Supporting Online Material for *Membrane Assembly Driven by a Biomimetic Coupling Reaction*

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Methods

Synthesis of alkyne lysolipid (3-(palmitoyloxy)-2-(pent-4-ynoyloxy)propyl (2-(trimethylammonio)ethyl) phosphate)

70 mg of 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (Avanti) was dissolved in 10 mL methylene chloride. To this, 70 mg of 4-pentynoic acid, 140 mg of 1,3-diisopropylcarbodiimide and 25 mg of 4-(dimethylamino)pyridine was added. The solution was stirred overnight, filtered and the solvent removed by rotary evaporation. The product was isolated by column chromatography ($CHCl_3$/MeOH/H_2O) as a white solid. Yield 70%. ^{1}HNMR (400 MHz $CDCl_3$): δ 5.25-5.2 (m, 1H), 4.4-4.3 (m, 3H), 4.2-4.1 (m, 2H), 4.05-4.0 (m, 1H) 3.95-3.9 (m, 2H), 3.4 (s, 9H), 2.6-2.4 (m, 4H), 2.35-2.25 (t, 2H), 2.05 (t, 1H), 1.6-1.5 (m, 2H), 1.35-1.2 (m, 24H), 0.9-0.8 (t, 3H). [M+H]+ calculated mass: 576.36; found mass: 576.48.

Synthesis of oleyl azide ((*Z*)-1-azidooctadec-9-ene)

Oleyl azide was synthesized using an adaptation of a previously reported procedure*(1).* 150 mg of oleyl bromide (*Z*)-1-bromooctadec-9-ene (Sigma) was dissolved in 1 mL anhydrous dimethylformamide and reacted overnight under nitrogen at 85^{o}C with 100 mg sodium azide. After the reaction, 5 mL water was added and the organic phase extracted using methylene chloride. After drying with $MgSO_4$, the solvent was removed by rotary evaporation and the product isolated by column chromatography as a pale yellow oil. Yield 80%. ^{1}HNMR (400 MHz $CDCl_3$): δ 5.4-5.3 (m, 2H), 3.3-3.2 (t, 2H), 2.1-1.95 (m, 4H), 1.7-1.5 (m, 2H) 1.4-1.2 (m, 22H), 0.9 (t, 3H).

Synthesis of triazole phospholipid ((*Z*)-3-(heptadecanoyloxy)-2-((3-(1-(octadec-9-en-1-yl)-1*H*-1,2,3-triazol-4-yl)propanoyl)oxy)propyl (2-(trimethylammonio)ethyl) phosphate)

13 mg of oleyl azide and 24 mg of alkyne lipid were dissolved in 2:1 dimethylformamide:water and stirred for 48 hours in the presence of a copper wire. The solvent was evaporated and the resulting triazole isolated by column chromatography as a white powder. Yield 65%. ^{1}HNMR (400 MHz $CDCl_3$): δ 7.5 (s, 1H), 5.4-5.3 (m, 2H)

5.25-5.2 (m, 1H), 4.5-4.25 (m, 5H), 4.2-4.1 (m, 1H), 4.05-4.0 (m, 2H) 3.9-3.8 (m, 2H), 3.4 (s, 9H), 3 (m, 2H), 2.8-2.7 (m, 2H), 2.6-1.0 (56H), 0.9-0.8 (t, 6H). [M+H]+ calc mass 869.64 found mass 869.73

Membrane Assembly

To 100 µL of either distilled water or appropriate buffer, 0.15 mg of oleyl azide and 0.3 mg alkyne phospholipid were added followed by 0.1 mg of sodium ascorbate and 6 µg of copper sulfate. The mixture was agitated overnight. Control experiments were prepared identically with the absence of copper catalyst.

Encapsulation Experiments

A 1 mM solution of 8-Hydroxypyrene-1,3,6-trisulfonic acid (HPTS) was made in 10 mM HEPES buffer. This solution was then used as the buffer for membrane assembly as described above. After overnight agitation, a vesicle sample was diluted 10 fold in buffer and observed using fluorescence microscopy (Figure S1A). Alternatively, the reaction mixture was separated via size exclusion chromatography (Sepharose 4B) and a fraction collector (Gilson). HPTS fluorescence (excitation 460 nm, emission 510 nm) was measured using a plate reader (Figure S1B, green). A control experiment lacking the copper catalyst was run similarly (Figure S1B, blue).

LC-MS of product formation

Membrane assembly was performed in distilled water as described above. A 5 µL sample was taken at various time points before and after addition of copper catalyst. This sample was diluted with 20 µL acetonitrile and analyzed using an analytical LC/MS with an Evaporative Light Scattering Detector from Waters Co. (Milford, MA), operated by Fractionlynx 4.0 or Masslynx software with Waters Xterra columns (C8) at a flow rate of 0.3 mL/min. For all LC/MS runs, solvent A consisted of water with 0.1% formic acid and solvent B of acetonitrile with 0.1% formic acid.

Anisotropy Measurements

Diphenylhexatriene (5 μM final concentration) was added to 100 nm extruded vesicles (2 mM phospholipid) as a 1% vol/vol concentrated ethanol stock, followed by an overnight incubation. Steady-state anisotropy was measured on a Cary Eclipse (Varian) spectrophotometer with a manual polarizer accessory and peltier temperature controller. Anisotropy (R) was calculated as a unit-less ratio *(2)* defined as $(I_{=} - I_{\perp})/(I_{=} + 2*I_{\perp})$, where $I_{=}$ and $I_{\perp}$ are the emission intensities at 430 nm (excitation 360 nm) parallel and perpendicular, respectively, to the direction of polarization of the excitation source. Measurements were taken at 23 °C, unless otherwise noted

Lipid Solubility Measurements

Serial dilutions of the given lipid were prepared and and tumbled overnight. Surface tensions of the solutions was measured by the Du Noüy ring method on a Model 20 (Fisher) surface tensiometer. Critical micelle concentrations (CMC) were extrapolated from the concentrations at which the surface tension plateaus.

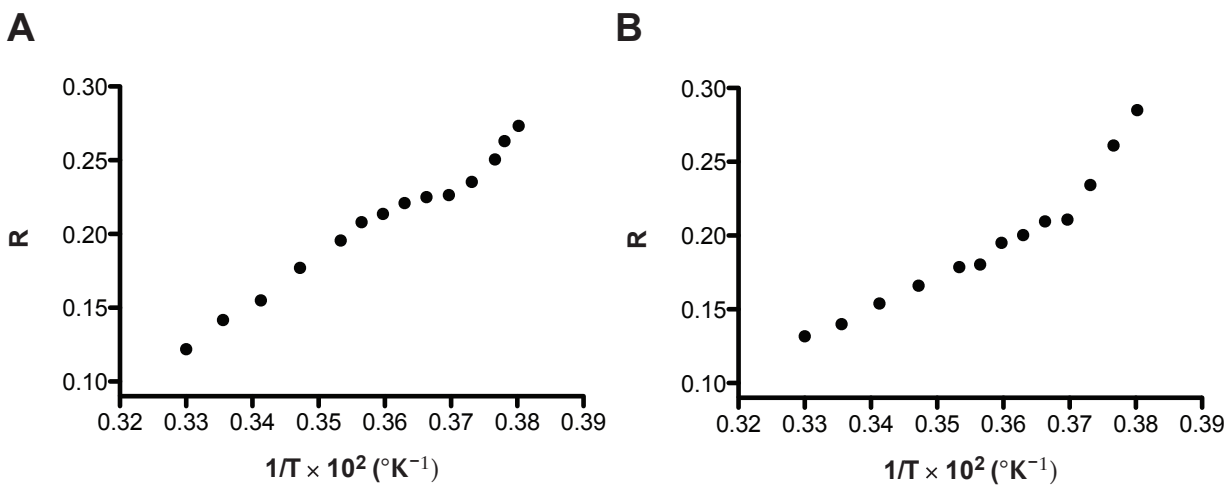

Figure S1. Chain melting temperature of triazole phospholipid. A) DPH anisotropy as a function of temperature was used to measure phase transitions of the lipid chains in triazole phospholipid vesicles as previously demonstrated (*3*). A sudden change in the slope of the anisotropy at 270 K indicates a transition from a gel to liquid-crystalline phase. B) The melting temperature of the lipid chains in POPC membranes was detected at 270 K, consistent with previous measurements (*4*).

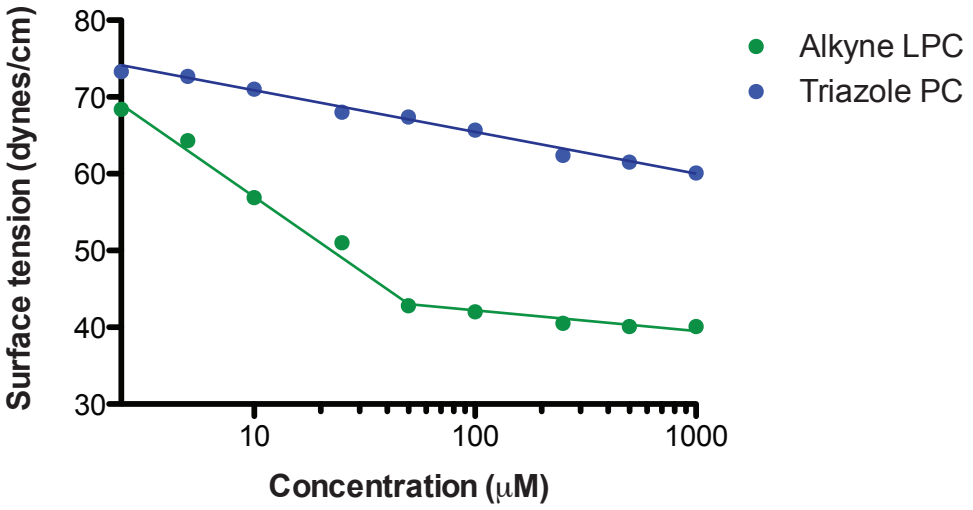

Figure S2. Aqueous solubilities of alkyne substrate and triazole product. Surface tension measurements were used to detect monomer to aggregate phase transitions. The alkyne lysolipid substrate (green) features a CMC of ~ 50 μM, consistent with that of other lysolipids (*5*). The triazole product (blue) does not show a concentration-dependent aggregation because of the extremely low (nM) solubility of diacyl phospholipids.

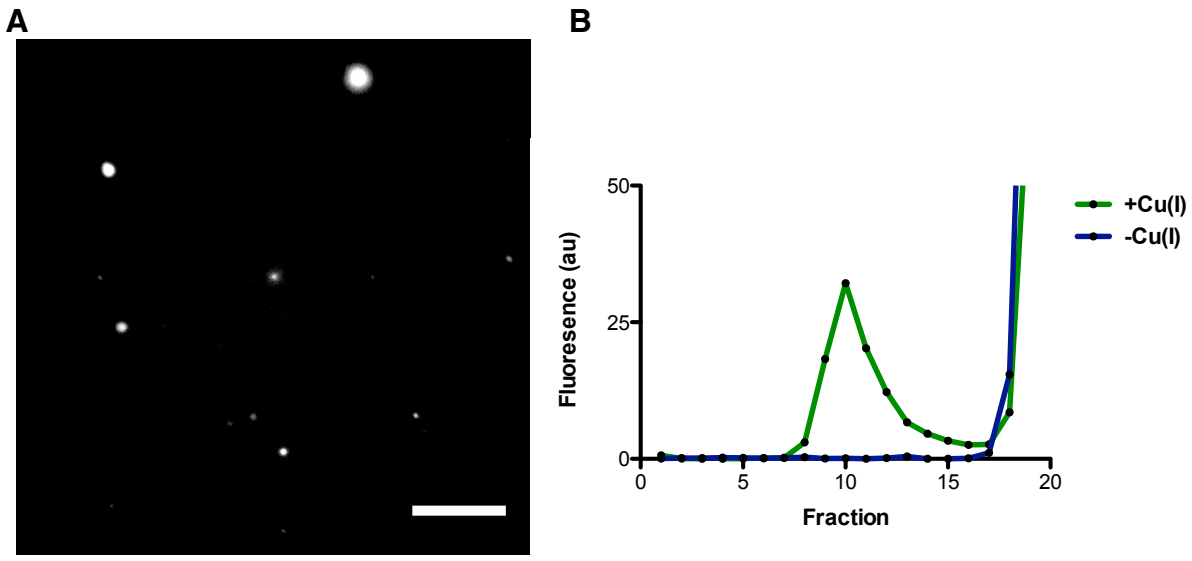

Figure S3. Encapsulation of polar fluorescent dye (8-Hydroxypyrene-1,3,6-trisulfonic acid) in triazole phospholipid membrane vesicles. A) Fluorescence microscopy of dye encapsulated in vesicles formed during copper-catalyzed reaction. Scale bar denotes 15 *μm*. B) fluorescence intensity versus collected fraction after size-exclusion chromatography of vesicles formed in the presence (green line) and absence (blue line) of the copper catalyst. All experiments were performed in 10 mM HEPES buffer.

Movie S1. Time lapse fluorescence microscopy of membrane formation. Copper catalyst (0.25 mM) was added to an aqueous emulsion of oleyl azide (5 mM) and alkyne lysolipid (5 mM) along with a membrane staining dye (Rh-DHPE, 2 μM). Sequential fluorescence images were taken near a non-fluorescent azide oil droplet (upper left) over a period of 3.5 minutes.

References

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