Marine Inorganic Biochemistry: From Photoreactive Siderophores to Iodide in Kelp

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shortened and updated by Hendrik Küpper

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

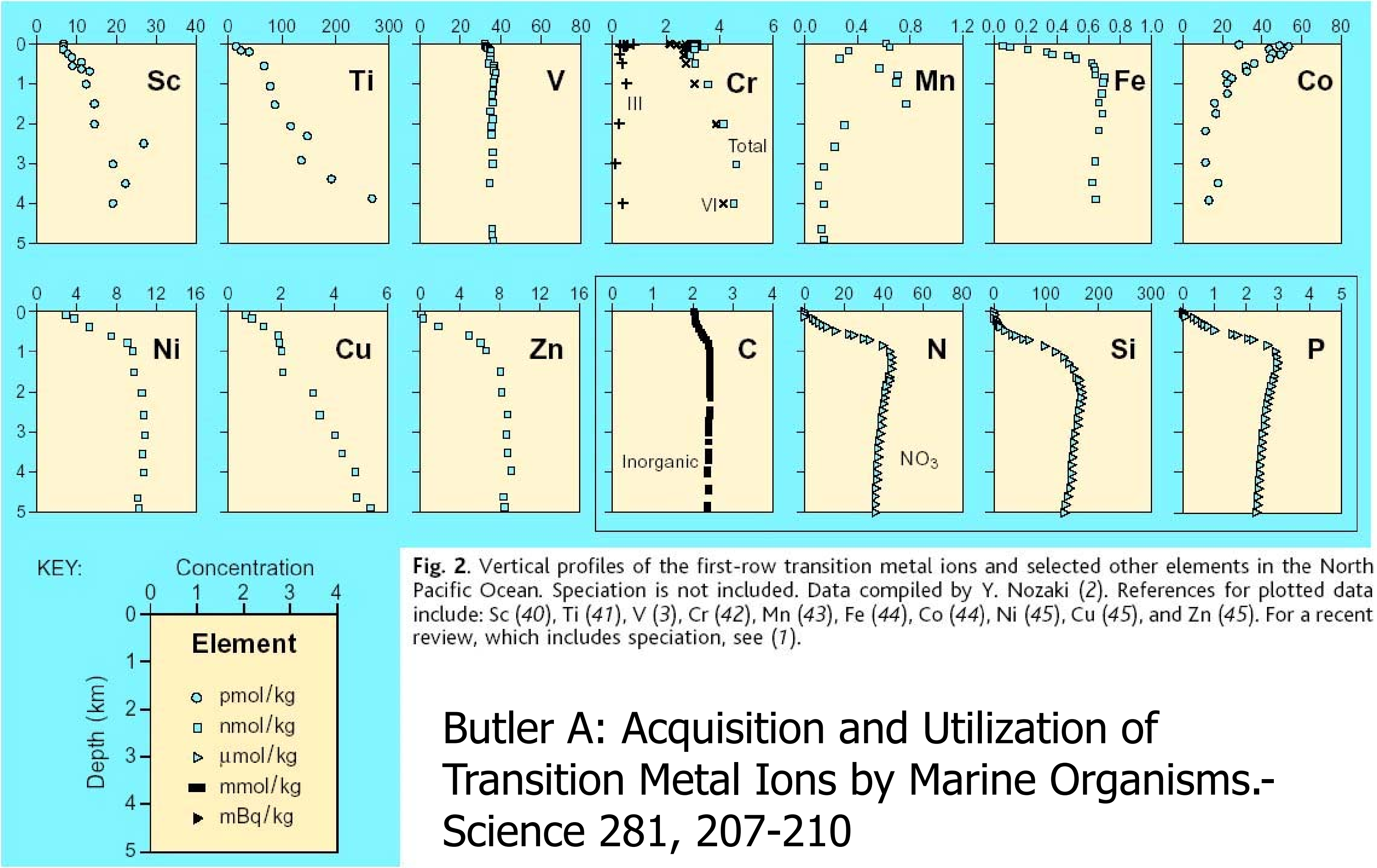
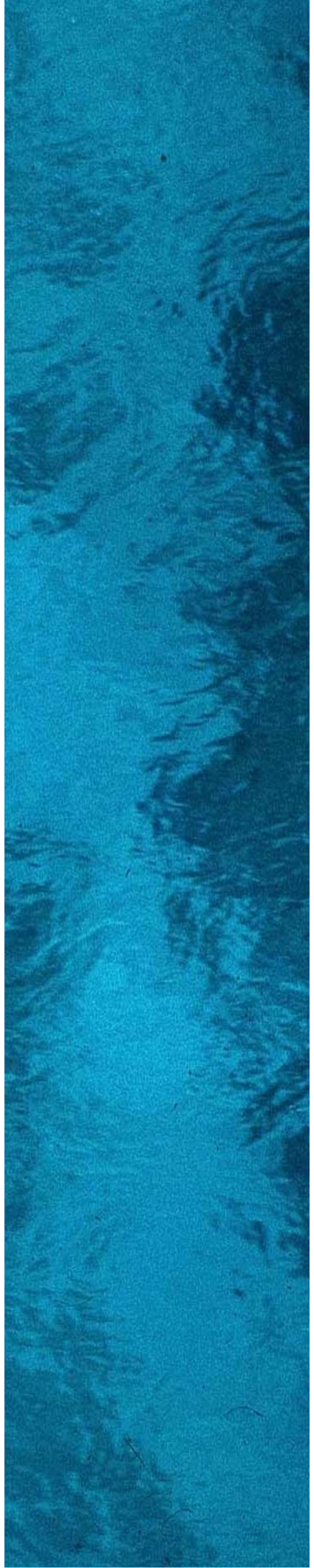


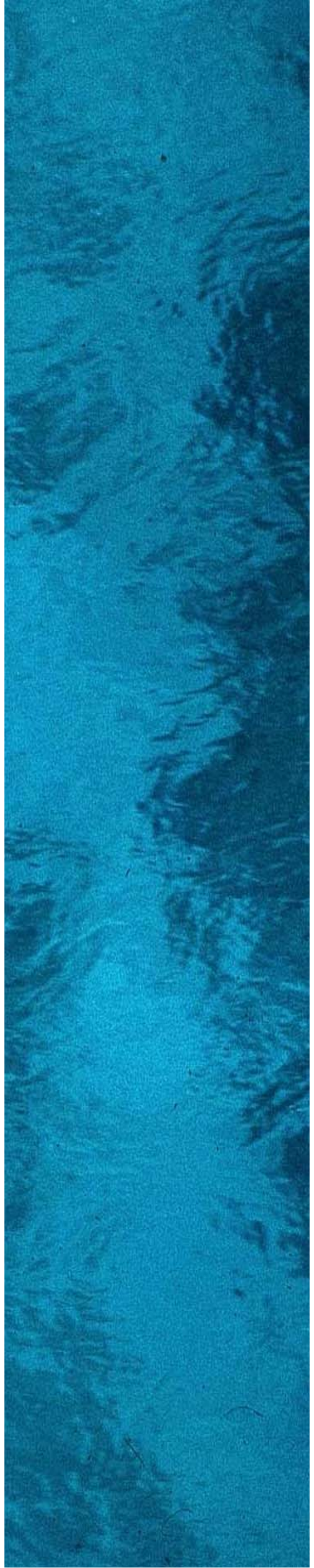
Fig. 2. Vertical profiles of the first-row transition metal ions and selected other elements in the North Pacific Ocean. Speciation is not included. Data compiled by Y. Nozaki (*2*). References for plotted data include: Sc (*40*), Ti (*41*), V (*3*), Cr (*42*), Mn (*43*), Fe (*44*), Co (*44*), Ni (*45*), 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, and Cu are much more abundant than iron

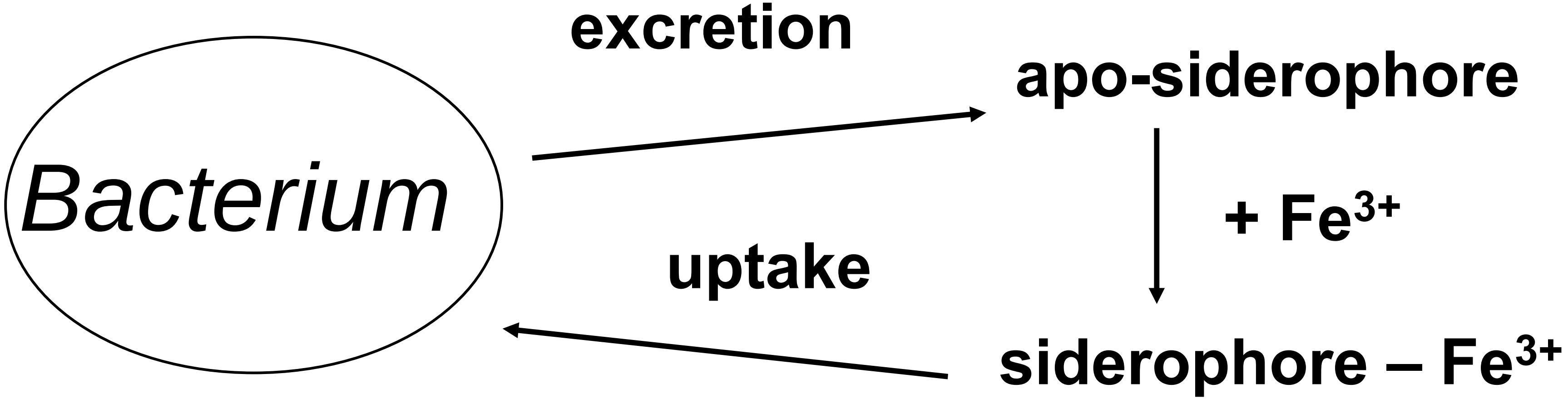


Strategies of marine organisms to cope with low iron availability

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

Siderophore-mediated metal uptake

Siderophore, *Greek*: “iron carrier”



excretion

apo-siderophore

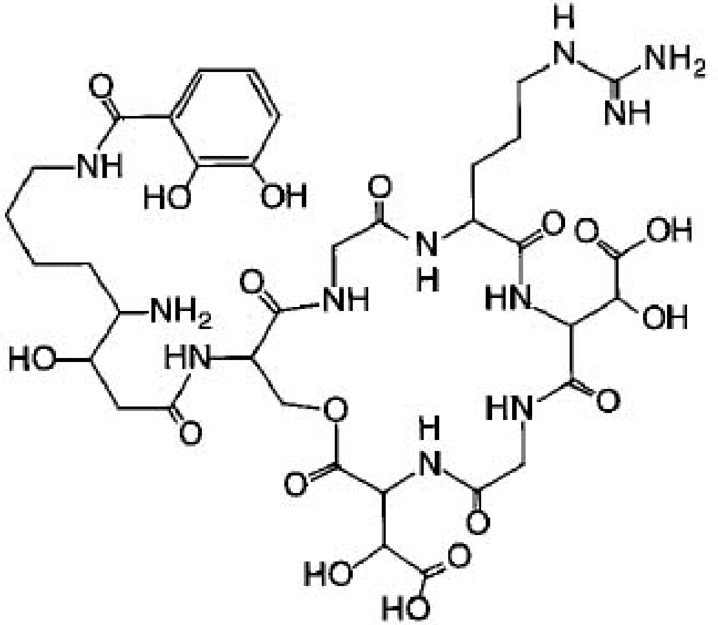
Bacterium

+ Fe^{3+}

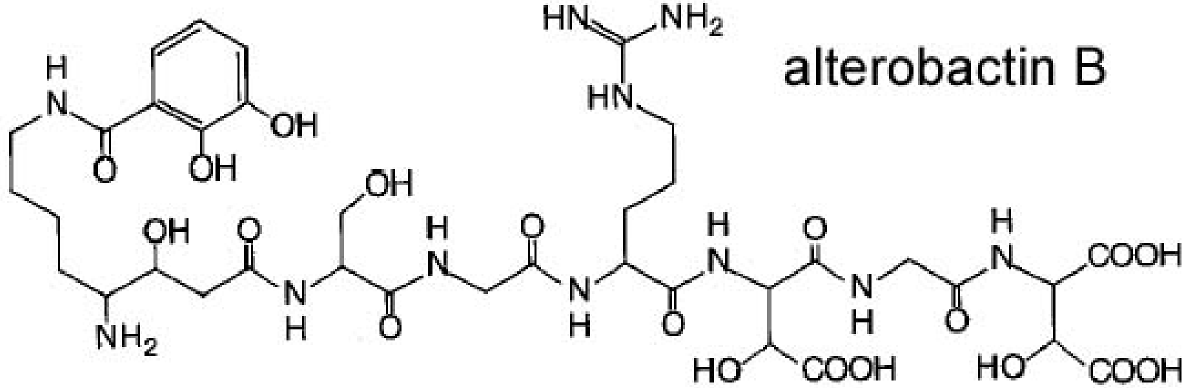
uptake

siderophore – Fe^{3+}

Marine siderophores

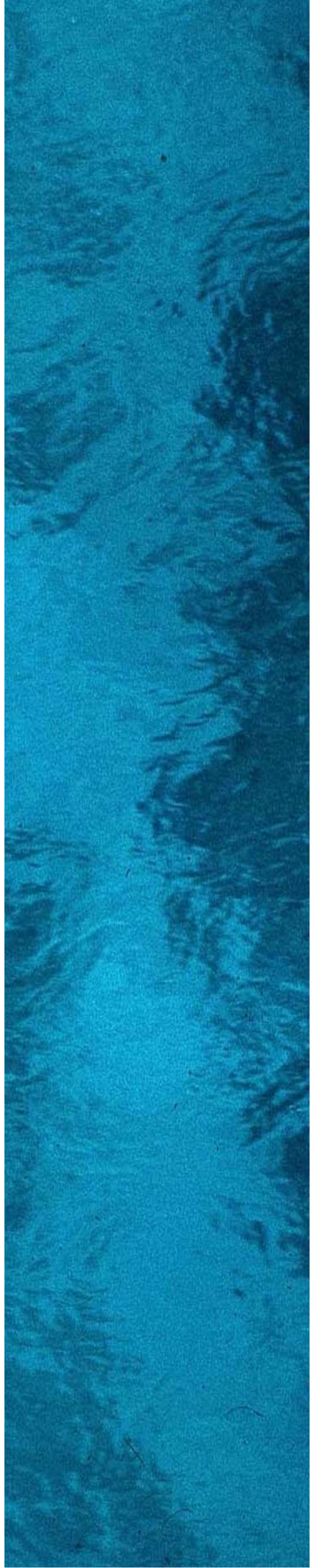


alterobactin A



alterobactin B

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



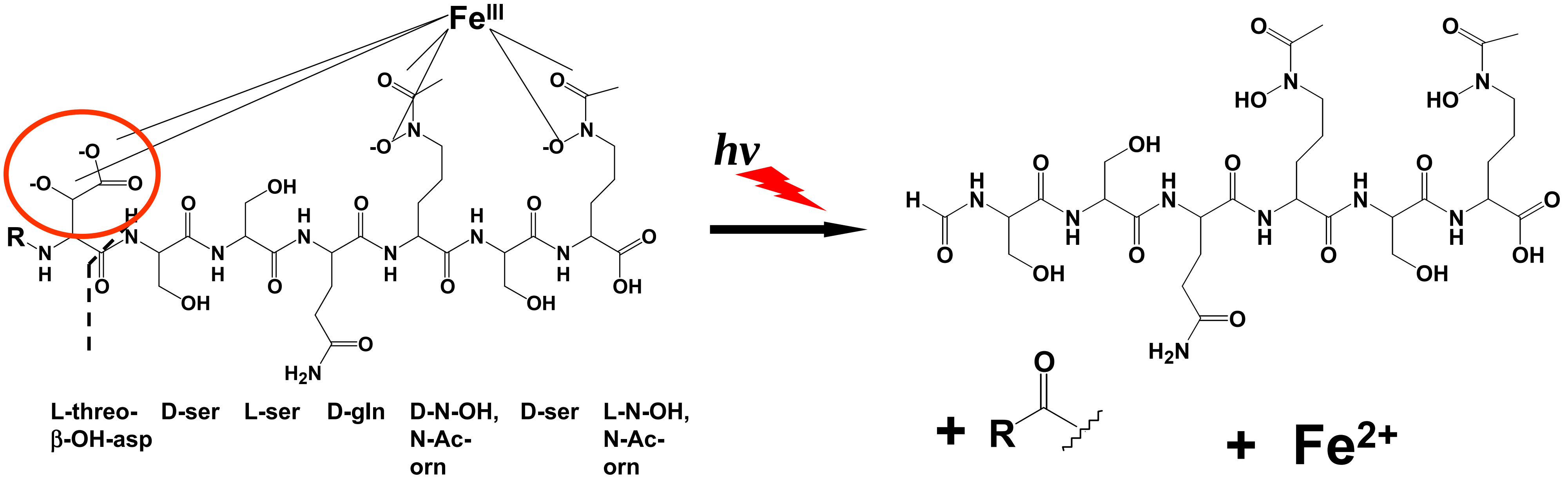
Marine siderophores

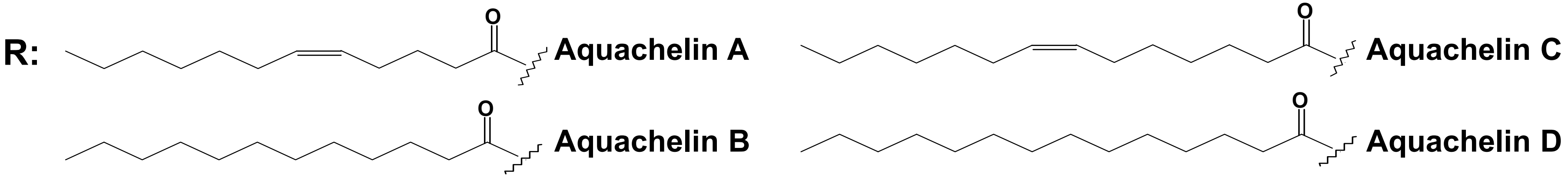
- Some well-known structures (e.g. aerobactin), but mostly many novelties
- Mediate prokaryotic Fe / metal uptake in marine systems: availability to eukaryotes not well established yet (e.g. in algal-bacterial symbioses)
- Most eukaryotic algae seem to have plasma membrane-bound ferrireductases
- Several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

Photolysis of aquachelin

Fe(III)-Aquachelin complex

Photolysis products





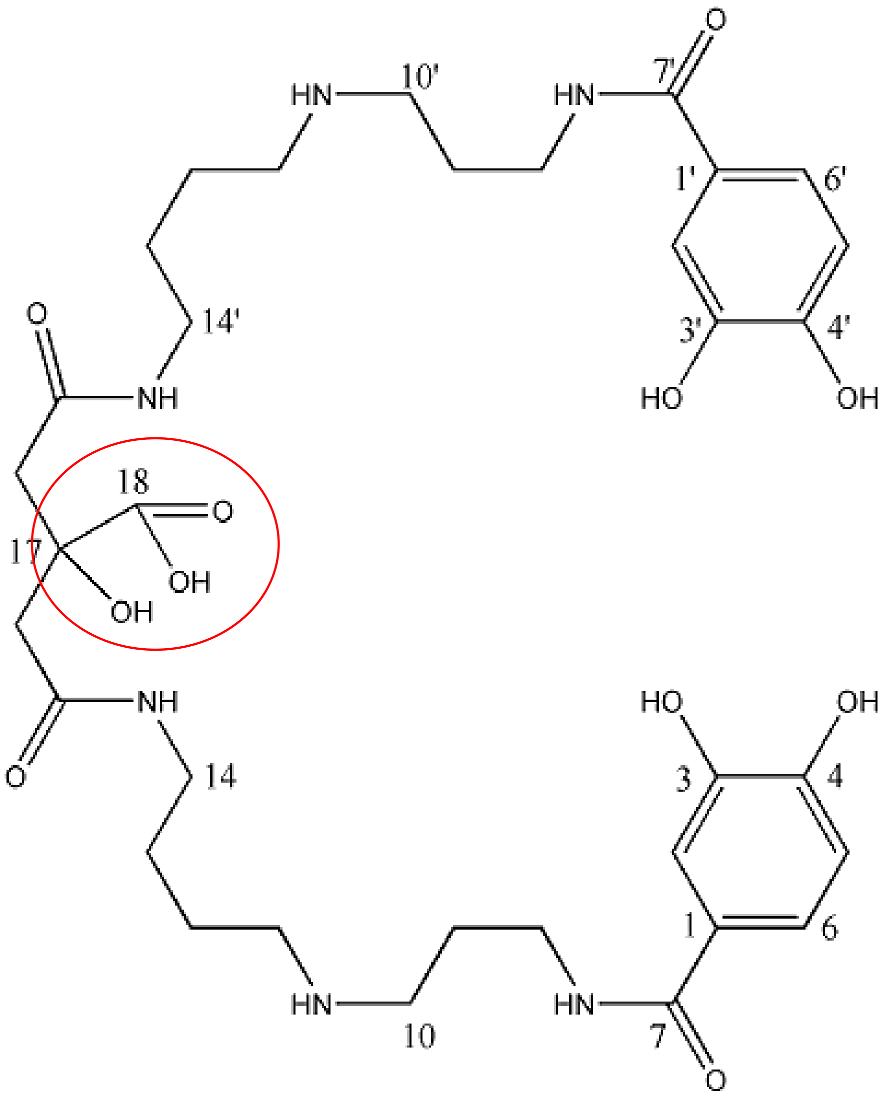
$K_{cond}^{Fe(III)'} = 10^{12.2}\ M^{-1}$

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

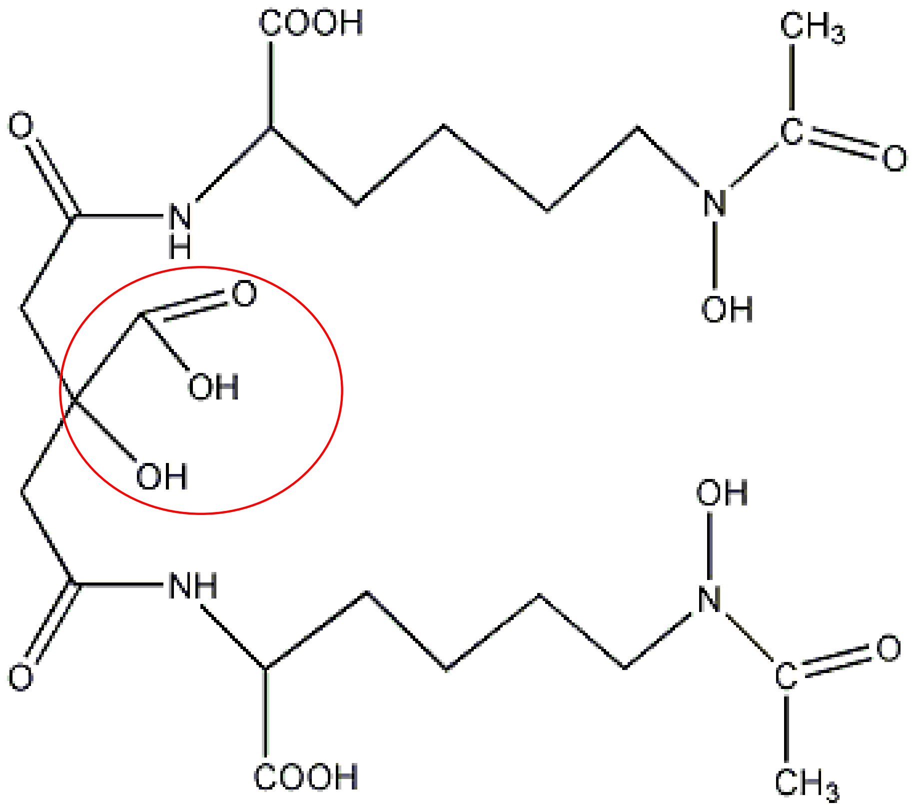
Barbeau, Rue, Bruland & Butler, 2001: Nature 413, 409-13

Other cases of photoreactive siderophores

Petrobactin



Aerobactin



Common feature:

α-hydroxy carboxylic acid moiety

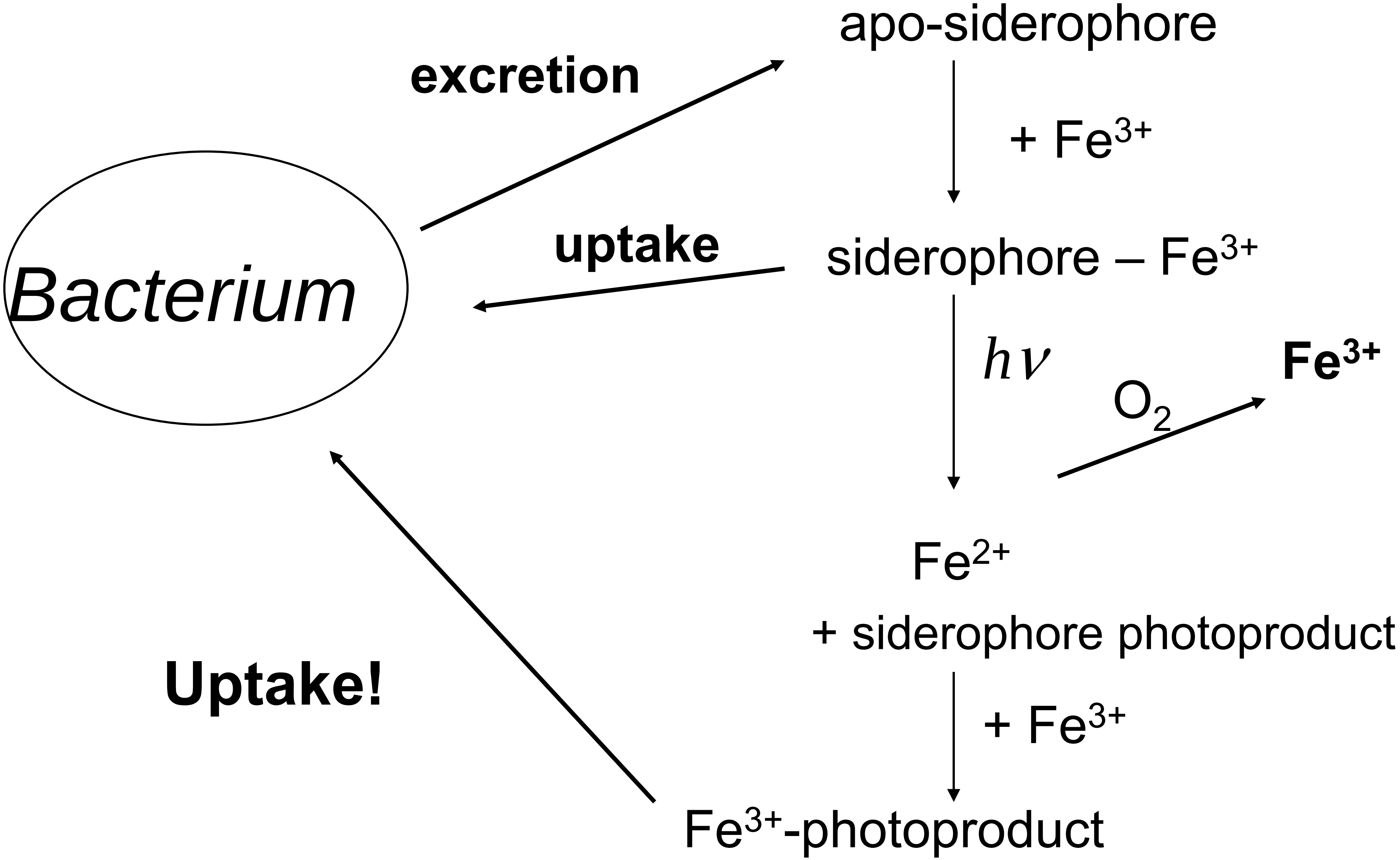
Barbeau *et al*., 2002: JACS 413, 409–13
Bergeron *et al*., 2003: Tetrahedron 59, 2007-14

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



apo-siderophore

excretion

+ Fe^{3+}

uptake

siderophore – Fe^{3+}

Bacterium

$h\nu$

O_2

Fe^{3+}

Fe^{2+}

+ siderophore photoproduct

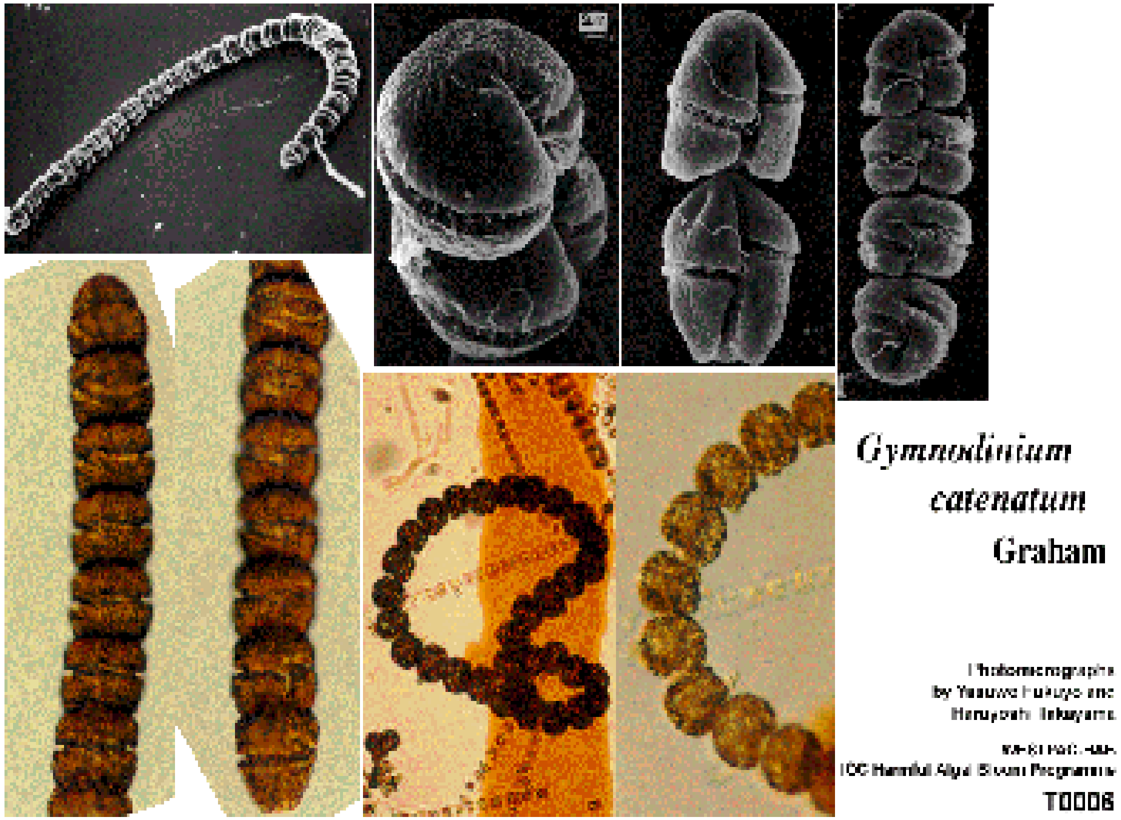
Uptake!

+ Fe^{3+}

Fe^{3+}-photoproduct

Algal-bacterial symbioses

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

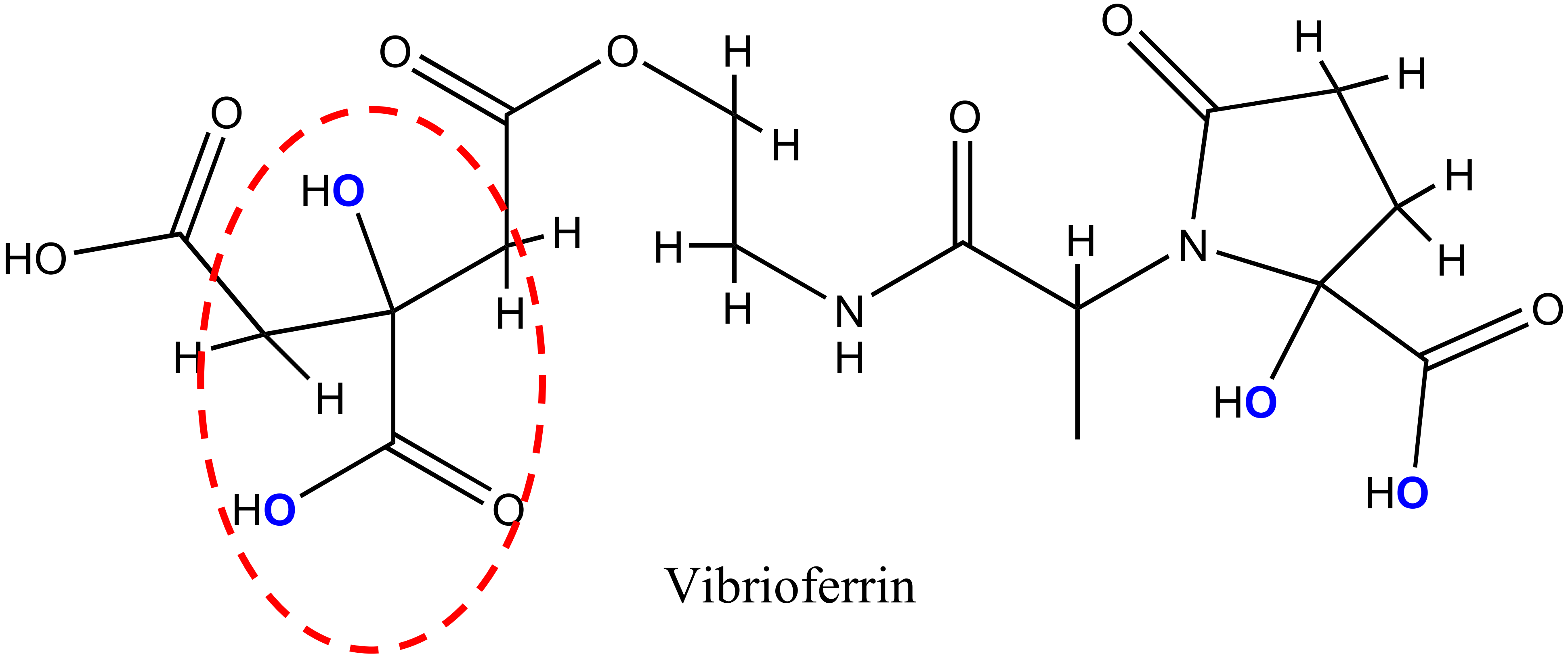


Gymnodinium catenatum Graham

Photomicrographs
by Yasuwo Fukuyo and
Haruyoshi Takayama

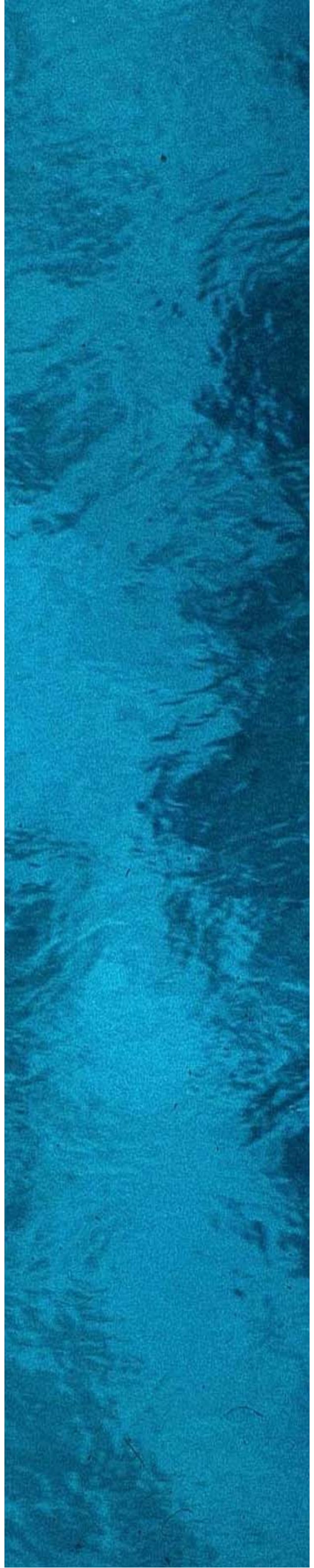
WESTPAC-HAB
IOC Harmful Algal Bloom Programme
T0006

Isolation of siderophores from culture media of bacterial symbionts



Vibrioferrin

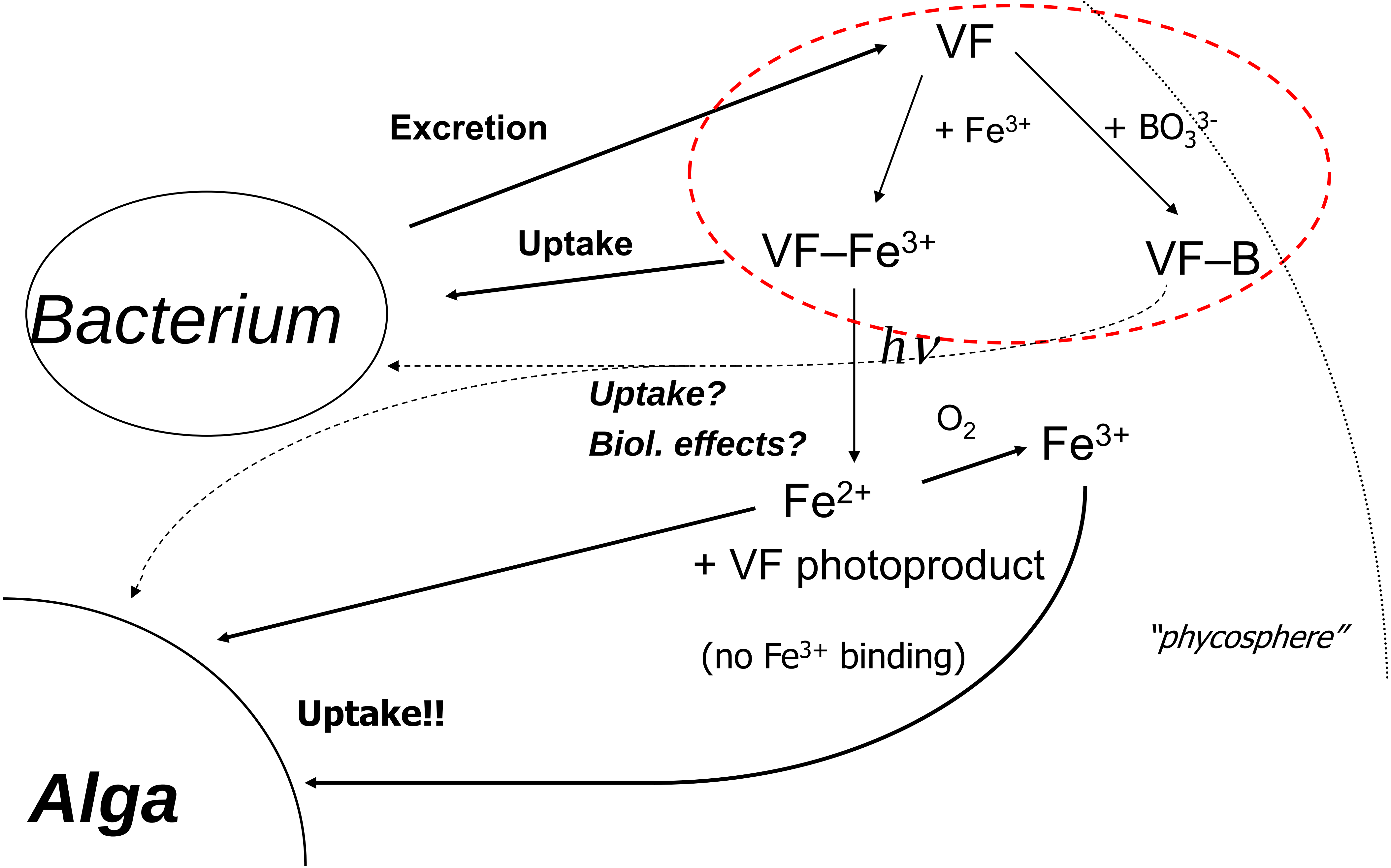
- Vibrioferrin



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)



VF

Excretion

+ Fe^{3+}

+ BO_3^{3-}

Uptake

Bacterium

$VF–Fe^{3+}$

VF–B

$h\nu$

Uptake?

Biol. effects?

O_2

Fe^{3+}

Fe^{2+}

+ VF photoproduct

(no Fe^{3+} binding)

"phycosphere"

Uptake!!

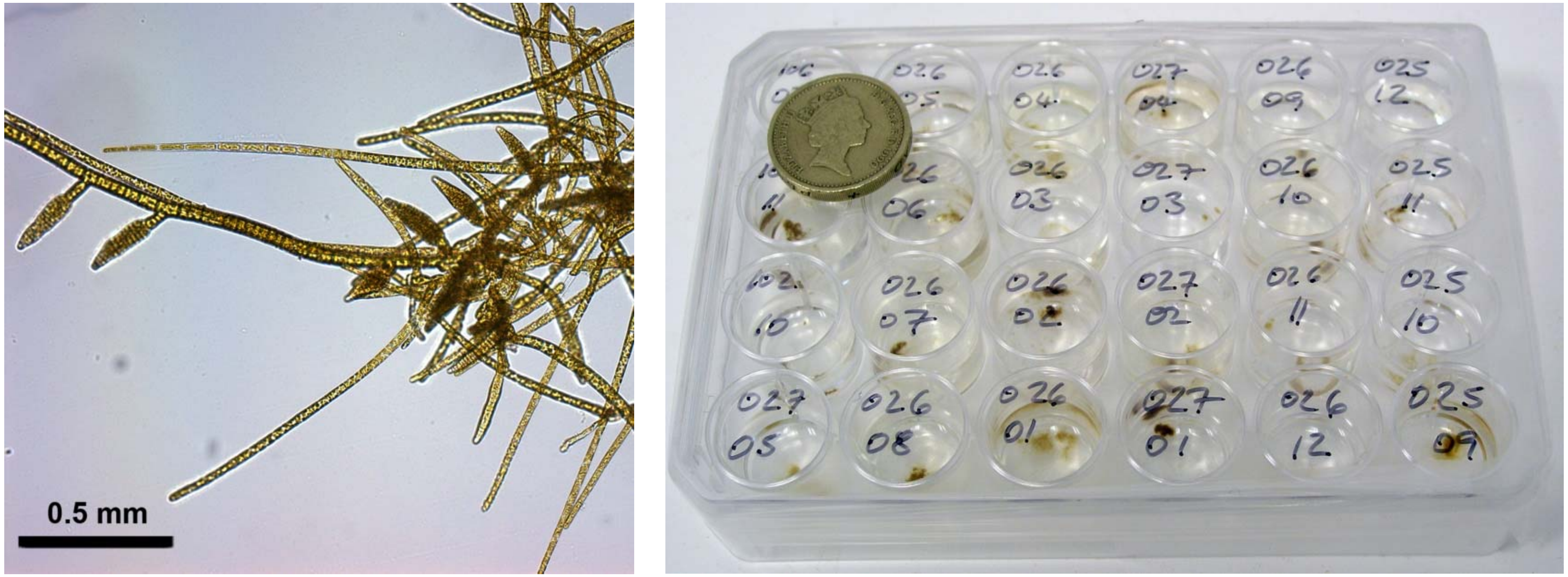
Alga

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

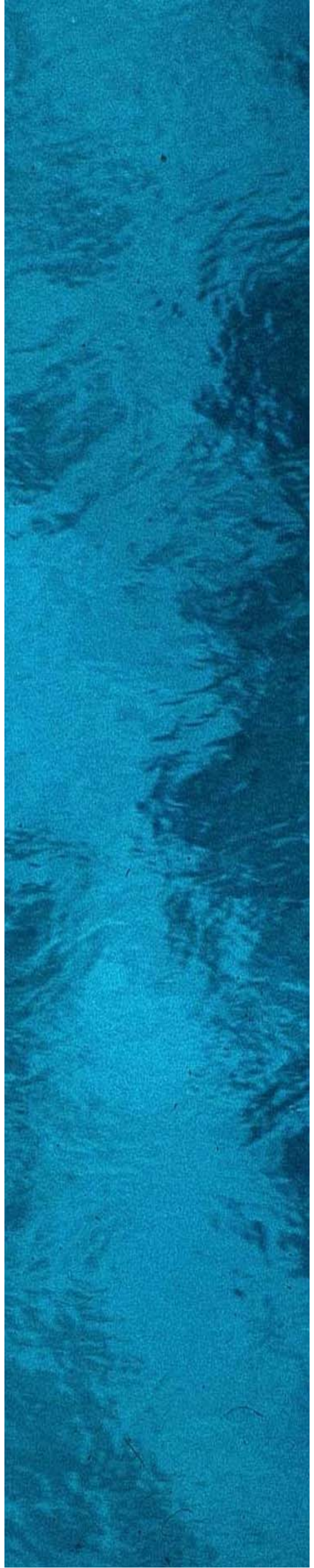
Amin SA, Green DH, Hart MC, Küpper FC, Sunda WG, Carrano CJ, 2009: Photolysis of iron–siderophore chelates promotes bacterial–algal mutualism.- Proceedings of the National Academy of Sciences of the USA 106(40), 17071–6

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

Ectocarpus siliculosus

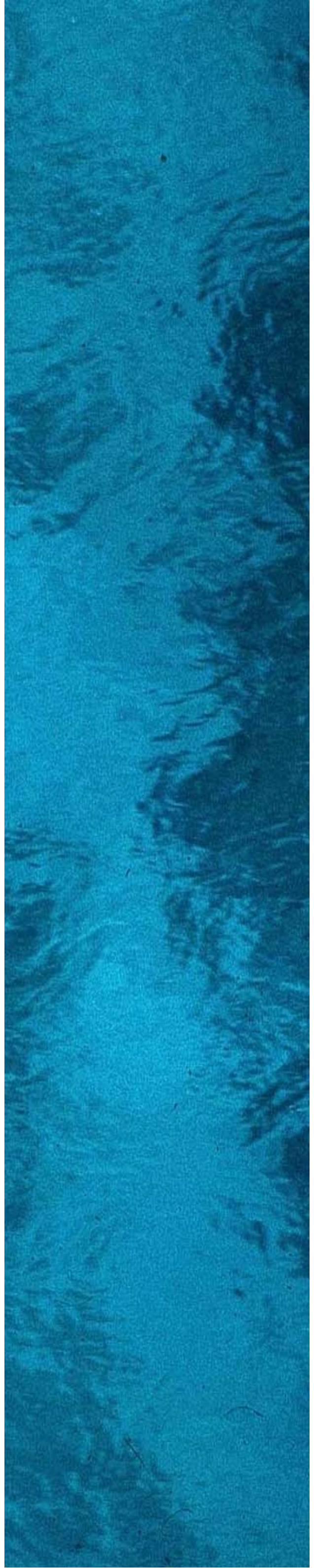


- Filamentous, cosmopolitan brown alga – mostly from temperate seas
- One of the best-studied seaweeds
- The first fully-sequenced multicellular alga! (Cock et al., Nature 465, 617-21)
- > 300 fully-characterized strains in public-domain culture collections (CCAP, KU-MACC)



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!

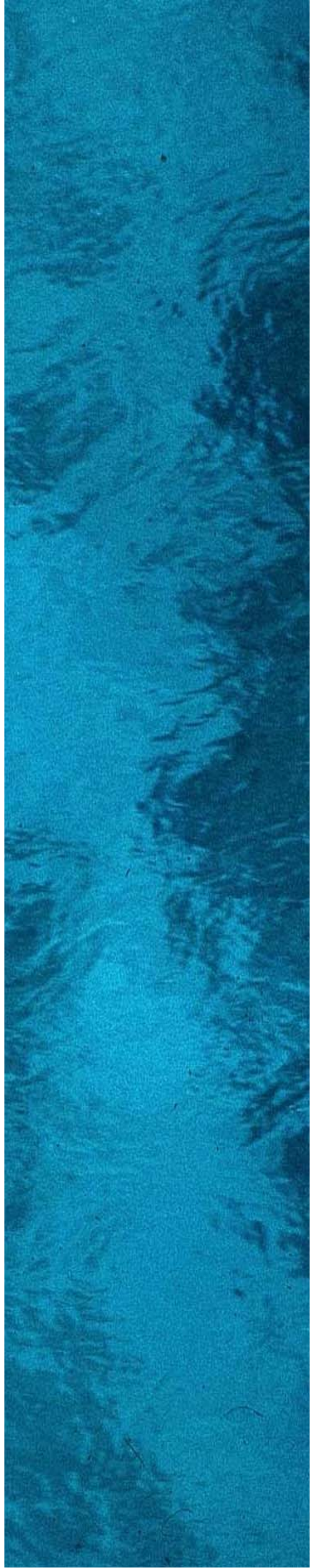


Features of the *Ectocarpus* genome from the bioinorganic perspective: Iron uptake

- 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))

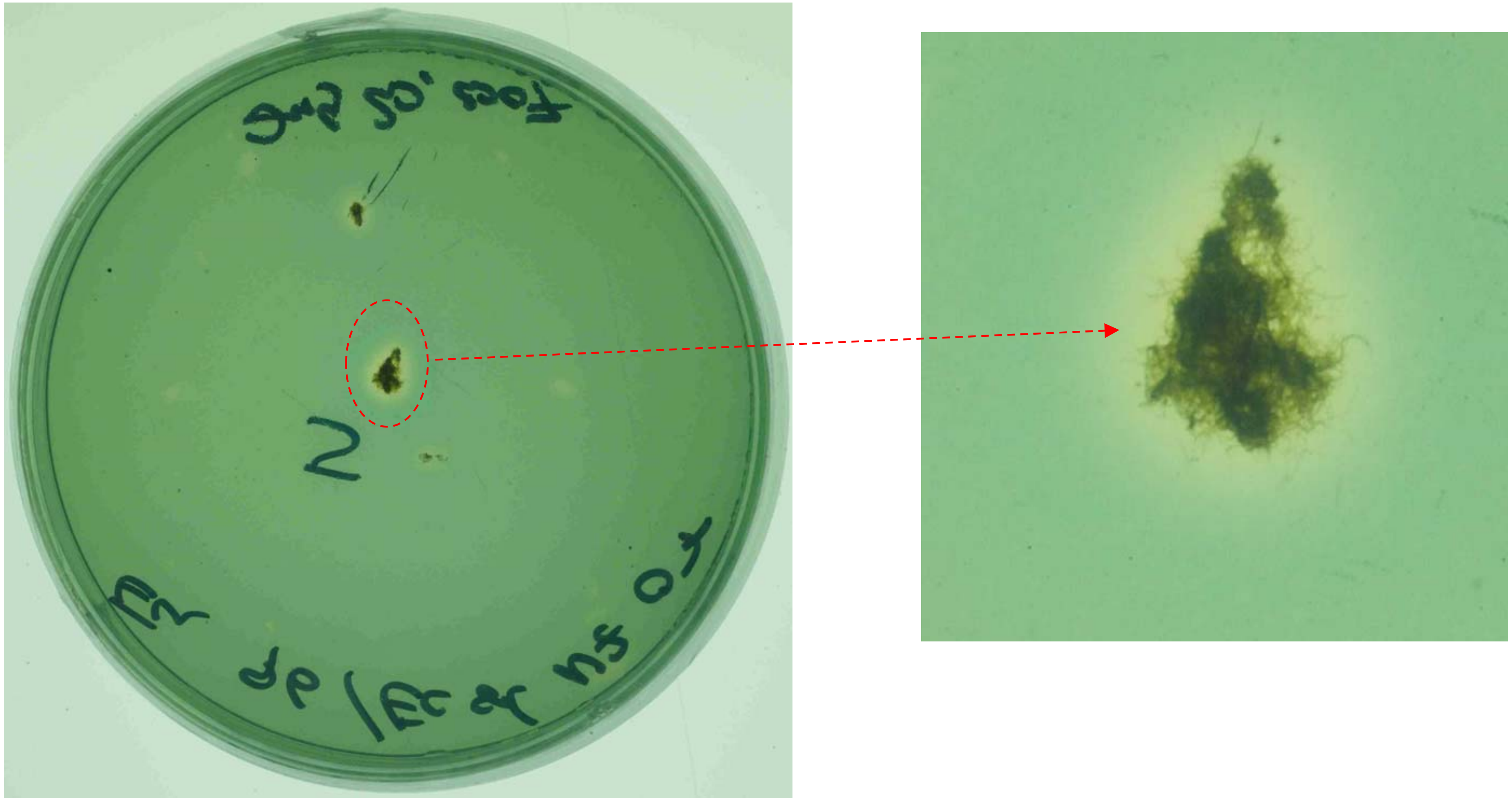
⇒ consistent with simple reductase/permease pathway

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



Ectocarpus and iron:

axenic cultures are CAS active!



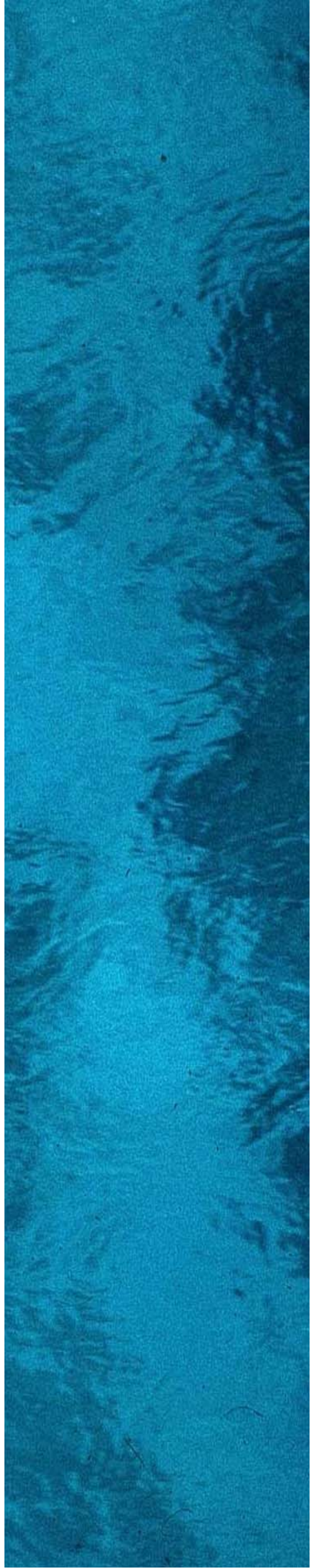
CAS = Chrome azurol S (a colorimetric assay for Fe^{3+}-binding activity)

- **CAS activity may be caused by secretion of strong Fe(III)-binding ligands – or (here) acidification as known from Fe uptake in plants!**

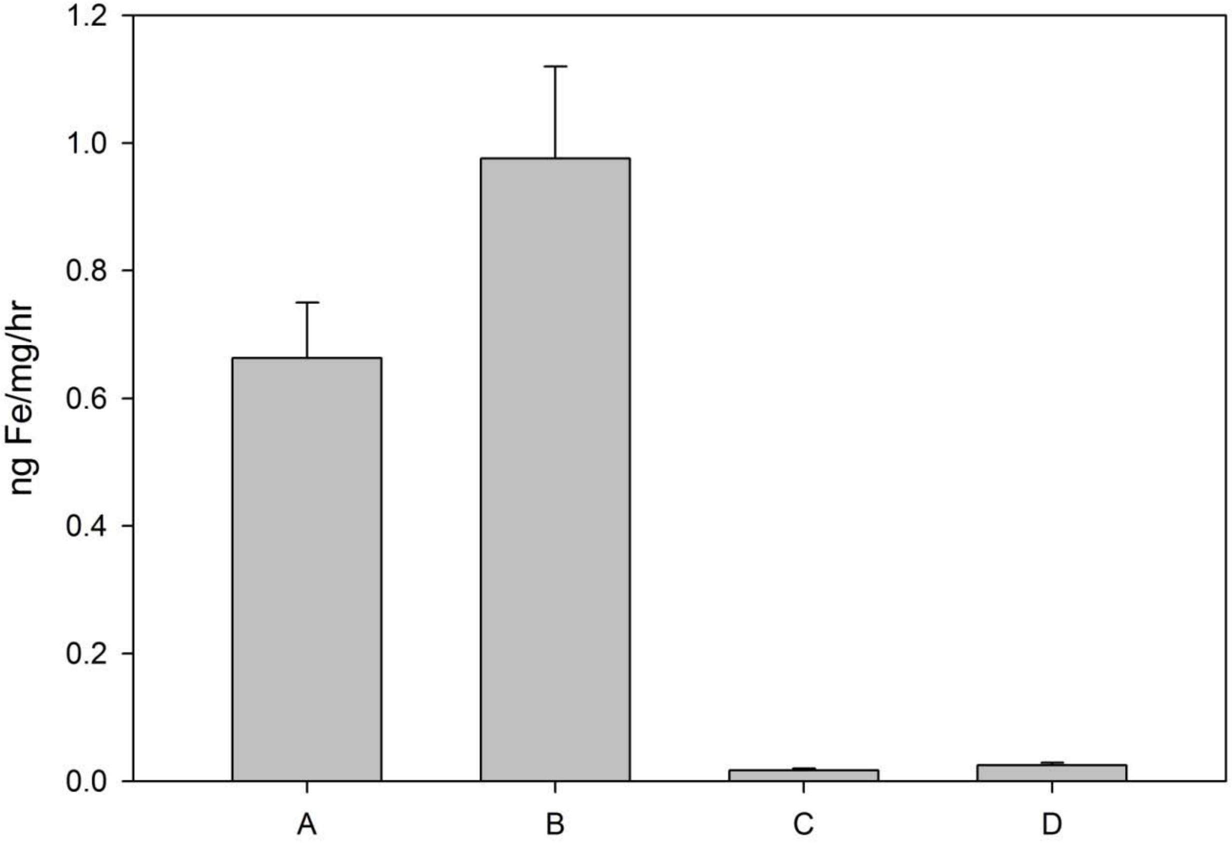
Iron uptake in *Ectocarpus*

- 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

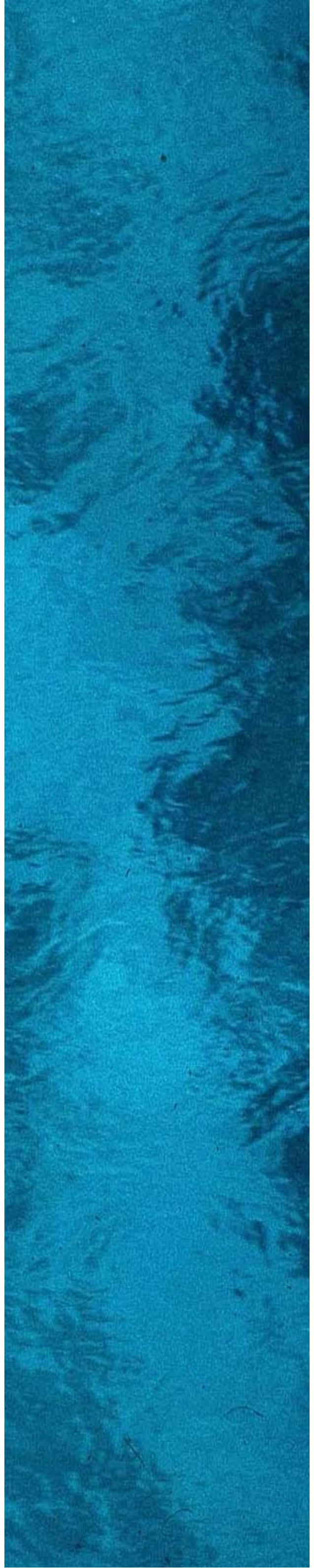
	Macrocystis	*Gracilaria*	*Laminaria*	*Undaria*	*Ectocarpus*
V_m	1.6 pmol/cm^2/hr	0.26 pmol/mg/hr	2.7 pmol/cm^2/hr	6.4 pmol/cm^2/hr	0.25 pmol/mg/hr
K_m	3.5 µM	0.6 µM	0.54 µM	6.4 µM	1.5 µM
Ref	Manley 1981	Liu 2000	Matsunaga 1991	Matsunaga 1991	This work



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



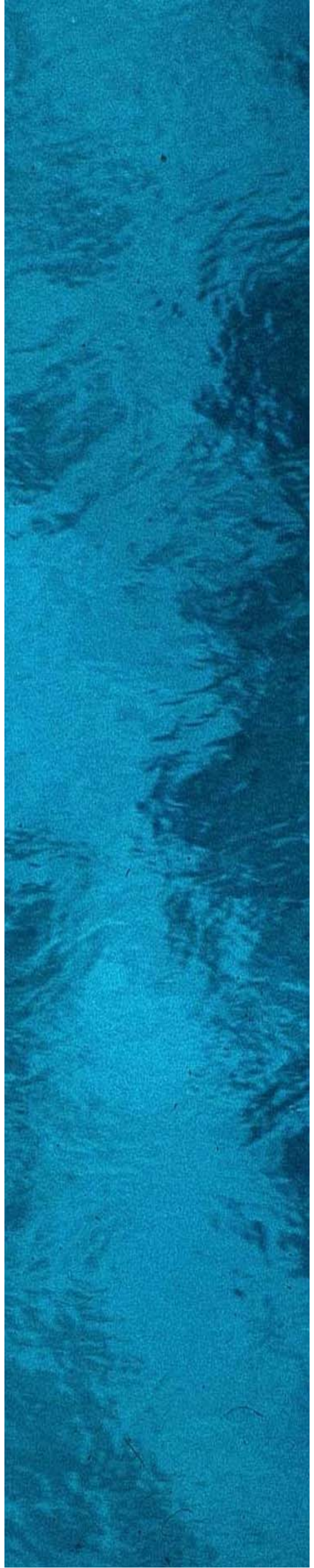
Features of the *Ectocarpus* genome from the bioinorganic perspective:
Iron storage

- **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)

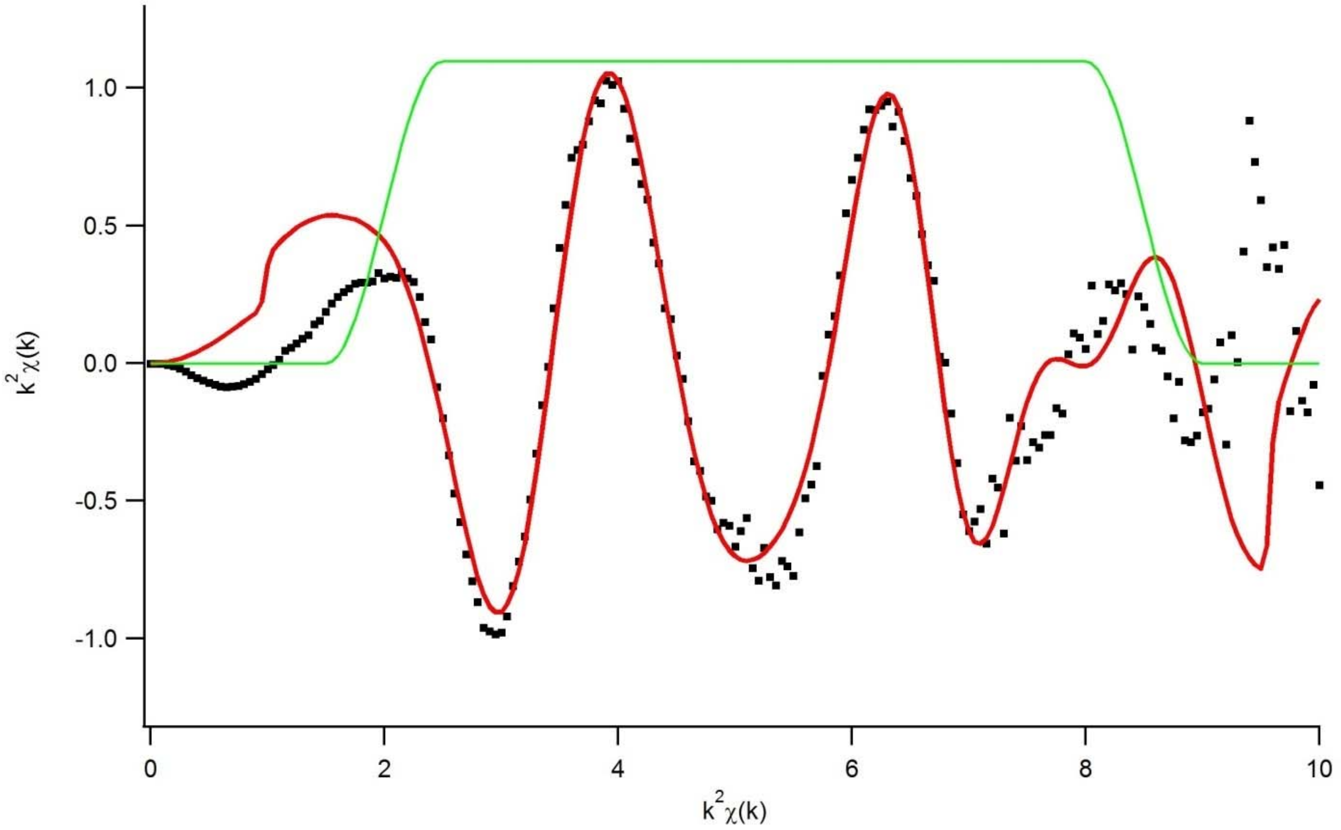
(2) a second component with spectra typical of a ($Fe^{3+}O_6$) system with parameters similar to the amorphous phosphorus-rich mineral core of bacterial and plant ferritins (approx. 74% of the cellular iron pool)

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

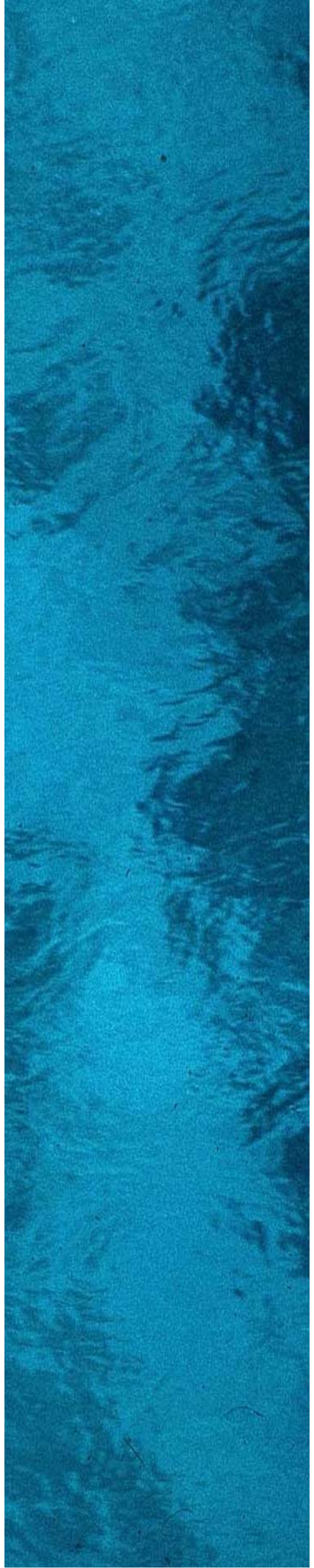
Brown algae and iron:

How to store iron without ferritin?

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



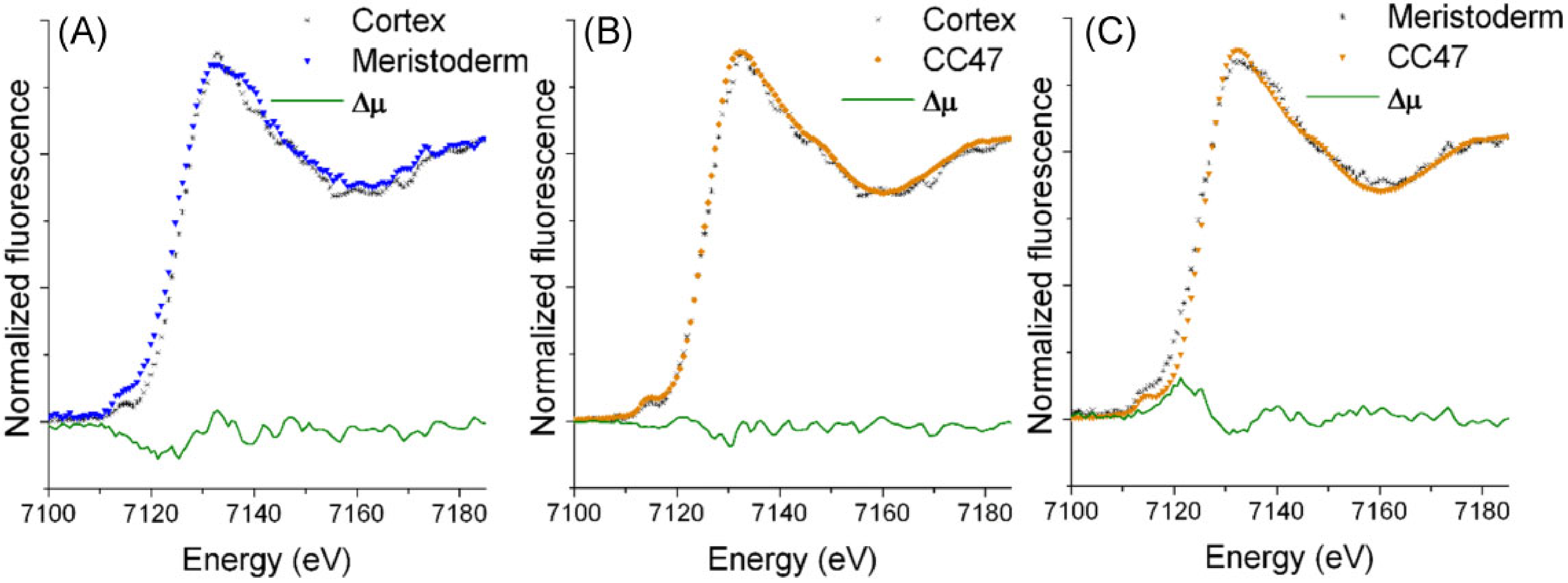
Extracted EXAFS spectrum of 52 merged spectra transformed in k-space. Black squares: experimental data; red line: fit with FeS-cluster and amorphous Fe phosphate. Green line: setting of the range.



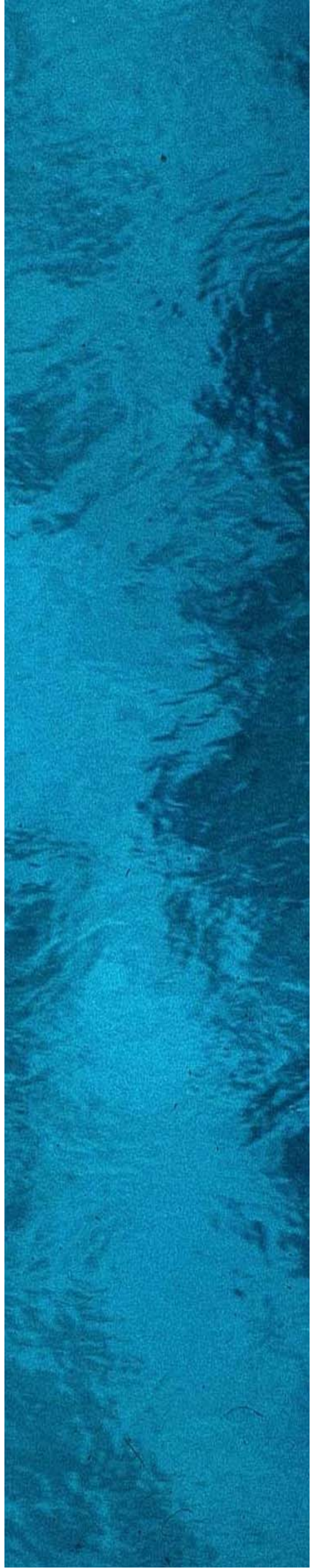
Brown algae and iron:

How to store iron without ferritin?

- **XANES spectra indicate that Fe in L. digitata is stored in a mineral non-ferritin core as shown by comparison with several models**



Mijovilovich A, Cloetens P, Lanzirotti A, Newville M, Wellenreuther G, Kumari P, Katsaros C, Carrano C, Küpper H, Küpper FC* (2023) Synchrotron X-rays reveal the modes of Fe binding and trace metal storage in the brown algae Laminaria digitata and Ectocarpus siliculosus. Metallomics 15 (10), mfad058, https://doi.org/10.1093/mtomcs/mfad058

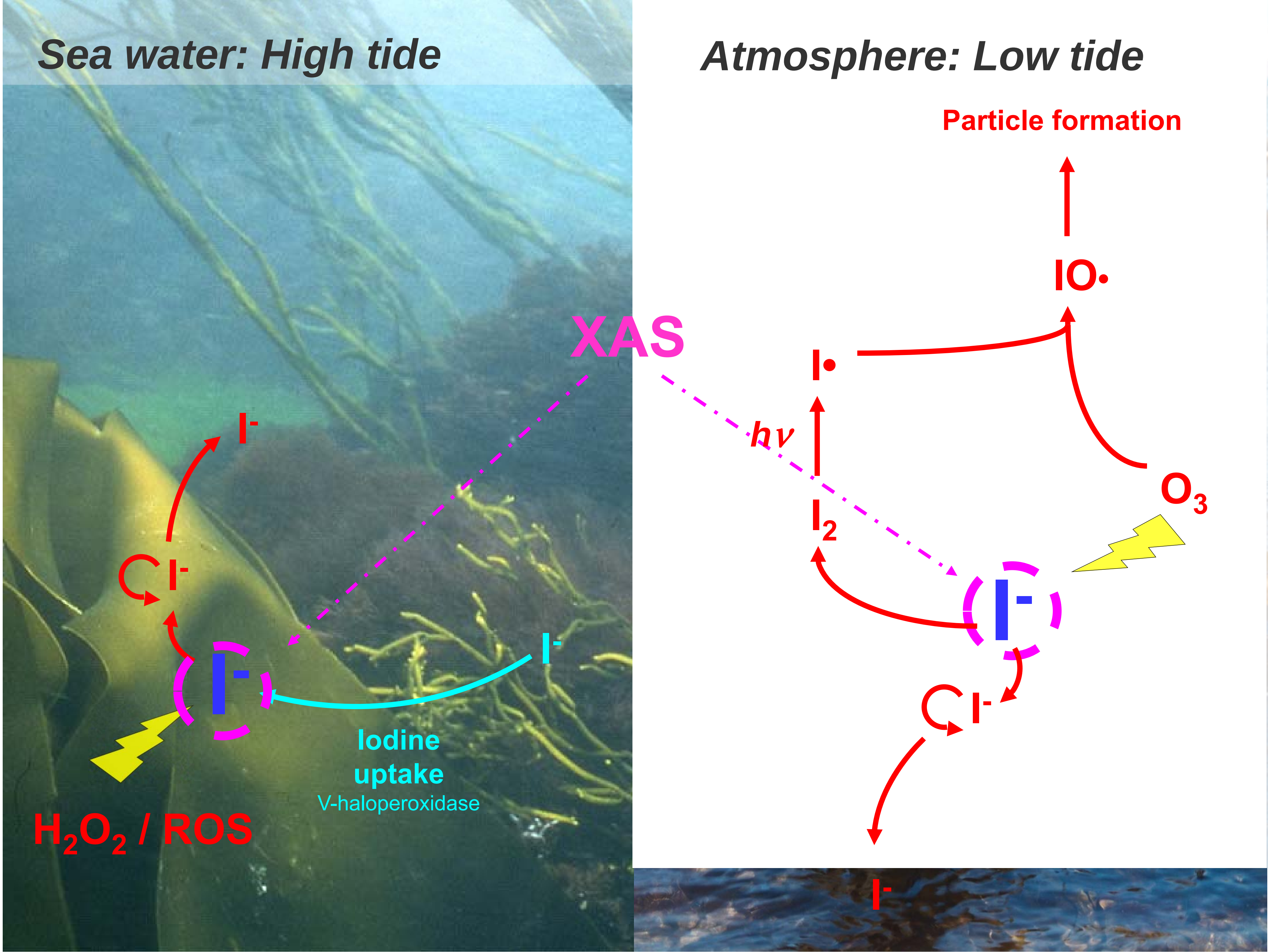


The World Ocean,
a halogen-rich environment!

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

Sea water: High tide

Atmosphere: Low tide



I^-

I^-

I^-

I^-

Iodine uptake

V-haloperoxidase

H_2O_2 / ROS

XAS

Particle formation

IO•

I•

hν

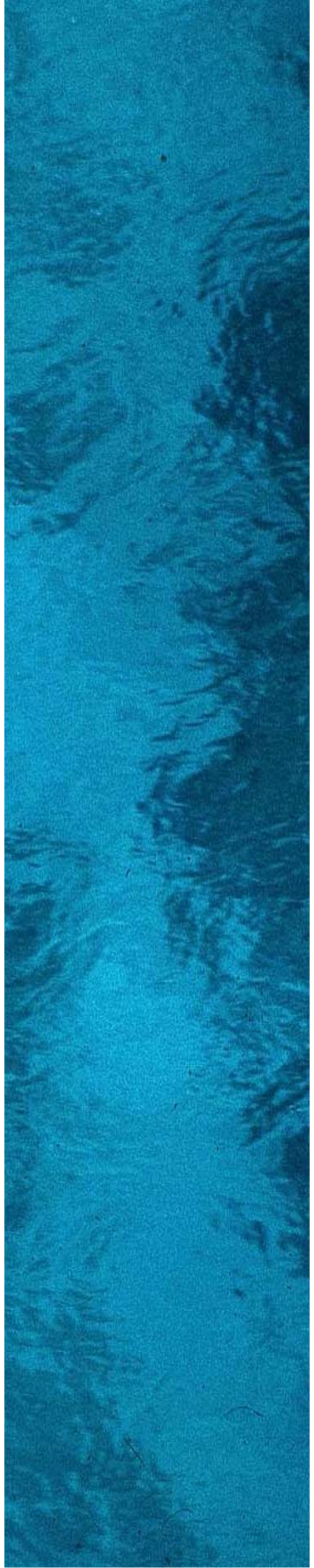
I_2

O_3

I^-

I^-

I^-



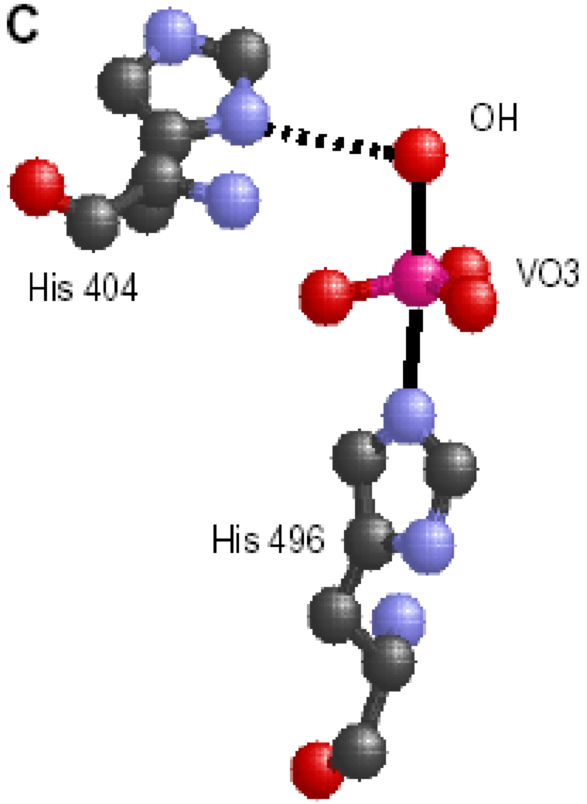
Marine metalloenzymes: Vanadium (V) haloperoxidases from marine red and brown algae

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

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

Vanadium haloperoxidases

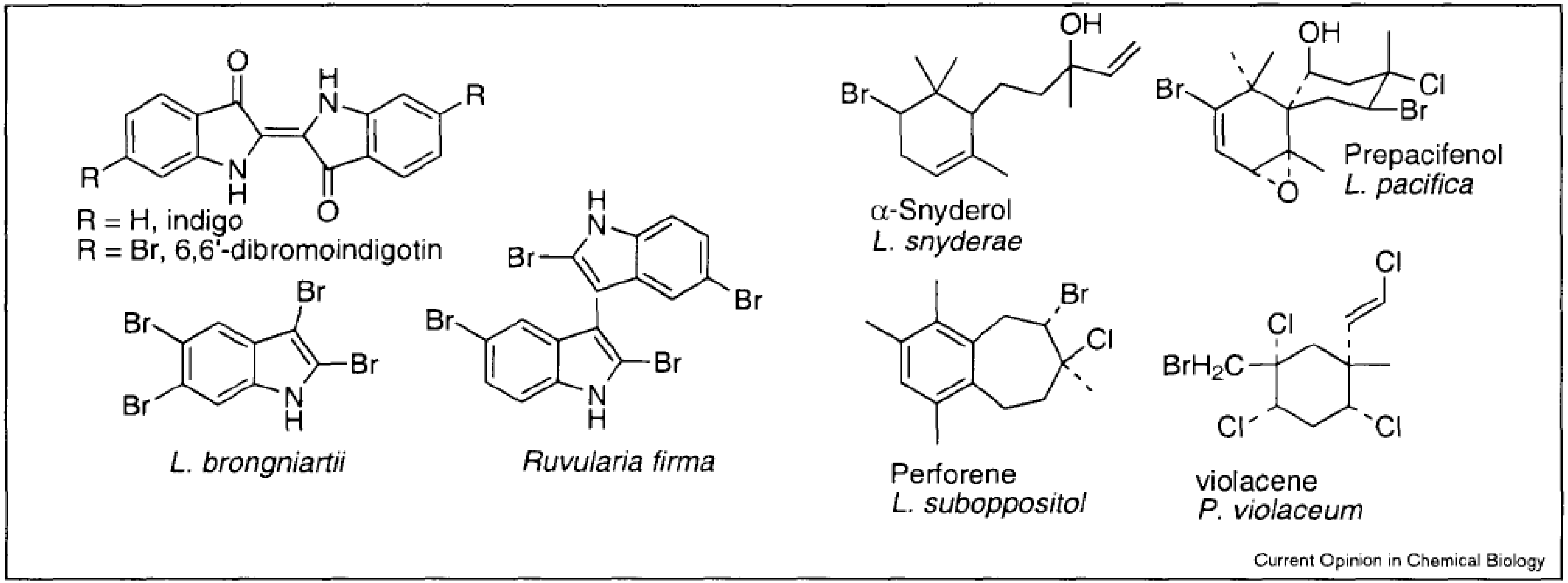
- Vanadium (V): vanadate
- Homology to acidic phosphatases



Butler A, 1998: Science 281, 207-10

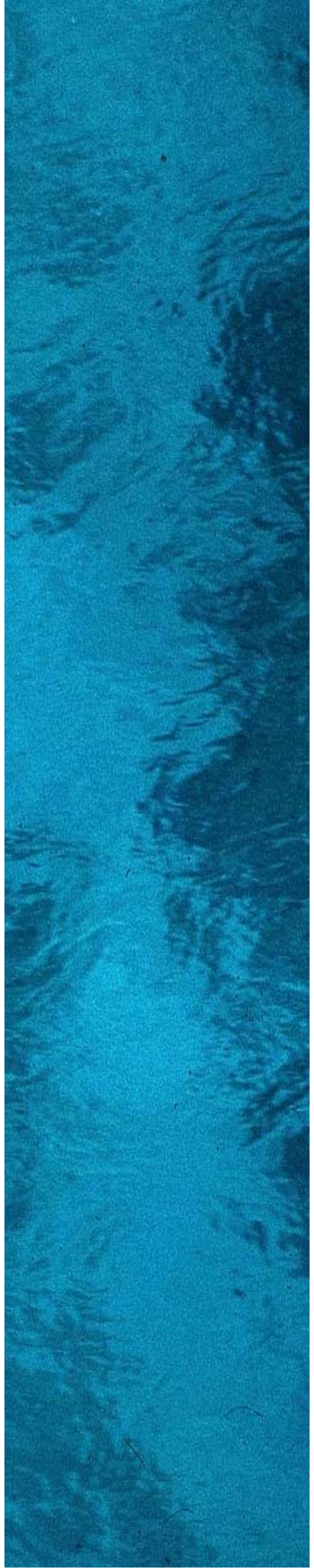
Vanadium haloperoxidases

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



Selected examples of halogenated marine natural products.

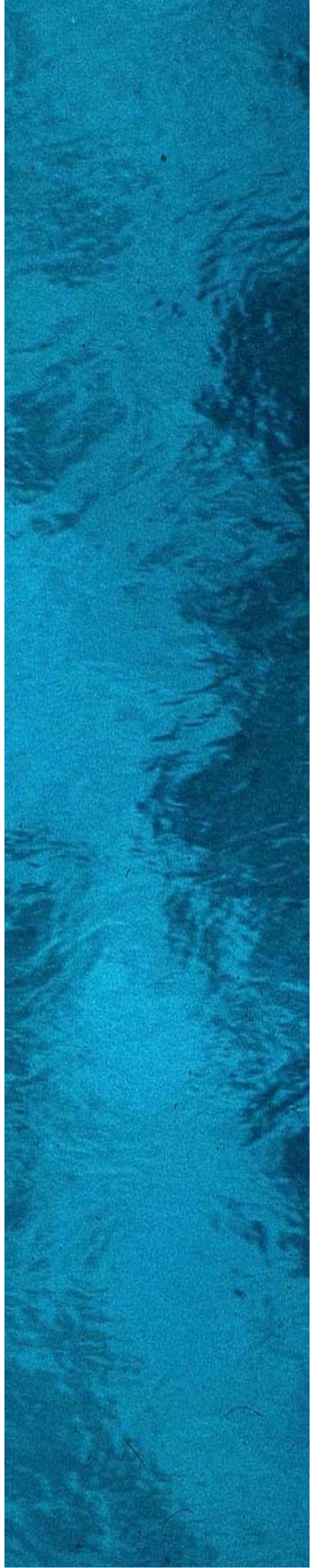
Butler A, 1998: Curr. Op. Chemical Biology 2, 279-85



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.]

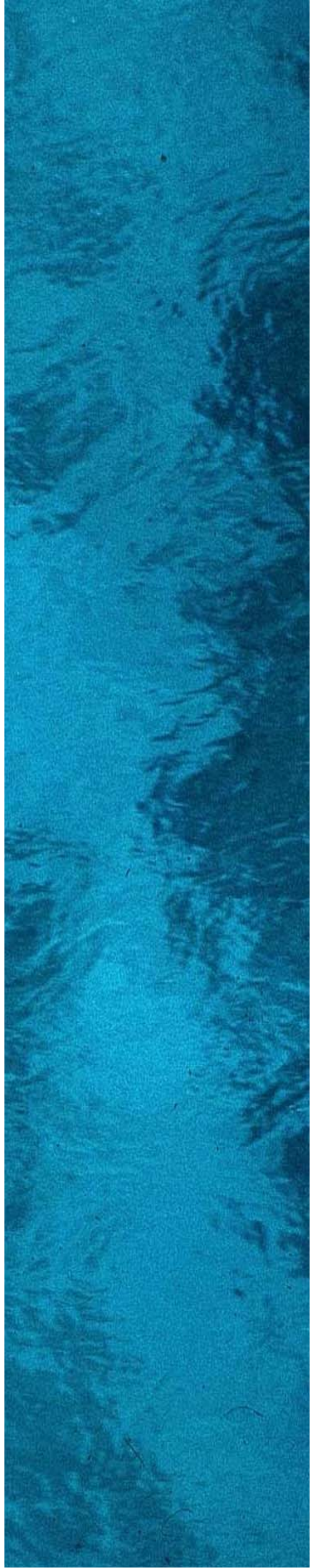


Iodine accumulation in *Laminaria*

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

Still unclear:

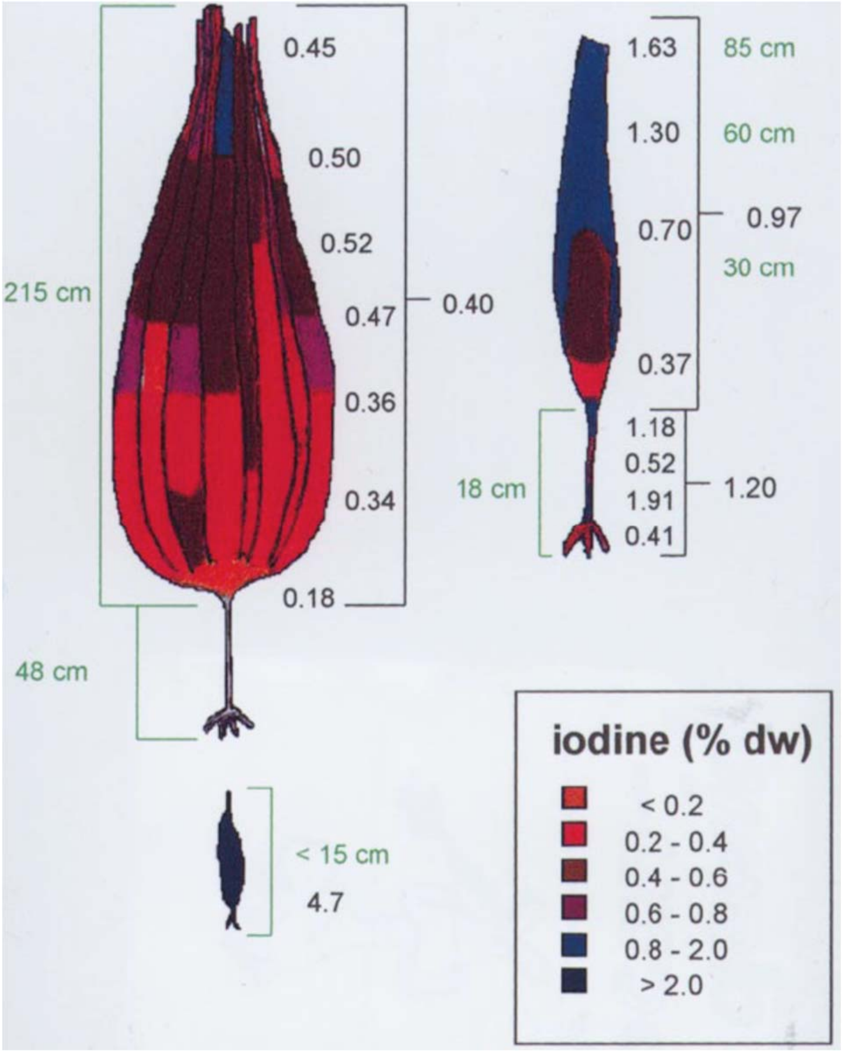
- Chemical form of accumulated iodine?
- **Biological significance?**



Iodine accumulation in *Laminaria*

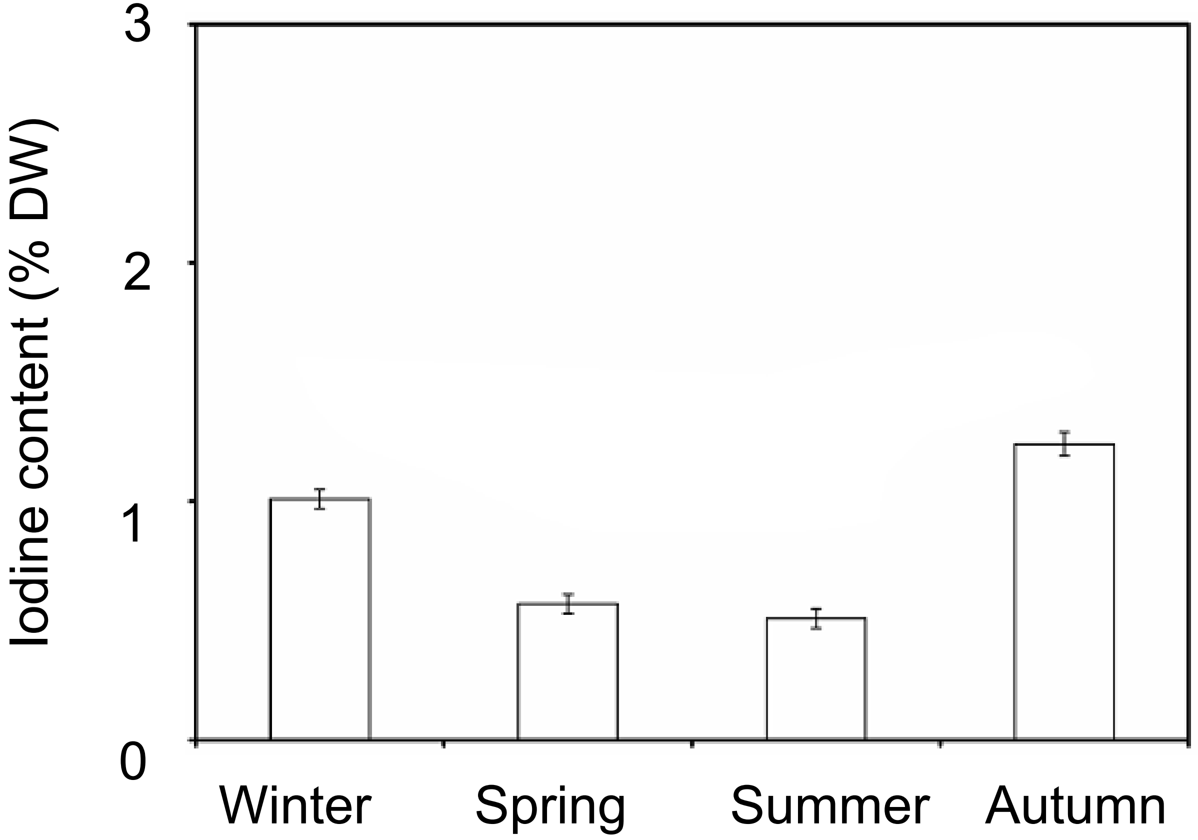
- Requirement of an intact cell wall (apoplast)
- Role of hydrogen peroxide and haloperoxidases in iodine uptake
- Iodine efflux upon oxidative stress

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

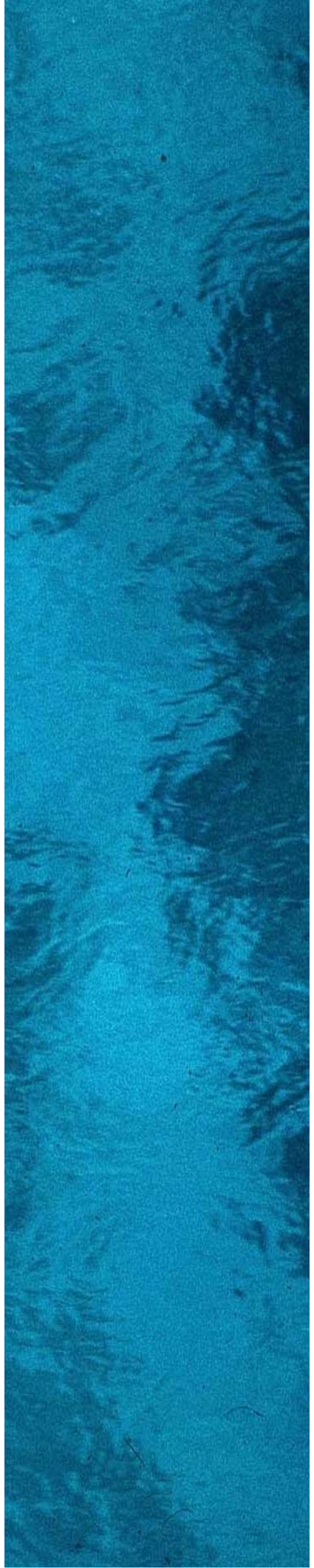


Iodine accumulation in *Laminaria*

- Strong seasonality!

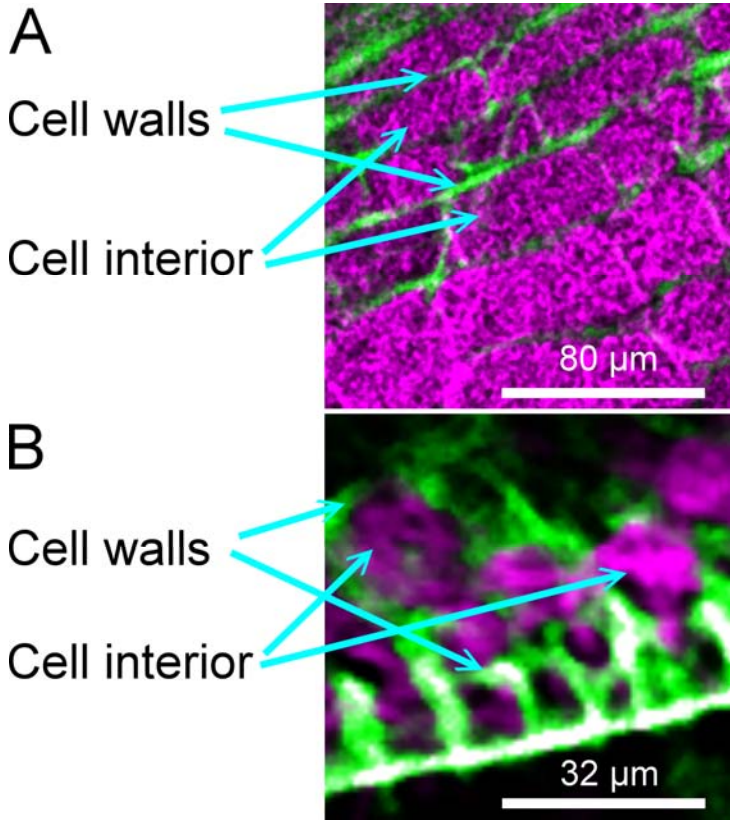


ArGall, E.; Küpper, F.C.; Kloareg, B., 2004: A survey of iodine contents in *Laminaria digitata*. *Botanica Marina* **47**, 30-37.

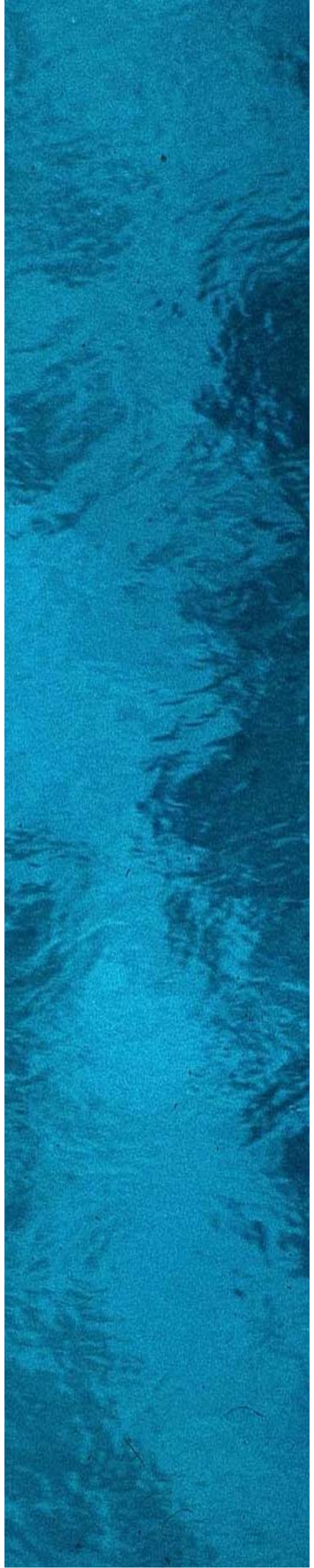


Iodine accumulation in *Laminaria*

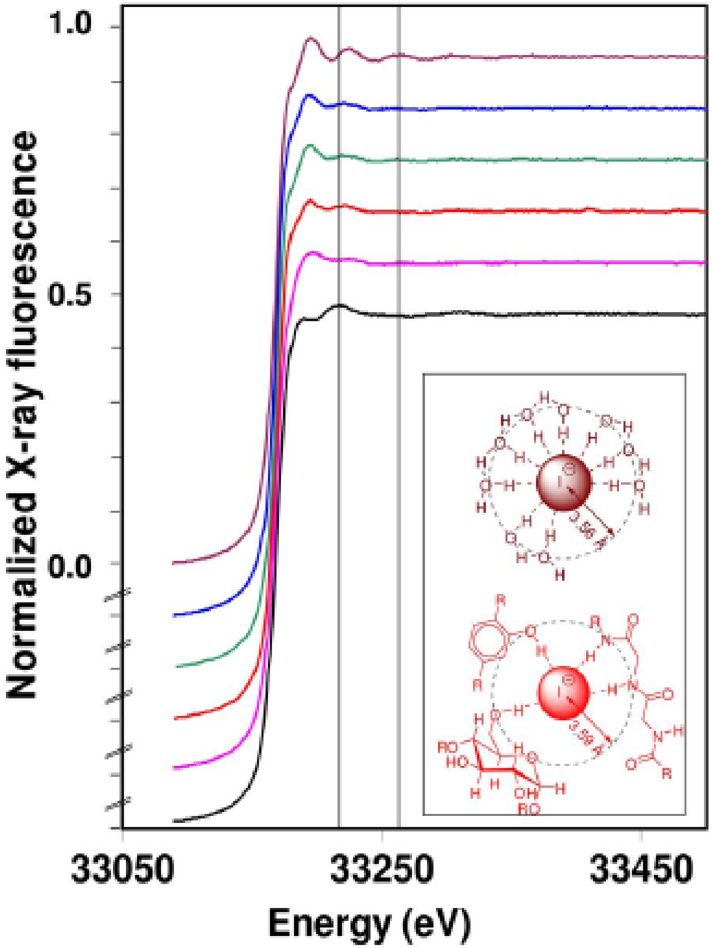
- storage in vacuoles as shown by µXRF tomography (pink: iodine; green: cell walls)

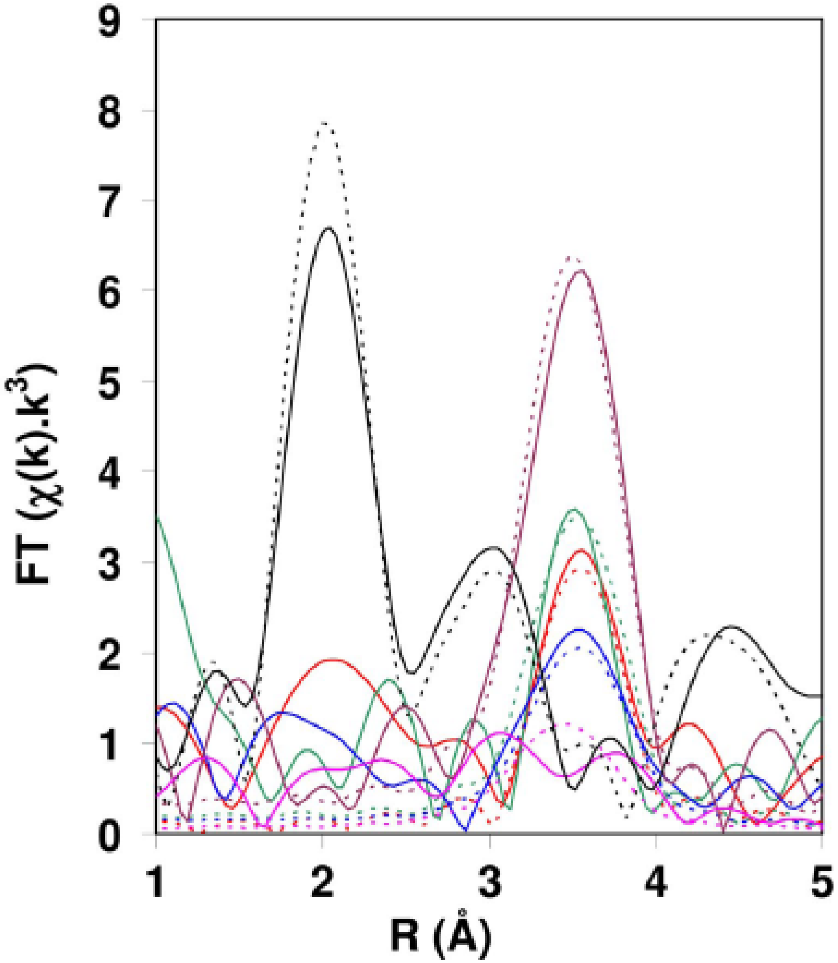


Küpper FC, Ana Mijovilovich A, Cloetens P, Kroneck PMH, Newville M, Lanzirotti T, Miller EP, Feiters MC, Meyer-Klaucke W, Christos Katsaros C, Küpper H, Carl J. Carrano CJ (2026) Synchrotron X-rays elucidate the intracellular storage of the iodide and bromide antioxidant system in the kelp Laminaria digitata. Royal Society Interface (in press)



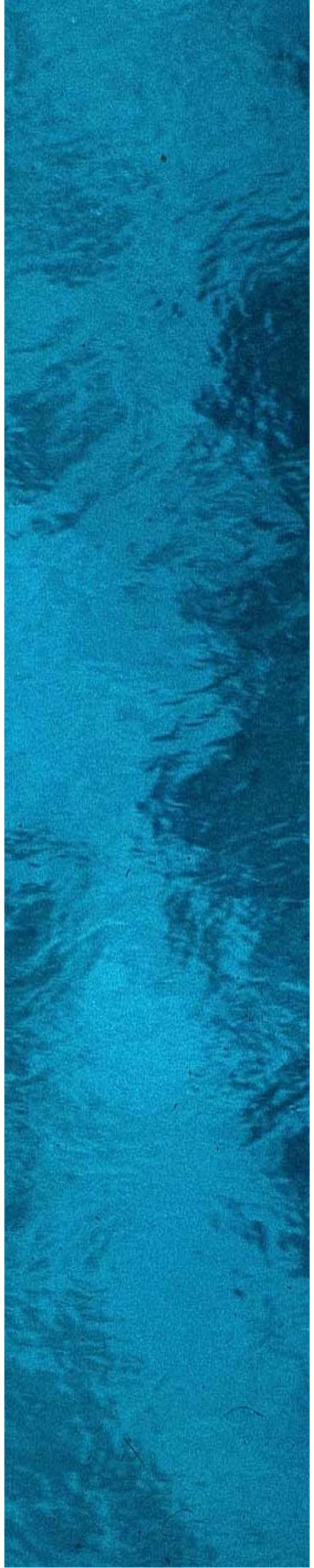
Iodine XAS of *Laminaria* tissues





(Iodine K-edge)

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



Iodine metabolism and oxidative stress

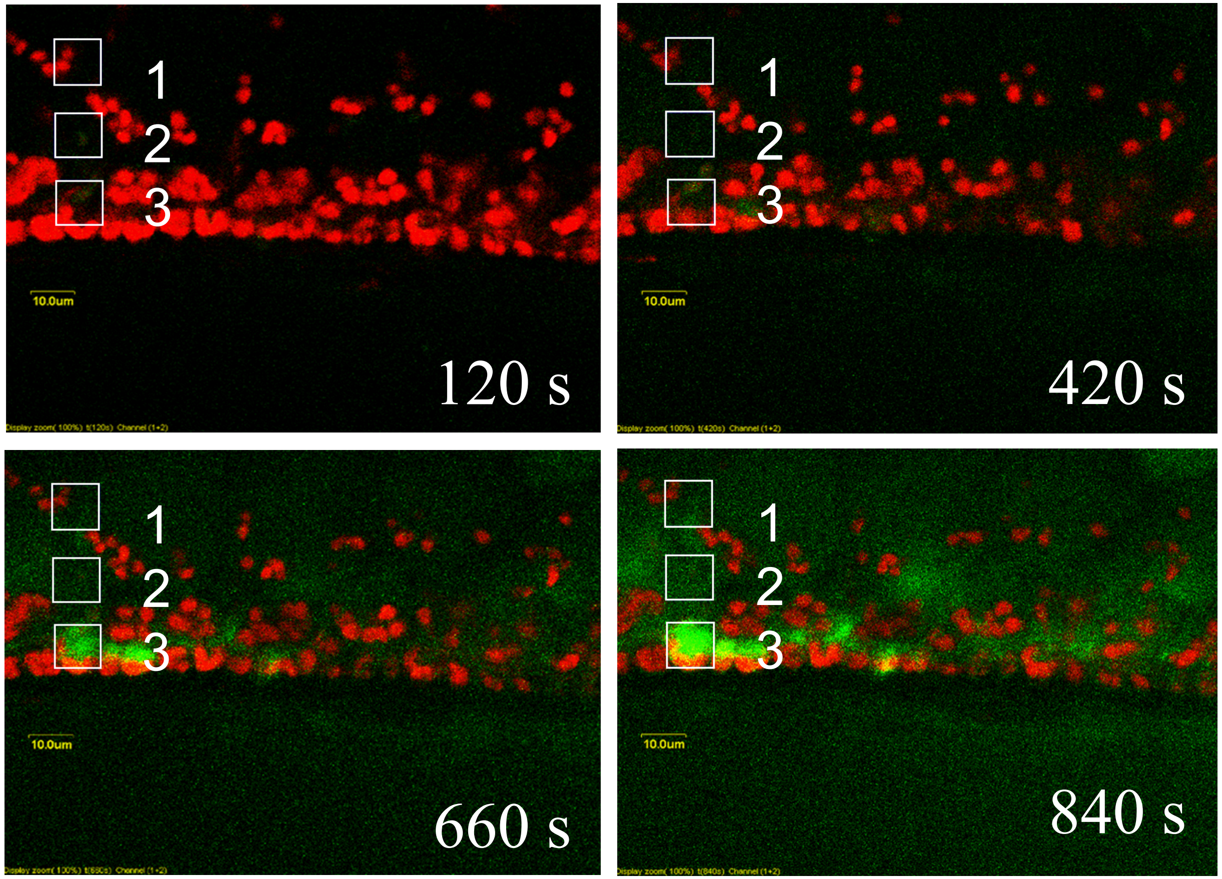
- Iodine **uptake** requires low H_2O_2 levels (< 25 μM)
- Higher concentrations of H_2O_2 result in iodine **efflux**

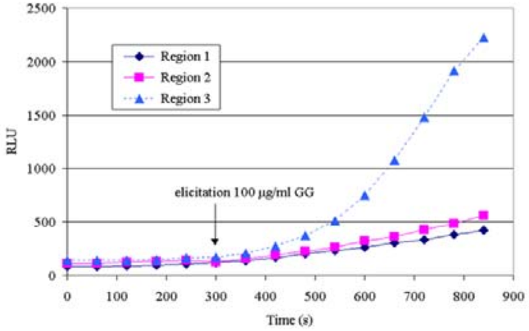
Oxidative stress in *Laminaria*:

- 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)





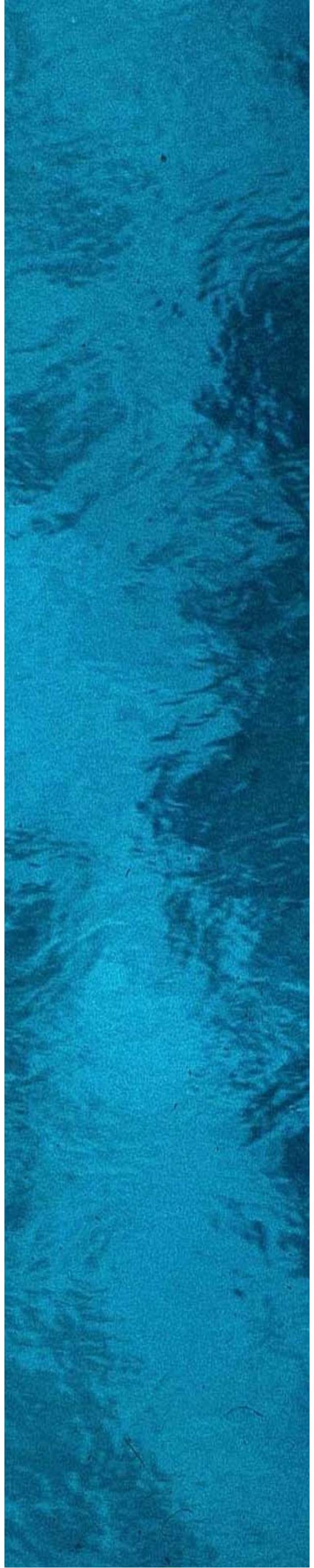
DCFH-DA :
dichlorohydro-fluorescein
diacetate

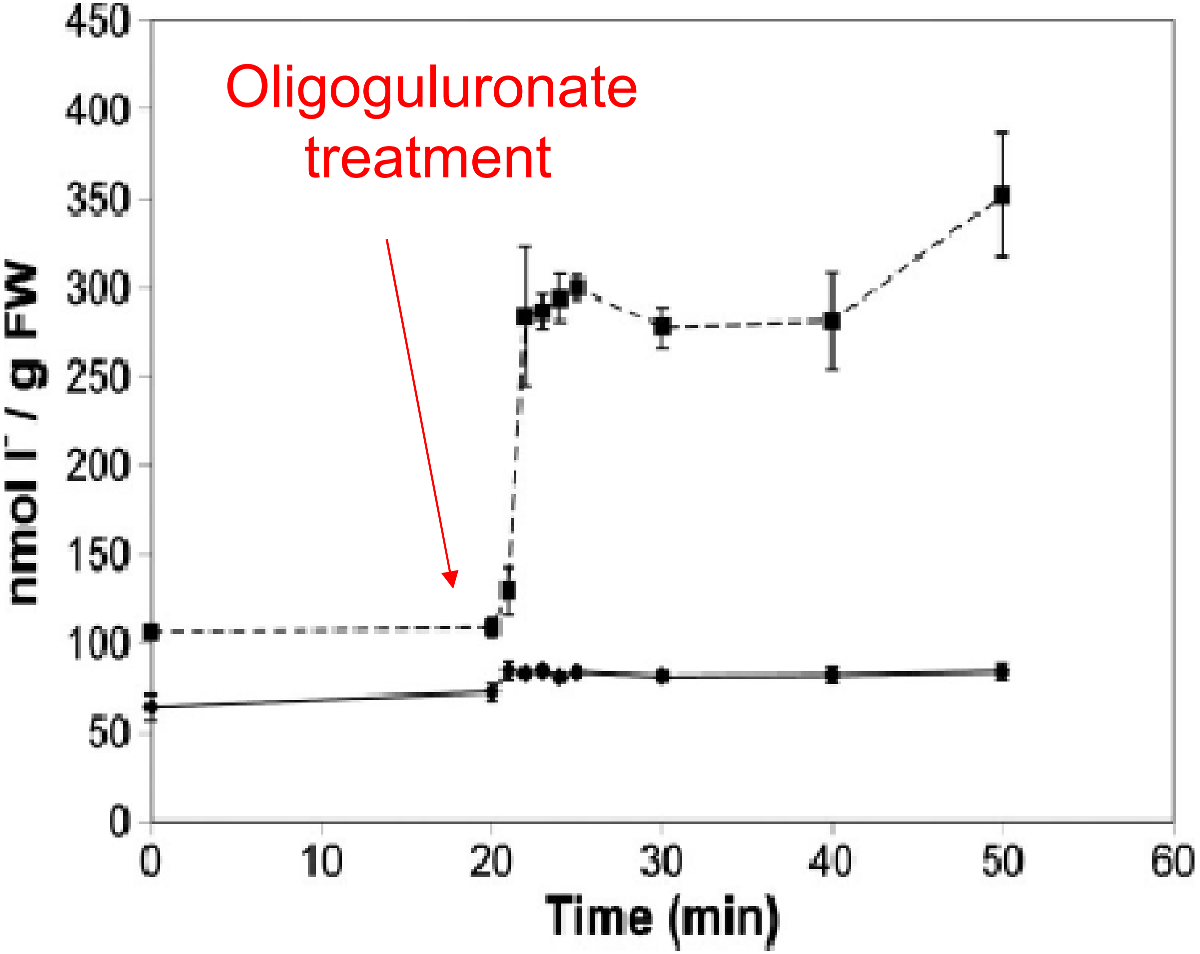
Küpper *et al.*, 2001: Plant Physiology **125**, 278-91

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

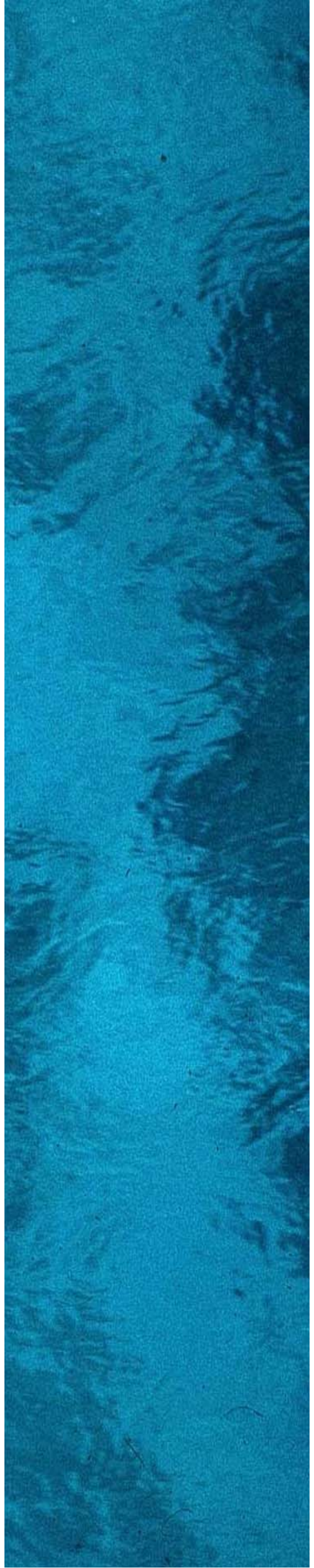
Cathodic stripping square wave voltammetry (CSSWV)



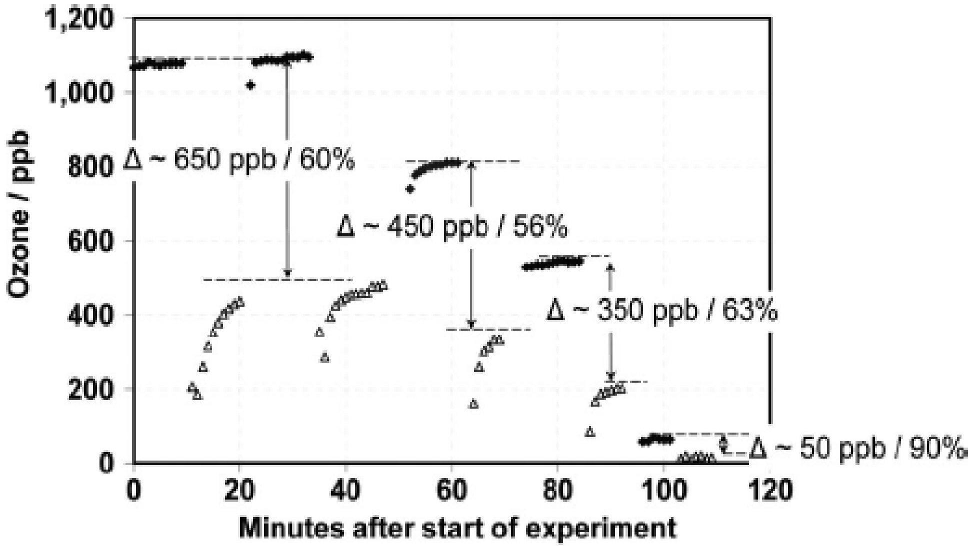


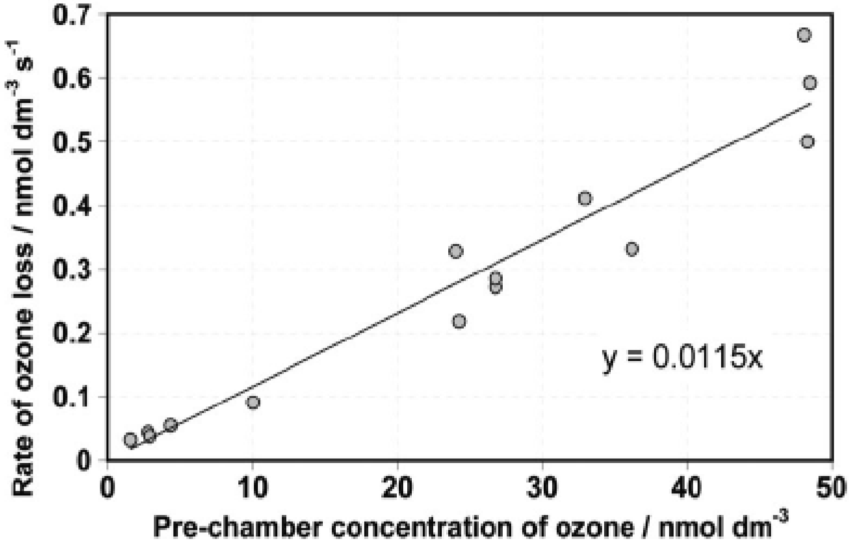
➢ **Strong iodide efflux upon oxidative stress**

- No increased levels of oxidized or organic iodine species



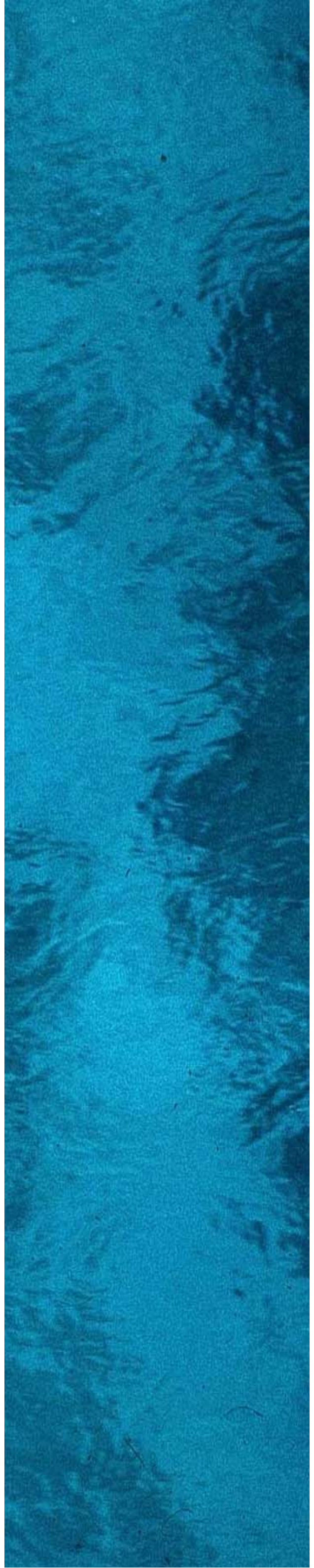
Scavenging of ozone (O_3) by *Laminaria*





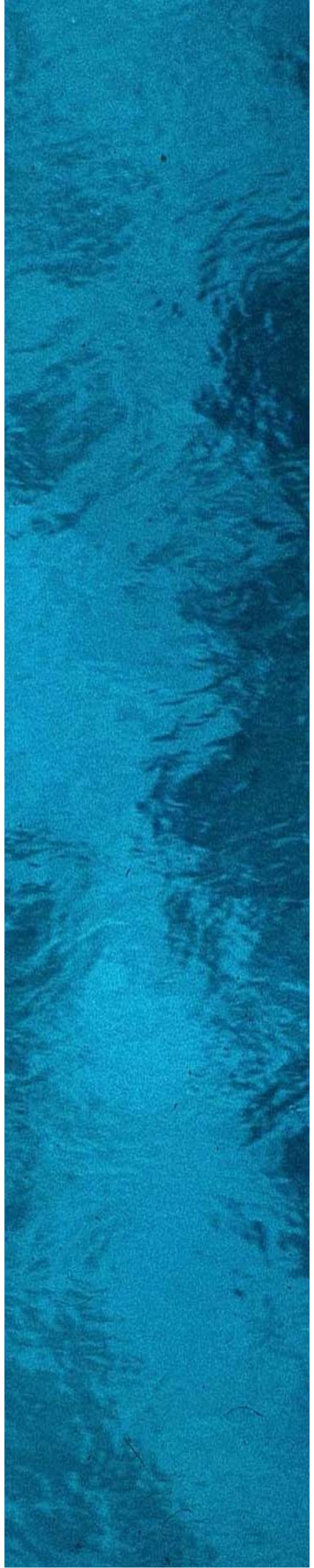
➢ ***Laminaria*** **thalli effectively scavenge ozone**

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



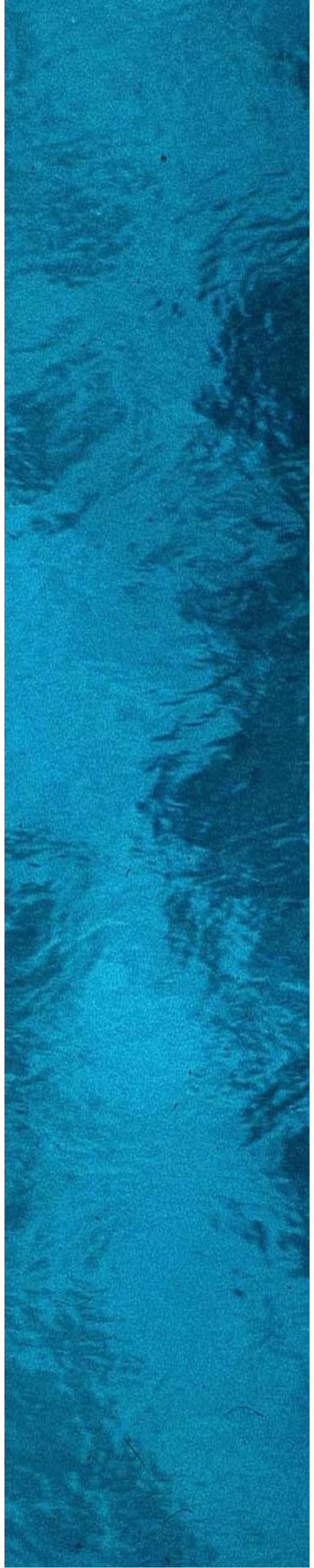
What makes iodide a suitable antioxidant?

- 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)



Kelp iodine emissions into the coastal atmosphere

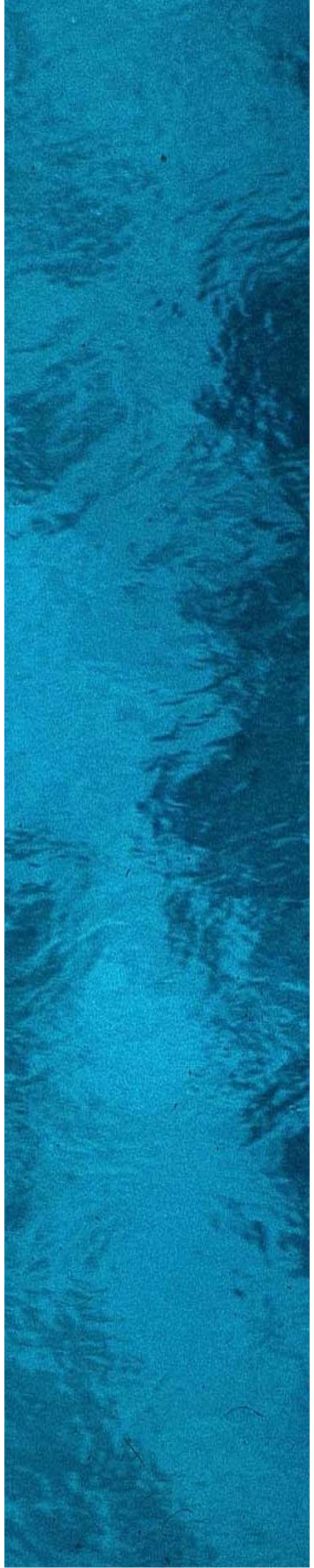
- "Iodovolatilisation" discovered in 1920s by Kylin and Dangeard: I_2 detected with starch paper
- J. Lovelock, 1973: Discovery of methyl iodide emissions from seaweeds
- B. Alicke et al., 1999: High IO levels above kelp beds at low tide
- L.J. Carpenter *et al*., 2000: CH_2I_2 main species emitted by *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})



Iodo(hydro)carbons) 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



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) → may still be true for iodo(hydro)carbons
- Implications for atmospheric & marine chemistry

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