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Reversible Ion Transportation Switch of ON-OFF-ON Type by a Ligand-Gated Calix[6]arene Channel

Xinyu Hu, [a] Nannan Liu, [b] Haishen Yang,[c] Fen Wu, [b] Xi Chen, [a] Canpeng Li *[d] and Xiaojing Chen* [a]

[a] *College of Mathematic and Electronic Information Engineering, Wenzhou University, Wenzhou 325035, P. R. China. E-mail: 00101094@wzu.edu.cn*

[b] *College of Chemistry and Materials Engineering, Wenzhou University, Wenzhou 325035, P. R. China.*

[c] *Shanghai Key Laboratory of Materials Protection and Advanced Materials in Electric Power, Shanghai University of Electric Power, Shanghai, 200090, China.*

[d] *School of Chemical Science and Technology, Yunnan University, Kunming 650091, P. R. China. E-mail: lcppp1974@sina.com*

Materials and equipments

Calixarenes: Calix[4]arene(CX4, >98%), calix[6]arene(CX6, 98%), 4-sulfonated calix[6]arene(SCX6), tert-butyl calix[6]arene (CX6-tBu), and calix[8]arene(CX8, 97%) were purchased from Alfa Aesar Chemical Co. Ltd. (Shanghai, China) without further purification.

Fluorescence spectra were conducted by HITACHI F-2500 fluorescence spectrophotometer. The conductance experiments were performed by Keithley 6487 picoammeter and Sutter P-97.

Preparation of HPTS-entrapped large unilamellar vesicles

1,2-diacyl-sn-glycero-3-phosphocholine (PC, 100 mg/ml, 0.15 mL) and 3-β-hydroxy-5-cholestene (CH, 3.75 mg) were dissolved in $CHCl_3$ (10 mL) in a round-bottom flask. The solvent was removed under reduced pressure (8 min, 25 ^{o}C) to produce a uniform thin film. The film was dried under high vacuum for 3 h at room temperature. Then the film was hydrated with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer solution (1.5 mL, 10 mM HEPES, 100 mM NaCl, pH = 7.0) containing a pH sensitive dye 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS, 0.1 mM) in thermostatic shaker-incubator at 37 ^{o}C for 2 h to give a milky suspension. The mixture was then subjected to ten freeze-thaw cycles: freeze in liquid N_2 for 30 s, warm it up at 37 ^{o}C for 1.5 min, then gentle vortex mixing for 3 min at room temperature. The suspension of LUVs was divided into two equal aliquots and dialyzed for 36 h with gentle stirring (200 r/min) using membrane tube (MWCO = 8000–14000) against the same HEPES buffer solution (300 mL, without HPTS) for eight times to remove free HPTS. [1]

Determination of proton transport activity through HPTS assay

The prepared LUVs suspension (100 µL, 13.3 mM, internal buffer: 10 mM HEPES, 100 mM NaCl, pH = 7.0) was added to HEPES buffer solution (total volume 2000 µL, 10 mM HEPES, 100 mM NaCl, pH = 7.6). The solution of macrocycle in THF (1.0 mM) was added with gentle mixing. The fluorescence intensity was immediately measured as a function of time to investigate the channeling activity of the macrocycles. Fluorescence intensity of HPTS (I_1) was continuously monitored at 510 nm (excitation at 454 nm) for 30 min. Aqueous solution of Triton X-100 (16 µL, 20% v/v) was added to achieve the maximum changes in dye fluorescence emission (I_2). The collected data were then normalized into the fractional change in fluorescence intensity according to the following equation: $R(\%) = (I_1-I_0)/(I_2-I_0) \times 100$, where I_0 is the initial intensity.

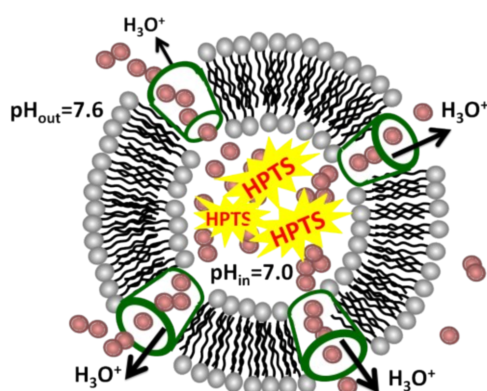

Figure S1. Schematic representation of the ion transport experiment using LUVs loaded with the pH-sensitive fluorophore HPTS and exposed to a pH gradient (inside pH = 7.0; outside pH = 7.6).

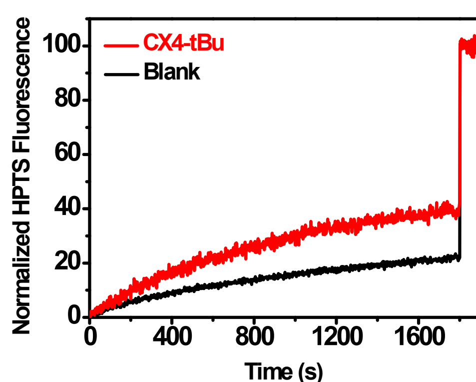

Figure S2. Normalized fluorescence traces of HPTS assay in the presence (red line) or absence (black line) of CX4-tBu (10 µM)

Calcein-encapsulated large unilamellar vesicles

PC (100 mg/mL, 0.10 mL) and CH (2.5 mg) were dissolved in $CHCl_3$ (20 mL). The solution was

evaporated under reduced pressure (8 min, 25 ^{o}C), and further dried under high vacuum for 3 h. The lipid film was then hydrated with HEPES buffer solution (1.0 mL, 10 mM HEPES, 100 mM NaCl, pH = 7.4) containing calcein (40 mM) at 37 ^{o}C for 2 h in thermostatic shaker-incubator to give a milky suspension. Ten freeze-thaw cycles (freeze in liquid nitrogen for 30 s, warm it up at 37 ^{o}C for 1.5 min, and then gentle vortex mixing for 3 min) were performed. The LUVs suspension was extruded through polycarbonate membrane (0.22 µm) to produce homogeneous suspension of LUVs. The suspension of LUVs was divided into two equal aliquots and dialyzed for 36 h with gentle stirring (200 r/min) using membrane tube (MWCO = 8000–14000) against the same HEPES buffer solution (300 mL, without calcein) for eight times to remove free calcein. [2]

Determination of calcein transport through calcein assay

The above suspension of LUVs with entrapped-calcein (20 µL) was added to HEPES buffer solution (total volume 2000 µL, 10 mM HEPES, 100 mM NaCl, pH = 7.4), followed by the solution of macrocycle (1.0 mM) in THF with gentle mixing. Fluorescence intensity of calcein (I_1) was continuously monitored at 505 nm (excitation at 493 nm) for 30 min. Then, aqueous solution of Triton X-100 (16 µL, 20% v/v) was added to the cuvette to achieve the maximum changes in dye fluorescence emission (I_2) at the end of experiment. The collected data were normalized into the fractional change in fluorescence according to the following equation: $R(\%) = (I_1-I_0)/(I_2-I_0) \times 100$, where I_0 is the initial intensity.

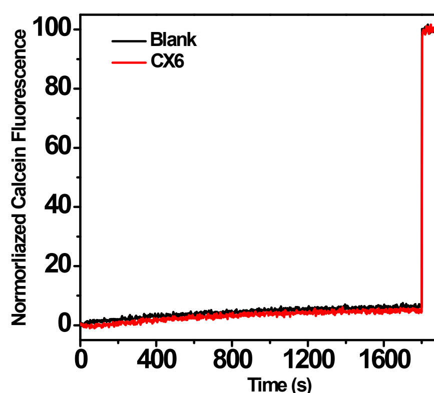

Figure S3. Normalized fluorescence traces of calcein assay in the presence (red line) or absence (black line) of CX6 (10 µM)

The addition of CX6 caused quenching of MB fluorescence intensities. Figure S4 shows the fluorescence titrations of MB (10 μM, λ_{ex} = 640 nm) upon successive addition of CX6 (up to 30 μM) in above mentioned pH = 7.0 HEPES buffer. We prepared MB stock solution (10 mM) in pH = 7.0 HEPES buffer and CX6 stock (1 mM) in THF due to its hydrophobicity. The MB stock solution was diluted with pH = 7.0 HEPES buffer to achieve final concentration of 10 μM. Then, CX6 stock was gradually added into MB solution and mixed by vortexing for 5 min before recording fluorescence titration.

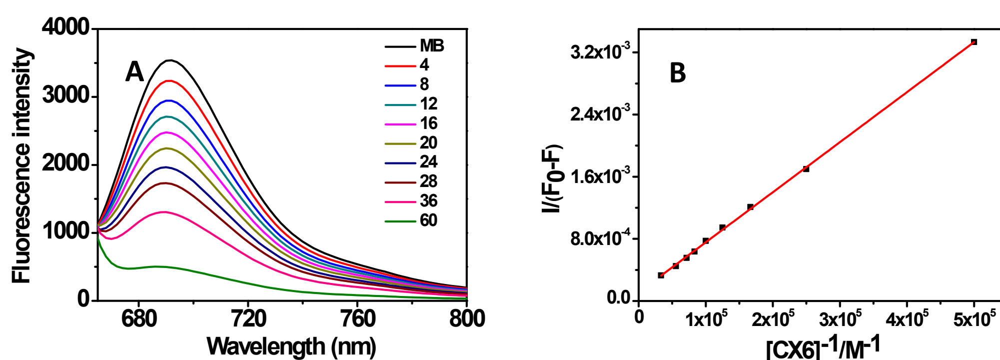

Figure S4. Fluorescence spectra of 10 μM MB upon successive addition of CX6 (up to 30 μM) (A) and plots of $1/(F_0 - F)$ *versus* 1/[CX6] for MB (B).

Herein, we report an electrochemical approach utilizing a nanopipet as a reliable and robust platform (Figure S5 and S6). The self-made current testing device used in this experiment is a double-electrode system in Figure S5 below. Ag/AgCl electrode was introduced in current experiments. We used +2V voltage and pH 7.0 Hepes buffer to detect current change due to ion transportation.

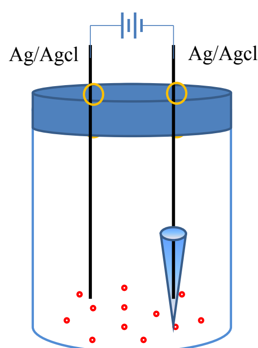

Figure S5. Experimental device for current testing

The nanocapillary was obtained after stretching by glass drawing device and characterized by electron scanning electron microscopy (SEM). The diameter of the nanocapillary tip was 1.58 μm.

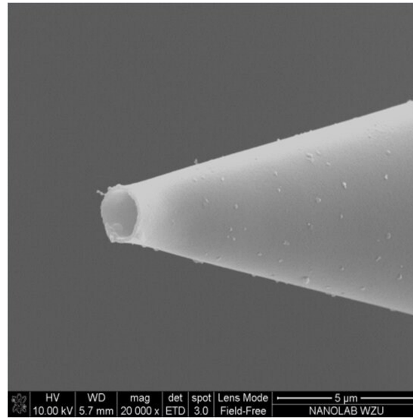

Figure S6. SEM image of nanocapillary after stretching

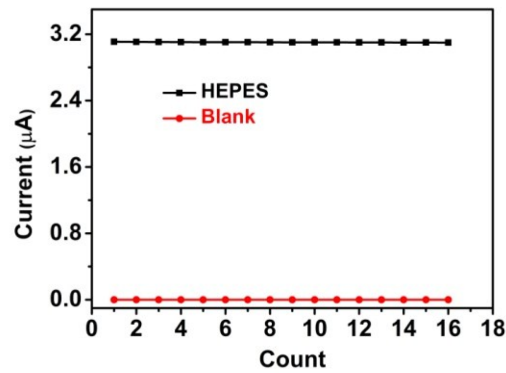

Figure S7. Current profiles under +2 V during 16 successive repeat experiments in the presence of lipid alone (red, Blank) or HEPES buffer (black, HEPES).

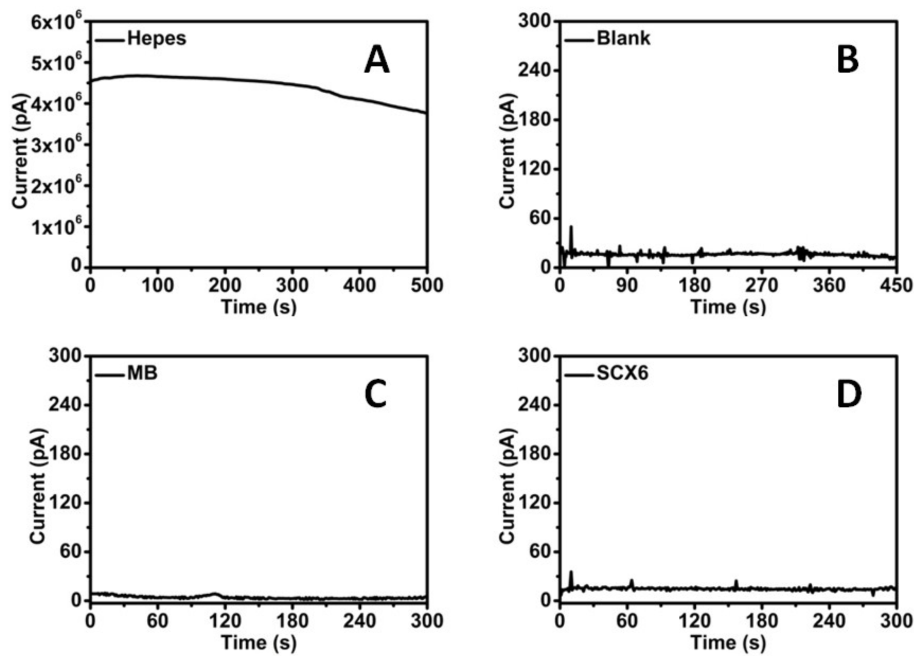

Figure S8. Conductance profiles at the applied voltage of +2 V at lipid bilayer membrane in HEPES buffer (10 mM HEPES, 100 mM NaCl, pH 7.0) (A) and (B) planar lipid membrane. Then, MB (C), SCX6 (D) was added into the chamber, respectively.

References

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2. M. Danial, C. M. N. Tran, K. A. Jolliffe and S. Perrier, *J. Am. Chem. Soc.*, 2014, **136**, 8018-8026.