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How SNARE molecules mediate membrane fusion: Recent insights from molecular simulations

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SNARE molecules are the core constituents of the protein machinery that facilitate fusion of synaptic vesicles with the presynaptic plasma membrane, resulting in the release of neurotransmitter. On a molecular level, SNARE complexes seem to play a quite versatile and involved role during all stages of fusion. In addition to merely triggering fusion by forcing the opposing membranes into close proximity, SNARE complexes are now seen to also overcome subsequent fusion barriers and to actively guide the fusion reaction up to the expansion of the fusion pore. Here, we review recent advances in the understanding of SNARE-mediated membrane fusion by molecular simulations.

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Membrane fusion is a fundamental process in cell biophysics, being involved in viral infection, endocytosis and exocytosis, and fertilization. Over the past 30 years it has become widely accepted that fusion proceeds through a hemifusion state where an initial hour-glass-shaped lipid structure, the so-called fusion stalk, is formed between the adjacent membrane leaflets [1•,5,6•]. This structure eventually evolves into a fusion pore [2•–6•]. SNARE molecules are the core constituents of the protein machinery that facilitates fusion of synaptic vesicles with the presynaptic plasma membrane, resulting in the release of neurotransmitter [7]. It remains unclear how SNARE complexes alter the energy landscape of fusion and steer the transition from the lipidic stalk to fusion pore on a molecular level [4]. Here, we review recent advances in the understanding of SNARE-mediated membrane fusion by molecular simulations. Because of computational cost required to cover the length and time scale of SNARE-mediated membrane fusion, simulations were typically applied to explore the membrane partitioning and dynamics of individual SNARE fractions [8,9,2•,3•], the stability of the coiled-coil complex [10•], or using a simplified representation of the SNARE complex to fuse two small vesicles [11].

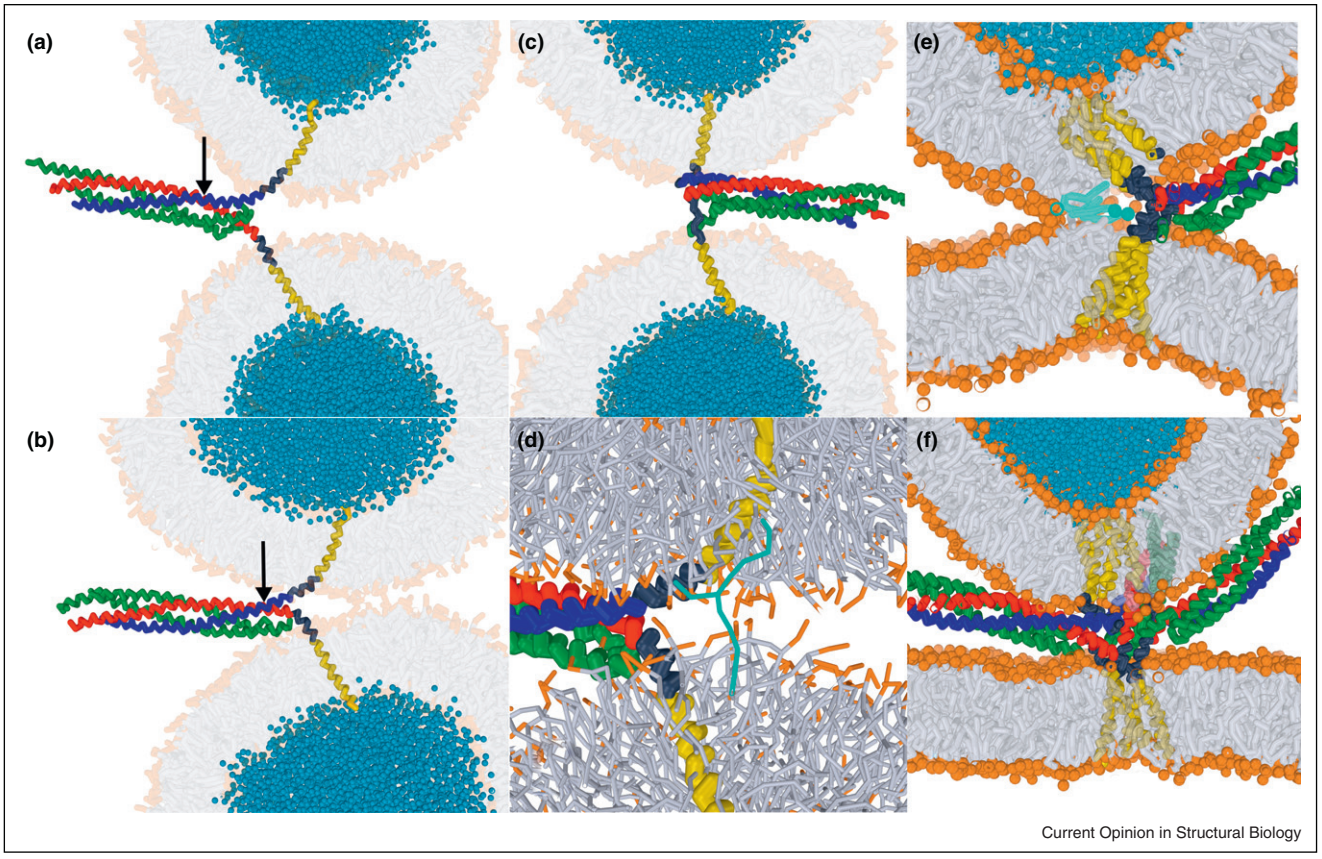
Recently, SNARE-mediated fusion events between two vesicles have also been simulated at near-atomic resolution [12]. Here, a coarse-grained model [13,14], where several atoms are represented by a single interaction site, was used to both capture the atomistic characteristics of the neuronal SNARE complex solved by X-ray [15•], such as secondary structure, and to simultaneously overcome the computational expense involved with SNARE-mediated membrane fusion. Combining the available experimental and simulation results, we will attempt to draw a consensus picture of how SNARE molecules might overcome the various lipidic fusion barriers and, especially, to identify these barriers.

Mechanical coupling between the SNARE complex and the bilayer

Atomistic simulations have revealed that the partly assembled coiled-coil complex forms a considerably stiff platform that allows force transduction between the SNARE complex and the membrane via the trans membrane domains (TMDs) of the SNARE molecules [10•]. During SNARE zipping a considerable fraction of the released energy is expected to dissipate, and the remaining fraction is stored as molecular bending stress in the individual SNARE molecules. This mechanical stress plays an important role in the self-organized arrangement at the fusion site and is minimized when the SNARE complex is located at the periphery of the contact region and thereby allows a closer proximity between the membranes (Figure 1a–c) [12]. A central positioning of the SNARE complex, where its excluded volume would rather hinder such close proximity (Figure 1c), requires stronger bending of the SNARE molecules and is therefore most likely unfavorable.

To be able to efficiently exert force on the membrane, the semi-flexible linkers of both the SNARE molecules *both* need to be sufficiently stiff. Despite a non-conserved α-helical structure upon bending, atomistic studies suggested a stiffness for the syntaxin linker of 1.7–50 cal mol^{-1} deg^{-2} (11 ± 0.2 cal mol^{-1} deg^{-2} for the coarse grained SNARE model [12]) [8]. Whereas syntaxin is readily α-helical before SNARE complex formation, synaptobrevin is unstructured [16]. Figure 1f illustrates a

Figure 1



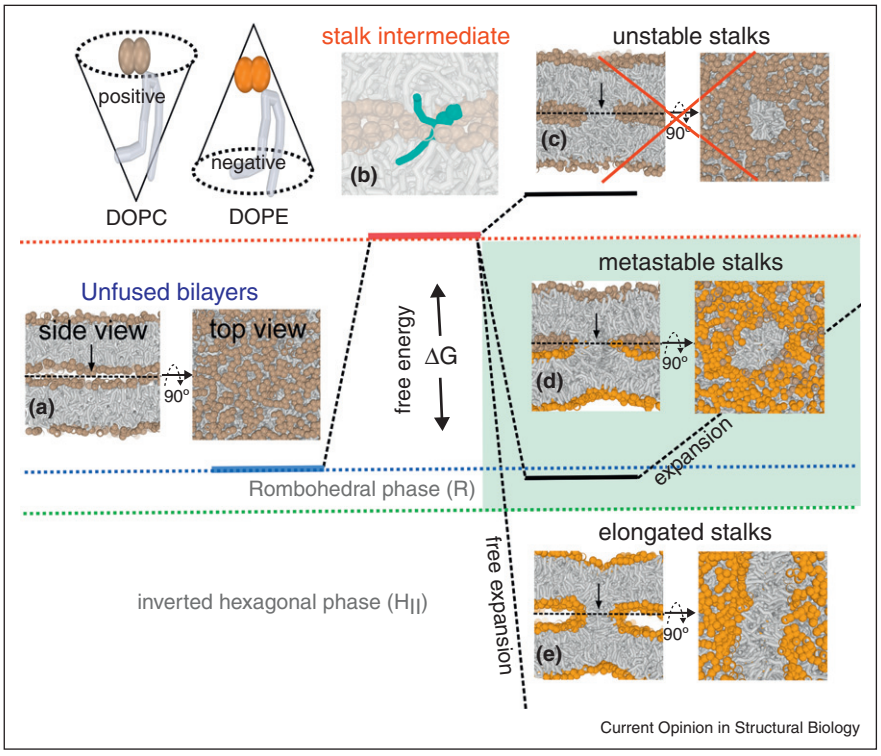
The SNARE complex before fusion. **(a)** and **(b)** SNARE zipping (arrows) brings two vesicles in close proximity. **(c)** Flexible linkers (colored black) allow a central positioning of the SNARE complex at the fusion site and thereby hinder a close proximity. **(d)** Splayed lipid intermediate formed at the onset of stalk formation (colored cyan) **(e)** SNARE-induced curvature in the target membrane. Note the hydrophobic nature of the adjacent fusion sites (colored cyan). **(f)** Example where the force transmission to the target membrane is impaired by the presence of unstructured (i.e. flexible) synaptobrevin (colored blue) linkers.

scenario where the synaptobrevin linker remains unstructured during SNARE zipping. In such a case, the transmission of force toward the membrane would be impaired because the bending stress that is otherwise stored in syntaxin now alternatively relaxes by additional 'kinking' of the more flexible synaptobrevin. Such 'kinking' of synaptobrevin would impose an additional barrier against subsequent α-helical nucleation [15•,17] and the progression of SNARE zipping [15•]. Thus, it seems essential that α-helical nucleation in synaptobrevin precedes SNARE zipping.

Stalk formation

When the membranes are brought into sufficiently close proximity, a stalk can be formed [1•]. Here, continuum descriptions tend to imply a transient stalk of infinitesimal radius before it expands into the well characterized hourglass-shaped stalk structure — in contrast to the molecular nature of the lipid membrane. In fact, there is a growing body of evidence from recent molecular simulations that stalk formation is neither the initial nor the rate limiting step in membrane fusion [3•]. These simulations show stalk intermediates, such as a single or several splayed lipids connecting the adjacent monolayers (Figure 1d), which lead to rapid stalk formation within several nanoseconds [12,18•–26•]. In a way, one might refer to such a splayed lipid (or a similar perturbation) as essentially the smallest 'stalk' that is possible in molecular terms. Importantly, once such splayed lipid state is reached, formation of the actual stalk is energetically downhill (i.e. spontaneous). Crucially, and in contrast to previous views, in this scenario the stalk structure does not represent the rate determining barrier, but rather a (local) free energy minimum. This view is strongly supported by the observation of stalk-like structures in recent X-ray studies [27•,28•]. One important consequence is that it is the energy barrier associated with the splayed lipid state that determines the kinetics of stalk formation, and which therefore needs to be overcome by the free energy released by SNARE complex formation (cf. Figure 2). Further, the free energy of the metastable stalk can either be larger [20•,29••] or, in the rhombohedral phase or stalk phase [27•,28•,30••], even slightly lower

Figure 2



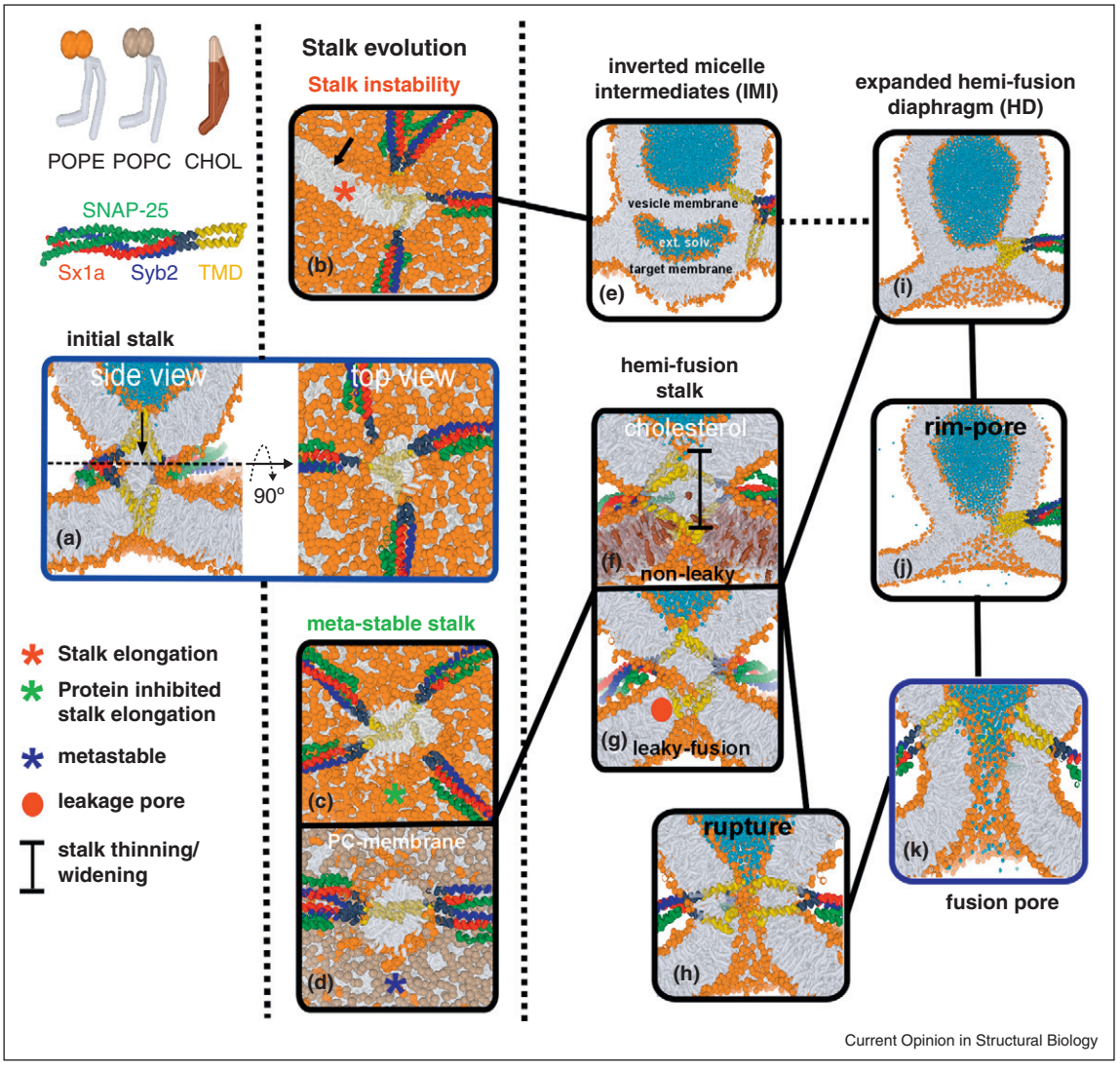
Energetics of stalk formation. (**a**) Unfused bilayers. **(b)** At a constant inter-membrane distance, the free energy of the splayed lipid (Δ*G*) is not expected to depend on the head-group type (spontaneous curvature). **(b)**, **(c)** and **(e)** The free energy of the stalk itself, however, depends on both the spontaneous curvature (plotted cone) and separation distance and these will determine whether a formed stalk is metastable or not [31•]. **(c)** At such an inter-membrane distance, the stalk formed between two DOPC membranes is an unstable transient intermediate. **(d)** The stalk is metastable when its free energy is lower than *both* the dissociation barrier and expansion barrier. **(e)** In the H_{II}-phase the formed hourglass-shaped (rhombohedral) stalk structure is unstable (no expansion barrier) and the stalk will continuously elongate to maximize its energetically favorable negatively curved perimeter. The barrier of stalk formation is determined by the free energy of the stalk intermediate (Δ*G*). However, this barrier *excludes* the additional energy that is required to bring the two membranes within such proximity (inter-membrane repulsion). This inter-membrane repulsion includes the known relation between spontaneous curvature and fusogenicity. Hence, an intrinsic negative curvature reflects the inability of the lipid head-group to sufficiently shield its hydrophobic moiety when arranged in a planar conformation. As a result the membrane surface becomes more hydrophobic which lowers the energetic cost of leaflet approach.

than that of the unfused bilayers. A stalk is metastable because it simultaneously faces a barrier to dissociate [20•,29••] and to expand [30••].

The free energy of the stalk intermediate (i.e. stalk barrier) is most directly determined by both the distance between the fusing leaflets and the length/solubility of the lipid tails [31•,32•,3•,23•,20•], and only indirectly by lipid shape or, equivalently, spontaneous curvature [31•,32•,3•,23•,20•]. Thus, one way to promote the formation of a sufficiently low energy stalk intermediate is to bring the adjacent leaflets within a critical distance. Indeed, we consider it not a coincidence that essentially all fusogenic conditions, such as a negative spontaneous curvature, tension/osmotic pressure, and positive membrane curvature (curvature stress), lower the energy of leaflet approach [33•–35•]. All these factors increase the hydrophobicity of the fusion site, and thereby lower inter-membrane repulsion by also reducing the energetic cost of solvent removal between the approaching leaflets. The positive membrane curvature in vesicles and 'dimples' decrease this repulsion even further by also reducing the exposed area of the fusion site.

In SNARE-mediated simulations, stalk formation requires a minimum distance between the head groups of the opposing bilayers of only about 1 nm. Similar distances were found by recent X-ray studies of stalk-phase formation [36] as well as by other simulation studies [23•]. Let us assume that most of the energy available for subsequent fusion is stored in the stiff linker regions of the SNARE complex. Because the angle between the transmembrane domains (TMDs) is about 120° [12], based on atomistic simulation-derived values of the linker stiffness [8], mechanical energy of up to $\approx 10 k_B T$ is stored in each SNARE complex during stalk formation. Only a small fraction of this energy, however, is released during stalk formation, because the angle between the TMD

Figure 3



Different pathways observed in the SNARE-mediated fusion between a 20 nm-sized vesicle and lipid bilayer of varying composition. **(a)** Initial stalk in the presence of four [74] SNARE complexes. **(b)** Stalk elongation (H_{II}-phase regime). **(c)** Protein inhibited stalk elongation (H_{II}-phase regime). **(d)** Metastable stalk (POPC target membrane). The formed stalk faces a substantial barrier against expansion [30••,40••] and progression of fusion does not occur in the course of the 6 μs simulations. **(e)** Inverted micelle intermediate (IMI). Progression of fusion does not occur in the course of the 6 μs simulations. **(f)** Non-leaky stalk expansion/widening in the presence of 40% cholesterol [75]. Sterols seem to specifically enhance non-leaky fusion [51•]. **(g)** Leaky stalk expansion in a pure POPE system. **(h)** Spontaneous stalk-pore transition. **(i)** Expanded hemifusion diaphragm (HD). **(j)** Pore formed at the rim of the HD. **(k)** Toroidal fusion pore.

remains nearly constant during that process [12]. Moreover, stalk formation was also observed in our simulations when force transmission to the membranes was blocked by artificially 'freezing' the bent conformation of the SNARE molecules. These results suggest that the energy released by SNARE zipping as well as the linker mainly serves to bring the opposing leaflets within critical distance — initially by an overall translation of the opposing bilayers (Figure 1a,b), and later likely by local membrane surface perturbation, for example, by forming a dimple (Figure 1e). Indeed, experiments have indicated that the TMDs play an important role in stalk formation. For example, SNARE molecules where the TMDs are replaced by lipids do not facilitate fusion [37], whereas isolated TMDs are able to induce fusion [38•]. Also recent simulations suggest an intrinsic propensity of TMDs to both perturb the lipid packing (enhancing lipid splaying) and thereby increase the hydrophobicity (reducing repulsions) of the fusion site [12]. According to this new consensus picture that emerges, stalk formation requires (a) proximity and (b) is driven by reducing the main free energy barrier at the splayed lipid state.

This view also explains why, apparently, a surprisingly broad variety of local lipid perturbations can serve that purpose, in particular those induced by TMDs.

Fusion pathways: the good, the bad, the ugly

After stalk formation, simulations have suggested mainly three pathways through which SNARE-mediated fusion can proceed [2•,3•]. As an example, Figure 3 shows these different pathways in the SNARE-mediated fusion between a lipid bilayer and 20 nm-sized vesicle, representing a synaptic vesicle (30–50 nm sized [39•]):

(i) Stalk elongation (Figure 3b,e). Stalk elongation relates to the inverted hexagonal phase transition (H_{II}-phase) [30••,40••–42•], where a stacked bilayer system spontaneously rearranges into inverted hexagonally packed cylinders. In the presence of sufficiently (small) rigid vesicles, however, the topology is limited to that of an inverted micelle (IMI) [12,40••,2•,3•] (Figure 3e).

(ii) Stalk widening (Figure 3c,f). A radial expansion (widening) can only occur when the stalk is metastable and, by definition, faces a barrier against expansion [30••,40••]. During stalk widening the *cis*-leaflets will eventually meet (black bar, 3f) to either form a metastable single bilayer, that is, the hemifusion diaphragm (Figure 3i), or to subsequently rupture (Figure 3h). Importantly, such widening faces a considerable free energy barrier of 15–63k_BT [30••]. This barrier can be reduced by increasing the negative spontaneous curvature of the *cis*-leaflets [43]; however, such increase is limited by the occurrence of the H_{II}-phase transition because at this point the stalk will spontaneously elongate (IMI-pathway) rather than expand radially [30••,40••,43]. In the latter case, stalk elongation might be impaired by a circumference of multiple SNARE complexes that 'lock' the stalk (Figure 3c vs b).

(iii) Leaky expansion (Figure 3g). On this pathway, the formed stalk is metastable and further expansion faces a substantial barrier [40••], that is, the barrier of competitive stalk widening is too large [44•,40••,2•]. Although *in vivo* synaptic fusion itself is believed to be a non-leaky process, *in vitro* SNARE-mediated fusion has often been observed to be transiently leaky [45–48]. Like stalk widening, also the leaky expansion can either stabilize a hemifusion diaphragm or instantaneously progress into a fusion pore [3•,2•]. There is accumulating evidence that the presence of a stalk dramatically increases the probability to nucleate a pore in its direct vicinity (stalk-pore complex), and vice versa [2•,40••,44•,49], that is, the favorable partitioning of a stalk at the rim of a pore renders the free energy of the resulting stalk-pore complex lower than that of isolated stalks or pores [44•,40••,2•]. The probability to form such stalk-pore complex is further increased by the presence of tension and intrinsically negatively curved lipids, such as PE-lipids [40••,47•] — quite in contrast to a 'usual' membrane pore that would rather be favored by the presence of positively curved lipids [6•].

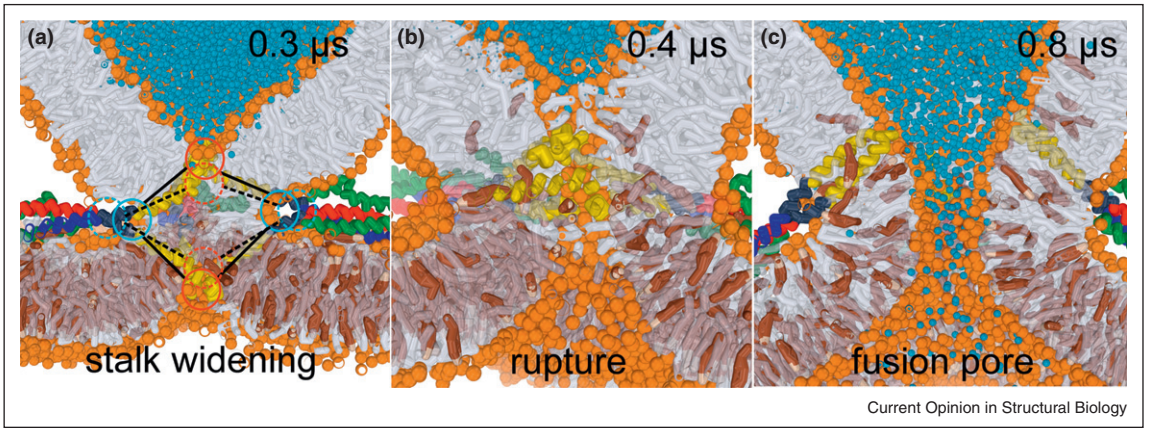
If the latter scenario were true, one would expect that transient leakage, that is, pore nucleation, can be reduced or even be inhibited by at least two modifications. (i) The opposing fusion-sites are made attractive, rather than repulsive (e.g., the presence of counter ions or hydrophobic polymers) [26•,25,50•]. In that case, pore nucleation would reduce the favorable membrane–membrane contact area at the fusion site. Molecular simulations have suggested that these attractions facilitate an alternative adhesion-condensation mechanism where a single membrane-thick hemifusion-diaphragm is instantaneously formed by a non-leaky lateral reorganization of the lipids [26•,25,50•]. (ii) The presence of hydrophobic membrane ordering/strengthening molecules such as sterols [51•] or carboxylic acids [18•] might specifically oppose pore nucleation. Notably, sterols are ubiquitous in the presynaptic plasma membrane [39•].

Formation of the fusion pore

Fusion pore formation seems related to both the presence of mechanical stress in the SNARE complex as well as the TMR ends. On one hand, the binding affinity between SNARE molecules and the nature of the linkers have been linked to fusion pore formation [52•,53]; on the other hand, deletions of TMR end residues and addition of polar amino acids to the Syb2 C-terminus have been shown to arrest fusion pore formation [54,55•]. Further, in agreement with current models [4,56•,57], conductance measurements suggested that the negatively charged C-termini of the TMDs reside in or near the appearing fusion pores [58•]. During hemifusion both synaptobrevin and syntaxin can only release their mechanical stress through widening of the stalk or formation of a fusion pore, due to the large barrier that prevents penetration of the charged C-termini in the TMRs through the membranes (Figure 4). This observation suggests that (i) the main action of SNARE complexes is to actively promote fusion pore opening, (ii) this process is driven by the stored mechanical stress in the SNARE complex that resulted from SNARE zipping, and (iii) the C-termini play an essential role in the underlying mechanism [12].

As a result of the mechanical stress stored within in the SNARE complex, the C-termini exert a 'squeezing' force on the *trans*-leaflets [12] of the hemifused membranes. Because stalk widening goes hand-in-hand with simultaneous stalk thinning (Figure 4), this force enhances such process. Self-consistent field calculations have suggested that stalk widening can require even more free energy than stalk formation [30••], which is also supported by our simulations (Figure 3d) as well as those of others

Figure 4



Detailed look at the non-leaky spontaneous stalk to pore transition. **(a)** Schematic illustration of a SNARE-mediated stalk expansion. The circles depict hydrophilic regions that interact with the stalk. Blue circles depict the N-terminal and red circles the C-terminal regions of the trans membrane domains. The zipping action of the SNARE complexes allows widening (blue circles) and simultaneously enforces thinning (red circles) of the stalk. In the presence of near C-terminal trans membrane attractions between opposing SNARE complexes (red circles), the motion between the blue and red circles become coupled (black frame-work). In such a case, expansion (blue circles) of a formed hemifusion diaphragm would directly translate into an additional squeezing force (red circles) that enhances premature rupturing of such diaphragm before it can expand to a less stressed and more stable size. **(b)** Rupture occurs when the stalk sufficiently widens/thins. Here, the C-termini slip through the initial pore. **(c)** Fusion pore.

[3•,2•,18•]. This finding might provide an explanation why much of the conserved mechanical stress in the SNARE complex is released during such process (Figure 4a).

This idea also raises the question, how much force the SNARES actually exert to drive stalk expansion? Because the distance between the separated C-termini is about 8 nm (i.e. the length of the stalk), and assuming that most of the stress arises from bending of the SNARE linkers (120°) with a maximal linker stiffness of 50 cal mol^{-1} deg^{-2} [8], one obtains an average force of 5 pN per SNARE complex. In this estimate we have allowed for an additional energy of ca. $<10k_BT$ that is released when the TMDs eventually assemble [15•,59]. With the α-helical TMDs being sufficiently stiff, however, this free energy is likely to be only available when the barrier of stalk widening is readily surpassed [12].

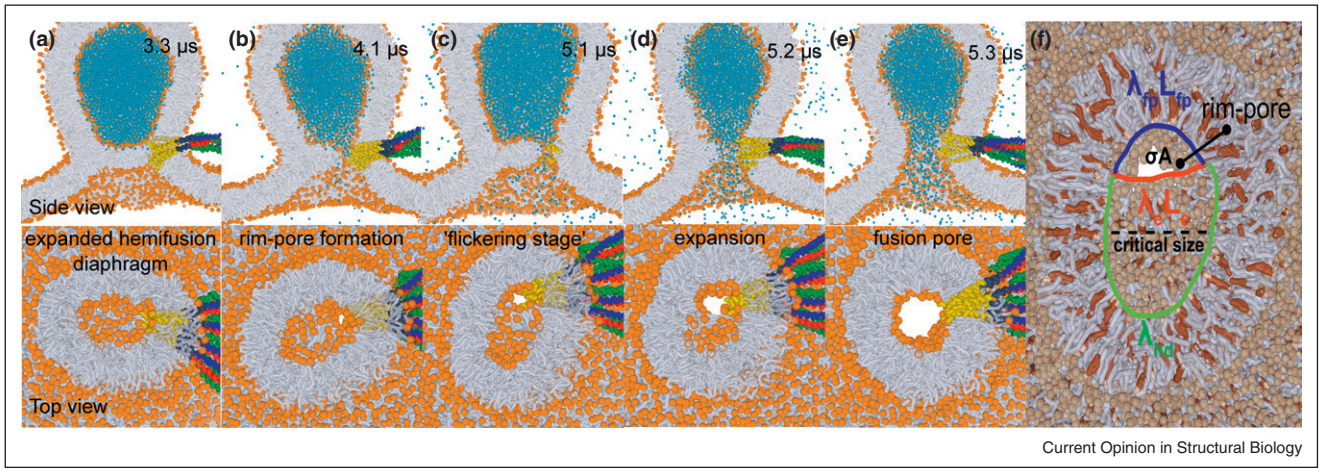
Further, the squeezing action that the TMD C-termini of partly assembled SNARE complexes exert on the trans-leaflets locally thins the leaflet (creates a well) and results in a hydrophobic mismatch. The latter enforces, aside from intrinsic TMD attractions [38•,60,15•], additional near C-termini attractions between the TMDs of multiple SNARE complexes [38•,60,15•] (Figure 3a,f). We will later rationalize why these C-terminal attractions might be important.

After the barrier against stalk expansion is overcome, fusion pore formation proceeds — either spontaneously (Figure 4) [61–63•,24•,11], or by an activated non-instantaneous transition via the formation of a metastable hemifusion diaphragm (HD, Figure 5) [3•,18•,26•,25,50•,62,63•]. The co-existence of these different pathways would result in a heterogeneous fusion kinetics [47•]. Metastable HDs have been observed in model membrane fusion [64•,65•,35•]. Further, the proximity of the initial pore to the rim of the HD [26•,25,50•,65•] suggests that, despite the large size of an HD, only a limited number of the potentially available SNARE complexes can actively participate in nucleating such rim-pore (Figure 5). In contrast, in the spontaneous mechanism, all available SNARE complexes can simultaneously release their mechanical energy, and thus cooperatively contribute to (toroidal) fusion pore opening [12] (Figure 4).

Once a HD has been formed, and despite being thermodynamically less stable than the toroidal fusion pore [25,30••,50•], the HD is kinetically stabilized by a kinetic barrier against the formation of a rim pore. Similar to the leakiness discussed above, such barrier is likely increased by sterols [66•–68•] and reduced by, for example, poly-unsaturated lipids [69] or lyso-lipids [70]. During expansion of the HD, its initial tension relaxes and, therefore, the probability of rim-pore nucleation and subsequent expansion decreases (Figure 5f).

If the expanded HD is in fact a metastable fusion intermediate with a slow escape rate [64•,66•,35•,26•,25,50•], this state needs to be circumvented by the fast synaptic fusion machinery. Indeed, simulations suggest an efficient strategy to both catch and rupture such 'slippery eel': If the stalk is circumvented by multiple opposing SNARE complexes, the presence of the above mentioned near C-termini attractions may not only limit further expansion of the HD, but also redirect these expanding forces into a

Figure 5



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(a)– **(e)** SNARE-mediated pore formation near the rim of an 8-nm sized expanded/equilibrated POPE hemifusion diaphragm (HD). The rim pore remains stable over a 1 μs (flickering stage) before it eventually expands into the (toroidal-shaped) fusion pore. Rim-pore expansion requires the absorption of excess material from the remaining HD. In turn, pore closure is opposed by the presence of the negatively charged TMD C-termini within the pore lumen [58•]. **(f)** Structure and energetics of a rim-pore (POPC:Cholesterol mixture). A rim-pore (top-view on the HD) is composed of an energetically favorable toroidal-shaped fusion pore edge (blue colored line) and a costly membrane edge (red colored line) and adopts half a circular shape to minimize its free energy. The free energy of a rim pore is considerably lower than that of a 'usual' circular-shaped membrane pore, which is only composed of the costly membrane edge. The free energy of such rim-pore is given by, $F(L_{fp}, L_e, A) = (\lambda_{fp} - \lambda_{hd})L_{fp} + L_e\lambda_e - \sigma A$, where L are the lengths and λ the line tensions (forces) of respectively the fusion pore (*fp*), hemifusion diaphragm (*hd*) and membrane edge (e) fractions, A is the area of the rim-pore, and σ the tension present. Below the critical size (black line), expansion is opposed by a costly increase in L_e ($\lambda_e > 0$), and is favored by an increase in L_{fp} (if $\lambda_{fp} - \lambda_{hd} < 0$, that is, the HD is thermodynamically unfavorable) and A (if $\sigma > 0$, that is, the remaining HD is under tension). Bystander SNARE complexes that exert force/stress on the remaining HD rim (green colored line), thereby increasing λ_{hd}, contribute to the expansion of the rim-pore.

'squeezing' force that enforces simultaneous thinning and rupture of the HD (Figure 4). Thus, the TMD attractions between multiple SNARE complexes seem to play an important role in preventing the expansion and stabilization of an HD, which would otherwise retard fusion pore formation [38•,60]. This idea can also explain why fast *in vivo* synaptic fusion requires at least three SNARE complexes, whereas fusion only requires one [71•,72•]. Further, to be able to hinder HD expansion, the TMD N-termini need to arrange at opposite sides of the stalk/HD such that their TMDs point away from each other (Figure 4). The presence of N-terminal attractions [73•] would oppose such positioning and rather enhance a parallel positioning where the TMDs are aligned next to each other (Figure 5), such that the HD could escape via expansion. As a possible test, therefore, the presence of N-terminal attractions should retard fusion pore formation by trapping the fusion reaction at the stage of an expanded HD.

Conclusions

In the last decennia molecular simulations have revisited the original stalk-pore hypothesis from different angles. Initially, and mainly motivated by continuum elastic models, it was the stalk that was believed to be the relevant fusion barrier. Accordingly, the main focus was on predicting the free energy of the stalk structure. The discovery of the rhombohedral phase (stalk phase) by X-ray experiments in 2003 proved that the stalk can be a stable structure, that is, a free energy minimum [27•]. In the same year, molecular simulations suggested that stalk formation is preceded by the formation of splayed lipid intermediates [19•]. Today, therefore, the focus has shifted to further identify, quantify, and structurally characterize the intermediates and molecular free energy barriers involved in stalk formation [3•,20•,22•,21•,23•,31•,32•]. In addition, the importance of subsequent fusion barriers, that is, the expansion of the stalk [2•,3•,30••,40••] and the formation of a fusion pore [64•,66•,35•,26•,25,50•], has now been recognized.

This paradigm shift has also changed our view on SNARE-mediated membrane fusion. In the original scaffold models, the role of SNARE molecules was confined to bringing the membranes into close apposition by exerting mechanical force to overcome the activation energy barrier [4]. In particular, the SNARE complex was not assumed to be involved in the transition states, which were considered to be exclusively lipidic [4]. The emerging view today is that such simple and clear-cut separation between the role of the SNARE complexes and that of the pure lipid membrane misses their close coupling, which turns out to be essential for a quantitative understanding of SNARE-induced membrane fusion.

First, whereas the SNARE complexes indeed bring the two membranes into close apposition, it is the lipids that determine the mechanical energy which is required to

overcome the inter-membrane repulsion. The smaller this required energy, the closer the resulting distance between the opposing leaflets, and thus the lower the free energy of the formed stalk intermediate [31•,3•,20•,23•,36]. All fusogenic conditions, such as a negative spontaneous curvature, tension/osmotic pressure, and positive membrane curvature (curvature stress), lower the energy of leaflet approach and thereby lower the initial fusion barrier [33•–36]. The new notion that it is not the stalk, but rather the splayed lipid state that forms the main fusion barrier, requires a reconsideration of previous explanations of fusogenicity in terms of stabilization of the overall negatively curved stalk structure. In fact, this new rate limiting barrier seems to be mainly determined by the inter-membrane distance [33•,31•,3•,20•,23•,36].

Second, whereas the transition states in membrane fusion are lipidic, experiments and molecular dynamics simulations suggest that the SNARE complexes are actively involved in several ways: (i) The formation of the stalk intermediate seems facilitated by the inherent propensity of the SNARE TMDs to distort the packing of the nearby lipid tails [12,38•,37]. (ii) Expansion of the stalk [30••] seems to be driven by mechanical stress which is released by the partly assembled SNARE complexes. (iii) SNARE complexes might prevent both the formation and expansion of a metastable hemifusion diaphragm that would otherwise impede the subsequent opening of the fusion pore [64•,66•,35•,26•,25,50•]. (iv) Both the mechanical stress stored in the SNARE complex and the resulting membrane penetration of the negatively charged TMD C-termini are associated with the opening of a fusion pore [12,55•,54,52•,53,58•].

In summary, SNARE complexes seem to play a quite versatile and involved role during all stages of fusion. In addition to merely triggering fusion by forcing the opposing membranes into close proximity, SNARE complexes are now seen to also overcome subsequent fusion barriers and to actively guide the fusion reaction up to the expansion of the fusion pore. Such functional diversity of just one core complex is staggering, but may also explain why so many more molecular components are recruited in the process [38•,37,55•,54,52•,53,58•,4,56•].

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