Supplementary Information

Energy filtering enables macromolecular MicroED data at sub-atomic resolution

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Supplementary Table 2. Comparison of data processing and refinement statistics of filtered and unfiltered MicroED data limited to 1.4 Å resolution.

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Supplementary Table 1. Data processing and refinement statistics.

Data collection				
No. of crystals	17			
Space group	$P4_{3}2_{1}2$			
Unit cell dimensions				
a, b, c (Å)	66.92, 66.92, 107.56			
α, β, γ (°)	90, 90, 90			
Resolution (Å)*	56.90-1.09 (1.12-1.09)			
Observed reflections	2,811,895 (95,185)			
Unique reflections	98,530 (6,664)			
Multiplicity	28.5 (14.3)			
Completeness (%)	97.5 (90.3)			
R _{merge}	0.284 (1.154)			
< <i>I/σI></i>	7.8 (1.4)			
CC _{1/2} (%)	99.5 (33.0)			
Refinement				
Resolution (Å)	56.82-1.09			
No. of reflections	98,240			
R _{work} /R _{free}	0.1498/0.1831			
No. of atoms				
Protein	2,197			
Ligand/ions	6			
Waters	334			
Hydrogen omit maps				
No. of hydrogen atoms	1,937			
Peaks at $\geq 3\sigma$	767 (39.6%)			
Peaks at $\geq 2\sigma$	1,354 (69.9%)			
 factors (Å²)				
Overall	11.29			
Protein	9.36			
Ligand/ions	10.86			
Waters	25.19			
R.m.s. deviations				
Bond lengths (Å)	0.012			
Bond angles (°)	1.80			

^{*}Values in parenthesis are for the highest resolution shell. Data were truncated at a mean $I/\sigma I \ge 1.0$ and a cross correlation between two random half sets that is statistically significant at $CC_{1/2} \sim 33\%$.

Supplementary Table 2. Comparison of data processing and refinement statistics of filtered and unfiltered MicroED data limited to 1.4 Å resolution.

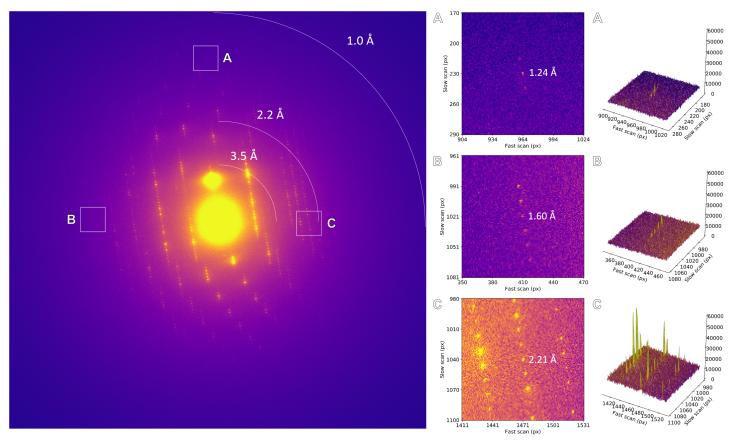
Data collection No. of crystals 5 12 17 Rotation range (°) 63.0 20.0 20.0 Flux density $(e^2/A^2/s)$ 0.002 0.002 0.002 Fluence (e^2/A^2) 1.00 0.84 0.84 Dose (MGy)* 3.66 3.08 3.08 Data integration Space group $P4;2;2$ <		Unfiltered [†]	Unfiltered [#]	Filtered		
Rotation range (°) 63.0 20.0 20.0 Flux density ($e/h^2/s$) 0.002 0.002 0.002 Fluence (e^2/h^2) 1.00 0.84 0.84 Dose (MGy) ‡ 3.66 3.08 3.08 Data integration Space group $P4_3212$ $P4_3212$ $P4_3212$ $P4_3212$ Unit cell dimensions $a, b, c(h)$ 67.02, 67.02, 107.53 67.62, 67.62, 106.59 66.92, 66.92, 107.56 $a, b, f(°)$ 90, 90, 90 90, 90, 90 90, 90, 90 90, 90, 90 Resolution (Å)* 19.55-1.40 (1.45-1.40) 57.10-1.40 (1.44-1.40) 56.90-1.40 (1.44-1.40) Observed reflections 1,231,502 (69,985) 1,101,042 (82,782) 1,371,884 (108,136) Unique reflections 47,744 (3,442) 47,420 (3,467) 47,927 (3,494) Multiplicity 25.8 (20.7) 23.4 (23.9) 28.6 (30.9) Completeness (%) 97.3 (96.2) 96.0 (96.3) 98.2 (98.4) R _{menge} 0.324 (1.784) 0.441 (1.987) 0.226 (0.598) $< V_{ab}^2$ 7.	Data collection					
Flux density $(e'/h^2/s)$ 0.002 0.002 0.002 Fluence (e'/h^2) 1.00 0.84 0.84 Dose (MGy)† 3.66 3.08 3.08 Data integration Space group $P4_3212$ $P4_3212$ $P4_3212$ $P4_3212$ Unit cell dimensions $a, b, c(h)$ 67.02, 67.02, 107.53 67.62, 67.62, 106.59 66.92, 66.92, 107.56 a, β, γ (°) 90, 90, 90 90, 90, 90 90, 90, 90 90, 90, 90 Resolution (Å)* 19.55-1.40 (1.45-1.40) 57.10-1.40 (1.44-1.40) 56.90-1.40 (1.44-1.40) Observed reflections 1,231,502 (69,985) 1,101,042 (82,782) 1,371,884 (108,136) Unique reflections 47,744 (3,442) 47,420 (3,467) 47,927 (3,494) Multiplicity 25.8 (20.7) 23.4 (23.9) 28.6 (30.9) Completeness (%) 97.3 (96.2) 96.0 (96.3) 98.2 (98.4) R _{merge} 0.324 (1.784) 0.441 (1.987) 0.226 (0.598) \sqrt{lot} 7,6 (1.4) 6.2 (1.4) 12.1 (5.3) CC _{1/2} (%) 99	No. of crystals	5	12	17		
Fluence (e' A^3) 1.00 0.84 0.84 Dose (MGy)² 3.66 3.08 3.08 Data integration Space group P43212 P43212 </td <td>Rotation range (°)</td> <td>63.0</td> <td>20.0</td> <td>20.0</td>	Rotation range (°)	63.0	20.0	20.0		
Dose (MGy)\$\frac{2}{2}\$ 3.66 3.08 3.08 Data integration Space group P43212 P43212 Unit cell dimensions	Flux density (e ⁻ /Å ² /s)	0.002	0.002	0.002		
Data integration Space group $P4_32_12$ $P4_32_12$ $P4_32_12$ $P4_32_12$ Unit cell dimensions a, b, c (Å) 67.02, 67.02, 107.53 67.62, 67.62, 106.59 66.92, 66.92, 107.56 a, β, γ (°) 90, 90, 90 90, 90, 90 90, 90, 90 90, 90, 90 Resolution (Å)* 19.55-1.40 (1.45-1.40) 57.10-1.40 (1.44-1.40) 56.90-1.40 (1.44-1.40) Observed reflections 1,231,502 (69,985) 1,101,042 (82,782) 1,371,884 (108,136) Unique reflections 47,744 (3,442) 47,420 (3,467) 47,927 (3,494) Multiplicity 25.8 (20.7) 23.4 (23.9) 28.6 (30.9) Completeness (%) 97.3 (96.2) 96.0 (96.3) 98.2 (98.4) Resegre 0.324 (1.784) 0.441 (1.987) 0.226 (0.598) $4/alz$ 7.6 (1.4) 6.2 (1.4) 12.1 (5.3) CC _{1/2} (%) 99.4 (27.0) 95.9 (23.2) 99.4 (85.7) Refinement 8 46,737 47,907 R _{cont} /κ _{Rec} 0.1374/0.1735 0.1538/0.1856 0.1208/0.1427 No. of atoms 10	Fluence (e ⁻ /Å ²)	1.00	0.84	0.84		
Space group $P4_32_{12}$ $P4_32_{12}$ $P4_32_{12}$ $P4_32_{12}$ Unit cell dimensions a, b, c (Å) 67.02, 67.02, 107.53 67.62, 67.62, 106.59 66.92, 66.92, 107.56 a, β , γ (°) 90, 90, 90 90, 90, 90 90, 90, 90 90, 90, 90 Resolution (Å)* 19.55-1.40 (1.45-1.40) 57.10-1.40 (1.44-1.40) 56.90-1.40 (1.44-1.40) Observed reflections 1,231,502 (69,985) 1,101,042 (82,782) 1,371,884 (108,136) Unique reflections 47,744 (3,442) 47,420 (3,467) 47,927 (3,494) Multiplicity 25.8 (20.7) 23.4 (23.9) 28.6 (30.9) Completeness (%) 97.3 (96.2) 96.0 (96.3) 98.2 (98.4) Remege 0.324 (1.784) 0.441 (1.987) 0.226 (0.598) $\sqrt{I/aI>}$ 7.6 (1.4) 6.2 (1.4) 12.1 (5.3) $\sqrt{I/aI>}$ 7.6 (1.4) 6.2 (1.4) 12.1 (5.3) $\sqrt{I/aI>}$ 7.6 (1.4) 6.2 (1.4) 12.1 (5.3) Refinement 8 46,737 47,907 Resolution (Å) 19.77-1.40 57.10-1.40	Dose (MGy) [‡]	3.66	3.08	3.08		
Unit cell dimensions 67.02, 67.02, 107.53 67.62, 67.62, 106.59 66.92, 66.92, 107.56 a, b, c (Å) 67.02, 67.02, 107.53 67.62, 67.62, 106.59 66.92, 66.92, 107.56 $a, β, γ$ (°) 90, 90, 90 90, 90, 90 90, 90, 90 Resolution (Å)* 19.55-1.40 (1.45-1.40) 57.10-1.40 (1.44-1.40) 56.90-1.40 (1.44-1.40) Observed reflections 1,231,502 (69,985) 1,101,042 (82,782) 1,371,884 (108,136) Unique reflections 47,744 (3,442) 47,420 (3,467) 47,927 (3,494) Multiplicity 25.8 (20.7) 23.4 (23.9) 28.6 (30.9) Completeness (%) 97.3 (96.2) 96.0 (96.3) 98.2 (98.4) Reactleties 97.3 (96.2) 96.0 (96.3) 98.2 (98.4) Remarge 0.324 (1.784) 0.441 (1.987) 0.226 (0.598) $\sqrt{a/a}$ 7.6 (1.4) 6.2 (1.4) 12.1 (5.3) CC _{1/2} (%) 99.4 (27.0) 95.9 (23.2) 99.4 (85.7) Refinement 7 7.0 -1.40 56.82-1.40 No. of reflections 47,738 46,737 47,907 Rwork/	Data integration					
a, b, c (Å) $67.02, 67.02, 107.53$ $67.62, 67.62, 106.59$ $66.92, 66.92, 107.56$ a, β, γ (°) $90, 90, 90$ $90, 90, 90$ $90, 90, 90$ $90, 90, 90$ Resolution (Å)* $19.55-1.40$ (1.45-1.40) $57.10-1.40$ (1.44-1.40) $56.90-1.40$ (1.44-1.40) Observed reflections $1.231,502$ (69.985) $1,101,042$ (82,782) $1.371,884$ (108,136) Unique reflections $47,744$ (3,442) $47,420$ (3,467) $47,927$ (3,494) Multiplicity 25.8 (20.7) 23.4 (23.9) 28.6 (30.9) Completeness (%) 97.3 (96.2) 96.0 (96.3) 98.2 (98.4) Recips 0.324 (1.784) 0.441 (1.987) 0.226 (0.598) $ I/or 7.6 (1.4) 6.2 (1.4) 12.1 (5.3) CC1/2(%) 99.4 (27.0) 95.9 (23.2) 99.4 (85.7) Refinement 88.00 (1.10) 86.82-1.40 Resolution (Å) 19.77-1.40 57.10-1.40 56.82-1.40 No. of reflections 47,738 46,737 47,907 Recolutions 10 6 $	Space group	P4 ₃ 2 ₁ 2	P4 ₃ 2 ₁ 2	P4 ₃ 2 ₁ 2		
α, β, γ (°) 90, 90, 90 90, 90, 90 90, 90, 90 Resolution (Å)* 19.55-1.40 (1.45-1.40) 57.10-1.40 (1.44-1.40) 56.90-1.40 (1.44-1.40) Observed reflections 1,231,502 (69,985) 1,101,042 (82,782) 1,371,884 (108,136) Unique reflections 47,744 (3,442) 47,420 (3,467) 47,927 (3,494) Multiplicity 25.8 (20.7) 23.4 (23.9) 28.6 (30.9) Completeness (%) 97.3 (96.2) 96.0 (96.3) 98.2 (98.4) R_{merge} 0.324 (1.784) 0.441 (1.987) 0.226 (0.598) $4/\sigma I^{>>}$ 7.6 (1.4) 6.2 (1.4) 12.1 (5.3) CC_{12} (%) 99.4 (27.0) 95.9 (23.2) 99.4 (85.7) Refinement Resolution (Å) 19.77-1.40 57.10-1.40 56.82-1.40 No. of reflections 47,738 46,737 47,907 R_{moth}/R_{free} 0.1374/0.1735 0.1538/0.1856 0.1208/0.1427 No. of atoms 10 6 6 Protein 2,063 2,197 2,197 Ligand/ions 10.41	Unit cell dimensions					
Resolution (Å)* 19.55-1.40 (1.45-1.40) 57.10-1.40 (1.44-1.40) 56.90-1.40 (1.44-1.40) Observed reflections 1,231,502 (69,985) 1,101,042 (82,782) 1,371,884 (108,136) Unique reflections 47,744 (3,442) 47,420 (3,467) 47,927 (3,494) Multiplicity 25.8 (20.7) 23.4 (23.9) 28.6 (30.9) Completeness (%) 97.3 (96.2) 96.0 (96.3) 98.2 (98.4) Renege 0.324 (1.784) 0.441 (1.987) 0.226 (0.598) $ 0.76 (1.4) 6.2 (1.4) 12.1 (5.3) 0.100 99.4 (27.0) 95.9 (23.2) 99.4 (85.7) Refinement Resolution (Å) 19.77-1.40 57.10-1.40 56.82-1.40 No. of reflections 47,738 46,737 47,907 Rwork/Rirce 0.1374/0.1735 0.1538/0.1856 0.1208/0.1427 No. of atoms 10 6 6 Waters 307 334 334 39-5 39-7 2,197 2,197 Ligand/ion$	a, b, c (Å)	67.02, 67.02, 107.53	67.62, 67.62, 106.59	66.92, 66.92, 107.56		
Resolution (Å)* 19.55-1.40 (1.45-1.40) 57.10-1.40 (1.44-1.40) 56.90-1.40 (1.44-1.40) Observed reflections 1,231,502 (69,985) 1,101,042 (82,782) 1,371,884 (108,136) Unique reflections 47,744 (3,442) 47,420 (3,467) 47,927 (3,494) Multiplicity 25.8 (20.7) 23.4 (23.9) 28.6 (30.9) Completeness (%) 97.3 (96.2) 96.0 (96.3) 98.2 (98.4) R_{merge} 0.324 (1.784) 0.441 (1.987) 0.226 (0.598) $\sqrt{I/\sigma I^2}$ 7.6 (1.4) 6.2 (1.4) 12.1 (5.3) $\sqrt{I/\sigma I^2}$ 99.4 (27.0) 95.9 (23.2) 99.4 (85.7) Refinemet Resolution (Å) 19.77-1.40 57.10-1.40 56.82-1.40 No. of reflections 47,738 46,737 47,907 R_{work}/R_{free} 0.1374/0.1735 0.1538/0.1856 0.1208/0.1427 No. of atoms 10 6 6 Waters 307 334 334 > 5 10.61 10.61 Protein 12.51 10.11 8.52	α, β, γ (°)	90, 90, 90	90, 90, 90	90, 90, 90		
Unique reflections 47,744 (3,442) 47,420 (3,467) 47,927 (3,494) Multiplicity 25.8 (20.7) 23.4 (23.9) 28.6 (30.9) Completeness (%) 97.3 (96.2) 96.0 (96.3) 98.2 (98.4) Rmerge 0.324 (1.784) 0.441 (1.987) 0.226 (0.598) <1/ol> 7.6 (1.4) 6.2 (1.4) 12.1 (5.3) CC1/2 (%) 99.4 (27.0) 95.9 (23.2) 99.4 (85.7) Refinement Resolution (Å) 19.77-1.40 57.10-1.40 56.82-1.40 No. of reflections 47,738 46,737 47,907 Rwork/Riree 0.1374/0.1735 0.1538/0.1856 0.1208/0.1427 No. of atoms	Resolution (Å)*	19.55-1.40 (1.45-1.40)	57.10-1.40 (1.44-1.40)	56.90-1.40 (1.44-1.40)		
Multiplicity 25.8 (20.7) 23.4 (23.9) 28.6 (30.9) Completeness (%) 97.3 (96.2) 96.0 (96.3) 98.2 (98.4) Rmerge 0.324 (1.784) 0.441 (1.987) 0.226 (0.598) <i ol=""> 7.6 (1.4) 6.2 (1.4) 12.1 (5.3) CC_{1/2}(%) 99.4 (27.0) 95.9 (23.2) 99.4 (85.7) Refinement Resolution (Å) 19.77-1.40 57.10-1.40 56.82-1.40 No. of reflections 47,738 46,737 47,907 Rwork/Rfree 0.1374/0.1735 0.1538/0.1856 0.1208/0.1427 No. of atoms Interpretation of the color of the color</i>	Observed reflections	1,231,502 (69,985)	1,101,042 (82,782)	1,371,884 (108,136)		
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	Unique reflections	47,744 (3,442)	47,420 (3,467)	47,927 (3,494)		
Rmerge $0.324 (1.784)$ $0.441 (1.987)$ $0.226 (0.598)$ $< I/o > 7.6 (1.4) 6.2 (1.4) 12.1 (5.3) CC1/2 (%) 99.4 (27.0) 95.9 (23.2) 99.4 (85.7) Refinement Resolution (Å) 19.77-1.40 57.10-1.40 56.82-1.40 No. of reflections 47,738 46,737 47,907 R_{work}/R_{free} 0.1374/0.1735 0.1538/0.1856 0.1208/0.1427 No. of atoms Protein 2,063 2,197 2,197 Ligand/ions 10 6 6 Waters 307 334 334 factors (Ų) 6 6 Overall 14.47 12.19 10.61 Protein 12.51 10.11 8.52 Ligand/ions 27.44 10.15 10.19 Waters 27.21 25.92 24.39 R.m.s. deviations Bond lengths (Å) 0.015 0.016 0.013 $	Multiplicity	25.8 (20.7)	23.4 (23.9)	28.6 (30.9)		
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	Completeness (%)	97.3 (96.2)	96.0 (96.3)	98.2 (98.4)		
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	R _{merge}	0.324 (1.784)	0.441 (1.987)	0.226 (0.598)		
Refinement Resolution (Å) 19.77-1.40 57.10-1.40 56.82-1.40 No. of reflections 47,738 46,737 47,907 Rwork/Rfree 0.1374/0.1735 0.1538/0.1856 0.1208/0.1427 No. of atoms		7.6 (1.4)	6.2 (1.4)	12.1 (5.3)		
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	CC _{1/2} (%)	99.4 (27.0)	95.9 (23.2)	99.4 (85.7)		
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	Refinement			•		
Rwork/Rfree 0.1374/0.1735 0.1538/0.1856 0.1208/0.1427 No. of atoms 2,063 2,197 2,197 Ligand/ions 10 6 6 Waters 307 334 334 factors (Ų) 0 10.61 Protein 12.51 10.11 8.52 Ligand/ions 27.44 10.15 10.19 Waters 27.21 25.92 24.39 R.m.s. deviations Bond lengths (Å) 0.015 0.016 0.013	Resolution (Å)	19.77-1.40	57.10-1.40	56.82-1.40		
No. of atoms 2,063 2,197 2,197 Ligand/ions 10 6 6 Waters 307 334 334 factors (Ų) 	No. of reflections	47,738	46,737	47,907		
Protein 2,063 2,197 2,197 Ligand/ions 10 6 6 Waters 307 334 334 factors (Ų) 12.19 10.61 Overall 14.47 12.19 10.61 Protein 12.51 10.11 8.52 Ligand/ions 27.44 10.15 10.19 Waters 27.21 25.92 24.39 R.m.s. deviations 0.015 0.016 0.013	Rwork/Rfree	0.1374/0.1735	0.1538/0.1856	0.1208/0.1427		
Ligand/ions 10 6 6 Waters 307 334 334 factors (Ų) 12.19 10.61 Overall 14.47 12.19 10.61 Protein 12.51 10.11 8.52 Ligand/ions 27.44 10.15 10.19 Waters 27.21 25.92 24.39 R.m.s. deviations 50.016 0.013	No. of atoms					
Waters 307 334 334 factors (Ų) 12.19 10.61 Protein 12.51 10.11 8.52 Ligand/ions 27.44 10.15 10.19 Waters 27.21 25.92 24.39 R.m.s. deviations Bond lengths (Å) 0.015 0.016 0.013	Protein	2,063	2,197	2,197		
 factors (Ų) 12.19 10.61 Protein 12.51 10.11 8.52 Ligand/ions 27.44 10.15 10.19 Waters 27.21 25.92 24.39 R.m.s. deviations 0.015 0.016 0.013	Ligand/ions	10	6	6		
Overall 14.47 12.19 10.61 Protein 12.51 10.11 8.52 Ligand/ions 27.44 10.15 10.19 Waters 27.21 25.92 24.39 R.m.s. deviations 0.015 0.016 0.013	Waters	307	334	334		
Protein 12.51 10.11 8.52 Ligand/ions 27.44 10.15 10.19 Waters 27.21 25.92 24.39 R.m.s. deviations	 factors (Å²)					
Ligand/ions 27.44 10.15 10.19 Waters 27.21 25.92 24.39 R.m.s. deviations	Overall	14.47	12.19	10.61		
Waters 27.21 25.92 24.39 R.m.s. deviations 0.016 0.013	Protein	12.51	10.11	8.52		
R.m.s. deviations 0.015 Bond lengths (Å) 0.015 0.016 0.013	Ligand/ions	27.44	10.15	10.19		
Bond lengths (Å) 0.015 0.016 0.013	Waters	27.21	25.92	24.39		
	R.m.s. deviations					
Bond angles (°) 1.10 1.80 1.79	Bond lengths (Å)	0.015	0.016	0.013		
	Bond angles (°)	1.10	1.80	1.79		

[†]Unfiltered MicroED data collected from 63° sweeps were previously published in Martynowycz *et al.*, 2023.

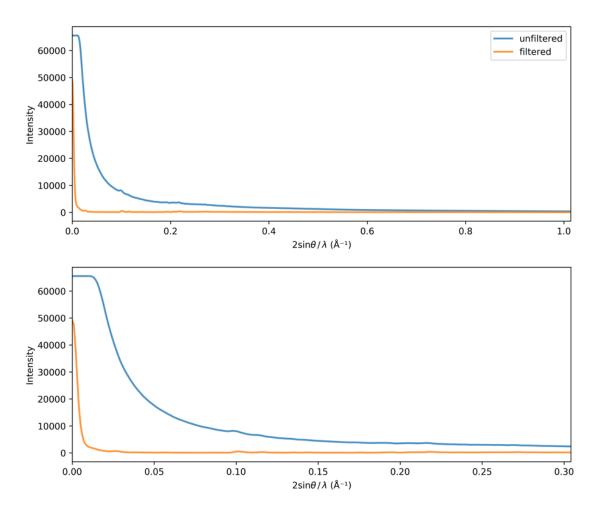
^{*}An equivalent unfiltered MicroED dataset collected utilizing a 20° sweeps strategy, identical to the filtered data.

^{*}Values in parenthesis are for the highest resolution shell. For an equal comparison, all data were truncated at 1.4 Å resolution.

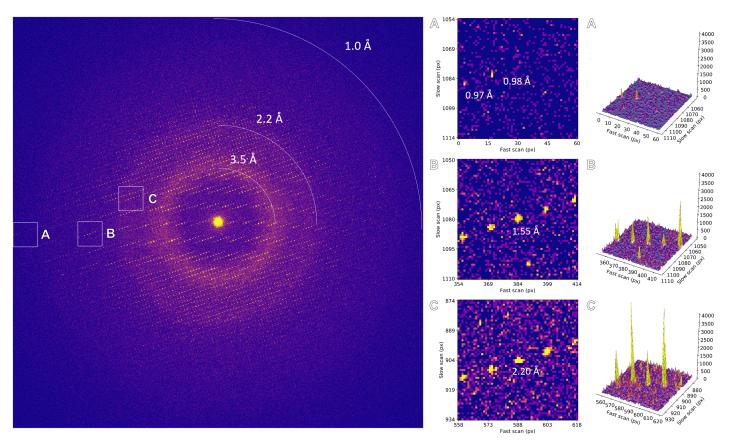
[‡] Calculated with the EMED subprogram of RADDOSE-3D (5.0.1047)²⁰.



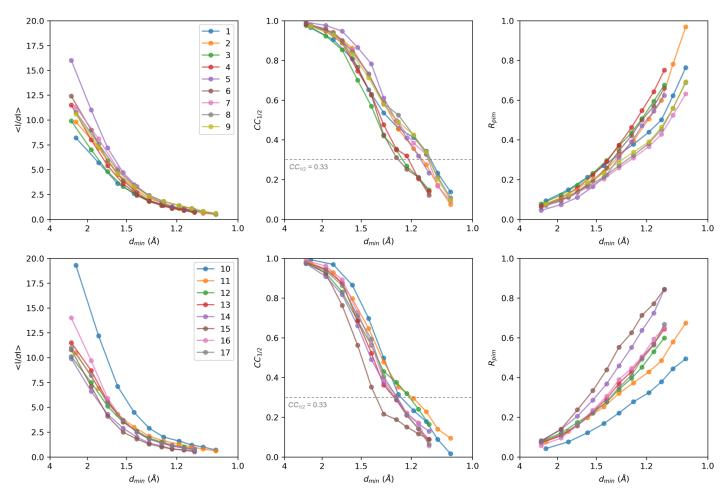
Supplementary Figure 1. Unfiltered MicroED data. Unfiltered diffraction pattern of a stationary proteinase K lamella from a 420 s exposure at a total fluence of $0.84 \text{ e}^{-}/\text{Å}^{2}$. Highlighted areas are magnified in the right panels and the corresponding peak profiles are plotted.



Supplementary Figure 2. Radial averages of unfiltered and filtered MicroED data. Radial averages of an energy-filtered diffraction frame (shown in Figure 1) and unfiltered data (shown in Supplementary Figure 1). Top: radial plots extending from the center peak to the edge of the detector, at approximately 1 Å resolution. Bottom: Same radial plots focusing on the lower angle data, extending to ~3 Å resolution.



Supplementary Figure 3. Energy-filtered continuous rotation MicroED data. Initial frame from energy-filtered and electron-counted MicroED data collection with a 5 s exposure time and a total fluence of 0.01 e⁻/Å². Highlighted areas are magnified in the right panels and the corresponding peak profiles are plotted.



Supplementary Figure 4. Intensity statistics for individual datasets. Intensity statistics for individual MicroED datasets, featuring from left to right $I/\sigma I$, $CC_{1/2}$, and R_{pim} as function of resolution. Individual datasets were truncated ranging from 1.13 to 1.06 Å resolution at a $CC_{1/2}$ that was still significant at the 0.1% level.