Supplemental Information

Need for Speed: Examining Protein Behavior during CryoEM Grid Preparation at Different Timescales

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

Figure S3, related to Figure 3. Comparing modelled data to experimental apoferritin partitioning. (A) Modelled distribution of particles (blue) in an ice layer (red), with coordinates indicated on axis (nm). AWIs were taken from an experimental tomogram and particle coordinates were randomly generated. This models a situation with no changes in concentration compared with sample applied, and no affinity for the AWI. (B) Comparison between modeled and experimental data for particle partitioning to the AWI. Particle concentration is 20 µM, P = 0.007. (C) Distributions of distances between particles and AWI for simulated (blue line) and experimental data (black bars) for apoferritin Vitrobot™ grids.
Figure S4, related to Figure 4. CryoEM image processing of HSPD1 data to yield angular distribution data and FSC curves for HSPD1 consensus structures. A Data processing pipelines for HSPD1 data showing particle numbers and corresponding 3D density maps and resolution for each sample preparation device and timescale analysed. Datasets have varying ice thicknesses, particle number and angular orientation and so resolutions cannot be directly compared. (B) FSC curves for masked maps of the consensus structure (all datasets combined) with and without symmetry. (C) 3D-FSC analysis of the same reconstruction, showing that resolution in the z-direction is limited through the lack of side views. Note that pixel size is 2.13 Å. (D) FSC curves for reconstructions for each individual dataset.
Figure S5, related to Figure 5. CryoEM image processing of ribosome data to yield angular distribution data. Data processing pipelines for ribosome angular orientation maps. Micrographs shown are representative of the type of micrograph used.

### Dataset Summary

| Dataset       | # Micrographs | # Picked Particles |
|---------------|---------------|--------------------|
| 13 ms TED     | 1,552         | 30,649             |
| 54 ms chameleon | 1,569       | 72,520             |
| 200 ms chameleon | 1,637       | 252,408            |
| Vitrobot™ 6 s | 1,826         | 201,519            |

| Dataset       | # Particles |
|---------------|-------------|
| 70S: 2,729    | 70S: 6,177  |
| 50S: 15,871   | 50S: 29,960 |
| 30S: 4,673    | 30S: 13,401 |

| Dataset       | # Particles |
|---------------|-------------|
| 70S: 6,177    | 70S: 29,667 |
| 50S: 29,960   | 50S: 127,946|
| 30S: 13,401   | 30S: 47,563 |

| Dataset       | 30S: 5.9 Å  | 50S: 3.9 Å  | 70S: 4.6 Å |
|---------------|-------------|-------------|------------|
| 1 class 30S  | 191,368     | 191,368     | 191,368    |
| 4 classes 50S| 283,851     | 283,851     | 283,851    |
| 2 classes 70S| 72,091      | 72,091      | 72,091     |

Note: 6,856 particles discarded.
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 4 subsets and corresponding FSC curves (D).
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 area for the different ribosomal proteins with the average buried surface area indicated by a dashed line.
Table S1, related to Figure 2. Summary table of tomograms analysed to produce partitioning and particle concentration data. “Concentration” is the estimated concentration from the tomogram, “Applied concentration” is the concentration of the sample applied. “Relative particle #” is the percentage of particles within ≤ 10 or 20 nm of the AWI.

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

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

| Time and vitrification device | Repeat | Concentration [µM] | Applied concentration [µM] | Ice thickness [nm] | # particles | Relative particle # (≤ 10nm) | Relative particle # (≤ 20nm) |
|------------------------------|--------|-------------------|-----------------------------|-------------------|-------------|-----------------------------|-----------------------------|
| Apoferritin                  | 1      | 1.0               | 20.0                        | 168               | 100         | 21*                         | 61                          |
|                              | 2      | 1.9               |                              | 96                | 111         | 77                          | 86                          |
|                              | 3      | 1.4               |                              | 78                | 64          | 73                          | 89                          |
|                              | 4      | 0.9               |                              | 97                | 53          | 74                          | 91                          |
|                              | 5      | 2.2               |                              | 139               | 186         | 75                          | 89                          |
| 50 ms TED                    | 1      | 3.4               | 20.0                        | 152               | 253         | 72                          | 77                          |
|                              | 2      | 7.7               |                              | 135               | 599         | 66                          | 78                          |
|                              | 3      | 2.1               |                              | 68                | 54          | 48                          | 72                          |
|                              | 4      | 1.7               |                              | 76                | 79          | 63                          | 82                          |
|                              | 5      | 1.8               |                              | 64                | 54          | 59                          | 76                          |
|                              | 6      | 119.2             |                              | 47                | 2160        | 90                          | 98                          |
|                              | 7      | 41.8              |                              | 90                | 1835        | 91                          | 97                          |
|                              | 8      | 1.3               |                              | 88                | 54          | 46                          | 52                          |
| 6 s Vitrobot™                | 1      | 53.9              | 20.0                        | 54                | 1751        | 90                          | 97**                        |
|                              | 2      | 69.1              |                              | 43                | 1622        | 82                          | 96**                        |
|                              | 3      | 83.8              |                              | 34                | 1582        | 87                          | 99**                        |
| HSPD1                        | 1      | 1.5               | 11.0                        | 95                | 70          | 77                          | 87                          |
|                              | 2      | 1.3               |                              | 73                | 69          | 46*                         | 81                          |
|                              | 3      | 0.9               |                              | 82                | 37          | 97                          | 97                          |
|                              | 4      | 2.3               |                              | 62                | 85          | 93                          | 96                          |
| 50 ms TED                    | 1      | 18.8              | 11.0                        | 60                | 680         | 99                          | 100                         |
|                              | 2      | 4.9               |                              | 67                | 204         | 85                          | 87                          |
|                              | 3      | 7.4               |                              | 73                | 325         | 100                         | 100                         |
| 6 s Vitrobot™                | 1      | 18.9              | 0.6                         | 39                | 193         | 100                         | 100**                       |
|                              | 2      | 10.0              |                              | 50                | 270         | 99                          | 100**                       |
|                              | 3      | 9.3               |                              | 64                | 156         | 98                          | 100**                       |
| Ribosome                     | 1      | 1.3               | 2.5                         | 113               | 57          | 19*                         | 100                         |
|                              | 2      | 0.3               |                              | 130               | 14          | 100                         | 100                         |
|                              | 3      | 3.2               |                              | 151               | 84          | 94                          | 98                          |
|                              | 4      | 3.9               |                              | 119               | 178         | 92                          | 99                          |
|                              | 5      | 5.9               |                              | 67                | 152         | 99                          | 99                          |
| 200 ms chameleon             | 1      | 11.9              | 2.5                         | 136               | 864         | 95                          | 96                          |
|                              | 2      | 12.5              |                              | 137               | 934         | 94                          | 96                          |
|                              | 3      | 15.8              |                              | 135               | 1255        | 92                          | 96                          |
| 6 s Vitrobot™                | 1      | 17.0              | 0.8                         | 92                | 601         | 79                          | 84                          |
|                              | 2      | 23.5              |                              | 93                | 843         | 84                          | 87                          |
|                              | 3      | 20.2              |                              | 97                | 787         | 78                          | 83                          |
Table S2, related to Figure 3. Microscope parameters for collection of cryo-ET data.

| HSPD1 | 6 ms TED | 50 ms TED | 54 ms chameleon | 6 s Vitrobot<sup>TM</sup> |
|-------|----------|-----------|-----------------|--------------------------|
| Microscope | Titan Krios I | 75,000 | 74 | 75 |
| Magnification | 75,000 | 74 | 75 |
| Voltage (kV) | 300 | 1.5 | 1.5 | 1.5 |
| Total electron dose (e/Å²) | 81 | 81 | 74 | 75 |
| Exposure time | 1.5 | 1.5 | 1.5 | 1.5 |
| Number of frames | 59 | 59 | 59 | 59 |
| Defocus range (µm) | -2 to -4 | -2 to -4.5 | -1.5 to -3.5 | -1.3 to -3.3 |
| Pixel size (Å) | 1.065 | 1.065 | 1.065 | 1.065 |

Table S3, related to STAR Methods. Data collection parameters for SPA datasets of HSPD1.

| ribosome | 13 ms TED | 54 ms chameleon | 200 ms chameleon | 6 s Vitrobot<sup>TM</sup> |
|----------|-----------|-----------------|-----------------|--------------------------|
| Microscope | Titan Krios I | 75,000 | 74 | 78 |
| Magnification | 75,000 | 74 | 78 |
| Voltage (kV) | 300 | 1.5 | 1.5 | 1.5 |
| Total electron dose (e/Å²) | 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 | 1.065 | 1.065 | 1.065 |

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