Sample preparation of biological samples for analysis with atom probe tomography

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Introduction

The development of atom probe tomography (APT) into using pulsed laser ion evaporation have made it possible to study non-conducting samples including ceramics and hard tissues. This also raised the potential to study biological samples, including proteins. However, lacking so far was a sample preparation method[1].

The crucial task was to prepare a needle-shaped APT specimen containing the biological specie, with its native structure intact. Biological species are fragile molecules or complexes sensitive to changes in pH, temperature and co-solutes like ions or organic species. Our approach was to embed the biological sample in an amorphous silica matrix, i.e. silicate glass, from which a specimen for atom probe tomography could be fabricated.

Silica is the most occurring inorganics in the animal and plant kingdom. Amorphous silicate glass can be synthesized by several methods and extensive research has showed that sol-gel derived silica can be used to embed proteins and other biological species with their native structure and functionality retained. The immobilization is accomplished as the silica forms a polymeric network exchanging the surrounding with a solid silica matrix.

In this work two silica embedding routes have been evaluated. By varying experimental parameters, like choice of silica precursor, pH and temperature, silicate glasses with different properties have been synthesized. A schematic overview of the two silica embedding procedures are presented in Figure 1. The produced glasses were evaluated in terms of protein stability, and feasibility for APT specimen preparation and analysis.

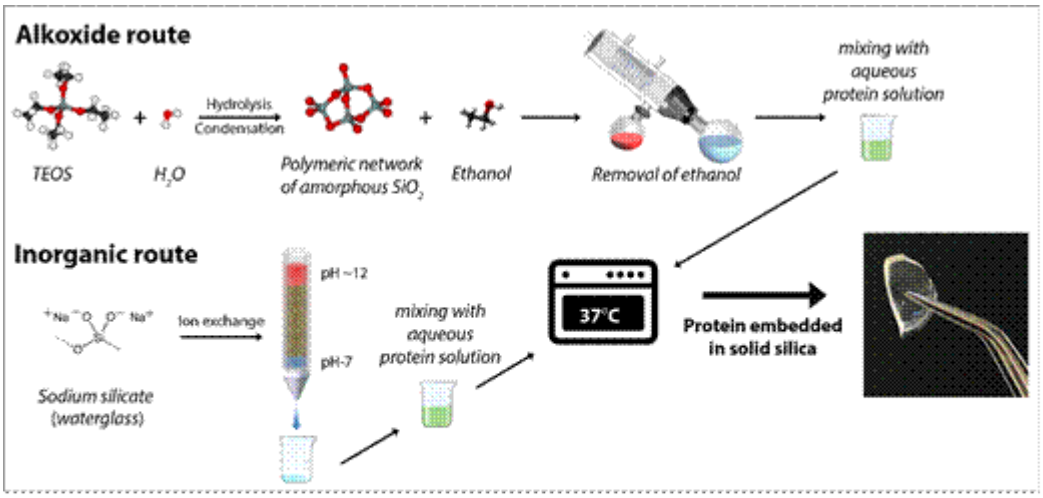

Figure 1. *Schematic overview of the organic (alkoxide) and inorganic routes for embedding of proteins in solid silica. Both routes share the same final curing step in 37°C to solidify the protein-containing silica gel.*

Results

The results show that biological samples could embedded in a silica matrix with its native structure intact and moreover, by using FIB-SEM specimen preparation a successful APT analysis could be accomplished of rabbit IgG antibody embedded in a water glass derived matrix. In Figure 2 transmission electron (TEM) images of a vesicle (a) and a rabbit IgG (b) antibody embedded in a silica matrix are shown. Image c in the same figure is showing an APT reconstruction heat map of rabbit IgG embedded in water glass derived silica matrix.

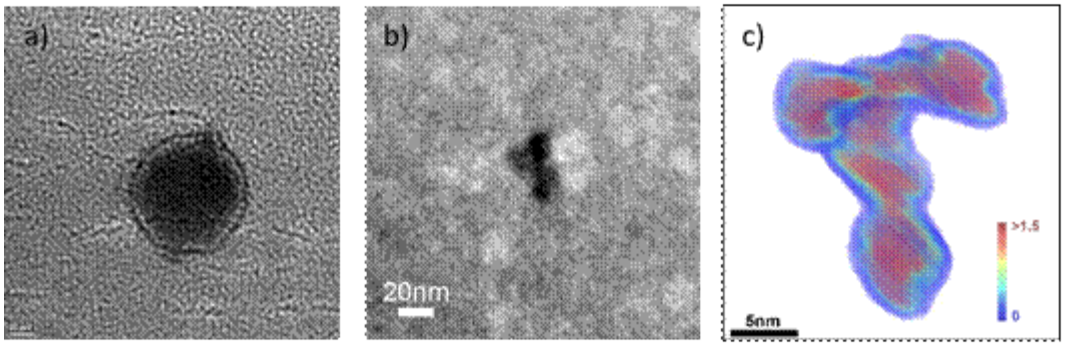

***Figure 2**. TEM-images showing vesicle (a) and rabbit IgG (b) embedded in a silica matrix. Image c is showing an APT reconstruction heatmap of rabbit IgG embedded in a silica matrix.*

Conclusion and future work

The results clearly state that biological samples can be embedded in a silica matrix with its native structure intact and subsequent APT can be used to determine its three-dimensional structure. The future work will include studies of additional biological samples and further improvement of experimental procedures to develop a methodology with higher hit rate and throughput.

Funding

This research is funded by Chalmers Area of Advance Nanoscience and Nanotechnology.

References

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