Structure, Volume 28

Supplemental Information

Need for Speed: Examining Protein

Behavior during CryoEM Grid

Preparation at Different Timescales

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Supplemental Material



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3 Figure S1, related to Figure 2. Partitioning of particles to the AWI. Fraction of particles 4 partitioning to the AWI within 10 nm (A) or 20 nm (B) for apoferritin (i), HSPD1 (ii) and 5 ribosomes (iii), with time and method of vitrification indicated. Some Vitrobot[™] values were 6 excluded from (B) because of low ice thickness. Shown are the individual data points, mean 7 value and standard deviation.



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Figure S2, related to Figure 2. Surface aggregates at varying timepoints. Sections through reconstructed tomograms from TED grids, showing morphology of apoferritin aggregates at the AWI at (A) 11 ms or (B) 50 ms, outlined in red. Some tomograms showed highly asymmetric distributions of particles, with two interfaces from the same tomogram shown in (Ci) and (Cii) or (Di) and (Dii) for the apoferritin 50 ms and ribosome 13 ms sample, respectively. Scale bars 50 nm.

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18 Figure S3, related to Figure 3. Comparing modelled data to experimental apoferritin

19partitioning. (A) Modelled distribution of particles (blue) in an ice layer (red), with coordinates20indicated on axis (nm). AWIs were taken from an experimental tomogram and particle21coordinates were randomly generated. This models a situation with no changes in22concentration compared with sample applied, and no affinity for the AWI. (B) Comparison23between modeled and experimental data for particle partitioning to the AWI. Particle24concentration is 20 μM, P = 0.007. (C) Distributions of distances between particles and AWI25for simulated (blue line) and experimental data (black bars) for apoferritin Vitrobot™ grids.



27 Figure S4, related to Figure 4. CryoEM image processing of HSPD1 data to yield angular 28 distribution data and FSC curves for HSPD1 consensus structures. A Data processing 29 pipelines for HSPD1 data showing particle numbers and corresponding 3D density maps and 30 resolution for each sample preparation device and timescale analysed. Datasets have varying 31 ice thicknesses, particle number and angular orientation and so resolutions cannot be directly 32 compared. (B) FSC curves for masked maps of the consensus structure (all datasets 33 combined) with and without symmetry. (C) 3D-FSC analysis of the same reconstruction, 34 showing that resolution in the z-direction is limited through the lack of side views. Note that 35 pixel size is 2.13 Å. (D) FSC curves for reconstructions for each individual dataset.



dataset	# micrographs
13 ms TED	1,552
54 ms chameleon	1,569
200 ms chameleon	1,637
Vitrobot [™] 6 s	1,826

particle picking

cryolo



70S 4.6 Å

> 50S 3.9 Å



6,856 particles discarded 3D classification & ----class selection 30S 5.9 Å select 554,166 particles 2D classification select 60,630 particles 3D refinement 2 classes 70S Relion 72,091 particles local resolution dataset # particles 13 ms TED 70S: 2,729 50S: 15,871 2D classification 30S: 4,673 select 258,448 particles 54 ms 70S: 6,177 zflip chameleon 50S: 29,960 4 classes 50S 3D refinement split into 30S: 13,401 283,851 particles original 200 ms 70S: 29,667 ----datasets & 50S: 127,946 chameleon 2D classification extract 30S: 47,563 2 rounds angles select 123,818 particles Vitrobot[™] 6 s 70S: 22,057 50S: 84,671 zflip 1 class 30S 30S: 58,181

36

191,368 particles

- 37 Figure S5, related to Figure 5. CryoEM image processing of ribosome data to yield
- 38 **angular distribution data.** Data processing pipelines for ribosome angular orientation maps.
- 39 Micrographs shown are representative of the type of micrograph used.

3D refinement



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Figure S6, related to Figure 5. CryoEM reconstructions of ribosome data at different time points and vitrification devices. (A) FSC curves for masked, consensus reconstructions of 70S, 50S and 30S ribosome. (B) Relative particle numbers for 30S, 50S and 70S by individual dataset. (C) Individual reconstructed maps for 70S, 50S and 30S for all 45 subsets and corresponding FSC curves (D).



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Figure S7, related to Figure 6. Analysis of the surface properties and buried surface area for the ribosomal proteins L9, L31 and S2. (A) Electrostatic surface potential of the ribosome with subunits L9, L31 and S2 highlighted showing their contrasting neutral/positive charge compared to the predominantly negative charge of the ribosome. (B) Buried surface are for the different ribosomal proteins with the average buried surface area indicated by a

51 are for the different ribosomal proteins with the average buried surface area indicated by a 52 dashed line.

Internationation dense Implement of unitation dense <thimplement dense<="" of="" th="" unitation=""> Implem</thimplement>			Popost	Concentration [uM]	Applied	Ico thicknoss [nm]	# particles	relative particle #	relative particle #
$ \begin{tabular}{ c c c c c c c } \hline 11 m s TED & 1 & 1 & 1 & 0 & 1 & 0 & 0 & 1 & 0 & 0$		Time and vitrification device	1			160	100	21*	(3 201111)
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$ \begin{tabular}{ c c c c c c c c c c c c c c c c c c c$		11 ms TED	2	1.5	20.0	78	64	73	80
$ \begin{tabular}{ c c c c c c c } \hline & & & & & & & & & & & & & & & & & & $		111115120		1.4	20.0	97	53	73	01
Apoferritin 3 1 3 1 3 1 3 1 1 3 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 3 2.1 1 <th1< th=""> 1 1 <t< td=""><td></td><td></td><td>5</td><td>2.5</td><td></td><td>130</td><td>186</td><td>74</td><td>80</td></t<></th1<>			5	2.5		130	186	74	80
$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$			5	2.2		135	100	75	85
$\begin{tabular}{ c c c c c c c } App {if a bound between the set of the set$			1	3.4		152	253	72	77
Apoferritin 3 2.1 3 2.1 3 2.1 5 1.4 5 7 6 7 6 7 6 7 6 7 6 7 6 7 7 4 8 7 7 4 8 7			2	7.7	20.0	135	509	66	74
$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$			3	2.1		68	54	48	72
$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	Aporerritin	FO ms TED	4	1.7		76	79	63	82
$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$		SO HIS TED	5	1.4	20.0	64	54	59	76
$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$			6	119.2		47	2160	90	98
$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$			7	41.8		90	1835	91	97
$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$			8	1.3		88	54	46	52
$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$									
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $			1	53.9		54	1751	90	97**
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$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$			3	83.8		34	1582	87	99**
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$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$		6 IIIS TED	3	0.9		82	37	97	97
HSPD1 1 18.8 60 680 99 100 50 ms TED 2 4.9 11.0 87 204 85 87 3 7.4 73 325 100 100** 6 s Vitrobot [™] 1 18.9 0.6 50 270 99 100** 3 9.3 0.6 50 270 99 100** 3 9.3 0.6 50 270 99 100** 3 9.3 9.3 100 100** 100** 100** 3 9.3 9.3 100 100** 100** 100** 3 9.3 9.3 100 100** 100** 100** 13 ms TED 1 1.3 2 0.3 130 14 100 100 13 ms TED 1 11.9 2.5 137 94 94 96 200 ms chameleon 1 11.9 2.5			4	2.3		62	85	93	96
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$\begin{array}{ c c c c c c c c c c c c c c c c c c c$			1	18.8		60	680	99	100
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$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$			3	7.4		73	325	100	100
Image: height of the structure Image:									
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Image: space of the sector of the s			2	10.0		50	270	99	100**
Image: space of the system of the s			3	9.3	1	64	156	98	100**
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13 ms TED 3 3.2 2.5 151 84 94 98 4 3.9 5 5.9 119 178 92 99 99 5 5.9 67 152 99 99 99 200 ms chameleon 2 12.5 2.5 136 864 95 96 3 15.8 135 1235 92 96 96 6 s Vitrobot [™] 1 17.0 92 601 79 84 2 23.5 0.8 93 843 87 83			2	0.3		130	14	100	100
A 3.9 119 178 92 99 5 5.9 67 152 99 99 67 152 99 99 99 1 11.9 178 92 99 200 ms chameleon 2 12.5 2.5 137 934 94 96 3 15.8 135 1235 92 96 96 6 s Vitrobot [™] 2 23.5 0.8 93 843 84 87 3 20.2 0.8 97 77 78 83			3	3.2		151	84	94	98
Sibosome S S.9 67 152 99 99 Ribosome 1 11.9 1 136 864 95 96 200 ms chameleon 2 12.5 2.5 137 934 94 96 3 15.8 135 1235 92 96 6 s Vitrobot™ 2 23.5 0.8 93 843 84 87 3 20.2 97 787 78 83 84 87			4	3.9		119	178	92	99
Ribosome 3 3.5 07 122 35 35 200 ms chameleon 1 11.9 2.5 136 864 95 96 3 15.8 135 135 1235 92 96 6 s Vitrobot™ 2 23.5 0.8 92 601 79 84 3 20.2 97 787 78 83			5	5.9		67	152	99	99
1 11.9 136 864 95 96 200 ms chameleon 2 12.5 2.5 137 934 94 96 3 15.8 135 1235 92 96 6 s Vitrobot [™] 2 23.5 0.8 92 601 79 84 3 20.2 97 787 78 83	Ribosome			5.5		0/	152	55	55
200 ms chameleon 2 12.5 2.5 137 934 94 96 3 15.8 1.35 1235 92 96 6 s Vitrobot™ 2 23.5 0.8 92 601 79 84 3 20.2 0.8 93 843 84 87		200 ms chameleon	1	11.9		136	864	95	96
1 17.0 33.4 34.4 30.4 3			2	12.5	2.5	137	934	94	96
1 17.0 92 601 79 84 2 23.5 0.8 93 843 84 87 3 20.2 97 787 78 83			3	15.8		135	1235	92	96
1 17.0 92 601 79 84 2 23.5 0.8 93 843 84 87 3 20.2 97 787 78 83			5	13.0		135	1255	52	50
6 s Vitrobot [™] 2 23.5 0.8 93 843 84 87 3 20.2 97 787 78 83		6 s Vitrobot [™]	1	17.0		92	601	79	84
3 20.2 97 787 78 83			2	23.5	0.8	93	843	84	87
			3	20.2	1	97	787	78	83

Table S1, related to Figure 2. Summary table of tomograms analysed to produce 56 **partitioning and particle concentration data**. "Concentration" is the estimated concentration 57 from the tomogram, "Applied concentration" is the concentration of the sample applied. 58 "Relative particle #" is the percentage of particles within \leq 10 or 20 nm of the AWI.

⁵⁹ * Values were excluded from analysis because of poor fitting of AWIs.

60 ** Values were excluded from analysis because of low ice thickness.

Titan Krios II
42,000
300
1.8 - 1.9
3
-60°, +60° (2°)
bidirectional
-5 to -10
3.4

Table S2, related to Figure 3. Microscope parameters for collection of cryo-ET data. 69

	HSPD1				
	6 ms TED	50 ms TED	54 ms chameleon	6 s Vitrobot™	
Microscope	Titan Krios I				
Magnification	75,000				
Voltage (kV)	300				
Total electron	81	81	74	75	
dose (e ⁻ /Ų)					
Exposure time	1.5	1.5	1.5	1.5	
Number of frames	59	59	59	59	
Defocus range	-2 to -4	-2 to -4.5	-1.5 to -3.5	-1.3 to -3.3	
(µm)					
Pixel size (Å)	1.065				

70 71 Table S3, related to STAR Methods. Data collection parameters for SPA datasets of

HSPD1.

	ribosome			
	13 ms	54 ms	200 ms	6 s
	TED	chameleon	chameleon	Vitrobot™
Microscope	Titan Krios I			
Magnification	75,000			
Voltage (kV)	300			
Total electron dose (e ⁻ /Å ²)	77	74	74	78
Exposure time	1.5	1.6	1.5	1.5
Number of frames	59	59	59	59
Defocus range (µm)	-1.3 to -3.3	-1.3 to -3.3	-1.3 to -3.3	-1.3 to -3.3
Pixel size (Å)	1.065			

72 Table S4, related to STAR Methods. Data collection parameters for SPA datasets of the

73 ribosome.