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Electronic Supplementary Information

Hydrogen Bond-Assisted Macrocylic Oligocholate Transporters in Lipid Membranes

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Abbreviation

ATP: adenosine 5´-triphosphoate; CF: carboxyfluorescein; HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; NADP: Nicotinamide adenine dinucleotide phosphate; NBD-DPPE: N-(7-nitro-2-1,3-benzoxadiazol-4-yl)-1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine ammonium salt; POPC: 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine; POPG: 1-palmitoyl-2-oleoyl-sn-glycero-3-[phospho-rac-(1-glycerol)] sodium salt; Rh-DPPE: N-(lissamine rhodamine B sulfonyl) -1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine ammonium salt; Tris: tris(hydroxymethyl)aminomethane.

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Preparation of the LUVs

CF-containing LUVs were prepared according to a slightly modified literature procedure.[1] A chloroform solution of POPC (25 mg/mL, 198 μL) and POPG (50 mg/mL, 10.0 μL) was placed in a 10 mL test tube and dried under a stream of nitrogen. The residue was dried further under high vacuum overnight. A solution of CF-HEPES buffer (0.5 mL, 50 mM CF, 10 mM HEPES, 10 mM NaCl, pH=7.4) was added. Rehydration of the lipids was allowed to continue for 30 min with occasional vortexing. The opaque dispersion was subjected to ten freeze–thaw cycles. The resulting mixture was extruded twenty-nine times through a polycarbonate filter (diameter = 19 mm, pore size = 100 nm) at room temperature using an Avanti Mini-Extruder. A portion (0.1 mL) of the liposome solution was passed through a column of Sephadex G-50 using HEPES buffer (10 mM HEPES, 107 mM NaCl, pH=7.4) as the eluent to remove the extravesicular CF. The liposome fractions were combined and diluted to 10.0 mL with the HEPES buffer. The concentration of phospholipids in the stock solution was 0.14 mM.

Glucose-leakage assay

Glucose-loaded LUVs were prepared according to a slightly modified literature procedure with 300 mM of D-(+)-glucose in 50 mM Tris buffer (0.5 mL, pH = 7.5).[2] The concentration of phospholipids in the stock solution was 0.86 mM. Glucose released from the liposomes was measured enzymatically by a slightly modified literature procedure.[3]Aliquots of the above LUV solution (250 μL), Tris buffer (750 μL, 50 mM Tris, pH = 7.5, 145 mM NaCl, 3.5 mM $MgCl_2$, and 0.15 mM $CaCl_2$), the enzyme solution (500 μL, 10 units/mL of hexokinase/glucose-6-phosphoate dehydrogenase and 2 mM ATP dissolved in the above Tris buffer), and NADP solution (500 μL, 1 mM dissolved in the above Tris buffer) were placed

in a series of cuvettes. The concentration of phospholipids in each cuvette was 107 μM. Aliquots of the oligocholate solution in DMSO were added to different cuvettes via a microsyringe. The amount of DMSO introduced to each sample was ≤20 μL. The absorbance of NADPH at 340 nm was monitored. To measure the nonspecific glucose leakage from the liposomes, the sample was prepared in an identical fashion and DMSO instead of the oligocholate solution was added. After 2 h, the liposomes were lysed by the addition of 100 μL of Triton X-100 (1% v/v) and the absorbance at 340 nm (A_{max}) was used to calculate the percent leakage [= $(A_t - A_0)/(A_{max} - A_0) \times 100$]. A_0 and A_t are the initial and intermediate absorbance, respectively.

Lipid-mixing assay

Unlabeled POPC/POPG LUVs were prepared with a mixture of POPC (25 mg/mL, 198 μL) and POPG (50 mg/mL, 10 μL) using HEPES buffer (10 mM HEPES, 107 mM NaCl, pH=7.4), following the procedure described above. Gel filtration was not needed in this experiment. Labeled POPC/POPG LUVs containing 1 mol % of NBD-DPPE and Rh-DPPE were prepared in the same manner. The labeled and the unlabeled LUVs were mixed in 1:4. An aliquot of the mixed LUVs (15 μL) was placed in a cuvette and diluted with the HEPES buffer to 2.0 mL. The concentration of lipids was 54 μM in the final mixture. The change of NBD fluorescence (λ_{ex} = 450 nm and λ_{em} = 530 nm) was measured upon injection of the oligocholate solution (0.5 mM in DMSO, 10 μL). An increase of NBD emission indicates dilution of membrane bound probes caused by membrane fusion. The percentage of fusion was determined using equation % Fusion = $(F_t - F_0)/(F_{max} - F_0) \times 100\%$, in which F_t is the emission intensity of NBD during the assay, F_0 the initial intensity, and F_{max} the maximum intensity (measured for LUVs containing 0.2 mol % each of NBD-DPPE and Rh-DPPE).

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CF leakage assay

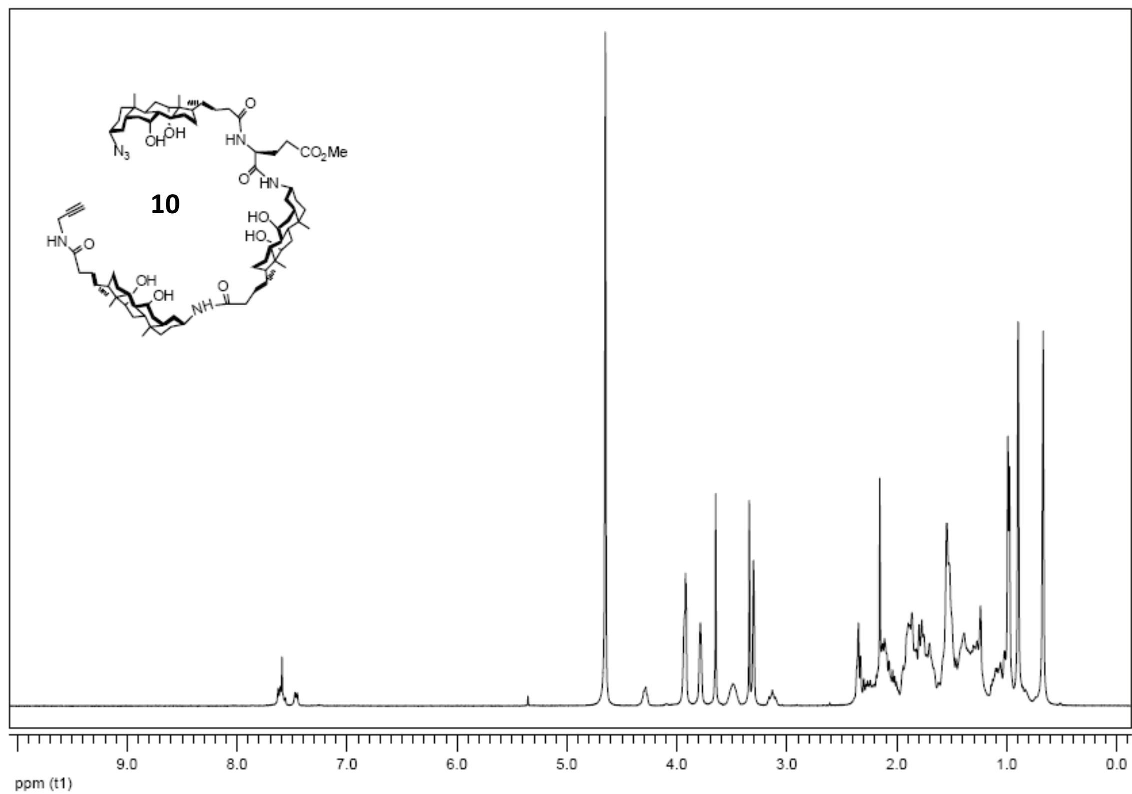
For fluorescence measurements, aliquots of the above LUV solution (40 μL) were diluted with the HEPES buffer (1.96 mL, 10 mM HEPES, 107 mM NaCl, pH=7.4) in separate cuvettes, resulting in a lipid concentration of 2.9 μM in each cuvette. Aliquots of the appropriate oligocholate in DMSO were added to different cuvettes via a microsyringe. The amount of DMSO introduced to each sample was ≤20 μL. The change of emission intensity at 520 nm (λ_{ex}= 492 nm) was monitored over time. After 2 h, 40 μL of Triton X-100 (1% v/v) was added, disrupting the vesicles and releasing the remaining CF (100% release). The percent leakage was defined as % leakage = $(F_t - F_0)/(F_{\max} - F_0) \times 100$, in which F_0 and F_t are the initial and intermediate emission intensity, respectively, and $F_{\max}$ was taken as the fluorescence intensity after lysis of the LUVs by Triton X-100.

References

(1) Tanaka, H.; Litvinchuk, S.; Tran, D.-H.; Bollot, G.; Mareda, J.; Sakai, N.; Matile, S. *J. Am. Chem. Soc.* **2006**, *128*, 16000-16001.

(2) Westmark, P. R.; Gardiner, S. J.; Smith, B. D. *J. Am. Chem. Soc.* **1996**, *118*, 11093-11100.

(3) Kinsky, S. C.; Haxby, J. A.; Zopf, D. A.; Alving, C. R.; Kinsky, C. B. *Biochemistry* **1969**, *8*, 4149-4158.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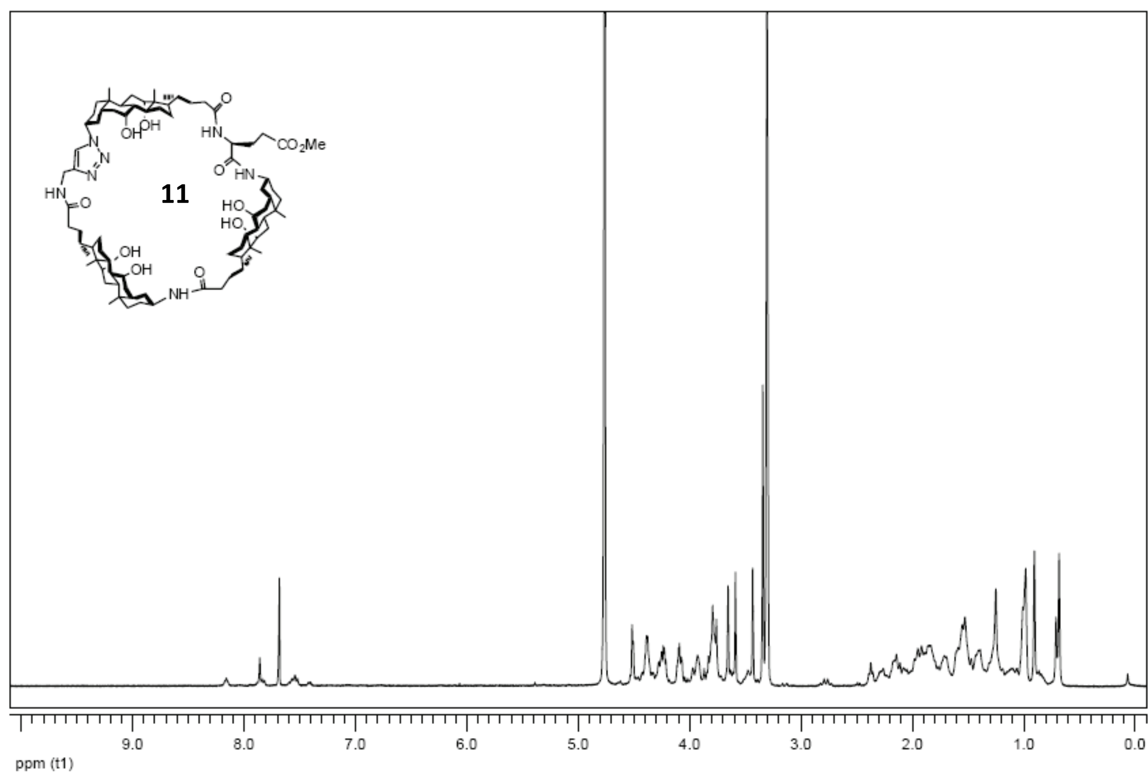
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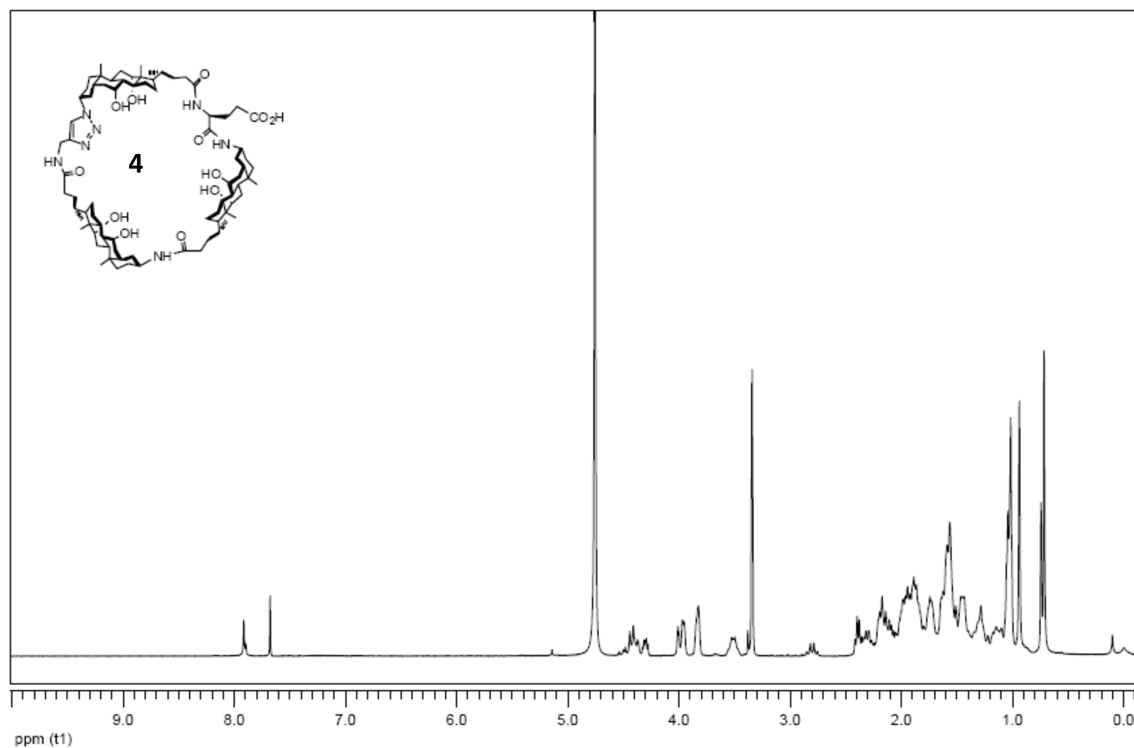
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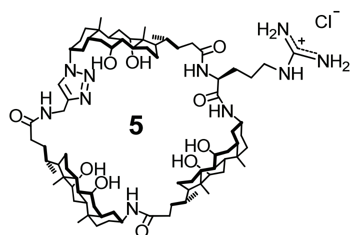

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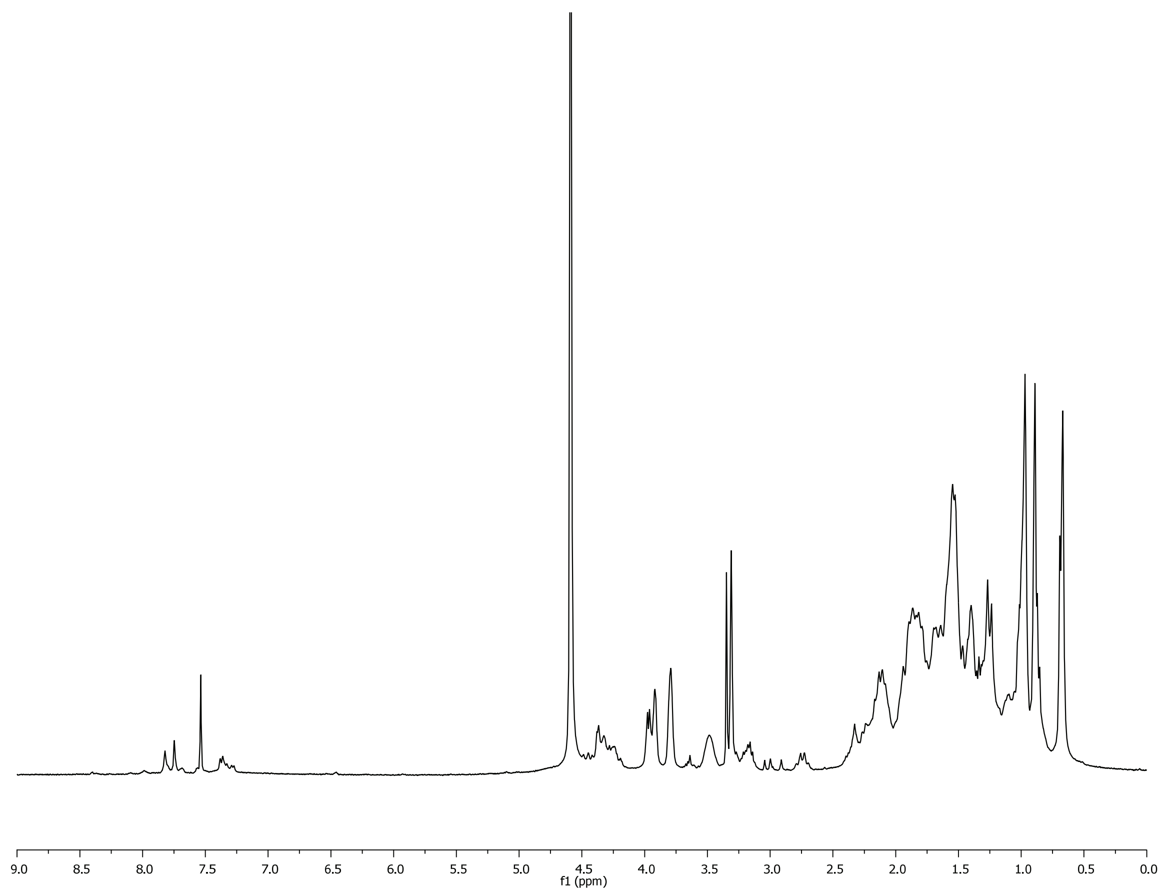

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