A Histidine- and Cysteine-rich Metal Binding Domain at the C-terminus of Heat-shock Protein A from *Helicobacter pylori*: IMPLICATION FOR NICKEL HOMEOSTASIS AND BISMUTH SUSCEPTIBILITY

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SUPPORTING INFORMATION

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ATG AAG TTT CAG CCA TTA GGA GAA AGG GTC TTA GTA GAA AGA CTT GAA GAA GAG AAC AAA    60
 M   K   F   Q   P   L   G   E   R   V   L   V   E   R   L   E   E   E   N   K    20
ACC AGT TCA GGC ATC ATC ATC CCT GAT AAC GCT AAG GAA AAG CCT TTA ATG GGC GTA GTC   120
 T   S   S   G   I   I   I   P   D   N   A   K   E   K   P   L   M   G   V   V    40
AAA GCG GTT AGC CAT AAA ATC AGT GAG GGT TGC AAA TGC GTT AAA GAA GGC GAT GTG ATC   180
 K   A   V   S  [H]  K   I   S   E   G   C*  K   C*  V   K   E   G   D   V   I    60
GCT TTT GGC AAA TAC AAA GGC GCA GAA ATC GTT TTA GAC GGC ACT GAA TAC ATG GTG CTA   240
 A   F   G   K   Y   K   G   A   E   I   V   L   D   G   T   E   Y   M   V   L    80
GAA CTA GAA GAC ATT CTA GGC ATT GTG GGC TCA GGC TCT TGT TGT CAT ACA GGT AAT CAT   300
 E   L   E   D   I   L   G   I   V   G   S   G   S   C*  C*  [H]  T   G   N  [H]  100
GAC CAT AAA CAT GCT AAA GAG CAT GAA GCT TGC TGT CAT GAT CAC AAA AAA CAC TAA       357
 D  [H]  K  [H]  A   K   E  [H]  E   A   C*  C*  [H]  D  [H]  K   K  [H]  *       118
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Fig. S1. Complete coding sequence and deduced amino acid sequence of *H. pylori hspA* gene. Nucleotide sequence is determined by DNA sequencing and is confirmed identical to the genomic sequence of *H. pylori* 26695 (id: HP0011). Protein sequence is translated from the gene sequence according to the universal genetic code. The histidine/cysteine-rich C-terminus is underlined. The histidines are marked with square brackets, and cysteines are indicated with asterisks. The fragment identified as the chaperonin Cpn10 signature is shown in bold font.

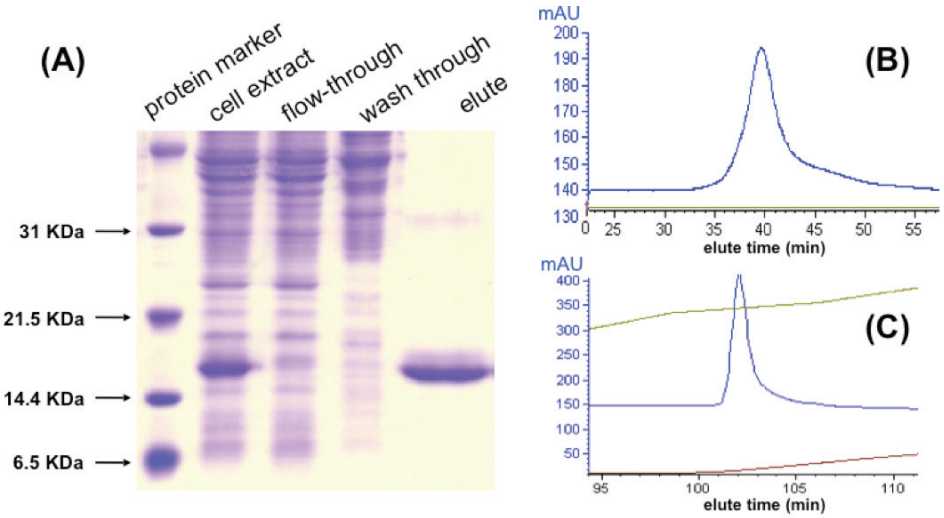

Fig. S2. Analyses of SDS-PAGE and purity examination for overexpressed HspA derived from *E. coli* BL21(DE3). (A) Electrophoretical analysis with 13% SDS-polyacrylamide gel stained by Coomassie brilliant blue. The apparent size of expressed protein (approx. 15.5 kDa) roughly corresponds to the predicted molecular mass of HspA (13 kDa). Elution profiles (monitoring at 280 nm) of HspA after gel filtration on size-exclusive column (B) and anion exchange column (C) in the presence of TCEP. A gradient with 0- 4 M NaCl was used for the latter.

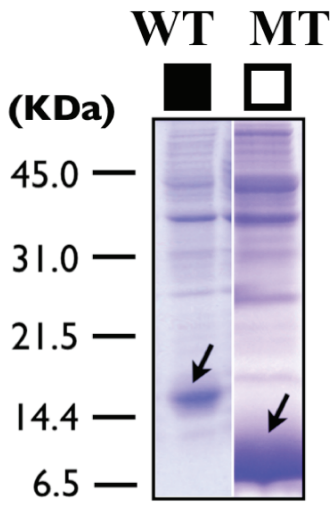

Fig. S3. SDS-PAGE analysis for the whole protein content of the start cultures, where the overexpressed wild-type (WT) and mutant (MT) HspA are indicated by arrows. Note that the apparent size (approx. 15.5 kDa) of the wild-type exhibits a slightly larger size than the predicted one (13 kDa), which is frequently observed for histidine-rich proteins.

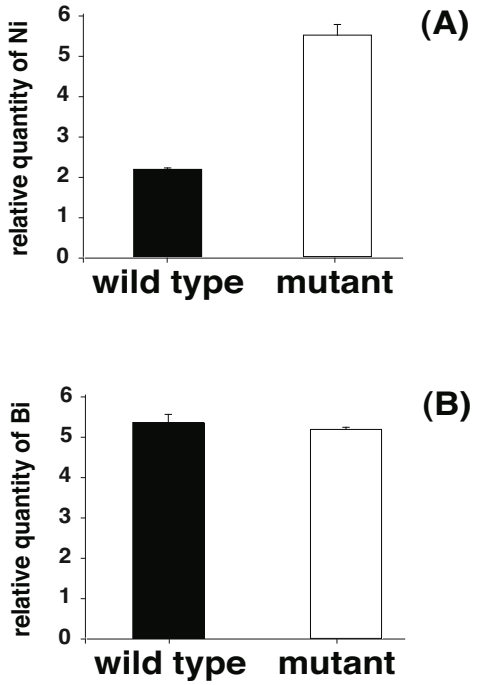

Fig. S4. Metal content in M9 minimal media that were used for metal resistance of *E. coli* BL21 (DE3) were cultured for about 18 hours in M9 minimal media supplemented with 5 μM $NiSO_4$ or 500 μM CBS. The metal content of cell-free media was determined by ICP-MS. Each sample was tested in independent triplicate, and the result was presented by mean ± SD.

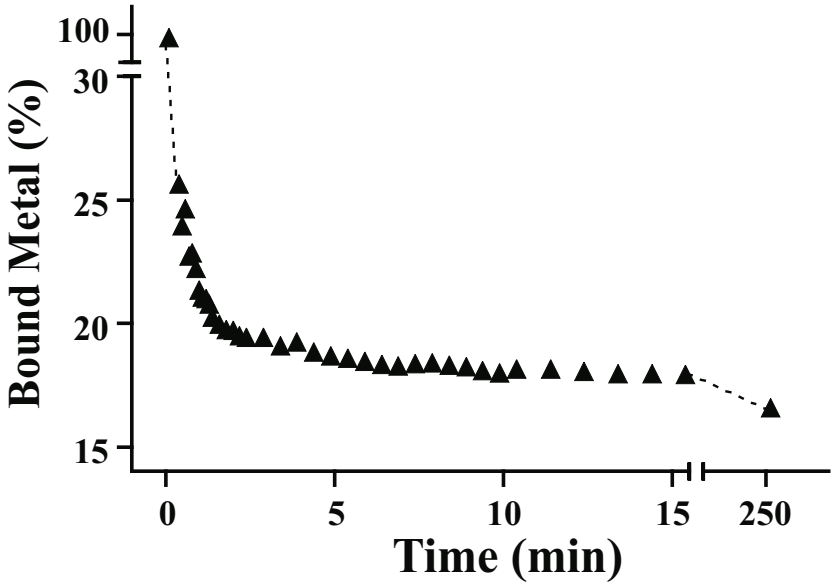

Fig. S5. Kinetics of Bi^{3+} release from HspA (200 μM). Time-dependent absorbance at 364 nm for Bi-bound HspA is monitored after addition of 40 molar equivalence of EDTA, pH 3.9 (sodium acetate- acetic acid buffer) at room temperature. The absorbance at each time point is normalized to percentages to represent the amount of bound metals.