

Metallomics in environmental and health related research: Current status and perspectives

HU LiGang^{1*}, HE Bin^{1,2}, WANG YuChuan¹, JIANG GuiBin² & SUN HongZhe^{1*}

¹ Department of Chemistry, the University of Hong Kong, Pokfulam Road, Hong Kong, China;

² State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

Received July 17, 2012; accepted September 6, 2012; published online September 30, 2012

Metals and metalloids play distinct roles in human health, either beneficial or toxic, depending on their concentrations and species. There is an increasing interest in metals uptake, trafficking, function, and exertion in microorganisms to maintain and advance human health. Metallomics, an emerging research area, focuses on elucidation of metals/metalloids location, distribution, speciation, and behavior in living organisms. This paper briefly summarized the recent progress on the methodology development of metallomics including various techniques, i.e. multiple dimensional liquid chromatography-inductively coupled plasma mass spectrometry (LC-ICPMS), gel electrophoresis-laser ablation-inductively coupled plasma mass spectrometry (GE-LA-ICPMS), synchrotron X-ray fluorescent spectroscopy (XFS), and the applications of metallomics in environmental and health care.

metallomics, environmental, health, metal

Citation: Hu L G, He B, Wang Y C, et al. Metallomics in environmental and health related research: Current status and perspectives. *Chin Sci Bull*, 2013, 58: 169–176, doi: 10.1007/s11434-012-5496-1

Metals (and metalloids) play crucial roles to lives. Currently, about twenty-eight elements are considered as essential or beneficial components, regulating a great number of biological processes and sustaining a healthy functional environment in living organisms. For example, copper is an essential trace element, serving as a cofactor in many redox enzymes, i.e., cytochrome c oxidase, that influence respiratory electron transport chain of mitochondria [1]. Selenium is an essential micronutrient for humans, usually incorporated into antioxidant enzymes such as selenocysteine, and plays a key role in host oxidative defense [2]. However, majority of the known metals and metalloids are potentially toxic to living organisms including those essential metals if not being regulated properly. Either metal deficiency or excess will exert damaging effects and cause a range of human diseases [3,4]. Iron deficiency is the most common nutritional deficiency leading to iron deficiency anemia;

dietary calcium deficiency can deplete calcium stores in the bones and poses potential risk for osteoporosis. Elevated levels of metals such as copper, zinc and iron are usually found in amyloid- β plaques within the brain of Alzheimer's disease, playing a role in the aggregation of A β peptides and the formation of reactive oxygen species [5]. Metal ion regulation can even be carried out at extremely low concentration, i.e., zinc concentrations in cells are tightly regulated at the picomolar to femtomolar level [6]. The oxidation state of a metal or metalloid may also determine whether it acts as a physiologically required or toxic element. Chromium is probably a well-known example, i.e., chromium(VI) is the most toxic form of chromium which classified as a carcinogen, whereas chromium(III) is a beneficial element required in trace amounts for glucose and lipid metabolism [7]. Moreover, the accumulation of toxic metals and metalloids in the environment naturally or anthropogenically pose significant potential risks on human health. Lead exposure is particularly influential to children, causing potentially

*Corresponding authors (email: hsun@hku.hk; liganghu@hku.hk)

permanent learning and behavior disorders [8]; whereas mercury toxicity mainly exerts damage to human kidney, lungs and central nervous system, mediated by different forms of mercury (i.e., organic mercuric compounds and inorganic forms) [9]. Therefore, systematic studies on the metal uptake, trafficking, and function in many basic and complex biological processes, will help us to understand the molecular mechanisms underlying the beneficial or toxic effects exerted by a given metal or metalloid, and further clarify the impact of metals/metalloids on human health.

The concept of metallome was firstly coined by Williams in 2001 [10,11], referring to an element distribution or a free element content in a cellular compartment or a whole cell, comparable to the genome or proteome characteristic for a living organism. The term was subsequently extended to the entirety of metal and metalloid species present in a cell or tissue type, described according to their identity, quantity and localization [12]. Characterization of the metallome, as well as its functional connections with the genome, transcriptome, proteome and metabolome was referred to as metallomics. As a complementary to genomics and proteomics, metallomics is an interdisciplinary area combining analytical, inorganic and biochemical studies, with an ultimate goal to elucidate the metal uptake, trafficking, accumulation and metabolism in biological systems [13,14]. Metalloproteomics is a new subset of proteomics focusing on the structural and functional characterization of all metalloproteins in proteome wide [15–17]. The specificity of metalloproteomics studies involves the description of the metal-binding sites, metal stoichiometry, metal-dependent conformation as well as the identification and quantification of the metalloproteins in a living organism [17]. Owing to the overlap of the research contents between metalloproteomics and metallomics, the former could be generally regarded as part of the metallomics study. Compared with genomics and proteomics, the metallomics community has not built up a robust and high-throughput experimental platform that could be widely accepted and utilized, which to some extent restricted the rapid development of this emerging field. To report the latest progress and state-of-the-art techniques for metallomics study, the journal “*Metallomics*” was initiated in 2009 by the Royal Society of Chemistry. This review is focused on recent advances in the methodologies used in this field, as well as recent applications of metallomics in the environmental and health related research. Relevant reviews in this field, either general [13,17–22] or more specific such as method-oriented [23–30], bioinformatics [16,31–34] and structure-related [35–37], are available elsewhere for readers.

1 Experimental approaches to metallomics

As an emerging research field, there is still lack of a well-established analytical platform to systematically decipher

the metallome and characterize the landscape of metalloproteins in a living organism. Typical proteomics strategies have been successfully employed in metalloproteomics. The continuous development of techniques by hyphenating a high resolution separating technique (e.g., HPLC, gel electrophoresis) with a high sensitive detection (elemental or molecular mass spectrometry) has led to new possibilities in these research fields [12]. To realize a comprehensive study of metalloproteins, complementary detection systems are necessary to obtain parallel information of the metal/metalloid associated with the organic moiety. Inductively coupled plasma mass spectrometry (ICP-MS) is the most commonly used approach to solve such challenges, and always performed in combination with different separation techniques [38]. Moreover, there is an increasing interest in the use of nuclear analytical techniques to study a number of basic issues in metalloproteomics. Neutron activation analysis, synchrotron radiation X-ray fluorescence (XRF) and isotopic tracer technique have significantly facilitated metalloprotein identification and characterization [27,39]. Metal distribution and localization in individual cells or cellular compartments can also be determined by SR-XRF microprobe analysis [40,41]. For the structural characterization of metalloproteins, Mössbauer spectrometry and X-ray absorption (XAS) are promising techniques to characterize the local structure of protein-associated metal ions [42,43]. Computational bioinformatics analysis, a complementary approach to experimental methods, can predict metal-binding proteins based on known consensus metal-binding pattern or metal-binding domain on a genome-wide scale [16]. Integrated applications of these techniques provide enormous possibilities to further investigate the function of the metallomes associated with human health.

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

As stated in some recent reviews [22,44], the combination

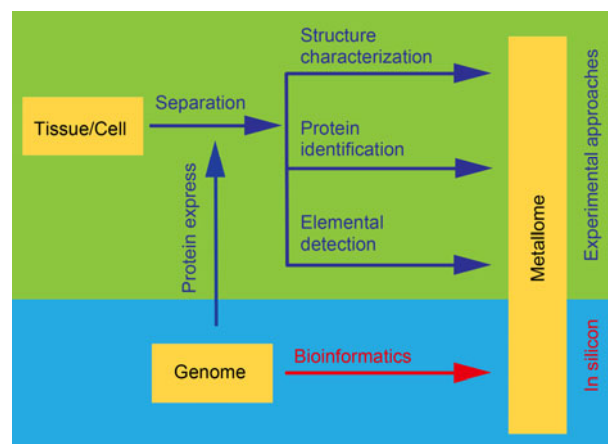


Figure 1 (Color online) Current approaches in metallomics.

of multidimensional LC and mass spectrometry is still a relatively mature and convincing technique now being widely applied to large-scale metalloprotein separation and characterization. Promising data on either all-element analysis of salmon egg cells [45] or exploration for full metalloproteome in bacterial cells [46] further confirmed the feasibility of this technique. However, it is worth noticing that different from the LC separation in normal proteomic research, metalloprotein separation generally requires a combination of ion-exchange and size-exclusion chromatography, rather than the combination of ion-exchange and reversed phase chromatograph. The application of size-exclusion chromatography would well preserve the non-covalent binding between metals and their associated proteins, and reduce the metal loss during separation under harsh conditions. Such a replacement would however sometimes compromise the separation capacity [47]. Currently, there is no systematic study on the stability of metal/protein complex during liquid chromatography separation, thus the metal losses from the complex or artificial gain/replacement during separation process remains to be investigated.

Apart from the above-mentioned chromatography methods, immobilized-metal affinity chromatography (IMAC) is a key technique commonly used to capture proteins with metal-binding ability, depending on their differential binding affinities of the surface exposed amino acids towards immobilized metal ion [48,49]. This approach could also efficiently enrich metal-binding proteins/peptides in order to systematically investigate the whole metalloproteome present in a biological sample, and has been used to identify hepatocellular proteins with copper-binding ability [50], Ni-interacting proteins in human B cells [51], copper-binding proteins in *Arabidopsis* roots [52], and the metalloproteins being targeted by bismuth in *Helicobacter pylori* [53]. As a bottom-up technique, IMAC is a promising tool for rapidly capturing proteins that are involved in metal sensing and trafficking, but it may not identify all proteins in a given metalloproteome. Proteins with a high metal affinity site will very likely pass through the column undetected as the metal sites are already occupied; or metalloenzymes with the metal-binding sites buried in the interior are not readily accessible for interaction with the immobilized metal ions [15,49]. The other drawback is that IMAC provides information on the presence of metal-binding sites in proteins but it may not be specific for physiologically active metal binding [54]. Therefore, great attention must be taken when interpreting the results from IMAC experiments to avoid premature conclusions.

Polyacrylamide gel electrophoresis (PAGE) employed either in the 1D (IEF, BN or SDS) or 2D mode (IEF-SDS) is considered the most adequate technique for protein separation, with the ability to improve the resolving power and reproducibility compared with liquid-based separation [55,56]. Metal-specific detection in the gels has enjoyed considerable interest for a long time, with recent applica-

tions referring to the analysis of Se-containing proteins in yeast cells on the 2D gel [57], metal imaging in non-denaturing 2D gels for the detection of metalloproteins from rat kidney [58] and Zn exchange by Cu in bovine serum albumin separated by 1D BN-PAGE gels [59]. However, the principle difficulty in its application for metalloprotein separation is also the requirement of the preservation of the integrity of metal-protein bond, especially in the commonly used 2D gel electrophoresis (IEF-SDS), which owns the advantage of simultaneously resolving thousands of proteins in a denatured separation procedure. Metal loss during the denatured separation seems inevitable due to application of the reducing agent, e.g. DL-dithiothreitol, and detergent used for improvement of gel resolution, as well as the high voltage used when performing IEF separation. Native PAGE (e.g. blue native-PAGE) can be performed in place of SDS-PAGE [58], but the resolution of the 2D separation would be significantly reduced and generate a great difficulty for the following protein identification. Therefore, the stability of metal-protein binding during gel separation may require some specific experimental evaluations. Another limitation in the use of PAGE techniques concerns the contamination issue. Many metal-protein complexes are labile and can be destroyed by exchange with the metal impurities in the gel during separation and staining [60]. Attention must be paid to avoid the presence of metal impurities in gels as well as in the buffer system especially when trace metal analysis was performed.

The subsequent detection techniques coupled to the above-mentioned separation systems are alike those high-quality bio-mass spectrometry applied to the traditional proteomics, varying slightly according to the analytical features which can be found in selected reviews [28,30,44]. For quantitative determination of trace metals in biological systems, ICP-MS and atomic spectrometry techniques are the best choices. ICP-MS, featuring high sensitivity and wide linear dynamic range, allows robust, accurate and multielemental detection for most metallic and metalloid elements [38,61]. By coupling with a front-end chromatography separation system, an on-line analysis could be performed for identifying and quantifying metal binding proteins in solution. For solid-based analysis, laser-ablation (LA)-ICP-MS, pioneered by Nielsen et al. [62], offers a competitive choice for *in situ* probing the protein spots with the presence of metals or metalloids on gels, by using a focused laser beam to ablate material from the surface of a solid sample [56]. LA-ICP-MS has been successfully applied to detect Se-containing proteins with a sensitivity of 6 pg/mm, promising the capability for quantitative measurement [63,64]. LA-ICP-MS offers a potentially fast and fairly robust technology, but the high cost of a powerful laser ablation system with sufficient spatial resolution restrained its application to a certain extent. Other atomic spectrometry techniques, e.g. atomic absorption spectrometry (AAS) [65] and atomic fluorescence spectrometry (AFS) [66,67] have been employed

in the early metalloproteomics studies, by digesting the separated protein spots on gels and inserting the resulting solution into the atomic analyzing system.

1.2 Application of nuclear analytical techniques in metallomics

Modern nuclear analytical techniques, including neutron activation analysis (NAA), synchrotron radiation X-ray fluorescence (SR-XRF), particle-induced X-ray emission (PIXE), have been applied extensively in metallomics for multielemental quantification and distribution analysis as well as for structural characterization of metallomes and metalloproteomes in a biological system, with their unique features of high sensitivity, noninvasive and less or none matrix effect [27,39]. NAA is a multielemental quantification technique with the ability to simultaneously measure more than thirty elements [68,69]. However, real time and on-line analysis are not achievable by this method, and the speed of the analysis is relatively slow [27]. The basic principle of XRF is that when materials are exposed to a high-energy radiation source, the component atoms will be irradiated. By detecting the fluorescent characteristic X-ray emitted by the excited atom, one could identify the specific element in a sample. The fluorescence peak is directly proportional to the concentration of the excited element, therefore XRF is a quantitative method that could provide a two/three-dimensional image of the metal distribution [24,27]. The early XRF technique was limited by its poor sensitivity and weakness in the determination of elements associated with protein spots [70]. Particle-induced X-ray emission spectrometry (PIXE) having a high-energy proton beam could provide a better analytical sensitivity [71]. Using synchrotron radiation (SR) as the exciting source can greatly improve the sensitivity and spatial resolution of XRF analysis. An absolute detection limit of 10^{-12} – 10^{-15} g and a relative detection limit as low as 10 ng/g can be achieved with only micrograms of sample required [19,27]. By combining gel electrophoresis with XRF, quantitative analysis of trace elements associated to proteins is possible and the combined technique possesses a great potential to facilitate metallomics and metalloproteomics research [23,24]. However, nuclear analytical techniques at the present stage can only be performed at off-line or quasi-on-line mode. In addition, the limited resources of synchrotron radiation sources have undoubtedly restrained the rapid development and widespread application of these techniques.

Besides chemical speciation analysis, nuclear analytical techniques can also be used in the structural characterization of metalloproteins. Techniques which are capable of offering a detailed structural information for the metal sites in a metalloprotein mainly include electron paramagnetic resonance (EPR), Mössbauer spectroscopy, and X-ray absorption spectrometry (XAS) [27]. These techniques are also applied to the traditional protein structural characterization. Here we

will mainly focus on the X-ray absorption technique that concerns more with the metallomics study. XAS is a well-developed tool for determining local structure around certain atoms without the requirement of crystalline samples. High-throughput X-ray absorptions spectrometry (HTXAS) including extended X-ray absorption fine structure (EXAFS) and X-ray absorption near edge structure (XANES) can be used to characterize the electronic configuration, site symmetry and the coordination environment of the absorbing atom [17,27]. HTXAS is a promising approach to elucidate metal insertion, cluster assembly and reaction mechanisms for metalloproteins, in order to obtain proteome-wide knowledge of the active-site structures. Separation technologies developed for proteomics and structural genomics have been fully utilized in HTXAS. Two potential workflows for characterizing the metalloproteome from a given genome were proposed [72]. One way is to perform separation at the gene level. Individual gene products are generated by high-throughput cloning, expression and purification. XAS characterization is then performed on separated metalloprotein samples. The other way is to delay the separation step to the proteome stage. After expression of the entire proteome (either from microorganisms or cells of a given organ or tissue), individual metalloprotein samples are generated from separated protein fractions and then characterize by XAS. In order to create a pipeline from genome to metalloproteome, automation of rapid data collection and analysis of multiple low-concentration samples must be developed. In recent years, significant progress has been made in high-throughput sample detection [43], theoretical XAS application [73,74] as well as automated data analysis [75]. Further, XAS can present its full advantages through combination with other techniques in metallomics and metalloproteomics. For example, the combination of crystallographic information and XAS promises a powerful tool to characterize unusual metal coordination and to refine models of complex structures [76–78].

1.3 Bioinformatic approaches

Due to the lack of well-developed experimental platform aiming at deciphering the complete metalloproteome encoded by an organism, information provided by computational approaches is thus essential for a comprehensive understanding of the functional characterization of metals and metalloids in the whole living system [16]. Bioinformatics can give valuable support to experimental methods in an effort to accelerate the investigation of metalloproteomes. By means of bioinformatic approaches, prediction of metal-binding proteins mainly based on known metal-binding domains or metal-binding patterns in their sequences, which resources can be extracted from a number of available databases [79]; while prediction of a metal binding site is based on a known 3D structure [80] or on the low-resolution structural data with sequence information [81]. However,

the accurate prediction of metalloproteins is generally limited to an deficient understanding of the complicated determinants of metal-binding specificity in proteins rather than a limitation of informatics methods [82]. The nearly infinite combinations of amino acid sequence result in highly variable sequences at metal binding sites and make it difficult to identify reliable sequence motifs. In addition, factors other than a protein's primary sequence may determine the metal binding specificity of a protein [46,83], thus the identification of specific metal binding site on a given protein is inherently limited. Additionally, with the incorporation of datasets from individual biological systems, 3D metalloprotein structures and other non-sequence-based datasets, the predictive capacity of bioinformatic approaches will definitely continue to be improved.

2 Application of metallomics in environmental/health related research

2.1 *In situ* imaging of metals/metalloids in living cell and tissue

Identification and quantification of metals in cells and tissues or even cellular compartments under the native conditions is the first important step towards elucidation of metal trafficking and their function at molecular level [84], which *in situ* imaging of metals/metalloids provides the most straightforward and comprehensive pathway. For example, using synchrotron X-ray fluorescence mapped the mercury distribution in zebrafish larvae after treated with MeHg-L-cysteine. The mercury could be imaged in micrometer resolution. As shown in Figure 2 [41], it was observed that the mercury distribution is not homogenous in fish, and mainly accumulated in brain, gastrointestinal tract, and especially the eye lens. High resolution image of zebrafish eye section revealed that Hg accumulated preferentially on the outer single cell layer of fish eyes epithelial tissue, and this layer also contained high content of S but not Zn (Figure 3) [41]. The high accumulation mercury in the fish eye implied that mercury impaired visual process might be led by the direct

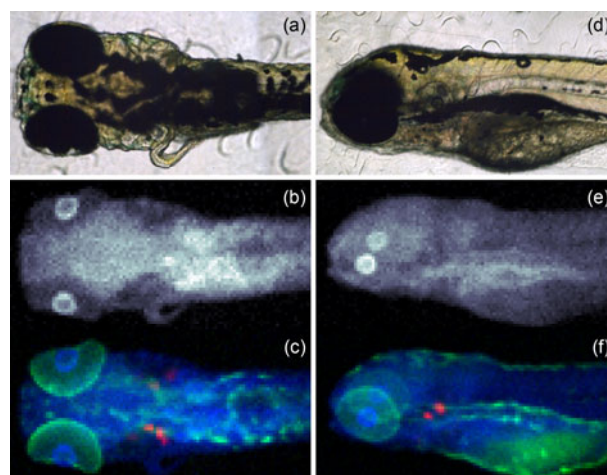


Figure 2 X-ray fluorescence images of MeHg-L-cysteine treated living zebrafish. (a),(d) were optical images; (b),(e) were images of mercury distribution; (c),(f) were the images of mercury (blue) merged with Zinc (green) and calcium (red). Reprinted with permission from [41]. Copyright (2008) National Academy of Sciences, USA.

effect on ocular tissue other than be originated from neurological effects. The imaging provided a straightforward way for investigation of metal toxicity on living cells and could screened out several leading targets for further study in detail. Currently, the XRF could provide the 3D image with spatial resolution up to 400 nm [85], which lateral might reach to tens of nanometers [86], and multiple elemental information, including not only metals/metalloids but also non-metals. In addition to metals/metalloids identification, the species of metals, i.e. chemical form and oxidative status, could also be characterized. For example, X-ray fluorescence imaging can be used to investigate arsenic adsorption on rice roots. The arsenic in different forms including arsenite [As(III)], arsenate [As(V)], arsenic triglutathione [As(GS)₃], and dimethylarsinic acid were observed (Figure 4) [87]. In addition to X-ray fluorescent imaging, others techniques, i.e. mass spectrometry imaging techniques (SIMS and LA-ICP-MS), magnetic resonance imaging, radioisotope imaging techniques and classical histochemical

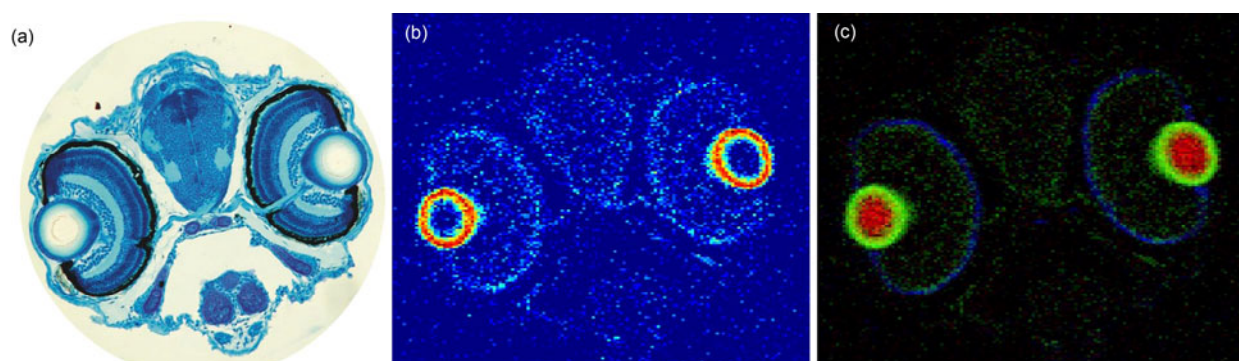


Figure 3 High resolution Hg, S, and Zn distribution of zebrafish head. (a) histological image, (b) mercury distribution at resolution of 2.5 μm , (c) merged image of Hg (green), S (red), and Zn (blue). Reprinted with permission from [41]. Copyright (2008) National Academy of Sciences, USA.

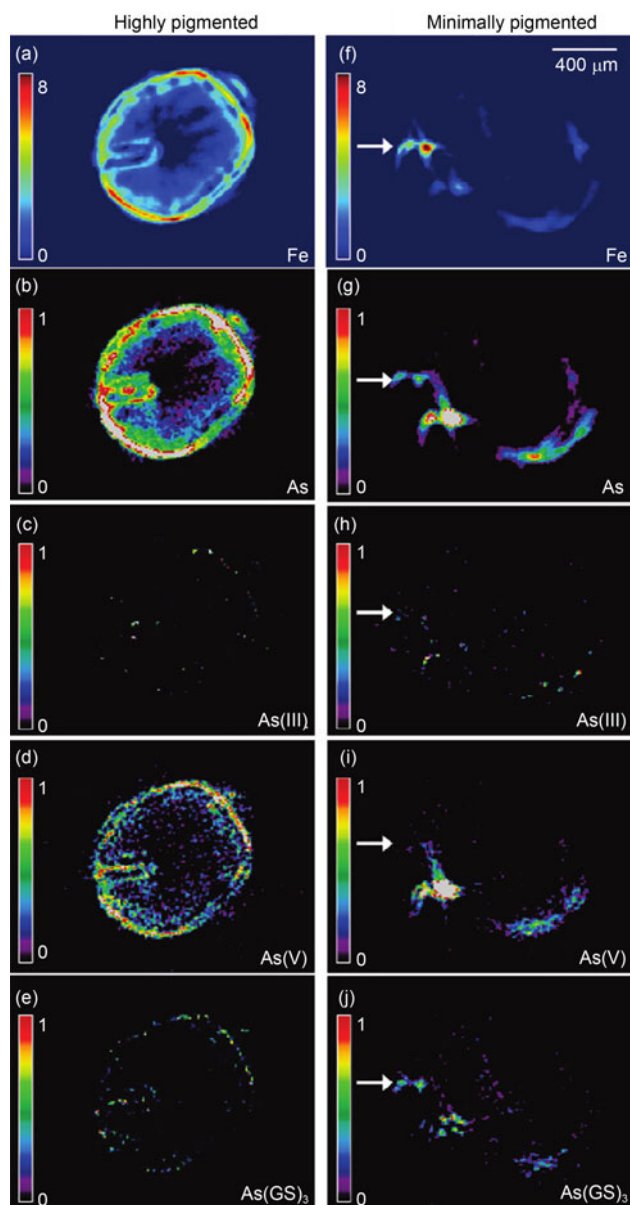


Figure 4 Cross-sectional image of rice roots obtained with X-ray fluorescence imaging showing iron, total arsenic, arsenite, arsenate and arsenic *tris*glutathione distribution. Reprinted with permission from [87]. Copyright (2010) American Chemical Society.

imaging techniques [84], could also be applied for *in situ* mapping metals in cells or tissues samples.

2.2 Profiling toxic metals associated proteins with metallomics approaches

Profiling metals associated proteins in proteome wide will facilitate elucidation of metals trafficking in biological systems and provide insight into molecular mechanisms of metals related biological function, although it is still a big challenge now. The strategies combining high resolution separation techniques and high sensitive element-specific detection i.e. multiple dimensional liquid chromatography-inductively

coupled plasma mass spectrometry/electrospray mass spectrometry, are predominant in recent studies. Limit reports have achieved the global analysis of metalloproteome in specific biological samples, for example investigation of metalloproteome of *Pyrococcus furiosus*, *Escherichia coli*, and *Sulfolobus solfataricus*, which clearly implied microbial metalloproteomes were largely unknown [46]. Most of works either monitored the metal contained proteins which however not characterized the proteins identity, or focused on particular proteins [45,88–90]. Uncharacterized insights into the behaviors and toxicity of metals were still provided. Wang et al investigated the metals contained proteins fractions in brain cytoplasm of mercury exposed rats and found that samples from maternal rats contained more mercury associated proteins and total mercury content than those from infant rats [91]. Multiple mercury contained protein peaks with molecular weight ranging from 12 to 300 kD also being monitored in cytoplasm from mercury treated salmon egg cells. Furthermore, these mercury containing peaks concurrently processed significant selenium and sulfur, indicating that mercury might binds the selenocysteine and/or cysteine residues in proteins [92]. For quantitative analysis of the metal containing proteins, post column isotope dilution provided a convenient and robust method for accurate evaluation their contents [93,94].

3 Perspectives

Recent results on metal related researches either on globally profiling the metallome in microbes or systematically elucidating the molecular mechanism of metal/metalloid based therapeutic agent revealed a promising future on metallomics [46,95,96], which also implied the hint of trends in both methodological development and functional studies. In current status, building up a robust experimental platform possessing high-throughput and high sensitivity of detection/ identification on metals/metalloids and associated biomolecules, is the primary task. In addition, experimentally based strategies in combination with bioinformatics will be a promising way to efficiently carry out metallomic studies.

This work was supported by the General Research Fund, the NSFC/RGC Joint Research Scheme of the Research Grants Council of Hong Kong (HKU7038/08P, HKU7049/09P and N_HKU75209), and the University of Hong Kong.

- 1 Cobine P A, Pierrel F, Winge D R. Copper trafficking to the mitochondrion and assembly of copper metalloenzymes. *Biochim Biophys Acta*, 2006, 1763: 759–772
- 2 Kryukov G V, Castellano S, Novoselov S V, et al. Characterization of mammalian selenoproteomes. *Science*, 2003, 300: 1439–1443
- 3 Chen P R, He C. Selective recognition of metal ions by metalloregulatory proteins. *Curr Opin Chem Biol*, 2008, 12: 214–221
- 4 Martinez-Finley E J, Chakraborty S, Fretham S J, et al. Cellular transport and homeostasis of essential and nonessential metals. *Metallomics*, 2012, 4: 593–605

- 5 Pithadia A S, Lim M H. Metal-associated amyloid-beta species in Alzheimer's disease. *Curr Opin Chem Biol*, 2012, 16: 67–73
- 6 Outten C E, O'Halloran T V. Femtomolar sensitivity of metalloregulatory proteins controlling zinc homeostasis. *Science*, 2001, 292: 2488–2492
- 7 Krzysik M, Grajeta H, Prescha A, et al. Effect of cellulose, pectin and chromium(III) on lipid and carbohydrate metabolism in rats. *J Trace Elem Med Biol*, 2011, 25: 97–102
- 8 Murata K, Iwata T, Dakeishi M, et al. Lead toxicity: Does the critical level of lead resulting in adverse effects differ between adults and children. *J Occup Health*, 2009, 51: 1–12
- 9 Carvalho C M, Chew E H, Hashemy S I, et al. Inhibition of the human thioredoxin system. A molecular mechanism of mercury toxicity. *J Biol Chem*, 2008, 283: 11913–11923
- 10 Williams R J P. Chemical selection of elements by cells. *Coord Chem Rev*, 2001, 216–217: 583–595
- 11 Frausto da Silva J J R, Williams R J P. *The Biological Chemistry of The Elements: The Inorganic Chemistry of Life*. 2nd ed. Oxford: Oxford University Press, 2001
- 12 Mounicou S, Szpunar J, Lobinski R. Metallomics: The concept and methodology. *Chem Soc Rev*, 2009, 38: 1119–1138
- 13 Sun H, Chai Z F. Metallomics: An integrated science for metals in biology and medicine. *Annu Rep Prog Chem, Sect A: Inorg Chem*, 2010, 106: 20–38
- 14 Ge R G, Sun H. Metallomics: An integrated biometal science. *Sci China Ser B: Chem*, 2009, 52: 2055–2070
- 15 Shi W, Chance M R. Metalloproteomics: Forward and reverse approaches in metalloprotein structural and functional characterization. *Curr Opin Chem Biol*, 2011, 15: 144–148
- 16 Andreini C, Bertini I, Rosato A. Metalloproteomes: A bioinformatic approach. *Acc Chem Res*, 2009, 42: 1471–1479
- 17 Shi W, Chance M R. Metallomics and metalloproteomics. *Cell Mol Life Sci*, 2008, 65: 3040–3048
- 18 Maret W. Metalloproteomics, metalloproteomes, and the annotation of metalloproteins. *Metallomics*, 2010, 2: 117–125
- 19 Lobinski R, Moulin C, Ortega R. Imaging and speciation of trace elements in biological environment. *Biochimie*, 2006, 88: 1591–1604
- 20 Bettmer J. Metalloproteomics: A challenge for analytical chemists. *Anal Bioanal Chem*, 2005, 383: 370–371
- 21 Szpunar J. Metallomics: A new frontier in analytical chemistry. *Anal Bioanal Chem*, 2004, 378: 54–56
- 22 Haraguchi H. Metallomics as integrated biometal science. *J Anal At Spectrom*, 2004, 19: 5–14
- 23 Finney L, Chishti Y, Khare T, et al. Imaging mass in proteins by combining electrophoresis with rapid X-ray fluorescence mapping. *ACS Chem Biol*, 2010, 5: 577–587
- 24 Ortega R. Synchrotron radiation for direct analysis of metalloproteins on electrophoresis gels. *Metallomics*, 2009, 1: 137–141
- 25 McRae R, Bagchi P, Sumalekshmy S, et al. *In situ* imaging of metals in cells and tissues. *Chem Rev*, 2009, 109: 4870–4827
- 26 Becker J S, Jakubowski N. The synergy of elemental and biomolecular mass spectrometry: New analytical strategies in life sciences. *Chem Soc Rev*, 2009, 38: 1969–1983
- 27 Gao Y, Chen C, Chai Z F. Advanced nuclear analytical techniques for metalloproteomics. *J Anal At Spectrom*, 2007, 22: 856–866
- 28 Szpunar J. Advances in analytical methodology for bioinorganic speciation analysis: Metallomics, metalloproteomics and heteroatom-tagged proteomics and metabolomics. *Analyst*, 2005, 130: 442–465
- 29 Prange A, Profrock D. Application of CE-ICP-MS and CE-ESI-MS in metalloproteomics: Challenges, developments, and limitations. *Anal Bioanal Chem*, 2005, 383: 372–389
- 30 Lobinski R, Schaumloffel D, Szpunar J. Mass spectrometry in bioinorganic analytical chemistry. *Mass Spectrom Rev*, 2006, 25: 255–289
- 31 Bertini I, Cavallaro G. Bioinformatics in bioinorganic chemistry. *Metallomics*, 2010, 2: 39–51
- 32 Andreini C, Banci L, Bertini I, et al. Occurrence of copper proteins through the three domains of life: A bioinformatic approach. *J Proteome Res*, 2008, 7: 209–216
- 33 Bertini I, Rosato A. From genes to metalloproteins: A bioinformatic approach. *Eur J Inorg Chem*, 2007, 2007: 2546–2555
- 34 Shi W, Zhan C, Ignatov A, et al. Metalloproteomics: High-throughput structural and functional annotation of proteins in structural genomics. *Structure*, 2005, 13: 1473–1486
- 35 Ma Z, Jacobsen F E, Giedroc D P. Coordination chemistry of bacterial metal transport and sensing. *Chem Rev*, 2009, 109: 4644–4681
- 36 Maret W, Li Y. Coordination dynamics of zinc in proteins. *Chem Rev*, 2009, 109: 4682–4707
- 37 Boal A K, Rosenzweig A C. Structural biology of copper trafficking. *Chem Rev*, 2009, 109: 4760–4779
- 38 Sanz-Medel A. ICP-MS for multiplex absolute determinations of proteins. *Anal Bioanal Chem*, 2010, 398: 1853–1859
- 39 Chen C, Chai Z F, Gao Y. *Nuclear Analytical Techniques for Metallomics and Metalloproteomics*. Cambridge: Royal Society of Chemistry, 2010
- 40 Kim A M, Vogt S, O'Halloran T V, et al. Zinc availability regulates exit from meiosis in maturing mammalian oocytes. *Nat Chem Biol*, 2010, 6: 674–681
- 41 Korbas M, Blechinger S R, Krone P H, et al. Localizing organomercury uptake and accumulation in zebrafish larvae at the tissue and cellular level. *Proc Natl Acad Sci USA*, 2008, 105: 12108–12112
- 42 Paulsen H, Schunemann V, Trautwein A X, et al. Mossbauer studies of coordination compounds using synchrotron radiation. *Coord Chem Rev*, 2005, 249: 255–272
- 43 Ascone I, Strange R. Biological X-ray absorption spectroscopy and metalloproteomics. *J Synchrotron Radiat*, 2009, 16: 413–421
- 44 Mounicou S, Lobinski R. Challenges to metallomics and analytical chemistry solutions. *Pure Appl Chem*, 2008, 80: 2565–2575
- 45 Haraguchi H, Ishii A, Hasegawa T, et al. Metallomics study on all-elements analysis of salmon egg cells and fractionation analysis of metals in cell cytoplasm. *Pure Appl Chem*, 2008, 80: 2595–2608
- 46 Cvetkovic A, Menon A L, Thorgersen M P, et al. Microbial metalloproteomes are largely uncharacterized. *Nature*, 2010, 466: 779–782
- 47 Jakubowski N, Lobinski R, Moens L. Metallobiomolecules: The basis of life, the challenge of atomic spectroscopy. *J Anal At Spectrom*, 2004, 19: 1–4
- 48 She Y M, Narindrasorasak S, Yang S, et al. Identification of metal-binding proteins in human hepatoma lines by immobilized metal affinity chromatography and mass spectrometry. *Mol Cell Proteomics*, 2003, 2: 1306–1318
- 49 Barnett J P, Scanlan D J, Blindauer C A. Protein fractionation and detection for metalloproteomics: Challenges and approaches. *Anal Bioanal Chem*, 2012, 402: 3311–3322
- 50 Smith S D, She Y M, Roberts E A, et al. Using immobilized metal affinity chromatography, two-dimensional electrophoresis and mass spectrometry to identify hepatocellular proteins with copper-binding ability. *J Proteome Res*, 2004, 3: 834–840
- 51 Heiss K, Junkes C, Guerreiro N, et al. Subproteomic analysis of metal-interacting proteins in human B cells. *Proteomics*, 2005, 5: 3614–3622
- 52 Kung C C, Huang W N, Huang Y C, et al. Proteomic survey of copper-binding proteins in Arabidopsis roots by immobilized metal affinity chromatography and mass spectrometry. *Proteomics*, 2006, 6: 2746–2758
- 53 Ge R, Sun X, Gu Q, et al. A proteomic approach for the identification of bismuth-binding proteins in *Helicobacter pylori*. *J Biol Inorg Chem*, 2007, 12: 831–842
- 54 Wheeler K E, Erickson B K, Mueller R, et al. Metal affinity enrichment increases the range and depth of proteome identification for extracellular microbial proteins. *J Proteome Res*, 2012, 11: 861–870
- 55 Pioselli B, Munro C, Raab A, et al. Denaturing and non-denaturing microsolution isoelectric focussing to mine the metalloproteome. *Metallomics*, 2009, 1: 501–510
- 56 Sussulini A, Becker JS. Combination of PAGE and LA-ICP-MS as an analytical workflow in metallomics: State of the art, new quantification strategies, advantages and limitations. *Metallomics*, 2011, 3: 1271–1279

- 57 Chassaing H, Chery C C, Bordin G, et al. 2-Dimensional gel electrophoresis technique for yeast selenium-containing proteins-sample preparation and MS approaches for processing 2-D gel protein spots. *J Anal At Spectrom*, 2004, 19: 85–95
- 58 Becker J S, Lobinski R, Becker J S. Metal imaging in non-denaturing 2D electrophoresis gels by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) for the detection of metalloproteins. *Metallomics*, 2009, 1: 312–316
- 59 Becker J S, Pozebon D, Dressler V L, et al. LA-ICP-MS studies of zinc exchange by copper in bovine serum albumin using an isotopic enriched copper tracer. *J Anal At Spectrom*, 2008, 23: 1076–1082
- 60 Chéry C C, Moens L, Cornelis R, et al. Capabilities and limitations of gel electrophoresis for elemental speciation: A laboratory's experience. *Pure Appl Chem*, 2006, 78: 91–103
- 61 Li Y F, Chen C, Qu Y, et al. *Metallomics*, *elementomics*, and analytical techniques. *Pure Appl Chem*, 2008, 80: 2577–2594
- 62 Neilsen J L, Abildtrup A, Christensen J, et al. Laser ablation inductively coupled plasma-mass spectrometry in combination with gel electrophoresis: A new strategy for speciation of metal binding serum proteins. *Spectrochim Acta Part B*, 1998, 53: 339–345
- 63 Claverie F, Pécheyran C, Mounicou S, et al. Characterization of the aerosol produced by infrared femtosecond laser ablation of polyacrylamide gels for the sensitive inductively coupled plasma mass spectrometry detection of selenoproteins. *Spectrochim Acta Part B*, 2009, 64: 649–658
- 64 Ballihaut G, Claverie F, Pecheyran C, et al. Sensitive detection of selenoproteins in gel electrophoresis by high repetition rate femtosecond laser ablation-inductively coupled plasma mass spectrometry. *Anal Chem*, 2007, 79: 6874–6880
- 65 Sidenius U, Gammelgaard B. Direct determination of selenoproteins in polyvinylidene difluoride membranes by electrothermal atomic absorption spectrometry. *Fresenius J Anal Chem*, 2000, 367: 96–98
- 66 Chen C, Zhang P, Hou X, et al. Subcellular distribution of selenium and Se-containing proteins in human liver. *Biochim Biophys Acta*, 1999, 1427: 205–215
- 67 Chen C, Zhao J, Zhang P, et al. Speciation and subcellular location of se-containing proteins in human liver studied by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and hydride generation-atomic fluorescence spectrometric detection. *Anal Bioanal Chem*, 2002, 372: 426–430
- 68 Hou X, Chen C, Ding W, et al. Study on chemical species of iodine in human liver. *Biol Trace Elem Res*, 1999, 69: 69–76
- 69 Chen C, Zhang P, Chai Z F. Distribution of some rare earth elements and their binding species with proteins in human liver studied by instrumental neutron activation analysis combined with biochemical techniques. *Anal Chim Acta*, 2001, 439: 19–27
- 70 Stone S F, Bernasconi G, Haselberger N, et al. Detection and determination of selenoproteins by nuclear techniques. *Biol Trace Elem Res*, 1994, 43–45: 299–307
- 71 Szökefalvi-Nagy Z, Bagyinka C, Demeter I, et al. Speciation of metal ions in proteins by combining PIXE and thin layer electrophoresis. *Fresenius J Anal Chem*, 1999, 363: 469–473
- 72 Scott R A, Shokes J E, Cosper N J, et al. Bottlenecks and roadblocks in high-throughput XAS for structural genomics. *J Synchrotron Radiat*, 2005, 12: 19–22
- 73 Krüger P, Natoli C. X-ray absorption spectra at the Ca L_{2,3} edge calculated within multichannel multiple scattering theory. *Phys Rev B*, 2004, 70: 245120
- 74 Hatada K, Hayakawa K, Benfatto M, et al. Full-potential multiple scattering for X-ray spectroscopies. *Phys Rev B*, 2007, 76: 060102
- 75 Wellenreuther G, Parthasarathy V, Meyer-Klaucke W. Towards a black-box for biological EXAFS data analysis. II. Automatic BioXAS Refinement and Analysis (ABRA). *J Synchrotron Radiat*, 2010, 17: 25–35
- 76 Hasnain S S, Hodgson K O. Structure of metal centres in proteins at subatomic resolution. *J Synchrotron Radiat*, 1999, 6: 852–864
- 77 Corbett M C, Hu Y, Fay A W, et al. Structural insights into a protein-bound iron-molybdenum cofactor precursor. *Proc Natl Acad Sci USA*, 2006, 103: 1238–1243
- 78 Loftin I R, Franke S, Blackburn N J, et al. Unusual Cu(I)/Ag(I) coordination of *Escherichia coli* CusF as revealed by atomic resolution crystallography and X-ray absorption spectroscopy. *Protein Sci*, 2007, 16: 2287–2293
- 79 Finn R D, Mistry J, Schuster-Bockler B, et al. Pfam: Clans, web tools and services. *Nucleic Acids Res*, 2006, 34: D247–251
- 80 Schymkowitz J W, Rousseau F, Martins I C, et al. Prediction of water and metal binding sites and their affinities by using the Fold-X force field. *Proc Natl Acad Sci USA*, 2005, 102: 10147–10152
- 81 Sodhi J S, Bryson K, McGuffin L J, et al. Predicting metal-binding site residues in low-resolution structural models. *J Mol Biol*, 2004, 342: 307–320
- 82 Yannone S M, Hartung S, Menon A L, et al. Metals in biology: Defining metalloproteomes. *Curr Opin Biotechnol*, 2012, 23: 89–95
- 83 Waldron K J, Rutherford J C, Ford D, et al. Metalloproteins and metal sensing. *Nature*, 2009, 460: 823–830
- 84 McRae R, Bagchi P, Sumalekshmy S, et al. *In situ* imaging of metals in cells and tissues. *Chem Rev*, 2009, 109: 4780–4827
- 85 de Jonge M D, Holzner C, Baines S B, et al. Quantitative 3D elemental microtomography of *Cyclotella meneghiniana* at 400-nm resolution. *Proc Natl Acad Sci USA*, 2010, 107: 15676–15680
- 86 Lombi E, de Jonge M D, Donner E, et al. Trends in hard X-ray fluorescence mapping: Environmental applications in the age of fast detectors. *Anal Bioanal Chem*, 2011, 400: 1637–1644
- 87 Seyffarth A L, Webb S M, Andrews J C, et al. Arsenic localization, speciation, and co-occurrence with iron on rice (*Oryza sativa* L.) roots having variable Fe coatings. *Environ Sci Technol*, 2010, 44: 8108–8113
- 88 Feng W Y, Meng W, Ming G A, et al. Mercury speciation and mercury-binding protein study by HPLC-ICP-MS on the estimation of mercury toxicity between maternal and infant rats. *J Anal At Spectrom*, 2011, 26: 156–164
- 89 Maltez H F, Tagle M V, de la Campa M D F, et al. Metal-metallothioneins like proteins investigation by heteroatom-tagged proteomics in two different snails as possible sentinel organisms of metal contamination in freshwater ecosystems. *Anal Chim Acta*, 2009, 650: 234–240
- 90 Gonzalez-Fernandez M, Garcia-Barrera T, Arias-Borrego A, et al. Metal-binding molecules in the organs of *Mus musculus* by size-exclusion chromatography coupled with UV spectroscopy and ICP-MS. *Anal Bioanal Chem*, 2008, 390: 17–28
- 91 Wang M, Feng W Y, Wang H J, et al. Analysis of mercury-containing protein fractions in brain cytosol of the maternal and infant rats after exposure to a low-dose of methylmercury by SEC coupled to isotope dilution ICP-MS. *J Anal At Spectrom*, 2008, 23: 1112–1116
- 92 Hasegawa T, Asano M, Takatani K, et al. Speciation of mercury in salmon egg cell cytoplasm in relation with metallomics research. *Talanta*, 2005, 68: 465–469
- 93 Li Y F, Hu L A, Li B, et al. Full quantification of selenium species by RP and AF-ICP-qMS with on-line isotope dilution in serum samples from mercury-exposed people supplemented with selenium-enriched yeast. *J Anal At Spectrom*, 2011, 26: 224–229
- 94 Shi J W, Feng W Y, Wang M, et al. Investigation of mercury-containing proteins by enriched stable isotopic tracer and size-exclusion chromatography hyphenated to inductively coupled plasma-isotope dilution mass spectrometry. *Anal Chim Acta*, 2007, 583: 84–91
- 95 Zhang X W, Yan X J, Zhou Z R, et al. Arsenic trioxide controls the fate of the PML-RAR alpha oncoprotein by directly binding PML. *Science*, 2010, 328: 240–243
- 96 Sun X S, Tsang C N, Sun H. Identification and characterization of metalloprotein binding proteins by (metallo)proteomics. *Metallomics*, 2009, 1: 25–31