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SUPPORTING INFORMATION for

Nickel translocation between Metallochaperones HypA and UreE in *Helicobacter pylori*

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Construction of Expression/Co-expression Plasmids. All plasmids and the primers used in this study are summarized in Table S1, S2. The DNA fragments for the expression of *N*-UreE (residues1-76), *C*-UreE (residues 77-170), UreEΔ158-170 (residues 1-157) and *C*-UreEΔ158-170 (residues 77-157) (Figure S3) were amplified using pET28a-*ureE* vector (full-length) as the template. pHP8080Δ*ureE* vector was generated using pHP8080 as a template. The *ureE* (or its variants) gene fragments were inserted into the MCS1 of pETduet-1 vector with the NcoI and EcoRI restriction sites, and *hypA* gene was amplified with NdeI and XhoI restriction sites for MCS2 of pETduet-1.

Protein Expression and Purification. HypA was overexpressed and purified similarly as previously described.[1] For comparison, HypA protein without extra amino acids GS at the N-terminal has also been prepared as following: *hypA* gene was inserted into vector pHisSUMO[2] for the expression of his-sumo tag fusion protein. After sumo cleavage, both sumo protease with His-tag and his-sumo tag were removed from $HypA_{(sumo)}$ protein by Histrap column. HypA protein was further purified by subjecting to superdex 75 10/60 column. For recombinant UreE and its variants, *Escherichia coli* BL21(DE3) (Stratagene) cells harboring a pET-28a recombinant plasmid were grown in 1 L of Luria-Bertani (LB) medium containing 50 μg/mL kanamycin at 37 °C to an OD_{600} of 0.6, followed by isopropyl β-D-thiogalactoside (IPTG) induction. The cells were harvested by centrifugation (4000 g, 20 min) upon further growth for 16 hrs at 25 °C, and resuspended in 20 ml buffer A (20 mM Hepes, 300 mM NaCl, pH 7.2) with 0.5 mM phenylmethanesulfonyl fluoride (PMSF) present. The following steps were performed at 4 °C unless stated otherwise. The cells were lysed by sonication, and centrifuged (15 000 g, 30 min) to remove cell debris and the supernatant was subjected to a HisTrap 5 mL column (GE Healthcare) equilibrated with buffer A supplemented with 30 mM imidazole. The proteins were eluted by 300 mM imidazole containing buffer A and subjected to a HiTrap Desalting column equilibrated with buffer B (20 mM Hepes, 100 mM NaCl, pH 7.2), followed by thrombin (50 NIH units) cleavage at 25 °C for 5 hrs to remove the His-tag. Recombinant plasmids pGEX-4T-1 *C-ureE* and pGEX-4T-1 *N-ureE* were used for overexpression of *C*-UreE (residues 77-170) and *N*-UreE (residues 1-76) proteins respectively. Similarly, the *E. coli* BL21 (DE3) cells over-expressing GST-fused *C*-UreE or *N*-UreE proteins were lysed in buffer B. Cell supernatant was loaded onto a GSTrap HP 5 ml column (GE Healthcare). The bound proteins were eluted by the same buffer in the presence of 10 mM glutathione (Sigma-Aldrich) and were pooled and subjected to a HiTrap Desalting column, followed by thrombin (50 NIH units) cleavage at 25 °C for 5 hrs to remove the GST-tag. The apo-proteins were produced by the overnight treatment with EDTA (20 mM) followed by further purification using a HiLoad 16/60 Superdex 75 column (GE healthcare) pre-equilibrated with buffer A in the presence of 1 mM tris(2-carboxyethyl)phosphine hydrochloride (TCEP). The metal-bound proteins were obtained by incubation of the apo-proteins with 10 molar equivalents of metal ions (i.e. Ni^{2+} or Zn^{2+}) at 4 °C for overnight, and the excess of metal ions was removed by SEC similarly. Protein concentrations were determined by the BCA assay (BCA Protein Assay Kit, Novagen). The metal contents of the proteins were determined by inductively-coupled plasma mass spectrometry (ICP-MS) (Agilent 7500 Series). The purity of proteins was assessed by 15% SDS polyacrylamide gel electrophoresis (SDS-PAGE) to be over 95%.

Chemical Cross-linking. Zn-HypA and the apo-form of UreE/mutant proteins (50 μM) were pre-incubated in 20 mM Hepes containing 100 mM NaCl, pH 7.2. Cross-linking reagent, bis(sulfosuccinimidyl) suberate (BS^3) (Sigma-Aldrich) was added into the protein mixtures to a final concentration of 0.1 mM, and the mixtures were further incubated at ambient temperature for 0.5 h. The

reaction was then terminated by addition of 1 M Tris solution to a final concentration of 50 mM and subjected to 12% SDS-PAGE analysis.

Static Light Scattering and Size Exclusion Chromatography. Typically, UreE or its mutants (50 μM) was pre-mixed with HypA and then loaded onto a Superdex 75 10/300 GL column (GE Healthcare) pre-equilibrated with buffer B in the presence of 0.5 mM TCEP. The molecular weights of the protein/complex in the elution peaks were measured by a multiple-angle laser light scattering detector (Mini-DAWN light scattering detector, Wyatt Technology). SEC/SLS data were analyzed by Astra version 5.3.4.18 (Wyatt Technology). Size exclusion chromatography analysis was carried out on an ÄKTA FPLC system (GE Healthcare) with a Superdex 200 10/300 GL column which was calibrated with Low Molecular Weight Gel-filtration Calibration Kit (GE Healthcare).

Isothermal Titration Calorimetry (ITC). Apo- or metal-bound proteins (apo-HypA, Zn-HypA, Ni-Zn-HypA, apo-UreE, Ni-UreE, Zn-UreE) were prepared freshly in the buffer B. For protein-protein interactions, HypA (0.2 mM) was titrated into UreE (20 μM as dimers). For metal-protein interactions, 0.4 mM of Ni^{2+} (as ligand in the same buffer) were titrated into 40 μM protein samples. All ITC experiments were performed at 25 °C on an ITC200 isothermal titration calorimeter (Microcal).

Urease Activity Assay. The two-plasmid system including plasmid pHP8080Δ*ureE* and pETduet-1 or one of its derivatives (i.e. pET-*ureE-hypA/*pET-*ureE variant-hypA,* pET-*ureE/*pET-*ureE variant*) was used for urease activity assay as described previously except that pHP8080Δ*ureE* instead of pHP8080 was used.[3] The different combinations of the two-plasmids were co-transformed into *E. coli* strain KMl603 [BL21(DE3) Δ*slyD*::*kan* (a gift from Prof. A.R. Davidson, University of Toronto)]. *E. coli* strains with co-expression plasmids were cultured in M9 minimal medium (6 g Na_2HPO_4, 3 g KH_2PO_4 0.5 g NaCl, 1g NH_4Cl, 4 g glucose, 1mM $MgSO_4$, 0.1 mM $CaCl_2$ per liter) at 37 °C in the presence of 100 μg·ml^{-1} ampicillin, 30 μg·ml^{-1} chloramphenicol and 2 μM IPTG, with or without 1 μM $NiSO_4$. Overnight-cultured *E. coli* cells were harvested and washed twice with 50 mM Hepes (pH 7.5) and re-suspended in the same buffer. Cells were lysed, and 50 μl supernatants of the lysates were diluted in 250 μl 50 mM Hepes buffer (pH 7.5) containing 25 mM urea and incubated in 37 °C for 20 mins to allow ammonia to be released. The phenol-hypochlorite assay was used to quantify the amount of ammonia.[3] The urease activity was defined as nmol ammonia produced per min per mg of total proteins (nmol · min^{-1} · mg^{-1}).

NMR Spectroscopy. The HypA-UreE interaction interfaces on *Hp*HypA were identified by NMR spectroscopy. NMR titration experiments were carried out at 25 °C on a Brüker Avance 600 MHz spectrometer, operating at a ^{1}H frequency of 600.13 MHz with a TCI cryogenic probe. 2D [^{1}H-^{15}N] HSQC spectra were acquired before and after each addition of UreE into *ca.* 0.3 mM ^{15}N-labeled HypA in 20 mM Tris, 100 mM NaCl, pH 7.4. Quantification of chemical shift perturbations was made similarly as previously described.[4] Briefly, the intensity of each well-resolved peak was plotted against molar percentages of the protein added and the resulting slopes were used to estimate the extent of line broadening induced by the binding. The residues experiencing large chemical shift perturbations were mapped onto the structure of HypA and the figure was generated using PyMOL software package.

Monitoring Nickel Transfer between HypA and UreE by *TRACER* probe. The ***TRACER*** is a heterobifunctional fluorescence probe and it was synthesized and employed in live-cell labeling His-tagged proteins in our laboratory.[7] It consists of a fluorophore, nitrilotriacetic acid moiety, which binds to metalloproteins, and arylazide as an anchor, which will fix the probe to its labeled protein *via* formation of covalent linkage through photo-activation even under denature condition. Therefore, binding of ***TRACER*** to nickel-bound protein will enable the labeling of the protein and the fluorescence will be visible even in SDS-PAGE. To investigate Ni^{2+} transfer, 30 μM of apo-UreE (or apo-UreEΔ158-170) were incubated with Ni-HypA samples with concentrations ranging from 7.5 μM, 15 μM, 22.5 μM to 30 μM at 4 °C for 2 h to allow the potential nickel transfer, followed by incubation with 18 μM ***TRACER*** probe for 0.5 h to label the Ni^{2+}-bound protein. The samples were subjected to ultraviolet radiation (wavelength 365 nm) for 10 min to "fix" ***TRACER*** probe on the Ni^{2+}-bound protein by photo-activation reaction, and then analyzed by 15% SDS-PAGE. Fluorescence gel images were captured and adjusted by ImageQuant 350 (GE Healthcare) (λ_{ex}=365 nm, λ_{em}=460-500 nm). It is noticeable that the probe exhibited higher sensitivity to Ni^{2+}-HypA than Ni^{2+}-UreE. The gels were stained by Coomassie blue afterwards. To examine the possibility of nickel transfer from UreE to HypA, the experiment was carried out similarly except 30 μM Ni-UreE and 30 μM apo-HypA were used.

Table S1 Strains and plasmids used.

Strain or Plasmid	Description	Source or reference
E. coli		
BL21(DE3)	Host strain for over-expression	
KMl603	BL21(DE3)Δ*slyD*, Host strain for two-plasmid system	A gift from AR Davidson, University of Toronto
XL1-Blue	Cloning Strain	Life Technology
Plasmid		
pET28a *hypA*	pET derivative plasmid for overexpression of HypA	Xia *et al.* (2009) [1]
pET28a *ureE*	pET derivative plasmid for overexpression of UreE	This study
pET28a *ureEΔ158-170*	pET derivative plasmid for overexpression of UreEΔ158-170	This study
pET28a *C-ureEΔ158-170*	pET derivative plasmid for overexpression of *C*-UreEΔ158-170	This study
pGEX-4T-1 *hypA*	pGEX derivative plasmid for overexpression of GST-HypA	This study
pHP8080	pACYC184 derivative with *H. pylori ureABIEFGH* and *nixA*	McGee *et al.* (1999) [5]
pHP8080Δ*ureE*	pACYC184 derivative with *H. pylori ureABIFGH* and *nixA*	This study
pETduet-1		Novagen
pET- *ureE+hypA*	pETduet-1 derivative plasmid for co-expression of UreE and HypA	This study
pET-*C-ureE+hypA*	pETduet-1 derivative plasmid for co-expression of *C*-UreE and HypA	This study
pET-*ureEΔ158-170+hypA*	pETduet-1 derivative plasmid for co-expression of UreEΔ158-170 and HypA	This study
pET- *ureE*	pETduet-1 derivative plasmid for expression of UreE	This study
pET-*C-ureE*	pETduet-1 derivative plasmid for expression of *C*-UreE	This study
pET-*ureEΔ158-170*	pETduet-1 derivative plasmid for expression of UreEΔ158-170	This study
pET- *hypA*	pETduet-1 derivative plasmid for expression of HypA	This study

Table S2 Primers used for PCR reactions and restriction sites are underlined.

For pET28a ureE	
UreE(NdeI)-for	GGAATTC**CATATG**ATCATAGAGCGTTTAGTTG
UreE(EcoRI)-rev	CG**GAATTC**CTATTTTACGACCACTTTAAAATCG
For pET28a N-ureE	
UreE(NdeI)-for	GGAATTC**CATATG**ATCATAGAGCGTTTAGTTG
N-UreE(EcoRI)-rev	CG**GAATTC**TTACAAGATATTAACGGCGATAAT
For pET28a C-ureE	
C-UreE(NdeI)-for	GGAATTC**CATATG**GATTCTGAAGTCATTCACATCCA
UreE(EcoRI)-rev	CG**GAATTC**CTATTTTACGACCACTTTAAAATCG
For pET28a ureEΔ158-170 and C-ureEΔ158-170	
UreE(NdeI)-for	GGAATTC**CATATG**ATCATAGAGCGTTTAGTTG

UreEΔ158(EcoRI)-rev	CG**GAATTC**CTAAAAATTAGGCTCACTATGGG
For pGEX-4T-1 hypA	
HypA(BamHI)-for	CG**GGATCC**ATGCATGAATACTCGGTC
HypA(EcoRI)-rev	CG**GAATTC**CTATTCCGCTAACATTTC
For pHP8080ΔureE	
pHPΔureE-for	TAGAAAAGAAATAGAAAAATAACAGA
pHPΔureE-rev	CATCTCACACCCAGTGTTGG
For pET-ureE+hypA	
UreE(NcoI)-for	CATG**CCATGG**GCATGATCATAGAGCGTTTAGTTG
UreE(EcoRI)-rev	CG**GAATTC**CTATTTTACGACCACTTTAAAATCG
HypA(NdeI)-for	GGAATTC**CATATG**CATGAATACTCGGTCG
HypA(XhoI)-rev	GGGAATTCCATATG**CTCGAG**TTATTCCGCTAACATTTCTAAAG
For pET-C-ureE+hypA	
C-UreE(NcoI)-for	CATG**CCATGG**ATTCTGAAGTCATTCACATCCA
UreE(EcoRI)-rev	CG**GAATTC**CTATTTTACGACCACTTTAAAATCG

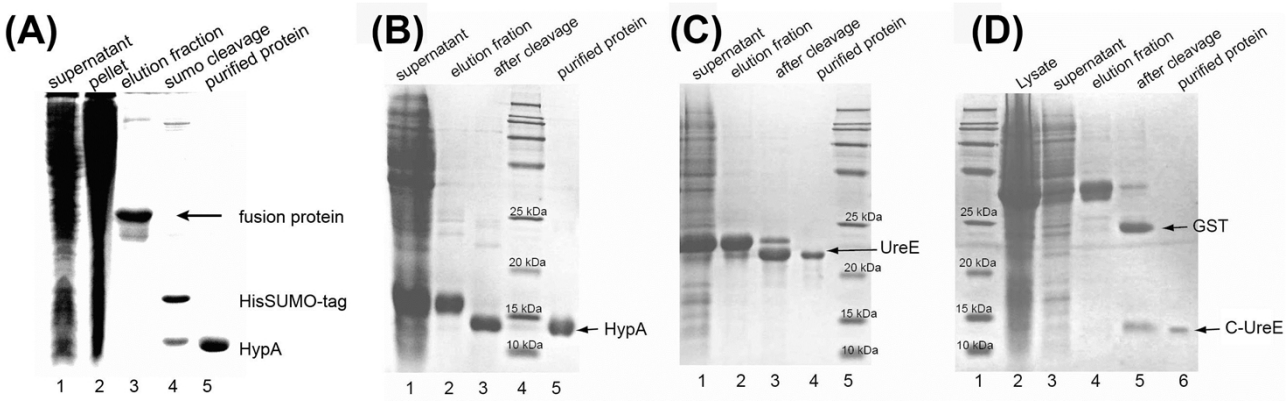

Figure S1 SDS-PAGE analysis of the purified HypA (A) (B); wild-type UreE (C) and *C*-UreE (D).

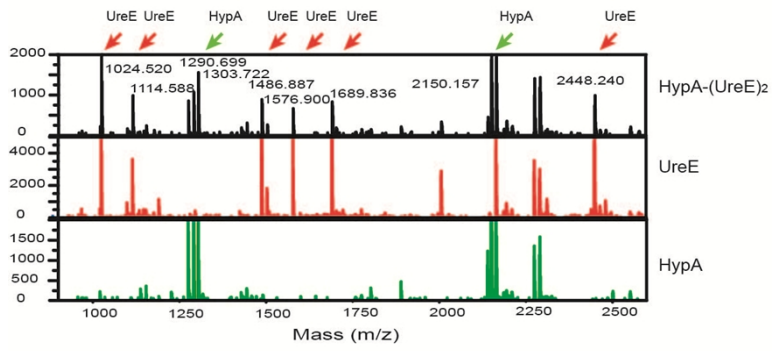

Figure S2 MALDI-TOF MS/MS fingerprinting for the HypA-$(UreE)_2$ complex band from SDS-PAGE. The MALDI-TOF MS/MS fingerprinting for the UreE and HypA bands were shown for comparison.

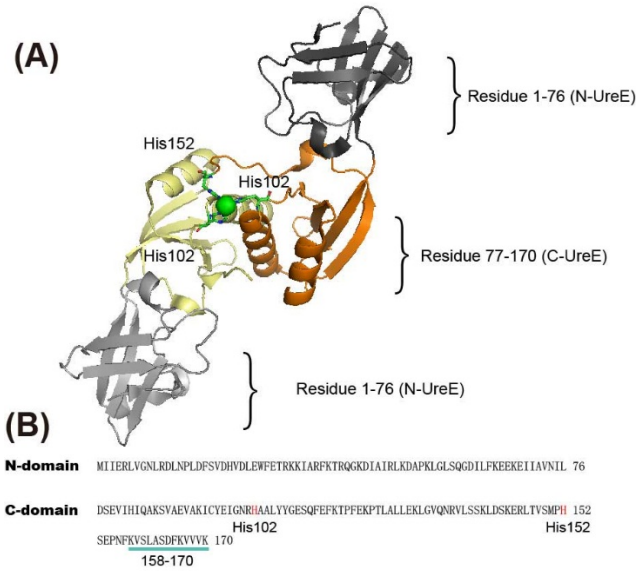

Figure S3 Structure and sequence of *Hp*UreE. (A) Crystal structure of *Hp*UreE (PDB code 3TJ8) shown as cartoon. *Hp*UreE dimer forms *via* the interaction between the two C-domains. His102 and His152 are involved in Ni^{2+} binding. Residues 153-170 are not defined.[6] (B) Sequence of *Hp*UreE with the N-domain (*N*-UreE: residues 1-76) and the C-domain (*C*-UreE: residues 77-170) is indicated. Residues of 158-170 are highlighted.

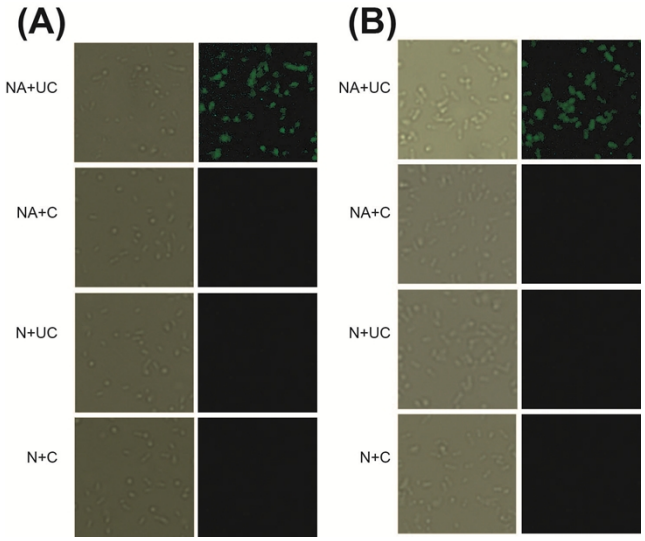

Figure S4 Intracellular HypA-UreE interaction by GFP-fragment reassembly. The bacteria were cultured in M9 minimal medium in the absence (A) or presence of 5 μM Ni^{2+} (B). The emitted green fluorescence of cells co-expressing proteins (NA+UC) indicates the formation of protein complex. Plasmids for expression of different proteins are indicated as follows. N: N-GFP; C: C-GFP; NA: N-GFP-*Hp*HypA; UC: *Hp*UreE-C-GFP.

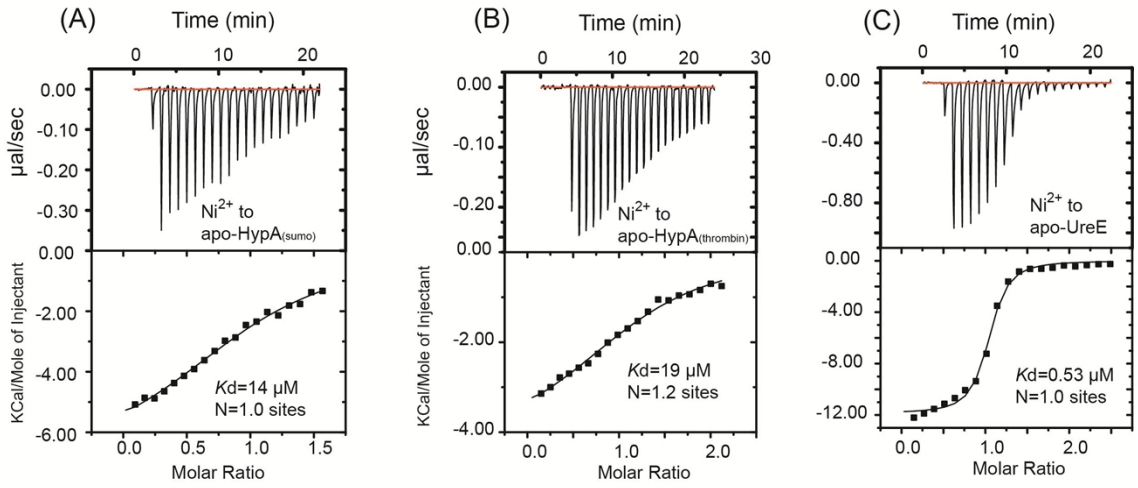

Figure S5 Binding of nickel to HypA (A) (B) and UreE (C) by isothermal titration calorimetry. $HypA_{(thrombin)}$ contains two extra amino acids (Gly-Ser) at N-terminal of protein after thrombin cleavage, compared with $HypA_{(sumo)}$. Ni^{2+} was titrated into $HypA_{(sumo)}$(A) and $HypA_{(thrombin)}$ (B) respectively. The extra amino acids did not have significant effect on nickel binding property of HypA. Note that nickel binding to UreE is much tighter than HypA with a dissociation constant (K_d) of 0.53 μM.

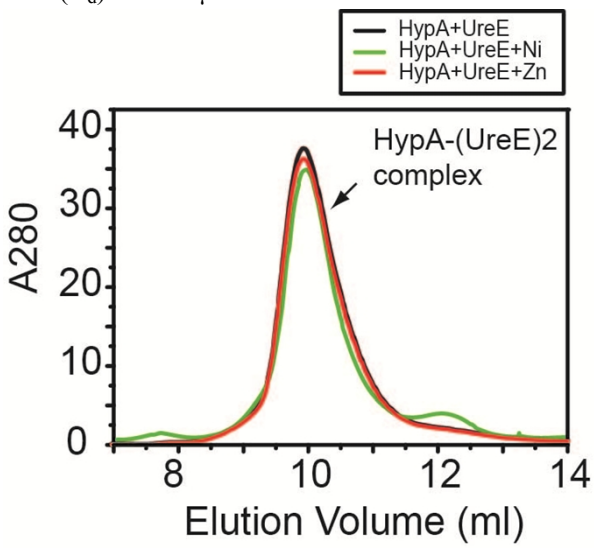

Figure S6 SEC profiles of HypA-$(UreE)_2$ complex in the absence and presence of metal ions.

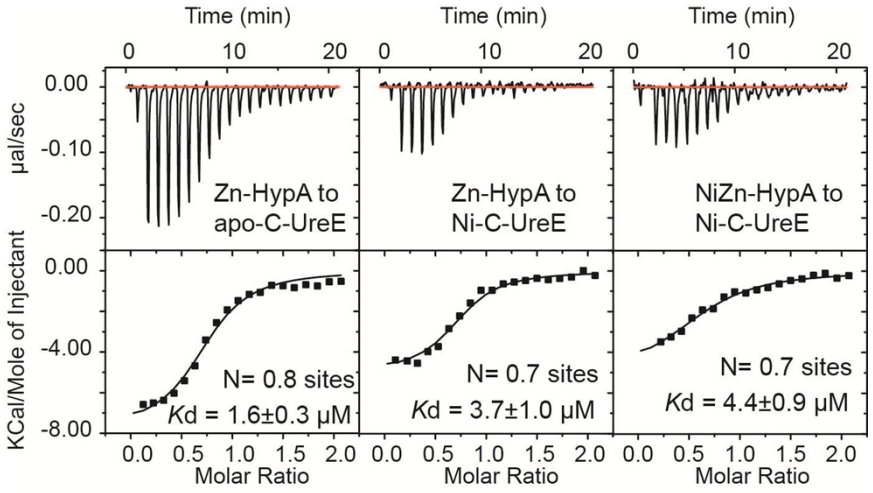

Figure S7 Binding affinities of HypA to *C*-UreE determined by isothermal titration calorimetry. Preloading of nickel ions to *C*-UreE led to reduction in the binding affinity of the protein-protein interaction.

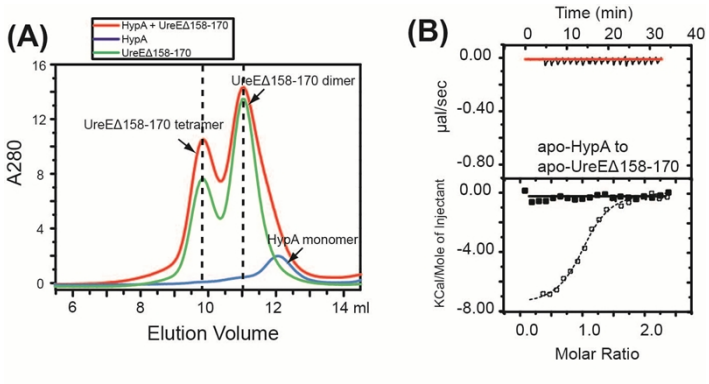

Figure S8 The C-terminal 158-170 residues of UreE are indispensable for HypA-UreE interaction. (A) SEC profiles of HypA, UreEΔ158-170 and their mixture did not show new peaks. (B) No interaction between HypA and UreEΔ158-170 was observed as adjudged by ITC (filled square). The titration curve of apo-HypA binding to apo-UreE is shown for comparison (opened circle).

References:

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(7) Unpublished data.