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Commentary and Perspective

Live-cell imaging of bio-metal species

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Bioinorganic species play essential roles in living organisms. Figure 1 shows the elements found in living things so far, and most of them are known as essential elements for human beings [1]. In particular, the concentrations of the inorganic species must be controlled within each appropriate range because the dysfunctions of the homeostasis of inorganic species often cause diseases. Revealing the relationship between the dysfunction of inorganic species' homeostasis and diseases must lead to the development of new drugs for the treatment of the diseases such as cancer and neurodegenerative diseases. Live-cell imaging is a powerful method to investigate the alteration of inorganic species in living cells and has contributed to elucidating the roles and functions of inorganic species in living systems. We organized the symposium, "Live-cell Imaging of Bio-metal Species," at the 60th Annual Meeting of the Biophysical Society of Japan to introduce recent advances in imaging technologies for metal ions and inorganic species. This symposium is co-supported by Scientific Research on Innovative Areas, "Integrated Bio-metal Science" [2]. One of the ultimate goals of this area is to decipher the behaviors of bioinorganic species from whole organisms to molecular levels. In this context, cutting-edge methods to visualize bioinorganic species are required. In this symposium, we invited six upcoming researchers working on imaging of inorganic species to present their recent achievements.

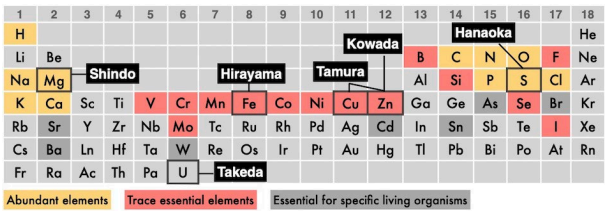

Figure 1 A periodic table highlighting abundant essential elements (yellow), trace essential elements (red), and essential elements for specific living organisms (dark gray). The target elements of each presenter are also highlighted.

Dr. Hirayama reported a Fe (II)-selective fluorescent probe and a heme-selective fluorescent probe. Iron is the most abundant transition metal in our body and is involved in various physiological activities such as oxygen transport and energy production. At the same time, its high redox activity triggers oxidative stress when an iron overload occurs. Thus, as in the case of other metal species, iron status must be controlled within an appropriate level. RhoNox-4, the most sensitive and robust fluorescent probe for labile Fe (II) ever, was presented [3]. The probe was applied to high-throughput screening (HTS) of compounds that fluctuate subcellular iron status. He also presented the development of a heme-selective fluorescent probe and its application to live-cell imaging. Heme is also essential chemical species to almost all living organisms, but its cellular trafficking remains unknown. The probe is highly sensitive and selective to heme and succeeded in visualizing the upregulation of labile heme during ferroptosis [4].

Dr. Kowada presented organelle-specific quantitative imaging of Zn (II) with their unique Zn (II) fluorescent probes fused with a HaloTag ligand. Zinc is the second most abundant transition metal ion and acts as a signaling mediator and enzyme cofactor. The concentrations of Zn (II) vary from organelle to organelle, and alterations in Zn (II) concentrations

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have been a target of interest to many researchers. In this symposium, organelle-specific Zn (II) fluorescent probes were presented to establish organelle-specific quantification of Zn (II) ion by taking advantage of a fine-tunable property of small-molecule probes and high subcellular localization selectivity of HaloTag technology [5]. He established a series of robust Zn (II) fluorescent probes, ZnDA series, having various affinities to fit the Zn concentrations of each organelle. The probes are inert against pH changes, which often interfere with the fluorescence readout of chelate-based probes. The precise control of localization and robust fluorescence readout in response to the change in Zn (II) status enabled the quantification of Zn (II) concentrations in the endoplasmic reticulum, Golgi apparatus, mitochondria, nucleus, and cytosol. The quantitative imaging revealed the subcellular distribution of Zn (II) as nucleus ~ cytosol > mitochondria > endoplasmic reticulum [6].

Dr. Tamura showed new chemical tools for conditional proteomics for metal-associating proteins. A Zn-selective proteomics probe, AIZin, is activated by Zn (II) to form a covalent bond with adjacent proteins [7]. Proteomics analysis of the labeled proteins reveals the proteomes locating subcellular domains where Zn (II) is upregulated. The probe can also be applied to image the subcellular distribution of Zn (II) in living cells. He presented the application of AIZin to image and omics analysis of nitric oxide (NO)-induced compartmentalization of Zn (II). An imaging study with AIZin revealed that the distribution of Zn (II) varies time-dependently from the whole cell to granular structures. The MS analysis of the proteomes demonstrated that the probe is mainly bound to ER- and Golgi apparatus-relevant proteins. Using this technique, we can know the relationship between the spatiotemporal distribution of Zn (II) and proteins surrounding the Zn (II) concentrated spots. He also presented Cu (I)-selective conditional proteomics probe, which was successfully applied to live-cell imaging and proteomics analysis of the cells over-expressing copper transporter.

Dr. Takeda gave a talk on quantum beam-based imaging technologies. Despite its incompatibility with live-cell imaging, quantum beam-based imaging technologies are powerful in knowing the distribution of elements, including metal ions and typical elements, simultaneously without any staining. She introduced the principles and applicability of PIXE (particle-induced X-ray emission), SR-XRF (X-ray fluorescence spectrometry using synchrotron radiation), and XAFS (X-ray absorption fine structure). PIXE and SR-XRF are used for mapping elements, and XAFS is used for determining chemical speciation, including redox states and coordination states. In this symposium, Dr. Takeda demonstrated uranium mapping in tissue samples to elucidate the chemical toxicity of uranium. The chemical mapping using μ-PIXE, SR-μ-XRF, and μ-XAFS with sub-micrometer resolution revealed that uranium was concentrated in the phosphorus-concentrated area as U(VI) state in the proximal tubules of the kidney of uranyl acetate-treated rats [8]. Single-cell analysis using μ-PIXE revealed the intracellular distribution of uranium in perinuclear regions [9].

Dr. Shindo presented live-cell imaging of magnesium ion (Mg (II)) with unique fluorescent probes. Mg (II) is highly abundant in cells (0.5~1.2 mM), but the biological meanings of its fluctuations remain unknown due to its relatively small change in concentration compared to Ca (II). His group developed a series of magnesium sensors, KMG probes, which show highly selective fluorescence turn-on in response to Mg (II). KMG-104 [10] was successfully applied to a simultaneous live-cell imaging study of Mg (II) and Ca (II) by virtue of the insensitivity to Ca (II), revealing that Ca (II) and Mg (II) change differently. He also talked about ongoing project of live-cell imaging study of Mg (II) and ATP by using a near-infrared Mg (II) fluorescent probe, KMG-501 [11] and a fluorescent protein-based FRET sensor. Monitoring the crosstalk of metal cations and other biological molecules would be one of the significant issues of live-cell imaging study.

Prof. Hanaoka presented his original research on developing fluorescent probes for hydrogen sulfide and their applications to high-throughput screening. Persulfides are important inorganic species that regulate redox signaling and sulfur signaling. In the catabolism of sulfur species, one of the initial sources of persulfides is provided as hydrogen sulfide, the biosynthesis of which involves three major enzymes, 3-MST (3-mercaptopyruvate sulfur transferase), CSE (cystathionine γ-lyase), and CBS (cystathionine β-synthase). He reported several fluorescent probes for hydrogen sulfide, one is based on sulfur-induced demetallation of copper complex, and the other is a sulfur-mediated reduction of azides to amines. HSip-1 [12] was successfully applied to high-throughput screening to find new selective inhibitors for 3-MST [13] and CSE. The found inhibitors showed selective inhibition activity to 3-MST and CSE, respectively. The co-crystal analysis of the inhibitor-bound 3-MST revealed that the enzymatic reaction stopped with its persulfide form. He has recently succeeded in the discovery of a new selective inhibitor to CBS. Since the discovered compounds are highly selective to their own target, these inhibitors will make it possible to elucidate the sulfur signaling.

To understand the homeostasis and pathogenetic roles of bioinorganic species, cutting-edge imaging technologies, including the development of new chemical probes and imaging modalities, must be required. The speakers presented their original techniques for imaging bioinorganic species in this symposium.

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