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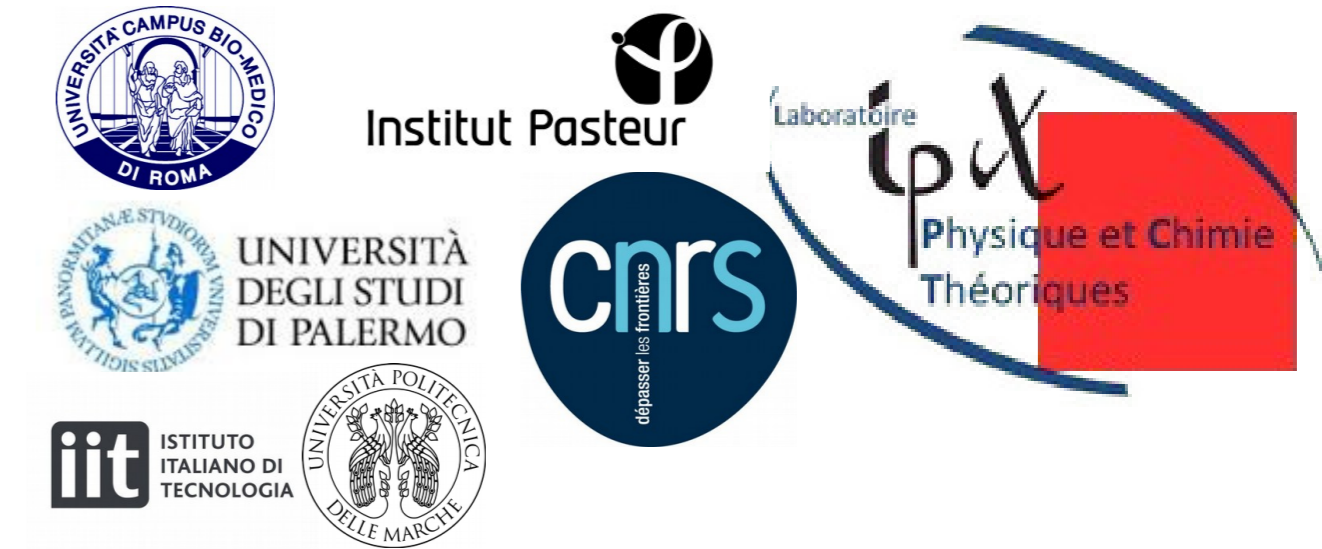
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# In silico analysis of the conformational features of botulinic toxins A1 and E1

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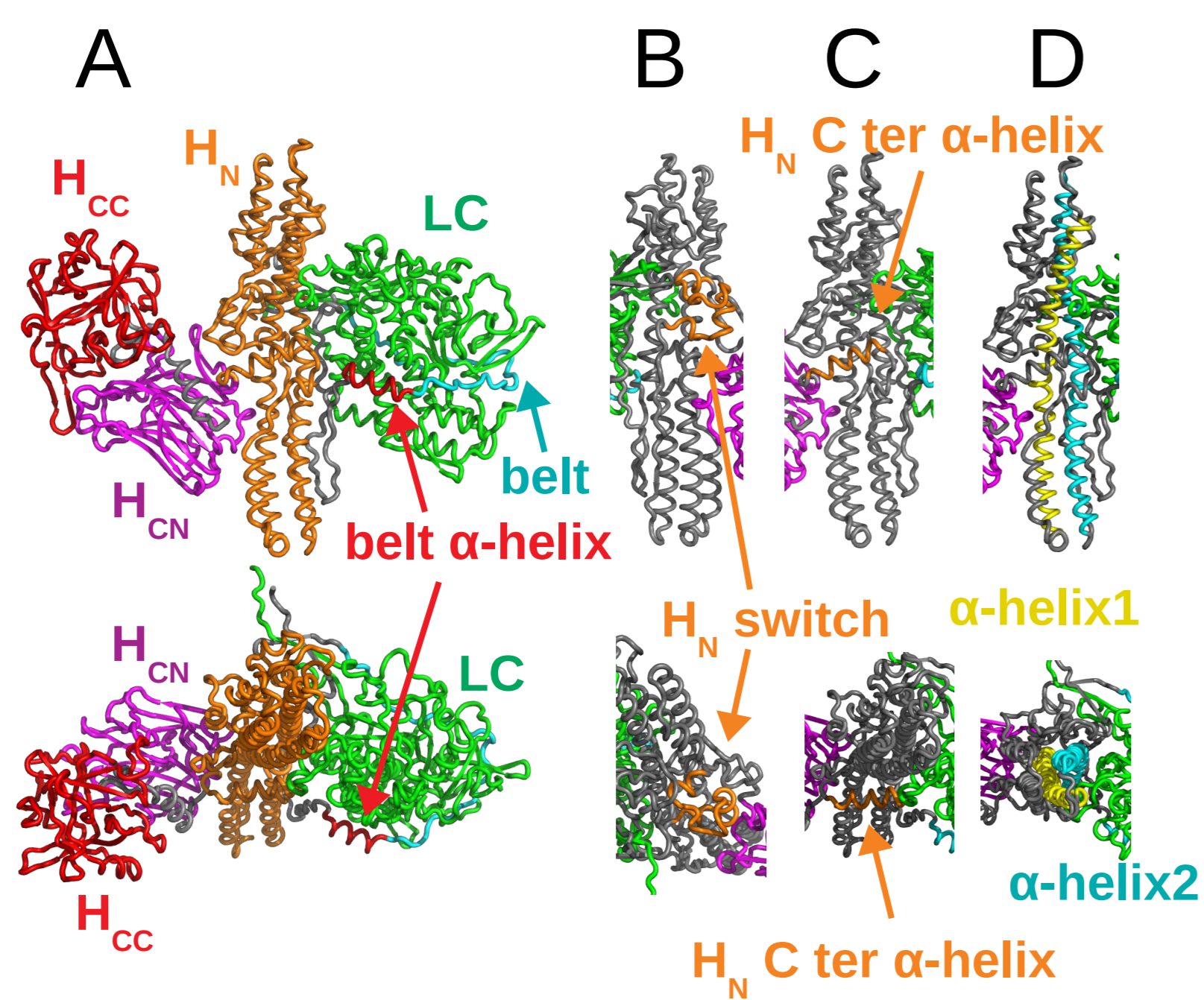
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## Abstract

Botulinum neurotoxins (BoNTs) are among the most powerful toxic compounds found in nature. They bind to pre-synaptic gangliosides and protein receptor complexes, which get internalized into recycling vesicles. Then, the inner acidification of vesicles induces a translocation of the catalytic domain into the cytosol, which next cleaves SNARE proteins to impair the action potential-mediated release of neurotransmitter into the neuromuscular cleft. The succession of these events is far from being elucidated at the atomic level, although numerous structures of the interaction partners have been determined. This is the reason for which we undertook a study of BoNTs by molecular dynamics simulations. Systems were prepared for the subtypes BoNT/A1 and BoNT/E1, with protonation levels defined by neutral and acidic pH values. The toxins flexibility is mainly described by the relative displacements of domains, variations of flexibility being observed in the domain H<sub>CC</sub>, the H<sub>N</sub> switch and the belt  $\alpha$  helix. The belt  $\alpha$  helix fluctuations are connected with the larger accessibility of residues in HN, allowing to propose a model for the translocation, in which the mobility of belt is transmitted to H<sub>N</sub>, inducing an easier interaction of H<sub>N</sub> residues with the non-polar membrane environment.

## Variations of overall organization of BoNTs

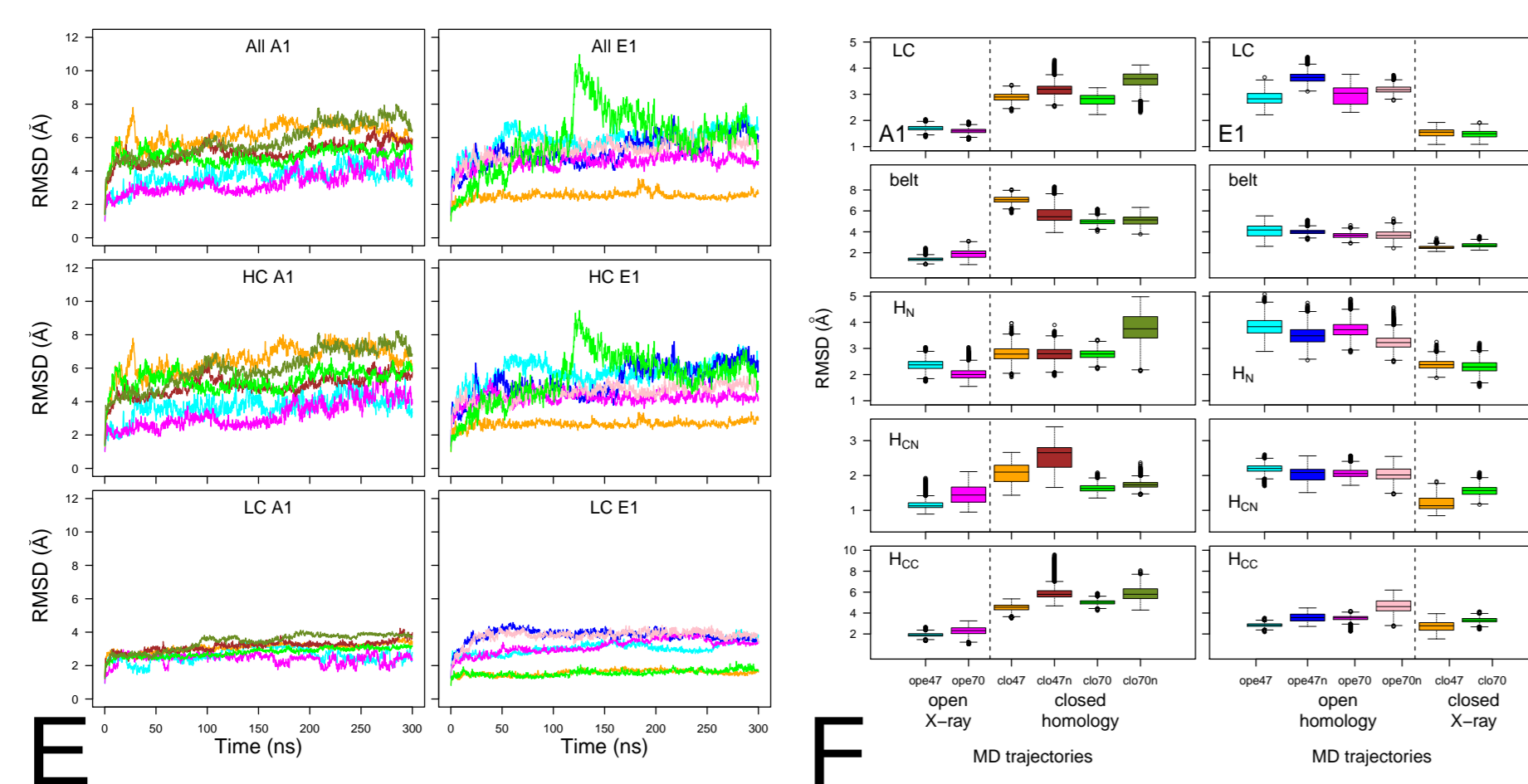


BoNTs are formed by two protein chains connected by one disulfide bridge: the light chain (LC, green) and the heavy chain (HC). The disulfide bridge is cleaved to convert the protoxin into its active form. The heavy chain is the delivery module comprised of an N-terminal domain (H<sub>N</sub>, orange) responsible for delivery of LC into the cytosol and a C-terminal domain (H<sub>C</sub>) responsible for recognizing specific cell-surface receptors. The X-ray crystallographic structures of BoNTs display the following organization (panel A): LC is essentially formed by the catalytic domain, whereas HC spans the belt (cyan) and the translocation domains (H<sub>N</sub>, orange) as well as the N terminal and C terminal receptor binding domains (H<sub>NC</sub>, magenta) and (H<sub>CC</sub>, red). The catalytic domain displays an  $\alpha$ - $\beta$  fold and the

translocation domain H<sub>N</sub> is formed of a bundle of  $\alpha$  helices. The N-terminal receptor-binding domain (H<sub>NC</sub>) contains predominantly  $\beta$ -sheets arranged into a jelly roll motif and the C-terminal receptor-binding domain (H<sub>CC</sub>) folds into a  $\beta$ -trefoil. The two long  $\alpha$  helices of H<sub>N</sub> will be denoted helix 1 (panel D, cyan) and helix 2 (panel D, yellow). Two other sub-domains of H<sub>N</sub> are the H<sub>N</sub> switch, formed by three  $\alpha$  helices, and located in the middle of H<sub>N</sub> (panel B) and on the other side of H<sub>N</sub>, the H<sub>N</sub> C terminal  $\alpha$  helix (panel C).

The BoNT structures display two distinct conformations, termed **open** and **closed**, characterized by different arrangement of the LC and receptor binding domain with respect to the H<sub>N</sub> domain. In the open state, the LC and the receptor binding regions are far apart, as wings open with respect to H<sub>N</sub> (see panel A). In the closed conformation, they come in close contact, as closing wings [14]. The transition between open and closed conformations is thought to facilitate membrane translocation [8].

In order to handle a BoNT defined by two chains connected by a disulfide bridge, the starting points for open and closed states have been prepared by cleaving the PDB entries 3BTA (A1) [7] and 3FFZ (E1) [6]. For BoNT/A1, the conformations of the open state were prepared from the cleaved X-ray crystallographic structure 3BTA and the conformations of the closed state using the closed structure 3FFZ of BoNT/E1. On the contrary, for BoNT/E1, the conformations of the closed state were prepared from the cleaved X-ray crystallographic structure 3FFZ and the conformations of the open state using open structure 3BTA of BoNT/A1.

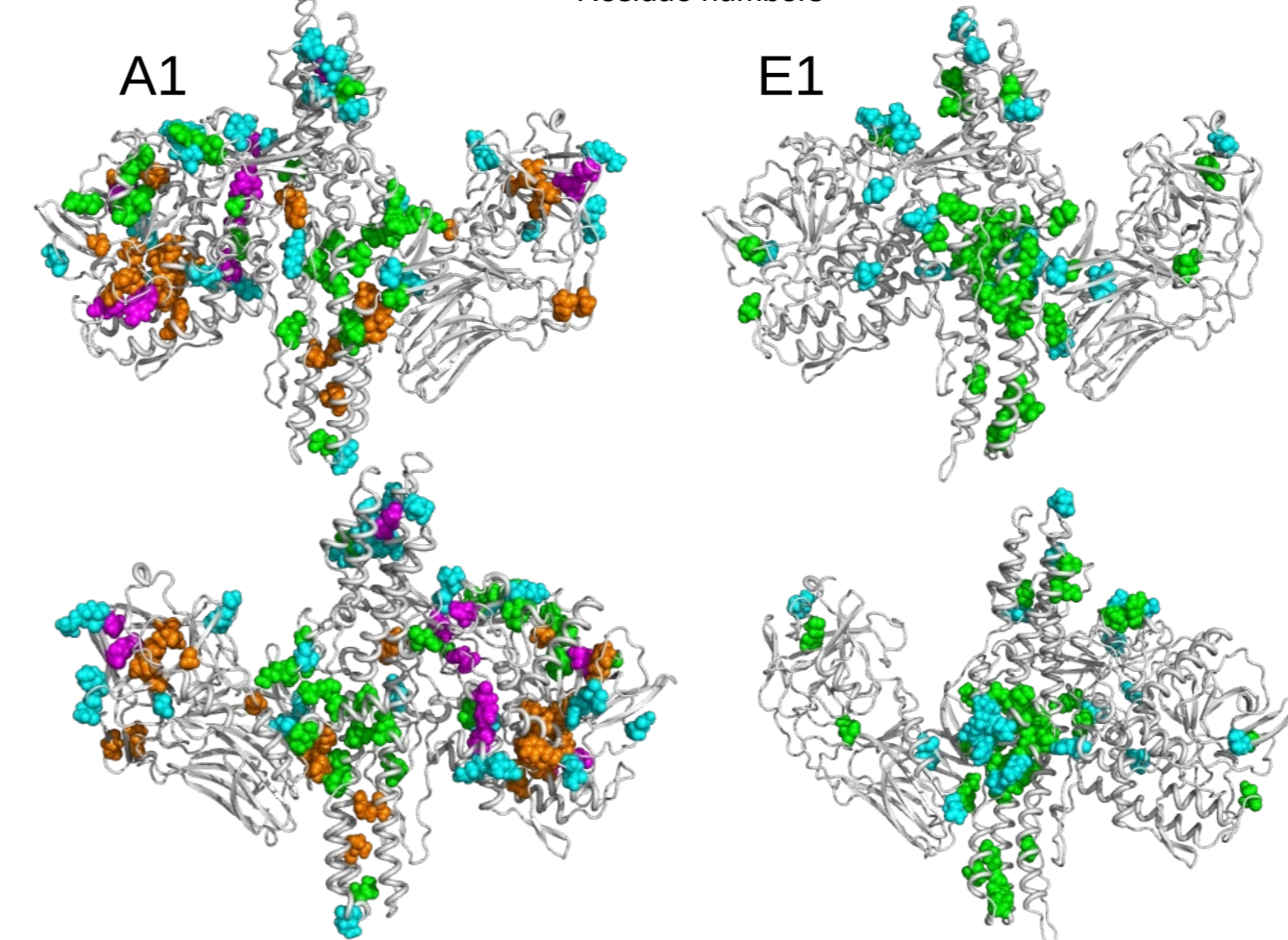
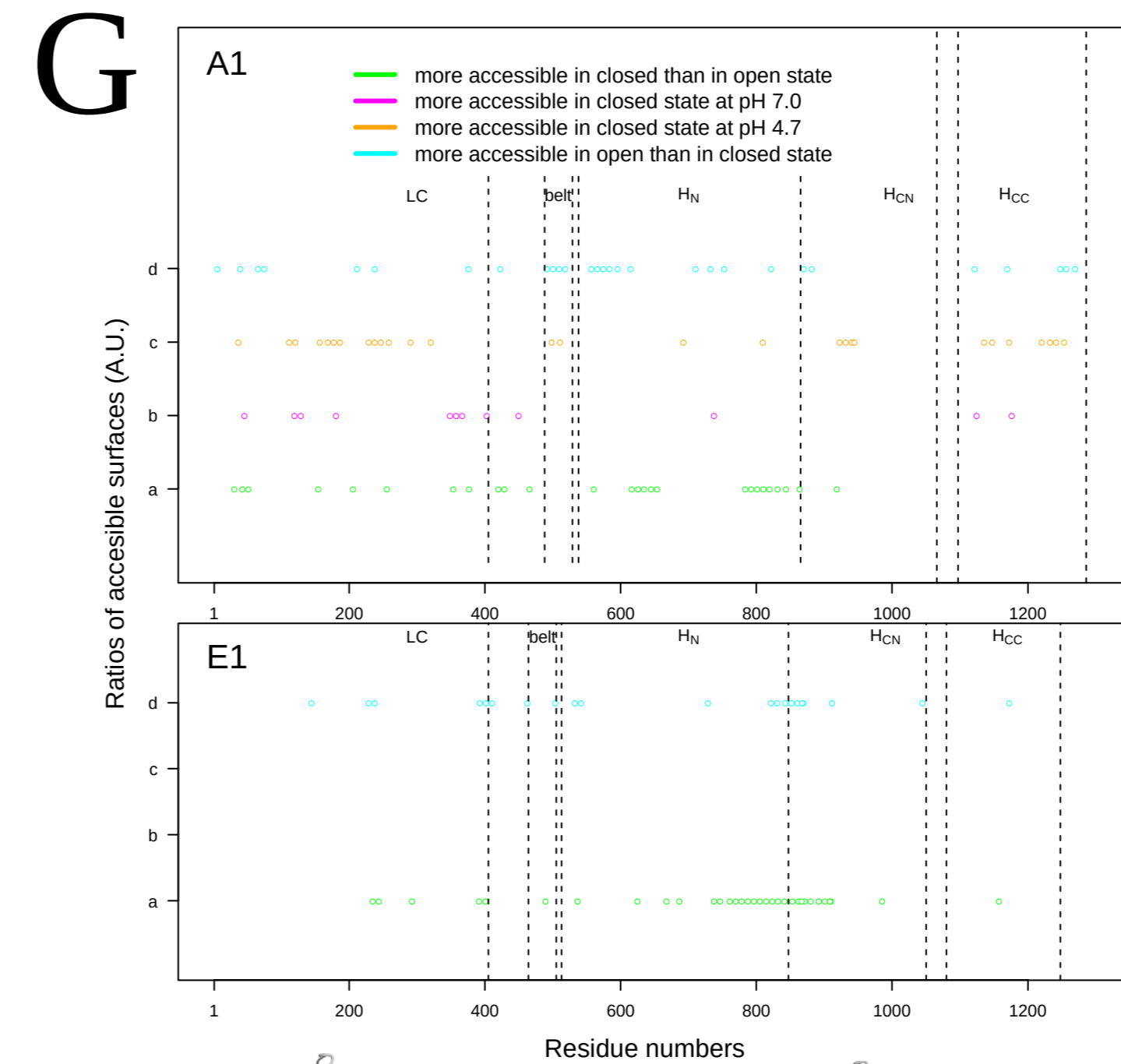


The closed A1 and open E1 conformations of BoNTs were built by homology modeling using Modeller [4] and sequence alignment between A1 and E1 obtained by T-Coffee [10]. Overall, three homology models were thus generated for A1 and for E1. Modeller was also used to generate open and closed conformations for BoNT/A1 and BoNT/E1 from the corresponding cleaved X-ray structures.

The motivation of using Modeller is to obtain starting points for MD energetically optimized with respect to the cleavage in two chains. Starting from the previously calculated models, the protonation of residues at neutral (7.0) and acidic (4.7) was predicted by using the web-based implementation of H++, a method for pKa calculations based on continuum electrostatic model [1]. Using the two different protonation levels on the six previously defined systems, twelve systems were finally set-up. The BoNT toxins were embedded in a large water box for a total number of atoms about 510000. Molecular dynamics (MD) trajectories were recorded using NAMD 2.13 [11], with the CHARMM36 force field [2] for protein and the TIP3P model for water [5]. For each trajectory, 300 ns of production were recorded for a cumulative trajectory duration of 3.6  $\mu$ s.

The coordinate root-mean-square deviations (RMSD, Å) along the MD trajectories (panel E) display quite stable plateaus after 50 ns. These plateaus display variations up to 8 Å (top plots), with a temporary jump up to 10 Å around 125 ns in the trajectory E1clo70 (green curve). **Very flat and low profiles around 2-4 Å are observed for the light chain (LC, bottom plots) whereas the RMSD calculated on the heavy chain (HC, middle plots) dominates the total RMSD values.** The light chain (LC), composed mostly of the catalytic domain, displays thus a stable conformation. The A1 trajectories recorded on the closed state not observed experimentally, but inferred by homology from the E1 structure, display RMSD values in the 4-6 Å range (green, orange, olivegreen, and brown curves), larger than what is observed for the open state (magenta and cyan curves). Similar large RMSD values are observed for E1 open state, with starting points generated by homology modeling (magenta and cyan curves). The trajectories starting from X-ray crystallography models (A1ope47, A1ope70, E1clo47, E1clo70) display different behaviors between A1 and E1. Indeed, the trajectories A1ope70 (magenta curve, panel E, left column) and A1ope47 (cyan curve, panel E, left column), as well as E1clo47 (orange curve,

panel E, right column) display the lowest RMSD values. More surprisingly, at pH 7.0, the trajectory E1clo70 displays a large jump around 125ns, in which the HC chain moves slightly apart from the LC chain.

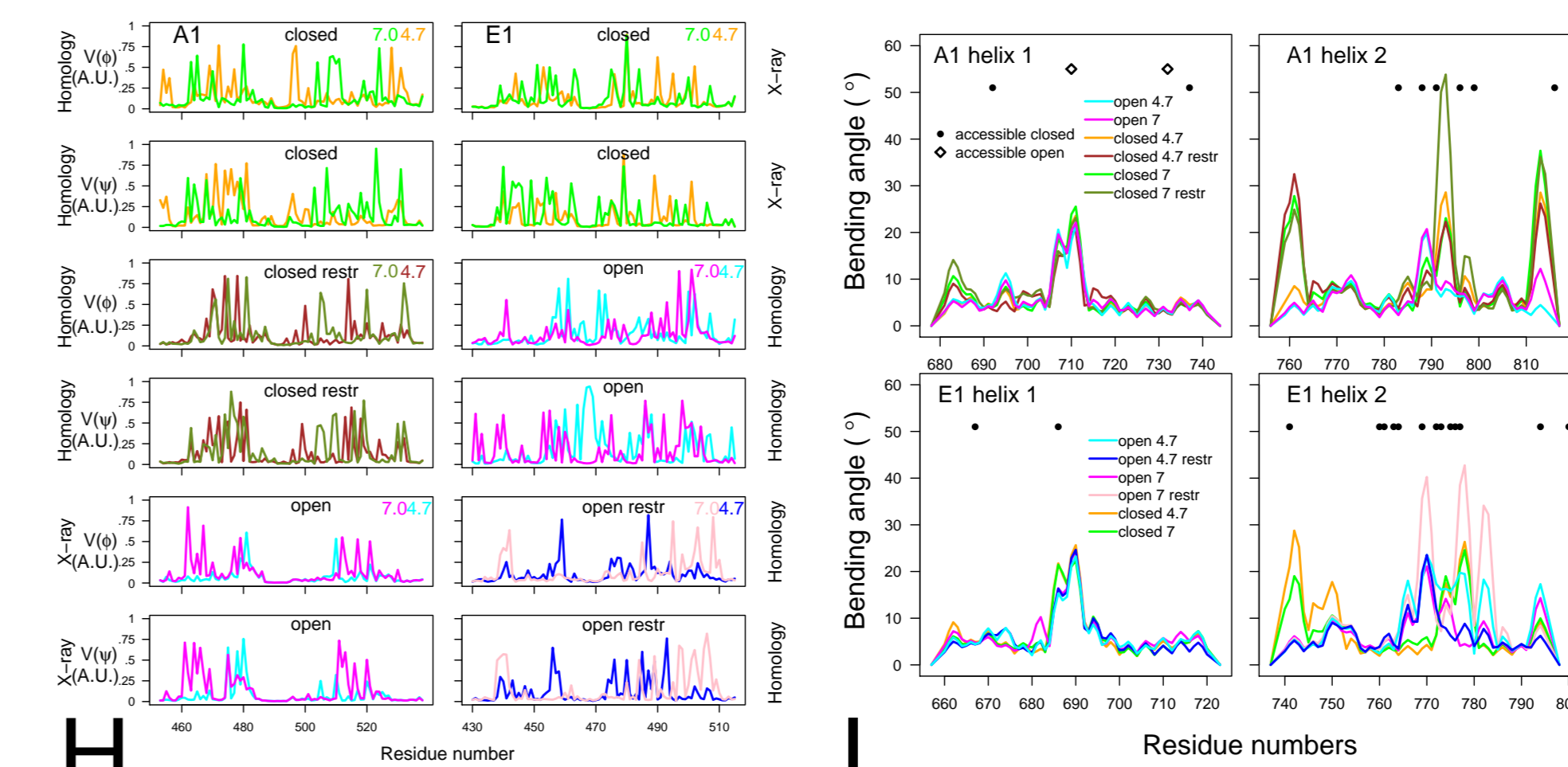


Coordinate RMSD calculated for the BoNT domains by superimposing each domain to its coordinates in the initial protein model, are displayed as boxplots (panel F). The RMSD values of individual domains except belt and H<sub>CC</sub>, are in much smaller range (1-4 Å for the medium values) than the global RMSD values. **This observation supports a model of mobility in which the individual domains fluctuate around stable conformations and most of the motions of the overall structure arise from relative displacements of the domains [12].** A repeated feature is the increase of RMSD values for MD trajectories starting from homology models with respect to MD trajectories starting from X-ray structures. This is related to the percentage of identity in the 35-45% range between the primary sequences of the two toxins.

## Connection of belt and translocation domains

The solvent accessible surfaces of residues were averaged along the time interval 150-300 ns in each trajectory (panel G). In order to anneal the influence of residue sizes on the surface values for each residue, average surface values of each trajectory were divided by the global average surface on all trajectories. The upper panel of Figure displays in various colors four sets of residues displaying different variations of surface ratios depending on the open/closed state and on the neutral/acidic pH: (1) residues more accessible in closed than in open states (green), (2)

residues more accessible in open than in closed states (cyan), residues more accessible only in closed state (3) at pH 4.7 (orange) or (4) at pH 7.0 (magenta). **For both BoNTs, clusters of residues more accessible in closed than in open conformations (green bullets) are observed in the translocation domain (H<sub>N</sub>) and to a lesser extent in the catalytic domain (LC).** At the contrary, the residues in H<sub>N</sub> more accessible in the open than in the closed conformation (cyan bullets) are more dispersed. The accessible residues observed in the domain H<sub>N</sub> can be related to experimental observations [9] showing that in BoNT/A LC-HCT, residues located in an  $\alpha$  helix close to the bottom extremity of H<sub>N</sub>, display increase in fluorescence intensity and blue shift to 530 nm when I830C-NBD binds to liposomes, in agreement with the transfer into a non-polar environment.



The circular variances [13] of the backbone dihedral angles  $\phi$  and  $\psi$  in the belt domain (panel H) display minimum values for most of the trajectories, in the center of the belt, for the  $\alpha$  helix 485-496 (A1) or 465-471 (E1). Otherwise, peaks of fluctuations are located mostly in the regions outside of the  $\alpha$  helix regions. The open state of A1 shows the largest interval of null values. Interestingly, peaks of circular variance are observed in the  $\alpha$  helix for protonation corresponding to the acidic pH for trajectories starting from homology models. This is specially visible for trajectories E1ope47 and E1ope70 (blue curves). Overall, the closed states globally display shorter ranges of minimal circular variances in  $\alpha$  helix. Similarly, the pH change as well as the change in internal mobility in  $\alpha$  helix induces displacements of fluctuation peaks outside of the  $\alpha$  helix region. **This could be a starting point of the belt destabilization at acidic pH, leading to its larger mobility before translocation.**

The bending of  $\alpha$  helices 1 and 2 located in H<sub>N</sub> (panel D) was analyzed (panel I) using Bendix [3]. In the helices 1 and 2, the maxima of bending angles are located in the residue ranges 705-715 and 777-797 (A1) and 698-718 and 737-798 (E1), corresponding to bend in the X-ray structures and initial models and are located slightly at the bottom of the H<sub>N</sub> switch. The local bending angles of  $\alpha$  helices 1 display similar profiles, with bending peaks at the middle of the helix. On the other hand, the helix 2 displays variations in intensities of bending angles. **The helices bending could be related to the initial events of H<sub>N</sub> interaction with the vesicle membrane.**

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