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**Supporting information for article:**

# High-resolution cryo-EM using beam-image shift at 200 keV

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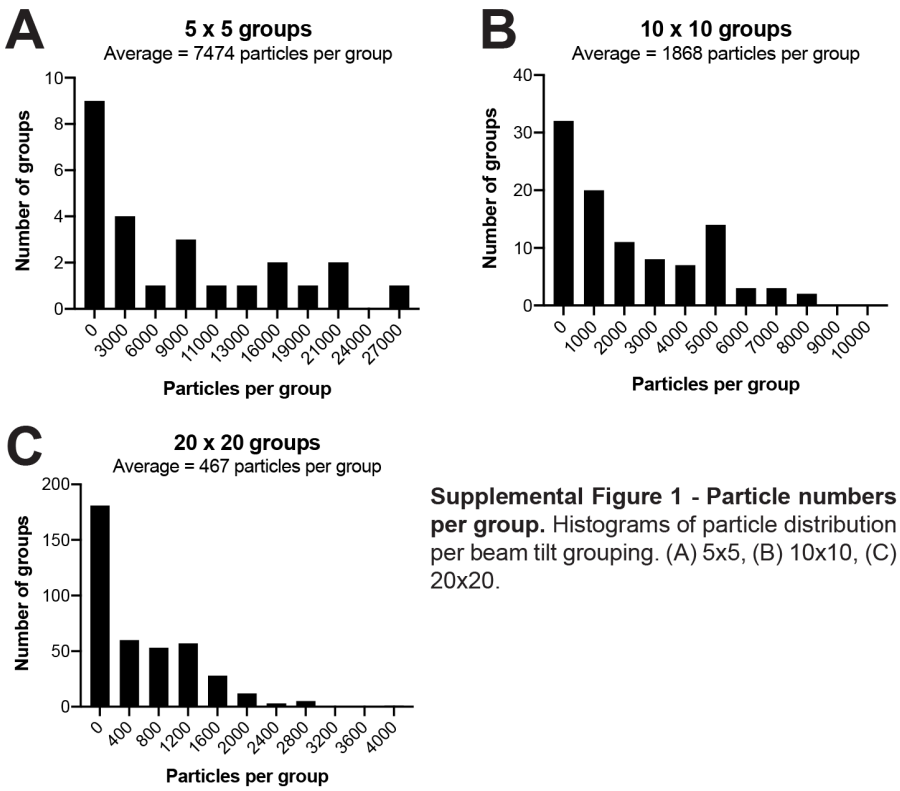
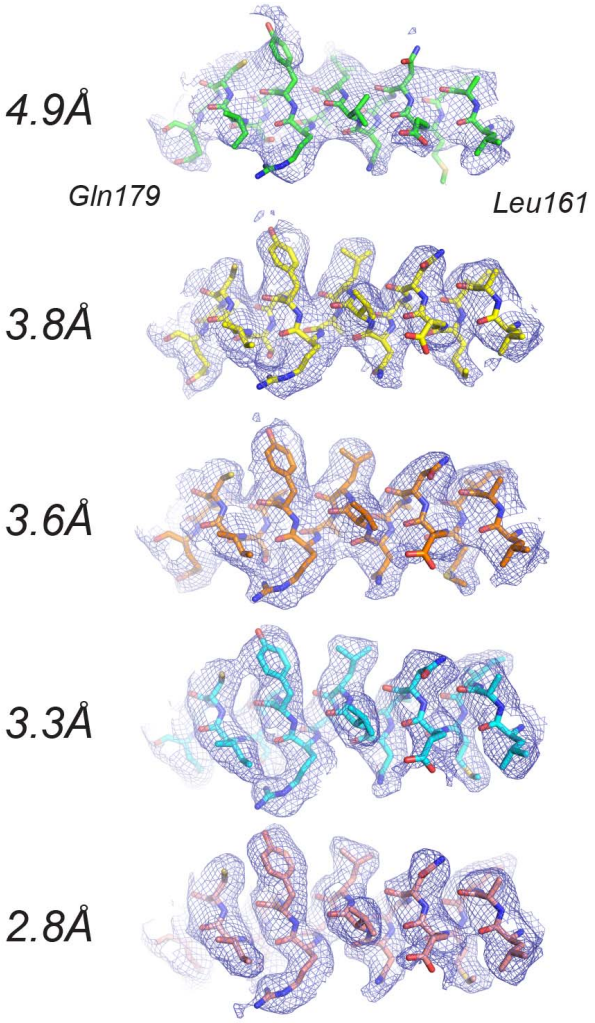
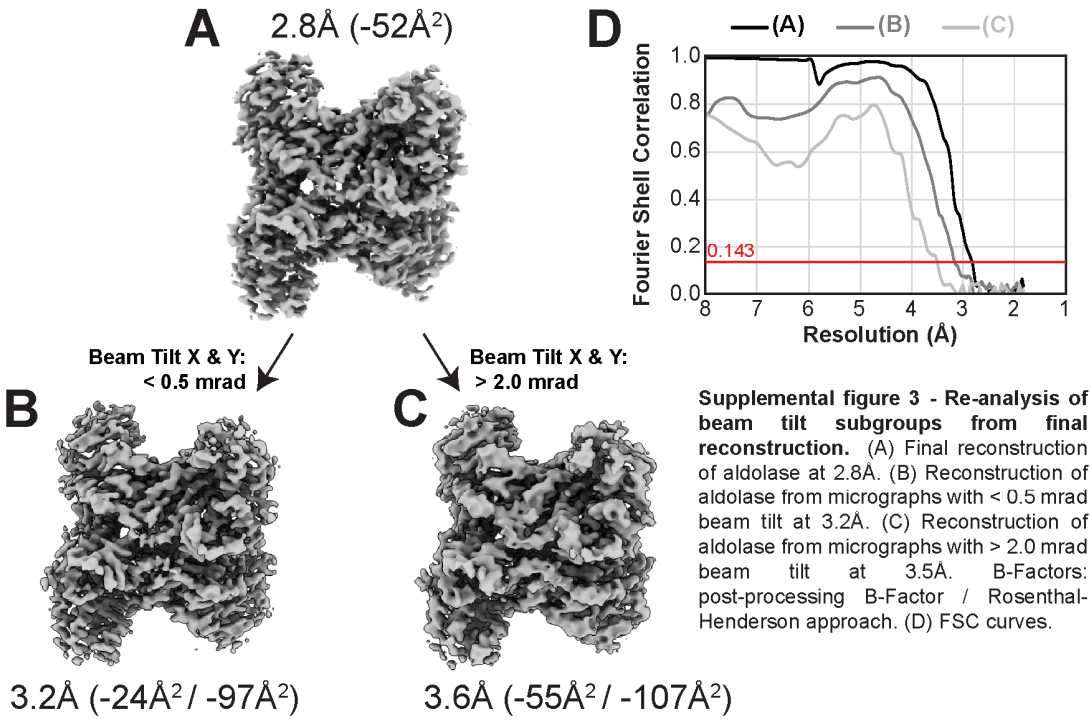


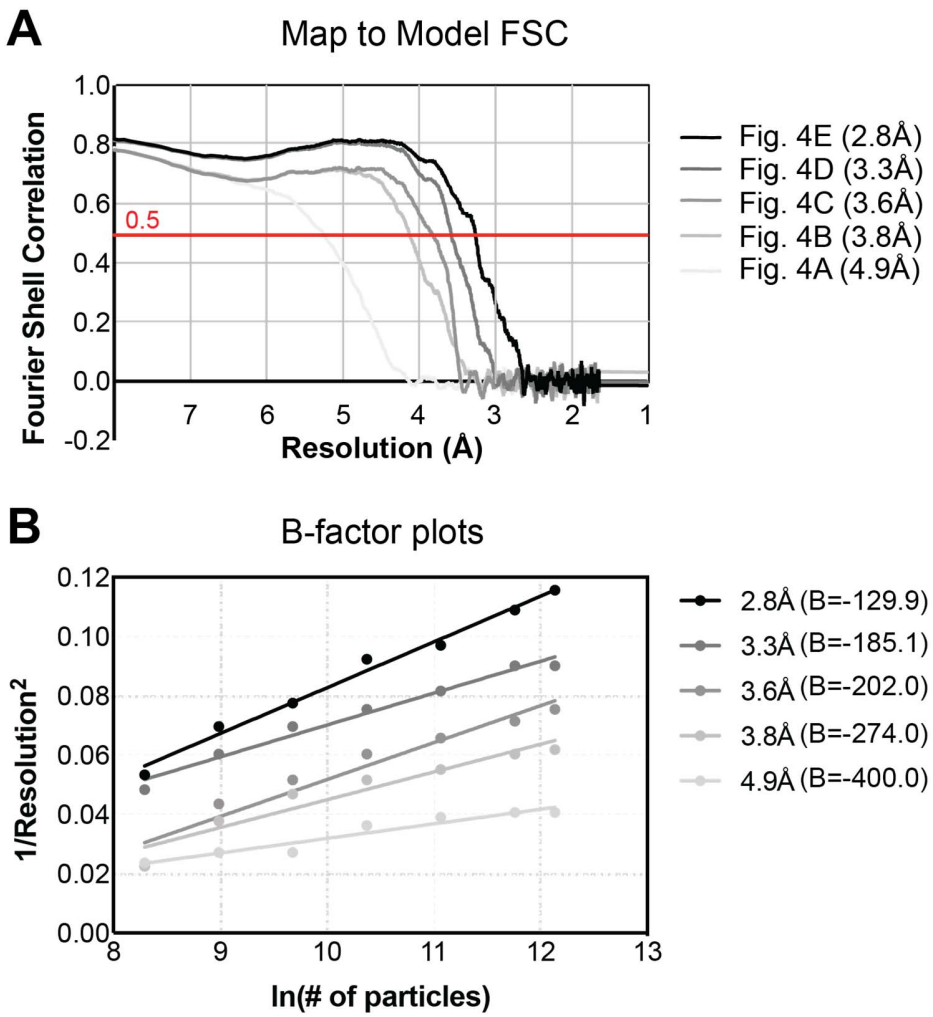

**Supplemental Figure 1 - Particle numbers per group.** Histograms of particle distribution per beam tilt grouping. (A) 5x5, (B) 10x10, (C) 20x20.



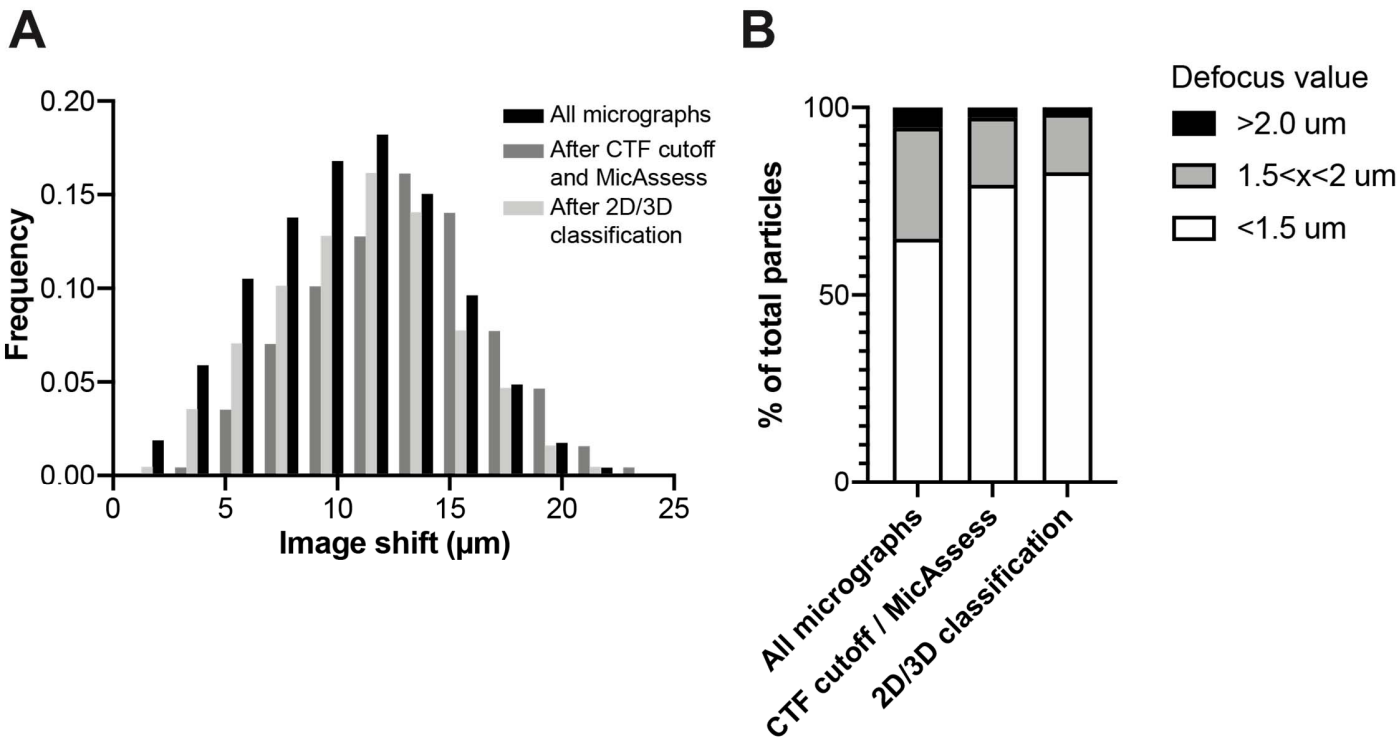

**Supplemental Figure 2 - Representative densities from iterative beam-tilt refinements.** Sharpened densities with associated models highlight changes in density quality through iterative rounds of beam-tilt refinement corresponding to structures from Figure 4A-4E.




**Supplemental figure 3 - Re-analysis of beam tilt subgroups from final reconstruction.** (A) Final reconstruction of aldolase at 2.8Å. (B) Reconstruction of aldolase from micrographs with < 0.5 mrad beam tilt at 3.2Å. (C) Reconstruction of aldolase from micrographs with > 2.0 mrad beam tilt at 3.5Å. B-Factors: post-processing B-Factor / Rosenthal-Henderson approach. (D) FSC curves.




**Supplemental figure 4 - Structure validation during data analysis.**
(A) Map-to-model FSC comparing our maps from Fig. 4A-E with 5vy5 as the model. (B) Rosenthal-Henderson plots showing particle number and resolution to determine B-factors for each reconstruction.




**Supplemental figure 5 - Quantification of micrograph image shift and particle defocus.** (A) For all micrographs from the dataset (black bars), the distance from (0,0) was determined and plotted as a function of frequency. The same was done for the subset of micrographs kept after CTF cut off and MicAsses (dark grey bars) and 2D/3D classification (light grey bars). (B) For every particle extracted, the defocus was binned into lower than 1.5 um (white), higher than 2 um (black), or inbetween (grey). The same was done for the subset if particles kept after CTFcutoff and MicAssess (500,001) and 2D/3D classification (186,471 particles).

**Supplemental Table S1 - Cryo-EM data collection, refinement, and validation statistics.**

| | **Aldolase (19apr12a)** |
|---|---|
| **Microscope** | Talos-Arctica |
| **Detector** | Gatan K2 |
| **Voltage (keV)** | 200 |
| **Electron exposure ($e^-/Å^2$)** | 43 |
| **Defocus range (μm)** | 0.8 - 2 |
| **Data collection mode** | Beam-image shift |
| **Micrographs collected (per hour)** | 73 |
| **Original pixel size (Å)** | 0.91 |
| **Symmetry imposed** | D2 |
| **Initial number of micrographs** | 2,111 |
| **Final number of micrographs** | 569 |
| **Initial particle images (no.)** | 718,578 |
| **Final pixel size** | 0.91 |
| **Final particle images (no.)** | 186,841 |
| **Number of optics groups** | 400 |
| **FSC threshold** | 0.143 |
| **Final map resolution (Å)** | 2.8 |
| **Final post-processing B-Factor ($Å^2$)** | -52 |

**Supplemental Table S2 - Model building statistics.**

| **Structure from Figure 4** | **4A** | **4B** | **4C** | **4D** | **4E** |
|---|---|---|---|---|---|
| **EMDB/PDB ID** | **22754/7K9L** | **22755/7K9X** | **22756/7KA2** | **22757/7KA3** | **22758/7KA4** |
| **Resolution (Å)** | 4.9 | 3.8 | 3.6 | 3.3 | 2.8 |
| Postprocessing B-Factor **(Å$^2$)** | -347 | -168 | -105 | -91 | -52 |
| **B-Factor (Å2)** (Rosenthal et. al. 2003) | -400.0 | -274.0 | -202.0 | -185.1 | -129.9 |
| **RELION Version** | 3.0.2 | 3.0.8 | 3.0.8 | 3.1 | 3.1 |
| **Bonds (RMSD)** | | | | | |
| Length (Å) | 0.007 | 0.008 | 0.008 | 0.004 | 0.005 |
| Angles (°) | 1.197 | 0.970 | 0.973 | 0.767 | 0.894 |
| **Molprobity score** | 2.18 | 2.21 | 1.94 | 1.35 | 1.86 |
| **Clash score** | 9.26 | 6.36 | 6.08 | 3.99 | 4.23 |
| **Ramachandran plot (%)** | | | | | |
| Outliers | 0 | 0 | 0 | 0 | 0 |
| Allowed | 3.52 | 3.81 | 4.40 | 2.93 | 3.23 |
| Favored | 96.48 | 96.19 | 95.60 | 97.07 | 96.77 |
| **Rotamer outliers (%)** | 3.96 | 6.12 | 2.52 | 0.36 | 3.96 |
| **CaBLAM outliers (%)** | 3.54 | 2.95 | 2.95 | 2.95 | 2.65 |

| | | | | | |
|---|---|---|---|---|---|
| **CC (mask)** | 0.78 | 0.81 | 0.83 | 0.82 | 0.82 |
| **CC (box)** | 0.67 | 0.79 | 0.87 | 0.76 | 0.77 |
| **CC (peaks)** | 0.58 | 0.71 | 0.80 | 0.72 | 0.73 |
| **CC (volume)** | 0.79 | 0.81 | 0.83 | 0.81 | 0.81 |
| **RMSD (Å) all atoms compared to Figure 4E** | 1.01 | 0.80 | 0.84 | 0.62 | - |
| **Resolution values of map-to-model FSC from Supplemental Figure 4 (Å)** | 5.3 | 4.1 | 3.8 | 3.6 | 3.2 |