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Mass spectrometry inside single proteins by atom probe tomography

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Atom probe tomography (APT) is considered as the only technique today to provide both the chemical composition and three-dimensional (3D) imaging at a near-atomic resolution [1]. We propose a new APT method to investigate the structure and chemical composition of single proteins maintained in their hydrated state. The specimens are prepared by encapsulating the solution containing proteins with graphene membranes on metal (W) tips (Fig. 1a), with the final tip diameter controlled at less than 100 nm (Fig. 1b). The hydrated specimens are then transferred to a laser-pulsed atom probe instrument for probing (Fig. 2a).

Ferritin from equine spleen was first imaged by TEM using negative staining protocol, to confirm the consistency of geometry. APT experiments were performed on a LEAP 5000XR instrument (Cameca Instruments, USA) in pulsed-laser mode under ultra-high vacuum (<1×10-11 Torr) with set-point temperature of 35 K. Results have shown that H_2O^+ (18 Da) peak can be clearly identified in the acquired mass-to-charge-state ratio spectrum, suggesting that the protein specimen was maintained in the frozen hydrated state (Fig. 2b). 3D maps of the spatial distributions of atomic and molecular species were also reconstructed (Fig. 2b), with the size of the ferritin core and shell consistent with that previously reported [2]. Compared to cryo-APT transfer as recently demonstrated [3], the proposed method demonstrates a new route to investigate the atomic and molecular structures of macromolecules in their near-native state at near-atomic resolution.

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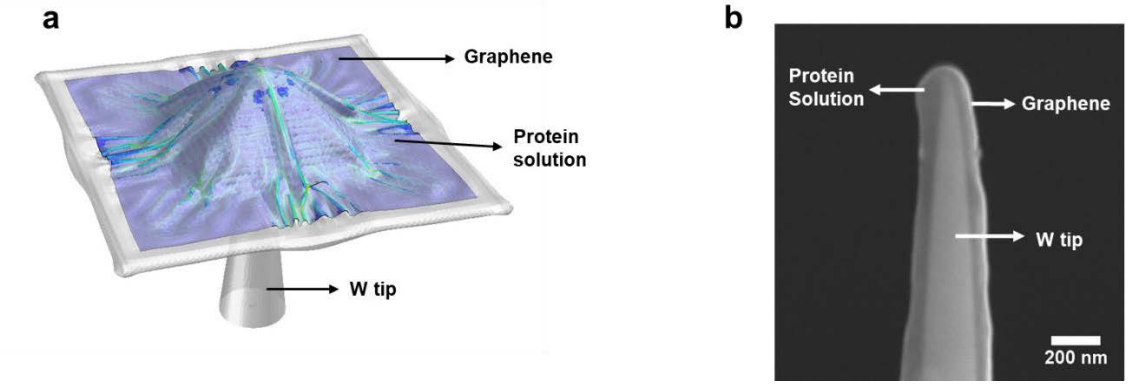

Figure 1. (a) Computer simulation of the dynamic process of encapsulation of protein solution with graphene membrane on an APT specimen tip using finite element method. (b) SEM image of the graphene-encapsulated protein specimen.

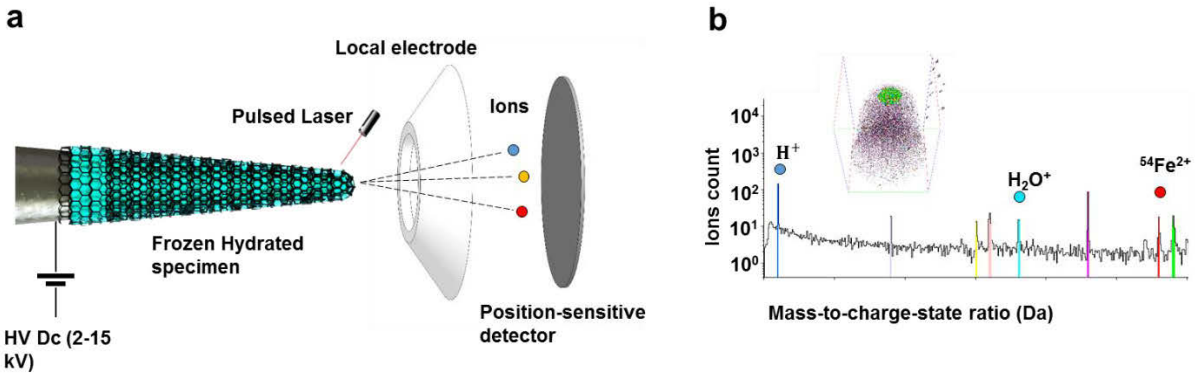

Figure 2. (a) Schematics of the protein specimen probed by laser-pulsed APT in the frozen hydrated state. (b) An acquired mass-to-charge-state ratio spectrum and the associated 3D reconstruction of the field evaporated volume of ferritin at near-atomic resolution.