Supporting Information for

A dynamic template complex mediates Munc18-chaperoned SNARE assembly

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The SI Appendix contains supplemental text and four supplemental figures.

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Kinetic model of the conformational transitions of the template complex and SNAP-25 binding. Suppose the template complex undergoes reversible and sequential conformational transitions among the unfolded state, the folded but inactive state, and the folded and activated open state to which SNAP-25 can bind, as shown in the inset in Fig. 1F with indicated transition rates. Then the probabilities to observe the unfolded template complex p_u, the closed or inactive template complex p_c, the open or activated template complex p_o, and the fully assembled SNARE complex p_s at any time t are determined by the Master equations:

$$\begin{cases} \dfrac{dp_u}{dt} = -k_f p_u + k_u p_c \\ \dfrac{dp_c}{dt} = k_f p_u - \left(k_o + k_u\right) p_c + k_c p_o \\ \dfrac{dp_o}{dt} = k_o p_c - \left(k_c + k_b c\right) p_o \\ \dfrac{dp_s}{dt} = k_b c p_o \end{cases} \tag{1}$$

where c is the concentration of SNAP-25 in the solution. Defining the probability vector

$$\boldsymbol{x} \equiv \begin{pmatrix} p_u \\ p_c \\ p_o \end{pmatrix} \tag{2}$$

and the rate matrix

$$\boldsymbol{A} \equiv \begin{bmatrix} -k_f & k_u & 0 \\ k_f & -k_o - k_u & k_c \\ 0 & k_o & -k_c - k_b c \end{bmatrix}, \tag{3}$$

we can rewrite Eq. (1) as

$$\frac{d\boldsymbol{x}}{dt} = \boldsymbol{A}\boldsymbol{x} \tag{4}$$

and

$$p_s\left(t\right) = 1 - \sum_{i=1}^{3} x_i. \tag{5}$$

Suppose the rate matrix has the eigenvalues $\lambda_i, i = 1,2,3$ and the corresponding eigenvectors $\boldsymbol{v}_i$ such that

$$\boldsymbol{A}\boldsymbol{V} = \boldsymbol{V}\boldsymbol{D} \tag{6}$$

where $\boldsymbol{V}$ is a matrix formed by all the eigenvectors

$$\boldsymbol{V} = \left(\boldsymbol{v}_1, \boldsymbol{v}_2, \boldsymbol{v}_3\right) \tag{7}$$

and $\boldsymbol{D}$ is a diagonal matrix

$$\boldsymbol{D} = \begin{bmatrix} \lambda_1 & 0 & 0 \\ 0 & \lambda_2 & 0 \\ 0 & 0 & \lambda_3 \end{bmatrix} \tag{8}$$

The solution to Eq. (4) is

$$\boldsymbol{x}(t) = \boldsymbol{V}e^{\boldsymbol{D}t}\boldsymbol{V}^{-1}\boldsymbol{x}_0 \tag{9}$$

where $\boldsymbol{x}_0$ is a constant vector whose elements represent the initial state probabilities at time zero.

In most of our SNAP-25 binding experiments, we held the Syx-VAMP conjugate at a constant mean force close to the equilibrium force of the template complex. In this case, the template complex folded and unfolded with equal rates, which were previously measured, i.e., $k_f = k_u = 3.2$ s^{-1} (1). Correspondingly, the initial state probabilities were chosen as $\boldsymbol{x}_0 = (0.5,\ 0.5,\ 0)^{\mathrm{T}}$. In a different experiment, we held the template complex in a fully folded state at a constant force and waited for SNAP-25 binding. In this case, the probability of the unfolded template complex $p_u = 0$ and Eq. (1) is simplified as

$$\begin{cases} \dfrac{dp_c}{dt} = -k_o p_c + k_c p_o \\ \dfrac{dp_o}{dt} = k_o p_c - \left(k_c + k_b c\right) p_o \\ \dfrac{dp_s}{dt} = k_b c p_o \end{cases} \tag{10}$$

The exact solution to this system of equations can be derived as

$$p_s(t) = 1 - \frac{1}{\lambda_+ - \lambda_-}\left(\lambda_+ e^{-\lambda_- t} - \lambda_- e^{-\lambda_+ t}\right), \tag{11}$$

where the two rate constants

$$\begin{aligned} \lambda_+ &= \frac{1}{2}\left[\left(k_o + k_c + k_b c\right) - \sqrt{\left(k_o + k_c + k_b c\right)^2 - 4k_o k_b c}\right], \\ \lambda_- &= \frac{1}{2}\left[\left(k_o + k_c + k_b c\right) + \sqrt{\left(k_o + k_c + k_b c\right)^2 - 4k_o k_b c}\right]. \end{aligned} \tag{12}$$

In the case of $k_o \ll k_c$, which corresponds to a high energy of the activated template complex relative to that of the inactive template complex, Eq. (11) can be approximated as

$$p_s(t) \approx 1 - e^{-\lambda_+ t}, \tag{13}$$

with

$$\lambda_+ \approx \frac{k_o c \eta}{1 + c\eta} \tag{14}$$

and

$$\eta = \frac{k_b}{k_c}. \tag{15}$$

The other rate constant $\lambda_- = k_c + k_b c$ is much greater than λ_+, leading to negligible contribution to the probability in a time region of our interest. Thus, in the presence of a high-energy activated template complex state, the probability of the assembled SNARE complex only depends upon two independent parameters k_o and η. As a result, the exact values for the model parameter k_c and k_b cannot be reliably determined.

Given model parameters, we numerically computed the state probabilities according to Eqs. (5), (9), and (13) using MATLAB. These calculations were fit to the corresponding experimental measurements to derive the rate constants in the model as best-fit parameters. Three sets of SNAP-25 binding probabilities measured under different conditions were included in the fitting: the first set with variable SNAP-25 concentration (40 nM – 1.2 μM) and the fixed time (t=120 s, Fig. 1F) and the second and third sets with fixed SNAP-25 concentrations (60 nM and 240 nM, respectively) but variable time (0-120 s, Fig. S1A). The initial conditions for the SNAP-25 binding were different: $p_c = 1/2$ for the first two sets of data and $p_c = 1$ for the third set of data. The model parameters were determined by nonlinear least-squares fitting with two independent fitting parameters. To this end, we varied the closing rate k_c from 0.2 s^{-1} to 100 s^{-1} and calculated the fitting error J with the model parameters k_o and k_b as fitting parameters. The fitting error J is defined as the sum of the square of the difference between the calculated (p_{sim}) and measured ($p_{\exp}$) probabilities of the assembled SNARE complex, i.e.,

$$J = \sum_{i=1}^{N_d} \left(p_{sim} - p_{\exp} \right)^2 \tag{16}$$

where $N_d = 57$ the total number of data points.

As k_c increases, the fitting error monotonically and quickly decreases as $k_c \le 1$ s^{-1} and then slowly converges to a minimum value as $k_c \ge 10$ s^{-1} (Fig. S1B, top panel). Similarly, the opening rate k_o and the rate ratio η converge to their best-fit values of 0.054 s^{-1} and 1.70 μM^{-1}, respectively (Fig. S1B, middle and bottom panels). To determine the standard deviation of the best-fit opening rate, we calculated the normalized fitting error χ^2 as a function of k_o around its best-fit value with k_c =10 s^{-1} and $k_b = k_c \eta = 17$ $\mu M^{-1} s^{-1}$ (Fig. S1C)(2). The normalized fitting error is defined as

$$\chi^2 \left(k_o \right) = \frac{J\left(k_o \right)}{\sigma^2} \tag{17}$$

where $\sigma^2 = J_{\min} / N_d$ with $J_{\min}$ the minimum fitting error calculated with the best-fit parameters. Fitting the normalized error to a quadratic function

$$\chi^2 \left(k_o \right) = \frac{\left(k_o - \overline{k_o} \right)^2}{\delta^2} + \chi_0^2 \tag{18}$$

with three fitting parameters $\overline{k_o}$, δ ,and χ_0^2 reveals the standard deviation of the best-fit opening rate as δ =0.0013 s^{-1}. The standard deviation of the best-fit rate ratio was similarly calculated. Thus, the best-fit opening rate k_o and rate ratio η are 0.054 ± 0.001 s^{-1} and 1.70 ± 0.05 μM^{-1}, respectively. The exact value for the inactivation rate of the activated template complex cannot be determined from our data but is estimated to be greater than 1 s^{-1} (Fig. S1B, top panel). Accordingly, the energy difference between the activated and inactivated template complex and the SNAP-25 binding rate constant are estimated be greater than $k_B T \ln\left(k_c / k_o \right) = 2.9$ $k_B T$ and $k_b = k_c \eta = 1.7 \times 10^6$ $M^{-1} s^{-1}$, respectively. The MATLAB scripts used for these calculations are available upon request.

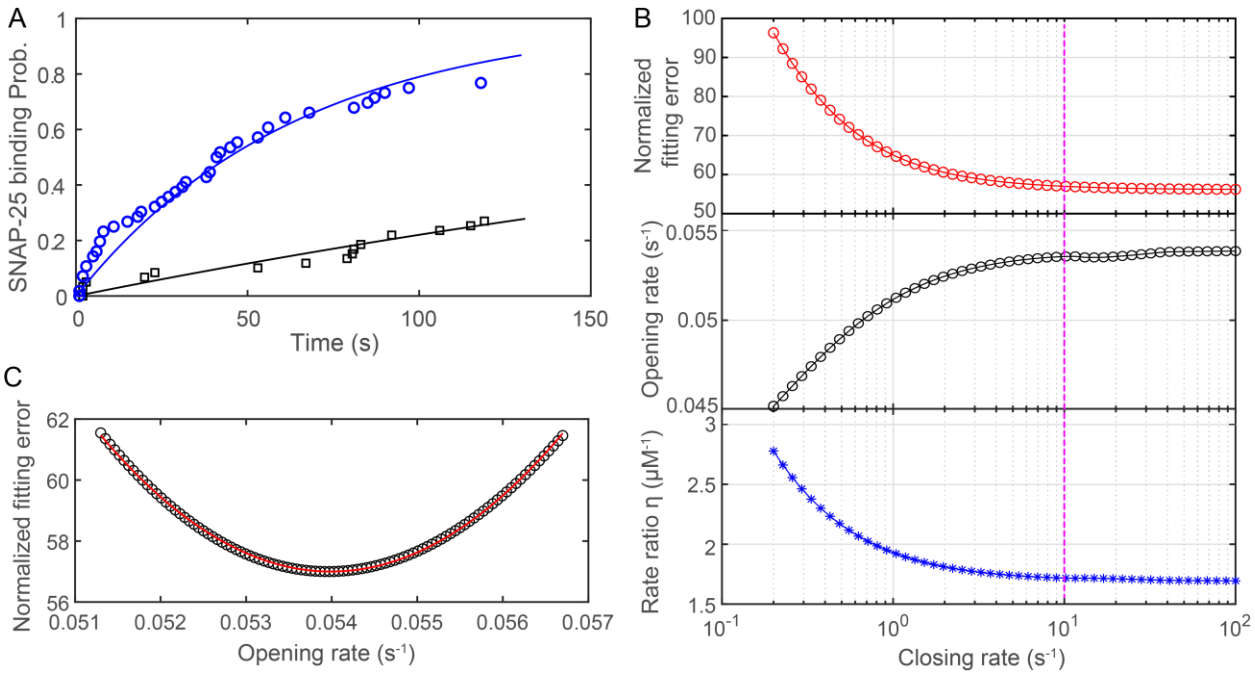

Fig. S1. Nonlinear least-squares fitting to determine the rate constants of the kinetic model for the template complex-mediated SNARE assembly. (A) Cumulative probabilities of SNAP-25 binding in the presence of 60 nM (black squares) or 240 nM (blue circles) SNAP-25 in the solution and their best model fits (black and blue curves). (B) The normalized fitting error χ^2 (top panel), the activation rate k_o (middle panel), and the rate ratio of the SNAP-25 binding rate to the inactivation rate η (bottom panel) as a function of the inactivation rate k_c. The magenta dashed line indicates the position where the best-fit values for the two independent model parameters k_o and η are evaluated. (C) The normalized fitting error χ^2 as a function of k_o around its best-fit value and the best fit of the fitting error with a quadratic function. See Supplementary Text in SI Appendix for details.

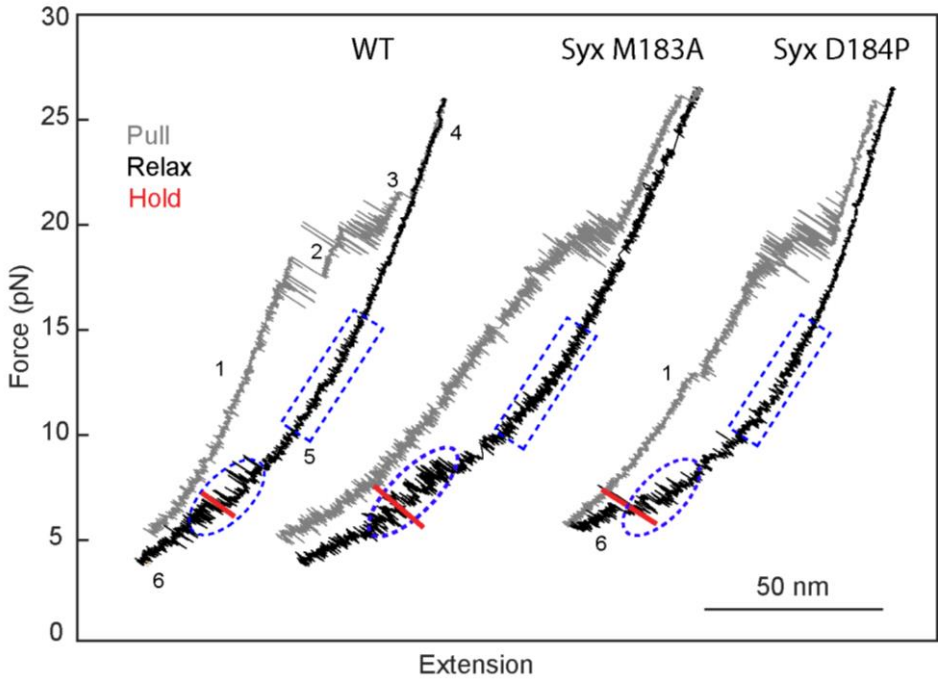

Fig. S2. FECs associated with syntaxin-1 with point mutation M183A or D184P in the linker in the presence of 1 µM Munc18-1 and 60 nM SNAP-25 in the solution. Single pre-assembled SNARE complexes were first pulled to high forces to detect their stepwise unfolding (grey FECs) and then relaxed to low forces to detect reversible refolding of open syntaxin (blue dashed rectangles) and template complexes (blue dashed ovals). Finally, the complexes were held around the equilibrium of the template complex transitions to detect SNAP-25 binding (red regions). The states associated with different FEC regions are labeled as in Fig. 1D.

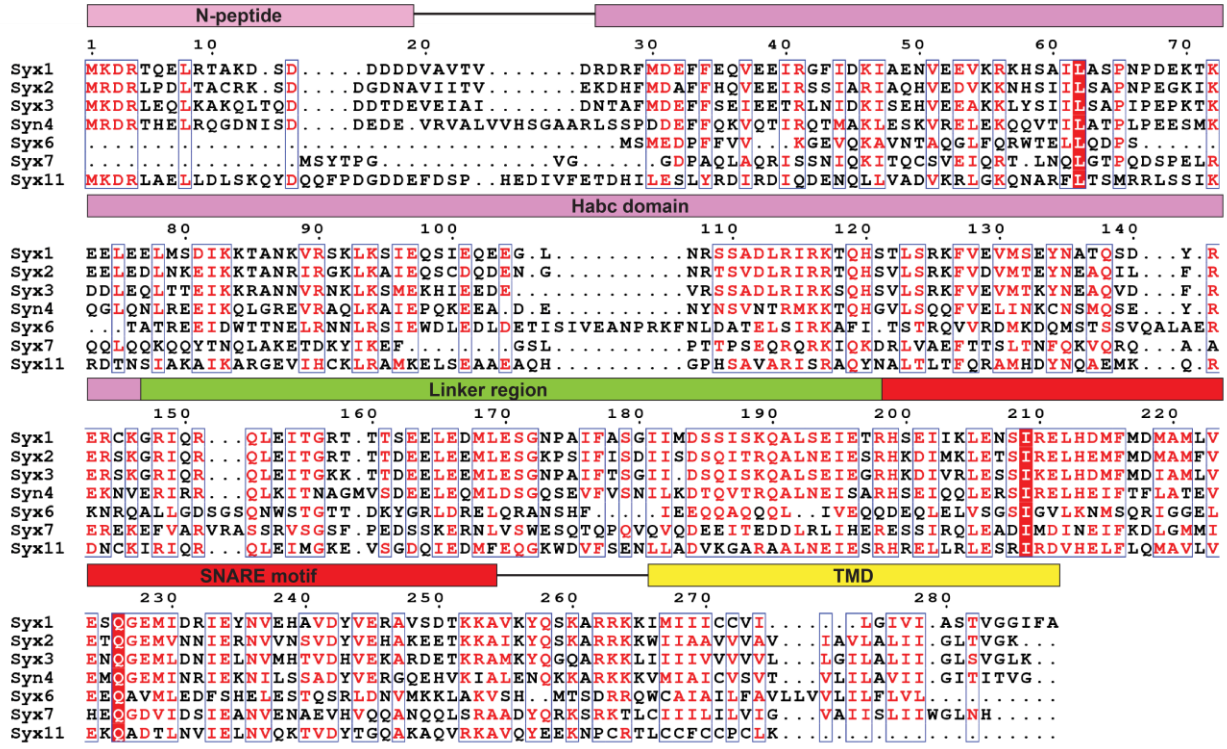

Fig. S3. Sequence alignment of human syntaxin proteins shows highly conserved amino acid sequences in the linker region and most of other regions.

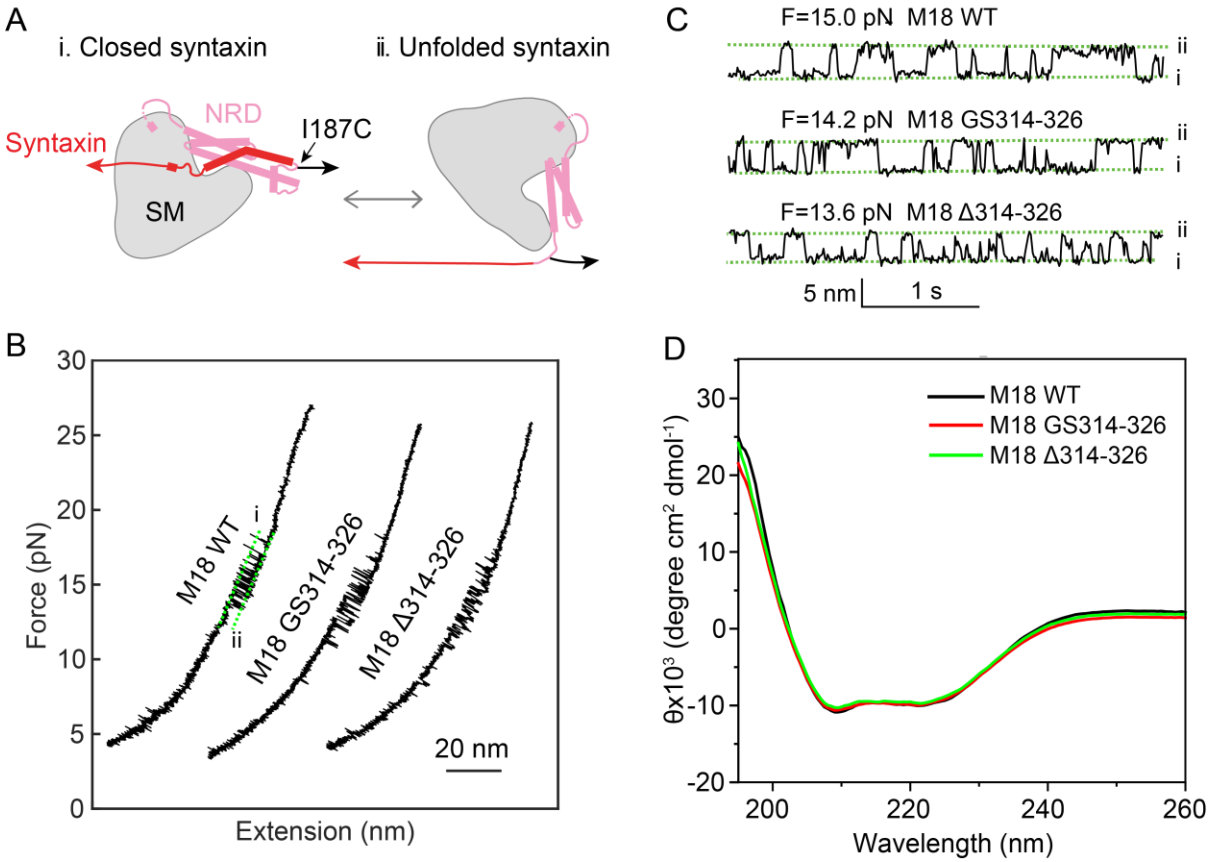

Fig. S4. The loop truncation or substitution does not significantly alter the Munc18-1 structure. (A) Diagram to illustrate the experimental setup to pull a single Munc18-1-bound syntaxin-1 and its unfolding and refolding transition (1). The SNARE motif of syntaxin-1 is pulled from its C-terminus and N-terminus at I187C. (B) Force-extension curves showing reversible syntaxin transition between the closed (i) and unfolded (ii) syntaxin states bound by WT Munc18-1 (M18 WT) or mutant Munc18-1 with loop truncation (M18 Δ314-326) or substitution (M18 GS314-326). (C) Time-dependent extension trajectories showing reversible syntaxin transition at constant force. Based on the measured average equilibrium forces and extension changes of the transition, we estimated the unfolded energy of the Munc18-1-bound closed syntaxin to be 7.2 (± 0.2, SEM) k_BT for M18 WT (1), 7.4 (± 0.3) k_BT for M18 Δ314-326, and 8.0 (± 0.3) k_BT for M18 GS314-326. (D) CD spectra of WT and mutant Munc18-1.

References

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2. Y. L. Zhang, D. M. Crothers, Statistical mechanics of sequence-dependent circular DNA and its application for DNA cyclization. *Biophys. J.* **84**, 136-153 (2003).