

# **Interpreting iron speciation and bioavailability in the marine environment from microbial genetics**

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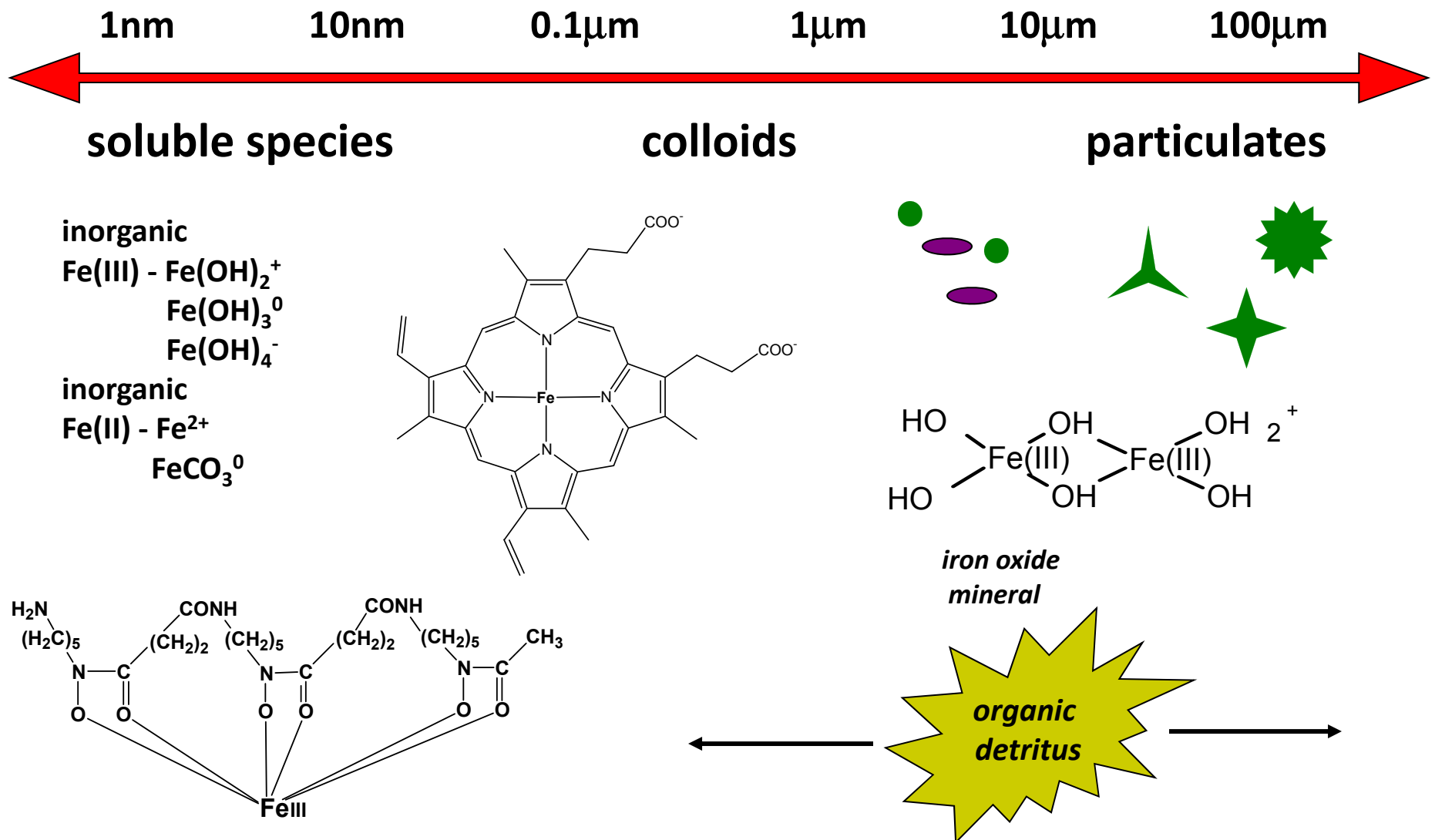
**<sup>2</sup>University of Georgia Marine Sciences Department**

**<sup>3</sup>Colorado School of Mines**



- **Iron availability is a potent control on marine phytoplankton and bacterioplankton productivity**
- **Availability of iron to marine microbiota is mediated not just by supply, but by *in situ* iron chemistry**

# Chemical forms of iron in seawater



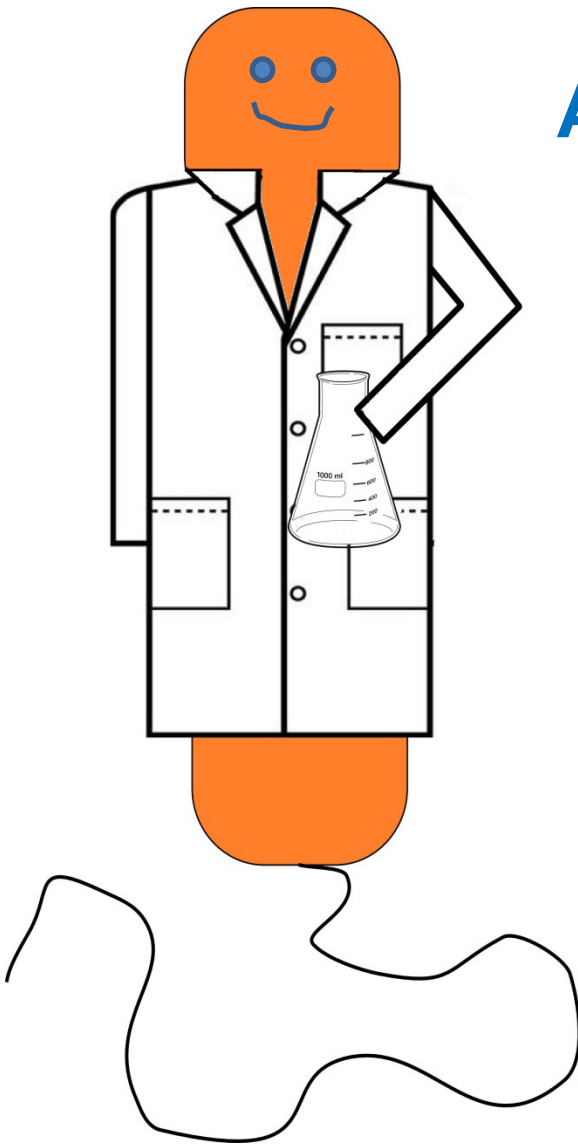
# **How do we address the chemical complexity of iron in seawater?**

- **Mass spectrometry techniques**
- **Advanced separation techniques**
- **Electrochemical techniques**

**This is challenging!**

# What can be gained from characterizing the Fe *transporter* pool?

A “biologically informed”  
approach to chemistry



- Siderophores

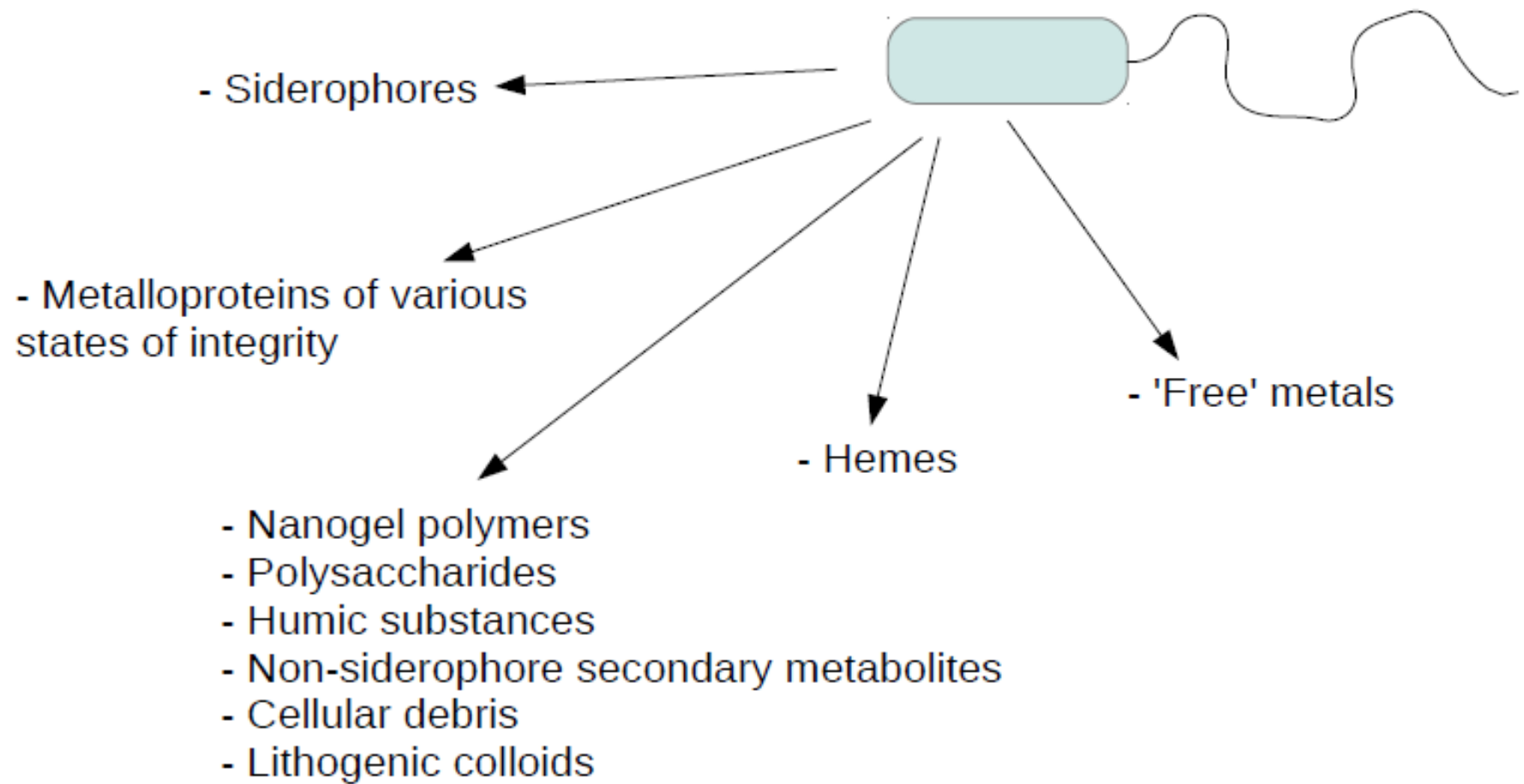


- Metalloproteins of various states of integrity

- 'Free' metals

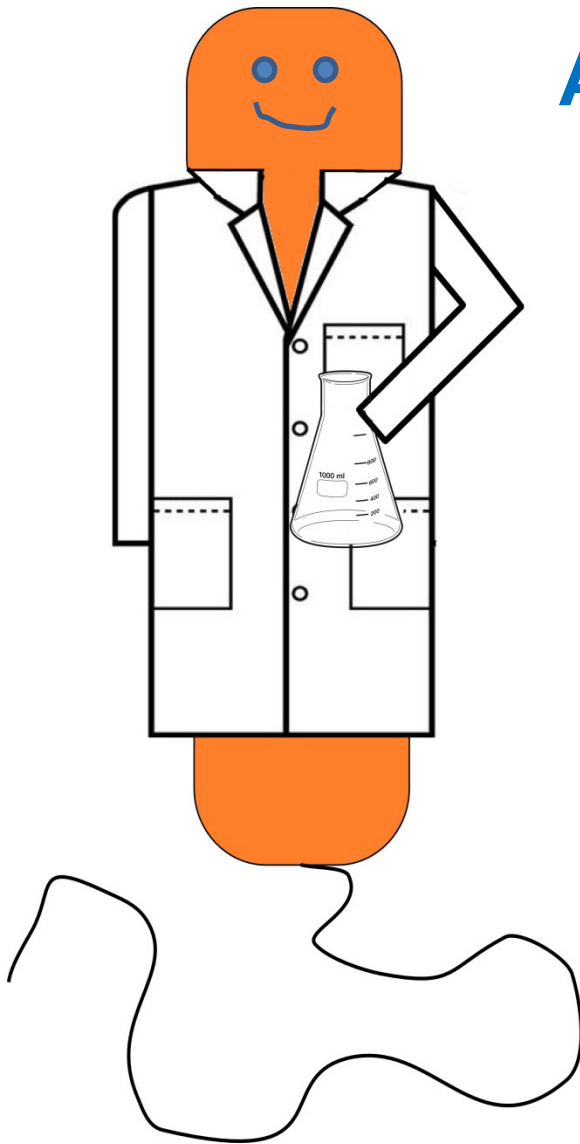
- Hemes

- Nanogel polymers
- Polysaccharides
- Humic substances
- Non-siderophore secondary metabolites
- Cellular debris
- Lithogenic colloids



# What can be gained from characterizing the Fe *transporter* pool?

A “biologically informed”  
approach to chemistry



**Model marine organisms**

- Growth studies
- Genomics
- Transcriptomics
- Gene Knockouts

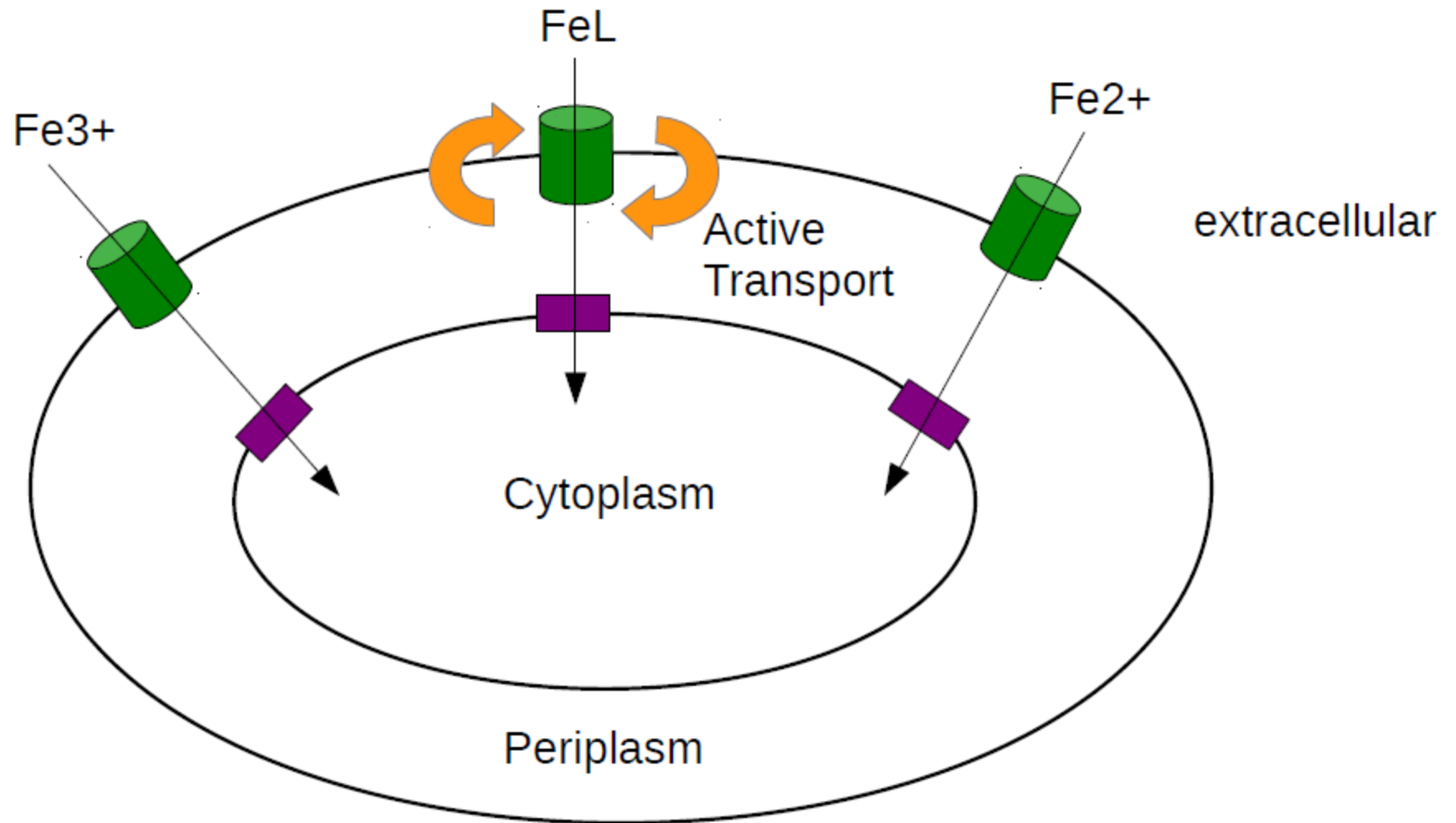
**Natural populations**

- - omics studies

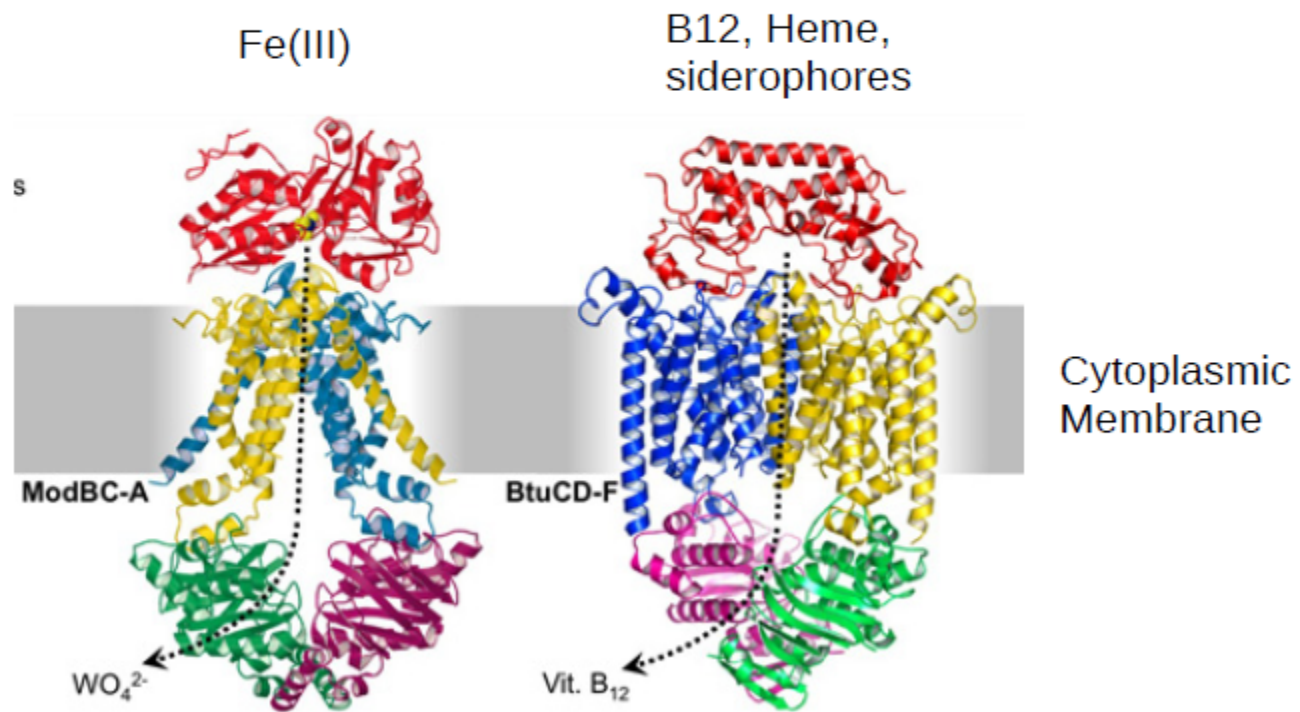


# **Iron uptake systems in marine bacteria**

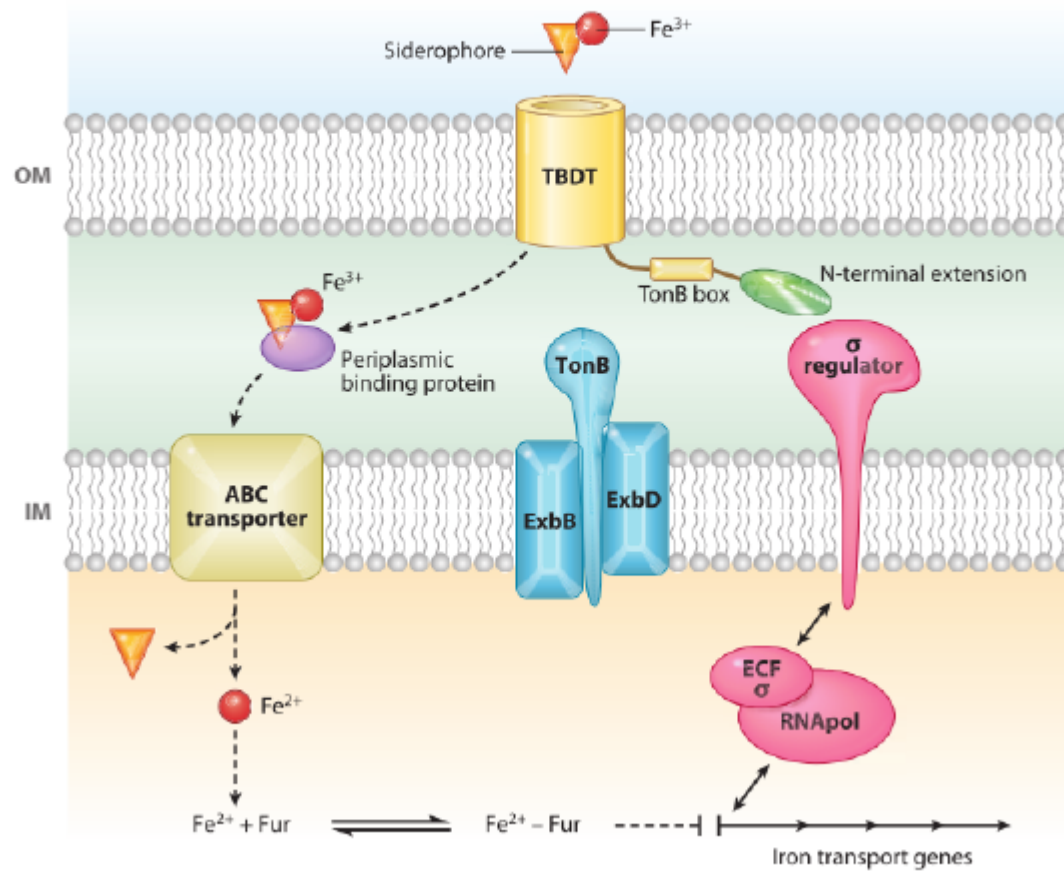
- Most of what is known about Fe transport comes from studies with model pathogenic bacteria



- ATP binding cassette transporters (ABCT)

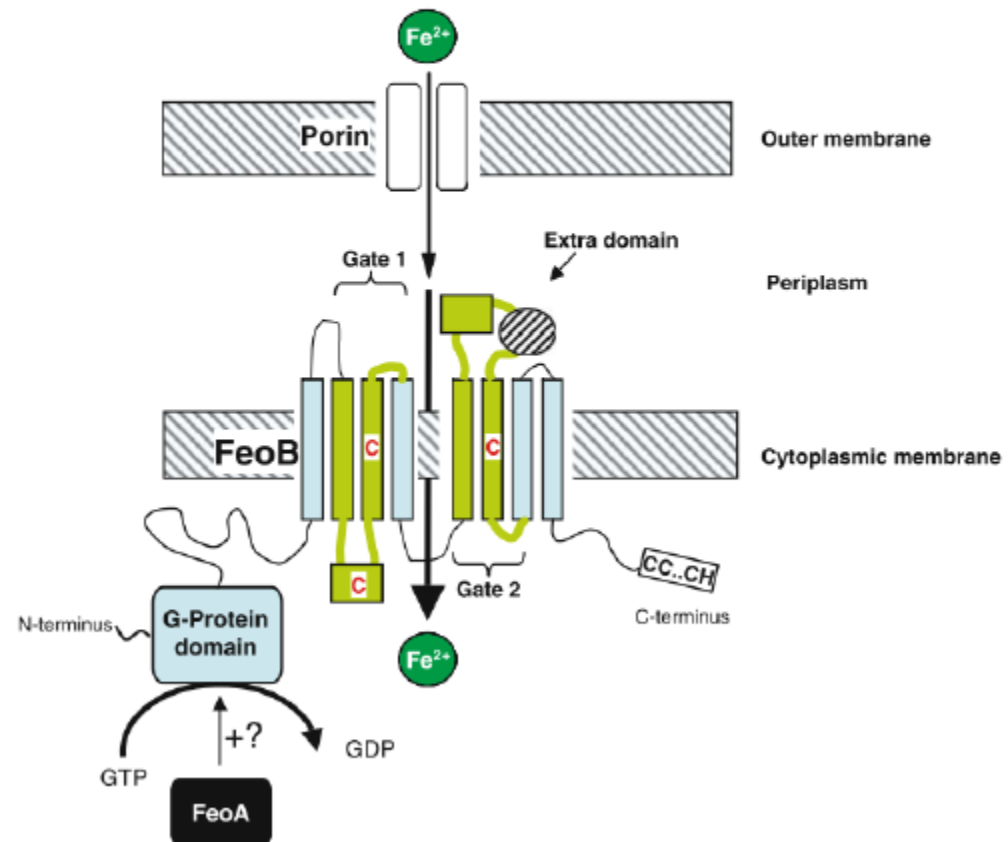


- TonB Dependent Transporters (TBDT)





- Feo transport



# Do we actually find these archetypal iron transporters in all *marine* bacteria?

First look: genome/metagenome survey

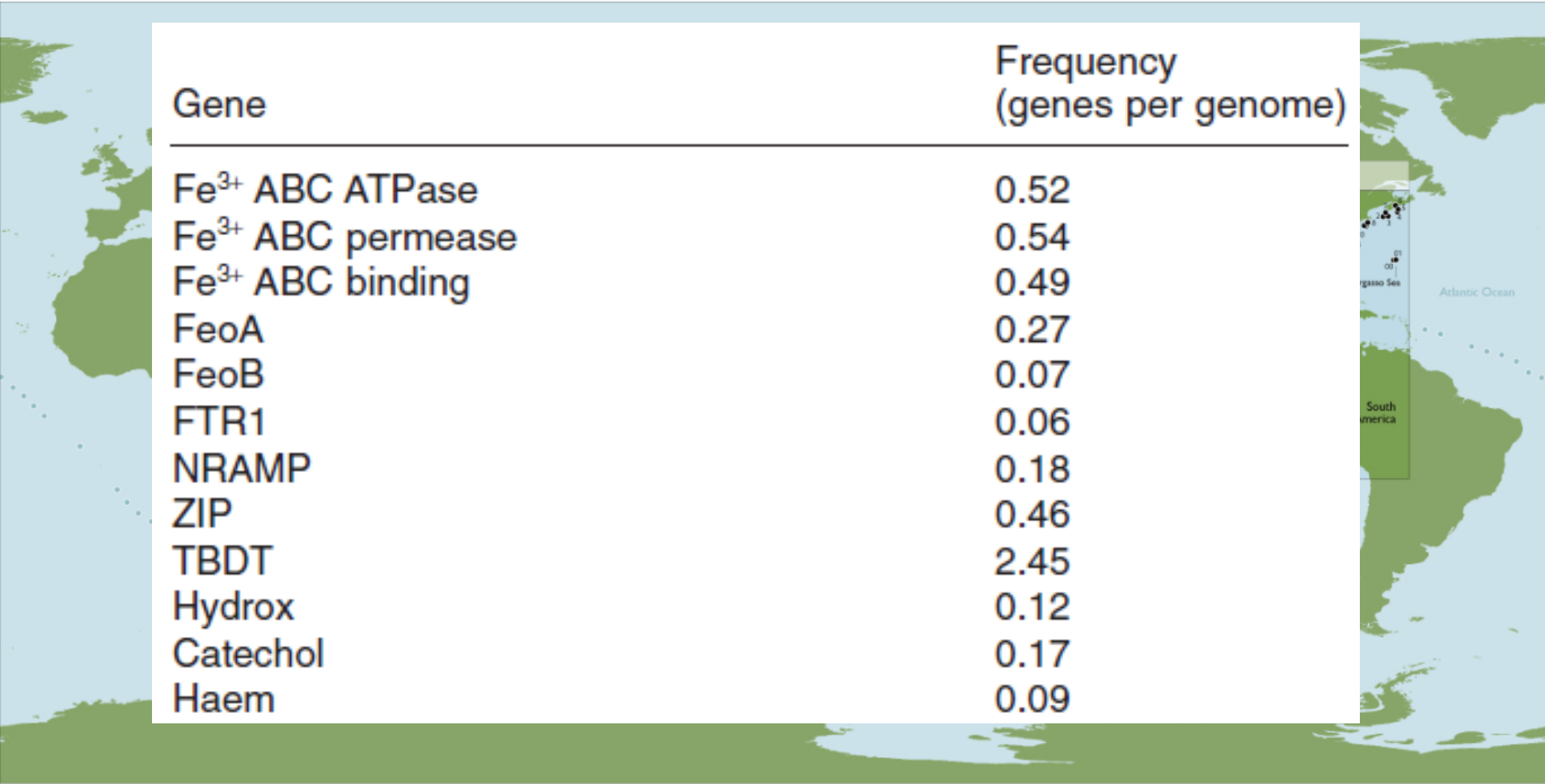
- Hopkinson and Barbeau *EMI* 2012
- Toulza et al. *PLOS One* 2012
- Desai et al. *Front. Microbiol* 2012

# Iron transporters in marine prokaryotic genomes

Taxa	No.	ATP	Perm	Bind	FeoA	FeoB	FTR1	NRAMP	ZIP	TBDT	Hydrox	Catechol	Haem
<i>Alphaproteobacteria</i>	61	51	54	40	8	4	4	5	27	43	28	9	31
<i>Gammaproteobacteria</i>	57	42	48	30	34	29	17	30	23	56	50	38	36
<i>Other Proteobacteria</i>	14	3	4	1	11	11	2	1	7	13	5	6	4
<i>Bacterioidetes</i>	16	1	1	1	16	15	0	15	16	16	8	4	14
<i>Picocyanobacteria</i>	22	22	22	7	2	1	0	15	2	1	0	0	1
<i>Other Cyanobacteria</i>	15	14	15	5	10	8	7	3	8	8	7	2	2
<i>Other Bacteria</i>	15	5	6	6	9	7	4	10	10	5	2	2	1
<i>Archaea</i>	6	0	1	0	4	4	2	1	4	0	0	0	0

Hopkinson and Barbeau EMI 2012

# Iron uptake genes in the GOS metagenomes



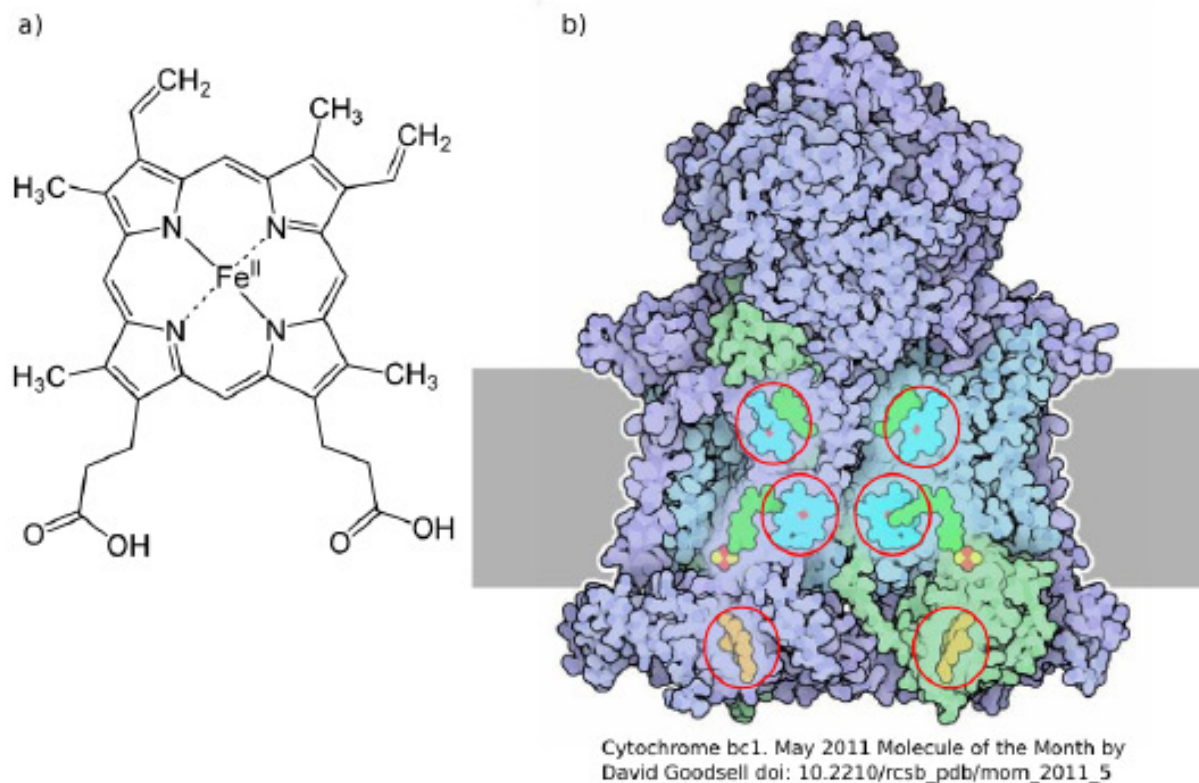
Gene	Frequency (genes per genome)
Fe <sup>3+</sup> ABC ATPase	0.52
Fe <sup>3+</sup> ABC permease	0.54
Fe <sup>3+</sup> ABC binding	0.49
FeoA	0.27
FeoB	0.07
FTR1	0.06
NRAMP	0.18
ZIP	0.46
TBDT	2.45
Hydrox	0.12
Catechol	0.17
Haem	0.09



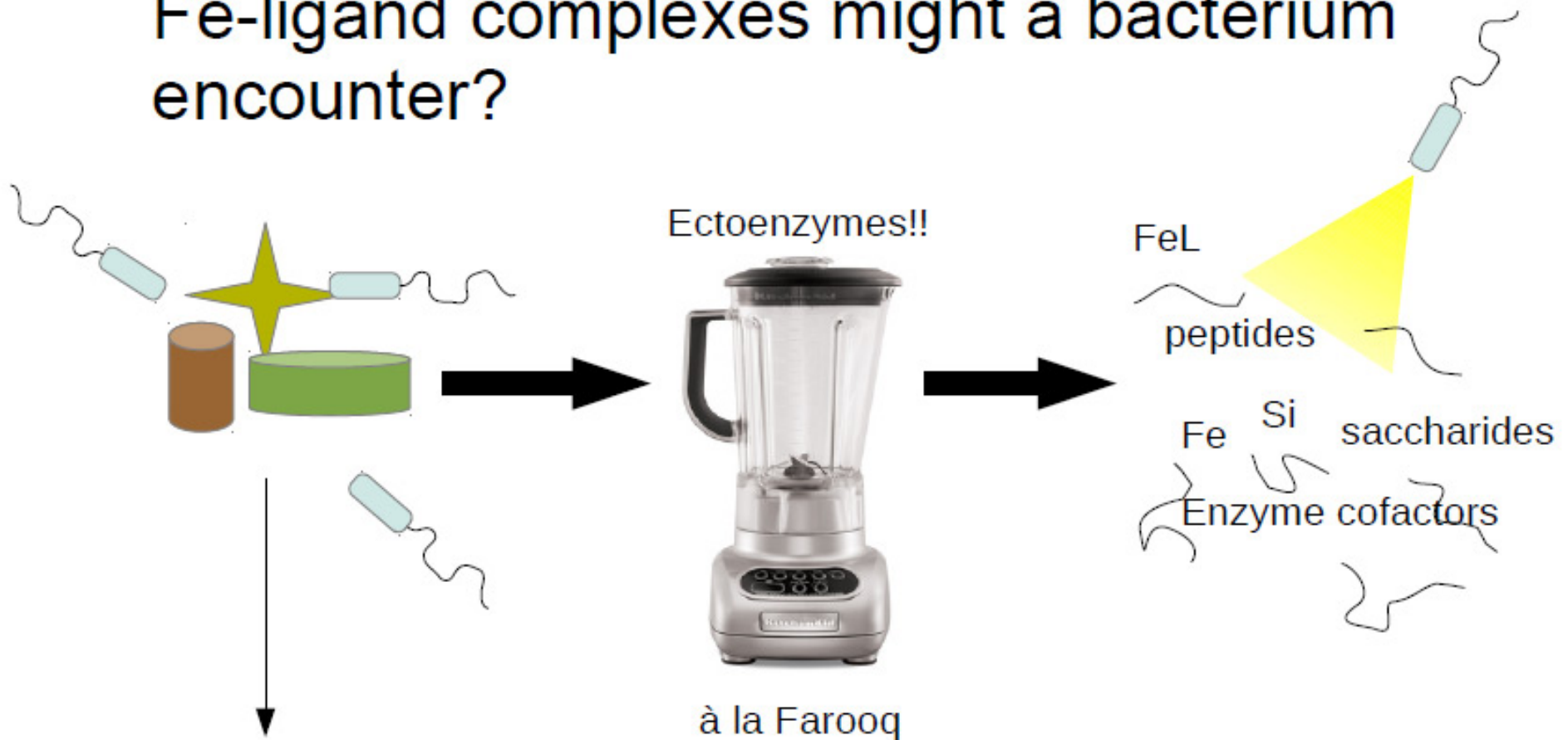
**Molecular mechanisms underlying microbial  
Fe acquisition in the marine environment:**

**A focus on transport of  
the model Fe-binding ligand heme**

- Heme is a fundamental building block in diverse enzymes across all domains of life



- In the process of 'regenerating' or 'recycling' Fe from sinking matter, what Fe-ligand complexes might a bacterium encounter?

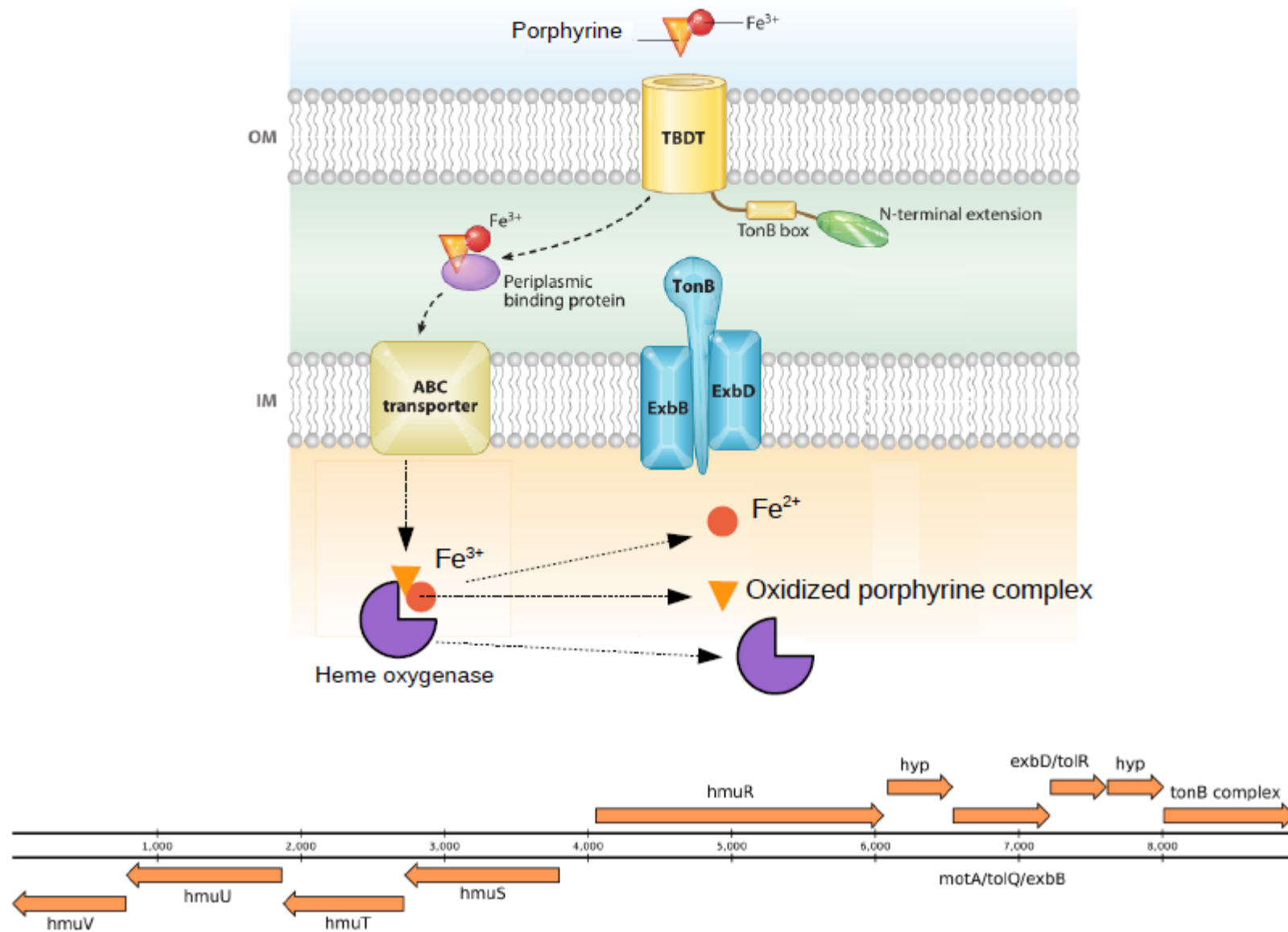


- Intracellular heme contents of common marine phytoplankton have been determined
- Heme concentrations in POM from the North Atlantic have also been determined
- Heme concentration in dissolved fraction ( $<0.2\text{ }\mu\text{m}$ ) have been determined for estuarine water but were below DL for oligotrophic seawater.

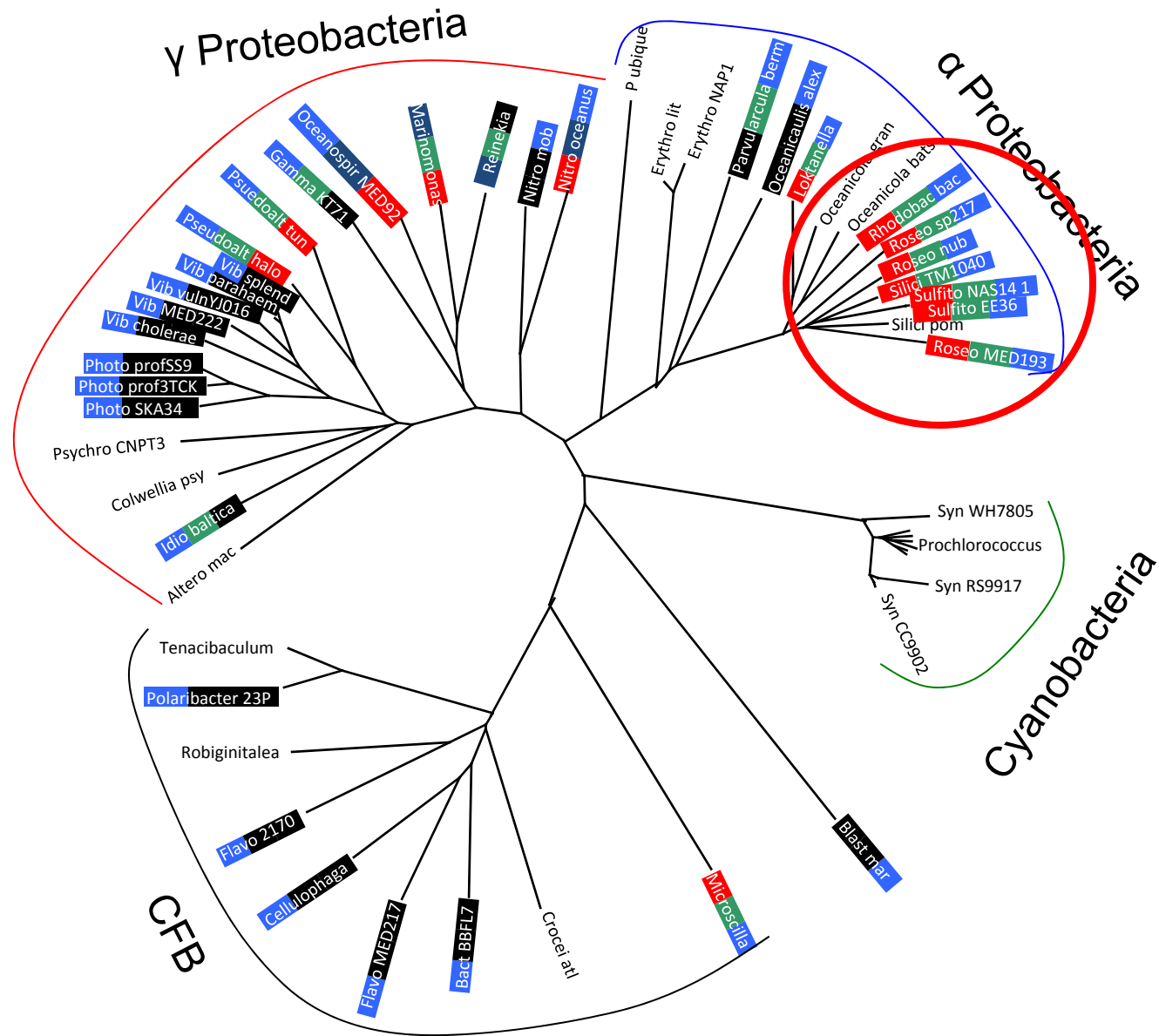
Honey et al. MEPS 2013; Gledhill Mar. Chem. 2007; Vong et al. ACA 2007



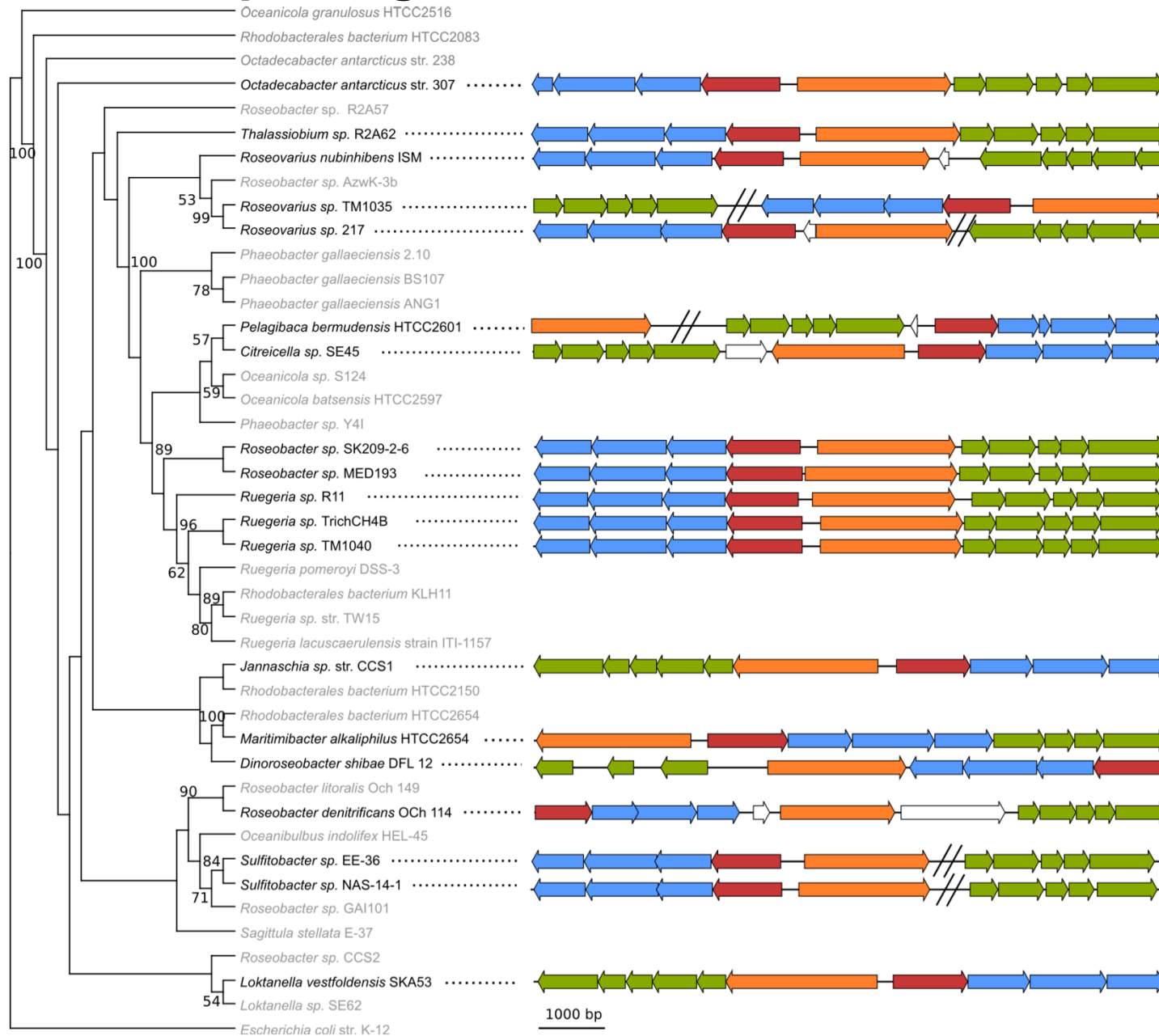
- Heme uptake in pathogens

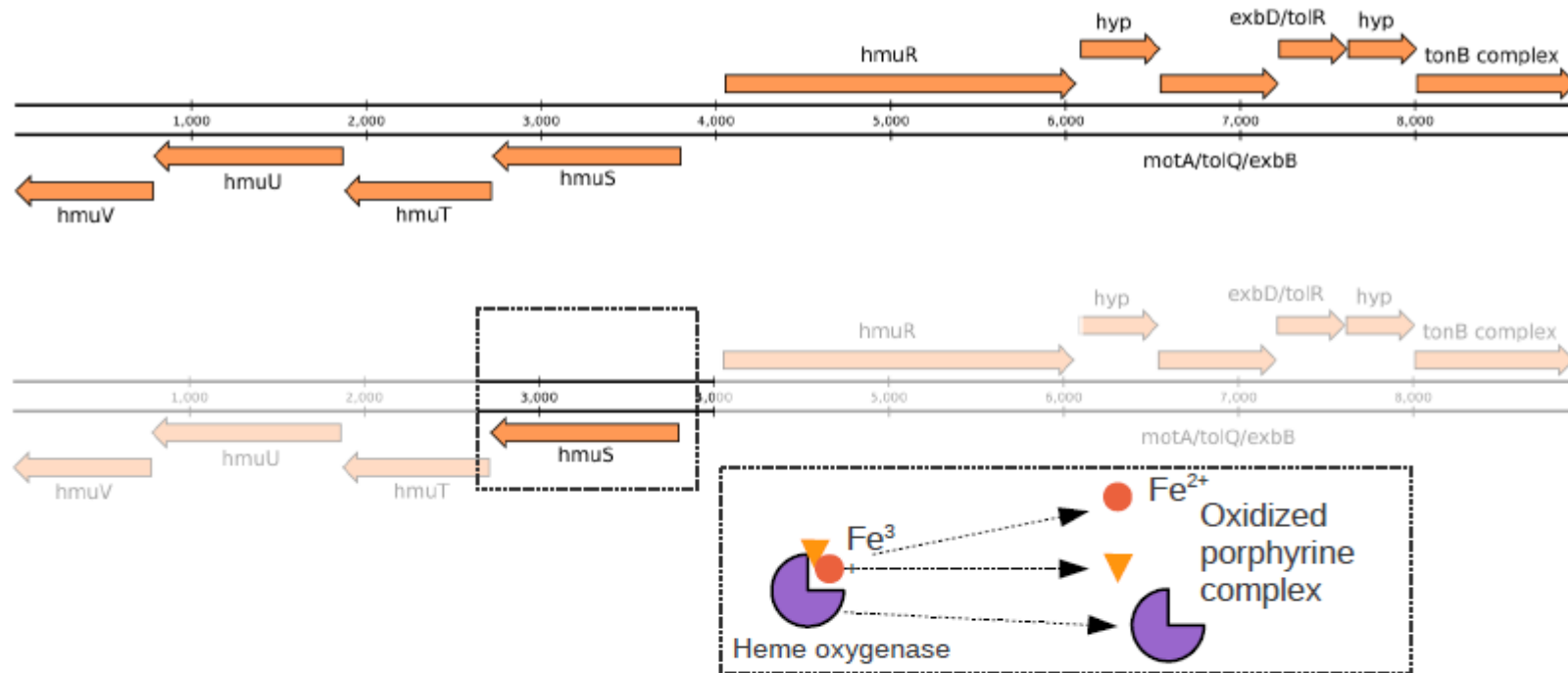


# Phylogenetic distribution of heme transport

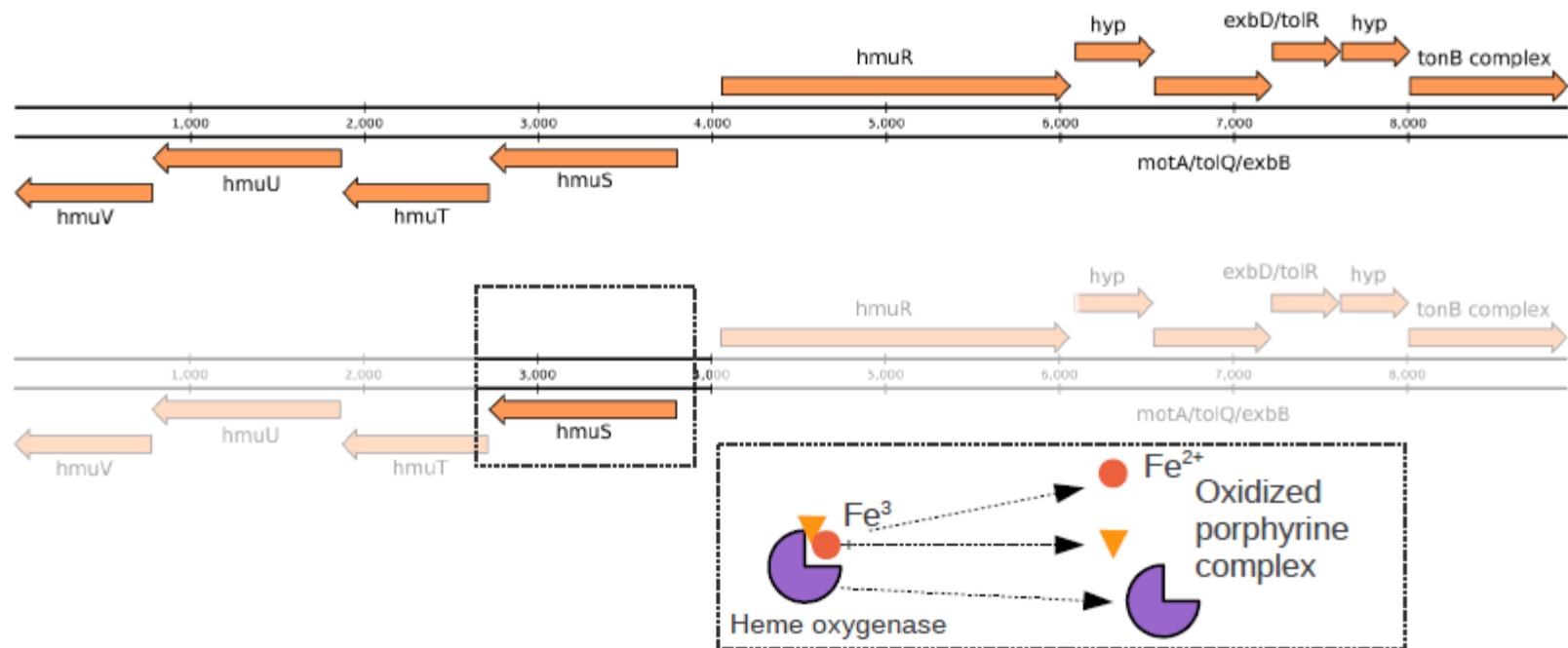


# Heme uptake genes in marine roseobacters



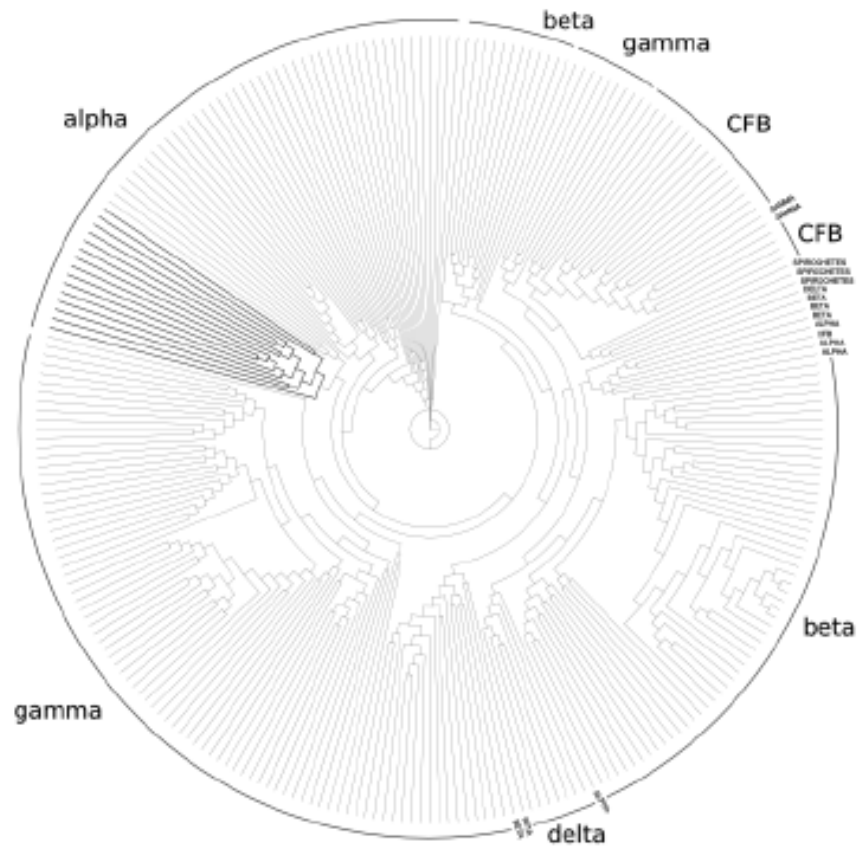


- Sequence conservation of hmuS gene makes it an appropriate subject for targeted metagenomics (degenerate primer based)



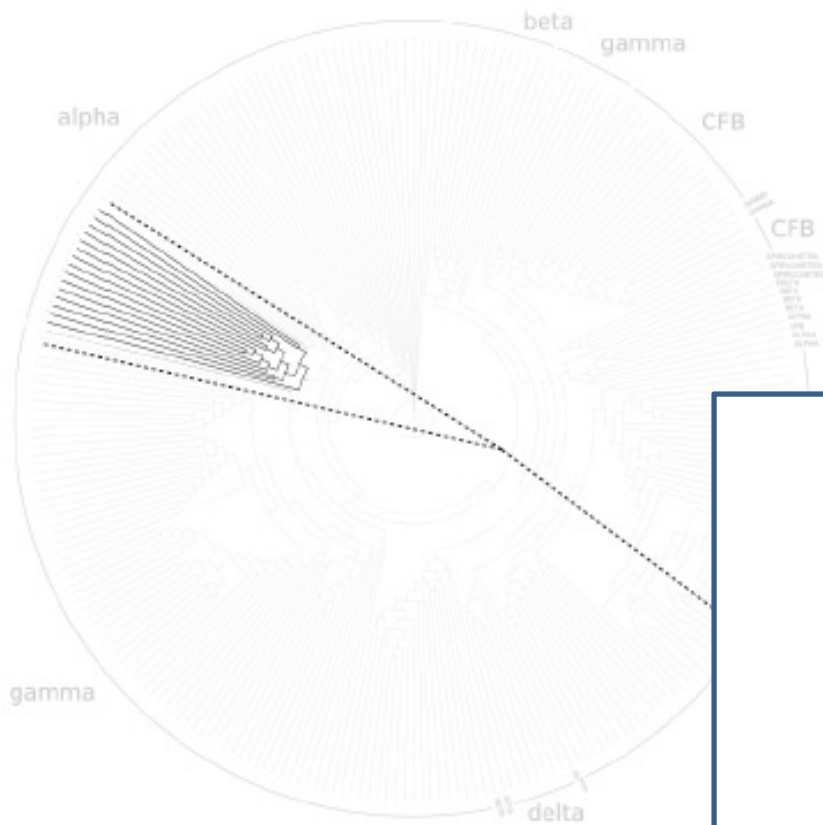
- Sequence conservation of hmuS gene makes it an appropriate subject for targeted metagenomics (degenerate primer based)
- Phylogeny of hmuS component of heme uptake pathway can provide insight to distribution/diversity of capability in the marine environment





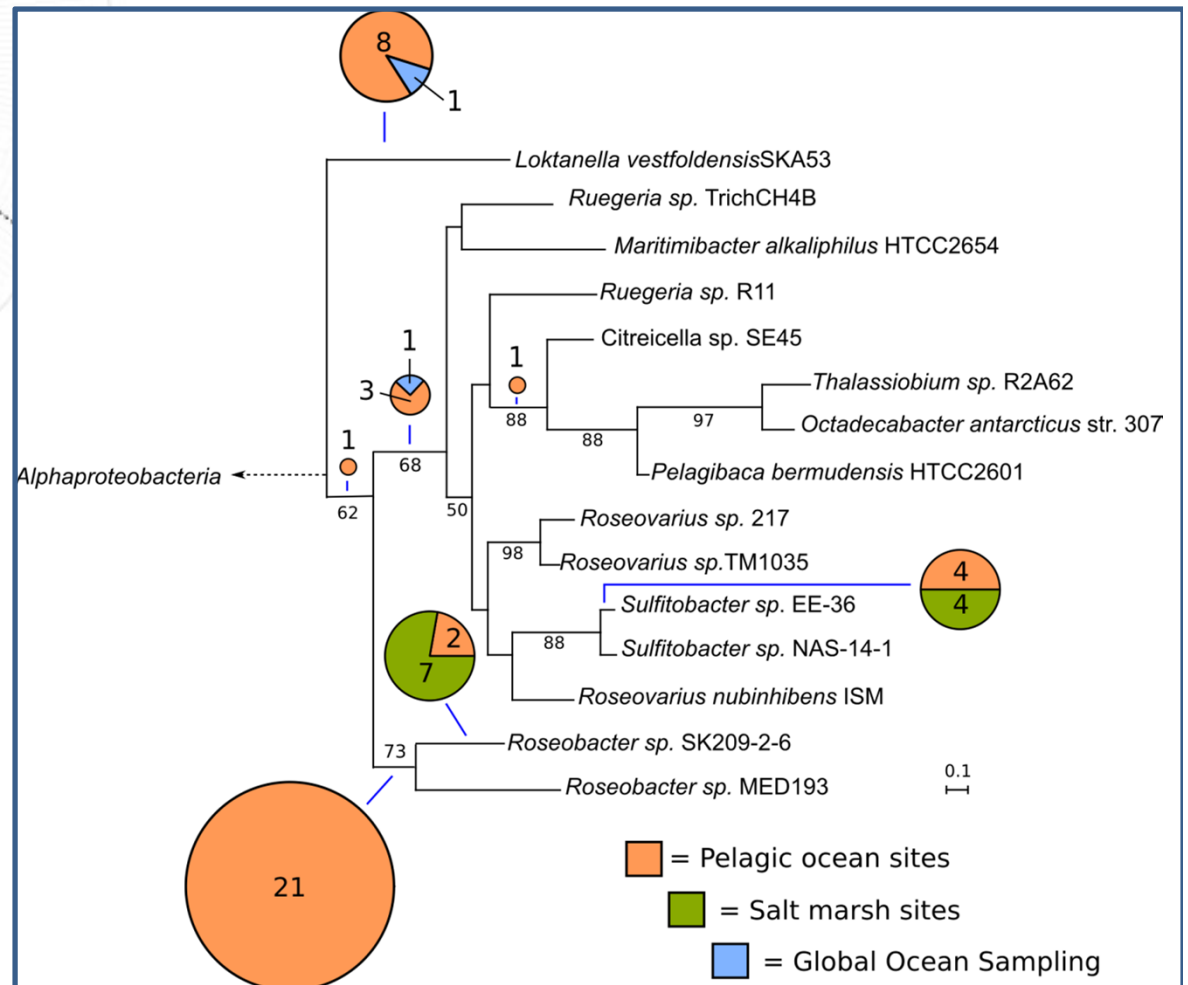
hmuS phylogeny is  
congruent with 16S  
taxonomy

Roe et al. AEM in press



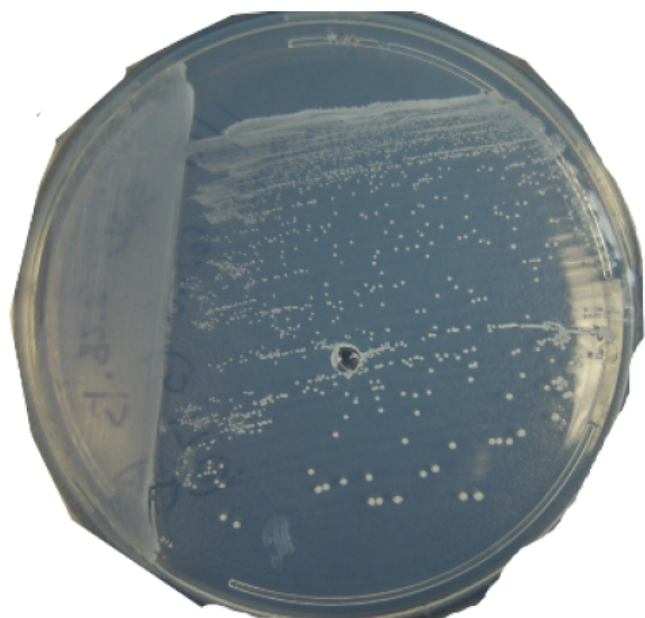
Primer system is specific for marine roseobacters

hmuS detected in the marine environment



**Work with roseobacter isolates**

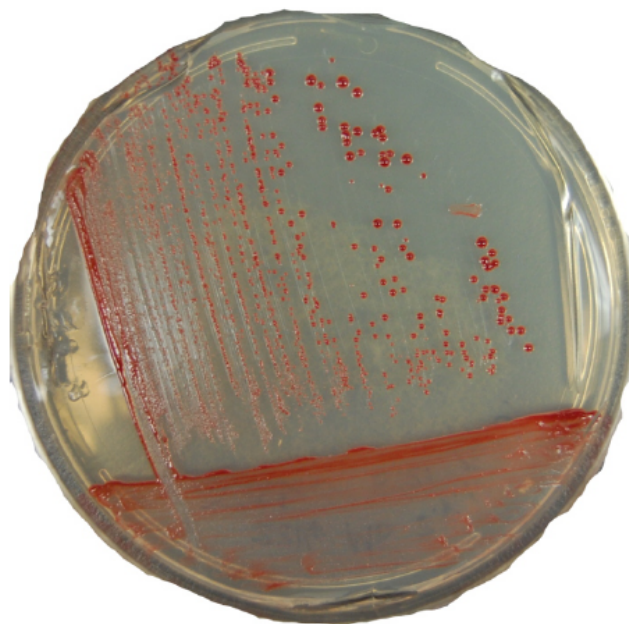
*Ruegeria* sp. TrichCH4B



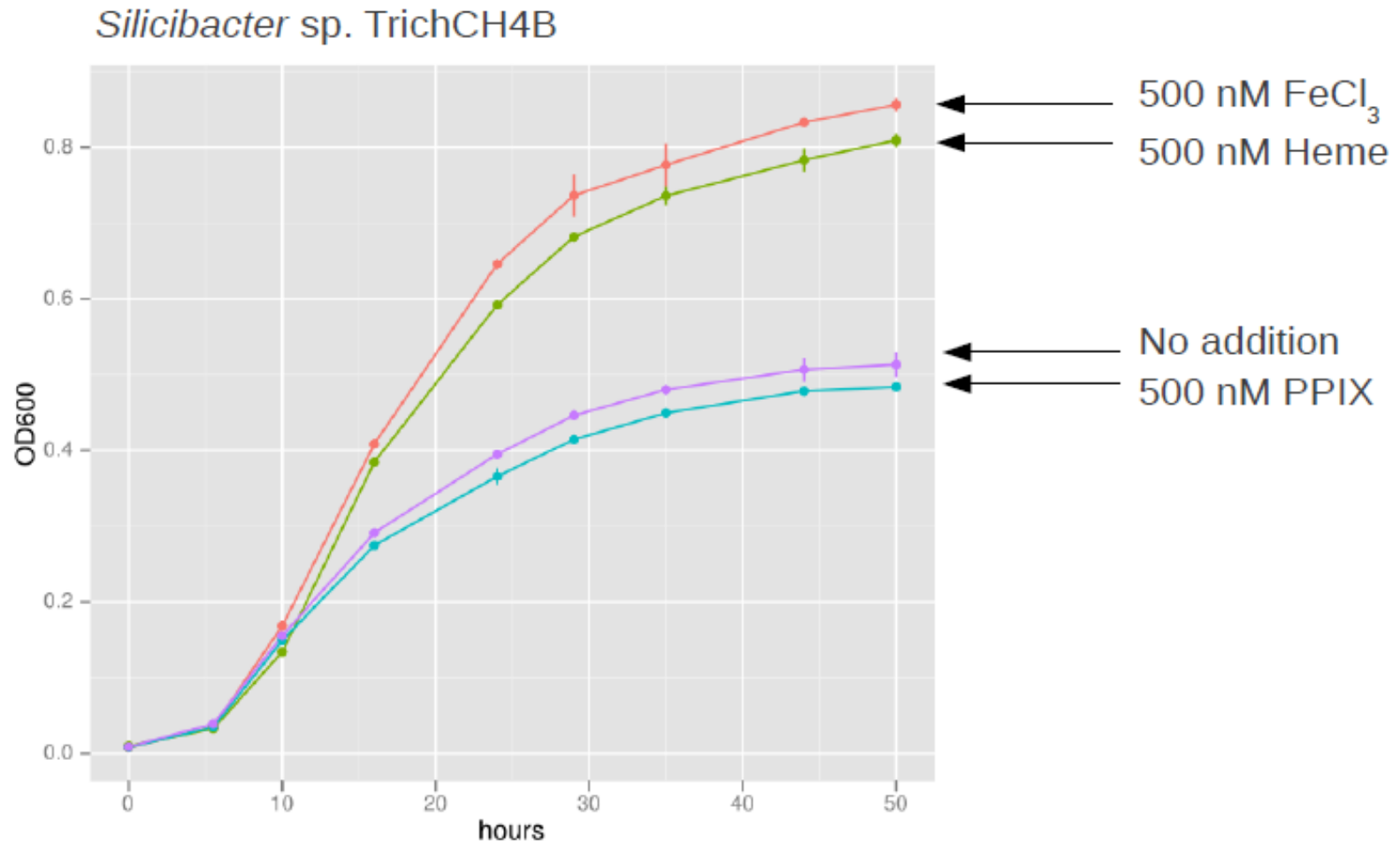
*Ruegeria* sp. TM1040



*Dinoroseobacter shibae* DFL-12

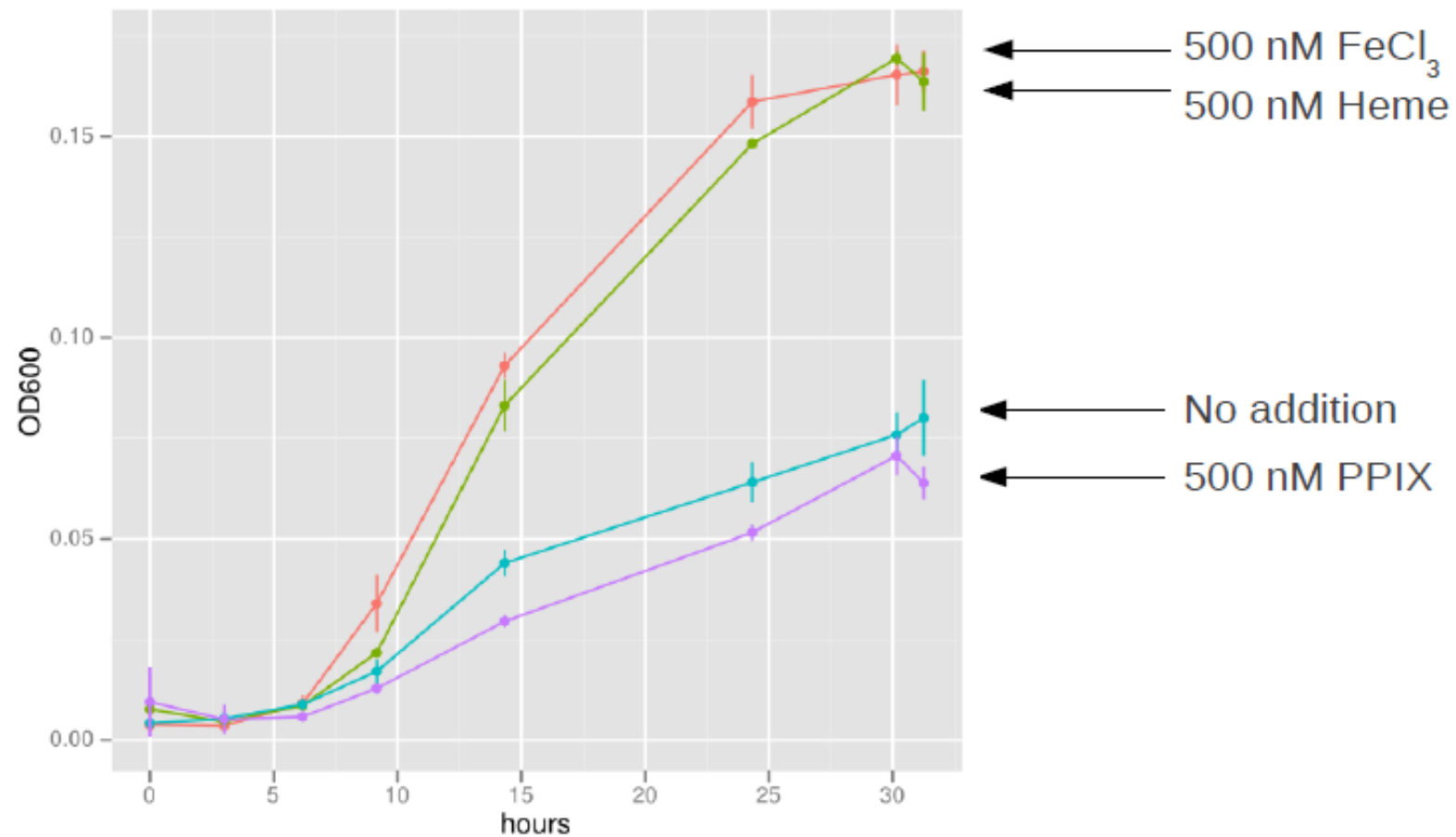


## Marine Isolates can grow on Ferric-porphyrin complexes as the sole iron source

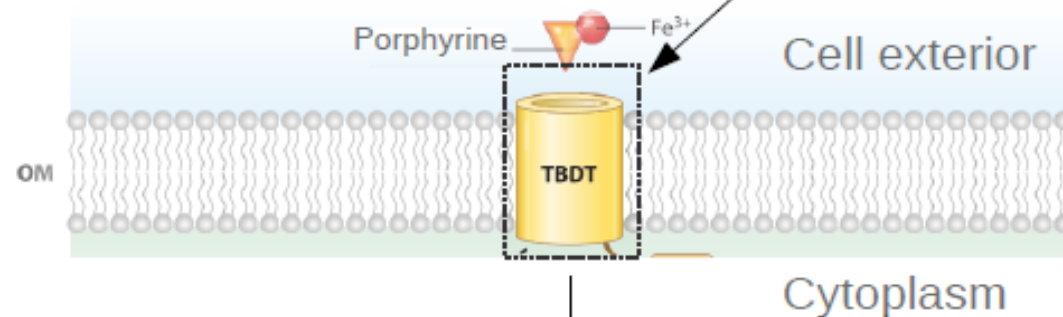
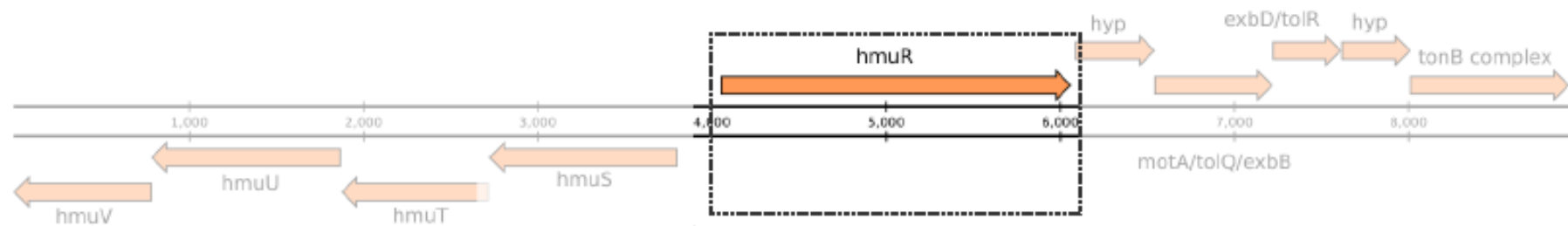


## Marine Isolates can grow on Ferric-porphyrin complexes as the sole iron source

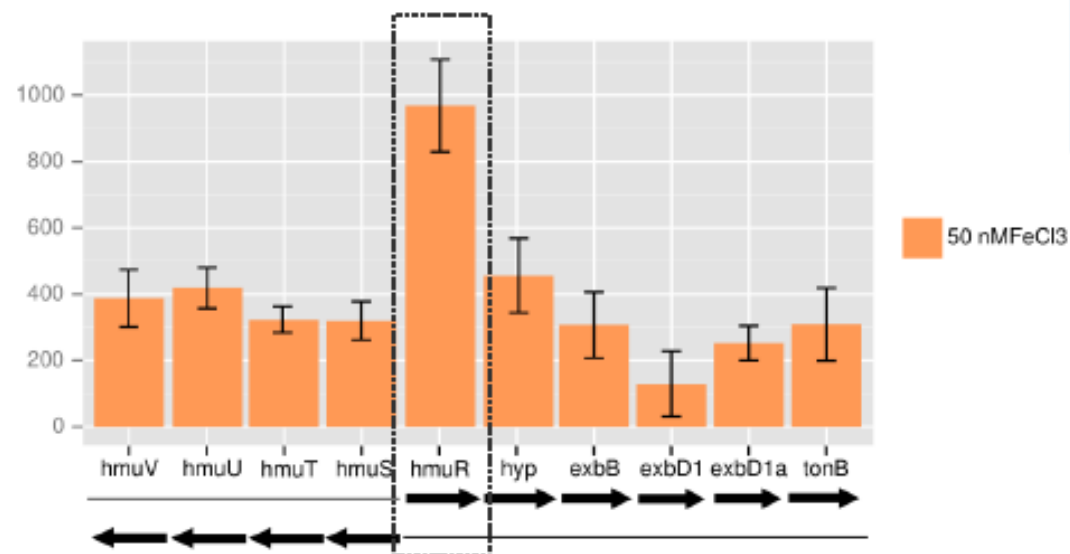
*Ruegeria* sp. TM1040

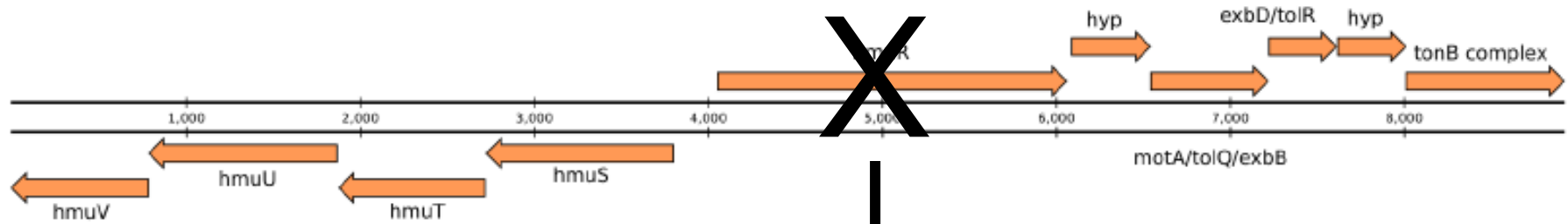






Putative heme tonB dependent transporter (TBDT) expression responds strongly to Fe source and concentration



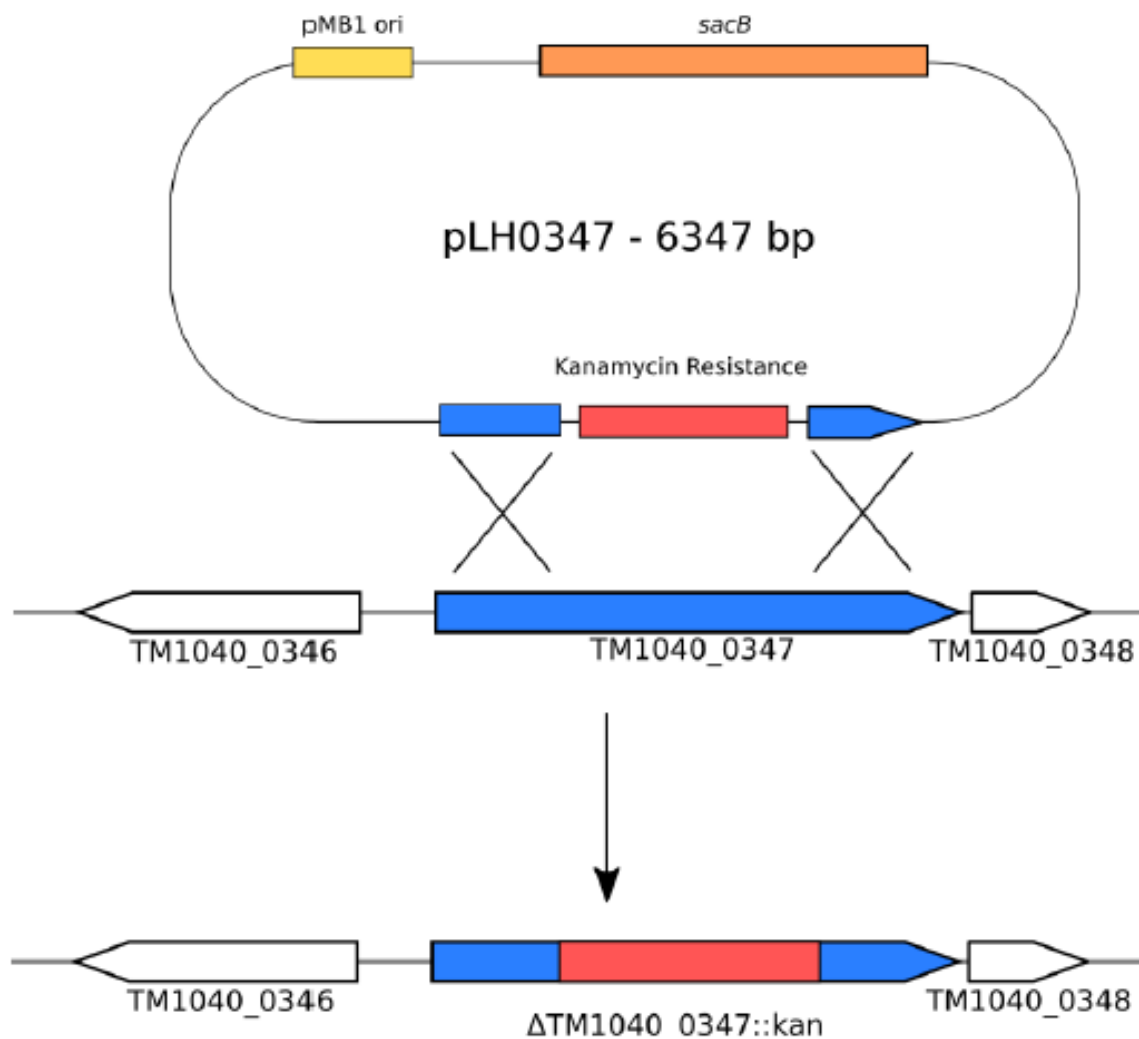


*Ruegeria* sp. TM1040

Biochemical  
confirmation of TBSDT  
substrates is critical!

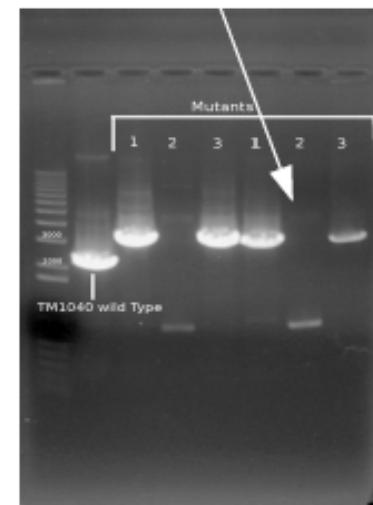
Functional characterization of  
“heme” TBSDT via knockout  
mutation

## Generation of TBDT knockout in TM1040 using Gibson cloned pRL271 derived suicide vector



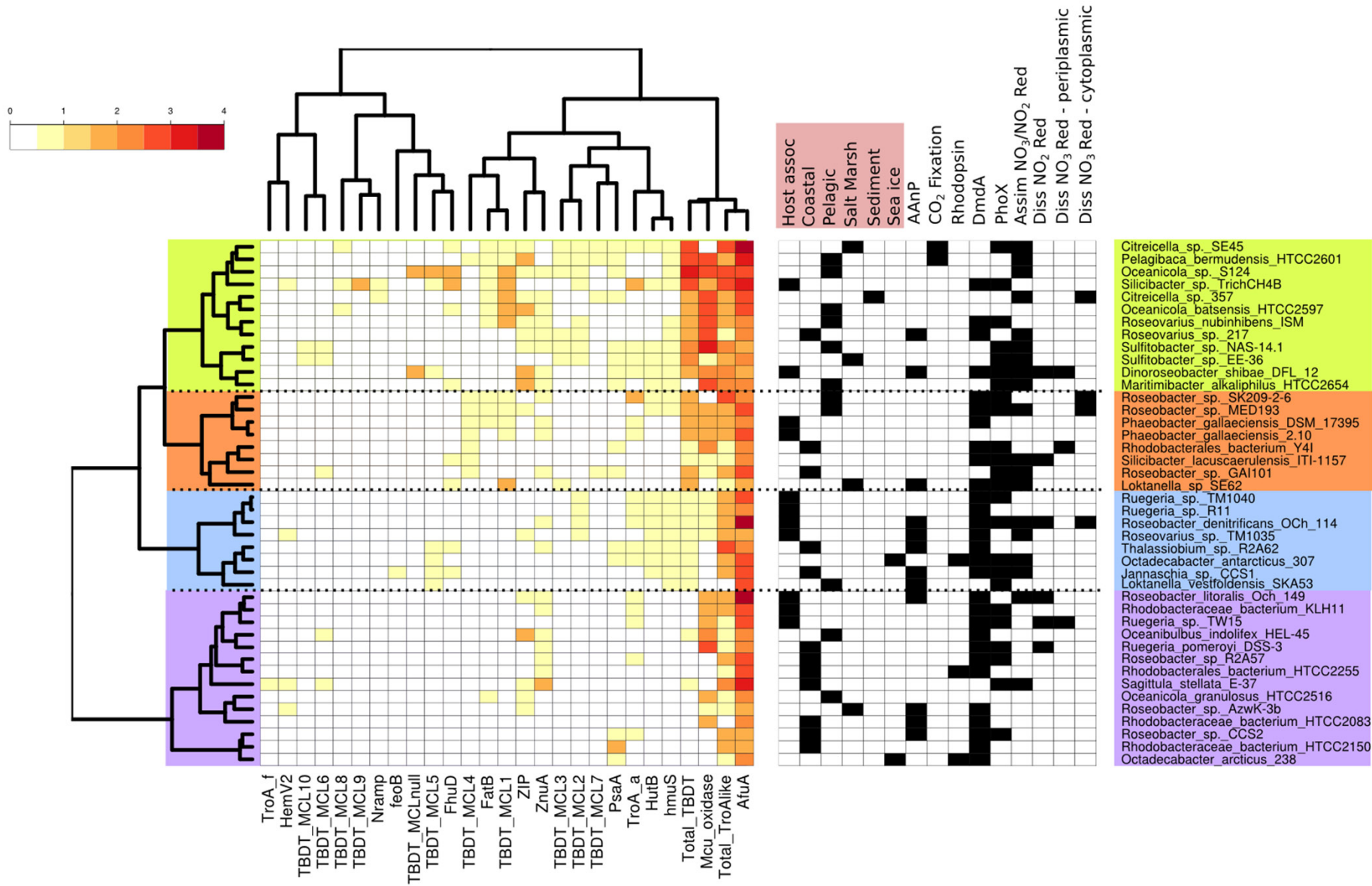
TM1040\_0347<sup>+</sup> 2210 bp  
TM1040\_0347<sup>-</sup> 3124 bp  
Deletion of 460 bp

$\Delta$ TM1040\_0347::kan

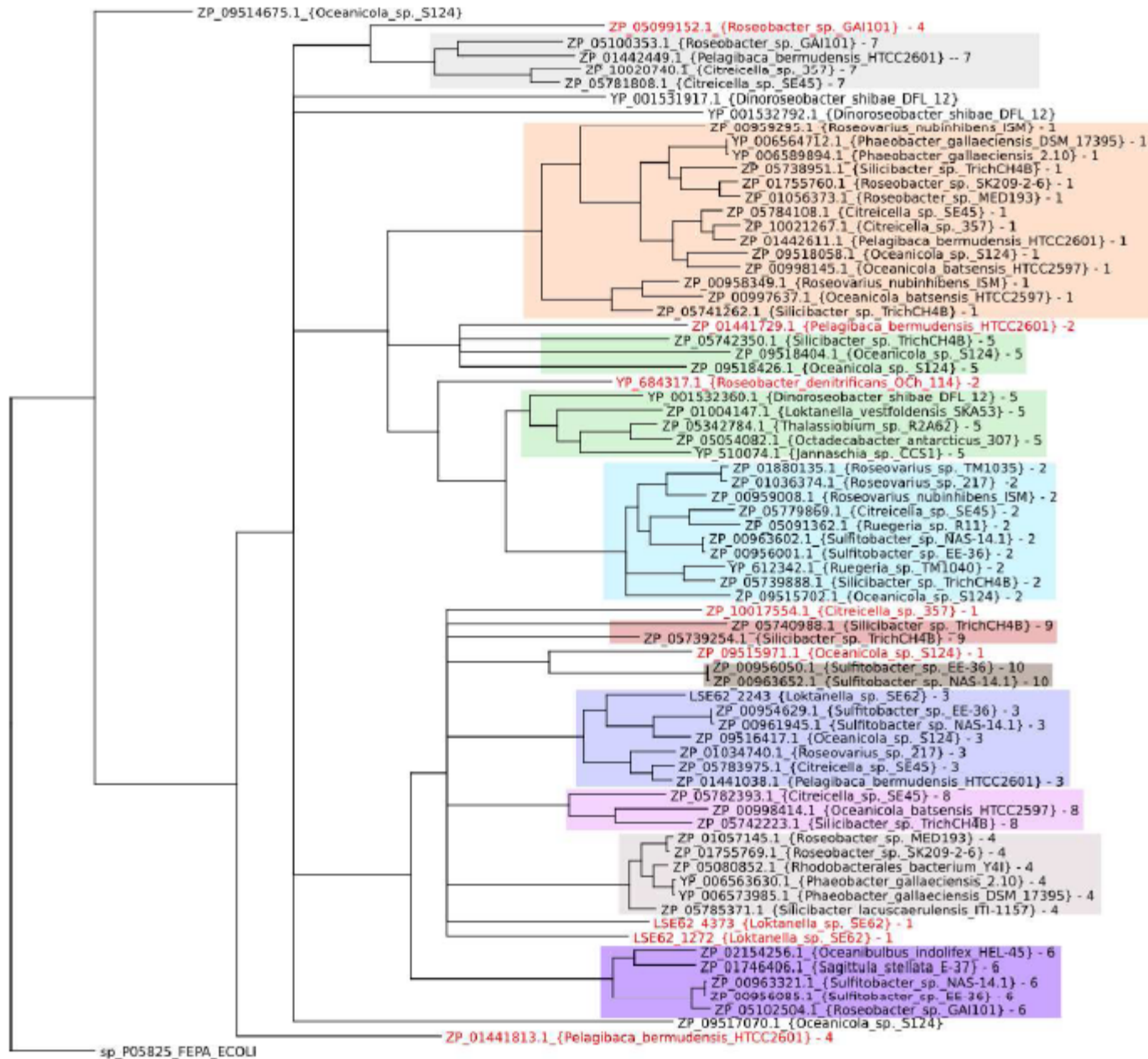


(Confirmed by sequencing)

# **Comparative analysis of metal uptake systems in marine *Roseobacter* genomes**

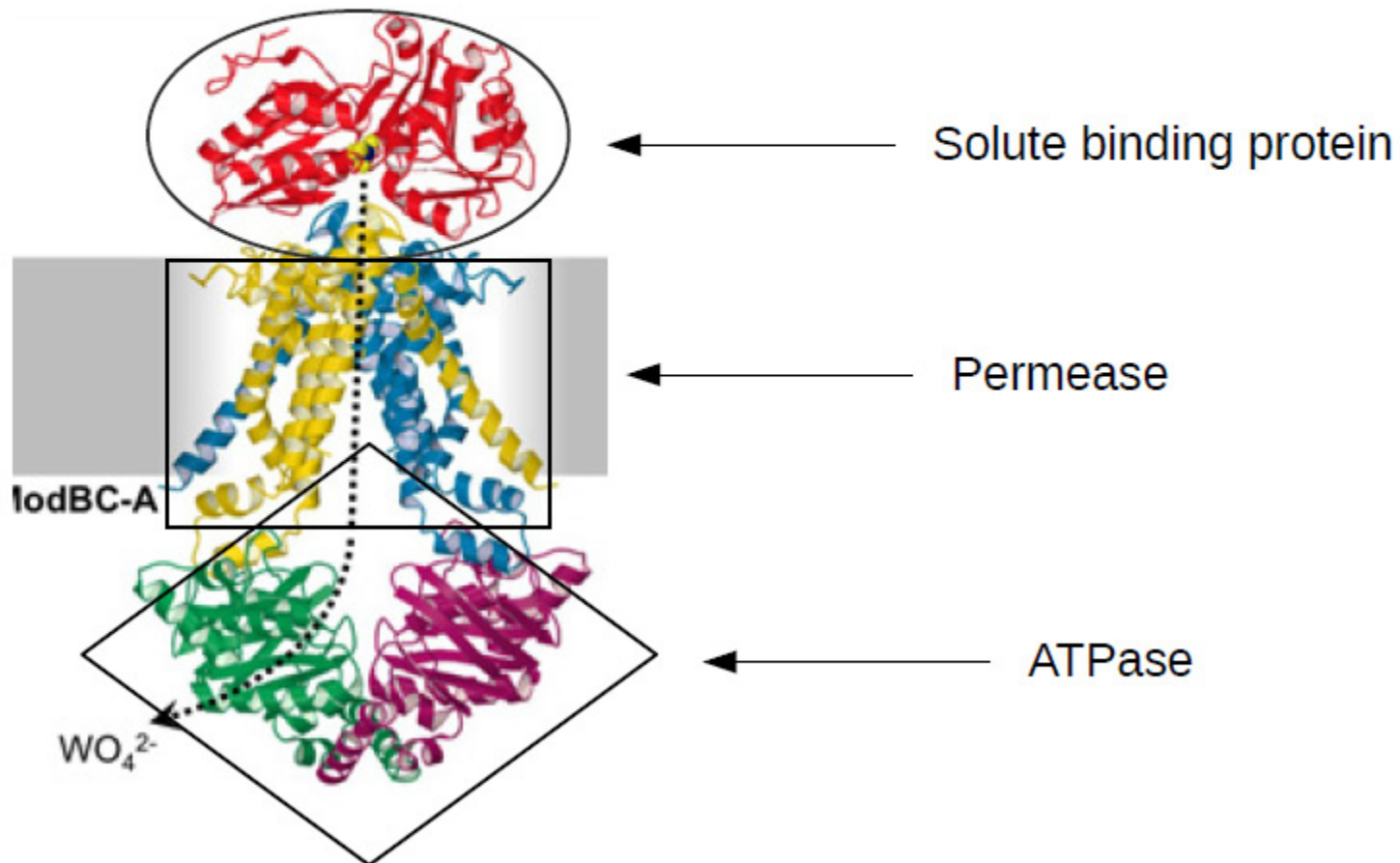


# Roseo TBDT phylogeny appears to be structured by putative substrate

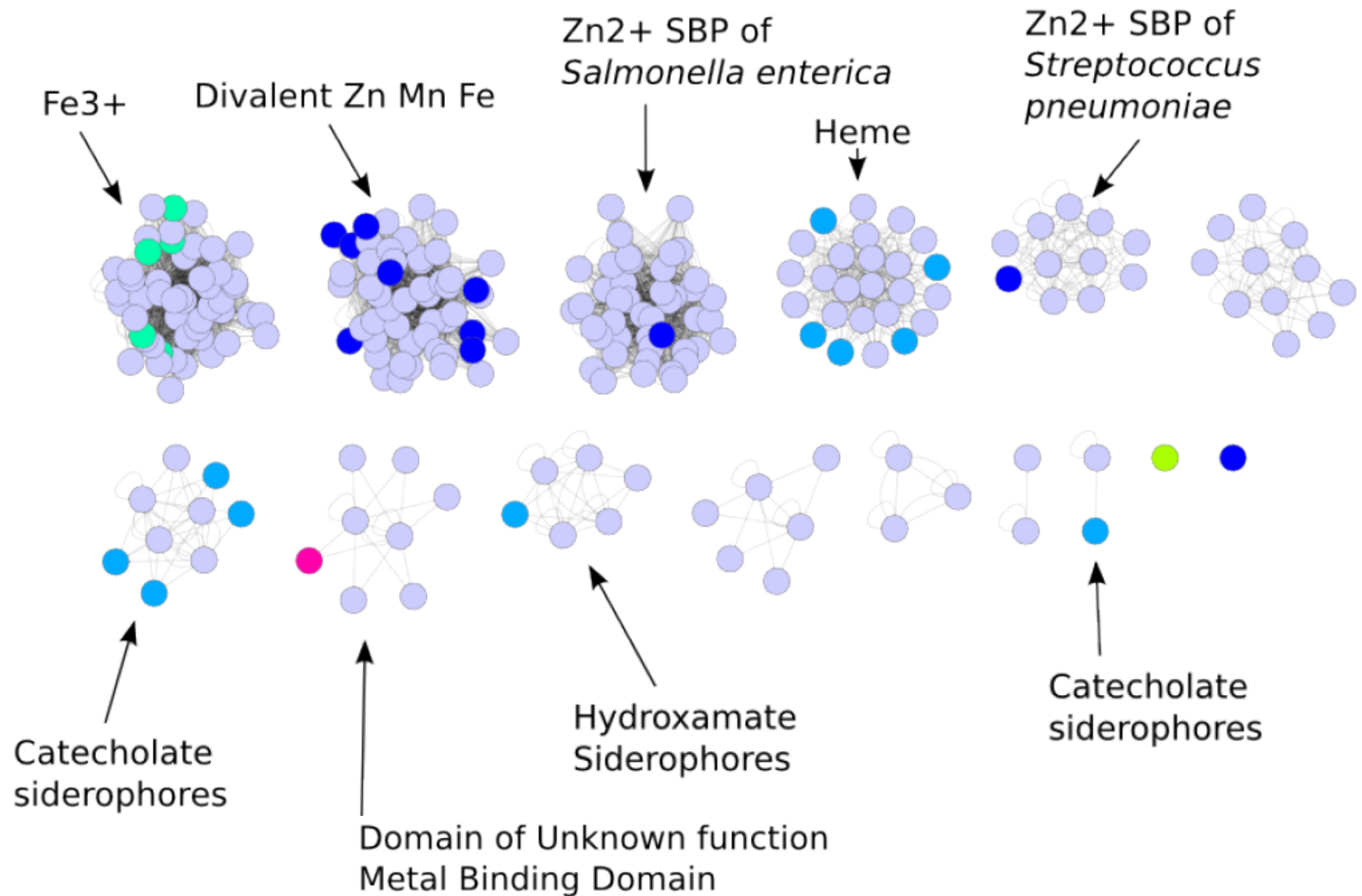




Solute binding protein of ABCT is most divergent in the whole complex...



Metal solute binding proteins of *Roseobacters* cluster by substrate with experimentally characterized SBPs from human pathogens

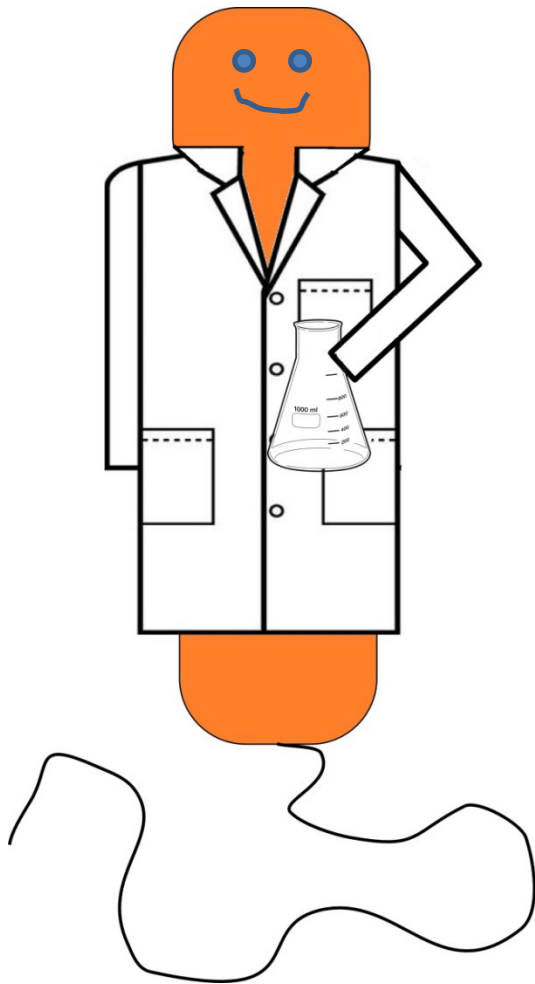


**Does the sequence diversity of metal uptake receptors reflect substrate diversity in the environment?**

 **e.g. – diversity of Fe-ligand complexes,  
and physico-chemical forms of Fe**

Does the sequence diversity of metal uptake receptors reflect substrate diversity in the environment?

↓  
e.g. – diversity of Fe-ligand complexes,  
and physico-chemical forms of Fe



**Increase understanding  
at the molecular level**

**Improve annotation capabilities  
for marine -omics studies**

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