



Supporting Information

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Synthetic Ion Channel Based on Metal-Organic Polyhedra

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Materials and Methods. All the reagents and solvents were purchased from commercial sources and used without further purification. Egg yolk *L*- α -phosphatidylcholine (EYPC) in chloroform was purchased from Avanti Polar Lipids, Inc. (USA) and stored in a refrigerator. All the vesicle solutions were extruded with a handheld extruder purchased from Avestin, Inc. MOP-18 was synthesized according to the literature¹ and recrystallized from acetonitrile and toluene. The structure of MOP-18 was confirmed by single-crystal X-ray crystallography using synchrotron radiation at Pohang Accelerator Laboratory (Beamline 6C1). Fluorescence experiments were carried out with a Shimadzu RF-5301PC ratiometric fluorimeter. FT-IR spectra were recorded on a Perkin-Elmer Spectrum GX FT-IR system. Dynamic light scattering (DLS) experiments were performed with an Otsuka ELS 8000 with a He-Ne laser ($\lambda = 632.8$ nm).

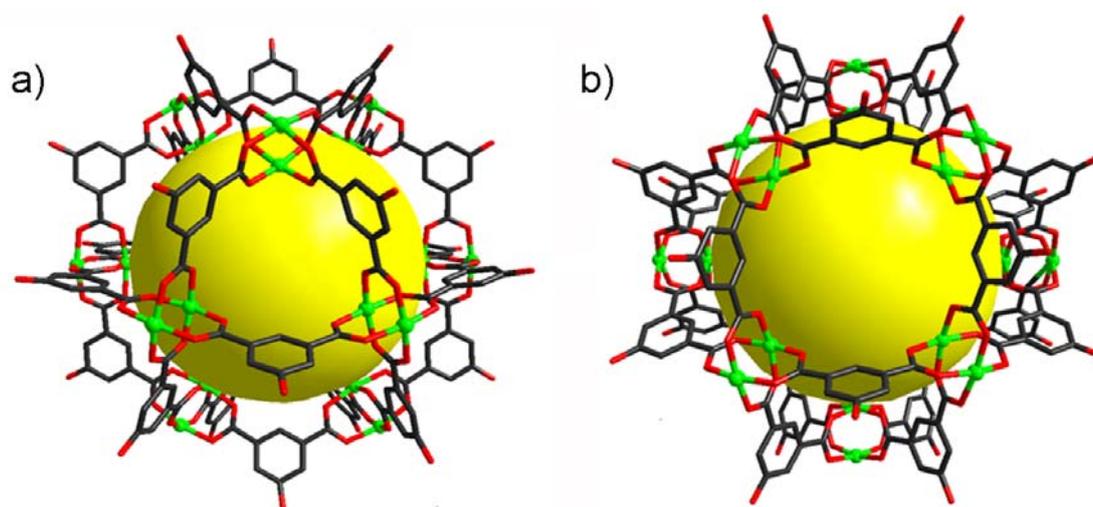


Figure S1. The core structure of MOP-18 (Cu, green; O, red; C, dark gray): (a) view down a 3-fold symmetry axis, and (b) view down a 4-fold symmetry axis. All hydrogen atoms, terminal ligands (H_2O) on the paddle-wheel units and the alkyl part of the organic ligands ($5\text{-OC}_{12}\text{H}_{25}\text{-}m\text{BDC}$) are omitted for clarity.

Infrared spectroscopy. One drop of the vesicle stock solution was topped on a BaF_2 plate and dried in the air. Spectra were recorded with a Perkin-Elmer[®] spectrum GX FT-IR system. FT-IR sample chamber was purged thoroughly with dry N_2 to remove the carbon dioxide and water signals before data acquisitions.

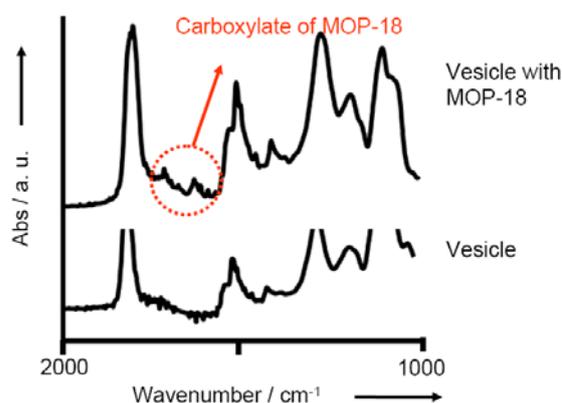


Figure S2. FT-IR Spectra of the vesicles (EYPC/cholesterol/dicetyl phosphate) prepared with and without 1 mol % MOP-18 and the subtraction FT-IR spectrum.

Proton transport experiments.

Preparation of vesicles. Stock solutions of EYPC (20.0 mg/mL) and cholesterol (1.0 mg/mL) and dicetyl phosphate (1.5 mg/mL) in CHCl_3 were prepared every 14 d and stored under refrigeration. In a typical experiment, EYPC (1.7 mL), cholesterol (2.0 mL) and sodium dicetyl phosphate (1.0 mL) stock solutions were mixed in a 100 mL round-bottomed flask and the chloroform was evaporated under a reduced pressure. The remaining thin film of the lipid mixture was dried *in vacuo*. After addition of PBS buffer solution containing HPTS (2 mL, 10 μM HPTS, pH = 7.0) and MOP-18 (1 mol %) in THF (50 μL), the lipid film was hydrated by sonication at 60 $^\circ\text{C}$. The milky suspension of vesicles was passed through a Sephadex[®] G-50 column to remove untrapped HPTS using PBS buffer as an eluent, which produced an approximately 10 mL vesicle solution. After dialysis against PBS buffer solution using a membrane (MWCO = 3500) for 4 h, the resulting vesicle solution was used in fluorescence experiments.

Fluorescence experiments. The vesicle solution (2 mL) prepared as described above and PBS buffer (1 mL) were mixed in a fluorescence cuvette. The emission of HPTS at 510 nm was monitored with an excitation wavelength at 460 nm. After starting time-based recording, 0.5 M HCl solution (30 μL) was added to lower the pH of the solution to 5.5 at 1 min. After the measurements were completed, 5 % Triton X-100 (50 μL) was added to lyse the vesicles.

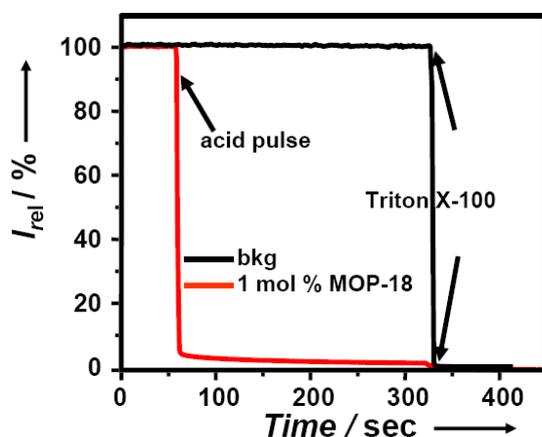


Figure S3. Changes in the fluorescence intensity of HPTS associated with the proton transport activity of MOP-18 in EYPC-LUVs as a function of time

Alkali metal ion transport.

Preparation of vesicles. EYPC (1.7 mL) in chloroform (20 mg/mL) was dried in a 100 mL round-bottomed flask with a rotary evaporator and then further dried under high vacuum for 1 d. After addition of phosphate buffer solution containing HPTS (2 mL, 100 mM NaCl, 10 mM phosphate, 10 μ M HPTS, pH = 6.4) and MOP-18 (1 mol %) in THF (50 μ L), the lipid film was hydrated by sonication at 60 °C. The suspension was extruded with an Avestin[®] handheld extruder to yield large unilamellar vesicles (LUVs) with a diameter of \sim 200 nm (characterized by DLS). The milky suspension of vesicles was passed through a Sephadex[®] G-50 column using phosphate buffer solution as an eluent to give approximately 10 mL vesicle solution. The suspension was dialyzed against phosphate buffer solution using a membrane (MWCO = 3500) for 4 h. The resulting vesicle solution was used in ratiometric fluorescence experiments.

Ratiometric fluorescence experiments. The emission of HPTS at 510 nm was monitored with two different excitation wavelengths (at 460 nm and 403 nm) simultaneously. The intensity ratio, I_{460}/I_{403} was measured with time to monitor the intravesicular pH change. In a typical experiment, the vesicle stock solution (200 μ L, pH = 6.4) containing HPTS prepared as described above was mixed with MCl phosphate buffer solution (1800 μ L, 100 mM MCl: M = Li⁺, Na⁺, K⁺, Rb⁺, Cs⁺, 10 mM sodium phosphate, pH = 6.4) in a fluorescence cuvette. During the experiment, 0.5 M NaOH solution (40 μ L) was added through an injection port to cause a pH increase from 6.4 to 7.4 in the extravesicular buffer solution. The maximal changes in the dye emission were obtained at the end of each experiment by lysis of the vesicles with 5 % Triton X-100 (50 μ L). The final transport trace was obtained as a ratio of the emission intensities (I_{460}/I_{403}) and normalized.

MOP-18 concentration dependent Na⁺ ion transport

Preparation of vesicles. A vesicle stock solution was prepared as described above in alkali metal ion transport experiments, but without addition of MOP-18 solution in THF.

Ratiometric fluorescence experiments. The emission of HPTS at 510 nm was monitored with two different excitation wavelengths (at 460 nm and 403 nm) simultaneously. The intensity ratio, I_{460}/I_{403} was measured with time to monitor the intravesicular pH change. In a typical experiment, vesicle stock solution (200 μ L, 100 mM NaCl, 10 mM sodium phosphate, pH = 6.4) containing HPTS dye (1.0×10^{-5} M) was mixed with phosphate buffer solution (1780 μ L, 100 mM NaCl, 10 mM sodium phosphate, pH = 6.4) in a fluorescence cuvette. Before fluorometric time-based scan, MOP-18 (0.4 – 1 mol %) in THF (20 μ L) was added. During the experiment, 0.5 M NaOH solution (40 μ L) was added through an injection port to cause a pH increase from 6.4 to 7.4 in the extravesicular buffer solution. The maximal changes in the dye emission were obtained at the end of each experiment by lysis of the vesicles with 5 % Triton X-100 (50 μ L). The final transport trace was obtained as a ratio of the emission intensities (I_{460}/I_{403}) and normalized.

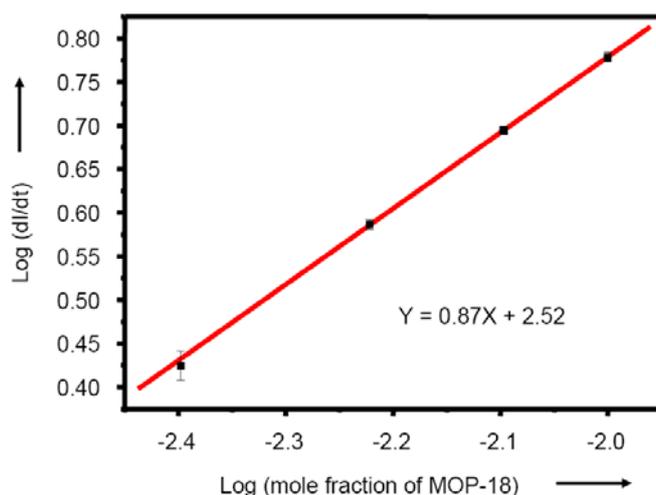


Figure S4. Log-log plot of the initial rate of Na⁺ transport (dI/dt) vs mol fraction of MOP-18 in the EYPC vesicle.

Planar Bilayer Lipid Membrane Voltage-Clamp Experiments. Single-channel conductance measurements were performed as described in the literature.² A solution of phosphatidylethanolamine (20 μg) and phosphatidylserine (20 μg) in *n*-decane (20 μL) was painted around a 200-μm diameter aperture of the bilayer “cup”. For incorporation of MOP-18, two chambers were initially filled with 0.25 – 2 M KCl, 10 mM Tris-HEPES. Thinning of the bilayer film was monitored by bilayer capacitance. A solution of MOP-18 and PS/PE (1:100 molar ratio) in *n*-decane was painted onto the aperture and gently stirred. The *cis* chamber was the voltage control side connected to the head stage of a BC-525D Warner amplifier, while the *trans* side was held at virtual ground. All the conductance measurements were performed at room temperature. Recording was filtered with an 8-pole low pass Bessel filter at 1 kHz and digitalized through a Digidata 1322A interface (Axon Instruments). Data acquisition and analysis were done with Axon Instruments software and hardware (“pClamp v9.0”). The conductance of channels was estimated from the slope of *IV* curve. Data calculations were done according to the literature.³

The inner channel diameter was calculated using Hille’s equation⁴ and the conductance at 100 mM KCl (13.8 pS) multiplied by a “correction factor” (5.6).⁵

$$g^{-1} = 4l\rho / \pi d^2 + \rho / d$$

The length of the channel $l = 34 \text{ \AA}$ and the resistivity of the recording solution $\rho = 77 \text{ \Omega cm}$ were used.

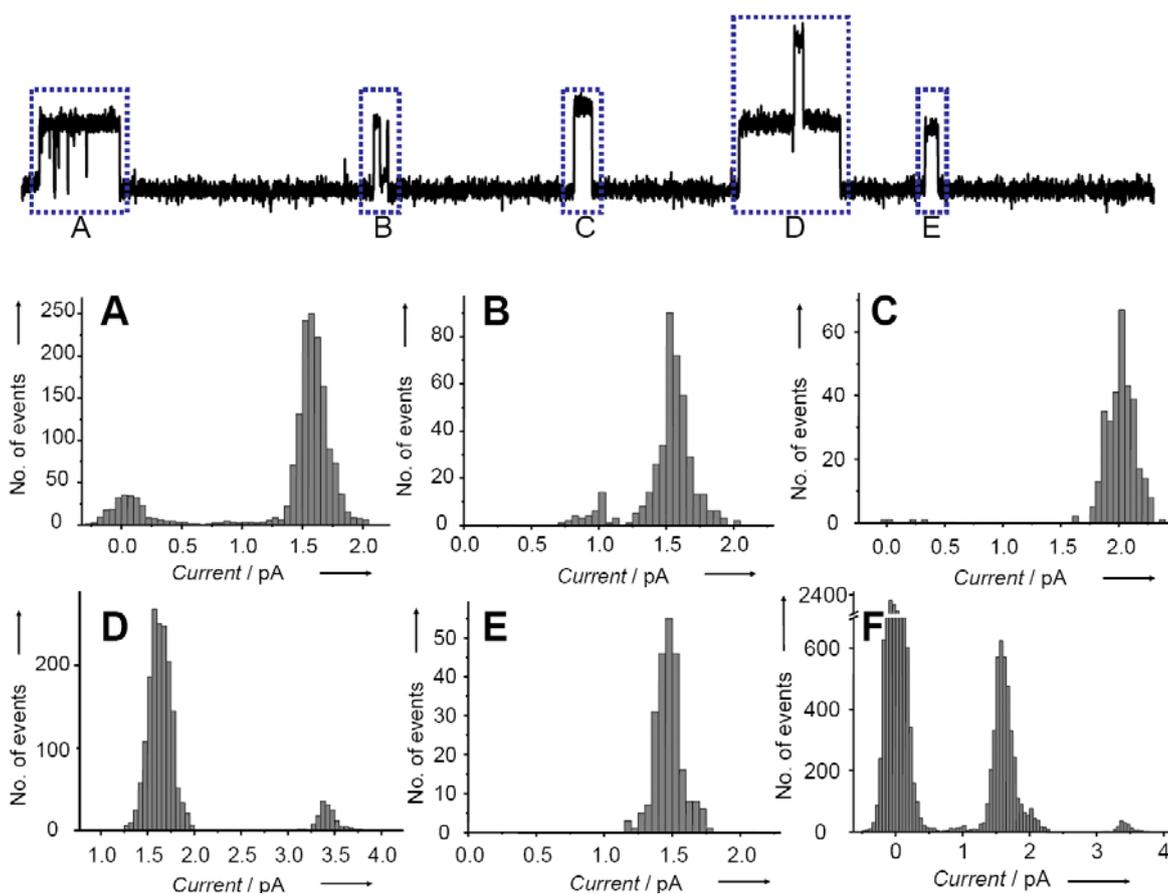


Figure S5. Typical current profile and histograms of the currents measured with MOP-18 at +60 mV in 2 M KCl. Histograms A – E: Histograms of the currents in sections A – E; F: Histogram of the currents for the whole currents profile.

References

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