

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



FUJITA, Makoto
Distinguished Professor
[mfujita@ims.ac.jp]

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 Prize

Member

IMS Research Assistant Professor
MITSUHASHI, Takaaki
Secretary
MASUDA, Michiko

Keywords Self-Assembly, Nano-Space, Coordination Chemistry

Our research is based on the design of new self-assembled molecular systems using coordination chemistry. We not only create the new self-assembled molecular systems but also research the application of them.

One example is a molecular system called crystalline sponge (CS). The CS is a porous crystal of a coordination network, into which various kinds of small molecules could be introduced. 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. Thus, the CS can be utilized for

the structure analysis, and this technique is called the CS method. This 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.

Besides structure analysis by the CS method, we also use the self-assembled molecular systems for various purpose, and try opening up new research field.

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).

Here, we show our recent progress. In the first case, we used the self-assembled molecular system for chemical reaction. In the second case, we applied the self-assembled molecular system to protein science.

1. Tetradehydro-Diels–Alder Reactions of Flexible Arylalkynes via Folding Inside the Self-Assembled Molecular Cage¹⁾

The tetradehydro-Diels–Alder reaction is useful, but not so straightforward, requiring careful substrate design and harsh reaction condition. Recently, we found that efficient and site-selective tetradehydro-Diels–Alder reaction can be achieved by using a kind of self-assembled molecular system, called a Pd₆L₄ cage. The Pd₆L₄ cage has a cavity, into which broad range of compound can be introduced.

In the reaction we found, the substrate was captured inside the Pd₆L₄ cage, and the conformation of the substrate was fixed. The control of the substrate conformation results in the efficient and site-selective reaction (Figure 1).

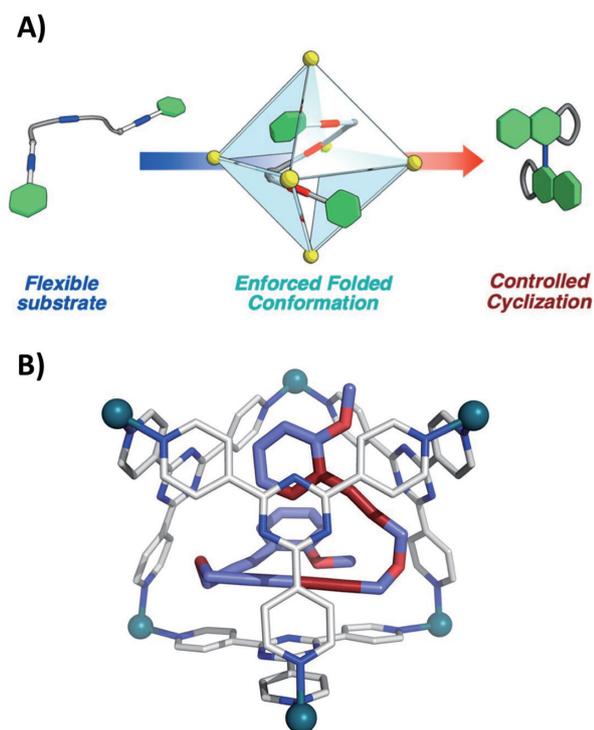


Figure 1. A) Concept of this study. B) Crystal structure of the substrate-Pd₆L₄ cage complex.

2. Hysteresis Behavior in the Unfolding/Refolding Processes of a Protein Trapped in the Self-Assembled Molecular Cage²⁾

The M₁₂L₂₄ self-assembled cage possesses a cavity large enough to accommodate proteins (Figure 2A).

Recently, we use this cage to analyze unfolding/refolding processes of a protein. When the concentration of organic solvent is increased in protein solution, the protein would be denatured. Then, the protein would be aggregated and precipitated normally. However, when the protein is captured in the self-assembled cage, the precipitation is prevented, because only single protein exists inside the cage. Thus, we can carry out the protein transient structure analysis, using this cage.

Concretely, we increase and decrease the concentration of acetonitrile of the protein solution, and the protein conformation was monitored by NMR analysis. As a result, it was revealed that the protein folding/unfolding process exhibited hysteresis behavior (Figure 2B).

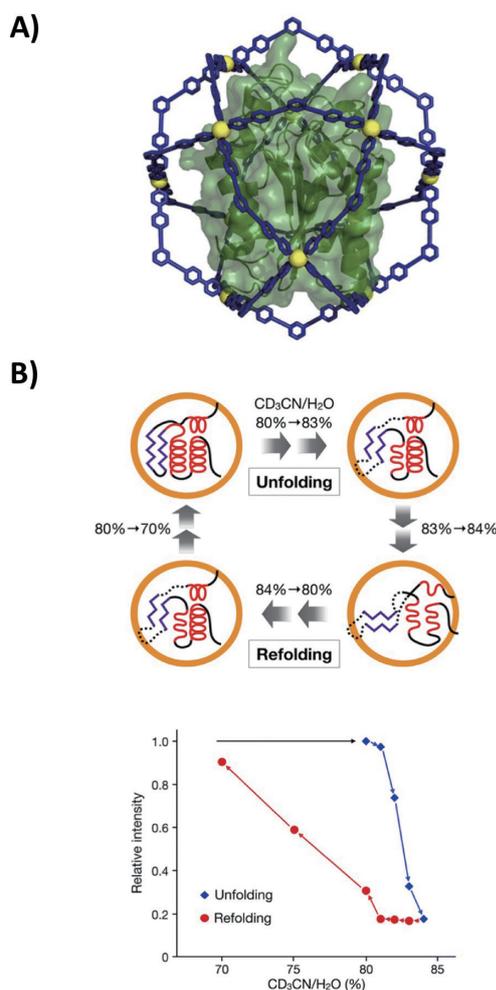


Figure 2. A) Molecular modeling of protein in the M₁₂L₂₄ self-assembled cage. B) Protein folding/unfolding process exhibited hysteresis behavior.

References

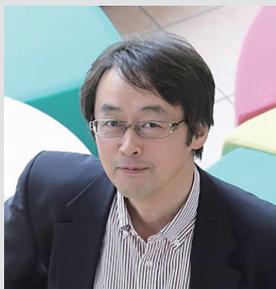
- 1) G. R. Genov, H. Takezawa, H. Hayakawa and M. Fujita, *J. Am. Chem. Soc.* **145**, 17013–17017 (2023).
- 2) T. Nakama, A. Rossen, R. Ebihara, M. Yagi-Utsumi, D. Fujita, K. Kato, S. Sato and M. Fujita, *Chem. Sci.* **14**, 2910–2914 (2023).

Award

FUJITA, Makoto; The Asahi Prize 2022 (2023).

Exploring Novel Physical Properties by Multi-Dimensional Spectroscopy

Division of Advanced Molecular Science
(Department of Materials Molecular Science, Electronic Structure)



KIMURA, Shin-ichi
Professor
(Cross Appointment)
[kimura@ims.ac.jp]

Education

1988 B.S. Tohoku University
1990 M.S. Tohoku University
1991 Ph.D. Tohoku University

Professional Employment

1991 JSPS Postdoctoral Fellow, Tohoku University
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

Member
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.

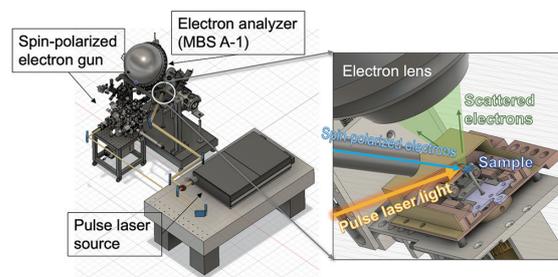


Figure 1. Time-, Spin-, and Angle-Resolved resonant Electron-Energy-Loss Spectroscopy (tSAR-rEELS) apparatus, which our group is now developing. The apparatus comprises a high-brilliant spin-polarized electron gun, a photoelectron spectrometer, and a femto-second pulse laser with an optical parametric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

Selected Publications

- Y. Ohtsubo, T. Nakaya, T. Nakamura, P. Le Fèvre, F. Bertran, F. Iga and S. Kimura, “Breakdown of Bulk-Projected Isotropy in Surface Electronic States of Topological Kondo Insulator $\text{SmB}_6(001)$,” *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).
- S. Kimura and H. Okamura, “Infrared and Terahertz Spectroscopy of Strongly Correlated Electron Systems under Extreme Conditions,” *J. Phys. Soc. Jpn.* **82**, 021004 (28 pages) (2013). [review]

1. Breakdown of Bulk-Projected Isotropy in Surface Electronic States of Topological Kondo Insulator $\text{SbB}_6(001)$ ¹⁾

The topology and spin-orbital polarization of two-dimensional (2D) surface electronic states have been extensively studied in this decade. One major interest in them is their close relationship with the parities of the bulk (3D) electronic states. In this context, the surface is often regarded as a simple truncation of the bulk crystal. Here we show a breakdown of the bulk-related in-plane rotation symmetry in the topological surface states (TSSs) of the Kondo insulator SbB_6 .²⁾ Angle-resolved photoelectron spectroscopy (ARPES) performed on the vicinal $\text{SbB}_6(001)-p(2 \times 2)$ surface showed that TSSs are anisotropic and that the Fermi contour lacks the fourfold rotation symmetry maintained in the bulk. This result emphasizes the important role of the surface atomic structure even in TSSs. Moreover, it suggests that the engineering of surface atomic structure could provide a new pathway to tailor various properties among TSSs, such as anisotropic surface conductivity, nesting of surface Fermi contours, or the number and position of van Hove singularities in 2D reciprocal space.

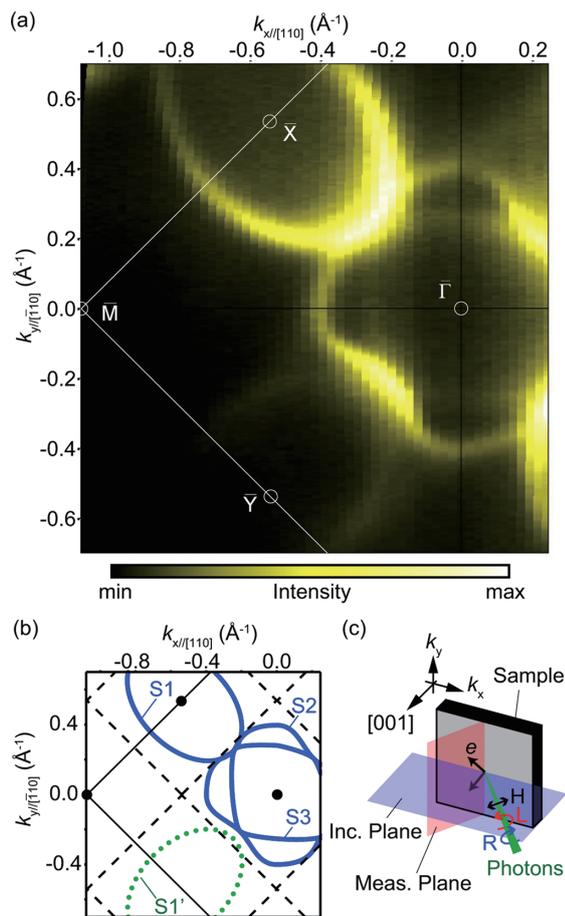


Figure 2. (a) ARPES Fermi contours (FCs) taken at the photon-incident plane of $(\bar{1}10)$ at 13 K. (b) Schematic drawing of the observed FCs together with the border of surface Brillouin zones; solid lines for bulk-truncated (1×1) and dashed for (2×2) . (c) Experimental geometry and definition of the in-plane wavevectors k_x and k_y . k_x and k_y are always in the photon-incident and photoelectron detection planes, respectively.

2. Fluctuating Spin-Orbital Texture of Rashba-Split Surface States in Real and Reciprocal Space³⁾

Spin-orbit interaction in low-dimensional systems, namely, Rashba systems and the edge states of topological materials, has been extensively studied in this decade as a promising source to realize various fascinating spintronic phenomena, such as the source of the spin current and spin-mediated energy conversion. Here, we show the odd fluctuation in the spin-orbital texture in a surface Rashba system on $\text{Bi}/\text{InAs}(110)-(2 \times 1)$ by spin- and angle-resolved photoelectron spectroscopy and a numerical simulation based on a density-functional theory (DFT) calculation. The surface state shows a paired parabolic dispersion with the spin degeneracy lifted by the Rashba effect. Although its spin polarization should be fixed in a particular direction based on the Rashba model, the observed spin polarization varies greatly. It even reverses its sign depending on the wave number. DFT calculations also reveal that the spin directions of two inequivalent Bi chains on the surface change from nearly parallel (canted parallel) to antiparallel in real space in the corresponding wave vector region. These results indicate an oversimplification of the nature of spin in Rashba and Dirac systems and provide more freedom than expected for spin manipulation of photoelectrons.

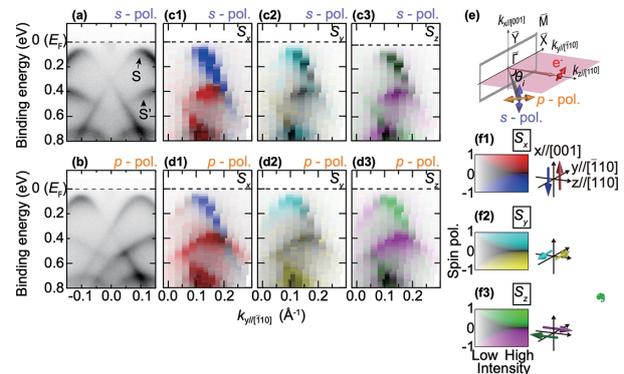


Figure 3. Spin-polarized surface band structures measured by spin- and angle-resolved photoelectron spectroscopy (SARPES) (a) and (b) ARPES and (c) and (d) SARPES 2D plots measured with s-polarized (top) and p-polarized (bottom) photons along $\bar{\Gamma}-\bar{X}$ (parallel to the Bi chains). (a) and (b) Spin-integrated ARPES intensity maps. SARPES maps polarized to (c1) and (d1) S_x , (c2) and (d2) S_y , and (c3) and (d3) S_z . The spin orientations are defined in (f). (e) Experimental geometry of the SARPES measurements and definitions of the coordinates. The (2×1) surface Brillouin zone and the common plane of the photon incidence and photoelectron detection are superposed simultaneously. (f) Definitions of the spin directions of the photoelectrons.

References

- 1) Y. Ohtsubo, T. Nakaya, T. Nakamura, P. Le Fèvre, F. Bertran, F. Iga and S. Kimura, *Nat. Commun.* **13**, 5600 (7 pages) (2022).
- 2) Y. Ohtsubo, S. Kimura and F. Iga, *Electron. Struct.* **4**, 033003 (13 pages) (2022).
- 3) T. Nakamura, Y. Ohtsubo, A. Harasawa, K. Yaji, S. Shin, F. Komori and S. Kimura, *Phys. Rev. B* **105**, 235141 (7 pages) (2022).

Operando Molecular Science in Liquid–Solid Interfaces of Finite Thickness

Division of Advanced Molecular Science (Department of Materials Molecular Science, Electronic Structure)



ONISHI, Hiroshi
Professor
(Cross Appointment)
[oni@ims.ac.jp]

Education

1985 B.S. The University of Tokyo
1993 Ph.D. The University of Tokyo

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

Member
Secretary
KURITA, Yoshiko

Keywords Reactions at Interfaces, Catalysis, Energy-Related Chemistry

We are proud of our internationally compatible studies of liquid–solid interfaces, e.g. photocatalysts for artificial photosynthesis and lubricants for smooth tribology. Characterization with frequency-modulation 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.

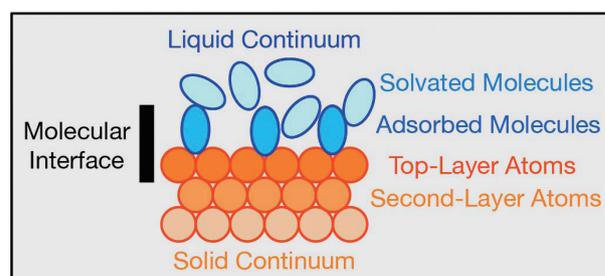


Figure 1. Liquid–Solid Interface of Finite Thickness.

Selected Publications

- 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, T. Ando, T. Hisatomi, H. Nishiyama, Y. Zhou, K. Domen, Y. Takahashi and H. Onishi, “Microelectrode-Based Transient Amperometry of O₂ Adsorption and Desorption on a SrTiO₃ Photocatalyst Excited under Water,” *Phys. Chem. Chem. Phys.* **23**, 19386–19393 (2021).
- T. Minato, K. Umeda, K. Kobayashi, Y. Araki, H. Konishi, Z. Ogumi, T. Abe, H. Onishi and, H. Yamada, “Atomic-Level Nature of Solid/Liquid Interface for Energy Conversion Revealed by Frequency Modulation Atomic Force Microscopy,” *Jpn. J. Appl. Phys.* **60**, SE0806 (2021).
- T. Kosaka, Y. Teduka, T. Ogura, Y. Zhou, T. Hisatomi, H. Nishiyama, K. Domen, Y. Takahashi and H. Onishi, “Transient Kinetics of O₂ Evolution in Photocatalytic Water-Splitting Reaction,” *ACS Catal.* **10**, 13159–13164 (2020).
- S. Xue, A. Sasahara and H. Onishi, “Atom-Scale Imaging of TiO₂(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. Infrared and Near-Infrared Spectrometry of Anatase and Rutile Particles Bandgap Excited in Liquid

The chemical transformation of materials is accomplished in milliseconds or seconds by assembling atoms over semiconductor photocatalysts. Bandgap-excited electrons and holes, which are reactive on this time scale, are key to the efficient assembly of atoms to yield the desired products. In this study,¹⁾ attenuated total reflection of infrared and near-infrared light was used to characterize and quantify the electronic absorption of TiO₂ photocatalysts excited under liquid. Nanoparticles of rutile or anatase were placed on a diamond prism, covered with liquid, and irradiated with continuous UV light through the prism. Electrons excited in rutile particles (JRC-TIO-6) formed small polarons characterized by a symmetric absorption band spread over 10000–700 cm⁻¹ with a maximum at 6000 cm⁻¹ (Figure 2). Electrons in anatase particles (JRC-TIO-7) formed large polarons and produced an asymmetric absorption band that gradually strengthened at wave numbers below 5000 cm⁻¹ and weakened sharply at 1000 cm⁻¹. The absorption spectrum of large electron polarons in TIO-7 was compared with the absorption reported in a Sr-doped NaTaO₃ photocatalyst, and it was suggested that excited electrons were accommodated as large polarons in NaTaO₃ photocatalysts efficient for artificial photosynthesis. The UV light power dependence of the absorption bands was observed in N₂-exposed decane liquid to derive the electron–hole recombination kinetics. At light power densities P greater than 200 W m⁻² (TIO-6) and 2000 W m⁻² (TIO-7), the polaron absorptions were enhanced with the absorbance proportional to $P^{1/2}$. The observed 1/2 power law suggested recombination of multiple electrons and holes moving randomly in each particle. Upon excitation with smaller P , the power law order increased to unity. The unity-order power law was interpreted as the recombination of an electron and a hole excited by the same photon. Furthermore, an average lifetime of 1 ms was estimated for electron polarons in TIO-6 when weakly excited at $P = 20 \text{ W m}^{-2}$ to simulate solar light irradiation.

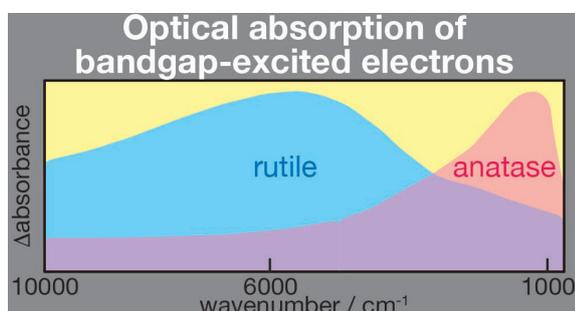


Figure 2. Optical absorption of electrons photoexcited in rutile and anatase TiO₂.

2. Three Dimensional Atom Imaging in KTaO₃ Photocatalysts Doped with Metal Cations

Potassium tantalate, KTaO₃, is a highly efficient semi-

conductor photocatalyst for the overall water splitting reaction. Doping a semiconductor photocatalyst with foreign metal cations typically increases the apparent quantum yield of the splitting reaction. In this study,²⁾ we constructed a single-crystal model of cation-doped photocatalysts that would be suitable for future investigation using advanced surface-sensitive methods. Centimeter-sized (001)-oriented KTaO₃ wafers were doped with Sr or La cations in KCl flux. X-ray diffraction revealed Sr- and La-containing perovskite-structured layers epitaxially covering the bulk KTaO₃. On the Sr-doped wafer, the surface layer lattice was expanded by 2% relative to the bulk lattice. X-ray fluorescence holography was used to determine the three-dimensional short-range ordered structure around the K and Sr cations (Figure 3). Holograms obtained with Sr K α fluorescence confirmed the simultaneous placement of Sr cations in the A and B sites. The placement of the Sr cations in the B sites was supported by the TaO₆ breathing vibration observed in Raman scattering. These experimental results suggest that a KTaO₃-Sr(Sr_{1/3}Ta_{2/3})O₃ solid solution is generated by doping. Two La-containing phases, one with a 2% lattice contraction and the other with a 0.4% expansion, were detected on the La-doped wafer. La L α fluorescence holograms indicated a complex type of doping. The obtained atom distribution around La cations was interpreted by the simultaneous La cation occupancy at the A site, B site and an interstitial site. Local lattice deformation was quantitatively deduced around the La cations occupying the interstitial site. Elemental composition determined by X-ray photoelectron spectroscopy revealed the enrichment of dopant elements on the wafer surface. Nanometer-scale topography observed by atomic force microscopy suggested that doping concentrations should be optimized to obtain flat, crystalline surfaces.

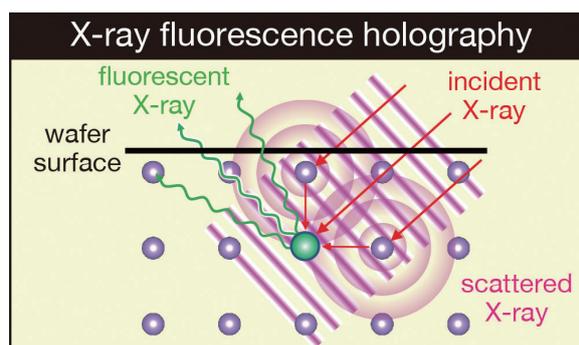


Figure 3. X-ray fluorescence holography.

References

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- 2) A. Sasahara, K. Kimura, H. Sudrajat, N. Happo, K. Hayashi and H. Onishi, *J. Phys. Chem. C* **126**, 19745–19755 (2022).

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

**Division of Advanced Molecular Science
(Department of Photo-Molecular Science, Photo-Molecular Science III)**



NAKAMURA, Akihiko
Associate Professor
(Cross Appointment)
[aki-naka@ims.ac.jp]

Education

2009 B.S. The University of Tokyo
2014 Ph.D. The University of Tokyo

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
NAKANE, Kaori
NOMURA, Junko

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 runoff into the environment (Figure 1).

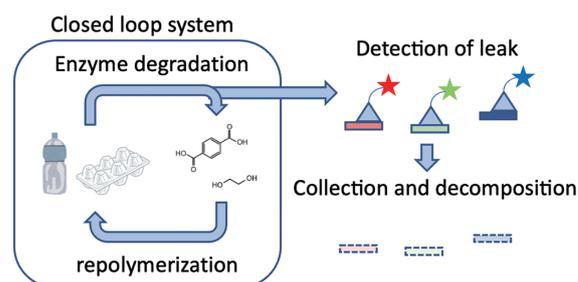


Figure 1. Scheme of plastic recycling and detection system.

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, the improvement was achieved with a combination of single mutations, but a saturation mutation, for example, in which a combination of all amino acids is tested in two locations, we need to test a library of 399 candidate mutants. Since it is difficult to purify and measure the activity of each mutant individually, a method was created to evaluate the activity without purification of the enzyme and without the use of HPLC.

We artificially prepared PET particulate suspensions by dissolving PET in Hexafluoro-2-propanol (HFIP) 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.

When the temporal changes in degradation rate and turbidity were analyzed by changing the concentration of PET degrading enzyme, it was observed that the turbidity decreased over time, and it was found that the high degradation activity could be evaluated from the rate of decrease in turbidity (Figure 2). 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 approximately 800 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 three mutants were produced and purified at large scale cultivation and the activities against PET film are under comparison

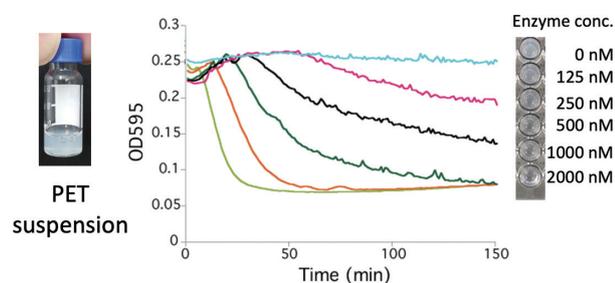


Figure 2. Results of PET degrading enzyme activity measurement using turbidity as an indicator.

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

To develop a method for detecting plastics in samples using plastic binding proteins, we first developed a protein

targeting PET. The template enzyme was a thermostable domain that adsorbs on chitin, which has a hydrophobic plane similar to PET.

Saturation mutations were introduced at four amino acid residues oriented on the adsorption surface and involved in chitin recognition. Since the number of candidate mutants is about 160,000 and it is difficult to verify them one by one, we created a library of phage vectors with adsorption 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, unbound phage were washed away, and phage bound on PET were recovered by trypsin digestion and re-amplified by infection with *E. coli*. The reproduced first-generation phage library was again mixed with PET and the number of washes was increased to 10 before PET adsorbed phage were collected and infected with *E. coli*. After the second-generation phage library was produced, the phage vectors were purified from *E. coli*, and the mutated portions were analyzed using a next-generation sequencer. In the pre-screening library, the percentage of amino acids that appeared for the four mutations was almost the same, but in the second-generation library, a large difference was observed in the probability of occurrence of different amino acids depending on the position of the mutation. This was thought to be the result of selection of amino acids structurally important for PET recognition. In order to select mutants with strong binding and high thermal stability, a third-generation screening was conducted with binding and washing temperatures set at 60 °C. As a result, 29 colonies were obtained, 27 of which were the same mutant. The mutant enzyme was conjugated with RFP, and when mixed with PET powder, the mutant complex stained the PET powder red, whereas the template enzyme did not (Figure 3). Currently, we are introducing further mutations into the mutant to improve the specificity of binding on PET.

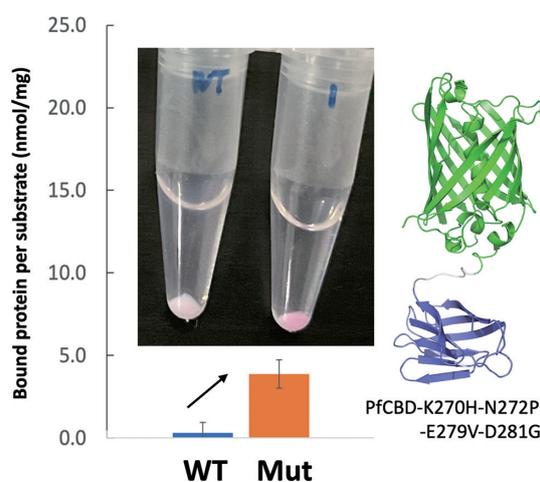


Figure 3. Binding measurement results using PET binding domain and RFP complex.

Reference

- 1) A. Nakamura, N. Kobayashi, N. Koga and R. Iino, *ACS Catal.* **11**, 8550–8564 (2021).