

Oral Presentations

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National BioResource Project-Yeast

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The National BioResource Project (NBRP) starts in FY 2002 to comprehensively promote life sciences. In the project, the systems of collection, preservation, and deposition have been established for bioresources, such as experimental animals, plants, and microbes (systems, groups, tissues, cells, and genetic materials of animals, plants, and microbes and their information as research and development materials) that are important for the nation to organize strategically.

Yeast is an important eukaryotic model organism. This is especially true of the fission yeast *Schizosaccharomyces pombe* and the budding yeast *Saccharomyces cerevisiae*, which are making significant contributions to research in a variety of areas within the life sciences. NBRP-Yeast has established a framework to collect, stock and distribute strain and DNA resources of mainly the two species. Through phases 1 and 4 (FY 2002 - 2018) of the NBRP, the NBRP-Yeast has become one of the top international yeast resource centers. Here, I will introduce the recent activity of NBRP-Yeast.

Contribution to Science using the Silkworm Bio-Resources



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The economic importance of silk has prompted exhaustive research on the Silkworm, *Bombyx mori*. There are also many kinds of silkworm strains, collected from world. The Japanese breeders and scientists have developed advanced strains for sericulture and academic resources. These strains are core resources in National-Bio-Resource Project (NBRP) started in 2002 with support by the Japanese government. Now, 29 species including silkworm are open for worldwide use. Purpose of NBRP is Collection, Preservation and Distribution of bio-resources that are basic materials for life science researches. Following resources are available by contact to NBRP silkworm (<http://silkworm.nbrp.jp/>). 1.

Domesticated silkworm (*Bombyx mori*): mutant strains, consomic strains and transgenic strains. 2. Wild silkworm: 8 species within 6 genus (*Bombyx*, *Antheraea*, *Samia*, *Rhodinia*, *Actias*, and *Trilocha*). 3. Genome: cDNA clone of *B. mori* and *S. cynthia ricini*, Fosmid clone of *B. mandarina*, Assembled RNA-seq data of *B. mori*, *S. cynthia ricini*, and *Trilocha varians*. Before starting this project, available season of resources supply of silkworm strains was limited in spring. However it is available to access to resources during all seasons with progress of NBRP. Development of genome editing technology in *Bombyx mori* has resulted in the rapid increase in number of strains. It is an urgent issue to establish effective systems for the preservation of increasing strains. Because diapause egg of *Bombyx mori* cannot be preserved more than one year. and all strains should be reared once a year. There are two kinds of long time preservation methods in *B. mori*. One uses frozen sperm and the other uses frozen ovaries. The former are artificially inseminated to female moths and the latter are implanted to castrated female larvae. In 2013, we have started to provide transgenic strains which were restored by using frozen ovaries. Practical applications of cryopreservation help us to reduce the labor of rearing silkworm and the risks of losing particular strains through unexpected accident such as disease or contamination.



Optical Tweezers for Manipulation of Nanomaterials

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Light can exert a mechanical force on a small object. It is called optical force or radiation pressure, by which light can trap and manipulate a small object. The optical manipulation science falls in 3 categories in accordance of the size of object. In the regime of smallest size, it is known as the laser cooling for trapping atoms, for which the Nobel prize in Physics 1997 was given. In a regime of a larger size where light wavelength is sufficiently larger than the object, nowadays optical tweezers are commercially available and widely used to manipulate living cells in medical science.

On the other hand, an optical manipulation study in an intermediate regime of nanomaterials has hitherto been limited. Optical manipulation of nanomaterials such as DNA, proteins, artificial polymers, molecular clusters, quantum dots and so on would be fruitful and intriguing, because various chemical processes involving phase transition, ordering, crystallization, molecular aggregation, patterning, chemical reactions, etc. should be induced and controlled due to intermolecular interactions in the manipulation processes. Under such situation, we expect the birth and growth of “the optical manipulation chemistry”.

However, it is rather difficult to stably trap nanomaterials since trapping potential energy ($U = (1/2) \alpha E^2$) should be much smaller than the thermal energy ($|U| \ll kT$). For stable and efficient optical manipulation of nanomaterials, we have proposed and developed novel techniques for optical manipulation, whose details are described in the following.

1) Resonant optical manipulation Using a traditional optical tweezers with a focused laser beam, we succeeded in optical trapping of a small protein (egg white lysozyme) and amino acid clusters in aqueous solution^[1]. However, trapping efficiency was low. Long time and intense laser light was necessary to detect a sign of trapping of these molecules. For enhancing the trapping force F , we proposed a method of resonant trapping whose concept is like this. When trapping laser light resonantly excites a targeted particle, F should be much enhanced due to an increase in α (or in other words, light-matter interactions should be enhanced). As a trapping target, we single out a heme protein, myoglobin. The trapping behavior was analyzed using a confocal Raman microscope. Only under a resonant condition where laser light electronically excite the cofactor heme, myoglobin molecules were obviously trapped at a focal point with great efficiency^[2]. Such behavior was well consistent with a theoretical model proposed by prof. Hajime Ishihara. These are direct evidence of the resonant optical manipulation.

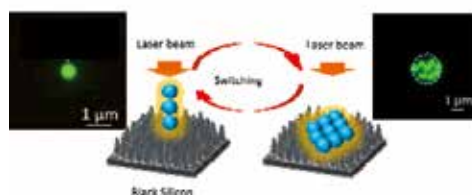
2) Plasmonic Optical Manipulation Plasmonic enhanced electromagnetic field is applicable not only to SERS but also to chemical reaction promotion^[3], and also even to optical trapping^[4]. Plasmon-based

optical trapping is quite intriguing and is currently attracting much attention in nano-photonics and related research fields, since optical trapping based on surface plasmon can potentially overcome several disadvantages of conventional optical trapping technique using a focused laser beam. For example, small palm-top lasers with low light intensity are applicable to plasmonic optical tweezers for trapping of nanomaterials.

So far, we have succeeded in efficient plasmonic trapping of quantum dots^[5], polymer beads^[6], artificial chain polymers^[7] and revealed trapping behaviors using microspectroscopies. Moreover, we developed a femtosecond plasmonic optical tweezer. This enables us switchable trapping for DNA in a persistent fixation mode or a trap-and-release mode^[8].

3) NASSCA Optical Manipulation According to these previous studies on plasmonic optical manipulation, it has several practical issues that need to be overcome. In many cases, it has suffered from thermal effects (Marangoni convection and thermophoresis) that frequently hindered stable trapping of nanomaterials^[9].

Here, we proposed an alternative approach using a nano-structured material that can enhance the optical force and be applied to optical tweezers^[10]. The material is metal-free black silicon (MFBS), the plasma etched nano-textured Si. We demonstrate that MFBS-based optical tweezers can efficiently manipulate small particles with characteristic features. The MFBS-based optical tweezers can overcome disadvantages of plasmonic optical tweezers, because of no detrimental thermal forces, and so on. We call this technique NASSCA optical tweezers (Nano-Structured Semiconductor Assisted optical tweezers). The concept of NASSCA optical tweezer is illustrated in the figure shown below.



Thus we have proposed novel concepts to enhance optical forces for manipulation of various molecular nanomaterials. These 3 optical manipulation techniques will open a new channel to the optical manipulation chemistry.

I would like to express my sincere thanks to all our collaborators, supervisors, and all the members of my lab.

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Photomechanical behavior of photochromic molecular crystals

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Photochromic compounds undergo a photochemically reversible transformation reaction between two isomers. Such molecules in organic crystals, which are regularly oriented and fixed in the crystal lattice, may be potentially useful for optoelectronic devices. To apply photochromic diarylethene crystals to photonics, electronics, mechanics, and medical fields, the materials are required to change large physical property by photoirradiation. We have so far reported on photoresponsive crystal shape change of diarylethene crystals [1]. In this paper, we have focused on recent development of novel photomechanical phenomena of crystals.

A crystal of diarylethene **1a** was found to undergo a reversible thermodynamic single-crystal-to-single-crystal phase transition accompanying a change in crystal length, which was clarified by differential scanning calorimetry measurement, X-ray crystallographic analysis, and direct microscopic observation of the crystal length. Furthermore, upon irradiation with UV light, the diarylethene crystal exhibited an unusual photomechanical behavior [2]. The photoinduced reversible crystal twisting of diarylethene **2a** was observed upon alternating irradiation with UV and visible light [3]. The crystal twisting takes place in both a left-handed helix and a right-handed helix. A ribbon-like crystal of diarylethene **3a** exhibited bending, cylindrical helix, and twisting depending on the illumination direction [4]. The control of photomechanical crystal deformation by illumination direction provides a convenient and useful way to generate a variety of photomechanical motions from a single crystal. Moreover, tiny nano-rod crystals with a thickness of a few hundreds nm were fabricated using an anodic aluminum oxide filter as a template, in which diarylethene molecules was crystallized [5,6]. The nano rod also underwent photoinduced bending.

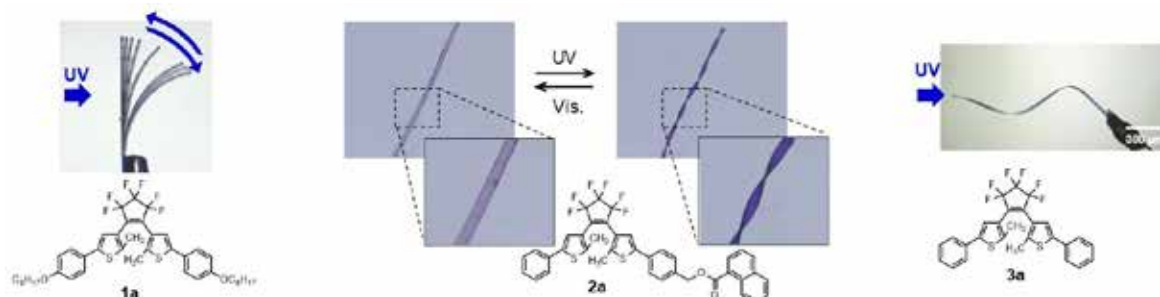


Figure 1. Unusual photomechanical behavior of diarylethene crystals.

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Development of Support Instrumentation in Regenerative Medicine applying Multi-functional OCT

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Abstract:

In recent years, the regenerative therapy of osteoarthritic cartilage [1] and skin equivalent [2] has attracted attention due to clinical transplantation of 3-dimensional autologous cultured cartilage and skin. However, a non-contact and invasive diagnosing method of bio-mechanical functions, e.g. viscosity and elasticity, has never been established yet. In this study, we have constructed and validated the ultrasonic-assisted Doppler OCT system (UA-OCDV), which can provide viscoelastic and pharmacokinetic characteristics inside tissue tomographically and non-contactly using a high intensity focused ultrasound transducer as a loading device. The proposed system was applied to 3-dimensional human dermal equivalent, then the tomographic phase map could display the discrimination of tissue viscoelasticity. This phase difference could be caused by the spatio-temporal interaction between elastic shear wave and acoustic streaming, which are generated by acoustic radiation pressure. In conclusions, UA-OCDV has good potential to visualize tissue function tomographically and non-contactly.

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Development of Aquifer Thermal Energy Storage System with High Performance Thermal Well and future spread measures

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Abstract:

Large urban areas in high energy consuming density are required to applicable practical scale renewable energy technologies without damaging urban environment like global warming and heat island problem. From 10 years before, we are trying to develop ground water utilization as 'ubiquitous' heat source.

Now, we can realize direct groundwater heat source utilization under iron and salt ion rich location in the coastal urban area, that leads hard well clogging easily and so on which was difficult to use, and this technical development project is assisted by Japanese Ministry of the Environment and Osaka city government.

We set up the well in the Umekita redevelopment area in the northern part of JR Osaka Station, and we can pump up groundwater total 550,000m³, get a heat power and immediately recharge the groundwater from another well to original same aquifer.

This is better way that reduce the influence of land subsidence problem in the urban area with keeping groundwater level in local area, and we confirmed it could be operated stably for a long period by using airtight structure.

In this presentation, we show the background and the outline of this technology development and introducing the next project in Maishima as Osaka bay area.



Promotion of aquifer thermal energy storage in Osaka metropolitan area

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Abstract:

Aquifer thermal storage (ATES) is a technology that extracts thermal energy from groundwater and efficiently air-condition the building, and is expected as a measure for energy saving, CO₂ emission reduction, and urban heat island. Osaka City, with its abundant groundwater in shallow aquifers, promotes aquifer thermal storage, taking advantage of the feature of the area that large commercial buildings are concentrated.

As a part of its efforts, Osaka City Government, in the commissioned research for the Ministry of the Environment, conducted a research project of ATES in the development district of Umekita, in collaboration with academia, industry and government. As a result, energy saving effect of 35% compared with the conventional air conditioning system has been confirmed without causing impact on the surrounding environment such as ground subsidence.

However, in Osaka, groundwater collection has been strictly restricted due to ground subsidence caused by pumping groundwater excessively in the past, so large-scale ATES cannot be installed. Accordingly Osaka City Government examined the data of the demonstration experiment in Umekita district with academic experts and submitted to the Ministry of the Environment the deregulation measures that allow groundwater collection subject to restoring same amount of groundwater to the same aquifer only for the purpose of heating and cooling the building.

When this deregulation is approved, large-scale aquifer thermal storage will be able to installed in Osaka metropolitan area where urban development will be planned in the near future such as Umekita or Yumeshima, and it is expected to contribute to significant energy saving and CO₂ reduction in Osaka.



Metal Oxide Photodeposition towards High Activity of Titanium Dioxide

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Heterogeneous photocatalysis is one promising approach to solve environmental problems via safe and green technology. Titanium dioxide (TiO₂) has been recognized as a highly active photocatalyst for degradation of organic pollutants. Various approaches have been made to improve the performance of TiO₂ photocatalysts such as by deposition of metal oxide as co-catalyst. One promising method is photodeposition method, which offers mild conditions as the process can be carried out at room temperature, and thus, would not affect the original properties of TiO₂ photocatalyst in terms of crystallinity, particle size, and anatase-rutile ratio. In this talk, the photodeposition of copper [1], lanthanum [2], and iron oxides [3] on the TiO₂ will be discussed at the point of view of properties and photocatalytic activity for degradation of 2,4-dichlorophenoxyacetic acid.

The TiO₂ was modified with copper species by the photodeposition method at room temperature under UV light irradiation [1]. The X-ray absorption near edge structure (XANES) analysis showed that copper species existed as Cu²⁺. Exploring the photocatalytic activities of copper oxide photodeposited on TiO₂ under UV, visible, and solar light irradiation resulted in different trends. Under UV light irradiation, the photocatalytic activity increased by a maximum factor of 4.3 compared to the unmodified TiO₂ when TiO₂ was loaded with 0.75 mol% CuO. In contrast, under visible light and solar simulator irradiation, the optimum loading of CuO was much lower (0.1 mol%) and enhancements of 22.5 and 2.4 times higher activities were observed, respectively. These results showed that the different masking effects due to the different light sources led to different optimum amounts of CuO, and the added CuO mostly contributed to the visible light activity of TiO₂. Therefore, in addition to the increased charge separation and improved visible light absorption, the masking effect shall also be considered in designing highly active photocatalysts under visible and solar light irradiation. In the proposed mechanism, the Cu²⁺ species was suggested to be the active sites to accept the excited electrons from TiO₂.

Different from copper species, in the case of using lanthanum species photodeposited on TiO₂, the lanthanum species only improved the UV photocatalytic activity of TiO₂ up to 5.5 times higher photocatalytic activity [2]. X-ray photoelectron spectroscopy (XPS) analysis showed

that photodeposition of lanthanum increased the formation of Ti^{3+} , while the electrochemical impedance and photocurrent results showed that the enhanced TiO_2 photocatalytic activity was caused by the increased charge separation in the TiO_2 photocatalyst, owing to the additional formation of Ti^{3+} states. Therefore, the optimum amount of lanthanum species that gave the optimum photocatalytic performance under UV light irradiation could be also related to the optimum amount of Ti^{3+} , which can additionally act as an acceptor for the excited electrons.

As for the iron oxide photodeposited on TiO_2 , it was confirmed that the iron species would exist as Fe_2O_3 [3]. The formation of a heterojunction between Fe_2O_3 and TiO_2 nanoparticles was suggested to promote the good charge transfer and suppressed electron-hole recombination. Under UV light irradiation, the photocatalytic activity of TiO_2 could be enhanced up to 3 times higher when 0.5 mol% Fe_2O_3 was photodeposited on the TiO_2 . The photocatalyst was shown to have good stability and reusability for the 2,4-D degradation. A further investigation on the role of the active species on Fe_2O_3/TiO_2 confirmed that the crucial active species were both holes and superoxide radicals. All these studies demonstrated that photocatalytic activity of TiO_2 can be enhanced by photodeposition of copper, lanthanum, or iron oxides. While the photodeposited copper oxides contributed to the visible light harvesting of TiO_2 , the addition of lanthanum and iron oxides gave higher enhancement on the activity of TiO_2 under UV light irradiation.

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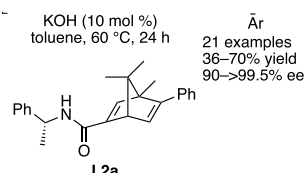
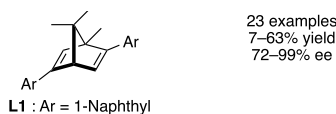
Enantioselective Rh-Catalyzed Syntheses of Chiral Nitrogen Containing Compounds



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Abstract: An enantioselective Rh(I)-catalyzed conjugate addition reaction of α -substituted β -nitroacrylates with various arylboronic acids using Rh(I)-chiral diene catalysts is described. The addition reaction proceeds under mild conditions in a range of common organic solvents and additives offers the corresponding quaternary carbon-containing α,α -disubstituted β -nitropropionate products in up to 63% yield with up to 99% ee (eq 1).^[1] While 2,5-dinaphthyl substituted chiral diene **L1** is proved optimal for the asymmetric arylation of α -substituted β -nitroacrylates, employing Rh/**L1** catalyst in the arylation of β -nitroacrylates sees only moderate selectivity. Enhanced asymmetric induction, in the arylation of β -nitroacrylates, is observed when applying Rh/**L2a** catalyst (eq 2).^[2,3]



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