# The interaction of metallochaperones HypA and UreE facilitates nickel transfer: a "cross-talk" between hydrogenase and urease

Xinming Yang[1], Tianfan Cheng, Wei Xia, Hongyan Li, Hongzhe Sun*

[1] *Department of Chemistry, The University of Hong Kong, HK, P.R. China, E-mail: hsun@hku.hk (Hongzhe Sun), yangxm@hku.hk (Xinming Yang)*

As a pathogen bacterium found in nearly half of the population, *Helicobacter pylori* relies heavily on proper function of urease and hydrogenase for its survival and successful pathogenesis[1-3]. Maturation of these two metalloenzymes requires a series of metallochaperones and accessory proteins. Among these, HypA and SlyD have been shown to form complexes with HypB and subsequently to help nickel insertion into hydrogenase[4-7]. Previous study showed that HypA was involved in the maturation of both hydrogenase and urease; unexpectedly, it was also suggested to interact with urease metallochaperone UreE[8,9].

In this work, we showed the $Ni^{2+}$-induced tetramerization of UreE in solution and investigated the interaction of HypA and UreE. By chemical cross-linking and static light scattering, we showed that one HypA binds to one UreE dimer to form a hetero-complex (i.e. HypA-$(UreE)_2$), with the dissociation constant ($K_d$) of 4 μM in the absence of nickel ions. Upon the binding of $Ni^{2+}$ on UreE, the stability of the complex decreased. The putative residues involving the binding on HypA are mainly located in the cleft between α1 and β1/β6 mapped by NMR chemical shift perturbation. The role of the flexible C-terminus (residues 158-170) of UreE in the discrimination of HypA was demonstrated by cross-linking, size exclusion chromatography and isothermal titration calorimetry. Deletion of C-terminus (residues 158-170) of UreE leads to the significant decrease of urease activity as well as nickel transfer from HypA to UreE. The HypA-UreE complex was also observed intracellularly by using GFP-fragment reassembly, and a model was proposed on the interaction.

**References**

[1] Garner, J. A.; Cover, T. L. *Infect. Immun*. **1996**, *64*, 4197-4203.
[2] Maier, R. J.; Fu, C.; Gilbert, J.; Moshiri, F.; Olson, J.; Plaut, A. G. *FEMS Microbiol*. Lett. **1996**, *141*, 71-76.
[3] McGee, D. J.; May, C. A.; Garner, R. M.; Himpsl, J. M.; Mobley, H. L. T. *J. Bacteriol*. **1999**, *181*, 2477-2484.
[4] Xia, W.; Li, H. Y.; Sze, K. H.; Sun, H. *J. Am. Chem. Soc.* **2009**, *131*, 10031-10040.
[5] Xia, W.; Li, H. Y.; Yang, X. M.; Wong, K. B.; Sun, H. *J. Biol. Chem.* **2012**, *287*, 6753-6763.
[6] Cheng, T. F.; Li, H. Y.; Yang, X. M.; Xia, W.; Sun, H. *Metallomics* **2013**, *5*, 804-807.
[7] Cheng, T. F.; Li, H. Y.; Xia, W.; Sun, H. *J. Biol. Inorg. Chem.* **2012**, *17*, 331-343.
[8] Olson, J. W.; Mehta, N. S.; Maier, R. J. *Mol. Microbiol.* **2001**, *39*, 176-182.
[9] Benoit, S. L.; Mehta, N.; Weinberg, M. V.; Maier, C.; Maier, R. J. *Microbiology* **2007**, *153*, 1474-1482.