Marine Inorganic Biochemistry: From Photoreactive Siderophores to Iodide in Kelp

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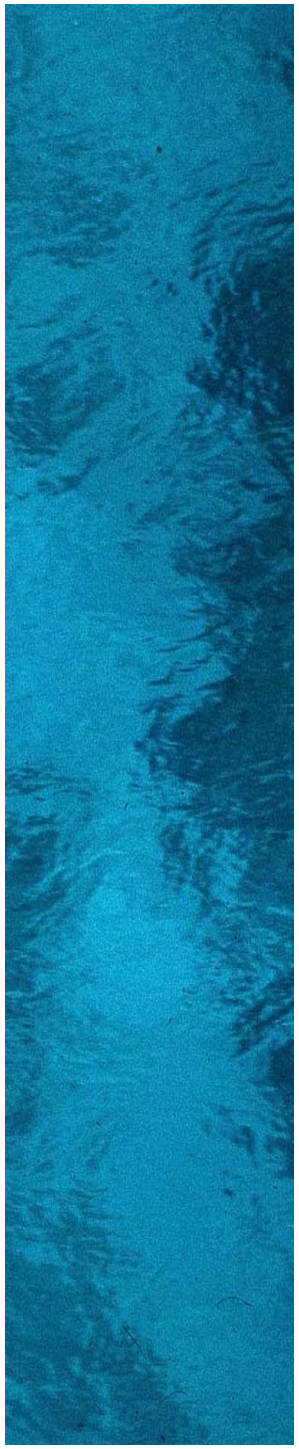
Given in shortened form by Hendrik Küpper,
Advanced Course on Bioinorganic Chemistry & Biophysics of Plants, summer semester 2021



Metals in the Ocean

Mo	100 nM
V	20 - 35 nM
Ni	2 - 12 nM
Zn	0.1 – 8 nM
Cu	0.6 - 5.7 nM
Cr	3 - 4.5 nM
Fe	0.05 – 0.7 nM
Mn	0.03 – 0.8 nM

Relative abundance of "exotic" biometals

Surface concentrations are often lower than in deeper waters! (Exceptions: Mn, Co)

Metals in the Ocean

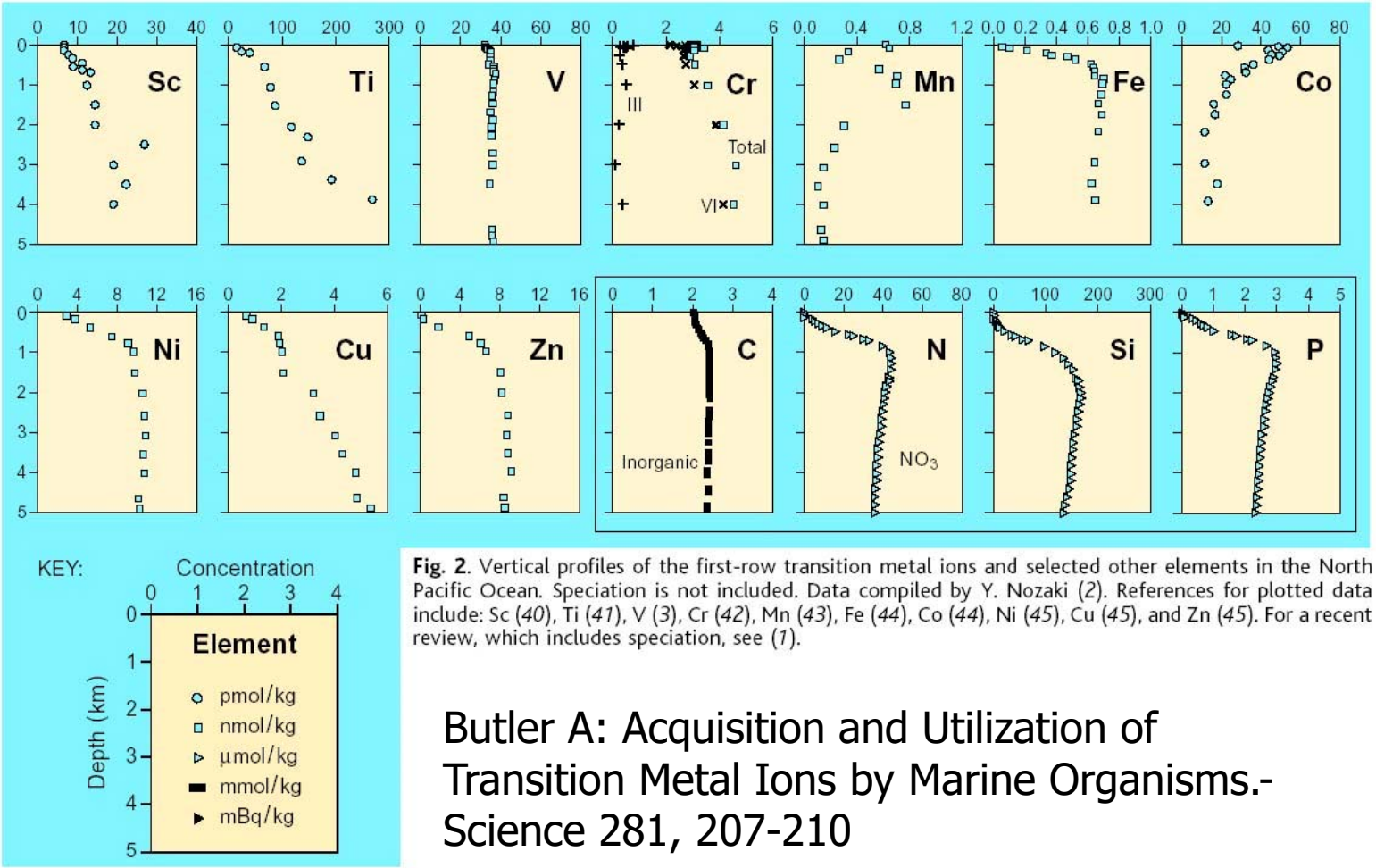


Fig. 2. Vertical profiles of the first-row transition metal ions and selected other elements in the North Pacific Ocean. Speciation is not included. Data compiled by Y. Nozaki (*2*). References for plotted data include: Sc (*40*), Ti (*41*), V (*3*), Cr (*42*), Mn (*43*), Fe (*44*), Co (*44*), Ni (*45*), Cu (*45*), and Zn (*45*). For a recent review, which includes speciation, see (*1*).

Butler A: Acquisition and Utilization of Transition Metal Ions by Marine Organisms.- Science 281, 207-210

Fundamental differences between the marine and terrestrial biosphere

- Iron tends to be scarce in the ocean!
- In fact, marine primary productivity is limited in HNLC (high nitrogen, low chlorophyll) regions by lack of iron.
- In the sea, Mo, V, Ni, Zn, Cu are much more abundant than iron!

Strategies of marine organisms to cope with low iron availability

- Highly efficient uptake and recycling systems
- Use of chemical alternatives to iron

The World Ocean, a halogen-rich environment!

- Marine organisms produce a plethora of halogenated natural products
- In many cases, metalloenzymes are involved in the biosynthesis

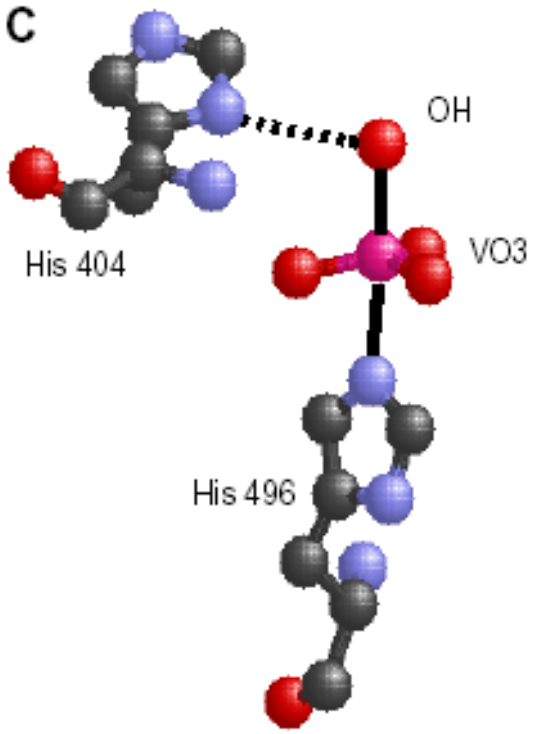
Marine metalloenzymes: Vanadium (V) haloperoxidases from marine red and brown algae

$$X^- + H_2O_2 + R\text{-}H + H^+ \longrightarrow R\text{-}X + 2\ H_2O$$

- Other peroxidases: heme (Fe) peroxidases in most terrestrial organisms, W peroxidases / oxidoreductases in hyperthermophilic archaea

Vanadium haloperoxidases

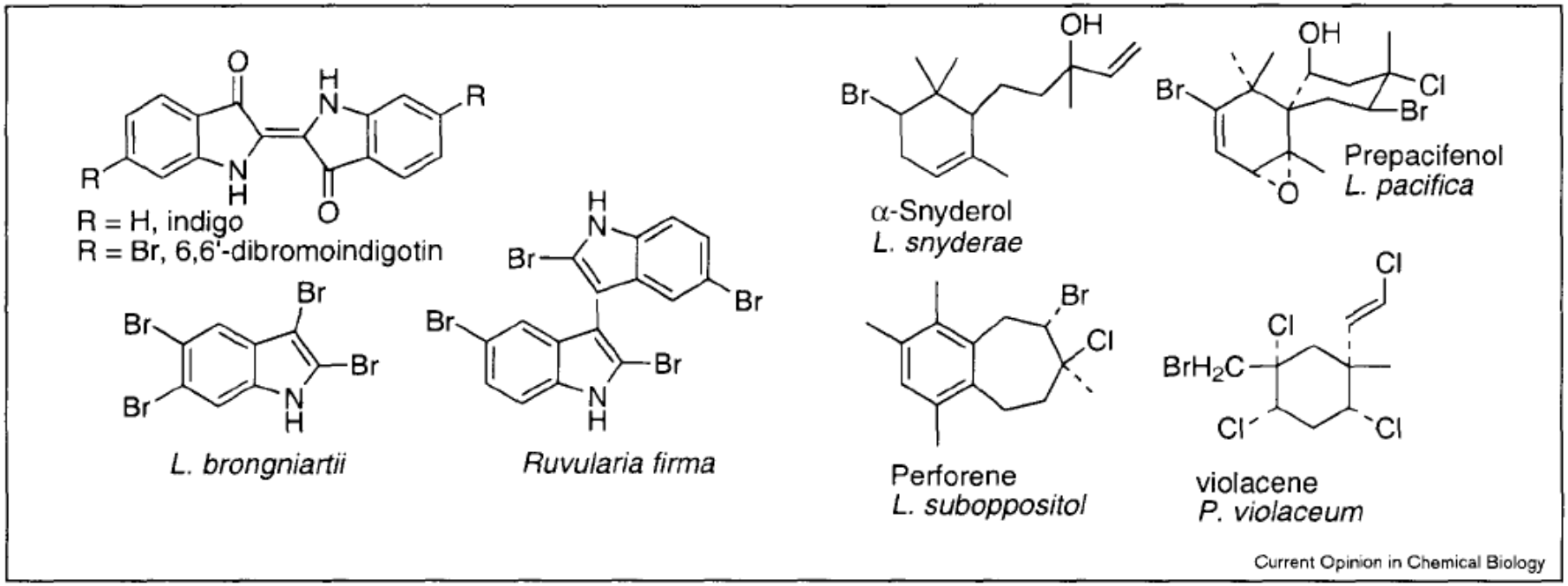
- Vanadium (V): vanadate
- Homology to acidic phosphatases



Butler A, 1998: Science 281, 207-10

Vanadium haloperoxidases

- A role in the synthesis of halogenated marine natural products (esp. red algae: *Laurencia* sp., *Plocamium cartilagineum*)

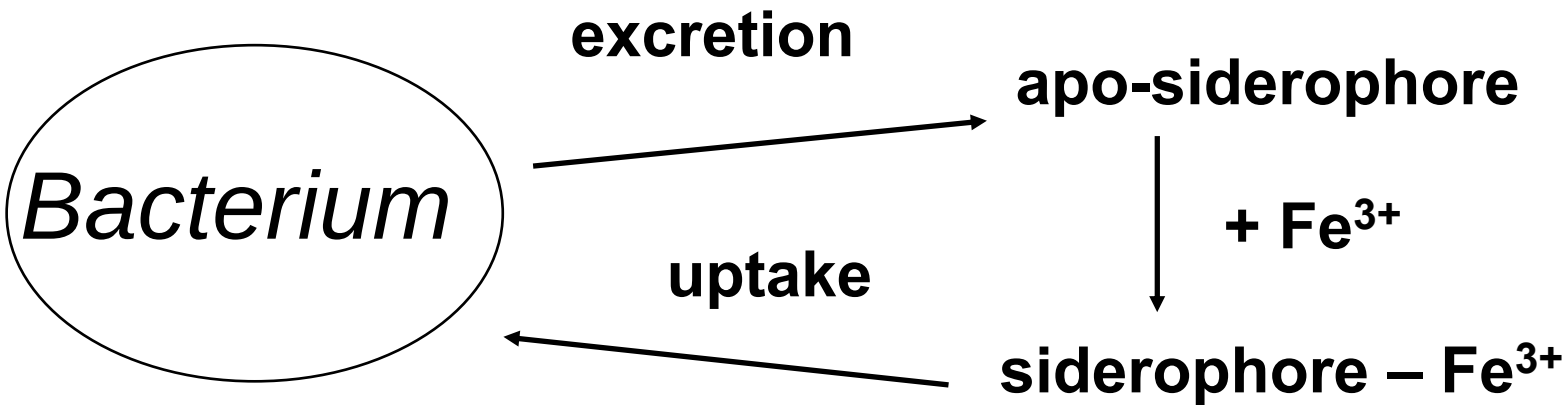


Selected examples of halogenated marine natural products.

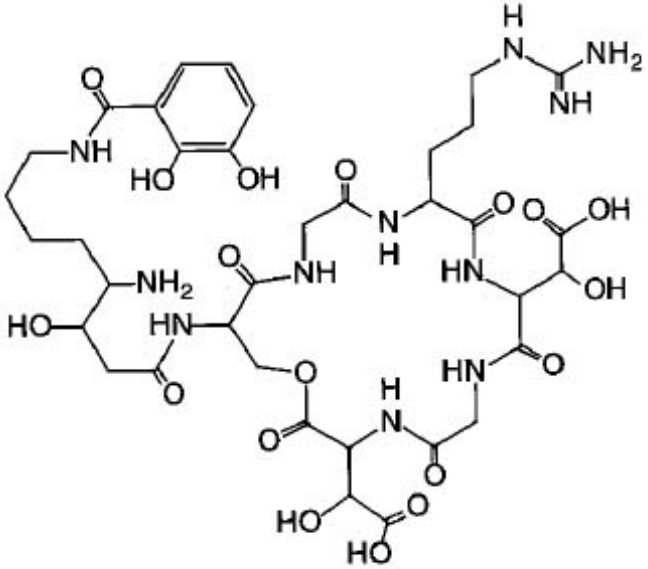
Butler A, 1998: Curr. Op. Chemical Biology 2, 279-85

Siderophore-mediated metal uptake

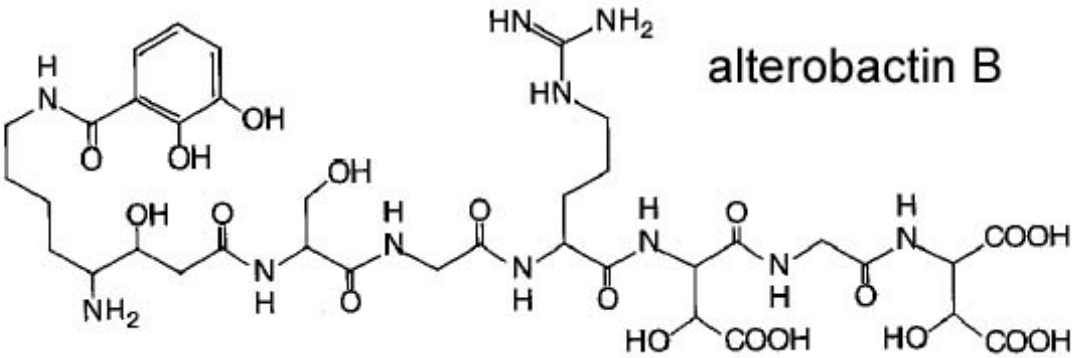
Siderophore, *Greek*: "iron carrier"



Marine siderophores



alterobactin A



alterobactin B

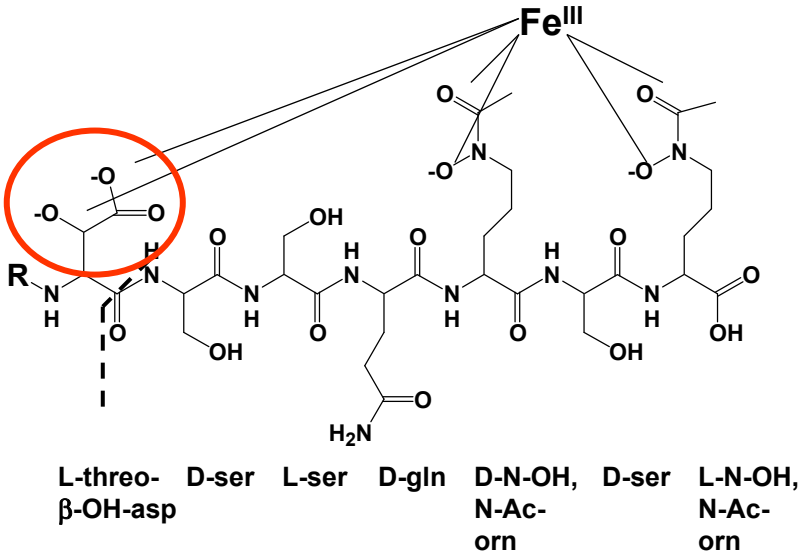
R. T. Reid, D. H. Live, D. J. Faulkner, A. Butler, *Nature* **366**, 455 (1993).

Marine siderophores

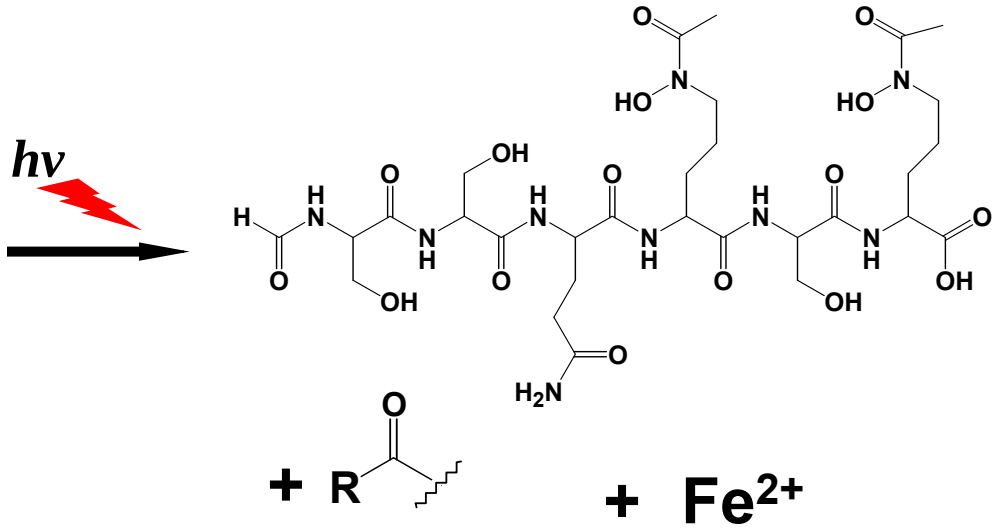
- Some well-known structures (e.g. aerobactin), but mostly many novelties
- Mediate prokaryotic Fe / metal uptake in marine systems: availability to eukaryotes not well established yet (e.g. in algal-bacterial symbioses)
- Most eukaryotic algae seem to have plasma membrane-bound ferrireductases
- Several marine siderophores are photoreactive!

Photolysis of aquachelin

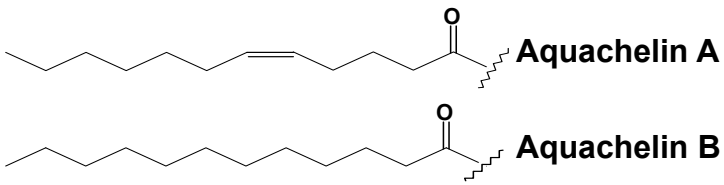
Fe(III)-Aquachelin complex

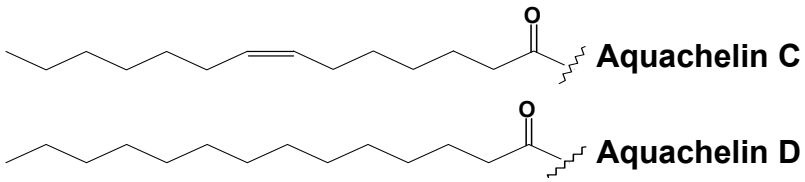


Photolysis products



R:





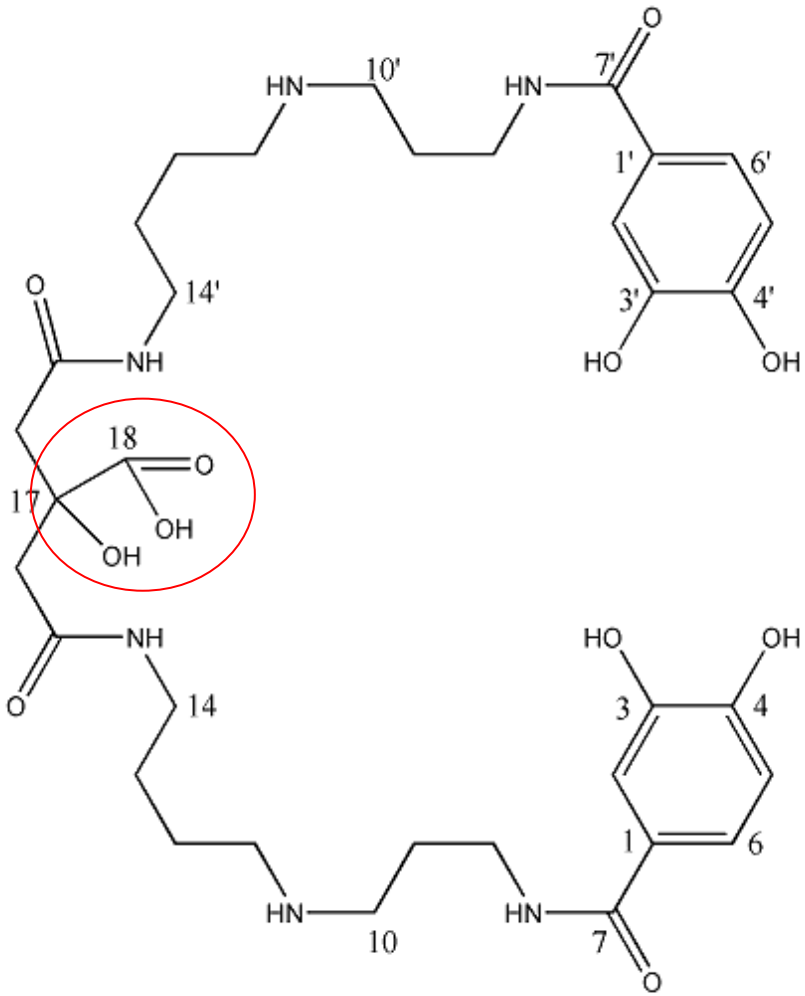
$K_{cond}^{Fe(III)'} = 10^{12.2}\ M^{-1}$

$K_{cond}^{Fe(III)'} = 10^{11.5}\ M^{-1}$

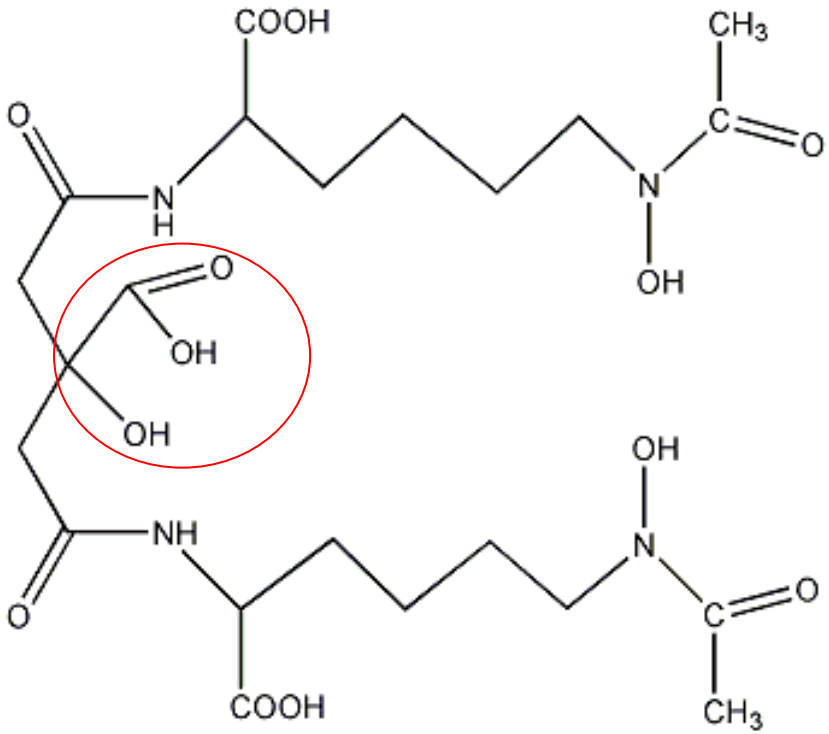
Barbeau, Rue, Bruland & Butler, 2001: Nature 413, 409-13

Other cases of photoreactive siderophores

Petrobactin



Aerobactin



Common feature:
α-hydroxy carboxylic acid moiety

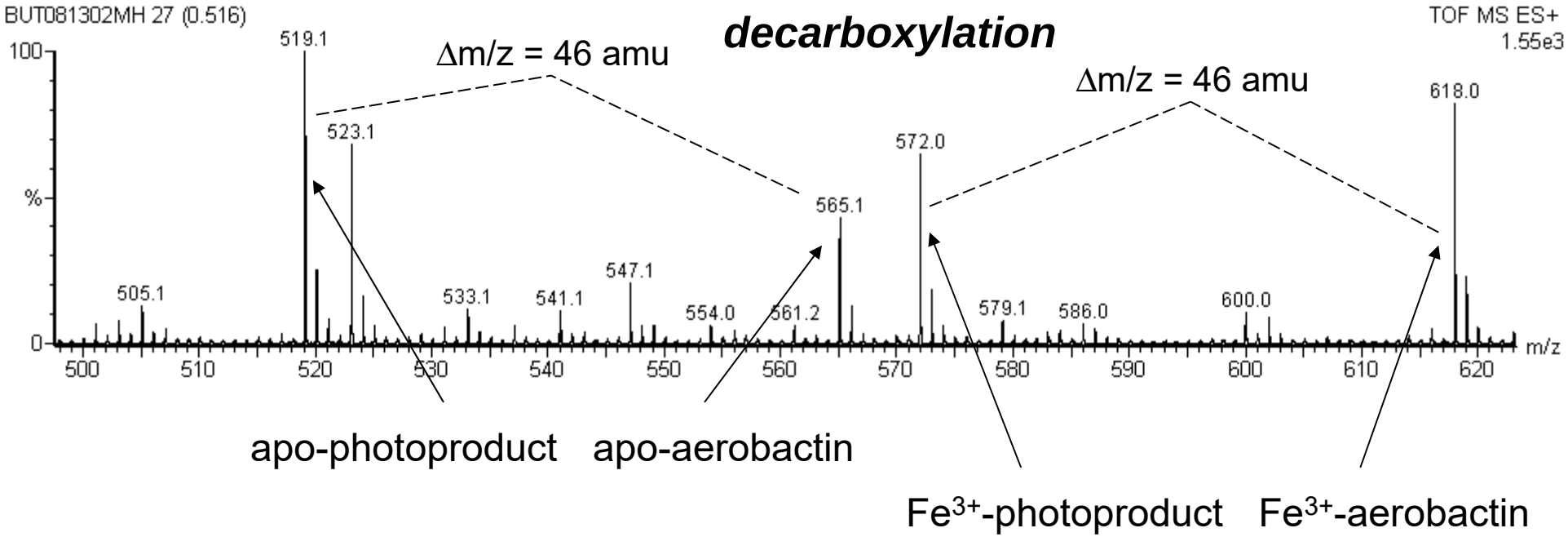
Barbeau *et al*., 2002: JACS 413, 409–13
Bergeron *et al*., 2003: Tetrahedron 59, 2007-14

Key questions

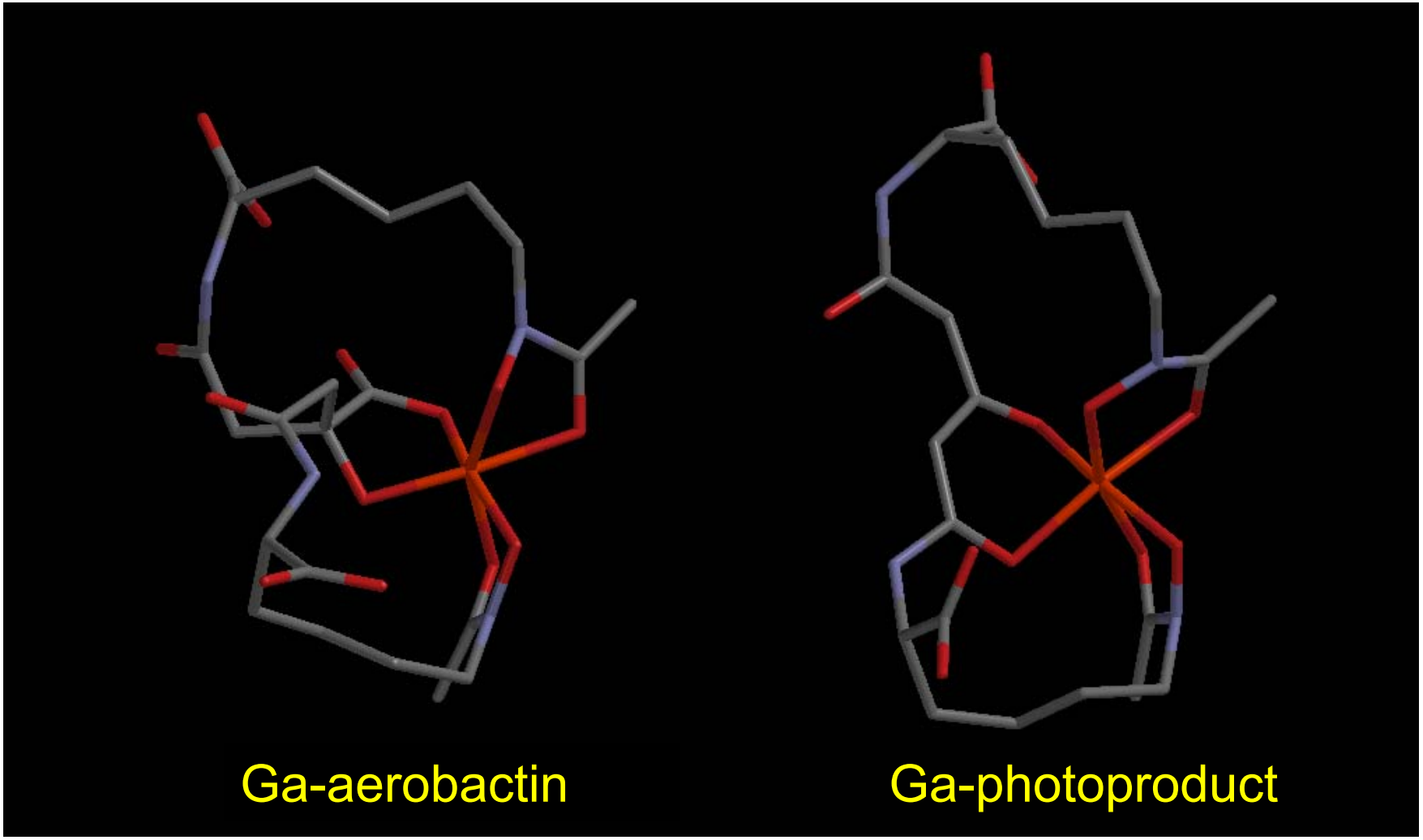
- Mechanistic aspects of photolysis
- Structure of photoproduct
- How do the physicochemical properties of the photoproduct compare to those of the original siderophore?
- Is photoproduct-bound Fe(III) as bioavailable as Fe(III) bound to the original siderophore?

=> Case study: AEROBACTIN

ESI-MS (positive ion mode): Fe-aerobactin photolysis



Quantum mechanical structure optimization of the Ga^{3+} complexes of aerobactin and its photoproduct



(Spartan 2000 - PM3 level of theory)

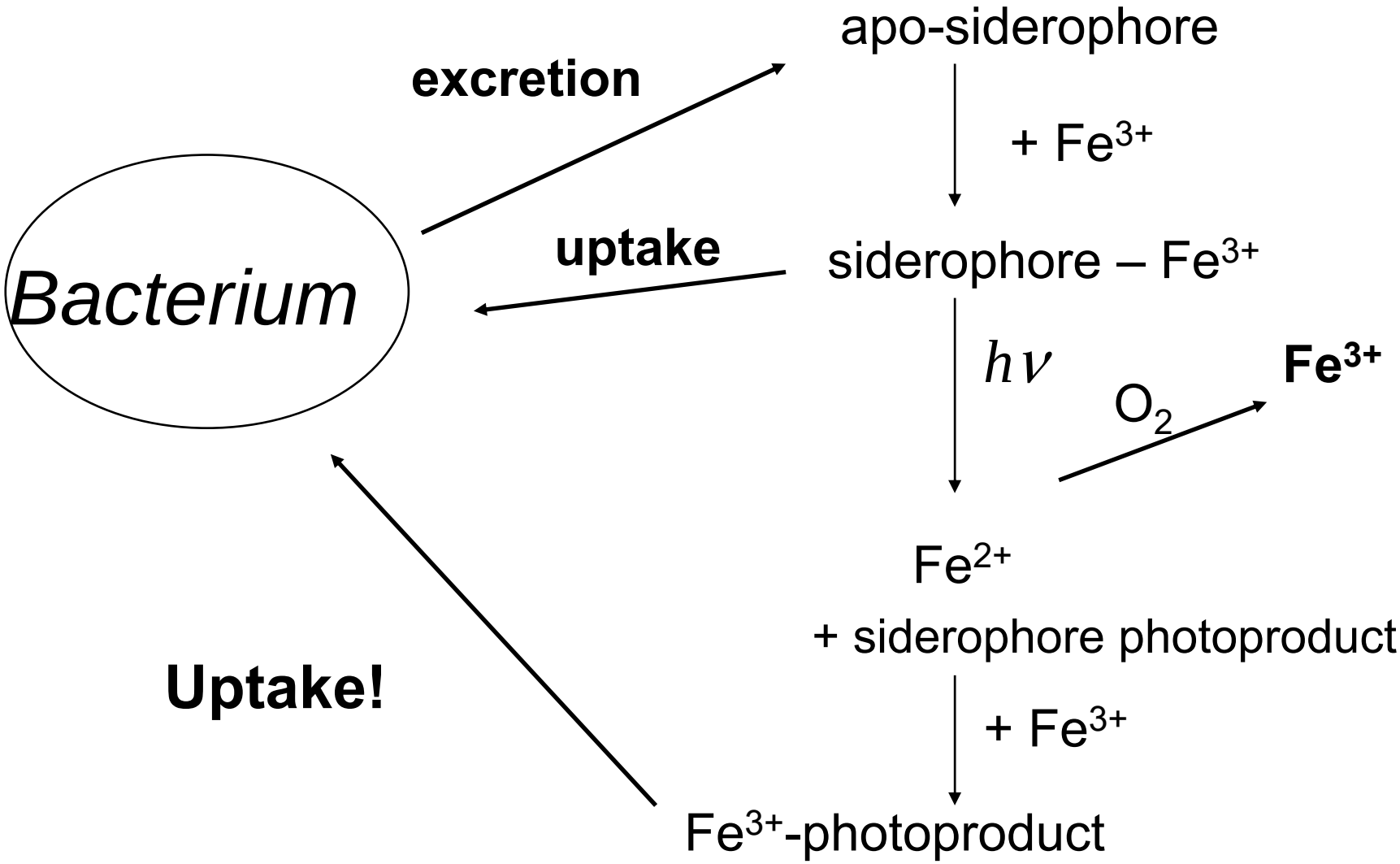
(C. J. Carrano)

Summary & conclusions: aerobactin photoproduct

- Photochemistry results in a **new ligand of similar physiological properties** in bacterial cells
- Photochemistry of Fe-aerobactin yields reactive Fe^{2+}, potentially available for a wide range of other organisms and ligands over a transient time span
- **Fe bound to the photoproduct can be considered to be of limited access** to marine biota
- Oceanographic / ecological implications (this is likely to be relevant only in euphotic open-Ocean conditions, yet not for Enterobacteria also producing aerobactin!)? Is there any **evolutionary advantage** of producing photoreactive siderophores?

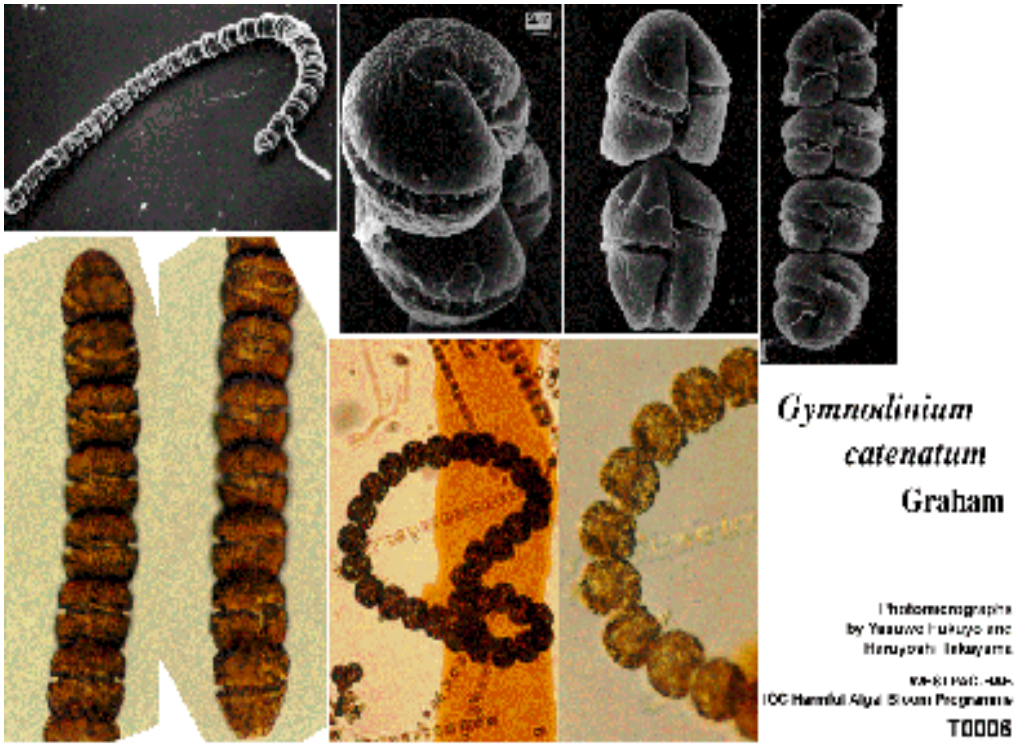
Küpper FC, Carrano CJ, Kuhn J-U, Butler A, 2006: Photoreactivity of Iron(III)-Aerobactin: Photoproduct Structure and Iron(III) Coordination.- Inorganic Chemistry 45(15), 6028-33

Summary:
Siderophore photochemistry

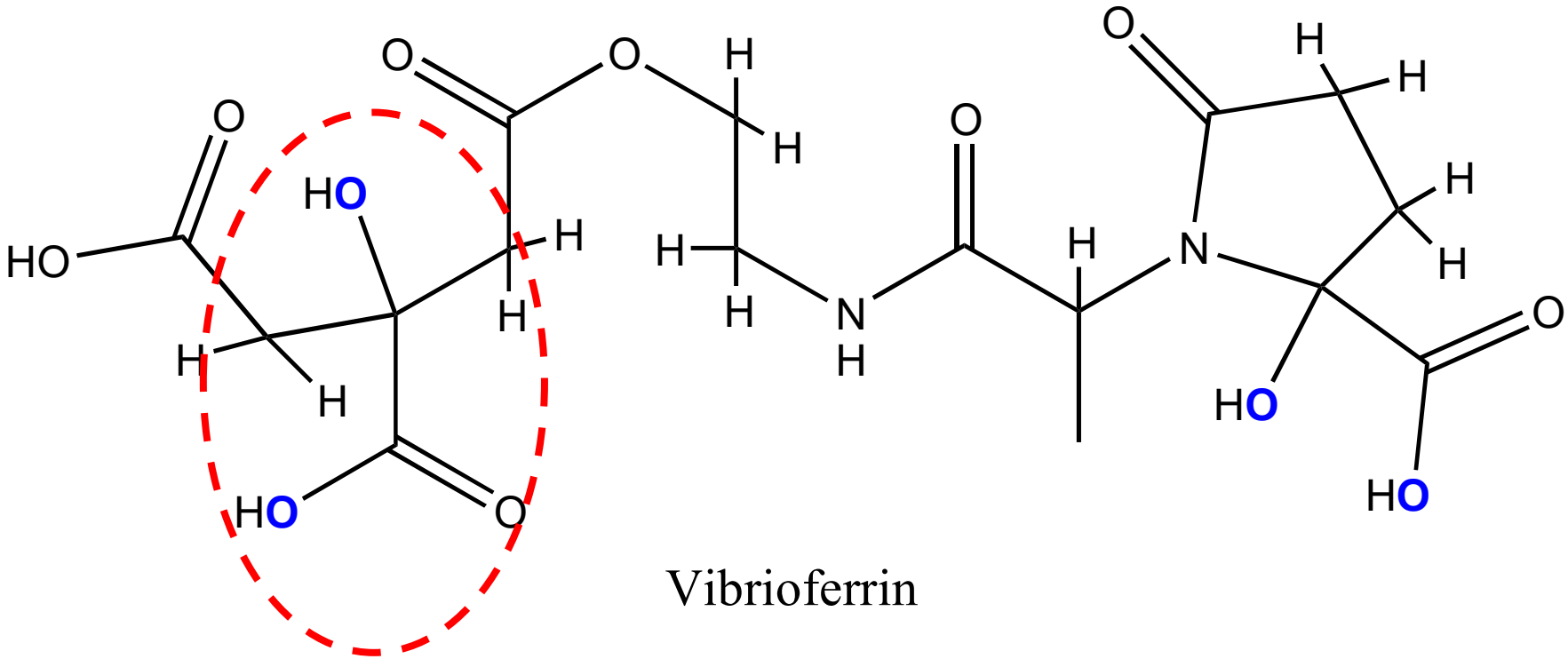


Algal-bacterial symbioses

- Bacterial symbionts of the dinoflagellate *Gymnodinium catenatum* (D.H. Green)



Isolation of siderophores from culture media of bacterial symbionts



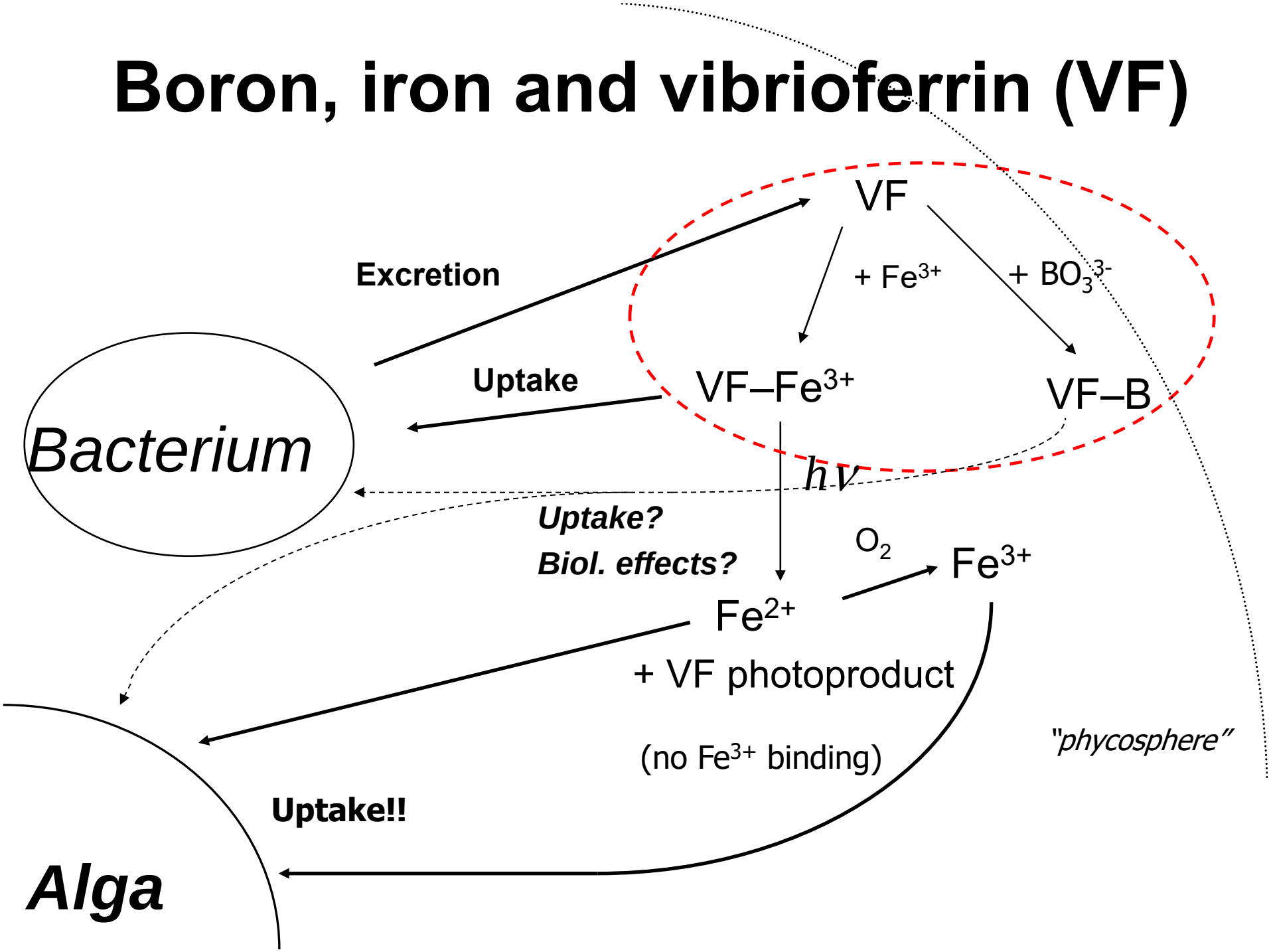
Vibrioferrin

- Vibrioferrin

Fe-vibrioferrin is highly photoreactive

- Unlike for aerobactin and all other photoreactive siderophores known so far, photochemistry destroys the Fe-binding backbone of VF
- VF photochemistry generates free reactive iron intermediates which enhance iron uptake of the algal symbiont

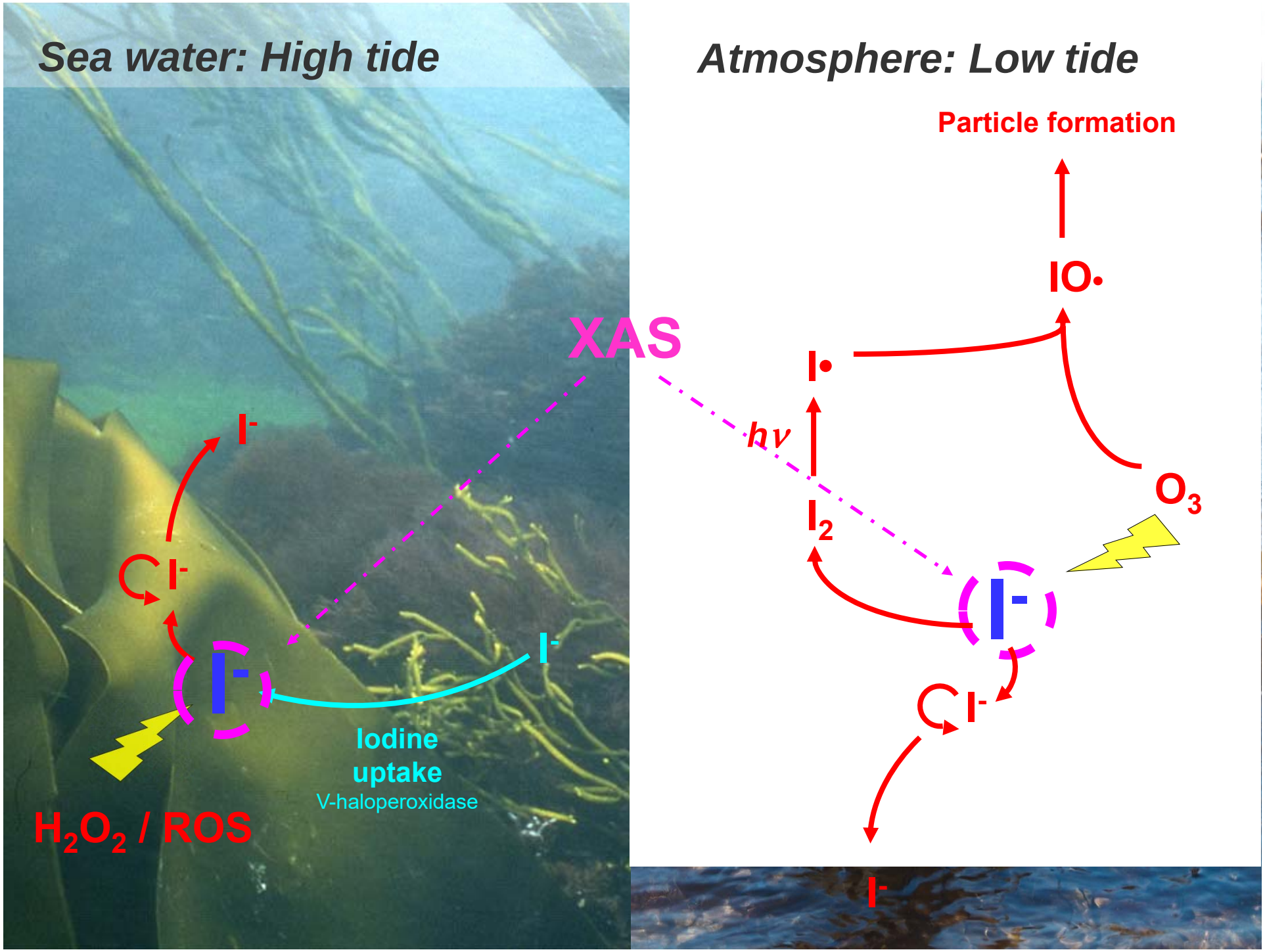
Boron, iron and vibrioferrin (VF)



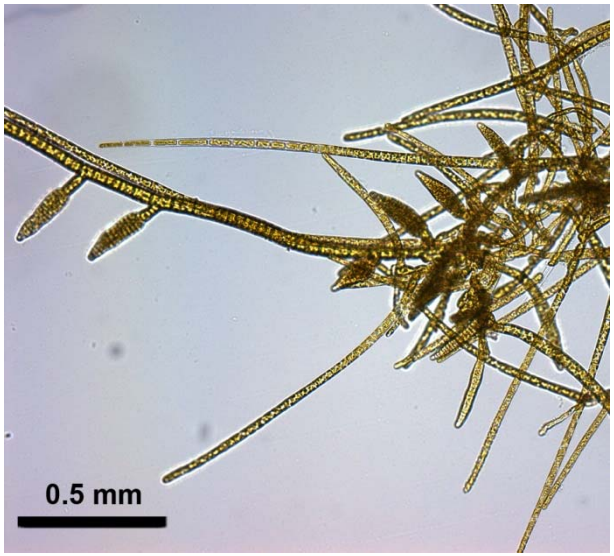
Vibrioferrin photochemistry
– a key factor in an algal-bacterial symbiosis

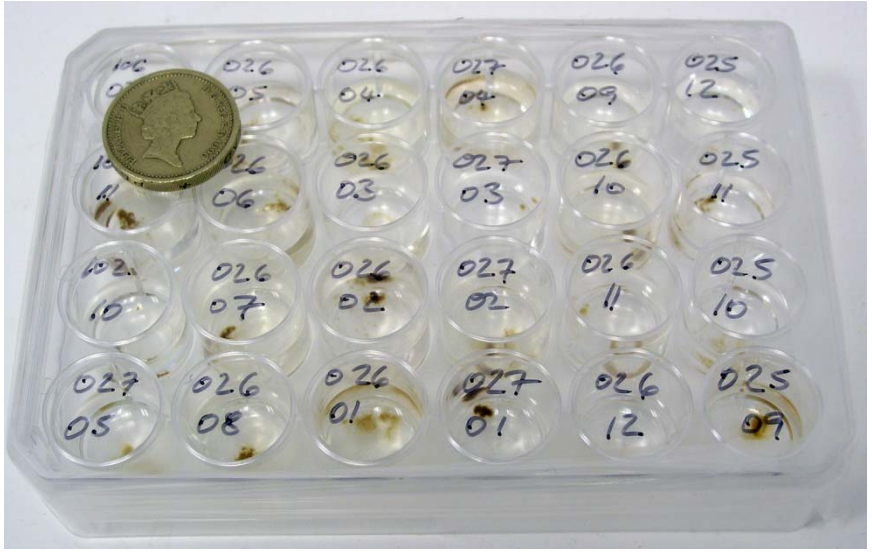
Amin SA, Green DH, Hart MC, Küpper FC, Sunda WG, Carrano CJ, 2009: Photolysis of iron–siderophore chelates promotes bacterial–algal mutualism.- Proceedings of the National Academy of Sciences of the USA 106(40), 17071–6

Amin SA, Green DH, Küpper FC, Carrano CJ, 2009: Vibrioferrin, an unusual marine siderophore: Iron binding, photochemistry, and biological implications.- Inorganic Chemistry 48, 11451-8


Sea water: High tide
Atmosphere: Low tide
Particle formation
IO•
XAS
I•
hν
I2
O3
I-
I-
I-
I-
I-
I-
Iodine
uptake
V-haloperoxidase
H2O2 / ROS
I-

Ectocarpus siliculosus





- Filamentous, cosmopolitan brown alga – mostly from temperate seas
- One of the best-studied seaweeds
- The first fully-sequenced multicellular alga!
- > 300 fully-characterized strains in public-domain culture collections (CCAP, KU-MACC)

Cock JM, Sterck L, Rouzé P, Scornet D, Allen AE, Amoutzias G, Anthouard V, Artiguenave F, Aury J-M, Badger JH, Beszteri B, Billiau K, Bonnet E, Bothwell JHF, Bowler C, Boyen C, Brownlee C, Carrano CJ, Charrier B, Cho GY, Coelho SM, Collén J, Corre E, Da Silva C, Delage L, Delaroque N, Dittami SM, Doulbeau S, Elias M, Farnham G, Gachon CMM, Gschloessl B, Heesch S, Jabbari K, Jubin C, Kawai H, Kimura K, Kloareg B, Küpper FC, Lang D, Le Bail A, Leblanc C, Lerouge P, Lohr M, Lopez PJ, Martens C, Maumus F, Michel G, Miranda-Saavedra D, Morales J, Moreau H, Motomura T, Nagasato C, Napoli CA, Nelson DR, Nyvall-Collén P, Peters AF, Pommier C, Potin P, Poulain P, Quesneville H, Read B, Rensing SA, Ritter A, Rousvoal S, Samanta M, Samson G, Schroeder DC, Ségurens B, Strittmatter M, Tonon T, Tregear J, Valentin K, von Dassow P, Yamagishi T, Van de Peer Y, Wincker P, 2010:

The *Ectocarpus* genome and the independent evolution of multicellularity in the brown algae.- Nature 465, 617-21

Vol 465|3 June 2010|doi:10.1038/nature09016 nature

LETTERS

June 3, 2010

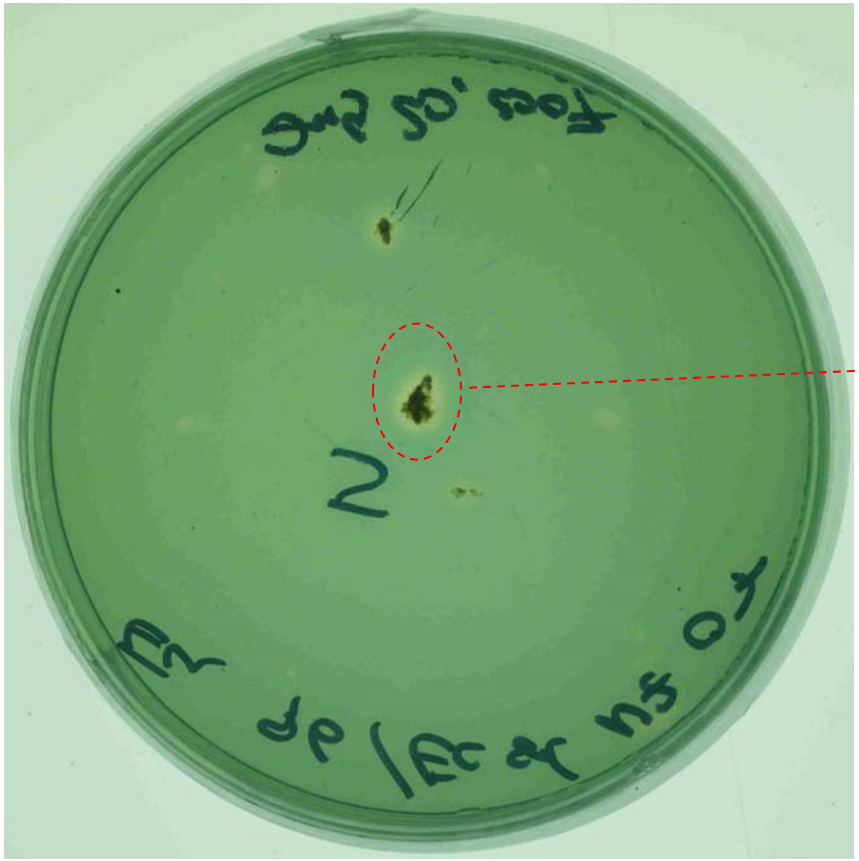
The *Ectocarpus* genome and the independent evolution of multicellularity in brown algae

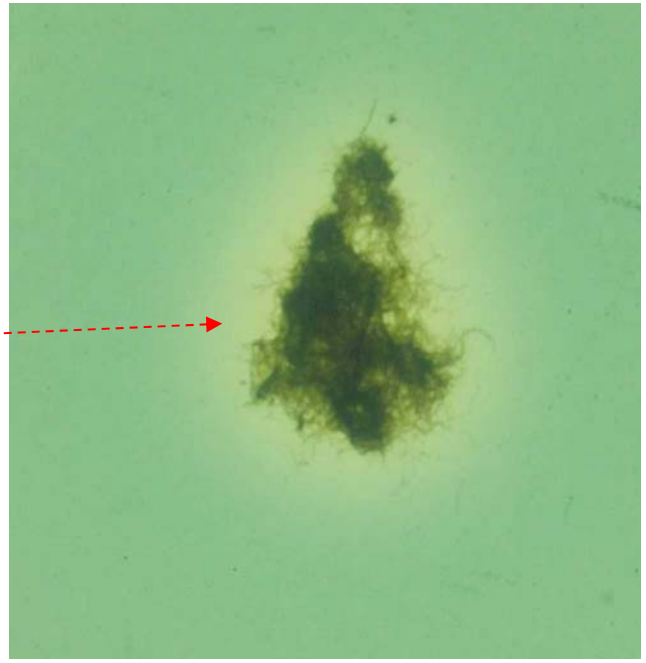
Features of the *Ectocarpus* genome

- 214 Mbp
- Very high number of introns
- Repeated sequences (Transposons, retrotransposons, helitrons) make up > 22% of the genome!
- No ferritins!!
- Halogen metabolism (1 V haloperoxidase, 21 putative dehalogenases and 2 haloalkane dehalogenases)
- Many bacterial genes
- … and a high percentage of genes of still unknown function!

Ectocarpus and iron:

axenic cultures are CAS active!





CAS = Chrome azurol S (a colorimetric assay for Fe^{3+}-binding activity)

- **CAS activity is caused by secretion of strong Fe(III)-binding ligands – or acidification**

Siderophores in *Ectocarpus*?

- Siderophore production is known to occur in bacteria, fungi and monocotyledons – but no eukaryotic algae so far!
(Only phytosiderophores from monocots / higher plants)
- CAS activity in axenic *Ectocarpus* cultures seemed like an exciting finding
- Still, no siderophores could be isolated so far
- CAS activity might be caused by external acidification

Features of the *Ectocarpus* genome from the bioinorganic perspective: Iron uptake

- homologs of *fro2*, a cell surface Fe(III) reductase
- several divalent metal transporters (ZIP type)
- NRAMP (M^{2+}-H^{+} symporter with preference for Fe(II))

$\Rightarrow$ consistent with simple reductase/permease pathway

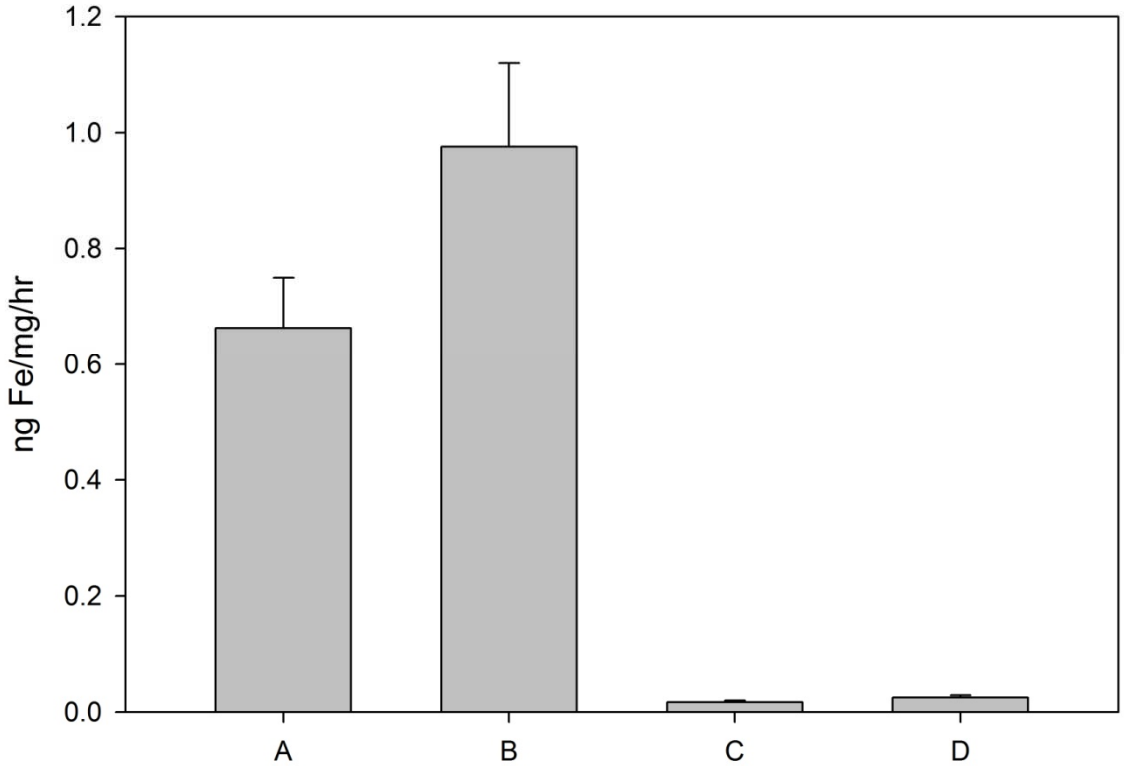
- absence of multicopper oxidases
- presence of siderophore biosynthetic pathway initially hypothesized, but not corroborated

Iron uptake in *Ectocarpus*

• Short term iron uptake studies: Fe is taken up in a time and concentration dependent manner
=> Consistent with an active transport process.
• Derived kinetic parameters: similar to those reported in the few available studies in other red, green and brown algae

	Macrocystis	*Gracilaria*	*Laminaria*	*Undaria*	*Ectocarpus*
V_m	1.6 pmol/cm^2/hr	0.26 pmol/mg/hr	2.7 pmol/cm^2/hr	6.4 pmol/cm^2/hr	0.25 pmol/mg/hr
K_m	3.5 µM	0.6 µM	0.54 µM	6.4 µM	1.5 µM
Ref	Manley 1981	Liu 2000	Matsunaga 1991	Matsunaga 1991	This work

Fe(III) chelate reductase activity in *Ectocarpus*



A) iron replete (30 µM) and B) iron starved (4 nM) cultures of *Ectocarpus siliculosus.*
Negative controls: C) dead cells and D) live cells minus FZ.
Error bars = ± 1 S.D. from triplicate measurements

Features of the *Ectocarpus* genome from the bioinorganic perspective: Iron storage

- **no ferritins!!**

⇒ typical feature of heterokont organisms

- No other iron store obvious from the *Ectocarpus* genome

⇒ need for physical techniques

Ectocarpus and iron:

How to store iron without ferritin?

Mössbauer and X-ray absorption spectroscopy show 2 main components of the cellular Fe pool:

(1) an iron-sulfur cluster (approx. 26% of the total intracellular iron pool)

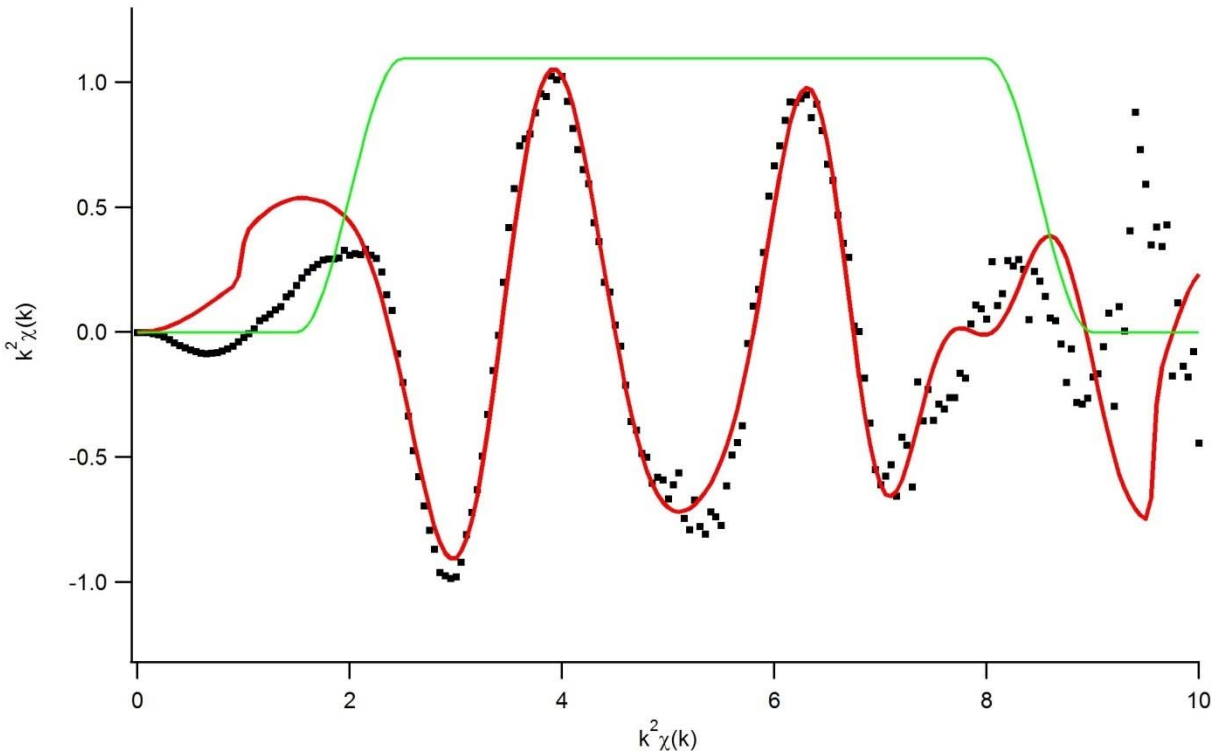
(2) a second component with spectra typical of a ($Fe^{3+}O_6$) system with parameters similar to the amorphous phosphorus-rich mineral core of bacterial and plant ferritins (approx. 74% of the cellular iron pool)

=> suggests that *Ectocarpus* contains a non-ferritin but mineral-based iron storage pool

Ectocarpus and iron:

How to store iron without ferritin?

- **probing the intracellular Fe pool by XAS**



Extracted EXAFS spectrum of 52 merged spectra transformed in k-space. Black squares: experimental data; red line: fit with FeS-cluster and amorphous Fe phosphate. Green line: setting of the range.

Iodine in seaweeds: A bit of historic background

- Goiter-preventing effects of seaweeds: Known to the Chinese emperor Shen-Nung (third millennium B.C.!)

- Use of burnt seaweeds and sponges as diet supplements for the same purpose: Common in ancient Greece at the time of physician Hippocrates [460-370 B.C.]

Iodine accumulation in *Laminaria*

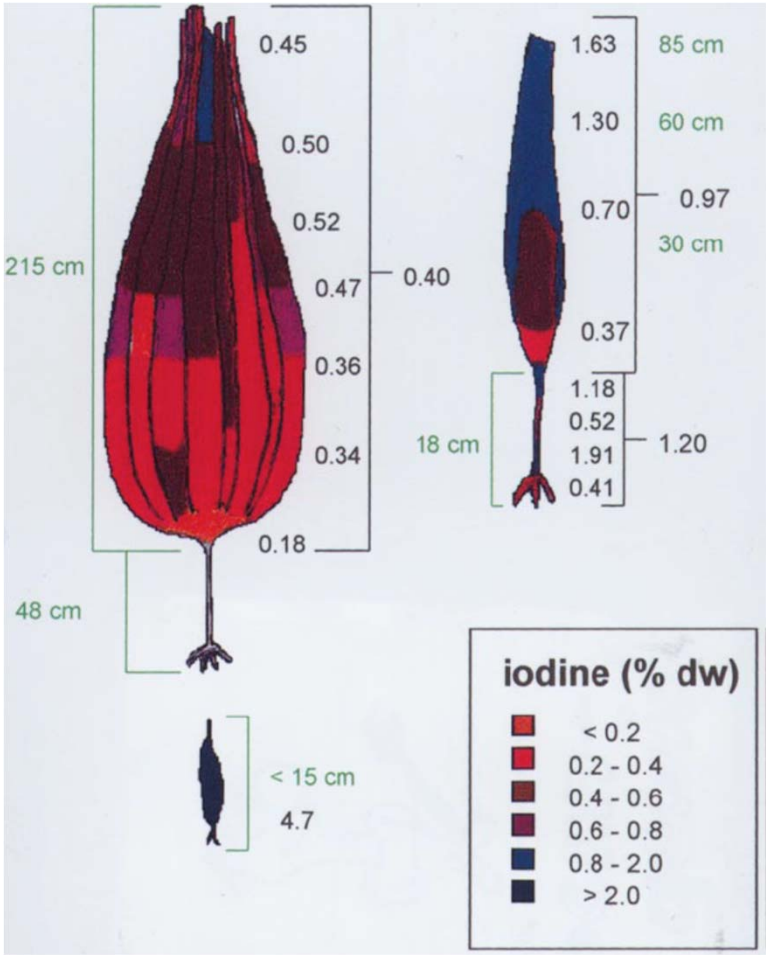
- Laminariales (kelps) are a major biogeochemical pump of iodine!
- *Laminaria* is the strongest iodine accumulator in life

Still unclear:

- Chemical form of accumulated iodine?
- **Biological significance?**

Iodine accumulation in *Laminaria*

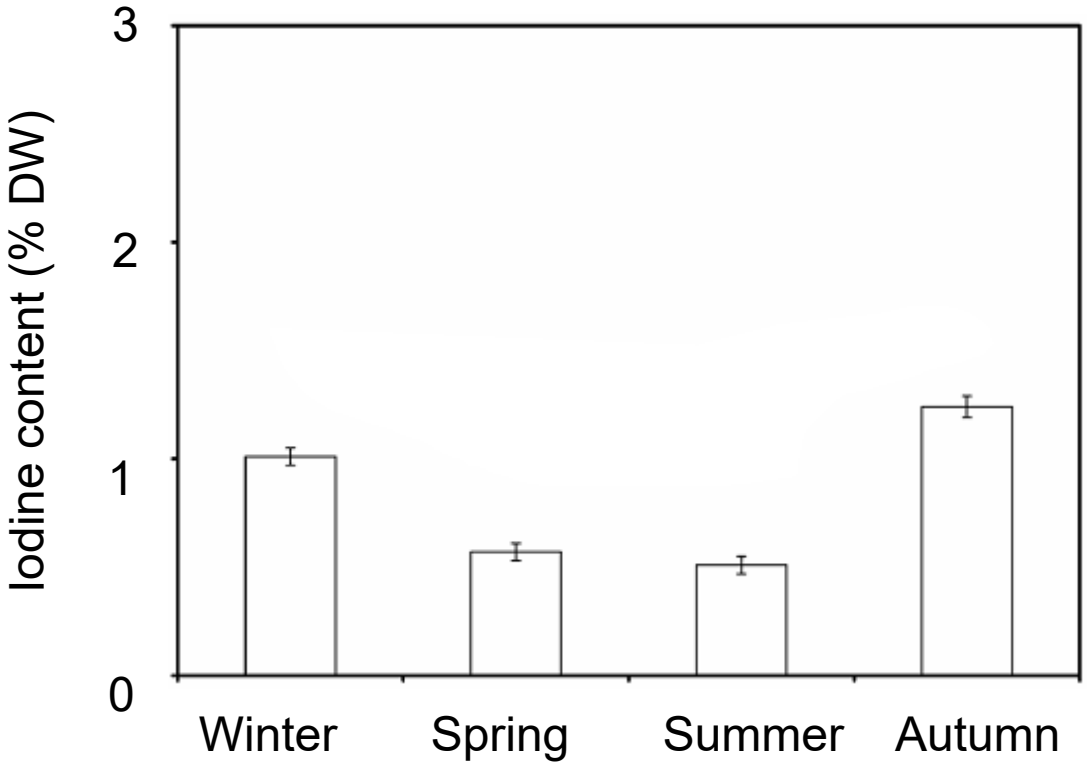
- Requirement of an intact cell wall (apoplast)
- Role of hydrogen peroxide and haloperoxidases in iodine uptake
- Iodine efflux upon oxidative stress



Küpper, F.C.; Schweigert, N.; ArGall, E.; Legendre, J.M.; Vilter, H.; Kloareg, B., 1998: Iodine uptake in Laminariales involves extracellular, haloperoxidase-mediated oxidation of iodide. *Planta* **207**, 163-171

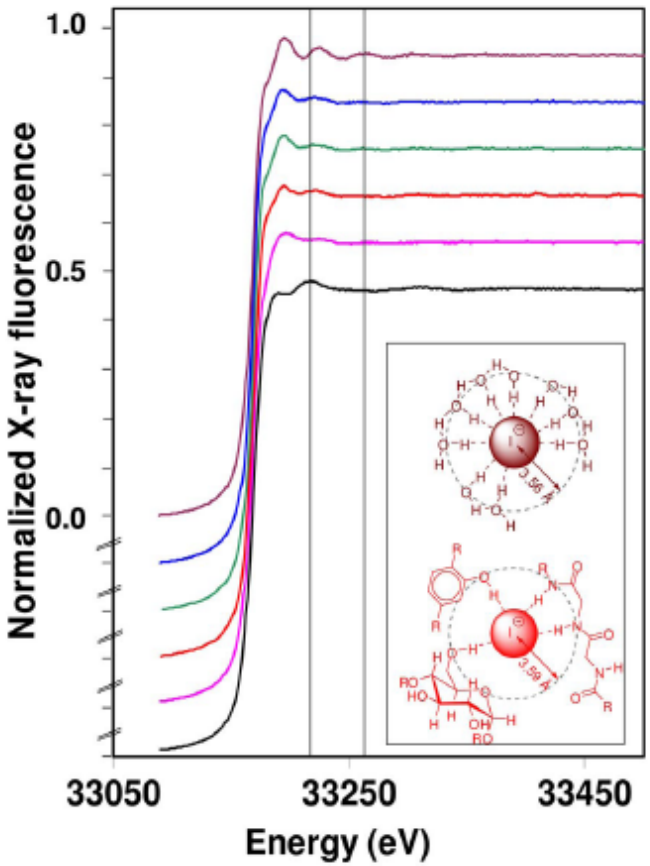
Iodine accumulation in *Laminaria*

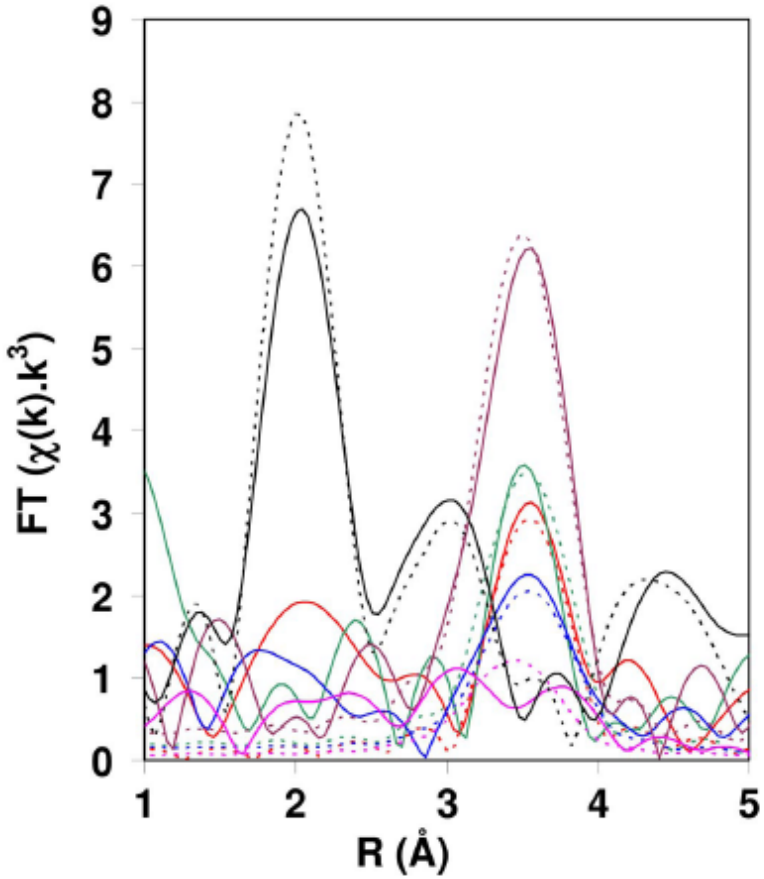
- Strong seasonality!



ArGall, E.; Küpper, F.C.; Kloareg, B., 2004: A survey of iodine contents in *Laminaria digitata*. *Botanica Marina* **47**, 30-37.

Iodine XAS of *Laminaria* tissues





(Iodine K-edge)

- **Iodide (I^-) is the accumulated form of iodine in *Laminaria*!**

Iodine metabolism and oxidative stress

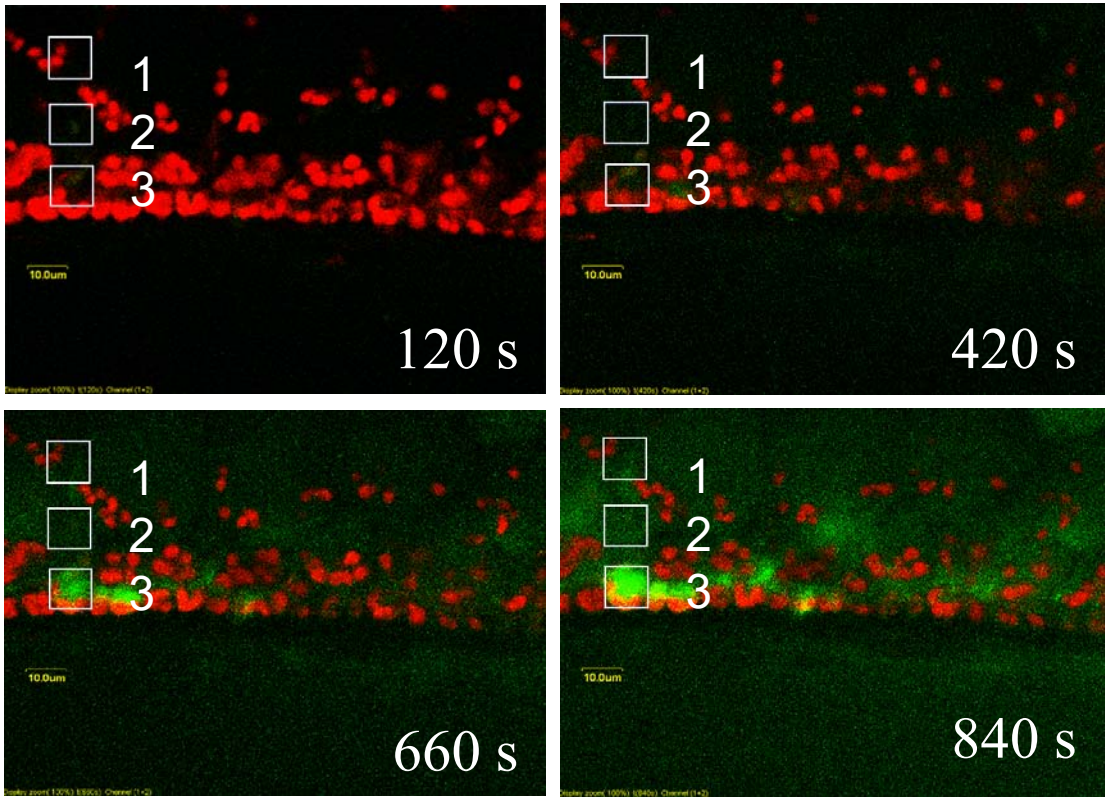
- Iodine **uptake** requires low H_2O_2 levels (< 25 μM)
- Higher concentrations of H_2O_2 result in iodine **efflux**

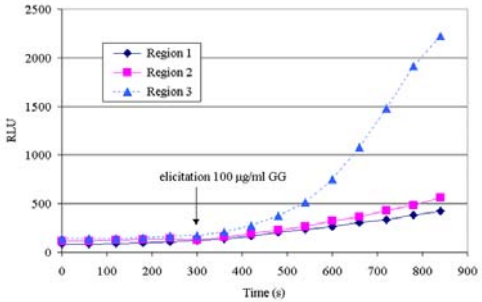
Oxidative stress in *Laminaria*:

- Oxidative (respiratory) burst – a defense reaction
- Desiccation, high temperatures, high irradiance and exposure to atmospheric oxidants at low tide

The oxidative burst in *Laminaria*

- A key element in eukaryotic innate immunity
- Triggers in *Laminaria*: bacterial endotoxins (LPS), oligoguluronates (oligoalginates)





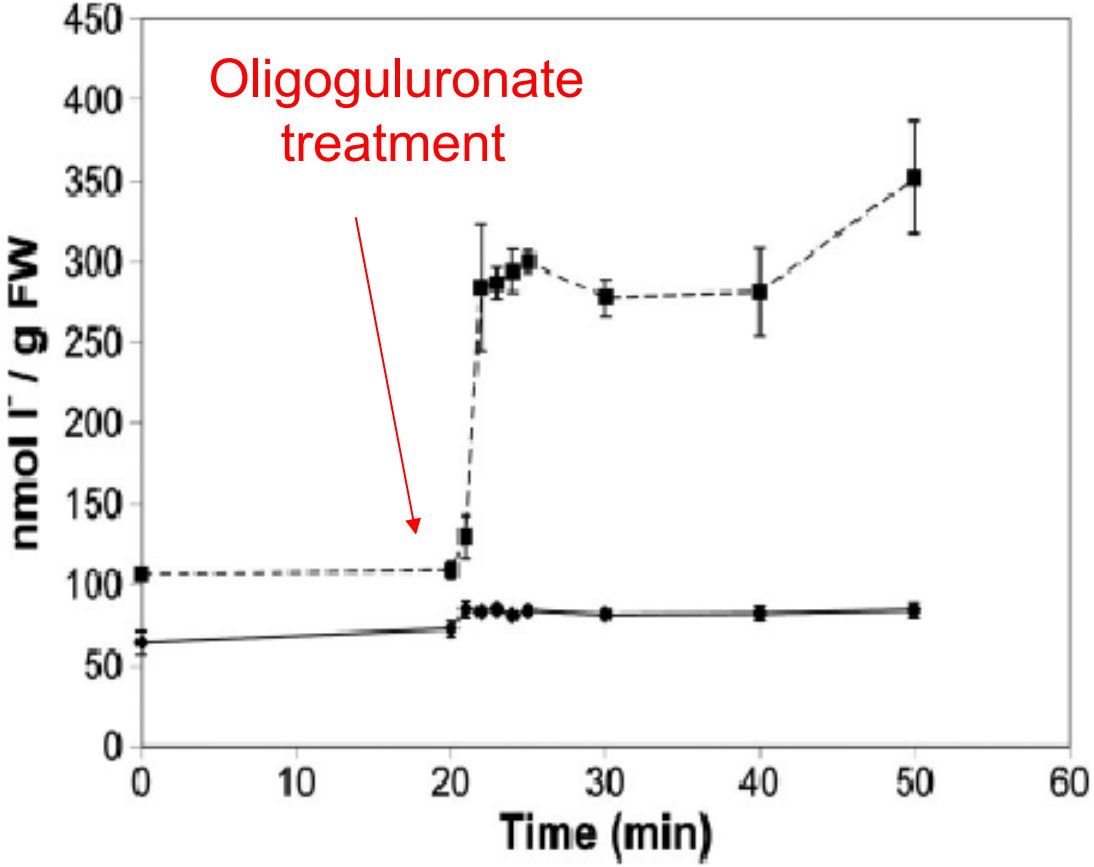
DCFH-DA : dichlorohydro-fluorescein diacetate

Küpper *et al*., 2001: Plant Physiology **125**, 278-91

Monitoring the iodine pool during the oxidative burst in *Laminaria* with XAS

- EXAFS: Oxidative stress results in a change of the solution environment of accumulated iodide (towards an aqueous, hydrated form)
- XANES: No changes in the redox state of iodine – only iodide is detectable

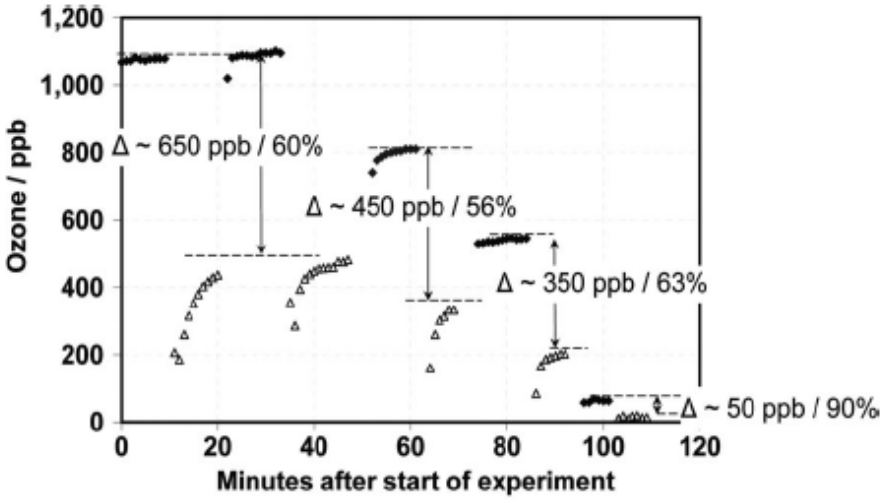
Cathodic stripping square wave voltammetry (CSSWV)

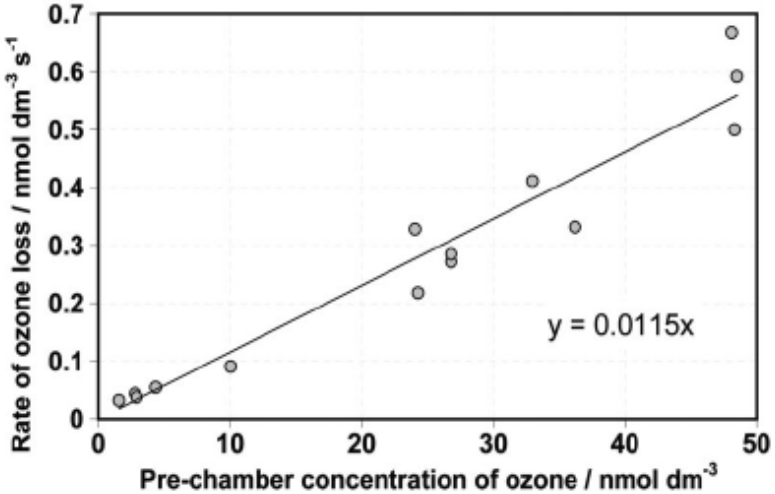


➢ **Strong iodide efflux upon oxidative stress**

• No increased levels of oxidized or organic iodine species

Scavenging of ozone (O_3) by *Laminaria*





- ***Laminaria*** **thalli effectively scavenge ozone**

- **When light is present: ultrafine particle formation**

Kelp iodine emissions into the coastal atmosphere

- "Iodovolatilisation" discovered in 1920s by Kylin and Dangeard: I_2 detected with starch paper
- J. Lovelock, 1973: Discovery of methyl iodide emissions from seaweeds
- B. Alicke et al., 1999: High IO levels above kelp beds at low tide
- L.J. Carpenter *et al*., 2000: CH_2I_2 main species emitted by *Laminaria*
  (total iodine emissions: 0.09 – 0.5 pmol g FW^{-1} min^{-1})
- This study: High I_2 fluxes due to reaction of O_3 with I^- on seaweed surface (130 pmol g FW^{-1} min^{-1})

Biological significance: Iodine accumulation in *Laminaria*

- ***Laminaria* accumulates iodide as an inorganic antioxidant: *the first case in a living system!***
- Other, previous hypotheses: chemical defense (grazers, pathogens)
- Implications for atmospheric & marine chemistry

Biological significance: Iodine accumulation in *Laminaria*

- Iodo(hydro)carbons: quantitatively insignificant as H_2O_2 scavenging products
- Must have another function – defense?!
- Iodine is a better leaving group than bromine or chlorine:
  I > Br > Cl

=> High reactivity / strong alkylating potential of iodinated compounds

What makes iodide a suitable antioxidant?

- Reactions with major oxidants (H_2O_2, O_2^-, $OH^\cdot$, $^1O_2$, O_3) are very favourable kinetically and thermodynamically
- Iodide compares well with established, organic biological antioxidants
- Iodide is the only halide with potential antioxidant properties
- Confirmed in a heterologous system: iodide quenches respiratory burst in human blood (IC_{50} = 2.9 mM)

Iodine accumulation in *Laminaria*

Küpper FC, Carpenter LJ, McFiggans GB, Palmer CJ, Waite T, Boneberg E-M, Woitsch S, Weiller M, Abela R, Grolimund D, Potin P, Butler A, Luther III GW, Kroneck PMH, Meyer-Klaucke W, Feiters MC, 2008:

Iodide accumulation provides kelp with an inorganic antioxidant impacting atmospheric chemistry.-

Proceedings of the National Academy of Sciences of the USA **105** (19), 5954-58

Acknowledgements

- Alison Butler (UC Santa Barbara)

- David H. Green (SAMS)
- Kathy Barbeau (formerly UCSB, now Scripps Institution of Oceanography)

- Wes Harris (University of Missouri, St. Louis)
- Carl J. Carrano (San Diego State University)

- Shady Amin (SDSU)

- Martina Strittmatter (SDSU & SAMS)

Acknowledgements

-- Peter M.H. Kroneck (University of Konstanz, Germany)
-- Sonja Woitsch, Markus Weiller (dto.)

-- Lucy J. Carpenter and Carl Palmer (University of York, UK)

-- Gordon McFiggans (University of Manchester, UK)

-- Bernard Kloareg and Philippe Potin (CNRS, Roscoff, France)

-- Martin Feiters (University of Nijmegen, The Netherlands)

-- Wolfram Meyer-Klaucke and Gerd Wellenreuther (European Molecular Biology Laboratory, Hamburg, Germany)

-- Eva M. Boneberg (Biotechnologie Institut Thurgau, Switzerland, formerly at University of Konstanz)

-- Alison Butler (University of California, Santa Barbara, USA)

-- George Luther & Tim Waite (University of Delaware, Lewes, USA)

-- Rafael Abela & Daniel Grolimund (Swiss Light Source, Paul Scherrer Institute, Villigen, Switzerland)