Microfluidic sample preparation for Time-resolved Cryo-EM analysis of biomolecular assemblies

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Cryo-electron microscopy (cryo-EM) based techniques for determining biomolecular structures have attracted much attention in the last decades. Here, we present a microfluidic 3D mixing-to-spraying device for achieving time-resolved sampling. Our system mainly features with the use of a 3D mixing and a custom-made gas nozzle to generate a small-angle spray of instantly well-mixed sample fluid. The 3D geometry provides forced-convective mixing that only takes 3.4 milliseconds to achieve above 90% mixing with our optimized number of junctions. A custum-made spray nozzle exploits a sheath-based flow to make plume narrow, removing contracted geometry of orifice. To confirm time-resolved sampling, we investigate the structural analysis of RecA-ssDNA interaction. We observed that the RecA proteins grow on the ssDNA in a time-dependent manner. We monitor the complex of RecA-ssDNA at different sampling times and obtain a growing length of filaments. Further, we perform the structural determination of apoferritin (480 kDa). We obtained the 3D reconstructed apoferritin structure with a near-atomic resolution of 2.77 Å.