Suppl. Materials

Figure S1 - System preparation. Truncated models of holo IscU_wt used in DFT calculations. Hydrogen atoms were added to compensate the valence of the truncated atoms.

Figure S2 - Location of the BCPs as respect to the atomic positions of the truncated models of IscU_wt and its mutants as in Figure 5 but assuming protonated cysteines. Top two panels: His105 of IscU_wt is assumed uncharged (left) and charged (right); Middle panels: the same for IscU_D39A. Lower panel left: IscU_H105A. Lower panel right: IscU_D39A_H105A. BCPs are shown as small red spheres. Zn, S, O, C, H, and N atoms are shown as white, yellow, red, black, grey, and blue spheres respectively.

Figure S3 – Effect of Zn^{2+} on the NMR HSQC spectrum of IscU_wt as in Figure 6D but in the presence of 150 mM NaCl. From top to bottom: Spectra of EDTA pre-treated IscU_wt, titrated with 1:1 and 5:1 Zn^{2+}: protein molar equivalents.

Table S1. Geometric parameters of the coordination site in zinc loaded IscU with all Cys protonated. The distances were taken from the structures optimized at the detailed QM/MM computational level. Numbering is according to Figure S1.

	Wt ($H105^+$)	Wt ($H105^0$)	D39A[a] ($H105^+$)	D39A[a] ($H105^0$)	H105A	D39A_H105A
Distances (Å)						
Zn-S(5)	2.604	4.252	2.484	2.633	2.974	2.495
Zn-S(7)	2.554	2.493	2.468	2.560	2.509	2.521
Zn-O(12)	2.092	2.045	--	--	2.023	--
Zn-O(13)	2.108	2.113	--	--	2.203	--
Zn-S(10)	2.746	4.081	2.467	2.582	2.580	2.489
Zn-N(9)	8.184	2.065	9.293	2.054	--	--
SH(5)-O(12)	4.488	2.954	--	--	2.459	--
SH(10)-O(13)	2.608	2.348	--	--	2.269	--
Angles (*deg*)						
O(12)-HS(5)-S(5)	42.6	151.0	--	--	118.6	--
O(13)-HS(10)-S(10)	120.4	143.8	--	--	121.4	--
O(13)-HS(7)-S(7)	33.1	143.8	--	--	--	--
S(7)-Zn-N(7)	144.1	110.6	--	136.15	--	--
O(12)-Zn-O(13)	62.73	63.6	--	--	62.2	--
Dihedral Angles (*deg*)						
S(7)-N(9)-O(13)-O(12)	87.1	84.9	--	--	--	--
S(5)-S(7)-Zn-S(10)	116.9	--	150.9	--	--	156.19
S(7)-N(9)-S(10)-S(5)	-5.6	--	--	51.5	--	--
S(7)-S(10)-O(13)-O(12)	117.9	--	--	--	88.2	--

Table S2. Geometric parameters of the coordination site in zinc loaded IscU when all Cys are deprotonated. The distances were taken from the structures optimized at the detailed QM/MM computational level. Numbering is according to Figure S1.

	Wt ($H105^+$)	Wt ($H105^0$)	D39A[a] ($H105^+$)	D39A[a] ($H105^0$)	H105A	D39A_H105A
Distances (Å)						
Zn-S(5)	2.396	5.706	2.389	2.518	2.585	2.419
Zn-S(7)	2.372	2.392	2.319	2.419	2.484	2.391
Zn-O(12)	2.202	2.050	--	--	2.095	--
Zn-O(13)	2.241	2.997	--	--	2.982	--
Zn-S(10)	4.826	2.414	2.479	2.485	2.541	2.405
Zn-N(9)	7.623	2.211	4.422	2.351	--	--
Angles (*deg*)						
S(5)-Zn-S(7)	121.2	73.3	126.2	101.1	96.3	107.1
N(9)-Zn-O(12)	81.9	93.5	--	--	--	--
N(9)-Zn-S(10)	47.0	92.2	44.0	86.06	--	--
S(7)-Zn-N(9)	146.7	105.4	62.8	117.5	--	--
O(12)-Zn-O(13)	59.6	48.1	--	--	48.6	--
Dihedral Angles (*deg*)						
S(7)-N(9)-O(13)-O(12)	102.1	120.5	--	--	--	--
S(5)-S(7)-Zn-S(10)	115.4	146.5	153.6	-171.9	130.5	165.6
S(7)-Zn-N(9)-S(10)	10.0	141.8	34.2	111.5	--	--
N(9)-Zn-O(12)-O(13)	41.3	168.8	--	--	--	--

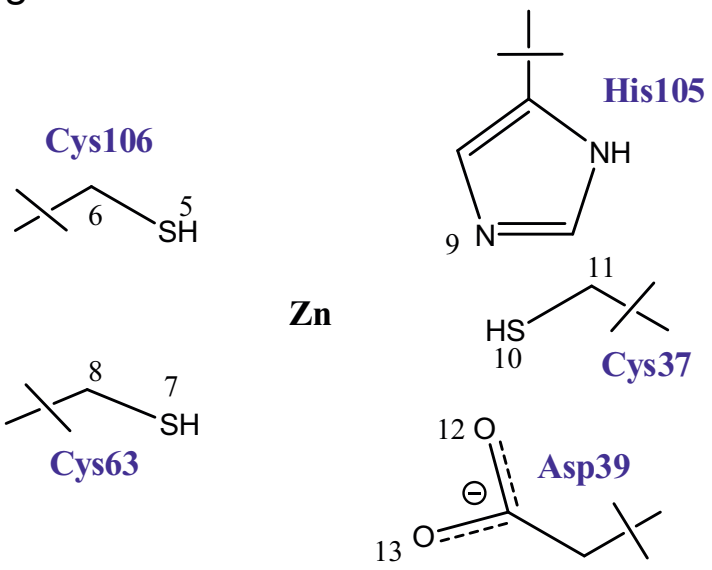
Table S3. Electron density at the bond critical points [$\rho(r_c)$ (au)] of IscU as calculated assuming protonated cysteines. BCP were derived from the wave function calculated at the SMD-B3LYP/6-311++G** computational level.

	BCP					
	Wt ($H105^+$)	Wt ($H105^0$)	D39A[a] ($H105^+$)	D39A[a] ($H105^0$)	H105A	D39A_H105A
Zn-S(5)	0.041	--	0.051	0.035	0.017	0.049
Zn-S(7)	0.046	0.050	0.055	0.045	0.050	0.049
Zn-O(12)	0.064	0.069	--	--	0.073	--
Zn-O(13)	0.061	0.058	--	--	0.049	--
Zn-N(9)	--	0.074	--	0.076	--	--
Zn-S(10)	0.030	--	0.055	0.044	0.044	0.053
SH(5)-O(12)	--	0.003	--	--	0.010	--
SH(10)-O(13)	--	0.011	--	--	0.016	--

Table S4. Electron density at the bond critical points [$\rho(r_c)$ (au)] of IscU as calculated assuming deprotonated cysteines. BCP were derived from the wave function calculated at the SMD-B3LYP/6-311++G** computational level.

	BCP					
	Wt ($H105^+$)	Wt ($H105^0$)	D39A[a] ($H105^+$)	D39A[a] ($H105^0$)	H105A	D39A_H105A
Zn-S(5)	0.062	--	0.061	0.046	0.042	0.056
Zn-S(7)	0.065	0.063	0.071	0.059	0.053	0.062
Zn-O(12)	0.049	0.067	--	--	0.061	--
Zn-O(13)	0.045	--	--	--	--	--
Zn-N(9)	--	0.054	--	0.040	--	--
Zn-S(10)	--	0.058	0.054	0.054	0.048	0.062
S(10)-O(13)	0.004	--	--	--	--	--
S(10)-NH^+(9)	--	--	0.053	--	--	--

Figure S1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SH
His105
NH
9
N
11
HS
10
Cys37
Zn
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12
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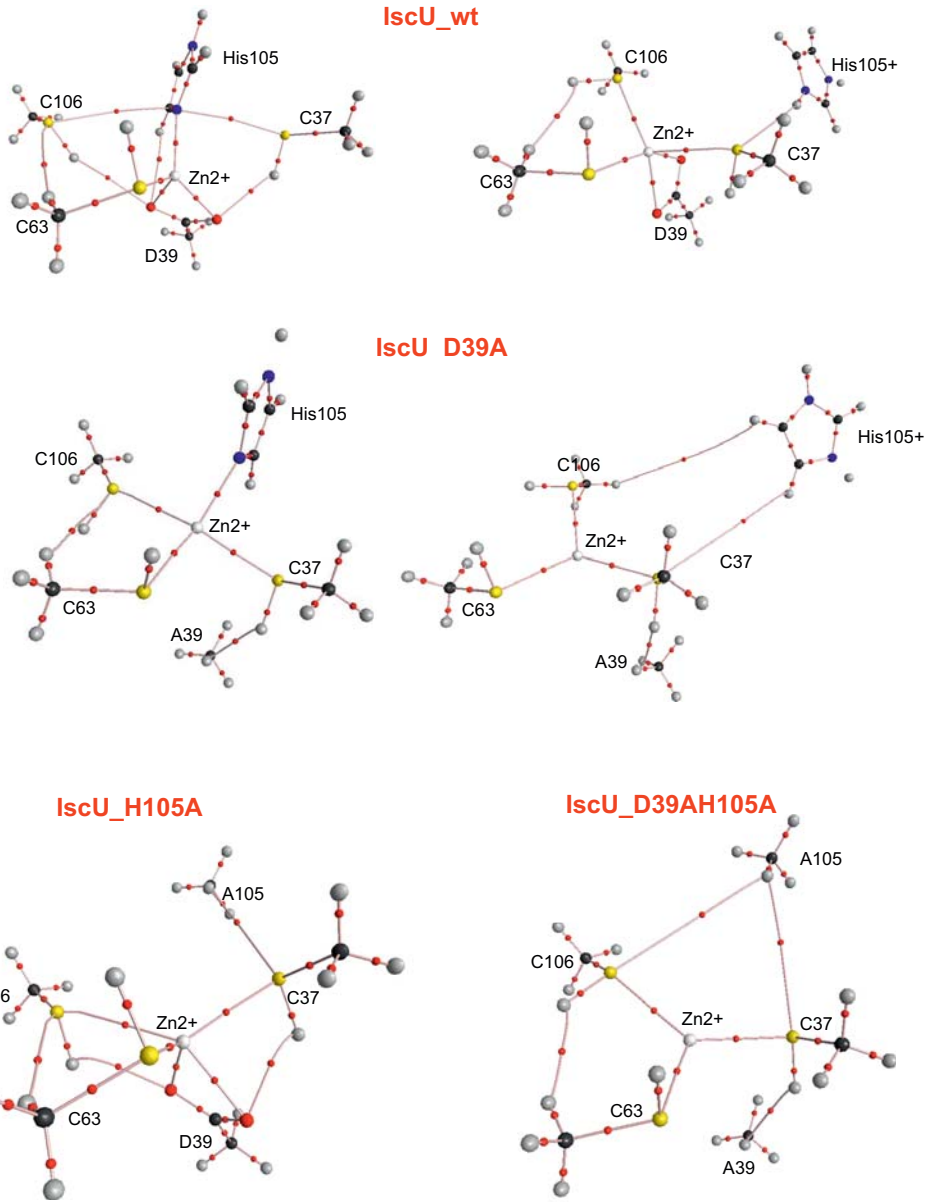
Asp39
13
O

Figure S2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C37
Zn2+
C63
D39
C106
His105+
Zn2+
C37
C63
D39
IscU D39A
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C106
Zn2+
C37
C63
A39
His105+
C106
Zn2+
C37
C63
A39
IscU_H105A
A105
C106
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D39
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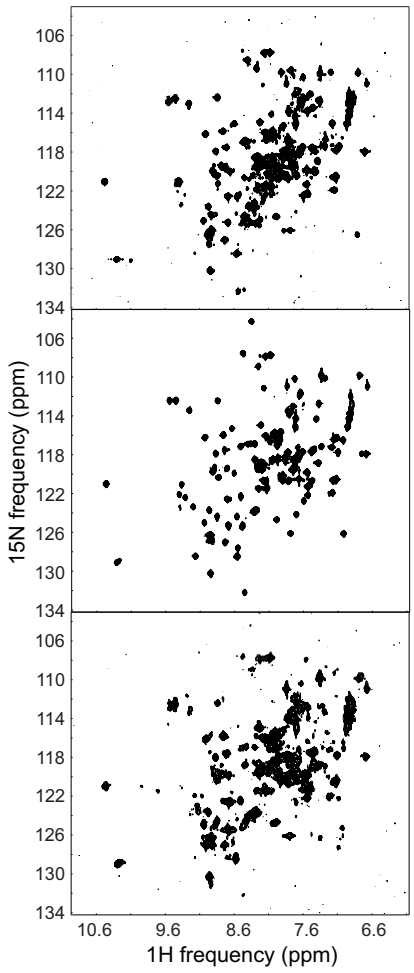
A105
C106
Zn2+
C37
C63
A39

Figure S3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126
130
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9.6
8.6
7.6
6.6
1H frequency (ppm)