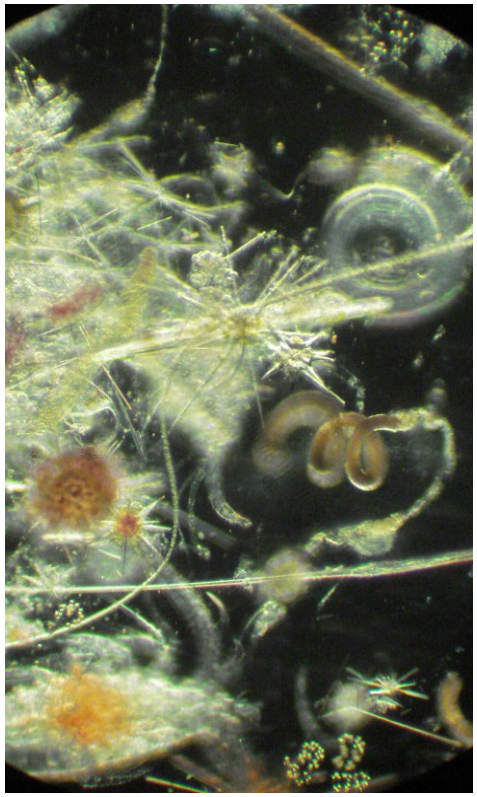
Coupling microbial diversity and activity with dissolved organic matter production and cycling within the microbial loop.

Autotrophs



Angel White, OSU

protozoa

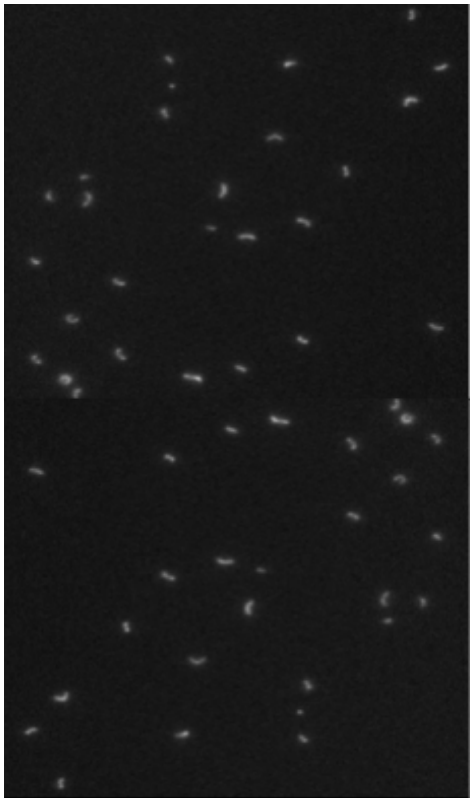
DOM

C/N/P/M/etc…

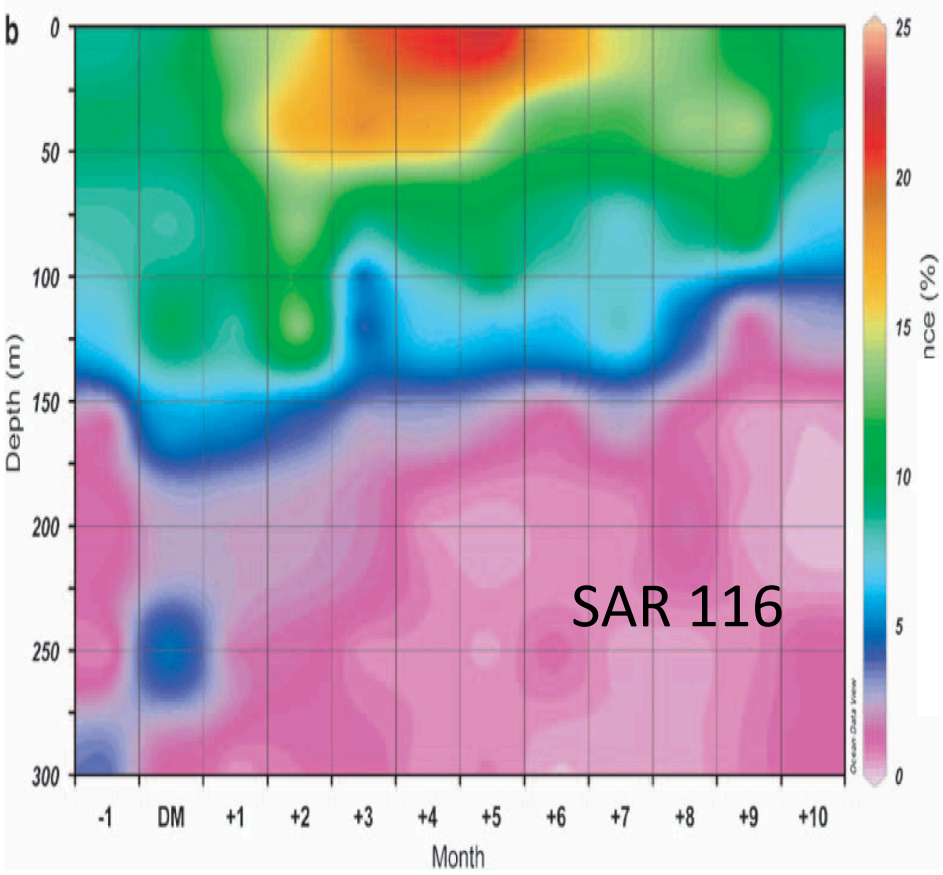
Proteins
Sugars
Nucleic acids
Lipids
Vitamins

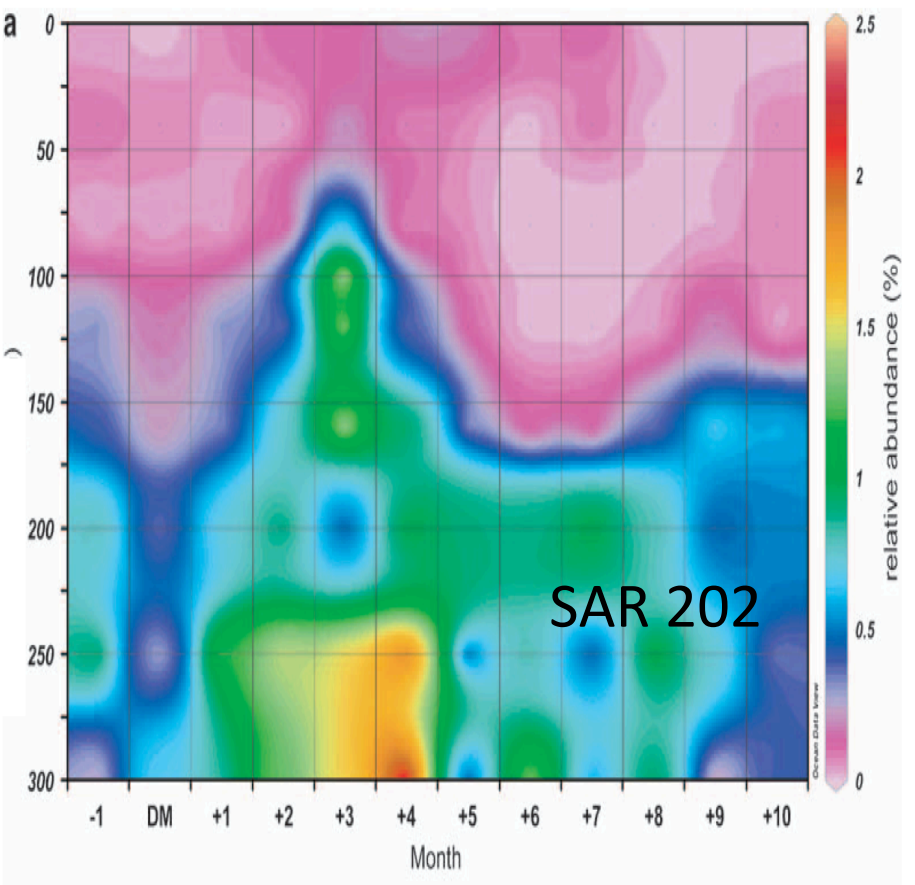
viruses

Heterotrophs



How do we explain the seasonal and vertical structure of heterotrophic microbial communities in the ocean?

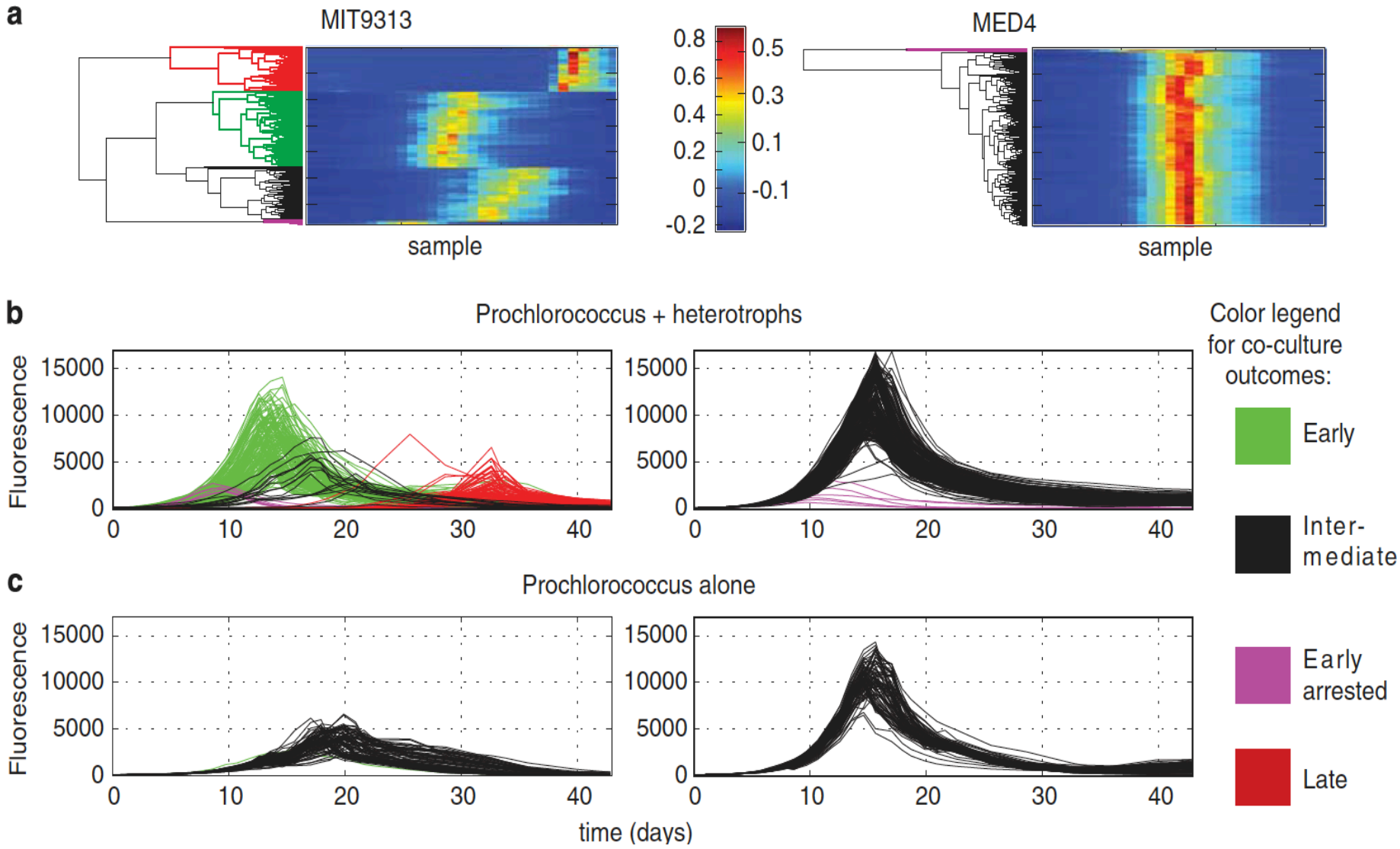




"Interactions between bacterioplankton cells and natural DOM pool are difficult to measure… Although it has not yet been possible to measure heterotrophic interactions between microbial populations and DOM at a comprehensive molecular level, described patterns of DOM concentration and reactivity at the surface layer suggest they are factors that influence the distributions of SAR 11 populations……" (Carlson et al., ISME 2009)

Treusch et al. ISME J. 2009

Do heterotrophs influence the growth of photoautotrophs?



Approaches

1) Perturbation experiments: Add a specific (known) organic substrate and measure changes in microbial diversity, abundance, etc.

2) High throughput screening of natural organic matter fractions via pure cultures of heterotrophs and photoautotrophs.

3) Use genomic based reconstruction of metabolic pathways in pure cultures to determine substrate specificity – confirm via pure culture experiments.

4) Whole community metabolism experiments- add natural organic matter to seawater and use community transcriptomics to deduce which metabolisms are up-regulated in which organisms.

Isolation of Phytoplankton derived DOM

Triplicate axenic cultures
(known biological source)

↓

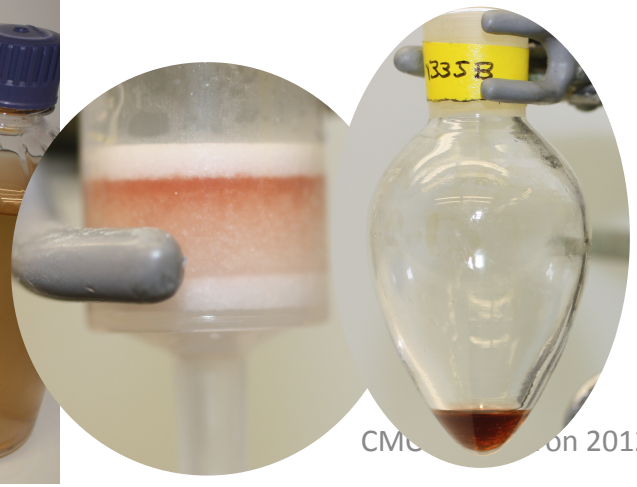
Biomass removal

↓

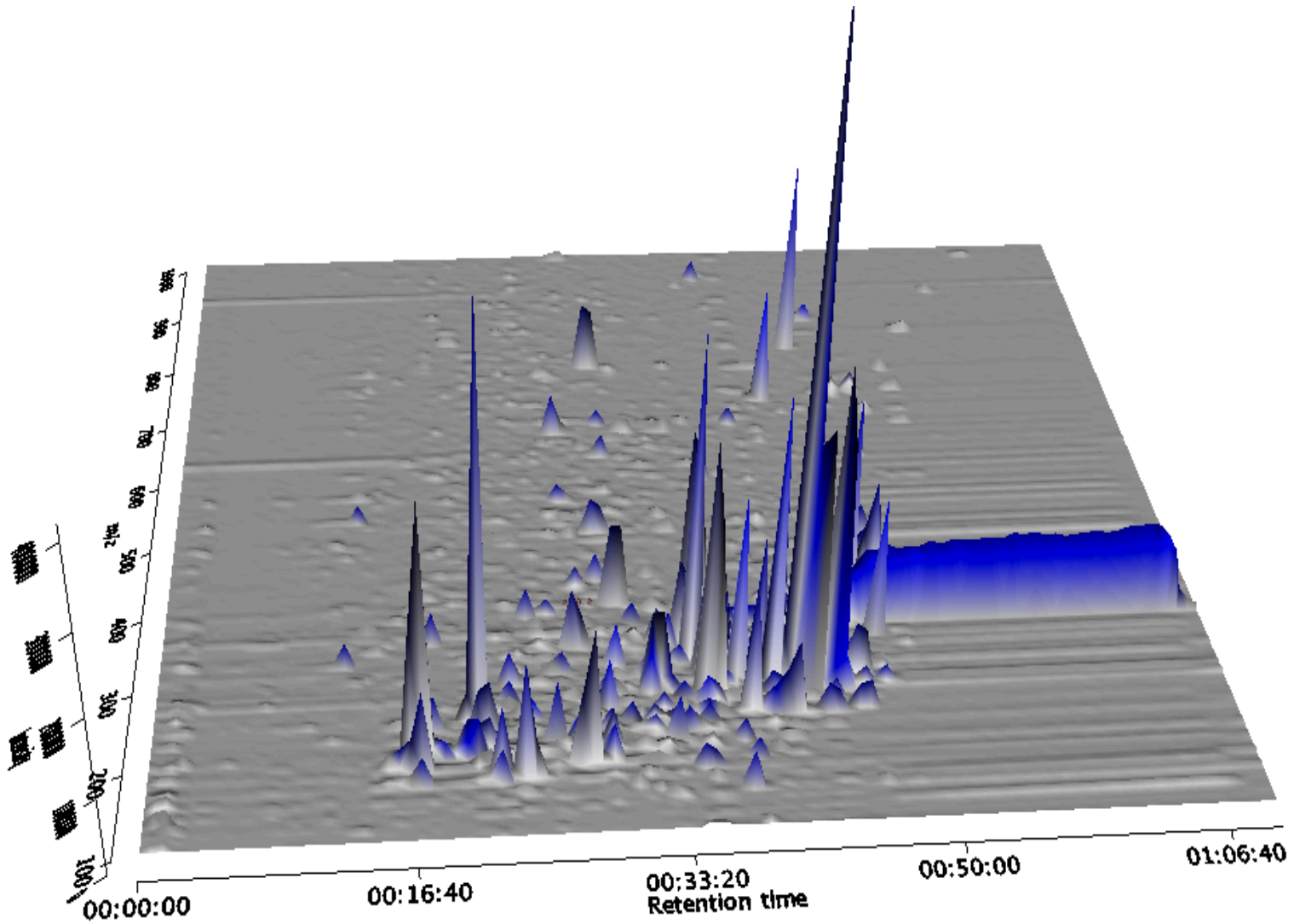
Solid-phase extraction

↓

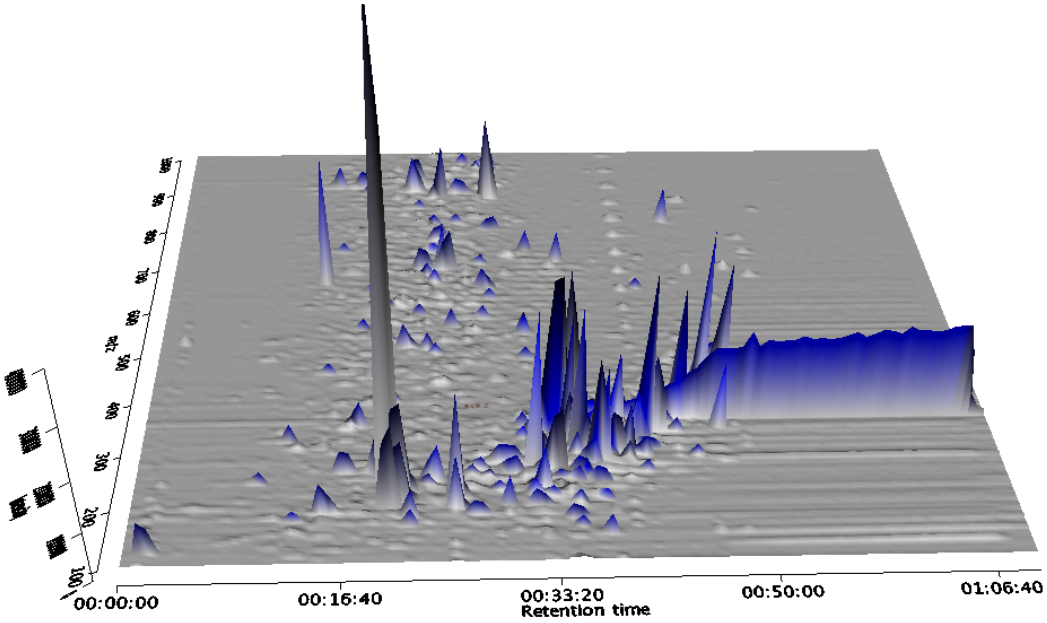
Labile material



Chemical Diversity by chromatography/mass spectrometry

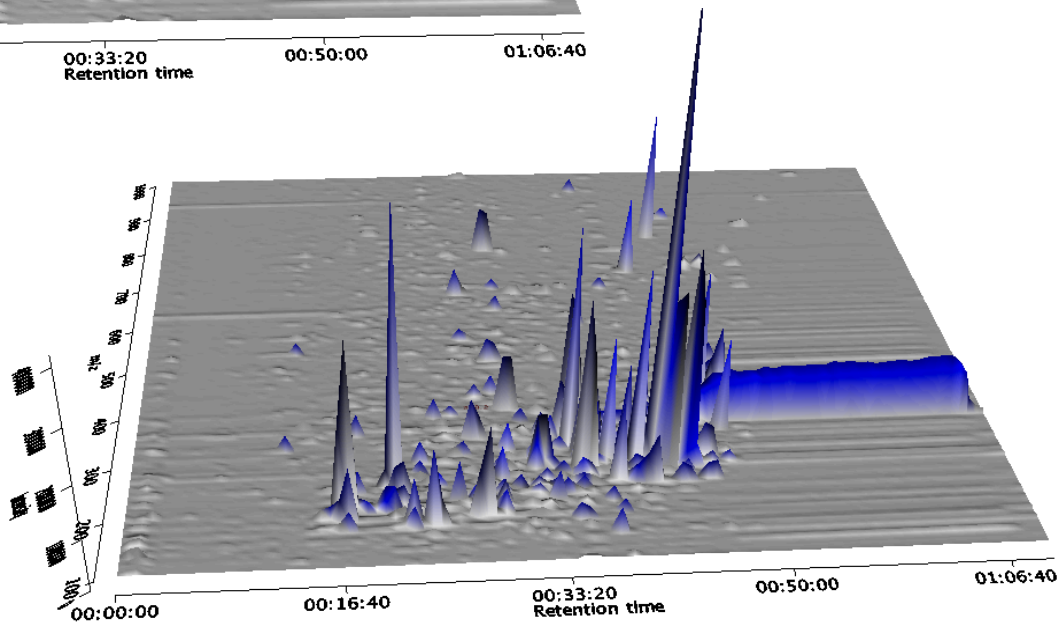


Chemical Diversity by chromatography/mass spectrometry

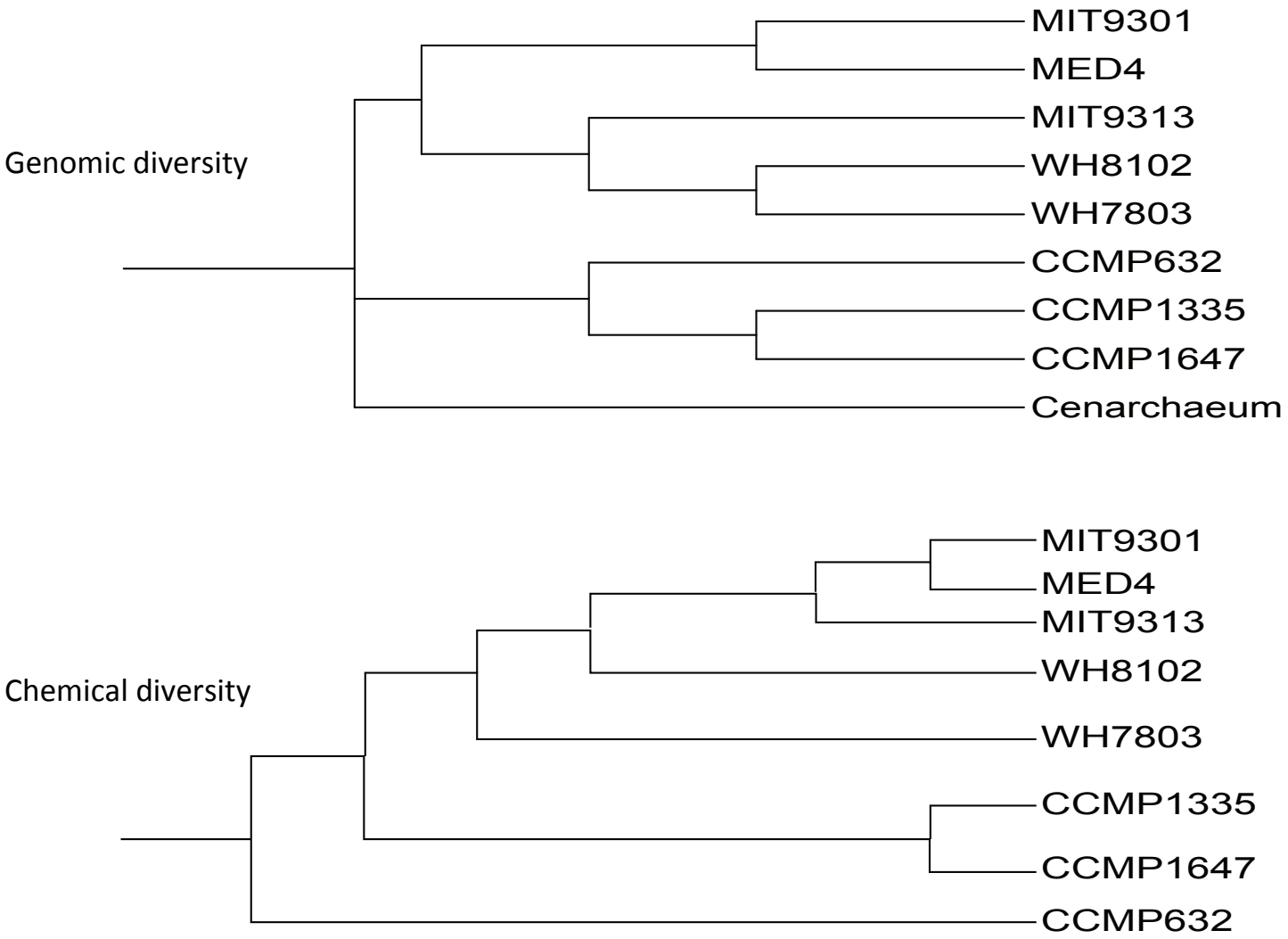


Diatom

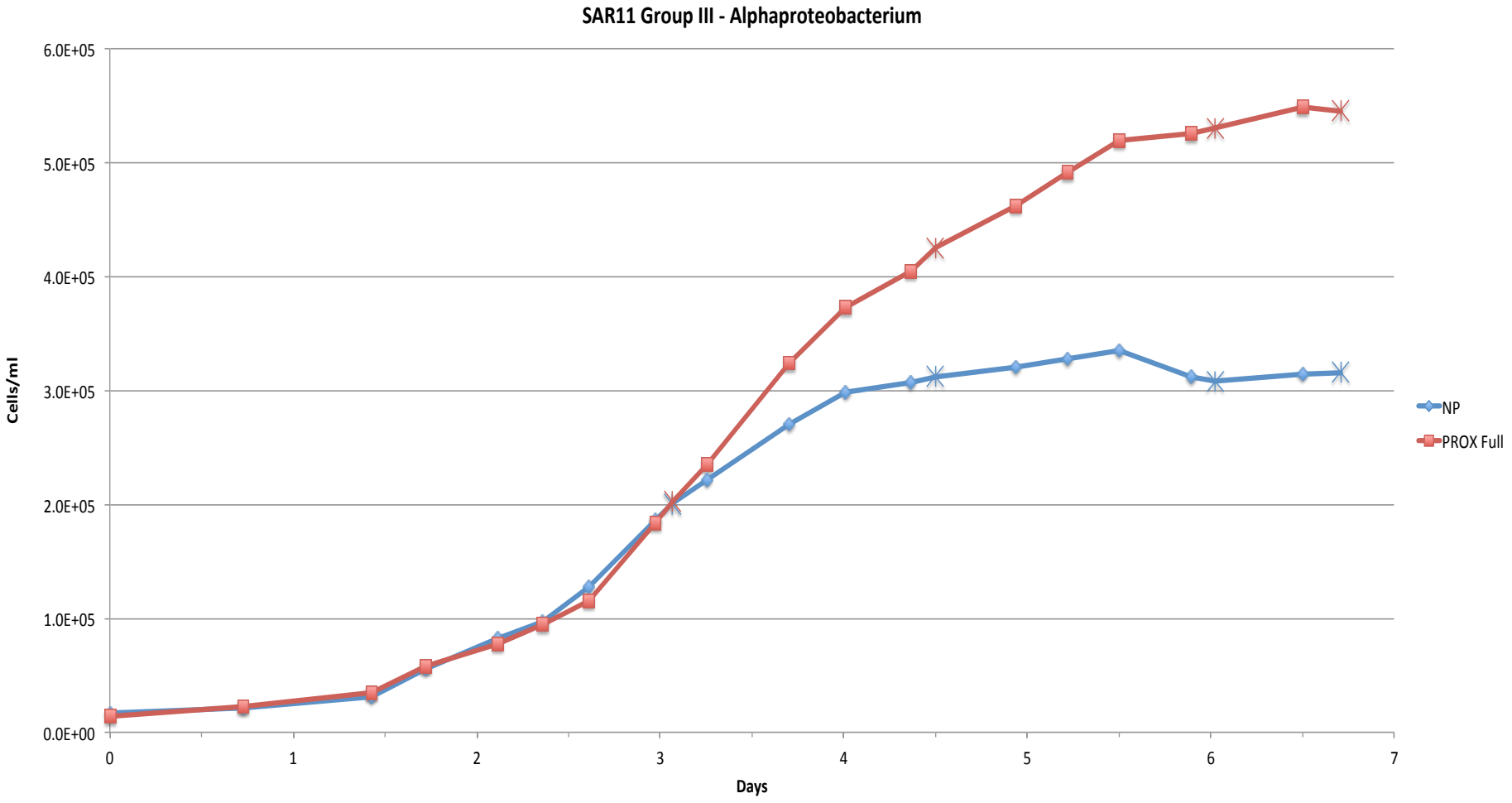
Cyanobacteria



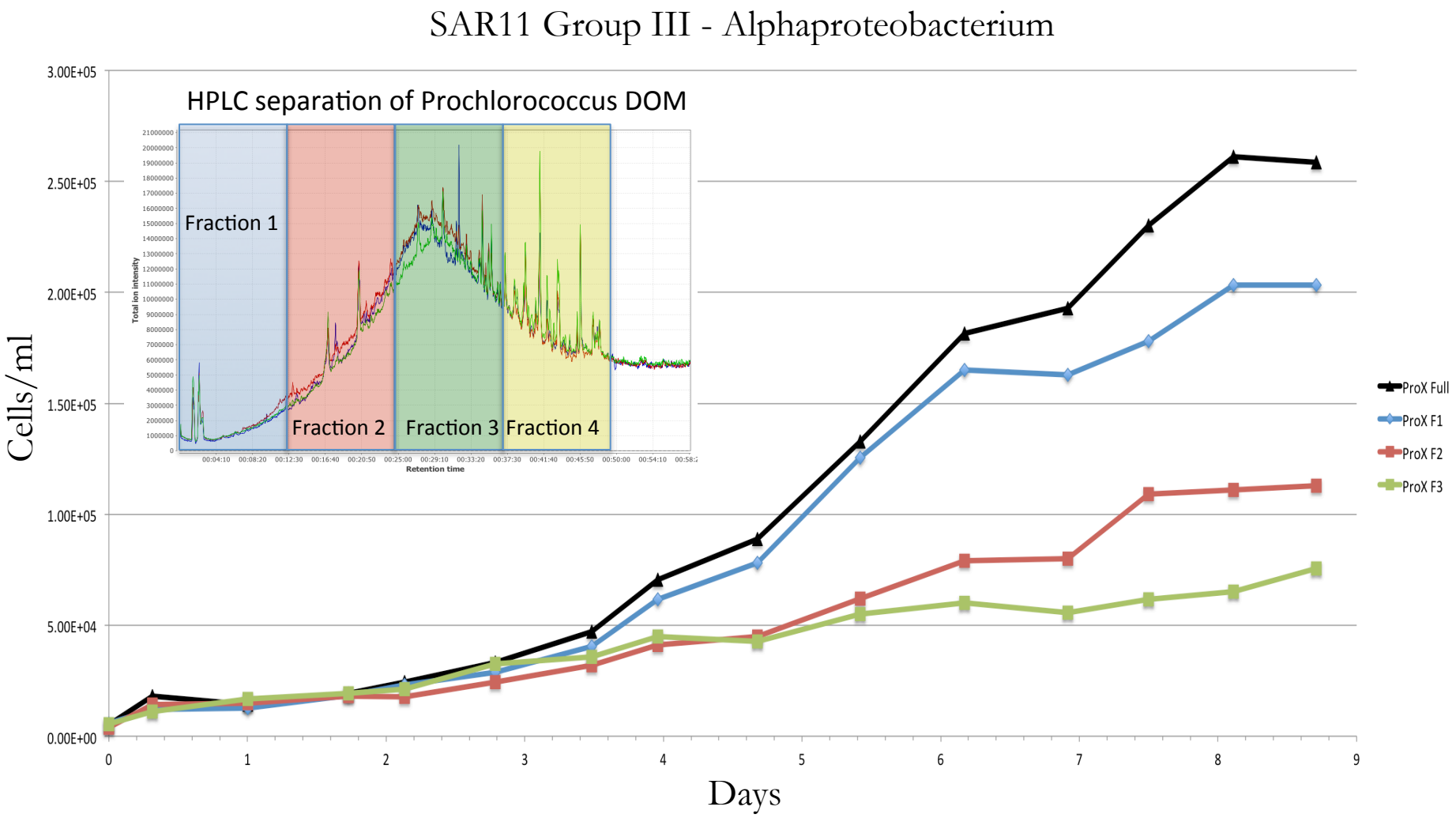
Chemical diversity tracks biological diversity



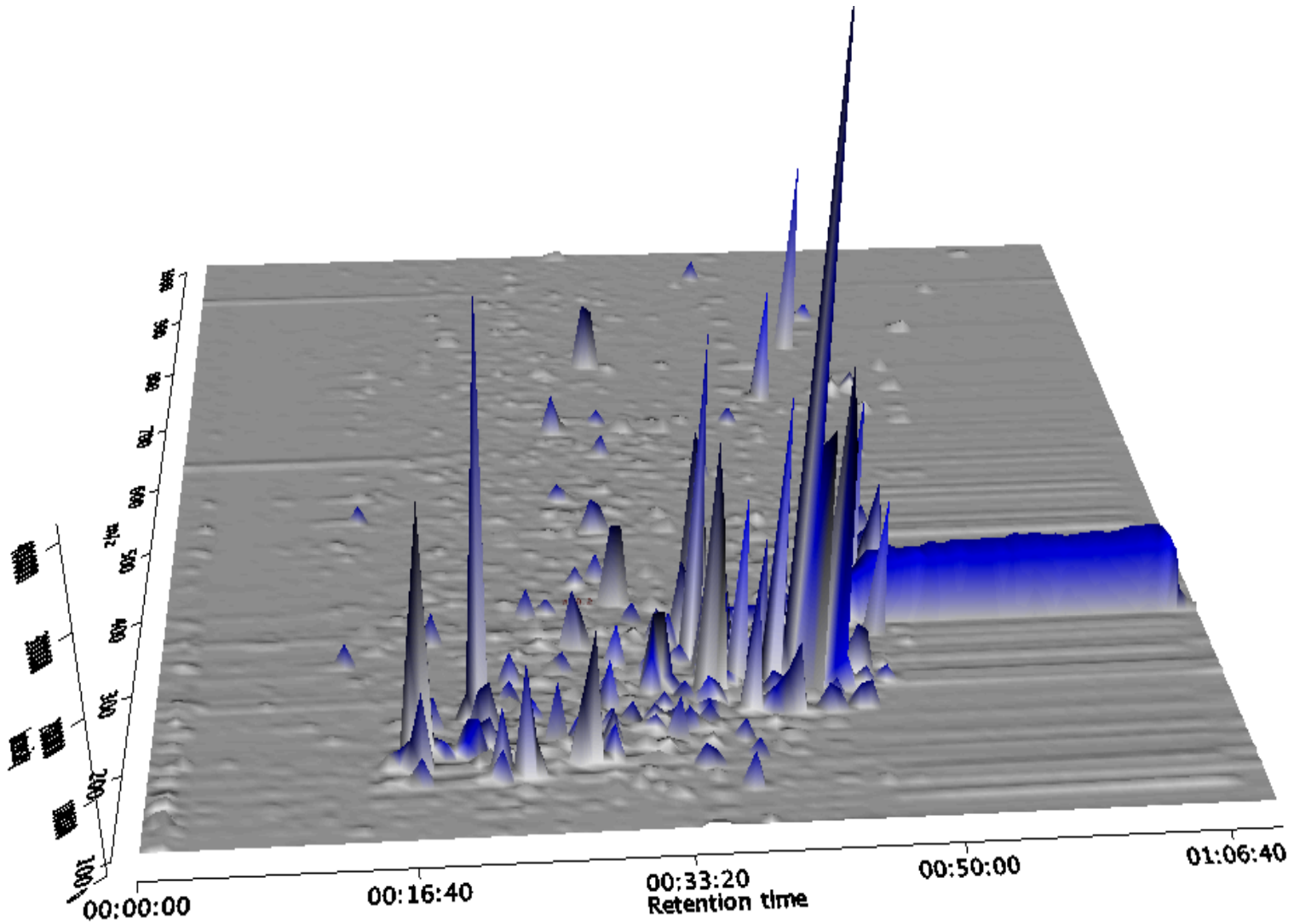
Lability Screening



Lability Screening

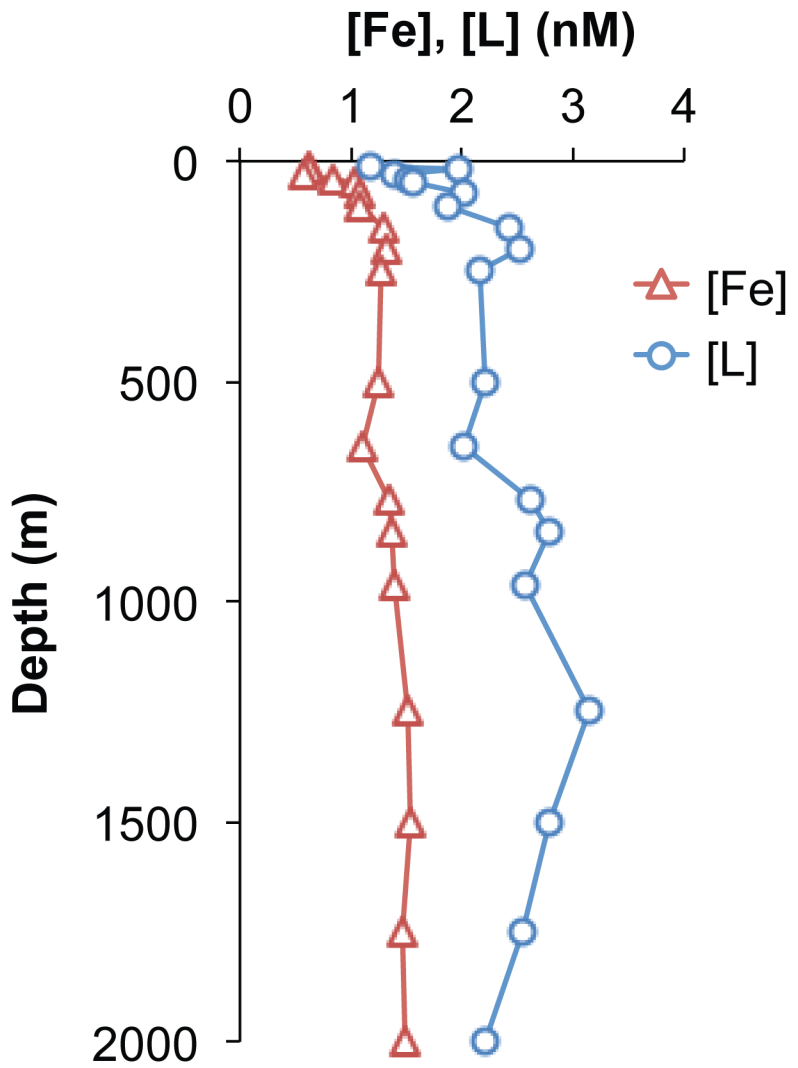


Chemical Diversity by chromatography/mass spectrometry



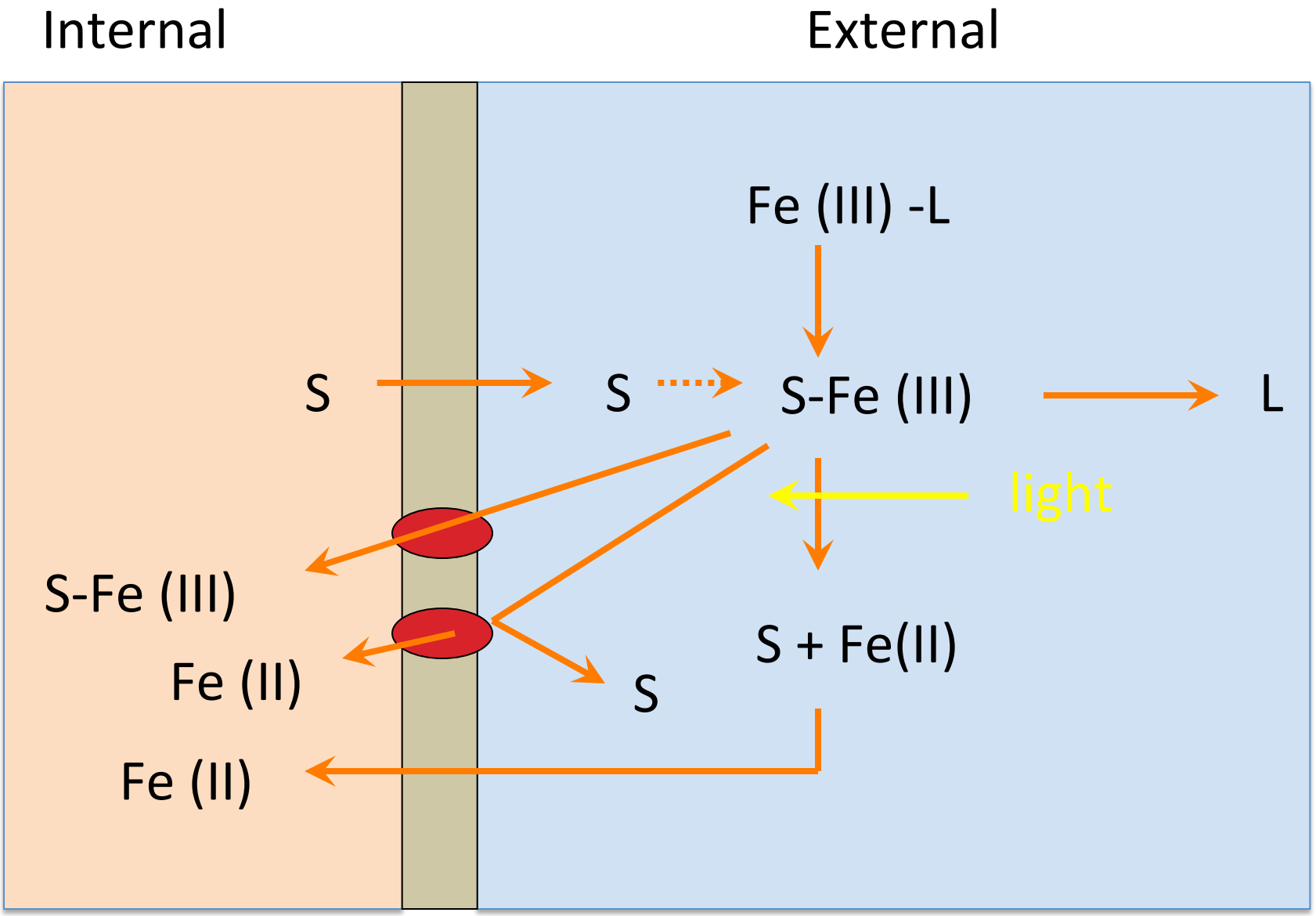
Marine Iron Ligands

- Concentration of Fe ligands nearly always exceeds dissolved Fe.
- Conditional stability constant $LogK_{FeL}$ between 10 -13.
- Suggests that >99% of dissolved Fe is complexed.

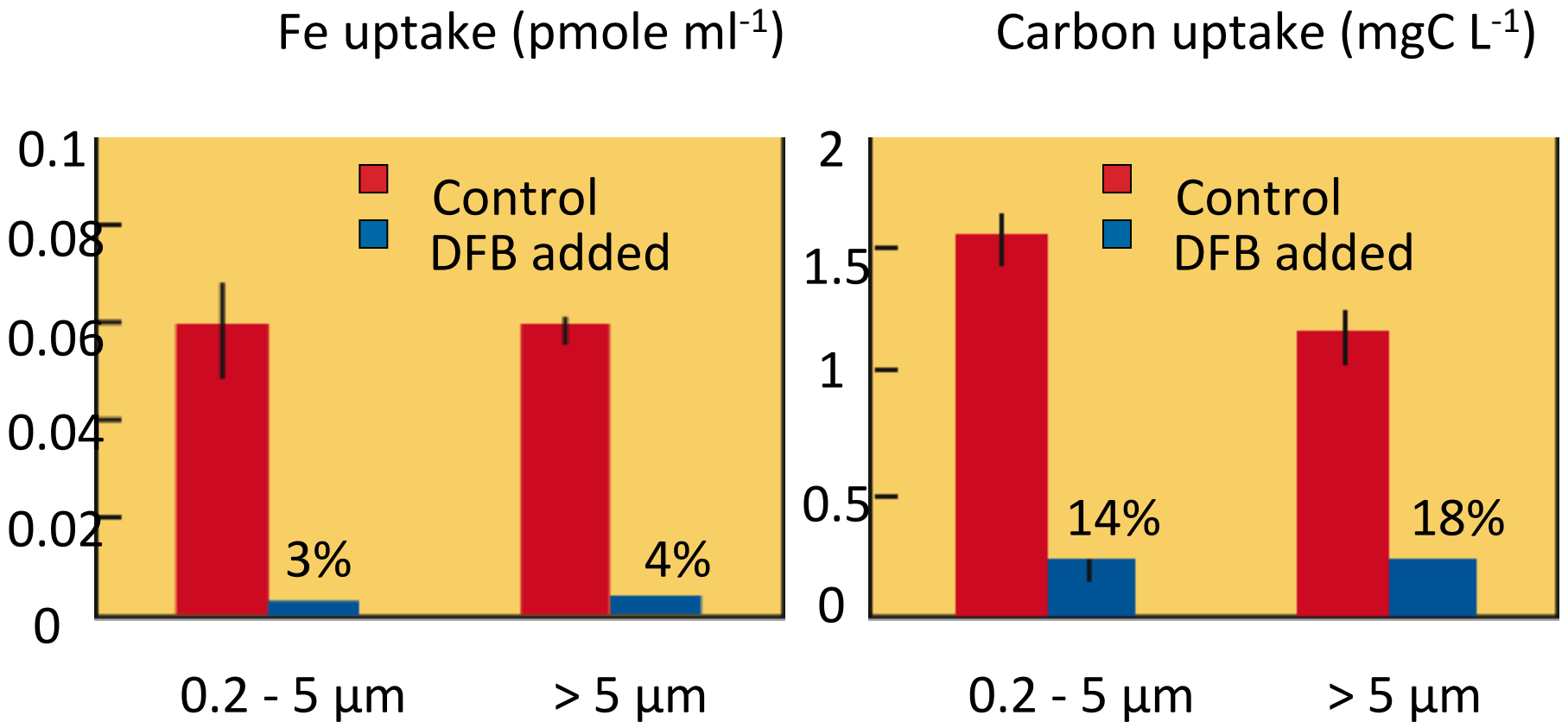


Boye et al., *Marine Chemistry*, 2006

Iron uptake mechanisms by bacterioplankton

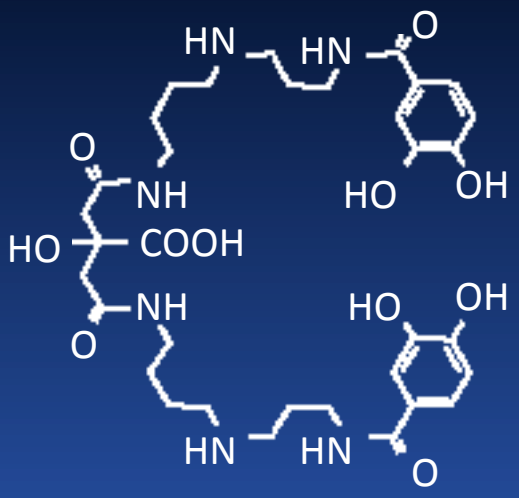


Manipulation of Fe availability in seawater by the addition of a siderophore (DFB)

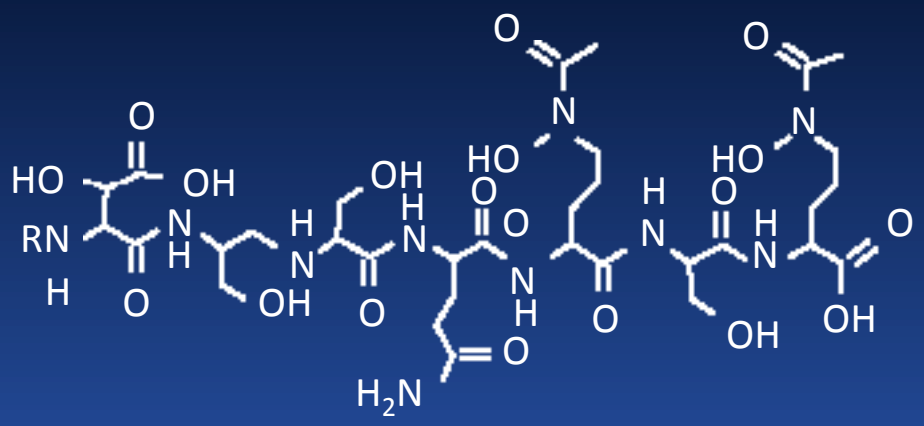


Wells, L&O 1999

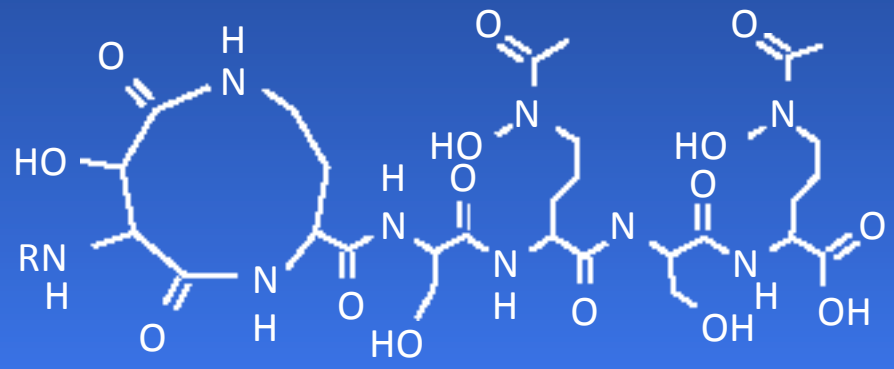
Siderophores in Marine Bacteria



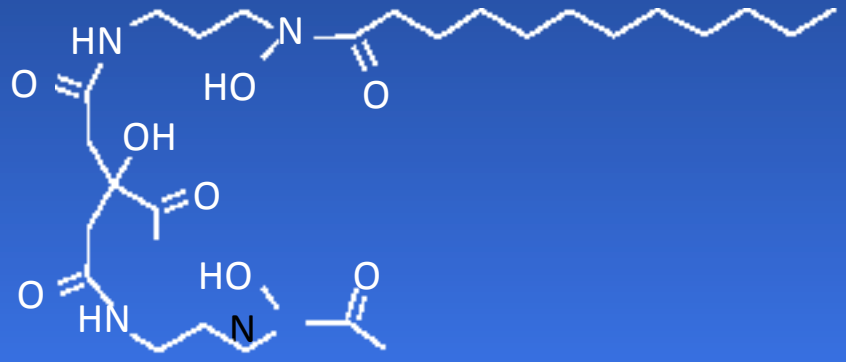
Petrobactin



Aquachelins

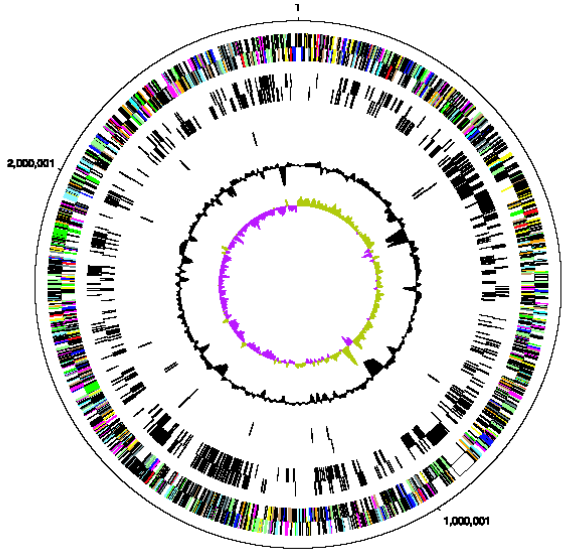


Marinobactins



Synechobactin

Then again there are good reasons to think that siderophores are not present as Fe-binding ligands in seawater…



Genomes of *Synechococcus* WH8102 and *Prochlorococcus* do not have detectable systems for siderophore production or uptake.

The energetics and efficiency of siderophore production and uptake in dilute media has been questioned in model calculations.

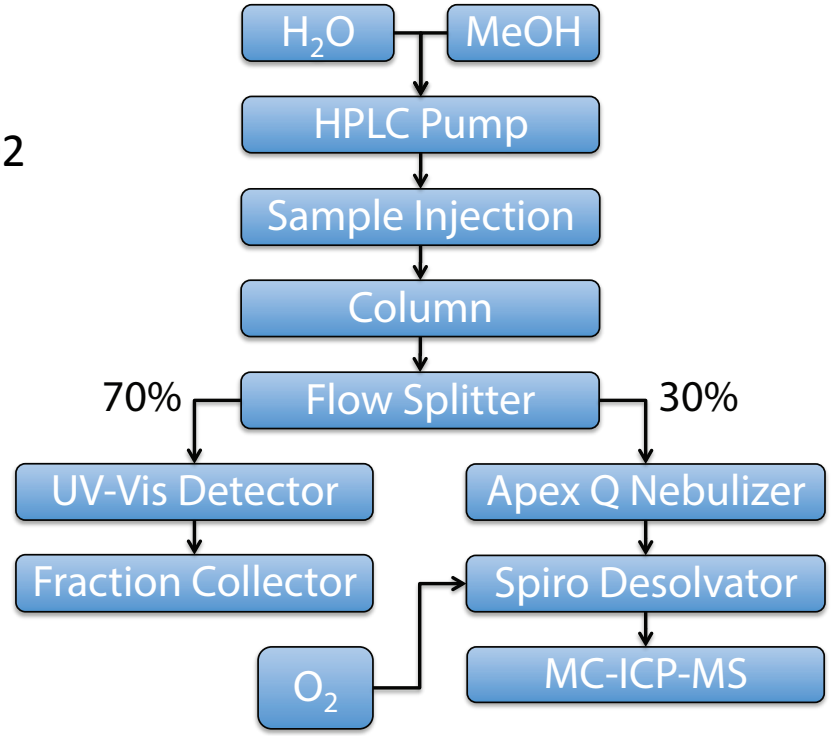
Only one marine cyanobacteria has been shown to make siderophores. NO siderophores have been isolated from seawater.

Palenik et al. Nature, 2003; Volker and Wolf-Gladrow, J. Plant Nutr. 1999

Applications

1. Cyanobacteria cultures
   - *Synechococcus* strain PCC 7002
   - *Prochlorococcus*

2. Natural Seawater
   - Subtropical Pacific



Applications

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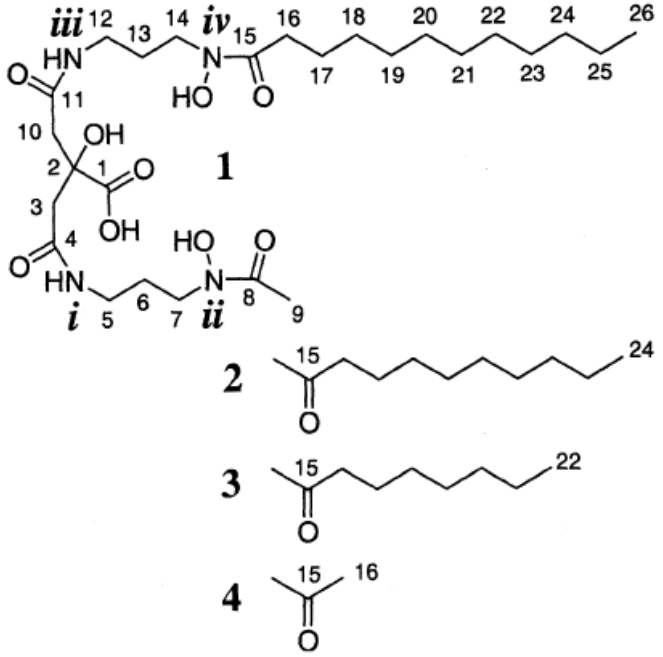
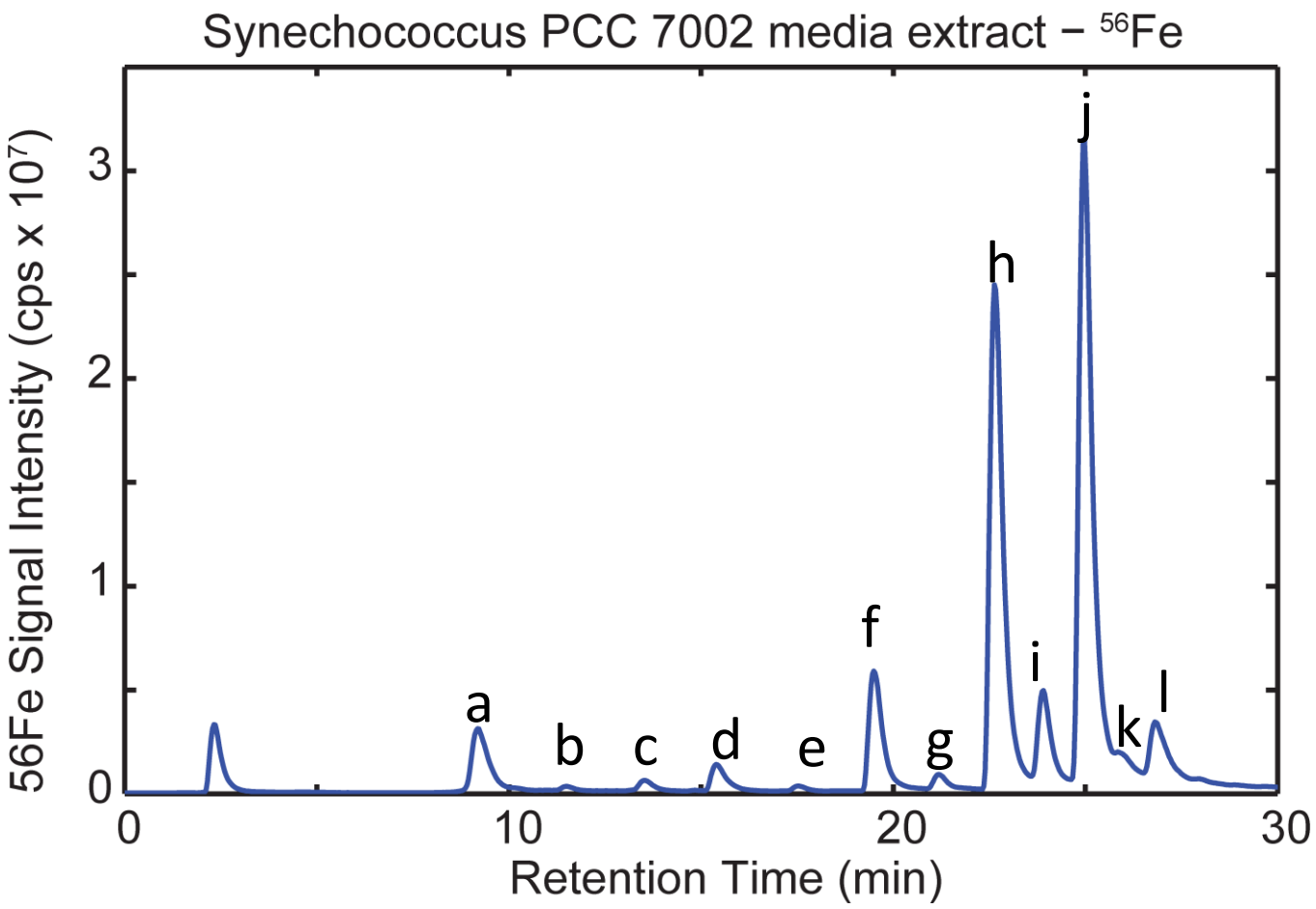
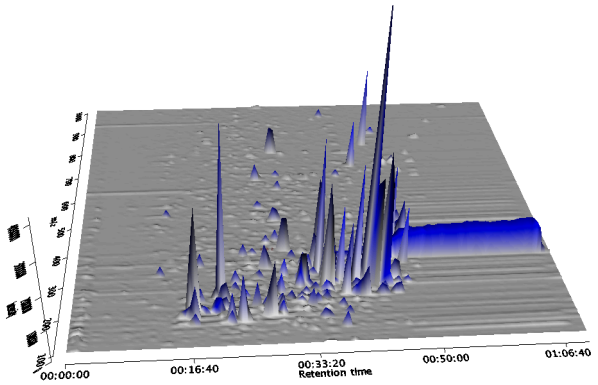


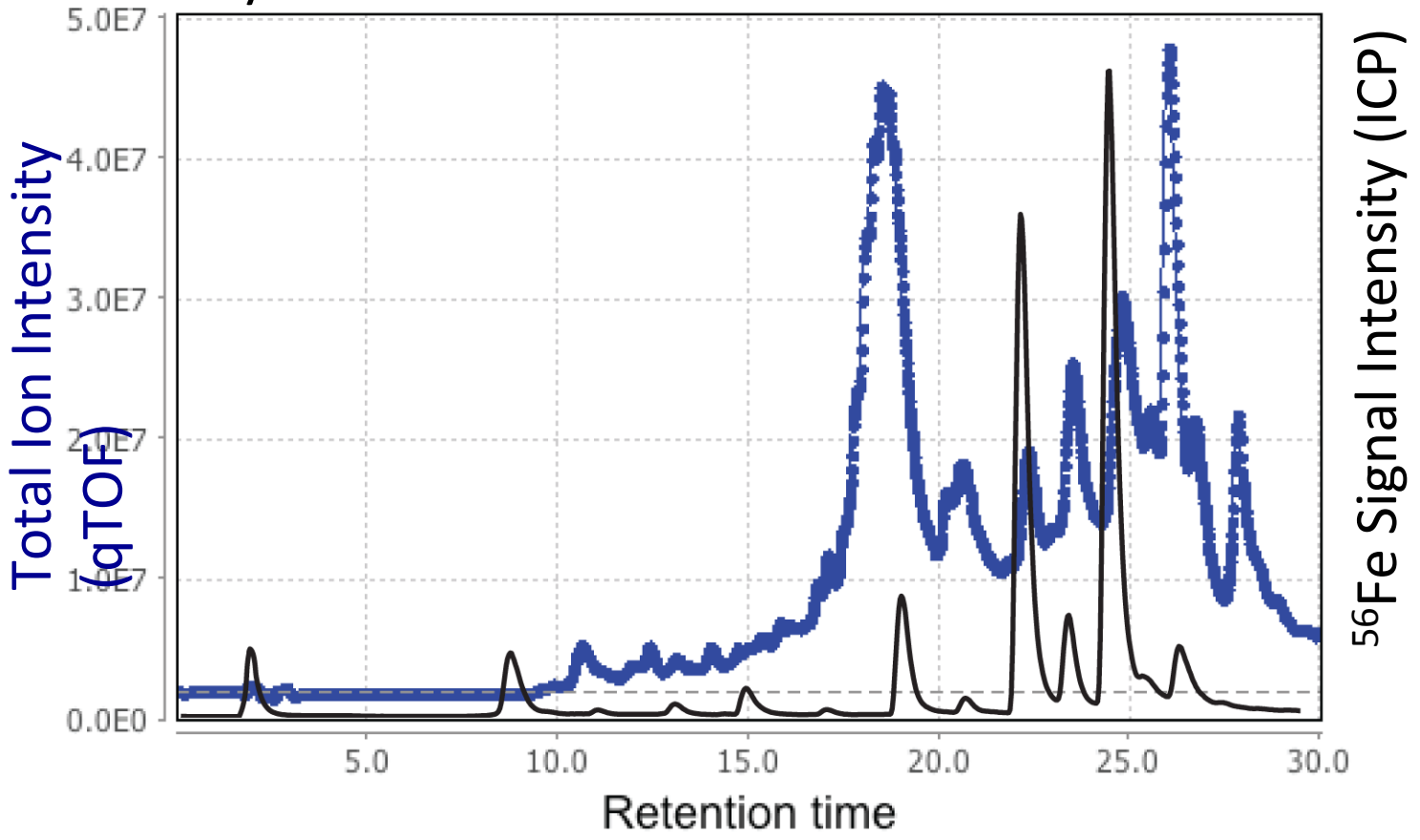
Synechobactins

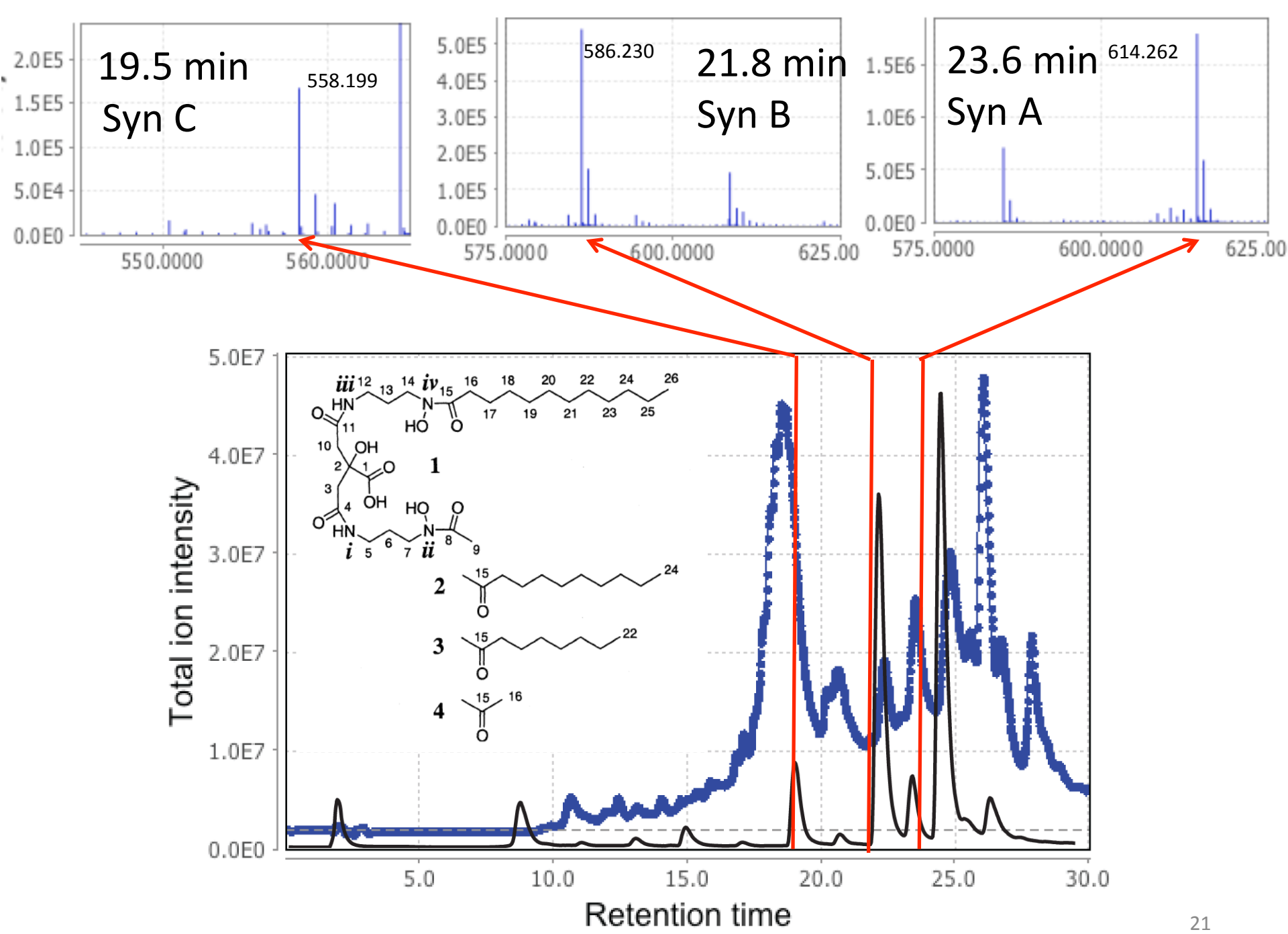


Synechobactins

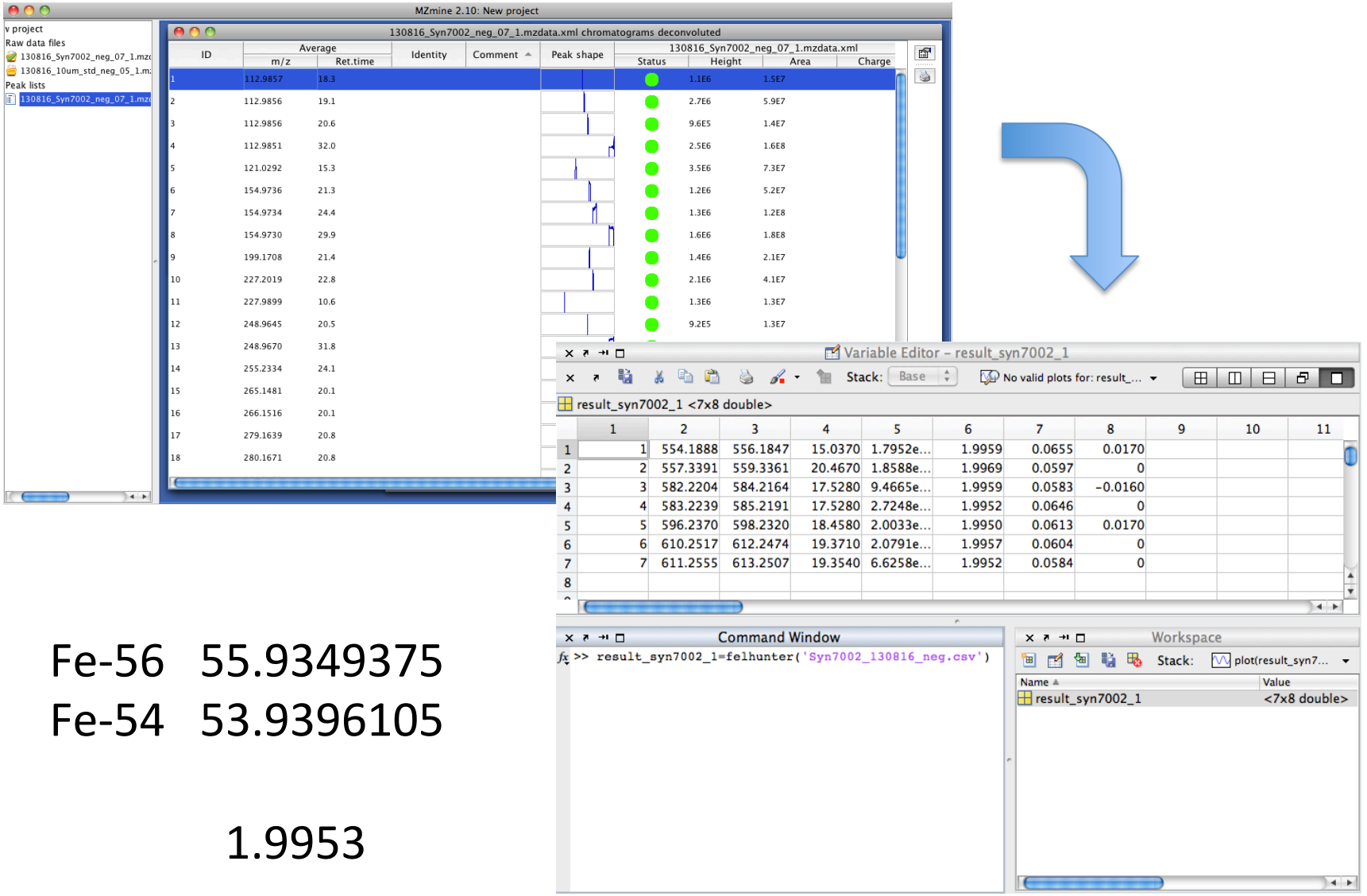


Synechococcus PCC 7002 media extract – ESI-MS




19.5 min
Syn C
558.199
21.8 min
Syn B
586.230
23.6 min
Syn A
614.262
Total ion intensity
Retention time
1
2
3
4

Data processing

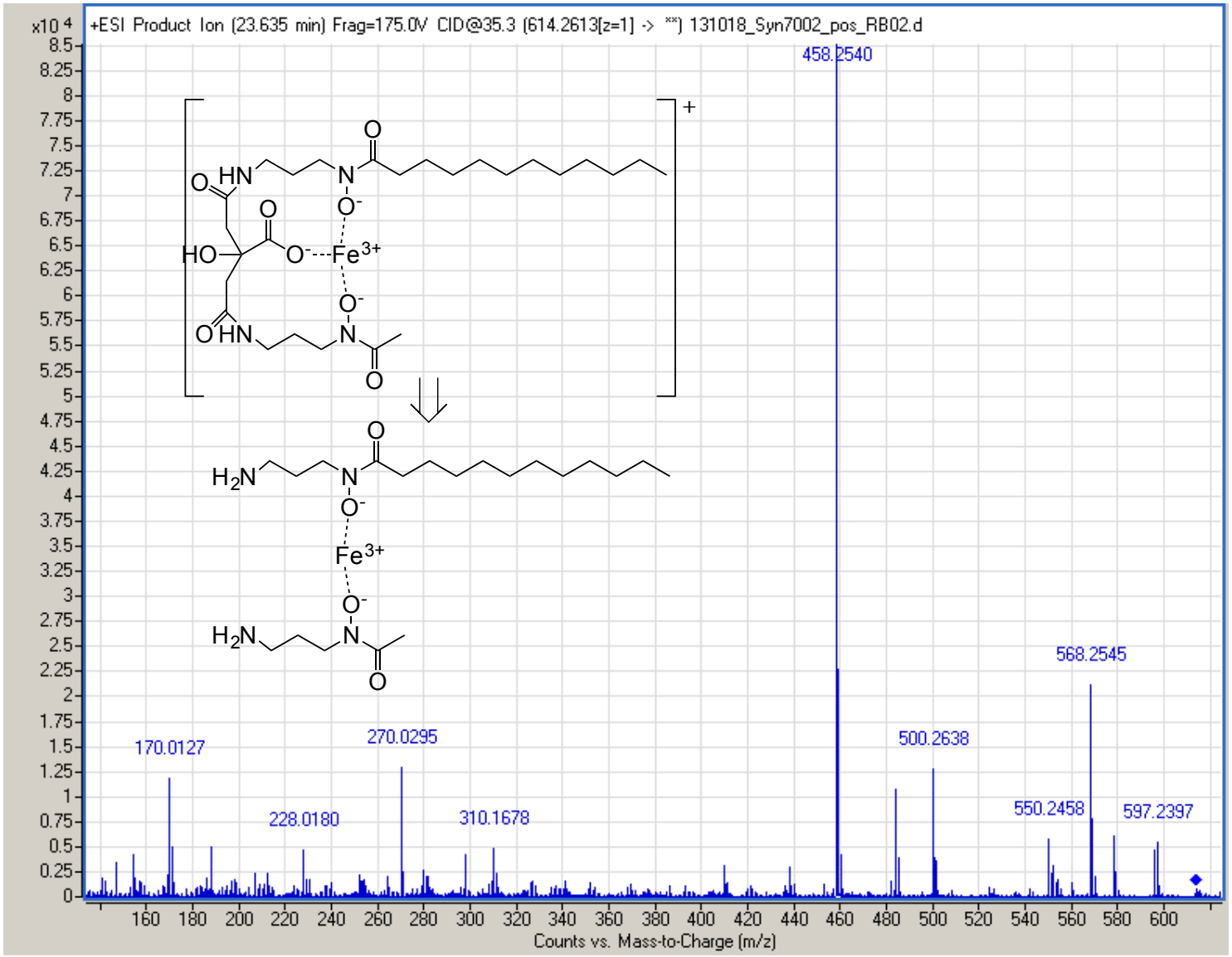


Fe-56 55.9349375

Fe-54 53.9396105

1.9953

MS/MS


+ESI Product Ion (23.635 min) Frag=175.0V CID@35.3 (614.2613[z=1] -> **) 131018_Syn7002_pos_RB02.d
x10 4
458.2540
568.2545
170.0127
270.0295
500.2638
228.0180
310.1678
550.2458
597.2397
Counts vs. Mass-to-Charge (m/z)

Novel Siderophore Discovery

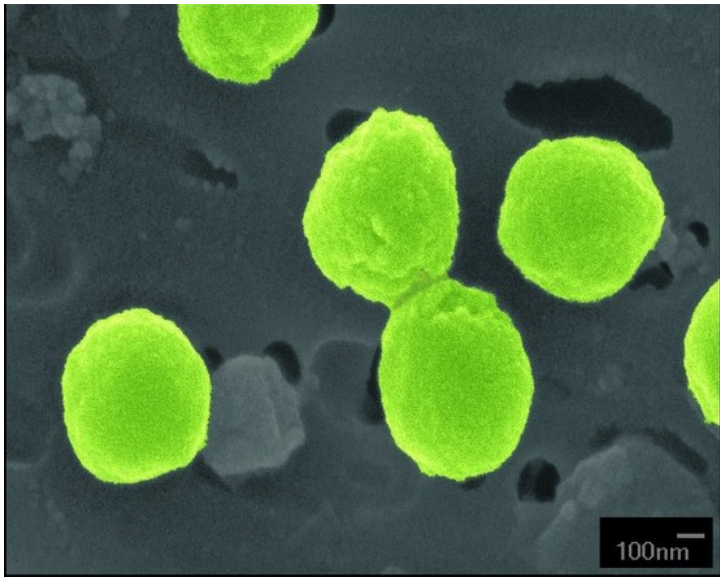
Synechococcus 7002 Ligand list

ICP-MS Peak	Mass (M/Z)	Retention Time (min)	MS/MS Fragments	Ligand I.D
a	474.1	9.5	428.1	**Schizokinan**
c	672.3	13.4	572.3	Unknown
d	654.3	15.5		Unknown
f	558.2	19.4	512.2	Synechobactin C
h	586.2	21.8	540.2	Synechobactin B
i	571.3	22.7		Syn A – CO_2
j	614.3	23.5	568.3	Synechobactin A
k	1198.5	23.6		Unknown
l	642.3	24.9	596.3	**Synechobactin D**

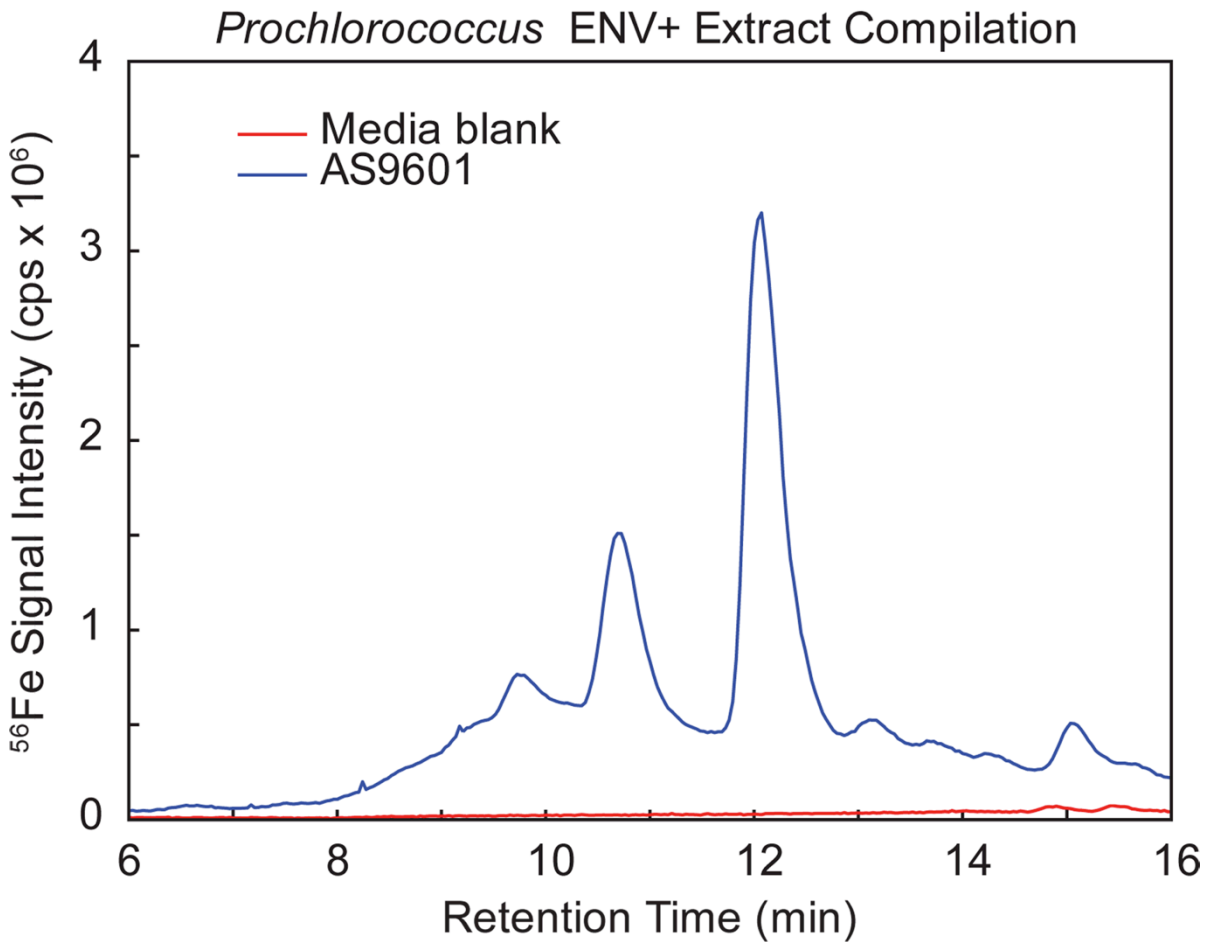
Applications

1. Cyanobacteria cultures
   - *Synechococcus* strain PCC 7002
   - ***Prochlorococcus***

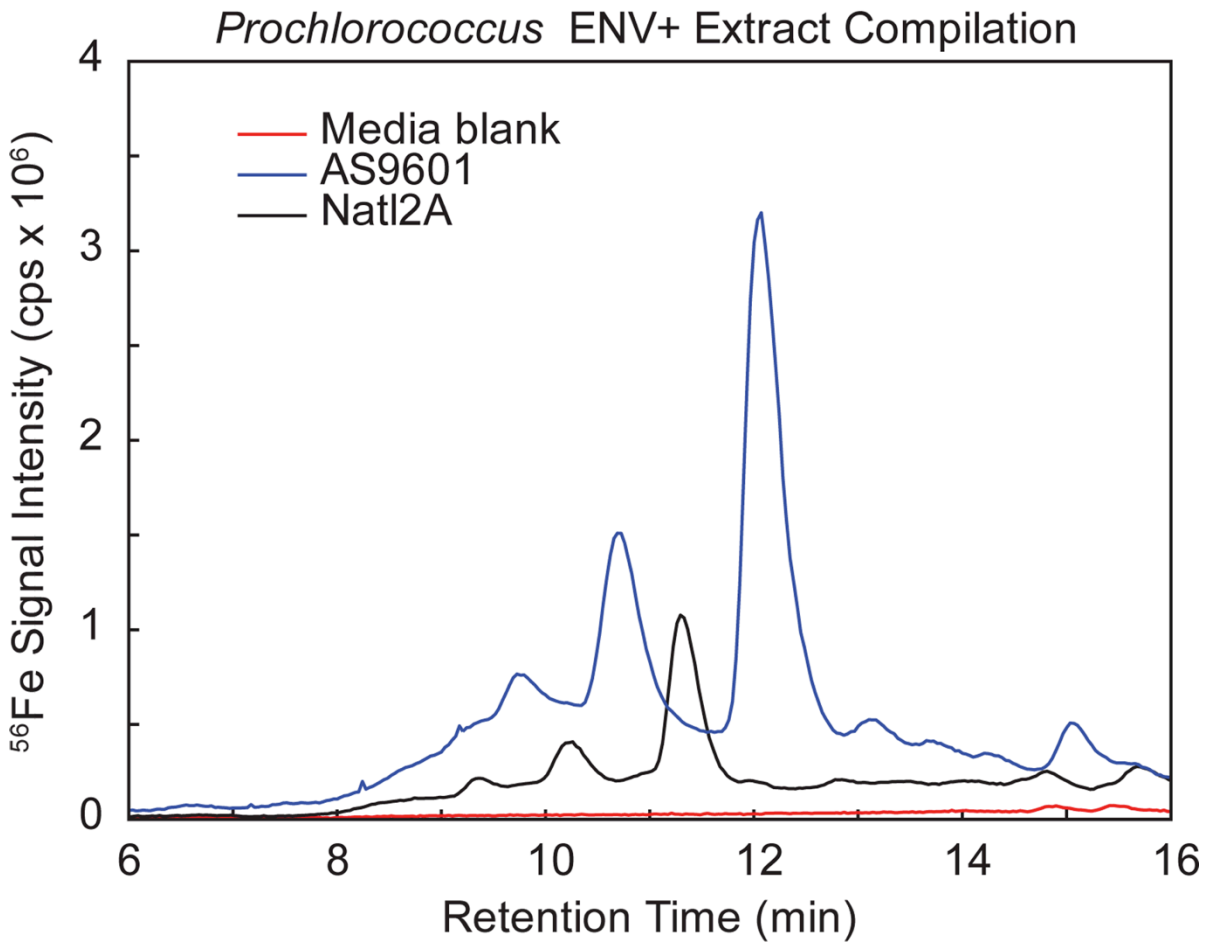
2. Natural Seawater
   - Subtropical Pacific



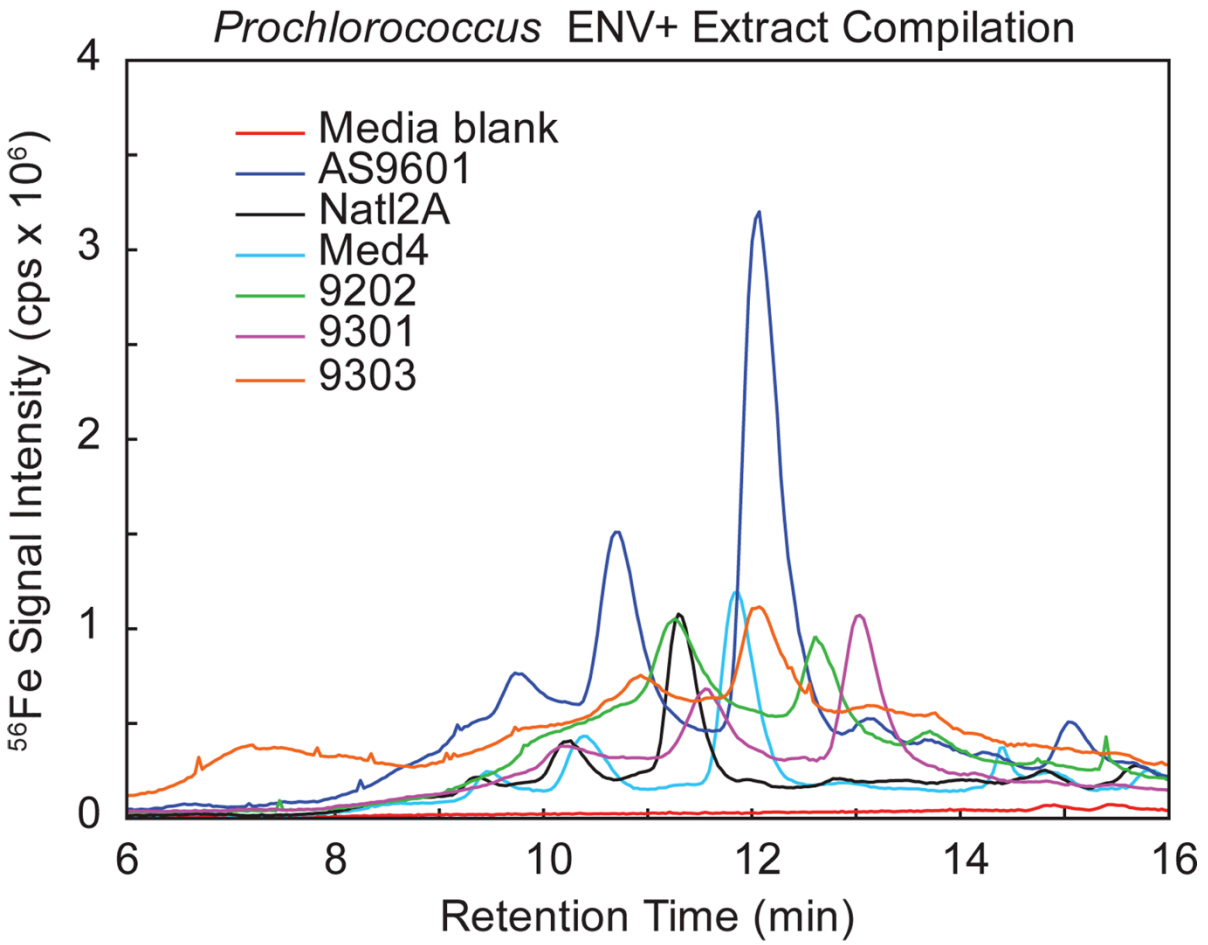
Prochlorococcus Fe Ligands



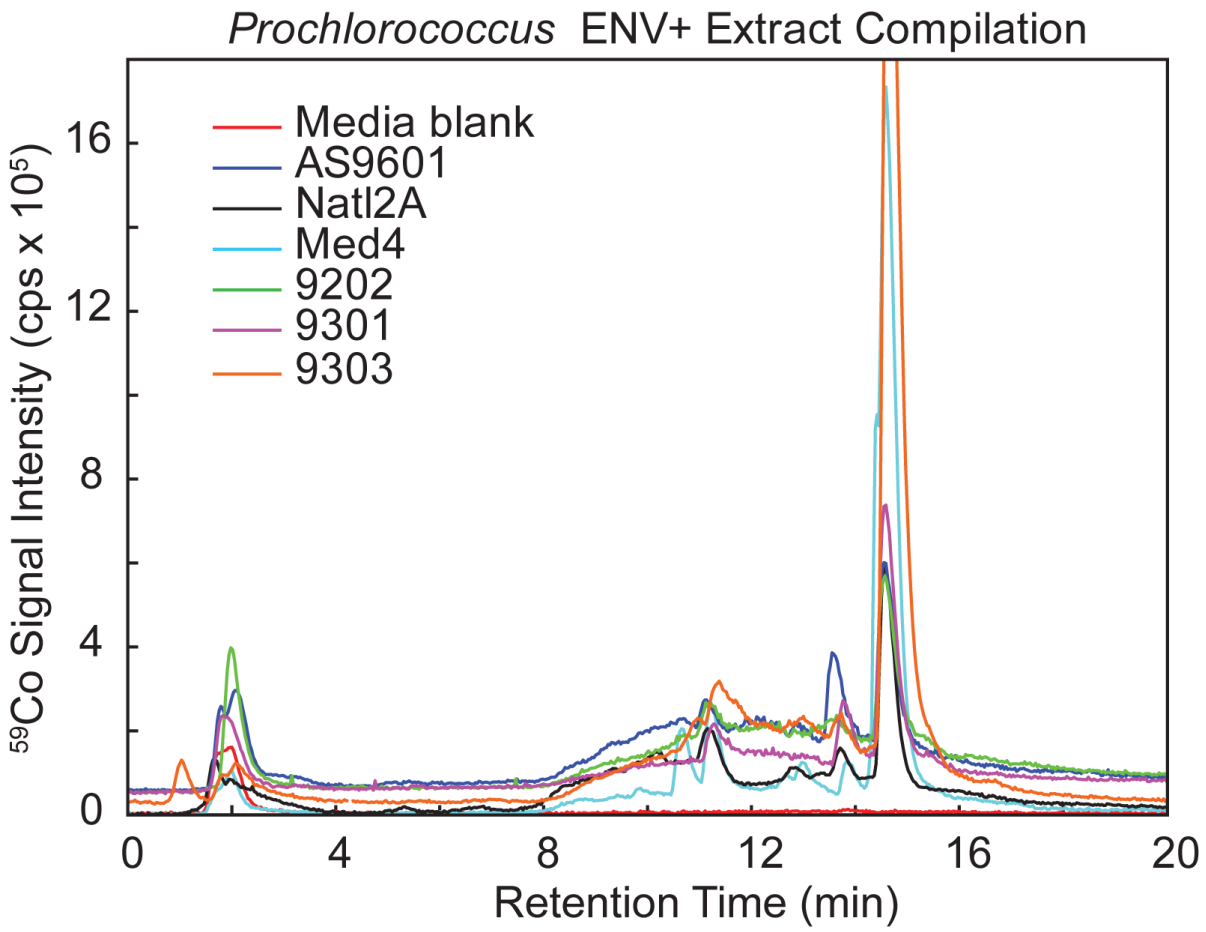
Prochlorococcus Fe Ligands

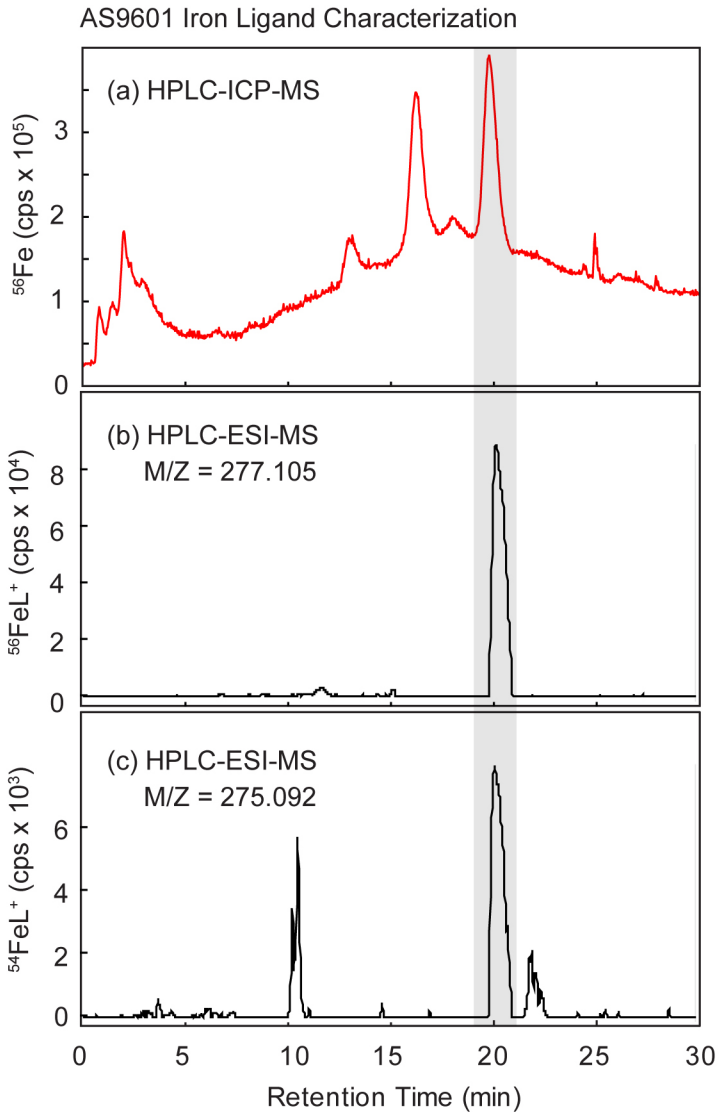


Prochlorococcus Fe Ligands

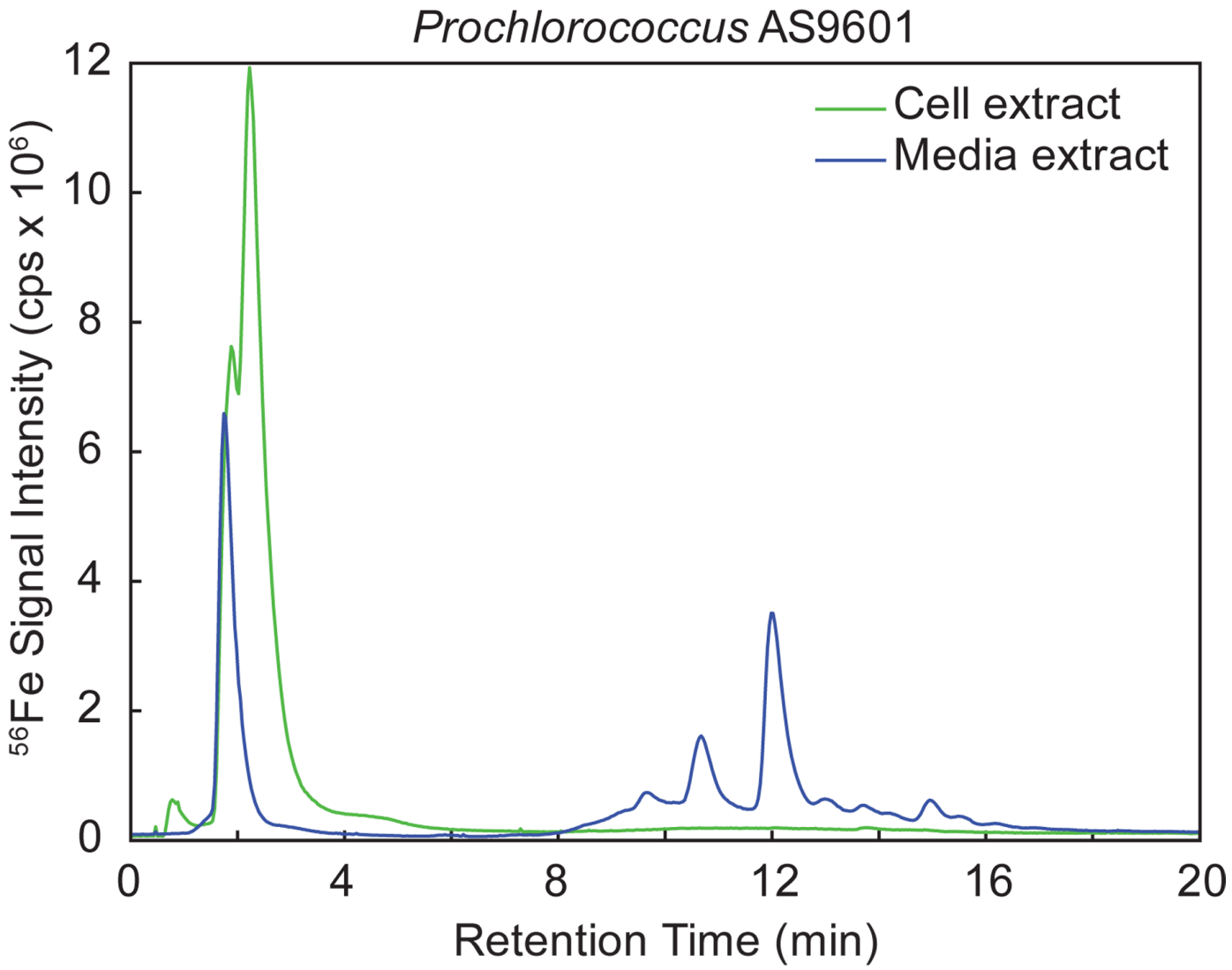


Prochlorococcus Co Ligands

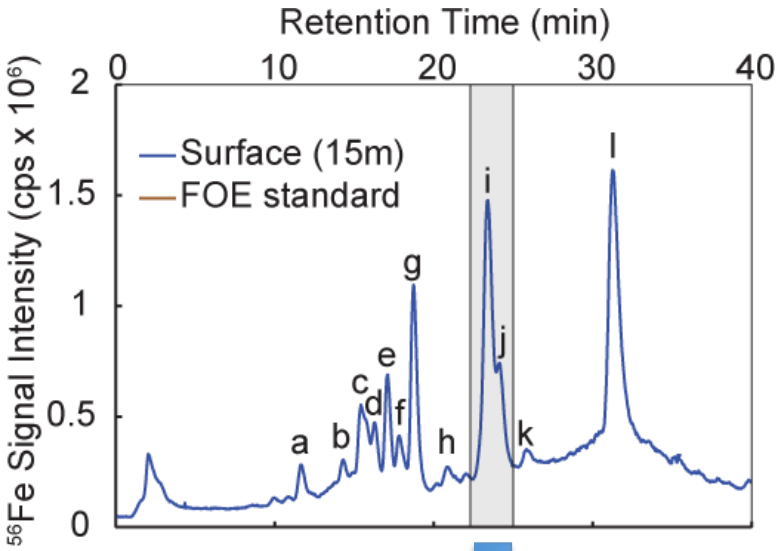



AS9601 Iron Ligand Characterization
(a) HPLC-ICP-MS
^{56}Fe (cps x 10^5)
0
1
2
3
(b) HPLC-ESI-MS
M/Z = 277.105
^{56}FeL$^+$ (cps x 10^4)
0
2
4
6
8
(c) HPLC-ESI-MS
M/Z = 275.092
^{54}FeL$^+$ (cps x 10^3)
0
2
4
6
0
5
10
15
20
25
30
Retention Time (min)

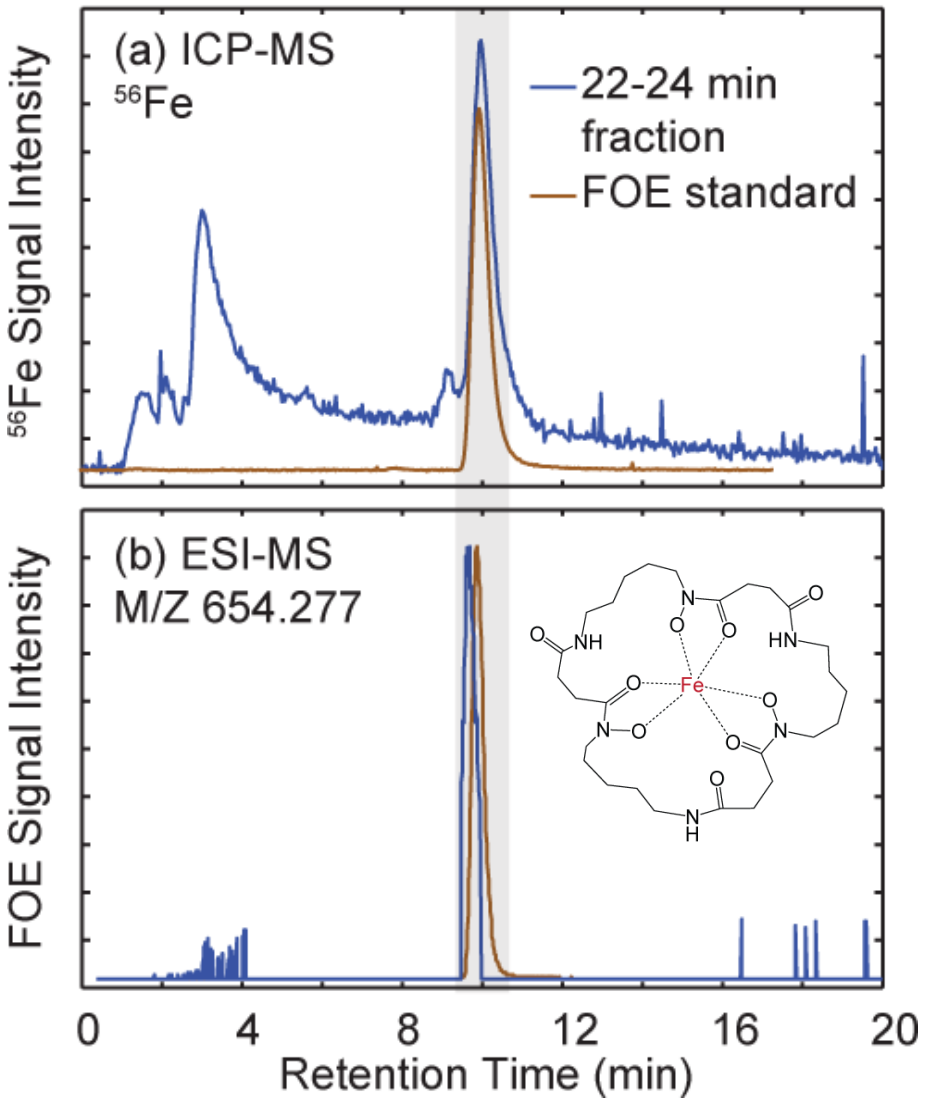
Prochlorococcus Fe Ligands



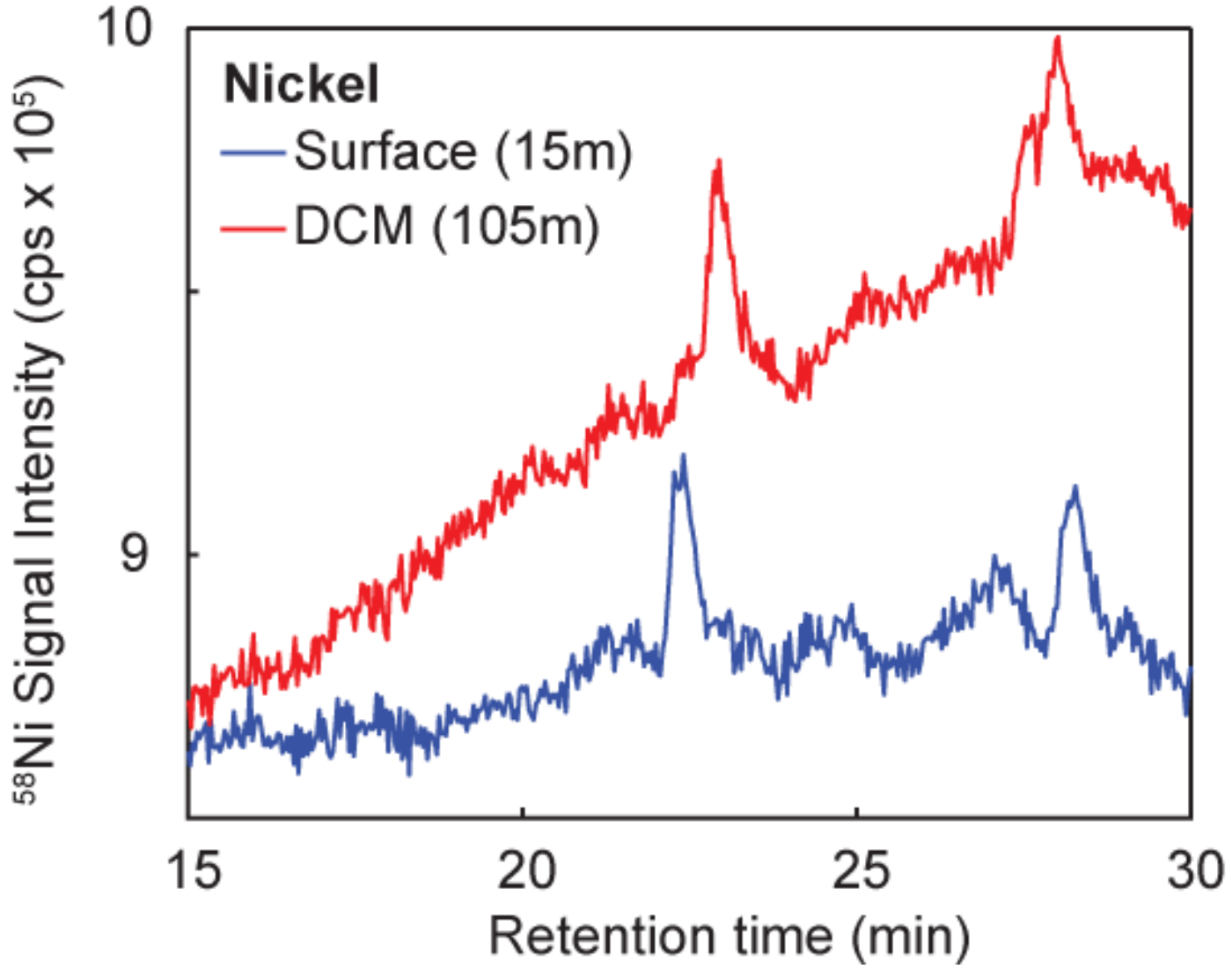
Subtropical North Pacific (Hoe-Phor)



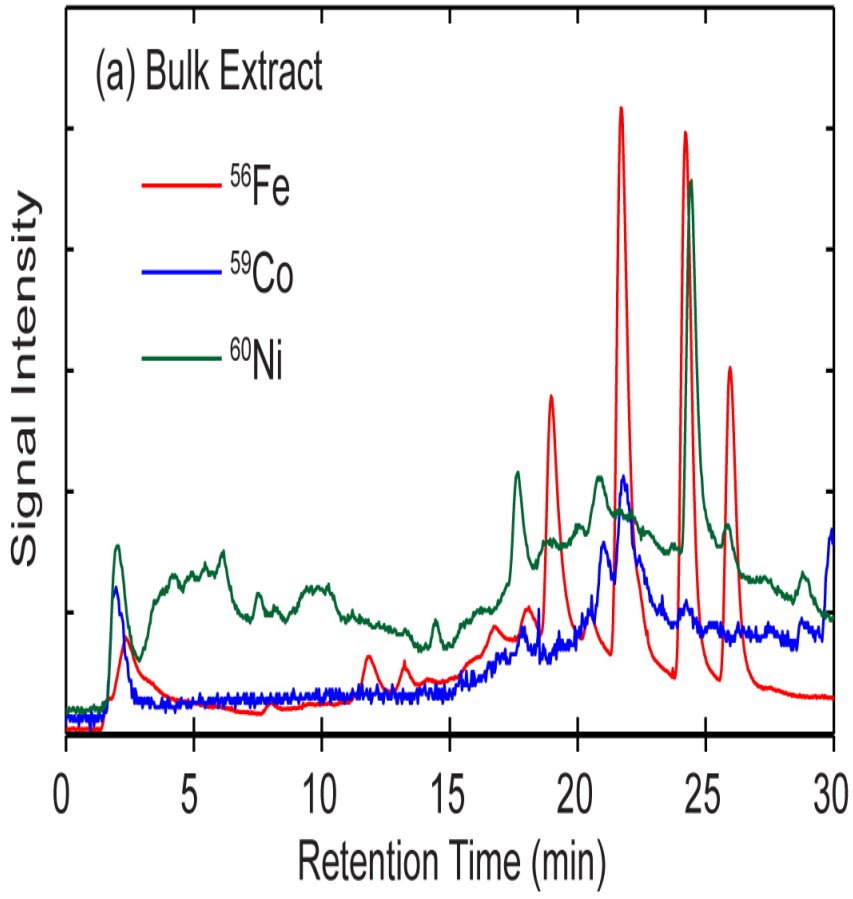
2nd separation on collected fractions

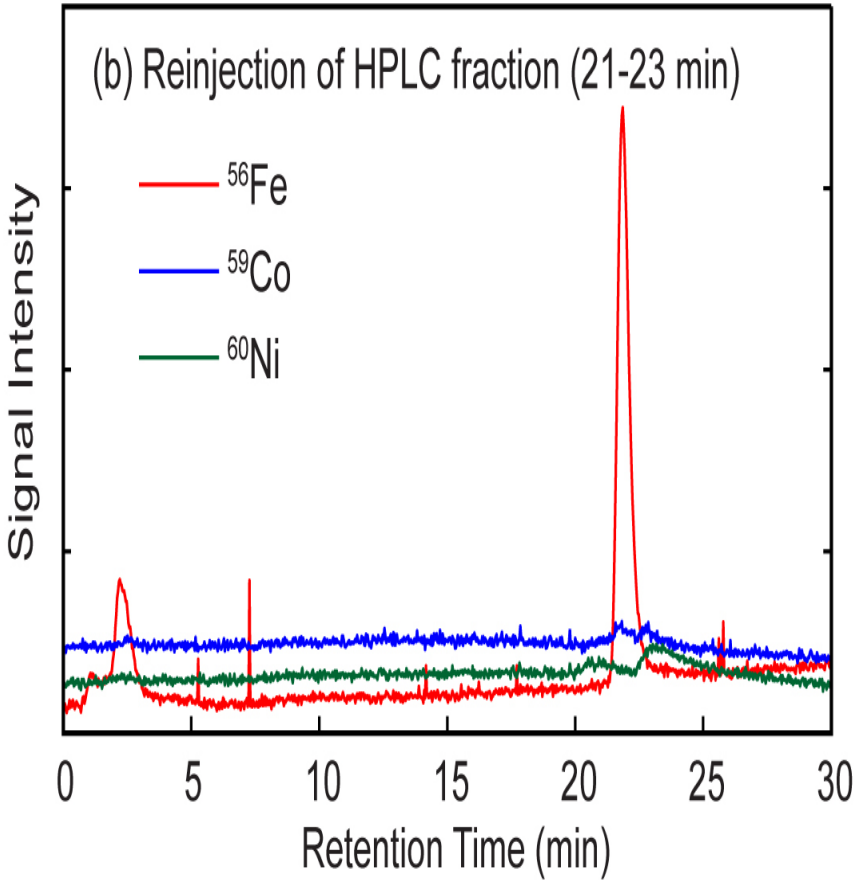


Subtropical North Pacific (Hoe-Phor)



Trace metal organic complexes in the South Pacific Subtropical Gyre





Summary:

- All marine microbes produce a very broad suite of organic compounnds that are released from the cell (exometabolome) 100's-1000's of compounds from a single pure culture.

- To understand microbial interactions we somehow need to identify which of these compounds matter, and which don't. There are two approaches to this, untargeted and targeted.

- We typically don't find compounds, we find "features". We can turn a feature into a compound through spectral analysis.

- In the case of organometallics, we can use the metals to "find" the feature and begin to track it to source and biosynthetic pathway: link "omics" to geochemical cycling.