Supporting Information

Controlled liposome fusion mediated by SNARE protein mimics

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CD of acetylated peptides in TES

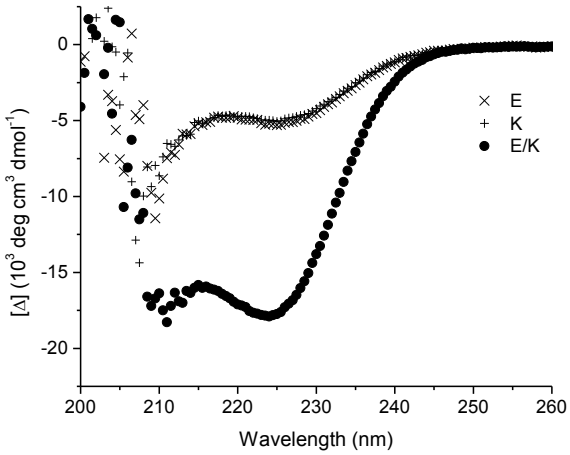

Figure S1. CD Spectrospcopic Data of E (Δ), K (+), and an equimolar mixture of E and K (●) in TES, pH 7.4, 25 °C. [Total Peptide] = 40 μM.

Temperature dependent CD data and analysis

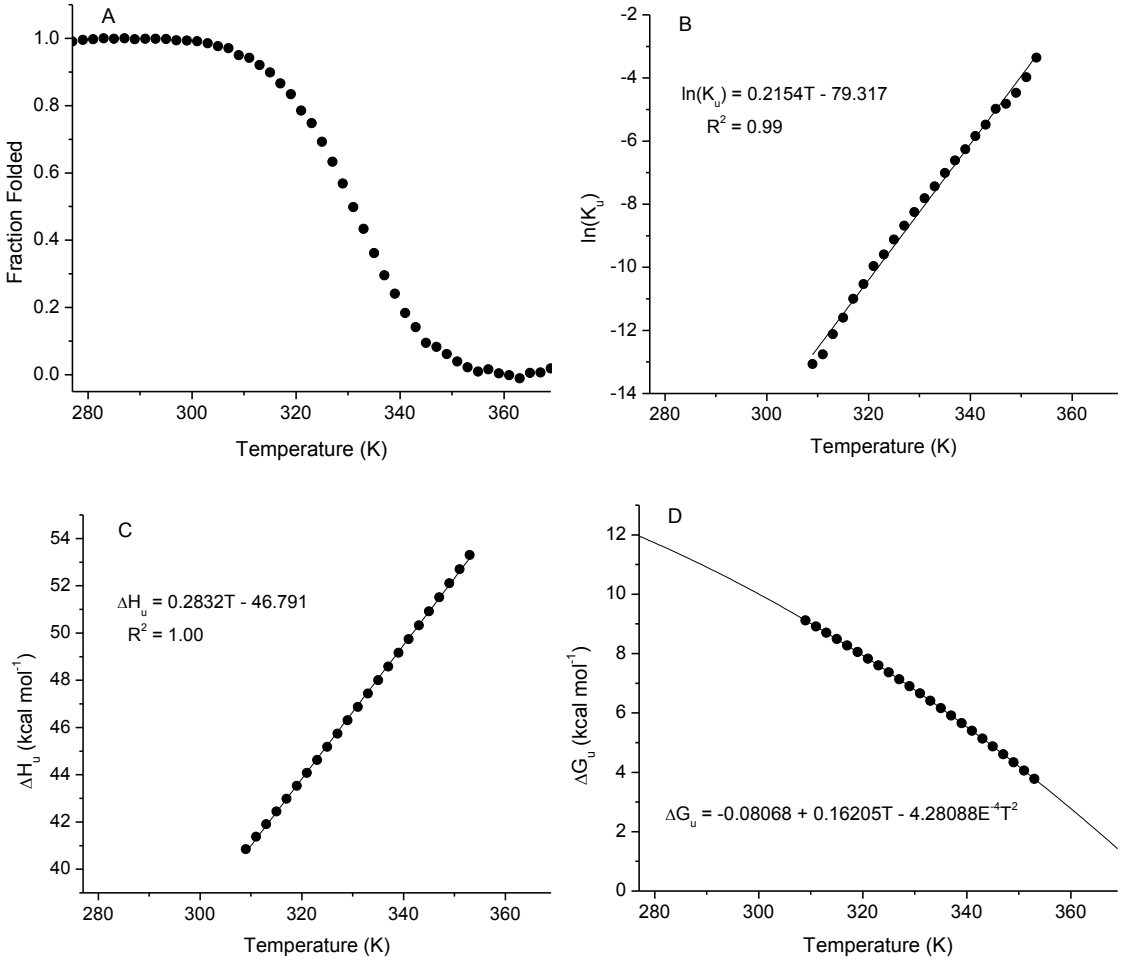

Figure S2. A) Thermal unfolding curve of unmodified E/K in TES buffer, pH 7.4, as followed by CD. [Total Peptide] = 40 μM. B) Van't Hoff plot of the thermal denaturation of E/K. C) Dependence of the enthalpy of unfolding of E/K on temperature. ΔH_u values were obtained from the derivative of the van't Hoff plot. D) Free energy associated with the unfolding of E/K as a function of temperature. The least-squares fit gives a ΔG_u value at 25 °C of 10.2 kcal mol^{-1}.

Temperature dependent CD spectra were obtained using an external temperature sensor immersed in the sample. The temperature was controlled with the internal sensor and measured with the external sensor. A 10 mm quartz cuvette was used, and the solutions were stirred at 900 rpm. Spectra were recorded from 260 nm to 200 nm, with data collected at 0.5 nm intervals with a 1 nm bandwidth and 1 s readings. Each spectrum was one scan. The temperature range was 4 °C to 96 °C with a temperature gradient of 2.0 °C/minute and a 60 s delay after reaching the set temperature. The spectrum of TES at 4 °C (average of 5 scans) was subtracted from each spectrum.

The data was analyzed using a two-state unfolding model to determine the fraction folded using Eqn. (1),

$$F_F = (\theta - \theta_u) / (\theta_f - \theta_u) \quad (1)$$

Where θ is the observed ellipticity at 222 nm, θ_u is the ellipticity at 222 nm of the denatured state, as determined from the plateau of the ellipticity vs. temperature curve, and θ_f is the ellipticity at 222 nm of the folded state at that temperature as determined from a linear fit of the initial stages of the ellipticity vs. temperature curve.

The fraction unfolded, F_u, was calculated by Eqn. (2),

$$F_u = 1 - F_f \quad (2)$$

The dimer dissociation constant in the transition zone was calculated using Eqn. (3),

$$K_u = 2 P_t F_u^2 / F_f \quad (3)$$

where Pt is the total peptide concentration. By taking the derivative of the $\ln(K_u)$ vs. temperature and using this in the van't Hoff equation, Eqn. (4), the change in enthalpy associated with unfolding with temperature can be plotted:

$$d\ln(K_u) / dT = \Delta H_u / RT^2 \quad (4)$$

The gradient of this plot, ΔCp, is the difference in heat capacity between the folded and unfolded forms, and can be used in the Gibbs-Helmholtz equation adapted to monomer-dimer equilibrium, Eqn. (5), to obtain the Gibbs free energy of unfolding as a function of temperature:

$$\Delta G_u = \Delta H_m (1 - T / T_m) + \Delta C_p [T - T_m - T \ln(T / T_m)] - RT \ln[P_t] \quad (5)$$

where T_m and H_m are the temperature and enthalpy at the midpoint of the transition, as determined by the maximum of the derivative of the ellipticity vs. temperature graph.

Calibration of fluorescence based lipid mixing assay

The lipid mixing assay monitors NBD fluorescence, which depends on the distance between the FRET pair NBD and LR. One liposome population contains 0.5 mol% of DOPE-NBD and DOPE-LR (headgroup labeled), while the other population is non-fluorescent. As non-fluorescent and fluorescent liposomes fuse the distance between the fluorophores increases, resulting in decreased FRET efficiency and an increase in NBD fluorescence. Using 0.5 mol% of each fluorophore the increase in NBD fluorescence is proportional to lipid mixing (Figure S3).

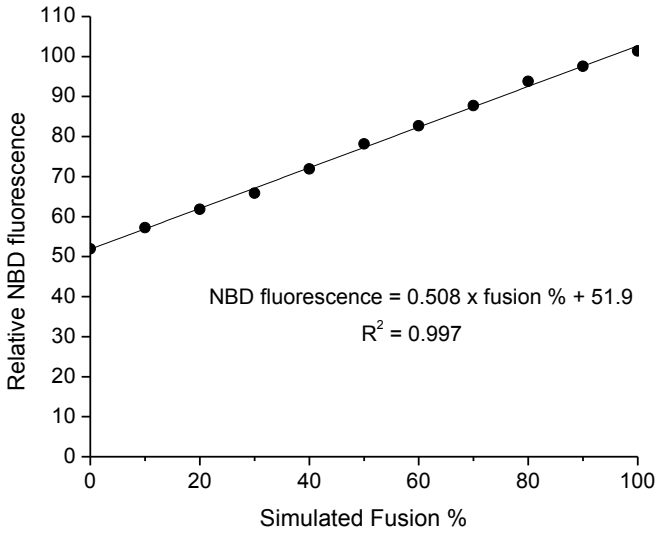

Figure S3. Samples which simulated 0-100% lipid mixing were prepared by combining the appropriate ratios of fluorescent and plain lipid stock solutions. The NBD fluorescence of these samples was monitored for 1 hr. The relative NBD fluorescence increases linearly with increasing fusion percent.

For the lipid mixing experiments each sample was calibrated by monitoring its fluorescence over time relative to an I_0 sample (the fluorescent liposomes were diluted 1:1 with TES) and an I_{100} sample (liposomes prepared from an equimolar mixture of fluorescent and plain lipid stock solutions). The NBD fluorescence of mixed liposomes is not affected by the lipopeptide, thus the I_{100} samples did not contain lipopeptide.

Using this assay the lipid mixing is calibrated using an "I_{100}" sample that simulates the distance between the fluorophores (and hence NBD intensity) if all of the original liposomes have fused. The most commonly used method for calibrating the fluorescence data is to add Triton-X to the sample after monitoring. This method results in a very large distance between the fluoropohores, much larger than is possible using 1:1 fluorescent and non-fluorescent liposomes. In that case the monitored fluorescence increase is not proportional to lipid mixing. Using Triton-X 100% lipid mixing corresponds to ~20-30% fluorescence increase.

Tryptophan fluorescence

The manner in which the coiled-coil formation between SNARE proteins leads to membrane distortion and fusion is unknown. A possible mechanism for both SNARE-mediated and LPK/LPE-mediated membrane fusion is molecular recognition followed by 'virus-like' membrane penetration and destabilization. This is of particular consideration for the minimal model as the E/K coiled-coil dimer is electrostatically neutral and hence may have a greater propensity for membrane insertion. In order to probe this possibility a tryptophan residue was included in the first generation LPK. Tryptophan fluorescence is highly sensitive to the polarity of its local environment, and to monitor environmental changes during the fusion process the LPK fusogen was synthesized containing tryptophan at the C-terminus (with the PEG spacer and lipid anchor at the N-terminus). Before fusion the C-terminus of LPK is in a relatively polar environment (the tryptophan emission maximum of LPK-decorated liposomes is at 342.5 nm, Figure S4).[1] Within one minute of mixing LPK- and LPE-modified liposomes the tryptophan emission maximum had red-shifted to 350 nm, indicating that upon E/K complex formation the C-terminus of the peptide moves from a relatively polar to a completely water-exposed environment.[1, 2] These results indicate that after the molecular recognition between E and K initiates membrane fusion the peptide complex is situated outside the liposomes, rather than buried in the liposome membrane. Thus, the membrane distortion required for membrane fusion does not appear to arise via penetration of the peptide complex into the bilayer. Having established that the E/K peptide complex is unlikely to cause liposome fusion by 'viral-like' membrane burial, the second generation LPK, as employed for all other experiments in this paper, does not contain the tryptophan residue.

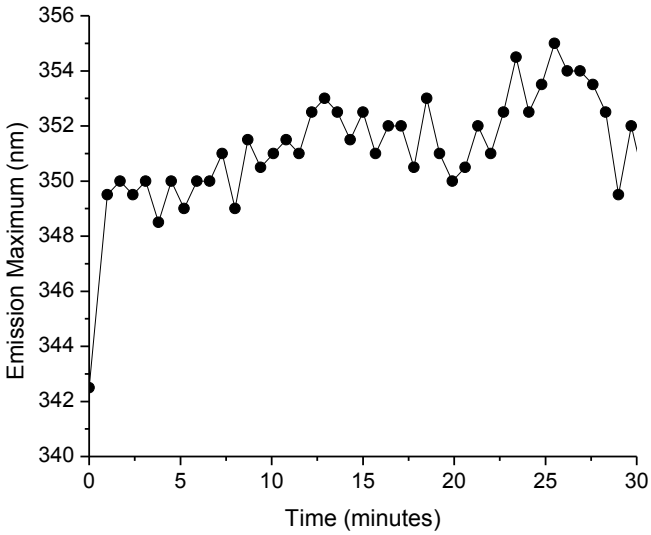

Figure S4. The wavelength of the tryptophan fluorescence maximum before (t = 0 minutes) and during lipopeptide induced fusion of liposomes. Lipid concentration 1mM, fusogen proportion 3%.

Tryptophan emission spectra were measured with a FS920 fluorometer from Edinburgh Instruments with a DTMS-300X excitation monochrometer and a peltier-controlled thermostatic cell. Spectra were obtained at 25 °C using a quartz cuvette with a 1 cm path length. The step size was 0.5 nm, with a sampling time of 0.5 s at each wavelength, and 1 scan was measured for each spectrum. The excitation and emission slits were 5 nm. Emission spectra were measured from 330 nm to 360 nm at a fixed excitation wavelength of 280 nm. Measurements were started immediately after mixing the solutions in the cuvette (30 seconds stirring at 1000 rpm).

Optical microscopy control

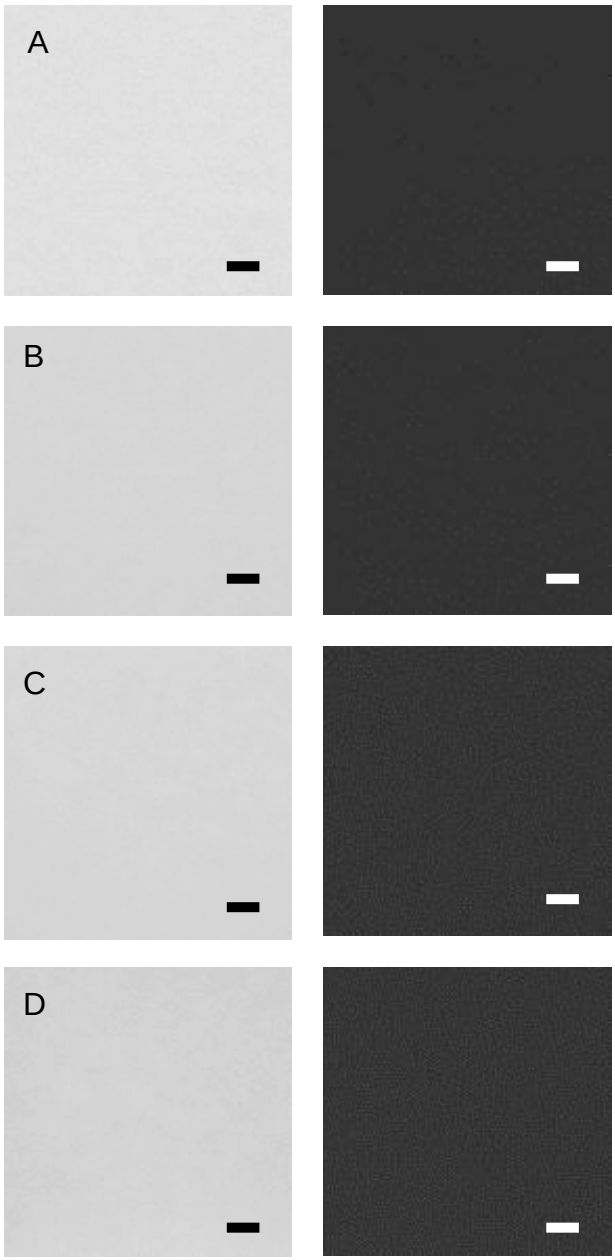

Figure S5. Optical and fluorescent microscopy images (left and right columns respectively) of liposomes without lipopdetide modification. A) 5 minutes after mixing plain and fluorescently labeled liposomes. B) 1 hour after mixing. C) 5 hours after mixing. D) 3 days after mixing. [Lipids] = 1mM lipids. Scale bars 10 μm.

Content mixing

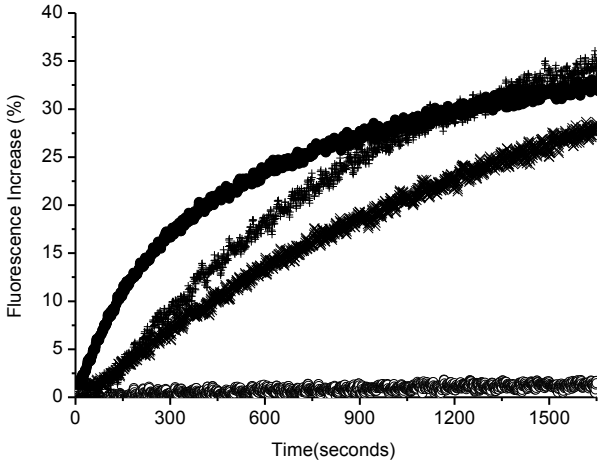

Figure S6. Content mixing recorded by fluorescence spectroscopy for liposomes modified with 1 mol% LPE and LPK. [lipid] 0.25 mM (●), 0.1 mM (+), 0.025 mM (×). Control with liposomes modified with 1 mol% LPE and 0 mol% LPK, [lipid] 0.1 mM (○).

To measure content mixing, LPE-modified liposomes containing sulforhodamine B at a self-quenching concentration were added to LPK-modified liposomes. Upon fusion the contents of the liposomes mix, resulting in an increased sulforhodamine B fluorescence signal due to the relief of self-quenching.[3,4] The fluorescence signal is calibrated by adding Triton-X to lyse the liposomes and reach the maximum dilution. The sulforhodamine B dilution is much greater than the two-fold dilution expected if all LPE- and LPK-modified liposomes fuse, therefore the extent of the content mixing cannot be compared with the extent of the lipid mixing. In previous experiments (3 mol% fusogen, [lipid] 0.4 mM, PBS buffer), it was observed that content mixing proceeded without leakage.[5]

For content mixing experiments a dried film containing DOPC/DOPE/CH 50:25:25 mol% and 1 mol % LPE was hydrated and sonicated (5 min at 50 °C) in TES buffer solution (*N*-Tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid sodium salt 10 mM, NaCl 100 mM, adjusted to pH 7.4) containing sulforhodamine B (20 mM). The final lipid

concentration was 1 mM. To remove the non-encapsulated dye, the liposome suspension was subjected to Sephadex (G50, Superfine) using TES buffer as the eluent. The fraction containing liposomes was collected and diluted to the final lipid concentration. Sulforhodamine fluorescence was measured with a Perkin Elmer Luminescence Spectrometer LS 50B at room temperature. The excitation and emission slits were 2.5 nm. The excitation wavelength was 520 nm and the emission wavelength was 580 nm. 800 µL of the LPE-decorated liposomes with encapsulated sulforhodamine B were added to a small volume disposable cuvette. The fluorescence signal of the sulforhodamine was detected and 800 µL of unmodified or LPK-modified liposomes (1:1 molar ratio with the LPE-modified liposomes) were added and the increase of sulforhodamine B fluorescence was detected due to a relief of self quenching. After 30 minutes 160 µL of 10% (v/v) solution of Triton X was added. To calculate the percentage of fluorescence increase the following equation was used:

$$F\% = (F_{(t)} - F_{(0)} / (F_{(\max)} - F_0) \times 100$$

where $F_{(t)}$ is the fluorescence at a certain time, $F_{(\max)}$ is the fluorescence after lyses of the liposomes with Triton X and $F_{(0)}$ is the starting fluorescence after addition of the LPK-modified liposomes.

References

1. Swaving Dijkstra, D.; Broos, J.; Lolkema, J. S.; Enequist, H.; Minke, W.; Robillard, G. T., *Biochemistry* **1996,** *35*, 6628-6634.
2. Ladokhin, A. S.; Jayasinghe, S.; White, S. H., *Analytical Biochemistry* **2000,** *285*, 235-245.
3. G. Pähler, C.Panse, U. Diederichsen, A. Janshoff, *Biophysical Journal*, **2012** , *103*, 2295–2303.
4. A. S. Lygina, K. Meyenberg, R. Jahn, U. Diederichsen, *Angew. Chem.*, **2011**, *123*, 8756-8760. *Angew. Chem. Int. Ed.*, **2011**, *50*, 8597-8601.
5. Robson Marsden, H.; Elbers, N. A.; Bomans, P. H. H.; Sommerdijk, N. A. J. M.; Kros, A. *Angew. Chem., Int. Ed.*, **2009,** *48*, 2330-2333.