Supplementary Information for:

Aqueous-Membrane Partitioning of β-Substituted Porphyrins Encompassing Diverse Polarity

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Table of Contents

I. RP-HPLC traces (controls).

The presence of trace peaks with retention times resembling those of the hydrophobic porphyrins (**P2**, **P0**) was a source of concern (Figure 2). All of the data reported in the body of the paper employed a single HPLC column, here termed HPLC column I.

A number of injections were performed under various conditions to assess the identity of such peaks. Representative results are shown in Figure S1. Panel A shows the same trace as is present in Figure 2 panel A, here with expanded y-scale, but without most of the * labels. In Figure 2 panel A, full scale is a signal of 15; here Figure S1 panel A has full scale of 0.6, an expansion of 25-fold. The expanded scale affords a better display of the peaks (* in Figure 2 panel A) that accompany porphyrins **P2** and **P0**.

A blank solution (15 μL of water, no porphyrins) was injected under otherwise identical conditions as in panel A, and the results are shown in panel B (HPLC column I). The chromatogram closely resembled that in panel A with the exception that peaks due to porphyrins **P8**, **P7**, **P6**, **P4**, **P2** and **P0** were absent (as expected).

We next turned to a newly purchased HPLC column (here termed HPLC column II), which in our hands was never exposed to porphyrins, and, hence, to our knowledge had never been exposed to porphyrins. A blank solution (15 μL of water, no porphyrins) was injected under otherwise identical conditions as in panel A, and the results are shown in panel C. Again, peaks similar to those of panel B were observed.

Finally, a blank solution (15 μL of water, no porphyrins) was injected into HPLC column II under identical conditions as in panel C except for excitation at 450 nm, a wavelength where porphyrins absorb very weakly. The results are shown in panel D. While the baseline shifted extensively, the elution time of the sharp peaks generally resembled those of panels B and C although the peaks were of greater intensity. In panel E, the chromatogram is displayed where no column at all was employed, and a blank solution (15 μL of water, no porphyrins) was injected. The absence of peaks shows that the HPLC system is clean; therefore, the peaks observed with blank injections into HPLC columns I and II stem from the columns themselves. That there are such peaks is not entirely surprising given the somewhat severe solvent conditions, which employ a gradient composed of tetrahydrofuran (THF) and 0.1% formic acid in water. The gradient extends from 20% THF (0 min) to 100% THF (20 min) to cause elution of all hydrophobic substances. The gradient is shown in panel F.

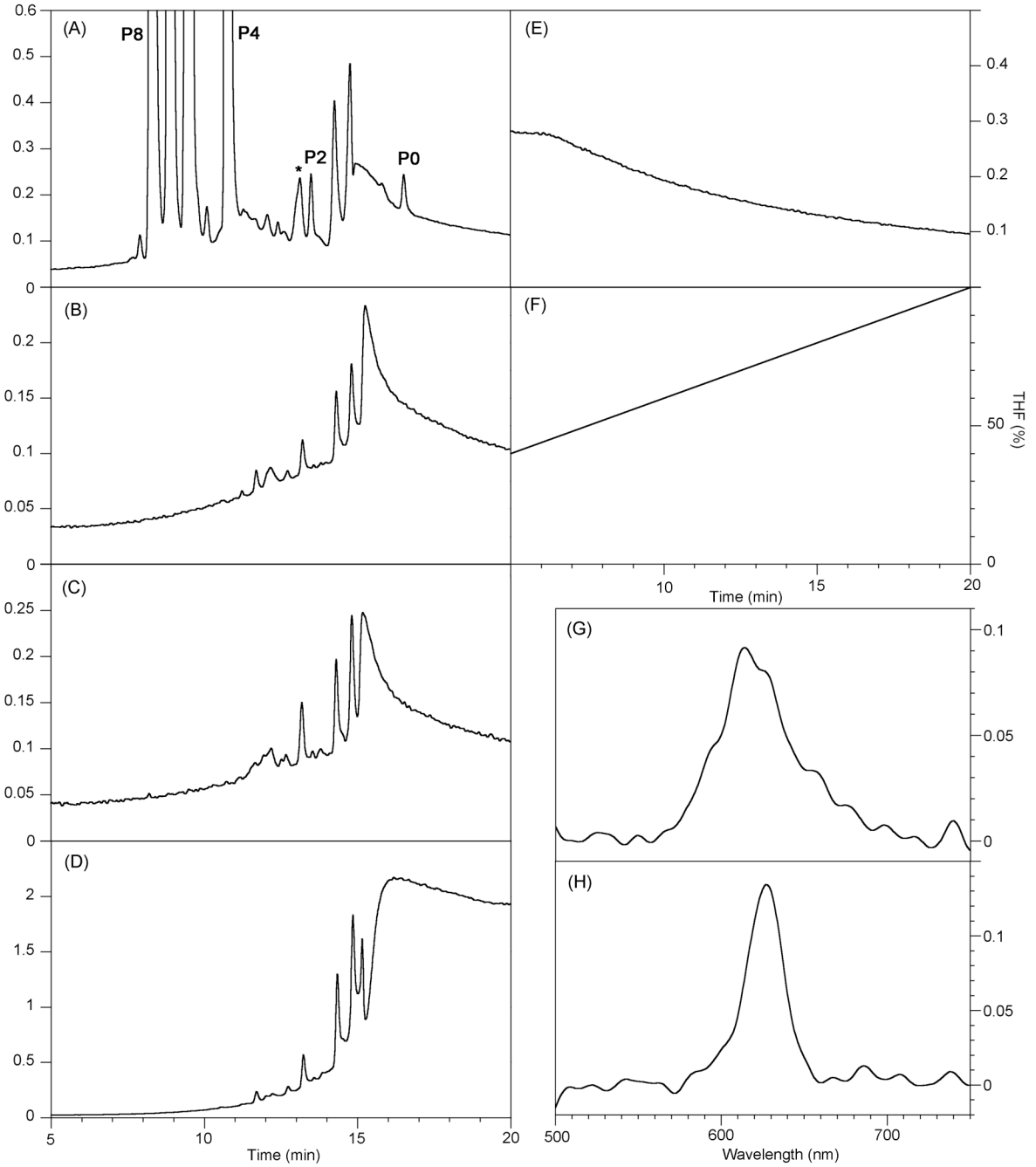

Figure S1. RP-HPLC chromatograms with fluorescence detection. (A) Analysis of porphyrins/aqueous fraction following SEC of the mixture (see Figure 2 panel A) using HPLC column I with fluorescence detection (λ_{exc} = 401, λ_{em} = 624 nm). (B) H_2O injection (15 µL) using HPLC column I with fluorescence detection (λ_{exc} = 401, λ_{em} = 624 nm). (C) H_2O injection (15 µL) using HPLC column II with fluorescence detection (λ_{exc} = 401, λ_{em} = 624 nm). (D) H_2O injection (15 µL) using HPLC column II with fluorescence detection (λ_{exc} = 450, λ_{em} = 624 nm). (E) H_2O injection (15 µL) without a column (λ_{exc} = 401, λ_{em} = 624 nm). (F) Gradient of THF and aqueous solution (0.1% formic acid) employed in all chromatograms. (G) Fluorescence spectrum collected online of the peak denoted with an asterisk (t_R = 13.1 min) from panel A. (H) Fluorescence spectrum collected online of the peak due to **P2** (t_R = 13.5 min) from panel A. For panels A-E, no peaks were observed before 5 min, hence this region is not displayed.

In all cases, the spectral properties of the unknown peaks did not resemble porphyrins as assessed by the fluorescence spectrum, collected with the online fluorescence detector during elution. A representative spectrum is shown in panel G for the peak at t_R = 13.1 min (denoted * in panel A). The broad spectrum is not typical for a porphyrin. The fluorescence emission spectrum of the peak due to **P2** is shown in panel H. The spectrum of the peak due to **P0** (not shown) was essentially identical to that of **P2**. None of the unassigned peaks (denoted with asterisks in Figure 2 panel A) gave spectra corresponding to porphyrins.

In summary, the unknown, typically tiny peaks (denoted * in Figure 2 panel A) are not due to the presence of residual porphyrinic species, but instead appear to stem from light-scattering from particles leaching from the column itself.

II. Calculated log*P* values for diverse porphyrins and coronene derivatives.

Table S1. clog*P* values of 13 porphyrins and 4 coronenes.

Comp.	clog*P* values							MW[h]
	ACD[a]	CD[b]	MI[c]	KW[d]	MSk[e]	ALOG[f]	XLOG[g]	
ZnP8	N/A	2.35	1.55	5.87	2.25	0.72	-0.94	894.12
P8	1.30	1.65	2.14	4.27	3.51	0.76	-1.01	830.75
P7	2.47	2.88	3.17	4.65	4.39	1.13	-0.11	786.74
P6	3.65	4.10	4.19	6.49	5.26	1.49	0.79	742.73
P4	5.75	6.39	6.24	7.25	7.02	2.53	2.60	654.71
P2	8.35	9.07	8.17	8.95	8.77	4.82	4.68	566.69
P0	10.95	11.75	9.14	11.55	10.52	6.70	6.45	478.67
P	4.63	6.47	5.25	4.48	4.63	3.04	3.90	310.35
P8'	0.79	N/A	3.12	4.17	2.58	0.99	0.15	718.88
P7'	2.11	N/A	4.02	4.62	3.57	1.59	0.93	688.85
P6'	3.43	3.01	4.92	6.12	4.57	2.24	1.72	658.83
P4'	5.69	5.92	6.72	8.06	6.55	3.73	3.30	598.77
P2'	8.32	8.84	8.36	10.01	8.53	5.05	5.03	538.72
MC	10.98	11.05	9.26	12.04	10.52	6.70	9.97	570.72
CC	7.71	7.98	8.11	10.06	8.32	3.60	7.99	644.71
UC	3.01	3.08	4.03	5.61	4.82	1.34	3.85	820.75
C	7.38	7.04	7.13	7.28	5.94	7.26	7.24	300.35

[a]ACD/Labs software. [b]ChemBioDraw software. [c]Molinspiration software. [d]KowWin software. [e]MarvinSketch software. [f]ALOGPS 2.1 software. [g]XLOGP3 software. [h]Molecular weight. N/A not available.

III. Calculated log*P* values for diverse organic compounds.

The partition coefficients were calculated for the compounds shown in Figure S2: phenol (**1**), benzoic acid (**2**), naphthalene (**3**), 2-phenylacetic acid (**4**), naphthalene-2,6-diol (**5**), adamantane-1-carboxylic acid (**6**), naphthalene-2,6-dicarboxylic acid (**7**), benzo[*a*]pyrene (**8**), benzo[*a*]pyrene-7,8-epoxide (**9**), benzo[*a*]pyrene-7,8-dihydrodiol (**10**), glutathione (**11**), coronene-2-methanol (**12**), coronene-2-acetic acid (**13**), cholecystokinin tetrapeptide (CCK-4, **14**). The clog*P* values are listed in Table S2 and shown in Figure S3.

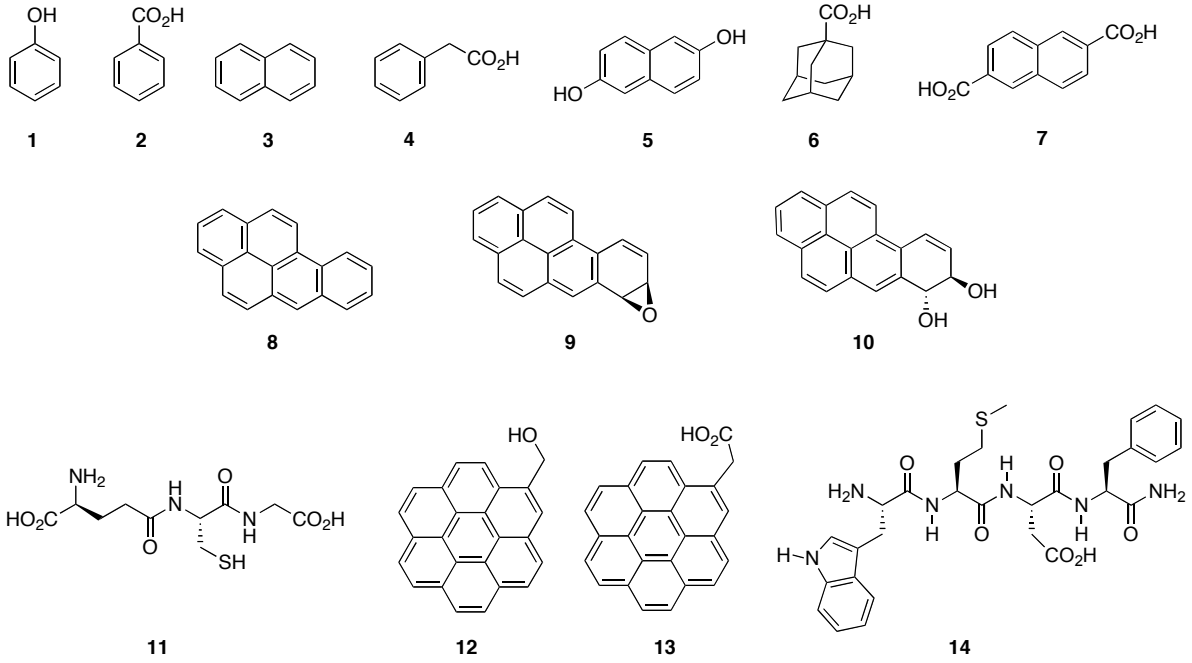

Figure S2. Organic compounds used for clog*P* calculations.

Table S2. clog*P* values of 14 organic compounds from different software programs.[a]

Comp.	clog*P* values							MW
	ACD	CD	MI	KW	MSk	ALOG	XLOG	
1	1.48	1.48	1.46	1.51	1.67	1.39	1.46	94.11
2	1.89	1.89	1.85	1.87	1.63	1.72	1.87	122.12
3	3.45	3.32	3.15	3.17	2.96	3.33	3.30	128.17
4	1.50	1.41	1.36	1.43	1.61	1.72	1.41	136.15
5	1.90	1.98	2.14	2.21	2.36	2.04	2.34	160.17
6	2.60	2.47	2.50	3.15	2.38	2.37	2.62	180.24
7	2.80	2.97	2.92	2.93	2.28	2.17	2.81	216.19
8	6.40	6.12	6.01	6.11	5.27	6.39	6.02	252.30
9	4.70	4.37	4.95	5.17	4.47	5.19	3.70	268.31

10	3.87	3.47	3.71	4.01	3.31	3.61	2.70	286.32
11	-0.87	-3.05	-4.97	-5.41	-2.53	-2.74	-4.50	307.32
12	6.20	6.01	6.40	6.37	5.17	6.54	6.09	330.38
13	6.67	6.32	6.48	6.72	5.57	6.19	6.43	358.39
14	1.95	-2.07	-0.18	0.26	0.43	-0.41	-2.13	596.70

[a] Abbreviations are identical with those in the preceding table.

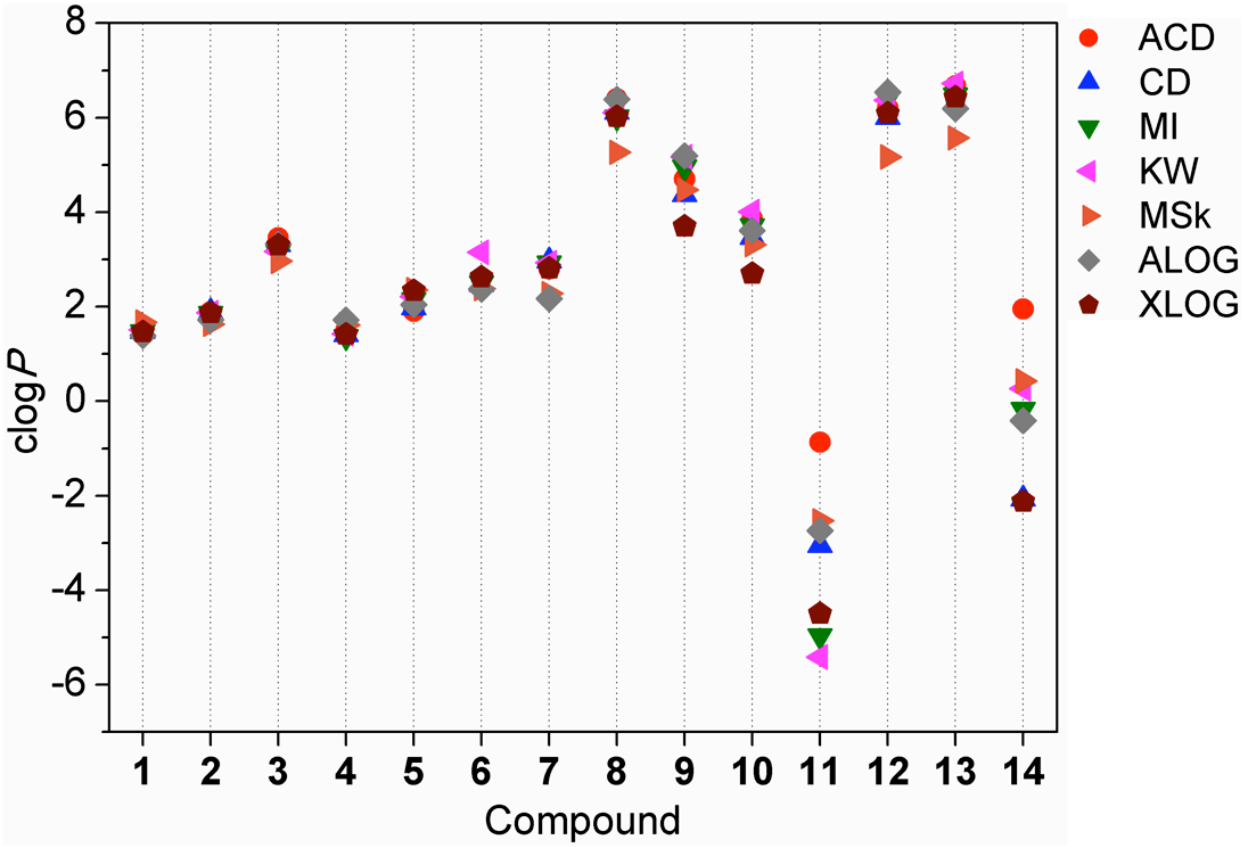

Figure S3. The clog*P* values of the 14 organic compounds from seven software programs.

IV. Absorption spectra of six standard porphyrins in acid.

The vesicles-aqueous partitioning of six standard porphyrins of diverse polarity was examined. The concentration of each porphyrin stock solution (**P8**, **P7**, **P6** and **P4** dissolved in 0.1 M potassium phosphate buffer (pH 7); **P2** and **P0** dissolved in THF) was determined by absorption spectroscopy in aqueous 1 M HCl using a molar absorption coefficient of 505,000 $M^{-1}cm^{-1}$ at the peak of the Soret band (~400 nm). The concentration of porphyrin in the cuvette was ~2.5 x 10^{-6} M. Absorption spectral peaks and fwhm (full-width-at-half-maximum) values of the standard porphyrins in acid are shown in Figures S4 and S5, and listed in Table S3.

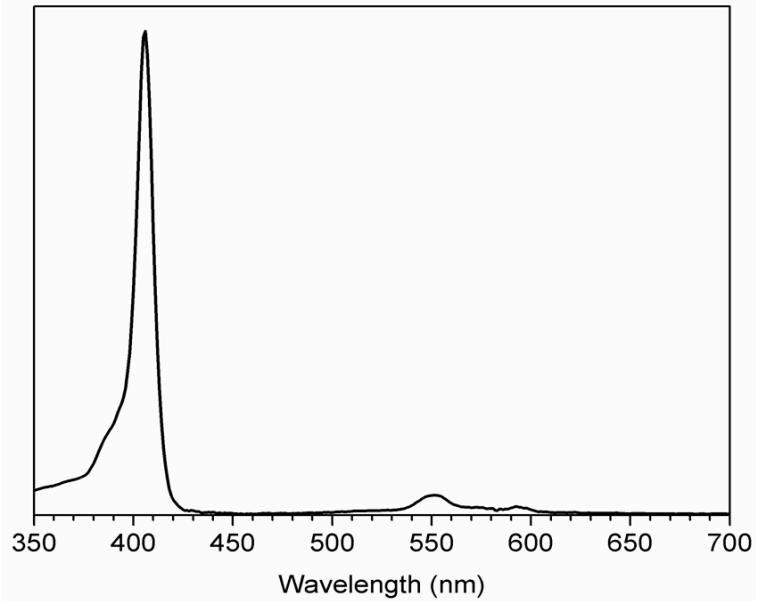

Figure S4. Absorption spectrum of uroporphyrin I (**P8**) in 1 M HCl at room temperature.

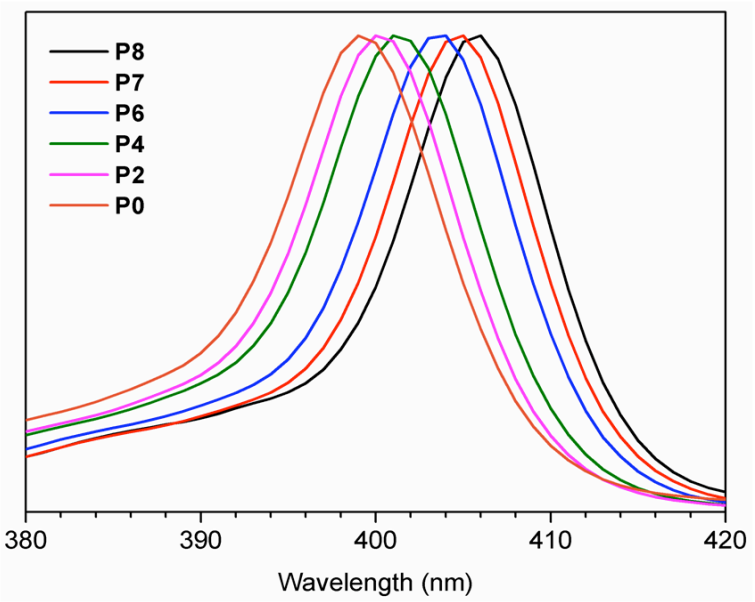

Figure S5. Absorption spectra of porphyrins in aqueous 1 M HCl. Legend: **P8** (uroporphyrin I), **P7** (heptacarboxylic acid porphyrin I), **P6** (hexacarboxylic acid porphyrin I), **P4** (coproporphyrin I), **P2** (mesoporphyrin IX), and **P0** (etioporphyrin I).

Table S3. Spectral properties of standard porphyrins in 1 M HCl at room temperature.

Porphyrin	# of CO_2H	λ_{max} (nm)	fwhm (nm)
Uroporphyrin I (**P8**)	8	406	10
Heptacarboxyporphyrin I (**P7**)	7	405	10
Hexacarboxyporphyrin I (**P6**)	6	404	11
Coproporphyrin I (**P4**)	4	401	10
Mesoporphyrin IX (**P2**)	2	400	10
Etioporphyrin I (**P0**)	0	399	10