

Supplementary Materials: Structural Analysis of Botulinum Neurotoxins Type B and E by cryo-EM

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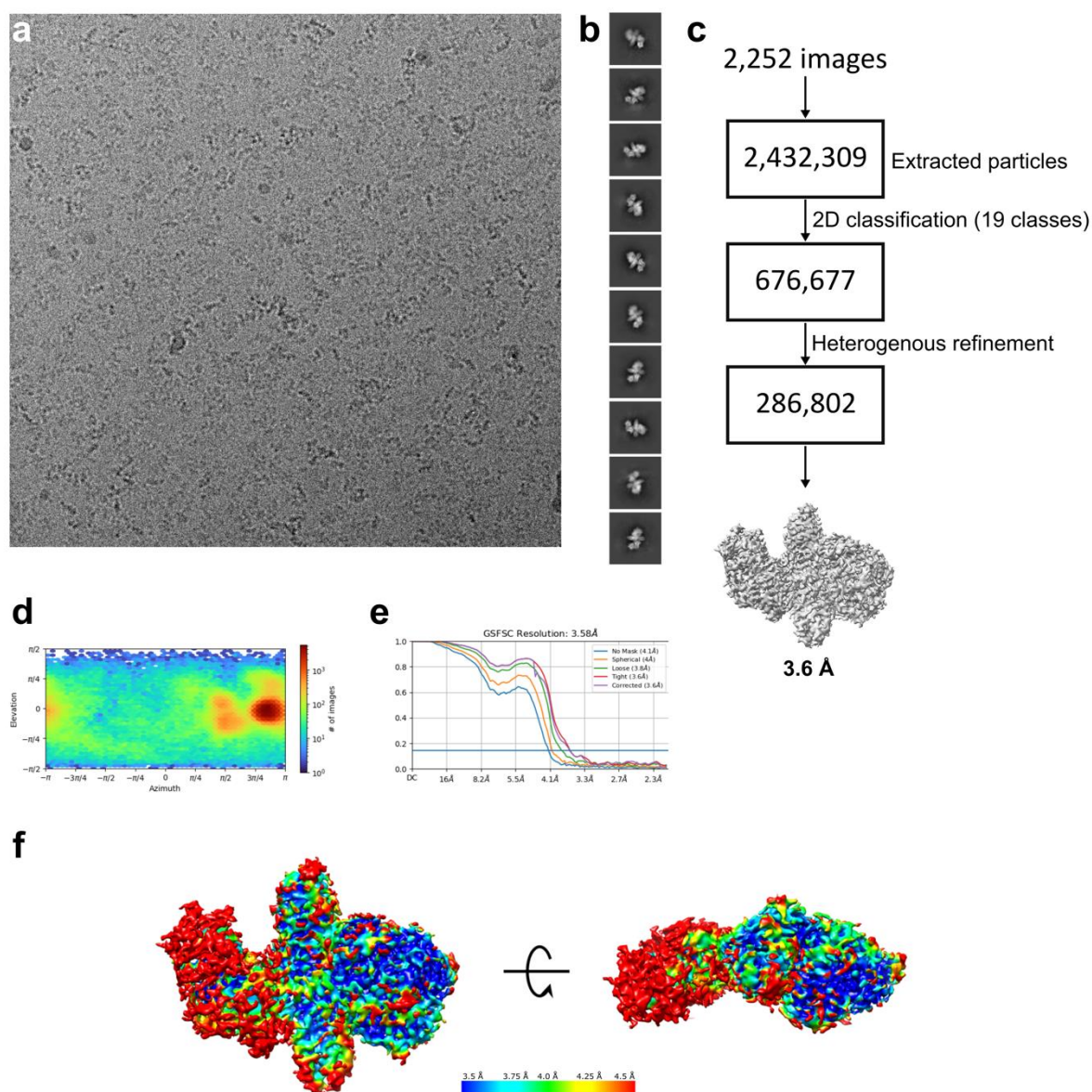


Figure S1. Image processing and evaluation of BoNT/B dataset. (a) Representative micrograph from a dataset of 2252 images. (b) Representative 2D class averages showing a range of angular orientations. (c) Image processing pipeline for BoNT/B, numbers indicate number of particles selected for each reconstruction step. (d) Reconstruction and corresponding angular distribution map, indicating BoNT/B has a strong preferred orientation. (e) Fourier shell correlation (FSC) curves. (f) EM density coloured by local resolution (colour bar indicates local resolution in Å).

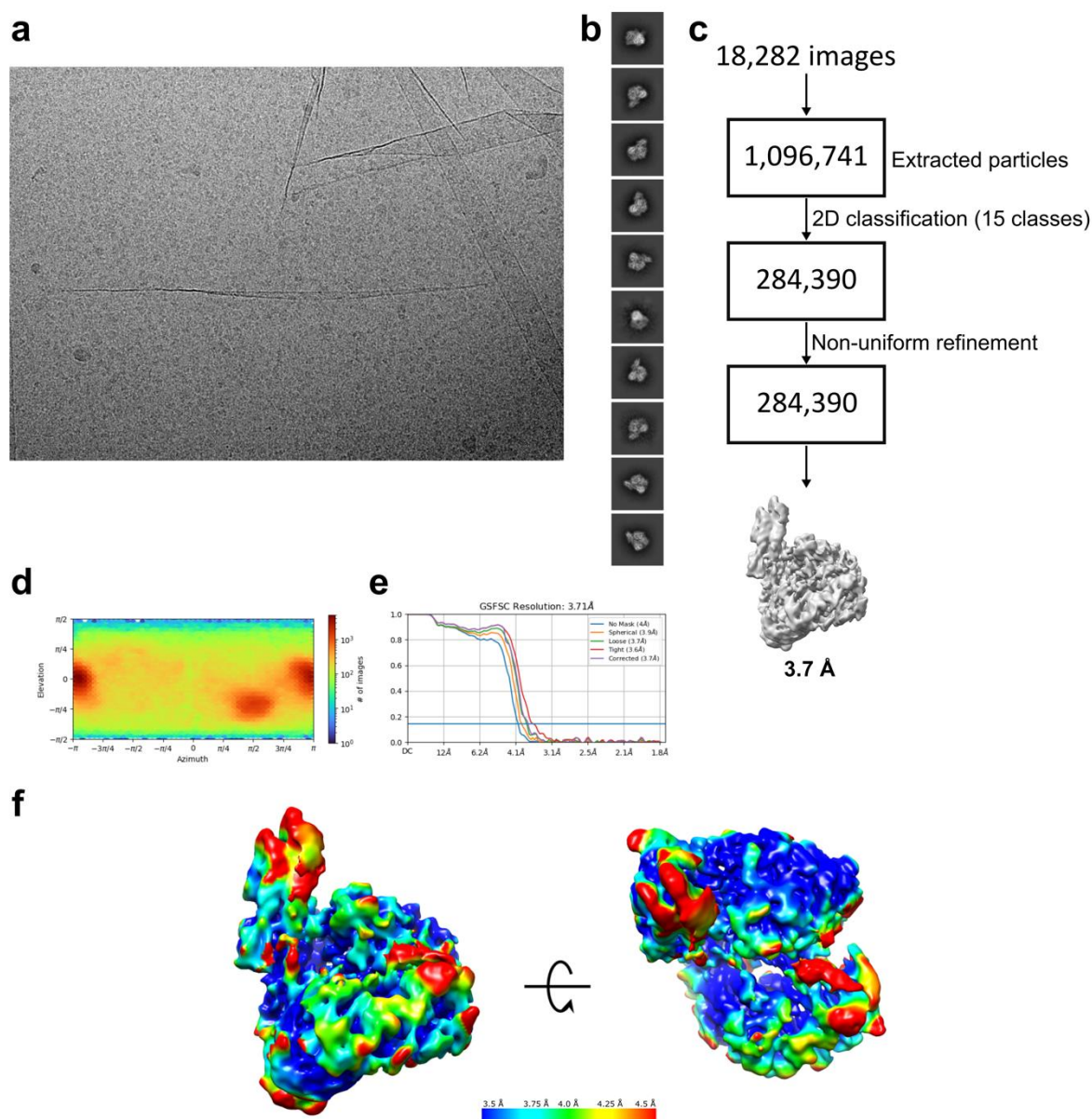


Figure S2. Image processing and evaluation of BoNT/E dataset. (a) Representative micrograph from a dataset of 18282 images. (b) Representative 2D class averages showing a range of angular orientations. (c) Image processing pipeline for BoNT/E, numbers indicate number of particles selected for each reconstruction step. (d) Reconstruction and corresponding angular distribution map, indicating BoNT/B has a strong preferred orientation. (e) Fourier shell correlation (FSC) curves. (f) EM density coloured by local resolution (colour bar indicates local resolution in Å).

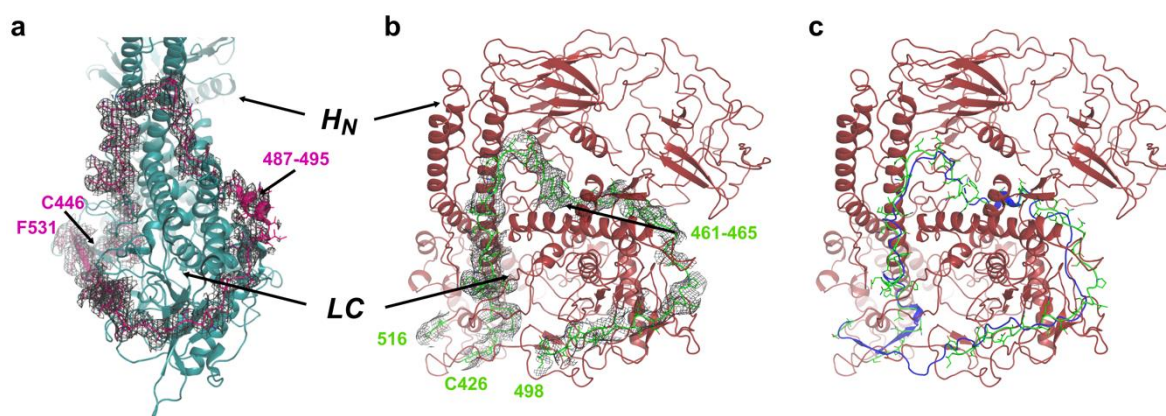


Figure S3. Belt region. (a) 3D reconstruction of BoNT/B (teal) and (b-c) BoNT/E (red), showing cryo-EM maps around the belt region (in pink and green for /B and /E respectively). Residues 499-515 of BoNT/E could not be modeled. In (c) the belt region of the crystal structure is shown in blue (PDB 3FFZ).

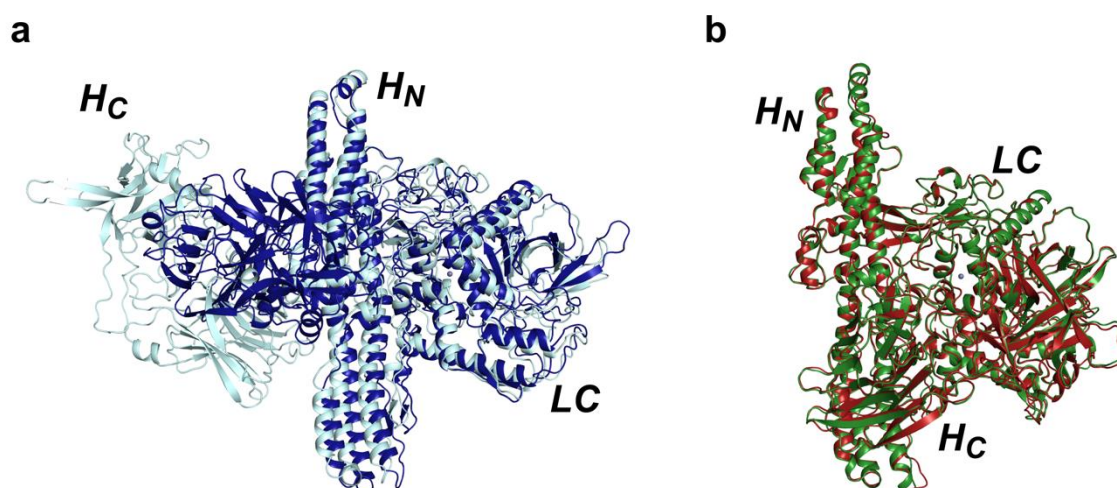


Figure S4. AlphaFold model predictions. (a) Superposition of the X-ray crystal structure (cyan) and the AlphaFold-predicted model (blue) of BoNT/B. (b) Superposition of the X-ray crystal structure (red) and the AlphaFold-predicted model (green) of BoNT/E. Prediction models were downloaded from the AlphaFold Protein Structure Database [<https://alphafold.ebi.ac.uk/entry/B1INP5>, and <https://alphafold.ebi.ac.uk/entry/Q00496>].