Supplementary Material

1   Supplementary Tables

Supplementary Table S1. Details of each Cryo-EM dataset.

Sample Conditions	Initial Dataset	ACSMB	eBIC	Optimized 1	Optimized 2	Optimized 3	Optimized 4
Purification method	Standard	Standard	Standard	Optimized	Optimized	Optimized	Optimized
Storage conditions	Fresh	Frozen	Frozen	Fresh	Fresh	Fresh	Fresh
Nucleotide added?	Yes	No	No	Yes	Yes	Yes	Yes
SEC hexameric fraction	Off-center	Pooled	Pooled	Center	Center	Center	Center
Grid Conditions							
Grid Type	C-flat Cu 300 mesh R1.2/1.3	Quantifoil Cu R1.2/1.3	Self-wicking nanowire Cu R1.2/0.8	C-flat Cu 300 mesh R1.2/1.3	C-flat Cu 300 mesh R1.2/1.3	C-flat Au 300 R1.2/1.3	C-flat Au 300 R1.2/1.3
Glow discharged?	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Sample concentration	2 mg/mL	2 mg/mL	5 mg/mL	2 mg/mL	2 mg/mL	2 mg/mL	2 mg/mL
Sample volume	2 µL	3 µL	~6 nL	2 µL	2 µL	2 µL	2 µL
Equipment	Vitrobot Mark IV	Vitrobot Mark IV	Chameleon	Vitrobot Mark IV	Vitrobot Mark IV	Vitrobot Mark IV	Vitrobot Mark IV

Blot time	3 s	3 s	-	3 s	3 s	3 s	3 s
Temperature	Ambient	4 ºC	Ambient	Ambient	Ambient	Ambient	Ambient
Humidity	100%	95%	75% - 85%	100%	100%	100%	100%
Data Collection							
Collection Facility	KAUST I&C Core Lab	University of Leeds, ACSMB	Diamond Light Source, eBIC	KAUST I&C Core Lab	KAUST I&C Core Lab	KAUST I&C Core Lab	KAUST I&C Core Lab
Microscope	Titan Krios G1	Titan Krios G2	Glacios	Titan Krios G1	Titan Krios G1	Titan Krios G1	Titan Krios G1
Operating voltage	300 kV	300 kV	200 kV	300 kV	300 kV	300 kV	300 kV
Energy filter	GIF Quantum968 Imaging Filter	GIF BioQuantum 967 Imaging Filter	-	GIF Quantum968 Imaging Filter	GIF Quantum968 Imaging Filter	GIF Quantum968 Imaging Filter	GIF Quantum968 Imaging Filter
Detector	K2 summit	K2 summit	Falcon IV	K2 summit	K2 summit	K2 summit	K2 summit
Magnification	130k	130k	120k	130k	130k	130k	130k
Pixel size	0.52 Å/pixel	1.07 Å/pixel	1.192 Å/pixel	0.52 Å/pixel	0.52 Å/pixel	0.52 Å/pixel	0.52 Å/pixel
Frames	32	60	50	32	32	32	32
Total dose	50 e/Å^2	75 e/Å^2	48.1 e/Å^2	50 e/Å^2	50 e/Å^2	50 e/Å^2	50 e/Å^2
Exposure time	5.6 s	10 s	10 s	5.6 s	5.6 s	5.6 s	5.6 s

Dataset Details							
Movies	1242	1311	1472	1073	797	2118	2284
Particles picked	177k	100k	500k	91k	111k	400k	550k
Particles in good 2D classes	-	47k	250k	71k	78k	270k	430k

2   Supplementary Figures
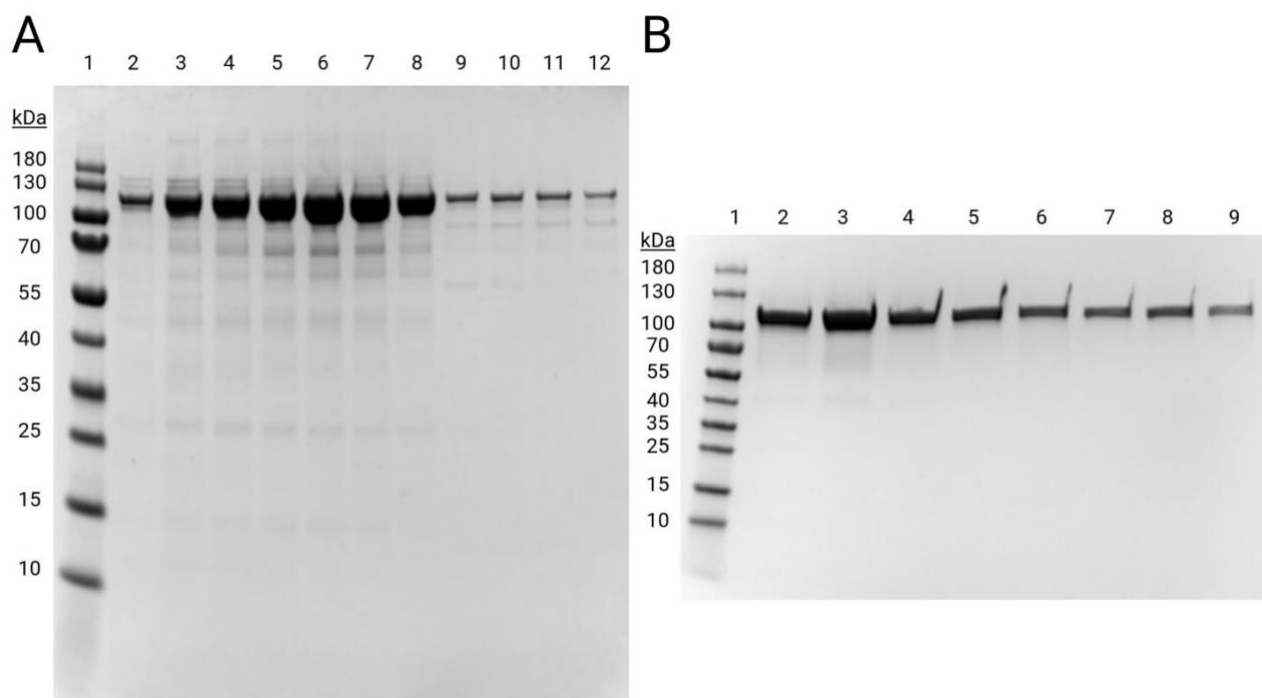
Supplementary Figure S1. SDS-PAGE of the standard and optimized purification methods. (A) SDS-PAGE gel corresponding to the SEC profile in Figure 1A. Lane 1 is the protein ladder, with molecular weights beside it. Lanes 2-4 correspond to peak 1. Lanes 5-9 correspond to peak 2. Lanes 9-12 correspond to peak 3. **(B)** SDS-PAGE gel corresponding to the SEC profile of the optimized method in Figure 3A. Lane 1 is the protein ladder, with molecular weights beside it. Lanes 2-9 correspond to fractions A10-B5, including fraction B3, respectively.