

Supplementary Material

Determining The Structure of the Bacterial Voltage-gated Sodium Channel NaChBac Embedded in Liposomes by Cryo Electron Tomography and Subtomogram Averaging

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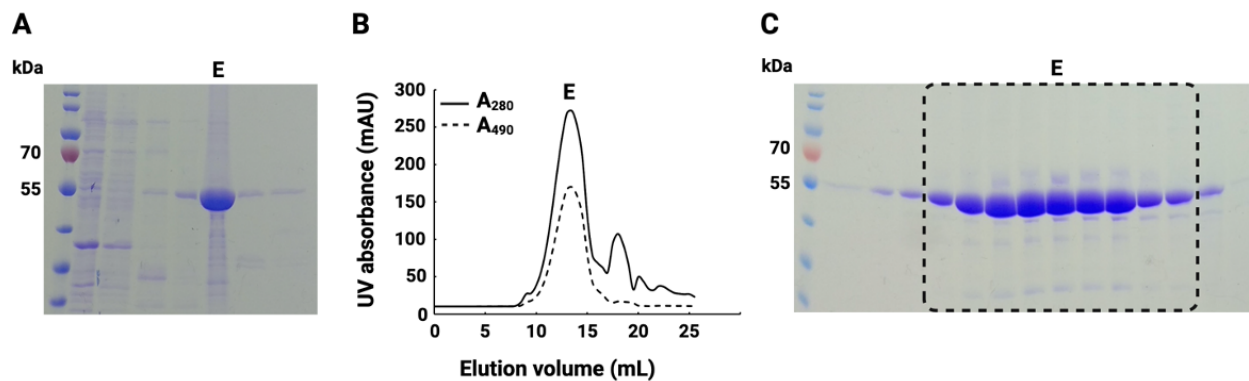


Figure S1 Purification of NaChBac. (A) SDS-PAGE of protein purification with Ni-NTA column. E, elution. (B and C) SEC purification of NaChBac in DDM detergent with a monodispersed peak. E, elution.

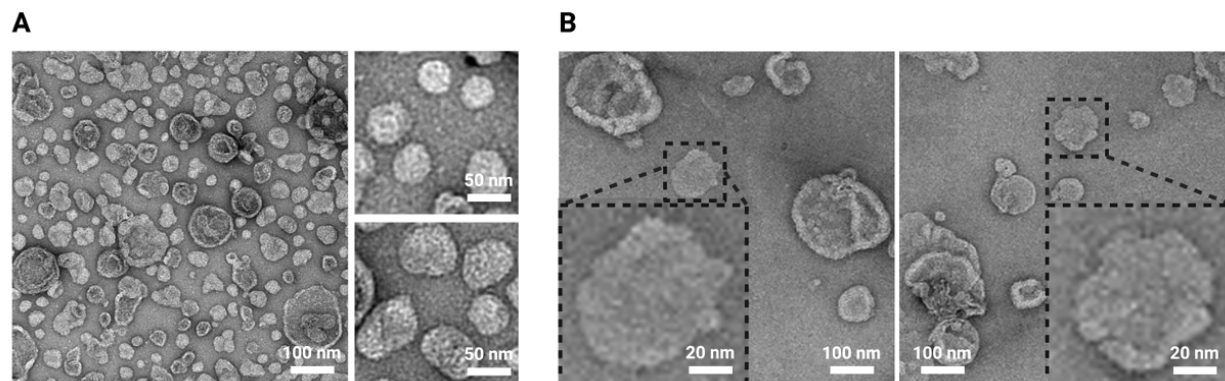


Figure S2 Negative-staining images of proteoliposomes. (A) A negative-staining image of proteoliposomes with detergent removal entirely. The insets showed proteoliposomes with “sharp edge” features. (B) Negative-staining images of proteoliposomes with residual detergent during dialysis. The insets showed proteoliposomes with “fluid edge” features.

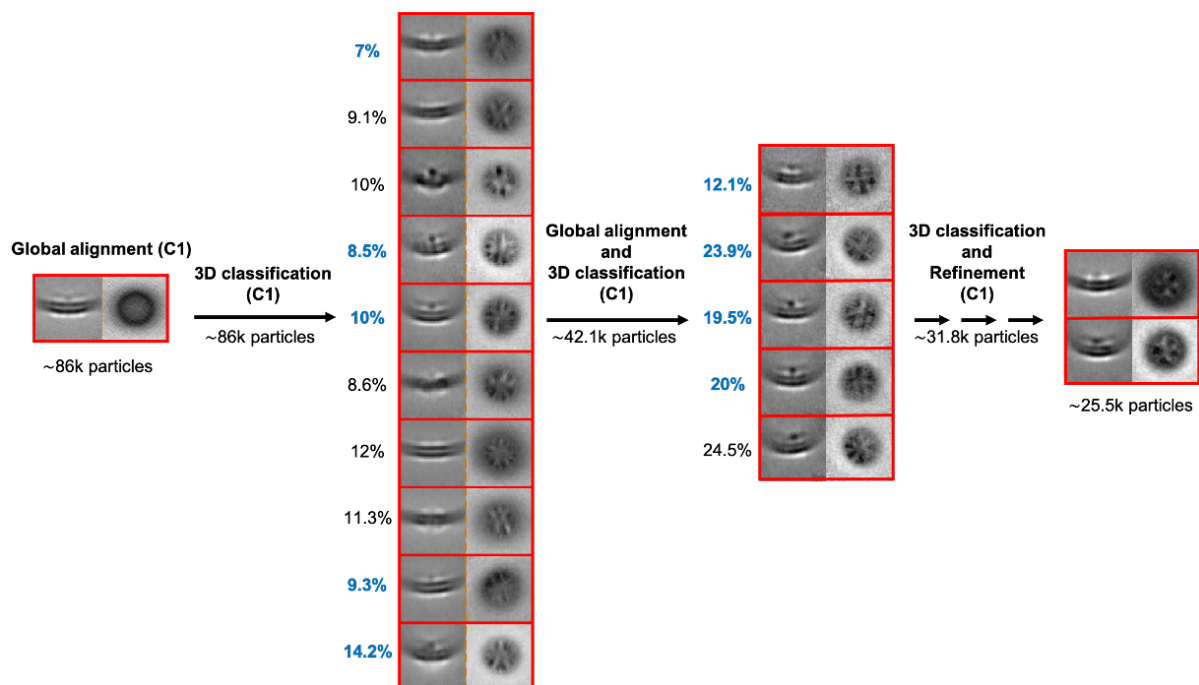


Figure S3 Strategy for the dataset cleaning by classification with Dynamo, without symmetry. The images show central slices through the averages in XZ and XY directions, produced by Dynamo (D. Castano-Diez et al, *J. Struct. Biol.*, 2012, 178:139-151). First, ~86,000 particles were aligned globally and were implemented to 3D classification for the particle cleaning. ~42,100 particles were selected to perform further classification. After collecting good particles (~31,800) from previous steps, multiple rounds of 3D classification and refinement were performed, and ~25,500 particles were then subjected to the RELION-4.0 for further 3D classification and refinement.

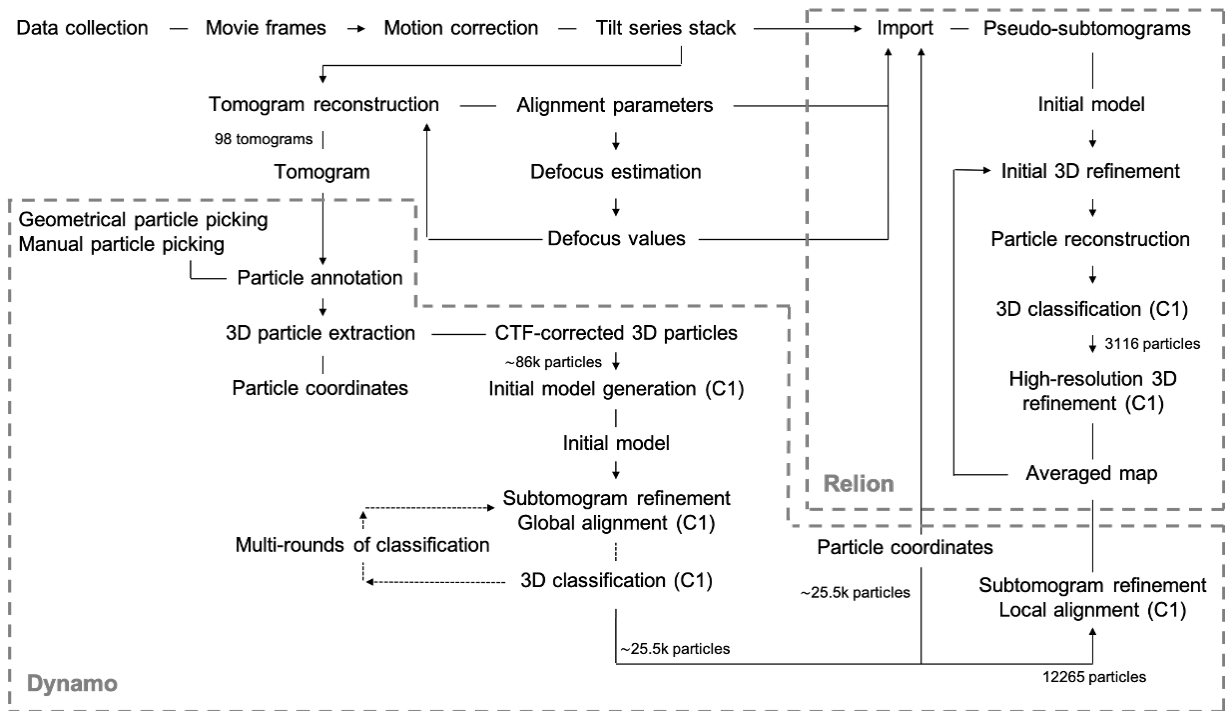


Figure S4 Workflow for tomogram reconstruction and subtomogram averaging. Tilt series stack alignment, tomographic reconstruction, particle annotation, 3D classification, and subtomogram averaging were shown in the workflow.

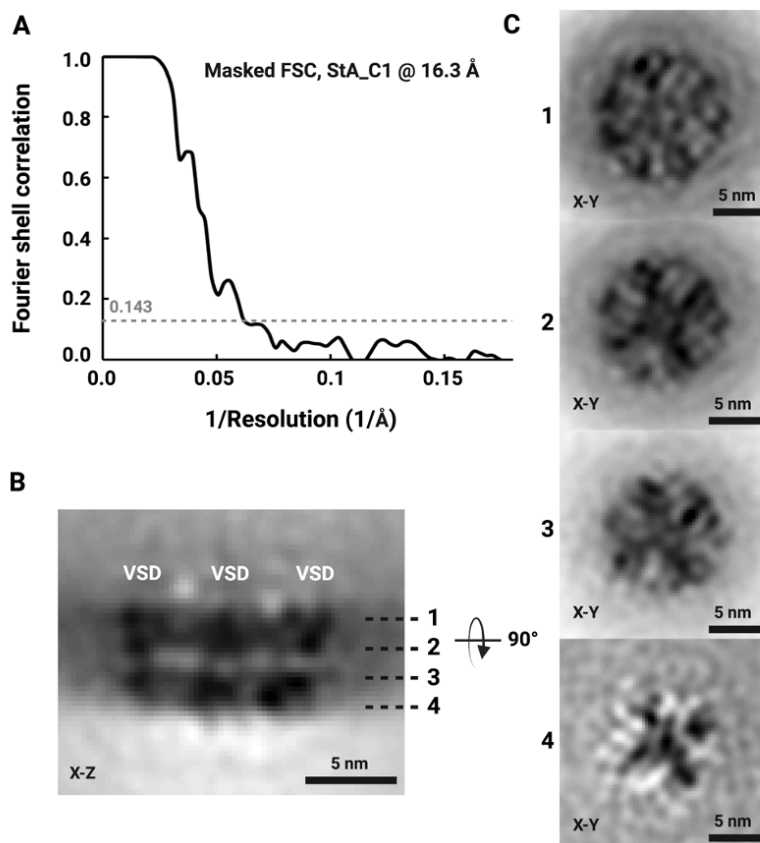


Figure S5 FSC curve of the masked map and slices through the structure of NaChBac map in liposomes along the z-axis. (A) Gold standard FSC (criteria 0.143) curve of the masked map with a reported resolution of 16.3 Å for the map with C1 symmetry. (B and C) The voltage sensing domain (VSD) and pore domain (PD) could be visualized as a tetrameric feature in the X-Y plane along the z-axis.

Table S1 Data Processing Statistics

| NaChBac in liposomes; EMD-17163 | |
|---|------------------------------------|
| Data collection and processing | |
| Microscope | Titan Krios G2 |
| Magnification | 81,000 x |
| Voltage (kV), Cs | 300 kV, 2.7 mm |
| Total electron dose (e ⁻ /Å ²) | ~130 |
| Defocus range (μm) | -2.5 to -3.5 |
| Camera | Gatan K3 + BioQuantum image filter |
| Pixel size (Å) | 1.393 |
| Number of tomograms | 98 |
| Symmetry imposed | C1 |
| Initial particles | ~86,000 |
| Final particles | 3,116 |
| Refinement method | Independent half-set |
| Map resolution (Å) | 16.3 |
| FSC threshold | 0.143 |
