Supplementary Information for:

Self-Organization of Tetrapyrrole Constituents to Give a Photoactive Protocell

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Table S1. Data for *in situ* oxidation of the combinatorial reaction products in the presence of vesicles and **NQS**.[a]

Entry	**NQS**, mM	hν[b]	Yield, %	
			Crude reaction mixture[c]	After I_2 oxidation[d]
1	0	No	1.2	7.6
2	0	Yes	3.8	11
3	4	No	1.4	5.4
4	4	Yes	4.5	8.7
5	6	No	2.3	5.3
6	6	Yes	2.6	4.2
7	12	No	1.9	3.4
8	12	Yes	2.1	2.8
9	16	No	1.5	2.7
10	16	Yes	1.5	1.9

[a] Each reaction mixture contains 30 mM of each of the four reactants, vesicles (20 mM [lipid]), the glucose oxidase/catalase system, and 0, 4, 6, 12 or 16 mM of **NQS**. The reactions were carried under anaerobic conditions for 24 h at 60 °C.
[b] Illumination was performed with the solar simulator using an AM 1D filter with total output power density of 3060 $W \cdot m^{-2}$. The chamber was illuminated for 39 min (corresponding to 1.4 sun hours).
[c] Yield of porphyrin *in situ* following stages 1–4 in Figure 1.
[d] Yield upon treatment of a reaction sample from the crude reaction mixture (following stages 1–4) with I_2 to give exhaustive conversion of porphyrinogens to porphyrins.

Table S2. Data for *in situ* oxidation of the combinatorial reaction products in the presence of vesicles.[a]

Entry	Quinone	hν[b]	Yield (%)		Percentage of oxidation[e] (%)
			Crude reaction mixture[c]	After I_2 oxidation[d]	
1	No	No	2.2	11	20
2	No	Yes	3.5	11	32
3	**NQS** + **TCQ**	No	3.3	8.7	38
4	**NQS** + **TCQ**	Yes	3.8	7.9	48

[a] Each reaction mixture was carried out at 30 mM for each of the four reactants for 24 h at 60 °C and pH 7, in the presence of vesicles (20 mM [lipid]), and 4 mM of hydrophilic (**NQS**) and hydrophobic (**TCQ**) quinones. The reactions were performed in the absence of the glucose oxidase/catalase system.
[b] Illumination was performed with the solar simulator for 69 min using an AM 0D filter.
[c] Yield of porphyrin *in situ* following stages 1–4 in Figure 1.
[d] Yield upon treatment of a reaction sample from the crude reaction mixture (following stages 1–4) with I_2 to give exhaustive conversion of porphyrinogens to porphyrins.
[e] Percentage of oxidation in the crude reaction mixture (following stages 1–4) versus that upon subsequent chemical oxidation (with I_2).

Spectroscopic studies of benchmark porphyrins in diverse media.

Spectroscopic data for the three mixtures were compared with data for four standard porphyrins in representative media. The four standard porphyrins include etioporphyrin I, mesoporphyrin IX, coproporphyrin I and uroporphyrin III, which encompass a broad range of polarity. Identical quantities of the four porphyrins were examined in the distinct media. The media include (i) DMF, where each porphyrin is expected to be monomeric; (ii) aqueous solution; (iii) aqueous solution followed by the addition of vesicles and 1 h incubation at 37 °C, which assesses whether an aggregated sample might disaggregate upon assembly into a membrane; and (iv) direct addition to an aqueous suspension of vesicles. To determine the absorbance of the porphyrins, Abs (λ_{max}), the absorption/scattering caused by the vesicles alone was subtracted from the overall absorption spectra.

Absorption spectra of the four porphyrins in each medium are shown in Figure S1. Emission spectra of the four porphyrins in each medium are shown in Figure S2. In DMF, all four porphyrins showed a sharp Soret band and typical porphyrin fluorescence emission bands, consistent with porphyrins in a monomeric form. The spectral parameters in conjunction with fluorescence quantum yield values are listed in Table S3. While most of the benchmark porphyrins have been employed over the years in a wide range of studies, few fluorescence quantitative yield measurements have been reported. Noteworthy points are as follows:

Etioporphyrin gave negligible fluorescence in aqueous solution, consistent with expectations for a high degree of aggregation. The addition of vesicles to the aggregated sample gave little recovery, yet the addition of etioporphyrin to the suspension containing vesicles resulted in a Φ_f value approximately ¼ that of etioporphyrin in DMF. This result suggests the importance of the presence of vesicles upon formation of hydrophobic porphyrins, at least on the timeframe examined herein.

Mesoporphyrin exhibited fluorescence emission in aqueous solution diminished by ~5-fold versus that in DMF. The addition of vesicles resulted in immediate recovery to half the fluorescence observed in DMF. Upon incubation for 1 h, or upon addition of mesoporphyrin to an aqueous suspension containing vesicles, the Φ_f value was comparable to that in DMF. Thus, the amphiphilic mesoporphyrin readily assembles from aqueous solution into vesicles. It should be noted that the emission spectrum of mesoporphyrin is shifted bathochromically in the presence of vesicles versus that in DMF.

Coproporphyrin exhibited fluorescence emission in aqueous solution diminished by only ~2-fold versus that in DMF. The addition of vesicles to the aqueous solution, or the addition of coproporphyrin to an aqueous suspension containing vesicles, resulted in a Φ_f value of ~0.07, to be compared with 0.11 in DMF. Regardless of order of addition, the emission spectrum was broadened and appeared to be the sum of emission from distinct species, a DMF-like monomer emission and a hypsochromically shifted emission. One possible interpretation is that a fraction of coproporphyrin associates as a monomer with the vesicle bilayer, while another fraction remains in a small aggregate in aqueous solution or associated with the vesicle bilayer.

Uroporphyrin exhibited fluorescence emission in aqueous solution (Φ_f ~0.12) that was relatively unchanged by the presence of vesicles (Φ_f ~0.1). Both values were greater than that in DMF (Φ_f = 0.078). The emission spectra in aqueous solution (with or without vesicles) are hypsochromically shifted versus that in DMF. The difference in spectra and emission yield may be due to a medium effect or electrostatics owing to more extensive ionization of uroporphyrin in aqueous solution. Regardless, the photochemical integrity of uroporphyrin, using fluorescence emission as a proxy, was not affected by the presence of vesicles in the aqueous solution.

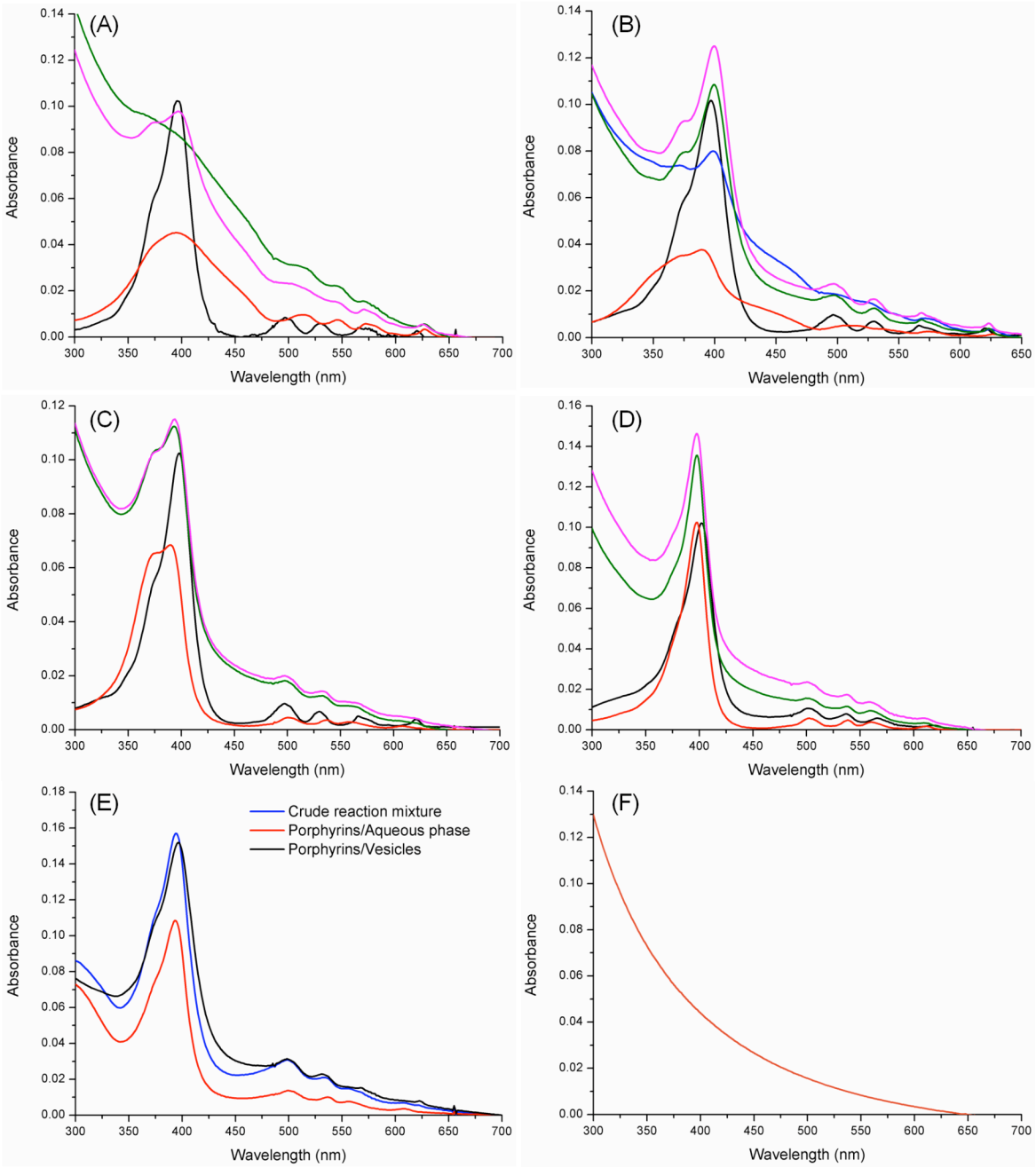

Figure S1. Absorption spectra of etioporphyrin I (A), mesoporphyrin IX (B), coproporphyrin I (C), and uroporphyrin III (D) at room temperature in various media: in DMF (black); in aqueous solution (0.1 M potassium phosphate buffer, pH 7; red), after addition of vesicles and incubation for 1 h at 37 °C (green); and after direct addition to vesicles (magenta). The spectrum in blue in panel B is immediately after addition of vesicles. The absorption spectra in panel E are from the crude reaction mixture and the two phases following SEC separation. The absorption (light-scattering) of vesicles is shown in panel F.

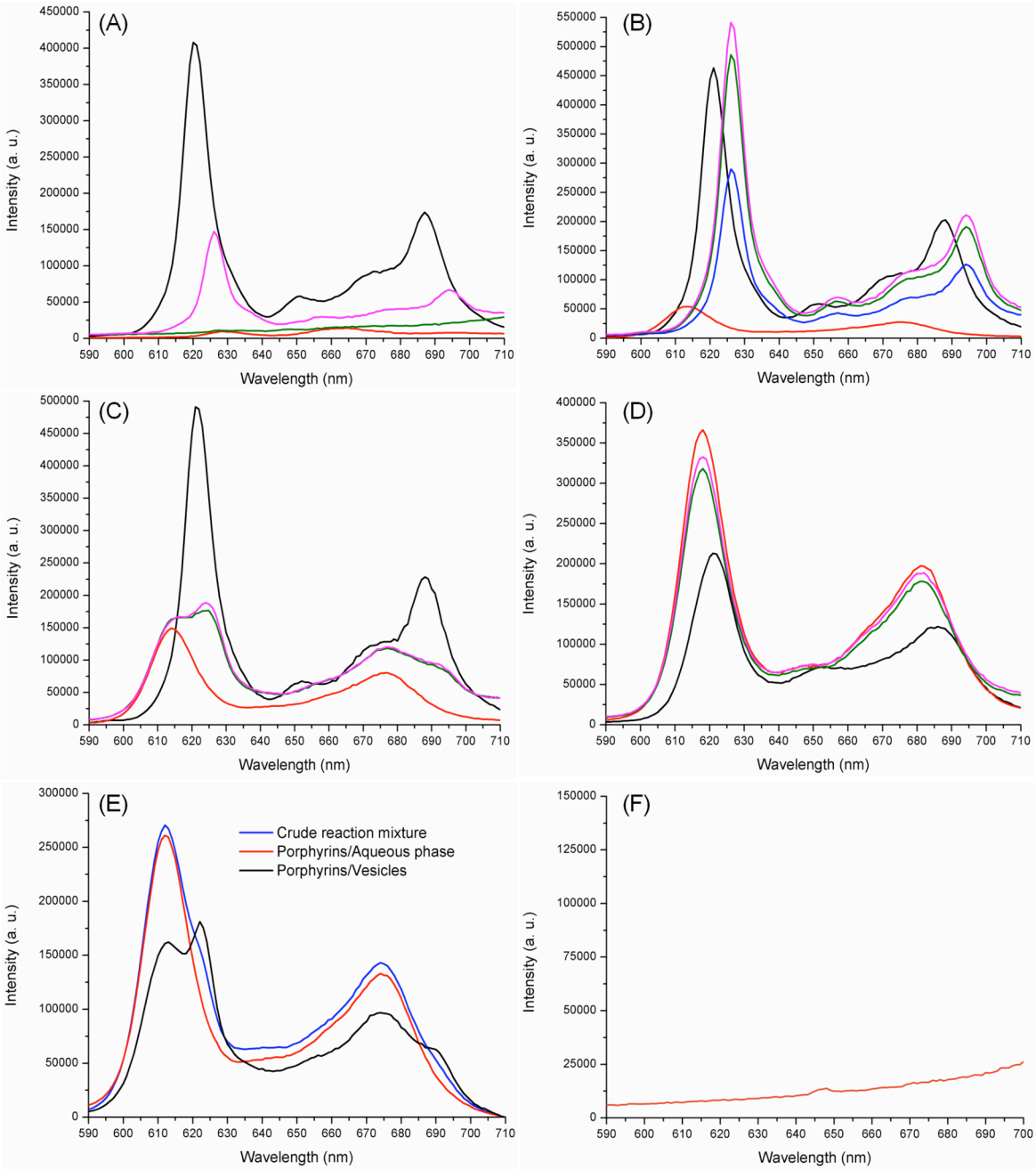

Figure S2. Fluorescence emission spectra of etioporphyrin I (A), mesoporphyrin IX (B), coproporphyrin I (C), and uroporphyrin III (D) at room temperature in various media: in DMF (black); in aqueous solution (0.1 M potassium phosphate buffer, pH 7; red), after addition of vesicles and incubation for 1 h at 37 °C (green); and after direct addition to vesicles (magenta). The spectrum in blue in panel B is immediately after addition of vesicles. The emission spectra in panel E are from the crude reaction mixture and the two phases following SEC separation. The emission (light-scattering) of vesicles is shown in panel F. The fluorescence emission spectra were obtained upon excitation at the maximum of the Soret band.

Table S3. Absorption and fluorescence data for porphyrins in various media.

Sample[a]	Medium	λ_{ex}(nm)[b]	Abs (λ_{ex})	λ_{em} (nm)	Φ_f
etio	DMF	396	0.102	620, 687	0.091
	Aq solution	396[c]	0.045	628, 662[d]	0.007
	Aq; then vesicles, 1 h	396[c]	0.042	[d]	0
	Aq + vesicles	397	0.052	626, 694	0.024
meso	DMF	397	0.102	621, 688	0.102
	Aq solution	390	0.038	613, 675[d]	0.018
	Aq; then vesicles, 0 h	399	0.036	626, 694	0.052
	Aq; then vesicles, 1 h	400	0.064	626, 694	0.087
	Aq + vesicles	400	0.081	626, 694	0.098
copro	DMF	398	0.102	621, 688	0.113
	Aq solution	390	0.068	614, 677	0.049
	Aq; then vesicles, 1 h	393	0.065	624,[e] 677	0.071
	Aq + vesicles	394	0.068	624,[e] 677	0.073
uro	DMF	402	0.102	621, 686	0.078
	Aq solution	397	0.102	618, 682	0.120
	Aq; then vesicles, 1 h	398	0.091	618, 682	0.096
	Aq + vesicles	397	0.101	618, 682	0.103
Crude rxn mixture	Aq + vesicles	394	0.116	612, 674	0.086
Por/aqueous phase	Aq + vesicles	394	0.108	612, 674	0.084
Por/vesicles	Aq + vesicles	397	0.105	622, 674[f]	0.072

[a] The four standard porphyrins are etioporphyrin I (etio), mesoporphyrin IX (meso), coproporphyrin I (copro) and uroporphyrin III (uro). The four porphyrins were employed in identical amounts.
[b] The λ_{ex} listed also is the Soret λ_{max} unless noted otherwise.
[c] A broad absorption was observed with no distinct λ_{max}; hence, the emission spectrum was obtained at the same excitation wavelength used for the DMF solutions.
[d] Broad featureless spectrum.
[e] A shoulder was present at ~614 nm.
[f] An additional band was present at 613 nm and a shoulder was present at ~690 nm.

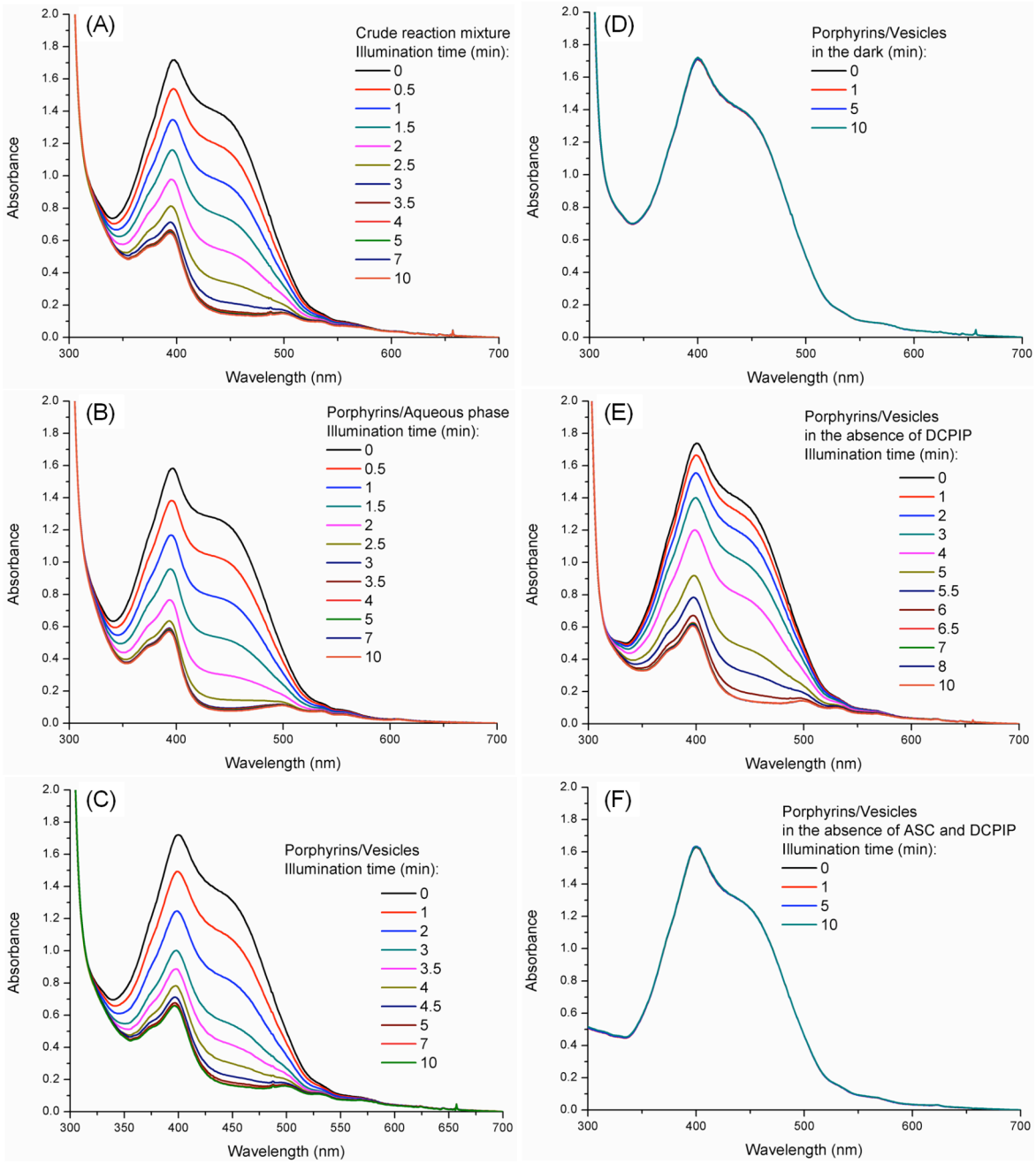

Figure S3. Spectral change upon illumination of various samples in the presence of the Krasnovsky reaction constituents. (A) Crude reaction mixture, (B) porphyrins/aqueous phase, and (C-F) porphyrins/vesicles. Panels D-F concern omission experiments of the porphyrins/vesicles: (D) in the dark, (E) in the absence of DCPIP, and (F) in the absence of DCPIP and ascorbic acid (ASC). Panel C is shown as Figure 5 in the paper and is repeated here to facilitate comparison.

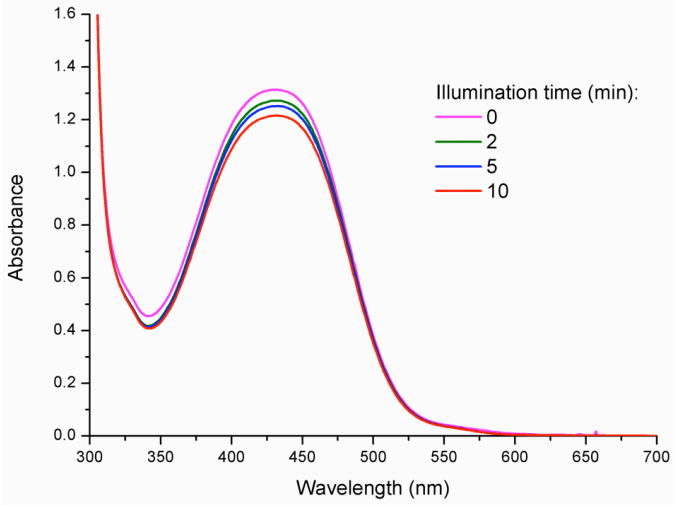

Figure S4. Spectral change upon illumination of a sample in the presence of the Krasnovsky reaction constituents with omission of the porphyrins.

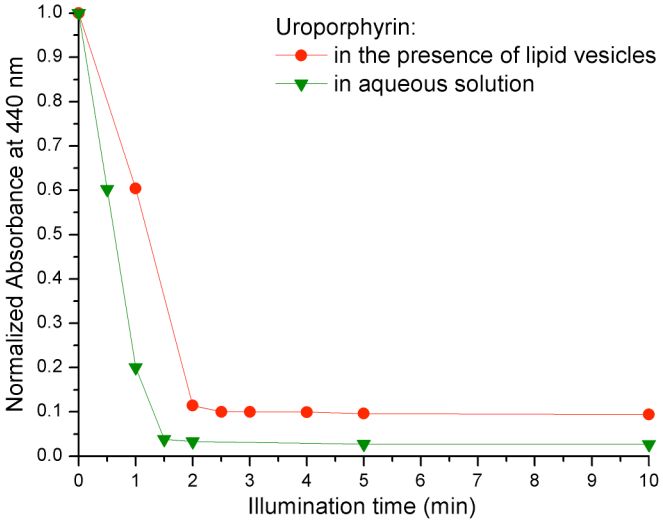

Figure S5. Time course of methyl red photoreduction (440 nm) in the Krasnovsky reaction with uroporphyrin I. The data are shown for the reaction in the presence of vesicles (red circles) or in 0.1 M potassium phosphate buffer (green inverted triangles).

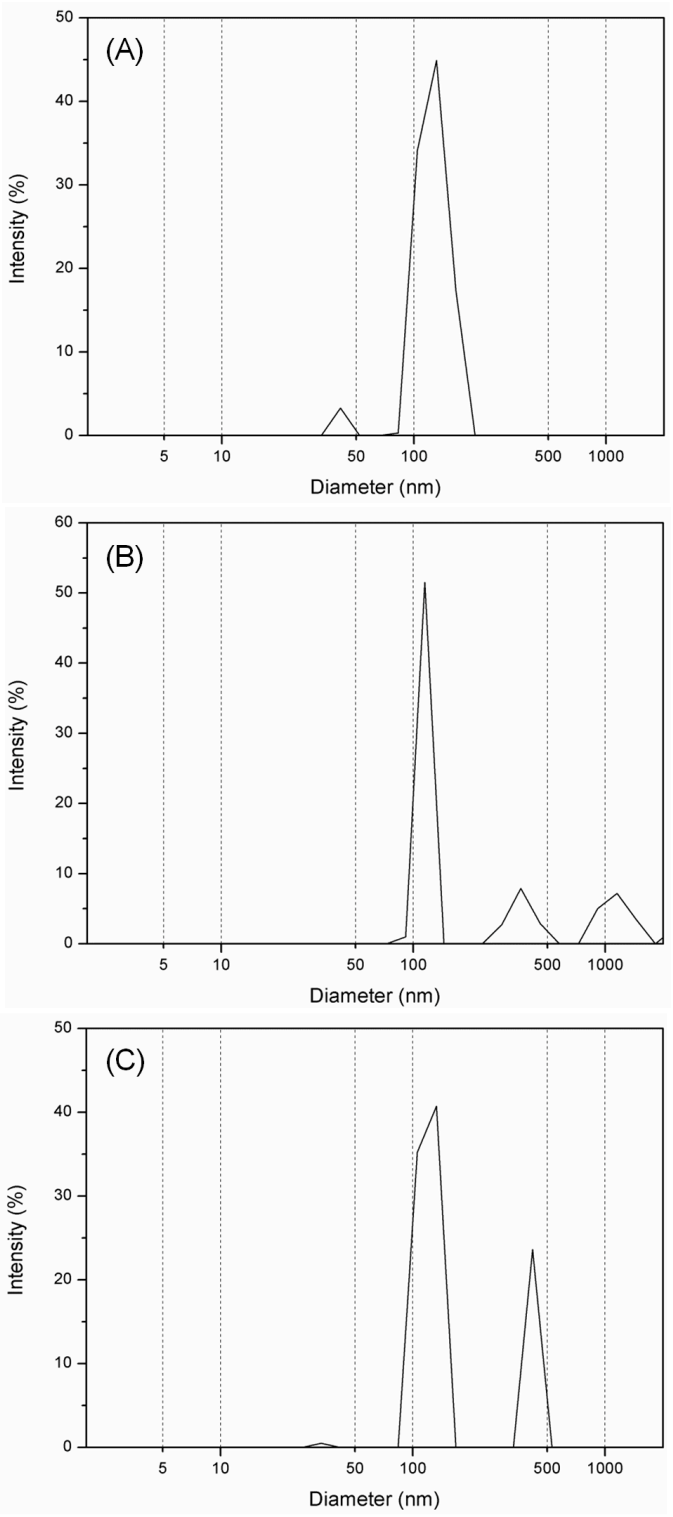

Figure S6. Size distributions of vesicles determined by DLS. (A) After extrusion. (B) In the crude reaction mixture (entry 8, Table 1). (C) Porphyrins/vesicles (the fraction after separation by SEC).