Novel Solutions to Accelerate Cryo-EM Single Particle Analysis Sample Screening

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Sample preparation currently remains a major bottleneck in cryo-electron microscopy (cryo-EM) single particle analysis (SPA). Even purified proteins that are stable and structurally intact can behave differently in a thin vitreous ice layer, exhibiting unwanted behavior such as denaturation, aggregation, or preferred orientation. Due to the unique properties of each protein, multiple rounds of optimization are often necessary, where vitrification parameters, grid types, or additives are adjusted before the optimal condition for high-resolution data collection are found. This is even further complicated as sample optimization is often performed in a non-systematic way, extending optimization time over multiple days or even weeks.

In this presentation we will discuss several approaches to streamline sample optimization for cryo-EM and increase throughput for screening samples. Firstly, we'll explore how a combination of buffer optimization and additive screening, similar to the commonly used screening approaches in X-ray crystallography, can help to overcome commonly observed problems for cryo-EM samples, such as protein aggregation and preferred orientation. We'll present a semi-automated screening strategy to arrive at an optimized sample in two days of screening on the microscope based on commercially available products. Finally, we'll show how native mass spectrometry can help to speed up the sample optimization process for cryo-EM, providing thorough characterization of samples prior to cryo-EM.