



Research article

Subtomogram averaging by cryo-electron tomography using CRYO ARM™ 300 II for purified and cellular samples

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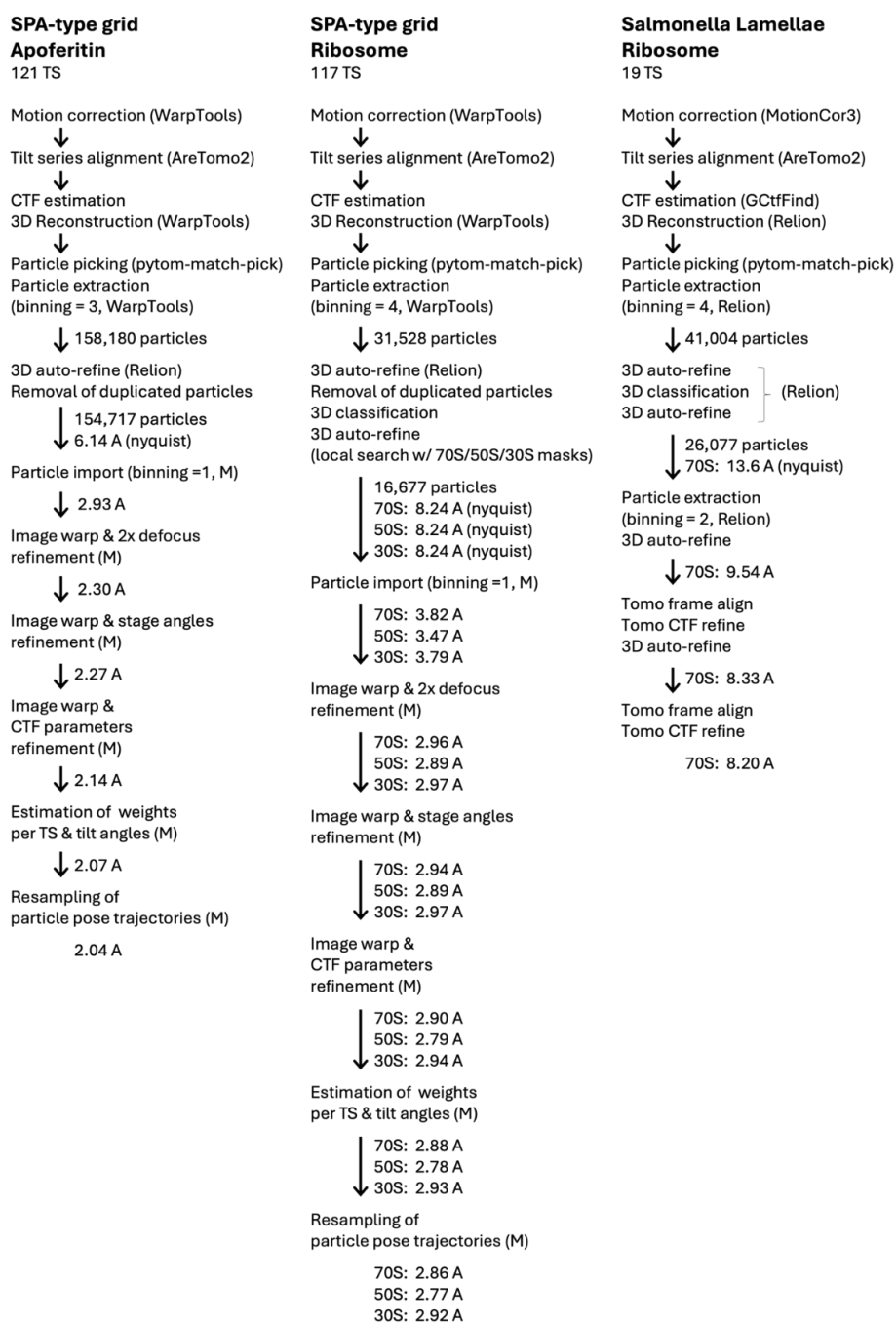
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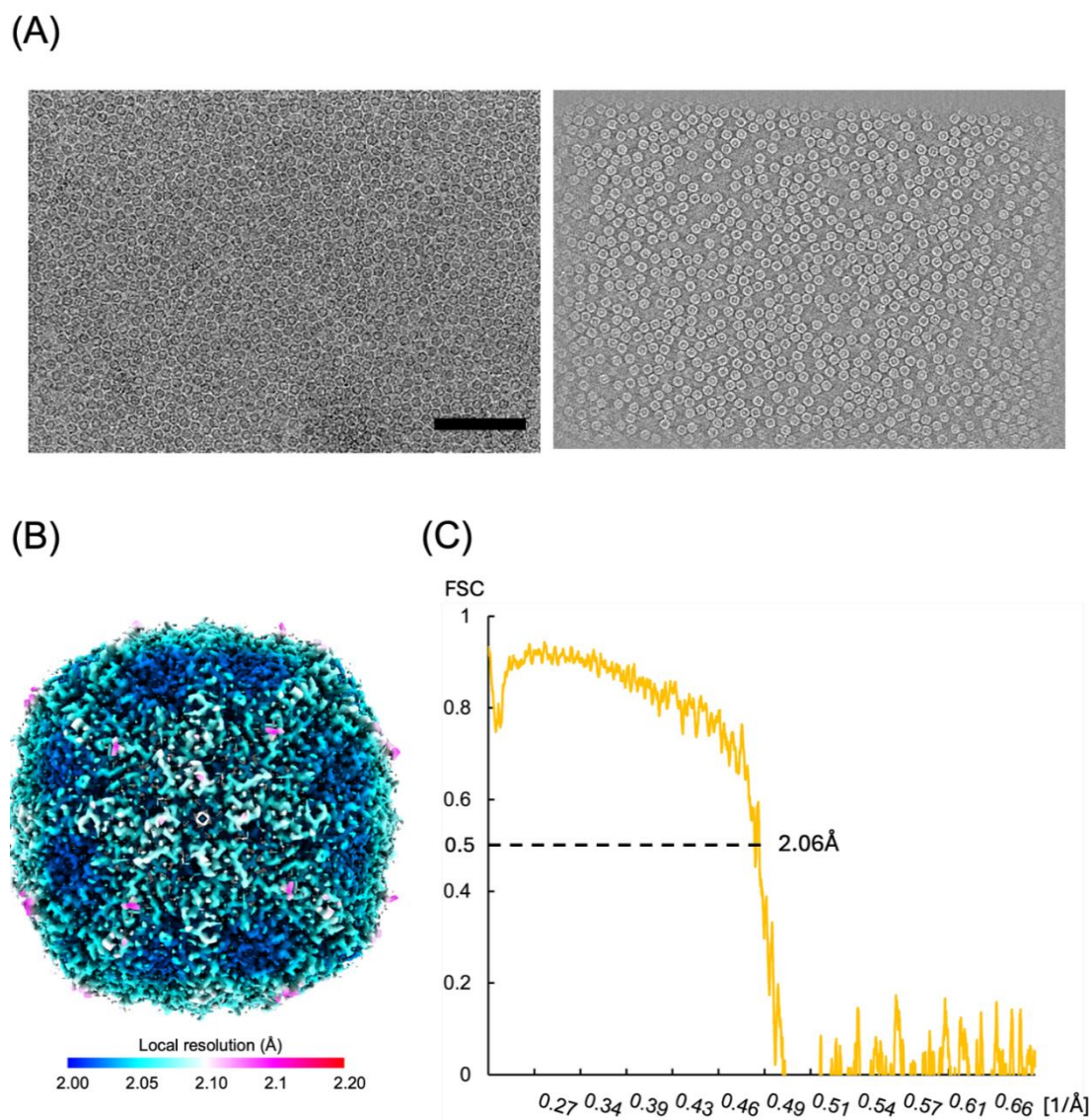
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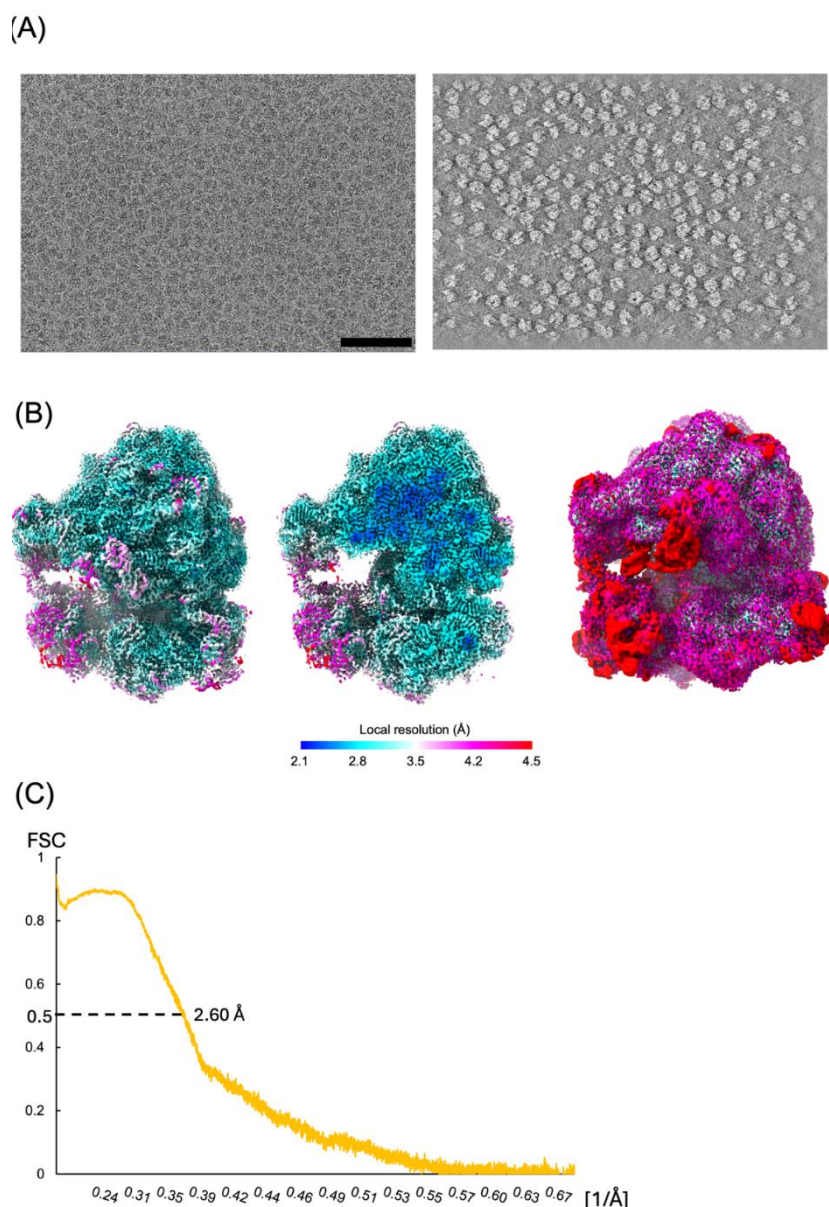
Supplementary



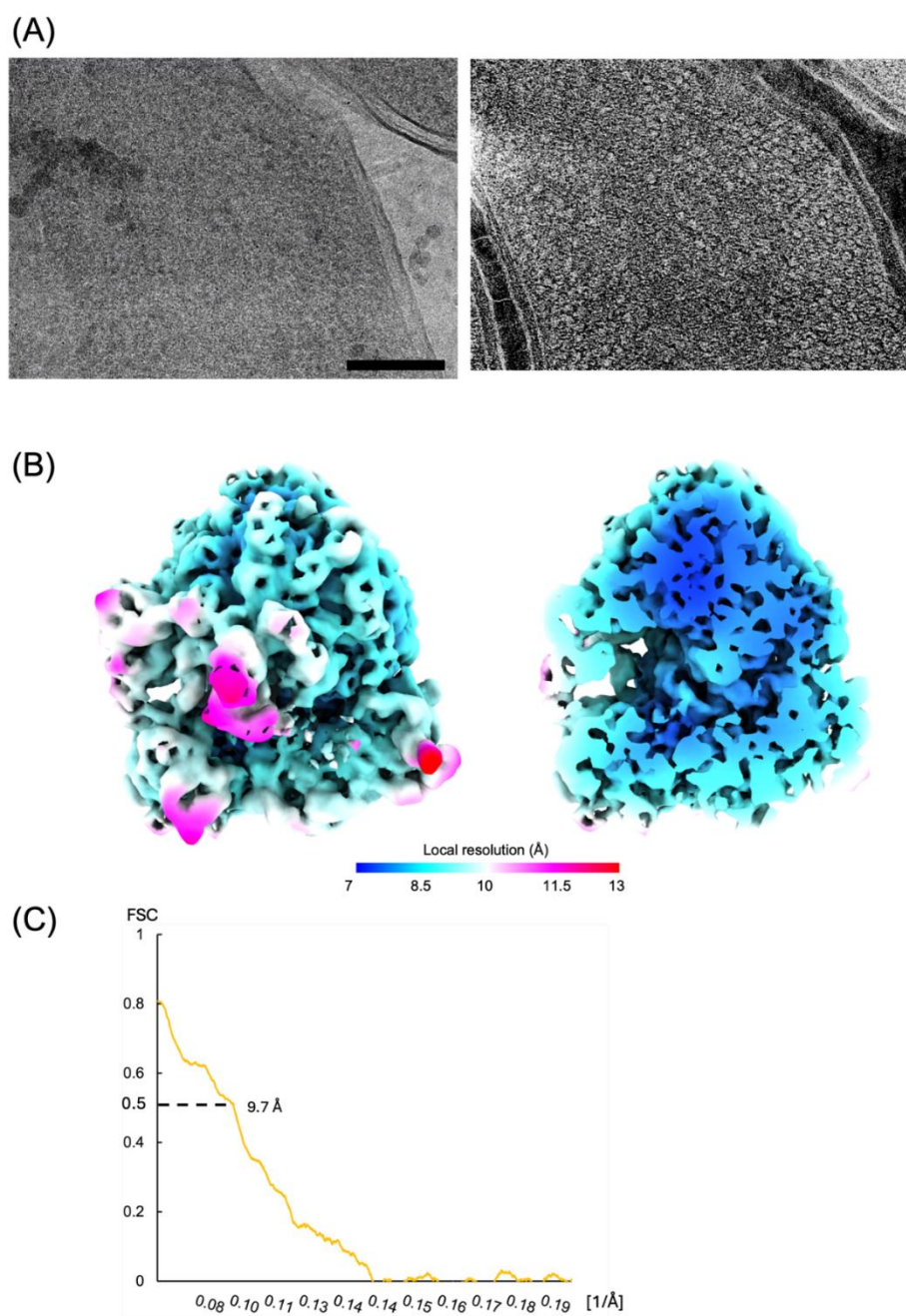
Supplementary Figure 1. STA image processing workflows for apoferritin and ribosomes on SPA-type grids, and *in situ* ribosomes on *Salmonella* lamellae. (A) Image processing workflow for apoferritin collected on an SPA-type grid (121 tilt series, 158,180 particles). The final resolution reached 2.04 Å after iterative refinement with Warp/M. (B) Image processing workflow for *E. coli* ribosomes collected on an SPA-type grid (117 tilt series). The final resolutions were 2.86 Å (70S), 2.77 Å (50S), and 2.92 Å (30S). (C) Image processing workflow for *in situ* ribosomes from a *Salmonella* lamella (19 tilt series, 26,077 particles). The final subtomogram average reached 8.20 Å resolution after Tomo-CTF refinement.



Supplementary Figure 2. Representative tilt series image and tomogram of apoferritin on SPA-type grids with local resolution of STA reconstruction and evaluation of FSC between the atomic model and map. (A) A representative tilt series image at 0 degree (left) and a slice from a tomographic reconstruction (right). Scale bar indicates 200 nm. (B) Local resolution map of apoferritin reconstructed by STA. The overall resolution based on the global FSC is 2.0 Å, and the local resolution was calculated using RELION. (C) The FSC curve between the atomic model and the STA map. The FSC threshold was set to 0.5. The atomic model was generated using the mouse apoferritin structure (PDB ID: 8RQB) as an initial model, followed by real-space refinement using Phenix, resulting in the deposited model (PDB ID: 23WI).



Supplementary Figure 3. Representative tilt series image and tomogram of ribosomes on SPA-type grids with local resolution of STA reconstruction and evaluation of FSC between the atomic model and map. (A) A representative tilt series image at 0 degree (left) and a slice from a tomographic reconstruction (right). Scale bar indicates 200 nm. (B) Local resolution map of the 70S ribosome reconstructed by STA. The resolution based on the global FSC is 2.9 Å, and the local resolution was calculated using RELION. The left panel shows an external view of the structure. The middle panel presents a central cross-section, highlighting the resolution distribution within the interior regions. The right panel shows an external view displayed with a lowered contour level to visualize regions with relatively low resolutions (contour level adjusted from 0.0023 to 0.0006). (C) The FSC curve between the atomic model and the STA map. The FSC threshold was set to 0.5. The atomic model was generated using the *Salmonella* 70S ribosome structure (PDB ID: 5AFI) as an initial model, followed by real-space refinement using Phenix, resulting in the deposited model (PDB ID: 23WJ).



Supplementary Figure 4. Representative tilt series image and tomogram of a *Salmonella* cell on a lamella with local resolution of *in situ* ribosome reconstruction and evaluation of FSC between the fitted atomic model and map. (A) A representative tilt series image at 0 degree (left) and a slice from a tomographic reconstruction (right). Scale bar indicates 200 nm. (B) Local resolution map of the *in situ* ribosome STA reconstruction. The resolution based on the global FSC is 8.2 Å, and the local resolution was calculated using RELION. The left panel shows an external view, while the right panel presents a central cross-section, highlighting the resolution distribution within the interior regions. (C) The FSC curve between the atomic model and the *in situ* ribosome map. The FSC threshold was set to 0.5. Rigid-body fitting was applied for model fitting. The atomic model was derived from a *Salmonella* ribosome structure (PDB ID: 5IMQ).



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