Sample Preparation Methods

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Osmium, epon and immunogold labeling - friend or foe?

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For studies concerning the ultrastructure of microorganisms (Bacteria, Archaea, and unicellular Eukarya), cells or tissues, it is essential to preserve them in a close to natural state [1]. This can be achieved by the application of high-pressure freezing, freeze substitution and subsequent embedding in a resin polymer [2]. In the special case of the diatom *Phaeodactylum tricornutum*, the combination of cryo-preparation with suitable freeze substitution media is crucial because of the high number of membranous structures within this organism [3]. As an example, the plastid of *P. tricornutum* is the result of a so called secondary endosymbiosis and is therefore surrounded by four membranes. For a better visibility of membranes, freeze substitution is performed with acetone containing 0.2 up to 2% OsO_4, 0.5% uranyl acetate and small amounts of water [4]. Additionally, and in contrast to the general procedure for immunogold labelling on thin sections, the cells were usually embedded in epon resins. After slight modifications of substitution protocols, the localization of proteins, GFP-fusion proteins and sugars is still possible after this treatment with the advantage of optimal structural preservation (Figure 1).

As a proof of principle, the above mentioned substitution medium as well as ethanol containing glutaraldehyde, formaldehyde and uranyl acetate were also used for the processing of high-pressure frozen eukaryotes like the plant pathogen *Ustilago maydis* and several Bacteria and Archaea. In the latter case for example, the localization of the major S-layer protein Msed_1806 of the crenarchaeal species *Metallosphaera sedula* TH2 at the surface of the coccoid cells was successful (Figure 2)[5].

It could further be shown, that high-pressure freezing seems to be a key step in preservation of epitopes to overcome the negative effects of OsO_4 and epon on antigenicity. In almost all cases, the localization of the respective proteins or sugars was not possible when cells were chemically fixed with glutaraldehyde.

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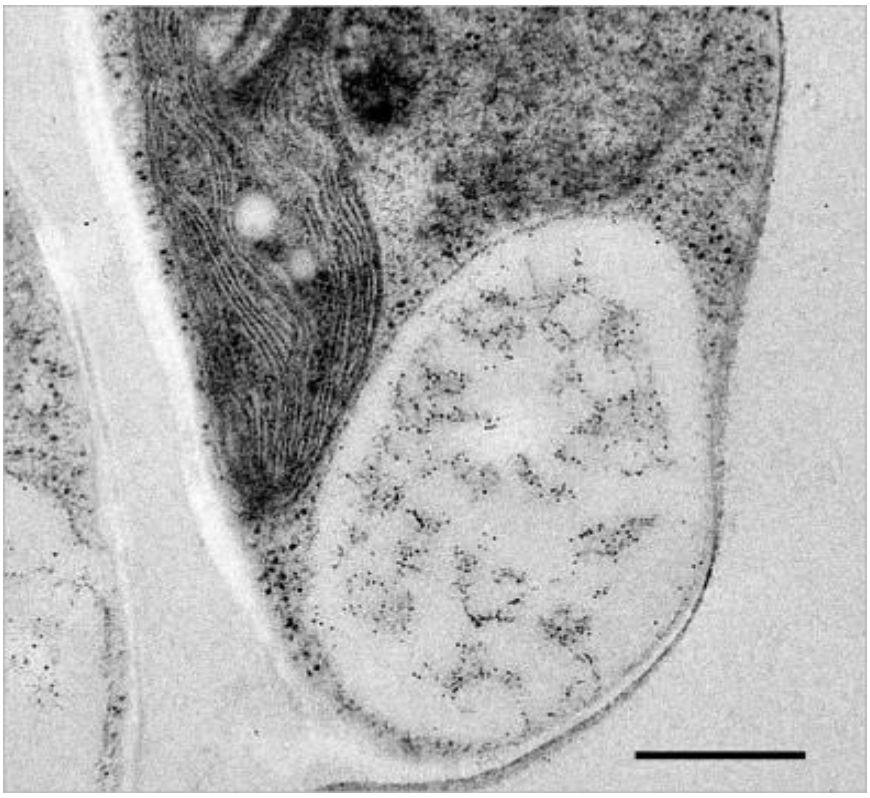

Figure 1. Transmission electron micrograph of a high-pressure frozen *P. tricornutum* cell showing the marbled vacuole. Within this compartment, the sugar chrysolaminarin could be localized with a mouse anti-betaglucan antibody (1:10000) in combination with a gold-coupled secondary antibody goat anti-mouse IgG + 5 nm gold (1:20). Freeze substitution: A.O.U.H.; scale bar: 500 nm.

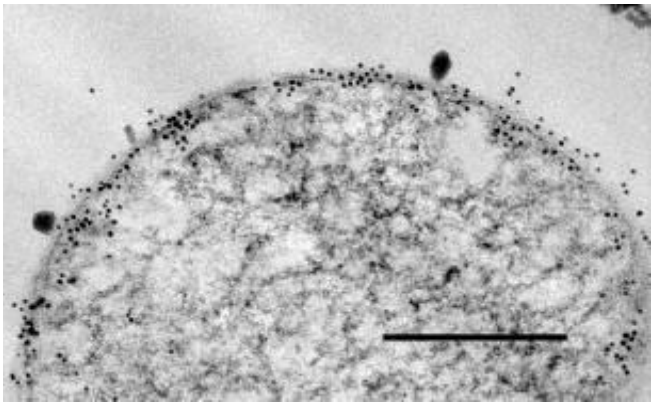

Figure 2. Ultrathin section of *M. sedula,* strain TH2. The major subunit of the S-layer protein Msed_1806 could be localized at the cell surface. Primary antibody: rabbit anti-Msed1806 (1:500); secondary antibody: goat anti-rabbit IgG + 6 nm gold (1:20). Freeze substitution: E.G.F.U.; scale bar: 200 nm.