Electronic Supplementary Material (ESI) for Chemical Communications

Supplementary Information for

Functional disruption of HypB, a GTPase of *Helicobacter pylori* by bismuth

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HypB Mutants Constructs

Constructs of HypB(C106A/C142A), HypB(C106A), HypB(C142A) and HypB(H107A) were generated using Phusion high fidelity DNA polymerase according to the provided protocol. Wild-type HypB construct was used as DNA template. The primers for mutants are listed in Table S1. The expression and purification of HypB mutants were similar to WT-HypB.

Table S1. Primers used for HypB mutants. The mutant residues are highlighted in purple.

HypB (C106A)-for	5' CGGCGAAGCAGCCCATTTGGAA 3'
HypB (C106A)-rev	5' GTGGTGATCTGGTGCGCACTCA 3'
HypB (H107A)-for	5' CGAAGCATGCGCTTTGGAAGCG 3'
HypB (H107A)-rev	5' CCGGTGGTGATCTGGTGCGC 3'
HypB (C142A)-for	5' TGGGGAATTTGGTTGCCCCCTCAAGCTA 3'
HypB (C142A)-rev	5' CGTTTTCAATGATTAAAAAATCGCTTTTTTCTAACG 3'

Bismuth nitrilotriacetate (Bi-NTA) Preparation

Bi-NTA was synthesized as described previsouly.[1] BiNTA was sythesized by gradually adding $(BiO)_2CO_3$ into NTA solution. After boiling for 3 h, the solution was filtered while hot. The crystals of Bi-NTA appeared after overnight incubation of filtrate at 4^{o}C. The 1mM Bi-NTA stock solution was prepared by dissovling BiNTA in H_2O.

Bismuth titration monitored by UV-vis spectra (CD)

Titration of apo-HpHypB with bismuth nitrilotriacetate (Bi-NTA) was carried out in a titration buffer (20 mM HEPES buffer at pH 7.0 supplemented with 100 mM NaCl and 1 mM TCEP) and monitored by UV-vis spectroscopy. Aliquots of Bi-NTA stock solution with 1 mM concnetration were added stepwise into 50 μM apo-HpHypB solution.

Circular Dichroism (CD)

Around 10 μM of apo-HypB with or without 1 molar equivalent of metal ion (Ni^{2+} or Bi^{3+}) was prepared in 10 mM HEPES buffer at pH 7.0 supplemented with 50 mM NaCl. CD experiments were carried out at ambient temperature on a JASCO 815 spectrophotometer using a quartz cuvette with a path length of 0.1 cm. CD spectra were recorded from 190 to 260 nm with data interval of 0.2 nm and scan rate of 50 nm/min. Three scans were averaged. CD spectra were smoothed using Savitsky-Golay method with a polynomial order of 3 and smoohting window of 15 points.[2] Protein secondary structure was analyzed by CDPro package.[3]

Table S2. Protein secondary structure analysis.

Sample	α helix	β sheet
Apo-HypB	24.4%	24.2%
Ni^{2+}-HypB	22.5%	26.6%
Bi^{3+}-HypB	18.9%	26.9%

Measurement of GTPase activity

Around 10 μM HypB with and without different amounts of Ni^{2+} or Bi^{3+} was incubated at 37˚C in a reaction buffer (20 mM HEPES, 100 mM NaCl, 200 μM GTP, 1 mM $MgSO_4$ and 1% glycerol, pH 7.0). Aliquots (50 μl) of the reaction mixture were taken out at different time intervals. The amounts of released phosphate were determined by Malachite Green phosphate assay kit (Cayman).

Binding constants (K_d') between HypB and Bi^{3+}

The binding constant between apo-HypB and Bi-NTA was determined by fitting UV-vis titration curve to the Ryan-Weber nonlinear equation below as described before.[4]

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

where I represents UV absorbance intensity; I_{max} is the maximal UV absorbance when all of the ligands are bound; C_p and C_m are the total concentration of protein and ligands, respectively and K_d is the dissociation constant. Given the log K_a of Bi-NTA is 17.55,[5] the apprarent constant of Bi^{3+} and HypB (K_d') was calculated to be K_d/K_a = 0.94(±0.25)×10^{-17} μM.

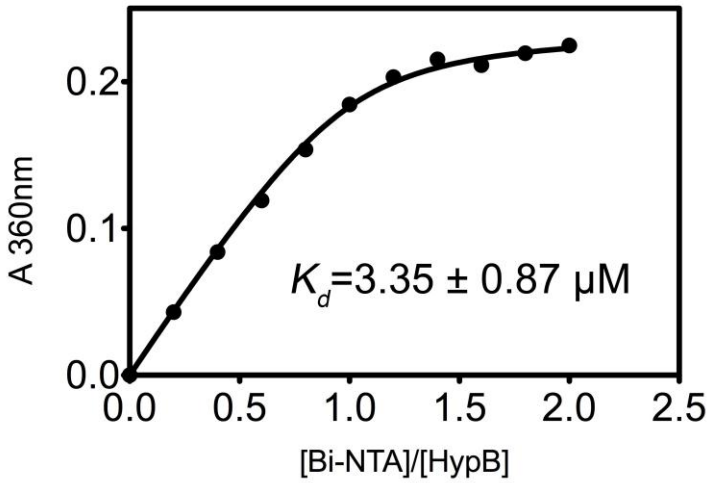

Fig S1. UV titration curve plotted at 360 nm against molar ratios of [Bi-NTA]/[HypB]. The curve was fitted to the Ryan-Weber nonlinear equation and K_d was determined accordingly.

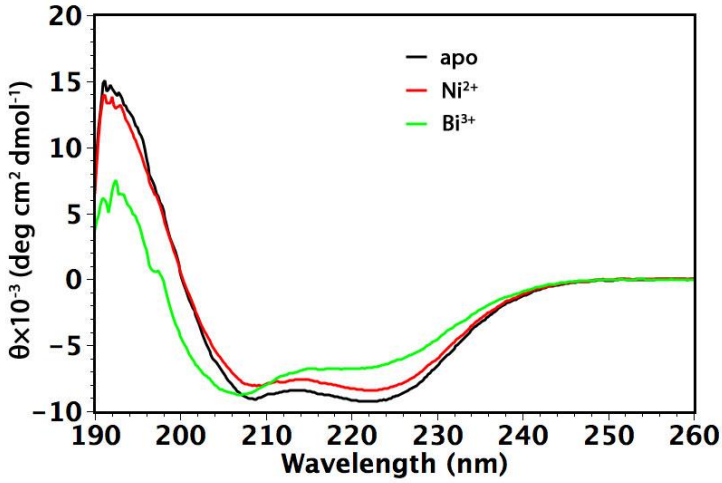

Fig S2. Circular dichroism spectra of apo-HypB (black), Ni^{2+}-HypB (red) and Bi^{3+}-HypB (green). No significant changes were observed upon Ni^{2+} binding while Bi^{3+}-binding perturbed HypB secondary structure.

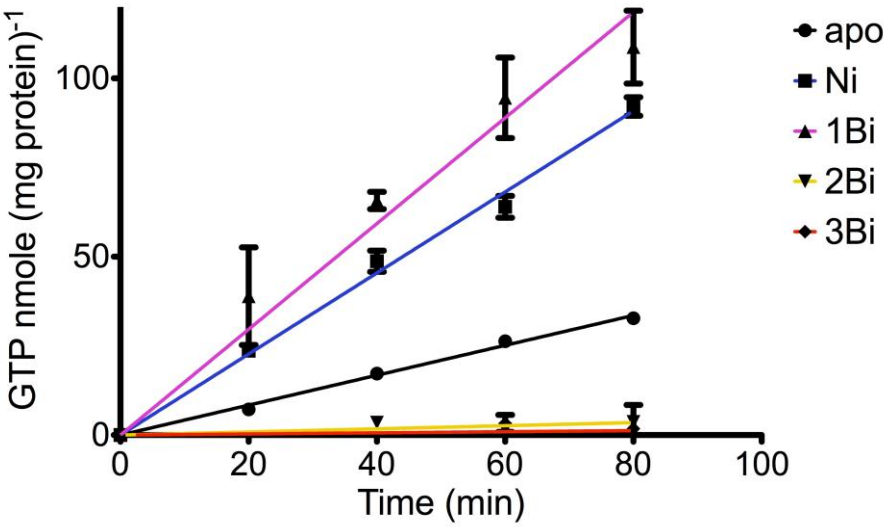

Fig S3. HypB GTPase activity assay. The GTP hydrolysis rates were linear within 80 min. Ni^{2+} (blue) and 1 equivalent Bi^{3+} (magneta) significantly enhanced apo-HypB (black) GTPase acitvity. Higher doses of Bi^{3+} (2 molar equivalents (yellow) or 3 eq. (red)) totally abolished the activity of HypB.

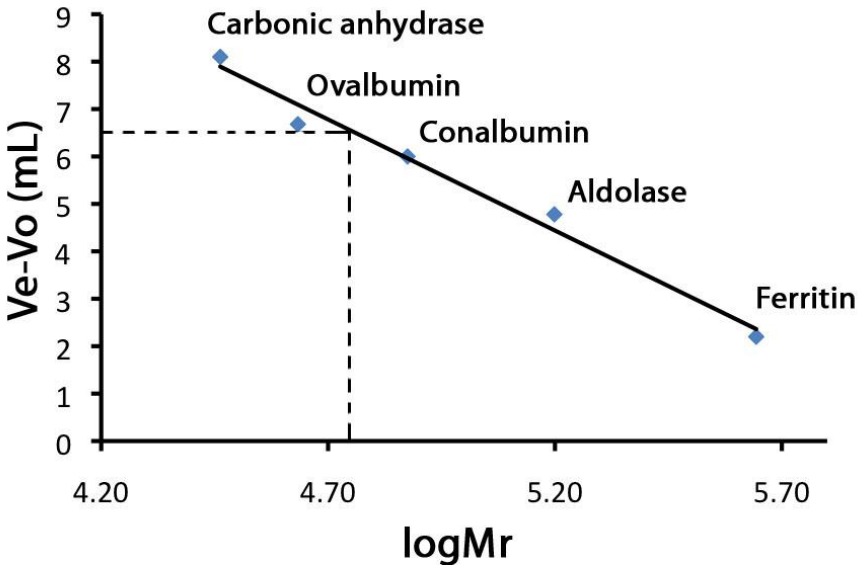

Fig S4. Calibration curve of Tricorn Superdex 200 10/300 (Amersham) using Gel Filtration calibration kit HMW (GE Healthcare). Ferritin (440 kDa), aldolase (158 kDa), conalbumin (75 kDa), ovalbumin (43 kDa), carbonic anhydrase (29 kDa) were used to obtain a standard curve.

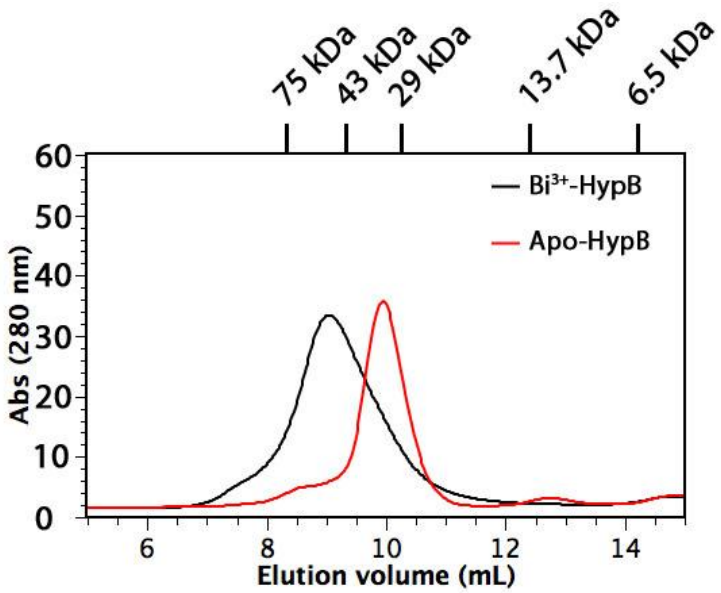

Fig S5. Gel filtration analysis of the WT-HypB oligomerization state upon Bi^{3+}-binding. The protein samples were analyzed by Tricron Superdex 75 10/300 column. WT-HypB was eluted as a dimer after incubation with 1 molar equivalent of Bi^{3+}. The column was calibrated by Gel Filtration calibration kit LMW (GE Healthcare).

References:

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5. G. Petit, and Petit, L. D., *IUPAC Stability Constant Database*, International Union of Pure and Applied Chemistry Academic Software, Otley, UK, 1997.