Supplemental Material

Hemagglutinin Fusion Peptide Mutants in Model Membranes: Structural Properties; Membrane Physical Properties and PEG-Mediated Fusion

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Methods

Vesicle Preparation. Vesicles were prepared from a 35/30/15/20 molar mixture of DOPC/DOPE/SM/CH . Lipids at this molar ratio in cyclohexane/methanol mixed solvent were freeze-dried under high vacuum overnight. The dried lipid powders were hydrated at 23 ºC for 1 hour in a buffer contained 100 mM NaCl, 1 mM EDTA and 10 mM MES, pH 5.0 preparing small, unilamellar vesicles (SUVs[1]) as documented previously (1). All contents mixing, leakage, and lipid mixing experiments were performed at 0.2 mM lipid.

Preparation of HA Peptides. The X-31 HA peptides (native and mutants) were chemically synthesized and purified by the peptide synthesis laboratory at UNC-CH (Director, David Klapper). The sequences of the peptides are GLFGAIAGFIENGWEGMIDG (X-31 HA, native), **E**LFGAIAGFIENGWEGMIDG (G1E mutant) and GLFGAIAGFIEN**L**WEGMIDG (G13L mutant). The peptides were synthesized by the standard solid phase method using fMOC chemistry (2, 3). Stock peptide solutions were prepared in DMSO solvent, and small aliquots of these solutions were added to vesicle suspensions. DMSO was always less than 1% of the buffer volume, and control experiments showed that this amount of DMSO had no detectable effect on either fusion or membrane structure.

CD Spectroscopy: Circular dichroism spectra were measured using an Applied Photophysics Model π* ® spectropolarimeter (Surrey, UK). Data were collected using 10 mm path-length cells at wavelengths from 250 to 200 nm with intervals of 0.2 nm. The peptide concentration was 40 μM, and the lipid concentration was 2 mM. Peptides were dissolved in DMSO and small amounts of peptide solutions were dried in thin films on the surface of 3.5 ml brown vials. The films were frozen using dry ice, and dried under high vacuum over night. Required amount of pre-formed SUVs were added to the vials and vortexed for 30 minutes to transfer peptides to the membranes. SUV without peptide was used as a control and the control spectrum was subtracted from peptide containing vesicle spectra. CD spectra were analyzed using the CD Pro package (http://lamar.colostate.edu/~sreeram/CDPro/main.html). Two basis sets (sets 4 and 7) used in CDPro are optimized for the spectral range of our measurements (195-260 nm). Of these, basis set 4 yielded the best agreement between analysis routines. Two of the three routines provided in that package (Selcon3 and Contin) gave consistent results, which are reported here.

Peptide Binding: Binding was monitored either by the increase in tryptophan (Trp) fluorescence observed when membranes were added to peptide in solution or by energy transfer from Trp to membrane-located DPH with addition of peptide to membrane samples. Experimental details as well as analysis of the data are given elsewhere (2).

ANTS/DPX contents mixing and leakage experiments: Mixing and leakage of the trapped contents of sonicated, small, unilamellar vesicles (SUVs) composed of PC/PE/SM/CH (35/30/15/20 mol ratio %) lipids were monitored by the ANTS/DPX assay, as described in detail elsewhere (4). All experiments were performed at pH 5.0.

Lipid Mixing Assay: Fluorescent lipid probes with fluorophores attached to their acyl-chains, BODIPY500-PC and BODIPY530-PE (Molecular probes, Eugene, OR), were used for measuring lipid transfer during PEG- and peptide- mediated vesicle fusion, with a probe-containing/probe-free vesicle ratio of 1:4, as described in detail elsewhere (5). Fraction of lipid mixing was calculated using a slightly modified form of the equation derived in our previous publications. Here we normalized the F_D/F_A fluorescence intensity with respect to the F_D/F_A in presence of the detergent, ideally a situation in which all lipids are mixed. In this method, the fraction of lipid mixing is:

$$LM(t) = \frac{1}{n}\exp\left(\left\{\frac{k\left[\left(F_D/F_{DA}\right)_t - \left(F_D/F_{DA}\right)_0\right]}{\left(F_D/F_{DA}\right)_{\text{det}\,ergent}}\right\} - 1\right),$$

where n is the ratio of probe-free to probe-containing vesicles in a sample, F_D is the fluorescence of donor taken at a wavelength (520nm) at which acceptor does not contribute, F_A is the fluorescence of acceptor taken at 560nm (where there is little contribution from donor), and *k* is a calibration constant obtained for each experimental system (for SUVs fusing with SUVs, it ranges between 3.5 and 3.8)(5). As noted previously, this somewhat unconventional quantity is related but not equal to FRET efficiency, but is ideally adapted to measurements in real time on a T-format fluorometer (5).

C_6-NBD-PC lifetime measurements: Small aliquots (0.04-0.08% of vesicle sample volume) of a stock solution of C_6-NBD-PC in methanol were added to vesicle suspensions to obtain a lipid to probe ratio of 200:1 (6). Phase shift and modulation ratios were collected using a Fluorolog-3 spectrofluorometer (Jobin Yvon Inc, Edison, NJ) with a 450W Xenon lamp. Phase resolved lifetime and mole fractions of each lifetime component were estimated using Global Analysis software package (Jobin Yvon Inc). The average lifetimes of the probe in the micellar and bilayer phases were used to estimate the partition function of the probe between these two phases, as described elsewhere (6).

PATIR-FTIR Experiments

Polarized attenuated total internal reflection Fourier-transform infrared (PATIR-FTIR) spectroscopy was performed using a FTS-60A spectrometer (BioRad Digilab, Waltham MA) equipped with a liquid-nitrogen cooled MCT detector coupled via a germanium internal reflection element to a lipid film balance as previously described (7) at pH 5.0. Monolayers were prepared by applying lipids dissolved in hexane:ethanol (9:1) to the buffer surface in a Langmuir trough. The lipid spread at the air-water interface was compressed to a surface pressure of 20 mN/m, and applied onto the internal reflection element by placing an octadecyltrichlorosilane-treated germanium internal reflection crystal flat onto the monolayer (8, 9). An enclosure around the Langmuir trough was filled with argon to avoid oxidation of lipids. After recording a base-

line single-beam spectrum, aliquots of the peptide (~ 4 μg) dissolved in deuterated DMSO were added to the continuously stirred sub-phase compartment containing 2.5 ml of 30 mM HEPES-NaOD buffer (pD 5.0) to a final concentration of ~ 0.8 μM. Minor contributions to the amide I' spectrum arising from SM are included in the base-line spectrum, and therefore do not contribute to the final amide I' spectrum of the peptide. The DMSO concentration in the buffer sub-phase was always less than 0.3 wt%. Polarized infrared absorption spectra were recorded a described previously (8). The depth of evanescent field penetration into the buffer sub-phase at the site of each internal reflection was approximately 0.5μm, so that the contribution of bulk solution peptide to the spectra is negligible. All spectra were collected in the rapid scan mode as 1024 co-added interferograms, with a resolution of 2 cm^{-1}, scanning speed of 20 kHz, triangular apodization, and one level of zero filling. All experiments were performed at 23˚C. A single horizontal baseline correction was applied to each spectrum, but no smoothing, deconvolution, or other aesthetic processing was employed. Dichroic ratios, $R_z = \int A_{\parallel} \Big/ \int A_{\perp}$, were evaluated using linked analysis (9) and integrated areas of the amide I' absorption bands, $\int A$, arising from the backbone peptide groups. For an angle θ between the absorption moment and the surface normal, $R_z = 2.34$ indicates either isotropic disorder, uniform orientation at $\theta = 54.7^{\circ}$ (the magic angle), or any distribution of orientations for which $\left\langle \cos^2 \theta \right\rangle = 1/3$. Accordingly, $R_z > 2.34$ indicates that absorption moments have preferential orientation more perpendicular to the plane of the membrane than the magic angle, while $R_z < 2.34$ indicates preferential orientation more parallel to the membrane than the magic angle. The range of possible values for the dichroic ratio has a physical minimum at $R_Z = 0.88$, corresponding to an orientation that is perfectly parallel to the membrane (10). The fractional contributions of different band components to the overall amide I' band were calculated for the conditions of internal reflection and polarized light using a band-fitting procedure related to "global analysis" approaches as previously described (11).

DPH and TMA-DPH fluorescence anisotropy. Measurement of DPH and TMA-DPH fluorescence anisotropy was carried out by adding a small volume (0.04-0.08% of vesicle sample volumes) of a stock solution of DPH and TMA-DPH in methanol to PC/PE/SM/CH SUV suspensions to a final lipid:probe ratio of 200:1. The mixtures were vortexed thoroughly and incubated for 30 minutes until fluorescence intensity stabilized. Aliquots of peptide were then incubated with the vesicle suspensions for 10 minutes prior to fluorescence measurements. The excitation wavelength was 360 nm, with excitation slits of 4 and 4 nm, and a 450 cutoff filter was used in the emission paths in the T-format.

Table S1: Recovered amide I′ components dichroic ratios for HA fusion peptides*

		HA wild type	G1E mutant	G13L mutant
1616 cm^{-1}	R_Z	0.0	0.0	0.84±0.76
	%	0.0	0.0	3.1±2.40
1625 cm^{-1}	R_Z	1.2 ± 0.0		
	%	19.0 ± 7.2		
1629 cm^{-1}	R_Z			1.51±0.46
	%			24.4±3.1
1632 cm^{-1}	R_Z		1.61±0.19	
	%		17.1±2.3	
1649 cm^{-1}	R_Z	1.7 ± 0.1	1.80±0.25	1.71±0.08
	%	73.1 ± 6.4	63.4±3.3	57.1±2.2
1668 cm^{-1}	R_Z		1.83±0.32	
	%		19.5±2.9	
1675 cm^{-1}	R_Z			2.34±0.73
	%			15.5±2.8
1677 cm^{-1}	R_Z	1.9±0.0		
	%	7.9±3.2		

* Values listed are the average and range of three to four measurements. The β sheet orientation, γ, is calculated as described in Methods. Percentages in each column are amide I' component areas with the total being 100%.

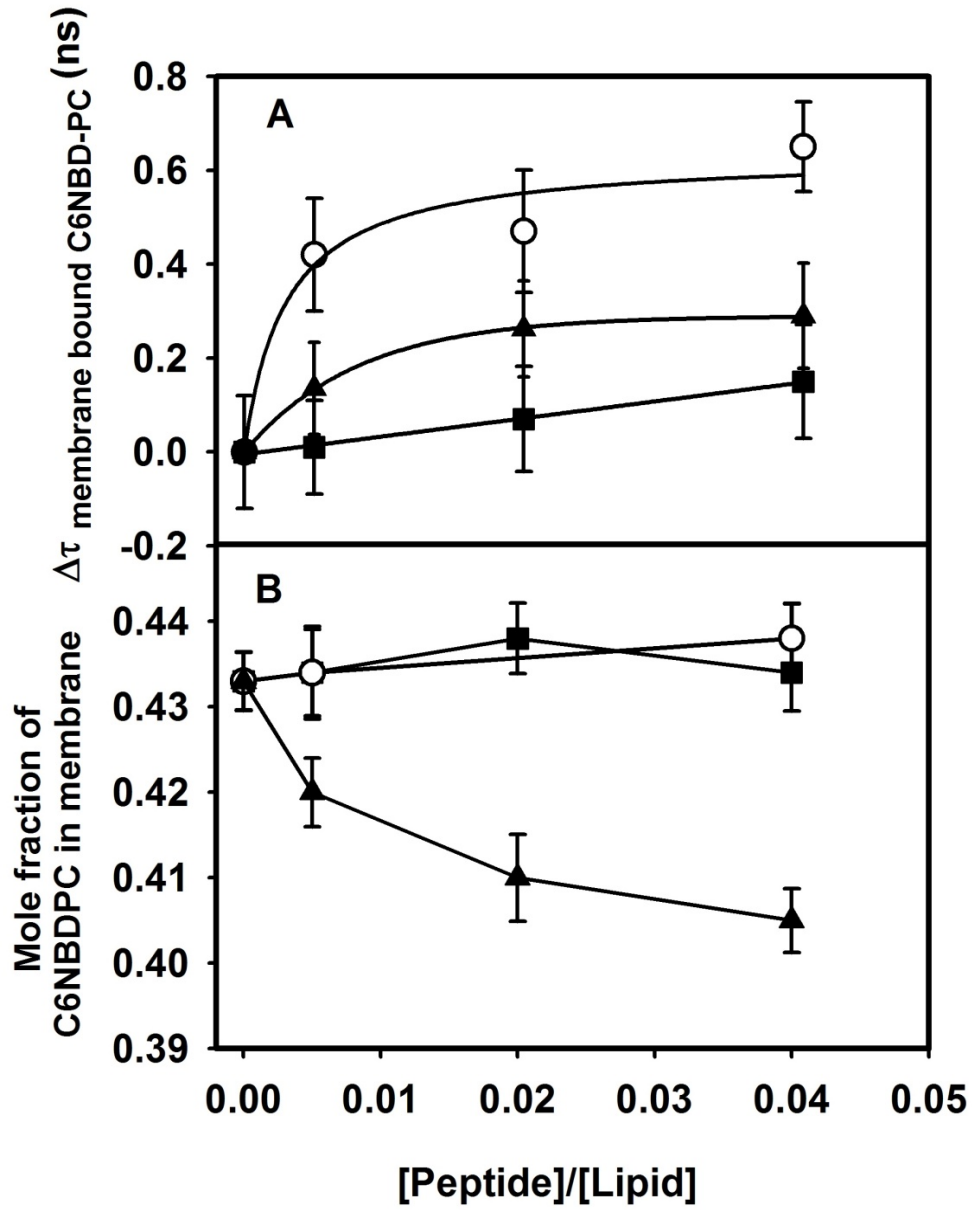

Fig. S1 Effect of fusion peptides (wild type and mutants) on the surface properties of PC/PE/SM/CH SUVs. (A) Delta lifetimes = {fluorescent lifetime in presence of peptide} – {fluorescent lifetime in absence of peptide} of membrane bound component of C_6-NBD-PC and (B) mole fraction of membrane bound C_6-NBD-PC as a function of peptide to lipid ratio in the presence of wild type fusion peptide (solid triangle), G13L (open circle), and G1E (solid square). Measurements were carried out in 10 mM MES, 100 mM NaCl, 1 mM $CaCl_2$, 1 mM EDTA, pH 5.0 at 23 ^{0}C. Total lipid concentration was 0.2 mM. Values presented here are the average of three independent measurements and the standard deviation of three results provides the error bars. The probe lifetime in its micellar phase was constant (0.95 ns ± 0.02) and independent of peptide concentration.

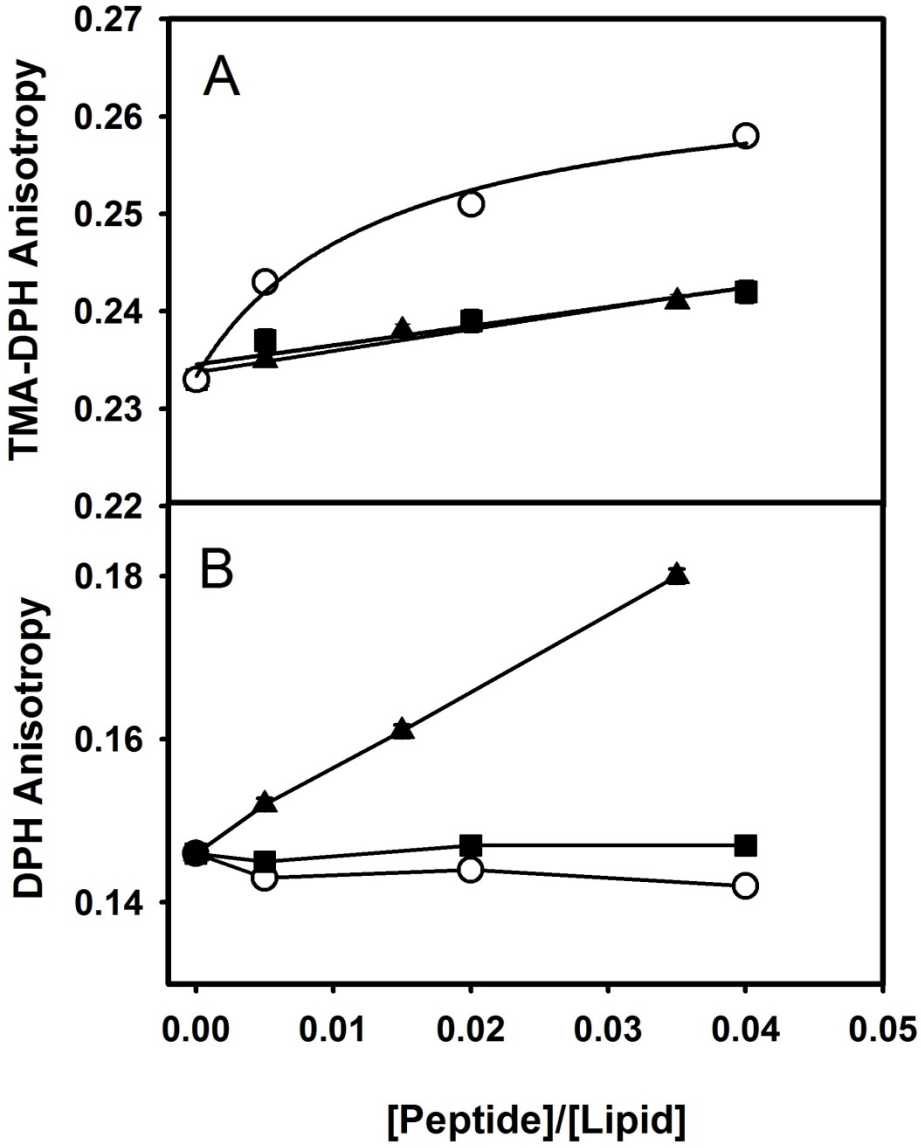

Fig. S2 Effect of wild type HA and mutant fusion peptides on the packing of acyl chain and interfacial regions of vesicle membrane. (A) TMA-DPH, (B) DPH fluorescence emission anisotropy in PC/PE/SM/CH (35/30/15/20) SUVs in presence of wild type fusion peptide (solid triangle), G13L (open circle), and G1E (solid square) are shown as a function of peptide to lipid ratio. Measurements were carried out using 0.2 mM SUVs in 10 mM MES, 100 mM NaCl, 1 mM $CaCl_2$, 1 mM EDTA, pH 5.0 at 23^0C. Values presented here are the average of three measurements, but the standard deviation of three results was almost always smaller than the symbol size used in the figure.

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