Supporting Information

A non-zipper-like tetrameric coiled coil promotes membrane fusion

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LC-MS spectra

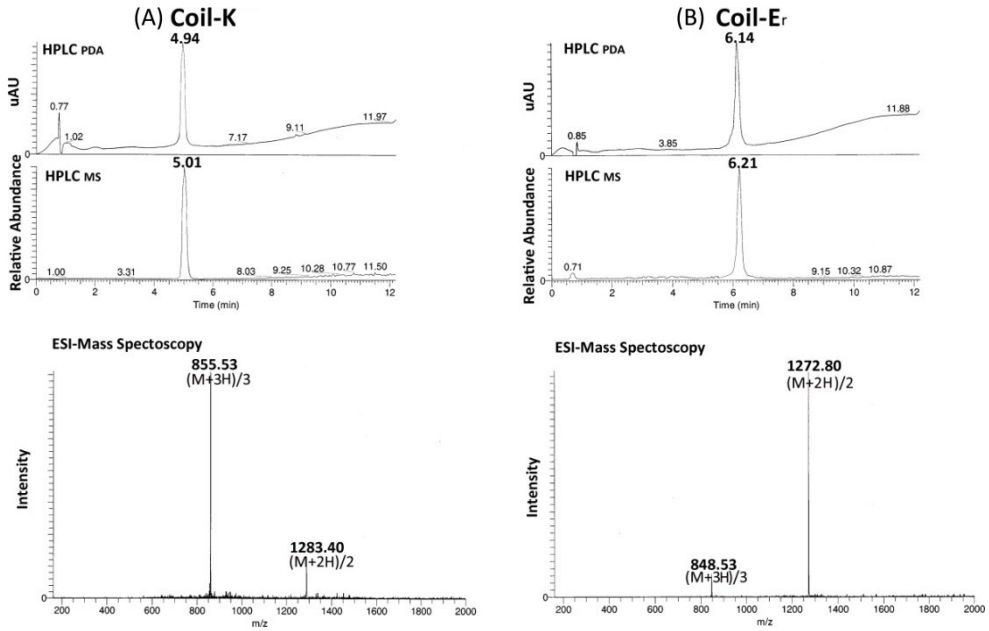

Figure S1. LC-MS spectra of: (A) Coil-K, (B) Coil-Er. Upper panels: UV-Vis (top) and MS (bottom) traces, lower panels: ESI (electrospray ionization) spectrum.

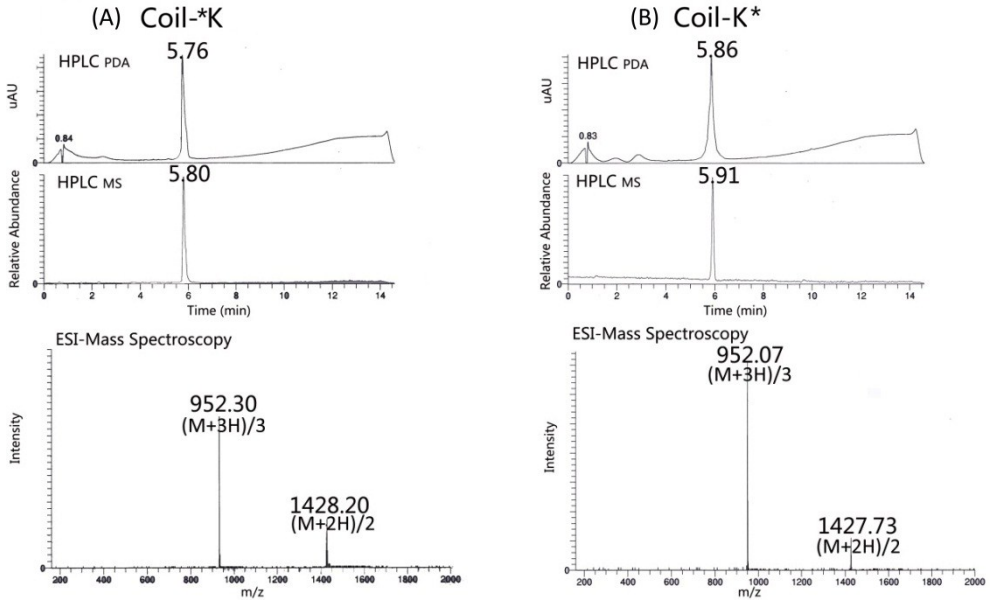

Figure S2. LC-MS spectra of: (A) Coil-*K, (B) Coil-K*. Upper panels: UV-Vis (top) and MS (bottom) traces, lower panels: ESI (electrospray ionization) spectrum.

Full range ^{1}H-NMR spectra of peptides

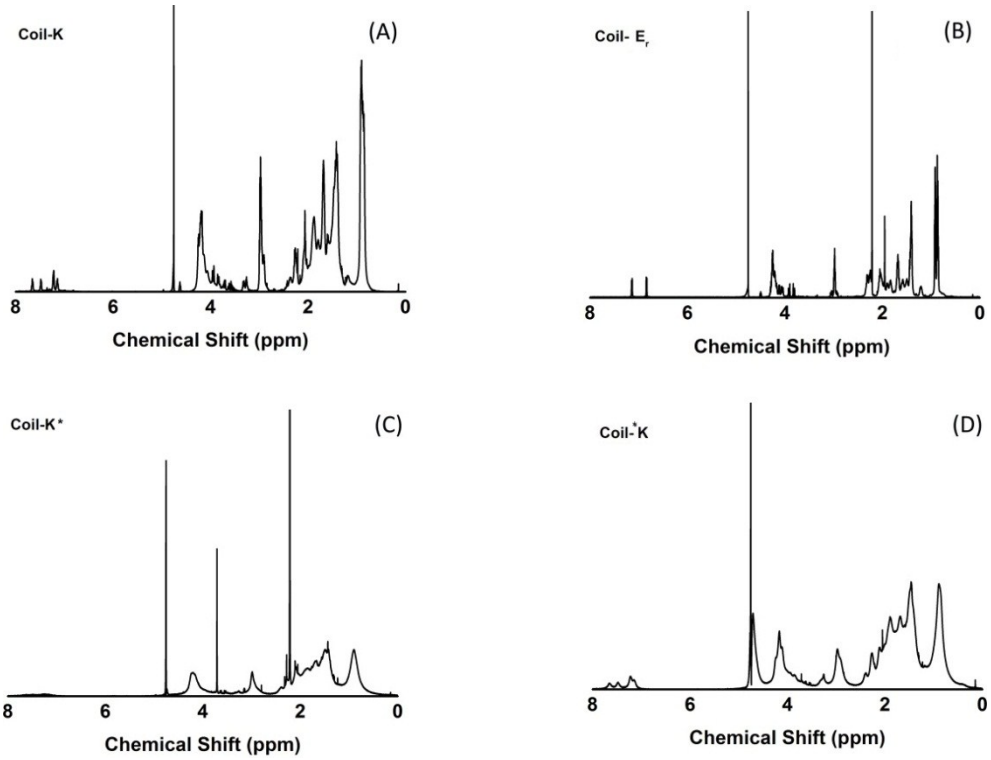

Figure S3. 600 MHz ^{1}H-NMR spectra (0-8 ppm) of the individual peptides. (A) Coil-K, (B) Coil-Er, (C) Coil-K*, (D) Coil-*K. [Total peptide]= 0.8 mM, PBS, pH=7.4.

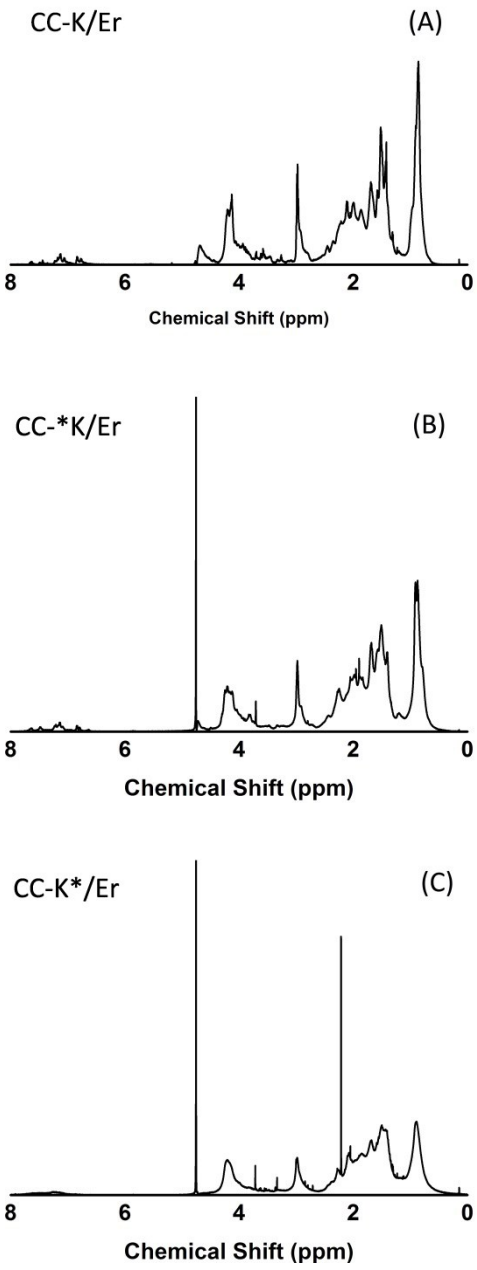

Figure S4. 600 MHz ^{1}H-NMR spectra, (0-8 ppm), of the various CC-K/Er complexes. (A) CC-K/Er, (B) CC-*K/Er, (C) CC-K*/Er. [Total peptide]= 0.8 mM, PBS, pH=7.4.

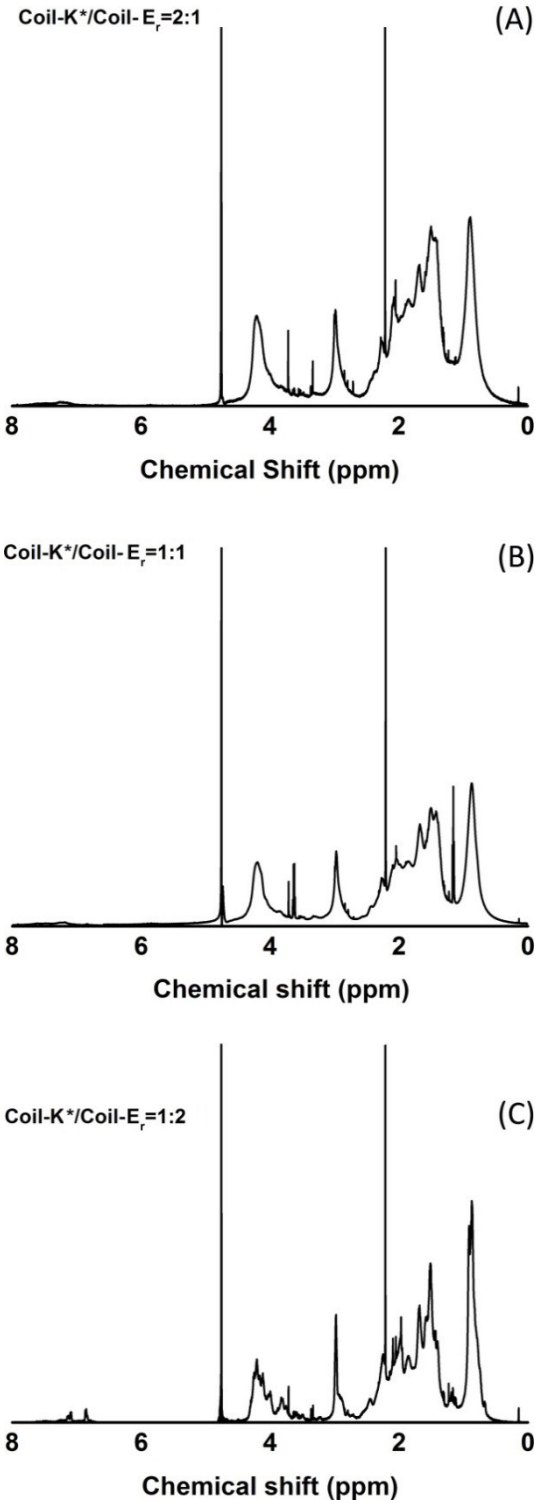

Figure S5. 600 MHz ^{1}H-NMR spectra of peptide complex stoichiometry measurements (0-8 ppm). (A) CC-K*:Er (2:1), (B) CC-K*:Er (1:1), (C) CC-K*:Er (1:2). [Total peptide]= 0.8 mM, PBS, pH=7.4.

Sedimentation Equilibrium

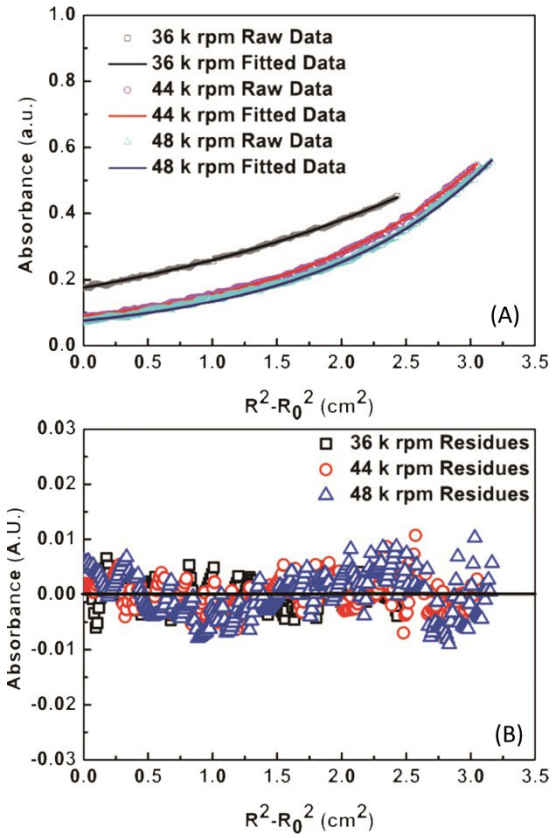

Figure S6. Analytical ultracentrifugation data for the CC-K/Er species (A) and residuals from the fits (B).

Molecular simulations

The coarse-grained molecular dynamics simulations were performed with the Gromacs package, version 4.5.3, using the known Martini force fields.[1] In the Martini model, groups of atoms (typically four) are united into specific interaction centres that absorb all the molecular detail of the replaced atoms. The coarse-grained particles interact via Lennard-Jones potentials (with different well-depth parameters depending on the specific pair type), and screened electrostatic Coulomb potentials, while the connectivity of the molecules is modelled by elastic bonds and angle potentials. By reducing the number of particles and the complexity of the interactions between them, longer simulation times can be achieved. Each of the five types of amino acids: I, L, A, E, and K, constituting either Coil-K or Coil-Er, is described at the coarse-grained level by an apolar interaction site representing the backbone and one or more interaction sites representing the side chains. The superposition of the atomistic and the coarse-grained representations of the CC-K/E and CC-K/Er heterodimers are shown in Figure S9. The interaction parameters (hydrophobicity and polarity) for the coarse-grained particles have been set to closely reproduce the difference between particle solvation free energy in polar and apolar media. The α-helicity of the peptides is imposed through dihedral potentials along the backbone beads during the simulations.

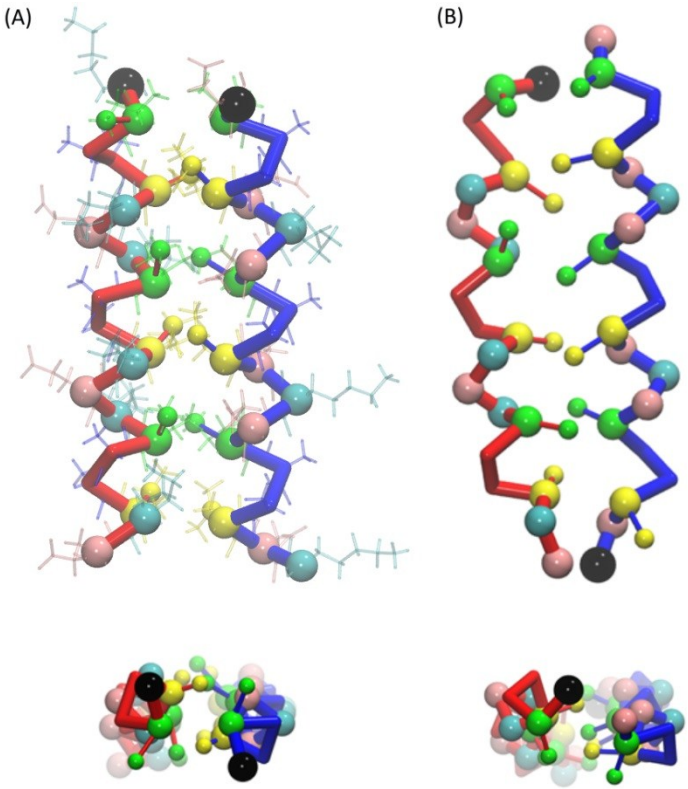

Figure S7. Models of the CC-K/E and CC-K/Er heterodimers. (A) Lateral and top views of CC-K/E, as reported by Hodges' group from NMR data.[2] (B) Lateral and top views of CC-K/Er, obtained from simulations. The red backbone represents Coil-K; while the blue backbone shows Coil-E or Coil-Er. Pink beads are glutamic acid (E); cyan beads represent lysine (K); green beads show isoleucine (I); and yellow beads give the positions of leucine (L) residues. Only the CG side-chains of I and L are shown, the rest are omitted for clarity. Alanine (A) is also omitted for clarity. For each peptide, the starting amino acid on N-terminus is coloured black. In the lateral view of CC-K/E, the atomistic structure is overlaid for completeness.

In a typical simulation for this study, two Coil-K and two Coil-Er peptides are randomly distributed (position and rotation randomness) in an 11 x 11 x 11 nm simulation box and solvated by water and ions, mimicking the buffer solution. At completion, the system consists of ~10000 coarse-grained particles: four peptides (21 amino acids for each), water particles (one coarse-grained water particle representing four real water molecules) and ions (Na^+ and Cl^-). Periodic boundary conditions in all directions were employed. Standard MARTINI simulations were used.[1b] The Berendsen thermostat and barostat kept the temperature (t = 300 K) and pressure (P = 1 atm) constant; the integration time step was t = 20 fs.

Fusion Data – Control Experiments

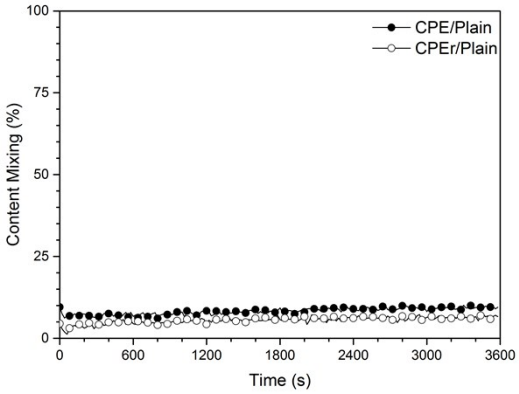

Figure S8. Control experiments for content-mixing. Sulforhodamine B filled Coil-E (solid circles) and Coil-Er (hollow circles) liposomes, mixed with plain liposomes, illustrating there is no fluorescence increase in the absence of Coil-K liposomes indicating the liposomes are not leaky and both peptides are required for fusion.

References

[1] a) B. Hess, C. Kutzner, D. van der Spoel, E. Lindahl, *J. Chem. Theor. Comput.* **2008**, *4*, 435-447; b) S. J. Marrink, H. J. Risselada, S. Yefimov, D. P. Tieleman, A. H. de Vries, *J. Phys. Chem. B,* **2007**, *111*, 7812-7824; c) L. Monticelli, S. K. Kandasamy, X. Periole, R. G. Larson, D. P. Tieleman, S. J. Marrink, *J. Chem. Theor. Comput.* **2008**, *4*, 819-834.

[2] J. R. Litowski, R. S. Hodges, *J. Biol. Chem.* **2002**, *277*, 37272-37279.