Molecular Assembly-Based Therapies and Scaffolds in the Central Nervous System

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New innovations have always driven the advancement of science and technology, including neurobiology. Our team aims to pioneer a novel drug discovery modality, proposed as “molecular assembly medicine”, through interdisciplinary collaboration with physics, chemistry, and engineering experts.

While molecular medicines such as antibody and nucleic acid drugs focus on modifying the functions of individual molecules, biological systems work through functional assemblies of molecules. To address this complexity, we have developed "JigSAP" (Jigsaw-Shaped Self-Assembling Peptide), an 11-amino-acid peptide that self-assembles into a structure mimicking the extracellular matrix (ECM). This artificial ECM uniquely facilitates the sustained release of bioactive molecules, offering exciting possibilities for therapeutic applications.

In this talk, I will introduce the concept of “molecular assembly medicine” and the interdisciplinary technologies driving its development. I will also highlight its potential applications in injured brain regeneration and its implications for uncovering novel mechanisms of brain repair. Finally, I would like to discuss with participants the key elements necessary for successful collaborative research across diverse fields, where differing values and backgrounds can often be challenging.

Development and application of photochemical ultra-fast DNA crosslinking

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Biotechnology using conventional enzymes has become indispensable for basic research in the fields of genetic diagnosis and biochemistry, etc. Nucleic acid manipulation using photo- crosslinking has the following features: 1) it can be used under a wide range of conditions without restrictions on pH, temperature, salt strength, etc., 2) no reagents need to be added, and 3) it is easy to control the reaction by timing and energy of photoirradiation. We have reported various photo-crosslinked artificial nucleic acids, and cyanovinylcarbazole nucleoside (^{CNV}K), a representative photo-crosslinker, can be photo-crosslinked with pyrimidine bases such as thymine or cytosine in complementary DNA or RNA strands by 366 irradiation for about 1 second. In addition, photocleavage reaction can be induced by 312 nm irradiation, and photo-reversible manipulation is possible. Compared to previously known psoralenes and coumarins, ^{CNV}K and its improved photo-crosslinker, ^{CNV}D, have high photoreactivity and are already on the market. Therefore, in this presentation, I intend to introduce the development of this ultrafast photo-crosslinking method and its application in the biotechnology field.

Seesaw protein: Design of a protein that adopts interconvertible alternative functional conformations and its dynamics.

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According to classical Anfinsen's dogma, a protein folds into a single unique conformation with minimal Gibbs energy under physiological conditions. However, certain natural proteins fold into two or more conformations from single amino acid sequences. For instance, metamorphic proteins reversibly change their topology in response to environmental factors leading to topology-dependent functional changes. While protein engineers have developed methods to design conformation-switching proteins, the creation of proteins with two distinct functional conformations remains elusive.

Here, we designed a protein that adopts interconvertible alternative functional conformations, termed "seesaw" protein (SSP). An SSP was engineered by fusing GFP lacking the C-terminal β-strand and DHFR lacking the N-terminal β-strand with an overlapping linker, which can be competitively incorporated into either the GFP or the DHFR moiety. In vivo and biochemical analyses, including AFM imaging, demonstrated that the SSP adopts two alternative conformations, which can be biased by point mutations and ligand binding. The drastic conformational change upon the ligand binding was directly visualized by high-speed AFM. Furthermore, the balance of the seesaw can be reversibly changed depending on buffer conditions. In summary, our design strategy for SSP provides a new direction for creating artificial proteins with on-off behaviors.

Innovative Boron Delivery System for Tumor Targeting: Utilizing Non-Covalent Albumin Ligands for Enhanced Neutron Capture Therapy

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Boron Neutron Capture Therapy (BNCT) is a minimally invasive cancer therapy that selectively destroys tumor cells through the nuclear reaction between boron and neutrons. In 2020, Japan approved accelerator-based BNCT for head and neck cancers using 4-borono-L-phenylalanine (BPA, Fig. 1A). BPA is taken up into cells primarily thorough L-types amino acid transporter 1, which is highly expressed in many types of cancer cells. However, BPA is not effective for all cancer types, highlighting the urgent need for the development of boron delivery agents with novel mechanisms of action to expand BNCT's clinical applications.

Our research group has developed an albumin-covalently-conjugated boron carrier and demonstrated its efficacy in BNCT for various cancers, including mouse colon cancer [1], rat glioma [2,3], and human glioblastoma [4]. Building on this success, we proposed a tumor-targeted boron delivery strategy that exploits endogenous albumin. To this end, we designed a low-molecular-weight boron carrier incorporating an albumin ligand (iodophenyl moiety) to non-covalently bind to albumin at its Sudlow site II [5]. More recently, we developed a novel small-molecule boron carrier, pteroyl-*closo*-dodecaborate conjugated with a 4-(*p*-iodophenyl)butyric acid moiety (PBC-IP, Fig. 1B). PBC-IP not only leverages endogenous albumin but also targets folate receptors, which are overexpressed on the surfaces of various cancer cells. Preclinical studies revealed that PBC-IP demonstrates remarkable efficacy in BNCT against malignant gliomas with low BPA uptake in animal models [6,7]. In addition, we recently developed a boron carrier featuring an albumin ligand targeting biotin receptors and validated its efficacy in BNCT for mouse colon cancer [8]. These advancements highlight the potential of albumin-based boron carriers to expand the therapeutic reach of BNCT and overcome limitations associated with BPA-based treatments.

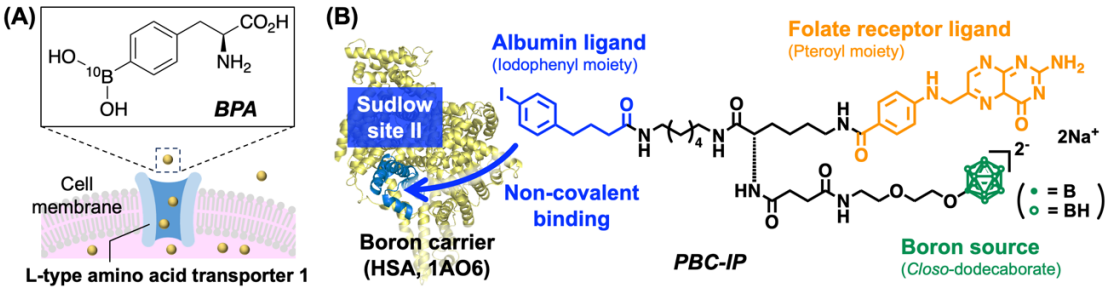

Fig. 1. (A) Approved boron drug for BNCT, BPA: Targets L-type amino acid transporter 1. (B) PBC-IP: Utilizes endogenous albumin as a carrier and targets folate receptors.

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Investigating skin photoaging by detecting dysregulations in circadian rhythms

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Photoaging is premature aging due to frequent exposure to high-energy light from the sun or artificial sources, especially from digital devices. Blue light has the highest energy within the visible light spectrum. While the effects of UV radiation on skin aging have been extensively studied, the effects of blue light are less understood. These effects not only result in morphological changes but also disrupt physiological homeostasis, including the circadian rhythm. However, the effects of blue light on circadian rhythm disruptions related to skin photoaging remain elusive.

Human keratinocyte cells and dermal fibroblasts were exposed to blue light in monolayer culture and spheroid coculture. Features of skin photoaging were observed as blue light increased the expression of *MMP2* and *NAMPT* genes while decreasing the expression of *COL3* and *SIRT1* genes in dermal fibroblasts. In addition, blue light disrupted the skin's circadian rhythm by delaying the trough time of *PER3* gene in dermal fibroblasts and the peak time of *SIRT1* gene in spheroid coculture. Moreover, blue light reduced the amplitude of tight junction genes expression in keratinocyte cells. In our *in vivo* study, blue light advanced the peak time of skin barrier performance in mice. To our knowledge, these findings provide initial evidence of the circadian profile of the skin under photoaging conditions. Our study introduces new perspectives for understanding photoaging phenomena and highlights the potential for establishing circadian medicine as an intervention for aging.

Design of intracellular antibody for live-cell imaging using AI tools

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Posttranslational modifications of histones are involved in the regulation of gene expression in various biological phenomena such as differentiation and development. While some histone modifications on specific genome loci are stably maintained to secure epigenomic gene regulation, others exhibit dynamic changes. To analyze histone modification dynamics, we have developed a genetically encoded live-cell imaging probe named mintbody (modification-specific intracellular antibody), which is a fusion protein consisting of the single chain variable fragment (scFv) of a modification-specific antibody and a fluorescent protein. However, most (>90%) of scFvs failed to be expressed as functional molecules to detect the target modification in cells, resulting in a low-throughput development. To improve the mintbody development throughput, we have established a platform for designing scFvs with high structural stability using ProteinMPNN, which is a deep leaning-based tool and predicts protein sequences expected to fold into the same 3D structure as the input. We synthesized DNA fragments coding designed mintbody and transfected into cells to evaluate whether the mintbody is localized to the nucleus. Unlike the original mintbodies that have the same amino acid sequences as the hybridoma genome, most ProteinMPNN-designed mintbodies showed nuclear enrichments, resulting in acquiring more than ten new functional mintbodies for different modifications. These new mintbodies will be useful for analyzing spatiotemporal dynamics of histone modifications that have not been previously observed in living cells. This general-purpose intracellular antibody development method can be applied to many antibody clones, and is expected to be applied to sensors and drug delivery.

Microbial Fuel Cells: Fundamental and Applied Research on Extracellular Electron Transfer

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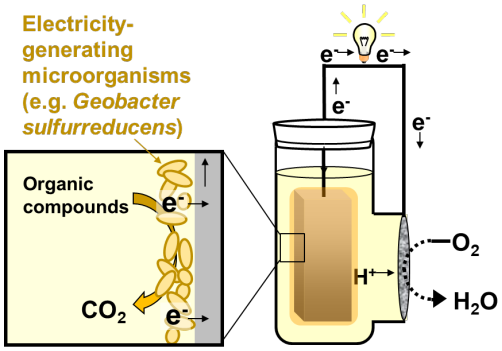
Bioelectrochemical systems are technologies that integrated biological and electrochemical processes to generate energy, treat wastewater, and produce valuable chemicals. Microbial fuel cell (MFC), one of the bioelectrochemical systems, directly converts the chemical energy of organic compounds into electrical energy using microorganisms. MFCs have been extensively studied as an energy-efficient water treatment technologies capable of both treating wastewater and generating electricity from wastewater.



When MFCs are used for wastewater treatment at near-neutral pH, *Geobacter* species, particularly those closely related to *G. sulfurreducens*, dominate the anode electrode surface. Thus, *G. sulfurreducens* is considered a representative electricity-generating microorganism in MFCs. *Geobacter* species have long been studied as obligate anaerobic iron-reducing bacteria, with extensive research on their mechanisms of electron transfer to extracellular insoluble Fe(III) and electrodes. This presentation introduces fundamental research on the extracellular electron transfer mechanisms of *G. sulfurreducens*.[1,2]

The performances of MFCs using livestock waste, brewery waste and soybean food wastewater will be also introduced.[3-4] Livestock waste and food waste are rich in organic matter, providing high energy density and significant potential as biomass energy sources. Our group has studied on treatment of cattle manure, swine farm wastewater, shochu (a traditional Japanese distilled liquor made from sweet potato, barley, or other crops) wastewater, and soybean food wastewater using MFCs. In MFCs using these different wastes, the microbial community structures varied, suggesting that the microorganisms involved in electricity generation and organic compound decomposition are diverse. This indicates a variety of mechanisms for extracellular electron transfer exist.

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Unraveling Medical and Biological Questions through Cryo-EM-Based Cross-Scale Measurements

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Advances in high-resolution imaging techniques such as cryo-electron microscopy (cryo-EM) have enabled quantitative cross-scale measurements from the molecular level to the organelle and cellular levels, making it feasible to seamlessly elucidate the fundamental question of how the origin of life phenomena and diseases is determined. In this presentation, I will introduce two examples of the biological and medical applications of cross-scale measurements: (1) the molecular mechanism of centrosome-independent microtubule network formation through the liquid-liquid phase separation (LLPS) and (2) the molecular pathological mechanism of hereditary dilated cardiomyopathy (DCM). In the first story, we have combined several cross-scale measurement techniques such as cryo-EM, Total Internal Reflection Fluorescence (TIRF) microscopy, and Atomic Force Microscopy (AFM) to elucidate how microtubule network is produced, triggered by the LLPS formation by the microtubule-associated protein CAMSAP2. In the second story, we have utilized seamless cryo-EM visualization techniques combined with single-cell analysis to elucidate the molecular pathological mechanisms of hereditary DCM caused by the Lamin A/C mutation.

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Oct. 20 2024

30 min zoom Lecture
"13th Tokyo Tech International Symposium on Life Science and Technology"
January 22nd, 2025, from 9:00 to 9:30 CET (17:00-17:30 JST).
[The symposium itself will be held from 5:00 to 10:00 CET (13:00-18:00 JST)].
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Enzymatic degradation of the polyester polyethylene terephthalate (PET): Enzyme kinetics and significance of PET crystallinity

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Products made of plastic have become a useful and ubiquitous part of our life, yet, the accumulation of plastics in landfills, soil and oceans has become a global environmental crisis. The desirable properties of plastics, notably the durability has now become a major drawback. One widely used plastic is poly(ethylene terephthalate) (PET), which currently constitutes ~9-10% of all plastic produced, equivalent to 35-45 million tons / year. Taking an estimated 450 years to decompose, it was a sensation in 2016[1] when Japanese scientists discovered that the microbe *Ideonella sakaienses* could grow on and hence degrade PET. Since then, research into enzymes that catalyze degradation of PET has surged and enzyme assisted PET recycling is moving into industrial reality. In this presentation, our recent results on the significance of PET substrate crystallinity on enzymatic PET degradation will be presented, and the unique enzyme kinetics of the endo-acting PET degrading enzymes will be discussed in relation to considering PET as an insoluble, semi-crystalline polymeric substrate[2-4]. In addition, a method for controlled modification of PET crystallinity for investigating the efficacy of PET degrading enzymes in response to PET crystallinity[5] will be outlined, and a new continuous assay for fast exploration and robust kinetic characterization of PET degrading enzymes will be presented[6]. The overall aim is to improve the understanding of enzymatic PET degradation in order to develop efficient enzymatic PET recycling processes.

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