Supporting information

Homogeneous Nanodiscs of Native Membranes Generated by Alternating Stilbene Maleic Acid Copolymer Derivatives

Experimental Section:

1. *Materials.*

SMA2000 (anhydride form) was purchased from Cray Valley (USA). (*E*)-Stilbene (EMS-I and Sigma-Aldrich, 96%), maleic anhydride (MA, Sigma-Aldrich, 99.0%), 2-methylbenzyl chloride (Sigma-Aldrich, 99%), 4-methylbenzyl chloride (Sigma-Aldrich, 98%), *o*-tolualdehyde (Sigma-Aldrich, 97%), benzaldehyde (Sigma-Aldrich, 99%), potassium *tert*-butoxide solution 1.0 M in tetrahydrofuran (KOtBu, Sigma-Aldrich), triethylphosphite (Sigma-Aldrich, 98%), 2,2'-azobis(2-methylpropionitrile) (AIBN, Sigma-Aldrich, 98%), and dicumyl peroxide (Sigma-Aldrich, 98%) were purchased and used as received, unless noted otherwise. Tetrahydrofuran (THF, Fisher, HPLC grade), hexanes (Fisher, HPLC grade), methylene chloride (Fisher, HPLC grade), and chloroform (Fisher, HPLC grade) were used as received. Water was deionized before use. As needed, MA was recrystallized from chloroform. Copolymers **(1)** and **(5)** were prepared as described, respectively.[1],[2] Apparent coupling constants, J_{app}, are reported. Copolymers were dissolved in THF and analyzed by size-exclusion chromatography to obtain M_n and PDI.

2. Synthesis of (E)-methylstilbenes

(*E*)-2,2′-dimethylstilbene. Diethyl (2-methylbenzyl)phosphonate (20.25 g, 83.59 mmol), 2-methylbenzaldehyde (10.18 g, 84.74 mmol) and dry THF (35 mL) were added to a 250-mL round bottom flask with a stir bar and an addition funnel sealed with a septum. The apparatus was flushed with N_2 and chilled in an ice bath for 30 min. Potassium *tert*-butoxide (~1.0 M in THF, 100 mL, ~100 mmol) was added dropwise over 1 h. During the addition, the reaction mixture changed from a pale-yellow liquid to a yellow slurry. The ice bath was removed. The reaction mixture slowly warmed to room temperature while stirring. After 24 h, the orange gelatinous liquid was poured into deionized water (800 mL). A brown liquid layer appeared above the liquid. The solution was stirred with a spatula for ~5 min and allowed to sit at room temperature for 1 h. A precipitate

formed, which was collected by filtration, washed with MeOH and dried *in vacuo* overnight to yield a white solid (11.79 g, 68%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 7.61 (t, 2H), 7.22 (m, 8H), 2.45 (s, 3H), 2.44 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm: 136.8, 135.83, 130.39, 130.38, 128.7, 128.01, 128.00, 127.54, 127.52, 126.6, 126.20, 126.18, 125.56, 125.54, 19.97, 19.96. IR: 968, cm^{-1}. mp: 82.0–82.4 °C; Lit. mp: 82–84 °C.[3]

Synthesis of (*E*)-4-methylstilbene. Diethyl benzyl phosphonate (17.62 g, 77.21 mmol) and 4-methylbenzaldehyde (9.28 g, 77.2 mmol) and dry THF (31 mL) were added to a 250-mL round bottom flask with a stir bar and an addition funnel sealed with a septum. The apparatus was flushed with N_2 and chilled in an ice bath for 40 min. Potassium *tert*-butoxide (~1.0 M in THF, 93 mL, ~93 mmol) was added to the pale-yellow solution dropwise over 1.5 h. During the addition, the reaction mixture changed from a light-yellow liquid to an orange slurry. The ice bath was removed. The slurry slowly warmed to room temperature while stirring. After 25 h, the orange slurry was poured into MeOH (200 mL). Deionized water (400 mL) was added to the solution, resulting in a white precipitate (~5 g), which was filtered. Adding CH_2Cl_2 (~200 mL) to the filtrate gave two layers. After separation, hexanes (~100 mL) was added to the yellow organic layer; no change occurred other than dilution. Then aq saturated NH_4Cl (400 mL) was added to this organic layer resulting in a suspension of white solid, which was filtered and washed with MeOH. The solids were combined, dissolved in CH_2Cl_2 (~15 mL), and placed in a dry ice–acetone bath. A solid precipitated from the solution, collected by filtration, washed with MeOH, and dried *in vacuo* overnight to yield a white solid (11.02 g, 73%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 7.49 (dd, 2H), 7.41 (d, 2H), 7.34 (t, 2H), 7.23 (tt, 1H), 7.16 (d, 2H), 7.09 (AB, 1H, J_{app} = 16 Hz), 7.05 (AB, 1H, J_{app} = 16 Hz), 2.35 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm: 137.49, 137.47, 137.5, 129.4, 128.62, 128.58, 127.7, 127.4, 126.39, 126.35, 21.2. mp: 118.5–119.0 °C; Lit. mp: 118–120 °C.[4]

Synthesis of (*E*)-2-methylstilbene. Diethyl benzylphosphonate (13.74 g, 60.21 mmol), 2-methylbenzaldehyde (7.23 g, 60.2 mmol), and dry THF (17 mL) were added to a 250-mL round bottom flask equipped with a stir bar and an addition funnel sealed with a septum. The apparatus was flushed with N_2 and chilled in an ice bath for 40 min. Potassium *tert*-butoxide (~1.0 M in

THF, 72 mL, ~72 mmol) was added to the pale-yellow solution dropwise over 1 h. During the addition, the reaction mixture changed from a pale-yellow liquid to a yellow slurry. After the addition, the ice bath was removed. The reaction mixture slowly warmed to room temperature while stirring. After 60 h, the reaction mixture was an orange gelatinous liquid. This liquid was poured into aq saturated NH_4Cl (600 mL); an oil formed above the aqueous layer. Adding CH_2Cl_2 (150 mL) gave two layers, which were separated. The aqueous layer was extracted with CH_2Cl_2 (1 × 150 mL, 1 × 100 mL). The organic layers were combined and washed with deionized water (300 mL), resulting in a cloudy emulsion. Aq saturated NH_4Cl (~50 mL) was added, resulting in clear organic and aqueous layers. The organic layer was separated and concentrated by rotary evaporation, resulting in an orange oil. The oil was dissolved in hexanes (~5 mL). With a pipette, the resulting solution was carefully spread on top of a column (8 × 4 cm) of wet-packed (hexanes) silica gel. Hexanes (200 mL) were pushed through the column with modest air pressure. Concentration of the hexanes solution yielded a clear oil. MeOH (~10 mL) was added to the oil, resulting in formation of a white solid. The mixture was placed in a freezer overnight. The liquid above the solid was decanted, and the solid was dried under vacuum overnight. (8.02 g, 68 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 7.59 (d, 1H), 7.50 (d, 2H), 7.28 (m, 7H), 7.00 (d, 1H), 2.43 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ ppm: 137.7, 136.4, 135.8, 130.4, 130.0, 128.7, 127.57, 127.53, 126.54, 126.52, 126.2, 125.3, 19.9. IR: 974 cm^{-1}. mp: 32.4–33.2 °C. Lit. mp: 30 °C.[5]

3. Polymer Synthesis

Free-radical Copolymerization of (*E*)-4-Methylstilbene and Maleic Anhydride.

Following the procedure,[6] (*E*)-4-methylstilbene (0.99 g, 5.1 mmol), recrystallized MA (0.50 g, 5.1 mmol), and AIBN (0.015 g, 1 wt%) were added to a 50 mL round bottom flask equipped with a stir bar and sealed with a septum. THF (7 mL) was added to the flask via syringe. The yellow reaction mixture was sparged with argon for 10 min and stirred for 16 h at 60 °C. The reaction solution was then precipitated into hexanes by slow addition via pipette. The white precipitate was re-dissolved in THF and then precipitated into hexanes. The white solid was collected by filtration and placed under vacuum for 16 h at 60 °C. (1.45 g, 73.1%, M_n = 5.4 kDa, PDI = 1.54)

Free-radical Copolymerization of(*E*)-2,2'-Dimethylstilbene and Maleic Anhydride.

Following the procedure,[6] (*E*)-2,2-dimethylstilbene (1.03 g, 5.0 mmol), MA (0.5 g, 5 mmol), and dicumyl peroxide (0.015 g, 1 wt%) were added to a 50 mL round bottom flask equipped with a stir bar and sealed with a septum. Anhydrous chlorobenzene (10 mL) was added to the flask via syringe. The reaction mixture was sparged with argon for 10 min and stirred for 24 h at 110 °C. The reaction solution was then precipitated into hexanes by slow addition via pipette. The precipitate was re-dissolved in THF and then precipitated into hexanes. The white solid was collected by filtration and placed under vacuum for 24 h at 60 °C. (0.74 g, 49%, M_n = 5.1 kDa, PDI = 1.52)

Free-radical Copolymerization of (*E*)-2-Methylstilbene and Maleic anhydride.

Following the procedure,[6] (*E*)-2-methylstilbene (0.99 g, 5.0 mmol), MA (0.5 g, 5 mmol), and dicumyl peroxide (0.015 g, 1 wt%) were added to a 50 mL round bottom flask equipped with a stir bar and sealed with a septum. Anhydrous chlorobenzene (10 mL) was added to the flask via syringe. The reaction mixture was sparged with argon for 10 min and stirred for 24 h at 110 °C.

The reaction solution was then precipitated into hexanes by slow addition via pipette. The precipitate was re-dissolved in THF and then precipitated into hexanes. The white solid was collected by filtration and placed under vacuum for 24 h at 60 °C. (0.56 g, 38%, M_n = 4.4 kDa, PDI = 1.19)

4. ***Activation of poly(stilbene-alt-maleic anhydride)s, STMAs.***

SMA2000 (anhydride form) and all STMA copolymers were hydrolyzed (in NaOH) and activated to their maleic acid forms as described.[7] Prior to lyophilization, each sample was adjusted to pH 8. Dried copolymers were stored at room temperature. The complete conversion of the anhydride into diacid was confirmed by FT-IR.

5. ***Membrane Isolation and preparation of native PagP in STMA nanodiscs***

A His_6-PagP construct in a pET21b vector was overexpressed in the outer membrane of *E.coli* BL21(DE3) *pLysS*. The crude membrane and outer membrane were isolated by high-speed centrifugations as described.[8] Briefly, the crude membrane was used for solubilization assays; the outer membrane was further used for the preparation of STMA nanodiscs containing PagP.[7]

The concentrations of hydrolyzed STMA copolymers were 0.5% w/v; that of hydrolyzed SMA2000 was 1% w/v. Copolymers (**3**) and (**4**) were incubated with outer membrane suspension (10 mL, ~80 mg/mL, in Tris 10 mM, 100 mM NaCl, 5% v/v glycerol pH 8) for 30 min at 37 °C followed by overnight incubation at cold room. The soluble fraction was next used for His-affinity batch purification by using Ni-NTA HisPur resins. After washing the column with 10- and 30-mM imidazole (in 10 mM Tris and 100 mM NaCl), the protein was eluted with 250 mM imidazole. Fractions of each step of purification were collected; the boiled samples in 2× sample buffer (BioRad) were analyzed by SDS-PAGE (12% precast gel; BioRad) and Western blot (PVDF membrane; BioRad) probed with anti-His tag probe (Pierce).

Using Supradex® S200 10/300 GL (GE Life Sciences), PagP–hydrolyzed-methylSTMA nanodiscs were further purified based on their size in Tris buffer 10 mM supplemented with 100 mM NaCl and glycerol (final concentration 5% v/v). Fractions were used for TEM imaging.

6. ***Electron microscopy of PagP–hydrolyzed-STMA nanodiscs***

Samples containing PagP–hydrolyzed-STMA nanodiscs were directly (no concentrating needed) used for negative-staining electron microscopy. Microliter amounts (5 μL) of sample were applied to already glow-charged 400 nm-mesh carbon-coated copper grids (Electron Microscopy Science, USA) and allowed to absorb for 1 min. The grids were then washed three times with deionized water and stained with 2% (w/v) uranyl acetate (filtered, 0.45 μm). The grids were air-dried for a minimum 2 h before imaging. EM micrographs were collected from a Tecnai G20 transmission electron microscope (FEI Eindhoven, NL; at an acceleration voltage of 200 kV) equipped with an Eagle 4k × 4k CCD camera (FEI).

7. NMR Data Acquisition

Large unilamellar vesicles of dimyristoylphosphatidylcholine (DMPC) (3.5 mM in Tris 10 mM, 100 mM NaCl) were incubated with 2% (w/v) of **(1)–(5)** at 37 °C; entirely clarified samples were used for data collection at 40 °C.

^{1}H NMR at 600 MHz and ^{31}P NMR at 241 MHz spectra were acquired on a Varian VNMRS 600 MHz NMR spectrometer equipped with a 5-mm high-field indirect detection broadband PFG probe. ^{31}P spectra were collected with 20-ms, 90° pulse, broadband ^{1}H WALTZ decoupling, 1024 scans, and a 1-s repetition delay. ^{31}P NMR spectra were calibrated by setting the ^{31}P chemical shift

of 100% H_3PO_4 sample to 0.0 ppm. Spectra were collected in HEPES buffer (10 mM, pH 8, 100 mM NaCl).

8. ***The sensitivity of stilbene-maleic acid copolymers to pH and divalent cations***

The concentration of polymers in each test was kept constant (0.5% w/v). All buffers were prepared as described.[9] Briefly, sodium acetate (50 mM; pH 4 and 5) and tris buffer (50 mM; pH values of 6, 8, 10) were supplemented with 100 mM NaCl and used for pH sensitivity assays. The sensitivities of (**3**) and (**4**) to divalent cations were tested in Tris buffer (pH 8, NaCl 100 mM) in the presence of increasing concentrations of $CaCl_2$ (2.5, 5, 7.5 and 10 mM).

Supplementary Figures.

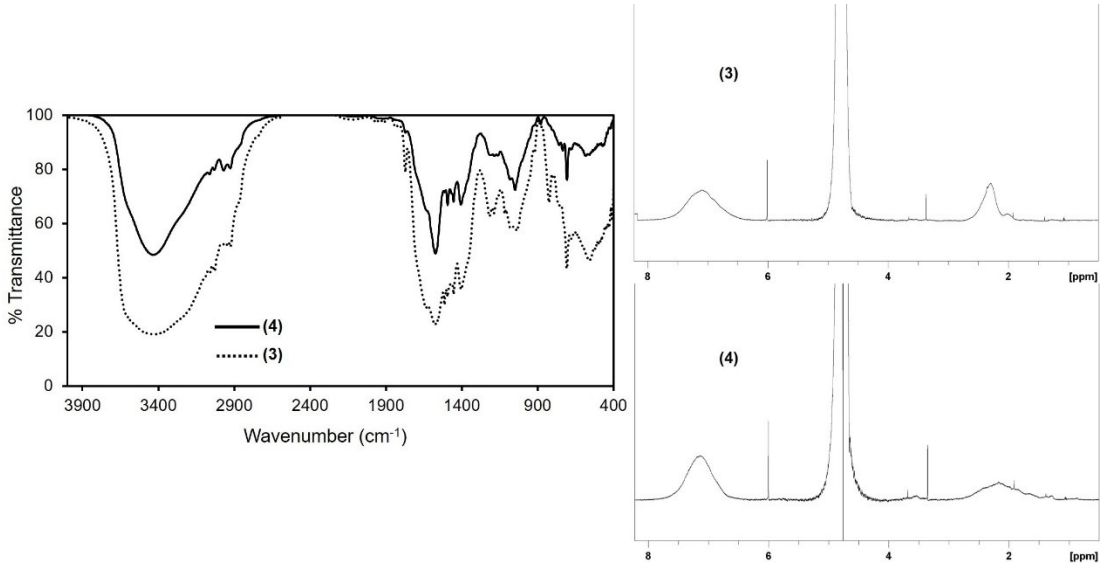

Fig. S1 (left) FT-IR spectra **(3)** and **(4)**; (right) 600 MHz ^{1}H NMR spectra in phosphate buffer pH 8.0, **(3)** (top) and **(4)** (bottom)**.**

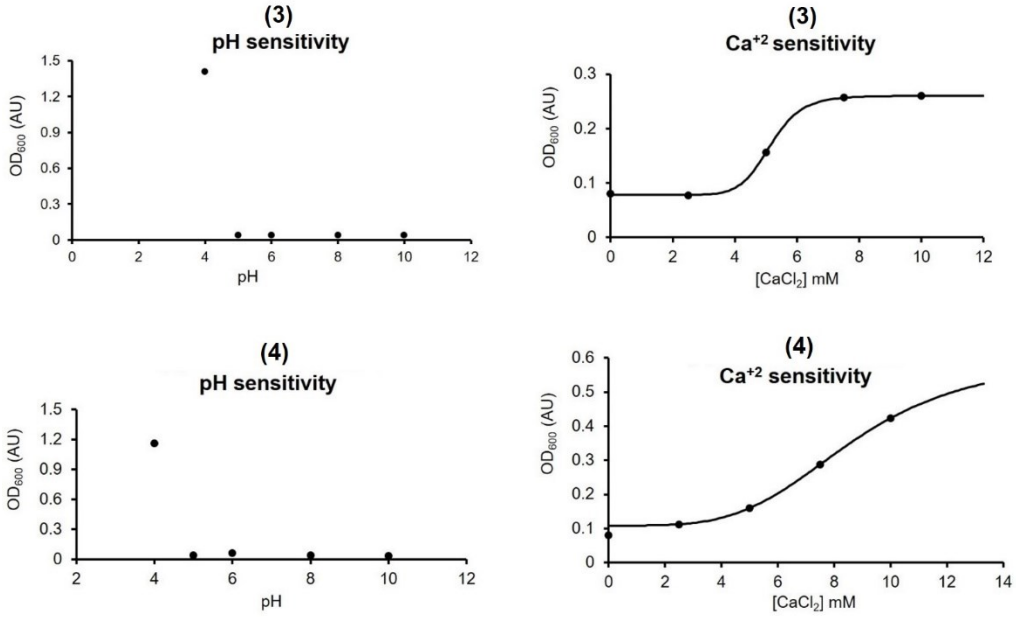

Fig. S2 The turbidity of samples used for calcium sensitivity and pH tolerance assays (see Fig. 2 in manuscript) was measured at 600 nm. Tris buffer (10 mM, pH 8.0) was used as a reference.

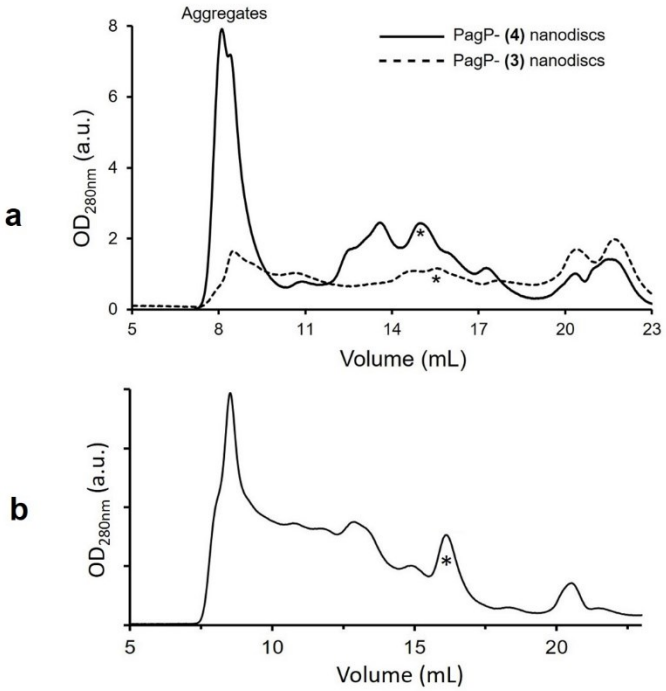

Fig. S3 Size exclusion chromatography profiles. (a) PagP–**(3)** and PagP–**(4)** nanodiscs; (b) PagP–hydrolyzed-SMA2000 nanodiscs. All nanodiscs were purified by a nickel affinity column.

Asterisks show fractions that contain the major amount of nanodiscs that were used for TEM imaging.

References

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