

1 **Title**

2 **Design and implementation of suspended drop crystallization**

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4 **Authors**

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16 cryogenic freezing, FIB/SEM, membrane protein

17 **Abstract**

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

31 **Introduction**

32 Crystallography is a widely used technique for determining the structures of both small and large
33 molecules such as proteins (McPherson & Gavira, 2014). Crystals, which possess repetitive
34 structural patterns, are utilized in this approach (McPherson, 1985). When a coherent beam of x-
35 rays or electrons is directed at a crystal, it is scattered in predictable ways that provide information
36 about the underlying structure of the molecule in the crystal (Bragg, 1912). Over the past century,

37 a number of crystal growth methods have been developed and refined, including liquid-liquid
38 diffusion (Salemme, 1972), vapor diffusion using hanging or sitting drops (McPherson, 1989), and
39 lipidic cubic phase (LCP) (Landau & Rosenbusch, 1996). Additionally, two-dimensional
40 crystallization utilizing dialysis and growth through evaporation and concentration has also been
41 explored and documented (Gonen *et al.*, 2005; Henderson & Unwin, 1975; Schmidt-Krey, 2007).

42 Vapor diffusion is the most commonly employed method for protein crystallization (Chayen &
43 Saridakis, 2008). In this method, the protein of interest is mixed with a mother liquor and placed
44 either in a small well or on a glass support that hangs above the solution. The mixture is then
45 sealed in a chamber with additional crystallization solution to allow for vapor diffusion. As the
46 vapors form, the effective concentration of the protein increases, causing the drop to shrink. Under
47 certain conditions, crystals may form, which are then detected using light, UV, or fluorescence.
48 Several automated instruments have been developed for crystal detection. Hanging drops are
49 typically used for soluble proteins in aqueous solution, while sitting drops are preferred for
50 membrane proteins that may be in a solution with detergent and lipids. Various conditions are
51 tested to optimize crystal growth, including pH, temperature, precipitants, and additives.

52 MicroED is a cryogenic electron microscopy (CryoEM) technique that utilizes electron diffraction
53 to determine the three-dimensional structure of proteins, peptides, and small molecules in
54 cryogenic conditions (Shi *et al.*, 2013; Jones *et al.*, 2018; Sawaya *et al.*, 2016; Xu *et al.*, 2019;
55 Gruene *et al.*, 2018). This method is suitable for crystals that are extremely small and typically
56 invisible to the naked eye, with a size a billionth that required for x-ray crystallography (Mu *et al.*,
57 2021; Nannenga & Gonen, 2019a). Once a crystal is obtained, it is transferred onto an electron
58 microscopy grid using a pipette and rapidly frozen in liquid ethane. The sample is then placed in
59 an electron microscope operating at liquid nitrogen temperatures to minimize radiation damage.
60 The electron beam is focused in diffraction mode onto the crystal when it is identified, and
61 MicroED data is collected on a fast camera while the stage is continuously rotating. X-ray data
62 reduction software is utilized to process the MicroED data, and established procedures are
63 employed to determine the structures (Hattne, Reyes, Nannenga, Shi, De La Cruz *et al.*, 2015).

64 In certain cases, it is advisable to avoid transferring crystals onto an electron microscopy grid.
65 Some protein crystals may be too delicate and have a large solvent fraction, which can result in
66 damage during the transfer process and render them unsuitable for MicroED. Additionally,
67 membrane protein crystals embedded in lipids, such as those formed through lipidic cubic phase
68 crystallization, are highly susceptible to damage from physical manipulation. For these sensitive
69 samples, new sample preparation techniques must be developed and optimized to ensure their
70 suitability for MicroED.

71 Recent studies have demonstrated successful determination of structures for membrane proteins
72 embedded in lipids using a novel approach for sample preparation (Martynowycz *et al.*, 2021,
73 2020, 2023). The method utilizes a scanning electron microscope coupled with a focused ion
74 beam (FIB/SEM) for sample preparation. In one example, the human adenosine receptor (A_{2A}AR)
75 was crystallized in LCP, and the crystal drop was transferred to an electron microscopy grid by
76 blotting and rapid freezing in liquid ethane. The sample was too thick for visualization by a
77 transmission electron microscope, so fluorescence was used to locate the nanocrystals within the
78 lipid matrix. Correlative light-EM was then utilized to expose the crystals with the FIB for MicroED
79 analyses, resulting in a high-resolution (2.0 Å) structure of the human receptor (Martynowycz *et al.*,
80 2023). A similar approach was also used to determine the structure of a functional mutant of
81 the mammalian voltage-dependent anion channel VDAC (Martynowycz *et al.*, 2020).

82 Although the aforementioned sample preparation methods have been successful, they rely on the
83 assumption that crystals are not damaged during the physical manipulation and transfer onto an
84 electron microscopy grid. Additionally, certain crystals, especially those that resemble sheets,
85 may exhibit a preferred orientation on the grid carbon support, which can limit the reciprocal space
86 available for sampling. Given these challenges, there is a need to develop alternative approaches
87 for sample preparation for MicroED, as well as for other imaging applications such as x-ray
88 crystallography.

89 Here we used conceptual design and 3D printing to create a suspended drop crystallization setup.
90 This is a novel approach for sample preparation for MicroED that eliminates the need for crystal
91 transfer and physical manipulation, offering an alternative to traditional crystallization methods.
92 The method involves allowing crystallization to occur directly on an EM grid without support,
93 enabling both sides of the drop to be exposed for uniform vapor diffusion. The absence of support
94 film on the grid eliminates preferred crystal orientations and enables complete reciprocal lattice
95 sampling. Crystal growth can be monitored visually, and the entire crystallization drop can be
96 plunge-frozen directly on the EM grid. The method was successfully demonstrated on proteinase
97 K crystals, resulting in a 2.1 Å resolution structure. This approach may have potential for other
98 imaging applications beyond MicroED.

99 **Results**

100 ***The 3D printed suspended drop screening tool.*** The suspended drop crystallization screening
101 tool is a screw cap that can mount pre-clipped EM grids and suspend them over a well reservoir.
102 The screw and mounting arms are made of a flexible rubber material made of thermoplastic
103 polyurethane (TPU) that applies gentle pressure on the clipped EM grid without the risk of bending
104 (Figure 1A). The screw also incorporates a clear glass coverslip that is securely tightened by a
105 3D printed plastic screw to create a viewing window. After dispensing sample onto a support-free
106 EM grid, the suspended drop is sealed into an incubation chamber containing mother liquor
107 (Figure 1B). Suspended crystallization drops can be monitored through the viewing window using
108 light and fluorescent microscopy. A screening tray has also been designed and 3D printed, which
109 can accommodate multiple incubation chambers for larger screening experiments (Figure 1C).
110 When suspended drop crystals are identified, the screening tool is unscrewed from the well,
111 tweezers are used to retrieve the grid, and the grid is rapidly plunged into liquid nitrogen or ethane
112 without blotting (Figure 1D). For MicroED, FIB/SEM milling is performed prior to TEM imaging
113 (Figure 1E). The suspended drop crystallization method can also be used directly for x-ray
114 analysis by mounting the grid directly onto the goniometer.

115 ***Protein crystals grown by suspended drop crystallization.*** We hypothesized that support-free
116 gold gilder grids with a low mesh count (50-200 mesh) could be used to suspend crystallization
117 drops during long incubation periods. Experimental results confirmed that suspended
118 crystallization drops could be stably retained by such grids. To prepare the grids, 3 mm diameter
119 gold gilder grids were clipped into autogrid cartridges for stability and rigidity, and glow-discharged
120 before being mounted horizontally between the mounting arms of the screw cap. Proteinase K
121 sample was mixed with mother liquor directly on the EM grid and the screening tool was tightened
122 into the well of a crystallization tray for incubation (Figure 1A). Light microscopy and UV
123 fluorescence was used to monitor the crystal growth through the coverslip at the top of the
124 screening tool (Figure 2B, C).

125 ***Machining crystal lamella.*** Grids containing suspended proteinase K crystallization drops were
126 retrieved from the screening apparatus and immediately plunged into liquid ethane. The grids

127 were loaded into a plasma beam FIB/SEM equipped with an integrated fluorescence microscope
128 (iFLM) at cryogenic conditions. The surface of the crystallization drop appeared smooth in the
129 SEM and crystal features could not be observed (Figure 2D). To visualize crystals below the
130 surface of the drop, the iFLM was used to detect crystal fluorescence (Figure 2E). A series of
131 images was acquired at different focal points between the grid bars and the surface of the drop,
132 and the depth at which the crystal appeared most in focus was taken to be the true depth of the
133 crystal. The stack of reflective images was correlated to the X-Y plane of the SEM images and a
134 three-dimensional representation of crystal locations inside the drop was generated.

135 The targeted crystal and surrounding media were milled into a thin lamella using a xenon plasma
136 beam (Figure 2F). We used the xenon plasma beam because it is the fastest and most gentle
137 option for milling crystals that are deeply embedded in solvent (Martynowycz *et al.*, 2023). The
138 final lamella was ~ 7 μm wide and 300 nm thick.

139 **MicroED analyses of suspended drop crystals.** The grid containing the crystal lamella was
140 transferred to a cryogenically cooled Titan Krios electron microscope operating at 300 kV. The
141 lamella site was identified with low magnification imaging and brought to eucentric height. A
142 diffraction preview of the lamella was acquired to confirm that it would diffract to high-resolution
143 (Figure 2G). Continuous rotation MicroED data was collected on a real space wedge from -40° to
144 $+40^\circ$ tilt using a Falcon4 direct electron detector set to counting mode. Data was collected with a
145 selected area aperture to reduce background dose. Strong and sharp reflections were visible to
146 2.1 \AA resolution and a clear lattice was visible.

147 MicroED data were converted to standard crystallographic formats using our online tools which
148 are freely available (<https://cryoem.ucla.edu/microed>). The data were indexed and integrated in
149 XDS to 2.1 \AA resolution. Phases for the MicroED reflections were determined by molecular
150 replacement. The space group was determined to be $P 4_32_12$ with a unit cell of (a, b, c) (\AA) =
151 (68.26, 68.26, 101.95) and (α , β , γ) ($^\circ$) = (90, 90, 90). The structure was refined using electron
152 scattering factors (Table 1). The structure of proteinase K that was determined matches other
153 MicroED structures of this protein that determined from crystals that were handled using
154 traditional MicroED sample preparation protocols (Figure 2H).

155 Discussion

156 In this study, we utilized suspended drop crystallization to grow crystals of a protein, and
157 subsequently determined its structure by MicroED. To optimize the conditions for suspended drop
158 crystallization, we developed a screening tool that features a screw cap with two extended arms
159 for clamping an EM grid and a clear glass coverslip that creates a viewing window (see Figure
160 1A). Once the sample was dispensed onto a support-free EM grid, the suspended drop was
161 sealed into an incubation chamber with mother liquor (see Figure 1B), and its growth could be
162 monitored using light and fluorescent microscopy. To harvest the crystals, the screening tool was
163 unscrewed from the incubation well, and the grid was retrieved with tweezers and rapidly frozen
164 in liquid nitrogen or ethane for cryo-preservation.

165 The process for preparing MicroED samples is akin to the standard procedure followed in other
166 cryoEM techniques like single particle analysis (SPA) and tomography. The sample is usually
167 dispensed onto an EM grid, excess solvent is blotted, and the grid is vitrified by immersing it in
168 liquid ethane (Nannenga & Gonen, 2019b). However, enhancing the preparation of samples for
169 MicroED experiments using this method can be challenging because of limited options for

170 improving crystal transfer and blotting conditions, which could cause damage to fragile crystals.
171 Nevertheless, suspended drop crystallization offers an alternative specimen preparation method
172 that eliminates the need for crystal transfer and blotting. This technique presents a promising
173 solution for crystallographers dealing with challenging crystals that are embedded in viscous
174 buffer (e.g., membrane proteins or crystals in high precipitant conditions), prone to mechanical
175 stress, toxic, volatile, or limited in number in the drop. We envisage that suspended drop
176 crystallization will be valuable in the preparation of recalcitrant crystals for MicroED experiments.

177 Crystals that adopt a preferred orientation on EM grids with carbon support can lead to incomplete
178 sampling of the reciprocal space, limiting the accuracy of structural determination. This is
179 especially common in plate-like sheet crystals, such as those of Catalase and Calcium-ATPase
180 (Nannenga *et al.*, 2014; Yonekura *et al.*, 2015). However, growing crystals using the support-free
181 suspended grid method can avoid preferred orientation. As there is no support film, crystals
182 cannot align themselves in a specific orientation, allowing for 100% sampling of the reciprocal
183 space for any crystal morphology and symmetry by merging data from several crystals. This
184 approach is particularly useful for crystallographers working with challenging samples, enabling
185 high-quality data collection and accurate structural determination.

186 The suspended drop crystallization tools described in this study enable crystal growth to be
187 assayed in a sparse matrix directly on grids without support. The modular design allows for a
188 large number of crystallization conditions to be assayed. While others have attempted to grow
189 crystals directly on grids, they typically use carbon support and cannot perform sparse matrix
190 crystallization assays (Li *et al.*, 2018). We found that gold grids were the most inert and produced
191 the most consistent results, as copper grids tend to oxidize and prevent crystal growth, and holey
192 carbon grids can make it difficult to monitor crystal growth. Using support-free gold grids with a
193 lower mesh count allows for easier monitoring of crystal growth, reduces the amount of material
194 in contact with the sample, and decreases the likelihood of obstruction by grid bars, which is
195 important for subsequent FIB milling. Additionally, because no blotting is required with this setup,
196 the initial position of the crystals remains unchanged after freezing, which facilitates targeting and
197 FIB milling.

198 Using a support-free grid and a sparse matrix approach, the suspended drop method allows for
199 easier monitoring of crystal growth and eliminates physical contact with the sample. Additionally,
200 the use of cryogenic plasma beam FIB/SEM enables efficient generation of sample lamellae,
201 while cryogenic TEM allows for high-quality MicroED data collection. One major advantage of this
202 approach is its potential applicability to membrane proteins, which are notoriously difficult to
203 crystallize due to their softness and fragility. Furthermore, the use of automation and robotics
204 could further streamline the process and make it more accessible to structural biologists. Overall,
205 the suspended drop crystallization method has the potential to become a routine approach in
206 structural biology and although not demonstrated in this study, suspended drop crystallization
207 could be employed in x-ray crystallography, as well as other microscopy and cryoEM applications.

208

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213 (Accession code XXXX) and the EM Data bank (Accession code YYYY).

214 **Figure Legends**

215 **Figure 1. Suspended drop crystallization** (a) A support-free EM grid is clipped into an autogrid
216 cartridge and mounted between the arms of the suspended drop screening tool. The sample and
217 crystallization solution are dispensed onto the grid. (b) The chamber is immediately sealed to
218 allow vapor diffusion. (b) The incubation chambers are inserted into a screening tray for efficient
219 storing and monitoring of crystallization progress by light, fluorescence and UV microscopy. (d)
220 EM grids containing crystals are retrieved from the screening tool and frozen. (e) The specimen
221 is then interrogated by MicroED or other methods such as tomography, x-ray crystallography, or
222 general microscopy. FIB milling is optional depending on the application.

223 **Figure 2. MicroED structure of suspended drop Proteinase K** (a) The suspended drop viewed
224 from the top and imaged by (b) Light microscopy or (c) UV. A frozen suspended drop specimen
225 was loaded into the FIB/SEM and imaged normal to the grid surface by (d) SEM and (e) iFLM
226 with the 385 nm LED to locate submerged crystals. (f) The targeted crystal site was milled into a
227 300 nm thick lamella. (g) Example of MicroED data acquired from the crystal lamella. The highest
228 resolution reflections visible to 2.1 Å (red arrow). Resolution ring is shown at 2.0 Å (blue). (H)
229 Cartoon representation of the Proteinase K colored by rainbow with blue N terminus and red C
230 terminus. The 2mF_o-DF_c map of a selected alpha-helix is highlighted, which was contoured at
231 1.5 σ with a 2-Å carve.

232 **Table 1. MicroED structure statistics of proteinase K crystallized by suspended drop**

233 **Methods and Materials**

234 **Materials.** Proteinase K from *Tritirachium album* was purchased from Fisher BioReagents
235 (Hillsborough, OR) and used without further purification. Ammonium sulfate and Tris buffer were
236 purchased from Sigma-Aldrich (St. Louis, MO). All reagents were made with MilliQ water. The
237 Ultimaker S5 3D printer and all filaments were purchased from MatterHackers (Lake Forest, CA).
238 Glass coverslips were purchased from Ted Pella (Redding, CA). The gold gilder grids were
239 purchased from Electron Microscopy Sciences (Hatfield, PA). The 100 nm fluorescent TetraSpeck
240 Microspheres were purchased from Thermo-Fisher.

241 **Object design and 3D printing.** All components of the screening tool were designed in the cloud-
242 based CAD program Onshape.com and exported in STL file format. To generate GCODE files for
243 3D printing, the STL files were imported into the slicer program Ultimaker Cura 5.0 and default
244 Ultimaker material profiles were used. All objects were printed at 0.1 mm layer height and 40
245 mm/sec print speed. All 3D printing was performed on an Ultimaker S5 3D printer equipped with
246 a 0.4 mm diameter nozzle and a glass build surface with a layer of glue applied. The main body
247 of the screening tool and the cover slip gasket were printed in thermoplastic polyurethane (TPU).
248 The cover slip retaining screw was printed in co-polyester (CPE). A single on-grid screening tool
249 takes approximately 1.5 hrs to print.

250 **Suspended drop crystallization.** Proteinase K was dissolved in 0.1 M Tris-HCl pH 8.0 at 25
251 mg/ml. A support-free gold gilder grid was clipped into an autogrid cartridge, negatively glow-
252 discharged for 1 min at 15 mA, and mounted in the screening tool. Equal volumes of proteinase
253 K and 1.5 M ammonium sulfate were mixed dispensed on the mounted grid (~0.3 μ L final drop

254 volume). The screening tool (with mounted grid and crystallization drop) was immediately screwed
255 into a well of the crystallization tray containing 300 μ L of 1.5 M ammonium sulfate in the reservoir.
256 Within 48 hrs, crystals of Proteinase K were observed in the hanging crystal drops.

257 **Sample preparation and cryo-preservation.** The EM grids supporting crystal drops were
258 carefully removed from the screening tool with tweezers and rapidly plunged into liquid ethane.
259 The grids were stored in liquid nitrogen until use.

260 **Machining proteinase K crystal lamellae using the plasma beam FIB/SEM.** The vitrified EM
261 grid was loaded into a Thermo-Fisher Helios Hydra dual-beam plasma beam FIB/SEM operating
262 at cryogenic temperature. A whole-grid atlas of the drop was acquired by the SEM operating at
263 an accelerating voltage of 0.5 kV and beam current of 13 pA using the MAPS v3.19 software
264 (Thermo-Fisher). The crystal drop was coated with platinum by beam-assisted (argon beam at 4
265 nA, 5 kV) GIS coating for 1 min to protect the sample from ion and electron beams. The drop was
266 then inspected using the iFLM with the 385 nm LED to locate crystals inside the drop at various
267 Z dimensions. The sample was presented normal to the FIB beam and small holes were milled
268 straight down the sample, around the crystal of interest, with the Xenon beam at 4nA for use as
269 “fiducials” for the later correlation step. A comprehensive fluorescence stack of the crystals of
270 interest was acquired with a binning of 2 (pixel size of 240 nm) and a step of 0.5 μ m (Figure 3B,
271 top panel). This stack was deconvolved using the DeconvolveLab Fiji plugin (Sage *et al.*, 2017).
272 An experimental Point Spread Function (PSF) was measured using sub-resolution 100nm
273 TetraSpeck microspheres. The processed PSF used for deconvolution was generated with the
274 Huygens software (<https://svi.nl/Huygens-Software>). Further preprocessing using the 3D-
275 Correlation Tool (3DCT) (Heymann *et al.*, 2006) was performed: 1- stack reslicing in order to
276 output isometric voxels 240 x 240 x 240 nm, and 2- intensity normalization. Low current FIB
277 (10pA) and low voltage SEM (2 kV) images were acquired at grazing incidence (milling angle 11°)
278 and were used to correlate against the fluorescent stack. 3DCT was used to correlate the
279 SEM/FIB views with the fluorescence images. To do so, the milled holes were located in 2D in
280 the SEM/FIB image and in 3D in the fluorescent stack. In the latter, the crystals of interest were
281 located by delineating them with markers. In our hands, as low as 6 fiducial holes, both visible in
282 fluorescence and SEM/FIB, were enough to correlate the two modalities with an error no less than
283 5 pixels.

284 This correlation process was performed during the milling procedure to make sure the final
285 lamellae were on target. During the final steps of milling (when the lamella was a 2-3 μ m thick),
286 the correlation precision in Z was no longer enough. Milling was performed from top to bottom
287 and the stage was brought back normal to the E-beam for checking the presence of the crystal at
288 the surface of the lamella. To do so, SEM settings were set to 1.2 kV, 13 pA. These settings
289 allowed scattering contrast between the crystal and the surrounding aqueous solvent. When the
290 contours of the crystal were visible, milling was performed from bottom to top until the final
291 thickness of 300 nm was reached. The xenon plasma beam (30 kV) was used for lamella milling
292 at an angle of 11°. For the first milling step, two boxes (20 x 35 μ m) separated by 5 μ m 4 nA.,
293 Second milling step a current of 1 nA was used to thin down the lamella to 3.5 μ m. Third milling
294 step a current of 0.3 nA was used to narrow the lamella to 10 μ m wide (X dimension of the milling
295 boxes) and thin it down to 2 μ m. Fourth milling step a current of 0.1 nA was used to thin down to
296 1 μ m. Final milling step a current of 30 pA was used to generate a 300 nm thick lamella. The final
297 lamella was 10 μ m wide, 20 μ m long, and 200 nm thick.

298 **MicroED Data Collection.** Grids with milled lamellae were transferred to a cryogenically cooled
299 Thermo-Fisher Scientific Titan Krios G3i TEM. The Krios was equipped with a field emission gun
300 and a Falcon4 direct electron detector, and was operated at an accelerating voltage of 300 kV. A
301 low magnification atlas of the grid was acquired using EPU (Thermo-Fisher) to locate milled
302 lamellae. The stage was translated to the lamellae position and the eucentric height was set. The
303 100 μm selected area aperture was inserted and centered on the crystal to block background
304 reflections. In diffraction mode, the beam was defined using a 50 μm C2 aperture, a spotsize of
305 11, and a beam diameter of 20 μm . MicroED data were collected by continuously rotating the
306 stage at 0.2 $^\circ / \text{s}$ for 400 s, resulting in a rotation range of 80 $^\circ$.

307 **MicroED data processing.** Movies in MRC format were converted to SMV format using MicroED
308 tools (Martynowycz *et al.*, 2019; Hattne, Reyes, Nannenga, Shi, Cruz *et al.*, 2015). The diffraction
309 dataset was indexed and integrated in XDS (Kabsch, 2010b). Integrated intensities from a single
310 crystal were scaled and merged in XSCALE (Kabsch, 2010a).

311 **Structure solution and refinement.** Phases for the MicroED reflections were determined by
312 molecular replacement in PHASER using Protein Data Bank (PDB) 6CL7 as the search model
313 (McCoy *et al.*, 2007; Hattne *et al.*, 2018). The solution was space group P4₃2₁2 and unit cell
314 dimensions 68.26, 68.26, 101.95 (a, b, c) (\AA) and 90, 90, 90 (α , β , γ) ($^\circ$). The first refinement was
315 performed with Coot and phenix.refine (Afonine *et al.*, 2012) using isotropic B-factors, automatic
316 water picking, and electron scattering factors. Occupancies were refined for alternative side chain
317 conformations and SO₄ and calcium were placed in coordination sites. The final refinement used
318 anisotropic B-factors, automatic water picking, and electron scattering factors and resulted in
319 Rwork/Rfree = 0.2442/0.2917 and resolution of 2.1 \AA .

320

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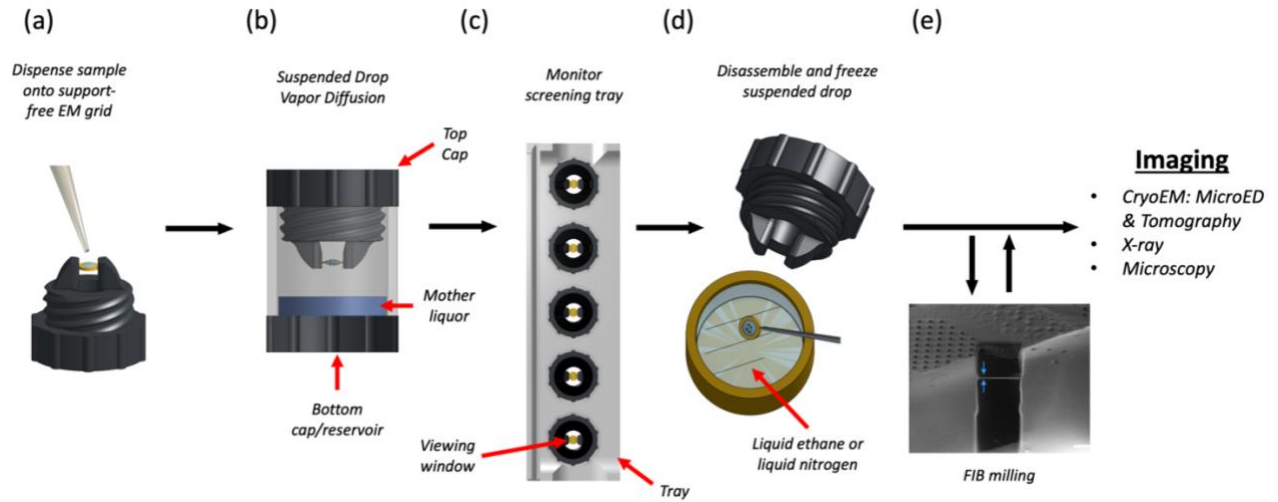
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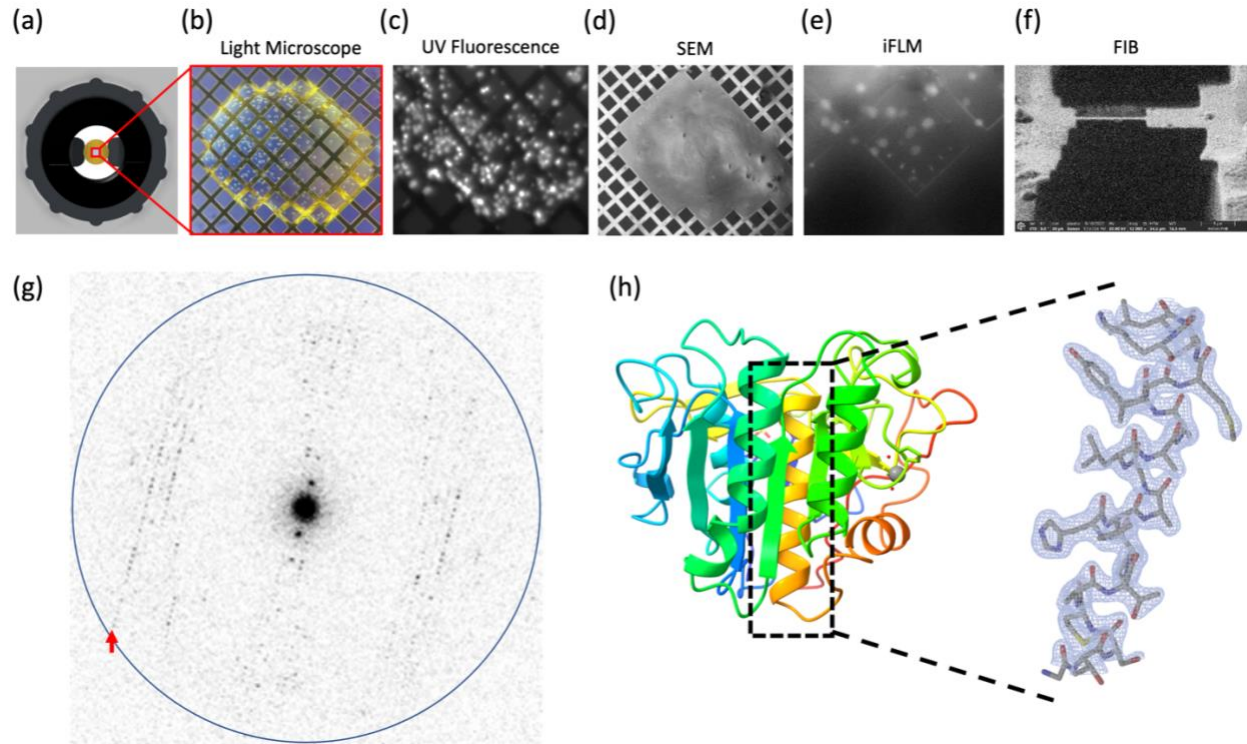
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Figure 1



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Figure 2

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Table 1

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Data	Parameter	Measure	
Data	accelerating voltage (kV)	300	
Collection	wavelength (Å)	0.019687	
	electron source	field emission gun	
	total accumulated exposure (e ⁻ Å ⁻²)	0.64	
	no. of crystals	1	
	microscope	Thermo Fisher Titan Krios	
	camera	Falcon 4 electron counting	
	rotation rate (deg/sec)	0.2	
	Data	resolution range (Å)	30.42-2.10
	analysis	space group	P4 ₁ 2 ₁ 2
		a, c, c (Å)	68.26, 68.26, 101.95
α, β, γ (°)		90, 90, 90	
reflections, total/unique		56301/12774	
multiplicity		4.41	
completeness (%)		87.0	
mean I/σ(I)		2.82	
CC1/2		92.5	
Rwork		0.2442	
Rfree	0.2917		

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