Supplemental Material for

The Transmembrane Domain Peptide of Vesicular Stomatitis Virus Promotes Both Intermediate and Pore Formation during PEG-mediated Vesicle Fusion.

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Samples	$k_1 \times 10^3$ (sec^{-1})	$k_3 \times 10^3$ (sec^{-1})	α	β	$\lambda_0 \times 10^4$	$\lambda_2 \times 10^4$	$\lambda_3 \times 10^4$	f_{CM}	f_{LM}
17°C_con	10.0± 0.5	1.08± 0.05	0.16± 0.01	0.49± 0.01	2.67 ±0.04	2.06± 0.02	0.52±0.06	0.15± 0.01	0.38± 0.01
17°C_vsv	19.3± 0.6	2.27± 0.02	0.41± 0.01	0.58± 0.01	5.49 ± .08	3.06± 0.03	0.57±0.15	0.24± 0.01	0.43± 0.01
17°C_hex	11.1± 0.6	1.54± 0.04	0.57± 0.01	0.41± 0.01	3.25 ±0.11	2.51± 0.05	0.21 ±0.22	0.35± 0.01	0.40± 0.01
17°C_hex+ vsv	22.0± 0.6	2.65± 0.04	0.58± 0.01	0.60± 0.01	11.36±0.23	3.85± 0.07	0.42±0.17	0.33± 0.02	0.39± 0.01
27°C_con	21.5± 0.9	2.22 ± 0.02	0.25± 0.01	0.62± 0.01	3.81± 0.10	3.00± 0.05	0.77±0.23	0.26± 0.01	0.46± 0.02
27°C_VSV	40.0 ± 1.0	3.06 ± 0.04	0.49± 0.01	0.69± 0.01	11.82±0.36	5.45± 0.03	0.74± 0.15	0.28± 0.01	0.47± 0.02
27°C_hex	27.5± 1.2	2.63± 0.03	0.70± 0.03	0.72± 0.03	15.86±0.27	5.37± 0.07	5.01±0.21	0.41± 0.02	0.44± 0.03
27°C_hex+ vsv	37.2 ± 1.1	2.96 ± 0.05	0.63 ± 0.02	0.62± 0.02	29.55±0.36	5.75± 0.09	5.57±0.23	0.39± 0.03	0.42± 0.01
32°C_con	33.1± 0.7	2.65 ±0.05	0.32± 0.01	0.72± 0.01	9.42± 0.31	4.69± 0.07	0.79± 0.25	0.36± 0.01	0.45± 0.01
32°C_VSV	75.0 ± 1.0	3.28 ±0.04	0.51± 0.01	0.75± 0.01	27.37±0.32	7.10 ± 0.04	9.26 ±0.23	0.35± 0.02	0.45± 0.01
32°C_hex	48.5± 1.2	3.57± 0.06	0.64± 0.02	0.75± 0.02	36.44±0.55	7.55± 0.13	3.45 ±0.23	0.41± 0.02	0.44± 0.01
32°C_hex+ VSV	57.9 ± 1.1	3.34± 0.07	0.64± 0.01	0.74± 0.01	51.94±0.04	6.81± 0.09	7.54±0.51	0.40 ±0.01	0.44± 0.03

Table S1. Different kinetic parameters of the fusion reaction in PC/PE/SM/CH (35/30/15/20) membrane system in presence of 2% hexadecane, VSV-TMD and hexadecane + VSV-TMD at different temperatures.

		y_0	a_i	b_i	c_i
Control	First Step	4762.4 ± 651.7	-47.90 ± 6.52	0.16 ± 0.022	$-2 \times 10^{-4} \pm 2.42 \times 10^{-5}$
Control	Second Step	1412.6 ± 89.86	-13.72 ± 0.89	0.045 ± 0.003	$-4.83 \times 10^{-5} \pm 3.33 \times 10^{-6}$
VSV-TMD	First Step	-3090.89 ± 200.63	30.74 ± 10.07	-0.10 ± 0.04	$1 \times 10^{-4} \pm 7.43 \times 10^{-5}$
VSV-TMD	Second Step	-1771.52 ± 142.33	17.89 ± 4.28	-0.06 ± 0.01	$6.72 \times 10^{-5} \pm 1.29 \times 10^{-5}$

Table S2. Parameters obtained by fitting the plot of ΔG_i^* vs. temperature (Figure 2) using the equation $\Delta G_i^* (kcal/mol) = y_{0,i} + a_i T + b_i T^2 + c_i T^3$ for the fusion of control vesicles and vesicles prepared in presence of VSV-TMD (Figure 4). The same parameters were used to obtain $T\Delta S_i^*$ ($-a_i T - 2b_i T^2 - 3c_i T^3$); ΔH_i^* ($y_{0,i} - b_i T^2 - 2c_i T^3$), and ΔCp_i^* ($-2b_i T - 6c_i T^2$).
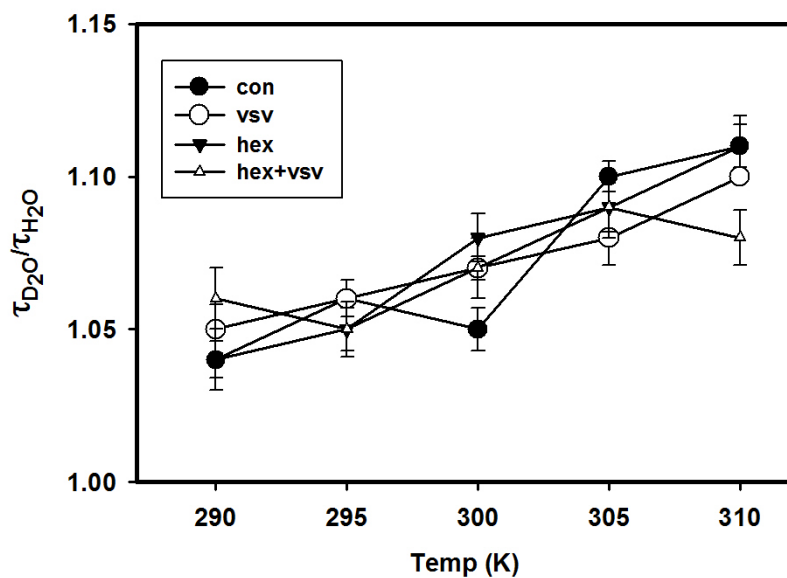
Figure S1. Plot of ratio of lifetime of TMA-DPH in membrane made in D_2O and H_2O buffer in control membrane (●) and membrane containing VSV-TMD (○) (L/P=600/1), 2% hexadecane (▼) and both hexadecane and VSV TMD (Δ) at five different temperature.
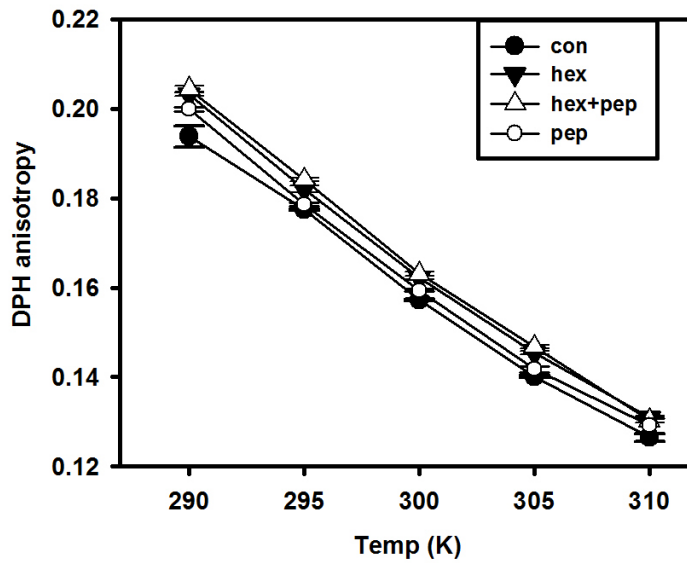
Figure S2. Effect of TMD, hexadecane, and hexadecane + TMD on acyl chain packing of membranes. DPH fluorescence emission anisotropy of control vesicles (●) and vesicles containing VSV (○), hexadecane (▼) and both hexadecane and VSV (Δ) different species are shown at different five temperatures (lipid: probe = 200:1).
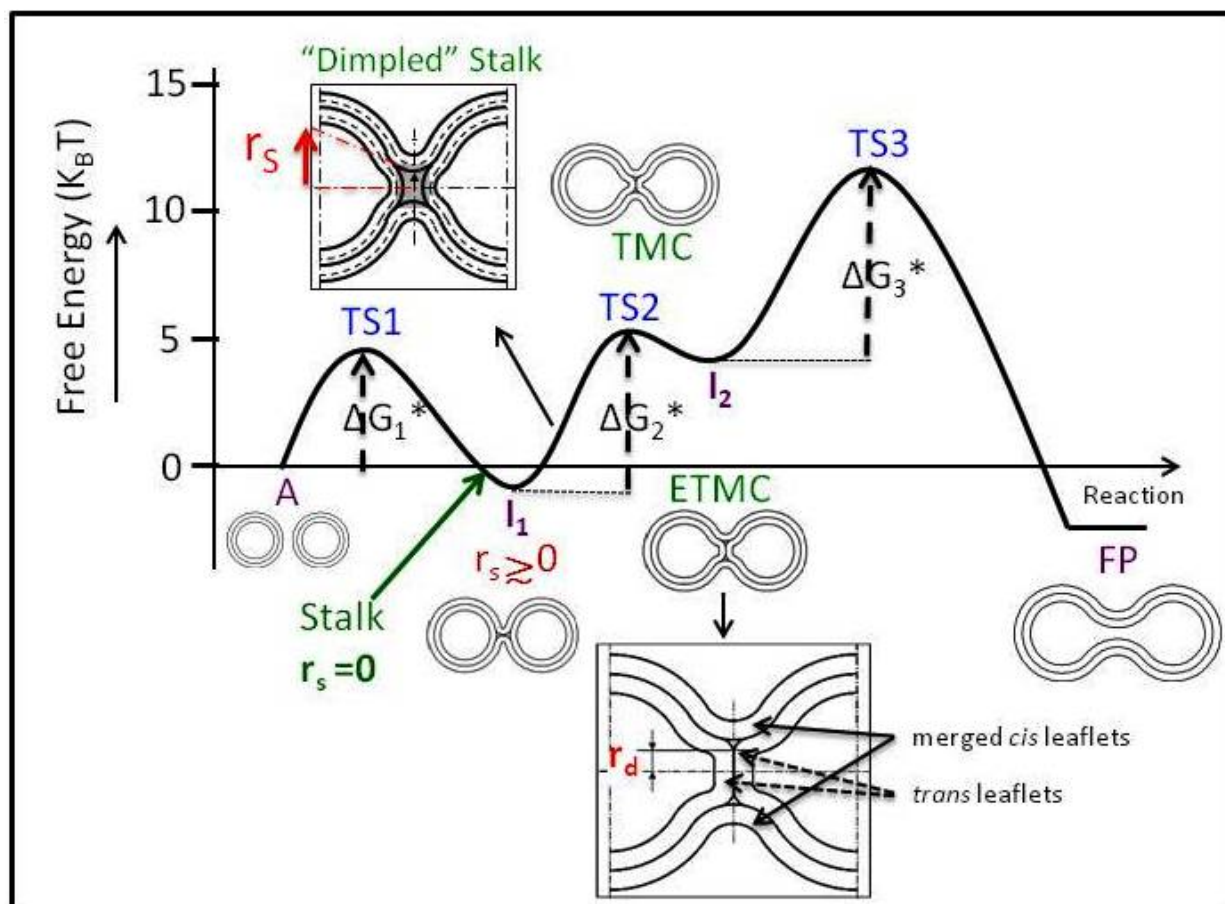
Figure S3. Our kinetic model (see Diagram) derives from the expanded-stalk structural model of Siegel for lamellar to non-lamellar phase transitions as applied to membrane fusion. We adapted this model in order to calculate the activation free energy path for the transition of an initial hemi-fused state (I_1, initial intermediate) between two highly stressed SUVs to a second semi-stable state (I_2) from which a fusion pore state (FP) can form. Two free energy minima confirmed the two-step nature of the fusion path as depicted in our model and as detected experimentally. The geometries (labeled in green) of the predicted intermediates are shown for intermediate structures derived from minimization of the free energies of structures at fixed stalk radii (1). Unstable "transition states" are labeled in blue and semi-stable intermediate "states" in purple. The "reaction coordinate" for this diagram is the "stalk radius" (r_s) that is illustrated in the "dimpled stalk" diagram and is defined as "0" when the two merged *cis*- leaflets first touch. Since our experiments are performed on vesicle ensembles, each "state" is a thermodynamic state and does not correspond to a single structure. In the context of the large-scale mechanical computation, the first state encountered as r_s increases is I_1, which is commonly called the stalk, but occurs at a slightly larger stalk radius than the stalk. The driving force for evolution of the system along its reaction coordinate is a reduction in positive curvature stress that overcomes an increase in unfavorable interstice energy as r_s increases. "Interstices" are regions at the edge of the hemi-fused region (shaded regions in figure) for which lamellar and non-lamellar lipid packing conflict. Because water-hydrocarbon interactions are required to compensate for this mismatch, this leads to an unfavorable "interstice" free

energy. The evolution from I_1 to I_2 proceeds through a *trans*-membrane contact (TMC) first described by Siegel that provides an unstable transition state to reaching the I_2 semi-stable intermediate, which corresponds to a slight minimum in free energy in a geometry described by Siegel as an "extended *trans*-membrane contact" (ETMC). The depth of the I_1 and I_2 free energy minima as well as the height of the TS2 barrier are all subject to variation with the particular natures of different membrane systems (*e.g.*, composition, pH, vesicle diameter, *etc.*). Thus, the membrane system DOPC/DOPE/SM/CH that we examine here passes through two intermediates when fusing at pH 5 but only one at pH 7.4, as we report here. The TS2 transition state is the only one appropriately predicted by the materials-scale materials model. The first and third steps involve changes from 2-compartment, to hemi-fused, to single-compartment topologies that require molecular rearrangements that cannot be described in terms of materials-scale mechanical models. We have used measured activation thermodynamics for each step and measured membrane structural properties to suggest possible mechanistic models for these molecular rearrangements, and here ask whether these are consistent with observations made in the presence VSV-TMD. The ΔG_1* and ΔG_3* magnitudes shown are illustrative of but not exact matches to experimental values obtained for k_1 and k_3.

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REFERENCES

1. Malinin, V. S., and B. R. Lentz. 2004. Energetics of vesicle fusion intermediates: comparison of calculations with observed effects of osmotic and curvature stresses. *Biophys. J.* 86:2951-2964.

2. Chakraborty, H., P. K. Tarafdar, M. J. Bruno, T. Sengupta, and B. R. Lentz. 2012. Activation Thermodynamics of PEG-Mediated Model Membrane Fusion Are Consistent with Mechanistic Models of Stalk and Pore Formation. *Biophys. J.* 102:2751-2760.