

Structurally simple trimesic amides as highly selective anion channels

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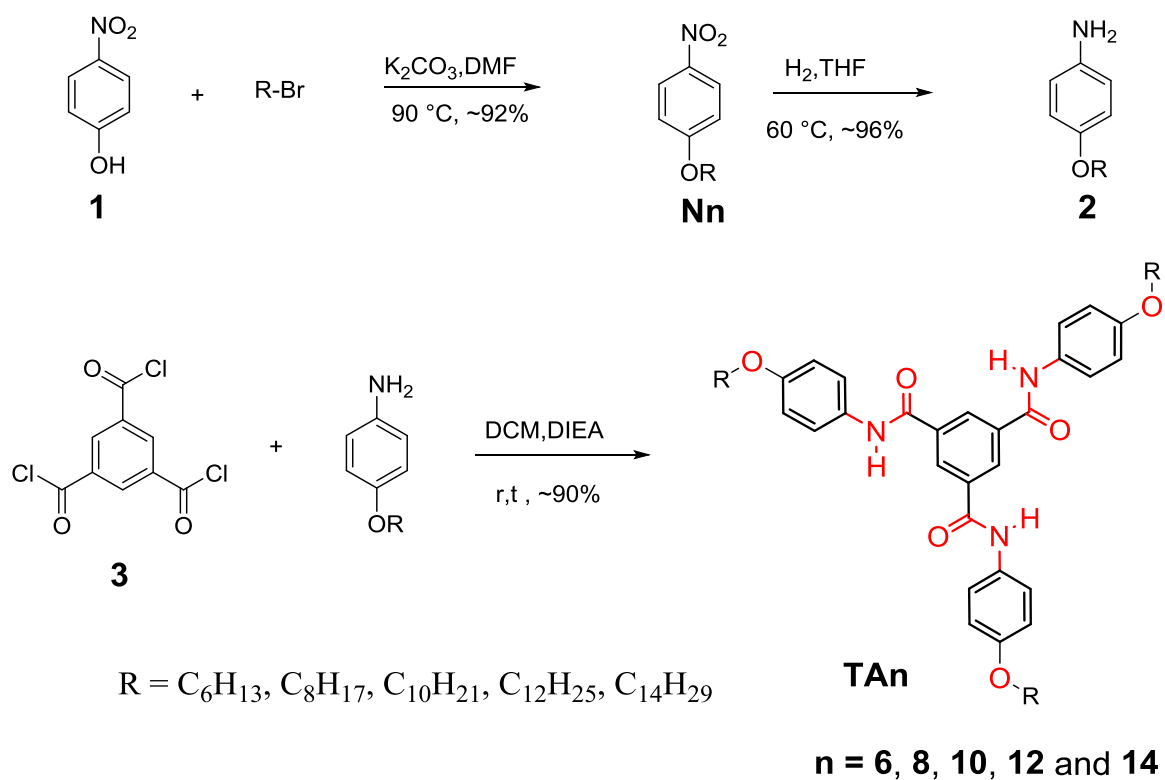
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General Remarks

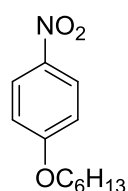
All the reagents were obtained from commercial suppliers and used as received unless otherwise noted. Aqueous solutions were prepared from MilliQ water. The organic solutions from all liquid extractions were dried over anhydrous Na₂SO₄ for a minimum of 15 minutes before filtration. Flash column chromatography was performed using pre-coated 0.2 mm silica plates from Selecto Scientific. Chemical yield refers to pure isolated substances. ¹H and ¹³C NMR spectra were recorded on either a Bruker ACF-400 spectrometer. The solvent signal of CDCl₃ was referenced at δ = 7.26 ppm. Coupling constants (*J* values) are reported in Hertz (Hz). ¹H NMR data are recorded in the order: chemical shift value, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad), number of protons that gave rise to the signal and coupling constant, where applicable. ¹³C spectra are proton-decoupled and recorded on Bruker ACF400 (400 MHz). The solvent, CDCl₃, was referenced at δ = 77 ppm. CDCl₃ (99.8%-Deuterated) was purchased from Aldrich and used without further purification. Mass spectra were acquired with Shimadzu LCMS-2010EV.

Scheme S1. Synthetic route that affords Trimesic amide-based pores



Experimental Procedures and Compound Characterizations

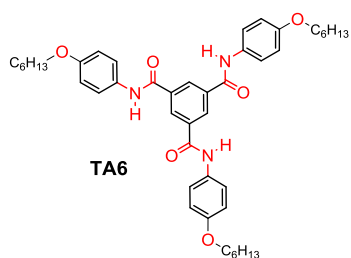
N6



Compound 1 (1.39 g, 10 mmol), 1-bromohexane (1.7 ml, 12 mmol) and K_2CO_3 (2.07 g, 15 mmol) were dissolved in DMF (50 mL). The reaction mixture was stirred for 24 h at 90 °C. The reaction mixture was then

N6 filtered, and solvent was removed in vacuo. Then the crude product was dissolved in CH_2Cl_2 (100 mL), and washed with water (2 x 100 mL) the crude product was purified by flash column chromatography (Hexane : Ethyl acetate = 50:1, v:v) to afford the target compound **N6** as a yellow oil. Yield: 2.05 g, 92%. 1H NMR (400 MHz, $CDCl_3$) δ 8.21 – 8.16 (m, 2H), 6.96 – 6.91 (m, 2H), 4.04 (t, J = 6.5 Hz, 2H), 1.81 (dt, J = 14.6, 6.6 Hz, 2H), 1.52 – 1.42 (m, 2H), 1.38 – 1.29 (m, 4H), 0.96 – 0.85 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.29, 141.27, 125.94, 114.41, 68.91, 31.51, 28.95, 25.61, 22.60, 14.05. MS-ESI: calculated for $[M+Na]^+$ ($C_{12}H_{17}O_3NNa$): m/z 246.11, found: m/z 246.17.

TA6

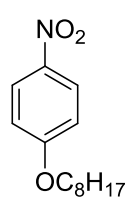


Compound N6 (1.12 g, 5 mmol) was dissolved in THF (30 mL), with an installation of H_2 balloon on top of the round bottom flask. After that the reaction was allowed to stir at 60 °C for 16 hours. The reaction mixture was then filtered, and solvent was removed in vacuo to give the pure product **2** as a brown solid, which was directly used in the next step without further purification. Yield: 0.93 g, 96%. Under the N_2 Protection, **Compound 3** (0.4 g, 1.5 mmol) and **Compound 2** (0.9 g, 4.65 mmol) were dissolved in CH_2Cl_2 (20 mL) to which N,N-

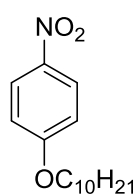
Diisopropylethylamine (1.57 ml, 9 mmol) was added. The reaction mixture was stirred for 24 h at room temperature. Solvent was removed in vacuo and the crude product was dissolved in CH₂Cl₂ (30 mL), and washed with water (2 x 40 mL), the crude product was purified by flash column chromatography (MeOH : DCM = 1:300, v:v) to afford the target compound **TA6** as a dark red solid. Yield: 0.99 g, 90%. ¹H NMR (400 MHz, DMSO) δ 10.46 (s, 3H), 8.65 (s, 3H), 7.70 (d, *J* = 9.0 Hz, 6H), 6.95 (d, *J* = 9.1 Hz, 6H), 3.95 (t, *J* = 6.5 Hz, 6H), 1.75 – 1.67 (m, 6H), 1.41 (dd, *J* = 10.0, 4.8 Hz, 6H), 1.31 (td, *J* = 7.0, 3.5 Hz, 12H), 0.88 (t, *J* = 7.0 Hz, 9H). ¹³C NMR (101 MHz, DMSO) δ 164.59, 155.63, 136.02, 132.36, 129.90, 122.39, 114.84, 68.00, 31.52, 29.19, 25.72, 22.59, 14.43. MS-ESI: calculated for [M+H]⁺ (C₄₅H₅₈O₆N₃) : *m/z* 736.42, found: *m/z* 736.49.

Preparation of N8,N10,N12 and N14 follows the same synthetic procedure as N6.

N8



¹H NMR (400 MHz, CDCl₃) δ 8.26 – 8.19 (m, 2H), 7.00 – 6.94 (m, 2H), 4.07 (t, *J* = 6.5 Hz, 2H), 1.85 (dq, *J* = 13.2, 6.6 Hz, 2H), 1.54 – 1.45 (m, 2H), 1.40 – 1.28 (m, 8H), 0.92 (dd, *J* = 8.7, 5.0 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 164.29, 141.28, 125.95, 114.41, 68.92, 31.81, 29.30, 29.23, 28.99, 25.94, 22.68, 14.14. MS-ESI: calculated for [M+Na]⁺ (C₁₄H₂₁O₃NNa) : *m/z* 274.14, found: *m/z* 274.27.

N10

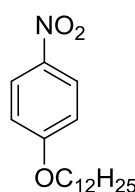
^1H NMR (400 MHz, CDCl_3) δ 8.19 (d, $J = 9.0$ Hz, 2H), 6.98 – 6.91 (m, 2H), 4.04 (t, $J = 6.5$ Hz, 2H), 1.86 – 1.77 (m, 2H), 1.50 – 1.42 (m, 2H), 1.38 – 1.24 (m, 12H), 0.88 (t, $J = 6.8$ Hz, 3H). ^{13}C NMR (101 MHz,

N10

CDCl_3) δ 164.28, 141.28, 125.95, 114.41, 68.92, 31.92, 29.56, 29.34,

28.99, 25.94, 22.71, 14.16. MS-ESI: calculated for $[\text{M}+\text{Na}]^+$ ($\text{C}_{16}\text{H}_{25}\text{O}_3\text{NNa}$) : m/z

302.17, found: m/z 302.09.

N12

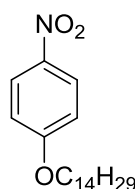
^1H NMR (400 MHz, CDCl_3) δ 8.23 – 8.16 (m, 2H), 6.96 – 6.90 (m, 2H), 4.04 (t, $J = 6.5$ Hz, 2H), 1.86 – 1.77 (m, 2H), 1.50 – 1.41 (m, 2H), 1.37 – 1.23 (m, 16H), 0.87 (t, $J = 6.7$ Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ

N12

164.29, 141.27, 125.94, 114.40, 68.92, 31.95, 29.68, 29.67, 29.61, 29.57,

29.39, 29.34, 28.99, 25.94, 22.73, 14.17. MS-ESI: calculated for $[\text{M}+\text{Na}]^+$

($\text{C}_{18}\text{H}_{29}\text{O}_3\text{NNa}$) : m/z 330.20, found: m/z 330.41.

N14

^1H NMR (400 MHz, CDCl_3) δ 8.22 – 8.17 (m, 2H), 6.96 – 6.91 (m, 2H), 4.04 (t, $J = 6.5$ Hz, 2H), 1.82 (dq, $J = 13.2, 6.6$ Hz, 2H), 1.50 – 1.42 (m, 2H), 1.36 – 1.23 (m, 20H), 0.88 (t, $J = 6.8$ Hz, 3H). ^{13}C NMR (101 MHz,

N14

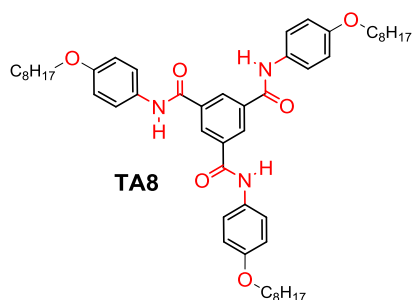
CDCl_3) δ 164.28, 141.29, 125.96, 114.41, 68.92, 31.96, 29.72, 29.70,

29.69, 29.61, 29.56, 29.40, 29.34, 28.99, 25.94, 22.73, 14.18. MS-ESI: calculated for

$[\text{M}+\text{Na}]^+$ ($\text{C}_{20}\text{H}_{33}\text{O}_3\text{NNa}$) : m/z 358.24, found: m/z 358.29.

Preparation of TA8,TA10,TA12 and TA14 follows the same synthetic procedure as TA6.

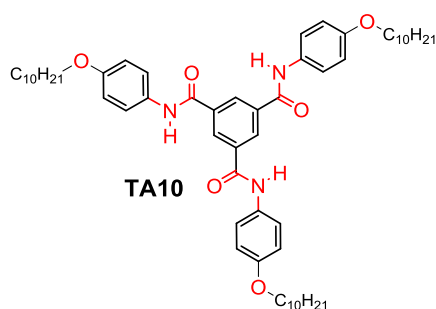
TA8



¹H NMR (400 MHz, DMSO) δ 10.45 (s, 3H), 8.64 (s, 3H), 7.72 – 7.67 (m, 6H), 6.97 – 6.92 (m, 6H), 3.95 (t, *J* = 6.5 Hz, 6H), 1.75 – 1.66 (m, 6H), 1.46 – 1.37 (m, 6H), 1.33 – 1.23 (m, 24H), 0.86 (dd, *J* = 8.6, 5.1 Hz, 9H). ¹³C NMR (101 MHz, DMSO) δ

164.61, 155.63, 136.02, 132.32, 129.90, 122.41, 114.85, 68.00, 31.73, 29.25, 29.21, 29.17, 26.04, 22.58, 14.47. MS-ESI: calculated for [M+H]⁺ (C₅₁H₇₀O₆N₃) : *m/z* 820.52, found: *m/z* 820.80.

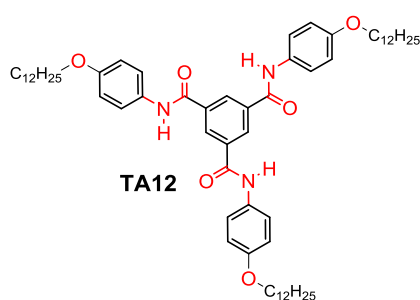
TA10



¹H NMR (400 MHz, DMSO) δ 10.45 (s, 3H), 8.64 (s, 3H), 7.69 (d, *J* = 9.1 Hz, 6H), 6.94 (d, *J* = 9.1 Hz, 6H), 3.94 (t, *J* = 6.5 Hz, 6H), 1.69 (dd, *J* = 14.5, 6.6 Hz, 6H), 1.46 – 1.37 (m, 6H), 1.32 – 1.22 (m, 36H), 0.85 (t, *J* = 6.9 Hz, 9H). ¹³C NMR

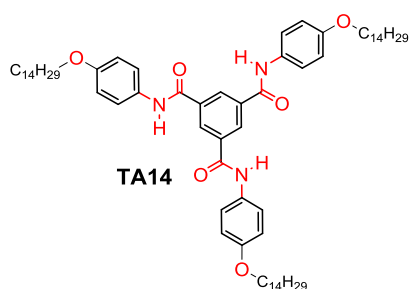
(101 MHz, DMSO) δ 164.61, 155.63, 136.01, 132.31, 129.89, 122.41, 114.85, 68.00, 31.78, 29.50, 29.45, 29.27, 29.19, 26.02, 22.59, 14.46. MS-ESI: calculated for [M+H]⁺ (C₅₇H₈₂O₆N₃) : *m/z* 904.61, found: *m/z* 904.77.

TA12



¹H NMR (400 MHz, DMSO/CDCl₃ = 6:1) δ 10.38 (s, 3H), 8.66 (s, 3H), 7.69 (d, *J* = 6.7 Hz, 6H), 6.87 (s, 6H), 3.92 (s, 6H), 1.70 (s, 6H), 1.41 (s, 6H), 1.23 (s, 48H), 0.84 (d, *J* = 3.5 Hz, 9H). ¹³C NMR (101 MHz, DMSO/CDCl₃ = 6:1) δ 164.58, 155.61, 135.96, 132.33, 129.88, 122.33, 114.66, 67.99, 31.82, 29.53, 29.34, 29.24, 26.05, 22.62, 14.42, 0.68. MS-ESI: calculated for [M+H]⁺ (C₆₃H₉₄O₆N₃) : *m/z* 988.71, found: *m/z* 988.81.

TA14



¹H NMR (400 MHz, DMSO/CDCl₃ = 6:1) δ 10.41 (s, 3H), 8.66 (s, 3H), 7.70 (d, *J* = 9.1 Hz, 6H), 6.92 – 6.87 (m, 6H), 3.93 (t, *J* = 6.5 Hz, 6H), 1.69 (dd, *J* = 14.4, 6.6 Hz, 6H), 1.40 (d, *J* = 8.0 Hz, 6H), 1.26 (d, *J* = 20.2 Hz, 60H), 0.84 (t, *J* = 6.8 Hz, 9H). ¹³C NMR (101 MHz, DMSO/CDCl₃ = 6:1) δ 164.57, 155.60, 135.96, 132.35, 129.89, 122.33, 114.69, 67.99, 31.81, 29.56, 29.53, 29.32, 29.25, 29.23, 26.04, 22.61, 14.42. MS-ESI: calculated for [M+H]⁺ (C₆₉H₁₀₆O₆N₃) : *m/z* 1072.80, found: *m/z* 1072.98.

Experimental methods for ion transport study

Ion transport study using the HPTS assay and EC_{50} measurements using the Hill analysis. Egg yolk L- α -phosphatidylcholine (EYPC, 1 ml, 25 mg/mL in $CHCl_3$, Avanti Polar Lipids, USA) and MeOH (1 mL) were mixed in a round-bottom flask. The mixed solvents were removed under reduced pressure at 40 °C. After drying the resulting film under high vacuum overnight at room temperature, the film was hydrated with 4-(2-hydroxyethyl)-1-piperazine-ethane sulfonic acid (HEPES) buffer solution (1 mL, 10 mM HEPES, 100 mM NaCl, pH = 7.0) containing a pH sensitive dye 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS, 1 mM) at room temperature for 60 minutes to give a milky suspension. The mixture was then subjected to 12 freeze-thaw cycles: freezing in liquid N_2 for 1 minute and heating 37 °C water bath for 1.5 minutes. The vesicle suspension was extruded through polycarbonate membrane (0.1 μ m) to produce a homogeneous suspension of large unilamellar vesicles (LUVs) of about 120 nm in diameter with HPTS encapsulated inside. The untrapped HPTS dye was separated from the LUVs by using size exclusion chromatography (stationary phase: Sephadex G-50, GE Healthcare, USA, mobile phase: HEPES buffer with 100 mM NaCl at pH 7.0) and diluted with the mobile phase to yield 12.8 mL of 2.5 mM lipid stock solution. This HPTS-containing LUV suspension (25 μ L, 2.5 mM) was added to a HEPES buffer solution (1.93 mL, 10 mM HEPES, 100 mM NaCl at pH = 8.0) to create a pH gradient for ion transport study. A solution of channel molecules in DMSO was then injected into the suspension under gentle stirring. Upon the addition of channels, the emission of HPTS was immediately monitored at 510 nm with excitations at both 460 and 403 nm recorded simultaneously for 300 seconds using fluorescence spectrophotometer (Hitachi, Model F-7100, Japan). At $t = 300$ s, an aqueous solution of Triton X-100 (20 μ L, 20% v/v) was immediately added to achieve the maximum change in dye fluorescence emission intensity. After subtracting background intensity at $t = 0$, the final transport trace was obtained as a ratiometric value of I_{460}/I_{403} and normalized based on the ratiometric value of I_{460}/I_{403} after addition of triton at $t = 300$ s. The fractional changes R_{Cl^-} was calculated for each curve using the normalized value of I_{460}/I_{403} at $t = 300$ s before the addition of triton, with ratiometric value of I_{460}/I_{403} at $t = 0$ s as 0% and that of I_{460}/I_{403} at $t = 300$ s (obtained after addition of triton) as 100%. Fitting the fractional transmembrane activity R_{Cl^-} vs channel concentration using the Hill equation: $Y=1/(1+ (EC_{50}/[C])^n)$ gave the Hill coefficient n and EC_{50} values.

Sulphate-containing HPTS assay for cation selectivity. Egg yolk L- α -phosphatidylcholine (EYPC, 1 ml, 25 mg/mL in $CHCl_3$, Avanti Polar Lipids, USA) solvents were removed under reduced pressure at room temperature. After drying the resulting film under high vacuum overnight at room temperature, the film was hydrated with 4-(2-hydroxyethyl)-1-piperazine-ethane sulfonic acid (HEPES) buffer solution (1.0 mL, 10 mM HEPES, pH = 7.0) containing a pH sensitive dye 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS, 1 mM) in thermostatic shaker-incubator at 37 °C for 2 hours to give a milky suspension. The mixture was then subjected to 10 freeze-thaw cycles: freezing in liquid N_2 for 1 minute and heating at 55 °C for 2 minutes. The vesicle suspension was extruded through polycarbonate membrane (0.1 μ m) to produce a homogeneous suspension of large unilamellar

vesicles (LUVs) of about 100 nm in diameter with HPTS encapsulated inside. The unencapsulated HPTS dye was separated from the LUVs by using size exclusion chromatography (stationary phase: Sephadex G-50, GE Healthcare, USA, mobile phase: HEPES buffer with , pH = 7.0) and diluted with the mobile phase to yield 13 mL of 2.5 mM lipid stock solution.

The HPTS-containing LUV suspension (25 μ L, 2.5 mM in 10 mM HEPES buffer, pH = 7.0) was added to a HEPES buffer solution (1.95 mL, 10 mM HEPES, 200 mM M_2SO_4 at pH = 7.0, where $M^+ = Li^+, Na^+, K^+, Rb^+$ and Cs^+) for ion transport study. A solution of channel molecule **6L₃10** at a final concentration of 4 μ M (or gramicidin A at 1 nM) in DMSO was then injected into the suspension under gentle stirring. Upon the addition of channels, the emission of HPTS was immediately monitored at 510 nm with excitations at both 460 and 403 nm recorded simultaneously for 300 seconds using fluorescence spectrophotometer (Hitachi, Model F-7100, Japan) after which time an aqueous solution of Triton X-100 (20 μ L, 20% v/v) was immediately added to achieve the maximum change in dye fluorescence emission. The final transport trace was obtained as a ratiometric value of I_{460}/I_{403} and normalized based on the ratiometric value of I_{460}/I_{403} after addition of triton.

Chloride transport using the SPQ Assay. Egg yolk L- α -phosphatidylcholine (EYPC, 1 ml, 25 mg/mL in $CHCl_3$, Avanti Polar Lipids, USA) and MeOH (1 mL) were mixed in a round-bottom flask. The mixed solvents were removed under reduced pressure at 40 $^{\circ}C$. After drying the resulting film under high vacuum overnight at room temperature, the film was hydrated $NaNO_3$ solution (1 mL, 200 mM) containing a Cl^- -sensitive dye 6-methoxy-N-(3-sulfopropyl)quinolinium (SPQ) (0.5 mM) in thermostatic shaker-incubator at room temperature for 60 minutes to give a milky suspension. The mixture was then subjected to 12 freeze-thaw cycles: freezing in liquid N_2 for 1 minute and heating at 37 $^{\circ}C$ in water bath for 1.5 minutes. The vesicle suspension was extruded through polycarbonate membrane (0.1 μ m) to produce a homogeneous suspension of large unilamellar vesicles (LUVs) of about 120 nm in diameter with SPQ encapsulated inside. The unencapsulated HPTS dye was separated from the LUVs by using size exclusion chromatography (stationary phase: Sephadex G-50, GE Healthcare, USA, mobile phase: 200 mM $NaNO_3$) and diluted with the mobile phase to yield 12.8 mL of 2.5 mM lipid stock solution.

The SPQ-containing LUV suspension (25 μ L, 2.5 mM in 200 mM $NaNO_3$) was added to a $NaCl$ solution (1.93 mL, 200 mM) to create an extravesicular chloride gradient. A solution of tripeptide molecule **6L₃10** in DMSO at different concentrations was then injected into the suspension under gentle stirring. Upon the addition of pore-forming mono-peptide molecules, the emission of SPQ was immediately monitored at 430 nm with excitations at 360 nm for 300 seconds using fluorescence spectrophotometer (Hitachi, Model F-7100, Japan) after which time an aqueous solution of Triton X-100 (20 μ L, 20% v/v) was immediately added to completely destroy the chloride gradient. The final transport trace was obtained by normalizing the fluorescence intensity using the equation of $I_f = [(I_t - I_1)/(I_0 - I_1)]$ where, I_f = Fractional emission intensity, I_t = Fluorescence intensity at time t , I_1 = Fluorescence intensity after addition of Triton X-100, and I_0 = Initial fluorescence intensity .

The HPTS assay in the presence of valinomycin (VA). The HPTS-containing LUV suspension (25 μ L, 2.5 mM in 10 mM HEPES buffer containing 100 mM NaCl at pH = 7.0) was added to a HEPES buffer solution (1.93 mL, 10 mM HEPES, 100 mM NaCl) to create a pH gradient for ion transport study. A solution of valinomycin (0.1 pM) and **6L₃10** (4.3 nM) in DMSO was then injected into the suspension under gentle stirring at 20 s and 70 s, respectively. Upon the addition of pore-forming mono-peptide molecules, the emission of HPTS was immediately monitored at 510 nm with excitations at both 460 and 403 nm recorded simultaneously for 300 seconds using fluorescence spectrophotometer (Hitachi, Model F-7100, Japan) after which time an aqueous solution of Triton X-100 (20 μ L, 20% v/v) was immediately added to achieve the maximum change in dye fluorescence emission. The final transport trace was obtained as a ratiometric value of I_{460}/I_{403} and normalized based on the ratiometric value of I_{460}/I_{403} after addition of triton.

The HPTS assay in the presence of FCCP (carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazine). The HPTS-containing LUV suspension (25 μ L, 2.5 mM in 10 mM HEPES buffer containing 100 mM NaCl at pH = 7.0) was added to a HEPES buffer solution (1.93 mL, 10 mM HEPES, 100 mM NaCl) to create a pH gradient for ion transport study. A solution of FCCP (10 pM) and **6L₃10** (4.3 nM) in DMSO was then injected into the suspension under gentle stirring at 20 s and 70 s, respectively. Upon the addition of pore-forming mono-peptide molecules, the emission of HPTS was immediately monitored at 510 nm with excitations at both 460 and 403 nm recorded simultaneously for 300 seconds using fluorescence spectrophotometer (Hitachi, Model F-7100, Japan). 300 s later, aqueous solution of Triton X-100 (20 μ L, 20% v/v) was immediately added to achieve the maximum change in dye fluorescence emission. The final transport trace was obtained as a ratiometric value of I_{460}/I_{403} and normalized based on the ratiometric value of I_{460}/I_{403} after addition of triton.

Single channel current measurement in planar lipid bilayers. The chloroform solution of 1,2-diphytanoyl-*sn*-glycero-3-phosphocholine (diPhyPC, 10 mg/ml, 20 μ L) was evaporated using nitrogen gas to form a thin film and re-dissolved in *n*-decane (8 μ L). 0.2 μ L of this *n*-decane solution was injected into the aperture (diameter = 200 μ m) of the Delrin[®] cup (Warner Instruments, Hamden, CT) with the *n*-decane removed using nitrogen gas. In a typical experiment for conductance measurement, both the chamber (*cis* side) and Delrin cup (*trans* side) were filled with an aqueous KCl solution (1.0 M, 1.0 mL). Ag-AgCl electrodes were inserted into the two solutions with the *cis* chamber grounded. Planar lipid bilayer was formed by painting 0.3 μ L of the lipid-containing *n*-decane solution around the *n*-decane-pretreated aperture. Successful formation of planar lipid bilayers can be established with a capacitance value ranging from 80-120 pF. Samples (**6L₃10**) in THF (0.3-1.0 μ L) were added to the *cis* compartment to reach a final concentration of around 10^{-8} M and the solution was stirred for a few min until a single current trace appeared. These single channel currents were then measured using a Warner BC-535D bilayer clamp amplifier, collected by PatchMaster (HEKA) with a sample interval at 5 kHz and filtered with an 8-pole Bessel filter at 1 kHz (HEKA). The data were analysed by FitMaster (HEKA) with a digital filter at 100 Hz. Plotting current trace vs voltage yielded chloride conductance (γ_{Cl}).

The HPTS assay for anion selectivity. The HPTS-containing LUV suspension (25 μ L, 2.5 mM in 10 mM HEPES buffer containing 100 mM NaX where $X^- = \text{Cl}^-, \text{Br}^-, \text{I}^-, \text{NO}_3^-, \text{and } \text{ClO}_4^-$ at pH = 7.0) was added to a HEPES buffer solution (1.93 mL, 10 mM HEPES, 100 mM NaX, where $X^- = \text{Cl}^-, \text{Br}^-, \text{I}^-, \text{NO}_3^-, \text{and } \text{ClO}_4^-$ at pH= 8.0) to create a pH gradient for ion transport study. A solution of peptides at specified concentrations in DMSO was then injected into the LUV suspension under gentle stirring. Upon the addition of pore-forming mono-peptide molecules, the emission of HPTS was immediately monitored at 510 nm with excitations at both 460 and 403 nm recorded simultaneously for 300 seconds using fluorescence spectrophotometer (Hitachi, Model F-7100, Japan) after which time an aqueous solution of Triton X-100 (20 μ L, 20% v/v) was immediately added to achieve the maximum change in dye fluorescence emission. The final transport trace was obtained as a ratiometric value of I_{460}/I_{403} and normalized based on the ratiometric value of I_{460}/I_{403} after addition of triton.

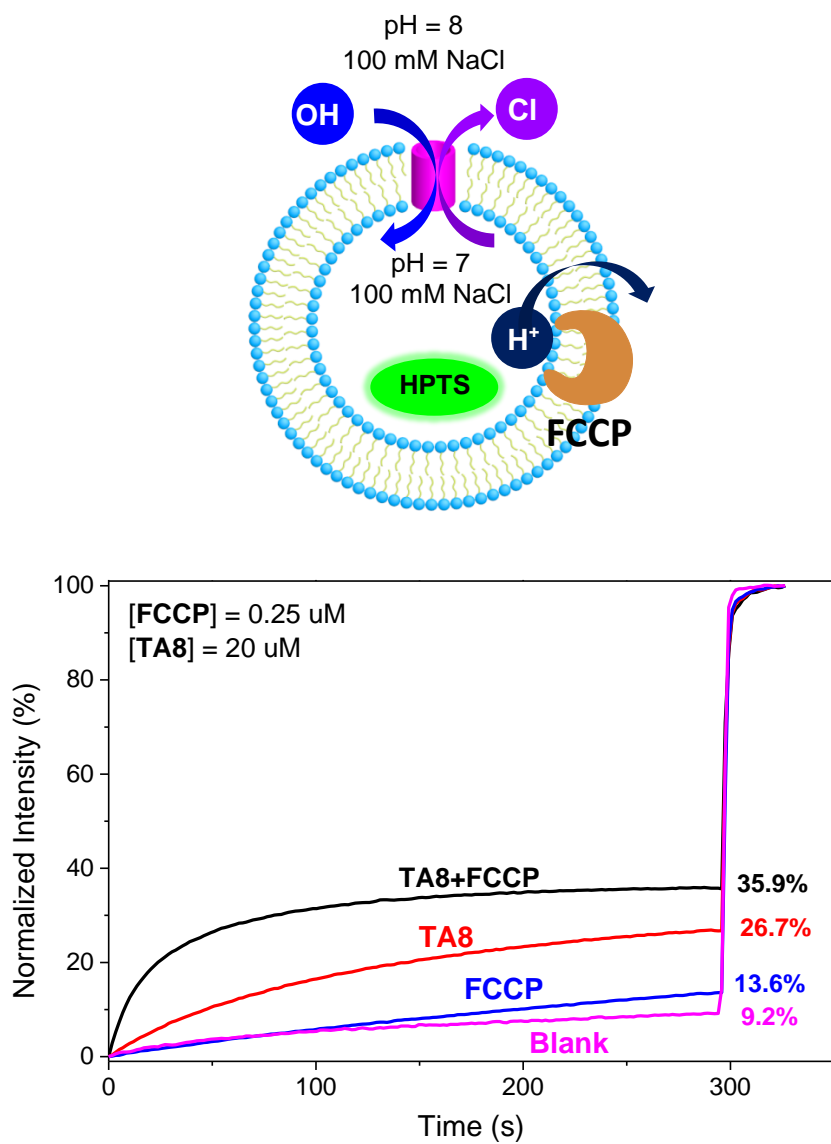


Figure S1. FCCP-based LUV assay that suggests transport rate of OH⁻ to be faster than H⁺.

Determination of Initial Rate Constants

Rate constant values were obtained by fitting transport curves to a single exponential decay equation S1 shown below.

$$\text{Single exponential: } I_F = A_1 e^{(-k_1 t)} \quad (\text{Equation S1})$$

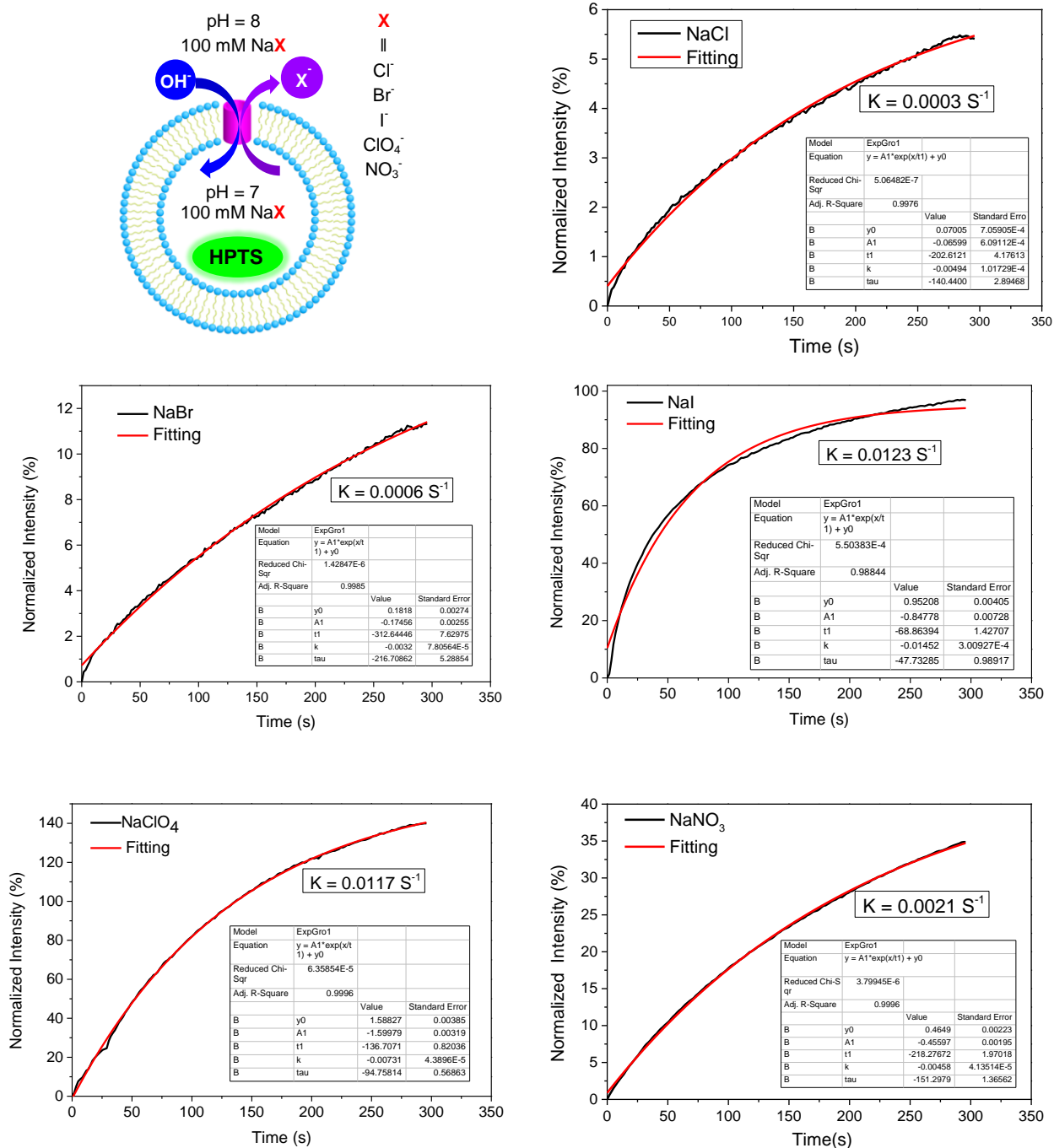


Figure S2. Fitted plots (red lines) to calculate initial rate constant (k_1) values of anion transport by channel TA12 at 20 μM by the HPTS assay.

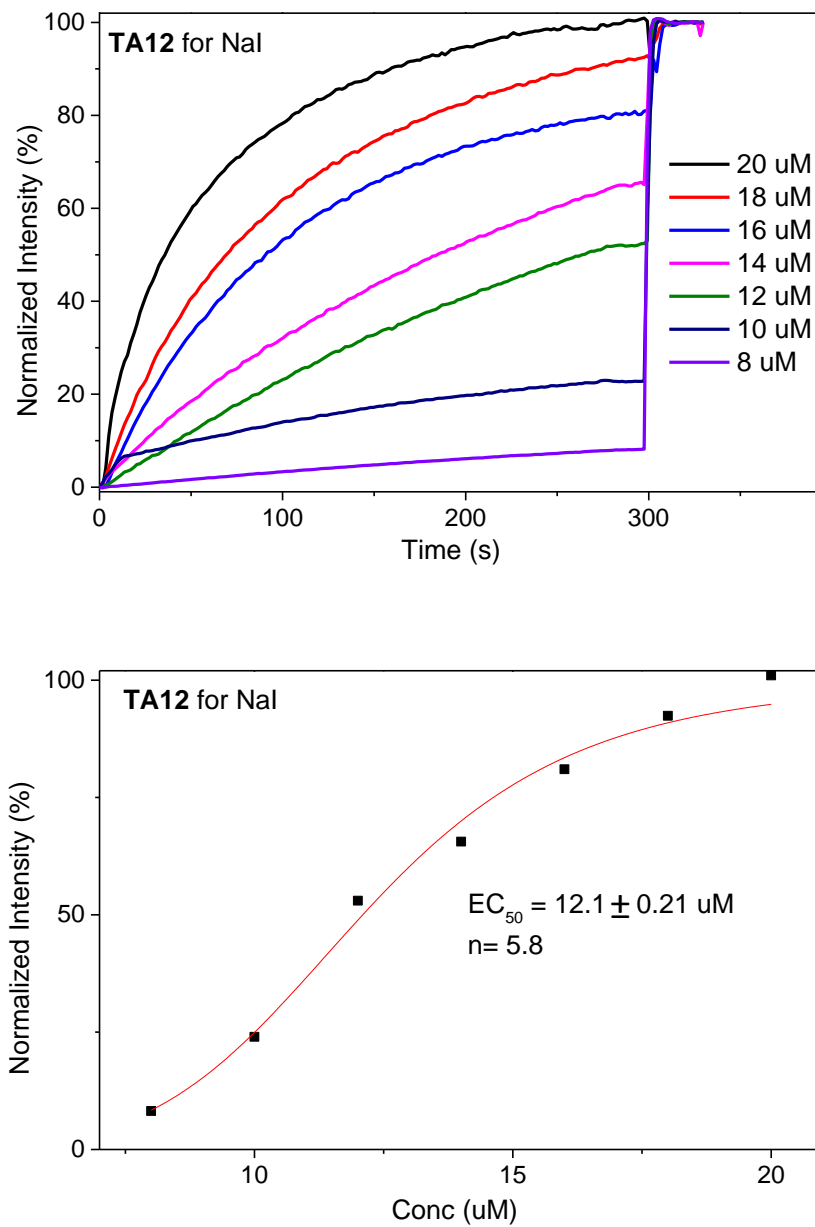


Figure S3. Determination of EC_{50} value for TA12-mediated transport of iodide anions.

Determination of Initial Rate Constants

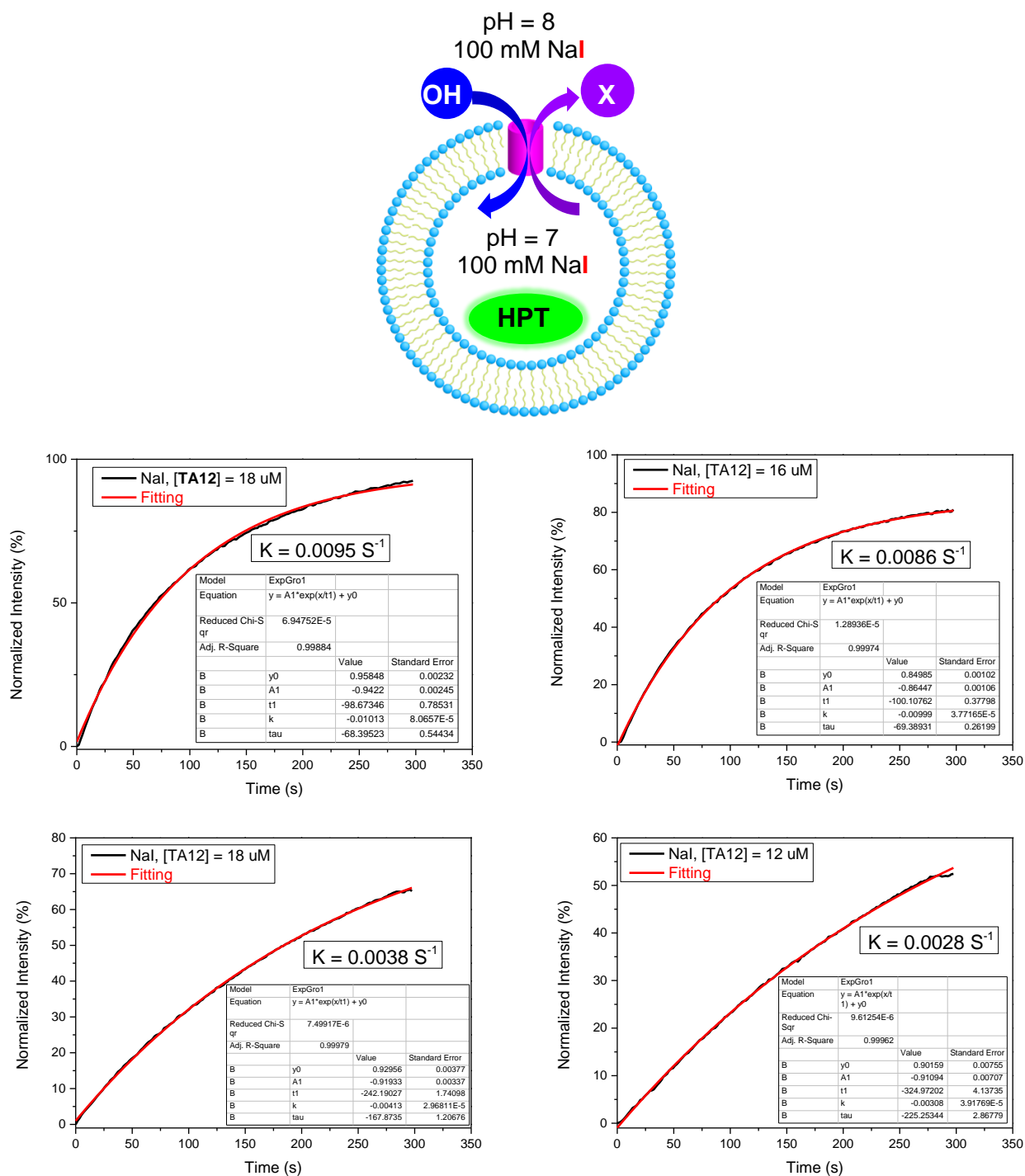


Figure S4. Fitted plots (red lines) to calculate initial rate constant (k_1) values of iodide transport by channel TA12 at various concentrations by the HPTS assay.

Determination of Initial Rate Constants

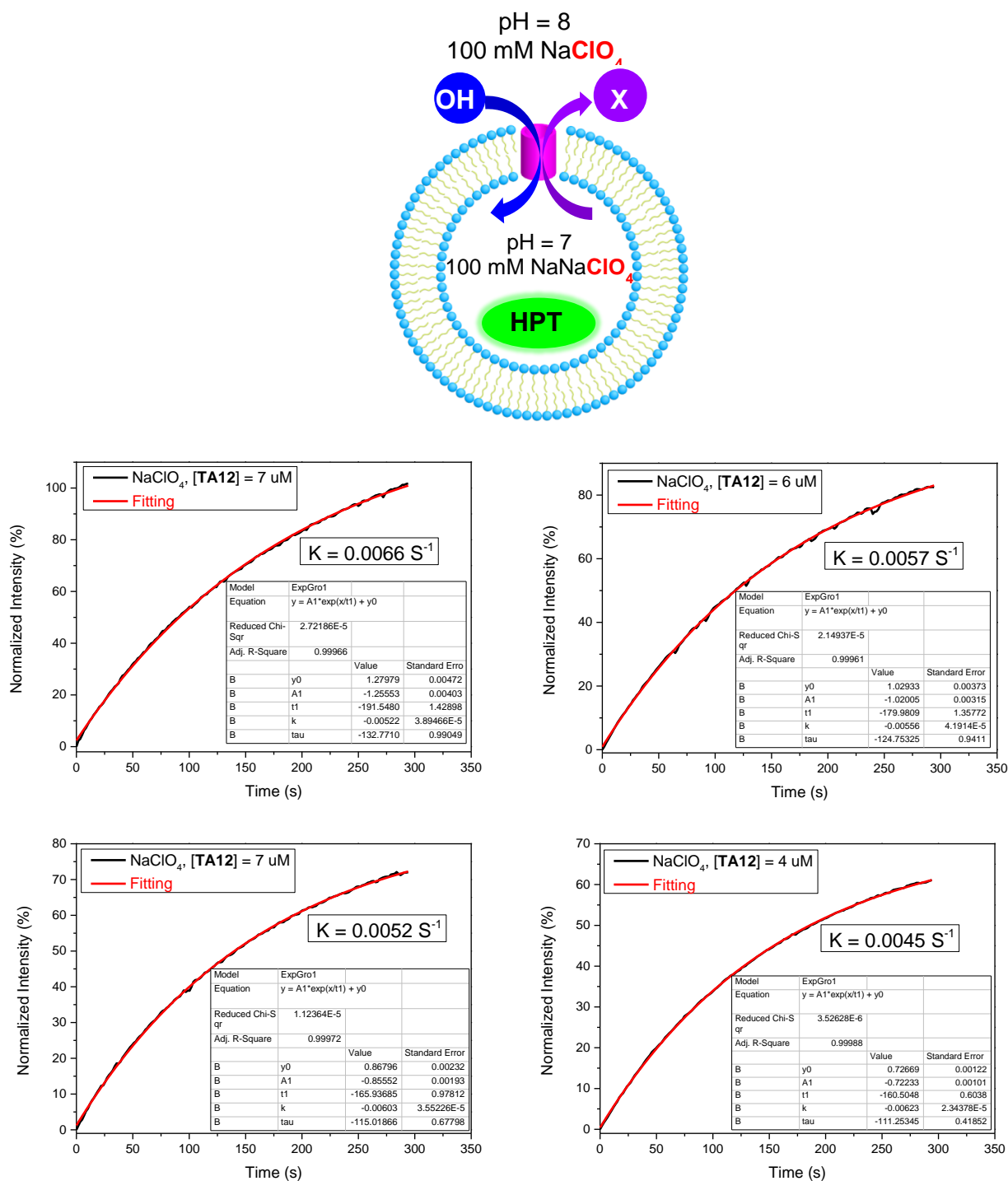


Figure S5. Fitted plots (red lines) to calculate initial rate constant (k_1) values of ClO_4^- transport by channel TA12 at various concentrations by the HPTS assay.

^1H NMR and ^{13}C NMR

