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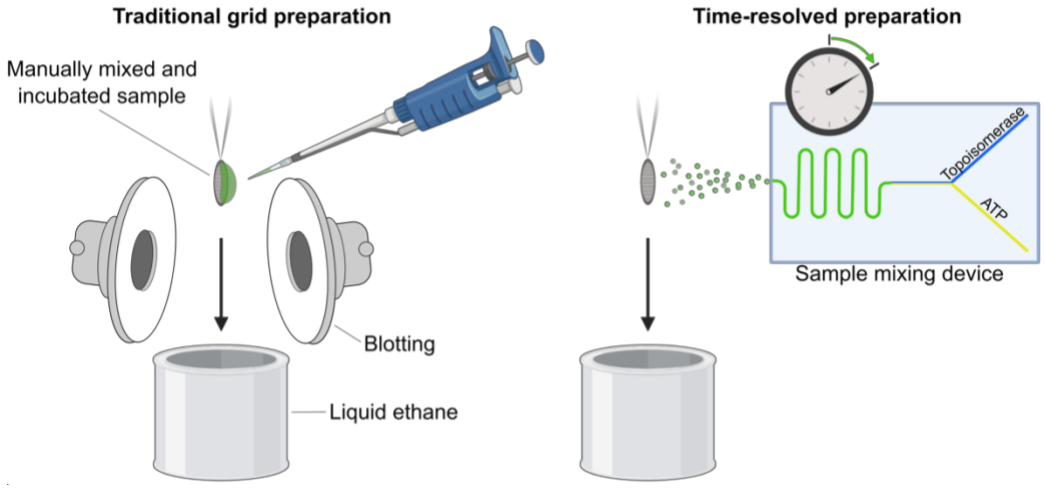
Time-resolved cryo-EM of protein complexes involved in pathogen replication

The function of a biological macromolecule is mediated by structural transitions that enable it to bind other molecules and/or catalyse a chemical reaction. Cryo-electron microscopy (cryo-EM) is nowadays routinely used to determine structures of biological macromolecules at near-atomic resolution; however, the traditional sample preparation approach using pipetting and blotting (figure, left) typically limits the number of structural states that can be captured. Many proteins perform their function within milliseconds. As such, new sample preparation approaches are needed to allow time-resolved studies that also capture the short-lived active intermediate conformations (reviewed in Mäeots and Enchev, 2022). Capturing these conformations is critical to understand the macromolecules' function completely and could potentially be key for developing novel therapeutics that target the transient states.

The Maia lab is developing a cryo-EM plunge-freezing device that will allow time-resolved experiments using the mixing-and-spraying approach (schematically shown in the figure, right). The projects aim to use this device to study two protein complexes involved in pathogen replication: the first being the replication-transcription complex of SARS-CoV-2 (reviewed in Malone et al., 2022), and the second being the bacterial topoisomerase enzyme gyrase (Vanden Broeck et al., 2019).

Interested students can choose to work on either of the two biological systems. Initially, the project will involve protein expression and purification. Individual proteins will then be combined to form functional complexes, which will be characterised (such as stability, activity and stoichiometry). Optimal freezing conditions for cryo-EM will be tested using the traditional grid preparation technique (Vitrobot), and cryo-EM data will be collected and analysed to verify sample integrity. If time permits, the samples can possibly also be tested using the in-house built plunge-freezer for time-resolved studies. Depending on the project's length and student interest, the described sample work can be combined with work on the plunge-freezing device since this still needs to be fully optimised. The latter could include optimising the ice thickness and testing the 3D-printed sample-mixing unit.



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