A Reversible Single-Molecule Ligand-Gating Ion Transportation Switch of ON–OFF–ON Type through a Photoresponsive Pillar[6]arene Channel Complex

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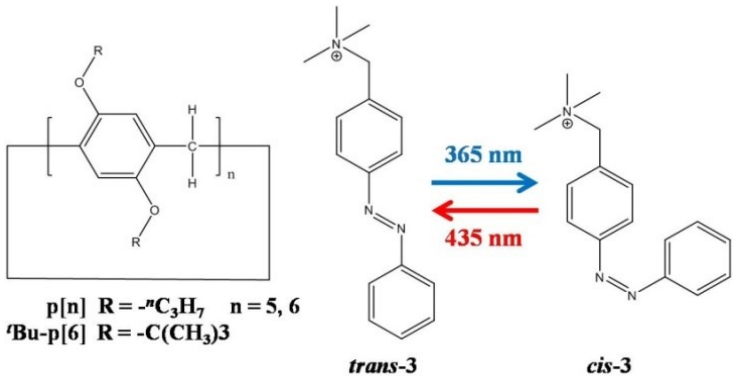
Materials and equipments

Materials： Solvents and reagents were purchased from commercial suppliers without further purification. Pillar[5]arene (P[5]), pillar[6]arene (P[6]), tert-butyl substituted pillar[6]arene (P[6]-^{t}Bu) and the azobenzene-containing guest 3 were synthesized following literature reported procedures[1]. In addition, the ^{1}H NMR spectra of pillararenes and guest 3 were also presented in the previous report[1].

Equipments: Fluorescence and UV-vis spectra were conducted by HITACHI U-3310 and HITACHI F-2500 fluorescence spectrophotometer, respectively. The conductance experiments were performed by Keithley 6487 picoammeter and Sutter P-97. The photoisomerization reaction of guest 3 was conducted by photothermal parallel reaction instrument.



Scheme 1. Molecular structure of pillararenes (P[5], P[6] and ^{t}Bu-P[6]) and *trans-cis* photoisomerization of 3 under UV (365 nm) and visible (435 nm) light irradiation.

Preparation of HPTS-entrapped large unilamellar vesicles

Firstly, 1,2-diacyl-sn-glycero-3-phosphocholine (PC, 15 mg) and 3-β-hydroxy-5-cholestene (CH, 3.75 mg) were dissolved in $CHCl_3$ (20 mL) in a round-bottom flask. The solvent was removed under reduced pressure (7 min, 25°C) to produce a uniform thin film. The film was dried under high vacuum for 3 h at 25°C. 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°C for 2 h to prepare a milky suspension. The mixture was then subjected to ten freeze-thaw cycles to achieve the HPTS-entrapped large unilamellar vesicles (LUVs): freeze in liquid N_2 for 30 s, warm it up at 37°C for 1.5 min, then gentle vortex mixing for 3 min at room temperature. Finally, the suspension of LUVs was divided into two equal aliquots and dialyzed over 36 h with gentle stirring (250 r/min) using membrane tube (MWCO = 8000–14000) against the same HEPES buffer solution (300 mL, without HPTS) for eight to ten times to remove free HPTS[2].

Experiment of proton transport activity through HPTS assay

The prepared LUVs suspension (13.3 mM, 100 µL, internal buffer: 100 mM NaCl, 10 mM HEPES, pH = 7.0) was added to HEPES buffer solution (external buffer: total volume 2000 µL, 10 mM HEPES, 100 mM NaCl, pH = 7.6). The solution of pillararene 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 pillararenes. Fluorescence intensity of HPTS (I_1) was continuously monitored under 510 nm emission and 454 nm excitation for 1800 s. The 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[2].

Calcein-encapsulated large unilamellar vesicles

PC (100 mg/mL, 0.10 mL) and CH (2.5 mg) were dissolved in CHCl3 (20 mL). The solution was evaporated under reduced pressure (8 min, 25°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°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°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 [3].

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[3].

Photoisomerization reaction of 3

The internal irradiation of UV and visible light caused photoisomerization of 3. Figure S1 shows the UV-Vis absorption spectra changes of 3 upon successive irradiation in mixed solution of chloroform/acetonitrile (10:1). The UV-Vis absorption at 318 nm of *trans*-3 decreased gradually with time extension of UV light irradiation under 365 nm. Meanwhile, the UV-Vis absorption at 435 nm increased slightly. The UV-Vis absorption changes can be attributed to photoisomerization of 3 from *trans* to *cis* conformation. Then, we used the above solution to investigate the photoisomerization from *cis* to *trans* conformation of visible light irradiation under 435 nm. UV-Vis absorption suggests that the absorbance increased significantly at 318 nm and decreased slightly at 435 nm along with time. The UV-Vis absorption changes demonstrate the reversible photoisomerization of 3.

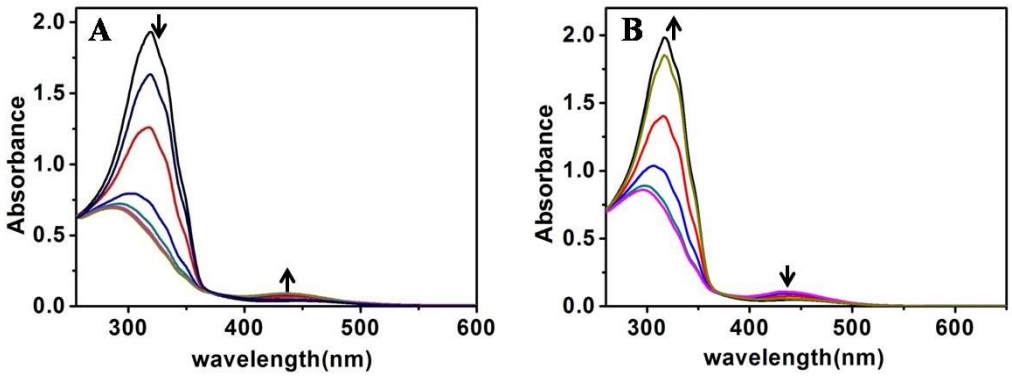


Figure S1. UV-Vis absorption spectra of 1.0 mM *trans*-3 in mixed solution (10:1 chloroform/acetonitrile) under UV light irradiation (365 nm) at different time: 0 s, 5 s, 20 s, 60 s, 120 s and 240 s (a) and later after visible light irradiation (435 nm) at different time: 0 s, 5 s, 10 s, 20 s, 60 s, 100 s and 150 s (b).

UV-Vis spectroscopic titrations

The successive addition of P[6] caused UV-Vis absorption changes of *trans*-3. Figure S3 shows the UV-Vis spectroscopic titrations of *trans*-3 (0.4 mM) upon successive addition of P[6] (up to 1.2 mM) in above mentioned chloroform/acetonitrile (10:1) mixed solution. We prepared *trans*-3 stock solution (10 mM) and P[6] stock (100 mM). The *trans*-3 stock solution was diluted to the final concentration of 0.4 mM. Then, P[6] stock was gradually added into *trans*-3 solution and mixed by vortexing for 3 min before recording UV-Vis absorption titration.

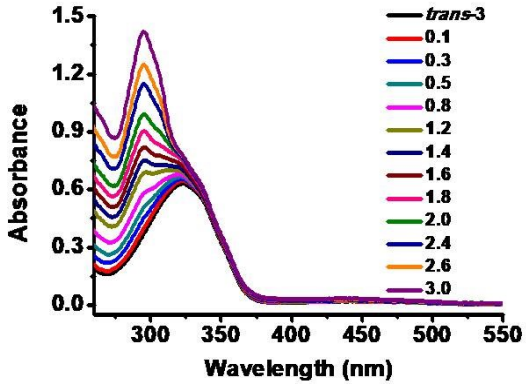


Figure S2. UV-Vis absorption spectral changes upon titration of P[6] (up to 1.2 mM) to a solution of *trans*-3 (0.4 mM) in mixed solution (10:1 chloroform/acetonitrile).

Current experiments

In the current experiments, picoammeter was utilized to investigate ion transportation activity. The self-made current testing device used in this experiment is a double-electrode (Ag/AgCl electrode) system in Figure S4 below. The nanopipet was attached to the terminal of an

electrode after stretching by glass drawing device, with 1.58 μm diameter (SEM image in Figure S3). The current changes were detected at +2V voltage and pH 7.0 HEPES buffer.

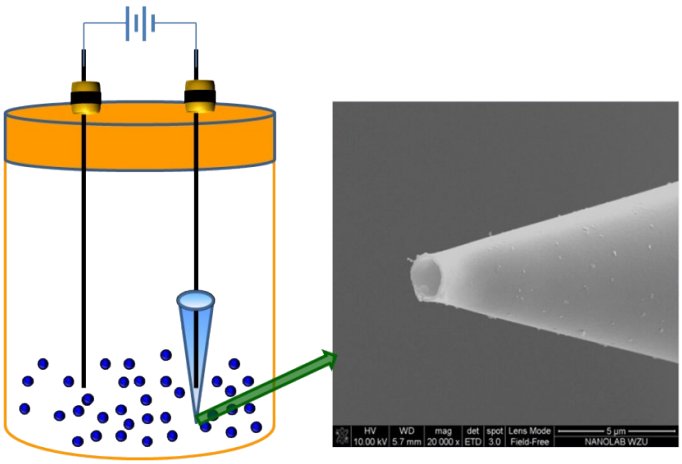


Figure S3. Picoammeter for current testing (left) and SEM image of nanocapillary after stretching (right).

Photoresponsive reversible switch

P[6] could act as an efficient transmembrane channel in lipid bilayer membrane, trans-3 could block P[6] channel through host-guest interaction. The blocked channel could be reopened after UV light irradiation at 365 nm by changing *trans*-3 to *cis*-3 due to photoisomerization and removing *cis*-3 out of internal cavity of P[6]. The reopened channel could be blocked again after visible light irradiation at 435 nm due to the transition from *cis*-3 to *trans*-3 conformation (Fig S4). This reversible single-molecule ligand-gating switch could be operated over three times (Fig S5).

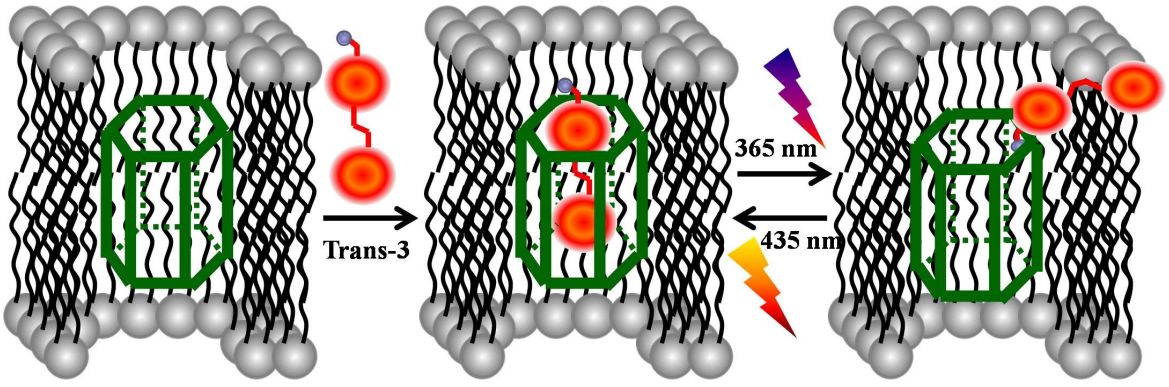


Figure S4. Schematic representation of the photoresponsive reversible switch utilizing P[6] (green hexagonal prism) and 3 (red meteor hammer) as channel and gate molecules under alternate UV and visible light irradiation (365 nm/435 nm) in lipid bilayer membrane.

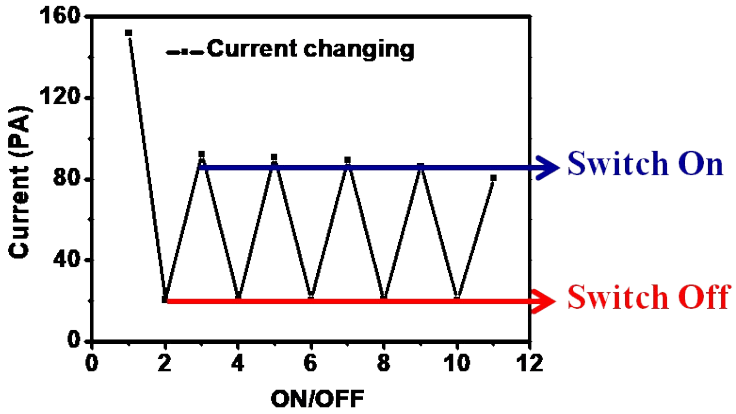


Figure S5. The reversible single-molecule ligand-gating switch could be operated five times and the current recordings could maintain about 80-90 pA and ～20 pA under switch on/off state.

References:

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