Supporting Information
Coiled coil driven membrane fusion: zipper-like vs. non-zipper-like peptide orientation

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Experimental section

Materials and Methods

Materials

The Fmoc-protected amino acids were purchased from Novabiochem. The Sieber Amide resin was purchased from Agilent Technologies. Fmoc-NH-PEG_{12}-COOH was purchased from IRIS Biotech. DOPE and DOPC were obtained from Avanti Polar Lipids and cholesterol was obtained from Sigma Aldrich. DOPE-NBD and DOPE-LR were obtained from Avanti Polar Lipids. Solvents were obtained from Biosolve Ltd.

G*eneral Methods*

The purification of the lipidated peptides was performed by RP-HPLC with a Shimadzu system with two LC-8A pumps and a SPD-10AVP UV-VIS detector. UV detection was performed at 214 nm. The peptide hybrids were dissolved in a mixture of tert-butanol:acetonitril:water (1:1:1 v/v) and eluted with a flow rate of 20 mL/min.and with a linear gradient from A to B, where A was H_2O with 0.1 vol% TFA and B was acetonitrile with 0.1 vol% TFA. Purification was performed on a Gemini C18 column. Initially, samples were eluted with a linear gradient from 10% to 90% B over 3 column volumes. After this initial run, the product peak was identified and the gradient was adjusted to run from x% to x + 10%. Analysis on the purity of synthesized compounds was performed via LCMS.

Table S1. Overview of observed masses (by LCMS) of all the lipidated peptides and their yields

Compound	Observed mass (g/mol)	% Yield
CPE	3351	11.3%
CPK	3348	9.8%
EPC	3478	8.2%
KPC	3475	8.0%
CPKPC	4542	4.7%

Liposome preparation

To prepare unlabelled liposomes, a 1 mM stock solution with the composition DOPC:DOPE:Cholesterol (50:25:25 mol%) in chloroform was used. For the lipid mixing assay, a stock solution with the compositition DOPC:DOPE:Cholesterol:DOPE-LR:DOPE-NBD (49.5:24.75:24.75:0.5:0.5 mol%) in chloroform was used. The lipidated peptides were dissolved in a mixture of Chloroform:Methanol (1:1 v/v), to a concentration of 50 μM. Typically, liposomes decorated with 1 mol% of the lipidated peptides were used and therefore the peptide hybrid and lipid stock solutions were mixed in equal amounts. The solvent was subsequently removed under a stream of air. For lipid mixing, DLS and CD experiments PBS was added to the dry lipid layer. For the content mixing experiments, PBS buffer containing 20 mM sulphorhodamine B was added to the dry lipid layer. Subsequent sonication yielded ~100 nm liposomes.

Characterization

Optical density measurements

Optical density measurements were carried out using a Cary UV-Visible spectrometer. A quartz cuvette with a 1cm pathlength was used. The wavelength was set to 400 nm and samples were continuously measured for 30 minutes, subsequent to combining K decorated liposomes with E decorated liposomes (total lipid concentration 0.25 mM and 1 mol% of lipidated peptide).

Circular Dichroism

CD spectra were measured using a Jasco J-815 spectropolarimeter. The observed ellipticity is given in millidegrees, the conversion to the mean residue molar ellipticity is performed by the following equation:

$$[\theta] = \theta_{obs} \frac{MRW}{10lc}.$$

Here, Θ_{obs} is the ellipiticity in millidegrees, MRW is the mean residue molecular weight, l is the path length of the cuvette in cm and c is the peptide concentration in mg/mL. Spectra were obtained with a total sample concentration of 0.5 mM with 1 mol% peptide in a 0.2 cm quartz cuvette at room temperature. The datapoints were collected at a 0.5 nm interval, at a scanning speed of 100 nm/min and a 1 nm bandwith. Each spectrum was the average of 10 scans.

Helical content was determined using the following formula:

$$f_H = \frac{\Theta_{(225nm)}}{-39500 \times (1 - \frac{2{,}57}{n})}$$

where f_H is the helical fraction, $\Theta_{(225nm)}$ is the ellipticity at 225 nm and n is the number of peptide bonds.

Dynamic Light Scattering

Particle size distributions were obtained with the aid of a Malvern Zetasizer Nano ZS which was equipped with a peltier controlled thermostatic holder. The laser wavelength was 633 nm and the scattering angle was 173º. To obtain an estimation of the hydrodynamic radius, D_h, the Stokes-Einstein relation was used:

$$D = \frac{k_B T}{3\pi\eta D_h}.$$

Here, k_B is the Boltzmann constant and η is the viscosity of the solvent. Measurements were carried out at room temperature.

Fluorescence spectroscopy

Fluorescence measurements for content mixing were performed using a luminescence spectrometer LS50B (Perkin Elmer). All spectra were obtained at room temperature using a cuvette with a 1 cm path length. Fluorescence time series measurements were started immediately after mixing 600 µL of the fluorescent-labeled liposome suspension with 600 µL of unlabeled liposome suspension in the cuvette. The sulphorhodamine fluorescence intensity at 580 nm was monitored in a continuous fashion for 1800 seconds. After that the liposomes were lysed by the addition of 150 mL of 10 wt% Triton X-100 in PBS to obtain 100% increments. The percentage of fluorescence increase (%F(t)) is calculated as: $\%F(t)=(F(t)-F_0)/(F_{max}-F_0)$ where F(t) is the fluorescence intensity measured

at time t, F_0 is the 0% fluorescence and F_{max} is the fluorescence intensity measured after addition of Triton X-100.

Fluorescence measurements for lipid mixing were performed on a Tecan Plate Reader Infinite M1000. NBD emission was measured continuously upon mixing fluorescent K decorated liposomes with non-fluorescent E decorated liposomes at 530 nm for 1800s. The 0% value was determined by measuring NBD emission of K liposomes to which an equal amount of PBS was added. The 100% value was determined by using liposomes which contained half the probe (NBD and LR) concentrations. The percentage of fluorescence increase (%F(t)) was calculated as: $\%F(t)=(F(t)-F_0)/(F_{max}-F_0)$ where F(t) is the fluorescence intensity measured at time t, F_0 is the 0% fluorescence and F_{max} is the fluorescence intensity measured after addition of Triton X-100.

CD experiments on the combination of E and K decorated liposomes

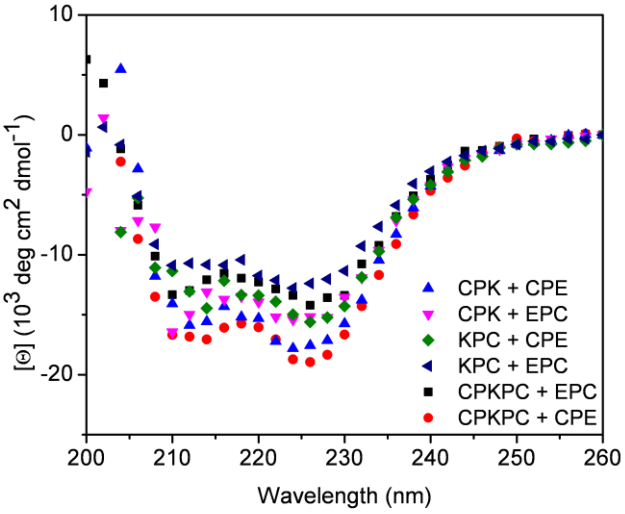

Figure S1. CD data for a 1:1 mixture of E and K decorated liposomes. Total lipid concentration 0.25 mM and 3 mol% lipidated peptide, in PBS.

Control experiments for lipid mixing

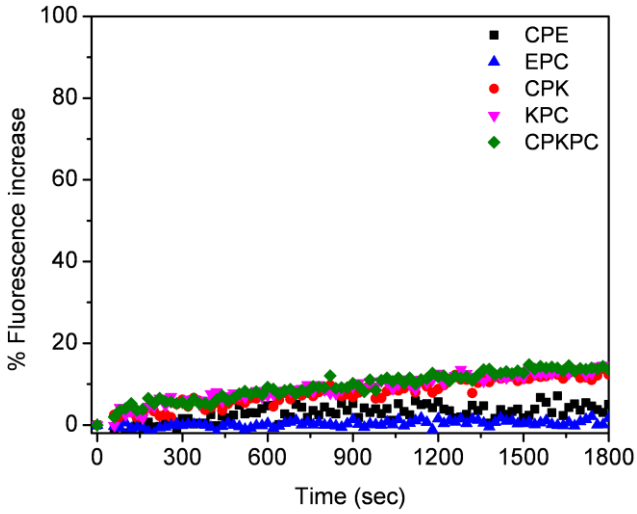

Figure S2. Lipid mixing between E or K decorated liposomes and plain liposomes, as indicated by an increase in NBD fluorescence. Non-fluorescent liposomes (0.1 mM) were added to fluorescent K liposomes (0.1 mM, 1% peptide E or K).

Control experiments for content mixing

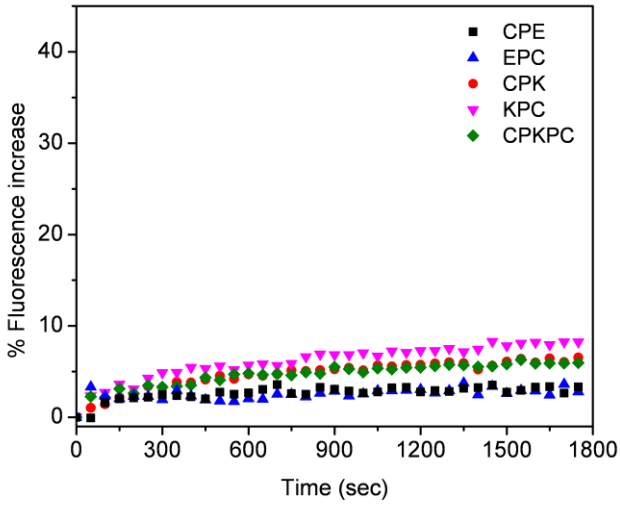

Figure S3. Content mixing between E or K decorated liposomes and sulphorhodamine loaded (20 mM) plain liposomes, as indicated by an increase in sulphorhodamine emission. Non-fluorescent liposomes (0.1 mM) were added to fluorescent K liposomes (0.1 mM, 1% peptide E or K).