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Optimization of CryoEM Sample Preparation: A New Freezing Strategy to Reduce the Time to Structure Loop

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The Resolution Revolution was triggered by great technological advanced in cryo-electron microscopes, from their electron source down to new electron-counting detectors, automated data-collection protocols and better software all together helped make molecular cryoEM the method of choice for high-throughput structure determination. However, sample preparation – a key determinant in the success of any cryoEM structural study – has seen little innovation since the introduction of commercial plunging devices, which still rely on paper-blotting and suffer from various unwanted side effects due to the slow freezing process [1].

Current sample preparation and optimization workflows are still of the "guess-and-check" type, using the electron microscope to determine the outcome. The chameleon instrument and self-wicking grids represent a paradigm shift towards a routine, automated, fast-plunge future for sample optimization.

On the chameleon, automation is achieved through two robotic systems to control grid and sample handling throughout the workflow. The process of thin film formation – "wicking" – is observed using an integrated camera and assessed using automated image processing throughout the freezing session. Visualization of the grid during the freezing process is correlated to the frozen grid observed on the electron microscope. The wicking process itself is controlled through changes in glow discharge strength using the on-board glow discharge unit. Results demonstrating this relationship and recommendations for its use in a standard freezing session to produce grids with appropriate ice will be presented.

Routine use is achieved through the integrated visualization options and the consistency of both the dispenser technology and the self-wicking grids. Results demonstrating the consistency and information indicating the careful quality control process undertaken during manufacture of the self-wicking grid will be presented. Additionally, prototype grid development work encompassing non-carbon support films on self-wicking grids and novel hole size and spacing patterns ("multi-hole grids") will be presented with a focus on integration of these technologies into standard chameleon workflows.

By improving both the freezing device and the sample supports, we are creating tools for faster and better optimisation of the sample preparation process to accommodate a wide range of samples. This is a paradigm shift from the current methods of modifying samples to fit flawed sample freezing techniques. Together, these improvements create a system with walk-up usability, democratizing opportunities to utilise cryo-EM across a broad range of cutting-edge research.

References:

[1] A Noble, et al. Nature Methods **15** (2018), p. 793.