Investigation of SNARE-Mediated Membrane Fusion Mechanism Using Atomic Force Microscopy

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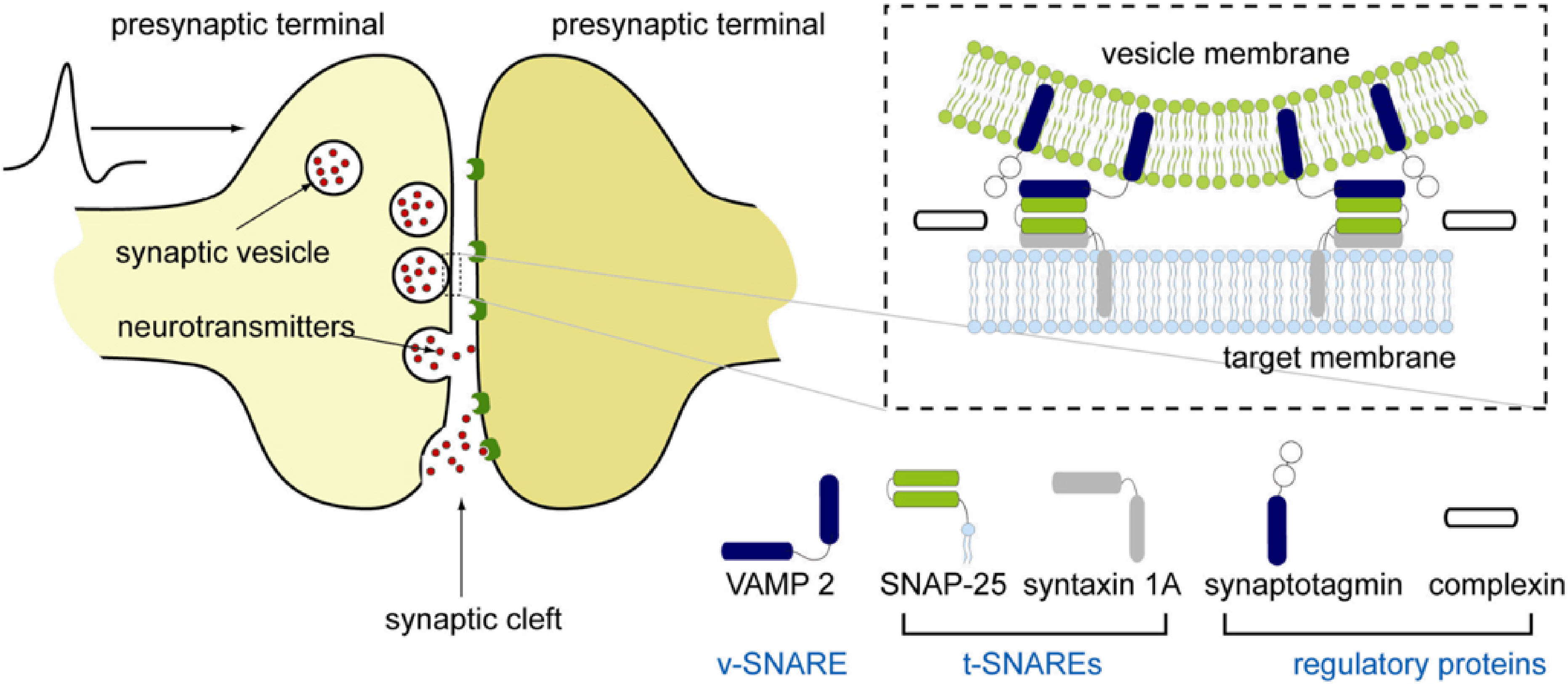
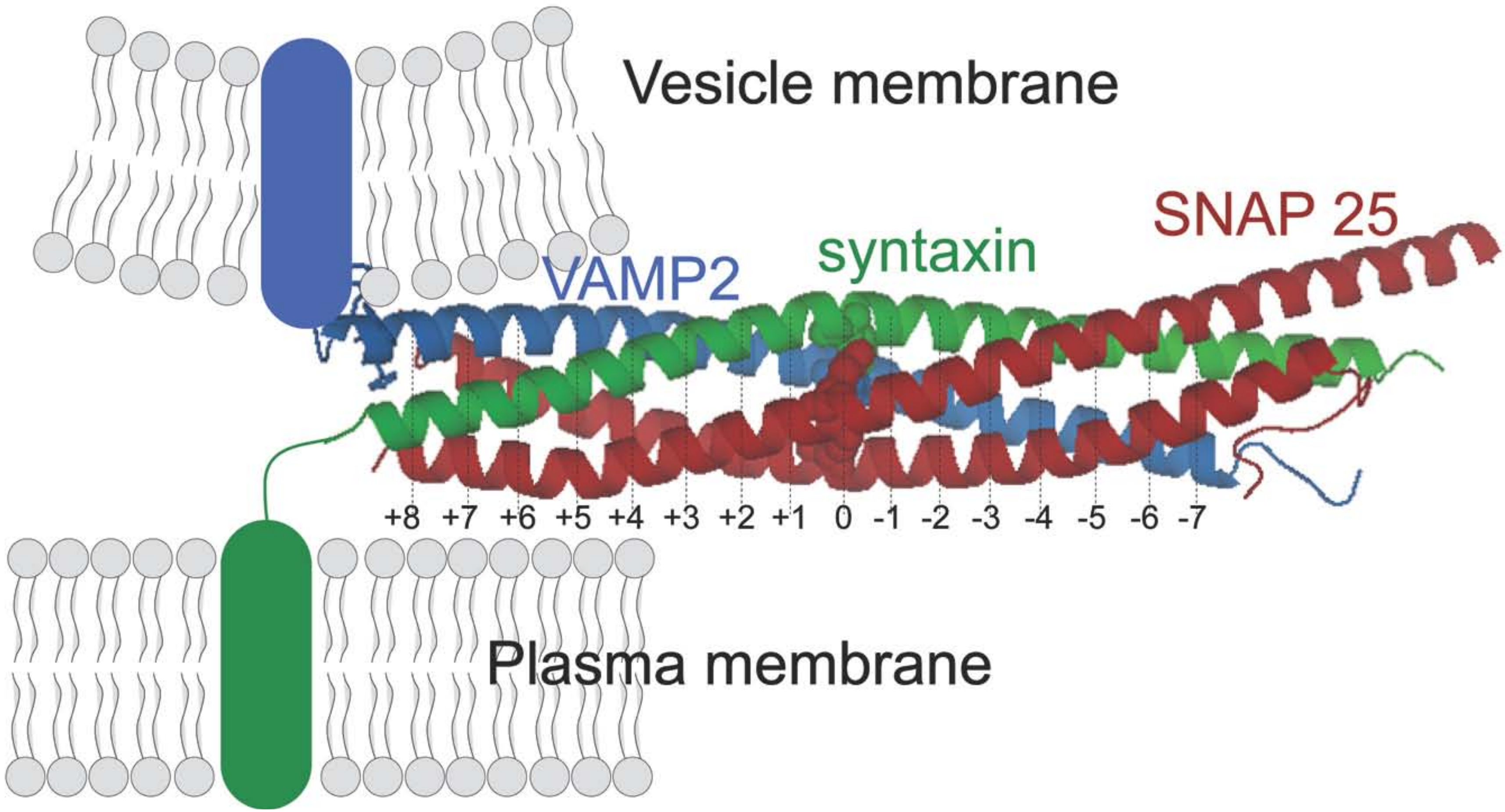
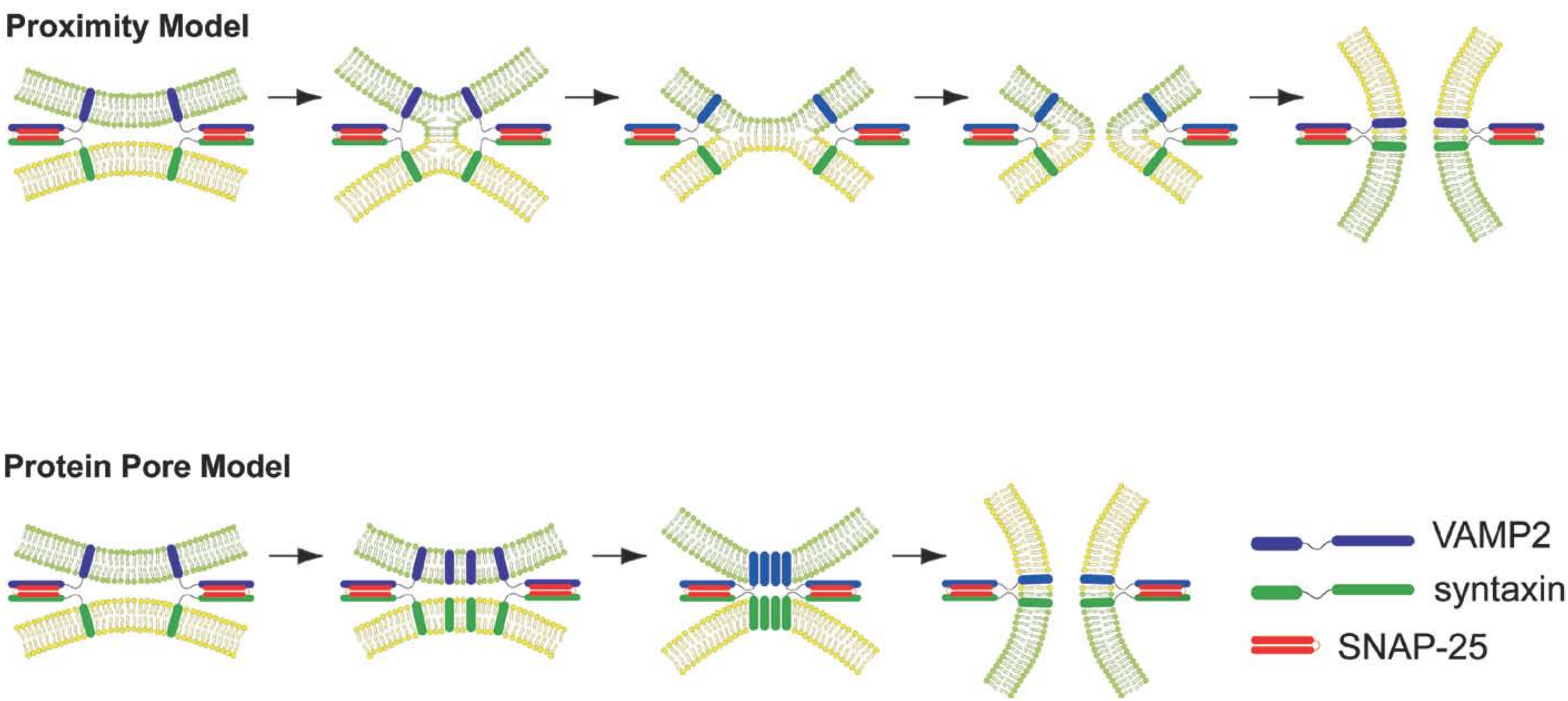
Synaptic vesicle fusion



Structure of the Synaptic SNARE Complex



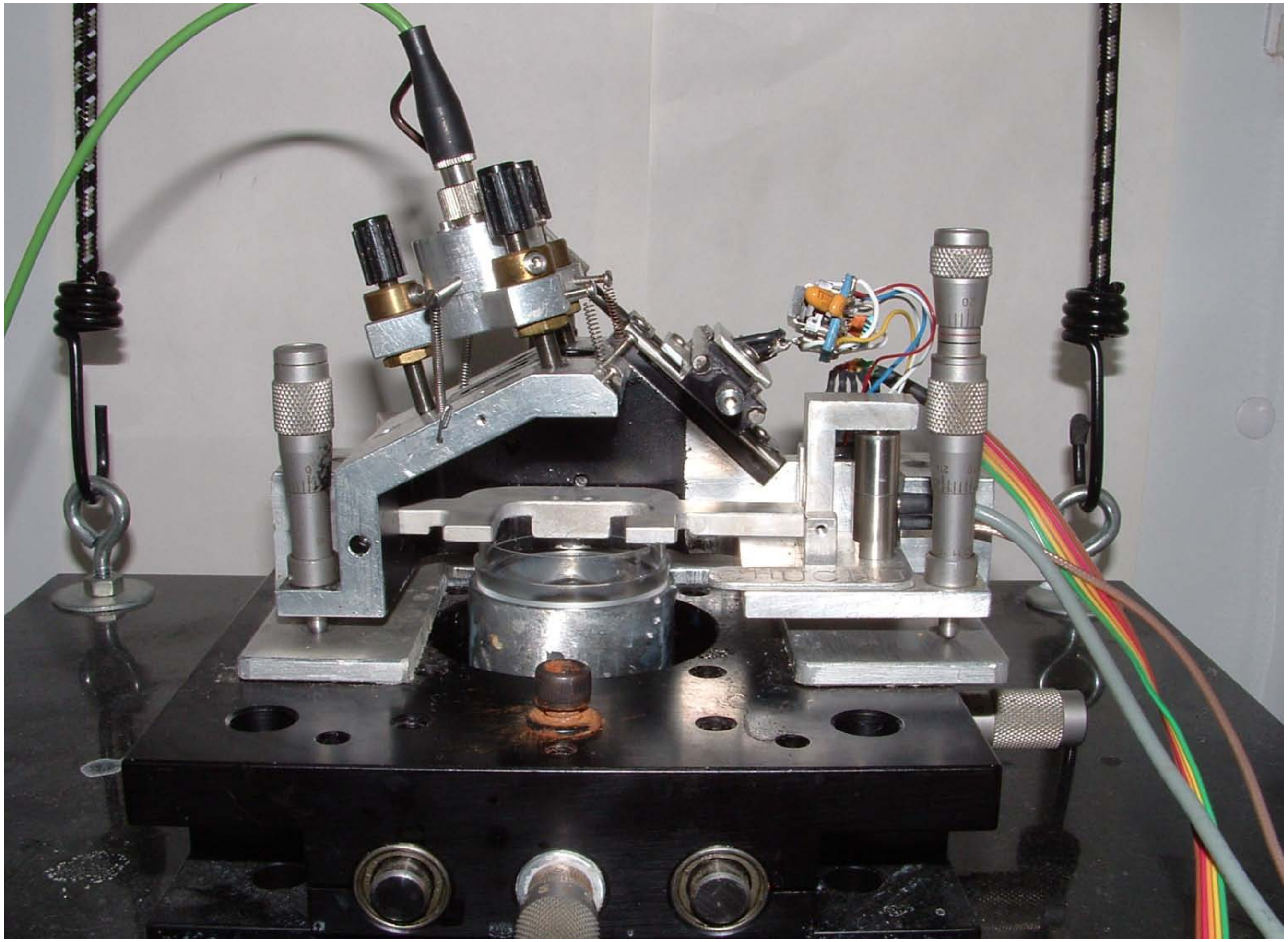
Models for SNARE-mediated membrane fusion



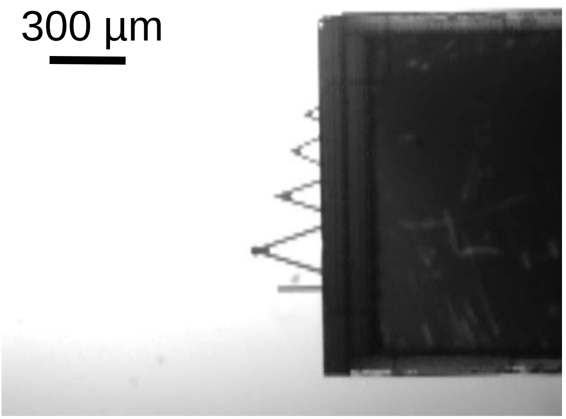
Aim 1:

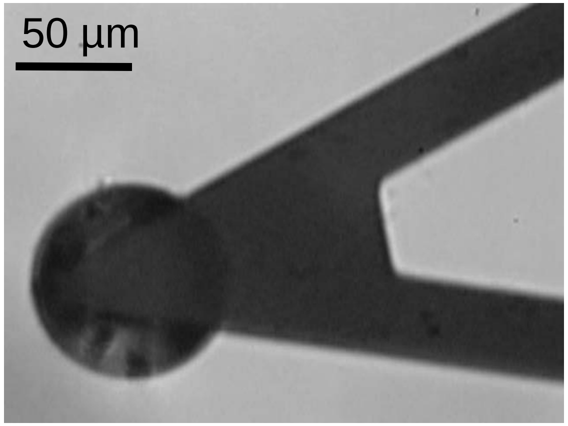
To develop an experimental system to investigate the effects of SNAREs on the energetics of membrane fusion.

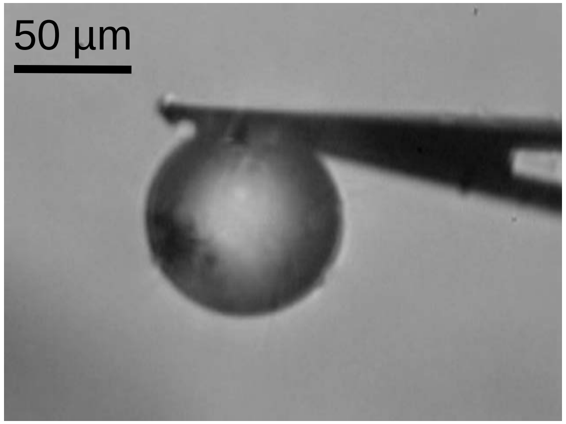
Atomic Force Microscopy (AFM)



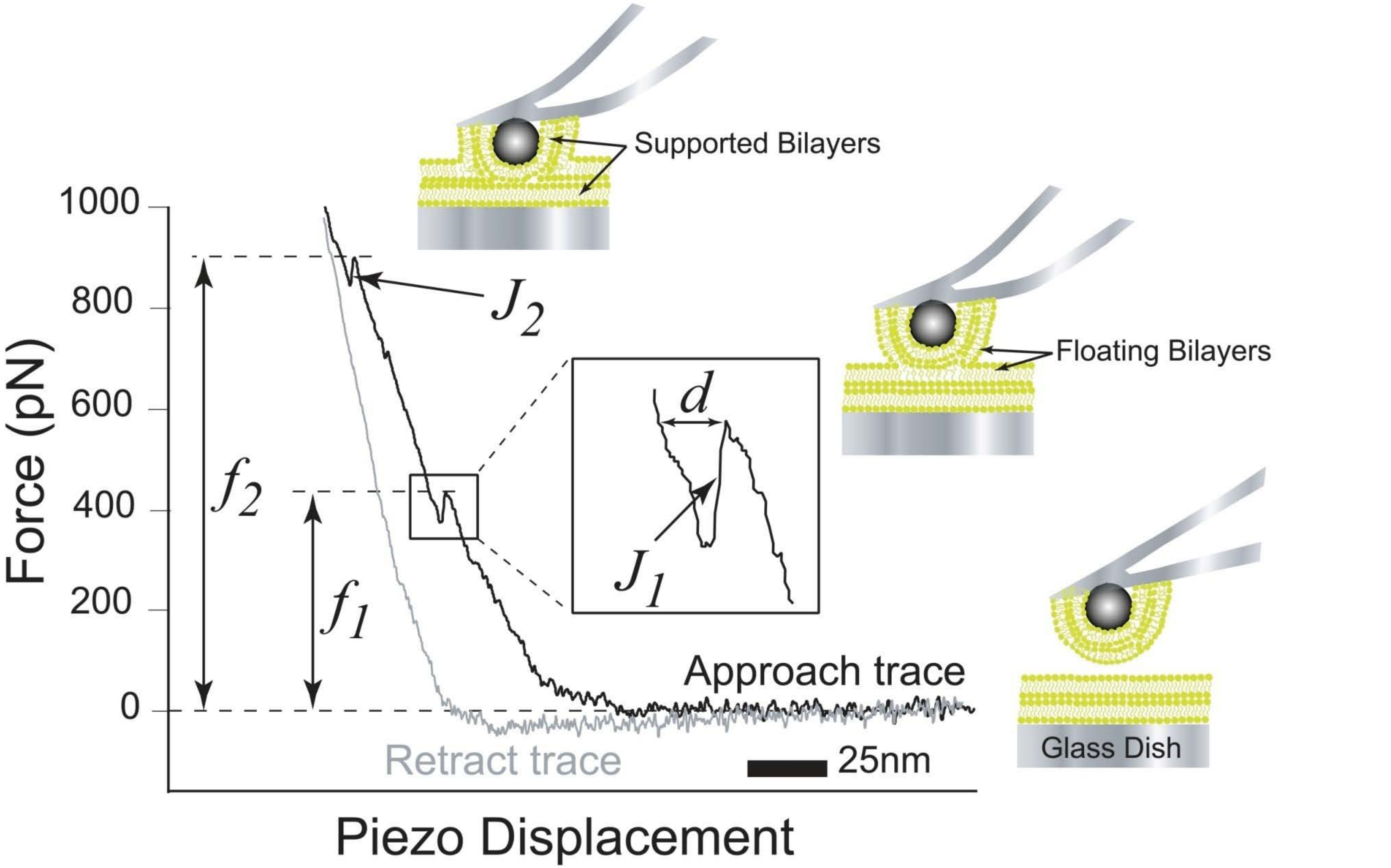
Lipid bilayer formation on hydrophilic glass surfaces



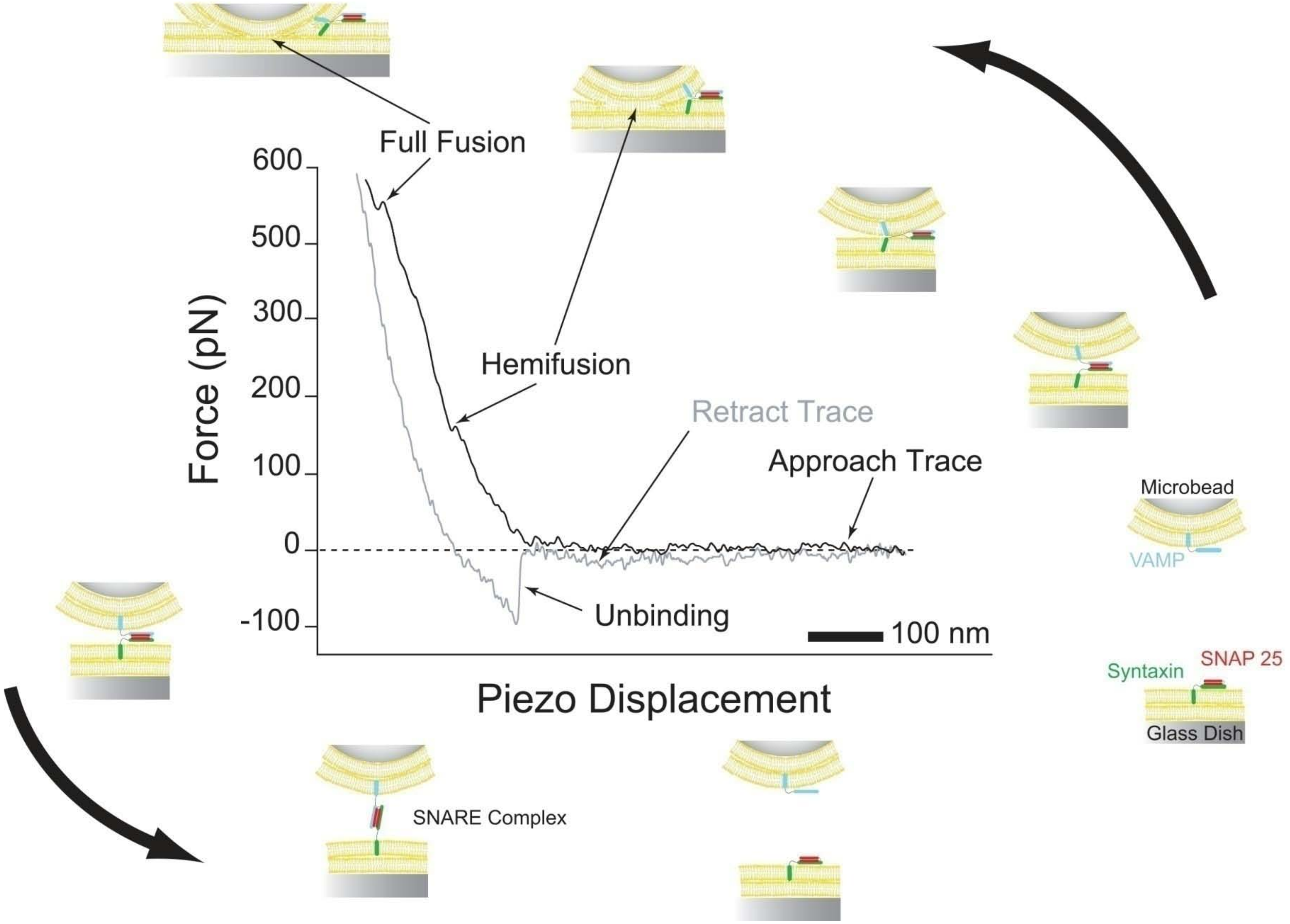




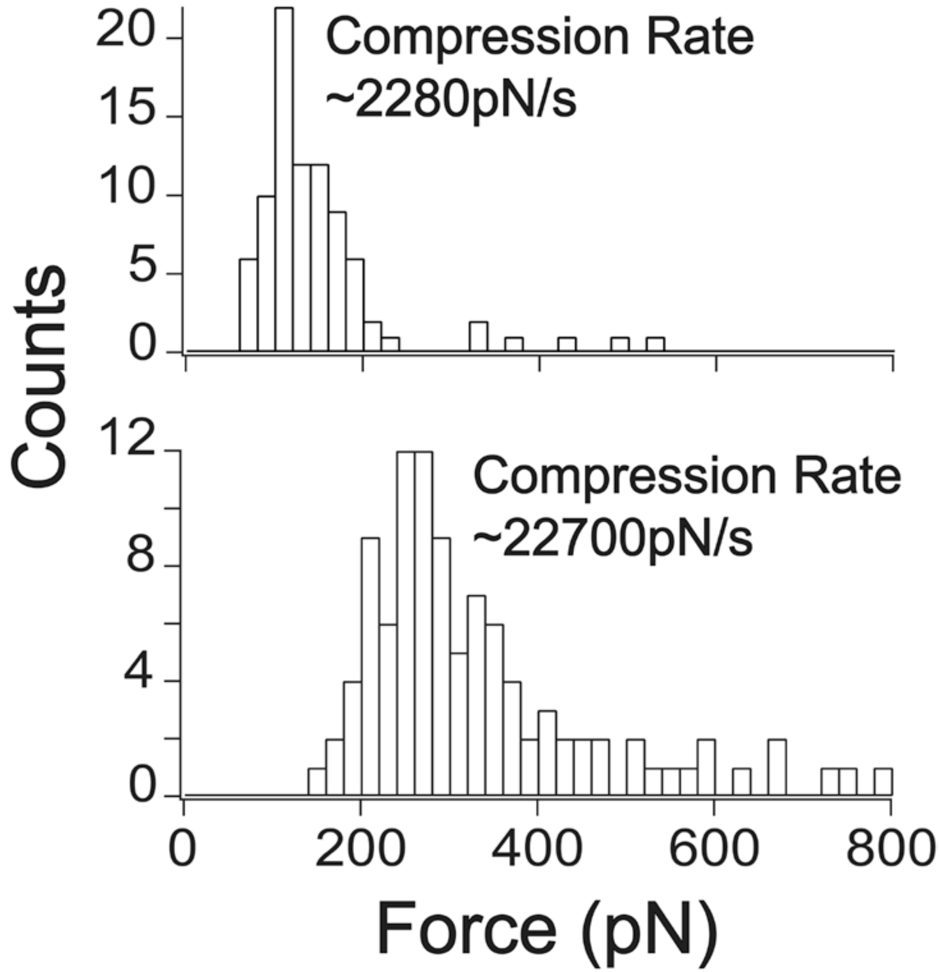
Detection and measurement of fusion force



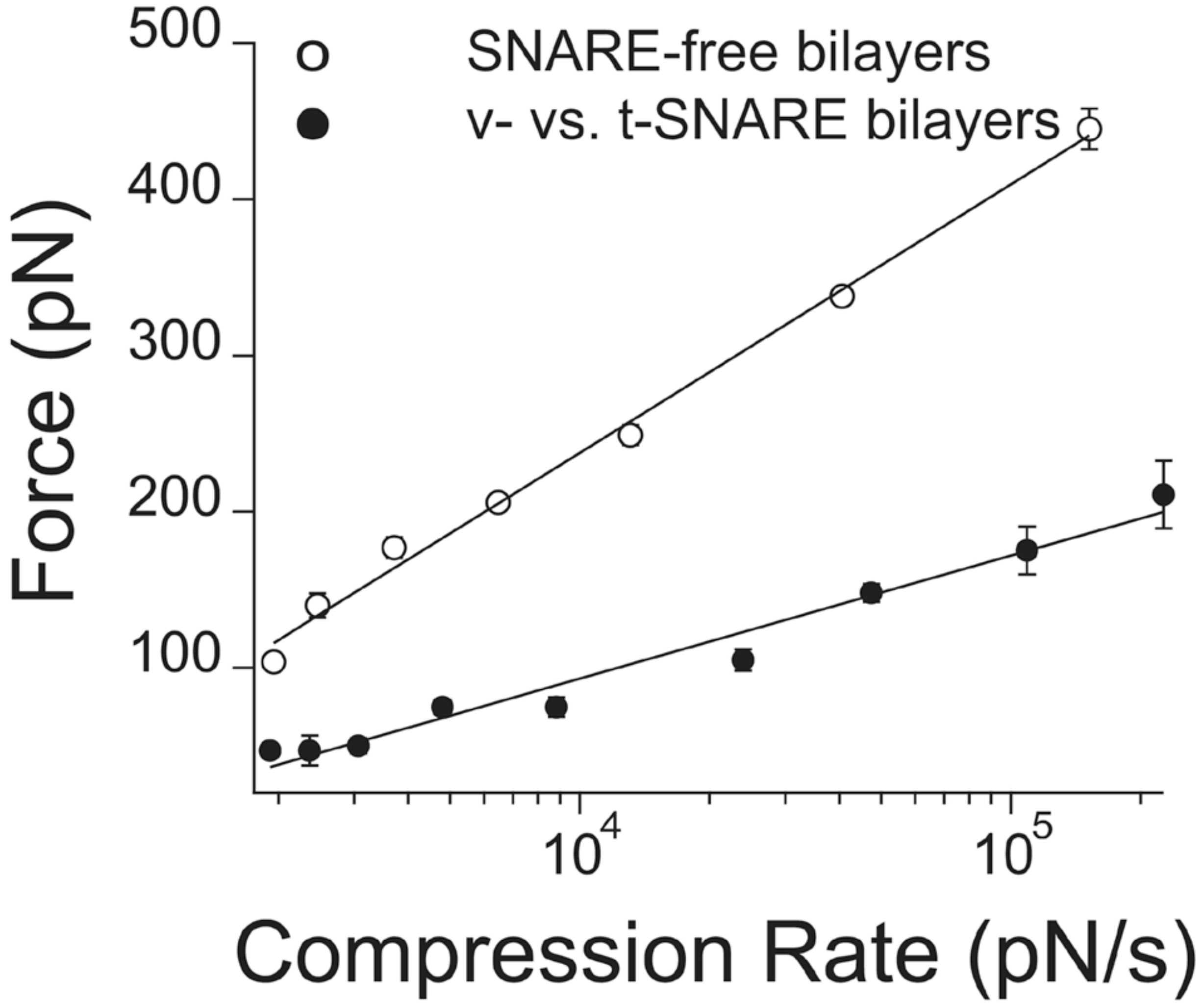
Measurements of SNARE-mediated fusion



Fusion force increases with compression rate

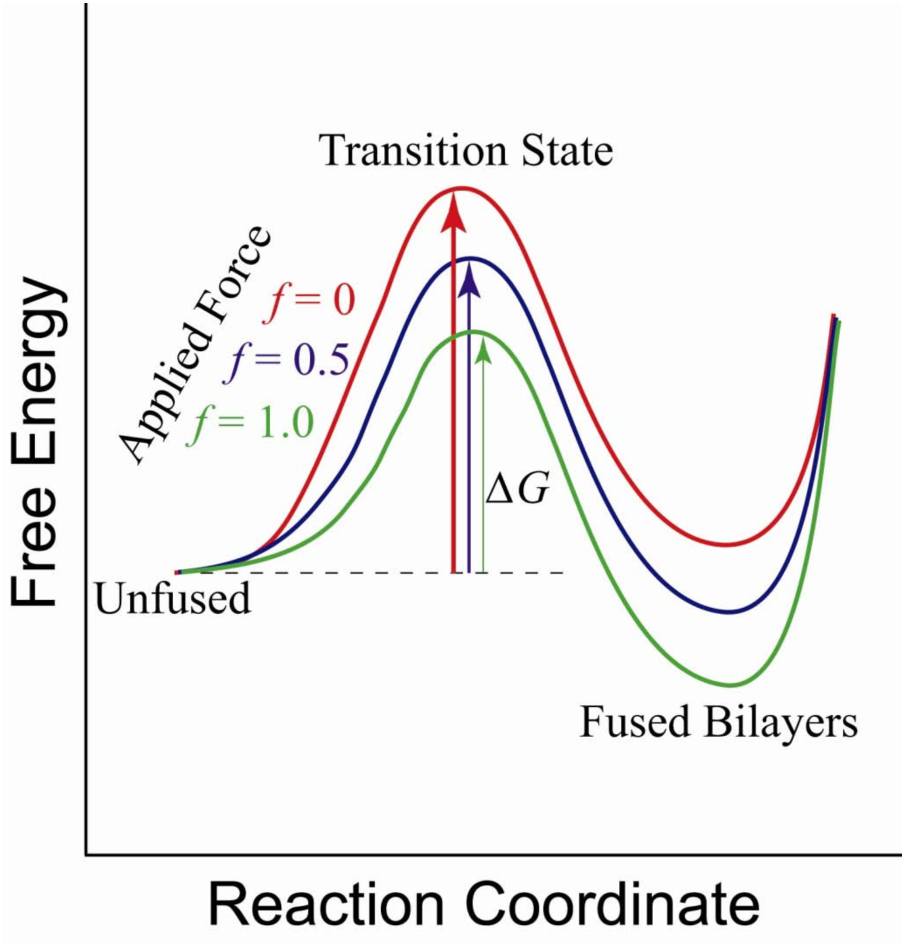


Fusion force decreases in presence of SNAREs



Effect of Applied Force on the Fusion Energy Barrier

Dynamic compression Model: The model is based on the transition state theory.



$$k_{fusion}(f) = k^{o} \exp\{\frac{f\gamma}{k_B T}\}$$

where

- k_B = Boltzmann's constant
- T = Absolute temperature
- k^{o} = Dissociation rate constant in the absence of external applied force which descibes the energy barrier height
- γ = Distance between the unfused and transition state which describes the energy barrier width
- r_f = Compression (loading) rate

with $f = (r_f)$ t: $$f = \frac{k_B T}{\gamma}\ln\{\frac{\gamma}{k^{o} k_B T}\} + \frac{k_B T}{\gamma}\ln\{r_f\}$$

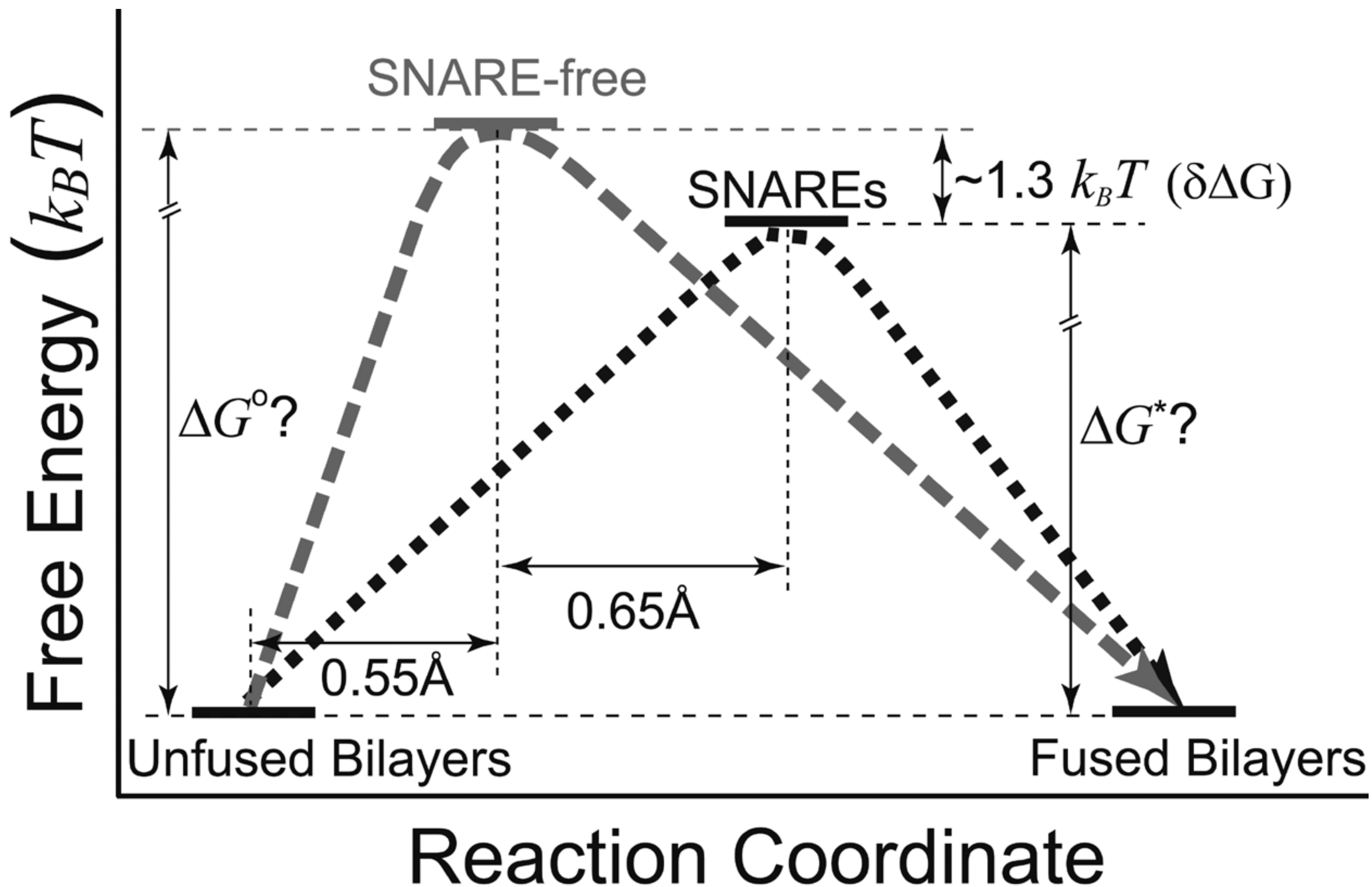
$$f = a + b \ln\{r_f\}$$

Fusion Energy barrier Parameters

Bilayers	γ (Å)	k^{o} (s^{-1})
SNARE-free vs. SNARE-free	0.55	5.52
v- vs. t-SNAREs	1.2	19.3

k° characterizes the height of the potential and γ describes its width.

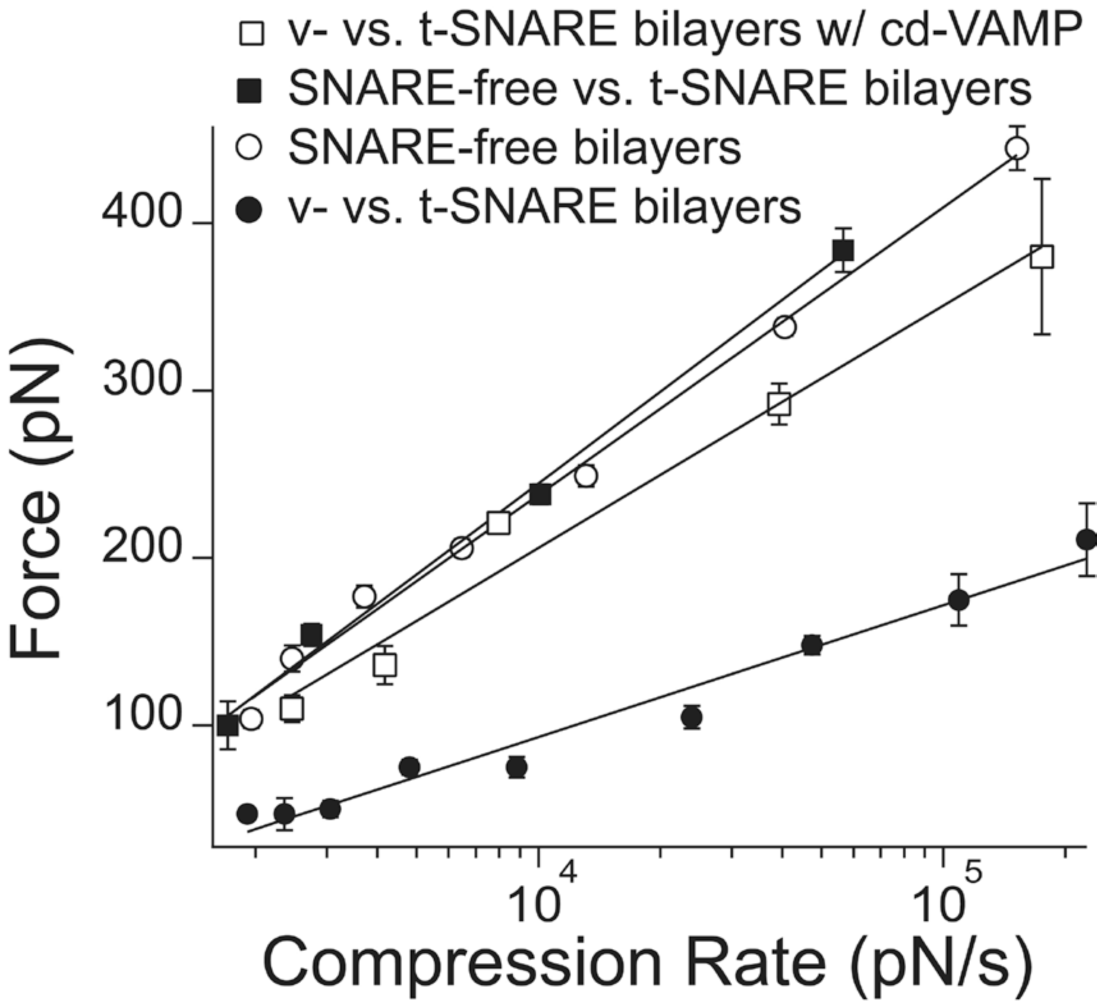
Fusion energy landscape

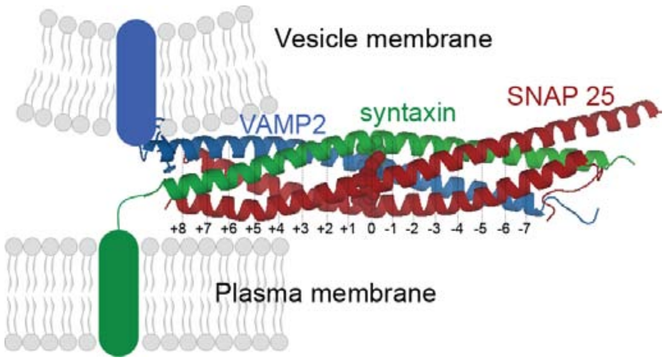


Aim 2:

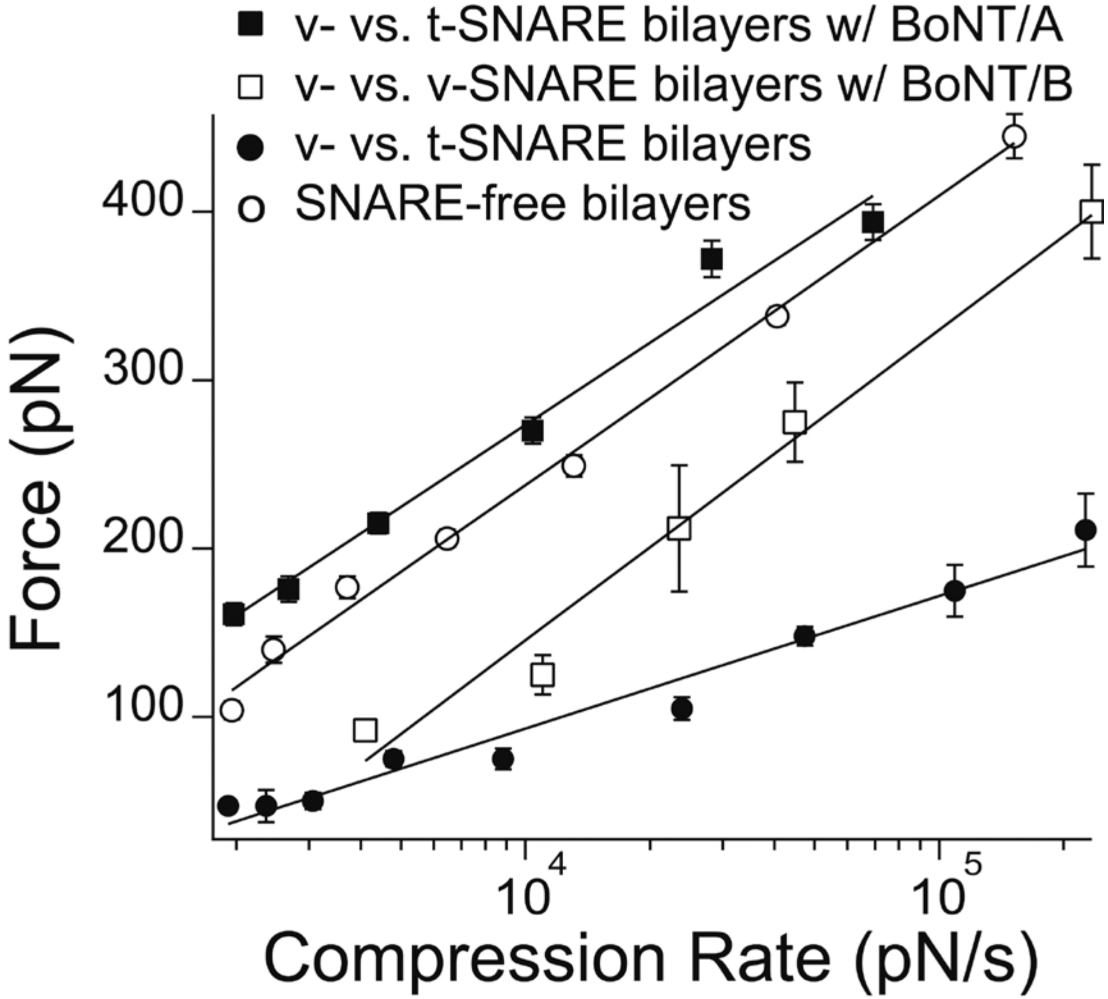
Identify the molecular determinants of SNARE-mediated membrane fusion.

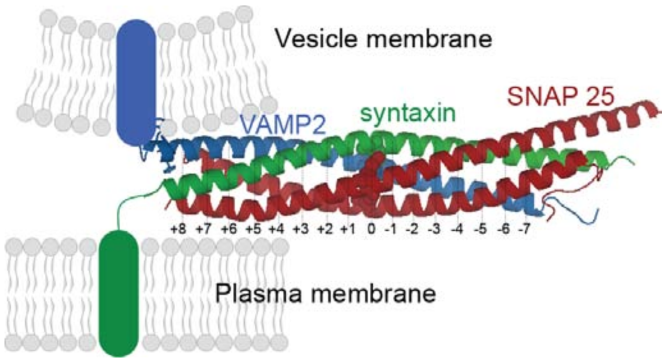
Interaction between SNARE cytoplasmic domains facilitates fusion



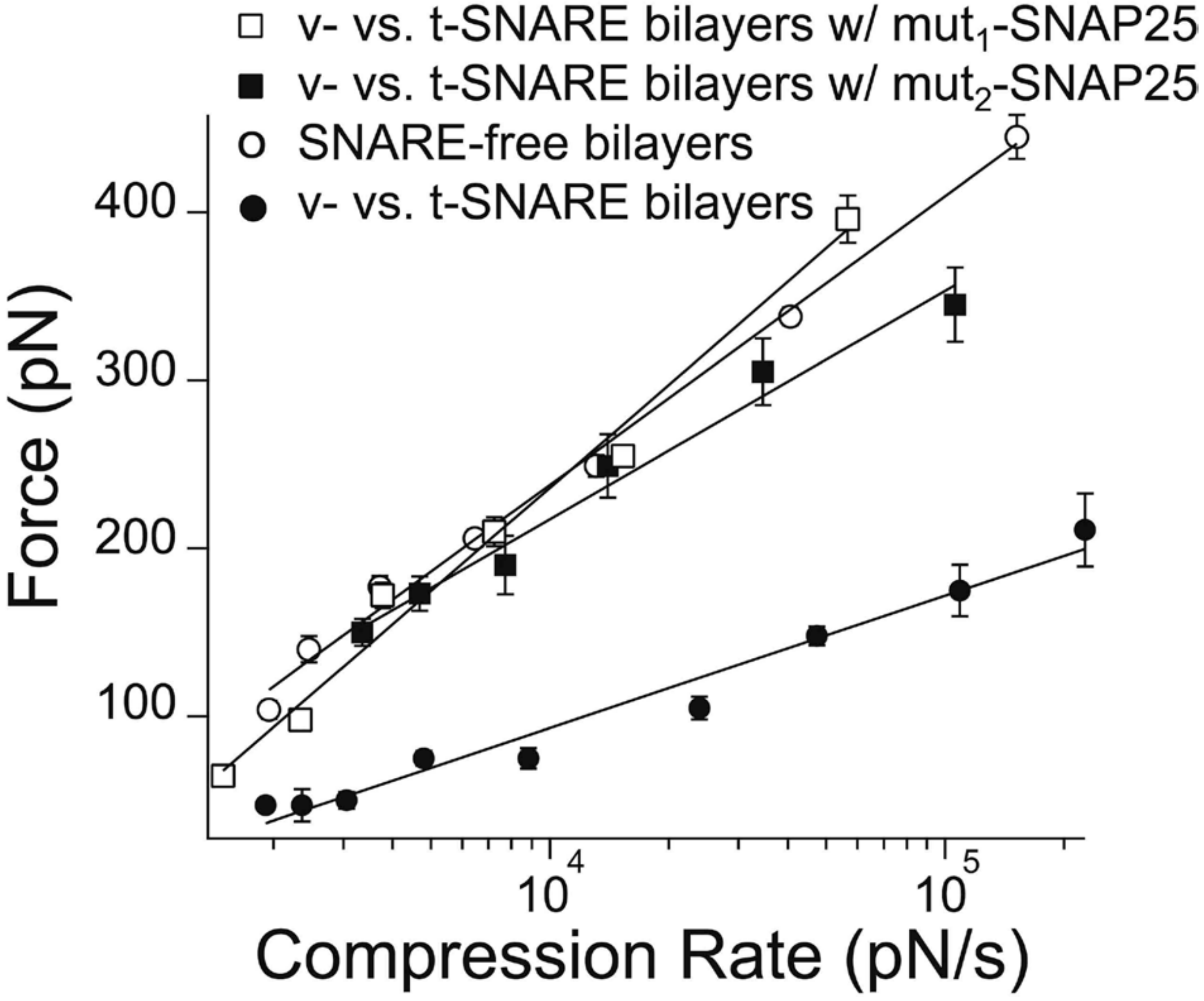


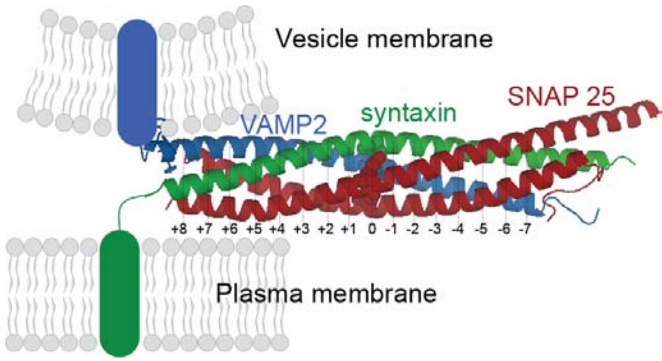
Full-length VAMP-2 is required for facilitated fusion





Full-length SNAP-25 is required to facilitate fusion





Fusion Energy barrier Parameters

Table 4: Energy barrier parameters for fusion of egg PC bilayers containing SNAREs under different combinations and conditions.

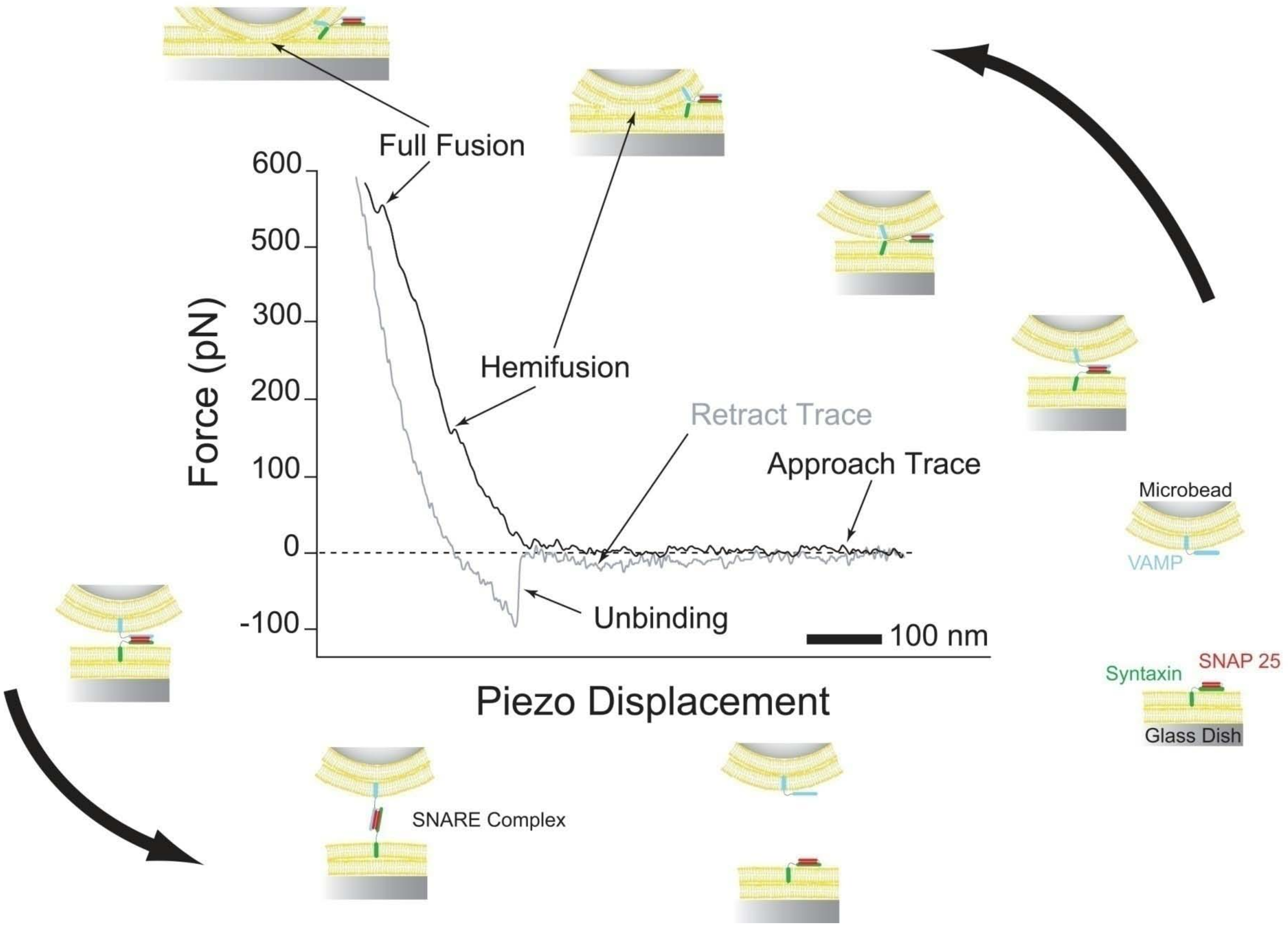
Bilayers	γ (Å)	k^o (s^{-1})
SNARE-free vs. SNARE-free	0.55	5.52
SNARE-free vs. t-SNAREs	0.52	5.67
v- vs. t-SNAREs	1.2	19.3
v- vs. t-SNAREs (after BoNT/B)	0.51	20.39
v- vs. t-SNAREs (after cd-VAMP)	0.65	6.0
v- vs. t-SNAREs (No SNAP 25)	0.63	9.96
v- vs. t-SNAREs (mut_1-SNAP 25)	0.46	7.86
v- vs. t-SNAREs (mut_2-SNAP 25)	0.69	4.25
v- vs. t-SNAREs (after BoNT/A)	0.58	2.91
v- vs. v-SNAREs	0.96	13.0
v- vs. v-SNAREs (after BoNT/B)	0.57	13.69
t- vs. t-SNAREs	0.96	8.86
t- vs. t-SNAREs (after BoNT/A)	0.41	7.6

k° characterizes the height of the potential and γ describes its width.

Aim 3:

Determine the pulling force generated by the interaction of v- and t-SNAREs.

Measurements of SNARE complex unbinding



Dynamic unbinding of individual SNARE complexes

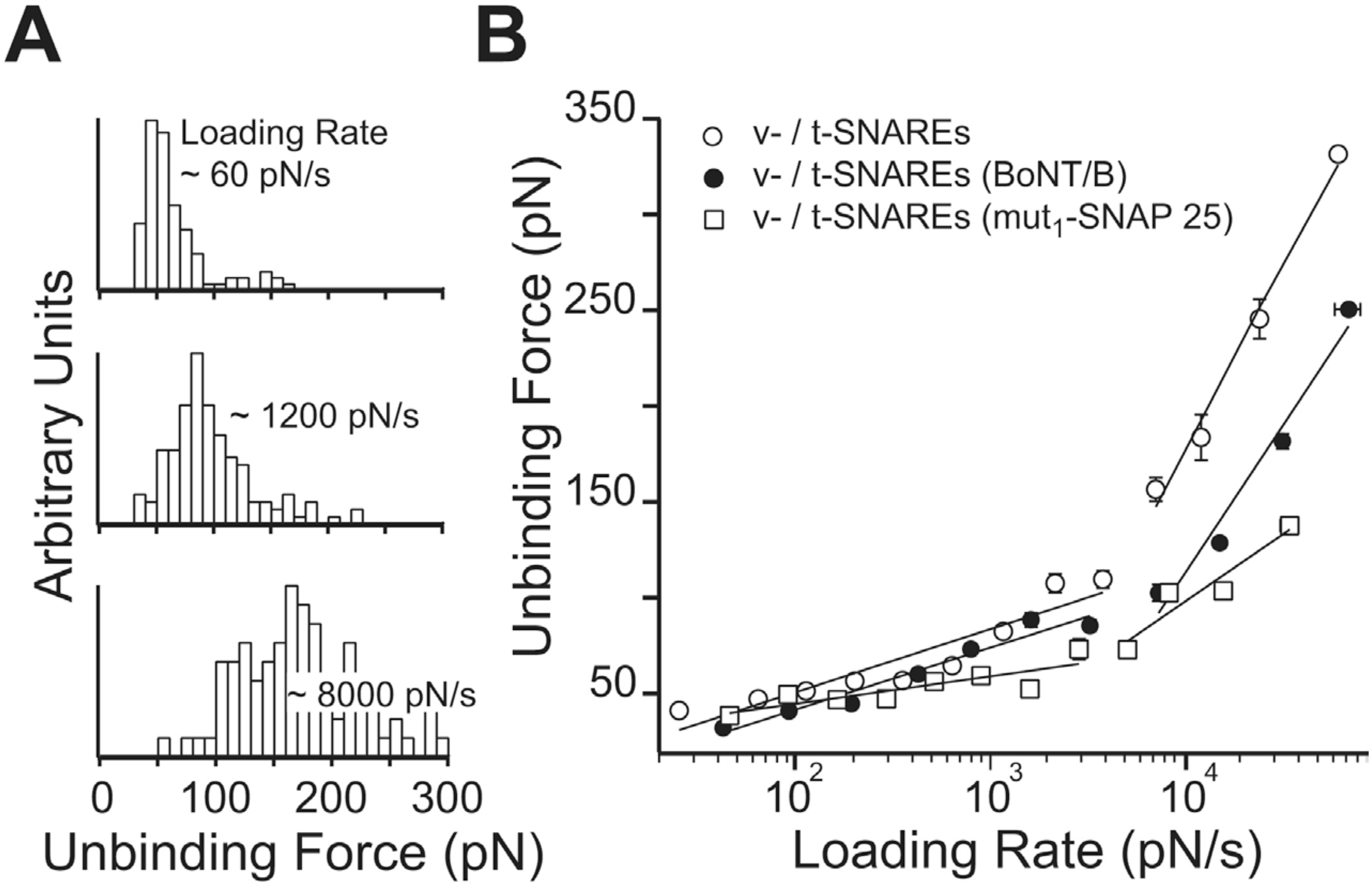
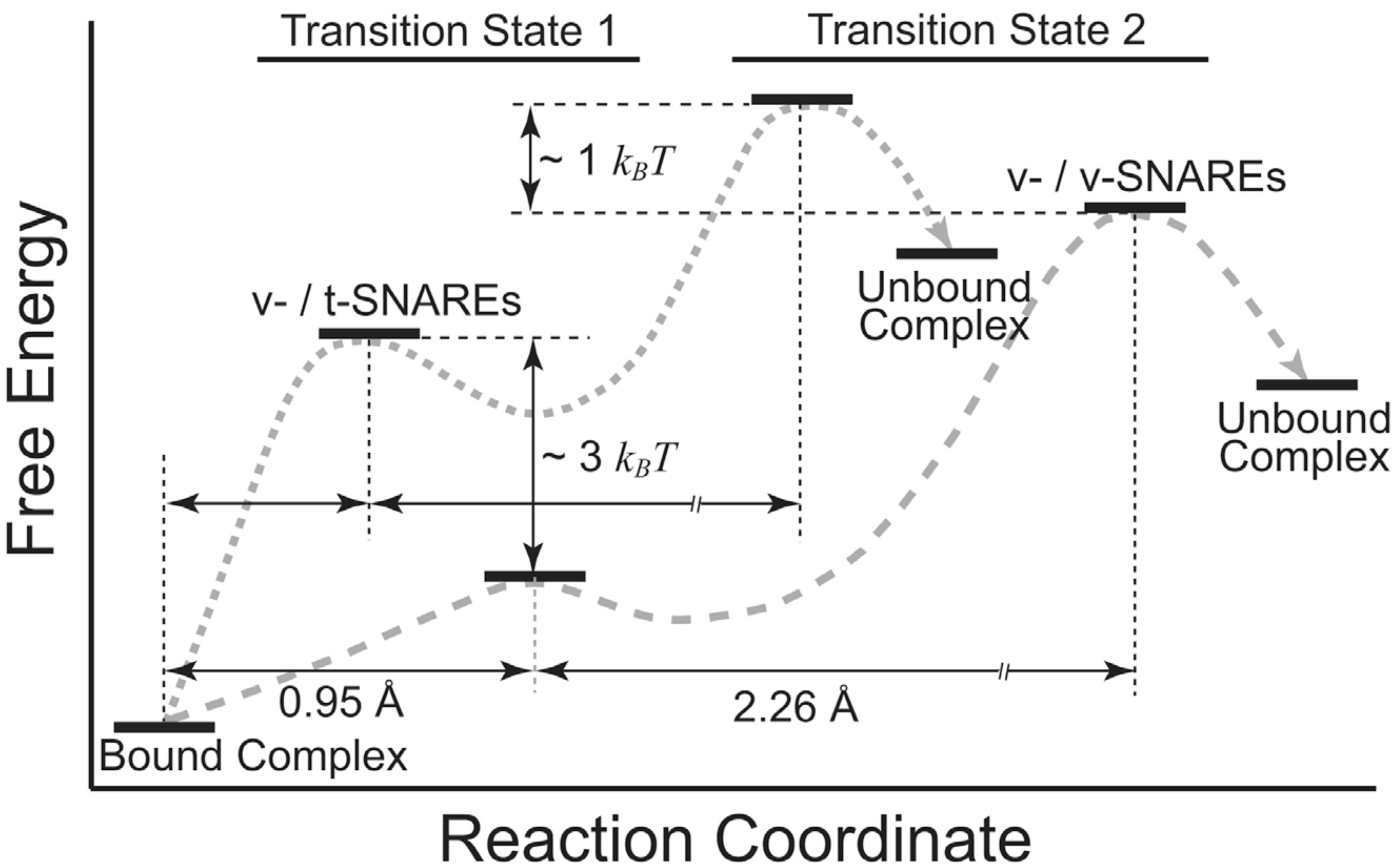


Table 1: Unbinding energy barrier parameters for the dissociation of different SNARE complexes present in egg PC bilayers.

Bilayers	# of Barriers	Barrier	γ (Å)	k° (s^{-1})
v- / t-SNAREs	2	Inner Outer	0.48 2.87	1.36 0.21
v- / t-SNAREs (No SNAP 25)	1	Single	3.5	0.03
v- / t-SNAREs (BoNT/A)	2	Inner Outer	1.26 5.1	2.49 0.09
v- / t-SNAREs (mut_1-SNAP 25)	2	Inner Outer	1.76 5.27	4.88 0.05
v- / t-SNAREs (mut_2-SNAP 25)	2	Inner Outer	0.89 4.47	17.3 0.05
v- / t-SNAREs (BoNT/B)	2	Inner Outer	0.61 2.9	27.25 0.39
v- / v-SNAREs	2	Inner Outer	0.95 3.21	11.16 0.54
t- / t-SNAREs	2	Inner Outer	0.51 3.61	17.04 0.09

k° characterizes the height of the potential and γ describes its width.

Dissociation (Unbinding) Energy Landscape



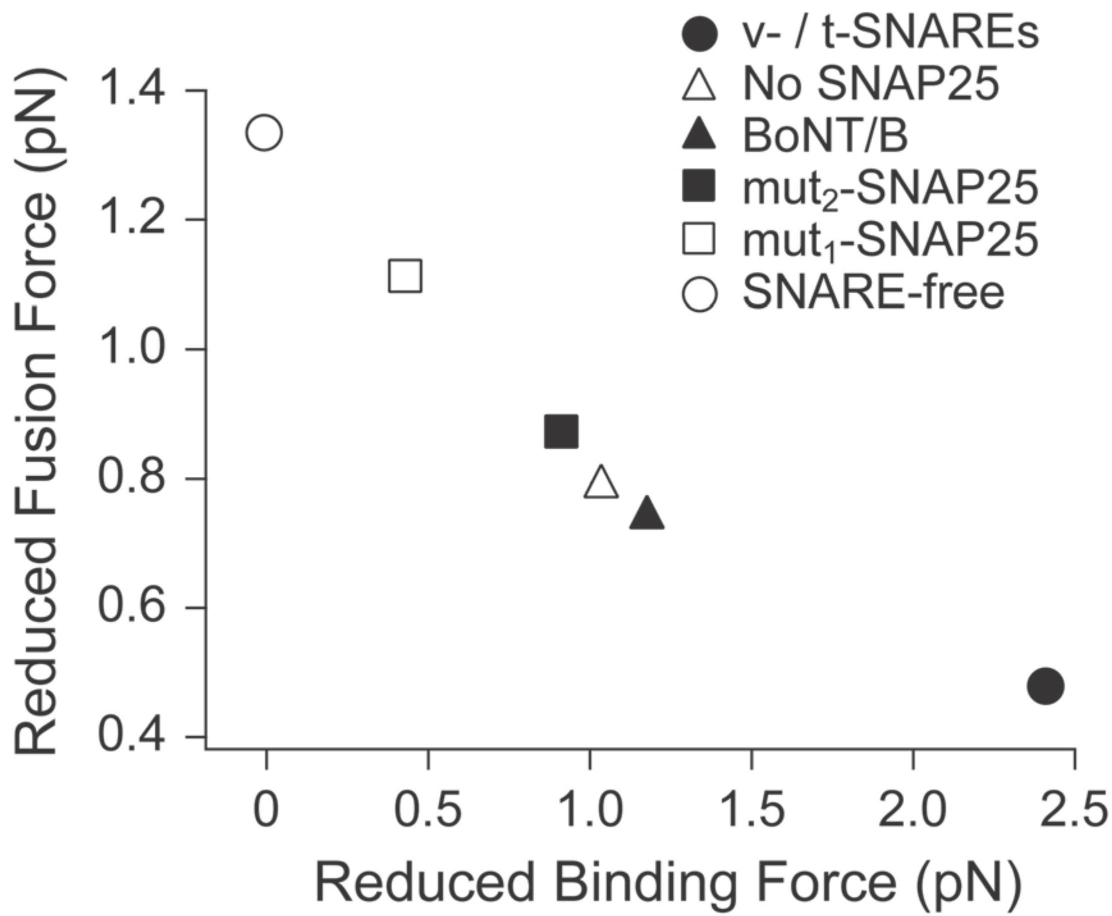
Aim 4:

Determine the pulling force generated by the interaction of v- and t-SNAREs correlates with facilitation of fusion.

To quantify the facilitation of bilayer fusion by SNAREs, we use the reduced fusion force (f_{ϕ}). This parameter corresponds to the compression force required to overcome 1 k_BT of the fusion energy barrier.

To quantify the strength of the interacting SNAREs, we use the reduced unbinding force (f_{β}) for the inner barrier of the SNARE complex. This parameter corresponds to the force required to suppress the inner activation barrier by 1 k_BT during forced unbinding.

Facilitation of membrane fusion is coupled to the pulling strength of interacting SNAREs.



Conclusions

- A correlation exists between the strength of SNARE-interactions and the degree of fusion facilitation.
- SNARE-mediated fusion transitions through an intermediate hemifused state.
- Interactions of SNARE cytoplasmic domains mediate membrane fusion by

1. Providing apposition, a “critical” proximity between the membranes.
2. Locally destabilizing the membranes by pulling on or tilting of their transmembrane segments.

- Midhat Abdulreda

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Thank You