Synthetic Pores with Sticky π-Clamps

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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 ^{o}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(pyridinum)bromide; EYPC LUVs: Egg yolk phosphatidylcholine large unilamellar vesicles; GDP: Guanosine 5′-diphosphate; *Gla*, *G*: Glycolate, -OCH_2CO- (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, π_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

$1^3,2^3,3^2,4^3,5^2,6^3,7^2,8^3$-Octakis(*Gla*-Leu-Lys-Leu-His-Leu-NH_2)-*p*-Octiphenyl 1^m. Monomer $\mathbf{1}^m$ for self-assembly into pore **1** was prepared in 19 steps following previously reported procedures (*S1*).

$1^3,2^3,3^2,4^3,5^2,6^3,7^2,8^3$-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 $CHCl_3$/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 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⊃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⊃CF**, Fig. 6, ●),
b) "intermediate-ionic-strength" buffer "+" (50 mM CF, 10 mM HEPES, 100 mM NaCl, pH 7.4) for **(++)-EYPC-LUVs⊃CF**, Fig. 6, ○) or
c) "high-ionic-strength" buffer "+" (50 mM CF, 10 mM HEPES, 200 mM NaCl, pH 7.4) for **(+++)-EYPC-LUVs⊃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⊃CF**, Fig. 6, ●)
b) buffer "++" (200 mM NaCl, 10 mM HEPES, pH 7.4) for **(++)-EYPC-LUVs⊃CF**, Fig. 6, ○), or
c) buffer "+++" (300 mM NaCl, 10 mM HEPES, pH 7.4) for **(+++)-EYPC-LUVs⊃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⊃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⊃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⊃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 ⊃ 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 (λ_{em} = 520 nm, λ_{ex} = 353 nm) was monitored as a function of time (t) during addition of 20 μl **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]

$$Y_t = [(I_t - I_0) / (I_\infty - I_0)] / [(I_t^{MAX} - I_0) / (I_\infty - I_0)] \qquad [S1],$$

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

$$Y = [(Y_{MAX} - Y_{MAX(0)}) / (Y_{MAX(\infty)} - Y_{MAX(0)})] \qquad [S2],$$

where $Y_{MAX(0)}$ is Y_{MAX} obtained under the conditions giving rise to lowest pore activity and $Y_{MAX(\infty)}$ is Y_{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)

(+)-, (++)-, or (+++)-EYPC-LUVs⊃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 (+), 200 (++) or 300 mM NaCl (+++), 10 mM HEPES]. Fluorescence emission intensity F_t (λ_{ex} 492 nm, λ_{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

$$Y = Y_\infty + (Y_0 - Y_\infty) / \{1 + (c_M / EC_{50})^n\} \qquad [S3],$$

where Y_0 is Y without pore, Y_∞ is Y with excess pore, EC_{50} the concentration for 50% pore activity and n the Hill coefficient.

2.5. Pore Blockage

(+)- or (++)-EYPC-LUVs⊃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 (+) or 200 mM

NaCl (++), 10 mM HEPES]. Fluorescence emission intensity F_t (λ_{ex} 492 nm, λ_{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 µl of concentrated stock solutions, for final concentrations, see Fig. 9, Tab. 1 and Tab. 2), pore **1** or **2** (20 µl stock solution, usually 200 nM final monomer concentration) 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 blocker concentration $c_{BLOCKER}$ and fitted to the Hill equation [S4] applied to self-assembly

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

where Y_0 is Y without blocker, Y_∞ 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 µm) 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

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