Synthetic Pores with Sticky π-Clamps

Hiroyuki Tanaka, Guillaume Bollot, Jiri Mareda, Svetlana Litvinchuk, Duy-Hien Tran, Naomi Sakai and Stefan Matile

1Department of Organic Chemistry, University of Geneva, Geneva, Switzerland.

stefan.matile@chiorg.unige.ch

Supporting Online Material

1. Materials and Methods. As in (S1), (S2) or (S3), Supporting Information. CF was from Fluka/Aldrich, EYPC from Avanti Polar Lipids, ANTS/DPX from Molecular Probes, all buffers, HEPES, MES, TES, Tris and inorganic salts were of the best grade available from Sigma. Triton X-100 and nucleotides were from Sigma. Large unimellar vesicles (LUVs) were prepared by the Mini-Extruder with polycarbonate membrane, pore size 100 nm, from Avanti Polar Lipids. Fluorescence spectra were recorded on either a Fluoromax 2 or Fluoromax 3 from Jobin Yvon-Spex equipped with an injector port, a magnetic stirrer and a temperature controller (25 °C). Planar bilayer conductance was measured on a bilayer apparatus (BCH-13, Warner) in a vibration isolated Faraday cage (house-made) with a bilayer clamp amplifier (BC-525c, Warner), low-pass filtered with a 8-pole Bessel filter (LPF-8, Warner), converted (DigiData 1200, Axon) and analyzed by computer (pClamp 8.0, Axon and QuB Software Suite, State University of New York).
**Abbreviations.** ADP: Adenosine 5′-diphosphate; AMP: Adenosine 5′-monophosphate; AMPSO: 1-[(4-Amino-2-propyl-5-pyrimidinyl)methyl]-2-methylpyridinium chloride; ANTS: 8-aminonaphtalene-1,3,6-trisulfonate; ATP: Adenosine 5′-triphosphate; CDP: Cytidine 5′-diphosphate; CF, 5(6)-carboxyfluorescein; DAN: 1,5-Dialkoxynaphthalene; DMF: N,N-Dimethylformamide; DMSO: Dimethylsulfoxide; DPX: p-xylenebis(pyridinium)bromide; EYPC LUVs: Egg yolk phosphatidylcholine large unilamellar vesicles; GDP: Guanosine 5′-diphosphate; Gla, G: Glycolate, -OCH2CO- (hydroxyglycine); GMP: Guanosine 5′-monophosphate; GTP: Guanosine 5′-triphosphate; HEPES: N-(2-hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid); His, H: L-Histidine; Leu, L: L-Leucine; Lys, K: L-Lysine; MES: 2-Morpholinoethanesulfonic acid monohydrate; NDI: 1,4,5,8-Naphthalenediimide; Ndi, A: Artificial amino acid in pore 2; TES: N-[Tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid; Tris: Tris(hydroxymethyl)aminomethane; UDP: Uridine 5′-diphosphate.

2. Supporting Text

2.1. Syntheses

1\(^{3,4,5,6,7,8}\)-Octakis(Gla-Leu-Lys-Leu-His-Leu-NH\(_2\))-p-Octiphenyl 1.\(^{m}\)

Monomer 1\(^{m}\) for self-assembly into pore 1 was prepared in 19 steps following previously reported procedures (S1).

1\(^{3,4,5,6,7,8}\)-Octakis(Gla-Leu-Ndi-Leu-Lys-Leu-NH\(_2\))-p-Octiphenyl 4. Monomer 4 for self-assembly into pore 2 was prepared in 24 steps as outlined in Scheme 1 following previously reported procedures (S2).
DAN Hydrazide 5. Hydrazide 5 was prepared in 2 steps as outlined in Scheme 2 following previously reported procedures (S2).

NDI Hydrazide 16. Hydrazide 16 was prepared in 4 steps as outlined in Scheme 2 following previously reported procedures (S2).

2.2 Vesicle Preparation

2.2.1. EYPC-LUVs ⊃ ANTS/DPX. As in ref (S1). Solutions of EYPC (25 mg) in CHCl₃/MeOH 1:1 (1 ml) were dried under a stream of nitrogen and then under vacuum (>2 h) to form thin films. The resulting films were hydrated with 1 ml buffer (12.5 mM ANTS, 45.0 mM DPX, 5 mM TES, 20 mM NaCl, pH 7.0) for more than 30 min, subjected to freeze-thaw cycles (5x) and extrusions (15x, Mini-Extruder with a polycarbonate membrane, pore size 100 nm). Extravesicular ANTS and DPX were removed by gel filtration (Sephadex G-50) with 10 mM TES, 100 mM NaCl, pH 7.0. The LUV fractions were combined and diluted to 6 ml with the corresponding buffer. Lipid concentrations were estimated from the amount of entrapped dye; the estimated values were consistent with earlier results from phosphate analysis. The final stock solutions had the following characteristics: ~2.5 mM EYPC; inside: 12.5 mM ANTS, 45.0 mM DPX, 5 mM TES, 20 mM NaCl, pH 7.0; outside: 10 mM TES, 100 mM NaCl, pH 7.0.
2.2.2. EYPC-LUVs\(\supset CF\). As in refs (S1) or (S4). Solutions of EYPC (25 mg) in CHCl\(_3\)/MeOH 1:1 (1 ml) were dried under a stream of nitrogen and then under vacuum (>2 h) to form thin films. The resulting films were hydrated with 1 ml

a) “low-ionic-strength” buffer “+” (50 mM CF, 10 mM HEPES, 10 mM NaCl, pH 7.4) for (+)-EYPC-LUVs\(\supset CF\), Fig. 6, ●),

b) “intermediate-ionic-strength” buffer “++” (50 mM CF, 10 mM HEPES, 100 mM NaCl, pH 7.4) for (++)-EYPC-LUVs\(\supset CF\), Fig. 6, ○) or

c) “high-ionic-strength” buffer “+++” (50 mM CF, 10 mM HEPES, 200 mM NaCl, pH 7.4) for (+++)-EYPC-LUVs\(\supset CF\), Fig. 6, □)

for more than 30 min, subjected to freeze-thaw cycles (5x) and extrusions (15x, Mini-Extruder with a polycarbonate membrane, pore size 100 nm). Extravesicular CF was removed by gel filtration (Sephadex G-50) with

a) buffer “+” (107 mM NaCl, 10 mM HEPES, pH 7.4) for (+)-EYPC-LUVs\(\supset CF\), Fig. 6, ●)

b) buffer “++” (200 mM NaCl, 10 mM HEPES, pH 7.4) for (++)-EYPC-LUVs\(\supset CF\), Fig. 6, ○), or

c) buffer “++” (300 mM NaCl, 10 mM HEPES, pH 7.4) for (+++)-EYPC-LUVs\(\supset CF\), Fig. 6, □).

The LUV fractions were combined and diluted to 6 ml with the corresponding buffer. Lipid concentrations were estimated from the amount of entrapped dye; the estimated values were consistent with earlier results from phosphate analysis. The final stock solutions had the following characteristics:

a) (+)-EYPC-LUVs\(\supset CF\), Fig. 6, ●: ~2.5 mM EYPC; inside, 50 mM CF, 10 mM NaCl, 10 mM HEPES, pH 7.4; outside, 107 mM NaCl, 10 mM HEPES, pH 7.4,
b) (++)-EYPC-LUVs\(\supset\)CF, Fig. 6, ○: ~2.5 mM EYPC; inside, 50 mM CF, 100 mM NaCl, 10 mM HEPES, pH 7.4; outside, 200 mM NaCl, 10 mM HEPES, pH 7.4,

c) (+++)-EYPC-LUVs\(\supset\)CF, Fig. 6, □: ~2.5 mM EYPC; inside, 50 mM CF, 200 mM NaCl, 10 mM HEPES, pH 7.4; outside, 300 mM NaCl, 10 mM HEPES, pH 7.4.

2.3 Pore Activity, pH Profile (ANTS/DPX assay)

EYPC-LUVs \(\supset\) ANTS/DPX (100 µl) were added to gently stirred, thermostated buffer (1.90 ml, 100 mM NaCl, 10 mM buffer: MES (pH = 4.5 ~ 6.5), HEPES (pH = 6.0 ~ 8.0), or AMPSO (pH = 8.0 ~ 10.0)) in a fluorescence cuvette. Fluorescence emission intensity \(I_t\) (\(\lambda_{\text{em}} = 520\) nm, \(\lambda_{\text{ex}} = 353\) nm) was monitored as a function of time (\(t\)) during addition of 20 µl of 4 (200 nM final concentration) and, after 5 min, 40 µl 1.2% aq triton X-100. Fluorescence kinetics were normalized to fractional intensity \(Y_t\) applying equation [S1]

\[
Y_t = \left[\frac{(I_t - I_0)}{(I_\infty - I_0)}\right] / \left[\frac{(I_t^{\text{MAX}} - I_0)}{(I_\infty - I_0)}\right] \tag{S1},
\]

where \(I_0 = I_t\) at pore addition, \(I_\infty = I_t\) at saturation after lysis, and \(I_t^{\text{MAX}} = I_t\) at maximal emission intensity before lysis. From the obtained curves, \(Y_{\text{MAX}} = Y_t\) at maximal fractional emission intensity before lysis was obtained for each measurement and converted into fractional pore activity \(Y\) applying equation [S2]

\[
Y = \left[\frac{(Y_{\text{MAX}} - Y_{\text{MAX}(0)})}{(Y_{\text{MAX}(\infty)} - Y_{\text{MAX}(0)})}\right] \tag{S2},
\]
where $Y_{\text{MAX}(0)}$ is $Y_{\text{MAX}}$ obtained under the conditions giving rise to lowest pore activity and $Y_{\text{MAX}(\infty)}$ is $Y_{\text{MAX}}$ of the highest activity ($= 1$). The obtained fractional pore activities $Y$ were plotted as a function of pH (Fig. 5).

2.4. Pore Activity, Hill Plot (CF assay)

$(\pm)$-, $(++)$-, or $(+++)$-EYPC-LUVs$\supset$CF (100 µl from above stock solutions) were added to gently stirred, thermostated buffer (1.90 ml) in a fluorescence cuvette [pH 6.5, 107 $(\pm)$, 200 $(++)$ or 300 mM NaCl $(+++)$, 10 mM HEPES]. Fluorescence emission intensity $F_t$ ($\lambda_{\text{ex}}$ 492 nm, $\lambda_{\text{em}}$ 517 nm) was monitored as a function of time during addition of monomer 4 (20 µl of 0 - 250 µM stock solution, compare Fig. 6) and 40 µl 1.2% aq triton X-100 for final calibration. Fractional pore activity $Y$ was determined as described in 2.3, plotted as a function of monomer concentration $c_M$ and fitted to the Hill equation [S3] applied to self-assembly

$$y = y_\infty + (y_0 - y_\infty) / \{1 + (c_M / EC_{50})^n\}$$  \hspace{1cm} \text{[S3]},

where $y_0$ is $y$ without pore, $y_\infty$ is $y$ with excess pore, $EC_{50}$ the concentration for 50% pore activity and $n$ the Hill coefficient.

2.5. Pore Blockage

$(\pm)$- or $(++)$-EYPC-LUVs$\supset$CF (100 µl from above stock solutions) were added to gently stirred, thermostated buffer (1.90 ml) in a fluorescence cuvette [pH 6.5, 107 $(\pm)$ or 200 mM
NaCl (++, 10 mM HEPES). Fluorescence emission intensity $F_t$ ($\lambda_{ex}$ 492 nm, $\lambda_{em}$ 517 nm) was monitored as a function of time during addition of blockers (nucleotides etc, see Fig. 9, Tab. 1 and Tab. 2; 20 $\mu$l of concentrated stock solutions, for final concentrations, see Fig. 9, Tab. 1 and Tab. 2), pore 1 or 2 (20 $\mu$l stock solution, usually 200 nM final monomer concentration) and 40 $\mu$l 1.2% aq triton X-100 for final calibration. Fractional pore activity $Y$ was determined as described in 2.3, plotted as a function of blocker concentration $c_{\text{BLOCKER}}$ and fitted to the Hill equation [S4] applied to self-assembly

$$Y = Y_0 + (Y_\infty - Y_0) / \{1 + (c_{\text{BLOCKER}} / IC_{50})^n\}$$  \[S4],

where $Y_0$ is $Y$ without blocker, $Y_\infty$ is $Y$ with excess blocker, $IC_{50}$ the concentration for 50% pore blockage and $n$ the Hill coefficient.

2.6. Planar Bilayer Conductance

General procedures have been described in (S1) and (S3). In brief, $n$-decane containing EYPC (33 mg/ml) and monomer 4 (0.08 mol%) was painted on an orifice (d = 150 $\mu$m) separating the two chambers of a planar bilayer cell. Measurements and analyses conditions are following. Symmetrical 2.0 M KCl; agar bridge with 2 M KCl; Ag / AgCl electrodes; holding potential as indicated; Bessel filter: 1 kHz; sampled at 10 kHz; 25 ± 1 °C.

3. Supporting References

