Supporting Information

Benefield et al. 10.1073/pnas.1222139110

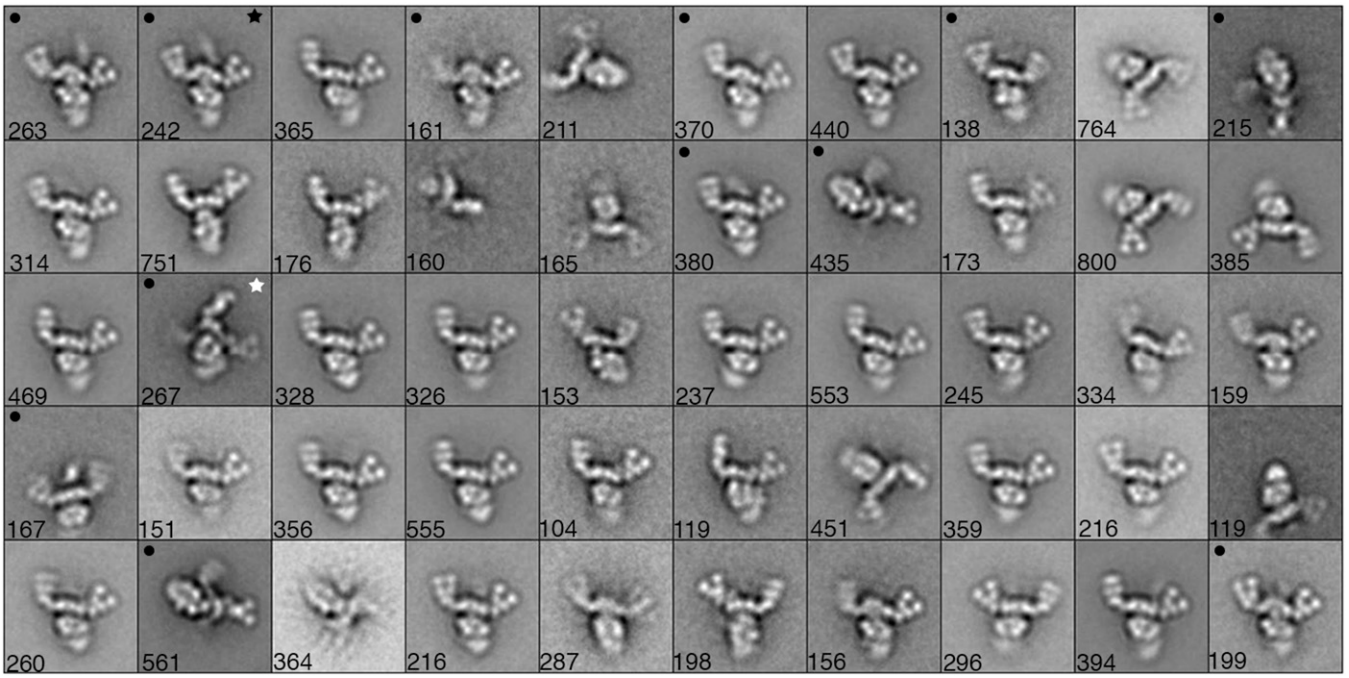


Fig. S1. Classes of botulinum neurotoxin (BoNT)/A1 progenitor complex (PC) three-armed species begin to emerge if the class size is expanded to 50. The two preferred orientations of the PC on the grid surface are indicated by either a black asterisk ("flat" view) or by a white asterisk ("prong" view). A "•" indicates class selected for the referenced-based alignment shown in Fig. 2*C*. Side length of panels, 420 Å.

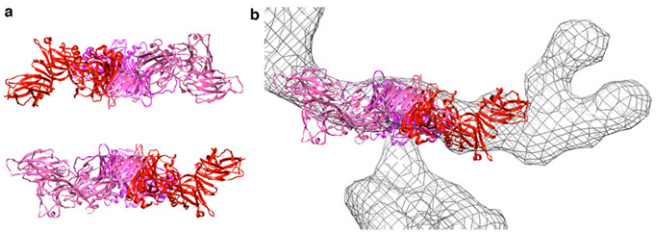


Fig. S2. Orienting the hemagglutinin 3 (HA3) trimer in the PC structure. (*A*) When viewed from the side, the HA3 trimer adopts either a "M" or "W" shape. (*B*) When the electron density is oriented with the pinchers at the top and the ovoid body at the bottom, the HA3 trimer can be confidently placed into the triangular plate in the W orientation.

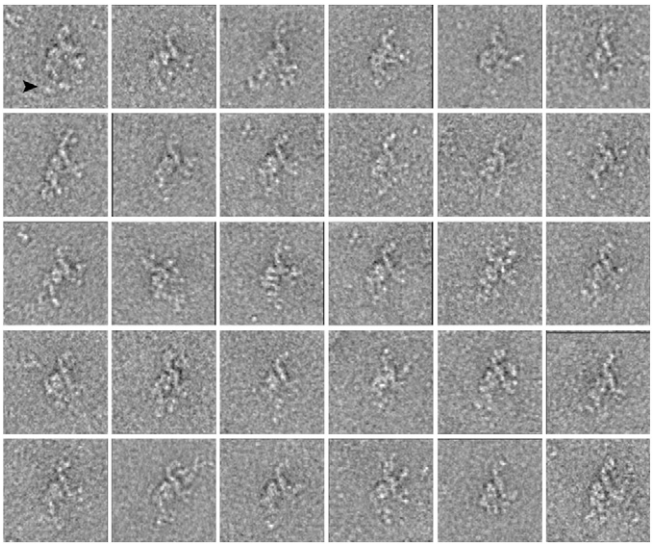


Fig. S3. Labeling the BoNT/A1 PC with an antibody against the neurotoxin's catalytic domain. The BoNT/A1 complex was incubated with a LC-specific monoclonal antibody (4LCA) and visualized by negative stain EM. A gallery of labeled single particle BoNT/A1 PCs is shown. Side length of panels, 525 Å.

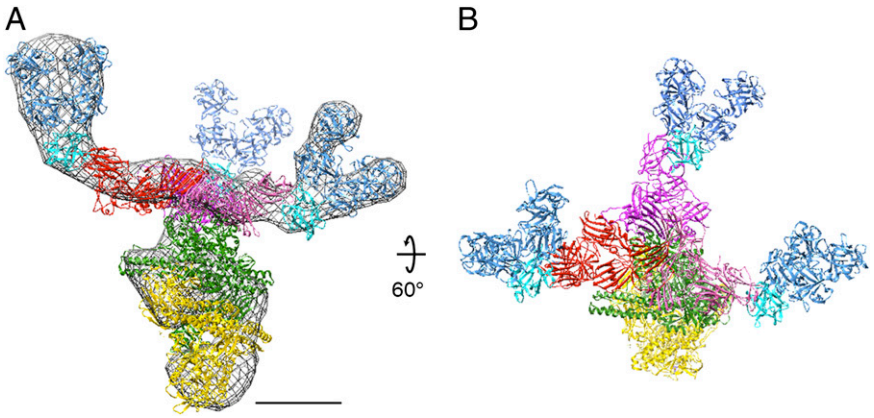


Fig. S4. Model of a three-armed BoNT/A1 PC. (*A*) Side view and (*B*) angled top view showing the HA3 trimer in between the BoNT/A1-nontoxic non-hemagglutinin (NTNH) main body and the HA1/HA2 pinchers. (Scale bar, 50 Å.)

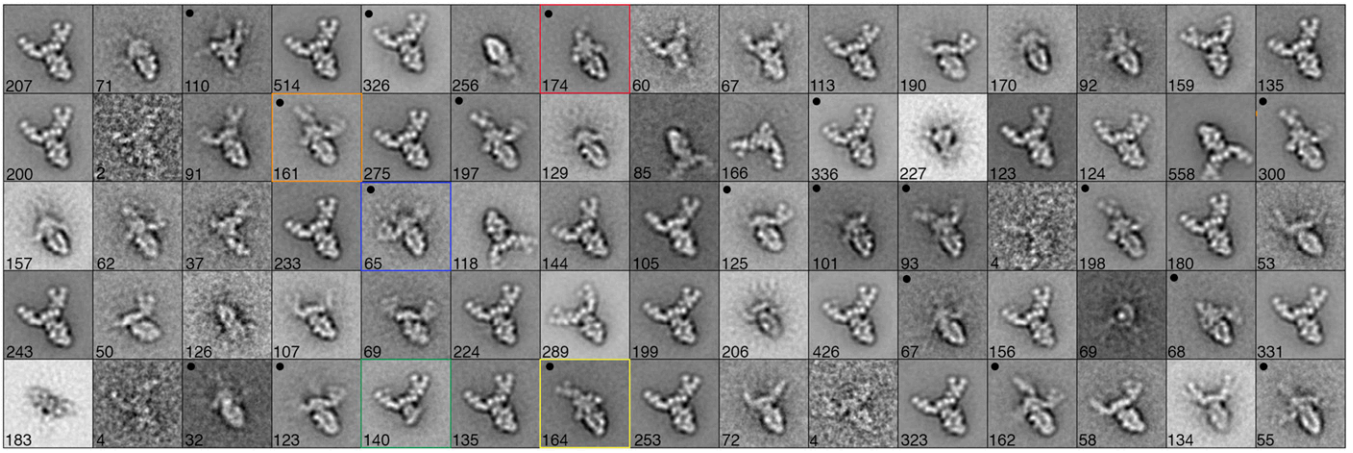


Fig. S5. The BoNT/B PC sample contains assembly/disassembly intermediates. • indicates class selected for the referenced-based alignment shown in Fig. 4*B*. The outlined boxes show different assembly/disassembly states in the sample: red, no HA1/HA2 pinchers; yellow, one pair of pinchers; orange, two pairs of pinchers; blue, three pairs of pinchers; and green, the NAP assembly without the neurotoxin. Many of the classes with two pairs of pinchers (including the one in orange) also include individual particles with three pinchers where the third pincher has not been resolved due to flexibility. Side length of panels, 420 Å.

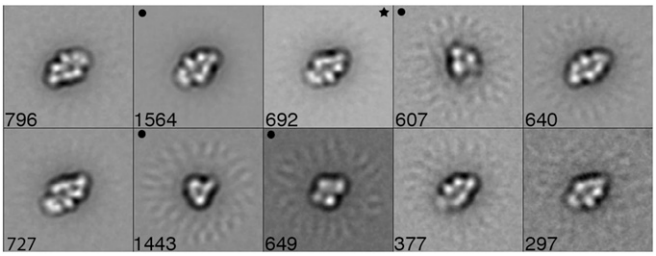


Fig. S6. A BoNT/E PC class average of 10 shows the two-protein complex and disassociated monomers. A "•" indicates class selected for the referenced-based alignment shown in Fig. 5*A*. Side length of panels, 420 Å.

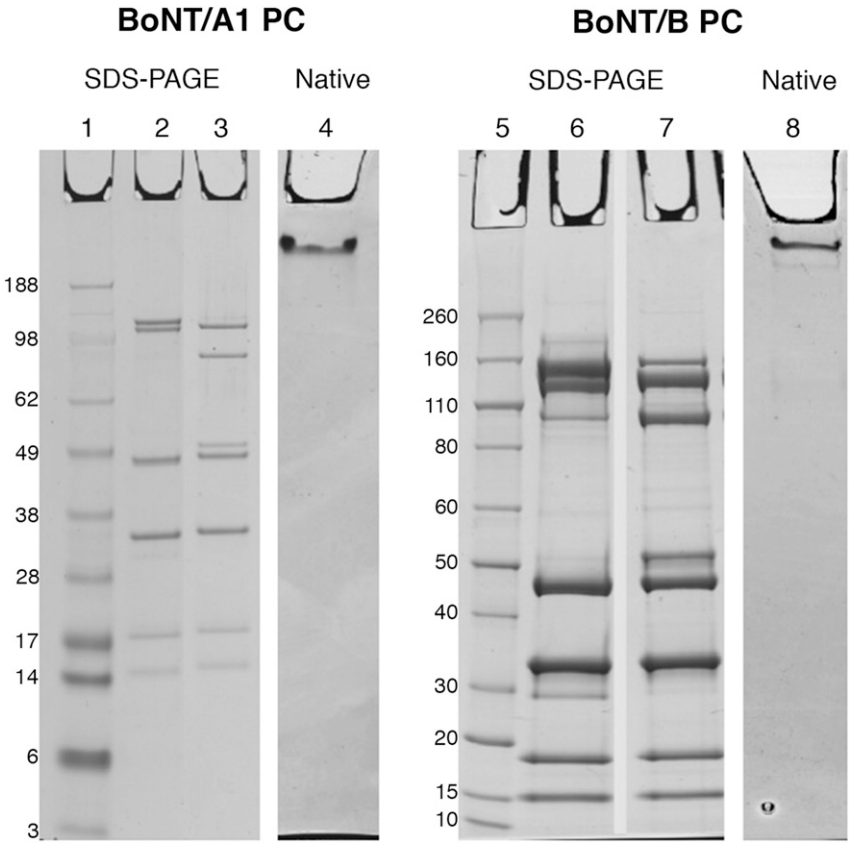


Fig. S7. Analysis of the BoNT/A1 and BoNT/B 16S PCs by SDS/ and native/PAGE. Lanes 2 and 6 correspond to nonreducing conditions. Lanes 3 and 7 correspond to reducing conditions. Lanes 4 and 8 were run in the absence of SDS and reductant.

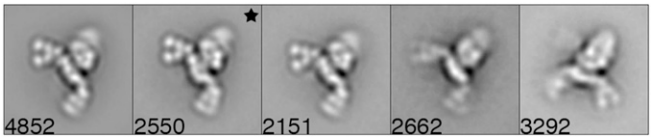


Fig. S8. The BoNT/A1 PC sorted into five classes. The tilted particles associated with the average marked with an asterisk were used for an initial 3D reconstruction by back projection of the two-armed complex shown in Fig. 3*C*. Side length of panels, 420 Å.

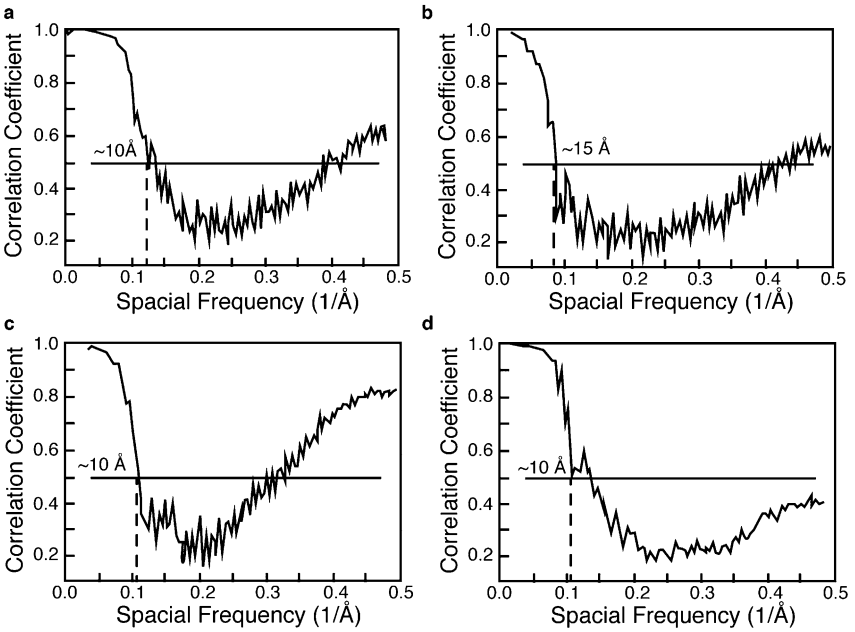
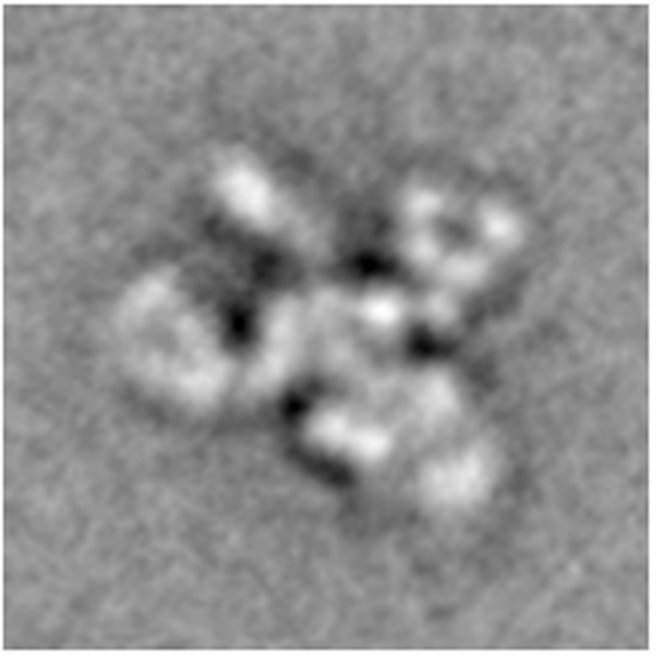
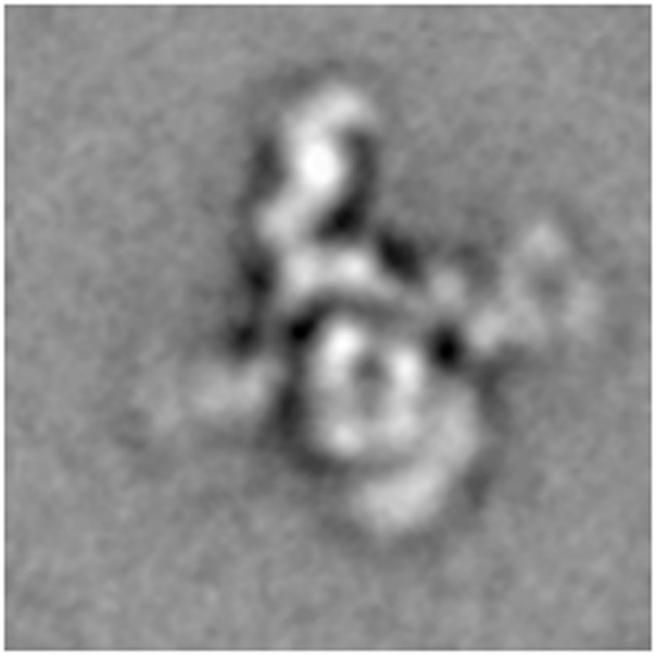


Fig. S9. The Fourier shell correlation (FSC) curve for (*A*) the BoNT/A1 PC "flat" view reconstruction from Fig. 3*A*, (*B*) the BoNT/A1 PC "prong" view reconstruction from Fig. 3*B*, (*C*) the BoNT/B reconstruction from Fig. 4*C*, and (*D*) the BoNT/E reconstruction from Fig. 5*B*.



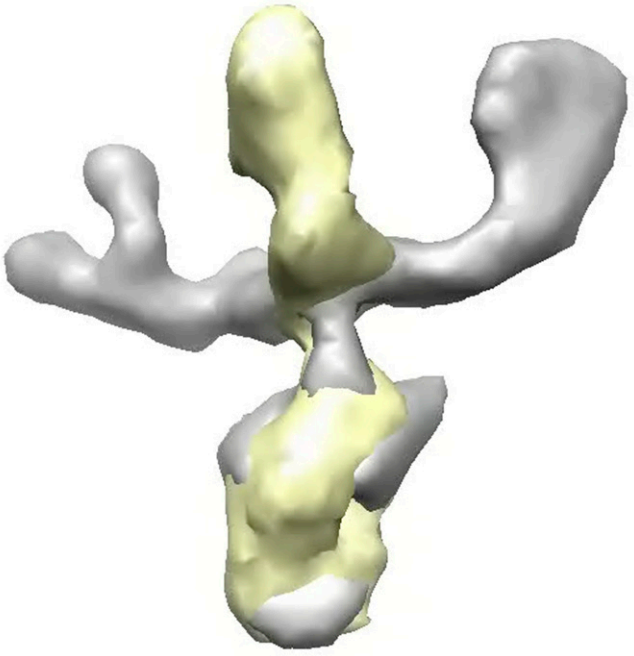
Movie S1. The "flat" view of the BoNT/A1 PC shows flexibility in the complex. Approximately 80 classes showing three-armed complexes in the flat orientation were selected from a total of 300. These classes were translationally aligned before putting them in a Quicktime movie format. Side length of panels, 420 Å.

Movie S1



Movie S2. The "prong" view of the BoNT/A1 PC shows flexibility in the complex. Approximately 30 classes showing three-armed complexes in the prong orientation were selected from a total of 300. These classes were translationally aligned before putting them in a Quicktime movie format. Side length of panels, 420 Å.

Movie S2



Movie S3. Alignment of the BoNT/A1 "flat" and "prong" 3D reconstructions forms a three-armed complex. The two models were aligned using Chimera and result in a complex representative of the single particles observed on the EM grid surface (Fig. 2*A*). A triangular assembly with V-shaped pinchers is associated with an ovoid body.

Movie S3