Supplementary Information (for proposal submission):

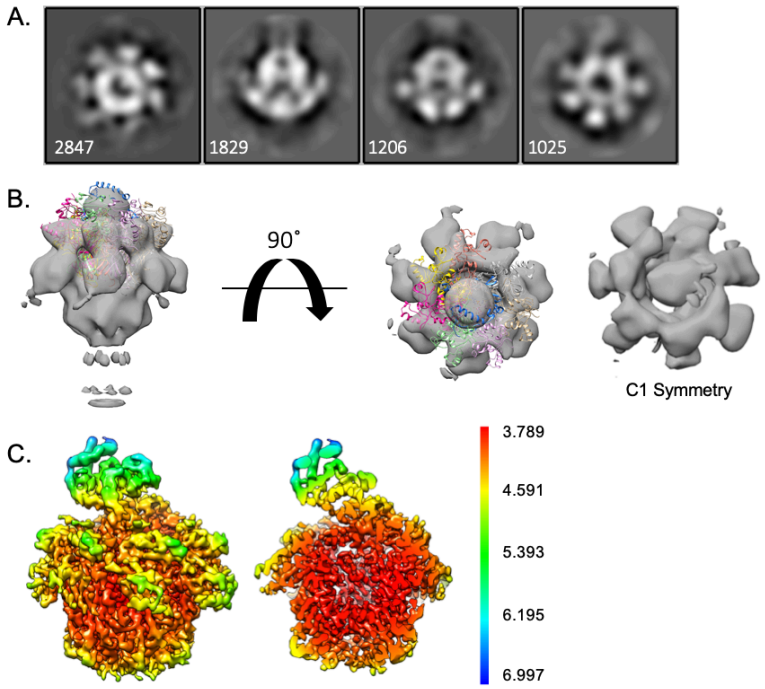

Figure 1. Preliminary structural characterization on *C. botulinum* C2 toxin. Both C2I and C2II were prepared in 0.01% TWEEN20 and combined at 1:1.2 ratio at room temperature for 15min to form the complex. *A*, representative 2D class averages. C2 toxin was applied 4µL at 0.5µM to glow discharged grids and absorbed for 45sec. The sample was stained using 0.75% (w/v) uranyl formate. Particles from 78 micrographs (10,457 particles) collected from FEI Tecnai F20 at x62000 magnification and -1.6µm defocus were extracted in 140x140 pixel boxes and masked at 240Å. *B*, C2 initial 3D model with C7 symmetry. 7 copies of the C2II partial crystal structure (PDB: 2J42) and a C2I (PDB: 2J3Z) were manually docked in to the negative stain density volume. C2 toxin was also reconstructed with C1 symmetry to confirm that C2IIa forms heptamers and C2I forms close contact to one protomer. The extra density on the side indicates the density from the D3' domain which is absent from the crystal structure. *C*, cryo-EM density map of *C. difficile* binary toxin (a similar toxin complex defined in our laboratory). The color scheme shows the local resolution.

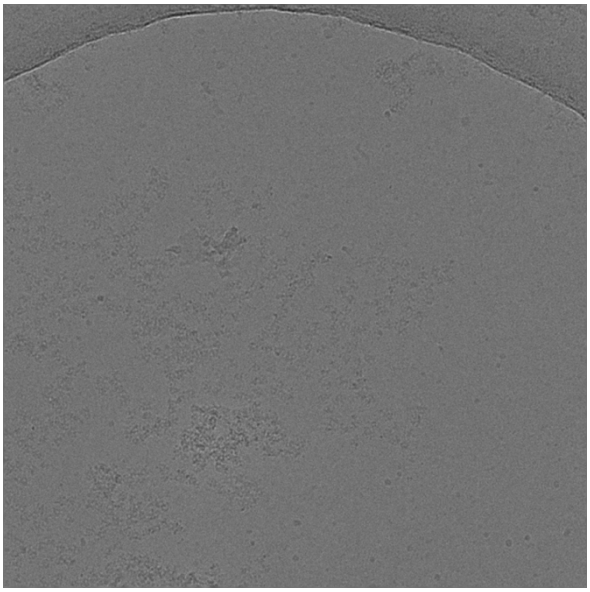

Figure 2. Representative micrograph of *C. botulinum* C2 toxin. C2 toxin was prepared in 0.01% TWEEN20 and applied 2µL at 0.65µM to the glow discharged 400-mesh Quantifoil r1.2/1.3 grids with 10sec wait time before plunging into liquid ethane. Initial screening was done on FEI Tecnai F20 equipped with a 4kx4k Gatan Ultrascan CCD camera. Images were collected at x62000 magnification and -3.5µm defocus.