Interaction of the Complexin Accessory Helix with the C-Terminus of the SNARE Complex: Molecular-Dynamics Model of the Fusion Clamp

Maria Bykhovskaia,[†]* Anand Jagota,[‡§] Agustin Gonzalez,[†] Alexander Vasin,[†] and J. Troy Littleton[¶||††]

[†]Neuroscience Department, Universidad Central del Caribe, Bayamon, Puerto Rico; [‡]Department of Chemical Engineering and [§]Bioengineering Program, Lehigh University, Bethlehem, Pennsylvania; and [¶]Picower Institute for Learning and Memory, [||]Department of Biology, and [††]Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts

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Supporting Material

1. Estimate of Electrostatic Force of Repulsion Between a Synaptic Vesicle and a Plasma Membrane

To calculate this force, we considered two polarized planes (the vesicle and the membrane) carrying different surface potentials and separated by a distance (*a*). We modeled the electrostatic potential (φ) along the coordinate (x) which connects the vesicle and the membrane, which can range between 0 and a. The electrostatic potential was calculated using the Debye-Huckel equation, which is a linearized version of the nonlinear Poisson-Boltzmann equation [40, 41]:

$$\frac{d^2\varphi}{dx^2} = \frac{\varphi}{l_D^2} \qquad (1)$$

where l_D is the Debye screening length:

$$l_D = \sqrt{\frac{\epsilon\epsilon_0 k_B T}{2q^2 z^2 c_0}},$$

ϵ is the dielectric constant of water, ϵ_0 is the permittivity of free space, q is the charge of an electron, z is the unsigned valence of each of the two ions, c_o is the concentration of the ions, T is temperature, and k_B is Boltzmann's constant. The solution of the equation (1) is:

$$\varphi(x) = A\cosh\left(\frac{x}{l_D}\right) + B\sinh\left(\frac{x}{l_D}\right) \qquad (2)$$

To calculate the electrostatic force between the vesicle and the membrane based on Eq. (2), we need to make an assumption about the relationship between the surface potential and surface charge. We have considered two limiting cases:

i. *Surface potential is fixed* and surface charge adjusts to keep it at a constant level. The surface charge could adjust to compensate for the potential change either via redistribution of ions, such as K^+, in the vicinity of the membrane, or via polar lipid groups adjusting their degree of ionization. Both mechanisms would work to minimize the change in the surface potential.

ii. *Surface charge is fixed* and surface potential adjusts. This is the limiting case corresponding to fully ionized groups with a fixed charge.

Fixed Potential

In this case the equation [1] is subject to boundary conditions

a. $\varphi = \varphi_1$ ($x=0$),

b. $\varphi = \varphi_2$ ($x=a$). (3)

where (*x=0*) corresponds to surface '1' and (*x=a*) corresponds to surface '2'. Surface potential on both the vesicle and plasma membrane surface are negative and, without loss of generality, we let $\varphi_2 < \varphi_1$ so that '2' corresponds to the plasma membrane (see Table 1).

Applying the boundary conditions, we find

$$A = \varphi_1$$
$$B = \left[\varphi_2 - \varphi_1 \cosh\left(\frac{a}{l_D}\right)\right] / \sinh(\frac{a}{l_D})$$

So that

$$\varphi(x) = \varphi_1 \cosh\left(\frac{x}{l_D}\right) + \left[\varphi_2 - \varphi_1 \cosh\left(\frac{a}{l_D}\right)\right] \sinh(\frac{x}{l_D}) / \sinh(\frac{a}{l_D}) \qquad (4)$$

Table 1. Parameters used for calculations of the electrostatic repulsion

	Parameter	Value	Reference	Comment
1	Surface Potential of Vesicle, φ_1	-25 mV	[3]	
2	Surface Potential of Membrane φ_2	-70 mV	[4]	
3	Permittivity ε_o	8.85 x 10^{-12} F/m		SI units
4	Dielectric constant of water ε	80		Dimensionless
5	Salt concentration	200 mM	(1-1 Electrolyte)	
6	Debye Screening Length, l_D	0.67 nm	[5]	$l_D = \sqrt{\frac{\epsilon\epsilon_0 k_B T}{2q^2 z^2 c_0}}$
7	Surface Charge of Vesicle σ_1	-0.0125 C/m^2	[3]	
8	Surface Charge of Vesicle σ_1, σ_2 estimated using assumed surface potential, φ_1	-0.025, -0.07 C/m^2	[3]	$\sigma_1 = \frac{\epsilon\epsilon_0\varphi_1}{l_D}$
9	Separation between vesicle and plasma membrane when SNAREs hold them together	1 nm		
10	Vesicle diameter	45 nm	[3]	

Given the potential, we calculate the force per unit area between the two flat planes using the result [1]

$$f = -\frac{\epsilon\epsilon_0}{2}\left(\frac{d\varphi}{dx}\right)^2 + \frac{\epsilon\epsilon_0}{2}\frac{\varphi^2}{l_D^2} \qquad (5)$$

which yields,

$$f = \frac{\epsilon\epsilon_0}{2l_D^2 \sinh^2(\frac{a}{l_D})}\left(2\varphi_1\varphi_2 \cosh\left(\frac{a}{l_D}\right) - \varphi_1^2 - \varphi_2^2\right) \quad (6)$$

The interaction energy between the two planes is calculated as

$$E \equiv -\frac{Work}{Area} = -\int_{\infty}^{a} f da' \quad (7)$$

which gives

$$E = \frac{\epsilon\epsilon_0}{2l_D}\left(2\varphi_1\varphi_2 /\sinh\left(\frac{a}{l_D}\right) - (\varphi_1^2 + \varphi_2^2)(\coth\left(\frac{a}{l_D}\right) - 1)\right) \quad (8)$$

Figure S1 plots the normalized interaction energy, $\frac{2l_D E}{\epsilon\epsilon_0 \varphi_1^2}$ as a function of a/l_D for different values of φ_2/φ_1.

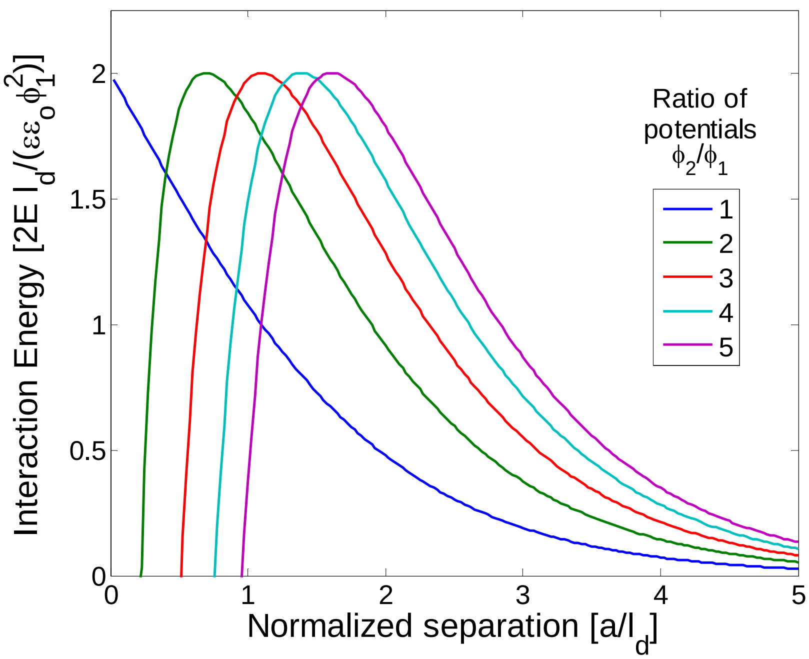

Figure S1. Interaction energy between two planes of charge as a function of ratio of potentials.

This shows some interesting peculiarities. Firstly, even for the same surface potential on both sides, the interaction energy (repulsive) remains bounded even for zero separation. This is because the surface charge reduces in magnitude to keep the surface potential fixed. In fact, at zero separation the surface charge density goes to zero just as the repulsion (for fixed charge) would be going to infinity and the two effects cancel out leaving a finite force. Secondly, for unequal surface potential, there is a maximum in the interaction energy *and this maximum is independent of the larger surface potential.* It depends only on the value of the smaller surface potential. It is given by a very simple expression (energy per unit area)

$$E_{max} = \frac{\varphi_1^2 \epsilon\epsilon_0}{l_D} \quad (9)$$

This is the interaction energy between two flat planes. We use Derjaguin's approximation [2], which relates the energy between two flat planes to the force of interaction between a spherical surface and a plane, which is what we need:

$$F_{max} = 2\pi R_v E_{max} = 2\pi R_v \frac{\varphi_1^2 \epsilon\epsilon_0}{l_D} \quad (10)$$

Fixed Charge

Let us now consider the case where surface charge is held fixed. In this case, the boundary conditions are:

$$-\epsilon\epsilon_0 \frac{d\varphi}{dx} = \sigma_1 \quad (x=0); \;\; \epsilon\epsilon_0 \frac{d\varphi}{dx} = \sigma_2 \quad (x=a). \quad (11)$$

Applying the boundary conditions, we obtain[1]

$$\varphi(x) = \frac{[\sigma_1 \cosh\left(\frac{a}{l_D}\right) + \sigma_2] l_D}{\epsilon\epsilon_0} \frac{\cosh(\frac{x}{l_D})}{\sinh(\frac{a}{l_D})} - \frac{\sigma_1 l_D}{\epsilon\epsilon_0} \sinh(\frac{x}{l_D}) \quad (12)$$

where σ_1 and σ_2 are the surface charge densities on the two surfaces. The force per unit area is again calculated using equation (6), which gives us

$$f = \frac{\sigma_1^2 + \sigma_2^2 + 2\sigma_1\sigma_2 \cosh\left(\frac{a}{l_D}\right)}{2\epsilon\epsilon_0 \sinh^2(\frac{a}{l_D})} \quad (13)$$

The interaction energy is again calculated using equation (7) and gives us

$$E = \frac{l_D}{2\epsilon\epsilon_0}\left(2\sigma_1\sigma_2 / \sinh\left(\frac{a}{l_D}\right) + (\sigma_1^2 + \sigma_2^2)(\coth\left(\frac{a}{l_D}\right) - 1)\right) \quad (14)$$

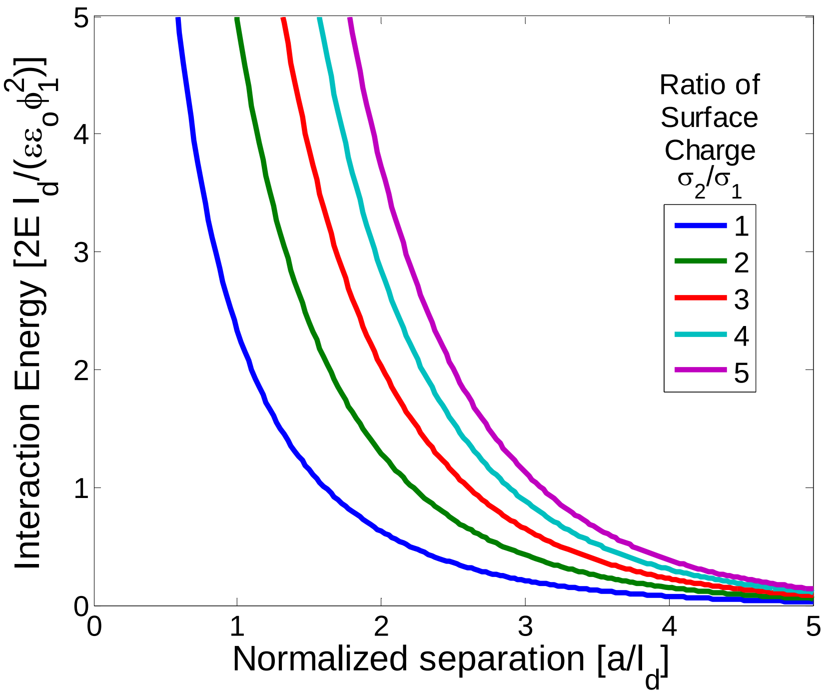

Figure S2 Interaction energy between two charged planes as a function of the ratio of surface charges

Replacing surface charge densities in equation (14) by surface potential at infinite separation, Figure S2 plots the normalized interaction energy, $\frac{2l_D E}{\epsilon\epsilon_0 \varphi_1^2}$ as a function of a/l_D for different values of φ_2/φ_1, w. Now we find that there is always repulsion and it diverges as the two surfaces are brought together. The force is again given by equation (10), i.e.,

$$F = 2\pi R_v E = \frac{\pi R_v l_D}{\epsilon\epsilon_0}\left(2\sigma_1\sigma_2 / \sinh\left(\frac{a}{l_D}\right) + (\sigma_1^2 + \sigma_2^2)\left(\coth\left(\frac{a}{l_D}\right) - 1\right)\right) \qquad (15)$$

To estimate the force, we assume a separation of about 1 nm and use other parameters given in Table 1.

Notably, the difference between the fixed potential and fixed charge calculations is significant only when the vesicle and membrane are within about a Debye length. For longer separations, the two converge. In fact, equations (8) and (14) for the energy of interaction both converge to a much simpler formula for large separations:

$$E = \frac{2\epsilon\epsilon_0}{l_D}\varphi_1\varphi_2 \exp\left(-\frac{a}{l_D}\right) \qquad (16)$$

and the force between the vesicle and membrane is given by

$$F = \frac{4\pi\,\epsilon\epsilon_0}{l_D}\varphi_1\varphi_2\,R_v\,\exp\left(-\frac{a}{l_D}\right) \tag{17}$$

Equation (17) is plotted in Figure S3.

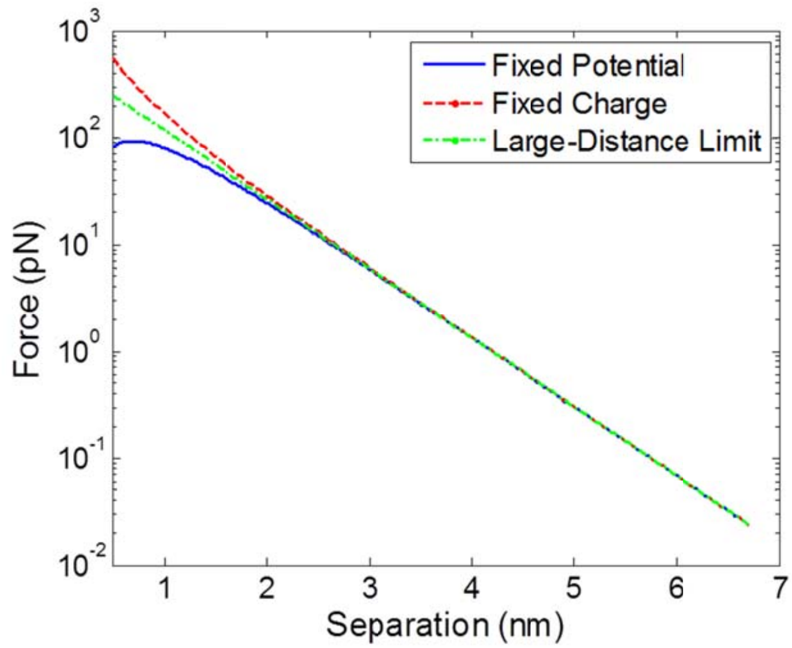

Figure S3. Distance dependence of the force produced by electrostatic repulsion between the vesicle and the membrane.

References

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2. Relaxation of the partially unzipped SNARE complex

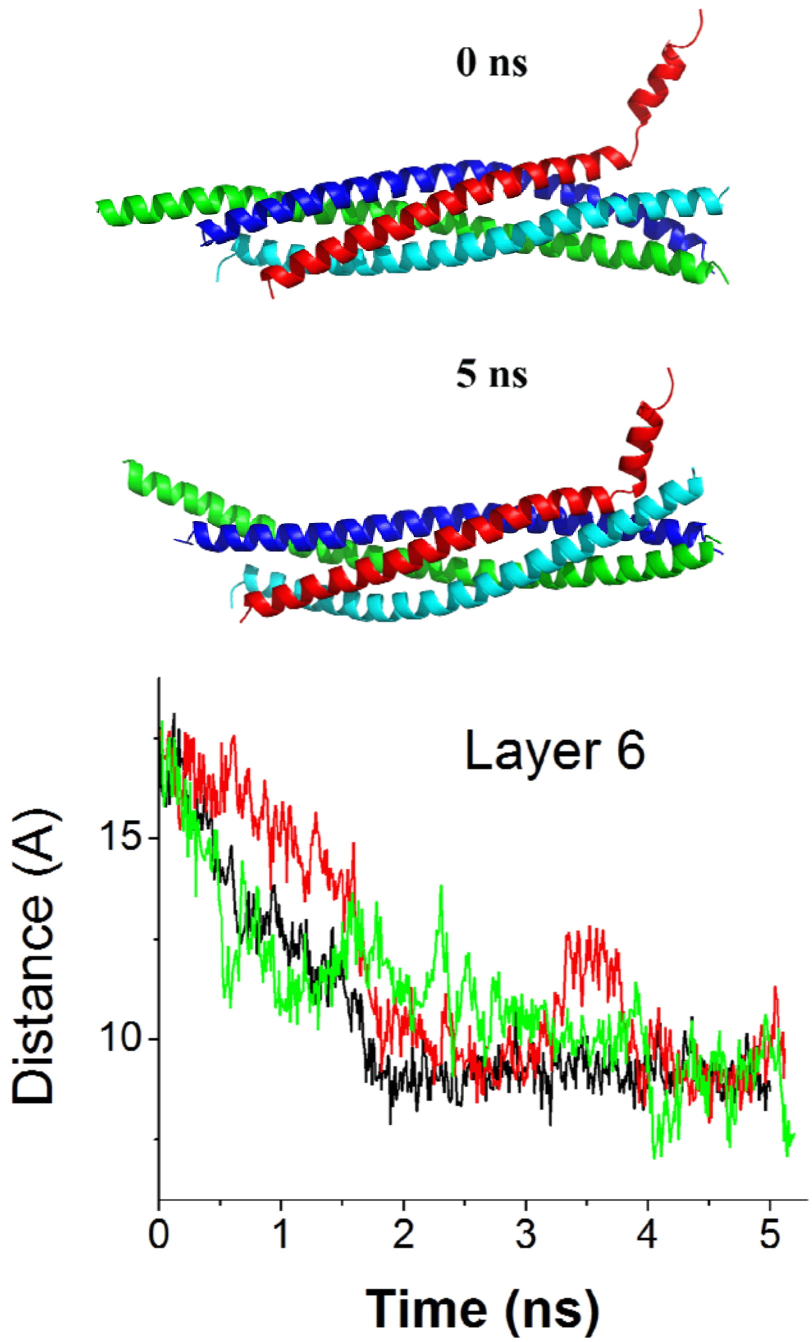

Figure S4. The SNARE conformation with layer 6 being separated from the rest of the SNARE bundle relaxes to the state with zippered layer 6 within 5 ns. Three lines (black, red, and green) correspond to three different runs. The separation of layer 6 was measured as a distance between Cα atoms of the residues F77 of Syb and A247 of Syx.

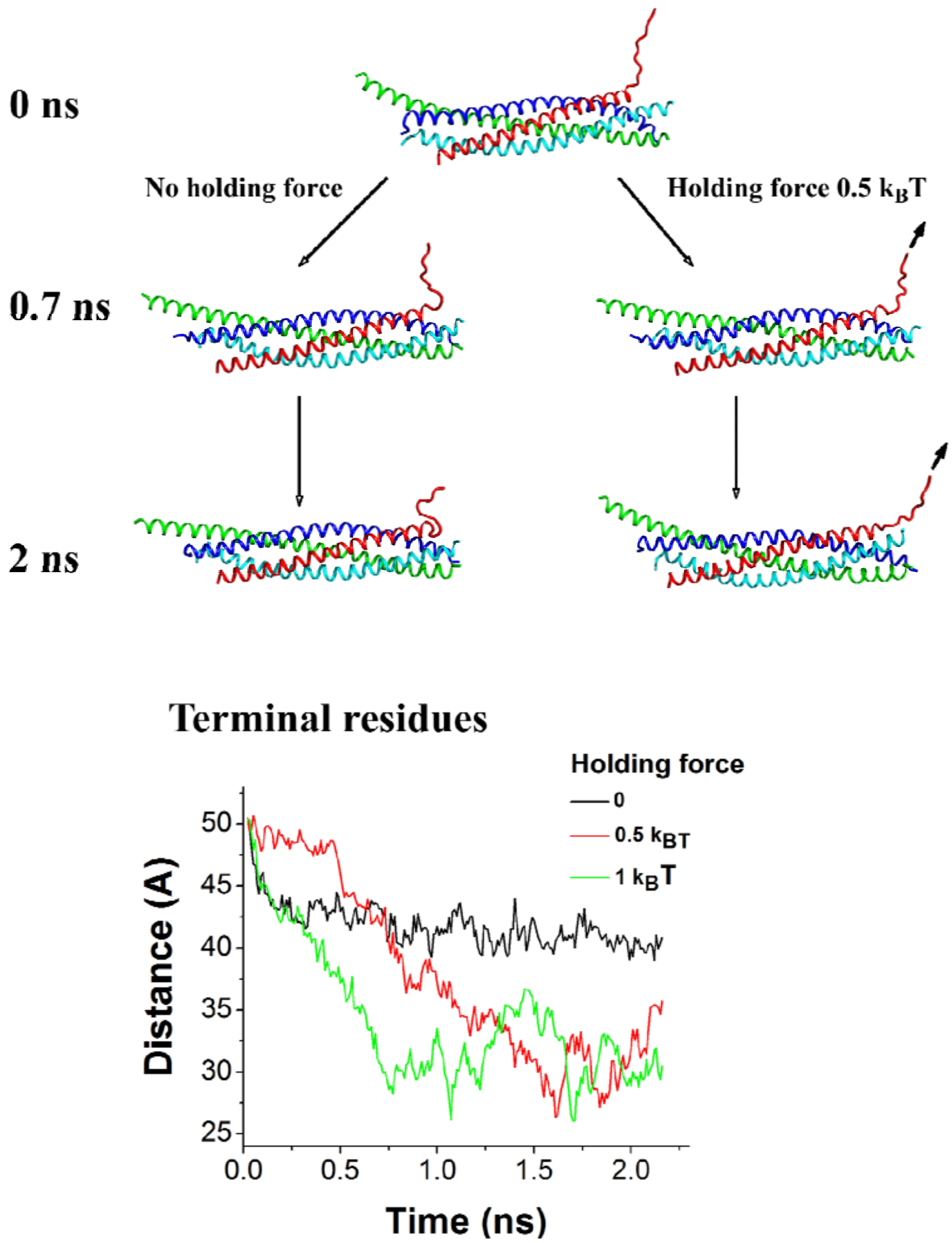

Figure S5. Holding force of 0.5-1 k_BT accelerates SNARE zippering

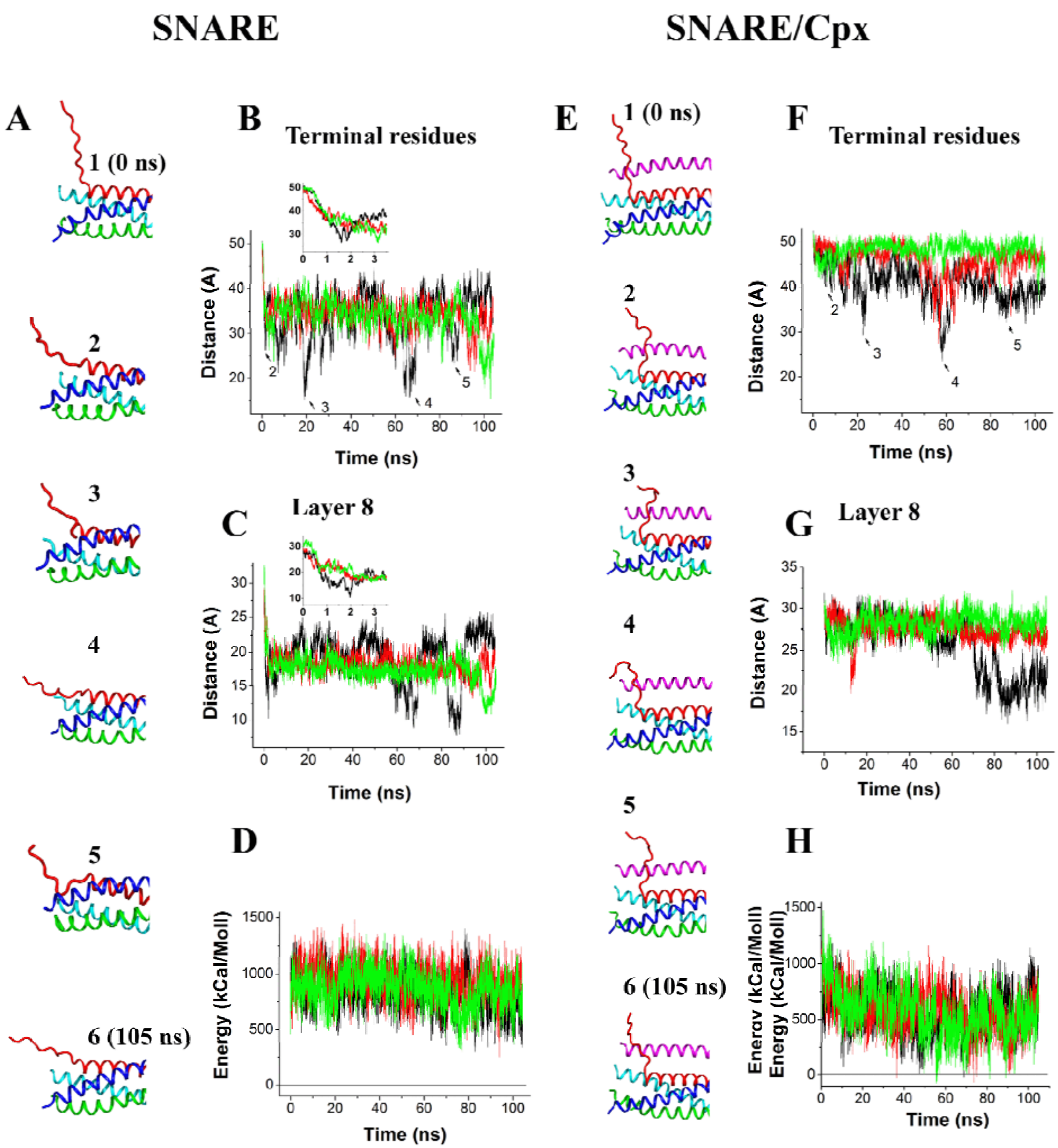

Figure S6. Relaxation of the SNARE complex without (A-D) or with (E-H) Cpx. Three independent runs are marked with different colors (black, red, green). Intermediate states (2-4, A, E) correspond to the trajectory points where the separation between Syb and Syx C-terminal residues diminishes (marked by arrows in B and F). Insets (B.C) show rapid decrease in the distance within the initial 3 ns.