

Assessment of New Cationic Porphyrin Binding to Plasma Proteins by Planar Microarray and Spectroscopic Methods

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Running title: *New cationic porphyrin interactions with plasma proteins*

Abbreviations: photodynamic therapy of tumors (PDT); serum albumin (SA); human serum albumin (HSA); bovine serum albumin (BSA); fatty acids (FA); hemoglobin (Hb); ultraviolet (UV), visible (VIS) and nuclear magnetic resonance (NMR) spectroscopy; phosphate buffer saline (PBS); infrared dye (IRDye); signal intensity (SI).

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Supplementary Material

Table S-1

Porphyrins and metalloporphyrins used in this work.

No.	Molecular formula of porphyrins and metalloporphyrins	Molecular weight, Dalton
	I. Peripheral group: hydroxyethyl ($R = CH_2-CH_2-OH$)	
	A) meso-tetra [4-N-(2'-hydroxyethyl) pyridyl] porphyrin (TOEt4PyP) and its metalloporphyrins	
1	TOEt4PyP	940
2	Ag-TOEt4PyP	1152
3	Zn-TOEt4PyP	1003
4	Cu-TOEt4PyP	1002
5	Co-TOEt4PyP	997
6	TOEt4PyP-indigo carmine	2750
	B) meso-tetra [3-N-(2'-hydroxyethyl) pyridyl] porphyrin (TOEt3PyP) and its metalloporphyrins	
7	TOEt3PyP	940
8	Ag-TOEt3PyP	1152
9	Zn-TOEt3PyP	1003
10	Cu-TOEt4PyP	1002
	II. Peripheral group: butyl ($R = CH_2-CH_2-CH_2-CH_3$)	
	A) meso-tetra [4-N-butyl pyridyl] porphyrin (TBut4PyP) and its metalloporphyrins	
11	TBut4PyP	1166
12	Ag-TBut4PyP	1200
13	Zn-TBut4PyP	1229
14	Mn-TBut4PyP	1219
	B) meso-tetra [3-N-butyl pyridyl] porphyrin (TBut3PyP) and its metalloporphyrin	
15	TBut3PyP	1166
16	Zn-TBut3PyP	1229
	III. Peripheral group: allyl ($R = CH_2-CH=CH_2$)	
	A) meso-tetra [4-N-allyl pyridyl] porphyrin (TAlI4PyP) and its metalloporphyrins	
17	TAlI4PyP	1102
18	Ag-TAlI4PyP	1136
19	Zn-TAlI4PyP	1165
20	Cu-TAlI4PyP	1164
21	Co-TAlI4PyP	1159
22	Fe-TAlI4PyP	1191.5
23	TriAlI4PyMVanP	1250
24	Ag-TriAlI4PyMVanP	1302
25	B) meso-tetra [3-N-allyl pyridyl] porphyrin (TAlI3PyP)	1102
	IV. Peripheral group: methylallyl ($R = CH_2-C(-CH_3)=CH_2$)	
	meso-tetra [4-N-methylallyl pyridyl] porphyrin (TMetAlI4PyP) and its metalloporphyrins	
26	TMetAlI4PyP	1158
27	Ag-TMetAlI4PyP	1192
28	Cu-TMetAlI4PyP	1220
29	Co-TMetAlI4PyP	1215
30	V. Chlorin e_6	639

Table S-2

Fluorescence signals recorded from 29 cationic porphyrins and chlorin e₆ after the binding reaction with proteins on microarrays*.

No.	Porphyrins and metalloporphyrins	HSA (SI)	BSA (SI)	BSA* (SI)	Hemoglobin (SI)
1	TOEt4PyP	1.12	1.38	1.11	1.36
2	Ag-TOEt4PyP	1.40	1.63	1.72	1.74
3	Zn-TOEt4PyP	0.23	0.22	0.32	0.84
4	Cu-TOEt4PyP	1.11	1.06	1.11	1.05
5	Co-TOEt4PyP	2.76	2.09	3.05	4.53
6	TOEt4PyP-indigo carmine	6.24	4.21	7.23	4.54
7	TOEt3PyP	1.07	1.25	1.76	1.29
8	Ag-TOEt3PyP	2.64	6.65	3.36	5.21
9	Zn-TOEt3PyP	1.01	1.39	1.83	1.31
10	Cu-TOEt3PyP	1.05	1.05	1.32	1.28
11	TBut4PyP	2.73	2.49	3.18	2.15
12	Ag-TBut4PyP	0.98	1.45	2.33	0.46
13	Zn-TBut4PyP	1.11	1.19	1.14	1.16
14	Mn-TBut4PyP	0.96	0.79	0.67	1.42
15	TBut3PyP	2.62	2.11	2.53	1.96
16	Zn-TBut3PyP	0.89	1.21	1.00	1.38
17	TAll4PyP	1.21	1.48	1.71	1.18
18	Ag-TAll4PyP	1.33	1.45	2.12	1.45
19	Zn-TAll4PyP	2.01	2.08	2.68	2.23
20	Cu-TAll4PyP	1.34	1.42	1.73	1.11
21	Co-TAll4PyP	6.23	5.58	4.36	4.93
22	Fe-TAll4PyP	0.99	0.83	1.18	1.32
23	TriAll4PyMVanP	3.55	9.16	9.97	9.01
24	Ag-TriAll4PyMVanP	1.15	1.23	1.56	1.41
25	TAll3PyP	1.13	1.21	1.00	1.48
26	TMetAll4PyP	0.41	0.74	0.39	0.63
27	Ag-TMetAll4PyP	1.10	1.28	1.51	1.93
28	Cu-TMetAll4PyP	1.05	1.18	1.29	1.07
29	Co-TMetAll4PyP	2.86	2.07	2.16	3.09
30	Chlorin e6	0.38	0.63	0.38	1.64

*Every value is shown as the average value of arbitrary SI from microspots. The standard deviation of values does not exceed 5%.

Table S-3

Characteristics of the positioning of 3 complexes [porphyrin + fatty acid]
onto subdomain IB of HSA.

Docking onto subdomain IB of the complexes [porphyrin + fatty acid]	The minimum binding energy of 50-step docking kcal/M	Number of steps in docking with negative binding energy	Number of steps in docking with the H-bond	Number of amino acids binding to porphyrins
TOEt4PyP + stearate	+111.97	0	1	14
TBut4PyP + palmitate	+120.49	0	0	15
TAll4PyP + palmitate	+71.86	0	0	15

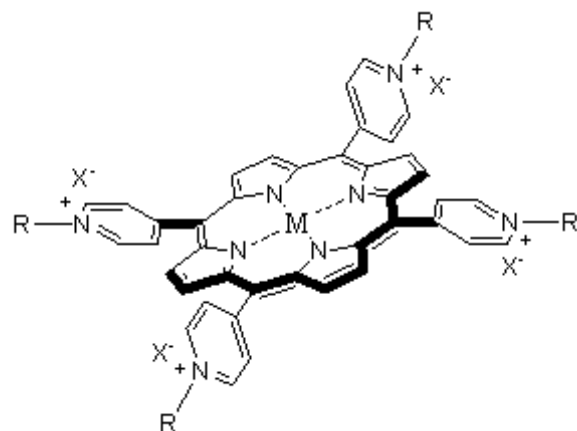


Figure S-1: Scheme of the cationic porphyrins. M indicates the central metal atom of the metalloporphyrin; R indicates the peripheral functional groups.

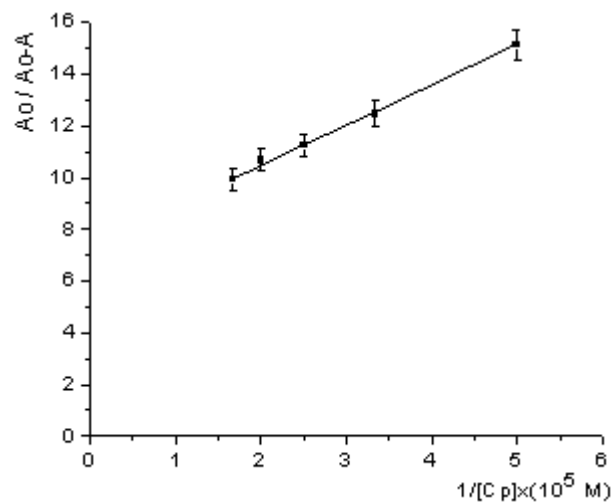


Figure S-2: Adsorption isotherm of porphyrin Zn-TOEt4PyP on BSA* (titration of the porphyrin solution (pH 7.2) with increasing concentrations of BSA*). The dots are experimental data for the values of A_0/A_0-A at a wavelength of 440 nm for different concentrations of protein.