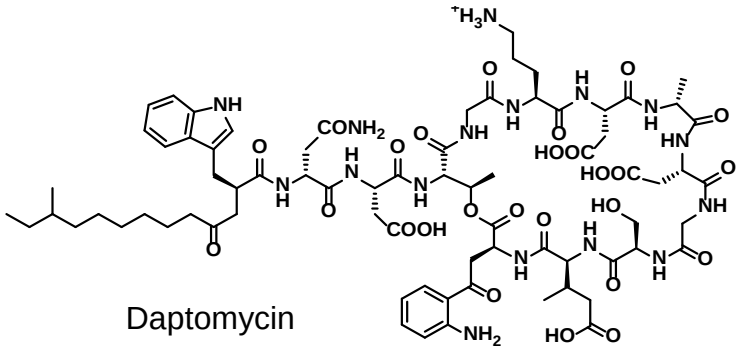
Unlocking chemical secrets of marine organisms with synthetic biology and the crystalline sponge method

Roland Kersten
Weng lab
Whitehead Institute for Biomedical Research

Natural products in life sciences

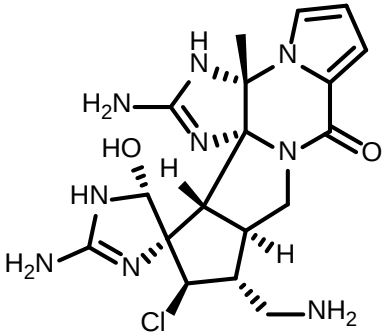
Pharmacy

>60% of small molecule drugs are natural product-derived [1]



Daptomycin

Organic and analytical chemistry

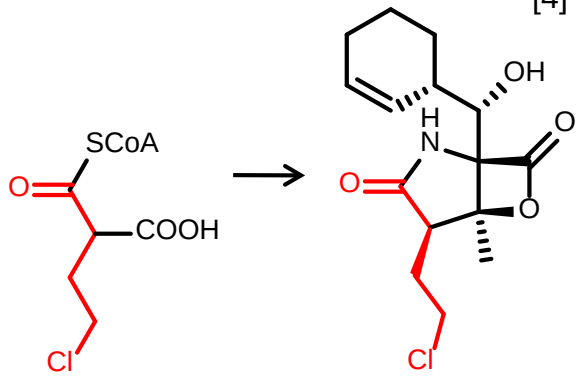


[2]

Palau'amine

Biochemistry

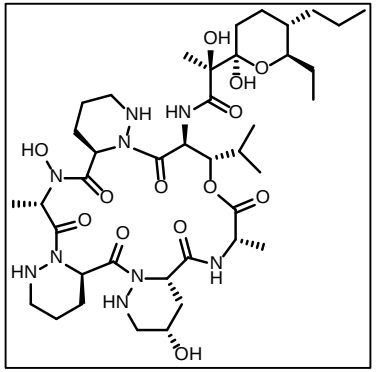
[4]

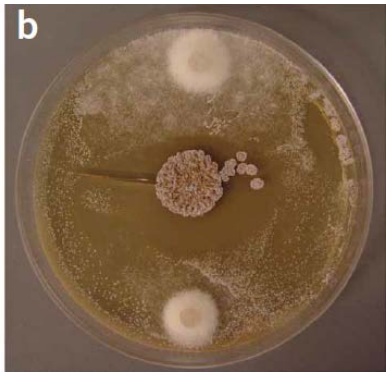


Salinosporamide A [3]

Biology

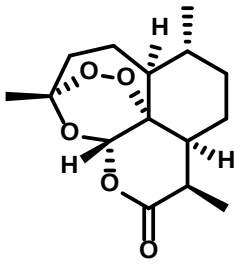
[5]





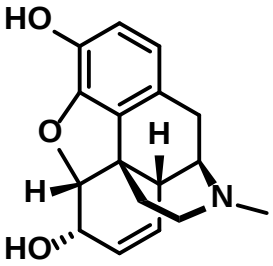
[1] Cubicin® (www.cubicin.com), [2] Seiple, I.B., *et al. Angew. Chem. Int. Ed. Engl*. (2010), [3] Feling R.H. *et al. Angew. Chem. Int. Ed. Engl.* (2003), [4] Eustáquio, A.S., Pojer, F., Noel, J.P., Moore, B.S. *Nat. Chem. Biol.* (2008). [5] Oh, D.C., Poulsen, M., Currie, C.R., Clardy, J. *Nat. Chem. Biol*. (2009).

Weng Laboratory



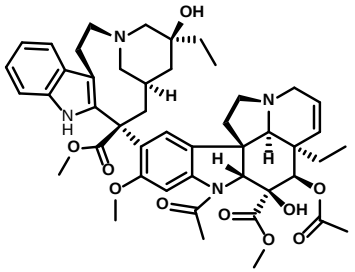
Artemisinin
(anti-malaria drug)

Artemisia annua



Morphine
(analgesic)

Papaver somniferum



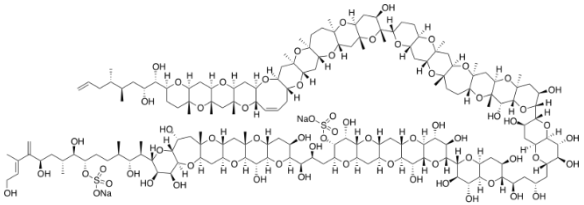
Vincristine
(anti-cancer drug)

Catharanthus roseus

Marine natural products

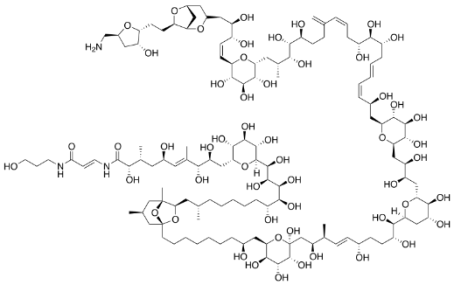
- Potent bioactivities and unique chemical structures

Gambierdiscus toxicus
(dinoflagellate)



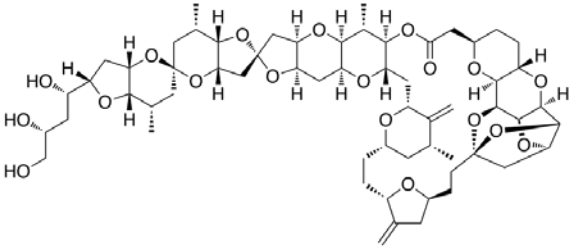
Maitotoxin
(neurotoxin)

Palythoa toxica
(soft coral,
'seaweed of death from Hana')

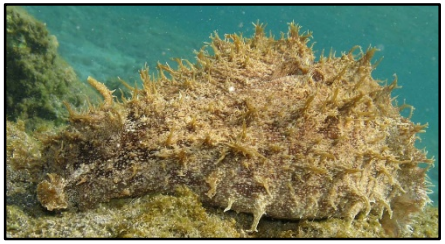


Palytoxin
(neurotoxin)

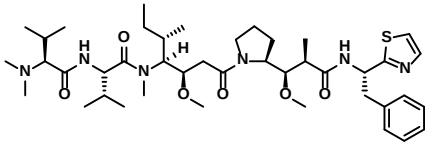
Halichondria okadai
(sponge)



Halichondrin B
(anti-cancer)

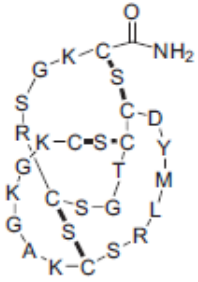


Dolabella auricularia
(sea hare)



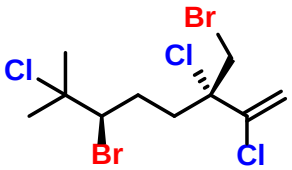
Dolastatin 10
(anti-cancer)

Conus magus
(cone snail)



Ziconotide
(analgesic)

Portieria hornemannii
(red alga)

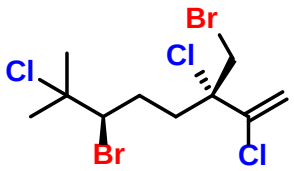


Halomon
(anti-cancer)

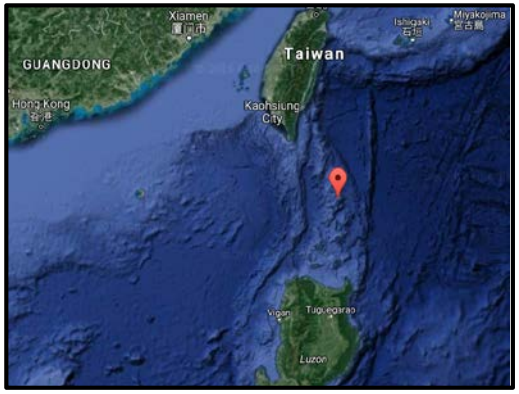
Challenges in drug discovery from marine natural products

1. Source limitation

Portieria hornemannii
(red alga)



Halomon
(anti-cancer)



- Difficult isolation and cultivation of source organism
- Low-yields from extraction
- Seasonal variability in chemotypes
- Symbiotic production possible
- Ecological stress by extensive collection

2. Structure elucidation

- Structure elucidation of complex natural products often requires kilogram starting materials
- 3D structure elucidation for some molecules only possible by total synthesis

3. Production

- Difficult production of complex natural products by total synthesis in amounts for drug development
- Total synthesis often involves unsustainable methodologies such as heavy metal catalysis

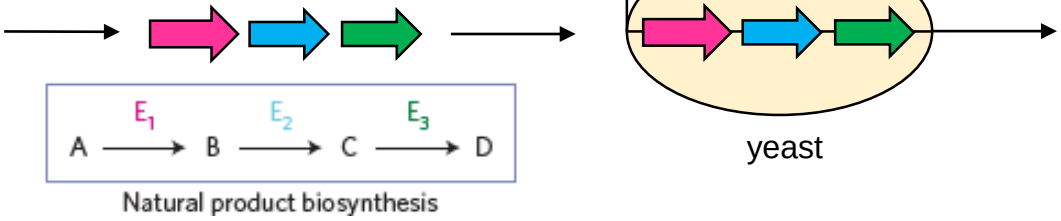
How Technology Drives (Biology) Natural product chemistry in the Weng lab

1. Synthetic biology

Source organism

Identification of candidate biosynthetic genes of a target natural product

Expression of candidate biosynthetic genes in a heterologous host organism



Target compound

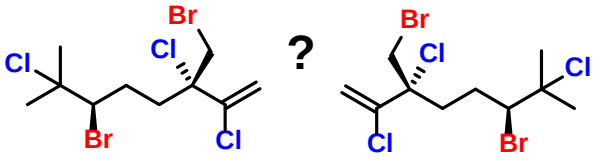
- No collection of source organism necessary
- No or reduced total synthesis

2. Crystalline sponge method

- Structure elucidation of natural products

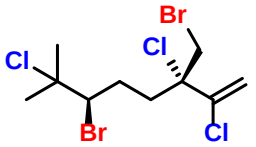
NMR Measurement of how nulei of an analyte behave in a magnetic field

- Planar structure
- Relative stereochemistry
- Milligram-scale



XRD Measurement of how a compound diffracts high energy light

- Absolute stereochemistry
- Milligram-scale
- Requires crystallization of analyte



Crystalline sponge method

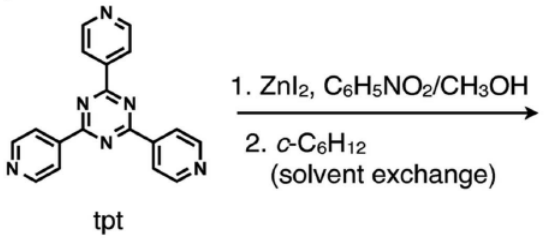
Absorption of analyte into a sponge crystal and subsequent XRD analysis

- Absolute stereochemistry
- Nano-to-microgram-scale
- No crystallization of compound required

How Technology Drives (Biology) Natural product chemistry in the Weng lab

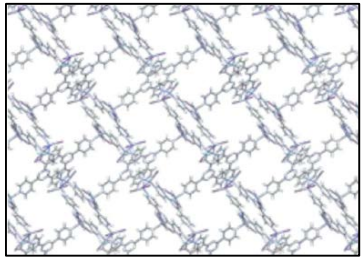
2. Crystalline sponge method

1. Crystalline sponge formation



$[(ZnI_2)_3(tpt)_2 \cdot x(c\text{-}C_6H_{12})]_n$

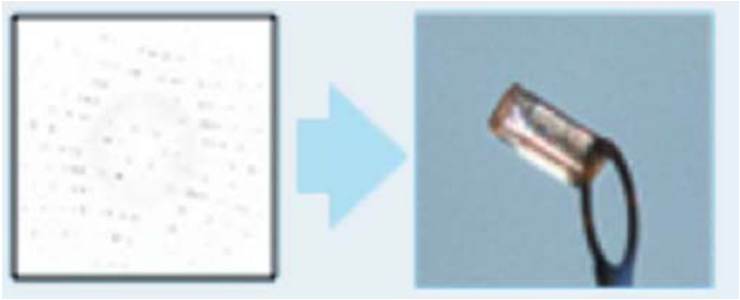
1



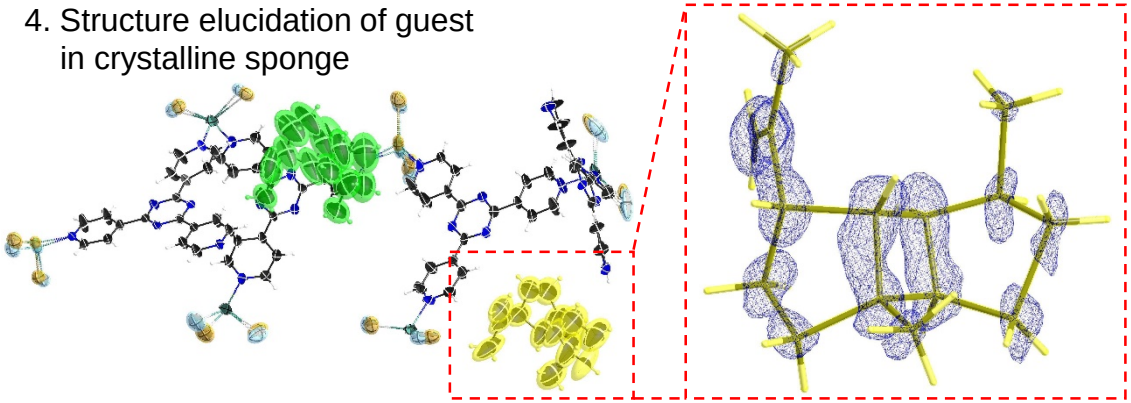
Makoto Fujita
(University of Tokyo)

2. Guest-soaking

3. XRD analysis



4. Structure elucidation of guest in crystalline sponge

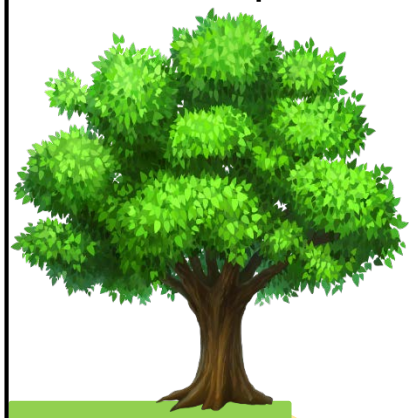


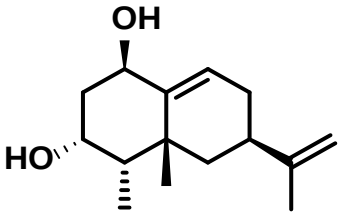
Hoshino, M., Khutia, A., Xing, H., Inokuma, Y. and Fujita, M. *IUCrJ* (2016)

Application of synthetic biology and crystalline sponge method to marine natural products

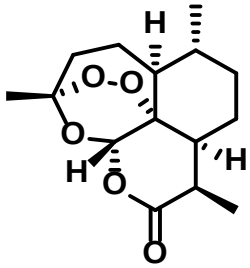
Terpene natural products from marine red macroalgae

Terrestrial plants

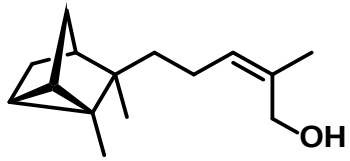




capsidiol
Phytoalexin



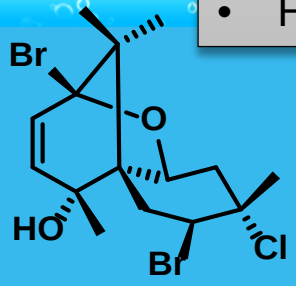
artemisinin
Anti-malarial drug



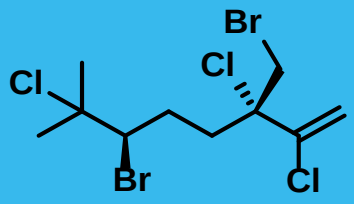
α-santalol
Sandalwood fragrance

Major differences between plant and algal terpenes:
- Cyclic terpenoid scaffolds
- Halogenation

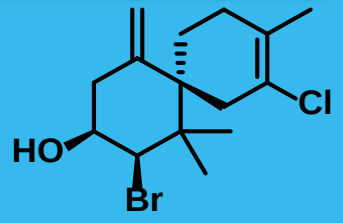
Red macroalgae



pacifenol
Anti-fouling agent



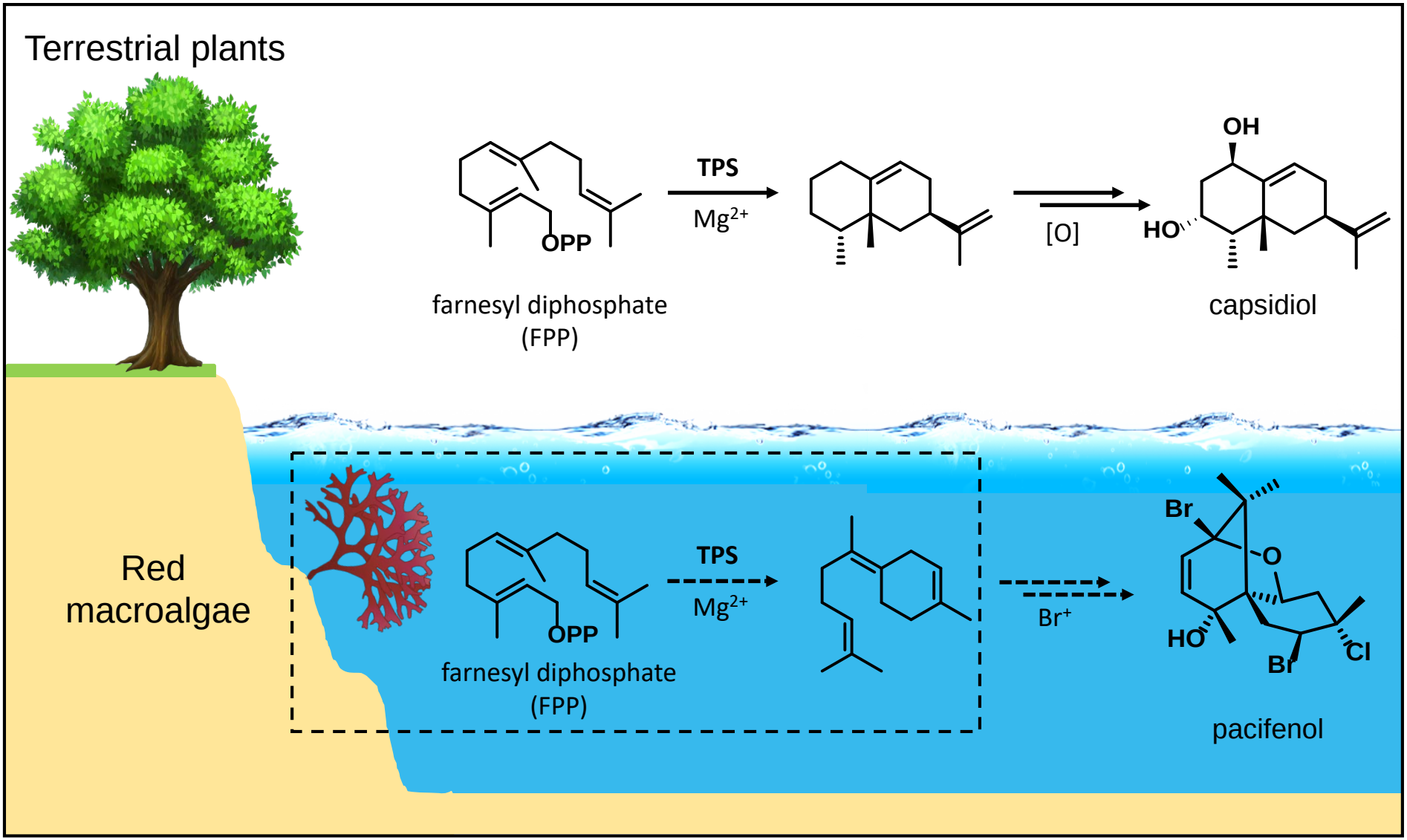
halomon
Anti-cancer agent



elatol
Anti-bacterial agent

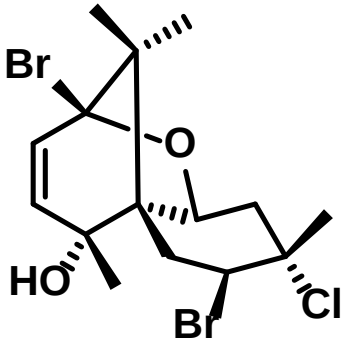
Identification of biosynthetic genes of red algal terpenes

- Biosynthesis of red algal terpenes is largely unknown



TPS – terpene synthase

Pacifenol from *Laurencia* pacifica as a model for red algal terpene biosynthesis



Pacifenol [1]

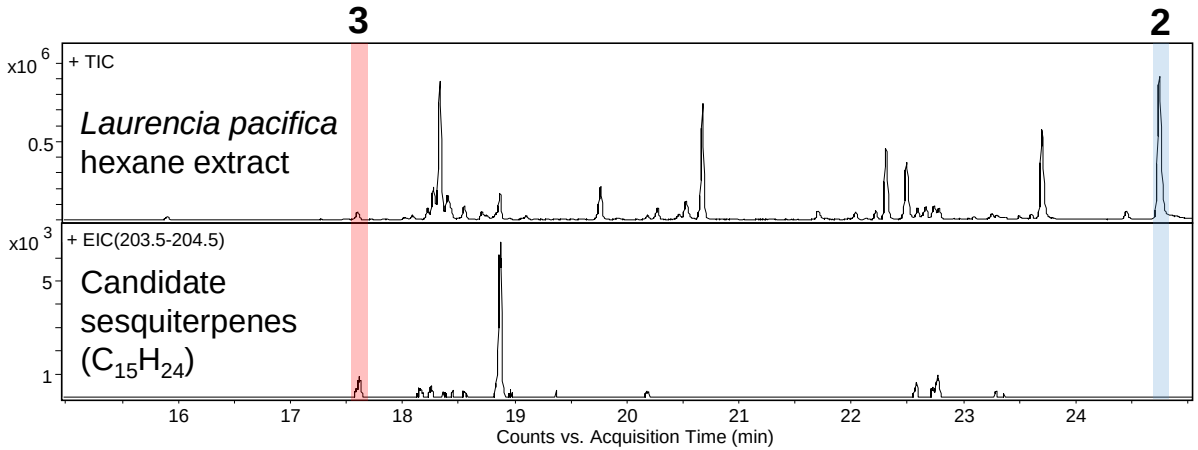
Laurencia pacifica
La Jolla, CA

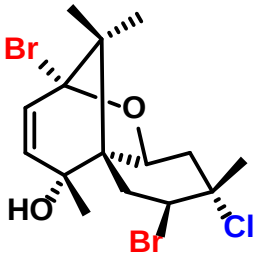
Windansea beach

[1] Sims JJ, Fenical W, Wing RM & Radlick P (1971) *J. Am. Chem. Soc.* 93(15), 3774-3775

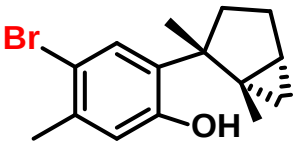
Characterization of sesquiterpene biosynthesis in *Laurencia pacifica* by synthetic biology

1. GC-MS-chemotyping





Pacifenol (**1**)



Laurinterol (**2**)

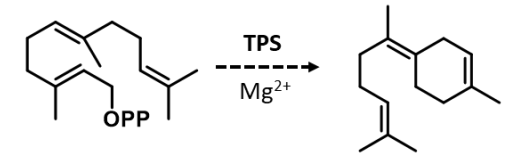
2. Transcriptome sequencing of ***L**aurencia **p**acifica* **h**olobiont (host organism + associated microbiome)

Laurencia pacifica

3. Transcriptome mining for candidate sesquiterpene synthases

Gene product	GC-content [%]	TPM value*	Length [aa]	Closest homolog (similarity/identity) [%/%]	GenBank ID
LphTPS-A	50.8	12.8	341	hypothetical protein SD80_35970 [*Scytonema tolypothrichoides* VB-61278] (48/28)	KIJ77002.1
LphTPS-B	44.8	1.7	341	Terpene synthase metal-binding [*Plesiocystis pacifica*] (46/29)	WP_006972929.1
LphTPS-C	45.8	22.2	338	Terpene synthase metal-binding [*Plesiocystis pacifica*] (46/31)	WP_006972929.1

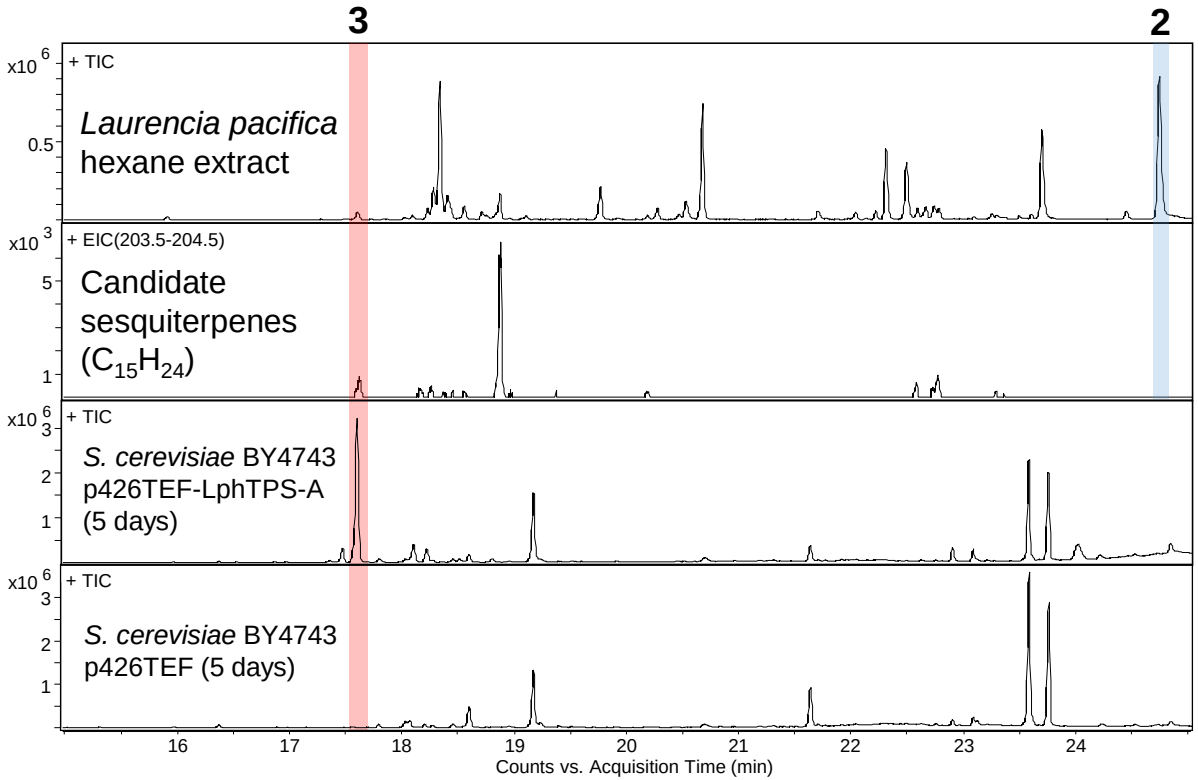
* TPM - transcripts per million reads

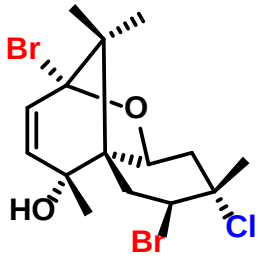


farnesyl diphosphate (FPP)

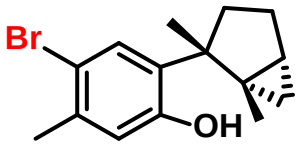
Characterization of sesquiterpene biosynthesis in *Laurencia pacifica* by synthetic biology

4. Heterologous expression of candidate LphTPS-A in yeast





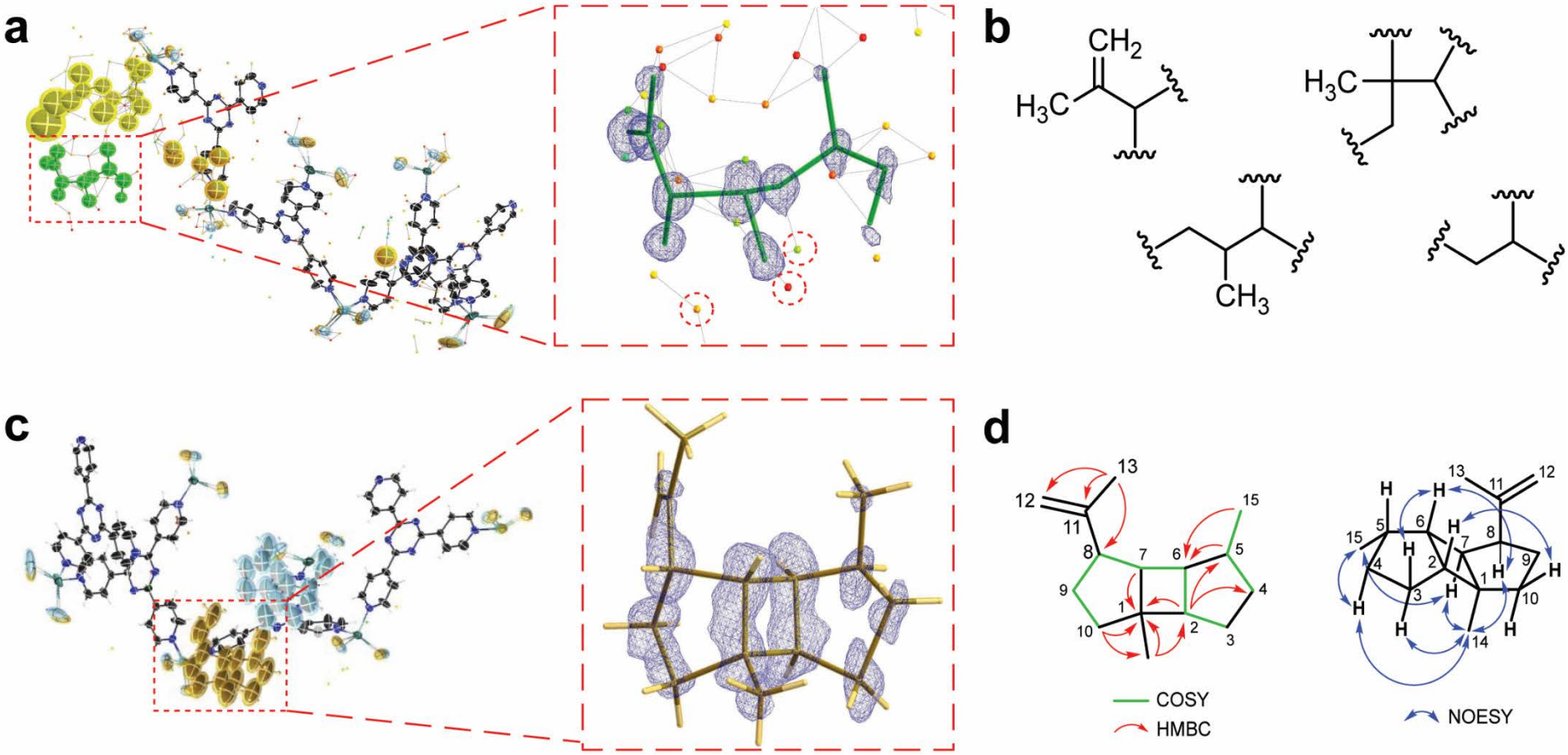
Pacifenol (**1**)



Laurinterol (**2**)

Characterization of a red algal terpene synthase by the crystalline sponge method

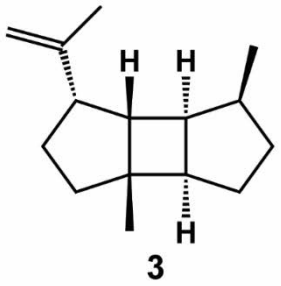
5. NMR-coupled crystalline sponge XRD analysis of **3** (0.8 mg)



Shoukou Lee, Takahiro Iwai (Fujita lab), [1] König, G. M.; Wright, A. D. *J. Org. Chem.* **1997**, *62*, 3837-3840, [2] Nabeta, K.; Yamamoto, M.; Koshino, H.; Fukui, H.; Fukushi, Y.; Tahara, S. *Biosci., Biotechnol., Biochem*. **1999**, *63*, 1772-1776.

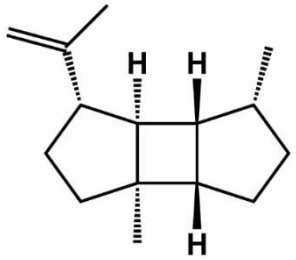
Characterization of a red algal terpene synthase by the crystalline sponge method

5. NMR-coupled crystalline sponge XRD analysis of **3** (0.8 mg)

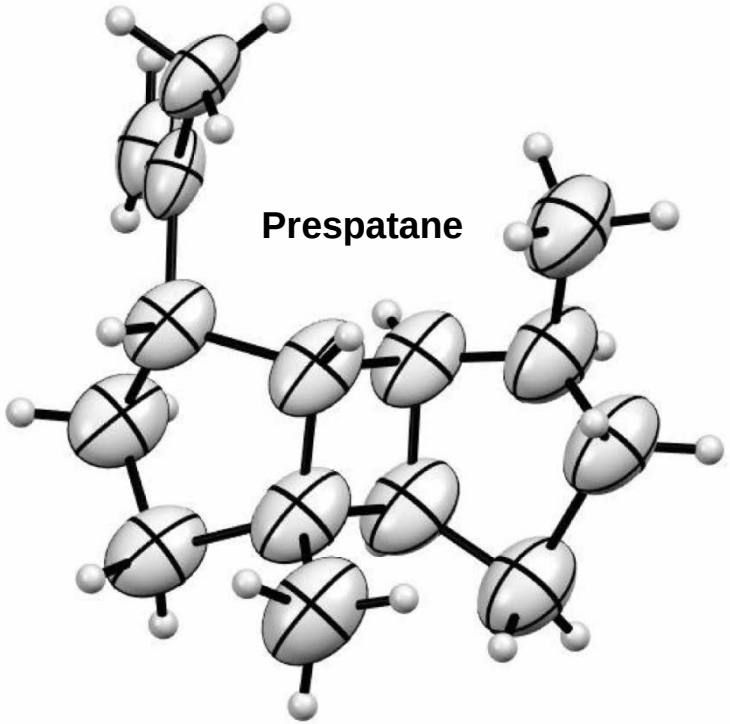


3

(revised structure of **4**)

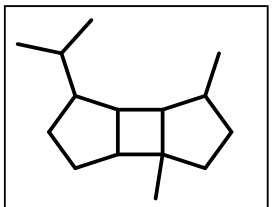


4

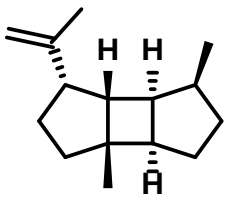


Cymbastela hooperi
(sponge)

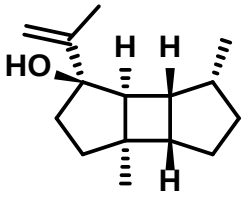
LphTPS-A is a bourbonane sesquiterpene synthase



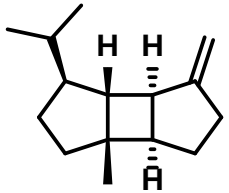
bourbonane scaffold



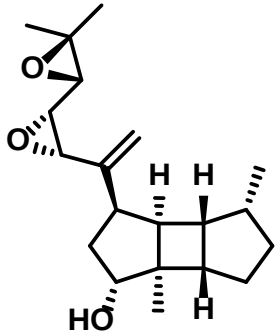
Prespatane (3)
(*Laurencia pacifica*)
NMR-coupled
crystalline sponge XRD



Bourbon-11-en-7-ol
(*Nephthea erecta*) [1]
NMR

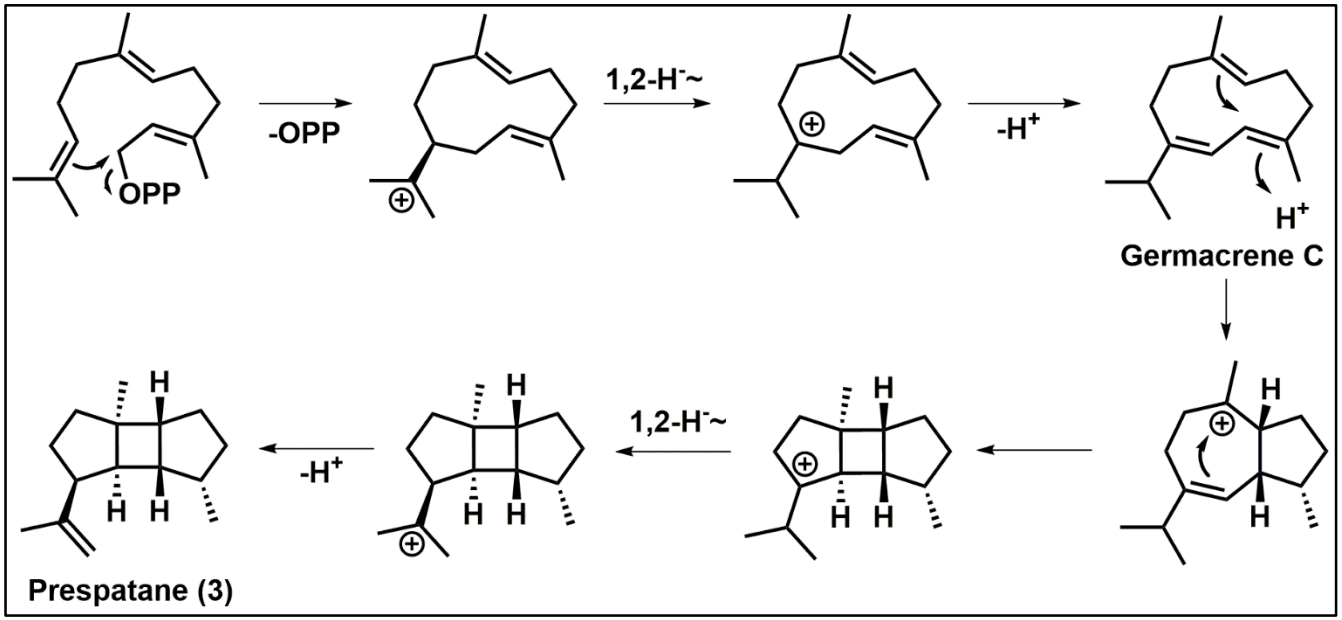


β-bourbonane
(*Geranium bourbon, Ceroplastes ceriferus*) [2,3]
NMR



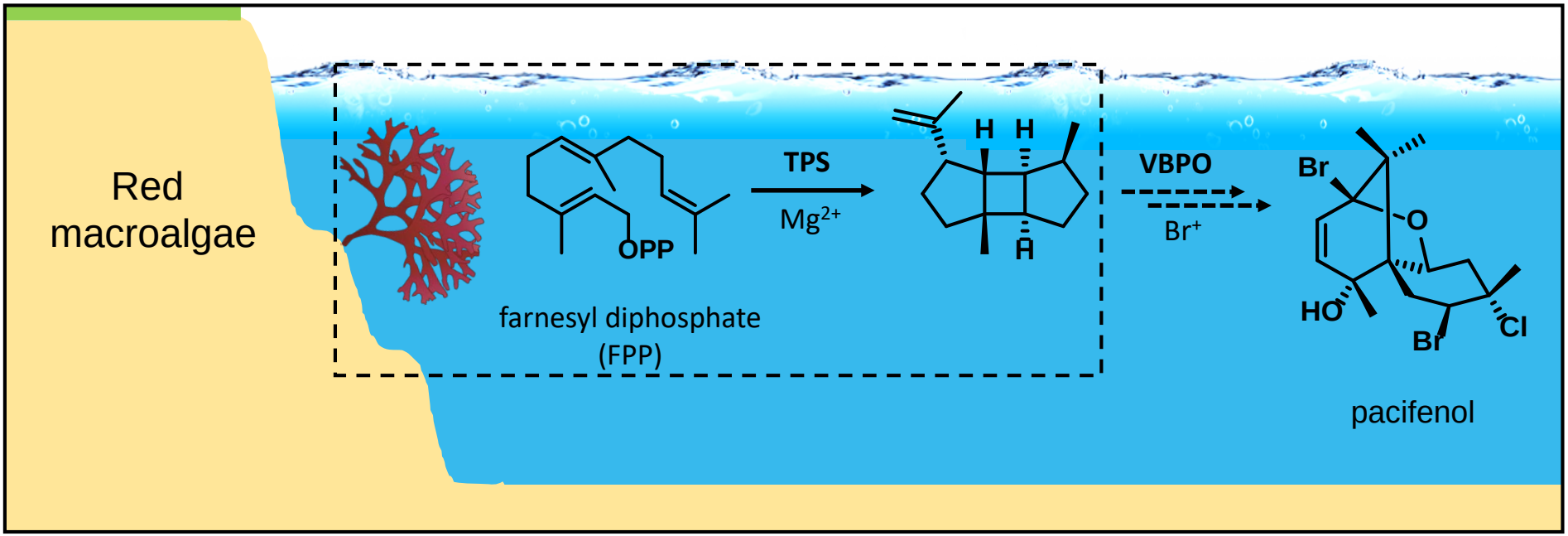
Spatol
(*Spatoglossum schmittii)* [4]
X-ray analysis of *p*-bromobenzylester-derivative

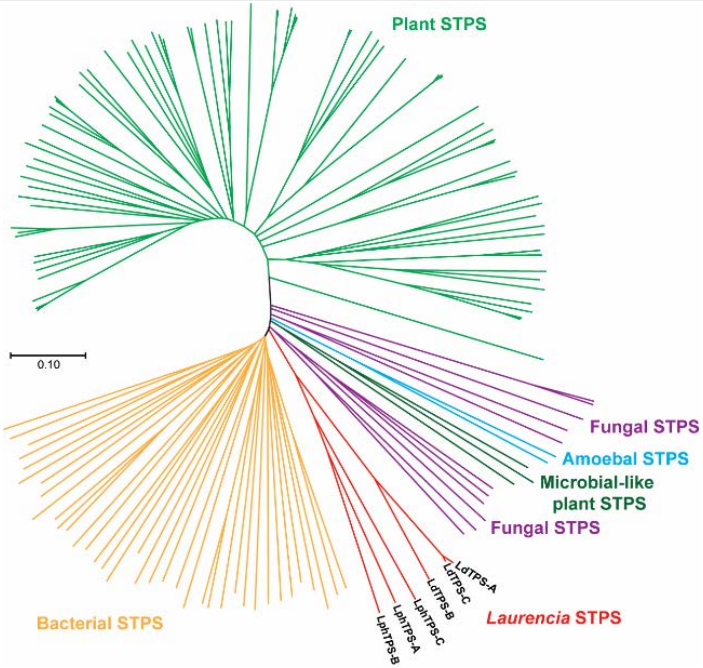
Proposed cyclization mechanism



[1] Cheng, S. Y.; Dai, C. F.; Duh, C. Y. *J. Nat. Prod.* **2007**, *70*, 1449-1453. [2] Křepinský, J.; Samek, Z.; Šorm, F.; Lamparsky, D.; Naves, Y. R. *Tetrahedron* 1966, *22*, 53-70. [3] Bohlmann, F.; Jakupovic, J.; Gupta, R. K.; King, R. M.; Robinson, H. *Phytochemistry*, 1981, *20*, 473-480. [4] Gerwick, W. H.; Fenical, W.; Van Engen, D.; Clardy, J. *J. Am. Chem. Soc.* **1980**, *102*, 7991-7993.

Identification of the first red algal sesquiterpene synthase by synthetic biology & crystalline sponge method



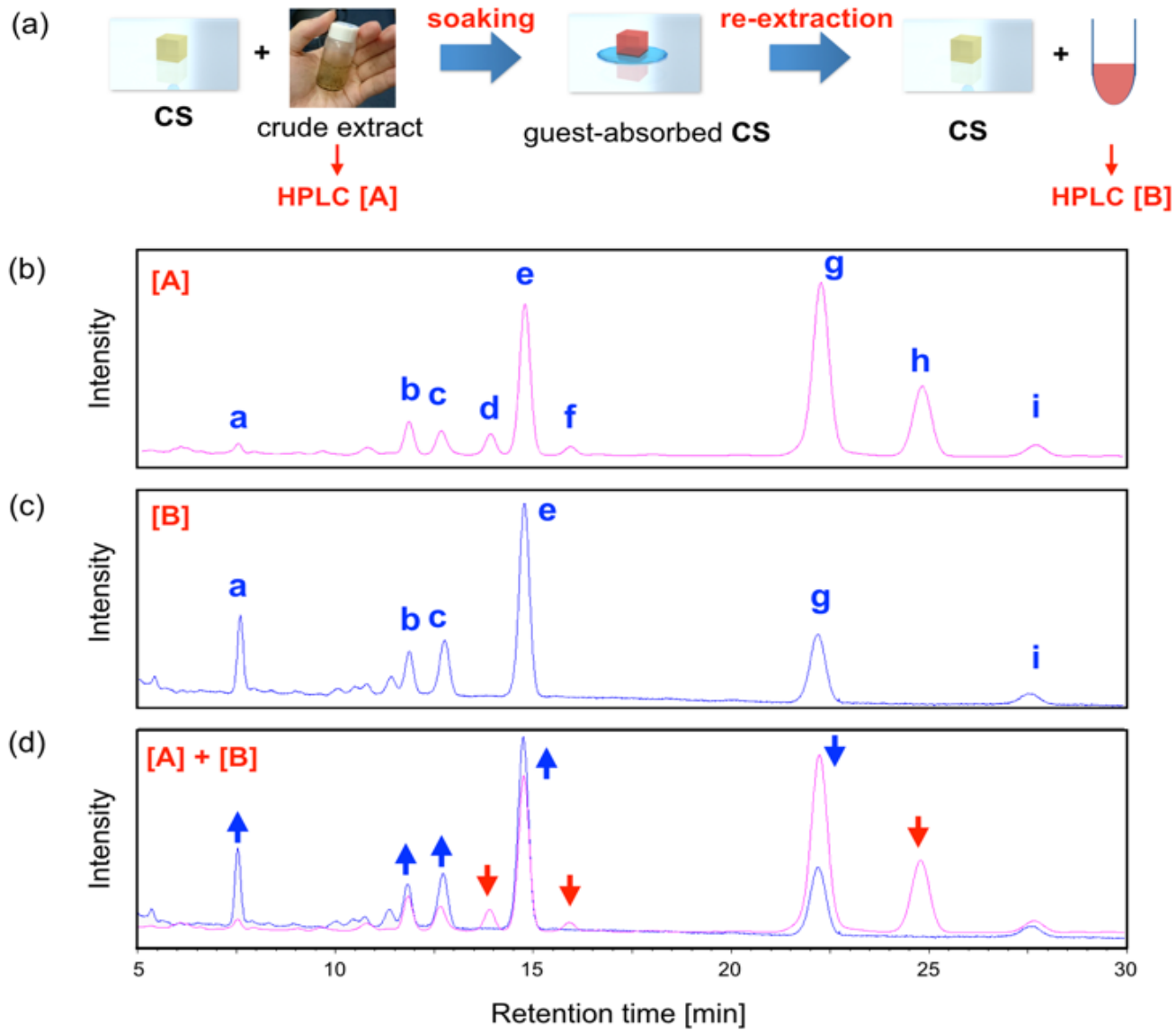


Outlook:

- Identification of halogenation and oxygenation steps in red algal sesquiterpene biosynthesis

Crystalline sponge-based chemotyping

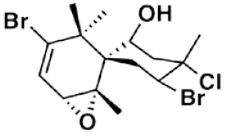
1. Affinity screening prior to CS-XRD analysis



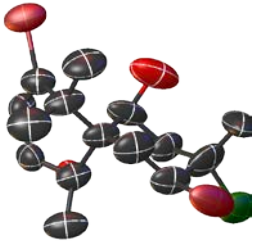
Crystalline sponge-based chemotyping

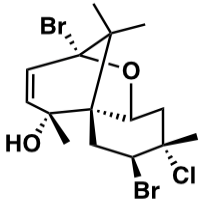
2. NMR-coupled crystalline sponge XRD analysis of prioritized analytes

→ Absolute structure elucidation of six sesquiterpene natural products from 10 mg of crude algal extract (<10 g starting material)

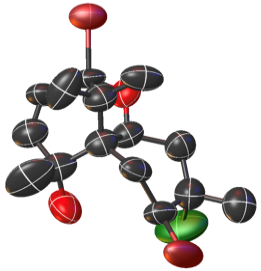


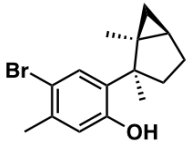
Prepacifenol



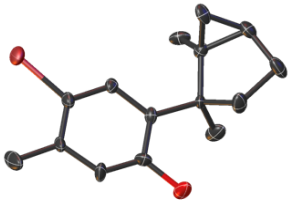


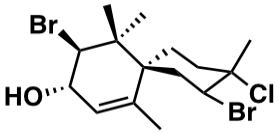
Pacifenol



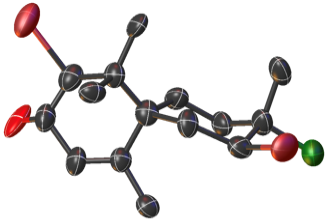


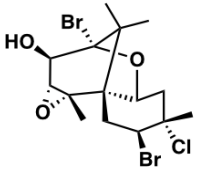
Laurinterol



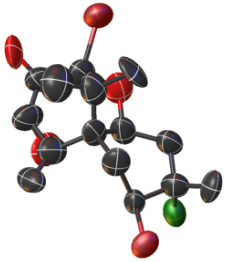


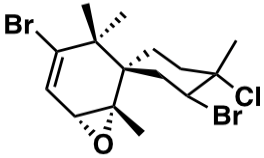
2,10-dibromo-
3-chlorochamigran-7-en-9-ol



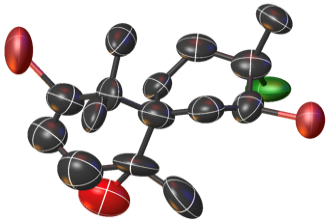


Johnstonol





Deoxyprepacifenol



Weng Laboratory

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Thanks!