# 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 proteins, biological-clock proteins, metalloproteins, glycoconjugates, molecular chaperone, and motor proteins. Coordination-complex divisions aim to develop molecular catalysts and functional metal complexes for transformation of organic molecules, water oxidation and reduction, and molecular materials with photonic-electronic-magnetic functions. Interdisciplinary alliances in this department aim to create new basic concepts for the molecular and energy conversion through the fundamental science conducted at each divisions.

# Bioinorganic Chemistry of Metalloproteins Responsible for Signal Sensing

**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 ( –2018)
Professor, The Graduate University for Advanced Studies
2018 Professor, Exploratory Research Center on Life and Living Systems

**Member**
Assistant Professor
YOSHIOKA, Shiro
MURAKI, Norifumi
Visiting Scientist
DOWE, Nicolas*
Technical Fellow
MURAKI, Megumi
Secretary
NAKANE, Kaori

**Keywords** Bioinorganic Chemistry, Metalloproteins, Sensor Protein

Gas molecules such as $O_2$, NO, CO and ethylene are present in the environment and are endogenously (enzymatically) produced to act as signaling molecules in biological systems. Sensing these gas molecules is the first step in their acting as signalling molecules. Sensor proteins are usually required. Input signals generated by gas sensing have to transduce to output signals that regulate biological functions. This is achieved by biological signal-transduction systems. Recognition of the cognate gas molecules is a general mechanism of functional regulation for gas-sensor proteins. This induces conformational changes in proteins that controls their activities for following signal transductions. Interaction between gas molecules and sensor proteins is essential for recognition of gas molecules. Metal-containing prosthetic groups are widely used. In my research group, our research focuses on heme-based gas-sensor proteins and the signalling systems working with them.

The prosthetic group heme acts as the active center of hemeproteins that show a variety of functions, including $O_2$ or NO storage/transport, electron transfer, redox catalysis of various substrate, and dehydration of aldoxime. In the present context, it acts as the active site for sensing of diatomic gas molecules such as NO, $O_2$, and CO. These gas molecules are able to bind to heme iron as an axial ligand, which is a reason why heme can be adapted as the active center for sensing gas molecules. Heme-based gas-sensor proteins constitute a major group in the gas-sensor proteins. Binding of a cognate gas molecule to heme is the initial step for gas sensing, which is followed by the signalling processes. The binding affinities of gas molecules, that measures of the sensitivities of the sensor proteins, can be controlled by heme environmental structures. Differences in the heme coordination structure of the axial ligand(s) and/or of interaction(s) between the heme-bound gas molecule and surrounding amino acid residue(s) in a heme pocket play important roles. They not only regulate the binding affinities of gas molecules but also discriminate one cognate effector gas molecule from others, allowing the sensor to respond with the proper signal transductions. We have been elucidating the relationships of structures and functions of heme-based sensor proteins by crystallographic, biochemical, biophysical, and molecular biological studies.

## Selected Publications


- A. Pavlou, H. Yoshimura, S. Aono and E. Pinakoulaki, "Protein Dynamics of the Sensor Protein HemAT as Probed by Time-Resolved Step-Scan FTIR Spectroscopy," *Biophys. J.* **114**, 584–591 (2018).
- A. Pavlou, A. Loullis, H. Yoshimura, S. Aono and E. Pinakoulaki, "Probing the Role of the Heme Distal and Proximal Environment in Ligand Dynamics in the Signal Transducer Protein HemAT by Time-Resolved Step-Scan FTIR and Resonance Raman Spectroscopy," *Biochemistry* **56**, 5309–5317 (2017).
- N. Muraki, C. Kitatsuji, M. Ogura, T. Uchida, K. Ishimori and S. Aono, "Structural Characterization of Heme Environmental Mutants of CgHmuT that Shuttles Heme Molecules to Heme Transporters," *Int. J. Mol. Sci.* **17**, 829 (2016).
- N. Muraki and S. Aono, "Structural Basis for Heme Recognition by HmuT Responsible for Heme Transport to the Heme Transporter in *Corynebacterium glutamicum*," *Chem. Lett.* **45**, 24–26 (2015).
- 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).

## 1. Molecular Mechanisms for Biosynthesis and Maturation of Hydrogen Sensing Regulatory Hydrogenase

Hydrogenases are metalloenzymes that catalyze the oxidation of $H_2$ into electrons and protons and the reduction of protons into $H_2$ reversibly, which are expected as biocatalysts for fuel cells and $H_2$ production for clean and sustainable energy. Based on the differences of metal content and the structure of the active site, they are classified into three groups: FeFe-, NiFe-, and Fe-hydrogenases containing a dinuclear Fe unit linked to a [4Fe-4S] cluster, a hetero dinuclear Ni-Fe cluster, and a mononuclear Fe center, respectively. In addition to the enzymatic function of hydrogenases, some hydrogenase that is classified as a regulatory hydrogenase (RH) acts as a molecular hydrogen sensor.

RH consists of two subunits, a large subunit containing the Ni-Fe dinuclear complex and a small subunit containing iron-sulfur clusters. Though the Ni-Fe dinuclear complex in the large subunit is assumed to be the active site for $H_2$ sensing by RH, the molecular mechanisms of biosynthesis and maturation of the Ni-Fe dinuclear complex are not clear yet.

CO and $CN^-$ ligands are coordinated to the Fe in the Ni-Fe dinuclear complexe in RH. These CO and $CN^-$ are biosynthesized and assembled into the metal clusters, for which several accessory and chaperone proteins are required. It is reported recentrly that HypX protein is responsible for CO biosynthesis for the maturation of Ni-Fe hydrogenases including RH, but the detailed molecular mechanism of CO biosynthesis by HypX is not elucidated. We are now elucidating the structural and functional relationships of the accessory protein HypX responsible for the construction of the Fe(CO) unit in the Ni-Fe dinulear complex in RH. We have obtained single crystals of HypX and determined the crystal structure.

HypX consists of the N-terminal (residues 1-270) and the C-terminal (residues 289-542) domains with the C-terminal tail (residues 543-562) as shown in Figure 1. The N- and C-terminal domains are linked by a loop (residues 271-288). The N-terminal domain is composed of two subdomains, subdomains A (residues 1-151) and B (residues 182-270), which are linked by a long loop (residues 152-181). The subdomain A consists of six β-strands and five α-helices. It forms a Rossmann-fold with a mixed parallel β-sheet, which is constructed by six β-strands that is sandwiched by two sets of two α-helices. The subdomain B has a barrel-helix framework as an oligonucleotide/oligosaccharide-binding (OB) fold consisting of six β-strands and one α-helix, in which six β-strands form an open barrel-like structure.

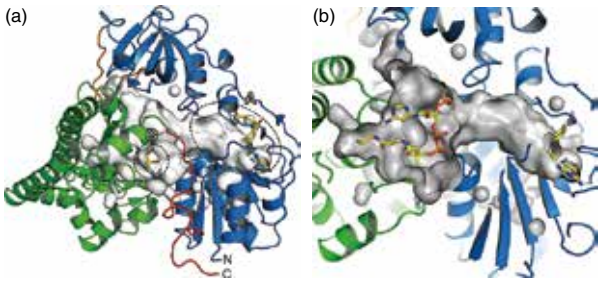



**Figure 1.** (a) Overall structure of HypX, in which the N-terminal domain (blue) and the C-terminal domain (green) are linked by a loop (orange). The cavity is shown in gray. (b) A sectional view of the cavity. CoA and THF are shown in the stick model.

A continuous cavity connecting the N- and C-terminal domains is present in the interior of HypX. Coenzyme A (CoA) is bound in the C-terminal region of the cavity with a "folded conformation" in which adenine and pantetheine groups are stacked in parallel. The HypX-THF complex has been obtained by soaking HypX crystals with THF. THF is bound at the N-terminal region inside the cavity.

The crystallographic analyses reveal that HypX consists of the N- and C-terminal domains that are structurally homologous to the hydrolase domain of $N^{10}$-formyl-tetrahydrofolate ($N^{10}$-formyl-THF) dehydrogenase (FDH) and enoyl-CoA hydratase/isomerase (ECH/ECI), respectively. The comparison of amino acid sequences and crystal structures between HypX and FDH reveals that His and Asp among the catalytic triad are conserved at the corresponding positions (His74 and Asp109 in HypX). Though Ser among the catalytic triad is not conserved in HypX, Asp80 forms a hydrogen bond with τN of His74, which fixes the orientation of His74 as does Ser106 in FDH. Asp80 not only sustain a functional role for the fixation of the orientation of His74 but may enhance the catalytic activity of Asp109 through the hydrogen bonding network among His74, Asp80, and Asp109. Thus, HypX adopts a slightly modified catalytic triad for formyl-group transfer reaction. While 4-phosphopantetheine in ACP accepts formyl group from $N^{10}$-formyl-THF in the case of FDH, CoA will do so for HypX because it has the phosphopantetheine moiety identical to ACP.

While CoA adopts the folded conformation in HypX, an "extended conformation" of CoA, in which the ADP and pantetheine moieties are extended in a linear fashion, is observed in some CoA-dependent enzymes. We examined whether the extended conformation of CoA was also available in HypX and found that CoA is able to adopt the extended conformation in the A392F-I419F variant. Ala392 and Ile419 are located near the pantetheine moiety of the folded form of CoA, whose positions correspond to "a neck of a bottle" accommodating the pantetheine moiety of CoA in the folded form. Replacing Ala392 and Ile419 with Phe will narrow "the neck of a bottle," which will destabilize the folded conformation of CoA by a steric hindrance to make the extended conformation of CoA more favorable because the residues 392 and 419 are no longer interacting with the pantetheine moiety of CoA in the extended conformation. In fact, we have found that CoA in the A392F-I419F variant adopts the extended conformation. Taken together, we propose the following reaction scheme of CO biosynthesis by HypX. HypX will catalyze two consecutive reactions, the formyl-group transfer from $N^{10}$-formyl-THF to CoA and decarbonylation of formyl-CoA, in the N- and C-terminal domains, respectively, to produce CO.

* IMS International Internship Program

# 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 ( –2018)
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"
2018 Professor, Exploratory Research Center on Life and Living Systems

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

**Member**
Assistant Professor
YAGI-UTSUMI, Maho
YANAKA, Saeko
Post-Doctoral Fellow
SUZUKI, Tatsuya
Visiting Scientist
KONGSEMA, Mesayamas*
SCHNAPKA, Vincent†
Graduate Student
HIRANYAKORN, Methanee
HONDA, Rena
SEKIGUCHI, Taichiro
YOGO, Rina‡
YUNOKI, Yasuhiro‡
SAITO, Taiki‡
KOFUJI, Kana‡
MATSUO, Muneyuki†
RATANABUNYONG, Siriluk‖
JUIPRASERT, Chanitha¶
Technical Fellow
ISONO, Yukiko
Secretary
TANAKA, Kei

**Keywords** Biomolecule Organization, 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.

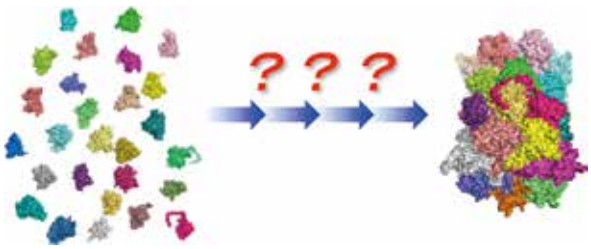



**Figure 1.** Formation of supramolecular machinery through dynamic assembly and disassembly of biomolecules.

## Selected Publications


- S. Yanaka, R. Yogo, R. Inoue, M. Sugiyama, S. G. Itoh, H. Okumura, Y. Miyanoiri, H. Yagi, T. Satoh, T. Yamaguchi and K. Kato, "Dynamic Views of the Fc Region of Immunoglobulin G Provided by Experimental and Computational Observations," *Antibodies* **8**, 39 (2019).
- T. Sekiguchi, T. Satoh, E. Kurimoto, C. Song, T. Kozai, H. Watanabe, K. Ishii, H. Yagi, S. Yanaka, S. Uchiyama, T. Uchihashi, K. Murata and K. Kato, "Mutational and Combinatorial Control of Self-Assembling and Disassembling of Human Proteasome α Subunits," *Int. J. Mol. Sci.* **20**, 2308 (2019).
- S. G. Itoh, M. Yagi-Utsumi, K. Kato and H. Okumura, "Effects of a Hydrophilic/Hydrophobic Interface on Amyloid-β Peptides Studied by Molecular Dynamics Simulations and NMR Experiments," *J. Phys. Chem. B* **123**, 160–169 (2019).
- H. Yagi, S. Yanaka and K. Kato, "Structure and Dynamics of Immunoglobulin G Glycoproteins," in *Glycobiophysics*, Y. Yamaguchi and K. Kato, Eds., Springer Nature; Singapore, pp. 219–235 (2018).
- T. Satoh and K. Kato, "Structural Aspects of ER Glycoprotein Quality-Control System Mediated by Glucose Tagging," in *Glycobiophysics*, Y. Yamaguchi and K. Kato, Eds., Springer Nature; Singapore, pp. 149–169 (2018).

## 1. Exploration of Protein Assembly Dynamics

We characterized a variety of protein assembly systems using an integrative biophysical approach to provide deeper insights into the biomolecular organization. One of the most striking examples of sophisticated protein assembly is the formation of the proteasome, which is a huge enzyme complex harboring four layers of heteroheptameric rings (*i.e.*, two α and two β rings). We focused on the mechanisms underlying the formation of the α ring that comprises seven different but homologous α subunits. They are correctly assembled via transient interactions with assembly chaperones. We comprehensively characterized the assembly states of the α subunits of the human proteasome, thereby controlled their assembly and disassembly using mutational and combinatorial techniques.[1] In addition, we provided a molecular and structural basis for the mechanism of α subunit assembly mediated by the chaperone complex as a molecular matchmaker[2] (Figure 2).

Our approach has been applied to other protein assembly systems.[3] We successfully characterized the interaction between cyanobacterial clock proteins through the combined use of NMR spectroscopy and native mass spectrometry.[4] Furthermore, we are addressing the molecular mechanisms underlying the environment-dependent self-assembly of proteins into filamentous structures, as exemplified by amyloid formation in microgravity and tardigrade protein assembly occurring during desiccation.


**Figure 2.** Three-dimensional model of the complex comprising the PAC3/PAC4 heterodimeric chaperon and proteasome subunits α4, α5, and α6.

## 2. Characterization of Structural Dynamics and Interactions of Immunoglobulin G Glycoproteins

Immunoglobulin G (IgG) molecules play pivotal roles in the immune system as multifunctional glycoproteins, coupling between antigen recognition and effector functions. The Fab region of each IgG binds to its specific antigen, whereas the Fc region interacts with the effector proteins typified by Fcγ receptors (FcγRs), depending on Fc glycosylation, particularly fucosylation. To date, crystallographic studies have been performed to elucidate the molecular mechanisms underlying IgG functions, primarily using isolated Fab and Fc fragments. We aimed to provide dynamic views of IgG-Fc by performing molecular dynamics (MD) simulations, which were experimentally validated using X-ray scattering and NMR spectroscopy. The results indicated that the dynamic conformational ensembles of Fc encompass most of the previously reported crystal structures in the free and FcγR-bound forms, although the major Fc conformers in solution significantly deviated from the crystal structures[5] (Figure 3). Furthermore, we found that glycans restrict the motional freedom of Fc and provide quaternary-structure plasticity via multiple intramolecular interaction networks. Particularly, the fucosylation of Fc glycans restricts the conformational freedom the proximal amino acid residue of functional importance, thereby preventing its interaction with FcγRIIIa, an FcγR isoform that mediates antibody-dependent cellular cytotoxicity. Moreover, based on integrated biophysical experiments, we demonstrated that the Fab portion of IgG is directly involved in its interaction with FcγRIIIa in addition to the canonical Fc-mediated interaction.[6] Our findings could inspire novel therapeutic antibody engineering targeting the previously unidentified receptor-interaction sites in IgG-Fab.

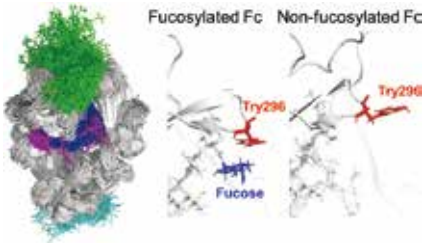



**Figure 3.** Dynamic views of the Fc region of IgG obtained by experimental and computational observations. This depiction shows the conformational ensemble of IgG-Fc derived from MD simulation (left) and the typical conformational snapshots of the functionally important tyrosine residue (Tyr296) derived from the major conformational states of the fucosylated (middle) and nonfucosylated (right) forms of Fc.

### References

1) T. Sekiguchi *et al.*, *Int. J. Mol. Sci.* **20**, 2308 (2019).
2) T. Satoh *et al.*, *Int. J. Mol. Sci.* **20**, 2231 (2019).
3) S. G. Itoh *et al.*, *J. Phys. Chem. B* **123**, 160–169 (2019).
4) Y. Yunoki *et al.*, *Life Sci. Alliance* **2**, e201900368 (2019).
5) S. Yanaka *et al.*, *Antibodies* **8**, 39 (2019).
6) R. Yogo *et al.*, *Sci. Rep.* **9**, 11957 (2019).

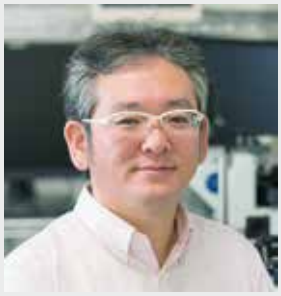
### Awards

YOGO, Rina; Excellent Student Presentation Award, the 139th Annual Meeting of the Pharmaceutical Society of Japan (2019).
HONDA, Rena; Tokai Branch Chief Award, The Chemical Society of Japan (2019).
SAITO, Taiki; Young Scientist Award, The 6th Joint Nagoya Meeting: Future perspectives on structural/functional analyses and molecular design of biomolecules (2019).
KOFUJI, Kana; The Best Presentation Award, The Tokai Branch Meeting of the Pharmaceutical Society of Japan (2018).
HONDA, Rena; Young Scientist award, The 15th Forum of the Glycoscience base for Chubu (2018).

* IMS-IIPA Program
† IMS International Internship Program
‡ carrying out graduate research on Cooperative Education Program of IMS with Nagoya City University
§ carrying out graduate research on Cooperative Education Program of IMS with the University of Tokyo
‖ carrying out graduate research on Cooperative Education Program of IMS with Kasetsart University
¶ carrying out graduate research on Cooperative Education Program of IMS with Srinakharinwirot 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 ( –2018)
Professor, The Graduate University for Advanced Studies

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

**Member**
Assistant Professor
NAKAMURA, Akihiko
ANDO, Jun
Post-Doctoral Fellow
TAKEDA, Kimitoshi
Visiting Scientist
MOHAMAD ZAKARIA, Siti Mastura Binti*
WANG, Tak-Wai*
Graduate Student
VISOOTSAT, Akasit
IIDA, Tatsuya
Technical Fellow
YAMAMOTO, Mayuko
OKUNI, Yasuko
Secretary
NAKANE, Kaori

**Keywords** Molecular Motors, Single-Molecule Analysis, Protein Engineering

Activity of life is supported by various molecular machines made of proteins. Protein molecular machines are tiny, but show very high performance, and are superior to man-made machines in many aspects. One of the representatives of protein 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 across the cell membrane.

We unveil operation principles of molecular motors with advanced single-molecule functional analysis and X-ray crystallographic structural analysis. With the help of computer science and robotic automation, we also engineer non-natural molecular motors to understand their design principles.

**Figure 1.** Protein molecular machines. (Left) A linear molecular motor chitinase A. (Center and Right) Rotary molecular motors $F_1$-ATPase and $V_1$-ATPase, respectively.

## Selected Publications


- J. Ando, A. Nakamura, A. Visootsat, M. Yamamoto, C. Song, K. Murata and R. Iino, "Single-Nanoparticle Tracking with Angstrom Localization Precision and Microsecond Time Resolution," *Biophys. J.* **115**, 2413–2427 (2018).
- A. Nakamura, K. Okazaki, T. Furuta, M. Sakurai and R. Iino, "Processive Chitinase is Brownian Monorail Operated by Fast Catalysis after Peeling Rail from Crystalline Chitin," *Nat. Commun.* **9**, 3814 (2018).
- A. Nakamura, T. Tasaki, Y. Okuni, C. Song, K. Murata, T. Kozai, M. Hara, H. Sugimoto, K. Suzuki, T. Watanabe, T. Uchihashi, H. Noji and R. Iino, "Rate Constants, Processivity, and Productive Binding Ratio of Chitinase A Revealed by Single-Molecule Analysis," *Phys. Chem. Chem. Phys.* **20**, 3010–3018 (2018).
- F. Kawai, A. Nakamura, A. Visootsat and R. Iino, "Plasmid-Based One-Pot Saturation Mutagenesis and Robot-Based Automated Screening for Protein Engineering,"*ACS Omega* **3**, 7715–7726 (2018).
- T. Uchihashi, Y. H. Watanabe, Y. Nakazaki, Y. Yamasaki, T. Watanabe, T. Maruno, S. Uchiyama, S. Song, K. Murata, R. Iino and T. Ando, "Dynamic Structural States of ClpB Involved in Its Disaggregation Function," *Nat. Commun.* **9**, 2147 (2018).
- H. Isojima, R. Iino, Y. Niitani, H. Noji and M. Tomishige, "Direct Observation of Intermediate States during the Stepping Motion of Kinesin-1," *Nat. Chem. Biol.* **12**, 290–297 (2016).
- A. Nakamura, T. Tasaki, D. Ishiwata, M. Yamamoto, Y. Okuni, A. Visootsat, M. Maximilien, H. Noji, T. Uchiyama, M. Samejima, K. Igarashi and R. Iino, "Direct Imaging of Binding, Dissociation, and Processive Movement of *Trichoderma reesei* Cel6A and Its Domains on Crystalline Cellulose," *J. Biol. Chem.* **291**, 22404–22413 (2016).

## 1. Rotational Substeps and Chemo-Mechanical Coupling Scheme of *Enterococcus hirae* $V_1$-ATPase[1)]

$V_1$-ATPase ($V_1$), the catalytic domain of an ion pump V-ATPase, is a rotary molecular motor and converts chemical energy of ATP hydrolysis into mechanical rotation. To understand chemo-mechanical coupling mechanism of *Enterococcus hirae* $V_1$ ($EhV_1$), we directly observed rotation of newly-constructed $EhV_1$ with gold nanoparticle probe. We found that 120° steps per ATP hydrolysis were divided into 40° and 80° substeps. In the main-pause before 40° substep, time constant was inversely proportional to ATP concentration ([ATP]) at low [ATP], indicating that ATP binds during the main-pause with rate constant of $1.0 \times 10^7$ $M^{-1}s^{-1}$. At high [ATP], [ATP]-independent two time constants (0.5, 0.7 ms) were obtained. One of two time constants was prolonged (143 ms) in rotation driven by slowly-hydrolyzable ATPγS, indicating that ATP cleavage occurs during the main-pause. In another subpause before 80° substep, [ATP]-independent time constant (2.5 ms) was obtained. Furthermore, in ATP-driven rotation of an arginine-finger mutant in the presence of ADP, −80° and −40° backward steps were observed. Time constants of the pauses before −80° backward and +40° recovery steps were inversely proportional to [ADP] and [ATP], respectively, indicating that these steps are triggered by ADP and ATP bindings. Assuming that backward steps are reverse reactions, we concluded that 40° and 80° substeps are triggered by ATP binding and ADP release, respectively. The remaining time constant in the main-pause was considered to be phosphate release. Combined with previous structural information, we propose a chemo-mechanical coupling scheme of $EhV_1$ including substeps, largely different from those of $F_1$-ATPases (Figure 2).

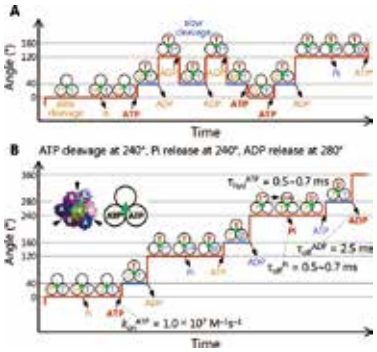



**Figure 2.** A model of chemo-mechanical coupling of $EhV_1$ including substeps and backward steps.

## 2. Multi-Color High-Speed Tracking of Single Biomolecules with Silver, Gold, Silver-Gold Alloy Nanoparticles[2)]

Gold nanoparticles have been used as an imaging probe to track motions of single biomolecules. Since they show high scattering signals, single-particle tracking has been performed with microsecond time resolution and nanometer localization precision. To investigate behaviors of various kinds of biomolecules simultaneously, increase of the color palette is necessary. Here we developed a multi-color, high-speed single-particle tracking system by using silver, gold, and silver-gold alloy nanoparticles. Peak wavelengths of plasmon resonance for silver and gold nanoparticles are around 400 nm and 530 nm, respectively, and those for silver-gold alloy nanoparticles can be modulated between 400 nm and 530 nm depending on their composition ratio. We constructed multi-color total internal reflection dark-field microscopy with multiple lasers at 404 nm for silver, 473 nm for silver-gold alloy, and 561 nm for gold nanoparticles. By using a spectrophotometer in the imaging optics, scattering images at each wavelength were projected onto the different portion of a two-dimensional detector (Figure 3 and 4). High contrast images of 30 nm silver, 30 nm silver-gold alloy, and 40 nm gold nanoparticles were simultaneously obtained at 404, 473, and 561 nm channels, respectively. With this system, diffusional motions of phospholipids in supported lipid membrane and stepping motions of kinesins along microtubules were observed with 2 nm localization precision and 100 μs time resolution. Furthermore, introduction of 649 nm laser enabled detection of plasmon coupling and transient dimer formation of two nanoparticles. Our method will pave the way to investigate operation mechanisms of complex biomolecular systems and multi-subunit biomolecular motors and machines.

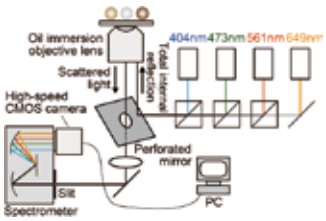



**Figure 3.** Spectrometer-based multi-color dark-field imaging system.

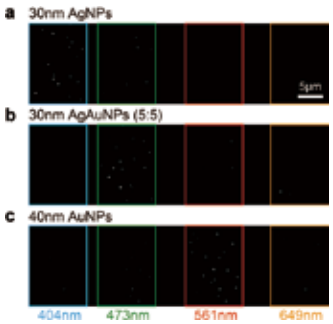



**Figure 4.** Spectrometer-based dark-field imaging of AgNPs, AgAuNPs (5:5), and AuNPs.

### References

1) T. Iida, Y. Minagawa, H. Ueno, F. Kawai, T. Murata and R. Iino, *J. Biol. Chem.* **294**, 17017–17030 (2019).
2) J. Ando, A. Nakamura, M. Yamamoto, C. Song, K. Murata and R. Iino, *ACS Photonics*, published online (2019).

* IMS International Internship Program

# A Supramolecular Chemical Approach to the Construction of Artificial Cells

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

**KURIHARA, Kensuke**
Research Associate Professor
[kkurihara@ims.ac.jp]

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

**Professional Employment**
2010 Technical Assistant, The University of Tokyo
2013 Postdoctoral Fellow, Research & Education Platform for Dynamics Living States, The University of Tokyo
2014 Research Associate Professor, Institute for Molecular Science
Research Associate Professor, Okazaki Institute for Integrative Bioscience (OKAZAKI ORION Project) ( –2018)
2018 Research Associate Professor, Exploratory Research Center on Life and Living Systems

**Award**
2018 Kurita Outstanding Research Award

**Member**
Post-Doctoral Fellow
MATSUO, Muneyuki
Visiting Scientist
NATSUME, Yuno
Secretary
TANAKA, Kei

**Keywords** Artificial Cell, Origin of Life, Vesicle

The cell is the smallest unit of life, and the first simple cells evolved from simple molecular assemblies on prebiotic earth. To understand this transition from non-living to living structures, we use a supramolecular chemical approach. As shown in Figure 1, the key elements of a cell are a compartment, information, and a catalyst (*i.e.*, metabolism). We have attempted to construct a chemically based artificial cell endowed with these three elements.

In our laboratory, we constructed two types of artificial cells by using giant vesicles (GVs) as the compartment. The first, developed in collaboration with the Sugawara group (Kanagawa Univ.), is an artificial cell that can proliferate from generation to generation. We have improved this model by constructing a recursive vesicular artificial cell system with proliferation cycles. After self-reproduction, these second-generation GVs contain no PCR reagents by consuming and therefore cannot reproduce for a second time. However, the reagents can be replenished by using the vesicular transport system and changing the pH of the dispersion, resulting in the fusion of the GVs with conveyer GVs bearing the PCR reagents. After the PCR reagents are replenished, the GV can self-reproduce again. This system could lead to an evolvable artificial cellular system. The second type of artificial cell contains a catalyst-producing system. The GV system can generate catalysts and membrane molecules by transforming their respective precursors. The catalysts that are produced facilitate the proliferation of the GVs.

We are now tackling the creation of artificial cells that mimic cellular dynamics, such as cytoskeleton formation within the cell.

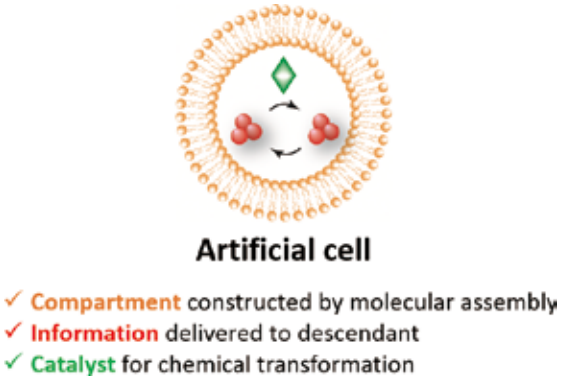



**Figure 1.** Artificial cell model. Materials containing heritable information are enclosed within a compartment. The reactions in the two replicating systems (compartment and information) are accelerated by appropriate catalysts The reactions in the two replicating systems are accelerated by appropriate catalysts.

## Selected Publications


- Y. Natsume, E. Noguchi and K. Kurihara, "Spontaneous Localization of Particles in Giant Vesicles Owing to Depletion Force," *J. Phys. Soc. Jpn.* **88**, 033001 (2019).
- M. Matsuo, Y. Kan, K. Kurihara, T. Jimbo, M. Imai, T. Toyota, Y. Hirata, K. Suzuki and T. Sugawara, "DNA Length-Dependent Division of a Giant Vesicle-Based Model Protocell," *Sci. Rep.* **9**, 6916 (2019).

## 1. Spontaneous Formation of Liquid–Liquid Phase-Separated Droplets with Amino Acid Polymerization

In the prebiotic era, cooperative interaction between self-producing molecular aggregates and peptide polymers led to the emergence of primitive cells. Although the advanced membrane provides a field for catalytic reaction, it remains a mystery how cooperation between polymers and molecular aggregates occurred even in membraneless organisms like coacervate droplets. Therefore, we attempted to construct a liquid–liquid phase-separated droplet that self-reproduces by constructing a reaction system in which a peptide is produced by spontaneous polymerization of an amino acid derivative in water.

We synthesized an amino acid derivative (monomer) with two cysteine reactive sites at the N-terminus and a thioester at the C-terminus that spontaneously polymerizes in water to form a peptide. In order to prevent undesirable oxidation, a monomer precursor was obtained by crosslinking monomers with disulfide. After the addition of a reducing agent (dithiothreitol, DTT) to the monomer precursor solution, we observed the formation of droplets. We then added more precursor and DTT and examined the changes in particle size. The mean particle size increased and decreased rapidly immediately after the addition, confirming the self-reproducibility of the formed droplets. When the precursor and DTT were continuously added every 20 hours, the particle size of the droplets fluctuated recursively, indicating autocatalytic self-reproduction of the formed liquid–liquid phase-separated droplets. This autocatalytic droplet formation in this system is considered to be due to a physical mechanism: When a molecular assembly is created as the dehydration condensation proceeds and it forms a hydrophobic field, the assembly functions as a site for promoting dehydration condensation, thereby allowing the autocatalytic dehydration condensation to proceed.

The behavior of the interface formed by this chemical reaction replicates the autocatalytic self-reproduction that might have occurred in droplets formed by liquid–liquid phase separation on the primitive, prebiotic earth. In the future, we aim to construct the Droplet World Hypothesis by inducing the emergence of the primordial cell membrane via an internal chemical reaction or by functionally expressing biologically active molecular species, such as ribozymes, inside the droplet.

## 2. Self-Reproduction Model Using Particle-Localized Vesicles

Biopolymers inside cells change their structures around themselves, thereby increasing the free movement area. This entropic action is called the excluded volume effect. We prepared phospholipid vesicles containing densely packed colloidal particles as a model to show the excluded volume effect. When polystyrene beads of two different sizes were confined in the GV, a novel phenomenon was observed: Smaller beads were localized in the vicinity of the vesicle membrane. To explain this phenomenon theoretically, we assumed that an equilibrium osmotic pressure was realized between an outer phase containing a relatively large number of small particles and a separate inner phase.[1] We constructed a second model based on the depletion effect.[2]

Considering the relation between vesicular membrane area and the volume fraction of the particles, we hypothesize that increased membrane area would cause the vesicle to become unstable, and that stability could be restored by division into two spherical vesicles. We confirmed this by showing that when a fatty acid similar to a vesicular membrane molecule is added to particle-containing vesicles, the vesicles divide easily and frequently. To further assess this self-reproducing vesicle model, we are constructing a system can track morphological changes and analyze microscopy images.

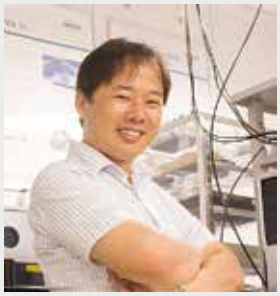
The purpose of this vesicular system is to achieve cellular behavior such as internal structure or membrane deformation without sophisticated biomaterials. Because this vesicular system is simple, it is possible to extract and analyze the contribution of the crowding effect to cell deformation. For this reason, applying data to simulation and modeling will be relatively easy in this system compared with other systems. Because this model excludes biopolymers with specific properties, the biopolymers' unique functions do not appear. Therefore, this vesicular system is expected to act as a primitive cell in which simple molecules interact loosely to express their function.

### References

1) Y. Natsume, Y. Komori, K. Itoh and K. Kurihara, *Trans. Mater. Res. Soc. Jpn.* **43**, 333–338 (2018).
2) Y. Natsume, E. Noguchi and K. Kurihara, *J. Phys. Soc. Jpn.* **88**, 033001 (2019).

# 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
( –September, 2018)
[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)
2018 Associate Professor, Nagoya Institute of Technology

**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
Graduate Student
TOMIDA, Sahoko*
Technical Fellow
MOTOMURA, Hiroe
INABA, Kayo
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.

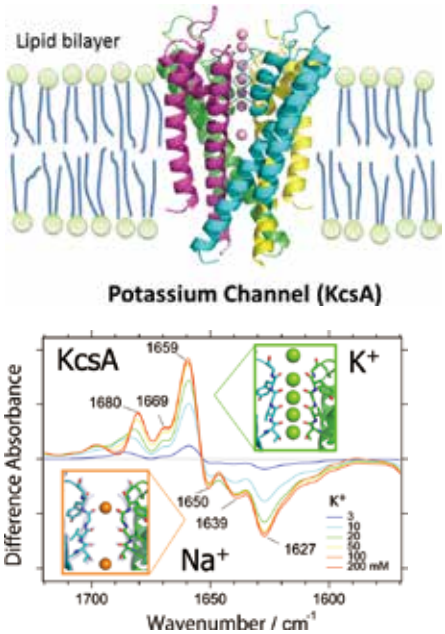



**Figure 1.** (top) X-ray crystal structure of a potassium ion channel, KcsA. (bottom) The ion-exchange induced difference infrared spectra of KcsA with different potassium ion concentration. The amide I bands are mainly originated from the carbonyl groups of the selectivity filter of KcsA.

## 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. Ion–Protein Interactions of MgtE Magnesium Channel with Magnesium or Calcium Ions and Its Implication for the Ion Selectivity[1)]

Magnesium ion ($Mg^{2+}$) is vital for living systems and utilized for various biological processes. Calcium ion ($Ca^{2+}$) is also important as a second messenger inside the cell. Thus, the selective permeation of $Mg^{2+}$ is not only prerequisite for homeostasis of internal $Mg^{2+}$ concentration and also for avoiding unintended induction of calcium signaling.

MgtE is an ion channel highly selective to $Mg^{2+}$. The crystal structure of MgtE showed a dimeric structure with transmembrane domain and two cytosolic domains [N- and cystathionine-β-synthase (CBS) domains]. The transmembrane domains constitute a pore with a central cavity which is important for the ion selectivity to $Mg^{2+}$. In addition, the crystal structure of the transmembrane region with higher resolution revealed that a $Mg^{2+}$ ion exists with hydrated water molecules in the cavity.

To elucidate the molecular mechanisms of the ion selectivity for $Mg^{2+}$ of MgtE in more detail, we applied ion-exchange induced difference FTIR spectroscopy with an aid of computational methods. By changing electrolyte solution containing $Mg^{2+}$ with that of $Ca^{2+}$, we obtained an infrared difference spectrum of MgtE which contains molecular information of the ion–protein interactions with $Mg^{2+}$ or $Ca^{2+}$. Comparing the difference spectra of several site-directed mutant proteins of MgtE, we assigned antisymmetric and symmetric $COO^-$ vibrations of Asp432 which was found to be crucial for the ion selectivity of MgtE.

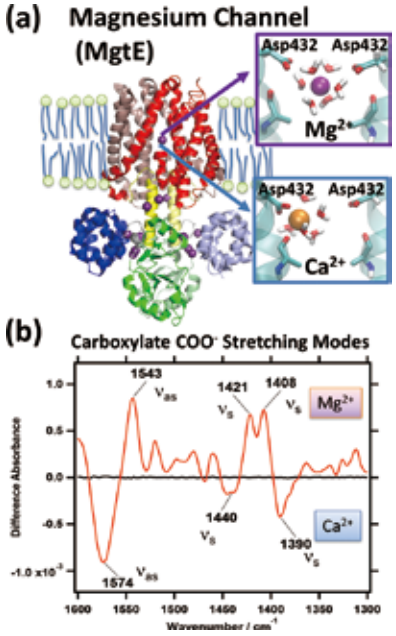



**Figure 2.** (a) The X-ray crystal structure of MgtE and snap shots of molecular dynamics simulation with $Mg^{2+}$ or $Ca^{2+}$ ion. (b) The ion-exchange induced difference infrared spectrum of MgtE in the carboxylate $COO^-$ stretching region.

Moreover, from systematic measurements with the different ion concentrations, we estimated the dissociation constant relating to the central cavity and found that the value is much lower for $Mg^{2+}$ (~0.3 mM) compared to that for $Ca^{2+}$ (~80 mM). The difference of affinity is well consistent with the high selectivity for $Mg^{2+}$ of MgtE elucidated by electrophysiological and biological methods. Difference in frequency of $COO^-$ stretching vibrations of Asp432 in the central cavity suggests that ion–protein interactions with $Mg^{2+}$ and $Ca^{2+}$ are different from each other. To get more information about energetics of the ion–protein interactions and dynamics, we applied molecular dynamics simulation and normal mode analysis with quantum chemical calculation on MgtE with hydrated $Mg^{2+}$ or $Ca^{2+}$ in the cavity. We found that $Mg^{2+}$ is more stable with hydrated configuration at the center between two Asp432 residues, but $Ca^{2+}$ is easily captured by either of the residues and forms the direct interaction. In this way, our experimental and computational approach provided new insights for the ion selectivity of MgtE.

## 2. Detection of Ligand- or Light- Induced Structural Changes in G Protein-Coupled Receptors Using ATR-FTIR Spectroscopy

My group has developed attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy to trace and reveal structural features of membrane proteins involved in various important biological functions. Based on IR spectral changes, we have reported how functional properties of ion channels (an ATP-sensitive P2X receptor and a potassium channel TWIK1) are regulated. In order to extend the applicability of ATR-FTIR spectroscopy to other important membrane proteins, we tried to measure IR spectral changes of G protein-coupled receptors (GPCRs) upon agonist-binding or light-absorption since GPCRs are involved in many important cellular processes such as neuro- transmission, hormone perception, vision and olfaction.

Besides ligand-binding GPCR, we also tried to measure IR spectral changes of light-sensitive GPCR opsin. We previously reported that an opsin in the brain of a zooplankton is activated by UV light and inactivated by visible light. Through the ATR-FTIR techniques, IR spectral changes of the opsin upon activation and inactivation were successfully detected. Time-resolved IR measurements of the opsin as well as ligand-binding GPCR would reveal how signal reception (UV light absorption for the opsin and ligand-binding for GPCRs) evokes conformational changes in the GPCRs toward activated states.

### Reference

1) T. Kimura, V. A. Lorenz-Fonfria, S. Douki, H. Motoki, R. Ishitani, O. Nureki, M. Higashi and Y. Furutani, *J. Phys. Chem. B* **122**, 9681–9696 (2018).

* carrying out graduate research on Cooperative Education Program of IMS with Nagoya Institute of Technology

# 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)
2014 Research Project Leader, JST ACCEL Project ( –2019)

**Awards**
1991 Eisai Award, Synthetic Organic Chemistry
1998 The Pharmaceutical Society of Japan Award for Young Scientist
2007 The Chemical Society of Japan (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 (Research Category)

**Member**
Visiting Professor
MASE, Toshiaki
Assistant Professor
OSAKO, Takao
HAMASAKA, Go
Post-Doctoral Fellow
PAN, Shiguang
HIRATA, Shuichi
PUTRA, Anggi Eka
KIM, Kiseong
SUGIYAMA, Yuya
Graduate Student
ICHII, Shun
SHEN, Guanshuo
NIIMI, Ryoko
TANI, Kazuki
Technical Fellow
TORII, Kaoru
TAZAWA, Aya
Secretary
SASAKI, Tokiyo
TANIWAKE, Mayuko

**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 transformations. In particular, we have recently been developing the heterogeneous aquacatalytic systems, continuous flow catalytic systems, and super active catalysts working at ppm-ppb loading levels. Thus, for example, a variety of palladium catalysts were designed and prepared promoting carbon–carbon bond forming reactions at ppm-ppb loading levels (Figure 1).

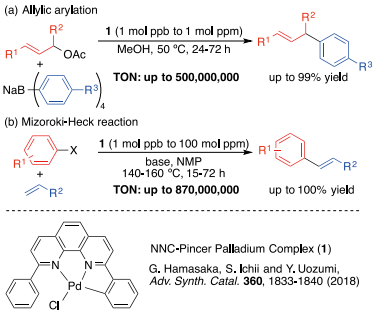



**Figure 1.** Typical Examples of Pd-Catalyzed Carbon–Carbon Bond Forming Reactions with ppm-ppb Loading Levels of an NNC-Pincer Pd Comples.

## Selected Publications


- R. David and Y. Uozumi, "Recent Advances in Palladium-Catalyzed Cross-Coupling Reactions at ppm-ppb Molecular Catalyst Loadings (review)," *Adv. Synth. Catal.* **360**, 602–625 (2018).
- T. Osako, K. Torii, S. Hirata and Y. Uozumi, "Chemoselective Continuous-Flow Hydrogenation of Aldehydes Catalyzed by Platinum Nanoparticles Dispersed in an Amphiphilic Resin," *ACS Catal.* **7**, 7371–7377 (2017).
- 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. Self-Assembled Polymeric Pyridine Copper Catalysts for the Huisgen Cycloaddition with Alkynes and Acetylene Gas: Application in Synthesis of Tazobactam[1)]

Novel convoluted polymeric pyridine copper(I) catalysts PVPy-Cu were developed for Huisgen cyclization of organic azides with alkynes and acetylene gas. They were readily prepared by our molecular convolution of $CuSO_4 \cdot 5H_2O$ and poly(4-vinylpyridine) (PVPy) in the presence of sodium ascorbate with/without various sodium salts in water. Their structural investigation was conducted with XANES and EXAFS as well as DFT calculation. The Huisgen cycloaddition of a variety of alkynes and acetylene gas was carried out with 100 to 800 mol ppm Cu of PVPy-Cu in water whose turnover numbers reached up to 10,000. This catalytic system was applied to synthesis of tazobactam, an inhibitor of bacterial β-lactamases.

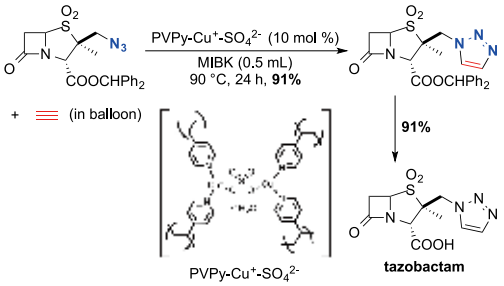



**Figure 2.** Synthesis of Tazobactam through the Huisgen Cyclization with Acetylene Gas Using a Polymeric Cu Catalyst.

## 2. Iterative Preparation of Platinum Nanoparticles in an Amphiphilic Polymer Matrix: Regulation of Catalytic Activity in Hydrogenation[2,3)]

We have demonstrated that iteration of the seeded preparation of platinum nanoparticles dispersed in an amphiphilic polystyrene–poly(ethylene glycol) resin (ARP–Pt) regulates the activity of the catalyst in the hydrogenation of aromatic compounds in water and is accompanied by a slight modification of its structural properties. Platinum nanoparticles dispersed in the amphiphilic polymer prepared through four iterations (ARP–Pt G5) showed a much higher catalytic activity than that of the initial ARP–Pt (G1) in the hydrogenation of aromatic compounds in water. These results suggest that iteration of the seeded preparation of nanoparticles can be an effective method for the precise regulation of the catalytic activity and the structural properties of the resulting catalyst.

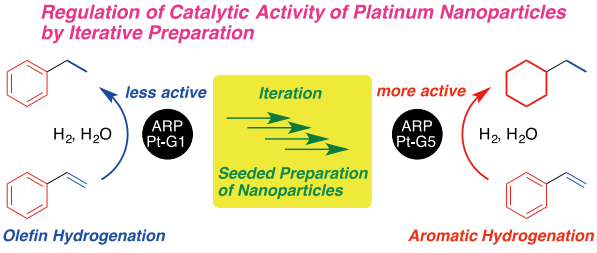



**Figure 3.** Regulated Hydrogenation with Iteratively Generated Polymeric nanoPt Catalysts.

## 3. Solvent-Free $A^3$ and $KA^2$ Coupling Reactions with Mol ppm Level Loadings of a Polymer-Supported Copper(II)-Bipyridine Complex for Green Synthesis of Propargylamines[4)]

A copper(II)–bipyridine complex immobilized on amphiphilic polystyrene–poly(ethylene glycol) (PS–PEG) resin (PS–PEG-BPy–$CuBr_2$) has been developed. The immobilized copper(II)–bipyridine complex at a mol ppm level of loading efficiently catalyzed the three-component coupling of aldehydes or ketones, amines, and alkynes ($A^3$ or $KA^2$ coupling) under solvent-free conditions to give the corresponding propargylamines in good-to-excellent yields. Moreover, a ten-gram-scale green syntheses of propargylamines proceeded with excellent atom economy (*E* factor ≥ 0.38) through a solvent-free $A^3$ coupling using 5 mol ppm of PS–PEG-BPy–$CuBr_2$. The total turnover number and turnover frequency of the catalyst reached as high as 178 800 and 7450 $h^{-1}$, respectively.


**Figure 4.** Preparation of Propargylamines by $A^3$ Coupling Reaction with ppm Loading Level of a Polymeric Cu(II) Catalyst.

### References

1) H. Hu, A. Ohno, T. Mase, Y. Uozumi and Y. M. A. Yamada, *Org. Pro. Res. Dev.* **23**, 493 (2019).
2) T. Osako, J. Srisa, K. Torii, G. Hamasaka and Y. Uozumi, *Synlett*, in press (2019). DOI: 10.1055/s-0037-1611813
3) T. Osako, K. Torii, S. Hirata and Y. Uozumi, *ACS Catal.* **7**, 7371–7377 (2017).
4) S. Yan, S. Pan, T. Osako and Y. Uozumi, *ACS Sus. Chem. Eng.* **7**, 9097 (2019).

# 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 Foundation Post Doctoral Research Fellowship
2008 Thieme Chemistry Journals Award
2014 The 17th Morita Science Research Award
Central Glass Co., Ltd. Award in Organic Chemistry, Japan

**Member**
Assistant Professor
IZUMISEKI, Atsuto
Post-Doctoral Fellow
FUJINAMI, Takeshi
OHTSUKA, Naoya
Graduate Student
JONGWOHAN, Chanantida
SUGAHARA, Yuto
HORI, Tatsuaki
OISHI, Shunya
OTA, Hino
KATO, Masayuki
SHIGEMITSU, Mai*
Technical Fellow
SUGIURA, Satoshi
Secretary
WATANABE, Yoko

**Keywords** Synthetic Chemistry, 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.

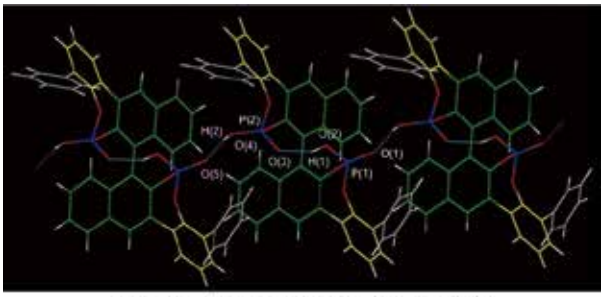



**Figure 1.** Hydrogen bonding network in chiral bis-phosphoric acid catalyst derived from (*R*)-3,3'-di(2-hydroxy-3 -arylphenyl)binaphthol. Hydrogen bond acts as activation unit for the substrate in asymmetric reaction space and controls atropisomeric behavior in naphthyl–phenyl axis.

## 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).
- N. Momiyama, H. Tabuse, H. Noda, M. Yamanaka, T. Fujinami, K. Yamanishi, A. Izumiseki, K. Funayama, F. Egawa, S. Okada, H. Adachi and M. Terada, "Molecular Design of a Chiral Brønsted Acid with Two Different Acidic Sites: Regio-, Diastereo-, and Enantioselective Hetero-Diels–Alder Reaction of Azopyridinecarboxylate with Amidodienes Catalyzed by Chiral Carboxylic Acid–Monophosphoric Acid," *J. Am. Chem. Soc.* **138**, 11353–11359 (2016).

## 1. Brønsted Acid Catalyzed Asymmetric Rearrangement: Asymmetric Synthesis of Linear Homoallylic Amines

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 homoallylic amines, we discovered chirality transferred formal 1,3-rearrangement of ene-aldimines in the presence of Brønsted acid, and developed it as synthetic method for variety of enantioenriched linear homoallylic amines.[1)] Furthermore, we studied details of reaction mechanism and succeeded catalytic asymmetric version of this rearrangement.[2)] To the best our knowledge, our discovery is the first example of asymmetric formal [1,3]-rearrangement 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)]

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.[4,5)] 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.[6)] 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.[7)]

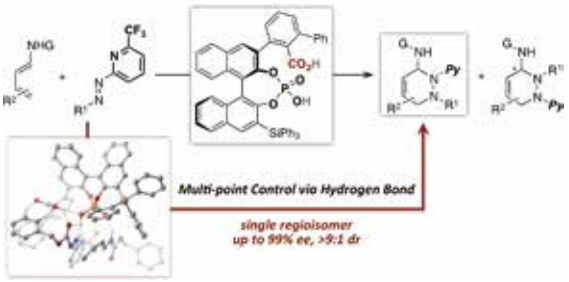



**Figure 2.** Chiral carboxylic acid–phosphoric acid-catalyzed azo-hetero-Diels–Alder reaction.

## 3. Design of Catalysis with Halogen Bond for Carbon–Carbon Bond Forming Reactions

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 LBs. 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 catalysis with halogen bond for carbon–carbon bond forming reactions.

We found that perfluorinated iodoaryls are able to catalyze the Mukaiyama-type Mannich reaction and allylation reaction.[8)]

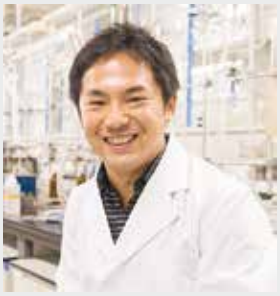
### References

1) C. Jongwohan, Y. Honda, T. Suzuki, T. Fujinami, K. Adachi and N. Momiyama, *Org. Lett.* **21**, 4991–4995 (2019).
2) N. Momiyama *et al.*, Manuscript in preparation.
3) N. Momiyama, H. Okamoto, J. Kikuchi, T. Korenaga and M. Terada, *ACS Catal.* **6**, 1198–1204 (2016).
4) N. Momiyama, T. Konno, Y. Furiya, T. Iwamoto and M. Terada, *J. Am. Chem. Soc.* **133**, 19294–19297 (2011).
5) N. Momiyama, K. Funayama, H. Noda, M. Yamanaka, N. Akasaka, S. Ishida, T. Iwamoto and M. Terada, *ACS Catal.* **6**, 949–956 (2016).
6) N. Momiyama, T. Narumi and M. Terada, *Chem. Commun.* **51**, 16976–16979 (2015).
7) N. Momiyama, H. Tabuse, H. Noda, M. Yamanaka, T. Fujinami, K. Yamanishi, A. Izumiseki, K. Funayama, F. Egawa, S. Okada, H. Adachi and M. Terada, *J. Am. Chem. Soc.* **138**, 11353–11359 (2016).
8) N. Momiyama *et al.*, Six manuscripts in preparation.

* Internship Student on Program of IMS with University of Fukui

# 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
( –March, 2019)
[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
2019 Professor, Osaka University

**Award**
2017 The 13th (FY 2016) JSPS Prize
2019 The 25th Gold Medal Prize

**Member**
Assistant Professor
KONDO, Mio
Post-Doctoral Fellow
OKAMURA, Masaya
VIJAYENDRAN, Praneeth
CHINAPANG, Pondchanok
Graduate Student
LEE, Sze Koon
IZU, Hitoshi
ENOMOTO, Takafumi
IWAMI, Hikaru
KACHI, Mami
TASAKI, Masahiro
AKAI, Takuya
FUJISAWA, Mayu
ISHIHARA, Mei
KATO, Soshi
TOMODA, Misa
Technical Fellow
MATSUDA, Miho
Secretary
TANIWAKE, Mayuko
NOGAWA, Kyoko

**Keywords** Metal Complex, Multi-Electron Transfer Reactions, 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 the research field. Our group studies the development of functional metal complexes toward the realization of artificial photosynthesis. Specific areas of research include (i) creation of cluster catalysts for multi-electron transfer reactions, (ii) frontier-orbital engineering of metal complexes for multi-electron transfer reactions, (iii) application of proton-coupled electron transfer toward multi-electron transfer reactions, (iv) electrochemical analysis of catalytic reactions, (v) development of novel photo-induced electron transfer systems, (vi) establishment of electrochemical method for the photoreactions of metal complexes in homogeneous solutions, and (vii) development of framework catalysts for small molecule conversion via the self-assembly of catalyst modules.


**Figure 1.** An overview of our work.

## Selected Publications


- V. K. K. Praneeth, M. Kondo, M. Okamura, T. Akai, H. Izu and S. Masaoka, "Pentanuclear Iron Catalysts for Water Oxidation: Substituents Provide Two Routes to Control Onset Potentials," *Chem. Sci.* **10**, 4628–4639 (2019).
- S. K. Lee, M. Kondo, M. Okamura, T. Enomoto, G. Nakamura and S. Masaoka, "Function-Integrated Ru Catalyst for Photochemical $CO_2$ Reduction," *J. Am. Chem. Soc.* **140**, 16899–16903 (2018).
- P. Chinapang, M. Okamura, T. Itoh, M. Kondo and S. Masaoka, "Development of a Framework Catalyst for Photocatalytic Hydrogen Evolution," *Chem. Commun.* **54**, 1174–1177 (2018).
- M. Okamura, M. Kondo, R. Kuga, Y. Kurashige, T. Yanai, S. Hayami, V. K. K. Praneeth, M. Yoshida, K. Yoneda, S. Kawata and S. Masaoka, "A Pentanuclear Iron Catalyst Designed for Water Oxidation," *Nature* **530**, 465–468 (2016).
- 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. 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).

## 1. Development of Function-Integrated Ru Catalyst for Photochemical $CO_2$ Reduction[1)]

The efficient conversion of solar energy into storable chemical fuels or useful chemicals is one of the major challenges in the 21st century. Particularly, visible-light driven photocatalytic reduction of $CO_2$ has attracted considerable attention because this technology can produce fuels and chemicals and counteract $CO_2$ emissions. The reaction is typically achieved by a system using a combination of two distinct functional units: A visible-light absorbing chromophore (photosensitizer, PS) and a catalyst (Cat). These systems require photoinduced electron transfer (ET) from the PS to the Cat to drive the reaction, and catalysis is largely affected by the efficiency of the ET process. Accordingly, optimization of the ET process with convergent modification of PS and Cat units is indispensable for efficient catalytic reactions.

A function-integrated catalyst, which can act as both PS and Cat, is a valuable alternative for photocatalytic $CO_2$ reduction. In this type of system, light absorption and subsequent $CO_2$ reduction proceed within one molecular unit. Thus, a reaction without an ET event between PS and Cat units can be achieved. However, the development of function-integrated catalysts that have (i) strong absorption in the visible-light region, (ii) high reaction rate, and (iii) high stability is still challenging.

In this study, we report efficient, visible-light-driven $CO_2$ reduction catalyzed by a function-integrated photocatalyst. The key to our success is the use of a phosphine-substituted Ru(II) polypyridyl complex, *trans(P,MeCN)*-$[Ru^{II}(tpy)(pqn)(MeCN)]^{2+}$ (**RuP**, tpy = 2,2′:6′,2″-terpyridine; pqn = 8-(diphenylphosphanyl)quinoline, Figure 2). **RuP** exhibits an intense band at 475 nm due to MLCT transitions. Additionally, **RuP** catalyzes electrochemical $CO_2$ reduction at one of the lowest overpotentials among homogeneous catalysts. These two characteristics of **RuP** enabled the development of a function-integrated system with a catalytic performance superior to that of best-in-class counterparts.

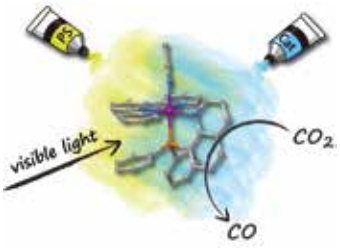



**Figure 2.** Function-integrated catalyst for visible-light driven $CO_2$ reduction.

## 2. Water Oxidation Reaction Catalyzed by Pentanuclear Iron Complexes[2)]

Water oxidation ($2H_2O \rightarrow O_2 + 4H^+ + 4e^-$) is considered the main bottleneck in the production of chemical fuels from sunlight and/or electricity. Recently, we demonstrated that a pentanuclear iron complex $[Fe^{II}_4Fe^{III}(\mu_3\text{-}O)(bpp)_6]^{3+}$, $[\mathbf{Fe_5\text{-}H}]^{3+}$ (Hbpp = bis(pyridyl)pyrazole), can serve as a highly active catalyst for electrocatalytic water oxidation. The reaction rate and durability of $[\mathbf{Fe_5\text{-}H}]^{3+}$ are the highest among iron-based water oxidation catalysts reported thus far. However, a relatively large onset potential is required for the catalysis. Therefore, the development of a novel strategy for designing catalysts that can drive the reaction at low onset potentials is essential.

Here, we report two approaches for decreasing the onset potential of pentairon water oxidation systems. Two approaches involving the installation of substituents onto the Hbpp ligand have been demonstrated. Two kinds of ligands, one with electron-donating and the other with electron-withdrawing groups at the 4-position of the Hbpp have been employed, and the new pentairon complexes were constructed utilizing these ligands. The newly synthesized complexes catalysed the oxidation of water with high Faradaic efficiencies, and the onset potentials of these complexes were lower than that of the parent complex. Mechanistic insights revealed that there are two methods for decreasing onset potentials: Control of the redox potentials of the pentairon complex and control of the reaction mechanism (Figure 3).

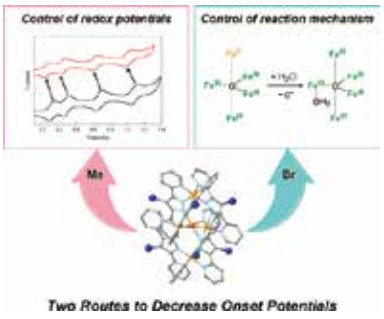



**Figure 3.** Two routes to control onset potentials of water oxidation in pentanuclear iron complexes.

### References

1) S. K. Lee, M. Kondo, M. Okamura, T. Enomoto, G. Nakamura and S. Masaoka, *J. Am. Chem. Soc.* **140**, 16899–16903 (2018).
2) V. K. K. Praneeth, M. Kondo, M. Okamura, T. Akai, H. Izu and S. Masaoka, *Chem. Sci.* **10**, 4628–4639 (2019).

### Awards

MASAOKA, Shigeyuki; The 25th Gold Medal Prize (2019).
IZU, Hitoshi; Elsevier Best Poster Prize, 15th International Symposium on Applied Bioinorganic Chemistry (2019).
ENOMOTO, Takafumi; The School of Physical Sciences Dean's Award, SOKENDAI (2018).
LEE, Sze Koon; SOKENDAI Award (2018).
CHINAPONG, Pondchanok; The School of Physical Sciences Dean's Award, SOKENDAI (2018)

# Creation of Novel Photonic-Electronic-Magnetic Functions Based on Molecules with Open-Shell Electronic Structures

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

**KUSAMOTO, Tetsuro**
Associate Professor
[kusamoto@ims.ac.jp]

**Education**
2003 B.S. The University of Tokyo
2010 Ph.D. The University of Tokyo

**Professional Employment**
2005 Sony Corporation
2010 Postdoctoral Fellow, RIKEN
2012 Project Assistant Professor, The University of Tokyo
2013 Assistant Professor, The University of Tokyo
2019 Associate Professor, Institute for Molecular Science
Associate Professor, The Graduate University for Advanced Studies

**Awards**
2011 Best Presentation Awards at the Annual Meeting, Japan Society for Molecular Science
2010 Research Award, Graduate School of Science, the University of Tokyo
2008 BCSJ Award, The Chemical Society of Japan

**Member**
Graduate Student
KIMURA, Shun*
KATO, Soshi
ISHIHARA, Mei
FUJISAWA, Mayu
Secretary
AOKI Junko

**Keywords** Radical, Open-Shell Electronic States, Photonic-Electronic-Magnetic Properties

The molecules with open-shell electronic states can exhibit unique properties, which are difficult to achieve for conventional closed-shell molecules. Our group develops new open-shell organic molecules (= radicals) and metal complexes to create novel photonic-electronic-magnetic functions.

While conventional closed-shell luminescent molecules have been extensively studied as promising components for organic light-emitting devices, the luminescent properties of radicals have been much less studied because of its rarity and low chemical (photo-)stability. We have developed a novel luminescent organic radical PyBTM, which is highly stable at ambient condition and in the photoexcited state. We have also discovered that (i) PyBTM-doped molecular crystals exhibit photoluminescence with a room-temperature emission quantum yield of 89%, which is exceptionally high in radicals, and (ii) the doped crystals show drastic changes in the emission spectra by applying a magnetic field. This is the first observation of the magnetoluminescence in organic radicals. Our studies provide novel and unique insights in molecular photonics, electronics, and spintronics, and also contribute to developing applied science for light-emitting devices.

Our group focuses on frustrated spins in molecular crystals. The anisotropic assembly of open-shell molecules in crystalline states can afford unusual electronic states attributed to the frustrated spins, providing exotic electrical and magnetic properties.

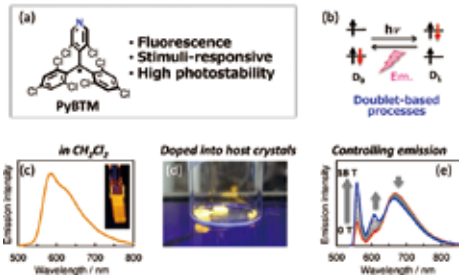



**Figure 1.** (a) Molecular structure of PyBTM and its characteristics. (b) Schematic photoexcitation-emission processes. (c) Emission in $CH_2Cl_2$. (d) Emission of PyBTM-doped molecular crystals. (e) Controlling emission by magnetic field.

## Selected Publications


- S. Kimura, T. Kusamoto, S. Kimura, K. Kato, Y. Teki and H. Nishihara, "Magnetoluminescence in a Photostable, Brightly Luminescent Organic Radical in a Rigid Environment," *Angew. Chem., Int. Ed.* **57**, 12711–12715 (2018).
- Y. Hattori, T. Kusamoto and H. Nishihara, "Enhanced Luminescent Properties of an Open-Shell (3,5-Dichloro-4-pyridyl)bis(2,4,6-trichlorophenyl)methyl Radical by Coordination to Gold," *Angew. Chem., Int. Ed.* **54**, 3731–3734 (2015).
- Y. Hattori, T. Kusamoto and H. Nishihara, "Luminescence, Stability, and Proton Response of an Open-Shell (3,5-Dichloro-4-pyridyl)bis(2,4,6-trichlorophenyl)methyl Radical," *Angew. Chem., Int. Ed.* **53**, 11845–11848 (2014).
- T. Kusamoto, H. M. Yamamoto, N. Tajima, Y. Oshima, S. Yamashita and R. Kato, "Bilayer Mott System with Cation···Anion Supramolecular Interactions Based on Nickel Dithiolene Anion Radical: Coexistence of Ferro- and Antiferro-Magnetic Anion Layers and Large Negative Magnetoresistance," *Inorg. Chem.* **52**, 4759–4761 (2013).

## 1. NIR Emission and Acid-Induced Intramolecular Electron Transfer Derived from a SOMO–HOMO Converted Non-Aufbau Electronic Structure

Some organic radicals violate the Aufbau principle and possess peculiar electronic structures in which the energy level of the SOMO (Singly Occupied Molecular Orbital) is formally lower than that of the highest occupied molecular orbital (HOMO). Radicals with such SOMO–HOMO converted electronic structures are attracting growing interest as promising candidates for unique stimulus-controlled molecular functions, which cannot be achieved using conventional radicals or closed-shell molecules. We prepared a novel organic radical with a SOMO–HOMO converted electronic structure, TPA-R$^{\bullet}$, a novel electron donor–acceptor hybrid of triphenylamine (an electron donor) and a stable polychlorinated diphenyl(4-pyridyl)methyl radical (an electron acceptor).[1] TPA-R$^{\bullet}$ exhibited fluorescence in the near-infrared region ($\lambda_{max}$ = 910 nm) in cyclohexane from a polar intramolecular charge-transfer excited state. Cyclic voltammetry, absorption spectroscopy, and DFT calculation revealed the inversion of the SOMO and HOMO levels in the electronic structure of TPA-R$^{\bullet}$. Addition of trifluoromethanesulfonic acid to TPA-R$^{\bullet}$ caused a two-step change. Protonation initially occurred on the diphenylpyridylmethyl radical moiety to form TPA-[RH]$^{\bullet+}$. Further addition of the acid caused unprecedented intramolecular electron transfer from the triphenylamine moiety to the protonated radical moiety, generating [TPA]$^{\bullet+}$-RH. TPA-[RH]$^{\bullet+}$ and [TPA]$^{\bullet+}$-RH could be switched by changing the acidity of the solution. These results constitute the first example of the multistep switching behavior stimulated by a single external stimulus in the SOMO–HOMO converted non-Aufbau electronic structure and demonstrate its great potential for realizing unique molecular photonic and electronic functions.

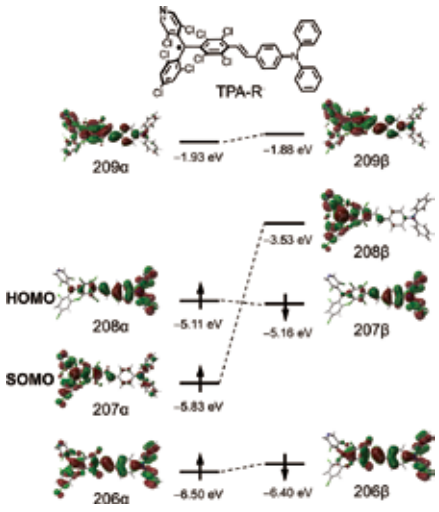



**Figure 2.** MO diagram of TPA-R$^{\bullet}$ calculated using DFT (uB3LYP/6-31G*).

## 2. 1D Cu$^{II}$–Radical Heterospin System: Temperature-Dependent Jahn–Teller Distortion Correlated to π-Conjugation and Magnetic Properties

Jahn–Teller (JT) and pseudo-JT distortions modulate coordination geometry around the metal ion to decrease the energy of an electronic system, thereby affecting the physical properties of materials. Controlling the JT distortion is one promising approach to develop tunable physical properties or to reveal structure–property relationships. We prepared a new class of 1D chain complexes $[Cu^{II}(hfac)_2(bisPyTM)]_n$ (hfac = hexafluoroacetylacetonato; bisPyTM[2] = bis(3,5-dichloro-4-pyridyl)(2,4,6-trichlorophenyl)methyl radical).[3] In the crystal, bisPyTM bridges two Cu$^{II}$ ions via the nitrogen atoms to form a 1D –Cu$^{II}$(hfac)$_2$–bisPyTM– type zigzag chain structure (Figure 3). Importantly, the coordination geometry around the Cu$^{II}$ atom continuously changes with temperature, where the JT axis rotates from the Cu–N1 bond direction at 298 K to the Cu–O1 bond direction at 93 K. This structural change induces changes in the mode of π-conjugation in the hfac moieties. Magnetic investigations revealed ferromagnetic (FM) interaction between spins on bisPyTM and Cu$^{II}$. The FM interaction was enhanced below 90 K due to the reorientation of the spin orbital ($d_{x2-y2}$ orbital) accompanied by the rotation of the JT axis on the Cu$^{II}$ atom. The reorientation of the spin orbitals was supported by ESR spectroscopy and DFT calculation. Namely, the JT distortion, degree of freedom of π-conjugation, and magnetic properties in $[Cu^{II}(hfac)_2(bisPyTM)]_n$ were coupled, resulting in unique temperature-dependent properties. The present study expands the scope of JT-active magnetic molecular compounds displaying controllable properties.

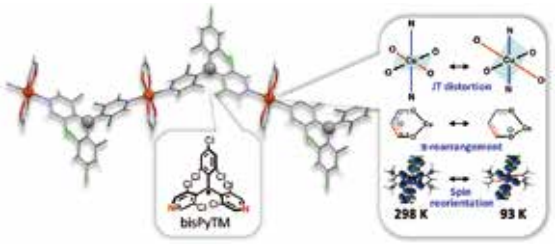



**Figure 3.** 1D chain structure of $[Cu^{II}(hfac)_2(bisPyTM)]_n$. $CF_3$ groups in the hfac ligands are omitted for clarity.

### References

1) A. Tanushi, S. Kimura, T. Kusamoto, M. Tominaga, Y. Kitagawa, M. Nakano and H. Nishihara, *J. Phys. Chem. C* **123**, 4417–4423 (2019).

2) S. Kimura, A. Tanushi, T. Kusamoto, S. Kochi, T. Sato and H. Nishihara, *Chem. Sci.* **9**, 1996–2007 (2018).

3) S. Kimura, H. Uchida, T. Kusamoto and H. Nishihara, *Dalton Trans.* **48**, 7090–7093 (2019).

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

# Control of Electron Transfer for Efficient Oxygenation Reactions

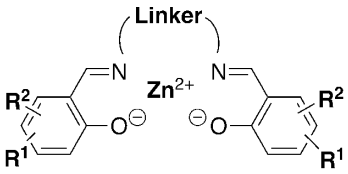
**Department of Life and Coordination-Complex Molecular Science**
**Division of Biomolecular Functions**

KURAHASHI, Takuya
Assistant Professor

Electron transfer is the most fundamental reaction to govern chemical reactions. To find an effective way to control electron transfer, electronic structures of key active species were investigated in detail with various techniques including absorption, $^{1}H$ and $^{2}H$ NMR, EPR, IR resonance Raman spectroscopy and magnetic susceptibility measurements. Correlations between electronic structures and electron transfer ability are the main focus. The insight obtained from electronic structural studies is utilized to create a new catalyst, which is applied for the reactions of gaseous methane under photoirradiation.

## 1. Development of Photoactive Schiff Base Ligands for Photocatalytic Reactions

A Schiff base ligand such as salen, which is prepared from salicylaldehydes and amines, could be easily obtained in a large scale and is also suitable for a wide variety of chemical modifications. This structural feature makes a Schiff base complex one of the most versatile framework for catalysts and materials. Some of Schiff base ligands shows relatively strong fluorescence, which has been used for sensory applications. But the photophysical properties of Schiff base ligands have not been fully exploited, and the mechanistic aspects of strong or weak fluorescence remains unknown. The present study explored a higher fluorescence quantum yield and longer emission wavelength by systematically changing the structure of salen-type Schiff base ligands with zinc ion shown in Figure 1.




**R$^{1}$**, **R$^{2}$** = MeO-, Me-, *t*-Bu-, Ph-, Cl-, MeCO-, $NO_2$-

**Figure 1.** Schiff base complexes for fluorescence studies.

# Visiting Professors

Visiting Professor
**SAKURAI, Hidehiro** *(from Osaka University)*

### Nanoscience Based on the Synthetic Organic Chemistry

Bowl-shaped π-conjugated compounds including partial structures of the fullerenes or the cap structure of carbon cnanotubes, which are called "buckybowls," are of importance not only as model compounds of fullerenes but also as their own chemical and physical properties due to their unique structure. We have developed the rational routes to the various buckybowls, including sumanene, a $C_{3v}$-symmetric pristine buckybowl framework, and also investigate their chemical and physical properties. We also investigate to develop novel catalytic properties of metal nanoclusters protected by organic polymers and/or molecules. We focus on the following projects: Preparation of size-selective gold and gold-based alloy nanoclusters supported by hydrophilic polymers and its catalytic activity: Development of designer metal nanocluster catalyst using the highly-functionalized protective polymers.

Visiting Professor
**UCHIHASHI, Takayuki** *(from Nagoya University)*

### High-Speed AFM Reveals Accelerated Binding of Agitoxin-2 to $K^+$ Channel by Induced-Fit

Agitoxin-2 (AgTx2) from scorpion venom is a potent blocker of Shaker-related $K^+$ channels. Docking model of them has been elucidated; however, whether binding dynamics is described by a two-state (AgTx2-bound and AgTx2-unbound) model or more complicated mechanism such as induced-fit or conformational selection is still unclear. Here, we observed the binding dynamics of AgTx2 to the Shaker-mimicking KcsA channel using high-speed atomic force microscopy. We imaged repeated binding and dissociation of AgTx2 to the channel, and found that the affinity of the channel for AgTx2 increases during persistent binding of AgTx2 and decreases during persistent dissociation. We propose a four-state model including high- and low-affinity states of the channel with relevant rate constants. Induced-fit pathway is dominant that accelerates binding event 400 times. This is the first analytical imaging of scorpion toxin binding in real time, which is applicable to various biological dynamics including channel-ligands, DNA-modifier proteins, and antigen-antibody complexes.

Visiting Associate Professor
**YAMADA, Teppei** *(from Kyushu University)*

### Thermocell and Ionic Motion in Soft Molecular Space

Recently we focus on the ionic motion in chiral nanospace. A porous metal–organic framework, Labtb, was synthesized with an enantioselective method. After the collaborative work with Prof. Okamoto and Dr. Narushima in IMS, high enantiomer-excess of Labtb in particle-level was visualized by circular dichroism imaging (*Chem. –Eur. J.* **10**, 6698–6702 (2019)). The obtained enantiomeric Labtb is highly stable from heat, chemicals and has 1D pore of ca. 13 Å in diameter, and we are searching the wide application of it.

I also intend to control the behavior of redox species by temperature response for constructing next-generation thermocell. Host–guest chemistry, (*Bull. Chem. Soc. Jpn.* **92**, 1142–1147 (2019); *Polymer J.* **50**, 761–769 (2019); *Chem. Sci.* **10**, 773–780 (2019)) and the chemistry of polythiolate (*ChemSusChem* **12**, 4014–4020 (2019)) were applied and high Seebeck coefficient was observed.