Structures of a Nickel Metallochaperone, HypA, from *Helicobacter pylori* Reveal Two Distinct Metal Binding Sites

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Supporting Information

Table 1. **Materials for molecular cloning.**

Primer-for	5' CGGGATCCATGCATGAATACTCGGTCGTT 3'
Primer-rev	5' CGGAATTCTTATTCCGCTAACATTTCTAAAG 3'
Plasmid	pGBHIS*
Bacteria strain	BL21(DE3)

*pGBHIS is a self-modified expression plasmid based on pET-22b (Novagen) which expressed a fusion protein with a GB1 tag and His tag at the N-terminus of the target protein.

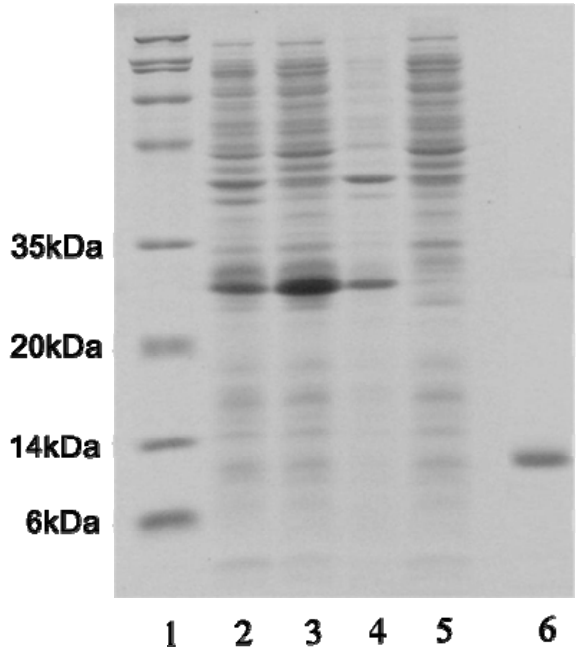

Figure S1. **12% SDS-PAGE of the purified HypA protein.**
Lane 1. Protein marker; *Lane 2.* Cell pellet after collection;
Lane 3. Soluble protein; *Lane 4.* Inclusion body;
Lane 5. Flow through after Ni column; *Lane 6.* Purified HypA after removing fusion tag.

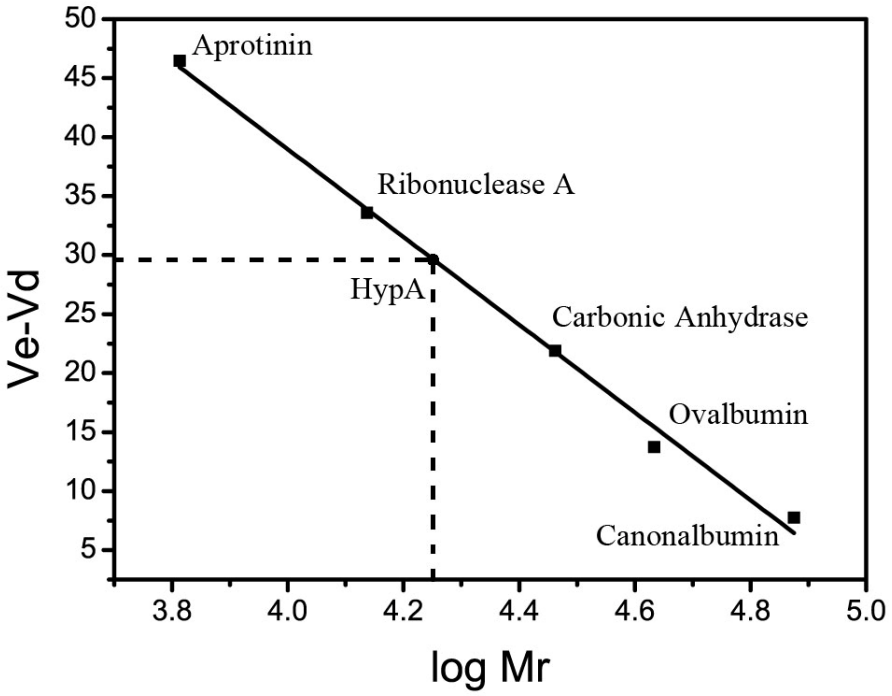

Figure S2. **Gel filtration analysis of the purified HypA protein.** Superdex 75 Hiload 16/60 (Amersham) was calibrated with Gel Filtration calibration kit LMW (GE Life Sciences). Caonalbumin (75 kDa), ovalbumin (43 kDa), carbonic anhydrase (29 kDa), ribonuclease A (13.7 kDa) and aprotinin (6.5 kDa) were used to obtain a standard curve. The purified HypA was calculated to be 16 kDa, in consistent with a monomer of the protein.

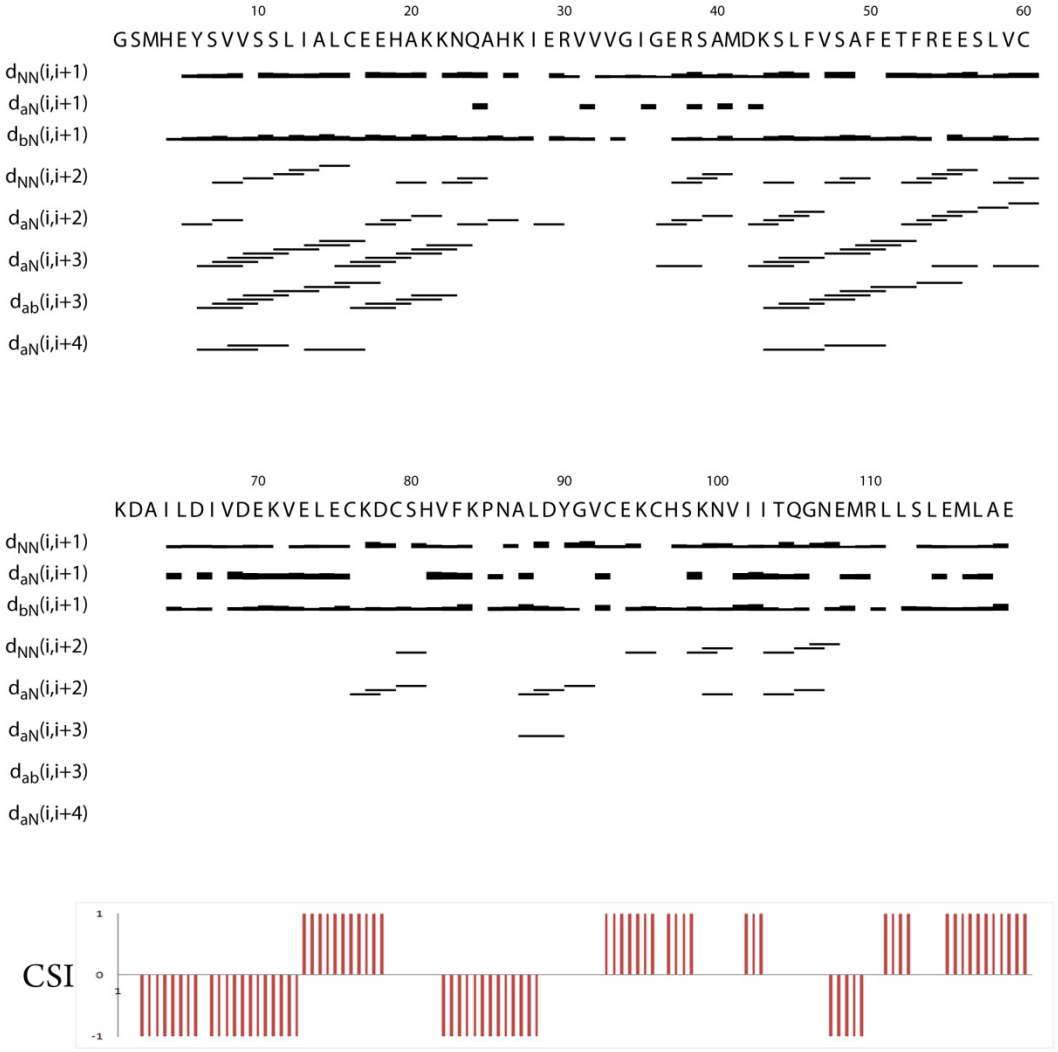

Figure S3. **NOE connectivity and chemical shift index of Zn-HypA protein.**

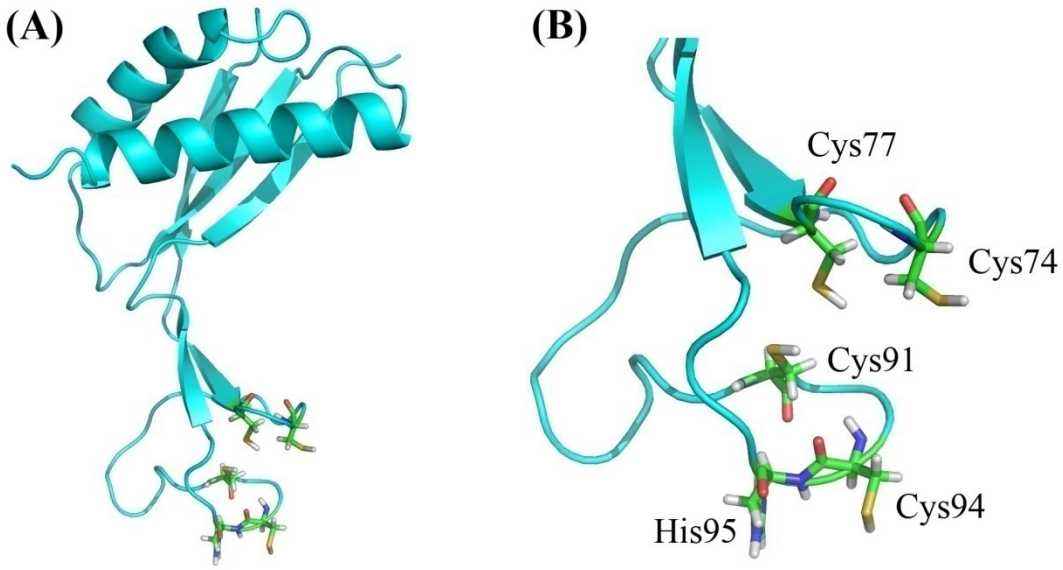

Figure S4. **Structure of *Hp*HypA without Zn-S cluster restraints.** (A)The ribbon representation of HypA with the lowest energy calculated without Zn-S cluster restraints. (B) The four conserved cysteines, Cys74, Cys77, Cys91, Cys94 and the His95 were shown in sticks model. The four cysteines are pre-organized to form a metal binding site.

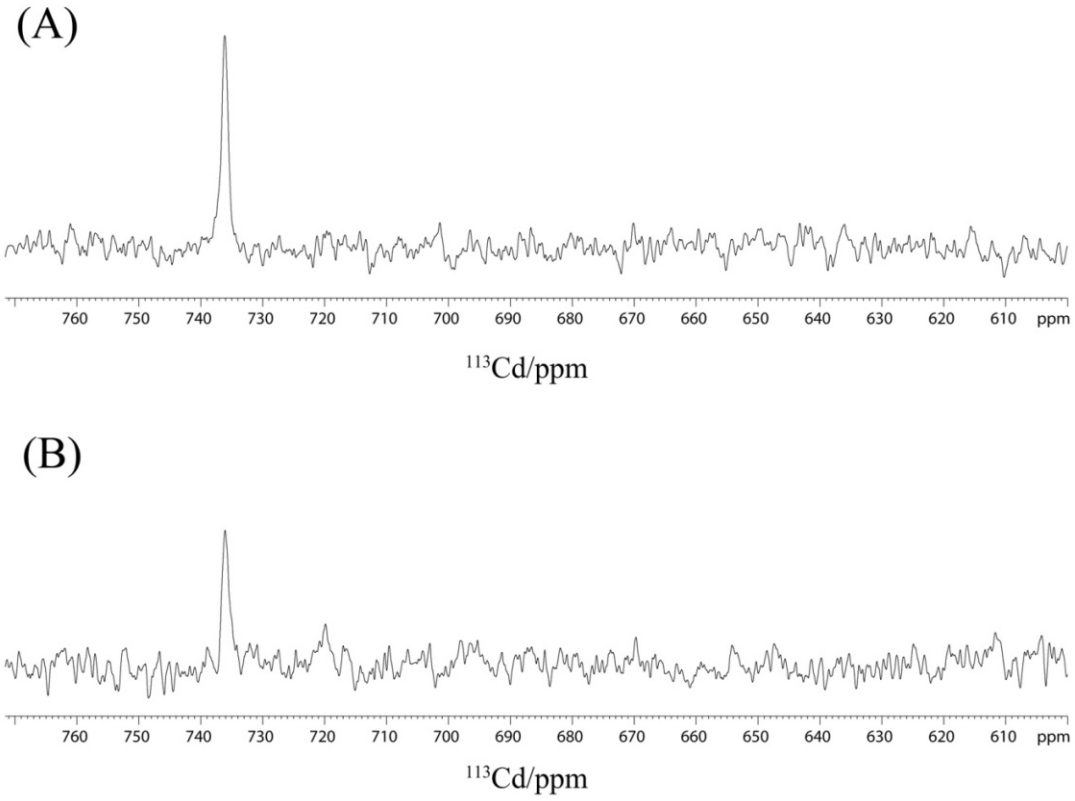

Figure S5. **^{113}Cd NMR spectra of 1 mM ^{113}Cd-reconstituted HypA.** In the absence (A) and the presence of 1 molar equivalence of Ni^{2+} (as $NiSO_4$) (B). The peak at 736 ppm is characteristics of CdS4 coordination. No changes were observed upon addition of 1 molar equivalence of Ni^{2+} for the ^{113}Cd signal in terms of both the chemical shift and linewidth.

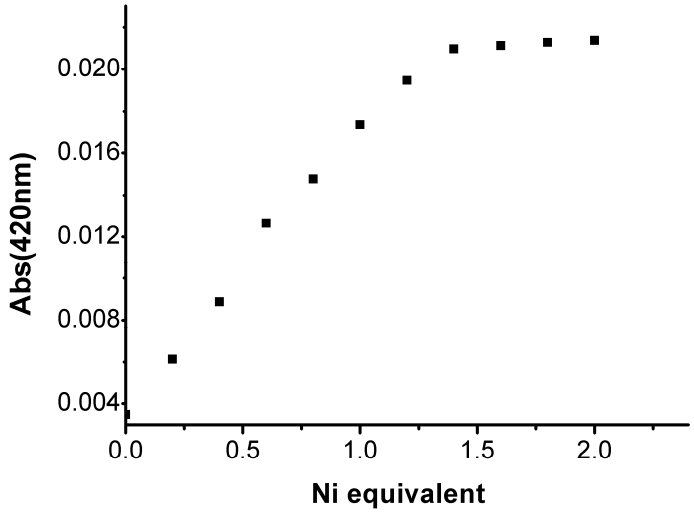

Figure S6. **UV absorbance at 420 nm of Zn-HypA upon Ni^{2+} titration.** Titration of Zn-HypA with up to 1 equivalent of Ni^{2+} results in a linear titration curve.

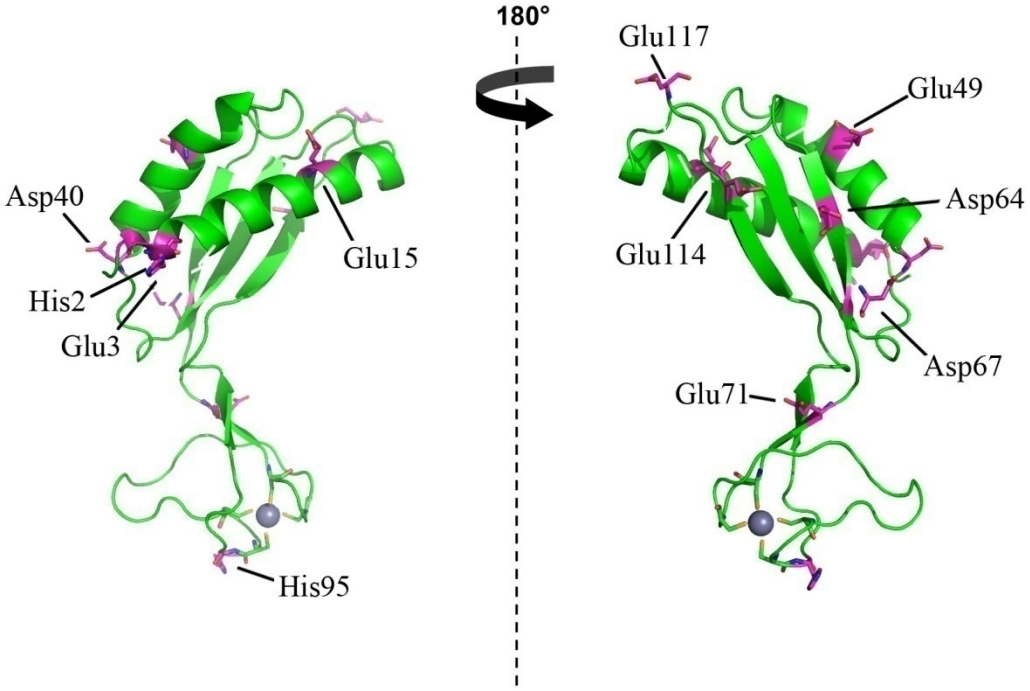

Figure S7. **Ribbon representation of Zn-HypA protein.** All conserved residues which are predicted to be nickel binding residues are highlighted in magentas. Except Asp40, all other residues are far away from the predicted N-terminal nickel binding site.

Apo-form HypA preparation

Apo-HypA was prepared by addition of EDTA and TCEP into a purified protein solution to a final concentration of 50 mM and 5 mM respectively. The protein sample was incubated at 4 ^{o}C overnight to fully remove bound metal ions, and then was applied to a HiTrap (GE Healthcare) desalting column to remove EDTA and other small molecules at 4^{o}C. No metal ion (Ni^{2+}, Zn^{2+}) was detectable by ICP-MS after treatment.

Full reference of 18 and 19:

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(21) Alm, R. A.; Ling, L. S. L.; Moir, D. T.; King, B. L.; Brown, E. D.; Doig, P. C.; Smith, D. R.; Noonan, B.; Guild, B. C.; deJonge, B. L.; Carmel, G.; Tummino, P. J.; Caruso, A.; Uria-Nickelsen, M.; Mills, D. M.; Ives, C.; Gibson, R.; Merberg, D.; Mills, S. D.; Jiang, Q.; Taylor, D. E.; Vovis, G. F.; Trost, T. J. *Nature* **1999**, *397*, 176-180.