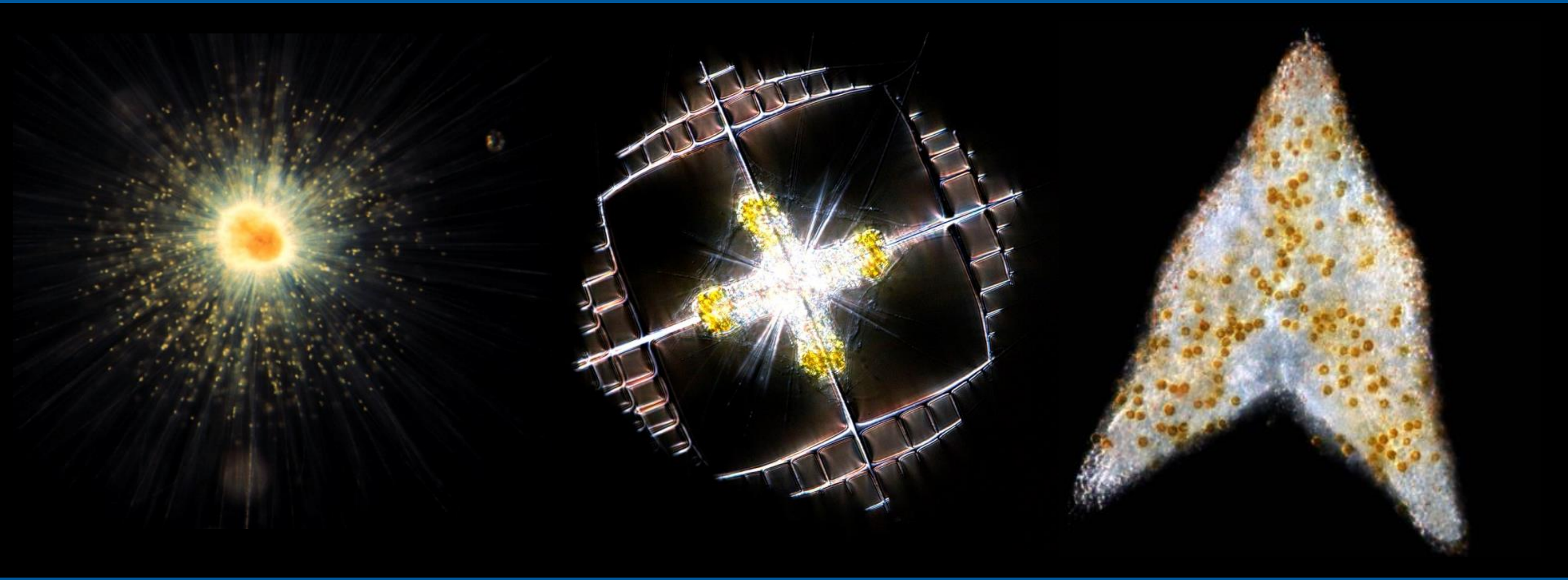
Visualizing the chemical landscape of planktonic photosymbioses using single-cell chemical imaging



Post-doctoral project
2016-2017

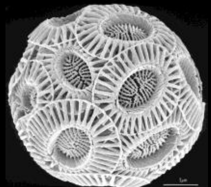
Johan Decelle

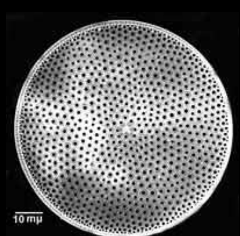
Hryhoriy Stryhanyuk, Benoit Gallet, Matthias Schmidt, Giulia Veronesi, Sergio Balzano, Sophie Marro, Hans Richnow, Niculina Musat

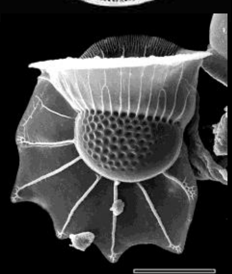
HELMHOLTZ CENTRE FOR ENVIRONMENTAL RESEARCH – UFZ
Leipzig

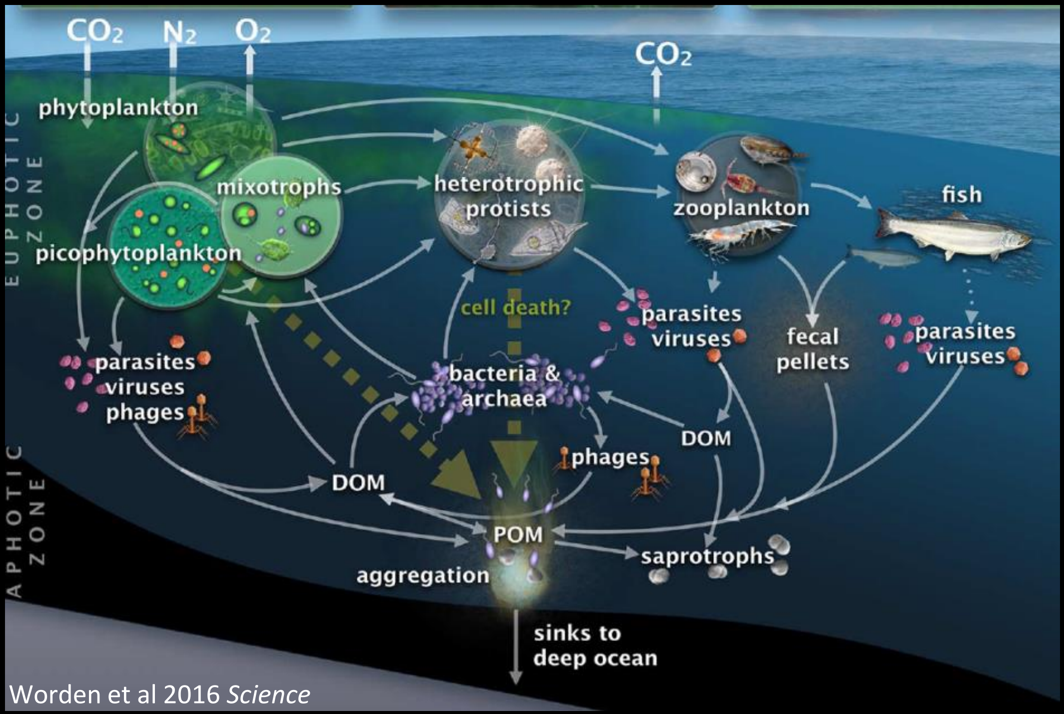
MARIE CURIE ACTIONS

Marine plankton is highly diverse and has complex trophic modes and life cycles

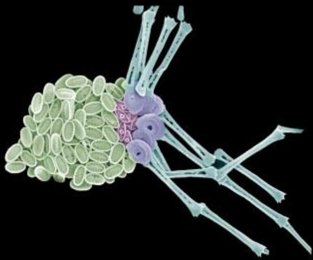


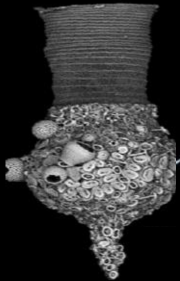


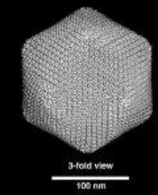


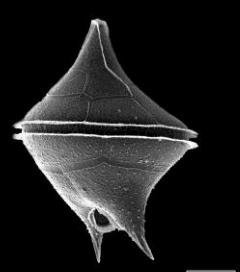


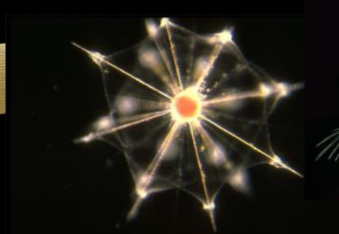
Worden et al 2016 *Science*





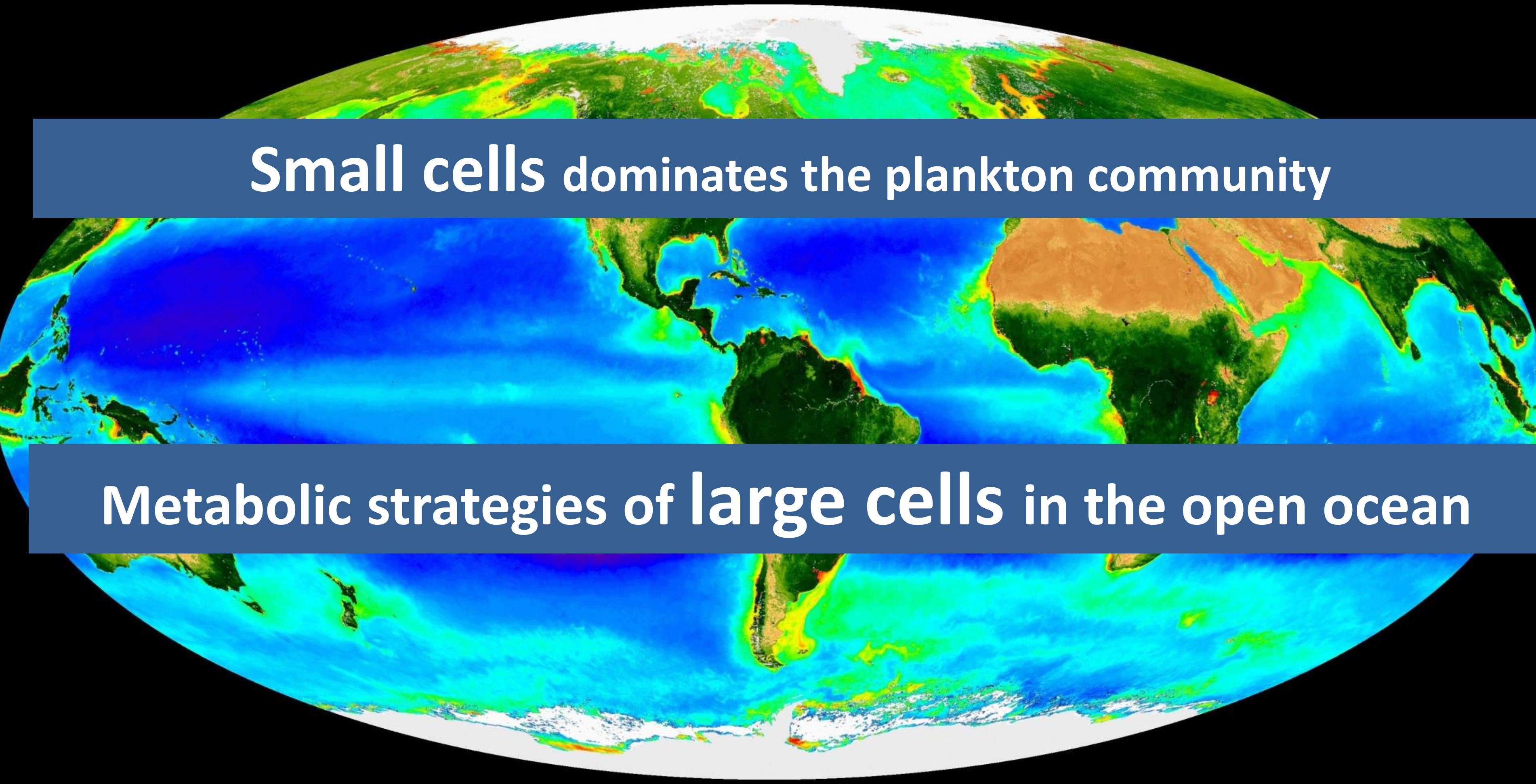






The ocean is mainly oligotrophic

(N, P, and trace metals)



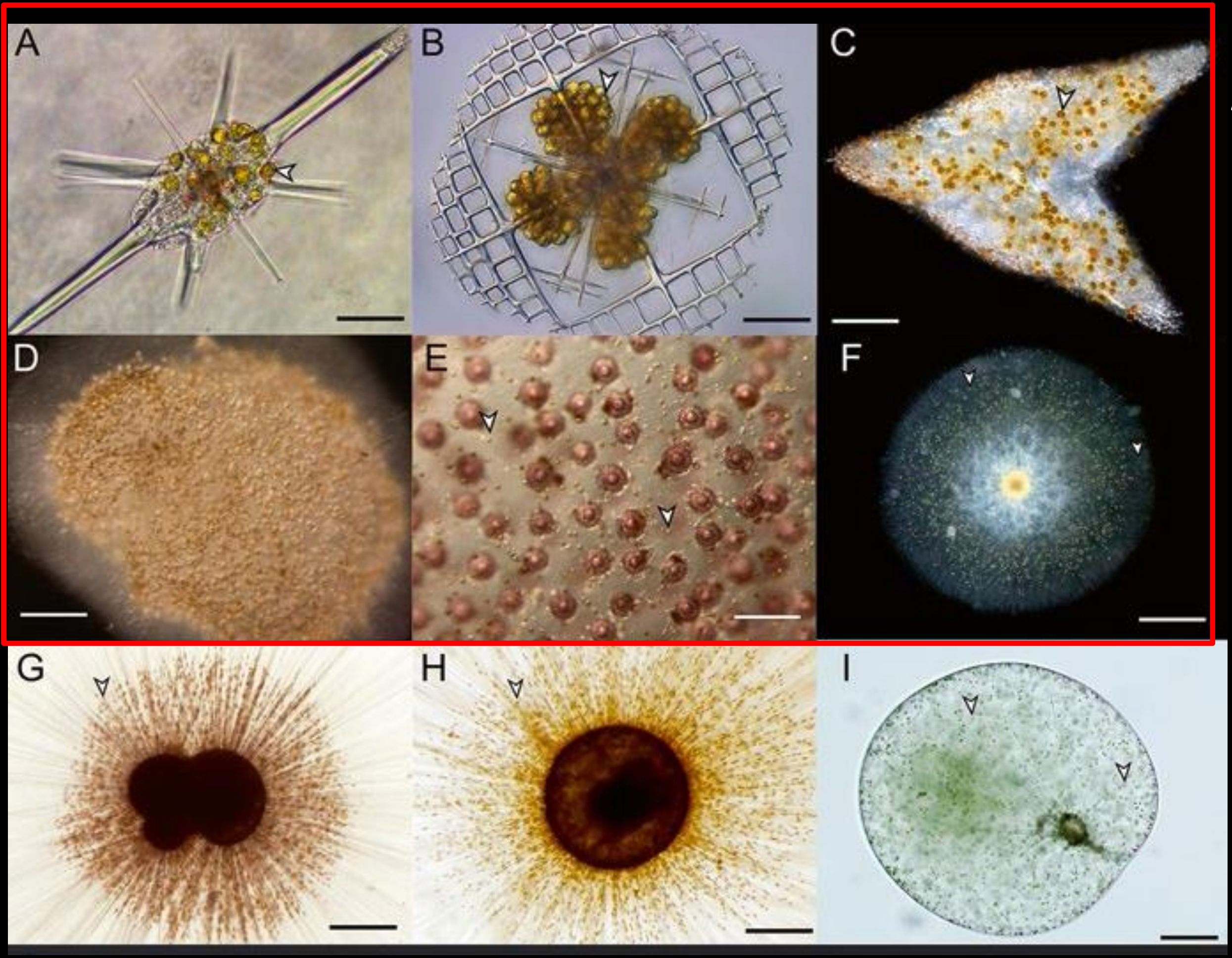
Small cells dominates the plankton community

Metabolic strategies of **large cells** in the open ocean

mixotrophy, nutrient storage, and <u>metabolic symbiosis</u>

Photosymbiosis between unicellular organisms in the oceanic plankton

Heterotrophic hosts + intracellular microalgae



A wide diversity of hosts:

(100-400 µm in size)

A-F: Radiolarians

(G-H) Foraminiferans

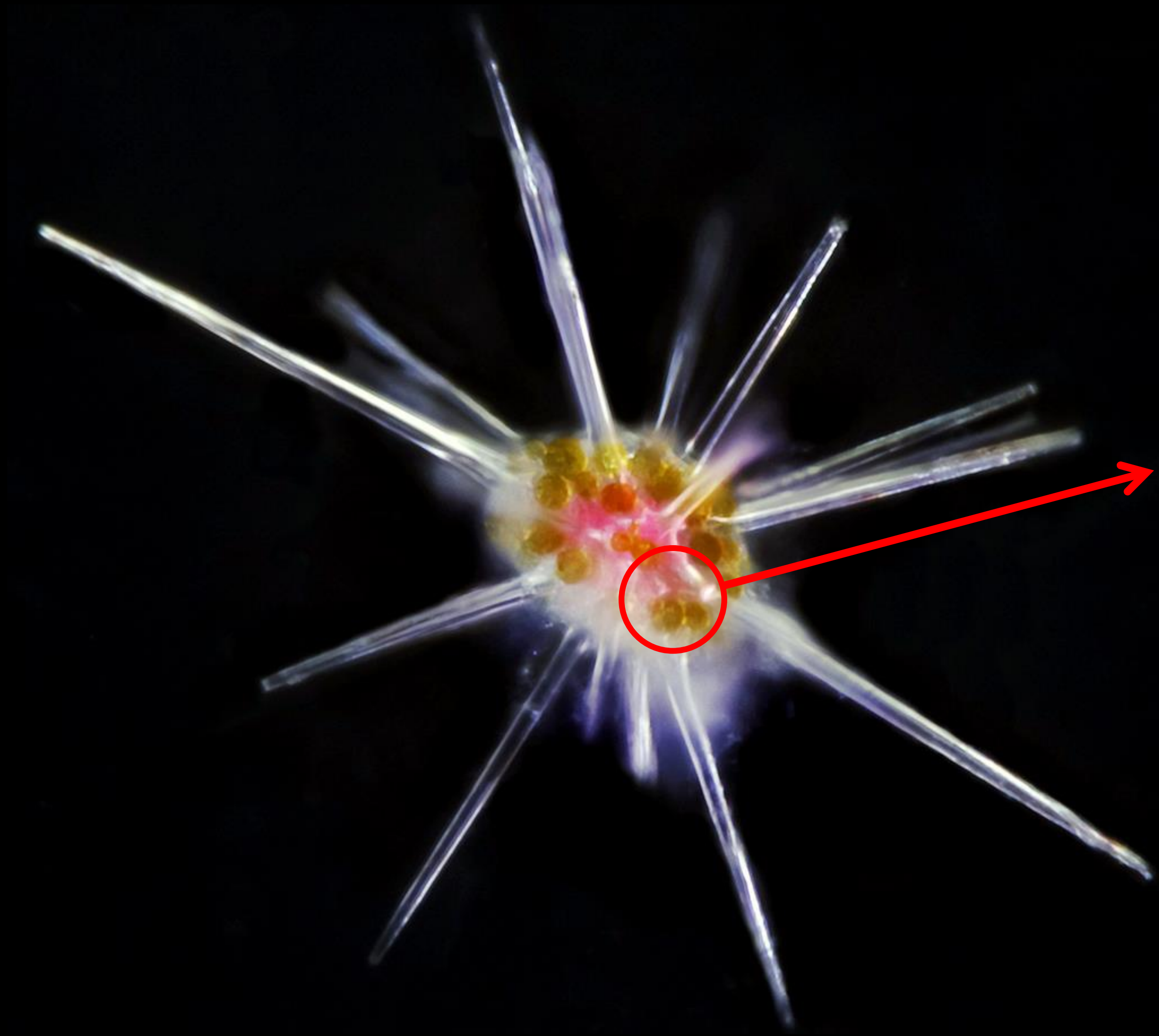
(I) Dinoflagellates

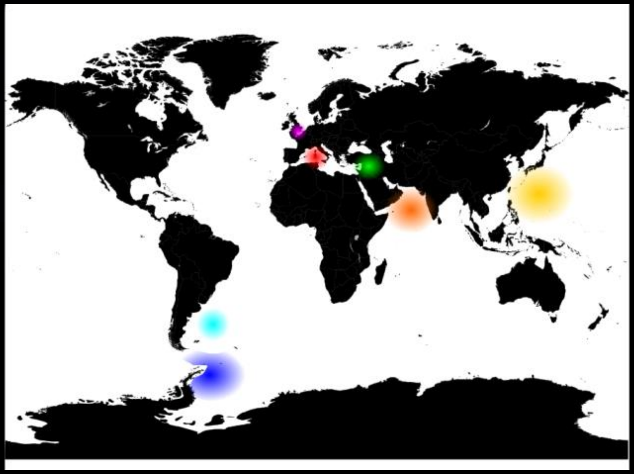
Symbiosis is obligatory for the host

Benefits for the symbiont?

Decelle J, Colin S and Foster R. 2015

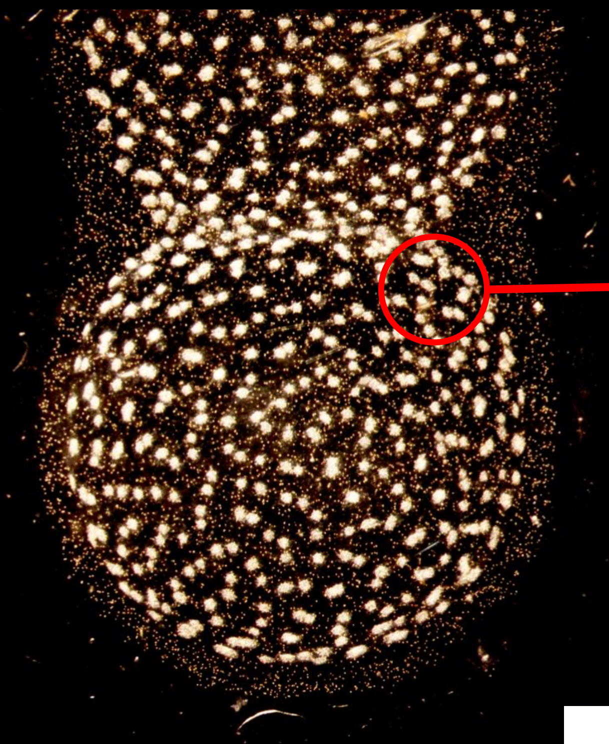
Acantharia host the microalga *Phaeocystis*

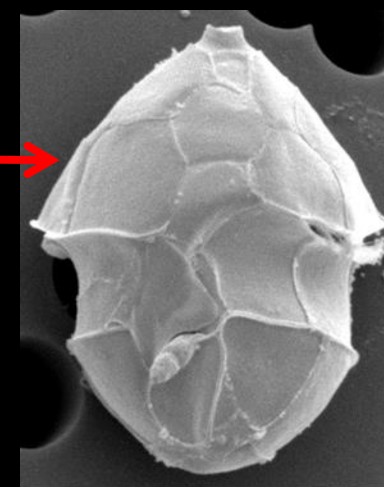




Decelle et al *PNAS* 2012

Collodaria host the microalga *Brandtodinium*





Free-living form of the dinoflagellate *Brandtodinium*

Probert et al *J Phycol* 2014

Ecological importance of Radiolaria

Eukaryotic plankton diversity in the sunlit ocean

Colomban de Vargas,[1,2*†] Stéphane Audic,[1,2†] Nicolas Henry,[1,2†] Johan Decelle,[1,2†] Frédéric Mahé,[3,1,2†] Ramiro Logares,[4] Enrique Lara,[5] Cédric Berney,[1,2] Noan Le Bescot,[1,2] Ian Probert,[6,7] Margaux Carmichael,[1,2,8] Julie Poulain,[9] Sarah Romac,[1,2] Sébastien Colin,[1,2,8] Jean-Marc Aury,[9] Lucie Bittner,[10,11,8,1,2] Samuel Chaffron,[12,13,14] Micah Dunthorn,[3] Stefan Engelen,[9] Olga Flegontova,[15,16] Lionel Guidi,[17,18] Aleš Horák,[15,16] Olivier Jaillon,[9,19,20] Gipsi Lima-Mendez,[12,13,14] Julius Lukeš,[15,16,21] Shruti Malviya,[8] Raphael Morard,[22,1,2] Matthieu Mulot,[5] Eleonora Scalco,[23] Raffaele Siano,[24] Flora Vincent,[13,8] Adriana Zingone,[23] Céline Dimier,[1,2,8] Marc Picheral,[17,18] Sarah Searson,[17,18] Stefanie Kandels-Lewis,[25,26] *Tara* Oceans Coordinators‡ Silvia G. Acinas,[4] Peer Bork,[25,27] Chris Bowler,[8] Gabriel Gorsky,[17,18] Nigel Grimsley,[28,29] Pascal Hingamp,[30] Daniele Iudicone,[23] Fabrice Not,[1,2] Hiroyuki Ogata,[31] Stephane Pesant,[32,22] Jeroen Raes,[12,13,14] Michael E. Sieracki,[33,34] Sabrina Speich,[35,36] Lars Stemmann,[17,18] Shinichi Sunagawa,[25] Jean Weissenbach,[9,19,20] Patrick Wincker,[9,19,20*] Eric Karsenti[26,8*]

Science **348**, (2015);
DOI: 10.1126/science.1261605

LETTER

doi:10.1038/nature17652

In situ imaging reveals the biomass of giant protists in the global ocean

Tristan Biard[1,2], Lars Stemmann[2], Marc Picheral[2], Nicolas Mayot[2], Pieter Vandromme[3], Helena Hauss[3], Gabriel Gorsky[2], Lionel Guidi[2], Rainer Kiko[3] & Fabrice Not[1]

Radiolaria represent a **high biomass** in the plankton community

ARTICLE

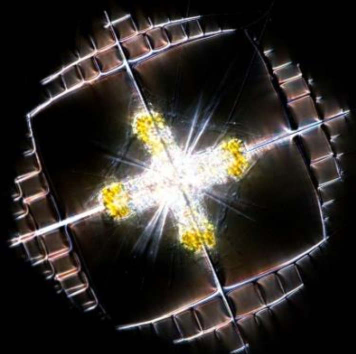
doi:10.1038/nature16942

Plankton networks driving carbon export in the oligotrophic ocean

Lionel Guidi[1,2*], Samuel Chaffron[3,4,5*], Lucie Bittner[6,7,8*], Damien Eveillard[9*], Abdelhalim Larhlimi[9], Simon Roux[10†], Youssef Darzi[3,4], Stephane Audic[8], Léo Berline[1†], Jennifer R. Brum[10†], Luis Pedro Coelho[11], Julio Cesar Ignacio Espinoza[10], Shruti Malviya[7†], Shinichi Sunagawa[11], Céline Dimier[8], Stefanie Kandels-Lewis[11,12], Marc Picheral[1], Julie Poulain[13], Sarah Searson[1,2], *Tara* Oceans Consortium Coordinators‡, Lars Stemmann[1], Fabrice Not[8], Pascal Hingamp[14], Sabrina Speich[15], Mick Follows[16], Lee Karp-Boss[17], Emmanuel Boss[17], Hiroyuki Ogata[18], Stephane Pesant[19,20], Jean Weissenbach[13,21,22], Patrick Wincker[13,21,22], Silvia G. Acinas[23], Peer Bork[11,24], Colomban de Vargas[8], Daniele Iudicone[25], Matthew B. Sullivan[10†], Jeroen Raes[3,4,5], Eric Karsenti[7,12], Chris Bowler[7] & Gabriel Gorsky[1]

Radiolaria are stongly involved in **carbon export** in oligotrophic waters

The physiology and functioning of photosymbiosis in plankton remains unknown



Their ecological success must rely on their efficiency to acquire, transfer and recycle nutrients

Questions

What are the metabolic strategies of the host ?

What is the metabolic role and needs of each partner ?

What is the metabolism of the microalga between the symbiotic and free-living stage **(outside the host) ?**

Studying physiology of uncultured microbial cells is highly challenging

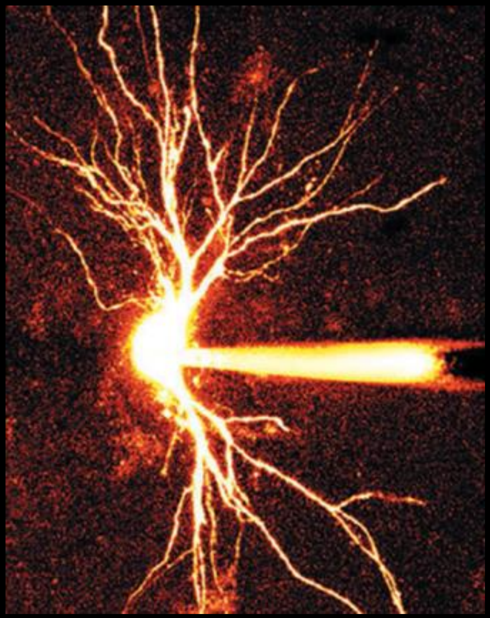
Bulk analyses

(transcriptomics, metabolomics, lipidomics)

↓

High cell biomass

No spatial information



Fessenden M, *Nature* 2016

➔ single-cell chemical imaging

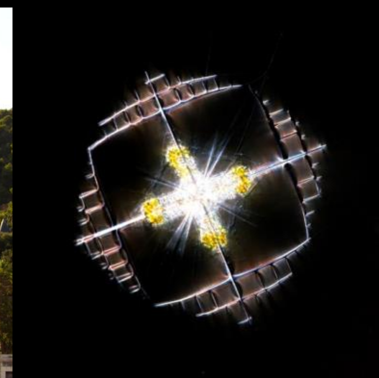
Single-cell approach: no need to have cultures

Maintain physical integrity (relevant for symbioses)

Spatial information: Localization of a metabolite or element

Sampling in the Mediterranean Sea

(Bay of Villefranche sur Mer, France)



➔ **Sampling in surface waters with a plankton net**

➔ **Rapid isolation of individual <u>host cells</u> in natural seawater**

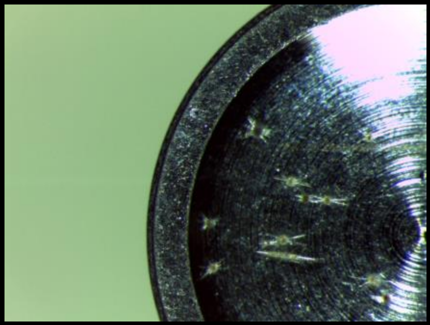
➔ **Cultures of *Phaeocystis* and *Brandtodinium* (free-living symbionts)**

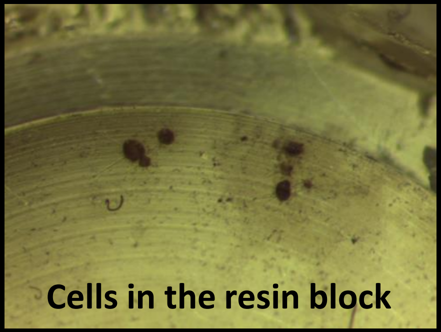
Collaboration with Sophie Marro and John Dolan (LOV: CNRS/UPMC)

Sample preparation for chemical imaging

1- Cryo-fixation with High-Pressure Freezing (Leica HPM100)

The best method for preserving the ultrastructure and native chemistry of cells





2- Freeze substitution -90°C to -30°C for 5 days with Acetone + osmium tetroxide (Leica AFS2)

3- Resin Embedding (Room temperature)

4- Ultra-Sectioning

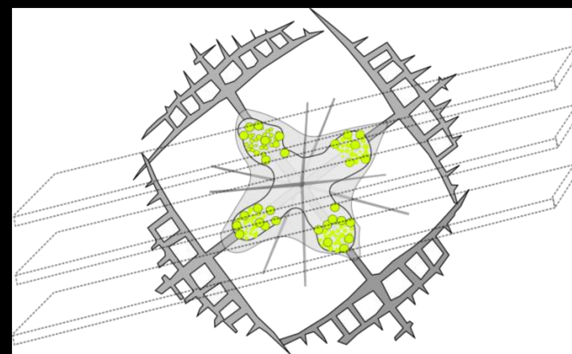
ibs Institut de Biologie Structurale

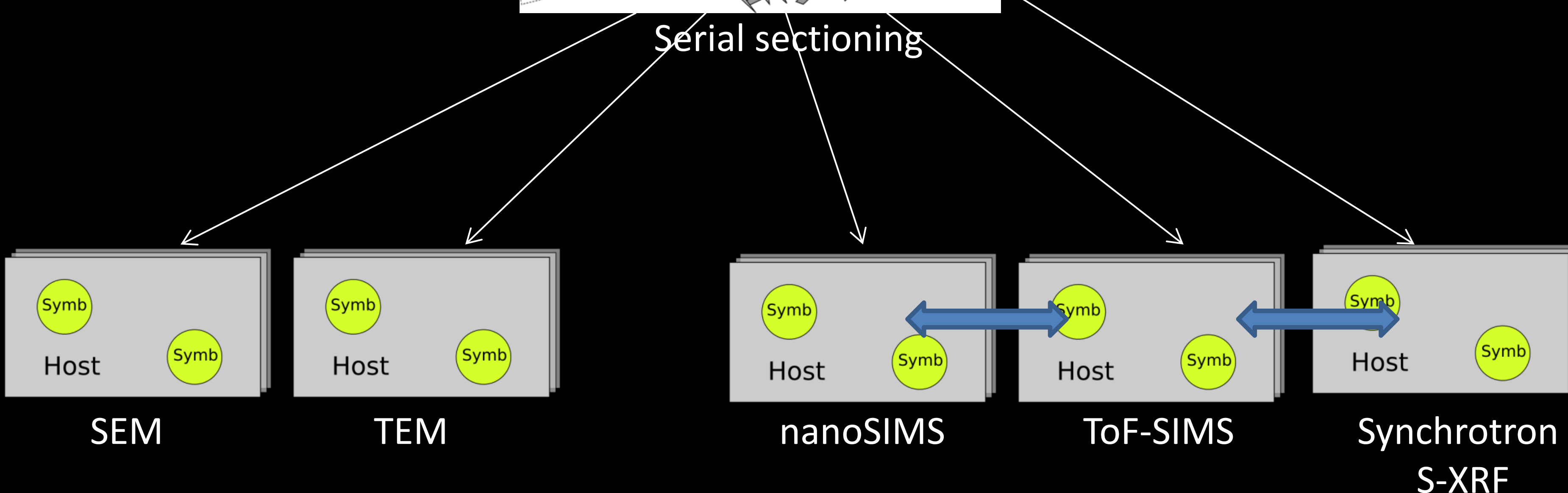
Collaboration with IBS, Grenoble (Benoit Gallet)

instruct Integrating Biology

Correlated approach between electron microscopy and chemical imaging

4- Ultra-Sectioning





Morphological information ⟷ Chemical information

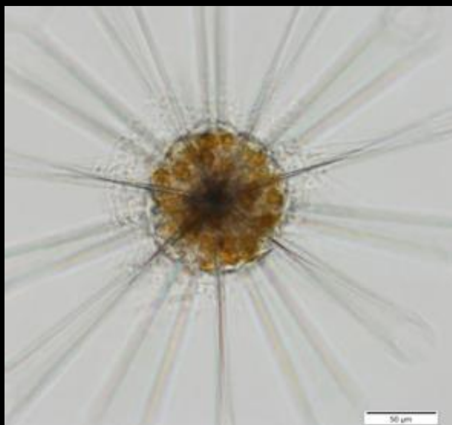
Results - I

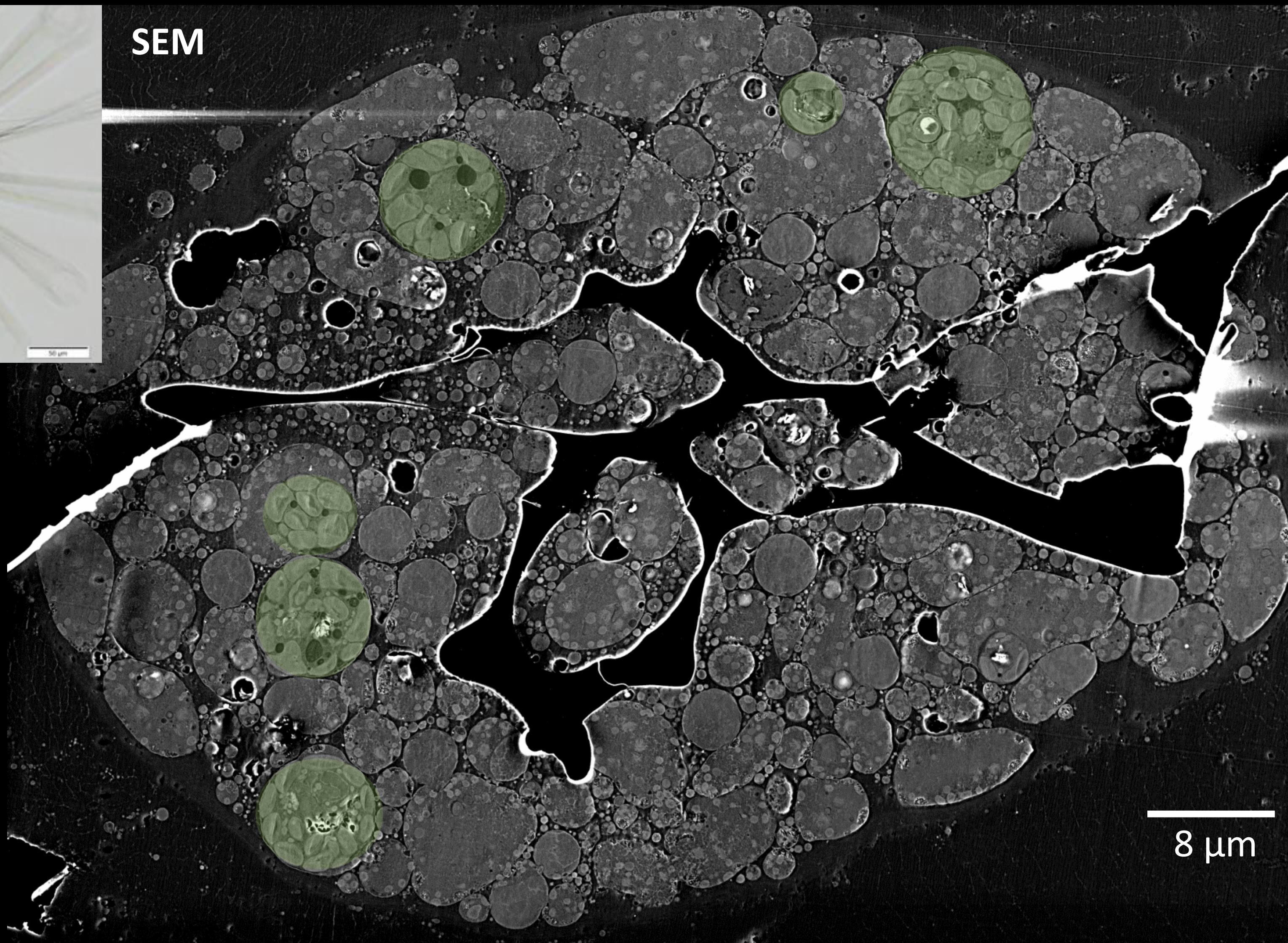
Ultratructure organisation of the host-symbiont integration

Morphology of the symbionts in the host *vs* free-living

Electron microscopy (SEM/TEM)

Acantharia- *Phaeocystis* (symbiont)

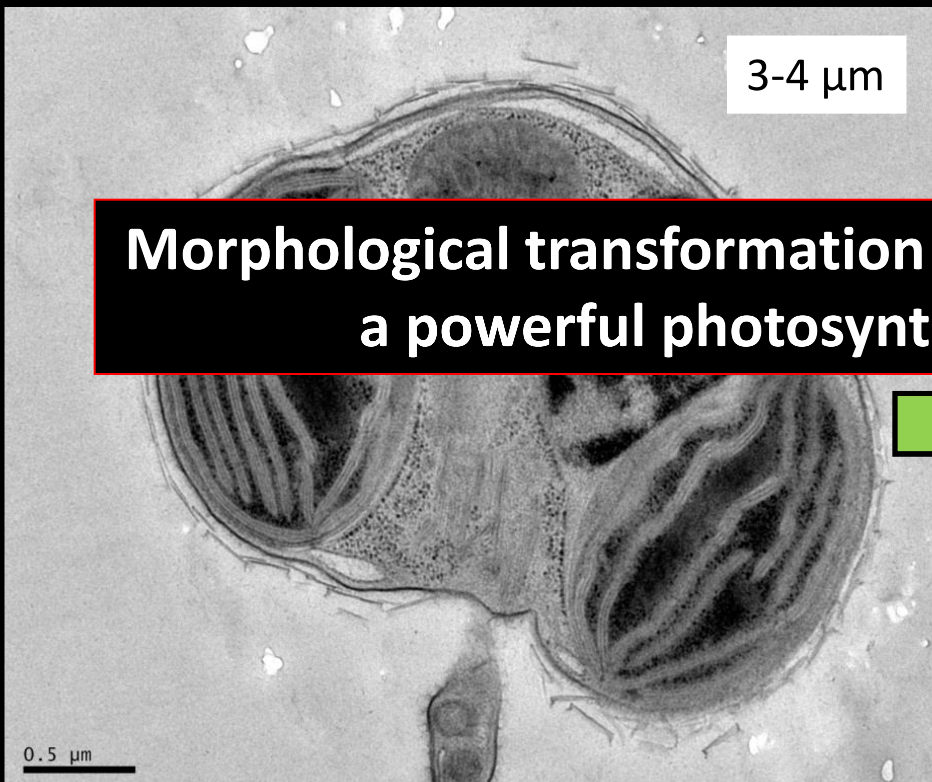




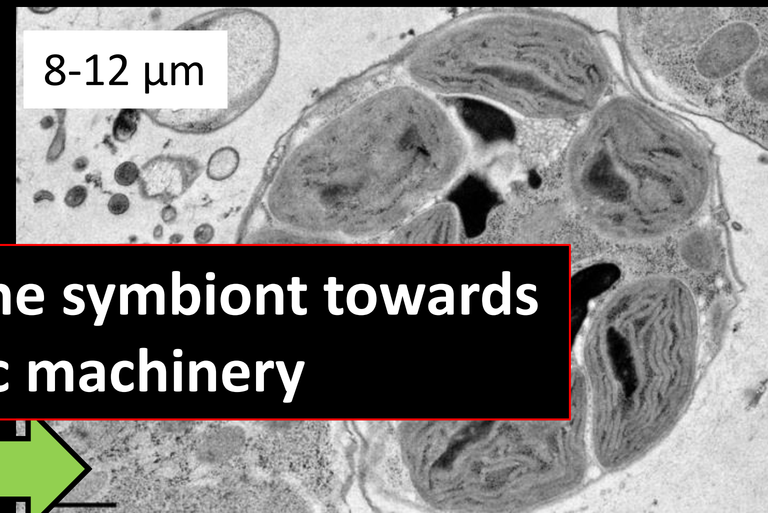
Acantharia- *Phaeocystis* (symbiont)

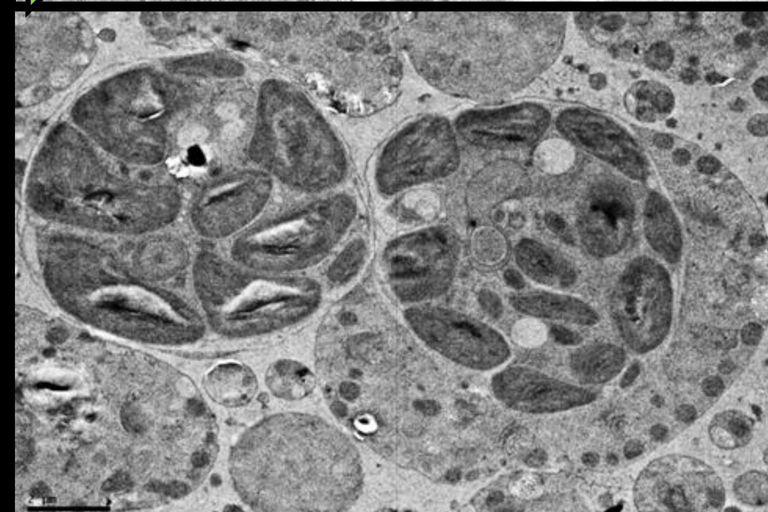
TEM

Outside the host



Inside the host

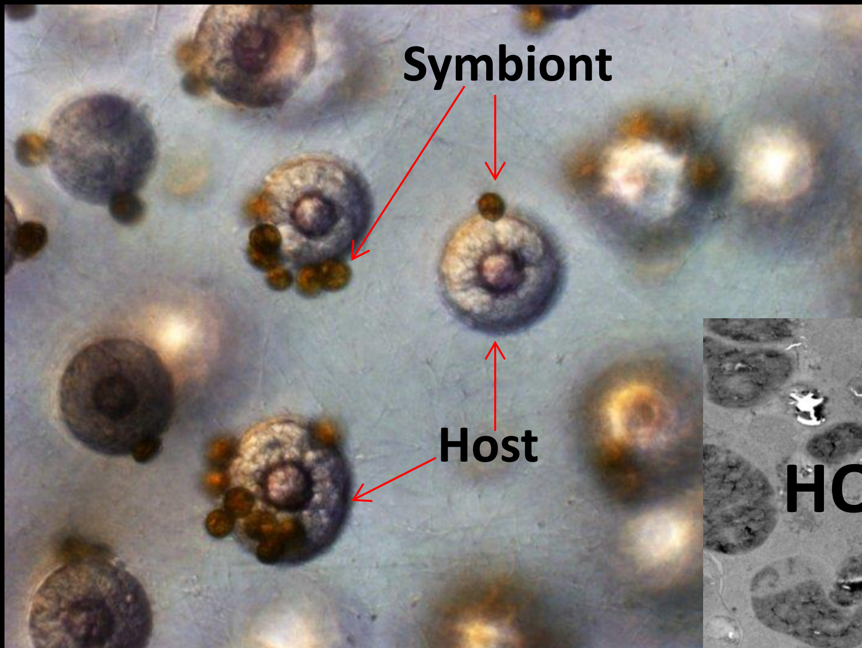




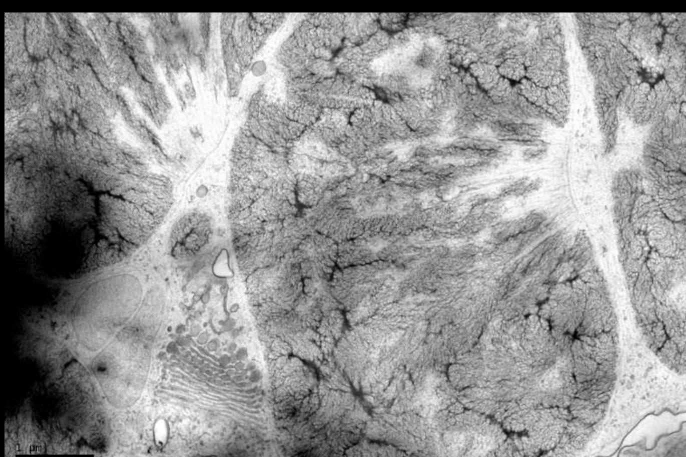
Morphological transformation of the symbiont towards a powerful photosynthetic machinery

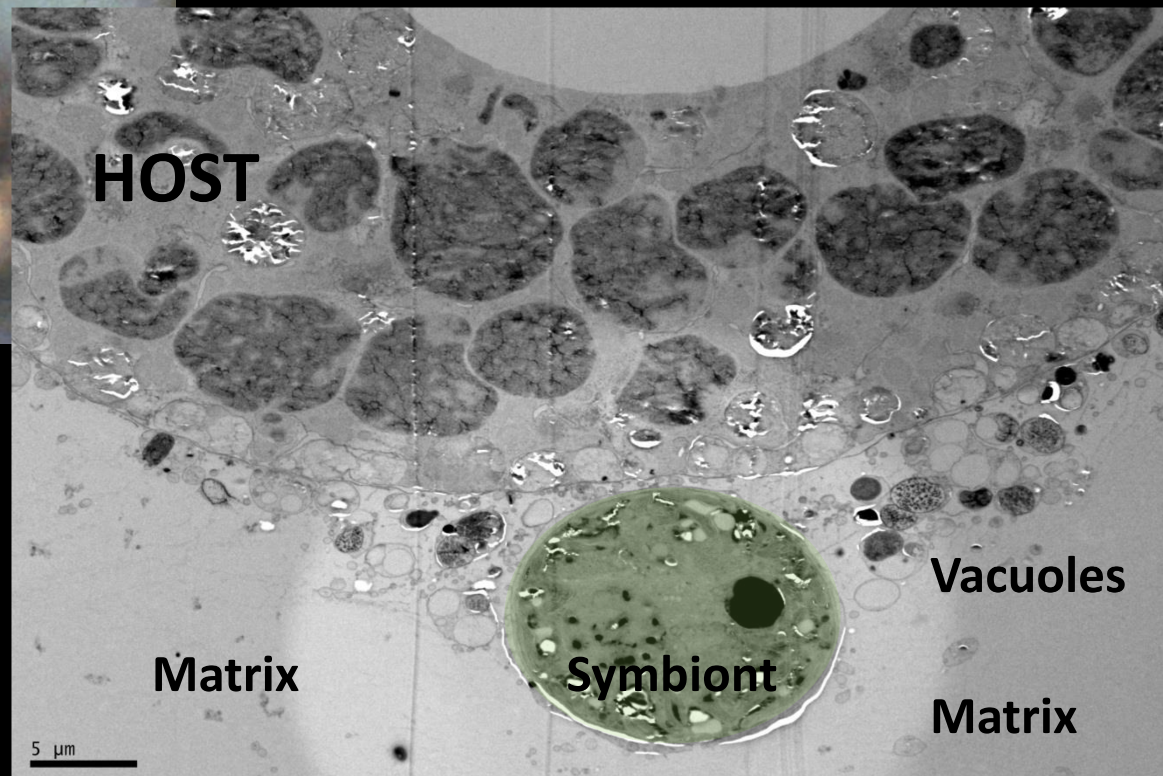
In the host: the volume of the microalga increases with <u>more chloroplasts and thylakoids</u>

Collodaria – *Brandtodinium* (symbiont)



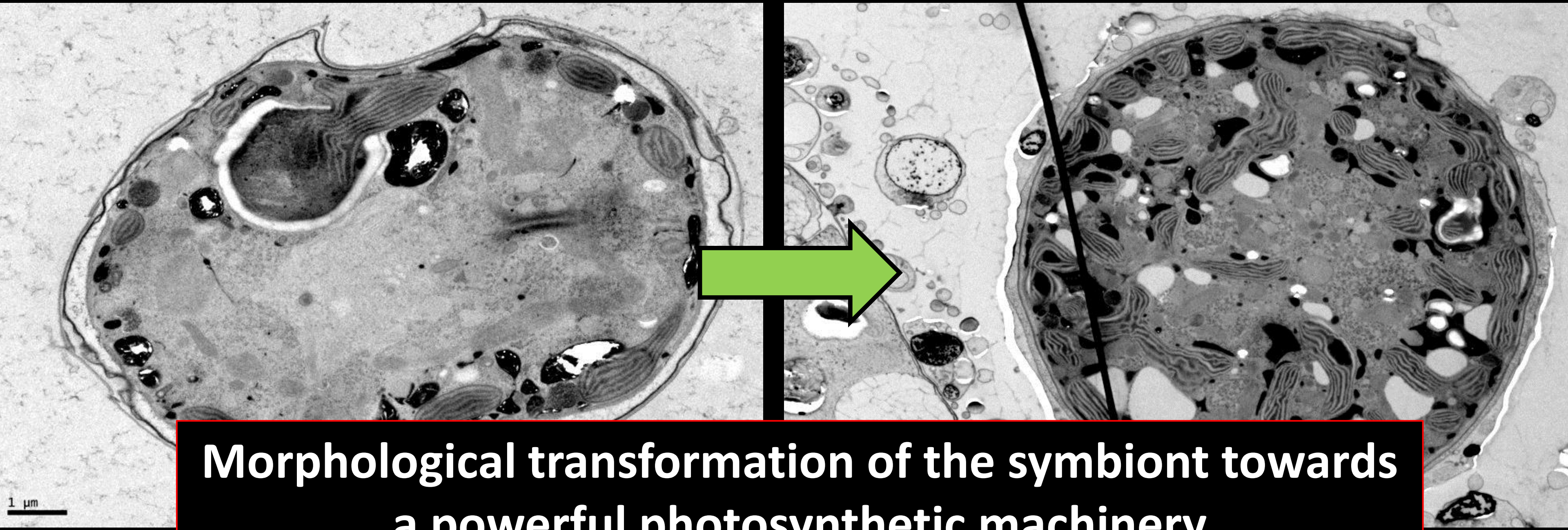
Symbionts are in the gelatinous matrix
(not in host cells)





Collodaria – *Brandtodinium* (symbiont)

Outside the host **Inside the host**



Morphological transformation of the symbiont towards a powerful photosynthetic machinery

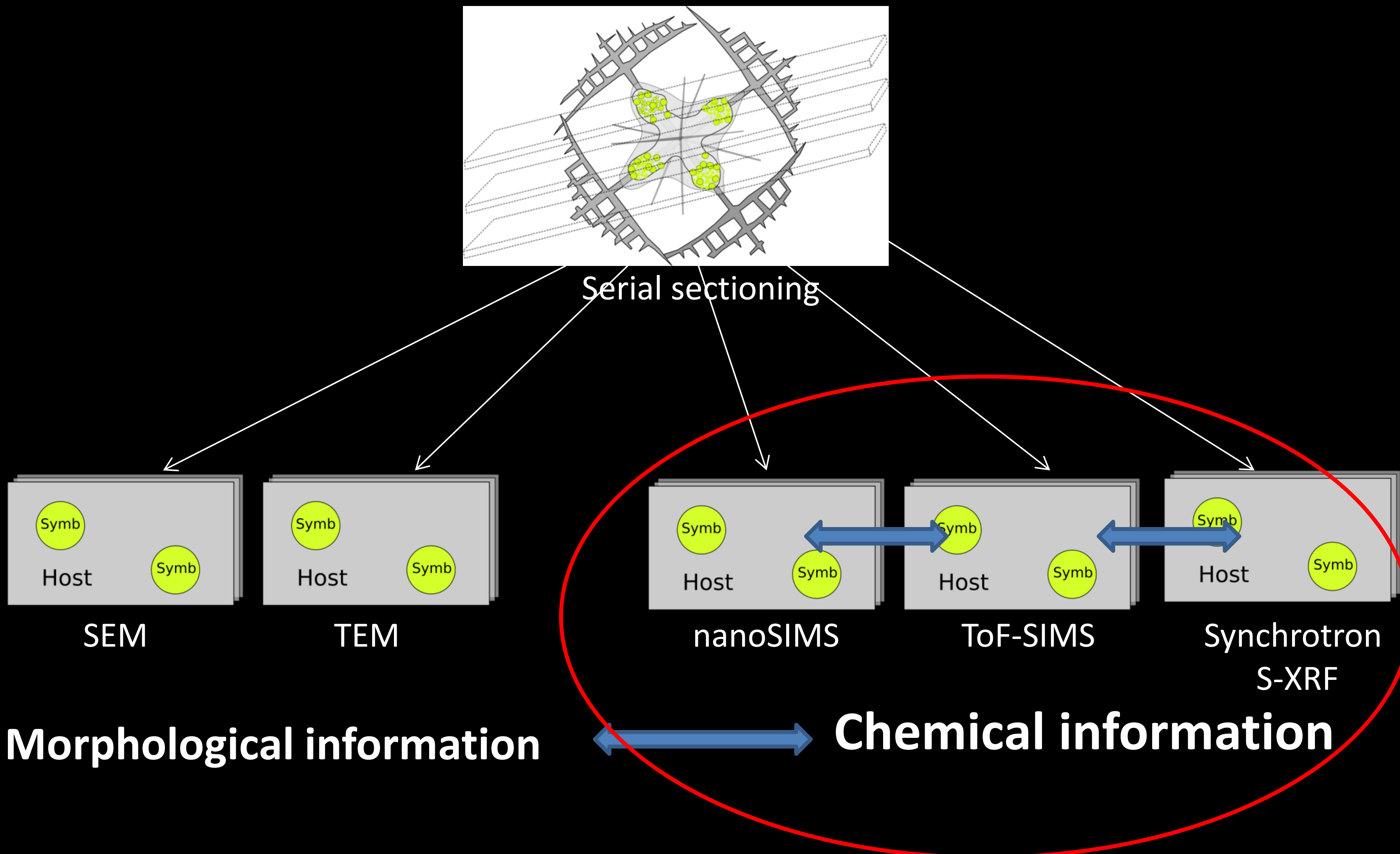
In the host: increase of the size of the symbiont and surface area of chloroplasts

Results- II

Metabolic transformation of the symbionts ?

Metabolic costs for the host ?

Correlated approach between electron microscopy and chemical imaging



ProVIS Centre Leipzig

HELMHOLTZ ZENTRUM FÜR UMWELTFORSCHUNG UFZ

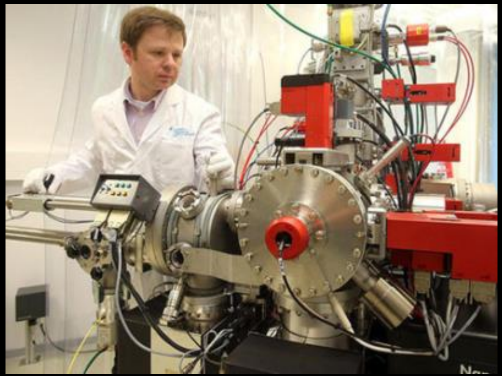
SIMS imaging

NanoSIMS

Mass species

$^{12}C^{14}N$ $^{31}P^{16}O2$ ^{31}P ^{16}O $^{12}C2$ ^{32}S

Analyses with look@nanosims software (Polerecky et al., 2012)



ToF-SIMS

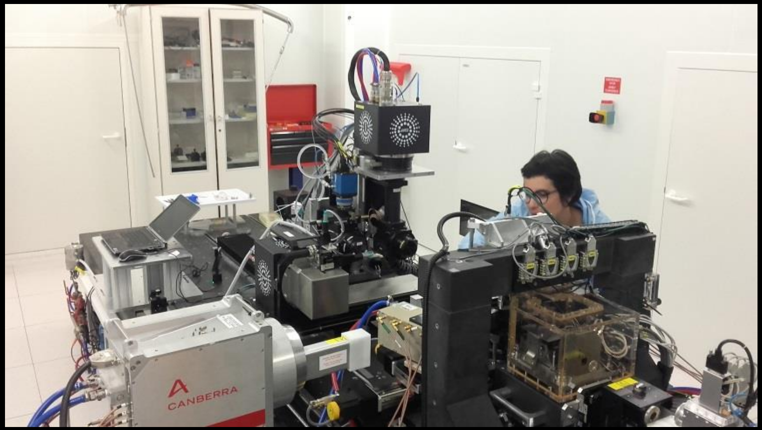
Analysis beam: Bi3
Sputter beam Ar-cluster

Mass spectrum (0-800 Da)

IonTof Surface Lab 6 software + reference database from literature

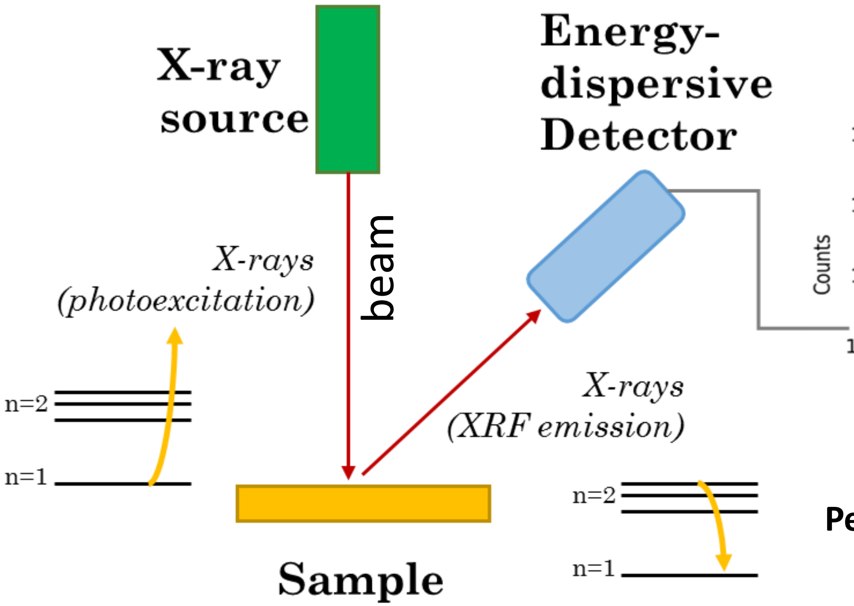
Synchrotron X-ray fluorescence
to visualize and quantify elements in cells

Synchrotron ESRF, Grenoble

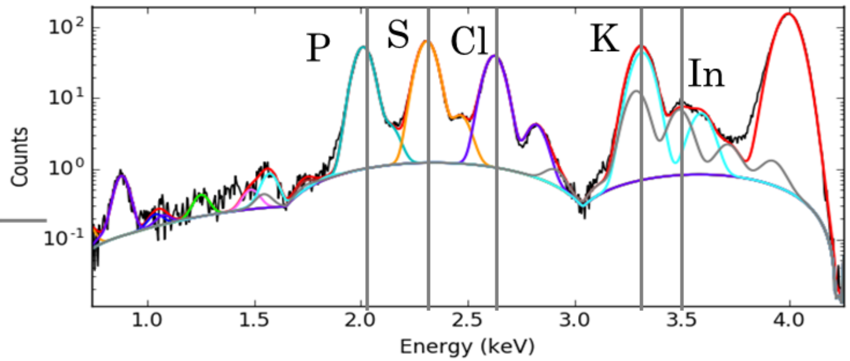


K
Ca
Cl
S
Fe
Mn
Cu

Beam lines: ID21 & ID16B



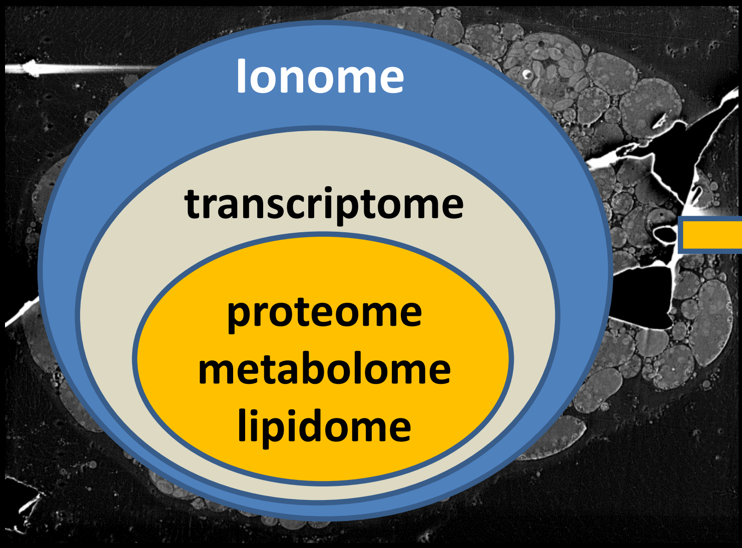
Peak position characteristic of a specific element

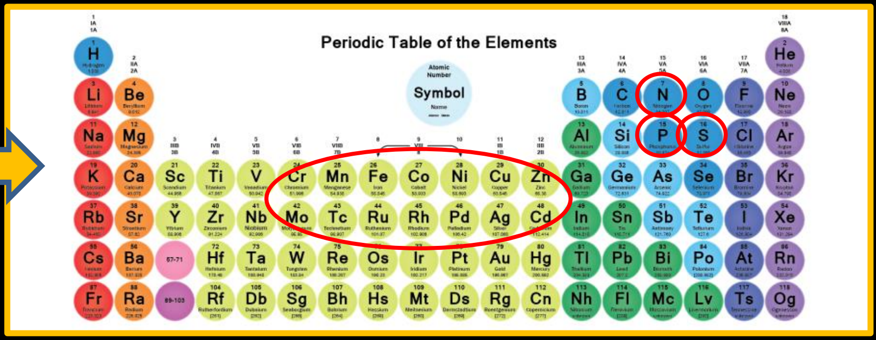


Peak area proportional to the concentration of the element

Visualization of the **ionome**

(elemental composition of a cell: macronutrients + trace metals)





Building blocks of molecules and metabolites

The ionome provides an additional view of the phenotypic state

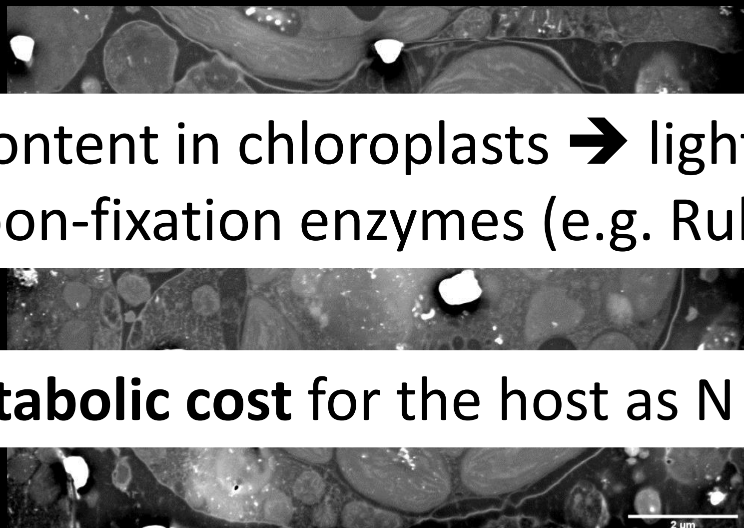
The ionome can reflect the metabolic capacity and needs of a cell

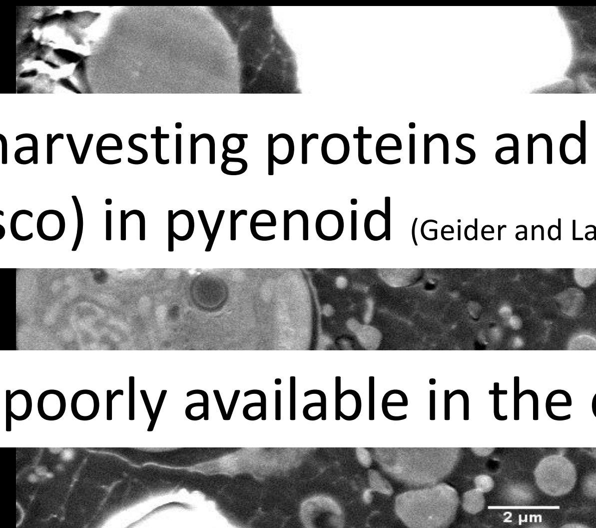
The ionome provides information about the biogeochemical impact

Nitrogen: a zoom-in into a single symbiont cell

High N content in chloroplasts ➔ light-harvesting proteins and pigments
And carbon-fixation enzymes (e.g. Rubisco) in pyrenoid (Geider and LaRoche 2002)

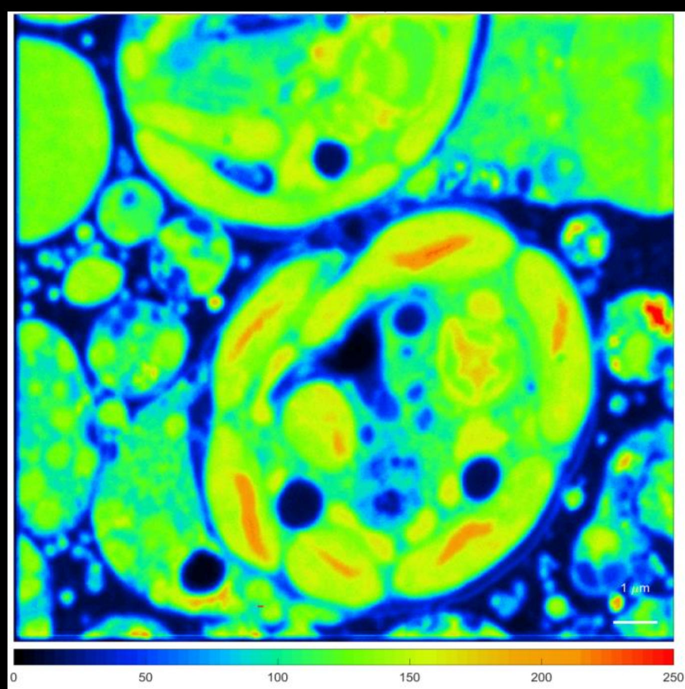
SEM

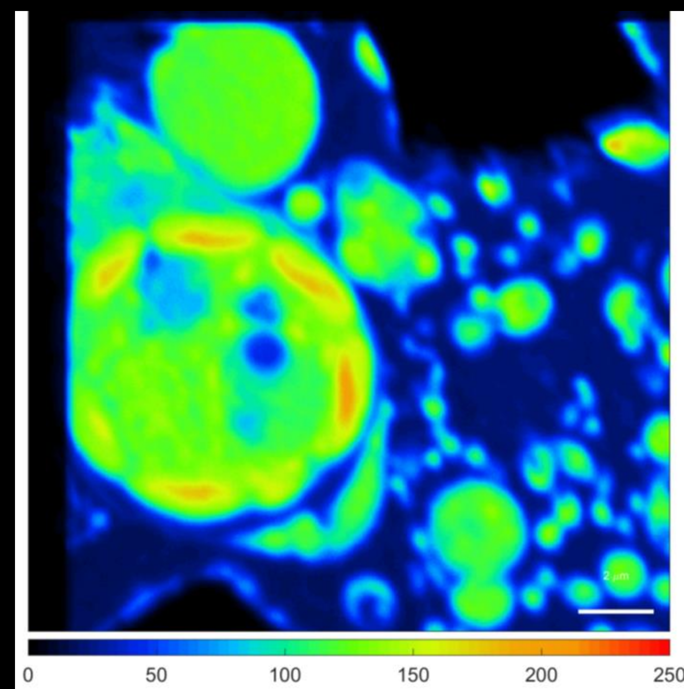




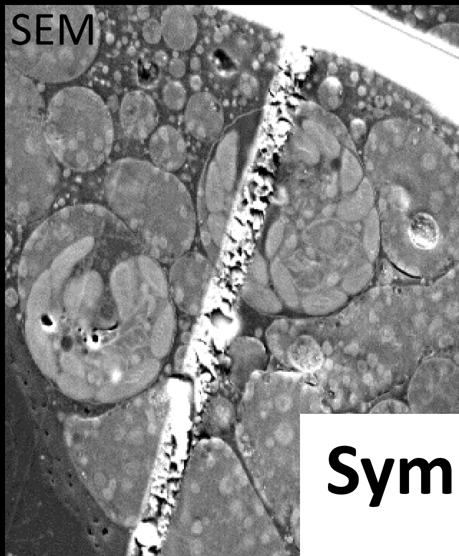
A **metabolic cost** for the host as N is poorly available in the ocean

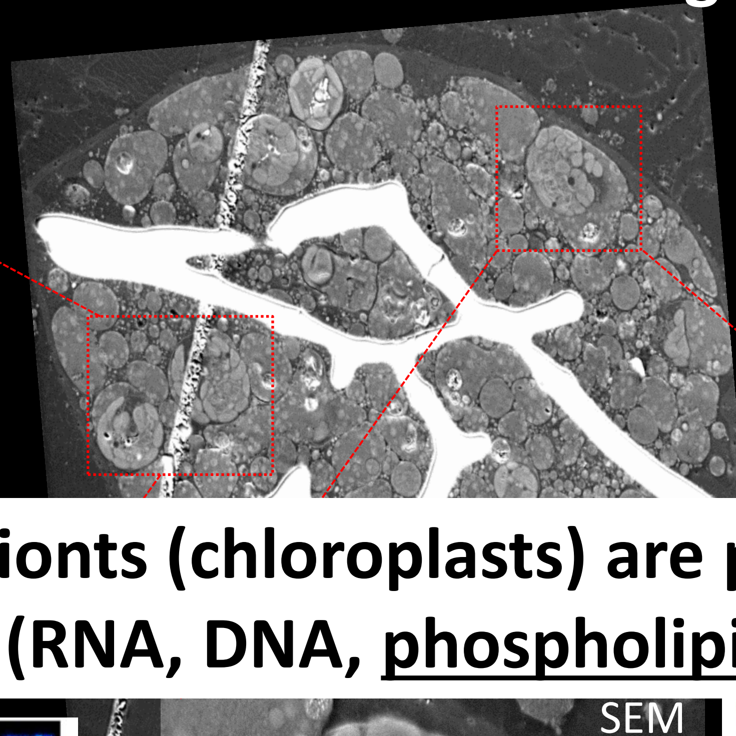
NanoSIMS
(CN^-)





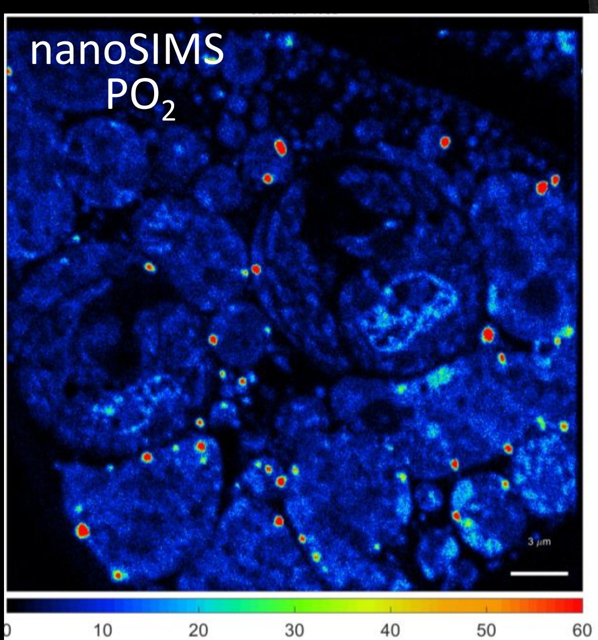
Phosphorous: a zoom-in into single symbiont cell

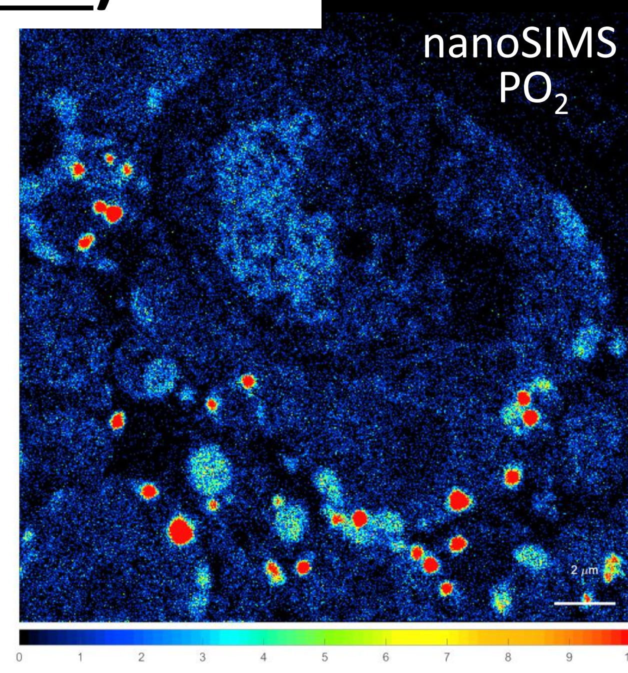




Acantharia- *Phaeocystis*

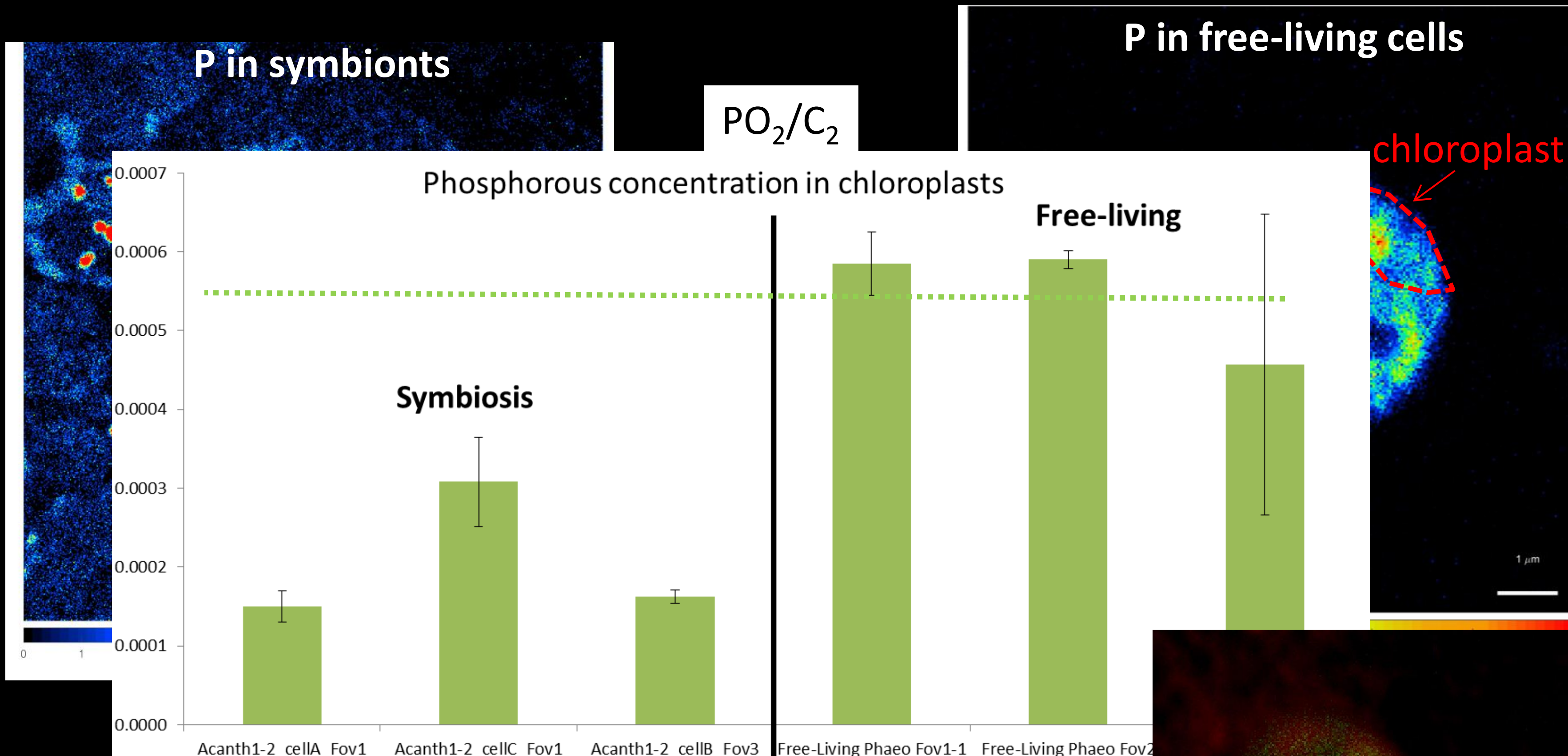
Symbionts (chloroplasts) are poor in P (RNA, DNA, phospholipids)

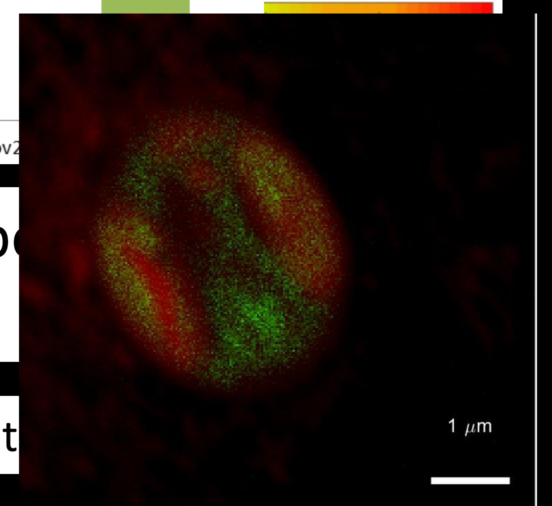




Phosphorous: a zoom-in into a single symbiont cell

<u>Symbiotic *vs* free-living stage</u>





P limitation by the host? To control symbiont p
spare the limiting P nutrient

P limitation can block cell division but does not inhibit the photosynt

Intracellular photosynthesis (numerous chloroplasts)

➔ source of ROS

existence of **antioxidant mechanisms** ?

➔ Sulfur metabolism

The chloroplast has a key role in sulfate reduction for the production of:

- The amino acids **cysteine** and **methionine**
- **Glutathione** and **phytochelatins** .
- **DMSP** **(Dimethylsulfopropionate), DMS (Dimethylsulfide) and DMSO (Dimethylsulfoxide)**

➔ These molecules play a role in antioxidant protection and global sulfur cycle

An antioxidant function for DMSP and DMS in marine algae

W. Sunda*, D. J. Kieber†, R. P. Kiene‡ & S. Huntsman*

LIMNOLOGY and OCEANOGRAPHY

ASLO

Limnol. Oceanogr. 00, 2017, 00–00

doi: 10.1002/lno.10491

Dimethylated sulfur compounds in symbiotic protists: A potentially significant source for marine DMS(P)

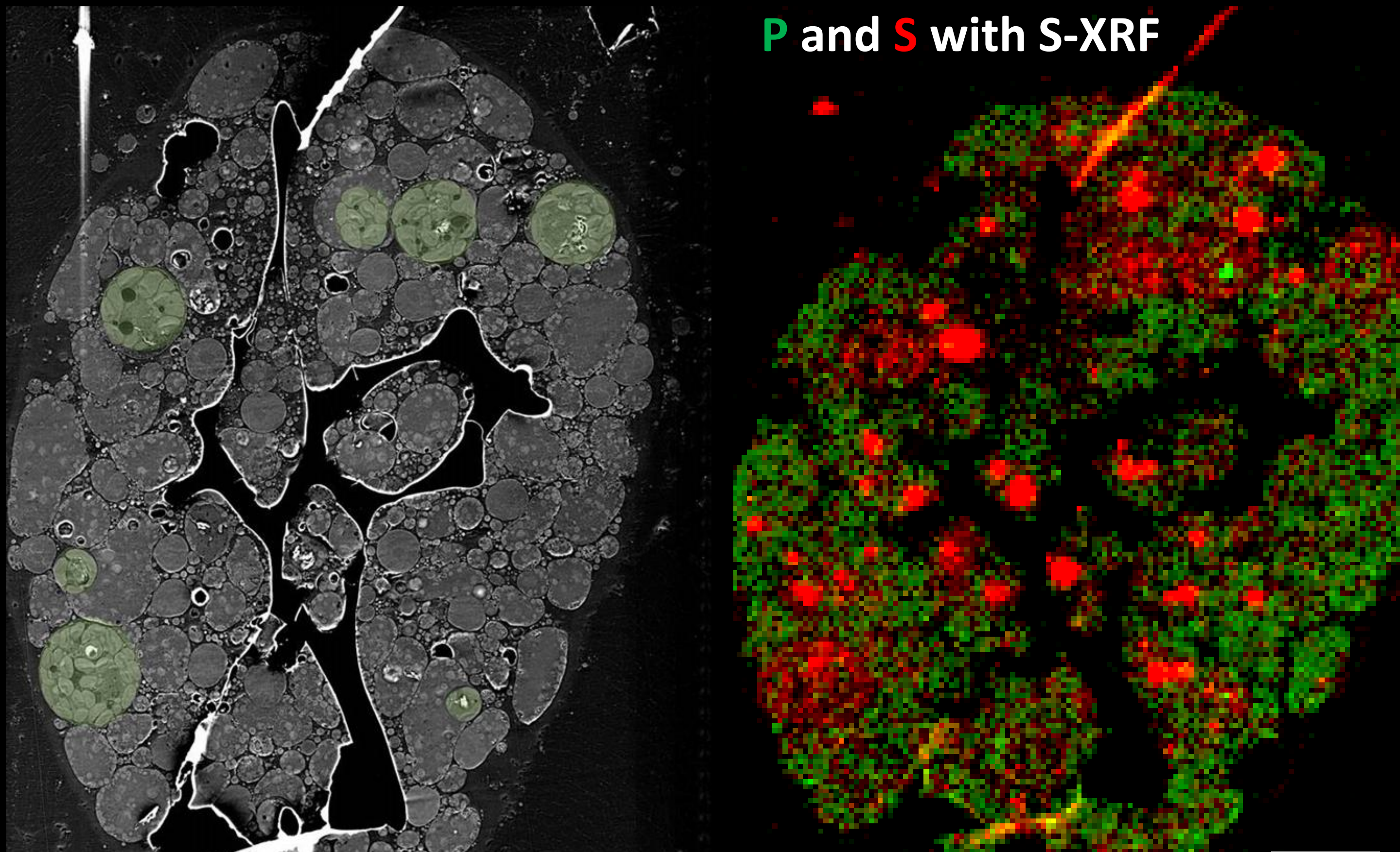
Andres Gutierrez-Rodriguez,[1,a*] Loic Pillet,[1,b] Tristan Biard,[1] Ward Said-Ahmad,[2] Alon Amrani,[2] Rafel Simó,[3] Fabrice Not[1]

[1]Sorbonne Universités, UPMC Université Paris 06, CNRS, Laboratoire Adaptation et Diversité en Milieu Marin UMR7144, Station Biologique de Roscoff, Roscoff, France
[2]The Institute of Earth Sciences, The Hebrew University, Jerusalem, Israel
[3]Department of Marine Biology and Oceanography, Institut de Ciencies del Mar (CSIC), Barcelona, Catalonia, Spain

Takahashi et al 2011; Meyer and Weis 2012; Sunda et al 2002; Deschaseaux, et al 2014

Subcellular mapping of sulfur

Acantharia - *Phaeocystis*



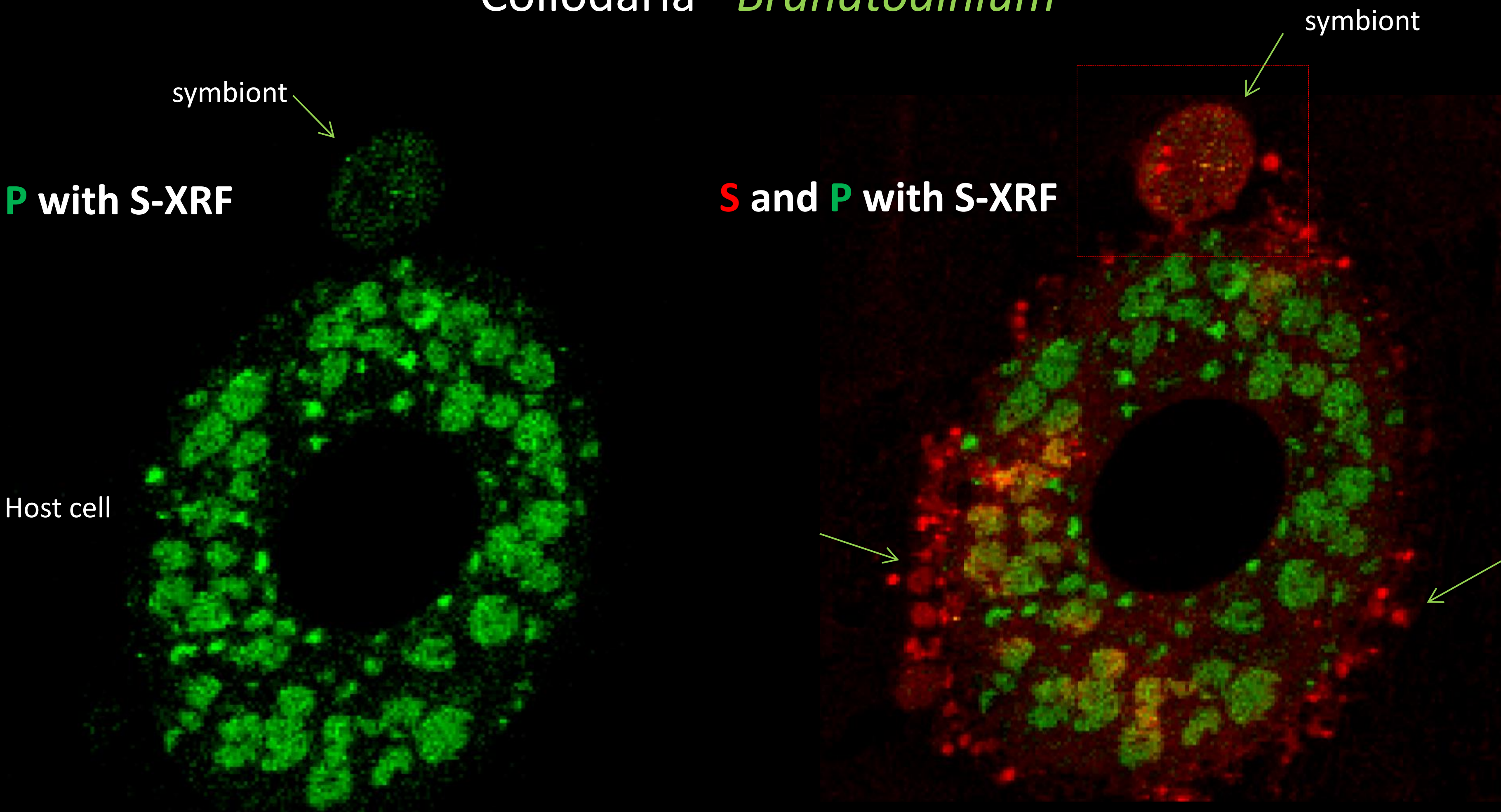
➔ High sulfur content in symbionts

(1.7 times more than in the host)

Collaboration with Giulia Veronesi (CEA – ESRF- Synchrotron Grenoble)

Subcellular mapping of sulfur

Collodaria - *Brandtodinium*



➔ High sulfur content in symbionts

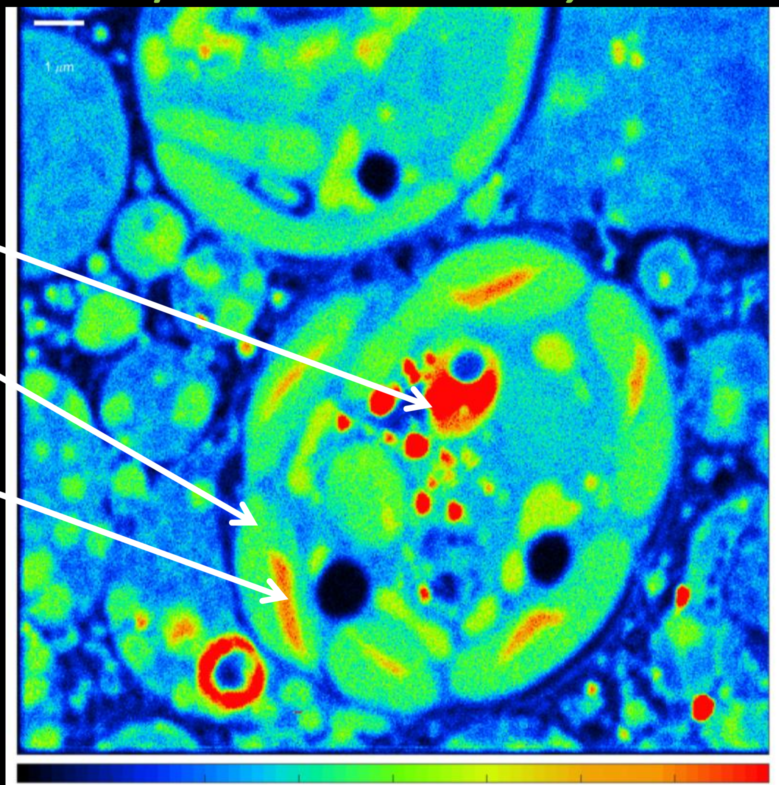
(2.8 times more than in the host)

Collaboration with Giulia Veronesi (CEA – ESRF- Synchrotron Grenoble)

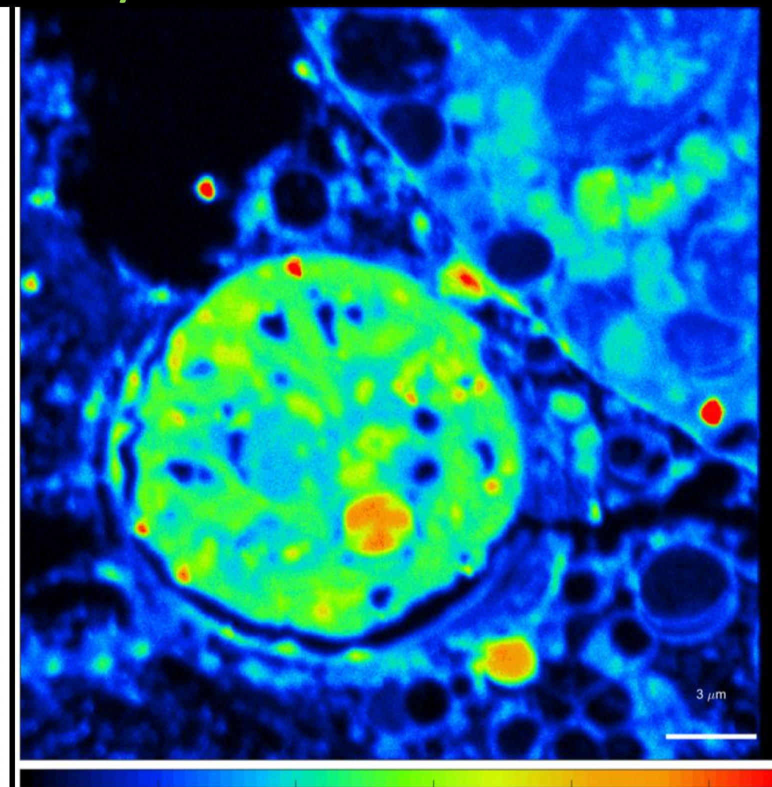
Subcellular mapping of sulfur in symbionts

^{32}S with nanoSIMS

Symbiont *Phaeocystis*



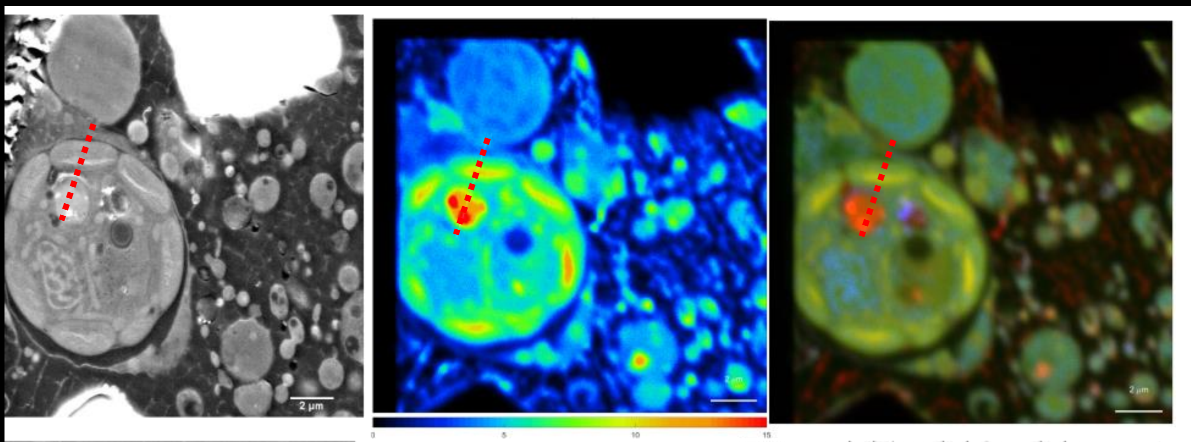
Symbiont *Brandtodinium*



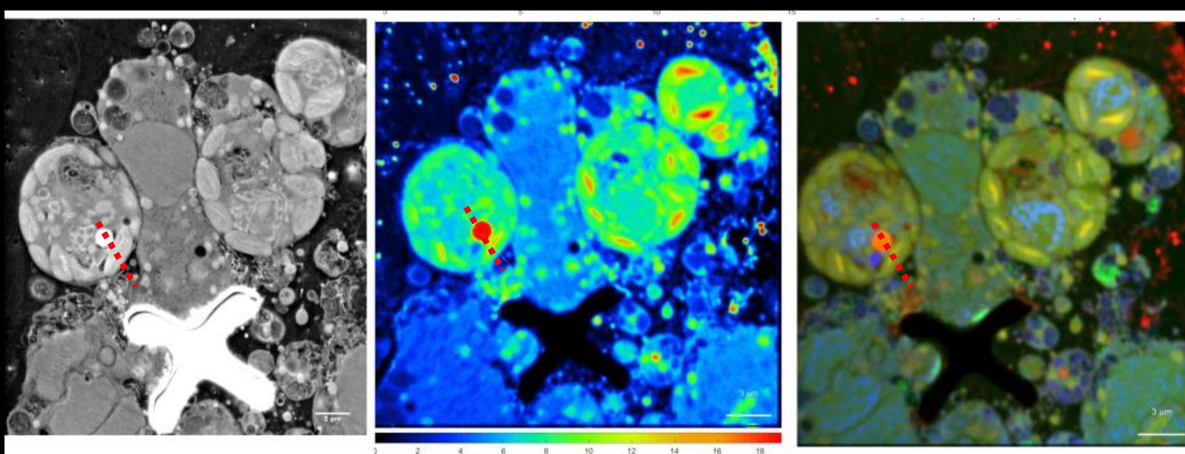
Sulfur in thylakoid membranes, pyrenoid, vacuoles

High sulfur content in vacuoles

SEM image S (nanoSIMS) S-N-P (nanoSIMS)



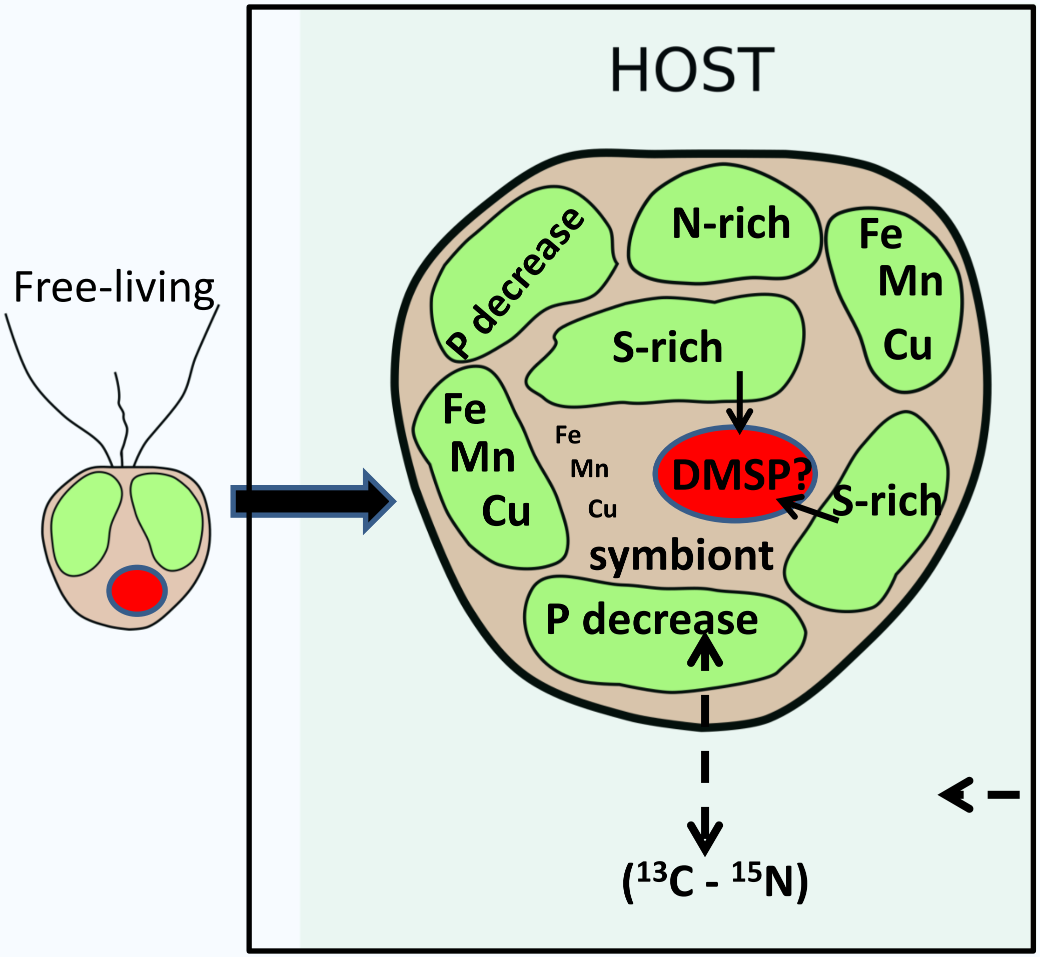
Vacuoles contain up to 6.5 times more S than in chloroplasts



S-rich vacuoles (with no P and no N) = DMSP storage?

Summary

Morphological and metabolic reconfiguration of the symbiont



Metabolic cost for the host

P limitation
Phospholipids synthesis?

Key source of reduced S

Needs for metals

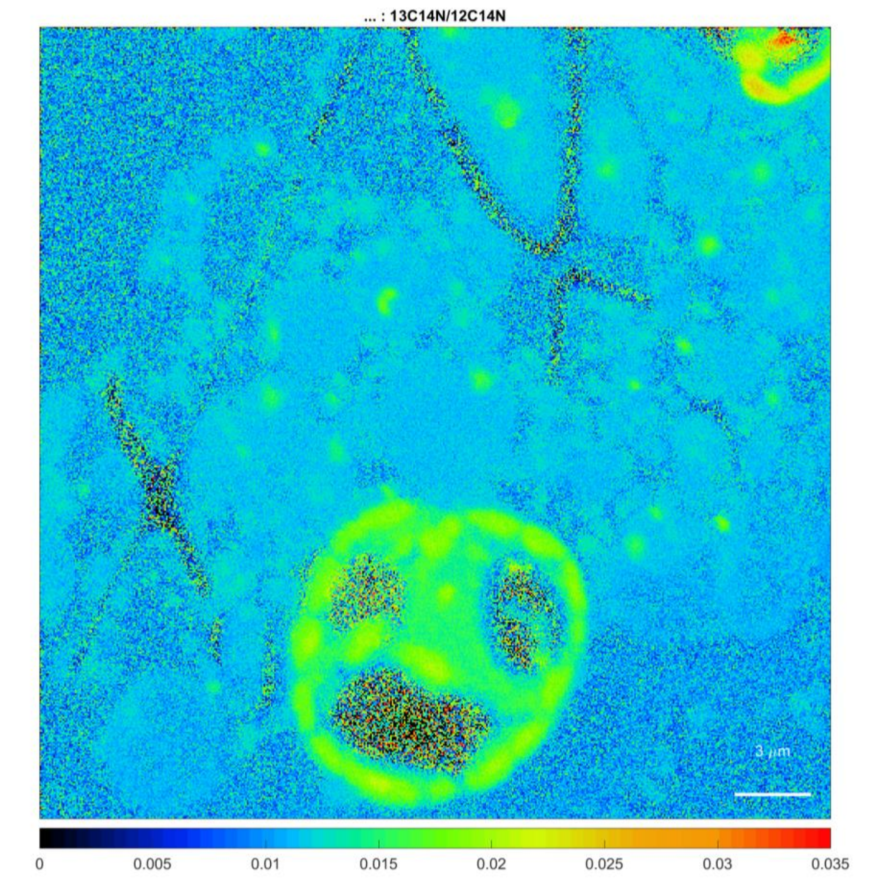
Ionome mapping
+
(^{13}C - ^{15}N)

...towards a more dynamic view of the metabolism

Perspectives

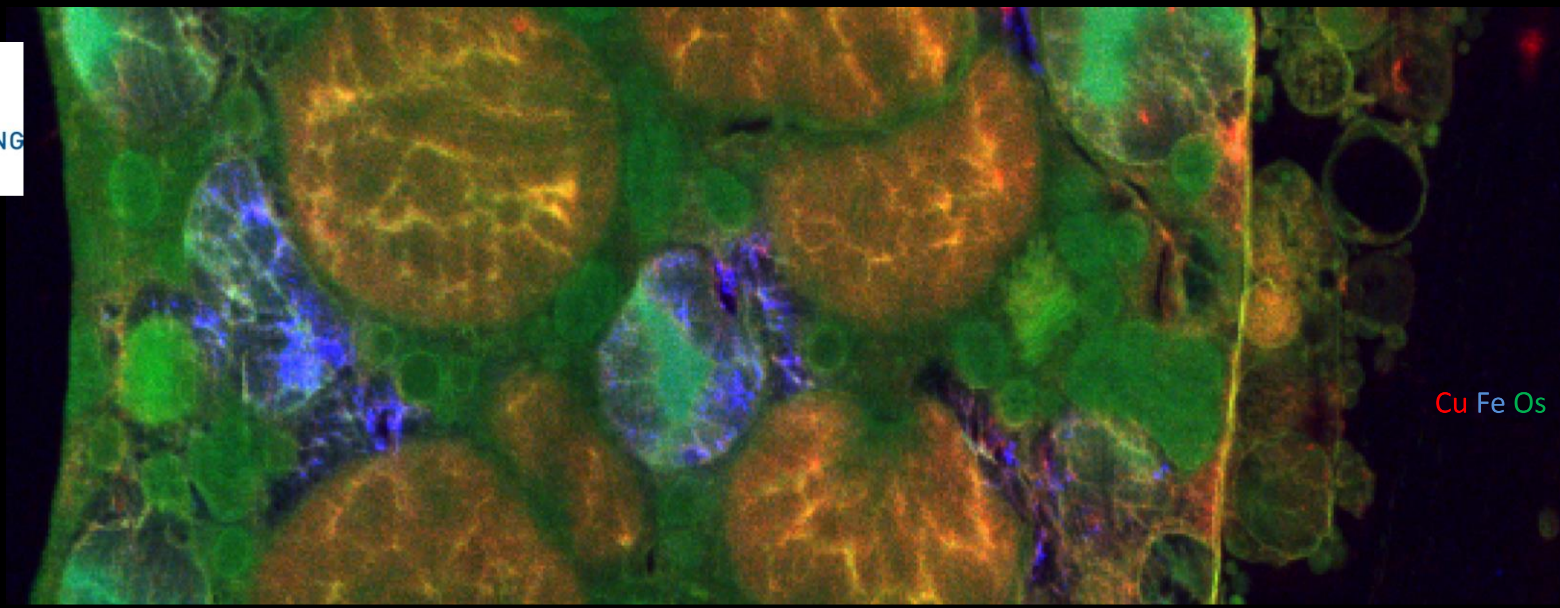
- **Visualize and quantify the uptake and transfer of C, N and S with stable isotopes (SIP-nanoSIMS) in correlation with ToF-SIMS**

^{13}C uptake - 5h incubation



Thank you for your attention

johan.decelle@ufz.de



- **PROVIS team**: Hryhoriy Stryhanyuk, Matthias Schmidt, Hans Richnow, Niculina Musat

- **Collaborators**: Benoit Gallet, Giulia Veronesi, Sergio Balzano, Alain Brunelle, Sophie Marro, John Dolan, Guillaume De Liege