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, associate professors Toshi Nagata, Hidehiro Sakurai (Research Center of Integrative Molecular Systems), Hiroshi Fujii (Okazaki Institute for Integrative Bioscience), who had joined this department under concurrent appointments, were promoted to move out from IMS. Dr. Norie Momiyama was recruited to be an associate professor of the Division of Complex Catalysis in June 2014. Dr. Ryota Iino was also recruited as a full professor of the Okazaki Institute for Integrative Bioscience in June, and simultaneously offered a concurrent appointment of this department.

Bioinorganic Chemistry of Metal-Containing Sensor Proteins

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

AONO, Shigetoshi
Professor
[aono@ims.ac.jp]

Education
1982 B.S. Tokyo Institute of Technology
1987 Ph.D. Tokyo Institute of Technology

Professional Employment
1988 Postdoctoral Fellow, Georgia University
1989 Assistant Professor, Tokyo Institute of Technology
1994 Associate Professor, Japan Advanced Institute of Science and Technology
2002 Professor, Institute for Molecular Science
Professor, Okazaki Institute for Integrative Bioscience
Professor, The Graduate University for Advanced Studies

Member
Assistant Professor
YOSHIOKA, Shiro
IMS Research Assistant Professor
MURAKI, Norifumi
Post-Doctoral Fellow
KITATSUJI, Chihiro
OKAMOTO, Yasunori
Secretary
TANIZAWA, Misako

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 mainly focused on the elucidation of the structure–function relationships of the biological systems in which a heme molecule takes part in maintaining cellular homeostasis.

Heme shows many biological functions. The most popular function is to be used as a prosthetic group in heme proteins. Heme proteins show a variety of functions including oxygen transport/storage, electron transfer, oxidase, peroxidase, oxygenase, catalase, and dehydratase. In addition to these functions, a new function of hemeprotein has been found recently, which is a sensor of diatomic gas molecules or redox change. In these heme-based sensor proteins, the heme acts as the active site for sensing the external signal such as gas molecules and redox change. Heme also shows a novel biological function as a signaling molecule for transcriptional and translational regulation. In these systems, heme-sensing proteins sense a heme molecule to regulate biological processes. We are now working on the heme-based gas sensor proteins, heme-dependent transcriptional regulation, and bacterial heme transport systems.

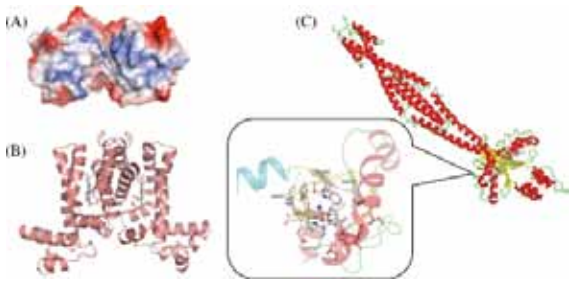


Figure 1. Structures of (A) the heme-transport protein HmuT, (B) the heme-sensing transcriptional regulator HrtR, and (C) the heme-based sensor protein Aer2.

Selected Publications

- 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. Molecular Mechanism for Heme-Mediated Inhibition of 5-Aminolevulinic Acid Synthase 1[1)]

Mammalian 5-aminolevulinic acid synthase 1 (ALAS1), an isozyme expressed in all cell types, catalyzes the first reaction in the heme biosynthetic pathway in mitochondria. Heme regulates ALAS1 function at multiple levels including the regulation of transcription, translation, mitochondrial import, protein degradation and enzyme activity to maintain intracellular heme concentrations at appropriate range. In this study, we elucidated the molecular mechanism of heme-mediated regulation of enzymatic activity for rat ALAS1. ALAS1 has three putative heme regulatory motifs (HRMs), two of which were found to be the ferric heme binding sites in ALAS1. Though the electronic absorption and resonance Raman spectroscopy have demonstrated that ^{110}Cys and ^{527}Cys are ferric heme binding sites in ALAS1, heme binding to ^{110}Cys and ^{527}Cys was not responsible for heme-mediated inhibition of ALAS1 activity. Rather, ALAS1 activity will be inhibited by heme binding with hydrophobic interactions to the succinyl-CoA binding pocket. The heme binding to the HRMs in ALAS1 was found not to be responsible for heme-mediated inhibition of ALAS1 activity. Though heme binding to ^{110}Cys and/or ^{527}Cys in the HRMs is not involved in the feedback regulation of ALAS1 activity, it would be responsible for the oxidative modification of ALAS1 that might regulate the heme-mediated proteolysis.

We also found that protoporphyrin IX (PpIX), a reaction intermediate of heme biogenesis, inhibited ALAS1 activity more efficiently compared with heme, indicating the presence of multiple pathways for the feedback regulation of ALAS1 activity. The intracellular accumulation of PpIX, which may be caused by unbalance between PpIX synthesis and iron chelation to PpIX to produce heme, causes severe damage to cells. Thus, it makes sense that PpIX functions as an effector of the feedback regulation of ALAS1 to shut down its activity to prevent from accumulation of PpIX under iron limitation.

2. Heme-Binding Properties of HupD Functioning as a Substrate-Binding Protein in a Heme-Uptake ABC-Transporter System in *Listeria monocytogenes*[2)]

Iron is an essential element for all organisms, which is used as a component of iron-containing proteins such as hemoproteins and iron-sulfur proteins responsible for a variety of biological processes. Pathogenic bacteria need to thieve iron from host organisms for their growth. Since heme iron involved in hemoglobin is the most abundant iron source for pathogenic bacteria infected in vertebrate, these bacteria have evolved sophisticated acquisition systems of heme from their host organisms.

Recently, it is reported that a Gram-positive pathogen *L. monocytogenes* that cause listeriosis has three Fur-regulated heme-uptake systems: the *srtB* region coding sortase-anchored proteins and a putative ABC transporter, the *fhu* and *hup* operons coding putative ABC transporters for ferric hydroxamates and hemin respectively. An ABC-type transporter system HupDGC is the primary heme transporter system and is most crucial to virulence for *L. monocytogenes*. HupD, the substrate-binding protein in the HupDGC transporter, is reported to be specific for heme, but the detailed properties of HupD for heme-binding remains to be elucidated. In this work, heme-binding properties of HupD from *L. monocytogenes* were characterized by spectroscopic and mutagenesis studies.

Titration of apo-tHupD with hemin revealed that apo-HupD takes up 1 mol equivalent of heme to form a 1:1 complex, as shown in Figure 2. UV-vis absorption, EPR, and resonance Raman spectroscopy have revealed that HupD binds a heme with two histidine residues as the axial ligand. ^{105}His and ^{259}His are identified as the axial ligands by site-directed mutagenesis. HupD is the first example of the heme-binding protein having bis-histidine coordination environment among heme-binding proteins working in the bacterial heme acquisition systems. While mutation of ^{259}His to Ala resulted in a loss of heme-binding ability of HupD, the H105A variant of HupD retained heme-binding ability with lower heme-binding affinity compared with wild type. These results suggest that ^{259}His is an essential ligand for heme acquisition by HupD and that ^{105}His might be responsible for the regulation of heme-binding affinity of HupD during heme transport process.

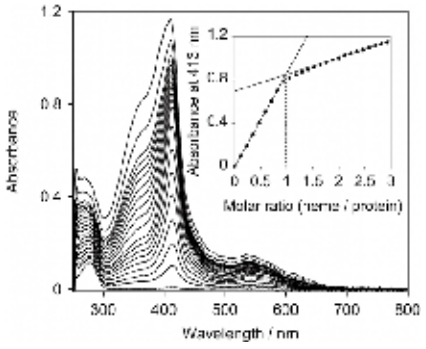


Figure 2. Spectral changes upon titration of apo-HupD with hemin. Inset: Titration curve of hemin binding to apo-HupD measured at 413 nm in 50 mM Tris-HCl (pH 7.4).

References

1) C. Kitatsuji, M. Ogura, T. Uchida, K. Ishimori and S. Aono, *Bull. Chem. Soc. Jpn.* **87** (2014), in press.

2) Y. Okamoto, H. Sawai, M. Ogura, T. Uchida, K. Ishimori, T. Hayashi and S. Aono, *Bull. Chem. Soc. Jpn.* **87** (2014), in press.

Dynamical Ordering of Biomolecular Systems for Creation of Integrated Functions

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

KATO, Koichi
Professor
[kkatonmr@ims.ac.jp]

Education
1986 B.S. The University of Tokyo
1991 Ph.D. The University of Tokyo

Professional Employment
1991 Assistant Professor, The University of Tokyo
1997 Lecturer, The University of Tokyo
2000 Professor, Nagoya City University
2008 Professor, Institute for Molecular Science
Professor, Okazaki Institute for Integrative Bioscience
Professor, The Graduate University for Advanced Studies
2006 Visiting Professor, Ochanomizu University
2013 Project Leader, JSPS Grant in Aid for Scientific Research on Innovative Areas "Dynamical Ordering of Biomolecular Systems for Creation of Integrated Functions"

Awards
2000 The Pharmaceutical Society of J apan Award for Young Scientists
2011 The Pharmaceutical Society of J apan Award for Divisional Scientific Promotions
2011 The 48th Baelz Prize

Member
Assistant Professor
YAMAGUCHI, Takumi
OIIB Research Assistant Professor
YAGI-UTSUMI, Maho
Post-Doctoral Fellow
WANG, Ying-Hui
ZHANG, Ying
NINAGAWA, Satoshi
Research Fellow
ANZAI, Takahiro
NASSIRI, Mohammadreza
Visiting Scientist
PADUNGROS, Panuwat*
LE GULLUCHE, Anne-Charlotte†
Graduate Student
CHANDAK, Mahesh
ZHU, Tong
WANG, Jinzheng
SIKDAR, Arunima
YAN, Gengwei
THAMMAPORN, Ratsupa‡
SEETAHA, Supaporn‡
SOMBOON, Tuanjai‡
INAGAKI, Kouya§
SUZUKI, Kousuke§
TOSHIMORI, Takayasu§
Technical Fellow
ISONO, Yukiko
IKEDA, Yukako
OKADA, Tomo
Secretary
TANAKA, Kei

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

- 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. Zhang, T. Yamaguchi and K. Kato, "New NMR Tools for Characterizing the Dynamic Conformations and Interactions of Oligosaccharides," *Chem. Lett.* **42**, 1455–1462 (2013).
- 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).
- K. Kato and Y. Yamaguchi, "Glycoproteins and Antibodies: Solution NMR Studies," in *Encyclopedia of Magnetic Resonance*, John Wiley; Chichester, **vol.3**, pp. 1779–1790 (2012).
- O. Serve, Y. Kamiya and K. Kato, "Redox-Dependent Chaperoning, Following PDI Footsteps," *Proteomics Res. J.* **3**, 69–79 (2012).
- Y. Kamiya, M. Yagi-Utsumi, H. Yagi and K. Kato, "Structural and Molecular Basis of Carbohydrate–Protein Interaction Systems as Potential Therapeutic Targets," *Curr. Pharm. Des.* **17**, 1672–1684 (2011).

1. Dynamic Orchestration of Proteasomes

Recently accumulated evidence has demonstrated that the assembly of the eukaryotic 26S proteasome is not due to spontaneous self-organization but due to an ordered process assisted by several proteins called 'proteasome assembly chaperones' that transiently associate with the assembly intermediates at certain steps in the proteasome assembly pathway.

To provide structural basis for quaternary structure formation of the proteasome and its consequent activation, we conducted structural study by employing X-ray crystallography and NMR spectroscopy. By inspection of our structural data, a working model is proposed in which the proteasome assembly chaperones Pba3-Pba4 and Nas2 act as molecular matchmakers and offer checkpoints, respectively, during the proteasome formation (Figure 2).[1,2] The proteasome assembly chaperones can be potential therapeutic targets for drug discovery.[3]

We also performed conformational characterization of an intrinsically disordered protein in complex with an archaeal proteasome activator, PbaB, by NMR spectroscopy combined with small-angle neutron scattering using an inverse contrast matching method.[4]

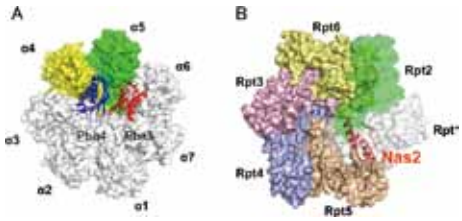


Figure 2. 3D models of (A) the proteasome α-ring complexed with the Pba3–Pba4 heterodimer and (B) the proteasome ATPase ring complexed with Nas2.

2. Functional Mechanisms of Glycans

The carbohydrate chains displayed on proteins play pivotal roles in a variety of molecular recognition events on cell surfaces as well as in intracellular environments. The intermolecular interaction systems involving the carbohydrate moieties could be potential therapeutic targets for various diseases.

In the early secretory pathway, *N*-glycans serve as tags recognized by cargo receptors having lectin activities. Our crystallographic data provide structural basis for disparate sugar-binding specificities in the homologous cargo receptors ERGIC-53 and VIP36, the former of which shows a broader specificity and lower binding affinity to the high-mannose-type oligosaccharides, irrespective of the presence or absence of the nonreducing terminal glucose residue at the D1 arm (Figure 3).[5]

Dystroglycanopathy is a major class of congenital muscular dystrophy that is caused by a deficiency of functional glycans on α-dystroglycan (α-DG) with laminin-binding activity. We demonstrated that a product of a recently identified causative gene for dystroglycanopathy, AGO61, is indispensable for the formation of laminin-binding glycans of α-DG. Furthermore, our results indicate that functional α-DG glycosylation was primed by AGO61-dependent GlcNAc modifications of specific threonine-linked mannosyl moieties of α-DG. These findings provide a key missing link for understanding how the physiologically critical glycan motif is displayed on α-DG and provides new insights on the pathological mechanisms of dystroglycanopathy.[6]

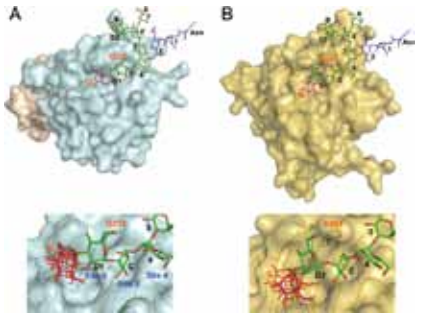


Figure 3. Structural models of the lectin domains of (A) ERGIC-53 and (B) VIP36 with monoglucosylated high-mannose-type oligosaccharides.

References

1) K. Takagi, Y. Saeki, H. Yashiroda, H. Yagi, A. Kaiho, S. Murata, T. Yamane, K. Tanaka, T. Mizushima and K. Kato, *Biochem. Biophys. Res. Commun.* **450**, 1110–1114 (2014).
2) T. Satoh, Y. Saeki, T. Hiromoto, Y.-H. Wang, Y. Uekusa, H. Yagi, H. Yoshihara, M. Yagi-Utsumi, T. Mizushima, K. Tanaka and K. Kato, *Structure* **22**, 731–743 (2014).
3) T. Doi, M. Yoshida, K. Ohsawa, K. Shin-ya, M. Takagi, Y. Uekusa, T. Yamaguchi, K. Kato, T. Hirokawa and T. Natsume, *Chem. Sci.* **5**, 1860–1868 (2014).
4) M. Sugiyama, H. Yagi, T. Yamaguchi, K. Kumoi, M. Hirai, Y. Oba, N. Sato, L. Porcar, A. Martele and K. Kato, *J. Appl. Crystallogr.* **47**, 430–435 (2014).
5) T. Satoh, K. Suzuki, T. Yamaguchi and K. Kato, *PLoS One* **9**, e87963 (2014).
6) H. Yagi, N. Nakagawa, T. Saito, H. Kiyonari, T. Abe, T. Toda, S. W. Wu, K. H. Khoo, S. Oka and K. Kato, *Sci. Rep.* **3**: 3288 (2013).

Awards

YAMAGUCHI, Takumi; Presentation Award, The 7th Symposium on Biofunctional Chemistry (2013).
YAMAGUCHI, Takumi; The 3rd NINS Prize for Young Scientists (2014).

* EXODASS Program
† IMS International Internship Program
‡ carrying out graduate research on Cooperative Education Program of IMS with Kasetsart University
§ carrying out graduate research on Cooperative Education Program of IMS with Nagoya City University

Operation and Design Principles of Biological Molecular Machines

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

IINO, Ryota
Professor
[iino@ims.ac.jp]

Education
1995 B.E. Kyoto University
1997 M.E. Kyoto University
2003 Ph.D. Nagoya University

Professional Employment
2000 Research Associate, Japan Science and Technology Cooperation
2002 Research Associate, Japan Science and Technology Agency
2005 Specially-Appointed Assistant Professor, Osaka University
2006 Assistant Professor, Osaka University
2011 Lecturer, The University of Tokyo
2013 Associate Professor, The University of Tokyo
2014 Professor, Institute for Molecular Science
Professor, Okazaki Institute for Integrative Bioscience
Professor, The Graduate University for Advanced Studies

Award
2012 Emerging Investigator. Lab on a Chip ., The Royal Society of Chemistry, U.K.

Member
Secretary
TANIZAWA, Misako

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 potential energy.

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 and H. Noji, "Intersubunit Coordination and Cooperativity in Ring-Shaped NTPases," *Curr. Opin. Struct. Biol.* **23**, 229–234 (2013).
- R. Iino and H. Noji, "Operation Mechanism of F_oF_1-Adenosine Triphosphate Synthase Revealed by Its Structure and Dynamics," *IUBMB Life* **65**, 238–246 (2013).
- 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).
- 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. Rotary Dynamics of *Enterococcus hirae* V_1-ATPase[1)]

V-ATPases are rotary molecular motors that generally function as proton pumps. We characterized the rotary dynamics of the V_1 moiety of *Enterococcus hirae* V-ATPase (EhV_1, Figure 2A) using single-molecule analysis employing a load-free probe (Figure 2B). EhV_1 rotated in a counterclockwise direction, exhibiting two distinct rotational states, namely clear and unclear, suggesting unstable interactions between the rotor and stator. The clear state was analyzed in detail to obtain kinetic parameters. The rotation rates obeyed Michaelis-Menten kinetics with a maximal rotation rate (V_{max}) of 107 revolutions/s and a Michaelis constant (K_m) of 154 μM at 26 °C. At all ATP concentrations tested, EhV_1 showed only three pauses separated by 120°/turn, and no substeps were resolved, as was the case with *Thermus thermophilus* V_1-ATPase (TtV_1). At 10 μM ATP ($<<K_m$), the distribution of the durations of the ATP-waiting pause fit well with a single-exponential decay function. The second-order binding rate constant for ATP was 2.3×10^6 M^{-1} s^{-1}. At 40 mM ATP ($>>K_m$), the distribution of the durations of the catalytic pause was reproduced by a consecutive reaction with two time constants of 2.6 and 0.5 ms. These kinetic parameters were similar to those of TtV_1. Our results identified the common properties of rotary catalysis of V_1-ATPases that are distinct from those of F_1-ATPases and furthered our understanding of the general mechanisms of rotary molecular motors.

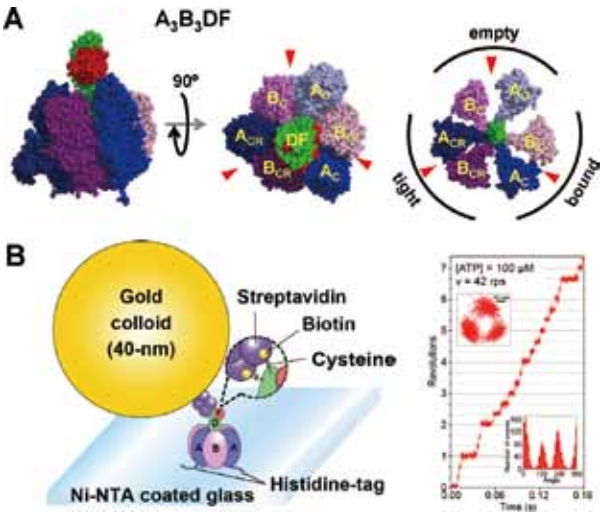


Figure 2. (A) Crystal structure of EhV_1. (B) Left, Schematics of the single-molecule rotation assay of EhV_1. Right, Example of the rotary motion.

2. Mechanism of Different Susceptibilities of Cellulose I_α and III_I to Hydrolysis by a Linear Molecular Motor Cellulase[2)]

A cellulase, *Trichoderma reesei* Cel7A (*Tr*Cel7A) is a linear molecular motor that directly hydrolyzes crystalline celluloses into water-soluble cellobioses. It has recently drawn attention as a tool that could be used to convert cellulosic materials into biofuel. However, detailed mechanisms of action, including elementary reaction steps such as binding, processive hydrolysis, and dissociation, have not been thoroughly explored because of the inherent challenges associated with monitoring reactions occurring at the solid/liquid interface. The crystalline cellulose I_α and III_I were previously reported as substrates with different crystalline forms and different susceptibilities to hydrolysis by *Tr*Cel7A. We observed that different susceptibilities of cellulose I_α and III_I are highly dependent on enzyme concentration, and at nanomolar enzyme concentration, *Tr*Cel7A shows similar rates of hydrolysis against cellulose I_α and III_I. Using single-molecule fluorescence microscopy and high-speed atomic force microscopy, we also determined kinetic constants of the elementary reaction steps for *Tr*Cel7A against cellulose I_α and III_I. These measurements were performed at picomolar enzyme concentration in which density of *Tr*Cel7A on crystalline cellulose was very low. Under this condition, *Tr*Cel7A displayed similar binding and dissociation rate constants for cellulose I_α and III_I and similar fractions of productive binding on cellulose I_α and III_I. Furthermore, once productively bound, *Tr*Cel7A processively hydrolyzes and moves along cellulose I_α and III_I with similar translational rates. With structural models of cellulose I_α and III_I, we proposed that different susceptibilities at high *Tr*Cel7A concentration arise from surface properties of substrate, including ratio of hydrophobic surface and number of available lanes (Figure 3).

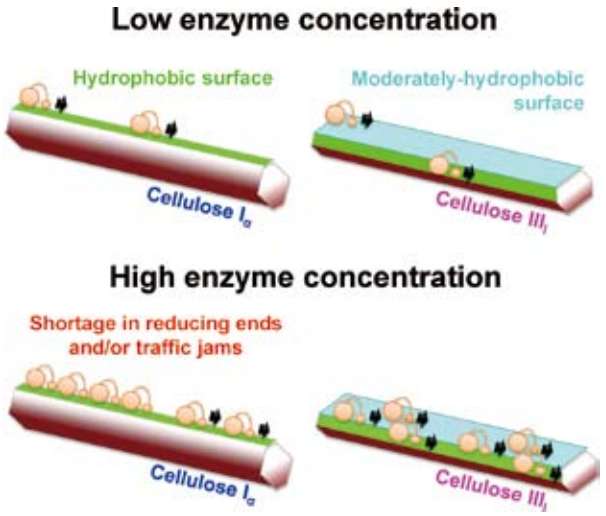


Figure 3. A model of different susceptibilities of cellulose I_α and III_I to hydrolysis by *Tr*Cel7A.

References

1) 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, *J. Biol. Chem.* **288**, 32700–32707 (2013).

2) 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, *J. Biol. Chem.* **289**, 14056–14065 (2014).

Structure-Function Relationship of Metalloenzymes

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

FUJII, Hiroshi
Associate Professor
(–March, 2014)
[hiro@ims.ac.jp]

Education
1985 B.E. Kanazawa University
1990 Ph.D. Kyoto University

Professional Employment
1990 Assistant Professor, Hokkaido University
1992 Postdoctoral Fellow, University of Minnesota
1994 Chief Scientist, Institute f or Life Support Technology, Yamagata Technopolis Foundation
1998 Associate Professor, Institute for Molecular Science
Associate Professor, The Graduate University for Advanced Studies
2001 Associate Professor, Okazaki Institute f or Integrative Bioscience
2014 Professor, Nara Women's University

Member
Assistant Professor
KURAHASHI, Takuya
IMS Fellow
KINEMUCHI, Haruki
Secretary
TANIZAWA, Misako

Keywords Metalloenzyme, Reaction Intermediate, Reaction Mechanism

Metalloproteins are a class of biologically important macromolecules, which have various functions such as oxygen transport, electron transfer, oxidation, and oxygenation. These diverse functions of metalloproteins have been thought to depend on the ligands from amino acid, coordination structures, and protein structures in immediate vicinity of metal ions. In this project, we are studying the relationship between the electronic structures of the metal active sites and reactivity of metalloproteins.

Selected Publications

- H. Fujii, "Effects of the Electron-Withdrawing Power of Substituents on the Electronic Structure and Reactivity in Oxoiron(IV) Porphyrin π-Cation Radical Complexes," *J. Am. Chem. Soc.* **115**, 4641–4648 (1993).
- H. Fujii, X. Zhang, T. Tomita, M. Ikeda-Saito and T. Yoshida, "A Role for Highly Conserved Carboxylate, Aspartate-140, in Oxygen Activation and Heme Degradation by Heme Oxygenase-1," *J. Am. Chem. Soc.* **123**, 6475–6485 (2001).
- H. Fujii, "^{13}C-NMR Signal Detection of Iron Bound Cyanide Ions in Ferric Cyanide Complexes of Heme Proteins," *J. Am. Chem. Soc.* **124**, 5936–5937 (2002).
- H. Fujii, X. Zhang and T. Yoshida, "Essential Amino Acid Residues Controlling the Unique Regioselectivity of Heme Oxygenase in *Psudomonas aeruginosa*," *J. Am. Chem. Soc.* **126**, 4466–4467 (2004).
- M. Kujime and H. Fujii, "Spectroscopic Characterization of Reaction Intermediates in Nitrite Reduction of Copper(I) Nitrite Complex as a Reaction Model for Copper Nitrite Reductase," *Angew. Chem., Int. Ed.* **45**, 1089–1092 (2006).
- C. Wang, T. Kurahashi and H. Fujii, "Structure and Reactivity of Iodosylarene Adduct of Manganese(IV) Salen Complex," *Angew. Chem., Int. Ed.* **51**, 7809–7811 (2012).
- A. Takahashi, D. Yamaki, K. Ikemura, T. Kurahashi, T. Ogura, M. Hada and H. Fujii, "The Effect of the Axial Ligand on the Reactivity of the Oxoiron(IV) Porphyrin π-Cation Radical Complex: Higher Stabilization of the Product State Relative to the Reactant State," *Inorg. Chem.* **51**, 7296–7305 (2012).
- Z. Cong, S. Yanagisawa, T. Kurahashi, T. Ogura, S. Nakashima and H. Fujii, "Synthesis, Characterization, and Reactivity of Hypochlorito-Iron(III) Porphyrin Complexes," *J. Am. Chem. Soc.* **134**, 20617–20620 (2012).

1. Oxygen-Atom Transfer from Iodosylarene Adducts of a Manganese(IV) Salen Complex: Effect of Arenes and Anions on I(III) of the Coordinated Iodosylarene[1)]

This paper reports preparation, characterization, and reactivity of iodosylarene adducts of a manganese-(IV) salen complex. In order to systematically investigate steric and electronic factors that control reactivity and selectivity, we prepared iodosylarene adducts from iodosylbenzene, iodosylmesitylene, 2,4,6-triethyliodosylbenzene, and pentafluoroiodosylbenzene. We also investigated the effect of anions on I(III) by using chloride, benzoate, and *p*-toluenesulfonate. Spectroscopic studies using ^{1}H NMR, electron paramagnetic resonance, infrared spectroscopy, and electrospray ionization mass spectrometry show that these iodosylarene adducts are manganese(IV) complexes bearing two iodosylarenes as external axial ligands. Reactions with thioanisole under the pseudo-first-order conditions show that the electron-withdrawing pentafluorophenyl group and the *p*-toluenesulfonate anion on I(III) significantly accelerate the oxygen-atom transfer. The high reactivity is correlated with a weakened I–OMn bond, as indicated by IR spectroscopy and mass spectrometry. Stoichiometric reactions with styrenes show that both enantioselectivity and diastereoselectivity are dependent on the arenes and anions on I(III) of the coordinate iodosylarenes. Notably, the pentafluorophenyl group and the *p*-toluenesulfonate anion suppress the *cis*-to-*trans* isomerization in the epoxidation of *cis*-β-methylstyrene. The present results show that iodosylarene adducts of manganese(IV) salen complexes are indeed active oxygen-atom-transfer reagents and that their reactivity and selectivity are regulated by steric and electronic properties of the arenes and anions on I(III) of the coordinated iodosylarenes.

2. Di-μ-oxo Dimetal Core of Mn^{IV} and Ti^{IV} as a Linker Between Two Chiral Salen Complexes Leading to the Stereoselective Formation of Different *M*- and *P*-Helical Structures[2)]

Because of restricted rotational freedom along the metal–metal axis, a di-μ-oxo dimetal core could be an excellent building block to create dinuclear compounds with well-defined stereochemistry, but their stereoselective synthesis remains a challenge. We herein report the formation of di-μ-oxo dimanganese(IV) complexes with tetradentate salen ligands bearing different degrees of steric bulk, in order to study stereochemical aspects of the dimerization reaction that potentially generates multiple stereoisomers. X-ray crystallography shows that the di-μ-oxo dimanganese(IV) complex with salen, where salen is (*R*,*R*)-*N*,*N*'-bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediamine, adopts a unique structure in which two salen complexes are arranged in an *M*-helical fashion. According to the solution study using ^{1}H, ^{2}H NMR, and circular dichroism spectroscopies, the dimerization reaction is highly diastereoselective in the presence of the *tert*-butyl group at the 3/3' position as a determinant steric factor. In contrast, the di-μ-oxo dititanium(IV) complex with the same salen ligand was previously reported to afford an opposite *P*-helical dimer. The present DFT study clarifies that a less-covalent Ti–O bonding causes a distortion of the di-μ-oxo dititanium(IV) core structure, generating a completely different framework for interligand interaction. The present study provides a solid basis to understand the stereochemistry for the formation of the di-μ-oxo dimetal core.

References

1) C. Wang, T. Kurahashi, K. Inomata, M. Hada and H. Fujii, *Inorg. Chem.* **52**, 9557–9566 (2013).

2) T. Kurahashi, M. Hada and H. Fujii, *Inorg. Chem.* **53**, 1070–1079 (2014).

Supramolecular Chemical Approach to Construction of Artificial Cell

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

KURIHARA, Kensuke
Research Associate Professor
(OKAZAKI ORION Project)
[kkurihara@ims.ac.jp]

Education
2005 B.S. The University of Tokyo
2010 Ph.D. The University of Tokyo

Professional Employment
2010 Postdoctoral Fellow, The University of Tokyo
2013 Postdoctoral Fellow, Research & Education Platf orm for Dynamics Living States, The University of Tokyo
2014 Research Associate Professor, Institute for Molecular Science
Research Associate Prof essor, Okazaki Institute f or Integrative Bioscience (OKAZAKI ORION Project)

Keywords Artificial Cell, Supramolecular Chemistry, Giant Vesicle

Exploring the boundary between a living and non-living matter is one of the most challenging problems for 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. By using a giant vesicle (GV), which is a supramolecular assembly of amphiphiles, as compartment, we constructed an artificial cellular system in which self-reproduction of GV and the amplification of internal DNA were combined. Such a constructive approach would be a powerful method of elucidating not only the boundary but also the origin of life.

In our laboratory, we aim to construct the following artificial cells: An artificial cell having a cycle of proliferation and an artificial cell which can be self-organized according to its environment.

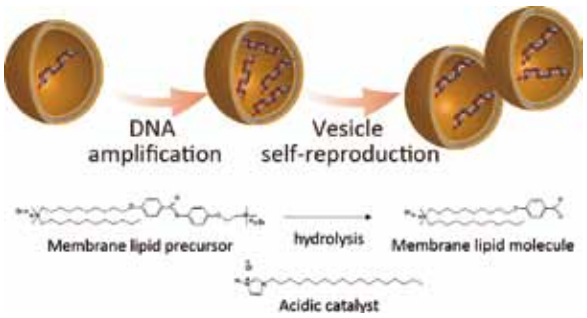


Figure 1. Proliferation of our artificial cellular system based on giant vesicle and structural formula of the molecules in the system.

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).
- K. Kurihara, K. Takakura, K. Suzuki, T. Toyota and T. Sugawara, "Cell-Sorting of Robust Self-Reproducing Giant Vesicles Tolerant to a Highly Ionic Medium," *Soft Matter* **6**, 1888–1891 (2010).

1. An Artificial Cell Incorporating a Proliferation 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 chemical approach.[1,2] We have constructed artificial cell which has three basic elements of a cell; information (DNA), compartment (giant vesicle: A supramolecular assembly of amphiphiles) and metabolism (synthetic catalyst).[3] The proliferation of the artificial cell was consisted of amplification of DNA and self-reproduction of GV. This vesicle is consisted of phospholipids, cationic synthesized molecules and cationic catalysts. Here, we added some phospholipids to the GVs for resistance of highly ionic medium and high temperature. First, we encapsulated template DNA and PCR reagents into the GVs and performed polymerase chain reaction to the GV dispersion and then the internal DNA was amplified. Second, we added a precursor of the GV membrane lipid molecule to the GV dispersion subjected to thermal cycles. After addition of the precursor, the GV proliferated accompanying with amplified DNA. From flow cytometric analysis, we found that the division of the GVs was accelerated by the amount of the amplified DNA in the GVs. We speculated that this complex formed by synthetic catalyst and the amplified DNA acted as an active scaffold of hydrolysis of membrane lipid precursor. This result means that information and compartment were combined.

However, this system ceased at the 2nd generation of GV because it does not have a cycle of growth and division. Now, we are constructing the recursive GV system with proliferation cycles, collaborating with Sugawara group (Kanagawa University). By using our 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. The replenished GV can amplify the internal DNA and yield 3rd generation of the GV after addition of membrane lipid precursor. This system would lead to an evolvable artificial cellular system.

2. An Artificial Cell Incorporating a Cross-Catalysis 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.

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. We are constructing an artificial cell incorporating a cross-catalysis system. 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 packing parameter of the catalysts on the membrane, the GV collapse when the number of the catalyst increased substantially. This means that the artificial cell incorporating the negative feedback is realized.

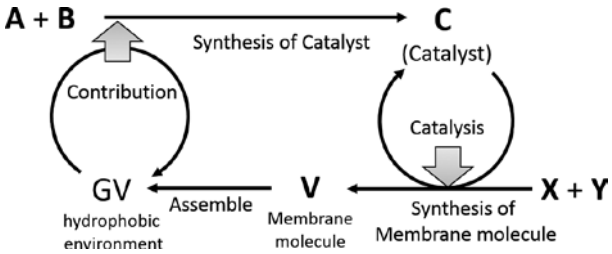


Figure 2. Scheme of new artificial cellular system. The membrane molecules of the GV was synthesized by the catalyst produced in the GV.

References

1) K. Takakura, T. Yamamoto, K. Kurihara, T. Toyota, K. Ohnuma and T. Sugawara, *Chem. Commun.* **50**, 2190–2192 (2014).
2) 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).
3) K. Kurihara, M. Tamura, K-I. Shohda, T. Toyota, K. Suzuki and T. Sugawara, *Nat. Chem.* **3**, 775–781 (2011).
4) K. Suzuki, R. Aboshi, k. Kurihara and T. Sugawara, *Chem. Lett.* **41**, 789–791 (2012).

Investigation of Molecular Mechanisms of Channels, Transporters and Receptors

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

FURUTANI, Yuji
Associate Professor
[furutani@ims.ac.jp]

Education
1999 B.S. Kyoto University
2004 Ph.D. Kyoto University

Professional Employment
2003 JSPS Research Fellow
2004 JSPS Postdoctoral Fellow
2006 Assistant Professor, Nagoya Institute of Technology
2009 Associate Professor, Institute for Molecular Science
Associate Professor, The Graduate University for Advanced Studies
2011 JST-PRESTO Researcher (concurrent post) (–2015)

Awards
2012 Morino Foundation for Molecular Science
2013 The 2013 Young Scientist Awards of the Japan Society for Molecular Science

Member
Assistant Professor
TSUKAMOTO, Hisao
Visiting Scientist
LÉCUYER, Thomas*
Technical Fellow
FUJIWARA, Kuniyo
Secretary
SHIMIZU, Atsuko

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.

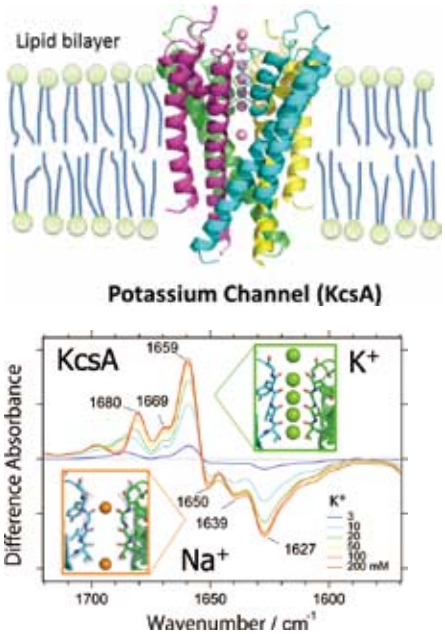


Figure 1. (top) X-ray crystal structure of a potassium ion channel, KcsA. (bottom) The ion-exchange induced difference infrared spectra of KcsA with different potassium ion concentration. The amide I bands are mainly originated from the carbonyl groups of the selectivity filter of KcsA.

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. Formation of Host–Guest Complexes on Gold Surface Investigated by Surface-Enhanced IR Absorption Spectroscopy[1)]

Surface-enhanced infrared absorption with attenuated total reflection (ATR-SEIRA) is a powerful tool for studying molecular systems at the monolayer level.

Ionophores capture guest ions selectively and carry them across interfaces efficiently. One of crown ethers, 18-crwon-6 (18C6) is one of well known ionophores for a potassium ion. Molecular mechanisms of the ion selectivity of 18C6 have been investigated by SEIRA spectroscopy as a cooperative research with Assoc. Prof. Inokuchi in Hiroshima University.

Thiol derivatives of 18C6 [2-(6-mercaptohexyloxy)methyl-18-crown-6 (18C6-C_1OC_6-SH) and 2-(mercaptomethyl)-18-crown-6 (18C6-C_1-SH)] were synthesized and chemisorbed on a gold surface (Figure 2). Aqueous solutions of MCl salts (M = alkali metals) were put on it to form M^+•18C6-C_1OC_6 and M^+•18C6-C_1 complexes. Infrared spectra of these complexes in the 2000–900 cm^{-1} region were obtained by SEIRA spectroscopy.

As a result, the SEIRA spectra of 18C6 with K^+ are very similar to those with Rb^+ and Cs^+, but largely different from those with Li^+ and Na^+. Moreover, it was demonstrated that the affinity for K^+ is higher than those for other alkali cations. Obtained results proved that SEIRA spectroscopy is a powerful method to examine the structure of host-guest complexes and the solvent effect on them in solutions.

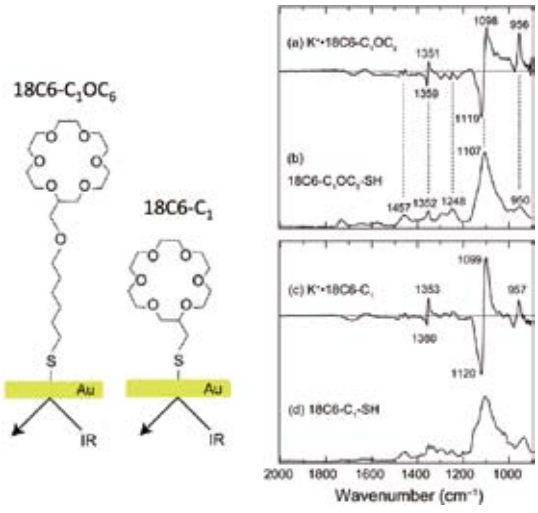


Figure 2. (left) Schematic figures of crown ethers immobilized on the gold surface through a S–Au bond. (right) SEIRA spectra of crown ethers 18C6-C1OC6 (a) and 18C6-C1 (c) recorded after addition of 0.1 M KCl solution. The absorption spectra of the crown ethers (b) and (d) were recorded by a conventional ATR-FTIR method. This figure is reproduced from ref. 1.

2. Deformation of β-Sheet Structures of the GroEL Apical Domain Induced at Sub-Micellar Detergent Condition[2)]

SEIRA spectroscopy is a useful tool to analyze protein structure as well. GroEL is a chaperonin which refolds denatured proteins with a cofactor GroES by utilizing hydrolysis energy of ATP. Dr. Jin Chen, who was an IMS research assistant professor in Prof. Kuwajima's Group in Okazaki Institute for Integrative Bioscience, studied the property of GroEL for formation of protein nanofibers at sub-micellar detergent condition. To understand the molecular mechanism of the fiber formation, SEIRA analysis on the GroEL apical domain was performed (Figure 3).

The data clearly showed SDS-dependent deformation of β-sheet structures in the GroEL apical domain, which would promote the formation of the nanofiber in the later stage.

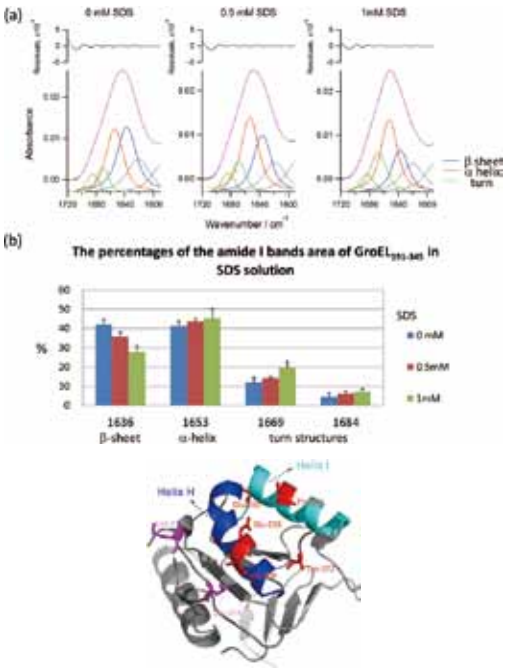


Figure 3. (a) The SEIRA spectra of GroEL apical domain recorded in SDS solution (0, 0.5, and 1 mM). The amide I bands are analyzed by band fitting method. (b) The effect of SDS on the secondary structures of GroEL apical domain. (c) The X-ray crystal structure of GroEL apical domain. This figure is adapted from ref. 2.

References

1) Y. Inokuchi*, T. Mizuuchi, T. Ebata, T. Ikeda, T. Haino, T. Kimura, H. Guo and Y. Furutani, *Chem. Phys. Lett.* **592**, 90–95 (2014).
2) J. Chen*, H. Yagi, Y. Furutani, T. Nakamura, A. Inaguma, H. Guo, Y. Kong and Y. Goto, *Sci. Rep.* **4**:5614 (2014).
(*; corresponding authors)

Awards

FURUTANI, Yuji; The 2013 Young Scientist Awards of the Japan Society for Molecular Science.
FURUTANI, Yuji; The 1st BIOPHYSICS Editors' Choice Award (2014).

* IMS International Internship Program

Development of Heterogeneous Catalysis toward Ideal Chemical Processes

Department of Life and Coordination-Complex Molecular Science
Division of Complex Catalysis

UOZUMI, Yasuhiro
Professor
[uo@ims.ac.jp]

Education
1984 B.S. Hokkaido University
1990 Ph.D. Hokkaido University

Professional Employment
1988 JSPS Research Fellow
1988 Research Associate, Hokkaido University
1990 Assistant Professor, Hokkaido University
1994 Research Associate, Columbia University
1995 Lecturer, Kyoto University
1997 Professor, Nagoya City University
2000 Professor, Institute for Molecular Science
Professor, The Graduate University for Advanced Studies
2007 Research team leader, RIKEN
2014 Distinguished Professor, Three George University
2003 Research Project Leader, JST CREST Project (–2008)
2008 Research Project Leader, NEDO Project (–2012)
2011 Deputy Research Project Leader, JST CREST (–2016)

Awards
1991 Eisai Award, Synthetic Organic Chemistry
1998 The Pharmaceutical Society of J apan Award for Young Scientist
2007 The Chemical Society of J apan (CSJ) Award for Creative Work
2007 MEXT Ministerial Award for Green Sustainable Chemistry
2010 Inoue Prize for Science
2014 The Commendation for Science and Technology by the Minister of MEXT, Prize for Science and Technology

Member
Assistant Professor
OSAKO, Takao
HAMASAKA, Go
Post-Doctoral Fellow
NAGAOSA, Makoto
PAN, Shiguang
Graduate Student
KOBAYASHI, Noboru
SAKURAI, Fumie
TSUJI, Hiroaki
YAN, Shuo
Technical Fellow
TORII, Kaoru
TAZAWA, Aya
Secretary
SASAKI, Tokiyo
FUKUSHIMA, Tomoko

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.

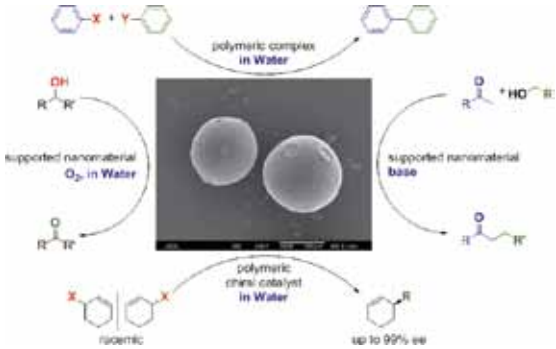


Figure 1. Typical Examples of Heterogeneous Aquacatalyses using Amphiphilic Polymer-Supported Metal Complexes and Metal Nanoparticles.

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).
- Y. M. A. Yamada, S. M. Sarkar and Y. Uozumi, "Self-Assembled Poly(imidazole-palladium): Highly Active, Reusable Catalyst at Parts per Million to Parts per Billion Levels," *J. Am. Chem. Soc.* **134**, 3190–3198 (2012).
- G. Hamasaka, T. Muto and Y. Uozumi, "Molecular-Architecture-Based Administration of Catalysis in Water: Self-Assembly of an Amphiphilic Palladium Pincer Complex," *Angew. Chem., Int. Ed.* **50**, 4876–4878 (2011).
- Y. Uozumi, Y. Matsuura, T. Arakawa and Y. M. A. Yamada, "Asymmetric Suzuki-Miyaura Coupling in Water with a Chiral Pallasium Catalyst Supported on Amphiphilic Resin," *Angew. Chem., Int. Ed.* **48**, 2708–2710 (2009).
- Y. M. A. Yamada, T. Arakawa, H. Hocke and Y. Uozumi, "A Nanoplatinum Catalyst for Aerobic Oxidation of Alcohols in Water," *Angew. Chem., Int. Ed.* **46**, 704–706 (2007).
- Y. Uozumi, Y. M. A. Yamada, T. Beppu, N. Fukuyama, M. Ueno and T. Kitamori, "Instantaneous Carbon–Carbon Bond Formation Using a Microchannel Reactor with a Catalytic Membrane," *J. Am. Chem. Soc.* **128**, 15994–15995 (2006).

1. A Palladium-Nanoparticles and Sillicon-Nanowire-Array Hybrid: A Platform for Catalytic Heterogeneous Reactions[1)]

We report the development of a silicon nanowire array-stabilized palladium nanoparticle catalyst, SiNA-Pd. Its use in the palladium-catalyzed Mizoroki-Heck reaction, the hydrogenation of an alkene, the hydrogenolysis of nitrobenzene, the hydrosilylation of an α,β-unsaturated aldehyde, and the C–H bond functionalization reactions of thiophenes and indoles achieved a quantitative production with high reusability. The catalytic activity reached several hundred-mol ppb of palladium, reaching a TON of 2,000,000.

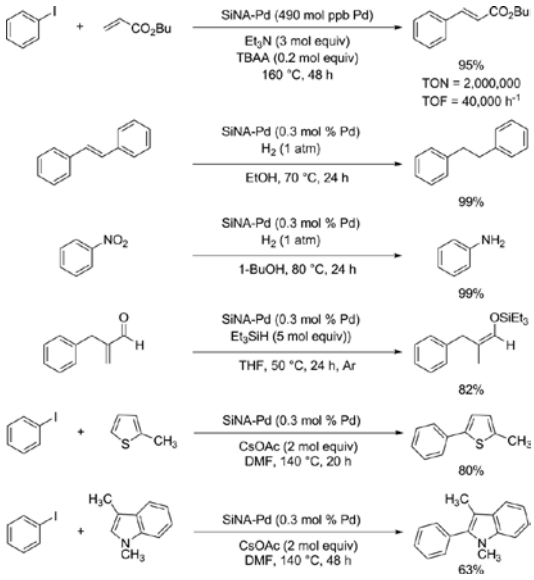


Figure 2. The Mizoroki-Heck reaction, the hydrogeneation of an alkene, the hydrogenolysis of nitrobenzene, the hydrosilation of an α,β-unsaturated aldehyde, and the C–H bond functionalization reactions of a thiophen and a indole catalyzed by SiNA-Pd.

2. Direct Dehydrative Esterification of Alcohols and Carboxylic Acids with a Macroporous Polymeric Acid Catalyst[2)]

A macroporous polymeric acid catalyst was prepared for the direct esterification of carboxylic acids and alcohols that proceeded at 50–80 °C without removal of water to give the corresponding esters with high yield. Flow esterification for the synthesis of biodiesel fuel was also achieved by using a column-packed macroporous acid catalyst under mild conditions without removal of water.

Figure 3. Direct dehydrative esterification of alchols and carboxylic acids with a macroporous polymeric acid catalyst.

3. Asymmetric Sonogashira Coupling with a Chiral Palladium Imidazoindole Phosphine Complex[3)]

The asymmetric Sonogashira coupling of 1-(2,6-dibromophenyl)naphthalene or 4,16-dibromo[2,2]paracyclophane with various terminal alkynes was carried out with a palladium complex of a homochiral imidazoindole phosphine, a derivative of a (3*R*,9a*S*)-2-aryl-[3-(2-dialkylphosphanyl)phenyl] tetrahydro-1*H*-imidazo[1,5*a*]indol-1-one, to give the corresponding axially chiral monoalkynylated biaryl products with up to 72% enantiomeric excess.

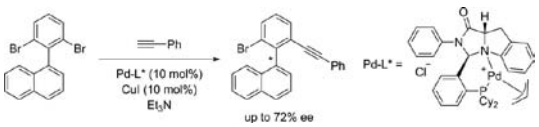


Figure 4. Asymmetric Sonogashira coupling with a chiral palladium imidazoindolephosphine complex.

References

1) Y. M. A. Yamada, Y. Yuyama, T. Sato, S. Fujikawa and Y. Uozumi, *Angew. Chem., Int. Ed.* **53**, 127–131 (2014).
2) M. Minakawa, H. Baek, Y. M. A. Yamada, J. W. Han and Y. Uozumi, *Org. Lett.* **15**, 5798–5801 (2013).
3) H. Zhou and Y. Uozumi, *Synlett* **24**, 2550–2554 (2013).

Award

UOZUMI, Yasuhiro; The Commendation for Science and Technology by the Minister of MEXT, Prize for Science and Technology (2014).

Design and Synthesis of Chiral Organic Molecules for Asymmetric Synthesis

Department of Life and Coordination-Complex Molecular Science
Division of Complex Catalysis

MOMIYAMA, Norie
Associate Professor
[momiyama@ims.ac.jp]

Education
2000 B.S. Nagoya University
2005 Ph.D. The University of Chicago

Professional Employment
2005 Postdoctoral Fellow, Harvard University
2006 Assistant Professor, Tohoku University
2014 Associate Professor, Institute for Molecular Science
Associate Professor, The Graduate University for Advanced Studies

Awards
2003 The Elizabeth R. Norton Prize for Excellence in Research in Chemistry, University of Chicago
2004 Abbott Laboratories Graduate Fellowship
2005 Damon Runyon Cancer Research F oundation Post Doctoral Research Fellowship
2008 Thieme Chemistry Journals Award

Member
Secretary
WATANABE, Yoko

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.

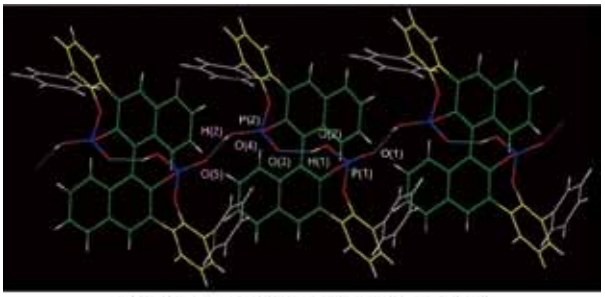


Figure 1. Hydrogen bonding network in chiral bis-phosphoric acid catalyst derived from (*R*)-3,3'-di(2-hydroxy-3 -arylphenyl)binaphthol. Hydrogen bond acts as activation unit for the substrate in asymmetric reaction space and controls atropisomeric behavior in naphthyl–phenyl axis.

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.

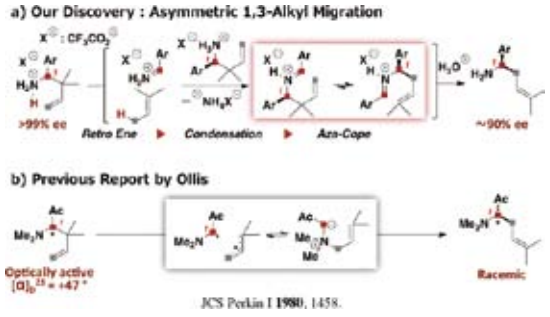


Figure 2. 1,3-Alkyl migration of 1-substituted-3-buten-1-amine. a) Our discovery, b) Previous report by Ollis *et al.*

2. Design of C_1 Symmetric Chiral Bis-Phosphoric Acid: Catalytic Enantioselective Diels–Alder Reaction of Acrolein with Amidodienes

We recently developed (*R*)-3,3'-di(2-hydroxy- 3-arylphenyl) binaphthol derived pseudo-C_2 symmetric chiral bis-phosphoric acid which efficiently catalyzed enantioselective Diels–Alder reaction of α,β-unsaturated aldehydes with amidodienes.[3)] Two cyclic phosphoric acid motifs introduced between the $C_{Naph}(2)$ and $C_{Ar}(2)$ positions and between the $C_{Naph}(2')$ and $C_{Ar}(2)$ positions represents a characteristic feature of our catalysts. On the basis of our early hypothesis and recent results, the intramolecular hydrogen bonding between two acidic moieties seems to be deeply related to control an atropisomeric behavior of catalyst structure; however, none of systematic study have been employed with respect to the importance of hydrogen bond in the molecular design of chiral catalysts.

We designed a new C_1 symmetric chiral bis-phosphoric acid that possesses an electron-withdrawing group at the $C_{Naph}(3')$–$C_{Ar}(3)$, $C_{Ar}(5)$.[4)] We found that (i) the stereodynamic behavior of atropisomeric biaryls was controlled by the intervention of hydrogen bond, (ii) the requisite catalyst activity was served by the electronic effect at the $C_{Naph}(3')$–$C_{Ar}(3)$, and (iii) the precise distinction of asymmetric reaction space was realized by the different substitution at the $C_{Naph}(3)$–$C_{Ar}(3)$ and the $C_{Naph}(3')$–$C_{Ar}(3)$.

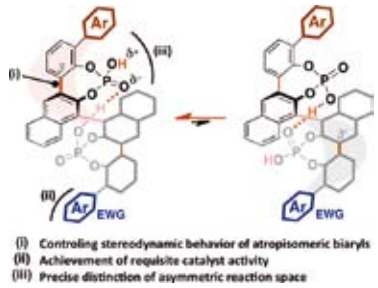


Figure 3. Molecular design of chiral C_1 symmetric bis-phosphoric acid.

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.[5)]

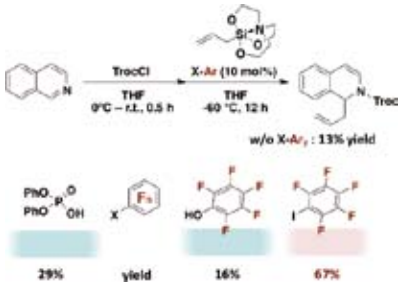


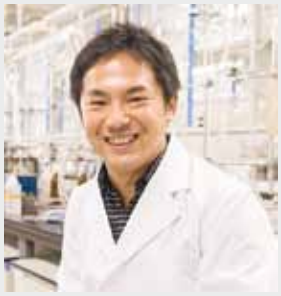
Figure 4. Halogen bond donor catalyzed allylation reaction. Comparison with Brønsted acid/hydrogen bond donor catalyst.

References

1) N. Momiyama, C. Kanai, A. Takei and M. Terada, Manuscript in preparation.
2) R. W. Jemison, T. Laird, W. D. Ollis and I. O. Sutherland, *J. Chem. Soc. Perkin Trans. 1* 1458–1461 (1980).
3) N. Momiyama, T. Konno, Y. Furiya, T. Iwamoto and M. Terada, *J. Am. Chem. Soc.* **133**, 19294–19297 (2011).
4) N. Momiyama, K. Funayama, H. Noda, M. Yamanaka, N. Akasaka, S. Ishida, T. Iwamoto and M. Terada, Manuscript in preparation.
5) N. Momiyama, H. Nishimoto, Y. Kamata and M. Terada, Manuscript in preparation.

Development of Functional Metal Complexes for Artificial Photosynthesis

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



MASAOKA, Shigeyuki
Associate Professor
[masaoka@ims.ac.jp]

Education
1999 B.S. Doshisha University
2004 Ph.D. Kyoto University

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
2011 Associate Professor, Institute for Molecular Science
Associate Professor, The Graduate University for Advanced Studies

Member
Assistant Professor
KONDO, Mio
Post-Doctoral Fellow
VIJAYENDRAN, Praneeth
Graduate Student
NAKAMURA, Go
OKAMURA, Masaya
ITOH, Takahiro
OKABE, Yuki
CHINAPANG, Pondchanok
LIU, Ke
FUKATSU, Arisa
FUKAHORI, Yukino
IZU, Hitoshi
ENOMOTO, Takafumi
Technical Fellow
KUGA, Reiko
KANAIKE, Mari
SHIBATA, Akane
Secretary
TANIWAKE, Mayuko

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.

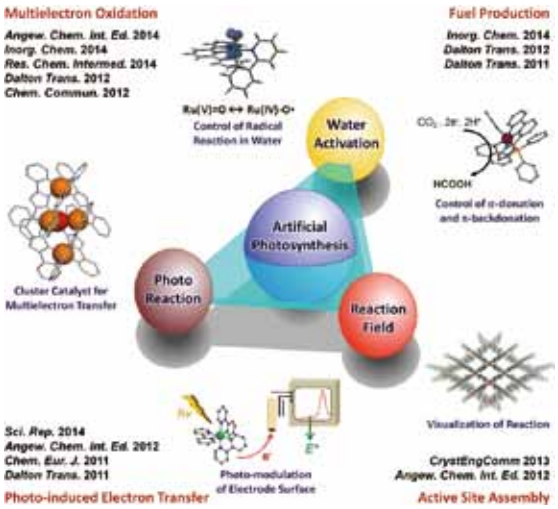


Figure 1. An overview of our work.

Selected Publications

- M. Yoshida, M. Kondo, 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).
- M. Okamura, M. Yoshida, R. Kuga, K. Sakai, M. Kondo and S. Masaoka, "A Mononuclear Ruthenium Complex Showing Multiple Proton-Coupled Electron Transfer toward Multi-Electron Transfer Reactions," *Dalton Trans.* **41**, 13081–13089 (2012).
- A. Kimoto, K. Yamauchi, M. Yoshida, S. Masaoka and K. Sakai, "Kinetics and DFT Studies on Water Oxidation by Ce^{4+} Catalyzed by $[Ru(terpy)(bpy)(OH_2)]^{2+}$," *Chem. Commun.* **48**, 239–241 (2012).

1. Electrochemical Response of Metal Complexes in Homogeneous Solution under Photoirradiation[1)]

Photoinduced electron transfer (PET) is a key process in reactions that convert light energy to electrical or chemical energy, both in natural and artificial systems. The efficiency of PET, which largely affects the performance of these systems, is correlated with the redox properties of the photoexcited molecule, which transfers electrons or holes during the PET reaction. Hence, determining the redox potentials of photoexcited molecules is of great significance not only for understanding the mechanisms of PET reactions but also for achieving highly efficient light-energy conversion systems. Electrochemical analysis under photoirradiation should enable the measurement of the redox potentials of excited species. However, reports of the direct electrochemical detection of photoexcited molecules have been limited to only a few examples in which specialized photoelectrochemical instrumentation was required. This limitation may be due to the difficulty to avoid the complication of voltammogram profiles that arises from the unintended side effects of photoirradiation, such as temperature increases and enhanced mass transfer. Thus, the redox potentials of excited states have more commonly been indirectly estimated using the 0-0 transition energy (E_{00}) or the quenching rate constant (k_q). Therefore, the establishment of a versatile methodology for electrochemical measurements under photoirradiation will provide new insights into PET phenomena.

To establish a simple method for directly determining the redox potentials of the photoexcited states of metal complexes, electrochemical measurements under several conditions were performed. The electrochemical response was largely influenced not only by the generation of photoexcited molecules but also by the convection induced by photoirradiation, even when the global temperature of the sample solution was unchanged. The suppression of these unfavourable electrochemical responses was successfully achieved by adopting well-established electrochemical techniques. Furthermore, as an initial demonstration, the photoexcited state of a Ru-based metal complex was directly detected, and its redox potential was determined using a thin layer electrochemical method.

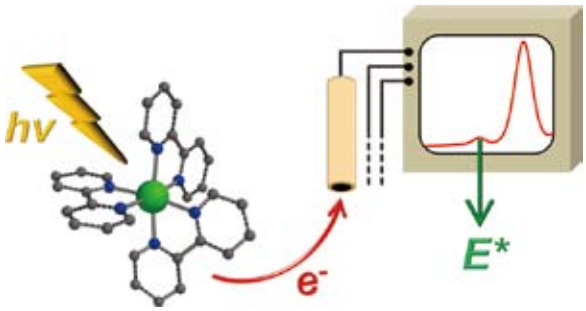


Figure 2. Schematic illustration of electrochemical measurement of a metal complex under photoirradiation.

2. Phosphine-Substituted Ruthenium(II) Polypyridine Complexes with a Single Labile Ligand[2)]

Ruthenium(II) polypyridine complexes with a single labile ligand have been widely studied as catalysts for many chemical reactions including water oxidation, CO_2 reduction, and photoinduced chemical conversions. One of the key strategies in developing ruthenium polypyridine catalysts with novel properties and reactivities is the introduction of phosphine ligands because their σ-donating and π-accepting abilities can influence electronic structures of the ruthenium center. However, few studies examining substitution of phosphine for pyridine moiety have been reported; there have been only a few works of diphosphine-coordinated ruthenium(II) polypyridine complexes, and no crystal structures have been reported. Thus, the investigation of phosphine-substituted ruthenium(II) polypyridine complexes with a single labile is important not only for the design and development of new catalysts but also for an understanding of their basic properties.

We report the synthesis, structural characterization, and electrochemical and spectroscopic properties of a series of ruthenium(II) polypyridine complexes containing 8-(diphenylphosphanyl)quinoline (Pqn), *trans*(P,MeCN)-[Ru(trpy)(Pqn)(MeCN)]$(PF_6)_2$ (*trans*-**PN**) and *cis*(P,MeCN)-[Ru(trpy)(Pqn)(MeCN)]$(PF_6)_2$ (*cis*-**PN**), or 1,2-bis(diphenylphosphanyl)benzene (dppbz), [Ru(trpy)(dppbz)(MeCN)]$(PF_6)_2$ (**PP**). Effects of the number and position of phosphine donors on the structures and electronic properties were investigated on the basis of comparisons with [Ru(trpy)(bpy)(MeCN)]$(PF_6)_2$ (**NN**). The electrochemical measurements of these complexes showed distinct behavior in their reduction reactions; reduction of *cis*-**PN** resulted in *cis–trans* isomerization to *trans*-**PN**, and that of **PP** proceeded via a two electron-transfer reaction. The mechanism of these electrochemical behaviors was explained in conjunction with the liberation of a monodentate labile ligand.

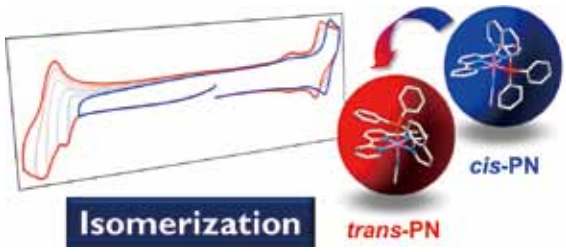


Figure 3. Cyclic voltammograms showing redox-induced *cis–trans* isomerization from *cis*-**PN** to *trans*-**PN**.

References

1) A. Fukatsu, M. Kondo, M. Okamura, M. Yoshida and S. Masaoka, *Sci. Rep.* **4**, 5327 (2014).
2) G. Nakamura, M. Okamura, M. Yoshida, T. Suzuki, H. D. Takagi, M. Kondo and S. Masaoka, *Inorg. Chem.* **53**, 7214–7226 (2014).

Award

OKAMURA, Masaya; Student Lecture Award, The 63rd Conference of Japan Society of Coordination Chemistry (2013).

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 r edox-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 Associate 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
KAMIKUKBO, Hironari *(from Nara Institute of Science and Technology)*

Excited-State Proton Transfer in Photoactive Yellow Protein

Green fluorescent protein (GFP) has been used as an effective tool in various biological fields. The large Stokes shift resulting from an excited-state proton transfer (ESPT) is the basis for the application of GFP. The chromophore of GFP is known to be involved in a hydrogen-bonding network. Previous X-ray crystallographic and FTIR studies suggest that a proton wire along the hydrogen-bonding network plays a role in the ESPT. In order to examine the relationship between the ESPT and hydrogen-bonding network within proteins, we prepared an artificial fluorescent protein using a light-sensor protein, photoactive yellow protein (PYP). Although PYP emits little fluorescence, we succeeded to reconstitute an artificial fluorescent PYP (PYP-coumarin) by substituting the pCA with its trans-lock analog 7-hydroxycoumarin. Spectroscopic studies with PYP-coumarin revealed that the chromophore takes an anionic form at neutral pH, but is protonated by lowering pH. Both the protonated and deprotonated forms of PYP-coumarin emit intense fluorescence, as compared with the native PYP. In addition, both the deprotonated and protonated forms show identical λ_{max} values in their fluorescence spectra, indicating that ESPT occurs in the artificial fluorescent protein.