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Tuning the Reactivity of Sp1 Zinc Finger with Platinum Complexes

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Supporting Information

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Experimental Section

1. Materials

The complexes [Pt(en)Cl_2] (en = ethylenediamine, common name for 1,2-diaminoethane) and *cis*-[$PtCl_2(NH_3)_2$] (*cis*-DDP) were prepared by literature methods. Purity was confirmed by ^{1}H and ^{195}Pt NMR Spectroscopy, and Elemental Analysis (performed by QTI Laboratory, USA). All reagents were purchased from Sigma Aldrich, USA and used without further purification. The Sp1-F3 peptide (KKFACPECPKRFMSDHLSKHIKTHQNKK) and its short peptide mimic (ACPECP) were purchased from GenScript Corporation.

2. Preparation and characterization of the zinc finger

The procedure followed published methods[1]. The apopeptide was dissolved in deionized water at a concentration of 1mM. Zinc acetate (1.2 molar eq.) was added to the solution and the pH was adjusted to 7.0 using NH_4OH. The zinc finger solution was incubated for 2 h at 37 °C before recording any experiment. Secondary structure characterization of the zinc finger (ZF) was monitored using ESI-MS and CD spectroscopy.

3. *Mass Spectrometry*

For mass spectrometry experiments, all zinc finger samples were prepared in an aqueous solvent at 1 mM and incubated immediately with the appropriate concentration of metal complex, also in water. The reaction solutions were incubated at 37^{o}C overnight and were sprayed using a final concentration of ~100 μM. Experiments were carried out on an Orbitrap Velos from Thermo Electron Corporation operated in positive mode. Samples (50 μL) were diluted with methanol (200 μL) and directly infused at a flow rate of 1 μL/min using a source voltage of 2.5 kV. The source temperature was maintained at 230 °C throughout.

4. {^{1}H,^{15}N} HSQC NMR Spectroscopy

For {^{1}H,^{15}N} HSQC NMR Spectroscopy the spectra were recorded at 22 ^{o}C on a Bruker AVANCE III 400 MHz and 600 MHz spectrometer fitted with a pulsed field gradient module. The ^{1}H NMR chemical shifts were internally referenced to TSP, the ^{15}N chemical shifts externally referenced to $^{15}NH_4NO_3$. The two-dimensional {^{1}H,^{15}N} HSQC spectra were recorded in phase sensitive mode using Echo/Antiecho-TPPI gradient selection. A total of 1024 points were acquired in the ^{1}H dimension and 96 complex points in the ^{15}N dimension with 16 transients. 3 mM [Pt(^{15}N-en)Cl_2] was allowed to react with 1 eq. of Sp1-ZF3 in 5% D_2O/95% H_2O, and the reaction was followed by {^{1}H,^{15}N} HSQC NMR spectroscopy on a Bruker Avance III 600 MHz NMR spectrometer (^{1}H, 600.1 MHz; ^{15}N, 60.8 MHz) with an inverse quadruple resonance (QXI) probe. {^{1}H,^{15}N} HSQC NMR spectra of ^{15}N-cisplatin with 1 eq. of Sp1-ZF3 at 2 mM in 5% D_2O/95% H_2O were recorded on a Bruker NanoBay Avance III 400 MHz NMR spectrometer (^{1}H, 400.0 MHz; ^{15}N, 40.5 MHz) with 5mm Multinuclear broadband Fluorine Observe (BBFO). Other parameters are the same.

5. Circular Dichroism Spectroscopy

CD spectra were obtained in a JASCO J-1500 Spectropolarimeter (Jasco Corp., Tokyo, Japan) under N_2 at a wavelength range 190–250 nm in a 0.1 cm cuvette path length at room temperature. Platinum complexes in different concentration were added to 50 μM zinc-bound Sp1-ZF3 sample in 10 mM phosphate buffer at pH 7.0. Samples were incubated for 30 h at 37°C prior to CD measurements.

To estimate secondary structure changes, circular dichroism spectra were deconvoluted using the webserver DichroWeb[2] following a protocol published previously[3]. Data acquired in the range 190-260 nm were used and ellipticity was converted to Δε. Deconvolution was obtained using CDSSTR and reference set #7[4]. CDSSTR is a modification of VARSLC4 which uses all possible combinations of a fixed number of proteins in the reference set. The parameters Helix1 and Helix 1 were combined, as well as Strand 1 and Strand 2.

6. Sp1-F2 and Sp1-F3 structural analysis

Figure S1 shows the sequence alignment between Sp1-ZF2 and SP1-ZF3. The descriptors selected for the analysis and comparison of Zn-bound residues are briefly explained. The amino acid **accessibility** is calculated according to SurfV program.[5] JPD shows 3 values: for the protein chain in isolation, for the protein chain in complex with the other chain (if) present in the PDB file and finally, a relative accessibility (the last one given by the table of absolute solvent accessible area for amino acids). Numerical values are expressed in Å^2. **Electrostatic Potential** values are calculated using Delphi[6] program according to the modifications done by Walter Rocchia[7] and further adapted to JPD requirements. The numerical values are expressed in kT/e.

7. Computational Modeling

The preliminary DFT-optimized structure was obtained after 32 geometry optimization steps using Orca 3.0. PBE0 was selected and def2-tzvp was used for Pt atoms. Solvent medium was taken into account using Cosmo (water). Chain of spheres (RIJCOSX) approximation was used for solving the Hartree-Fock exchange term and level shift was turned on.

Supporting Information - Tables

Table S1: Main species observed by mass spectrometry for the 1:1 reaction of *cis*-DDP with the ZF3 of Sp1.

Species	Charge State	Observed m/z	Calculated m/z
Pt/ apopeptide	5+	713.15	713.15
Pt_2 / apopeptide	5+	751.74	751.74
$Pt(NH_3)$ / apopeptide	5+	716.35	716.55
$Pt_2(NH_3)$ / apopeptide	5+	755.14	755.14
Pt / ZF3	5+	725.73	725.73
$Pt(NH_3)_2$ / apopeptide	5+	719.96	719.96
$Pt_2(NH_3)_2$ / apopeptide	5+	758.35	758.55
$Pt(NH_3)$ / ZF3	5+	729.14	729.13

Table S2: Main species observed by mass spectrometry for the 1:1 reaction of [$PtCl_2$ (en)] with the ZF3 of Sp1.

Species	Charge State	Observed m/z	Calculated m/z
$\{Pt(en)\}_2$ / apopeptide	5+	775.77	775.76
2[Pt(en)] /ZF3	5+	788.55	788.55
2[Pt(en)]Cl /ZF3	5+	795.75	795.74
Pt(en) / apopeptide	5+	724.96	725.16
2[Pt(en)Cl] / ZF3	5+	803.34	803.14
2[Pt(en)]Cl / apopeptide	5+	782.96	782.96
3[Pt(en)] / apopeptide	5+	826.37	826.37
3[Pt(en)]Cl/ apopeptide	5+	833.77	833.76

Table S3. Comparison of relevant {^{1}H, ^{15}N} and ^{15}N NMR chemical shifts for Pt-S species formed from reactions of cisplatin and [Pt(en)Cl_2] with biomolecules.

Pt Reactant	S Reactant	Pt-S Product	{^{1}H, ^{15}N} HSQC Shift	Ref.
[Pt(en)Cl_2]	GSSG	[{Pt(en)(μ_2-SG)}$_2$]	-10.0, 5.1	8
		Bridged Pt-S-Pt Macrochelate	-5.5, 6.0/5.2 -13.4, 5.4/5.1	
[PtCl(H_2O)(en)]$^+$	N-Ac-L-Met	[Pt([^{15}N]en)(MeCO-Met-S)C1]$^+$	-8.7, 5.4	9
		[Pt([^{15}N]en){MeCO-Met(2-)-S,N}]	-8.2, 5.4/5.1 -10.9, 5.3	
		[Pt([^{15}N]en){MeCO-Met(1-)-S,O}]$^+$	-6.7, 6.0/5.6	
cis-[Pt(NH_3)$_2$(H_2O)$_2$]$^{2+}$	GSH	[Pt($^{15}NH_3$)$_2$(μ-GS)}$_2$]$^{2+}$	-41.7 [a]	10
cis-DDP	NCP7-ZF2	S-Pt-NH_3	-41.6, 3.6 -40.6, 3.8	11
[Pt(en)Cl_2]	ZF3	S-Pt-NH_2	-10.5, 5.2 -8.2, 5.3 -8.0, 5.2	This work
cis-DDP	ZF3	S-Pt-NH_3	-41.7, 4.0	This work

[a] ^{15}N observed directly

Supporting Information - Figures

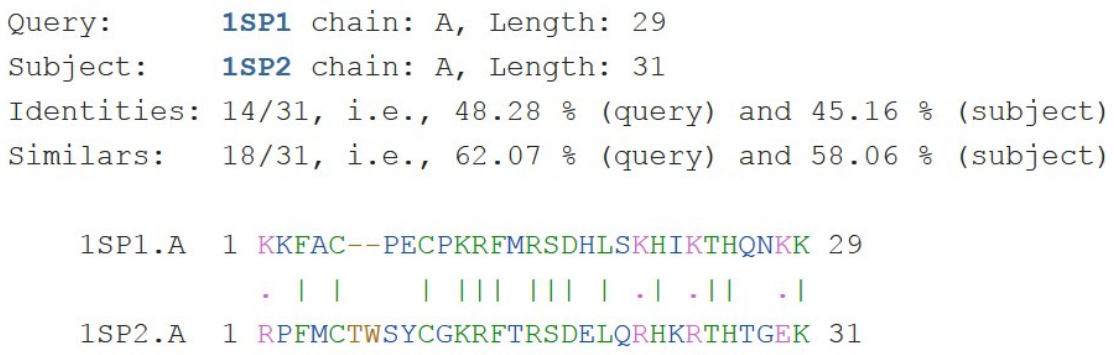

Figure S1. Smith-Waterman sequence alignment comparison for ZF2 and ZF3 (PDB entries 1SP2 and 1SP1 respectively). F3 is two residues shorter. Caption: Green – identical residues; Pink – similar residues; Blue – sequence mismatch; Brown – insertion/deletion.

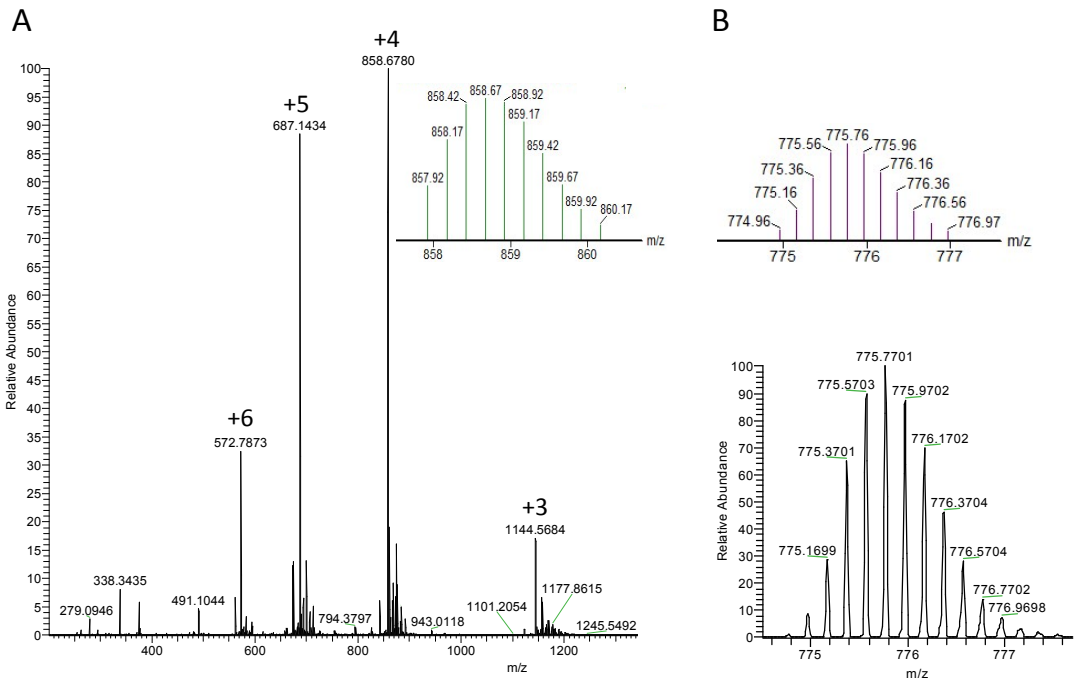

Figure S2. ESI-MS spectra of A) Sp1-ZF3, the inset shows the theoretical isotope distribution of the peak at m/z 858.67, B) [{Pt(en)}$_2$]/apopeptide species, the inset shows the theoretical isotope distribution of the peak.

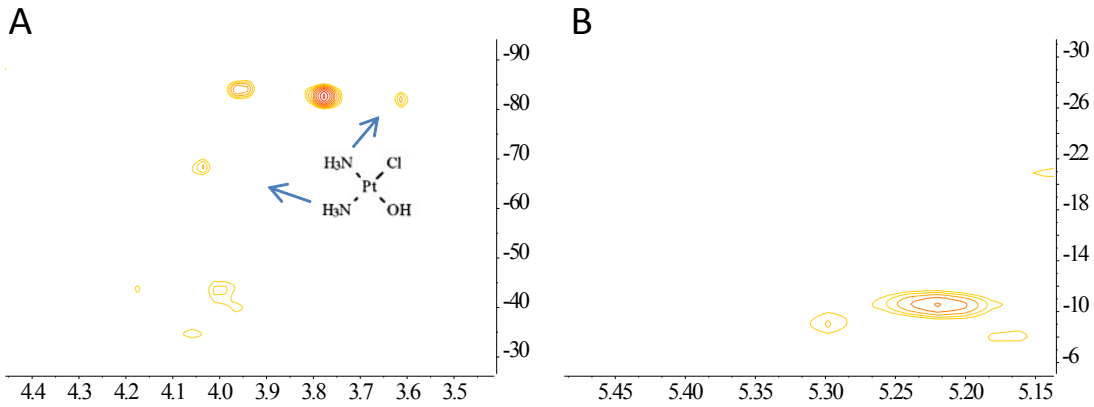

Figure S3. {^{1}H,^{15}N} HSQC NMR spectra of 1:1 reaction of A) ^{15}N-*cis*-DDP with Sp1-ZF3 for 4 days. B) [$PtCl_2$ (^{15}N-en)] with Sp1-ZF3 for 24h.

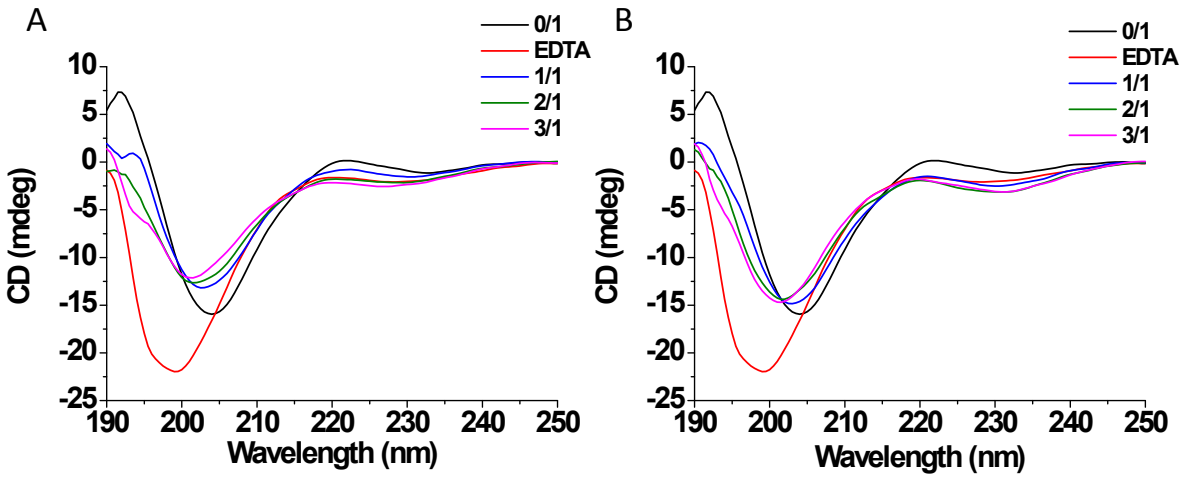

Figure S4. Circular dichroism spectra of the reaction of Sp1-ZF3 with A) cisplatin and B) [$PtCl_2$(en)] after 30 h incubation at 37 °C. Ratio of [Pt]/[protein]: 0:1, 1:1, 2:1, 3:1. The red line shows the spectrum of apo-Sp1-ZF3 after addition of EDTA to remove zinc from the protein.

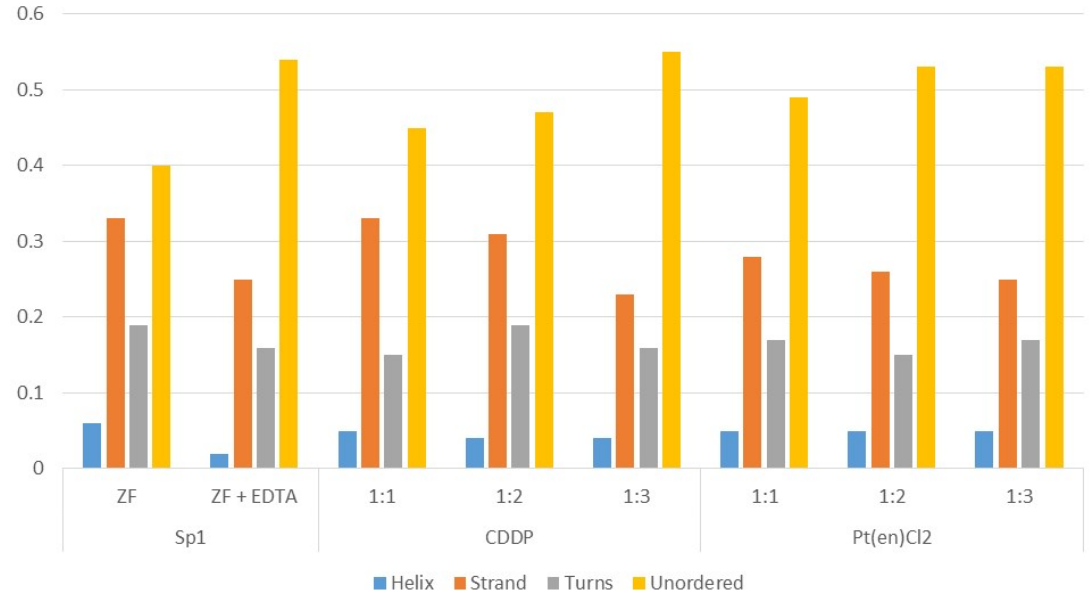

Figure S5. Secondary structure from CD spectrum deconvolution for Sp1-ZF3 incubated with platinum compounds in different molar ratios.

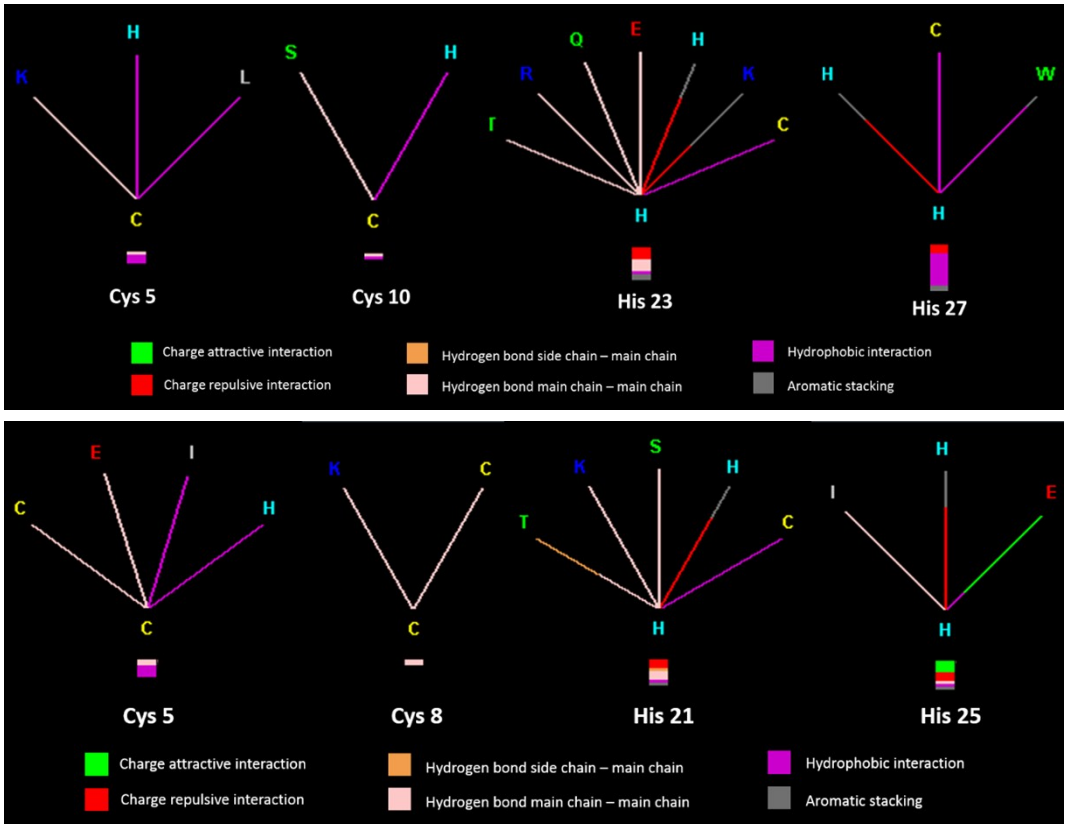

Figure S6. Contact interaction map for the zinc-coordinated residues of Sp1 Finger 2 (top) and Finger 3 (bottom).

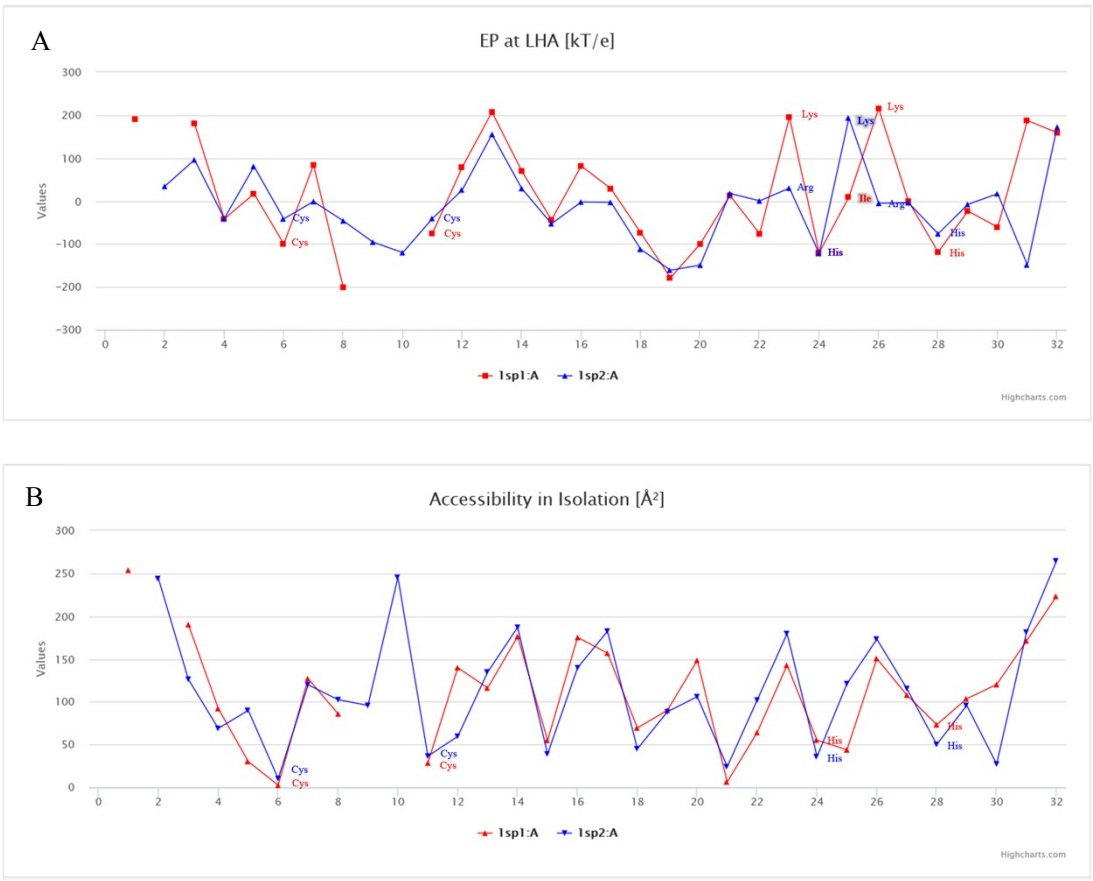

Figure S7. MSSP analysis showing structurally aligned residues (MUSTANG 3.2.2) and comparing the descriptors (A) EP @ LHA and (B) Accessibility in isolation for every residues in the sequences of Sp1-ZF2 (PDB 1SP2) and Sp1-ZF3 (PDB 1SP1)

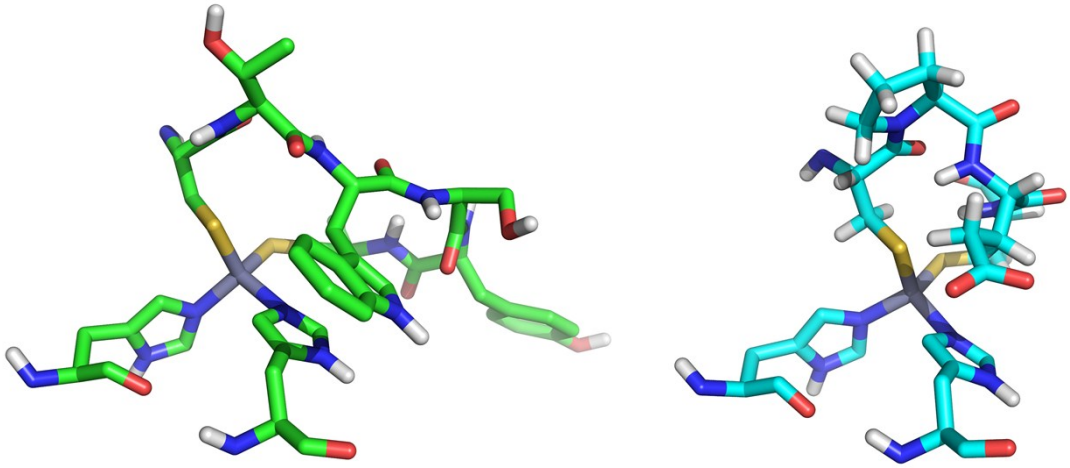

Figure S8. Detailed view of the Cys-X_n-Cys spacer region for Sp1-F2 (green) and Sp1-F3 (blue).

References

1. Q. A. de Paula, J. B. Mangrum and N. P. Farrell, *J Inorg Biochem*, 2009, **103**, 1347-1354.
2. L. Whitmore and B. A. Wallace, *Nucleic Acids Res.*, 2004, **32**, W668-W673.
3. N. J. Greenfield, *Nat. Protocols*, 2007, **1**, 2876-2890.
4. N. Sreerama and R. W. Woody, *Anal. Biochem.*, 2000, **287**, 252-260.
5. S. S., A. Nicholls and B. Honig, *Biophys. J.*, 1992, **61**, A174.
6. B. Honig and A. Nicholls, *Science*, 1995, **268**, 1144-1149.
7. W. Rocchia, S. Sridharan, A. Nicholls, E. Alexov, A. Chiabrera and B. Honig, *J Comput Chem*, 2002, **23**, 128-137.
8. P. del Socorro Murdoch, N. A. Kratochwil, J. A. Parkinson, M. Patriarca and P. J. Sadler, *Angew. Chem. Int. Ed.*, 1999, **38**, 2949-2951.
9. K. J. Barnham, Z. Guo and P. J. Sadler, *J. Chem. Soc., Dalton Trans.*, 1996, 2867-2876.
10. T. G. Appleton, J. W. Connor, J. R. Hall and P. D. Prenzler, *Inorg. Chem.*, 1989, **28**, 2030-2037.
11. S. D. Tsotsoros, Y. Qu and N. P. Farrell, *J Inorg Biochem*, 2015, **143**, 117-122.