Supporting Information for

Small-Molecule Fluorescent Sensors for Investigating Zinc Metalloneurochemistry

Elizabeth M. Nolan and Stephen J. Lippard*

Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139

This Supporting Information includes:

Table S1: Spectroscopic and Thermodynamic Data

Table S2: Kinetic Data

Table S1. Spectroscopic and Thermodynamic Data[a]

	Absorption λ (nm), ε x 10^4 ($M^{-1}cm^{-1}$)		Emission λ (nm), Φ[b]		pK_{a1}[c]	pK_{a2}[d]	pK_{a3}[e]	K_d[f]	~DR[g]	Ref.
	Unbound	Zn(II)	Unbound	Zn(II)						
ZP1	515, 7.9	507, 8.4	531, 0.38	527, 0.87	7.0	4.0		0.7 ± 0.1 nM	2.5	22,46
ZP2	498, 4.4	490, 5.3	522, 0.25	517, 0.92	9.4	n.o.	3.9	0.5 ± 0.1 nM	4	22
ZP3	502, 7.5	492, 8.5	521, 0.15	516, 0.92	6.8	4.0	2.0	0.7 ± 0.1 nM	6	23, 46
ZP4	506, 6.1	495, 6.7	521, 0.06	515, 0.34	7.2	10.0	4.0	0.65 ± 0.1 nM	4.4	25
ZP5	504, 8.3	495, 9.1	520, 0.29	517, 0.48	9.6	10.9	4.7	0.50 ± 0.1 nM	1.6	26
ZP6	506, 8.9	495, 9.8	519, 0.10	517, 0.34	6.3	11.2	4.7	0.50 ± 0.1 nM	4	26
ZP7	505, 6.8	495, 7.7	521, 0.04	517, 0.05	6.9	n.o.	4.6	n.d.	1	26
ZP8	500, 8.1	489, 7.8	516, 0.03	510, 0.35	6.5	n.o.	3.8	0.60 ± 0.1 nM	11	26
ZP9	505, 5.1	495, 4.4	526, 0.02	521, 0.41	7.2	n.o.	5.0	0.69 ± 0.04 μM	12	29
ZP10	506, 5.4	497, 4.5	523, 0.08	516, 0.32	7.0	n.o.	4.8	1.9 ± 0.2μM	7	29
ZPF1	533, 9.9	525, 12.0	547, 0.11	544, 0.55	6.9	4.2	1.8	0.9 ± 0.1 nM	6	23
ZPCl1	534, 9.7	527, 12.0	550, 0.22	549, 0.50	7.0	4.3	1.9	1.1 ± 0.1 nM	2.5	23
ZPBr1	534, 4.5	528, 8.6	549, 0.25	547, 0.36	7.3	4.5	2.1	0.9 ± 0.1 nM	2.2	23
ZPF3	520, 8.7	510, 9.3	537, 0.14	533, 0.60	6.7	4.0	1.9	0.8 ± 0.1 nM	5	23
ZP1(5-CO_2H)	520, 8.1	509, 8.8	0.17	0.62	7.1	3.7	1.6	0.22 ± 0.04 nM	8	24
ZP1(5-CO_2Et)	517, 6.6	506, 7.1	0.14	0.58	7.0	4.0	1.5	0.26 ± 0.03 nM	8	24
ZP1(6-CO_2H)	516, 7.6	506, 8.1	531, 0.21	528, 0.63	7.1	4.1	2.1	0.16 ± 0.02 nM	8	24
ZP1(6-CO_2Et)	519, 6.1	509, 7.2	0.13	0.67	7.0	4.0	2.1	0.37 ± 0.04 nM	8	24

Me_2ZP1	515, 7.4	505, 8.06	528, 0.18	524, 0.61	7.8	4.8	1.7	3.3 ± 0.2 nM	4	28
Me_4ZP1	516, 5.6	506, 4.74	529, 0.17	527, 0.35	7.3	4.1	1.5	0.63 ± 0.05 μM	2.5	28
ZAP1	506, 8.3	500, 5.46	525, 0.82	n.d.	8.3	n.o.	2.0	10.2 ± 1.4 μM	1	35
ZAP2	508, 7.8	503, 5.78	527, 0.74	n.d.	8.3	n.o.	2.7	109 ± 20 μM	1	35
ZAP3	510, 8.04	504, 5.68	527, 0.52	n.d.	7.9	n.o.	3.2	9.3 ± 0.7 μM	1	35
ZS1	510, 8.39	501, 7.52	531, 0.50	526, 0.70	7.7	n.o.	2.0	n.d.	1.4	36
ZS2	499, 6.69	489 6.76	523, 0.39	516, 0.69	7.7	n.o.	2.7	n.d.	2	36
ZS3	500, 8.69	n.d.	525, 0.71	n.d.	9.3	n.o.	4.5	n.d.	1	36
ZS4	507, 8.11	495, n.d.	522, 0.12	520, 0.50	7.6	n.o.	5.1	n.d.	4.5	36
ZS5	497, 3.3	490, 4.2	522, 0.36	517, 0.80	8.0	n.o.	4.6	1.5± 0.2 μM	4.6	37
ZS6	515, n.d.	505, n.d.	533, 0.44	527, 0.64	7.8	n.o.	3.3	n.d.	3.3	37
ZS7	500, 6.2	490, 6.6	524, 0.25	518, 0.79	7.0	n.o.	2.0	3.7± 0.2 μM	2.7	37
ZSF6	532, 6.3	522, 7.0	549, 0.19	545, 0.63	7.1	n.o.	2.7	4.6 ± 0.2 μM	2.0	37
ZSF7	521, 6.2	511, 7.0	535, 0.24	527, 0.68	6.9	n.o.	2.5	5.0 ± 0.3 μM	2.5	37
QZ1	505, 6.89	498, 6.98	524, 0.024	524, 0.78	6.1	n.o.	5.0	33 ± 2 μM	42	33
QZ2	499, 3.72	489 3.36	~520, 0.005	518, 0.70	7.0	n.o.	4.9	41 ± 3 μM	150	33

[a]Measurements were performed at pH 7 (50 mM PIPES, 100 mM KCl). Thermodynamic data are derived from fluorescence measurements (see footnote, main text). The references correspond to those in the main text. [b] Fluorescein in 0.1N NaOH was used as the standard. [c] The pK_a value corresponding to a fluorescence increase when going from high to low pH. [d] In some instances, a second pK_a transition corresponding to a fluorescence increase is observed when going from high to low pH. [e] The pK_a value associated with fluorescence quenching when moving from high to low pH. The ZP1 are revised from those in ref. 23 (B. A. Wong, personal communication). [f] The K_d value is for the Zn(II) binding event observable by fluorescence titration for sensors with two chelates. The ZAP K_d values were determined by using optical absorption spectroscopy. [g] DR is the dynamic range, or the increase in brightness (Φ x ε), that results from Zn(II) coordination.

Table S2. Kinetic and Thermodynamic Parameters for Zn(II) Binding to ZP, ZS and QZ Sensors[a]

	k_{on} ($M^{-1}s^{-1}$)[b] 4.3 °C	k_{on} ($M^{-1}s^{-1}$)[c] 25 °C	K_d[d] 4.3 °C	K_d[e] 25 °C	k_{off} (s^{-1})[f] 4.3 °C	~k_{off} (s^{-1})[g] 25 °C	$\Delta H^{\ddagger}$ [h] (kcal/mol)	$\Delta S^{\ddagger}$ [h] (cal/mol-K)
ZP1	(6.3±0.1) x 10^5	(3.3±0.4) x 10^6		0.7±0.1 nM		(2.3±0.4) x 10^{-3}	12.5±0.1	13.2±0.3
ZP3	(7.8±0.1) x 10^5	(4.3±0.3) x 10^6		0.7±0.1nM		(2.9±0.4) x 10^{-3}	11.6±0.2	10.7±0.7
ZP4	(8.5±0.4) x 10^5	(5.2±0.1) x 10^6		0.65±0.1 nM		(3.4±0.5) x 10^{-3}	12.2±0.3	13.1±0.1
ZP9	(3.00±0.09) x 10^5	(2.2 ±0.1) x 10^6		0.69±0.04 μM		1.5±0.1	14.4 ± 0.1	18.3± 0.9
ZP10	(2.04 ±0.04) x 10^5	(2.1± 0.1) x 10^6		1.9±0.2 μM		4.0±0.1	14.6 ±0.1	20.3 ±2.0
ZS5	(3.6±0.2) x 10^5	(2.2±0.1) x 10^6		1.5±0.2 μM		3.3	13.3±0.1	15.2±0.2
ZS6	(3.7±0.1) x 10^5	(2.1±0.1) x 10^6		n.d.		n.d.	12.9±0.4	14.0±0.7
ZS7	(5.9±0.1) x 10^5	(3.0±0.1) x 10^6		3.7±0.2 μM		11	12.5±0.3	12.9±1.3
ZSF6	(3.3±0.1) x 10^5	(1.8±0.1) x 10^6		4.6±0.2 μM		8.3	12.6±0.2	12.4±0.9
ZSF7	(5.0±0.1) x 10^5	(2.7±0.1) x 10^6		5.0±0.3 μM		13.5	12.6±0.2	13.0±0.7
QZ1	(3.1±0.2) x 10^6	(3.7±0.2) x 10^7	48±3 μM	33±2 μM	1.5±0.3 x 10^2	1.2±0.1 x 10^3	10.7±0.1	12.1±0.1
QZ2	(3.9±0.3) x 10^6	(4.5±0.3) x 10^7	41±3 μM		1.6±0.2 x 10^2		10.7±0.1	12.4±0.1

[a]All measurements were made at pH 7 (50 mM PIPES, 100 mM KCl). [b] Experimentally determined values for k_{on} at 4.3 ± 0.1 °C. [c] Experimentally determined k_{on} for ZP and ZS sensors at 25 ± 1 °C. The k_{on} values for QZ were estimated by extrapolation of the Eyring plots (E. M. Nolan, Ph. D. Thesis, MIT, 2006, Figure 7.13). [d] Dissociation constants for QZ1 and QZ2 calculated from stopped-flow kinetic data obtained at 4.3 ± 0.1 °C. [e]Dissociation constants for ZP, ZS and QZ1 obtained experimentally by fluorimetric titration at 25 ± 1 °C. See Table S1 for references. [f] Experimentally determined k_{off} at 4.3 ± 0.1 °C. [g]Calculated k_{off} for ZP and at 25 ± 1 °C. [g]Activation parameters were determined over a temperature range of ~4 to ~20 °C for QZ and from ~4 to ~40 °C for ZP and ZS.