Structure *16*

Supplemental Data

Solution NMR Structure of a Designed Metalloprotein and Complementary Molecular Dynamics Refinement

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Table S1. Interatomic Distances (Å) at the Metal Center in di-Zn(II) DF Proteins**

Atoms	NMR	QM/MM -MD	classic-MD	DF2	DF1
OE2(E44)—OE2(E104)	1.93	3.26	3.57	2.92	3.31
OE1(E44)—OE2(E104)	3.25	4.05	4.17	3.04	3.73
OE1(E11)—OE1(E104)	2.97	3.72	3.47	3.35	2.40
OE2(E11)—OE1(E104)	1.60	3.13	3.16	2.68	3.55
OE1(E44)—OE1(E74)	2.46	3.14	2.94	3.22	2.79
OE2(E44)—OE1(E74)	3.74	4.26	3.96	2.66	4.05
OE1(E11)—OE2(E74)	2.40	3.47	3.35	3.00	3.71
OE2(E11)—OE2(E74)	3.74	3.25	3.16	2.91	2.87
OE1(E104)—OE1(E74)	4.19	3.85	3.32	4.09	3.54
OE2(E104)—OE1(E74)	3.74	3.25	2.96	3.01	3.62
OE1(E104)—OE2(E74)	3.18	3.57	3.96	2.92	3.55
OE2(E104)—OE2(E74)	3.80	4.25	4.53	3.03	3.74

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DF2 E11'=DFsc11, DF2 E11=DFscE44
DF2 E37=DFscE74, DF2 E37'=DFscE104
DF1 PDB 1EC5, DF2 1u7J1

Table S2. Upper and Lower Limits of Bond Lengths Used to Calculate the di-Zn(II) DFsc Structure (All Measurements in Å)

Zn1		Zn2	
E44-OE1	2.10 ± 0.40	E11-OE1	2.10 ± 0.40
E44-OE2	2.10 ± 0.40	E11-OE2	2.10 ± 0.40
E74-OE1	2.10 ± 0.40	E74-OE2	2.10 ± 0.40
H77-ND1	2.10 ± 0.20	H107-ND1	2.10 ± 0.20
E104-OE2	2.10 ± 0.40	E104-OE1	2.10 ± 0.40

Zn-Zn bond length 4.0 ± 0.20

Table S3. Average Zn(II) Ligand Distances after Classical MD Simulation (See for Comparison with Table 1 in the Manuscript)

Zn 1	Distance (Å)	Zn 2	Distance (Å)
E44-OE1	2.19 ± 0.10	E11-OE1	3.37 ± 0.33
E44-OE2	2.10 ± 0.07	E11-OE2	2.06 ± 0.06
E74-OE1	2.10 ± 0.06	E74-OE2	2.07 ± 0.06
H77-ND1	2.32 ± 0.13	H107-ND1	2.29 ± 0.12
E104-OE2	2.07 ± 0.07	E104-OE1	2.11 ± 0.07

Zn1-Zn2 =3.59 ± 0.19

Table S4. Charges at the Dizinc Site

Zn	1.35 (2.0)		
His		**Glu**	
ND1	-0.6532 (-0.5432)	OE1	-0.7400 (-0.8188)
CE1	0.1835 (0.1635)	OE2	-0.8400 (-0.8188)
HE1	0.1535 (0.1435)	CD	0.7600 (0.8054)
NE2	-0.3395 (-0.2795)	CG	0.1200 (0.0136)
HE2	0.3339 (0.3339)	HG	-0.0200 (-0.0425)
CD2	-0.1407 (-0.2207)	CB	0.0860 (0.0560)
HD2	0.1962 (0.1862)	HB	-0.0053 (-0.0173)
CG	0.2568 (0.1868)	CA	0.0597 (0.0397)
CB	0.0874 (-0.0074)	HA	0.1305 (0.1105)
HB2	0.0467 (0.0367)	Total charge	-0.74 (-1.0)
Total charge	0.13 (0.00)		

Original parm99 (AMBER) charges are shown in parenthesis.

Table S5. Distances between Helix 1 and 2 and Helix 3 and 4 for Central 5 Origins [Å] (see Figure S2 and Experimental Procedures)

(a) Helix 1-2

Origins	DFsc-NMR	DFsc MD	DFsc target designed model	DF2
6-10	8.9	11.1 ± 0.4	10.5	10.3
6-9	9.2	11.0 ± 0.4	10.5	10.7
7-9	8.7	11.0 ± 0.4	10.6	10.4
7-8	8.9	10.8 ± 0.4	10.4	10.6
8-8	8.8	10.8 ± 0.4	10.4	10.3
8-7	8.3	10.7 ± 0.4	10.4	10.5
8-6	**8.0**	**10.8 ± 0.4**	**10.5**	**11.0**
9-7	8.6	10.6 ± 0.4	10.6	10.3
9-6	8.0	10.6 ± 0.4	10.5	10.6
10-6	8.4	10.5 ± 0.4	10.6	10.5
10-5	8.2	10.4 ± 0.4	10.6	10.4

(b)Helix 3-4

Distance between origins [Å]	DFsc-NMR	DFsc MD	DFsc target designed model	DF2
6-10	10.6	11.6 ± 0.3	11.3	10.4
6-9	**10.6**	**11.5 ± 0.3**	**11.2**	**10.8**
7-9	11.1	11.6 ± 0.3	11.3	10.5
7-8	11.1	11.6 ± 0.3	11.2	10.7
8-8	11.3	11.7 ± 0.3	11.4	10.4
8-7	11.3	11.5 ± 0.3	11.2	10.6
9-7	11.0	11.6 ± 0.3	11.4	10.3
9-6	10.9	11.4 ± 0.3	11.2	10.7
10-6	10.9	11.6 ± 0.3	11.3	10.6
10-5	11.0	11.3 ± 0.3	11.1	10.8

The closest distance found in the NMR-model is indicated in bold.

NOE violations: A total of sixteen violations of over 1 Å were observed relative to the NMR restraints. Of these, most all involved the sidechains of surface-exposed residues, although a few involved buried residues, which helped identify particularly flexible regions, in which sidechain conformations vary in different snapshots of the MD simulation. Table S6 lists the nine deviations that are greater than 2 Å. In particular, the flexibility of Y51 is interesting, as this residue appears to form an interaction with Fe(III) under some conditions (unpublished results).

Table S6. NOE Violations Observed in NMR Structure Determination

Atoms	Distance violation	Apparent cause
V41 (HG1) – K82 (HA)	2-3 Å	Surface-exposed
Q13 (H) – A43(HB)	2-3 Å	Surface-exposed
K76 (HE2) – H107(HE1)	3-4 Å	Surface-exposed
G88 (H) – L33(HD22)	3-4 Å	Gly in flexible, surface-exposed loop
N115(H) – D2(HA)	3-4 Å	N- and C-terminal residues, surface-exposed
I37(HA) – S85(HB)	2-3 Å	Internal dynamics
H107(H) – Y51(HE)	2-3 Å	Internal dynamics
L36(HD2) – F93(HE1)	2-3 Å	Internal dynamics
L101(HD2) – Y18(HE2)	3-4 Å	Internal dynamics, alternate rotamer of L101 satisfies NOE restraint.

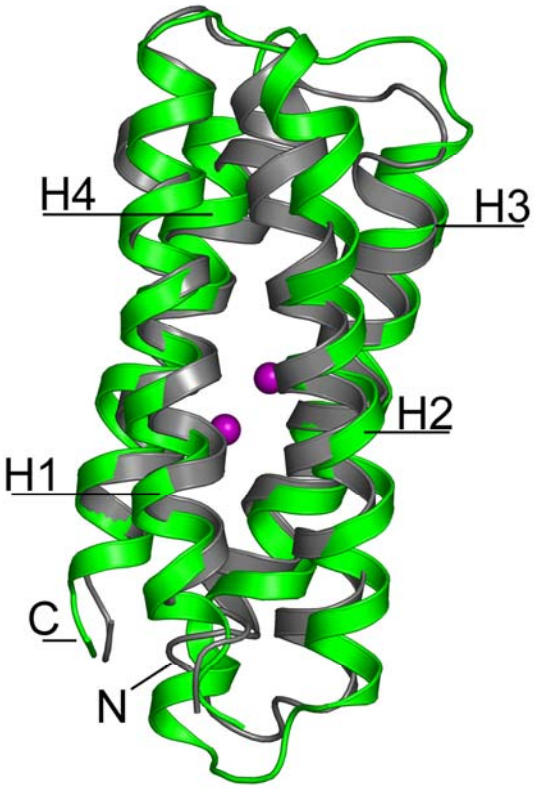

Figure S1. Superposition of di-Zn(II) DFsc Calculated NMR Structure (Gray) and MD Model of di-Zn(II) DFsc (Green)
The helices are labeled H1-H4. The backbone rmsd for the calculated NMR structure and MD model is 1.1 Å.

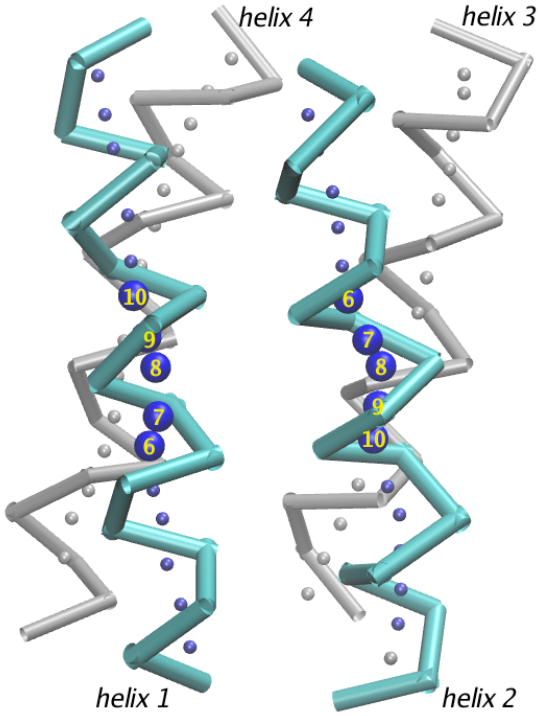

Figure S2. DFsc Calculated NMR Structure
Trace of Cα of residues 4-21 (helix 1), 37-54 (helix 2), 67-84 (helix 3), and 95-112 (helix 4). Axis points are shown as spheres, and the central five axis points of helix 1 and 2 are enlarged and numbered.