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A Well-defined Unimolecular Channel Facilitates Chloride Transport

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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 carried out 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. Dry tetrahydrofuran (THF) was distilled from sodium benzophenone; dry dichloromethane was distilled from calcium hydride; and dry 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 $mg \cdot mL^{-1}$) and stored at -20 °C prior to use. 8-hydroxy-1,3,6-pyrenetrisulfonate (HPTS), carbonyl cyanide-4-(trifluoromethoxy)-phenylhydrazone (FCCP) and Trixon-100 were obtained from Sigma-Aldrich and used without further purification.

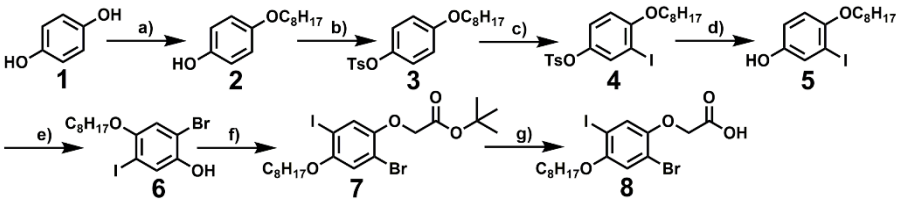
2. Characterizations.

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 ^1H NMR spectra. Mass spectra were recorded on a Micromass GCTTM and a Micromass LCTTM. Fluorescence measurements were performed on a Varian Cary Eclipses fluorescence spectrometer equipped with a stirrer and a temperature controller (kept at 25 °C unless otherwise noted). Absorption spectra were recorded on a Shimadzu UV-2550 UV-vis spectrometer. 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).

3. Synthesis and characterization of compounds.

3.1 The synthesis of the monomer for OPEs.



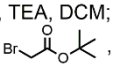
Reagents and reaction conditions: a) bromooctane, K_2CO_3, CH_3CN; b) Tosyl chloride, TEA, DCM; c) I_2, KIO_3, H_2SO_4, CH_3COOH,CCl_4, H_2O; d) KOH, H_2O, tert-Butanol; e) Br_2, DCM; f)  , K_2CO_3, CH_3CN; g) CF_3COOH, DCM.

Figure S1. The synthetic route for the monomer of OPEs.

Compound 2: Bromooctane (17.45 g, 90.90 mmol) was added to a solution of hydroquinone (10.00 g, 90.90 mmol) and potassium carbonate (18.82 g, 136.37 mmol) in CH_3CN (300 mL). The solution was stirred for 24 h at 95 °C. After cooling to room temperature, the mixture was filtered and washed with DCM (2 × 100 mL). The filtrate was rotary evaporated to dryness and the residue was purified by column chromatography on silica using petroleum ether/ethyl acetate (5 : 1) as an eluent to obtain **2** as a white solid

(19.76 g, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.87-6.67 (m, 4H), 3.89 (t, *J* = 6.6 Hz, 2H), 1.75 (dq, *J* = 8.2, 6.7 Hz, 2H), 1.51-1.39 (m, 2H), 1.29 (m, 8H), 0.93-0.83 (m, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ: 153.35, 149.31, 115.98, 115.61, 68.74, 31.83, 29.39, 29.26, 26.06, 22.67, 14.11. LR-MS (ESI-TOF): Calcd. For $C_{14}H_{23}O_2$ $[M+H]^+$: 223.2. Found: 223.2.

Compound 3: To a round bottomed flask, **2** (10.00 g, 45.05 mmol), triethylamine (9.32 mL, 67.58 mmol), and dichloromethane (400 mL) were mixed, and the obtained solution was stirred at 0 °C. Then, another dichloromethane solution (80 mL) of p-toluene sulfonyl chloride (9.42 g, 49.56 mmol) was added dropwise in 2h, and the reaction mixture was stirred for 12 h at room temperature. The reaction mixture was washed with aqueous hydrochloric acid, and organic phase was dried with $MgSO_4$. The solvent was evaporated, the crude product was purified by column chromatography on silica gel to afford **3** as a colorless oil (13.72 g, 81%).^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.68 (d, *J* = 8.3 Hz,2H), 7.29 (d, *J* = 8.1 Hz, 2H), 6.90-6.81 (m, 2H), 6.79-6.70 (m, 2H), 3.88 (t, *J* = 6.6 Hz, 2H), 2.44 (s, 3H), 1.74 (dq, *J* = 8.5, 6.6 Hz, 2H), 1.42 (td, *J* = 11.4, 9.2, 4.5 Hz, 2H), 1.39-1.22 (m, 8H), 0.92-0.84 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 157.80, 145.21, 142.89, 132.38, 129.69, 128.58, 123.30, 114.97, 68.39, 53.46, 31.81, 29.34, 29.23, 29.19, 26.01, 22.66, 21.71, 14.11. LR-MS (ESI-TOF): Calcd. For $C_{21}H_{28}O_4SNa$ $[M+Na]^+$: 399.1. Found: 399.1.

Compound 4: 3 (12.00 g, 31.91 mmol) was added to a stirred mixture of I_2 (6.48 g, 25.52 mmol), sulfuric acid (1.25 mL), CCl_4 (6.75 mL), water (8 mL), and acetic acid (16.25 mL) at room temperature. Then, KIO_3 (2.73 g, 12.76 mmol) was added, and the mixture was

heated at reflux for 48 h. The reaction was cooled to room temperature, and chloroform (20 mL) was added. The solution was washed with saturated aqueous Na_2SO_3 (100 mL) followed by 10% (w/w) aqueous NaOH (2 × 100 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure and the crude product was purified by column chromatography on silica gel to give yellow oil iodide **4**. (12.05g, 75%) ^{1}H NMR (400 MHz, Chloroform-*d*) δ: 7.72-7.65 (m, 2H), 7.36-7.29 (m, 3H), 6.93 (dd, *J* = 8.9, 2.9 Hz, 1H), 6.64 (d, *J* = 8.9 Hz, 1H), 3.94 (t, *J* = 6.4 Hz, 2H), 2.46 (s, 3H), 1.55-1.43 (m, 2H), 1.42 -1.20 (m, 10H), 0.93-0.82 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 156.63, 145.54, 142.85, 133.08, 132.03, 129.80, 128.60, 123.16, 111.32, 85.68, 69.69, 31.80, 29.25, 29.22, 29.01, 26.05, 22.67, 21.75, 14.13. LR-MS (ESI-TOF): Calcd. For $C_{21}H_{27}IO_4SNa$ $[M+Na]^+$: 525.4. Found: 525.4.

Compound 5: 20% (w/w) aqueous NaOH (15 mL) was added to the stirred solution of **4** (10.00 g, 19.92 mmol) in tertiary butanol (150 mL), and the mixture was heated at 95 °C for 6 h. After cooling, the solution was neutralized by HCl aq (50 mL, 1 mol/L), the solvent was evaporated and the crude material was re-dissolved in dichloromethane (500 mL) washed with water and dried over $MgSO_4$, filtered and concentrated to dryness. The resulting material was purified by column chromatography over silica gel to give pale yellow oil **5** (5.68g, 82%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ: 7.28 (d, *J* = 2.9 Hz, 1H), 6.77 (dd, *J* = 8.8, 2.9 Hz, 1H), 6.68 (d, *J* = 8.8 Hz, 1H), 3.92 (t, *J* = 6.5 Hz, 2H), 1.79 (dq, *J* = 8.3, 6.5 Hz, 2H), 1.55-1.41 (m, 2H), 1.41-1.22 (m, 8H), 0.93-0.83 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 152.24, 150.17, 126.12, 116.02, 113.43, 87.01, 60.84, 31.84, 29.51, 29.32, 29.26, 26.11, 22.69, 14.15. LR-MS (ESI-TOF): Calcd. For $C_{14}H_{21}IO_2Na$

$[M+Na]^+$: 371.0. Found: 371.3.

Compound 6: Bromine (4.13 g, 25.85 mmol) in DCM (25 mL) was added dropwise to a stirred solution of **5** (6.00 g, 17.24 mmol) in DCM (20 mL) at 0 °C with spontaneous liberation of HBr. After addition, the mixture was warmed to room temperature and stirred for a further 30 min. The solution was washed with saturated aqueous $Na_2S_2O_3$ (100 mL) and dried over $MgSO_4$. Removal of the solvent in vacuum afforded the crude product which was chromatographed on silica gel (hexane/EtOAc = 8/1) to give a yellow solid **6** (5.73g, 78%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ: 7.45 (s, 1H), 6.88 (s, 1H), 5.21 (s, 1H), 3.91 (t, *J* = 6.4 Hz, 2H), 1.86-1.74 (m, 2H), 1.56-1.43 (m, 2H), 1.30 (dddd, *J* = 24.8, 19.8, 9.4, 3.6 Hz, 8H), 0.93-0.85 (m, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ: 152.38, 147.07, 125.97, 115.24, 109.65, 86.17, 70.37, 31.82, 29.26, 29.23, 29.09, 26.05, 22.68, 14.14. LR-MS (ESI-TOF): Calcd. For $C_{14}H_{20}BrIO_2Na$ $[M+Na]^+$: 449.1. Found: 449.3.

Compound 7: A suspension of **6** (5.72 g, 13.38 mmol), bromoacetic acid tert-butyl ester (2.59 mL, 16.06 mmol), K_2CO_3 (2.77 g, 20.07 mmol) in acetonitrile (120 mL) was stirred at 60 °C for 16 h under protection of N_2. The reaction mixtures were filtered, and the solvents were removed under reduced pressure to provide the crude product that was purified by flash chromatography and afforded the product as white solid **7** (6.29 g, 87%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.36 (s, 1H), 7.21 (s, 1H), 4.71 (s, 2H), 3.97 (d, *J* = 6.3 Hz, 2H), 1.68 (dt, *J* = 8.2, 6.3 Hz, 2H), 1.49 (s, 9H),1.45 (t, *J* = 7.8 Hz, 2H), 1.29 (dp, *J* = 16.0, 3.7 Hz, 8H), 0.90-0.82 (m, 3H).^{13}C NMR (100 MHz, DMSO) δ: 169.84, 152.24, 148.94, 123.43, 116.83, 111.17, 85.47, 69.51, 66.07, 55.99, 31.19, 28.66, 28.55, 28.51, 25.48, 22.07, 18.53, 13.95. LR-MS (ESI-TOF): Calcd. For $C_{20}H_{30}BrIO_4Na$ $[M+Na]^+$: 563.0.

Found: 563.1.

Compound 8: A solution of **7** (6.00 g, 11.65 mmol) in dichloromethane (100 mL) and trifluoroacetic acid (40 mL) was stirred at room temperature for 2 h. Acetone (80 mL) was added to the reaction mixture. Excess trifluoroacetic acid and dichloromethane were evaporated to provide the white solid **8** without any purification (5.30 g, 94%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.34 (s, 1H), 7.20 (s, 1H), 4.69 (s, 2H), 3.97 (t, J = 6.3 Hz, 2H), 1.68 (dt, J = 8.1, 6.3 Hz, 2H), 1.45 (p, J = 7.0 Hz, 2H), 1.36-1.20 (m, 8H), 0.91-0.79 (m, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 169.77, 152.33, 148.81, 123.46, 116.81, 111.23, 85.46, 69.51, 65.82, 31.20, 28.67, 28.57, 28.52, 25.49, 22.08, 13.95. LR-MS (ESI-TOF): Calcd. For $C_{16}H_{22}BrIO_4$ $[M]^+$: 507.0. Found: 507.3.

3.2 The synthesis of the macrocycle for chloride recognition.

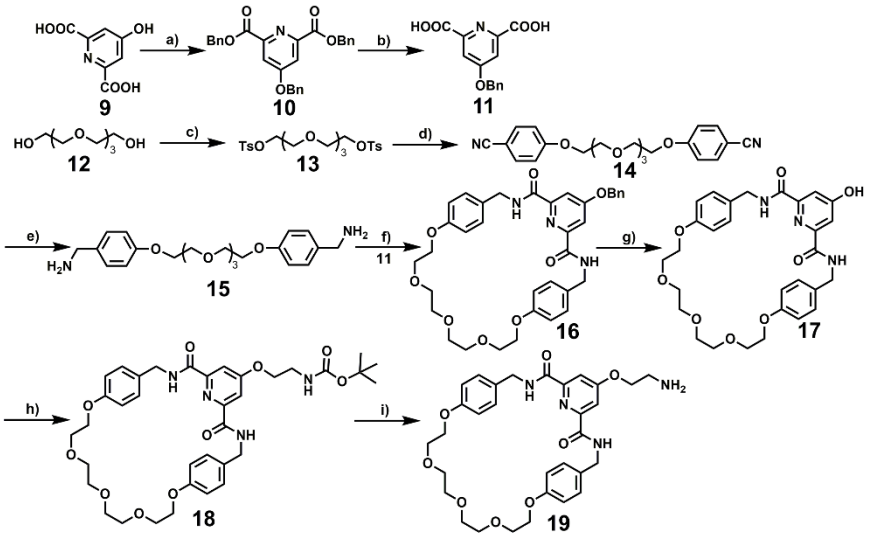


Figure S2. The synthetic route for PDA macrocycle.

Compound 10: A 500 mL two-necked flask equipped with a stir bar and a condenser coil was charged with anhydrous potassium carbonate (37.70 g, 273.20 mmol), evacuated and filled with nitrogen. Then, a solution containing 4-hydroxypyridine-2,6-dicarboxylic acid

(10.00 g, 54.64 mmol), acetone (250 mL), and (2-bromoethyl)benzene (20.82 mL, 174.85 mmol) were added by a syringe. The mixture was refluxed at 70 °C for 12 h. The reaction mixture was cooled to room temperature and filtered. The filtrate was evaporated and dissolved in DCM (500 mL). The resulting solution was washed with 5% aqueous solution of potassium hydroxide (300 mL) and brine (200 mL) and dried over anhydrous sodium sulfate. After evaporation, the residue was purified by column chromatography (hexane/ethyl acetate 8:1) to give a pale yellow solid **10**. (20.29 g, 82%) ^{1}H NMR (400 MHz, Chloroform-*d*) δ: 7.84 (s, 2H), 7.47-7.45 (m, 5H), 7.42-7.29 (m, 10H), 5.43 (s, 4H), 5.17 (s, 2H).^{13}C NMR (100 MHz, Chloroform-*d*) δ: 166.52, 164.39, 150.04, 135.04, 130.28, 124.56, 114.81, 69.25. LR-MS (ESI-TOF): Calcd. For $C_{28}H_{23}NO_5Na$ $[M+Na]^+$: 476.1. Found: 476.4.

Compound 11: KOH (2.50 g, 44.60 mmol) was added to a stirred solution of **10** (2.50 g, 5.50 mmol) in EtOH (200 mL), the mixture was heated to reflux overnight. After cooling to room temperature, the mixture was filtered and washed with EtOH, the filter cake was dissolved in 100 mL H_2O, followed by adding trifluoroacetic acid until the white solid was precipitate completely. Then the mixture was filtered and washed with H_2O, collect the filter cake as white solid to give compound **11**. (1.47g, 98%) ^{1}H NMR (400 MHz, Chloroform-d) δ: 7.77 (s, 2H), 7.58-7.28 (m, 5H), 5.41 (s, 2H). ^{13}C NMR (100 MHz, Chloroform-d) δ: 164.3, 162.1, 150.2, 135.9, 129.0, 128.7, 128.3, 113.6, 70.8. LR-MS (ESI-TOF): Calcd. For $C_{14}H_{11}NO_5K$ $[M+K]^+$: 312.0. Found: 312.0.

Compound 13: Tetraethylene glycol (4 mL, 23 mmol) was dissolved in anhydrous DCM (30 mL). The solution was cooled to 0 °C in ice bath. Tosyl chloride (13 g, 69 mmol) and

anhydrous triethylamine (24 mL) were added sequentially, then the mixture was stirred 4h at room temperature. DCM and TEA were removed under reduced pressure. Ice water (250 mL) was added and the solution was extracted with CH_2Cl_2 (200 mL each) three times. The combined organic phase was washed twice with HCl (2N, 250 mL), $NaHCO_3$ solution (5%, 200 mL) and water (200 mL) sequentially. After the organic layer was dried over Na_2SO_4, the solvent was removed under reduced pressure. The residue was subjected to silica chromatography using EtOAc/hexane (from 2/8 to 5/5, V/V) to give product **13** in 95 % yield (11.05 g, 22.07 mmol) as a colorless oil. ^{1}H NMR (400 MHz, Chloroform-d) δ: 2.45 (s, 6H), 3.57 (s, 8H), 3.68 (t, J=4.7 Hz, 4H), 4.16 (t, J=4.7 Hz, 4H), 7.35 (d, J=8.2Hz, 4H), 7.80 (d, J=8.2Hz, 4H). ^{13}C NMR (100 MHz, Chloroform-d) δ: 144.8, 133.0, 129.8, 128.0, 70.7, 70.5, 69.3, 68.7, 21.6. LR-MS (ESI-TOF): Calcd. For $C_{22}H_{30}O_9S_2$ $[M]^+$:502.1. Found: 502.1

Compound 14: K_2CO_3 (7.05 g, 50.00 mmol) was added to a acetonitrile solution (260 mL) containing 4-hydroxybenzonitrile (2.24 g, 18.80 mmol) and tetra-(ethyleneglycol)di-p-toluene sulfonate (4.64 g, 9.25 mmol), and the obtained mixture was heated to reflux for 3 days. After filtration, the remained solvent was removed under reduced pressure, and the crude was purified by column chromatography (CH_2Cl_2: EtOAc =9:1) to obtain the colorless solid **14**. (6.08 g, 83%) ^{1}H NMR (400 MHz, Chloroform-d) δ: 7.62-7.53 (m, 4H), 7.00-6.92 (m, 4H), 4.20-4.13 (m, 4H), 3.90-3.83 (m, 4H), 3.77-3.64 (m, 8H). ^{13}C NMR (100 MHz, Chloroform-d) δ: 162.08, 133.97, 119.17, 115.32, 104.17, 70.91, 70.66, 69.44, 67.76. LR-MS (ESI-TOF): Calcd. For $C_{22}H_{24}N_2O_5Na$ $[M+Na]^+$: 419.2. Found: 419.3.

Compound 15: To a solution of 1M $LiAlH_4$ in THF (75 mL, 75 mmol) at 0 °C was added

a solution of **14** (4.77 g, 16.00 mmol) in THF (80 mL) dropwise. Following the precipitation of a yellow solid, the mixture was heated at reflux for 3 h. The flask was then cooled to 0 °C and water (2.85 mL) was added, followed by 15% aqueous NaOH solution (2.85 mL) and finally water (8.55 mL). The aluminum salts were removed by filtration and the filtrate concentrated under reduced pressure to give **15** as a colorless solid which was used without further purification. (3.74 g, 79%) ^{1}H NMR (400 MHz, Chloroform-*d*) δ: 7.23-7.16 (m, 4H), 6.89-6.84 (m, 4H), 4.10 (dd, *J* = 5.7, 4.1 Hz, 4H), 3.84 (dd, *J* = 5.8, 4.0 Hz, 4H), 3.75-3.65 (m, 8H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ: 157.74, 135.52, 128.27, 114.69, 70.82, 70.67, 69.76, 67.48, 45.81. LR-MS (ESI-TOF): Calcd. For $C_{22}H_{33}N_2O_5$ $[M+H]^+$: 405.2. Found: 405.2.

Compound 16: To a solution of **15** (5.27 g, 13.08 mmol) and NEt_3 (3.5 mL) in CH_2Cl_2 (2.50 L) at 0 °C was added a solution of pyridine-4-acrinyl-2,6-dicarbonyl chloride (3.58 g, 13.08 mmol) in CH_2Cl_2 (24 mL) dropwise over a period of 4 h. The solution was then stirred at room temperature for 12h. After removal of solvent in vacuum, the obtained crude product was purified by flash chromatography (CH_2Cl_2:MeOH=96:4) to give **16** as a colorless solid (3.84 g, 55%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ: 7.99 (s, 2H), 7.89 (s, 2H), 7.50-7.32 (m, 5H), 7.11 (d, *J* = 8.5 Hz, 4H), 6.76 (d, *J* = 8.4 Hz, 4H), 5.28 (s, 2H), 4.56 (d, *J* = 5.5 Hz, 4H), 4.08 (dd, *J* = 5.8, 3.5 Hz, 4H), 3.94-3.84 (m, 4H), 3.77-3.64 (m, 8H).^{13}C NMR (100 MHz, Chloroform-*d*) δ: 163.13, 158.28, 150.65, 135.15, 129.90, 128.84, 128.57, 127.77, 114.72, 111.64, 77.23, 70.90, 70.63, 69.66, 67.64, 43.00. LR-MS (ESI-TOF): Calcd. For $C_{36}H_{39}N_3O_8Na$ $[M+Na]^+$: 664.3. Found: 664.4.

Compound 17: Pd/C (10%, 0.025 g) and **16** (0.32 g, 0.50 mmol) were combined in

methanol (7 mL) and dichloromethane (7 mL). The mixture was stirred for 12h at room temperature under 70 atm of hydrogen pressure. Then, the catalyst was filtered and washed with tetrahydrofuran, and the solvents of the filtrate were removed under reduced pressure to give **17** as a white solid which was used without further purification. (0.27 g, 98%) ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.44 (s, 1H), 9.69 (t, J = 6.3 Hz, 2H), 7.57 (s, 2H), 7.20 (d, J = 8.3 Hz, 4H), 6.88 (d, J = 8.4 Hz, 4H), 4.54 (d, J = 6.4 Hz, 4H), 4.09-3.96 (m, 4H), 3.78-3.67 (m, 4H), 3.58-3.48 (m, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.03, 157.38, 150.72, 131.20, 128.11, 114.20, 111.45, 69.89, 68.83, 67.16, 40.97. LR-MS (ESI-TOF): Calcd. For $C_{29}H_{33}N_3O_8Na$ $[M+Na]^+$: 574.2. Found: 574.6.

Compound 18: To a solution of **17** (5.28 g, 9.58 mmol) and tert-Butyl N-(2-bromoethyl) carbamate (3.84 g, 17.25 mmol) in dimethylformamide (300 mL) was added K_2CO_3 (7.05 g, 50.00 mmol) and catalytic KI (0.005 g). The mixture was heated to 90 °C for 2 days. After filtering K_2CO_3, the filtrate was evaporated under vacuum, and the crude was purified by column chromatography (CH_2Cl_2: MeOH =98:2) to obtain the white solid **18** (4.99 g, 75%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.69 (s, 2H), 7.21 (d, J = 8.4 Hz, 4H), 6.88 (d, J = 8.7 Hz, 4H), 4.56 (d, J = 6.3 Hz, 5H), 4.23 (t, J = 5.5 Hz, 2H), 4.09-3.98 (m, 4H), 3.75 -3.67 (m, 4H), 3.60-3.47 (m, 8H), 1.37 (s, 9H). ^{13}C NMR (100 MHz, Chloroform-d) δ: 167.32, 163.60, 162.55, 157.93, 130.26, 128.70, 114.25, 111.26, 70.81, 70.63, 69.65, 68.05, 67.41, 42.75, 39.71, 36.49, 28.44. LR-MS (ESI-TOF): Calcd. For $C_{36}H_{46}N_4O_{10}Na$ $[M+Na]^+$: 717.3. Found: 717.5.

Compound 19: A solution of **18** (0.66 g, 0.95mmol) in dichloromethane (25 mL) and trifluoroacetic acid (5 mL) was stirred at room temperature for 1 h. Acetone (20 mL) was

added to the reaction mixture. Excess trifluoroacetic acid and dichloromethane were evaporated to provide the white solid **19** without any further purifications. (0.53 g, 94%) ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.77 (t, J = 6.4 Hz, 2H), 7.77 (s, 2H), 7.27-7.14 (m, 4H), 6.95-6.81 (m, 4H), 4.56 (d, J = 6.3 Hz, 4H), 4.44 (t, J = 5.0 Hz, 2H), 4.08-3.96 (m, 4H), 3.75-3.66 (m, 4H), 3.59-3.47 (m, 8H), 3.31 (q, J = 5.4 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 166.57, 162.69, 157.43, 150.91, 131.10, 128.17, 114.22, 110.56, 69.90, 68.83, 67.17, 65.17, 41.08, 38.05. LR-MS (ESI-TOF): Calcd. For $C_{31}H_{38}N_4O_8$ $[M]^+$: 594.2. Found: 594.5.

3.3 The synthesis of OPE1-4.

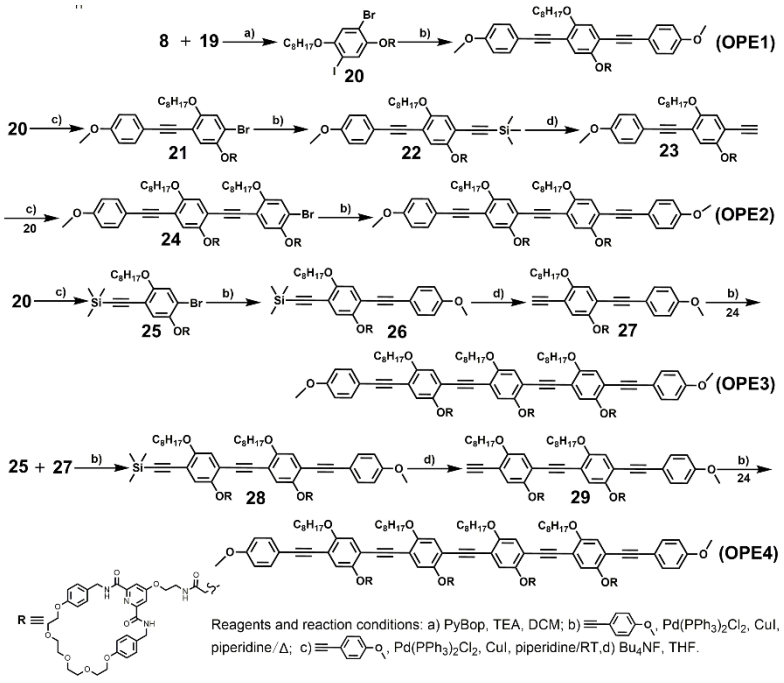


Figure S3. The synthetic route for **OPE1-4**.

Compound 20: A solution of **8** (0.58 g, 1.20 mmol) in 10 mL of DCM was added to a suspension containing PyBop (0.94 g, 1.80 mmol) and TEA (0.99 mL, 5.40 mmol) in 20 mL DCM at room temperature. The mixture was stirred for 5 min and a solution of **19** (0.71 g, 1.20 mmol) in 5 mL of DCM was added, and the reaction was kept stirring at room

temperature overnight. The reaction was quenched with ice cold water and extracted with DCM (3×10 mL). The combined organics was washed with 1 M Na_2CO_3 (5 × 10 mL), brine (2 × 10 mL) and dried over Na_2SO_4. Solvent was evaporated under vacuum to yield crude product which was purified by column chromatography (CH_2Cl_2:MeOH=97:3) to give **20** as a pale yellow solid (0.99g, 78%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.73 (t, J = 6.4 Hz, 2H), 8.16 (t, J = 5.7 Hz, 1H), 7.71 (s, 2H), 7.42 (s, 1H), 7.23-7.17 (m, 5H), 6.91-6.83 (m, 4H), 4.61-4.52 (m, 6H), 4.31 (t, J = 5.4 Hz, 2H), 4.06-4.00 (m, 4H), 3.96 (t, J = 6.3 Hz, 2H), 3.74-3.68 (m, 4H), 3.60 (q, J = 5.3 Hz, 2H), 3.56-3.50 (m, 8H), 1.67 (p, J = 6.6 Hz, 2H), 1.42 (q, J = 7.2 Hz, 2H), 1.31-1.22 (m, 8H), 0.89-0.81 (m, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 167.01, 162.77, 157.40, 150.83, 131.10, 128.11, 124.66, 116.60, 114.19, 111.72, 110.42, 69.90, 69.51, 68.84, 67.16, 45.84, 45.80, 45.73, 41.07, 37.58, 31.19, 28.66, 28.56, 28.50, 25.92, 25.85, 25.47, 22.07. LR-MS (ESI-TOF): Calcd. For $C_{47}H_{58}BrIN_4O_{11}Na$ $[M+Na]^+$: 1083.2. Found:1083.5.

Compound OPE1: Compound **20** (0.3 g, 0.28 mmol) and 4-ethynylanisole (92 mg, 0.70 mmol) were dissolved in dry piperidine (5 mL) and the system was flushed with argon. Bis(triphenylphosphine)-palladium dichloride (12.29 mg, 0.05 mmol) and copper(I) iodine (7.65 mg, 0.04 mmol) was added, and the mixture was stirred at 60 °C for 5h. Dichloromethane was added to the residue and washed with saturated NH_4Cl, HCl (10%) and NaCl. The organic phase was dried with Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (CH_2Cl_2: MeOH=97:3) to afford **OPE1** (0.27 g, 86%) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.72 (t, J = 6.4 Hz, 2H), 8.16 (s, 1H), 7.66 (s, 2H), 7.48-7.43 (m, 2H),

7.42-7.36 (m, 2H), 7.20 (d, *J* = 8.5 Hz, 4H), 7.13 (d, *J* = 6.7 Hz, 2H), 6.99-6.94 (m, 2H), 6.94-6.89 (m, 2H), 6.87 (d, *J* = 8.6 Hz, 4H), 4.64 (s, 2H), 4.55 (d, *J* = 6.3 Hz, 4H), 4.30 (t, *J* = 5.3 Hz, 2H), 4.02 (td, *J* = 6.3, 5.3, 3.3 Hz, 6H), 3.77 (s, 3H), 3.75-3.68 (m, 7H), 3.62 (d, *J* = 5.7 Hz, 2H), 3.57-3.48 (m, 8H), 1.72 (q, *J* = 6.8 Hz, 2H), 1.48 (p, *J* = 7.0 Hz, 2H), 1.33 (s, 2H), 1.23 (s, 6H), 0.86-0.78 (m, 3H). HR-MS (ESI-TOF): Calcd. For $C_{65}H_{73}N_4O_{13}$ $[M+H]^+$: 1117.5174. Found: 1117.5176.

Compound 21: Compound **20** (3.00 g, 2.80 mmol) and 4-ethynylanisole (0.46 g, 3.50 mmol) were dissolved in dry piperidine (30 mL) and the system was flushed with argon. Bis(triphenylphosphine)-palladium dichloride (61.50 mg, 0.25 mmol) and copper(I) iodine (38.25 mg, 0.2 mmol) was added, and the mixture was stirred at room temperature for 3h. Dichloromethane was added to the residue and washed with saturated NH_4Cl, HCl (10%) and NaCl, respectively. The organic phase was dried with Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (CH_2Cl_2: MeOH=97:3) to afford **21** (1.97 g, 65%) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.73 (t, *J* = 6.4 Hz, 2H), 8.14 (t, *J* = 5.7 Hz, 1H), 7.71 (s, 2H), 7.41-7.34 (m, 2H), 7.31 (s, 1H), 7.24-7.16 (m, 4H), 7.15 (s, 1H), 6.99-6.91 (m, 2H), 6.91-6.82 (m, 4H), 4.60 (s, 2H), 4.55 (d, *J* = 6.4 Hz, 4H), 4.34 (dt, *J* = 10.5, 5.2 Hz, 2H), 4.07-3.93 (m, 6H), 3.76 (s, 3H), 3.75-3.66 (m, 4H), 3.62 (t, *J* = 5.4 Hz, 2H), 3.57-3.48 (m, 8H), 1.71 (p, *J* = 6.3 Hz, 2H), 1.46 (p, *J* = 7.2 Hz, 2H), 1.37-1.17 (m, 8H), 0.86-0.74 (m, 3H).^{13}C NMR (100 MHz, DMSO-d_6) δ: 167.59, 162.75, 159.50, 157.40, 154.17, 150.86, 147.96, 132.60, 131.10, 128.11, 117.86, 117.54, 114.34, 114.20, 110.38, 69.90, 69.07, 68.83, 68.65, 67.16, 55.20, 41.06, 38.94, 37.58, 31.16,

28.74, 28.69, 28.63, 25.51, 22.07, 13.88. LR-MS (ESI-TOF): Calcd. For $C_{47}H_{58}BrIN_4O_{11}Na$ $[M+Na]^+$: 1087.4. Found: 1087.4.

Compound 22: The synthetic processes of compound **22** were similar as the synthesis of compound **OPE1** except using trimethylsilylacetylene and **21** as the corresponding original materials. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.73 (t, J = 6.4 Hz, 2H), 8.08 (t, J = 5.7 Hz, 1H), 7.71 (s, 2H), 7.42-7.35 (m, 2H), 7.23-7.17 (m, 4H), 7.09 (s, 1H), 7.04 (s, 1H), 6.99-6.92 (m, 2H), 6.90-6.83 (m, 4H), 4.59 (s, 2H), 4.55 (d, J = 6.4 Hz, 4H), 4.33 (dt, J = 16.0, 5.2 Hz, 2H), 4.06-3.96 (m, 6H), 3.76 (s, 3H), 3.74-3.67 (m, 4H), 3.61 (p, J = 6.3, 5.8 Hz, 2H), 3.58-3.48 (m, 8H), 1.70 (p, J = 6.4 Hz, 2H), 1.46 (p, J = 7.1 Hz, 2H), 1.30 (d, J = 8.6 Hz, 2H), 1.22 (tq, J = 10.3, 6.3, 5.6 Hz, 6H), 0.84-0.77 (m, 3H), 0.23 (s, 9H).^{13}C NMR (100 MHz, DMSO) δ: 167.89, 162.73, 159.57, 157.39, 153.55, 152.39, 150.85, 132.67, 131.10, 128.09, 114.35, 114.27, 114.19, 112.87, 110.34, 95.17, 69.89, 68.82, 67.15, 55.99, 55.21, 41.04, 39.16, 31.15, 28.73, 28.68, 25.51, 22.06, 18.52, 13.87, -0.18. LR-MS (ESI-TOF): Calcd. For $C_{61}H_{74}N_4O_{12}SiNa$ $[M+Na]^+$: 1105.5. Found: 1105.5.

Compound 23: Tetrabutylammonium fluoride (0.85g, 3.27mmol) was added to a solution of **22** (1.20 g, 1.09 mmol) in 20 mL THF, the solution was stirred at room temperature for 2h. The mixture was extracted with dichloromethane and washed with water (2 × 50 mL). And the organic phase was dried with Na_2SO_4 and the solvent was removed under vacuum. The product was purified by flash chromatography by column chromatography to afford compound **23** as a yellow solid. (1.00 g, 90.0%) ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.73 (t, J = 6.4 Hz, 2H), 8.16 (t, J = 5.7 Hz, 1H), 7.72 (s, 2H), 7.41-7.34 (m, 2H), 7.24-7.16

(m, 4H), 7.08 (d, J = 5.4 Hz, 2H), 6.98-6.92 (m, 2H), 6.90-6.84 (m, 4H), 4.60 (s, 2H), 4.55 (d, J = 6.3 Hz, 4H), 4.45 (s, 1H), 4.33 (dt, J = 16.1, 5.2 Hz, 2H), 4.06-3.96 (m, 6H), 3.76 (s, 3H), 3.73-3.68 (m, 4H), 3.61 (q, J = 5.5 Hz, 2H), 3.58-3.48 (m, 8H), 1.70 (p, J = 6.5 Hz, 2H), 1.51-1.42 (m, 2H), 1.36-1.17 (m, 8H), 0.85-0.78 (m, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 167.02, 162.78, 159.57, 157.40, 153.44, 152.69, 150.85, 132.67, 131.10, 128.11, 117.24, 117.13, 114.35, 114.28, 114.20, 113.87, 112.29, 110.40, 86.34, 84.28, 69.90, 68.83, 68.35, 67.16, 55.21, 41.06, 39.60, 39.38, 31.17, 28.74, 28.70, 28.68, 25.53, 22.07, 18.53, 13.88. LR-MS (ESI-TOF): Calcd. For $C_{58}H_{67}N_4O_{12}$ $[M+H]^+$: 1011.5. Found: 1011.5.

Compound 24: The synthetic processes of compound **24** were similar as the synthesis of compound **21** except using **20** and **23** as the corresponding original materials. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.69 (d, J = 9.1 Hz, 4H), 8.11 (dd, J = 10.1, 5.4 Hz, 2H), 7.70 (d, J = 7.1 Hz, 2H), 7.59 (s, 2H), 7.42-7.34 (m, 2H), 7.27 (s, 1H), 7.18 (m, 9H), 7.13 (s, 1H), 7.01 (s, 1H), 6.99-6.92 (m, 2H), 6.90-6.81 (m, 8H) , 4.62 (d, J = 14.2 Hz, 4H), 4.54 (m, 8H), 4.38-4.22 (m, 6H), 4.05-3.96 (m, 12H), 3.76 (m, 3H), 3.73-3.67 (m, 8H), 3.60 (t, J = 5.5 Hz, 2H), 3.57-3.48 (m, 16H) , 1.79-1.60 (m, 8H), 1.51-1.10 (m, 16H), 0.79 (m, 6H).^{13}C NMR (100 MHz, DMSO-d_6) δ: 167.92, 162.74, 157.38, 154.31, 150.82, 150.74, 132.66, 131.10, 128.09, 128.06, 114.38, 114.18, 69.89, 68.82, 67.15, 55.99, 55.22, 41.06, 31.23, 31.16, 28.76, 28.71, 22.07, 13.87. LR-MS (ESI-TOF): Calcd. For $C_{105}H_{124}BrN_8O_{23}$ $[M+H]^+$: 1943.8. Found: 1943.8.

Compound OPE2: The synthetic processes of compound **OPE2** were similar as the synthesis of compound **OPE1** except using 4-ethynylanisole and **24** as the corresponding

original materials. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.70 (t, J = 6.1 Hz, 4H), 8.20-8.09 (m, 2H), 7.69 (s, 1H), 7.63 (d, J = 5.7 Hz, 2H), 7.59 (s, 1H), 7.45 (d, J = 8.7 Hz, 2H), 7.41-7.36 (m, 2H), 7.23-7.12 (m, 12H), 6.98-6.94 (m, 2H), 6.91 (d, J = 8.8 Hz, 2H), 6.89-6.82 (m, 8H), 4.66 (s, 4H), 4.54 (d, J = 6.2 Hz, 8H), 4.28 (s, 4H), 4.02 (dd, J = 6.8, 3.4 Hz, 12H), 3.77 (s, 3H), 3.74-3.66 (m, 11H), 3.66-3.58 (m, 4H), 3.58-3.47 (m, 16H), 1.78-1.63 (m, 4H), 1.44 (dd, J = 16.4, 8.4 Hz, 4H), 1.22 (q, J = 17.5, 13.8 Hz, 16H), 0.90-0.74 (m, 6H). HR-MS (ESI-TOF): Calcd. For $C_{114}H_{131}N_8O_{24}$ $[M+H]^+$: 1996.9276. Found:1996.9290.

Compound 25: The synthetic processes of compound **25** was similar as the synthesis of compound **21** except using trimethylsilylacetylene and **20** as the corresponding original materials. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.71 (t, J = 6.4 Hz, 2H), 8.08 (t, J = 5.7 Hz, 1H), 7.68 (s, 2H), 7.24 (s, 1H), 7.21-7.15 (m, 4H), 7.04 (s, 1H), 6.88-6.81 (m, 4H), 4.58-4.49 (m, 6H), 4.29 (t, J = 5.4 Hz, 2H), 4.04-3.96 (m, 4H), 3.93 (t, J = 6.0 Hz, 2H), 3.71-3.64 (m, 4H), 3.58 (q, J = 5.5 Hz, 2H), 3.54-3.45 (m, 8H), 1.62 (dt, J = 11.3, 6.1 Hz, 2H), 1.42 (dq, J = 14.9, 7.1 Hz, 2H), 1.27-1.19 (m, 8H), 0.86-0.77 (m, 3H), 0.15 (s, 9H).^{13}C NMR (100 MHz, DMSO-d_6) δ: 167.56, 162.75, 157.41, 150.87, 131.11, 128.12, 118.21, 117.47, 114.21, 113.11, 110.37, 99.17, 69.90, 68.89, 68.83, 68.63, 67.17, 56.00, 41.06, 39.36, 39.14, 37.55, 31.20, 28.73, 25.49, 22.07, 18.52, 13.88, -0.27. HR-MS (ESI-TOF): Calcd. For $C_{52}H_{67}BrN_4O_{11}SiNa$ $[M+Na]^+$: 1053.3657. Found: 1053.3671.

Compound 26: The synthetic processes of compound **26** was similar as the synthesis of compound **22** except using 4-ethynylanisole and **25** as the corresponding original materials. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.74 (t, J = 6.4 Hz, 2H), 8.15 (t, J = 5.7 Hz, 1H),

7.71 (s, 2H), 7.30 (s, 1H), 7.24-7.18 (m, 4H), 7.10 (s, 1H), 6.91-6.83 (m, 4H), 4.61-4.52 (m, 6H), 4.31 (d, J = 11.5 Hz, 3H), 4.06-4.01 (m, 4H), 3.97 (t, J = 6.4 Hz, 2H), 3.74-3.68 (m, 4H), 3.61 (q, J = 5.4 Hz, 2H), 3.58-3.49 (m, 8H), 1.66 (q, J = 6.9 Hz, 2H), 1.40 (tq, J = 9.6, 4.7, 3.0 Hz, 2H), 1.30-1.22 (m, 8H), 0.88-0.82 (m, 3H), 0.195 (s, 9H).^{13}C NMR (100 MHz, DMSO-d_6) δ: 167.55, 167.03, 162.78, 157.41, 154.69, 150.86, 147.84, 131.11, 128.13, 118.88, 117.46, 114.22, 110.39, 85.22, 79.21, 69.90, 68.98, 68.83, 68.64, 67.17, 41.07, 37.57, 31.18, 29.20, 28.97, 28.63, 28.58, 28.48, 25.31, 22.05, 13.93. LR-MS (ESI-TOF): Calcd. For $C_{61}H_{74}N_4O_{12}SiNa$ $[M+Na]^+$: 1105.5. Found: 1105.5.

Compound 27: The synthetic processes of compound **27** was similar as the synthesis of compound **23** except using tetrabutylammonium fluoride and **26** as the corresponding original materials. ^{1}H NMR (400 MHz, DMSO-d6) δ: 9.74 (t, J = 6.4 Hz, 2H), 8.15 (s, 1H), 7.67 (s, 2H), 7.50-7.42 (m, 2H), 7.26-7.17 (m, 4H), 7.13 (s, 1H), 7.07 (s, 1H), 6.95 -6.90 (m, 2H), 6.91-6.85 (m, 4H), 4.62 (s, 2H), 4.56 (d, J = 6.3 Hz, 4H), 4.36 (s, 1H), 4.29 (t, J = 5.3 Hz, 2H), 4.07-3.97 (m, 6H), 3.76-3.68 (m, 7H), 3.61 (q, J = 5.4 Hz, 2H), 3.58-3.49 (m, 8H), 1.69 (p, J = 6 .6 Hz, 2H), 1.42 (s, 2H), 1.26 (q, J = 6.7, 5.6 Hz, 8H), 0.89-0.81 (m, 3H). LR-MS (ESI-TOF): Calcd. For $C_{58}H_{67}N_4O_{12}$ $[M+H]^+$:1011.5. Found: 1011.5.

Compound OPE3: The synthetic processes of compound **OPE3** were similar as the synthesis of compound **OPE1** except **27** and **24** as the corresponding original materials. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.76-9.62 (m, 6H), 8.23-8.11 (m, 3H), 7.64-7.63 (m, 4H), 7.60 (s, 2H), 7.46 (d, J = 8.5 Hz, 2H), 7.39 (d, J = 8.4 Hz, 2H), 7.21-7.15 (m, 16H), 7.05 (s, 1H), 7.02 (s, 1H), 6.98 (s, 1H), 6.95 (s, 1H), 6.91 (d, J = 8.7 Hz, 2H), 6.88-6.83

(m, 12H), 4.67 (s, 6H), 4.53 (s, 12H), 4.27 (s, 6H), 4.01 (q, *J* = 5.7, 5.2 Hz, 18H), 3.77 (s, 3H), 3.74-3.67 (m, 15H), 3.61 (s, 6H), 3.56-3.48 (m, 24H), 1.72 (s, 6H), 1.44 (dd, *J* = 15.7, 7.7 Hz, 6H), 1.29-1.14 (m, 24H), 0.78 (td, *J* = 7.2, 4.6 Hz, 9H). HR-MS (ESI-TOF): Calcd. For $C_{163}H_{188}N_{12}O_{35}Na$ $[M+Na]^+$: 2896.3198. Found: 2896.3374.

Compound 29: The synthetic processes of compound **28** was similar as the synthesis of compound **22** except using **25** and **27** as the corresponding original materials. The obtained crude product of **28** was deprotected directly to obtain compound **29**. The reaction process and compound purification were similar as compound **23**. ^{1}H NMR (400 MHz, DMSO-d6) δ: 9.72 (q, J = 6.0 Hz, 4H), 8.177-8.098 (m, 2H), 7.70 (d, J = 2.8 Hz, 2H), 7.65 (d, J = 4.6 Hz, 2H), 7.46 (dd, J = 8.8, 3.1 Hz, 2H), 7.24-7.16 (m, 10H), 7.12 (dd, J = 12.9, 9.0 Hz, 2H), 6.92 (dd, J = 8.9, 1.8 Hz, 2H), 6.90-6.82 (m, 8H), 4.67-4.50 (m, 12H),4.32-4.24 (m, 4H), 4.02-3.98 (m, 12H), 3.76-3.67 (m, 11H), 3.65-3.59 (m, 4H), 3.58-3.49 (m, 16H), 1.69-1.68 (m, 4H), 1.42-1.39 (m, 4H), 1.32-1.18 (m, 16H), 0.81-0.76 (m, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 167.5, 163.23, 157.89, 154.40, 152.83, 151.34, 133.42, 131.61, 128.60, 114.86, 114.69, 79.05, 70.39, 69.33, 67.65, 55.63, 41.55, 40.53, 31.69, 29.19, 25.87, 22.56, 14.37. MS (MALDI-TOF): Calcd. For $C_{107}H_{124}N_8O_{23}Na$ $[M+H]^+$: 1887.9059. Found: 1887.9467.

Compound OPE4: The synthetic processes of compound **OPE4** were similar as the synthesis of compound **OPE1** except **29** and **24** as the corresponding original materials. ^{1}H NMR (500 MHz, DMSO-d6) δ: 12.21-11.90 (m, 8H), 9.79-9.72 (m, 4H), 7.70 (d, J = 3.3 Hz, 2H), 7.66 (d, J = 3.7 Hz, 2H), 7.53(s, 1H), 7.51(s,1H), 7.48 (s, 2H), 7.46 (s, 2H), 7.43 (s, 2H), 7.40 (s, 2H), 7.33 (dd, J = 8.6, 2.5 Hz, 4H), 7.24 (m, 16H), 7.11 (s, 1H),

7.08 (s, 1H), 7.03-6.96 (m, 4H), 6.99-6.86 (m, 8H), 6.74-6.66 (m, 8H), 4.78-4.68 (m, 8H), 4.67-4.50 (m, 16H), 4.37-4.27 (m, 8H), 4.14-3.99 (m, 24H), 3.85-3.50 (m, 62H), 1.65-1.50 (m, 8H), 1.42-1.20 (m, 40H), 0.94-0.91(m, 12H). MS (MALDI-TOF) Calcd. For $C_{212}H_{246}N_{16}O_{46}$ $[M+Na]^+$:3774.7294. Found: 3774.6206.

3.4 Absorption and fluorescence spectra and quantum yields determination of OPE1-4.

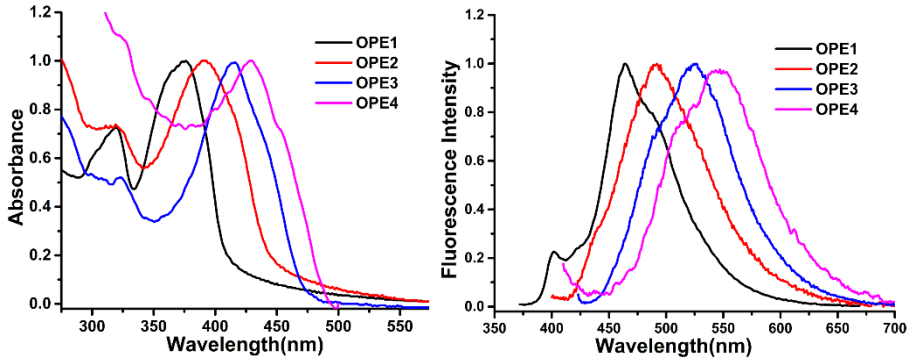


Figure S4 Normalized absorption (left) and fluorescence (right) spectra of **OPE1-4** in aqueous solution (1.0×10^{-6} M). The excitation wavelength in fluorescence spectra is 360 nm for **OPE1**, 390 nm for **OPE2**，416 nm for **OPE3** and 425 nm for **OPE4**, respectively.

The fluorescence quantum yield of **OPE1-4** in water was determined according to standard methods using quinine bisulfate in 0.1 M sulfuric acid as a reference.[1]

4. Ion transporting activity studies across LUVs⊃HPTS assay.

Preparation of EYPC vesicles enwrapped with HPTS (LUVs⊃HPTS)**:** A solution of 400 μL EYPC (EYPC, 25 mg/mL, 10 mg) in deacidified chloroform was mixed with 100 μL of cholesterol (10 mg/mL, 1 mg) in deacidified chloroform. The solvents were evaporated by a slow stream of nitrogen, followed by drying under vacuum for 12 hours. Then the lipid membrane was hydrated by overtaxing with 500 μL buffer (1 mM HPTS,

10 mM HEPES, 100 mM KCl, pH = 7.0). Then, the suspension was subjected to seven freeze–thaw cycles and allowed to age for 30 min at room temperature before extruding 25 times through a 100 nm polycarbonate membrane. The excess HPTS was separated from the vesicles by size exclusion column chromatography (SephadexG-25) using 100 mM KCl, 10 mM HEPES buffer (pH = 7.0) as eluent. The vesicles were further diluted to reach a total lipid concentration of 1 mM, assuming 100% retention of lipid during the gel filtration process.

Preparation of EYPC vesicles without HPTS (LUVs⊅HPTS) as a control: Vesicles were prepared in the same way as stated above except the step of hydrating with 500 μL buffer (10 mM HEPES, 100 mM KCl, pH = 7.0).

Ion transport activity: In a typical experiment, 2900 μL of HEPES buffer (10 mM HEPES, 100 mM KCl, pH = 7.0) was transferred to a quartz cuvette followed by addition of 100 μL of LUVs⊃HPTS (1 mM). The cuvette was placed in the fluorescence instrument with slow stirring condition by a magnetic stirrer equipped in the instrument (at t = 0s). The time-dependent change in fluorescence intensity F_t' (λ_{em} = 510 nm) was monitored at an excitation wavelength at λ_{ex} = 450 nm, during the addition of base (30 μL, 0.5 M KOH) at t = 50 s, transporter (10 μL stock solution in DMSO, 0-50 μM final concentration) at t = 100 s, and 60 μL of 5% Triton X-100 aqueous solution at t = 550 s.

Given **OPE1-4** have innate emission at λ_{em} = 510 nm upon the excitation at λ_{ex} = 450 nm, we also monitored the fluorescence background $F_t''$of the transporters from LUVs⊅HPTS assay in the same process as stated above. Then $F_t = F_t' - F_t''$was used for

further data analysis to deduct the native fluorescence interference of OPEs

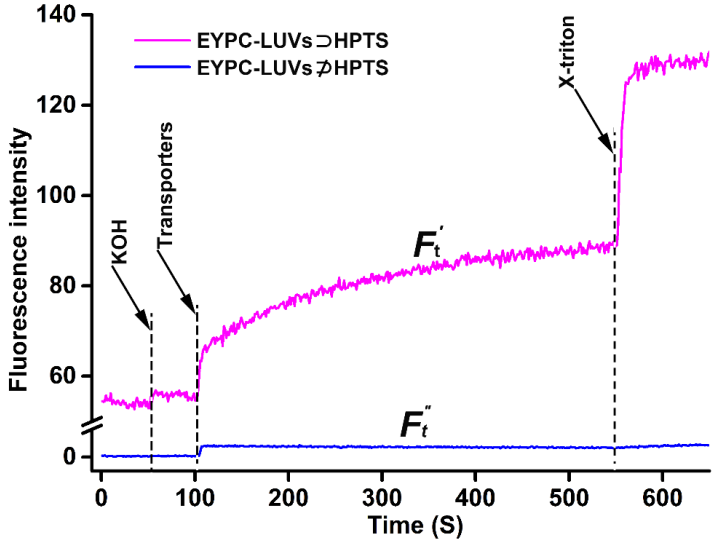


Figure S5 Illustration of ion transport kinetics using both LUVs⊃HPTS and LUVs⊅HPTS vesicles. **OPE3** at 0.31 μM was presented as example.

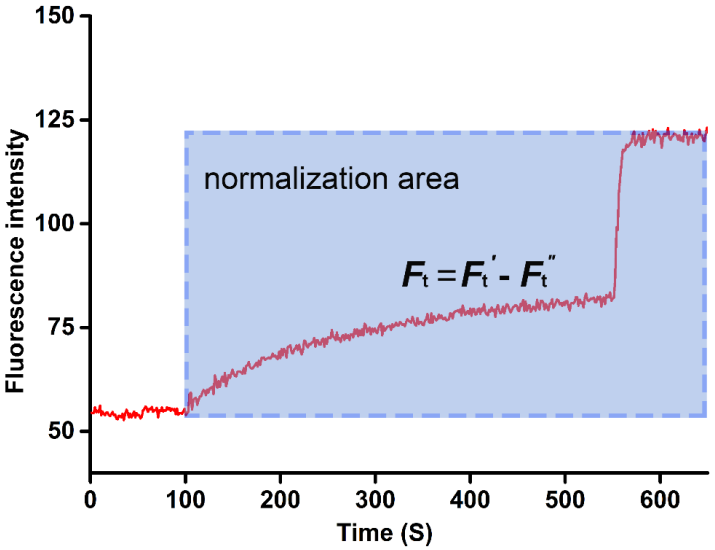


Figure S6 Illustration of data analysis by deducting the native fluorescence interference of OPEs. The blue window marks the data region for following normalized treatment.

Then, the data located in the blue window as illustrated in Figure S6 were futher analyzied by normalized treatment. Fluorescence intensity F_t were normalized to fractional emission intensity I_F using Equation S1:

$$I_F = [(F_t - F_0)]/[(F_\infty - F_0)] \qquad \text{Equation S1}$$

Where F_0 represents the value before addition of transporters, F_∞ represents the value after addition of Triton X-100 (reach ultimate with complete leakage). I_F at 450 s just before addition of Triton X-100 was defined as transmembrane activity. The represented concentrations for transporters in Figure 1 in the manuscript are the final concentrations: 0, 0.023, 0.33, 0.67, 1.67, 3.33, 4.17 and 5.00 μM for **OPE1**; 0, 1.00, 1.67, 3.33, 10.00 and 16.67μM for **OPE2** and 0, 0.037, 0.077, 0.22, 0.31, 0.44, 0.71, 1.07 and 2.22 μM for **OPE3**, and 0, 1.67, 3.33, 4.67, 6.67 μM for **OPE4**, respectively. "0.00 μM" means the blank experiments with addition of 10 μL DMSO.

Quantification of observed transport rates (k_{obs}) based on fluorescence data:[2,3] As described above, the fluorescence curves that were normalized to fractional activity I_F were then fitted to a single exponential decay function:

$$I_F = 1 - e^{-k_{obs}t} \qquad \text{Equation S2}$$

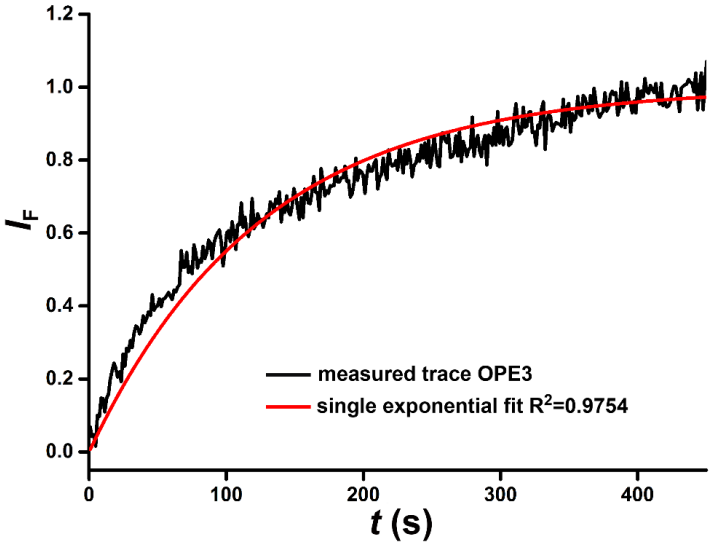


Figure S7 HPTS fluorescence assay plot and single exponential fit of I_F by **OPE3.** Taking **OPE3** at 2.20 μM as example.

Computer fitting of each fluorescence curve to the first order decay equation provided an observed rate constant (k_{obs}). The points in Figure 1 (right) of the maintext are the average of three individual experiments with S.D. of ± 4.0%. The dependence of the observed rate of Cl^- exchange (k_{obs}) on the concentration of transporters can be used to determine the aggregation state of the channel, or the number of monomers that form the active structure [equation S3].[3]

$$k_{obs} \propto [monomer]^n \qquad \text{Equation S3}$$

Figure 1c and 1d showed that the increased concentration of transporter **OPE3** and **OPE4** produced an linear increase (n=1) in k_{obs} which was indicative of active structure formed from **OPE3** and **OPE4** were monomolecular structures.

5. Patch-clamp measurements and structure optimization of PDA macrocycle.

5.1 General process. DPhPC lipids at 50 mg/mL in chloroform were dried under a stream of nitrogen and under vacuum for 4 h and then dispersed in decane at 20 mg/mL. This solution was used to precoat a 200 μm hole of a polystyrene cup held by a chamber upon which a planar lipid bilayer membrane was formed. The cup (cis, ground) and chamber (trans) was filled with 1mL 1M KCl solution. Formation of membrane was monitored by measuring membrane capacitance. The transporter in DMSO (0.5 mM) 5 μL was added to both the *cis/trans* side of the chamber (final concentration 2.5 μM) and the solution was stirred for 2 minutes. A holding potential of +100 mV was applied and the channel responses were recorded. Ag/AgCl electrodes were used to impose voltages and record currents across the membrane. The patch clamp workstation (Warner

Instruments) was used for all experiments. The currents were measured by a Warner BC-535 bilayer clamp amplifier and collected using the Digidata 1550A data acquisition system. All data was filtered at 1 kHz with 8-pole Bessel filter.

5.2 Estimation of Pore Size Using Hille's Equation.[4-6]

The pore size formed by **OPE3** could be estimated based on the Hille's equation as following:

$$g^{-1} = (4L\rho/\pi D^2) + \rho/D \qquad \text{Equation S4}$$

where g is the measured channel conductance, L is length of the ion channel, D is the pore diameter and ρ is the bulk resistivity of the ionic solution. For our case, ρ is estimated to be 0.1 Ω•m at 1 M for KCl. L was estimated as the hydrophobic membrane length at around 3.4 nm. Then, the above equation would predict a pore diameter of about 5.4 ± 0.34 Å for compound **OPE3**.

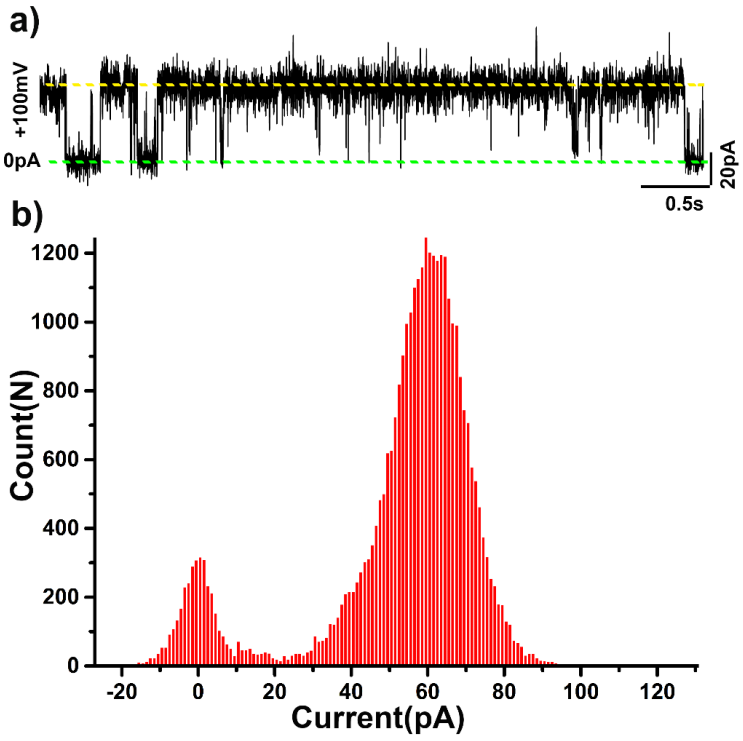


Figure S8 Typical single-channel current recording a) and a histogram of the currents

b) at 100 mV of **OPE3** measured by planar bilayer conductance measurements.

5.3 Energy-minimized (DFT B3LYP/6-31G*) calculation of PDA macrocycle.[7]

Geometry optimization of PDA macrocycle was performed with Spartan'14. An MMFF Monte Carlo search was performed on 10000 conformers and the lowest-energy conformer found was further optimized by DFT at the B3LYP/6-31G* level.

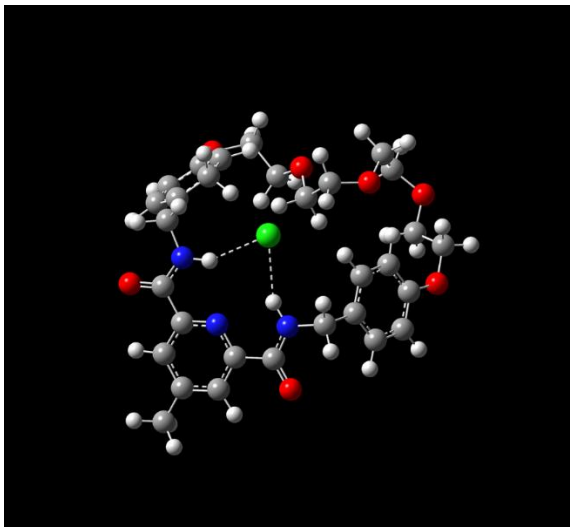


Figure S9 DFT-optimized structure of **PAD macrocycle • Cl^-**, the chloride ion is shown as a green sphere.

6. Fluorescence Resonance Energy Transfer (FRET) assay and fluorescence imaging for channel insertion.

General procedure: The Nile red contained vesicles (LUVs⊃NR) were prepared as those of LUVs⊃HPTS except NR dye (1mM) were dissolved in chloroform with the lipids. The resulting vesicle suspension was diluted with the buffer to give a lipid concentration of 33 μM. To investigate the energy transfer between **OPE3** and NR, the solution of **OPE3** (10 μL, 0.13 mM DMSO solution, final concentration 0.43 μM) was added to the LUVs⊃NR vesicle suspension (3 mL), and the emission spectra were recorded with excitation at 350 nm.

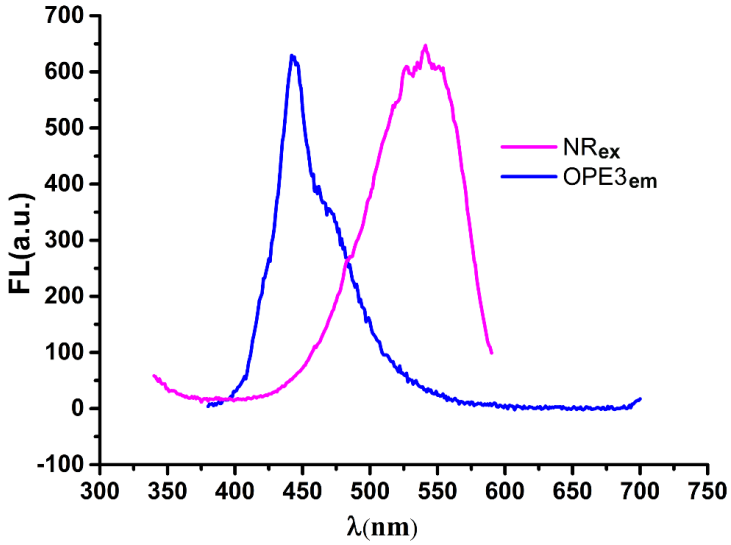


Figure S10 Spectral properties of **OPE3** and Nile Red in $CHCl_3$: Blue, emission spectrum of **OPE3** (excitation at 350 nm); Red, excitation spectrum of Nile Red (emission at 640 nm).

Preparation of giant unilamellar vesicles (GUVs) for fluorescence imaging. GUVs were prepared as following: in brief, 0.3 mg EYPC were first dissolved in 3 mL chloroform and dried with N_2 flow to form a thin lipid film. The resulting film was subsequently dried under vacuum for 3h, and then the film was hydrated at 37 ºC overnight with 2 mL sucrose solution (0.2 M). Fluorescence imaging was performed on a Nikon A1R Laser Scanning Confocal microscope, the bright field images were obtained in the monochromatic mode and fluorescence images were taken with the addition of **OPE3** (2 μM) excited at 405 nm.

7. Ion selectivity studies across LUVs⊃HPTS.

Preparation of EYPC Vesicles: LUVs⊃HPTS and LUVs ⊅ HPTS vesicles were prepared in the same way as stated above (in section 4).

Cation selectivity: In a typical experiment, 2900 μL of HEPES buffer (10 mM HEPES,

100 mM MCl, pH = 7.0, where, M^+ =Li^+, Na^+, K^+, Rb^+ and Cs^+) was transferred to a quartz cuvette followed by addition of 100 μL of LUVs⊃HPTS (1mM) or LUVs⊅HPTS (1mM). The cuvette was placed in the fluorescence instrument with slow stirring condition by a magnetic stirrer equipped in the instrument (at t = 0s). The time-dependent change in fluorescence intensity F_t' or F_t'' (λ_{em} = 510 nm) was monitored at excitation wavelength λ_{ex} = 450 nm, during the addition of base (30 μL, 0.5 M MOH, M^+ =Li^+, Na^+, K^+, Rb^+ and Cs^+) at t = 50 s, transporter (10 μL stock solution in DMSO) at t = 100 s, and 60μL of 5% Triton X-100 aqueous solution at t = 550 s. The data treatment was the same as those HPTS activity assays. The points in Figure 4 of the maintext are the average of three individual experiments with S.D. of ± 5.0%.

Anion selectivity: In a typical experiment, 2900 μL of HEPES buffer (10 mM HEPES, 100 mM K_nA, pH = 7.0, where, A^- =F^-, Cl^-, Br^-, I^- and SO_4^{2-}) was transferred to a quartz cuvette followed by addition of 100 μL of LUVs⊃HPTS (1mM) or LUVs⊅HPTS (1mM). The cuvette was placed in the fluorescence instrument with slow stirring condition by a magnetic stirrer equipped in the instrument (at t = 0 s). The time-dependent change in fluorescence intensity F_t' or F_t'' (λ_{em} = 510 nm) was monitored at excitation wavelengths λ_{ex} = 450 nm, during the addition of base (30 μL, 0.5 M KOH) at t = 50 s, transporter (10 μL stock solution in DMSO) at t = 100 s, and 60μL of 5% Triton X-100 aqueous solution at t = 550 s. The data treatment was the same as those activity assays. The points in Figure 4 of the maintext are the average of three individual experiments with S.D. of ± 5.0%.

FCCP Assay: In a typical experiment, 2900 μL of HEPES buffer (10 mM HEPES, 100

mM KCl, pH = 7.0) was transferred to a quartz cuvette followed by addition of 100 μL of LUVs⊃HPTS (1mM) or LUVs⊅HPTS (1mM). The cuvette was placed in the fluorescence instrument with slow stirring condition by a magnetic stirrer equipped in the instrument (at t = 0s). The time-dependent change in fluorescence intensity F_t' or F_t'' (λ_{em} = 510 nm) was monitored at excitation wavelengths λ_{ex} = 450 nm, during the addition of base (30 μL, 0.5 M KOH) at t = 25 s, FCCP (10 μL stock solution in HEPES buffer) at t = 50s, transporter (10 μL stock solution in DMSO) at t = 100 s, and 60 μL of 5% Triton X-100 aqueous solution at t = 550 s. Similarly, the initial 100s data was removed after normalization. The data analysis and comparison was in the same way as stated above.

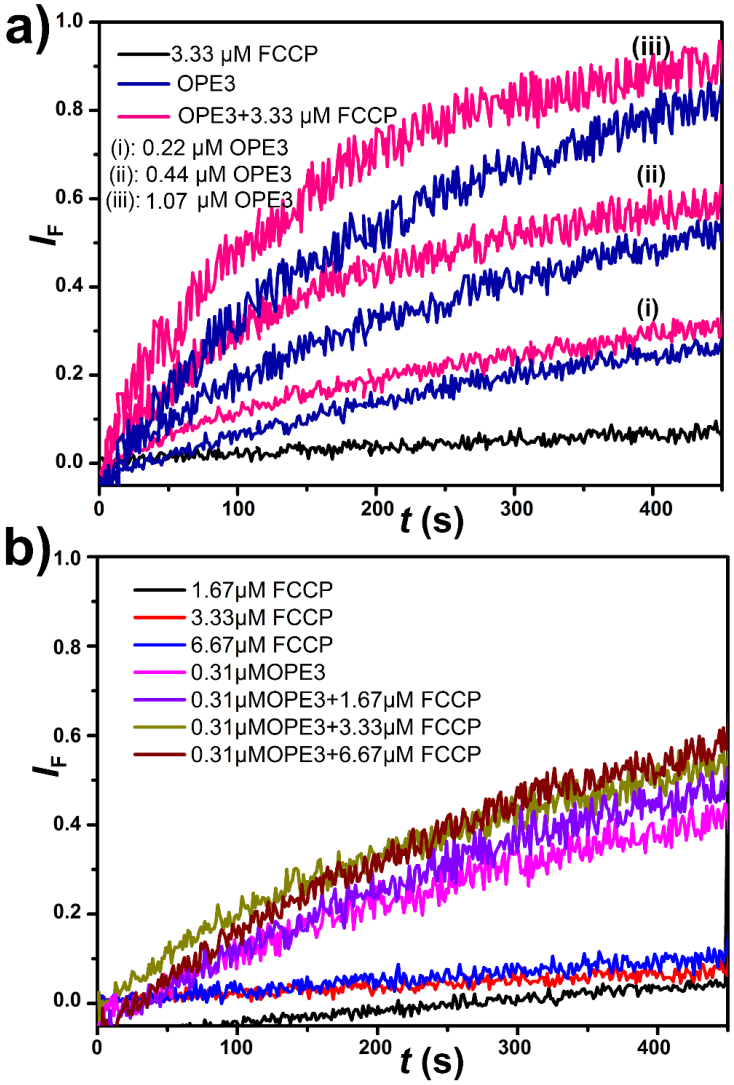


Figure S11 Ion transport activity of **OPE3** in presence and absence of FCCP: a) a fixed FCCP concentration (3.33 μM) with varied **OPE3** concentrations (0.22, 0.44, and 1.07 μM); b) a fixed **OPE3** concentration (0.31 μM) with varied FCCP (1.67, 3.33 and 6.67 μM).

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