doi:10.1017/S1431927622001301
Microsc. Microanal. 28 (Suppl 1), 2022

Advancing High-resolution Imaging of Human Viruses in Liquid

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Interest in liquid-Electron Microscopy (liquid-EM) has skyrocketed in recent years as scientists can now observe real-time processes at the nanoscale [1-4]. It is becoming highly desirable to pair high-resolution structural information with dynamics as many events occur at rapid timescale – in the millisecond-range or faster. Such rapid movements may include changes in virus surface proteins that serve to evade the immune system [4]. Improved knowledge of flexible structures may also benefit the design of anti-viral reagents to combat emerging diseases, such as SARS-CoV-2. Importantly, viewing biological materials in a fluid environment provides a unique glimpse of their performance in cells and tissues.

We aimed to investigate the dynamic properties of human viruses in liquid at the atomic scale. To accomplish this goal, we used Adeno-associated Virus (AAV) as a model system. A side-by-side comparison of three-dimensional (3D) structures obtained using cryo-EM and liquid-EM showed better thermal stability in the liquid samples. Side-chain densities and helical features could be traced in the multiple liquid-EM structures that resolved between 3.5 Å – 7.2 Å [4]. These correlative applications also advanced our ability to quantify molecular dynamics in the context of high-resolution structural results.

To extend the use of liquid-EM as a high-throughput tool for the scientific community, we have modified specimen preparation protocols to employ autoloader clipping devices. The clipping devices produced for the Titan Krios autoloader can effectively prepare microchip-based liquid assemblies prior to loading them into an EM. The assemblies are mechanically sealed using standard grid clips and the autoloader can accommodate up to 12 samples per imaging session. The clear advantage of automated specimen production is that many samples can be quickly assessed for optimum liquid thickness and electron dose conditions. One disadvantage is the inability to flow analytes or other substances into the liquid chambers during an imaging session. For 3D structure determination purposes, however, the automated microchip technique easily competes with high-throughput routines implemented for cryo-EM workflows.

Overall, by incorporating automated routines into current imaging procedures we can elevate the field. Recent results that support this frame-work include our high-resolution and dynamic insights of viruses, vaccine candidates, and antibody-based therapies aimed at improving human health and disease [3, 4].

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