MS25-1-2 Protein crystallization 'de-optimization' for microED
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Abstract
Understanding interactions between the protein-active sites and small molecule ligands will provide insights into structure-based drug discovery and following drug development[1]. Recently, the micro-crystal electron diffraction (MicroED) method, developed on the widely available transmission electron microscope (TEM), has shown advantages in providing high-quality structural information[2,3]. Compared with the traditional single X-ray diffraction (SXRD) method, it only needs tiny crystals and enables the investigation of proteins that are difficult to crystalize. Furthermore, micro-crystals may have fewer defects and lower mosaicity than larger ones, which improves stability during ligand soaking or rapid cooling[4–6].
In the previous study, SXRD researchers focused on growing a formidable and well-ordered protein crystal, while protein crystals used for MicroED, on the other hand, are supposed to be small and thin. These plate-like crystals allow the penetration of electrons and minimize multiple scattering. To this aim, methods such as cryo-FIB[7] and fragmentation[8] were introduced to obtain protein crystals with suitable size and morphology. However, direct crystallization without mechanical modification is still challenging. Here we developed a 'de-optimization' crystallization strategy to grow micro-crystals from the protein solution. This general protocol shows the potential to prepare a large concentration of micro-crystals for MicroED experiments.

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