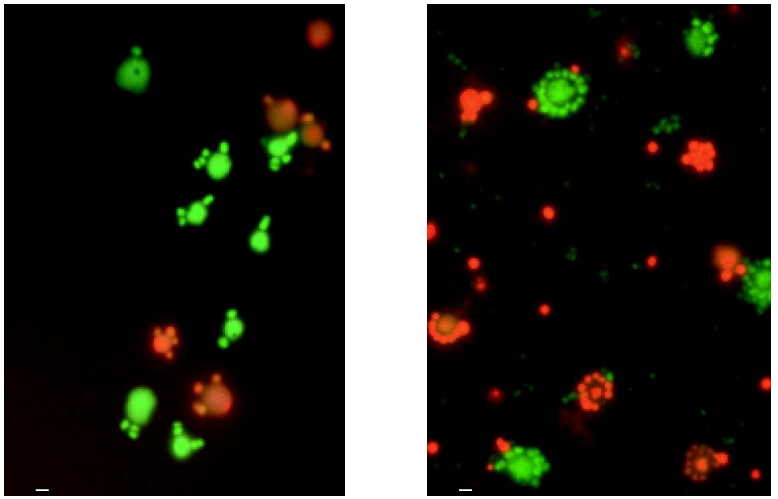
Analysing protein exchange between *Ignicoccus hospitalis* KIN4/1T and *Nanoarchaeum equitans*



Epifluorescence micrographs of *Ignicoccus/Nanoarchaeum* coculture stained with BacLight (Boulos *et al.,* 1999) adapted from (Jahn *et al*., 2008) (Scale bar: 1µm)

Summary

- Objectives of research
- Relevance and importance of research
- Discovery of co-culture
- Biology of *I. hospitalis* and *N. equitans*
- Current understanding of association
- Experimental limitations of biological system
- Proposed methodologies to investigate protein exchange between *I. hospitalis* and *N. equitans*
- Conclusions

Objectives

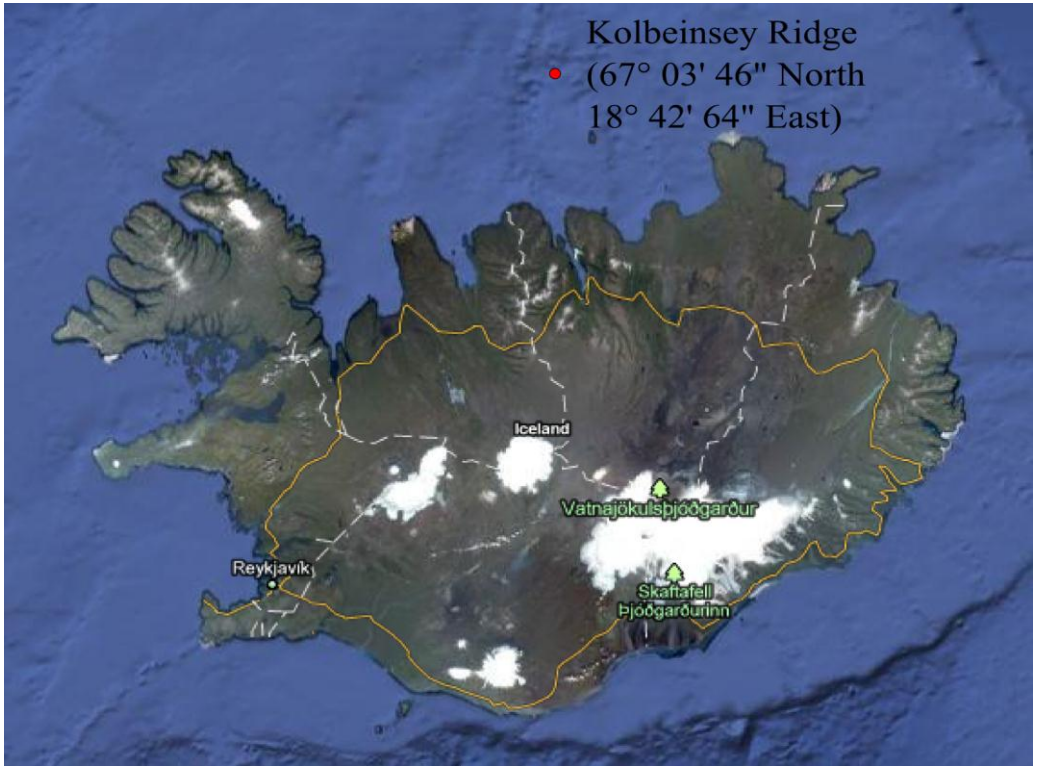
- Determine the role of Secretory (Sec) and Twin-Arginine-Transporters (TAT) in the exchange of proteins between *Nanoarchaeum equitans* and *Ignicoccus hospitalis*
  - Identify candidate proteins for Sec or TAT transport
  - Determine the localisation of TAT transporters in *I. Hospitalis*
  - Test competence of *I. hospitalis* Sec and TAT complexes for export of identified candidate proteins
  - Test competence of *N. equitans* SecDF complex for candidate protein uptake
  - Identify further avenues of research

Relevance

- Why are *Ignicoccus hospitalis* and *Nanoarchaeum equitans* of interest?
  - Hyperthermophiles (Leigh *et al.,* 2011)
  - Novel proteins (Podar *et al.,* 2008a)
  - Very ancient lineages? (Podar *et al.,* 2008a)
  - Novel phyla in case of *Nanoarchaeum equitans*? (Huber *et al.*, 2003)
  - Evolution of the eukaryotic cell? (Kuper *et al*., 2010)
  - Evolution of a vesicle trafficking system (Podar *et al.,* 2008b)
  - Evolution of species co-associations (Mevarech and Allers, 2007)

Discovery of organisms

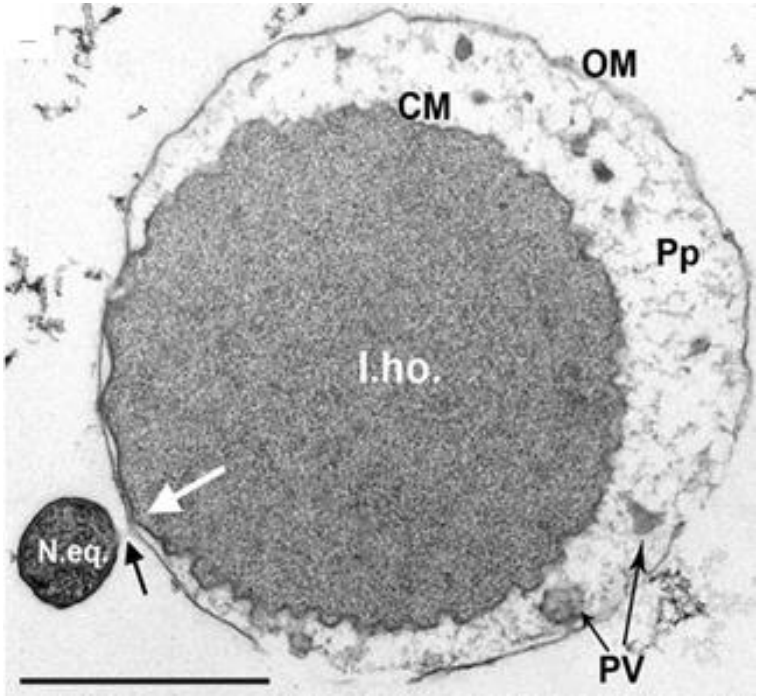
- Hydrothermal system at Kolbeinsey Ridge from depth of 106m (Fricke *et al.*, 1989)
- *Ignicoccus hospitalis* KIN4/I isolate
- Discovery of *Nanoarchaeum equitans* by Karl Stetter in 2002
- Unique relationship (Burghardt *et al.*, 2009)
- Stable co-culture established at University of Regensburg



Map showing location of Kolbeinsey Ridge

Ignicoccus hospitalis

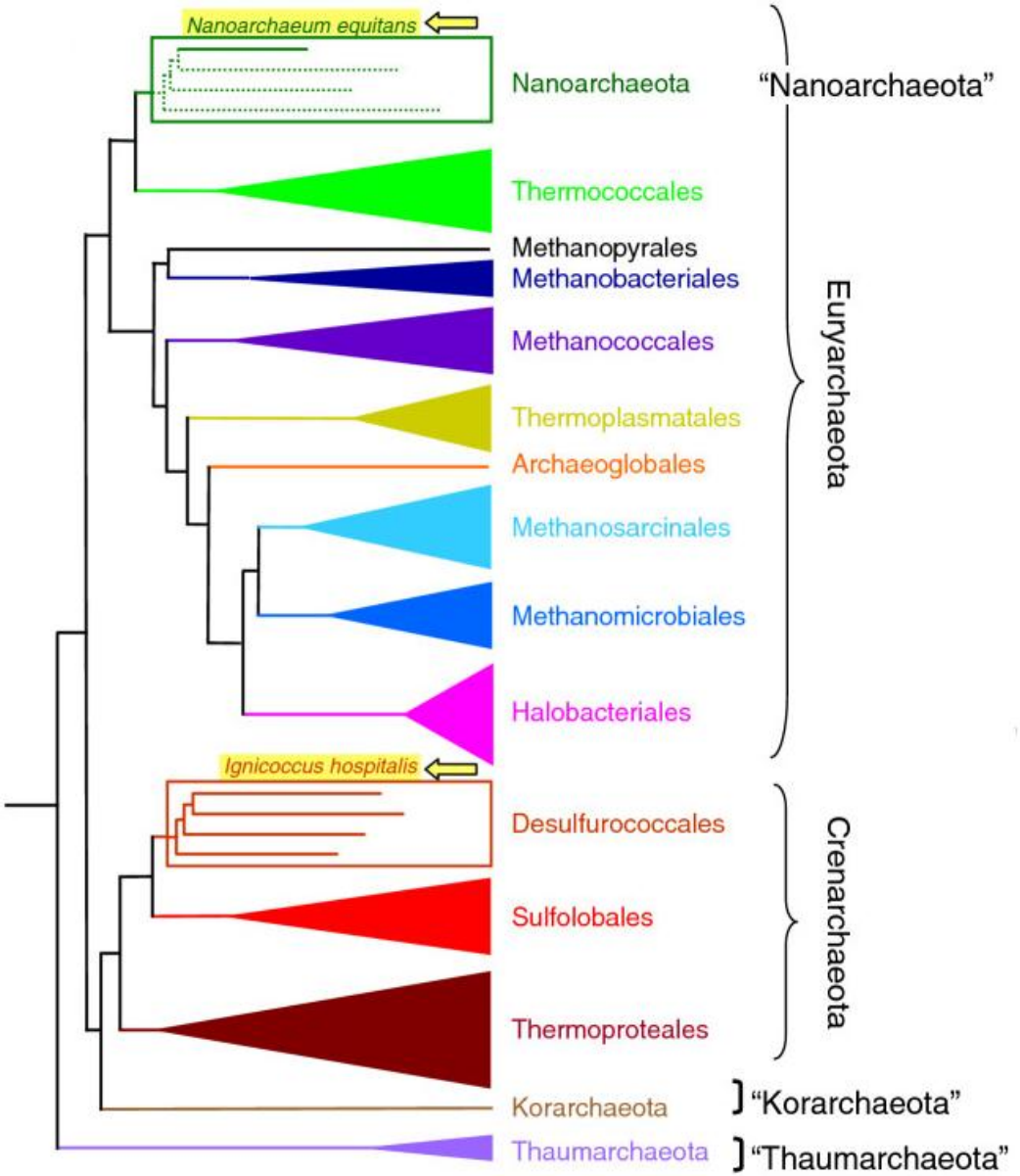
- Obligate anaerobe (Forterre *et al.,* 2009)
- Hyperthermophile (Forterre *et al.,* 2009)
- Ancient organism? (Podar *et al.,* 2008a)
- Unusual morphology (Paper *et al.,* 2007, Burghardt *et al.,* 2007)
- Unusual metabolism (Junglas *et al.,* 2008)
- Unique carbon assimilation (Junglas *et al.,* 2008)
- Smallest free-living genome (Podar *et al.,* 2008)



Transmission electron micrographs of ultrathin sections of *I. hospitalis* and *N. equitans*
CM: Cytoplasmic membrane
OM: Outer membrane
Pp: Periplasm
Figure from (Jahn *et al*., 2008)
(Scale Bar: 1µm)

Nanoarchaeum equitans

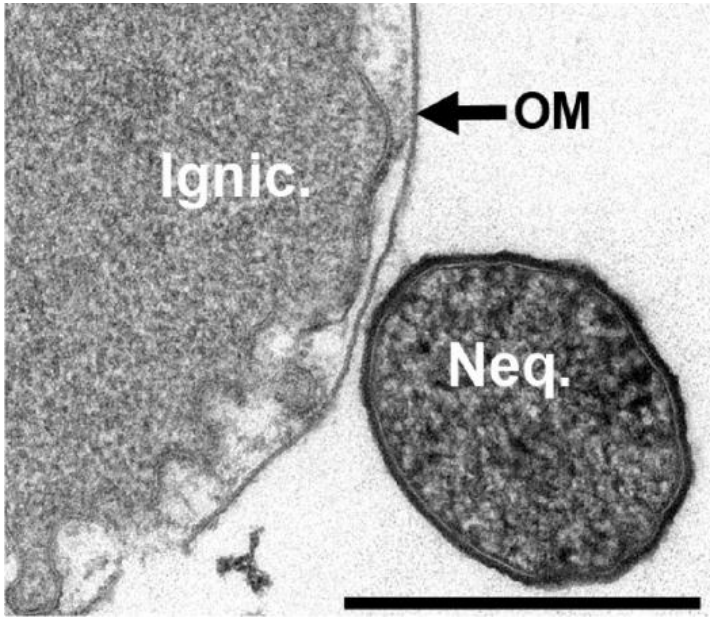
- Nanoarcheota (Huber *et al.*, 2002)
- Smallest genome in archaea (Huber *et al.*, 2003)
- Obligate symbiont /parasite (Waters *et al.*, 2003)
- Lacks key genes (Podar *et al.* 2008a)
- Unknown metabolism (Lewalter and Muller, 2006)



Archael Phylogeny from (Forterre *et al*., 2009)

Physiological dependence

- Host-derived
  - Amino acids (Jahn *et al*., 2008)
  - Lipids (Jahn *et al*., 2004)
- *Ignicoccus* protein exporters:
  - SecYE/61β complex (Burghardt *et al*., 2009)
  - Twin-arginine translocation (TAT) system (Podar *et al*., 2008a)
- *Nanoarchaeum* putative protein importer:
  - SecDF complex (Burghardt *et al*., 2009)



Electron micrograph showing *Nanoarchaeum equitans* attached to *Ignicoccus hospitalis*
OM: Outer membrane

Figure from (Forterre *et al*., 2009)
(Scale bar: 100nm)

Limitations of experimental system

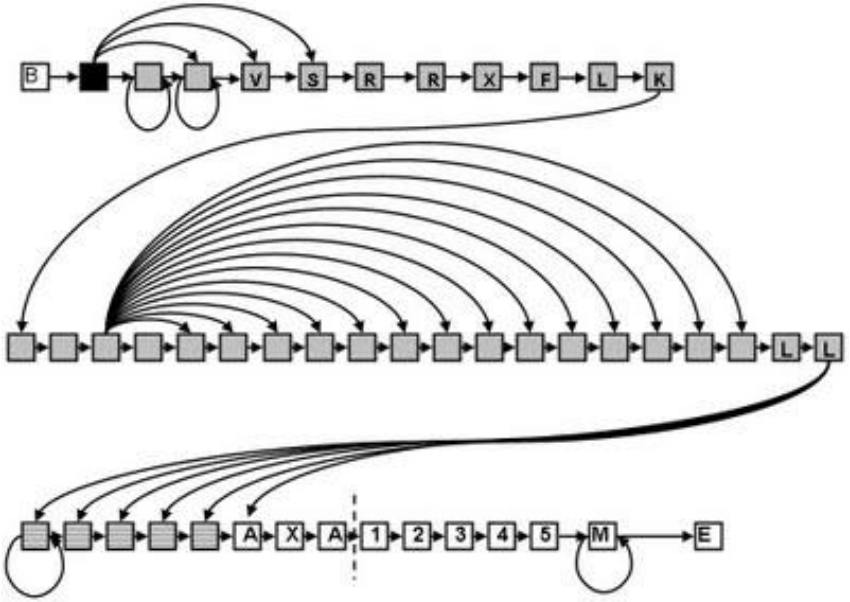
- Genetic methods unavailable (Burghardt *et al.,* 2009)
- Key difficulties: (Mevarech and Allers, 2007)
  - Solid media cultivation
  - Transformation systems
  - Enrichment
  - RNAi unavailable
- Divergent from the standard genetic models (Leigh *et al.,* 2011)
- Enigmatic genes (Podar *et al.,* 2008a)
- Culture density (Huber *et al.*, 2003)

BD BioSciences FACSAria-II cell sorter

From (http://www.bdbiosciences.com)

Identification of candidate transferred proteins

- Combination survey using existing bioinformatic tools and heuristic approaches:
  - PRED-TAT (Bagos *et al.*, 2010)
  - TatP (Bendtsen *et al*., 2005)
  - TATFIND (Rose *et al.,* 2002)
  - SignalP 3.0 (Bendtsen *et al*., 2004)
  - Phobius (Kall *et al*., 2004)



PRED-TAT Hidden Markov Model diagram
Figure from (Bagos *et al*., 2010)

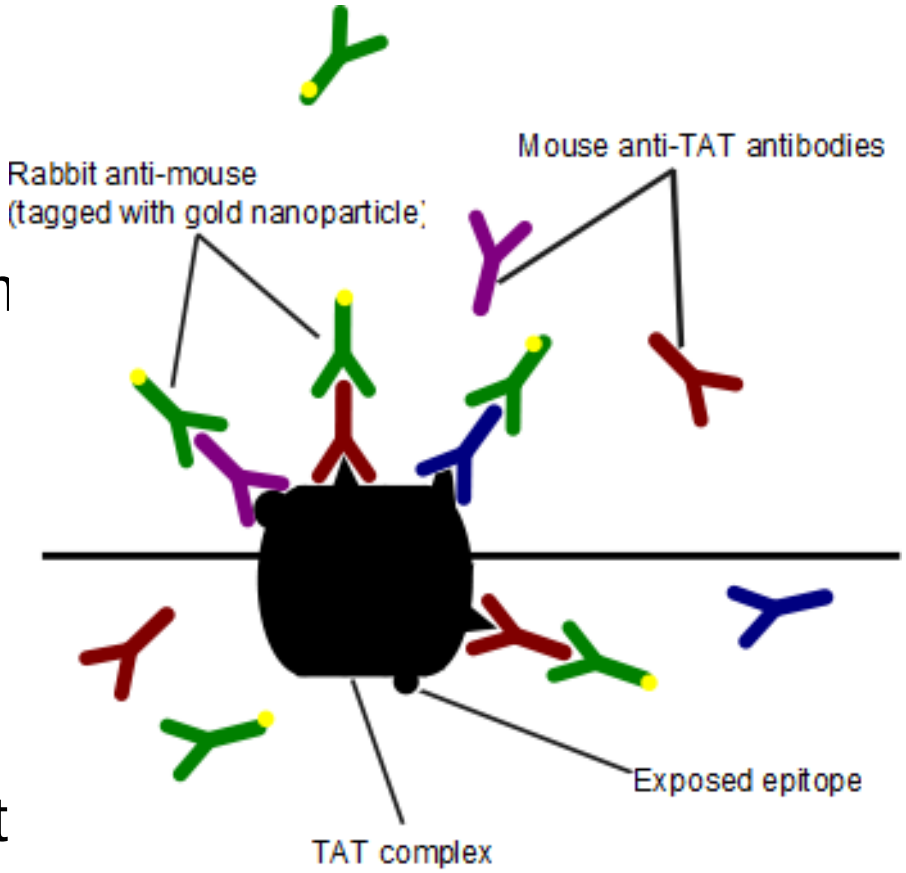
- Preliminary survey of *I. hospitalis* protein database:
  - 8 Sec signal peptide-containing proteins
  - 3 TAT signal peptide-containing proteins

Culturing organisms

- Basic growth conditions:
  - Seawater medium (Huber *et al.,* 2000)
  - Anoxic: Gas phase of H_2-CO_2 (80/20 vol/vol) at 300kPa (Paper *et al.,* 2007)
  - pH 5.5-6.0 (Paper *et al.,* 2007)
  - Temperature: 90ºC (Mevarech and Allers, 2007)
- Final cell densities: $2x10^7$ cells ml^{-1} (Huber *et al.,* 2003)
- Modifications to increase cell density:
  - Cellulose capillaries (increase to $3x10^7$ cells ml^{-1}) (Paper *et al.*, 2007, Kuper *et al.*, 2009)
  - H_2S stripping (increase of *Nanoarchaeum* density to $3x10^8$ cells ml^{-1}) (Mevarech and Allers, 2007)

Localisation of complexes

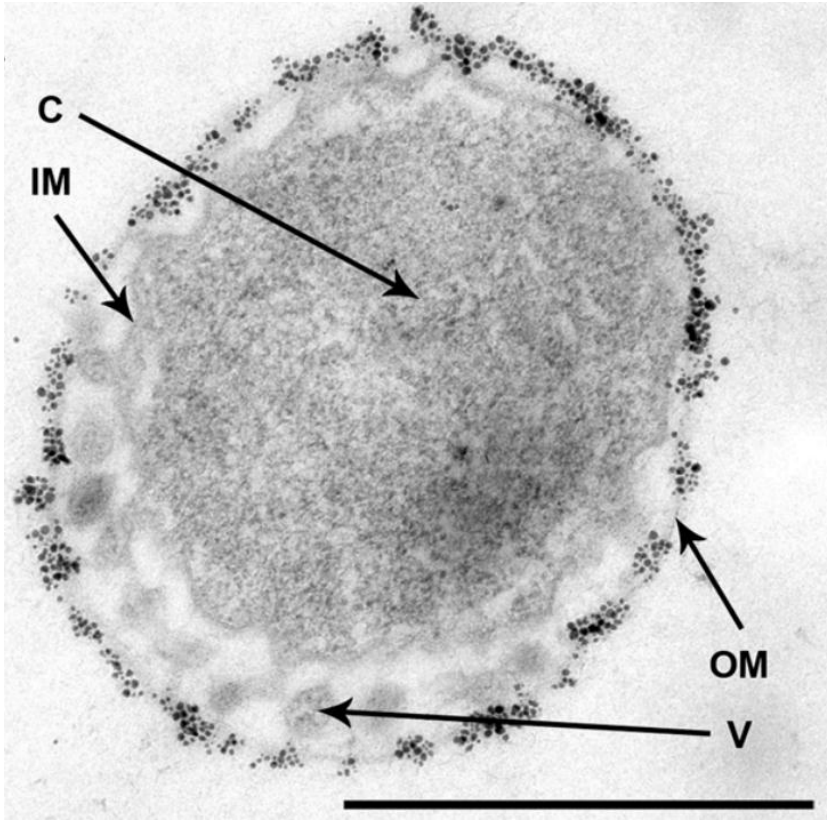
- Sec complexes previously isolated at interaction site (Burghardt *et al.*, 2009)
- Isolate and purify TAT complex from *I. hospitalis* via procedure used in (Porcelli *et al.*, 2002)
  - Membrane solubilisation
  - Ultracentrifugation
  - SDS-PAGE
- Raise polyclonal antibodies against purified TAT protein using mouse system



Immunolocalisation using polyclonal antibodies and secondary antibody markers

Sectioning and labelling

- Cryoimmobilisation via high-pressure freezing (Kuper *et al*., 2009)
- Freeze-substitution dehydration (Walther and Ziegler, 2002)
- Embed in Epon resin (Junglas *et al*., 2008)
- Serial ultrathin sections (70nm) (Junglas *et al*., 2008)
- Incubate with primary rabbit anti-TAT antibody
- Incubate with secondary anti-rabbit antibody with gold nanoparticles
- Transmission electron micrography (Kuper *et al*., 2009)

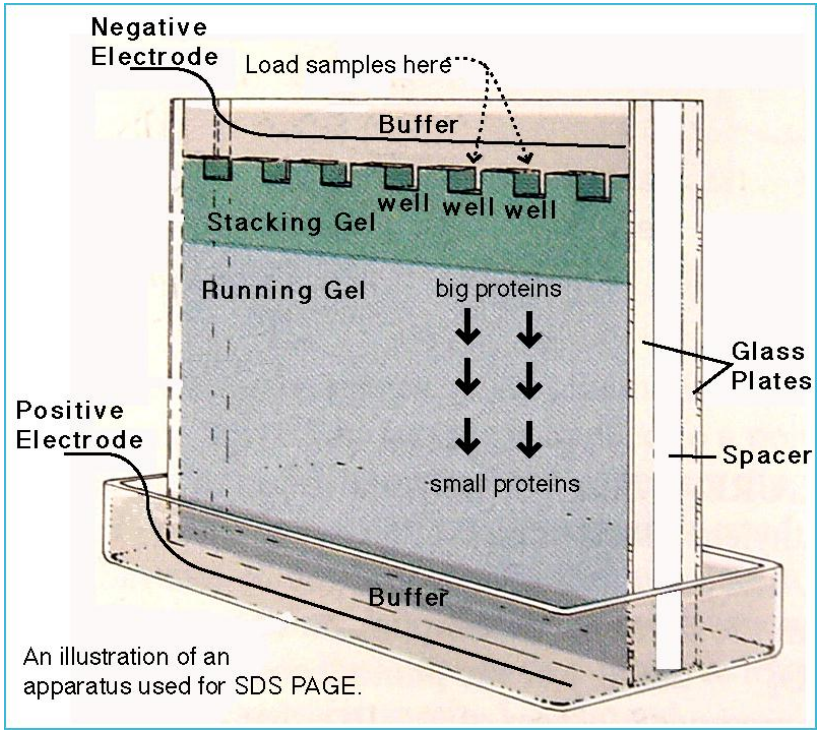


Immunolabelled A_1A_0 ATP-synthase

Figure from (Kuper *et al.*, 2009)
(Scale Bar 1 µm)

Ignicoccus hospitalis Sec and TAT export competence

- Generation of *Ignicoccus* inverted membrane vesicles (Ring and Eichler, 2001)
  - French Press
  - Centrifugation and resuspension
- Isolation and purification of candidate proteins
  - Size-exclusion chromatography
  - Centrifugation
  - SDS-PAGE
  - Protein-specific biophysical separation

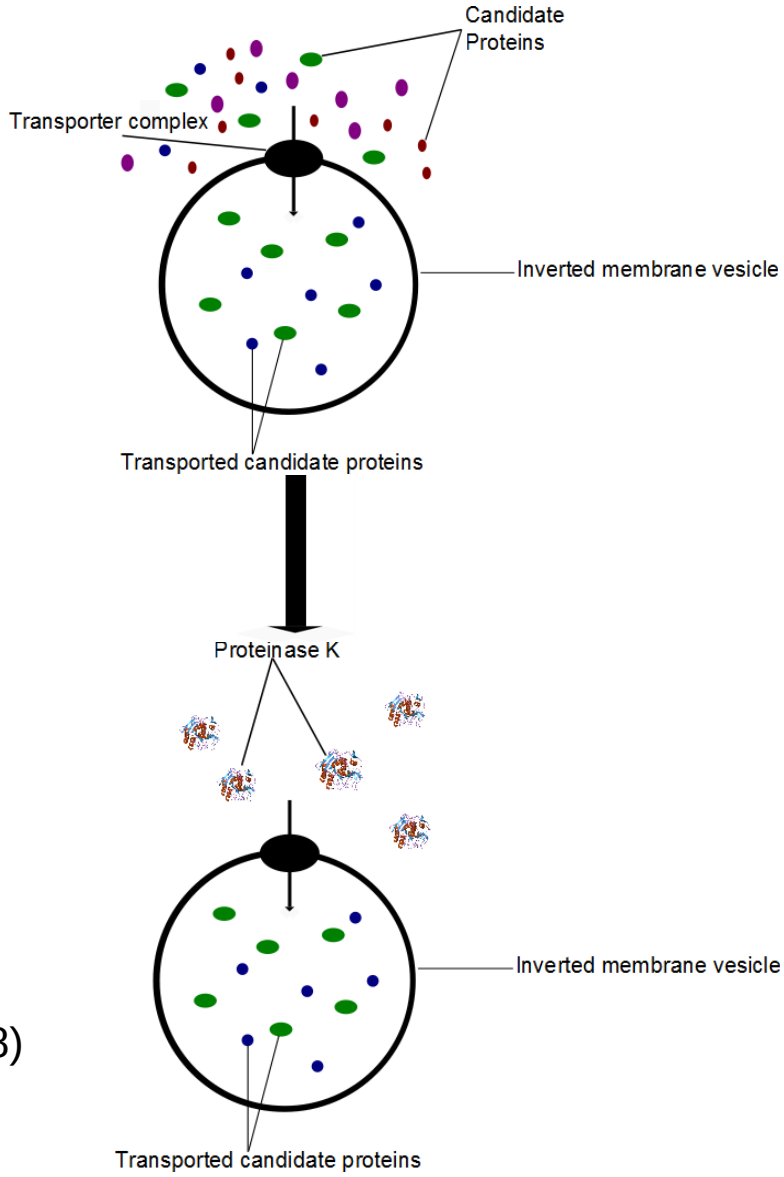


SDS-PAGE diagram

From (Georgia Institute of Technology)

Protection Assay

- Proteinase K treatment
- Lyse liposomes
- Re-isolate and purify candidate proteins
- Controls:
  - Treat candidate proteins with archael signal peptidases: Igni153 and Neq432 (Podar *et al*., 2008a)
  - Trimethylene *N*-oxide reductase (TorA) TAT inhibitor (Chanal *et al.*, 2003)
  - Sec small peptide inhibitors (Li *et al*., 2008)



Proteinase K protection assay

Nanoarchaea equitans SecDF import

- Problematic S-layer (Ring and Eichler, 2001)
- Isolate and purify SecDF complex (Nouwen *et al.*, 2005)
- Formation of liposomes (Cladera et al., 1997)
- Reconstitution of SecDF complex into liposomes (Nouwen *et al.*, 2005)
- TEM validation
- Proteinase K protection assay
- Controls:
  - Treat candidate proteins with Igni153 and Neq432 (Podar *et al*., 2008a)
  - Sec small peptide inhibitors (Li *et al*., 2008)

Conclusions

- Enigmatic relationship
- Genetically intractable organisms
- Potentially important and interesting
- Investigation of TAT and Sec mediated protein exchange between *Nanoarchaeum equitans* and *Ignicoccus* requires:
  - Identification of potential transported proteins
  - Demonstration of transporter localisation to interaction site
  - Demonstration of transporter competence for candidate proteins
- Further work

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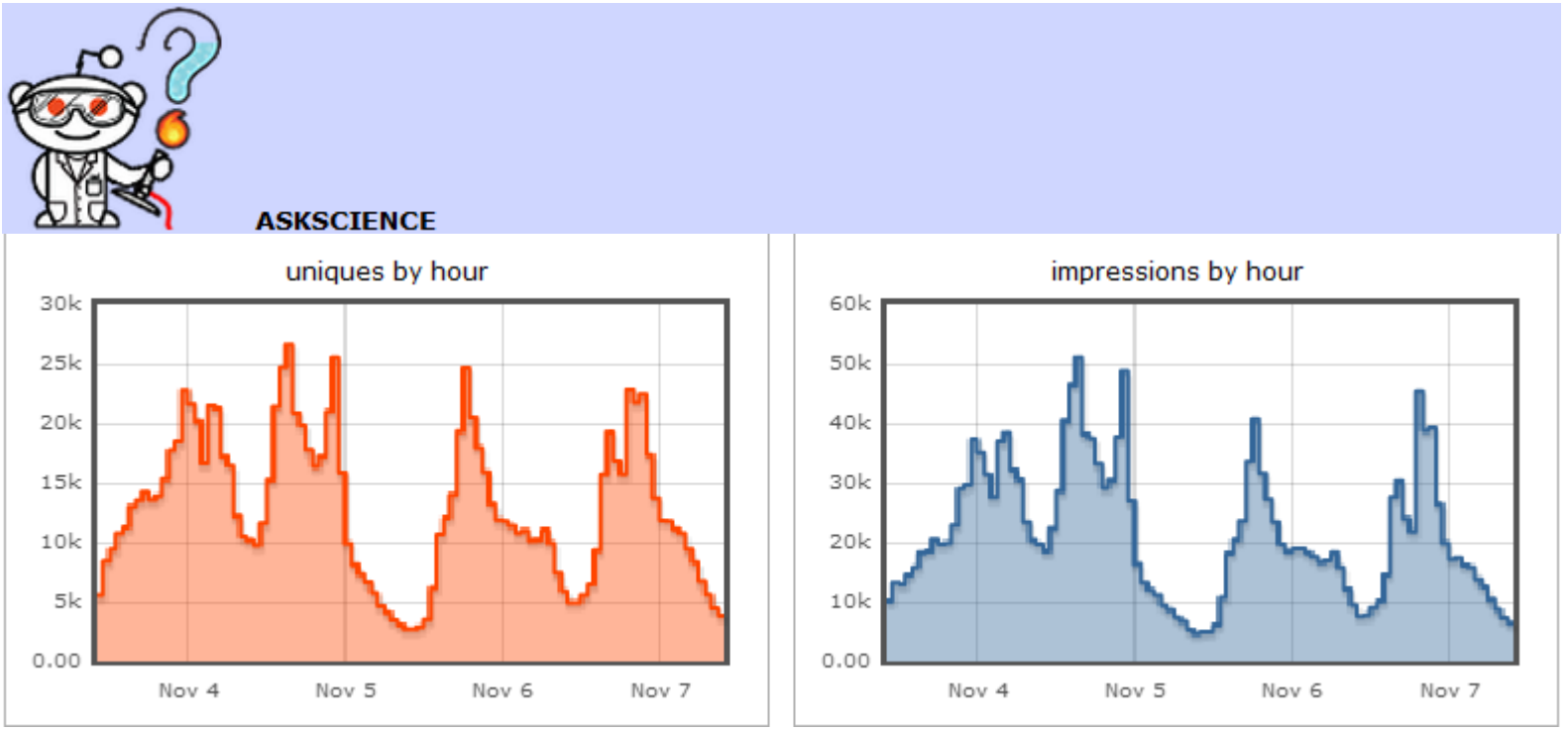

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