



Advances in Atom Probe Tomography Analysis of Silica Embedded Proteins

Downloaded from: <https://research.chalmers.se>, 2026-10-12 19:58 UTC

Citation for the original published paper (version of record):

Eriksson, G., Mayweg, D., Hulander, M. et al (2025). Advances in Atom Probe Tomography Analysis of Silica Embedded Proteins. *Microscopy and Microanalysis*, 31.
<http://dx.doi.org/10.1093/mam/ozaf048.131>

N.B. When citing this work, cite the original published paper.

Advances in Atom Probe Tomography Analysis of Silica Embedded Proteins

Gustav Eriksson, David Mayweg, Mats Hulander, Mattias Thuvander, Martin Andersson

DECTRIS QUADRO

The flexible standard for **electron microscopy**
Maximizing performance on a single platform



Proceedings

Advances in Atom Probe Tomography Analysis of Silica Embedded Proteins

Gustav Eriksson¹, David Mayweg², Mats Hulander¹, Mattias Thuvander², and Martin Andersson^{1,*}

¹Department of Chemistry and Chemical Engineering, Chalmers University of Technology, Göteborg, Sweden

²Department of Physics, Chalmers University of Technology, Göteborg, Sweden

*Corresponding author: martin.andersson@chalmers.se

The determination of the three-dimensional (3D) structure of proteins remains a longstanding challenge in biology. It is essential for understanding fundamental biological processes and has applications such as pharmaceutical development. Historically, X-ray diffraction, NMR spectroscopy, cryo-electron microscopy, and most recently, machine learning methods, have all been used successfully to determine the structure of many proteins. However, there remains a need for new methodologies, as the established methods often struggle with the determination of the 3D structure of membrane and dynamic or intrinsically disordered protein structures. Predicting the structure of these proteins also remains a challenge for the new machine learning methods [1].

Atom probe tomography (APT) has potential to contribute to the studies of proteins, as it can reveal the chemical composition of materials in 3D with near atomic resolution, potentially yielding more information from each single protein molecule than other characterization techniques. However, the inherent limitations of APT, namely the requirements of the material to survive ultra-high vacuum, cryogenic temperatures and extensive electrical fields, make soft biological materials challenging to study using the technique. Lately, some progress has been made towards enabling such studies using different strategies [2–4]. Despite this, no methodologies have yet demonstrated high throughput and reliability in obtaining the 3D structure of proteins using APT.

In this work, the concept of embedding proteins in silica to obtain a material that retains the biomolecule's native structure while being compatible for APT analysis [5]. This methodology utilizes a sol-gel synthesis of silica from a sodium silicate precursor, which is carried out at room temperature under conditions suitable for the biomolecules (Fig 1). Previously, this method has been shown to enable analysis of proteins, and the known structure of an antibody was successfully obtained. However, due to amorphous silica's poor absorption of the laser wavelengths used in previous APT instruments, the amount of data obtained was limited by premature specimen fractures, putting a limit on the detection of proteins by the method. Using the newest generation of commercially available APT instruments, equipped with a deep-UV laser ($\lambda = 257.5$ nm), the yield when analyzing amorphous silica samples is greatly improved. This allows for a large number of protein molecules to be detected within a single APT measurement.

The protein used in this work was Green Fluorescent Protein (GFP), enabling the use of fluorescence microscopy to identify high protein concentration regions in the silica sample for site specific lift-out and APT specimen preparation (Fig. 2A). The localization of proteins in the silica material was also verified by Time-of-Flight Secondary Ion Mass Spectroscopy (TOF-SIMS) (Fig 2B). GFP is a small barrel shaped protein at 28 kDa, which is below the general lower size limit for cryo-EM. The protein was also ¹³C isotope-labelled, allowing for unambiguous identification of protein originating ions in the APT data obtained distinct from carbon-based contaminations from other sources.

In deep-UV laser assisted APT analyses of the silica embedded GFP, carbon was mainly detected at 13 m/z, at levels up to ten times higher than the signal at 12 m/z (Fig 3A), confirming that the origin was isotope-labelled proteins rather than contaminations from other sources. The number of ¹³C-ions detected in the longest analysis corresponds to the expected number of detected carbon ions from 10,000 individual GFP molecules. The proteins are primarily detected as elementary carbon ions, enhancing the potential spatial resolution that can be achieved of the protein's 3D structure compared to earlier results, where molecular fragments such as CNH₂⁺ and CO₂⁺ were mainly detected.

In the reconstructed 3D atom maps, isolated ¹³C-ion clusters were observed with the number of carbon ions expected to be detected from each protein (Fig 3B). The shape and dimensions somewhat resemble the known structure of GFP. While further work is required to obtain high resolution information on protein, these results demonstrate a step forward for protein structure studies using APT and encourage continued pursuit of enabling APT as a method for structural biology.

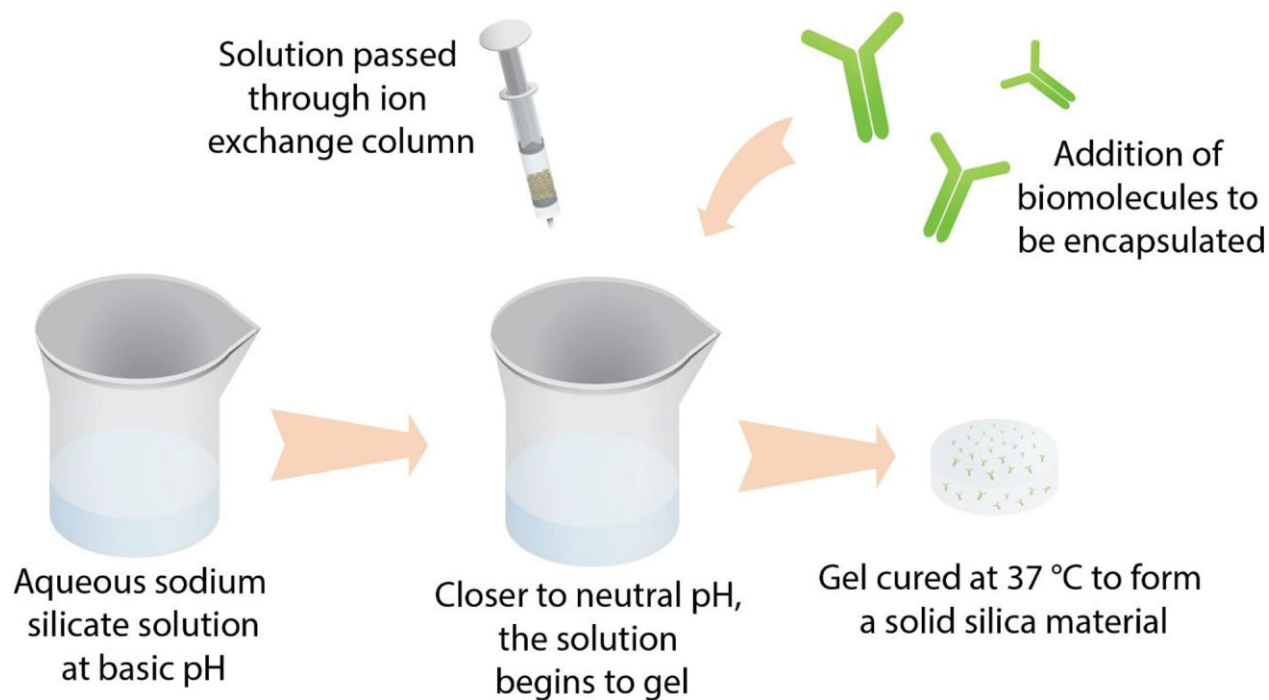


Fig. 1. Schematic overview of the silica embedding of proteins process.

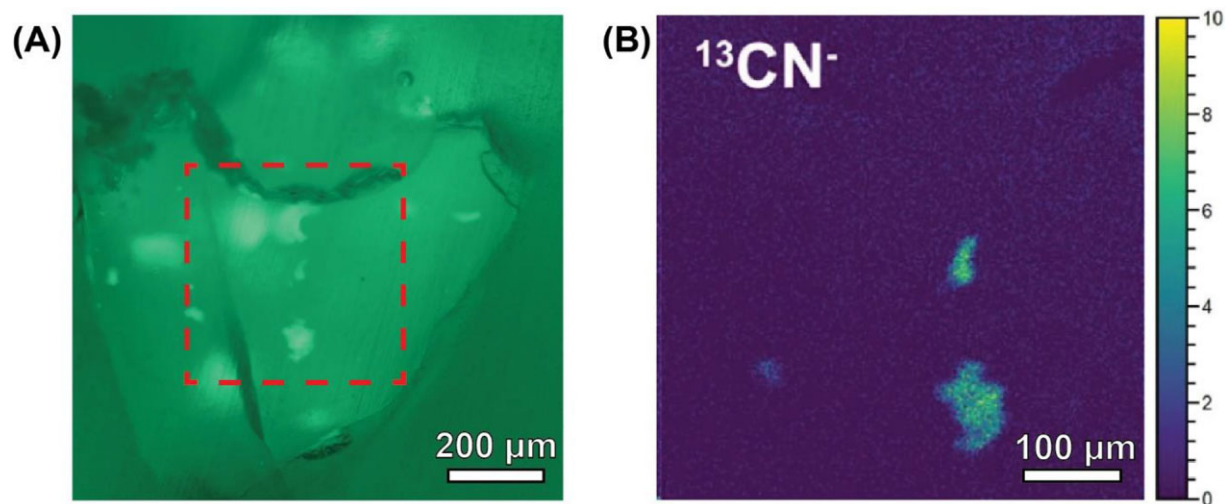


Fig. 2. A: Fluorescent microscopy image of GFP embedded in silica. Scale bar: 200 μm . B: TOF-SIMS map from the region highlighted in A, showing a clear signal from the fluorescent regions. Scale bar: 100 μm .

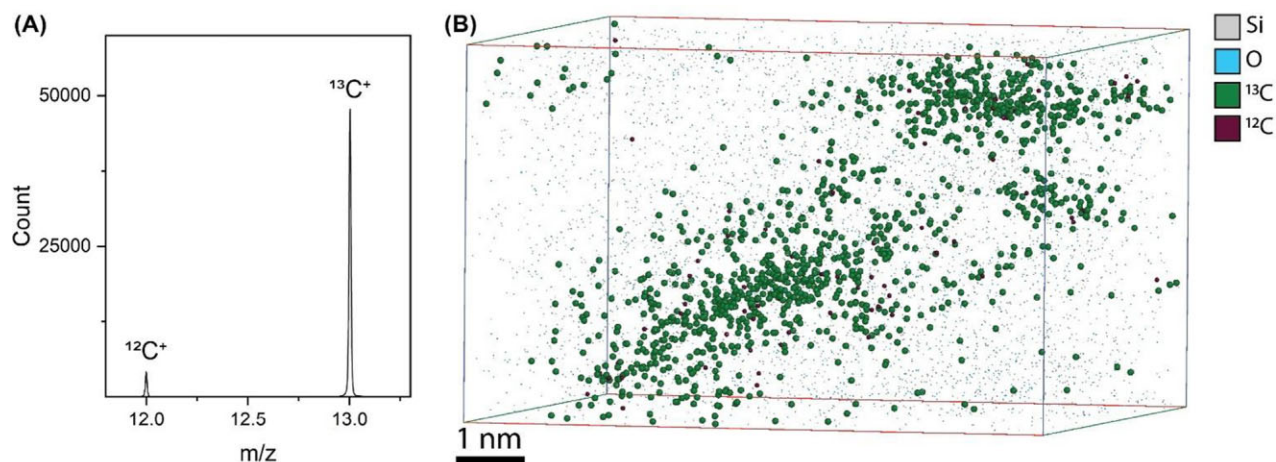


Fig. 3. A: Section of a mass spectrum obtained from APT analysis of silica embedded proteins. B: Atom map with Si and O shown as grey and blue points, respectively. ^{13}C and ^{12}C are shown as green and brown spheres, respectively.

References

1. Kiersten RM and Rohit PV. *J. Mol. Biol.* (2021) 433, 167208, <https://doi.org/10.1016/j.jmb.2021.167208>.
2. Perea DE *et al.*. *Sci. Rep.* (2016) 6, 22321, <https://doi.org/10.1038/srep22321>.
3. Rusitzka KAK *et al.*. *Sci. Rep.* (2018) 8, 17615, <https://doi.org/10.1038/s41598-018-36110-y>.
4. Qiu S *et al.*. *Anal. Chem.* (2020) 92, 5168-5177, <https://doi.org/10.1021/acs.analchem.9b05668>.
5. Sundell G *et al.*. *Small* (2019) 15, 1900316, <https://doi.org/10.1002/sml.201900316>.