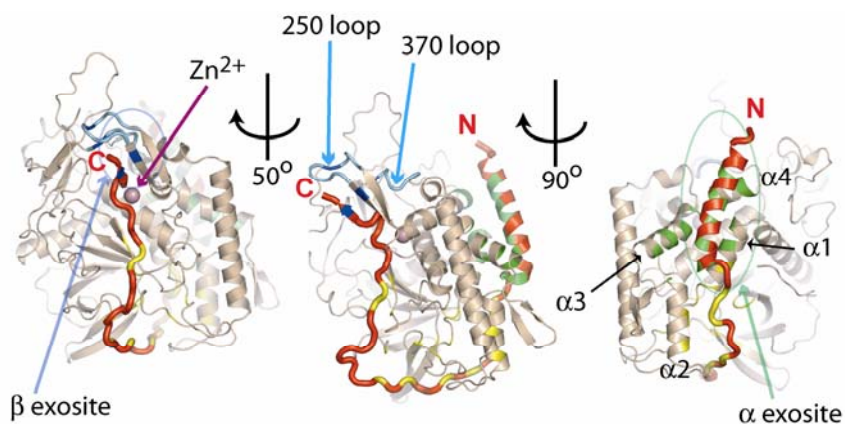


## Substrate Recognition Strategy for Botulinum Neurotoxin Serotype A

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Clostridal neurotoxins (CNTs) are the causative agents of the neuroparalytic diseases botulism and tetanus<sup>1,2</sup>. CNTs impair neuronal exocytosis (a process by which neurotransmitter is released into a synapse) through specific proteolysis of essential proteins called SNAREs (soluble N-ethylmaleimide-sensitive factor attachment protein receptors)<sup>3</sup>. SNARE assembly into a low-energy ternary complex is believed to catalyse membrane fusion, precipitating neurotransmitter release; this process is attenuated in response to SNARE proteolysis<sup>4-7</sup>. Site-specific SNARE hydrolysis is catalysed by the CNT light chains, a unique group of zinc-dependent endopeptidases (enzymes that catalyse hydrolysis of peptide bonds within other proteins)<sup>3</sup>. The means by which a CNT properly identifies and cleaves its target SNARE has been a subject of much speculation; it is thought to use one or more regions of enzyme-substrate interaction remote from the active site (exosites)<sup>8-10</sup>. Using x-ray diffraction data collected in part at SSRL beamline 9-2, we have determined the first structure of a CNT endopeptidase in complex with its target SNARE at a resolution of 2.1 Å: botulinum neurotoxin serotype A (BoNT/A) protease bound to human SNAP-25. The structure, together with enzyme kinetic data, reveals an array of exosites that determine substrate specificity (figure 1).

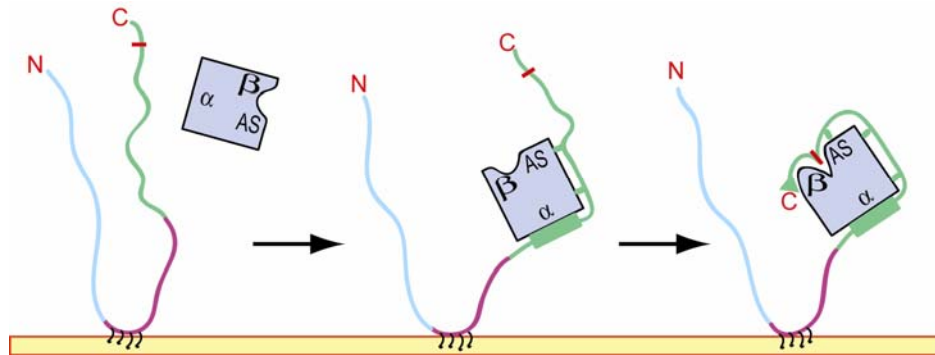


**Figure 1.** Three different views of the SNAP-25-BoNT/A complex are shown; these are related by the specified rotations around a vertical axis in the plane of the figure that goes through the centre of the complex.

The  $\alpha$ -exosite (indicated by a green arrow in Fig. 1) is formed by BoNT/A light-chain helices (tan)  $\alpha$ 1- $\alpha$ 4 that bind to the helical N-terminus of the substrate (red). Green areas indicate the approximate locations of contacting side chains involved in the  $\alpha$ -exosite. On the opposite face of BoNT/A, the  $\beta$ -exosite is indicated by a blue arrow (Fig. 1). The C-terminus

of SNAP-25 forms an anti-parallel  $\beta$  sheet along with a portion of the '250 loop' (light-blue), which is separated from the active site (indicated by  $Zn^{2+}$ , purple sphere) by the '370 loop' (light-blue). Dark-blue areas indicate the approximate locations of contacting side chains involved in the  $\beta$ -exosite. Yellow areas indicate the approximate locations of other exosites (anchor points) involved in side-chain contacts between the SNAP-25 substrate and the BoNT/A light chain.

Based on our structure and available kinetic data for several mutant SNAP-25 substrates, we conclude that most of this unusually large enzyme-substrate interface serves to provide a substrate-specific boost to catalytic efficiency by reducing  $K_m$  (the Michaelis constant). We also observe significant structural changes near the toxin's catalytic pocket upon substrate binding, probably serving to render the protease competent for catalysis. A general model of the strategy used by BoNT/A to recognize and cleave SNAP-25 is presented in figure 2. SNAP-25 is shown attached to a presynaptic membrane via palmitoylation sites (shown in black) on its linker domain (purple). The N-terminal (sn1, cyan) and C-terminal (sn2, green) domains are unstructured or flexible in uncomplexed SNAP-25 (ref. 11). Binding of BoNT/A (blue) is probably initiated by helix formation at the  $\alpha$ -exosite, and anchor points along the extended portion of SNAP-25 (green notches) are additional determinants of substrate specificity. These sites reduce  $K_m$  and enhance binding at the  $\beta$ -exosite, inducing conformational changes at the active site, which render the endopeptidase competent to cleave its substrate.



**Figure 2.** Exosite-based model of BoNT/A substrate recognition.

Ultimately, the novel structures of the substrate-recognition exosites could be used for designing inhibitors specific to BoNT/A.

#### Primary Citation:

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