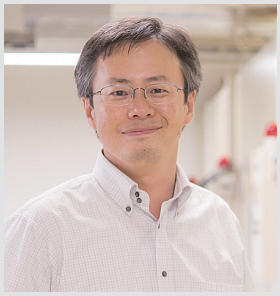
RESEARCH ACTIVITIES
Research Center of Integrative Molecular Systems

The mission of CIMoS is to analyze molecular systems in nature to find the logic behind the sharing and control of information between the different spatiotemporal hierarchies, with the ultimate goal of creating novel molecular systems on the basis of these findings.

Biological Rhythm and Dynamics through Chemistry

Research Center of Integrative Molecular Systems
Division of Trans-Hierarchical Molecular Systems



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The Young Scientists' Prize
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2006 SAS Young Scientist Prize, IUCr Commission on Small-angle Scattering
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Keywords Biological Rhythm, Circadian Clock, Cyanobacteria

Living organisms on Earth evolved over time to adapt to daily environmental alterations, and eventually acquired endogenous time-measuring (biological clock) systems. Various daily activities that we perform subconsciously are controlled by the biological clock systems sharing three characteristics. First, the autonomic rhythm repeats with an approximately 24-hour (circadian) cycle (self-sustainment). Second, the period is unaffected by temperature (temperature compensation). Third, the phase of the clock is synchronized with that of the outer world in response to external stimuli (synchronization). We seek to explain these three characteristics, and consider the biological clock system of cyanobacteria to be an ideal experimental model.

The major reason that cyanobacteria are considered to be the ideal experimental model is that the core oscillator that possesses the three characteristics of the clock can be easily reconstructed within a test tube. When mixing the three clock proteins KaiA, KaiB, and KaiC with ATP, the structure and enzyme activity of KaiC change rhythmically during a circadian cycle. Taking advantage of this test tube experiment, we used an approach combining biology, chemistry, and physics to elucidate the means by which the clock system extends from the cellular to atomic levels.

Among the three Kai proteins, KaiC is the core protein of the oscillator. In the presence of KaiA and KaiB, KaiC revelas the rhythm of autophosphorylation and dephosphorylation; however, the cycle of this rhythm depends on the ATPase activity of KaiC independent of KaiA or KaiB. For example, when the ATPase activity of KaiC doubles as a result of amino acid mutations, the frequencies of both the *in vitro* oscillator and the intracellular rhythm also double (the cycle period is reduced to half). This mysterious characteristic is called a transmural hierarchy, in which the cycle (frequency) and even the temperature compensation both *in vitro* and *in vivo* are greatly affected (controlled) by the function and structure of KaiC.

How are the circadian activities and temperature compensation features encoded in KaiC and then decoded from it to propagate rhythms at the cellular level? We are committed to better understanding biological clocks and other dynamic systems through the chemistry of circadian ***rhythm***, ***structure***, and evolutionary ***diversity***.

Selected Publications

- Y. Furuike, A. Mukaiyama, S. Koda, D. Simon, D. Ouyang, K. Ito-Miwa, S. Saito, E. Yamashita, T. Nishiwaki, K. Terauchi, T. Kondo and S. Akiyama, *Proc. Natl. Acad. Sci. U. S. A.* **119**, e2119627119 (2022).
- Y. Furuike, A. Mukaiyama, D. Ouyang, K. Ito-Miwa, D. Simon, E. Yamashita, T. Kondo and S. Akiyama, *Sci. Adv.* **8**, eabm8990 (2022).
- Y. Furuike, D. Ouyang, T. Tominaga, T. Matsuo, A. Mukaiyama, Y. Kawakita, S. Fujiwara and S. Akiyama, *Commun. Phys.* **8**, 75 (2022).
- J. Abe, T. B. Hiyama, A. Mukaiyama, S. Son, T. Mori, S. Saito, M. Osako, J. Wolanin, E. Yamashita, T. Kondo and S. Akiyama, *Science* **349**, 312–316 (2015).
- Y. Murayama, A. Mukaiyama, K. Imai, Y. Onoue, A. Tsunoda, A. Nohara, T. Ishida, Y. Maéda, T. Kondo and S. Akiyama, *EMBO J.* **30**, 68–78 (2011).
- S. Akiyama, A. Nohara, K. Ito and Y. Maéda, *Mol. Cell* **29**, 703–716 (2008).

1. *Structure*: Reasons for Seeking Structure and Dynamics of Circadian Clock Components in Cyanobacteria[1–4)]

A great deal of effort has been devoted to characterizing structural changes in the clock proteins along the circadian reaction coordinate. However, little is known about the mechanism driving the circadian cycle, even for the simple cyanobacterial protein KaiC that has ATPase and dual phosphorylation sites in its N-terminal C1 and C-terminal C2 domains, respectively. Nearly all KaiC structures reported to date share a nearly identical structure, and they do not appear to be suggestive enough to explain the determinants of circadian period length and its temperature compensation. We are studying the structural and dynamical origins in KaiC using high-resolution x-ray crystallography,[1–4)] real-time fluorescence detection,[5)] and quasielastic neutron scattering.[6)]

2. *Rhythm*: Cross-Scale Analysis of Cyanobacterial Circadian Clock System[6–8)]

KaiC ATPase is of particular interest here, as it finely correlates to the frequencies of *in vivo* as well as *in vitro* oscillations and also it is temperature compensated. This unique property has inspired us to develop an ATPase-based screening[7)] for KaiC clock mutants giving short, long, and/or temperature-dependent periods.[8)] A developed HPLC system with a 4-channel temperature controller has reduced approximately 80% of time costs for the overall screening process (Figure 1). Using the developed device, we are screening a number of temperature-dependent mutants of KaiC.[6,7)]

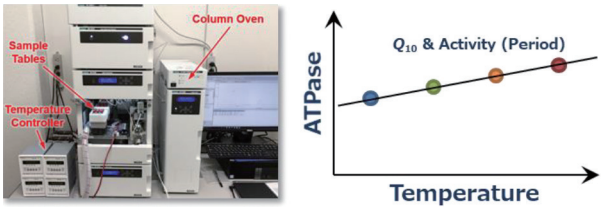

Figure 1. Development of a quick ATPase assay system.

3. beyond Evolutionary *Diversity*[9)]

In the presence of KaiA and KaiB, the ATPase activity of KaiC oscillates on a 24-hour cycle. KaiC is not capable of maintaining a stable rhythm on its own, but its activity was observed to fluctuate with reduced amplitude over time (Figure 2A). We have identified a signal component that is similar to damped oscillation, and propose that it encodes the specific frequency, equivalent to a 24-hour cycle.

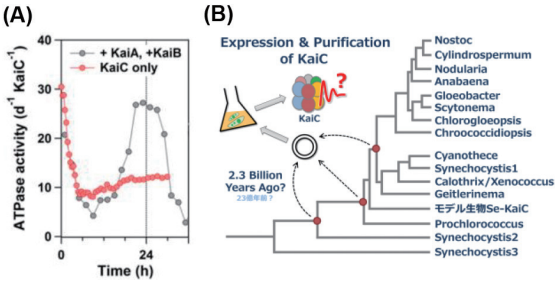

Figure 2. Damped oscillation of KaiC ATPase activity (**A**) and evolutionary diversity of cyanobacteria (**B**).

The habitats of cyanobacteria are diverse, so the space of their sequence is immense.[9)] Furthermore, some KaiA and KaiB genes are missing in several strains of cyanobacteria. This is understandable to some extent if KaiC possesses the specific frequency. Given this assumption, *what specific frequencies are possessed by KaiC homologues in other species and ancestral cyanobacteria?* (Figure 2B) If you strain your ears, the rhythms of the ancient Earth may be heard from beyond evolutionary diversity.

4. Bio-SAXS Activity in IMS[10)]

We have supported SAXS users so that they can complete experiments smoothly and publish their results.[10)]

References

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2) Y. Furuike, A. Mukaiyama, S. Koda, D. Simon, D. Ouyang, K. Ito-Miwa, S. Saito, E. Yamashita, T. Nishiwaki-Ohkawa, K. Terauchi, T. Kondo and S. Akiyama, *Proc. Natl. Acad. Sci. U. S. A.* **119**, e2119627119 (2022).
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8) D. Simon, A. Mukaiyama, Y. Furuike and S. Akiyama, *Biophys. Physicobiol.* **19**, e190008 (2022)..
9) A. Mukaiyama, D. Ouyang, Y. Furuike and S. Akiyama, *Int. J. Biol. Macromol.* **131**, 67–73 (2019).
10) M. Okumura, S. Kanemura, M. Matsusaki, Y. H. Lee, S. Akiyama and K. Inaba, *Structure* **29**, 1–14 (2021).

Award

FURUIKE, Yoshihiko; The Progress Award of the Crystallographic Society of Japan (2022).

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Elucidation of Function, Structure, and Dynamics of Condensed-Phase Molecular Systems by Advanced Ultrafast Laser Spectroscopy

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2019 RSC PCCP Prize
2020 The Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology
The Young Scientists' Award
2020 Morino Foundation for Molecular Science
2020 The 13th Young Scientist Awards of the Japan Society for Molecular Science
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Keywords Ultrafast Spectroscopy, Nonlinear Spectroscopy, Chemical Reaction Dynamics

We develop and apply advanced ultrafast laser spectroscopy based on state-of-the-art optical technology to study the chemical reaction dynamics of the condensed-phase molecules. In particular, we focus on exploiting unique methodologies based on few-cycle ultrashort pulses (*e.g.*, time-domain impulsive vibrational spectroscopy and multidimensional spectroscopy) and tracking molecular dynamics from electronic and structural viewpoints throughout the chemical reaction with exquisite temporal resolution. We also develop a novel methodology and light source to probe ultrafast dynamics of single molecules in the condensed phase at room temperature, with the aim to understand chemical reaction dynamics at the single-molecule level. Our particular interest rests on elucidating sophisticated molecular mechanisms that underlie the reactions of functional molecular systems such as proteins, molecular assemblies, and metal complexes. On the basis of new insights that can be gained from our advanced spectroscopic approaches, we aim to establish a new avenue for the study of chemical reaction dynamics.

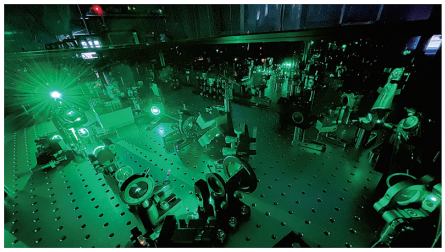

Figure 1. Setup for advanced ultrafast spectroscopy based on sub-10-fs pulses.

Selected Publications

- Y. Yoneda, and H. Kuramochi, "Rapid-Scan Resonant Two-Dimensional Impulsive Stimulated Raman Spectroscopy of Excited States," *J. Phys. Chem. A* **127**, 5276–5286 (2023).
- H. Kuramochi and T. Tahara, "Tracking Ultrafast Structural Dynamics by Time-Domain Raman Spectroscopy," *J. Am. Chem. Soc.* **143**, 9699–9717 (2021).
- H. Kuramochi, S. Takeuchi, M. Iwamura, K. Nozaki and T. Tahara, "Tracking Photoinduced Au–Au Bond Formation through Transient Terahertz Vibrations Observed by Femtosecond Time-Domain Raman Spectroscopy," *J. Am. Chem. Soc.* **141**, 19296–19303 (2019).
- H. Kuramochi, S. Takeuchi, H. Kamikubo, M. Kataoka and T. Tahara, "Fifth-Order Time-Domain Raman Spectroscopy of Photoactive Yellow Protein for Visualizing Vibrational Coupling in Its Excited State," *Sci. Adv.* **5**, eaau4490 (2019).
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- T. Fujisawa, H. Kuramochi, H. Hosoi, S. Takeuchi and T. Tahara, "Role of Coherent Low-Frequency Motion in Excited-State Proton Transfer of Green Fluorescent Protein Studied by Time-Resolved Impulsive Stimulated Raman Spectroscopy," *J. Am. Chem. Soc.* **138**, 3942–3945 (2016).

1. Rapid-Scan Resonant Two-Dimensional Impulsive Stimulated Raman Spectroscopy of Excited States

Photochemical reactions occur in the electronically excited state, which is effectively represented by a multi-dimensional potential energy surface (PES) with a vast degree of freedom of nuclear coordinates. The elucidation of the intricate shape of the PES constitutes an important topic in the field of photochemistry and has long been studied both experimentally and theoretically. Recently, fully time-domain resonant two-dimensional Raman spectroscopy has emerged as a potentially powerful tool to provide unique information about the coupling between vibrational manifolds in the excited state.[1,2] However, the wide application of this technique has been significantly hampered by the technical difficulties associated with experimental implementation and remains challenging. We demonstrated fully time-domain resonant two-dimensional impulsive stimulated Raman spectroscopy (2D-ISRS) of excited states using sub-10-fs pulses based on the rapid scan of the time delay, which facilitates the efficient collection of time-domain signals with high sensitivity. As a proof-of-principle experiment, we performed 2D-ISRS of TIPS-pentacene in solution. Through 2D Fourier transformation of the high-quality time–time oscillatory signal, we obtained a 2D frequency–frequency correlation map of excited-state TIPS-pentacene in the broad frequency window of 0–2000 cm^{-1}. The data clearly resolve a number of cross peaks that signify the correlations among excited-stated vibrational manifolds. The high capability of the rapid-scan-based 2D-ISRS spectrometer presented in this study enables the systematic investigation of various photochemical reaction systems, thereby further promoting the understanding and applications of this new multi-dimensional spectroscopy.[3]

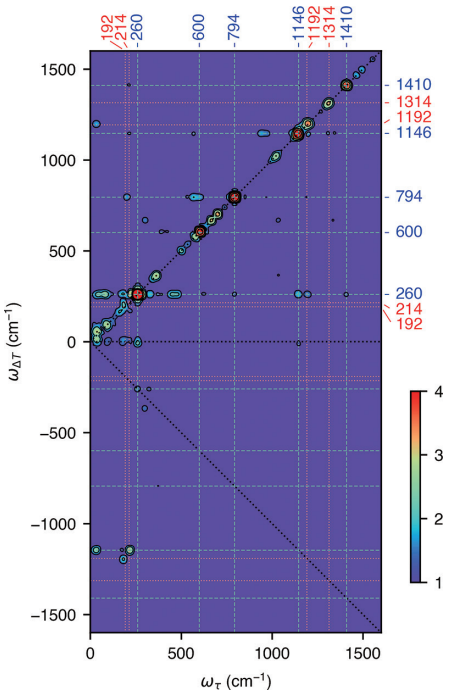

Figure 2. Two-dimensional frequency-frequency correlation map of excited-state TIPS-pentacene in chloroform.

2. Fourier Transform Excitation-Emission Spectroscopy with Phase-Locked Pulse Pairs

Polyatomic molecules in condensed phases undergo constant fluctuations in molecular structure and solvent environment. These fluctuations can lead to variations in the physical properties, reactivities, and functionalities. However, conventional ensemble measurements only provide statistically averaged information, making it challenging to observe the properties of individual molecules and transitions between subensembles. In order to overcome this limitation, we aim to develop new single-molecule spectroscopic techniques capable of observing the fluctuations of electronic and vibrational transitions and reaction dynamics. Recently, we developed Fourier transform excitation-emission spectroscopy in a room-temperature solution. We send a phase-locked pulse pair of broadband pulses to a home-built confocal microscope and detect the fluorescence with time-correlated single photon counting. Fourier transform of the fluorescence interferogram obtained by scanning the interpulse delay provides a fluorescence excitation spectrum, which shows an identical spectral shape to the bulk absorption spectrum. We aim to achieve single-molecule sensitivity in this experiment and interrogate how the excitation energy of chromophores fluctuates with large amplitude spontaneous fluctuation of photoresponsive proteins.

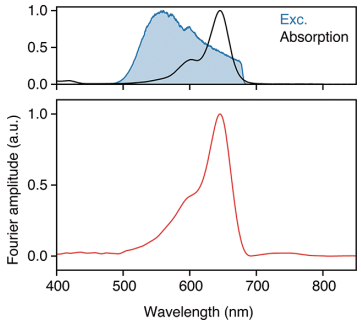

Figure 3. Fluorescence excitation spectrum of ATTO647N aqueous solution measured with broadband pulses. The excitation spectrum and bulk absorption spectrum are shown at the top.

References

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3 Y. Yoneda, H. Kuramochi, S. Takeuchi and T. Tahara, *J. Phys. Chem. A* **127**, 5276–5286 (2023).

* IMS International Internship Program

Open up Future Electronics by Organic Molecules

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Keywords Organic Spintronics, Chirality, Organic Superconductor

Spintronics is a new indegredient of electronics in which a magentic moment of an electron is utilized as an information carrier together with its charge. Spin-polized current is one of the most important resources in spintronics, because it can drive devices such as ferromagnetic memory with spin angular momentum. In convetional spintronics, such a spin-polarized current is generated by passing a charge current through ferromagnetic metals. However, recently, researchers are finding other ways of spin-polarized current genertation by using topological insulators and non-collinear antiferromagnets, which can sometimes be more efficient than those with ferromagnets.

Chiral molecules are attracting recent attention as a new source of spin-polarized current. Chirality-Induced Spin Selectivity (CISS) effect generates spin polarization parallel to or antiparallel to the electron's velocity depending on the handedness of the chiral molecule that is being passed through by a tunneling electron (Figure 1). Although the mechanism of CISS effect is still under debate, it seems to create spin-polarization higher than those of ferromagnets, which is suprisingly large when the small spin–orbit coupling energy of organic molecules is considered. In order to rationalize such a large effect, some microscopic hyptheses are proposed based on experimental results, whose proofs are being waited for. Our group is trying to unveil such mechanisms that drive CISS effect by using chiral crystalline materials.

The use of crystalline materials has serveral advantages. For example, one can employ theoretical framework with well-difined wave number of electrons. Another advantage is the size of the chiral material which allows direct attachment of detection electrodes in different positions. With these merits in mind, we are fabricating spintronic devices suitable for the CISS investigations.

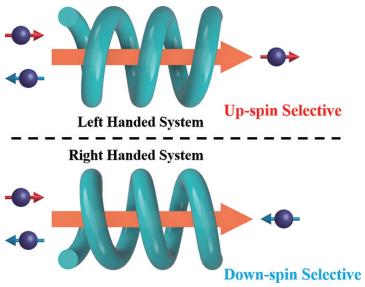

Figure 1. Conceptual schematic for CISS effect. P-helix molecule (lower panel) can transmit more electrons with spins antiparallel to the velocity (negative helicity electrons) than the other, while M-helix molecule (upper panel) favors transmission of electrons with parallel spin (positive helicity electrons).

Selected Publications

- R. Nakajima, D. Hirobe, G. Kawaguchi1, Y. Nabei1, T. Sato, T. Narushima, H. Okamoto and H. M. Yamamoto, *Nature* **613**, 479 (2023).
- Y. Nabei, D. Hirobe, Y. Shimamoto, K. Shiota, A. Inui, Y. Kousaka, Y. Togawa and H. M. Yamamoto, *Appl. Phys. Lett.* **117**, 052408 (2020).
- A. Inui, R. Aoki, Y. Nishiue, K. Shiota, Y. Kousaka, H. Shishido, D. Hirobe, M. Suda, J.-i. Ohe, J.-i. Kishine, H. M. Yamamoto and Y. Togawa, *Phys. Rev. Lett.* **124**, 166602 (2020).
- M. Suda, Y. Thathong, V. Promarak, H. Kojima, M. Nakamura, T. Shiraogawa, M. Ehara and H. M. Yamamoto, "Light-Driven Molecular Switch for Reconfigurable Spin Filters," *Nat. Commun.* **10**, 2455 (7 pages) (2019).

1. Spin Current Generation in a Chiral Organic Superconductor

Although *s*- and *d*-wave superconductors are in a spin singlet state at its ground state, a superconductor with broken mirror symmetry is expected to show spin triplet state when supercurrent is flowing, according to a theory developed by Edelstien.[1)] This means spin polarization can be generated by applying supercurrent in a chiral superconductor. The magnetization direction that depends on the lattice symmetry has been recently calculated by group theory.[2)] We have tested this idea by employing κ-(BEDT-TTF)$_2$Cu(NCS)$_2$ (hereafter, κ-NCS) which is an organic superconductor with chiral and polar crystal lattice. The space group of this crystal is $P2_1$, and its handedness is defined by the relative arrangement between the anionic Cu(NCS)$_2$ and cationic BEDT-TTF. This handedness can be experimentally determined by X-ray diffraction or circular dichroism (CD).

After confirming pure enantiomeric lattice system with CD microscope, a thin crystal of κ-NCS has been laminated onto a resin substrate with prepatterned gold and nickel electrodes. At temperature lower than superconducting T_c, an a.c. electrical excitation was applied to induce spin polarization (Figure 2). The spin polarization accumulated at the interface between κ-NCS and the magnetic electrode was detected as a built-up voltage that is dependent on the relative angle between the accumulated and ferromagnetic spins. We have compared the observed voltage with theoretical estimation and found that it exceeds the value predicted by Edelstein effect more than 1000 times. This surprising result suggests that there is a spin enhancement effect other than Edelstein effect, implying existence of an effect analogous to CISS for a chiral superconductor.

By measuring the angle dependency of this magneto–voltaic signal, the direction of accumulated spin could be determined. The observed spin polarization direction was dependent on the location of the detection electrode inside the crystal, and its arrangement was consistent with a magnetic monopole structure which has been hypothesized in a chiral molecule under non-equilibrium state with CISS effect. More specifically, the spin accumulation was forming an antiparallel pair on the upper and lower sides of the κ-NCS crystal. With a right-handed crystal, the accumulated spins showed outward spin pairs.

To our surprise, this spin accumulation could be observed in nonlocal measurements where the excitation and detection electrodes are separated by 600 μm. We have also fabricated a nonlocal detection device with a crystal possessing two chirality domains where right- and left-handed crystal structures are spatially separated. By exciting this crystal at two different positions with opposite handednesses, we have observed a switching of spin pairing mode from outward to inward. This corresponds to the sign reversal of magnetic monopole in the language of multipole expression.[3)] An interesting point here is that the sign of magnetic monopole, which shows time-reversal-odd (*T*-odd) characteristics, is connected to the chirality of underlying crystal lattice so that representing *T*-odd chirality. Although this *T*-odd chirality is a metastable state and disappears at ground state, its relevance to the enantio-separation experiments in CISS effect is directly implied in this experiment. If one accepts the fact that a sign of such a metastable magnetic monopole at excitation can represent the sign of chirality (electric toroidal monopole) at ground state lattice, both the large enhancement of spin polarization and the enantio-separation of chiral molecules at non-equilibrium state observed in CISS experiments can be naturally understood, because such a monopole can interact with magnetic substrate in a handedness-specific manner. In this sense, this experiment provides the first direct observation of spin pair (or magnetic monopole) formation from coherent chiral system and provides proof of concept for microscopic CISS mechanism. Although the Hamiltonians describing the chiral superconductor and chiral molecules are quite different, there are many common features such as singlet ground state, chiral lattice and quantum coherence over the entire body. Therefore, we believe the present result provides a lot of stimulating insights for microscopic understanding of CISS. Since the conversion from *T*-even spin current to *T*-odd spin accumulation requires time integration with an existence of spin reservoir, the spin carriers in chiral molecules and superconductors should be identified in future studies. We also expect emergence of superconducting spintronics once a sourcing of spin-polarized current in superconductor is established by chiral superconductors.

(BEDT-TTF = bis(ethylenedithio)tetrathiafulvalene)

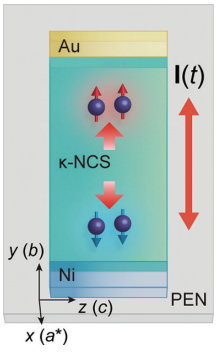

Figure 2. Device schematic for the detection of spin polarization in a chiral superconductor κ-NCS. By applying electrical current, electron spins are polarized along the current direction by CISS-like effect which can be detected as voltage across the κ-NCS/Ni interface. The amplitude of the signal is proportional to the accumulated spins at the interface.

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* IMS International Internship Program

Design of Protein Functions Using Computational and Experimental Approaches

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Division of Trans-Hierarchical Molecular Systems

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Our research is to design a variety of protein functions using computational and experimental approaches. We try to (1) design enzymes from scratch and reveal the origin of the enzymatic activity, (2) control concerted functions by rationally engineering protein complexes and understand their mechanisms and (3) uncover roles of protein complexes in cells and control cellar functions by creating several customized proteins or protein complexes.

1. Design of Allosteric Sites into a Rotary Molecular Motor by Restoring Lost Function of Pseudo-Active Sites

We have succeeded in designing artificial allosteric sites (where by binding an effector molecule, activity at the distal active site is regulated) into a rotary molecular motor, *Enterococcus hirae* V_1-ATPase.[1] The allosteric sites were created by restoring lost functions of pseudo-active sites in a pseudo enzyme, of which function is predicted to have been lost during the evolution. Single-molecule experiments together with X-ray crystallography analyses revealed that the rotational rate of the designed V_1-ATPase, which was restored the lost ATP binding ability at the pseudo-active sites, is allosterically accelerated. In principle, our strategy enables us to create allosteric sites into various kinds of protein complexes and to artificially control the concerted functions.

Reference

1) T. Kosugi, T. Iida, M. Tanabe, R. Iino and N. Koga, *Nat. Chem.* **15**, 1591–1598 (2023).