Botulinum Neurotoxin: Unique Folding of Enzyme Domain of the Most Poisonous Poison

Supporting Material

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Figure S1. Secondary structure dynamics of the BoNT/A in urea solution from MD simulations at 25 °C. Shown are the secondary structure propensities of various residues as a function of time. Color denotation for α -helices, β -sheets, and random coil is presented in the graph (turns, 3_{10} -helices, and π -helices are shown in blue, red and gray color, respectively).

Figure S2. Tertiary structure dynamics of the BoNT/A in urea solution from MD simulations at 25 °C. Shown are the profiles of the structure overlap ξ as a function of time (see Experimental Methods in main text).

Figure S3. ANS (8-anilino-1-naphthalene sulphonic acid) binding to urea denatured BoNT/A LC at room temperature (25 °C). Here, I_{488} is the measured fluorescence emission intensity of ANS at $\lambda = 488$ nm after exciting the solution at $\lambda = 388$ nm. ANS is a dye which binds to hydrophobic residues of a protein molecule. In this experiment, 70 μ M of ANS was titrated into 1 ml of 1 μ M solution of BoNT/A LC dissolved in 10 mM sodium phosphate buffer (pH = 7.4), containing 50 mM NaCl, 1 mM DTT, and urea at the required concentration in a 1 cm path-length cuvette.

Figure S4. Equilibrium fluctuations of amino acid residues forming BoNT/A in 0 M, 3.75 M, 5 M, and 7 M urea solution at room temperature ($T=25^{\circ}\text{C}$). Shown are the profiles of the root-mean-square-deviations (RMSD values) for residues 10 – 400 of BoNT/A LC. Colored strips represent amino acids in the α -exosite (shown in yellow), β -exosite (shown in green), and in the active site (shown in brown). We used same color denotation as in Figure 1 in the main text.

Table S1. Model systems for the BoNT/A light chain in urea solution in terms of ambient conditions (concentration of urea, pH and temperature), system size (number of amino acid residues in proteins, number of counterions, number of urea molecules, and number of water molecules), dimensions of the solvation box, the total length of a simulation run, and the CPU time expended.

[Urea], M	pH; $T,^{\circ}\text{C}$	No.of counter- ions Na^+/Cl^-	No.of urea molecules	No.of water molecules	Solvation box (x, y, z)	Length of trajectory, ns	CPU time, hours
0	7; 25	10/9	0	20,407	90 Å, 90 Å, 90 Å	100	~2,640
3.8	7; 25	8/7	1,707	13,958	90 Å, 90 Å, 90 Å	100	~2,640
5	7; 25	7/6	2,864	15,235	90 Å, 90 Å, 90 Å	100	~2,640
7	7; 25	6/5	2,764	10,841	90 Å, 90 Å, 90 Å	100	~2,640