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Keynote 2: Microsecond time-resolved cryo-electron microscopy

Monday, May 15, 2023 7:15 PM (45 minutes)

While cryo-electron microscopy (cryo-EM) is rapidly gaining in popularity, its time resolution is currently insufficient to directly observe proteins in action, leaving our understanding of these nanoscale machines fundamentally incomplete. We have recently introduced a novel approach to time-resolved cryo-EM that affords microsecond time resolution and thus promises to overcome these limitations. Our method involves melting a cryo sample with a laser beam, which allows dynamics of the embedded particles to occur in liquid once a suitable stimulus is provided. While the dynamics occur, the heating laser is switched off at a well-defined point in time, causing the sample to rapidly cool and revitrify. The particles are thus trapped in their transient configurations, in which they can subsequently be imaged. We demonstrate that our approach affords a time resolution of 5 μ s or better. Moreover, near-atomic resolution reconstructions can be obtained from revitrified samples, showing that the revitrification process leaves the protein structure intact. Finally, I will present a microsecond time-resolved pH jump experiment, in which we observe the dynamics of the capsid of CCMV, an icosahedral plant virus. These results highlight the potential of our method to fundamentally advance our understanding of protein function through direct observation of dynamics.

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Modern Methods in Structural Biology and Dynamics

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Bioimaging

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