EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:
https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Deadlines for submission of Experimental Reports

Experimental reports must be submitted within the period of 3 months after the end of the experiment.

Experiment Report supporting a new proposal ("relevant report")

If you are submitting a proposal for a new project, or to continue a project for which you have previously been allocated beam time, you must submit a report on each of your previous measurement(s):

- even on those carried out close to the proposal submission deadline (it can be a *"preliminary report"),*
- even for experiments whose scientific area is different form the scientific area of the new proposal,
- carried out on CRG beamlines.

You must then register the report(s) as "relevant report(s)" in the new application form for beam time.

Deadlines for submitting a report supporting a new proposal

- 1st March Proposal Round - **5th March**
- 10th September Proposal Round - **13th September**

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report in English.
- include the experiment number to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Exploring the localization and potential functional links between stored iodine and iron in brown algae	**Experiment number**: LS-2772
Beamline: ID16A-NI	**Date of experiment**: from: 2 to: 8 June 2018	**Date of report**:
Shifts: 6 days	**Local contact(s)**: Dr. Peter Cloetens	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

Background. Brown algae of the Laminariales (kelps) are the strongest accumulators of iodine among living organisms. They represent a major pump in the global biogeochemical cycle of iodine and in particular, the major source of iodocarbons in the coastal atmosphere. Nevertheless, the chemical state and biological significance of accumulated iodine have remained unknown. Elucidation of these questions was the objective of this study. Using an interdisciplinary array of techniques, chiefly relying on synchrotron X-ray absorption spectroscopy, we showed that the accumulated form is iodide [1], which readily scavenges a variety of reactive oxygen species (ROS; Fig. 1). We have previously shown that its biological role is that of an inorganic antioxidant, the first ever to be described in a living system. Upon oxidative stress, iodide is effluxed. On the thallus surface and in the apoplast, iodide detoxifies both aqueous oxidants and ozone, the latter resulting in the release of high levels of molecular iodine and consequent formation of hygroscopic iodine oxides leading to particles, which are precursors to cloud condensation nuclei. When kelp thalli are submerged, this process impacts iodine speciation in seawater [1].- In several aspects, iodide is unique as a biological antioxidant. Among the halides, it has by far the best antioxidant properties; yet, bromide complements it for the detoxification of superoxide [2].-

Results and Discussion. Our recent µXRF nanoprobe tomography work at the ESRF yielded findings concerning the subcellular localization of iodine in cells, with implications for its mechanism of uptake, storage and efflux, alongside with localization information for a range of other elements including strontium and bromine (Fig. 1). In part it confirms previous work [3] that iodine is concentrated in the cortical (surface) cell layer. However, the tomography images show clearly (Fig. 1 center left and right) that the storage of iodine is

intracellular, likely in the vacuoles (or physodes, their brown algal variants) and not in the cell wall, contrary to previously published work [3]. This was most important, because in the previously published work samples were prepared by chemical fixation, which had led to artefactual re-localization of dissolved elements to the cell wall, leading to entirely wrong conclusions about the mechanism of iodine storage and mobilization in brown algae. In the current study, samples had been prepared by shock-freezing in supercooled isopentane and were measured in frozen-hydrated state.

Conversely, Sr is strongly associated with alginate in brown algal cell walls (Fig. 1 left), de facto providing a useful histochemical marker for them. Interestingly, bromine shows a somewhat different tissular and intracellular distribution pattern than iodine (Fig. 1 right). It is less concentrated in the cortical cells, but more throughout the medulla, and apparently also throughout the entire vacuole of medullar cells.

Outlook and wider context. Currently, we are complementing this work by transmission electron microscopy using chemically fixed vs. cryofixed *L. digitata* tissues in order to unambiguously establish the nature of the vesicles containing the intracellular iodine store (in particular, whether these are indeed vacuoles). We are planning to publish this in an interdisciplinary paper together with results of radiolabeling experiments showing that iodide is mobilized upon oxidative stress by a hitherto-uncharacterized anion transporter different from the well-known eukaryotic chloride channels.

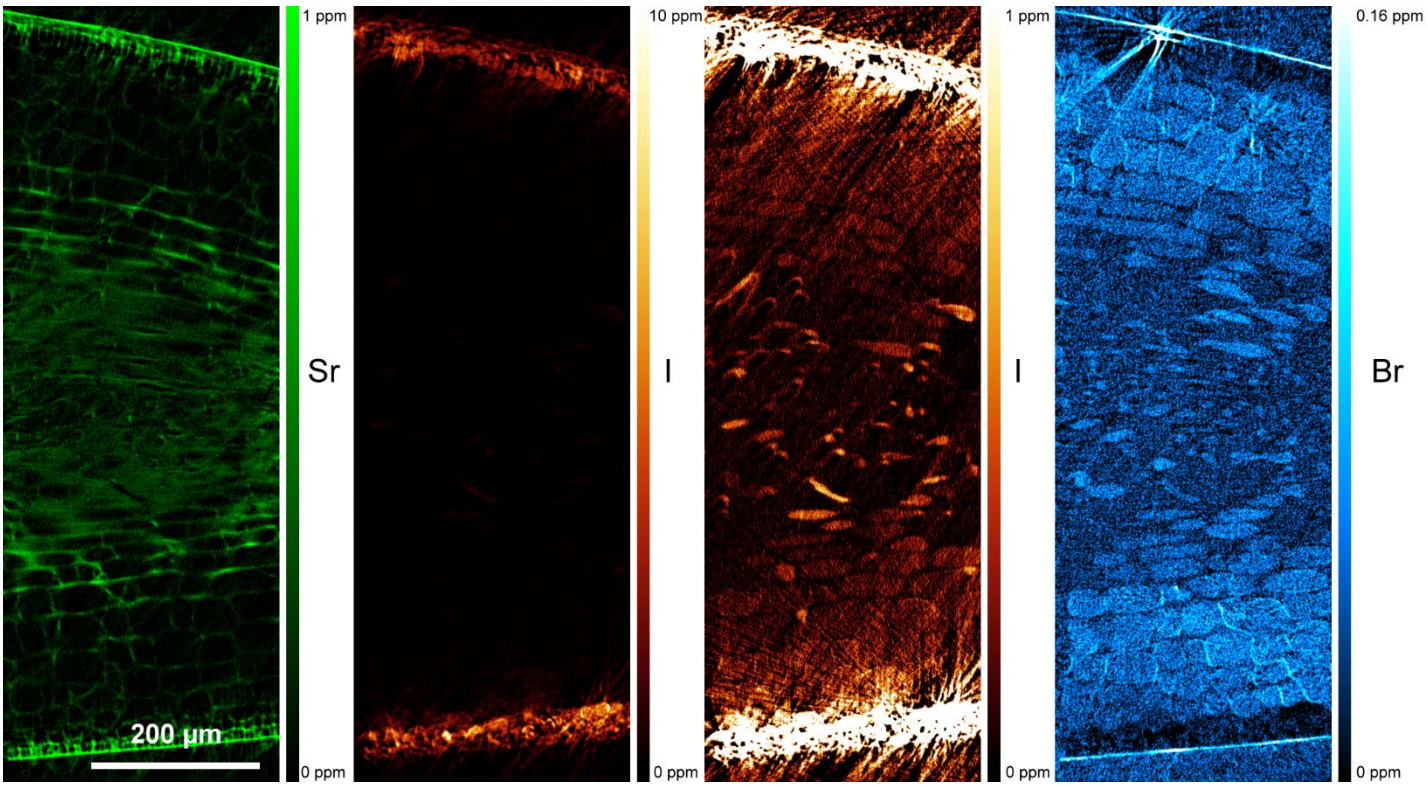

Fig. 1. Sub-cellular distribution of I and Sr in *Laminaria digitata* (measured at Sr Kα and I Kα with 0.8 µm resolution at beamline ESRF ID16A; usable resolution limited by readout electronics at beamsize 0.013µm). Opposite subcellular distribution patterns for three elements
- Strontium is mostly in cell walls, like by passive adsorption from seawater
- Bromine is in vacuoles mostly in the inner cell layers of the thallus
- Iodine is in vacuoles mostly of the outer cell layers of the thallus

Contrary to earlier reports based on chemically fixed samples, in shock-frozen hydrated samples bromine and iodine are localized not in the cell wall but in the vacuole. This demonstrates the importance of measuring properly prepared samples, which means for plant samples in synchrotrons usually shock-frozen hydrated.

References

[1] Küpper, F.C., et al., *Iodide accumulation provides kelp with an inorganic antioxidant impacting atmospheric chemistry.* Proceedings of the National Academy of Sciences of the United States of America, 2008. **105**(19): p. 6954-6958.

[2] Küpper, F.C., et al., *Speciation studies and antioxidant properties of bromine in Laminaria digitata reinforce the significance of iodine accumulation for kelps.* Journal of Experimental Botany, 2013. **64**(10): p. 2653-2664.

[3] Verhaeghe, E.F., et al., *Microchemical imaging of iodine distribution in the brown alga Laminaria digitata suggests a new mechanism for its accumulation.* Journal of Biological Inorganic Chemistry, 2008. **13**: p. 257-269.