Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Light-controlled Ion Channel Formed by Amphiphilic Small Molecule Regulate Ion Conduction via *cis-trans* Photoisomerization

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1 Experimental Section.

1.1 General materials. All starting materials were obtained from commercial suppliers and were used without further purification unless otherwise stated. All air- or moisture-sensitive reactions were performed using oven-dried or flame-dried glassware under an inert atmosphere of dry argon. Air- or moisture-sensitive liquids and solutions were transferred via syringe. Tetrahydrofuran (THF) was distilled from sodium benzophenone; dichloromethane was distilled from calcium hydride; triethylamine (TEA) was redistilled and stored over KOH pellets prior to use. Egg yolk phosphatidylcholine (EYPC) was obtained from Avanti Polar lipids as a solution in chloroform (25 mg/mL). 8-hydroxy-1,3,6-pyrenetrisulfonate (HPTS) and Trixon-100 were obtained from Sigma-Aldrich and used without further purification.

1.2 Characterizations. Proton and carbon magnetic resonance spectra (^{1}H, ^{13}C NMR) were recorded on a Bruker Avance 500 (400 MHz) spectrometer. Chemical shifts were reported in parts per million (ppm) downfield from the Me_4Si resonance which was used as the internal standard when recording ^{1}H NMR spectra. Mass spectra were recorded on a Micromass GCTTM and a Micromass LCTTM. Absorption

spectra were recorded on a Shimadzu UV-2550 UV-vis spectrometer. Fluorescence measurements were performed on a Varian Cary Eclipses fluorescence spectrometer equipped with a stirrer and a temperature controller (kept at 25 ℃ unless otherwise noted). Small angle X-ray diffraction (XRD) assay was performed on a on a Japan Rigaku D/max-γA. XRD equipped with graphite monochromatized $Cu_{K\alpha}$ radiation (λ=1.5418 Å), employing a scanning rate of $0.02^{\circ}s^{-1}$ in the 2θ range from 0.7 to 10°. A Mini-Extruder used for the preparation of large unilamellar vesicles (LUVs) was purchased from Avanti Polar lipids. The size of EYPC vesicles was determined using a Delsa™ Nano Submicron Particle Size and Zeta Potential Particle Analyzer (Beckman Coulter Inc., USA). A CHF-XM-500w lamp with filters (365 nm or 450 nm) made by Beijing Trusttech Co., Ltd was used for azobenzene isomerization and photo-controlled experiments.

1.3 Synthesis of compounds.

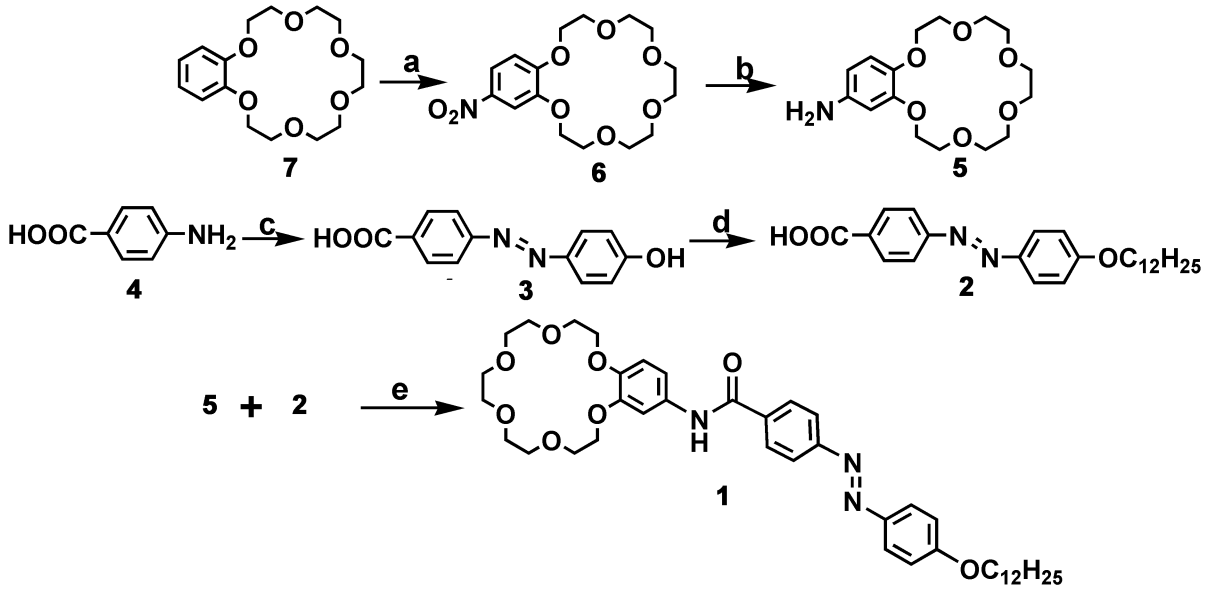


Fig. S1 Synthesis of compound **1**. Reagents and conditions: (a) 70% HNO_3, HOAC/$CHCl_3$, 78% yield. (b) 80% $N_2H_4{\cdot}H_2O$, 10% Pd/C, 1, 4-dioxane, 90% yield. **(**c) $NaNO_2$/HCl aq, phenol/NaOH, 82% yield. (d) $BrC_{12}H_{25}/K_2CO_3$/KI/DMF, KOH/EtOH, 56% yield. (e) $SOCl_2$ reflux, then TEA/CH_2Cl_2, 0-5 ℃ for 0.5 h

and rt overnight, 80% yield.

Compound 6: To a mixture of 18-benzocrown-6 ether **7** (2.0 g, 6.4 mmol) and acetic acid (30 mL) in chloroform (25 mL), nitric acid (7 mL, 70%) was added dropwise over 30 mins. The obtained mixture was stirred for 24 h at room temperature, and then neutralized with aqueous Na_2CO_3. The organic layer was separated, and the aqueous layer was again extracted with $CHC1_3$ twice. The combined chloroform extracts were dried over $MgSO_4$. After evaporation of $CHC1_3$, a yellow solid was obtained which, upon recrystallization from ethanol, yielded 1.78 g (78%) of pure compound **6**.

^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.89 (dd, J = 8.8 Hz, 2.4 Hz, 1 H), 7.74 (d, J = 2.8 Hz, 1 H), 6.89 (d, J = 9.2 Hz, 1H), 4.24(q, J = 2.4 Hz, 4 H), 3.97~3.94 (m, 4 H), 3.78~3.69 (m, 12 H). ^{13}C NMR ($CDCl_3$, 100 MHz), δ (ppm): 154.3, 148.4, 141.4, 117.9, 111.1, 107.9, 70.9, 70.8, 70.7, 70.6, 70.5, 70.4, 69.1, 69.0, 68.9. MS(ESI): m/z : Calcd. For $C_{16}H_{23}NO_8Na^+$ $[M+Na]^+$: 380.1. Found: 380.1.

Compound 5: A solution of **6** (1.25 g, 3.5 mmol) and 10% Pd/C (0.12 g) in 20 mL of 1,4-dioxane was heated to reflux. Then 10 mL of hydrazine hydrate was added dropwise, and the reaction kept refluxing for another 3 h. After cooling to room temperature, the remained and insoluble catalyst was filtered off and the solvent was removed to obtain compound **5** (1.04 g, 90%) as a pale yellow oil which was used without further purification.

^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.73 (d, J = 8.4 Hz, 1 H), 6.29 (d, J = 2.8 Hz, 1 H), 6.21 (dd, J = 8.4 Hz, 2.8 Hz, 1 H), 4.12~4.07 (m, 4 H), 3.93~3.86 (m, 4 H), 3.77~3.68 (m, 12 H). ^{13}C NMR ($CDCl_3$, 100MHz), δ (ppm): 150.1, 142.0, 141.3, 117.2, 107.1, 102.6, 70.7, 70.6, 70.3, 69.9, 68.6. MS(EI): m/z : Calcd. For $C_{16}H_{25}NO_6^+$ $[M]^+$: 327.2. Found: 327.2.

Compound 3: A solution of $NaNO_2$ (4.14 g, 60 mmol) in 60 mL H_2O was added dropwise to a cooled solution of 4-aminobenzoic acid (6.86 g, 50 mmol) in 14 mL of concentrated HCl at 0-5 ℃. The mixture was stirred for another 30 min and then poured into an ice-cooled aqueous solution of phenol (4.71 g, 50

mmol) and NaOH (4.00 g, 100 mmol). The reaction was kept stirring at 5 ℃ for 1 hour, and then neutralized. The generated yellow precipitate was collected, washed with water, and recrystallized from H_2O:C_2H_5OH (1:1, V/V) solution to yield 9.88 g compound **3**, Yield= 82 %. ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 8.0 (d, J = 8.4 Hz, 2 H), 7.79 (d, J = 8.8 Hz, 2 H), 7.71 (d, J = 8.4 Hz, 2 H), 6.96 (d, J = 8.8 Hz, 2 H). ^{13}C NMR (DMSO-d_6, 100 MHz), δ (ppm): 166.7, 161.6, 154.6, 145.3, 131.8, 130.5, 125.3, 122.1, 116.1 MS (ESI): m/z: Calcd. For $C_{13}H_9N_2O_3^-$ [M-H] $^-$: 241.1. Found: 241.1.

Compound 2: Compound **3** (2.42 g,10 mmol), potassium carbonate (2.76 g, 20 mmol), 1-bromododecane (9.97 g, 40 mmol) and a trace of potassium iodide were dissolved in 50 mL dry DMF and refluxed for 48 h. After the reaction was completed, 100 mL water was added, and the product was extracted with dichloromethane. The separated organic layer was dried over MgSO4, and concentrated in vacuum. The obtained intermediate was further hydrolyzed with KOH (5.8 g, 90 mmol) in ethanol (150 mL) by refluxing overnight. After cooling to room temperature, the reaction was continued by acidation with 30% aqueous HCl, the generated precipitate was filtered and recrystallized from ethanol to obtain 1.5 g compound **2** as a bright orange solid, Yield = 56 %. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 8.12 (d, J = 8.4 Hz, 2 H), 7.92 (q, J = 4Hz, 4 H), 7.15 (d, J = 8.8 Hz, 2 H), 4.09 (t, J = 6.4 Hz, 2 H), 1.79~1.72 (m, 2 H), 1.46~1.39 (m, 2 H), 1.35 ~1.24 (m, 16 H), 0.85 (t, J = 6.4 Hz, 3 H). The ^{13}C NMR spectrum was hard to obtain due to the bad solubility in most deuterium solvents. MS (ESI): m/z : Calcd. For $C_{25}H_{33}N_2O_3^-$ [M-H] $^-$: 409.3. Found: 409.3.

Compound 1: Under Ar gas protection, compound **2** (0.82 g, 2 mmol) was dissolved and refluxed in $SOCl_2$ (15 mL) for 5 h. After removing the $SOCl_2$ in vacuum, the obtained acid chloride was dissolved in fresh distilled dry CH_2Cl_2 for the amidation coupling reaction. Under anhydrous condition, to a mixture of compound **5** (0.65 g, 2 mmol) and TEA (7.23 mL, 5 mmol) in dry CH_2Cl_2 (20 mL) at 0-5 ℃, the above prepared acid chloride was added dropwise and the reaction was kept at 0 ℃ for another 30 mins and then

at RT overnight. The reaction mixture was evaporated to dryness and chromatographed on a column of silica (silica gel, 10% methanol/ CH_2Cl_2) to obtain 1.2 g (80%) compound **1** as an orange powder.

^{1}H NMR (400 MHz, CDCl3), δ (ppm):7.92~ 8.04 (m, 6H), 7.80 (s, 1H), 7.50 (s, 1H), 7.0~7.04 (m, 3H), 6.88 (d, J=7.6 Hz, 1H), 4.18~4.22 (m, 4H), 4.07 (t, J=6.8 Hz, 2H), 3.94 (s, 4H), 3.70~3.78(m, 12H), 1.79~1.86 (m, 2H),1.45~1.52 (m, 2H), 1.27 ~1.39 (m, 16H), 0.90 (t, J=6.8 Hz, 3H). ^{13}C NMR (CDCl3, 100 MHz), δ (ppm): 165.0, 162.3, 154.6, 149.2, 146.8, 146.0, 136.0, 132.0, 128.0, 125.2, 122.8, 114.8, 114.6, 112.9, 107.4, 71.0, 70.9, 70.8, 70.7, 70.6, 69.7, 69.6, 69.5, 69.0, 68.5, 31.9, 29.7, 29.6, 29.5, 29.4, 29.2, 26.0, 22.7, 14.1. MS (HR-ESI): m/z Calcd. For $C_{41}H_{57}N_3O_8Na^+$ $[M+Na]^+$: 742.4043; $C_{41}H_{57}N_3O_8K^+$ $[M+K]^+$: 758.3777. Found: 742.4022; 758.3785.

2 The studies of self-assembled properties for compound 1 in both solution and solid state.

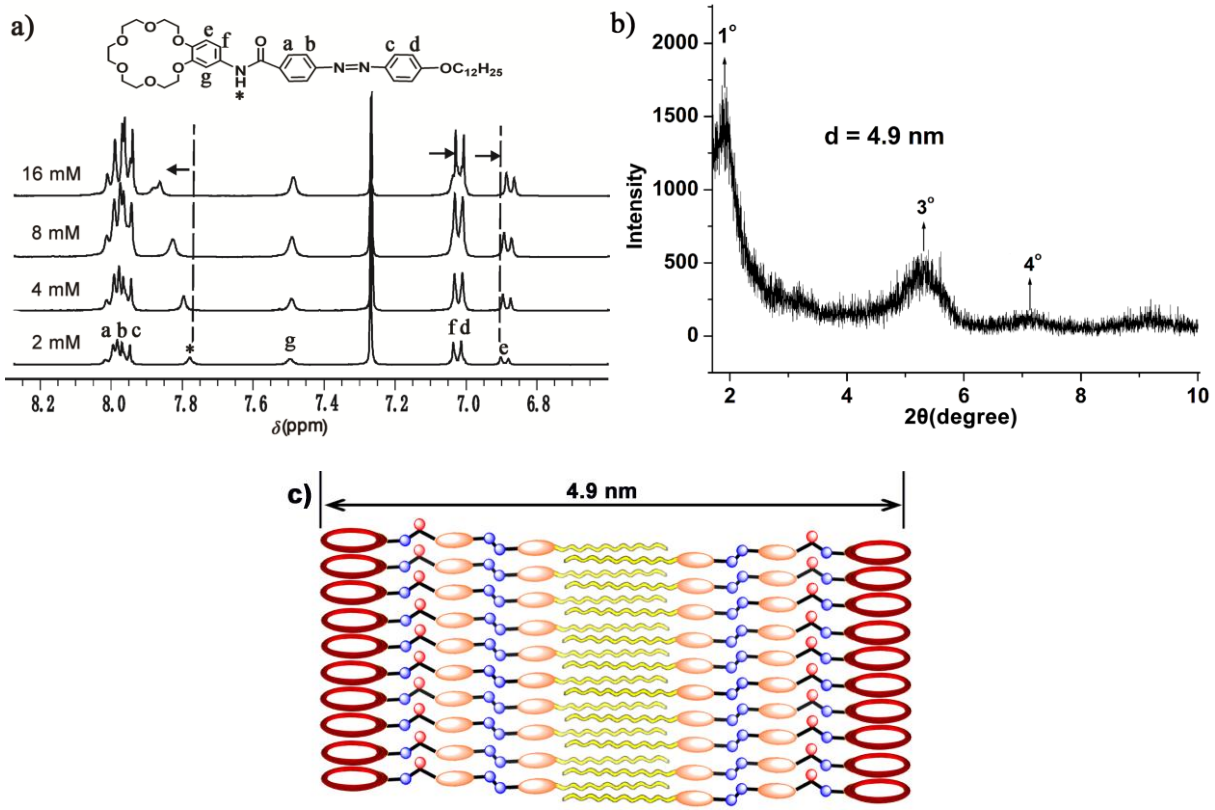


Fig. S2 a) Partial ^{1}H NMR spectra for compound **1** at different concentrations in $CDCl_3$ at 25 ^{o}C. b) X-Ray small angle thin-film diffraction pattern of compound **1** from concentrated chloroform solution. c) Schematic presentation of self-assembly mode of compound **1**.

3 UV-vis and NMR spectroscopic studies on photoisomerization of compound 1 in solution.

Isomerization of trans-cis and cis-trans under irradiation over time in THF solution.

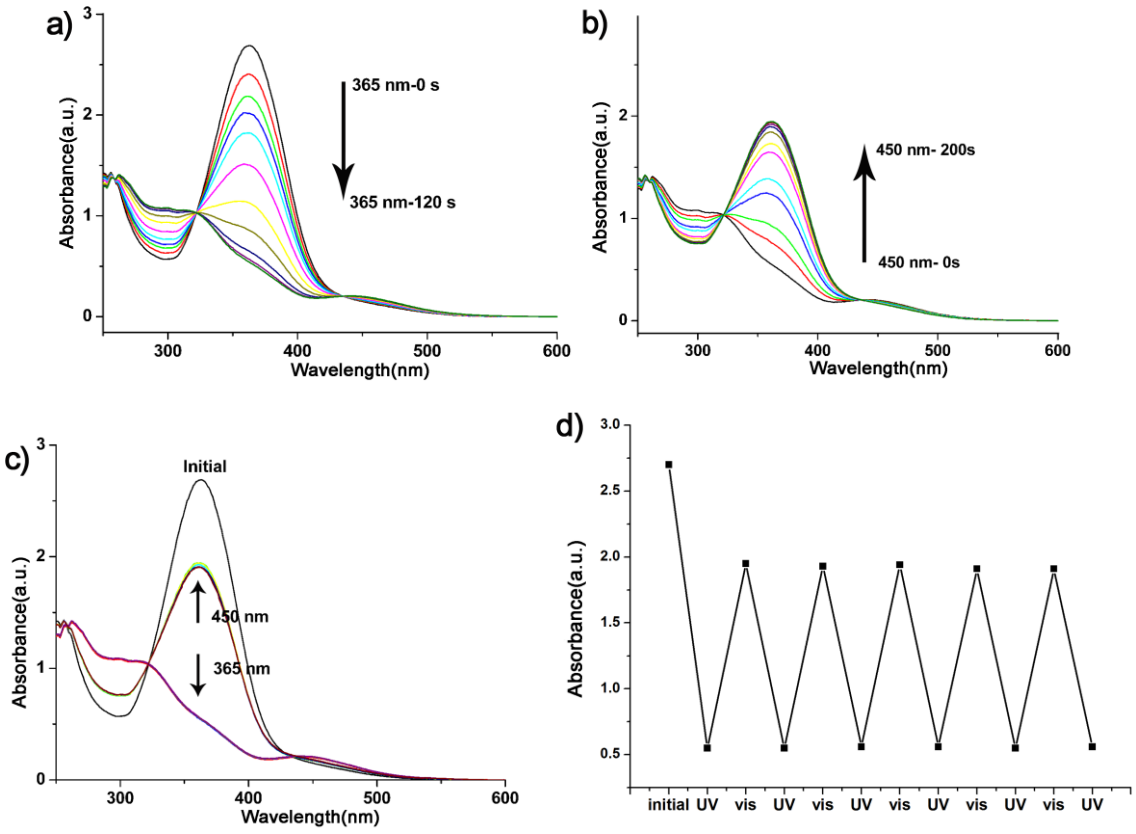


Fig. S3 Evolution of the UV-vis absorption spectra of compound **1** solution (0.25 mM in THF) as a function of irradiation time a) upon the irradiation of 365 nm UV light (2.5 mW/cm^2), t (sec) = 0, 5, 10, 15, 20, 30, 45, 60, 80, 100, 120. b) upon the irradiation of 450 nm light (5 mW/cm^2), t (sec) = 0, 5, 10, 25, 30, 45, 60, 80, 100, 120, 150, 200 s. c) switching cycles under alternating irradiation with 365 nm UV light and 450

nm visible light (2 min each). And d) the absorbance at 362 nm from c) procedure.

The synchronous NMR and UV-vis spectra detection under irradiation over time in deuterated THF solution.

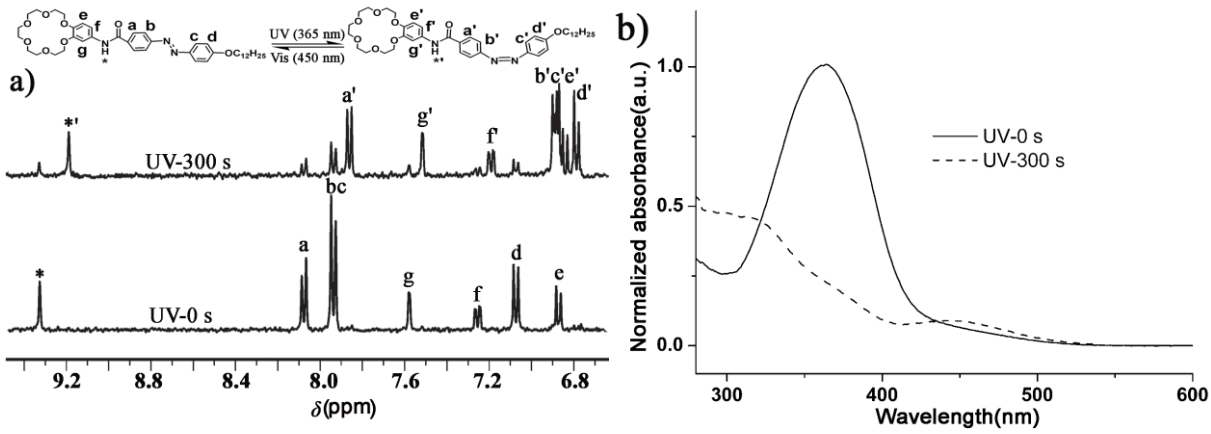


Fig. S4 The NMR a) and UV b) spectra for compound **1** solution before irradiation and after 300 s exposure to UV light (365 nm, 2.5 mW/cm^2). The concentrations are 0.5 mM in D_4-THF for NMR experiments and 0.03 mM in THF for UV experiments which was taken from above 0.5 mM D_4-THF solution by diluting just before testing.

From the NMR spectra, the final conversion for the azobenzene conformation from *trans* to *cis* under UV irradiation is 88 %. Then, by calculating form the corresponding UV spectra, the molar absorptivity for *trans* compound is about 3.6×10^4 L/mol cm and the molar absorptivity for *cis* compound is about 3.2×10^3 L/mol cm, respectively.

4 Single-channel Patch-clamp measurements.[1]

4.1 General process.

DPhPC lipids at 50 mg/mL in chloroform were dried under a stream of nitrogen for 4 h and then

dispersed in decane at 20 mg/mL. This solution was used to precoat a 150 μm hole in the side of a Delrin® cup (Warner Instruments, Hamden, CT) upon which a planar lipid bilayer membrane was formed across within a chamber having 1 mL of 1 M KCl (10 mM HEPES, pH 7.0) on both sides. All the operation temperature was kept at 25 ˚C (room temperature) by a temperature controller. Formation of membrane was monitored by measuring membrane capacitance. The transporter in DMSO (0.16 mM) 5μL was added to both the *cis/trans* side of the chamber (final concentration 0.8 μM) and the solution was stirred for 2 minutes. A holding potential of +100 mV was applied and the channel responses were recorded. Channel activity was measured with respect to the *trans* (ground) side. Ag/AgCl electrodes were used to impose voltages and record currents across the membrane. The Axon patch clamp workstation was used for all experiments. Data were amplified (Chem-Clamp; Dagan Corp. USA), digitized (DigiData 1322A; Axon Instruments, Foster City, CA), and stored on a Celeron PC using the Clamplex program (version 9.2; Axon Instruments, Foster City, CA). Data Analysis was performed using the Clampfit suite software (version 9.2; Axon Instruments, Foster City, CA) and OriginLab 8.0 (OriginLab Corporation, North-ampton, MA, USA).

4.2 Estimation of Pore Size Using Hill's Equation.[2-5]

The pore size formed by compound **1** could be estimated based on the Hille's equation as following:

$$g^{-1} = (4L\rho/\pi D^2) + \rho/D \quad (S1)$$

where g is the measured channel conductance, L the pore length, D the pore diameter and ρ the bulk resistivity of the ionic solution. For our case, ρ is estimated to be 0.081 Ω•m at 1 M for KCl. 3.5 nm can be assigned to L for a bilayer membrane.

4.3 Photo-regulated single-channel current recording of compound 1 on planar bilayers by Patch-clamp technology.

The in situ photo-regulated experiments were carried out by introducing an optical fiber in the cis side of the chamber and directly irradiating with 365 nm UV light and 450 nm visible light alternatively. The recording signals over a period were also analyzed by statistics and Gaussian fitting.

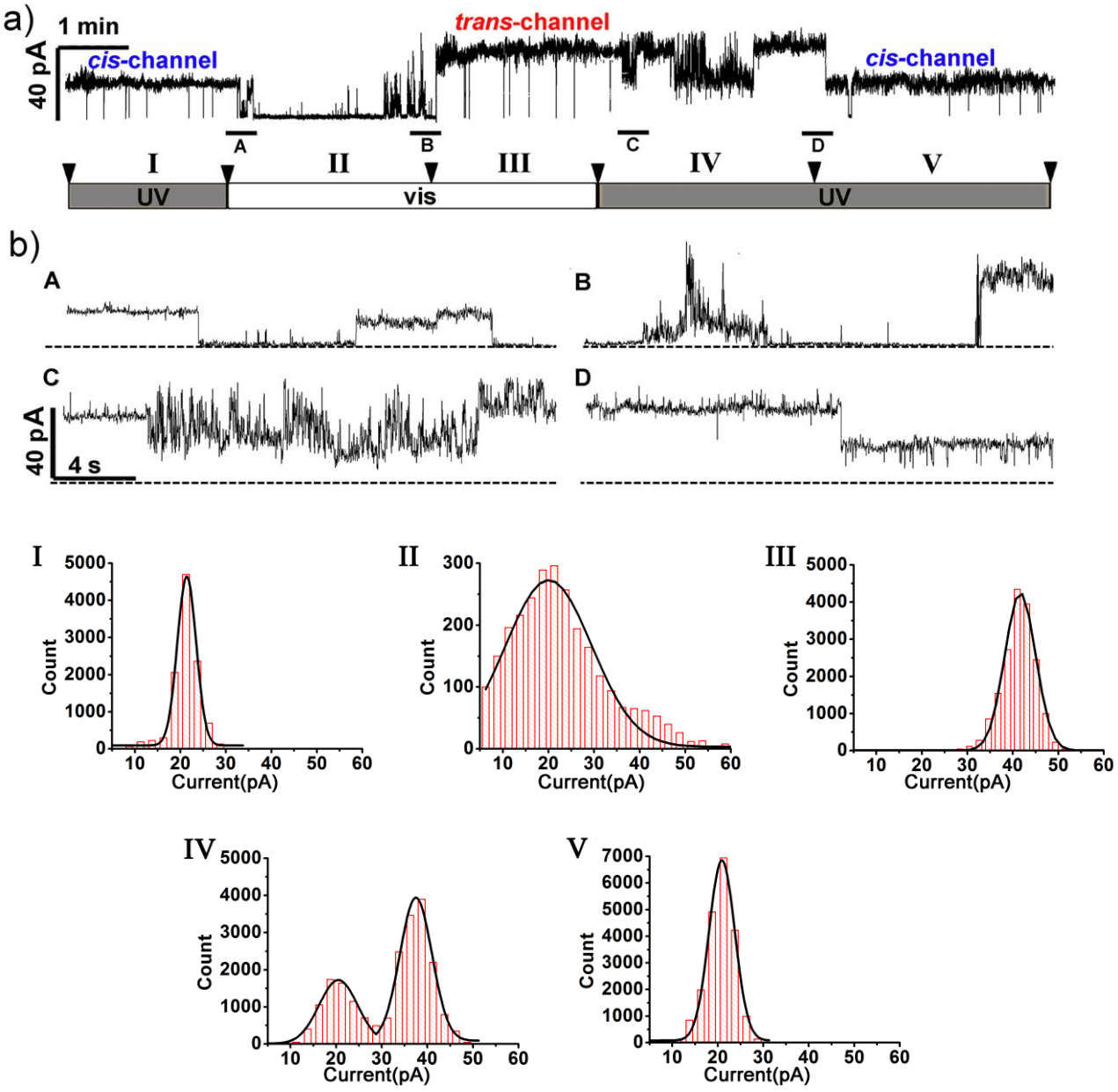


Fig. S5 a) *In situ* photoswitching of a single ion channel. A single *cis*-ion channel was irradiated with UV light, then visible light irradiation photoswitched off the channel and later caused a single *trans*-ion

channel to emerge. After this *trans*-ion channel was established, UV irradiation switched it back to the *cis*-ion channel. Current recordings were taken with compound 1 at 0.8 μM at 50 mV. b) Enlarged views of the critical photoswitching moments (A-D) reveal sharp on-and-off or *cis*-*trans* switching. Bottom is the corresponding current statistic histograms of the five parts (I-V) in a), the signals were processed by the Gaussian statistics and fitting using software OriginLab 8.0.

5 Study of transmembrane transport and ion selectivity of compound 1 with the liposome experiments.[6]

5.1 Vesicle preparation and size determination.

General preparation of large unilamellar vesicles (LUVs). A chloroform solution of EYPC (400 μL, 10 mg) and cholesterol (100 μL, 1 mg) was first evaporated with Ar-flux to form a thin film and then dried under high vacuum overnight. The lipid cake was hydrated in 0.5 mL of HEPES buffer (HEPES 10 mM, 100 mM NaCl or KCl , pH 7.0) containing 1 mM HPTS for 2 h at 40 ℃. The lipid suspension was submitted to 6 freeze-thaw cycles (-196 ℃/40 ℃) using liquid nitrogen and a thermostatic bath, and then 21 times extruded through a 0.1 μm polycarbonate membrane at room temperature. The LUV suspension was separated from extravesicular dye by size exclusion chromatography (SEC) (stationary phase: pre-packed column Sephadex™ G-25, mobile phase: HEPES buffer) and diluted with HEPES buffer to give a stock solution with a lipid concentration of 1 mM (assuming 100% of lipids were incorporated into liposomes). The size of the vesicles was determined by DLS experiments with mean diameter of 133.4 nm, and P. I. value of 0.071.

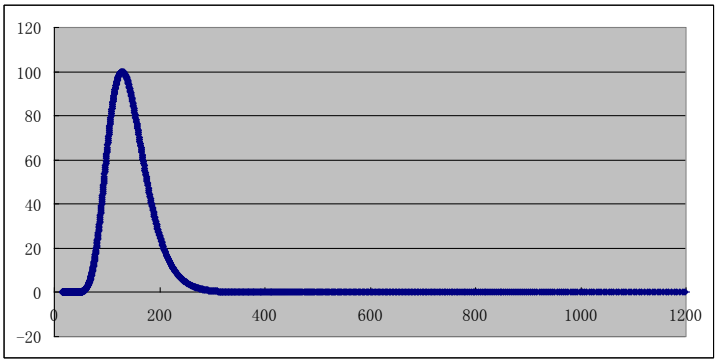


Fig. S6 Size distribution of EYPC-LUV vesicles determined by dynamic light scattering.

5.2 Determination of transport activity with the HPTS assays.[6]

Generally, 100 μL of the lipid suspension were added to 2900 μL gently stirred, thermostatic buffer in a fluorimetric cell. The total lipid concentration in the fluorimetric cell was about 33 μM. The time-dependent change in fluorescence intensity (λ_{em} = 510 nm) was monitored at two excitation wavelengths simultaneously ($I_{t,450}$: λ_{ex} = 450 nm, $I_{t,405}$: λ_{ex} = 405 nm) during the addition of base (30 μL, 0.5 M NaOH) at t = 50 s, transporter (30 μL stock solution in Tetrahydrofuran, 10 nM - 100 μM final concentration) at t = 100 s, and 60 μL of 5% Triton X-100 aqueous solution at t = 350 s. All the temperature was kept at 25 ˚C by a stirrer and a temperature controller. Time courses of fluorescence intensity I_t were obtained by first, ratiometric analysis ($R = I_{t,450} / I_{t,405}$) and second, normalization according to equation S2,

$$I_t = (R - R_{100})/(R_\infty - R_{100}) \quad \text{(S2)}$$

where R_{100} = R before addition of transporter and R_∞ = R after addition of Triton X-100. I_t at 350 s just before addition of Triton X-100 was defined as transmembrane activity Y, and analyzed with the Hill equation S2 to give effective concentration EC_{50} and the Hill coefficient n,

$$Y = Y_\infty + (Y_0 - Y_\infty)/(1 + (c/EC_{50})^n) \quad \text{(S3)}$$

where Y_0 is Y in absence of transporter, Y_∞ is Y with excess transporter, and c is the transporter concentration.

For all ion transport experiments, the HPTS assays were repeated for three times and the error

bars were less than 5%.

5.3 Determination of cations selectivity with the HPTS assays.

100 μL EYPC-LUVs contain HPTS prepared as described above were added to 2900 μL gently stirred, thermostatic buffer (10 mM HEPES, 100 mM M^+Cl^-, pH 7.0, where M^+ = Li^+, Na^+, K^+, Rb^+, Cs^+) in a fluorimetric cell. The time-dependent change in fluorescence intensity was monitored and analyzed as described above (final concentration of transporter is 5 μM) to obtain the fractional transmembrane activity Y dependent on the externally added cation.

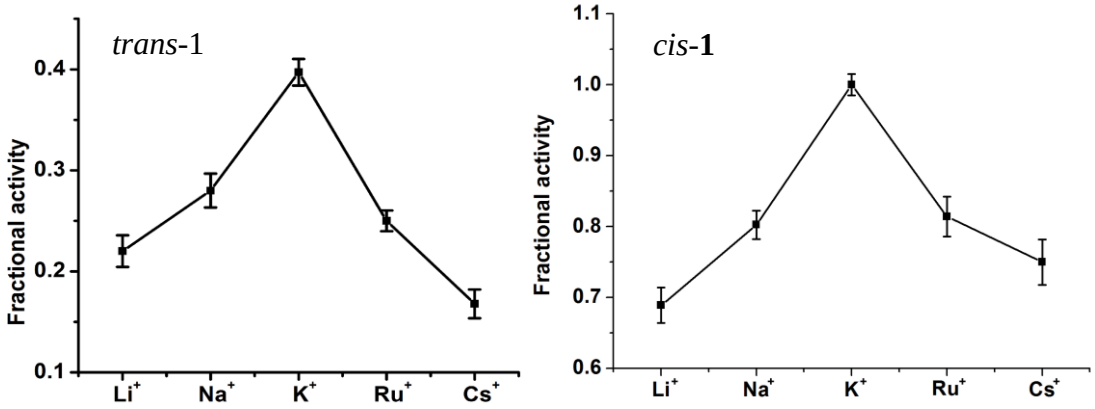
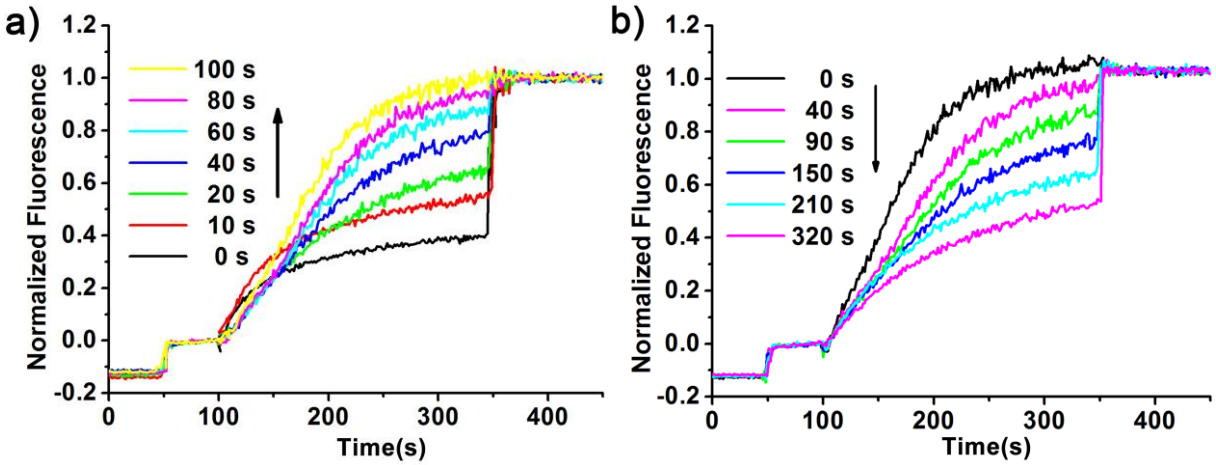


Fig. S7 Assays of cation selectivity of compound **1** (left: *trans*-**1**; right: *cis*-**1**) (5 μM, final concentration). Internal buffer (1 mM HPTS, 10 mM HEPES, 100 mM NaCl, pH 7.0), external buffer (10 mM HEPES, 100 mM MCl, pH 7.0).

5.4 Light-regulated transmembrane activity between *tran*-1 and *cis*-1 with HPTS assay.



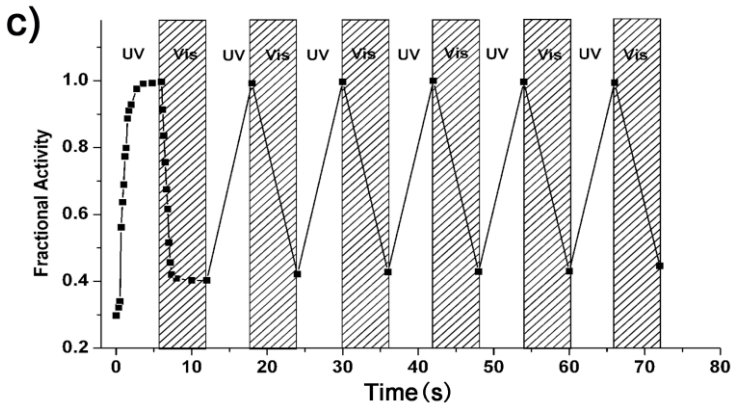


Fig. S8 Observed change in transmembrane activity of HPTS assay of compound **1** (5.0 μM, final concentration) upon the irradiation of light: a) upon the irradiation of 365 nm UV light (8 mW/cm^2); b) upon the irradiation of 450 nm light (11 mW/cm^2); c) cycles of transmembrane activity under alternating irradiation with 365 nm UV light and 450 nm visible light (6 min each).

*Determination of transmembrane activity of cis-**1** with HPTS assays.* 100 μL LUV solution prepared as described above was added to 2900 μL gently stirred, thermostatic buffer (10 mM HEPES, 100 mM KCl, pH 7.0) in a cuvette. The stock solutions with different concentrations of *trans*-**1** were dealt with 365 nm irradiation (8 mW/cm^2) for 6 min to ensure the completed photo-isomerization. The contents of *cis*-**1** in these solutions were determined by NMR spectra, which suggested that all the solutions completed the biggest isomerization and all of the contents of *cis*-compound were around 88%. Then, the time-dependent change in fluorescence intensity was monitored as normal; it could be found that the transport activity arrived 100% when the concentration of the *cis*-**1** (88% *cis*-compound) was increased to 5 μM. Under these concentrations, the concentration of *trans*-**1** inside was less than 0.60 μM, in which the contribution on transport activity was ignorable. Thus the obtained transport activity should be mostly contributed from the *cis*-**1**, as well as for the Hill curve fitting as below.

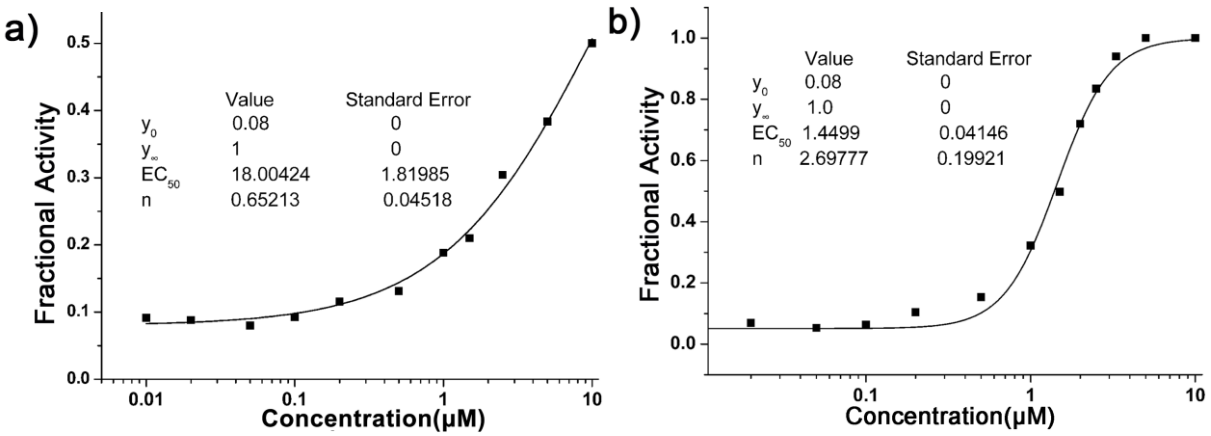
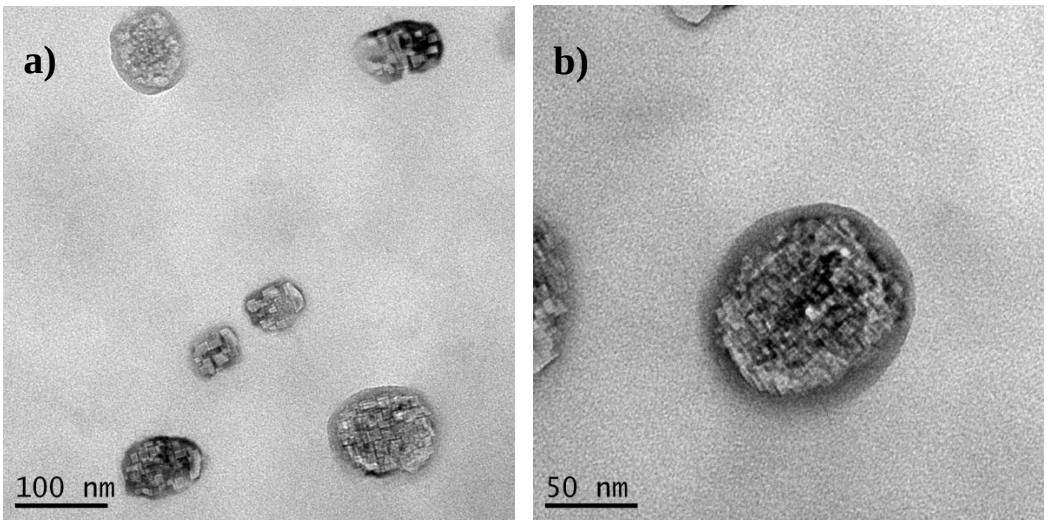


Fig. S9 Representative Hill plot of transmembrane activity of a) *trans*-**1** and b) *cis*-**1** in 10 mM HEPES, 100 mM KCl, pH 7.0 as external buffer.

6 Photo-regulated self-assembly of compound 1 in solution.



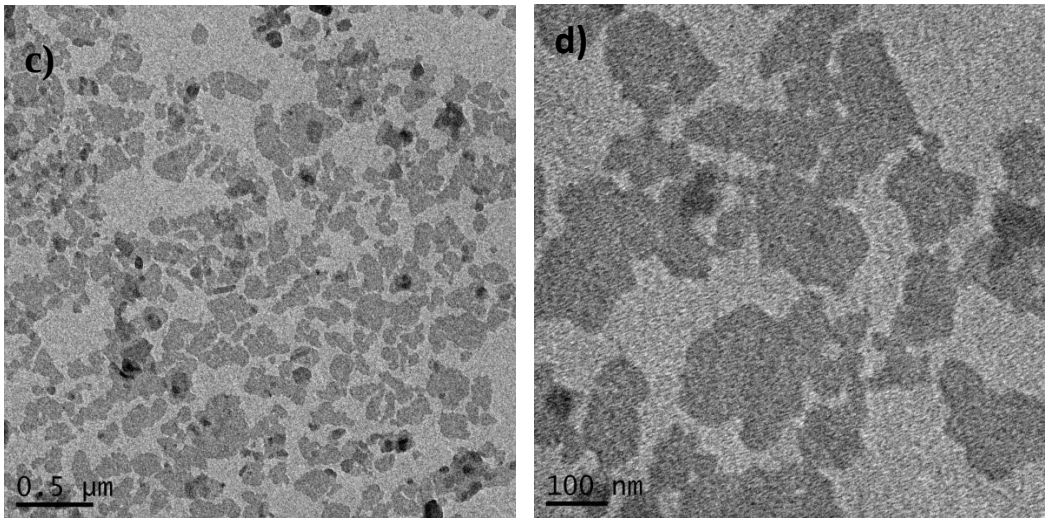


Fig. S10 Direct observation of the self-assembly of compound **1** in 10 mM HEPES solution: TEM images of *trans*-**1** (a-b) and *cis*-**1** (c-d). The *trans*-**1** sample was prepared by dropping the THF solution into 10 mM HEPES solution. The *cis*-**1** sample was obtained by irradiating the *trans*-**1** sample in HEPES solution with 365 nm light (8 mW/cm^2) for 10 min, which was frozen immediately in liquid nitrogen and submitted to freeze-drying in vacuum overnight.

7 The photo-regulated doping measurements of compound 1 with LUVs in situ.

To 3 mL of EYPC-LUVs without HPTS (with a lipid concentration of 1 mM) prepared as described above was added a THF solution of compound **1** (4 mM, 200 μL) and stirred for 10 mins. Then, an aliquot of the obtained composite LUVs were taken out and purified by minicolumn centrifugation with pre-packed Sephadex™ G-25. The compound contained vesicle solution was collected, and the doping amount was quantified by the UV analysis.

Photo-regulated doping amounts *in situ* were measured, in which the *cis* doping amount was obtained by irradiation of the *trans* solution *in situ*. The switching cycles were obtained by the irradiation of alternative UV 365 nm (8 mW/cm^2) and visible 450 nm (11 mW/cm^2) light. The irradiation time was kept at 10 mins each for completed isomerization of compound. After each irradiation, an aliquot (0.5 mL) of

the suspension was taken out, purified and analyzed. Due to the weak molar absorptivity, the doping amount of *cis*-isomer in LUVs was evaluated by photo-switching the purified *cis*-composite LUVs with 365 nm light again.

8 The photo-regulated solubility of compound 1 in LUVs-contained buffer solution.

Fig. S11 Solubility variation of compound **1** in liposome contained HEPES buffer solution before (left) and after the irradiation of 365 nm light (right) (8 mW/cm^2, 10 min).

The detailed process for the hydrophilicity comparison was performed as described as following: To a 3 mL of EYPC-LUVs without HPTS (with a lipid concentration of 1 mM) was added a THF solution of *trans*-**1** or *cis*-**1** (4 mM, 200 μL). The *cis*-**1** THF solution was obtained by irradiating the *trans*-**1** THF solution with 365 nm light for 10 min (8 mW/cm^2).

9 The studies of self-assembled properties for *cis*-1 by concentrated NMR.

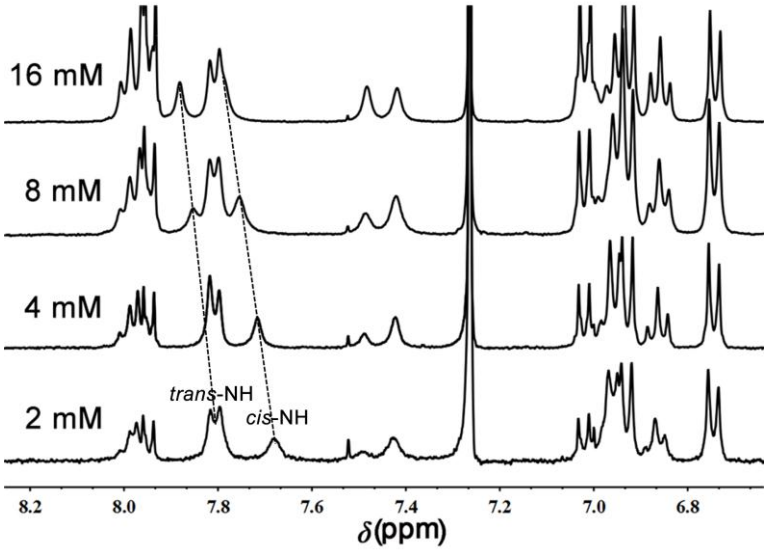


Fig. S12 Partial ^{1}H NMR spectra for the irradiated compound **1** (365 nm light, 8 mW/cm^{2} for 6 min) at different concentrations in $CDCl_3$ at 25 ^{o}C. Both of the amide proton signals that attributed to *trans*-**1** and *cis*-**1** exhibited downfield shift, which suggested the self-assembly by intermolecular hydrogen bonding actions.

10 References:

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