Vol. 17, pp. 94–97 (2020)
doi: 10.2142/biophysico.BSJ-2020017

Biophysics and Physicobiology
https://www.jstage.jst.go.jp/browse/biophysico/

Commentary and Perspective

Integrated bio-metal science: New frontiers of bio-metal science opened with cutting-edge techniques

Hitomi Sawai[1] and Koichiro Ishimori[2]

[1] *Graduate School of Life Science, University of Hyogo, 3-2-1 Kouto, Kamigori-cho, Ako-gun, Hyogo 678-1297, Japan*
[2] *Department of Chemistry, Faculty of Science, Hokkaido University, Kita 10, Nishi 8, Kita-ku, Sapporo, Hokkaido 060-0810, Japan*

Received August 7, 2020; accepted August 24, 2020; Released online in J-STAGE as advance publication August 28, 2020

Trace amounts of "bio-metals" are essential for maintaining our life, but we have not yet fully understood mechanisms of how they function in proteins, cells, organs, and bodies. The Scientific Research on Innovative Areas "Integrated Bio-metal Science" aims to reveal regulatory mechanisms of the maintenance, failure and disturbance of the bio-metals by integrating several research fields dealing with the bio-metals into a new inter-disciplinary research field. Extensive researches of the bio-metals have been explored by the development of precise biophysical measurements and visualization of trace metals in biological materials. Cutting-edge developments of nuclear magnetic resonance (NMR), electron paramagnetic resonance (EPR), native mass spectrometry (Native MS), chemical imaging, and nuclear resonance vibrational spectroscopy (NRVS) are now opening the door for new strategies to understand various kinds of bio-metals essential for life and to establish "integrated bio-metal science". For the issue, we had a symposium at the 58th Annual Meeting of the Biophysical Society of Japan held in September 2020. Six pioneers of the aforementioned measurements and exploitation of bio-metals were invited to introduce their marvelous techniques and discussed recent achievements toward medical and environmental applications. In this paper, their talks with figures provided from the speakers are summarized.

The simplest and direct method to identify metal binding to biomolecules is "Native MS", which mildly ionizes metal binding biomolecules including proteins, nucleic acids, and their complexes in crowded biosystems or without purification and detects them without dissociation even in the gas phase. With these characteristics, Native MS has been utilized to determine the molecular mass of intact metal binding proteins and to analyze their biophysical properties. The first speaker of this symposium, Dr. Satoko Akashi from Yokohama City University, talked about "Native MS for bio-metal science", demonstrating the recent advancement of application of "Native MS" to protein complexes [1,2].

To characterize the environments of the metal binding site in biomolecules, several new methodologies have developed over the past few decades. The second speaker in this symposium, Dr. Masaki Horitani from Saga University, presented a combinational study of X-ray crystallography and EPR spectroscopy on a manganese-binding protein, cold-adapted inorganic pyrophosphatase (PPase). PPase from a psychrophilic bacterium, *Shewanella* sp. AS-11 (Sh-PPase), activated by manganese, catalyzes hydrolysis of inorganic pyrophosphate to phosphates. The characteristic temperature dependence of the activity showing an optimum at 5°C suggested a specific molecular mechanism for cold adaptation of Sh-PPase. To examine the environmental changes of the metal binding site associated with the substrate binding, he combined X-ray crystallography and EPR spectroscopy with rapid mixing freeze-quench technique, revealing that unique active site rearrangement of Sh-PPase is induced by the substrate binding (Fig. 1) [3].

The metal binding can be also used for one of the structural markers to structural dynamics of proteins, particularly multi-domain proteins. The third speaker, Dr. Tomohide Saio from Tokushima University, recently moved from Hokkaido University, introduced the exploitation of paramagnetic metal ions for protein structural

Corresponding author: Hitomi Sawai, Graduate School of Life Science, University of Hyogo, 3-2-1 Kouto, Kamigori-cho, Ako-gun, Hyogo 678-1297, Japan.
e-mail: sawai@sci.u-hyogo.ac.jp

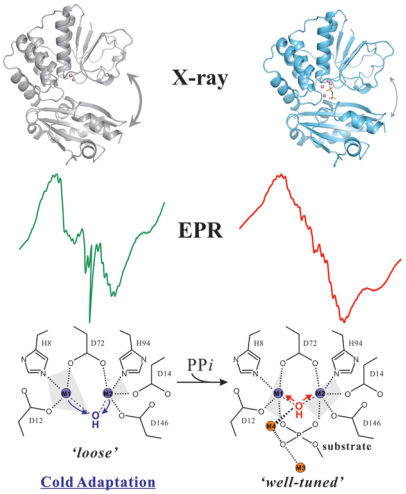

Figure 1 Overall conformational changes of Sh-PPase upon metal binding (top), EPR spectra (middle) and coordination sphere of the metal binding sites (bottom) of Sh-PPase in the absence (left) and presence (right) of the substrate (PDB IDs: 6LL7 for the Mn-bound form and 6LLB for the Mg and PNP-bound form) [3].

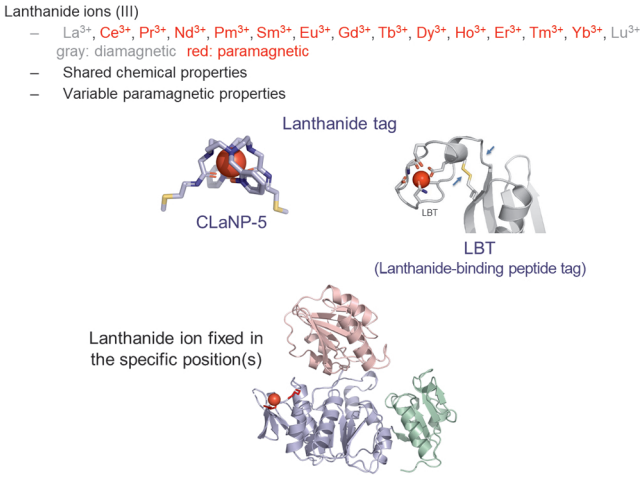

Figure 2 Paramagnetic lanthanide ions for protein structural study. Top panel is GB1 attached with a lanthanide-binding peptide tag (PDB ID: 2RPV) [4]. The bottom panel is a lanthanide ion fixed to MurD, UDP-N-acetylmuramoyl-L-alanine: D-glutamate ligase (PDB ID: 3UAG) [5].

studies in solution. Long-reach (<40 Å) paramagnetic effects provide positional information of the observed nuclei with respect to the paramagnetic center [4]. Using paramagnetic NMR, he revealed structural dynamics of a multi-domain protein enzyme in solution (Fig. 2) [5]. Paramagnetic lanthanide ions fixed at the surface of one of the domains induces significant paramagnetic effects, providing a detailed view of the conformational states of the protein and allowing detection of conformational changes induced by ligand binding.

Considering that protein dynamics is substantially

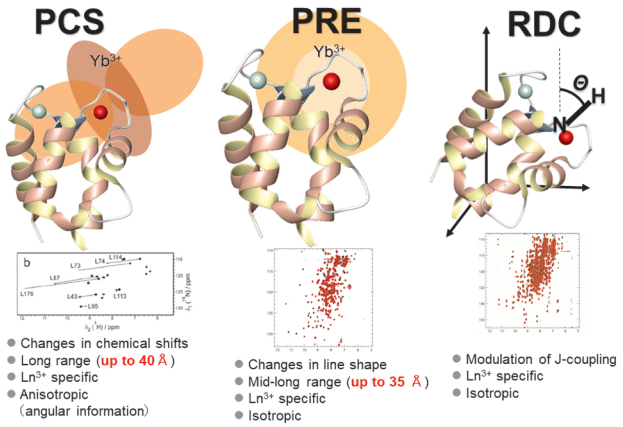

Figure 3 Long-range information on paramagnetic NMR of calbindin (PDB ID: 1CLB) in solution.

sensitive to its surrounding environment, 3D structures under physiological conditions are indispensable. Solution NMR is currently one of the valid techniques to investigate the dynamics and conformational changes of biomacromolecules under physiological conditions or even in living cells. To date, various NMR measurements using paramagnetic effects and magnetic anisotropy have been developed, allowing us to extract more accurate structural ensemble information (Fig. 3). The fourth speaker, Dr. Teppei Ikeya from Tokyo Metropolitan University, developed new computational methodologies for NMR data analysis and structure calculation. He recently integrated these paramagnetic NMR data to the new system and demonstrated multi-state structures of several model proteins in solution. He discussed further potential applications, such as in-cell protein structure determinations [6,7].

While combination of structural and dynamic investigations of proteins has provided new insights into molecular mechanisms of protein functions as shown in NMR studies presented here, dynamic property of bio-metal itself has not been extensively investigated. The fifth speaker, Dr. Yoshitaka Yoda from SPring-8/JASRI, showed the recent progress of the NRVS technique using the long undulator beamline BL19XU at SPring-8. NRVS is a unique technique using high intensity X-ray, produced by synchrotron radiation, to investigate the atomic vibration in molecules. Vibration modes of a specific atom, selectively excited via the nuclear level, give quite different and complementary information from that taken by Raman or infrared (IR) spectroscopy in the examination of the active center of metalloenzyme (Fig. 4) [8–12].

X-rays from the synchrotron radiation can be also utilized for light source of new imaging systems that visualize bio-metal distribution and chemical status in tissues or even in cells. The last speaker, Dr. Shino Takeda from National Institute of Radiological Sciences, is trying

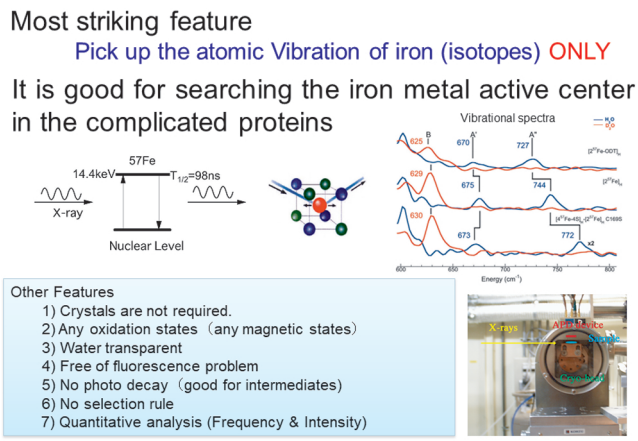

Figure 4 Introduction to NRVS. The vibrational spectra (right top panel) was published in [9].

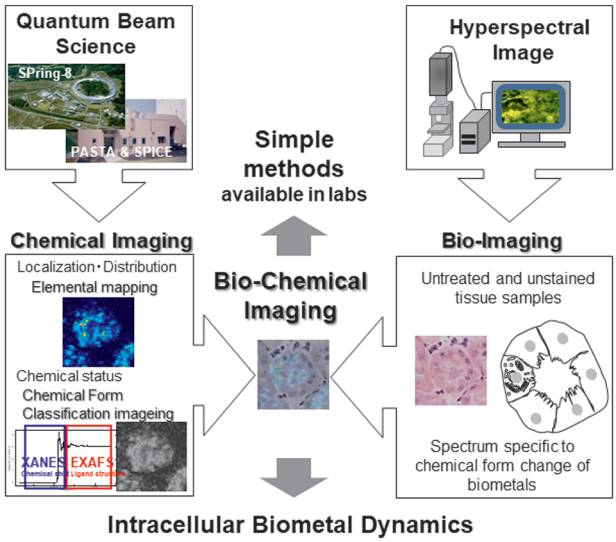

Figure 5 Conceptual diagram of Dr. Shino Takeda's research.

to establish bio-chemical imaging using quantum nano-beam and construction for intracellular bio-metal dynamics *in situ* (Fig. 5). She presented some results of elemental characteristics and formation mechanisms of localized and concentrated bio-metals in tissues by combination of pathological observation and the *in situ* elemental analyses such as particle induced X-ray emission (PIXE), synchrotron radiation X-ray fluorescence (SR-XRF) and X-ray absorption fine structure (XAFS) [13–19].

To understand molecular mechanisms for metal homeostasis and metal-related diseases, detailed structural and functional characterizations of metal binding biomolecules at atomic levels in cells, tissues or organs, as well as under physiological conditions, must be revealed by cutting-edge techniques presented in this symposium. We expect that developments of these techniques accelerate the progress of the researches in "Integrated Bio-metal Science", which can pave the way for fully deciphering why and how life utilizes the specific transition metals as the "bio-metals".

References

[1] Takano, K., Arai, S., Ushijima, H., Ikegami, T., Saijusa, K., Konuma, T., *et al.* Screening of protein-ligand interactions under crude conditions by native mass spectrometry. *Anal. Bioanal. Chem.* **412**, 4037–4043 (2020). DOI: 10.1007/s00216-020-02649-x

[2] Saikusa, K., Kato, D., Nagadoi, A., Kurumizaka, H. & Akashi, S. Native mass spectrometry of protein and DNA complexes prepared in nonvolatile buffers. *J. Am. Soc. Mass Spectrom.* **31**, 711–718 (2020). DOI: 10.1021/jasms.9b00145

[3] Horitani, M., Kusubayashi, K., Oshima, K., Yato, A., Sugimoto, H. & Watanabe, K. X-ray crystallography and electron paramagnetic resonance spectroscopy reveal active site rearrangement of cold-adapted inorganic pyrophosphatase. *Sci. Rep.* **10**, 4368 (2020). DOI: 10.1038/s41598-020-61217-6

[4] Saio, T. & Ishimori, K. Accelerating structural life science by paramagnetic lanthanide probe methods. *Biochim. Biophys. Acta Gen. Subj.* **1864**, 129332 (2020). DOI: 10.1016/j.bbagen.2019.03.018

[5] Saio, T., Ogura, K., Kumeta, H., Kobashigawa, Y., Shimizu, K., Yokochi, M., *et al.* Ligand-driven conformational changes of MurD visualized by paramagnetic NMR. *Sci. Rep.* **5**, 16685 (2015). DOI: 10.1038/srep16685

[6] Ikeya, T., Güntert, P. & Ito, Y. Protein structure determination in living cells. *Int. J. Mol. Sci.* **20**, 2442 (2019). DOI: 10.3390/ijms20102442

[7] Tanaka, T., Ikeya, T., Kamoshida, H., Suemoto, Y., Mishima, M., Shirakawa, M., *et al.* High-resolution protein 3D structure determination in living eukaryotic cells. *Angew. Chem. Int. Ed. Engl.* **58**, 7284–7288 (2019). DOI: 10.1002/anie.201900840

[8] Reijerse, E. J., Pham, C. C., Pelmenschikov, V., Gilbert-Wilson, R., Adamska-Venkatesh, A., Siebel, J. F., *et al.* Direct observation of an iron-bound terminal hydride in [FeFe]-hydrogenase by nuclear resonance vibrational spectroscopy. *J. Am. Chem. Soc.* **139**, 4306–4309 (2017). DOI: 10.1021/jacs.7b00686

[9] Pham, C. C., Mulder, D. W., Pelmenschikov, V., King, P. W., Ratzloff, M. W., Wang, H., *et al.* Terminal hydride species in [FeFe]-hydrogenases are vibrationally coupled to the active site environment. *Angew. Chem. Int. Ed. Engl.* **57**, 10605–10609 (2018). DOI: 10.1002/anie.201805144

[10] Birrell, J. A., Pelmenschikov, V., Mishra, N., Wang, H., Yoda, Y., Tamasaku, K., *et al.* Spectroscopic and computational evidence that [FeFe] hydrogenases operate exclusively with CO-bridged intermediates. *J. Am. Chem. Soc.* **142**, 222–232 (2020). DOI: 10.1021/jacs.9b09745

[11] Gee, L. B., Pelmenschikov, V., Wang, H., Mishra, N., Liu, Y.-C., Yoda, Y., *et al.* Vibrational characterization of a diiron bridging hydride complex—a model for hydrogen catalysis. *Chem. Sci.* **11**, 5487–5493 (2020). DOI: 10.1039/D0SC01290D

[12] Rodríguez-Maciá, P., Galle, L. M., Bjornsson, R., Lorent, C., Zebger, I., Yoda, Y., *et al.* Caught in the H_{inact}: crystal structure and spectroscopy reveal a sulfur bound to the active site of an O_2 – stable state of [FeFe] hydrogenase. *Angew. Chem. Int. Ed. Engl.* published online (2020). DOI: 10.1002/

anie.202005208
[13] Homma-Takeda, S., Nishimura, Y., Watanabe, Y., Yukawa, M. & Ueno, S. Lobe-specific changes in zinc levels in the prostate of rats exposed to tributyltin chloride. *Int. J. PIXE* **15**, 131–138 (2005). DOI: 10.1142/S012908350500043X
[14] Homma-Takeda, S., Terada, Y., Iso, H., Ishikawa, T., Oikawa, M., Konishi, T., *et al.* Rubidium distribution in kidneys of immature rats. *Int. J. PIXE* **19**, 39–45 (2009). DOI: 10.1142/S0129083509001722
[15] Homma-Takeda, S., Kokubo, T., Terada, Y., Suzuki, K., Ueno, S., Hayao, T., *et al.* Uranium dynamics and developmental sensitivity in rat kidney. *J. Appl. Toxicol.* **33**, 685–694 (2013). DOI: 10.1002/jat.2870
[16] Homma-Takeda, S., Kitahara, K., Suzuki, K., Blyth, B. J., Suya, N., Konishi, T., *et al.* Cellular localization of uranium in the renal proximal tubules during acute renal uranium toxicity. *J. Appl. Toxicol.* **35**, 1594–1600 (2015). DOI: 10.1002/jat.3126
[17] Kitahara, K., Numako, C., Terada, Y., Nitta, K., Shimada, Y. & Homma-Takeda, S. Uranium XAFS analysis of kidney from rats exposed to uranium. *J. Synchron Radiat.* **24**, 456–462 (2017). DOI: 10.1107/S1600577517001850
[18] Homma-Takeda, S., Numako, C., Kitahara, K., Yoshida, T., Oikawa, M., Terada, Y., *et al.* Phosphorus localization and its involvement in the formation of concentrated uranium in the renal proximal tubules of rats exposed to uranyl acetate. *Int. J. Mol. Sci.* **20**, 4677 (2019). DOI: 10.3390/ijms20194677
[19] Homma-Takeda, S., Uehara, A., Yoshida, T., Numako, C., Sekizawa, O., Nitta, K., *et al.* Two-dimensional μXAFS analysis for accumulated uranium in kidneys of rats exposed to uranyl acetate. *Radiat. Phys. Chem.* **175**, 108147 (2020). DOI: 10.1016/j.radphyschem.2019.02.006

(*Edited by Haruki Nakamura*)