Microsc. Microanal. 27 (Suppl 2), 2021
doi:10.1017/S1431927621013076

Imaging Protein Dynamics in Liquid Water

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Recent developments on electron-transparent materials have facilitated the advancement of liquid-phase electron microscopy (LP EM) leading to an unprecedented understanding of the structure and dynamics of specimens in their liquid environment [1]. LP EM offers remarkable capabilities with regards to imaging label-free, time resolved-structures in liquid by removing the artifacts caused by traditional drying or cryogenic treatments. One of the most attractive applications of LP EM is the investigation of cell molecular machinery structures such as proteins. The liquid nature of the sample presents exciting new opportunities such as accessing dynamic processes or the possibility of 3D structure reconstruction by applying tomographic methods [2]. Image reconstruction in liquid-state poses several challenges, and most importantly, it undermines the single-particle analysis assumption that the three-dimensional objects captured on the image sensor are identical over time. The free movement of soft objects in LP EM may very well provide a unique selling point of the technique for structural biology by granting the opportunity to monitor the protein structural landscape during the imaging process.

We propose the combination of all-atom simulations with LP EM to complement structural studies with dynamic investigations. In this work, we exploited LPEM to image the dynamics of proteins undergoing Brownian motion, using their natural rotation to access the particle structural landscape for reconstructing its architecture in 3D using tomographic techniques. We have selected two test proteins for our approach: archaeal RNA polymerase and apoferritin. RNA polymerase (RNAP) from the archaea *Sulfolobus acidocaldarus* is both asymmetrical and very flexible [3] allowing us to both properly assign profiles and screen the effects of dynamics. Apoferritin is a cage-like spherical particle with outer and inner diameter of 12 and 8nm respectively [4]. It also displays 24-fold octahedral symmetry, this makes it an appealing test case for cryo-EM as the data can be multiplied by 24, a key reason for its selection. We show that the adopted approach allows to achieve sub-nanometer spatial resolutions of protein structures, either imaging proteins one by one and assessing different conformational states using Brownian Tomography (BT) or combining several proteins into one statistical conformational ensemble using Brownian Particle Analysis (BPA). The use of LTEM for investigation of proteins is not limited to 3D reconstruction and structural analysis, hence to explore another use we have imaged amyloid-β (Aβ) aggregation. Aβ is a small, disordered peptide with 36-43 amino acids. Aβ accumulates into stages of microscopic amyloid fibres and plaques that are found in brains affected by Alzheimer's disease (AD) [6]. Preliminary investigations on

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https://doi.org/10.1017/S1431927621013076 Published online by Cambridge University Press

Aβ aggregation, via LTEM will be presented. This work, although still in early stages, promises to provide relevant and novel biological information on Aβ aggregation.

These findings set the foundation for future dynamic studies and time-resolved 3D structure reconstruction of biological materials in their native environment. Moreover, we show that LP EM is a powerful tool for addressing current challenges in soft matter and molecular biology.

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[6] Authors acknowledge funding from the EPSRC (grant EP/N010906/1), Jeol UK and DENSsolutions for sponsoring part of this work. HEC-BioSim (EP/R029407/1), PRACE (BSC, Project BCV-2019-3-0010 and CSCS ProjectS847), CSCS (Project 86), and the Leibniz Supercomputing Center (SuperMUC, Project pr74su) are acknowledged for their allocation of supercomputer time. Acknowledgement for the Spanish Supercomputer Network, RES, for their allocation of HPC resources, project BCV-2020-3-0009 (Tirant UV).

https://doi.org/10.1017/S1431927621013076 Published online by Cambridge University Press