Molecular Science of Bio-Metal Dynamics: Understanding and Regulation of Metals in the Cells

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Metals play important roles in sustaining life. Cells are mainly composed of water, proteins, and lipids, but they also contain small amounts of metals that help maintain health by being acquired from food. Those metals have been known for many years to be used as active centers of enzymes that carry out functions essential to sustaining life, *e.g.* transport and storage of oxygen, energy production, gene synthesis. However, the series of molecular mechanisms underlying metal dynamics in the body (absorption, sensing, transport, storage, and excretion of metals) and selectivity for individual metals to maintain the metal homeostasis remain unknown (Figure 1). We focus on "iron," which is the most important metal among the essential metals for sustaining life of living things, and various proteins that play a role in the selective absorption, sensing, and intracellular transport of iron in food. We are not only elucidating the structure of related proteins but also exploring their relationship with their functions in human cells.

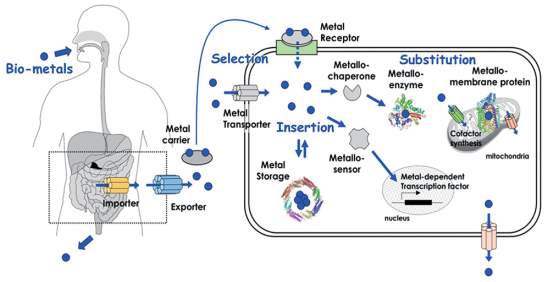

Figure 1. Our aim is to understand the uptake, trafficking, and regulation of "bio-metals" through the relay of protein–protein interactions.

Selected Publications

- M. Ganasen, H. Togashi, H. Takeda, H. Asakura, T. Tosha, K. Yamashita, K. Hirata, Y. Nariai, T. Urano, X. Yuan, I. Hamza, A. G. Mauk, Y. Shiro, H. Sugimoto and H. Sawai, "Structural Basis for Promotion of Duodenal Iron Absorption by Enteric Ferric Reductase with Ascorbate," *Commun. Biol.* **1**, 120 (2018). DOI: 10.1038/s42003-018-0121-8
- G. S. A. Wright, A. Saeki, T. Hikima, Y. Nishizono, T. Hisano, M. Kamaya, K. Nukina, H. Nishitani, H. Nakamura, M. Yamamoto, S. V. Antonyuk, S. Samar Hasnain, Y. Shiro and H. Sawai, "Architecture of the Complete Oxygen-Sensing FixL-FixJ Two-Component Signal Transduction System," *Sci. Signaling* **11**, eaaq0825 (2018). DOI: 10.1126/scisignal.aaq0825
- M. Nishinaga, H. Sugimoto, Y. Nishitani, S. Nagai, S. Nagatoishi, N. Muraki, T. Tosha, K. Tsumoto, S. Aono, Y. Shiro and H. Sawai, "Heme Controls the Structural Rearrangement of Its Sensor Protein Mediating the Hemolytic Bacterial Survival," *Commun. Biol.* **4**, 467 (2021). DOI: 10.1038/s42003-021-01987-5
- H. Sawai, "Molecular Science of Biological Iron: Dynamics and Regulation of Iron Ions and Heme Iron," *Artif. Blood* **33**, 55–63 (2025).

1. Development of Intracellular Fe Imaging in Living Cells Using Soft X-Ray Microscopy

The eminent physician of ancient Greece, Hippocrates, is reputed to have stated that "iron acts as a medicine," thereby underscoring the maintenance of metal homeostasis within the human body, as well as the pathological consequences arising from altered metal quantities and distributions, which remain enigmatic to this day. Therefore, the regulatory mechanisms of metals in the body and the factors of diseases related to altered metal concentrations are not yet fully understood. In response to these issues, our group, together with Dr. Iwayama's group at UVSOR, has started to develop new techniques to visualize metals in living cells by chemical species using soft X-rays from this year. Dr. Iwayama has developed the "contact-type soft X-ray microscope"[1] for biological samples. This technique allows the measurement of transmitted X-ray images of biological samples at the K- or L-edge energies of metal elements. Biological samples are placed on a Ce:YAG scintillator and covered with a Si_3N_4 membrane. The transmitted X-ray image is converted into a visible-light image by the scintillator and then captured by a CMOS camera. By changing the photon energies, we can obtain photon-energy dependence of transmitted images. From the Lambert-Beer's law, we can obtain a XANES spectrum for each pixel of the image. The experiment was performed on the beamline BL4B at UVSOR.

Our group is conducting research on iron in living organisms. Iron is an essential metal for all living organisms because of its unique chemical properties that control physiological functions essential for life.[2] Conversely, iron within cells is predominantly found in the reduced form of iron ions (Fe^{2+}). However, in a cellular environment where oxygen is present, excess iron can promote reactions that generate hydroxyl radicals, the most potent reactive oxygen species. Oxidized iron ions (Fe^{3+}) have extremely low water solubility and low toxicity.[3] However, in a reducing intracellular environment, they are easily converted to Fe^{2+}, indicating that the accumulation of Fe^{3+} can also be a factor in non-alcoholic steatohepatitis, multi-organ dysfunction due to iron overload (hereditary hemochromatosis) and neurodegenerative diseases. Therefore, the technique that can distinguish between Fe^{2+} and Fe^{3+} in living cells and tissues and simultaneously observe their distribution and local concentrations would be useful, but such technology has not yet been developed. First, we tried to observe intracellular iron levels in MDCK cells. However, due to the low iron concentration, we were unable to obtain soft X-ray transmission images with sufficient intensity for analysis. Therefore, in this study, we attempted iron imaging using red blood cells, which have the highest iron content in the body, as a model cell. We succeeded in observing the localized distribution of Fe^{2+} and Fe^{3+} in red blood cells isolated from preserved bovine blood (Figure 2). Moving forward, we will develop a comprehensive library of XANES spectra of hemoglobin to visualize the distribution of iron in hemoglobin based on their states, such as oxygen-bound or met form. We will also conduct similar iron imaging studies in cells with high iron content, such as liver cells.

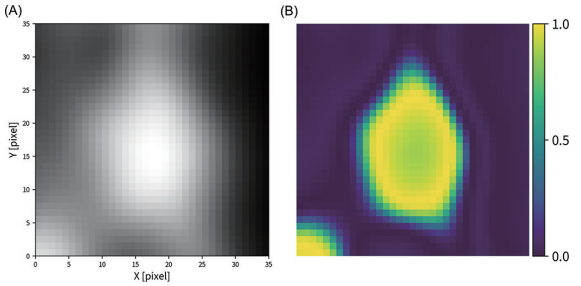

Figure 2. Fe imaging results of a single red blood cell from bovine blood. (A) XANES image of the cell detected at 700 eV. (B) Maps of spectral features calculated from the XANES spectra at each pixel position. This map shows that the Fe^{2+} species are located in the yellowish area.

In addition to developing this new technology, our group has been engaged in research for a few years on the interactions of proteins involved in the absorption, concentration sensing, transport, and storage of iron in cells. We also investigated the maturation process of iron-binding proteins using intracellular iron delivery chaperones.[4] This year, we initiated research endeavors aimed at identifying novel genes (proteins) that are influenced by fluctuations in intracellular metal levels. This objective will be achieved by CRISPR screening in cases where intracellular metal levels are high.

Our research has begun to focus on establishing a framework for the future, in which diseases stemming from disruptions in metal homeostasis can be identified at an early stage. This objective will be pursued by leveraging the application of soft X-ray spectroscopy, a technique employed in materials science, to develop novel methodologies within the realm of life sciences (Figure 3).

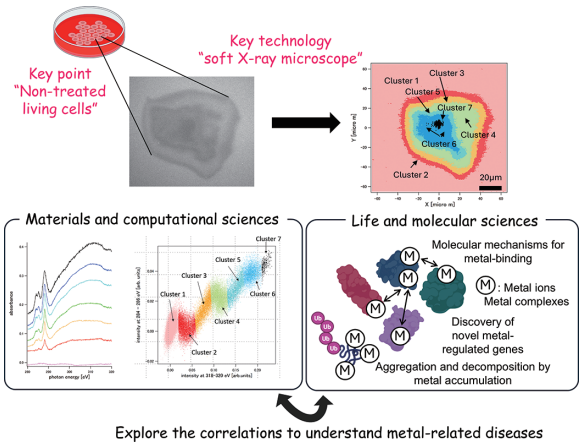

Figure 3. Concept diagram of this study.

References

1) T. Ejima *et al.*, *J. Phys.: Conf. Ser.* **463**, 1 (2013).
2) N. C. Andrews, *Nat. Rev. Genet.* **1**, 208–217 (2000).
3) H. Sawai, *Artif. Blood* **33**, 55–63 (2025).
4) H. Sawai *et al.*, *Iron in Biology: Molecular Structures, Cellular Processes and Living Systems*, Royal Society of Chemistry, 75–88 (2025).