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Utilizing Liquid-Electron Microscopy to Visualize SARS-CoV-2 Assemblies from COVID-19 Patients

Samantha Berry[1,2], Liza-Anastasia DiCecco[1,3], William J. Dearnaley[1,2], Maria Solares[1,4], Jennifer Gray[2], and Deborah F. Kelly[1,2*]

[1.] Department of Biomedical Engineering, Pennsylvania State University, University Park, PA, USA.
[2.] Materials Research Institute, Pennsylvania State University, University Park, PA, USA.
[3.] Department of Materials Science and Engineering, McMaster University, Hamilton, ON, Canada.
[4.] Molecular, Cellular, and Integrative Biosciences Graduate Program, Huck Institutes of the Life Sciences, Pennsylvania State University, University Park, PA, USA.
* Corresponding author: Debkelly@psu.edu

The SARS-CoV-2 pandemic has upended the global healthcare system, resulting in over 5.9 million deaths worldwide [1]. This prompted a race to develop effective vaccines and therapeutics; however, two years later much of the virus's characteristics, such as its rapid transmissibility and assembly mechanisms, remain an enigma. Researchers have attempted to unravel these mysteries of SARS-CoV-2 using x-ray crystallography and cryo-electron microscopy to model its static structures [2,3]. The innovative liquid-electron microscopy (liquid-EM) technique enables researchers to study biological structures in more a dynamic, native environment. This is of great relevance to biologists, since side-chain structures can be very flexible and go through a variety of conformational changes that may not be captured in a solid-state experiment. By studying samples such as viruses in liquid, real-time processes and flexible conformations can be observed at the nanoscale [4]. The need for high-resolution, dynamic models of SARS-CoV-2 has never been greater.

In this work, we aimed to create an accurate, high-resolution viral capsid model from PCR+ SARS-CoV-2 patient serum using an innovative microchip sandwiching technique. To achieve this goal, we isolated SARS-CoV-2 viral proteins from patient serum using a Ni-NTA purification method. These wet, purified samples were then clipped in our novel microchip assembly, using gold foil grids and silicon nitride (SiN) microchips (Fig.1)[5]. Images from the Talos200C electron microscope revealed large sub-viral assemblies. Imaging session results remained consistent across multiple days, with little to no signs of sample degradation (Fig. 2B). These virions were modeled to reveal an 8.25 Å spherical structure (Fig. 2A). Findings confirmed our ability to extract and image native SARS-CoV-2 proteins in a unique way.

It is suspected that by using our microchip assemblies in liquid-EM, the fluid environment facilitated sub-viral particle interactions leading to larger, more stable assemblies. This is supported by the consistency of particles that were observed across multiple imaging sessions, and indicates the opportunity to further explore dynamic interactions within SARS-CoV-2. Liquid-EM has such capability and more, with possible research avenues including heated experiments and real-time interactions with combative agents. In addition to this technology, the microchip assemblies are an efficient, economically viable option for those wishing to get their hands wet and dive into the field of liquid-EM. Ultimately, liquid-electron microscopy may hold the key to understanding infectious mechanisms and finally put an end to the SARS-CoV-2 pandemic.

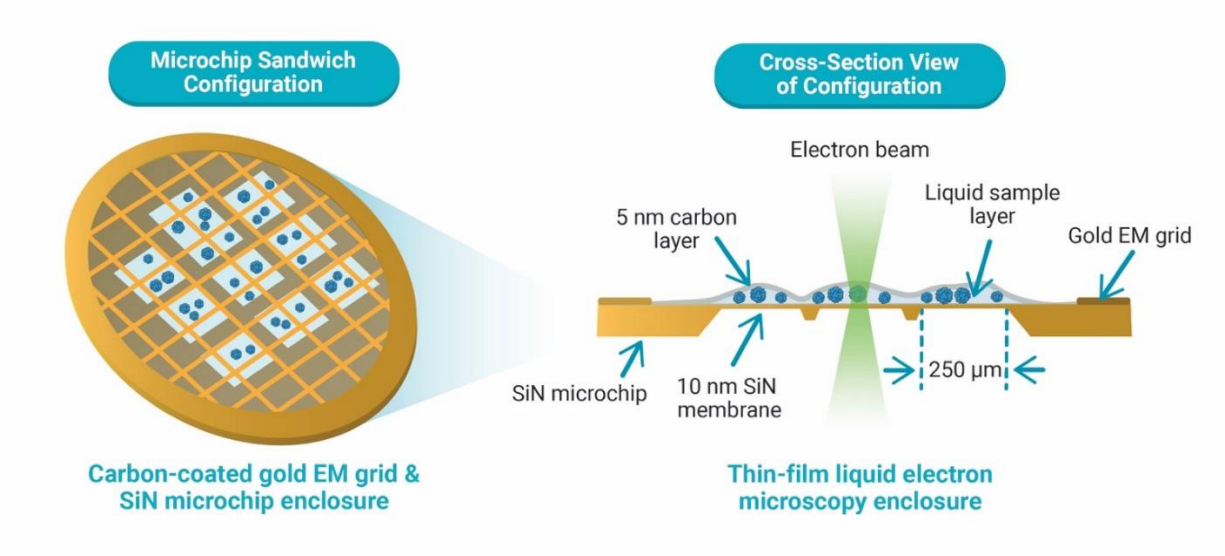

Figure 1. Microchip assembly for liquid-EM samples. Biological samples are prepared on glow-discharged gold foil grids before being sandwiched with a SiN microchip. Liquid pockets form within the microwells so that areas overlapping with the SiN membrane windows may be imaged.

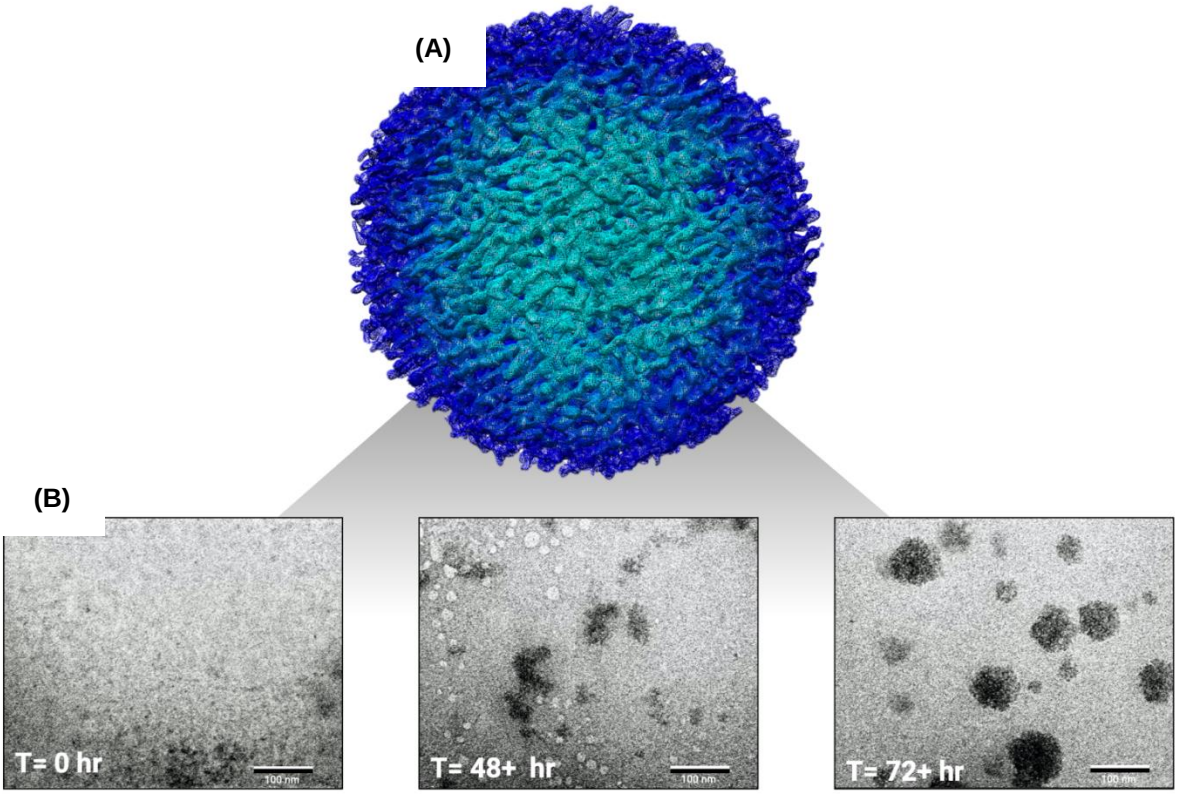

Figure 2. SARS-CoV-2 sub-viral model from COVID-19 patient serum. (A) Subviral 3-D reconstruction at 8.25 Å obtained from micrographs. Model uses C2 symmetry and has a diameter of ~100 nm. (B) Micrographs taken during imaging sessions across multiple days show continuous sample presence with few signs of degradation.

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