Electronic Supplementary Material (ESI) for Metallomics.

Electronic Supporting Information

Metal binding properties of zinc fingers with a naturally altered metal binding site

Katarzyna Kluska, Justyna Adamczyk and Artur Krężel*

Contribution from

Department of Chemical Biology, Faculty of Biotechnology, University of Wrocław, Joliot-Curie 14a, 50-383 Wrocław, Poland

*Corresponding author.

E-mail address: artur.krezel@uwr.edu.pl (A. Krężel)

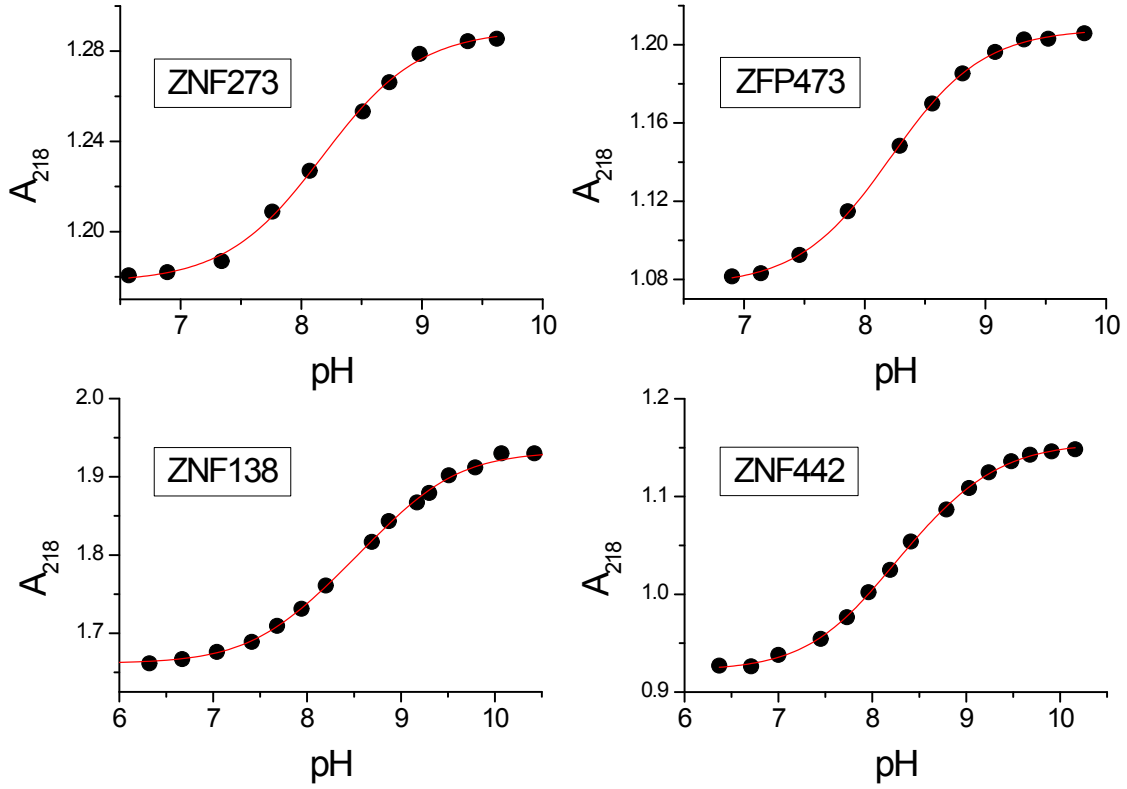
Materials

The following reagents were purchased from Sigma-Aldrich: *p*-nitrophenyl acetate, $ZnSO_4 \cdot 7H_2O$, 4-(2-pyridylazo)resorcinol (PAR), 2-carboxy-2′-hydroxy-5′-sulfoformazyl-benzene monosodium salt (Zincon, ZI), 1,2-ethanedithiol (EDT), thioanisole, anisole, triisopropylsilane (TIPS), nitrilodiaceticpropionic acid (NDAP), *N*-(2-hydroxyethyl)ethylenediamine-*N*,*N*′,*N*′-triacetic acid (HEDTA), *N*,*N*′-ethylenedi-(aspartic acid)trisodium salt (EDDS), and ethylene-bis(oxyethylenenitrilo)tetraacetic acid (EGTA). Sodium perchlorate was purchased from Acros Organics. The metal-chelating resin Chelex 100 was acquired from Bio-Rad. Tris(hydroxymethyl)aminomethane (Tris base) and 4-(2-hydroxyethyl)-1 piperazineethanesulfonic acid (HEPES) were obtained from ROTH and BioShop, respectively. *N*,*N*-dimethylformamide (DMF) and 98% HCl were purchased from VWR Chemicals. Acetonitrile (ACN) and $Co(NO_3)_2 \cdot 6H_2O$ were acquired from Merck Millipore. NaCl, acetic anhydrine, diethyl ether, dichloromethane (DCM) and dimethyl sulfoxide (DMSO) were purchased from Avantor Performance Materials Poland (Gliwice, Poland). Tris(2-carboxyethyl)phosphine hydrochloride (TCEP), 1-methyl-2-pyrrolidinone (NMP), *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU), trifluoroacetic acid (TFA), *N*,*N*-diisopropylethylamine (DIEA), piperidine, TentaGel S Ram and Fmoc-protected amino acids were obtained from Iris Biotech GmbH (Marktredwitz, Germany). The concentration of metal ion salt stock solutions was 0.05 M and was confirmed by a representative series of ICP-MS measurements. All pH buffers were treated with Chelex 100 resin to eliminate trace metal ion contamination. PAR and Zincon 20 mM stock solutions in DMSO were prepared freshly before each experiment and were not stored at room temperature longer than one week due to degradation.[1,2]

Table S1. Calculated and experimental molecular masses of synthesized zinc finger peptides. MW_{cal} (calculated) and MW_{exp} (experimental) values refer to averaged not monoisotopic values.

ZF peptide	MW_{cal}	MW_{exp}
ZNF273	3531.0	3529.6
ZNF729	3475.0	3475.2
ZScan20	3299.7	3300.0
ZFP473	3507.9	3507.3
ZNF138	3459.9	3460.8
ZNF732	3526.1	3526.5
PRZ1	3399.8	3400.0
ZNF442	3294.7	3295.6
ZNF43	3610.1	3610.2

Figure S1. The pH-dependent absorption increase at 218 nm of 30 μM of selected zinc finger peptides (ZNF273, ZFP473, ZNF138, ZNF 442), 25°C, I = 0.1 M (from $NaClO_4$).



Depending on zinc finger peptides two different equations were used for absorption data fitting.[3,4]

Peptides with one Cys residue were fitted to the logarithmic form of Hill's equation:

$$A = A0 \times \frac{H^n}{H^n + K^n} + A1 \times \frac{K^n}{H^n + K^n} \qquad \text{(Eq. S1)}$$

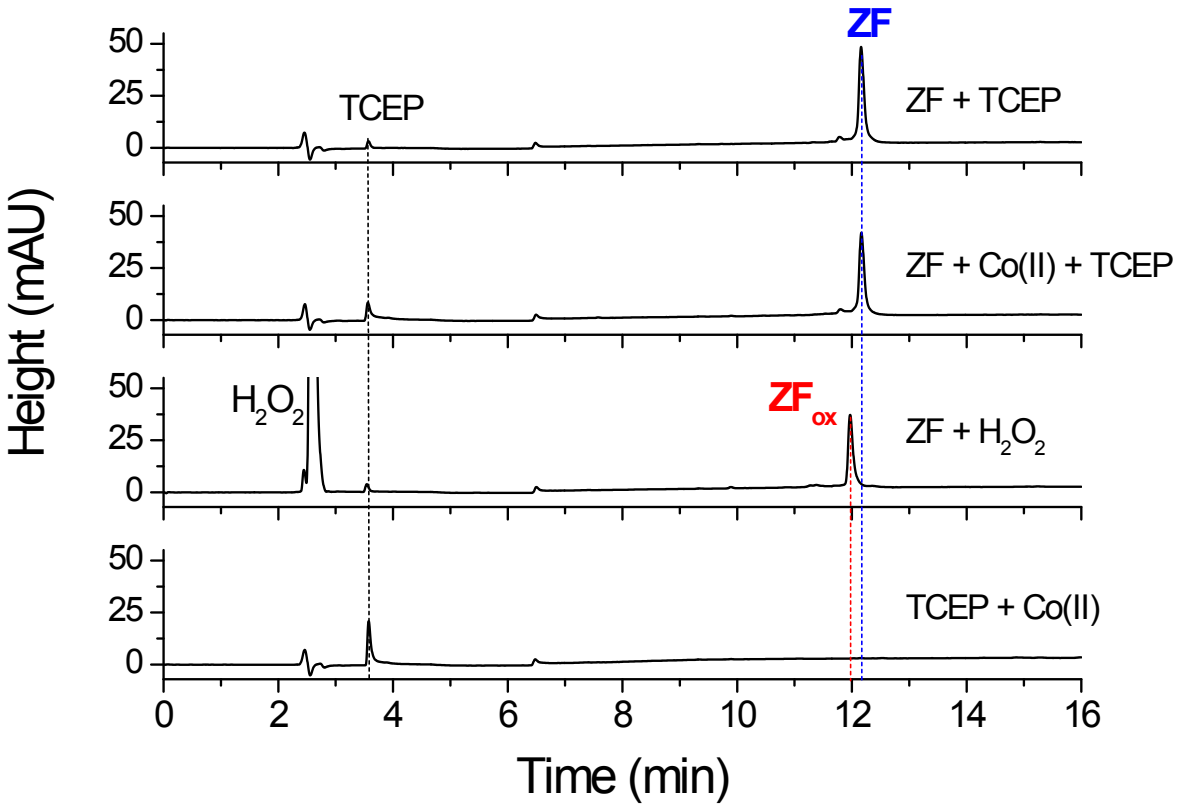
where n is Hill's coefficient, H is 10^{-pH}, and K corresponds to the K_a^{CysH} value. A, A0, A1 show experimental, minimal and maximal absorbance values, respectively.

Peptides with two Cys residues were fitted to the following equation:

$$A = \frac{A0 + A1 \times 10^{pH - pK1} + A2 \times 2 \times 10^{2 \times pH - pK1 - pK2}}{1 + 10^{pH - pK1} + 2 \times 10^{2 \times pH - pK1 - pK2}} \qquad \text{(Eq. S2)}$$

Where pK1 and pK2 are pK_{a1}^{CysH} and pK_{a2}^{CysH}, respectively. A0, A1, A2 correspond to experimental, minimal, intermediate, and maximal absorbance values, respectively.

Figure S2. HPLC monitoring of the oxidation reaction of PRZ1 ZF (60 μM) in a chelexed 50 mM HEPES buffer (100 mM NaCl, pH 7.4) with 1 h exposure to O_2 or H_2O_2 with presence or absence of TCEP and/or Co(II). The reverse-phase HPLC experiment was performed on a Phenomenex C18 column using a gradient of ACN in 0.1% TFA/water from 0% to 70% over 30 min.



Determination of the dissociation constants of Zn(II) complexes with ZF peptides (L) determined by competition with the chromophoric chelators probes Zincon and PAR.

Competition with Zincon[1]

$$ZnZI + L \rightleftharpoons ZI + ZnL \qquad K_{ex} = \frac{[ZnL][ZI]}{[Zn(ZI)][L]}$$

$$K_d^{ZnL} = K_d^{ZnZI} \frac{1}{K_{ex}}$$

where K_d^{ZnZI} (2.09×10^{-6} M) is the dissociation constants of ZI complex at pH 7.4,

$$[ZnZI] = \frac{A_{618}}{\varepsilon_{ZnZI}}$$

where ε (24 200 M^{-1} cm^{-1}) is the molar extinction coefficient of ZI complex at 618 nm,

$[ZI] = C_{ZI} - [ZnZI]$

$[ZnL] = C_{ZnZI} - [Zn(ZI)]$

$[L] = C_L - [ZnL]$

where C_{ZI}, C_{ZnZI}, C_L are total Zincon, ZnZI complex and ligand concentrations, respectively.

Competition with PAR[2]

$$Zn(PAR)_2 + L \rightleftharpoons 2PAR + ZnL \qquad K_{ex} = \frac{[ZnL][PAR]^2}{[Zn(PAR)_2][L]}$$

$$Zn(PAR)_2 + 2L \rightleftharpoons 2PAR + ZnL_2 \qquad K_{ex} = \frac{[ZnL][PAR]^2}{[Zn(PAR)_2][L]^2}$$

where $K_d^{Zn(PAR)_2}$ (7.08×10^{-13} M^2) is the dissociation constants of PAR complex at pH 7.4,

$$[Zn(PAR)_2] = \frac{A_{492}}{\varepsilon_{Zn(PAR)_2}}$$

where ε (71 500 M^{-1} cm^{-1}) is the molar extinction coefficient of PAR complex at 492 nm,

$[PAR] = C_{PAR} - 2[Zn(PAR)_2]$

$[ZnL] = C_{Zn(PAR)2} - [Zn(PAR)_2]$

$[L] = C_L - [ZnL]$

where C_{PAR}, $C_{Zn(PAR)2}$, C_L are total PAR, $Zn(PAR)_2$ complex and ligand concentrations, respectively.

Table S2. Determination of the K_d of the Zn(II) complex with ZNF273 zinc finger (L) in 50 mM HEPES buffer (I = 0.1 M from NaCl), pH 7.4, 25°C. 100 μM ZI was partially saturated with 10 μM Zn(II) and titrated with 500 μM peptide. The absorbance was monitored spectrophotometrically at 618 nm. The reported values are the averages of three independent samples.

L_{total} (μM)	A_{620}	[Zn(ZI)] (μM)	[ZI] (μM)	[L] (μM)	[ZnL] (μM)	K_{ex} (M)	K_d^{ZnL} ($\times 10^{-7}$ M)	-log K_d^{ZnL}
0.00	0.223	9.21	90.8	-	-	-	-	-
1.00	0.2083	8.61	91.4	0.391	0.607	16.5	1.27	6.90
1.99	0.1938	8.00	92.0	0.785	1.21	17.7	1.18	6.93
2.98	0.1820	7.52	92.5	1.29	1.69	16.2	1.29	6.89
3.97	0.1704	7.04	93.0	1.80	2.17	16.0	1.31	6.89
4.95	0.1606	6.63	93.4	2.37	2.58	15.3	1.37	6.87
5.93	0.1540	6.36	93.6	3.08	2.85	13.6	1.53	6.81
6.90	0.1458	6.02	94.0	3.72	3.19	13.4	1.56	6.81
7.87	0.1388	5.74	94.3	4.40	3.48	13.0	1.61	6.79
8.84	0.1329	5.49	94.5	5.12	3.72	12.5	1.67	6.78
9.80	0.1270	5.25	94.8	5.84	3.97	12.3	1.70	6.77
10.76	0.1212	5.01	95.0	6.56	4.20	12.2	1.72	6.76
11.72	0.1169	4.83	95.2	7.33	4.38	11.8	1.77	6.75
12.67	0.1115	4.61	95.4	8.07	4.61	11.8	1.77	6.75
13.62	0.1083	4.48	95.5	8.88	4.74	11.4	1.84	6.74
14.56	0.1045	4.32	95.7	9.67	4.89	11.2	1.86	6.73
15.50	0.1013	4.18	95.8	10.5	5.03	11.0	1.90	6.72
16.44	0.0987	4.08	95.9	11.3	5.14	10.7	1.95	6.71
17.37	0.0957	3.95	96.0	12.1	5.26	10.6	1.98	6.70
18.30	0.0930	3.84	96.2	12.9	5.37	10.4	2.01	6.70
19.23	0.0915	3.78	96.2	13.8	5.43	10.0	2.09	6.68
20.15	0.0891	3.68	96.3	14.6	5.53	9.91	2.11	6.68
21.07	0.0881	3.64	96.4	15.5	5.57	9.53	2.19	6.66
21.99	0.0853	3.52	96.5	16.3	5.69	9.56	2.19	6.66
22.90	0.0816	3.37	96.6	17.1	5.84	9.82	2.13	6.67
							Average -log K_d^{ZnL} = 6.7 ± 0.1	

Table S3. Determination of the K_d of the Zn(II) complex with ZScan20 zinc finger (L) in 50 mM HEPES buffer (I = 0.1 M from NaCl), pH 7.4, 25°C. 100 μM ZI was partially saturated with 10 μM Zn(II) and titrated with 500 μM peptide. The absorbance was monitored spectrophotometrically at 618 nm. The reported values are the averages of three independent samples.

L_{total} (μM)	A_{492}	[Zn(ZI)] (μM)	[ZI] (μM)	[L] (μM)	[ZnL] (μM)	K_{ex} (M)	K_d^{ZnL} ($\times 10^{-7}$ M)	-log K_d^{ZnL}
0.00	0.2193	9.06	90.9	-	-	-	-	-
1.00	0.2058	8.50	91.5	0.440	0.558	13.6	1.53	6.81
1.99	0.1940	8.02	92.0	0.947	1.05	12.7	1.65	6.78
2.98	0.1826	7.54	92.5	1.46	1.52	12.7	1.64	6.78
3.97	0.1712	7.07	92.9	1.98	1.99	13.2	1.59	6.80
4.95	0.1617	6.68	93.3	2.57	2.38	13.0	1.61	6.79
5.93	0.1525	6.30	93.7	3.17	2.76	13.0	1.61	6.79
6.90	0.1443	5.96	94.0	3.80	3.10	12.9	1.62	6.79
7.87	0.1364	5.63	94.4	4.45	3.43	12.9	1.62	6.79
8.84	0.1287	5.32	94.7	5.10	3.74	13.1	1.60	6.80
9.80	0.1214	5.01	95.0	5.76	4.05	13.3	1.57	6.80
10.76	0.1149	4.75	95.3	6.45	4.31	13.4	1.56	6.81
11.72	0.1081	4.46	95.5	7.12	4.60	13.8	1.51	6.82
12.67	0.1030	4.25	95.7	7.86	4.81	13.8	1.52	6.82
13.62	0.0983	4.06	95.9	8.62	5.00	13.7	1.52	6.82
14.56	0.0944	3.90	96.1	9.40	5.16	13.5	1.55	6.81
15.50	0.0899	3.72	96.3	10.2	5.35	13.6	1.53	6.81
16.44	0.0852	3.52	96.5	10.9	5.54	13.9	1.50	6.82
17.37	0.0816	3.38	96.6	11.7	5.68	13.9	1.51	6.82
18.30	0.0790	3.26	96.7	12.5	5.80	13.7	1.52	6.82
19.23	0.0771	3.19	96.8	13.4	5.88	13.4	1.56	6.81
20.15	0.0749	3.10	96.9	14.2	5.97	13.2	1.59	6.80
21.07	0.0725	3.00	97.0	15.0	6.07	13.1	1.60	6.80
21.99	0.0694	2.87	97.1	15.8	6.19	13.3	1.57	6.80
22.90	0.0679	2.80	97.2	16.6	6.26	13.0	1.60	6.80
								Average -log K_d^{ZnL} = 6.8 ± 0.1

Table S3. Determination of the K_d of the Zn(II) complex with PRZ1 zinc finger (L) in 50 mM

L_{total} (μM)	A_{492}	$[ZnH_x(PAR)_2]$ (μM)	[PAR] (μM)	[L] (μM)	[ZnL] (μM)	K_{ex} ($\times 10^{-2}$ M)	K_d^{ZnL} ($\times 10^{-11}$ M)	-log K_d^{ZnL}
0.00	0.7023	9.82	100.0	-	-	-	-	-
1.00	0.6345	8.87	82.3	0.0498	0.948	1.45	4.87	10.31
1.99	0.5711	7.99	84.0	0.157	1.83	1.03	6.86	10.16
2.98	0.5109	7.15	85.7	0.305	2.68	0.902	7.85	10.11
3.97	0.4512	6.31	87.4	0.456	3.51	0.931	7.60	10.12
4.95	0.3949	5.52	89.0	0.651	4.30	0.946	7.49	10.13
5.93	0.3377	4.72	90.6	0.830	5.10	1.07	6.63	10.18
6.90	0.2816	3.94	92.1	0.102	5.88	1.24	5.69	10.25
7.87	0.2303	3.22	93.6	1.27	6.60	1.41	5.02	10.30
8.84	0.1893	2.65	94.7	1.67	7.17	1.46	4.85	10.31
9.80	0.1477	2.07	95.9	2.05	7.76	1.69	4.20	10.38
10.76	0.1122	1.57	96.9	2.51	8.25	1.97	3.60	10.44
11.72	0.0847	1.18	97.6	3.08	8.64	2.26	3.14	10.50
12.67	0.0649	0.908	98.2	3.76	8.91	2.52	2.81	10.55
13.62	0.0520	0.727	98.5	4.52	9.10	2.68	2.64	10.58
14.56	0.0424	0.593	98.8	5.33	9.23	2.85	2.48	10.60
15.50	0.0375	0.524	99.0	6.21	9.30	2.80	2.53	10.60
16.44	0.0343	0.480	99.0	7.10	9.34	2.69	2.63	10.58
17.37	0.0294	0.411	99.2	7.96	9.41	2.83	2.50	10.60
18.30	0.0279	0.390	99.2	8.87	9.43	2.68	2.64	10.58
19.23	0.0229	0.320	99.4	9.73	9.50	3.01	2.35	10.63
20.15	0.0214	0.299	99.4	10.6	9.52	2.96	2.39	10.62
21.07	0.0182	0.255	99.5	11.5	9.57	3.23	2.19	10.66
21.99	0.0165	0.231	99.5	12.4	9.59	3.32	2.13	10.67
22.90	0.0165	0.231	99.5	13.3	9.59	3.09	2.29	10.64
							Average -log K_d^{ZnL} = 10.4 ± 0.2	

HEPES buffer (I = 0.1 M from NaCl), pH 7.4, 25°C. 100 μM PAR was partially saturated with 10 μM Zn(II) and titrated with 500 μM peptide. The absorbance was monitored spectrophotometrically at 492 nm. The reported values are the averages of three independent samples.

Table S4. Determination of the K_d of the Zn(II) complex with ZNF43 zinc finger (L) in 50 mM HEPES buffer (I = 0.1 M from NaCl), pH 7.4, 25°C. 100 μM PAR was partially saturated with 10 μM Zn(II) and titrated with 500 μM peptide. The absorbance was monitored spectrophotometrically at 492 nm. The reported values are the averages of three independent samples.

L_{total} (μM)	A_{492}	$[ZnH_x(PAR)_2]$ (μM)	[PAR] (μM)	[L] (μM)	[ZnL] (μM)	K_{ex} ($\times 10^{-3}$ M)	K_d^{ZnL} ($\times 10^{-11}$ M)	-log K_d^{ZnL}
0.00	0.7010	9.80	80.4	-	-	-	-	-
1.00	0.6346	8.88	82.2	0.071	0.927	9.99	7.09	10.15
1.99	0.5731	8.02	84.0	0.204	1.79	7.71	9.18	10.04
2.98	0.5171	7.23	85.5	0.411	2.57	6.33	11.2	9.95
3.97	0.4668	6.53	86.9	0.693	3.27	5.47	12.9	9.89
4.95	0.4189	5.86	88.3	1.01	3.95	5.22	13.6	9.87
5.93	0.3708	5.19	89.6	1.31	4.62	5.45	13.0	9.89
6.90	0.3269	4.57	90.9	1.67	5.23	5.65	12.5	9.90
7.87	0.2861	4.00	92.0	2.07	5.80	5.93	11.9	9.92
8.84	0.2549	3.56	92.9	2.60	6.24	5.80	12.2	9.91
9.80	0.2207	3.09	93.8	3.09	6.72	6.21	11.4	9.94
10.76	0.1902	2.66	94.7	3.62	7.14	6.65	10.6	9.97
11.72	0.1619	2.26	95.5	4.18	7.54	7.26	9.75	10.01
12.67	0.1382	1.93	96.1	4.80	7.87	7.85	9.02	10.04
13.62	0.1190	1.66	96.7	5.48	8.14	8.34	8.49	10.07
14.56	0.1009	1.41	97.2	6.17	8.39	9.11	7.77	10.11
15.50	0.0880	1.23	97.5	6.93	8.57	9.57	7.40	10.13
16.44	0.0752	1.05	97.9	7.69	8.75	10.4	6.83	10.17
17.37	0.0635	0.888	98.2	8.46	8.92	11.4	6.18	10.21
18.30	0.0557	0.778	98.4	9.28	9.03	12.1	5.85	10.23
19.23	0.0457	0.639	98.7	10.1	9.16	13.9	5.10	10.29
20.15	0.0387	0.541	98.9	10.9	9.26	15.4	4.60	10.34
21.07	0.0310	0.434	99.1	11.7	9.37	18.1	3.90	10.41
21.99	0.0252	0.352	99.3	12.5	9.45	21.1	3.35	10.48
22.90	0.0223	0.311	99.4	13.4	9.49	22.5	3.15	10.50
								Average -log K_d^{ZnL} = 10.1 ± 0.2

Determination of the dissociation constants of Zn(II) complexes with ZF peptides (L) determined using a series of metal buffers to maintain constant free Zn(II) concentrations ($[Zn(II)]_{free}$).

In order to obtain the apparent formation constants, we determined the normalized isotherms corresponding to complex formation by fitting with the following Hill's equation:[5-7]

$$\Theta = \Theta_{min}\left(\frac{x^n}{x^n + [Zn(II)_{0.5}]^n}\right) + \Theta_{max}\left(\frac{[Zn(II)_{0.5}]^n}{x^n + [Zn(II)_{0.5}]^n}\right)$$

where:

Θ_{min} and Θ_{max} are the observed minimum and maximum ellipticities, respectively,

n is the cooperativity index (Hill's coefficient),

x is the free Zn(II) concentration at a specific experimental point,

$[Zn(II)_{0.5}]$ is the free Zn(II) concentration at the half-point saturation of the ZnL complex.

The obtained concentrations of free Zn(II), which correspond to the half-point complex saturation $[Zn(II)_{0.5}]$ where half of the total peptide is in the form of ZnL_2, were subsequently used to calculate the apparent formation constants K_d of the ZnL_2 based on the following equation:

$$Zn(II) + 2L \rightleftharpoons ZnL_2 \qquad K_d = \frac{[Zn(II)]\,[L]^2}{[ZnL]}$$

References:

1. A. Kocyła, A. Pomorski and A. Krężel, Molar absorption coefficients and stability constants of Zincon metal complexes for determination of metal ions and bioinorganic applications, *J. Inorg. Biochem.*, 2017, **176**, 53-65
2. A. Kocyła, A. Pomorski and A. Krężel, Molar absorption coefficients and stability constants of metal complexes of 4-(2-pyridylazo)resorcinol (PAR): Revisiting common chelating probe for the study of metalloproteins, *J. Inorg. Biochem.*, 2015, **152**, 82–92.
3. J. Adamczyk, W. Bal and A. Krężel, Coordination properties of dithiobutylamine (DTBA), a newly introduced protein disulfide reducing agent, *Inorg Chem.*, 2015, **54**, 596–606.
4. G. Bulaj, T. Kortemme and D. P. Goldenberg, Ionization-reactivity relationships for cysteine thiols in polypeptides, *Biochemistry*, 1998, **37**, 8965–8972.
5. A. Pomorski, T. Kochańczyk, A. Miłoch and A. Krężel, Method for accurate determination of dissociation constants of optical ratiometric systems: chemical probes, genetically encoded sensors, and interacting molecules, *Anal. Chem.,* 2013, **85**, 11479–11486.
6. A. Miłoch and A. Krężel, Metal binding properties of the zinc finger metallome--insights into variations in stability, *Metallomics*, 2014, **6**, 2015–2024.
7. O. Sénéque, and J.-M. Latour, Coordination properties of zinc finger peptides revisited: Ligand competition studies reveal higher affinities for zinc and cobalt, *J. Am. Chem. Soc.*, 2010, **132**, 17760–17774.