2-6. Young Researchers supported by "Research Funding Support for Young Researchers"

No.	Name	Project Title
2-6-1	**Yusuke INOMATA** FAST	Temperature-Dependent Spontaneous Resolution of CsCuCl3
2-6-2	**Yoshihiro SEKINE** POIE	Development of functional compounds based on electron transfer
2-6-3	**Yi-Lun TSAI** FAST	Soil chemotactic signal perception and response of plant-parasitic nematodes
2-6-4	**Akira UEDA** FAST	Development of molecular design principles for novel chiral molecular conductors
2-6-5	**Wei XU** FAST	Membrane vesicles-modified graphene quantum dots for specific antibacterial activity against drug-resistant bacteria

FAST: Faculty of Advanced Science and Technology
POIE: Priority Organization for Innovation and Excellence

No. 2-6-1	Temperature-Dependent Spontaneous Resolution of $CsCuCl_3$		
Name	Yusuke INOMATA		
Affiliation	Faculty of Advanced Science and Technology Email: inomata@kumamoto-u.ac.jp	Title	Assistant Professor
Research Field	Advanced materials		

[Details of activities]

1. Research outline

The relationship between crystallization temperature and the resolution of a chiral inorganic crystal is examined. Spontaneous resolution is the easiest way to obtain enantiopure compounds. In the crystallization of well-known Pasteur's salt, the spontaneous resolution happens below 28°C. Despite the importance of crystallization temperature, only a few examples of organic crystals that show temperature-dependent spontaneous resolution have been reported. The behavior has not been confirmed for inorganic crystals. In this work, the inorganic crystal that exhibits temperature-dependent racemate-conglomerate crystallization is shown. Chiral $CsCuCl_3$ crystallizes as a racemate or enantiopure crystal depending on the crystallization temperature. Spontaneous resolution of $CsCuCl_3$ occurs at 65°C or more. Enantiopure chiral inorganic crystals are obtained simply by changing the crystallization temperature.

2. Research progress and results

When the crystallizations were performed at 80°C, hexagonal prism-shaped crystals were obtained after a day (Figure 1a). The sizes of the single crystals were 3-5 mm. Single crystals of $CsCuCl_3$ were collected from a batch crystallized at 80°C and the crystal structures were determined by single crystal XRD observations. Since the size of the crystals was large for the single crystal XRD observations, the crystals were cut into small pieces (< 0.3 mm) for the measurements. The XRD observations confirmed that both single crystals of $CsCuCl_3$ belonging to $P6_122$ (right-handed crystal, Figure 1b) and $P6_522$ (left-handed crystal, Figure 1c) crystallized in a beaker. The Flack parameters of the crystals were -0.02 for the right-handed ($P6_122$) crystal and 0.00 for the left-handed ($P6_522$) crystal, indicating that the crystals were enantiopure and the

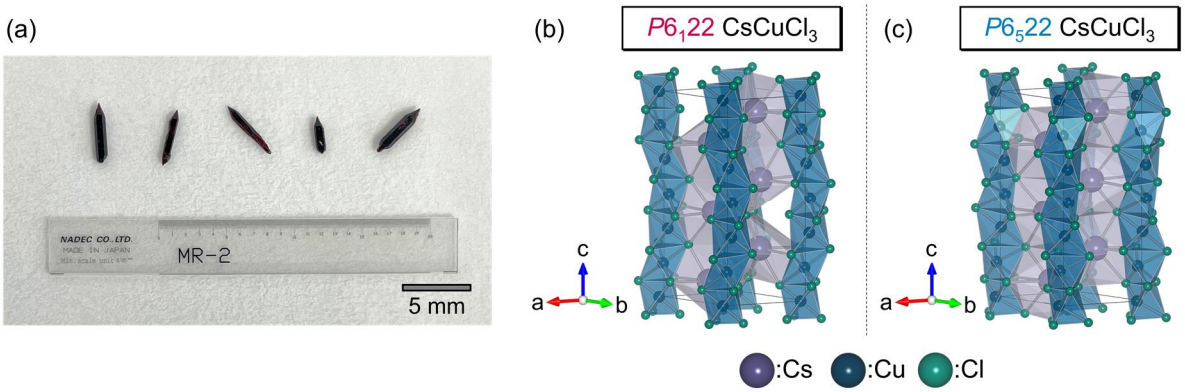


Figure 3. (a) Single crystals of $CsCuCl_3$ obtained by slow evaporation from aqueous solution at 80°C. (b) Crystal structures of right-handed $CsCuCl_3$ with clockwise 6-fold screw axis (space group: $P6_122$). (c) Crystal structures of left-handed $CsCuCl_3$ with counter-clockwise 6-fold screw axis (space group: $P6_522$).

crystals were composed of a single domain. The handedness of five small pieces cut from the original $CsCuCl_3$ crystal was checked to confirm the uniformity of the chirality. For all the small pieces, the space groups were the same and the Flack parameters were almost 0. The data confirmed that the entire crystal is enantiopure. These results show that $CsCuCl_3$ can be resolved into enantiopure homochiral crystals (conglomerates) in the crystallization at 80ºC and thus crystallization at high temperature poses spontaneous resolution of $CsCuCl_3$.

3. Research plan for the next year

・To investigate the functionalities of $CsCuCl_3$

4. List of journal papers

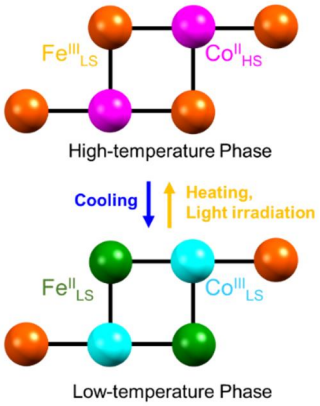
1. "Pt-Decorated ZnO Nanorods for Light-Assisted Ethanol Sensing and In Situ Analysis of the Sensing Mechanism under Light Irradiation"
   Y Inomata*, K Koga, T Shinkai, T Kida*
   ACS Applied Materials & Interfaces, **2024**, in press
   **Included the description of IROAST in the "Acknowledgments"*

No. 2-6-2	Development of functional compounds based on electron transfer		
Name	Yoshihiro SEKINE		
Affiliation	Priority Organization for Innovation and Excellence Email: sekine@kumamoto-u.ac.jp	Title	Associate Professor
Research Field	Advanced materials		

[Details of activities]

1. Research outline and its perspective

The new approach to developing external-stimuli-responsive materials based on electron transfers was demonstrated. The external-stimuli-responsive materials exhibited their electronic and spin state switching by temperatures and light irradiation. In this study, I could control electron transfers through chemical substitution, modifying the intermolecular interactions.



2. Research progress and results in the fiscal year

Ordinary molecules have a single structure, color, electronic state, and magnetic properties, but molecules whose electronic states can be controlled by external stimuli can be considered functional molecules because they exhibit switching functions. For example, if it is possible to create molecules that can reversibly convert their spin states in response to external stimuli, it is expected that they will be applied to molecular magnetic sensors and molecular magnetic memory in the future. In this study, we conducted experiments with the aim of synthesizing cyanide-bridged CoFe metal complexes that can be used to create external field-responsive electronic materials.

We focused on the phenomenon of intramolecular electron transfer as a switching behavior, and worked on metal complexes that can flexibly convert spin states. Both Co and Fe ions are redox-active, and the structural changes before and after electron transfer are small, so we designed the molecules with consideration for repeated switching durability.

By mixing metal ions and organic ligands in an organic solvent and investigating various methods for preparing single crystals, we succeeded in isolating complex molecules with different types and numbers of substituents in the ligands. As a result of single crystal X-ray structure analysis and magnetic measurement, we succeeded in creating complexes **1** and **2**, which do not show external stimulus responsiveness and a new molecular compound (complex **3**) that shows temperature- and light-induced magnetic switching by chemical modification of the ligand. The differences in external stimulus responsiveness between complexes **1**-**3** can be understood by single crystal structure analysis and the difference in redox potential of the metal ions. In other words, it can be said that we have succeeded in rationally creating the desired external stimulus-responsive switching molecules by introducing slight structural differences in the ligands introduced by chemical modification in molecules **1**-**3**, which have the same basic backbone structure. In addition, since the phase transition temperature at which electron transfer occurs can be controlled by chemical modification, we believe that there is potential for development in terms of creating molecules that can be magnetically switched at arbitrary temperatures.

3. Research plan for the next fiscal year

I am planning to construct electronically flexible molecular materials toward advanced materials. The series of compounds, which I have synthesized as switching materials, are soluble in solution. The key factor for fabricating advanced materials is efficiency for solution processing. Therefore, I will investigate the switching properties using solution samples.

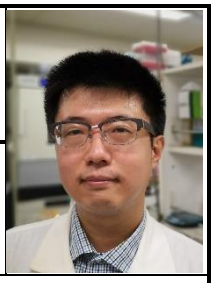
4. List of awards, grants, and patents

Poster Award, Yoshihiro Sekine, The 48th Annual Conference on Magnetics in Japan
Young Scholar Lecture, Yoshihiro Sekine, The 105th CSJ Annual Meeting (2025)

5. List of journal papers in international journal as of the end of December; (excluding conference papers and proceedings)

1) issued with volume numbers from April 2024 to December 2024
R. Fukushima, Y. Sekine,* Z. Zhang, S. Hayami*
Assembling Smallest Prussian Blue Analogs Using Chiral Hydrogen Bond-Donating Unit toward Complete Phase Transition, J. Am. Chem. Soc. 2024, 146, 35, 24238–24243 (Supplemental Cover)

2) scheduled to be issued with volume numbers from January 2025 to March 2025 and confirmed to be issued thereafter as of the end of December
Two papers are under review, but it is not decided when these are accepted. One paper is under preparation by the end of March 2025.

No. 2-6-3	Soil chemotactic signal perception and response of plant-parasitic nematodes		
Name	Yi-Lun TSAI		
Affiliation	Faculty of Advanced Science and Technology Email: tsai-yilun@kumamoto-u.ac.jp	Title	Assistant Professor
Research Field	Environmental bioscience / Environment-friendly technology / Strengthening resilience		

[Details of activities]

1. Research outline and its perspective

Plant pathogenic root-knot nematodes (RKN, *Meloidogyne incognita*) (Fig. 1, left) are prominent agricultural pests that infect many crop plants worldwide, and cause substantial economic loss annually. In nature, RKN are not the only soil microorganism that interact with plants. Rather, many species of bacteria and fungi, both pathogenic and symbiotic, share the same soil environment and host plants with the RKN. We are interested in how RKN interact with these microorganisms, and how these interactions affect RKN infections and pathogenesis (Fig. 1, right).

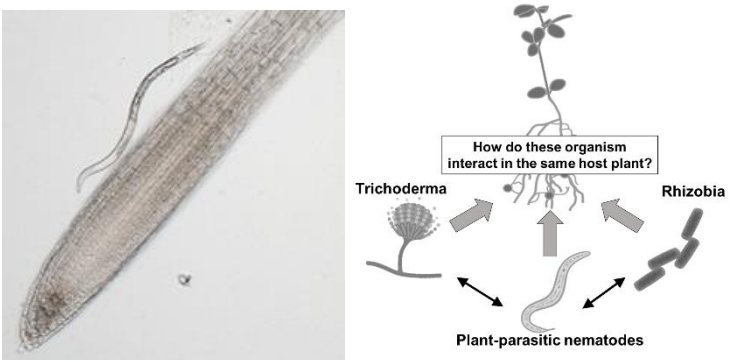


Fig. 1. Root-knot nematode (left panel) and the overall research plan (right panel)

2. Research progress and results in the fiscal year

2-1. Competitive infection behaviors between RKN and rhizobia

Rhizobia refer to a group of bacteria that colonize legume plant roots and induce the formation of specialized organs known as nodules. RKN adapt a similar life style, as they also invade plant roots and induce the formation of specialized organs called galls. However, rhizobia are considered beneficial in agriculture for providing the host with organic nitrogen, whereas RKNs causes diseases and are considered pests. Since both rhizobia and RKN target the same host tissue but produce different infection outcomes, we're interested in how these organisms compete when infecting the same host plant.

Previously we were able to show that RKN strongly suppress rhizobia (*Mesorhizobium loti*)-induced nodulation in bird's foot trefoil (*Lotus japonicus*). This suppression occurs as early as infection thread formation, possibly through mobile signaling molecules. One of the early hallmark feature of rhizobia infection is root hair curling (Fig. 2). To determine whether RKN infections also influences *L. japonicus* root hair curling, we examined root hair morphologies of plants infected with either rhizobia, RKN, or both simultaneously. We were able to detect increase in root hair curling induced by rhizobia infection, which is indeed suppressed by RKN infection (Fig. 2). This suggests RKN infection blocks nodulation at as early as rhizobia perception by root hairs.

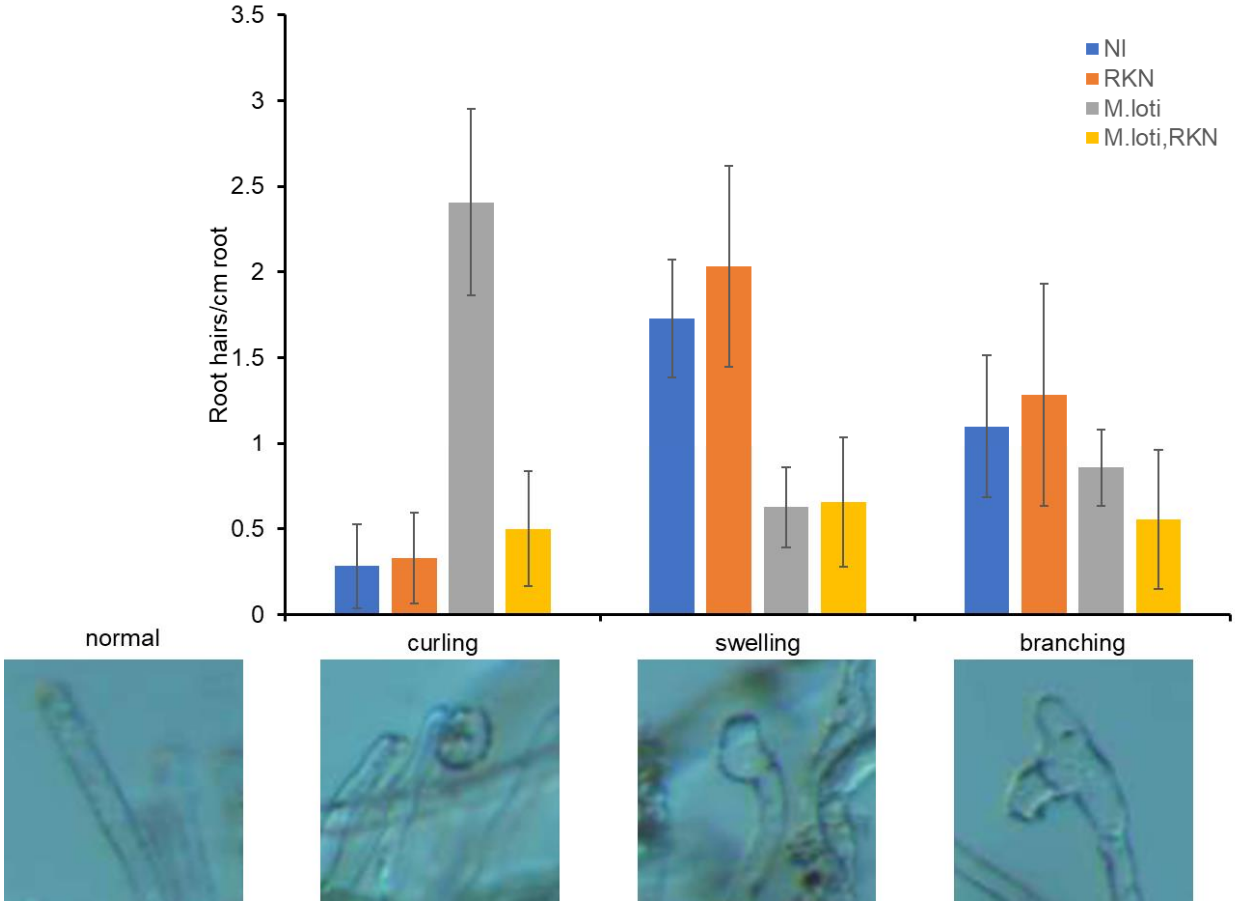


Fig. 2. Root hair morphology response to RKN and rhizobia infection. Numbers of root hairs with curling, swelling and branching morphological defects per cm of root in *L. japonicus* plants infected with RKN, rhizobia (*Mesorhizobium loti*) or both. Averages of n=3 ± SD are shown. Experiment was done twice with similar results.

2-2. Identification of endophytic fungi that interact with RKN

Many soil fungi are known to colonize plant roots, with some being able to promote plant growths. In order to identify such useful fungi species, we screened soil samples collected from various locations in Kumamoto. We were able to isolate seven strains belong the genus *Trichoderma* that show high competitiveness against pathogens, thereby having potentials as biocontrol agents in agriculture. These strains include *T. asperellum* (Tas), *T. atroviride* (Tat), *T. harzianum* (Tha) and *T. virens* (Tvi) isolated from Kumamoto Castle (KC), Suizenji Park (SZJ), Kumamoto University (KU) and Kumamoto University test field (KUF).

Previously we reported that Tat-SZJ and Tat-KU strains secrete nematicidal compounds active against RKN larvae. However, we were unable to reproduce these results and have decided to invest other properties of these strains. Interestingly, we found that Tat-SZJ and Tat-KU secrete volatile organic compounds (VOCs) that inhibit Arabidopsis seedling growth (Fig. 3AB). We were able to identify other *T. atroviride* strains from another collection with similar activities, suggesting this growth inhibition may be evolutionary conserved in *T. atroviride*. Surprisingly, we found that Tat-SZJ and Tat-KU promote tomato growth (Fig. 3CD), making this VOC-mediated growth inhibition more complex than expected.

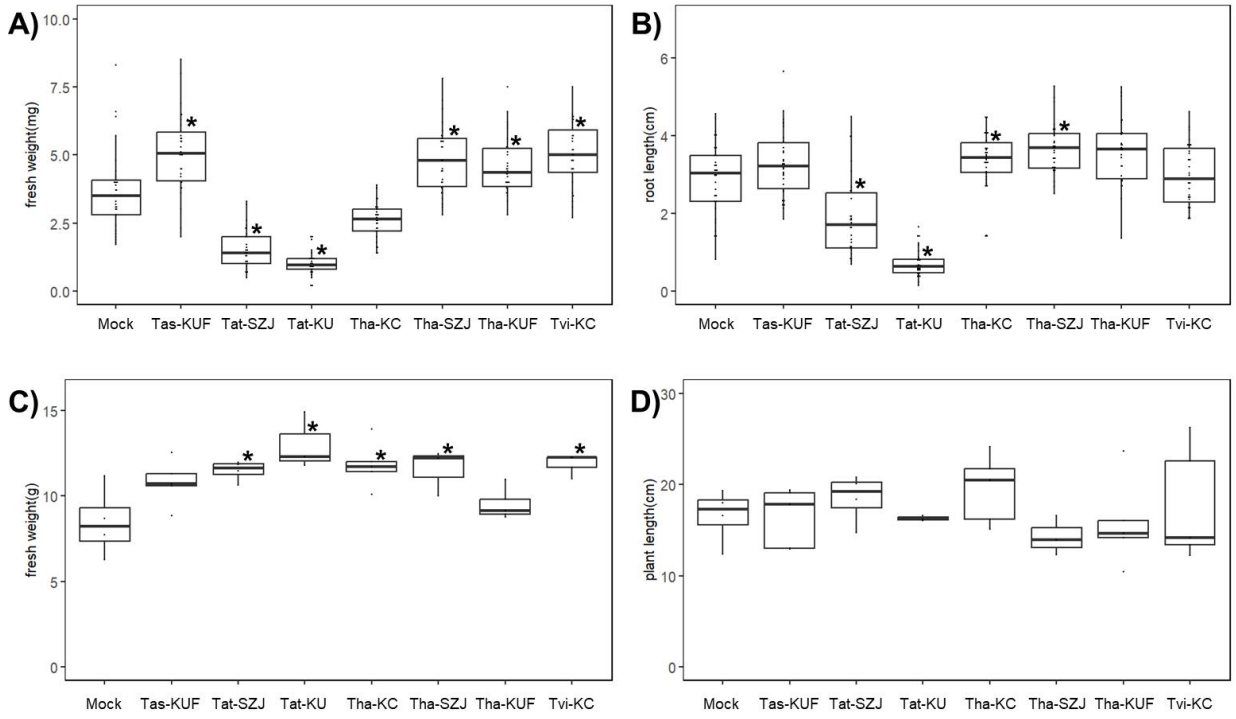


Fig. 3. Fresh weight (A, C) and root lengths (B, D) of Arabidopsis seedlings treated with *Trichoderma* VOC for 2 weeks (A, B) or tomato plants with root and soil inoculated with *Trichoderma* for 6 weeks. Averages of n=3 are shown. *denotes significant difference from mock-treated plants, Dunnett's test, $P<0.05$. Experiment was done at least twice with similar results.

2-3. Characterization of spermosphere microbiome

Other than plant roots, soil microorganisms are also known to colonize surfaces of plant seeds, or the spermosphere. The seed coats of many angiosperms, including the model plant Arabidopsis, secrete a layer of viscous polysaccharide mixture known as mucilage (Fig. 4A). Despite its widespread occurrence among plants, the biological relevance of mucilage remains unclear. We suspect mucilage may determine the spermosphere microbial community structures, since mucilage alters the physical properties of the seed surface, and may contain compounds that attract or repel specific microbes.

To test this hypothesis, we compared the microbes from the surfaces of wild-type and the *mucilage modified 2-10* (*mum2-10*) mutant seeds, which fail to extrude mucilage (Fig. 4A). The seeds were incubated on wet soil for 48 hours to allow mucilage extrusion and microbe colonization, then the seeds were recovered microbes were extracted from the seed surfaces. These microbes are enumerated by culturing on four different rich media – lysogeny broth (LB), tryptic soy agar (TSA), Reasoner's 2A agar (R2A) and nutrient broth (NB), then identified by analyzing their ribosomal DNA through next-generation sequencing. We were able to show that Col-0 seed surfaces contain more culturable microbes than *mum2-10* seeds (Fig. 4B-F). Whether these microbes are beneficial or harmful to the host plant remain to be determined, in general mucilage likely positively regulates microbial colonization in the spermosphere.

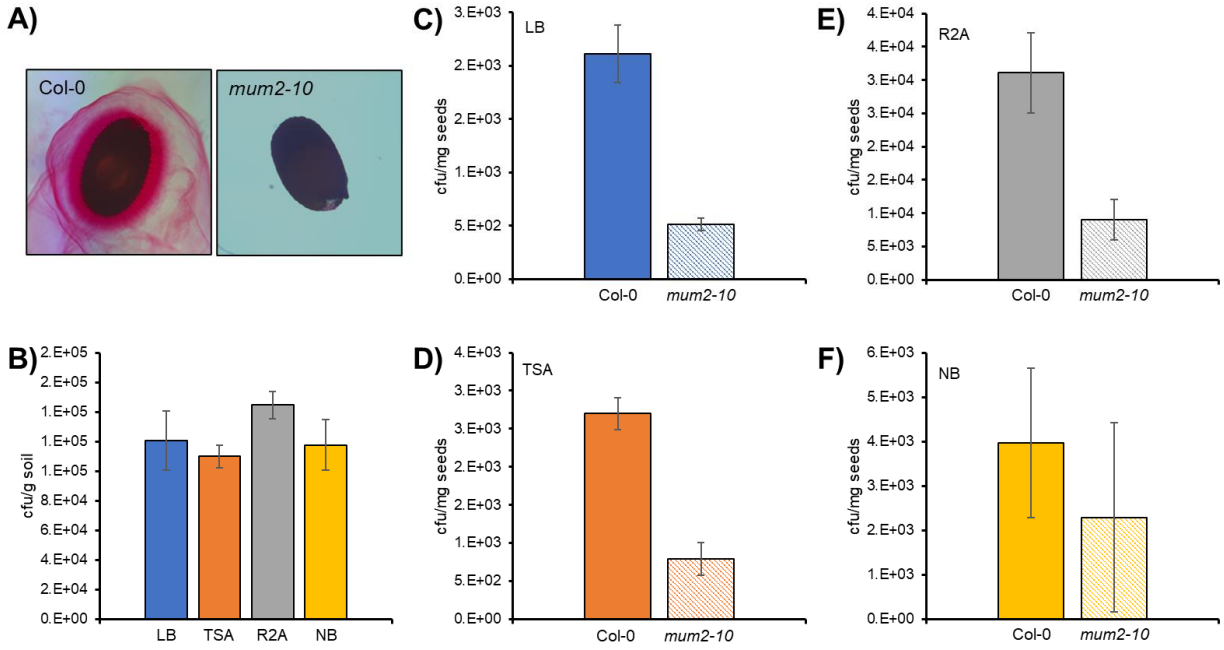


Fig. 4. A) Seed coat mucilage morphologies of Col-0 and mum2-10 seeds as stained by Ruthenium Red. B) CFUs of culturable bacteria in bulk soil grown on LB, TSA, R2A and NB media. C~F) CFUs of culturable bacteria extracted from Col-0 and mum2-10 spermospheres after 48 hours of incubation, grown on LB (C), TSA (D), R2A (E) and NB (F). Averages of n=3 ± SD are shown.

3. Research plan for the next fiscal year

3-1. Systemic transcriptomic shifts induced by RKN infection

We plan to perform transcriptomic analyses on roots of *L. japonicus* challenged with RKN, to identify genes that respond to RKN infection locally or globally, and determine whether these genes suppress nodulation. The expression patterns and identities of *L. japonicus* genes respond to RKN infection can help us identify the mobile signal induced by RKN to suppress nodulation. For preliminary analyses, we plan to focus on major regulatory genes of nodulation, as their expression levels most likely fluctuate in response to nodulation. If possible, we'd like to perform RNA-seq analysis to more comprehensively analyze the shift patterns of all transcripts in roots.

3-2. Characterization of RKN-antagonistic *Trichoderma* fungal species

Trichoderma are known to promote plant growth through modulating the phytohormone auxin, therefore we suspect the Arabidopsis growth suppression induced by *T. atroviride* VOCs may also act through auxin. As such, we plan to examine the patterns of auxin response gene expression and auxin accumulation in plants exposed to *T. atroviride* VOCs. Ultimately, we hope to perform RNA-seq to globally analyze transcripts affected by *T. atroviride* VOCs, and gas chromatography to identify the chemical structure of the T. atroviroide VOCs.

In addition, we're also curious to find the reason why *T. atroviride* have different plant growth regulatory activities for Arabidopsis and tomato. It is possible that Trichoderma have different effects on plants of different species or developmental stages. We'd also like to test whether the *Trichoderma* strains we collected suppress RKN infection.

3-3. Characterization of spermosphere microbiome

We are currently in the process of analyzing the culturable microbes isolated from Col-0 and mum2-10 seed surfaces. Once they are identified, we will have a better idea of which microbes are attracted or repelled by seed coat mucilage, and how they affect the host plant. Furthermore, we'd like to test whether the culturable microbes we isolated affect nematodes, in hope to identify strains that can suppress RKN infections.

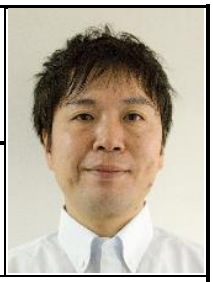
On the other hand, we've also submitted total microbial DNA collected from Col-0 and *mum2-10* seed surfaces, which include both culturable and unculturable microbes, for next-generation sequencing analysis. This will offer us a global understanding of all microbes in the spermosphere beyond just the culturable microbes we isolated, and help us characterize the microbial community profiles in the presence/absence of seed coat mucilage.

4. List of awards, grants, and patents, if any

(N/A)

5. List of journal papers in international journal as of the end of December; (excluding conference papers and proceedings)

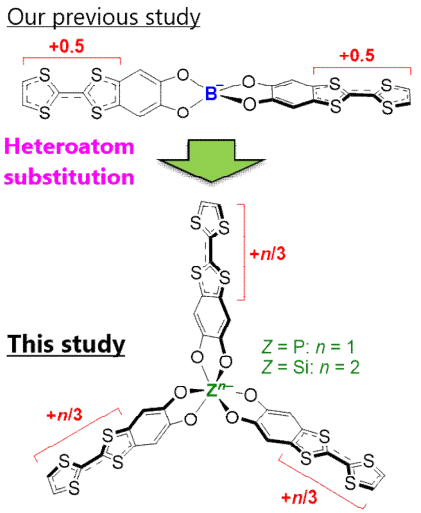
Gushino S, **Tsai AY**, Otani M, Demura T, Sawa S. (2024) VND genes redundantly regulate cell wall thickening during parasitic nematode infection. *Plant Cell Physiol*. **65(8)**: 1224-1230.

No. 2-6-4	Development of molecular design principles for novel chiral molecular conductors		
Name	Akira UEDA		
Affiliation	Faculty of Advanced Science and Technology Email: aueda@kumamoto-u.ac.jp	Title	Associate Professor
Research Field	Advanced materials		

[Details of activities]

1. Research outline and its perspective

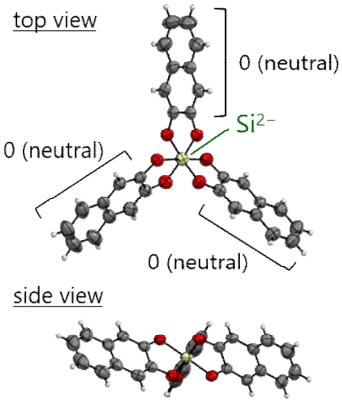
This research aims to develop innovative molecular design principles for low-molecular-weight organic conductors with unique chirality, building upon previous successful work funded by the IROAST Young Researchers Support Program. Namely, as shown in the right figure, we have explored the replacement of the boron atom in the molecules developed in our previous study with other heteroatoms, such as phosphorus or silicon, to create propeller-shaped chiral zwitterionic molecular structures with conducting carriers. This approach could lead to novel chiral molecular conductors, potentially influencing electronic and magnetic properties and advancing the discovery of new phenomena in molecular solids.



Propeller-shaped chiral zwitterionic molecule with conducting carriers

2. Research progress and results in the fiscal year

As a first step of this research, in this fiscal year we have decided to explore a synthetic methodology to construct the abovementioned propeller-shaped zwitterionic molecular structures with a phosphorus or silicon atom as the central atom (*Z* in the above figure). Screening of the reaction conditions revealed that the silicon-centered propeller structure can be readily constructed by the reaction of catechol derivatives with tetraalkoxysilane. On the basis of this methodology, we have successfully synthesized some silicon-centered propeller molecules with three aromatic blades. Among them, the one with three naphthalene blades was obtained as single crystals, enabling us to reveal the detailed molecular structure by X-ray diffraction (right figure). Namely, we see that this molecule certainly has a propeller-like chiral structure centered on an octahedral silicon atom with a valence of 2–, and has two enantiomers with right-handed and left-handed propeller conformations (only the right-handed one is shown in the figure). Therefore, in this study we have successfully established a general synthetic method to construct the propeller-shaped molecular structure with the central atom of Si^{2-} and three aromatic blades.



3. Research plan for the next fiscal year

As can be seen from the above figure, the blades of the obtained propeller molecule are now in a neutral state and thus have no conducting carriers (*i.e.*, a closed-shell electronic configuration). Therefore, we will oxidize this kind of propeller molecule in the next fiscal year, to introduce carriers (or holes) into this system and to study the transport properties in the solid state. In fact, we have already confirmed that this type of molecule has a sufficiently low oxidation potential by

electrochemical measurements and theoretical calculations. Furthermore, optical resolution of the enantiomers will be also examined to obtain novel molecular conductors without inversion symmetry. In addition, the exploration of phosphorus-centered analogues of the abovementioned propeller molecules will be performed in the near future.

4. List of awards, grants, and patents

Awards

· Kumamoto University Research Achievement Award FY2024 (熊本大学研究業績表彰)

Grants

· Grant-in-Aid for Transformative Research Areas (A) 23H04035, FY2023-2024 (Principle Investigator), Direct Cost: ¥6,000,000, "Zwitterionic neutral radical-based strongly correlated electron systems with high-dimensional condensed conjugation"

· Grant-in-Aid for Scientific Research (C) 23K04692, FY2023-2025 (Principle Investigator), Direct Cost: ¥3,700,000, "Modulation of band filling and exploration of novel electronic phases in purely organic neutral radical solids"

5. List of journal papers in international journal as of the end of December; (excluding conference papers and proceedings)

· "Boron-bridged bis(tetrathiafulvalene) zwitterionic neutral radical conductors: substituent effects on intramolecular and intermolecular electronic interactions and physical properties"
K. Sonoda, S. Shimokawa, S. Suzuki, T. Kusamoto, A. Ueda*
Bulletin of the Chemical Society of Japan, **97**, uoae107 (2024). DOI: 10.1093/bulcsj/uoae107

No. 2-6-5	Membrane vesicles-modified graphene quantum dots for specific antibacterial activity against drug-resistant bacteria		
Name	Wei XU		
Affiliation	Faculty of Advanced Science and Technology Email: xuwei@kumamoto-u.ac.jp	Title	Associate Professor
Research Field	Biotechnology & healthcare technology		

[Details of activities]

1. Research outline and its perspective

Similar to mammalian cells, most bacteria can release nano-sized membrane vesicles (MVs) into the extracellular environment. MVs contain lipids, bioactive proteins, nucleic acids, and metabolites, and play important roles in microbial physiology. MVs have great potential for immunotherapeutic applications, such as bacterial vaccines and cancer immunotherapy. However, because of the diversity in content and heterogeneity in size of MVs, the clinical application of MVs has been limited. Recently, the use of MVs combined with nanoparticles (NPs) has been shown to be effective in improving the homogeneity, stability and function of MVs.

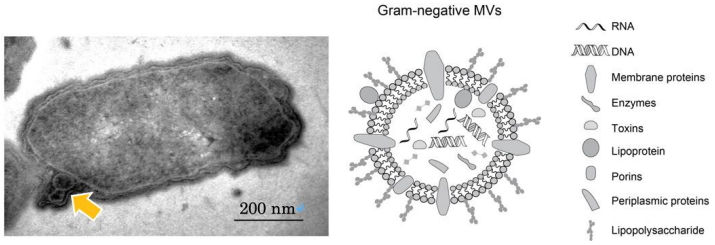


Fig.1. Architecture and composition of MVs from Gram-negative bacteria

In addition, graphene quantum dots (GQDs), nanostructures of graphene with non-zero band gap show unique optical and electronic properties. However, most of reported GQDs are water-soluble, which limits their applications as antimicrobial materials. We found a simple method to synthesize water-insoluble hydrophobic GQDs, and we investigated their antibacterial and antiviral activities, as reported at last year (Fig.2).

To improve the antibacterial activity and specificity of GQDs, I considered to combine MVs and GQDs. Because GQDs are hydrophobic, we imaged that GQDs could be combined with MVs just by sonication.

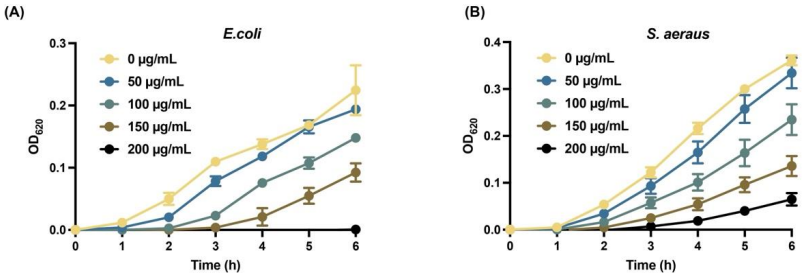


Fig.2. Antibacterial activity of GQDs against to gram-negative *E.coli* and Gram-positive *S. aureus*.

2. Research progress and results in the fiscal year

First, we obtained MVs from several kinds of bacterials, such as gram-negative Salmonera (*S. typhimurium*), and Escherichia (*E. coli*) and measured their size and observed by TEM. As shown in Fig.3., we observed the vesicle shape of MVs, and they showed the size around 100~150 nm. Compared to MVs from *S. typhimurium*, the size of MVs from *E. coli* are smaller.

Next, we measured the cytotoxicity of MVs and evaluated the roles of MVs in the immune system (immune response from magrophage). As result, we investigated that MVs have high safety with no cytotoxicity. According to real-time PCR analysis, MVs from *E. coli* howed a higher immuno simulation, compared to MVs from *S. typhimurium.* Further, we evaluated the cellular uptake of MVs from *E. coli* and *S. typhimurium. S. typhimurium* as intracelular bacteria which can enter and multiply in macrophage, as an important intracellular pathogen that cuase gastroenteritis and life-threatening systemic disease. However, different with my speculation, MVs from *E. coli* howed a higher cellular take behavior, compared to MVs from *S. typhimurium.*

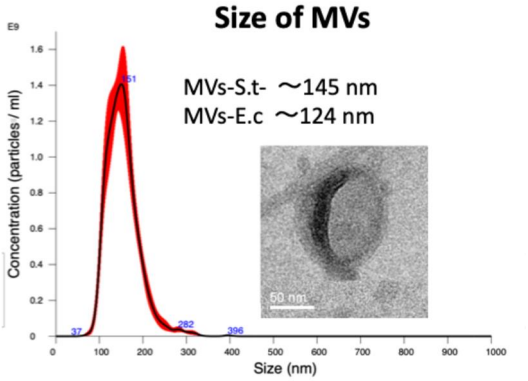


Fig.3. TEM images and size distribution of MVs.

For surface modification of GQDs with MVs, we mixed GQDs and MVs together and sonicated for 5 min. After removing the excess MVs, the samples were collected by centrifugation. As shown in Fig.4., we observed the fluoresence of MVs-modified GQDs under UV light irradiation, and protein absorption by BCA assay.

Therefore, we confirmed that we succeeded to prepare the MVs-modified GQDs.

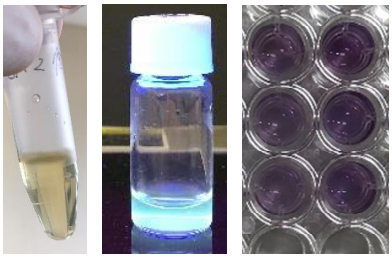


Fig.4. MVs-modified GQDs showed fluorescence under UV light irradiation (middle), and protein absorption (Right).

3. Research plan for the next year

Next year, first, I will evaluate the effect (immune response and cytotoxicity) of MVs *in vivo* to confirm the safety of MVs from different bacterial species. In addition, I will evaluate the accumulation of MVs in mice.

In addition, I will evaluate the anti-bacterial activity of MVs-modified GQDs against to different species of bacteria to confirm the specify properties of MVs-modified GQDs.

4. List of awards, grants, and patents

- Best presentation Award, 40th Annual Meeting of the Japan Society of Drug Delivery System, 9-11 July 2024, Tsukuba, Japan

5. List of journal papers (with IROAST as your affiliation) published between April 2024 and March 2025.

- Wei Xu, Sayo Maruyama, Akito Sato, Takuro Niidome, Bacterial membrane vesicles combined with nanoparticles for bacterial vaccines and cancer immunotherapy, Colloids and Surfaces B: Biointerfaces 243 (2024) 114125.