Supplementary Material (ESI) for Molecular BioSystems

Supplementary data

The isolated Cys_2His_2 site in E_C metallothionein mediates metal-specific protein folding

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Table S1: Theoretical and observed masses of neutral zinc- and cadmium-loaded recombinant wheat E_C species. Errors of observed masses are <1.0 Da, except for low-abundance peaks, where the mass error may reach 1.5 Da.

protein	Metalloform	Observed Mass	Theoretical Mass
WT-apo	M_0-Met	7735.5	7735.7
	M_0+Met	7866.4	7866.9
	M_0+DTT	8000.9	8002.7
WT-Zn	Zn_5 –Met	8051.5	8052.1
	Zn_6 – Met	8116.1	8115.5
	Zn_5+Met	8182.6	8183.3
	Zn_6 +Met	8247.8	8246.7
	Zn_5+Met +Tris	8287.1	8286.6
	Zn_6 +Met +Tris	8350.7	8350.0
	Zn_6 +Met+DTT	8383.4	8383.0
WT-Cd	Cd_5 -Met	8287.0	8287.3
	Cd_5 +Met	8417.8	8418.5
	Cd_5Zn +Met	8481.9	8481.8
	Cd_6 +Met	8528.8	8528.9
	Cd_5 +Met+DTT	8551.7	8554.8
	Cd_5Zn +DTT	8617.4	8618.1
	Cd_6 +Met+DTT	8664.1	8665.2

Table S2: Theoretical and observed masses of neutral zinc-loaded mutant wheat E_C species. Errors of observed masses are <1.0 Da, except for low-abundance peaks, where the mass error may reach 1.5 Da.

protein	Metalloform	Observed Mass	Theoretical Mass
H32A	Zn_5 -Met	7986.5	7986.0
	Zn_6 -Met	8051.0	8049.4
	Zn_5 +Met	8120.7	8117.2
	Zn_6 +Met	8180.1	8180.6
	Zn_5 +Met+DTT	8252.1	8253.5
	Zn_6 +Met +Tris	8285.4	8283.9
	Zn_6 +Met+DTT	8318.6	8316.9
	Zn_7 +Met+DTT	8380.7	8380.3
H40A	Zn_4 -Met	7920.0	7922.6
	Zn_5 -Met	7985.8	7986.0
	Zn_6 -Met	8052.0 [b)]	8049.4
	Zn_4 +Met	8052.0 [b)]	8053.8
	Zn_5 +Met	8120.3	8117.2
	Zn_6 +Met	8184.8	8180.6
	Zn_6 +Met +Tris	8284.8	8283.9
	Zn_6 +Met+DTT	8317.6	8316.9

	Zn_7 +Met+DTT	8378.5	8380.3
H32A/H40A	Zn_4 -Met	7854.7	7856.7
	Zn_5 -Met	7921.2	7920.1
	Zn_4 +Met	7986.8 [b)]	7987.9
	Zn_6 -Met	7986.8 [b)]	7983.5
	Zn_5 +Met	8054.7	8051.3
	Zn_6 +Met	8120.3	8114.77
	Zn_5 +Met+DTT	8186.8	8187.6
	Zn_6 +Met+DTT	8250.1	8251.0
	Zn_7 +Met+DTT	8312.6	8314.4
H32C [c)]	Zn_5 -Met	8016.1	8018.4
	Zn_6 -Met	8082.3	8081.8
	Zn_5Cd -Met	8128.7	8128.8
	Zn_5 +Met	8146.8	8149.6
	Zn_6Cd -Met	8192.8	8192.2
	Zn_6 +Met	8213.0	8213.0
	Zn_5Cd +Met	8260.6	8260.0
	Zn_6Cd +Met	8322.9	8323.4
	Zn_6 +Met+DTT	8347.7	8349.2
	Zn_5Cd +Met+DTT	8394.9	8396.3
H40C [c)]	Zn_4 -Met	7953.0	7955.0
	Zn_5 -Met	8017.6	8018.4
	Zn_4Cd -Met	8063.4	8065.4
	Zn_4 +Met	8083.1 [b)]	8086.2
	Zn_6 -Met	8083.1 [b)]	8081.8
	Zn_5Cd +Met	8128.1	8128.8
	Zn_5 +Met	8149.6	8149.6
	Zn_4Cd +Met	8192.7	8196.6
	Zn_6 +Met	8214.3	8213.0
	Zn_5 +Met+DTT	8279.0	8285.8
	Zn_6 +Met+DTT	8349.0	8349.2
	Zn_4Cd +Met+DTT	8324.7	8332.9

Notes:

a) The species -Met+DTT are not considered, as their abundance is expected to be minor, and their masses are similar to the species +Met.

b) These peaks are composed of more than one species. The mass of a Methionine (131.2 Da) and the mass for the addition of $2Zn^{2+}$-$4H^{+}$ = 126.8 Da are relatively close.

c) Sample contaminated by small amounts of Cd^{2+}. This contamination does not affect conclusions reached in the manuscript.

Table S3. Chemical shifts for H32C mutant of wheat E_C with bound Zn^{2+}

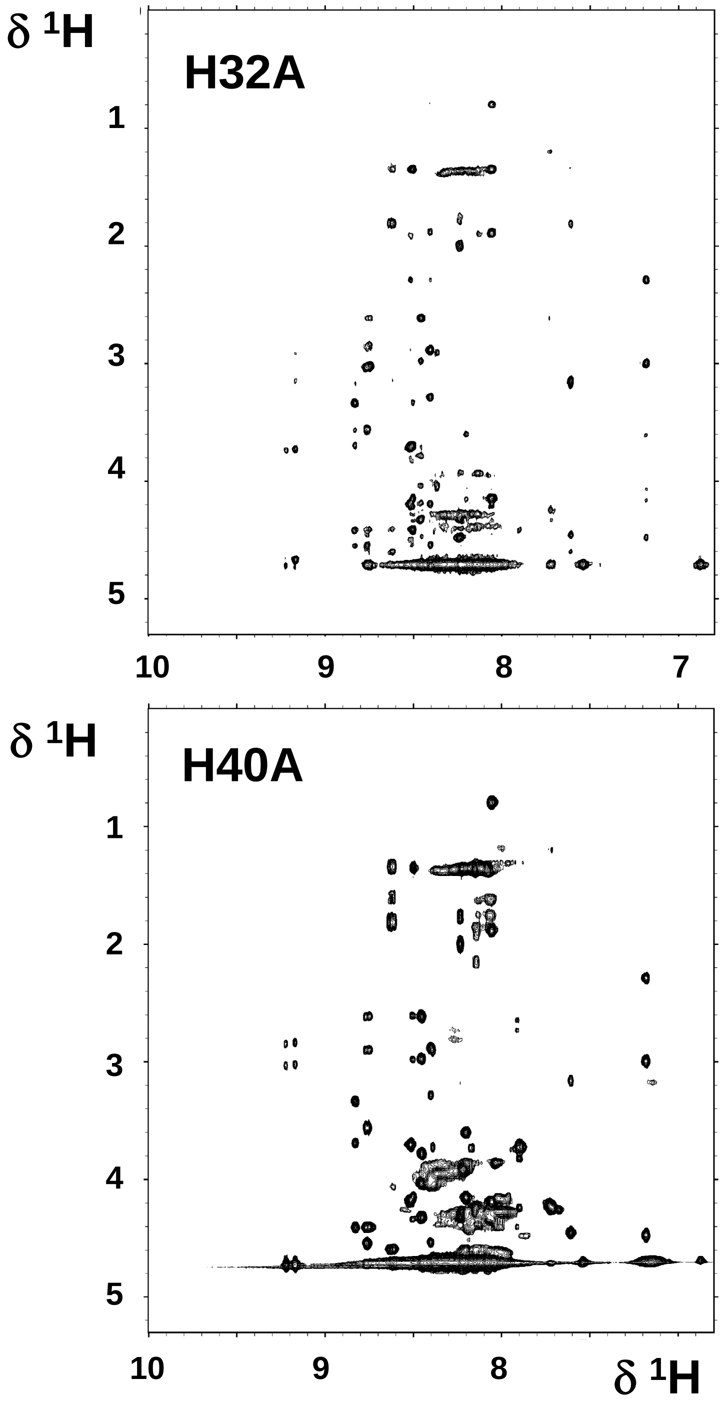
(700 MHz, 50 mM Tris-D_{11}, 50 mM NaCl, 10% D_2O, pH 7.3).

	NH	H(α)	H(β)	H(γ), H(δ), H(ε)
GLY1	-	3.72	--	
CYS2	9.17	4.71	3.02 2.83	
CYS2'	9.22	4.71	3.02 2.87	
ASP3	n.d.	n.d.	n.d.	
ASP4	8.77	4.40	2.90;2.61	
LYS5	8.63	4.59	1.79	1.34; 1.58
CYS6	7.60	4.45	3.15	
GLY7	8.76	4.54; 3.55		
CYS8	8.83	4.40	3.68; 3.33	
ALA9	8.50	4.14	1.34	
VAL10	8.06	4.19	1.89	0.80
PRO11	-	4.72	2.27; 1.87	1.78; 3.47; 3.56
CYS12	8.40	4.53	3.27; 2.87	
PRO13	-	4.20	2.20; 1.92	2.06; 3.81
GLY14	8.51	4.17; 3.69		
GLY15	8.45	4.01; 3.80		
THR16	8.03	4.26	4.13	1.16
GLY17	8.20	4.15; 3.59		
CYS18	7.18	4.47	2.98; 2.28	
ARG19	8.23	4.34	2.10; 2.00	1.79; 1.72
CYS20	8.46	4.32	2.97; 2.60	
THR21	7.71	4.24	4.14	1.19
ARG24	8.07	4.32	1.75; 1.61	
GLY26	8.40	3.90		
ALA27	8.08	4.27	1.34	
GLY30	8.24	3.93		
GLU31	8.13	4.39	2.06; 1.89	2.24
CYS32	8.38	4.46	2.66	
THR33	9.54	4.36	3.86	1.04
THR34	8.15	4.62	4.01	0.46; 4.23(OH)
CYS35	8.41	4.12	3.16; 2.95	
GLY36	9.39	4.04; 3.96		
CYS37	7.25	4.58	3.29; 3.00	
GLY38	8.27	4.53; 3.58		
GLU39	8.25	4.57	2.09; 1.99	2.46; 2.30
HIS40	9.15	4.42	2.99; 2.85	7.10; 7.86
CYS41	8.45	4.43	3.07; 2.87	
GLY42	8.49	3.89; 3.81		
CYS43	7.52	4.38	3.01	
ASN44	7.24	4.89	2.50	7.44; 6.81
PRO45	-	4.15	2.23; 2.02	1.92; 3.56; 3.45
CYS46	8.55	4.46	3.57; 3.08	
ALA47	8.91	4.29	1.48	
CYS48	9.38	4.45	3.21; 2.94	
GLY49	7.78	4.19; 3.75		
ARG50	8.48	4.09	1.52; 1.34	1.23; 2.65
GLU51	8.41	4.36	2.05; 1.90	2.22
GLY52	8.29	4.15; 3.92		

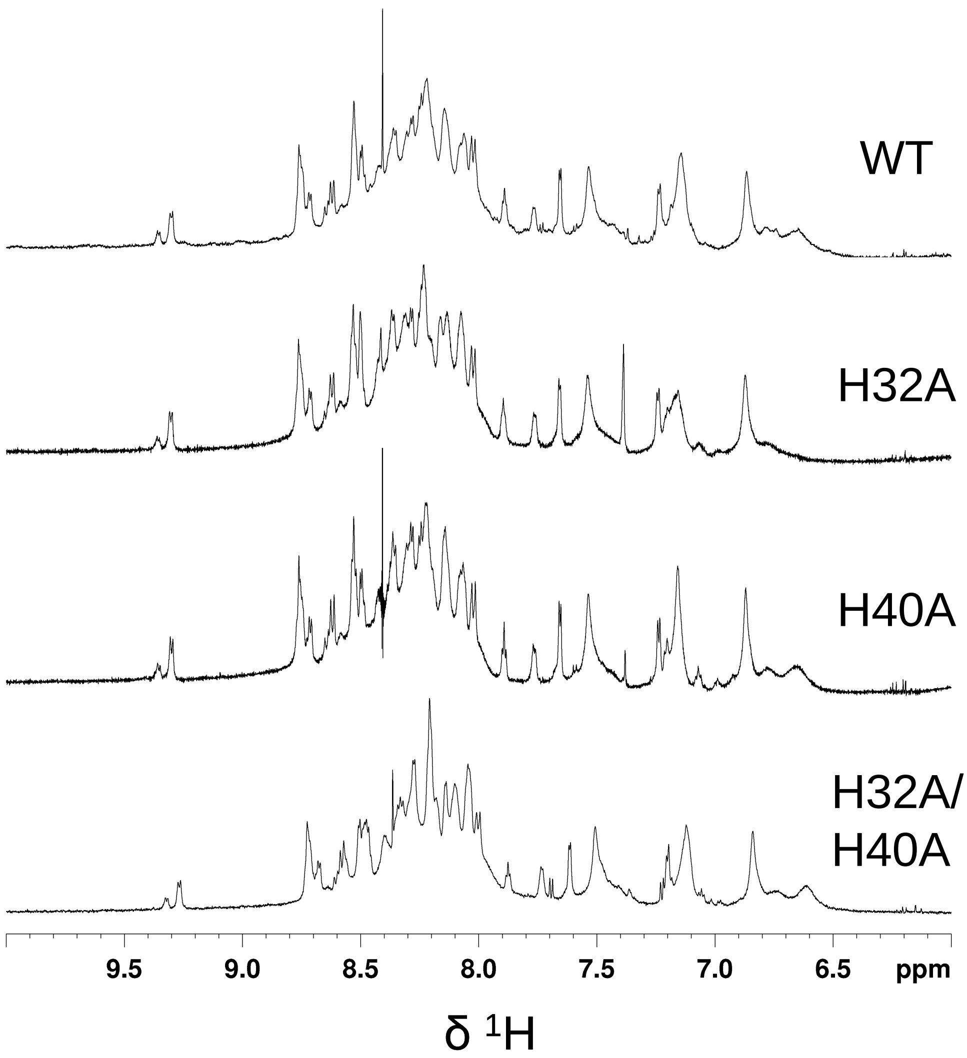
THR53	8.46	4.59	3.98	1.20
PRO54	-	4.17	2.34	1.87; 3.65
SER55	8.87	4.29	3.93; 3.85	
GLY56	8.92	3.94; 3.81		
ARG57	8.00	4.13	1.78; 1.63	1.54
ALA58	8.52	3.82	1.37	
ASN59	8.51	4.47	2.99; 2.87	7.53; 6.86
ARG60	7.35	4.89	1.94; 1.71	1.72; 1.41
ARG61	8.26	4.10	1.86; 1.82	1.73; 1.69
ALA62	8.58	4.07	1.38	
ASN63	8.07	4.55	3.03; 2.79	7.55; 6.79
CYS64	7.76	4.69	3.07; 2.98	
SER65	9.48	4.82	4.20; 3.60	
CYS66	9.71	4.02	3.00; 2.86	
GLY67	9.05	4.29; 3.83		
ALA69	n.d.	4.27	1.31	
CYS70	7.02	3.96	2.83	
ASN71	8.65	4.95	2.76	7.44; 6.74
CYS72	8.74	4.11	2.92; 2.83	
ALA73	9.21	n.d.	1.48	
SER74	8.45	4.39	3.89	
CYS75	8.27	4.22	2.76	
GLY76	7.61	4.25; 3.81		
SER77	7.55	4.39	3.80; 3.71	
ALA80	n.d.	n.d.	n.d.	
PRO81	n.d.	n.d.	n.d.	
Gly82	n.d.	n.d.		

Table S4. Selected distance restraints derived from 2D NOESY spectrum (120 ms mixing time) of Zn-H32C.

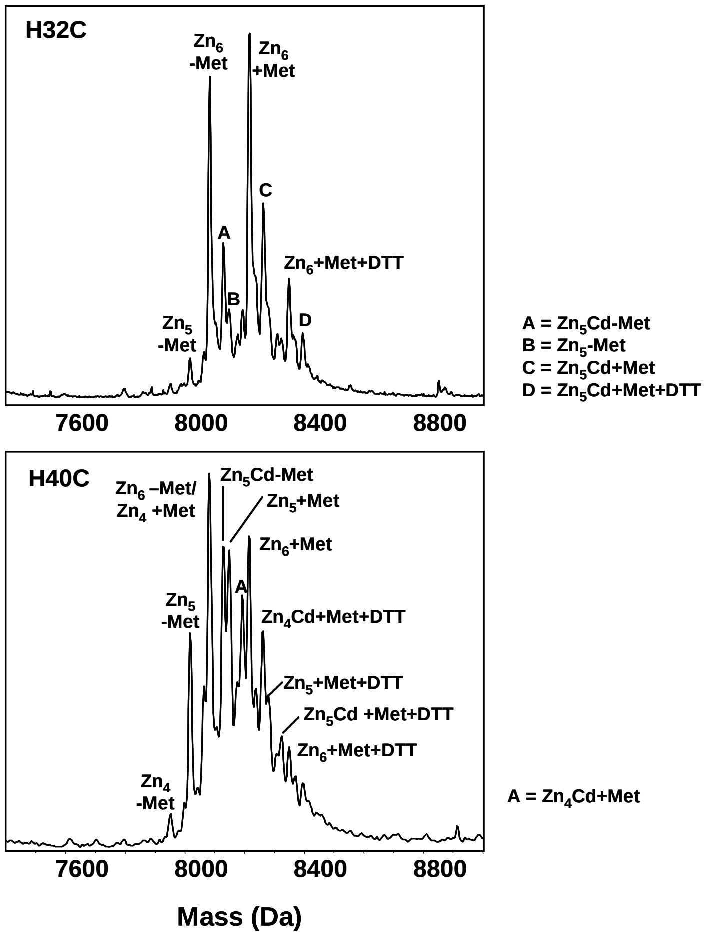
Atom 1			Atom 2			Restraint (Å)
34	THR	HA	40	HIS	HE1	6.04
50	ARG	CG	40	HIS	HD2	6.50
32	CYS	H	40	HIS	HE1	5.50
40	HIS	HD2	50	ARG	H	5.40
32	CYS	HA	40	HIS	HE1	5.05
39	GLU	HG3	40	HIS	H	4.80
32	CYS	CB	40	HIS	HD2	5.75
46	CYS	HB2	48	CYS	H	4.65
31	GLU	H	32	CYS	H	4.60
40	HIS	HD2	46	CYS	H	4.30
46	CYS	H	40	HIS	HD2	4.25
41	CYS	H	40	HIS	HD2	4.20
39	GLU	HB3	40	HIS	H	4.10
46	CYS	HB3	33	THR	H	4.04
48	CYS	HA	49	GLY	H	3.80
40	HIS	HA	40	HIS	H	3.80
46	CYS	HB2	33	THR	H	3.70
46	CYS	HB3	48	CYS	H	3.70
45	PRO	HB2	46	CYS	H	3.65
47	ALA	CB	48	CYS	H	4.60
39	GLU	HG2	40	HIS	H	3.55
40	HIS	HB3	40	HIS	HD2	3.45
40	HIS	HB2	40	HIS	H	3.40
32	CYS	HA	32	CYS	H	3.40
39	GLU	HB2	40	HIS	H	3.30
34	THR	CG2	40	HIS	HE1	4.30
46	CYS	HA	46	CYS	H	3.05
48	CYS	HA	48	CYS	H	3.05
50	ARG	HB3	40	HIS	HD2	3.05
46	CYS	HB2	46	CYS	H	3.00
32	CYS	CB	33	THR	H	4.00
46	CYS	HB2	40	HIS	HD2	2.85
46	CYS	HB3	46	CYS	H	2.80
31	GLU	HB2	32	CYS	H	2.65
46	CYS	HA	47	ALA	H	2.60
32	CYS	CB	40	HIS	HE1	3.55
39	GLU	HA	40	HIS	H	2.50
46	CYS	HB3	40	HIS	HD2	2.45
31	GLU	HA	32	CYS	H	2.17
32	CYS	HA	33	THR	H	2.16
45	PRO	HA	46	CYS	H	2.12
40	HIS	HB2	40	HIS	HD2	1.88



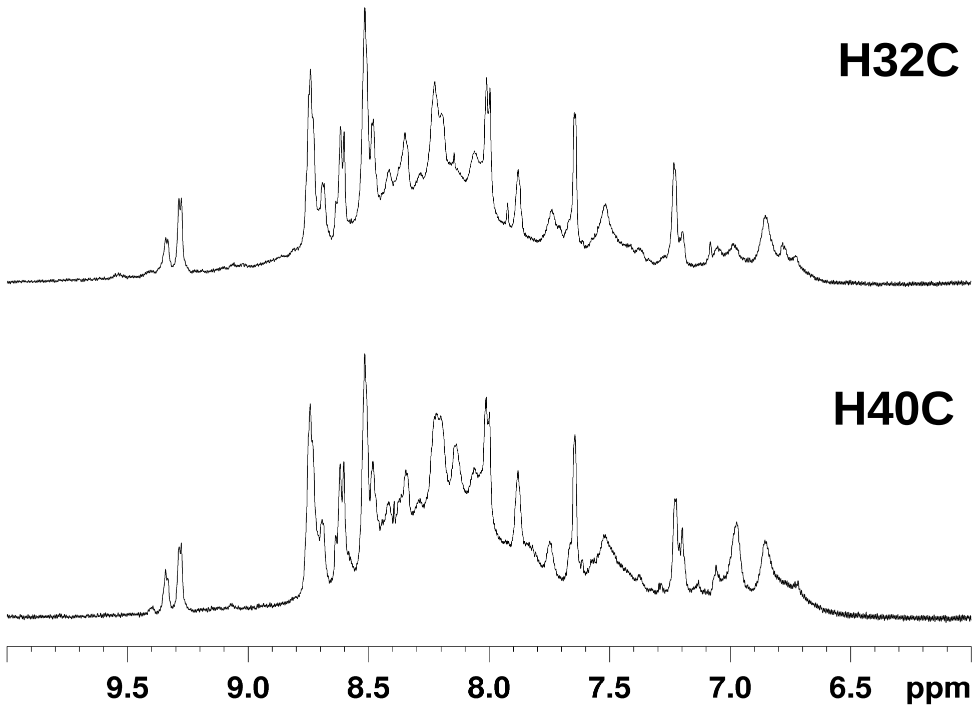
Supplementary Figure S1. Fingerprint regions of 2D TOCSY spectra of Zn-bound H32A and H40A mutants.



Supplementary Figure S2. 1D ^{1}H spectra (fingerprint region) of Cd-bound His-to-Ala mutants, in comparison with the spectrum for Cd-bound wild-type E_C.



Supplementary Figure S3. Deconvoluted ESI-Mass spectra of His-to-Cys mutants expressed in the presence of Zn^{2+}. The spectrum for H32C is dominated by M_6 species, whereas the increased presence of undermetallated species is evident from the spectrum for H40C. The presence of small amounts of Cd^{2+} is due to sample contamination, but does not affect conclusions drawn.



Supplementary Figure 4. Fingerprint region of 1D ^{1}H NMR spectra for Cd-bound His-to-Cys mutants.