

Electronic Supplementary Information

Cholate-Derived Amphiphilic Molecular Baskets as Glucose Transporters across Lipid Membranes

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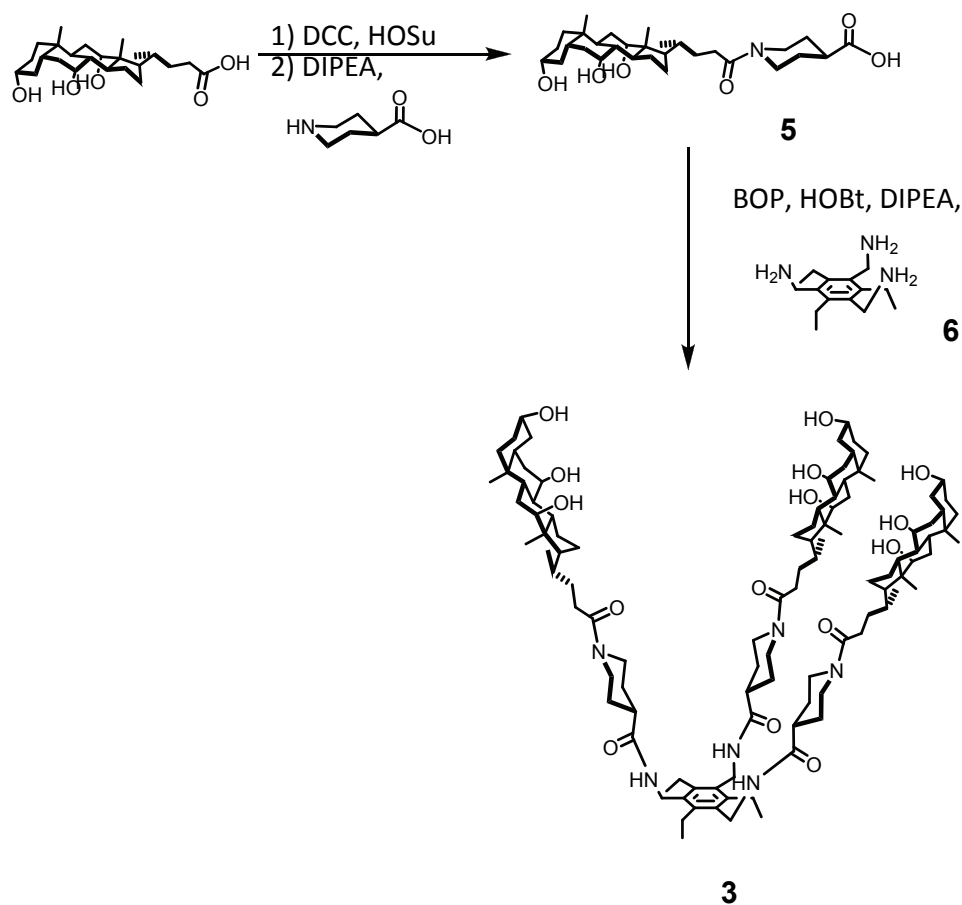
General Method

Lipids for this study were purchased from Avanti Polar Lipids and stored at -20 °C. All other reagents and solvents were of ACS-certified grade or higher, and were used as received from commercial suppliers. Millipore water was used to prepare buffers and the liposomes. Routine ¹H and ¹³C NMR spectra were recorded on a Bruker DRX-400 or on a Varian VXR-400 spectrometer. MALDI-TOF mass was recorded on a Thermobioanalysis Dynamo mass spectrometer. UV-vis spectra were recorded at ambient temperature on a Cary 100 Bio UV-visible spectrophotometer. Fluorescence spectra were recorded at ambient temperature on a Varian Cary Eclipse Fluorescence spectrophotometer.

Abbreviation

ATP: adenosine 5'-triphosphate; 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.

Scheme 1S



Synthesis

The syntheses of compounds **1** and **2** were previously reported.¹

Compound 5. Cholic acid (1.593 g, 3.77 mmol) and *N*-hydroxy succinimide (HOSu, 477 mg, 4.14 mmol) were dissolved in CH₃CN/THF (1:5, 60 mL). Dicyclohexylcarbodiimide (DCC, 840 mg, 4.07 mmol) was added in portions. The reaction mixture was stirred at room temperature under N₂ for 24 h. The white solid was filtered off and washed with EtOAc (3 × 20 mL). The combined filtrate was concentrated in vacuo to give a white foam (1.81 g, quant.). Part of the foam (700 mg, 1.46 mmol) was dissolved in CH₃CN (7 mL). The solution was added to a solution of isonipecotic acid hydrochloride (291 mg, 1.76 mmol) and *N,N*-Diisopropylethylamine (DIPEA, 1.27 mL, 7.32 mmol) in

¹ Ryu, E.-H.; Yan, J.; Zhong, Z.; Zhao, Y. *J. Org. Chem.* **2006**, *71*, 7205-7213.

H₂O (3 mL). After stirred under N₂ for 15 h at room temperature, the organic solvent was removed in vacuo and the residue was poured into a dilute HCl solution (100 mL, 0.1 M). The precipitate was collected by suction filtration and purified by column chromatography over silica gel with CH₂Cl₂/EtOAc/MeOH (5:5:2) as the eluent to give an off-white powder (504 mg, 66%). ¹H NMR (400 MHz, CD₃OD, δ): 4.33 (br, 1H), 3.93 (br, 2H), 3.80 (br, 1H), 3.37 (m, 1H), 3.21 (m, 1H), 2.80 (m, 1H), 2.59 (br, 1H), 2.46 (m, 1H), 2.24 (m, 3H), 2.05–0.82 (series of m, 30H), 0.71 (s, 3H). ¹³C NMR (100 MHz, CD₃OD, δ): 174.7, 173.1, 74.1, 73.0, 69.1, 65.5, 61.7, 48.0, 47.6, 46.6, 43.3, 43.1, 42.4, 42.0, 41.1, 40.6, 37.2, 36.6, 36.0, 33.1, 31.9, 31.3, 30.1, 29.7, 29.3, 28.9, 28.0, 24.4, 23.3, 21.0, 20.3, 18.0, 14.6, 14.2, 13.2. MALDI-TOFMS (*m/z*): [M + H]⁺ calcd for C₃₀H₅₀NO₆, 520.7; found, 520.9, [M + Na]⁺ calcd for C₃₀H₄₉NO₆Na, 542.7; found, 542.8.

Compound 3. Compound **5** (122 mg, 0.235 mmol), amine **6** (15 mg, 0.060 mmol), 1-hydroxybenzotriazole hydrate (HOBt, 24 mg, 0.178 mmol), and benzotrazol-1-yloxytris (dimethylamino) phosphonium hexafluorophosphate (BOP, 133 mg, 0.301 mmol) were dissolved in anhydrous DMF (2.0 mL). DIPEA (0.104 mL, 0.601 mmol) was added. The reaction mixture was heated to 110 °C for 40 min in a CEM Discover microwave reactor. After cooled to room temperature, the reaction mixture was poured into a diluted HCl solution (50 mL, 0.05 M). The precipitate was collected and purified by column chromatography over silica gel with CH₂Cl₂/EtOAc/MeOH (1:1:1) as the eluent to give an off-white powder (74 mg, 71%). ¹H NMR (400 MHz, CD₃OD, δ): 4.53 (br, 3H), 4.44 (s, 6H), 3.99 (br, 6H), 3.79 (br, 3H), 3.37 (m, 3H), 3.11 (m, 3H), 2.75 (br, 6H), 2.60 (m, 3H), 2.47 (br, 6H), 2.28 (br, 9H), 2.10–0.82 (series of m, 99H), 0.71 (s, 9H). ¹³C NMR (100 MHz, CD₃OD, δ): 176.9, 174.7, 145.5, 133.2, 74.1, 73.0, 69.2, 50.0, 48.0, 47.7, 46.7, 43.8, 43.3, 43.2, 42.6, 41.2, 40.6, 39.1, 37.2, 37.2, 36.7, 36.1, 33.1, 31.3, 30.5, 29.9, 29.7, 29.0, 28.0, 24.4, 24.1, 23.4, 18.0, 16.8, 13.2. MALDI-TOFMS (*m/z*): [M + H₃O]⁺ calcd for C₁₀₅H₁₇₁N₆O₁₆, 1773.5; found, 1775.0.

Liposome preparation

Glucose-loaded LUVs were prepared according to a slightly modified literature procedure.² A chloroform solution of POPC (25 mg/mL, 198 μ L) and POPG (50 mg/mL, 10.0 μ L) 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 D-(+)-glucose (300 mM) in 50 mM Tris buffer (0.5 mL, pH = 7.5) 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 (0.3 mL) of the liposome solution was passed through a column of Sephadex G-50 using Tris buffer (50 mM Tris, 150 mM NaCl, pH=7.5) as the eluent to remove the extravesicular glucose. The liposome fractions were combined and diluted to 5.0 mL with the Tris buffer. The concentration of phospholipids in the stock solution was 0.86 mM.

Glucose leakage assay

Glucose released from the liposomes was measured enzymatically by a slightly modified literature procedure.³ Aliquots of the above LUV solution (250 μ L), Tris buffer (750 μ L, 50 mM Tris, pH = 7.5, 145 mM NaCl, 3.5 mM MgCl₂, and 0.15 mM CaCl₂), the enzyme solution (500 μ L, 10 units/mL of hexokinase/glucose-6-phosphoate dehydrogenase and 2 mM ATP dissolved in the above Tris buffer), and NADP solution (500 μ L, 1 mM dissolved in the above Tris buffer) were placed in a series of cuvettes. The concentration of phospholipids in each cuvette was 107 μ M. 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 μ L. 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 1 h,

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**, *10*, 4149–4158.

the liposomes were lysed by the addition of 100 μL of Triton X-100 (1% v/v) and the absorbance at 340 nm (A_∞) was used to calculate the percent leakage $[= (A_t - A_0)/(A_\infty - A_0) \times 100]$. 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 a 1:4 ratio. 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 ($\lambda_{\text{ex}} = 450$ nm and $\lambda_{\text{em}} = 530$ 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

$$\% \text{ Fusion} = (F_t - F_0)/(F_{\text{max}} - F_0) \times 100\%$$

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).

Na⁺-transport assay

Na⁺ transport across lipid membrane was monitored by ²³Na NMR experiment according to a slightly modified literature procedure.⁴ A chloroform solution of POPC (25 mg/mL, 2.0 mL) was placed in a 10 mL test tube and dried under a stream of nitrogen. The residue was dried further under high vacuum overnight, resulting in a thin film. The dried film was rehydrated in KCl solution (150 mM, 1.0 mL) for at least 30 min with occasional vortexing. The dispersion was subjected to five freeze–

⁴ Zhang, J.; Jing, B.; Regen, S. L. *J. Am. Chem. Soc.* **2003**, *125*, 13984-13987.

thaw cycles, followed by extrusion (29 times) through a polycarbonate filter (diameter = 19 mm, pore size = 100 nm) at room temperature using an Avanti Mini-Extruder. An aliquot of the liposome solution (0.20 mL) was placed in a NMR tube and diluted with 0.50 mL of 150 mM NaCl. To this solution was added 0.30 mL of the shift reagent solution (10 mM DyCl_3 and 30 mM $\text{Na}_5\text{P}_3\text{O}_{10}$). The concentration of phospholipids in the final solution was 13 mM. NMR Spectra were recorded upon injection of the oligocholate solution (0.5 mM in DMSO, 10 μL) on a Bruker DRX-400 spectrometer.

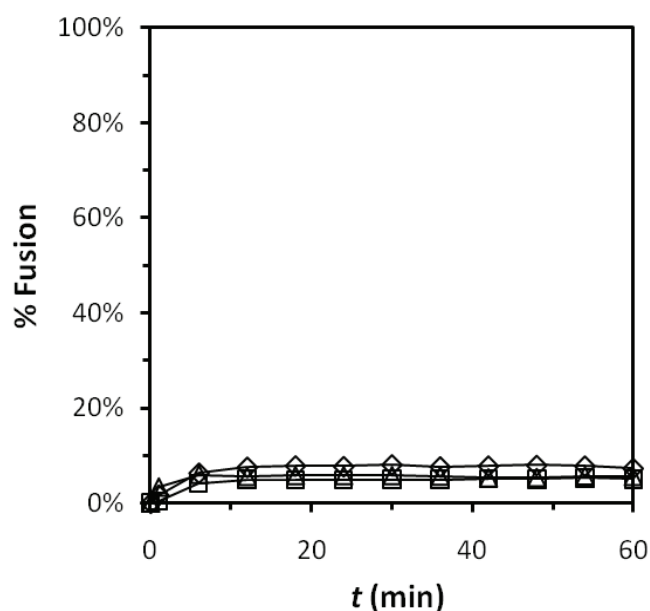


Figure 1S. Percent fusion of LUVs as a function of time **1** ($\triangle$), **2** ($\square$), and **3** ($\diamond$). The data points are connected to guide the eye. [basket] = 2.5 μM , [lipid] = 54 μM .

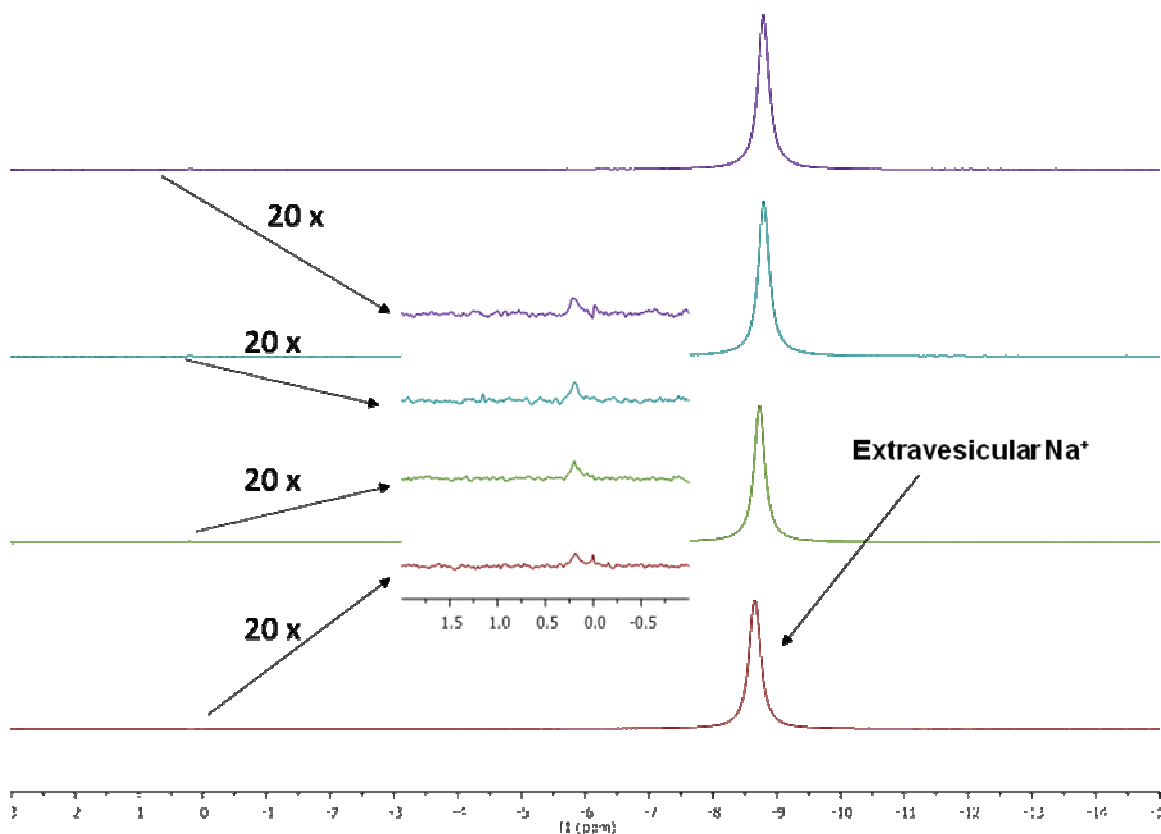


Figure 2S. ^{23}Na NMR spectra of POPC LUVs upon injection of **1** (5 mol% to the phospholipids). The spectra from bottom to top correspond to 0, 1.5 h, 12 h, and 48 h after the addition of the basket compound. The insets are magnified spectra (20 times) near 0 ppm. The main peak at -9 ppm was from extravesicular Na^+ . The constant small peak at 0.2 ppm was probably from Na^+ associated with the membranes. [basket] = 70 μM , [lipid] = 13 mM.

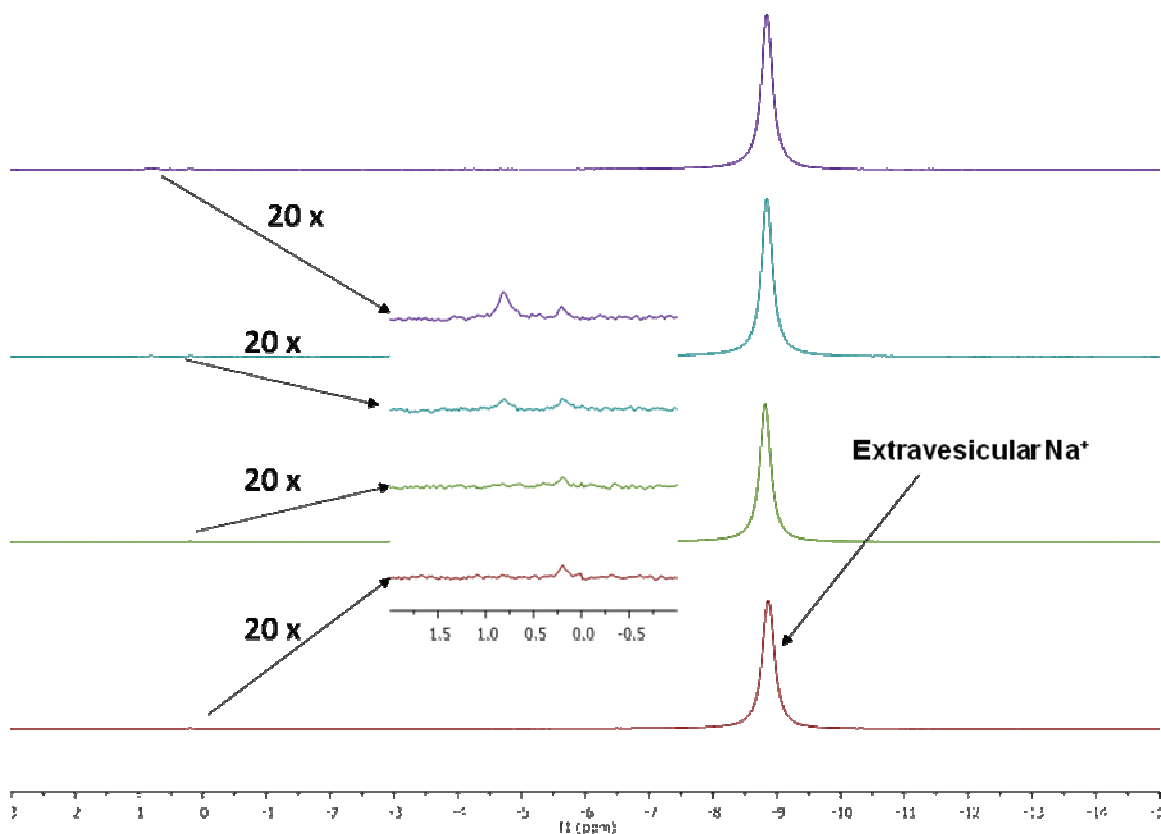


Figure 3S. ^{23}Na NMR spectra of POPC LUVs upon injection of **2** (5 mol% to the phospholipids). The spectra from bottom to top correspond to 0, 1.5 h, 12 h, and 48 h after the addition of the basket compound. The insets are magnified spectra (20 times) near 0 ppm. The main peak at -9 ppm was from extravesicular Na^+ and the slowly growing small peak at 0.7 ppm was from Na^+ transported into the LUVs. The constant small peak at 0.2 ppm was probably from Na^+ associated with the membranes. [basket] = 70 μM , [lipid] = 13 mM.

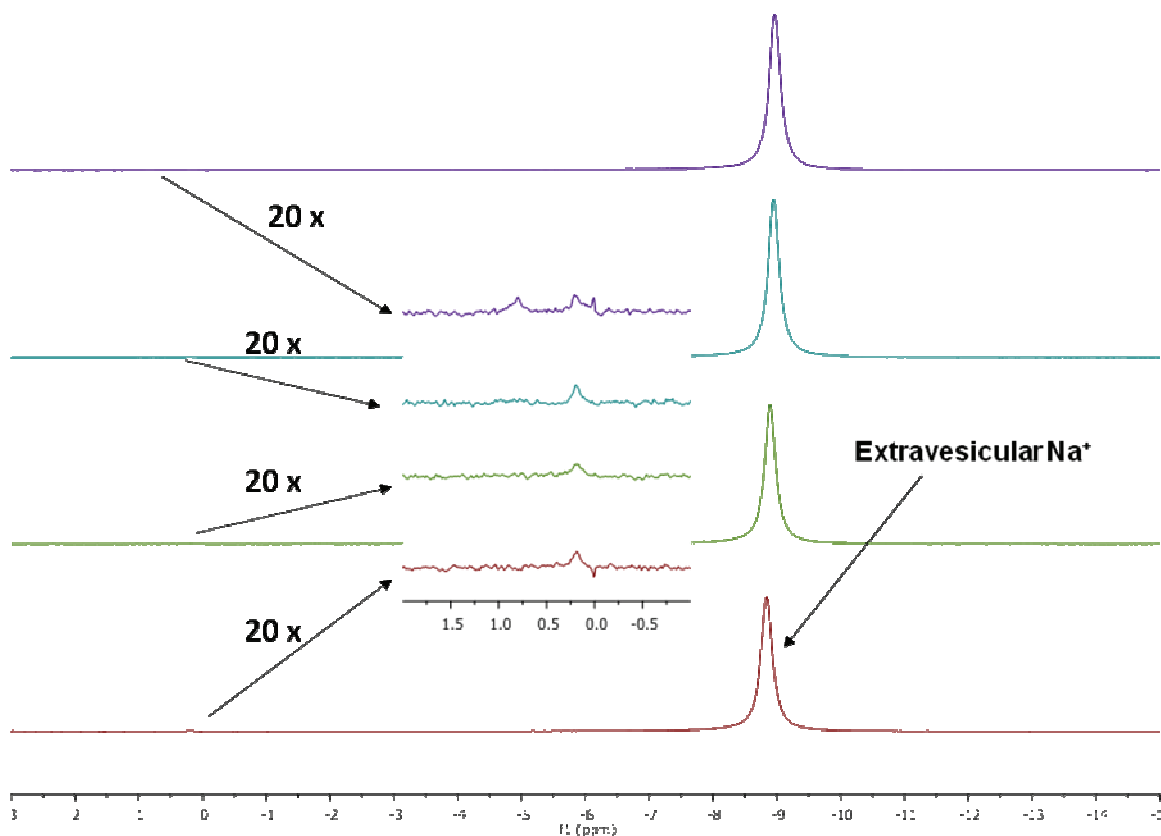


Figure 4S. ^{23}Na NMR spectra of POPC LUVs upon injection of **3** (5 mol% to the phospholipids). The spectra from bottom to top correspond to 0, 1.5 h, 12 h, and 48 h after the addition of the basket compound. The insets are magnified spectra (20 times) near 0 ppm. The main peak at -9 ppm was from extravesicular Na^+ and the slowly growing small peak at 0.7 ppm was from Na^+ transported into the LUVs. The constant small peak at 0.2 ppm was probably from Na^+ associated with the membranes. [basket] = 70 μM , [lipid] = 13 mM.

