Supplementary Information

Ruthenium Photoredox-Triggered Phospholipid Membrane Formation

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Materials

Triethylamine (≥99%), Copper sulfate pentahydrate (≥98%), and $Ru(bpy_3)^{2+}$ were purchased from Sigma Aldrich. Rhodhamine DHPE was purchased from Invitrogen. Alkyne lysolipid and alkyl azide were synthesized as reported previously (1, 2).

Methods

$Ru(bpy_3)^{2+}$ Photoredox-Triggered Triazole Phospholipid Synthesis

Preparation of a 1 mM dodecane azide and 650 µM alkyne lysolipid solution was prepared by adding 43.3 µL of a 15 mM alkyne lysolipid in chloroform stock and 27 µM of 40 mM alkyl azide in chloroform stock to a 2 mL LCMS vial. The chloroform was evaporated under a gentle stream of nitrogen. To the remaining residue of pure alkyne lysolipid and alkyl azide, 1 ml of water was added. The solution was briefly vortexed and then sonicated in a bath sonicator (3510 Branson) for 15 minutes.

Reactions were prepared by mixing together the alkyne-azide solution, a triethylamine in ethanol solution, a copper sulfate in water solution, and a $Ru(bpy_3)^{2+}$ in water solution in a 2 mL LCMS vial containing a 250 µL insert. The 75 µL reaction consisted of 72 µL of the alkyne-azide solution, 0.5 µL of 30 mM triethylamine, 1.0 µL of 15 mM copper sulfate and 1.5 µL of 2 mM $Ru(bpy_3)^{2+}$. The reaction was placed on a shaker rotating at 100 rev/min and placed ~8'' from an 11 watt CFL. Triethylamine, $Ru(bpy_3)^{2+}$ and alkyne-azide solutions were prepared fresh daily.

Starting reaction conditions: 40 µM $Ru(bpy_3)^{2+}$, 200 µM triethylamine, 200 µM copper sulfate pentahydrate, 1 mM dodecane azide and 650 µM alkyne lysolipid.

Effect of triethylamine of on Triazole Phospholipid Synthesis

Reactions were prepared as described in the previous section with the following changes. Serial dilutions of a 30 mM triethylamine in ethanol solution were performed to create stock solutions of 22.5 mM, 15 mM and 7.5 mM, corresponding to reaction concentrations of 200 µM, 150 µM, 100 µM and 50 µM. For the 0 µM TEA control, 0.5 µL of pure ethanol was added in place of a triethylamine in ethanol solution.

Starting reaction concentrations: 40 μM $Ru(bpy_3)^{2+}$, 200 μM, 150 μM, 100 μM, 50 μM or 0 μM triethylamine, 200 μM copper sulfate pentahydrate, 1 mM dodecane azide and 650 μM alkyne lysolipid.

Liquid Chromatography/Mass Spectrometry/Evaporative Light Scattering Detection (LC/MS/ELSD)

Protocols were based upon previously reported settings and procedures (2). Mass identification of alkyne lysolipid and alkyl azide were reported previously(1, 2).

Cryo-EM microscopy

Vesicles for Cryo-EM were prepared with the following conditions: 0 or 40 μM $Ru(bpy_3)^{2+}$, 200 μM triethylamine, 200 μM copper sulfate pentahydrate, 1 mM dodecane azide and 750 μM alkyne lysolipid. No vesicles were observed in the negative control (reaction without $Ru(bpy_3)^{2+}$). For additional information on negative controls, please see the previously reported discussion on azide emulsions(2). Conditions for freezing: blot time = 5 seconds, glow discharge time = 25 seconds, sample incubation time = 60 seconds.

Fluorescent Microscopy

Reaction conditions: 40 μM $Ru(bpy_3)^{2+}$, 200 μM triethylamine, 200 μM copper sulfate pentahydrate, 2.5 mM dodecane azide and 2.5 mM alkyne lysolipid. Reactions were exposed to 20 mW/cm^2 for 1 hour or to 50 mW/cm^2 for 30 min.

Supporting Figures

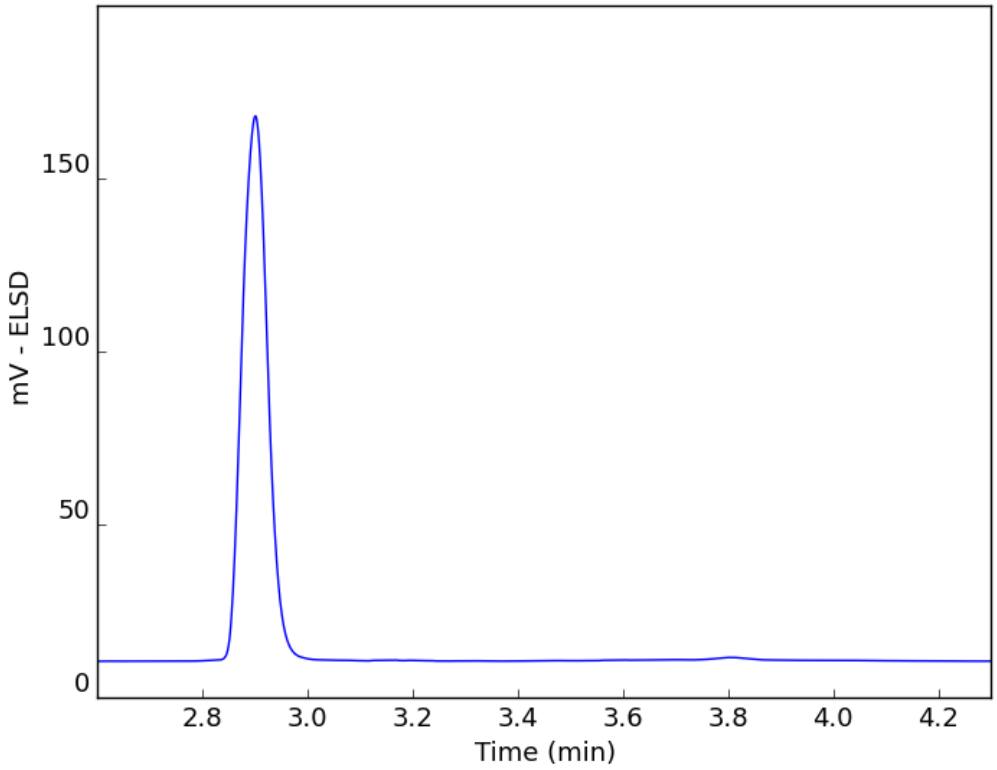

Figure S1. Triazole lipid Synthesis in the absence of $Ru(bpy_3)^{2+}$. Conditions: 200 µM $CuSO_4$, 200 µM TEA, 625 µM alkyne lysolipid, and 1 mM alkyl azide. Alkyne lysolipid retention time = 2.9 min; triazole phospholipid rentention time = 3.8 min.

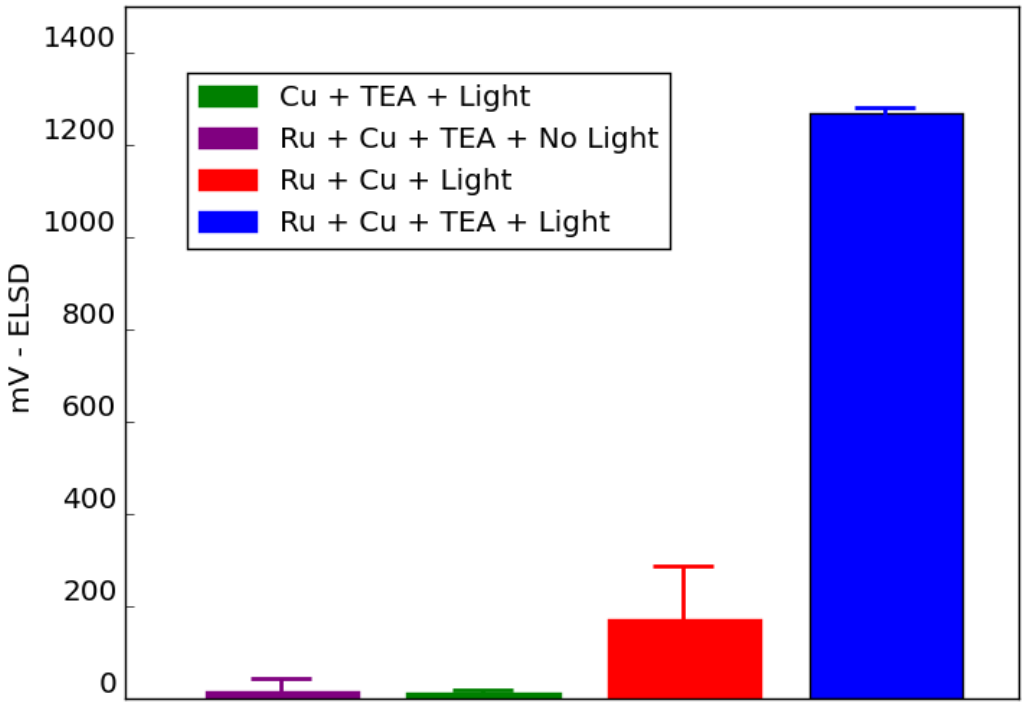

Figure S2. Triazole Lipid Synthesis monitored via ELSD. Conditions: 215 μM $CuSO_4$, 33 μM $Ru(bpy_3)^{2+}$, 200 μM TEA, 625 μM alkyne lysolipid, and 1 mM alkyl azide. Results recapitulate absorbance data shown in Figure 1 of the main text.

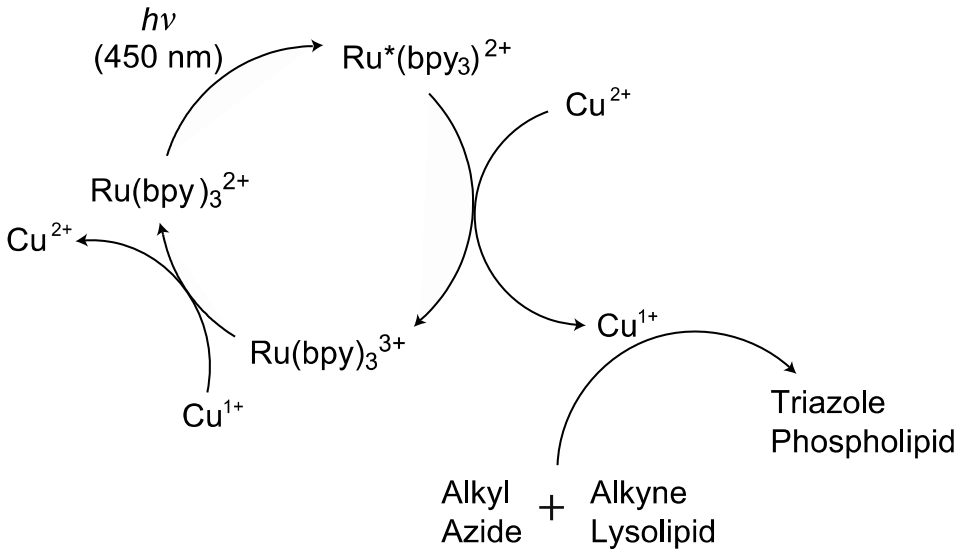

Figure S3. An oxidative quenching $Ru(bpy_3)^{2+}$ scheme. The photoexcited $Ru^*(bpy_3)^{2+}$ is quenched by copper (II), yielding copper(I) and $Ru(bpy_3)^{3+}$. $Ru(bpy_3)^{3+}$ is a strong oxidant (+1.29V) and can oxidize copper(I) back to copper(II).

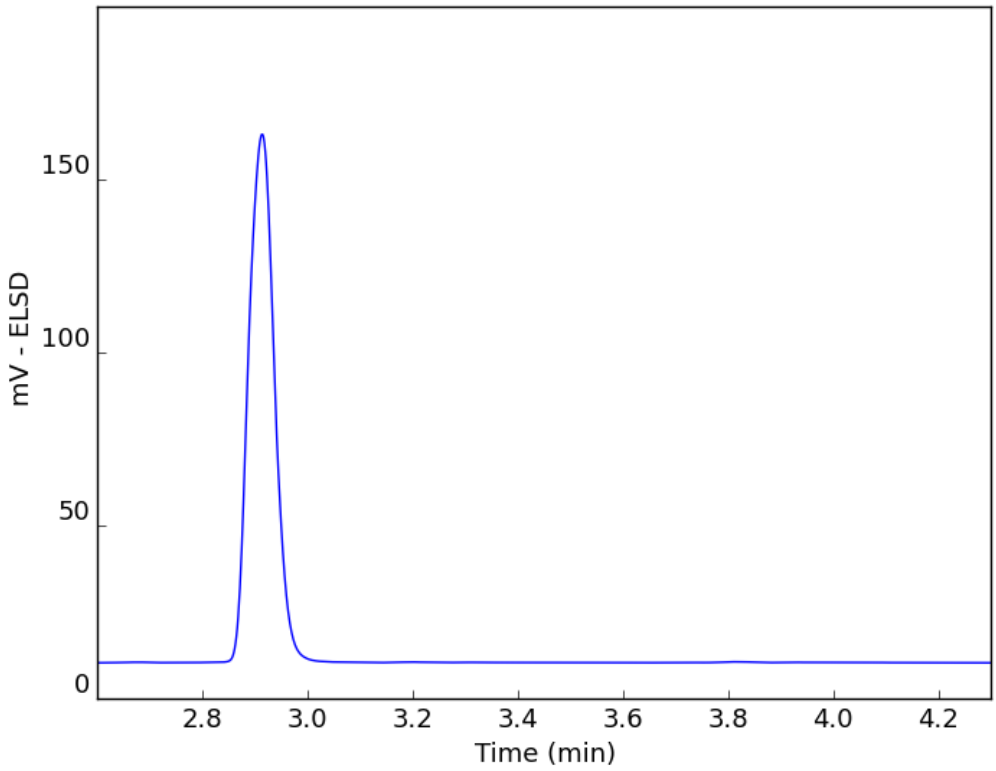

Figure S4. Triazole Lipid Synthesis with 0 µM TEA. Conditions: 200 µM $CuSO_4$, 40 µM $Ru(bpy_3)^{2+}$, 625 µM alkyne lysolipid, and 1 mM alkyl azide. Alkyne lysolipid retention time = 2.9 min; triazole phospholipid rentention time = 3.8 min.

References

1. Budin I & Devaraj NK (2012) Membrane assembly driven by a biomimetic coupling reaction. *Journal of the American Chemical Society* 134(2):751-753.
2. Hardy MD*, et al.* (2015) Self-reproducing catalyst drives repeated phospholipid synthesis and membrane growth. *Proceedings of the National Academy of Sciences of the United States of America* 112(27):8187-8192.