RESEARCH ACTIVITIES

Division of Advanced Molecular Science

The division consists of two sections. In the first one, scientists of the first water are invited as "distinguished professors," and the environment, in which they can devote themselves to their own research, is provided. The research in this section should be the last word in the field of molecular science.

In the second section, we invite researchers in the universities performing unique researches in the field of molecular science as cross-appointment faculty members, and provide the research environment to enable research activity with advanced facilities in IMS.

Self-Assembling Molecular Systems Based on Coordination Chemistry

Division of Advanced Molecular Science

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Education

1980	B.S. Chiba University
1982	M.S. Chiba University
1987	Ph.D. Tokyo Institute of Technology

Professional Employment

1982	Researcher, Sagami Chemical Research Center
1988	Assistant Professor to Associate Professor, Chiba University
1997	Associate Professor, Institute for Molecular Science
1999	Professor, Nagoya University
2002	Professor, The University of Tokyo
2018	Distinguished Professor, Institute for Molecular Science
2019	Distinguished Professor, The University of Tokyo

Awards

1994	Progress Award in Synthetic Organic Chemistry, Japan
2000	Division Award of Chemical Society of Japan (Organic Chemistry)
2001	Tokyo Techno Forum 21 Gold Medal
2001	Japan IBM Award
2003	Nagoya Silver Medal
2004	Izatt-Christensen Award
2006	G. W. Wheland Award (Chicago University Lectureship Award)
2010	The Reona Esaki Award
2010	The JSCC Award
2011	3M Lectureship Award (University of British Columbia)
2012	Thomson Reuters Research Front Award 2012
2013	The Chemical Society of Japan (CSJ) Award
2013	Arthur C. Cope Scholar Award (ACS National Award)
2013	Merck-Karl Pfister Visiting Professorship (MIT Lectureship Award)
2014	ISNSCE 2014 Nanoprize
2014	Medal with Purple Ribbon
2014	Fred Basolo Medal (Northwestern University)
2018	Wolf Prize in Chemistry
2019	The Imperial Prize and the Japan Academy Prize
2020	The 73rd Chunichi Cultural Award
2020	Clarivate Citation Laureates (Chemistry)
2020	“Major Results” of Nanotechnology Platform, MEXT
2022	Le Grand Prix 2022 de la Fondation de la Maison de la Chimie
2023	Asahi Prize2023
2023	2022 Natta Award (Politecnico di Milano)
2024	Van't Hoff Award

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MITSUHASHI, Takaaki
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MASUDA, Michiko

Keywords Self-Assembly, Nano-Space, Coordination Chemistry

We are developing new self-assembled molecular systems using coordination chemistry and researching the application of the developed molecular systems. One representative application of the molecular system developed by us is the crystalline sponge (CS) method, which enables the rapid structure elucidation of small molecules. In this method, a porous crystal of a coordination network called CS, which was developed by us and can accommodate various kinds of small molecules, is used. Notably, we can know structures of the small molecules accommodated in the pore of the CS by X-ray crystallography, because the accommodated small molecules periodically aligned in the CS. The CS method has some advantages; i) only nanogram to microgram scale of analytes is required, ii) the absolute stereochemistry can be determined, iii) even oily substances can be analyzed by X-ray crystallography. Because of these fascinating features, the CS method attracts the interests of many people not only in academia but also in industry.

Selected Publications

- Y. Inokuma, S. Yoshioka, J. Ariyoshi, T. Arai, Y. Hitora, K. Takada, S. Matsunaga, K. Rissanen and M. Fujita, “X-Ray Analysis on the Nanogram to Microgram Scale Using Porous Complexes,” *Nature* **495**, 461–466 (2013).
- D. Fujita, Y. Ueda, S. Sato, N. Mizuno, T. Kumasaka and M. Fujita, “Self-Assembly of Tetravalent Goldberg Polyhedra from 144 Small Components,” *Nature* **540**, 563–566 (2016).

We now apply the CS method in the biological studies, especially studies on the natural product biosynthesis. Scientists in this field often obtain small molecules as products of natural product biosynthetic enzymes. However, it is often difficult to elucidate the complex structures of the enzyme products. We thus consider that the CS method, which enables the rapid structure elucidation of small molecules, is helpful, and can accelerate the studies in this field.

1. Analysis of a Terpene Synthase from a Giant Virus[1–3]

Giant viruses are unique existence. The size of giant viruses is as large as that of bacteria, whereas normal viruses are much smaller than bacteria. Because of the unique feature of the giant viruses, many researches on the giant viruses have been reported. However, it was unknown whether the giant viruses were producers of natural products or not.

We thus investigated whether the giant viruses have the natural product biosynthetic enzymes, and noticed that a gene coding a protein relatively similar to a terpene synthase exists in the genome of a giant virus Orpheovirus IHUMI-LCC2. The terpene synthase is a kind of natural product biosynthetic enzymes, and responsible for the biosynthesis of the terpenoids, one of the largest groups of the natural products.

We named the terpene synthase from Orpheovirus IHUMI-LCC2 as OILTS (Orpheovirus IHUMI-LCC2 terpene synthase), and analyze it. As a result, we revealed that OILTS can work as the terpene synthase, and found an interesting feature of OILTS (Figure 1). Terpene synthases normally require Mg^{2+} as metal cofactor, and OILTS also requires Mg^{2+} to produce an enzyme product **1**. However, when the metal cofactor was changed to Mn^{2+}, Co^{2+}, or Ni^{2+}, the profile of the enzyme products was changed, and not only **1** but also **2** was produced. In addition, we found that **1** was relatively unstable and easily converted to four degradation products (**3**–**6**) in weakly acidic condition. Notably, the structures of **1**–**6** were successfully analyzed by the CS method.

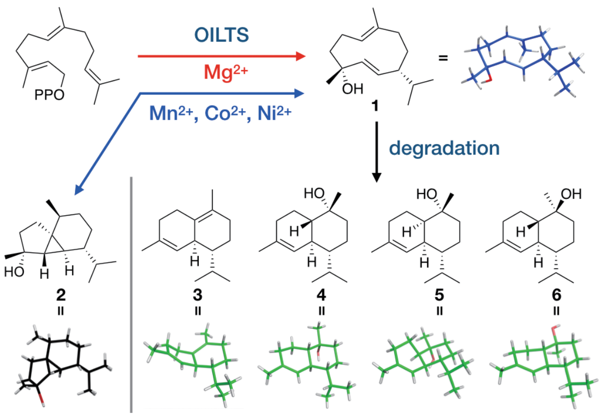

Figure 1. Reactions catalyzed by OILTS, a terpene synthase from a giant virus. Three dimensional structures of **1**–**6** were obtained by the CS method analysis.

We also performed the phylogenetic analysis, comparing terpene synthases from giant viruses and other organisms (Figure 2A). The terpene synthases from giant viruses are revealed to be phylogenetically separated from other terpene synthases. This result suggests the unique evolution of the terpene synthases from the giant viruses.

In addition, we solved the structure of OILTS by protein X-ray crystallography (Figure 2B). Whereas the structure of OILTS was similar to the structures of bacterial terpene synthases, OILTS was a little smaller than other terpene synthases whose structures have been known.

In summary, we reported the first functional analysis of the terpene synthase from the giant virus. We would like to analyze more enzymes from giant viruses in future, to more deeply understand the natural product biosynthesis in the giant viruses. In addition, the CS method could solve the structures of the enzyme products (**1** and **2**) and the degradation products (**3**–**6**) obtained in this study, showing the potential of the CS method to facilitate the studies in the field of natural product chemistry.

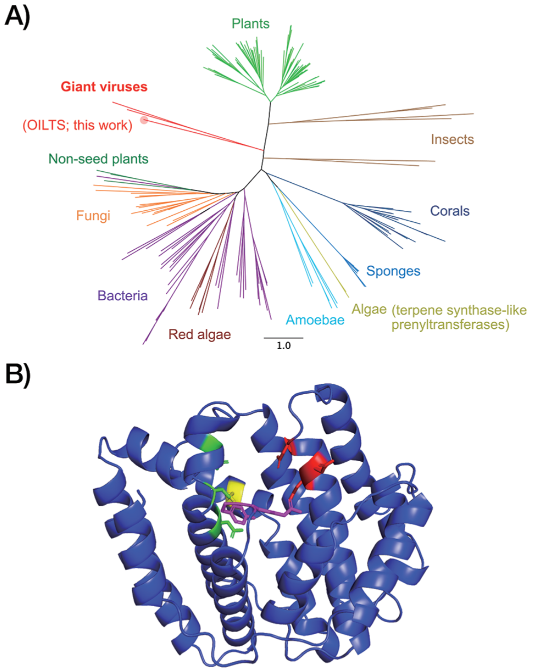

Figure 2. A) Phylogenetic analysis of the terpene synthases from giant viruses and other organisms. The figure is reprinted with permission from ref. 1). Copyright 2023 American Chemical Society. B) Structure of OILTS solved by protein X-ray crystallography.

References

1) Y. Jung, T. Mitsuhashi, S. Sato, M. Senda, T. Senda and M. Fujita, *J. Am. Chem. Soc.* **145**, 25966–25970 (2023).

2) Y. Jung, T. Mitsuhashi, T. Kikuchi and M. Fujita, *Chem. –Eur. J.* **30**, e202304317 (2024).

3) Y. Jung, T. Mitsuhashi, K. Kageyama, T. Kikuchi, S. Sato and M. Fujita, *Chem. –Eur. J.* **39**, e202400512 (2024).

Exploring Novel Physical Properties by Multi-Dimensional Spectroscopy

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Education

1988	B.S. Tohoku University
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1991	Ph.D. Tohoku University

Professional Employment

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1993	Research Associate, Kobe University
1993	Research Associate, Institute for Molecular Science
1998	Associate Professor, Kobe University
2002	Associate Professor, Institute for Molecular Science
2013	Professor, Osaka University
2020	Professor (Cross Appointment), Institute for Molecular Science

Awards

2001	Young Incentive Award, Japanese Society for Synchrotron Radiation Research
2008	The Commendation for Science and Technology by MEXT, Japan Science and Technology Prize (Research Field)
2008	Morita Memorial Prize

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Secretary
KURITA, Yoshiko

Keywords Condensed Matter, Electronic Structure, Synchrotron Radiation

Physical and chemical properties of solids, such as conductivity, magnetism, superconductivity, and chemical reactions, originate from microscopic electronic structure, lattice/molecular vibrations, and molecular movements based on quantum mechanics in materials and their interactions. By revealing the microscopic states and their evolution, we can learn about the origin of physical and chemical properties and hidden functionalities. Also, the microscopic information is helpful for the creation of novel functional properties. To visualize hidden microscopic information, we develop novel spectroscopic techniques using synchrotron radiation, high brilliant electron beams, and other so-called quantum beams. We have started a novel electron spectroscopy technique, Spin-Resolved resonant Electron-Energy-Loss Spectroscopy (SR-rEELS), with bulk-sensitive primary energies of 0.3–1.5 keV. At present, we combine it with a time- and angle-resolved technique, shown in Figure 1, to simultaneously observe both the changing electronic structure and collective excitations and the lattice and magnetic structure relaxation. Based on the obtained information on electronic structures, we aim to develop novel physical properties of new materials.

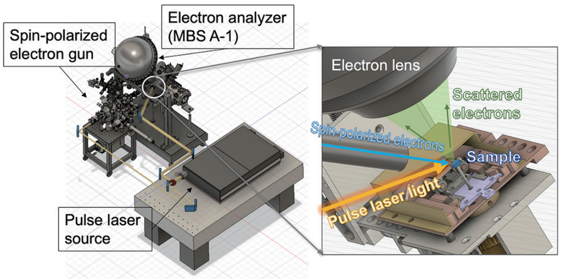

Figure 1. Time-, Spin-, and Angle-Resolved resonant Electron-Energy-Loss Spectroscopy (tSAR-rEELS) apparatus, which our group is now developing. The apparatus comprises a high-brilliant spin-polarized electron gun, a photoelectron spectrometer, and a femto-second pulse laser with an optical parametric amplifier.

Selected Publications

- T. Nakamura, H. Sugihara, Y. Chen, R. Yukawa, Y. Ohtsubo, K. Tanaka, M. Kitamura, H. Kumigashira and S. Kimura, "Two-Dimensional Heavy Fermion in Monoatomic-Layer Kondo Lattice $YbCu_2$," *Nat. Commun.* **14**, 7850 (7 pages) (2023).
- Y. Ohtsubo, T. Nakaya, T. Nakamura, P. Le Fèrve, F. Bertran, F. Iga and S. Kimura, "Breakdown of Bulk-Projected Isotropy in Surface Electronic States of Topological Kondo Insulator SmB_6(001)," *Nat. Commun.* **13**, 5600 (7 pages) (2022).
- S. Kimura, T. Kawabata, H. Matsumoto, Y. Ohta, A. Yoshizumi, Y. Yoshida, T. Yamashita, H. Watanabe, Y. Ohtsubo, N. Yamamoto and X. Jin, "Bulk-Sensitive Spin-Resolved Resonant Electron Energy-Loss Spectroscopy (SR-rEELS): Observation of Element- and Spin-Selective Bulk Plasmons," *Rev. Sci. Instrum.* **92**, 093103 (8 pages) (2021).
- K. Hagiwara, Y. Ohtsubo, M. Matsunami, S. Ideta, K. Tanaka, H. Miyazaki, J. E. Rault, P. Le Fèvre, F. Bertran, A. Taleb-Ibrahimi, R. Yukawa, M. Kobayashi, K. Horiba, H. Kumigashira, K. Sumida, T. Okuda, F. Iga and S. Kimura, "Surface Kondo Effect and Non-Trivial Metallic State of the Kondo Insulator YbB_{12}," *Nat. Commun.* **7**, 12690 (7 pages) (2016).

1. Two-Dimensional Heavy Fermion in Monoatomic-Layer Kondo Lattice $YbCu_2$[1)]

The Kondo effect between localized *f*-electrons and conductive carriers leads to exotic physical phenomena. Among them, heavy-fermion (HF) systems, in which massive effective carriers appear due to the Kondo effect, have fascinated many researchers. Dimensionality is also an important characteristic of the HF system, especially because it is strongly related to quantum criticality. However, the realization of the perfect two-dimensional (2D) HF materials is still a challenging topic. Here, we report the surface electronic structure of the monoatomic-layer Kondo lattice $YbCu_2$ on a Cu(111) surface observed by synchrotron-based angle-resolved photoemission spectroscopy. The 2D conducting band and the Yb 4*f* state are observed very close to the Fermi level. These bands are hybridized at low temperatures, forming the 2D HF state, with an evaluated coherence temperature of about 30 K. The effective mass of the 2D state is enhanced by a factor of 100 by the development of the HF state. Furthermore, clear evidence of the hybridization gap formation in the temperature dependence of the Kondo-resonance peak has been observed below the coherence temperature. Our study provides a new candidate as an ideal 2D HF material for understanding the Kondo effect at low dimensions.

2. Observation of Electronic Structure Modification in the Hidden Order Phase of CeCoSi[2)]

CeCoSi with no local inversion symmetric crystal structure (*P*4/*nmm*) exhibits a phase transition of unknown origin (Hidden Order: HO) at about 12 K (T_0) above the antiferromagnetic transition temperature (T_N = 9.4 K). The electronic structure change across T_0 was investigated with high-precision optical reflection spectroscopy. The optical spectrum changed from a typical metallic behavior above T_0 to a gap-like structure at around 15 meV below T_0. The gap-like structure was unchanged across T_N except for the narrowing of the Drude component of carriers due to the suppression of magnetic fluctuations. This result suggests a slight change from the typical metallic electronic structure above T_0 to that with an energy gap near the Fermi level in the HO phase. The change in electronic structure in the HO phase was concluded to be due to electron/valence instability.

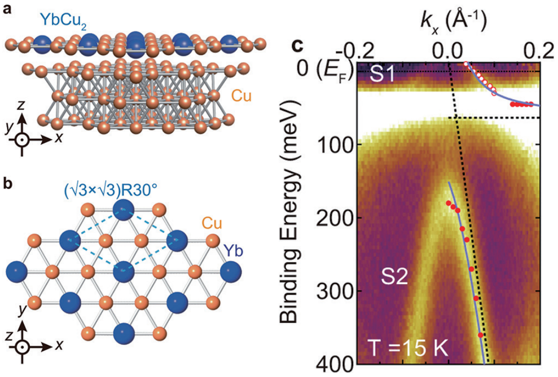

Figure 2. (a) A surface atomic structure of $YbCu_2$/Cu(111). (b) Top view of monoatomic-layer $YbCu_2$. The dashed line indicates the unit cell of $YbCu_2$. (c) ARPES image near the center of the surface Brillouin zone taken with circularly polarized 35-eV photons at 15 K. ARPES intensities are divided by the Fermi–Dirac distribution function convolved with the instrumental resolution. The filled and break lines indicate the simulated *c*-*f* hybridization band dispersions with the hybridization energy of 120 meV (solid lines) and 0 meV (dashed lines) by the periodic Anderson model. The open and filled circles indicate the peak positions from energy distribution curves (EDCs) and MDCs, respectively.

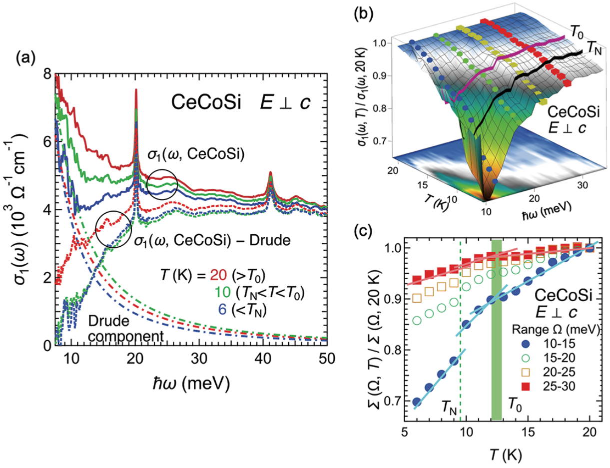

Figure 3. (a) Optical conductivity [$\sigma_1(\omega)$] spectra of CeCoSi at representative temperatures of 20 K (> T_0), 10 K ($T_N < T < T_0$), and 6 K (< T_N) (solid lines). Dot-dashed lines are Drude curves obtained from the values of electrical resistivity and the $\sigma_1(\omega)$ values at $\hbar\omega \sim 5$ meV. The interband components evaluated by subtracting the Drude components from the $\sigma_1(\omega)$ spectra are shown by dashed lines. (b) Temperature-dependent $\sigma_1(\omega)$ spectra normalized by that at 20 K of CeCoSi. The marks are the same as those in (c). (c) Temperature dependence of the spectral integrations of the range Ω of 10–15 meV (solid circle), 15–20 meV (open circle), 20–25 meV (open square), and 20–40 meV (solid square) in (b), which are representative regions of relative $\sigma_1(\omega)$ spectra in (b). The integrated intensity at each Ω is normalized by the value at T = 20 K. Solid lines are guides for the eye. T_0 and T_N are shown by vertical solid and dashed lines, respectively.

References

1) T. Nakamura, H. Sugihara, Y. Chen, R. Yukawa, Y. Ohtsubo, K. Tanaka, M. Kitamura, H. Kumigashira and S. Kimura, *Nat. Commun.* **14**, 7850 (7 pages) (2023).

2) S. Kimura, H. Watanabe, S. Tatsukawa and H. Tanida, *J. Phys. Soc. Jpn.* **92**, 043704 (5 pages) (2023).

Operando Molecular Science in Liquid–Solid Interfaces of Finite Thickness

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Education

1985	B.S. The University of Tokyo
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Professional Employment

1989	Assistant Professor, The University of Tokyo
1997	Associate Professor, The University of Tokyo
1999	Project Leader, Kanagawa Academy of Science and Technology
2004	Professor, Kobe University
2021	Professor (Cross Appointment), Institute for Molecular Science

Awards

2001	Young Scientist Award , Catalysis Society of Japan
2002	Nano-Probe Technology Award, Nano-Probe Technology Committee, JSPS
2003	Technical Award, Surface Science Society of Japan
2004	Yazaki Award, Yazaki Foundation
2019	Society Award, Japan Society of Vacuum and Surface Science

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Keywords Reactions at Interfaces, Catalysis, Energy-Related Chemistry

We are proud of our internationally compatible studies of liquid–solid interfacess; photocatalysts for artificial photosynthesis, lubricants for smooth tribology, and ice in antifreeze liquids. Characterization with advanced AFM, time-resolved ATR-IR spectroscopy, soft X-ray absorption and microelectrode-based amperometry are being developed. We look forward to collaborating with researchers in academic and industrial organizations to unravel the science behind material conversion and energy dissipation at liquid–solid interfaces.

A new era of molecular science will be revealed at liquid-solid interfaces of finite thickness (Figure 1). The molecular interface is the site of reaction where molecules of interest collide or interact with other molecules. We need to observe individual molecules there. On the other hand, the molecular interface is connected to the liquid and solid. Materials and energy come from/to the two condensed phases, since functional interfaces are always open to the environment. Operando characterization is absolutely necessary to study the interface in its working state.

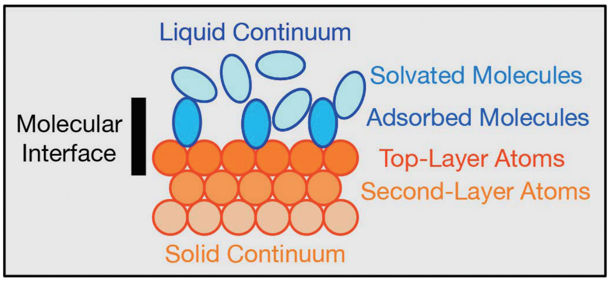

Figure 1. Liquid–Solid Interface of Finite Thickness.

Selected Publications

- Y.-H. Chew and H. Onishi, "Infrared Absorption of $Zn_{0.5}Cd_{0.5}S$ Photocatalyst Bandgap-Excited under an Aqueous Environment," *J. Phys. Chem. C* **128**, 4535–4543 (2024).
- M. Buessler, S. Maruyama, M. Zelenka, H. Onishi and E. H. G. Backus, "Unravelling the Interfacial Water Structure at the Photocatalyst Strontium Titanate by Sum Frequency Generation Spectroscopy," *Phys. Chem. Chem. Phys.* **25**, 31471–31480 (2023).
- Z. Fu, T. Hirai and H. Onishi, "Long-Life Electrons in Metal-Doped Alkali-Metal Tantalate Photocatalysts Excited under Water," *J. Phys. Chem. C* **125**, 26398–26405 (2021).
- T. Kosaka, Y. Teduka, T. Ogura, Y. Zhou, T. Hisatomi, H. Nishiyama, K. Domen, Y. Takahashi and H. Onishi, "Transient Kinetics of O_2 Evolution in Photocatalytic Water-Splitting Reaction," *ACS Catal.* **10**, 13159–13164 (2020).
- S. Xue, A. Sasahara and H. Onishi, "Atom-Scale Imaging of $TiO_2(110)$ Surface in Water by Frequency-Modulation Atomic Force Microscopy," *J. Chem. Phys.* **152**, 054703 (7 pages) (2020).
- S. Moriguchi, T. Tsujimoto, A. Sasahara, R. Kokawa and H. Onishi, "Nanometer-Scale Distribution of Lubricant Modifier on Iron Films: A Frequency-Modulation Atomic Force Microscopy Study Combined with Friction Test," *ACS Omega* **4**, 17593–17599 (2019).

1. Ice in Antifreeze Liquids Characterized by Atomic Force Microscopy

Ice in nature is surrounded by liquid most of the time, and therefore it is key to understand how ice and liquid interact. We could now directly observe the precise shape of ice at the interface between ice and liquid, by using antifreeze and a refrigerated microscope.

Ice provides the most important crystalline molecules in our lives. Because of its critical role in science and engineering, a number of experimental studies have been conducted in bulk ice and also on ice–vacuum interfaces. On the other hand, molecular-scale knowledge of the ice–water interface is still quite limited. The problem is that an ice–water interface fluctuates in space, even when the interface is held exactly at the freezing point.

Here we performed atomic force microscopy (AFM) imaging of ice surfaces immersed in antifreeze organic solvents. Ice in contact with liquid 1-octanol, for example, is stable at temperatures lower than 0 °C and higher than the freezing point of 1-octanol (−16 °C). We expect to have a good chance of mimicking some features, hopefully important features, of the ice–water interface, even though antifreeze solvents are not equivalent to water.

We did some preliminary research in 2022 to find a way to keep the ice–liquid interface below 0 °C on a Bruker AFM (Dimension XR Icon Nano Electrochemical Microscope). The best way we found was to cool the entire microscope in a soundproof box. A liquid nitrogen vessel was pressurized to provide a steady flow of cold nitrogen vapor, and the vapor flow was introduced into the box equipped with copper pipe works cooled with antifreeze fluid (Figure 2). The temperature inside the box was controlled to a desired temperature in a range from RT to −10 °C, stable enough to record topographic images of ice under antifreeze liquids.[1)]

Figure 2. The Bruker microscope at the Instrument Center devised for operation at −10 °C.

Ice films were prepared from ultrapure water. After eliminating residual gases by heating to 100 °C, 100 µl of the water was dropped onto a cleaved mica substrate on the microscope cooled in the nitrogen vapor environment. After the water was dropped on mica, the water spread on the surface. This was followed by a gradual phase change of the water from liquid to solid. After waiting about 30 minutes from the initial drop, the ice surface could be scanned using a cantilever in amplitude modulation mode. The images obtained showed that the ice surface is not atomically flat, but rather has a rough structure such as nanometer-scale frost pillars.

The imaging liquid, 1-octanol for the results shown in Figure 3, was then applied to the ice film. The thickness of the octanol layer was about 1 mm. Panel (a) in Figure 3 shows a topographic image obtained at the ice–octanol interface. Two flat terraces were separated by a straight step. A cross section was constructed along the light blue line and shown in panel (b). The height of the step was 0.10 nm, as shown in the cross section. The change of the ice surface in octanol liquid from the rough surface in N_2 vapor would be caused by the partial dissolution of the surface in octanol. A limited concentration of water dissolves in octanol (the solubility of water in 1-octanol is 2 mol l^{-1} at 25 °C in ref. 2).

Considering the various roles of ice, this study concludes that it opens up new possibilities for the study of surface and interfacial phenomena associated with ice in liquids. In addition, the microscope devised in this study is functional for imaging ice and also other materials cooled at −10 °C. It is available for use at the Instrument Center through the Advanced Research Infrastructure for Materials and Nanotechnology (ARIM).

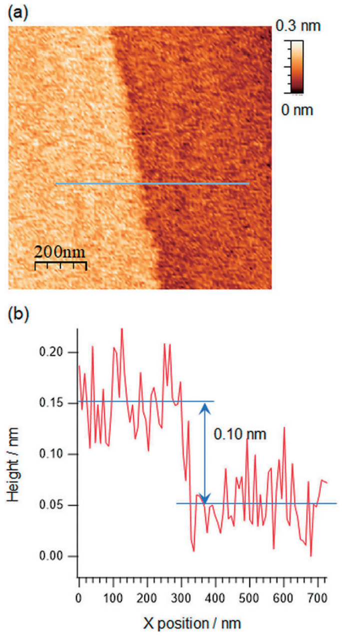

Figure 3. A topographic image of an ice film in 1-octanol liquid at −7 °C. A cross section determined along the line in panel (a) is shown in panel (b).

References

1) R. Yanagisawa, T. Ueda, K. Nakamoto, Z. Lu, H. Onishi and T. Minato, *J. Chem. Phys.* **161**, 024702 (2024).

2) I. Matsuoka, T. Naito and H. Yamada, *Bunseki-Kagaku*, **51**, 759 (2002).

Engineering of PET Hydrolase for Plastic Recycling and Environmental Remediation, and Engineering of Plastic Binding Domains for Detection and Quantification of Particles

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Education

2009	B.S. The University of Tokyo
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Professional Employment

1995	Postdoctoral Fellow, The University of Tokyo
2015	Assistant Professor, Okazaki Institute for Integrative Bioscience
2018	Assistant Professor, Institute for Molecular Science
2020	Tenure-track Associate Professor, Shizuoka University
2022	Associate Professor (Cross Appointment), Institute for Molecular Science

Awards

2015	HAMMON President Choice
2019	ATI Research Encouragement Award
2019	Early Career Award in Biophysics
2021	8th Biophysics and Physicobiology Editors' Choice Award

Member
Secretary
NOMURA, Junko
KAWAGUCHI, Ritsuko

Keywords Solid-Liquid Interfacial Reaction, Protein-Engineering, Single-Molecule Observation

Plastic is an indispensable material in our daily lives. It is used in a wide variety of products because it is low-cost, mass-producible, and easy to mold and process. However, due to its convenience, the amounts of plastic used and disposed are increasing every year, raising concerns about its environmental impact and sustainability. Therefore, there is a need to develop efficient and environmentally friendly recycling methods, as well as simple and rapid methods to detect plastics that have leaked into the environment.

We are attempting to obtain more active mutants by exhaustively mutating amino acid residues on the enzyme surface and screening their activity using a dispensing robot. We are also attempting to create plastic adsorption domains by modifying the amino acids constituting the adsorption surface of the carbohydrate binding domain and using a phage display method.

These studies will promote the recycling of used plastics by enzymatic degradation and promote more sustainable use of plastics. In addition, by creating a protein that detects and stains plastics, we will contribute to the protection of the natural environment by monitoring the small plastics runoff into the environment (Figure 1).

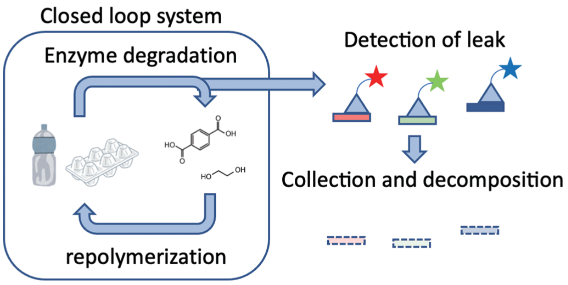

Figure 1. Scheme of plastic recycling and detection system.

Selected Publications

- A. Nakamura, N. Kobayashi, N. Koga and R. Iino, "Positive Charge Introduction on the Surface of Thermostabilized PET Hydrolase Facilitates PET Binding and Degradation," *ACS Catal.* **11**, 8550–8564 (2021).
- A. Nakamura, D. Ishiwata, A. Visootsat, T. Uchiyama, K. Mizutani, S. Kaneko, T. Murata, K. Igarashi and R. Iino, "Domain Architecture Divergence Leads to Functional Divergence in Binding and Catalytic Domains of Bacterial and Fungal Cellobiohydrolases," *J. Biol. Chem.* **295**, 14606–14617 (2020).
- 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. Ishida, K. Kusaka, T. Yamada, S. Fushinobu, I. Tanaka, S. Kaneko, K. Ohta, H. Tanaka, K. Inaka, Y. Higuchi, N. Niimura, M. Samejima and K. Igarashi, "'Newton's Cradle' Proton Relay with Amide-Imidic Acid Tautomerization in Inverting Cellulase Visualized by Neutron Crystallography," *Sci. Adv.* **1**, e1500263 (2015).

1. High Throughput Screening Method of PET Hydrolase Activity

Mutations were introduced into PET hydrolytic enzyme PET2 derived from a metagenomic library to create a mutant with improved thermostability and PET degrading activity.$^{1)}$ In this study, we tried to further improve the activity of PET2 with a combination of saturation mutations. For a combination of saturation mutation at two positions, we need to test 399 mutants. Since it is difficult to purify and measure the activity of each mutant individually, a screening method was created to evaluate the activity without purification of the enzyme and without the use of HPLC (Figure 2).$^{1)}$

We prepared PET particulate suspensions by dissolving PET in Hexafluoro-2-propanol and regenerating it in water. Since the concentration of the suspension correlates with the intensity of light scattering at 595 nm, we attempted to measure PET degradation activity using turbidity as an indicator.

The correlation between the reduction of turbidity and concentration of soluble product was analyzed by HPLC. At the first stage of degradation, the two parameters showed linear correlation. Therefore, we created a library in which saturation mutations were introduced at two substrate recognition sites of the PET2 mutant and performed selection. colonies were cultured in 96-well plates, and after one night of enzyme induction, the culture medium was collected and mixed with the PET suspension. The rate of decrease in turbidity was analyzed in the culture medium of 720 colonies, and 13 enzymes were found to be more than twice as active as the PET2 mutant that used as the template.

To further verify thermostability, activity measurements were performed using heat-treated enzymes. The use of 384 wells for activity measurement enabled analysis of many enzymes at one time and efficient screening. The best H229T-F233M mutant showed 3.4 times higher degradation rate against PET film than the template enzyme at the initial time. The molecular dynamics simulation implied that F233M mutation make the space for making alpha helix and H229T mutation resolved steric hindrance with Trp199. These mutations were speculated to change the angle of the Trp199 side chain of PET2 to an angle similar to that of the Trp185 of IsPETase, making it suitable for PET binding to the active center. Screening of activity using PET suspensions is compatible with robotic automation and is expected to be useful for validating computationally predicted mutations.

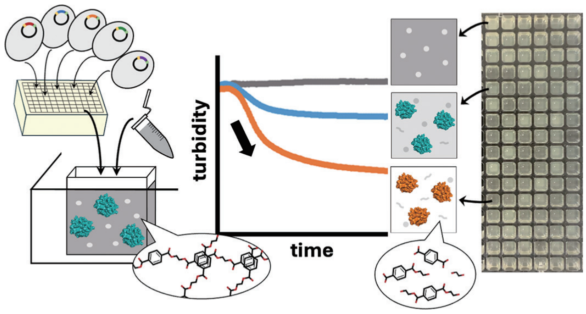

Figure 2. Scheme of PET degrading enzyme activity measurement using turbidity as an indicator.

2. Artificial Evolution of Carbohydrate Binding Domain to PET Binding Domain

To develop a method for detecting plastics using plastic binding proteins, we first developed a protein targeting PET. The template enzyme was a thermostable domain that binds on chitin, which has hydrophobic planes similar to PET.

Saturation mutations were introduced at four amino acid residues oriented on the binding surface and involved in chitin recognition. Since the number of candidates of mutants is about 160,000 and it is difficult to verify them one by one, we created a library of phage vectors with binding domain mutant genes inserted into, which were then used to create a library of M13 phage presenting adsorption domain mutants on their surface. The M13 phage library was incubated with PET film, and unbound phage were washed away. The phage bound on PET were recovered by trypsin digestion and re-amplified by infection with *E. coli*. The concentrated library of phage was further screened with more strong condition of washing.

The 3 cycles of screening of the first-generation phage library resulted in the 29 colonies of PET binding domain candidates. The 27 colonies were the PfCBD-K270H-N272P-E279V-D281G mutant (PfCBD-4M) and showed higher binding affinity to PET than WT. But the binding affinities to chitin and cellulose were remained. The other two mutant were rejected because the RFP fusion proteins were insoluble.

To improve the binding specificity, three mutations were further added to PfCBD-4M. After the 3 cycles of screening, the 425 colonies were obtained. The colonies were mixed, and the CBD genes were amplified by PCR. The CBD genes were ligated with RFP gene and the fusion proteins were produced in small scale. The solubilities of proteins were analyzed based on the RFP fluorescence signal in the solution to remove the insoluble proteins. The gens of the top 10 proteins were sequenced, and the 6 candidates were obtained. The binding amount of 2nd generation of 6 mutants, 4M and PfCBD-WT against chitin, cellulose and low and high concentrations of PET were measured. The all of 6 mutants showed almost no binding to chitin and cellulose but higher binding amount than 4M and WT (Figure 3). Therefore, we succeeded to change the binding specificity of the domain from chitin/cellulose to PET. Now we tried to make a fusion enzyme with PET hydrolase to increase the affinity to PET.

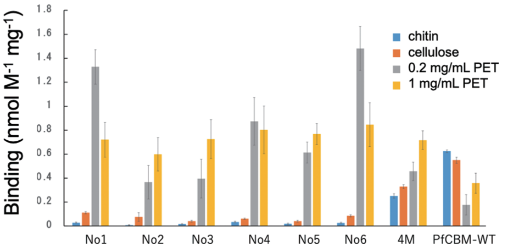

Figure 3. Binding measurement of 2nd generation of mutants against chitin, cellulose and PET.

Reference

1) Y. Ogura, Y. Hashino and A Nakamura, *ACS Omega* (2024), in press.

Molecular Science of Bio-Metal Dynamics: Understanding and Regulation of the Strategies of Metal Utilization in Living Cells

Division of Advanced Molecular Science
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Education

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Professional Employment

2003	Junior Research Associate, RIKEN
2006	IMS Fellow, Institute for Molecular Science
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2010	Research Assistant Professor, Okazaki Institute for Integrative Bioscience
2013	Assistant Professor, University of Hyogo
2022	Associate Professor, Nagasaki University
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Awards

2010	Yamamura Fellow, Fumi Yamamura Memorial Foundation for Female Natural Scientists, Chuo Mitsui Trust and Banking
2012	Shiseido Female Researcher Science Grant, SHISEIDO Company, Limited
2018	Excellent Research Award, The Japanese BioIron Society

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Keywords Iron Dynamics in Human Cells, Integrated Bio-Metal Sciences, Biological Inorganic Chemistry

Metals play important roles in sustaining life. Cells are mainly composed of water, proteins, and lipids, but they also contain small amounts of metals that help maintain health by being acquired from food. Those metals are used as active centers of enzymes that carry out functions essential to sustaining life, *e.g.* transport and storage of oxygen, energy production, gene synthesis, has been known for many years. However, the series of molecular mechanisms underlying metal dynamics in the body (absorption, sensing, transport, storage, and excretion of metals) (Figure1) and selectivity for individual metals remain unknown. Our group focuses on "iron," which is the most important metal among the essential metals for sustaining life of living things, focusing on various proteins that play a role in the selective absorption, sensing, and intracellular transport of iron in food. We are not only elucidating the structure of related proteins but also exploring their relationship with their functions in human cells.

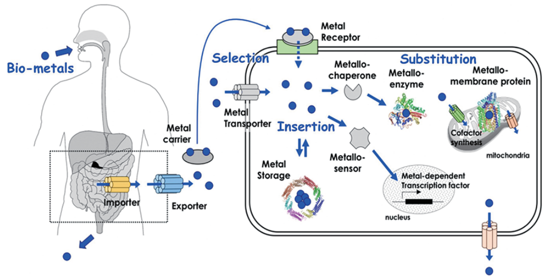

Figure 1. Our aim is to understand the uptake, trafficking, and regulation of "bio-metals" through the relay of protein–protein interactions.

Selected Publications

- M. Ganasen, H. Togashi, H. Takeda, H. Asakura, T. Tosha, K. Yamashita, K. Hirata, Y. Nariai, T. Urano, X. Yuan, I. Hamza, A. G. Mauk, Y. Shiro, H. Sugimoto and H. Sawai, "Structural Basis for Promotion of Duodenal Iron Absorption by Enteric Ferric Reductase with Ascorbate," *Commun. Biol.* **1**, 120 (2018). DOI: 10.1038/s42003-018-0121-8
- G. S. A. Wright, A. Saeki, T. Hikima, Y. Nishizono, T. Hisano, M. Kamaya, K. Nukina, H. Nishitani, H. Nakamura, M. Yamamoto, S. V. Antonyuk, S. Samar Hasnain, Y. Shiro and H. Sawai, "Architecture of the Complete Oxygen-Sensing FixL-FixJ Two-Component Signal Transduction System," *Sci. Signaling* **11**, eaaq0825 (2018). DOI: 10.1126/scisignal.aaq0825
- H. Sawai and K. Ishimori, "Integrated Bio-Metal Science: New Frontiers of Bio-Metal Science Opened with Cutting-Edge Techniques," *Biophys. Physicobiol.* **17**, 94–97 (2020).
- M. Nishinaga, H. Sugimoto, Y. Nishitani, S. Nagai, S. Nagatoishi, N. Muraki, T. Tosha, K. Tsumoto, S. Aono, Y. Shiro and H. Sawai, "Heme Controls the Structural Rearrangement of Its Sensor Protein Mediating the Hemolytic Bacterial Survival," *Commun. Biol.* **4**, 467 (2021). DOI: 10.1038/s42003-021-01987-5

1. Molecular Mechanism of Intracellular Fe^{2+} Regulation by the Iron-Delivery Chaperone PCBP

Iron is an essential “nutrient” for supporting life. In humans, iron nutrients (*e.g.*, heme iron and iron ions) ingested from food are absorbed from duodenum (upper small intestine) and after intracellular sensing, transport and storage, are eventually distributed as serum iron to organs and muscles throughout the body.$^{1)}$ Iron ions can undergo redox, and although Fe^{2+} is water-soluble and easily accessible, it readily reacts with O_2 to produce reactive oxygen species, which are cytotoxic. On the other hand, Fe^{3+} is not a source of reactive oxygen species, but is less water-soluble. Therefore, the body uses a variety of proteins to avoid Fe^{2+} toxicity and strictly regulates iron homeostasis. Failure of this regulatory system to sustain iron homeostasis can lead to iron overload or deficiency. To help introduce the protein framework that dictates human iron dynamics, we focused on dietary iron dynamics via the intracellular iron-delivery chaperone, PCBP (poly rC binding protein)$^{2)}$ (Figure 2).

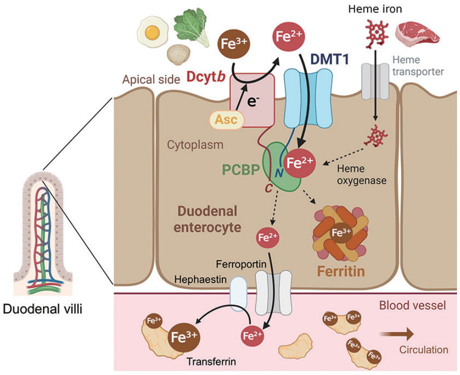

Figure 2. Dietary iron absorption in duodenum enterocyte. The first step of duodenal iron absorption is the transport of Fe^{2+} by DMT1 (divalent metal transporter 1) at the apical plasma membrane of duodenal enterocytes. Reduction of the predominant Fe^{3+} by Dcyt*b* (duodenal cytochrome *b*$_{561}$) using cytoplasmic electron doner, Asc (ascorbate), is necessary for Fe^{2+} transport by DMT1.

PCBP has been proposed to play a role in supplying iron ions to the iron storage protein ferritin,$^{3)}$ but the molecular mechanism of iron trafficking from PCBP to ferritin remains elusive. To unveil these mechanisms, we prepared recombinant human PCBP and investigated its properties. Iron binding assays using apo-PCBP revealed that one molecule of apo-PCBP can bind one Fe^{2+}, while Fe^{3+} does not bind, clearly showing PCBP can only bind Fe^{2+}. The complexation of Fe^{2+}-bound PCBP and ferritin H- or L-chain were investigated by SEC-SAXS (size-exclusion chromatography integrated small-angle X-ray scattering) and mass photometry (Figures 3 and 4). Based on these results, we proposed that Fe^{2+}-bound PCBP preferentially binds the ferritin H-chain compared to the L-chain. Since the ferritin H-chain acts as a ferroxidase, converting Fe^{2+} to Fe^{3+} for the safe storage of iron in ferritin, PCBP thus mediates safe and effective iron trafficking to ferritin in the cytoplasm.

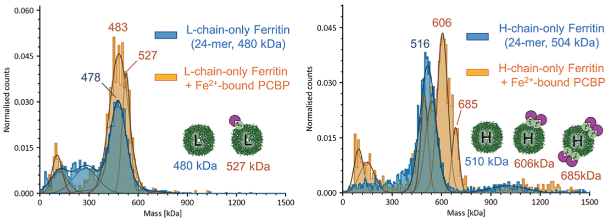

Figure 3. Mass photometry of ferritin and its mixture with Fe^{2+}-bound PCBP.

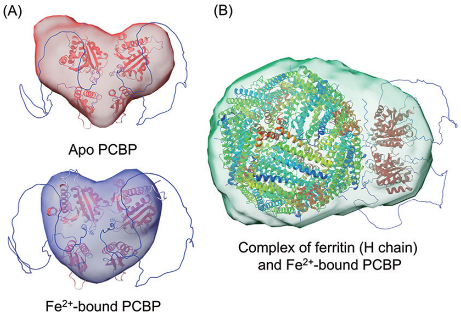

Figure 4. Space-filling models and pseudoatomic models of PCBPs (A) and its complex with H-chain only ferritin (B). Space-filling models were created based on SAXS data. Pseudoatomic models of PCBP in apo and Fe^{2+}-bound forms were predicted by AlphaFold3.

2. Cooperative Fe^{2+} Transfer between Dcyt*b* and DMT1 in the Plasma Membrane of Intestinal Living Cells

Because Fe^{2+} produced by the enzymatic reaction of Dcyt*b* is a source of reactive oxygen species, for safe and efficient trafficking of Fe^{2+}, Dcyt*b* must be close to or form a complex with DMT1 and transfer the produced Fe^{2+} to DMT1 as quickly as possible. To demonstrate that, we confirmed the localization of Dcyt*b* and DMT1 by immunofluorescence observation and proximity ligation assay between Dcyt*b* and DMT1 using Caco2-kh cells,$^{3)}$ a human intestinal model cell. We further found that the *C*-terminal region of Dcyt*b* also binds to PCBP by biochemical analysis of complex formation using wild-type and deletion mutant of the *C*-terminal loop of Dcyt*b*. These data imply that not only the interaction between Dcyt*b* and DMT1 in the apical membrane of the cells, but also PCBP interacts with these membrane proteins to ensure safe and efficient Fe^{2+} transport. Therefore, it is possible that Dcyt*b*-DMT1-PCBP forms a ternary complex to play a cooperative function for the Fe^{2+} transport in the cell. Further studies at molecular and cellular levels will provide new insights into the mechanisms of ternary complex formation for dietary iron absorption in human duodenum.

References

1) N. C. Andrews, *Nat. Rev. Genet.* **1**, 208–217 (2000).
2) I. Yanatori *et al.*, *Biochim. Biophys. Acta, Gen. Subj.* **1864**, 129685 (2020).
3) H. Shi *et al.*, *Science* **320**, 1207–1210 (2008).
4) H. Fujishiro *et al.*, *J. Toxicol. Sci.* **42**, 559–567 (2017).

Integration of Quantum Chemistry and Machine Learning for a Deeper Understanding and Rational Design of Functional Materials

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Education

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Professional Employment

2009	Research Associate (fixed term/research incentive), Keio University
2011	Fukui fellow, Kyoto University
2015	Assistant Professor, Kindai university
2015	Japan Science and Technology Agency (JST) PRESTO Researcher
2017	Associate Professor, Nara Institute of Science and Technology
2020	Associate Professor, Keio University
2024	Associate Professor (Cross Appointment), Institute for Molecular Science

Awards

2017	11th PCCP Prize
2019	12th Young Scientist Award of the Japan Society for Molecular Science
2021	The Chemical Society of Japan Award for Outstanding Young Women Chemists for 2021
2021	MEXT National Institute of Science and Technology Policy (NISTEP), NISTEP Selection (The Researchers with Nice Step) 2021

Member
Secretary
MASUDA, Michiko

Keywords Materials Informatics, Lanthanide, Computational Chemistry

We develop computational methods, apply them to elucidate mechanisms, and design materials using data science techniques such as machine learning (ML). Currently, our research focuses on the following subjects:

(1) Lanthanide Photofunctional Materials

Lanthanide (Ln) luminescence, originating from 4f–4f transitions, has been applied in various optical materials. While the 4f–4f emission wavelengths of Ln complexes are almost independent of the surroundings, the emission intensities are heavily influenced, making the design of appropriate ligand crucial. To get deeper insights into Ln luminescence, we proposed the energy shift method, which has contributed to the understanding and design of various Ln materials and has also been applied to non-Ln systems. However, it could not handle 4f–5d and charge transfer excited states, which are particularly important for cerium and europium complexes. To address this limitation, we are developing an updated method. In addition, we have constructed a database of cerium complexes containing 1.7k geometries and electronic properties. We plan to make this database public and further develop it into a platform for optical function prediction *via* ML.

(2) Transition Metal Catalysts

Transition metal (TM) complexes play a crucial role in organic synthesis, catalyzing a wide variety of chemical reactions. The catalytic abilities of TM complexes can be finely tuned by manipulating ligand-induced electronic and steric effects, which are key to controlling the reactivity and selectivity of specific reactions. In recent years, however, chemical products are being produced in smaller quantities and with greater variety, reducing the time available to study the synthesis conditions for each product. To address this challenge, we are constructing a database of TM complexes with various organophosphorus ligands, ranging from monodentate to multidentate skeletons. For descriptors applicable to different skeletons, we propose using the reaction energies of elementary reactions, such as oxidative addition and transmetallation. Our goal is to accelerate catalyst design by developing an ML model where our descriptors serve as explanatory variables and experimental catalytic activity results are used as objective variables.

Selected Publications

- S. Gocho, H. Nakamura, Q. Gao, T. Kobayashi, T. Inagaki and *M. Hatanaka, "Excited State Calculations Using Variational Quantum Eigensolver with Spin-Restricted Ansätze and Automatically-Adjusted Constraints," *npj Comput. Mater.* **9**, 13 (2023).
- X.-F. Wei, T. Wakaki, T. Itoh, H.-L. Li, T. Yoshimura, A. Miyazaki, K. Oisaki, *M. Hatanaka, *Y. Shimizu and *M. Kanai, "Catalytic Regio- and Enantioselective Proton Migration from Skipped Enynes to Allenes," *Chem* **5**, 585 (2019).
- *M. Hatanaka, Y. Hirai, Y. Kitagawa, T. Nakanishi, *Y. Hasegawa and *K. Morokuma, "Organic Linkers Control the Thermosensitivity of the Emission Intensities from Tb(III) and Eu(III) in a Chameleon Polymer," *Chem. Sci.* **8**, 423 (2017).

1. Geometry Exploration Using Bayesian Optimization

Conventional molecular geometry searches on a potential energy surface utilize energies and energy gradients from quantum chemical calculations. However, replacing energy calculations with noisy quantum computer measurements generates errors in the energies, which makes geometry optimization using the energy gradient difficult. One gradient-free optimization method that can potentially solve this problem is Bayesian optimization. To use Bayesian optimization in geometry search, a suitable acquisition function must be defined. In this study, we propose a strategy for geometry searches using Bayesian optimization and examine the appropriate acquisition functions to explore the global minimum (GM) and the most stable conical intersection (CI). The acquisition functions for the GM and CI searches were defined as the probability improvements of the energy with opposite sign and the cost function C in eq (1), respectively.

$$C(\boldsymbol{x}) = -\left\{\frac{E_{S0}(\boldsymbol{x}) + E_{S1}(\boldsymbol{x})}{2} + \frac{\{E_{S0}(\boldsymbol{x}) - E_{S1}(\boldsymbol{x})\}^2}{\alpha}\right\} \cdots (1)$$

Here, $E_{Sn}(x)$ and α are the electronic energy of the n-the singlet state at the geometry x and the hyperparameter, respectively. We applied our strategy to two molecules, formaldehyde and ethylene, and found the GM and the most stable CI geometries with high accuracy for both molecules.

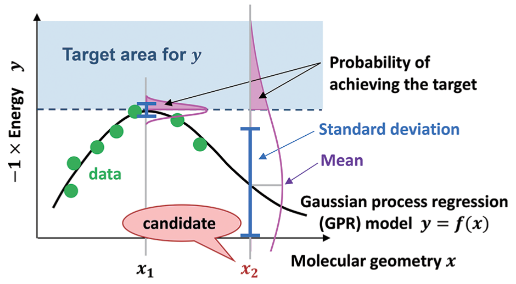

Figure 1. Schematic illustration of global minimum search using Bayesian optimization.

2. Copolymer Descriptor Database and Its Application to Machine Learning

Polymer informatics, which applies data-driven science to polymers, has attracted significant research interest. However, developing suitable descriptors for polymers, particularly copolymers, to enable machine learning models with limited data sets remains a challenge. To address this, we computed a range of parameters, including reaction energies and activation barriers of elementary reactions in the early stage of radical polymerization, for 2,500 radical–monomer pairs derived from 50 commercially available monomers. We then created an open-access database called the "Copolymer Descriptor Database (CopDDB)." Additionally, we constructed machine learning models using these descriptors as explanatory variables and various physical properties—such as the reactivity ratios, monomer conversion rates, monomer composition ratios, and molecular weights—as objective variables and achieved high predictive accuracies. We also conducted process optimization for the copolymerization of untested monomers. Typical Bayesian optimization requires an initial training set of target molecules, which entails significant experimental cost. However, our approach overcomes this challenge by using data from previously tested molecules that do not contain the target molecules for the initial training dataset. These models achieved high predictive accuracy, demonstrating the potential of our descriptors to advance polymer informatics.

3. Mechanism of Formation of Thermally Stable Cyclotrisilanyl Radicals

A halogen-substituted cyclic disilene compound, bromocyclotrisilene, $Si_3Br(Eind)_3$ (**3a**) bearing the fused-ring bulky Eind groups, was synthesized as an extraordinarily air-stable compound by our collaborators (Prof. Matsuo group at Kindai university). Even though **3a** is quite air-stable both in solutions and in the solid state, its Si–Br bond is reactive under reducing conditions. The further treatment of **3a** with Li metal leads to the formation of room-temperature thermally-stable silicon homologues of the cyclopropyl radical, *i.e.*, the cyclotrisilanyl radicals (**6a**), via intramolecular C–H bond activation in a transient silicon homologue of the cyclopropenyl radical, *i.e.*, the cyclotrisilenyl radical, $[Si_3(Eind)_3]^\bullet$ (**5a**). To elucidate the formation mechanism of **6a** from **5a**, we performed the reaction path search using the artificial force induced reaction (AFIR) method at the ONIOM (B3LYP-D3/PM6-D3) level. The reaction started from the approach of silyl radical center of **5a** to one of the H atoms in the Eind group, which afforded carbon-centered radical specie. The carbon-centered radical attacked Si_3, which induced the formation of C–Si and formed a ring-opened radical species. Then silylene center approaches the Si–H bond, which induces the formation of the Si–Si and Si–H bonds and results in the formation of the stable product **6a**. To the best of our knowledge, derivatives of the cyclopropyl radical have not yet been isolated and remained elusive. The cyclotrisilanyl radicals **6a** are the first room-temperature thermally stable homologues of the cyclopropyl radical, whose structural features and electronic properties have been experimentally and theoretically investigated.

References

1) R. Ohno, K. Ota, N. Nishimura, K. Taniguchi, S. Kurokawa, T. Wakabayashi, M. Hatanaka, A. Rosas-Sánchez, D. Hashizume and T. Matsuo, *J. Am. Chem. Soc.* **146**, 24911–24924 (2024).
2) K. Muto, M. Hatanaka, F. Kakiuchi and T. Kochi, *Organometallics* **43**, 1528–1535 (2024).
3) S. Kanno, H. Nakamura, T. Kobayashi, S. Gocho, M. Hatanaka, N. Yamamoto and Q. Gao, *npj Quantum Inf.* **10**, 56 (2024).
4) A. Wakiuchi, S. Jasial, S. Asano, R. Hashizume, M. Hatanaka, Y. Ohnishi, T. Matsubara, H. Ajiro, T. Sugawara, M. Fujii and T. Miyao, *MRS Commun.* **14**, 439–444 (2024).
5) K. Muto, M. Hatanaka, F. Kakiuchi and T. Kochi, *J. Org. Chem.* **89**, 4712–4721 (2024).
6) K. Terasaka, T. Kamoshida, T. Ichikawa, T. Yokoyama, M. Shibuta, M. Hatanaka and A. Nakajima, *J. Am. Chem. Soc.* **146**, 9605–9613 (2024).
7) T. Inoue, M. Hatanaka and A. Nakajima, *J. Am. Chem. Soc.* **145**, 23088–23097 (2023).