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Supporting Information

Coiled Coil Driven Membrane Fusion between Cyclodextrin Vesicles and Liposomes

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Table of contents

Materials 2

Methods 2

Figure S1 Structure of APK 2

Figure S2 Structure of CPE 2

Figure S3 Structure of amphiphillic CD's 2

Table S1: Mass spectrometric data 3

Characterisation 3

Figure S4: LC data of APK and CPE 5

Figure S5: ITC titration curves of APK/β-CD binding 5

Figure S6: Structures of DOPE-LR and DOPE-NBD 5

Figure S7: Content mixing data 6

Figure S8: Inner leaflet content mixing 6

Figure S9 & Figure S10: Cryo-TEM tilt series of liposome/CDV hybrids 7

References 8

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. Cholesteryl hemisuccinate and adamantane 1-carboxylic acid were purchased from Sigma Aldrich. Solvents were obtained from Biosolve Ltd and used as received.

METHODS

Synthesis of APK [1], CPE[1] and amphiphilic β-CD[2]

The synthesis of the lipidated peptides CPE and APK has been described in detail previously.[1] Briefly, peptides E and K were synthesized on a fully automated peptide synthesizer, using a sieber amide resin and standard solid phase peptide chemistry. The peptides were pegylated by addition of Fmoc-PEG_{12}-COOH to the resin, using HOBT and DIC as reactants. Using the same reactants the hydrophobic anchors adamantane and cholesterol were covalently conjugated to the pegylated peptides. Purification of the lipidated peptides was performed by reverse phase HPLC using a C18 column.
The synthesis of the amphiphilic cyclodextrin has been reported previously.[2] The primary hydroxylfunctionality of the β-CD rim was first converted into the corresponding per-chlorinated species using MsCl followed by a thioetherification with dodecylthiol in the presence of NaH. The amphiphillic cyclodextrin was finally obtained by a grafts polymerization with ethylenecarbonate. All spectroscopic data were in good agreement with those reported before.

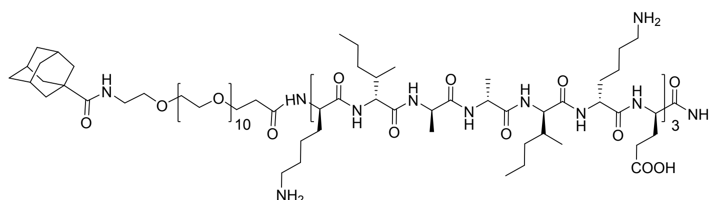

Figure S1: Structure of APK

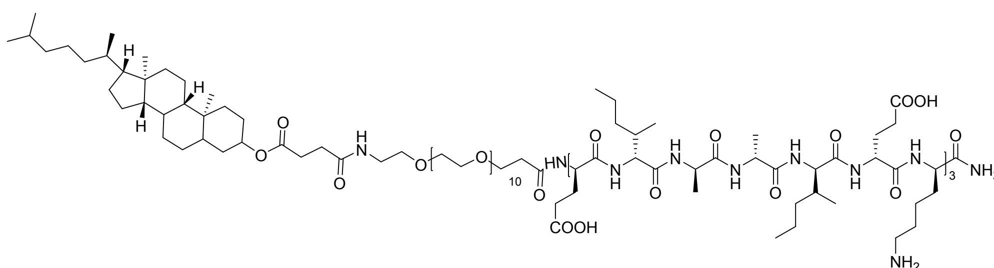

Figure S2: Structure of CPE.

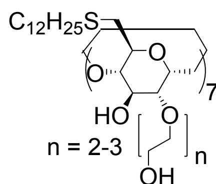

Figure S3: Structure of the amphiphillic cyclodextrins

Compound	Molecular formula	z	calculated	found
APK	$[C_{140}H_{244}N_{26}O_{47}H_3]^{3+}$	3	1013.91	1014.27
CPE	$[C_{160}H_{278}N_{26}O_{49}]^{2+}$	2	1675.00	1675.40
Amphiphillic CD	$[C_{162}H_{310}O_{46}S_7Na_2]^{2+}$ (for 18 ethyleneglycol units)	2	1632.30	1632.00

Table S1: Overview over obtained mass spectrometric values of all compounds used in this study.

Preparation of CPE decorated liposomes and APK decorated CDVs

The CPE decorated liposomes used in this study were composed of a ternary lipid mixture of DOPC/DOPE/CH (50:25:25 mol %) and 1 mol% of CPE. Appropriate amounts of lipid and lipidated peptide stock solutions (in $CHCl_3$ and a 1:1 mixture of $CHCl_3$: MeOH respectively) were mixed, the organic solvent was evaporated and the dry lipid layer was hydrated with phosphate buffer saline (PBS). Subsequent sonication for 1 min at 50°C yielded uniform distributions of liposomes with a diameter of ~100 nm, as shown by dynamic light scattering.
APK decorated liposomes were prepared by dissolving an appropriate amount of amphiphilic β-CD in chloroform, removing the solvent and hydration of the lipid layer with PBS. Sonication for 5 min at 50°C, followed by extrusion through a polycarbonate filter yielded vesicles, at a 0.1 mM concentration, with an average diameter of ~80 nm. Surface functionalization of these CDVs was achieved by the addition of an appropriate amount APK stock solution in PBS to the CDVs. Typically, 0.1 mM CDVs were used with 0.5 mol% APK, effectively decorating 1 mol% of the CD cavities at the outer layer of the vesicles.

CHARACTERISATION

Fluorescence measurements

Lipid mixing[1]

All spectra were obtained at room temperature using a quartz cuvette with a 1 cm path length. Liposomes consisting of DOPC/DOPE/CHOL/NBD-DOPE/RHD-DOPE (49.5/24.75/24.75/0.5/0.5 mol %) and 1 % of CPK where mixed with CDV's mixed with 0.5% of APK. The NBD fluorescence was used to calculate the lipid mixing percentage with time. Fluorescence time series measurements were started immediately after mixing 750uL of the fluorescent-labeled liposome suspension with 750uL of unlabeled liposome suspension in the cuvette. The NBD fluorescence intensity at 530 nm was monitored in a continuous fashion for 3000 seconds. After that the liposomes were lysed by the addition of 150 µL of 10 wt % Triton X-100 in PBS to obtain 100 % increments.
The values measured after lysis were multiplied by 1.82 to take into account the effect of Triton X-100 on the NBD fluorescence and dilution, which was obtained from a separate lysis experiment of a liposome solution that only contained DOPE-NBD. The percentage of fluorescence increase (%) is calculated as:

$$F(\%) = (F(t) – F0) / (Fmax \times 1.82 – F0) \times 100$$

where F(t) is the fluorescence intensity measured at time t, F0 is the 0% fluorescence and Fmax is the fluorescence intensity measured after addition of Triton X-100.

Content Mixing[1]

Content mixing experiments were carried out as follows: A dried film containing DOPC/DOPE/CH 50:25:25 mol% and CPE (1 % of CPE) were hydrated and sonicated (5 min at 50°C) with a sulforhodamine B (20 mM) containing HEPES buffer solution (20 mM HEPES, 90 mM NaCl) at pH 7,2. The final lipid concentration was 1 mM. To get rid of non encapsulated dye the liposomal solution was subjected to Sephadex (G50, Superfine) using HEPES (20 mM HEPES-Na, 90 mM NaCl) buffer as eluent. The fraction containing liposomes was collected and

diluted to a final liposome concentration of 0.1 mM. 400 µL of the E-Peptide containing liposomes with encapsulated sulforhodamine B were added to a small volume disposable cuvette. The fluorescence signal of the Sulforhodamine ($\lambda em = 580$ nm) was detected and another 400 µL of the corresponding CDV's (0.1 mM + 0.5% APK) in HEPES-buffer at pH = 7,2 were added and the increase of sulforhodamine B fluorescence, due to a relief of self quenching, was detected. After a certain time 100 µL of 10% (v/v) solution of Triton X was added to lyse the liposmes and reach the maximum dilution.

$F\% = (F(t) – F(0) / (F(max) - F0) \times 100$

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 K-Peptide containing liposomes.

Inner leaflet mixing

The inner leaflet lipid mixing assay was performed as follows. CPE decorated liposomes (2.5 mM final concentration, 1 mol% CPE), containing 0.5 mol% DOPE-LR and 0.5 mol% DOPE-NBD, were prepared as described earlier. The sample was transferred to a cuvette and the NBD fluorescence was measured (λ_{ex}=465 and λ_{em}=520). Next, a 20 mM solution of $Na_2S_2O_4$ in PBS was added to the cuvette. This resulted in a decrease of NBD fluorescence of around 60%, due to inactivation of NBD moieties in the outer lipid leaflet of the liposomes. Subsequently, the liposomes were purified with the aid of a sephadex G-50 column. Finally, these liposomes (diluted to 0.1 mM) were mixed with CDVs and APK (1 mol%) was added. The fluorescence was monitored for 30 minutes.
DLS measurements and CD measurements were performed as reported elsewhere.[1]

Cryo-TEM measurements

Sample vitrification was performed using an automated vitrification robot (FEI Vitrobot™ Mark III) for plunging in liquid ethane. Cu grids, R2/2 Quantifoil Jena grids (Quantifoil Micro Tools GmbH) were surface plasma treated for 40 seconds using a Cressington 208 carbon coater prior to use. For 2D imaging and tomography, samples were studied on the TU/e CryoTitan (FEI, www.cryotem.nl), equipped with a field emission gun (FEG) operating at 300 kV and a post column Gatan Energy Filter (GIF). Images were recorded using a post-GIF 2k x 2k Gatan CCD camera.

The alignment and 3-dimensional reconstructions of the data sets were performed in IMOD or Inspect3D v.3.0 (FEI Company). For the segmentation and visualization of the 3D volume, Amira 4.1.0 (Mercury Computer Systems) was used.

LC data for APK and CPE

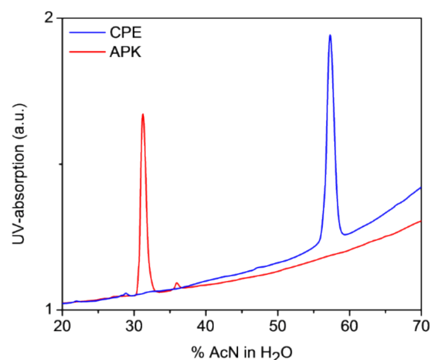

Figure S4. LC data of CPE and APK, showing the purity of the materials and their relative hydrophobicity.

ITC titration curves of APK/ β-CD binding

ITC titrations was performed on a MicroCal© isothermal titration calorimeter. The titration was performed in the inverse titration mode by filling a 1 mM β-CD solution in the syringe which is titrated into a 0.1 mM solution of APK. 25 injections were performed every 5 minutes at 25°C.

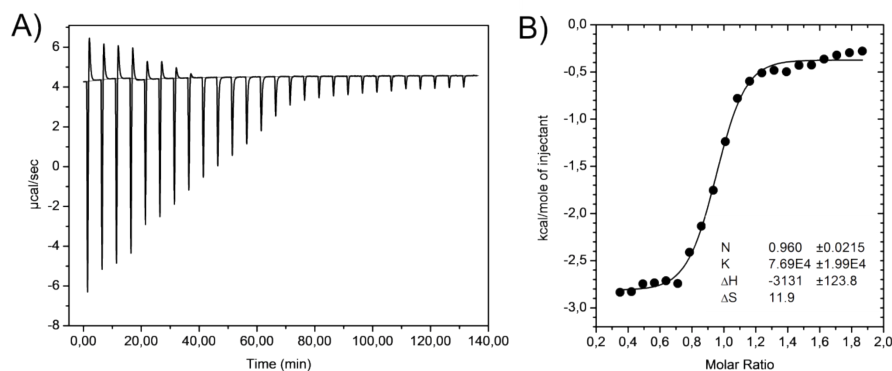

Figure S5. ITC titration curve obtained by titrating β-CD into a solution of APK. A) Raw injection peaks B) Integrated injection peaks and obtained thermodynamic parameters.

Chemical structures of DOPE-NBD and DOPE-LR

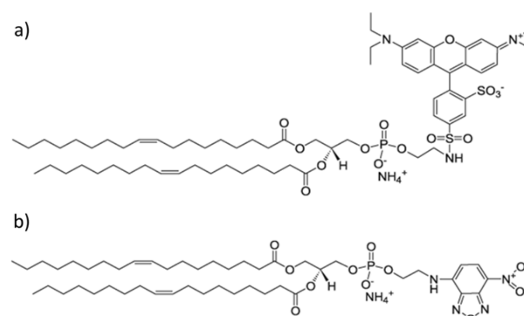

Figure S6. Chemical structures of the FRET pair that was used in the lipid mixing assays, a) DOPE-lissamine rhodamine (1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (ammonium salt) and b) DOPE-NBD (1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl) (ammonium salt).

Content mixing data

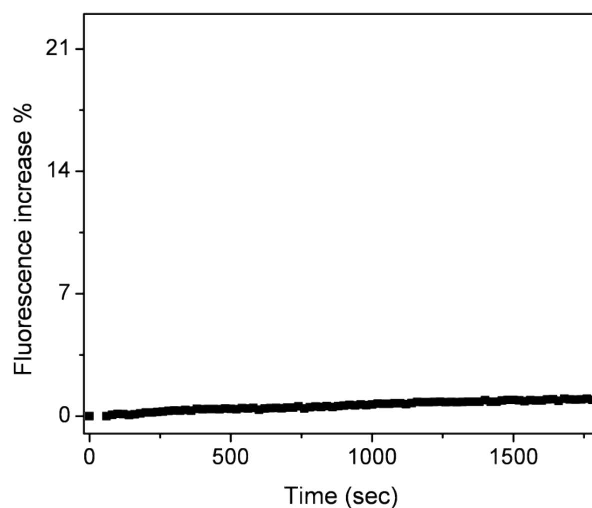

Figure S7. Fluorescence graph indicating the extend of content mixing between liposomes and cyclodextrin vesicles. CPE decorated liposomes (0.1 mM, 1 mol% CPE) were loaded with 20 mM sulphorhodamine and mixed with APK decorated CDVs (0.1 mM, 1 mol% APK).

Inner leaflet lipid mixing

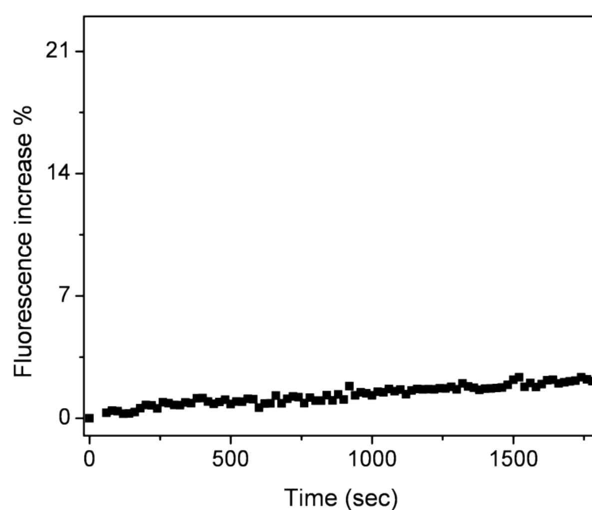

Figure S8. Fluorescence graph indicating inner leaflet lipid mixing between liposomes and cyclodextrin vesicles. CPE decorated liposomes (0.1 mM, 1 mol% CPE) were also decorated with 0.5 mol% DOPE-LR and 0.5 mol% DOPE-NBD. The NBD probes residing in the outer lipid leaflet were quenched by addition of sodium dithionite. The liposomes were mixed with APK decorated CDVs (0.1 mM, 1 mol% APK).

Cryo-TEM tilt series of liposome/CDV hybrid structures

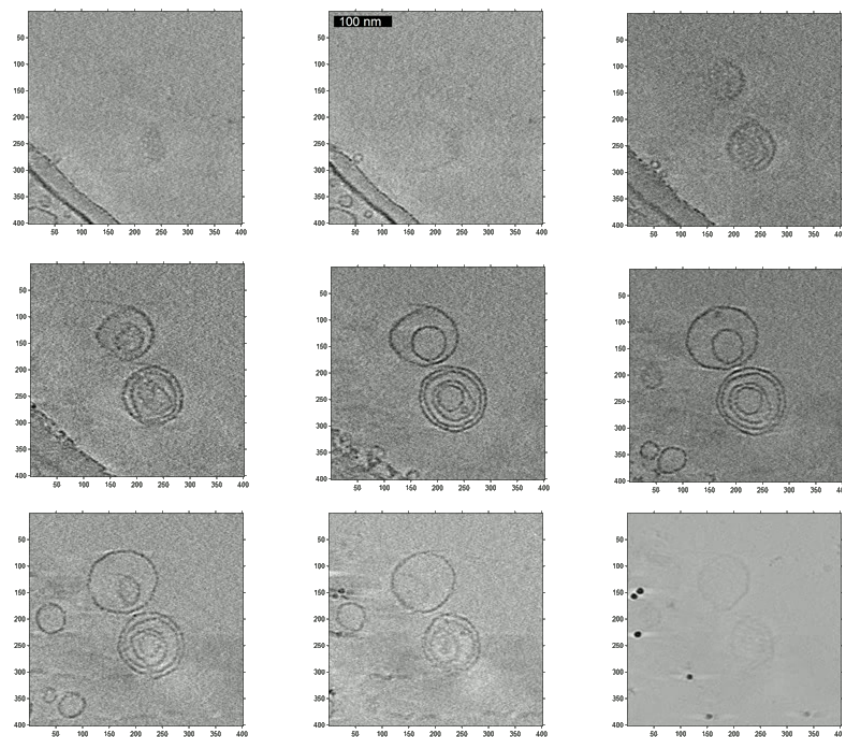

Fig. S9: Selected z-slices from a reconstruction of a cryo-electron tomogram of a 1:1 mixture of the peptide decorated CDVs and liposomes. Total amphiphile concentrations were 0.5 mM with 1% lipidated peptide in PBS at pH 7.3. Scale bar is 100 nm.

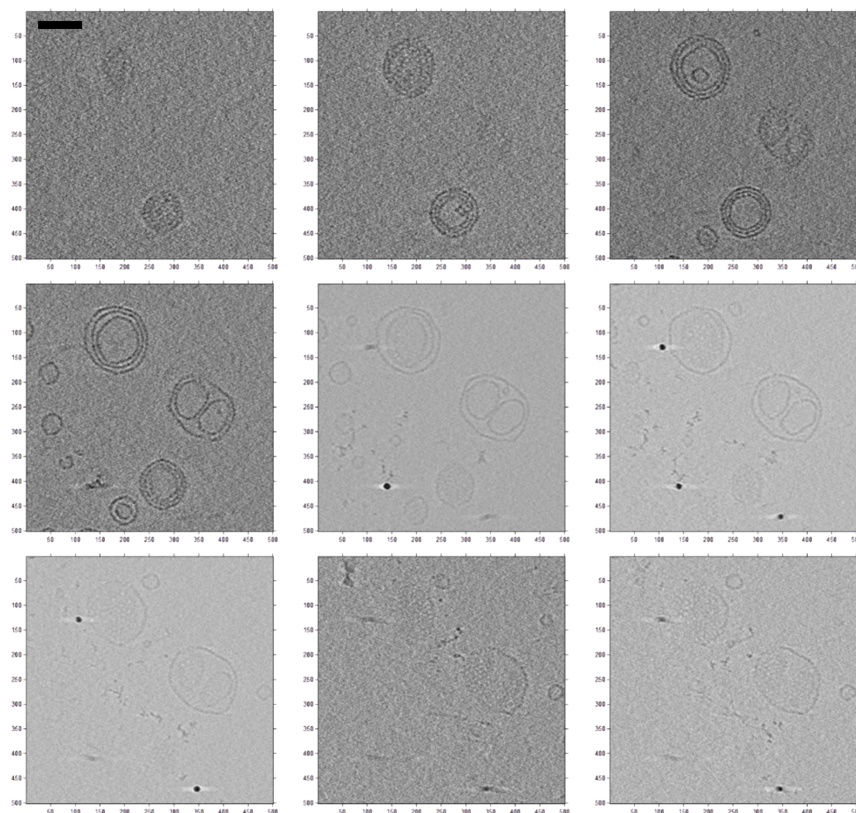

Fig. S10 Selected z-slices from a reconstruction of a cryo-electron tomogram of a 1:1 mixture of the peptide decorated CDVs and liposomes. Total amphiphile concentrations were 0.5 mM with 1% lipidated peptide in PBS at pH 7.3. Scale bar is 100 nm.

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[1] F. Versluis, J. Voskuhl, B. van Kolck, H. Zope, M. Bremmer, T. Albregtse, A. Kros, *J. Am. Chem. Soc.* 2013, **135**, 8057-8062.
[2] B. J. Ravoo, R. Darcy, *Angew. Chem.* 2000, **112**, 4494–4496.