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, metalloproteins, biological-clock proteins, glycoconjugates, antibodies, and motor proteins. Coordination-complex divisions aim to develop molecular catalysts and functional metal complexes for transformation of organic molecules, and molecular materials with three-dimensional complex structures. 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.

Bioinorganic Chemistry of Metalloproteins Responsible for Metal Homeostasis and Signal Sensing

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

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Keywords Bioinorganic Chemistry, Metalloproteins, Sensor Protein

Transition metal ions and metalloproteins play crucial roles in meeting the energy demands of the cell by playing roles in intermediary metabolism and in signal transduction processes. Although they are essential for biological function, metal ion bioavailability must be maintained within a certain range in cells due to the inherent toxicity of all metals above a threshold. This threshold varies for individual metal ions. Homeostasis of metal ions requires a balance between the processes of uptake, utilization, storage, and efflux and is achieved by the coordinated activities of a variety of proteins including extracytoplasmic metal carriers, ion channels/pumps/transporters, metal-regulated transcription and translation proteins, and enzymes involved in the biogenesis of metal-containing cofactors/metalloproteins. In order to understand the processes underlying this complex metal homeostasis network, the study of the molecular processes that determine the protein-metal ion recognition, as well as how this event is transduced into a functional output, is required. My research interests are focused on the elucidation of the structure and function relationships of metalloproteins responsible for the regulation of biological homeostasis.

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

Selected Publications

- H. Matsuura, N. Sakai, S. Toma-Fukai, N. Muraki, K. Hayama, H. Kamikubo, S. Aono, Y. Kawano, M. Yamamoto and K. Hirata, “Elucidating Polymorphs of Crystal Structures with Intensity-Based Hierarchical Clustering Analysis on Multiple Diffraction Datasets,” *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **79**, 909–924 (2023).
- D. Matsui, N. Muraki, K. Chen, T. Mori, A. A. Ingram, K. Oike, H. Gröger, S. Aono and Y. Asano, “Crystal Structural Analysis of Aldoxime Dehydratase from *Bacillus sp.* OxB-1: Importance of Surface Residues in the Optimization for Crystallization,” *J. Inorg. Biochem.* **230**, 111770–111779 (2022).
- Y. Ikenoue, Y. Tahara, M. Miyata, T. Nishioka, S. Aono and H. Nakajima, “Use of a Ferritin L134P Mutant for the Facile Conjugation of Prussian Blue in the Apoferritin Cavity,” *Inorg. Chem.* **60**, 4693–4704 (2021).
- 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 Bacterial Survival,” *Commun. Biol.* **4**, 467 (12 pages) (2021).
- N. Muraki, K. Takeda, D. Nam, M. Muraki and S. Aono, “Structural Characterization of Thermoglobin from a Hyperthermophilic Bacterium *Aquifex aeolicus*,” *Chem. Lett.* **50**, 603–606 (2021).
- N. Muraki, K. Ishii, S. Uchiyama, S. G. Itoh, H. Okumura and S. Aono, “Structural Characterization of HypX Responsible for CO Biosynthesis in the Maturation of NiFe-Hydrogenase,” *Commun. Biol.* **2**, 385 (12 pages) (2019).

1. Structural and Functional Analysis of Heme-Based Oxygen Sensor Protein HemAT

Aerotaxis is a typical biological signal transduction system that consists of a signal transducer protein (MCP), CheA, CheY, and other Che proteins. Signal transducer proteins, sometimes called as MCPs (methyl-accepting chemotoxis proteins), bind a repellant or attractant in their sensor domain. Many chemical and physical stimuli act as a repellant or attractant, among which molecular oxygen is a typical gaseous signaling molecule. HemAT is a MCP responsible for aerotaxis control, which consists of two domains, the sensor domain and the signaling domain. Though the sensor domain of HemAT shows structural homology to myoglobin, it has a different heme environmental structure in the distal heme pocket from myoglobin. In the case of myoglobin, a distal His forms a hydrogen bond with the heme-bound oxygen to stabilize the heme-oxygen complex. However, there is no distal His in HemAT, in which a Thr is involved in the formation of a hydrogen bonding network upon oxygen binding to HemAT.

HemAT forms HemAT/CheA/CheW complex, in which the intramolecular signal transduction takes place upon O_2 binding to HemAT. In this work, we have tried to determine the HemAT/CheA/CheW complex by cryo-electron microscopy (cryoEM) to understand the molecular mechanisms of O_2 sensing and signal transduction of HemAT and HemAT/CheA/CheW complex (Figure 1). We have carried out cryoEM single particle analysis to determine the structure of HemAT/CheA/CheW complex, which revealed that BsmHemAT, CheA, and CheW formed the complex in 2:1:1 ratio.

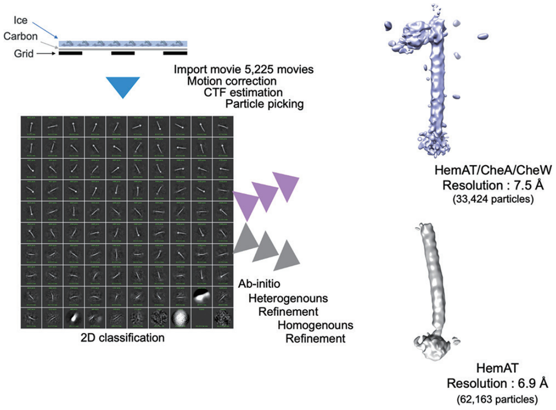


Figure 1. Structural determination of the HemAT/CheA/CheW complex by cryoEM.

2. CO Biosynthesis for the Construction of the Active Site in [NiFe]-Hydrogease

Hydrogenase, an enzyme that catalyzes the oxidation of hydrogen gas and the reduction of protons, plays a central role in hydrogen metabolism in bacteria and other microorganisms. Recently, it is also expected to be utilized as a catalyst for fuel cells. There are three types of hydrogenases classified based on the structure of their active centers: [NiFe]-, [FeFe]-, and [Fe]-hydrogenases. In all cases, carbon monoxide (CO) is coordinated to the Fe in the active center. While it is known that CO is biosynthesized through enzyme reactions, the molecular mechanism of CO generation has been unclear. In this work, the crystal structure of the enzyme HypX involved in CO biosynthesis used by [NiFe]-hydrogenase was determined. It was revealed that HypX synthesizes CO through a completely novel reaction. HypX consists of two domains (N-terminal domain and C-terminal domain), and within the molecule, there is a cavity spanning across these two domains. It was also found that coenzyme A (CoA) binds to the cavity on the C-terminal domain side.

Based on the obtained crystal structure, the following CO biosynthesis reaction mechanism was proposed: Two different chemical reactions occur in the N-terminal domain and the C-terminal domain of HypX. In the N-terminal domain, a formyl transfer reaction from formyl-tetrahydrofolate (formyl-THF), which is bound in the cavity of the N-terminal domain, to CoA takes place. During this process, CoA in the cavity adopts an extended linear conformation, and the -SH group at the end of CoA is positioned adjacent to the formyl group in formyl-THF. As a result of the formyl transfer reaction, formyl-CoA is generated as an intermediate. The generated formyl-CoA undergoes a significant conformational change within the cavity to position the formyl group at the end of the CoA molecule towards the enzyme active site in the C-terminal domain of HypX. In the C-terminal domain, the CO release reaction from formyl-CoA occurs, resulting in the production of CO and CoA.

The N-terminal domain of HypX is structurally homologous to the proteins that catalyze formyl-group transfer reaction with N^{10}-formyl-THF as a formyl-group donor such as the hydrolase domain of FDH, methionyl-t-RNA formyltransferase (FMT), and UDP-glucuronic acid dehydrogenase (ArnA). We also obtained HypX/THF complex by soaking HypX crystals with THF and solved its structure at a resolution of 2.1 Å. The THF binding sites are conserved among HypX, FDH, and ArnA (Figure 2), which supports the notion that N^{10}-formyl-THF is the substrate of HypX as is the case of FDH and ArnA.

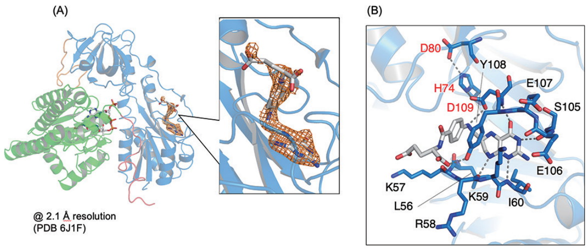
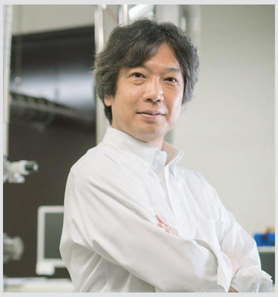


Figure 2. (A) Crystal structure of THF-bound HypX, in which the electron densities of THF is shown in orange mesh. (B) Closed-up view of THF binding site. Hydrogen bonding interactions are shown in dotted line. His74, Asp80, and Asp109 in HypX act as the catalytic triad to catalyze the formyl-group transfer from N^{10}-formyl-THF to CoA bound in HypX.

Dynamical Ordering of Biomolecular Systems for Creation of Integrated Functions

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



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Professional Employment

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2008	Professor, Institute for Molecular Science Professor, Okazaki Institute for Integrative Bioscience (–2018) Professor, The Graduate University for Advanced Studies
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2018	Professor, Exploratory Research Center on Life and Living Systems (ExCELLS)

Awards

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Keywords Biomolecule Organization, NMR

Living systems are characterized as dynamic processes of assembly and disassembly of various biomolecules that are self-organized, interacting with the external environment. The omics-based approaches developed in recent decades have provided comprehensive information regarding biomolecules as parts of living organisms. However, fundamental questions still remain unsolved as to how these biomolecules are ordered autonomously to form flexible and robust systems (Figure 1). Biomolecules with complicated, flexible structures are self-organized through weak interactions giving rise to supramolecular complexes that adopt their own dynamic, asymmetric architectures. These processes are coupled with expression of integrated functions in the biomolecular systems.

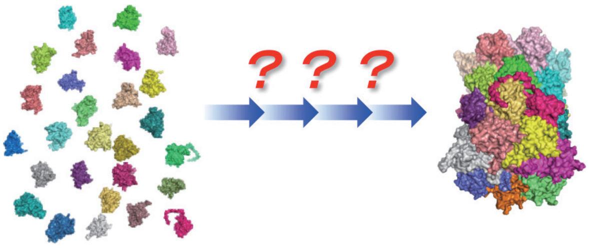

Figure 1. Formation of supramolecular machinery through dynamic assembly and disassembly of biomolecules.

Toward an integrative understanding of the principles behind the biomolecular ordering processes, we conduct multidisciplinary approaches based on detailed analyses of dynamic structures and interactions of biomolecules at atomic level, in conjunction with the methodologies of molecular and cellular biology along with synthetic and computational technique.

Selected Publications

- K. Kato, S. Yanaka and H. Yagi, “Glycoprotein Preparation by Heterologous Expression,” in *NMR of Glycoproteins*, J. Jiménez-Barbero and O.Millet, Eds., *Methods in Molecular Biology*, Humana; New York, **vol. 2961**, pp. 53–67 (2025).
- K. Kato, S. Yanaka and T. Yamaguchi, “The Synergy of Experimental and Computational Approaches for Visualizing Glycoprotein Dynamics: Exploring Order within the Apparent Disorder of Glycan Conformational Ensembles,” *Curr. Opin. Struct. Biol.* **92**, 103049 (2025).
- K. Kato, S. Yanaka and H. Yagi, “Technical Basis for Nuclear Magnetic Resonance Approach for Glycoproteins,” in *Experimental Approaches of NMR Spectroscopy II*, The Nuclear Magnetic Resonance Society of Japan, Ed., Springer Nature; Singapore, pp. 169–195 (2025).
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- D. Koga, S. Kusumi, H. Yagi and K. Kato, “Three-Dimensional Analysis of the Intracellular Architecture by Scanning Electron Microscopy,” *Microscopy* **73**, 215–225 (2024).
- K. Kato and H. Yagi, “Current Status and Challenges in Structural Glycobiology,” *Trends Carbohydr. Res.* **15**, 38–46 (2023).

1. Structural and Kinetic Modulation of Amyloid β Fibrils: Insights from Antibody Recognition and Familial Mutation

The dynamic interaction between amyloid β (Aβ) fibrils and antibodies during fibril elongation was investigated at the single molecule level.[1)] Real-time observations revealed that Aβ fibril elongation alternates between an "elongation phase," in which two protofilaments grow in a staggered manner, and a "pause phase," during which growth temporarily halts. Notably, Aβ monomers were found to alternately attach to the two protofilaments during elongation, whereas growth paused when both protofilament tips became aligned. Furthermore, a monoclonal antibody 4396C selectively bound to fibril ends in this paused state, effectively suppressing further elongation. By integrating high-speed atomic force microscopy with molecular simulations, this study provided a detailed understanding of the dynamic assembly mechanism underlying Aβ fibril formation.

In parallel, we harnessed the unique microgravity environment aboard the International Space Station to elucidate, for the first time, the detailed structure of amyloid β fibrils bearing the Tottori-type familial mutation (D7N), a rare variant linked to Alzheimer's disease.[2)] Under Earth gravity, Tottori-type Aβ40 predominantly forms amorphous aggregates, hindering structural analysis. In contrast, microgravity suppressed amorphous aggregation and promoted efficient fibril formation, enabling high-resolution cryo-electron microscopy (Figure 2). The resulting structures revealed a flexible, disordered N-terminal region, suggesting that the D7N mutation disrupts the stabilizing role of the N-terminus observed in wild-type Aβ fibrils. Under microgravity, the absence of convection and sedimentation limited off-pathway aggregation and facilitated the emergence of structurally convergent, high-quality fibrils.

These two perspectives jointly deepen our mechanistic understanding of Aβ fibril formation and offer new avenues for identifying therapeutic targets associated with Alzheimer's disease pathogenesis.

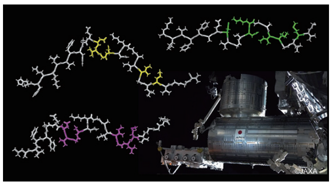

Figure 2. Fibril formation of the Aβ Tottori variant (D7N) was investigated under microgravity on the International Space Station.[2)]

2. Decoding Glycan Dynamics and Engineering Precision in Glycoprotein Function

Glycans play vital roles in cellular recognition, immune signaling, and protein stability, yet their dynamic and heterogeneous structures remain difficult to characterize. We reported a series of studies that deepen our understanding of glycan conformational behavior, its influence on protein function, and its potential in glycoengineering and biopharmaceutical design.

To explore glycan dynamics, we developed an analytical framework integrating molecular dynamics (MD) simulations with NMR-guided constraints. Using nonlinear correlation metrics such as the Hilbert-Schmidt Independence Criterion and Maximal Information Coefficient, we uncovered hidden structural relationships beyond conventional intuition.[4)] This data-driven approach reveals how glycan flexibility modulates protein interactions.

We next examined how glycosylation affects the structure and immune functions of human IgG antibodies.[5)] Through isotope-assisted NMR and MD simulations, we analyzed four IgG1-Fc glycoforms differing in galactose and fucose content. Galactose residues acted as molecular "anchors" and "wedges," stabilizing the Fc domain and enhancing binding to Fcγ receptors and complement C1q (Figure 3). In contrast, the absence of core fucose altered dynamics of residues critical for ADCC, offering atomic-level insights for antibody optimization.

In parallel, we identified a 10-amino-acid "passport sequence" that enhances N-glycan maturation as well as secretion. When appended to therapeutic glycoproteins such as erythropoietin, the passport sequence is recognized by NUCB1, which resides near B4GALT1 in the Golgi and promotes its enzymatic activity. This facilitates N-glycan maturation and, in turn, enhances pharmacokinetics and therapeutic efficacy.[6)]

Additional studies revealed temporal and sex-dependent N-glycosylation changes in rat serum[7)] and unusual glycan structures in tardigrades,[8)] underscoring the evolutionary diversity of glycosylation.

Together, these findings offer new strategies for precision glycoengineering and deepen our understanding of glycan-mediated regulation.

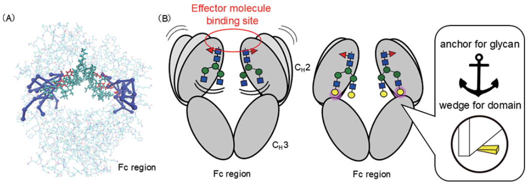

Figure 3. (A) Structural propagation pathways originating from galactose residues within the Fc-linked glycan. (B) Galactose residues (indicated by yellow circles) suppress glycan mobility by acting as molecular anchors, and constrain Fc domain dynamics by serving as molecular wedges—together enhancing the stability of the functional site and promoting effector molecule binding.[5)]

References

1) M. Yagi-Utsumi *et al.*, *J. Am. Chem. Soc.* **146**, 31518–31528 (2024).
2) M. Yagi-Utsumi *et al.*, *ACS Chem. Neurosci.* **16**, 2682–2690 (2025).
3) R. N. Burton-Smith *et al.*, *Int. J. Mol. Sci.* **26**, 1179 (2025).
4) K. Kato *et al.*, *Chem. Pharm. Bull.* **73**, 639–644 (2025).
5) S. Yanaka *et al.*, *Proc. Natl. Acad. Sci. U. S. A.* **122**, e2505473122 (2025).
6) H. Yagi *et al.*, *iScience* **27**, 111457 (2024).
7) H. Yagi *et al.*, *Int. J. Mol. Sci.* **26**, 7266 (2025).
8) H. Yagi *et al.*, *Mol. Cell Proteomics* **24**, 100974 (2025).

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Operation and Design Principles of Biological/Artificial Molecular Machines

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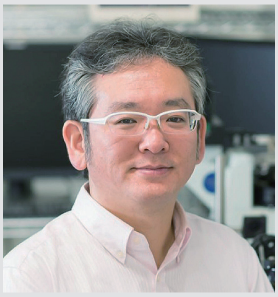

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Education

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Professional Employment

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2014	Professor, Institute for Molecular Science Professor, Okazaki Institute for Integrative Bioscience (–2018) Professor, The Graduate University for Advanced Studies

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Keywords Molecular Motors, Single-Molecule Biophysics, Protein Engineering

Life is supported by protein molecular machines which show better performance than man-made machines. One representative of the protein molecular machines is molecular motors (Figure 1). Molecular motors show autonomous unidirectional motions using the energy of chemical reaction. We unveil operational principles of molecular motors with advanced single-molecule analysis. In addition, with protein engineering, we create non-natural/hybrid molecular motors to understand their design principles. Furthermore, we create DNA/RNA-based artificial molecular motors and motor systems that mimic and outperform biological ones.

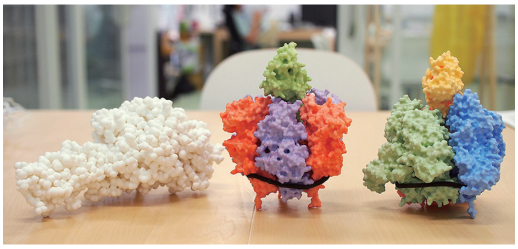

Figure 1. Protein molecular machines. (Left) A linear molecular motor chitinase A. (Center and Right) Rotary molecular motors F_1-ATPase and V_1-ATPase, respectively.

Selected Publications

- A. Otomo, L. G. Hui Zhu, Y. Okuni, M. Yamamoto and R. Iino, “ATP Synthesis of *Enterococcus hirae* V-ATPase Driven by Sodium Motive Force,” *J. Biol. Chem.* **301**, 108422 (2025).
- K. Suzuki, Y. Goto, A. Otomo, K. Shimizu, S. Abe, K. Moriyama, S. Yasuda, Y. Hashimoto, J. Kurushima, S. Mikuriya, F. L. Imai, N. Adachi, M. Kawasaki, Y. Sato, S. Ogasawara, S. Iwata, T. Senda, M. Ikeguchi, H. Tomita, R. Iino, T. Moriya and T. Murata, “Na^+-V-ATPase Inhibitor Curbs VRE Growth and Unveils Na^+ Pathway Structure,” *Nat. Struct. Mol. Biol.* **32**, 450–458 (2025).
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- 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).
- R. Iino, K. Kinbara and Z. Bryant, “Introduction: Molecular Motors,” *Chem. Rev.* **120**, 1–4 (2020).
- J. Ando, A. Nakamura, M. Yamamoto, C. Song, K. Murata and R. Iino, “Multicolor High-Speed Tracking of Single Biomolecules with Silver, Gold, Silver-Gold Alloy Nanoparticles,” *ACS Photonics* **6**, 2870–2883 (2019).
- 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).

1. ATP Synthesis of *Enterococcus hirae* V-ATPase Driven by Sodium Motive Force1)

V-ATPases generally function as ion pumps driven by ATP hydrolysis in the cell, but their capability of ATP synthesis remains largely unexplored. Here we show ATP synthesis of Na^+-transporting *Enterococcus hirae* V-ATPase (EhV_oV_1) driven by the electrochemical potential gradient of Na^+ across the membrane (sodium motive force, *smf*). We reconstituted EhV_oV_1 into liposome and performed a luciferin/luciferase-based assay to analyze ATP synthesis quantitatively. Our result demonstrates that EhV_oV_1 synthesizes ATP with a rate of 4.7 s^{-1} under high *smf* (269.3 mV). The Michaelis constants for ADP (21 µM) and inorganic phosphate (2.1 mM) in ATP synthesis reaction were comparable to those for ATP synthases, suggesting similar substrate affinities among rotary ATPases regardless of their physiological functions. Both components of *smf*, Na^+ concentration gradient across the membrane (ΔpNa) and membrane potential (Δψ), contributed to ATP synthesis. At the equilibrium points where *smf* and Gibbs free energy of ATP synthesis are balanced, EhV_oV_1 showed reversible reactions between ATP synthesis and hydrolysis (Figure 2). The obtained Na^+/ATP ratio (3.2 ± 0.4) closely matched the value expected from the structural symmetry ratio between EhV_o and EhV_1 (10/3 = 3.3), indicating tight coupling between ATP synthesis/hydrolysis and Na^+ transport. These results reveal the inherent functional reversibility of EhV_oV_1. We propose that the physiological function of EhV_oV_1 *in vivo* is determined by relatively small *smf* against large Gibbs free energy of ATP synthesis.

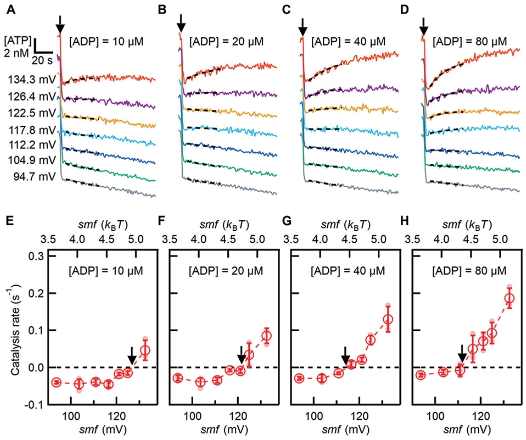

Figure 2. Determination of equilibrium points between ATP synthesis and hydrolysis. *A–D*, time courses of ATP synthesis and hydrolysis at different *smf* (93.5–132.6 mV). Reaction was initiated by adding proteoliposome as indicated by black arrow. The black dashed lines represent the fitting with a single exponential function for Δt = 35 s after the addition of PL. *E–H*, *smf* dependence of ATP synthesis and hydrolysis rates. Black arrows indicate equilibrium points obtained by linear fitting between two data points across the catalysis rate of zero. The reaction solution contains 25 nM ATP, 9.95 mM Pi, and ADP at 10 µM (*A* and *E*), 20 µM (*B* and *F*), 40 µM (*C* and *G*), and 80 µM (*D* and *H*), respectively.

2. Na^+-V-ATPase Inhibitor Curbs VRE Growth and Unveils Na^+ Pathway Structure2)

Vancomycin-resistant *Enterococcus faecium* (VRE) is a major cause of nosocomial infections, particularly endocarditis and sepsis. With the diminishing effectiveness of antibiotics against VRE, new antimicrobial agents are urgently needed. Our previous research demonstrated the crucial role of Na^+-transporting V-ATPase in *Enterococcus hirae* (EhV_oV_1) for growth under alkaline conditions. In this study, we identified a compound, V-161, from 70,600 compounds, which markedly inhibits EhV_oV_1 activity (Figure 3). V-161 not only inhibits VRE growth in alkaline conditions but also significantly suppresses VRE colonization in the mouse small intestine. Furthermore, we unveiled the high-resolution structure of the membrane V_o part due to V-161 binding. V-161 binds to the interface of the c-ring and a-subunit, constituting the Na^+ transport pathway in the membrane, thereby halting its rotation. This structural insight presents potential avenues for developing therapeutic agents for VRE treatment and elucidates the Na^+ transport pathway and mechanism.

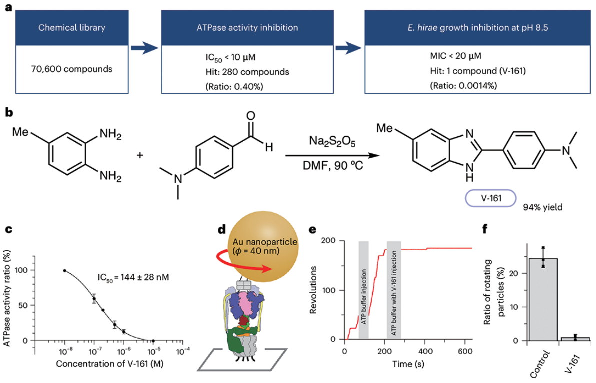

Figure 3. Schematic of screening process for EhV_oV_1 inhibitors. **b**, Chemical synthesis route of V-161. **c**, Inhibition of ATPase activity of EhV_oV_1 by V-161. Results are shown as mean ± s.e.m., derived from three separate experiments. **d**, Schematic of single-molecule rotation assay for EhV_oV_1. **e**, Rotation time course. After an initial 1 min of rotation recording, a buffer with 10 µM ATP was injected (indicated by the first gray square). After another several minutes of recording, a buffer containing both 2 µM V-161 and 10 µM ATP was injected (indicated by the second gray square). The recording speed was set to 50 frames per second. **f**, Ratio of rotating particles relative to the total particles attached to the glass surface. Results are shown as mean ± s.d., derived from three separate experiments.

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* IMS International Internship Program

Biomolecular Science Based on In Situ Observation by Magnetic Resonance

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Protein structures and dynamics are closely related to physiological functions, but are strongly affected by temperature, pH, ionic strength, pressure, and molecular crowding. Within cells, biomolecules are densely packed, and the intracellular space is compartmentalized by cytoskeletal structures and organelles. Furthermore, this environment is non-equilibrium, constantly adapting to external stimuli. To accurately understand protein function, it is therefore essential to investigate biomolecules in their native contexts. We apply magnetic resonance techniques—particularly in-cell NMR—to observe the atomic-level structures and dynamics of proteins directly in living cells, where they perform their natural roles. Our group also develops methodological innovations to improve sensitivity and resolution, expanding the scope of in-cell NMR. By correlating structural and dynamic information with cellular physiology, we aim to reveal the molecular basis of complex biological systems and establish a foundation for biomolecular science based on in situ magnetic resonance observation.

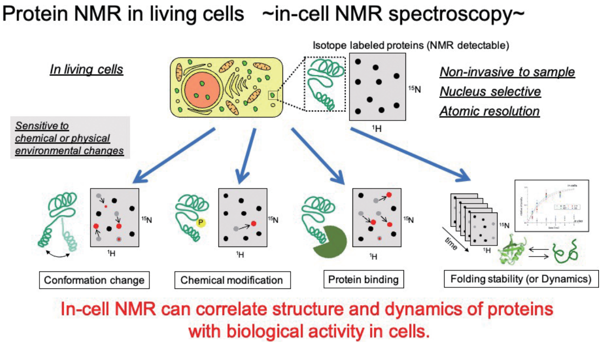

Figure 1. Overview of in-cell NMR spectroscopy.

Selected Publications

- M. Ikari, H. Yagi, T. Kasai, K. Inomata, M. Ito, K. Higuchi, N. Matsuda, Y. Ito and T. Kigawa., "Direct Observation of Membrane-Associated H-Ras in the Native Cellular Environment by In-Cell ^{19}F-NMR Spectroscopy," *JACS Au* **3**, 1658–1669 (2023).
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- K. Inomata, A. Ohno, H. Tochio, S. Isogai, T. Tenno, I. Nakase, T. Takeuchi, S. Futaki, Y. Ito, H. Hiroaki and M. Shirakawa., "High-Resolution Multi-Dimensional NMR Spectroscopy of Proteins in Human Cells," *Nature* **458**, 106–109 (2009).

1. In-Cell NMR Analysis of Keap1-Nrf2 Interactions Enabled by Controlling Intracellular Degradation

We investigated the interaction between Nuclear factor erythroid 2-related factor 2 (Nrf2) and its regulator Keap1 in living mammalian cells using in-cell NMR spectroscopy. Nrf2 is a transcription factor that protects cells from oxidative stress by inducing antioxidant response genes, and its stability is tightly controlled by Keap1-mediated ubiquitination and proteasomal degradation. Dysregulation of this system is implicated in cancer, neurodegeneration, and other diseases.

A major challenge we faced was the rapid intracellular degradation of delivered ^{15}N-labeled Nrf2-Neh2, which hindered direct NMR observation. To overcome this, we optimized cell preparation conditions by applying proteasome inhibitors such as MG132 and bortezomib. This successfully stabilized Nrf2-Neh2 after electroporation into HeLa and other cell lines. By contrast, mutating ubiquitination sites (Nrf2-Neh2 R7) or deleting Keap1 alone did not prevent degradation, showing that proteasomal pathways beyond Keap1-dependent ubiquitination contribute significantly.

Using degradation-controlled conditions, we observed clear in-cell NMR spectra of Nrf2-Neh2, which provided atomic-level evidence of its interaction with endogenous Keap1. We detected strong signal attenuation around the DLG and ETGE motifs, confirming their role in binding. By comparing HeLa cells with A549 lung carcinoma cells harboring the Keap1 G333C mutation, we revealed that this mutation weakens binding in dilute solution but displays selective effects inside cells, particularly near the ETGE motif. This demonstrates that the intracellular environment modulates the consequences of disease-associated mutations in ways not apparent from in vitro experiments.

We further evaluated the small-molecule inhibitor KI696, a nanomolar-affinity binder of Keap1. In dilute solution, KI696 efficiently disrupted the Nrf2-Keap1 complex, restoring Nrf2-Neh2 signals. In living mouse embryonic fibroblast cells reconstituted with Keap1, KI696 also increased Nrf2-Neh2 signal intensity, though to a lesser degree than in vitro. These results highlight how molecular crowding and intracellular kinetics reduce inhibitor potency, underscoring the importance of evaluating drug action directly in the cellular context.

Our study also revealed that the Nrf2-Keap1 system in cells exhibits rapid association–dissociation dynamics, producing extensive NMR signal broadening not predicted by concentration ratios alone. We interpret this as ubiquitination-dependent cycling of Nrf2, even under proteasome inhibition, reflecting the highly dynamic nature of the intracellular environment.

Taken together, we established a robust methodology to overcome proteasome-mediated degradation, enabling in-cell NMR spectroscopy of unstable proteins. Applying this platform to the Nrf2-Keap1 system, we uncovered unique insights into binding dynamics, mutation effects, and inhibitor activity in living cells. These findings not only advance mechanistic understanding of Nrf2 regulation but also demonstrate the broad potential of in-cell NMR as a tool for probing protein–protein interactions and evaluating therapeutic compounds under physiologically relevant conditions.

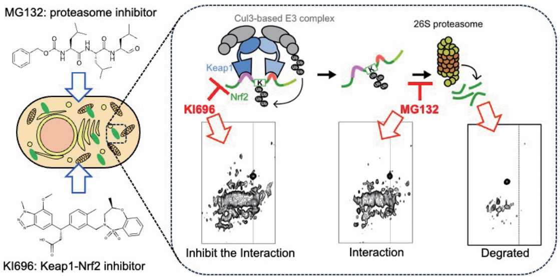

Figure 2. Schematic representation of Keap1-Nrf2 interactions in protein degradation-controlled mammalian cells.

2. In-Cell NMR Study of Protein Diffusion In Mammalian Cells

Protein diffusion in living cells is a crucial factor that determines localization, interactions, and functional regulation of biomolecules. Unlike dilute solution conditions, the intracellular environment is crowded and viscous, leading to significant restrictions on molecular mobility. To quantitatively characterize such behavior, we are developing an in-cell NMR platform that enables direct measurement of translational and rotational diffusion of proteins in mammalian cells.

As an initial step, we implemented fast, high-sensitivity NMR pulse sequences for intracellular diffusion measurements. Using ^{15}N-labeled ubiquitin mutants (L8A, I44A, V70A) introduced into HeLa cells, we successfully applied DOSY-SOFAST-HMQC experiments. Data acquisition was performed under optimized conditions, which enabled the detection of intracellular diffusion signals with sufficient sensitivity.

Diffusion analysis revealed a clear contrast between intracellular and dilute solution conditions. The translational diffusion coefficient was 2.18×10^{-10} m^2/s in buffer, but decreased to 4.96×10^{-11} m^2/s in HeLa cells—approximately a fourfold reduction. This result demonstrates that the dense intracellular milieu strongly impedes protein motion. Importantly, the ability to capture this quantitative difference confirms that our in-cell NMR setup is capable of monitoring protein dynamics under physiologically relevant conditions.

Looking forward, we plan to expand this model system to measure rotational diffusion in addition to translational motion. Rotational diffusion reflects molecular shape and local interactions and will complement the information gained from translational mobility. By integrating both, we aim to obtain a broader picture of protein dynamics across multiple timescales. In the coming year, we will refine our methodology and apply it to other protein systems, seeking to establish a versatile platform for in-cell structural biology.

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Development of Novel Catalytic Organic Transformations

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2008	Research Project Leader, NEDO Project (–2012)
2011	Deputy Research Project Leader, JST CREST (–2016)
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Keywords Transition Metal Catalysis, Green Chemistry, Photocatalysis

Our research interests lie in the development of catalytic reaction systems toward ideal SDGs-conscious (highly efficient, selective, green, safe, simple, *etc.*) organic molecular transformations. In particular, development of a wide variety of the heterogeneous in-water catalytic systems, continuous flow catalytic systems, and super active catalysts working at ppm-ppb loading levels, have been achieved. Furthermore, we have recently been studying on the novel photocatalysis where, for example, carbonyl groups underwent two successive single-electron reduction to generate carbinol anion species achieving electrophilic carbonyl substitution. Along this line, in 2024, we have developed a series of novel diazabenzacenaphthenium photocatalysts, denoted as ***N*-BAP**s, which promoted the unprecedented 4-electron photoreduction of esters to form the corresponding alcohols with visible LED light irradiation under aqueous conditions.

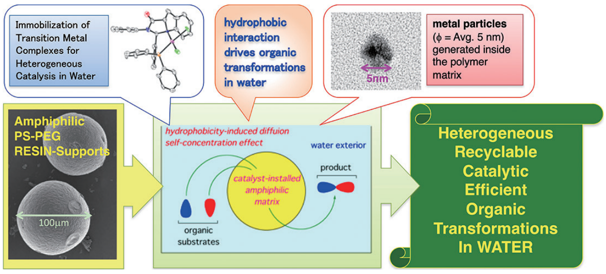

Figure 1. The typical concept of heterogeneous in-water catalyses using amphiphilic polymer-supported complex and nanoparticles catalysts.

Selected Publications

- S. Okumura, K. Torii and Y. Uozumi, "Electrophilic 1,4-Addition of Carbon Dioxide and Aldehydes to Enones," *Org. Lett.* **25**, 5226–5230 (2023).
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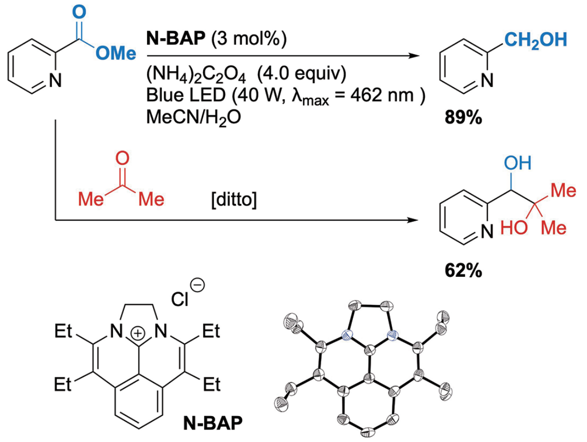
- Y. Uozumi, Y. Matsuura, T. Arakawa and Y. M. A. Yamada, "Asymmetric Suzuki-Miyaura Coupling in Water with a Chiral Palladium Catalyst Supported on Amphiphilic Resin," *Angew. Chem., Int. Ed.* **48**, 2708–2710 (2009).

1. Multielectron-Reduction with a Novel Photocatalyst *N*-BAP[1)]

In 2024, we have developed a novel diazabenzacenaphthenium photocatalyst ***N*-BAP** which was designed with a view to its use as a photocatalyst under visible-light irradiation in photoinduced multielectron-transfer reactions. Indeed, under visible-light irradiation, ***N*-BAP** promoted the four-electron reduction of esters, via the carbinol anion intermediates, to give the corresponding alcohols.[1a)] The intermediates, carbinol anions, also underwent a 1,2-addition to a second carbonyl compound, affording unsymmetric 1,2-diols. Furthermore, the ***N*-BAP**-catalyzed multielectron reduction is now applied to a variety of carbonyl compounds extensively.[1b)]



Scheme 1. Photocatalytic Reduction of Methyl Picolinate.

2. Silver-Mediated Homocoupling of Arylboronic Acids[2)]

We collaborated with Prof. Ohtaka's group at Osaka Institute of Technology to develop a homocoupling of arylboronic acids to form the corresponding biaryls. The coupling reaction proceeded with a catalytic amount of silver carbonate, where silver nanoparticles were generated to promote the reaction.

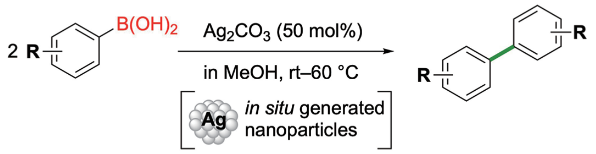
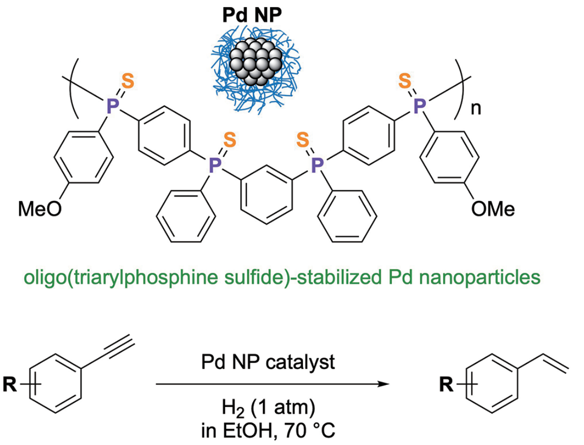


Figure 2.

TEM examination of the reaction mixture revealed that silver nanoparticles were generated in situ under the reaction conditions which should promoted the homocoupling reaction.

3. Oligo(Triarylphosphine Sulfide)s-Stabilized Pd Nanoparticles for Controlled Hydrogenation of Terminal Aryl Alkynes[3)]

In the development of high-performance metal nanoparticle (NP) catalysts, the exploration of new classes of multidentate organic stabilizers is crucial. Prof. Ohta and his co-workers at Ehime University and we have developed a series of structurally diverse oligo(triarylphosphine sulfide)s through the Pd-catalyzed P–C cross-coupling reactions of hydroxymethylphosphine sulfide derivatives with aryl halides. The oligomers were employed as stabilizing agents for Pd NP catalysts. These catalysts were characterized by TEM, EDS, and ICP-MS analyses to determine the average Pd particle size and the constituent elements on the catalyst. We evaluated their catalytic activity in the semihydrogenation of phenylacetylene to styrene in EtOH at 70 °C for 3 h under atmospheric pressure of H_2 with a catalyst loading of 0.5 mol% Pd. It was revealed that Pd NPs stabilized with oligo(triarylphosphine sulfide)s, featuring a high number of coordination sites and a combination of p-phenylene and m-phenylene linkers, exhibited high selectivity for styrene and low Pd leaching.



Scheme 2. Synthesis of oligo(triarylphosphine sulfide)s.

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Design and Synthesis of Organic Molecules for Catalysis

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Our research aims to establish new principles for molecular catalysis and functional molecule discovery by integrating halogen-driven molecular design with digitalized synthesis frameworks. We have focused on two unique classes of halogen-based molecular systems. The first is *perfluorohalogenated arenes*, which exhibit unprecedented cooperative σ-hole and π-hole bonding interactions and provide new insights into organic materials. The second is *halenium complexes* featuring three-center-four-electron (3c4e) halogen bonds, which serve as strong and tunable non-metallic driving forces in catalysis. These studies highlight the potential of non-covalent halogen interactions to expand the chemical space of molecular catalysts and functional small molecules.

Furthermore, we are advancing the digitalization of organic synthesis through collaborations that combine *augmented intelligence* and *automated synthesis systems*. This approach allows accurate prediction of reaction outcomes under untested conditions, thereby accelerating the exploration of novel functional molecules. At the same time, our batch-type automated organic synthesis platform integrates reaction execution, purification, and analysis into a unified process, enabling Human-in-the-Loop collaboration between chemists and AI systems. Together, these approaches pave the way toward a new paradigm of “AI- and data-driven molecular discovery.”

Selected Publications

- T. P. Yoon and E. N. Jacobsen, *Science* **299**, 1691–1693 (2003).
- N. Momiyama and H. Yamamoto, “Brønsted Acid Catalysis of Achiral Enamine for Regio- and Enantioselective Nitroso Aldol Synthesis,” *J. Am. Chem. Soc.* **127**, 1080–1081 (2005).
- N. Momiyama, T. Konno, Y. Furiya, T. Iwamoto and M. Terada, “Design of Chiral Bis-Phosphoric Acid Catalyst Derived from (*R*)-3,3’-Di(2-hydroxy-3-arylphenyl)binaphthol: Catalytic Enantioselective Diels–Alder Reaction of α,β-Unsaturated Aldehydes with Amidodienes,” *J. Am. Chem. Soc.* **133**, 19294–19297 (2011).
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- S. Oishi, T. Fujinami, Y. Masui, T. Suzuki, M. Kato, N. Ohtsuka and N. Momiyama, “Three-Center-Four-Electron Halogen Bond Enables Non-Metallic Complex Catalysis for Mukaiyama-Mannich-Type Reaction,” *iScience* **25**, 105220 (2022).
- N. Ohtsuka, H. Ota, S. Sugiura, S. Kakinuma, H. Sugiyama, T. Suzuki and N. Momiyama, “Perfluorohalogenated Naphthalenes: Synthesis, Crystal Structure, and Intermolecular Interaction,” *CrystEngComm* **26**, 767–772 (2024).

1. Design of Perfluorohalogenated Arenes

Halogens form attractive non-covalent interactions between terminal halogen atoms in compounds of the type R—X (X = Cl, Br, I) and Lewis bases. This non-covalent interaction is known as either halogen bonding or σ-hole bonding, which occurs when R is a highly electronegative substituent, such as perfluorinated alkyl or aryl substituents. Based on the electrophilic feature of the halogen atom, we have investigated perfluorohalogenated arenes, aimed at creating functional molecules.[1,2]

Perfluorohalogenated naphthalenes (PFXNaPs) are unique small molecules with great potential to exhibit a new type of σ-hole and π-hole bonding, owing to the incorporation of multiple F atoms onto the naphthalene ring. We developed a synthetic protocol for PFXNaPs, conduct crystal engineering investigations, and explored the intermolecular interactions of PFXNaPs through π-hole and σ-hole bondings. We successfully synthesized PFXNaPs using Mg amide-mediated halogenation reactions of electron-deficient F_7 and F_6 naphthalenes, achieving good to excellent yields. Crystal structure analyses of 3,6-I_2F_6 naphthalene unveiled intermolecular π-hole stacking between two C atoms in the naphthalene ring, in cooperation with the σ-hole bonding of two I atoms. This mode of intermolecular interaction has not been classified in previous reports. Computational studies show that the π-hole bonding in PFXNaPs is substantially enhanced compared to corresponding benzene molecules without diminishing the σ-hole bonding. The unique stacked interaction in PFXNaPs is primarily governed by electrostatic interaction and dispersion correction energies, with the contribution of C···C contacts being 10 times greater than that in benzene analogs. The results enable further applications of PFXNaPs in the fields of perfluorohalogenated arenes and organic crystalline materials.[2]

2. Design of Halenium Complexes

In the filed of perfluorohalogenated arenes, the two-center-two-electron (2c2e) bond is a key feature. Similarly, halogen(I), generally X^+ (X = I, Br, Cl), acts as a strong halogen bond donor site. In contrast to the 2c2e halogen bond, halogen(I) simultaneously interacts with two Lewis bases. This bond is recognized as a three-center-four-electron (3c4e) halogen bond. Importantly, successful examples have consistently required the use of stoichiometric amounts of 3c4e complexes. Despite the utility of the 3c4e halogen bond in synthetic chemistry, its potential for non-metallic complex catalysis had not been thoroughly investigated until our report.[3,4]

We discovered that the 3c4e halogen bond can serve as a new driving force for catalysis. By integrating halogen(I) (X^+: I^+ or Br^+), the bis-pyridyl ligand *NN*, and a non-nucleophilic counter anion Y, we developed non-metallic complex catalysts, $[N\cdots X\cdots N]$Ys, that exhibited outstanding activity and facilitated the Mukaiyama–Mannich-type reaction of *N*-heteroaromatics with parts-per-million-level catalyst loading. NMR titration experiments, CSI-MS, computations, and UV-vis spectroscopic studies suggest that the robust catalytic activity of $[N\cdots X\cdots N]$Y can be attributed to the unique ability of the 3c4e X-bond to bind chloride: i) the covalent nature transforms the $[N\cdots X\cdots N]^+$ complexation to sp^2 CH as a hydrogen-bonding donor site, and ii) the noncovalent property allows for the dissociation of $[N\cdots X\cdots N]^+$ for the formation of $[Cl\cdots X\cdots Cl]^-$. This study introduces the application of 3c4e X-bonds in catalysis *via* halogen(I) complexes.[4]

3. Digitalization of Organic Synthesis

Recent advances in our group have been directed toward the digitalization of organic synthesis. In collaboration with Professor Kazuhiro Takeda (Shizuoka University), we explored the use of generative machine learning with virtual variables (GMLV) to predict reaction outcomes under untested conditions. In particular, a predictive framework was developed for the deuteration of polyfluoroperylene (PFDPR), a promising luminescent material, based on small experimental datasets. By introducing virtual descriptors that capture the intrinsic relationships between reactants, the model achieved accurate estimation of reaction yields and provided mechanistic insights into reaction processes. This study highlights the potential of digitalized data and AI frameworks to accelerate the discovery of functional molecules.[5]

Furthermore, we have developed a batch-type automated organic synthesis system. Unlike previously developed systems, our platform integrates reaction execution, work-up, purification, and analysis into a unified automated process. The system comprises modular equipment, including a multi-reaction station, robotic arms for liquid handling, automated purification, and mass spectrometry analysis. Importantly, it enables non-experts in organic synthesis to access a comprehensive experimental environment—"pushing a button does almost everything." This infrastructure provides a foundation for Human-in-the-Loop collaborations, where organic chemists and AI agents can work synergistically to accelerate reaction development and molecular discovery.[6]

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3) S. Oishi, T. Fujinami, Y. Masui, T. Suzuki, M. Kato, N. Ohtsuka and N. Momiyama, *iScience* **25**, 105220 (2022).
4) S. Oishi, T. Fujinami, Y. Masui, T. Suzuki, M. Kato, N. Ohtsuka and N. Momiyama, *STAR Protocols* **4**, 102140 (2023).
5) K. Takeda, N. Ohtsuka, T. Suzuki and N. Momiyama, *Comput.-Aided Chem. Eng.* **53**, 2689 (2024).
6) Automated organic synthesis system: This platform was developed with support from the Institute for Molecular Science (IMS), Moonshot Goal 3 (Ushiku Project), and Transformative Research Areas (A) "Digitalization-driven Transformative Organic Synthesis (Digi-TOS)."

Design and Synthesis of Three-Dimensional Organic Structures

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The Young Scientists' Prize
2019 Nozoe Memorial Award for Young Organic Chemists
2021 Chemist Award BCA

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Aromatic compounds are potentially useful as functional electronic materials. However, the controlled synthesis and assembly of three-dimensional complex molecules are still very difficult, especially for the crystal engineering of organic molecules. This group aims to create novel topological and reticular organic structures by using synthetic organic chemistry and geometric insights (Figure 1).

To achieve our purpose, this group will start electron-diffraction crystallography (MicroED) for the rapid structure determination of organic compounds. While X-ray crystallography is a general and reliable method for structure determination, it requires ~0.1 mm single crystals and making such crystal sometimes needs tremendous times and efforts. Since electron beam has much higher diffraction intensity than X-ray, structural analysis can be performed even with ultra-small crystals (1 µm or less). There are many fields such as covalent organic crystals with a three-dimensional structure and molecules with complex molecular topologies, where structural analysis has not been sufficiently developed.

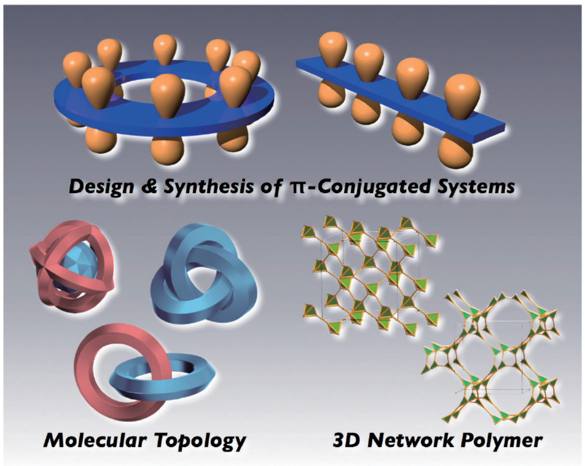

Figure 1. Design and synthesis of π-conjugated organic molecules (top); Development of novel molecular topology (bottom left); Construction of three-dimensional network polymers (bottom right).

Selected Publications

- M. Nagase, R. Yoshida, S. Nakano, T. Hirose and Y. Segawa, *Chem. Commun.* **61**, 11187–11190 (2025).
- K. Watanabe, H. Sugiyama and Y. Segawa, *CrystEngComm* **27**, 3552–3559 (2025).
- K. Watanabe, T. Toya, Y. Toyota, Y. Kobayashi, J. Usuba, Y. Hijikata, R. Matsuda, K. Nishimura, H. Sugiyama and Y. Segawa, *Chem. Commun.* **61**, 2822–2825 (2025).
- H. Sugiyama, K. Watanabe, C. Song, K. Murata and Y. Segawa, *Chem. Lett.* **53**, upae192 (2024).
- R. Yoshida, H. Sugiyama and Y. Segawa, *Chem. Lett.* **53**, upae048 (2024).
- S. Hirota, S. Nakano, H. Sugiyama and Y. Segawa, *Org. Lett.* **25**, 8062–8066 (2023).
- M. Nagase, S. Nakano and Y. Segawa, *Chem. Commun.* **59**, 11129–11132 (2023).
- Y. Segawa, *Chem* **9**, 2725–2727 (2023).
- Y. Segawa, *Bull. Chem. Soc. Jpn.* **95**, 1600–1610 (2022).

1. Twisted π-Conjugated Molecules Featuring 3D π–π Interactions in Solid States

Electronic devices based on organic materials are lightweight, flexible, and can display a wide variety of properties by subtle changes in molecular structure, making them promising environmentally friendly next-generation devices. Most organic electronic materials developed to date are made of planar molecules, so charge transport is confined to limited directions; as a result, devices require strict control of molecular orientation. The team wondered whether “twisting” molecules could yield a new material architecture in which charge carriers move easily in three dimensions.

We attached methyl groups to molecules containing multiple thiophene units, thereby synthesizing twisted molecules. X-ray crystallography confirmed the twisted geometry and revealed that, in the solid state, the molecules stack in a three-dimensional fashion. Computational analysis of charge-transport pathways predicted an aggregated structure in which holes can migrate in several directions. When the molecule was fabricated into an organic field-effect transistor, it exhibited a hole mobility of 1.85×10^{-4} cm^2 V^{-1} s^{-1}, experimentally confirming its behavior as an organic semiconductor.

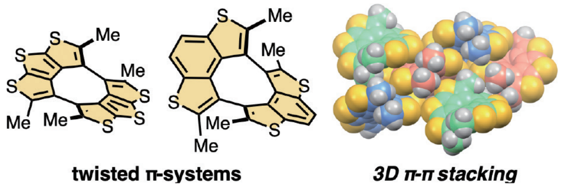

Figure 2. Structures of twisted π-conjugated molecules, and their 3D π–π stacking mode.

2. Diverse Clathrate Crystals Assembled from Weak Intermolecular Interactions

Tetracyanodihydrodipyrazinopyrazines with two mesityl (2,4,6-trimethylphenyl) groups formed clathrate crystals with 15 kinds of organic solvents. Two common types of host molecular networks were observed in the crystals. Theoretical calculations indicated that these host networks are constructed from π–π and CN–π interactions. As these intermolecular interactions are relatively weak, the host network can change flexibly in response to guest molecules. Guest-free crystals can be reversibly transformed into clathrate crystals through crystal-to-crystal phase transitions via the adsorption/desorption of solvent vapor.

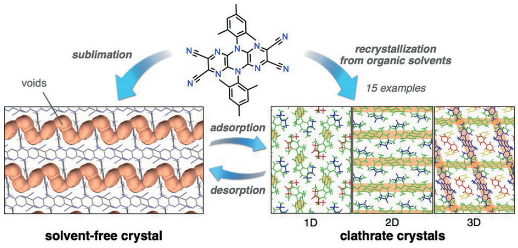

Figure 3. Solvent-free and clathrate crystals of tetracyanodihydrodipyrazinopyrazines.

3. Fully Fused 3D π-Conjugated Polymers Controlled by Steric Repulsion

The synthesis and characterization of fused aromatic networks composed of zinc tetrapyrazinoporphyrazines are reported. The steric repulsion of bulky substituents induced the formation of three-dimensional structures. Thus-obtained insoluble polymers adsorbed CO_2 and had near-infrared absorption indicating their porosity and extended π-conjugation.

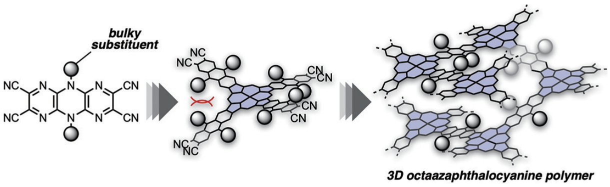

Figure 4. Synthesis of fully fused 3D π-conjugated polymers.

4. Structure Determination of π-Extended Tetraphenylenes by MicroED

The structure determination of large π-conjugated molecules using microcrystal electron diffraction (MicroED) was demonstrated. The poorly soluble solids of the tweezer-shaped molecules were subjected directly from the reaction vessel to MicroED to determine their structures and packing modes. This work validated the efficiency of MicroED in determining the structures of such compounds.

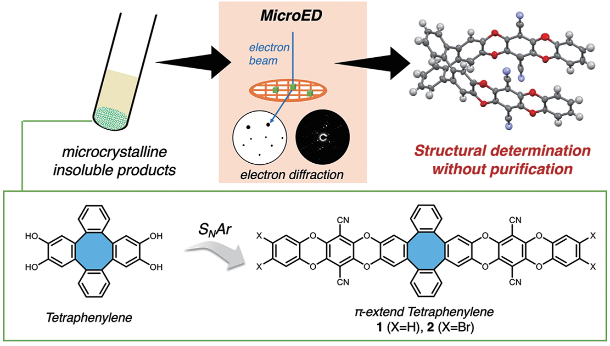

Figure 5. The synthesis and structure determination of π-extended tetraphenylene **1** and **2**.

References

1) M. Nagase, R. Yoshida, S. Nakano, T. Hirose and Y. Segawa, *Chem. Commun.* **61**, 11187–11190 (2025).

2) K. Watanabe, H. Sugiyama and Y. Segawa, *CrystEngComm* **27**, 3552–3559 (2025).

3) K. Watanabe, T. Toya, Y. Toyota, Y. Kobayashi, J. Usuba, Y. Hijikata, R. Matsuda, K. Nishimura, H. Sugiyama and Y. Segawa, *Chem. Commun.* **61**, 2822–2825 (2025).

4) H. Sugiyama, K. Watanabe, C. Song, K. Murata and Y. Segawa, *Chem. Lett.* **53**, upae192 (2024).

Visiting Professors

Visiting Professor

SATO, Sota *(from The University of Tokyo)*

Integrated Molecular Structure Analysis Through Industry-Academia Collaboration

Elucidating molecular structures is crucial in various fields of molecular science, regardless of academia or industry. In addition to NMR and mass spectrometry, X-ray/electron diffraction is a powerful analytical technique that can directly determine atomic positions, enabling clear determination of three-dimensional structures. We are actively pursuing the “crystalline sponge method” as one of core technologies, which eliminates the need for the crystallization process and completes sample preparation by simply soaking the target molecules into crystalline sponge. Recently, we achieved to reduce sample amount into only 3 ng using small-wedge synchrotron crystallography method. Furthermore, we are building collaborative relationships with numerous companies to promote research aimed at creating new industries. Also, we have been dedicated to fostering future talent who will support the scientific community in Japan and the world. We organized mock lectures and research experiences for junior high and high school students in collaboration with corporate researchers, aiming to nurture the next generation of scientists.

Visiting Professor

HAYASHI, Kumiko *(from The University of Tokyo)*

Interdisciplinary Research on Motor Proteins

Motor proteins move and carry out their functions by using the energy obtained from the hydrolysis of adenosine triphosphate (ATP). Our group has conducted research on motor proteins such as F_1-ATPase, a component of F_oF_1-ATP synthase that produces ATP in cells, and the kinesin motor KIF1A, which is responsible for axonal transport in neurons. To study these systems, we employ single-molecule experiments, cellular experiments, and theoretical analyses based on extreme-value statistics and nonequilibrium statistical mechanics. These approaches have enabled physical measurements of motor proteins under nonequilibrium conditions, which had been difficult to achieve with equilibrium statistical mechanics. At present, through collaborations with the National Institute of Information and Communications Technology (NICT) and the Institute for Molecular Science (IMS), we are pursuing the development of high-precision force measurements of these motor proteins using DNA origami technology. In the future, we aim to elucidate the in-cell mechanisms of motor protein motility through the development of this approach.

Visiting Associate Professor

SATO, Shinichi *(from Tohoku University)*

Development of Protein Labeling Methods and Applications to Chemical Proteomics

Our research tackles the fundamental challenge of understanding protein behavior in living systems through the development of organic chemistry-based tools. To address questions such as post-translational modifications, protein–protein interactions, and protein conformational changes that are difficult to investigate using biological methods alone, we have established unique chemical methodologies including protein labeling techniques that function specifically in nanometer-scale spaces within living systems, methods for detecting changes in amino acid residue (Tyr, His) exposure on protein surfaces, and technologies for selectively labeling aggregated proteins. By combining these novel chemical approaches with rapidly advancing proteomics techniques using mass spectrometry—capable of simultaneously analyzing thousands to tens of thousands of proteins in a single experiment—we aim to advance our understanding of biological phenomena, develop new manipulation technologies, and contribute to drug discovery applications.