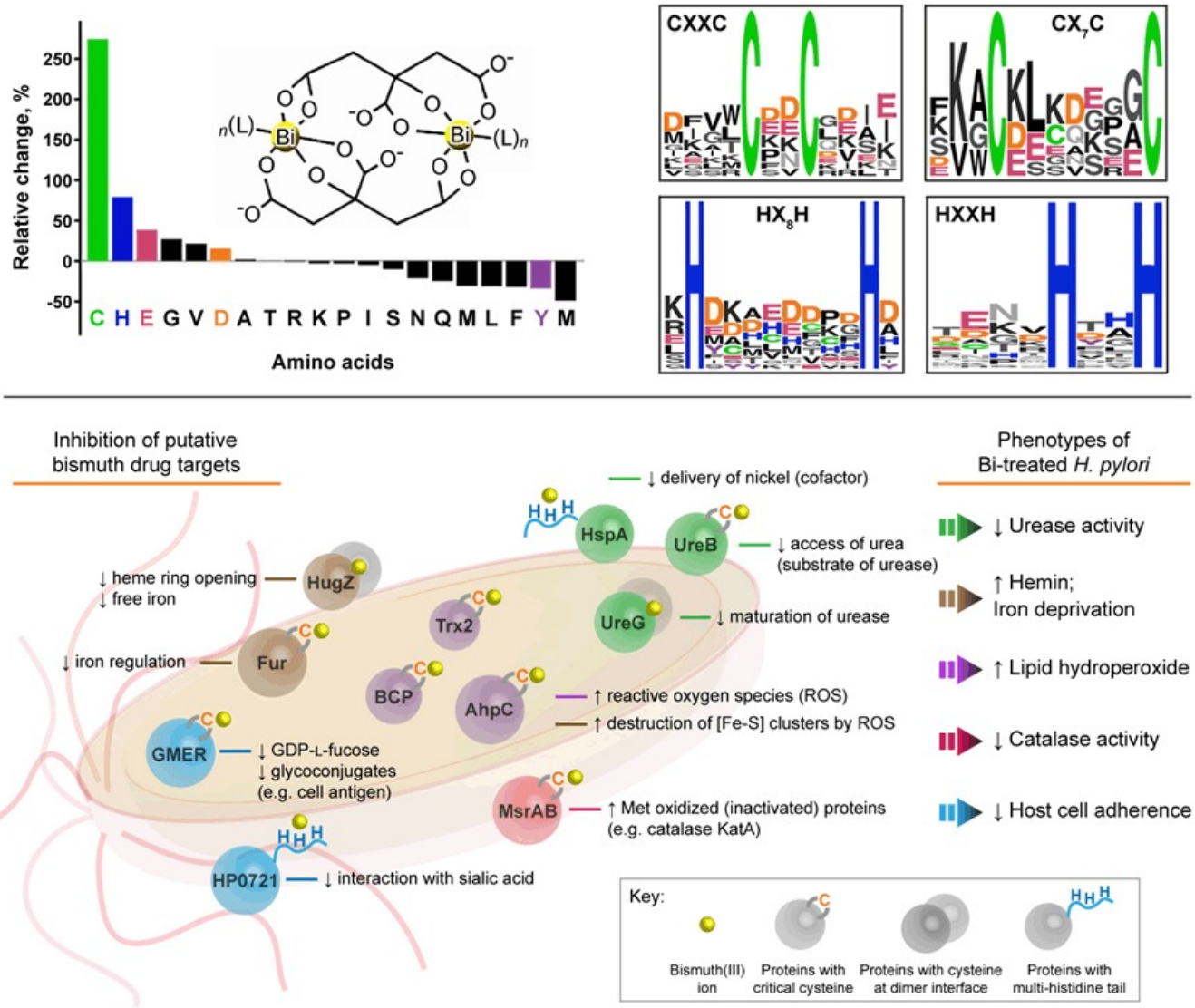
Bio-coordination of bismuth in Helicobacter pylori tells the inhibitory mechanisms of metallodrugs

Helicobacter pylori (*H. pylori*), the dominant bacterial pathogen in human stomach, is the leading risk factor for the development of gastric cancer and is now infecting over half of the worlds' population. Bismuth compounds have been used in medicine for over two centuries, which exhibit low toxicity to humans but relatively high drug selectivity towards pathogens that lack of glutathione such as *H. pylori*. Presently, three bismuth drugs, bismuth subsalicylate (BSS), colloidal bismuth subcitrate (CBS) and ranitidine bismuth citrate (RBC) are used in clinic in combination with antibiotics for the treatment of *H. pylori* infection, and resistance to bismuth has not been reported. It has been demonstrated that bismuth drugs exhibit various binding modes with proteins and enzymes, and inhibit the growth of *H. pylori* in a unique manner through acting on multiple targets. However, the underlying mechanisms of bismuth against the pathogen are still not fully understood.



Bismuth exhibits cysteine- and histidine-oriented bio-coordination in *H. pylori* and may broadly interfere with protein functions.

To understand where Bi-protein interactions occur in *H. pylori,* we unequivocally identified over 300 Bi-binding peptides from 166 proteins in *H. pylori* using Bi-IMAC (immobilized metal affinity chromatography) in combination with high-throughput LC-MS (liquid chromatography-mass spectrometry). Bioinformatics analysis indicated that bismuth exhibited high selectivity towards peptides enriched by cysteines and histidines with dominated motif patterns of CX_nC, CX_nH and HX_nH. The preferential binding of soft thiolate sulfur of cysteine and imidazolium nitrogens of histidine reflects the soft feature of bismuth, which is consistent with the *in vitro* evaluations.

Moreover, some of the identified Bi-binding peptides were mapped to the protein structures retrieved from Protein Data Bank (PDB) for rationalization, which provided direct view of the Bi-binding motifs in the pathogen.

To further understand the biological functions of the identified putative Bi-binding proteins, we then performed GO term enrichment based on the newly constructed GO annotations to the gene products in *H. pylori* 26695. Results revealed that the putative Bi targets were enriched in several key functional categories, including translation, transcription, oxidation-reduction process, protein folding and transition metal ion binding; any malfunctions of the relevant proteins might lead to the bacterium to be eradicated.

In conclusion, the present study generated information-rich resources on bismuth-protein interfaces in *H. pylori*, which provided a rational basis in understanding the high efficacy and low antibiotic resistance of bismuth-based metallodrugs, and further guide in bismuth-based drug design. We provided the first example of using IMAC-based approach to explore the bio-coordination of a metallodrug, and the methodology could be readily extended to other organisms for the investigation of the antimicrobial and anticancer activities of metallodrugs.

Publication

Bio-coordination of bismuth in Helicobacter pylori revealed by immobilized metal affinity chromatography.
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