SUPPORTING INFORMATION

Tuning Liposome Membrane Permeability by Competitive Coiled Coil Heterodimerization and Heterodimer Exchange

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1. Peptide sequences and helical wheel

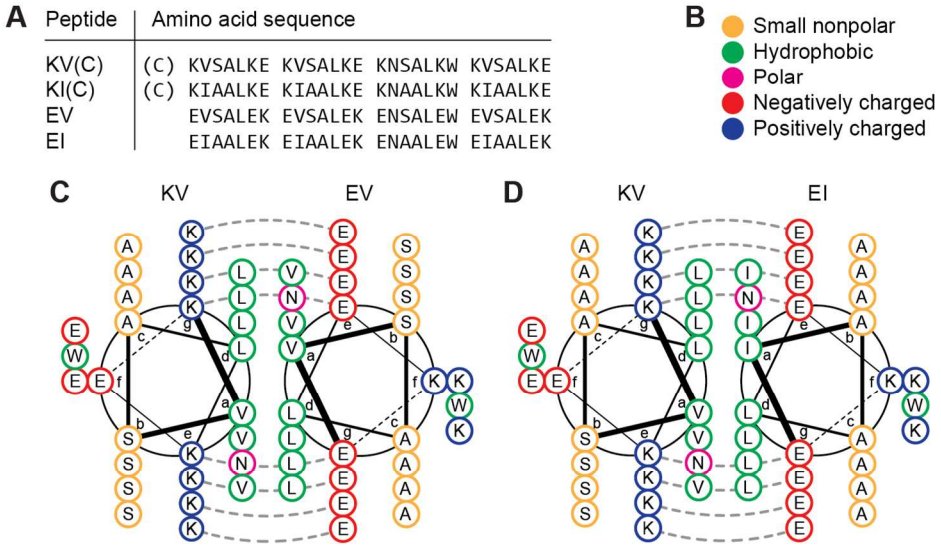


Figure S1: A) Primary structure of the peptides KV/KVC, KI/KIC, EV and EI. B) Amino acid color coding according to Lesk.[1] C) Helical wheel diagram of the heterodimer EVKV and D) EIKV drawn with DrawCoil.[2]

2. Lipids and liposome composition

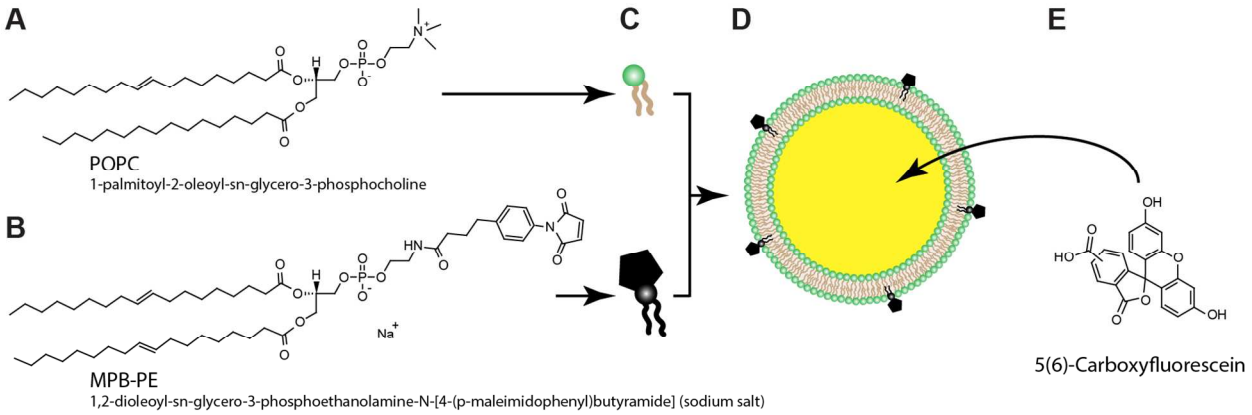


Figure S2: Chemical structures of the lipids A) POPC and B) MPB-PE used to form liposomes in compositions of 0, 1 and 5 mol% MPB-PE. C) Illustrations symbolizing the two lipids and D) a 2D representation of the formed liposome and the yellow interior represents the encapsulated fluorophore E) 5(6)-carboxyfluorescein (CF). This illustration is a simplification and not to scale.

3. CF release of KIC and KVC during 2 h

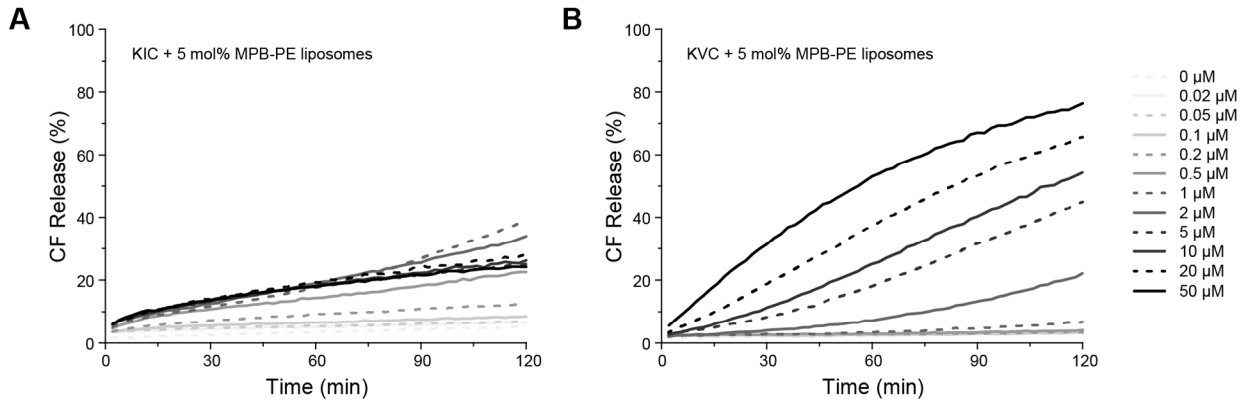


Figure S3: The CF release for A) KIC and B) KVC allowed to conjugate to 5 mol% MPB-PE liposomes measured every 2 min for 2 h in PBS. These measurements are single measurements from Figure 1B-C. The total lipid concentration was 40 µM with a peptide:maleimide ratio ranging from 0:0-25:1.

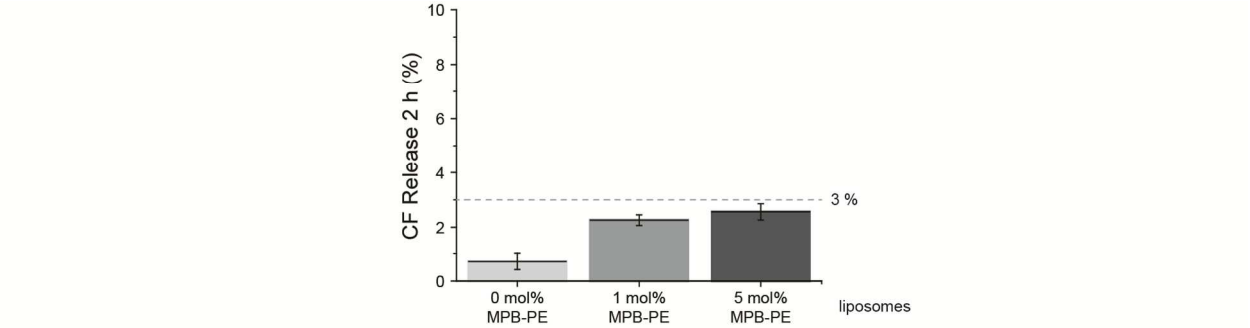


Figure S4: Spontaneous release of CF after 2 h from liposomes with 0, 1 and 5 mol% MPB-PE.

4. CF release with peptides without Cys

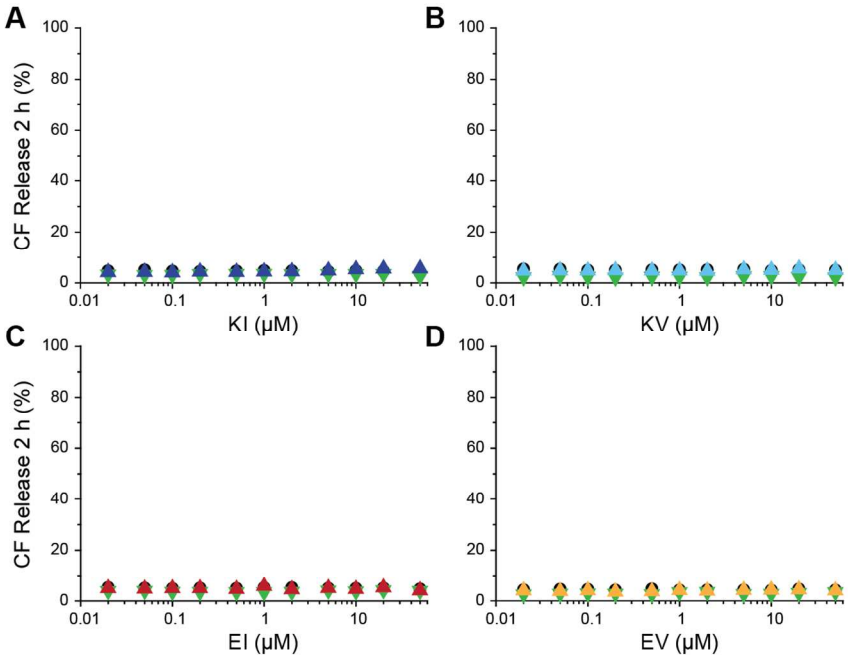


Figure S5: Control experiments showing release of CF after 2 h upon addition of 0.01-50 μM A) KI, B) KV, C) EI and D) EV to 5 mol% (blue/light blue/red/orange triangle), 1 mol% (green triangle) and 0 mol% (black circle) MPB-PE liposomes and a total lipid concentration of 40 μM in PBS.

5. CF release with partly oxidized KIC and KVC

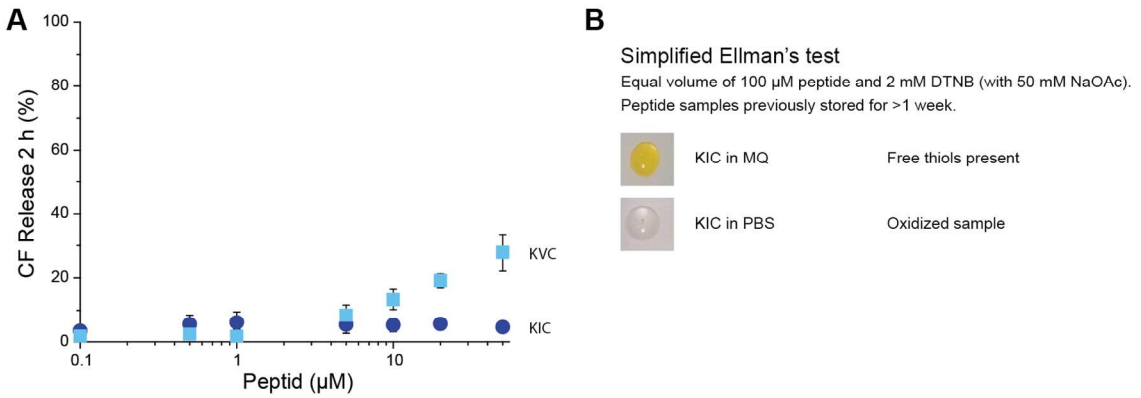


Figure S6: A) CF release after 2 h incubation with oxidized peptides using KIC (dark blue circle) and KVC (light blue square) incubated in PBS > 1 week prior to the experiment. B) Ellman's test showing the extent of oxidation of KIC after incubation in slightly acidic conditions (MQ water) or PBS. The total lipid concentration was 40 μM with a peptide:maleimide ratio ranging from 0:0-25:1.

6. Oxidation rate and folding of KIC and KVC

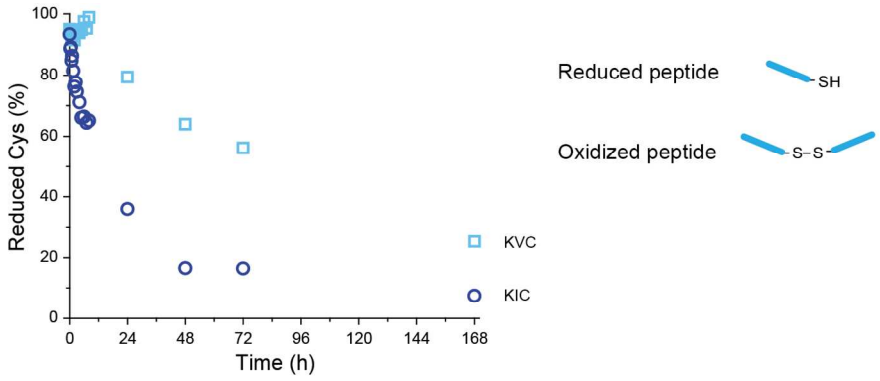


Figure S7: The amount of reduced Cys in the peptide was measured with an Ellman's test and a calibration curve of pure Cys was used to calculate the percentage of reduced Cys in KIC (dark blue circles) and KVC (light blue squares).

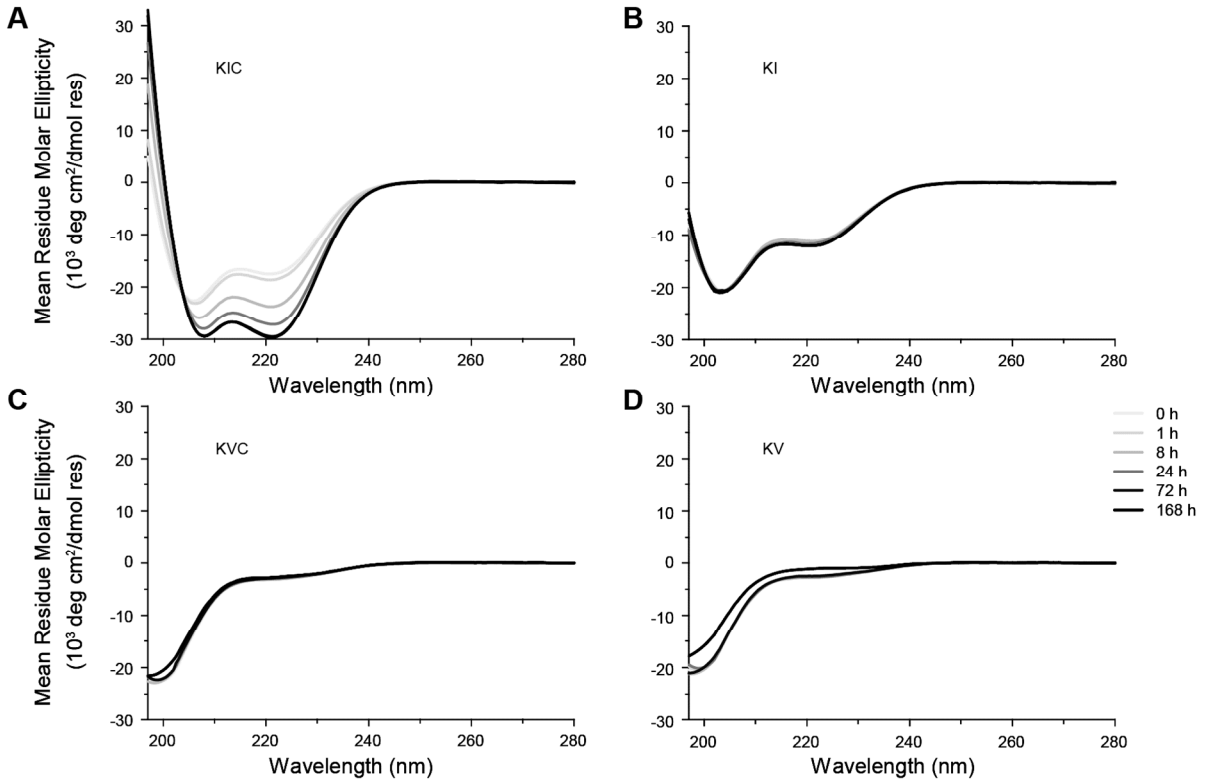


Figure S8: Circular dichroism (CD) measurements of 100 µM A) KIC, B) KI, C) KVC and D) KV in PBS after 0, 1, 8, 24, 72 and 168 h incubation.

7. Size distribution of 5 mol% MPB-PE liposomes with KVC

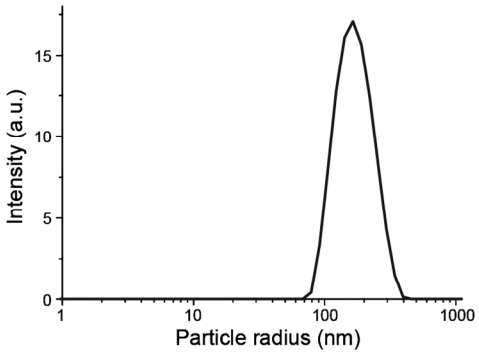
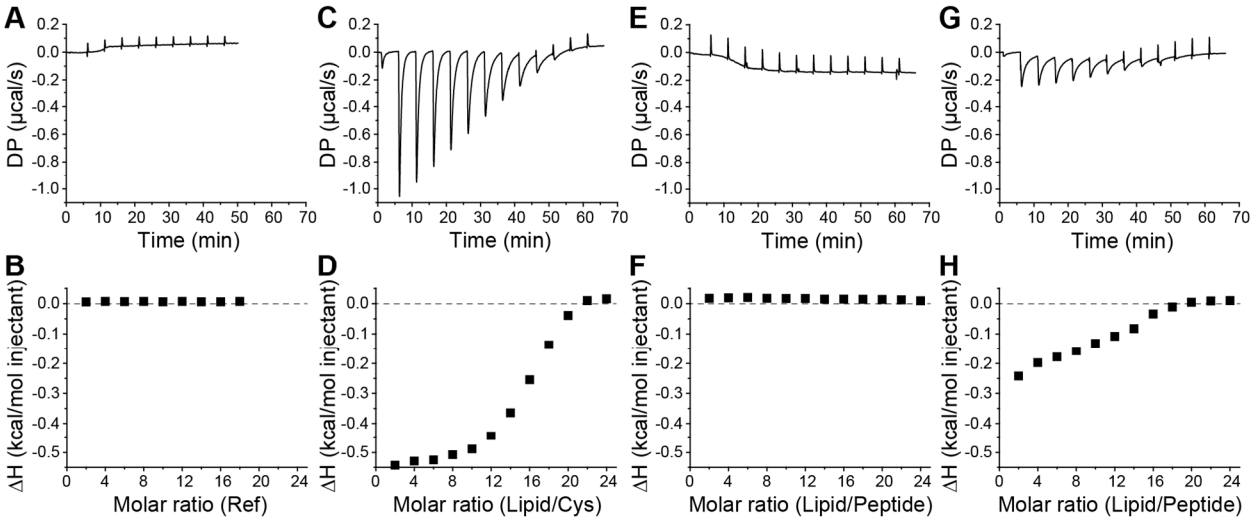


Figure S9: DLS measurement of liposomes with 5 mol% MPB-PE and 95% POPC incubated with 50 µM KVC for 4 h. The total lipid concentration was 40 µM and the peptide:maleimide ratio was 25:1. The size distribution was calculated using the cumulant model and the plotted data was number fitted to better show presence of any potential smaller fragments.

8. Interaction evaluation of KVC + liposomes



Figur S10: ITC measurements with 3 µL injections. A, B) PBS titrated into PBS and C, D) 150 µM Cys titrated into 30 mM 5 mol% MPB-PE liposomes. Samples 150 µM KVC titrated into E, F) 30 mM 0 mol% MPB-PE and G, H) 30 mM 5 mol% MPB-PE liposomes. The molar ratio described in the graphs were of total lipid content. The point corresponding to 1:1 peptide:maleimide was a molar ratio of 20 (5 mol% MPB-PE liposomes).

9. Estimated free KVC in heterodimer formation

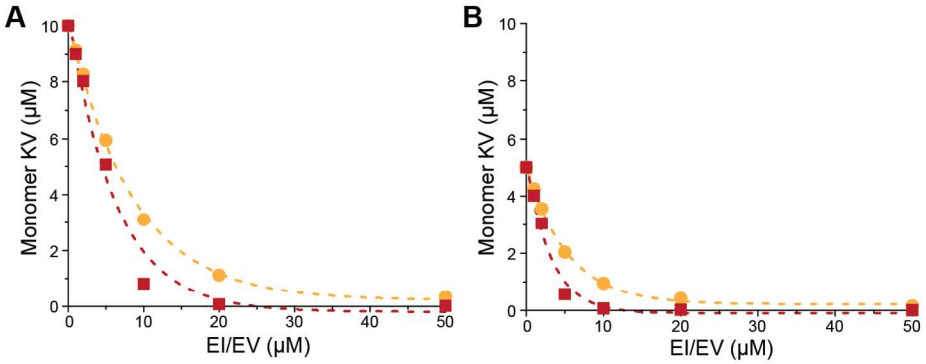


Figure S11: Estimation of free monomer KV when EI (red) or EV (orange) was added to A) 10 µM KV and B) 5 µM KV. The calculations were based on K_d (EIKV) = 0.072 µM and K_d (EVKV) = 1.4 µM at 20 °C.[3]

1. Heterodimerization of KVC-functionalized liposomes and EV

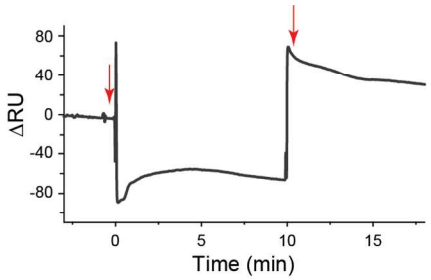


Figure S12: SPR data showing EV interacting with KVC-functionalized liposomes extracted from Figure 3A. The reference was the EV injection before KVC-functionalization, i.e. the interaction of EV with unfunctionalized liposomes, which was subtracted from the EV injection after KVC-functionalization to generate this graph where t = 0 min was the start of each EV injection. All SPR data was treated similarly to generate Figure 3C. The amount of associated EV was calculated as the difference in ΔRU before and after the injection, as indicated by the arrows.

2. Heterodimer exchange EVCKI + EI/EV → EVC + EIKI/EVKI

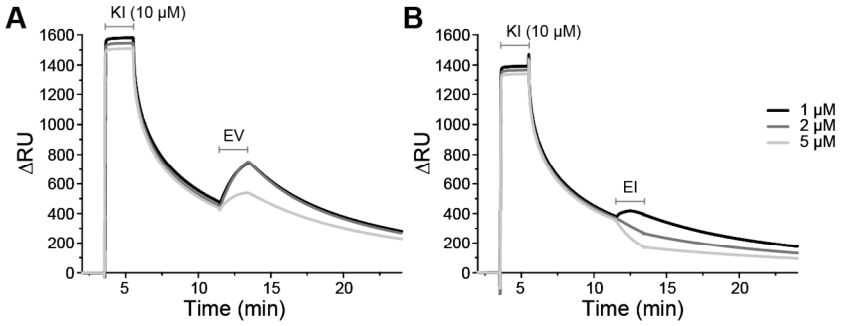


Figure S13: KI (10 μM) introduced to a Biacore CM5 chip with immobilized with EVC followed by a subsequent addition of 1, 2 and 5 μM EI/EV. Heterodimers are present on the sensor chip after injection of KI. The subtracted reference with only Cys on the surface showed no binding of either EI, EV or KI (not shown).

[1] Lesk, A. M. *Introduction to bioinformatics*. 1st edn (Oxford University Press, 2002)

[2] Grigoryan, G.; Keating, A. E. Curr. Opin. Structural Specificity in Coiled-Coil Interactions *Curr. Opin. Struct. Biol.* **2008**, 18 (4), 477–483.

[3] Aronsson, C.; Dånmark, S.; Zhou, F.; Öberg, P.; Enander, K.; Su, H.; Aili, D. Self-sorting Heterodimeric Coiled Coil Peptides with Defined and Tuneable Self-assembly Properties *Sci. Rep.* **2015**, 5, 14063