Supporting Information

Metal-mediated affinity and orientation specificity in a computationally designed protein homodimer.

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Table S1. Computed parameters for eight designs selected for experimental testing

scaffold	Å	#res	#mut	C/H	metal	ΔG_{bind}	ΔSASA	ratio	pack	unsat	polar	S.S.
1RZ4	2.10	213	13	0/4	2.12	-28	1490	-0.019	0.52	2	apolar	H
1YZM	1.50	46	10	0/4	0.84	-23	1230	-0.019	0.58	0	apolar	H
1G2R	1.35	94	15	4/0	0.17	-33	1930	-0.017	0.70	6	mix	H, E, L
2IL5	2.30	162	18	4/0	0.42	-37	2430	-0.015	0.76	6	apolar	E
2A9O	1.65	117	8	4/0	0.24	-26	1360	-0.019	0.67	4	mix	H, L
2Q0V	2.40	140	6	4/0	0.53	-39	1490	-0.026	0.57	4	polar	L
2D4X	1.90	214	7	4/0	0.09	-48	2300	-0.021	0.61	6	polar	H, L
1HE9	2.40	131	12	2/2	0.74	-38	2140	-0.018	0.59	4	apolar	H

Column headings:

Å: resolution of crystal structure
#res: number of residues
#mut: number of mutations in the design
C/H: how many cysteines versus histidines in designed the zinc binding sites
metal: computed geometric score of zinc binding site
ΔG_{bind}: computed binding energy
ΔSASA: computed interface size
ratio: ΔG_{bind} / ΔSASA
pack: computed packstat score (0 to 1, where 1 is best)
unsat: number of buried polar atoms without a hydrogen bond
polar: qualitative statement of polar vs. nonpolar character of the designed interface
S.S.: predominant secondary structure elements at the designed interface (H = helix, E = strand, L = loop)

Table S2. Experimental end-results for the eight tested designs

scaffold	# zinc sites in model	Experimental result
1RZ4	2	No expression
1YZM	2	Dimer with zinc, weak dimer without zinc
1G2R	2	Higher-order oligomer with and without zinc
2IL5	2	Higher-order oligomer with and without zinc
2A9O	1	~1/3 dimeric without zinc, ~2/3 dimeric with zinc, poor solubility of the MBP fusion protein)
2Q0V	1	Monomer without zinc, small oligomer (larger than dimer) with zinc, poor expression
2D4X	1	Monomer with and without zinc
1HE9	1	Higher-order oligomer with and without zinc

Table S3. Crystallographic data collection and refinement statistics[1]

Data collection Protein	**MID1-apo1**	**MID1-apo2**	**MID1-zinc**	**MID1-cobalt**	**MID1-H12E-zinc**	**MID1-H35E-zinc**
PDB code	3V1A	3V1B	3V1C	3V1D	3V1E	3V1F
Wavelength	0.9794	0.9794	0.9180	0.9494	1.000	1.000
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	P1	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions *a*, *b*, *c* (Å) α, β, γ (degrees)	25.3, 33.0, 42.9 90.0, 90.0, 90.0	25.4, 41.9, 67.2 90.0, 90.0, 90.0	25.3, 29.8, 105.4 90.0, 90.0, 90.0	27.7, 45.5, 62.1 90.04, 90.0, 90.0	27.1, 47.4, 55.8 90.0, 90.0, 90.0	37.2, 46.5, 62.3 90.0, 90.0, 90.0
Resolution (Å)	18.18 – 0.98	21.7 – 1.28	19.3 – 1.13	45.5 – 1.24	23.72 – 1.00	26.35 – 1.15
R_{merge} (%)	6.9 (18.0)	5.0 (37.4)	8.9 (49.0)	6.8 (42.9)	11.3 (59.3)	5.9 (65.0)
I/s*I*	11.1 (4.6)	38.8 (2.5)	31.4 (2.0)	18.1 (2.0)	35.6 (2.8)	42.8 (2.1)
Unique reflections	20,751	19,067	30,296	81,186	36,098	38,608
Completeness (%)	97.2 (77.6)	99.8 (99.8)	97.8 (97.8)	94.3 (94.3)	91.4 (49.4)	98.6 (83.1)
Redundancy	10.0 (3.6)	8.2 (8.2)	7.3 (7.3)	2.5 (2.5)	7.2 (4.2)	6.3 (4.6)
Wilson B-factor ($Å^2$)	7.3	11.3	12.4	10.2	10.7	14.1
Refinement						
Resolution (Å)	18.18 – 0.98	21.7 – 1.28	19.3 – 1.13	45.5 – 1.24	23.72 – 1.00	26.35 – 1.15
No. of reflections work/free	20,588 / 1,057	18,922 / 973	30,257 / 1,523	81,041 / 1,136	34,274 / 1,824	37,684 / 787
Cut-off (s)	None	None	None	None	None	None
R_{work} / R_{free}	0.0938 / 0.1134	0.1623 / 0.2027	0.1468 / 0.1763	0.1467 / 0.1941	0.1455 / 0.1591	0.1608 / 0.1863
No. of atoms						
Protein	468	833	819	3449	766	692
Ions + ligands	0	6	19	72	2	18
Water	58	63	115	410	111	153
B-factors ($Å^2$)						
Overall	7.8	16.1	14.1	11.6	11.3	20.8
Protein	6.3	5.6	12.7	10.3	9.2	17.4
Water	17.8	29.1	24.3	22.2	23.6	34.4
R.m.s. deviations						
Bond lengths (Å)	0.017	0.016	0.013	0.012	0.009	0.010
Bond angles (°)	1.640	1.535	1.241	1.323	1.316	1.244
Ramachandran						
Favored (%)	100	100	100	98.3	97.83	97.65
Generally Allowed (%)	0	0	0	1.7	2.17	2.35
Disallowed (%)	0	0	0	0	0	0
Missing residues	0	2	3	0	10	9

Table S4. Zinc-coordination geometry as observed in the MID1-zinc crystal structure compared to the designed MID1-zinc model

Residue number	**12**	**16**	**35**	**36**		
Distance to zinc $_{xtal}$	2.01	2.02	1.97	2.02		
Distance to zinc $_{model}$	2.07	2.08	2.07	2.07		
Angle at nitrogen $_{xtal}$	127	128	112	129		
Angle at nitrogen $_{model}$	125	128	117	126		
Dihedral $_{xtal}$	157	177	176	4		
Dihedral $_{model}$	179	180	180	176		
Residue number pairs	**12 & 16**	**12 & 35**	**12 & 39**	**16 & 35**	**16 & 39**	**35 & 39**
Angle at zinc $_{xtal}$	101	97	116	116	108	118
Angle at zinc $_{xtal}$	102	105	107	119	109	114
Angle at zinc $_{model}$	107	109	111	113	111	104

Table S5. Number of occurrences of ligating-residue combinations in known zinc-binding sites

Histidine	**# occurrences**	**Cysteine**	**# occurrences**	**Asp/Glu**	**# occurrences**
4H	7	4C	668	4DE	11
3H, 1C	9	3C, 1H	311	3DE, 1H	67
3H, 1DE	185	3C, 1DE	28	3DE, 1C	0
2H, 2C	191	2C, 2H	191	2DE, 2H	162
2H, 2DE	162	2C, 2DE	1	2DE, 2C	1
2H, 1C, 1DE	4	2C, 1H, 1DE	42	2DE, 1H, 1C	6

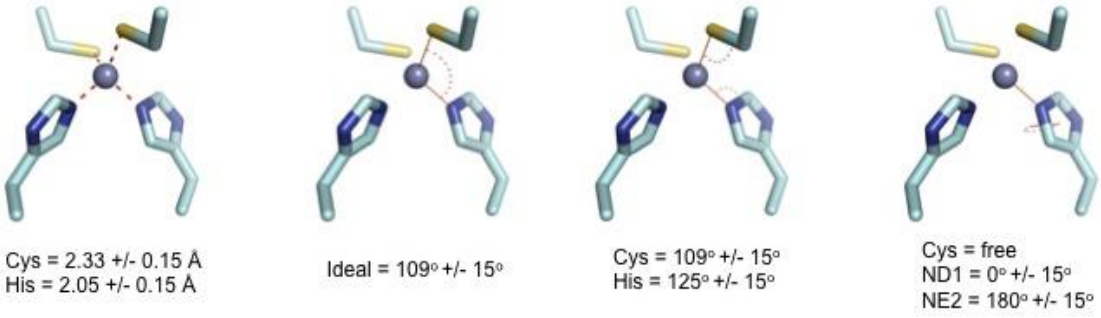
Notation:

4H: zinc binding site with 4 histidines

3H, 1C: zinc binding site with 3 histidines and 1 cysteine

3H, 1DE: zinc binding site with 3 histidines and 1 aspartate or glutamate

Supplemental Figures:



$$metal\_score = \sum \frac{(dist - ideal)^2}{stdev^2} + \sum \frac{(tetr\_angle - ideal)^2}{stdev^2} + \sum \frac{(angle - ideal)^2}{stdev^2} + \sum \frac{(dihed - ideal)^2}{stdev^2}$$

Figure S1: Ideal geometry for zinc coordination by histidine and cysteine. Coordination geometry is defined by coordination bond lengths, angles about zinc, angles about the ligating atom, and dihedral angles that put the zinc in the same plane as the histidine ring. Standard deviations are based on statistics we obtained from 1705 four-coordinated zinc binding sites in structures deposited in the Protein Data Bank. To evaluate the quality of zinc-binding geometry, the differences between actual and ideal values are normalized by standard deviation, squared and summed. Thus, for a 4-residue zinc-binding site, a score less than 4 would reflect a metal site that on average is within standard deviation.

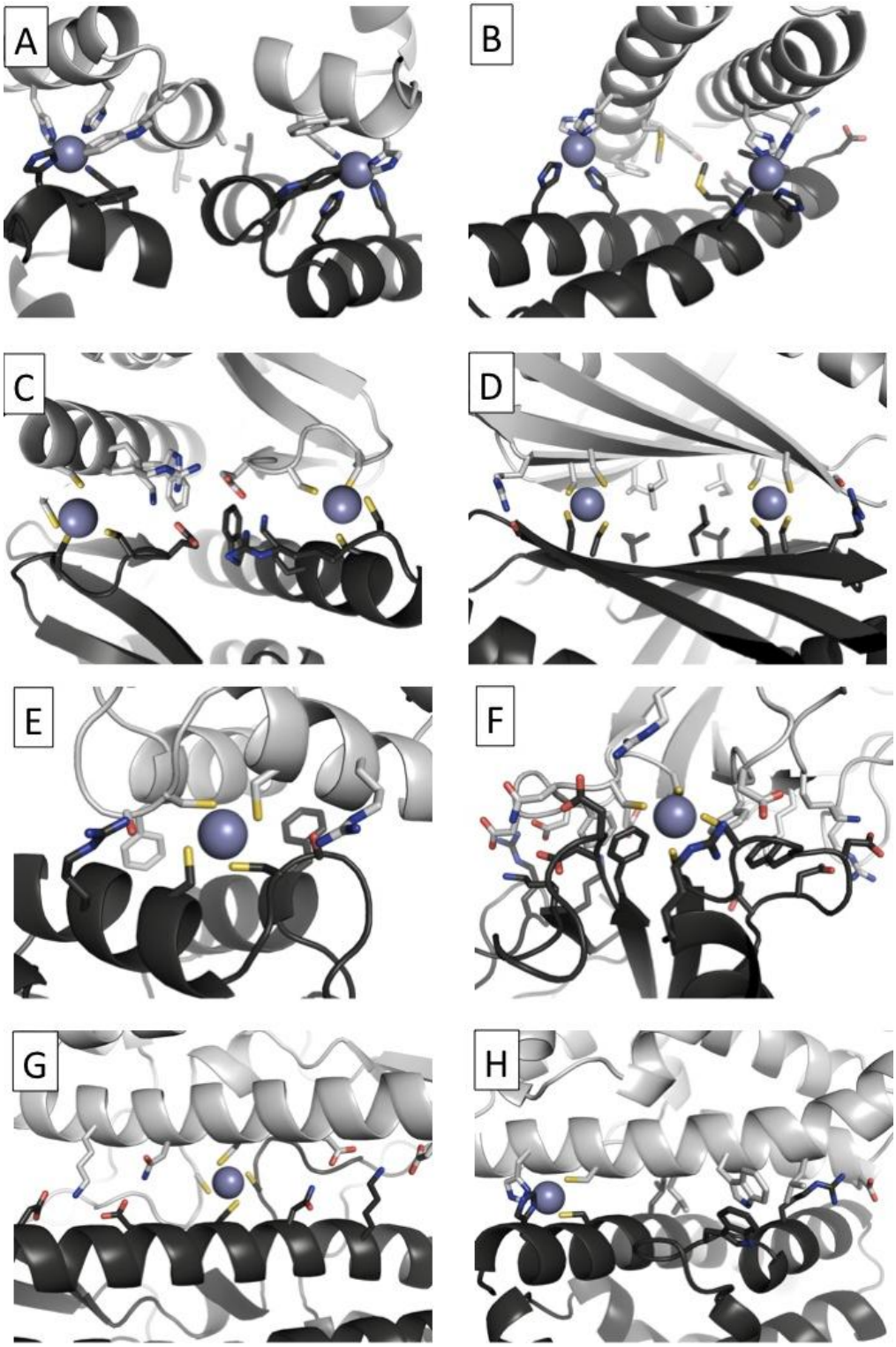


Figure S2: Ribbon diagrams of the eight experimentally tested designs. Four designs have two interface zinc sites, and four have one interface zinc site. The binding orientations are shown in cartoon, and significant intermolecular interactions are shown in sticks. Wild-type scaffold PDB codes of each design are A) 1RZ4. B) 1YZM. C) 1G2R. D) 2IL5. E) 2A9O. F) 2Q0V. G) 2D4X. H) 1HE9.

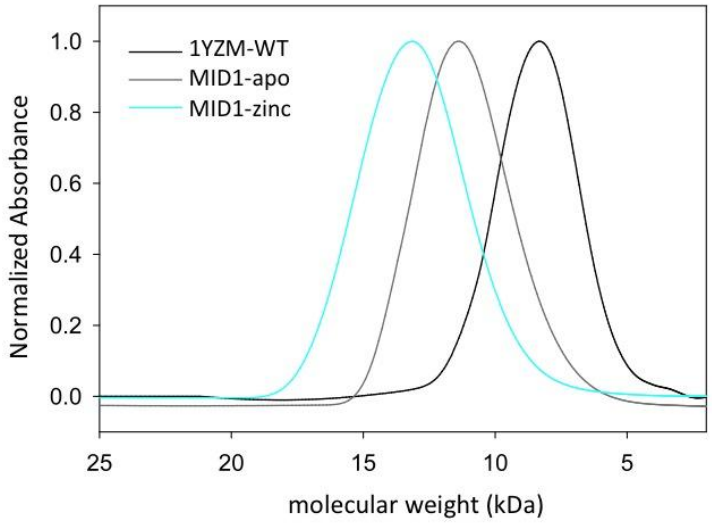


Figure S3: Size exclusion chromatography of 1YZM-WT, MID1-apo, and MID1-zinc provides an initial indication of dimer formation. PDB code 1YZM was previously characterized as a monomer (5.3 kDa). MID1-apo and MID1-zinc elute slightly earlier than 1YZM-WT, likely due to dimer formation.

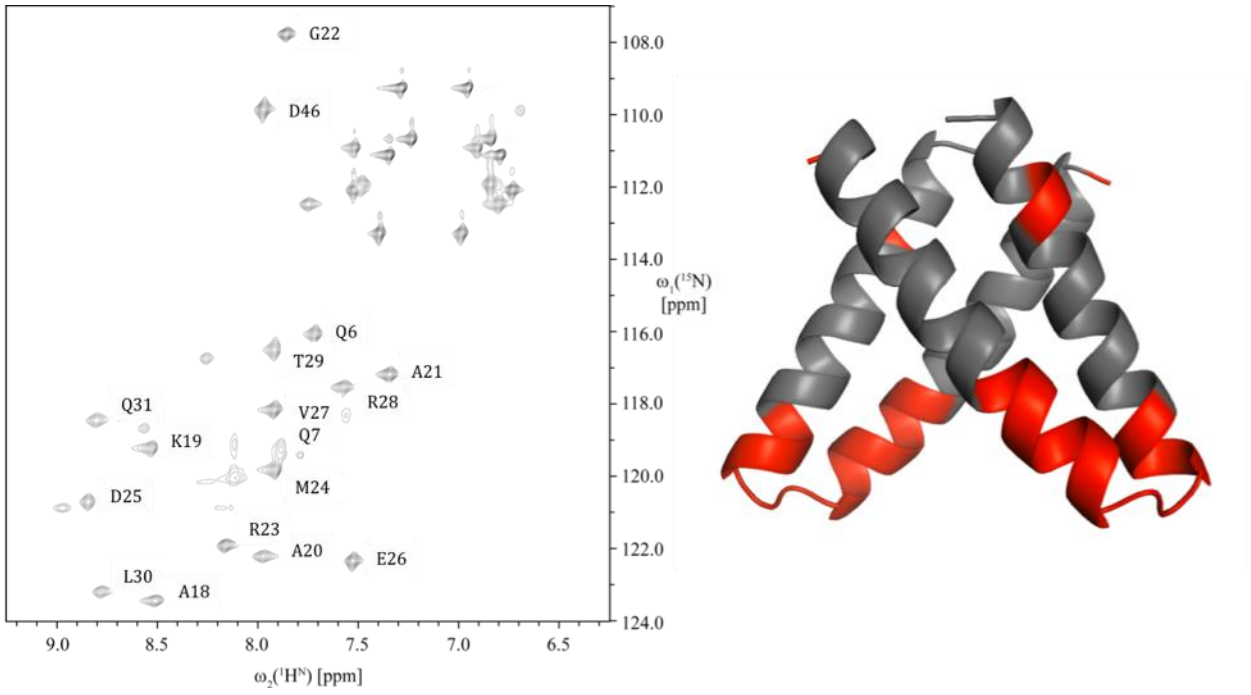


Figure S4: NMR $^{1}H^{15}N$ HSQC of MID1-zinc. MID1-zinc has 48 residues, but only 26 backbone amide peaks were observed. Only residues near the helical hairpin were assignable. These residues (colored red) are not at the interface, suggesting possible plasticity at the interface that causes broadening and loss of other backbone amide peaks.

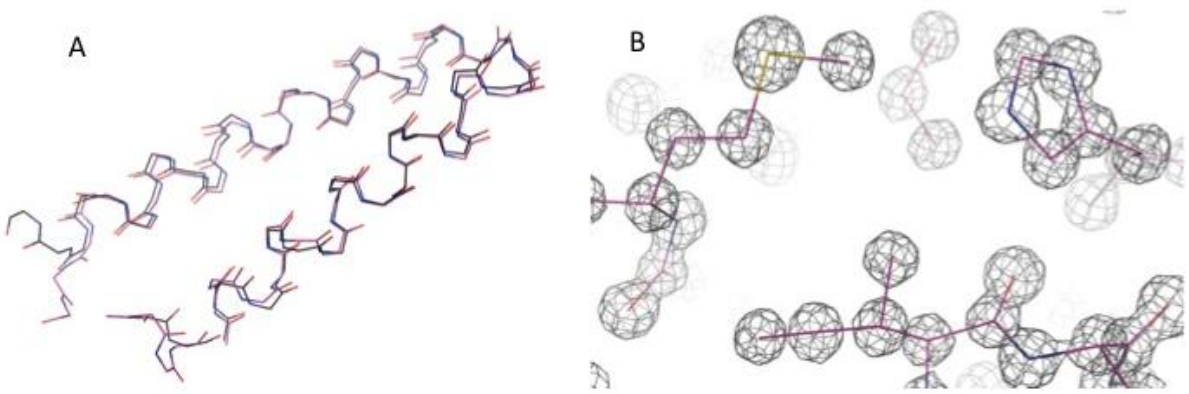


Figure S5: High-resolution crystal structure of MID1-apo1. **A)** The structure of MID1-apo1 confirms that mutations did not significantly alter the backbone conformation. **B)** The MID1-apo1 monomer structure was determined using reflections to 0.98 Å. The electron density is contoured at 2.5σ and resolves individual atoms.

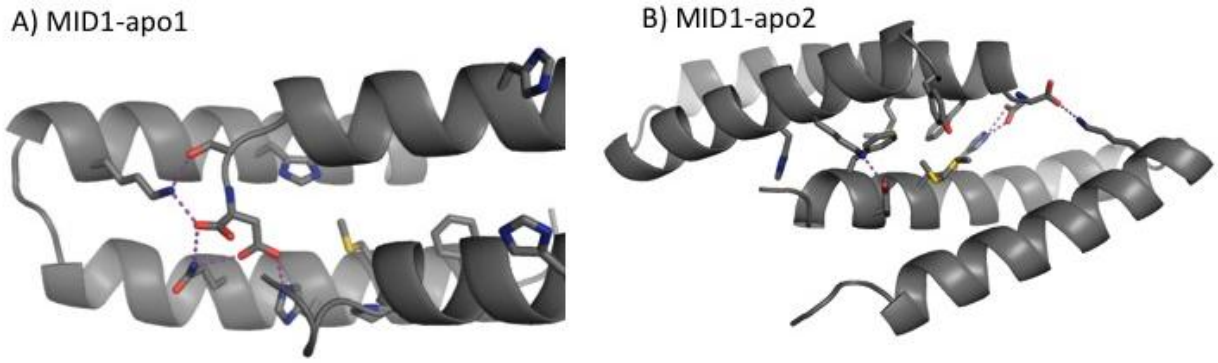


Figure S6: Two possible dimerization modes for MID1-apo. MID1-apo crystallized in two different crystal forms. The two crystal forms are related, but the arrangement of the molecules in the crystal lattices is slightly different. **A)** In MID1-apo1, the asymmetric unit contains one monomer. A dimer is formed by a symmetry-related molecule through a 1000-$Å^2$ interface that features several hydrogen bonds. The histidines are very distant from each other and do not create metal-binding sites. B) In MID1-apo2, the asymmetric unit contains two monomers that form a dimer through a 1050-$Å^2$ interface that also features several hydrogen bonds and good packing interactions. These two binding modes were unpredicted and do not resemble the design model.

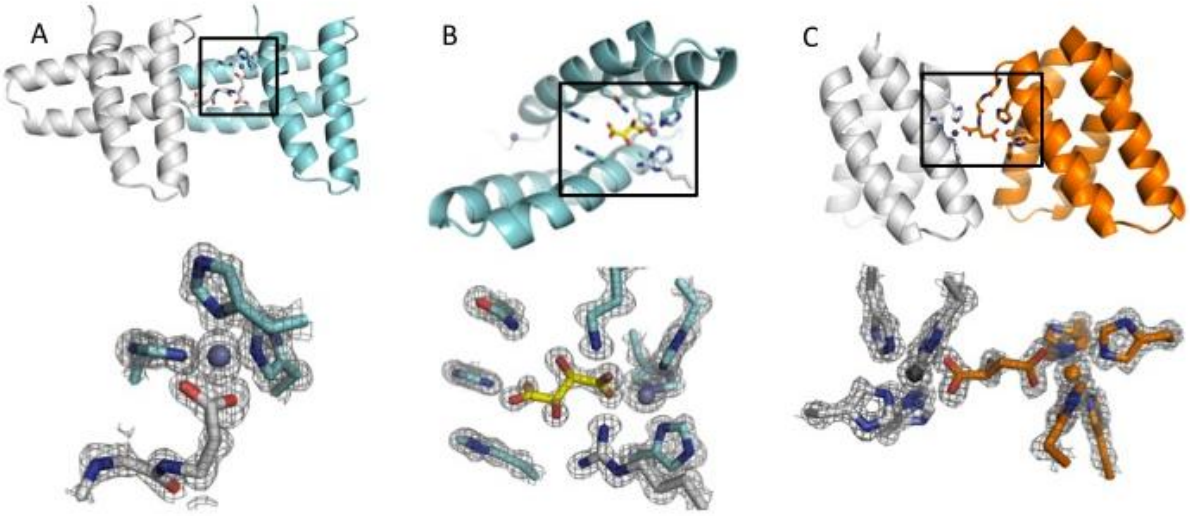


Figure S7: High-resolution electron density reveals carboxylate-metal interactions in the zinc- and cobalt-coordination spheres. 2Fo-Fc electron density (contouring level = 2.0σ). **A)** In the MID1-zinc crystal structure (cyan), one zinc is coordinated by the C-terminal aspartate from a symmetry-related molecule. **B)** The second zinc is coordinated by a tartrate molecule from the crystallization buffer (yellow). **C)** In the MID1-cobalt crystal structure (orange), one cobalt is coordinated by the C-terminal aspartate. The other cobalt is coordinated by the C-terminal carboxyl group from a symmetry-related molecule.

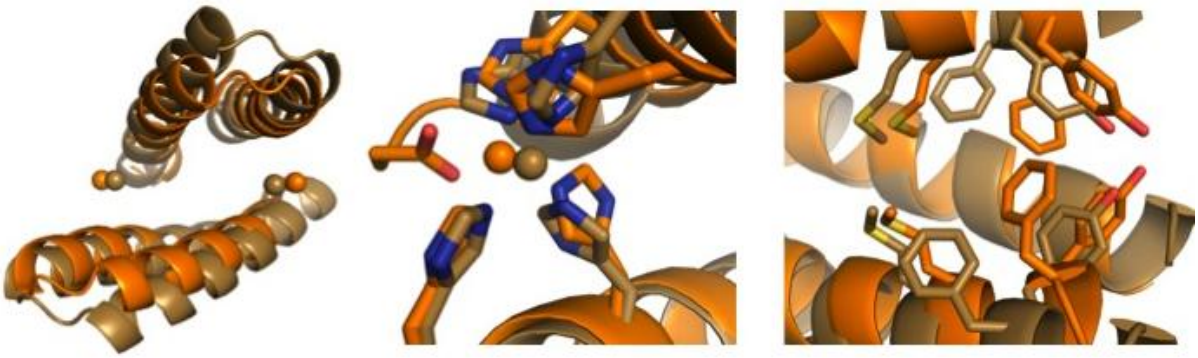


Figure S8: Comparison of the MID1-zinc model to the MID1-cobalt crystal structure. Right panel: the global alignment of metal-bound dimers, spheres indicate metal ions. **Center panel:** the observed metal-coordination geometry compared to the model. **Right panel:** observed interface sidechain contacts compared to the model.

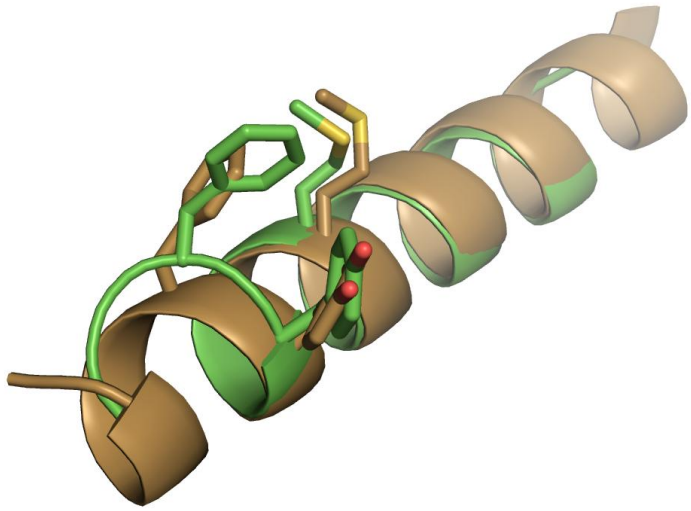
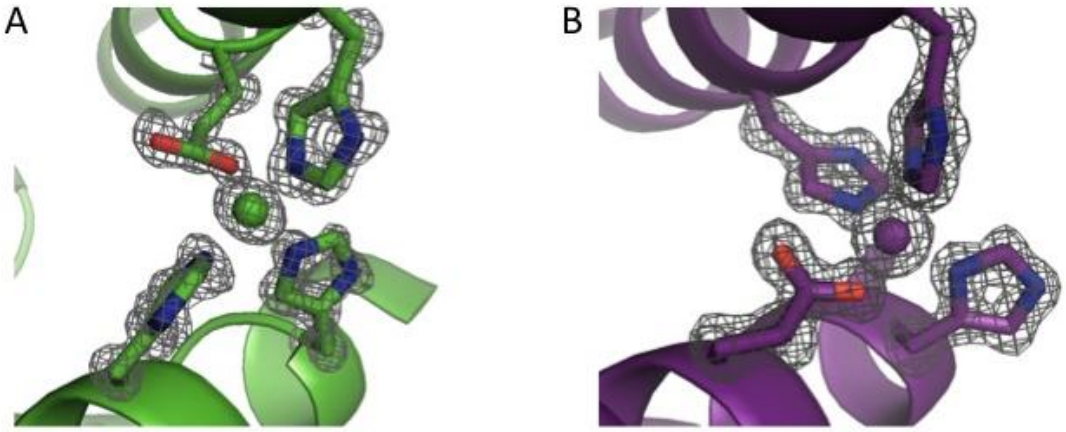


Figure S9: The position of phenylalanine at residue 42 deviates from the computational model due to helix unwinding. This unexpected backbone movement allows Phe42 from the MID1-H12E-zinc crystal structure (green) to fill empty space present at the interface in the design model (tan). The empty space in the design model is apparent in Figure 1B of the main article. Thus, instead of Phe42 only contacting the opposing helix as predicted, Phe42 directly interacts with its symmetric counterpart in the MID1-zinc, MID1-cobalt, and MID1-H12E-zinc crystal structures.



Figures S10: Glutamate point mutations result in four-coordination of zinc. Electron density (contour level = 2.0σ) at the coordination sphere of zinc in MID1-H12E (green) and MID1-H35E (purple) conclusively shows glutamate-zinc coordination and four-coordination of zinc.

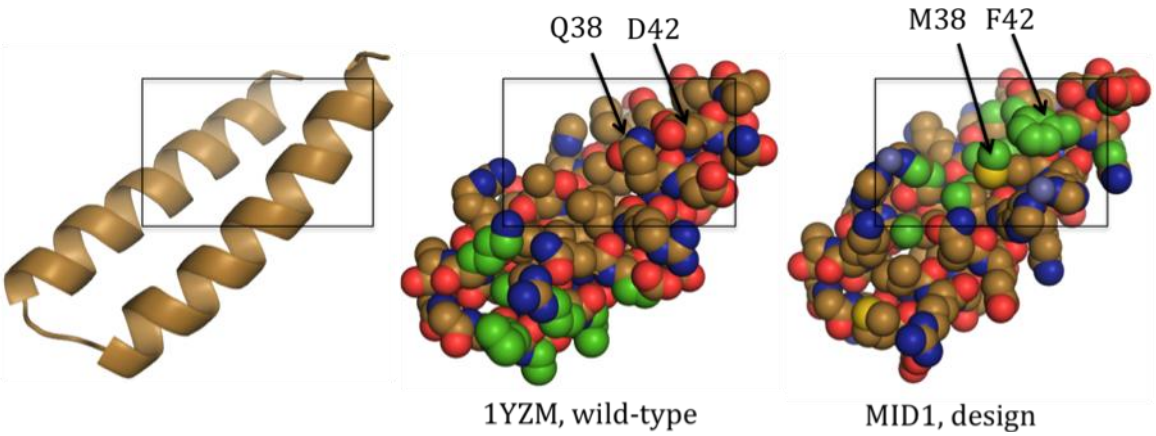


Figure S11: QUILT [2] analysis of hydrophobic patch size of the 1YZM wild-type scaffold compared to the MID1 design. The position of the MID1 interface is outlined in a black square, and the largest hydrophobic patch identified by QUILT is shown in green. The designed interface residues of MID1 form the largest hydrophobic patch (right), whereas the largest hydrophobic patch of the wild-type 1YZM scaffold is located elsewhere (center).

Supplementary Methods:

scaffold pdb code list

These scaffolds were obtained from a query of the Protein Data Bank, specifying no disulfides, no nucleic acid, less than 250 residues, resolution < 2.5 Å, and eliminating proteins with >70% sequence identity.

1A58, 1A7S, 1AKY, 1ALY, 1AYE, 1C81, 1CKE, 1E58, 1E5K, 1E9M, 1EX7, 1F4P, 1F9Y, 1FDR, 1FKB, 1FM4, 1FMK, 1G2R, 1G8A, 1GMI, 1GMX, 1GNU, 1GWM, 1H0P, 1H1D, 1H68, 1H6H, 1H7C, 1HBK, 1HE9, 1HH8, 1HQV, 1HTJ, 1HXI, 1HZ5, 1I1N, 1I2A, 1I39, 1I5G, 1I76, 1I8A, 1IAP, 1ID0, 1IFR, 1IJT, 1IKT, 1IM5, 1IMJ, 1IO2, 1IPC, 1IQZ, 1IU9, 1IUH, 1IUK, 1IXV, 1J2A, 1J3A, 1J84, 1JBE, 1JF8, 1JG1, 1JHS, 1JJV, 1JL1, 1JMW, 1JOS, 1JRL, 1JUV, 1JVW, 1JWQ, 1K1B, 1K6K, 1K7J, 1KGS, 1KMQ, 1KMV, 1KON, 1KR7, 1KSK, 1KW4, 1KY3, 1KZF, 1KZL, 1L2H, 1L3K, 1LB4, 1LFP, 1LM6, 1LMB, 1LQY, 1LU4, 1LVG, 1M2K, 1MB3, 1MG4, 1MIJ, 1MJ4, 1MK0, 1MQO, 1MR3, 1MVE, 1MVO, 1N3Y, 1NB9, 1NEG, 1NH9, 1NIO, 1NNX, 1NQZ, 1NWZ, 1NZN, 1O08, 1O1Z, 1O4R, 1O6D, 1O8V, 1O8X, 1O9G, 1OAP, 1OCS, 1OD3, 1OH4, 1OJQ, 1OPD, 1OSH, 1P2F, 1P4P, 1P4X, 1P5F, 1P5S, 1P90, 1PA7, 1PAQ, 1PBK, 1PI1, 1PKO, 1PMH, 1PZ4, 1Q1U, 1Q7H, 1Q7R, 1Q8B, 1QCY, 1QF9, 1QV1, 1QWZ, 1QZM, 1R18, 1R26, 1R2D, 1R2Q, 1R6J, 1R6N, 1R9H, 1R9W, 1RIS, 1RKB, 1RLJ, 1RM8, 1ROC, 1RW1, 1RW7, 1RWJ, 1RYB, 1RZ3, 1RZ4, 1S1E, 1S21, 1S29, 1S2O, 1S2X, 1S35, 1S3G, 1S3P, 1S68, 1S69, 1S7Z, 1S8N, 1S9U, 1SAU, 1SBX, 1SDI, 1SEN, 1SGW, 1SH6, 1SQW, 1SU0, 1SVI, 1T00, 1T3Y, 1T4W, 1T95, 1TA0, 1TEN, 1TEV, 1TFF, 1TKE, 1TOV, 1TQ3, 1TQ5,

1TQH, 1TS9, 1TT8, 1TTZ, 1TUH, 1TUV, 1TYJ, 1U02, 1U2P, 1U3G, 1U61, 1U6T, 1U7O, 1U7U, 1U84, 1U9C, 1U9P, 1UHN, 1UI0, 1UJC, 1ULR, 1UMH, 1UNQ, 1UOH, 1UOW, 1UPQ, 1URN, 1URR, 1UX8, 1UXX, 1UY4, 1UYL, 1UZ0, 1V0A, 1V5H, 1V77, 1V7R, 1VAJ, 1VE4, 1VG1, 1VJF, 1VJK, 1VJX, 1VK1, 1VK2, 1VKB, 1VKK, 1VKU, 1VMB, 1VR3, 1VR8, 1VSR, 1VYF, 1VZW, 1W0H, 1W0N, 1W1D, 1W24, 1W2L, 1W41, 1W4S, 1W66, 1W8G, 1W9W, 1WBE, 1WD5, 1WJ9, 1WJX, 1WL8, 1WLF, 1WLJ, 1WOJ, 1WPA, 1WQG, 1WR2, 1WRI, 1WRM, 1WS0, 1WS6, 1WU3, 1WUB, 1WV3, 1WVH, 1WVN, 1WZW, 1X0T, 1X1R, 1X3O, 1X6O, 1X6Z, 1X8H, 1XBI, 1XBN, 1XBS, 1XCL, 1XDZ, 1XE1, 1XJ3, 1XK5, 1XKR, 1XMT, 1XS5, 1XT0, 1XTQ, 1XW3, 1XWW, 1Y02, 1Y63, 1Y6I, 1Y81, 1Y88, 1Y8C, 1Y93, 1Y9Q, 1YD0, 1YE8, 1YIO, 1YN4, 1YQB, 1YSP, 1YSQ, 1YUL, 1YVD, 1YZL, 1Z06, 1Z0F, 1Z0I, 1Z0W, 1Z1S, 1Z2A, 1Z2M, 1Z2U, 1Z3X, 1Z4R, 1Z67, 1Z6G, 1Z95, 1ZAT, 1ZD8, 1ZD9, 1ZDE, 1ZI8, 1ZMA, 1ZN6, 1ZV9, 1ZZK, 1ZZO, 2A0J, 2A1I, 2A1V, 2A2K, 2A4V, 2A5J, 2A7B, 2A7M, 2A8E, 2A90, 2A9O, 2AAK, 2AAN, 2ACY, 2AF0, 2AJ6, 2AMH, 2AMY, 2AP3, 2AR1, 2AR5, 2ATZ, 2AVK, 2AVR, 2AWG, 2AWK, 2AZW, 2B0C, 2B5H, 2BBR, 2BDV, 2BEP, 2BF0, 2BFW, 2BH4, 2BK8, 2BK9, 2BKF, 2BL1, 2BL7, 2BL9, 2BM3, 2BMD, 2BMM, 2BMV, 2BOO, 2BRF, 2BSN, 2BWQ, 2BYO, 2BZ7, 2BZG, 2C1F, 2C2P, 2C3G, 2C53, 2C60, 2C71, 2CAL, 2CB9, 2CBZ, 2CDN, 2CE2, 2CFE, 2CHD, 2CJJ, 2CKK, 2CKX, 2CM5, 2CMT, 2CU9, 2CUL, 2CWR, 2CWS, 2CWY, 2CX1, 2CXH, 2CXV, 2CY2, 2CYJ, 2CYY, 2D1E, 2D2E, 2D3D, 2D3Y, 2d4x, 2D4X, 2D58, 2D6O, 2DCH, 2DHO, 2DJH, 2DWR, 2E1F, 2E6M, 2EI9, 2ESA, 2ESB, 2ETD, 2ETJ, 2EVE, 2EW0, 2EW1, 2EW5, 2EXU, 2EYI, 2F1W, 2F21, 2F9L, 2FCF, 2FCK, 2FCL, 2FDJ, 2FDR, 2FE5, 2FF7, 2FFQ, 2FGO, 2FI1, 2FIW, 2FJ9, 2FL7, 2FM9, 2FN4, 2FQ3, 2FSQ, 2FSX, 2FU2, 2FUF, 2FUK, 2FUP, 2FVV, 2FWH, 2FYG, 2FZ4, 2G3R, 2G3Y, 2G6B, 2G7B, 2G9F, 2GBN, 2GF9, 2GKG, 2GKP, 2GO2, 2GU3, 2GUI, 2GW2, 2GWM, 2GWR, 2H17, 2H5P, 2HAZ, 2HB5, 2HBW, 2HCF, 2HCU, 2HDO, 2HDZ, 2HE4, 2HHZ, 2HIA, 2HJE, 2HNX, 2HP7, 2HPJ, 2HPK, 2HQK, 2HS5, 2HSB, 2HW4, 2HWV, 2HXM, 2HXP, 2HZC, 2I0M, 2I5H, 2I5U, 2I6C, 2I6J, 2I6V, 2I88, 2I9C, 2I9W, 2IA7, 2IAF, 2IAY, 2IBB, 2IBJ, 2ICI, 2IDV, 2IGP, 2IHD, 2IJE, 2IL1, 2IL5, 2IMG, 2IN3, 2IOR, 2IPQ, 2IQC, 2IS9, 2IU1, 2IUG, 2IWD, 2IWN, 2IYV, 2J13, 2J1A, 2J1L, 2J22, 2J44, 2J49, 2J5A, 2J6A, 2J8K, 2J9V, 2JC7, 2JD9, 2JDC, 2JEK, 2JEX, 2JFR, 2JG6, 2JHS, 2JIN, 2NLY, 2NN5, 2NN8, 2NQ3, 2NQW, 2NR9, 2NS0, 2NS6, 2NSQ, 2NSZ, 2NYV, 2O2G, 2O2X, 2O37, 2O71, 2O7A, 2O9U, 2OC5, 2OCS, 2ODH, 2ODV, 2OFZ, 2OGQ, 2OJ4, 2OLM, 2OML, 2ONU, 2OPC, 2OQK, 2OQZ, 2OSS, 2OT9, 2OVJ, 2OZF, 2P0D, 2P0T, 2P2E, 2P3H, 2P57, 2P8G, 2PA1, 2PAG, 2PC1, 2PCS, 2PE8, 2PHC, 2PKT, 2PL1, 2PL3, 2PLU, 2PLW, 2PNM, 2PNY, 2POE, 2POI, 2PPX, 2PV4, 2PWQ, 2PWW, 2PXX, 2Q0V, 2Q3H, 2Q7B, 2Q9V, 2QG1, 2QGG, 2QGU, 2QJL, 2V0S, 2V1L, 3EUG

Running SurfaceGroups in Rosetta

File: mini/src/apps/pilot/rjha/SurfaceGroups.cc

Command line: `./SurfaceGroups.linuxgccrelease -database /path_to/rosetta_database -jd2:no_output -l pdblist.txt -`

`local:surface_residue 18` (cutoff for number of neighbors by distance that qualifies a residue as surface)

Running ZincMatchFilter in Rosetta

File: mini/src/apps/pilot/rjha/MatchFilter.cc

Command line: `./ZincMatchFilter.linuxgccrelease -database /path_to/rosetta_database -jd2:no_output -l matchlist.txt`

Running RosettaMatch in Rosetta

The zinc-binding constraint file used in RosettaMatch is given below. For a detailed explanation of this file, refer to the Rosetta documentation in rosetta/rosetta_source/doc/public/enzyme_design.dox

ZNX.cst (matcher geometric constraint file for zinc binding sites)

```
#block 1 of 2
VARIABLE_CST::BEGIN
#Block 1 His, trying to make it work for both NE2 and ND1 at the same time
(torsion_AB = 0 +/- 180)
CST::BEGIN
  TEMPLATE::   ATOM_MAP: 1 atom_name: ZN V1 V2
  TEMPLATE::   ATOM_MAP: 1 residue3:  ZNX

  TEMPLATE::   ATOM_MAP: 2 atom_type: Nhis
  TEMPLATE::   ATOM_MAP: 2 residue3:  HIS

  CONSTRAINT:: distanceAB:   2.05   0.15  40.0  0
  CONSTRAINT::    angle_A:   109.5  15.0  40.0  360.
  CONSTRAINT::    angle_B:   125.0  15.0  40.0  360.
  CONSTRAINT::  torsion_A:   0.0    15.0  40.0  10.0
  CONSTRAINT:: torsion_AB:   60.0   15.0  40.0  120.
  CONSTRAINT::  torsion_B:   0.0    15.0  40.0  180.
CST::END

#Block 1 Cys
CST::BEGIN
  TEMPLATE::   ATOM_MAP: 1 atom_name: ZN V1 V2
  TEMPLATE::   ATOM_MAP: 1 residue3:  ZNX

  TEMPLATE::   ATOM_MAP: 2 atom_type: S
  TEMPLATE::   ATOM_MAP: 2 residue1:  C

  CONSTRAINT:: distanceAB:   2.33   0.15  40.0  0
  CONSTRAINT::    angle_A:   109.5  15.0  40.0  360.
  CONSTRAINT::    angle_B:   109.5  15.0  40.0  360.
  CONSTRAINT::  torsion_A:   0.0    15.0  40.0  10.0
  CONSTRAINT:: torsion_AB:   60.0   15.0  40.0  120.
  CONSTRAINT::  torsion_B:   0.0    15.0  40.0  10.0
CST::END
VARIABLE_CST::END
```

ZNX.params (parameter file that describes the zinc transition state with zinc and virtual atoms)

```
NAME ZNX
IO_STRING ZNX Z
TYPE LIGAND
AA UNK

ATOM ZN   Zn2p  X   2.0
ATOM  V1  VIRT  X   0.0
ATOM  V2  VIRT  X   0.0
ATOM  V3  VIRT  X   0.0
ATOM  V4  VIRT  X   0.0

BOND  ZN   V1
BOND  ZN   V2
BOND  ZN   V3
BOND  ZN   V4

NBR_ATOM  ZN
NBR_RADIUS 0.0

ICOOR_INTERNAL    V1     0.000000    0.000000    0.000000   V1    ZN    V2
ICOOR_INTERNAL    ZN     0.000000    0.000000    1.000000   V1    ZN    V2
ICOOR_INTERNAL    V2     0.000000   70.500000    1.000000   ZN    V1    V2
ICOOR_INTERNAL    V3  -120.000000   70.500000    1.000000   ZN    V1    V2
ICOOR_INTERNAL    V4   120.000000   70.500000    1.000000   ZN    V1    V2
```

RosettaMatch options file (command line)

-database /ifs1/home/bder/minirosetta_database
-match::lig_name ZNX
-match::grid_boundary /path_to/gridlig.txt
-match::scaffold_active_site_residues /path_to/pos.txt
-match::geometric_constraint_file /path_to/ZNX.cst
-match::output_matchres_only
-extra_res_fa /path_to/ZNX.params
-output_matches_per_group 10
-ex1, ex2
-euclid_bin_size 1.0
-euler_bin_size 10.0
-bump_tolerance 0.5
-match:output_format PDB
-match:consolidate_matches
-match:output_matchres_only

More detailed documentation for RosettaMatch can be found in rosetta/rosetta_source/src/protocols/match.

Running SymMetalInterface_TwoZN_setup

File: rosetta/rosetta_source/src/apps/pilot/bder/SymmMetalInterfaceDesigner_TwoZN_setup.cc

Command line options:
-database /path_to/rosetta_database
-jd2:no_outupt
-s 1YZM_WT.pdb
-match1 1YZM_1_H12H16_1_ZNX.pdb
-match2 1YZM_1_H35H39_1_ZNX.pdb
-angle_rotation_increment 5.0
-ddG_centroid_cutoff 0.0
-zn_zn_distance_cutoff 10.0
-tetrahedral_angle_sumsq_cutoff 1800

Running SymMetalInterface_TwoZN_design

File: rosetta/rosetta_source/src/apps/pilot/bder/SymmMetalInterfaceDesigner_TwoZN_design.cc
Command line options:
-database /path_to/minirosetta_database
-jd2:no_output
-symmetry:symmetry_definition symmdef.txt
-s 1YZM_1_H12H16_1_ZNX.1YZM_1_H35H39_1_ZNX_360_INPUT.pdb
-repackmin_iterations 5
-fav_nat_bonus 1.5
-nstruct_iterations 10

Fluorescence polarization homodimeric equilibrium binding equations

To obtain apparent K_d's from fluorescence polarization titration experiments, polarization values were correlated to fraction-bound values using the following equations in SigmaPlot. These equations assume that affinity is equivalent between labeled and unlabeled MID1, and they assume that the concentration of labeled protein remains constant throughout the titration (~20 ml added to 3000 ml starting volume).

; variables are Kd, Polmax, Polmin
; X data column is final concentration of titrant
; Y data column is polarization

labled=0.01; [MID1-GC-Bodipy]=10 nM, assumed constant
Ptotal=labled+X; Ptotal=total protein, X=[titrant]
deltaPol=Polmax-Polmin; polarization change
a=2, b=Kd, c=-Ptotal*Kd
MonomerConc=(-b+sqrt(b*b-4*a*c))/(2*a)
DimerConc=(Ptotal-MonomerConc)/2
;write heterodimer conc as fraction of total protein
HeteroDimer=2*(labled / Ptotal)*(X/Ptotal)*DimerConc fractionHet=HeteroDimer / labled
Y=deltaPol*fractionHet+Polmin

PDB survey of zinc binding sites

The Protein Data Bank was queried for entries containing zinc atoms and <70% homology. For each zinc atom, the number of histidine, cysteine, aspartate, and glutamate sidechains with a non-carbon atom within 3 Å of the zinc atom was counted. If this number equaled four, it was marked as a four-residue zinc binding site, and the coordinating residue types are given in Supplemental Table SV.

(1) Adams, *et al*. *Acta Crystallographica Section D-Biological Crystallography* **2010**, *66*, 213-221.
(2) Lijnzaad, P.; Berendsen, H. J.; Argos, P. *Proteins* **1996**, *26*, 192-203.