RESEARCH ACTIVITIES

Life and Coordination-Complex Molecular Science

Department of Life and Coordination-Complex Molecular Science is composed of two divisions of biomolecular science, two divisions of coordination-complex molecular science, and one adjunct division. Biomolecular science divisions cover the studies on functions, dynamic structures, and mechanisms for various biomolecules such as sensor proteins, membrane-anchored proteins, biological-clock proteins, metalloproteins, glycoconjugates, and molecular chaperone. Coordination-complex divisions aim to develop molecular catalysts and functional metal complexes for transformation of organic molecules, water oxidation and reduction, and molecular materials such as molecular wires. Interdisciplinary alliances in this department aim to create new basic concepts for the molecular and energy conversion through the fundamental science conducted at each division. During this year, professor Tetsuro Murahashi (Research Center of Integrative Molecular Systemes) was moved out from IMS.

Bioinorganic Chemistry of Metalloproteins Responsible for the Homeostasis Control

Department of Life and Coordination-Complex Molecular Science
Division of Biomolecular Functions

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Professor, Okazaki Institute for Integrative Bioscience
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Keywords Bioinorganic Chemistry, Metalloproteins, Sensor Protein

Transition metal ions and metalloproteins play crucial roles in meeting the energy demands of the cell by playing roles in intermediary metabolism and in signal transduction processes. Although they are essential for biological function, metal ion bioavailability must be maintained within a certain range in cells due to the inherent toxicity of all metals above a threshold. This threshold varies for individual metal ions. Homeostasis of metal ions requires a balance between the processes of uptake, utilization, storage, and efflux and is achieved by the coordinated activities of a variety of proteins including extracytoplasmic metal carriers, ion channels/pumps/transporters, metal-regulated transcription and translation proteins, and enzymes involved in the biogenesis of metal-containing cofactors/metalloproteins. In order to understand the processes underlying this complex metal homeostasis network, the study of the molecular processes that determine the protein–metal ion recognition, as well as how this event is transduced into a functional output, is required. My research interests are focused on the elucidation of the structure and function relationships of metalloproteins responsible for the regulation of biological homeostasis.

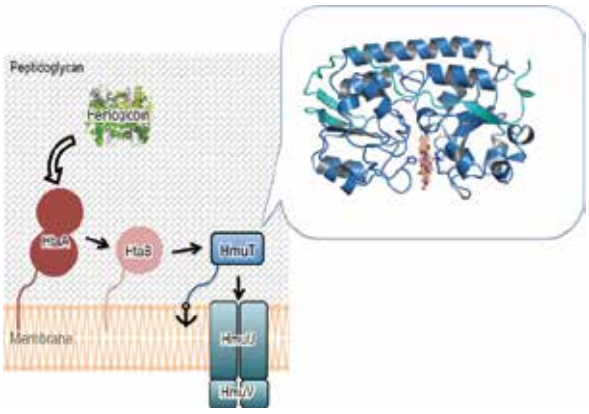

Figure 1. Schematic view of heme uptake system in *Corynebacterium glutamicum* and the crystal structure of HmuT that transports heme to the heme transporter HmuUV.

Selected Publications

- C. Kitatsuji, M. Ogura, T. Uchida, K. Ishimori and S. Aono, "Molecular Mechanism for Heme-Mediated Inhibition of 5-Aminolevulinic Acid Synthase 1," *Bull. Chem. Soc. Jpn.* **87**, 997–1004 (2014).
- Y. Okamoto, H. Sawai, M. Ogura, T. Uchida, K. Ishimori, T. Hayashi and S. Aono, "Heme-Binding Properties of HupD Functioning as a Substrate-Binding Protein in a Heme-Uptake ABC-Transporter System in *Listeria monocytogenes*," *Bull. Chem. Soc. Jpn.* **87**, 1140–1146 (2014).
- S. Aono, "The Dos Family of Globin-Related Sensors Using PAS Domains to Accommodate Haem Acting as the Active Site for Sensing External Signals," *Adv. Microb. Physiol.* **63**, 273–327 (2013).
- H. Sawai, M. Yamanaka, H. Sugimoto, Y. Shiro and S. Aono, "Structural Basis for the Transcriptional Regulation of Heme Homeostasis in *Lactococcus lactis*," *J. Biol. Chem.* **287**, 30755–30768 (2012).
- H. Sawai, H. Sugimoto, Y. Shiro and S. Aono, "Structural Basis for Oxygen Sensing and Signal Transduction of the Heme-Based Sensor Protein Aer2 from *Pseudomonas aeruginosa*," *Chem. Commun.* **48**, 6523–6525 (2012).
- S. Aono, "Novel Bacterial Gas Sensor Proteins with Transition-Metal-Containing Prosthetic Groups as Active Sites," *Antioxid. Redox Signaling* **16**, 678–686 (2012).

1. Structure and Function of CgHmuT that is a Heme Binding Protein for the ABC-Type Heme Transporter CgHmuUV

As iron is an essential trace element for most of organisms, they develop sophisticated iron acquisition systems. Pathogenic bacteria can use heme as an iron source partly because heme is the most abundant iron species in their host. However, there is little free heme molecule as most of heme molecules are tightly bound to hemoproteins as a prosthetic group. Therefore, some heme acquisition system is required to use heme in hemoproteins as an iron source.

In Gram-negative bacteria, hemophores that are secreted to the extracellular medium acquire heme from hemoproteins and transport it to a specific outer membrane receptor. The outer membrane receptor transports heme across the outer membrane to the periplasmic space, where a periplasmic heme-binding protein binds heme to transport it to an ABC-type heme transporter. On the other hand, in Gram-positive bacteria, heme uptake occurs by direct interaction between hemoproteins or heme and the membrane anchored proteins responsible for heme binding and transport. In a Gram-positive bacterium *Corynebacterium glutamicum*, heme is captured by the membrane anchored heme binding proteins, HtaA and HtaB proteins, and then heme is transferred to HmuT, which is a heme-binding protein for the ABC-type heme transporter HmuUV. Heme is transported into cytoplasm by this ABC transporter. While this heme uptake process is proposed based on the genetic and microbiological studies, the molecular mechanisms of heme uptake/transport are not obvious mainly due to a lack of structural information of these proteins. We have characterized HmuT from *Corynebacterium glutamicum* (CgHmuT) by X-ray crystallography to elucidate the molecular mechanism of heme transport by CgHmuT.

The structure of CgHmuT was determined at a resolution of 1.42 Å. CgHmuT showed a basket handle shape, where a long α helix is connected the N- and C-terminal domains (Figure 1). There was a cleft between the N- and C-terminal domains, in which one heme molecule was accommodated with His141 and Tyr240 as axial ligands that were located at the loop regions in the N- and C-terminal domains, respectively. Intriguingly, it was shown that heme was accommodated in the heme-binding site of CgHmuT with two different orientations. As protoheme bound to CgHmuT has an asymmetric structure, there are two possible orientations of heme when it is accommodated in the heme-binding site of CgHmuT. When a single orientation of heme was assumed in the model refinement, the residual electron densities were observed in the F_O-F_C map. On the other hand, good fitting of the model into the electron densities was obtained without any residual electron densities when 1:1 mixture of two orientations of heme was assumed, indicating the existence of the two different orientation of heme in CgHmuT.

2. A Novel Photosensor Protein CarH Using Vitamin B12 as a Photosensing Unit

Vitamin B12 is well known as a cofactor for the B12-dependent enzymes that catalyze carbon skeleton rearrangement or elimination reactions, where Co–C bond hemolysis takes place to form the radical species as the reaction intermediate. Recently, a novel biological function of vitamin B12 has been reported: A photosensor protein CarH utilizes adenosylcobalamin (vitamin B12) as its senor unit for light sensing. We are now working on CarH from *Thermus thermophilus* to elucidate the molecular mechanisms of photosensing and signal transduction of CarH.

* IMS International Internship Program

Dynamical Ordering of Biomolecular Systems for Creation of Integrated Functions

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Awards
2000 The Pharmaceutical Society of Japan Award for Young Scientists
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Keywords Biomolecule, Dynamical Ordering, NMR

Living systems are characterized as dynamic processes of assembly and disassembly of various biomolecules that are self-organized, interacting with the external environment. The omics-based approaches developed in recent decades have provided comprehensive information regarding biomolecules as parts of living organisms. However, fundamental questions still remain unsolved as to how these biomolecules are ordered autonomously to form flexible and robust systems (Figure 1). Biomolecules with complicated, flexible structures are self-organized through weak interactions giving rise to supramolecular complexes that adopt their own dynamic, asymmetric architectures. These processes are coupled with expression of integrated functions in the biomolecular systems.

Toward an integrative understanding of the principles behind the biomolecular ordering processes, we conduct multidisciplinary approaches based on detailed analyses of dynamic structures and interactions of biomolecules at atomic level, in conjunction with the methodologies of molecular and cellular biology along with synthetic and computational technique.

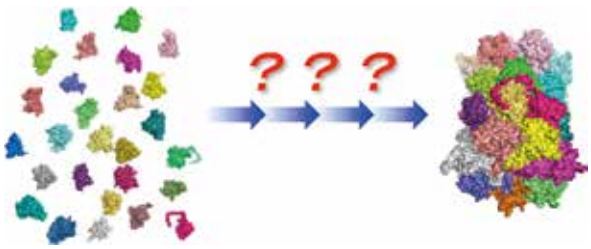

Figure 1. Formation of supramolecular machinery through dynamic assembly and disassembly of biomolecules.

Selected Publications

- M. Yagi-Utsumi and K. Kato, "Structural and Dynamic Views of GM1 Ganglioside," *Glycoconjugate J.* **32**, 105–112 (2015).
- T. Satoh, T. Yamaguchi and K. Kato, "Emerging Structural Insights into Glycoprotein Quality Control Coupled with *N*-Glycan Processing in the Endoplasmic Reticulum," *Molecules* **20**, 2475–2491 (2015).
- Y. Zhang, T. Yamaguchi, M. Yagi-Utsumi, Y. Kamiya, Y. Sakae, Y. Okamoto and K. Kato, "Conformational Dynamics of Oligosaccharides Characterized by Paramagnetism-Assisted NMR Spectroscopy in Conjunction with Molecular Dynamics Simulation," in *Advances in Experimental Medicine and Biology*, Springer; Switzerland, **842**, pp. 217–230 (2015).
- T. Yamaguchi and K. Kato, "Paramagnetism-Assisted Nuclear Magnetic Resonance Analysis of Dynamic Conformations and Interactions of Oligosaccharides," in *Glycoscience: Biology and Medicine*, Springer; Japan, **1**, pp. 137–145 (2014).
- Y. Kamiya, T. Satoh and K. Kato, "Recent Advances in Glycoprotein Production for Structural Biology: Toward Tailored Design of Glycoforms," *Curr. Opin. Struct. Biol.* **26**, 44–53 (2014).
- Y. Kamiya, T. Satoh and K. Kato, "Molecular and Structural Basis for *N*-Glycan-Dependent Determination of Glycoprotein Fates in Cells," *Biochim. Biophys. Acta, Gen. Subj.* **1820**, 1327–1337 (2012).

1. Exploration of Conformational Spaces of Flexible Oligosaccharides

Conformational dynamics are essential properties of biomacromolecules that are involved in molecular recognition events in living systems. The motional freedom of three-dimensional structures can endow them with adaptability to various interaction partners, occasionally in promiscuous fashions. We employed stable isotope- and lanthanide-assisted NMR approaches in conjunction with replica-exchange molecular dynamics (REMD) simulations to obtain atomic descriptions of the conformational dynamics of high-mannose-type oligosaccharides, which harbor intracellular glycoprotein-fate determinants in their triantennary structures.[1] The experimentally validated REMD simulation provided quantitative views of the dynamic conformational ensembles of the complicated, branched oligosaccharides, and indicated significant expansion of the conformational space upon removal of a terminal mannose residue during the functional glycan-processing pathway (Figure 2).

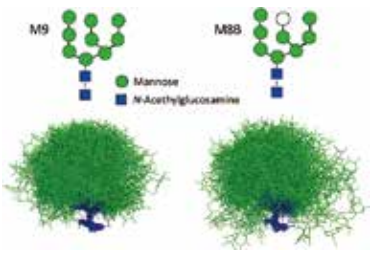

Figure 2. Superimpositions of 240 conformers derived from NMR-validated replica exchange MD simulations of the high-mannose-type M9 (left) and M8B (right) oligosaccharides.

2. Structural Characterization of Biomolecular Interactions Involved in Protein Fate Determination

Using NMR spectroscopy and X-ray crystallography, we characterized structures and interactions of multidomain proteins involved in fate determination of other proteins in living systems. In the endoplasmic reticulum, folding of newly synthetized proteins is facilitated through interaction with various proteins including molecular chaperones. We determined three-dimensional structures of the putative substrate-binding domains of UDP-glucose:glycoprotein glucosyltransferase (UGGT), a folding sensor enzyme, and protein disulfide isomerase (PDI), a folding catalyst, underscoring the importance of conformational changes in substrate recognition.[2,3]

Many of proteins in cells are destroyed primarily by ubiquitin-/proteasome-mediated protein degradation system. We applied a paramagnetic NMR technique to determine the mode of substrate recognition by the Josephin domain of ataxin-3, which has an endo-type deubiquitinase activity.[4] Moreover, our NMR study revealed that Ump1, a proteasome assembly chaperone, is an intrinsically unstructured protein and largely devoid of secondary structural elements.[5]

Our NMR data also contributed to providing structural bases of interactions of amyloidogenic proteins with self-assembled spherical complex displaying a gangliosidic glycan cluster (collaboration with Dr. Sota Sato, Tohoku University and Dr. Makoto Fujita, the University of Tokyo) and with SorLA, a neuronal sorting receptor considered to be a major risk factor for Alzheimer's disease (in collaboration with Dr. Junichi Takagi, Osaka University).[6,7]

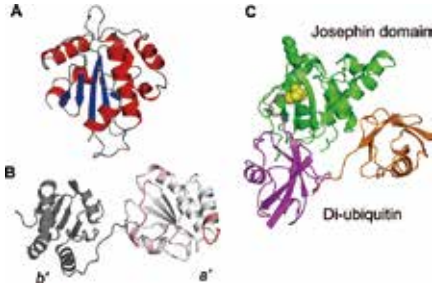

Figure 3. 3D Structures of (A) the Trx3 domain of UGGT, (B) PDI *b'-a'* domains, and (C) the Josephin domain of ataxin-3 complexed with di-ubiquitin.

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3) K. Inagaki, T. Satoh, S. G. Itoh, H. Okumura and K. Kato, *Chem. Phys. Lett.* **618**, 203–207 (2015).
4) T. Satoh, A. Sumiyoshi, M. Yagi-Utsumi, E. Sakata, H. Sasakawa, E. Kurimoto, Y. Yamaguchi, W. Li, C. A. P. Joazeiro, T. Hirokawa and K. Kato, *FEBS Lett.* **588**, 4422–4430 (2014).
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7) Y. Kitago, M. Nagae, Z. Nakata, M. Yagi-Utsumi, S. Takagi-Niidome, E. Mihara, T. Nogi, K. Kato and J. Takagi, *Nat. Struct. Mol. Biol.* **22**, 199–206 (2015).

Awards

TONG, Zhu; Young Presentation Award, The 87th Annual Meeting of the Japanese Biochemical Society (2014).
YAGI-UTSUMI, Maho; Poster Presentation Award, The 3rd International Symposium of "Dynamical ordering of biomolecular systems for creation of integrated functions" (2015).
SIKDAR, Arunima; Poster Presentation Award, The Winter School of Sokendai/ Asian CORE Program (2015).

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Operation and Design Principles of Biological Molecular Machines

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Keywords Single-Molecule Biophysics, Molecular Machines, Molecular Motors

Activity of life is supported by various molecular machines made of proteins and nucleic acids. These biological molecular machines show high performance such as reaction specificity and energy conversion efficiency, and are superior to man-made machines in some aspects.

One of the representatives of the molecular machines is linear and rotary molecular motors (Figure 1). Molecular motors generate mechanical forces and torques that drive their unidirectional motions from the energy of chemical reaction or the electrochemical potential.

We will unveil operation principles of biological molecular motors and machines with single-molecule techniques based on optical microscopy. We will also try to create new biological molecular motors and machines to understand their design principles. Our ultimate goal is controlling living organisms with created molecular machines.

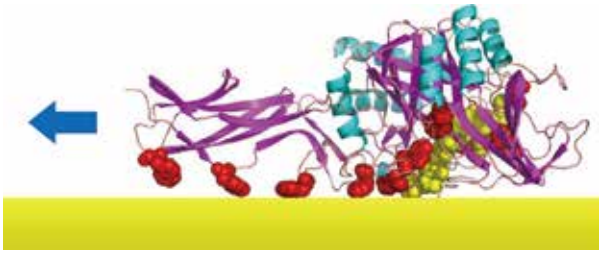

Figure 1. A linear molecular motor chitinase. Chitinase moves on the substrate crystalline chitin unidirectionally and processively, driven by the energy of hydrolysis of the chain end of the chitin.

Selected Publications

- R. Iino, H. Ueno, Y. Minagawa, K. Suzuki and T. Murata, "Rotational Mechanism of *Enterococcus hirae* V_1-ATPase by Crystal-Structure and Single-Molecule Analyses," *Curr. Opin. Struct. Biol.* **31**, 49–56 (2015).
- Y. Shibafuji, A. Nakamura, T. Uchihashi, N. Sugimoto, S. Fukuda, H. Watanabe, M. Samejima, T. Ando, H. Noji, A. Koivula, K. Igarashi and R. Iino, "Single-Molecule Imaging Analysis of Elementary Reaction Steps of *Trichoderma reesei* Cellobiohydrolase I (Cel7A) Hydrolyzing Crystalline Cellulose I_α and III_I," *J. Biol. Chem.* **289**, 14056–14065 (2014).
- R. Iino and H. Noji, "Intersubunit Coordination and Cooperativity in Ring-Shaped NTPases," *Curr. Opin. Struct. Biol.* **23**, 229–234 (2013).
- Y. Minagawa, H. Ueno, M. Hara, Y. Ishizuka-Katsura, N. Ohsawa, T. Terada, M. Shirouzu, S. Yokoyama, I. Yamato, E. Muneyuki, H. Noji, T. Murata and R. Iino, "Basic Properties of Rotary Dynamics of the Molecular Motor *Enterococcus hirae* V_1-ATPase," *J. Biol. Chem.* **288**, 32700–32707 (2013).
- R. Watanabe, K. V. Tabata, R. Iino, H. Ueno, M. Iwamoto, S. Oiki and H. Noji, "Biased Brownian Stepping Rotation of F_oF_1-ATP Synthase Driven by Proton Motive Force," *Nat. Commun.* **4**, 1631 (2013).
- T. Uchihashi, R. Iino, T. Ando and H. Noji, "High-Speed Atomic Force Microscopy Reveals Rotary Catalysis of Rotorless F_1-ATPase," *Science* **333**, 755–758 (2011).

1. Key Chemical Factors of Arginine Finger Catalysis of F_1-ATPase Clarified by an Unnatural Amino Acid Mutation[1)]

A catalytically important arginine, called Arg finger, is employed in many enzymes to regulate their functions through enzymatic hydrolysis of nucleotide triphosphates. F_1-ATPase, a rotary molecular motor, possesses Arg fingers which catalyze hydrolysis of adenosine triphosphate (ATP) for efficient chemo-mechanical energy conversion. In this study, we examined the Arg finger catalysis by single-molecule measurements for a mutant of F_1-ATPase in which the Arg finger is substituted with an unnatural amino acid of a lysine analogue, 2,7-diaminoheptanoic acid (Lyk). The use of Lyk, of which the side chain is elongated by one CH_2 unit so that its chain length to the terminal nitrogen of amine is set to be equal to that of arginine, allowed us to resolve key chemical factors in the Arg finger catalysis, *i.e.*, chain length matching and chemical properties of the terminal groups. Rate measurements by single-molecule observations showed that the chain length matching of the side-chain length is not a sole requirement for the Arg finger to catalyze the ATP hydrolysis reaction step, indicating the crucial importance of chemical properties of the terminal guanidinium group in the Arg finger catalysis. On the other hand, the Lyk mutation prevented severe formation of an ADP inhibited state observed for a lysine mutant and even improved the avoidance of inhibition compared with the wild-type F_1-ATPase. The present study demonstrated that incorporation of unnatural amino acids can widely extend with its high "chemical" resolution biochemical approaches for elucidation of the molecular mechanism of protein functions and furnishing novel characteristics.

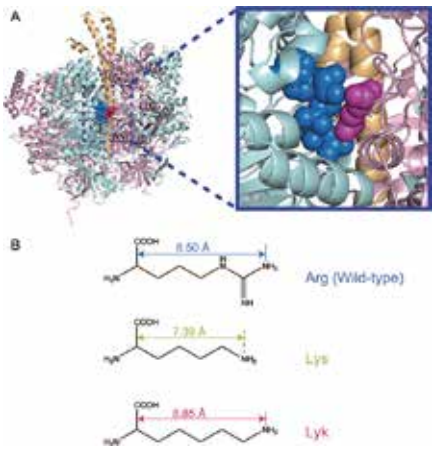

Figure 2. (A) Crystal structure of mitochondrial F_1–ATPase viewed from the side, β_{DP}/α_{DP} catalytic interface. The α, β, and γ subunits are shown in pearl pink, pearl blue, and pearl yellow, respectively. The "arginine finger" in the α subunit is shown by pink space-filling model. AMP-PNP bound to the catalytic site are shown by blue space-filling model. (B) Chemical structures and side-chain length of arginine (Arg, top), lysine (Lys, middle), and 2,7-diaminoheptanoic acid (Lyk, bottom).

2. High-Speed Angle-Resolved Imaging of Single Gold Nanorod with Microsecond Temporal Resolution and One-Degree Angle Precision[2)]

We developed two types of high-speed angle-resolved imaging methods for single gold nanorods (SAuNRs) using objective-type vertical illumination dark-field microscopy and a high-speed CMOS camera to achieve microsecond temporal and one-degree angle resolution. These methods are based on: (i) an intensity analysis of focused images of SAuNR split into two orthogonally polarized components and (ii) the analysis of defocused SAuNR images. We determined the angle precision (statistical error) and accuracy (systematic error) of the resultant SAuNR (80 nm × 40 nm) images projected onto a substrate surface (azimuthal angle) in both methods. Although both methods showed a similar precision of ~1° for the azimuthal angle at a 10 μs temporal resolution, the defocused image analysis showed a superior angle accuracy of ~5°. In addition, the polar angle was also determined from the defocused SAuNR images with a precision of ~1°, by fitting with simulated images. By taking advantage of the defocused image method's full revolution measurement range in the azimuthal angle, the rotation of the rotary molecular motor, F_1-ATPase, was measured with 3.3 μs time resolution. The time constants of the pauses waiting for the elementary steps of the ATP hydrolysis reaction and the torque generated in the mechanical steps have been successfully estimated. The high-speed angle-resolved SAuNR imaging methods will be applicable to the monitoring of the fast conformational changes of many biological molecular machines.

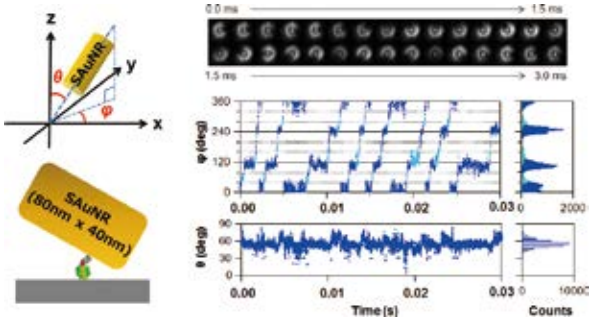

Figure 3. (Left) Schematic image of experimental system of rotation assay of F_1-ATPase using single gold nanorod (SAuNR) as a probe. (Right) Example of rotation of F_1-ATPase probed at 3.3 μs time resolution.

References

1) A. Yukawa, R. Iino, R. Watanabe, S. Hayashi and H. Noji, *Biochemistry* **54**, 472–480 (2015).
2) S. Enoki, R. Iino, Y. Niitani, Y. Minagawa, M. Tomishige and H. Noji, *Anal. Chem.* **87**, 2079–2086 (2015).

Supramolecular Chemical Approach to Construction of Artificial Cell

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Professional Employment
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Keywords Artificial Cell, Origin of Life, Vesicle

Exploring the boundary between a living and non-living matter is one of the most challenging problems for contemporary scientists. In order to understand a cell, which is a minimum unit of life, synthesis of an artificial cell from supramolecular chemical approach is a plausible strategy, because simple molecular assemblies evolved to a simple cell on prebiotic earth. As shown in Figure 1, the key elements of a cell are compartment, information and catalyst, *i.e.* metabolism. We have tackled the construction of a chemical artificial cell endowed with these three elements.

In our laboratory, we aim to construct the two artificial cells using giant vesicles (GV) as compartment. One is an artificial cell which can proliferate from generation to generation. This work is a collaboration with Sugawara group (Kanagawa Univ.). The other research is an artificial cell incorporating catalyst producing system. The GV system can generate catalyst and membrane molecule by transforming each precursors, which makes it possible for GVs to proliferate with producing catalyst.

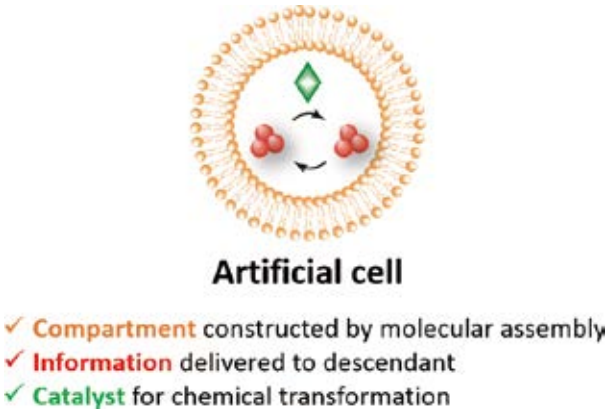

Figure 1. Artificial cell model
The replicating systems of compartment and the replicating system of information materials are combined. The reactions in the two replicating systems are accelerated by each proper catalysts.

Selected Publications

- K. Kurihara, M. Tamura, K-I. Shohda, T. Toyota, K. Suzuki and T. Sugawara, "Self-Reproduction of Supramolecular Giant Vesicles Combined with the Amplification of Encapsulated DNA," *Nat. Chem.* **3**, 775–781 (2011).
- T. Sugawara, K. Kurihara and K. Suzuki, "Constructive Approach towards Protocells," in *Engineering of chemical complexity*, world scientific lecture notes in complex systems, World Scientific Pub. Co. Inc., pp. 359–374 (2013).

1. An Artificial Cell with a Primitive Cell Cycle

One of the approaches for exploring the origin of life or elucidating of the functions of life is construction of an artificial cell from supramolecular chemical approach.[1,2] In collaboration with Sugawara's group, artificial cells which have three basic elements of a cell; information (DNA), compartment (giant vesicle (GV): A supramolecular assembly of amphiphiles) and metabolism (synthetic catalyst) have been constructed.[3] The artificial cellular system consisted of amplification of DNA by polymerase chain reaction and self-reproduction of GV by addition of membrane lipid precursor. Although this GV proliferated with distribution of internal amplified DNA, it ceased at the 2nd generation because of depletion of internal information substances.

Now, we construct a recursive vesicular artificial cell system with proliferation cycles, collaborating with Sugawara group. By using the vesicular transport system,[4] the 2nd generation of GVs which have no PCR reagents after self-reproduction was replenished by fusing with the conveyer GVs encapsulating the PCR reagents (Figure 2). The replenished GV can amplify the internal DNA and yield 3rd generation of the GV after addition of membrane lipid precursor. The GV system with replenishing system was constructed.[5] This system would lead to an evolvable artificial cellular system.

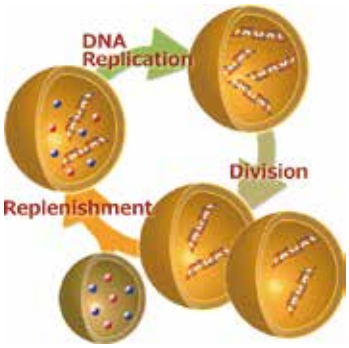

Figure 2. An artificial cell system with premitive cell cycle. After growth and division of GV, the substance-depleted GV was replenished by the vesicular fusion.

2. An Artificial Cell Containing a Catalyst-Producing System

A cell is a self-organized system which is able to maintain its state due to metabolism. The previous artificial cellular system have been so robust that it can self-reproduce only specific state in the any environments.

Here, we aim to realize a new artificial cellular system in which the GV self-organize its own composition spontaneously according to the environment. In order for GV to self-reproduce and self-maintain, it is necessary to combine metabolism and compartment. By introducing the cross-catalysis system (Figure 3), we construct an artificial cell in which catalysts are produced. After addition of membrane precursor aldehyde, the production of catalyst and membrane molecule was confirmed by NMR, microscopy observation. In this system, the GV was reproduced by the catalyst which catalyze the production of the GV membrane lipid molecule. The GV membrane provides the field where the catalyst is synthesized.

In addition, by changing the composition of the vesicular membrane, the production of catalyst and membrane molecule was oscillated by interacting each other. This means that the artificial cell incorporating the negative feedback is realized.

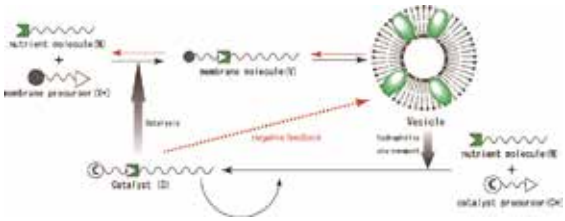

Figure 3. Scheme of new artificial cellular system. The membrane molecules of the GV was synthesized by the catalyst produced in the GV.

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Investigation of Molecular Mechanisms of Channels, Transporters and Receptors

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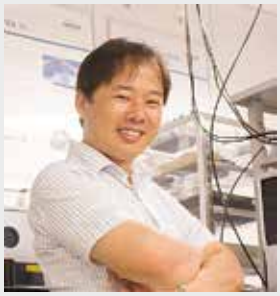

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Keywords Infrared Spectroscopy, Membrane Protein, Ion Channel

Membrane proteins are important for homeostasis and signaling of living cells, which work as ion channel, ion pump, various types of chemical and biophysical sensors, and so on. These proteins are considered as one of important targets for biophysical studies. Our main goal is to clarify molecular mechanisms underlying functions of the channels, transporters and receptors mainly by using stimulus-induced difference infrared spectroscopy, which is sensitive to the structural and environmental changes of bio-molecules.

We applied attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy to extract ion-binding-induced signals of various kinds of membrane proteins. For example, KcsA is a potassium channel, which is highly selective for K^+ over Na^+, and the selectivity filter binds multiple dehydrated K^+ ions upon permeation. Shifts in the peak of the amide-I signals towards lower vibrational frequencies were observed as K^+ was replaced with Na^+ (Figure 1). These vibrational modes give us precise structural information of the selectivity filter. Moreover, by changing concentrations of K^+ in buffer solutions, we can estimate affinity of the selectivity filter for K^+ ions.

Recently, we have developed a rapid-buffer exchange apparatus for time-resolved ATR-FTIR spectroscopy, which can be utilized for studying dynamics of structural transition in membrane proteins.

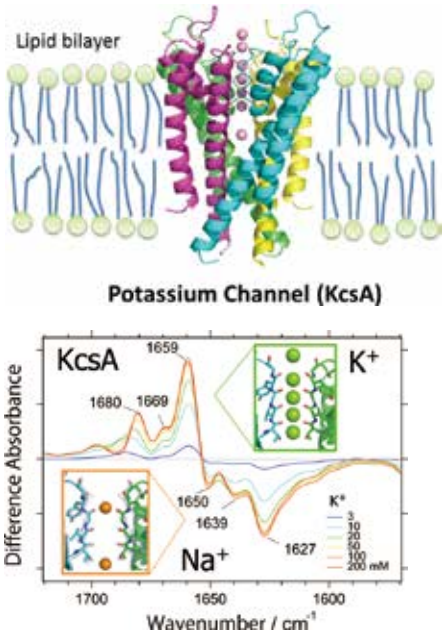

Figure 1. (top) X-ray crystal structure of a potassium ion channel, KcsA. (bottom) The ion-exchange induced difference infrared spectra of KcsA with different potassium ion concentration. The amide I bands are mainly originated from the carbonyl groups of the selectivity filter of KcsA.

Selected Publications

- Y. Furutani *et al.*, "ATR-FTIR Spectroscopy Revealed the Different Vibrational Modes of the Selectivity Filter Interacting with K^+ and Na^+ in the Open and Collapsed Conformations of the KcsA Potassium Channel," *J. Phys. Chem. Lett.* **3**, 3806–3810 (2012).
- Y. Furutani *et al.*, "Development of a Rapid Buffer-Exchange System for Time-Resolved ATR-FTIR Spectroscopy with the Step-Scan Mode," *Biophysics* **9**, 123–129 (2013).

1. Light-Induced Structural Changes of Chimeras of Channelrhodopsin-1 and -2 from *Chlamydomonas reinhardtii*[1)]

Optogenetics is a powerful technique for manipulating specific neural activities by light stimulation, which has been rapidly growing up since discovery of light-gated cation channel, channelrhodopsin. There are two kinds of channelrhodopsin called channelrhodopsin-1 and -2 (ChR1 and ChR2) which are expressed in the eyespot of *Chlamydomonas reinhardtii*. Among them, ChR2 and its derivatives have been extensively utilized in optogenetics application. Alteration of channelrhodopsins to achieve a favorable electrophysiological response could be rationally applied when the molecular mechanisms of channelrhodopsin are understood well.

The basic architecture of channelrhodopsin is similar to other microbial rhodopsins which are composed of seven transmembrane helices with an all-*trans* retinal as the chromophore. Photoisomerization of the retinal chromophore upon light absorption causes conformational changes of the protein that result in opening of the channel gate and the influx of cations. The time course of the photocurrent upon continuous illumination of ChR2 shows a peak-and-plateau, while that of ChR1 shows a rectangular shape. The suppression just after the transient maximum photocurrent seen in ChR2 is denoted the "desensitization."

In 2009, several types of ChR1/ChR2 chimeras were characterized using electrophysiological techniques. One of these chimeras consists of the first five transmembrane helices (TM1 to TM5) from ChR1 and the last two transmembrane helices (TM6 and TM7) from ChR2. This chimera is referred to as $ChR_{5/2}$. Another chimera consists of TM1 and TM2 from ChR1 and TM3 to TM7 from ChR2. This chimera is referred to as $ChR_{2/5}$ (for details, see Figure 2). These ChR1/ChR2 chimeras show larger photocurrents than the wild types, and their desensitization is significantly reduced upon continuous illumination. However, the molecular mechanism of suppression of desensitization has remained unknown.

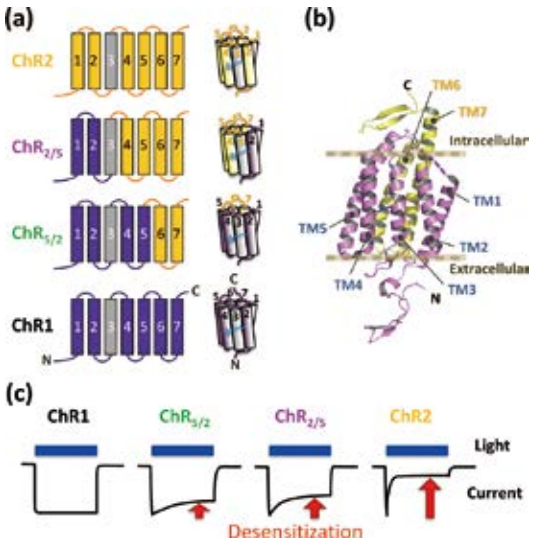

Figure 2. (a) Schematic representation of the chimeric channel rhodopsins. (b) The X-ray crystal structure of a chimeric channelrhodopsin called C1C2, which is nearly identical to $ChR_{5/2}$. (c) The photocurrent profiles of ChR1, ChR2and the chimeras. This figure is adapted from ref. 1.

Fourier-transform infrared (FTIR) spectroscopy has revealed the molecular mechanisms underlying the photo-induced structural dynamics of various microbial rhodopsins, such as bacteriorhodopsin and halorhodopsin.[2)] We applied light-induced difference FTIR spectroscopy on ChR1/ChR2 chimeras and ChR2 with an aim to reveal the molecular basis underlying the differences in electrophysiological properties between them.

As a consequence, we found that ChR1/ChR2 chimeras exhibited structural changes distinct from those in ChR2 upon continuous illumination. In particular, the protonation state of a glutamate residue, Glu129, (Glu90 in ChR2 numbering) in

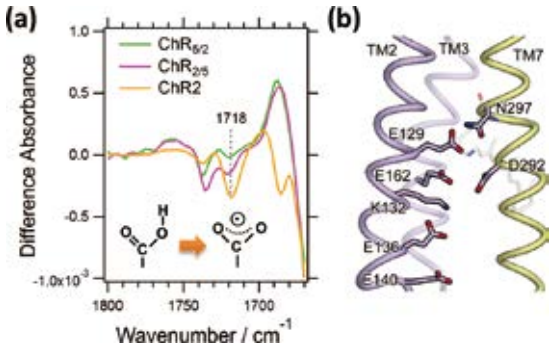

Figure 3. (a) Light-induced difference spectra in the C=O stretching region of carboxylic acid side chains. (c) The X-ray crystal structure of C1C2 shown along TM2 and TM7 helices. This figure is adapted from ref. 1.

the ChR chimeras is not changed as dramatically as seen in ChR2 as a negative band at 1718 cm^{-1} (Figure 3). Moreover, using mutants stabilizing particular photointermediates as well as time-resolved measurements, we identified some differences between the major photointermediates of ChR2 and ChR1/ChR2 chimeras. We couldn't see any substantial change in the protonation state of Glu129 in $ChR_{5/2}$ during the photocycle. Taken together, our data indicate that the gating and desensitizing processes in ChR1/ChR2 chimeras are different from those in ChR2 and that these differences should be considered in the rational design of new optogenetic tools based on channelrhodopsins.

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* IMS International Internship Program

Development of Heterogeneous Catalysis toward Ideal Chemical Processes

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Keywords Transition Metal Catalysis, Green Chemistry, Organic Synthesis

Our research interests lie in the development of transition metal-catalyzed reaction systems toward ideal (highly efficient, selective, green, safe, simple, *etc*.) organic transformation processes. In one active area of investigation, we are developing the heterogeneous aquacatalytic systems. Various types of catalytic organic molecular transformations, *e.g*. carbon–carbon bond forming cross-coupling, carbon–heteroatom bond forming reaction, aerobic alcohol oxidation, *etc*., were achieved in water under heterogeneous conditions by using amphiphilic polymer-supported transition metal complexes and nanoparticles (**Figure 1**), where self-concentrating behavior of hydrophobic organic substrates inside the amphiphilic polymer matrix played a key role to realize high reaction performance in water.

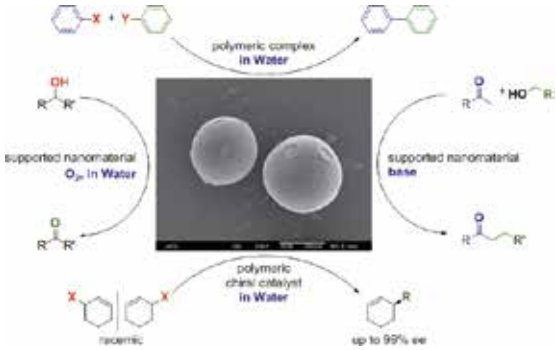

Figure 1. Typical Examples of Heterogeneous Aquacatalyses using Amphiphilic Polymer-Supported Metal Complexes and Metal Nanoparticles.

Selected Publications

- Y. M. A. Yamada, S. M. Sarkar and Y. Uozumi, "Amphiphilic Self-Assembled Polymeric Copper Catalyst to Parts per Million Levels: Click Chemistry," *J. Am. Chem. Soc.* **134**, 9285–9290 (2012).
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1. Enantioposition-Selective Copper-Catalyzed Azide–Alkyne Cycloaddition for Construction of Chiral Biaryl Derivatives[1,2)]

A highly enantioposition-selective copper-catalyzed azide–alkyne cycloaddition (CuAAC) of dialkynes bearing prochiral biaryls has been developed for the construction of 1,2,3-triazoles bearing axially chiral biaryl groups in up to 76% yield and up to 99% ee.

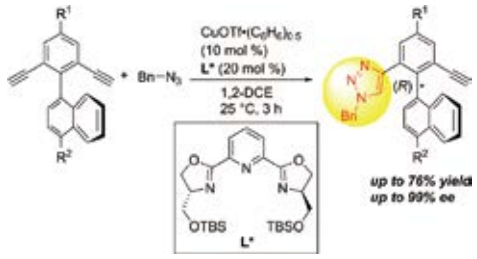

Figure 2. Enatioposition-selective copper-catalyzed azide–alkyne cycloaddition.

2. Continuous-Flow Oxidation of Alcohols and Hydrogenation of Olefines and Nitrobenzenes Catalyzed by Platinum Nanoparticles Dispersed in an Amphiphilic Polymer[3,4)]

We have developed a continuous-flow reaction system containing amphiphilic polymer-dispersion of platinum nanoparticles (ARP-Pt) packed in a catalyst cartridge to catalyze the aerobic oxidation of alcohols and the hydrogenation of olefins and nitrobenzenes. In the flow system using O_2, various alcohols were fully oxidized within 73 seconds (100–120 °C, 40–70 bar of the system pressure, 5 vol% of O_2) in water to give the corresponding carbonyl products in up to 99% yield. Olefins and nitrobenzenes underwent hydrogenation with the same flow system under H_2 (25 °C, 5–15 bar of the system pressure, 5 vol% of H_2) within 31 seconds to afford the corresponding hydrogenated products in up to 99% yield.

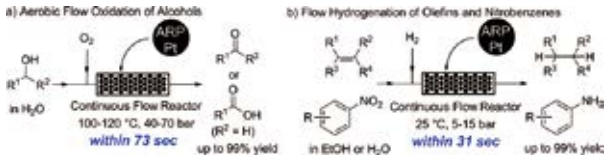

Figure 3. Continuous-flow oxidation of alcohols and hydrogenation of olefins and nitorobenzenes.

3. Palladium NNC-Pincer Complex: An Efficient Catalyst for Allylic Arylation at Perts Per Billion Levels[5)]

Allylic arylation of allylic acetates by sodium tetraarylborates in the presence of ppb to ppm (molar) loadings of a palladium NNC-pincer complex catalyst in methanol at 50 °C gave the corresponding arylated products in excellent yields. Total turnover numbers of up to 500,000,000 and turnover frequencies of up to 11,250,000 h^{-1} were achieved.

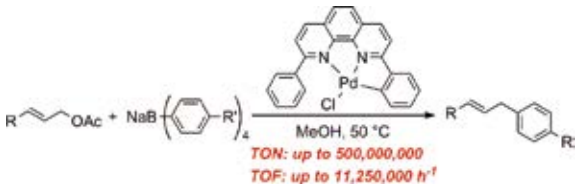

Figure 4. Allylic arylation of allylic acetates with sodium tetraarylborates in the presence of a palladium NNC-pincer complex.

4. Development of an Aquacatalytic System Based on the Formation of Vesicles of an Amphiphilic Palladium NNC-Pincer Complex[6)]

Two amphiphilic palladium NNC-pincer complexes bearing hydrophilic tri(ethylene glycol) chains and hydrophobic dodecyl chains were designed and prepared for the development of a new aquacatalytic system. In water, these amphiphilic complexes self-assembled to form vesicles, the structures which were established by means of a range of physical techniques. When the catalytic activities of the vesicles were investigated in the arylation of terminal alkynes in water, they were found to catalyze the reaction of aryl iodides with terminal alkynes to give good yields of the corresponding internal alkynes. The formation of a vesicular structure was shown to be essential for efficient promotion of this reaction in water.

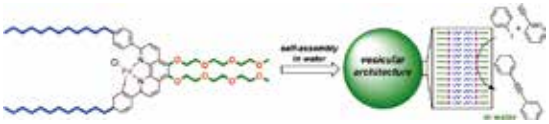

Figure 5. Cu-free Sonogashira reaction in water in the presence of a self-assembled vesicular amphiphilic palladium NNC-pincer complex.

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4) T. Osako, K. Torii, A. Tazawa and Y. Uozumi, *RSC Adv.* **5**, 45760–45766 (2015).
5) G. Hamasaka, F. Sakurai and Y. Uozumi, *Chem. Commun.* **51**, 3886–3888 (2015).
6) F. Sakurai, G. Hamasaka and Y. Uozumi, *Dalton Trans.* **44**, 7828–7834 (2015).

Award

OSAKO, Takao; The 4th NINS Prize for Young Scientists (2015).

Design and Synthesis of Chiral Organic Molecules for Asymmetric Synthesis

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Keywords Organic Synthesis, Molecular Catalyst, Non-Covalent Interaction

The field of molecular catalysis has been an attractive area of research to realize efficient and new transformations in the synthesis of functional molecules. The design of ligands and chiral molecular catalysts has been recognized as one of the most valuable strategies; therefore, a great deal of effort has been dedicated to the developments. In general, "metal" has been frequently used as the activation center, and conformationally rigid, and C_2- or pseudo C_2 symmetry has been preferably components for the catalyst design. To develop new type of molecular catalysis, we have focused on the use of hydrogen and halogen atom as activation unit, and have utilized conformationally flexible components in the molecular design of catalyst, which had not received much attention until recently. We hope that our approach will open the new frontier in chiral organic molecules from chiral molecular chemistry to chiral molecular science.

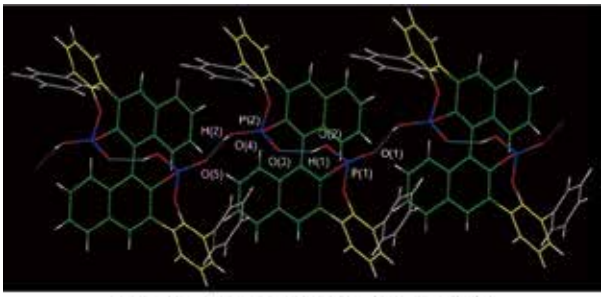

Figure 1. Hydrogen bonding network in chiral bis-phosphoric acid catalyst derived from (*R*)-3,3'-di(2-hydroxy-3 -arylphenyl)binaphthol. Hydrogen bond acts as activation unit for the substrate in asymmetric reaction space and controls atropisomeric behavior in naphthyl–phenyl axis.

Selected Publications

- T. P. Yoon and E. N. Jacobsen, *Science* **299**, 1691–1693 (2003).
- N. Momiyama and H. Yamamoto, "Brønsted Acid Catalysis of Achiral Enamine for Regio- and Enantioselective Nitroso Aldol Synthesis," *J. Am. Chem. Soc.* **127**, 1080–1081 (2005).
- N. Momiyama, H. Tabuse and M. Terada, "Chiral Phosphoric Acid-Governed Anti-Diastereoselective and Enantioselective Hetero-Diels–Alder Reaction of Glyoxylate," *J. Am. Chem. Soc.* **131**, 12882–12883 (2009).
- N. Momiyama, T. Konno, Y. Furiya, T. Iwamoto and M. Terada, "Design of Chiral Bis-Phosphoric Acid Catalyst Derived from (*R*)-3,3'-Di(2-hydroxy-3-arylphenyl)binaphthol: Catalytic Enantioselective Diels–Alder Reaction of α,β-Unsaturated Aldehydes with Amidodienes," *J. Am. Chem. Soc.* **133**, 19294–19297 (2011).

1. Brønsted Acid Catalyzed Asymmetric 1,3-Alkyl Migration of 1,2,2-Substituted Butenyl Amines: Asymmetric Synthesis of Linear Homoprenylamines

Allylation of imines with allylic metal reagents has been one of the most valuable tools to synthesize enantioenriched homoallylic amines. Due to the inherent nature of allylic metal reagent, however, regioselectivity has been a long-standing subject in this area. To develop the synthetic reaction for enantioenriched linear homoprenylic amines, we discovered chirality transferred 1,3-alkyl migration of 1,2,2-substituted butenyl amines in the presence of trifluoromethyl acetic acid, and developed it as synthetic method for variety of enantioenriched linear homoprenylic amines.[1)] In sharp contrast, Ollis *et al.* previously reported that chirality was significantly dropped in 1,3-alkyl migration of *N*,*N*-dimethyl-1-substituted-3-buten-1-amine.[2)] To the best our knowledge, our discovery is the first example of chirality transferred 1,3-alkyl migration and the new entry of the synthetic methodology for the linear enantioenriched homoallylic amines.

2. Design of Chiral Brønsted Acid Catalyst

Chiral Brønsted acid catalysis has been recognized as one of the useful tools in asymmetric synthesis. We have contributed to this area by focusing on the use of perfluoroaryls and C_1-symmetric design.

Perfluorinated aryls have emerged as an exquisite class of motifs in the design of molecular catalysts, and their electronic and steric alterations lead to notable changes in the chemical yields and the stereoselectivities. However, unfortunately, the distinctive potential of perfluorinated aryls has not been fully exploited as design tools in the development of chiral Brønsted acid catalysts. We developed the perfluoaryls-incorporated chiral mono-phosphoric acids as chiral Brønsted acid catalysts that can deriver high yields and stereoselectivities in the reactions of imines with unactivated alkenes. We have described the first example of a diastereo- and enantioselective [4+2] cycloaddition reaction of *N*-benzoyl imines, as well as the enantioselective three-component imino–ene reaction using aldehydes and $FmocNH_2$.[3,4)]

We have developed (*R*)-3,3'-di(2-hydroxy- 3-arylphenyl) binaphthol derived chiral bis-phosphoric acid which efficiently catalyzed enantioselective Diels–Alder reaction of acroleins with amidodienes.[5,6)] We demonstrated that two phosphoric acid groups with individually different acidities can play distinct roles in catalyst behavior through hydrogen bonding interactions. Hence, we were interested to explore whether a combination of *different acidic functional groups*, in particular an aryl phosphinic acid-phosphoric acid, would function as an efficient Brønsted acid catalyst. We developed a Brønsted acid with two different acidic sites, aryl phosphinic acid-phosphoric acid, and its catalytic performance was assessed in the hetero-Diels–Alder reaction of aldehyde hydrates with Danishefsky's diene, achieving high reaction efficiency.[7)] Furthermore, molecular design of a chiral Brønsted acid with two different acidic sites, chiral carboxylic acid–cyclic mono-phosphoric acid, was identified as a new and effective concept in asymmetric hetero-Diels–Alder reaction of 2-azopyridinoester with amidodienes.[8)]

3. Halogen Bond Donor Catalyzed Allylation Reaction of Isoquinoline with Allylsilatrane

Halogen bonds are attractive non-covalent interactions between terminal halogen atoms in compounds of the type R–X (X = Cl, Br, I) and Lewis bases LB. It has been known that strong halogen bonds are realized when "R" is highly electronegative substituents such as perfluorinated alkyl or aryl substituents. We recently developed synthetic methodology for perfluorinated aryl compounds, and applied it for the development of chiral Brønsted acid catalysts. On the basis of our achievements, we have examined it to develop halogen bond donor catalyzed allylation reaction.

We found that pentafluoroiodebenzene was able to catalyze the allylation reaction of isoquinoline with allylsilatrane to give the corresponding product in good yield.[9)]

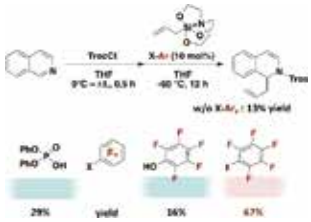

Figure 2. Halogen bond donor catalyzed allylation reaction. Comparison with Brønsted acid/hydrogen bond donor catalyst.

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9) N. Momiyama *et al.*, Manuscript in preparation.

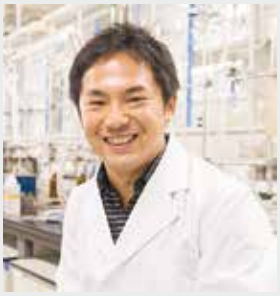
Awards

MOMIYAMA, Norie; The 17th Morita Science Research Award (2014).
MOMIYAMA, Norie; Central Glass Co., Ltd. Award in Organic Chemistry, Japan (2014).

* carrying out graduate research on Cooperative Education Program of IMS with Tohoku University

Development of Functional Metal Complexes for Artificial Photosynthesis

Department of Life and Coordination-Complex Molecular Science
Division of Functional Coordination Chemistry



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Professional Employment
2002 JSPS Research Fellow (DC2)
2004 Research Assistant (Postdoc), University of Liverpool
2005 Research Associate, Kyushu University
2007 Assistant Professor, Kyushu University
2009 JST PRESTO Researcher
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Associate Professor, The Graduate University for Advanced Studies

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Keywords Metal Complex, Water Oxidation, Artificial Photosynthesis

Artificial photosynthesis is a solar energy conversion technology that mimics natural photosynthesis, and considered to be one of the next big breakthroughs in energy. Our group studies the development of functional metal complexes toward the realization of artificial photosynthesis. Specific areas of research include (i) synthesis of ruthenium-based molecular catalysts for water oxidation and carbon dioxide reduction, (ii) creation of cluster catalysts for multi-electron transfer reactions, (iii) mechanistic investigation into water oxidation catalyzed by metal complexes, (iv) application of proton-coupled electron transfer toward multi-electron transfer reactions, (v) electrochemical evaluation of the activity of molecular catalysts for water oxidation and carbon dioxide reduction, (vi) electrochemical measurement of metal complexes in homogeneous solutions under photoirradiation, and (vii) development of reaction fields via self-assembly of molecular catalysts.

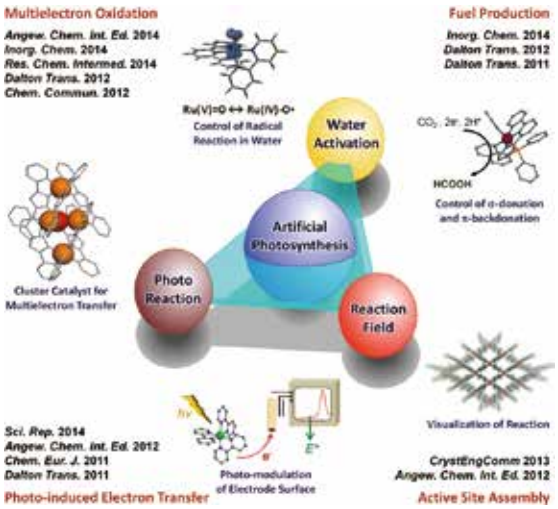

Figure 1. An overview of our work.

Selected Publications

- M. Yoshida, M. Kondo, S. Torii, K. Sakai and S. Masaoka, "Oxygen Evolution Catalysed by a Mononuclear Ruthenium Complex bearing Pendant -SO_3^- Groups," *Angew. Chem., Int. Ed.* **54**, 7981–7984 (2015).
- M. Okamura and S. Masaoka, "Design of Mononuclear Ruthenium Catalysts for Low-Overpotential Water Oxidation," *Chem. –Asian J. [Focus Review]* **10**, 306–315 (2015).
- M. Yoshida, M. Kondo, T. Nakamura, K. Sakai and S. Masaoka, "Three Distinct Redox States of an Oxo-Bridged Dinuclear Ruthenium Complex," *Angew. Chem., Int. Ed.* **53**, 11519–11523 (2014).
- G. Nakamura, M. Okamura, M. Yoshida, T. Suzuki, H. D. Takagi, M. Kondo and S. Masaoka, "Electrochemical Behavior of Phosphine-Substituted Ruthenium(II) Polypyridine Complexes with a Single Labile Ligand," *Inorg. Chem.* **53**, 7214–7226 (2014).
- A. Fukatsu, M. Kondo, M. Okamura, M. Yoshida and S. Masaoka, "Electrochemical Response of Metal Complexes in Homogeneous Solution under Photoirradiation," *Sci. Rep.* **4**, 5327 (2014).
- T. Itoh, M. Kondo, M. Kanaike and S. Masaoka, "Arene-Perfluoroarene Interactions for Crystal Engineering of Metal Complexes: Controlled Self-Assembly of Paddle-Wheel Dimers," *CrystEngComm* **15**, 6122–6126 (2013).

1. Oxygen Evolution Catalysed by a Mononuclear Ruthenium Complex bearing Pendant -SO_3^- Groups[1)]

Rational molecular design of catalytic systems capable of smooth O–O bond formation is critical to the development of efficient catalysts for water oxidation. In this work, we developed a new ruthenium complex which bears pendant SO_3^- groups in the secondary coordination sphere: [Ru(terpy)(bpyms)(OH_2)] (terpy = 2,2':6',2''-terpyridine, bpyms = 2,2'-bipyridine-5,5'-bis(methanesulfonate)). Water oxidation driven by a Ce^{4+} oxidant is distinctly accelerated upon introduction of the pendant SO_3^- groups in comparisons to the parent catalyst, $[Ru(terpy)(bpy)(OH_2)]^{2+}$ (bpy = 2,2'-bipyridine). Spectroscopic, electrochemical, and crystallographic investigations concluded that the pendant SO_3^- groups promote the formation of an O–O bond via the secondary coordination sphere on the catalyst, whereas the influence of the pendant SO_3^- groups on the electronic structure of the [Ru(terpy)(bpy)(OH_2)]$^{2+}$ core is negligible. The results of this work indicate that modification of the secondary coordination sphere is a valuable strategy for the design of water oxidation catalysts.

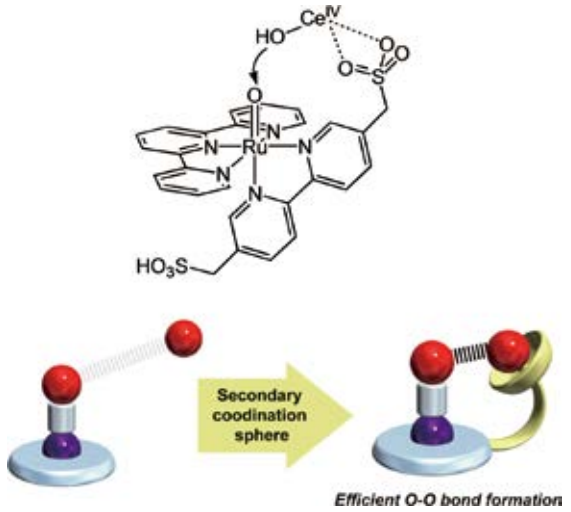

Figure 2. Schematic illustration of efficient O–O bond formation through modification of the secondary coordination sphere.

2. Three Distinct Redox States of an Oxo-Bridged Dinuclear Ruthenium Complex[2)]

Mixed-valence (MV) complexes are excellent model systems for the investigation of electron-transfer phenomena in biophysical processes such as photosynthesis and in artificial electronic devices based on conjugated materials. Given that the electronic properties of MV states could be strictly controlled by the oxidation state of the dinuclear core, systematic investigations on the several oxidation states of dinuclear metal complexes are an interesting and important research topic. In this work, a series of [{(terpy)(bpy)Ru}(μ-O){Ru(bpy)(terpy)}]$^{n+}$ (**[RuORu]$^{n+}$**, terpy = 2,2';6',2''-terpyridine, bpy = 2,2'-bipyridine) was systematically synthesized and characterized in three distinct redox states (n = 3, 4, and 5 for $Ru^{II,III}_2$, $Ru^{III,III}_2$, and $Ru^{III,IV}_2$, respectively). The crystal structures of **[RuORu]$^{n+}$** (n = 3, 4, 5) in all three redox states were successfully determined. X-ray crystallography showed that the Ru–O distances and the Ru–O–Ru angles are mainly regulated by the oxidation states of the ruthenium centers. X-ray crystallography and ESR spectra clearly revealed the detailed electronic structures of two mixed-valence complexes, **[$Ru^{III}ORu^{IV}$]$^{5+}$** and **[$Ru^{II}ORu^{III}$]$^{3+}$**, in which each unpaired electron is completely delocalized across the oxo-bridged dinuclear core. These findings allow us to understand the systematic changes in structure and electronic state that accompany the changes in the redox state.

Figure 3. Two distinct MV states derived from a homovalent dimer.

References

1) M. Yoshida, M. Kondo, S. Torii, K. Sakai and S. Masaoka, "Oxygen Evolution Catalysed by a Mononuclear Ruthenium Complex bearing Pendant -SO_3^- Groups," *Angew. Chem., Int. Ed.* **54**, 7981–7984 (2015).

2) M. Yoshida, M. Kondo, T. Nakamura, K. Sakai and S. Masaoka, "Three Distinct Redox States of an Oxo-Bridged Dinuclear Ruthenium Complex," *Angew. Chem., Int. Ed.* **53**, 11519–11523 (2014).

Awards

FUKATSU, Arisa; Excellent Poster Award, International Conference on Artificial Photosynthesis (2014).
IZU, Hitoshi; Excellent Poster Award, The 4th CSJ Chemistry Festa (2014).
ITOH, Takahiro; CrystEngComm Poster Prize (2014).
ITOH, Takahiro; Poster Award, The 64th Conference of Japan Society of Coordination Chemistry (2014).

Control of Electron Transfer for Efficient Oxygenation Reactions

Department of Life and Coordination-Complex Molecular Science
Division of Biomolecular Functions

KURAHASHI, Takuya
Assistant Professor

Electron transfer is the most fundamental reaction to govern chemical reactions. To find an effective way to control electron transfer, transient active species were prepared at low temperature under inert atmosphere. Electronic structures of these active species were investigated with various techniques including absorption, ^{1}H and ^{2}H NMR, EPR, IR resonance Raman spectroscopy and magnetic susceptibility measurement. Correlations between electronic structures and electron transfer ability are investigated in detail.

1. Dioxygen Activation via Two-Electron Transfer from Hydroxide to Dioxygen Mediated By a Manganese(III) Complex

Although atmospheric dioxygen is regarded as the most ideal oxidant, O_2 activation for use in oxygenation reactions intrinsically requires a costly sacrificial reductant. This study investigated the use of inexpensive aqueous alkaline solution for O_2 activation. This study has clarified that a manganese (III) salen complex mediates O_2 activation in the presence of OH^- from 2 M KOH aqueous solution (Figure 1). Mechanistic investigation have shown that the reaction of Mn^{III}(salen)(Cl) with OH^- generates a transient species with strong reducing ability, which effects the reduction of O_2 by means of a manganese(II) intermediate.

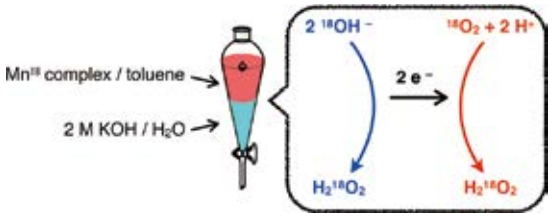

Figure 1. Isotope experiments to verify two-electron transfer from OH^- to O_2.

Visiting Professors

Visiting Professor
KATO, Masako *(from Hokkaido University)*

Construction of Photofunctional Metal Complexes and the Elucidation of Their Properties

In our research group, we focus on the creation of photofunctional metal complexes. **Fabrication of new multichromic materials:** Platinum(II) complexes exhibit characteristic luminescence by assembling. Taking advantage of the characteristic metallophilic interactions between Pt ions, our laboratory have developed new Pt(II) complexes with diimine or cyclometalating ligands exhibiting unique multichromic behaviors. **Fabrication of novel 3d-metal complexes with intense luminescence:** In order to effectively utilize elements, it is important that common metals should be used to fabricate materials with strong emissivity. We have developed various Cu(I) complexes exhibiting intense luminescence. **Fabrication of new photocatalysts based on redox-active organic ligands:** The strategy of our group to contribute to the energy issues is to construct novel photocatalytic systems using common metals instead of precious metals. By using a redox-active ligand, *o*-phenylenediamine, we found a simple metal-complex system for photochemical hydrogen evolution without extra photosensitizers.

Visiting Professor
YORIMITSU, Hideki *(from Kyoto University)*

Synthesis of π-Conjugated Molecules by Means of Organometallics

Porphyrins are an important class of compounds that occur in nature, playing the vital roles in biologically important phenomena such as oxygen transport, oxygen storage, and photosynthesis. Additionally, they constitute useful functional molecules in the field of advanced organic material sciences including organic photovoltaics. These important functions largely rely on their highly conjugated, 18π electronic, aromatic core. Peripheral functionalizations of the core have hence been attracting considerable attentions since they effectively alter the electronic and steric natures of the parent porphyrins to create new π-rich molecules and properties. Along this line, we have been interested in the following topics. 1) Catalytic selective direct arylation of porphyrin periphery, 2) Oxidative fusions of *meso*-(diarylamino)porphyrins and the properties of nanoazagraphene products, 3) Generation and reactions of porphyrinyl Grignard reagents, 4) Synthesis and properties of porphyrin oligomers.

Visiting Associate Professor
KAMIKUBO, Hironari *(from Nara Institute of Science and Technology)*

Development of an Auto-Sampling System Designed for Titration-SAXS Measurements

Various protein molecules concert with each other to express various biological functions. Because these multicomponent biological molecules weakly interact with each other, they can undergo regulatory dissociation and association upon inducing biological stimuli. In order to understand biological systems, we must, at first, aim to identify every possible unstable complex involved in the given multicomponent system, and then quantitatively analyze the interactions of these complex molecules. However, because of the complexity, it is generally difficult to apply conventional analytical methods to analyze such multi-component equilibrium systems. We have realized a new analytical method that would enable us to perform structure and interaction analyses on multi-component equilibrium systems. This was achieved by developing an auto-sampling system equipped with micro-fluidics technology. Applying this newly designed equipment to SAXS measurements, we can automatically collect numerous scattering profiles while altering the molar ratios of each component involved in the multi-component equilibrium; thus, enabling us to determine the system's free energy landscape of the multi-component equilibrium.