



Research Presentation of Sun's lab

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SUN's Laboratory



Department of Chemistry, HongKong University



Professor Sun's research interests:

Biological inorganic chemistry

Recognition of metallodrugs by biomolecules.

Structure and function of metal transport and storage proteins

Medicinal chemistry and inorganic structural biology.

**Metalloproteins;
Metallomics and Metalloproteomics**



Background

- **Metallomics:**

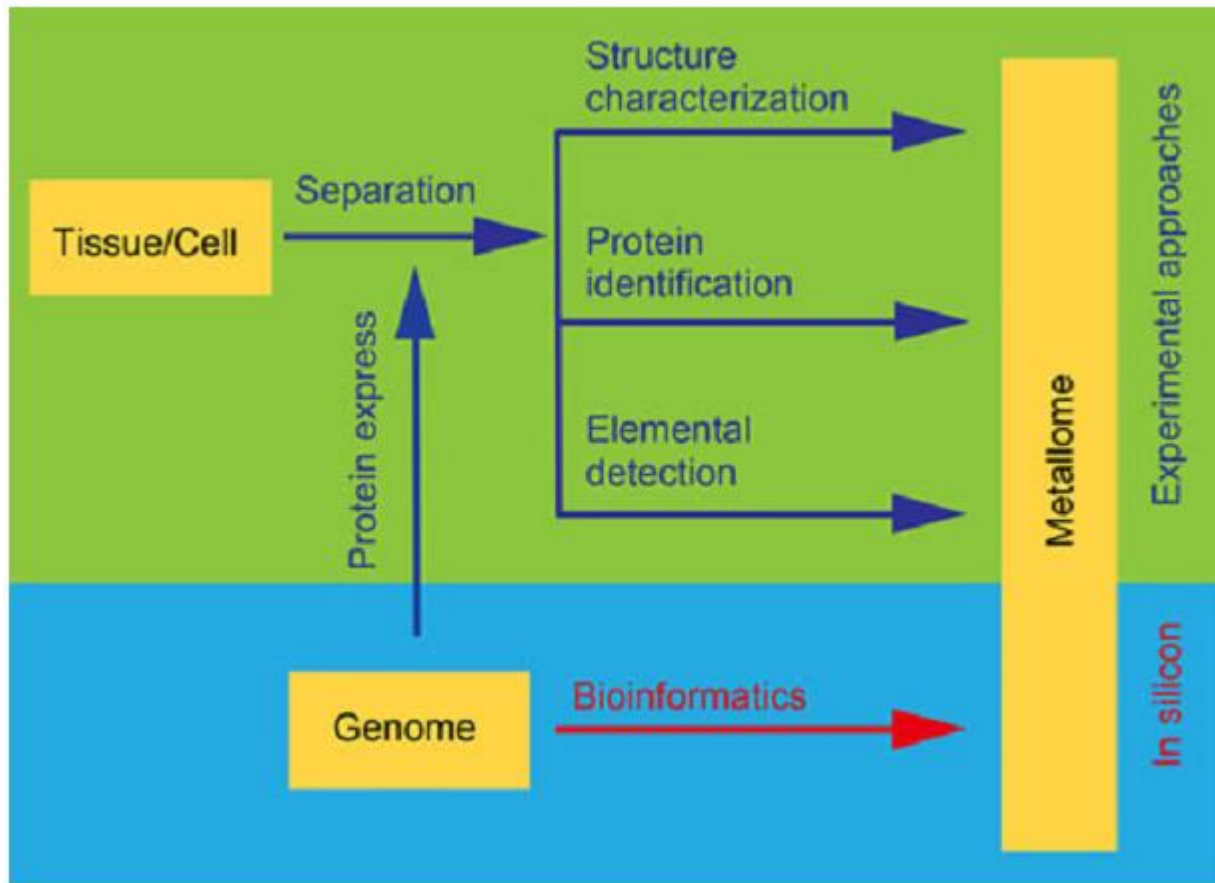
an emerging scientific area, focuses on elucidation of metals/metalloids location, distribution, speciation, and behavior in living organisms.

- **Metalloproteomics:**

a new subset of proteomics focusing on the structural and functional characterization of all metalloproteins in proteome wide.



Experimental pathway in metallomics





Experimental approaches in metallomics

Application of liquid chromatography (LC)/gel electrophoresis-mass spectrometry(GE-MS)/atomic spectroscopy in metallomics

LC/MS/GE/IMAC/LA/ICP

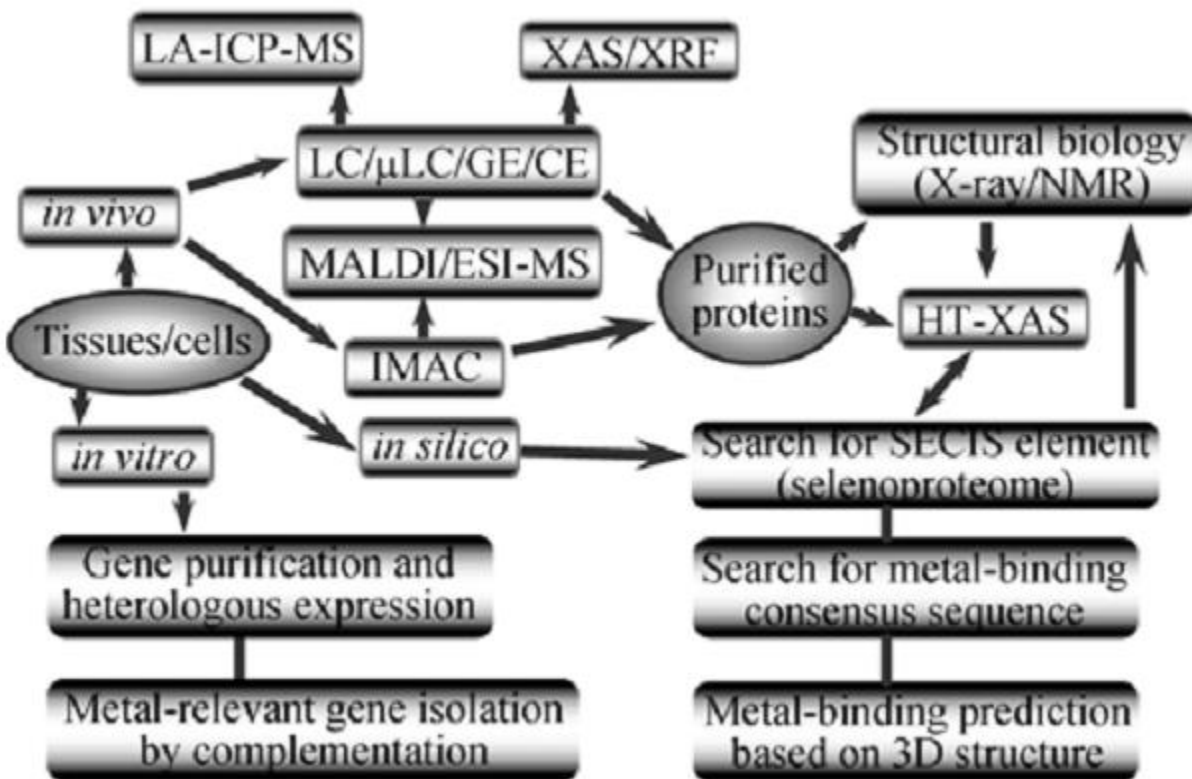
Application of nuclear analytical techniques in metallomics

NMR/XAS/HTXAS

Bioinformatic approaches



Experimental approaches in metallomics



ICP-MS: inductively coupled plasma mass spectrometry

LA-ICP-MS: laser-ablation-ICP-MS

IMAC: immobilized-metal affinity chromatography

XAS: X-ray absorption spectrometry

HT-XAS: High-throughput X-ray absorptions spectrometry

XRF: X-ray fluorescence

LC: liquid chromatography

GE: gel electrophoresis

Sci China Ser B-Chem, Dec. 2009, vol. 52 , no. 12 , 2055-2070



Application of metallomics in environmental/ health related research

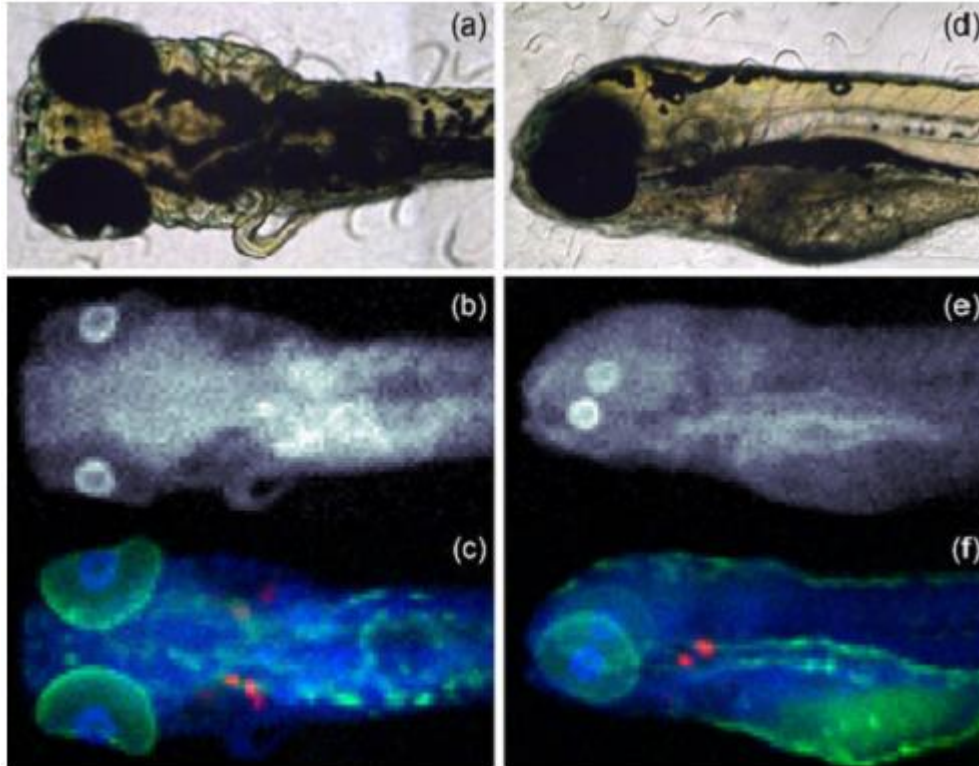
In situ-imaging of metals/metalloids in living Cell and tissue

Profiling toxic metals associated proteins with metallomics approaches

Sci China Ser B-Chem, Dec. 2009, vol. 52 , no. 12 , 2055-2070



Examples



optical images

images of mercury
Distribution

images of mercury (blue)
merged with Zinc (green)
and calcium (red)

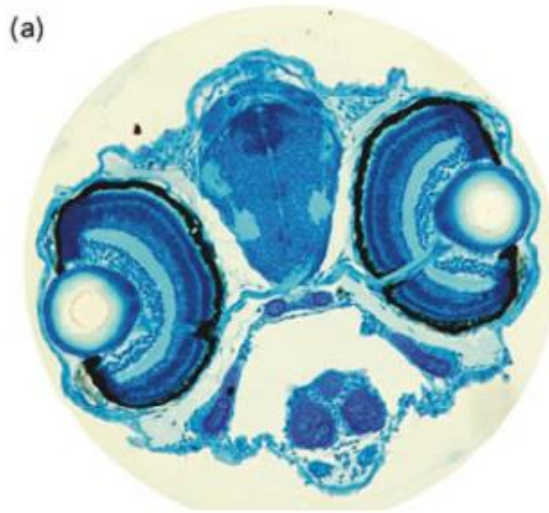
synchrotron X-ray fluorescence images of MeHg-L-cysteine treated living zebrafish

Proc Natl Acad Sci USA, 2008, 105: 12108–12112

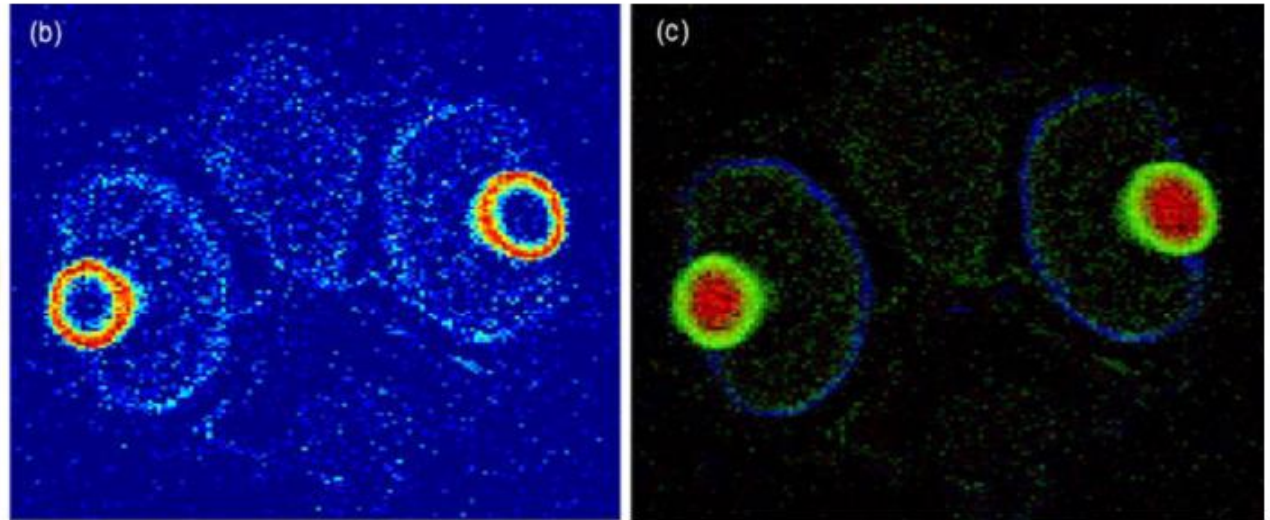


Examples

histological image



merged image of Hg (green), S (red), and Zn (blue)



mercury distribution at resolution of 2.5um

High resolution Hg, S, and Zn distribution of zebrafish head

Proc Natl Acad Sci USA, 2008, 105: 12108–12112



Clinically used metallodrugs

Anti-Helicobacter pylori bismuth drugs

- Bismuth-based triple therapies have been commonly recommended for the treatment of Helicobacter pylori (H. pylori)-related ulcers and chronic gastritis

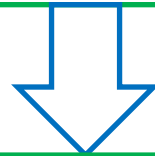
Anti-cancer platinum drugs

- Cisplatin and carboplatin have been used clinically as anti-cancer drugs worldwide for the treatment of various malignancies, especially for testicular and ovarian cancers



Bismuth

Bismuth has been used in medicine for over two centuries for the treatment of various diseases



syphilis, hypertension, infections, skin conditions, and **gastrointestinal disorders**



high effectiveness , low toxicity , antimicrobial activity, anticancer activities, reduce the side-effects of cisplatin in cancer therapy



Structure modeling of bismuth drugs

Bismuth subsalicylate (BSS)

- one of bismuth drugs in the treatment of a variety of gastrointestinal ailments including duodenal and peptic ulcers, ulcerative colitis, and diarrhea.

Colloidal bismuth subcitrate (CBS) Ranitidine bismuth citrate (RBC)

- the most widely used bismuth drugs in many countries. The function of CBS possibly involves the formation of bismuth citrate “polymeric coating” on ulcer craters to prevent the erosion by gastric acid.



Tracking Bismuth Antiulcer Drug Uptake in Single *Helicobacter pylori* Cells

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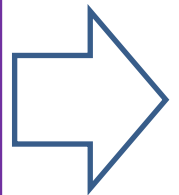
Background

- *Helicobacter pylori*:

is a major human pathogen that can cause peptic ulcers and chronic gastritis.

- Inductively coupled plasma mass spectrometry (ICP-MS)

Its inherent properties of element selectivity combined with high sensitivity and structure-independent response provide unambiguous qualitative and quantitative results.





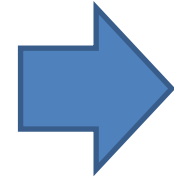
Background

ICP-MS: acid digestion

tedious and prone to
contamination and sample loss



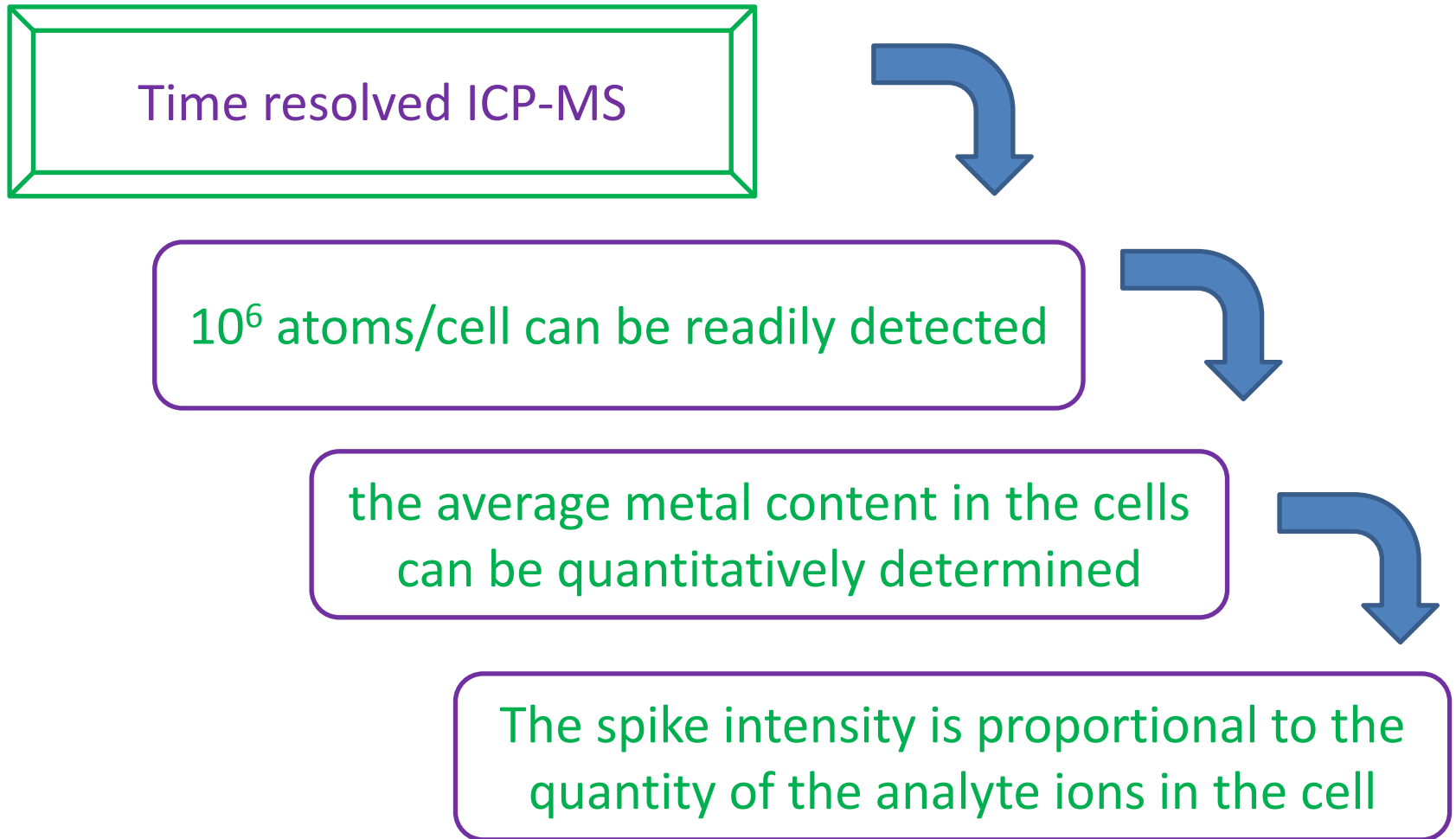
Time resolved ICP-MS for
Single-cell analysis



minimal sample preparation, intact cells, immediate
information on the metal content, high sensitivity

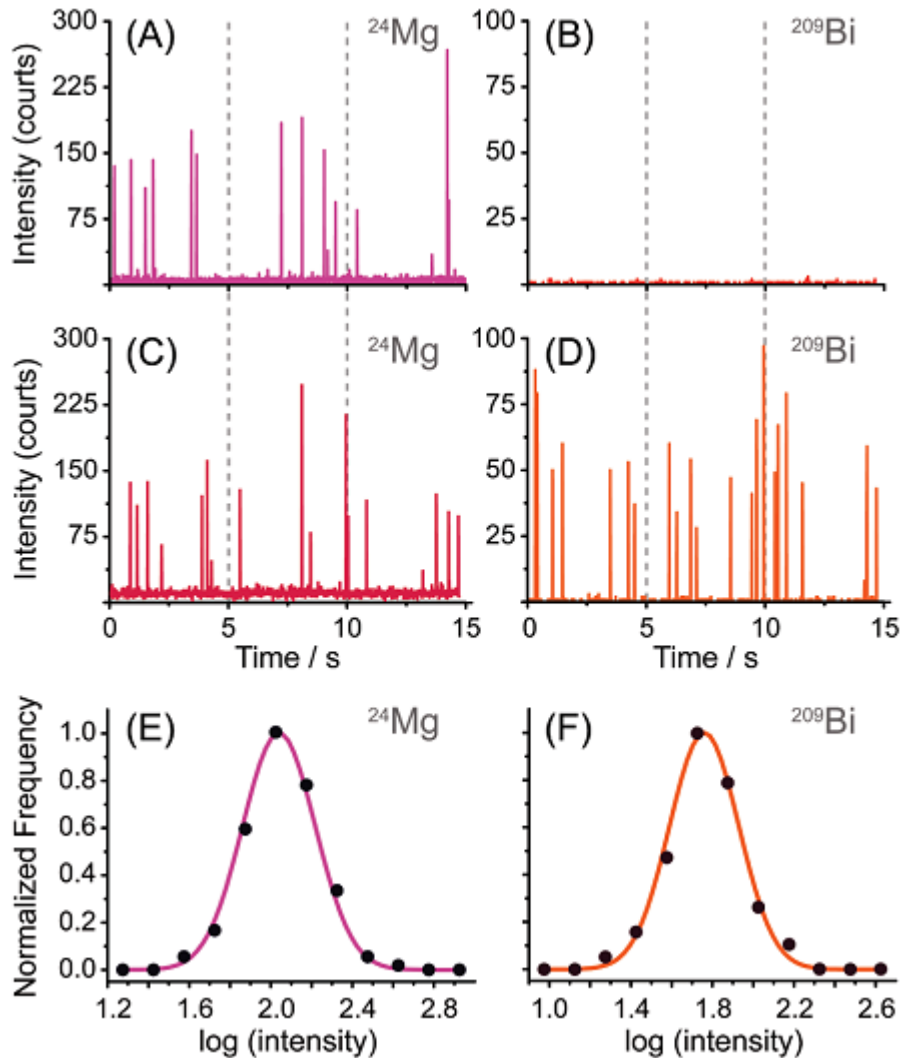


Background





Experiments and Results



without CBS.

Suggesting that no Bi is present in the pathogen under normal conditions

with 20 $\mu\text{g/mL}$ CBS

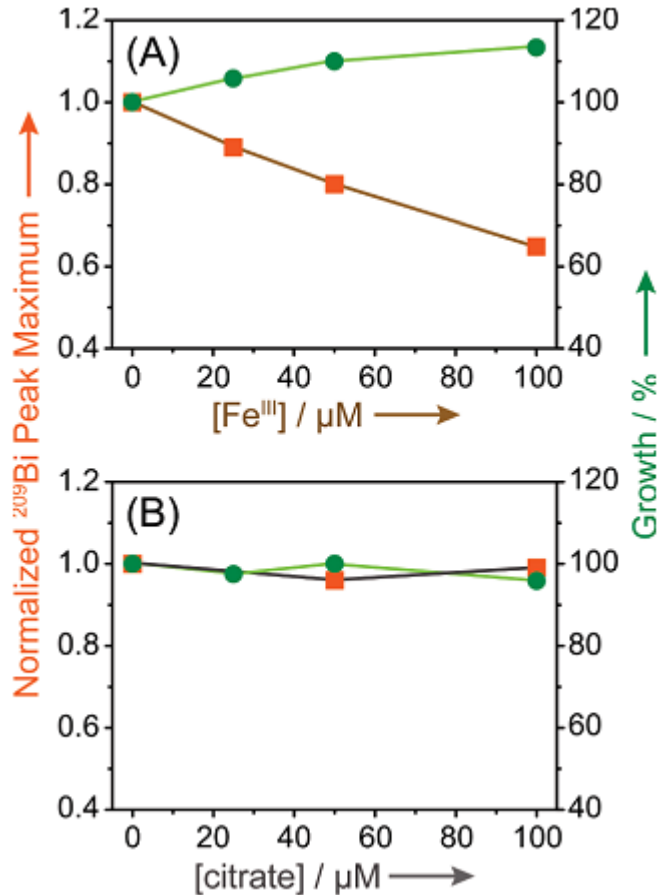
The reduction was probably due to the loss of live and/or intact *H. pylori* cells

Corresponding distributions of ICP-MS spike intensities for ^{24}Mg and ^{209}Bi

Feasibility of using time-resolved ICP-MS for tracking the presence of individual cells and metaldrug uptake



Competition Study



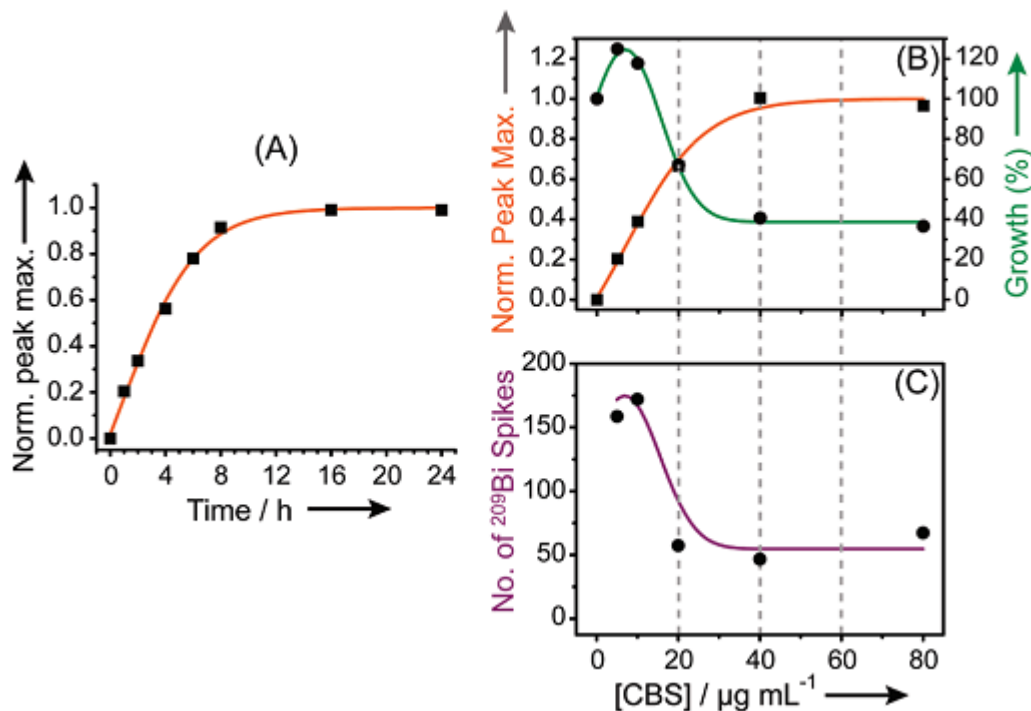
Fe(III) protects *H. pylori* from Bi stress in a concentration-dependent fashion

Indicating that citrate was responsible for neither the growth nor the interference with the metallodrug uptake.

Protective effect of ferric citrate against CBS accumulation in *H. pylori*



Kinetics of CBS uptake in *H. pylori*



The time- and dose-related bismuth uptake upon CBS

Uptake of a small amount of Bi (i.e., <50% of Bi uptake limit) stimulates the growth of *H. pylori*, and the growth is inhibited afterward.



Summary

1. the changes in bismuth content in bacteria with volumes in femtoliter range can be rapidly Monitored

2. single-cell analysis offers valuable biologically relevant insights into the uptake of bismuth-based drugs

3. the study has launched new directions in using time-resolved ICP-MS for extensive single-cellbased applications



J | A | C | S

A R T I C L E S

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Structure of a Nickel Chaperone, HypA, from *Helicobacter pylori* Reveals Two Distinct Metal Binding Sites

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Received January 23, 2009; E-mail: hsun@hku.hk



Background

Metallochaperones:

bind metals and ensure the safe delivery of metals to the targets play an important role in the transport and trafficking metals, and assist the assembly of metallocenter in metalloenzymes.

HypA

Is a metallochaperone, found to be essential to facilitate nickel delivery to hydrogenase together with its partner HypB

HypB

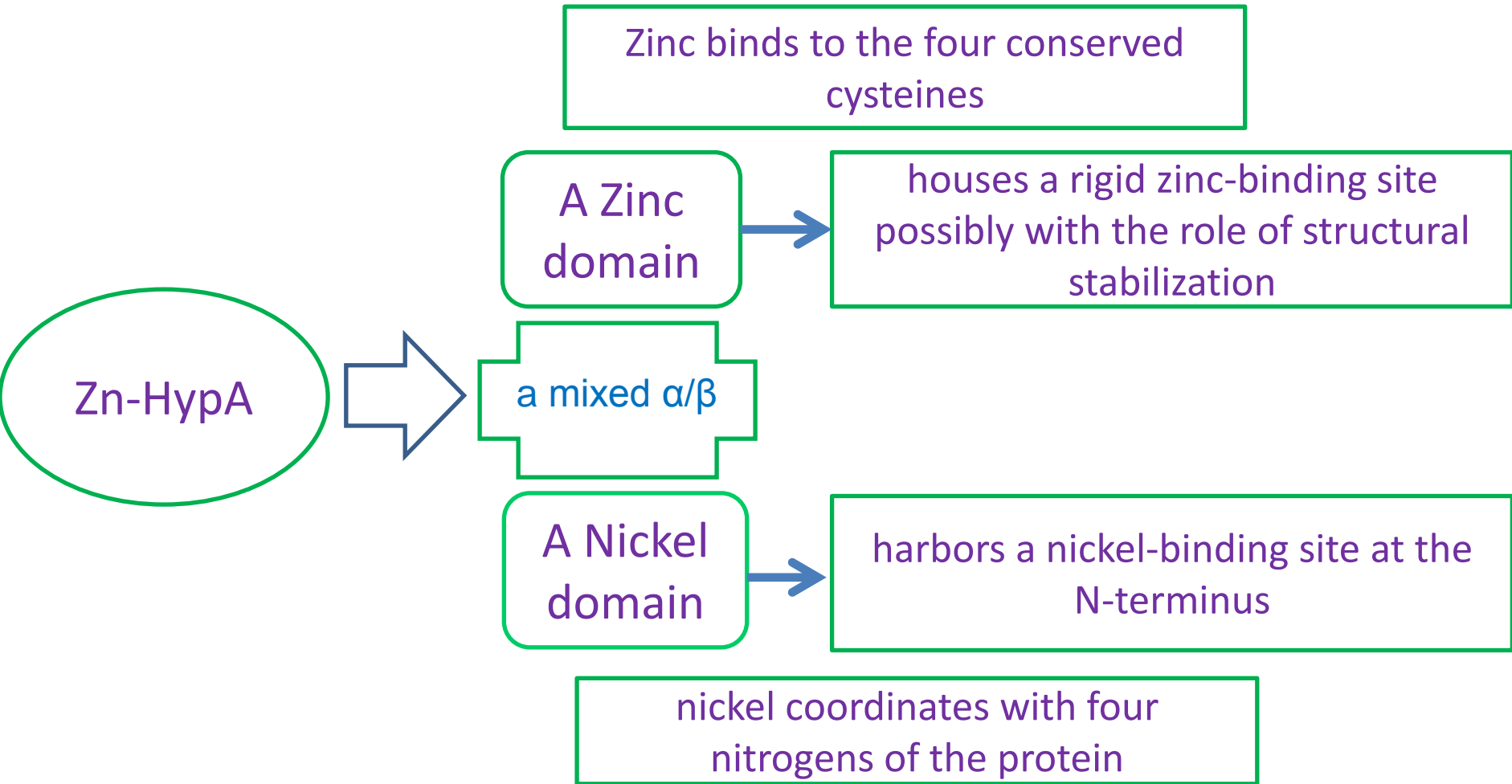
hydrogease-related proteins, a small GTPase to deliver nickel ions to the apo-hydrogeanse.

Urease

Catalyzes the hydrolysis of urea into carbamate and ammonia, helping to maintain the bacterial cytoplasm at neutral pH



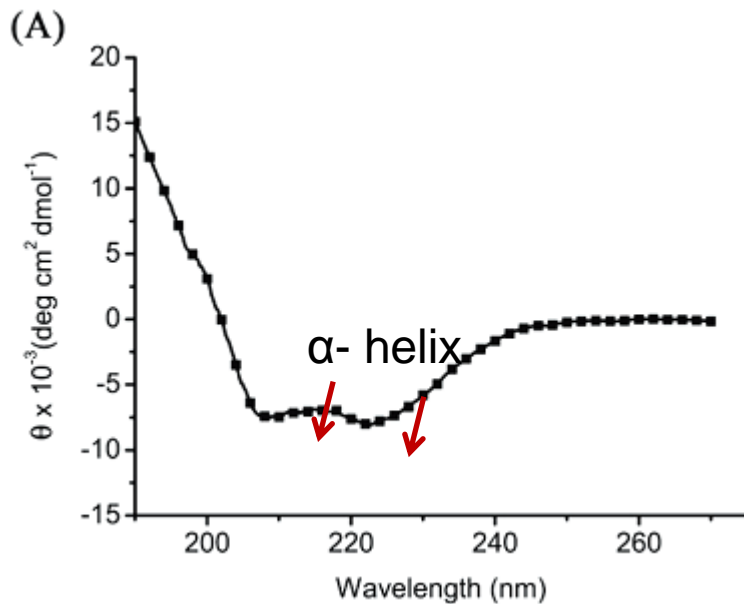
Introduction



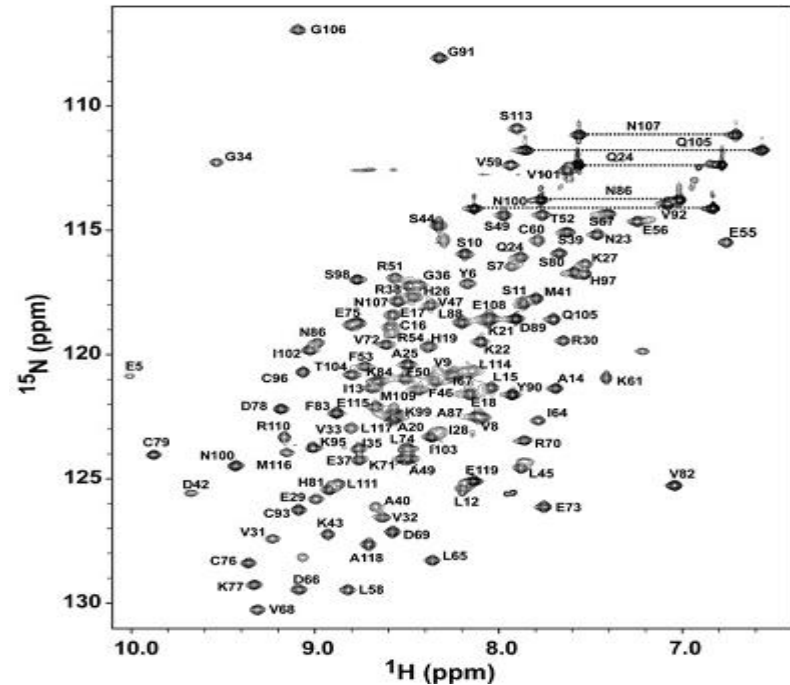
How the metallochaperone-HypA exerts its functions in intracellular nickel delivery?

Results and Discussions

- Characterization of *H. pylori* HypA Protein



Secondary structure and protein stability of *H. pylori* HypA measured by CD spectroscopy.

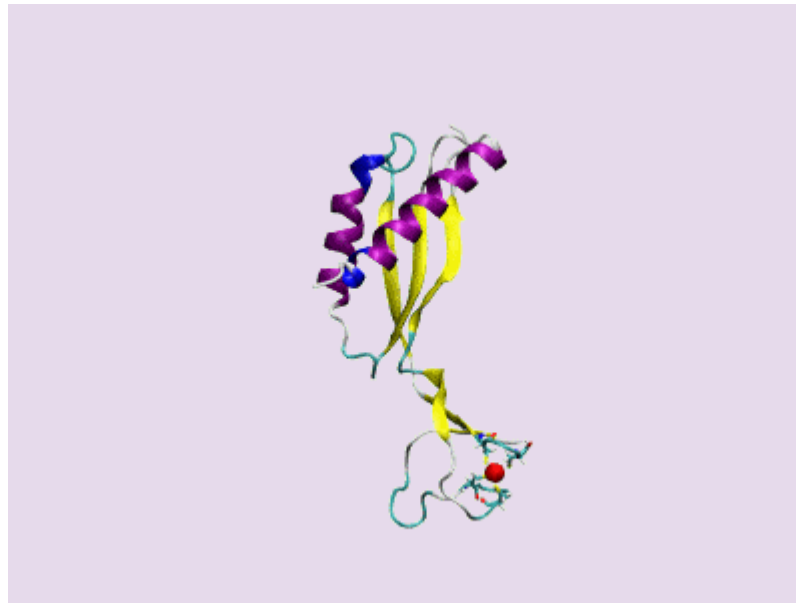


2D ^1H - ^{15}N HSQC spectrum of Zn-HypA in 20 mM Tris-HCl buffer at pH 7.4

The well-dispersed signals suggest a well-folded protein.



Structure Determination and Description



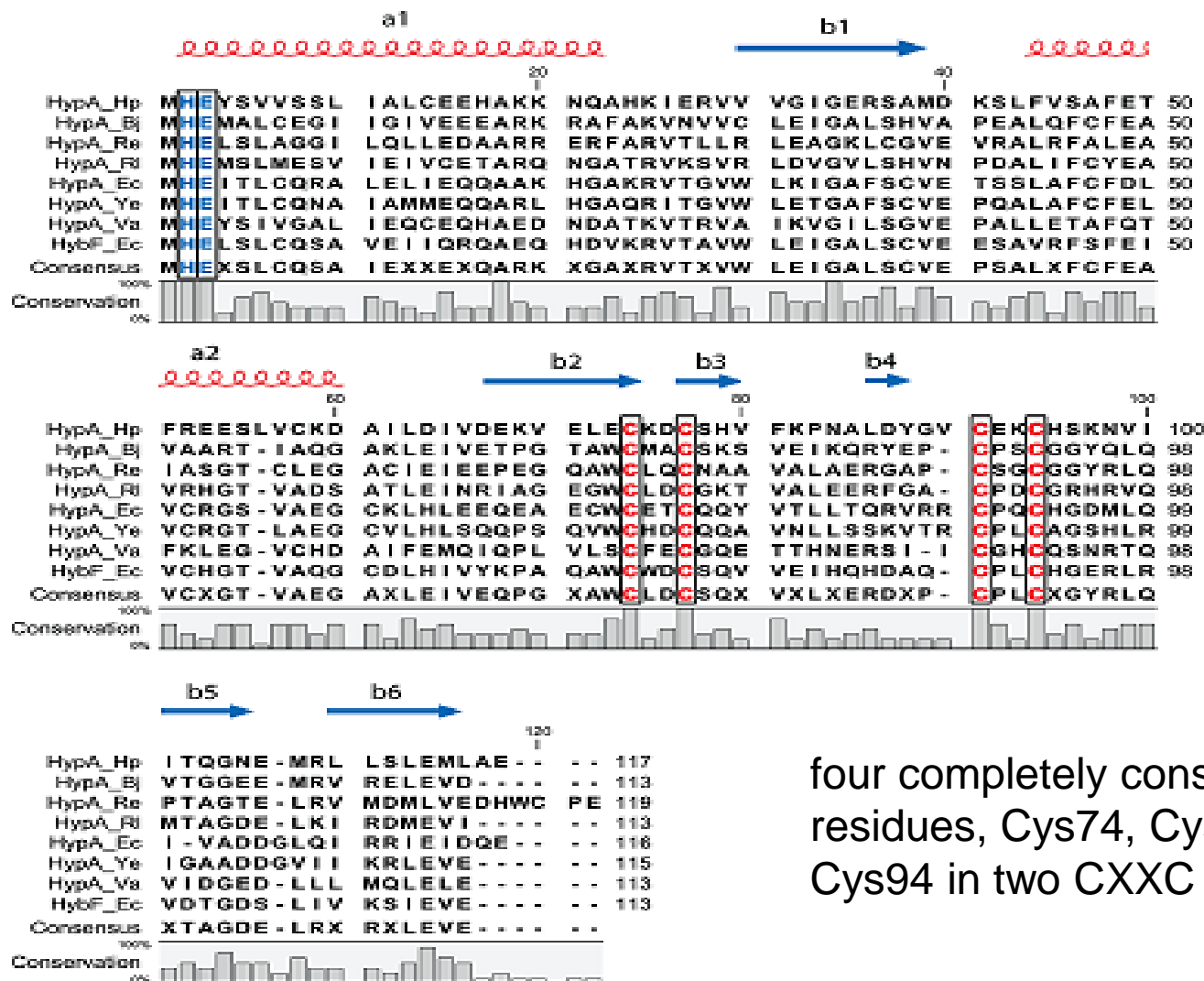
Solution structures of HpHypA

A: The protein folds into an elongated structure consisting of two domains (nickel domain and zinc domain) with $\alpha 1-\beta 1-\alpha 2-\beta 2-\beta 3-\beta 4-\beta 5-\beta 6$ topology.

B: The segments that connect to the two domains of HypA comprise Lys69-Val70 and Gly104-Met107



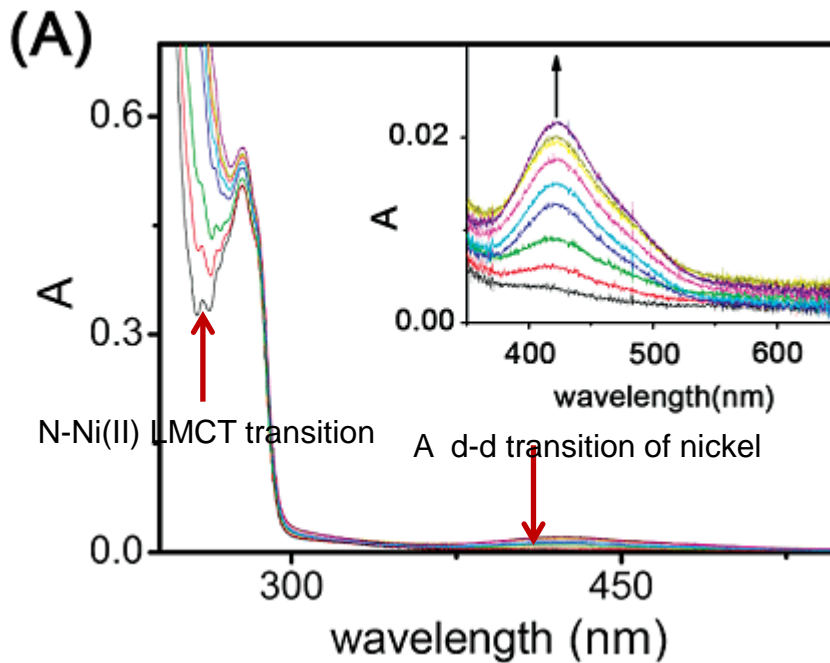
Zinc Coordination



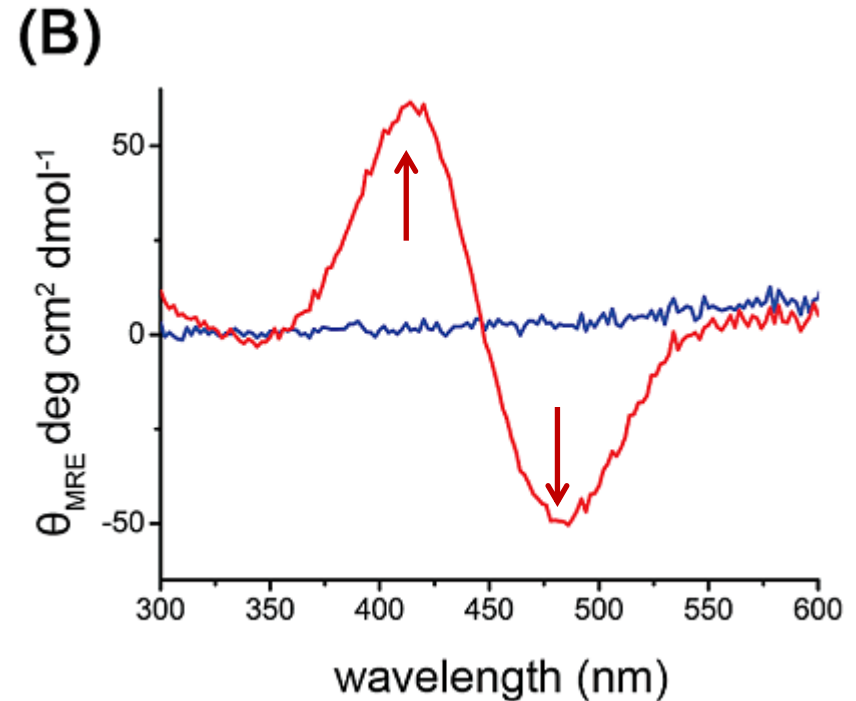
four completely conserved cysteine residues, Cys74, Cys77, Cys91, and Cys94 in two CXXC motifs



Nickel Coordination



UV-vis spectra of HypA (0.2 mM) upon addition of Ni²⁺.

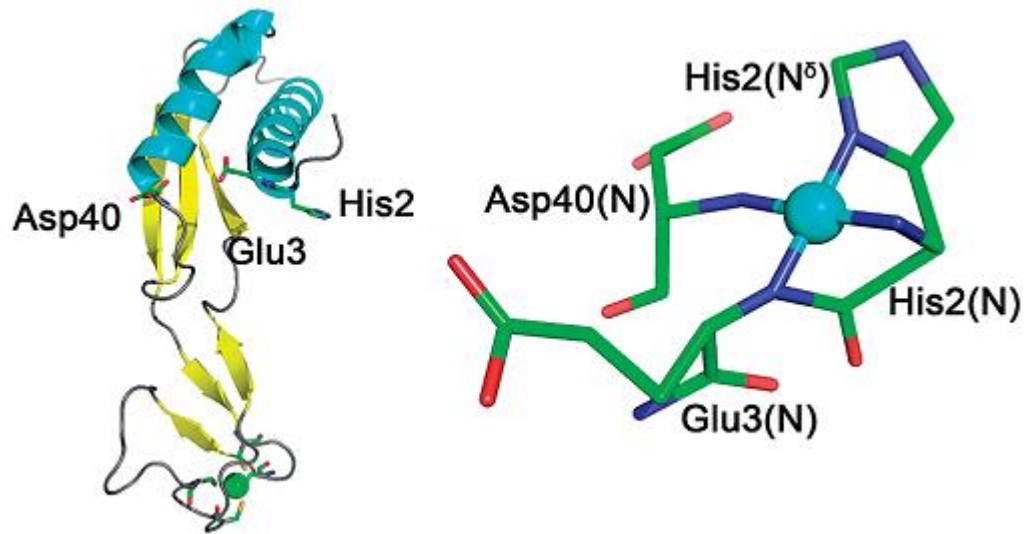


CD spectrum of 0.25 mM Zn-HypA (blue) and nickel-bound Zn-HypA protein (red)

It is characteristic for a planar coordination of Ni²⁺ in a 4N chromophore



Nickel Coordination



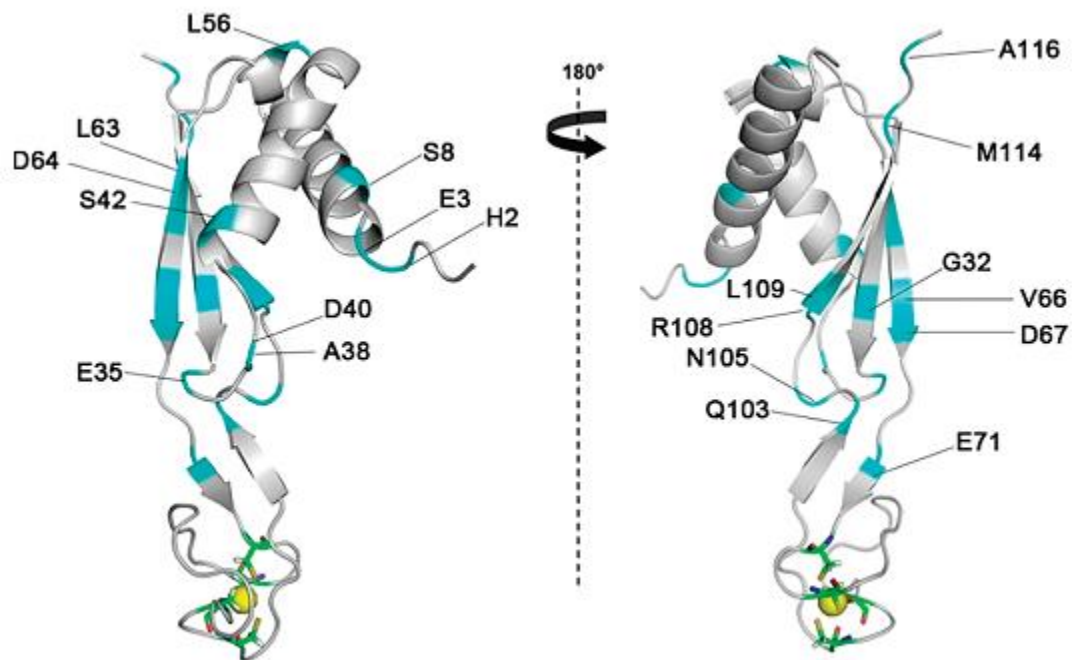
Characterization of Ni-binding site of HypA

A: Structure of HpHypA with putative nickel-binding residues His2, Glu3, and Asp40 highlighted.

B: Proposed square-planar Ni site with four binding ligands, His2 (N), His2 (N δ), Glu3(N), and Asp40 (N)



Nickel Coordination



HypA structures showing the location of residues (in cyan) perturbed upon Ni^{2+} binding based on nickel titration experiments, indicating conformational changes of the protein



Summary

Determined the solution structure of *H. pylori* HypA and characterized its binding properties to both Zn^{2+} and Ni^{2+} .

The protein exhibits a unique two-domain architecture with a distinct metal binding site in each domain

our structure will provide important new insight into nickel trafficking and subsequently the cell biology of nickel