Metallomics

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Metallomics: the history over the last decade and a future outlook†

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In 2004, the term "metallomics" was coined to describe integrated biometal science (H. Haraguchi, Metallomics as Integrated Biometal Science, *J. Anal. At. Spectrom.*, 2004, **19**, 5–14). Around 10 years have passed since then, and the history of metallomics over the last decade is reviewed here, discussing the development of metallomics before and after the proposal. Furthermore, the future outlook of metallomics research will be considered, in terms of topics such as the organization of platforms for metallomics research related to trace metal sciences, a simplified model of the biological system and *omics*-sciences, research subjects in metallomics, recent trends of metallomics research, and the challenge of single biological cell analysis.

Introduction

Since the 1970s, ICP-AES and ICP-MS have been developed as highly sensitive analytical methods with excellent feasibility for simultaneous multielement detection.[2–4] Therefore, these analytical methods have been extensively applied to the elemental analysis of biological samples such as blood, cells, organs, and organisms, as well as to geochemical, environmental, and industrial samples.[1–3] Furthermore, it should be stressed here that nowadays the simultaneous multielement detection capability of analytical plasma spectrometry, such as ICP-AES and ICP-MS, seems to allow the analysis of almost all elements in diverse samples. I thus proposed the concept of the "Extended All Present Theory of the Elements", from consideration of the distribution and circulation of the elements on Earth;[1,2] this is an extension of the hypothesis known as the "All Present Theory of the Elements" proposed by I. Noddack in the 1930s.[5,6] More recently, chemical speciation for the identification of chemical species in biological and environmental samples has also been explored as an important analytical strategy to elucidate the biological functions of trace metals.[1,7–10]

It should be mentioned here that genomics,[11,12] proteomics[13] and metabolomics,[14] as *omics*-sciences, have newly evolved in the life sciences and molecular cell biology since the late 1990s, and now these terms are commonly used to indicate the integrated biological sciences for genes, proteins and metabolites, respectively. In fact, genes and proteins are the key biomaterials in the construction, regulation and/or maintenance of the life systems of animals, plants, and microorganisms. Even so, it should also be noted here that the biological functions and physiology of life systems cannot be maintained solely with genes and proteins; they also require the aid of various trace metals to express their functions. Thus, it was highly desirable to establish a new scientific field for biometals, which might be complementary to genomics and proteomics. On considering these research trends in the life sciences, the concept of "metallomics" came to mind; it was just like an inspiration.[1] While deliberating on the matter for several months, the conclusion was finally reached that a new concept for trace metal science should be proposed to establish metallomics as integrated biometal science, in which various independent fields in trace metal sciences and their communities would be integrated in order to cooperate with genomics, proteomics, and other *omics*-sciences. This was the reason that "metallomics as integrated biometal science" was proposed in 2004.[1]

In the following sections, firstly the historical processes for establishing metallomics as an academic scientific field in the last decade will be described in detail, and secondly an outlook of metallomics research will be considered for further development in the future.

The history of metallomics over the last decade

A challenge to pico-world science and metallomics: a new frontier of trace element chemistry

The title of this subsection was the title of the invited lecture given at the Tokushima Seminar on Chemical Engineering, held in Tokushima, Japan, on June 19, 2002.[15] It was the first time that I mentioned the idea of "metallomics" as a new scientific field. Since it was the seminal lecture on metallomics,

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the abstract of the lecture at the Tokushima Seminar is quoted here,[15] which is translated from Japanese into English.

In recent years, analytical detection sensitivities have been increasingly improved, to pico (10^{-12}) grams in absolute amounts, or sub-ppt (10^{-12} g ml^{-1}) in terms of concentration, due to the development of ICP-MS. As a result, we now have a good chance to challenge research into pico-technology or pico-science, which may be called ''Pico-World Science''.

Such progress in analytical atomic spectrometry will lead to another interesting and important area of research, that of bio-trace elements in biological systems, including ''human beings'', because all elements might be contained in all biological systems. This concept is referred to as the ''Extended All Present Theory of the Elements''. Furthermore, various trace elements play important roles in biological systems, as metalloproteins and/or metalloenzymes. Thus, now is a good time to challenge trace element biochemistry to open up our new scientific world: ''metallomics''.

As described in the Introduction, in the last few decades, various scientific fields such as analytical atomic spectrometry and *omics*-science as well as cell biology have progressed rapidly, and they have also had a great impact in promoting the study of metals in biology to a new stage.[16] Deliberating such advances in the biological sciences, it was thought that the study of metals in biology should be taken on as a challenge for future development. The term ''metallomics'' came to mind in the spring of 2002, with the suggestion that metallomics should be a new scientific field as one of the *omics*-sciences. As can be seen from the above abstract at the Tokushima Seminar, the first step in the approach towards metallomics was significantly influenced by the progress of analytical atomic spectrometry.

Trace element speciation for metallomics

The International Symposium on Bio-Trace Elements 2002 (BITREL 2002) was held in Woko, Saitama, Japan, as the Joint Symposium of RIKEN (Institute of Physical and Chemical Research) and YIES (Yamanashi Institute of Environmental Sciences) from October 28–November 2, and was co-organized by Shuichi Enomoto at RIKEN (he is now a Professor at Okayama University) and Yoshiyuki Seko at YIES. I delivered the invited lecture under the title of ''Trace Element Speciation for Metallomics'' at this symposium, in which metallomics was proposed as a new scientific term for the first time in English. The abstract of the invited lecture is shown below, which is quoted from the Proceedings of BITREL 2002.[17]

In this paper, ''metallomics'' is newly proposed as a new scientific field in order to integrate the research fields related to bio-trace metals. Metallomics might be the scientific field of post-genomics and post-proteomics, where metal-containing compounds are defined as metallomes, in a similar manner to genome in genomics and proteome in proteomics. Since the elucidation of the biological or physiological functions of metal-containing species in biological systems is the main research target of metallomics, elemental speciation is important as one of analytical technologies to promote metallomics.

As can be seen from the abstract above, the importance of speciation analysis (chemical speciation) for trace biometals in biological samples as well as in biological systems was especially emphasized in the lecture, because most bio-trace metals (actually existing as ionic forms in biological systems) are found in metalloproteins and/or metalloenzymes in biological systems. It is well known that various metal ions play essential roles as the active centers of metalloenzymes for the expression of their biological and physiological functions.[18] On the other hand, it is also known in environmental science and toxicology that some metals and metalloids are seriously toxic or hazardous to humans and living organisms, resulting in serious dysfunction of the biological systems. Thus, it should be understood that ''trace element chemistry'' is concerned with both natures of the elements: one essential and the other toxic, depending on their chemical forms.

At BITREL 2002, distinguished scientists working in the field of biometal science were invited from various countries; they were Ryszard Lobinski (Warsaw University of Technology, Warsaw, Poland; now CNRS, Pau, France), Zhifang Chai (Institute of High Energy Physics, Chinese Academy of Sciences, Beijing, China), Wolfgang Maret (Harvard Medical School, USA; now King's College London, UK), Joanna Szpunar (Group of Bioinorganic Analytical Chemistry, CNRS, Pau, France), Bibudhendra Sarkar (University of Toronto, Ontario, Canada), David Brown (University of Bath, UK) and so forth. At the symposium, I was the first speaker. Later, several participants gave their lectures using the term ''metalloproteomics'', because they were thinking that most heavy metals exist as metalloproteins in biological systems. After the session closed, a hot discussion about the new scientific term for biometal science followed among the participants both inside and outside of the conference room. Finally, the term ''metallomics'' seemed to be accepted by most participants.

There was an interesting story about the terminology for metallomics during BITREL 2002. It happened on the excursion bus to Hakone near Mount Fuji. Maret described the scenery of the excursion in his recent publication.[18]

Hiroki Haraguchi is credited with coining the word ''metallomics''. While travelling with highly inspired scientists on a high-spirited bus excursion to Mount Fuji in 2002, he envisioned metallomics as an integrative biometal science.

Metallomics as integrated biometal science

On the occasion of BITREL 2002, as discussed above, I was asked by Lobinski to submit a paper about metallomics to *J. Anal. At. Spectrom.*, published by the Royal Society of Chemistry (RSC), UK, because the journal was planning to publish a themed issue on ''Metals in Biology''[16] and he was one of the guest editors of the themed issue. The paper on metallomics as a new science was submitted to the editorial office of the RSC the following year, 2003. Titled ''Metallomics as Integrated Biometal Science'', the paper appeared in the January issue of *J. Anal. At. Spectrom.* in 2004,[1] being a summary of the lecture presented in BITREL 2002. Since this was an epoch-making article for metallomics, the whole abstract of the paper in *J. Anal. At. Spectrom.*[1] is quoted below.

In this paper, ''Metallomics'' is proposed as a new scientific field in order to integrate the research fields related to biometals.

Metallomics should be a scientific field in symbiosis with genomics and proteomics, because syntheses and metabolic functions of genes (DNA and RNA) and proteins cannot be performed without the aid of various metal ions and metalloenzymes. In metallomics, metalloproteins, metalloenzymes and other metal-containing biomolecules are defined as ''metallomes'', in a similar manner to genomes in genomics as well as proteomes in proteomics. Since the identification of metallomes and the elucidation of their biological or physiological functions in the biological systems is the main research target of metallomics, chemical speciation for specific identification of bioactive metallomes is one of the most important analytical technologies to establish metallomics as integrated biometal science. In order to rationalize the concept of metallomics, the distributions of the elements in man, human blood serum and sea-water, a challenge to all-elements analysis of one biological cell, and some other research topics are introduced with emphasis on recent development of chemical speciation of trace metals in some biological samples.

After this publication, metallomics was formally coined as a scientific field in 2004.

Creation of metallomics as a new scientific field

Since 2004, metallomics has been increasingly receiving great attention as a newly emerging scientific field,[19–22] and great progress has been achieved as one of the *omics*-sciences. The situation was the same in Japan.[23,24] Thus, in 2004, I applied for a research grant in the category of Grant-in-Aid of the Specially Promoted Research, supported by the Ministry of Education, Science, Culture and Sports of Japan. The title of the project was ''Creation of Metallomics as the New Scientific Field''. Fortunately, this was approved as a 3 year project (2004–2007). This project was evaluated highly by the Selection Committee of the Specially Promoted Research to establish a new scientific field for the life sciences. It was very fortunate and helpful that the original paper[1] had been published before the final meeting of the Selection Committee. In the grant proposal, the organization of the International Symposium on Metallomics was also scheduled, when the project would be terminated. The plan for the International Symposium was also appreciated by the Selection Committee.

International Symposium on Metallomics

In 2007, the International Symposium on Metallomics 2007 (ISM 2007) was organized by the Chemical Society of Japan, with the support of IUPAC and the Science Council of Japan, of which I was the chairman. It was held in Nagoya from November 28–December 1 as the first international symposium on metallomics. Professors Kazuo Suzuki, Hiromu Sakurai and Naoki Furuta were the vice-chairmen of the symposium. In this symposium, about 350 scientists participated and more than 250 lectures and posters were presented.[25]

At the International Advisory Board Meeting of ISM 2007, it was concluded that ISM 2007 was a successful meeting and that ''metallomics'' should be promoted as a newly emerging scientific field thereafter. Furthermore, it was also agreed in the advisory board meeting that the symposium would be held regularly, every 2 years, at different locations in the world. In addition, the Proceedings of ISM 2007 were published as a special issue of *Pure Appl. Chem.* (vol. 12, 2008) from IUPAC.[25,26] The publication of the proceedings was a requirement of IUPAC, because they had supported the ISM 2007 symposium.

The ISM meetings have been held five times in the following cities: Nagoya, Japan, in 2007; Cincinnati, USA, in 2009; Münster, Germany, in 2011; Oviedo, Spain, in 2013; and Beijing, China, 2015, as listed in Table 1. (It was sad that Prof. J. A. Caruso, who was the organizer of the second symposium and the first Editorial Board Chair of the journal *Metallomics*, passed away in November 2015.) The reports of these symposia were published in the RSC journal *Metallomics*.[27–30] These symposia were very helpful to envision the future of metallomics research and to accelerate the diffusion of metallomics into other scientific communities.

Metallomics: guidelines for terminology and critical evaluation of analytical chemistry approaches (IUPAC technical report)

Prof. Lobinski showed a great interest in metallomics following BITREL 2002, in which metallomics was proposed for the first time. On that occasion, he asked me to write a paper concerned with metallomics, as I have already mentioned. He also provided great help and contribution in obtaining support from IUPAC when ISM 2007 was organized. In those days, he was the President of Scientific Division V (Analytical Chemistry) on the IUPAC Committee. Taking advantage of his position in IUPAC, at that time, he also applied funding support from IUPAC as a three year project (2007–2009) to summarize the IUPAC Technical Report for Metallomics. The sub-committee members of the project were R. Lobinski (France), J. S. Becker (Germany), H. Haraguchi (Japan) and B. Sarkar (Canada). The IUPAC Technical Report entitled ''Metallomics: guidelines for terminology and critical evaluation of analytical chemistry approaches'', which is the title of this sub-section, was published in the IUPAC journal *Pure Appl. Chem.* in 2010.[31] In the report, the concept and terminology of metallomics and a critical evaluation of the analytical chemistry approach to metallomics were summarized as the IUPAC recommendation.

Table 1 A list of the meetings of the International Symposium on Metallomics (ISM); years, cities and organizers

Year	City, country	Organizers (Chairperson(s))
2007	Nagoya, Japan	H. Haraguchi, K. T. Suzuki, H. Sakurai, N. Furuta
2009	Cincinnati, USA	J. A. Caruso, G. M. Hieftje
2011	Münster, Germany	U. Karst, M. Sperling
2013	Oviedo, Spain	A. Sanz-Medel, M. Montes-Bayón
2015	Beijing, China	Z. Chai, X. Zhang
2017	Wien, Austria (scheduled)	G. Köllensperger, B. Keppler

Publication of *Metallomics* as the international academic journal

It was really surprising news, and was a great pleasure, that the academic journal *Metallomics: Integrated Biometal Science* (hereafter referred to as *Metallomics*) was launched in January 2009, by the RSC. At the beginning of the first issue of *Metallomics*, Prof. J. A. Caruso (the chair of the Editorial Board) and Dr N. O'Connor (of the RSC, the Editor of the journal) gave a welcome message to celebrate the publication of the new journal,[32] in which they mentioned the aim of the journal as well as the significance of establishing the newly emerging scientific field of metallomics. The first part of their message is quoted below.

Welcome to the first issue of Metallomics: Integrated Biometal Science. The study of metals in biological systems is an increasingly important area of research. Metallomics is a newly emerging scientific field that is receiving great attention as a new frontier in the study of trace elements in the life sciences. It is a global discipline encompassing many areas including biology, chemistry, geology, medicine, physics, and pharmacy.

As this field brings together researchers from such diverse areas, we anticipate that Metallomics will help to bridge the gap between researchers from different backgrounds so that ideas can be shared and the field progresses to the benefit of all. Our journal will serve as a focus for the community of metallomics researchers to come together and gain new perspectives and insights. It is our aim to reflect the interests of the emerging community and to support you as your community grows.

Then, they added the following message as an acknowledgement.

*We would like to acknowledge the work of those whose vision has led to the establishment of this field, including Bob Williams (R. J. P. Williams, Coord. Chem. Rev., 2001, **216–217**, 583–595), Hiroki Haraguchi (H. Haraguchi, J. Anal. At. Spectrom., 2004, **19**, 5–14) and Joanna Szpunar (J. Szpunar, Anal. Bioanal. Chem., 2004, **378**, 55–56), and to all those who continue to contribute to the emergence of metallomics, without whom we would not be launching this exciting new journal.*

Caruso and O'Connor appreciated the works by Williams, Haraguchi and Szpunar for their contribution to establishing metallomics. However, it may be appropriate to provide some comments around the above description. Williams published his paper entitled ''Chemical selection of elements by cells'' in *Coord. Chem. Rev.* in 2001.[33] This paper was the summary of his lecture at the 34th International Conference on Coordination Chemistry (ICCC34) held at the University of Edinburgh, Edinburgh, Scotland, July 09–14, 2000, and he represented his viewpoint of ''metallome'' in the abstract, as follows:[33]

The selection of the chemical elements by a particular cell from the environment involves a series of steps, the complexity of which depends upon the organism.—(intermediate sentences are omitted)—The variety of paths which individual elements follow in any organism adds to the specific character of the organism. Clearly the paths have evolved to create an element distribution which we shall call the metallome, to parallel the nomenclature of protein distribution, the proteome.

In fact, Williams described the term ''metallome'' for the first time in 2000, but he never mentioned ''metallomics'' in his papers and books. Of course, his great and long-standing contribution to complex chemistry as well as bioinorganic chemistry has been highly appreciated in scientific communities. Therefore, it is a great pleasure to cite his publications in the present Perspective. However, it was strange that nobody commented on the term ''metallome'' on the occasion of BITREL 2002. It might be guessed that nobody knew the term at the time of the conference; frankly speaking, I also did not know the term ''metallome'' at that time. Williams' paper[33] was added to the references cited in the original paper on metallomics[1] during a literature survey after the BITREL symposium.

Szpunar published a paper entitled ''Metallomics: a new frontier in analytical chemistry'' in *Anal. Bioanal. Chem.* in 2004.[34] Part of her paper is quoted below:

Recently, Haraguchi and Matsuura suggested the term "metallomics" to denote metal-assisted function biochemistry and postulated it to be considered at the same level of scientific significance as genomics or proteomics.[17] The metallomic information will comprise the identities of the individual metal species (qualitative metallomics) and their concentrations (quantitative metallomics). As such, metallomics can be considered as a subset (referring to cellular biochemistry) of speciation analysis understood as the identification and/or quantification of elemental species.[7]

Szpunar was a participant in BITREL 2002. She was therefore aware of the proposal of metallomics in the symposium as well as the publication of the symposium proceedings, and so, within her own paper, she was able to cite the present author's paper in the conference proceedings.[17]

Up until the end of 2016, more than 1000 articles and reviews have been published in 8 volumes of *Metallomics*. It is highly considered that the publication of *Metallomics* has accelerated the establishment and progress of metallomics research as a multidisciplinary science. Thus, the hard work of the successive Chairs of the Editorial Board of *Metallomics*, the late Prof. Joseph A. Caruso (Cincinnati University, USA), Prof. Wolfgang Maret (King's College London, UK) and Prof. David Giedroc (Indiana University, USA), should be much appreciated with great respect.

Future outlook of metallomics research

From the historical progress of metallomics mentioned thus far, it can be seen that metallomics has grown rapidly as a newly emerging scientific field in the last decade.[19,20,22,35] Some excellent review papers have also been published to interpret the terminology, concepts and research achievements in metallomics.[22,26,31] In addition, reference books dealing with metallomics have now been published, which cover various research areas of the subject.[18,36–40] Thus, in the second part of this Perspective, an outlook of the recent trends of metallomics research will be discussed to promote further development in the future.

Organization of a platform for promotion of metallomics research

Biological sciences dealing with trace biometals have been studied widely in various scientific fields such as chemistry,

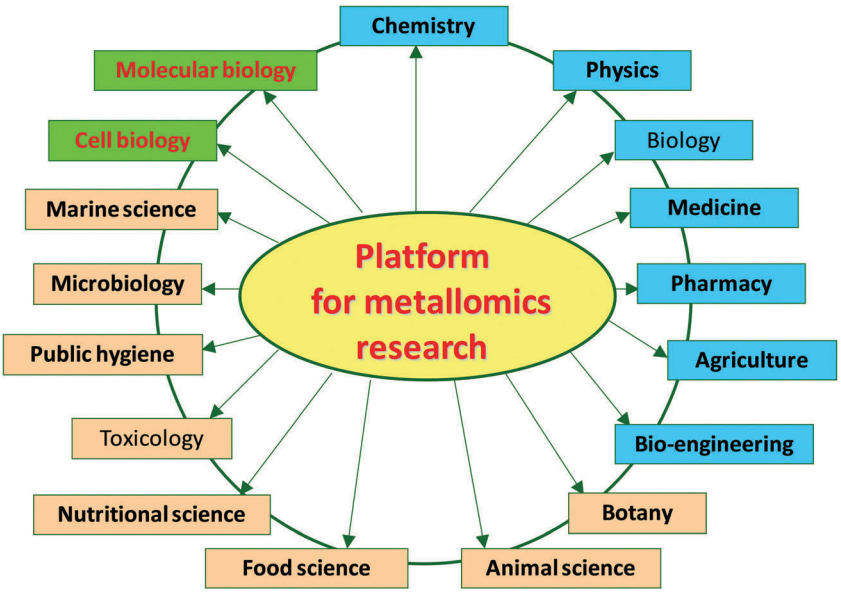

Fig. 1 The platform for metallomics research in cooperation with the diverse scientific fields dealing with biometals.

biology, physics, medicine, pharmacy and agriculture, as well as environmental science and other mission-oriented (applied) scientific fields. Biometal sciences belong within diverse scientific fields, as is shown in Fig. 1, where the various scientific fields are divided into three groups; (1) life sciences such as molecular biology and cell biology (green boxes); (2) basic sciences such as chemistry, biology, physics and others (blue boxes); and (3) mission-oriented sciences such as toxicology, food science, nutritional science, public hygiene and others (orange boxes). It should be stressed here that health science and environmental/green science are very important research fields in metallomics in relation with the sustainability of humans and nature, although they are not shown in Fig. 1. As is well recognized, molecular science and modern cell biology are now basic subjects in life science, where genomics and proteomics have been extensively developed since the 1990s. Considering such present situations, of course, it is desirable for metallomics to develop by maintaining a close relationship with molecular biology and modern cell biology. Thus, molecular biology and cell biology are illustrated in Fig. 1, together with other basic and applied biometal scientific fields.

A platform for metallomics research is indicated at the location of the center circle in Fig. 1 to show that cooperation with all biometal sciences is required for metallomics research to progress in the future. If such a platform can be organized, the information and technology for metallomics research can be shared, encompassing the diverse scientific communities. As a result, metallomics would play an important role as the integrated biometal science for the development of biometal sciences. The Steering Committee of Metallomics was organized on the occasion of the 5th International Symposium on Metallomics held in Beijing in 2015, according to the suggestion of Prof. Zhifang Chai. At present, Prof. R. Lobinski (France) is the chairman of the Steering Committee, and the organizers of past Metallomics Symposia are the committee members. Then, it is greatly expected that the Steering Committee of Metallomics will take leadership for the organization of the platform for metallomics, with the support of the editorial office of the journal *Metallomics*.

Omics-sciences and a simplified model of the biological system

Biological cells, composed of various organelles, construct characteristic internal structures depending on their functions in organs. Even so, the cell structures, as complex assemblies, are well organized to express their intrinsic functions in the biological system (either prokaryotes or eukaryotes). Taking into account such cell structures and functions, a simplified model of a biological system is schematically illustrated in Fig. 2 in order to clarify the biological standpoint of "metallomics", cited from the original paper in 2004 with some revision.[1] In Fig. 2, the dotted line (inside) and continuous line (outside) indicate a cell unit and an organ/whole body, respectively, where biological fluids (*e.g.*, blood) are circulating between cell membrane and organ. Some biological substances (components) and their functions (*e.g.*, biosynthesis, conversion, transportation and metabolism) in the biological system are also indicated in Fig. 2. Although the cell model shown in Fig. 2 is very simple and primitive, it is helpful to understand cell community.

On the left hand side of the simplified model in Fig. 2, various *omics*-sciences (such as genomics, proteomics, glycomics, metabolomics and metallomics) are illustrated together with their corresponding components (genome, transcriptome, proteome, glycome, metabolome and metallome) to indicate their research areas and mutual relations in the biological system.[18] As is well known, the terms of these *omics*-science have been developed since the late 1980s, and now they are widely used to consider each research area comprehensively, maybe in correlation with molecular biology and cell biology, for example, as shown in Fig. 1. Genomics stores the genetic information of DNAs and

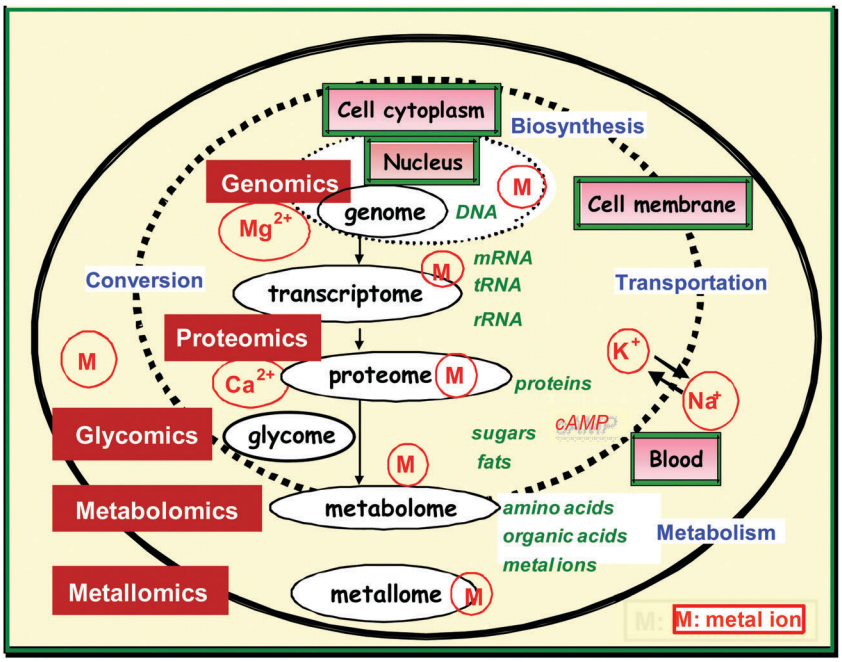

Fig. 2 Simplified model of a biological system, showing the relationship of *omics*-sciences (revised and adapted from ref. 1 with permission from the Royal Society of Chemistry).[1] The outer line and the inner dotted line indicate the organ or the whole body and the biological cell, respectively.

RNAs encoded as the sequences of nucleic bases, required for biosynthesis of proteins as well as for regulation of protein structures/functions. A large number of proteins are distributed inside and outside the cells as well as in the membranes. Most of them are structural proteins, but some of them work as enzymes for biological and/or physiological functions such as biosynthesis, material conversion, transportation and metabolism of various biological substances inside the cell. It is well known, for example, that DNAs and RNAs are synthesized in the nucleus with the aid of DNA polymerase and RNA polymerase, both of which are zinc enzymes. When the Human Genome Project was almost complete, proteomics (protein science) received great attention as a post-genome science.

Many biological substances as well as metal ions are stored and transported inside and outside cells through the membrane.[33] Such dynamic processes of transportation for material storage and exclusion are important to regulate the biological systems. Such a scientific field is now called metabolomics. As is seen from Fig. 2, metal ions are ubiquitously distributed inside and outside the cell to assist the physiological functions of the genome, transcriptome, proteome, glycome and metabolome. Later, two examples for transportation processes will be described.

Research subjects in metallomics

In order to envision the research direction in the early stages, the research subjects for metallomics were provided in the first paper in 2004.[1] They are summarized in Table 2. These research subjects were selected taking into account the biological roles of biometals in the life sciences, referring to the relationship between metallomics and other biometal sciences in Fig. 1 as well as to the simplified model of biological systems in Fig. 2. It can be seen from Table 2 that the research subjects are roughly divided into several groups: Subject 1 and Subject 2 from the analytical chemistry approach, Subject 3 and Subject 4 from the bioinorganic chemistry approach, Subject 5 and Subject 6 from the biological (and/or biochemistry) approach, Subject 7 and Subject 8 from the medical/pharmaceutical approach, Subject 9

Table 2 Research subjects in metallomics[a]

(1)	Quantitative distribution and imaging analysis of elements in biological fluids, cells, organs *etc.*
(2)	Speciation of the elements and the metallome (metal-binding molecules) in biological systems
(3)	Structural analysis of the metallome
(4)	Elucidation of reaction mechanisms of the metallome using model metal complexes (bioinorganic chemistry)
(5)	Identification of unknown metalloproteins and metalloenzymes
(6)	Metabolism of biological molecules and metals (metabolome and metabolites)
(7)	Medical diagnosis of health and disease related to trace metals
(8)	Metallodrug design for chemotherapy
(9)	Chemical evolution of living systems and organisms on Earth
(10)	Mission-oriented biometal sciences: medicine, environmental science, food science, agriculture, toxicology, biogeochemistry *etc.*

[a] The list of the research subjects above is partly revised from the original one.[1]

from chemical evolution, and Subject 10 from the diverse mission-oriented biosciences.

The analytical chemistry approach is still very important to obtain fundamental information on the elemental distributions, chemical speciation of metals and metalloids (metallome) in the biological systems.[41,42] Recently, imaging analysis of the metallome in cells and organs has been greatly required. All-element analysis of single biological cells is an important research subject[43] as will be described later. The studies on Subjects 3–5 in Table 2 have been extensively performed mainly in the field of bioinorganic chemistry. We can find many excellent achievements concerning metalloproteins and metalloenzymes in bioinorganic research, especially regarding the structures and reaction mechanisms of the metallome. As for Subject 6, significant progress has been achieved by applying analytical techniques such as GC-MS and LC-MS to the profiling analysis of metabolites (metabolome) in urine and blood serum. Such results can also be applicable to the medical diagnosis of health and disease in Subject 7. Subject 8, *i.e.*, the design of inorganic drugs for chemotherapy, is one of the most active research areas in metallomics. Such situations will be explained in the next sub-section. Of course, many scientists have been carrying out mission-oriented biometal science research related to Subject 10. Such mission-oriented biometal sciences – in another words, traditional trace metal sciences – have a close relationship with almost all the research fields in Subjects 1–9. Looking over the articles published in the last 8 years in *Metallomics*, it can be found that the research subjects in Table 2 are quite well covered in metallomics research, therefore Table 2 seems to be quite a good guideline for research.

Recent trends of metallomics research examined by literature survey

In order to ascertain recent trends in metallomics research, a literature survey of metallomics has been performed using the Web of Science provided by Thomson Reuters. The results are reported first for the journal *Metallomics*. The total number of articles published in the journal *Metallomics* is about 1080 (2009–2016) at present. The impact factor of *Metallomics* is 3.54 (2015). It is interesting that the number of articles in which ''metallomics'' is indicated as the keyword was only 331 (*ca.* 30%) among 1080 articles in total. It is quite difficult to understand why the citation of the keyword ''metallomics'' in the articles published in *Metallomics* is so small. My simple speculation is that the authors submitting their papers do not have any obligation to actively cite the keyword ''metallomics'' when submitting articles to the journal *Metallomics*.

Another trend can be seen from the literature survey for all scientific journals, where the survey was performed for journals published after 2004. The results are shown in Table 3. As is expected, *Metallomics* was top-ranked, and the second- and third-ranked journals were *Journal of Analytical Atomic Spectrometry* and *Analytical and Bioanalytical Chemistry*, respectively. It is seen in Table 3 that the journals in the higher ranks are major journals in analytical chemistry, but citations are also found in the more biology-oriented journals such as *BioMetals*, *Journal of Proteomics* and *FEBS Journal*, although their citation totals were not so high. It can be understood from these results that the analytical chemistry approach is still predominant in metallomics research.

Table 3 The numbers of articles published in various journals surveyed using the keyword ''metallomics''; total publications 317

Journal	No. of articles	Impact factor (2015/2016)
Metallomics	58	3.54
Journal of Analytical Atomic Spectrometry	28	3.379
Analytical and Bioanalytical Chemistry	19	3.125
Talanta	8	4.035
Analytica Chimica Acta	7	4.712
Analytical Chemistry	6	5.886
BioMetals	4	2.134
Journal of Proteomics	4	3.867
Analytical Sciences	4	1.174
Journal of Chromatography A	3	3.936
FEBS Journal	3	4.237
Analyst	3	4.033
Analytical Biochemistry	2	2.219
Analytical Methods	2	1.915

Next, a similar literature survey was performed again for *Metallomics*, to learn the citation ranking of the articles published in the journal. The articles ranked top-10 in terms of citations are shown in Table 4, along with their topic area and their number of citations. The results in Table 4 indicate that the articles concerned with drug design for cancer, Alzheimer's disease and other human diseases occupy the higher citation rankings, indicating that drug design and chemotherapy for serious diseases are receiving great interest in metallomics. These studies correspond to Subject 8 in Table 2: design of inorganic drugs for chemotherapy. Some recent research will be introduced in the next section.

Topics of recent research in metallomics

So far, the recent trends in metallomics research have been examined mainly by literature survey. In the following sections, some topics in metallomics research are considered and reviewed briefly.

a. Quantitative and speciation analysis of the elements in a single cell. In recent years, quantitative analysis of the elements (often together with proteins) in a single biological cell has been carried out using various measurement systems. Huang's group at Xiamen University investigated single-cell elemental analysis *via* high irradiance femtosecond laser ionization orthogonal time-of-flight mass spectrometry (fs-LI-O-TOFMS), where paramecium cells were used for investigation.[55] The schematic diagram of fs-LI-O-TOFMS is shown in Fig. 3. They could determine elements such as Na, Mg, Al, K, Ca, Mn, Fe, Cu, Zn, Cs as well as some non-metallic elements such as P, S, Cl and I in paramecium at the pg per cell level. Ceko *et al.* analyzed Se in bovine ovarian granulosa cells by X-ray fluorescence spectrometry to evaluate bovine female reproductive function.[56] Shigeta *et al.* reported an interesting trial of sample introduction of single cells by micro droplet generation into an ICP-sector field mass spectrometer for label-free detection of trace elements.[57] In this experiment, the single selenized yeast

Table 4 The topic areas of the top-10 cited articles published in *Metallomics*[a]

Topic of article	Citations[b]	Ref.
Ruthenium anticancer drugs	303	45
Platinum antitumor compounds, inhibition of transcription	214	46
Cytosolic zinc buffering and muffling, intracellular zinc homeostasis	171	47
Gold compounds as therapeutic agents	154	48
Epigenetics in metal carcinogenesis	145	49
Metal–carbene complexes, potent anticancer agents	131	50
Metal dyshomeostasis, Alzheimer's disease	112	51
Zn(II) and Cu(II) ions with Alzheimer's amyloid-beta peptide	101	52
Selenium biochemistry, role for human health	91	53
Zinc transporters in physiology and pathogenesis	90	54

[a] The total number of articles published in *Metallomics* from 2009 to 2016 was 1069 as of January 22, 2017. [b] Number of citations obtained from the Web of Science, Thomson Reuters.

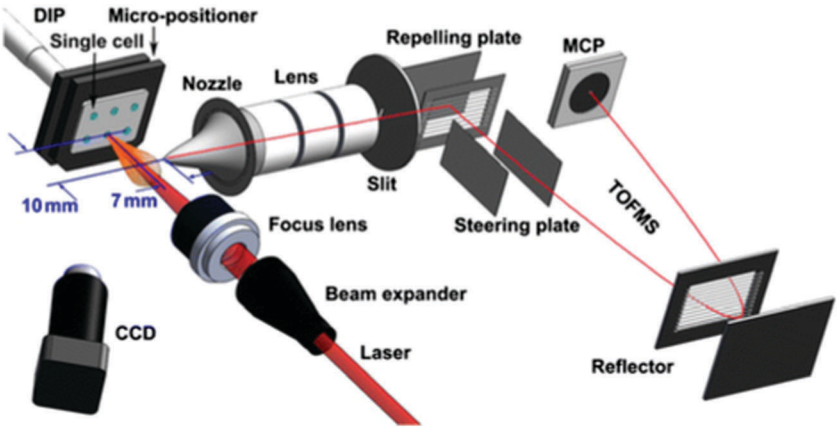

Fig. 3 Schematic diagram of the fs-LI-O-TOFMS platform.[55] Reprinted with permission from Gao *et al., Anal. Chem.*, 2013, **85**, 4268–4272. Copyright © 2013 American Chemical Society.

cells were embedded into droplets and introduced into the plasma by a pulsed droplet introduction device for measurement. Although their results were still preliminary, the following results were obtained: signal intensities from single cells were measured for the elements Cu, Zn and Se from the histograms for about 1000 cell events. The mean elemental sensitivities measured were in the range of 0.7 counts per ag (Se) to 10 counts per ag (Zn) with RSDs from 49% (Zn) to 69% (Se) for about 1000 cell events. In addition, they performed open vessel digestion of washed yeast cells for multielement analysis and obtained the estimated analytical values of absolute amounts per single cell of Na 0.91 fg, Mg 9.4 fg, Fe 5.9 fg, Cu 0.54 fg, Zn 1.2 fg and Se 72 fg.

Recently, Umemura *et al.* reported multielement analytical data for prokaryotic and eukaryotic cells as well as organelles, measured by micro-flow injection (μFI)-ICP-MS.[58] They employed a convenient digestion method, not the single cell analysis method. The data for *E. coli*, synechocystis, chlorella, chloroplast from spinach, mitochondria from potato tuber and bovine live were summarized as the number of atoms (normalized values) per cell. These data will be helpful when studies on single cell analysis are carried out more extensively in the future. Many experiments on single-cell elemental analysis using different measurement systems have been reported so far, but still the number of elements determined or detected in those reports is limited.

Next, our study on multielement (challenged to all-elements) analysis as well as speciation analysis for salmon egg cell is introduced here.[44] The aim of this research was to obtain multielement data for salmon egg cells for proof of the Extended All Present Theory of the Elements and cell microcosm.[1,2] In the experiment, 2 or 3 salmon eggs were decomposed by the microwave digestion method. In the measurement, 78 elements – excepting rare gas elements and radioactive elements – were the targets of analysis, because for the quantitative analysis of rare gas elements and radioactive elements special experimental facilities or equipment are required for the sample preparation of such elements. So far, 66 elements among 78 elements have been determined in a single salmon egg cell by ICP-AES and ICP-MS.[44,59,60] 7 other elements (Li, Zr, Nb, Hf, Ir, Bi), because of their low abundances, were only detected close to their analytical detection limits, and the remaining 5 elements (F, Rh, Te, Ta, Re) were below their detection limits. Non-metallic elements such as H, C, N and O were measured by the conventional elemental analysis method.

Speciation analysis of the elements in salmon egg cell cytoplasm was performed by an HPLC/ICP-MS system with simultaneous/multielement detection.[44] The element-selective chromatograms are shown in Fig. 4, where the CHAPS-coated ODS column was prepared by dynamically coating the ODS (octadecyl silica) column (4.6 mm i.d. × 250 mm long) with

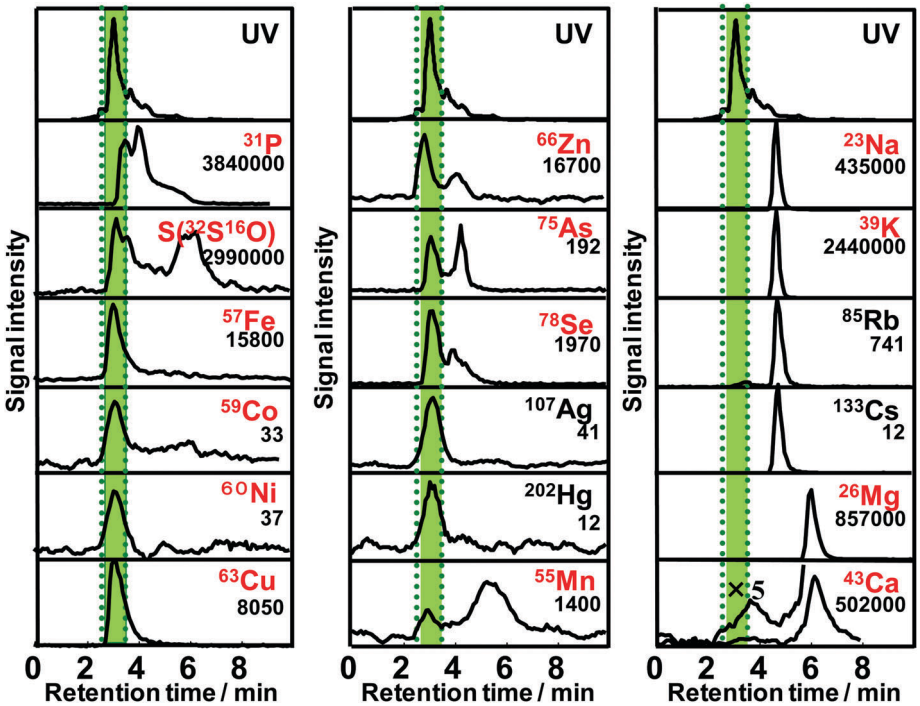

Fig. 4 The element-selective chromatograms for salmon egg cytoplasm obtained by HPLC/ICP-MS.[44] (Elements in red are essential elements for humans.) Sample: salmon egg cell cytoplasm diluted 5-fold with 0.1 M Tris buffer (pH 7.4), column: CHAPS-coated ODS column, mobile phase: 0.1 M Tris buffer solution (pH 7.4), UV absorption detection: 254 nm. The numbers below the elements indicate the concentrations (ng g^{-1}) of the elements in salmon egg cell cytoplasm. The retention time range between 3.0–3.5 min (green region) corresponds to the protein elution zone. Reprinted with permission from Haraguchi, *et al., Pure Appl. Chem.*, 2008, **80**, 2595–2608. Copyright © 2009 Walter de Gruyter GmbH.

CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; zwitterionic surfactant). The CHAPS-coated ODS column provided unique characteristics for the simultaneous separation of ions/small molecules and large molecules (*e.g.*, proteins),[60] and thus the binding or non-binding of the elements with proteins can be easily determined on the chromatograms.[61]

In Fig. 4, the chromatograms with the UV-absorption detection at 254 nm are also shown on the top of each column together with the element-selective chromatograms. It should be noted here that the retention ranges of 3.0–3.5 min, shown as the green regions in Fig. 4, correspond to the elution zones for proteins (large molecules with molecular weight (MW) greater than *ca.* 10 000 Da). On the other hand, the retention range after 3.5 min corresponded to the elution region for small ions and molecules. However, information about molecular weights could not be obtained from the chromatograms measured by the present CHAPS-coated ODS column because it had no size exclusion characteristics.

Heavy metals such as Fe, Co, Ni, Cu, Ag, and Hg, which are essential or toxic elements, gave broad elution peaks within the protein elution region. These results indicate that these heavy metals mostly exist as protein-binding species. Zinc and Mn, however, gave two large broad peaks within and after the protein-elution region, indicating that these elements exist partly as small molecules/ions, maybe free ions or amino acid complexes, in addition to protein-binding species. On the contrary, it is clearly seen in Fig. 4 that alkali and alkaline earth elements, such as Na, K, Rb, Cs and Mg, gave sharp single peaks in the elution region for small molecules/ions, indicating that Na, K, Rb, Cs and Mg in salmon egg cells exist solely as the ionic forms. However, Ca gave small peaks in the large molecular region (see expanded chromatogram), which suggests that Ca was partly binding with proteins.

As for arsenic and selenium, two separate peaks in the small and large molecular ranges were observed in a similar manner to the cases of Zn and Mn. These results suggest that arsenic and selenium in the salmon egg cell cytoplasm exist not only as small inorganic species (*e.g.*, arsenate and selenate), but also as protein-binding molecules. In the case of arsenic, the existence of small arsenic molecules such as dimethylarsenate and arsenobetaine was confirmed experimentally in the cytoplasm of salmon egg cell,[37] which were detected in the small molecule region of Fig. 4, while the existence of protein-binding arsenic molecules in the cell cytoplasm could not be ascertained from the experiment of Fig. 4. Such arsenic-binding proteins have been investigated from the viewpoint of toxicity and drug design.[62,63] In the case of selenium, glutathione peroxidase, which is an antioxidant, is known as a large molecule (tetramer; MW *ca.* 84 kDa) in animals.

Furthermore, in Fig. 4, the elution peaks of Hg overlap with the elution peaks of both S and Se. These results indicate that Hg is binding not only with S-containing proteins (cysteine residue-sites), but also with Se-containing proteins (selenocysteine residue-sites). It was confirmed in a separate experiment using a size exclusion column that Hg in salmon egg cell cytoplasm binds with proteins containing selenocysteine and/or cysteine residues in proteins.[64] Furthermore, the existence of alkaline phosphatase,

which is a zinc enzyme, was experimentally elucidated in the protein fraction in Fig. 4.[65]

b. Transportation of zinc to amniotic fluid in pregnant women. In blood serum, albumin, α-macroglobulin, ceruloplasmin and transferrin exist as transport proteins for Zn and Cu, Zn, Cu and Fe, respectively. Among them, the binding strengths of each protein with metal ions are different, depending on the combination of metal ions and proteins. For example, albumin binds quite weakly with Zn, while α-macroglobulin binds strongly with Zn. Such transportation processes of metal ions with proteins are very important to maintain homeostasis in biological cells as well as to maintain biological functions. In Fig. 5, the correlations between the concentration of Zn and albumin in blood sera of pregnant women are shown together with the correlations for healthy volunteers; examined through the courtesy of *ca.* 100 healthy volunteers and 4 pregnant women.[66]

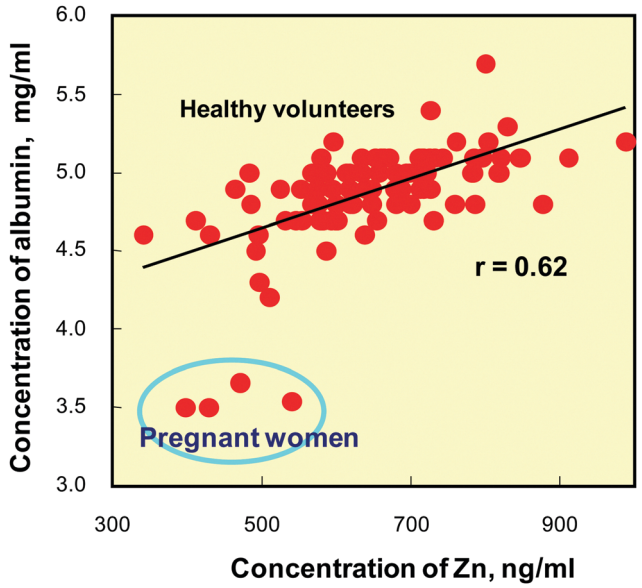

Fig. 5 The correlation between the concentrations of Zn and albumin in blood sera of healthy volunteers and pregnant women.[66]

It can be seen from Fig. 5 that the albumin concentrations in blood sera of pregnant women were much lower than those in blood sera of healthy volunteers. At the same time, the Zn concentrations in blood sera of pregnant women were significantly lower compared to those of healthy volunteers. On the other hand, the Cu concentrations in blood sera of pregnant women were significantly higher by almost 2-times, although the results are not shown in Fig. 5. During pregnancy, a lot of Zn is required for fetal (embryo) growth in amniotic fluid because Zn is an important cell growth factor. Then, it can be understood that the lower concentrations of Zn and albumin in blood sera of pregnant women are caused by the supply of Zn with albumin from serum to amniotic fluid. Since pregnant women in these situations are nearly within the zinc-deficient classification, they should take care of their health.

c. Regulation of zinc homeostasis in biological cells. Recently, Maret and his colleagues published an interesting article concerned with the molecular mechanisms of intracellular homeostatic control of zinc ions,[47] as listed in Table 4. They performed a study of zinc proteomes and metallomes, zinc transporters, and insights from the use of computational approaches. They proposed the following two mechanisms to regulate zinc homeostasis in cells. They are: (A) under steady state conditions (zinc buffering), a primary function of cytosolic zinc-binding proteins is to buffer the relatively large zinc content found in most cells to a cytosolic zinc(II) ion concentration in the picomolar range, and (B) under non-steady state conditions (zinc muffling), zinc-binding proteins and transporters act in concert to modulate transient changes in cytosolic zinc ion concentration in a process that is called zinc muffling. Those processes are schematically illustrated in Fig. 6.[47] It is interesting that they found for the first time that metallothionein binding its seven zinc ions with

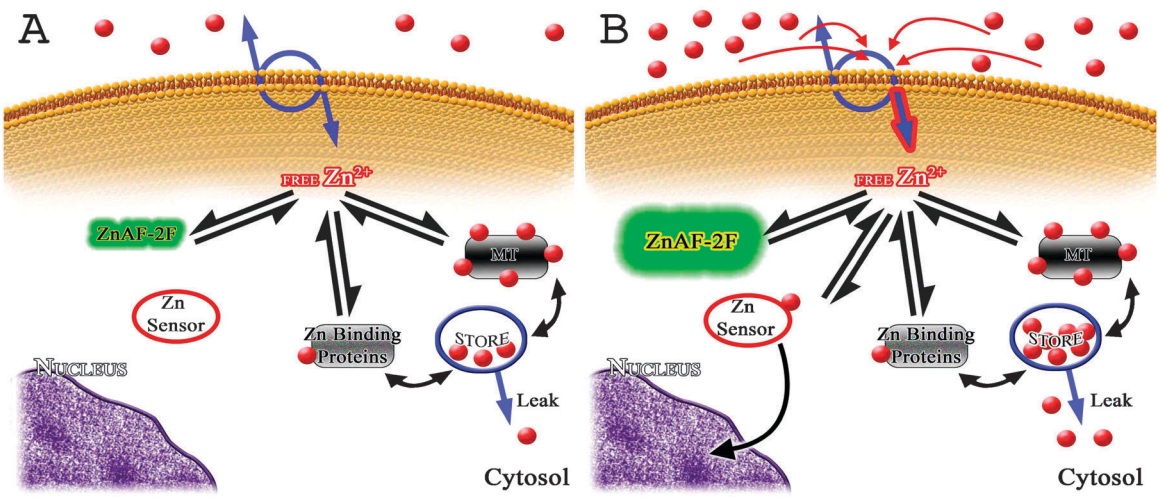

Fig. 6 Model schematic of the key elements of intracellular zinc ion homeostasis.[47] (A) Zinc buffering; steady state cytosolic free zinc ion concentrations are maintained at picomolar to single-digit nanomolar concentrations primarily by the buffering action of cytosolic zinc ion-binding proteins that include metallothionein (MT). (B) Zinc muffling; net zinc ion influx is increased as a way to illustrate the functioning of muffling reactions and zinc ion sensing under non-steady state conditions. Reproduced from ref. 47 with permission from the Royal Society of Chemistry.

different affinities plays an important role in zinc buffering and muffling reactions. Also, they suggested that metallothionein is a source of zinc ions under the conditions of redox signalling. If cytosolic free zinc ion concentrations reach a sufficiently high level, zinc ions may bind to zinc ion sensors in the cytoplasm. It is further mentioned there that zinc ion sensors discovered to date are the transcription factors that, upon binding zinc ions, translocate to the nucleus and effect changes in the expression of genes encoding zinc homeostatic proteins.

d. Recent developments in metallodrugs. As listed in Table 4, studies on drug design and chemotherapy for serious diseases such as cancer, Alzheimer's disease, rheumatoid arthritis and so forth are receiving great interest even in metallomics. Thus, recent developments in metallodrugs for chemotherapy will be briefly reviewed here.

It is well known that the anticancer effect of cisplatin (*cis*-diamminedichloroplatinum(II)) was found by Rosenberg in 1965,[67] and it was first successfully administered as an anticancer drug for testicular tumors. However, cisplatin has toxic side effects including nephrotoxicity and neurotoxicity, and thus it was of limited use for chemotherapy.[68] Subsequently, the development of other platinum anticancer compounds with less toxic side effects has been extensively explored over the last few decades. Chemical structures of platinum anticancer agents are shown in Fig. 7.[46] Among them, cisplatin, carboplatin, and oxaliplatin are FDA-approved for chemotherapy use in the United States and many other countries. The mechanisms of action of platinum anticancer compounds remained a long-term problem. More recently, it has been elucidated that these compounds induce apoptosis in tumor cells by binding to nuclear DNA, forming a variety of structural adducts and triggering cellular responses, one of which is the inhibition of transcription.[46] Knowledge obtained from the detailed study of structural adduct formation will promote the development of new compounds in this family.

In addition to platinum anticancer drugs, ruthenium anticancer drugs have been recently receiving interest, as is seen

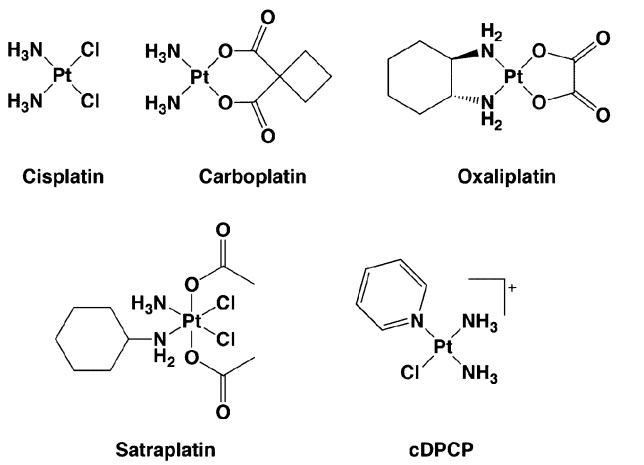

Fig. 7 Chemical structures of platinum anticancer agents used in chemotherapy for various cancers. Reproduced from ref. 46 with permission from the Royal Society of Chemistry.

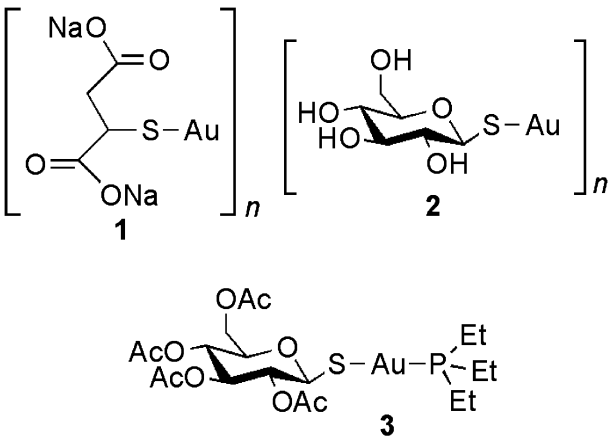

Fig. 8 Gold(I) drugs used for the treatment of rheumatoid arthritis; (**1**) sodium aurothiomalate (Myocrisin), (**2**) aurothioglucose (Solganol), (**3**) tetraacetyl-β-D-thioglucose gold(I) triethylphosphine (auranofin). Reproduced from ref. 48 with permission from the Royal Society of Chemistry.

in Table 4. Levina *et al.* published a review article concerned with the recent development of ruthenium anticancer drugs.[45] Their anticancer activities had already been noted in the 1980s, but in those days platinum anticancer drugs were receiving great interest and the study of ruthenium anticancer drugs was not explored again at the time. Since the 1990s, the organometallic chemistry of ruthenium has been extensively progressing for exploitation of the synthesis of new ruthenium compounds and their application to catalysts. Such ruthenium complexes have been tested for their cytotoxicity in cancer cells, but it seems that their application to clinical administration is still ongoing.

Gold was used in medicine in ancient times, and Au(I) compounds have been used clinically to treat rheumatoid arthritis since the last century. Gold drugs for the treatment of rheumatoid arthritis are shown in Fig. 8.[48] Among them, **1** and **2** in Fig. 8 are thiolate compounds, developed in the 1960s. Auranofin, **3** in Fig. 8, was developed as an orally active Au(I) phosphine compound for the treatment of rheumatoid arthritis, which was approved for clinical use in 1985. In recent years, Au(I) and Au(III) compounds as anticancer drugs have been under investigation. Moreover, studies on gold compounds for the treatment of diverse human diseases, such as cancer, acquired immune deficiency syndrome (AIDS) caused by the human immunodeficiency virus (HIV), and parasitic diseases are in progress.[48]

Conclusion

The history of metallomics over the last decade has been summarized in this Perspective. It is confirmed that metallomics has been developing as an interdisciplinary or multidisciplinary field in cooperation with a variety of biometal sciences, as well as with genomics and proteomics. For further development, it is highly expected that metallomics research will be promoted in terms of the biological functions of metallomes on the basis of molecular science and cell biology. In order to accelerate the development of metallomics, the

organization of a scientific platform is greatly required to share information and technology among mission-oriented sciences such as medicine, pharmacy, toxicology, food and nutrition and so forth. Single-cell elemental analysis and the imaging of cell and organs, cell biology of metals from multielemental aspects, molecular and genetic features of diverse metals, and inorganic drug design for chemotherapy, together with health science and environmental science, might be the major research fields in the next decade.

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