Electronic Supplementary Material (ESI) for Metallomics.

Supplementary Information

On-line coupling of continuous-flow gel electrophoresis with inductively coupled plasma-mass spectrometry to quantitatively evaluate intracellular metal binding properties of metallochaperones *Hp*HypA and *Hp*HspA in *E. coli* cells

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Supplementary methods

UV-vis Spectroscopy

All UV-vis spectra were recorded on a Varian Cary 3E spectrophotometer at ambient temperature. Aliquots of Bi-NTA solution (2 mM) were titrated into freshly prepared *Hp*HypA (20 μM) solution in Hepes buffer (20 mM Hepes, 100 mM NaCl, 1 mM TCEP, pH 7.4). To oxidize the two free cysteines (Cys14 and Cys58) of *Hp*HypA, 20 μM *Hp*HypA solution was treated with 1 mM hydrogen peroxide (H_2O_2) and incubated at 4°C overnight. After desalting, the resulting protein solution was titrated with Bi-NTA solution similarly. The UV-vis absorption spectra were recorded at wavelengths ranging from 200 to 600 nm. The dissociation constant (K_d) of *Hp*HypA to Bi-NTA was determined by fitting the UV titration data to the Ryan-Weber nonlinear equation[1] as shown below:

$$I = \frac{I_{max}}{2C_p}((C_p + C_l + K_d) - \sqrt{(C_p + C_l + K_d)^2 - 4C_pC_l})$$

where I is the UV absorbance intensity; I_{max} represents the maximum UV absorbance; C_p and C_l are the final concentrations of protein and ligand respectively; K_d is the dissociation constant between protein and ligand.

By fitting the UV data, the dissociation constants of Bi-NTA to *Hp*HypA (K_{d1}) and H_2O_2-treated *Hp*HypA (K_{d2}) were determined to be 2.43±0.53 μM and 1.05±0.28 μM, respectively. Given the formation constant of Bi-NTA is $\log K_a$ = 17.55,[2] the dissociation constants of Bi^{3+} to *Hp*HypA (K_{d1}') and H_2O_2-treated *Hp*HypA (K_{d2}') were calculated to be $K_{d1}' = K_{d1}/K_a = 6.85(\pm1.49)\times10^{-18}$ μM and $K_{d2}' = K_{d2}/K_a = 2.96(\pm0.79)\times10^{-18}$ μM, respectively.

For the binding of Cu^{2+} to *Hp*HypA, 50 μM Zn-HypA was titrated stepwise by Cu^{2+} (as $CuCl_2$) and monitored by UV spectroscopy from 200 to 600 nm similarly.

Size-exclusion chromatography

Size exclusion chromatography analysis was performed on ÄKTA FPLC system (GE Healthcare). Freshly prepared *Hp*HypA (20 μM) was incubated with different molar equivalents of Bi-NTA at 4°C for 1 h. Protein samples (500 μL) were then loaded onto a Superdex 75 10/300 GL column (GE Healthcare) pre-equilibrated with Hepes buffer. Proteins were eluted with the same buffer at a flow rate of 0.25 mL/min, and the eluent was monitored at 280 nm. The column was calibrated with LMW gel filtration calibration kit (GE Healthcare).

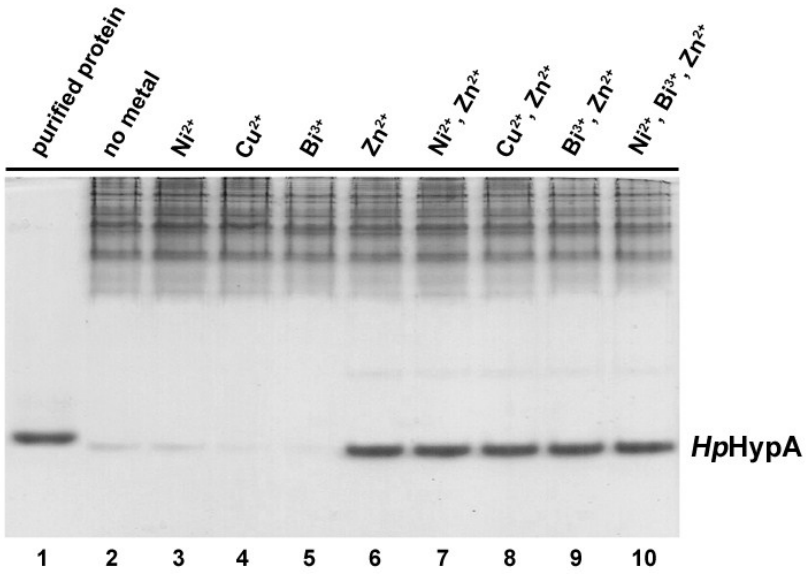

Fig. S1 Native-PAGE analysis of purified *Hp*HypA (*lane 1*) and lysed *E. coli* cells overexpressing *Hp*HypA (*lanes 2-10*). *Hp*HypA is not successfully overexpressed in M9 medium without Zn^{2+} (*lanes 2-5*). Supplementation of either Zn^{2+} alone or in combination with other metal ions to the culture medium (10 μM of each metal) resulted in similar levels of the protein overexpressed at the same molecular weight, i.e. *Hp*HypA.

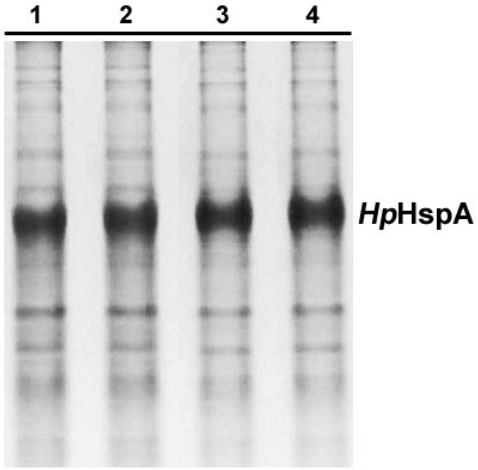

Fig. S2 Native-PAGE analysis of lysed *E. coli* cells overexpressing *Hp*HspA. Lanes 1-4 correspond to supplementation of no metal (*lane 1*), essential metals (Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} and Mo^{2+}, 10 μM each; *lane 2*), essential metals plus 10 μM Bi^{3+} (*lane 3*), essential metals plus 20 μM Bi^{3+} (*lane 4*) respectively during protein overexpression. Note that neither the levels, nor the molecular weight of the protein overexpressed were affected upon supplementation of metal ions to M9 medium.

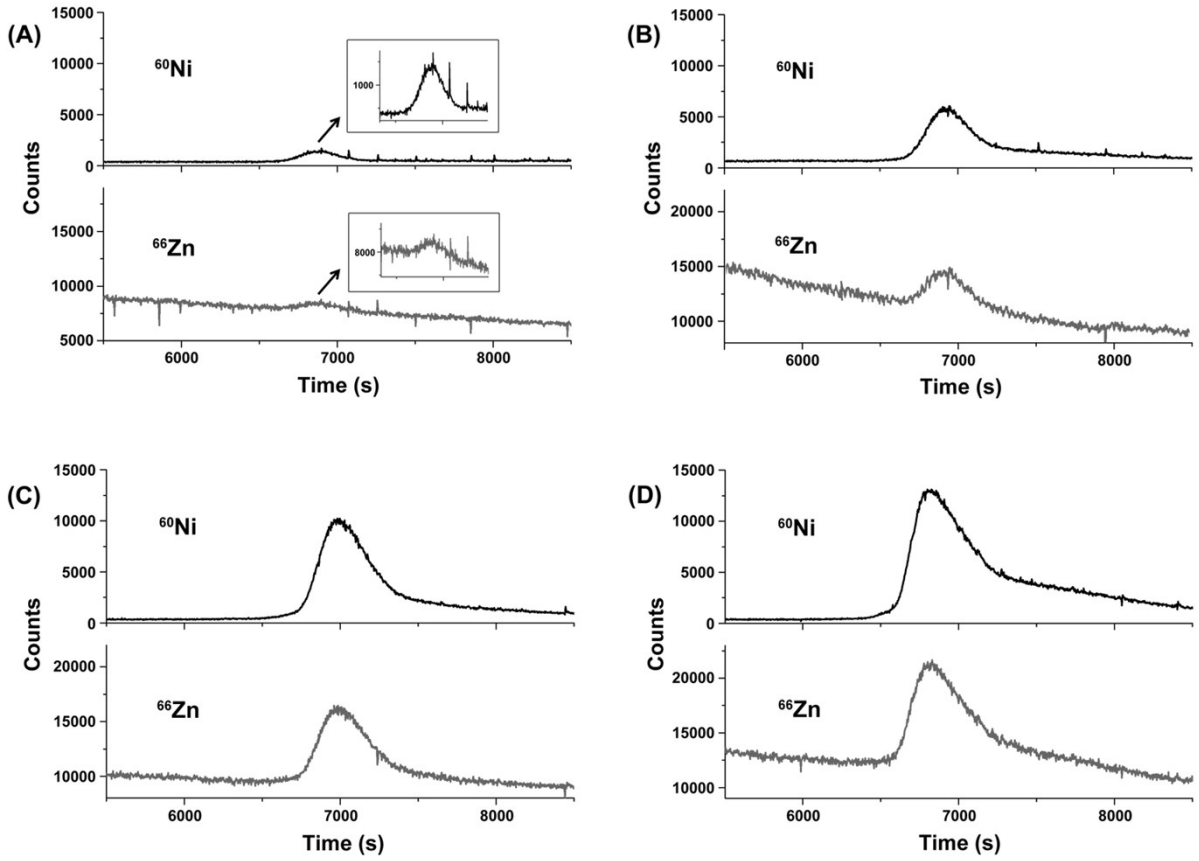

Fig. S3 GE-ICP-MS profiles of ^{66}Zn and ^{60}Ni associated with different concentrations of purified *Hp*HypA. The concentrations of Zn, Ni-*Hp*HypA subjected to GE-ICP-MS analysis were: (A) 15 μM, (B) 30 μM, (C) 60 μM and (D) 90 μM. For each analysis, 16 μL of the proteins were loaded.

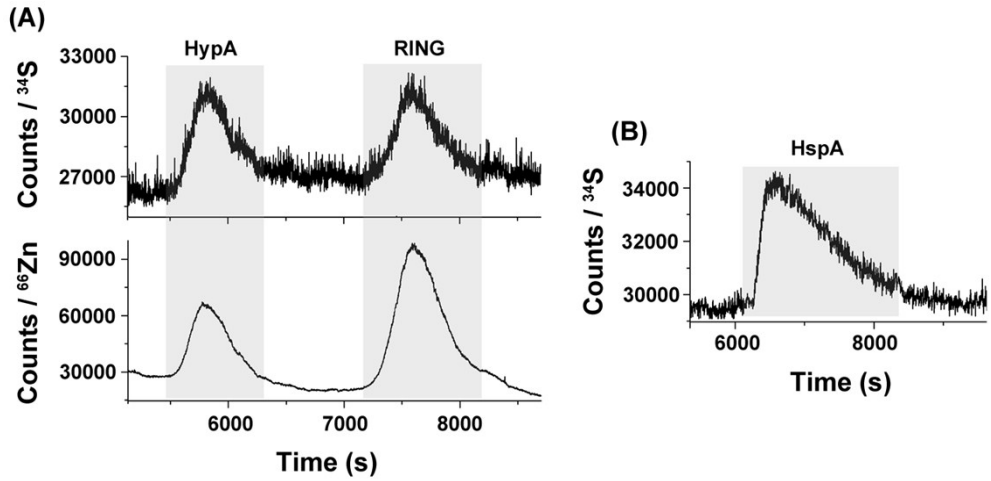

Fig. S4 Sulfur profiles of the proteins for determination of protein amounts *via* GE-ICP-MS analysis. (A) ^{34}S and ^{66}Zn signals of *Hp*HypA and RING. (B) ^{34}S signals of *Hp*HspA.

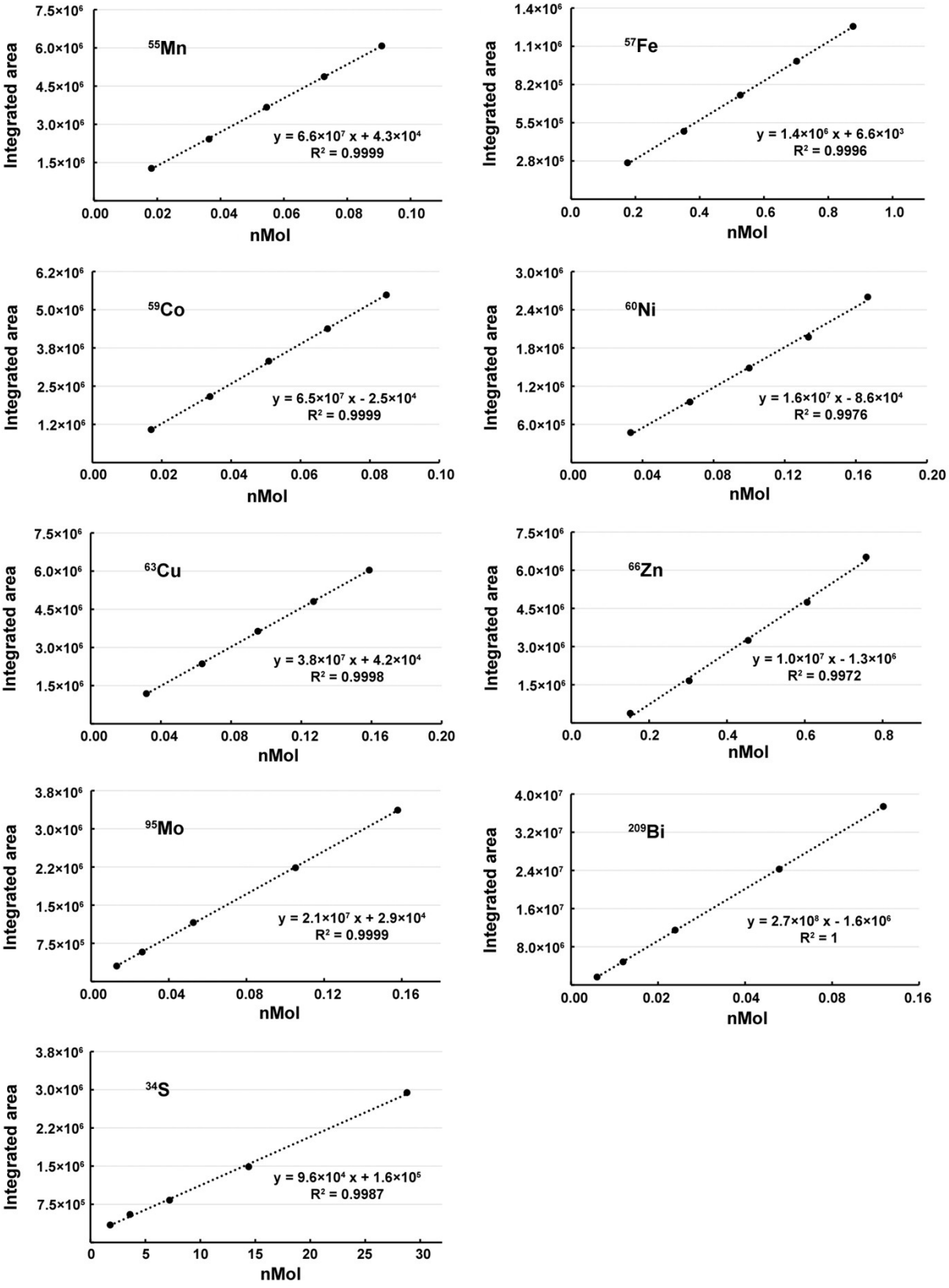

Fig. S5 Calibration curves of ^{55}Mn, ^{57}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{95}Mo, ^{209}Bi and ^{34}S for elemental quantification in GE-ICP-MS system. All the standard curves of the elements analyzed gave rise to good linearities ($R^2 > 0.99$).

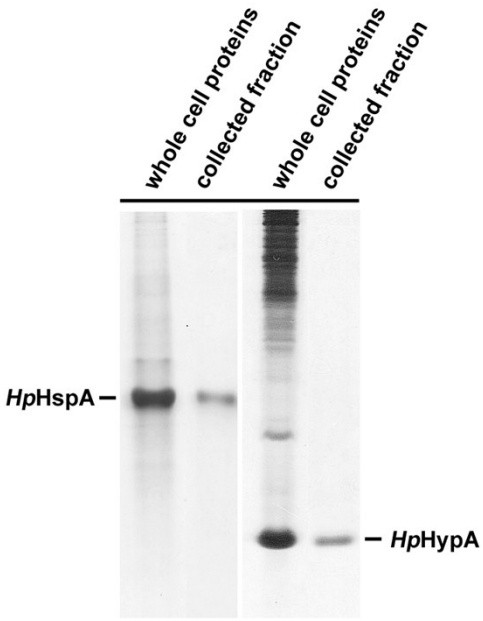

Fig. S6 Native-PAGE analysis of lysed *E. coli* cells overexpressing *Hp*HspA (lane 1) or *Hp*HypA (lane 3). Lane 2 and lane 4 are protein fractions corresponding to the major metal peaks collected during GE-ICP-MS analysis, indicating that the overexpressed proteins are the main components associated with the major metal peaks in cells.

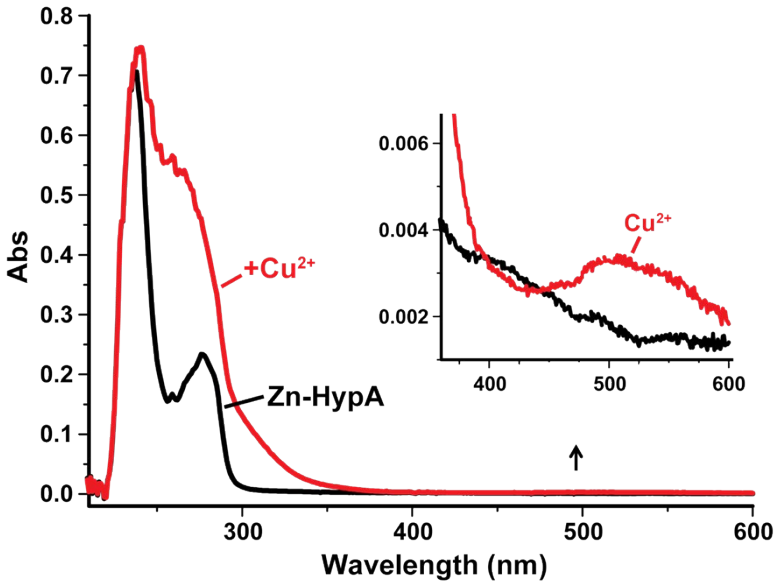

Fig. S7 Binding of Cu^{2+} to *Hp*HypA. UV/Vis spectra of 50 μM Zn-HypA and Cu^{2+}-bound Zn-HypA. *Inset*: expansion of the spectra at 400-600 nm. Upon the addition of Cu^{2+}, the absorbance of two peaks at 250 nm and 280 nm increased significantly, which could be assigned to the ligand-to-metal charge-transfer bands; a new and weak peak at *ca.* 510 nm was observed, which could be assigned to Cu^{2+} *d-d*' transition bands,[3] indicative of the involvement of imidazole nitrogen (histidines) in Cu^{2+}-HypA binding.[4]

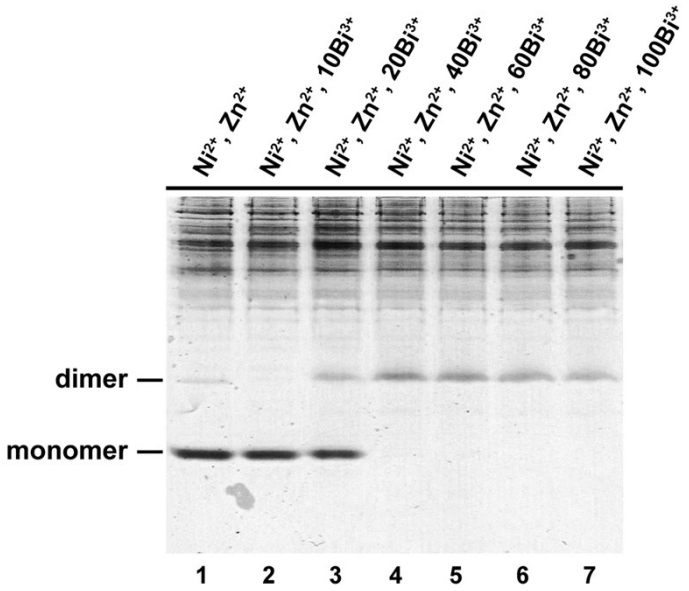

Fig. S8 Native-PAGE analysis of lysed *E. coli* cells overexpressing *Hp*HypA. CBS solutions at concentrations of 0, 10, 20, 40, 60, 80 and 100 μM were supplemented to the M9 medium under the same condition for essential metals (Ni^{2+} and Zn^{2+}, 10 μM each metal). The changes on the oligomeric state of the overexpressed proteins were observed with the increase in CBS concentrations.

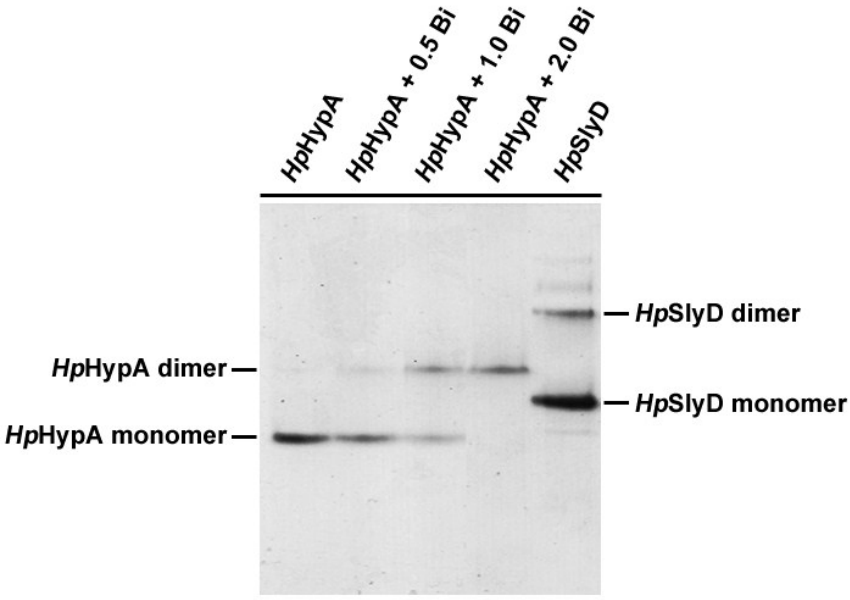

Fig. S9 Native-PAGE analysis of *Hp*HypA upon incubation with Bi-NTA. Freshly prepared *Hp*HypA was incubated with different molar equivalents of Bi-NTA prior to separation. Purified *Hp*SlyD with a molecular weight of 25 kDa on polyacrylamide gel[5] was used as a molecular weight marker. Upon loading of two molar equivalents of Bi-NTA, *Hp*HypA migrated at a larger molecular weight (*ca.* 28 kDa estimated from the molecular weight of *Hp*SlyD), indicative of the formation of *Hp*HypA dimer upon Bi^{3+} binding.

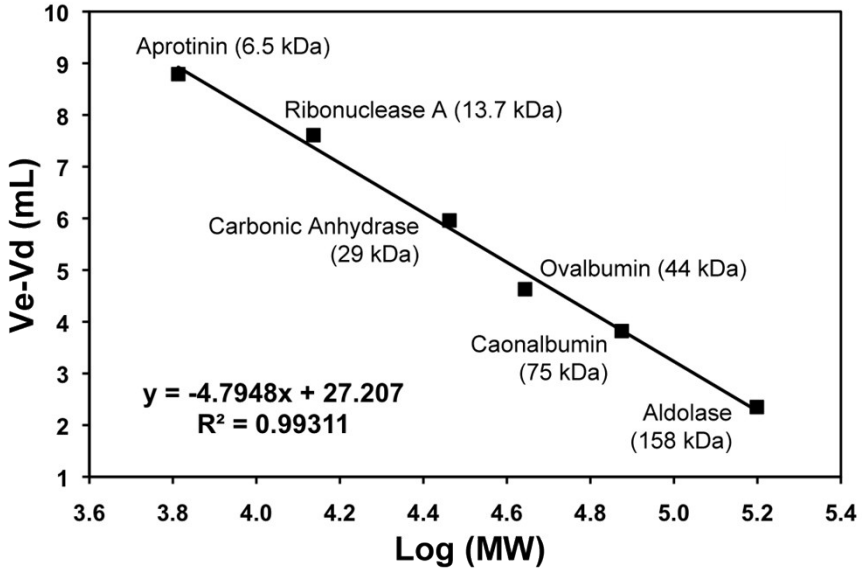

Fig. S10 Calibration curve of Superdex 75 100/300 GL column. Proteins used for calibration are indicated in the figure. The dead volume (V_d) of the column was determined to be 5.54 mL (determined from the elution volume of Blue Dextran 2000).

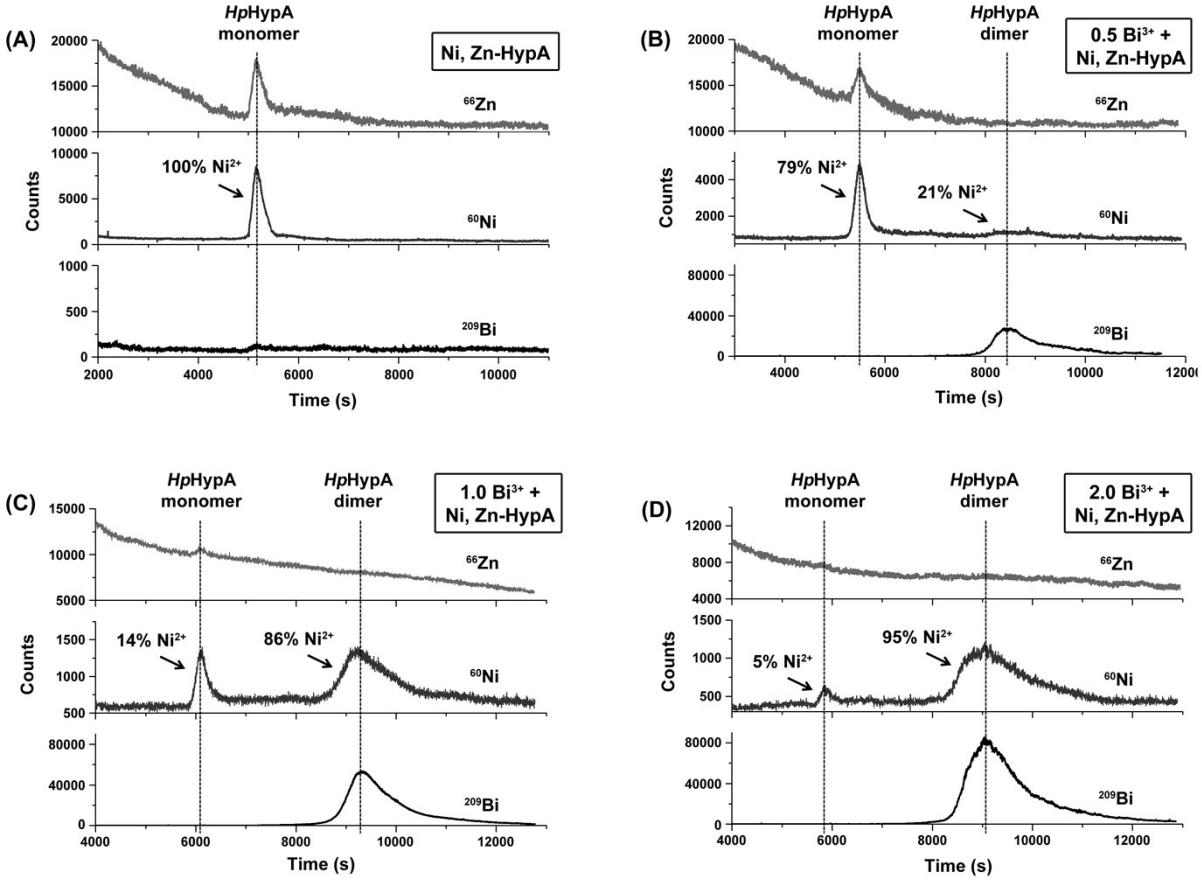

Fig. S11 GE-ICP-MS profiles of ^{66}Zn, ^{60}Ni and ^{209}Bi associated with recombinant Ni, Zn-*Hp*HypA incubated with different molar equivalents of Bi-NTA.

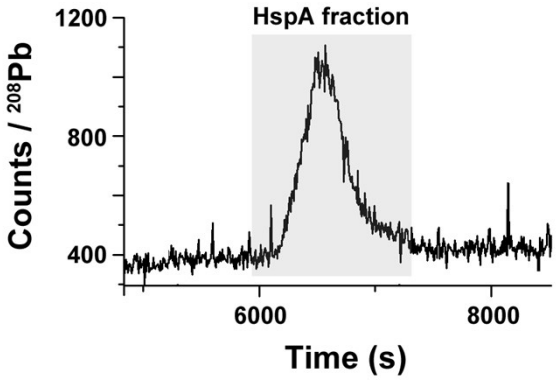

Fig. S12 GE-ICP-MS profile of ^{208}Pb binding to *Hp*HspA. *E. coli* cells harboring *hspA* gene were cultured in LB medium without supplementation of any extra metal ions. The concentration of Pb^{2+} in LB medium was determined to be 0.01 μM (*ca.* 0.01% of total metals in LB medium).[6] The association of *Hp*HspA with Pb^{2+} suggests its potential role as a metal detoxifier in cells.

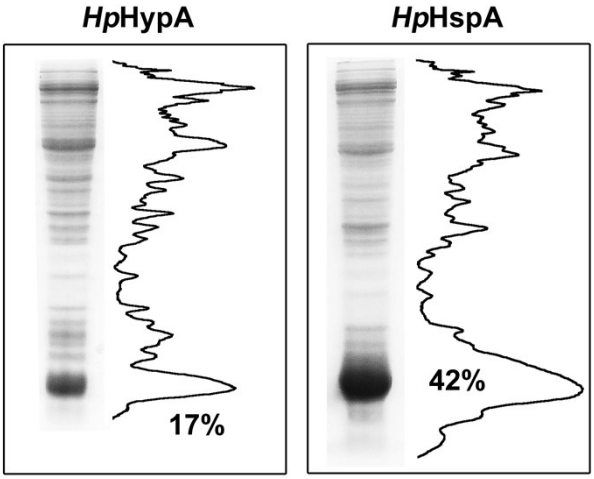

Fig. S13 The levels of overexpressed *Hp*HypA or *Hp*HspA in *E. coli* cells. Soluble fractions of *E. coli* cells overexpressing *Hp*HypA or *Hp*HspA were separated by SDS-PAGE. Image J was used to analyze the scanned images of the polyacryamide gels stained by Coomassie Blue. The proportions of the proteins overexpressed to total soluble proteins were quantified by pixel density.

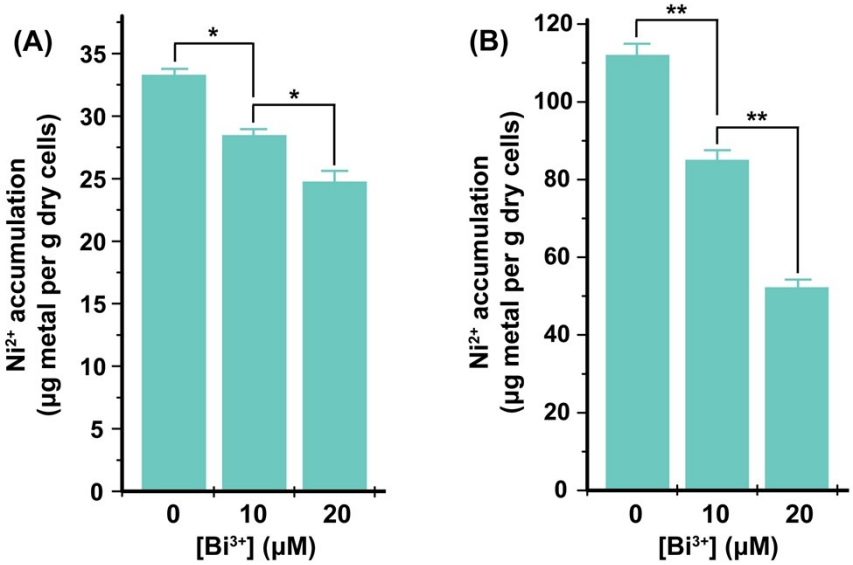

Fig. S14 Ni^{2+} accumulation in *E. coli* cells harboring *hypA* or *hspA* gene. Cells were cultured in M9 medium supplemented with essential metals (10 μM each metal), and different concentrations of Bi-NTA (10 μM and 20 μM). Each column represents the average ± standard deviation from triplicate measurements. $P < 0.05$ was determined by Student's t-test (*, $0.01 < P < 0.05$; **, $0.001 < P < 0.01$). The comparing groups are shown in solid lines.

Table S1 Operating parameters of ICP-MS

RF Power	1300 W
RF Matching	1.6 V
Carrier gas flow rate (Ar)	1.00 L/min
Makeup gas flow rate (Ar)	0 L/min
Type of nebulizer	Babbington high solids
Spray chamber	Quartz Scott type
Sampling depth	5 mm
Data acquisition mode	Time-resolved analysis
Data sampling rate	10 Hz
Measurement duration	10000 s

Table S2 Dissociation constants (K_d) of standard proteins and the proteins studied

Metal ion	**Protein**	**K_d, M**	**Ref**
Zn^{2+}	SOD	4.2×10^{-14}	7
Cu^{2+}	SOD	6.0×10^{-18}	7
Cu^{2+}	BSA	7.6×10^{-12}	8
Fe^{3+}	Transferrin	5.0×10^{-21}	9
Zn^{2+}	*Ec*HypA [a]	9.0×10^{-10} [a]	10
Ni^{2+}	*Hp*HypA	1.3×10^{-6}	11
Zn^{2+}	*Hp*HspA	1.2×10^{-10}	12
Ni^{2+}	*Hp*HspA	1.1×10^{-6}	13
Bi^{3+}	*Hp*HspA	5.9×10^{-25}	13
Bi^{3+}	*Hp*HypA	6.8×10^{-24}	This study

[a] As K_d value of Zn-*Hp*HypA is not available, the value listed here is for Zn binding to *E. coli* HypA.

Table S3 Peptide mass fingerprints of *Hp*HypA

Protein name	Accession No.	Protein Score	Protein Score C. I. %	Protein MW	Peptide Count
hydrogenase nickel insertion protein HypA [*Helicobacter pylori*]	gi|487872234	136	100	13507.7	5

Peptide information						
Calc. Mass	Obsrv. Mass	Start Seq.	End Seq.	Sequence	Ion Score	Modification
1130.594	1130.7599	60	69	DAILDIVDEK		
1290.6471	1290.8348	98	108	NVIITQGNEMR		Oxidation (M)[10]
1290.6471	1290.8348	98	108	NVIITQGNEMR	26	Oxidation (M)[10]
1303.6682	1303.8524	42	52	SLFVSAFETFR		
1888.9573	1889.2344	60	75	DAILDIVDEKVELECK		Carbamidomethyl (C)[15]
2149.0635	2149.3508	42	59	SLFVSAFETFREESLVCK		Carbamidomethyl (C)[17]
2149.0635	2149.3508	42	59	SLFVSAFETFREESLVCK	70	Carbamidomethyl (C)[17]

Table S4 Peptide mass fingerprints of *Hp*HspA

Protein name	Accession No.	Protein Score	Protein Score C. I. %	Protein MW	Peptide Count
heat shock protein A [*Helicobacter pylori*]	gi|357530156	347	100	13385.6	7

Peptide information						
Calc. Mass	Obsrv. Mass	Start Seq.	End Seq.	Sequence	Ion Score	Modification
935.4833	935.4723	56	64	EGDVIAFGK		
974.5417	974.5418	2	9	KFQPLGER		
1000.5859	1000.5790	33	41	EKPLMGVVK		
1000.5859	1000.5790	33	41	EKPLMGVVK	60	
1016.5809	1016.5753	33	41	EKPLMGVVK		Oxidation (M)[5]
1105.5823	1105.5916	1	9	MKFQPLGER		
1105.5823	1105.5916	1	9	MKFQPLGER	54	
1215.6580	1215.6506	21	32	TSSGIIIPDNAK		
1215.6580	1215.6506	21	32	TSSGIIIPDNAK	60	
1357.7322	1357.7432	10	20	VLVERLEEENK		
1958.0077	1957.9896	15	32	LEEENKTSSGIIIP DNAK		
1958.0077	1957.9896	15	32	LEEENKTSSGIIIP DNAK	110	

Table S5 Metal contents of *Hp*HspA overexpressed in *E. coli*. [*]

Sample	Percentages of associated metals					Total molar equivalents of metal/protein
	Co^{2+}	Ni^{2+}	Cu^{2+}	Zn^{2+}	Bi^{3+}	
HspA - essential metals	12.76%	12.76%	4.26%	70.21%	ND [a]	0.47 ± 0.04
HspA - essential metals + 10 μM Bi^{3+}	10.42%	4.17%	4.17%	47.92%	33.33%	0.48 ± 0.01
HspA - essential metals + 20 μM Bi^{3+}	4.17%	2.08%	10.42%	35.42%	47.92%	0.48 ± 0.01

[*] The averages (±standard deviation) from at least triplicate measurements are shown.

[a] ND, non-detectable.

References

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