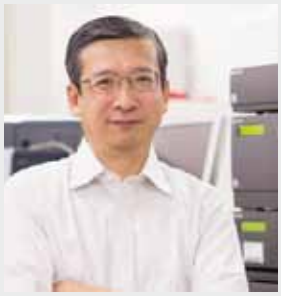
RESEARCH ACTIVITIES

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. Professor Kunihiro Kuwajima was retired at the end of March, 2013. Professors Shuji Akiyama and Tetsuro Murahashi (Division of Biomolecular Sensing and the Division of Functional Coordination Chemistry, respectively) have moved to the Research Center of Integrative Molecular Systems in April 2013, who have been contributing this department under concurrent appointments.

Bioinorganic Chemistry of Metal-Containing Sensor Proteins

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



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The widely studied biological function of heme is to act as a prosthetic group in hemeproteins that show a variety of functions, including oxygen storage and transport, electron transfer, redox catalysis, and sensing of gas molecules. Besides acting as a prosthetic group in a protein matrix, it has become apparent that free heme molecules can act as physiological effectors of several proteins, including transcriptional regulators, heme-regulated eIF2α kinase, and sensor kinases in two-component signal transduction systems. Reversible heme binding regulates the physiological function of these proteins. Though research on these proteins has shown new physiological functions of heme as a signaling molecule, the detailed molecular mechanisms by which heme regulates the functions of these proteins remain to be elucidated, mainly because the three-dimensional structures of these regulatory proteins have not yet been solved. Our research interests are focused on the elucidation of the molecular mechanisms of how heme molecule acts as a signaling molecule. We are also studying about the structure–function relationships of the heme-based gas sensor proteins.

1. Structural Basis for the Transcriptional Regulation of Heme Homeostasis in *Lactococcus lactis*[1)]

Though heme is a crucial element for many biological processes including respiration, heme homeostasis should be regulated strictly due to the cytotoxicity of free heme molecules. Numerous lactic acid bacteria, including *Lactococcus lactis*, acquire heme molecules exogenously to establish an aerobic respiratory chain. A heme efflux system plays an important role for heme homeostasis to avoid cytotoxicity of acquired free heme, but its regulatory mechanism is not clear. Here, we report that the transcriptional regulator HrtR senses and binds a heme molecule as its physiological effector to regulate the expression of the heme-efflux system responsible for heme homeostasis in *L. lactis*. To elucidate the molecular mechanisms of how HrtR senses a heme molecule and regulates gene expression for the heme efflux system, we determined the crystal structures of the apo-HrtR/DNA complex, apo-HrtR, and holo-HrtR at a resolution of 2.0, 3.1, and 1.9 Å, respectively. These structures revealed that HrtR is a member of TetR family of transcriptional regulators. The residue pair Arg46 and Tyr50 plays a crucial role for specific DNA-binding through hydrogen-bonding and a CH–π interaction with the DNA bases (Figure 1).

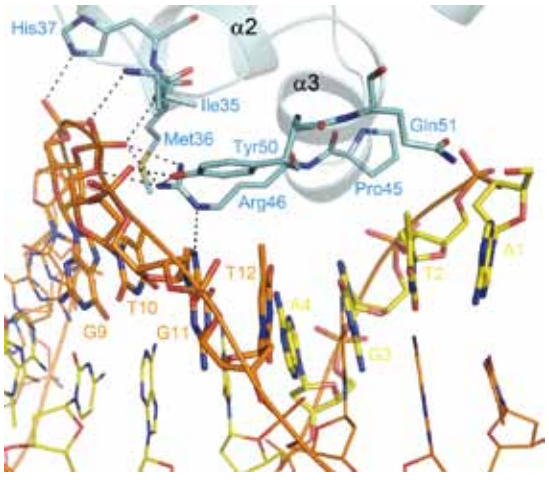

Figure 1. Interaction between apo-HrtR and DNA.

HrtR adopts a unique mechanism for its functional regulation upon heme-sensing. A comparison of the apo-HrtR/DNA complex and holo-HrtR structures revealed that heme-binding triggers a coil-to-helix transition at the α4a-α4b region (Figure 2 (A), (B)). In apo-HrtR, a loop (residues 68-71) intervenes between α4a and α4b helices. Glu70 in the middle of this intervening loop forms a hydrogen bond with Trp123 in apo-HrtR (Figure 2 (A)). Upon heme-binding, a coil-to-helix transition occurs in this intervening loop, which results in the formation of a long α4 helix in holo-HrtR. As the location of Glu70 is largely altered by this coil-to-helix transition, the hydrogen bond between Glu70 and Trp123 is lost in holo-HrtR as shown in Figure 2B. Heme-binding to HrtR causes a coil-to-helix transition of the α4 helix in the heme-sensing domain, which triggers a structural change of HrtR causing it to dissociate from the target DNA for de-repression of the genes encoding the heme efflux system. HrtR uses a unique heme-sensing motif with *bis*-His (His72 and His149) ligation to the heme, which is essential for the coil-to-helix transition of the α4 helix upon heme-sensing.

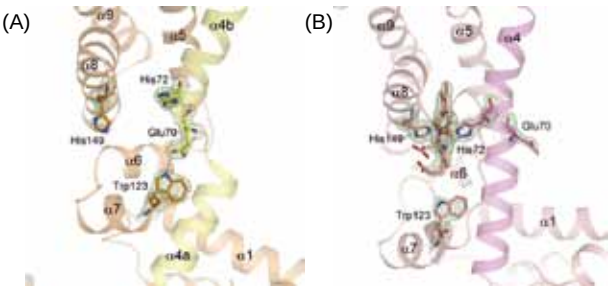

Figure 2. (A) Crystal structure of apo-HrtR. (B) Crystal structure of holo-HrtR.

2. A Model Theoretical Study on Ligand Exchange Reactions of CooA[2)]

Rr-CooA is a CO-sensor heme protein, where binding of CO with the heme group stimulates a transcriptional activator activity of CooA. In this process, the heme undergoes a series of ligand exchanges. In the ferric form, the heme has Cys75 and Pro2 as the axial ligands. In the reduced ferrous form, the heme has His77 instead of Cys75 as an axial ligand with Pro2. Only in the reduced form, CooA can bind CO that replaces Pro2. Model calculations are carried out to elucidate the ligand exchange reactions of CooA. The coordinated proline is found to be the neutral, protonated form. The ligand exchange of cysteine for histidine is reproduced by a relatively small model. This exchange would be mainly due to difference in stability of the non-bonding sulfur p-orbital in Cys75 between the ferric and ferrous states. The selectivity of gas molecules among CO, NO, and O_2 in the proteins is explained by the relative stability of products for Rr-CooA. This is also the case for Ch-CooA, where the amino group of the N-terminus and a histidine are coordinated to the iron ion both in the ferric and ferrous states. The ability to bind the gas molecules is a little stronger in Rr-CooA than in Ch-CooA. In the ferric form of Rr-CooA, heme is deformed to a ruffled form whereas heme is planar in the ferrous form, which leads to a red-shifted Q-band in the former.

3. Molecular Mechanisms of Heme Transport in Gram-Positive Bacteria

Iron, the second-most abundant metal in the Earth's crust, forms a water insoluble oxyhydroxide polymer in water under aerobic conditions, resulting in poor bio-availability. As it is an essential nutrient for prokaryotes and eukaryotes, they develop sophisticated iron acquisition systems to overcome this problem. A gram-positive bacterium *Listeria monocytogenes* has several iron (and iron complexes) transport systems, one of which is an ABC-type transporter (HupCGD) that transports heme as an iron source for this bacterium. HupC, HupG, and HupD proteins are an ATPase component, a membrane permease, and substrate (heme) biding protein, respectively. We have charezterized HupD protein to elucidate the molecular mechanisms of heme transport in *L. monocytogenes*.

HupD contains a typical N-terminal sequence of lipoprotein signal peptide. The amino acid sequence of LLASC is present at the C-terminus of the signal peptide, of which the cysteine residue is the target for lipid modification and becomes the first residue of the mature lipoprotein after cleavage by SPase II protease. When HupD with the signal peptide is expressed in *E. coli*, the recombinant HupD forms aggregates probably due to a hydrophobic signal peptide. Mature HupD lacking the signal peptide is expressed as a soluble protein, which is a mixture of apo- and holo-forms. Purified holo-HupD shows the Soret, α, and β peaks at 413, 565, and 530 nm, respectively, which are typical for 6-coordinated ferric heme proteins with two His as the axial ligands. Ferric HupD shows EPR signals with g = 3.27 and 2.08, which are consistent with a bis-His type ligation of the heme. Site-directed mutagenesis studies suggest that His105 and His259 act as the axial ligands of the heme in HupD. Crystallization experiments are now in progress to obtain structural information of HupD.

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Elucidation of the Molecular Mechanisms of Protein Folding

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Kuwajima group is studying mechanisms of *in vitro* protein folding and mechanisms of molecular chaperone function. Our goals are to elucidate the physical principles by which a protein organizes its specific native structure from the amino acid sequence. In this year, they studied molecular mechanisms of the cytotoxicity of human α-lactalbumin made lethal to tumor cells (HAMLET) and other protein-oleic acid complexes, in which a protein folding intermediate forms a complex with oleic acid, and this complex has a unique apoptotic activity for the selective killing of tumor cells.

1. Molecular Mechanisms of the Cytotoxicity of Human α-Lactalbumin Made Lethal to Tumor Cells (HAMLET) and Other Protein-Oleic Acid Complexes

Although HAMLET (human α-lactalbumin made lethal to tumor cells), a complex formed by human α-lactalbumin and oleic acid, has a unique apoptotic activity for the selective killing of tumor cells, the molecular mechanisms of expression of the HAMLET activity are not well understood. Therefore, we studied the molecular properties of HAMLET and its goat counterpart, GAMLET (goat α-lactalbumin made lethal to tumor cells), by pulse field gradient NMR and 920-MHz two-dimensional NMR techniques. We also examined the expression of HAMLET-like activities of complexes between oleic acid and other proteins that form a stable molten globule state. We observed that both HAMLET and GAMLET at pH7.5 were heterogeneous, composed of the native protein, the monomeric molten globule-like state, and the oligomeric species. At pH 2.0 and 50 °C, HAMLET and GAMLET appeared in the monomeric state, and we identified the oleic acid-binding site in the complexes by two- dimensional NMR. Rather surprisingly, the binding site thus identified was markedly different between HAMLET and GAMLET. Furthermore, canine milk lysozyme, apo- myoglobin, and β_2-microglobulin all formed the HAMLET- like complex with the anti-tumor activity, when the protein was treated with oleic acid under conditions in which their molten globule states were stable. From these results, we conclude that the protein portion of HAMLET, GAMLET, and the other HAMLET-like protein-oleic acid complexes is not the origin of their cytotoxicity to tumor cells and that the protein portion of these complexes plays a role in the delivery of cytotoxic oleic acid molecules into tumor cells across the cell membrane.

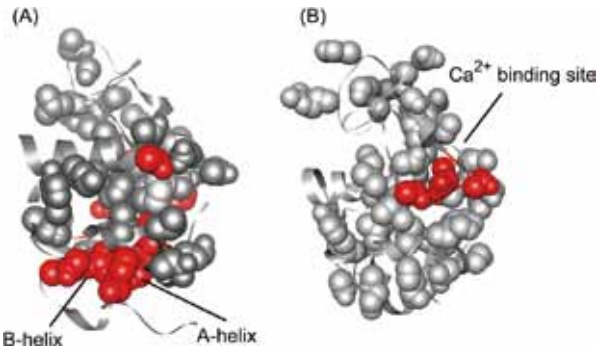

Figure 1. The oleic acid-binding sites of human α-lactalbumin in HAMLET (A) and of goat α-lactalbumin in GAMLET (B) as determined by differences in cross-peaks between the free molten globule state and the α-lactalbumin-olecic acid complex (HAMLET or GAMLET). The amino acid residues represented by the space-filling model are those whose cross-peaks in the ^{1}H-^{15}N HSQC spectra are assigned. The red residues indicate the oleic acid-binding site of each protein.

2. Native-State Heterogeneity of β_2-Microglobulin as Revealed by Kinetic Folding and Real-Time NMR Experiments

The kinetic folding of β_2-microglobulin from the acid-denatured state was investigated by interrupted-unfolding and interrupted-refolding experiments using stopped-flow double-jump techniques. In the interrupted unfolding, we first unfolded the protein by a pH jump from pH 7.5 to pH 2.0, and the kinetic refolding assay was carried out by the reverse pH jump by monitoring tryptophan fluorescence. Similarly, in the interrupted refolding, we first refolded the protein by a pH jump from pH 2.0 to pH 7.5 and used a guanidine hydrochloride (GdnHCl) concentration jump as well as the reverse pH jump as unfolding assays. Based on these experiments, the folding is represented by a parallel-pathway model, in which the molecule with the correct Pro32 *cis* isomer refolds rapidly with a rate constant of 5–6 s^{-1}, while the molecule with the Pro32 *trans* isomer refolds more slowly (pH 7.5 and 25 °C). At the last step of folding, the native-like *trans* conformer produced on the latter pathway isomerizes very slowly (0.001–0.002 s^{-1}) into the native *cis* conformer. In the GdnHCl-induced unfolding assays in the interrupted refolding, the native-like *trans* conformer unfolded remarkably faster than the native *cis* conformer, and the direct GdnHCl-induced unfolding was also biphasic, indicating that the native-like *trans* conformer is populated at a significant level under the native condition. The one-dimensional NMR and the real-time NMR experiments of refolding further indicated that the population of the *trans* conformer increases up to 7–9% under a more physiological condition (pH 7.5 and 37 °C).

3. Structural Insights into the Stability Perturbations Induced by N-Terminal Variation in Human and Goat α-Lactalbumin

Addition of an extra methionine at the N-terminus by recombinant expression of α-lactalbumin in *Escherichia coli* significantly destabilizes the protein, and this destabilization has hampered mutational analyses such as the mutational phi-value analysis of the protein. Deletion of residue 1 from the recombinant form recovers the stability in human and goat α-lactalbumin. Here, we thus determined the crystal structures of the residue 1-deletion variants of recombinant human and goat α-lactalbumin, and compared the structures with those of the authentic and recombinant forms. The results demonstrate the importance of the N-terminal backbone structure and hydrogen- bonding pattern for the stability of α-lactalbumin.

4. The H/D-Exchange Kinetics of the *Escherichia coli* Co-Chaperonin GroES Studied by 2D NMR and DMSO-Quenched Exchange Methods

We studied hydrogen/deuterium-exchange reactions of peptide amide protons of GroES using two different techniques: (1) two-dimensional ^{1}H–^{15}N transverse-optimized NMR spectroscopy and (2) the dimethylsulfoxide-quenched hydrogen-exchange method combined with conventional ^{1}H–^{15}N heteronuclear single quantum coherence spectroscopy. By using these techniques together with direct heteronuclear single quantum coherence experiments, we quantitatively evaluated the exchange rates for 33 out of the 94 peptide amide protons of GroES and their protection factors, and for the remaining 61 residues, we obtained the lower limits of the exchange rates. The protection factors of the most highly protected amide protons were on the order of 10^6–10^7, and the values were comparable in magnitude to those observed in typical small globular proteins, but the number of the highly protected amide protons with a protection factor larger than 10^6 was only 10, significantly smaller than the numbers reported for the small globular proteins, indicating that significant portions of free heptameric GroES are flexible and natively unfolded. The highly protected amino acid residues with a protection factor larger than 10^5 were mainly located in three β-strands that form the hydrophobic core of GroES, while the residues in a mobile loop (residues 17–34) were not highly protected. The protection factors of the most highly protected amide protons were orders of magnitude larger than the value expected from the equilibrium unfolding parameters previously reported, strongly suggesting that the equilibrium unfolding of GroES is more complicated than a simple two-state or three-state mechanism and may involve more than a single intermediate.

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Elucidation of Dynamical Structures of Biomolecules toward Understanding the Mechanisms Underlying Their Functions

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Our biomolecular studies are based on detailed analyses of dynamical structures of various biological macromolecules and their complexes at atomic level, using NMR spectroscopy in conjunction with other biophysical, biochemical and molecular biology techniques. Here we report our recent structural studies of biomolecular complexes for exploring their functional relevance.

1. NMR Characterization of Specific Glycolipid-Protein/Peptide Interactions

Lipid membranes provide active platforms for dynamic interactions of a variety of biomolecules on cell surfaces, where oligosaccharides covalently modifying lipids are involved in divergent molecular recognition events. Sarcotoxin IA, a ecropin-type peptide from *Sarcophaga peregrine*, exhibits antibacterial activity against Gram-negative bacteria through its interaction with lipid A, a core component of lipopolysaccharides. To acquire detailed structural information on this specific interaction, we performed NMR analysis using bacterially expressed sarcotoxin IA analogs with isotope labeling along with lipid A-embedding micelles composed of dodecylphosphocholine. By inspecting the NMR data, we revealed that the N-terminal segment of sarcotoxin IA formed an amphiphilic α-helix upon its interaction with the aqueous micelles, thereby identifying key lysine residues in the interaction with lipid A and the consequent antibacterial activity.[1)] These results offer unique information for designing chemotherapeutics based on antibacterial peptide structures.

Gangliosides, glycosphingolipids possessing a sialyl oligosaccharide moiety, can be targets for various amyloidogenic proteins that are associated with neurodegenerative disorders. We successfully displayed a series of gangliosides on small bicelles with a uniform confined size, offering nanoscale standardized membrane mimics for spectroscopic characterization of weak encounter complexes formed between ganglioside clusters and amyloidogenic proteins.[2)] This enabled probing of initial membrane-landing processes of α-synuclein as therapeutic target by NMR spectroscopy.

2. NMR Characterization of Interactions of Molecular Chaperones with Model Ligands

Molecular chaperones are involved in various cellular processes in which proteins undergo folding, unfolding, and/or refolding under physiological and stress conditions. It has been proposed that molecular chaperones actively contribute to the suppression of toxic aggregate formation of various amyloidogenic proteins associated with neurodegenerative disorders. Furthermore, molecular chaperones have attracted attention due to their potential applicabilities as intelligent nanodevices in bioenginnering fields.

The chaperonin of *Escherichia coli* GroEL forms a large cylindrical complex that assists in the folding of nascent polypeptides. We characterized GroEL–protein interactions by stable isotope-assisted NMR spectroscopy using chemically

denatured bovine rhodanese and an intrinsically disordered protein, α-synuclein, as model ligands. NMR data indicated that proteins tethered to GroEL remain largely unfolded and highly mobile, enabling identification of the interaction hot spots displayed on intrinsically disordered proteins.[3] Furthermore, we found that GroEL could suppress Aβ amyloid formation by interacting with its two hydrophobic segments involving key residues in fibril formation. The binding site of Aβ was mapped on a pair of α-helices located in the GroEL apical domain.[4] These results provide insights into chaperonin recognition of amyloidogenic proteins of pathological interest.

Moreover, we successfully identified ligand-binding site of Hsp47, a client-specific chaperone for collagen, by NMR spectroscopy in conjunction with mutational analysis.[5] Our findings provide a molecular basis for the design of drugs that target the interaction between Hsp47 and procollagen in therapeutics for fibrotic diseases.

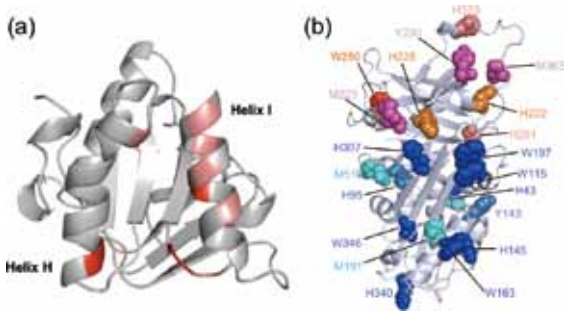

Figure 1. Mapping of (a) the Aβ-binding site on the GroEL apical domain and (b) the collagen-binding site of Hsp47.

3. Elucidation of the Functional Relevance of Quaternary Structures of Proteasome-Associated Proteins

Assembly of the eukaryotic 20S proteasome is an ordered process involving several proteins operating as proteasome assembly factors including PAC1-PAC2 but that of the archaeal 20S proteasome involves spontaneous self-assembly. Recent bioinformatic analysis identified archaeal PAC1-PAC2 homologs PbaA and PbaB. However, it remains unclear whether such assembly factor-like proteins play an indispensable role in orchestration of proteasome subunits in archaea. We revealed that PbaB forms a homotetramer and exerts a dual function as an ATP-independent proteasome activator and a molecular chaperone through its tentacle-like C-terminal segments.[6] Our findings provide insights into molecular evolution relationships between proteasome activators and assembly factors.

In mammals, a major form of proteasome activator PA28 is a heteroheptamer composed of interferon-γ-inducible α and β subunits, which share approximately 50% amino acid identity and possess distinct insert loops. Using deuteration-assisted small-angle neutron scattering, we demonstrated three α and four β subunits are alternately arranged in the heteroheptameric ring.[7] In this arrangement, PA28 loops surround the central pore of the heptameric ring, suggesting that the flexible PA28 loops act as gatekeepers, which function to select the length of peptide substrates to be transported between the proteolytic chamber and the extra-proteasomal medium.

We have also succeeded in encapsulation of ubiquitin, a protein modifier recognized by the proteasome, within synthetic hosts in collaboration with Dr. Makoto Fujita (The University of Tokyo) and his colleagues.[8]

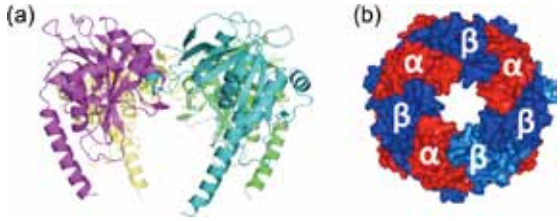

Figure 2. Quaternary structures of (a) the PbaB homotetramer and (b) the PA28 heteroheptamer.

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Award

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Structure-Function Relationship of Metalloenzymes

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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.

1. Comparative Spectroscopic Studies of Iron(III) and Manganese(III) Salen Complexes Having a Weakly-Coordinating Triflate Axial Ligand[1)]

Salen and porphyrin are among the most versatile synthetic ligands that are widely utilized as catalysts and materials. Both ligands are four-coordinate dianionic ligands that bind a metal ion in a tetradentate fashion, forming a similar square-planar metal complex. Prominently, iron and manganese, which are abundant transition metals in nature, become functional in combination with these ligands. In the case of porphyrin complexes, spectroscopic properties and electronic structures of iron(III) and manganese(III) complexes have been extensively studied, and some unique properties have been found, such as an intermediate spin state of an iron(III) ion in the presence of weak-field anionic axial ligands such as perchlorate (ClO_4^-) and triflate ($CF_3SO_3^-$).

However, relatively less has been investigated for salen complexes bearing these weak-field anionic axial ligands, while spectroscopic and magnetic properties of iron and manganese salen complexes with strongly-coordinating axial ligands such as imidazole are well documented. This is mostly because a monomeric iron(III) salen complex is quite prone to dimerization in the absence of a strongly-coordinating axial ligand. We previously designed a sterically-hindered salen ligand, and successfully synthesized a monomeric iron(III) and manganese(III) complex bearing a weakly-coordinating ClO_4^- ligand. But the sterically-hindered salen ligand is not necessarily suited for electronic tuning by modification of the phenolate rings, which prevented us to obtain in-depth insight into their spectroscopic properties.

We herein prepare mononuclear manganese(III) and iron(III) salen complexes bearing a weakly-coordinating triflate axial ligand, using salen ligands with differing electron-donating properties (Chart 1). We also synthesize nonsymmetrical salen ligands (Chart 1), in order to precisely understand spectroscopic properties. Magnetic susceptibility and dual-mode electron paramagnetic resonance (EPR) data show that iron(III) and manganese(III) salen complexes adopt high-spin d^5 (S = 5/2) and d^4 (S = 2) electronic configurations, respectively, in all the cases in Chart 1. Further insights into electronic structures of central metal are obtained from ^{2}H NMR spectra of selectively-deuterated complexes, in which Fe^{III}(salen)(OTf) and Mn^{III}(salen)(OTf) exhibit well-resolved paramagnetic NMR signals of quite different shift patterns, due to the presence or absence of the unpaired electron in the dx^2–y^2 orbital.

2. Synthesis, Characterization, and Reactivity of Hypochlorito-Iron(III) Porphyrin Complexes[2)]

Myeloperoxidase (MPO) and chloroperoxidase (CPO) are unique heme peroxidases that catalyze oxidation of chloride ion to hypochlorite ion (OCl^-). MPO is loctaed in azurophil

granules of neutrophils and produces OCl^- or hypochlorous acid (HOCl), which works as an antimicrobial agent, from hydrogen peroxide and chloride ion. On the other hand, CPO is an enzyme of *Caldariomyces fumago* and catalyzes chlorination reactions in the biosynthesis of the chlorinated metabolite caldariomycin. It has been proposed that ferric MPO and CPO initially react with hydrogen peroxide to form an oxoiron(IV) porphyrin π-cation radical species known as compound I. Compound I then reacts with chloride ion to form a transient hypochlorito-iron(III) porphyrin intermediate, which finally releases HOCl with the protonation of the heme-bound hypochlorite. In addition, hypochlorito-metal complexes have been proposed as key intermediates in catalytic oxygenation reactions catalyzed by transition-metal complexes. Because of its significant importance, a hypochlorito-iron(III) porphyrin intermediate has been examined to detect in MPO and CPO reactions and to synthesize its model complex. Although previous reports have indicated the possibility of formation of hypochlorito-iron(III) porphyrin intermediates, spectroscopic evidence for the formation of such species has not been reported until now. The reactivity of a hypochlorito-iron(III) porphyrin complex has been also received much attention in relation to those of other terminal oxidant-metal complexes such as hypochlorite, hydroperoxide, and iodosylarene. Herein, we report the preparation, spectroscopic characterization, and reactivity of hypochlorito-iron(III) porphyrin complexes, including a bis-hypochlorite complex, $[(TPFP)Fe^{III}(OCl)_2]^-$ (**1**), and imidazole-hypochlorite complexes, $(TPFP)Fe^{III}(OCl)$(1-R-Im), where TPFP is 5,10,15,20-tetrakis(pentafluorophenyl) porphyrinate and R is $–CH_3$ (**2**), –H (**3**), or $–CH_2CO_2H$ (**4**). (see Figure 1).

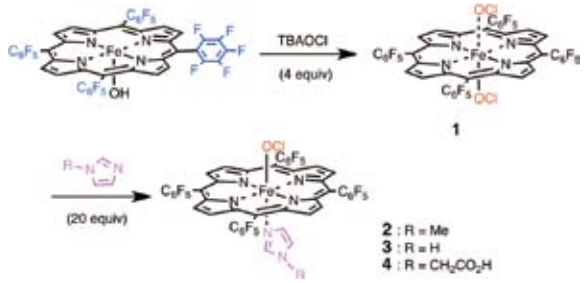

Figure 1. Preparation of hypochlorito-iron(III) porphyrin complexes.

3. Unique Ligand Radical Character of an Activated Cobalt Salen Catalyst that Is Generated by Aerobic Oxidation of a Cobalt(II) Salen Complex[3)]

The Co(salen)(X) complex, where salen is chiral *N*,*N*'-bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediamine, and X is an external axial ligand, has been widely utilized as a versatile catalyst. The Co(salen)(X) complex is a stable solid that has been conventionally described as a Co^{III}(salen)(X) complex. Recent theoretical calculations raised a new proposal that the Co(salen)$(H_2O)(SbF_6)$ complex contains appreciable contribution from a $Co^{II}(salen^{+\bullet})$ electronic structure (Kochem, A.; Kanso, H.; Baptiste, B.; Arora, H.; Philouze, C.; Jarjayes, O.; Vezin, H.; Luneau, D.; Orio, M.; Thomas, F. *Inorg. Chem.* **51**, 10557–10571 (2012)), while other theoretical calculations for Co(salen)(Cl) indicated a triplet Co^{III}(salen) electronic structure (Kemper, S.; Hrobárik, P.; Kaupp, M.; Schlörer, N. E. *J. Am. Chem. Soc.* **131**, 4172–4173 (2009)). However, there has been no experimental data to evaluate these theoretical proposals. We herein report key experimental data on the electronic structure of the Co(salen)(X) complex (X = $CF_3SO_3^-$, SbF_6^-, and *p*-$MeC_6H_4SO_3^-$). The X-ray crystallography shows that Co(salen)(OTf) has a square-planar N_2O_2 equatorial coordination sphere with OTf as an elongated external axial ligand. Magnetic susceptibility data indicate that Co(salen)(OTf) complexes belong to the S = 1 spin system. ^{1}H NMR measurements provide convincing evidence for the $Co^{II}(salen^{+\bullet})$(X) character, which is estimated to be about 40% in addition to 60% Co^{III}(salen)(X) character. The CH_2Cl_2 solution of Co(salen)(X) shows an intense near-infrared absorption, which is assigned as overlapped transitions from a ligand-to-metal charge transfer in Co^{III}(salen)(X) and a ligand-to-ligand charge transfer in $Co^{II}(salen^{+\bullet})$(X). The present experimental study establishes that the electronic structure of Co(salen)(X) contains both $Co^{II}(salen^{+\bullet})$(X) and Co^{III}(salen)(X) character.

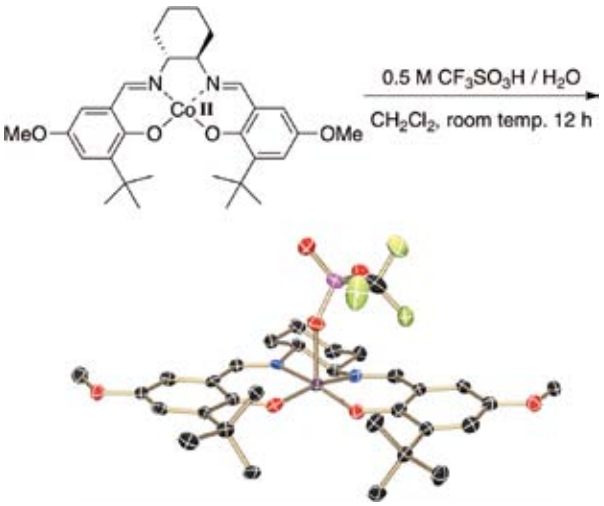

Figure 2. Preparation and Structure of Co(salen)(OTf) complex.

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Award

KURAHASHI, Takuya and FUJII, Hiroshi; BCSJ Award Article, September, 2012.

Investigation of Molecular Mechanisms of Channels, Transporters and Receptors in Cell Membrane

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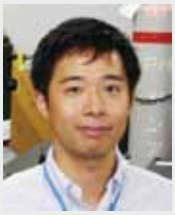
Associate Professor



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MEENA SINGH, Yatindra	Visiting Scientist		
FUJIWARA, Kuniyo	Graduate Student		

Membrane proteins are important for homeostasis of living cells, which work as ion channels, transporters, various types of chemical and biophysical sensors, and so on. These proteins are considered as important targets for biophysical studies.

Our main goal is to clarify molecular mechanisms of channels, transporters and receptors in cell membrane mainly by using stimulus-induced difference infrared (IR) spectroscopy, which is sensitive to the structural and environmental changes of bio-molecules.

1. O–H Stretching Vibrations of Dangling Bonds of Water Molecules in *pharaonis* Halorhodopsin Studied by Time-Resolved FTIR Spectroscopy

Ion transportation via the chloride ion pump protein *pharaonis* halorhodopsin (*p*HR) occurs through the sequential formation of several intermediates during the photocyclic reaction. Although the structural details of each intermediate state have been studied, the role of water molecules in the translocation of chloride ions inside the protein at physiological temperatures remains unclear. To analyze the structural dynamics of water inside the protein, we performed time-resolved Fourier transform infrared (FTIR) spectroscopy under H_2O or $H_2{}^{18}O$ hydration and successfully assigned water O–H stretching bands.[1)] We found that a dangling water band at 3626 cm^{-1} in *p*HR disappears in the L_1 and L_2 states. On the other hand, relatively intense positive bands at 3605 and 3608 cm^{-1} emerged upon the formation of the N and O states, respectively, suggesting that the chloride transportation is accompanied by dynamic rearrangement of the hydrogen-bonding network of the internal water molecules in *p*HR (Figure 1) .

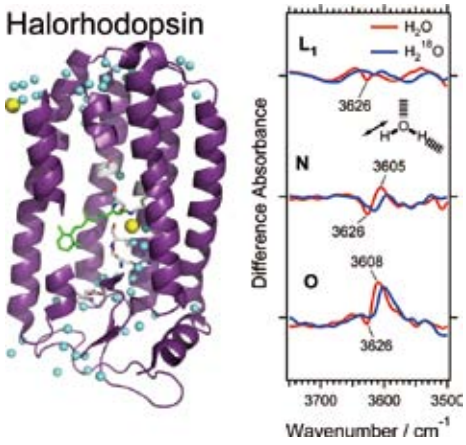

Figure 1. X-ray crystal structure of halorhodopsin (left). The O–H stretching bands of dangling bonds of water molecules inside the protein(right). The figure is adapted with permission from ref. 1. Copyright 2012 American Chemical Society.

2. Perfusion-Induced ATR-FTIR Spectroscopy for Studying Ion-Protein Interactions of a Potassium Ion Channel, KcsA

The potassium channel is highly selective for K^+ over Na^+,

and the selectivity filter binds multiple dehydrated K^+ ions upon permeation. Here, we applied attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy to extract ion-binding-induced signals of the KcsA potassium channel at neutral pH.[2] Shifts in the peak of the amide-I signal towards lower vibrational frequencies were observed as K^+ was replaced with Na^+ (Figure 2). These ion species-specific shifts deduced the selectivity filter as the source of the signal, which was supported by the spectra of a mutant for the selectivity filter (Y78F). The difference FTIR spectra between the solution containing various concentrations of K^+ and that containing pure Na^+ demonstrated two types of peak shifts of the amide-I vibration in response to the K^+ concentration. These signals represent the binding of K^+ ions to the different sites in the selectivity filter with different dissociation constants (K_D = 9 or 18 mM).

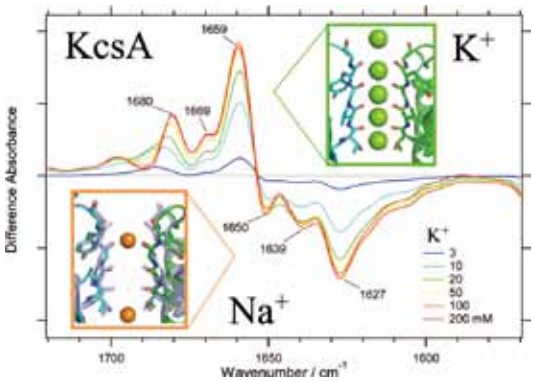

Figure 2. The K^+-minus-Na^+ difference spectra measured with different K^+ concentration. The insets show the X-ray crystal structures of KcsA. The figure is reprinted with permission from ref. 2. Copyright 2012 American Chemical Society.

3. Development of the Measurement Condition of Surface-Enhanced Infrared Absorption Spectroscopy on Membrane Proteins

Surface-enhanced infrared absorption with attenuated total reflection (ATR-SEIRA) is a powerful tool for exploring molecular mechanisms of membrane proteins at the monolayer level. However, the band intensity, position, and direction can be largely influenced by the presence of a thin gold film, as observed for small molecules existing in close proximity to the surface. Here we investigated influence on the band shapes of an α-helical membrane protein, *p*HR, attached on the gold surface through a complex formation between a six-histidines tag and a Ni-nitrilotriacetic acid (Ni-NTA) linker.[3] Normal, bipolar, and inverted shapes of amide-I and -II bands were observed with an increase in film thickness, although *p*HR molecules would locate relatively far from the surface. The physical origin of this interesting phenomenon has been identified by changing incident angle, polarization, and film deposition rate. We find the observed absorption anomalies are due to the influence of perpendicularly polarized light. Furthermore, it is shown that the band shapes are normal below the percolation threshold, and bipolar ones occur when an anomalous absorption by the films is strong, while the inverted ones develop with films in which surface scattering is predominant.

4. Development of a Rapid Buffer-Exchange System for Time-Resolved ATR-FTIR Spectroscopy with the Step-Scan Mode

Attenuated total reflectance (ATR)-FTIR spectroscopy has been widely used to probe protein structural changes under various stimuli, such as light absorption, voltage change, and ligand binding, in aqueous conditions. Time-resolved measurements require a trigger, which can be controlled electronically; therefore, light and voltage changes are suitable. Here we developed a novel, rapid buffer-exchange system for time-resolved ATR-FTIR spectroscopy to monitor the ligand- or ion-binding reaction of a protein.[4] By using the step-scan mode (time resolution; 2.5 ms), we confirmed the completion of the buffer-exchange reaction within ~25 ms; the process was monitored by the infrared absorption change of a nitrate band at 1350 cm^{-1} (Figure 3). We also demonstrated the anion-binding reaction of a membrane protein, *p*HR, which binds a chloride ion in the initial anion-binding site near the retinal chromophore. The formation of chloride- or nitrate-bound *p*HR was confirmed by an increase of the retinal absorption band at 1528 cm^{-1}. It also should be noted that low sample consumption (~1 μg of protein) makes this new method a powerful technique to understand ligand–protein and ion–protein interactions, particularly for membrane proteins.

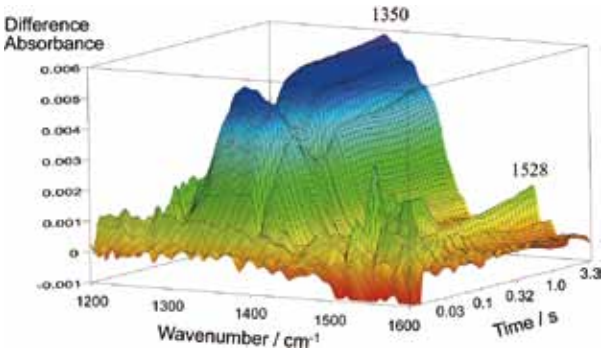

Figure 3. The time-resolved FTIR spectra of the rapid-buffer exchange reaction of pHR. The figure is reprinted with permission from ref 4. Copyright 2013 The Biophysical Society of Japan.

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4) Y. Furutani *et al.*, *Biophysics* **9**, 123–129 (2013).

Heterogeneous Catalytic Systems for Organic Chemical Transformations in Water

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		FUKUSHIMA, Tomoko	Secretary

Various transition metal-catalyzed organic molecular transformations in water were achieved under heterogeneous conditions by use of a self-assembled polymeric imidazole-copper catalyst, a boron-iridium heterobimetallic polymeric catalyst, or resin-supported iron nanoparticles which were designed and prepared by this research group. In particular, development of a highly active reusable poly(imidazole-copper) and an iron-catalyzed hydrogenation of under flow conditions are highlights among the achievements of the 2012–2013 period to approach what may be considered ideal chemical process of next generation. Representative results are summarized here under.

1. Amphiphilic Self-Assembled Polymeric Copper Catalyst to Parts per Million Levels: Click Chemistry[1)]

Self-assembly of copper sulfate and a poly(imidazole–acrylamide) amphiphile provided a highly active, reusable, globular, solid-phase catalyst for click chemistry. The self-assembled polymeric Cu catalyst was readily prepared from poly(*N*-isopropylacrylamide-co-*N*-vinylimidazole) and $CuSO_4$ via coordinative convolution. The surface of the catalyst was covered with globular particles tens of nanometers in diameter, and those sheetlike composites were layered to build an aggregated structure. Moreover, the imidazole units in the polymeric ligand coordinate to $CuSO_4$ to give a self-assembled, layered, polymeric copper complex. The insoluble amphiphilic polymeric imidazole Cu catalyst with even 4.5–45 mol ppm drove the Huisgen 1,3-dipolar cycloaddition of a variety of alkynes and organic azides, including the three-component cyclization of a variety of alkynes, organic halides, and sodium azide. The catalytic turnover number and frequency were up to 209000 and 6740 h^{-1}, respectively. The catalyst was readily reused without loss of catalytic activity to give the corresponding triazoles quantitatively.

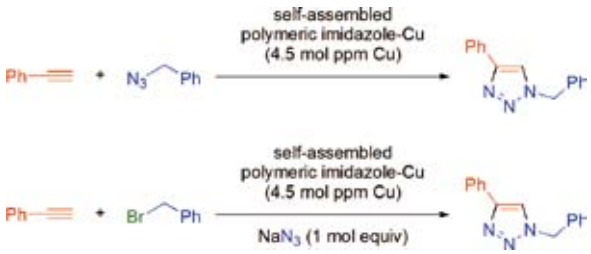

Figure 1. Click reaction catalyzed by self-assembled polymeric imidazole-Cu.

2. Polymeric Bimetallic Catalyst-Promoted In-Water Dehydrative Alkylation of Ammonia and Amines with Alcohols[2)]

A dehydrative alkylation with three kinds of Ir/B heterobimetallic polymeric catalysts in water is reported. The poly-

meric heterobimetallic catalysts were readily prepared by ionic convolution of a poly(catechol borate) and iridium complexes. The N-alkylation of ammonia and amines with alcohols, as alkylating agents, was carried out with a heterogeneous catalyst (1 mol% Ir) at 100 °C without the use of organic solvents under aerobic and aqueous conditions to afford the corresponding alkylated amines in high yield.

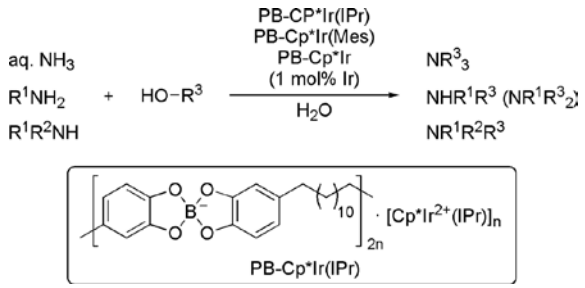

Figure 2. In-water dehydrative alkylation of ammonia and amines with alcohols using polymeric bimetallic catalysts.

3. Highly Efficient Iron(0) Nanoparticle-Catalyzed Hydrogenation in Water in Flow[3)]

Highly efficient catalytic hydrogenations are achieved by using amphiphilic polymer-stabilized Fe(0) nanoparticle (Fe NP) catalysts in ethanol or water in a flow reactor. Alkenes, alkynes, aromatic imines and aldehydes were hydrogenated nearly quantitatively in most cases. Aliphatic amines and aldehydes, ketone, ester, arene, nitro, and aryl halide functionalities are not affected, which provides an interesting chemoselectivity. The Fe NPs used in this system are stabilized and protected by an amphiphilic polymer resin, providing a unique system that combines long-term stability and high activity. The NPs were characterized by TEM of microtomed resin, which established that iron remains in the zero-valent form despite exposure to water and oxygen. The amphiphilic resin-supported Fe(0) nanoparticles in water and in flow provide a novel, robust, cheap and environmentally benign catalyst system for chemoselective hydrogenations.

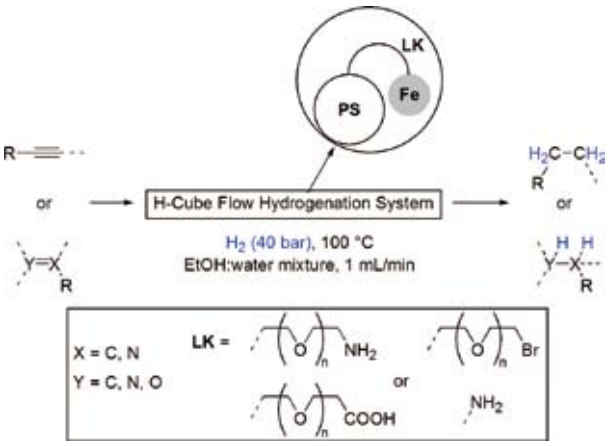

Figure 3. Schematic of hydrogenation reactions undertaken with polymer supported iron nanoparticles, under flow conditions (PS = polystyrene).

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Awards

OSAKO, Takao; Shionogi & Co. Ltd., Award in Synthetic Organic Chemistry, Japan.
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Development of Functional Metal Complexes for Artificial Photosynthesis

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ITOH, Takahiro	Graduate Student
FUKAHORI, Yukino	Graduate Student
FUKATSU, Arisa	Graduate Student
LIU, Ke	Graduate Student
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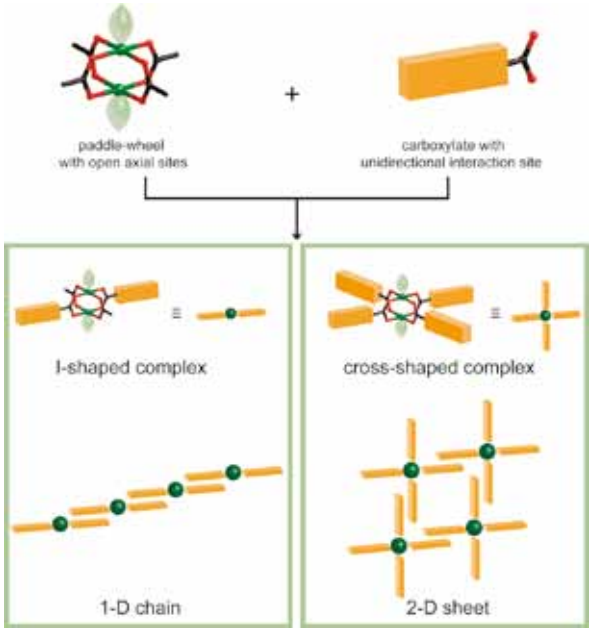
WAKABAYASHI, Kaori	Technical Fellow
SHIBATA, Akane	Technical Fellow
TANIWAKE, Mayuko	Secretary

1. Controlled Self-Assembly of Paddle-Wheel Dimers via Multipoint Arene-Perfluoroarene Interactions[1)]

Control over the self-assembling process of metal complexes is of key importance to construct supramolecular materials in which desirable bulk properties emerge as a consequence of specific intermolecular orientations. Paddle-wheel complexes which are described as M_2L_4 (M = metal ion, L = monoanionic bidentate ligand) attract much attention because of their highly symmetric (D_{4h}) structures suitable for the construction of continuous structures. Moreover, the existence of free coordination sites at the axial positions (open axial sites) and their Lewis acidity play a crucial role in catalysis or selective guest recognition. Therefore, the construction of continuous structures of paddle-wheel units with open axial sites is of significance to develop functional materials.

In this study we firstly report the self-assembly of Rh(II) and Cu(II) paddle-wheel complexes with open axial sites controlled via *unidirectional* interaction realized by multipoint arene-perfluoroarene interactions (Scheme 1). Two kinds of paddle-wheel dimers, the I-shaped complex (**1**), which has only two *unidirectional* interaction sites and is expected to one-dimensional chain assembly, and the cross-shaped complex (**2**), which has four *unidirectional* interaction sites in one molecule and is expected to have two-dimensional sheet-structure, are chosen to examine the molecular arrangements in the crystalline state (Scheme 1).



Scheme 1. Schematic illustration of I- and cross-shaped paddle-wheel complexes with open axial sites and their self-assembled structures.

In the crystal packing structures of **1** and **2**, intermolecular multipoint arene-perfluoroarene interactions are observed. **1** is arranged in one-dimensional chain due to face-to-face overlap of phenylene and perfluorophenyl rings. Interchain stacking are stabilized by π–π interactions between perfluorophenyl

rings to form the two-dimensional sheet structure. In the crystal packing structures of **2**, an infinite two-dimensional square-grid sheet structure is formed via multipoint arene-perfluoroarene interaction between ligands.

Moreover, porous structure was formed by the stacking of the two dimensional sheets via π–π interaction between ligands along the *a* axis and open axial sites are oriented to the channel.

The results presented in this contribution offer a new strategy to assemble paddle-wheel units of various metal ions with open axial sites at room temperature. This can be a powerful tool to construct supramolecular structures applied for heterogeneous catalytic system or sensor.

2. Photoinduced Hydrogen Evolution from Water by a Simple Platinum(II) Terpyridine Derivative[2)]

Hydrogen energy has been one of the most important targets as a renewable clean energy. Particularly, hydrogen generation based on water splitting by solar energy has attracted considerable attention for many years. Up to now, photochemical hydrogen production catalyzed by metal complexes has been extensively studied using a so-called three-component system consisting of tris(2,2'-bipyridine)ruthenium(II) ($Ru(bpy)_3^{2+}$) as a photosensitizer, methylviologen (N,N'-dimethyl-4,4'- bipyridinium, MV^{2+}) as an electron relay, and ethylenediaminetetraacetic acid disodium salt (EDTA) as a sacrificial electron donor, for which colloidal platinum was often employed as a H_2-evolving catalyst. Previously, we reported on the photochemical hydrogen production from water catalyzed by $[PtCl(terpy)]^+$ (terpy = 2,2':6',2"-terpyridine) in the presence of EDTA, where the platinum(II) complex is known to serve as a photosensitizer as well as a H_2-evolving catalyst. Thus this work was considered as the first example of 'bifunctional single-component photocatalysts' driving visible light-induced H_2 production from water (Figure 1).

In this work, we reported the novel platinum(II) complex (PV^{2+}) as the first example of 'trifunctional single-component photocatalysts' for visible light-induced H_2 production from water (Figure 1).

The H_2-evolving activity of PV^{2+} evaluated in the presence of EDTA showed that the stability of the PV^{2+} catalyst during the photolysis is much higher than that of the parent compound $[PtCl(tpy)]^+$. This is due to the improved electron-accepting ability of PV^{2+} compared with $[PtCl(tpy)]^+$. It is also found that the photolysis of PV^{2+} first generates a one-electron-reduced species (PV^+) as an initial photoproduct and this further undergoes a photoinduced process leading to H_2 generation from water. Therefore, this is the first demonstration of Z-scheme photosynthesis within the family of artificial molecular systems, although these two-step reductive quenching processes do not perfectly match with the oxidative ones in natural photosynthesis. The present study also demonstrates that the PHE activity can be dramatically enhanced by the presence of a Pt^{II}-based molecular co-catalyst, such as *cis*-$[PtCl_2(NH_3)_2]$.

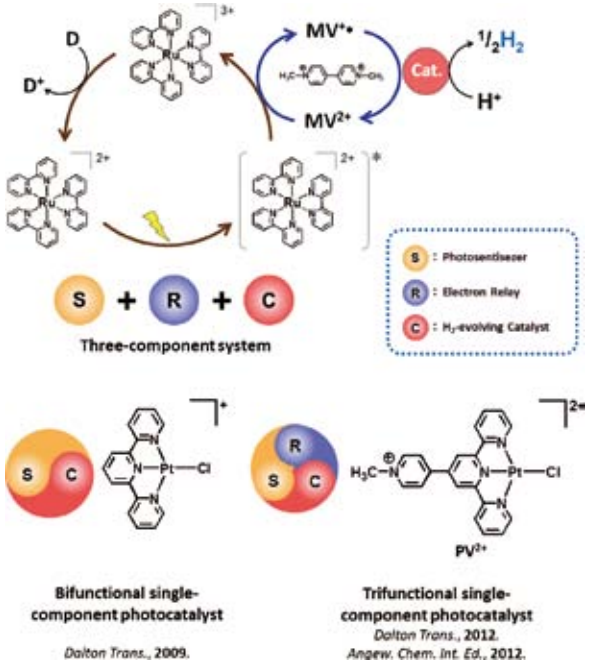


Figure 1. Three-component, bifunctional single-component and trifunctional single-component photocatalysts for hydrogen production from water.

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Awards

NAKAMURA, Go; FY2013 (the 4th) Sokendai President's Award (2013).
NAKAMURA, Go; Student Poster Award, 2nd CSJ Chemistry Festa (2012).
YOSHIDA, Masaki; Student Poster Award, 2nd CSJ Chemistry Festa (2012).
MURASE, Masakazu; Student Poster Award, 2nd CSJ Chemistry Festa (2012).
OKAMURA, Masaya; Student Poster Award, 2nd CSJ Chemistry Festa (2012).

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Visiting Professors

Visiting Professor
SASAI, Hiroaki *(from Osaka University)*

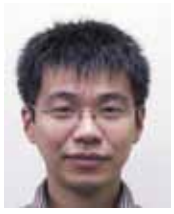
Design and Synthesis of Novel Enantioselective Catalysts

Synthesis of optically active complex molecules using catalytic amount of chiral compounds plays an important role in pharmaceutical industrial processes. Our group engages in the development of novel enantioselective catalyses which involve asymmetric domino reaction promoted by an acid-base type organocatalyst, oxidative coupling of 2-naphthol derivatives using dinuclear vanadium(V) catalysts, spiro bis(isoxazoline) ligand (SPRIX) accelerated transition metal catalyses, *etc.* Recently we have realized a highly enantioselective Pd(II)/Pd(IV) catalysis, formal [n+2] type cycloadditions of a ketimine with an alkyl 2,3-butadienoate, and an enantioselective Friedel-Crafts type reaction using chiral dinuclear vanadium catalyst.

Visiting Associate Professor
UEMURA, Takashi *(from Kyoto University)*

Highly Ordered Polymers by Host-Guest Cross-Polymerization

Chain alignment can deeply influence the ultimate macroscopic properties of a polymeric material; however, a general and versatile methodology for attaining highly ordered crystalline packing of polymer chains with high stability has not been reported so far. We have disclosed a strategy to produce polymeric materials that exhibit a crystalline arrangement promoted by “ordered cross-links.” Divinyl cross-linkers were embedded into a porous coordination polymer (PCP). During the polymerization of vinyl monomers in the channels, the divinyl species crosslink vinyl polymer chains that have formed within adjacent channels of the PCP. This bridging ensures that, on selective removal of the PCP, the polymer chains remained aligned even in the absence of stereoregularity.



Visiting Associate Professor
SUDO, Yuki *(from Nagoya University)*

Understanding and Controlling the Photoactive Proteins

Light absorbing photoactive proteins show characteristic colors originating from a species specific energy gap between their ground state and excited state, which leads to different characteristic absorption maxima (λ_{max}). Among these proteins and their cognate chromophores, the rhodopsins are known to show a large variation in their absorption spectra depending on the interaction between the apoprotein (opsin) and the retinal chromophore. Another striking characteristic of the rhodopsins is their wide range of seemingly dissimilar functions. Our research are roughly divided into three topics as follows; i) Discovery of novel microbial rhodopsins from the nature, ii) Identification of the biological function and investigation of the structure and the structural change during the photoreaction, and iii) Development of the rhodopsin-based optical tools for the life scientists.