



POSTER #6

## CHARACTERIZING MINERAL-BEARING VESICLES IN SEA URCHIN EMBRYOS

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Sea urchin embryos have endoskeletons comprised of two calcitic spicules. Spicule growth takes place by the initial deposition of amorphous calcium carbonate (ACC)<sup>1</sup> in vesicles inside the spicule-forming cells<sup>2</sup>(PMCs). The calcium in the mineral-bearing vesicles was recently reported to originate from body fluid internalization and in part from calcium channels<sup>3</sup>. Using cryo-scanning electron microscopy (SEM) to image high pressure frozen and cryo-sectioned samples of embryos, we were able to detect several types of vesicles inside the PMCs. Some vesicles have granulated texture, some appear smooth, some have backscattered electrons signal, and some contain lipids or proteins (Fig. 1). Performing energy dispersive spectroscopy (EDS) measurements under cryogenic conditions revealed that some of these vesicles are rich in sodium, which indicates sea water internalization. Other vesicles give signals for potassium and calcium and are still under investigation.

In order to perform quantitative Ca mapping of the cell content in 3D we apply cryo-soft X-ray tomography (SXT). Performing cryo-SXT using energies in the Ca near-edge region will allow us to determine the Ca concentrations in intra-cellular vesicles in PMCs. Preliminary results obtained by this method provided a proof of concept of the feasibility of the technique. Figure 2 shows a slice through a 3D reconstruction of a tilt series obtained by cryo-SXT. In this image the many vesicles around the nucleus have different grey levels, indicating different levels of carbon and perhaps even different levels of calcium. We conclude that the compositional landscape of the vesicles in the PMCs, some of which fulfil a fundamental role in the mineralization of the spicules, is complex.

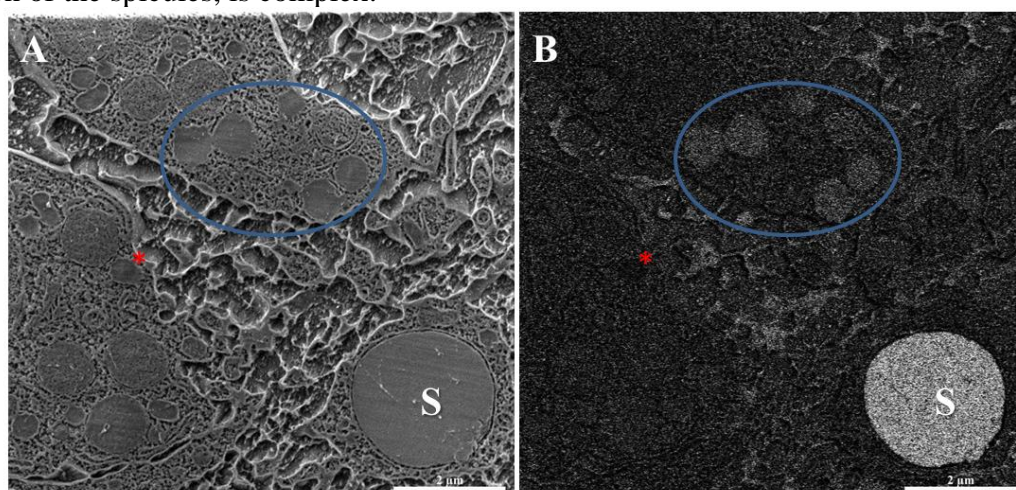


Figure 1 – Cryo-SEM images of a high pressure frozen and cryo-sectioned sample showing a spicule (S) with two adjacent PMCs. (A) SE image, (B) BSE image. Six vesicles (in the circle) with different textures have BSE signals, which indicate electron dense contents. The vesicle marked by an asterisk appears darker than the surrounding, indicating carbon rich content such as in lipids or proteins.

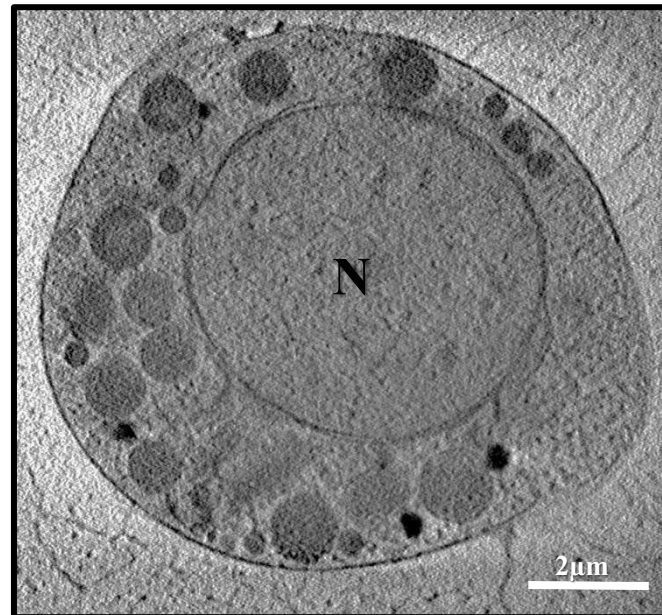


Figure 2 - A slice through a 3D reconstruction of soft X-ray tomogram of a cell disaggregated from a sea urchin embryo. The different levels of grey in the vesicles around the nucleus (N) are indicative of variations in carbon and calcium content.

#### References

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