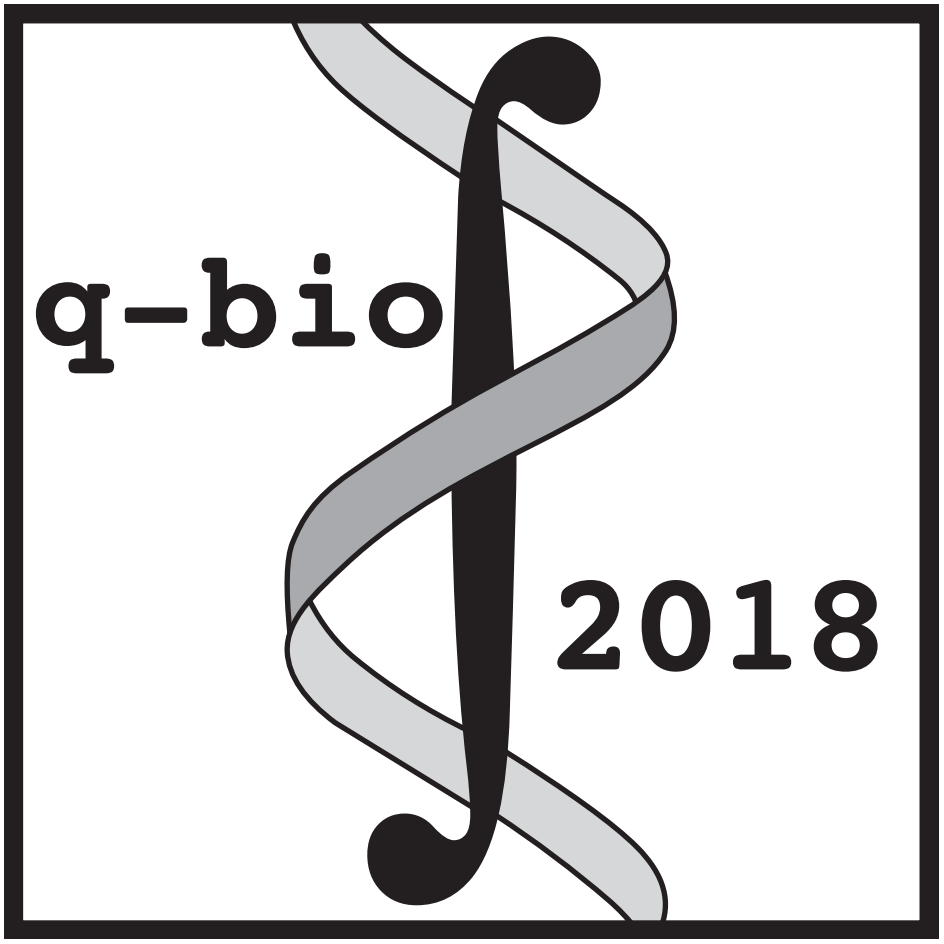
RICE | CTBP
Center for Theoretical Biological Physics

12th Annual q-bio Conference

June 26 - 29, 2018



BioScience Research Collaborative (BRC)
Rice University
Houston, TX

12th Annual q-bio Conference

JUNE 26 - 29, 2018
BioScience Research Collaborative (BRC)
Rice University, Houston, TX
PROGRAM

Unless otherwise noted, all talks are held in the BRC Auditorium (Room 103)

TUESDAY, JUNE 26

12:00 - 4:00 PM - CONFERENCE ATTENDEE CHECK-IN

BRC LOBBY – AUDITORUM 103 (PRE-FUNCTION SPACE)

1:00 - 3:30 PM - TUTORIALS/PARALLEL SESSIONS

1:00 – 3:30 PM	"Introduction to Rule-Based Modeling with BioNetGen"	BRC 282
1:00 – 3:30 PM	"Tutorial on StochSS: An Integrated Development Environment for Simulation and Analysis of Discrete Stochastic Biochemical Models"	BRC 284
4:00 - 4:30 PM	**Opening Remarks**	
4:30 - 5:30 PM	**Keynote Talk:** "Thinking About the Cancer-Immune Interaction"	Herbert Levine, *Rice University*
6:30 - 10:00 PM	**Night at the Museum Reception**	**Houston Museum of Natural Science**

WEDNESDAY, JUNE 27

SESSION I

Session Chair: Fred Mackintosh, *Rice University*

9:00 - 9:30 AM	**Invited Talk:** "Quantitative Behavior: Understanding What Animals Do and How Brains Control Them"	Joshua Shaevitz, *Princeton University*
9:30 - 9:50 AM	**Contributed Talk:** "Sign Epistasis Induced by Ribosome Collisions During Eukaryotic mRNA Translation"	Arvind Subramaniam, *Fred Hutchinson Research Center*
9:50 - 10:10 AM	**Contributed Talk:** "Properties of Gene Expression and Chromatin Structure with Mechanically Regulated Transcription"	Stuart Sevier, *Rice University*
10:10 - 10:30 AM	**Contributed Talk:** "Phase Transitions and Critical Phenomena in Mutualistic Communities under Invasion"	Bo Wang, *Stanford University*

10:30 - 11:00 AM - COFFEE BREAK

Program continued…

12th Annual q-bio Conference
PROGRAM, *continued…*

SESSION II

Session Chair: Oleg Igoshin, *Rice University*

11:00 - 11:30 am	**Invited Talk:** "Evolutionary Search and Races in the Adaptive Immune System"	Shenshen Wang, *University of California, Los Angeles*
11:30 – 11:50 am	**Contributed Talk:** "Precision in a Rush: Hunchback Pattern Formation in a Limited Time"	Huy Tran, *École Normale Supérieur*

11:50 - 12:20 AM - Spotlight Talk Session I

"Anomalous Diffusion, Spatial Coherence, and Viscoelasticity from the Epigenetic Energy Landscape of Human Chromosomes"	Michelle Di Pierro, *Rice University*
"Quantifying Epistatic Conservation Across Genetic and Environmental Backgrounds"	Andrew D. Mathis, *University of Texas Southwestern Medical Center*
"Modeling the Regulation of Cancer Metabolism: Interplay Between Glycolysis and OXPHOS"	Dongya Jia, *Rice University*
"Antigen Recognition at Immune-Cell Interfaces: Probing the Role of Mechanical Forces"	Steven M. Abel, *University of Tennessee, Knoxville*
"Agent-Based Model for Developmental Aggregation in *Myxococcus xanthus* Bacteria"	Zhaoyang Zhang, *Rice University*
"Effects of Cell Cycle Noise on Excitable Circuits"	William Ott, *University of Houston*

12:20 - 1:30 PM – LUNCH

BioScience Research Collaborative, Event/Exhibition Hall, Room 120

12:20 – 1:30 PM	**Special Session:** "NIH Peer Review and Research Initiatives for Quantitative Biologists"	Craig, N Giroux, *Center for Scientific Review/NIH* **(10th Floor, BRC Room 1060 A/B)**

SESSION III

Session Chair: Ido Golding, *Baylor College of Medicine*

1:30 - 2:00 PM	**Invited Talk:** "Hyaluronan Glycocalyx Physically Modulates Cell Adhesion and Migration"	Jennifer Curtis, *Georgia Institute of Technology*
2:00 - 2:30 PM	**Invited Talk:** "Comprehensive, High-Resolution Binding Energy Landscapes of Transcription Factor Binding"	Polly Fordyce, *Stanford University*

Program continued…

12th Annual q-bio Conference
PROGRAM, *continued…*

2:30 - 3:00 PM - SPOTLIGHT TALK SESSION II

"Using the Automated Building of Computational Models to Understand Cardiotoxic Drug Responses"	Robert P. Sheehan, *Harvard Medical School*
"Modulation of Conjugation in Pathogenic *Escherichia coli"*	Jonathan Bethke, *Duke University*
"The Role of Metabolic Spatiotemporal Dynamics in Modulating Biofilm Colony Expansion"	Federico Bocci, *Rice University*
"Spatiotemporal Dynamics of Phage-Biofilm Interactions"	Hemaa Selvakumar, *Georgia Institute of Technology*
"Ensemble response of immune repertoires to Vaccination"	Maximilian Puelma Touzel, *École Normale Supérieur*
"Modularity of the Metabolic Gene Network as a Prognostic Biomaker for Hepatocellular Carcinoma"	Fengdan Ye, *Rice University*

3:00 - 3:30 PM - COFFEE BREAK

SESSION IV

Session Chair: Joshua Shaevitz, *Princeton University*

3:30 - 3:50 PM	**Contributed Talk:** "An Artificial Cell-Cycle System: How Network Structures Modulate the Clock Functions"	Qiong Yang, *University of Michigan*
3:50 - 4:10 PM	**Contributed Talk:** "How to Model Cell Decision Making Errors"	Ali Abdi, *New Jersey Institute of Technology*
4:10 - 4:30 PM	**Contributed Talk:** "Design principles of Binding-Induced Selective Transport Through the nuclear Pore Complex"	Loren Hough, *University of Colorado, Boulder*
4:30 - 4:50 PM	**Contributed Talk:** "Increased Mortality Favors Fast-Growing Species in Microbial Communities"	Clare Abreu, *Massachusetts Institute of Technology, Gore Lab*
4:50 - 5:10 PM	**Contributed Talk:** "Synthetic NF-κB: A Building Approach to Study Complex Signaling Behaviors"	Ping Wei, *Center for Quantitative Biology, Peking University*

7:30 – 10:00 PM - POSTER SESSION

BioScience Research Collaborative, Event/Exhibition Hall, Room 120

THURSDAY, JUNE 28

SESSION V

Session Chair: Linchong You, *Duke University*

9:00 - 9:30 AM	**Invited Talk:** "The Outer Membrane is an Essential Load-Bearing Element in Gram-Negative Bacteria"	KC Huang, *Stanford University*
9:30 -10:00 AM	**Invited Talk:** "Ant Rafts and Maggot Flows"	David Hu, *Georgia Institute of Technology*

Program continued…

12th Annual q-bio Conference
PROGRAM, *continued…*

10:00 - 10:30 AM - SPOTLIGHT TALK SESSION III

"Dynamic Interrogation of the *Bacillus Subtilis* Sporulation Network using an Engineered Light-Switchable Two-Component System"	Sebastian Castillo-Hair, Rice University
"Temporal Precision of Regulated Gene Expression"	Shivam Gupta, Purdue University
"Carbohydrate Storage Determines Cell Size and Cell Fate"	Yanjie Liu, University of Texas Southwestern Medical Center
"Survival of the Chiral"	Ashish Bino George, Boston University
"Causes and Consequences of Asynchrony in *D. discoideum* Multicellular Development"	Ricardo Martinez-Garcia, *Princeton University*
"Measuring Transcription from a Single Gene Copy in Live *Escherichia coli* Cells"	Jing Zhang, *Baylor College of Medicine*

10:30 - 11:00 AM - COFFEE BREAK

SESSION VI

Session Chair: Yi Jiang, *Georgia State University*

11:00 - 11:30 AM	**Invited Talk:** "Regulatory Networks in Synthetic Microbial Consortia"	Matthew Bennett, *Rice University*
11:30 - 11:50 AM	**Contributed Talk:** "Impact of a Periodic Presence of Antimicrobial on Resistance Evolution"	Anne-Florence Bitbol, *Institut Curie*
11:50 - 12:10 PM	**Contributed Talk:** "A High Throughput Optogenetic System to Interrogate MAPK Signaling Network Dynamics at the Single Cell Level"	Coralie Dessauges, *University of Bern*
12:10 - 12:30 PM	**Contributed Talk:** "In-Sequence Coding of Noise in Gene Expression"	Enrique Balleza, *Harvard University*

12:30 – 1:45 PM - LUNCH

BioScience Research Collaborative, Event/Exhibition Hall, Room 120

SESSION VII

Session Chair: Elena Koslover, *University of California, San Diego*

1:45 - 2:15 PM	**Invited Talk:** "The Microbial Brain: Electrochemical Signaling and Higher-Order Coordination in Bacterial Communities"	Gurol Suel, *University of California, San Diego*
2:15 - 2:45 PM	**Invited Talk:** "When Things Go Wrong: A Breakdown of Breakdowns in Optimally Resilient Vascular Networks"	Eleni Katifori, *University of Pennsylvania*

Program continued…

12th Annual q-bio Conference
PROGRAM, *continued…*

2:45 - 3:15 PM - SPOTLIGHT TALK SESSION IV

"Multiplexing Cell-Cell Communication"	John Sexton, *Rice University*
"Role of Stochasticity in Mammalian Drug Resistance"	Kevin Farquhar, *Laufer Center for Physical and Quantitative Biology*
"Regulation of T Cell Expansion by the Dynamics of Antigen Presentation"	Andreas Mayer, *Lewis-Sigler Institute, Princeton University*
"Stochastic Modeling Quantifies Tumor Elimination and Evasion in the Setting of Immunotherapy"	Jason George, *Rice University*
"Optimal Sensory Network for the Unfolded Protein Response"	Wylie Stroberg, *University of Michigan*
"Intracellular Bistable Signaling in *Streptococcus mutans* Competence Regulation"	Simon Underhill, *University of Florida*

3:15 – 3:45 PM - COFFEE BREAK

SESSION VIII

Session Chair: Michael Diehl, *Rice University*

3:45 - 4:05 PM	**Contributed Talk:** "Metabolic Organization Through Glucose-Mediated Regulation of Mitochondrial Transport"	Elena Koslover, *University of California, San Diego*
4:05 - 4:35 pm	**Invited Talk:** "Membrane Criticality and Ion Channel Function"	Ben Machta, *Yale University*
4:35 - 4:55 PM	**Contributed Talk:** "Emergent Versus Individual-Based Multicellular Chemotaxis"	Sean Fancher, *Purdue University*
4:55 -5:15 PM	**Contributed Talk:** "Evolution of New Regulatory Functions"	Tamar Friedlander, *Hebrew University of Jerusalem*

7:30 – 10:00 PM - POSTER SESSION

BioScience Research Collaborative, Event/Exhibition Hall, Room 120

FRIDAY, JUNE 29

SESSION IX

Session Chair: Dmitry Makarov, *University of Texas*

9:00 - 9:30 am	**Invited Talk:** "Binary Transcriptional Control of Pattern Formation in Development"	Hernan Garcia, *University of California, Berkeley*
9:30 -10:00 am	**Invited Talk:** "Crowd Dynamics on a Lively Bridge"	Igor Belykh, *Georgia State University*
10:00 - 10:20 am	**Contributed Talk:** "*In situ* Analysis of Microbial Communities Using Expansion Microscopy"	Youngbin Lim, *Stanford University*
10:20 -10:40 am	**Contributed Talk:** "Homeostasis of Protein and mRNA Concentrations in Growing Cells"	Jie Lin, *Harvard University*

10:40 - 11:10 AM - COFFEE BREAK

Program continued…

12th Annual q-bio Conference
PROGRAM, *continued…*

SESSION X

Session Chair: Margaret Cheung-Wyker, *University of Houston*

11:10 - 11:40 am	**Invited Talk:** "Integrating Multiplex Single-Molecule Pull-Down (SiMPull) Data and Computational Modeling to Understand EGFR Signaling"	Diane Lidke, *University of New Mexico*
11:40 - 12:00 PM	**Contributed Talk:** "Biphasic Translation During Viral Infection"	Tomasz Lipniacki, *Polish Academy of Sciences*
12:00 - 12:20 PM	**Contributed Talk:** "Four Simple Rules That Are Sufficient to Generate the Mammalian Blastocyst"	Silas Boye Nissen, *Niels Bohr Institute*

12:20 - 1:30 PM – LUNCH

BioScience Research Collaborative, Event/Exhibition Hall, Room 120

SESSION XI

Session Chair: Cecilia Clementi, *Rice University*

1:30 - 2:00 PM	**Invited Talk:** "Biomolecular Folding and Dynamics from Single-Molecule Measurements"	Dmitrii Makarov, *University of Texas at Austin*
2:00 - 2:20 PM	**Contributed Talk:** "Partial Inhibition of HIV Cell-to-Cell Spread Results in More HIV Infected Lymph Node Cells"	Alex Sigal, *KwaZulu-Natal Research Institute for Tuberculosis and HIV*
2:20 - 2:40 PM	**Contributed Talk:** "Quantifying Physiology-Ecology Feedback Enables Prediction of Microbial Community Dynamics"	Wenying Shou, *Fred Hutchinson Research Center*
2:40 - 3:00 PM	**Contributed Talk:** "Predicting Influenza Vaccine Effectiveness from Evolution of the Dominant Epitope"	Melia Bonomo, *Rice University*

3:00 – 3:30 PM COFFEE BREAK

SESSION XII

Session Chair: Anatoly Kolomeisky, Rice University

3:30 - 4:00 PM	**Invited Talk:** "Bacterial Viruses Organize Subcellular Environments to Mediate Heterogeneous Development"	Lanying Zeng, *Texas A&M University*
4:00 - 4:20 PM	**Contributed Talk:** "Precision Measurements of Regulatory Energetics in Living Cells"	Justin Kinney, *Cold Spring Harbor Laboratory*
4:20 - 4:40 PM	**Contributed Talk:** "Cellular Sorting and Trafficking Mediated by Membrane Microdomains"	Ilya Levental, *University of Texas Health Science Center at Houston*
4:40 - 5:00 PM	**Contributed Talk:** "Biophysics of Adversarial Examples"	Thomas Rademaker, *McGill University*
6:30 - 9:30 PM	**Closing Banquet** "Wisdom of Hives and Mounds: Collective Problem Solving by Super-Organisms"	**Rice Memorial Center - Banquet Speaker:** Lakshminarayanan Mahadevan, *Harvard University*

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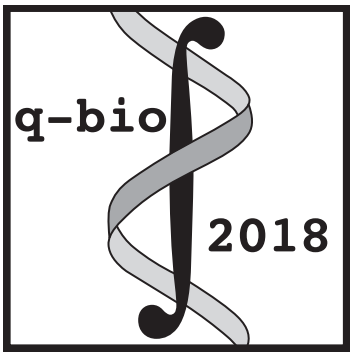
List of Posters



POSTER SIZE RESTRICTIONS AND MOUNTING INSTRUCTIONS

All posters **must not** exceed 48" x 48" (4ft. x 4ft.).
RECOMMEND poster size is 40" wide and 36" tall.

All posters can be taken to the Event/Exhibition Hall (BRC 120) for mounting
NO EARLIER than 7:00 AM on Wednesday, June 27th.

All posters must be mounted
NO LATER than 11:00 AM on Wednesday, June 27th.

TO AVOID VIOLATING FIRE AND SAFETY CODES PLEASE DO NOT MOVE THE ROLLING POSTER BOARDS

Please locate your assigned poster space based on the corresponding number in the list on the following pages.
ALL posters must be removed **NO LATER** than 11:00 AM on June 29th.

POSTER PRESENTATIONS

The Poster Sessions will be held in the BRC Event/Exhibition Hall, Room 120 on
Wednesday, June 27th and Thursday, June 28th.
ODD number posters will be presented on June 27th.
EVEN number posters will be presented on June 28th.

ALL POSTERS MUST BE REMOVED FROM BRC 120
NO LATER THAN 11:00 AM, FRIDAY, JUNE 29TH.

List of q-bio Posters

1. Steven M. Abel et al., *Antigen Recognition at Immune-Cell Interfaces: Probing the Role of Mechanical Forces*
2. Torin J. Adamson et al., *DockIt: Crowdsourcing Molecular Docking*
3. Kunal Aggarwal et al., *Investigating Potential Mechanisms for Negative Regulation of T Cell Activation*
4. Razan Alnahhas et al., *Culture Environment Impacts Synthetic Microbial Consortia Behavior*
5. Argenis Arriojas et al., *Quantifying Noise in General Stochastic Models of Post-Transcriptional Regulation of gene expression*
6. David E. Axelrod et al., *Computer Simulation of Colon Cancer Chemoprevention*
7. Jonathan Bethke et al., *Modulation of Conjugation in Pathogenic Escherichia coli*
8. Ashish Bino George et al., *Survival of the Chiral*
9. Federico Bocci et al., *The Role of Metabolic Spatiotemporal Dynamics in Modulating Biofilm Colony Expansion*
10. Ryan Butcher et al., *Optogenetic Investigation of B. subtilis Sporulation Network at the Single Cell Level*
11. Matthew Carpenter et al., *Probing ER-Related Stress with Lipid Markers*
12. Sebastian Castillo-Hair et al., *Dynamic Interrogation of the Bacillus Subtilis Sporulation Network Using an Engineered Light-Switchable Two-Component System*
13. Ankit Chandra et al., *Integrative Model of Actin, Adhesion, and Signaling Dynamics at the Leading Edge of Migrating* Cells
14. Srikiran Chandrasekaran et al., *Infection Risk of Antibiotic Resistant Bacteria*
15. Ke Chen et al., *Proteome Allocation Determines Thermosensitivity of Growth and Structure of the Evolutionary Landscape*
16. Ryan R. Cheng et al., *De novo Prediction of Human Chromosome Structures: Epigenetic Marking Patterns Encode Genome Architecture*
17. Sapna Chhabra et al., *Role of Paracrine Signaling and Cell Movements in the Self-Organization of Micropatterned Human Embryonic Stem Cell Colonies*
18. Selahittin Cinar et al., *Predicting Transcriptional Output of Synthetic Multi-Input Promoters*
19. Seth Coleman et al., *Modeling Viral Copy Number Dynamics During Infection by Bacteriophage lambda*
20. Vinícius G. Contessoto et al., *Protein Folding at Constant pH: Salt Concentration and pH Changing Protein Stability*
21. Keisha Cook et al., *Decreasing the Computational Time of Biochemical Systems via Parallelism*
22. Ojan Khatib Damavandi et al., *Inflationary Embryology*
23. Jon Christian L. David et al., *Modeling Molecular Motor* Procession
24. Aram Davtyan et al., *Unveiling Molecular Mechanisms of Kinesin-5*
25. Michele Di Pierro et al., *Anomalous Diffusion, Spatial Coherence, and Viscoelasticity from the Epigenetic Energy Landscape of Human Chromosomes*
26. Kevin Farquhar et al., *Role of Stochasticity in Mammalian Drug Resistance*
27. Daniel Gamermann et al., *Caracterization of PPI and Metabolic Networks*
28. Jason George et al., *Stochastic Modeling Quantifies Tumor Elimination and Evasion in the Setting of Immunotherapy*

29. Gennady Gorin et al., *Simulating and Fitting Stochastic Models of RNA Transcription via the Gillespie Algorithm*
30. Yueyang Eric Gou et al., *Characterization and Application of Fluorescent Indicators for Imaging Neuronal Voltage Computations*
31. Sanjana Gupta et al., *Evaluation of Parallel Tempering to Accelerate Bayesian Parameter Estimation in Systems Biology*
32. Shivam Gupta et al., *Temporal Precision of Regulated Gene Expression*
33. Kelsey Hallinen et al., *Population Dynamics of Cooperative Resistance in E. faecalis*
34. Jungmin Han et al., *Merging Multiple Data Sets to Study HCV*
35. Rasoul Hekmati et al., *Machine Learning to Evaluate fMRI Recordings of Brain Activity in Epileptic Patients*
36. R. Antonio Herrera et al., *Cliffs & Canals in Waddington's Landscape*
37. Andrew J. Hirning et al., *Dynamic Responses of LacI/GalR Chimera-Based Transcriptional Logic Gates*
38. Danh-Tai Hoang et al., *Network Inference with Latent Variables*
39. Kabir Husain et al., *Programming Fitness Landscapes by Sparse Epistasis*
40. Dumitru A. Iacobas et al., *The GMR Approach of Cancer Gene Therapy*
41. Dongya Jia et al., *Modeling the Regulation of Cancer Metabolism: Interplay between Glycolysis and OXPHOS*
42. Lavisha Jindal et al., *Plasmid Behavior under Par System Control*
43. Bhargav Karamched et al., *Stochastic Model of Cell Alignment in Traps*
44. Marek Kochańczyk et al., *Information Processing in the NF-κB Pathway*
45. Pawel Kocieniewski et al., *RAF1 Coordinates Proliferation and Motility*
46. Daria Kogut et al., *Modeling miRNA-Mediated Translation Control*
47. Vivek Kohar et al., *Effect of Noise and Parametric Variations on Gene Regulatory Circuit Dynamics*
48. Jesse M. Kreger et al., *Mathematical Models of Virus Infections*
49. Dana Krepel et al., *Deciphering the Structure of the Condensin Protein Complex*
50. Niraj Kumar et al., *Stochastic Modeling of Post-Transcriptional Regulation of Gene Expression by Non-Coding RNAs*
51. Ariel Langevin et al., *Stress Introduction Rate Impacts Acquisition of Antibiotic Tolerance and Resistance*
52. Yerim Lee et al., *Quantification of Ras Membrane Diffusion and Multimer Formation in Live Cells*
53. Kyunghyun (Katie) Lee et al., *Differences Between Telomerase Activation and ALT Based on the G-Networks*
54. Chung Yin (Joey) Leung et al., *The Synergistic Effect of Host Immunity with Phage and Probiotic Therapy Against Bacterial Pathogens*
55. Xuefei Li et al., *Spatial Profiles of Tumor-Infiltrating T Cells*
56. Xiaona Li et al., *Mechanics Before Chemistry: Tensile Stress Induced Cytoskeletal Reorganization*
57. Xingcheng Lin et al., *High-resolution Prediction and Refinement of Protein Structures*
58. Yanjie Liu et al., *Carbohydrate Storage Determines Cell Size and Cell Fate*
59. Zhuohe Liu et al., *Automated Multimodal Screening of Fluorescent Biosensors of Membrane Potential*
60. Leonardo López-Ortiz et al., *Stability and Accuracy Analysis of the Circadian Clock Couple with Metabolism*
61. Xiaoyu Lu et al., *Structure Guided Genetically Encoded Voltage Indicator Engineering*

62. Nan Luo et al., *Stress-Induced Division of Labor Underlies Bacterial Colony Branching*
63. Sridevi Maharaj et al., *BLANT: Sampling Graphlets in a Flash*
64. Ricardo Martinez-Garcia et al., *Causes and Consequences of Asynchrony in D. discoideum Multicellular Development*
65. Andrew D. Mathis et al., *Quantifying Epistatic Conservation Across Genetic and Environmental Backgrounds*
66. Andreas Mayer et al., *Regulation of T Cell Expansion by the Dynamics of Antigen Presentation*
67. James McCormick et al., *Determining the Internal Allosteric Architecture of DHFR With Saturation Mutagenesis*
68. Joseph P. McKenna et al., *Modeling Adipose Tissue Hormone Regulation*
69. R. Tyler McLaughlin et al., *Multiplexed Live-Cell Signaling Dynamics of the Cytoskeletal and Phospholipid Scaffold, IOGAP1*
70. Kareem Mehrabiani et al., *Predicting Actin Interfaces from Genomic Data*
71. Justyna Mika et al., *Does Diversity of T Cell Receptors Functionality Depend on Age and Sex?*
72. Robert Mines et al., *Wnt-Notch Crosstalk Tunes Intestinal Crypt Spatial Patterning*
73. Chaitanya Mokashi et al., *Cellular Responses to Dynamic Patterns of Cytokine Stimulation*
74. Thuy N. Nguyen et al., *Amino Acid Sequence Constraints and Co-Evolution across a Metabolic Enzyme Pair*
75. Jamie Nosbisch et al., *Feedback Loops at the Level of Lipid Metabolism Enhance Sensitivity and Robustness in Models of Chemotactic Gradient Sensing*
76. Jackson O'Brien et al., *Decoding High-dimensional Temporal Dynamics in Gene-regulatory Networks*
77. William Ott et al., *Effects of Cell Cycle Noise on Excitable Circuits*
78. Weerapat Pittayakanchit et al., *Trade-off between Resistance to External and Internal Fluctuation in Biophysical Sensing*
79. Maximilian Puelma Touzel et al., *Ensemble Response of Immune Repertoires to Vaccination*
80. Alberto Ramírez-Hurtado et al., *Synchronization Modes of the Mechanical Response in Mouse Heart*
81. Satyajit Rao et al., *Dynamics of Stress Response in Bacteria*
82. Brandon Reid et al., *Tensor-Based Approximation of the Stationary Solution to the Chemical Master Equation*
83. Vedant Sachdeva et al., *Tuning Evolution Towards Generalists by Resonant Environmental Cycling*
84. Andrew Schober et al., *Collective Genetic Units in Bacterial Metabolism*
85. Hemaa Selvakumar et al., *Spatiotemporal Dynamics of Phage-Biofilm Interactions*
86. John Sexton et al., *Multiplexing Cell-Cell Communication*
87. Robert P. Sheehan et al., *Using the Automated Building of Computational Models to Understand Cardiotoxic Drug Responses*
88. Jaeoh Shin et al., *How to Find a Small Target on a Surface? Surface-Assisted Search Dynamics*
89. Matthew Smart et al., *Population Dynamics of Epigenetic Oncogenesis*
90. Jaroslaw Smieja et al., *Modeling Adjuvant Chemo- and Radiotherapy*
91. Mara P. Steinkamp et al., *Quantification of Tumor Burden in Patient-Derived Orthotopic Models of Ovarian Cancer by Fluorescent and Bioluminescent 3-D Imaging*
92. Wylie Stroberg et al., *Optimal Sensory Network for the Unfolded Protein Response*
93. Peter Suzuki and Jihwan Lee et al., *High-Throughput Screening of Fluorescent Probes for in vivo Imaging*

94. Tavella Franco et al., *Coarse Grained Constant-pH Protein Dynamics*
95. Nihal Ezgi Temamogullari et al., *A Yeast Segmentation and Tracking Algorithm*
96. Shubham Tripathi et al., *Analysis of Hierarchy in Gene Expression Reveals Principles Underlying Metastatic Aggressiveness of Inflammatory Breast Cancer*
97. Karolina Tudelska et al., *Secreted INFβ Coordinates Antiviral Response*
98. Simon Underhill et al., *Intracellular Bistable Signaling in Streptococcus mutans Competence Regulation*
99. John Vastola et al., *Towards a Coherent Theory of Stochastic Gene Dynamics: from Landscapes to Green Field Theory*
100. Qian Wang et al., *Inter-Head Tension of Cytoplasmic Dynein Regulates the Coordination between Two Heads*
101. Mengyu Wang et al., *Measuring Transcription at a Single Gene Copy Illuminates RNA Dynamics and Reveals Intracellular Correlations*
102. Huijing Wang et al., *Differentially Regulated Pathway Analysis of RNA-Seq by Deep Learning*
103. Teng Wang et al., *Predicting Plasmid Maintenance and Abundance in Complex Microbial Community*
104. Zikai Xu et al., *Noise Analysis in Biochemical Complex Formation*
105. Fengdan Ye et al., *Modularity of the Metabolic Gene Network as a Prognostic Biomaker for Hepatocellular Carcinoma*
106. Jin Yang et al., *An Integrative Computational Approach for Engineering Genetically Encoded Voltage Indicators*
107. Jin Yang et al., *A Synthetic Gene Circuit with Tunable Expression Level and Dosage Compensation for Mammalian Cells*
108. Jing Zhang et al., *Measuring Transcription from a Single Gene Copy in Live Escherichia coli Cells*
109. Zhaoyang Zhang et al., *Agent-based Model for Developmental Aggregation in Myxococcus xanthus Bacteria*
110. Maxwell De Jong et al., *Tuning Spatial Profiles of Selection Pressure to Modulate the Evolution of Resistance*

RICE | CTBP
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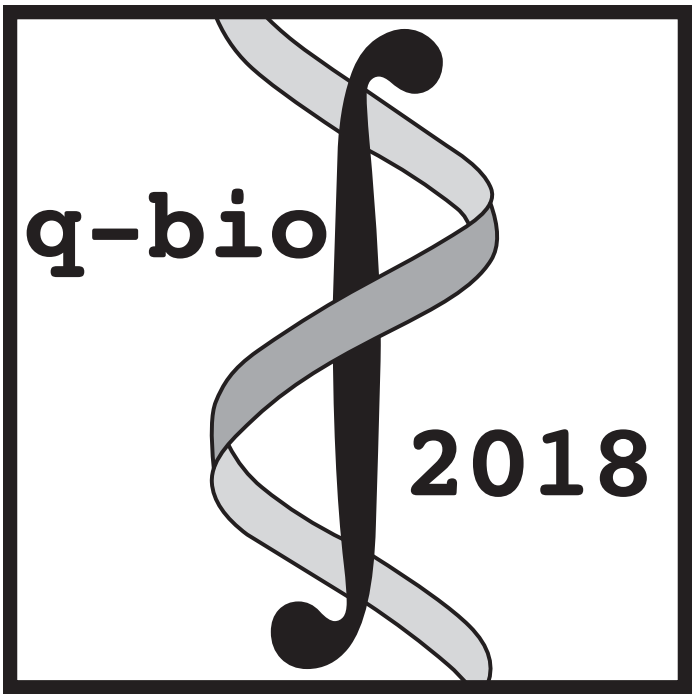
12th Annual q-bio Conference

Invited Speaker Abstracts



The invited speaker abstracts are presented in alphabetical order by the presenters' last name.

Crowd dynamics on a lively bridge

Igor Belykh[1], Russell Jeter[1], and Vladimir Belykh[2]

Several modern footbridges around the world have experienced large lateral vibrations during crowd loading events. The onset of large-amplitude bridge wobbling has generally been attributed to crowd synchrony; although, its role in the initiation of wobbling has been challenged. In this talk, we will introduce biomechanically-inspired models of human locomotion and use them (i) to study the contribution of a single pedestrian into overall, possibly bistable, crowd dynamics [1] and (ii) to investigate to what degree pedestrian synchrony must be present for a bridge to wobble significantly and what is a critical crowd size [2]. The pedestrian models can be used as "crash test dummies" when numerically probing a specific bridge design. This is particularly important because the U.S. code for designing pedestrian bridges does not contain explicit guidelines that account for the collective pedestrian behavior.

REFERENCES

[1] Belykh I, Jeter R, Belykh V (2016) Bistable gaits and wobbling induced by pedestrian-bridge interactions. *Chaos* **26**, 116314.
[2] Belykh I, Jeter R, Belykh V (2017) Foot force models of crowd dynamics on a wobbly bridge. *Science Adv.* **3**, e1701512

Acknowledgements: This work was funded by NSF grant DMS-1616345.

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Regulatory networks in synthetic microbial consortia

Matthew R. Bennett[1,2]

Synthetic biology aims to engineer biological systems for practical purposes through the manipulation of gene regulation and enzymatic processes within the host. The vast majority of synthetic gene circuits operate within a single cell or isogenic colony of bacteria. However, utilizing multiple strains or species of bacteria simultaneously greatly expands the possibilities of synthetic biology. These systems, called synthetic microbial consortia, more closely resemble the naturally heterogeneous environments of bacteria, such as gut microbiomes or biofilms. Here, I will describe our recent efforts to design, construct, and analyze multi-scale regulatory structures in synthetic microbial consortia. This work includes: 1) creating novel transcription factors and promoters that allow cells to sense and respond to complex environmental conditions; 2) engineering multiple intercellular signaling mechanisms to create population-level regulatory pathways; and 3) developing mathematical techniques that accurately model and predict the dynamics of gene regulation in competing bacterial strains. Using these techniques, we have been able to engineer novel synthetic microbial consortia that exhibit complex emergent behaviors, such as oscillations and global bistability. For example, in one system we used two different bacterial quorum sensing systems to construct an “activator” strain and a “repressor” strain that respectively up- and down-regulate gene expression in both strains. When co-cultured in a microfluidic device, the two strains form coupled positive and negative feedback loops at the population-level. The interacting strains exhibit robust, synchronized oscillations that are absent if either strain is cultured in isolation.

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Hyaluronan Glycocalyx Physically Modulates Cell Adhesion and Migration

Shlomi Cohen [1,2], Rebecca Keate [1,2], Patrycja Kotowska [1,2], Patrick Chang [1,2], Dennis Zhou [2,3], Andres Garcia [2,3], Jennifer E. Curtis[1,2],

Polymer brushes are used to tailor and engineer fundamental surface properties in a wide range of applications. Biological organisms may leverage the same strategy at cell surfaces via the presentation of the glycocalyx, a sugar-rich cell surface bound polymer matrix. In particular, we focus on the glycocalyx associated with biological processes involving cell dynamics and rearrangements – events that require exquisite control of cell adhesion and migration. From embryogenesis to wound healing to synaptogenesis to cancer metastasis, changes in the hyaluronan-rich glycocalyx and HA milieu is connected to these processes. Hyaluronan (HA) is a linear polyelectrolyte whose gigantic size (up to 20 microns) creates significant physical effects when it is bound to the cell surface. These impacts are enhanced by its binding to and potential for dense aggregation of bottlebrush proteoglycans.

Our lab is interested in the consequences of maintaining a bulky sugar matrix on the surface of cells, whether it is neurons in the brain, cancer cells in a tumor, or fibroblasts in a wound. How does the glycocalyx interfere with or alter receptor-ligand binding and cell-cell contacts? How is it possible that integrins manage to bind to extracellular matrix (ECM) proteins? When the glycocalyx is compressed at an interface after cell-ECM binding, how do the forces alter the cell adhesion strength? Here we present quantitative measurements demonstrating that indeed, compressed HA glycocalyx reduces cell adhesion strength. Further, we show how manipulating cell adhesion strength with HA glycocalyx is an independent parameter to tune cell migration speed. Together these data suggest that HA glycocalyx works in concert with adhesion receptors to modulate the strength cell adhesion thru physical repulsion. This is an interesting outcome because it provides evidence that cell integration into tissues – a fundamental aspect of multicellular organisms – is controlled not just via adhesion, but in some cases, via an interplay of adhesive and repulsive elements.

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Comprehensive, High-Resolution Binding Energy Landscapes of Transcription Factor

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Gene expression *in vivo* is regulated primarily by transcription factor (TF) proteins that bind regulatory sequences within genomic DNA, thereby recruiting or blocking the transcriptional machinery to either activate or repress transcription. However, it remains difficult to predict TF occupancy *in vivo* based on regulatory sequence. Thermodynamic models that explicitly consider TF concentrations and the change in Gibbs free energy upon binding have shown the greatest success; however, a scarcity of energetic data has precluded their broad implementation. Although high-throughput *in vitro* transcription factor (TF) binding site characterization techniques have greatly increasing the speed of TF target site discovery, these techniques sacrifice the ability to measure binding energies in order to query a large sequence space. Here, we present a novel high-throughput experimental assay and analysis pipeline capable of estimating changes in binding energies for > 1 million sequences in parallel at high resolution. To demonstrate the capabilities of the assay, we took advantage of existing specificity information to refine the binding motifs of two model TFs from S. cerevisiae, Pho4 and Cbf1. By coupling the existing MITOMI microfluidic platform to a DNA sequencing-based readout and high-capacity neural network models, we generated comprehensive thermodynamic landscapes for an exhaustive library of all 10-mer sequences flanking the shared Pho4 and Cbf1 consensus motif. These measurements reveal that sequence specificity extends far beyond the known consensus to distal flanking positions and that the extended binding specificity of Cbf1, in particular, is surprisingly epistatic. When combined with existing in vivo datasets, we find that sites occupied by TFs in vivo are both energetically and mutationally distant from the lowest-energy sequences, providing evidence that even small differences in binding energies can provide a basis for evolutionary selection.

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Binary Transcriptional Control of Pattern Formation in Development

Nicholas Lammers[1], Vahe Galstyan[2], Armando Reimer[1], Sean A. Meddin[3], Chris Wiggins[4], and Hernan G. Garcia[1,3,5,6]

During embryonic development, tightly choreographed patterns of gene expression specify cell fate. Output transcriptional activity is characterized by bursts of gene expression, where promoters stochastically transition between transcriptional ON and OFF states. Here, we quantitatively test the hypothesis that transcriptional bursts are the main drivers of pattern formation. We quantify the transcriptional activity that leads to the formation of the widely studied stripe 2 of the *even-skipped* gene in living embryos of the fruit fly at the single cell level. We develop a novel memory-adjusted hidden Markov model to extract the parameters governing transcriptional bursting and show that that promoter switching dynamics cannot quantitatively explain pattern formation in the embryo. We discover that, in addition to bursting, the window of time over which genes engage in transcription is also regulated along the embryo, and that this digital regulation of when promoters become competent for transcription is the main driver of pattern formation. Thus, in order to reveal the molecular rules behind the transcriptional control of pattern formation and reach a predictive understanding of development, a non-steady-state and quantitative description of both the regulation of promoter bursting and the transcriptional time window needs to be adopted.

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Ant rafts and maggot flows

David L. Hu[1] and Olga Shishkov[1]

We present two model systems for studying swarm behavior in insects. Fire ants link their bodies together to build waterproof rafts to survive floods. We conduct mechanical tests to show that ant rafts can flow like a liquid or spring back like a solid. These properties can enhance their chance of survival on rough waters. In contrast, the black soldier fly larva is an insect that lives on dry land, but deals with large numbers of its neighbors as it feeds on rotting fruit and carcasses. When feeding around food objects, we show that the maggots generate coherent flows, which can increase the average feeding rate of the colony. In both systems, the continuous motion of insects leads to many of the desirable properties observed.

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The outer membrane is an essential load-bearing element in Gram-negative bacteria

Kerwyn Casey Huang[1]

Gram-negative bacteria have a complex cell envelope consisting of a plasma membrane, a peptidoglycan cell wall, and an outer membrane. The envelope is a selective chemical barrier that defines cell shape and allows the cell to sustain large mechanical loads such as turgor pressure. It is widely believed that the covalently cross-linked cell wall grants the envelope its mechanical properties. We have recently demonstrated that the stiffness and strength of *Escherichia coli* cells are largely due to the outer membrane. Compromising the outer membrane, chemically or genetically, greatly increased deformation of the cell envelope in response to stretching, bending, and indentation forces, and induced elevated levels of cell lysis upon mechanical perturbation and L-form proliferation. Both lipopolysaccharides and proteins contributed to outer membrane stiffness. These findings overturn the prevailing dogma that the cell wall is the dominant mechanical element within the Gram-negative bacterial cell, instead demonstrating that the outer membrane is at least as stiff as the cell wall and that mechanical loads are often balanced between these structures.

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When things go wrong: A breakdown of breakdowns in optimally resilient vascular networks

Tatyana Gavrilchenko[1] and Eleni Katifori[2]

Life above a certain size relies on a circulatory system for oxygen and nutrient delivery; without it, no complex animal would exceed a few millimeters. As a result, plants, animals and fungi have developed circulatory systems of striking complexity. Typically, these systems have to satisfy competing demands to operate efficiently and robustly while confronted with an ever-changing environment [1,2]. The architecture of these networks, as defined by the topology and edge weights, determines how efficiently the networks perform their function and represents a trade-off between optimizing power dissipation, construction cost, and tolerance to damage [3,4].

In this work we delve further in the vascular network's tolerance to damage. Loosely modeling ischemic strokes, we quantify the extent of functional disruption a vascular network undergoes when a vessel is occluded. We study how the topology and hierarchy of the network can influence the extent of the disruption. We find that a highly conducting vessel establishes a region around it where, if an occlusion where to occur, the effects on the global flow profile are minimized. We discuss what these results mean for the design of optimally tolerant vascular networks.

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Thinking about the cancer-immune interaction

J. George, X. Li, D. Kessler and H. Levine

The world of cancer research is excitedly pursuing the idea that the immune system can be harnessed to attack cancer cells and thereby provide long term remission for heretofore untreatable cases [1]. In order for this work, T-lymphocytes must detect the cancer cell via its aberrant proteins, the T-cells must be able to infiltrate the tumor so as to reach the cancer cells, and the cancer cells must not be able to inhibit immune response or evolve immune resistance [2]. This talk will describe our initial efforts [3,4,5] to create simple models of these processes to enable the beginnings of a quantitative approach to this entire field of study. Along the way, we encounter many interesting examples of the application of statistical physics to both cellular and tissue-level biology.

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Integrating Multiplex Single-Molecule Pull-Down (SiMPull) Data and Computational Modeling to Understand EGFR Signaling

Emanuel Salazar-Cavazos[1], Bridget S. Wilson[1], Keith A. Lidke[2], William S. Hlavacek[3] and Diane S. Lidke[1*]

The Epidermal Growth Factor Receptor (EGFR) plays an important role in both physiological and cancer-related processes. To study the factors that influence EGFR phosphorylation, we have coupled single-molecule microscopy experiments with rule-based modeling of EGFR signaling. We have made technical improvements over the previously described Single-Molecule Pull-down (SiMPull) assay to facilitate direct detection of the phosphorylation state of thousands of individual receptors. We monitored the phosphorylation of EGFR-GFP expressed in CHO cells. By counting the number of GFP molecules colocalized with a red-emitting fluorescent antibody, the fraction of receptors phosphorylated at a specific tyrosine residue was determined. We found that only a subpopulation of EGFR become phosphorylated under what is considered maximal activation conditions and that the extent of phosphorylation varies by tyrosine residue. Three-color imaging of EGFR-GFP with antibodies directed to two distinct phospho-sites revealed that multi-site phosphorylation frequently occurs.

To better understand the implications of these results, we created a computational model of EGFR signaling. In our model, a phosphorylated site cannot be dephosphorylated if it is bound by one of its protein binding partners, such as the adaptor protein Grb2. Our model predicted that an increase in the abundance of Grb2 would result in a higher percentage of receptors phosphorylated at sites to which Grb2 binds. In agreement with this prediction, overexpression of Grb2 caused a dramatic increase in the phosphorylation levels of a Grb2-binding site in EGFR (Y1068), but not in a site which Grb2 does not bind (Y1173). These results demonstrate the importance of receptor:adaptor protein ratios in modulating receptor phosphorylation patterns. Since protein abundance varies across cell types and is often altered in cancer, we are currently extending these studies to cancer cells lines with markedly different EGFR:Grb2 ratios.

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Membrane criticality and ion channel function

Benjamin B Machta[1,2]

The plasma membrane surrounding animal cells is a two-dimensional liquid that is home to many of the complex processes that carry out biological function. Recent experiments have demonstrated that this two-dimensional liquid is close to a miscibility critical point, distinguished by emergent time and length scales much larger than individual molecules. I will talk about what this critical point means for the function of membrane bound proteins, and especially ion channels, mediating long-ranged forces, sensitive allosteric regulation and non-Markovian dynamics. I'll also give some qualitative comparisons to phase separation of proteins into coexisting three dimensional fluid phases in the cytoplasm, which has emerged as a common theme in diverse cellular processes. I will also report on our recent experimental progress demonstrating that anesthetics move membranes away from criticality, and that anesthetic reversers also reverse effects on membrane criticality. Our results suggest a picture in which membrane bound proteins are highly sensitive to the near-critical solvent properties of the membrane in which they are embedded

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Biomolecular folding and dynamics from single-molecule measurements

Dmitrii E. Makarov[1]

Biological function of proteins and other biomolecules is commonly based on their ability to undergo large conformational changes via activated barrier crossing. Recent single-molecule experiments achieved time resolution sufficient to catch biomolecules in the act of crossing free energy barriers as they fold or undergo other structural changes, offering a window into the elusive reaction “mechanisms”. At the same time, molecular simulations have attained timescales where they begin to overlap with single-molecule measurements. These developments put common models and theories of activated barrier crossing to test. In this talk I will describe our recent efforts to understand, using theory, simulations, and experimental information, what happens during the most interesting part of a single-molecule trajectory where it undergoes a large thermal fluctuation allowing it to transition between two distinct conformations (such as the folded and unfolded states).
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Quantitative Behavior: Understanding what animals do and how brains control them

Joshua W. Shaevitz [1]

I will present recent work from my group and our collaborators on the use of unsupervised machine learning to define and quantify stereotyped behaviors performed by animals. Through the use of real-time optogenetic readout and control of neuronal activity, we have made progress on understanding the systems that convert external stimuli into behavioral changes and those that allow animals to dynamically select from a suite of hundreds of unique actions.

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The microbial brain: Electrochemical signaling and higher-order coordination in bacterial communities

G. Süel[1]

Abstract:
Most bacteria on our planet reside in densely packed communities, yet we have little understanding of bacterial behavior in such communities. My laboratory has uncovered ion channel-mediated long-range signaling within such bacterial communities. This electrochemical cell-to-cell signaling gives rise to unexpected emergent behaviors that are organized in space and time. We are working to understand the underlying electrophysiology of bacterial communities that allows them to cope with stress (such as antibiotic exposure) as a collective. I will discuss our recent efforts to develop new devices, techniques and theoretical frameworks to understand and control the electrophysiology and behavior of bacterial communities.

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Evolutionary search and races in the adaptive immune system

Shenshen Wang[1]

Biological evolution is a search process: microbes rapidly evolve to seek variants capable of surviving antibiotics; antibody-expressing B cells undergo accelerated Darwinian evolution to discover high-affinity mutants that bind and neutralize pathogens. Such rapid evolutionary search takes place in environments that are neither static nor completely unrelated in time, which poses a challenge to our understanding of the emergent properties of evolving systems. What might be useful spaces to describe adaptive "moves" in changing "landscapes"? How would correlation across environments impact the capacity of evolutionary novelty? Can environmental dynamics turn evolutionary constraints into opportunities of speedy paths? In this talk, I will present our recent attempts, using theoretical descriptions and numerical schemes, toward addressing these questions in the context of evolutionary races in the adaptive immune system, where immune repertoires evolve in a varying environment that is either programmed or adaptively changing. We find that epistatic interactions, generally regarded as constraining the set of adaptive paths, may play a beneficial role in evolving generalist solutions. Furthermore, new pathways toward generalists – the desirable outcome of vaccine strategies – may open as a result of meeting the demands for survival and adaptation that operate on different time scales.

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Bacterial Viruses Organize Subcellular Environments to Mediate Heterogeneous Development

Lanying Zeng[1]

Spatial organization underpins biological processes in lifeforms. For complex organisms, body parts must develop properly in space, and within the cells making up these organisms, membranes separate organelles with different functions. Simpler organisms, like bacteria, lack intracellular membranes, but possess alternate strategies for organization, where certain proteins are confined to specific cellular locations to consolidate their functions. Viruses, among the simplest lifeforms, have also been reported to display organized development, further underscoring the essentiality of structure in biology.

Bacterial viruses, known as bacteriophages or phages, are utilized as model systems for studying advanced cellular functions. Phage lambda infects *Escherichia coli* and propagates via distinct pathways, either lysis or lysogeny, where this fate-selection is a paradigm for cellular decision-making. Recent efforts probing decision-making at the subcellular level have discovered that phages behave as individual interacting intracellular entities when processing decisions, generating considerable insights into how complex decision-making may occur [1-4]. However, little is known about this phage's spatial development within its host and how spatial organization might affect cellular decision-making.

Here, we characterize how phage lambda develops in subcellular space, from infection through lysis, using multiple fluorescence reporters targeting phage DNA replication, essential host resources, phage transcription, and phage assembly. We observe that these processes coalesce into distinct areas of the host cell during infection to coordinate phage development, reminiscent of virus factories, where multiple factories may be present in single cells. Remarkably, different intracellular factories quantitatively diverge at the viral DNA, mRNA and protein levels, suggesting the existence of bona-fide microenvironments within cells. We propose that individual viruses drive the formation of disparate territories, implying that phages may sense separate environments during decision-making and development. The conclusions from this work may shed significant light on the detailed molecular mechanisms of cell-fate bifurcation.

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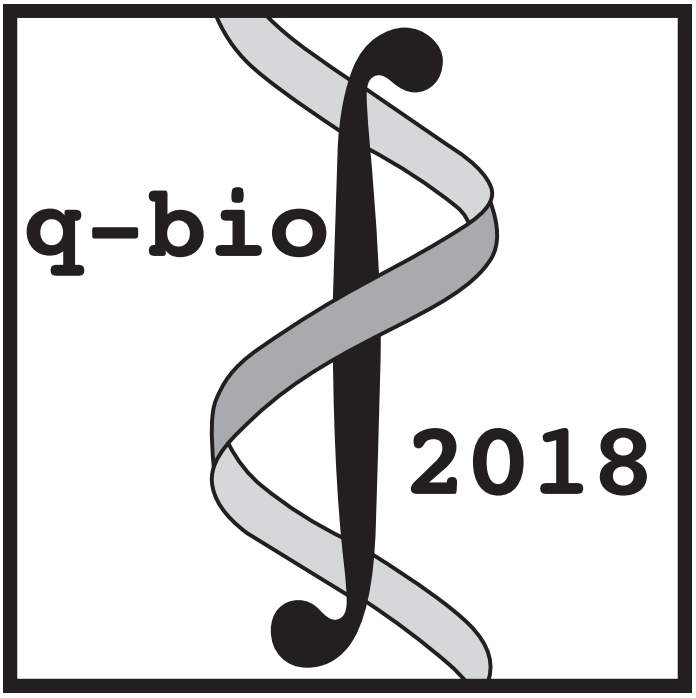

These abstracts are presented in alphabetical order by last name.

How to Model Cell Decision Making Errors

Iman Habibi[1], Raymond Cheong[2], Tomasz Lipniacki[3], Andre Levchenko[4], Effat S. Emamian[5] and Ali Abdi[1,6]

***Short Abstract* — Due to signal transduction noise, cells respond differently to similar inputs, which may result in incorrect cell decisions. Here we present a method for modeling decision making processes in cells, and apply it to an important signaling pathway that is involved in cell survival. Our method reveals that cells can make two types of incorrect decisions. We compute the likelihood of these decisions using single cell experimental data, and demonstrate how these incorrect decisions are affected by the noise. We also study the connection between information transmission capacity of the pathway and decision making errors using experimental data.**

***Keywords* — Cell decision making, noise, signaling errors, signaling networks, information capacity, TNF, NF-κB.**

I. Introduction

EACH cell continuously receives signals from its surrounding environment and is supposed to make correct decisions, i.e., respond properly to various signals and initiate certain cellular functions. In various organisms ranging from viruses to bacteria, yeast, lower metazoans and finally complex organisms such as mammals, various decisions are made in the presence of noise [1]. The noise, however, causes the cell to respond differently to the same input, which may result in incorrect (unexpected) cell responses [2]. Here we introduce a method for modeling and measurement of decision making processes in cells, using statistical signal processing and decision theory concepts. Given the importance of single cell modeling approaches to understand the biochemical processes in each individual cell [3], we use single cell experimental data [2].

II. The Method and the Results

Details of the method and mathematical formulation can be found in [4]. Analysis of the tumor necrosis factor (TNF) signaling pathway which regulates the transcription factor Nuclear Factor κB (NF-κB) using this method has identified two types of incorrect cell decisions called false alarm and miss. These two events represent, respectively, declaring a signal which is not present and missing a signal that does exist. Using single cell experimental data and the developed method, we have computed false alarm and miss error probabilities in wild-type cells and have investigated how they depend on the signal transduction noise level [4].

We also have shown that in the presence of abnormalities in a cell, decision making processes can be significantly affected, compared to a wild-type cell, and the method is able to model and measure such effects. In the TNF-NF-κB pathway, the method has revealed changes in false alarm and miss probabilities in A20-deficient cells, caused by cell's inability to inhibit TNF-induced NF-κB response [4]. In biological terms, a higher false alarm metric in this abnormal TNF signaling system indicates perceiving more cytokine signals which in fact do not exist at the system input, whereas a higher miss metric indicates that it is highly likely to miss signals that actually exist.

In addition to these findings, we have also studied how the developed cell decision making model relates to the information transmission capacity and dynamical modeling of the signaling pathway [4].

III. Conclusion

This study demonstrates the ability of a new method for computing cell decision making error probabilities under normal and abnormal conditions, and in the presence of transduction noise uncertainty. Using the method, decision making errors of molecular networks can be modeled. Such models are useful for understanding and developing treatments for pathological processes such as inflammation, various cancers and autoimmune diseases.

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Antigen Recognition at Immune-Cell Interfaces: Probing the Role of Mechanical Forces

Robert H. Pullen III[1,2], Bing Li[1], and Steven M. Abel[1-3]

***Short Abstract* — One of the central problems in immunology involves molecular recognition at cell-cell interfaces. Fascinating recent experiments have revealed that mechanical forces regulate processes by which T cells and B cells identify molecular signatures of pathogens. In this work, we develop hybrid computational models that account for key biophysical properties of immune cell interfaces, including stochastic receptor-ligand binding kinetics, membrane mechanics, and actin-mediated forces on the membrane. We use these models to investigate how mechanical forces modulate the interactions of T cells and B cells with surface-presented antigens.**

I. Introduction

Understanding mechanical forces and dynamics at membranes is critical for understanding how T cells and B cells distinguish between self and foreign ligands, and how they subsequently respond to antigens [1]. Both cell types use surface receptors to identify molecular signatures of pathogens. Because T cells and B cells physically engage other cells in direct contact, they are subject to a variety of mechanical forces. For example, their surface receptors experience forces due to membrane deformations, cell motion, and coupling to the dynamic actin cytoskeleton. Mechanical forces play important roles in the regulation of immune cell activation [1,2], and new experimental probes are beginning to provide details about forces at cell-cell interfaces [3].

II. Results

In this work, we use computational and theoretical methods to investigate the role of mechanical forces at T cell and B cell surfaces. Our methods combine a continuum description of membranes with stochastic reaction-diffusion kinetics of surface receptors [4,5]. We use the methods to study two related problems.

A. T cells: Force-dependent dissociation kinetics

T cells use the T cell receptor (TCR) to identify peptide fragments presented by surface molecules (pMHC) on other cells. Experiments have shown that stimulatory TCR-pMHC bonds behave as catch bonds [2], with an average bond lifetime that initially increases with an increasing tensile force. Because T cells are initially stimulated by small numbers of TCR-pMHC complexes, it is important to understand how bond formation drives dynamic changes in membrane organization and shape, how these changes affect forces experienced by the bonds, and how these forces affect bond lifetimes.

We characterize time-dependent forces on TCR-pMHC bonds in response to dynamic membrane changes [4]. We then determine the distributions of bond lifetimes using force-dependent lifetime data for TCRs bound to various ligands. Strong agonists, which exhibit catch bond behavior, are markedly more likely to remain intact than antagonists. Thermal fluctuations of the membrane shape enhance the decay of the average force on a bond, but also lead to fluctuations of the force. When more than one bond is present, the bonds experience reduced average forces, leading to changes in lifetimes.

B. B cells: Membrane-dependent affinity threshold

The activation of B cells is controlled largely by the B cell antigen receptor (BCR). Although B cells can be activated by soluble antigens, B cells *in vivo* are activated predominantly by antigen attached to membrane surfaces. Experiments have revealed that B cells use mechanical forces transmitted by the actin cytoskeleton to discriminate between antigens of similar binding affinity and to internalize portions of the antigen-presenting surface [6].

We study dynamics of BCRs at an intermembrane junction and show that the bending rigidity of the antigen-presenting membrane influences the affinity at which antigens are internalized through a mechanism involving BCR clustering. The clustering and membrane invagination occur with marked stochasticity near the affinity threshold.

Taken together, our results highlight the importance of forces at immune-cell interfaces, and we conclude by discussing our results in the context of antigen discrimination by T cells and B cells.

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Increased mortality favors fast-growing species in microbial communities

Clare Abreu[1], Billy Woltz[1], Jonathan Friedman[2], and Jeff Gore[1]

***Short Abstract* — A challenge in ecology is to understand how communities will change in deteriorating environments, in which increased mortality can result from events such as global warming. Simple phenomenological models predict that increased mortality will favor the fast-growing species, potentially reversing the competitive outcome. We use an experimental microbial system to observe reversal of pairwise outcomes from dominance of the slow grower to dominance of the fast grower, with an intermediate coexisting or bistable phase, consistent with expectations from theory. Our results show that simple models can provide insight into the effects of deteriorating environments on community structure.**

***Keywords* — competition, ecology, microbial communities, environmental fluctuations, model system**

I. Purpose

In the world of microbes and beyond, deteriorating environments have been shown to lead to biodiversity loss [1-2]. The consequences of a harsh environment on populations that do not go extinct, however, are equally important. Community structure can change radically as a result of events such as antibiotic use on gut microbiota [3], ocean warming in reef communities [4], overfishing [5], and habitat loss [6]. Such disturbances can change the members of a community as well as their interactions. With such examples in mind, we sought to predict how increased mortality would alter a community.

II. Methods

Using an experimentally tractable model system of soil bacteria subject to daily growth/dilution cycles, we tuned environmental harshness by spanning a range of dilution factors. The magnitude of the dilution factor determines the amount of added mortality, and can be incorporated into the Lotka-Volterra (LV) two-species competition model as a uniform death rate added to both species. We used this model to guide our intuition about how the experimental outcomes might change with increasing mortality. We determined the experimental outcome of pairwise competition by tracking population dynamics with daily plating and colony-counting.

We also explored two extensions to the pairwise experiments: we oscillated the environment between two dilution factors that led to two qualitatively different outcomes, and we competed multispecies communities.

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III. Results

Our experiments confirmed two interesting predictions from the model about pairwise competition. First, we saw that increased mortality/dilution does indeed favor the fast grower. The slow grower often displayed a tradeoff between growth and competitive ability, excluding the fast grower at low dilution factors (~10/day), while the fast grower dominated at high dilution rates (~10^4/day). Second, we observed coexistence or bistability of alternate stable states at intermediate dilution rates (~10^2 – 10^3/day). The confirmation of both predictions persuaded us of the model's relevance, despite its simplicity.

After demonstrating varying outcomes across dilution factors, we applied environmental fluctuations. The simplicity of the LV model leads to a prediction that in a fluctuating environment, the outcome will be that of the time-averaged environment [7]. Thus by oscillating between high and low dilution factors, we expected to see the intermediate outcome: coexistence or alternate stable states. This also proved true, giving the model more credibility.

Finally, we expanded from pairwise to multispecies competitions, including up to five species at once. Previous work in the lab showed that pairwise outcomes provided a good set of rules for predicting multispecies outcomes [8]. Our new experiments recapitulated the rules' success, showing that they can be applied in different environments. Altogether, our results argue that simple models and rules can accurately predict microbial community states.

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DockIt: Crowdsourcing Molecular Docking

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***Short Abstract* — Molecular binding is an important part of many biological mechanisms including immune system recognition and cell signaling. However, existing methods to predict bound states can be computationally expensive. We present DockIt, a computer game where players dock ligands to receptor proteins and find potentially bound states. Users are guided both visually and with haptic feedback. Ligand state transition roadmaps can be constructed incrementally using data crowdsourced from multiple players. The game also supports colored surface analysis displays. Our studies have shown users can find low potentially docked states and contribute toward refining motion planning queries.**

***Keywords* — Molecular docking, crowdsourcing**

I. Motivation

MOLECULAR BINDING has implications in the study of diseases, allergic responses, and many other biological processes. Molecular dynamics simulations can predict interactions between ligand and receptor proteins accurately at a high computational cost [1,2]. Probabilistic roadmaps built for protein binding can provide results at a reduced cost, but still require initial low potential energy states [3]. In this work, we aim to find potential ligand-receptor docked states by collecting data from *DockIt*, a crowdsourced interactive molecular docking puzzle game. The game allows users to fit ligands to receptors [4], where players are given both visual and haptic tactile feedback based on the potential energy between the molecules. The quality of the current fit is shown via a score based directly of the all-atom intermolecular energy. Surface analysis coloring, such as depicting surface electrostatics, can be implemented for use within the game.

II. DockIt

The molecular models used in *DockIt* were converted from RCSB data and assigned van der Waals and electrostatics parameters using the AMBER99 force field [2]. The all-atom model is used for potential energy calculation within the game but shown visually using an isosurface representation. Roadmaps are constructed from ligand states recorded as the user moves the ligand. Each ligand state is considered a roadmap node, and the edges represent transitions between two ligand states. Edge weights are given based on the difference in potential energy between two states. Shortest path queries are performed between two ligand states to predict ligand motion pathways.

A. User Study

A pilot study was performed with 24 participants assigned to one of four input devices. Users were aged from 18 to 66, with an average age of 30.2 years. The number of users with or without a technical background (biology or computation) was evenly split, 12 in each group.

B. Game Mechanics

Players can translate and rotate the ligand around the environment, move the camera for a better view, or let the game perform gradient descent toward the nearest minima. The user can see their current score and the best score for the current session. The best score encourages players to continue finding better potentially docked ligand states.

C. Input Devices

The four input devices used in the study vary by degrees of freedom for input and haptic feedback. First was the standard mouse and keyboard lacking any haptic feedback and only two degrees of manipulation at one time. The game controller had haptic vibration feedback and two degrees of manipulation. The Novint Falcon is a device that allows three degrees of haptic force feedback and manipulation, and the PHANToM input device allows six degrees of haptic force feedback and manipulation.

III. Results and Conclusions

DockIt users were able to find low energy potentially docked ligand states and ligand motion queries were refined as more user data was incorporated. Low energy states were found by users of all four devices, including the commodity game controller input device. *DockIt* can be adapted for use on mobile devices for future crowdsourcing scale studies.

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Investigating Potential Mechanisms for Negative Regulation of T Cell Activation

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***Short Abstract* — Anti-tumor T cells quickly reach a state of unresponsiveness to antigenic stimuli in the tumor microenvironment. Inhibitory receptors, also known as immune checkpoints, are critically linked to this anergy. Yet, a definitive model of T cell activation that incorporates the effects of these molecules has not been established. Here, we have devised a minimal model of T cell activation that captures the spatial effects of LAG3, a negative regulator of TCR signaling. We show that our model successfully replicates experimental observations and provides insights into the mechanism by which LAG3 functions to limit T cell activity.**

***Keywords* — T Cell Activation, Immunological Synapse, LAG3, Exhaustion, Immunotherapy, Mathematical Modeling.**

T lymphocytes are a major class of immune cells responsible for the recognition of pathogens and other potentially harmful substances that may enter the body. Upon activation T cells proliferate, a process that is required for a successful defense against infections and other diseases, most importantly, cancer. T cell proliferation is tightly regulated because excessive proliferation can lead to systemic damage, such as occurs in auto-immune disease [1]. Inhibitory receptors, like PD1, CTLA4, LAG3, play a crucial role in regulating T cell activation. Persistent exposure to antigen in the tumor microenvironment can lead to a phenomenon called "exhaustion" [2], a state of immune cell dysfunction marked by sustained expression of inhibitory receptors. Exhaustion is thought to be an important reason anti-tumor T cells fail to contain tumors, making these inhibitors a potential target for cancer immunotherapy treatments. Here, we have developed a minimal spatial model, calibrated against experimental data, to investigate potential mechanisms for negative regulation of T cell activation by LAG3.

T cells are activated following successful recognition of a foreign antigen on the surface of antigen-presenting cells (APCs) by T cell receptors (TCRs). Triggering of TCRs leads to phosphorylation of tyrosine residues on the cytoplasmic portions of the receptor subunits by the tyrosine kinases LCK and FYN. Considerable phosphorylation of these immunoreceptor tyrosine-based activation motifs (ITAMs) occurs only when several TCR-containing complexes aggregate into a condensed structure known as the immunological synapse [3]. Another tyrosine kinase, ZAP70, is recruited to phosphorylated ITAMs and is also phosphorylated by LCK. Phosphorylated ZAP70 (pZAP70) then activates a downstream signaling cascade that leads to T cell proliferation [4].

LAG3 is an activation-induced cell surface molecule [5] that inhibits T cell activation in a currently unknown manner. Our experimental data shows that LAG3 rapidly localizes to the synapse by associating with TCRs upon antibody stimulation. To understand the spatial effects of this reorganization, we have formulated a compartmental model of early T cell signaling events that models the immune synapse as a partition on the cell surface. We hypothesize that TCR-mediated aggregation of LAG3 can result in significant CD4-LCK dissociation in the synapse. LCK separated from CD4 may not be suitably positioned to phosphorylate the ITAMs limiting downstream signal transduction [6]. Furthermore, the synapse excludes the phosphatase CD45, allowing sustained phosphorylation of ITAMs only in this region [7]. We simulated the model for both WT and LAG3-deficient cells and used pZAP70 and CD4-LCK association levels to calibrate our model. We also performed sensitivity analysis to determine the impact of various model parameters on pZAP70 levels.

Our model replicated trends in pZAP70 levels across experimental timepoints while maintaining the appropriate ratio between WT and LAG3 deficient regimes. While LAG3 is expected to cause dissociation of CD4-LCK, experimental findings showed that the ratio of CD4-LCK association increased in favor of WT cells upon stimulation. This counterintuitive behavior could be explained by trafficking of a majority of LAG3 into the synapse allowing CD4 and LCK, largely present outside, to form a stable association. Another surprising prediction of the model is that pZAP70 levels show a biphasic response to variation in the affinity of CD4-LCK binding. This behavior arises in the model because of the interplay between ZAP70 phosphorylation, which is activating on the one hand and targets it for degradation on the other. It is our hope that further refinement and analysis of the model will lead to a better understanding of the working of LAG3 and may lead to novel strategies for targeting it to boost immune responses to cancers of various types.

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Culture Environment Impacts Synthetic Microbial Consortia Behavior

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***Short Abstract* — In nature, microorganisms grow in consortia that help them adapt to changing environments by cooperating and communicating. Expanding traditional synthetic biology gene circuits to two or more bacterial strains can lend the same advantages. These synthetic microbial consortia can be used to produce desired products in an assembly line fashion, measure intercellular communication, study the evolution of natural consortia, and eventually lend insights to engineering probiotics. To successfully engineer cooperative synthetic microbial consortia, we must examine how engineered strains communicate and cooperate in different environments.**

***Keywords* — Synthetic microbial consortia, microfluidics**

I. Purpose

TRADITIONALLY synthetic gene circuits have been studied in a single engineered bacterial strain. Expanding these gene circuits to multiple cooperating strains in synthetic microbial consortia can yield more robust population level phenotypes, enhance bioprocessing, and reveal insights to the evolution of natural consortia [1, 2]. We aim to examine the effect of different growth environments on the cooperation and communication of strains in synthetic microbial consortia.

Microfluidic devices allow for measuring single cell gene expression over time using fluorescent proteins as reporters [3]. However, they may introduce spatial patterns when used to grow multiple bacterial strains. Here, we examine which cell trapping regions provide a better microfluidic environment for synthetic microbial consortia to allow for cooperative growth and proper communication. The main attribute being overall size and shape of the cell trap.

In addition, we examine how the same microbial consortium behaves differently when grown in a fluidic device, in bulk culture, and on agar plates. The main differences that affects the population phenotype in these three environments is the presence of spatial patterns and any limitations on communication via small molecule diffusion.

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II. Results

Multiple synthetic microbial consortia are studied in this work including non-communicating strains, communicating strains, and strains that exchange nutrients. Their growth rates, communication distances, and cooperation are measured and compared in different environments.

A. Microfluidic devices affect strain stability and spatial patterning

We used non-communicating strains to determine that microfluidic devices with larger cell trapping areas allow for greater stability of two strains over time. We also observed that in these larger cell traps, spatial patterns arise that depend on the number of cells seeded into the trap.

B. Spatial patterns affect communication

We then examined how these spatial patterns affect communicating strains in microfluidic devices. We measured how far quorum sensing molecules can diffuse in the cell trapping region to determine the necessary proximity of cells from different strains for proper consortia behavior.

C. Synthetic consortia in different environments

We grew the consortia in microfluidics, bulk culture, and on agar plates and compared growth, cooperation, and communication. While bulk culture does not allow for single cell measurements, it eliminates spatial patterning. In agar plates, we can measure population level phenotypes and control the spatial patterns. In each environment the distance of quorum sensing signaling and nutrient exchange differs.

III. Conclusion

Synthetic microbial consortia need to be grown in environments that allow for strain stability over time, intercellular communication, and exchange of nutrients. Microfluidic devices, bulk culture, and agar plates each have attributes that can affect these. Here we've quantified the limitations and strengths of each environment depending on the synthetic microbial consortia being evaluated.

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Quantifying noise in general stochastic models of post-transcriptional regulation of gene expression

Argenis Arriojas[1], Sho Inaba[2] and Rahul Kulkarni[3]

***Short Abstract* — Gene expression is an stochastic process, and fluctuations in protein levels are often critical for generating phenotypic heterogeneity within a population of isogenic cells. There is thus considerable interest in quantifying how fluctuations (noise) in gene expression are impacted by cellular control mechanisms such as post-transcriptional regulation. In previous work, a general framework for *promoter-based regulation* has been developed [1], which leads to exact results for the moments of mRNA distributions. However, a similar framework for protein statistics in models with *post-transcriptional regulation* is currently lacking. In this work we develop an analytical framework that maps a general class of models of post-transcriptional regulation into models with promoter-based regulation, leading to exact analytical results for the moments of protein distributions. This mapping is based on the partitioning of Poisson arrivals (PPA) approach developed in recent work [2]. The proposed framework can be used to model complex schemes of post-transcriptional regulation and to evaluate its effects on variability in protein distributions.**

***Keywords* — Gene expression, post-transcriptional regulation, Markovian Arrival Process, stochastic modeling, promoter-based regulation, protein variability**

I. INTRODUCTION

THE stochastic nature of gene expression leads to fluctuations of mRNA and protein levels among isogenic cell populations. Modeling and quantifying this variability is important, since it has a significant impact on the resulting phenotypic variability of cell populations (e.g. emergence of bacterial persisters, drug-tolerant cancer cells). To address this, an analytical framework for mRNA distributions have been previously developed, in particular for general models of promoter-based transcriptional regulation [1]. In this framework, mRNA creation is represented by a Markovian Arrival Process (MAP) and obtaining exact results for moments only requires finding solutions for linear algebraic equations. However, a similar framework is currently lacking for determining protein statistics in general models of post-transcriptional regulation. Meanwhile, recent work has developed a novel approach that enables to obtain exact result for protein distributions in coarse-grained stochastic models of gene expression. This is achieved through the so called Partitioning of Poisson Arrivals mapping (PPA mapping) [2]. By using this approach, we show that a general class of models of post-transcriptional regulation can be mapped onto reduced models with promoter-based regulation. By combining the two previous approaches, we have developed a framework that can be used to derive exact analytical expressions for the moments of protein distributions for general models of post-transcriptional regulation.

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II. MAPPING TO REDUCED MODELS

A general class of models with post-transcriptional regulation can be mapped to a reduced model by means of a PPA mapping [2, 3]. The generating function for proteins can be written as in equation (1),

$$G(z,t) = \lim_{N\to\infty} \exp\{N[g(z,t)-1]\} \tag{1}$$

where $g(z,t)$ is the generating function for proteins in the corresponding reduced model. In the reduced model, a single mRNA can transition through a finite set of states, with each state having its own protein production rate. This is now equivalent to a promoter-based model with the mRNA transitions analogous to promoter-state transitions. Correspondingly, its MAP representation can be used to determine the moments for the protein distribution using linear algebra.

III. CONCLUSIONS

The PPA mapping is a powerful tool that can be used to derive and compute moments of protein distributions, given a model of post-transcriptional regulation. This general class of models can be used for analyzing combinations of different processes, i.e. mRNA senescence and interactions with different RNA-binding proteins. The developed framework is general, and can be used to design strategies for controlling variability of protein levels. This work is currently being extended to consider more general cases wherein mRNA arrivals are not a simple Poisson process (e.g. to include production of mRNAs in bursts) and to analyze rare events and large deviations in the rate of protein production [4].

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Computer Simulation of Colon Cancer Chemoprevention

David E. Axelrod[1] and Rafael Bravo[2]

***Short Abstract* — A calibrated computer model was developed for cell kinetics in human colon crypts. Simulations indicated that colon cancer may be prevented by intermittent doses of a drug that induces cell death at the top of the crypt.**

***Keywords* — colon cancer, chemoprevention, intermittent treatment, adenoma, computer simulation**

I. Purpose

Effective chemoprevention of cancer requires choices of (*i*) an agent(s) that is effective with little or no undesirable effects, (*ii*) patients who would benefit from the exposure to the agent, and (*iii*) dose intensity and dose schedules that maximize the prevention effect and minimize negative side effects. The purpose of this project was use a computer model of cell kinetics in human colon crypts to determine the maximum dose intensity and intermittent pulse dose schedules of a chemoprevention drug that would be effective in preventing colon cancer.

II. Methods

An agent based-computer model of cell dynamics in human colon crypts was developed. The model was calibrated with the number of quiescent stem cells, proliferating cells, and non-proliferating differentiated cells measured in human biopsy specimens. Details of image acquisition, measurements by image analysis, reliability of measurements, and confirmed behavior of the model were previously described [1]. The "Colon Crypt Model 031215.nlogo" is available to download at http://dx.doi.org/doi:107282/T3TQ638W. The model program runs on the open-source multi-platform NetLogo application version 4.1.3, or 5.3.1 available to download at http:ccl.northwestern.edu/netlogo/. The model allowed simulation of continuous and intermittent dose schedules of a chemoprevention drug, such as sulindac that induces apoptosis at the lumen surface of the crypt. Parameter sweeping indicated the effect of various dose durations, intervals, and intensities on the effectiveness of drug treatment in removing mutant cells before they can form an adenoma, while retaining crypt function.

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III. Results

In normal colon crypts, quiescent stem cells at the bottom of the crypt may become active stem cells and divide. The progeny cells move up the crypt, continue to divide, differentiate, and are removed at the top of the crypt. Mutant cells, if they have a higher probability of dividing than normal cells, may proliferate and fill the crypt before they can be removed at the top, forming an adenoma that is an early stage of colon cancer. Sulindac is a drug that alters crypts by increasing the probability that both normal cells and mutant cells at the top of the crypt will die by apoptosis and be removed. Sulindac can be applied to crypts as a chemopreventive drug before the appearance of mutants. It makes crypts inhospitable to mutant cells rather than killing mutant cells. Simulation results indicated that treatment of a crypt before a mutant cell arises can decrease the probability that a mutant cell will proliferate, fill the crypt and form an adenoma. Crypts treated with intermittent pulse schedules have three times the maximum tolerated dose than crypts treated with constant dose schedules, and have a 10-year delay in the appearance of adenomas. This results in chemoprevention by delay.

IV. Conclusions

Intermittent pulses of a drug that induces apoptosis at the top of a colon crypt could allow an increased maximum tolerated dose compared to a constant dose, and could result in chemoprevention of colon cancer.

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In-sequence coding of noise in gene expression

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It is well established that, under nutrient rich conditions, stochastic transcription governs noise in gene expression. Surprisingly, we have identified a novel source of noise during translation elongation when bacteria grow in nutrient limited conditions. We demonstrate that the observed noise can be modulated by the choice of synonymous codons. Using a two-color reporting system, we show that this source of noise does not depend on fluctuations of common resources available to the translation machinery. Rather, we propose that ultrasensitivity in the tRNA charging/discharging cycle is the mechanism responsible for the observed noise.

***Keywords* — selective charging, nutrient limitation, gene expression noise**

I. Background

GENETICALLY identical cells exposed to homogeneous environmental conditions can exhibit dramatically different levels of gene expression [1,2]. High quality data combined with mathematical modeling has now yielded a common physical framework for cell-to-cell variability in gene expression [3,4]. This variability—termed *noise*—was found to arise from Poisson statistics reflecting bursts of transcription. These bursts are governed by either small numbers of mRNA being transcribed [4] or infrequent activation of promoters [3,5]. In all of these stochastic models, it is generally assumed that transcription but not translation governs noise.

Unexpectedly, our results reveal that, during nutrient limitation, *translation*, and not transcription, can instead be the dominant source of noise.

II. Results

At the population level, we demonstrated that the usage of "*robust*-to-starvation" or "*sensitive*-to-starvation" synonymous codons can modulate translation rate by up to two orders of magnitude when cells starve for the cognate amino acid [6]. With single-cell experiments, we show that not only the average expression changes but that there is an associated noise that depends on the synonymous codon and the concentration of the cognate amino acid. We exclude the possibility that transcription noise underlies the observed variability with two control experiments in which, using a single type of robust codon and a fixed amino acid concentration, we change i) the translation initiation rate and ii) the transcription rate of a fluorescent reporter. The control experiments confirm the expectation that noise driven by transcription bursts follow Poisson statistics *even* during nutrient limitation: i) noise is independent from translation initiation rate and ii) noise increases as transcription decreases. *A priori* the method for changing translation rate should not affect the previous results. However, when we change translation rate by using different synonymous codons the associated noise varies. In fact, the noise associated to sensitive codons displays a magnitude comparable to the transcription noise obtained in the control experiment.

In order to decipher the origin of the observed noise we used a dual-reporter: a polycistronic transcript composed of a "sensitive" (blue) and a "robust" (yellow) fast maturing fluorescent proteins [7]. We find that as the limiting amino acid concentration becomes lower, extrinsic noise dominates but decreases and, when the total noise is maximal, the intrinsic noise presents a maximum indicating that the observed single-cell variability comes from a codon-specific effect.

To explain these results, we hypothesize that the observed noise takes its origin in the ultrasensitivity of the tRNA charging/discharging cycle. We show that in fact the observed noise from a sensitive codon can be shifted to different amino acid concentrations by overexpressing the cognate tRNAs.

III. Conclusion

Life in resource-rich conditions is more the exception than the rule. Cells compete and strive in resource-limited environments, thus making the translation noise here uncovered an alternative bet-hedging strategy.

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Modulation of conjugation in pathogenic *Escherichia coli*

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***Abstract* — As resistance outpaces new antibiotics, evolutionary mechanisms like horizontal gene transfer (HGT) become targets for resistance prevention and reversal therapies. Discrete modulation of HGT is difficult to quantify, however, because unequal fitness across mixed populations confounds HGT with selection dynamics. To address this, we present a high-throughput assay that uses time to optical density threshold for conjugation quantification. We apply this method to study antibiotic modulation in pathogenic *E. coli* strains collected from patient cultures. In contrast with previous literature, we find that sublethal antibiotics exert little to no effect on conjugation, although exceptions with biologically significant modulation may exist.**

***Keywords* — Horizontal gene transfer, Conjugation, Antibiotic, Resistance**

I. Introduction

Horizontal gene transfer (HGT) is a fundamental process by which bacteria adapt to their environment. It is especially pertinent in recent decades, with sublethal antibiotic gradients permeating throughout diverse environments and selecting for antibiotic resistance genes (ARGs) [1]. Conjugation, the direct transfer of genetic material between a donor and recipient, has drawn particular attention for its ability to spread ARGs due to broad host range, high rates of transfer, and close association of ARGs with plasmids [2]. In certain cases, environmental stimuli appear to influence HGT [4]. Antibiotics may have a similar effect—accelerating their own demise by promoting the spread of ARGs.

Most antibiotics are natural in origin, multifaceted in purpose, and bacteria have been exposed to them for millions of years prior to human use [4]. Past studies have generally concluded that antibiotics can promote horizontal gene transfer [1]. However, selection dynamics may have overshadowed any true modulation of conjugation [5]. Moving forward, a framework for accurate and high-throughput quantification is needed to understand modulation of conjugation across a wide variety of conditions.

II. Results

In conjugation, three cell populations are at play: plasmid donors (D), recipients (R), and transconjugants (T). We define the conjugation rate (η_c) as:

$$\eta_C = \frac{T}{DR\Delta t}$$

with transconjugants being the critical measurement. To decouple selection from conjugation dynamics, we prevent growth during a limited conjugation period. This restricts conjugation, reduces transconjugants, and makes cell quantification in mixed culture more difficult.

We resolve these issues by taking advantage of exponential bacterial growth:

$$T_C = T_0 e^{\mu t}$$

where the time (t) it takes to reach an optical density threshold (T_c) is proportional the initial inoculum (T_0). A small initial population of transconjugants can therefore be amplified and easily quantified with corrections for further conjugation.

As a clinically relevant model, we characterized 219 pathogenic *E. coli* strains isolated from bloodstream infections. Of these, 65% displayed extended spectrum beta-lactamase resistance and 24% were able to transfer it to recipient *E. coli*. Using beta-lactamase as a marker for conjugation, we then tested for antibiotic modulation of conjugation with five classes of antibiotics at three sublethal concentrations. By and large, antibiotics exerted no significant effect on conjugation rates, although exceptions may exist. We follow-up on significant effects with whole-genome long-read sequencing capable of capturing difficult mobile elements and shedding light on potential mechanisms for modulation.

III. Conclusion

Identifying and understanding factors that modulate conjugation is key for addressing issues of bacterial evolution, notably antibiotic resistance. To this end, the time to threshold method enables high-throughput, selection-decoupled conjugation quantification in natural populations.

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Impact of a periodic presence of antimicrobial on resistance evolution

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Short Abstract — **Antimicrobial resistance is a major public health issue. It is crucial to determine which conditions accelerate or slow down its evolution. We study theoretically the impact of a periodic alternation of phases of absence and presence of antimicrobial on the time necessary for resistance to take over. We demonstrate that alternations strongly accelerate the evolution of resistance, especially for fast alternations and large microbial populations. For antisymmetric alternations, the time taken by the population to fully evolve resistance features a striking minimum. The corresponding parameters should preferably be avoided in clinical situations, while they could be harnessed in evolution experiments.**

Keywords — **Antimicrobial resistance, evolution, population, stochastic model, fitness landscape, fitness cost, analytical results, numerical simulations**

I. Introduction

THE discovery of antimicrobials is one of the greatest medical advances of the twentieth century, allowing many major infectious diseases to be treated. However, with the increasing use of antimicrobials, pathogenic microorganisms become resistant to these drugs. Antimicrobial resistance has become a major and urgent problem of public health worldwide [1].

Mutations that confer antimicrobial resistance are often associated with a fitness cost, i.e. a slower reproduction [2]. Indeed, resistance acquisition generally involves either a modification of the molecular target of the antimicrobial, which often alters its biological function, or the production of specific proteins, which entails a metabolic cost. However, resistant microorganisms frequently acquire subsequent mutations that compensate for the initial cost of resistance. These microorganisms are called "resistant-compensated" [3]. Resistance acquisition is therefore often irreversible, even upon removal of the antimicrobial from the environment [2].

In the absence of antimicrobial, the adaptive landscape of the microorganism, which represents its fitness (i.e. its reproduction rate) as a function of its genotype, involves a valley, since the first resistance mutation decreases fitness. However, this fitness valley disappears above a certain concentration of antimicrobial, as the growth of the antimicrobial-sensitive microorganism is impaired. Thus, the adaptive landscape of the microorganism depends drastically on whether the antimicrobial is present or absent. Taking into account these effects constitutes a fundamental problem, which has been little studied so far, in particular because experimental works have traditionally focused on comparing different mutants in a unique environment.

II. Questions and model

How do the time scales of the evolution and of the variation of the adaptive landscape couple together? What is the impact of the time variability of the adaptive landscape on antimicrobial resistance evolution ?

To answer these questions, we construct a minimal model retaining the fundamental aspects of antimicrobial resistance evolution. Focusing on the case of a homogeneous microbial population of fixed size, which is described in the framework of the Moran model, we perform a complete stochastic study of *de novo* resistance acquisition in the presence of binary alternations of phases of presence and absence of antimicrobial, which may represent, for example, a treatment where the concentration within the patient falls under the Minimum Inhibitory Concentration (MIC) between drug intakes [4].

III. Results

Combining analytical and numerical approaches, we show that alternations of phases of antimicrobial absence and presence substantially accelerate the evolution of resistance, especially for larger populations. We fully quantify this effect and shed light on the different regimes at play. We also compare the alternation-driven acquisition of resistance to the spontaneous evolution of resistance by fitness valley crossing, and extend previous results on valley crossing [5]. We then generalize our study to the case of asymmetric alternations, featuring a different duration of the phases with and without antimicrobial. We demonstrate the existence of a broad minimum of the time taken by the population to fully evolve resistance, occurring when both phases have durations of the same order. This situation dramatically accelerates the evolution of resistance. We show that this worst-case scenario may unfortunately be quite realistic in clinical settings, and argue for a modification of antimicrobial dosage strategy. We also show how our results could be exploited to accelerate evolution experiments investigating resistance.

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The role of metabolic spatiotemporal dynamics in modulating biofilm colony expansion

Federico Bocci[1,4], Yoko Suzuki[2], Mingyang Lu[3] and José Onuchic[1]

***Short Abstract* — A recent microfluidic experiment showed that the metabolic co-dependence of two cell populations generates a collective oscillatory dynamic during the expansion of a *Bacillus subtilis* biofilm. We develop a modeling framework for the spatiotemporal dynamics of the associated metabolic circuit for cells in a colony. We elucidate the role of metabolite diffusion and the need of two distinct cell populations to observe oscillations. Uniquely, this description captures the onset and thereafter stable oscillatory dynamics during expansion and predicts the existence of damping oscillations under various environmental conditions.**

***Keywords* — biofilm expansion, phenotypic differentiation, metabolic co-dependence, oscillations, reaction-diffusion system**

I. Introduction

Cell fate determination is typically regulated by biological networks[1][2], yet increasing evidences suggest that cell-cell communication and environmental stresses play crucial roles in the behavior of a cell population[3][4].

The oscillatory expansion of bacterial colonies recently observed in a biofilm system exemplifies how intercellular communication plays a central role [5]. Liu et al supplied a B. subtilis colony with glutamate and observed a transition from steady expansion to oscillatory growth when the biofilm reached a threshold size. Detailed analysis revealed that the spatial regulation of the glutamate biochemical pathway for cell metabolism is critical for the formation of this specific dynamics.

We introduced a scheme to study both temporal and spatial dynamics of the metabolic interactions within the biofilm. This model considers the diffusion of small metabolites by incorporating the spatial dynamics of the bacterial colony. Such features allow us to investigate the spatial organization of cells with different phenotypes, and directly explore the repercussions of the glutamate synthesis pathway on the biofilm development.

II. Results

The proposed model can explain the recurrent oscillatory cycles of the growth rate in terms of the space-dependent interplay between the internal and peripheral phenotypes and reproduce the observed growth dynamics in presence of altered conditions of the growth media. Moreover, we show that he occurrence of oscillations is insensitive to the radius of the biofilm; instead, it is sensitive to the width of the peripheral layer. These findings suggest that the initial onset of the oscillation of the biofilm expansion rate is due to the switch of the bacterial cells from interior to peripheral phenotype, and it is specifically triggered when the peripheral layer increases its width to a certain level. Finally, various types of growth dynamics, including dampened, stable, dissipating dynamics, are revealed by varying the ratio between interior and peripheral cells in the biofilm and modifying the biofilm's external conditions.

III. Conclusion

This modeling scheme provides insights to understand how cells integrate the information from external signaling and cell-cell communication to determine the optimal survival strategy and/or maximize cell fitness in a multi-cellular system.

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Predicting influenza vaccine effectiveness from evolution of the dominant epitope

Melia E. Bonomo[1] and Michael W. Deem[2]

***Short Abstract* — We predict influenza A(H3N2) vaccine effectiveness in humans using a novel measure of antigenic distance, $p_{epitope}$, between the vaccine and circulating virus strains. $p_{epitope}$ is based on the evolution of the hemagglutinin protein's dominant epitope, which is the viral site to which an antibody binds. This measure originates as an order parameter from our statistical mechanics model of the antibody-mediated response to infection following vaccination. During 2016-2017, our model predicts 19% effectiveness compared to 20% observed. This robust tool aids vaccine selection by rapidly predicting human protection against all circulating strains.**

***Keywords* — influenza, vaccine effectiveness, pEpitope, antigenic distance**

I. Background

SEASONAL influenza constitutes a significant disease burden worldwide, with three to five million cases of severe illness and an estimated annual death toll of 290,000 to 650,000; however, vaccination can provide protection [1]. For the 2016-2017 influenza season, the World Health Organization chose an A(H3N2) vaccine reference strain that was well-matched to the dominant infecting viruses. Results from conventional ferret models however did not explain why effectiveness of the manufactured vaccine was unusually low, at only 20± 8% for adults aged 18-64 [2].

Influenza type A viruses are primarily recognized by the immune system via two proteins on their surface, hemagglutinin (HA) and neuraminidase [3]. These viruses constantly evolve to evade human antibody binding, most notably by introducing amino acid substitutions into the HA binding sites. In addition to increased antigenic distance due to virus evolution, the vaccine strain may also diverge from circulating strains due to substitutions acquired during egg passaging [4]. Egg adaptations have posed an issue when manufacturing vaccines for A(H3N2) viruses in particular.

Here we develop a comprehensive model that utilizes the primary protein structure of the antibody binding sites to quantify the vaccine's antigenic drift [5]. We define an antigenic distance between influenza A(H3N2) viruses and vaccines based on amino acid substitutions in the dominant epitope to predict vaccine effectiveness.

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II. Summary of Results

We have derived a statistical mechanics model that captures the dynamics of human antibody-mediated response to viral infection following vaccination [6]. We generalize the model to data both from the 1971-1972 to 2015-2016 influenza seasons and from laboratory-confirmed studies over the past decade, and $p_{epitope}$ has $r^2 = 0.77$ in both cases.

We employ this theoretical method to predict how well the administered A(H3N2) vaccine protects humans. We identify $p_{epitope} = 0.111$ and 19± 4% average effectiveness for the vaccine against all circulating A(H3N2) strains during the 2016-2017 and early 2017-2018 seasons**.** We establish that this low vaccine performance was largely caused by the substitutions that occurred in the dominant HA epitope *B* during egg passaging of the vaccine strain.

We compare this method with the typical measure of antigenic distance d_1, defined in ferret animal models as the $\log_2$ difference between vaccine antiserum titer against itself and the vaccine antiserum titer against a strain representative of the dominant circulating viruses [7]. The conventional ferret antisera d_1 has $r^2 = 0.42$ on data since 1971, and over the past 10 years this has dropped to $r^2 = 0.23$

III. Conclusion

Our model can rapidly calculate $p_{epitope}$ for a vaccine paired with an individual strain or averaged over many circulating viruses, whereas ferret models are time consuming and restricted to a few analysis pairs. While other factors influence vaccine effectiveness in humans, the $p_{epitope}$ theory has accounted for most of the variance over the past 10 and 45 years ($r^2 = 0.77$). This work showcases a robust tool for ensuring optimal reference strain selection and predicting manufactured vaccine effectiveness.

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Optogenetic investigation of *B. subtilis* sporulation network at the single cell level

Ryan J. Butcher[1], Sebastian M. Castillo-Hair[1], and Jeffrey J. Tabor[1, 2]

Upon starvation, *Bacillus subtilis* cells will employ a sporulation mechanism to form spores that endure harsh environments. This mechanism is operated by a phosphorelay network, culminating in the activation of a master regulator, Spo0A. Sporulating cells display pulses of Spo0A that gradually increase in amplitude, but the purpose of these specific dynamics is unknown. Using recently developed optogenetic methods from our lab, we apply temporally varied Spo0A inputs to a population of cells. We assess the phenotypic response at the single cell level to determine the role of Spo0A pulsing in sporulation.

Keywords: Sporulation, gene dynamics, optogenetics

I. Introduction

Cellular decision making, the process that governs whether or not a cell will differentiate, is governed by the dynamics of gene networks that process internal and external information [1]. One example of cellular decision making is the sporulation process in *Bacillus subtilis,* in which DNA is preserved in a stress-resistant spore until a favorable environment is found. Under limited nutrient conditions, a phosphorelay network gradually activates a "master regulator", Spo0A [2]. Sporulation can be artificially triggered by chemical induction of phosphorelay components [2], but very high induction levels decrease sporulation efficiency [3]. Further studies have shown that the chromosomal arrangement of the components in the phosphorelay network results in pulses of Spo0A activation [4]. The amplitude and period of this pulsing have been shown to increase in response to decreases in cellular growth rate (during starvation, for example) [5]. One of the major questions surrounding the sporulation network is if the pulsing dynamics of activated Spo0A are necessary for sporulation or not. The purpose of this work is to probe the sporulation network with synthetically induced Spo0A dynamics to determine which temporal features are required to induce sporulation at the single cell level.

Recently, our lab has developed a model to predict the translational output of a green and red light controlled two component system (TCS). We use the model to achieve a wide variety of synthetic protein dynamics by calculating the light input signal needed for the desired output [6]. We have ported the TCS into *B. subtilis*, allowing us to program custom Spo0A dynamics. The TCS acts as a "biological function generator", meaning that the gene network is investigated by applying various Spo0A input signals and assessing the overall phenotypic result or specific gene output via fluorescent reporters.

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II. Results

A. Single-cell characterization of optogenetic system

We examine the previously characterized optogenetic system again in singular *B. subtilis* cells. The output of the system, measured on a single cell basis, determines the error between individual cell expression and the population mean, which correctly matches the programmed signal.

B. Determining the necessity of Spo0A pulsing for successful sporulation

We are studying the sporulation efficiency for static, ramped, and pulsed Spo0A inputs. In doing so, we determine if linear inputs are sufficient for sporulation, or if pulsing is required. The sporulation failures, such as forespore formation without separation versus no forespore formation at all, provide important information about the extent that each type of signal can activate the network.

III. Conclusion

By studying the output of the optogenetic system at the single cell level, we gain a better understanding of what determines a complete or incomplete sporulation response. This investigation answers the question of which dynamic features of Spo0A input are actually required to induce sporulation and why.

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Probing ER-related Stress with Lipid Markers

Matthew D. Carpenter[1,2], Orlando Argüello-Miranda[1], and Andreas Doncic[1,3*]

Robust stress response pathways are essential for survival and fitness in all cells. Here we investigate a, to the best of our knowledge, previously uncharacterized stress response in budding yeast. Specifically, we observed an unexpected high-intensity, intermittent fluorescence signal indicative of stress response in *S. cerevisiae* grown in the presence of fluorescently-conjugated lipids. Using single cell microscopy-based analysis, we determined that this signal originates from the endoplasmic reticulum (ER), and is related to changes in membrane potential. Here we quantify this phenomenon with the aim to characterize a novel mechanism for responding to ER-associated stress.

I. Introduction

STRESS response pathways enable life to persist in dynamic environments. An important class of stress responses control and maintain the homeostasis of cells' cytoplasm and organelles, e.g. by controlling the intracellular osmolarity [1]. While responses to such stresses may be short-lived, manifest as severe changes in physiology and morphology, and even fire irregularly, failure to respond can be fatal. Here, we develop new methods to characterize stress-responses using lipid probes, and use our new methods to investigate what might be a novel ER-mediated stress response.

II. Investigation

Fluorescently-conjugated lipids are used as reporters in many different contexts and can be used to gain insight into the function and composition of lipid membranes, including in *S. cerevisiae*. Here we investigate the effects of fluorescently-conjugated lipids on yeast cells grown in its presence. Surprisingly, we noted that cells grown in the presence of the fluorescently-conjugated lipids display intermittent bursts of short-lived, high intensity fluorescence. Importantly, a minor fraction of come populations of cells remained in a high fluorescent state and died, as is characteristic of a population enduring a stress response.

Next, we determined the subcellular localization of the phenomenon. Colocalization of the signal from the fluorescently-conjugated lipids with a signal from fluorescently-tagged Erg6, which served as a marker for the ER, clearly demonstrates that the stress and/or stress-response are associated with the ER. This result also suggests that the ER membrane, in which the fluorescently-conjugated lipids may be incorporated, undergoes perturbations during this stress event.

Interestingly, we also observed that the intermittent fluorescent signal was sometimes accompanied by a decrease in cell volume. Rapid perturbations of cell volume in yeast are normally seen when cells respond to changes in the extracellular osmolarity, a response mediated by the high-osmolarity glycerol (HOG) pathway. To test if the HOG pathway also governs the observed bursts of fluorescence, we tagged Hog1 with a fluorescent reporter and observed its dynamics while cells were exposed to fluorescently-conjugated lipids. Surprisingly, we noted that the HOG pathway did not appear to be induced in cells displaying the bursting phenotype. This suggests that we are observing a novel stress response.

Since the observed fluorescence phenomenon may be related to cell volume changes and is localized to the ER membranes, we next investigated whether changes in membrane potential are related to the bursting phenotype. We found that it was possible to modulate the frequency of the intermittent blinking signal via incorporation of different ions in the media, indicating that the fluorescence phenomenon is indeed related to changes in membrane potential.

III. Future Directions

To understand the mechanism that controls this phenomenon, we will monitor major signaling/stress response pathways (including PKA, TORC1, etc.) to determine which pathway(s) control this phenomenon. Here, we will capitalize on our recently developed system of fluorescently-tagged proteins that allows us to tag and monitor up to six fluorophores at the same time, allowing us to simultaneously monitor multiple pathways in single cells. By quantifying this signal and its relationship with changes in membrane potential and signaling, we will determine whether this is indeed a *bona fide* new stress response in yeast. Moreover, we will characterize the spatial and temporal organization of important stress response pathways surrounding an ER-associated event and describe novel methods for interrogating stress response at the single cell level.

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Dynamic Interrogation of the *Bacillus Subtilis* Sporulation Network using an Engineered Light-Switchable Two-Component System

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***Short Abstract* — Under starvation, *Bacillus subtilis* differentiates into metabolically-inert spores. This process is triggered by master response regulator Spo0A via activity pulses of increasing amplitude. Simple overexpression of Spo0A does not result in successful spore formation, however, suggesting that Spo0A dynamics are important. To study this further, we develop the first *B. subtilis* optogenetic system based on a cyanobacterial Two-Component System (TCS), and demonstrate its usage in generating time-varying gene expression signals. We use it to express Spo0A under different dynamics and evaluate its effects on sporulation. This novel approach helps us understand the significance of Spo0A dynamics at an unprecedented level.**

***Keywords* — Optogenetics, Sporulation, Synthetic Biology, Bacillus subtilis, Two-Component Systems, Dynamical Systems.**

I. INTRODUCTION

COMPLEX, time-varying, heterogeneous gene regulation has been found to occur in several stages of *B. subtilis* endospore formation. Initiation of this process proceeds via increasingly larger pulses of activity of the master response regulator Spo0A [1]. Interestingly, triggering sporulation via overexpression of a constitutively active Spo0A mutant is not possible [2]. It has been proposed that fast Spo0A activation can lead to early repression of essential sporulation genes, thus leading to non-viable spore formation [3]. To fully understand how Spo0A dynamics affect downstream sporulation processes, the ability to manipulate Spo0A dynamics is desirable.

CcaS/R is a photoreversible TCS from *Synechocystis PCC6803*, in which expression of an output gene is controlled by green and red light. We have previously transferred this system into *E. coli*, described its dynamic response using an ODE model, and showed that arbitrary gene expression dynamics can be precisely generated by using the model to calculate a corresponding green light intensity signal [4]. The availability of a similar system in *B. subtilis* would be useful in studying not only sporulation, but other dynamic and heterogeneous processes such as the general stress response and colony formation.

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II. RESULTS

Here, we transport the CcaS/R TCS to *B. subtilis*, demonstrate control of gene expression dynamics, and use it to investigate the effect of different Spo0A dynamics on different elements of the sporulation network.

A. Engineering an optogenetic tool for B. subtilis

We divide the CcaS/R system into three "modules" – chromosomally integrated DNA sequences encoding the sensor kinase CcaS, the response regulator CcaR, and the enzymes that produce CcaS' chromophore – and separately optimize expression and performance of each one. The resulting system can regulate gene expression in response to light with a 70-fold range. We measure the steady state and dynamic responses, and show that they can be described using our previously developed model [5]. Finally, we use this system to generate complex gene expression dynamics.

B. Dynamic Interrogation of the Sporulation Network

We use the CcaS/R system to induce expression of Spo0A under different dynamics, including ramps of different slopes and pulses. We measure the effects of these on the sporulation network, including expression of downstream genes and sporulation efficiency, and identify the mechanism by which Spo0A dynamics are decoded by the network.

III. CONCLUSION

Introducing time-varying perturbations to the sporulation network allows us to elucidate the significance of Spo0A dynamics. Our novel approach based on optogenetics can be directly applied to other *B. subtilis* processes as well.

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Integrative Model of Actin, Adhesion, and Signaling Dynamics at the Leading Edge of Migrating Cells

Ankit Chandra and Jason M. Haugh[1]

***Short Abstract* — We have constructed a spatiotemporal model that incorporates adhesion, cytoskeletal and signaling dynamics governing protrusion of lamellipodia in mesenchymal cells. The model includes actin polymerization at the leading edge and the resulting retrograde flow of the F-actin network. Nascent adhesions promote actin polymerization via Rac signaling and interact with the F-actin network to activate RhoA. Consequently, myosin II is activated and applies contractile stress on the F-actin network. Our model predicts an optimal adhesion density for maximal protrusion velocity. Moreover, myosin contractility is limited in its ability to control protrusion velocity, unless global tension of the membrane boundary is considered.**

I. Extended Abstract

Cell migration plays a crucial role in a wide variety of biological processes. It is essential in wound healing, embryonic development, cancer metastasis as well as innate and adaptive immunity. Amongst the different cell migration phenotypes, amoeboid and mesenchymal motility modes lie at opposite extremes. The mesenchymal migration phenotype is characterized by slow locomotion, strong adhesions mostly with extracellular matrix (ECM), and a distinct actin cytoskeletal and myosin spatial profile [1]. In mesenchymal and epithelial cells, integrins orchestrate the dynamics of the actin cytoskeleton, responsible for force generation, adhesion complexes, responsible for force transduction, and biochemical regulatory networks, responsible for signal transduction [2]. Nascent adhesions form at the leading edge of migrating cells, where transmembrane integrins form attachments to ECM and to actin filaments during membrane protrusion in a myosin II-independent manner [3]. Nascent adhesions play an important signaling role in migrating cells. They activate Rac and other signaling pathways that further promote barbed end polymerization and protrusion, forming a positive feedback loop [4]. Moreover, adhesions under tension promote the activation of RhoA/ROCK signaling, which in turn activates myosin II [5]. Nascent adhesions also play a crucial role in force transduction. They bind with F-actin and create a mechanical clutch, allowing polymerizing actin to overcome membrane stress push the membrane forward [6].

Specific aspects of this system have been explored in previous models [7–10]. In this study, however, we have constructed a model that integrates and spatially resolves adhesion, cytoskeletal, and signaling dynamics in the lamellipodia of mesenchymal cells. We have modeled actin polymerization at the leading edge and the resulting retrograde flow of the F-actin network. Nascent adhesions promote actin polymerization via Rac signaling as well as interact with the F-actin network to activate RhoA. Subsequently, active myosin II engages and applies stress to the F-actin network. Our model predicts an optimal ECM (adhesion) density for maximal protrusion velocity. At lower ECM densities, not enough adhesions are formed, and most of the actin polymerization results in retrograde flow. At higher ECM densities, competition among increased barbed end density for G-actin and increased myosin activity reduce protrusion below optimum levels. Moreover, at low ECM densities, increasing total G-actin has limited effect on protrusion velocity as compared to increasing total G-actin at higher ECM densities. Lastly our model predicts that myosin is limited in its ability to limit protrusion, unless global tension of the membrane boundary is considered.

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Infection Risk of Antibiotic Resistant Bacteria

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***Short Abstract* — Antibiotic resistant bacteria (ARB) are a growing problem due to rapid evolution of resistance to existing drugs and concomitant void in drug discovery. To estimate the size of their threat and manage the risk, it is useful to understand the probability of a person falling ill from a given bacterial dose. Studies producing such data involve inoculating volunteers with ARB and raise ethical concerns. Here we present a stochastic modeling approach that leverages data from *in-vitro* experiments to predict the relationship between infection probability and number of ARB. Specifically, we discuss results for *E. coli* and its Gentamicin resistant strain.**

***Keywords* — Antibiotic resistant bacteria, stochastic, birth and death process, dose response model**

I. Introduction

THE emergence of antibiotic resistance and its subsequent escalation is well documented. Among reasons for this are the overuse/misuse of antibiotics (ABs) and the greater difficulty in discovering new drugs [1–3).

A necessary step in evaluating the size of the problem and predicting the efficacy of risk management measures is understanding the probability of a person falling ill ($P_i(N_d)$) from a given dose of bacteria (N_d). Models built for this purpose are termed dose response models (DRMs) (4). To understand risk in the ARB context, existing DRMs require data from studies where volunteers are inoculated with ARB. Such studies are currently infeasible due to ethical and practical difficulties. This calls for DRMs that can effectively integrate data from *in-vitro* studies involving ARB to address the issue.

II. Methods

A dose of bacteria once inoculated in a person can face one of two fates. Either it dies out not causing any symptoms or it survives, causing illness. Thus, a stochastic model of bacterial growth can establish a relationship between N_d and $P_i(N_d)$. Such a growth model can then be extended to two bacterial populations, one susceptible and one resistant to AB. The relationship between the growth constants of the populations can be obtained from relevant experiments done *in-vitro*. Here we apply this concept to *Escherichia coli* that causes diarrhea.

A. *Escherichia coli*

A stochastic birth and death process (discrete bacterial numbers, continuous time) was assumed to arrive at a simple analytical expression (5) relating $P_i(N_d)$ to the dose (N_d), birth rate (λ) and death rate (μ). Fitting of this relationship to 4 datasets from human volunteer studies indicates that new model outperforms existing DRMs in 2/4 cases.

Exploratory analyses (with relevant parameters from (6)) on *E.coli* and Gentamicin resistant *E. coli* revealed the alarmingly small doses of the latter required to outcompete its susceptible counterpart in the presence of residual antibiotic in the body. Bayesian data fitting was performed with R(7) and STAN(8).

III. Conclusion

Stochastic modeling of bacterial growth dynamics appears to be as good as, if not better than, current DRMs. They have the added advantage of extending naturally to mixed doses of antibiotic sensitive bacteria and ARB.

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Proteome allocation determines thermosensitivity of growth and structure of the evolutionary landscape

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***Short Abstract* — We reconstruct an integrated genome-scale protein-folding network for *Escherichia coli* termed FoldME. FoldME simulations delineate the multi-scale strategies cells use to resist unfolding stresses induced by high temperature or destabilizing mutations in a single gene. Furthermore, FoldME enables sampling of the solution space with diverse genotypes, thus reveals the intrinsic structure of the fitness landscape constrained by the cost of expression for the large energy production protein complexes. The results provide a system-level understanding of the regulatory relationship between global proteome allocation and bacterial stress response. The method is readily extended to study cell's complex responses to multiple stresses simultaneously.**

***Keywords* — Proteome Allocation, Bacterial Growth Law, Evolutionary Landscape, Proteostasis, Thermoadaptation, Genome-scale Model.**

Gene expression is intimately coupled to the growth physiology of the cell, and regulated by global allocation of the cellular resource and energy. Such relationship has been quantitatively captured by the empirical bacterial growth law [1], which successfully explain how bacterial growth is affected by a wide range of biological processes, including molecular crowding, protein overexpression, cAMP-signaling, overflow metabolism [2], and growth transition kinetics. However, these coarse-grained models lack the necessary details to address the underlying molecular mechanisms that drive regulation. It is not clear how to combine different regulatory mechanisms into one composite model, describe the behavior of more complex systems under diverse environmental perturbations, and provide insights into the structure of fitness landscape instead of simple linear physiological correlations. To address these challenges, an integrated genome-scale model is calling to connect our understanding of molecular stress response mechanisms with phenotypic adaptation.

We started by modeling bacterial thermoadaptation [3-5], because temperature is one of the most important environmental parameters that dictate the evolution. First, we calculated the temperature-dependent biophysical profile of the proteome using thermodynamic principles based on protein sequences and structures [6]. This profile serves as an internal "sensor" to reflect the environmental and genetic perturbations. Second, we design a mathematical formulation to describe how the molecular chaperones respond to the folding request of each protein [7]. Third, we integrate the kinetic protein folding network into the genome-scale reconstruction of metabolism and protein expression for *Escherichia coli* [8], by enabling competition among the spontaneous and multiple chaperone-assisted folding pathways. As such, changes in the proteostatic state of the cell induced by environmental and genetic perturbations can be calculated based on first principles, evaluated by the protein quality-control machinery, and coupled to the whole cell's economics.

FoldME simulations reproduce the asymmetrical temperature response of *E. coli*, and the proteomic changes upon destabilizing mutation in a single gene [3]. The results highlight the system-level regulatory role of chaperones beyond efficient folding of any single protein. Rather, chaperones participate in the global proteome reallocation to balance between the need for folding and the complex machinery synthesizing the proteins in response to the perturbations. The ability of FoldME to delineate multi-level cellular responses to a variety of perturbations encouraged further sampling of the solution space using a large number of random genotypes. Preliminary results reveal a rugged fitness landscape defined by discreteness of ATP production strategies [9]. Overall, these results expand our view of cellular regulation, from targeted specific control mechanisms to global regulation through a web of nonspecific competing interactions that modulate the optimal reallocation of cellular resources. The methodology developed enables genome-scale integration of environment-dependent protein properties and a proteome-wide study of cellular stress responses.

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De novo prediction of human chromosome structures: Epigenetic marking patterns encode genome architecture

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Short Abstract — **We show that this chromatin architecture can be predicted *de novo* using epigenetic data derived from ChIP-Seq. We exploit the idea that chromosomes encode a 1D sequence of chromatin types, analogous to a sequence of amino acids for a protein. A neural network is used to infer the relation between the epigenetic marks present at a locus and the genomic compartment in which those loci reside. The sequence of types inferred from this neural network is used as an input to an energy landscape model for chromatin organization (MiChroM) in order to generate an ensemble of 3D chromosome conformations.**

***Keywords* — Epigenetics, Genome Architecture, Machine Learning, Energy Landscape Theory, Hi-C, FISH**

I. PURPOSE

The use of high-resolution contact mapping experiments (Hi-C) has revealed that, at the large scale, genome structure is dominated by the segregation of human chromatin into compartments. Analysis of Hi-C experiments revealed that loci exhibit at least six long-range contact patterns, indicating the presence of at least six sub-compartments (A1, A2, B1, B2, B3, and B4) in human lymphoblastoid cells (GM12878) [1]. Further, the long-range contact pattern seen at a locus is cell-type specific, and is strongly associated with particular chromatin marks.

To model chromosome structure, an effective energy landscape model for chromatin structure called the Minimal Chromatin Model (MiChroM) was previously introduced [2]. This model combines a generic polymer potential with additional interaction terms governing compartment formation, the local helical structural tendency of the chromatin filament, and the chromatin loops associated with the presence of CCCTC-binding factor (CTCF). The formation of compartments (as well as any other interaction in MiChroM) is assumed to operate only through direct protein-mediated contacts bringing about segregation of chromatin types through a process of phase separation. MiChroM shows that the compartmentalization patterns that Hi-C maps reveal can be transformed into 3D models of genome structure at 50 kb resolution.

We extend upon the earlier work [3] by demonstrating that the structure of chromosomes can be predicted, *de novo*, by inferring chromatin types from ChIP-Seq data and then using these inferences as an input into MiChroM.

We first obtained ChIP-Seq profiles available from the ENCODE project for the GM12878 lymphoblastoid cell line, encompassing protein-binding experiments and histone marks. We then constructed a neural network to uncover the relationship between compartment annotations and epigenetic markings. This neural network allowed us to predict the chromatin type of a locus, provided biochemical data for that locus.

The predicted sequence of chromatin types for a chromosome then serves as direct input for molecular dynamics simulations using the MiChroM potential, which generates an ensemble of 3D structures. The de novo prediction of chromosome architecture for human lymphoblastoid cells was extensively validated against DNA-DNA ligation and fluorescence in situ hybridization data, demonstrating that there is sufficient information encoded in the biochemical data to accurately predict chromosomal structures. The broad agreement between theory and experiment point to the existence of a sequence-to-structure relationship between epigenetic modifications and chromosomal structure.

Furthermore, since the MEGABASE annotation is made from biochemical data alone, it supports the idea that phase separation of distinct chromatin types is carried out by proteins and regulated by epigenetics.

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Role of paracrine signaling and cell movements in the self-organization of micropatterned human embryonic stem cell colonies

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Human embryonic stem cells (hESCs) offer a unique window into early stages of our own development. In a previous study, we showed that spatially confined hESCs treated with Bone Morphogenic Protein 4 (BMP4) ligand, self-organize to form spatial patterns of differentiation, thus recapitulating gastrulation *in vitro*. In the current study, we quantitatively examined the role of cell movements and cell communication through paracrine signals in this self-organization. Our results show that waves of paracrine signals, moving from colony edge inwards, are essential for hESCs self-organization. Based on experimental results, we propose a reaction-diffusion based mathematical model that recapitulates the signaling wave and correctly predicts the self-organized patterning of spatially confined hESCs.

Keywords **— Embryonic development, stem cells, self-organization**

Gastrulation is a stage in embryonic development when a homogeneous population of stem cells self-organizes into the three germ layers: endoderm, mesoderm, and ectoderm. These germ layers eventually form all the cells of the developed embryo. Despite its importance, the mechanisms underlying gastrulation are not completely understood. Genetic studies in mouse embryos have revealed the signaling pathways involved in gastrulation, however, we still do not understand how these signaling pathways function together to initiate differences in a homogeneous population of cells. The interplay between signaling and cell movement during gastrulation is also not well understood [1].

Human embryonic stem cells (hESCs) offer a good model to investigate the mechanisms underlying gastrulation. As an *in vitro* system, they can be used for quantitative studies, which are very difficult to perform in a developing mammalian embryo. In a previous study, we showed that when hESCs grown in circular micropatterned colonies are differentiated by Bone Morphogenic Protein 4 (BMP4) ligand, they self-organize to form robust spatial patterns of differentiation. These patterns comprise consecutive radial rings of differentiated cells, with cells in each ring representative of a distinct germ layer [2]. Thus, in response to minimal cues - spatial confinement and BMP4, hESCs undergo gastrulation-like events in vitro.

In the current study, we examined the role of cell movements and paracrine signaling to better understand this self-organized pattern formation. We computationally tracked sparsely labeled cells to determine their movement trajectories during differentiation. We also studied the temporal and spatial evolution of secondary paracrine signaling pathways, Wnt and Nodal, which are necessary for this self-organization. Comparing the signaling data with cell movement data, and coupling it with cell fates revealed that the spatial patterning is due to an expanding wave of paracrine signals than moves from the edge of the colony towards its center and not due to the movement of cells. Taking cues from experimental results, we formulated a simple mathematical model based on reaction-diffusion that correctly predicts the self-organized spatial patterning of hESCs in micropatterned colonies.

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Predicting Transcriptional Output of Synthetic Multi-Input Promoters

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***Short Abstract* — Recent advances in synthetic biology have led to a wealth of well characterized parts. Considering these developments, the potential to create novel multi-input inducible promoters with a greater number of inputs has become simpler. However, the potential combinations of inputs rapidly outpace our capability to fully characterize every possible combination. In this study, we describe two methods to predict the output of multi-input systems as a function of varying concentrations of multiple inducing ligands by combining characterization data of single input systems. These methods can be used by synthetic biologists to better design systems that utilize multi-input promoters**.

I. Purpose

As our ability to design and construct sophisticated synthetic circuits continues to grow, so too must our ability to predict the performance of such circuits *in silico* [1]. In our poster, we will present results of two predictive modeling frameworks. The naïve framework is philosophically ideal in that our naïve models accurately predict multi-input system responses using only single-input data (and knowledge of promoter architecture). The energy modeling framework provides an even higher level of accuracy but requires a small amount of multi-input data.

Importantly, both modeling frameworks scale to systems with large numbers of inputs. In particular, the amount of multi-input data needed to train our energy models scales linearly with the number of inputs, while the number of inducer combinations that such models predict scales exponentially: Suppose we wish to predict a D-input system, where each input takes V possible values. Our energy modeling framework accurately predicts system output for all V input combinations, while requiring only D(V 1)+2 values for model specification. When using a small amount of multi-input data to train our energy models, one natural question arises: How have we chosen the particular 'one-dimensional' subset of the multi-input data? Would not another subset work just as well, or perhaps better? Answering the first question, by selecting the 'one-dimensional' subset of multi-input data by varying one inducer at a time while holding the others at full induction, we thereby probe the full dynamic range of each individual inducer. To validate our choice, we compared the error associated with our energy model prediction to the error produced by using all of the multi-input data to fit the energy model. Importantly, these error values essentially match for all of the systems we tested. (This observation leaves open the possibility that other subset choices may work just as well).

II. Conclusion

Practitioners should consider the following when selecting a method. The naïve model is ideal when the user has a vast library of well-characterized single-input devices and wishes to evaluate the digital (on/off) behavior of potential designs without having to perform additional lab work. The naïve framework provides predictions that will aid the user in narrowing down the large design space of potential multi-input combinations and select candidates to build or analyze further. The energy model excels when the user has already constructed a multi-input system and wishes to probe the entire induction space. By collecting a small set of induction data, the rest of the induction space can be predicted to a high degree of accuracy.

Our predictive energy modeling framework captures the analog nature of the inputs/output of multi-input promoters. Such promoters are often treated as digital devices, because it can be too resource intensive to test the entire input space. Digital inputs/output approximation works in certain situations. However, when designing microbes for complex environments such as the gut microbiome or soil, relevant signals may be in constant flux. An analog predictive approach is therefore necessary, as it facilitates the design of circuits that can accommodate a range of signals [2]. The analog approach assists with the parts problem as well: Analog circuits can require fewer parts than their digital counterparts to compute a given function [3].

Overall, our predictive methodologies facilitate the design of synthetic microbes that can operate in complex, dynamic environments.

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Modeling viral copy number dynamics during infection by bacteriophage lambda

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***Short Abstract* — Infection of an *E. coli* cell by the bacteriophage (virus) lambda results in a cell-fate decision between two outcomes: cell death following rampant viral replication, or viral dormancy and integration into the host genome. While much attention has been focused on the effect of the initial number of co-infecting viruses on the decision, the role and regulation of post-infection replication is still poorly understood. We are developing a model of the post-infection decision focusing on regulation of replication, with the goal of achieving a clearer understanding of the role of viral copy number in the decision.**

***Keywords* — Cell-fate decision, replication, viral copy number**

THE infection of *E. coli* by the bacteriophage lambda is a paradigm for cell-fate decisions [1]. During infection, a choice is made between two pathways: rampant viral replication leading to cell death (lysis), or dormancy and passive viral replication (lysogeny) [2].

While the decision outcome is influenced by many factors [3], the number of simultaneously infecting viruses (multiplicity of infection, or MOI) was one of the first identified [4] and remains the one of the most studied [5]. During infection, high MOI biases the decision towards lysogeny, whereas low MOI biases it towards lysis — a curious reversal of the relative viral copy number levels at the end of the decision for each fate. However, the mechanism by which viral copy number is sensed and integrated into the decision is not well understood [6]. More broadly, the mapping of copy number to expression dynamics in gene regulatory networks remains an active area of research [7].

We are currently developing a kinetic model to explore the role of viral copy number in the lambda cell-fate decision. The model consists of a deterministic system of ordinary differential equations describing a simplified lambda gene regulatory network, focusing on the regulation of replication by key proteins, and is able to describe infection both in wild type and replication-deficient mutants. After calibrating the model with experimental data describing viral replication kinetics, we will make testable predictions and elucidate the mechanism by which viral copy number biases the decision. The knowledge we gain from the lambda system may provide additional insights into how gene copy number affects network dynamics.

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Protein Folding at Constant pH: Salt Concentration and pH Changing Protein Stability

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***Short Abstract* — The understanding of electrostatic interactions in protein folding process and, in native state stability is important to molecular biophysics area. The charge interactions are affected by changes in pH and salt concentration of the environment which may alter the protein stability. Here it is implemented the Structure-Based Models with a Constant pH Molecular Dynamics (SBM-CpHMD) to investigate the protein folding in different pH and salt concentration conditions. The simulations present qualitative and quantitative results that agree with experimental data and these studies help in the understanding of pH and salt dependence effects in protein stability.**

***Keywords* — Constant pH Molecular Dynamics, Structure-Based Models, pH Dependence, Ionic Strength**

I. Introduction

An understanding of factors which affect the stability and the folding of proteins is of fundamental importance in the comprehension of principles that govern the behavior of these macromolecules as well as in the development of biotechnological applications. Charged residues may play an essential role in protein stability and function, especially when they are related to binding site recognition. Electrostatic interactions during the protein folding process have been extensively investigated by experiments and theory. pH and ionic strength play a central role in these electrostatic interactions, as well as in the arrangement of ionizable residues in each protein-folding stage.

Most of the computational studies a fixed charge has been adopted to investigate the contribution of the electrostatic interaction. In biological systems, The charge distribution in a protein may change following the protonation/deprotonation of the charged residues based on the ionic strength and the pH of the environment.

In this study, a Molecular Dynamics simulation with Constant pH was implemented in the Structure-Based Model (CpHMD-SBM) to investigate the pH and Salt concentration effects on the folding of different proteins[1–3].

II. Methods

The proteins were modeled using a Structure-based model (SBM) in which the protein is coarse-grained in a Cα atom level of simplification. The residues are represented by beads in α carbon positions, and the Hamiltonian that gives the protein energy interaction is based on the geometry of its native state[4].

The electrostatic interactions were introduced through the inclusion of point charges at the center of the beads representing basic/acidic residues and treating the electrolyte solution according to the Debye-Hückel theory. Thus, a Coulomb screening potential gave the charge-charge interaction[5].

The constant-pH molecular dynamic method (CpHMD) adopted in this study combines a standard molecular dynamics simulation with the Metropolis Monte Carlo method for sampling protein protonation states[6]. Basically, at each given number of molecular dynamics steps, a titratable residue is randomly chosen, and the changing of its protonation state is accepted or rejected according to the Metropolis criterion.

III. Conclusion

The simulations were performed for different proteins capturing the essential features of the pH and salt concentration dependence. The protein stability calculation shows a good agreement between simulations and experiments.

The implementation of CpHMD-SBM reveals to be a powerful tool which helps in the evaluation of salt and pH effects throughout the folding process.

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Decreasing the Computational Time of Biochemical Systems via Parallelism

Keisha Cook[1], Roger B. Sidje[2]

***Short Abstract* — Existing stochastic simulation and tau-leaping methods are frequently used to simulate the transient solutions of biochemical reaction systems. These Monte Carlo type methods require averaging their results over many time-consuming runs to obtain a probability distribution. Therefore, we want to speed up the computational time without compromising the accuracy of the transient solutions. By implementing parallel processes, we can spread the work among many threads simultaneously, and thereby decrease the computational time. Here we describe some strategies used for that purpose.**

***Keywords* — Stochastic Simulation Algorithm, SSA, Parallel, OpenMP, Tau-Leap, First Reaction Method**

I. Introduction

In biochemistry, species/molecules undergo randomly occurring population changes due to chemical reactions. We want to examine the probability distribution of a system. We explore the behavior and performance of simulation methods on a number of biological models. The following models/systems will be used in this study: Gene Toggle Model, Michaelis-Menten System, Schlogl Reactions, p53 and Map-K. Examining the transient solutions of each method offers practical insight into the behavior of the system, i.e. what happens to the population of molecules in a given system over a period of time. One simulation tells us one possible randomization of the given model. For this study, we run simulations many times in order to examine the probability distribution of the final population of each element. We seek to expand upon the well-known stochastic simulation and tau-leaping methods [1,2]. The goal is to extend to large or stiff models; models that take a long time to compute. In order to decrease the computational time, we implement parallel processes. This will significantly decrease the computational time.

II. Biochemical Systems

We consider a chemical reaction system of N molecular species $\{S_1, \dots, S_N\}$ and N reaction channels $\{R_1, \dots, R_M\}$. The state vector of the system is defined as $\mathbf{X}(t)=\{X_i(t),\dots,X_N(t)\}$ where $X_i(t)$ is the number of molecules of species S_i at time t. The propensity functions $a_j(x)\tau$ tells us the probability that reaction R_j will occur in $[t,t+\tau)$ and v_{ij} gives the change in the population after each reaction. The system is updated by $X(t+\tau)=x+v_j$.

A. SSA

The Stochastic Simulation Algorithm (SSA) is used to simulate the random behavior of the species and reactions using one reaction at a time [1]. Multiple runs can be performed in parallel.

B. Tau-Leap

The Tau-Leap method speeds up the SSA by simulating multiple reactions in each time interval. This method starts by determining how many times a reaction will fire in a subinterval. The system is then updated after simulating the group of reactions [1]. Multiple runs can also be performed in parallel.

C. First Reaction Method

The classic SSA and Tau-Leap methods assume that the reaction rates are constant. When reaction rates are time varying, other methods such as the First Reaction Method that take into account variable rates are more appropriate [3].

D. Parallel Computing

Because Monte Carlo methods such as the SSA and Tau-Leap require many runs that are time-consuming, parallelizing them can spread their workload across multiple processors. To do so, we use the OpenMP Fortran Application Program Interface, which allows the use of directives to implement parallelism.

III. Conclusion

Preliminary results show that the parallelization of complicated systems decreases the computational time significantly. Thus, allowing for larger systems with a large number of realizations to be simulated quickly. In the experiments, we use 1,2,4 and 8 processing cores. This work provides insight into how efficient parallelization can be beneficial to many different models.

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Inflationary Embryology

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***Short Abstract* — Soft tissues can build up residual stresses as they grow if their growth rate contains spatio-temporal variations due to local fluctuations in cell division rate. These stresses in turn can feed back on the local growth resulting in nontrivial growth dynamics. We model the growth of epithelial sheets subject to local noise and mechanical feedbacks, and study the density correlations across the tissue. We find that generically these show power law scaling.**

***Keywords* — epithelial growth, morpho-elasticity, target metric, mechanical feedback**

I. Background

Growth of epithelial tissues often involves a level of stochasticity due to noise in cell division rates [1]. Local fluctuations in growth rate can lead to uneven accumulation of mass, which in turn can cause mechanical stresses to build up. It is been known that such stresses can affect cell growth and division rates via the Hippo pathway [2,3]. For example, areas with high density (high compression) are expected to show a decreased growth rate, while areas with low cell density should show increased growth. How strong this stress response is and how much it affects growth in normal conditions are not entirely known.

Experimentally, one can look at the density profile and correlations at different time points, or study how the shape of tagged clones evolves in time. Then, from the theoretical point of view, the question becomes, given a growing tissue with local noise in growth rate and a specific mechanical feedback, what is the time evolution of the density profile, and what can we predict about the shape of clones undergoing growth? Answering these questions can help us better understand the growth dynamics and possible stress response mechanisms underlying the development of epithelial tissues.

II. Model

We model the epithelium as a continuum, elastic sheet undergoing noisy, exponential growth. The challenge is that a non-uniform growth can lead to "incompatible" configurations that cannot be embedded in $\mathbb{R}^3$ [4,5]. Thus, the tissue deforms into a stressed configuration. Tackling the growth incompatibility is in general not an easy task. To make the problem tractable, we work in the target metric formalism [5], and linearize about the average growth rate. We consider both isotropic and anisotropic growth. Anisotropic growth can occur due to oriented cell divisions [6-8], which in turn can be due to stress anisotropy. In each of these two cases, we study the time evolution of density-density correlation functions in the presence of two biologically sensible stress feedbacks. We also look at initially circular clones undergoing non-uniform growth, and study the clone shape statistics.

III. Conclusion

We find that the initial fluctuations in the tissue are correlated on an intrinsic length scale. These fluctuations can then be advected over the tissue as the tissue is dilated, leading to a non-trivial power-law behavior for the density-density correlators at long times. These results are interestingly reminiscent of inflationary cosmology where quantum fluctuations in the early universe are believed to have caused the formation of large-scale structures [9]. Our model also predicts specific clone shape statistics for isotropic and anisotropic growths. By comparing our predictions with experimental findings, we could constrain the underlying mechanisms of mechanical feedback on growth in epithelia.

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Modeling Molecular Motor Procession

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***Short Abstract* — Kinesins are molecular motors that transport cargo along cellular microtubules by transducing chemical energy into forward motion. However, the mechanism by which this is accomplished is not fully understood. The purpose of this study is to explore the kinesin stepping mechanism using a robotics-inspired, physics-based model. In this work, we model a kinesin head and a microtubule surface. We use a motion planning technique to generate kinesin conformations and calculate their respective energies. A transition graph is constructed and used to simulate kinesin dynamics, and identify low energy paths in the graph from a starting point to the native state.**

***Keywords* — Molecular walkers; kinesin; motion planning; OBPRM; energy landscape; protein-protein interaction; motor protein**

I. Introduction

Kinesins are molecular motor proteins that transport cargo while performing a hand-over-hand procession on the surface of microtubules. Each 8-nm step uses the energy of 1 ATP hydrolysis. Much work has been done to understand the mechanism by which these motor proteins convert chemical energy from ATP hydrolysis to mechanical energy. However, the process underlying kinesin's navigation to the plus end of microtubules during cargo transport has not yet fully elucidated. In our work [1], we explore how a kinesin motor domain traverses the molecular interaction energy landscape to find the low energy binding sites to anchor itself on the microtubule surface.

II. Methods

A. Models and generating samples with OBPRM

Kinesins are protein dimers consisting of two motor domains (heads) that bind to the microtubule during stepping, a stalk, and a cargo domain. Microtubules are biopolymers constituted of protofilaments of tubulin heterodimers. We created a model of the kinesin-microtubule system based on the PDB structure 4LNU that includes a single kinesin head interacting with a tubulin heterodimer. A small patch of the microtubule surface with 9 heterodimers and 3 protofilaments was created by aligning the PDB structure with an EM map of a microtubule. Samples were generated using Obstacle Based Probabilistic Roadmaps (OBPRM) [2], in which the microtubule is treated as an obstacle. We produced samples at 5 Å intervals along vectors generated at random directions from the microtubule surface. The benefit of this method is that samples can follow the contours of the microtubule patch more closely.

B. Energy calculations

For each sample, we calculated the non-bonded interaction energy between the kinesin head and the microtubule patch surface based on the Amber94 force field. Both the kinesin and microtubule are treated as rigid bodies, so only inter-molecular interactions are considered in the energy evaluation.

C. Roadmap construction and searching for a path

We constructed a graph from the set of samples, where each sample corresponds to a vertex. For each pair of samples that are within 5 Å of each other, we added an edge connecting those two vertices. We used a graph-based approach to search for a path from a given start state to a goal state, the native state in the PDB structure. Beginning with the start state, we chose the best vertex for a transition, defined as the one that maximally decreases the energy. This is repeated after each transition until the goal is reached.

III. Conclusions and Future Work

Our energy calculations show the presence of low energy regions at the positions on the microtubule surface where kinesin is known to bind. However, we also find low energy regions in between binding sites, which could indicate the existence of metastable states. We also find that there exists a low energy path from a start state to the native state along a single protofilament with no sidestepping. Future work will include random walks on the roadmap and will investigate how adding obstacles to the model can affect the kinesin stepping mechanism.

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Unveiling Molecular Mechanisms of Kinesin-5

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***Short Abstract* — Molecular motor protein Kinesin-5 (Eg5) is a member of kinesin superfamily that is critical for bipolar spindle assembly and spindle maintenance during mitosis. As a result it is a promising chemotherapeutic target for cancer treatment. While a number of small-molecule drugs that interact with Eg5 have been identified, little is known about the molecular mechanisms by which they inhibit Eg5 function. Furthermore, multi-motor systems can exhibit qualitatively diverse behavior for different drugs, in some cases showing non-linear dependence of motor velocity on drug concentration. We study molecular mechanisms behind function of individual Eg5 and multi-motor systems involving it using computational modeling techniques. Besides apparent fundamental value this work has direct implications for clinical applications, where in depth understanding of Eg5-drug interaction is important.**

***Keywords* — Motor proteins, Kinesin-5, computational modeling.**

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Tuning Spatial Profiles of Selection Pressure to Modulate the Evolution of Resistance

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***Short Abstract* — Spatial heterogeneity plays an important role in the evolution of drug resistance. While recent studies have indicated that spatial gradients of selection pressure can accelerate resistance evolution, much less is known about evolution in more complex spatial profiles. Here we use a stochastic toy model of drug resistance to investigate how different spatial profiles of selection pressure impact the time to fixation of a resistant allele. Using mean first passage time calculations, we show that spatial heterogeneity accelerates resistance evolution when the rate of spatial migration far exceeds that of mutation but slows fixation when mutation dominates. Interestingly, there exists an intermediate regime — characterized by comparable rates of migration and mutation — in which the rate of fixation can be either accelerated or decelerated depending on the spatial profile, even when spatially averaged selection pressure remains constant. Finally, we demonstrate that optimal tuning of the spatial profile can dramatically slow the spread and fixation of resistant subpopulations, which may lay the groundwork for optimized, spatially-resolved drug dosing strategies for mitigating the effects of drug resistance.**

***Keywords* — antibiotic resistance, spatial heterogeneity, evolution, modeling, mean first passage time, master equation**

I. Background

ANTIBIOTIC resistance is a central impediment to the treatment of microbial infections. While most of the work on understanding resistance has been performed at the molecular level, resistance is a fundamentally stochastic process governed by the complex interplay between microbial evolution and evolutionary selection. Evolution in natural settings takes place in heterogeneous environments characterized by spatial fluctuations in multiple factors, such as drug concentrations, pH, and host immune responses, all of which potentially affect cellular growth. Recent experimental [1] and theoretical [2] results show that understanding evolution and ecology in such spatially-extended systems is crucial for understanding resistance.

In this work, we use stochastic models of evolution along with a mean first passage time from statistical physics to calculate the mean time required for an initially wild-type population to be composed entirely of mutants. For tractability, we restrict our system to having three connected microhabitats and allow cells to replicate according to a simple Moran process. Our primary interest is calculating the fixation time from an initially wild-type population for a given selection pressure landscape and comparing these fixation times across different selection pressure landscapes with the spatially-averaged selection pressure fixed. In addition to the specific selection pressure landscape, the mutation rate and migration rate of the system will determine the fixation time.

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II. Results

We find that the fixation time for a population of initially wild-type cells varies significantly with the spatial distribution of selection pressure, even when the spatially-averaged selection pressure remains fixed. Interestingly, we observe that resistance can be either accelerated or decelerated by spatial heterogeneities in selection pressure.

We observe that there are three different regimes for our system. In the limit where the mutation rate is much smaller than the migration rate, spatial heterogeneity speeds fixation for the system. In the limit where the mutation rate is much larger than the migration rate, any spatial heterogeneity slows fixation. And between these two limits exists an intermediate regime, in which heterogeneity can either speed or slow fixation. While the mutation rate and migration rate are often inherent to the specific system of interest, the selection pressure landscape can potentially be modulated to speed or slow the evolution of resistance in the system.

We also use this method to look at fixation starting with an initial mutant subpopulation. We demonstrate that tuning the spatial distribution of selection pressure can dramatically slow fixation when the resistant subpopulation is not uniformly distributed in space.

III. Conclusion

Using a simple toy model to investigate the evolution of antibiotic resistance in a spatially-extended system, we demonstrate that the selection pressure landscape can be tuned to slow or speed the emergence of antibiotic resistance.

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A high throughput optogenetic system to interrogate MAPK signaling network dynamics at the single cell level

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***Short Abstract* — We have coupled the FGF/MAPK pathway to an optogenetic FGF receptor and an ERK activity reporter. This system allows us to activate the pathway with light in a highly automated and reproducible way and to measure ERK signaling output in hundreds of single cells with high temporal resolution. By combining dynamic INPUT stimulation patterns together with drug and siRNA perturbations and by recording ERK activity OUTPUTs, we aim to identify molecular players and feedback wiring involved in the regulation of the MAPK network.**

***Keywords* — MAPK signaling, ERK, optogenetic, single-cell measurements, drug perturbations, siRNA screen.**

I. BACKGROUND

RECEPTOR tyrosine kinases (RTK) convert extracellular inputs such as a growth factor binding into specific cellular outputs through the activation of signaling networks [1]. Despite numerous studies of the MAPK pathway, we still miss crucial information about how network components are wired, and how various dynamic responses of the network orchestrate the cell fate choice.

Due to this complexity, we need to combine live ERK activity measurement with network perturbations to elucidate network wiring and how network components affect dynamic responses.

This approach has already allowed to discover new pathway connections as recently demonstrated by Pertz et al. [2]. Mathematical models of different pathway topologies could be discriminated by measuring dynamic single-cell ERK activity responses to growth factors delivered in a pulsatile manner using microfluidic devices.

II. RESULTS

To further elucidate the MAPK pathway topology, we have built a synthetic system where the MAPK pathway can be activated with light of different intensity, duration, or stimulation pattern (single- or multi-pulse) using an optogenetic FGF receptor (optoFGFR1). The use of dynamic light input provides high temporal and spatial resolution and enables to fully automatize the experiments. We then measure ERK signaling output in hundreds of single cells at the same time with an ERK activity reporter (ERK-KTR).

The optoFGFR1 receptor activates specifically and reversibly the ERK, AKT and PLCγ MAPK pathways with 488nm light pulses. The receptor activation is based on the self-dimerization of CRY2PHR, a photosensitive domain fused to the cytosolic part of the receptor [3]. ERK-KTR is a substrate of ERK that gets exported from the nucleus to the cytosol upon its phosphorylation by activated ERK [4]. This translocation event is used as a proxy to measure ERK activity without modifying ERK protein itself.

We generated a stable NIH3T3 cell line expressing the optoFGFR1 and the ERK-KTR and characterized ERK activity in response to light stimulations. To increase the throughput of our experiments, we established a pipeline to highly automatize the image acquisition and the stimulations, as well as the data processing. Using this pipeline, we could observe that light triggers specific and reproducible ERK responses depending on the pulses intensity and frequency.

As a proof of concept, this system was used to study the effect of two well-known MEK inhibitors, UO126 and PD0325901, on the activation of the pathway. Using time series classification and clustering we could identify subpopulations of cells with distinct dynamic patterns despite high variability in ERK activation levels in response to the same treatment conditions.

III. CONCLUSION

Our system is a tool to study the effects of targeted system perturbations. It enables triggering of homogenous and reproducible ERK activity dynamics in hundreds of single cells with a high temporal resolution. Such measurements are essential for building and calibrating predictive mathematical models of the MAPK pathway.

Combined with a highly-automatized image analysis workflow, this system provides the required throughput to screen for proteins involved in the network regulation using drugs and siRNAs targeting selected molecular players [5].

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Anomalous Diffusion, Spatial Coherence, and Viscoelasticity from the Epigenetic Energy Landscape of Human Chromosomes

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***Short Abstract* — Chromosome architecture originates from non-equilibrium, active processes that continuously rearrange chromatin over the lifetime of cells; optical experiments report sub-diffusive dynamics and spatially coherent motion. Using energy landscape theory, we built a model for chromosomes that takes into account biochemical interactions mediated by proteins and regulated by epigenetic markers. We study the dynamics of interphase human chromosomes as generated by this quasi-equilibrium energy landscape. Using numerical simulations of two interacting human chromosomes, we show that the epigenetic energy landscape naturally explains the physical mechanism leading to spatial coherence, viscoelasticity and sub-diffusive behavior in interphase chromosomes as observed in numerous experiments.**

Abstract

Chromatin consists of DNA and hundreds of associated proteins. In eukaryotic nuclei, the interactions between proteins and DNA generate organized structures, which are characteristic of both cell state and type [1,2]. This organization is a key element of transcriptional regulation, and its disruption often leads to disease.

Recently, we introduced a physical model for chromatin folding [3] that is able to predict the structural ensembles of human chromosomes using as input the sequence of epigenetic markings obtained by chromatin immunoprecipitation-sequencing. We exploited the idea that chromosomes encode a 1D sequence of chromatin structural types. Interactions between these chromatin types determine the 3D structural ensemble of chromosomes through a process similar to phase separation. Chromatin types are distinct from DNA sequence and change during cell differentiation, thus constituting a link between epigenetics, chromosomal organization, and cell development.

We demonstrated that it is possible to predict the sequence of chromatin structural types and, consequently, how a genome will fold, based on the epigenetic marks that decorate chromatin [4]. The structural ensembles resulting from this theory of genome folding were extensively validated by the results of DNA-DNA ligation assays and fluorescence microscopy.

Here, we revisit the results of several experimental observations regarding chromatin dynamics in light of the new theory for genome organization outlined above. By using molecular simulation, we analyze the implications of the model without any tuning and we show that it naturally explains and reproduces anomalous diffusion, viscoelasticity, and spatially coherent dynamics as observed in chromosomes. All of these phenomena were previously analyzed only through phenomenological models. We show that the very same interactions that account for genome organization in interphase also naturally explain several non-trivial features of genome dynamics, namely, spatial coherence, viscoelasticity and sub-diffusivity.

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Emergent versus Individual-Based Multicellular Chemotaxis

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***Short Abstract* — Multicellular chemotaxis can occur via individually chemotaxing cells that are mechanically coupled. Alternatively, it can emerge collectively, such as from cells on the exterior of the collective responding to chemical signals while bulk cells remain uninvolved. We find that the precision of this type of emergent chemotaxis is higher than that of individual-based chemotaxis for one-dimensional cell chains and two-dimensional cell sheets, but not three-dimensional cell clusters. We describe the physical origins of these results, discuss their biological implications, and show how they can be tested using experimental measures. Lastly, we discuss expansions to this work through the use of long distance chemical signaling rather than mechanical coupling.**

***Keywords* — Chemotaxis, collective migration, sensing**

I. Background

Collective migration is ubiquitous in cell biology, occurring in organism development [1], tissue morphogenesis [2], and metastatic invasion [3]. Collective migration often occurs in response to chemical cues in the environment, a process known as chemotaxis. The simplest way for cells to collectively chemotax is by individual detection and response: each cell measures and moves in the perceived direction of a chemical gradient, while mechanical coupling keeps the group together. This individual-based chemotaxis (IC, Fig. 1, left) is found throughout cell biology [4]. However, recent experiments have uncovered an alternative type of chemotaxis in which cells on the exterior of a group polarize while interior cells do not, a mechanism observed in neural crest cells [1]. This type of emergent chemotaxis (EC, Fig. 1, right) presupposes a machinery within cells that allows for behavior to change once a cell is in a group. Since this machinery may come at a cost, this raises the question of whether EC offers any fundamental advantages over IC.

II. Results

To address this question, we compare groups of cells undergoing IC and EC in 1D chains, 2D sheets, and 3D clusters. For cells undergoing IC, each cell is assumed to make measurements of a local gradient and polarize completely independently of all other cells. The polarization vector of the group is then taken to be sum of all individual polarization vectors. For cells undergoing EC, exterior cells

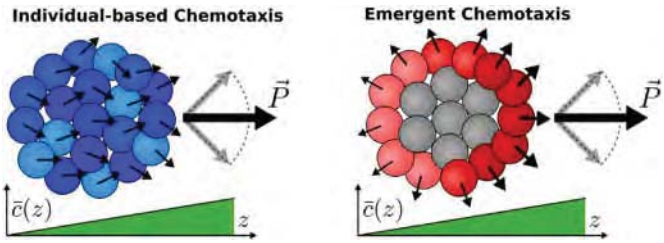

FIG. 1. Groups of cells undergoing IC (left) and EC (right).

polarize outward with a weight proportional to the local concentration. This allows cells in areas of higher concentration to dominate the group polarization vector. We find that both mechanisms cause the mean group polarization vector to scale linearly with population size. However, due to the stochastic nature of diffusion and cell sensing, these methods are shown to have different noise properties. In particular, we show that for 1D chains and 2D sheets, the relative noise in the group polarization vector decreases more rapidly with population size for cells undergoing EC, while no difference in scaling is seen for 3D clusters [5].

III. Future Works

Currently, we are expanding this work to look at groups of cells interacting not through mechanical coupling, but rather through long range chemical signaling. We will show preliminary work in which we model cells that through a combination of secreted attractant and repellent molecules create a preferred separation between themselves and neighboring cells. In a manner similar to the IC model, these cells measure and chemotax individually while being coupled to their neighbors via these secreted molecules. We will show the effects of population size, preferred separation length, and secretion rate on the precision with which these groups can successfully track a chemical gradient.

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Role of Stochasticity in Mammalian Drug Resistance

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***Short Abstract* — How cell-to-cell expression variability or noise contributes to chemotherapy survival and resistance independently from mean expression in cancer is unclear. As a model to this end, we built two synthetic noise-controlling gene circuits based on feedback in Chinese Hamster Ovary (CHO) cells. To decouple noise from mean expression, we chemically controlled the noise of a puromycin resistance gene at a similar mean. Noise delayed the adaptation time, ranging from initial cell death to regrowth, under low drug concentrations, while the opposite was true under high drug concentrations. Mathematical modeling explained these evolutionary dynamics based on the severity of cell drug-sensitivity.**

I. Purpose

Chemotherapy resistance can arise from genetic mutations, but the role of nongenetic processes, including cell-to-cell gene expression variability or noise [1], in survival and evolution is unclear. Gene expression noise can aid yeast cells to survive drug treatment [2], but little evidence exists for subsequent evolutionary effects, especially in genetically-identical mammalian cells. To properly address this question, one must decouple noise (typically quantified as the standard deviation divided by the mean) by experimentally manipulating noise for a drug resistance gene at a similar mean.

The field of synthetic biology rationally designs and builds gene regulatory networks from the ground-up. Synthetic gene circuits can then be engineered to manipulate noise in bacteria [3], yeast [4], and mammalian cells [5] using, for example, positive feedback to amplify noise [6] and negative feedback to minimize noise [7]. However, synthetic gene circuits that can control decoupled gene expression noise in genetically-identical mammalian cells are lacking.

Here, we constructed two synthetic gene circuits that amplify or minimize gene expression noise in Chinese Hamster Ovary (CHO) cells based on positive or negative feedback. We controlled gene expression noise of a puromycin drug resistance gene while maintaining comparable means, thereby allowing a controlled study of noise and mean expression in mammalian drug resistance.

II. Results

With time-lapse microscopy, we measured the adaptation time, spanning from initial cell death to the start of exponential regrowth. Noise delayed the adaptation time under low drug levels, while the reverse was true under high drug levels. A modified population genetics model [8] explained these drug dose- and expression noise-dependent evolutionary dynamics. We found that the evolutionary dynamics depends on the severity of cellular drug-sensitivity. Most of the regrowing cells maintained their resistance after drug withdrawal. In rare cases, cells reverted to their pre-treatment expression levels and regained drug sensitivity.

III. Conclusion

Gene expression noise contributes independently of the mean to the timing of adaptive drug resistance in mammalian cells. Cells with higher levels of noise that are transiently protected from drug may then acquire mutations leading to resistance. This synthetic system indicates that gene expression noise should in general contribute to cancer chemotherapy resistance.

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Coarse grained Constant-pH protein dynamics

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***Short Abstract* — Protein's environment is critical to define its structure and function. One critical variable is pH and some constant pH molecular dynamics methods already exist. In our work we want to systematically study long timescale events. To achieve this we implemented and tested a constant pH module to the existing coarse grained potential AWSEM. We obtain the parameters of our model, calculate native state pKa shifts of proteins and study changes in stability as a function of pH. Our results show a promising tool to study global behaviour of proteins coupling dynamics and pH.**

***Keywords* — Constant-ph, Dynamics, Coarse-grained, AWSEM, LAMMPS**

Based on the energy landscape theory [1], protein structure fluctuates among minimal frustrated conformations. The dynamics between these conformers and the relative stability depend on the protein sequence and the surrounding media. Just like temperature, pH is a key macroscopic variable that living systems regulate and take advantage from. Some of the protein residues are titratable making these macromolecules coupled to pH.

To study the coupling between the energy landscape and pH, several constant pH molecular dynamic methods have been implemented in recent years [4-8]. However these tools aren't well suited to systematically study long timescale events because of the restrictive computer time required. To tackle this problem we implemented and tested a constant-pH module in the transferable potential AWSEM [2] which has been previously used to predict many unknown protein structures and to describe protein's energy landscapes. It is inside LAMMPS [9], a molecular dynamics suite written in C++, which gives many advantages at the hour of developing new features.

Our main goal is to couple long scale conformational changes in proteins with pH and use this as a tool to explore pH stability, dynamics and protein-protein interactions.

The existing AWSEM code has an electrostatic module which enables the use of Debye Huckel screened interaction [3]. We added to this a Monte Carlo discrete charge sampling algorithm to account for pH behaviour. We modelled each residue behaviour with local and long range contributions to the free energy [11].

To refine our method we optimized our parameters with an analytical version of the model exploring different approximations and approaches to test its limits. We compared our method with an already existent Go-model [10] and classical MD constant-pH and with experimentally determined pKa. We found that this method improves the Go-model constant-pH pKa prediction and, in the worst of the cases, the difference between experimental and predicted value is around 1.5 pH units.

This method would aid in the study of the coupling between protein thermodynamics, kinetics and pH.

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Evolution of new regulatory functions

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***Short Abstract* — Gene expression is controlled by regulatory proteins interacting specifically with external signals and DNA regulatory sequences. These interactions force the network components to co-evolve to maintain functionality. Yet, existing evolutionary models mostly focus on isolated genetic elements. Here we construct a network model to study the evolutionary expansion of gene regulatory networks via duplication and subsequent specialization. We synthesize a biophysical model of molecular interactions with the evolutionary framework to find the conditions and pathways by which new regulatory functions emerge. We show that specialization is usually slow, but is accelerated by regulatory crosstalk and mutations that promote promiscuous interactions.**

***Keywords* — biological networks, molecular evolution, gene regulation.**

I. Introduction

GENE regulation is flexible and its evolution is thought to be more rapid than the evolution of the coding sequences. The case that we focus on here is the divergence of gene regulation, via expansion of transcription factor (TF) families. Following such expansion, a regulatory function is carried out by a larger number of TFs than before, allowing for additional fine-tuning or for an expansion of the regulatory scope. The main avenue for such expansions are TF duplications. Subsequent specialization of TFs often involves divergence in both their inputs (e.g., ligands) and outputs (regulated genes). Despite its key role, theoretical understanding of TF duplication is still incomplete. Existing models predominantly belong to two categories: gene duplication–differentiation models study sub-functionalization of isolated proteins with no regulatory role; Biophysical models use a thermodynamic description of TF–BS (binding site) interactions accounting for the broad DNA binding repertoire of TFs [1], but disregard gene duplications. Here we synthesize these two frameworks to construct a biophysically realistic description of gene regulatory network evolution.

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II. Results

We assume two TF copies, potentially responding to two signals and regulating two or more downstream genes**.** A genotype in our model is specified by the regulatory DNA binding site sequences, TF binding preferences and signal sensitivities. Using the thermodynamic model of gene expression [1] we can calculate for each genotype its gene expression in response to the input signal(s). Genotypes evolve via combinations of mutations affecting TF binding preferences, signals sensitivities or binding sites. These mutations are then either fixed or lost depending on their fitness effect, whereas fitness is determined by the network gene expression. This defines a huge, yet finite fitness landscape. Using Markov chain framework, we are able to fully calculate its steady state, dynamics and evolutionary trajectories. Importantly, network functionality in our model is determined not by any particular sequence, but rather by the match or mismatch between sequences of distinct components. This enables us to coarse-grain the huge genotype space into only six "macro-states" based on interaction intensities. Such mapping significantly simplifies the analysis and demonstrates the huge dimensionality reduction between genotype to phenotype. We find two possible evolutionary trajectories to specialization: either going via intermediate configurations of partial specificity or via temporary loss and re-gain of TF specificity.

As TFs and BS should co-evolve, they constrain each other. We find that TF evolution becomes slower and more constrained the more downstream genes it regulates. We propose that mutations that reversibly broaden the TF binding scope ("promiscuity-promoting") can alleviate these constraints and shorten evolutionary times [2].

III. Conclusions

The novelty of our work is both conceptual and methodological. While most evolutionary models focus on single genes, here we demonstrate that network evolution is radically different. We develop methodologies to analyze high-dimensional genotype spaces and interpret network phenotypes, that are more broadly applicable [3].

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Caracterization of PPI and Metabolic Networks

D. Gamermann[1], J. Triana[2], R. Jaime[3]

Short Abstract — **In this work we perform a systematic statistical analysis of thousands of graphs representing metabolic and protein-protein interaction (PPI) networks. The focus of the analysis is to identify properties that deviate from the expected values had the networks been build by randomly linking nodes with the same degree distributions. The survey identifies the properties of biological networks which are not solely the result of the degree distribution of the networks, but emerge from the evolutionary pressures under which the network evolves. We also investigate the quality of fits obtained for the nodes degree distributions to power-law functions.**

Keywords — **Graphs, biological networks, PPI networks, Metabolic networks, scale-free networks, small-world**

I - Motivation

It is often claimed that biological networks are scale-free (meaning that their node's degree distributions follow a power-law function) though most works that fit a power-law to the degree distribution of a given network overlook the quality of the fit.

In the present work we study a sample of thousands of networks representing organisms' metabolisms and proteomes. We evaluate the main graph topological characteristics of the networks and perform fits to the graphs' degree distributions, evaluating also the quality of the fits (p-value).

II - Data

The metabolic network for an organism is a graph representing its metabolism based on the biochemical reactions that keep its cells (or cell) alive. Two metabolites in the graph are connected if they appear as a substrate-product pair in any chemical reaction in its metabolism. The PPI network of an organism is the graph where each protein present in a proteome represents a node and a link between two nodes indicates the existence of some interaction between the respective proteins.

Data from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database [1, 2] was downloaded and we successfully reconstructed the metabolic networks for 3481 organisms.

In order to reconstruct the PPI networks, we used data from the STRING database [3]. We reconstructed 1073 graphs representing PPI networks.

III - Analysis

The theory on measurements related to graphs and the study of network characteristics and parameters can be found in several books and reviews such as, for example, [4, 5].

For each reconstructed network, we evaluated the graphs average local and global clustering coefficients, assortativity, average nodes distances, number of two-paths and triangles. Also, we fitted each graph degree distribution to a power-law function by the maximum likelihood method and we evaluated the p-value for the fit with the χ^2 statistic.

Moreover, for each graph, we evaluated all above mentioned topological characteristics for 10 randomized versions of each network (graphs with the same degree distribution, but with the nodes linked randomly. We then evaluated the student's t statistic for the difference of each parameter between the real and the randomized versions, obtaining in this way the statistical significance of the deviations.

IV - Results

Our findings suggest that, while PPI networks have properties that differ from their expected values in their randomized versions with great statistical significance, the differences for metabolic networks have a smaller statistical significance, though it is possible to identify some drift.

About the quality of the fits obtained for the nodes degree distributions to power-law functions, our work indicates that metabolic networks do describe the distributions only if one disregards nodes with degree equal to one, but in the case of PPI networks the power-law distribution poorly describes the data except for the far right tail covering around half or less of the total distribution.

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Survival of the chiral

Ashish Bino George[1] and Kirill S. Korolev[2]

***Short Abstract* — The origin of chirality has intrigued generations of scientists. The role of natural selection, however, has been largely overlooked. Yet, chirality in shape and motility readily evolve in bacteria and cancer cells. We find that cells gain a substantial fitness advantage by increasing their chirality, or switching handedness. Selection occurs via bulges along the colony edge in regions where cells with different chiralities meet. We developed an analytical framework that explains these bulges and their effect on selection. Overall, our work suggests that chirality could be an important ecological trait that mediates competition, invasion, and spatial structure in cellular populations.**

***Keywords* — evolution, chirality, population dynamics, cancer, range expansions, pattern formation, KPZ equation.**

I. Introduction

CHIRALITY exists at all scales: from DNA and flagellar motion to embryogenesis and bacterial swarms. The classic explanation for molecular chirality is a fluctuation that slightly breaks the left-right symmetry, magnified by a self-amplifying process [1]. The many chiral components in the cell then serves as a natural explanation for macroscopic chirality. This existing theory explains how, but not why, chirality emerges. Several lines of evidence suggest that a change in chirality could be advantageous. Experiments with *Arthrospira* observed switching from a right-handed to left-handed helix following exposure to grazing by a ciliate [2]. Extensive work with *Paenibacillus* demonstrated that this microbe switches between chiral and non-chiral forms to optimize its fitness in different environments [3]. A study of spatial patterns made by human and mouse cells across tissue types found that all cells tested produced chiral patterns [4]. Additionally, skin cancer cells displayed a chirality opposite to that of human cells, including skin cells derived from the same patient.

II. Results

Motivated by these experimental observations, we asked whether chirality could be related to natural selection in the context of growing cellular populations. We developed a minimal reaction-diffusion model of chiral growth in compact colonies. For strains with equal chiralities, our model quantitatively reproduced logarithmic twisting of boundaries between the strains and other spatial patterns observed in experiments [5]. Colonies of a chiral strain and a non-chiral strain expanded at the same rate when grown separately. However, the chiral strain was more fit when grown together. We found that competition always favored the more chiral strain when two strains with the same handedness, but different magnitude of chirality were competed. In contrast, the competition between two strains with different handedness often resulted in stable coexistence. To understand our observations, we developed an effective theory of chiral growth. For chiral strains, the population dynamics is described by the chiral KPZ equation coupled to the Burger's equation with multiplicative noise. The theory shows that selection for a specific chirality is mediated by bulges along the colony edge that appear in regions where the strains with different chiralities meet. Additionally, we observe that strains of opposing chiralities can overcome the effects of genetic drift which causes sharp boundaries between strains in growing colonies [6]. For sufficiently strong chirality, we observe a transition to a completely intermixed state.

III. Conclusion

Using a minimal reaction-diffusion model, we captured experimental observations of logarithmic boundaries formed by chiral bacteria. Extending this, we have shown that selection for chirality is mediated by the formation of bulges. We have also shown that chirality can significantly alter the spatial structure within a colony. The intermixed phase can facilitate and stabilize interactions such as nutrient exchange. We developed an analytical framework to study the two-way coupling between selection and colony front. The theory could also be valuable for studies of competition in microbial colonies accounting for the effect of front undulations, which are known to fundamentally change the nature of competition. Our work suggests that some changes in cellular chirality could mediate invasion, coexistence and spatial structure and, therefore, deserve further study.

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Stochastic Modeling Quantifies Tumor Elimination and Evasion in the Setting of Immunotherapy

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***Short Abstract* — We propose a mathematical model between tumor and CD8+ T-cell adaptive immune compartments. This framework accurately models relevant empirical features, including the growth-threshold conjecture of immune activation, and predicts experimental observations including 'sneak-through,' wherein intermediate growth threats are penalized relative to their slower and faster counterparts. We find agreement between our model and AML age-dependent incidence as a function of decreasing immune turnover and repertoire size. Lastly, we quantify therapeutic efficacy of neoadjuvant immunotherapeutic strategies in the setting of an immune evading threat. Our model serves as a first attempt at modeling stochastic cancer evolution alongside an adaptive immune compartment.**

***Keywords* — Cancer immunotherapy, applied probability, acquired immune evasion.**

I. Introduction

THE adaptive immune system plays an integral role in immuno-editing, and cancer progression occurs only if a tumor successfully evades immune detection [1]. Immunotherapy is responsible for recent breakthroughs in cancer treatment and encompasses strategies aimed at enhancing the patient's immune system via a number of mechanisms, including tumor antigen vaccines [2], immune checkpoint inhibition [3], and Chimeric Antigen Receptor T-cell (CAR-T) therapy [4].

Despite this improvement, durable clinical outcomes are still limited as tumors are capable of acquiring treatment-resistant clones during disease progression [5], and cancer cells exploit a variety of strategies to avoid CD8+ T-cell elimination [6], including downregulation MHC-I [7], preventing CD8+ recognition altogether. Prior studies have considered systems level interactions between the tumor and host adaptive immune system [8,9]. An independent research effort has investigated acquired drug-resistance during clonal evolution [5,10]. At present, the time-dynamic effect of acquired immune evasion on tumor development under adaptive immune surveillance remains uncharacterized. Understanding the successes, and failures, of adaptive immune system co-evolution with tumor cells from a population dynamical level would enhance our understanding of immunotherapy, enabling quantitative predictions of treatment success.

Here, we describe a foundational model between cancer and the adaptive immune system wherein tumor cells may be recognized by the immune cells but may also acquire an immune-evasive phenotype. We show that our model predicts empirically observed 'sneak-through' in that threats with large and small net growth rates have a preferential advantage over those with intermediate growth rates [11]. We characterize AML incidence as a function of immune turnover and repertoire diversity and conclude by quantifying the benefit of immunotherapy and predicting treatment-specific advantages based on tumor growth rates and immune competency.

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II. Results

A. Cancer sneak-through predicted via increased immune evasion

Preferential immune escape and immune evasion are predicted for threats with small and large growth rates over those of intermediate growth rates.

B. Age-specific AML incidence characterized by diminishing adaptive immune system

AML incidence data is quantified by an immune system that diminishes in age and is therefore at increased risk of tumor escape and evasion.

C. Enhanced survival predicted for immunotherapy

We end by estimating treatment success probabilities for CAR-T and tumor vaccine immunotherapies.

III. Conclusion

Our model is consistent with empirical observations, predicts the likelihood of cancer progression due to immune escape and evasion, and may be used in the future to quantify immunotherapy's enhanced tumor control.

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Simulating and fitting stochastic models of RNA transcription via the Gillespie algorithm

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***Short Abstract* — Testing mechanisms of RNA transcription requires predictive models and experimental data. We present an implementation of the Gillespie algorithm that simulates the stochastic kinetics of nascent (actively transcribed) and mature RNA, including two- and three-state gene regulation, RNA synthesis initiation and stepwise elongation, release to the cytoplasm, and stepwise degradation. To facilitate comparison with experimental data, the algorithm predicts probe signals measurable by single-cell imaging. By minimizing statistical distance from experimental signal distributions, we can estimate underlying parameters.**

***Keywords* — Transcription kinetics, single-cell imaging, parameter estimation.**

I. Background

THE transcription of RNA is the product of both stochastic and deterministic dynamical processes in the cell [1]. The wealth of hypotheses about these processes motivates the development of a modular framework to test proposed mechanisms and quantify their kinetic parameters. Due to challenges in producing closed-form solutions to arbitrary kinetic models [1], simulations offer an attractive alternative. We present a simulation platform that easily incorporates new mechanisms, offers a graphical user interface (GUI), provides outputs comparable to experimental measurements, and efficiently scales when scanning kinetic parameters.

II. Methods

We modified the Gillespie algorithm to simulate the kinetics of nascent and mature RNA. Stochastic reactions model initiation of transcription, stepwise elongation along the DNA strand, release to the cytoplasm, attachment of RNase and stepwise degradation [2], while stochastic or deterministic schema model gene regulation. The algorithm is implemented in MATLAB and can be scaled using distributed computing, such as the Amazon Web Services (AWS) cloud. Probe signals predicted by the simulation are verified using differential equation solutions and the finite state projection (FSP) algorithm [3]. Parameter estimation uses the genetic algorithm to fit synthetic FSP data and single-molecule fluorescence *in situ* hybridization (smFISH) measurements of nascent and mature RNA in cells [4].

III. Results

The simulation reproduces the average smFISH probe signals of the nascent and mature RNA populations predicted by the differential equation solution and the corresponding copy-number histograms predicted by the FSP algorithm. It has already been modified to incorporate multiple gene copies, deterministic changes in kinetic parameters, variable transcription speeds, co-transcriptional degradation by RNase, and other features. A GUI is available, enabling parameter input and inspection of graphical outputs.

Using cloud computation, the algorithm can generate (within minutes to hours) time-dependent signal predictions for hundreds of kinetic parameter sets, each applied to thousands of cells. For parameter estimation, we bin the predicted measurements to generate time-dependent histograms for each parameter set, calculate their statistical distance away from "target" histograms, and generate new parameter sets by using the genetic algorithm to minimize the statistical distance. We currently fit synthetic data to determine the best methods for fitting to experiments.

IV. Conclusion

The platform facilitates testing and fitting of transcription models, as well as casual use via the GUI. These capabilities make it a useful addition to the toolbox of quantitative biology.

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Characterization and application of fluorescent indicators for imaging neuronal voltage computations

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***Short Abstract* — Neurons integrate inputs and generate outputs by regulating the voltage across their plasma membrane. Understanding these computations requires tools to monitor membrane potential (voltage) with high spatial and temporal resolution. Here, we quantitative characterize and apply a new generation of protein-based biosensors that report voltage dynamics as changes in brightness. We demonstrate that these genetically encoded voltage indicators (GEVIs) can report action potential with millisecond time-scale and subcellular spatial resolution in cultured neurons and brain slices with two-photon microscopy. We also show we can track action potential backpropagation through dendritic arbors, an important step towards studying how neuronal morphology tunes neuronal computations.**

***Keywords*— Fluorescence Microscopy, Neuronal computation, GEVI**

I. Background

How the brain regulates behavior in response to environmental stimuli is a longstanding question in neuroscience. Neurons are generally thought to be the basic computational elements in the brain: they receive chemical signals called neurotransmitters at specialized structures named synapses, and integrate and transmit this information as voltage changes that propagate across spines, dendrites, the cell body, and the axon. Electrical activity at axonal termini can result in release of neurotransmitters, thereby activating downstream neurons. How individual neurons integrate multiple inputs remains poorly understood given the unavailability of tools to monitor voltage dynamics with sufficient spatiotemporal resolution [1].

Here, we describe advances in using protein-based indicators of voltage for monitoring computations in single neurons. Specifically, we propose to use Genetically Encoded Voltage Indicators (GEVIs) to quantitatively image membrane potential dynamics with subcellular resolution and millisecond-timescale resolution in genetically defined cell type [2-3]. We could potentially measure the membrane potential of tiny but critical structures of neurons such as an individual spine or the axon initial segment, which are typically inaccessible by other strategies. We also can record the membrane voltages of multiple locations simultaneously to stress how electrical signals change during propagation [4]. Voltage imaging also allows us to track long-term changes of activities of the same neuron to investigate how development, aging, and disease can alter the computation properties.

II. Results

We have previously reported a voltage indicator ASAP1, a chimeric protein with a GFP variant inserted in an avian voltage sensitive domain (VSD). Voltage changes result in a conformational change in the VSD, thereby modulating the brightness of the coupled GFP [5]. Here, we report an improved voltage indicator and demonstrate that it increases detectability of action potentials, sub-threshold membrane potentials, and hyperpolarization events. We quantitatively characterize the performance of this new indicator in dissociated neurons and in brain slices under fluorescence microscopy. We further show that this indicator can track backpropagating action potentials in neuronal dendrites. We anticipate that further development of this methodology will enable quantitative modelling of computations by individual neurons.

III. Conclusion

We demonstrate that we can image voltage dynamics with high spatiotemporal resolution and show strong potential to understand the role of each neuronal structure in processing information.

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Evaluation of Parallel Tempering to Accelerate Bayesian Parameter Estimation in Systems Biology

Sanjana Gupta[1], Liam Hainsworth[1], Justin S. Hogg[1], Robin E.C. Lee[1], and James R. Faeder[1]

Short Abstract — **Bayesian parameter estimation (BPE) is popular in systems biology, where often a large number of correlated model parameters have to be estimated from limited experimental data. Commonly-used Markov chain Monte Carlo (MCMC) methods for BPE often suffer from slow convergence. Here[1] we evaluate the performance of parallel tempering (PT), a physics-based MCMC method designed to accelerate convergence by swapping between multiple MCMC chains run in parallel at different temperatures.**

I. Introduction

Computational models are used to describe biological systems and make testable predictions[2]. Parameter estimation is the calibration of a model to data by searching for parameterizations that minimize the discrepancy between the data and model output. MCMC is a Bayesian parameter estimation method commonly used in systems biology, but standard algorithms such as the Metropolis-Hastings (MH) suffer from slow convergence. Parallel tempering (PT) is a physics-based method that accelerates sampling of probability distributions by swapping between parallel MCMC chains run at different temperatures[3]. While PT has been commonly used in molecular dynamics simulations to accelerate sampling the conformational space of biomolecules[3], it has sparsely been used in systems biology[2].

In this work[1] we evaluate the performance of PT relative to MH on six biological models of increasing complexity. We include a comparison with Approximate Bayesian Computation – Sequential Monte Carlo (ABC-SMC), another common Bayesian parameter estimation method.

II. Methods

We performed all the MCMC fits using pTempEst, our MATLAB-based tool for parameter estimation using PT. The models were specified in the BioNetGen language (BNGL)[4], and exported as ODE models in MATLAB's MEX-file format that are called by pTempEst, which invokes the CVODE library for efficient integration of high dimensional models. We used the tool ABC-SysBio to perform fits using ABC-SMC[5].

In our analyses we fit ODE models to synthetic data generated using known parameters. For smaller models (3-6 parameters), both MH and PT found the global minimum and we compared the algorithms using convergence time and sampling efficiency. For more complex models (11-25 parameters) we did not always obtain parameter sets that fit the data. In this case we compared the algorithms using the likelihood of the best-fit parameters.

We compared fits from ABC-SMC and PT by allowing each algorithm a specified number of model integrations, fixing the total amount of computational resource used.

III. Results and Future work

For simple models with 3-6 parameters (Michaelis-Menten model, mRNA self-regulation[5], simple negative feedback loop), PT accelerated convergence and improved sampling over MH. For bigger models with 12-25 parameters (calcium signaling, negative feedback oscillator[6], growth factor signaling[7]) PT more consistently found the global optimum, while MH frequently got trapped in local optima. Finally, we found that for a fixed number of integrations, PT outperformed ABC-SMC for parameter sampling on a relatively simple ODE model of mRNA self-regulation.

A current limitation of PT is that it is only moderately parallel across a small number of chains and does not fully leverage the large number of nodes on typical modern day clusters. We are currently investigating ways to increase the parallelizability of the algorithm, for example by running multiple chains at each temperature level. Another area of improvement that we are pursuing is in the proposal function, which currently does not leverage known parameter correlations to improve sampling efficiency. We will investigate whether previous work in this area[8,9] would benefit from a parallel tempering approach.

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Temporal precision of regulated gene expression

Shivam Gupta[1], Julien Varennes[1], Hendrik C. Korswagen[2] and Andrew Mugler[1,*]

Cells trigger events with high timing precision, but how do they so with inherently noisy molecular components remains unclear. We investigate this question using a first-passage-time approach, for an event triggered by a molecule that crosses an abundance threshold and that is regulated by either an accumulating activator or a diminishing repressor. We find that the optimal strategy arises from a tradeoff between minimizing the extrinsic noise of the regulator and minimizing the intrinsic noise of target molecule itself. Our results explain the low noise of mig-1 gene expression in migrating neuroblast cells during *Caenorhabditis elegans* development, and suggest that mig-1 regulation is dominated by repression for maximal temporal precision.

***Keywords* — First-passage time | Gene regulation | Cell migration.**

I. Background

Proper timing is crucial for biological processes, including cell division [1], cell differentiation [2], cell migration [3], and embryonic development [4]. These processes are governed by molecular events inside cells, i.e., production, degradation, and interaction of molecules. Molecular events are subject to unavoidable fluctuations, because molecule numbers are small and reactions occur at random times [5]. Cells combat these fluctuations using networks of regulatory interactions among molecular species. This raises the fundamental question of whether there exist regulatory strategies that maximize the temporal precision of molecular events and, in turn, cellular behaviors. A canonical mechanism by which a molecular event triggers a cellular behavior is accumulation to a threshold [2, 6]: molecules are steadily produced by the cell, and once the molecule number crosses a particular threshold, the behavior is initiated. Recent work has investigated the impact of auto-regulation (i.e., feedback) on the temporal precision of threshold crossing [6]. Interestingly, it was found that auto-regulation generically decreases the temporal precision of threshold crossing, meaning that the optimal strategy is a linear increase of the molecule number over time with no auto-regulation [6]. However, in many biological processes, such as the temporal control of neuroblast migration in *Caenorhabditis elegans* [3], the molecular species governing the behavior increases nonlinearly over time. This suggests that other regulatory interactions beyond auto-regulation may play an important role in determining temporal precision. In particular, the impact of activation and repression on temporal precision, where the activator or repressor has its own stochastic dynamics, remains unclear.

II. Results

Here we investigate the temporal precision of threshold crossing for a molecule that is regulated by either an accumulating activator or a degrading repressor. Using a first-passage-time approach [6] and a combination of computational and analytic methods. We find that the optimal regulatory strategy for either an activator or a repressor corresponds to a non-linear increase in the regulated molecule number over time. We elucidate the physical mechanism behind these optimal strategies, which stems from a tradeoff between reducing the noise of the regulator and reducing the noise of the target molecule. Motivated by data from migrating neuroblast cells in *C. elegans* larvae [3], we also consider the effects of cell division, and find that activation (repression) is optimal if cell division occurs early (late) in the temporal process. Our results are quantitatively consistent with both the temporal precision and nonlinearity of the *mig-1* mRNA dynamics in the macroblasts, and we predict that *mig-1* regulation is dominated by repression for maximal temporal precision.

III. Conclusion

We demonstrate that regulation increases the timing precision of threshold crossing by a target molecule beyond the precision achievable with constitutive expression alone. Our minimal model is sufficient to explain both the nonlinearity rise in molecules and low degree of noise in dynamics of mig-1 in *C. elegans*.

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Population Dynamics of Cooperative Resistance in *E. faecalis*

Kelsey Hallinen[1], Jason Karslake[1], and Kevin Wood[1]

***Short Abstract* — *E. faecalis* is a gram-positive bacterial species among the leading causes of nosocomial infections. In this work, we investigate the dynamics of enzyme-mediated cooperative, drug resistance in *E. faecalis* populations exposed to temporally varying influx of β-lactam antibiotics. By combining experiments in computer-automated bioreactors with mathematical models, we show that drug-treated populations exhibit a range of dynamic behaviors, including bistability between population extinction and survival depending on the flow rate of antibiotic, the initial population density, and ratio of sensitive and resistant cells.**

***Keywords* — Population Dynamics, Cooperation, Antibiotic Resistance, Modeling, Chemostat**

I. Background

Antibiotic resistance is an urgent threat to public health and has garnered significant research interest. While the molecular mechanisms of resistance are increasingly understood, the picture of how resistance determinants are spread in complex microbial populations remains incomplete. While selection driven fixation of a single resistance phenotype is perhaps the simplest route to a resistant population, recent work has highlighted that resistance may also be a collective phenomenon depending in complex ways on the competition and cooperation between sensitive and resistant cells [1-4].

In this work, we investigate the population dynamics of β-lactamase mediated drug resistance in *E. faecalis*, a common source of hospital-acquired infections. β-lactamase is responsible for the degradation of antibiotics of the β-lactam class [5-6]. Recent work has shown that drug deactivation, even if intracellular [4], can lead to counterintuitive population dynamics by promoting the survival of drug sensitive cells [1-4]. For example, adding an enzyme inhibitor to disrupt β-lactamase function may lead to populations increasingly dominated by enzyme-producing resistant cells [3]. In addition, we've recently shown [7] that the efficacy of β-lactams increases with population density in *E. faecalis*, a surprising pH-mediated effect that occurs in the absence of β-lactamase production. Taken altogether, these results indicate that β-lactamase resistance may induce rich dynamical behavior in bacterial populations, particularly in the presence of time-dependent flows of antibiotics.

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II. Results

Here we combine experiments using computer-automated bioreactors and flow cytometry with mathematical models to investigate β-lactamase resistance in mixed populations of sensitive and resistant (enzyme-producing) cells in the presence of time-dependent influx of antibiotic. We observe a wide range of dynamical behavior, including bistability between population extinction and survival, that depends on flow rate of drug, the initial (total) population density, and the ratio of sensitive to resistant cells. Using a simple mathematical model, we derive and experimentally validate a full phase diagram that predicts regimes of population survival, extinction, and bistability that arise from the interplay between drug degradation, temporal dosing dynamics, and density-dependent efficacy of the antibiotic. In addition, we experimentally modulate pH using different growth media to tease apart contributions to these dynamics from density-dependent drug activity (modulated by pH), and density-dependent drug degradation (modulated by enzyme production). Finally, we discuss ongoing work to optimize dynamical drug dosing strategies for maximizing population extinction.

III. Conclusion

Our findings uncover rich dynamics of *E. faecalis* populations exposed to β-lactams. These results underscore the need for quantitative understanding of cooperative resistance in the systematic optimization of antimicrobial treatment strategies.

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Merging Multiple Data Sets to Study HCV

Jungmin Han[1], Rabab Ali[2], Grace Zhang[2], Elizabeth Townsend[2], Gabriella Quinn[2], Kareen Hill[2], Ohad Etzion[2], Theo Heller[2], and Vipul Periwal[1]

***Short Abstract* — Hepatitis C virus (HCV) is a pervasive public health problem that infects three to four million every year worldwide, many of whom will develop cirrhosis [1]. We aim to better understand the key attributes of HCV infection, which in turn may give insight into the disease prognosis. Here, we show how we applied similarity network fusion (SNF), a recently developed computational method that is particularly suited to obtaining a comprehensive integrated understanding of multiple types of measurements, to HCV clinical data collected from 29 patients.**

***Keywords* — HCV infection, similarity network fusion, cirrhosis.**

I. Purpose

Approximately 170 million people suffer from chronic hepatitis C infection worldwide. There is no preventive vaccine for HCV. Although in some the infection has a good prognosis, a significant number of infected people develop more lethal health conditions such as cirrhosis or liver cancer [1]. To better understand the pathogenesis of HCV infection, we collected a range of clinical data from a group of patients with a spectrum of disease stages. We not only measured several different classes of biomarkers including cytokines, metabolites, and bacterial genomics, but also documented the patients' diet records. Given the diverse data types, it was not obvious how to collate embedded information and draw integrated conclusions. In order to overcome this challenge, we employed SNF, a method developed for coalescing multiple data sets emerged from a common source [2, 3, 4].

For the preliminary analysis, we focused our attention to the three biomarkers mentioned above and inspected for any separations within the group using spectral clustering. There was no common clustering pattern across the data sets. However, some of the clusters showed a positive association with the degree of liver damage, which emphasizes the necessity of an exhaustive analysis.

II. Data and Method

The data set consisted of traces of 64 different cytokines, the total of 1546 metabolites from 25 subgroups, and bacteria counts spanning 13 phyla. There were some missing entries, which were imputed using the R software package, *softImpute* [3, 5]. Since the metabolite data were partitioned into 25 subgroups, they required amalgamation into a single network through SNF, before it could be combined with other networks.

For each biomarker data set, the method computes an affinity matrix that quantifies the global similarities between the subjects and a kernel matrix representing local similarities between *k*-neighboring patients. As the final step, the SNF method iteratively updates each affinity matrix through a matrix multiplication of its kernel matrix with the other affinity matrices to obtain a unified network that conveys both shared and complementary information. All the sets had an equal weight towards the final network. We then applied the spectral clustering algorithm on the fused network to determine clusters among patients, and the same method was used on each data set for the comparison.

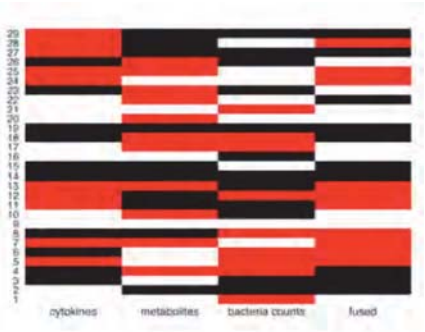

Figure 1. The clustering patterns within each biomarker data set, which are not consistent across the whole set. The clusters are represented in three colors. The last column shows the clusters identified from the converged network of all three data sets. The numbers on the left are patient numbers.

III. Conclusion

The information in the cytokine data set seems to be well carried through, compared to the other data sets. This suggests that the network structure underlying the cytokine data is dominant over the others.

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Machine Learning to Evaluate fMRI Recordings of Brain Activity in Epileptic Patients

R. Hekmati[1], R. Azencott[2], W. Zhang[3], MJ. Paldino[4]

***Short Abstract* — We analyze fMRI recordings of brain activity in epileptic infants. These recordings provide roughly 1500 recorded time series mapping the activity of 1500 very small cortex patches. The ultimate goal of our study is to facilitate noninvasive localization of the epileptic focus. We have implemented several Automated Classifiers of fMRI recordings into 5 classes of patients. Here we outline how one can use a Multi-Layer Perceptron (MLP) with highly restricted number of nodes to mitigate the currently small number of diagnosed patients. We introduce a novel multiscale analysis to select Cortex Regions with high discriminating power between patients classes. Another key point is our systematic use of very large matrices of Mutual Information (MI) between pairs of recorded time series. We generalize the MI concept to evaluate the interactivity between pairs of cortex regions of arbitrary size. Our MLP classifier performance is quite good, but will need validation on larger data sets.**

***Keywords* — fMRI Brain Recordings, Robust Classifiers, Deep Learning, Mutual Information, Epilepsy Focus.**

I. Mutual Information and Regional Cortex Connectivity

An ongoing study at Texas Children's Hospital gathers sequences of fMRI 3D-images recording cortex activity for young epileptic patients [1]. Each fMRI recording generates 295 3D-images algorithmically registered onto a pre-segmented cortex atlas and thus partitioned into 780 disjoint "parcels", 148 "cortex regions", and 10 "lobes".

For each patient, there are roughly 780 cortex parcels and fMRI data provide for each parcel one time series with 295 points. To characterize brain interactivity, we compute for each patient the 780 x 780 matrix of Mutual Information MI(m,n) between all pairs of time series. MI quantifies non-linear information links between two time series and has often outperformed correlations in the analysis of cerebral activity [2].

We have 148 anatomically identified cortex regions R_j We quantify the "connectivity" $c(R_j)$ of any region R_j by computing the 75% quantile of all the MI(m,n) corresponding to parcels "m" and "n" belonging to R_j. This defines a vector V of 148 regional connectivities $c(R_j)$. Each patient Pat_k is characterized by its vector V_k of regional connectivities.

Diagnosed patients belong to 5 classes defined by the epileptic focus localization, positioned by neuro-surgeons within 5 distinct large cortex zones. Our database provides 19 diagnosed and 13 non-diagnosed patients. This very restricted dataset imposes a strong parsimony for the number of parameters in our classifier. So even moderately large MLPs are not usable, because they involve large numbers of unknown weights. We radically reduce the input dimension for our MLP by selecting highly discriminating cortex regions. We also minimize the size of our MLP classifier to ensure robustness of classification.

II. Highly discriminating Cortex Regions

Fix any two classes CL_p and CL_q . For any region Rj, we quantify its power to discriminate CL_p vs CL_q by comparing the distributions of c(Rj) values for patients in CL_p and patients in CL_q. Call $S_{p,q}$ the region having highest power to discriminate CL_p vs CL_q The 10 pairs CL_p vs CL_q thus generate 10 cortex regions $S_{p,q}$. For each patient, the vector of 10 connectivities $c[S_{p,q}]$ becomes the short input for our MLP classifier, for which we impose a single hidden layer of size 5 to minimize the number of MLP parameters. After automatic learning the classification performance estimated by "leave-one-out" is 93% +/- 3%.

III. Conclusion

We introduce and test efficient new methods to implement robust classification for fMRI recordings of brain activity.

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Cliffs & canals in Waddington's landscape

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***Short Abstract* — Mature multicellular organisms reproduce by transmitting genetic information onto progeny, which requires successful parental somatic and germline formation. Different strategies to develop to reproductive maturity exist, yet a common thread is that major developmental events are coordinated in time. Given that multiple cell fates are regulated by one genome, we hypothesize that temporal coordination arises from complex gene regulatory network (GRN) dynamics. To test how GRNs affect coordinated multicellular development, we are using a computer simulation based on a standard model of GRN evolution to examine how dynamics and attractor states evolve with stabilizing selection.**

***Keywords* — Waddington's Canalization, Heterochrony, Cell Fate Acquisition, Attractor Networks, Discrete Dynamical Systems.**

I. Background

IN multicellular organisms, reproduction is ultimately achieved by the transmission of genetic material onto progeny, which depends on the successful somatic and germline development in parent(s).

Although there are many strategies used during the development towards reproductive maturity, multicellular organisms tend to have major life history events which are coordinated in time[1,2]. For example, gonads produce sex hormone in vertebrates in response to long range induction by gonadotropin releasing hormone (GNRH), which is first released by neurons within the hypothalamus[3]. In this case, the capacity for the gonad to respond to GNRH is coordinated in time with the ability of the somatic cell to release GNRH. Despite the prevalence of this pattern, how life has evolved to exhibit temporal coordination during development remains unknown[2,4].

II. Hypothesis

We posit that temporal coordination is a common feature of developmental programs where one genome regulates cell fate acquisition and does so robustly from a single cell zygote across multiple daughter lineages. This is supported by the discovery of a genetic pathway in the roundworm nematode *Caenorhabditis elegans* dedicated to regulating the timing of larval development across multiple tissues and cell types[5-8]. In fact, many *C. elegans* developmental timing genes also participate in the onset of human pubertal timing by regulating the GNRH release[6,9-11].

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III. Results

To test how GRNs and gene expression states affect the development of multiple stable cell types, we are using a computer simulation based on a standard model of GRN evolution[12-14]. This model recapitulates how GRNs become more robust to perturbation (i.e. mutational & environmental) when evolved under a stabilizing selection regime. For our analysis, we select GRNs with multiple stable attractor states and impose the constraint that they stabilize within similar time frames.

Here, we present data on how GRN structure and dynamics evolve under a stabilizing selection regime requiring temporal coordination. Our findings indicate that GRNs increase in robustness when they change to take advantage of new inputs from the state space, sculpting, molding, and canalizing the ancestral GRN basins of attraction.

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Dynamic Responses of LacI/GalR Chimera-Based Transcriptional Logic Gates

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***Short Abstract* — Genetically encoded logic is central to the function of living organisms. Synthetic biology has created many orthogonal genetic logic systems, but these systems are usually studied at steady state. We use microfluidic devices to study the dynamic responses of genetic logic gates. The specific gates in this work are constructed using chimeric transcriptional repressors, so logic can be created by controlling induction or production of these repressors. Controlling induction yields IMPLY or AND logic; controlling production yields NOT or NOR logic. We find that the speed at which gates reliably respond to environmental changes depends on their mechanism of induction.**

***Keywords* — Genetic Logic Gates, Microfluidics, Dynamics**

I. Purpose

IN synthetic biology, genetic logic gates have been constructed with applications in mind such as biological computing and sensors in a dynamic environment. These logic gates may be integrated into larger networks, mediating response or coupling dynamics of modular genetic circuits based on environmental conditions. However, less work has been done to understand the limitations of transcriptional logic gates in these applications. It is clear that reliable logic gates should respond on a faster time-scale than time-varying environmental conditions. Therefore, we aim to measure the limitations on the dynamic range of transcriptional logic gates.

The focus of this study is chimera-based transcriptional logic gates. Previous work showed that chimeric proteins derived from the LacI/GalR family of transcriptional repressors can be used to create transcriptional AND gates *in vivo* [1]. These chimeric proteins have the same operator (DNA) binding domain but different ligand binding domains; hence, they will bind to the same operator site but are induced by different sugars. By changing the production of the chimeras to inducible promoters (from constitutive promoters), NOT and NOR logic is implemented.

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II. Results

Two types of logic gates are described in this work – "ligand" gates (AND, IMPLY) and "inducible" gates (NOT, NOR). "Inducible" gates include an extra production step – that of the repressor – when compared to "ligand" gates. We find that transcriptional logic gates generally function as low-pass filters, responding faithfully to low frequency (long period) signals and unfaithfully to high frequency (short period) signals.

A. "Ligand" gates respond over a wide range of driving frequencies

Both AND and IMPLY gates were tested over a range of driving periods from 20 to 240 minutes. Both gates show robust output at periods from 40 to 240 minutes – that is, a clear threshold for ON and a clear threshold for OFF can be set in all these experiments. At a 20 minute driving period, the outputs of these gates do not reach a clear ON or OFF thresholds.

B. Responses in the NOT gate are delayed compared to "ligand" gates

While the AND and IMPLY gates are robust at periods greater than 40 minutes, the NOT gate tested here is only robust in its response at periods greater than 120 minutes. This difference in behavior is attributed to the extra time required to produce and degrade the repressor proteins, steps not required in the "ligand" gates.

III. Conclusion

While genetic logic can be implemented by controlling the production of a transcription factor, we show that robust responses are generated at faster time scales when the levels of regulatory proteins do not need to be controlled.

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Network inference with latent variables

Danh-Tai Hoang[1], Junghyo Jo[2], and Vipul Periwal[1]

***Short Abstract* — We recently developed an entire data-driven approach for network inference based on free energy minimization. The method outperformed performance with published methods in predicting coupling strengths of stochastic processes, especially in the regimes of little observed samples and large variance of couplings. Using latent variables, we extend the method to recover the network when only a partial system is available. Our method can infer accurately the states of latent variables, the coupling strengths and the number of latent variables.**

***Keywords* — Network reconstruction, kinetic Ising model, latent variables, neural network.**

I. Purpose

PREDICTING network connections from observed data is a critical topic, not only in quantitative biology but also in other areas, more generally, data science [1]. Statistical methods have been developed based on naïve mean field [2], Thouless-Anderson-Palmer mean field [2], exact mean field [3], and maximum likelihood [4]. However, these methods work well only in the weak-coupling and large number of observed sample regimes. We recently developed an approach based on free energy minimization (FEM) and demonstrated that our method has a better performance than previous methods for strong-coupling regimes, especially in the limit of few observed samples [5].

Our aim is to extend the FEM method to infer network connections when available data does not contain every variable but some of them are unobserved.

II. Method

Our method combines FEM and maximum likelihood for latent variables and contains the following steps: (i) Assign the state of latent variables as random; (ii) Infer interaction between variables based on FEM [5]; (iii) Update the state of latent variables with a probability $P_2/(P_1 + P_2)$ where P_1 and P_2 represent the likelihood of systems before and after the updating; and (iv) Repeat steps (ii) and (iii) until the discrepancy of actual and its expectation value of variables becomes saturated. The number of latent variables can be estimated from the minima of the average of the discrepancy over the total number of variables.

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III. Results

We first tested our method to infer the coupling strengths in the kinetic Ising model in a system of 100 variables, using the states of only 60 variables. We could recover successfully the states of the 40 latent variables and the coupling strengths. We then applied our method to recover a neural network from neuronal activities in the salamander retina [6]. After inferring the neural dynamics couplings and the external local fields and the state of latent variables, we recovered the neuronal activities with an accuracy of 80% (Figure 1).

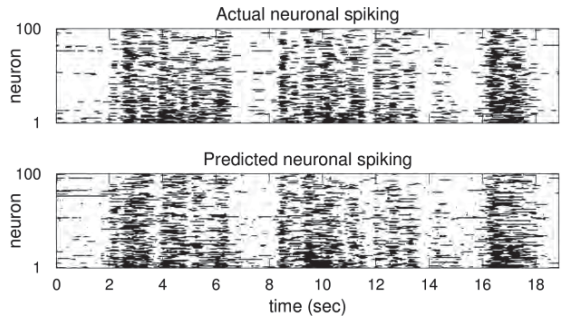

Figure 1. Inference of neuronal activity. Raster of 100 neuronal activities from experimental data (top) and our prediction (bottom).

IV. Conclusion

Extending our FEM method, we proposed an iterative algorithm with latent variables to infer the coupling strengths and the configuration of latent variables. Applying this to neuronal data, we can infer accurately activities. Besides better performance, our method is parameter-free and generalizes to many data types.

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Design principles of binding-induced selective transport through the nuclear pore complex

Laura Maguire[a,b], Michael Stefferson[a], Meredith D. Betterton[a], and Loren Hough[a,b]

A key step in a wide variety of cellular signaling cascades is selective entry into the nucleus. This regulatory step requires the nuclear pore complex (NPC) to act as a selective filter allowing the passage of only a subset of macromolecules. In many biological filters, such as mucus, binding inhibits transport. In contrast, in the NPC binding of specialized proteins called transport factors to disordered proteins called FG Nups specifically enhances their transport. We developed a quantitative theory of the minimal ingredients sufficient for binding-induced selective transport. Our model provides a framework to study how biological filters regulate access to key macromolecules.

Selective filters made of biopolymers are used in living and synthetic systems to control the localization and movement of molecules, nanoparticles, viruses and other organisms [1]. One of these filters, the nuclear pore complex, or NPC, regulates access to cells' genetic material. Controlled passage through the NPC to the nucleus is a key features of many signaling cascades. For example, transcription factor activation typically includes its nuclear localization. In plants, signaling can also directly affect properties of the nuclear pore, regulating nuclear access for a wide range of pathways [2].

Selective biopolymer filters regulate access to genetic material (the nuclear pore complex, or NPC), cells (the pericellular matrix), tissues (the extracellular matrix), and organs (mucus). How particle binding affects motion and filtering is unclear. Binding of transport factors that bind to proteins in the NPC move rapidly through it. In contrast, binding inhibits the uptake of nanoparticles that bind to airway mucus and many viruses minimize binding interactions [3]. While particle size, charge, and binding interactions are known to affect filtering, the physical principles that underlie mobility and transport in polymeric biomaterials are not fully understood.

Among these filters, the NPC is tuned for selective passage enabled by binding. The NPC selectively filters molecular traffic between the nucleus and cytoplasm of eukaryotic cells, making it important for diverse processes including gene regulation and translation [4]. In particular, it is a key regulatory step in cellular signaling. Post translational modification or the presence of binding partners can expose a nuclear localization signal (NLS). This marks the protein as a cargo, recognized by a transport factor and so selectively transported.

Transport occurs through the central channel of the NPC, ∼50 nm in diameter and ∼100 nm long. The selective barrier filling the central channel is made from disordered proteins, the FG nucleoporins (FG Nups), which contain repeated phenylalanine-glycine (FG) motifs. Transport factors (TFs) that directly bind to the FG repeats can cross the NPC and carry cargo with them. Transport through the NPC is remarkably fast, with pore residence times ∼10 ms [5]. Binding between FG Nups and TFs shows diffusion-limited on-rates and transient binding of individual FG repeats to TFs [6,7]. How the FG Nups both block passage (of non-binding molecules) and facilitate passage (of binding molecules) is not fully understood, making the NPC an ideal system to dissect the principles of binding-controlled selective transport.

We address the central contradiction of selective transport through the NPC: how does binding of TFs to FG Nups within the pore increase the flux rather than decreasing it? Using a biophysical model, we demonstrate that TF diffusion and binding are sufficient for selective transport, as long as binding only partially immobilizes TFs. Binding increases the local concentration, and these molecules contribute to the flux if mobile. Thermally-driven diffusion of TFs bound to flexible tethers gives sufficient particle mobility to produce selectivity similar to experimental measurements. Tether flexibility also allows bound TFs to hop between tethers, further enhancing selectivity.

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[1]

Programming Fitness Landscapes by Sparse Epistasis

Kabir B Husain[1] and Arvind Murugan[1]

***Short Abstract* —The effect of a mutation in a genetic sequence depends on the context in which it is made. This phenomenon, known as epistasis, leads to rugged fitness landscapes that proteins explore over evolutionary timescales. The form of evolutionary pathways in these landscapes are not well characterised. Inspired by recent experimental findings, here we construct a class of fitness landscapes characterised solely by their epistatic statistics. We analyse the structure of mutational pathways in this space, and investigate both its global and local topology. Our results serve as theoretical baselines against which experimental data may be assessed.**

***Keywords* — Epistasis, Protein evolution, Statistical landscapes, Mutational pathways**

I. Background

Proteins evolve towards fitter variants by a process of mutation and selection. In principle, these local transformations occur in the background of a fitness landscape defined over an underlying sequence space. Global features of this landscape are therefore key in determining how well and how quickly proteins evolve. However, we do not quite understand what the defining structure of a `generic' or representative fitness landscape is -- this is related to the problem of predicting the form of the genotype-phenotype map.

Nonetheless, experiments have, by exhaustive mutagenesis, begun to map this out. In particular, a recent study [1] found that the landscape could be encoded by a small number of appropriately-defined epistatic contributions. This sparsity suggests a simple but non-trivial structure, and leads to the question of whether particular mutational pathways in genotypic space can be understood as arising just from this underlying epistatic structure.

II. Methods and Results

To address this question, we resort to a minimal, statistical approach. We represent a genotype as a bit string, so that the genotypic space is a Boolean hypercube. On this space, we generate an ensemble of fitness landscapes by randomly generating a (small) number of epistatic terms; as described in [2], this is analogous to specifying a landscape by its Fourier modes. Our main control parameter is the extent of sparsity: the fraction of epistatic terms that we allow to be non-zero.

We first consider local properties of the resulting ensemble, measuring correlations lengths as a function of the sparsity and magnitude of epistasis. We then go on to characterise more global properties: in particular, the 'ruggedness' of the landscape (intuitively, the number of local fitness maxima), and the connectivity of mutational pathways in this space [3]. We contrast our results with prior statistical landscapes, such as the NK model, and discuss the effects of higher-order epistatic contributions. Finally, we consider potential applications to existing and future experiments [4].

III. Outlook

The form of the sequence-function map, and its connection with the mutational pathways undertaken by an evolving protein, has been a long standing puzzle. Our theory provides a simple prescription to generate ensembles of rugged landscapes, and therefore a systematic investigation of the link between epistasis, ruggedness, and evolutionary paths. Our results may be important for identifying a minimal, conserved set of features shared by diverse fitness landscapes.

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The GMR Approach of Cancer Gene Therapy

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***Short Abstract* — We prove that cancer nodules and surrounding normal tissue are governed by distinct Gene Master Regulators (GMR) and that smart manipulation of a GMR's expression selectively affects cancer cells. The method, consistent with our Genomic Fabric Paradigm, relies on an original mathematical algorithm that establishes the gene hierarchy from the transcriptomic profiles of tumor biopsies based on their Gene Commanding Height (GCH). GCH is a composite measure of gene expression control and coordination with major functional pathways. We present validation of the approach using microarray data obtained in our NYMC laboratory by profiling human thyroid, kidney and prostate cancer samples.**

***Keywords* — gene master regulators, genomic fabric, transcriptomic topology, microarray, thyroid cancer, kidney cancer, prostate cancer.**

I. Introduction

NUMEROUS groups race to discover the gene biomarkers whose alteration (as sequence or expression) alone is indicative of a particular disease in all humans. However, thousands other genes (whose contribution is neglected but not necessarily negligible) are affected. In each person a similar disease results from a unique, never-repeatable combination of gene alterations. As selected from the most frequently altered genes in large populations (indicating little protection by the homeostatic mechanisms like for low-key players) biomarkers are of little therapeutic value. Instead, our Genomic Fabric Paradigm [1] identifies in the cancer nodules of **each** patient the GMRs whose highly protected expression governs major functional pathways by controlling the expression of numerous other genes. The genomic fabric is defined as the transcriptome associated with the most interconnected and stably expressed network of genes responsible for a particular functional pathway. The fabric exhibits specificity with respect to race/strain, sex, age, tissue/cell type, and lifestyle and environmental factors. It remodels during development, progression of a disease, and in response to external stimuli. GFP is powered by mathematically advanced analytical tools (e.g. [2]) that are presented together with the experimental protocol to collect and profile the cancer nodules from a heterogeneous tumor.

II. Results

Our experimental protocol requires that biopsies from cancer nodules and surrounding normal tissue (best reference for malignancy) are split in four, quarters profiled separately as biological replicas of the same transcriptomic machinery subjected to slightly different local conditions. Thus, we get for every single gene three independent measures: average expression level (L), expression variability (V) and expression coordination (C) with each other gene. "L" is used to determine what gene is up/down-regulated in malign vs normal tissue, "V" to estimate the control of transcript abundance in each condition and "C" how the genes are networked in functional pathways to satisfy a kind of "transcriptomic stoichiometry" [3]. Together, these results are uploaded in an algorithm that constructs the genomic fabrics of major functional pathways [4] and their interplays [5], determines the GCH scores of individual genes and establishes the gene hierarchy in each part of the tumor. We have published recently proves that malign and normal regions of kidney [6] and thyroid [7] tissues are governed by different GMRs. Here, we provide additional evidence for several cases of prostate cancer and validate the therapeutic value of the GMR approach in standard cell lines of human cancers of thyroid, lung and blood.

III. Conclusion

GMR approach identifies for each patient the most legitimate gene targets for cancer gene therapy. Smart manipulation (e.g. up-regulation if pro-apoptotic or silencing if anti-apoptotic) of the personalized GMR would have the best result not for everybody but for that person.

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Modeling the Regulation of Cancer Metabolism: Interplay between Glycolysis and OXPHOS

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Short Abstract:

Abnormal metabolism is a hallmark of cancer. In a traditional view, cancer cells largely utilize glycolysis for energy production irrespective of the presence of oxygen. Recently, increasing experimental evidence shows a critical role of actively functional mitochondria and oxidative phosphorylation (OXPHOS) in tumorigenesis and metastasis. However, how cancer cells orchestrate glycolysis and OXPHOS to facilitate malignancy is largely unknown. Through integrating mathematical modeling, bioinformatics with experiments, we show that cancer cells can acquire a stable hybrid glycolysis/OXPHOS phenotype, characterized by high activity of AMPK and HIF-1 and high metabolic activity of glycolysis and glucose/fatty acid oxidation.

Keywords **— Cancer metabolism, metabolic plasticity, hybrid metabolic phenotype, AMPK and HIF-1 signatures, gene regulatory network, metabolic pathway activity**

I. Background

ABNORMAL metabolism is an emerging hallmark of cancer [1]. Unlike normal cells, cancer cells largely depend on glycolysis to produce energy even in presence of oxygen. Emerging evidence shows that mitochondria are actively functioning in cancer cells and OXPHOS may be specifically associated with metastasis [2,3]. However, it remains elusive how cancer cells take advantage of both glycolysis and OXPHOS to facilitate malignancy.

To capture the two regimes of cancer metabolism, we develop a coarse-grained model on a core metabolism regulatory circuit, composed of AMPK, a master regulator of OXPHOS, HIF-1, a master regulator of glycolysis and ROS that can mediate the interplay between AMPK and HIF-1 [4]. Computational modeling of the AMPK:HIF:ROS circuit shows that in addition to the glycolysis and OXPHOS phenotypes, which are adopted by normal cells, cancer cells can acquire a hybrid glycolysis/OXPHOS phenotype, that can be promoted by elevated ROS production rate, stabilization of HIF-1 and regulation of oncogenes, such as MYC and c-SRC [4].

To quantify the activity of OXPHOS and glycolysis, we developed the AMPK and HIF-1 signatures by evaluating the expression of their downstream targets. Strikingly, even though the AMPK and HIF-1 gene sets are independent, we observed strong anti-correlation between AMPK and HIF-1 activities in multiple cancer types. The AMPK and HIF-1 signatures can capture the significant metabolic features of both bulk tumors and single cells [4].

To further characterize cancer metabolic activity, we extended our AMPK:HIF-1:ROS model by integrating three metabolic pathways, glucose oxidation, glycolysis and fatty acid oxidation. Our results unraveled a direct association between gene activity and metabolic pathway activity.

II. Results

A. Coupling the AMPK:HIF:ROS circuit with metabolic pathways

The modeling simulation revealed the coupling between the AMPK/HIF-1 activity and the metabolic pathway activity in different metabolism phenotypes. Particularly, the hybrid metabolic phenotype has been shown to be capable of using both glycolysis and OXPHOS for ATP production. The model further help elucidate the similarity and difference of HIF-1 and ROS in regulating the stability of various metabolism phenotypes of cancer.

B. Association of the AMPK/HIF-1 activity with metabolic pathway activity

The modeling predicted association of the AMPK/HIF-1 activity with metabolic pathway activity were supported by bioinformatics analysis of gene expression and metabolite abundance in breast and hepatocellular carcinoma samples. We showed that the evaluation of metabolic pathway activity by enzyme gene expression has more robust performance than the evaluation by metabolite abundance.

III. Conclusion

Our systems biology analysis provides signatures to assess the metabolic states of tumor samples using gene expression. Further studies are needed to evaluate the roles of the hybrid metabolic state and therapeutic strategies targeting the hybrid state.

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Plasmid behaviour under Par system control

Lavisha Jindal and Eldon Emberly

The underlying mechanism of the ParA-ParB protein interaction that causes plasmid translocation and equidistant spacing between multiple plasmid foci has been widely discussed but a comprehensive study that describes how system parameters lead to either oscillatory behaviour or stable plasmid arrangements requires further explanation. In this study we provide a deterministic model that includes finite substrate size effects on protein concentrations that can reproduce a wide range of the observed plasmid behaviour.

***Keywords* — ParA-ParB system, Plasmid translocation, spatial oscillations, deterministic model**

I. Review

The spatial organization of low-copy plasmids in bacteria is mediated by the Par protein system consisting of two proteins, ParA and ParB, and a *cis*-acting centromere-like site on the plasmid, *parS*. ParB specifically binds to *parS* and by interacting with ParA bound to the nucleoid, is able to orchestrate the movement and positioning of plasmids inside the cell [1]. Various theoretical models have been proposed that explain how plasmid translocation arises from ParA-ParB interactions [2,3] and recently simulations based on one such model have successfully achieved the required positioning of multiple plasmids at equi-distant locations along the length of the cell [3].

However, live imaging has revealed complex plasmid behaviours ranging from pole to pole oscillations in cells with single plasmids, to states with stably positioned plasmids within cells with inhibited division [4]. Given these observations, it is unexplained whether the plasmids are undergoing sustained oscillations or settling towards fixed point positions within the cell. Additionally, super-resolution microscopy has revealed differences in the stable positions of F plasmids in *E. Coli* and chromosomal foci in *B. Subtilis* both of which are controlled by the same Par system [5].

It also remains to be understood how system parameters like cell length and plasmid number affect the organization and dynamics of plasmid foci. Since these parameters change during the cell-cycle, simulations that include cell-cycle durations and plasmid replication events are required to provide a complete explanation of the observed plasmid behaviour in-vivo.

II. Results

Previously we developed model that was able to describe the locomotion of an in-vitro system that was actively driven by the Par system [6]. Here we combine it with finite substrate length to obtain equi-distant plasmid positioning and further extend it to provide a comprehensive description of all factors that affect plasmid dynamics within the cell. These key parameters are the number of plasmids within the same cell, the length of the nucleoid to which ParA is bound, the rate at which ParA hydrolyzation is mediated by ParB, the rate at which ParA rebinds (closely related to total ParA within the cell), and the ratio of the length scale over which the plasmid hydrolyzes ParA to the length scale over which it is tugged by ParA dimers.

From the deterministic model we are able to analytically calculate the boundary in parameter space that separates oscillatory plasmid behaviour from stable, equi-distantly spaced fixed points. We find important differences in the location of this boundary depending on whether ParA resources are unlimited (in-vitro) or limited (in-vivo). We also find that increasing the number of plasmids pushes a system with oscillatory behaviour towards stable fixed points while increasing nucleoid length has the opposite effect on the system. Furthermore, we outline the in-vivo conditions required to observe plasmid oscillations and find that realistically it is impossible to observe three plasmid oscillation in a bacterial cell of regular size. Interestingly, finite size effects are observed as the distance separating two oscillatory plasmid trajectories is reduced as system size is increased. We show through simulations how plasmid segregation is robust to replication events during a single cell-cycle. Finally, we discuss the parameter ranges within which the plasmid positions in the super-resolved images provided by [5] can be obtained.

III. Conclusion

Our results show that plasmid number and the length of the nucleoid can drive the system to switch from oscillatory dynamics to stable equi-distantly placed fixed points along the cell. Bacterial systems might be poised near this boundary to achieve the faithful segregation of genetic material. We suggest further experiments that could probe the changeover from oscillatory to stable plasmid dynamics.

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Stochastic Model of Cell Alignment in Traps

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***Short Abstract* — Experiments with *E. coli* cells growing in open-walled extended microfluidic traps with have shown that cells orient themselves orthogonally to the long side of the trap. We develop a stochastic model of cell dynamics that explains this phenomenon. We find that steady-state cell alignment is a function of the aspect ratio of the trap as well as the cells' ability to sense their position within the trap. In the absence of this sensing, boundary effects dominate and steady-state cell orientation becomes exactly opposite of what is experimentally seen.**

***Keywords* — Cell Alignment, Aspect Ratio, Spatial Moran Model, Mean-Field Approximation, Phase Transition, Transcritical Bifurcation, Synthetic Biology**

Extended Abstract

EMERGENT patterns and structures are ubiquitous in biology: leopards develop spotted patterns on their skin [1], mushrooms form fairy rings, and specialized cells conglomerate to form tissues with specific forms. The question of *how* these structures emerge has been a research focus in recent years from both the biological and mathematical perspectives. Examples include the study of Turing instabilities [2], pattern formation on the skin of several animals [1], and tumor initiation and growth [3,4].

Recently, experimentalists have observed an emergent structure in the synthetic biology laboratory. *E. coli* cells growing in open-walled, extended microfluidic traps as a monolayer align orthogonally to the long side of the trap. In this synthetic setting, cellular movement is driven by the growth of the cells along the major axis of their capsule-like body. Experimental evidence also shows that growth-induced cell movement is preferential toward the nearest boundary in the trap, so as to minimize the number of cells a given cell needs to push in order to grow. That is, cells grow in directions where physical resistance is minimal.

To understand the mechanism behind this emergent behavior, we model the trap as an M x N lattice and treat the cells as oriented (vertical or horizontal) particles growing along their major axis on the lattice according to a spatial Moran process. These are typically used as a modeling framework for tumor initiation and growth [3,4]. One differentiating factor between our Moran process formulation and others' is that the growth rates for cells in our model are space-dependent. This reflects a cell's tendency to grow toward the nearest boundary.

Monte Carlo simulations of this model show steady-state cell alignment orthogonal to the long boundary of the lattice, as in experiments. That is, for $M/N > 1$, cells orient horizontally whereas for $M/N < 1$, cells orient vertically. In the interesting case where $M = N$, the system reaches a quasi-equilibrium wherein cells orient orthogonally to the boundary nearest them; however, cells equidistant from the two boundaries constantly switch between orientations. Specifically, the aspect ratio M/N acts as a bifurcation parameter for a transcritical bifurcation in the system at the critical value $M/N = 1$.

Removing space-dependence from the growth rates yields steady states that have cell alignment *parallel* to the long boundary. This is due to boundary effects. There are more chances for cells oriented orthogonally along the long boundary to exit the trap than the short boundary. Boundary size dichotomy dampens cellular growth orthogonally to the long boundary when growth is space-independent. Cell alignment at steady state is therefore a balance between boundary effects and growth dampening caused by physical resistance from other cells.

We make this balance explicit by characterizing lattice dynamics with Heisenberg equations [5] and invoking a mean field approximation to derive a single effective equation for the dynamics of the fraction of cells in a given orientation. The latter is a logistic equation whose growth term reflects the tug-of-war between boundary effects and physical resistance between cells. In this analytically tractable framework, we show the existence of critical parameter values that dictate a phase transition between bulk cell alignments at steady state.

Steady-state cell alignment in traps is the result of a balance between boundary effects and physical resistance to cellular growth. The latter is crucial for cells to align orthogonally to the long side of the trap, as in experiments. We find that even small differences in growth rate at the level of individual cells can translate to large differences at the population level.

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Precision measurements of regulatory energetics in living cells

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Transcription in all organisms is regulated by weak protein-DNA and protein-protein interactions, but measuring these interactions in living cells remains exceedingly difficult. Here we show how reporter assays can be used to measure the Gibbs free energy of critical regulatory interactions in vivo and with high precision (~0.1 kcal/mol). We further demonstrate how this approach can be used to distinguish which kinetic steps in the transcription initiation pathway are regulated. The prospects for a massively parallel implementation of this approach are promising, and we will discuss ongoing work in this direction.

I. Background

Despite an imposing arsenal of experimental techniques for studying transcriptional regulation genome-wide, knowledge of what regulatory proteins actually do when bound to individual regulatory sequences remains highly limited. Indeed, an understanding of how elemental protein-DNA and protein-protein interactions mechanistically control transcription has been established for only a small handful of intensively studied bacterial promoters. New experimental methods are needed if we are to understand the mechanistic basis for transcriptional regulation more broadly.

The weak nature of regulatory interactions presents a major experimental difficulty. Transcription is controlled by multi-protein-DNA complexes that continually form and break apart due to thermal fluctuations. The dynamic nature of these complexes, and thus their physiological function, is strictly governed by the quantitative strength of the interactions that hold them together. For example, a transcription factor (TF) can up-regulate transcription as much as 5-fold through a stabilizing interaction with RNA polymerase (RNAP) of as little as 1 kcal/mol (1.6 k_BT at 37 C, ~1/4 of a hydrogen bond). Even a crude mechanistic understanding of such interactions therefore requires knowing their energies to a precision of substantially less than 1 kcal/mol. Standard biochemical methods, however, cannot measure such interactions in living cells to this precision.

II. Results

Here we show how reporter assays can be used as a general method for measuring protein-DNA and protein-protein interactions in living cells. Each Gibbs free energy is measured by quantifying expression from a variety of synthetic regulatory sequences, the activities of which form a one-dimensional "expression manifold" embedded in a two-dimensional space. Quantitative energy values are then obtained by mathematically modeling this manifold. This modeling task is far simpler and more transparent than other model-based approaches to transcriptional biophysics (e.g., [1-4]). Indeed, accurate free energy values can often be discerned by eye from raw data.

We demonstrate this approach by measuring TF-DNA, RNAP-DNA, and TF-RNAP interactions in *Escherichia coli*. In doing so, we demonstrate the ability to robustly measure free energies to high precision (~0.1 kcal/mol). We find that the well-studied transcription factor CRP can activate transcription far more strongly than has long been thought [5,6]. This expression manifold strategy can further distinguish which kinetic steps in the transcript initiation pathway CRP activates when bound to DNA at varying positions upstream of RNAP.

III. Outlook

Because this approach requires only mRNA expression measurements, it has the potential to be applied in high throughput using massively parallel reporter assays in a wide variety of systems, including human cells [7]. Progress toward this goal will be discussed, as will outstanding challenges. The potential utility of studying expression manifolds in higher dimensions will also be discussed.

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Information Processing in the NF-κB Pathway

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***Short Abstract* — We combined experimentation with mathematical modeling to elucidate how information about TNF concentration is turned into a binary decision in the NF-κB pathway. Using Kolmogorov-Smirnov distance, we quantified the ability of a cell to discern 8 TNF concentrations at each step of the pathway. Discernibility of low TNF concentrations is restricted by noise at the TNF receptor level, whereas discernibility of high TNF concentrations is restricted by saturation/depletion of downstream signaling components. The impact of co-stimulation with a translation inhibitor shows that the NF-κB network not only relays ~1 bit of information to coordinate expression of early genes, but also over a longer time course integrates information about other stimuli.**

I. Background

BINARY cell fate decisions result from integration of information obtained from several sources and subsequent information processing. These processes should be distinguished from trivial information decay to ultimately 1 bit just by noise.

A key pathway of innate immunity, the NF-κB pathway, is known to transmit merely 1 bit of information about the level of a biochemical stimulus [1,2]. Here, we analyze how information transmitted through subsequent steps of the signaling cascade from TNF receptor (TNFR) to NF-κB is reduced or turned into a binary decision [3]. We employ our recently calibrated [4] mathematical model of the NF-κB network and focus on the first pulse of NF-κB nuclear translocation. In addition to estimating upper bound of mutual information (max MI), we quantify the ability of the system to discern the neighboring concentrations of a stimulus ("dose discernibility") at each step of the core pathway by means of the Kolmogorov–Smirnov (KS) distance, a measure of distance between two probability distributions.

II. Results

A. Transmission of information and dose discernibility are reduced by extrinsic noise

The model system simulated stochastically without extrinsic noise transmits about 1.3 bit of information about TNF concentration. When extrinsic noise, associated with heterogeneity in the levels of TNF receptor and total NF-κB, is considered, both dose discernibility and max MI are reduced; the latter, to ~1 bit.

B. Mutual information and dose discernibility decrease as the signal propagates through the pathway

Information transmission at low TNF concentrations is mostly limited by molecular noise at the TNFR level, whereas at high TNF concentrations it is mostly limited by saturation of active IKK. As a result, the highest discernibility of adjacent TNF doses by NF-κB is observed at intermediate TNF doses: between 0.03 and 0.3 ng/ml.

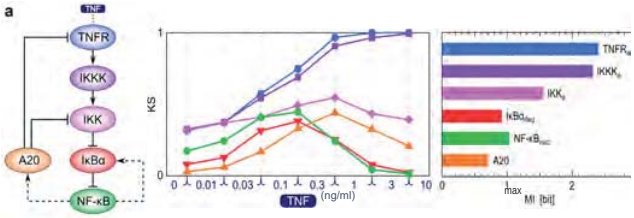

Figure 1. When passing through the NF-κB pathway (**A**), dose discernibility (**B**) and mutual information (**C**) are reduced.

C. NF-κB network can integrate signals from different sources over time

Cycloheximide (CHX) that blocks the synthesis of NF-κB inhibitors, IκBα and A20, allows NF-κB to remain in the nucleus for at least two hours. Responses to the combined lipopolysaccharide (LPS) + CHX stimulation, although almost indistinguishable from responses to pure LPS at 30 min, are clearly distinct at 120 min. This shows that LPS-stimulated cells are able to integrate additional signal from CHX over a longer time course.

III. Conclusion

The NF-κB system should be perceived as a feedback-controlled decision-making module rather than a simple information transmission channel.

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RAF1 Coordinates Proliferation and Motility

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***Short Abstract* — RAF1 and BRAF are two main RAF kinase isoforms in the ERK pathway. We have constructed detailed computational model of the ERK pathway, accounting for the regulatory sites of both RAF1 and BRAF, and their effects on assembly and coordination of RAF1-BRAF and RAF1-ROKα complexes, promoting respectively survival/proliferation and motility.**

***Keywords* — RAF1, BRAF, isoforms, ROKα, cell fate, proliferation, migration, MAPK, MEK, ERK, rule-based modeling**

I. Background

THE RAF/ERK cascade plays a principal role in the signal transduction of growth factors and initiating proliferation. The RAF kinase possess three isoforms: RAF1, BRAF, and ARAF that share similar homologous regulatory phosphorylation sites and activation mechanism but differ in activity and protein-protein interactions (PPIs) [1]. Specifically, RAF1 interacts with BRAF promoting ERK activation and proliferation but also with ROKα, which promotes cytoskeleton rearrangement and cell motility. These interactions are mutually exclusive and depend on the phosphorylation status of RAF1-Ser621 In this study we explored the coordination of the two responses [2].

II. Results

A. Computational Model

We have constructed a model of the cascade that accounts for the regulation of RAF1 and BRAF by phosphorylation and protein-protein interactions. The model accounts for the phosphorylation state of the N- and C-terminal 14-3-3 binding sites, the NtA domain, and dimerization interface (by a negative feedback from ERK). Of particular importance is the N-terminal 14-3-3 binding site of RAF1 – Ser621; when phosphorylated 14-3-3 stabilizes RAF1-BRAF complex via crosslinking, or crosslinks RAF1 in an inactive form, when unphosphorylated RAF1 binds ROKα

The model features a detailed mechanism of RAF1 and BRAF activation by RAS-GTP recruitment, homo- and heterodimerization, and stabilization by 14-3-3. The model comprises a system of 277 ODEs generated from the system of 85 rules specified in BNGL. The model was manually fit to reproduce the observed time profiles of (1) the phosphorylation of RAF1 sites, (2) ERK activation, (3) RAF1-BRAF and RAF-ROKα complex formation and effects of single and multiple mutations of the regulatory sites of RAF1 and BRAF on the ERK activity and protein-proteins interactions.

B. Model Analysis

The analysis of the model demonstrated the mechanisms of coordinating protein-protein interactions in response to growth factor stimulation.. The model indicated that the recruitment of RAF1 to RAS-GTP dimer is critical for Ser621 phosphorylation and heterodimerization with BRAF. This favors early ERK activation with maximal response at 5 min after the stimulation, which coincides with the maximal level of RAF1-BRAF heterodimer and promotes cell proliferation in the initial phase of signaling. The subsequent dimer disassembly and dephosphorylation at RAF1-Ser621 promotes RAF1-ROKα complex assembly with the maximal level at 15 min post-stimulation, inducing cytoskeletal rearrangements and promoting cell motility.

Sensitivity analysis confirmed the importance of the RAF1-BRAF stability and regulation thereof in determining the time profile of RAF1-ROKα complexes. the model predicts that the negative feedback from ERK to RAF isoforms terminates ERK activity and actively promotes RAF1-ROKα assembly by prior disruption of RAF1-BRAF heterodimers, thus establishing a temporal sequence of cellular responses.

III. Conclusions

We have formulated and analyzed the first comprehensive model of the RAF/ERK cascade, accounting for the combinatorial complexity of the RAF1 and BRAF activation and interactions, and explaining coordination of proliferation and motility due to growth factor signaling.

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Modeling miRNA-mediated translation control

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***Short Abstract* — This work is concerned with modeling of miRNA-mediated regulation of gene expression. We propose a novel approach based on queuing systems theory. The model describes changes of probabilities of different numbers of ribosomes associated with a given transcript. This allows to compare simulation results with experimental observations of polysome profiles and determine the type of control exerted by miRNA on the translation process.**

***Keywords* — miRNA, translation control, queuing systems.**

I. Introduction

ONE of the molecular components regulating gene expression is microRNA – a small (21-25 nt) single-stranded non coding RNA molecule. The main function of this molecule is post-transcriptional regulation of gene expression through gene silencing. It is achieved either by inhibition of translation or by degradation of mRNA [1]. The detailed mechanisms employed include inhibition of attaching the 60s ribosomal subunit, premature ribosome drop-off or inhibition of protein elongation process, cleavage of mRNA or destabilization of mRNA [2-3].This regulation appears to be used in control of cellular responses to stress, e.g. induced by irradiation. Despite many efforts, however, detailed mechanisms employed in such case are still not fully understood. Development and analysis of mathematical model of miRNA-mediated mechanisms of control of gene expression should advance the research in this field.

II. Preparation of Abstracts

In the literature, one can find several models of regulatory networks, in which miRNAs are involved. Some of them describe specific miRNA interactions, while others attempt at creating a generic model of these processes [3-5]. In this work, a different approach is proposed, inspired by our own experimental results, showing different polysome profiles observed for reporter genes containing miRNA binding sites (using the technique described in [6]). The model is focused on the mRNA processing by the ribosome complexes, a number of which may produce proteins from one mRNA template in parallel. When there is more than one ribosome on a mRNA, such construct is called a polysome [7]. Measuring polysome, together with respective protein levels may lead to conclusions about the type and power of miRNA-mediated control specific gene expression as well as provide information about highly processed transcripts.

The approach proposed in this paper is based on a queuing systems theory. Each transcript is processed in such system, with ribosomes viewed as service stations. There are discrete events of ribosome binding, movement along a mRNA strand, finishing translation, thus producing a single protein molecule, and ribosome stop, either random or caused by miRNA complex attached to a respective binding site. Binding of miRNA molecules to mRNA is also modeled as a discrete event, probability of which depends on the level of mRNA and its type.

The system state is defined by a n-dimensional vector P. Each variable $P_i(t)$ represents probability of i ribosomes being attached to mRNA at the moment t. Thus, simulation results can be directly compared with the experimentally observed distribution of polysome fractions. The dimension of the state vector is defined by the size of mRNA,

III. Conclusion

In this work a novel approach of modeling regulatory mechanisms of gene expression is proposed. It consists in describing a process of translation within the framework of queuing systems theory. Stationary distribution of polysomes observed in biological experiments were well represented by the simulation results in preliminary studies.

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Effect of Noise and Parametric Variations on Gene Regulatory Circuit Dynamics

Vivek Kohar[1], and Mingyang Lu[1]

***Short Abstract* — We study the effect of both the noise and the parametric variations on the gene regulatory circuit (GRC) dynamics by integrating stochastic analysis in the *Ra*ndom *Ci*rcuit *Pe*rturbation (RACIPE) method. Our two sampling schemes, one based on constant noise simulations and the other based on simulated annealing, enable estimation of both the basins of attraction as well as the relative stability of different gene expression states of the GRC. This systematic investigation of various GRC topologies sheds light on noise-induced hybrid states.**

***Keywords* — gene regulatory circuits, gene expression noise, simulated annealing, network robustness, sRACIPE.**

I. Introduction

Noise or stochastic fluctuations in molecular components play an important role in biological systems [1-3]. The dynamics of a gene regulatory circuit (GRC), a functional regulatory network motif of a small set of interconnected regulators, can be modeled using different mathematical frameworks like stochastic simulation algorithms such as Gillespie algorithm, stochastic differential equation-based methods, asynchronous random Boolean network models, and hybrid methods [4]. Most of these methods require a fixed set of kinetic parameters whose estimations are difficult and thus any uncertainty in these parameters limits the accuracy of the models as well.

*Ra*ndom *ci*rcuit *pe*rturbation (RACIPE) is an ordinary differential equation-based method that overcomes the uncertainty in parameter estimation by generating an ensemble of models with random kinetic parameters [5-6]. Gene expression patterns obtained from the statistical analysis of this ensemble of models correspond to the distinct functional states of the GRC. To generalize the RACIPE method to capture the stochasticity of cellular processes, we have incorporated stochastic analysis in RACIPE in our method, sRACIPE [7]. Such stochastic analysis allows us to analyze changes in the gene expression patterns in different gene expression clusters at different noise levels. Crucially, sRACIPE also allows us to compare the stability of various states, which is not feasible in RACIPE in which all states are considered equally probable. This is accomplished by a simulated annealing-based approach where the GRC is first simulated at a high noise, and then the noise is decreased gradually to zero. Next, we discuss the results obtained by application of sRACIPE to various GRCs.

II. Results

Statistical analysis of sRACIPE models revealed that the changes in gene expression patterns due to parametric variations are qualitatively different from those arising due to stochasticity of molecular components. While parameter variations result in an increase in the spread of the gene expression clusters, large noise alters both the number of clusters as well as the gene expression patterns within the clusters. We observed merger of distinct states at high noise levels and creation of new hybrid states. The basin of attraction of the states can be estimated by using multiple initial conditions and simulating the GRC at a constant noise. Similar findings were observed for cascaded toggle switches and other large networks. Simulated annealing revealed relative stability of the states as well as the most stable state of a GRC as high noise enables the GRC to access all possible states and then it gets trapped in the most stable state as the noise is reduced [7].

III. Conclusion

We have developed a method to study the gene expressions of stochastic GRCs and provided a publically available R-package for applying this method to any GRC.

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Metabolic Organization Through Glucose-Mediated Regulation of Mitochondrial Transport

Anamika Agrawal[1], Gulcin Pekkurnaz[2], and Elena F. Koslover[1]

***Short Abstract* — Metabolism in large eukaryotic cells can be enhanced by spatial localization of key enzymes to regions with the highest metabolic demands. In mammalian neurons, mitochondria have been shown to respond to glucose levels by halting active transport preferentially in high glucose regions. We use reaction-diffusion models to explore the physical limits on spatial organization of organelles through such regulated stopping of processive motion. Our results highlight that the effectivity of this regulatory mode is limited to a range of glucose concentrations, but that glucose-dependent mitochondrial halting provides a plausible mechanism for enhancing metabolic flux under hypoglycemic conditions.**

***Keywords* — intracellular transport; cytoplasmic organization; reaction-diffusion; metabolism**

I. Introduction

EXTENSIVE cells such as neurons often have spatially heterogeneous metabolic requirements. For instance, myelinated cells in the central nervous system tend to restrict glucose entry into the cell to narrow nodes of Ranvier that can be separated by hundreds of micrometers. The ability of mitochondria, the energetic powerhouses of the cell, to sense and respond to changing glucose levels is known to be critical to cellular health [1]. Recent experiments in cultured neurons have demonstrated that mitochondria can localize preferentially to subcellular regions with high glucose levels [2]. This localization is achieved through the glucose-dependent activation of *O*-GlcNAc transferase (OGT) and *O*-GlcNAc modification of Milton, an adaptor protein that links mitochondria to the molecular motors responsible for their long-range transport through the cell. Upon modification by OGT, Milton triggers a halting of the mitochondria, causing them to stop disproportionately in glucose-rich regions of the cell [2].

We leverage continuum physical models to investigate the efficiency of organelle localization by enhanced halting in response to a diffusive signal that is itself consumed by the organelles.

II. Results

We develop a simplified quantitative model for mitochondrial distribution in a long narrow cellular projection in the context of glucose-mediated halting of active transport. Our model postulates a fixed glucose concentration at localized regions corresponding to the nodes of Ranvier, combined with glucose diffusion and consumption through initial metabolism by hexokinase enzymes that are localized to mitochondria. The mitochondria are assumed to engage in bidirectional processive transport, initiating walks at a constant rate, and halting walks with a rate dependent on the local glucose levels. Steady-state glucose and mitochondrial distributions in this model are governed by just three dimensionless parameters: the glucose decay length scale (which quantifies the balance between diffusion and consumption) relative to the internode distance, the external glucose concentration relative to the Michaelis-Menten constant K_M for the utilization of glucose by hexokinase, and the equilibrium constant defining the balance between stopping and walking rates of mitochondria.

We demonstrate that efficient localization of mitochondria to the nodes requires intermediate external glucose concentrations. Overly high concentrations saturate hexokinase kinetics, resulting in high levels of glucose throughout the cell. Concentrations that are too low lead to insufficient stopping of the mitochondria, also allowing them to disperse throughout the domain. Quantitative analysis of our model indicates that external glucose concentrations must be below approximately 1mM to achieve mitochondrial accumulation at the glucose source. Comparison to physiological brain glucose (measured at 0.7-1.3mM depending on brain region [3]) implies that this spatial organization mechanism is expected to be significant under both physiologic and hypoglycemic conditions. Furthermore, we find that total glucose flux in a myelinated neuron under such conditions can be increased two-fold by the proposed mechanism of glucose-dependent tethering of mitochondria.

We additionally develop an analogous model for non-myelinated cells, incorporating spatially heterogeneous external glucose and limited glucose permeability into the cell via facilitated transport through glucose channels. This model is leveraged for quantitative comparison with experimental data on mitochondrial localization in cultured neurons exposed to a glucose gradient [2].

III. Conclusion

We outline the physical limits on spatial organization of organelles by regulated halting of active transport, highlighting the interplay between diffusion and decay of the signaling molecule and the organelle stopping rates. In particular, we demonstrate the potential for enhancing metabolic rate in myelinated neurons at low glucose conditions by glucose-dependent modification of the Milton adaptor protein.

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Mathematical models of virus infections

Jesse M. Kreger[1], Natalia L. Komarova[2], and Dominik Wodarz[3]

Short Abstract — **Motivated by recent experimental data, we investigate mathematical models regarding the evolutionary outcomes of viral infections, specifically human immunodeficiency virus (HIV), in humans. We analyze how the interplay between multiplicity of infection, synaptic cell-to-cell transmission of the virus, and free virus transfer can affect the dynamics of an infection taking place. Finally, we will discuss how recombination between virus strains can change the evolutionary outcomes of infection and influence the course of disease. The overall goal of the project is to better understand the dynamics of viral infections, specifically HIV, and to help design more effective healthcare and vaccination approaches.**

Keywords **— HIV, recombination, synaptic cell-to-cell transmission, free virus transmission, multiplicity of infection.**

I. Extended Abstract

Motivated by recent experimental data [1,2,4], we investigate mathematical models regarding the evolutionary outcomes of viral infections, specifically human immunodeficiency virus (HIV), in humans [5]. We will analyze how the interplay between synaptic cell-to-cell transmission of the virus and free virus transfer can affect the dynamics of an infection taking place.

We consider models that take into account multiplicity of infection, where a cell can be infected multiple times by different strains of the virus. Further, we also consider models with competition between virus strains, characterized by different mutations, to see how each strain's infection strategy can affect outcome.

Finally, we will discuss how recombination between virus strains can change the evolutionary outcomes of infection and influence the course of disease. Multiple mathematical models for HIV including synaptic cell-to-cell transmission have been put forth [3], however we now make use of an agent based model to study recombination and the dynamics of recombinant strains of the virus in the presence of both transmission modes. We show that a combination of both free virus transmission and synaptic cell-to-cell transmission minimizes the time to recombinant virus formation. This is because non-spatial free virus transmission ensures different strains will meet in the same cell and spatial synaptic cell-to-cell transmission then repeatedly transmits the different strains together, resulting in more opportunities for recombination to occur.

The overall goal of the project is to better understand the dynamics of viral infections, specifically HIV, and to help design more effective healthcare and vaccination approaches.

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Deciphering the Structure of The Condensin Protein Complex

D. Krepel[1], R.R. Cheng[1], M. Di Pierro[1] and J.N. Onuchic[1]

Short Abstract — **Understanding chromatin organization within the nucleus is a key question in cell biology. Despite recent progress, little is known about the mechanisms underlying chromatin structure and how it can be established. One prominent hypothesis suggested is that loop-extrusion motors bind and translocate segments of chromatin to form structural loops. Such motors are proposed to be Structural Maintenance of Chromosomes (SMC) - Kleisin protein complexes.**

Here, we use Direct Coupling Analysis (DCA) to reveal the full structure of the condensin protein complex. We demonstrate that this methodology predicts coevolved residue-residue interactions between the different components of the complex. These are used to predict the full structure of SMC complexes, for which only limited experimental data exists. This work serves as pioneering study to understand the establishment of chromatin architecture.

Keywords **— condensin, cohesion, chromosome, cell replication, direct coupling analysis, co-evolution**

I. BACKGROUND

MEMBERS of the structural maintenance of chromosomes (SMC) and kleisin families of proteins are conserved in all domains of life and have key roles in the maintenance of chromosomes [1]. One such protein complex is cohesin, which has several critical biological roles. It has been suggested that cohesin organizes DNAs into chromatids by capturing small loops of DNA and then extruding them in a processive manner [2]. Similar to eukaryotes, the condensin complex in prokaryotes is also part of SMC-kleisin family. Condensin is formed by SMC-kleisin proteins SMC and ScpA, respectively, and a third subunit, ScpB [2]. Despite major progress in recent years, many questions related to the structure and function of SMC-kleisin complexes remain open. Importantly, the complete structure of SMC-kleisin complexes has yet to be established. The two main models of the structure of these complexes are: (1) two rings acting as a pair of molecular "handcuffs" in which each embrace one DNA single, or (2) a single ring that embraces two DNA strands. Although limited structural data exist for each subunit of the condensin protein complex [3-4], these cannot capture the dynamics and reveal interaction surfaces in details, and therefore are limited in their ability to disentangle the various controversies of the complex.

II. RESULTS

Here, an integrative approached was used by combining previously known crystallographic data with evolutionary information in order to study a series of controversial features of the condensin protein complex [5-6]. Combined with molecular dynamics (MD) simulations, we are able to predict the full structure of the previously unknown condensin complex. This allows us to:

A. *Construct the full structure of condensin*

Taking advantage of existing experimental data, we use extract both homologous sequences and experimentally known structures. Direct coupling analysis (DCA) then run predicts a contact map, revealing the interprotein residue contacts between all constituents. To obtain the complete structure of the condensin complex, we use the subcomponents and dock them guided by the DCA top contact predictions.

B. Examine the plausibility of higher-order stoichiometries

Having established a full DCA contact map, we examine whether our DCA contact predictions remain preserved in higher order stoichiometries of condensing while comparing their stability with the previously obtained structure.

C. Reveal alternative configurations

Specific domains in the complex are suggested to serve as entry and exit gates for DNA, respectively. Our results manage to predict the rearrangement of these domains, which may lead to the opening of the ring in the presence of DNA. Moreover, the flexibility of ScpA subunit projects on the overall structure of the condensin complex, allowing possible interaction with the DNA strand and its release from the ring.

III. CONCLUSION

In this project the full all-atom structure of the condensing protein complex is revealed. Using our integrated methodology of existing experimental data with co-evolution information, we are able to study a series of controversial features of the condensin protein complex. Hence, this study serves as key foundation for the system-level study of the various SMC-kleisin protein complexes.

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Stochastic modeling of post-transcriptional regulation of gene expression by non-coding RNAs

Niraj Kumar[1], Kourosh Zarringhalam[2], and Rahul V. Kulkarni[3]

***Short Abstract* — We study the canonical model of stochastic gene expression wherein non-coding small RNAs (sRNAs) and mRNAs interact with each other leading to stoichiometric mutual degradation. Due to the presence of the nonlinear interaction term between sRNAs and mRNAs, the exact solution of the model is analytically intractable and theoretical analysis typically involves mean-field approaches. However, mean-field results are inaccurate in the limit of strong interactions and low abundances; thus alternative theoretical approaches are needed. In this work, we obtain analytical results for the canonical model of regulation of stochastic gene expression by sRNAs in the strong interaction limit. We derive analytical results for the steady-state generating function of the joint distribution of mRNAs and sRNAs in the limit of strong interactions and use the results derived to obtain analytical expressions characterizing the corresponding protein steady-state distribution. Furthermore, under simplifying assumptions, we also derive the large deviation rate function characterizing rare events associated with the rate of protein production.**

***Keywords* — Stochastic gene expression, Post-transcriptional regulation, Non-coding small RNAs, Rare events.**

I. Introduction

Intrinsic randomness in gene expression process can play an important role in stochastic cell fate decisions [1-2]. Cellular control of such intrinsic randomness (noise) depends on the regulation of gene expression which occurs at various stages. While considerable research has focused on regulation of gene expression by transcription factors, there has been growing interest in understanding gene regulation at the post-transcriptional level by non-coding sRNAs (sRNAs). Non-coding sRNAs are known to play key roles in regulating diverse cellular processes [3-4] and dysregulation of and by sRNAs is linked to various diseases including cancer [5]. Correspondingly, there is significant interest in quantitatively modeling the effect of post-transcriptional regulation on fluctuations [6] and rare events in gene expression [7].

In the present work, we focus on quantitative understanding of gene regulation due to these sRNAs. For this, we use the canonical model [8] of stochastic gene expression that incorporates post-transcriptional regulation. In this model both sRNAs and mRNAs interact with each other leading to stoichiometric mutual degradation. The presence of nonlinearity due to this interaction makes the model analytically intractable. However, in the limiting case of strong interactions, we have derived analytical results for this model.

II. Results

For the canonical model studied in this work, we derive analytical results for the steady-state joint distribution of mRNAs and sRNAs in the limit of strong interactions. The results derived at the level of mRNAs are then extended to derive analytical expressions for the corresponding protein distribution. Characterization of protein probability distribution in this limit is an important step in quantifying post-transcriptional regulation of noise in gene expression. Furthermore, within the framework of canonical model and under biologically relevant simplifying assumptions, we also derive the large deviation rate function characterizing rare events in the rate of protein production.

III. Conclusion

The derived analytic results for the probability distribution of mRNAs and proteins in the strong interaction limit and derived expression for large deviation function for protein production rate can provide significant insights into the role of sRNAs in regulating stochastic gene expression. These results can serve as building blocks for the analysis of more complicated genetic circuits involving sRNAs and the approach developed can potentially be generalized to analyze stochastic models of gene regulation with non-linear interaction.

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Stress Introduction Rate Impacts Acquisition of Antibiotic Tolerance and Resistance

Ariel Langevin[1,2,3], Imane El Meouche[1,2], and Mary Dunlop[1,2]

***Short Abstract* — Stress tolerance studies are typically conducted in an all-or-none fashion. However, in realistic settings, cells may encounter stresses, including antibiotics, at different rates. Thus, how cells tolerate stress may depend on its rate of appearance. To address this, we study how the rate of introduction affects tolerance using a key stress response mechanism, efflux pumps. We found that slower rates of introduction provide a disproportionate benefit to efflux pump-containing strains, allowing cells to survive beyond their original inhibitory concentrations. We hypothesize cells surviving these stressful environments for longer periods will be predisposed to acquire mutations for antibiotic resistance.**

***Keywords* — dynamic environments, stress response, *E. coli*, antibiotics, efflux pumps**

I. Background

ANTIBIOTIC resistance has become a major public health concern as bacteria produce strategies to evade drugs, leading to recurring infections and overuse of antibiotics. Understanding the complex strategies bacteria employ to resist treatment could provide insight into different methods of targeting these evasive cells. Recent literature suggests that antibiotic resistance may be achieved after cells have gained tolerance in particular environments [1].

As a case study, we use efflux pumps to investigate how antibiotic resistance may be more likely to occur based on the environmental dynamics. Efflux pumps, such as AcrAB-TolC from *Escherichia coli*, are membrane transporters well known for their ability to export a wide variety of substrates, including antibiotics and biofuels [2-3]. As such, efflux pumps may be able to maintain a low intracellular concentration in stressful environments; and by doing so, enable long term survival and perhaps mutations.

II. Results

Our goal is to quantitatively determine the impact of short-term and long-term environmental dynamics on bacterial populations. To achieve this we co-culture cells with and without the efflux pumps. The relative fraction of cells with and without the pumps can change with time under variable antibiotic conditions.

A. Short-term dynamic environments

We hypothesized that the ideal pump expression level would involve a rate-dependent trade-off between the benefit of pumps and the cost of their expression. To test this, we evaluated the benefit of the AcrAB-TolC pump under different rates of stress introduction, including a step, fast ramp, and gradual ramp using the antibiotic chloramphenicol. A mathematical model describing these effects predicted the benefit as a function of the rate of stress introduction. [4]

B. Long-term dynamic environments

Next, we hypothesized that long-term environmental perturbations enabling survival could produce permanent changes through mutations. Using a modular turbidostat, the eVOLVER, we were able to apply long-term dynamics to populations of bacteria [5]. We then quantify the population changes over several days to a week and check when mutations began to emerge.

III. Conclusion

Thus far, we have demonstrated that the benefit of efflux pumps depends heavily on the rate of stress introduction. We found that strains exposed to slower stress introduction rates were able to survive cumulative concentrations well beyond what they could survive if the stress appeared suddenly. This led us to investigate the long-term consequences of this increased tolerance through acquired resistance. Overall, this work will provide insight into how bacteria optimize the use of efflux pumps as strategic mechanisms for surviving and mutating in stressful conditions.

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Differences between telomerase activation and ALT based on the G-Networks

Kyunghyun Lee, Marek Kimmel

The length of telomeres, protective structures at the end of chromosomes, positively correlates with the survival ability of carcinogenic cells. The length in human tumors is maintained by two known mechanisms, active telomerase and the alternative lengthening of telomeres (ALT). However it is known that compelled repression of telomerase-related genes may induce the cells to use ALT for immortalization. Using the Abnormal Pathway Detection Algorithm based on the theory of G-network, our analysis shows a distinguishable pattern of the hTERT down-regulation in the ALT cell lines compared to the normal cell lines. Moreover the algorithm detects a number of genes positively activated by c-Myc, which is found in many malignant tumors in a mutated form, have significantly reduced mRNA expression levels in the ALT cell lines.

I. Introduction

Telomeres, which are composed of TTAGGG repeats at the end of chromosomes (Blackburn, 2001), informs of replicative senescence as they are shortened per normal cell division (Harley, 1991). Maintaining telomere lengths, which is accomplished by the activation of telomerase or ALT, is positively associated with one of the hallmarks of cancers (Hirashima et al., 2013), which is resisting cell death (Hanahan and Weinberg, 2000). However hTERT, one of the most important protein components of telomerase, tends to be suppressed in the ALT-expressed cell lines (Atkinson et al., 2005), implying that deliberate repression of telomerase is a potential catalyst for cell immortalization by ALT [1]. Moreover, most genes positively interacting with c-Myc, which is found in many malignant tumors in a mutated form, are repressed in the ALT-expressed cell lines, suggesting that ALT may reduce the role of c-Myc.

G-network [2], one of the stochastic models motivated by queuing theory, introduces a new notion of *inhibition* to conventional queuing theory. It allows us to explore gene regulatory networks using queuing models. Based on interactions between known gene-regulation pathways (Metacore Analytical Suite), here we detect the significant abnormal pathways [3] among 29 genes present in the ALT-expressed cell lines. Furthermore, in order to uncover the roles of each targeted gene, we compare multiple gene regulatory networks with different combinations of genes.

II. Methods

Identifying significant abnormal pathways using the G-network mainly focuses on estimation of transition probabilities (p_{ij}^{+}, p_{ij}^{-}), a probability that gene *i* activates gene *j* and a probability that gene *i* inhibits gene *j*, respectively. We adopt and modify the algorithm illustrated in [3] for estimating the negative customer input rates under a normal condition, optimizing the transition probabilities in an ALT-expressed condition and determining the significant abnormal pathways under the steady state. Moreover the theory of Stochastic Automata Networks [4] and spectral gap are employed to explore the transient state using the global infinitesimal generator and the rate of convergence of the chain to its steady state for the last step of the analysis.

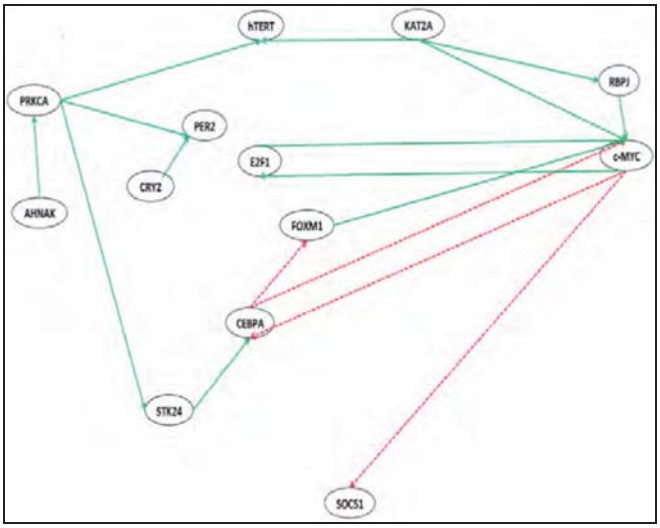

Figure 1. The significant gene regulatory pathways consisting 13 genes. From GSE14533 dataset in Gene Expression Omnibus, 4 normal and 18 ALT-expressed cell lines were analyzed. Green (solid) and red (dashed) lines represent positive and negative interactions between two genes, respectively. Consistent with the result from [1], E2F1, a known repressor of hTERT, maintains its pathways related to c-Myc in ALT-expressed cell lines, whereas the association between c-Myc and hTERT was identified as being insignificant.

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Quantification of Ras membrane diffusion and multimer formation in live cells

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***Short Abstract* — Ras small GTPases are key regulators of cell signaling. Recent evidence suggests a critical role of Ras dimers and clusters in cell signaling, but the mechanisms of how these structures form and function in cells remain unclear. Here we describe nanoscopic sites on the cell membrane that can transiently trap and enrich Ras to potentially facilitate dimer and cluster formation, as revealed by single-molecule tracking, quantitative trajectory analysis, and simulations. Our results demonstrate the importance of membrane heterogeneity in regulating biological processes in cells.**

***Keywords* — single-molecule tracking, quantitative imaging, Ras, diffusion, dimer, membrane**

I. Purpose

Ras is predominantly monomeric in solution but readily forms dimers and clusters in cells where it is membrane-bound, suggesting a role of the membrane in promoting Ras-Ras interaction [1,2]. To understand how Ras forms dimers and clusters on the membrane, we used single particle tracking photoactivated localization microscopy (spt-PALM) to quantitatively measure the diffusion of Ras in living cells at the single-molecule and nanometer scales [3]. spt-PALM yields tens of thousands of single-molecule trajectories with 10-35 millisecond time resolution and 20-30 nm spatial precision, from which Ras membrane diffusion models could be inferred via statistical analysis. We quantified the diffusion, occupancy, and transition rates for each state, which were in simulations to test the hypothesis that *Ras dimers and clusters primarily form in specialized membrane compartments.*

II. Results

A. Ras exhibits three distinctive diffusion states

The large spt-PALM dataset enabled the use of statistical analysis such as variational Bayes SPT to derive the diffusion states, occupancy, and state-transition rates unavailable through bulk measurements or conventional SPT [4]. We observed three distinctive diffusive states of Ras on living U2OS cell membranes as listed below (D: diffusion coefficient, error: standard deviation):

	State 1	State 2	State 3
D ($\mu m^2/s$)	0.07 ± 0.01	0.26 ± 0.05	0.84 ± 0.07
Occupancy (%)	17.4 ± 6.8	38.9 ± 9.0	43.7 ± 13.5

Further, Ras switches between the fast diffusive state (state 3) and the immobile state (state 1) predominantly by going through the intermediary state (state 2).

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B. Membrane nanodomains trap and enrich Ras

We identified membrane regions that are about 40 nm in radius and are correlated with the slowest diffusive state of Ras; these regions are termed Ras-associated nanodomains (RANDs). Trajectory analysis also indicated at least two populations of RANDs, a transient population lasting for 1-2 s on average and a stable population lasting up to 15 minutes. Importantly, each RAND can contain multiple Ras molecules, providing a mechanism for Ras to be locally enriched, which potentially facilitates Ras-Ras interactions.

C. Initial simulations show RANDs promote Ras multimers

To further determine the role of RANDs in facilitating Ras dimer and cluster formation, we have run computer simulations with experimentally derived Ras diffusion and RAND parameters to compare Ras interactions in fast and immobile (inside RANDs) diffusive states. Preliminary results suggest that in general, Ras molecules trapped in RANDs are more likely to yield dimers and clusters than those that are in the fast diffusive state. There is an optimal RAND to Ras ratio for maximal Ras dimer and cluster formation, with a low ratio yielding a low fraction of total Ras in RANDs and a high ratio yielding too few Ras per RAND. Additional simulations will be tested with the Ras diffusion parameters from section A.

III. Conclusion

Our work suggests that membrane nanodomains (RANDs) can increase the local concentration of Ras to potentially facilitate Ras dimer and cluster formation. Ras molecules can interact with multiple types of RANDs, indicative of parallel mechanisms that could regulate Ras dimer and cluster formation. Lastly, the majority of Ras molecules enter an intermediary state before interacting with RANDs, implying that RANDs are likely associated with specialized membrane compartments. The same paradigm may apply to other membrane molecules.

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The synergistic effect of host immunity with phage and probiotic therapy against bacterial pathogens

Chung Yin Leung[1] and Joshua S. Weitz[2]

***Short Abstract*— The rise of antibiotic resistance in bacterial pathogens is a major public health concern. Existing alternatives to antibiotics include the use of phage (bacterial viruses) and probiotic bacteria. However, these therapies have not demonstrated consistent efficacy comparable to antibiotics, possibly due to heterogeneity in the host immune response against the pathogen. Through analysis of a combination of population models and data from animal experiments, we show that host immunity works synergistically with phage to cure an acute respiratory infection. We extend our modeling framework to show that the same principle of immunological synergy may also be applicable to probiotic therapy.**

***Keywords*— Bacteriophage, Phage therapy, Probiotics, Commensal bacteria, Antibiotic resistance, Mathematical model**

I. INTRODUCTION

The spread of antibiotic-resistant pathogens has become a major public health crisis. Without urgent intervention in antibiotic stewardship and development, the world may be approaching a post-antibiotic era where common infections can become fatal [1]. The plight of antibiotic resistance has stimulated interest in developing alternative or adjunct antimicrobial therapies to antibiotics, including the use of phage (viruses that exclusively infect bacteria) [2] and competitive exclusion by commensal or probiotic bacteria [3]. Unfortunately, these alternatives have yet to achieve the level of robust efficacy on par with antibiotics.

To understand the variations in clinical outcomes of these antimicrobial therapies, it is essential to consider contributions from the host immune system, which is a critical driver of the *in vivo* dynamics of pathogens. The *in vivo* host-pathogen interactions as well as *in vitro* pathogen-antimicrobial interactions have been relatively well studied. However, research into the tripartite interactions between pathogenic bacteria, antimicrobials and host immunity has remained scarce. Recent advances in mathematical modeling have suggested that host immunity can have a significant effect on the pharmacodynamics of antibiotics [4]. Here, we explore the role of host immunity in the effectiveness of novel antimicrobial therapies including phage therapy and probiotics therapy.

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II. RESULTS

We propose a nonlinear population model of phage therapy that considers the interactions between pathogenic bacteria, phage and host immune response [5]. Our model accounts for bacterial growth, phage predation, and immune killing of pathogenic bacteria. Crucially, we include the key immunological features of saturation of the immune response and immune evasion by bacteria. The model predicts a synergistic effect between host immunity and phage that eliminates bacterial pathogens, even when neither of which can do so when acting alone. This synergism is validated in animal experiments of acute pneumonia under different immunological backgrounds, and adaptation of the model to the *in vivo* conditions show that host immunity can prevent the emergence of phage resistance during therapy [6].

We extend our theoretical framework to incorporate competition between bacterial pathogen and commensal or probiotic bacteria. Our results indicate that host immunity may also act synergistically with probiotic therapy to prevent and cure bacterial infections. We systematically explore the effects of different competition strengths between the pathogenic and probiotic bacteria, and find that host immune killing promotes competitive exclusion of the pathogen by stabilizing the pathogen-free state of the system.

III. CONCLUSION

We demonstrate that synergy with host immune response can be a general mechanism of antimicrobial therapy applicable to phage and probiotic therapies. Our results highlight the need to characterize the host immune status when evaluating the effectiveness of novel antimicrobial therapies.

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Cellular sorting and trafficking mediated by membrane microdomains

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***Short Abstract* — Ordered membrane domains known as rafts are believed to serve crucial functional roles in mammalian cells; however, their investigation has been hampered by poor methodologies. We have developed a quantitative framework for investigating raft affinity of transmembrane proteins and used it to define the structural determinants of transmembrane protein partitioning between coexisting membrane phases. We relate this partitioning to subcellular trafficking, establishing a direct, quantitative role for raft domains in membrane protein sorting. We are establishing the molecular mechanisms of this domain-mediated traffic using systems approaches and super-resolution quantitative imaging.**

I. Introduction

Eukaryotic cells are organized into spatially and functionally distinct membrane-bound organelles, whose functions are defined by their lipid and protein composition. Accurate and robust sorting of membrane components between these compartments is necessary for the maintenance of organelle identity. For most membrane proteins, the determinants of their steady-state subcellular localization remain unknown (1).

Lateral membrane domains known as lipid rafts provide an ideal platform for membrane sorting processes, and have been widely implicated in post-Golgi sorting and endocytosis/recycling. However, the structural determinants of protein association with such domains are almost entirely unknown. We have developed and characterized a robust experimental system for direct, quantitative measurements of raft affinity in intact plasma membranes and used it to explore the determinants of transmembrane protein recruitment into raft domains and the consequences of this recruitment on subcellular traffic.

II. Results

A. Structural determinants of raft affinity

Using our quantitative platform, we quantified ordered domain affinity for >100 transmembrane proteins and identified three physical features – transmembrane domain surface area, length, and palmitoylation – that independently affect raft partitioning (2). Specifically, long, palmitoylated TMDs with smaller surface areas partition efficiently to the more ordered raft domains. These findings were rationalized with a mechanistic, physical model wherein raft affinity is determined by the interfacial energy between a protein TMD and the surrounding lipid matrix. This model was shown to be capable of correctly predicting raft affinity solely from protein sequence. Using bioinformatics, we generated proteome-wide predictions of raft affinity and observed that PM proteins have higher predicted raft affinity than those of intracellular membranes.

B. Functional consequences of raft affinity

We established a quantitative and functional relationship between raft association and subcellular protein localization. Specifically, we observed that raft association is fully sufficient for plasma membrane recycling of certain proteins, and that abrogation of raft partitioning for these proteins led to their degradation in the lysosomes. These findings support the conclusion that ordered membrane domains mediate recycling of specific membrane components from the endosomal compartments to the PM. We have proceeded to define the molecular machinery that mediates raft lipid and protein sorting and recycling to the PM. Using a set of orthogonal transmembrane proteins as probes of raft and non-raft domains, we developed a high throughput siRNA screen to dissect the molecular machinery and dynamics for raft-mediated sorting. We identified a number of validated hits including known players of the early endocytic traffic, but also novel players that appear to define a distinct class of trafficking mediators specific for raft-associated proteins. This pathway is not dependent on the classical recycling pathways, rather defining a novel route for PM recycling of raft-preferring cargo.

III. Conclusion

Our findings define the structural features that determine protein association with ordered membrane microdomains, and validate the key role of these sub-microscopic domain in sub-cellular sorting and trafficking. However, vital questions remain unanswered regarding the underlying mechanistic principles and molecular mechanisms by which these domains serve as cellular sorting hubs. These questions can only be answered by quantitative microscopy and mechanistic modeling approaches.

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Mechanics before Chemistry: Tensile Stress Induced Cytoskeletal Reorganization

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***Short Abstract* — Cellular remodeling in response to mechanical stimuli is critical for understanding mechano-signal transduction. We hypothesize that external stress induced subcellular adaption is accomplished through dynamical cytoskeletal reorganization. To study the interactions between subcellular structures involved in transducing mechanical signals, we combined experimental and modeling approaches to measure real-time structural and mechanical adaption of the actin cytoskeletal network. In vitro, we imaged the actin cytoskeleton as tensile stress was applied to live vascular smooth muscle cells (VSMC) using an ECM-functionalized atomic force microscope probe. In silico, we modeled the mechanochemical coupling of the actin cytoskeleton network. Both experimental and modeling results agree that under tensile stress, mechanical structural adaptation occurs before chemical adaptation: actin filaments align first, then actin polymerization takes place to further restructure the cytoskeleton.**

***Keywords* — Cytoskeletal Network, Tensile Stress, Filament Alignment, Actin Polymerization**

I. Introduction

CELLS interact with a complex microenvironment. Among all the microenvironmental stimuli, mechanical stress [1, 2] is important in many biological and physiological processes. Vascular smooth muscle cells (VSMC) are subjected to the cyclic stretch of pulsatile blood pressure that deforms the extracellular matrix and induces axial and circumferential wall stresses [3]. However, how VSMC responds to the mechanism of axial stress in the vessel wall, which can be considered as tensile stress applied to cell, is not well-understood [4]. We combine experimental and modeling approaches to investigate the effects of tensile stress on the dynamic remodeling of the cytoskeleton network.

II. Methods

The tensile stress was applied to live VSMC using an atomic force microscope probe functionalized with extracellular matrix proteins. Mechanical stimulation of the cell at low (~0.5 nN) and high (~1 nN) magnitude forces was applied every 3–5 min for 20–25 min each and the actin cytoskeleton was imaged by spinning-disk confocal microscopy after each force application [3].

A computational model for mechanochemical dynamics of active network (MEDYAN) [5] was used to simulate the actin network with an external pulling force. The model considers actin fibers as semi-flexible polymers embedded in a solution of actin monomers, alpha-actinin cross-linking proteins, and non-muscle myosin II (NMII) motors. A system of reaction-diffusion equations describes the spatiotemporal dynamics of actin polymerization and actomyosin network formation. In a simulation volume of $1\times1\times1$ μm^3, a randomly initialized actin filament network was subjected to an external pulling force. We varied the strength of force, and measured the resulting fiber alignment, polymerization, and uniformity.

III. Results

Both experimental and simulation results show that tensile stress has significant effect on the dynamics of the cytoskeleton network: as the tensile stress increases, the fibers rearrange to increase alignment along the direction of the external stress, before fiber polymerization takes place. This result suggests mechanical structural adaptation operates at a shorter timescale than biochemical processes, which can have important implications to mechano-signal transduction.

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Spatial profiles of tumor-infiltrating T cells

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***Short Abstract* — Higher amount of tumor infiltrating T cells has been demonstrated to associate with better prognosis in various types of cancer. However, the mechanism underlying different infiltration levels is still not clear. Here we focus on charactering the spatial infiltration pattern of T cells around tumor-cell clusters in patients with triple negative breast cancer. Combining mathematical modeling with patient data analysis, we propose that there exists an undefined factor that repels T cells away from tumor-cell clusters.**

***Keywords* — tumor-infiltrating T cells, spatial profiles, tumor-cell clusters, repellent**

I. Background

ACTIVATED T lymphocytes have been demonstrated to be able to kill antigen-specific cancer cells via various mechanisms [1]. Not-surprisingly, stronger infiltration of cytotoxic T cells into tumor/tumor-cell-clusters generally associates with better prognosis, which has been demonstrated in various cancer [2-5].

There have been efforts on quantifying the distribution of cytotoxic T cells on the whole tumor level [6]. On the other hand, a solid tumor is usually composed by many tumor-cell clusters as well as stromal contents in gaps between those clusters. It has been noticed that T cells can be mostly constrained in the stromal regions of a solid tumor [7]. Therefore, it is also important to quantify the spatial pattern of T cells on the tumor-cell cluster level and further investigate the mechanism underlying the observed limited infiltration.

II. Results

In this work, based on images of immune cells and cancer cells (triple negative breast cancer, patient samples), we developed a procedure to estimate the spatial profile of immune-cell density and a few properties of those spatial profiles were observed

A. T-cell profiles correlate well with potential Antigen-Presenting cells

First, we simply calculated the fraction of each type of immune cells inside tumor-cell clusters. The fraction of T cells is plotted against that of potential antigen-presenting cells (two types investigated here). It is observed that the fraction of T cells inside tumor-cell clusters can separate 18 patients into 2 groups, with 4 patients having the fraction above 0.4 and others below 0.2, whereas the fraction of each antigen-presenting cells is rather continuous for different patients. One interpretation of the plot is that the infiltration of T cells is bistable as a function of the infiltration of the potential antigen-presenting cells.

Secondly, we further investigated the detailed spatial profile of those cells inside/outside tumor-cell clusters. In 12 out of 15 patient samples analyzed, spatial profiles of T-cells correlate very well with at least one type of potential antigen-presenting cells.

B. T-cell density profile is better explained by a mathematical model hypothesizing a global attraction and local repulsion between T cells and cancer cells

Two types of mathematical models were developed: i) the motility of T cells decreases around tumor-cell clusters, or ii) the direction of motion of T cells is manipulated by tumor-cell clusters. One major difference between the two types of models is that the second model can generate a steady-state profile of T cells around tumor-cell clusters, which is favored since we did not observe any cancer-stage-dependent trend on the infiltration pattern.

III. Conclusion

Combining data analysis with mathematical modeling, we propose that innate immune cells actually promote the infiltration of T cells into tumor-cell islands and there exist a factor that repels T cells away from tumor-cell islands.

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In Situ Analysis of Microbial Communities Using Expansion Microscopy

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***Short Abstract* — We demonstrate the utility of expansion microscopy (ExM) in visualizing and analyzing spatial distributions of microbial species in gut microbiota of several organisms. First, we developed a new super-resolution imaging method to improve the photostability and brightness in expansion microscopy. Second, by tuning the enzymatic digestion of microbial cell walls, we use the expansion ratios in ExM as an in situ measure of bacterial physiology. This method enables multiplex imaging of complex bacterial communities while simultaneously providing spatial and cell physiological information.**

***Keywords* — Expansion microscopy, Microbial community, Bacterial physiology**

I. Background

THERE is a global race in microbiome research to map spatial organization of microbial species in densely packed communities. This information is critical for understanding the physiological and molecular interactions among species. The rapid progress in adapting fluorescence microscopy [1,2] and super-resolution imaging techniques [3] have greatly advanced the characterization of spatial structures in microbial communities and bacterial physiology in individual cells at the molecular level. However, progress is limited in linking these two scales due to lack of methods to characterize the diversity in physiology of microbial species within a population in situ.

II. Results

Here we describe a new super-resolution imaging method, based on expansion microscopy (ExM) [4], to achieve super-resolution imaging of dense bacterial populations in two organisms, planarian flatworms and mice, and simultaneous measurement of bacterial mechano-physiology.

A. Locked expansion microscopy

Expansion microscopy is an optical imaging technique based on physical expansion of tissues anchored to a hydrogel. The current method relies on a polyelectrolyte hydrogel that is expanded by electrostatic repulsion; as a result, the hydrogel shrinks in ionic buffers. This technical limitation precludes the possibility of using most anti-photobleaching systems, which typically require buffering. This restricts the application of ExM in thick tissues with weak fluorescence signals, as these applications typically demand long imaging times and suffer from photobleaching. Here, we developed a novel method, locked ExM, in which the first expanded polyelectrolyte hydrogel is embedded in a second interpenetrating hydrogel mesh that expands through entropic forces. The second interpenetrating mesh retains the size of expanded tissues even in buffers with extreme ionic strengths. This method has allowed us to use anti-photobleaching systems in ExM to achieve sub-diffraction-limit resolution with high photostability in order to capture features that are otherwise impossible to image.

B. Differentiation of bacterial cell wall properties by expansion ratios in situ

In locked ExM, the mechanical properties of a specimen are critical to the extent of expansion. In particular, bacteria rely on their cell walls to bear mechanical stress and maintain cell shape. The mechanical property of the cell wall also reflects the physiological states of bacteria. We found that the expansion ratios of microbial species depend on their specific cell wall structures, which is characteristic to species and cell physiology. By using this information, our method allows multiplex imaging to identify different bacterial species, characterize their physiological states, and measure their spatial organization in complex communities in vivo. We use this method to study the gut microbiota in two organisms: the planarian flatworm, as a model for regeneration, and mice.

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Homeostasis of Protein and mRNA Concentrations in growing cells

Jie Lin[1], Ariel Amir[1]

***Short Abstract* — Many experiments show that the concentrations of protein and mRNA fluctuate but on average are constant in growing cells, independent of the genome copy number. However, models of stochastic gene expression often assume constant gene expression rates that are proportional to the gene copy numbers, which are therefore incompatible with experiments. Here, we construct a minimal gene expression model to fill this gap. We show that (1) because the ribosomes translate all proteins, the concentrations of proteins are regulated in an exponentially growing cell volume; (2) the competition between genes for the RNA polymerases makes the gene expression rate independent of the genome number; (3) the fluctuations in ribosome level and cell density can generate a global extrinsic noise in protein concentrations; (4) correlations between mRNA and protein levels can be quantified.**

***Keywords* — Gene expression; Cell size regulation; Biophysics**

Despite the noisy nature of gene expression [1,2], various aspects of single cell dynamics, such as volume growth, are effectively deterministic. Recent single-cell measurements show that the growth of cell volumes is often exponential. These include bacteria [3], budding yeast [4] and mammalian cells [5]. Moreover, the mRNA and protein numbers are on average proportional to the cell volume throughout the cell cycle [5-7]. Therefore, the homeostasis of mRNA concentration and protein concentration is maintained in an exponentially growing cell volume. The exponential growths of mRNA and protein number indicate dynamical transcription and translation rates proportional to the cell volume, and also independent of the genome copy number. However, current gene expression models often assume a fixed cell volume with constant transcription and translation rates, which are proportional to the gene copy number. Therefore, fixed cell volume models fail to reveal how cells keep constant mRNA and protein concentrations as the cell volume grows and the genome is replicated.

The homeostasis of protein and mRNA concentrations imply that there must be a regulatory mechanism in place to prevent the accumulation of noise over time and to maintain a bounded distribution of concentrations. The goal of this work is to identify such a mechanism by developing a genome-wide coarse-grained model taking into account explicitly cell volume growth. We will consider an idealized cell in which genes are constitutively expressed for simplicity. The ubiquity of homeostasis suggests that the global machineries of gene expression, RNA polymerases (RNAPs) and ribosomes, should play a central role within the model. Indeed, the exponential growth of cell volume, protein and mRNA number originates from the auto-catalytic nature of ribosomes, the limiting factor in the translational process. The bounded distributions of concentrations are a result of the fact that ribosomes make all proteins. The independence of the mRNA concentration of the genome copy number is a natural result of the limiting nature of RNAP in the transcriptional process in which genes are competing for this global resource. Furthermore, we attempt to identify candidates for the global extrinsic noise, which sets the lower bound of noise in protein concentrations. Within our model, the only two possibilities are the fluctuations in ribosome levels and cell volume growth rate. We show that these two mechanisms lead to distinct correlation patterns between protein levels, therefore providing a method to determine the dominant contribution to the global extrinsic noise from experimental data.

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High-resolution Prediction and Refinement of Protein Structures

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***Short Abstract* — A precise understanding of cellular regulatory system hinges on an accurate prediction of protein structures. Although protein structures can be solved experimentally, *de novo* predictions by computers will be much cheaper. However, high-resolution predictions of protein structures have been challenging throughout the history. Recently, we have implemented multiple methods to achieve correct folds of proteins using AWSEM, a coarse-grained model feasible for personal computers. Based on our initial predictions, atomistic simulations were used to further refine the model prediction. Our results show a further improvement over existing methods and indicate a new avenue for the prediction of protein structures.**

***Keywords* — Structure Prediction, AWSEM, Refinement.**

A precise understanding of cellular regulatory system hinges on an accurate description of the functions of the participating proteins, which in turn, relies on an accurate prediction of protein structures. Although protein structures can be solved experimentally, an accurate *de novo* prediction of protein by computers will be much more feasible and cheaper. However, a high-resolution prediction of protein structures has been challenging throughout the history of protein folding studies [1]. Especially, to reach a high-resolution structure, expensive computational resources are needed [2, 3]. Recently, we have implemented multiple methods to achieve high-resolution structures of proteins using AWSEM (The associative memory, water mediated, structure and energy model) [4, 5, 6], a coarse-grained model that is computationally manageable for personal computers. Our initial predictions already reach sub-angstrom regime in the best cases. Based on our first-level predictions, atomistic simulations with multiple enhance-sampling techniques were used to further refine the model predictions. Our results show a further improvement over the existing methods, and indicate a new avenue for the prediction of protein structures.

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Biphasic Translation during Viral Infection

Z. Korwek[1], M. Czerkies[1], J. Jaruszewicz-Błońska[1], M. Kochańczyk[1], W. Prus[1] and T. Lipniacki[1]

***Short Abstract* — During viral infection cells have to execute transcription and translation programs to secret IFNβ and IL6 in order to inform other cells about pathogen threat. They should also degrade RNAs and inhibit translation in order to limit virus replication. By analyzing responses to RSV (respiratory syncytial virus) at the single-cell level we found that these two opposing programs are executed simultaneously within cell population.**

I. Background

THE innate immune responses are regulated by interlinked feedback loops mediated by three potent transcription factors: NF-κB, IRF3, and STAT1/STAT2. At the cell population level, cells are coordinated by IFNβ, which is secreted by infected or poly(I:C)-stimulated cells, and via paracrine signaling activate STAT1/2, triggering synthesis of antiviral proteins including RIG-I (sensor of dsRNA), PKR (kinase responsible for inhibition of protein translation) and OAS1A (responsible for mRNA degradation). In response to poly(I:C), IFNβ-primed cells rapidly produce antiviral responses and commit to apoptosis [1].

II. Results

Here, by analysis of A549 cell responses to RSV and poly(I:C) at small Multiplicities Of Infection (MOI=0.001–0.1), we found a distinct protein synthesis regulation in naïve and IFNβ-primed cells (Fig. 1).

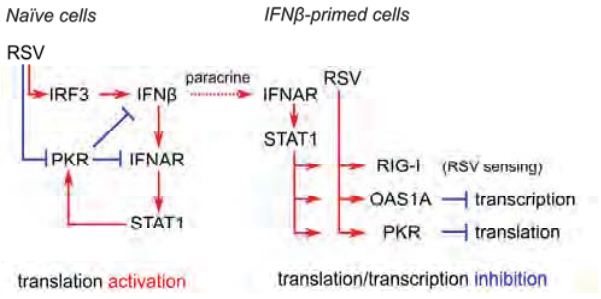

Fig. 1. Regulation of translation in naïve and IFNβ-primed cells upon RSV.

In naïve cells, RSV/poly(I:C) lead to downregulation of PKR (observed at both mRNA and protein level) and activation of IRF3 (and NF-κB), leading to massive IFNβ synthesis and secretion. By autocrine regulation IFNβ activates STAT1/2 allowing for PKR resynthesis. This in turn shuts down autocrine IFNβ signaling by depletion of IFNβ receptor (IFNAR) due to its endocytosis and inhibited translation.

In IFNβ-primed cells, STAT1/2 triggers synthesis of RIG-I, OAS1A and PKR. Upon subsequent contact with RSV, both OAS1A and PKR are activated enabling primed cells to degrade viral RNA and inhibit protein synthesis. In this way, at small MOI, almost all cells may can have their translation inhibited shortly after contact with virus. 16 h after infection at MOI=0.01, all but RSV infected cells show STAT1 activation (phosphorylation and nuclear translocation).

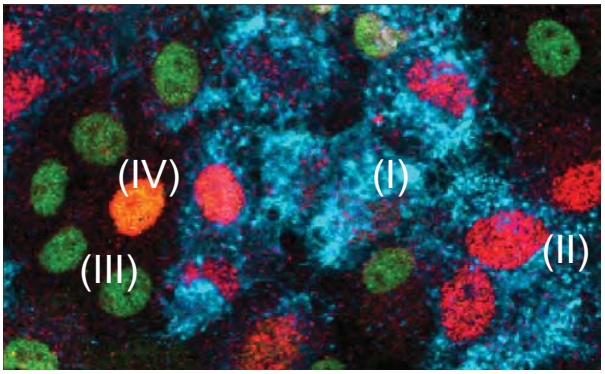

Fig. 2. Heterogeneous regulation of IRF3 (red) and STAT1 (green) 24 hours after RSV (blue) stimulation at MOI=0.1.

The discussed biphasic regulation can be observed through immunostaining. In Fig. 2, RSV-infected cells are stained blue (I). IRF3 (together with NF-κB, not stained here, whose activation is well-correlated with that of IRF3) is activated in a fraction of infected cells (II), leading to IFNβ secretion and STAT1 activation in neighboring cells (III). RSV-expressing cells show almost no STAT1 activation (due to IFNAR depletion). Some IFNβ-primed cells (IV) show IRF3 activation but no expression of viral proteins, suggesting the occurrence of RSV infection not followed by virus replication due to both its RNA degradation and inhibition of protein synthesis. Thus, differential translation regulation in naïve and IFNβ-primed cells slows down the spread of infection.

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Carbohydrate storage determines cell size and cell fate

Yanjie Liu, N Ezgi Temamogullari, Orlando Mirando, Piya Kositangool and Andreas Doncic

Cell fate determination is critical for cell survival and development. Importantly, cell fates are shaped by the integration of external cues, intracellular nutrient storage, and the overall state of the cell. Yeast cell enters into meiosis/sporulation in depletion of nitrogen and fermentable carbon. Even though larger yeast cells tend to sporulate more and that the accumulation of storage carbohydrates is necessary for meiosis [1], it remains unclear how the storage carbohydrate and size interact to shape the cell fate. Here we use biochemical methods, live cell imaging, and genetics to investigate the relationship between storage carbohydrates, cell size and cell fate.

Keywords **— cell fate, meiosis/sporulation, carbohydrates, size, single cell**

I. Introduction

In the absence of nitrogen and a fermentable carbon source, yeast cells starts accumulate carbohydrates and will eventually stop cycling and either become quiescent or enter meiosis/sporulation [2]. Two major factors have independently been implicated in this process: cell size and storage carbohydrates. Specifically, cells need to reach a certain size and accumulate trehalose and glycogen to sporulate.

II. Results

To find the link between carbohydrates, cell size and cell fate, we created a library of trehalose and glycogen deficient mutants and determined their spore frequency, number of spores per ascus, spore viability, amounts and concentrations of trehalose and glycogen by using biochemical, genetic, and live-cell imaging approaches. Specifically, we found that:

A. Cell Size scales with amount of carbohydrates:

We performed size fractionation of cells by using sucrose gradient and measured the amount of trehalose and glycogen in cell fractions. Doing this we found that cell size scales with the amount of carbohydrates, while the concentration of storage carbohydrates remains approximately constant. This result, together with the fact that cell size and nutrient stores can be decoupled in the context of the Meiosis/Quiescence decision [manuscript submitted] suggesting that size is passive readout of storage carbohydrates in the context of this cell fate decision.

B. Glycogen and trehalose play differential role for sporulation:

The following attributes were measured for all strains in our library: the ability to generate spores, the number of spores, the spore viability and the ability store trehalose and glycogen. Doing so, we found that glycogen and trehalose compensate to each other for sporulation. Furthermore, glycogen and trehalose mutants showed differential defects with respect to their ability to store carbohydrates and to initiate sporulation. Specifically, cells that could not produce trehalose had a higher sporulation frequency than cells that could not produce glycogen. On the other hand, glycogen mutants had larger defects with respect to the number of spores per ascus, i.e. a higher percentage of dyads and triads instead of tetrads. These results suggest that glycogen is more important for spore formation while trehalose may play a larger role ensuring spore viability.

III. Conclusion & future plans

We conclude that during sporulation cell size is likely a readout of storage carbohydrate pools which in turns are necessary to reach a certain threshold for sporulation.

We are now quantifying the cell size of carbohydrate mutants. We will confirm these results using a live cell trehalose sensor [3], in combination with markers for trehalose and glycogen metabolisms.

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Automated Multimodal Screening of Fluorescent Biosensors of Membrane Potential

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***Short Abstract* — A quantitative understanding of neuronal computations can be achieved by monitoring membrane potential reported by genetically-encoded voltage indicators (GEVIs). The fluorescent biosensors enable recordings of cellular electrical activity *in vivo* with subcellular resolution and cell type specificity. However, current indicators are not photostable and bright enough for long-term recording, and their sensitivity and kinetics are not satisfactory for detection of fast voltage dynamics. We report a high-throughput platform to screen mutagenesis libraries of GEVIs by analyzing microscopy videos of HEK293 cells during electric field stimulation. The platform quantitatively ranks candidate GEVIs based on performance scores across multimodal imaging methods. We anticipate that the approach can be extended to the screening of other fluorescent biosensors.**

***Keywords* — Genetically-encoded voltage indicator (GEVI), High-throughput screening.**

I. Purpose

GENETICALLY encoded voltage indicator (GEVI) provides a new scheme of probing neuron circuits by reporting membrane potential changes with fluorescence. Compared to electrophysiological methods, it enables readout of neuronal electrical activity with subcellular resolution. Its ability of achieving cell-type specificity facilitates targeting and differentiating neurons *in vivo*. However, the first generation of GEVIs still suffer from low brightness and/or photostability [1], which prevent their wider applications for prolonged recordings. Moreover, the sensitivity and kinetics of GEVIs still have ample room of improvement to match that of the traditional electrode-based recording [2].

We have previously developed a voltage indicator, ASAP1, by coupling a green fluorescent protein (GFP) to the voltage sensitive domain (VSD) of a voltage sensitive phosphatase [3]. Further improvements of the sensor were achieved by rationally introducing mutations or deletions to the sensitive domains of the protein [4, 5]. This approach is considered low-throughput because each variant must be tested by labor-intensive electrophysiological methods, so we seek faster ways of discovering new GEVIs at a rate of at least thousand constructs per day.

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II. Result

We report here the development of a high-throughput platform to screen mutagenesis libraries in a 96-well format. Our platform automatically acquires and analyzes multi-channel high speed microscopy videos of GEVI-expressing cells during electric field stimulation, and ranks variants quantitatively based on multiple performance parameters, such as sensitivity, kinetics, photostability, for both one-photon and two-photon excitations. We create and adapt HEK293 cell lines to better simulate the electrophysiological properties of neurons, while avoiding the excessive costs and labor from culturing neuron. The selected new GEVI candidates are further verified by gold standard voltage clamping.

III. Conclusion

We anticipate that this new directed evolution screening platform will accelerate development of indicators optimized for accurate and sensitive detection of voltage dynamics *in vivo*, laying the foundation of optical investigation of population of cells. The resulting GEVIs and fluorescent biosensors in general should be of broad utility for elucidating complex interactions among neurons that underlie behavior.

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Stability and accuracy analysis of the circadian clock coupled with metabolism

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***Short Abstract* — Circadian rhythms are near 24-hour oscillations, these oscillations are generated by molecular circadian clocks formed by biochemical networks with negative feedback loops. However, although the coupling has been studied, little attention has been placed on the precision of the circadian clock coupled with metabolism. In this work, the accuracy of the circadian clock coupled with metabolism in hepatocyte is studied. We developed a minimal nonlinear model. The local stability and limit cycles are studied. Our predictions suggest that the metabolism has an essential role in the performance of the daily rhythms.**

***Keywords* — Nonlinear circadian model, Systems biology, Dynamics analysis, Robustness.**

I. Introduction

Physiological rhythms are of vital importance to life. These rhythms arise from nonlinear biological mechanisms interacting with a fluctuating environment. Mathematical analysis of physiological rhythms shows that nonlinear equations are necessary to describe it [1]. They are controlled by many feedback loops that enable life. Abnormal rhythms are associated with the disease. Many of these rhythms follow day and night cycle produced by the rotation of the earth. They are called circadian rhythms. It is well known that these rhythms are controlled by a molecular clock. It is called the circadian clock, and in mammals it is in every tissue with a master clock in a region of the hypothalamus called the suprachiasmatic nucleus (SCN). The neurons in the SCN are synchronized, and experimentally it has been found that, if they are dissociated, each cell can present autonomous oscillations at slightly different periods, from 20 to 28 h [2]. The architecture of the clock network is well known, consist of transcription-translation feedback loops that oscillate the concentration of proteins every 24 hours and control a wide variety of physiological events, including metabolism. Energetic cycles are one type of physiological process that shows transcription-dependent circadian periodicity. The liver plays a central role in maintaining energy homeostasis. Accordingly, the feeding and fasting cycle is a potent zeitgeber for the liver clock than systemic cues controlled by the circadian clock in the SCN [3]. Circadian clocks have been studied with a mathematical approach. There exists a substantial body of literature regarding the molecular mechanisms of the circadian clock from mathematical point of view. Recently, it has been reported a mathematical model that links circadian rhythm with liver metabolism [4]. However, little attention has been paid to accuracy. We use mathematical modeling to address the following question: whether the accuracy of the liver clock cells coupled with metabolism is sensitive to parametric variation.

II. Results

We considered a reduced model of six variables and two coupled oscillators. Using stability analysis, we found a stable equilibrium point from the linearization of the proposed model. Then we performed simulations of the coupled model with a forced function that represents feeding-fasting periods under the effect of parametric variation. Each parameter value was scaled by multiplying by a factor of 0.5, 2, 10, and 50, one factor at a time, while the rest of the parameters was kept without change. Then, the period was measured and the accuracy evaluated based on a criterion of ±10% around 24-hour period following [5][2]. The model was strongly sensitive to variations in parameters associated to circadian but mostly with metabolic molecules.

III. Conclusion

According to [6] organisms are subject to a series of entraining agents that promote the synchronization and resonance of different clocks in peripheral tissues. We can conclude from these facts that perturbations of the metabolic cycles affect the accuracy of the circadian clock.

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Structure Guided Genetically Encoded Voltage Indicator Engineering

Xiaoyu Lu[1], Harvey Yang[2], Yueyang Gou[3], Zhuohe Liu[4], Sihui Guan[3], Jihwan Lee[1] and François St-Pierre [3,4]

***Short Abstract* — Genetically encoded voltage indicators (GEVIs) are protein sensors that transform cell membrane voltage signals into fluorescence changes. They are promising tools for simultaneously recording from large populations of neurons with cell type specificity. However, currently- available GEVIs have insufficient brightness, photostability, response amplitude and kinetics. To overcome these limitations, we used structural approaches to identify the important residues for voltages sensing, mutated these positions, and screened for variants with improved properties. We also used these screening results in a machine learning approach to refine our strategy for further improvements.**

***Keywords* — Structure guided screening, machine learning, Genetically encoded voltage indicators**

I. Background

MONITORING neuronal collaborations in circuits in vivo is a central goal in neuroscience but remains challenging. A critical technology gap is the lack of tools that can quantitatively monitor neuronal electrical (voltage) dynamics with single-cell or even subcellular resolution from a large and genetically-defined populations of neurons. Genetically encoded voltage indicators (GEVIs) are a promising solution to achieving this goal, yet current versions exhibit insufficient in kinetics, response amplitude, brightness and photostability for detecting fast voltage transients in vivo [1].

Accelerated Sensor of Action Potentials 1 (ASAP1) [2] is a GFP-based GEVIs with a circularly permuted GFP (cpGFP) inserted in the voltage sensitive domain. ASAP1 is a suitable starting templates for further GEVI improvements because they have fast (millisecond-timescale) kinetics and compatibility with two photon imaging methods (ASAP2s) [3]. However, its sensitivity (response amplitude) to voltage transients remains small, motivating further optimization.

Since it is impossible to screen the entire sequence space, we turned to semi-rational screening methods, supported by the accumulation of sequence and structural data, and improvements in machine learning algorithms. For example, 3D structural prediction using Rosetta [4] and existing physical model on voltage-sensing domain (VSD) movements during membrane depolarization [5] suggest promising mutation sites. By targeting specific residues, we can focus our screening efforts on smaller and more productive screening libraries. Performing machine learning on screening results will reveal the relative importance of each position in ASAP and guided the rational design of new variants [6].

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II. Results

A. Structure-guided GEVI screening at single positions

We used 3D structure alignment to locate amino acids predicted to be important in physical models of orthologous voltage-sensing domain. Mutating these residues in voltage indicator ASAP1 led to mutants with larger sensitivity.

B. Structure -guided optimization of interacting residues

Using data from single-position screening, we figured out the positive and negative mutations at each important site. We did multi-position screening to combine the most promising variants and determined whether mutations produced additive, subtractive, or synergistic effects on indicator performance. We applied machine learning algorithms to quantify the relative importance of each variant on individual performance metrics: kinetics, response amplitude, and brightness.

III. Conclusion

Using semi-rational protein engineering and high-throughput screening pipeline, we developed genetically encoded voltage indicators with improved properties, which better meet the need for large-population voltage dynamics quantification in neuroscience.

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Stress-Induced Division of Labor Underlies Bacterial Colony Branching

Nan Luo[1] and Lingchong You[1]

***Short Abstract* — Branching patterns of *Pseudomonas* colonies have been shown to depend on the secretion of biosurfactant. Here we show that starvation induces the differentiation of a *Pseudomonas* population to slow-growing cells that produce biosurfactant as a public good and fast-growing non-producers. Mathematical modeling indicates that this division of labor strategy is critical for explaining the diverse morphologies of branching patterns observed. We propose that the division of labor of branching bacterial colonies optimizes nutrient utilization and maximizes the population fitness by balancing between cell growth and motility. This collective mechanism for colony branching implies the role of population heterogeneity in pattern formation.**

***Keywords* — Patterning, division of labor.**

I. Introduction

THE branching pattern is one of the most prevalent patterns found in nature [1]. How branching patterns emerge in living organisms has been a long standing question. The study of branching morphogenesis in bacterial colonies serves as a step stone to the understanding of branching pattern formation in multicellular organisms and also allows us to rationally design and control branching patterns using synthetic biology.

A number of mechanistic models for branching pattern formation in bacterial colonies have been proposed [2-5], but they are either not tested by experiments or not able to account for the diverse patterns under different experimental conditions. Based on mathematical modeling and experimental observations, here we show that under stress, *Pseudomonas aeruginosa* cells employ a division-of-labor strategy, which is necessary for explaining the various complex branching patterns observed in experiments.

II. Results

The formation of branching patterns of *Pseudomonas* colonies has been shown to depend on the secretion of biosurfactants (such as rhamnolipids), which facilitates local cell swarming [6]. The expression of the rhamnolipid synthesis operon *rhlAB* is regulated by quorum sensing signaling and nutrient availability [7]. Flow cytometry of *Pseudomonas aeruginosa* PA14 cells expressing the *rhlAB* reporter showed that when nutrient was abundant, surfactant synthesis was turned off. Under starvation, however, a subpopulation of Pseudomonas cells with high biosurfactant expression quickly emerged. The metabolic burden of biosurfactant synthesis suggests that the population differentiates into cells producing biosurfactant as a public good at the expense of cell growth (Producers) and non-producers with higher proliferation rate (Growers).

Based on this observation, we developed a diffusion-reaction model that incorporates nutrient consumption, surfactant production, and the spatial-temporal dynamics of both subpopulations, while the transition rates between the two cell states are modulated by environmental conditions.

Simulations reveal that the division-of-labor scheme is essential for reproducing the wide range of branching patterns of *Pseudomonas* colonies under different conditions, as well as the unexpected observation of colony expansion against a nutrient gradient. Future work is needed to determine the evolutionary significance of this strategy and we propose that division-of-labor underlies the efficient adaptation of bacterial colony to various environments and ensures optimal resource exploitation which is manifested as colony branching.

III. Significance

Division of labor and cell differentiation within communities of unicellular organisms were proposed to be the prelude to the emergence of multicellularity [8]. The population-level regulation during the morphogenesis of colony branching patterns may shed light on the design principles of pattern formation and provides insights to the control of heterogeneous cell communities such as biofilms and tumors.

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BLANT: Sampling Graphlets in a Flash

Wayne Hayes[1], Sridevi Maharaj[2]

***Abstract* — BLAST is an invaluable tool to compare and align sequences. Its efficiency comes from *k*-mers—short *k*-length subsequences—that are indexed across a larger corpus, which allows identical *k*-mers from different regions to aid finding longer matches. Analogously, networks could be indexed by *k*-node graphlets. Unfortunately, existing graphlet counting methods enumerate *all* the graphlets, which is infeasible on large networks. We introduce BLANT (*Basic Local Alignment Network Tool*) which randomly samples and indexes graphlets. BLANT samples *millions* of graphlets in seconds, which aids search and local alignment via indexing, and provides a statistical sample of both global graphlet distribution and local orbit degree vectors.**

***Keywords* — biological network alignment, local network alignment, network database, network classification, network search, network function, network topology.**

I. PURPOSE

Networks are used to represent biological interactions such as protein-protein, gene-uRNA, brain connectomes, and enzymes; their topology (the structure of connectivity between nodes) is related to function [1]. Matching local structures also helps identify similar functional modules in other larger networks. In order to find these modules and understand the details of their structural components, several graph topological features have been studied but none appear to give as robust results as graphlets [2]. Graphlets have been used to quantify the local structure of biological networks via global alignments, alignment-free comparison, analysis of brain connectomes, and in recovering functional and phylogenetic information [3].

Existing graphlet counting methods [4] perform exhaustive enumeration of graphlets and are infeasible on large networks. We propose that statistical sampling [5] can produce a satisfactory approximation. Here, we introduce BLANT, which samples and indexes millions of graphlets in seconds. We show that the sampled distribution agrees with the true graphlet distribution.

II. METHOD – NODE BASED EXPANSION

A *k*-graphlet is an induced, connected subgraph of *k* nodes taken from a graph *G(V, E)*. We construct a sampled *k*-graphlet *g* as follows. Initially, we select an edge (u_1, u_2) uniformly at random from *G* and add u_1 and u_2 to *S*, the set of nodes that will become *g*. We iteratively add nodes to *S* by picking from all nodes outside *S* adjacent to a node inside *S*, until we have *k* nodes.

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III. EXPERIMENTAL RESULTS

A. We sampled 10,000 *k*-graphlets (taking a fraction of a second) for *k* = {3,4,5} from a total of 1540 synthetic networks of Geometric, Erdös-Rényi, Scale Free, Small World and Sticky graphs of varying sizes (1000, 2000, 4000, 6000 nodes) and densities (0.005, 0.0075, 0.01). We computed their full graphlet counts using ORCA [4], which took weeks of CPU time. Figure 1 shows that the mean relative graphlet frequency from our method agrees well with the true mean relative graphlet frequency both in accuracy and intrinsic variation.

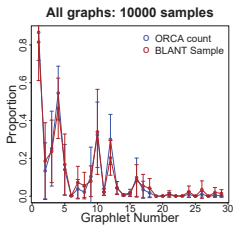

Figure 1

B. We also sampled 10^7 7-graphlets from Enzyme, Brain-ADHD, Gene-µRNA, and Facebook networks. Multi-dimensional scaling on pairwise graphlet correlation distances obtained from our graphlet sample shows that sampling clearly distinguishes between different network types (Figure 2) as well as exhaustive enumeration [6].

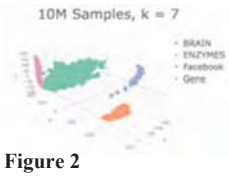

Figure 2

IV. CONCLUSION

Random graphlet sampling is orders of magnitude faster than existing exhaustive enumeration methods and produces distributions of graphlets that are close to the true distribution. This promises to revolutionize network analysis by allowing graphlet analyses on networks of arbitrary size.

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Causes and consequences of asynchrony in *D. discoideum* multicellular development

Ricardo Martinez-Garcia[1], Fernando W. Rossine[1], Allyson E. Sgro[2,3], Thomas Gregor[2], Corina E. Tarnita[1]

***Short Abstract* — The life cycle of the social amoeba *Dictyostelium discoideum* includes a starvation-induced transition to aggregative multicellularity. During aggregation, however, amoebae do not coordinate perfectly and some of them remain solitary (loners). Combining experiments and modeling, we show that the loner-aggregator partitioning behavior is characteristic of each genetic variant and could be, potentially, shaped by natural selection. Finally, we discuss how interactions between partitioning behaviors could affect *D. discoideum* diversity when two genetic variants co-occur and develop in mixes as compared to segregated development.**

***Keywords* — Multicellular development, cell aggregation, quorum sensing, synchronization**

I. Purpose

In the social amoebae *Dictyostelium discoideum,* starvation triggers the partitioning of a population of free-living cells into aggregators, which ultimately develop a multicellular fruiting body made of dead stalk cells and reproductive spores, and non-aggregators, which remain as vegetative cells. While the emergence and functioning of the multicellular phase has been extensively investigated [1-3], it has been only recently that the asocial component, represented by non-aggregating (loner) cells, has captured some attention [4-6]. Loners are less resistant to starvation than spores, but they persist for some time and will eventually recover the multicellular stage in a subsequent starvation event. In addition, according to recent theoretical work, loners could provide selective benefits in stochastic environments and, when coupled with environmental heterogeneity (seasonality or spore dispersal across different patches) could contribute toward the rich diversity of *D. discoideum* observed in nature [4,7].

However, despite this potential importance of loners, the question of whether they are an unavoidable consequence of large-scale synchronization events or a heritable component of *D. discoideum* life-history remains unanswered. In this presentation, I will first show, using experimental results, that aggregation, and therefore the population partitioning between aggregating and non-aggregating cells, is a heritable population-partitioning process, with different genetic variants differing in their partitioning behavior. To explain these experimental results, I will introduce an individual-based model for the aggregation process, showing that the loner-aggregator partitioning could be the result of the population responding in an imperfectly synchronized manner during the developmental process. Finally, I will move the discussion to scenarios in which two genetic variants co-occur and mix, which results in interactions between their developmental programs that could profoundly impact the diversity of the species.

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Quantifying epistatic conservation across genetic and environmental backgrounds

Andrew D. Mathis[1], Judith Boldt[1,3], and Kimberly A. Reynolds[1,2]

***Short Abstract* — Epistasis is a measure of how the effect of one gene is influenced by other genes. Quantifying epistasis will improve genotype-to-phenotype predictions, but our ability to generalize these results beyond model systems is dependent on (1) how much epistasis is conserved and (2) the sufficiency of conserved epistasis to improve phenotype prediction. To determine this, we are measuring how epistasis varies across *E. coli* strains and nutrient conditions. Additionally, we infer conserved epistasis statistically by analyzing gene co-evolution across bacterial species. Combining these data may point to a general strategy for predicting conserved epistasis and estimating phenotype in non-model organisms.**

***Keywords* — Epistasis, *E. coli*, evolutionary statistics, genetic background, environmental background, genotype-to-phenotype.**

I. Environment, genetic background, and epistasis

Epistatic interactions between genes are the basis for the complex traits, and a careful mapping of these genetic interactions is an important step towards making better genotype-to-phenotype predictions. Prior work has focused on mapping epistasis computationally via flux-balance analysis or experimentally using high throughput cell growth assays [1-3]. However, it is unclear how epistasis measurements made *in silico* or within a given model organism will generalize across different genetic and environmental backgrounds. Additionally, how well can conserved epistatic interactions be predicted? And is knowledge of only conserved epistatic interactions sufficient to improve genotype-to-phenotype predictions? We are developing complementary experimental and computational strategies to address these questions.

II. Experimental measurements of variation in epistasis

We selected 22 enzymes from bacterial folate and purine metabolism as a model system for measuring epistatic conservation. Folate and purine metabolism are conserved pathways, which allows for more powerful statistical co-evolution analyses, and perturbations in these pathways result in experimentally measureable growth phenotypes. To measure epistasis, we use CRISPR interference (CRISPRi) to knockdown gene expression [4]. CRISPRi specificity is directed using a guide RNA (gRNA) library that targets our 22 selected enzymes individually and in all possible combinations. We then grow this library in a continuous culture device (turbidostat), regularly sample the bacterial population, and count allele frequencies using next generation sequencing. Our method can resolve a 2% difference in relative doubling time per hour, is applicable in a variety of *E. coli* strains (and other organisms), simplifies targeting essential genes, and easily scales for studies in different environments. Additionally, we have developed a strategy for post hoc removal of "escapers", which occur when an adaptive mutation renders CRISPRi ineffective. Using this method, we are currently measuring epistasis in seven strains of non-pathogenic *E. coli* and in multiple environmental conditions (variations in media, culture density) [5].

III. A statistical model of conserved epistasis

An alternative approach is to estimate epistatic interactions using quantitative statistical models of co-evolution [6]. The basic premise is that epistatic constraints between genes will drive their co-evolution, leading to detectable statistical correlations like conserved proximity on the chromosome (synteny), joint presence and absence, or amino acid sequence covariation. Because we search for these correlations in large and diverse genomic databases, the interactions we find likely represent conserved epistasis, rather than background specific idiosyncrasies. We have conducted statistical co-evolution analyses across folate and purine metabolism using a database of ~2000 bacterial species, and hypothesize that these data will be more like the conserved epistatic interactions we find experimentally than those sampled from any single organism.

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Andreas Mayer[1], Yaojun Zhang[2], and Ned S. Wingreen[1,3]

Regulation of T cell expansion by the dynamics of antigen presentation

***Short Abstract* — During an immune reaction, specific lymphocytes proliferate multiple times to form a large pool of effector cells. Adoptive cell transfer experiments have recently demonstrated that the magnitude of T cell expansion is related to the initial number of cells via an inverse power law [1]. Here, we show that such a relationship arises naturally from models in which T cell expansion is limited by decaying antigen availability, and we relate the power law exponent to the proliferation rate of lymphocytes and the rate of antigen decay. Our model identifies the dynamics of presented antigen as a key regulator of the size of an immune response.**

I. Background

THE rapid expansion of specific immune cells forms the basis of the effectiveness of the adaptive immune response despite the low initial number of cells specific to any particular antigen. The proliferation of cells in response to antigenic stimulation is one of the key dynamic processes shaping the immune repertoire, whose composition determines how well an organism is protected against various pathogens. Understanding how this expansion is regulated is thus an important question, with applications, e.g., in the design of vaccines. Recently, adoptive cell transfer experiments, in which transgenically labeled T cells are transferred between mice, have provided new quantitative data to study the regulation of T cell expansion in controlled conditions [1-3]. Quiel et al. have found that fold expansion upon stimulation with cognate antigen depends on the number of transferred T cells as a power law with exponent ~ -1/2 over four decades [1]. In this work we present a physically motivated model to explain this striking observation.

II. Results and Discussion

In our model an exponentially growing number of T cells compete for an exponentially decaying number of antigens. This model incorporates two experimental observations: First, the continued proliferation of T cells is dependent on sufficient signaling from presented antigens [4]. Second, there is antigen turnover, so presented antigen levels decay over time [5]. The model closely fits experimental data for the dependence of fold expansion on initial cell numbers (Fig.1A) and for the time course of expansion (Fig.1B).

In the model, proliferation proceeds until the number of T cells reaches some relative level with respect to the number of presented antigens. From this we derive the key result that fold expansion is proportional to some power of the ratio of the initial number of presented antigens and the initial number of T cells. Using experimental data on antigen turnover rate and T cell proliferation rate, our theory predicts an exponent consistent with the observed exponent.

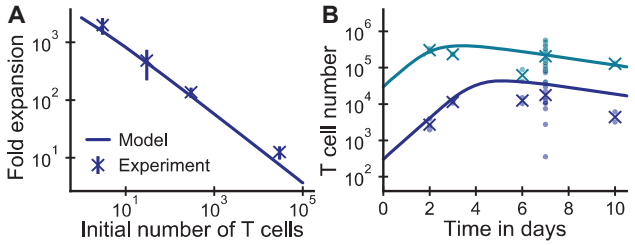

Figure 1: Comparison of experimental data from [1] with model predictions. (A) Factor of expansion at day 7 as a function of the initial number of T cells. (B) T cell number versus time for 300 and 30,000 initial T cells.

Using the same model we quantitatively explain the dependence of T cell expansion on antigen affinity [2] and the influence of antigen dosing kinetics on expansion [3] observed in other adoptive transfer studies.

III. Conclusion

Our model provides a simple explanation for the experimentally observed dependence of T cell expansion on initial cell numbers, supplementing those provided elsewhere [6, 7]. Importantly, the new model dispenses with the need for fine-tuned parameters to explain the power law relationship. Our results suggest that T cell expansion might be regulated by the dynamical turnover of presented antigens across a range of conditions.

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Determining the Internal Allosteric Architecture of DHFR With Saturation Mutagenesis

James W. McCormick[1], Samuel Thompson[2], and Kimberly A. Reynolds[1]

Allosteric regulation involves the transfer of information between distant regions of a protein. However, the thermodynamic coupling pattern between amino acids that enables this transmission remains unclear. Statistical analyses of amino acid co-evolution suggest one hypothesis: that allostery is mediated by sparse, cooperative networks of amino acids embedded in the protein structure. To test this idea, I am using deep mutational scanning to comprehensively map the positions contributing to allostery in a synthetic allosteric switch: the metabolic enzyme Dihydrofolate Reductase (DHFR), which is regulated by the light-sensing domain LOV2.

***Keywords* — Dihydrofolate Reductase, DHFR, Allostery, LOV2, Saturation Mutagenesis, Deep Mutational Scanning**

LONG range interactions between amino acids are important for allosteric regulation, information transmission, and catalysis. One approach for inferring the underlying pattern of interactions between amino acids is Statistical Coupling Analysis (SCA). The premise behind SCA is that functional interactions between amino acids link the evolution of those residues, and this co-evolution can be detected in multiple sequence alignments that are representative of a protein family's long-term evolutionary record. In prior research, SCA was used to identify a co-evolving network of residues inside DHFR (the sector), and it was shown that sector-connected suface sites are hotspots for introducing allosteric regulation [1]. However, further work is needed to test if sector positions do indeed form the structural basis for allosteric information transfer within a protein.

One method for testing these proposed allosteric pathways is through double-mutant cycles. In this experiment, a wild-type protein, two single mutants, and a protein containing both mutants are compared. If the change in free energy associated with the double mutant protein differs from the sum of changes in the single mutants, then the two mutations are thermodynamically coupled [2]. However, the difficulty of examining all residue combinations in a protein increases exponentially with the number of residues. One study on a small, 9-residue section of a protein involved 65,840 double-mutant cycles [3]. To overcome this combinatorial complexity, we have designed a new strategy to measure the effect of all single mutations in DHFR within the context of a light-induced allosteric perturbation.

Specifically, we generated saturation mutagenesis libraries for the DHFR enzyme in the context of two DHFR/LOV2 fusions: one in which LOV2 is connected at a sector position and provides ~2-fold regulation of DHFR activity by light, and one in which LOV2 is not sector-connected and does not regulate DHFR activity. Library competition in light/dark environments allows the detection of mutant fitness (and contribution to allostery) through the application of next-generation sequencing, where the relative frequency of each mutation is coupled to its fitness.

The results of these experiments in progress will provide a complete map of which positions inside DHFR contribute to allostery in the DHFR/LOV2 switch. Furthermore, it will provide insight into how allostery evolves: is there a path of single mutant variation that can lead to optimization of regulation in the sector-connected and/or non-sector-connected fusions? Finally, these data will provide a rich test set for evaluating computational methods to model allosteric pathways inside the protein.

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Modeling Adipose Tissue Hormone Regulation

Joseph P. McKenna[1], Xiangpeng Li[2], Christopher Easley[3], Vipul Periwal[1]

Short abstract — **Motivated by global obesity and type 2 diabetes epidemics, researchers have realized the integral role of adipocytes in energy balance. In addition to their role as lipid stores, adipocytes participate in shaping the milieu of circulating hormones in the blood. Knowledge of adipocyte biology is therefore crucial to understanding the pathophysiology of metabolic diseases. In this poster, we present a spatial model of the dynamics of fatty acid uptake by adipose tissue informed by data collected from mouse explants using a microfluidics platform combined with a recently developed fluorescent reporter of fatty acids. We demonstrate that fatty acid uptake is hormone-dependent on a short timescale. We also address whether diffusion of fatty acids through adipose tissue is mediated through gap junctions by comparing the model diffusion pattern to the data.**

Keywords — **adipocyte, hormone, insulin, glucose, microfluidics, microscopy, fluorescence, finite element method.**

I. BACKGROUND

As calorie stores, adipocytes are well suited to regulate energy balance. They play this crucial role through a number of mechanisms such as secretion of adipose-derived molecules (adipokines), initiation of neural signals via the peripheral nervous system, and breakdown of fat (lipolysis) and release of fatty acids for direct use as an energy source [1].

Although adipocyte mass represents excess energy intake relative to energy expenditure, the rate of adipogenesis is not merely proportional to ingested calories. Indeed, there are hormonal effects that complicate a strict interpretation of energy balance by the First Law of Thermodynamics. For example, insulin, which is secreted by pancreatic β-cells, is required for glucose uptake by adipose tissue and has the effect of repressing lipolysis [3]. To determine the extent to which hormones affect fatty acid uptake and release, Easley et al developed a fluorescent sensor of intracellular free fatty acid (FFA) that is used in combination with a microfluidics platform [2].

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To quantify the rate of fatty acid uptake, they pulsed square waves of fluorescent FFA to mouse adipose explants with various concentrations of insulin and glucose in the bath. Here, we show that the rate of fatty acid uptake can change rapidly with an increase in insulin. We also address whether diffusion of fatty acids through adipose tissue is mediated through gap junctions.

II. MODEL

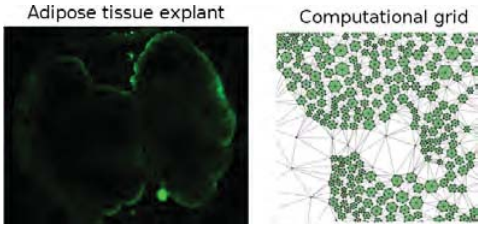
We model the concentration $u(x,t)$ of fatty acid in the microfluidic chamber containing adipocytes by

$$\begin{cases} u_t + du_{xx} = gWu & B(0,R)\times(0,T] \\ u = f(t,x) & \partial B(0,R)\times(0,T] \\ u_x\cdot\vec{n} = [u(c_i)-u(\partial C_i)]/r_i & \partial C_i\times(0,T]\ \forall i \end{cases}$$

where variables have the following meanings

- $C=\cup C_i$: collection of adipocyte cells,
- $d>0$: fatty acid diffusion coefficient,
- $g\geq 0$: gap junction fatty acid diffusion coefficient,
- W: cell coupling matrix.

To create a computational grid that mimics the geometry of the adipose explant, we segment microscopy images and triangulate the result.



III. CONCLUSION

We constructed a model of adipose tissue informed by experiments with state-of-the-art microscopy and fluorescence techniques to characterize the rate of fatty acid uptake in various metabolic conditions. This understanding should be useful for developing interventions to mitigate a global obesity epidemic.

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Multiplexed Live-Cell Signaling Dynamics of the Cytoskeletal and Phospholipid Scaffold, IQGAP1

R. Tyler McLaughlin[1,2], Nicholaus J. Trenton[2], Satya K. Bellamkonda[2], David S. Tsao[2], and Michael R. Diehl[1,2,3]

***Short Abstract* —Regulation by the scaffold protein IQGAP1 underlies the coordination of the actin cytoskeleton and phospholipid membranes. Live-cell dynamics studies of endosomal compartments revealed a temporal sequence of scaffold dissociation and actin bursting events. Domain-level mutations and statistical modeling have lead to an understanding of the multifaceted tethering modes of IQGAP1, including mutually opposing forces mediated by two distal protein domains.**

***Keywords* — Scaffold proteins, live-cell protein dynamics, endosomes, IQGAP1, actin, phosphoinositides, fluctuation analysis, statistical modeling.**

I. Purpose

UNDERSTANDING the adaptive material properties of the eukaryotic cell and its spatiotemporal regulation by complex networks of signaling macromolecules remains a major unsolved scientific problem. Full characterization of adaptive subcellular systems requires new quantitative descriptions and new experimental frameworks.

The actin cytoskeleton is an adaptive, mechanical network known for its ability to change cell morphology. The deformation of the phospholipid membrane via actin polymerization is crucial in cellular processes like phagocytosis, cell migration, and immune synapse formation. In each of these contexts, actin is regulated by multiple protein species of the Rho GTPase family, whose local, collective spatial assembly is dependent on organelle-specific phospholipid signatures such as the abundant phosphatidylinositol (4,5)-bisphosphate (PIP_2).

Also critical to the regulation of actin-membrane structures are scaffold proteins, which function as biomolecular 'circuit boards' and template the flow of intracellular information [2]. The best studied cytoskeletal-regulating scaffold protein, IQGAP1, binds Rho GTPases, PIP_2, and filamentous actin through multiple protein domains, while also facilitating Raf-MEK-ERK and PI3K-Akt-mTOR signaling pathways which are major signaling axes for growth and proliferation [3].

II. Results

We use a combined live-cell imaging and statistical modeling approach to characterize the micron-scale, endosomal recruitment dynamics of the cytoskeletal and phospholipid scaffold protein, IQGAP1.

A. Actin and IQGAP1 correlations at endosomes

Through super-resolution and time lapse epifluorescent microscopy, we have characterized the scaffold-regulated maturation process of endosomes at the basal actin cortex of human mammary epithelial cells. Here, the dissociation of IQGAP1 is always followed by excitable bursts of actin polymerization. By analyzing multi-protein trajectories from time lapse movies, we found that at the 60-minute time scale, actin and IQGAP1 are positively correlated, whereas on 60 second timescales this pair is anti-correlated. This suggests the scaffold plays activating and inhibitory roles in actin polymerization.

B. Domain-level contributions to dynamics

Via a series of protein domain- and residue-level mutations, we identify the regions of IQGAP1 responsible for the anti-correlations and endosomal tethering [5].

C. Statistical modeling of protein fluctuations

We construct multiple linear regression models of the pairwise-correlative structure of actin, membrane, and scaffold fluctuations. By model selection we conclude that using wild type and mutant scaffolds in conjunction yields the best prediction of actin fluctuations, whereas a scaffold mutant alone sufficiently predicts membrane fluctuations [5].

III. Conclusion

Our combination of live-cell microscopy and statistical modeling yielded vital insights into the adversarial dynamics of a scaffold-regulated, membrane and cytoskeletal system.

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Predicting Actin Interfaces from Genomic Data

Kareem M. Mehrabiani[1,2], Ryan R. Cheng[2], Jose N. Onuchic[1,2]

***Short Abstract* — Actin is one of the most abundant proteins found in eukaryotic cells. It plays a crucial role in cell motility, division, and forms the cytoskeleton. The actin filament is a helical polymer and its crystal structure has yet to be solved. Low resolution atomic-models of the filament, constructed using crystallographic data and known G-actin crystal structure, provide useful, but limited, clues as to the interactions involved in filament formation and regulation. Current statistical modeling tools can predict residue pair coevolution from genomic information. Here, we utilize both structural and genomic data to elucidate the functional interactions of actin.**

I. Purpose

Actin is a highly conserved, abundant protein found in every eukaryotic cell where it plays a critical role in cell motility, flexibility, division, and forms the core of the cytoskeleton. It is found in two forms: globular (G) actin, which is unbound and undergoes random diffusion in the cytoplasm, and filamentous (F) actin, which forms when G-actin polymerizes into a long filament. The filament is dynamically regulated by actin-binding proteins (ABPs), which induce viscoelastic changes to the cell. Filament regulation is poorly understood due to a restricted knowledge about its formation, which requires high-resolution structural information. The structure of G-actin was experimentally determined using x-ray crystallography [1]. Conversely, the crystallization of F-actin has remained a significant challenge because actin dimers and trimers are kinetically unstable and rapidly polymerize into filaments [2]. Previous groups have used experimental methods such as x-ray fiber diffraction [3, 4] and cryo-electron microscopy [5] to propose various atomic models of F-actin. While these models have shown that the transition from G- to F-actin is accompanied by a significant structural flattening of G-actin, they are of limited resolution and lack the details needed regarding the functionally relevant DNase I binding loop (D-loop). The D-loop has been implicated in playing a crucial role in the polymerization of actin as well as ensuring filament stability [6]. By determining a high-resolution structure of F-actin and its functional interfaces with ABPs, a great wealth of information regarding how actin functions in cells can be gleaned.

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Does diversity of T cell receptors functionality depend on age and sex?

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***Short Abstract* — T lymphocytes play an essential role in defence of an organism against pathogens and cancers through their clonally distributed T cell receptors (TCR). TCR gene sequences, which are randomly assembled during T lymphocyte ontogeny, can be either functional or nonfunctional. Here we examined effects of age on the functional status of TCR genes in men and women separately. Our results show that the diversity of functional rearranged TCR sequences significantly decreases with age only in women. The similar, but not significant trend was observed among men.**

***Keywords* — TCR repertoire, ageing, immunosenescence**

I. INTRODUCTION

T lymphocytes are one of the cells responsible for an adaptive immune response. Each of them expresses a unique heterodimeric T cell receptor (TCR) able to recognize a unique set of antigens. TCR genes are assembled from discrete V, D and J gene segments in developing lymphocytes. Due to the random nature of the V(D)J recombination process, only one-third of the rearranged TCR genes are functional. The remaining 2/3 are non-functional because of the loss of an open reading frame between the V and J genes, or the introduction of a stop codon. Hence, the TCR gene repertoire comprises functional and non-functional genes. Upon T cell activation, naïve T cells proliferate and differentiate into memory T cells. The production of naïve T lymphocytes declines with age [1], causing decreased TCR repertoire diversity and impaired immunity [2]. On the other hand, the size of naïve T cell clones increases in the elderly [3]. Even though the optimal size of TCR repertoire required to maintain efficient protection is not known, a decrease of TCR diversity is associated with impaired immune defences in mice [4]. Age-associated constriction of the TCR repertoire most probably participates in the increased susceptibility to infectious and non-infectious diseases in the elderly.

Here we intend to determine the impact of age on the functional status of TCR sequences. Due to the different ageing of men and women [5] and the influence of sex hormones on the immune system [6], we model the TCR repertoire status diversity for men and women separately.

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II. METHODS

We used a collection of 587 human TCRB repertoires obtained from healthy donors [7] publically available on the Adaptive Biotechnologies website [8], and a dataset of TCR repertoires obtained from 6 donors sampled three times 10 years apart [9]. Previously proposed analysis pipeline, including usage of Pielou's J index, was used to determine functional status diversity [10]. Weighted linear regression was employed to model functionality status diversity in age, with total counts of sequences serving as weighs. Separate models were created for men and women for two datasets. T-test compared regression slopes of created models.

III. RESULTS

We observed a negative correlation between age and diversity index among women. The age has a very small, but significant, effect on the functional status of TCR genes; the slope coefficients vary from -0.0006 to -0.0007 depending on the model. Furthermore, the rate of decline of the diversity index in women for the two datasets was not different (p-value = 0.64). The similar dependence was not detected among men, although the decreasing trend was also noticed.

These results illustrate a new aspect of the differences in biological ageing of the immune system in men and women.

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Wnt-Notch Crosstalk Tunes Intestinal Crypt Spatial Patterning

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***Short Abstract* — The intestinal epithelium is the fastest regenerative tissue, yet it maintains a remarkably consistent structure. While Notch signaling is implicated in the formation of the crypt base checkerboard arrangement of stem and Paneth cells, recent works suggests that ß-catenin can form a complex with Notch Intracellular Domain (NICD) and upregulate expression of Hes1 on its own or through this complex. Additionally, numerous questions exist about short-range Wnt secretion by Paneth cells at the crypt base. To address these questions, we perform bifurcation analysis on a dynamical model of this gene circuit, suggesting that the crosstalk may facilitate transition from the stem niche to transit amplifying region and that short range Wnt secretion may spatially constrain the size of the stem niche.**

***Keywords* — Intestinal Stem Cell, Paneth Cell, Wnt Signaling, Notch Signaling, Hes1, Systems Biology**

I. Introduction

THE crypts of the small intestinal epithelium are the fastest regenerating cell population in the body, turning over almost all cells every 2 – 6 days [1]. However, precise control of the crypt structure is maintained by the Wnt and Notch pathways. They are integrated through the transcription factor Hes1 to control differentiation the stem or Paneth cell type and the rate of stem cell proliferation [2].

Wnt proteins, such as mesenchymal Wnt 2b and Paneth-produced Wnts 3 and 11, increase accumulation of ß-catenin which upregulates Hes1 [3]. When a stem cell's Notch receptor is activated by a Paneth cell's Delta-like ligand, NICD translocates to the nucleus where it upregulates production of Notch receptors (positive feedback) and of Hes1 which inhibits the production of the cell's own Delta like ligands (lateral inhibition). This combined PFLI mechanism drives the formation of the characteristic checkerboard pattern of stem cells and Paneth cells [4].

Recently, two intriguing observations have been made: First, NICD may form a complex with ß-Catenin instead of directly upregulating Hes1 [2]. Second, the Paneth cells appear to only secrete Wnt to their immediate neighbors [5]. To understand the roles of crosstalk in Hes1 regulation and of short-range Paneth-mediated Wnt secretion, we modeled the integrated gene circuit considering these effects.

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II. Results

To compare competing models of the pathways, we employed tuning parameters that allowed us to vary the relative control of ß-catenin and ß-catenin-NICD complex control over the Hes1 promoter without altering the steady state [2]. Additionally, three Wnt secretion models were considered: mesenchymal (constant), paracrine, and juxtacrine.

In two cell simulations, bifurcation analysis of Wnt production rate and percent control of the Hes1 promoter by the ß-cat/NICD complex was conducted. With greater than 80% control by the complex, the models exhibited bistability of cell fates at both low and supraphysiologic Wnt concentrations. Below 50%, the cells failed to differentiate. From 50 – 80%, the model exhibited desirable switching behavior. At high Wnt concentrations (crypt base), the model demonstrated bistability, while at lower concentrations (TA region) the model predicted a monostable population. Accordingly, the mesenchyme may provide a constant level of Wnt to drive proliferation in the stem niche and TA region, but short range Wnt secretion by Paneth cells may exist to drive differentiation at the crypt base by reinforcing Hes1-mediated lateral inhibition.

Last, the models were implemented in a multi-cell simulation. All three models exhibited formation of a characteristic checkerboard pattern. While the juxtacrine model produces stem and Paneth cells with less variance in the final protein concentrations, the concentrations are less polarized than in the constant and paracrine models, and it takes significantly longer to achieve steady state.

III. Conclusion

Via crosstalk, short range Wnt secretion by the Paneth cells defines the size of the stem niche since it reinforces the Hes1 mediated lateral inhibition necessary to promote differentiation in a spatially constrained region.

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Cellular responses to dynamic patterns of cytokine stimulation

Chaitanya Mokashi[1*], David Schipper[1*] and Robin E C Lee[1]

***Short Abstract* — Activation of NF-kB through exposure to inflammatory cytokines mediates signals for migration, differentiation, cell-cycle progression, and apoptosis. Time-varying properties in the concentration of cytokine, such as the duration of stimulus, influence the type of response elicited in a cell. To study the extent to which temporal properties of cytokine stimulation mediate cell fate decisions, we have developed a microfluidic flow system for precise control of cytokine concentration as a user-defined time varying function. By imaging cells that express reporters for NF-kB activation and exposing them to different patterns of stimulation in the microfluidic system, we aim to understand how cells decode time-varying cues from their environment to make cell fate decision.**

***Keywords* — NF-kB signaling pathway, TNF, microfluidics, environmental cues, temporal signaling patterns**

I. Introduction

Responding appropriately to molecular signals from the extracellular milieu, such as cytokines or growth factors, is essential for cellular adaptation and viability. The NF-kB signaling pathway, for instance, upon activation with tumor necrosis factor (TNF) activates transcription for a myriad of genes [1] responsible for anti-apoptotic and pro-inflammatory responses [2]. Additionally, the same pathway through its non-canonical branch activates genes inducing the opposite i.e. pro-apoptotic and anti-inflammatory response [3]. Thus, cells must decode and extract information from external signals to produce the corresponding response. It is apparent that time-varying properties of molecular signals play an important role in this information transfer. For example, short duration exposure to high concentrations of TNF can be more effective at killing than a TNF-pulse of longer duration [4], and the subsequent dynamics of NF-kB localization within the cell encodes multiple levels of responses that reflect the strength of stimulation[4]. Although these studies have begun to scratch the surface [5], cells are using molecular switches and dials that we do not currently understand to transmit information about TNF in their environment. Here, we develop a framework to more broadly define the capabilities of single cells when exposed to arbitrary user-defined patterns of TNF stimulation and use it to uncover molecular circuits that encode this information.

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II. Methods

To explore the role of temporal dynamics of external signals in cellular response, precise control of extracellular environment is needed. We have developed a microfluidic system for analog control over the cellular environment, exposing cells to user-defined temporal patterns of stimulation. The system consists of a hydrostatic pump connected to a microfluidic cell-culture chip. The hydrostatic pressure and hence the flow rates of cytokine and media are controlled by manipulating the heights of the corresponding reservoirs through the Arduino microprocessor. A passive mixer in the chip then dilutes the cytokine concentration as desired before the cell-culture chamber. We have developed a framework to control the device by a Hagen-Poiseuille equation based mathematical model and CFD simulations in Ansys-Fluent software. Given a user-defined input of concentration, time and position in the chip, our system generates the corresponding positions of heights to generate the desired concentration profile. We use live-cell imaging to track single-cells expressing a GFP reporter of NF-kB and measure the corresponding nuclear localization as a proxy for response to TNF stimulation. The system can hypothetically achieve an arbitrary-fold dynamic range in concentration, depending on parameters of the cell-culture chip, and we are working on adding extra functionalities to our system that permit simultaneous and independent control of multiple cytokines in the same chip.

III. Progress

We are testing a preliminary version of our system by monitoring the NF-kB response in cells exposed to linear and exponential ramps of TNF stimulation. Our early results show that ramp stimulations produce NF-kB responses that are distinct from those seen after a pulse or step increase in TNF concentration. These results may hint at the molecular architecture of circuits that transmit information about extracellular TNF into the NF-kB system. We envision that our microfluidic system may be adopted with remarkably low cost by labs without specialized expertise to enable studies of cellular systems in dynamic microenvironments.

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Amino acid sequence constraints and co-evolution across a metabolic enzyme pair

Thuy N. Nguyen[1], Saumel M. Thompson[2], and Kimberly A. Reynolds[1,3]

***Short Abstract* — Several studies which have investigated the pattern of amino acid interactions between proteins in physical complexes show that a subset of contacting interfacial residues are thermodynamically coupled and co-evolve. However, it is less clear how the amino acid sequence of one protein is constrained by another protein to which it is functionally coupled, but does not directly bind. Here I describe a strategy and initial data for experimentally quantifying the sequence constraints imposed by functional coupling between metabolic enzymes. These measurements are then compared to sequence variation across homologs.**

***Keywords* — epistasis, co-evolution, statistical coupling analysis, fitness, saturation mutagenesis, next-generation sequencing**

I. Epistasis beyond physical interfaces

The amino acid sequence of an enzyme encodes information necessary for it to fold, catalyze biochemical reactions, and interact with partners in the cell. Maintaining these properties over evolutionary time constrains sequence variation, and can drive co-evolution between functionally coupled (or epistatic) positions. For example, positions at the interface of two-component signal transduction systems are thermodynamically coupled and co-evolve [1,2]. However, epistasis between proteins is not limited to physical complexes. In the general case of functionally coupled, but not necessarily physically interacting proteins, the pattern of epistasis between amino acids is poorly characterized. I am using: (1) experimental deep mutational scanning and (2) statistical analysis of sequence variation to map the constraints introduced by coupling in metabolism. Together, these data will provide a comprehensive and quantitative illustration of how epistasis between proteins shapes amino acid sequence variation.

II. The model system

To study the structural basis of epistasis, I am using the model system of dihydrofolate reductase (DHFR) and thymidylate synthase (TYMS), two enzymes in the folate metabolic pathway. Statistical analysis of gene presence/absence and synteny across 1445 bacterial genomes show that they are highly co-evolving with each other but are independent from the rest of the genome [3]. Though the two enzymes do not physically interact, recent experimental measurements of metabolomics and epistasis indicate that the relative biochemical activities of DHFR and TYMS are constrained by a need to limit accumulation of the metabolic intermediate dihydrofolate (DHF) [3]. In particular, mutations in DHFR that reduce catalytic activity are buffered by loss-of-function mutations in TYMS.

III. Quantitative measurements of epistasis

To determine how sequence variation in DHFR is modulated by the catalytic activity of TYMS, I am conducting fitness measurements for a saturation mutagenesis library of DHFR in the context of several TYMS mutants. The library contains all possible single point mutations at every position of DHFR (of 5,088 total). To assay fitness, I transform the library into *E. coli* and grow this mixed culture in continuous culture. I take samples of this culture during the experiment and use next-generation sequencing to track allelic frequencies of each mutant. The fitness of each mutant in the library is then calculated from a linear fit of the allelic frequency over time [3]. These data will reveal a fitness landscape across the entire structure of DHFR. I am currently repeating the assay in the background of five TYMS that range in catalytic activity from WT to catalytically dead. For each TYMS mutant, I will calculate epistasis from these fitness measurements and map these epistatic couplings onto the structure of DHFR. At the end, these data will reveal how the fitness landscape of DHFR is modulated by TYMS activity.

IV. Comparing Epistasis to co-evolution

I am comparing the pattern of epistasis from the experiments described above to the pattern of co-evolution between DHFR and TYMS homologs. To compute co-evolution, I am using the Statistical Coupling Analysis [4], which has recently been shown to reasonably estimate pair-wise couplings between positions in a single protein [5]. This comparison of experimental and statistical couplings will test the hypothesis that co-evolution can be used to infer sequence constraints linking non-binding but functionally coupled proteins.

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Four simple rules that are sufficient to generate the mammalian blastocyst

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***Short Abstract* — Early mammalian development is a fascinating example of how deterministic spatiotemporal patterns emerge at the level of cell populations from highly stochastic regulatory components, but why is this process so successful, and what ensures the stability of the blastocyst? With four simple rules, we get cavity formation, salt and pepper pattern, and three distinct lineages. In addition, we get most phenotypes by eliminating one rule at a time. Finally, we show that time from post-fertilization regulates the cells' competence of FGF. This could provide the robustness necessary for the evolutionary diversification of the preimplantation gene regulatory network.**

***Keywords* — Early embryonic development, blastocysts, cell polarity, cell communication, fibroblast growth factor (FGF), segregation, differential adhesion, apoptosis.**

I. Purpose

EARLY mammalian development is both highly regulative and self-organizing [1]. It involves the interplay of cell position, gene regulatory networks, and environmental interactions to generate the physical arrangement of the blastocyst with precise timing [2-3]. However, this process occurs in the absence of maternal information and in the presence of transcriptional stochasticity [4-5].

How does the preimplantation embryo ensure robust, reproducible development in this context? It utilizes a versatile toolbox that includes complex intracellular networks coupled to cell-cell communication, segregation by differential adhesion, and apoptosis.

Here, we ask whether a minimal set of developmental rules based on this toolbox is sufficient for successful blastocyst development, and to what extent these rules can explain mutant and experimental phenotypes.

II. Results

We implemented experimentally reported mechanisms for polarity, cell-cell signaling, adhesion, and apoptosis as a set of four developmental rules in an agent-based *in silico* model of physically interacting cells.

First rule, surface cells develop polarity at E3.0. Second rule, inner cells surrounded by too many cells of the same type, switch fate. Third rule, primitive endoderm (PrE) progenitors get less adhesion. Fourth and final rule, PrE progenitors in a wrong position undergo apoptosis.

We find that this model quantitatively reproduces specific mutant phenotypes and provides an explanation for the emergence of heterogeneity without requiring any initial transcriptional variation [6].

It also suggests that a fixed time point for the cells' competence of fibroblast growth factor (FGF)/extracellular signal-regulated kinase (ERK) sets an embryonic clock that enables certain scaling phenomena, a concept that we evaluate quantitatively by manipulating embryos *in vitro*.

Embryos were obtained at 8-cell stage and placed in pairs or triplets to make aggregates. Controls were cultured in KSOM only, while a Mek inhibitor was added to delay experiments [7]. At E4.5, embryos were fixed and stained, and analyzed with a custom-built MATLAB script.

III. Conclusion

Based on these observations, we conclude that the minimal set of rules enables the embryo to experiment with stochastic gene expression and could provide the robustness necessary for the evolutionary diversification of the preimplantation gene regulatory network.

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Feedback Loops at the Level of Lipid Metabolism Enhance Sensitivity and Robustness in Models of Chemotactic Gradient Sensing

Jamie L. Nosbisch[1], Krithika Mohan[2], James E. Bear[3], Timothy C. Elston[4], and Jason M. Haugh[2]

***Short Abstract* — The phospholipase C (PLC)/protein kinase C (PKC) signaling pathway is required for chemotaxis of fibroblasts biased by a gradient of platelet-derived growth factor (PDGF), as during wound healing. Experiments further demonstrated that abundance of the lipid intermediate, diacylglycerol (DAG), is polarized in a shallow PDGF gradient. To identify mechanisms capable of amplifying the sensitivity of this signaling pathway, reaction-diffusion models were formulated, and simulations show that inclusion of putative feedback loops at the level of lipid availability and metabolism yields a polarization circuit that is both sensitive and robust to varying gradient conditions. Thus, we offer a framework for understanding chemotactic gradient sensing in fibroblasts and for designing experiments to reveal and characterize sources of nonlinearity.**

***Keywords* — Reaction-diffusion modeling, cell signaling, polarization, chemotaxis, wound healing**

I. Background

In fibroblasts responding to gradients of platelet-derived growth factor (PDGF), an important chemoattractant in development and wound healing, signaling through the phospholipase C (PLC)/protein kinase C (PKC) pathway proved necessary for chemotaxis, whereas pathways that collaborate to activate the Arp2/3 complex were found to be dispensable [1,2]. PKC is activated through its binding to the lipid second messenger diacylglycerol (DAG), which is formed from hydrolysis of phosphatidylinositol (4,5)-bisphosphate (PIP_2) by PLC. Strikingly, in fibroblasts exposed to a shallow PDGF gradient, the density of DAG in the plasma membrane is focally enriched at the up-gradient leading edge [1], suggesting an internal amplification mechanism is at play.

In previous work, a mechanistic, reaction-diffusion model of the PLC/PKC signaling pathway was developed to identify possible mechanisms responsible for signal amplification [3]. We found that phosphorylation of myrisotylated alanine-rich C kinase substrate (MARCKS) by membrane-localized PKC constituted a positive feedback loop sufficient for local amplification of DAG and active PKC at the up-gradient end of the cell. By itself, the MARCKS feedback only weakly amplifies the signal in shallow PDGF gradients, however [3]. The system also lacks robustness to modest changes in the midpoint concentration of PDGF.

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II. Model Development and Results

The new model includes phosphatidic acid (PA), a lipid intermediate in the metabolism of DAG. PA is interconvertible with DAG by way of DAG kinases and phosphatidate phosphatases. Feedback loops incorporating PA were added to the model based on evidence that PA increases the rate of PIP_2 hydrolysis by stabilizing the recruitment of PLC [4] and that active PKC can enhance the activity of phospholipase D, another enzyme that produces PA [5]. Model simulations show that the MARCKS feedback mechanism synergizes with these new feedback loops for increased amplification even at shallow PDGF gradients and over an appreciable range of midpoint PDGF concentrations. Simulations with variations of parameter values or cell geometry further indicate that this signaling network is a highly sensitive and robust gradient sensing circuit.

III. Conclusion

Experiments will need to be performed, in concert with refinement of our modeling framework, to validate the source(s) of nonlinearity in this signaling pathway. We are currently exploring the effects of the cell's geometry on the polarization of the signaling pathway and assessing the effects of stochasticity on the performance of this system. In the future, this model will be linked to models describing the organization of the actin cytoskeleton and directionality of cell migration for a more comprehensive understanding of how fibroblast chemotaxis proceeds during physiological processes such as wound healing.

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Decoding High-dimensional Temporal Dynamics in Gene-regulatory Networks

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Recent advances in visualization of single molecules, such as transcription factors with temporal resolution in single cells, have shown that information is transmitted through time-varying dynamics of components shared between multiple pathways. This phenomenon stands in contrast to the typical paradigm where information is transmitted via structurally specific interactions (e.g. the lock and key model). Consequently, signaling through time dynamics via shared components raises natural questions about how such interactions can effect only the intended response. By analyzing realistic, coarse-grained regulatory networks informed by experimental studies, we find the design principles for regulatory circuits that respond to specific characteristics of an input time-series, such as frequency, duty cycle or pulse number, while buffering variations in other aspects. Our results show which aspects of time-varying input patterns need to varied independently in experiments to fully understand the signaling capacity of a multiplexed regulatory pathway.

I. Purpose

CELLS are constantly exposed to a dynamically changing environment; often survival hinges on the capability to extract meaningful information from this environment and respond appropriately. The capability of biochemical circuits to respond to temporal patterns in a specific manner has been explicitly established experimentally in a wide range of systems, ranging from bacterial to mammalian cells [1]. Here, we aim to understand the origins of these networks' capability of faithful temporal decoding mechanistically. By analyzing experimental data and numerically simulating coarse-grained gene-regulatory network models, we describe what aspects of temporal data are accessible to these networks. Further, we identify key mechanisms for recognizing specific features in a time-series and construct explicit models capable of such processing.

II. Results

A. Dimensionality in the Yeast Msn2 System

We introduce our own model-independent quantification of network decoding fidelity which we have termed *specificity*. By calculating this quantity for a set of genes coupled to *Msn2* dynamics [2] and operating under the reasonable assumption of monotonicity of response to larger doses of the input, we are able to definitively state that this system is able to access more than a single dimension of the temporal input.

B. Mechanisms for Temporal Decoding

In contrast, any linear time-invariant (LTI) system must have its integrated output proportional to the area of the input time-series (in accordance with the Convolution Theorem). Motivated by this divergence between LTI systems and the observed processing in the *Msn2* system, we chose four features (amplitude, period, duty fraction, and pulse number) to characterize a space of time-varying inputs and built nonlinear models able to respond to each feature independently.

C. Temporal Decoding and Circuit Topology

In establishing our mechanisms for temporal decoding, the importance of circuit topology was evident. For example, adaptation was found to be central to sensing period and pulse number, and it is known that only specific network topologies are capable of such dynamics [3]. To generalize our findings, we numerically optimized sets of networks using our *specificity* as an objective function for the task of decoding a fixed set of input pulse-trains with restrictions on the type of connections allowed (thus restricting accessible topologies over the course of the optimization procedure). The optimum obtained is strongly dependent on both the allowed topologies of the search and the distinguishing features of the input pulse trains, exhibiting the critical relationship between encoding method and decoder design.

III. Conclusions

By introducing specificity, a model-independent quantification of decoding efficacy, we are able to observe multidimensional temporal decoding in data from the *Msn2* system. Informed by this, we explicitly construct models capable of decoding in a four-dimensional space of pulsatile inputs. Our results suggest when

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Effects of cell cycle noise on excitable circuits

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***Short Abstract* — We assess the impact of cell cycle noise on gene circuit dynamics. For bistable genetic switches and excitable circuits, we find that transitions between metastable states most likely occur just after cell division and that this concentration effect intensifies in the presence of transcriptional delay. We explain this concentration effect with a 3-states stochastic model. For genetic oscillators, we quantify the temporal correlations between daughter cells induced by cell division. Temporal correlations must be captured properly in order to accurately quantify noise sources within gene networks.**

***Keywords* — Bistable switch, cell cycle noise, excitable system, metastability, synthetic genetic oscillator, transcriptional delay**

I. Background

CELLULAR noise and transcriptional delay shape the dynamics of genetic regulatory circuits. Stochasticity in cellular processes has a variety of sources, ranging from low molecule numbers, to variability in the environment, metabolic processes, and available energy. Such fluctuations can drive a variety of dynamical phenomena, including oscillations, stochastic state-switching, and pulsing. Microbial and eukaryotic cells make use of such dynamics in probabilistic differentiation strategies to stochastically switch between gene expression states, and for transient cellular differentiation.

How cell cycle noise shapes dynamics is only partially understood. The cycle of cell growth and division results in a distinct noise pattern: Intrinsic chemical reaction noise decreases as cells grow before abruptly jumping following cell division. The partitioning of proteins and cellular machinery at division also induces a temporally localized, random perturbation in the two daughter cells. These perturbations are correlated, as a finite amount of cellular material is divided between the two descendant cells. Such correlations can propagate across multiple generations within a lineage.

II. Results

We find that cell cycle noise can strongly impact the dynamics of bistable and excitable systems. In both cases, transitions out of metastable states are concentrated within a short time interval just after cell division. Interestingly, this effect intensifies as transcriptional delay (the time required for a regulator protein to form and signal its target promoter) increases. We show that this concentration effect results primarily from the random partitioning of cellular material upon cell division, and explain the underlying mechanisms via a 3-states reduced model.

For genetic oscillators, we find that cell cycle noise plays an important role in shaping temporal correlations along descendant lineages. In particular, for a dual feedback genetic oscillator, we show that temporal correlations between daughter cells decay significantly faster when the cell cycle is modeled explicitly.

In models of genetic networks the effects of cell growth are frequently described by a simple dilution term, which does not capture the distinct temporal characteristics of cell cycle noise. We conclude that in order to accurately describe gene circuit dynamics, such models should include both cell cycle noise and transcriptional delay.

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Trade-off between resistance to external and internal fluctuation in biophysical sensing

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***Short Abstract* — Cells must extract relevant information from time-varying external signals while ignoring a sea of other irrelevant fluctuations in those signals. We find that biophysical mechanisms that are most effective at projecting out irrelevant external fluctuations are most vulnerable to the internal noise originated from the mechanisms themselves. We show this trade-off relationship in biochemical receptors that measure ligand concentrations, in regulatory circuits that measure rate of concentration changes and in circadian clocks that measure the phase of the external day-night cycle. We trace this trade-off to a fundamental tension in the length of time used to make a measurement; slow measurements average out external fluctuations but make the system vulnerable to internal fluctuations and vice-versa. We show how such a trade-off emerges in the non-linear dynamical systems that underlie these diverse biological mechanisms.**

***Keywords* — Noise, Dynamical Systems, Computation in Cells, Circadian clocks, Chemosensing, Adaptation**

I. Purpose

Extracting information from a noisy external signal is fundamental to the survival of organisms in dynamic environments [1]. From yeast anticipating the length of starvation and bacteria estimating sugar availability, to dictyostelium counting cAMP pulses, organisms must often filter noisy irregular aspects of the environment while inferring parameters about a regular aspect in order to be well-adapted . However, in addition to external fluctuations, organisms must also deal with internal fluctuations due to finite copy number effects, bursty transcription and other biomolecular fluctuations. While such the impact of noise has been extensively documented, it is not clear whether different sources of noise are equivalent and whether strategies to mitigate them are compatible.

II. Method

We start by analytically showing the trade-off between resistance to external and internal fluctuation in the abstract context of Bayesian estimators – we consider a simple moving average estimator, a slope estimator, and a phase estimator. Then, we use simulations and theory using realistic biophysical mechanisms to show how such trade-offs emerge in non-linear dynamical systems viewed as Bayesian estimators.

III. Result

For all the cases we consider, we found the a trade-off along a Pareto front defined by:

$$\sigma_{int}^2 \sigma_{ext}^2 \sim 1$$

,where σ_{int}^2 and σ_{ext}^2 are the variance of the outputs due to internal and external fluctuation respectively. We arrived at this equation separately from mean estimator, slope estimator, and phase estimator. Here, we also present the simulations of 3 main biological estimators in the push-pull network in chemoreception [3] for moving average estimators, the adaptive network in concentration ramp sensing [2] for slope estimators, and the circadian clock network in [4] for phase estimators.

The universal reasons that the trade-off holds in non-linear biological estimators as well relates to the geometry of the attractor system. To be robust against external fluctuation, the geometry of the estimator's dynamics needs to be flat in the projected dimension of the information in the signal and curved along the direction of the external noise. However, this flat direction makes internal fluctuation diffuse faster, resulting in poor accuracy. Intuitively, the curvature of this flat direction relates to the time scale of the estimator's measurement that trace the performance along the Pareto front. We discuss how result is related to gain-bandwidth trade-offs in some contexts and discuss speed-error trade-offs in these systems. We find that while slow estimators are more accurate when subject to external fluctuations, such a speed-error tradeoff is absent in the context of internal fluctuations – fast estimators are the most accurate estimators.

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Ensemble response of immune repertoires to vaccination

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Short Abstract — **The T-cell repertoire response to the yellow fever vaccine builds near total immunity. To quantify the response we developed a statistical model of differential T-cell proliferation and used it as a basis for inference from high-throughput receptor sequencing data obtained from individuals pre and post vaccination. While the learned model for replicate statistics varies little across time points, the repertoire ensemble parameters vary, consistent with YF response timescales. Finally, we identify candidate clones responsive to the vaccine by their posterior expansion probability. These candidates are experimentally validated.**

***Keywords* — Immune repertoire, inference, vaccine response.**

I. Background

THE efficacy of a vaccine depends strongly on its interactions with the immune repertoire: a vast set of receptors capable of focusing immune defenses on infectious agents. These interactions leave signatures in the sequences and relative frequencies of immune clones. High throughput sequencing now provides measurements of millions of receptors, allowing for characterizing the repertoire at the level of the ensemble [1]. In spite of large scale efforts [2], how repertoire statistics respond to infection is unknown. This limits our ability to predict which TCR sequence will respond to a given antigen. The attenuated yellow fever (YF) vaccine induces near total immunity in humans and serves as human model for acute viral infection [3]. We developed a methodology for identifying responding clonotypes from time-dependent repertoire-sequencing data and applied it to sequencing measurements before and after YF vaccination.

II. Methods

We developed accurate models of the variation in sampled receptors molecules between a pair of replicates. We then assessed functional forms for a prior on the log fold-change of clone frequency via the likelihood-based criterions of the corresponding observed marginal distribution of pair counts. We combined these to obtain a model of differential expression. Inverting this model gave the posterior log fold-change probability of a clone given an observed count pair. The latter served to make inferences about the response of individual clones. Finally, we ran an experimental validation assay and compared these to those identified by our method.

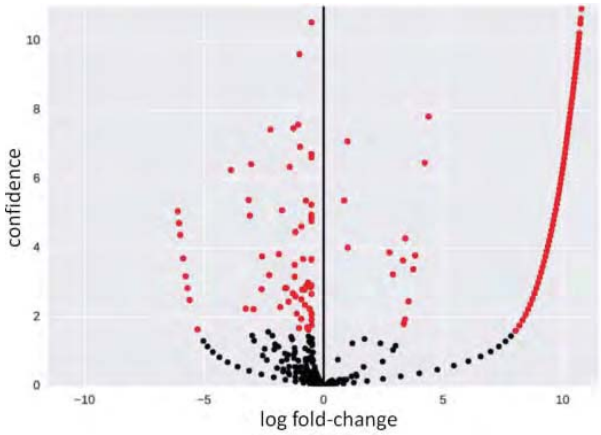

Fig. 1. Confidence of clone contraction (left) and expansion (right) as a function of fold-change of the hidden clone frequency. Circles denote pairs of measured cell counts. Red circles denote clones significantly affected by vaccination.

III. Results

A two-step replicate model best fit our data, giving similar parameter values across days, suggesting a unique natural replicate statistics. By combining this model with the best fitting log fold-change prior, we found correlated variation in the learned prior parameters values that systematically varied in time after vaccination. For each time point, the list of significantly expanded clones (see Fig. 1) correlated highly to the results of our experimental validation assay.

IV. Conclusions

Inferred changes in ensemble parameters of repertoire statistics reflect repertoire-level response dynamics. They are consistent with the known time scales of the response to YF and suggest temporally-sensitive ensemble features subject to homeostatic constraints. Finally, our validated method can detect significantly expanded clones by accounting for the natural variation in clone statistics.

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Biophysics of adversarial examples

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***Short Abstract* — Neural networks consistently misclassify adversarial examples, images overlaid with a small and specific perturbation. Similarly, immune cells misclassify agonist ligands in the presence of antagonist ligands in a phenomenon called ligand antagonism. We discovered a mathematical relation between ligand antagonism and adversarial examples, and show how the decision boundary tilts and better approximates the true decision boundary with increasing nonlinearity in both the immune and the neural network.**

***Keywords* — Machine learning, adversarial examples, immune recognition, ligand antagonism, decision boundary**

I. Background

In recent times, neural networks have been immensely successful in performing diverse tasks like object detection, speech recognition, and language translation [1]. Surprisingly, neural nets intrinsically suffer from blind spots, so-called adversarial examples [2]. An imperceptibly small, well-designed perturbation laid over an image will cause the neural net to misclassify the image, while it remains unchanged to the human eye. It has been proposed that adversarial examples are caused by the linearity of neural networks and the high-dimensionality of the data. Indeed, small changes in many pixels can add up to a macroscopic change in the classifier [3]. Others have argued that adversarial examples exist only when the decision boundary lies close to the sampled data, depending on the regularization used during training [4]. At the decision boundary, images are classified with equal probability in either category. At the true decision boundary, images are ambiguous, even for us, whereas at a suboptimal decision boundary we expect to find adversarial examples. It remains an open question on how precisely adversarial effects arise and how more robust neural nets can be designed.

T cells are faced with similar classification tasks as neural networks. They specialize in triggering an immune response when presented with minute amounts of not self ligands while ignoring a vast number of self ligands. Differentiation between ligands is based on the ligand receptor binding kinetics. It is known that antagonist ligands with a dissociation time just below the detection threshold impede the T cell's response to not self ligands via a phenomenon called ligand antagonism [5]. Nature's solution to overcome severe antagonism is to include kinetic proofreading (KPR) and biochemical adaptation in the immune network.

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We discovered that antagonism in immunology and adversarial examples in machine learning are instances of the same class of problems. Via an analytically tractable model of immune recognition, we established mathematical connections between antagonism and adversarial examples, and explored consequences that until now have been confined to a machine learning context.

II. Results

We applied the Fast Gradient Sign Method [3] to the immune classifier and found that the maximum adversarial perturbation comprises a global decrease of binding times, a decrease in agonist number and an increase in antagonist number, as expected from immunology. Next, we observed when the decision boundary is tilted stronger, the effects of ligand antagonism are weakened, conform [4].

Recent work on Hopfield networks [6,7] discusses the implications of learning with rectified polynomials (RePns), higher order nonlinear activation functions based on Rectified Linear Units. They find their analogue in immune networks via KPR. We showed that prototypic learning is enforced with high order RePns and many KPR steps.

Finally, we demonstrated how networks with higher order RePns or more KPR steps visually and quantitatively better approach the optimal decision boundary. In such networks, adversarial examples and mixtures of antagonists are more robustly classified.

III. Conclusions

Immune networks use proofreading and adaptation to lessen antagonistic effects. Training neural networks with equivalent nonlinear activation functions make them less sensitive to adversarial effects. Our work demonstrates how problems in two very different fields belong to the same class, motivating future studies on the connection between machine learning and biology.

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Synchronization modes of the mechanical response in mouse heart

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***Short Abstract* — The heart rhythm as a system of weakly coupled oscillators was studied recently, but little attention has been paid to the synchronization modes of the electrical stimulation-contractile response coupling in isolated mouse heart. This study aimed to describe with a minimalistic mathematical model the synchronization modes on the electrical stimulation-contractile response coupling in the whole heart. We propose a minimal linear coupled oscillator model to study the synchronization modes, which is validated with experimental results. The local stability is studied. We predict through in-silico experiments the presence of several synchronization modes, and these could be associated with arrhythmias.**

***Keywords* — Linear model, synchronization modes, systems biology, stability and complex dynamics, modeling and identification of nonlinear systems.**

I. Introduction

THE cardiovascular system has been studied with a substantial body of mechanistic mathematical models [1–5].

Christie et al., recently modeled the cardiovascular system as a series of weakly coupled oscillators. The interactions between these oscillators generate a chaotic blood pressure waveform signal. A minimal linear model to identify different dynamical scenarios as a function of the parameters values is presented [6]. However, although the interaction between heart rate, sympathetic nervous, parasympathetic nervous, and respiratory systems were analyzed, little attention has been paid to the synchronization modes in electrical stimulation-contractile response coupling.

In this paper, we propose a minimal linear mathematical model of the electrical stimulation-contractile response coupling to address the following question: whether the synchronization modes can be obtained with a model of weakly coupled oscillators. Using stability analysis, we found a stable equilibrium point to the proposed linear model. Then, we performed simulations of the coupled model with a forced function that represents electrical stimulus.

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II. Results

The model shown in (1) where k_C is the coupling parameter, ω_E is the sinoatrial node frequency, ω_M is the final contraction frequency, $f(t)$ is the external electrical stimulation function which was chosen as a square signal with 10% of the duty cycle.

$$\begin{aligned} \dot{Z}_1 &= Z_2, \\ \dot{Z}_2 &= f(t) - (\omega_E{}^2 + k_c)\, Z_1 + k_c\, Z_3, \\ \dot{Z}_3 &= Z_4, \\ \dot{Z}_4 &= k_c\, Z_1 - (\omega_M{}^2 + k_c)\, Z_3, \end{aligned} \tag{1}$$

We present five cases that describe five synchronization modes for different parameter settings:

Parameter	Value				
Mode	**1:1**	**2:1**	**1:2**	**3:2**	**2:3**
ω_E [rad/s]	37.7				
ω_M [rad/s]	37.7				
k_C [Hz^2]	900	260	3157	1280	3680
$Z_{1\ o}$ [$A.U.$]	0.1				
$Z_{2\ o}$ [$A.U.$]	0.5	1.2	0.4	3.5	3.5
$f(t)$ *amplitude* [$A.U.$]	16000	7020	3000	4500	390
$f(t)$ *freq* [Hz]	9	14	7	15	10

III. Conclusion

The simplicity of the model precludes it from capturing the molecular events at the intracellular level. We have illustrated how changes in the coupling parameter control the synchronization modes. The model reproduces 1:1, 1:2 and 2:1 synchronization modes, experimentally obtained. The main prediction of the model is that 2:3 and 3:2 synchronization modes can be obtained changing the coupling parameter.

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Dynamics of Stress Response in Bacteria

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***Short Abstract* — In order to successfully colonize a host, bacterial pathogens must sense and adapt to stress conditions such as damage to cell membrane. In human pathogen *Mycobacterium tuberculosis*, the disease causing agent of Tuberculosis, response to cell membrane damage is regulated by MprA/B two-component signaling system, and the alternative sigma factor σ^E. The stress regulatory network features multiple layers of regulation including transcriptional feedback and post-translational regulation. Using time-course qRT-PCR data for *sigE* and *mprA* genes following exposure to membrane damaging stress, we aim to uncover how network architecture shapes the observed dynamic response.**

***Keywords* — dynamical properties, feedback, networks.**

I. Introduction

THE pathogen *Mycobacterium tuberculosis* (Mtb) can cause a latent tuberculosis (TB) infection by reprogramming its metabolism and gene expression to a persistent, non-replicating state. To successfully colonize human hosts, Mtb must sense and adapt to stresses generated by the host immune system [1]. One such stress, damage to cell membrane, is regulated by transcriptional master regulators – MprA/B two-component system (TCS) and alternative sigma factor σ^E [2].

Exposure of Mtb cells to membrane damaging stress leads to activation of the MprA/B TCS. MprA is autoregulatory, and upregulates the *mprA-mprB* operon. In addition, it activates transcription of *sigE*, whose gene product σ^E in turn upregulates *mprA-mprB* [4]. This sets up a network with two transcriptional positive feedback loops – direct (autoregulation) and indirect. Further complexity is added to the network by post-translational sequestration of σ^E by its cognate anti-sigma factor RseA, preventing downstream gene activation [3].

II. Results

Previous theoretical work from our lab has suggested that the MprA/B- σ^E network described above can be bistable [3]. Specifically, the positive feedback loops combined with ultrasensitivity generated by strong σ^E-RseA interaction give rise to bistability in some parameter ranges. However, a step change from unstressed to stressed conditions with this bistable model results in a large activation delay. To verify this experimentally, we quantified transcript abundance of *mprA*, *sigE* and *sigB* (reporter for σ^E activity) in Mtb cells exposed to surfactant SDS. Time course of the transcripts was collected along with dose-response measurements at increasing and decreasing SDS doses. While time-course measurements reveal surprisingly rapid accumulation given the positive feedback circuit, dose-response measurements reveal hysteresis in *sigE, mprA* transcript levels between previously unstressed and previously stressed Mtb cells.

Utilizing this data, we have succeeded in constructing a model that explains the dynamical properties of MprA/B-σ^E stress response pathway. We report that a chaperone, DnaK, known to suppress MprB autokinase activity plays a role in generating bistability. In absence of this chaperone mechanism, our models display a trade-off between short transcript accumulation time and hysteresis in transcript levels. Short transcript accumulation time is observed in absolute concentration robustness (ACR) regime of MprA/B TCS wherein, the level of phosphorylated MprA depends only on the signal level, provided enough MprA protein is present [4]. In this regime, bistability is ruled out and simulations do not display dose-response hysteresis. On the other hand, outside this regime in bistable conditions, dose-response hysteresis is observed but the models display high transcript accumulation times. We report that the network model including DnaK-MprB interaction does not possess ACR. The model is bistable at intermediate SDS concentrations, giving rise to hysteresis in transcript levels. Interestingly, we can still maintain short transcript accumulation times, avoiding the previous models' trade-off.

III. Conclusion

We find that a trade-off exists between short accumulation time and hysteresis in transcript levels in the classical two-component system model. In contrast, using a DnaK-dependent activation mechanism of MprA/B, we can explain the hysteresis in dose-response while avoiding the previous trade-off. Stress-independent, constitutive production of DnaK, along with positive feedback to MprA/B are critical for hysteretic bistability.

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Tensor-Based Approximation of the Stationary Solution to the Chemical Master Equation

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***Short Abstract* — We can model the dynamics of certain cellular processes via the chemical master equation. Analyzing the system becomes especially burdensome as the number of chemical species being tracked increases. Numerous recent attempts have been made to alleviate this so-called curse of dimensionality using tensors (i.e., multidimensional arrays), because tensor-based approaches have been found to scale linearly with species count. We develop tensor-based strategies to approximate the probability distribution in the long run, after the transient behavior of the system has dissipated, with an emphasis on constructing iterative methods that scale efficiently as more chemical species are introduced to the system.**

***Keywords* — chemical master equation, stationary solution, limiting behavior, iterative methods, tensors**

I. Motivation

THE chemical master equation yields a system of linear equations that is frequently represented in matrix form:

$$A\mathbf{p} = \dot{\mathbf{p}}$$

where A is the n x n sparse transition rate matrix and $\mathbf{p}$ is an n x 1 vector enumerating the (time-dependent) probability of the n possible chemical configurations of the system. The derivative of the probability vector, $\dot{\mathbf{p}}$, will simply be the zero vector when the system is in statistical equilibrium. Solving such a system using traditional methods requires $O(n^3)$ steps [1]. Numerous approaches have been implemented to arrive at a solution more efficiently, but they tend to suffer from the curse of dimensionality to varying extents.

II. Tensor Approach

In recent years, tensors have been touted as a means of vastly reducing the computational burden involved in analyzing systems modeled by the chemical master equation. A straightforward means of converting the problem from matrix format to tensor format is to reconfigure $\mathbf{p}$ into a multidimensional array of dimension

$$n_1 \times n_2 \times \ldots \times n_d$$

where $n_j - 1$ is the maximum copy number of the j^{th} chemical species and d is the number of chemical species being tracked in the system [2-3]. The transition matrix A is likewise reformatted to tensor form and a multiplication operation relating A and $\mathbf{p}$ is defined accordingly.

Unlike the matrix formulation, tensor representation holds the advantage of preserving in its structure information concerning the underlying geometry of the system. Vast computational savings are realized when the multidimensional arrays are factored into a low-rank approximation using methods such as tensor train decomposition [4-8]. Tensor-based approaches have already been applied to the study of the chemical master equation's stationary solution [9]. Here, we present new iterative techniques to approximate the stationary solution using tensors and compare their computational efficiency to more traditional methods.

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Tuning Evolution Towards Generalists by Resonant Environmental Cycling

Vedant Sachdeva[1], Kabir Husain[2], Shenshen Wang*[3], Arvind Murugan*[2]

***Short Abstract* —Some evolutionary conditions result in `generalists' that are well-adapted to a common aspect of environments in evolutionary history and can thus deal with unseen environments. Such `generalists' can be hard to evolve if they are less fit or are entropically disfavored. We show that time-dependent evolutionary protocols, such as environmental cycling on an intermediate timescale or a chirp can select for generalists in spite of high fitness or entropic costs. These time-dependent strategies can be successful even when all static protocols fail. We show the regimes of the landscape ruggedness, selection pressure, and other parameters in which time-dependent protocols can discover generalist solutions.**

***Keywords* — changing environments, immunology models, population genetics models**

I. Purpose

UNDERSTANDING the conditions under which a system is able to generalize the experience of past challenges to solve novel challenges is a theme at the heart of diverse fields, ranging from statistical learning theory to evolutionary biology. Arriving at such a generalist solution is especially difficult since `specialist' solutions might be entropically favored or generally outperform (i.e., have higher fitness) any generalist solution. In addition, this task can be made more difficult by the ruggedness of the fitness landscape, as the system may become trapped in a local well and fail to successfully evolve a solution to the environment.

The emergence of generalists has been linked to the idea of changing environments. Simplified models[1] of evolutionary dynamics in low-dimensional spaces have indicated that environmental cycling at a specific frequency could select (or `localize') a population at a `generalist' phenotype or genotypes. Such emergence of generalists has also been thought about in the context of modularity[2,3].

Recent experiments in immunology have provided a quantitative handle on such questions. The observed emergence of broadly neutralizing antibodies (bnAbs) suggests that by exposing the germinal center to a variety of mutants of a given antigen, the immune system can develop antibodies that succeed against mutants not previously seen in spite of the fact that these antibodies are not entropically favored. In addition, epistatic interaction between the sites increases the difficulty of the problem by enhancing degeneracies of antibodies[4]. Discovery of bnAbs is promoted by switching between antigens, rather than simultaneous presentation of multiple strains of antigen [5].

These examples raise critical questions about exactly what circumstances make the emergence of generalists difficult and necessitate temporal strategies.

II. Methods and Results

The works above suggest three critical parameters to be studied: the switching rate of the environment, the cost of the generalist strategy relative to specialized strategies, and the ruggedness of the fitness landscapes. We performed Wright-Fisher simulations and varied the above parameters.

We find that generalists can emerge in static environments only if their fitness cost (relative to specialists) is below a critical value. For larger fitness costs, static environments or rapid cycling of environments cannot produce generalists. In parts of this regime, we find that cycling through different environments favors the emergence of generalists. However, the timescale of environmental cycling must be tuned to match the timescale of the evolutionary dynamics itself, set by the mutation rate, population size and selection pressure. If the environmental cycling is faster or slower, we do not recover generalists. We summarize our results in the form of a phase diagram.

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Collective genetic units in bacterial metabolism

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Metabolic enzymes must reliably catalyze the conversion of substrate to product, but are also constrained to function properly in the context of their pathway. Here we show that constraint on the accumulation of a metabolic intermediate leads to epistasis and co-evolution of two enzymes in folate metabolism: Dihydrofolate reductase (DHFR) and Thymidylate synthase (TYMS). These two enzymes co-evolve: (1) experimentally during forward evolution and (2) statistically across 1445 bacterial genome sequences. Moreover, these two enzymes evolve relatively independent from the remainder of the pathway. These results motivate the development of new co-evolutionary analyses for examining sequence constraints within metabolism.

Keywords **— coevolution, statistical genomics, folate metabolism, dihydrofolate reductase (DHFR), epistasis, experimental evolution**

I. Introduction

In metabolism, the coordinated activity of multiple enzymes produces the substrates necessary for cell growth and division. Though a large body of prior work has elucidated the molecular components and biochemical reactions comprising central metabolism, it remains unclear what evolutionary and functional constraints act on metabolic enzymes. For example, if one enzyme in a pathway is inhibited or otherwise reduced in activity, what (if anything) has to happen in the rest of the pathway to compensate? And to what extent are the activities of metabolic enzymes coupled or entirely independent from one another? We chose folate metabolism, a well-characterized and highly conserved pathway, as a model system to study these questions. Our approach combines coevolutionary analyses, epistasis measurements, and experimental forward evolution in order to understand the evolutionary constraints on this system.

II. Results

We conducted an analysis of thirteen enzymes comprising the core one-carbon folate metabolic pathway across 1445 bacterial genomes. Two measures of coevolution were considered: 1) synteny, the conservation of chromosomal proximity between genes and 2) co-occurrence, the coordinated loss and gain of genes across species. The results indicate a sparse architecture of interactions, in which most genes evolve independently of one another, with several small groups that coevolve modularly.

Expectedly, one of these groups is composed of the glycine cleavage system proteins H, P and T, which make up a physical complex. The second coevolving unit is made up of two enzymes, dihydrofolate reductase (DHFR) and thymidylate synthase (TYMS), which catalyze sequential reactions but are not known to physically bind. We chose DHFR and TYMS for detailed experimental study in *E. coli*. Quantitative epistasis measurements reveal that DHFR and TYMS are coupled such that a decrease in the activity of one enzyme can be compensated for by lowering the activity of the other. In concordance with this, metabolomic measurements suggest that this epistasis is driven by a constraint on their relative activities, which must be balanced to prevent the accumulation of a toxic intermediate. We evolved wild-type *E. coli* in the presence of trimethoprim, a competitive inhibitor of DHFR. Whole genome sequencing reveals that resistance is obtained by mutations in both DHFR and TYMS, but not other genes in the pathway. Thus, the enzyme pair shows a capacity for adaptation that is independent from the rest of folate metabolism, which is supported by both statistical and experimental evidence.[2]

III. Discussion

This work suggests that complex systems such as folate metabolism may be subdivided into functional units that act collectively and adapt relatively independent of one another. We provide one such example, which was predicted by statistical analysis of genomic data. Our results motivate a global analysis of coevolution within metabolism, to be followed by comprehensive experimental testing. This strategy has the potential to provide important insights toward rationally engineering new systems, and predicting the combined effects of mutations.

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Spatiotemporal dynamics of phage-biofilm interactions

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Short Abstract— Bacteriophage ('phage') - viruses that infect and lyse bacteria - can be deployed therapeutically to treat infections caused by bacterial pathogens. However most reported studies of the therapeutic potential of phage neglect the spatial heterogeneity in bacterial communities, e.g., in microcolonies and biofilms. Here, we present experiments, theory, and simulations that investigate the spatiotemporal dynamics arising from interactions between P. aeruginosa and phage. Time-dependent high resolution confocal imaging is used to examine how phage propagate through spatial domains of bacteria. Together with a three dimensional multi-scale modeling approach, our results shed light on how phage shape the emergence and collapse of microcolonies and biofilms.

Keywords— Biofilm, Bacteriophage, Phage therapy, Antibiotic resistance, Pseudomonas aeruginosa, Pattern formation

I. INTRODUCTION

Bacteriophages are ubiquitous in natural systems and provide an alternate cure for infections caused by antibiotic-resistant strains of bacteria. Infections in cystic fibrosis and wound patients often contain clusters of bacteria which secrete a protective exopolymeric substance creating a biofilm-like environment [1]. Though the non-linear population dynamics between planktonic bacteria and phage is well studied, the interactions between phages and spatially organized bacterial aggregations are underexplored. Mean-field dynamics need not recapitulate spatial dynamics, particularly given that local aggregates modulate the strength of phage diffusivity [2], latent period of phage incubation, and other physiological properties of bacteria. Therefore, it is essential to understand the emergent spatial structure and reorganization of bacteria in biofilms in the presence of phage in order to better understand the therapeutic potential of phages [3].

II. RESULTS

Using fluorescent strains of Pseudomonas aeruginosa and its phage PeV2 as model organisms, we observe their spatial evolution through spinning disk confocal microscopy. We quantify the characteristics of the biofilm such as density, volume of live and dead bacteria, size and abundance of bacterial clusters and zones of bacterial elimination, and typical inter-cluster distances through the evaluation of pair-correlation function. We compare these findings with the results of our simulation of bacteria-phage dynamics, based on an individual-based molecular dynamics framework.

III. CONCLUSION

Through theory, experiments, and simulation, we investigate the nature of interactions in phage-biofilm systems. The complex non-linear population dynamics gives rise to various emergent spatial patterns which is dependent on the initial conditions of phage exposure.

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Properties of Gene Expression and Chromatin Structure with Mechanically Regulated Transcription

Stuart A. Sevier[1], Herbert Levine[1]

***Short Abstract* — The mechanical properties of transcription have emerged as central elements in our understanding of gene expression. Recent work has been done introducing a simple description of the basic physical properties of transcription. Here we generalize this framework to accommodate the behavior of many RNAPs operating on multiple genes on a shared piece of DNA. The resulting framework offers a preliminary explanation a number of previously unexplained an unrelated phenomena in gene expression and DNA organization.**

***Keywords* — Gene Expression, Transcription, Chromatin, Mechanics**

I. Purpose

It has long been recognized that during transcription a elongation a combination of RNA polymerase (RNAP) rotation and DNA twist would have to occur. This simple fact has the potential to effect many aspects of transcription and gene expression [1]. However, only recently has a physical model of this process been developed.

Recent work has been done introducing a simple description of the basic physical elements of transcription where RNA elongation, RNA polymerase (RNAP) rotation and DNA super-coiling are coupled [2]. Here we generalize this framework to accommodate the behavior of many RNAPs operating on multiple genes on a shared piece of DNA. The resulting framework is combined with well-established stochastic processes of transcription resulting in a model which characterizes the impact of the mechanical properties of transcription on gene expression and DNA structure. Transcriptional bursting readily emerges as a common phenomenon with origins in the geometric nature of the genetic system and results in the bounding of gene expression statistics. Properties of a multiple gene system are examined with special attention paid to role that genome composition (gene orientation, size, and intergenic distance) plays in the ability of genes to transcribe. The role of transcription in shaping DNA structure is examined and the possibility of transcription driven domain formation is discussed [3].

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Multiplexing Cell-Cell Communication

John T. Sexton[1], Jeffrey J. Tabor[1,2,3]

***Short Abstract* — To coordinate complex behaviors, living cells transmit and receive many pieces of information with one another. However, a small limited number of engineered cell-cell communication systems currently exist, limiting our ability to engineer multicellular behaviors. Here, we overcome this limitation by constructing a genetically-encoded Multiplexer-Demultiplexer (MUX-DEMUX) system that enables *Escherichia coli* to have two separate conversations over a single chemical channel.**

***Keywords* — cell-cell communication, genetically-encoded Multiplexer-Demultiplexer, MUX-DEMUX, multicellular behaviors, transcriptional logic gates, CRISPRi**

I. Purpose

Communication among cells enables complex multicellular behaviors and is employed by many natural systems. Examples include the Notch-Delta systems in animals, which coordinate multiple cellular differentiation processes, auxin systems in plants, which orchestrate growth and development, and quorum-sensing systems in bacteria, which regulate bacterial gene expression based on population density. Several engineered systems have also been presented that utilize cell-cell communication, including a bacterial edge detector [1], multicellular NOR gates [2], and an oscillating microbial consortium [3]. However, the most complex engineered multicellular systems currently use at most two communication channels, due in part to the limited availability of engineered cell-cell communication systems. Expanding the effective capacity of existing cell-cell communication systems overcomes this limitation and enables increasingly complex engineered multicellular behaviors.

Constraints on the transmission of information are also prevalent in electrical systems, where digital logic devices are employed to manage access to finite communication resources. These logic devices, known as multiplexers, enable multiple independent conversations to occur over the same communication channel. A multiplexer (MUX) outputs one of multiple input signals based on a "select" signal. Similarly, a demultiplexer (DEMUX) routes a single input signal to one of multiple outputs based on a select signal. If a MUX and a DEMUX are juxtaposed across a communication channel, they can be used to arbitrate access to the channel. Furthermore, if their select signals are derived from the same source, the MUX-DEMUX system can select and transmit one of multiple simultaneous independent signals, effectively increasing the capacity for communication across the channel. We thus used standard logic design techniques to design a MUX circuit and a DEMUX circuit, and we built on existing techniques for constructing genetically-encoded logic circuits in living cells [4] to implement a MUX-DEMUX system that enables *Escherichia coli* to transmit two independent signals over a single chemical channel.

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II. Results

We have constructed a novel library of 9 orthogonal CRISPRi-based NOT and NOR gates, and we optimized 3 small-molecule sensors and 1 quorum-sensing cell-cell communication channel to interface with them. From this library of parts, we assembled a chemically-inducible genetically-encoded 2-to-1 MUX from 3 sensors, 4 CRISPRi NOT gates, and 3 CRISPRi NOR gates and a chemically-inducible genetically-encoded 1-to-2 DEMUX from 2 sensors, 3 CRISPRi NOT gates, and 2 CRISPRi NOR gates. We introduced the MUX and DEMUX circuits into separate *E. coli* strains, validated the performance of every regulated promoter in each circuit, and cocultured both strains to show that two different small-molecule inducers, detected only by the MUX strain, can independently control the activity of two transcription units in the DEMUX strain. We also characterized the dynamics of signal propagation in the MUX circuit and established a gate delay time for CRISPRi logic circuits in *E. coli*.

III. Conclusions

This work represents one of the largest synthetic systems constructed to date with 20 regulated transcription units and 8 layers of logic computation. This work also expands the capacity for communication over existing engineered cell-cell communication systems and enables increasingly complex multicellular behaviors.

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Using the automated building of computational models to understand cardiotoxic drug responses

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Sorafenib, a tyrosine kinase inhibitor used in the treatment of a number of cancers, has been shown to cause cardiotoxic side effects. "Omics" level profiling of cardiomyocytes showed significant changes in glycolysis and amino acid metabolism following treatment, however the mechanistic connection to Sorafenib is still unclear. We hope to better understand the mechanistic dynamics through a novel computational model, using the INDRA (Integrated Network and Dynamical Reasoning Assembler) software to automate the model building process. INDRA allows us to read published literature, extract mechanistic information, and build a rule-based model. With added functionality to improve rule context generation and constrain model scope for small driven models, we built a model that connects known Sorafenib targets to enzymes that participate in glycolysis and amino acid metabolism.

I. Background

TYROSINE kinase inhibitors, including Sorafenib, have been shown to induce of cardiac side effects including heart attack, high blood pressure, QT prolongation, and cardiomyopathy [1]. Using human induced pluripotent stem cells (hiPSC)-derived cardiomyocytes we found that Sorafenib treatment induces metabolic changes, including changes in oxygen consumption, glucose uptake, lactate production, and serine production, as well as causing differential expression of genes and proteins enriched for GO terms related to key metabolic pathways.

To explain how Sorafenib causes these effects, we aim to build a computational model that can connect canonical targets of Sorafenib to metabolic pathways. However, modeling such a broad hypothesis is a difficult task, since mechanistic models are typically less exploratory and building such a far-reaching model would be a slow and error-prone process. To address these issues we are using the INDRA [2] software to automate the process. INDRA allows us to read thousands of published papers, extracting any mechanistic statements. This results in a large corpus of detailed information which can be automatically assembled into many types of models, including an executable rule-based model [3]. However, these models are often so large and highly connected that they are difficult for a human to parse, and computationally difficult or impossible to simulate. Additionally, key context governing when a model rule fires is often missing or incomplete, leading to unexpected and incorrect dynamics. To use these models to answer highly specific questions about a system, we need additional tools to help us reduce and focus our model.

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II. Results

A. By inferring rule context and identifying meaningful pathways from an assembled set of rules, we can build an accurate and simulatable model with minimal intervention.

To build use-case driven models, we need to improve automated model assembly by improving the context governing when a model rule fires, and by limiting the scope of the model. By enforcing appropriate activating and inhibitory conditions for each model rule, we improve model accuracy and reduce simulation time by reducing the number of possible model species. We are able to infer many of these conditions by assuming directionality in a signaling cascade, and enforcing that events happen in order, initiated downstream of a receptor or specified starting point. This results in a model that is better behaved and quicker to simulate. To improve model scope definition, we leverage graph analysis and path finding tools to identify short paths between elements of interest, such as drug targets and experimental outputs. This allowed us to automate the building of a model that is easier to visualize and simulate.

B. Automated model construction and simulation shows that Sorafenib perturbs regulation of PKM2 through phosphorylation and transcription

We used INDRA to build a model that connects targets of Sorafenib to enzymes that participate in glycolysis. This model, which focuses on events downstream of the FLT3, KDR, and PDGFRA tyrosine kinases, is able to recreate our data, notably showing that Sorafenib treatment leads to an increase in PKM2 activity, causing increased flux through glycolysis and reduced serine synthesis. Model calibration using high dimensional, single-cell immunofluorescence data [4] will allow us to make quantitative predictions on how Sorafenib causes changes in glycolysis that are absent following treatment with less toxic drugs.

III. Conclusion

Automated construction of models allows us to quickly and quantitatively explore new areas of biology. Using new model assembly tools allows the user to reduce a huge amount of mechanistic information mined from the literature to a smaller set of pathways of interest. This results in a model that can be simulated and calibrated to data.

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How to find a small target on a surface? Surface-assisted search dynamics

Jaeoh Shin[1] and Anatoly Kolomeisky[1, 2]

***Short Abstract* — The process of a reactant search for a target located on a surface is ubiquitous in Nature. We study the dynamics of a reactant search for a small target on a two-dimensional surface from the bulk using both a continuum and discrete model. We find that depending on the scanning length λ of the reactant on the surface, which is determined by the reactant-surface interactions, the search dynamics are different and the combination of 3D and 2D diffusion can minimize the search time T. We discuss the relevance of our results with some recent experiments.**

***Keywords* — search dynamics, intermittent search.**

I. Extended Abstract

In many natural and technological systems, participating particles switch between spatial regions that are characterized by different geometries and dimensionalities. Examples include heterogeneous chemical catalysis when reacting molecules have to be absorbed to the solid catalysts before the reaction can take place [1]; transcription proteins finding the specific target sequences on DNA at the start of most major biological processes [2]. Such complex processes with intermittent search dynamics have been intensively studied in recent years, but molecular mechanism of underlying phenomena still remain not well understood.

We present a comprehensive theoretical approach to describe the search process for the target, which is localized on two-dimensional (2D) surface, while the reacting species start in the bulk solution around the surface segment. Both continuum and discrete-state stochastic methods are developed and compared with each other. We identified three major search regimes, which are characterized by comparing the surface scanning length of the reacting molecules with the size of the surface segment. A full analytical description of the search dynamics is obtained for all ranges of parameters, which is also tested by extensive Monte Carlo computer simulations. In addition, the role of the surface segment size and the location of the target are evaluated. It is found that the application of the continuum model provides only qualitative but not quantitative description of the process. The mean search times calculated in the continuum model are significantly underestimated, and the largest difference is observed in the realistically most significant 2D+3D search regime. These arguments suggest that it is not reasonable to apply the continuum model framework for understanding the molecular mechanisms of the surface-assisted search phenomena, especially for the quantitative analysis.

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Quantifying physiology-ecology feedback enables prediction of microbial community dynamics

Samuel F. Hart[1], Hanbing Mi[1], Robin Green[1], Chichun Chen[1], Jose Pineda[1], Babak Momeni[2], Wenying Shou[1]

***Short Abstract* — Predicting microbial community dynamics is critical for controlling communities. It should be trivial to predict the growth rate of a simplified community of two cross-feeding *S. cerevisiae* strains. However, a model based on parameters measured from batch cultures matches experiments poorly despite its previous success in predicting for example steady-state strain ratio. Here, we find that metabolite release rate can vary significantly with growth rate. Thus, we need to consider how physiology (e.g. growth rate) impacts ecology (e.g. metabolite release rate) in addition to how ecology affects physiology. Incorporating this ecology-physiology feedback enables prediction of community dynamics.**

***Keywords* — Microbial community dynamics; Microbial physiology and ecology**

I. Purpose

Microbial communities are ubiquitous, and impact us and our ecosystems. Commensal gut microbiota influence our body weight and immune system [1]. Microbial communities are also used in pollutant degradation [2] and in industrial production of important compounds [3]. To control microbial communities, we need to understand how interactions between species lead to community dynamics.

Here, we attempt to predict the growth rate of an engineered yeast community CoSMO (Cooperation that is Synthetic and Mutually Obligatory") [4]. CoSMO comprises two differentially-fluorescent non-mating haploid strains. The A^-L^+ strain requires adenine due to deletion of *ADE8*, and over-activates the lysine biosynthetic pathway due to a feedback-resistant *LYS21* mutation. The L^-A^+ strain requires lysine due to deletion of *LYS2*, and over-activates the adenine biosynthetic pathway due to a feedback-resistant *ADE4* mutation. Overproduced metabolites are released into the environment. In minimal medium lacking adenine and lysine supplements, the two strains engage in obligatory cooperation.

In a well-mixed environment, CoSMO dynamics can be modeled by ordinary differential equations. In our earlier study on CoSMO [4], we quantified some of the model parameters and "borrowed" others from the literature. Model parameters correspond to strain phenotypes such as metabolite release rate, metabolite consumption per new cell, death rate, and birth rates at various concentrations of the required metabolite. Our model correctly predicted, for example, the steady state strain ratio [4] and qualitative features of community spatial organization [5]. However, predictions on steady state CoSMO growth rate poorly matched experiments.

Here, we re-measure each strain's phenotypes in chemostat cultures where growth rates are set at various levels that span the steady state CoSMO growth rate. We find that both populations rapid evolve during measurements, and we devise methods to mitigate the effects of rapid evolution.

We find that within the range of environments that CoSMO experiences, lysine release rate of A^-L^+ varies significantly with growth rate. Thus, besides modeling how ecological interactions affect cell physiology (growth rate), we need to consider how cell physiology (growth rate) affects ecological interactions (release rates). When we incorporate growth rate-dependent release rate (physiology-ecology feedback), our model agrees with experimental results.

II. Conclusion

Quantitative prediction of microbial community dynamics may require quantifying physiology-ecology feedback. When model parameters are measured (instead of "borrowed"), model-experiment discrepancy motivates new discoveries.

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Partial inhibition of HIV cell-to-cell spread results in more HIV infected lymph node cells

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***Short Abstract* — HIV is known to cause cell death. In infections with multiple HIV transmissions per cell, partial inhibition may lead to increased numbers of live infected cells as eliminating surplus viral copies reduces cell death. Using a cell line and lymph node cells, we observed increased numbers of live infected cells when infection was partially inhibited with the antiretroviral efavirenz or antibody. We observed more live infected cells, but fewer HIV DNA copies per cell, relative to no drug. Hence, reduction in HIV transmissions per cell may increase live infected cell numbers in environments where the force of infection is high.**

***Keywords* — HIV, cell-to-cell spread, multiple infections per cell, cell death.**

I. Background

HIV infection is known to result in extensive cell depletion in lymph node environments [1], where infection is most robust [2]. Cell death occurs by several mechanisms. For example, double strand breaks in the host DNA caused by integration of the reverse transcribed virus results in cell death by the DNA-PK mediated activation of the p53 response [3].

Multiple infections per cell have been reported in cell-to-cell spread of HIV In this mode of HIV transmission, an interaction between the infected donor cell and the uninfected target results in directed transmission of large numbers of virions [4-7]. Here we examined the effect of reducing the number of HIV transmissions per cell on the number of live infected cells in cell-to-cell spread.

II. Results and Conclusion

We introduce a model of infection where each donor to target transmission leads to an infection probability r and death probability q per infection attempt, and a number of infection attempts per cell n. In our experimental system, one infection attempt is measured as one HIV DNA copy. The probability of a cell to be infected and not die after it has been exposed to n infection attempts is:

$$P_n = (1-(1-r)^n)(1-q)^n. \quad (1)$$

We experimentally measured parameter values for this relation. n was measured by PCR to detect the number of reverse transcribed copies of viral DNA in the cell by splitting each individual infected cell over multiple wells. n=15 in RevCEM cell line infection and 20 in primary human lymph node cells. We also experimentally measured r and q, which were observed to be 0.28 and 0.15 respectively.

We observed that Equation (1) resulted in a peak in live infected cells when the value of n was decreased from that measured in the absence of inhibitor. We therefore dialed down the number of HIV DNA copies per cell using the reverse transcriptase inhibitor efavirenz or a neutralizing antibody and obtained an increase in live infected cells with partial inhibition, followed by a decrease in infection once inhibition was further increased.

Partial inhibition of HIV infection may therefore provide a surprising advantage to the virus as it may reduce infection mediated cell death and hence increase the number of live infected cells.

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Population dynamics of epigenetic oncogenesis

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***Short Abstract* — The role of the tumour microenvironment in cancer initiation is poorly understood. We present and analyze a microenvironment population dynamics model motivated by an attractor landscape view of cellular states. Cell types are regarded as stable minima of a quasi-potential function that harbours additional spurious minima, some of which may be pre-cancerous. Stochastic transitions between healthy and mutagenic minima enable mutation acquisition at the population level (oncogenesis). This transition rate may be amplified by tumour signals, causing positive feedback. A three-state ODE model describes this process. We analyze its dynamics with and without tumour feedback, discussing implications for cancer initiation.**

I. Purpose

THE role of the tumour microenvironment in the early stages of cancer initiation remains poorly understood. We rigorously investigate its potential role using a model inspired by an attractor landscape view of cellular phenotypes. This view associates a cell's "state" with its set of gene transcript levels [1,2]. Physiological cell types are interpreted as minima of a quasi-potential function that captures epigenetic regulatory interactions. Due to the high-dimensionality of the corresponding landscape, it is generally the case that unexpected or "spurious" stable points will exist, some of which could exhibit deleterious phenotypes and have been hypothesized to be pre-cancerous [2,3].

The subset of spurious phenotypes with mutagenic potential (e.g. via p53 dysregulation) is hypothesized to be a precursor to the emergence of cells with cancer driver mutations [2,3]. Accordingly, the role of tumour microenvironment signalling is of particular interest, as evidence suggests tumour signals may amplify the transition rate from healthy to spurious cell types (e.g. via tumour exosomes [4]). Our modelling formalizes these hypotheses and investigates their effects on cancer initiation.

II. Model

A minimal three-state ODE system is constructed by considering three cell classes: x (the set of non-mutagenic or healthy attractors), y (the mutagenic spurious attractors), and z (cells which have acquired irreversible cancer driver mutations). The total population $N = x + y + z$ is dynamically conserved, representing pre-cancerous metaplasia (i.e. no outgrowth). Cells can switch reversibly (epigenetically) between the x and y attractor sets, but only cells which have entered the y state can acquire mutations at non-negligible rates. Our model accounts for state transitions, growth, immigration, and population-regulating cell death.

III. Analysis

We consider the model's behaviour in two scenarios: 1) fixed transition rates, and 2) positive feedback in the form of mutant population-dependent transition rate between x and y.

Without feedback there is exactly one physical and stable fixed point of the dynamics for all physical parameter values. As parameters such as the growth rates of states y or z are varied, the stable fixed point undergoes a continuous transcritical bifurcation between two regimes: "all-z" ($z_\infty = N$) and "low-z" ($0 \leq z_\infty < N$). These regimes can be interpreted as pre-cancer and cancer-free states, respectively.

Positive feedback on the x-to-y transition rate leads to rich dynamics, including bistability (via a pitchfork bifurcation) of the all-z and low-z fixed points over a wide range of parameters. We investigate switching between the monostable and bistable regimes as a function of y and z growth rates. The resulting "phase diagram" is reminiscent of a liquid-gas transition (discontinuous phase switching below a critical point but continuous switching beyond this point). Stochastic simulation of the first-passage time to acquire a second driver mutation in each regime indicates that small deviations in mutation and subpopulation growth rates can accelerate or inhibit tumour development.

IV. Conclusion

Analysis of our minimal population dynamics model reveals significant consequences of positive feedback in the tumour microenvironment. Namely, if tumour signals can induce reprogramming to spurious phenotypes in healthy surrounding tissue, then mutated cells can dominate the total population even if they grow much more slowly than healthy cells. In addition, mutants can fixate with a mutation rate that is orders of magnitude lower than what is needed in the absence of signalling. This suggests that blocking pro-tumour microenvironment signals alone (i.e. without direct targeting of tumour cells) could inhibit tumour development.

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Modeling adjuvant chemo- and radiotherapy

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***Short Abstract* — Surgery that removes solid tumors can be preceded or followed by adjuvant radio – chemo- or radiochemotherapy. These therapies can be administered in a different order. In this work we present analysis of different therapy protocols, aimed at finding a hypothetical optimal one and checking what results it yields in a heterogeneous population of patients. Moreover, we discuss differences in modeling results between a standard linear-quadratic model of radiotherapy and an approach that takes into account dynamic effects of radiation.**

***Keywords* — adjuvant therapy, radiotherapy, chemotherapy, Kaplan-Meier curves, optimal control.**

I. Background

THE The development of adjuvant anticancer therapies is largely empirical, based on the outcome of a prospectively designed randomized clinical trials. One of the most intensively explored areas in the clinic is comparison of the effectiveness of postoperative (or preoperative) radiotherapy to the effectiveness of postoperative (or preoperative) radiotherapy combined with chemotherapy.

Dynamical models of cancer growth employ a variety of modeling techniques, from ordinary differential equations to agent-based models. Therapy is incorporated in tem in the form of control variable, thus facilitating formal approaches to optimize therapy protocols or comparison of different ways to model chemo- and radiotherapy [1-2]. In this work, we focus on two different approaches to model radiotherapy and include it in a combined protocol with chemotherapy. The comparison is based on patient survival curves obtained through simulation of tumor growth for a population of heterogeneous patients, differentiated by parameters corresponding to the tumor growth rate and responsiveness to therapy.

II. The Model

One of the most widely used models, generally accepted by clinicians, is the Gompertz model. In this work, chemotherapy is incorporated in it in the simplest form, without taking into account cell-cycle specificity nor drug resistance phenomenon, as one of the control variables $\gamma(t)$ and the simplest pharmacokinetics model. The effects of radiation therapy are most often described by the so called Linear-Quadratic (LQ) model and switches in state trajectory, following radiation events. While this has been tested as well, in an alternative model version we also take into account repair of radiation-induced DNA damage with the half time μ then the decay rate R, following the line of reasoning presented in [3]. Denoting by $N(t)$ tumor size and by $d(t)$ the radiation dose rate at time t, and the system under consideration is described by the following set of equations:

$$\dot{N} = -\rho N log\frac{N}{N_\infty} - c\gamma N - (\alpha d + \beta d f)N \quad (1)$$

$$\dot{\gamma} = -\lambda\gamma + u \quad (2)$$

$$\dot{f} = -\mu f + d \quad (3)$$

Where (2) represents pharmacokinetics and (3) fast repair mechanisms, following irradiation events.

III. Model Analysis and Conclusions

Two main questions arise in such approach to modeling: (i) what are the differences in tumor growth dynamics under standard therapy protocols between standard LQ model and the one given by (3) and (ii) how should intertumor heterogeneity be incorporated for analysis of protocols efficacy for a given population of patients.

In recent years many reports have showed a correlation between a metabolism of cancer cells and tumor malignancy (e.g., [6]). In the case of breast cancer it has been found that isoleucine, threonine, glutamine and linoleic acid, measured in serum before therapy had started, allow to differentiate patients with respect to predicted outcome of adjuvant chemotherapy [7]. Therefore, we used patient blood morphology data to create a distribution from which model parameters, corresponding to tumor growth rate and its responsiveness to therapy, were sampled. Thus, a population of virtual patients was created. Therapy protocols were simulated for each patient, with a predefined threshold of tumor size defining patient death. Subsequently, survival curves were calculated. The results showed that blood morphology can be used to estimate parameters in tumor growth models, making it possible to model a heterogeneous population of patients.

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Quantification of Tumor Burden in Patient-Derived Orthotopic Models of Ovarian Cancer by Fluorescent and Bioluminescent 3-D Imaging

Mara P. Steinkamp[1,2], Irina Lagutina[2], Bridget S. Wilson[1,2]

***Short Abstract* – Ovarian cancer is often diagnosed at a late stage when cancer cells have disseminated throughout the peritoneal cavity and formed tumors on multiple organs. We have established orthotopic models of disseminated ovarian cancer from patient-derived cancer spheroids to test novel therapies in the context of the peritoneal microenvironment. Fluorescent and bioluminescent imaging and 3-D reconstruction allows us to estimate changes in tumor burden in these patient-derived orthotopic models. The pan-ErbB inhibitor, afatinib, alone or in combination with paclitaxel, was tested in these models.**

Keywords — ovarian cancer, in vivo imaging, patient-derived xenografts, afatinib

I. Background

Preclinical studies in mouse models of cancer are important for initial validation of novel therapies. It has become increasingly apparent that the tumor microenvironment plays an important role in the growth and metastasis of tumors as well as in the response of cancer cells to treatment[1]. Therefore, orthotopic tumors growing in their preferred microenvironment may best reflect human cancer. We have used an orthotopic model of disseminated ovarian cancer to demonstrate that ovarian cancer tumors exhibit different morphology depending on the site of metastasis[2]. These studies were used to parameterize a 3-D cellular Potts model and examine drug penetration in ovarian tumors[3]. To better represent tumor heterogeneity, we have now developed orthotopic patient-derived xenograft models. The development of new techniques in *in vivo* imaging are improving the estimation of tumor burden in these models. Here, fluorescent and bioluminescence imaging was used to assess changes in tumor burden during treatment with a pan-ErbB receptor inhibitor compared to chemotherapy or combination therapy.

II. Methods

Patient-derived xenograft (PDX) models were established by isolating cancer spheroids from fresh patient ascites fluid obtained at the time of surgery. Spheroids were injected into the peritoneal cavity (IP) of NOD-*scid* gamma (NSG) immunocompromised mice to model disseminated ovarian cancer. Five PDX lines have been established. PDX lines are maintained passaging them into new mice.

Tumor burden was assessed in non-transduced PDX models using a human-specific anti-HLA-ABC antibody conjugated to the near-infrared dye CF750. For bioluminescence imaging, cancer cells from PDX models were cultured as spheroids and transduced with an R-luciferase reporter carrying puromycin resistance. After expansion in a mouse, transduced spheroids were selected for puromycin resistance in culture before passaging in mice.

Five mice/group were inoculated with PDX cancer spheroids and treated with paclitaxel, afatinib or in combination for three weeks. Tumors were imaged pretreatment and every week during treatment. Tumor burden at necropsy was compared to *in vivo* imaging. Imaging was performed on the IVIS Spectrum *in vivo* imaging system. Fluorescent imaging tomography (FLIT) or diffuse luminescence tomography (DLIT) were used to construct 3-D images of tumor positions. DLIT images can be calibrated to the luminescence rate (photons/cell/sec) to estimate the number of cells in a given tumor.

III. Results

Peritoneal tumors were visible one to two weeks post-inoculation using either fluorescent or bioluminescent probes. Treatment with afatinib, paclitaxel, or the combination reduced tumor burden over time with the greatest affect seen in combination-treated animals. Imaging of control mice at later time points was complicated by the development of ascites fluid that appears to dampen or dilute the fluorescent signal. In this case, imaging at necropsy revealed more tumors than could be seen in the live image.

IV. Conclusion

The use of 3-D in vivo imaging techniques with fluorescent or bioluminescent reporters provides a more quantitative way to assess tumor burden in orthotopic models of cancer. These techniques may provide unique data sets that could be used to parametrize multiscale models of tumor growth and response to therapies.

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Optimal sensory network for the unfolded protein response

Wylie Stroberg[1], Hadar Atkin[2], Yonatan Savir[2], and Santiago Schnell[1,3]

***Short Abstract* —Protein homeostasis requires continuous monitoring of stress in the endolplasmic recticulum. Stress detection networks control protein homeostasis by mitigating the deleterious effects of aberrant protein accumulation, such as excessive unfolded protein, protein aggregates, and misfolded proteins, with precise modulation use of chaperone production. Here, we develop a coarse model of the unfolded protein response in yeast and use multi-objective optimization to determine efficient sensing and activation strategies that optimally balance the trade-off between unfolded protein accumulation and chaperone production.**

***Keywords* — Unfolded protein response, endoplasmic reticulum stress, feedback, Pareto optimization.**

I. Background

THE unfolded protein response is a collection of cellular responses that maintain protein homeostasis in the endoplasmic reticulum (ER) [1,2]. Initiation of the response requires activation of transmembrane proteins that detect the level of stress placed on the folding machinery within the ER lumen. The detection of stress, and activation of the transcriptional responses that mitigate ER stress, are essential to the control of protein folding and malfunction of the ER stress response is related to numerous diseases [3]. At their most basic level, stress detection networks [4,5] act as controllers of protein homeostasis that seek to mitigate the deleterious effects of aberrant protein accumulation, which can lead to aggregation and misfolding, with a precise and efficient use of chaperone production [6]. Aberrant protein accumulation leads to protein misfolding and aggregation of toxic oligomers. Evidence suggests that the response is sensitive to concentrations of both unfolded protein and chaperone in the ER lumen. In this work, we employ a multi-objective optimization algorithm to balance the trade-off between the metabolic cost of chaperone production and the deleterious effects of unfolded protein accumulation in the ER. Using this framework, we analyze a set of biologically-relevant sensory networks that integrate signals from both unfolded protein and chaperone concentrations in the ER lumen to determine underlying rules governing the effectiveness of sensing network designs.

II. Results

For a sensor that detects the level of unfolded protein directly, we find that the level at which the response is activated dictates the balance of costs, but all costs can be reduced if the magnitude of the response scales gradually with the strength of the stimulus. Additionally, we show that a sensing mechanism that responds to the level of free chaperone in the ER offers more efficient control than sensors that detect unfolded proteins directly. This is the result of the chaperone-detection mechanism having asymmetric activation and deactivation thresholds. Lastly, we demonstrate that a sensor whose activation requires a combination of unfolded protein and free chaperone provides an extra degree of freedom that the cell can use to further optimize homeostatic control.

III. Conclusions

Our results suggest a strategy for the optimal design of stress sensors and provide a possible explanation as to why BiP-mitigated ER stress sensing networks have evolved. By unraveling the different factors regulating the control of the ER stress sensor, our approach can guide the design of homeostatic controllers in other biological contexts as well as synthetic biology.

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Sign epistasis induced by ribosome collisions during eukaryotic mRNA translation

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Ribosome stalling reduces protein expression in eukaryotes by activating quality control pathways. We used an inverse approach combining protein expression measurements with computational modeling to study the kinetics of ribosome stalling in budding yeast. Our expression measurements reveal a counterintuitive sign epistasis between mutations that cause ribosome stalling and those that decrease initiation and elongation rate. This sign epistasis is recapitulated by a kinetic model in which collision with a trailing ribosome stimulates abortive termination of a leading stalled ribosome. Our results illustrate the utility of quantitative modeling for dissecting the *in vivo* kinetics of co-translational quality control in eukaryotes.

Ribosomes move at an average speed of 3–20 codons per second during translation elongation *in vivo*. Since this rate is higher than the typical initiation rate of ribosomes on mRNAs [less than $1 s^{-1}$], elongation is often assumed not to affect the expression level of most proteins. Nevertheless, the elongation rate of ribosomes can decrease significantly at specific locations on an mRNA due to low abundance of aminoacyl-tRNAs. Ribosome profiling — the deep sequencing of ribosome-protected mRNA fragments — has enabled the identification of additional factors that induce slowing or stalling of ribosomes during elongation (*1*). An important question emerging from these studies is the extent to which ribosome stalling affects the expression of the encoded protein, since initiation might still be the slowest step during translation.

Predicting the effect of ribosome stalling on protein levels has been challenging because of uncertainty in our knowledge of *in vivo* kinetic parameters such as the duration of ribosome stalling and the rate of abortive termination at stall sites. While we have a detailed understanding of the kinetic steps and structural changes that occur during the normal elongation cycle of the ribosome, the 'off-pathway' events that occur at stalled ribosomes have been elucidated in only a few specific cases (*2*). Thus, development of complementary approaches, which can quantitatively constrain the *in vivo* kinetics of stalled ribosomes without precise knowledge of rate parameters, will be useful for bridging the gap between the growing list of ribosome stall sequences and their effect on protein expression (*3*).

Ribosome stalling reduces protein expression in eukaryotes by activating co-translational quality control pathways (*4*). However the kinetic events leading to recruitment of quality control factors to stalled ribosomes have not been clearly delineated. We used an inverse approach combining protein expression measurements with computational modeling to study the kinetic events that occur upon ribosome stalling in the budding yeast, *S. cerevisiae*. Surprisingly, our measurements revealed that protein expression increased from stall-containing reporters when the initiation rate or the elongation rate in the 5' region of the mRNA was decreased through 5' UTR or synonymous mutations. The same mutations decrease protein expression from control reporters that do not contain ribosome stalls. This counterintuitive sign epistasis is recapitulated by a kinetic model in which collision with a trailing ribosome stimulates abortive termination of a leading stalled ribosome. By contrast, canonical models of quality control without a stimulatory role for ribosome collisions do not exhibit sign epistasis. Modeling shows that sign epistasis requires multiple successive stalls and a collision-stimulated abortive termination rate that exceeds the forward elongation rate. Hel2, an E3 ubiquitin ligase implicated in co-translational quality control (*5*, *6*), is necessary for the sign epistasis in our protein expression measurements, which suggests that Hel2's activity is specifically stimulated by ribosome collisions. Together, our results illustrate the utility of quantitative modeling for dissecting the *in vivo* kinetics of co-translational quality control in eukaryotes.

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High-throughput screening of fluorescent probes for *in vivo* imaging

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***Short Abstract* — Fluorescent proteins (FPs) are commonly used as quantitative reporters of biological structures and events. However, their application *in vivo* is greatly limited by the low brightness and photostability of the current generation of FPs. It is therefore imperative to engineer brighter and more photostable FPs. FP properties measured in bacteria or *in vitro* often do not extend to their performance in mammalian cells. Moreover, the traditional approach to engineer new fluorescent proteins via directed evolution in *Escherichia coli* is low-throughput. To address these issues, we developed an automated microscopy-based FP screening platform. We use *Saccharomyces cerevisiae*, a eukaryotic organism that is a better proxy for mammalian cells than bacteria, to express FP variants at a single cell level. FP variants are imaged and analyzed under a microscope and the desirable variants are recovered for further screening. With our method, we can screen thousands of variants in a well of a 96-well plate in a matter of minutes. We anticipate that this platform will enable rapid development of brighter and more photostable FPs across the color spectrum.**

Keywords* — Fluorescent proteins, High-throughput screening, *Saccharomyces cerevisiae

SINCE the discovery and engineering of Green Fluorescent Protein from *Aequorea victoria* over 15 years ago, fluorescent proteins (FPs) and the tags and sensors derived from them have become ubiquitous imaging tools across biology. FPs are commonly used as reporter proteins to study gene regulatory networks, and have also become integral to genetically encoded sensors such as genetically encoded indicators of voltage and calcium. These sensors allow unprecedented cellular-level resolution of neural activity, but are yet limited in brightness and photostability [5]. Given the wide array of applications, engineering of brighter and more photostable FPs has far-reaching impact. Directed evolution in *Escherichia coli* is the standard approach to develop new fluorescent proteins. Bacteria are used because of their fast growth and low cost of maintenance. Variant libraries of FPs are created by mutagenesis and screened for desired properties, usually by picking colonies on agar plates. There are several limitations to this approach for optimizing FPs for mammalian expression; the properties of FPs which are expressed in bacteria and characterized *in vitro* do not translate directly in mammalian cells. Also, the current method of FP screening is low-throughput making the screening process slow and cumbersome.

To address the limitations of current approaches, we developed an FP screening platform that converts an epifluorescent microscope setup into an automated high-throughput FP screening platform. To facilitate the expression of the FP variants, we chose *Saccharomyces cerevisiae* (yeast) as a 'middle ground' between bacterial and mammalian systems. As eukaryotes, yeast use protein expression and folding machinery more closely resembling those in mammalian cells. Yet, yeast is also cheap and easy to work with it. Also, yeast can overexpress exogenous proteins for *in vitro* characterization [6].

To utilize the yeast system for screening, mutagenesis is performed using degenerate primers to create a library of FP variants housed in vectors designed for expression in yeast. Yeast-transformed cells are plated on a glass bottom plate and imaged under a common fluorescent light microscope set up. Captured images are segmented and analyzed, ranking cells by fluorescent intensity. Top-ranked cells are selected, and the corresponding X and Y locations of the cells are sent to the microscope. The selected cells can then be picked by thin glass pipettes and grown in liquid media. With our method, we managed to screen several hundred thousand of variants in a matter of minutes. This would be equivalent to testing a thousand 96-well plates. We anticipate that this platform will enable rapid development of brighter and more photostable FPs across the color palette.

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A Yeast Segmentation and Tracking Algorithm

N Ezgi Temamogullari[1] and Andreas Doncic[1]

Accurate segmentation and tracking of individual cells from time lapse live microscopy is essential to understand single cell behavior and signaling. Here we introduce a fully automated segmentation and tracking algorithm for budding yeast that can accurately segment and track individual yeast cells for at least 60h. The algorithm does not require any specific biomarkers and can segment cells with arbitrary morphologies (e.g. sporulating, and pheromone treated cells), with high efficiency. In addition, the algorithm is largely independent of the specific imaging method (bright field / phase), objective or image resolution.

***Keywords* — Segmentation, Yeast, Watershed, Morphological Image Processing**

I. INTRODUCTION

RECENT technological advances enabled us to follow gene expression and protein dynamics in single cells using time-lapse microscopy. To generate such data for some given cell, two requirements have to be met: First, cell boundaries have to be identified (segmentation), and second, the cell has to be tracked through time (tracking).

Here, we introduce a yeast segmentation and tracking algorithm [1], which improves the accuracy and speed of our previously published method [2]. In particular, we drastically improve its performance with respect to yeast cells with irregular boundaries such as spores and pheromone treated cells. In addition, we introduce a novel automatized seeding step, which replaces the semi-automatic seeding of the previous method and enables us to have a fully automatic segmentation algorithm.

II. RESULTS

A. Seeding

Our algorithm segments images backwards in time, i.e. it starts from the last time point and goes to the first and uses the segmented image of the previous image to track and segment the subsequent image. The first step in this process, called seeding, involves providing the algorithm the segmentation of the last image, the seed. This step was a bottleneck in the previous version of our algorithm since it was only semi-automated.

To resolve this bottleneck, we developed a fully automated seeding step that (1) pre-processes the image using morphological image analysis and applies watershed transform to the processed image, and then (2) fine-tunes the cell boundaries and automatically corrects under- and over-segmentation.

B. Segmentation and Tracking

In addition to automatizing seeding, we introduced the following improvements to our previously published algorithm [2]:

Parallelization

The new algorithm is fully parallelizable, which significantly decreases the run-time.

Cell Intersections

Imaging artifacts may create the impression that two cells are overlapping, which results in contiguous cells claiming the same area. Instead of discarding the pixels that appear to be overlapping, in this algorithm we accurately distribute them between cells.

Using existing fluorescent channels

Live cell imaging applications often have fluorescent channels in addition to the phase or the bright field image, which harbor information about the cell location that the previous algorithm did not exploit. In this algorithm, we improve segmentation accuracy by using these arbitrary fluorescent channels to generate composite images, which have higher contrast between cell and non-cell pixels.

Applications

Using our algorithm, we segment and track time lapse movies of (1) cycling cells imaged with 40X objective and (2) 63X objective, (3) of sporulating cells imaged with 40X objective, (4) pheromone treated cells imaged with 63X objective, and (5) bright field images of cycling cells imaged with 40X objective.

III. CONCLUSION

Here we introduce a fully automated and parallelizable algorithm that can segment yeast cells with arbitrary morphologies and imaging conditions.

IV. METHODS

The algorithm is implemented in MATLAB and is available upon request.

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Precision in a rush: *hunchback* pattern formation in a limited time

Huy Tran[12], Carmina A Perez[3], Jonathan Desponds[5], Mathieu Coppey[14], Cecile Fradin[3], Nathalie Dostatni[14] and Aleksandra M. Walczak[24]

Fly development amazes us by the precision and reproducibility of gene expression, especially since the initial expression patterns are established during very short nuclear cycles. Recent live imaging of *hunchback* promoter dynamics shows a stable sharp binary expression pattern established within the 3 minute interphase of nuclear cycle 11. Considering expression models of different complexity we show how a limited readout time imposed by short developmental cycles affects the ability of the gene to read positional information and express reliably. Comparing our theoretical results to real-time monitoring of the *hunchback* transcription dynamics in live flies we discuss possible regulatory strategies.

***Keywords* — Drosophila melanogaster, hunchback, gene expression regulation, positional sensing**

I. Introduction

The *hunchback* pattern formation in *Drosophila melanogaster* has been extensively studied as a model to understand how cell identity is established reliably at the correct time, correct location in space [1-2]. In this system, the *hunchback* gene extracts the positional information from transcription factor gradients, including Bicoid, and forms a sharp binary-like expression pattern that is key for the development of properly proportioned individuals. Driven by recent real-time monitoring of the *hunchback* transcription activity in live fly embryos [3-4] using the MCP-GFP system [5], we explore the constraints that a limited readout time coming from the short developmental nuclear cycles (3-10 mins in cycle 11-13) imposes on the ability of the gene network to readout positional information.

II. Results

Using a theoretical model of gene expression regulation at the molecular level [6], we show that the steep expression pattern can emerge from the cooperative interactions between transcription factors and the operator sites of the *hunchback* promoter. However, this cooperativity comes at the cost of a long pattern formation time and an increased heterogeneity of gene expression at the pattern boundary. The identified trade-off has an impact on the system's "positional resolution", which corresponds to the minimum morphogen concentration difference at the expression boundary that neighboring nuclei are able to detect. We find that the kinetic parameters controlling gene expression yielding the highest positional resolution depend on the nuclear cycle duration (Fig. 1).

Our results show that if transcription regulation is done by a single transcription factor (e.g. Bicoid) via the operator sites of the *hunchback* promoter alone, the *hunchback* readout is likely to adopt a pattern of moderate steepness, much less than the experimentally observed steepness in flies [7]. We hypothesize that multiple transcription factors are required to cooperate with one another to achieve such high steepness while ensuring that future cell identity can be correctly encoded in the *hunchback* readout.

Finally, we present our first attempts to validate the hypothesis experimentally using synthetic promoters derived from the *hunchback* promoter, where the operator sites of known transcription factors (Bicoid, Hunchback, Caudal, Zelda) are present/absent.

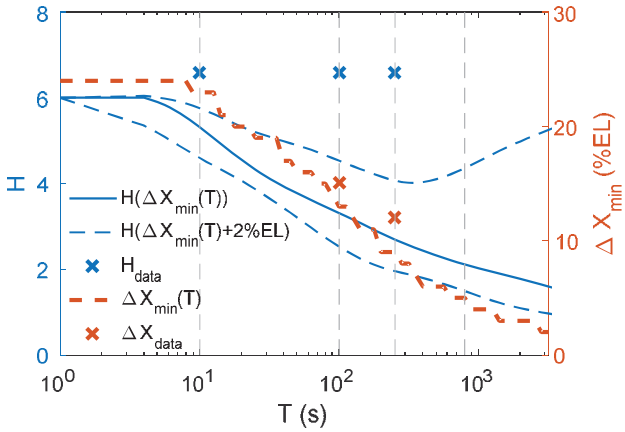

Figure 1. Optimal Hill coefficient H (solid blue line) yielding the best positional resolution (ΔX) (dashed orange line) for the varying readout time T, along with the confidence interval (blue dashed) with 2% EL (embryo length) tolerance. Also shown are the observed Hill coefficient (blue crosses) and positional resolution (orange crosses) in live flies.

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Analysis of Hierarchy in Gene Expression Reveals Principles Underlying Metastatic Aggressiveness of Inflammatory Breast Cancer

Shubham Tripathi[1,2], Mohit Kumar Jolly[2], Wendy A. Woodward[3], Herbert Levine[2], and Michael W. Deem[2]

***Short Abstract* — We quantified the hierarchical organization in the networks of collective dissemination-associated and inflammatory breast cancer (IBC)-associated genes in multiple cell lines and tumor samples. CCC, a measure of hierarchical organization, was found to be higher in epithelial versus mesenchymal cell lines and in IBC versus non-IBC breast tumor samples, for both sets of genes. Our results suggest a role for retention of some epithelial traits by disseminating tumor cells in the metastatic aggressiveness of IBC and a correlation of hierarchy in expression of collective dissemination-associated genes with high rates of metastatic relapse. Our analysis indicates that the CCC encodes additional information regarding the complexity of gene expression in cancer cells and can be a prognostic factor for IBC and other aggressive cancer sub-types.**

***Keywords* — collective dissemination, inflammatory breast cancer, EMT, hierarchy, E/M hybrid, CCC.**

I. Introduction

CLUSTERS of circulating tumor cells (CTCs), despite being rare, may account for more than 95% of metastases [1, 2]. Cells in these clusters do not undergo a complete epithelial-to-mesenchymal transition (EMT) but retain some epithelial traits as compared to individually disseminating tumor cells [3]. Determinants of single cell dissemination versus collective dissemination remain elusive. Inflammatory breast cancer (IBC), a highly aggressive breast cancer subtype that chiefly metastasizes via CTC clusters, is a promising model for studying mechanisms of collective tumor cell dissemination [4]. Previous studies on breast cancer and adult acute myeloid leukemia, motivated by a theory that suggests physical systems with hierarchical organization tend to be more adaptable, have found that the expression of metastasis associated genes is more hierarchically organized in cases of successful metastases [5, 6].

II. Methods

We used the cophenetic correlation coefficient (CCC) to quantify the hierarchical organization in the expression networks of two distinct gene sets, collective dissemination associated genes and IBC associated genes, in cancer cell lines and in tumor samples from breast cancer patients. Hypothesizing that a higher CCC for collective dissemination associated genes and for IBC associated genes would be associated with a more evident epithelial phenotype and with worse outcomes in breast cancer patients, we evaluated the correlation of CCC with different phenotypic groups.

III. Results

The CCC of both gene networks, the collective dissemination associated gene network and the IBC associated gene network, was higher in (a) epithelial cell lines as compared to mesenchymal cell lines and (b) tumor samples from IBC patients, as compared to samples from non-IBC breast cancer patients. A higher CCC of both networks was also correlated with a higher rate of metastatic relapse in breast cancer patients. Neither the levels of *CDH1* gene expression, nor gene set enrichment analysis could provide similar insights.

IV. Conclusions

These results suggest that retention of some epithelial traits in disseminating tumor cells as IBC progresses promotes successful breast cancer metastasis to distant organs. The CCC provides additional information regarding the organizational complexity of gene expression in comparison to differential gene expression analyses. We have shown that the CCC may be a useful metric for investigating the collective dissemination phenotype and a prognostic factor for IBC.

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Secreted INFβ Coordinates Antiviral Response

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Nature Communications **9**, 493 (2018).

***Short Abstract* — Virus-infected or poly(I:C)-stimulated cells secrete IFNβ, which coordinates population antiviral responses By combining experiments and our mathematical model of the NF-κB–IRF3–STAT1/2 signalling network, we show that IFNβ priming increases apoptosis in MEFs responding to poly(I:C) by initiating activation of STAT1/2, which in turn induces expression of antiviral components, RIG-I, PKR and OAS1A.**

I. Background

PROGRAMMED cell death, or apoptosis, is a key cellular mechanism protecting against the spread of viral infection. Virus-infected cells can activate transcription factors NF-κB and IRF3, both of which are required for the production of IFNβ. A cell receiving secreted IFNβ responds by activation of transcription factor STAT1/2 and consequent upregulation of its antiviral components. Among them are RIG-I, cytosolic receptor for viral dsRNA, PKR, inhibitor of translation, and OAS1A, functioning in mRNA degradation.

Using experiments and our stochastic model of the NF-κB–IRF3–STAT1/2 signalling network (Fig. 1), we elucidate how IFNβ coordinates population antiviral responses to poly(I:C), an analog of viral dsRNA

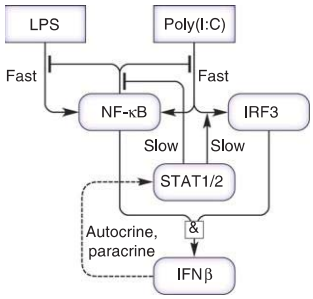

Fig. 1. Simplified diagram of the mathematical model of the NF-κB–IRF3–STAT1/2 signalling network.

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II. Results

Priming MEF cells with IFNβ on its own does not activate NF-κB or IRF3 and does not cause apoptosis (Fig. 2). Stimulation of these cells with poly(I:C) transiently activates NF-κB and/or IRF3 and causes apoptosis in approximately 25% of the population.

IFNβ priming followed by stimulation with poly(I:C) increases the fraction of cells that activate both NF-κB and IRF3, prolongs this activity, and increases the fraction of apoptotic cells over three-fold, compared to stimulation with poly(I:C) alone (Fig. 2).

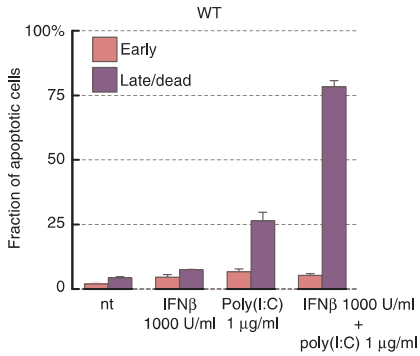

Fig. 2. Effect of INFβ priming on responses to poly(I:C) in WT MEFs.

In addition, IFNβ priming in MEF *Stat1*$^{-/-}$ cells has no effect on the activation of NF-κB and/or IRF3 in response to poly(I:C), and no effect on the fraction of apoptotic cells, compared to stimulation with poly(I:C) alone [1]. Activation of NF-κB and/or IRF3 in response to poly(I:C) alone occurs in fraction of MEF *Stat1*$^{-/-}$ cells smaller than in WT MEFs [1].

III. Conclusion

IFNβ priming sensitises naïve cells to poly(I:C) through expression of STAT1/2-dependent genes, such as RIG-I, PKR and OAS1A. When subsequently activated by poly(I:C), their protein products override the negative feedbacks on NF-κB and initiate a positive feedforward to IRF3 (Fig. 1). As a result of prolonged activity of NF-κB and IRF3, more cells commit to apoptosis, thus limiting infection spread.

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Intracellular bistable signaling in *Streptococcus mutans* competence regulation

Simon A.M. Underhill[1] and Stephen J. Hagen[1]

The regulation of genetic competence in the bacterium *Streptococcus mutans* is sensitive to quorum sensing signals, environmental factors, and stochastic gene expression. The master competence regulator ComX is directly regulated by the *comRS* system, which is viewed as a novel type of Gram-positive quorum sensing system based on a diffusible signal derived from ComS. However it has also been posited that intracellular autofeedback in *comS* is a source of bistability in competence. We combine experiments and modeling to show that the *comRS* mechanism provides both intercellular and intracellular signaling, so that its quorum sensing is enhanced by positive feedback amplification.

***Keywords* – stochasticity, microfluidics, quorum sensing**

I. Background

Bistability in gene expression, which causes a population of cells to form two subpopulations of different phenotype, is often a consequence of positive transcriptional feedback. In the bacterial pathogen *Streptococcus mutans,* entry into the state of genetic competence (transformability) has a bimodal character under certain environmental conditions. Stimulation by exogenous CSP (the 18-residue competence stimulating peptide) causes a subpopulation of cells to activate expression of *comX* (also called *sigX*), which encodes a master regulator for genetic transformability (1). Alternativ[1]ely, homogenous or unimodal expression of *comX* can be induced by providing a different exogenous peptide, XIP (comX/sigX-inducing peptide). The 7-residue XIP is the intercellular signal of a novel Gram positive quorum sensing system known as *comRS*: The XIP peptide, which is derived from ComS, binds with the cytosolic receptor ComR to form a transcriptional activator for both *comX* and *comS*. Mechanisms that process ComS and export it as extracellular XIP remain unknown. Therefore the relation between the bimodal and unimodal modes of *comX* activation, the ComRS system, and intercellular XIP signaling have been unclear.

We have argued that bimodal *comX* expression can be understood as resulting from positive intracellular transcriptional feedback via *comS*: If ComR binds endogenously produced ComS (or XIP) to activate both *comX* and *comS,* then each individual cell can autoactivate (or not) *comX,* depending on its intracellular ComS level (*comX* bimodality). By contrast, extracellular XIP gives unimodal *comX* expression because it readily enters the cell, interacts with ComR, and drives all cells in a population to express *comX* at roughly similar levels (2,3).

Here we have combined microfluidic and single cell methods with quantitative modeling to test the relationship between intercellular XIP signaling, *comS*, and *comX* activation. We used signaling mutants and reporter strains of *S. mutans* in co-cultures and under microfluidic flow to test the efficacy of intercellular signaling and its dependence on environment and on the *comS* gene.

II. Results

Our data show that possession of the *comS* gene under native control has a distinct effect on the behavior of *comX*, over a range of different environmental conditions and modes of circuit stimulation. Although extracellular XIP can stimulate *comX*, the presence of *comS* boosts the *comX* response of individual cells to the XIP signal. Further, deletion of *comS* impairs *comX* response in ways that cannot be fully corrected either by addition of exogenous XIP or by overexpression of ComS from a plasmid. These data indicate that the cell's own control of endogenous ComS synthesis always plays a role in the control of *comX*. Our data also show that neither export nor import of extracellular XIP necessarily accompanies *comX* activation, so that intracellular signaling can be more important than *comRS* quorum sensing in competence.

III. CONCLUSIONS

Our data show that a cell's own *comS* generates an intracellular feedback signal that boosts quorum sensing response in the competence circuit of *S. mutans*. The dual role of ComS as an internal signal and a quorum signal provides positive feedback amplification (4) in the competence pathway.

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Towards a coherent theory of stochastic gene dynamics: from landscapes to green field theory

John J. Vastola[1], William R. Holmes[1]

***Short Abstract* — Introducing stochasticity into previously deterministic models of gene regulatory dynamics leads to a rich phenomenology that is qualitatively different, and has greater predictive power. But how should one study the effects of stochasticity on the Waddington landscape and cell state transitions? We assess the validity of stochastic differential equations models and associated Fokker-Planck, path integral, and Lagrangian descriptions for analyzing stochasticity in gene regulation, and discuss our results on noise in simple networks and preexisting data. We also discuss a fascinating analogy with physics that may lead to a 'green' quantum field theory.**

***Keywords* — stochastic models, noise, Fokker-Planck, path integral, Lagrangian, quantum field theory**

I. Introduction

Gene expression dynamics is often treated as *deterministic*, and modeled using systems of coupled ordinary differential equations. But gene expression is actually *stochastic*, and so-called *gene expression noise* has interesting biological consequences—beyond just being something that must be buffered against. For example, noise facilitates error correction in early mammalian development by allowing wrongly differentiated cells to fix their identity via a noise-induced transition [1].

In stochastic differential equation (SDE) models, extrinsic noise seems to be well-treated by an additive stochastic term, while some experiments have suggested that intrinsic noise should be modeled by a linear multiplicative stochastic term, which can lead to very different qualitative behavior. Even if we did have the data to parameterize these models, an important theoretical question is: what tools can one use to understand the influence of additive and linear multiplicative noise, and how does each kind of noise affect the Waddington landscape and cell state transitions?

II. Results

One theoretical tool associated with stochastic dynamics is the Fokker-Planck equation: a partial differential equation that describes the time evolution of the probability distribution associated with an ensemble of cells exploring the Waddington landscape. We have found approximate solutions for fairly general dynamics which describe how steady state gene expression distributions should qualitatively depend on both intrinsic and extrinsic noise; in particular, high intrinsic noise leads to *non-Gaussian* behavior. We suspect that probing this non-Gaussianity in experimental data will allow experimentalists to determine the relative importance of extrinsic and intrinsic noise.

The Fokker-Planck equation is equivalent to a path integral [2], a tool often used in nonequilibrium statistical mechanics to describe the probability of transitions between two states in a stochastic system. We use path integral methods to calculate the probability of transitions between different states, and the relative occupancies of those states, in simple models of gene regulatory network motifs like the bistable switch. The results agree with the results obtained via brute-force simulations and the Fokker-Planck equation.

Those tools lead one to consider the idea of a Lagrangian describing average stochastic gene expression dynamics. We have identified several candidate Lagrangians, which are more or less equivalent, and will discuss some of the physical and biological principles that may be behind them. With them, one can begin to discuss approximately conserved quantities analogous to energy and momentum. We speculate that one can draw an analogy between the Lagrangian/average cell description and classical mechanics; the Fokker-Planck/single cell description and quantum mechanics; and the complicated stochastic dynamics of many interacting cells and quantum field theory. Perhaps something like a quantum field theory will allow one to describe many cells interacting, being created, and being destroyed in a developmental process.

III. Conclusion

We believe that SDEs with linear multiplicative noise terms well describe intrinsic noise, and that the phenomenology associated with these equations can be systematically studied using Fokker-Planck, path integral, and Lagrangian descriptions. These tools all suggest provocative analogies with physics, which may lead to a quantum field theory analogue for interacting cells.

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Phase Transitions and Critical Phenomena in Mutualistic Communities under Invasion

Samuel R. Bray[1], Yuhang Fan[1], and Bo Wang[1,2]

***Short Abstract* — Natural ecological communities often experience tipping points – where little changes can make a big, abrupt difference in community composition in response to species invasion and changing environments. The nature of these tipping points is still poorly understood. By analytically solving for species-resource interactions in a mutualistic community, we demonstrate these tipping points represent phase transitions similar to those described in many physical systems. The order of these transitions offers insight to the underlying community network and suggests strategies to construct barriers in combating generalized invasions such as antibiotic resistant pathogens and cancer cells.**

***Keywords* — Synthetic ecosystem, microbial communities, population modeling**

INTRODUCING novel species to an ecosystem is a fundamental problem that has many practical applications, including probiotic therapy, collective antibiotic resistance, biodiversity preservation, and ecological engineering. Introduction of "invader" species can lead to a variety of outcomes, ranging from native community stabilization to complete community collapse and replacement by invader [1]. While empirical studies exist [2], it remains a challenge to predict invasion outcome based on species' interaction network topography and invader strategy. Previous theoretical work on community structure have mostly focused on either analyzing the stability of an ecosystem under small perturbations around a certain equilibrium (so-called dynamical stability) [2], or defining the range of parameters in which the system remains stable and always returns to a fixed point (so-called structural stability) [3]. However, these studies have not yet addressed how and when an ecosystem may switch from one stable point to another under strong perturbations. The challenge of species invasion is one obvious perturbation of such kind.

Transitions between stable states are often characterized by phase transitions, a universal phenomenon in the physical world. Phase transitions include first-order transitions, during which the system can change only abruptly from one state to another, and second-order transitions, when systems can change continuously but discontinuity, or divergence, exists in the susceptibility of the system to external perturbations. The nature of the phase transition reflects the organization and interactions within the system. The conceptual difficulty here is that physical phase transitions typically emerge in systems with long-range correlations, which are difficult to capture and often analytically intractable with classical ecological models that primarily rely on pairwise species interactions [4]. Here we explore the impact of boundary conditions, specifically the availability of resources, on ecosystems under invasion. Our mechanistic approach builds on a simple mutualistic community that contains cross-feeding species with limiting resources explicitly modeled. This model builds on the classic consumer-resource model, in which resources are required for species growth, and no other nonlinear or long-range interactions are considered [2]. The stability and dynamics of such systems have been extensively characterized both theoretically and experimentally [2,4], providing a well-defined start point to solve for their behaviors under invasion.

The analytical tractability of this model enables us to show that community composition undergoes phase transitions in response to species invasion. The exact outcome can be predicted based on community topography and constraints of environmental resources. The high susceptibility of the system around phase boundaries also predicts nonlinear amplifications of variations during community assembly and species invasion, a phenomenon that has been widely reported in empirical studies [2,5]. The discontinuous nature of the transitions offers a new explanation for the unpredictability in adaptation, where small beneficial changes can build up to catastrophic outcomes. The observed phase transitions have implications in a broad practical problem set, including spatial patterning arising from ecological interactions, rejection of antibiotic resistant bacterial strains using commensal communities, and early preventive strategies to stop invasion of healthy tissue by cancer cells [6].

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Differentially Regulated Pathway Analysis of RNA-Seq by Deep Learning

Huijing Wang[1] and Christian Ray[2]

***Short Abstract* — High throughput RNA sequencing technology is widely used as a tool for transcriptomic analysis in recent years. Large network inference from traditional RNA-Seq differential expression analysis can be limited and biased by researcher subjectivity. As deep learning plays an advantageous role in motif recognition and classification for big data, here we propose training artificial neural networks (ANN) on RNA-Seq abundance data for studying a bacterial phenotypic transition into the persister state. Automated pathway activation recognition by ANN may potentially provide an efficient, accurate, and adaptive tool for network inference.**

***Keywords* — High throughput, RNA-Seq, data inference, deep learning, neural network**

I. Introduction

Bacteria are everywhere and integral to human life. Slow growing pathogenic bacteria are able to tolerate long-term treatment without a cure because of so-called persister cells. Contrary to the common mechanism of stringent response-mediated persister formation, our lab's previous findings show that a novel type of persister occurs in the *E. coli* strain B REL606 when cultured in minimal media containing excess lactose. This effect may arise from fluctuations in critical metabolic pathways [1].

High throughput RNA sequencing technology is essential to biological fields such as epigenomics and transcriptomics [2]. As machine learning (ML) has rapidly evolved into a popular tool in many fields, ML implementations for analyzing RNA-Seq are being developed for sequence analysis and differential expression analysis [3, 4]. However, no implementation of ML is available for pathway analysis, which is necessary for identifying regulatory and metabolic pathways involved in novel phenotypes such as lactose-mediated persister formation. We applied deep learning to unveil the underlying pathway dispersion responsible for the novel persister phenotype.

II. Methods

Unlike image data, transcription counts are independent of data orientation, but connected by the underlying regulatory network. The counts are affected by multiple factors, such as noise, enzyme efficiency, and gene regulation. Here we use TensorFlow [5], a python ML package, to create ANN for RNA-Seq count profile.

A. ANN for identifying differentially regulated pathways (DRP)

We used two methods for DRP detection.

The first method involves using KEGG, a knowledge-based pathway database [6]. We trained an ANN to identify known KEGG pathways and modules. This classifier probabilistically recognizes or rejects gene clusters that have similar transcriptional patterns without explicitly considering regulatory network topology because the pathways and modules are given.

The second method considers regulatory network topology as a component for depicting pathway interactions. Without knowledge-based pathway information, we train the ANN to identify different network motifs with increasing complexity. In this way, comparing existing pathways with the machine-learned pathways, we can validate the ANN as well as potentially discover unknown pathway interactions.

B. Non-ML differential expression analysis

We also performed non-ML DRP analysis by locating differentially expressing genes, which might be responsible for bacterial phenotypic switches in known regulatory pathways in KEGG. This step allows us to compare our machine learning methods with well-established RNA-Seq analysis methods for large network inference.

III. Conclusion

Deep learning by artificial neural network is an unbiased method for processing high-dimensional reduction problems. Once a proper ANN is established for cell expression profiles, it can reveal hidden regulatory patterns in large networks, with which we expect to find clues about bacterial persister formation.

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Measuring transcription at a single gene copy illuminates RNA dynamics and reveals intracellular correlations

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***Short Abstract* — Gene regulation consists of a series of stochastic, single-molecule events, resulting in substantial randomness in RNA production between individual cells, and even between the individual copies of the same gene within a single cell. To characterize the stochastic kinetics of transcription in *E. coli* at the resolution of individual gene copies, we combine RNA and gene-locus labeling to simultaneously detect a gene of interest and measure its transcriptional activity, in individual bacteria.**

***Keywords* — Transcription, transcription kinetics, single cell, single gene copy, smFISH, RNA life history, correlation, cell cycle.**

I. BACKGROUND

Stochastic gene expression gives rise to population heterogeneity, which has been extensively studied using single-cell measurements for more than a decade [1]. However, some of the observed cell-to-cell variability may not be casued by stochasticity, but by deterministic factors such as cell size [2], gene copy number [3], correlation between gene copies in the same cell, and the cell-cycle phase [4]. Measuring these "hidden variables" will thus reveal a less random picture of transcription. Towards removing cellular hidden variables, we aimed to measure transcription from a single gene copy in individual cells.

II. RESULTS

A. Single-molecule FISH and gene locus detection

To detect individual gene copies, a set of *tetO* operators is inserted near the gene of interest in the *E. coli* chromosome [5]. The RNA transcribed from the gene is labeled by a set of fluorophore-labeled DNA oligo probes [6]. The observed co-localization of RNA signal and gene signal enables us to measure active transcription at the resolution of individual gene copies, in individual cells.

B. Kinetic model describing RNA dynamics

We developed a theoretical model describing RNA dynamics. By fitting the model (in both deterministic and stochastic formalisms) to our single-cell experimental data, we are able to obtain the kinetic parameters characterizing RNA life history: the probabilistic rate of promoter switching, transcription initiation and elongation, RNA release and degradation.

C. Correlation between different copies in the same cell

We examined the activity of individual copies of the lactose promoter P_{lac} in cells having two copies of the gene. We found that the two copies were either highly correlated or almost independent, depending on the growth conditions.

D. Correlation of gene activity to the cell cycle

We examined the transcriptional activity of P_{lac} and P_R at different times along the cell cycle, at different expression levels and growth conditions. We found that the transcription of a strong "constitutive" promoter (P_R) closely follows the gene dosage. However, for a promoter under tightly-repressed conditions (P_{lac}), we observed a transient increase of transcriptional activity upon gene replication, as has been speculated before [7].

III. CONCLUSION

The existence of these gene-copy and cell-cycle correlations demonstrates the limits of mapping whole-cell RNA numbers to the underlying stochastic gene activity and instead highlights the contribution of previously hidden variables to the observed population heterogeneity.

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Inter-head tension of cytoplasmic dynein regulates the coordination between two heads

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***Short Abstract* — Studying the coordination between two heads of a motor protein is crucial to understand the walking mechanism of the motor protein on cytoskeletal tracks. Previous experiments found that inter-head tension of a cytoplasmic dynein was able to regulate the coordination between its two heads. However, the molecular origin is largely unknown. Here we utilized a structure-based coarse-grained model to investigate the conformational changes of a cytoplasmic dynein monomer responding to opposite forces. Our simulation successfully explained the experimental observations and thus provide a molecular basis to understand the walking pattern of cytoplasmic dynein.**

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Predicting Plasmid Maintenance and Abundance in Complex Microbial Community

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***Short Abstract* —Predicting whether a plasmid can be maintained in heterogeneous microbial communities is challenging. This is due to the large number of subpopulations and complexity of conjugation networks. Here, we establish a general theoretical framework to describe the dynamics of plasmid distribution across species. With this framework, we derive a general metric to predict plasmid maintenance and abundance in arbitrary microbial community. We validate the predictive power of this metric with simulations of random conjugation networks as well as experimental data found in the literature.**

***Keywords* — plasmid abundance, conjugation, microbial community, segregation, growth burden, plasmid-centric model, species, mathematical modelling.**

I. INTRODUCTION

Plasmids are one of the most important carriers of accessory genes in microbial communities, and reside at the core of horizontal gene transfer [1,2]. A plasmid can replicate itself, transfer from one species to another through conjugation, or be incorporated into the genome of its host [3,4]. Whether a plasmid is maintained or lost within a microbial population, determines whether the functions carried by the plasmid, such as antibiotic resistance, are exhibited [4,5].

Despite its importance, the general understanding of the criterion for plasmid maintenance in heterogeneous microbial communities is lacking. The conventional approach of modelling the plasmid-carrying population, which we call a 'population-centric model' [3], models all possible species-plasmid combinations. Using this approach is challenging because the complexity of the model increases exponentially with increasing numbers of plasmids.

Here, we establish a plasmid-centric model. Compared with the conventional approach, this new framework greatly reduced the complexity of model formulation and mathematical simulation.

II. MATHEMATICAL MODEL

Four dynamic processes are described in this model: (1) species growth, (2) plasmid conjugation, (3) plasmid segregation, (4) system dilution. For a m-species and n-plasmid population, the framework contains two groups of ordinary differentiation equations (ODEs) which describe the population size of each species and the abundance of each plasmid in each species. These equations can be combined into two in the form of matrices, regardless of the numbers of species and plasmid in the community:

$$\frac{dP}{dt} = A \circ U_p \circ P \circ C - D \cdot P,$$

$$\frac{dQ}{dt} = B \circ U_Q \circ Q \circ \bar{C} + (\bar{P} - Q) \circ H \circ \bar{Q} - D \cdot Q - K \circ Q.$$

Here, $\circ$ is the Hadamard multiplication between the matrices. P and Q are the matrices representing species sizes and plasmid abundance, respectively. A and B are the burden or benefit caused by the plasmids. U_p and U_Q are the maximum growth rates. C is the matrix of growth capacity. K is the matrix of plasmid segregation rate constants. H is the matrix of conjugation rate constants.

The population-centric model requires $m2^n$ ODEs and involves $nm^2 2^{2n-2}$ conjugation pairs, while the plasmid-centric model only requires $m(n+1)$ ODEs and nm^2 conjugation pairs.

III. CONCLUSIONS

Despite the complexity of the communities, there exists a universal indicator, ω, for plasmid abundance. ω is defined as:

$$\omega = \frac{\bar{\eta}}{\frac{\bar{\mu}}{\bar{\mu} - \bar{m}D}\left(D + \bar{\kappa} - \frac{D}{1+\bar{\lambda}}\right)}$$

$\bar{\eta}$, $\bar{\mu}$, $\bar{\kappa}$ and $\bar{\lambda}$ are the weighted average of the conjugation rates, maximum growth rates, segregation rates and burdens, respectively. Simulations with randomized parameters show that $\omega > 1$ is the criterion for plasmid maintenance, which provides a simple reference of the fate of each plasmid. The values of ω correspond well with the final abundance of the plasmids, regardless of species number, plasmid number, community connectivity, or species coexistence.

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Synthetic NF-κB: A Building Approach to Study Complex Signaling Behaviors

Ping Wei[1]

***Short Abstract* — The precise dynamic features in cell signaling play crucial roles in regulating various cellular functions. Due to the complexity and redundancy in natural cells, it remains challenging to completely understand how the complex temporal behaviors are programmed in parameters or structures of the signaling circuits. To overcome such problems, we took a synthetic approach to reconstitute the human nuclear factor κB (NF-κB) system in S. cerevisiae. This simple but highly tunable circuit allows us to systematically explore the design principles of oscillatory signaling dynamics.**

***Keywords* — Signaling Dynamics, NF-κB, Oscillation, Waveform, Frequency, Circuit, Negative Feedback, Synthetic Biology.**

Quantitative features of cellular signaling behaviors have drawn much attention due to their capabilities to carry extra information to regulate comprehensive downstream cellular events [1-2]. More recently, it has been found that different temporal behaviors or signaling dynamics of Msn2 in yeast and p53 in mammals resulted in dramatically different cell fates [3-5]. Remarkably, such quantitative signaling behaviors were often oscillatory or pulsatile. The particular properties of oscillatory signaling dynamics (e.g., amplitude, frequency) have shown great benefit to coordinate or differentially regulate gene expression in stress signaling and inflammatory response [6]. It remains incompletely understood how the properties of such oscillatory dynamics are controlled in natural systems and how they could be tailored synthetically.

The NF-κB in immune response is one of the well-known signaling systems to be highly dynamic, responding to various extracellular antigens or cytokines. Notably, the fluorescence tagged NF-κB protein was found to be activated in a damped oscillatory fashion in response to tumor necrosis factor α (TNF-α) [7-8]. The core design of the circuit underlying this oscillator is similar to that of many biological oscillators; a negative feedback loop with time delay, usually consisting of a transcription factor (NF-κB) and an inhibitory protein (IκB). However, in practice, because the native NF-κB system exists in the complicated context of mammalian cells, it is difficult to specifically experimentally probe and rewire the circuit components of such oscillatory signaling networks.

Here, we designed and built a robust and tunable synthetic oscillatory signaling circuit in *Saccharomyces cerevisiae*. By recapitulating a human NF-κB module in yeast, the design emphasized orthogonality and predictable tuning performances. Additionally, the circuit contains a synthetic promoter module that can program oscillatory dynamics at the transcriptional level and importantly also contains a synthetic phospho-degron module that allows for programming at the post-translational level. We initially operated the circuit with a single negative feedback loop and found that the peak shape and the period of the oscillatory waveforms could be tuned using a combination of three parameters, including the protein level of RelA, negative feedback strength, and the protein stability of IκBα. Guided by model prediction, we next found a unique circuit structure with two-layers of negative feedback loops enabled frequency-only tuning of oscillatory waveforms.

We conclude that the signaling dynamics in such synthetic NF-κB circuit could be quantitatively customized through carefully selected circuit parameters and circuit structures. We propose that the design principles established here enable function-guided design of signaling controllers to meet the requirement of the increasing sophisticated and precise regulation of diverse synthetic biology applications.

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Noise Analysis in Biochemical Complex Formation

Zikai Xu [1], Khem Raj Ghusinga [1], and Abhyudai Singh [1]

***Short Abstract* — Several biological functions are carried out via complexes that are formed via multimerization of either a single species (homomers) or multiple species (heteromers). Given functional relevance of these complexes, it is presumably desired to maintain their level at a set point and minimize fluctuations around it. Here we consider two simple models of complex formation -- one for homomer and another for heteromer of two species -- and analyze effect of important model parameters on the noise in complex level. In particular, we study the effect of (i) sensitivity of the complex formation rate with respect to constituting species' abundance, and (ii) relative stability of the complex as compared with that of the constituents. By employing an approximate moment analysis, we find that for a given steady state level, there is an optimal sensitivity that minimizes noise (quantified by fano-factor; variance/mean) in the complex level. Furthermore, the noise becomes smaller if the complex is less stable than its constituents. Finally, for the heteromer case, our findings show that noise is enhanced if the complex is comparatively more sensitive to one constituent. We briefly discuss implications of our result for general complex formation processes.**

***Keywords* — complex noise, stochastic process, linearization**

I. Introduction

Formation of biochemical complexes plays vital role for most cellular processes, including gene regulation, signal transduction [1]. Given their importance, it can be argued that their level must be closely regulated so as to achieve robust function. However, many species are present at low copy numbers in cells, and thereby stochasticity (or noise) in the reactions involving them is unavoidable. Previous work indeed has shown that complex formation might be an important mechanism in control of noise in gene regulation [2].

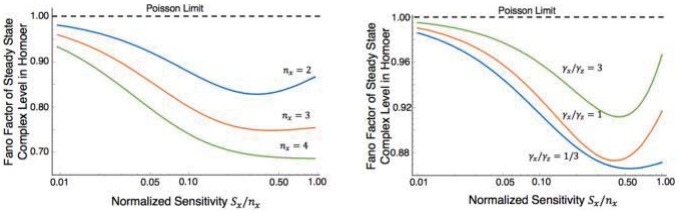
Here we investigate the effect of various attributes of the complex formation process on the noise in the complex level. We focus on the steady-state noise in two toy models of complex formation. In the first model, a single species forms a homomer whereas in the second model two species interact to form a heteromer. Not only these toy models themselves are appropriate for analysis of some real biological examples [3], but they also hint towards what behaviors might arise in more complicated scenarios.

Our strategy to analyze the noise behavior relies upon using moment dynamics of these toy models. Due to nonlinearities in these models, however, the moment dynamics is not closed and we use a linear approximation of the system at its steady-state to estimate the noise behavior.

Interestingly, our analysis reveals that noise in complex level has a U-shape profile with respect to sensitivity for both homomer and heteromers. Moreover, we find that for these toy models, if the complex is relatively unstable (i.e., it degrades faster) as compared with its constituents, the overall noise profile shifts downwards. We also analyze the heteromer with different sensitivities for each species. In this case, the overall noise profile shifts upwards.

II. Main Results

For a homomer, its steady state noise shows a non-monotonic behavior as the sensitivity of the complex formation rate to the species level is changed. Moreover, the noise in complex level reduces when the complex is relatively unstable as compared to the species.



For a heteromer, the noise exhibits similar behavior as in the homomer case if sensitivities and other parameters of both species are exactly same. However, when the complex formation rate is more sensitive to one species, then noise in the complex increases.

III. Summary, and Future Work

We analyzed two simple models of biochemical complex formation and explored the effect of different parameters in the models. In the future, it would be interesting to explore reversible kinetics for the complex formation (i.e., dissociates to its constituents), and also self-regulation in production of the species as found in production of a range of proteins.

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An Integrative Computational Approach for Engineering Genetically Encoded Voltage Indicators

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***Short Abstract* — Neurons compute by regulating the electrical potential (voltage) across their plasma membrane. Promising tools to quantitatively monitor voltage dynamics are fluorescent biosensors called Genetically Encoded Voltage Indicators (GEVI). However, currently available GEVIs are limited by combinations of factors including sensitivity, kinetics, brightness, photostability and compatibility with two-photon microscopy. The size of the sequence space precludes saturation mutagenesis across all GEVI residues, motivating the development of quantitative approaches to guide mutagenesis to those residues most likely to tune GEVI performance. Here we report a novel computational method to identify critical biosensor residues and demonstrate its application for improving voltage indicators. We anticipate that this methodology will be of broad utility for biosensor engineering.**

***Keywords* — Genetically encoded voltage indicator, ASAP, Hidden Markov Model, evolutionary trace, electrostatic interaction, covariance**

I. Background

MONITERING neural electrical activity *in vivo* with single neuron resolution is a longstanding goal in neuroscience, but with the fast development of Genetically Encoded Voltage Indicators (GEVIs), we are getting ever closer to achieving that goal. However, aside from deficits in sensitivities and kinetics, many existing GEVIs have poor performance under two-photon microscopy, which limits their application for deep tissue imaging. Indicators of the Accelerated Sensor of Action Potentials (ASAPs) family, on the other hand, shows moderate sensitivity and millisecond-timescale kinetics under both one-photon and two-photon microscopy. The most recent variant of ASAP family-ASAP2s demonstrates higher signal-to-noise ratio than ASAP1 at all frequencies but also shows slower kinetics than ASAP1 and thus has room for further improvement [1].

Here we report a computational approach to guide mutagenesis of the voltage sensing domain (VSD) of ASAPs and thus improve their sensitivity and kinetics systematically, enabling their application in mammalian models *in vivo*.

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II. Results

While the crystal structures of ASAP and its underlying VSD are not currently available, the structure of a homologous domain from the sea squirt *Ciona intestinalis* has recently been solved. However, homology modeling the ASAP VSD is complicated by repeated patterns of positively-charged arginines in the fourth transmembrane helix, which makes it challenging to determine the correct alignment between the sea squirt and the ASAP VSD. Here we choose Hidden Markov Model, a statistical model that shows promising VSD alignment results previously [2].

Second, although crystallized structure for Ciona VSD is solved, it remains a challenge to localize functionally important sites [3]. Here we use evolutionary trace (ET), a method capable of distinguishing functionally essential residues from residues that regulate specific functional features [3], to locate candidate residues for mutation.

Third, VSD is distinct from other domain in that electrostatic interaction dominates preferable resting and depolarized conformation. Therefore, an adequate model of electrostatic interaction is required to predict the direction and magnitude of point mutation. Here we propose a model that considers VSD charged residues in the surrounding electric field.

Fourth, single-residue mutation may disrupt the intramolecular interaction and thus affect protein folding. Here we include covariance analysis to retain proper folding.

With this integrative computational approach, we successfully identified residues that tune ASAP sensitivities and kinetics.

III. Conclusion

This integrative computational approach proves to be an effective method to decrease variant library size and to identify variants with desired features. We can generalize this approach to the engineering of other GEVIs to obtain improved variants.

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A Synthetic Gene Circuit with Tunable Expression Level and Dosage Compensation for Mammalian Cells

Jin (Harvey) Yang[1], Oleg Igoshin[1], Dmitry Nevozhay[2] and François St-Pierre [3,4]

***Short Abstract* — The ever-expanding synthetic biology toolbox has seen the fast development of synthetic gene circuits due to their ability to implement diverse functions in living cells. Tunable gene circuits have been given particular attention because they enable the implementation of different output states in response to user-defined input signals through a single circuit. Previous studies have optimized the tunability by achieving linear input-output relationship [1]. The circuit, however, shows compromised tunability and substantial heterogeneity among cells under transient plasmid transfection. Transient plasmid transfection or viral transduction without genomic integration usually results in a broad distribution of DNA copy number, and cells with higher DNA copy number tend to have undesirable overexpression, leading to cytotoxicity and/or impaired transgene function. Combining experiments with computational simulations and mathematical analysis, we developed a second-generation linearly tunable circuit that applies to a broader audience with robust dosage compensation capacity to achieve homogeneous expression across individual cells and better protein localization.**

***Keywords* — synthetic biology, tunable circuit, dosage compensation, expression level, linearizer, localization**

I. Background

TUNABLE circuits are widely applied because they enable the regulation of output through an external input signal. The toggle switch circuit is one of the most well-known tunable circuits with the capability of switching between ON and OFF states depending on whether the input exceeds the activation threshold. The tunability of the toggle switch is low because the output is all or none, and a ON-state optimized for one application may not be applicable for another, so much effort is often required to adapt the circuit for different applications. In contrast, continuously tunable circuit is more user-friendly and adaptable as one can obtain a continuous spectrum of output simply by varying the input intensity. A linear input-output relationship can further improve the tunability by avoiding regions of steep change in output with little change in input level or vice versa. A synthetic circuit named the "linearizer" meets these design criteria [1]. However, the linearizer circuit showed compromised tunability and dosage compensation when transiently transfected (see Results). Therefore, we engineered a new version called Linearizer 2.0 with more robust linear tunability and improved dosage compensation.

There is a broad application for this novel gene circuit. The state-of-the-art gene delivery using viral vector in gene therapy or other applications often results in a non-homogenous expression profile as a function of distance from the injection site: cells closer to the injection site have higher expression level, while expression level decreases further away from the injection site [2]. These variations in expression level are typically undesirable, yielding many cells that exhibit either inadequate or excessive transgene expression. The Linearizer 2.0 circuit can be tuned to achieve the best balance between maximal expression and minimal cytotoxicity. We also demonstrate the potential of using this circuit to improve localization of membrane proteins that formed intracellular aggregation when overexpressed.

II. Results

A. Linearizer 1.0 improves the localization of membrane proteins when genome integrated but showed reduced tunability and no dosage compensation in transient transfection

We observed that chromosomally-integrated Linearizer 1.0 can be tuned to obtain improve plasma membrane localization of multiple membrane proteins including natural and light-gated ion channels. However, transient transfection of the same circuit produced large cell-to-cell variation and compromised tunability.

B. Development of Linearizer 2.0

Combining experiment results with computational simulations and mathematical analysis, we engineered an improved circuit design, Linearizer 2.0, that retains its linear tunability while achieving dosage compensation by combining negative feedback and incoherent feedforward loops. We will present the performance of this circuit under various expression contexts including genomic integration, transient transfection, and viral transduction.

III. Conclusion

Linearizer 2.0 is a versatile synthetic circuit for tunable expression level and dosage compensation. We anticipate it will be of broad utility in applications that benefit from tunable expression levels and reduced cell-to-cell variation.

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An artificial cell-cycle system: how network structures modulate the clock functions

Zhengda Li[1], Shiyuan Wang[2], Meng Sun[2], Owen Puls[3], Ye Guan[4], and Qiong Yang[5]

***Short Abstract* — Although central architectures drive robust oscillations, networks containing the same core vary drastically in their potential to oscillate. What peripheral structures contribute to the variation remains elusive. Systematically analyzing network structures and functions showed that, while certain core topologies are essential for robust oscillations, local structures substantially modulate the degree of robustness. Strikingly, nodes receiving incoherent or coherent inputs promote or attenuate the robustness, additively. These may explain why auxiliary structures not required for oscillation are evolutionarily conserved. We developed an artificial mitotic oscillator, combined with single-cell analysis and modeling, to understand how network structures are linked to clock functions.**

***Keywords* — Biological oscillators, robustness, network structures, motifs, microfluidics, cell cycle, synthetic circuit, time-lapse fluorescence microscopy.**

I. Introduction

In principle, a single negative feedback is required and sufficient to generate self-sustained oscillations [1]. However, known biological oscillators are organized into more complex network structures. Some of the additional structures, such as positive feedback loops, are not required for generating oscillations but are evolutionary conserved, which has raised a question of what functional role they may play. Computational studies on several biological oscillators such as cell cycles have shown that adding a self-positive feedback loop to a core oscillatory circuit can increase the oscillator's robustness [2-4]. However, whether positive feedback is necessary or sufficient to increase robustness has remained controversial. While both Wee1 and Cdc25 form positive feedbacks in embryonic cell cycles, only the one from Cdc25 is critical for robustness of oscillation period [5]. A recent study using synthetic circuits [6], has shown that adding a negative feedback to an oscillator could also increase its robustness. These studies reveal the difficulty of identifying generalizable mechanisms through analyzing only a subset of oscillators. To obtain a complete picture beyond any chosen systems, a comprehensive mapping from the entire topology space to the function space is necessary. Moreover, it is challenging but critical to test the computational predictions by experimentally dissecting clock circuit and analyzing clock behaviors at the single-cell level.

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II. Results

We computationally generate an atlas of oscillators and systematically analyzed robustness of all oscillatory topologies. We found that, two key local structures, incoherent inputs and coherent inputs, can modify a core topology to increase and decrease its robustness respectively [7] (*Cell Systems 2017*), underscoring the important role of local modifications in robustness. It also suggests a convenient way to design robust synthetic circuits. Experimentally, we develop an artificial cell-cycle system to mimic the real cell mitotic oscillatory processes in microfluidic droplets [8] (*eLife 2018*). With nanofabrication and long-term time-lapse fluorescence microscopy, this system has enabled high-throughput single-cell analysis of clock dynamics and functions. We now apply the experimental platform together with mathematical modeling to investigate how network structures are linked to the essential functions of early embryonic cell cycles, such as tunability and robustness.

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Modularity of the Metabolic Gene Network as a Prognostic Biomarker for Hepatocellular Carcinoma

Fengdan Ye[1,2*], Dongya Jia[2,3*], Mingyang Lu[4], Herbert Levine[1,2,5,6] and Michael W. Deem[1,2,3,5]

***Short Abstract* — We analyzed the modular expression patterns of metabolism genes for 371 hepatocellular carcinoma samples from the Cancer Genome Atlas. We found that higher modularity significantly correlated with glycolytic phenotype, later tumor stages, higher metastatic potential, and cancer recurrence, all of which contributed to poorer prognosis. Among patients with recurred tumor, we found the correlation of higher modularity with worse prognosis during early to mid-progression. Furthermore, we developed metrics to calculate individual modularity, which was shown to be predictive of cancer recurrence and patients' survival. Our conclusion is that more aggressive HCC tumors had more modular expression patterns of metabolic genes.**

***Keywords* — modularity; metabolism; hepatocellular carcinoma; HCC; HIF-1; AMPK; prognosis**

I. Background

HEPATOCELLULAR carcinoma (HCC) is a primary malignancy of the liver, and it is the third leading cause of cancer mortality worldwide [1]. We here aimed to analyze cancer-associated gene networks of HCC samples to gain insight into the complex biological systems underlying tumor progression.

We chose the previously identified AMPK and HIF-1 downstream genes to quantify the activities of the two main metabolism phenotypes in HCC, OXPHOS and glycolysis [2]. Modularity was chosen to quantify the modular gene expression patterns. Previous theory developed by Deem predicts that more modular gene expression pattern corresponds to more aggressive tumor [3].

II. Results

There existed a strong anti-correlation between the AMPK activity and HIF-1 activity across all 371 samples. The expression pattern of these genes was highly modular and consisted of two modules, one containing mainly AMPK-downstream genes and the other HIF-1-downstream genes.

A. Modularity and cancer status

Group modularity calculation showed that the OXPHOS group had the lowest mean modularity and glycolysis group had the highest mean modularity. Survival analysis showed that glycolysis group had the worst survival and OXPHOS had the best survival.

Similarly, samples at stage II-IV had higher modularity than those at stage I. Stage II-IV samples also had worse survival than stage I samples. Samples with higher metastatic potential had higher modularity and worse survival. Samples that recurred in a certain time had higher modularity and worse survival than those did not in the same amount of time. Among patients with recurred tumor, there was correlation of higher modularity with worse prognosis during early to mid-progression, up until about 8 months before recurrence.

B. Individual modularity and prediction

We defined individual modularity and correlated it with probability of no recurrence in 12 months and probability of survival within 24 months. A high value of individual modularity was shown to be predictive of poor prognosis. Individual modularity values of $M > 0.6$ correlated to survival and non-recurrence probabilities less than 0.4.

III. Conclusion

Higher modularity of cancer-associated gene network of HCC samples significantly correlated with glycolytic phenotype, later tumor stages, higher metastatic potential, and cancer recurrence, all of which contributed to poorer prognosis. Among patients with recurred tumor, higher modularity correlated with worse prognosis during early to mid-progression. The developed individual modularity was shown to be predictive of cancer recurrence and patients' survival. Our conclusion is that more aggressive HCC tumors had more modular expression patterns of metabolic genes.

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Measuring transcription from a single gene copy in live *Escherichia coli* cells

Jing Zhang[1,2*], Mengyu Wang[1,2,3*], Heng Xu[4,5], and Ido Golding[1,2,3]

***Short Abstract* — Single-cell measurements of transcriptional activity inform our understanding of stochastic gene expression, but these measurements coarse-grain over the individual copies of the gene, where transcription and its regulation stochastically take place. In this study, we follow the transcriptional activity of individual gene copies in live *E. coli* cells, by labeling the gene locus of interest and measuring its transcriptional activity at the same time.**

***Keywords* — Transcription kinetics, individual gene copies, two-color labeling, live-cell imaging.**

Gene expression and its regulation are traditionally studied by averaging over large cell populations. These "bulk" measurements mask the heterogeneity in gene expression between individual cells. Single-cell measurements have informed us on the inherent stochasticity of the reactions involved in gene regulation [1]. However, the whole-cell measurement is still limited in many ways: i) It typically represents the summation over the contributions from multiple copies of the same gene, each of which is independently regulated, and whose number doubles during the cell cycle [2, 3]; ii) It fails to distinguish RNA molecules that are being transcribed from those already completed.

In this study, we set to follow the active transcription of an individual gene copy within an individual *E. coli* cell in real-time. We hypothesized that active transcription can be quantified by measuring the amount of RNA that is localized to the transcribed gene [4]. We therefore developed a two-color labeling system to simultaneously mark the gene locus and the RNA produced from the gene. The gene locus was labeled using the Fluorescent Repressor Operator System (FROS), which combines fluorescently-tagged DNA binding proteins with a synthetic array of cognate binding sites [5]. The RNA produced from the gene was tracked using an analogous method, where a fluorescently-tagged MS2 bacteriophage coat protein labels an array of MS2 binding sites [6]. By taking time-lapse movies, we are able to track the transcriptional activity of a single gene copy over multiple cell generations. Using automated image analysis, we can directly measure the kinetic parameters of transcription (e.g., the probabilistic rate of promoter state switching). Moreover, this method allows us to assess the dependence of gene activity on the instantaneous activity of other copies of the same gene in the same cell, and on the event of gene replication during the cell cycle.

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*Equal contribution

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Agent-based Model for Developmental Aggregation in *Myxococcus xanthus* Bacteria

Zhaoyang Zhang[1], Christopher Cotter[2], Lawrence Shimkets[2], and Oleg Igoshin[1]

Using a mechanistic agent-based model, we uncover the physical basis of behaviors that control aggregation of *M. xanthus* cells into multicellular mounds. The observed bias in reversal times with respect to cell's direction relative to the aggregates can be explained by chemotaxis but not by contact-dependent signaling model. On the other hand, a combination of local alignment by steric interactions and the ability of cells to lay and follow slime trails explains observed patterns of cell orientation. Incorporating these effects in our model leads to the formation of stable aggregates in quantitative agreement with the experiments.

I. BACKGROUND

SPATIAL multicellular self-organization is widely studied due to its biological significance across all kingdoms of life. *Myxococcus xanthus* is a rod-shaped soil bacterium that serves as a simple model system to study self-organization. Under different environmental conditions, *M. xanthus* cells self-organize into distinct dynamical patterns [1]. For example, starving cells execute a complex multicellular developmental program by aggregating into multicellular mounds, termed fruiting bodies [1,2]. Despite decades of research, the mechanistic basis of self-organization in *M. xanthus* is not yet fully understood. In particular, how cells aggregate into fruiting bodies is still not clear.

Recently, an approach that combined fluorescence microscopy with data-driven modeling uncovered the set of cellular behaviors required for aggregation: decreased cell motility inside the aggregates, a biased walk toward aggregate centroids, and alignment among neighboring cells and in a radial direction to the nearest aggregate [3]. Here we use a mechanistic agent-based model to test possible biological mechanisms of these behaviors.

II. RESULTS

To test aggregation mechanisms, we developed an agent-based model. In this model, each cell is represented by a point-agent characterized by its position and moving direction. Each agent actively aligns its direction with nearby agents and secrete slime trails. Other agents can sense and follow these trails by turning towards high slime density regions. At low cell density, our model matches the patterns of collective alignment observed in experiments [3] and reproduced by detailed biophysical model [4]. At high cell density, the model can efficiently simulate alternative hypotheses on cell behaviors.

A. A contact signaling based model does not lead to stable aggregation.

In the first model, we assumed that the reversal period of each cells is dependent on a contact dependent signal that conveys the direction of the surrounding cells. The model using this contact-based signal is unable to form stable aggregates, even if the parameters in this mechanism are fitted to the bias data [3].

B. A chemotaxis model produces biased movement similar to the experiment and helps with aggregation.

In the second model, we assumed a chemotaxis mechanism, i.e. cells produce a diffusible chemical signal that affects the reversal period via an intracellular chemotaxis network with adaptation. Applying this mechanism into our model, we find that the resulting model can replicate the experimentally observed bias and aggregation patterns [3].

C. Local cell alignment and slime-trail following can explain observed cell-orientation patterns.

Using our chemotaxis model with local cell alignment and slime-trail following, we compare the simulated distributions of agents' orientation with those observed in experiments [3]. The results are in qualitative agreement: near aggregate boundaries, cells move in circumferential direction rotating around aggregates whereas further away cells align radially to aggregate centers. These patterns are partially driven by cell alignment and slime trails prior to aggregation initiation.

III. CONCLUSION

We propose that *M. xanthus* cells use a chemotaxis mechanism to form aggregates. Combined with local alignment and slime-trail following, these mechanisms are sufficient to explain the observed aggregation patterns.

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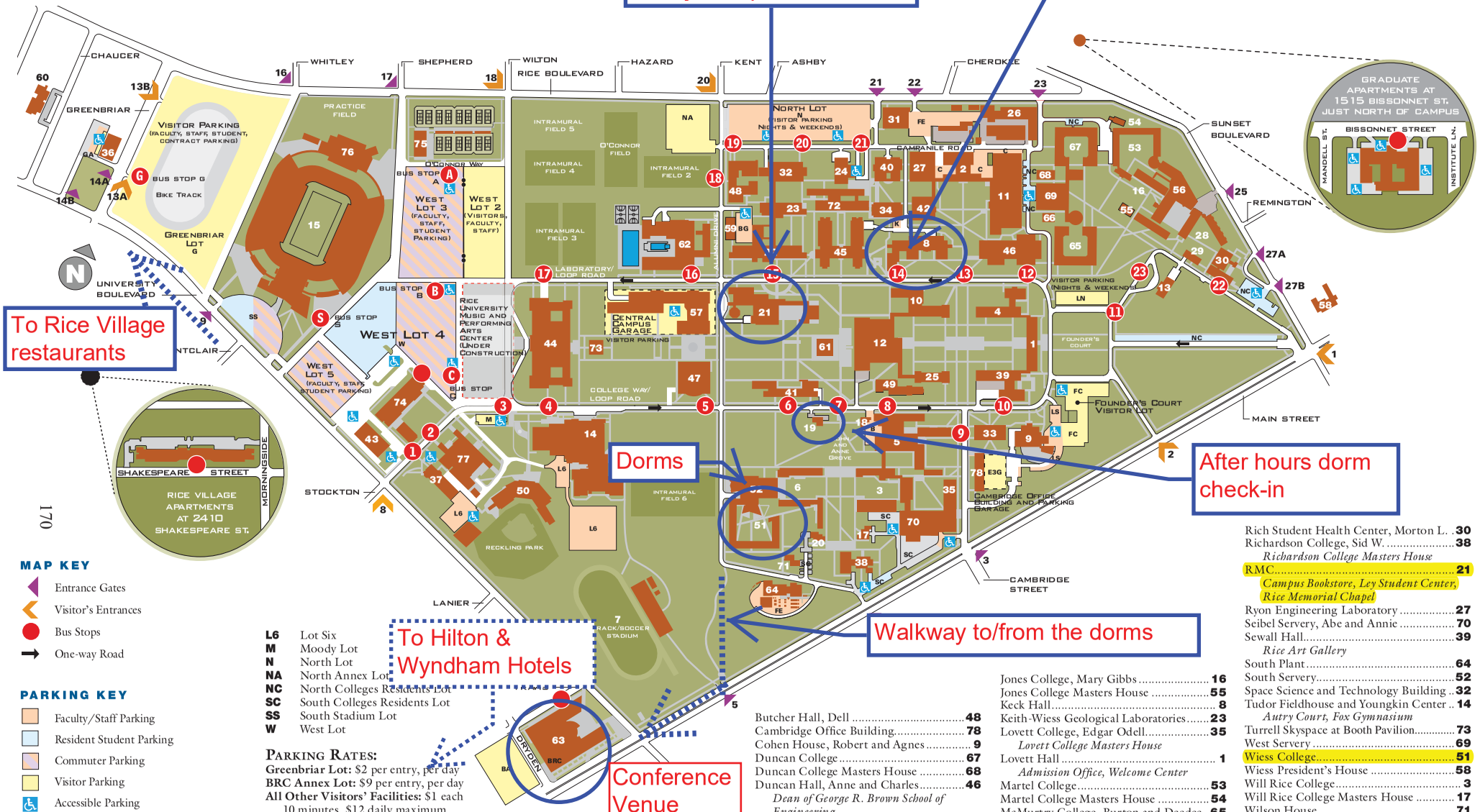
2 Department of Microbiology, University of Georgia

FOR NOTES

Rice University Campus Map

10/13/17



170

MAP KEY

- Entrance Gates
- Visitor's Entrances
- Bus Stops
- One-way Road

PARKING KEY

- Faculty/Staff Parking
- Resident Student Parking
- Commuter Parking
- Visitor Parking
- Accessible Parking

Parking Lots:

B Baker College–Housing & Dining Lot
BA BioScience Research Collaborative (BRC) Annex
BG Biology–Geology Lot
BRC BioScience Research Collaborative Garage
C Campanile Lot
CG Central Campus Garage
E3G Entrance 3 Garage
FC Founder's Court Visitor Lot
FE Facilities, Engineering and Planning Lot
G Greenbriar Lot
GA Greenbriar Annex
K Keck Lot
LN Lovett North Lot
LS Lovett South Lot
L6 Lot Six
M Moody Lot
N North Lot
NA North Annex Lot
NC North Colleges Residents Lot
SC South Colleges Residents Lot
SS South Stadium Lot
W West Lot

Parking Rates:

Greenbriar Lot: $2 per entry, per day
BRC Annex Lot: $9 per entry, per day
All Other Visitors' Facilities: $1 each 10 minutes, $12 daily maximum

Payment Methods:

BioScience Research Collaborative Garage and Central Campus Garage: cash or credit card
BRC Annex, Founder's Court, Greenbriar Lot, North and West Lots Visitor Section: credit card

BUILDINGS

ACADEMIC SCHOOLS

OFF-CAMPUS FACILITIES

Rice Graduate Apartments
Rice Village Apartments

LOCAL DINING OPTIONS

This partial list should serve as a guide to local restaurants and cafes and does not include all the dining options in the area surrounding Rice University, the BioScience Research Collaborative or the Texas Medical Center

(UPDATED: June 19, 2018)

Medical Center (Closest to Rice Campus)

Cliff's Grill
6605 Main Street
Houston, TX 77030
Hours: Fri. – Sat.: 7AM – 8PM
CLOSED SUNDAY
$ - American, Burgers, Stuffed Baked Potatoes, Vegetarian Friendly, Salads, Mediterranean, Sandwiches,
2 min. walk from BRC Bldg.

Chipotle Mexican Grill
6600 Main St. SPC A
Houston, TX 77030
Hours:
Mon. – Sun.: 10:45 AM – 10PM
2 min. walk from BRC Bldg.

Starbuck's
6600 Main St.
Houston, TX 77030
Hours: Open Daily: 5AM – 9PM

Subway
Medical Office Tower
Houston Medical Ctr.
6618 Fannin St.
Houston, TX 77030
Hours: Mon. – Fri.: 7AM – 10PM
Sat.: 8AM – 9PM
Sun.: 9AM – 9PM
4 min. walk from BRC Bldg.

Miller's Café
Scurlock Tower
6560 Fannin Street, Ste. 110
Houston, TX 77030
$ - Burgers, Salads, Sandwiches,
Hours: Fri.: 10AM – 6PM
Sat.: 10AM – 3PM
CLOSED SUNDAY
3 min. walk from BRC Bldg.

Al Vetro Coffee and Espresso Bar
Medical Center in Scurlock Tower 2nd floor, Suite 245
6560 Fannin St.
Houston, TX 77030
$ - Coffee, Espressos, Breakfast Items
Hours:
Mon. – Fri.: 6:30AM – 4:30PM
NOT OPEN ON WEEKENDS
3 min. walk from BRC Bldg.

RICE VILLAGE (20 – 25 Minute Walk/Each Way)

Black Walnut Café
5510 Morningside Dr.
Houston, TX 77005
$$ - American
Hours:
Sun. – Thur.: 7AM – 10PM
Fri. – Sat.: 7AM – 11PM
Popular Menu Items: Black Walnut Salad, Pot Roast, Grilled Cheese, French Dip, Desserts, Fish Tacos
19 min. walk from BRC Bldg.
3 min. drive from BRC Bldg.

Brown Bag Deli
2540 Amherst St.
Houston, TX 77005
$ - Sandwiches, Burger, Deli
Hours: Open Daily:
10:30AM – 4PM
23 min. walk from BRC Bldg.
4 min. drive from BRC Bldg.

Starbucks
2531 Amherst St, Houston, TX 77005
Hours: Open Daily: 5AM – 9PM
21 min. walk from BRC Bldg.
5 min. drive from BRC Bldg.

Istanbul Grill & Deli
5613 Morningside Dr.
Houston, TX 77005
$$ - Turkish, Mediterranean, Deli
Hours:
Tue. – Thurs. 11AM – 10PM
Fri. – Sat. 11AM – 10:30PM
CLOSED MONDAY
Popular Menu Items: Rice, Baklava, Adana Kebab, Turkish Coffee
18 min. walk from BRC Bldg.
4 min. drive from BRC Bldg.

More on following page…

Local Foods
2424 Dunstan Rd
Houston, TX 77005
$$ - Sandwiches, Vegetarian, Deli
Hours:
Open daily 10AM – 8:30PM
25 min. walk from BRC Bldg.
6 min. drive from BRC Bldg.

Sud Italia
2347 University Blvd.
Houston, TX 77005
$$$ - Southern Italian Cuisine
Hours:
Mon. – Sat. Lunch: 11AM – 2PM
Mon. – Wed. Dinner:
5PM – 9:30PM
Thurs. – Sat. Dinner:
5PM – 10PM
CLOSED SUNDAY
17 min. walk from BRC Bldg.
4 min. drive from BRC Bldg.

Chicken Kitchen
2516 Rice Blvd.
Houston, TX 77005
$ - Fast Food, American, Southwestern
Hours: Mon. – Sat.: 11AM – 9PM
CLOSED SUNDAY
Popular Menu Items: Black Beans, Cuban Chop-Chop, Cobb Salad, Baked Plantains
24 min. walk from BRC Bldg.
4 min. drive from BRC Bldg.

Hopdoddy Burger Bar
5510 Morningside Dr. #100,
Houston, TX 77005
$$ - Burgers, Cocktail Bars, Salad
Hours:
Sun. – Wed.: 10AM – 10PM
Thurs. – Sat. 10AM – 12AM
Vegetarian & Vegan friendly, Fresh baked breads. Daily ground meats. Hand-cut fries. Supporting local purveyors. No antibiotics or hormones. Scratch-made sauces.
19 min. walk from BRC Bldg.
5 min. drive from BRC Bldg.

NAM: Noodles and More
2512 Rice Blvd.
Houston, TX 77005
$ - Vietnamese, Chinese, Korean
Hours: Open Daily: 11AM – 9PM
Popular Menu Items: Hue Spicy Noodle Soup, Vietnamese Iced Coffee, Viet-Ton Soup, Rice Platter
24 min. walk from BRC Bldg.
4 min. drive from BRC Bldg.

Zoe's Kitchen
5215 Kelvin Dr.
Houston, TX 77005
$$ - Mediterranean, Salad, Sandwiches
Hours: Open Daily
11AM – 9:30PM
24 min. walk from BRC Bldg.
6 min. drive from BRC Bldg.

Island Grill
2365 Rice Blvd.
Houston, TX 77025
$$ - Juice Bars & Smoothies, Mediterranean, American (Traditional)
Hours: Mon. – Sat.: 7AM – 9PM
Sun.: 8AM – 4:30PM
Vegetarian friendly
19 min. walk from BRC Bldg.
4 min. drive from BRC Bldg.

Punk's Simple Southern Food
5212 Morningside Dr.
Houston, TX 77005
$$ - Southern
Hours:
Tues. – Thurs.: 11AM – 10PM
Fri. – Sat.: 11AM – 10PM
Sun.: 10AM – 9PM
CLOSED MONDAY
22 min. walk from BRC Bldg.
5 min. drive from BRC Bldg.

Pasha Restaurant
2325 University Blvd.
Houston, TX 77005
$$ - Mediterranean, Turkish, Irish
Hours:
Sun. – Thurs.: 11AM – 10PM
Fri. & Sat.: 11AM – 11PM
CLOSED MONDAY
Popular Menu Items: Dolma, Yogurt, and Hummus
17 min. walk from BRC Bldg.
3 min. drive from BRC Bldg.

Thai Village Restaurant
2512 Times Blvd.
Houston, TX 77005
$$ - Thai, Vegetarian, Vietnamese
Hours:
Lunch (Daily): 11AM – 2:30PM
Dinner:
Mon. – Thurs.: 5PM – 9:30PM
Fri.: 5PM – 10PM
Sat.: 12PM – 10PM
Sun.: 12PM – 9PM
Popular Menu Items: Pad Thai, Green Curry, Yum, Red Curry
23 min. walk from BRC Bldg.
4 min. drive from BRC Bldg.

Coppa Osteria
5210 Morningside Dr.
Houston, TX 77005
$$ - Italian
Hours:
Mon. – Thurs.: 11AM – 10PM
Fri. – Sat.: 11AM – 11PM
Sun.: 11AM – 9PM
22 min. walk from BRC Bldg.
5 min. drive from BRC Bldg.

More on following page…

D'Amico's Italian Market Café
5510 Morningside Dr. Ste. 140
Houston, TX 77005
$$ - Italian, Café
Hours:
Mon. - Thurs.: 11AM – 10PM
Fri.: - Sat.: 11AM – 11PM
Sun.: 12PM – 9PM
20 min. walk from BRC Bldg.
3 min. drive from BRC Bldg.

Cyclone Anaya's Mexican Kitchen
5214 Morningside Dr.
Houston, TX 77005
$$ - Mexican
Hours:
Mon. – Thurs.: 11AM – 10PM
Fri.: 11AM – Midnight
Sat.: 10AM – Midnight
Sun.: 10AM – 9PM
22 min. walk from BRC Bldg.
5 min. drive from BRC Bldg.

Hungry's Café & Bistro
2356 Rice Blvd.
Houston, TX 77005
$$ - Burger, Café, American, Vegetarian, Pizzas, Sandwiches, Wraps, Salads and more…
Hours: Mon. - Fri.: 11AM – 10PM
Sat. - Sun.: 9AM – 10PM
Popular Menu Items: Eggs Benedict, Croissant French Toast
20 min. walk from BRC Bldg.
4 min. drive from BRC Bldg.