Membrane-Fusogen Distance is Critical for Efficient Coiled-Coil-Peptide-Mediated Liposome Fusion

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Supporting Information

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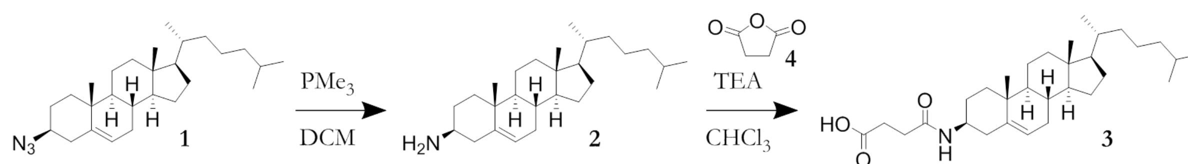
Synthetic procedure for Cholesteryl-amino-hemisuccinate



Scheme S1. Synthetic procedure towards cholesteryl- 4-amino-4- oxobutanoic acid (3). 3-azido-5-cholestene (1) is reduced to 3-amino-5-cholestene (2), which in turn is condensated with succinic anhydride (4) to yield final cholesterol anchor 3.

3-Amino-5-cholestene (2): To a solution of 3-Azido-5-cholestene (1) (15.8 g, 38.4 mmol) in dichloromethane, 4 equivalents of trimethylphosphine (1M solution in toluene) was added and the reaction was stirred overnight. When 3-Azido-5-cholestene was consumed as evidenced by TLC (eluent: hexane), the reaction was concentrated *in vacuo* to yield 2 (14.8 g, 100%) as a pure compound. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.27 (s, 1H), 2.60 (m, 1H), 2.17-0.85 (m, 40H), 0.64 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 141.8, 120.7, 56.8, 56.2, 52.0, 50.3, 43.4, 42.3, 39.8, 39.5, 38.2, 36.6, 36.2, 35.8, 32.7, 31.9 (× 2), 28.3, 28.0, 24.3, 23.9, 22.8, 22.6, 21.0, 19.5, 18.7, 11.9; IR (film) ν 3354, 3260, 3154, 2936, 2896, 2849, 1464, 1437, 1381 cm-1; LRMS (ESI^{+}) *m/z* 386.4 ([M+H]$^{+}$, $C_{27}H_{45}N$ calcd. 386.4).

Cholesteryl-4-amino-4-oxobutanoic acid (3): To a solution of **2** (10g, 26 mmol) in chloroform (150mL), **4** (7.8g, 78 mmol) was added. Triethylamine (8mL, 57 mmol) was added drop-wise to the solution and the reaction mixture was stirred overnight under nitrogen atmosphere at room temperature, until the starting material was completely consumed as evidenced by TLC (eluent: hexane: ethylacetate 1:1). The reaction mixture was transferred to a separatory funnel, and the organic layer was washed with water and brine, was dried with $MgSO_4$ and was concentrated in *vacuo* to afford cholesterol amide acid (10g) as a yellow solid. The crude cholesterol amide acid was purified by recrystallization in Acetonitrile: MeOH (1:1) at 4°C and was vacuum filtered to afford **3** as an off-white solid (8.7g, 76%) ^{1}H NMR (400MHz, $CDCl_3$) δ 5.36 (s, 1H), 3.69 (s, 1H), 3.60 (m, 1H) 2.62 (m, 2H), 2.44 (m, 2H), 2.20-1.72 (m, 7H), 1.67-1.03 (m, 20H)1.01 (s, 3H) 0.92 (d, *J* = 6.5 Hz, 3H), 0.87 (dd, *J* = 6.6, 1.7 Hz, 6H), 0.69 (s, 3H).TLC-MS (ESI^{+}) *m/z* 486.4 ([M+H]$^{+}$, $C_{31}H_{51}NO_3$ calcd. 486.4 g/mol).

Lipopeptide analysis

Purity was confirmed using LC-MS analysis equipped with Gemini 3µ C18 column coupled with Finningan LCQ advantage max (Thermo) ESI-MS analyzer and the results are summarized in Table S1. LCMS eluents were A) H2O + 0.1% v/v TFA, B) MeCN + 0.1 v/v TFA, with a flow rate of 1 mL min^{-1}. Gradient was applied between 2 and 12 min, from 10% B in A to 90% B in A.

Table S1. Formulae and masses of of synthesized lipopeptides.

peptide	formula	Calc. mass	Found mass
CP_0E_3	$C_{144}H_{236}N_{26}O_{40}$	2971.57	2972.14, $[M+H]^+$
CP_0K_3	$C_{149}H_{252}N_{30}O_{33}$	2991.78	2992.60, $[M+H]^+$
CP_4E_3	$C_{154}H_{255}N_{27}O_{45}$	3204.83	3206.63, $[M+H]^+$
CP_4K_3	$C_{159}H_{271}N_{31}O_{38}$	3225.04	1611.48, $[M+2H]^{+2}$
CP_8E_3	$C_{163}H_{273}N_{27}O_{49}$	3395.06	1696.40, $[M+2H]^{+2}$
CP_8K_3	$C_{168}H_{289}N_{31}O_{42}$	3415.28	1706.40, $[M+2H]^{+2}$
$CP_{12}E_3$	$C_{171}H_{289}N_{27}O_{53}$	3571.27	1190.53, $[M+3H]^{+3}$
$CP_{12}K_3$	$C_{176}H_{305}N_{31}O_{46}$	3591.49	1197.07, $[M+3H]^{+3}$
$CP_{16}E_3$	$C_{179}H_{305}N_{27}O_{57}$	3747.48	1249.27, $[M+3H]^{+3}$
$CP_{16}K_3$	$C_{184}H_{321}N_{31}O_{50}$	3767.70	1255.93, $[M+3H]^{+3}$
LP_0E_3	$C_{147}H_{256}N_{26}O_{43}P^-$	3106.78	1563.44, $[M+H+NH_4]^{+2}$
LP_0K_3	$C_{150}H_{271}N_{29}O_{37}P^-$	3103.96	1553.01, $[M+2H]^{+2}$
LP_2E_3	$C_{153}H_{267}N_{27}O_{46}P^-$	3251.94	1084.98, $[M+3H]^{+3}$
LP_2K_3	$C_{156}H_{282}N_{30}O_{40}P^-$	3249.12	1084.03, $[M+3H]^{+3}$
LP_4E_3	$C_{157}H_{275}N_{27}O_{48}P^-$	3340.04	1114.33, $[M+3H]^{+3}$
LP_4K_3	$C_{160}H_{290}N_{30}O_{42}P^-$	3337.22	1113.72, $[M+3H]^{+3}$
LP_8E_3	$C_{166}H_{293}N_{27}O_{52}P^-$	3530.28	1178.04, $[M+3H]^{+3}$
LP_8K_3	$C_{169}H_{308}N_{30}O_{46}P^-$	3527.46	1177.09, $[M+3H]^{+3}$
$LP_{12}E_3$	$C_{174}H_{309}N_{27}O_{56}P^-$	3706.49	1236.74, $[M+3H]^{+3}$
$LP_{12}K_3$	$C_{177}H_{324}N_{30}O_{50}P^-$	3703.67	1235.79, $[M+3H]^{+3}$

Full data of 30min content mixing assay

Content mixing experiments were followed for 30 minutes in a continuous fashion and showed characteristic 1st order kinetics in the initial minutes (*Figure S1, and insets*). Therefore, fluorescence increase after 4 minutes is used to estimate the relationship of L_{PEG} and the fusion efficiency. Error bars and absolute values after 4 and 30 minutes were obtained by at least 3 individual experiments and are shown in Figure S, as a function of *m, n*. In representative negative control experiments, peptide-containing liposomes (m, n = 4 or 8) were mixed with plain liposomes and the fluorescence increase was recorded for 30 minutes.

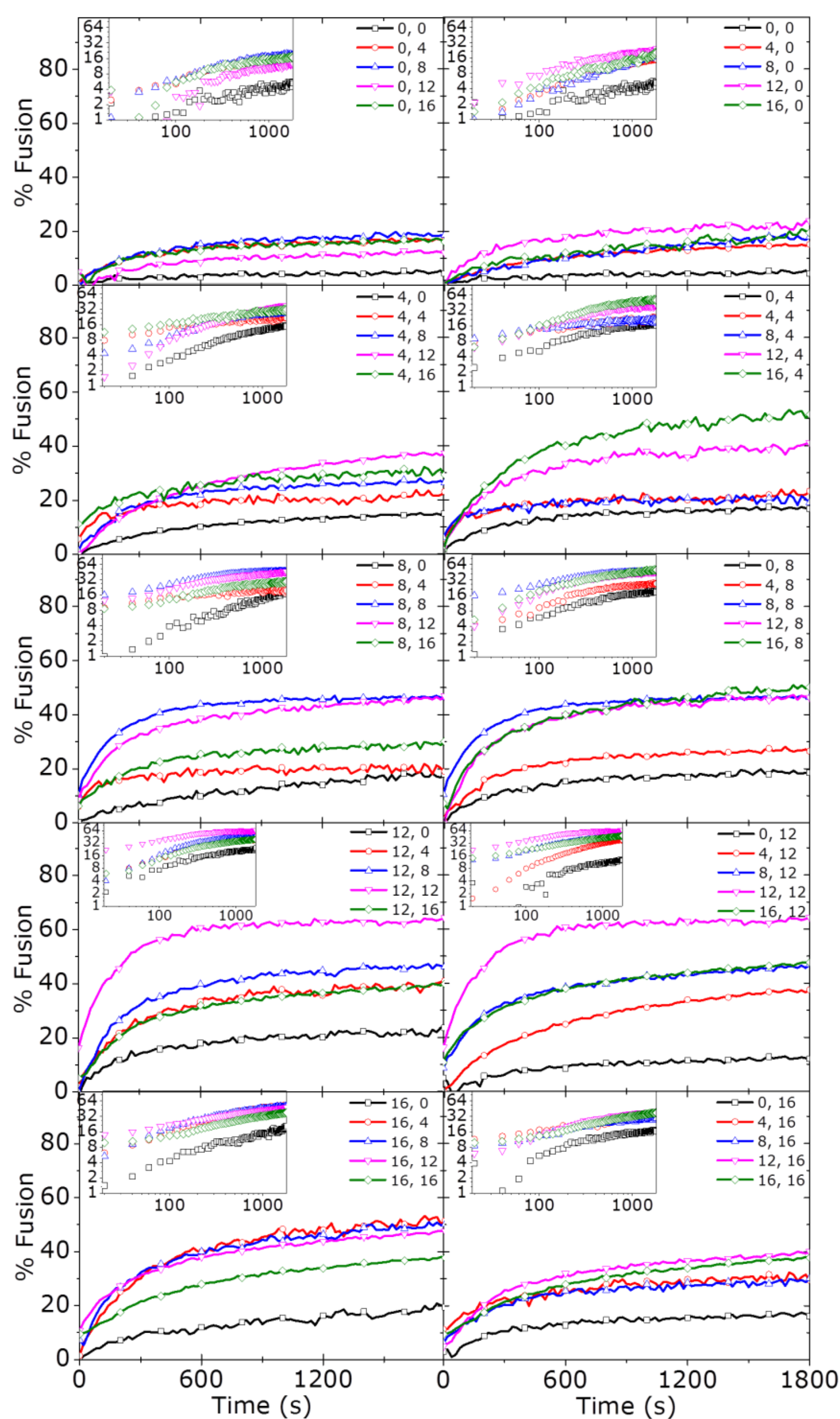


Figure S1. Content mixing of CP_mE_3 and CP_nK_3 decorated liposomes as a function of *m*, *n*. Insets with log scale illustrate 1st order kinetics until at least the 4th minute. [Total lipid] = 0.1 mM with 1 mol% lipopeptide, PBS pH 7.4.

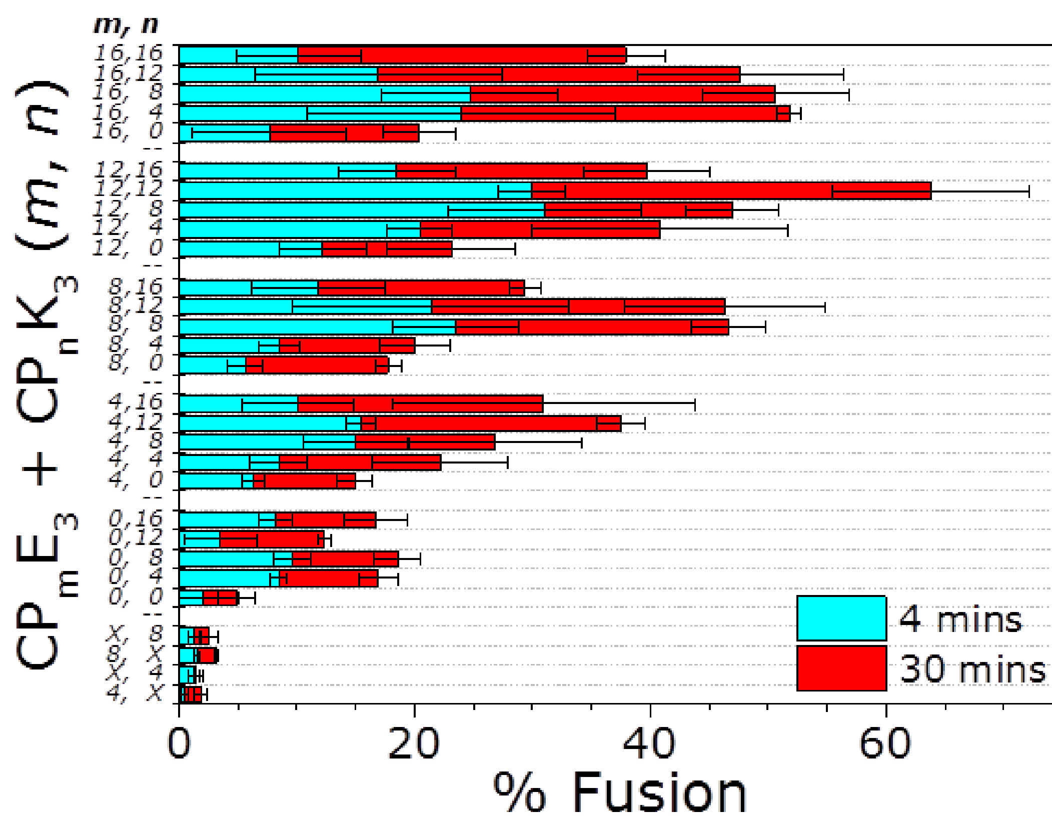


Figure S2. Time resolved rate of fusion (content mixing) with lipopeptide CP_mE_3 or CP_nK_3 decorated liposomes as a function of *m*, *n*, indicated by an increase in sulforhodamine B emission after 4 and 30minutes. For control experiments the omitted peptide-functionalized liposomes are represented by *X*. [Total lipid] = 0.1 mM with 1 mol% lipopeptide, in PBS pH 7.4.

Influence of CPE / CPK ratio on fusion efficiency

A change in peptide functionalization ratio reveals the influence of peptide equilibria on the fusion process. Again, results are shown as a function of *m, n*. (Figure S2)

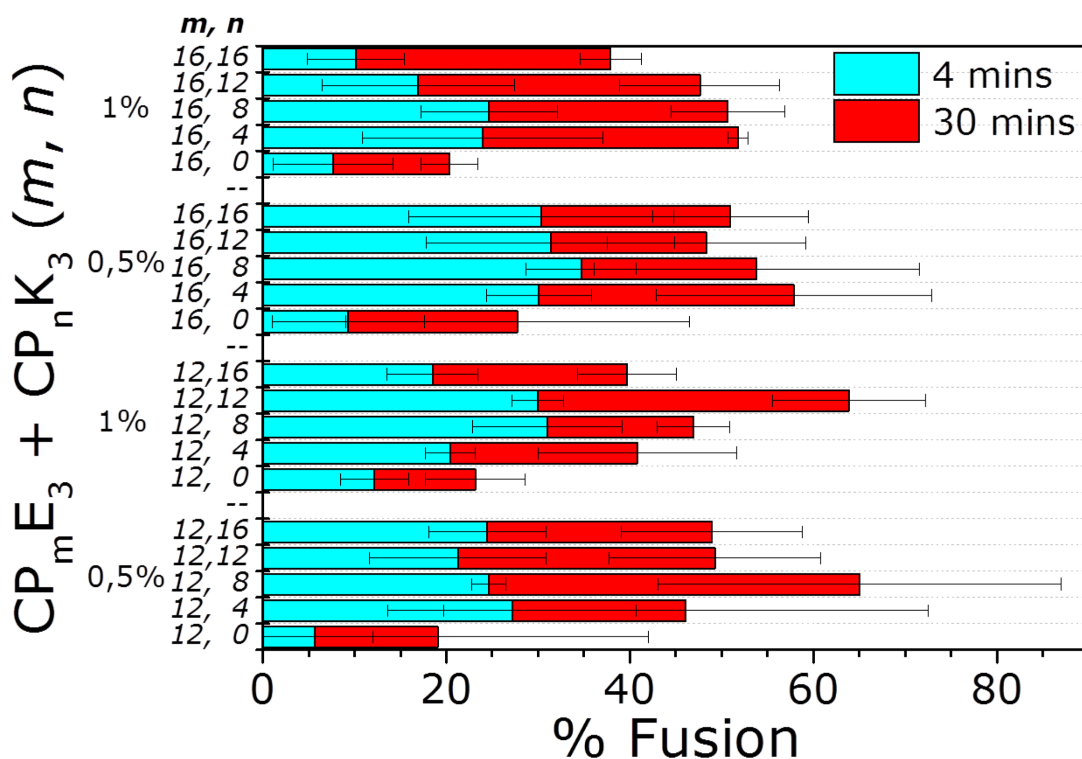


Figure S2. Time resolved rate of fusion (content mixing) with peptide CP_mE_3 and CP_nK_3 decorated liposomes as a function of m,n, and [CP_mE_3], as indicated by an increase in sulforhodamine B emission after 4 and 30 minutes. [Total lipid] = 0.1 mM with 1 mol% CP_nK_3 and 1 mol% or 0.5 mol% CP_mE_3, PBS pH 7.4.

CD spectroscopy

Characteristics ($\theta_{222}/\theta_{208}$ and percentage helicity at θ_{222}) of the spectra as summarized in Table 2, are supported with the recorded spectra between 207nm and 250 nm (*Figure S3*). Below 207 nm, spectra were too noisy due to scattering of the signal by liposomes.

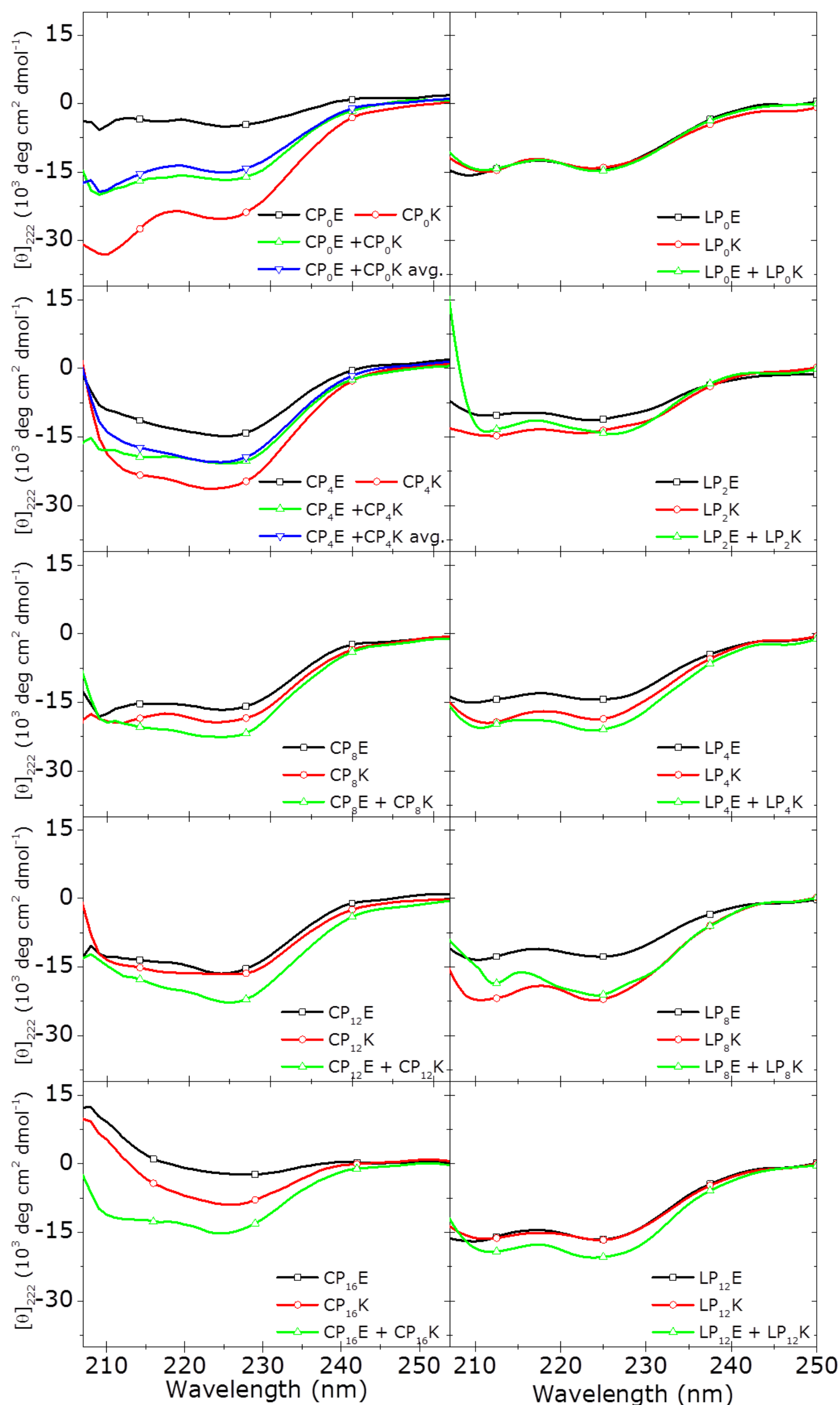


Figure S3. Peptide secondary structure of E- (black) and K- (red) decorated liposomes before and after mixing (green), measured by circular dichroism. Left series is with cholesterol anchor, right series contain a DOPE anchor. Only symmetric pairs ($m = n$) are used in this study. With CP_0 and CP_4 anchor coiled coil formation is not evident, so the calculated average of the individual E_3 and K_3 spectra is added (blue). [Total lipid] = 0.5 mM with 1mol% lipopeptide, PBS pH 7.4, and cuvette path length of 5mm.