

Design And Implementation of Suspended Drop Crystallization

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We have developed a novel crystal growth method known as suspended drop crystallization. Unlike traditional methods, this technique involves mixing protein and precipitant directly on an electron microscopy grid without any additional support layers. The grid is then suspended within a crystallization chamber which we designed, allowing for vapor diffusion to occur from both sides of the drop. A UV transparent window above and below the grid enables the monitoring of crystal growth via light, UV, or fluorescence microscopy. Once crystals have formed, the grid can be removed and utilized for x-ray crystallography or microcrystal electron diffraction (MicroED) directly without having to manipulate the crystals. To demonstrate the efficacy of this method, we grew crystals of the enzyme proteinase K and determined its structure by MicroED following FIB/SEM milling to render the sample thin enough for cryoEM. Suspended drop crystallization overcomes many of the challenges associated with sample preparation, providing an alternative workflow for crystals embedded in viscous media, sensitive to mechanical stress, and/or suffering from preferred orientation on EM grids.

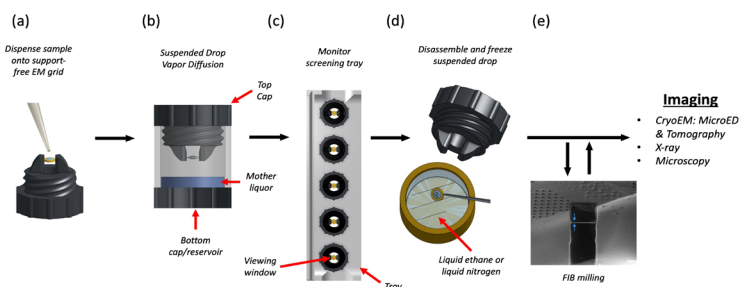


Figure 1

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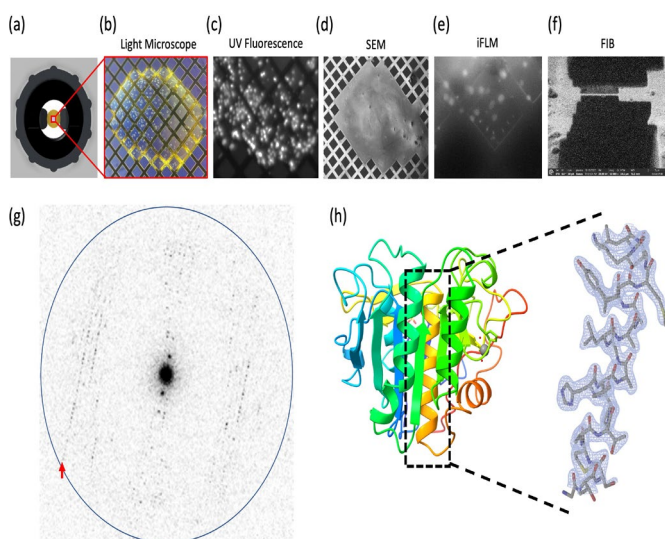


Figure 2

Figure 2