


2-5. Young Researchers supported by "Research Funding Support for Young Researchers"

No.	Name	Project Title
2-5-1	Muhammad Sohail AHMAD IINa	Selective hydrogenation of 5-(hydroxymethyl) furfural to 5-methyl furfural over Mn nanoparticles supported on graphene oxide
2-5-2	Yusuke INOMATA FAST	Fe-Cu Complex Oxide Catalysts for Aerobic Oxidative Coupling of 2-naphthol
2-5-3	Yoshihiro SEKINE POIE	Development of external-stimuli-responsive metal complexes
2-5-4	Yi-Lun TSAI FAST	Rhizosphere microbiomes dynamics during root symbiosis and parasitism
2-5-5	Akira UEDA FAST	Development of superconducting or highly conducting molecular materials soluble in organic solvents
2-5-6	Wei XU FAST	Antibacterial and antiviral of graphene quantum dots

IINa: Institute of Industrial Nanomaterials

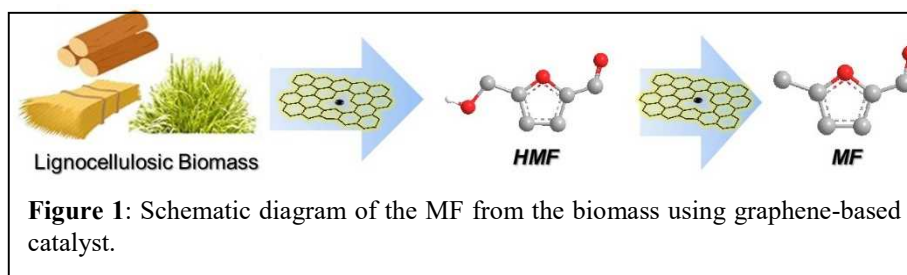
POIE: Priority Organization for Innovation and Excellence

FAST: Faculty of Advanced Science and Technology

No.5-1	Selective hydrogenation of 5-(hydroxymethyl) furfural to 5-methylfurfural over Mn nanoparticles supported on graphene oxide			
Name	Muhammad Sohail AHMAD	Title	Especially Appointed Assistant Professor	
Affiliation	Institute of Industrial Nanomaterials (IINa) Email: sohail@kumamoto-u.ac.jp			
Research Field	Environment-friendly technology / Advanced materials			

[Details of activities]

Developing precious metal-free catalysts to promote the sustainable production of fuels and chemicals from biomass remains an important and challenging task. In this context, the selective reduction of the biomass-derived molecule



5-(hydroxymethyl)

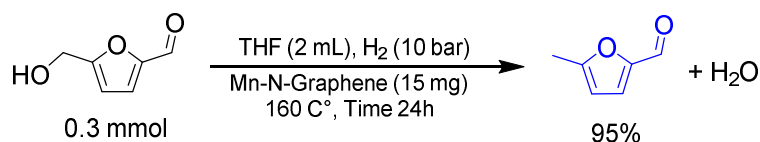
furfural (HMF) to 5-methylfurfural (MF) with molecular hydrogen as a reducing agent is one of the most important transformations but a challenging target because the C=O bond reduction in HMF is more susceptible than C-OH (**Figure 1**). Recent research has demonstrated the conversion of HMF to MF using direct and indirect routes. However, the use of noble metal catalysts such as Pt anchored on Nb₂O₅ is costly and energy-intensive due to the separation and purification processes involved. (*Nat. Commun.* **2021**, 12 (1), 584) Therefore, there is a need to develop cost-effective and environmentally friendly methods to achieve direct conversion of HMF to MF while utilizing H₂ as the reducing agent. To address this issue, highly active and selective Mn-based graphene catalysts for the hydrogenolysis of HMF to MF under mild conditions, utilizing molecular hydrogen as a hydrogenation source, were investigated. This research has the potential to contribute significantly to the sustainable production of fuels and chemicals from biomass, which is relevant to various industries including pharmaceuticals, petrochemistry, and organic synthesis.

2. Research progress and results in the fiscal year

Manganese-based catalysts are widely utilized for oxidation reactions while seldom applied in catalytic reduction reactions because they cannot activate and dissociate molecular hydrogen. We show that the doping of the nanosize of manganese on graphene oxide (GO) can work as catalyst for the conversion of HMF to MF. Typically, the catalyst material was synthesized by wet impregnation method of 1:1 molar ratio of manganese precursor and 1,10-phenanthroline on GO, followed by pyrolysis under an argon atmosphere. The Mn_NGraphene was then tested for its reactivity toward the selective hydrogenation of HMF. Almost no product was formed when the reaction was performed without a catalyst (data not shown here). Interestingly, when HMF in THF was subjected to hydrogenation (H₂ 10 bar) at 160 °C with Mn_NGraphene (15 mg) as the catalyst >99 % selectivity at the complete conversion of HMF in 24 hrs (**Scheme 1**). We observed the formation of MF as the reaction product by GC-MS, and ¹HNMR revealing the C-OH bond breaking to be the susceptible pathway under these reaction conditions.

Furthermore, FT-IR spectroscopy was used to investigate the reason for the high selectivity of MF over Mn_N_Graphene in the HMF hydrogenation reaction. Basically, methanol and butanal were used as example substrates, and it was observed that the methanol goes into methoxy species in the presence of Mn_N_Graphene, and the band was observed at 1155 cm^{-1} can be allocated to the (C–O) bands (**Figure 2**). These results show that the chemical adsorption of C–OH occurred over the Mn_N_Graphene Catalyst, while Mn_N_Graphene could not adsorb C=O functional group. The FT-IR data shows that the Mn_N_Graphene Catalyst may selectively activate the C–OH bond in the HMF hydrogenation reaction.

In summary, a new Mn nanoparticle-based heterogeneous catalytic system for the selective hydrogenation reaction of HMF has been developed. This is the first report on Mn nanoparticles for the selective hydrogenolysis of HMF in the presence of H_2 . The excellent properties of the Mn NPs for the hydrogenation reaction are due to the cooperation of graphene and Mn sites in the catalyst. Besides, the catalyst can be recycled without any decrease in selectivity.



Scheme 1: selective hydrogenation of HMF to MF.

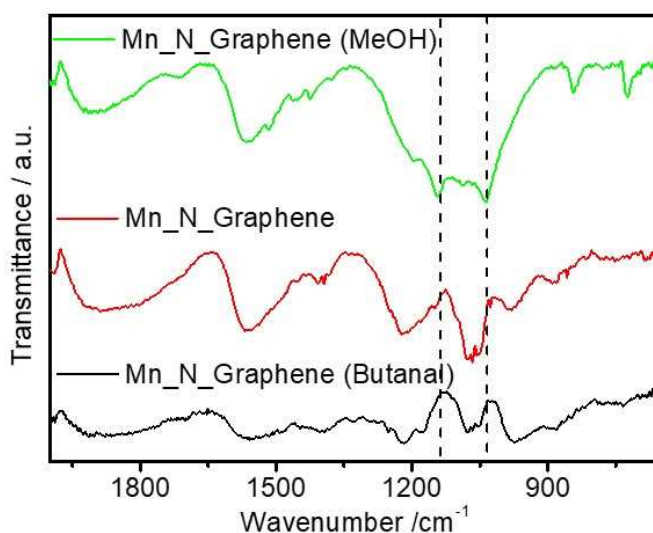
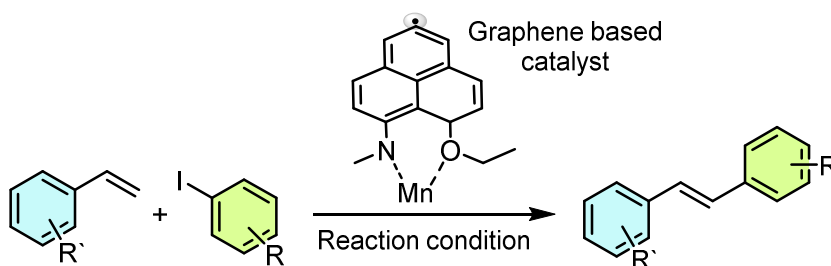


Figure 2: Characterization results from FT-IR, methanol, and butanal adsorbed Mn_N_Graphene surface

3. Research plan for the next year

In the future, we are going to tune the graphene-based materials as catalyst for the Matsuda-Heck and Mizoroki-Heck coupling reactions. The redox state of the graphene oxide materials by doping foreign atoms such as Mn may act as an active site for the C-C coupling reactions (**Scheme 2**).




Scheme 2: Tuning the functionalities of graphene oxide for the C-C coupling reaction

Furthermore, the catalyst will be characterized through various spectroscopic techniques to reveal the active sites.

5. List of journal papers (with IROAST as your affiliation) published between April 2022 and March 2023.

- Ahmad, Muhammad Sohail, Yusuke Inomata, and Tetsuya Kida. "Heterogenized manganese catalyst for C-, and N-alkylation of ketones and amines with alcohols by pyrolysis of molecularly defined complexes." *Molecular Catalysis* 526 (2022): 112390.

No.5-2	Fe-Cu Complex Oxide Catalysts for Aerobic Oxidative Coupling of 2-naphthol			
Name	Yusuke INOMATA	Title	Assistant Professor	
Affiliation	Faculty of Advanced Science and Technology (FAST) Email: inomata@kumamoto-u.ac.jp			
Research Field	Environment-friendly technology / Advanced materials			

[Details of activities]

● Research outline and its perspective

BINOL (1,1'-Bi-2-naphthol) is an axially chiral compound with C_2 symmetry and is an important precursor for chiral molecules and chiral ligands. In the current process, BINOL is synthesized by using oxidizing reagents or homogeneous catalysts that have problems with waste and recyclability. Heterogeneous catalysis is a practical way for producing chemicals because heterogeneous catalysts are easily separated by their filtration and are reusable. Aerobic oxidation reactions are an environment-friendly process because an abundant oxygen molecule is used for an oxidizing reagent and its by-product is water. In this work, an Fe-Cu complex oxide catalyst is developed for a cost-effective method for aerobic catalytic coupling reaction of 2-naphthol for the synthesis of BINOL.

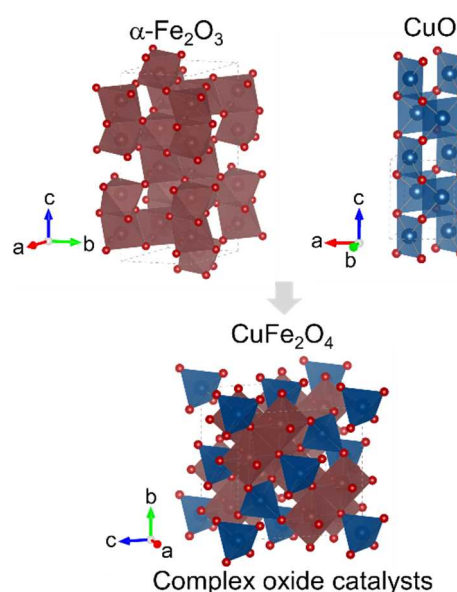
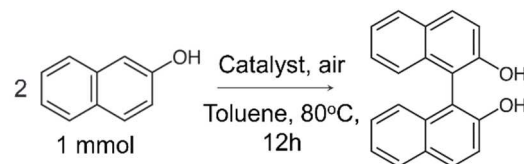


Fig. 1 Crystal structures of Fe_2O_3 , CuO and $CuFe_2O_4$.

● Research progress and results

Fe-Cu complex oxide catalysts were synthesized by the oxalate method using copper nitrate and iron nitrate as precursors. The obtained precursor powder was calcined at $300^\circ C$ in air to get catalysts. The catalysts with different composition were obtained by changing the ratio of precursors. When the molar ratio of precursors was $Cu:Fe = 33:66$, a spinel type $CuFe_2O_4$ complex oxide was obtained as a single phase compounds (**Figure 1**).

BET specific surface area was checked by N_2 adsorption measurement. The value of specific surface area was $128 \text{ m}^2/\text{g}$ for $CuFe_2O_4$ and this value was higher than the previously reported values for $CuFe_2O_4$ synthesized by the different methods (solid state method, sol-gel method and co-precipitation method). The catalytic activity of Fe-Cu complex oxides was investigated for the oxidative coupling of 2-naphthol (**Scheme 1**). Within the Fe-Cu complex oxide catalysts, $CuFe_2O_4$ showed the best catalytic activity and the highest BINOL yield (yield: 60%, $80^\circ C$, 12h). The catalytic activity of $CuFe_2O_4$ was greater than the catalytic activities of single-phase oxides (Fe_2O_3 and CuO). The reaction mechanism of oxidative coupling of 2-naphthol over $CuFe_2O_4$ is expected as follows: (1) C-H cleavage at 1 position of 2-naphthol, (2) one electron reduction of metal sites ($M^{n+} \rightarrow M^{(n-1)+}$), (3) C-C bond formation and (4) reoxidation of metal sites ($M^{(n-1)+} \rightarrow M^{n+}$). According to the previous work, the rate-determining step is the reoxidation of metal sites. The coordinative environments of Fe and Cu units in Fe_2O_3

Scheme 1. Oxidative coupling reaction of 2-naphthol.

and CuO are distorted octahedral (FeO_6) and square planer (CuO_4) while CuFe_2O_4 is composed of symmetric octahedral units (MO_6 , $\text{M} = \text{Fe}$ and Cu) and tetrahedral units (MO_4 , $\text{M} = \text{Fe}$ and Cu). Such structural difference would contribute to the enhancement of redoxability and thus the catalytic activity increases. This catalytic system is heterogeneous and utilize abundant Cu and Fe as active species and air as an oxidizing reagent that produce only water as a by-product. Therefore, this method would be the sustainable and eco-friendly method for the production of BINOL.

- Research plan for the next year


(i) To investigate the structural effect of CuFe_2O_4 on the catalytic activity.

(ii) Other aerobic oxidation reactions with CuFe_2O_4 (ex. alcohol oxidation and epoxidation of olefins).

- List of journal papers (with IROAST as your affiliation) published between April 2022 and March 2023

Ahmad, Muhammad Sohail Yusuke Inomata, and Tetsuya Kida. "Heterogenized manganese catalyst for C-, and N-alkylation of ketones and amines with alcohols by pyrolysis of molecularly defined complexes." *Molecular Catalysis* 526 (2022): 112390

S.A. Fauzi, N.L. Hamidah, S. Kitamura, T. Kodama, K. Sonda, G.K. Putri, T. Shinkai, M. S. Ahmad, Y. Inomata, A. T. Quitain, T. Kida., "Electrochemical Detection of Ethanol in Air Using Graphene Oxide Nanosheets Combined with Au- WO_3 " *Sensors*, 22(9) (2022): 3194

No.5-3	Development of external-stimuli-responsive metal complexes			
Name	Yoshihiro SEKINE	Title	Associate Professor	
Affiliation	Priority Organization for Innovation and Excellence (POIE) Email: sekine@kumamoto-u.ac.jp			
Research Field	Advanced materials			

[Details of activities]

1. Research outline and its perspective

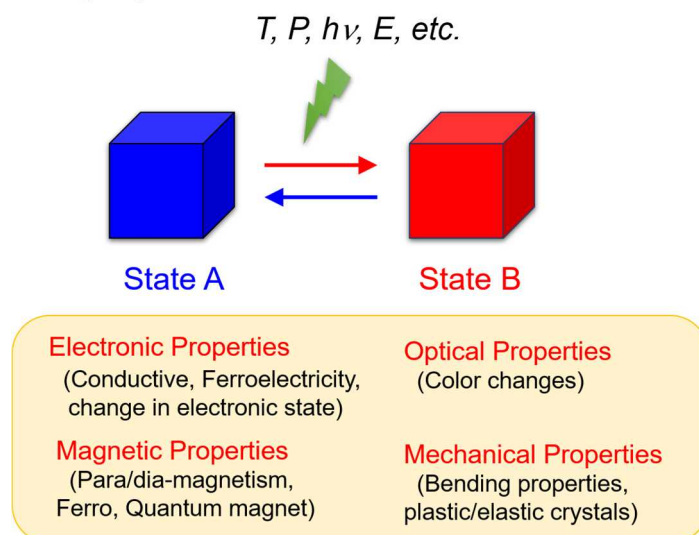


Fig.1 Schematic illustration of external-stimuli-responsive materials.

In this study, we aimed to synthesize molecular crystals that can reversibly transform their structure, electronic state, and crystal morphology in response to external stimuli (Fig.1). Such molecular crystals have the potential to be developed as switchable molecular materials and may have applications in future molecular sensors and memories.

2. Research progress and results in the fiscal year

Metal complex crystals are attracting attention as materials with unique mechanical properties due to their flexible deformation behavior under external stimuli such as heat, pressure, and light. In this study, we aimed to develop metal complex crystals that exhibit flexible mechanical properties and to investigate the control of their elasticity by changing the metal ion type.

Metal complex crystals consisting of salophen ligands with planar tetradentate coordination and Pt or Pd metal ions with d^{10} electron configuration were crystallized using various organic solvents, such as MeOH, CH_2Cl_2 (Fig. 2). Elastic metal complex crystals were isolated and their crystal structures were determined by X-ray crystal structure analysis. The elasticity of the crystals was measured using a stress-strain apparatus. The energy framework calculations were performed based on the obtained crystal structures.

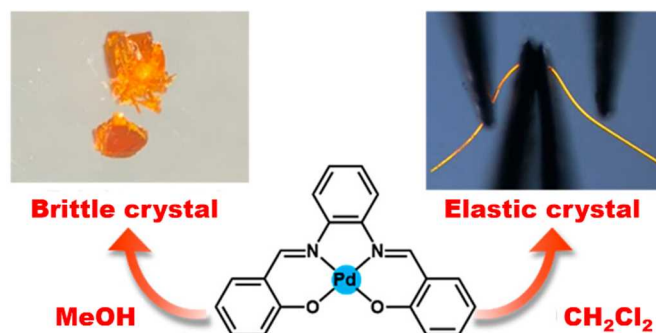


Fig.2 Structure of [Pd(salophen)] and the photo of brittle and elastic crystals.

Elastic metal complex crystals were successfully obtained by crystallizing salophen-Pt and salophen-Pd complexes using various organic solvents. X-ray crystal structure analysis showed that the crystals had a one-dimensional anisotropic molecular alignment based on intermolecular interactions and both Pd and Pt complexes had the same crystal structure. Elasticity measurements revealed that the metal complex crystals had about 10% elasticity. Interestingly, the flexibility of the metal complex crystals varied depending on the type of central metal ion, and the control of flexibility according to the intermolecular distance was possible. The energy framework calculations based on crystal structures supported these findings. This study revealed for the first time that it is possible to design the flexibility of elastic crystals by controlling the metal ion type. In conclusion, we developed metal complex crystals with flexible mechanical properties by physical perturbation and investigated the control of their elasticity by changing the metal ion type. The obtained elastic metal complex crystals showed about 10% elasticity and their flexibility varied depending on the type of central metal ion. This study revealed for the first time that it is possible to design the flexibility of elastic crystals by controlling the metal ion type. These findings provide new insights into the design of elastic materials and may have potential applications in various fields such as sensing, actuation, and energy harvesting.

3. Research plan for the next year

Our research this year successfully developed flexible metal complex crystals that exhibit mechanical flexibility through physical perturbation, and provided guidelines for their synthesis via molecular design. Furthermore, we discovered that by controlling the type of metal center ions, it is possible to design the flexibility of these elastic crystals based on the distance between molecules, which was supported by energy framework calculations. Going forward, we plan to focus on developing molecular crystals that enable electronic function switching through mechanical perturbation, as well as constructing reversible molecular electronic states through temperature and light control.

4. List of awards, grants, and patents

Award

The 17th International Student Conference on Advanced Science and Technology, Best Presentation Award,

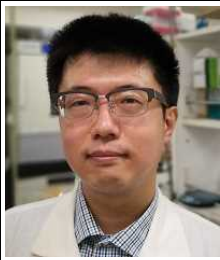
Crystal structure and magnetic properties of cobalt(II) complexes with π -conjugated substituents
Best Presentation Award

Keita Soejima, Ryuya Tokunaga, Yoshihiro Sekine, Shinya Hayami

The 17th International Student Conference on Advanced Science and Technology, Best Presentation Award,

Synthesis of new organic ligand for Ultimate Functional Materials
Best Presentation Award

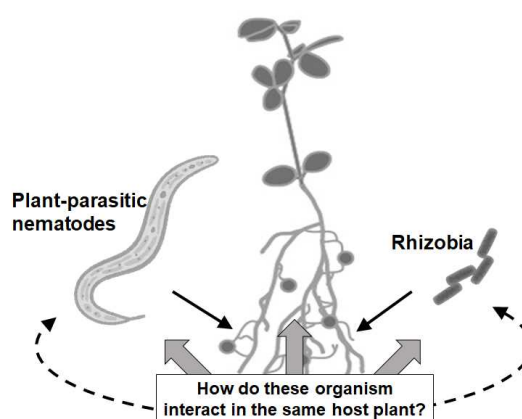
Kenta Hirakawa, Yoshihiro Sekine, Shinya Hayami

No.5-4	Rhizosphere microbiomes dynamics during root symbiosis and parasitism			
Name	Yi-Lun TSAI	Title	Assistant professor	
Affiliation	Faculty of Advanced Science and Technology(FAST) Email: tsai-yilun@kumamoto-u.ac.jp			
Research Field	Environmental bioscience / Environment-friendly technology			

[Details of activities]

1. Research outline and its perspective

Plants encounter myriads of environmental microorganisms that influence the plant's growth. However, it remains difficult to study these microbial communities in the laboratory. Here we introduce a tripartite system using bird's foot trefoil (*Lotus japonicus*) and two microbes: rhizobia (*Mesorhizobium loti*) and root-knot nematodes (RKN, *Meloidogyne incognita*), both of which infect plant roots but with different outcomes. Using this system, we hope to investigate how different microbes interact in plants, and determine the biological significance of these interactions.



2. Research progress and results in the fiscal year

M. loti is susceptible to infection by both rhizobia and RKN, which form nodules and galls, respectively, on plant roots. We tested 3 different co-infection scenarios, and counted nodules and galls as proxies for infection rates.

1. Inoculate plants already infected with rhizobia with RKN.
2. Inoculate plants already infected with RKN with rhizobia.
3. Inoculate plants with rhizobia and RKN simultaneously.

In addition, we tested whether RKN and rhizobia infection rates change in *M. loti* mutants known to have rhizobia nodulation defects, including *NODULATION SIGNALING PATHWAY (NSP1)*, *CYCLOPS*, *Nodules Inception (NIN)* and *Ethylene Response Factor Required for Nodulation1 (ERN1)*, which show reduced nodule formation, as well as *HYPERNODULATION AND ABERRANT ROOT1 (HAR1)* and *TOO MUCH LOVE (TML)*, which produce excessive nodules.

RKN infection rates do not appear to change much regardless whether the host plant is already infected with rhizobia. *tml* plants infected with rhizobia do show a modest, but nevertheless significant increase in RKN infection compared to uninfected *tml* plants. This suggests *TML* may suppress RKN infection in plants already infected with rhizobia, even though the effect may be minute.

On the other hand, plants already infected with RKN develop almost no rhizobia nodules (Fig. 1), suggesting RKN infection may trigger certain signaling mechanism to suppress subsequent rhizobia infection. Interestingly, this suppression was also observed in the *tml* and *har1* mutants known to process excess nodules. *TML* and *HAR1* are known to suppress nodule formation, yet RKN does not appear to suppress rhizobia nodule formation through the activities these genes, at least not exclusively. It is also possible that RKN utilizes other unknown signaling pathways to inhibit rhizobia nodulation.

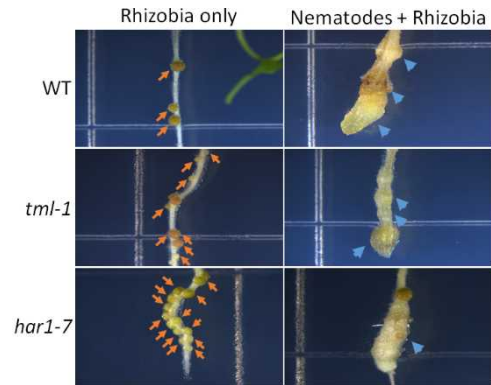


Fig. 1: Nodules (arrowheads) and galls (triangles) of plants inoculated with both RKN and rhizobia

Similarly, plants simultaneously inoculated with both RKN and rhizobia show primarily RKN galls but very little rhizobia nodules (Fig. 2), confirming that RKN infection likely actively suppresses rhizobia infection, and that RKN is likely dominant over rhizobia in nature. Since rhizobia are beneficial for agriculture due to their nitrogen-fixing ability, while RKN are known agricultural pest, our findings show microbial community interactions can strongly influence the host plant's physiology and pathology.

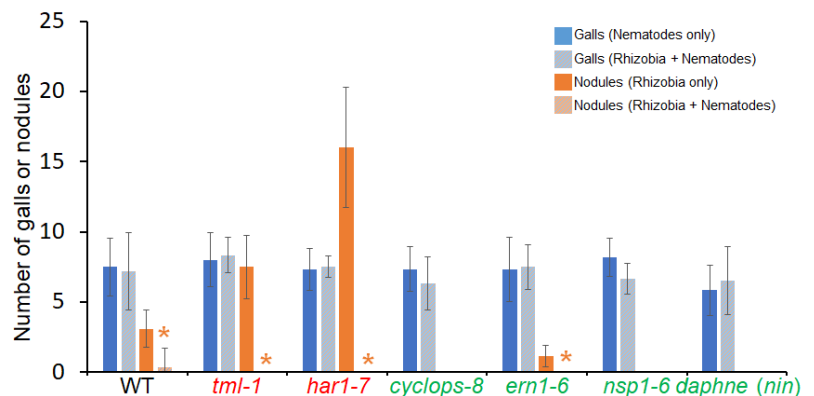


Fig. 2: Nodule and gall numbers of plants co-inoculated with rhizobia and RKN. Averages of $N=3 \pm SD$ are shown, * $P < 0.05$, student's T-test

3. Research plan for the next year

1. Determine whether the nodulation suppression signal induced by RKN is systemic, i.e. affects the entire plant. To accomplish this, *M. loti* roots from one plant will be split into two equal portions in separate compartments, to be inoculated with rhizobia and RKN independently. This will confirm whether RKN infection can suppress rhizobia nodulation in tissues that are not in direct contact with RKNs. Systemic nodule suppression would suggest the presence of a mobile signaling released during RKN infection, and the next objective would be to identify this signaling molecule.

2. Characterize the cellular response of RKN-induced nodulation suppression. Rhizobia with fluorescent marker will be used to infect plants with or without RKN infection. This will allow the observation of rhizobia infection at the cellular level, and identify the mechanism of how RKN suppresses nodulation.

3. Quantify changes in nitrogen fixation efficiency during RKN infection. Since RKN infection suppresses nodulation, it is also possible that RKN also affects nitrogen fixation of nodules that have already been formed. To test this, plants infected with rhizobia will be challenged with RKN, then the nitrogen fixation efficiency of the root tissues will be measured using the acetylene reduction assay, which quantifies nitrogenase activities in rhizobia. This will provide a more comprehensive outlook on how RKN infections affect the host plants' overall physiology.

4. List of awards, grants, and patents


FY2022 理学部基礎研究助成（熊本大学）

FY2022 めばえ研究推進事業 NEO（熊本大学）

FY2023 Grant-in-Aid for Scientific Research C

5. List of journal papers (with IROAST as your affiliation) published between April 2022 and March 2023.

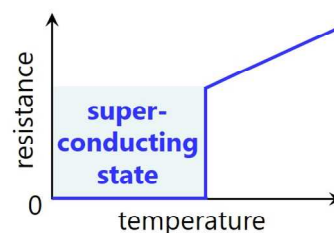
(Not available)

No.5-5	Development of superconducting or highly conducting molecular materials soluble in organic solvents			
Name	Akira UEDA	Title	Associate Professor	
Affiliation	Faculty of Advanced Science and Technology (FAST) Email: aueda@kumamoto-u.ac.jp			
Research Field	Advanced materials			

[Details of activities]

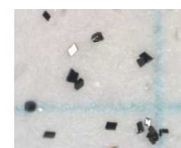
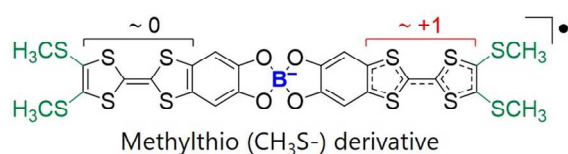
1. Research outline and its perspective (in approx. 50-80 words and attach 1-2 relevant photographs)

Superconductors have various peculiar properties, such as zero electrical resistance (shown right), and thus have been actively studied for many years in terms of both basic research and application development. In this study, this researcher has attempted to develop a new type of molecular superconductors/high conductors soluble in organic solvents by his original molecular design, aiming to bring a new breakthrough in superconductor research and to open the door to the creation of innovative science and technology for building a well-being society.

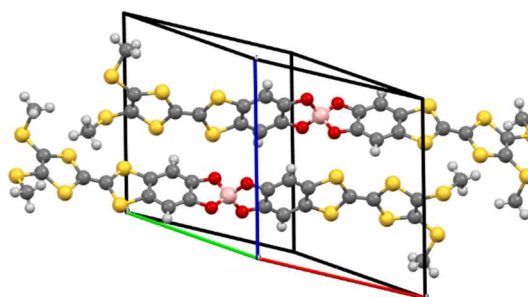


2. Research progress and results in the fiscal year (attach 1-2 related photos)

In this Young Researchers Support Program in FY2021, this researcher has successfully developed an innovative molecular design for high conductive purely organic materials that can be formed with a single kind of molecule (this result was published in 2022; see the section 5). In this fiscal year of 2022, on the basis of this molecular design, he has aimed to obtain superconducting or highly conducting molecular materials soluble in organic solvents. Specifically, he has designed several kinds of new analogues molecules with chain-type substituents and attempted to synthesize and crystallize them. As a result, the methylthio ($\text{CH}_3\text{S}-$) derivative was successfully obtained as air-stable crystals (shown right) and its structure and properties were experimentally revealed.



Interestingly, this crystalline material is found to show fairly high conductivity (10^{-4} S/cm at room temperature) in single-component purely organic materials, which demonstrates that the molecular design proposed by this researcher is certainly effective for developing low-molecular-weight superconductors/high conductors. On the other hand,



unfortunately this material is not soluble in common organic solvents, similar to the parent one with ring-type substituents reported in the paper shown below. In general, molecules with chain-type substituents have higher solubility in organic solvents than the corresponding ones with ring-type substituents; this is why this researcher designed the analogues molecules with chain-type substituents in this fiscal year. However, considering the present results, chain-substituents longer

than the methylthio group are needed to make this kind of molecule soluble in organic solvents. Therefore, in this fiscal year this researcher has successfully obtained important findings to achieve the development of superconducting or highly conducting molecular materials soluble in organic solvents, the goal of this project.

3. Research plan for the next year

As discussed in the section 2, solubilization of the designed molecules in organic solvents is the most important issue at this stage to achieve the goal of this project, and thus this researcher will work on the introduction of longer chain-substituents ($\text{CH}_3(\text{CH}_2)_n\text{S}-$; $n \geq 2$) into the molecules in the next year (FY2023). Since such substituents affect not only the solubility of molecules but also the intermolecular interactions, molecular arrangements and conductivity in the solid/aggregation states, it is important to find substituents with appropriate chain lengths which provide both solubility and conductivity, rather than simply increasing the chain length. The molecular level discussion on the structure and properties of the obtained molecules/molecular solids will be performed. A part of these studies in FY2023 will be supported by JSPS KAKENHI Grant Numbers JP23K04692 and JP23H04035.


It should be noted that there are no examples of molecular superconductors/high conductors soluble in organic solvents; therefore, if such organic materials are really developed in this project, new chemistry, physics, and materials science of superconductors/high conductors will emerge, leading to the creation of innovative science and technology, such as solution-processable superconducting materials/devices. This researcher believes that this project has the potential to open the door to a “super” energy-efficient, sustainable, and well-being society using organic materials.

4. List of awards, grants, and patents

Not applicable

5. List of journal papers (with IROAST as your affiliation) published between April 2022 and March 2023.

“Partially Oxidized Purely Organic Zwitterionic Neutral Radical Conductor: Multi-step Phase Transitions and Crossover Caused by Intra- and Intermolecular Electronic Interactions”
Taro Suemune[#], Keita Sonoda[#], Shuichi Suzuki, Hiroyasu Sato, Tetsuro Kusamoto, Akira Ueda*, *Journal of the American Chemical Society*, **144**, 21980–21991 (2022).

No.5-6	Antibacterial and antiviral of graphene quantum dots			
Name	Wei XU	Title	Assistant Professor	
Affiliation	Faculty of Advanced Science and Technology(FAST) Email: xuwei@kumamoto-u.ac.jp			
Research Field	Biotechnology & healthcare technology			

[Details of activities]

1. Research outline and its perspective

Coronavirus disease 2019 caused a significant threat to human health, and increased the needs of antimicrobial materials which can inhibit the growth or kill bacteria and virus. 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. In this study, we found a simple method to synthesize water-insoluble hydrophobic GQDs (Fig.1), and their antibacterial and antiviral activities were examined.

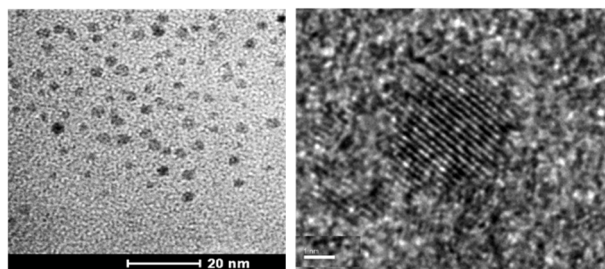


Fig.1. TEM images of GQDs

2. Research progress and results in the fiscal year (attach 1-2 related photos)

As traditional antibacterial and antiviral materials, photocatalyst (such as TiO₂) and silver materials are widely studied. However, light irradiation and environment problems have to be considered. Graphene, a single layer of graphite shows carbon atoms arranged in a honeycomb structure, exhibits high thermal, mechanical and electrical properties. Nanostructures of graphene, GQDs are attracting attention due to the superior properties.

In this study, GQDs was prepared with a bottom-up method by heating acetic anhydride with sulfuric acid at 160°C for 1 h. Then, GQDs were dispersed into chloroform and washed with water, sodium bicarbonate and saturated saline. Under UV light irradiation, GQDs showed yellow-green fluorescence. On excitation of GQDs at 440 nm light, strong fluorescence peak at 530 nm was observed.

For evaluation of antibacterial activity, gram-positive bacteria, *S. aureus* and gram-negative bacteria, *E. coli* was cultured with GQDs and measured concentration of the bacteria within 6 h. As shown in Fig.2, the antibacterial activity of GQDs against *E. coli* was enhanced by increasing

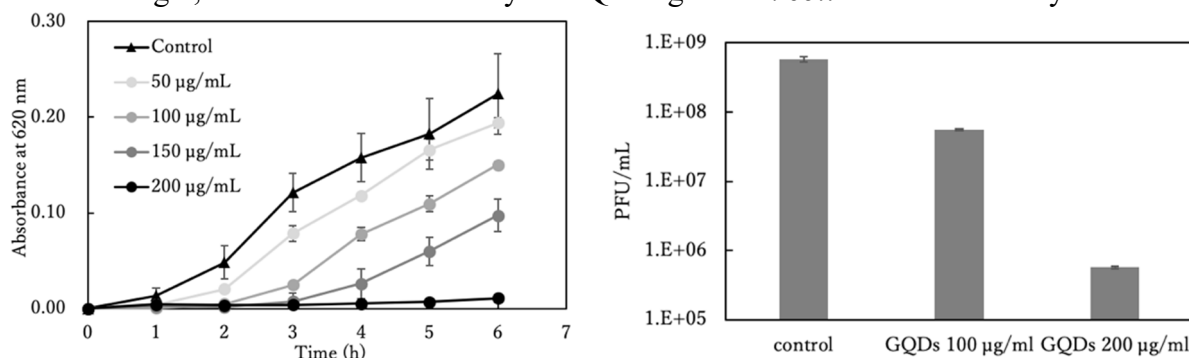


Fig.2. Antibacterial and antiviral activities of GQDs

concentration of GQDs. At 200 µg/ml, bacterial growth was inhibited entirely. For bacterial *S. aureus*, GQDs exhibited slimily antibacterial activity. It means GQDs inhibit the growth of both gram-positive and gram-negative bacteria. According to microscope observation, we found GQDs adhered on the surface of bacteria, however, water-soluble GQDs did not show the adhesion behavior. Therefore, we suggested that this adhesion inhibited bacterial growth and exhibited antibacterial activity.

For evaluation of antiviral activity, bacteriophage Q β , a positive-strand RNA virus which infects *E. coli* bacteria was cultured with GQDs for 3 h and counted plaques formed by co-culturing bacteriophage and *E. coli* on agar plates. For antiviral activity, 5% of bacteriophage Q β was survived when added 100 µg/ml of GQDs added and cultured for 3 h. At 200 µg/ml, survival of the virus was 0.02%. From these results, GQDs are expected as a candidate of antibacterial and antiviral materials.

3. Research plan for the next year

Next year, I will mix GQDs with several kinds of polymers, especially with biodegradable polymers. Biodegradable polymers are widely used in biomedical and pharmaceutical fields. They can be roughly divided into synthetic and natural biodegradable polymers. For synthetic polymers, poly-L-lactic acid (PLLA) will be used. It is a semi-crystalline polymer with random and amorphous segments that is known for its high degree of crystallinity. The amorphous segments and molecular weight determine its degradation rate and influence its mechanical properties. Whereas, for natural polymers, silk fibroin will be used. Silk fibroin (SF) is an attractive natural protein produced by the silkworm (*Bombyx mori*) for biomedical application because of its permeability to oxygen and water, controlled biodegradability, good cell adhesion, low inflammatory response, and high tensile strength. After preparing the GQDs-contained polymer film with drop and dip methods, the antibacterial and antiviral activities of GQDs-contained polymer film will be evaluated as mentioned above. Moreover, the suitable concentration of mixed GQDs will be confirmed.

4. List of awards, grants, and patents

None

5. List of journal papers (with IROAST as your affiliation) published between April 2022 and March 2023.

None