



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



ELSEVIER

Recent advances in bioinorganic chemistry of bismuth

Hongyan Li and Hongzhe Sun

Bismuth has been used in medicine for over two centuries for the treatment of various diseases, in particular for gastrointestinal disorders, owing to its antimicrobial activity. Recent structural characterization of bismuth drugs provides an insight into assembly and pharmacokinetic pathway of the drugs. Mining potential protein targets inside the pathogen via metallomic/metalloproteomic approach and further characterization on the interactions of bismuth drugs with these targets laid foundation in understanding the mechanism of action of bismuth drugs. Such studies would be beneficial in rational design of new potential drugs.

Address

Department of Chemistry, The University of Hong Kong, Pokfulam Road, Hong Kong, PR China

Corresponding author: Sun, Hongzhe (hsun@hku.hk)

Current Opinion in Chemical Biology 2012, 16:74–83

This review comes from a themed issue on
Bioinorganic Chemistry
Edited by Fraser Armstrong and Lawrence Que

Available online 7th February 2012

1367-5931/\$ – see front matter

© 2012 Elsevier Ltd. All rights reserved.

DOI 10.1016/j.cbpa.2012.01.006

Introduction

Bismuth compounds have been used in medicine for more than 200 years for the treatment of various diseases including syphilis, hypertension, infections, skin conditions, and gastrointestinal disorders [1,2^{*}]. The discovery of *Helicobacter pylori* (*H. pylori*), a Gram-negative bacterium from gastric mucosa that is responsible for gastric and duodenal ulcers [3], has further promoted both research and medical applications of bismuth. Currently, three bismuth drugs, that is, bismuth subsalicylate (Pepto-Bismol[®]), colloidal bismuth subcitrate (De-Nol[®]), and ranitidine bismuth citrate (Tritec[®] and Pylorid[®]) are being used worldwide in combination with antibiotics to eradicate *H. pylori* infection. In addition, many new bismuth compounds with different structures and activities as well as bismuth nanotubes have been synthesized [2^{*},4,5].

For the past few years, enormous efforts have been made towards understanding the structures [6,7^{**},8] as well as the mechanism of actions of the bismuth drugs [2^{*}]. Recent advances in biophysics and molecular biology

have provided invaluable tools necessary to study the bioinorganic chemistry of bismuth. For example, time-resolved inductively coupled plasma mass spectrometry enables bismuth antiulcer drugs to be tracked in single *Helicobacter pylori* cells (ca. 1.0×10^6 Bi atoms/cell). The uptake of bismuth by *Helicobacter pylori* is retarded by ferric ions (Fe^{3+}) suggesting that bismuth drugs may utilize certain iron-transport pathways in the pathogen [9]. A number of protein targets of bismuth drugs, in particular those from the *H. pylori* have been identified by metallomic and metalloproteomic approach [10^{*},11]. Several comprehensive reviews summarized the chemistry and biological chemistry of bismuth as well as its medical applications are available recently [1,2^{*},6,11,12]. Here we will only focus on some recent advances in the structures and protein targets of bismuth drugs as well as design of potentially active new bismuth complexes.

Structural models of bismuth drugs

In spite of extensive clinical usage of bismuth drugs, the structures of these drugs have not been unveiled until the past two decades. Various bismuth citrate complexes with different bismuth to citrate ratios have been crystallized at different pH values. Among these, the structure obtained under acidic condition (pH 3) most probably represents real situation in the stomach [13]. Colloidal bismuth subcitrate (CBS) is likely to assemble into sheets and then three-dimensional polymers using bismuth citrate dinuclear units $[\text{Bi}(\text{cit})_2\text{Bi}]^{2-}$ as building blocks to form a protective coating on the ulcer craters [13].

Crystal structures of four bismuth citrate complexes obtained at the acidic pH values in the presence of either ethylenediamine or pyridine [14] reveal that bismuth citrate dimeric units $[\text{Bi}(\text{cit})_2\text{Bi}]^{2-}$ (Figure 1a) serve as the basic building blocks leading to polymeric structures with regular meshes and internal cavities. The protonated ethylenediamine, despite unobservable in the structure (Figure 1b), and pyridine moieties (Figure 1c) are embedded probably by diffusion or electrostatic interaction in the polymeric framework to achieve charge balance since all citrate anions found in the structures are deprotonated leading to citrate tetrannions $[\text{C}_6\text{H}_4\text{O}_7]^{4-}$. The composition of the bismuth citrate complex frameworks depends on the size and level of protonation of the inserted cations, the pH values as well as the time of crystallization. The structural model of ranitidine bismuth citrate (RBC) was constructed based on the framework of crystal structure of complex 1 [14]. Ranitidine molecules can be readily embedded into the cavities perpendicular to the *bc* plane with H-bonds formed between ranitidine and the bound citrate ligands,

Figure 1d. In addition, the sulfur atoms of ranitidine form H-bonds with a water molecule that coordinates to bismuth. Based on X-ray crystal structures as well as ESI-MS of bismuth citrate complexes, it is likely that bismuth citrate-based drugs degrade under gastric acidic condition (pH \sim 3), from 3-D polymer framework to 2-D polymeric sheets and finally to a basic dimeric unit such as $[\text{Bi}(\text{cit})_2\text{Bi}]^{2-}$ that can be absorbed or transported by membrane receptors.

Bismuth subsalicylate (Pepto-Bismol[®], BSS) is one of the most commonly used bismuth drugs [11]. Structural characterization of bismuth carboxylates is challenging owing to difficulties in controlling hydrolysis of these complexes or the formation of coordination oligomers/polymers. Recently, structures $[\text{Bi}_{38}\text{O}_{44}(\text{Hsal})_{26}(\text{Me}_2\text{CO})_{16}(\text{H}_2\text{O}_2)] \cdot (\text{Me}_2\text{CO}_4)$ and $[\text{Bi}_9\text{O}_7(\text{Hsal})_{13}(\text{Me}_2\text{CO})_5] \cdot (\text{Me}_2\text{CO})_{1.5}$ (Figure 1e) may provide an insight into the nature of BSS and a basis for studies the mode of action of the drug [8]. The crystals of $[\text{Bi}_9\text{O}_7(\text{Hsal})_{13}(\text{Me}_2\text{CO})_5] \cdot (\text{Me}_2\text{CO})_{1.5}$ initially predominate with only relative small amounts of the large cluster with 38 bismuth atoms that appeared to be the least soluble and most thermodynamically stable form when extending crystal growth times. The structure contains a basic building block $[\text{Bi}_6\text{O}_8]^{2+}$ polyhedron as found in other bismuth oxo clusters [15], which have six octahedral Bi atoms with the eight O atoms located over all of the triangular faces. The cores of the two structures are the Bi_9 clusters comprised of a central Bi_6 octahedron but with only seven of the eight trigonal faces capped by an O atom as shown in Figure 1f. In both structures, bismuth also coordinated to the solvent molecules of acetone, which can be replaced by other solvent molecules such as DMF (dimethylformamide) when crystallized in this solvent [7^{••}]. Interestingly, the core of $[\text{Bi}_9\text{O}_7(\text{Hsal})_{13}(\text{Me}_2\text{CO})_5] \cdot (\text{Me}_2\text{CO})_{1.5}$ lies at the heart of $[\text{Bi}_{38}\text{O}_{44}(\text{Hsal})_{26}(\text{Me}_2\text{CO})_{16}(\text{H}_2\text{O}_2)] \cdot (\text{Me}_2\text{CO}_4)$ combined with the presence of Bi-coordinated solvent molecules in these structures reveals a possible process for hydrolysis and core formation, and indicates that the complex $[\text{Bi}_9\text{O}_7(\text{Hsal})_{13}(\text{Me}_2\text{CO})_5] \cdot (\text{Me}_2\text{CO})_{1.5}$ gave rise to the complex $[\text{Bi}_{38}\text{O}_{44}(\text{Hsal})_{26}(\text{Me}_2\text{CO})_{16}(\text{H}_2\text{O}_2)] \cdot (\text{Me}_2\text{CO}_4)$.

The first structure of BSS without organic auxiliaries obtained from X-ray diffraction data of bismuth disalicylate powder [7^{••}] gave rise to two-dimensional polymers held by Bi-O linkages and O-H...O hydrogen bonds with one bismuth atom, one salicylate mono-anion and one salicylate dianion in each unit. The monoanionic salicylate coordinates to a single Bi^{3+} ion through its carboxylate group only and the dianionic salicylate employs the phenoxide oxygen atoms as bridging ligands to form four-membered Bi_2O_2 rings. An additional oxygen atom from a water molecule is bonded to bismuth. Such a structure of BSS resembles bismuth complexes with substituted benzoic acids [16].

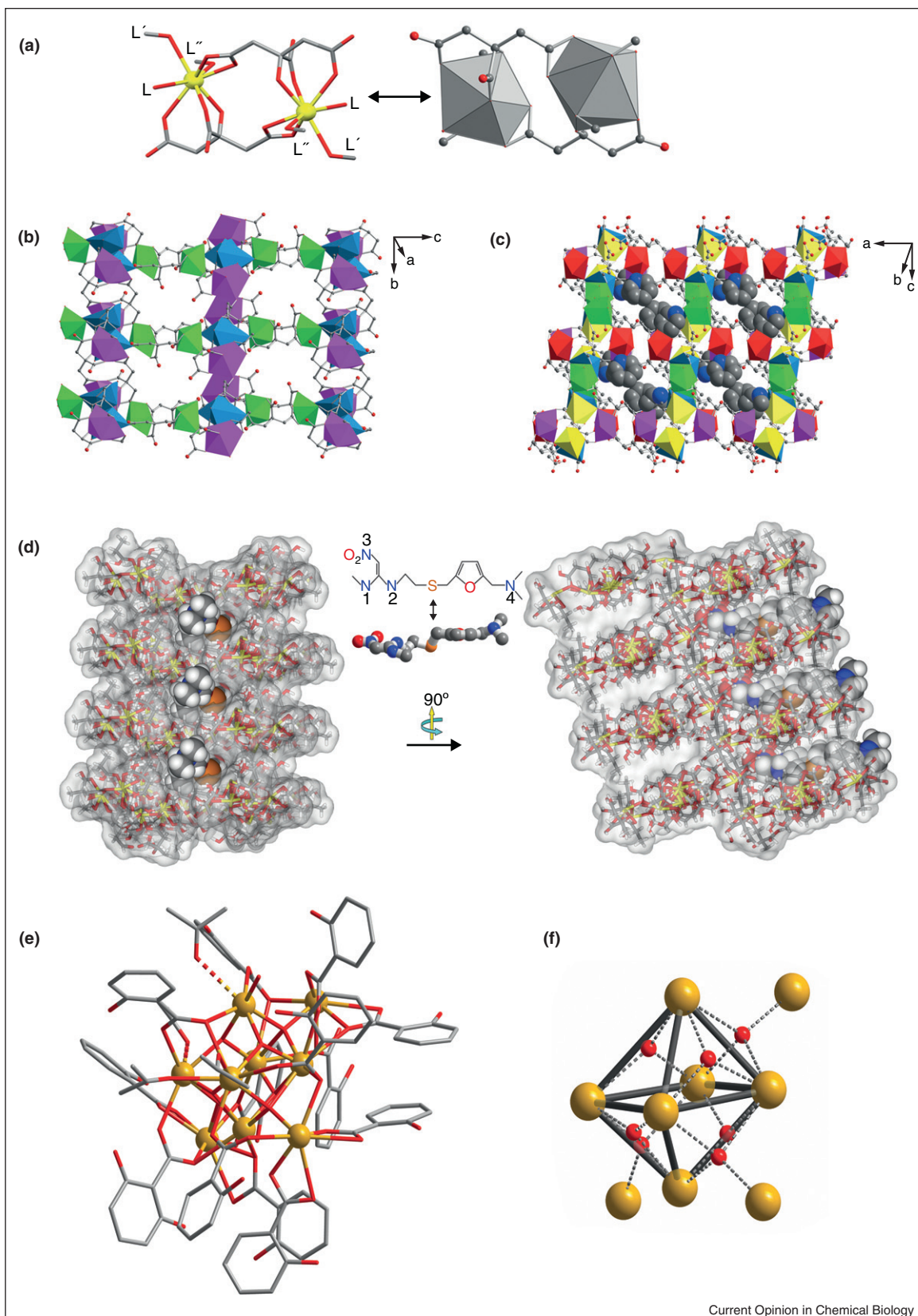
New bismuth complexes and their activities

Over the past decade, significant work has been devoted into the development of new bismuth drugs [2[•],17,18]. Various new bismuth-containing complexes have been synthesized and showed promising *in vitro* activities against *H. pylori* [17,18]. Importantly, some bismuth-containing complexes have been demonstrated to exhibit new *in vitro* activities including antifungal, antiviral or even anticancer activities [19–24].

Bismuth drugs such as BSS, CBS and RBC are effective in treating and eradicating *Helicobacter pylori* together with antibiotics. However, these non-steroidal anti-inflammatory drugs (NSAIDs) may also cause gastrointestinal damage. Bismuth derivatives of NSAIDs exhibited good *in vitro* activity against the three strains of *H. pylori* with the minimum inhibitory concentrations (MIC) \geq 6.25 $\mu\text{g}/\text{mL}$, which are better than commercially used BSS (8 $\mu\text{g}/\text{mL}$), laboratory prepared bismuth salicylate (\geq 12.5 $\mu\text{g}/\text{mL}$) and CBS (\geq 12.5 $\mu\text{g}/\text{mL}$) [18]. Therefore, these compounds may have great potential in the treatment of *H. pylori* infection while allowing the concomitant therapeutic benefits of NSAID treatment. Similarly, heteroleptic bismuth sulfosalicylate complexes $[\text{PhBi}(\text{HSsal})\text{H}_2\text{O}]_\infty$ and $[\text{PhBi}(\text{HSsal})\text{H}_2\text{O}]_\infty$ as well as *bis*-phenylbismuth sulfonates $[\text{Ph}_2\text{Bi}(\text{O}_3\text{SR})]_\infty$ (R = *p*-tolyl, mesityl or S-(+)-10-camphoryl) [17,25] showed significant activities against *H. pylori* with MIC $<$ 6.25 $\mu\text{g}/\text{mL}$. Some cyclic organobismuth compounds bearing a nitrogen or sulfur atoms as additional ring member also exhibit various antibacterial activities including Gram-positive and Gram-negative bacteria [26]. Although these bismuth-containing complexes showed *in vitro* activities against *H. pylori* and other pathogens, there appears to be lack of *in vivo* data and more works are warranted to promote medical application of these complexes.

Beside antibacterial activity, bismuth complexes of substituted benzoic acids also exhibit significant anti-Leishmanial activity against the promastigotes of *L. major* V121 [27]. Heterocyclic organobismuth compounds $[\text{ClBi}(5\text{-R-C}_6\text{H}_3\text{-2-SO}_2\text{C}_6\text{H}_4\text{-1'})]_\infty$ (R = Me, Ph, MeO, Cl, H, *t*-Bu, CF_3 , F, Me_2N) exert antifungal activities against *Saccharomyces cerevisiae* and the activity depends on the lipophilicity of the compounds: the higher the lipophilicity, the lower the antifungal activity [19]. Some organobismuth compounds have been also found to exhibit anti-tumor potentials [2[•],24]. Bismuth xanthate complexes $[\text{Bi}(\text{S}_2\text{COR})_3]$ (R = Et, *i*-Pr, cyclohexyl) were shown to exert cytotoxic activities against Calu-6 (lung adenocarcinoma) with a similar IC_{50} values of cisplatin [28], indicating that these compounds have potency comparable to cisplatin. Moreover, these bismuth complexes also exerted cytotoxic activities against cisplatin-insensitive MCF-7 (mammary carcinoma) [28]. Bismuth dithiocarbamate complexes with general formula of $[\text{Bi}(\text{S}_2\text{CNR}_2)]$ were also demonstrated to exhibit potent

Figure 1



in vitro cytotoxicity against a panel of seven human cancer cell lines [23]. Some heterocyclic organobismuth compounds have potent cytotoxic activities against various cancer cell lines with IC_{50} in the range of 0.059–5.1 μ M, in particular, are sensitive towards leukemic cell lines [20,29]. These bismuth compounds such as bichlorodibenzo[c,f][1,5]thiabismocine may induce apoptosis in HL-60 cells through the activation of caspase, production of ROS, and perturbation of mitochondria [20], they may also target tubulin to induce G2/M arrest in HeLa cells probably by interacting with the colchicines-binding site through its thiolate (–SH) groups [29]. This type of organobismuth compounds may be utilized as antimetabolic agents and in the treatment of refractory acute promyelocytic leukemia similar to arsenic trioxide.

The antiviral activity of bismuth complexes was explored over the severe acute respiratory syndrome coronavirus (SARS-CoV), an enveloped, single-stranded RNA positive-strand virus that killed hundreds of people worldwide when it was first identified in 2003 [30]. A series of bismuth complexes including bismuth nitrilotriacetate Bi(NTA), bismuth tricysteine Bi(Cys)₃, RBC, bismuth ethylenediaminetetraacetate (Bi(EDTA)) as well as bismuth complexes with various N,O-containing chelate ligands including bismuth porphyrin complexes were examined against the SARS-CoV helicase [21,22], which was postulated to be the potential target for anti-SARS therapy [30]. The majority of bismuth complexes tested exhibited activities against SARS-CoV helicase ATPase and duplex-unwinding activities with the highest activities found for RBC and bismuth porphyrin complexes at IC_{50} values of less than 1 μ M. Bi(EDTA) and other two bismuth complexes with N,O-containing chelate ligands showed almost no activity, indicating that different inhibition activities of these complexes are correlated to their different coordination environments, that is, the higher affinity of bismuth towards the chelate ligands, the lower activities. The treatment of SARS-CoV infected cells by bismuth-containing complexes such as RBC and Bi₂-hTF (hTF = human transferrin) confirmed an inhibitory role of bismuth during later stages of the replicative cycle [22]. The studies represent the first attempt in using bismuth as anti-virus agent although further work is required to elucidate such activities.

Potential protein targets of bismuth drugs

The mechanism of action of bismuth drugs is complicated and not fully understood. It is generally believed that bismuth drugs are taken up into gastric mucus to form a

protective coating probably as BiOCl and bismuth citrate complexes on the ulcer crater. They may inhibit *Helicobacter pylori* adherence and also bind strongly to connective tissue proteins, mucus glycoproteins and enzymes [1,2*]. Accumulative studies demonstrated that proteins (peptides) are likely to be the potential targets of the drugs. Bismuth drugs have been shown to interact with a range of proteins such as human serum transferrin [31,32], lactoferrin [33], serum albumin [32,34], and metallothionein [35]. The binding of bismuth to lactoferrin may deprive of iron acquisition of *H. pylori* since the bacteria utilize host-specific lactoferrin for iron acquisition [36]. The level of bismuth uptake by *H. pylori* single cell is also found to be reversibly correlated to the level of iron [9]. Bismuth drugs have also been demonstrated to inhibit several enzymes from *H. pylori*. It inhibits the activity of yeast alcohol dehydrogenase by interfering with the zinc site and altering enzyme native structures [37]. Once bismuth is up-taken by the pathogen *H. pylori*, it may target several proteins inside the pathogen to inhibit synthesis of essential enzymes such as urease and hydrogenase or other nickel-binding proteins, which are critical for bacterial survival. Very recently, the metallomics/metalloproteomics approach has been used to investigate the role of metals in biological systems [38*,39*] and to search for putative binding proteins (targets) of bismuth drugs inside the bacterium [10*,40,41]. The recent advances in this area are updated since other information has been summarized elsewhere [1,2*,6,11].

Urease, accountable for up to 10% of the total cellular proteins, is an essential nickel-containing enzyme for *H. pylori* colonization and virulence. It catalyzes the hydrolysis of urea to yield carbamate and ammonia and thus neutralizes its immediate environment of the bacterium to aid its survival under acidic conditions of the gastric lumen and mucosa. Bismuth complexes such as, RBC as well as some triarylbismuthanes can inhibit urease activity [42,43]. Inhibition of jack bean urease by the triarylbismuthanes compounds is in good agreement with observed antibacterial activity of the compounds against *H. pylori* [43]. Both Bi(EDTA) and Bi(Cys)₃ are competitive inhibitors of jack bean urease, while RBC is a non-competitive inhibitor. Kinetic analysis demonstrated that Bi(EDTA) is both a competitive inhibitor and a time-dependent inactivator of the recombinant *Klebsiella aerogenes* urease. Such an inhibition is probably due to the binding of bismuth to the cysteine residues of the enzyme (Cys319 in *Klebsiella aerogenes* and probably Cys592 in jack bean urease) at the entrance of the active site [42].

(Figure 1 Continued) Structures and structural models of bismuth citrate and bismuth subsalicylate (BSS). **(a)** Dimeric bismuth citrate building block with ball-stick model and polyhedral; **(b)** the polymeric framework of bismuth citrate linked by three types of bismuth citrate building blocks represented by adjacent green, blue and purple polyhedral respectively; **(c)** the two-dimensional polymeric structure of the polymeric framework with pyridine rings in the channels (represented as space-filling mode); **(d)** structural model of ranitidine bismuth citrate with ranitidine inserted in the channel; **(e)** core structure of bismuth subsalicylate $[Bi_9O_7(HSal)_{13}((CH_3)_2CO)_3]$ showing the shrouding of the Bi_9O_7 core by 13 salicylate ligands (stick structures) and **(f)** structure of the Bi_9O_7 core showing the octahedral arrangement of the bismuth atoms (ark brown balls) with seven octahedral faces capped by an oxygen atom (red balls).

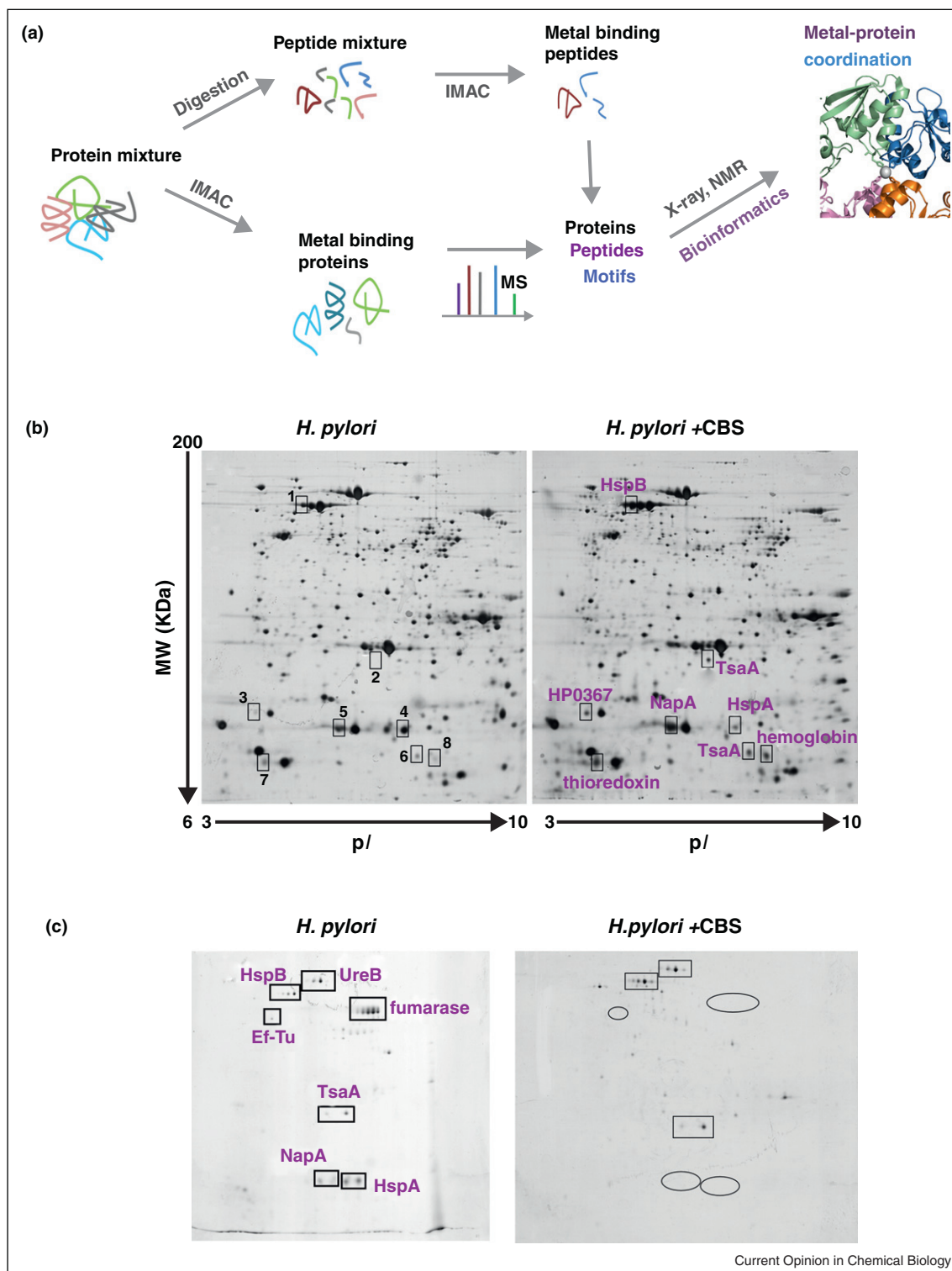
The survival of *H. pylori* requires a constant supply of Ni^{2+} ions for the synthesis and activities of the Ni^{2+} -containing enzymes. Like other bacteria, *H. pylori* has to be able to strike a delicate balance between the import of Ni^{2+} ions, their efficient intracellular storage and delivery to Ni^{2+} -dependent metalloenzymes when required. *H. pylori* is able to synthesize a small histidine-rich cytoplasmic protein Hpn (28 histidine out of 60 amino acids) and histidine-rich glutamine-rich proteins HpnI (18 histidine and 30 glutamine out of 71 amino acids), which may play roles of storage of nickel ions as a 'reservoir', donation of nickel to other proteins and detoxification via sequestration of excess nickel ions depending on the exogenous nickel levels [44–46]. Both *in vitro* and *in vivo* experiments suggested that Hpn and HpnI sequester nickel ions at neutral pH but donate them probably for urease activation under acidic condition [44,45,47]. *H. pylori* lacking hpn/hpnI gene, cultured *in vitro*, are more susceptible to Ni^{2+} and Bi^{3+} than the wild-type strain. Both essential metal ions such as Ni^{2+} , Zn^{2+} , Cu^{2+} and therapeutic metal ions, for example, Bi^{3+} bind to Hpn *in vitro*, indicating that Hpn may serve as a potential target for bismuth therapy. Hpn binds ca. $4.8 \pm 0.2 \text{ Ni}^{2+}$ and $3.8 \pm 0.2 \text{ Bi}^{3+}$ per monomer probably via multiple imidazole groups and four Cys residues with dissociate constants (K_d) of 7.1 and 11.1 μM respectively [47,48], while Hpn-like (HpnI) binds ca. $2.0 \pm 0.1 \text{ Ni}^{2+}$ per monomer (K_d of $3.8 \pm 0.2 \mu\text{M}$) and two histidine residues (His29 and His31) were identified to play a critical role in binding of Ni^{2+} [45,49]. Whether HpnI interacts with bismuth drugs is not known and may warrant for studies. Recently, fluorescence resonance energy transfer (FRET) approach has allowed studies of metal binding properties of Hpn (where the protein sequence is inserted between the FRET partners CFP and YFP) both *in vitro* and *in vivo*. It was found that when Hpn-FRET was expressed in a model system, for example, *E. coli*, FRET change was only observed with addition of bismuth subsalicylate (Pepto Bismol) but not Ni^{2+} and Zn^{2+} , clearly indicating that Hpn is a potential target for bismuth *in vivo* [50]. The binding of bismuth to the protein may therefore interfere with its normal functions.

Systematic identification of potential targets of metallo-drugs is achievable nowadays by metallomic/metalloproteomic approach as shown in Figure 2a. Immobilized-metal affinity chromatography (IMAC) in combination with two dimensional electrophoresis (2-DE) and MALDI-TOF mass spectrometry is a particularly useful tool for elucidating the metabolism of intracellular metal ions and identifying molecular targets or binding proteins associated with the disease etiology and pathology for metallodrugs [10,40]. This approach has been used for the first time to search the potential protein targets of bismuth drugs in *H. pylori* on a genome-wide scale. A comparative proteomic analysis of *H. pylori* cells before and after treatment of CBS was performed [41]. Eight

proteins were found to be significantly upregulated or downregulated, Figure 2b. These proteins are mainly involved in either cellular processes (HspA, HspB, putative alkyl hydroperoxide reductase TsaA and neutrophil-activating protein NapA), or oxidative stress resistance (thioredoxin), and hemoglobin. The upregulated expression of thioredoxin, a low redox potential reductant, may reflect the response of *H. pylori* to the high level of oxidative stress. Using immobilized-bismuth affinity chromatography (Bi-IMAC), seven bismuth-binding proteins were subsequently identified from *H. pylori* cell extracts Figure 2c. Interestingly, the intracellular levels of four proteins, for example, HspA, HspB, NapA, TsaA were decreased upon addition of CBS, Figure 2c, suggesting that these proteins directly bind to bismuth. The other bismuth-binding proteins identified include fumarase and urease subunit UreB and a translational factor Ef-Tu. Furthermore, it was found that bismuth led to around 8-fold decrease in cellular protease activity and elevated the levels of lipid hydroperoxide and hemin in the whole cell extract of bismuth-treated *H. pylori* cells than those untreated cells [41].

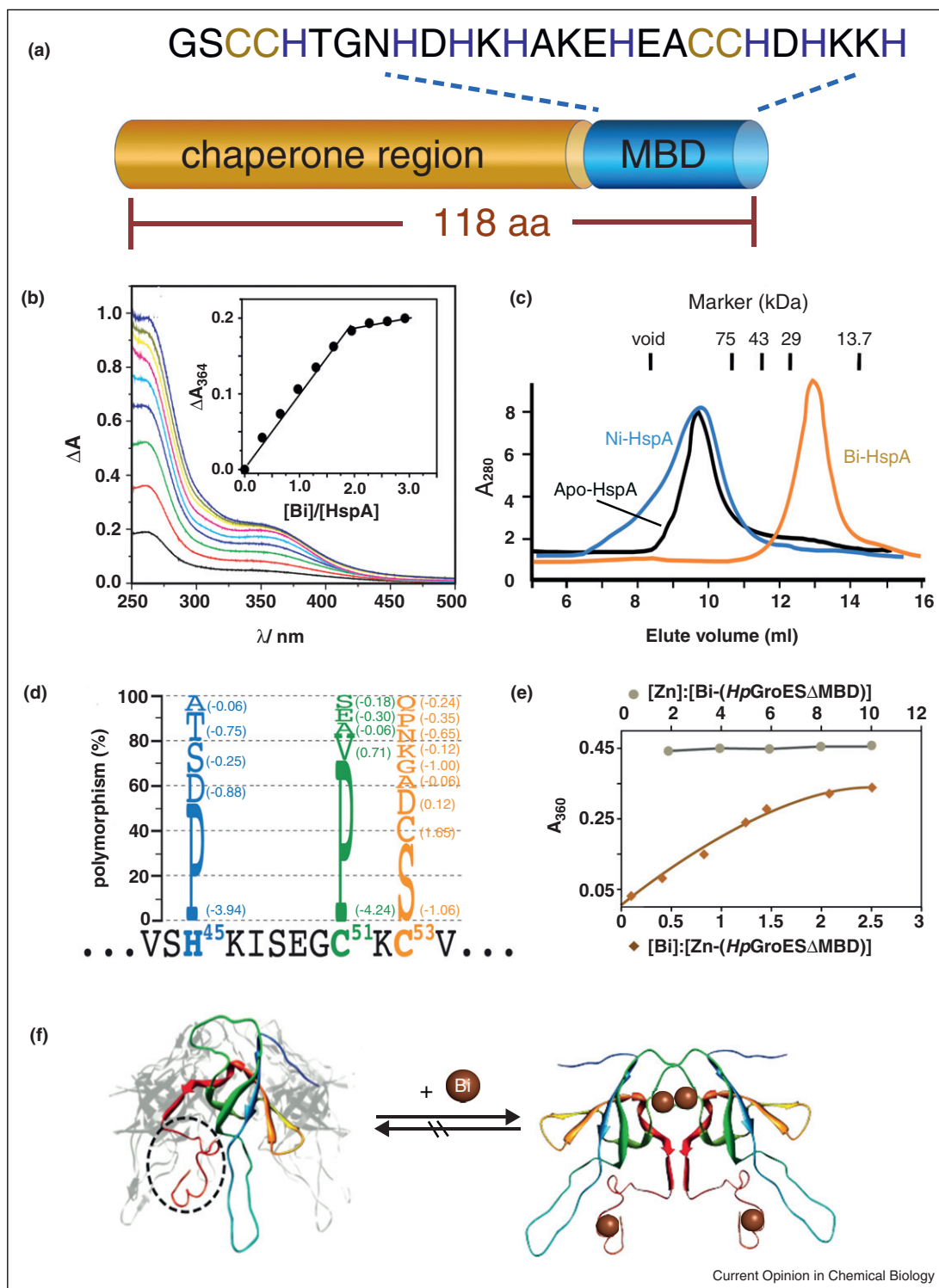
To validate the potential targets of bismuth drugs identified by metalloproteomic approach subsequent studies have been carried out [51,52,53]. For example, fumarase, an enzyme that catalyzes the reversible hydration of fumarate to malic acid, binds one Bi^{3+} per monomer, leading to an apparent non-competitive inhibition of the enzyme [53]. HspA, a member of the GroES chaperonin family, is a small protein with a unique histidine/cysteine-rich domain at the C terminus with the sequence shown in Figure 3a. Apart from the normal function of GroES, for example, aiding protein folding in conjunction with GroEL, it also plays a role in nickel regulation: to facilitate nickel acquisition by donation of nickel to appropriate proteins (and enzymes), and to detoxify excess nickel [51]. Recombinant HspA from *H. pylori* was found to bind about two Bi^{3+} ions (or two Ni^{2+}) per monomer of the protein, Figure 3b, and the C-terminal Cys–Cys motif and histidine residues are probably involved in the binding, similar to Hpn [44,48,51,54]. The potential roles of the His-rich and Cys-rich terminus *in vivo* was examined by comparison of the growth of *E. coli* cells expressing the wild type or C-terminal deletion mutant in M9 minimal medium supplemented with metal ions in a concentration-dependent manner [51]. *E. coli* cells expressing the C-terminal deletion mutant were more susceptible to increasing concentrations of nickel ions. By contrast, Bi^{3+} retarded the growth of the wild type-containing cells [51]. Surprisingly, apart from the C-terminal metal binding domain, three residues (His45, Cys51, and Cys53) are found to be critical for Zn^{2+} binding *in vivo* but not for other transition metal ions such as Ni^{2+} , Cu^{2+} , Fe^{3+} , Mn^{2+} [52]. These residues are located at the N-terminal apical domain and were probably originated from negative selection (Figure 3d) and make up an

Figure 2



(a) Schematic chart of metalloproteomics in identification of bismuth-binding (including bismuth) proteins and motifs. **(b)** Two-dimensional gel electrophoresis image of cell extracts of *H. pylori* (strain 11637) in the absence and presence of bismuth drugs. **(c)** Two-dimensional gel electrophoresis image of fractions of cell extracts eluted from bismuth column from untreated and bismuth drug treated *H. pylori* cells, showing bismuth binding proteins, that is, UreB, HspA, HspB, TsaA, NapA and fumarase.

Figure 3



(a) Scheme of HspA with the putative metal binding domain highlighted. **(b)** Binding of bismuth to the protein was demonstrated by difference UV-vis spectra. **(c)** Bismuth but not nickel induces HspA from a heptamer to a dimer. **(d)** Negative selection of the three metal-binding residues, His49, Cys51 and Cys53 as demonstrated in the position-specific polymorphism. The substitution modes are identified from the alignment of multiple Hspa (GroES) homologs, and the corresponding proportions of each substitute are shown by the height of the letters. **(e)** Proposed scheme showing the oligomeric state of HspA in the presence of bismuth. For clarity, only the front subunit is highlighted, and the rest are shaded in gray.

oxidation-sensitive zinc binding site since the Zn²⁺-bound HpGroESΔMBD restored the activity of enzyme alkaline phosphatase (AP). Equilibrium dialysis study showed that bismuth binds to the C-terminal deleted HpGroES (HpGroESΔMBD) in a similar pattern to Zn²⁺ with an affinity 40-fold higher than that bismuth binding to the C-terminal metal binding domain [52^{••}]. Competition experiments between Bi³⁺ and Zn²⁺ reacting with HpGroESΔMBD indicated that bismuth is able to replace the bound zinc from the protein, Figure 3e. Significantly, the binding of bismuth to GroES altered its quaternary structure from its native heptamer to a dimer, which is attributable to bismuth binding to the N-terminal zinc-site, not the C-terminal metal binding domain, Figure 3f. Taken together, once bismuth drugs entered the *H. pylori*, they may interfere with nickel homeostasis by binding to nickel storage proteins (Hpn/Hpnl), and accessory proteins (HspA) responsible for hydrogenase or urease synthesis. The bismuth drugs are also likely to inhibit protease and urease activity; and modulate cellular oxidative stress.

Conclusions and perspectives

Bismuth drugs have been used clinically for its antimicrobial activity. Structural characterization of bismuth drugs shed light on assembly and decomposition of the drugs. Extensive studies have been carried out to explore other potentials of bismuth drugs and amazingly, bismuth also exhibits *in vitro* activities such as anti-Leishmanial activity, anti-tumor activity that both antimony and arsenic possess. Currently, no bismuth-containing compounds are in clinical trial for treatment of either cancer or viral infection. Nevertheless, some recent *in vitro* data are quite encouraging [20]. Further extensive explore may be needed in future. Moreover, pretreatment of bismuth drugs can reduce the side-effects of anticancer drugs such as cisplatin [55], which may also offer a potential for the application of bismuth in the treatment of cancer.

In spite of extensive studies, our knowledge on the mechanism of action of bismuth drugs is still very limited. Accumulated data indicated that proteins (and enzymes) are likely to be the targets of bismuth drugs. The interactions of bismuth drugs with a number of proteins have been characterized individually. Recently, an integrated view on potential bismuth drugs inside *H. pylori* was given with the aid of metallomics/metalloproteomics approach [41,56]. The strategy can readily be extended to study the mechanism of other metallodrugs as well as to evaluate uptake, storage and excursion of transition metal ions [10[•],38[•]].

Acknowledgements

This work was supported by the Research Grants Council of Hong Kong (HKU1/07C, HKU7042/07P, HKU7049/09P, N_HKU752/09), the Croucher Foundation and the University of Hong Kong.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Sun H, Zhang L, Szeto KY: **Bismuth in medicine**. *Met Ions Biol Syst* 2004, **41**:333-378.
 2. Sun H (Ed): *Biological Chemistry of Arsenic, Antimony and Bismuth*. John Wiley & Sons; 2011.
- This book summarizes the latest progress of biological chemistry of arsenic, antimony and bismuth. New techniques such as metallomics and metalloproteomics were introduced.
3. Marshall BJ, Warren JR: **Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration**. *Lancet* 1984, **1**:1311-1315.
 4. Chen R, So MH, Yang J, Deng F, Che CM, Sun H: **Fabrication of bismuth subcarbonate nanotube arrays from bismuth citrate**. *Chem Commun (Camb)* 2006:2265-2267.
 5. Chen R, Cheng G, So MH, Wu JL, Lu Z, Che CM, Sun H: **Bismuth subcarbonate nanoparticles fabricated by water-in-oil microemulsion-assisted hydrothermal process exhibit anti-*Helicobacter pylori* properties**. *Mat Res Bull* 2010, **45**:654-658.
 6. Yang N, Sun H: **Biocoordination chemistry of bismuth: recent advances**. *Coord Chem Rev* 2007, **251**:2354-2366.
 7. Andre V, Hardeman A, Halasz I, Stein RS, Jackson GJ, Reid DG, Duer MJ, Curfs C, Duarte MT, Friscic T: **Mechanosynthesis of the metallodrug bismuth subsalicylate from Bi₂O₃ and structure of bismuth salicylate without auxiliary organic ligands**. *Angew Chem Int Ed* 2011, **50**:7858-7861.
- Mechanochemical methods were used to give the bismuth subsalicylate (BSS), as well as bismuth disalicylate and trisalicylate directly from bismuth oxide. Structure determination by powder X-ray diffraction gave the first structure of a bismuth salicylate without auxiliary organic ligands, in agreement with a previous study [8].
8. Andrews PC, Deacon GB, Forsyth CM, Junk PC, Kumar I, Maguire M: **Towards a structural understanding of the anti-ulcer and anti-gastritis drug bismuth subsalicylate**. *Angew Chem Int Ed* 2006, **45**:5638-5642.
 9. Tsang CN, Ho KS, Sun H, Chan WT: **Tracking bismuth antiulcer drug uptake in single *Helicobacter pylori* cells**. *J Am Chem Soc* 2011, **133**:7355-7357.
 10. Sun XS, Tsang CN, Sun H: **Identification and characterization of metallodrug binding proteins by (metallo)proteomics**. *Metallomics* 2009, **1**:25-31.
- This review provides an excellent introduction to the characterization of metallodrug binding proteins by metalloproteomics.
11. Ge R, Sun H: **Bioinorganic chemistry of bismuth and antimony: target sites of metallodrugs**. *Acc Chem Res* 2007, **40**:267-274.
 12. Yang N, Sun H: **Bismuth: environmental pollution and health effects**. In *Encyclopedia of Environmental Health*. Edited by Nriagu JO. Elsevier; 2011:414-420.
 13. Li W, Jin L, Zhu N, Hou X, Deng F, Sun H: **Structure of colloidal bismuth subcitrate (CBS) in dilute HCl: unique assembly of bismuth citrate dinuclear units ([Bi(cit)₂Bi]²⁻)**. *J Am Chem Soc* 2003, **125**:12408-12409.
 14. Yang N, An Y, Cai JW, Hu LH, Zeng YB, Mao ZW, Chen GH, Sun H: **Polymeric architectures of bismuth citrate based on dimeric building blocks**. *Sci Chin-Chem* 2010, **53**:2152-2158.
 15. Mehring M: **From molecules to bismuth oxide-based materials: potential homo- and heterometallic precursors and model compounds**. *Coord Chem Rev* 2007, **251**:974-1006.
 16. Andrews PC, Deacon GB, Junk PC, Kumar I, MacLellan JG: **Synthesis, ethanolsis, and hydrolysis of bismuth(III) ortho-nitrobenzoate complexes en route to a pearl necklace-like polymer of Bi₁₀ oxo-clusters**. *Organometallics* 2009, **28**:3999-4008.

17. Andrews PC, Busse M, Deacon GB, Ferrero RL, Junk PC, Huynh KK, Kumar I, MacLellan JG: **Structural and solution studies of phenylbismuth(III) sulfonate complexes and their activity against *Helicobacter pylori***. *Dalton Trans* 2010, **39**:9633-9641.
18. Andrews PC, Ferrero RL, Junk PC, Kumar I, Luu Q, Nguyen K, Taylor JW: **Bismuth(III) complexes derived from non-steroidal anti-inflammatory drugs and their activity against *Helicobacter pylori***. *Dalton Trans* 2010, **39**:2861-2868.
19. Murafuji T, Fujiwara Y, Yoshimatsu D, Miyakawa I, Migita K, Mikata Y: **Bismuth heterocycles based on a diphenyl sulfone scaffold: synthesis and substituent effect on the antifungal activity against *Saccharomyces cerevisiae***. *Eur J Med Chem* 2011, **46**:519-525.
20. Yagura T, Iuchi K, Hatano Y: **Heterocyclic organobismuth(III) induces apoptosis of human promyelocytic leukemic cells through activation of caspases and mitochondrial perturbation**. *Biochem Pharmacol* 2008, **76**:974-986.
21. Yang N, Tanner JA, Zheng BJ, Watt RM, He ML, Lu LY, Jiang JQ, Shum KT, Lin YP, Wong KL *et al.*: **Bismuth complexes inhibit the SARS coronavirus**. *Angew Chem Int Ed* 2007, **46**:6464-6468.
22. Yang N, Tanner JA, Wang Z, Huang JD, Zheng BJ, Zhu N, Sun H: **Inhibition of SARS coronavirus helicase by bismuth complexes**. *Chem Commun (Camb)* 2007:4413-4415.
23. Tiekink ERT, Li HY, Lai CS, Wu JZ, Ho PC, de Vos D: **Cytotoxicity, qualitative structure-activity relationship (QSAR), and anti-tumor activity of bismuth dithiocarbamate complexes**. *J Inorg Biochem* 2007, **101**:809-816.
24. Tiekink ERT: **Antimony and bismuth compounds in oncology**. *Crit Rev Oncol Hematol* 2002, **42**:217-224.
25. Andrews PC, Deacon GB, Ferrero RL, Junk PC, Karrar A, Kumar I, MacLellan JG: **Bismuth(III) 5-sulfosalicylate complexes: structure, solubility and activity against *Helicobacter pylori***. *Dalton Trans* 2009:6377-6384.
26. Yagura T, Kotani T, Nagai D, Asahi K, Suzuki H, Yamao F, Kataoka N: **Antibacterial properties of some cyclic organobismuth(III) compounds**. *Antimicrob Agents Chemother* 2005, **49**:2729-2734.
27. Andrews PC, Frank R, Junk PC, Kedzierski L, Kumar I, MacLellan JG: **Anti-Leishmanial activity of homo- and heteroleptic bismuth(III) carboxylates**. *J Inorg Biochem* 2011, **105**:454-461.
28. Friebolin W, Schilling G, Zoller M, Amtmann E: **Antitumoral activity of non-platinum xanthate complexes**. *J Med Chem* 2005, **48**:7925-7931.
29. Iuchi K, Akagi K, Yagura T: **Heterocyclic organobismuth(III) compound targets tubulin to induce G2/M arrest in HeLa cells**. *J Pharmacol Sci* 2009, **109**:573-582.
30. Tanner JA, Watt RM, Chai YB, Lu LY, Lin MC, Peiris JS, Poon LL, Kung HF, Huang JD: **The severe acute respiratory syndrome (SARS) coronavirus NTPase/helicase belongs to a distinct class of 5' to 3' viral helicases**. *J Biol Chem* 2003, **278**:39578-39582.
31. Li H, Sadler PJ, Sun H: **Unexpectedly strong binding of a large metal ion (Bi³⁺) to human serum transferrin**. *J Biol Chem* 1996, **271**:9483-9489.
32. Sun H, Li H, Mason AB, Woodworth RC, Sadler PJ: **Competitive binding of bismuth to transferrin and albumin in aqueous solution and in blood plasma**. *J Biol Chem* 2001, **276**:8829-8835.
33. Zhang L, Szeto KY, Wong WB, Loh TT, Sadler PJ, Sun H: **Interactions of bismuth with human lactoferrin and recognition of the Bi(III)-lactoferrin complex by intestinal cells**. *Biochemistry* 2001, **40**:13281-13287.
34. Sun H, Szeto KY: **Binding of bismuth to serum proteins: implication for targets of Bi(III) in blood plasma**. *J Inorg Biochem* 2003, **94**:114-120.
35. Sun H, Li H, Harvey I, Sadler PJ: **Interactions of bismuth complexes with metallothionein(II)**. *J Biol Chem* 1999, **274**:29094-29101.
36. Senkovich O, Ceaser S, McGee DJ, Testerman TL: **Unique host iron utilization mechanisms of *Helicobacter pylori* revealed with iron-deficient chemically defined media**. *Infect Immun* 2010, **78**:1841-1849.
37. Jin L, Szeto KY, Zhang L, Du W, Sun H: **Inhibition of alcohol dehydrogenase by bismuth**. *J Inorg Biochem* 2004, **98**:1331-1337.
38. Mounicou S, Szpunar J, Lobinski R: **Metallomics: the concept and methodology**. *Chem Soc Rev* 2009, **38**:1119-1138.
This critical review addresses the concept and methodology of metallo-mics that may be important for mechanism study of metallo-drugs.
39. Cvetkovic A, Menon AL, Thorgersen MP, Scott JW, Poole FL 2nd, Jenney FE Jr, Lancaster WA, Prassman JL, Shanmukh S, Vaccaro BJ *et al.*: **Microbial metalloproteomes are largely uncharacterized**. *Nature* 2010, **466**:779-782.
Metalloproteomic approach was used to identify metalloproteins on a genome-wide scale, and will be very useful for evaluation of metal uptake, storage, excretion and metal remediation.
40. Sun H, Chai ZF: **Metallomics: an integrated science for metals in biology and medicine**. *Ann Rep Prog Chem Sec A Inorg Chem* 2010, **106**:20-38.
41. Ge R, Sun X, Gu Q, Watt RM, Tanner JA, Wong BC, Xia HH, Huang JD, He QY, Sun H: **A proteomic approach for the identification of bismuth-binding proteins in *Helicobacter pylori***. *J Biol Inorg Chem* 2007, **12**:831-842.
42. Zhang L, Mulrooney SB, Leung AF, Zeng Y, Ko BB, Hausinger RP, Sun H: **Inhibition of urease by bismuth(III): implications for the mechanism of action of bismuth drugs**. *Biometals* 2006, **19**:503-511.
43. Murafuji T, Azuma T, Miyoshi Y, Ishibashi M, Rahman AFMM, Migita K, Sugihara Y, Mikata Y: **Inhibition of jack bean urease by organobismuth compounds**. *Bioorg Med Chem Lett* 2006, **16**:1510-1513.
44. Ge R, Watt RM, Sun X, Tanner JA, He QY, Huang JD, Sun H: **Expression and characterization of a histidine-rich protein, Hpn: potential for Ni²⁺ storage in *Helicobacter pylori***. *Biochem J* 2006, **393**:285-293.
45. Zeng YB, Zhang DM, Li HY, Sun H: **Binding of Ni²⁺ to a histidine- and glutamine-rich protein, Hpn-like**. *J Biol Inorg Chem* 2008, **13**:1121-1131.
46. Seshadri S, Benoit SL, Maier RJ: **Roles of His-rich hpn and hpn-like proteins in *Helicobacter pylori* nickel physiology**. *J Bacteriol* 2007, **189**:4120-4126.
47. Ge R, Zhang Y, Sun X, Watt RM, He QY, Huang JD, Wilcox DE, Sun H: **Thermodynamic and kinetic aspects of metal binding to the histidine-rich protein, Hpn**. *J Am Chem Soc* 2006, **128**:11330-11331.
48. Kozlowski H, Rowinska-Zyrek M, Witkowska D, Bielinska S, Kamysz W: **The -Cys-Cys- motif in *Helicobacter pylori*'s Hpn and HspA proteins is an essential anchoring site for metal ions**. *Dalton Trans* 2011, **40**:5604-5610.
49. Zeng YB, Yang N, Sun H: **Metal-binding properties of an Hpn-like histidine-rich protein**. *Chemistry* 2011, **17**:5852-5860.
50. Wegner SV, Ertem E, Sunbul M, He C: **Metal-binding properties of Hpn from *Helicobacter pylori* and implications for the therapeutic activity of bismuth**. *Chem Sci* 2011, **2**:451-456.
Histidine-rich protein Hpn from the pathogen was converted to a FRET-based fluorescent probe to help to find the mechanism of action of the metallo-drug. Using *E. coli* as a model system, significant amounts of Bi³⁺ but not Ni²⁺ was found, indicating that Hpn is a potential target of bismuth drug.
51. Cun S, Li H, Ge R, Lin MC, Sun H: **A histidine-rich 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**. *J Biol Chem* 2008, **283**:15142-15151.
52. Cun S, Sun H: **A zinc-binding site by negative selection induces metallo-drug susceptibility in an essential chaperonin**. *Proc Natl Acad Sci USA* 2010, **107**:4943-4948.
HspA, a form of GroES found in *H. pylori* uniquely has one histidine and two cysteines at particular positions of its N-terminus. These residues (H45, C51 and C53), evolved from negative selection, form an oxidation-sensitive

Zn-binding site, and importantly bismuth antiulcer drugs displace Zn^{2+} from this site, disrupting the protein's structure.

53. Chen Z, Zhou Q, Ge R: **Inhibition of fumarase by bismuth (III): implications for the tricarboxylic acid cycle as a potential target of bismuth drugs in *Helicobacter pylori***. *Biometals* 2012, **25**:95-102.
54. Kozłowski H, Rowinska-Zyrek M, Witkowska D, Valensin D, Kamysz W: **The C terminus of HspA-a potential target for native Ni(II) and Bi(III) anti-ulcer drugs**. *Dalton Trans* 2010, **39**:5814-5826.
55. Kondo Y, Himeno S, Satoh M, Naganuma A, Nishimura T, Imura N: **Citrate enhances the protective effect of orally administered bismuth subnitrate against the nephrotoxicity of cis-diamminedichloroplatinum**. *Cancer Chemother Pharmacol* 2004, **53**:33-38.
56. Tsang CN, Bianga J, Sun H, Szpunar J, Lobinski R: **Probing of bismuth antiulcer drug targets in *H. pylori* by laser ablation-inductively coupled plasma mass spectrometry**. *Metallomics* 2012 doi: [10.1039/C2MT00169A](https://doi.org/10.1039/C2MT00169A).