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

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• 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 \textit{in situ} iron chemistry
Chemical forms of iron in seawater

1nm  10nm  0.1μm  1μm  10μm  100μm

soluble species  colloids  particulates

inorganic
Fe(III) - Fe(OH)$_2^+$
Fe(OH)$_3^0$
Fe(OH)$_4^-$
inorganic
Fe(II) - Fe$^{2+}$
FeCO$_3^0$

iron oxide mineral
organic detritus
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

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

- 'Free' metals
- Siderophores

- Metalloproteins of various states of integrity

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- 'Free' metals

- Hemes
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
Iron transporters in marine prokaryotic genomes

<table>
<thead>
<tr>
<th>Taxa</th>
<th>No.</th>
<th>ATP</th>
<th>Perm</th>
<th>Bind</th>
<th>FeoA</th>
<th>FeoB</th>
<th>FTR1</th>
<th>NRAMP</th>
<th>ZIP</th>
<th>TBDT</th>
<th>Hydrox</th>
<th>Catechol</th>
<th>Haem</th>
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Hopkinson and Barbeau EMI 2012
Iron uptake genes in the GOS metagenomes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Frequency (genes per genome)</th>
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<td>Fe$^{3+}$ ABC ATPase</td>
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<tr>
<td>Fe$^{3+}$ ABC permease</td>
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<td>FeoA</td>
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<td>Catechol</td>
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<tr>
<td>Haem</td>
<td>0.09</td>
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</table>

Hopkinson & Barbeau *EMI* 2012
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 um) 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
Heme uptake genes in marine roseobacters

Roe et al. AEM in press
• Sequence conservation of hmuS gene makes it an appropriate subject for targeted metagenomics (degenerate primer based)
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• 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
hmuS detected in the marine environment

Primer system is specific for marine roseobacters

Roe et al. AEM in press
Work with roseobacter isolates
Marine Isolates can grow on Ferric-porphyrin complexes as the sole iron source

Silicibacter sp. TrichCH4B

- 500 nM FeCl$_3$
- 500 nM Heme
- No addition
- 500 nM PPIX

Roe et al. AEM in press
Marine Isolates can grow on Ferric-porphyrin complexes as the sole iron source
Putative heme tonB dependent transporter (TBDT) expression responds strongly to Fe source and concentration.
Functional characterization of "heme" TBDT via knockout mutation

Biochemical confirmation of TBDT substrates is critical!
Generation of TBDT knockout in TM1040 using Gibson cloned pRL271 derived suicide vector

<table>
<thead>
<tr>
<th>Description</th>
<th>Size</th>
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<tbody>
<tr>
<td>TM1040_0347</td>
<td>2210 bp</td>
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<td>TM1040_0347::kan</td>
<td>3124 bp</td>
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<tr>
<td>Deletion of 460 bp</td>
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ΔTM1040_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.

- Fe³⁺
- Divalent Zn Mn Fe
- Zn²⁺ SBP of *Salmonella enterica*
- Zn²⁺ SBP of *Streptococcus pneumoniae*

- Catecholate siderophores
- Hydroxamate Siderophores
- Domain of Unknown function Metal Binding Domain
- Catecholate siderophores
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
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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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