doi:10.1017/S143192761900610X
Microsc. Microanal. 25 (Suppl 2), 2019

Visualization of the Ionome in Planktonic Symbioses

Johan Decelle[1*], Giulia Veronesi[2,3], Benoit Gallet[4], Hryhoriy Stryhanyuk[5], Sophie Marro[6], Rémi Tucoulou[3], Niculina Musat[5]

[1.] Cell and Plant Physiology Laboratory – University of Grenoble Alpes, CNRS, CEA, INRA; Grenoble, France.
[2.] Laboratoire de Chimie et Biologie des Métaux - University of Grenoble Alpes, CNRS, CEA ; Grenoble, France.
[3.] ESRF, The European Synchrotron Radiation Facility; Grenoble, France.
[4.] Institut de Biologie Structurale, University of Grenoble Alpes, CNRS, CEA; Grenoble, France.
[5.] Helmholtz Centre for Environmental Research – UFZ, Department of Isotope Biogeochemistry; Leipzig, Germany.
[6.] Sorbonne Universités, UPMC Université Paris 06, CNRS, Laboratoire d'Océanographie de Villefranche (LOV), Villefranche-sur-Mer, France.
* Corresponding author: johan.decelle@univ-grenoble-alpes.fr

In the ocean, planktonic microorganisms display a wide range of trophic modes, such as symbiotic associations [1]. The association between the single-celled radiolarians (host) and their symbiotic microalgae is one of the most prevalent eukaryotic symbioses in surface waters where nutrients are poorly available. However, despite their key ecological role, the functioning and physiology of the partnership remain poorly understood. The study of microbial symbioses where partners cannot be separated calls for dedicated high-resolution imaging methods that maintain the physical and chemical integrity of the host-symbiont association. More particularly, chemical imaging can provide information about the homeostasis of the nutrients (e.g. ionome) at the subcellular level, therefore highlighting the metabolic capacity and needs of the host and the symbiont [2]. The ionome can also provide information about the quality of the energy that is transferred up the food web and exported to the deep ocean.

Here, we visualized the ionome (composition and distribution of nutrients) in intact planktonic symbioses at the subcellular level, using a combination of chemical imaging instruments, such as secondary ion mass spectrometry (NanoSIMS) and synchrotron X-ray fluorescence (S-XRF). Live cells (host and the symbiotic microalgae *Brandtodinium nutricula*) were collected in the Mediterranean Sea and cryo-fixed with high-pressure freezing. Vitreous cells were then freeze-substituted and embedded in resin. Consecutive semi-thin sections were analyzed in parallel on nanoSIMS and S-XRF to unveil the macronutrients (nitrogen and phosphorous) and trace metals (iron and manganese), respectively, in organelles of each partner. Additional consecutive ultra-thin sections were also used for electron microscopy to obtain the ultrastructure of the regions of interest.

The subcellular distribution of nitrogen (nanoSIMS: $^{12}C^{14}N^-$), which is limiting in the ocean and mainly contained in proteins and amino acids, shows that symbiotic microalgae are N-rich, particularly their photosynthetic machinery (Figure 1). Sulfur ($^{32}S^-$) is also highly concentred in algal cells especially in vacuoles (Figure 1). By contrast, phosphorous content unveiled by nanoSIMS ($^{32}P^{16}O_2^-$) and S-XRF is lower in microalgae than in the host (Figures 1 and 2). Iron (Fe) exhibits a high concentration in specific vacuoles of the host, presumably as a source for the symbiotic microalgae, while manganese is homogeneously distributed in both partners (Figure 2). Our nanoscale elemental mapping paves the way for complementary studies to understand the metabolic interactions in planktonic symbioses.

References:
[1] J Decelle J, S Colin, R Foster, Marine Protists: Diversity and Dynamics. Springer: Tokyo, Japan (2015), p. 465–500.
[2] J Decelle *et al*., Current Biology **29** (2019), p.1-11. https://doi.org/10.1016/j.cub.2019.01.073
[3] The authors acknowledge funding from the European Union's Horizon 2020 research and innovation programme (Marie Sklodowska-Curie grant agreement 658442: MSCA-IF-2014). J.D was also supported by the LabEx GRAL (ANR-10-LABX-49-01) and Pôle CBS from the University of Grenoble Alpes. We are thankful for using the analytical facilities of the Centre for Chemical Microscopy (ProVIS) at UFZ Leipzig. The authors acknowledge the ESRF for providing beamtime and the platforms of the Grenoble Instruct centre (ISBG; UMS 3518 CNRS-CEA-UJF-EMBL).

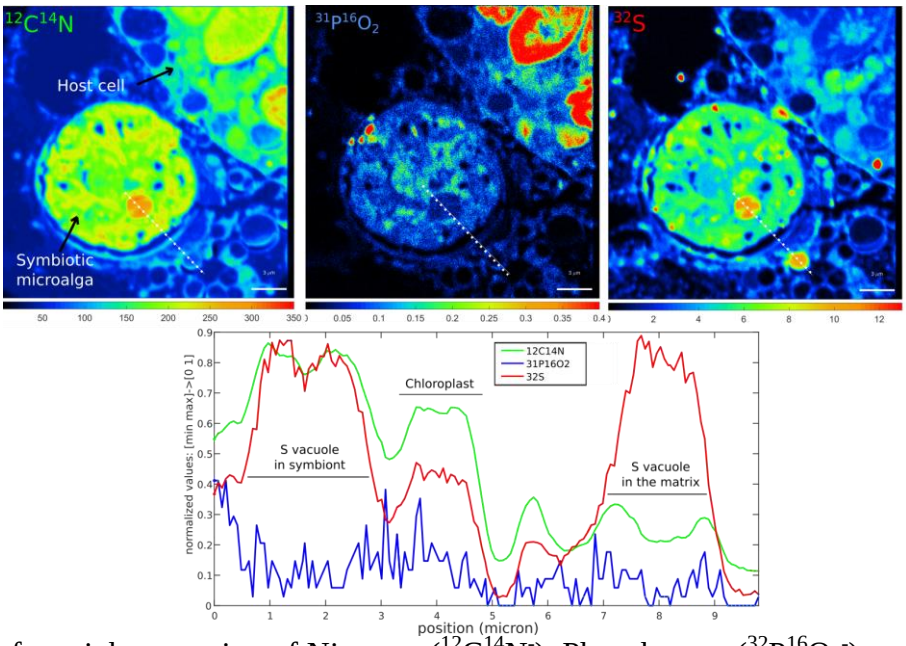

Figure 1. From left to right, mapping of Nitrogen ($^{12}C^{14}N^-$), Phosphorous ($^{32}P^{16}O_2^-$) and Sulfur ($^{32}S^-$) in the symbiotic microalga and host cell unveiled by nanoSIMS. Graph below: The content of these three secondary ions were compared across the dashed line in the organelles of the symbiont.

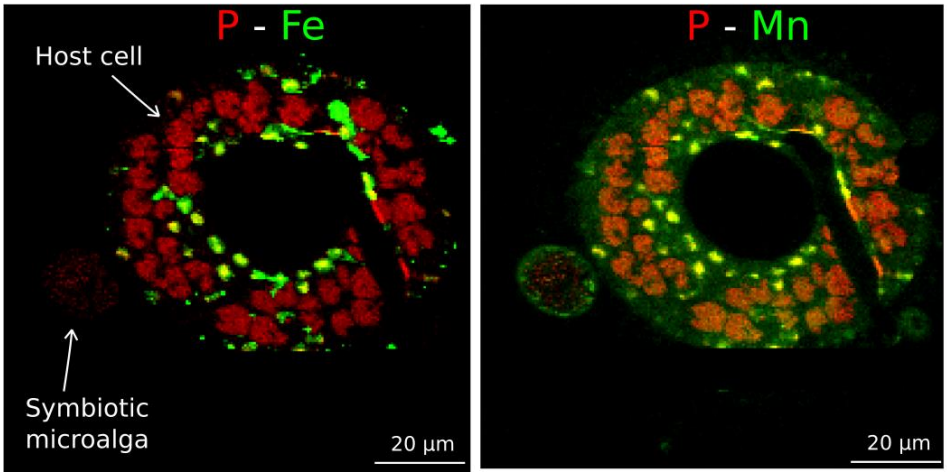

Figure 2. Quantitative mapping of Phosphorous (red) and the trace metals Iron (green, left image) and Manganese (green, right image) in the host and symbiotic microalgal cell unveiled by S-XRF.

https://doi.org/10.1017/S143192761900610X Published online by Cambridge University Press