Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information for:

Paley's Watchmaker Analogy and Prebiotic Synthetic Chemistry in Surfactant Assemblies. Formaldehyde Scavenging by Pyrroles Leading to Porphyrins as a Case Study

Eric J. Alexy, Carl W. Hintz, Hubert M. Hughes,
Masahiko Taniguchi, and Jonathan S. Lindsey*

Table of Contents **Page**

1. Reaction setup table

A typical spreadsheet for reaction setup and analysis is shown in Table S1.

Table S1. Specific reaction quantities and analysis protocol.

Rxn	[Pyrrole] stock[a] (mM)	Pyrrole volume (µL)	[Pyrrole] in RXN (mM)	[H_2CO] stock[b] (mM)	H_2CO volume (µL)	[H_2CO] in RXN (mM)	Buffer/SDS[c] volume (µL)	Water volume (µL)	Total volume (µL)	Analysis	
										Dilution factor[d]	Abs.[e]
1	0.8	25	0.01	0.8	25	0.01	1200	750	2000	1	0
2	0.8	25	0.01	0.8	250	0.1	1200	525	2000	1	0.041
3	0.8	25	0.01	80	25	1.0	1200	750	2000	1	0.137
4	0.8	25	0.01	80	250	10	1200	525	2000	1	0.192
5	3.712	25	0.0464	4	23.2	0.0464	1200	751.8	2000	1	0.008
6	3.712	25	0.0464	4	232	0.464	1200	543	2000	1	0.332
7	3.712	25	0.0464	400	23.2	4.64	1200	751.8	2000	1	0.686
8	3.712	25	0.0464	400	232	46.4	1200	543	2000	1	0.654
9	17.2	25	0.215	20	21.5	0.215	1200	753.5	2000	1	0.090
10	17.2	25	0.215	20	215	2.15	1200	569	2000	2	0.748
11	17.2	25	0.215	200	21.5	21.5	1200	753.5	2000	2	0.685
12	80	25	1.0	80	25	1.0	1200	750	2000	10	0.103
13	80	25	1.0	80	250	10	1200	525	2000	10	0.603
14	80	25	1.0	800	25	100	1200	750	2000	10	0.722

[a]In methanol. [b]In water. [c]0.5 M potassium phosphate (pH 7) containing 50 mM SDS in water. [d]For absorption spectroscopy. [e]Absorption after baseline correction.

Table S2. Specific reaction quantities and analysis protocol.

Rxn	[Pyrrole] stock[a] (mM)	Pyrrole volume (μL)	[Pyrrole] in RXN (mM)	[H_2CO] stock[b] (mM)	H_2CO volume (μL)	[H_2CO] in RXN (mM)	Buffer/CTAC[c] volume (μL)	Water volume (μL)	Total volume (μL)	Analysis	
										Dilution factor[d]	Abs.[e]
1	0.8	25	0.01	0.8	25	0.01	1200	750	2000	1	0
2	0.8	25	0.01	0.8	250	0.1	1200	525	2000	1	0
3[f]	0.8	25	0.01	80	25	1.0	1200	750	2000	1	0.007
4	0.8	25	0.01	80	250	10	1200	525	2000	1	0.043
5	3.712	25	0.0464	4	23.2	0.0464	1200	751.8	2000	1	0
6	3.712	25	0.0464	4	232	0.464	1200	543	2000	1	0.033
7	3.712	25	0.0464	400	23.2	4.64	1200	751.8	2000	1	0.320
8	3.712	25	0.0464	400	232	46.4	1200	543	2000	1	0.265
9	17.2	25	0.215	20	21.5	0.215	1200	753.5	2000	1	0.019
10	17.2	25	0.215	20	215	2.15	1200	569	2000	1	0.496
11	17.2	25	0.215	200	21.5	21.5	1200	753.5	2000	1	0.489
12	80	25	1.0	80	25	1.0	1200	750	2000	2	0.364
13	80	25	1.0	80	250	10	1200	525	2000	10	0.269
14	80	25	1.0	800	25	100	1200	750	2000	10	0.270

[a]In methanol. [b]In water. [c]0.5 M potassium phosphate (pH 7) containing 50 mM CTAC in water. [d]For absorption spectroscopy. [e]Absorption after baseline correction. [f]Reaction 3 was used arbitrarily as the representative procedure in the main paper.

2. Vesicles preparation and characterization

The procedure for vesicle preparation closely resembles that in the literature[7,20] and is provided here for clarity and clear segue with the follow-on procedures: Large, unilamellar vesicles of L-α-phosphatidylcholine (chicken egg, 770 Da) were prepared by extrusion (Avanti mini-extruder). A $CHCl_3$ solution (2 mL) of the phosphatidylcholine (50 mg) was treated to a stream of argon to give a partially dried lipid film, which was thoroughly dried overnight under vacuum. The resulting dried film was hydrated in 1 mL of 0.1 M potassium phosphate buffer (pH 7) with moderate agitation (benchtop vortexer) for 10 min. The resulting hydrated, large multilamellar vesicles were subsequently frozen in liquid nitrogen and then thawed in a 50 °C water bath. The freeze-thaw cycle was repeated 10 times. The vesicles were extruded 11 times through a polycarbonate filter (0.1 μm pore size) to yield large unilamellar vesicles. The inner vesicle volume consisted of aqueous phosphate solution. The vesicle suspension was stored at 4 °C. The resulting suspension was 65 mM [lipid]. Then, an aliquot of this vesicle suspension was diluted into 0.1 M potassium phosphate buffer (pH 7) to give the desired concentration of vesicles for reactions.

The vesicles (0.3 mM in 0.1 M phosphate buffer, pH 7) were examined by dynamic light scattering (DLS) using a Zetasizer 1000 HS_A (Malvern Instruments, Malvern UK) equipped with a 5 mW helium-neon laser (633 nm) at 25 °C. The number distribution was peaked at a vesicle diameter of 106 nm (Figure S1, black trace). The constituents for reaction were then added (0.046 mM **1-Et** and 4.6 mM formaldehyde). The reaction was then carried out for 24 h at 25 °C. The vesicles, now slightly colored, were again subjected to DLS characterization. The results are shown in Figure S1 (red trace). No new peaks or substantial tailing of the size distribution was observed. The apparent change in size distribution is typical from day-to-day experimentation and, via control experiments, does not appear to reflect aging or a reaction-derived increase in size.

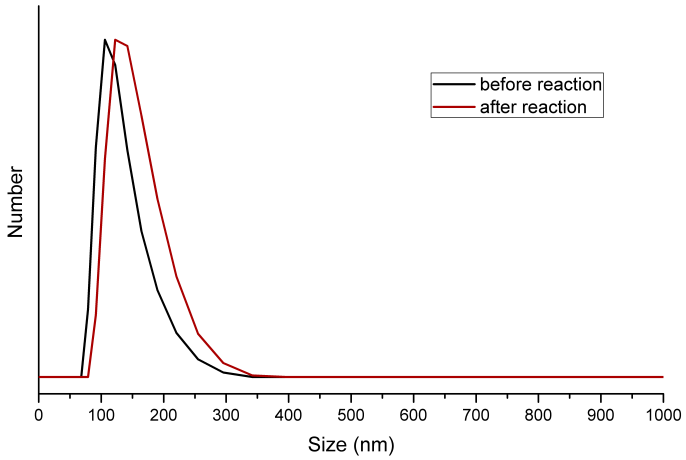


Figure S1. Number of vesicles of a given size obtained by dynamic light scattering. The peak is at ~106 nm before reaction and ~125 nm after reaction.

3. Fluorescence data

Fluorescence spectroscopy was carried out to characterize **Et_8P**. The reaction mixture (derived from the timecourse experiment at 25 °C) was diluted with the same medium employed for the reaction (potassium phosphate buffer containing SDS or CTAC) until the absorption of the Soret band was ~0.1. Fluorescence emission and fluorescence excitation spectroscopy was performed in the standard way.

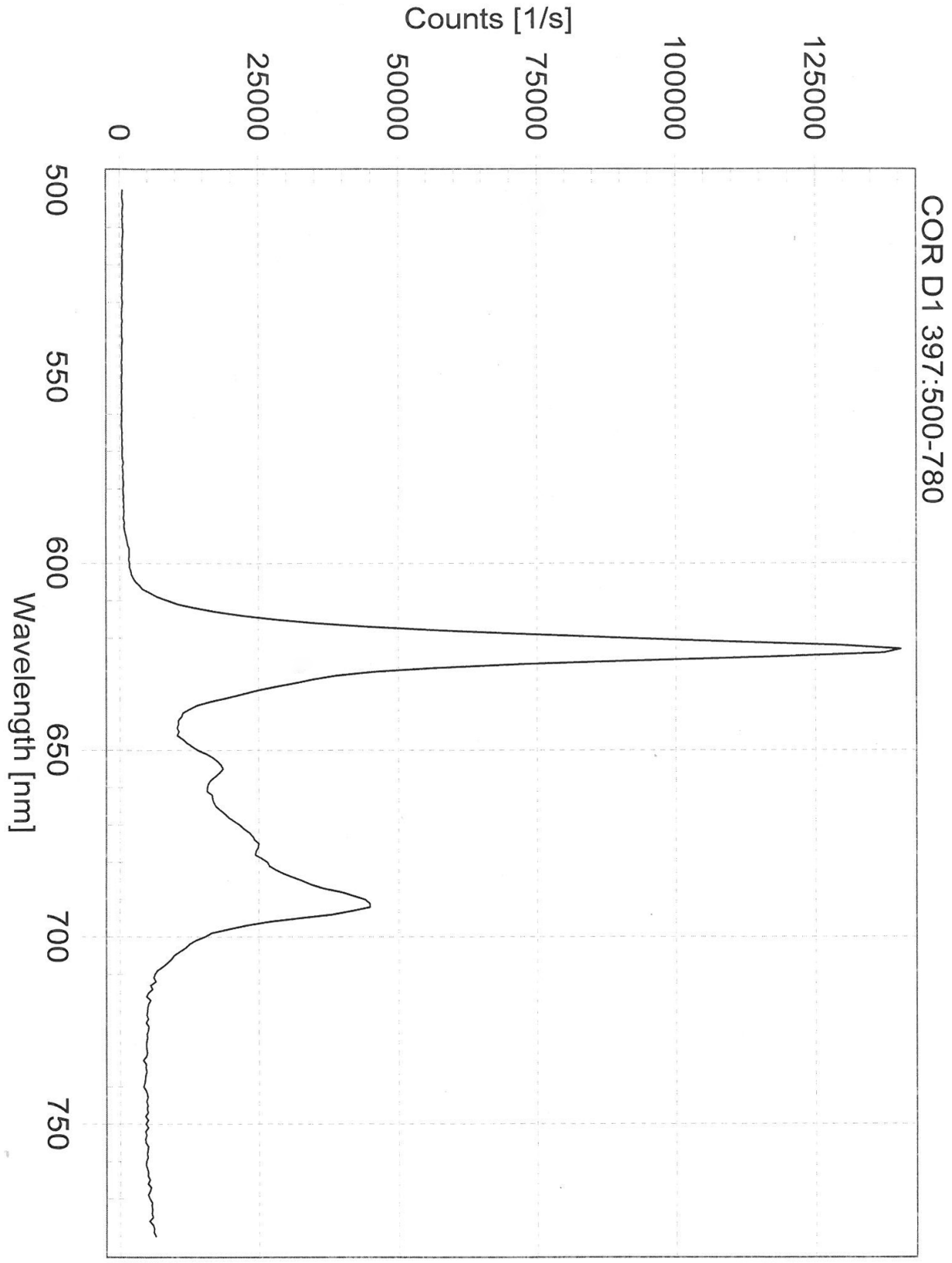


Figure S2. Fluorescence emission (corrected) of **Et_8P** in aqueous CTAC (pH 7) at room temperature (from a reaction in CTAC); λ_{exc} = 397 nm.

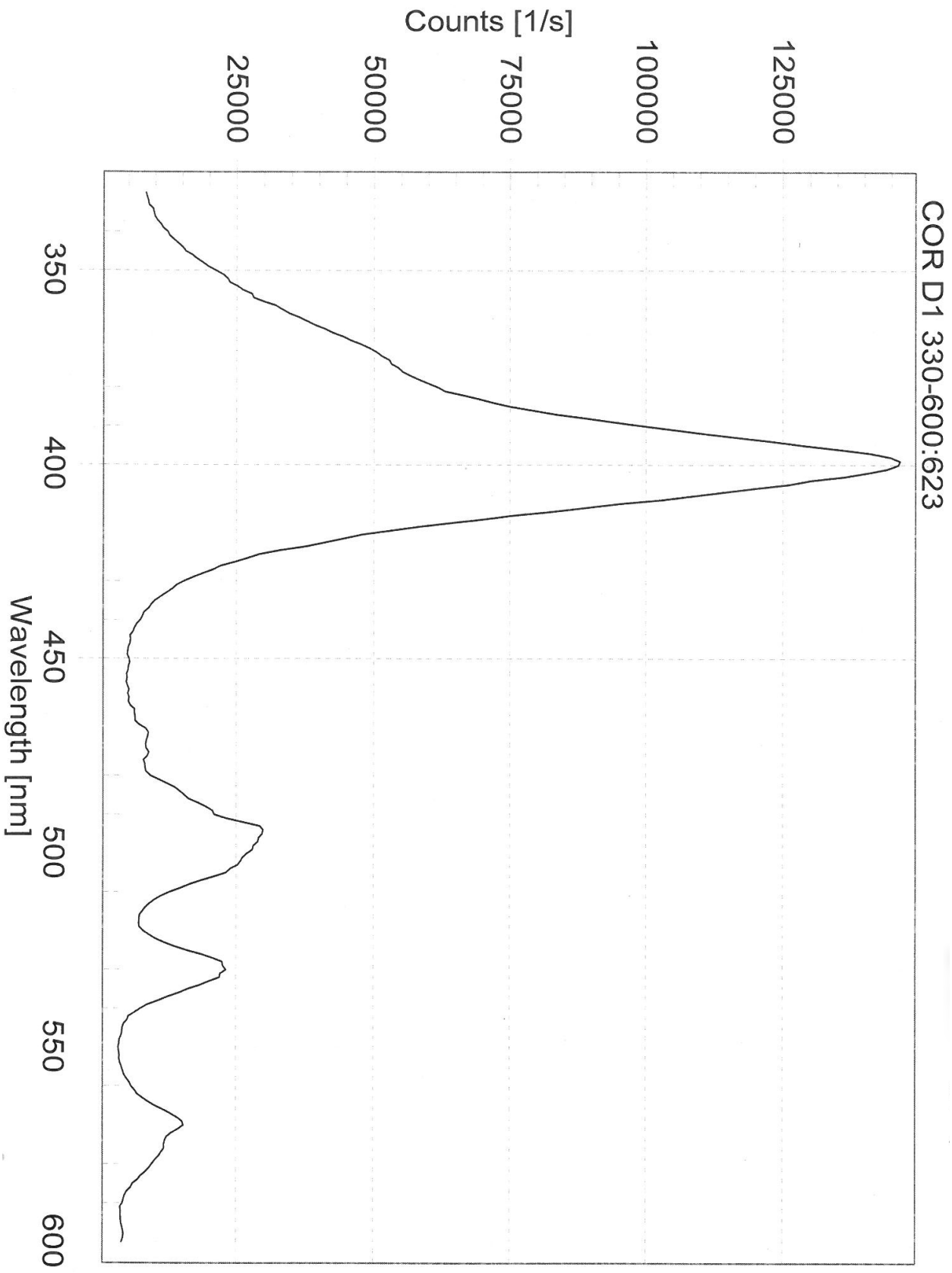


Figure S3. Fluorescence excitation of **Et_8P** in aqueous CTAC (pH 7) at room temperature (from a reaction in CTAC); λ_{em} = 623 nm.

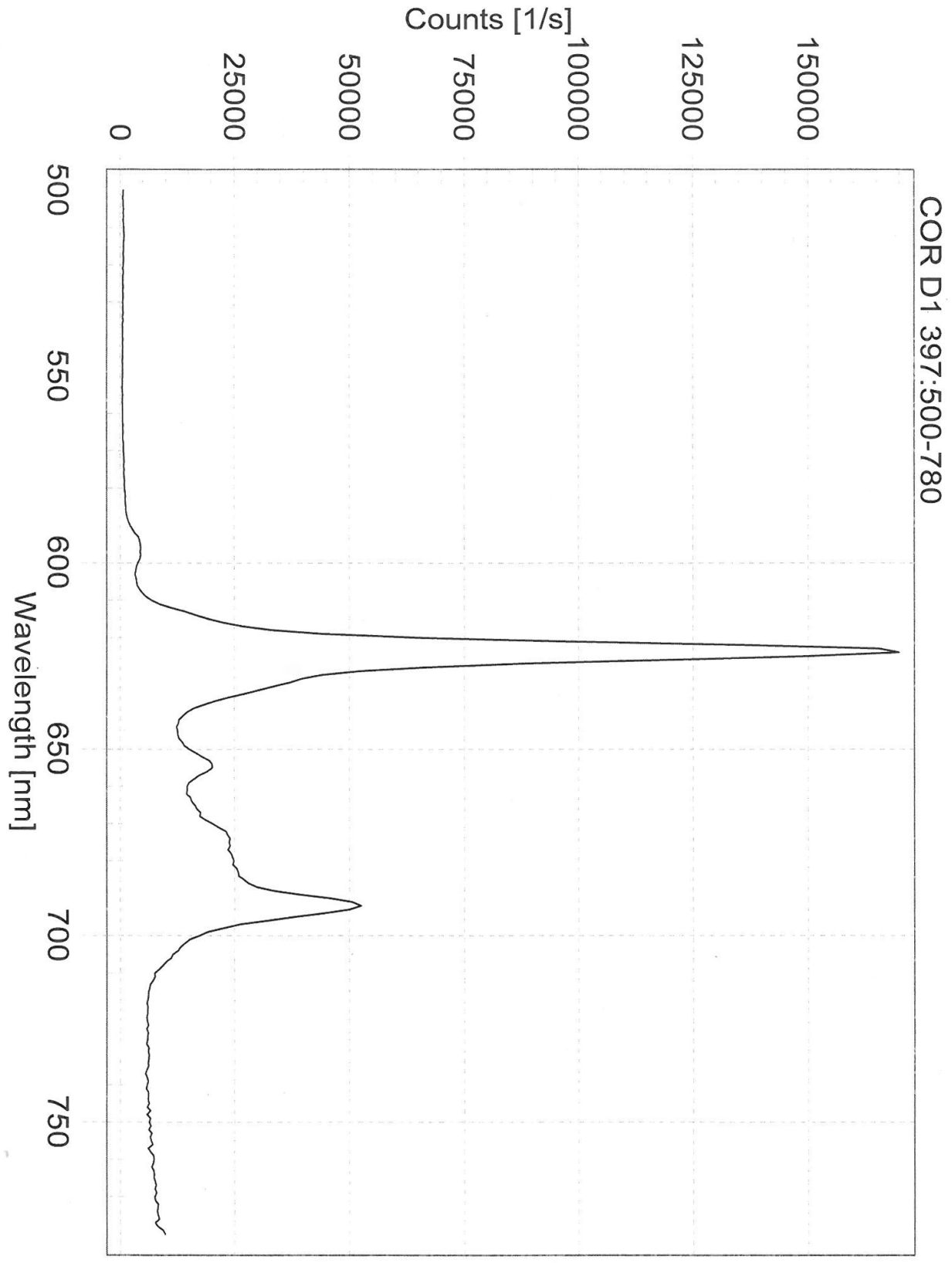


Figure S4. Fluorescence emission (corrected) of **Et_8P** in aqueous SDS (pH 7) at room temperature (from a reaction in SDS); λ_{exc} = 397 nm.

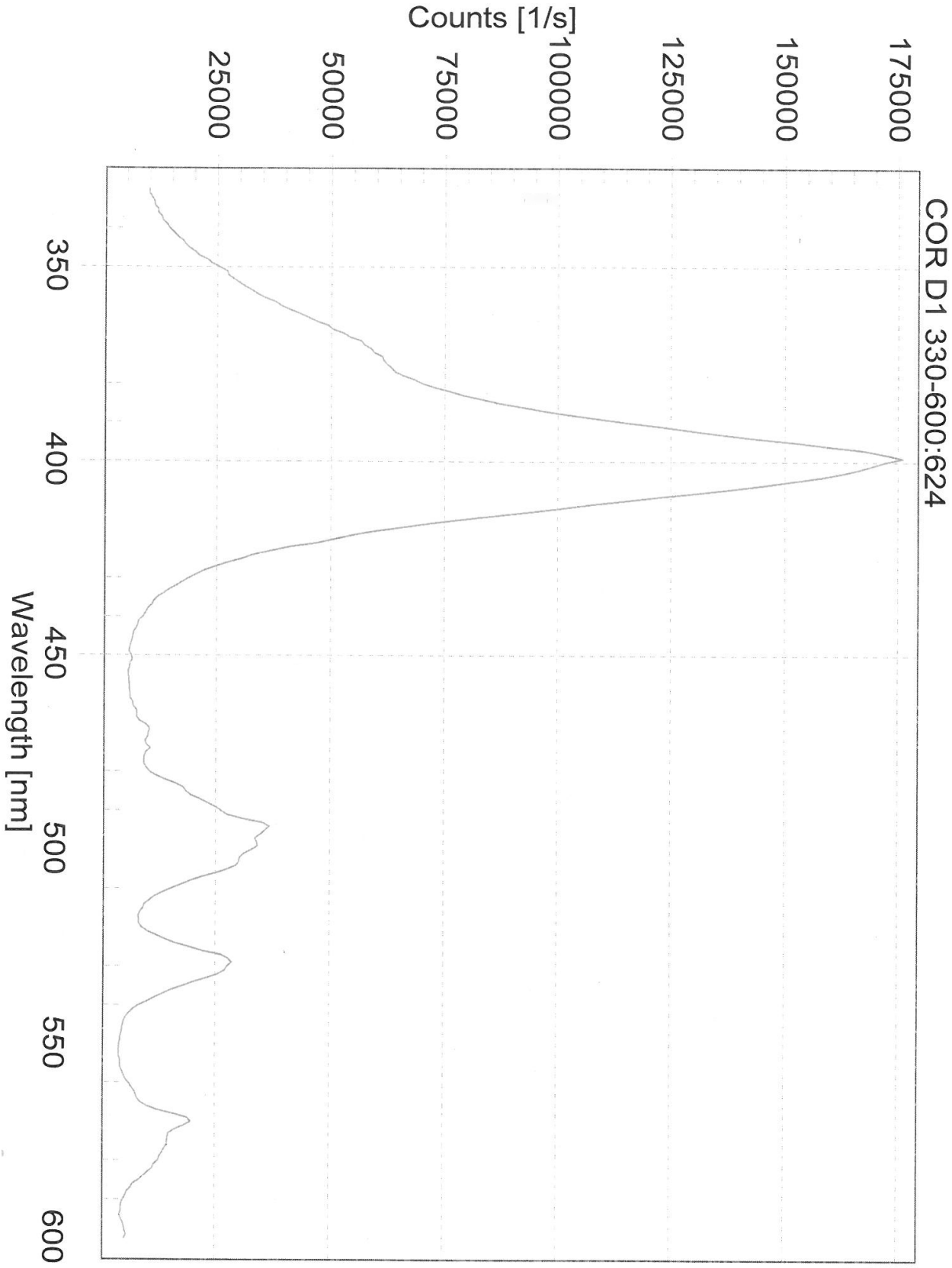


Figure S5. Fluorescence excitation of **Et_8P** in aqueous SDS (pH 7) at room temperature (from a reaction in SDS); λ_{em} = 624 nm.

4. Timecourse data

The timecourse was examined for reactions at selected concentrations in aqueous micelles that provided yields >10%. The results shown in Figure S6 concern use of 1000 equiv of formaldehyde at 25 °C, to be compared with the data in Figure 1. The reaction at a given temperature with 1000 equiv of formaldehyde is faster than that with 100 equiv, as expected, yet the ultimate yield of porphyrin is essentially the same and did not decline over the period examined. Figure S6 also shows reactions with 100 equiv of formaldehyde at 50 °C. Together, the results show that the yield obtained after 24 h of reaction likely provides a reasonable measure of the final yield for the given condition examined.

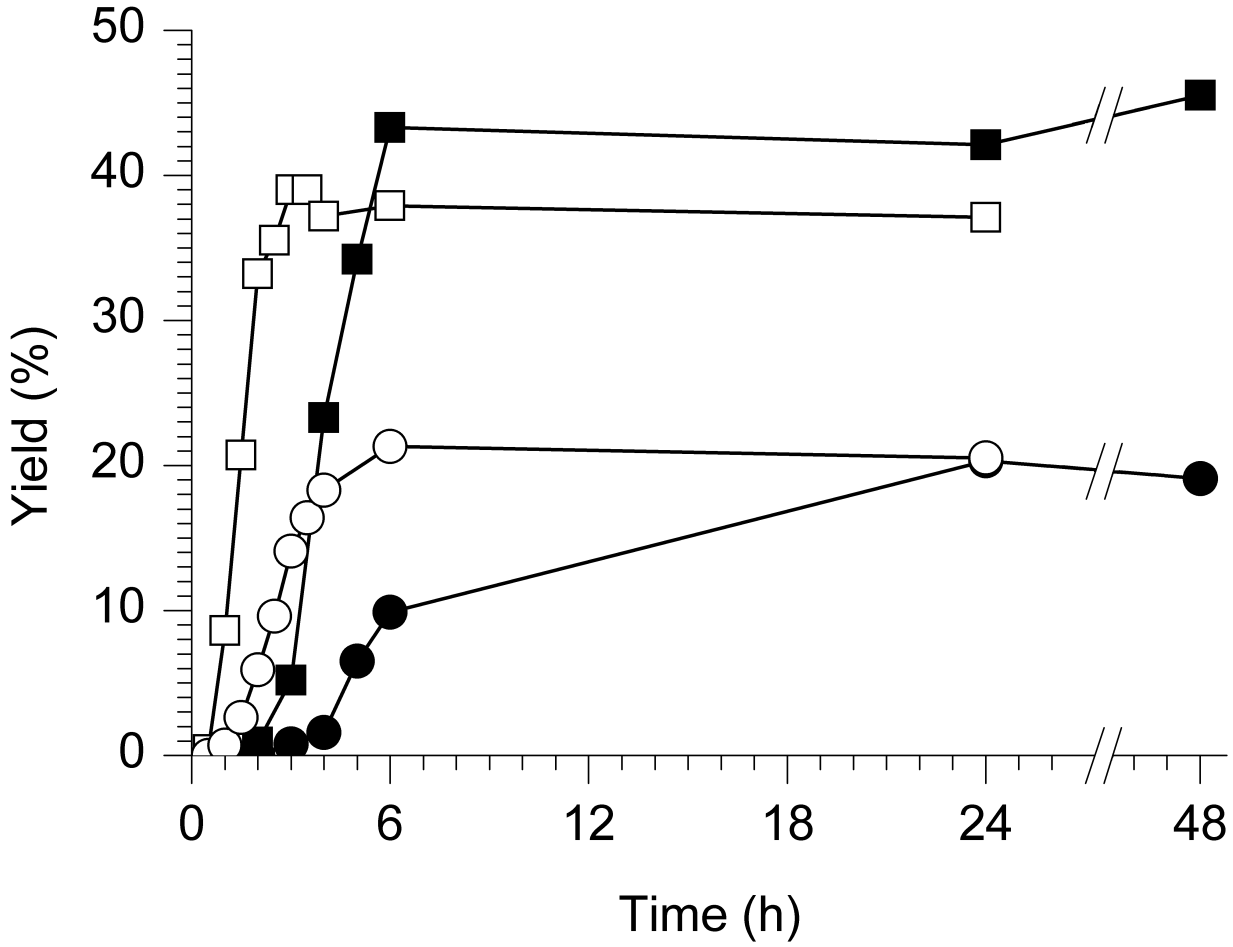


Figure S6. Yield of porphyrin $\mathbf{Et_8P}$ versus time for 0.046 mM **1-Et** and 100 or 1000 equiv of formaldehyde at 25 or 50 °C in aqueous micelles. Legend: SDS with 1000 equiv of formaldehyde at 25 °C, solid squares; CTAC with 1000 equiv of formaldehyde at 25 °C, solid circles; SDS with 100 equiv of formaldehyde at 50 °C, open squares; CTAC with 100 equiv of formaldehyde at 50 °C, solid circles.

5. Double-labeling crossover experiments

Double-labeling crossover experiments were carried out under a variety of conditions. The results from four such experiments are shown below. Figure S7 shows the double-labeling crossover experiment in the presence of CTAC that corresponds to the experiment in the main paper (Figure 3) in SDS.

For this study, we first examined the reactivity of 3,4-dimethylpyrrole (**1-Me**) in 30 mM CTAC. The reaction of **1-Me** at 0.046 mM and 100 equiv of formaldehyde at 25 °C gave **Me_8P** in 17%, respectively, to be compared with 15% for **Et_8P**. For the crossover experiment, CTAC micelles were separately treated with the respective pyrrole (**1-Me** versus **1-Et**), then mixed ("post-mixed"), treated with formaldehyde, and allowed to react. A control ("pre-mixed") experiment entailed adding the two pyrroles together to the surfactant followed by formaldehyde. After 24 h, examination of the pre-mixed reaction mixture in micelles revealed a porphyrin yield of 15%, in accord with expectation for the separate reactions.

Mass spectral analysis of the pre-mixed reaction mixture revealed the presence of **Et_8P** (*m/z* = 534.3) and **Me_8P** (422.2) in addition to the products **Et_6Me_2P** (506.3), **Et_4Me_4P** (478.3), and **Et_2Me_6P** (450.3), in agreement with calculated values for the porphyrin cation radicals. The post-mixed reaction mixture gave essentially identical yield (15%) and distribution of species upon MALDI-MS analysis.

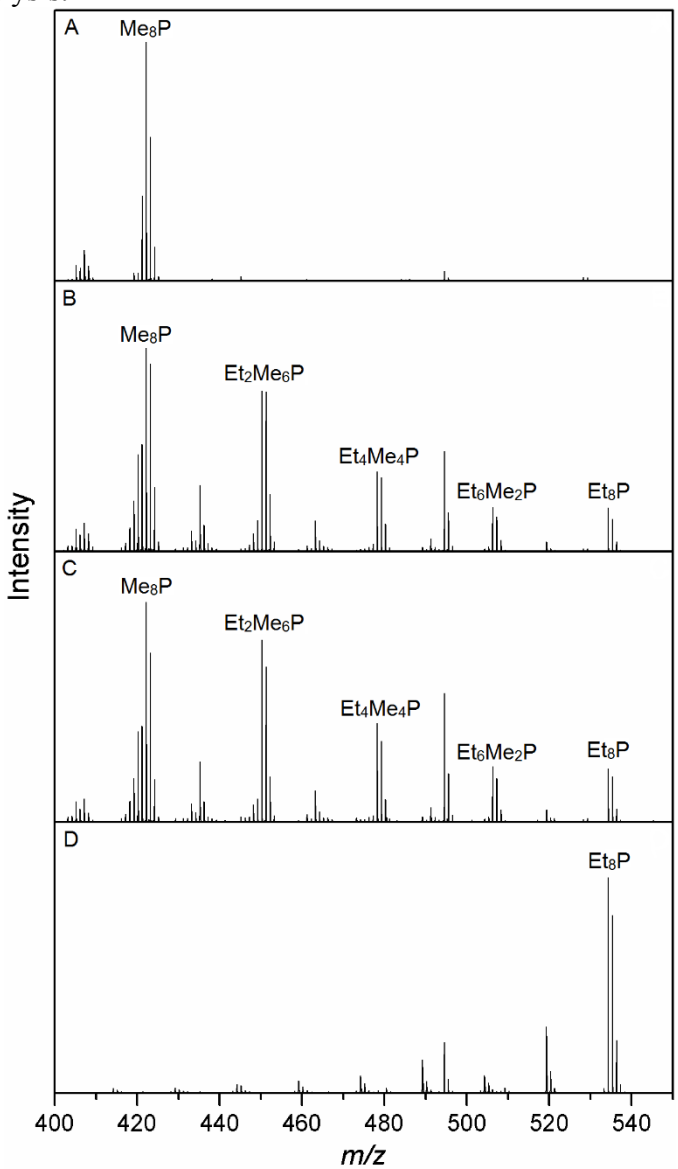


Figure S7. MALDI-MS spectra for double-labeling crossover experiment in 30 mM CTAC (pH 7) at 25 °C. Panels A, B, C, and D correspond to **1-Me** only (yield = 17%), pre-mix control (15%), post-mix (15%), and **1-Et** only (15%), respectively.

Figure S8 shows the double-labeling crossover experiment in 50 mM SDS with 100 mM NaCl (no phosphate buffer) at 25 °C. The reaction of **1-Et** at 0.046 mM and 100 equiv of formaldehyde at 25 °C in 50 mM SDS and 100 mM NaCl for 24 h gave 37% yield. Under the same conditions the reaction of **1-Me** at 0.046 mM and 100 equiv of formaldehyde gave 26% yield. The pre-mix control reaction gave a 37% yield and the post-mix reaction gave a 35% yield.

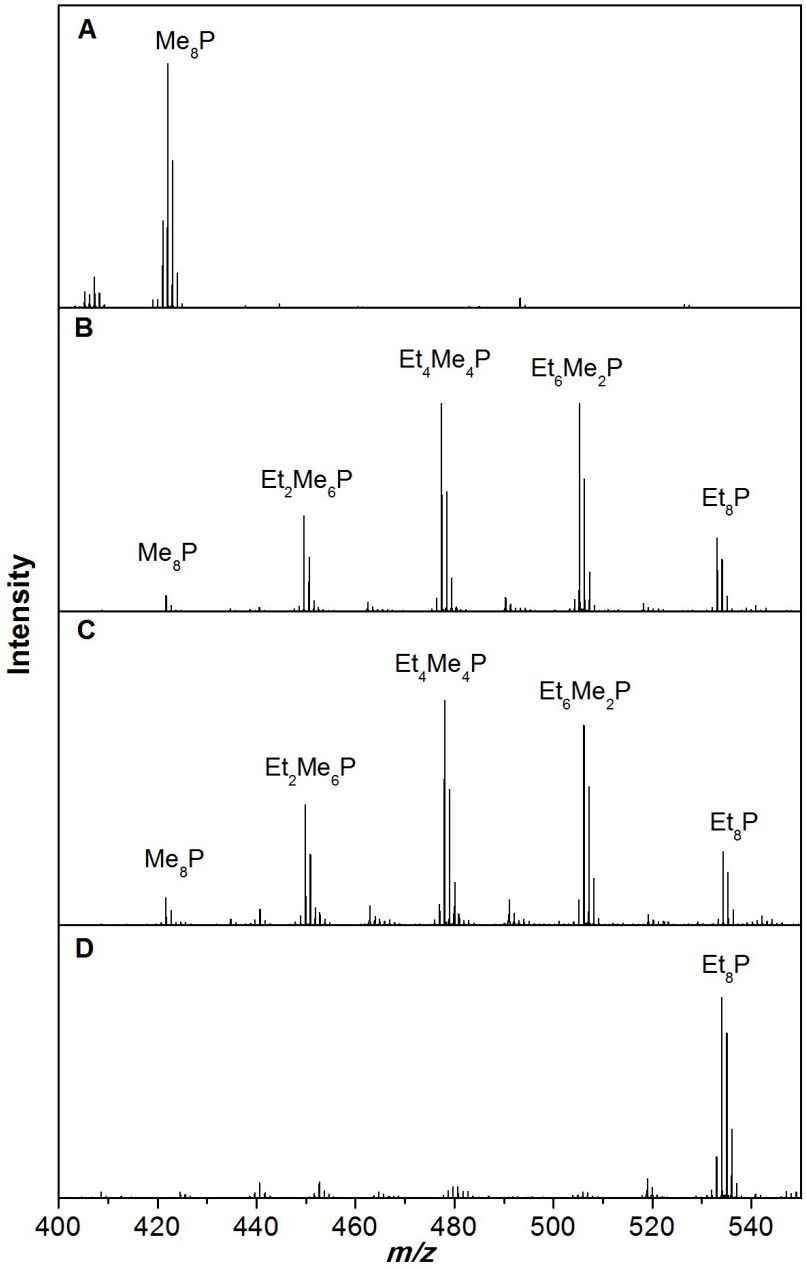


Figure S8. MALDI-MS spectra for double-labeling crossover experiment in 50 mM SDS with 100 mM NaCl (and no phosphate buffer) at 25 °C. Panels A, B, C, and D correspond to **1-Me** only (yield = 26%), pre-mix control (37%), post-mix (35%), and **1-Et** only (37%), respectively.

Figure S9 shows the double-labeling crossover experiment in 50 mM SDS using 20 mM NaCl in aqueous solution (no phosphate buffer) at 25 °C. The reaction of **1-Et** at 0.046 mM and 100 equiv of formaldehyde at 25 °C in 50 mM SDS and 20 mM NaCl for 24 h gave 48% yield. Under the same conditions the reaction of **1-Me** at 0.046 mM and 100 equiv of formaldehyde gave 27% yield. The pre-mix control reaction gave a 38% yield and the post-mix reaction gave a 40% yield.

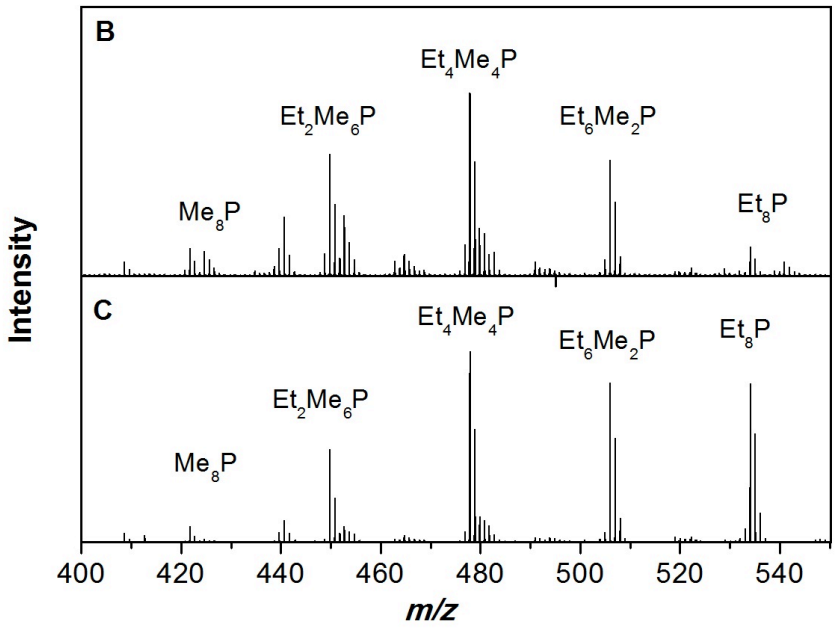


Figure S9. MALDI-MS spectra for double-labeling crossover experiment in 50 mM SDS with 20 mM NaCl (and no phosphate buffer) at 25 °C. Panel B corresponds to the pre-mix control (yield = 38%) whereas panel C corresponds to the post-mix experiment (40%).

Figure S10 shows the double-labeling crossover experiment in phosphatidylcholine vesicles.

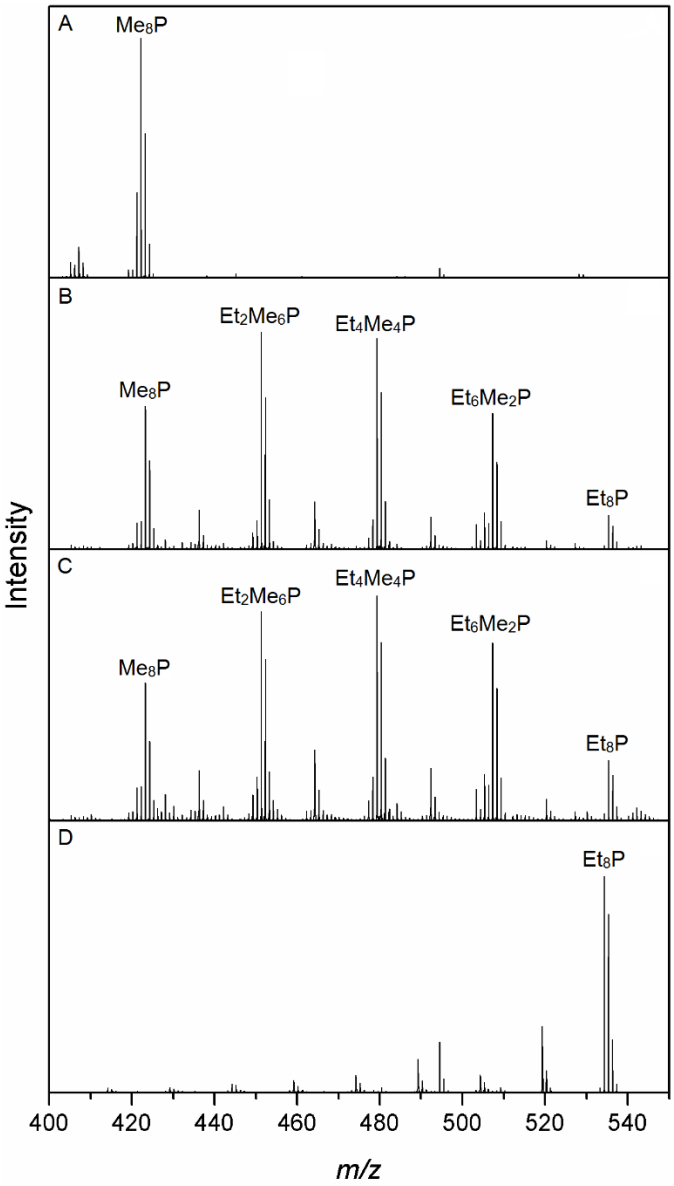


Figure S10. MALDI-MS spectra for double-labeling crossover experiment in vesicles derived from 0.3 mM phosphatidylcholine (pH 7) at 25 °C. Panels A, B, C, and D correspond to **1-Me** only (not detected), pre-mix control (20%), post-mix (18%), and **1-Et** only (19%), respectively.

6. Methanol control experiments

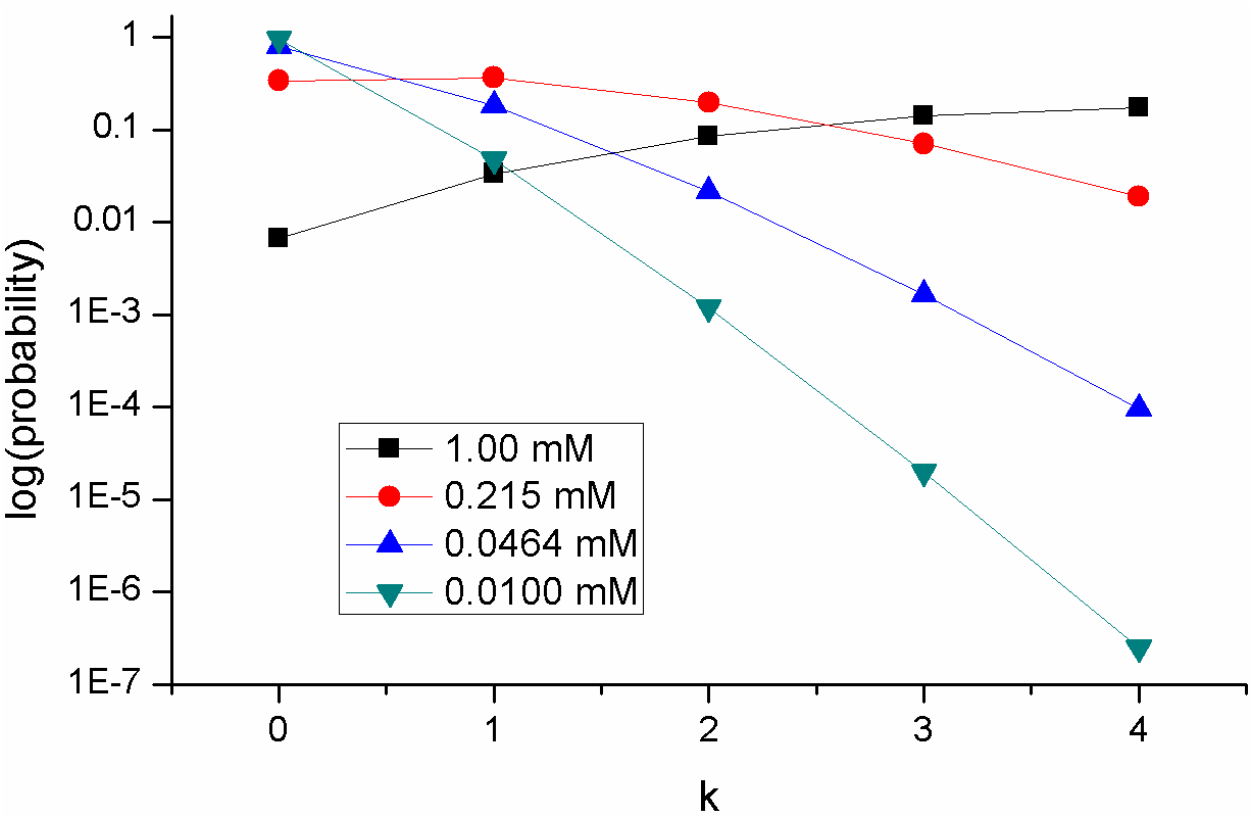
For all reactions, the pyrrole stock solution (**1-Et**, **1-Me**) was prepared in methanol, owing to the limited solubility of the pyrrole in water. Methanol was chosen due to its complete miscibility with water and its presence already as a stabilizer in commercially available aqueous formaldehyde solutions. The aqueous formaldehyde reagent (37 wt%) contains 10–15% methanol. Assuming 15% methanol in the formaldehyde, at the highest concentration of formaldehyde (100 mM) employed, the amount of methanol added to the reaction vial from the formaldehyde source was 1.1 μL. The contribution from the pyrrole stock was 25 μL (and this was constant across all reactions). Thus, the maximum methanol concentration was 0.32 M, and the contribution of methanol from the formaldehyde aliquot was miniscule.

To test the effect of methanol on porphyrin formation, several reactions were set up with increasing amounts of added methanol (up to 160 μL in addition to the **1-Et** stock). The data are shown in Table S3. From these results, it can be seen that there is a small effect of methanol on porphyrin yields. Specifically, the yield is lower when more methanol is added, compared to that of a standard reaction.

Table S3. Yield of **Et_8P** for reactions with varying amounts of added CH_3OH. All reactions were performed with 0.046 mM **1-Et** and 4.64 mM formaldehyde.

Added CH_3OH (μL)	[CH_3OH], M	Yield (%)
20	0.25	11.2
40	0.49	12.5
80	0.99	9.7
160	1.98	9.4

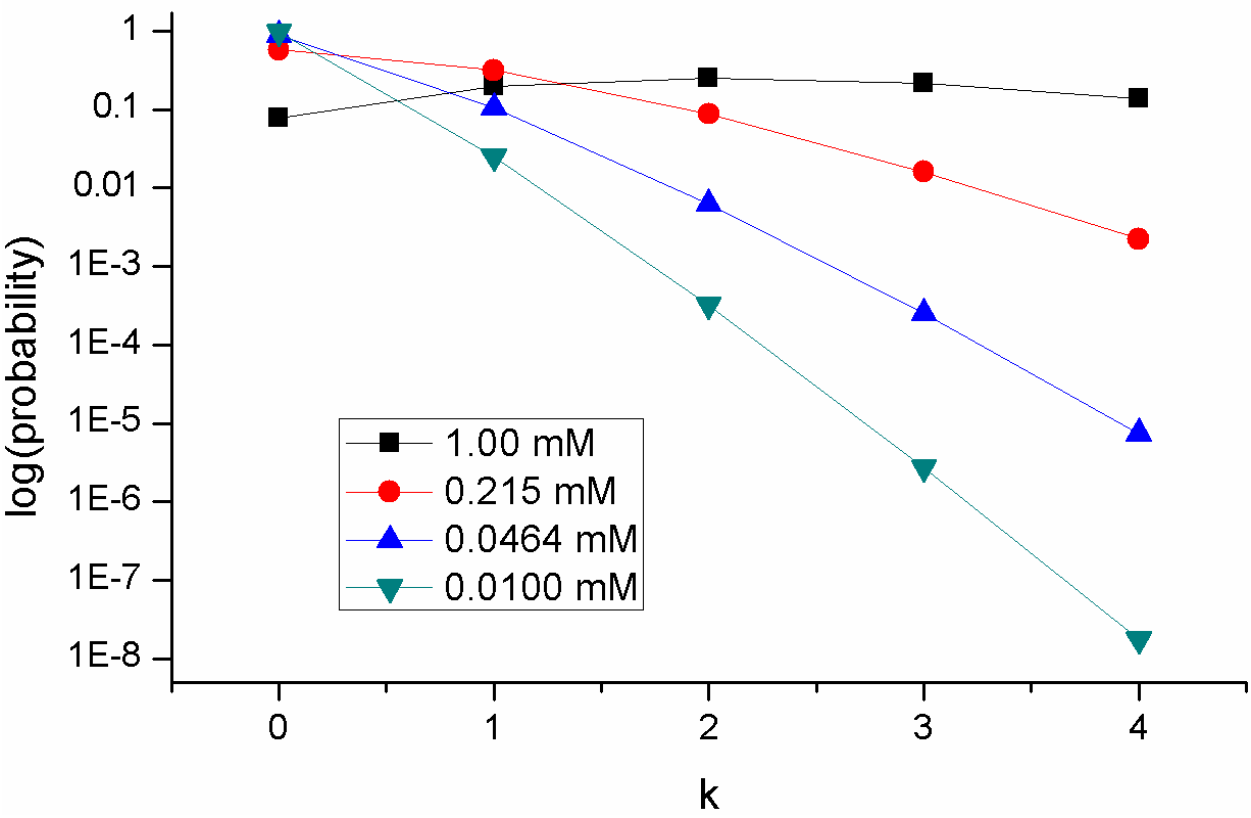
7. Poisson calculations



Poisson statistics for a micelle concentration of 0.20 mM

k, number of pyrroles/micelle	Pyrrole concentration			
	1.00 mM	0.215 mM	0.0464 mM	0.0100 mM
0	0.006738	0.341298	0.792946	0.951229
1	0.03369	0.366895	0.183964	0.047561
2	0.084224	0.197206	0.02134	0.001189
3	0.140374	0.070666	0.00165	1.98E-05
4	0.175467	0.018991	9.57E-05	2.48E-07

Figure S11. Poisson distribution of the fraction of micelles having k = 0–4 pyrroles over a 100-fold range of pyrrole concentrations (for 0.20 mM micelles), in graphical and tabular display.

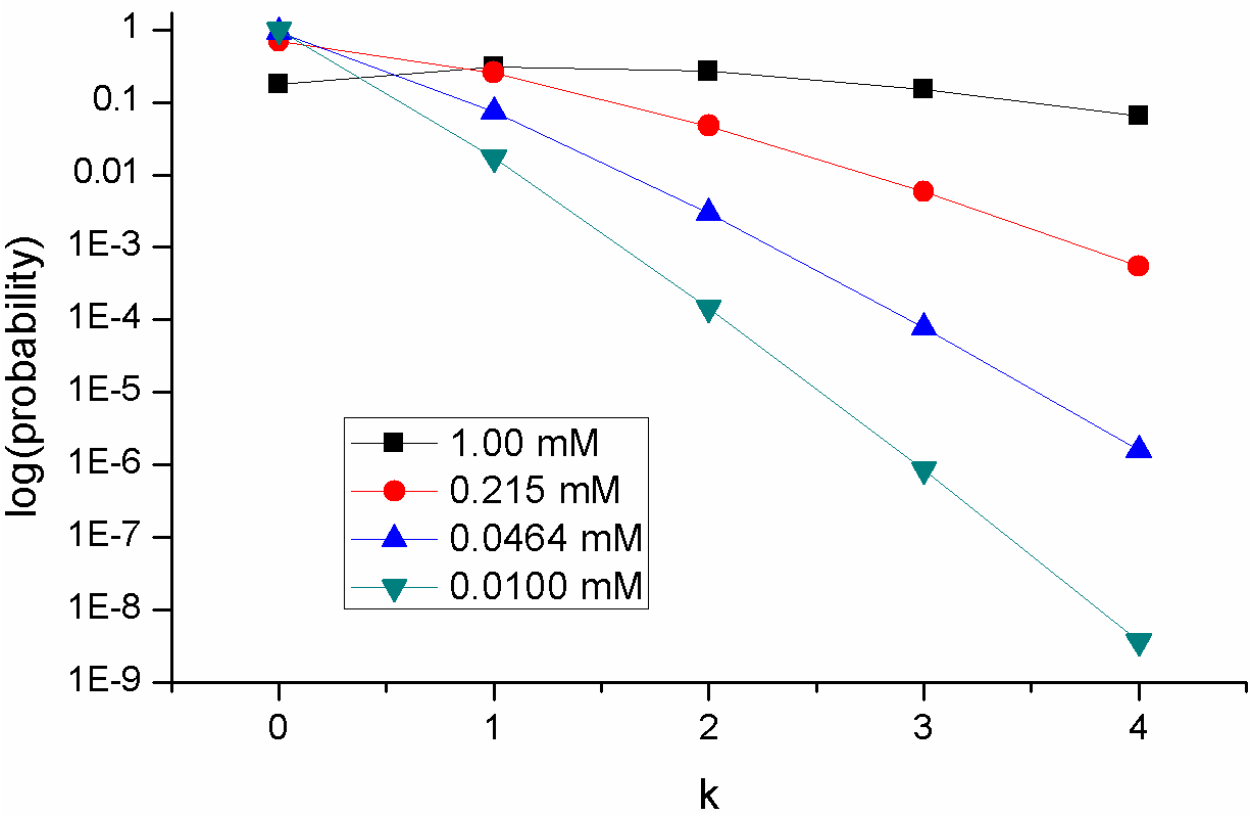


Poisson statistics for a micelle concentration of 0.39 mM

k, number of pyrroles/micelle	Pyrrole concentration			
	1.000 mM	0.215 mM	0.0464 mM	0.0100 mM
0	0.076988	0.576211	0.887831	0.974685
1	0.197406	0.317655	0.105629	0.024992
2	0.253084	0.087559	0.006284	0.00032
3	0.216311	0.01609	0.000249	2.74E-06
4	0.138661	0.002218	7.41E-06	1.76E-08

Figure S12. Poisson distribution of the fraction of micelles having k = 0–4 pyrroles over a 100-fold range of pyrrole concentrations (for 0.39 mM micelles), in graphical and tabular display. This figure is identical to Figure 4 in the body of the paper and is included here for completion.

Note that consideration of micelles with ≥5 pyrrole molecules in this regime (for m = 0.12) appears inconsequential. For example, the fraction of micelles that contain k = 5 pyrroles is 1.8×10^{-7}; and for k = 6, the fraction is 3.7×10^{-9}.



Poisson statistics for a micelle concentration of 0.58 mM

k, number of pyrroles/micelle	Pyrrole concentration			
	1.00 mM	0.215 mM	0.0464 mM	0.0100 mM
0	0.178327	0.690258	0.923116	0.982906
1	0.30746	0.255872	0.073849	0.016947
2	0.265052	0.047424	0.002954	0.000146
3	0.152329	0.00586	7.88E-05	8.4E-07
4	0.065659	0.000543	1.58E-06	3.62E-09

Figure S13. Poisson distribution of the fraction of micelles having k = 0–4 pyrroles over a 100-fold range of pyrrole concentrations (for 0.58 mM micelles), in graphical and tabular display.

8. Partition coefficient calculations

The molecules shown in Figure S14 were employed in calculations of the *n*-octanol/water partition coefficient (P). The "Log P" and "ClogP" results stem from different calculation methods, not experimental data. Note the profound variation in values for a given compound.

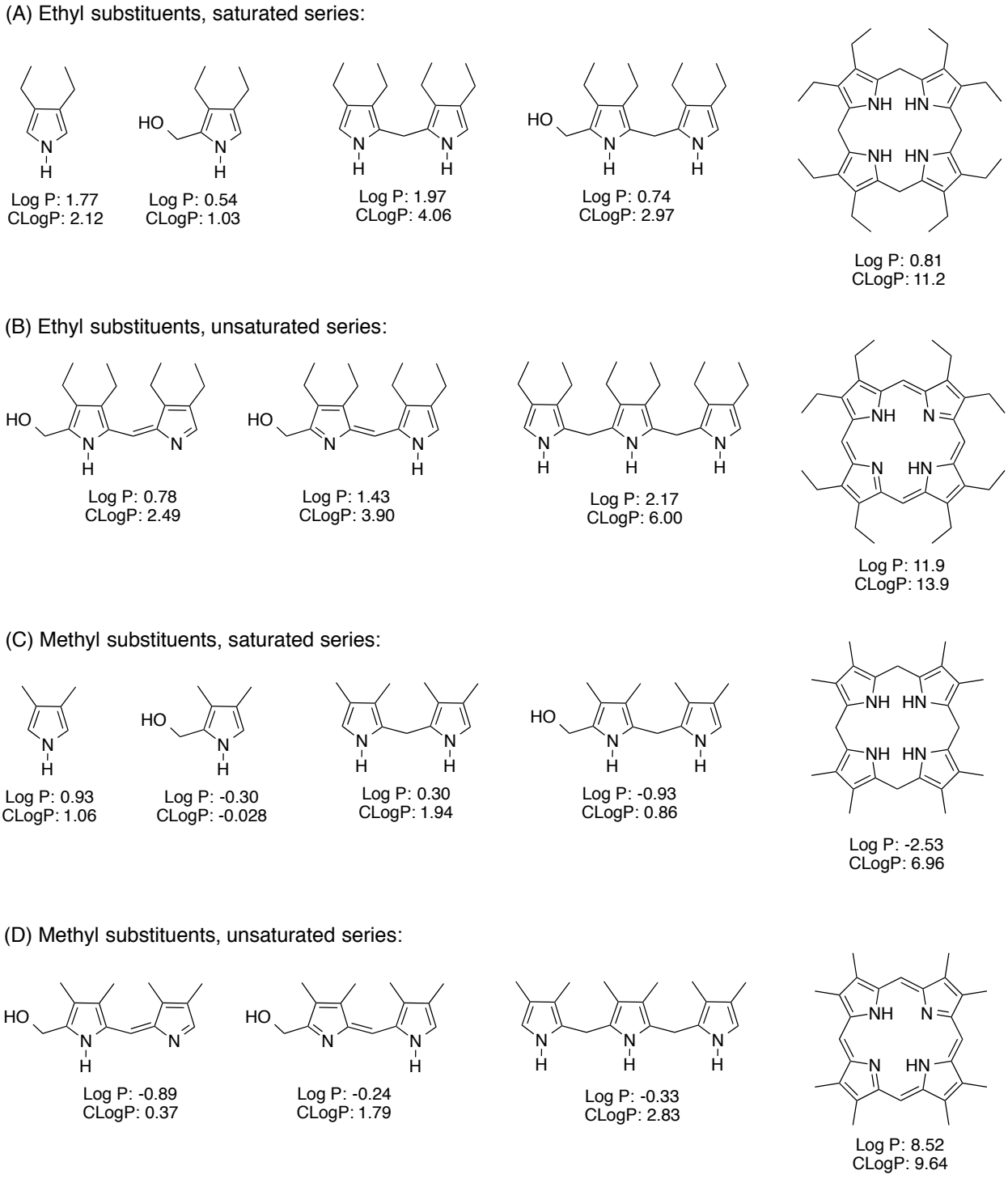


Figure S14. Calculated partition coefficients for putative reactants and products.