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Electronic Supporting Informations :

2,4,7-triphenylbenzimidazole : the monomeric unit of supramolecular helical rod-like transmembrane transporters

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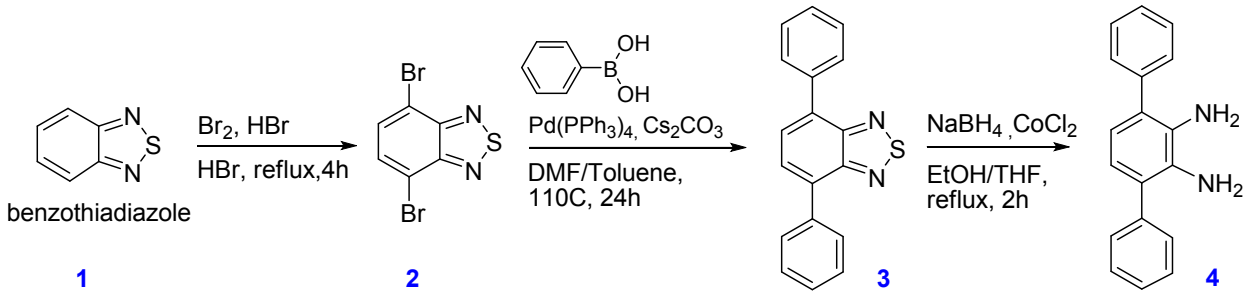
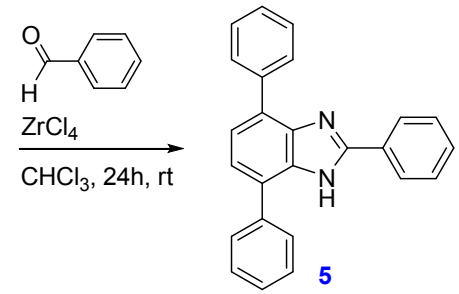
1. General information

All chemicals were purchased from Aldrich Chemicals in their highest purity and used without further purification. CD_3OD or $CDCl_3$ were also purchased from CDN Isotopes. NMR experiments were recorded on Bruker Advance 400 instruments. High-resolution mass spectra (HRMS) were recorded on a TSQ Quantum Ultra (thermo Scientific) triple quadrupole with accurate mass option (Université de Montréal Mass Spectrometry Facility). L-α-Phosphatidylcholine was purchased from Avanti Polar Lipids. Fluorimetric studies were performed on a Varian Cary Eclipse Fluorescence spectrophotometer equipped with a temperature controller.

2. Synthesis

The general procedure to the synthesis of the 2,4,7-triphenylbenzimidazolium family was previously reported by the Loeb. [1]





Compound **5** was prepared as previous reported and the characterization data was in accordance with the literature.[1]

1. N. Noujeim, K. Zhu, V. N. Vukotic and S. J. Loeb, *Org. Lett.*, 2012, 2484-2487.

3. Ion transport

3.1 Preparation of EYPC large unilamellar vesicles (LUVs) for lucigenin based assays

A phospholipid film was formed by evaporating 1 ml chloroform solution containing 50 mg of EYPC, under vacuum at 25°C during 2 hours. The lipid film was then hydrated with 1 mL of a 2 mM lucigenin solution diluted in $NaNO_3$ (100 mM) and phosphate buffer (10 mM, pH = 6.4). The obtained suspension was subjected to 8 freeze/thaw/vortex cycles (1 cycle = 1 minute at -78°C followed by 1 minute at 35°C and 1 minute in vortex). The solution was then extruded through a 100 nm polycarbonate membrane 21 times until the solution was transparent and passed down a Sephadex G-25 column to remove extravesicular lucigenin dye. The liposomes were eluted with a solution containing 100 mM of $NaNO_3$ and 10 mM of phosphate buffer. 6.4 mL of liposomes solution were isolated after separation. The stock solution was 10 mM in lipid, assuming all EYPC was incorporated into the liposomes.

3.2 Chloride transport assays with EYPC LUVs

A 50 µL aliquot of the solution of EYPC LUVs were added to a 2.5 mL gently stirred thermostated buffer solution containing100 mM $NaNO_3$ and 10 mM phosphate salt (pH = 6.4). 100µL of NaCl solution 4M was added to the cuvette. The lucigenin fluorescence was monitored by excitation at λ_{ex} = 372 nm and the emission was recorded at λ_{em} = 503 nm. At t = 50 s, 50µL of solution of transporter at different concentrations in MeOH were added. At t = 300 s, 100 µL of a Triton-X 4% solution were added to lyse all liposomes and obtained the minimum of lucigenin fluorescence. The temperature was set to 37°C.

Experiments were repeated in triplicate and all the reported traces are the average of the three independent trials.

3.3 Preparation of DPPC large unilamellar vesicles (LUVs) for lucigenin based assays

A phospholipid film was formed by evaporating under vacuum at 25°C during 2 hours, a solution of 50 mg of DPPC in 1 ml of chloroform. The lipid film was then hydrated with 1 mL of a 2 mM lucigenin solution diluted in $NaNO_3$ (100 mM) and phosphate buffer (10 mM, pH = 6.4). The obtained suspension was subjected to 8 freeze/thaw/vortex cycles (1 cycle = 1 minute at 0°C followed by 1 minute at 45°C and 1 minute in vortex). The solution was then extruded through a 100 nm polycarbonate membrane 21 times until the solution was transparent. If the extrusion is too hard, the extruder can be placed in the oven during few minutes to be under the phase transition of the lipids. Then, liposomes are passed down in a Sephadex G-25 column to remove extravesicular lucigenin dye. The eluant was a solution containing 100 mM of $NaNO_3$ and 10

mM of phosphate buffer. 5.9 mL of liposome solution were isolated after separation. The stock solution was diluted to obtain a concentration of10mM in lipid, assuming all DDPC was incorporated into the liposomes.

3.4 Chloride transport assays with DPPC LUVs

A 50 µL aliquot of the solution of DDPC LUVs were added to a 2.5 mL gently stirred thermostated buffer solution containing 100 mM $NaNO_3$ and 10 mM phosphate salt (pH = 6.4). 100µL of NaCl solution 4M was added to the cuvette. The lucigenin fluorescence was monitored by excitation at λ_{ex} = 372 nm and the emission was recorded at λ_{em} = 503 nm. At t = 50 s, 50µL of solution of transporter at different concentrations in MeOH were added. At t = 300 s, 100 µL of a Triton-X 4% solution were added to lyse all liposomes and obtained the minimum of lucigenin fluorescence. Four tests at different temperatures were performed to obtain chloride transport above and below the phase transition temperature of the DPPC (30°C, 35°C, 40°C, 45°C).

Experiments were repeated in triplicate and all the reported traces are the average of the three independent trials.

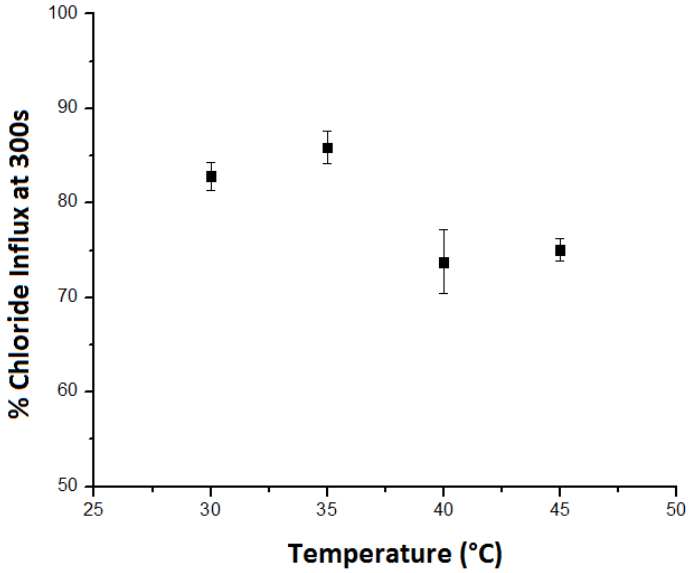


Figure 1: Comparison of chloride transport of 10 mol% of **2** in DPPC LUVs containing lucigenin**.**

3.5 Preparation of EYPC/Cholesterol 7/3 Large unilamellar vesicles (LUVs) for the lucigenin based assays

An EYPC/cholesterol film (7/3, w/w, 60.8mg) was formed by evaporating 1 ml chloroform solution under vacuum at 25°C during 2 hours. The lipid film was then hydrated with 1 mL of a 2 mM lucigenin solution diluted in $NaNO_3$ (100 mM) and phosphate buffer (10 mM, pH = 6.4). The obtained suspension was subjected to 8 freeze/thaw/vortex cycles (1 cycle = 1 minute at -78°C followed by 1 minute at 37°C and 1 minute in vortex). The solution was then extruded through a 100 nm polycarbonate membrane 21 times until the solution was transparent. Then, the liposomes were passed o a Sephadex G-25 column to remove theextravesicular lucigenin. Liposomes were eluted with was a solution containing 100 mM of $NaNO_3$and 10 mM of phosphate buffer. 5.4 mL of liposome solution were isolated after separation. The stock solution was diluted to obtain a concentration of 10 mM in lipid, assuming all EYPC and cholesterol were incorporated into the liposomes.

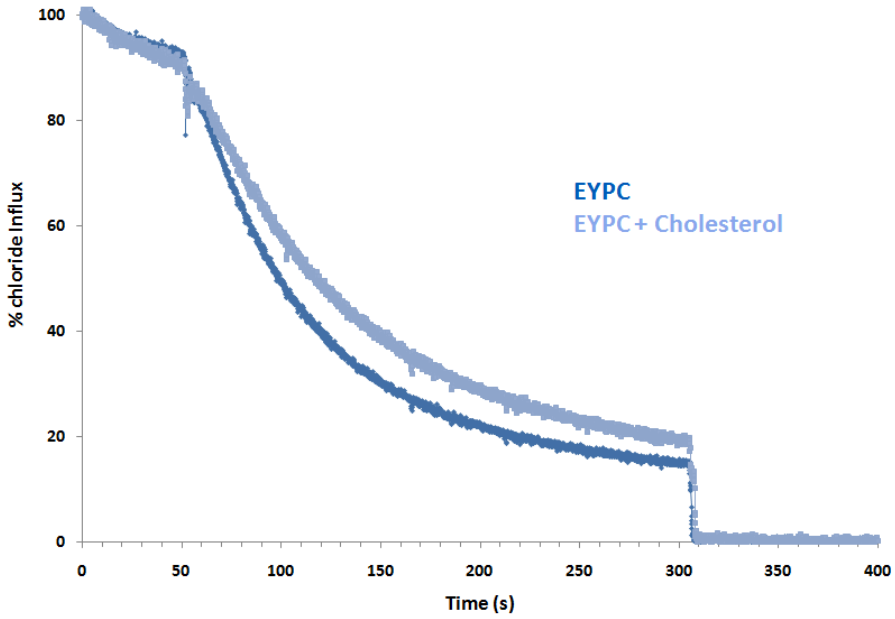


Figure 2: Comparison of chloride transport of 10 mol% of **2** in EYPC LUVs and in EYPC/cholesterol LUVs containing lucigenin**.**

3.6 Preparation of EYPC large unilamellar vesicles (LUVs) for the HPTS based assays

A phospholipid film was formed by evaporating under vacuum at 25°C during 2 hours a solution of 50 mg of EYPC in 1 ml of chloroform. The lipid film was then hydrated with 1 mL of a 2 mM lucigenin solution diluted in NaCl (100 mM) and phosphate buffer (10 mM, pH = 6.4). The obtained suspension was subjected to 8 freeze/thaw/vortex cycles (1 cycle = 1 minute at -78°C followed by 1 minute at 35°C and 1 minute in vortex). The solution was then extruded through a 100 nm polycarbonate membrane 21 times until the solution was transparent and separated on Sephadex G-25 column to remove the extravesicular lucigenin. The liposomes were eluted with a solution containing 100 mM of NaCl and 10 mM of phosphate buffer. 5.2 mL of liposome solution were isolated after separation. The stock solution was diluted with the buffer to obtain a concentration of 5mM in lipid, assuming all EYPC was incorporated into the liposomes.

3.7 HPTS assays with EYPC LUVs

A 100 µL aliquot of the previous solution of EYPC LUVs were added to a 2.5 mL gently stirred thermostated buffer solution containing100 mM NaX (X=NO_3^-, SO_4^{2-}, ClO_4^-) and 10 mM phosphate salt (pH = 6.4). The HPTS fluorescence was monitored by excitation at λ_{ex} = 403 nm and λex = 460 nm and the emission was recorded at λ_{em} = 510 nm. At t = 50 s, 50µL of a solution of transporter at different concentrations in MeOH were added. At t = 300 s, 100 µL of a solution 5%Triton-X were added to lyse all liposomes. The temperature was set to 37°C.

Experiments were repeated in triplicate and all traces reported are the average of the three trials.

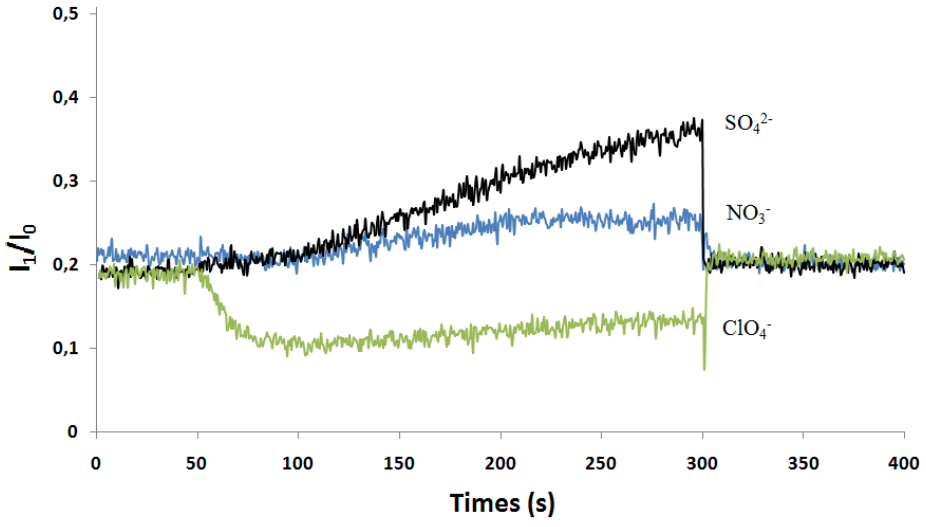


Figure 3: Comparison of chloride transport of 10 mol% of **2** in HPTS LUVs containing lucigenin, in different external phosphate buffer.

3.8 Chloride transport assays with EYPC LUVs in different phosphate buffers

A 50 µL aliquot of the solution of EYPC LUVs (10mM) were added to a 2,5 mL gently stirred thermostated buffer solution containing100 mM XNO_3 (X= Na^+, NH_4^+, NMe_4^+) and 10 mM phosphate salt (pH = 6.4). 100µL of NaCl solution 4M was added to the cuvette. The lucigenin fluorescence was monitored by excitation at λex = 372 nm and the emission was recorded at λem = 503 nm. At t = 50 s, 50µL of solution of transporter at different concentrations in MeOH were added. At t = 300 s, 100 µL of a Triton-X 5% solution were added to lyse all liposomes and obtained the minimum of lucigenin fluorescence. The temperature was set to 37°C.

Experiments were repeated in triplicate and all traces reported are the average of the three trials.

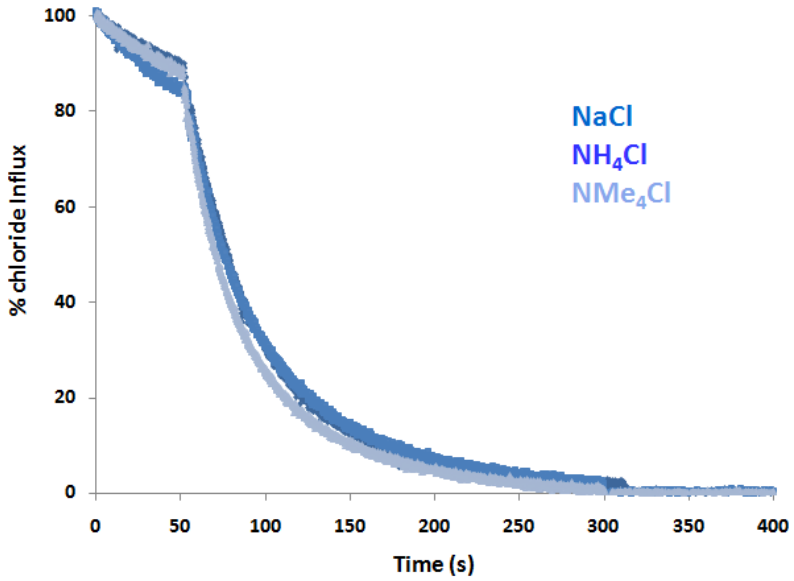


Figure 4: Comparison of chloride transport of 10 mol% solution of **2** in EYPC LUVs containing lucigenin, in different external phosphate buffers

4. Conversion of fluorescence data into % of chloride Influx:

The residual fluorescence of the transporter was subtracted from each fluorescence curve obtained for the transport assays and the curves were normalized using the following equation:

$$\% Cl_{influx} = 100 * \frac{I - I_{mini}}{I_0 - I_{mini}}$$

where I = fluorescence intensity

I_0 = fluorescence intensity before the addition of the transporter

I_{mini} = fluorescence intensity at the end of the experiment.

5. Dynamic Light Scattering (DLS)

500 µL of a phosphate buffer ($NaNO_3$, 100mM and 10mM phosphate buffer), 10 µL of EYPC LUVs (without fluorescent dye) and 10 µL of studied molecules were placed in a plastic cuvette. After shaken the sample by hand for 10 s, DLS measurements were taken.
The concentration of each molecule was given compare to lipid concentration.

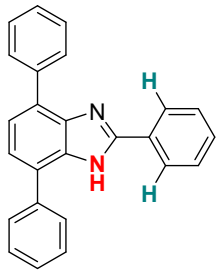
Intensity data from each sample were collected in three replicates.

Experiments	Liposomes	Liposomes + MeOH	Liposomes + triton X	**2** (30 mol%)
Diameter (nm)	116.0 ± 0.1	120.3 ± 0.1	8.9 ± 0.6	134.6 ± 0.2

6. Kinetic studies in EYPC liposomes

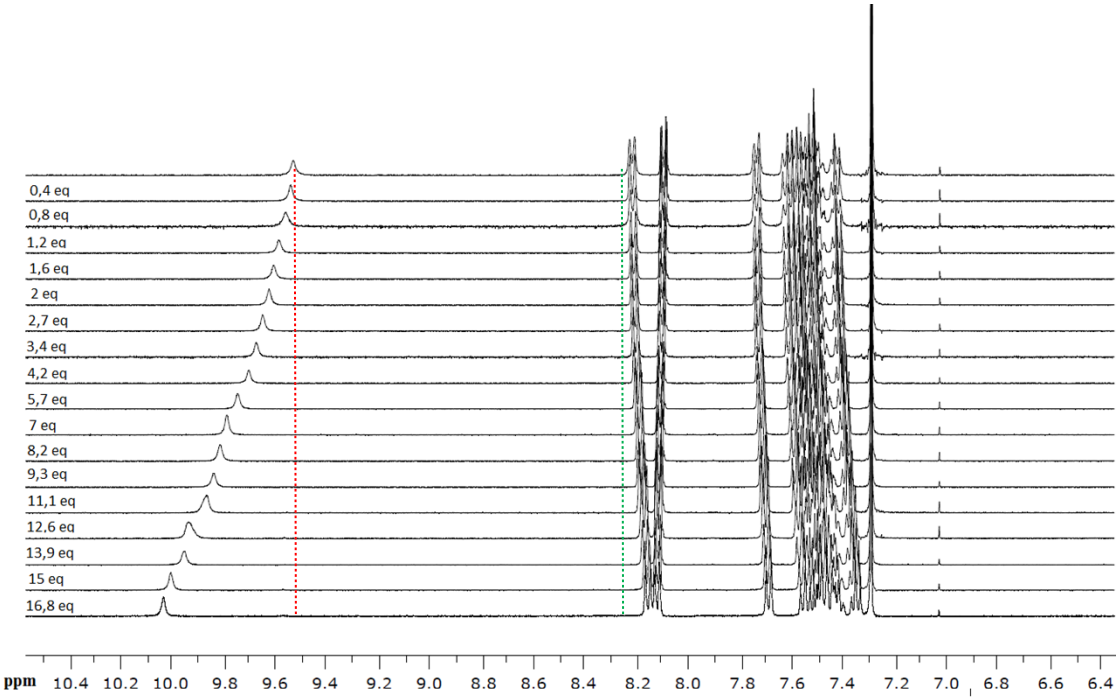
A 50 µL aliquot of the solution of EYPC LUVs (10mM) were added to a 2.5 mL buffer solution containing 100 mM $NaNO_3$ and 10 mM phosphate salt (pH = 6.4). 100µL of NaCl solution 4 M was added to the cuvette. 50 µL of solution of transporter 10 mol % in MeOH, relative to liposome concentration, were added. The fluorescence was monitored by excitation at λex = 372 nm and the emission was observed between λem = 400 nm and λem = 800 nm. Fluorescence spectra were taken at different time. The temperature was set to 37°C.

7. ^{1}H NMR titrations of 2 with (a) TBACl and (b) $TBAClO_4$

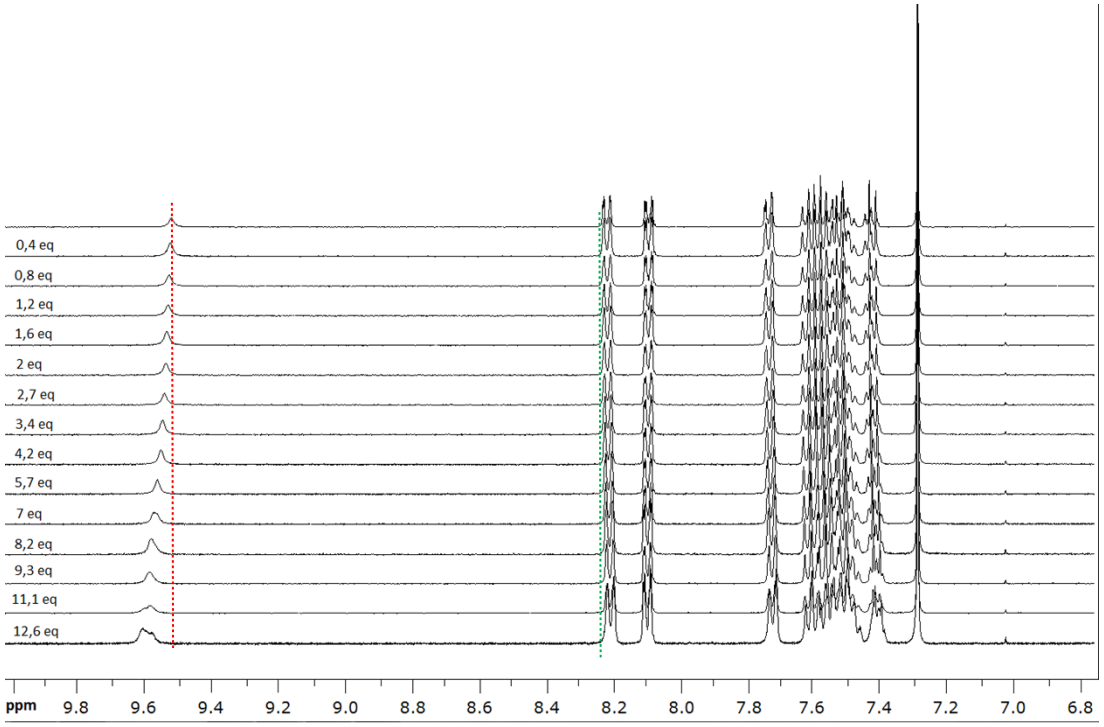


NMR titrations showed a downfield shift of the secondary amine NH proton of the benzimidazole moeity in the presence of TBACl (a) and $TBAClO_4$ (b). The lower chemical scift (0.0862 ppm) obtained for the NH proton in the presence perchlorate can be explained by the size of the anion which prevents its proximity to the benzimidazole moeity.

a)



b)



8. Molecular modeling

Calculations were performed on a Windows® XP workstation with HyperChem 7.5 software. The initial configuration of **2** was obtained from PM3 semi-empirical calculations. A model of the EYPC bilayer was constructed using 200 molecules of phosphatidylcholine, with different conformations and 15 Å layers of water molecules on each side, after a 500 ps molecular dynamics (MD) simulation at 1000 K. To investigate the self assembly of 2, 4 monomers of 2 were positioned inside the EYPC bilayer and 200 ps MD simulation with periodic boundary conditions at 300 K was performed. The cutoff for non-bonded interactions was taken to be 12 Å throughout all simulations. At the beginning, we carried out high temperature annealed MD simulations starting at 1000 K (2 ps) annealing to 0 K (10 ps). Heating to 1000 K was necessary to enable the molecules to overcome energy barriers between different conformations and to prevent the system from getting stuck in a particular region of the conformational space. The simulations in aqueous solution were relaxed using the steepest descent method until a gradient difference of 0.01 kcal/mol was reached. After energy minimization of the system at 0 K, the MD simulation was initialized using a time step of 1 fs for 200 ps. The temperature was kept constant at 300 K yielding a canonical ensemble (NVT).