Supplemental Material

pH Alters PEG-mediated Fusion of Phosphatidylethanolamine-Containing Vesicles

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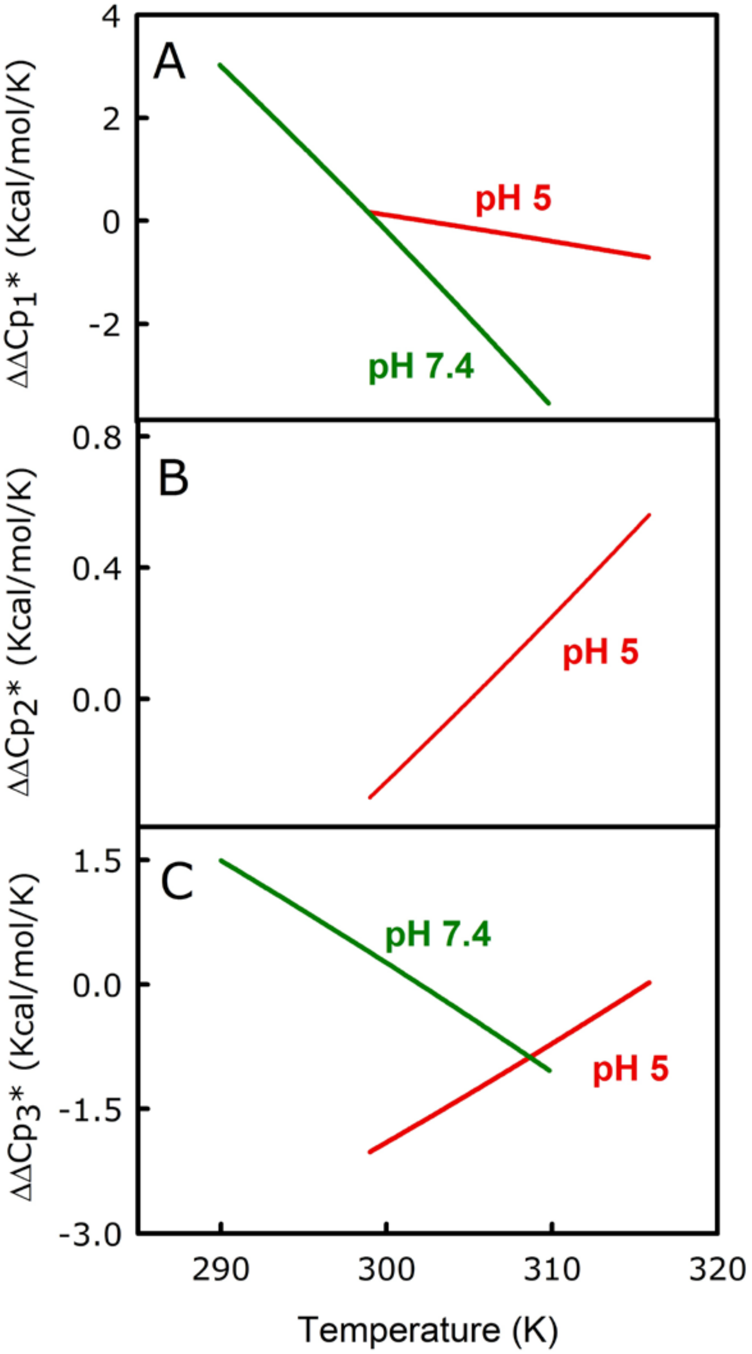

Figure S1. Temperature dependence of change in ΔCp_i^* ($\Delta\Delta Cp_i^*$) in presence of hexadecane for the formation of (A) I_1 state, (B) I_2 state and (C) pore at pH 5.0 (red) and pH 7.4 (green)

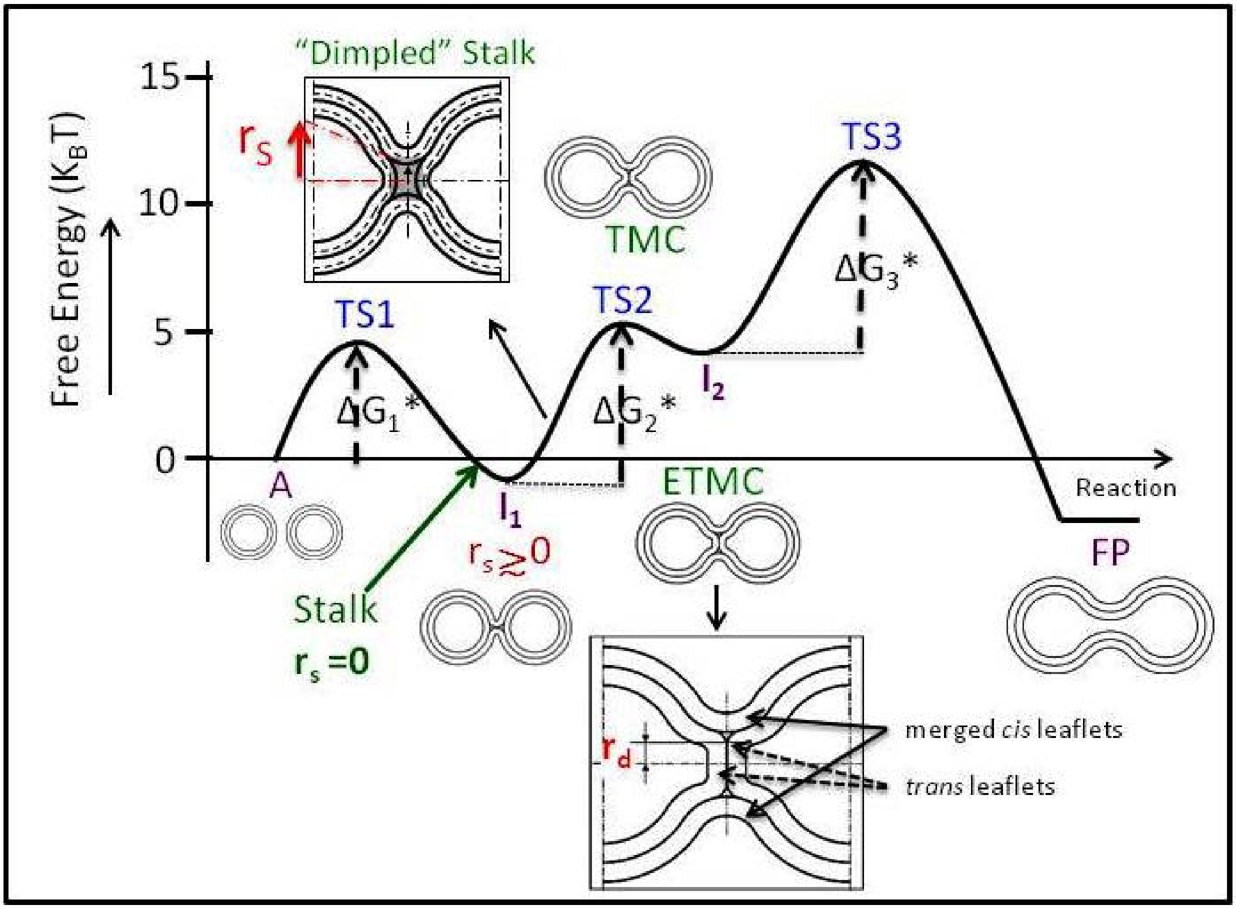

Figure S2. Our kinetic model (see Diagram) derives from the expanded-stalk structural model of Siegel for lamellar to non-lamellar phase transitions as applied to membrane fusion. We adapted this model in order to calculate the activation free energy path for the transition of an initial hemi-fused state (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 of hexadecane (or VSV-TMD in the companion paper (2)). 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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Table S1. Parameters obtained by fitting the ΔG_i^* vs. temperature (Fig. 2) using the equation $\Delta G_i^* = y_0 + aT + bT^2 + cT^3$ for the fusion of vesicles at pH 5.0 and pH 7.4.

		y_0	a	b	c
pH 5.0	First step	-1670	16.25	-5.27×10^{-2}	5.69×10^{-5}
	Second step	-194	2.22	-8.06×10^{-3}	9.58×10^{-6}
	Third step	-742	7.46	-2.48×10^{-2}	2.74×10^{-5}
pH 7.4	First step	4760	-47.9	0.16	-1.80×10^{-4}
	Second step	1412	-13.72	4.56×10^{-2}	-4.83×10^{-5}

REFERENCE.

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2. Sengupta, T., H. Chakraborty, and B. R. Lentz. 2014. The Transmembrane Domain Peptide of Vesicular Stomatitis Virus Promotes Both Intermediate and Pore Formation during PEG-mediated Vesicle Fusion. *Biophys. J.* (Submitted)

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