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Artificial Leaf Based on Artificial Photosynthesis for Solar Fuel Production

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Final Report

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14. ABSTRACT The main contribution of this system is the demonstration that the conversion efficiency of CO ₂ to formic acid in a photo reduction reactor combining a photosensitizer and formate dehydrogenase (FDH) was improved 9-fold when compared with a solution system. This new device was constructed by immobilizing the photosensitizer, methyl viologen, and FDH in nanoporous glass plates with an inner nanopore diameter of 50 nm. The improvement of the efficiency was achieved through three-dimensional high-density immobilization of the photoreactants in the nanocavity inside PGP. The maximum production efficiency of formic acid inside the nanocavity was estimated to be at least 50-fold higher than that of conventional methods using FDH (e.g. homogenous solution systems and reaction systems on substrates). We believe that this contribution is theoretically and practically relevant because the artificial photosynthetic system developed has potential applications for the production of renewable energies, such as solar fuel. These methods of approach using enzyme model assembled in pore glass and on electrode with pattern will be very useful for construction of artificial leaf with the functions of solar to fuel, hydrogen or oxygen production and CO ₂ reduction to chemical resource from water.					
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Title of Proposed Research:

“Artificial Leaf” Based on Artificial Photosynthesis for Solar Fuel Production

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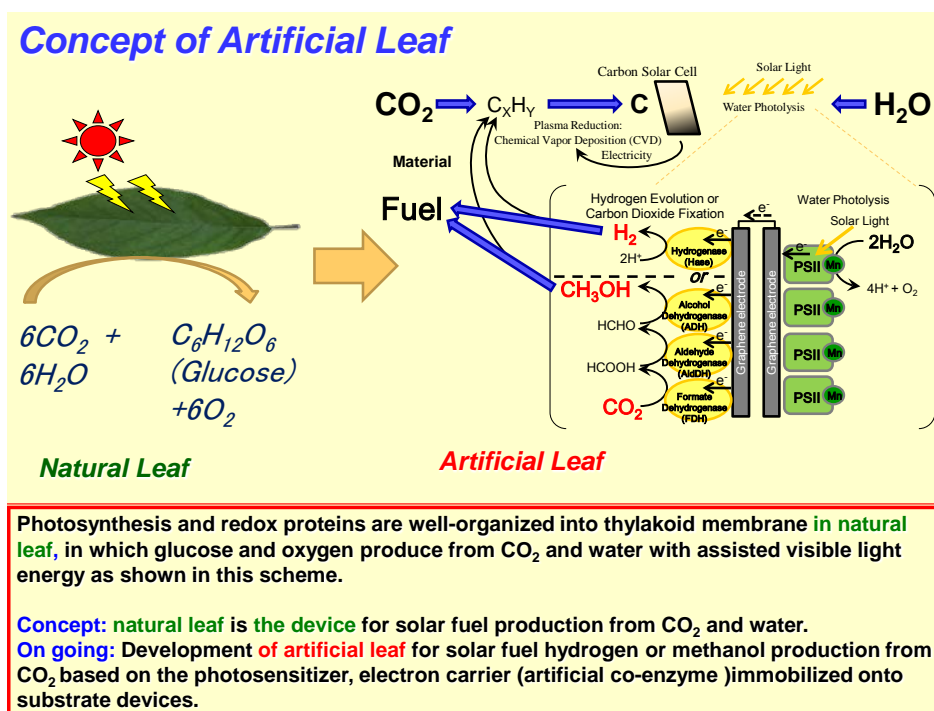
Contents

I . Abstract of the project results -----	3 p
II . Result of the project-----	3 p
1. Introduction -----	3 p
2. Experiment-----	6p
3. Results & Discussion -----	6 p
4. List of Publications -----	13p
A. Journal paper publications	13p
B. Conference presentation	14p

I. Abstract of the project results

Carbon dioxide fixation is a potential technology for the carbon dioxide reduction and synthesis of organic compounds from carbon dioxide and water as a starting material in environmental science and technology. Development of the creative system for production from carbon dioxide to low carbon such as methanol and hydrogen has received much attention. Especially, solar fuel production from carbon dioxide and water based on artificial photosynthesis has received significant attention in recent years. In this project, carbon dioxide reduction and hydrogen evolution systems based on the artificial photosynthesis, “**artificial leaf**” are proposed as for an example illustrated in Scheme 1: 1) CO₂ reduction to chemical resource, liquid fuel. 2) Solar fuel production from water.

We proposed a scenario where the construction of electron transfer system analogous to artificial photosynthetic system. This system is expected to start from molecular and supra-molecular entities in a variety of smart matrices that collect light energy and separate charge for developing new types of nanobiodevices to construct “**artificial leaf**” from solar to fuel.



Scheme 1.

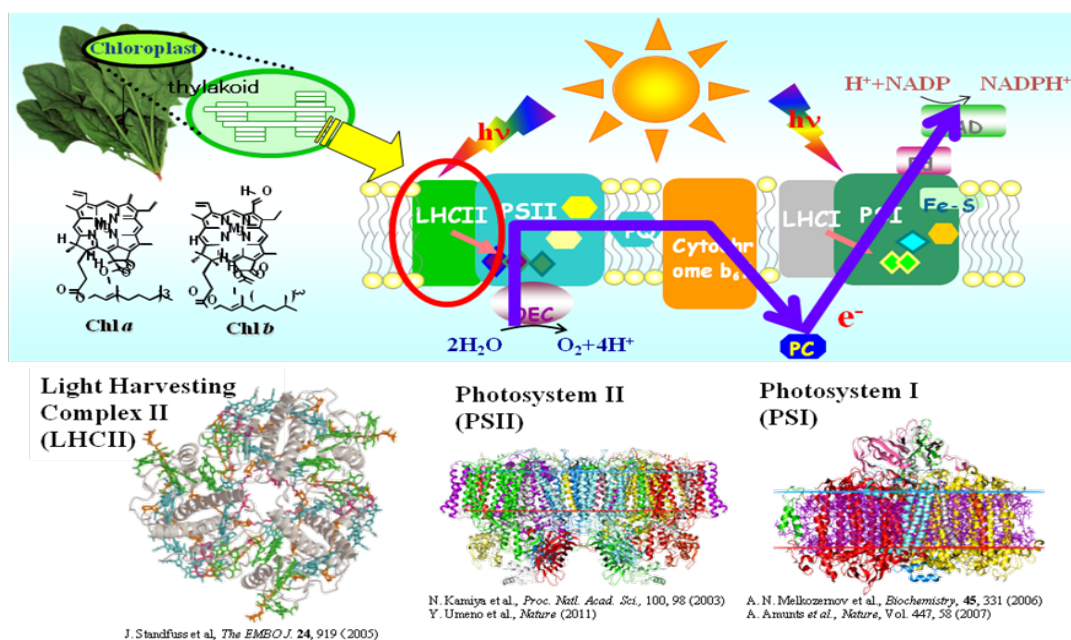
II. Result of the project.

1. Introduction

Nature provides a number of examples, in which processes of energy conversion, storage and transport are combined and optimized through ‘smart matrices’ at various levels, going from molecular to cellular or higher organisms. Based on biological design principles, future biology-based photonics or their synthetic organic materials could form clean and inexpensive future alternatives for productions of

nano-semiconductors and constructing fuel production system from solar with water and carbon dioxide (the “artificial leaf”).

It is important to understand not only the mechanisms of efficient light-harvesting but also those of photo-protection. In order to understand these reactions both structural and functional information are required. The data on how the energy levels and intermolecular interactions of the pigments affect their energy-transfer properties, and how the ‘durability’ of the complexes is required for rational design of novel biodevices. Based on the experiments using the native photosynthetic antenna complexes illustrated in Scheme 2, a variety of modified complexes will be synthesized and tested for their usefulness in artificial nanobiodevices, “artificial leaf”. After elucidation of the mechanisms of harvesting, transferring, usage and dissipation of light energy, our aim is to optimize under a given light intensity the energy-conversion efficiency and the durability of the core and the antenna complexes by modifying the pigment carotenoids (Cars) and chlorophylls (Chls) as well as the supporting peptides. These modified photosynthetic protein-mimic complexes was assembled on electrodes or into a pore glass as a light-induced redox component to produce a new type of nanobiomaterials from solar to fuel production. These approaches provided a foundation for using the artificial domains of photosynthetic core-antenna and antenna complexes, isolated from algae or plants for the development of new type of nanobiodevices (the artificial leaf).



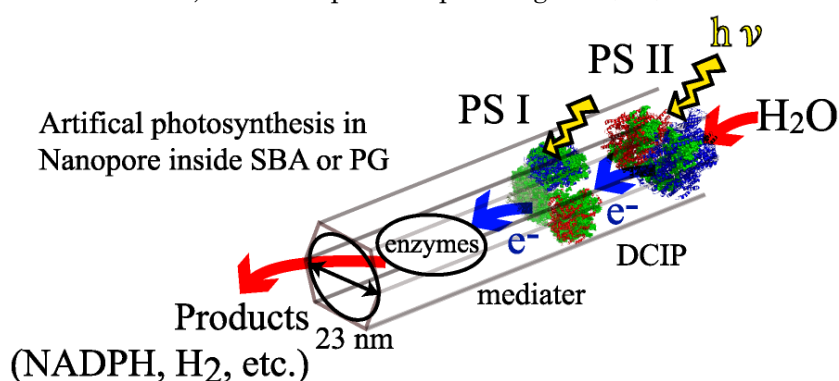
Scheme 2. Compartmentalization of light harvesting (LHCII) and charge separation (PSII & PSI). The antenna complexes (LHCII, PSII and PSI) efficiently realize various photosynthetic functions using cofactors (Chl a and carotenoids) assembled in the apoproteins.

The purpose of this project is to use artificial photosynthetic systems to develop the

systems from carbon dioxide to low carbon such as formic acid(HCOOH),formaldehyde (HCHO),methanol (CH₃OH) and hydrogen (H₂) evolution as shown in Scheme 1.

Our goal is to use photosynthetic pigment complex from algae, LHCII from plants, and PSII and PSI from algae or plants or their model complexes as a light harvester of the well-established cell to convert light energy in the ultraviolet and visible region into that in the near infrared region for development of new type of nanobiodevices (artificial leaf). The advantage of the light-harvesting complex is its efficient capture of photons throughout the near UV to near IR region and much higher durability than ordinary isolated dyes supported by its inherent photo-protective function. Thus, the results of the above grounds can be directly applied to the development of nanobiodevices using modified photosynthetic pigments or their model light-harvesting materials.

In this project, we focus to the assembly of photosynthetic pigment complexes on electrodes and then, into nanopores of porous glass (PG) as shown in Schemes 1 & 3.



Scheme 3. A schematic drawing of artificial photosynthesis in a nanopore of mesoporous silica (SBA) or porous glass (PG). Electrons are obtained from water by the photoreaction of Photosystem II (PSII) inside the nanopore. Then, electrons are transferred to Photosystem I (PSI) mediated by DCIP in the pore. Finally, NADPH or H_2 are produced by the photoreaction of PSI that reduces FNR and ferredoxin or hydrogenase inside the nanopore.

The majority of the aim is construction of the array of artificial photosynthetic system with various substrates using photosynthetic pigment complexes and their model complexes.

We propose a scenario where the construction of artificial photosynthetic systems on various electrodes and in PG is expected to start from molecular and supramolecular entities in a variety of smart matrices that collect light energy and separate charge for developing new types of nanobiodevices.

[A challenging task is to mimic the principle of the function in artificial photosynthesis devices \(artificial leaf\).](#)

During development of these projects, several useful chemical materials for physiologically useful chemical materials and functional polymer materials from bio-resource will be produced as challenging tasks to several applications.

2. Experiment

More details are presented in our papers in the list of publication below.

3. Results & Discussion

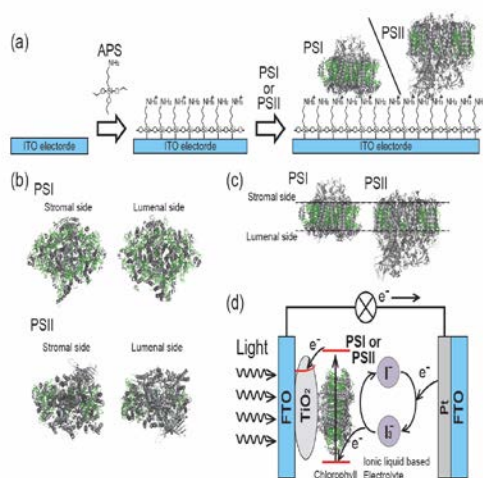
Our goal is to provide insight into the systems “how nature establish the efficient energy transfer systems from solar to fuel production as well as to construct efficient energy transfer systems for development of new type of nanobiodevices, “artificial leaf “ (Refs. 1) & 7). As indicated by Tasks below, we performed these objects in the proposed project period.

1) Artificial domain assembly of the LH to construct efficient energy harvesting and electron transfer systems (Refs. 2)-5), 7)-9),11),14)-15) & 16), 2D.6).

In the current of our previous study (Apply Phys. Lett. , 100,233701 (2012), J. Phys. Chem. Lett. 4, 1087–1092 (2013) ACS Macro Lett. , 1, 28-22(2012), & *Langmuir*, 29,5104-5109(2013) , we further prepared modified LH model complex to control the orientation and direction of the complex onto electrodes for hydrogen production to develop new types of nanobiodevices as Tasks 1-5 below.

Task 1: Assembly of PSI or PSII with tag on various electrodes (Ref. 3)).

Photosystems, PSI and PSII isolated from *Thermosynechococcus elongatus* were successfully immobilized onto a TiO₂ nanostructured film for use in dye-sensitized biosolar cells (DSBCs) as shown in Scheme 4. The photosystem complexes were also assembled on an ITO electrode modified with 3-aminopropyltriethoxysilane by utilizing interactions between the electrode and the surface of the PSI or PSII polypeptide. Illumination of the PSI and PSII complexes immobilized on the ITO electrode resulted in a cathodic photocurrent, which corresponded to the absorption spectra of the complexes. Compared to the ITO electrode, PSI or PSII complexes assembled on the TiO₂ electrode showed a remarkable enhancement in the energy-conversion efficiency in the presence of an iodide/triiodide redox system of an ionic-liquid based electrolyte. This systems could act as useful energy-harvesting materials in dye-sensitized biosolar cells (DSBCs).



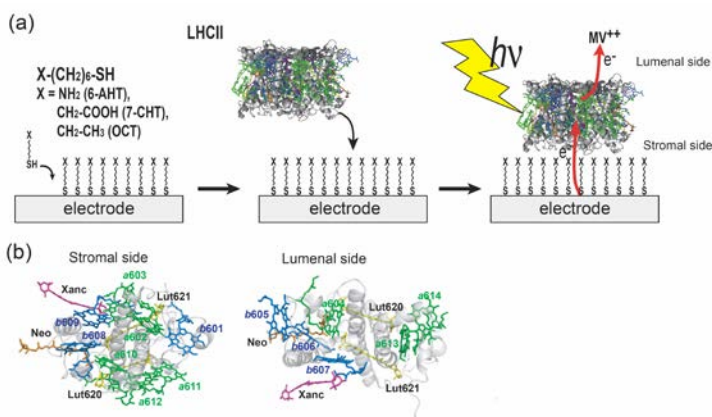
Scheme 4.

Our understanding of charge separation and energy transfer in these complexes has enabled us to

generate artificial systems that convert light energy into usable electrical current.

Task 2: Artificial domain assembly of LHCII pigment complexes or LH model pigments to construct energy harvesting systems (Refs.4) & 16,6D. 7).

A light-harvesting complex of photosystem II (LHCII), isolated from spinach, was immobilized onto a gold electrode modified with self-assembled monolayers (SAMs) of alkanethiols, $\text{NH}_2\text{-(CH}_2\text{)}_n\text{-SH}$, $n = 2, 6, 8, 11$; $\text{HOOC-(CH}_2\text{)}_7\text{-SH}$; and $\text{CH}_3\text{-(CH}_2\text{)}_7\text{-SH}$; and a bare electrode as shown in Scheme 5. The extent of LHCII complex adsorption according to surface treatment decreased in the order amino groups > carboxylic acid groups > methyl groups and increased with the methylene chain length in $\text{NH}_2\text{-(CH}_2\text{)}_n\text{-SH}$. Interestingly, the photocurrent density depended on the terminal group and the methylene chain length in $\text{NH}_2\text{-(CH}_2\text{)}_n\text{-SH}$ and decreased in the order amino groups > methyl groups > carboxylic acid groups. An efficient photocurrent response of the LHCII complex on SAMs of $\text{NH}_2\text{-(CH}_2\text{)}_n\text{-SH}$, $n = 8$ was observed upon illumination at 680 nm. These results indicated that the LHCII complexes were well organized on the cationic surfaces of the gold electrodes modified with amino alkanethiols. The quantum yield depended on the methylene chain length (n), where the maximum photocurrent response was observed at $n = 8$, which corresponded to a distance of 1.7 nm between the terminal amino group in $\text{NH}_2\text{-(CH}_2\text{)}_8\text{-SH}$ and the gold surface.



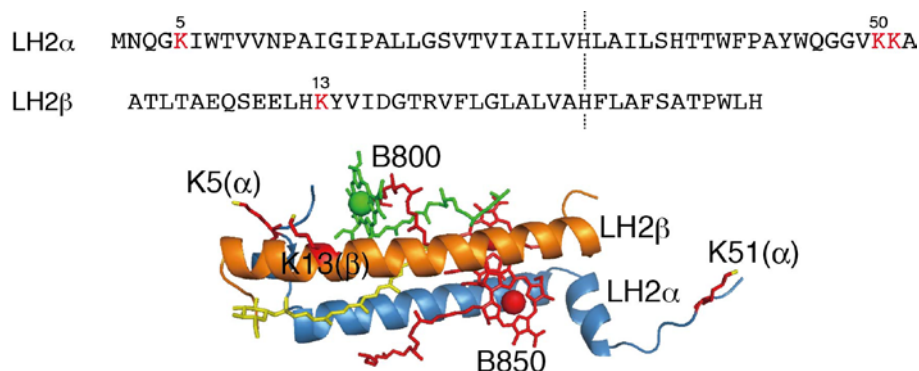
Scheme 5.

These results provide useful information about the effects of the surface modification of electrodes on the performance of the LHCII complex as a photo energy converter as well as its stability.

Task 3: Extension of light-harvesting ability of photosynthetic light-harvesting complex 2 (LH2) through ultrafast energy transfer from covalently attached artificial chromophores (Ref. 8)).

Introducing appropriate artificial components into natural biological systems could enrich the original functionality. In order to expand the available wavelength range of photosynthetic bacterial light-harvesting

complex 2 (LH2 from *Rhodospseudomonas acidophila*), artificial fluorescent dye (Alexa Flour 647: A647) was covalently attached to N- and C-terminal Lys residues in LH2 α -polypeptides with a molar ratio of A647/LH2 = \sim 9/1 (Scheme 6). Fluorescence and transient absorption spectroscopies revealed that intra-complex energy transfer from A647 to intrinsic chromophores of LH2 (B850) occurs in a multiexponential manner, with time constants varying from 440 fs to 23 ps through direct and B800-mediated indirect pathways. Kinetic analyses suggested that B800 chromophores mediate faster energy transfer, and the mechanism was interpretable in terms of Förster theory. This study demonstrates that a simple attachment of external chromophores with a flexible linkage can enhance the light harvesting activity of LH2 without affecting inherent functions of energy transfer, and can achieve energy transfer in the subpicosecond range. Addition of external chromophores, thus, represent a useful methodology for construction of advanced hybrid light-harvesting systems that afford solar energy in the broad spectrum. This systems could act as useful energy-harvesting materials and our understanding of energy transfer in these complexes has enabled us to generate artificial systems that convert light energy into usable electrical current.

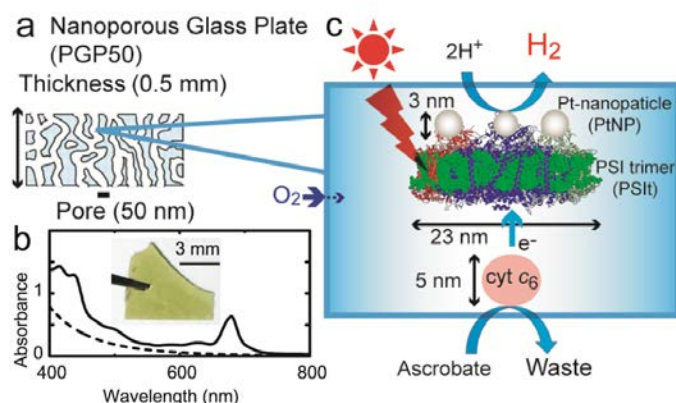


Scheme 6.

Task 4: Stabilization of Charge-Separated State and Photocurrent Generation Activity of LH1-RC in Lipid Environments (Ref. 16), 2D.6)

Purple bacterial light-harvesting/reaction center complex (LH1-RC) possesses a high potential for photovoltaic devices, however, it has been still challenge to bring out the potential ability of LH1-RC invitro. In this study, we examined effects of phospholipids on the stability of charge-separated state of LH1-RC, which may influence photocurrent generation activity of LH1-RC assembled onto an electrode. Photocurrent generation of LH1-RC incorporated in planar lipid membranes composed of either DOPG or phosphatidylcholine (DOPC) that were assembled onto amino-functionalized ITO electrodes was observed in the presence of ubiquinone (UQ0) and cytochrome c as electron mediators. Significant photocurrent generation was observed for LH1-RC in DOPG membrane with a prominent quantum efficiency (26%) compared to that in DOPC (10%) and without a lipid system (\sim 2%). These results suggest that the LH1-RC with more stable charge-separated state tuned by phospholipids functions as an efficient photoelectric conversion device. We show a novel approach to exerting potential of LH1-RC.

hydrogen production system by immobilizing PSIt-PtNP conjugate inside porous glass plate having pore of 50 nm (PGP50) (Scheme 9). A reaction medium containing PSIt-PtNP-PGP50 or PSI-PtNP conjugate was irradiated under anaerobic and aerobic conditions. The O₂-resistivity (ratio of light-induced hydrogen evolution activity under aerobic and anaerobic condition) of PSIt-PtNP conjugate in solution and PSIt-PtNP-PGP50 were 0.8 and 16%, respectively, showing that this PGP system proposed represents a promising first step toward the development of an O₂-tolerant solar energy conversion system.



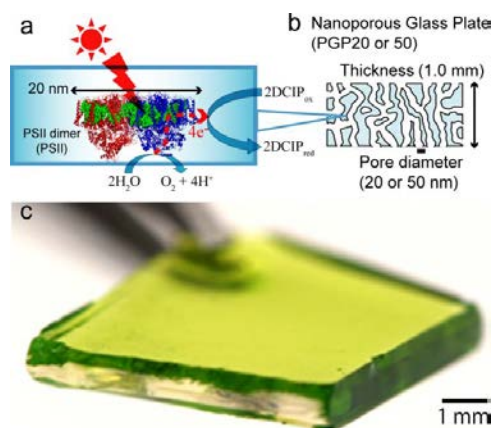
Scheme 9. Light-driven H₂ production by PSIt-PtNP conjugate inside nanopores in PGP50. Schematic of nanoporous glass plate (PGP50) with pore diameter of 50 nm (a). cytochrome c₆(cyt c₆), photosystem I trimer (PSIt), and Pt-nanoparticle (PtNP) were immobilized in the PGP50, where efficient H₂ production took place via sequential electron transfer by redox components (b). (c) Solid and broken lines show absorbance of PSIt-PtNP-PGP50 and PGP50 in MES-NaOH (pH6.2), respectively. Inset shows a photographic image of PSIt-PtNP-PGP50. Scale bar: 3 mm.

Now, light-induced oxygen-hydrogen production systems via optimization of LHCII, PSI and PSII-PGP conjugates are on-going for development of “artificial leaf”.

Task 7: Oxygen-Evolving Porous Glass Plates Containing the Photosynthetic Photosystem Pigment–Protein Complex (PSII) (Refs. 12) & 16), 6D.7).

The development of artificial photosynthesis has focused on the coupling of a photo-anode and cathode, wherein the production of hydrogen (or energy carriers) is coupled to the electrons derived from water-splitting reactions. The natural photosystem II (PSII) complex split water efficiently using light energy. The complex is a large pigment-protein complex containing manganese cluster with a 20 nm diameter. A new photoanodic device has been constructed with the stable PSII purified from *Thermosynechococcus vulcanus* by immobilizing it in the 20- or 50-nm nanopores in porous glass plates (PGPs)(Scheme 10). PSII in the nanopores retained its native structure and high photo-induced water splitting activity. The photocatalytic rate (turnover frequency) of PSII in PGP was enhanced 11-fold compared to that in solution, giving the rate of 50–300 mol e⁻/(mol PSII•s) with 2,6-dichloroindophenol (DCIP) as an electron acceptor. The PGP system realized the high local concentrations of PSII and DCIP and enhanced the collisional reactions in nanotubes with low

disturbance of light penetration.

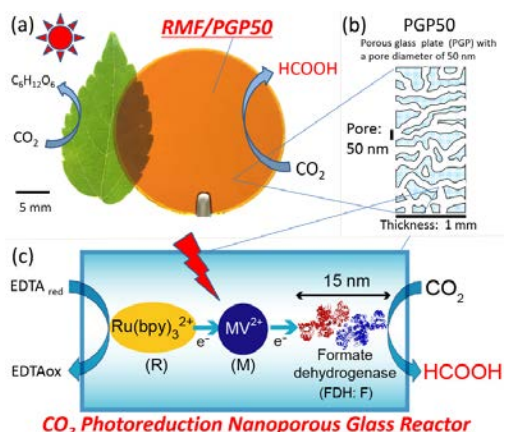


Scheme 10. Immobilization of PSII in nanoporous PGPs. Schematic illustrations of PGP (a) and photoreactions of PSII with water (electron donor) and DCIP (electron acceptor) (b). (c) Photographs of PSII-containing PGP50. The PSII structure was drawn using PyMOL software with the pdb file 3WU2.3

The system allows direct visualization/determination of the reaction inside nanotubes. This contributed to the optimization of the local reaction conditions. The PSII/PGP device will contribute to the construction of artificial photosynthesis using water as the ultimate electron source.

Task 8: Artificial assembly of LH device for fixation of carbon dioxide in pore glass and on electrodes (Ref. 13), Ongoing).

Fuelization from carbon dioxide to formic acid was successfully observed by selecting optimum electron transfer reactions with proton coupling system as shown in Scheme 11, collaborated with Prof. Yutaka Amai, Osaka city University.



Scheme 11. Light-induced formic acid production in nanopores. (a) Photographic image of a leaf and Ru(bpy)₃²⁺ (R)/MV²⁺ (M)/formate dehydrogenase (FDH: F)-immobilized PGP50 (RMF/PGP50). Scale bar: 5 mm. (b) Schematic of a porous glass plate (PGP) with a nanopore diameter of 50 nm (PGP50). (c)

$\text{Ru}(\text{bpy})_3^{2+}$ (R, photosensitizer), MV^{2+} (M, electron mediator), and FDH immobilized in PGP50, where efficient formic acid production takes place via sequential electron transfer by the redox components.

The main contribution of this system is the demonstration that the conversion efficiency of CO_2 to formic acid in a photoreduction reactor combining a photosensitizer and formate dehydrogenase (FDH) was improved 9-fold when compared with a solution system. This new device was constructed by immobilizing the photosensitizer, methyl viologen, and FDH in nanoporous glass plates with an inner nanopore diameter of 50 nm. The improvement of the efficiency was achieved through three-dimensional high-density immobilization of the photoreactants in the nanocavity inside PGP. The maximum production efficiency of formic acid inside the nanocavity was estimated to be at least 50-fold higher than that of conventional methods using FDH (e.g. homogenous solution systems and reaction systems on substrates). We believe that this contribution is theoretically and practically relevant because the artificial photosynthetic system developed has potential applications for the production of renewable energies, such as solar fuel.

Summary:

In this project, we propose a scenario for the construction of artificial photosynthetic system (the artificial leaf) with the subject options, starting from molecular and supramolecular entities in a variety of smart matrices to lead an electrochemical potential for development of new types nanobiodevices and fuelizing carbon dioxide. These methods of approach using enzyme model assembled in pore glass and on electrode with pattern will be very useful for construction of “artificial leaf” with the functions of solar to fuel, hydrogen production and CO_2 reduction to chemical resource from water .

As indicated by the Tasks of this project, we performed these objects in the project period below.

Task 1: Assembly of PSI or PSII with tag on various electrodes (Ref. 3)).

(Project period : 12 months from June 11, 2014 to June 10, 2015)

Task 2: Artificial domain assembly of LHCII pigment complexes or LH model pigments to construct energy harvesting systems (Refs.4) & 16), 6D. 7 & 6D. 12).

(Project period : 10 months from June 11, 2014 to June 10, 2015)

Task 3: Extension of Light-Harvesting Ability of Photosynthetic Light-Harvesting Complex 2 (LH2) through Ultrafast Energy Transfer from Covalently Attached Artificial Chromophores (Ref. 8)).

(Project period : 24 months from June 11, 2014 to June 10, 2016)

Task 4: Stabilization of Charge-Separated State and Photocurrent Generation Activity of LH1-RC in Lipid Environments (Ref. 2), 14)-16), 2D.6)

(Project period : 24 months from June 11, 2014 to June 10, 2017)

Task 5: A Artificial assembly of Light-energy conversion systems for hydrogen production and photocurrent generation by Zn chlorin derivatives (Refs. 9) &11)).

(Project period : 24 months from June 11, 2015 to June 10, 2017)

Task 6: Light-induced hydrogen production of Photosystem I-Pt nanoparticle conjugate inside nano-pores of porous glass plate (Ref. 10)).

(Project period : 24 months from June 11, 2015 to June 10, 2017)

Task 7: Oxygen-Evolving Porous Glass Plates Containing the Photosynthetic Photosystem Pigment–Protein Complex (PSII) (Refs. 12) & 16), 6D.7).

(Project period : 24 months from June 11, 2015 to June 10, 2017)

Task 8: Artificial assembly of LH device for fixation of carbon dioxide in pore glass and on electrodes (Ref. 13), Ongoing).

(Project period :24 months from June 11, 2015 to June 10, 2017)

Pay-off

Effects of dissemination of research results are as follows,

1) Assembly of photosynthetic antennas and their protein-mimic complexes on electrodes and into pore glass. This proposal aims to assemble photosynthetic core complexes (PSII and PSII) and the antenna (LHCII) complex, and their model complexes onto ITO and TiO₂, and into pore glass to solar to fuel production from water, with the reduction of carbon dioxide. If this trial becomes successful, it can trigger the development of a new information technology, IT industry as well as development of a new type of nanobiodevices.

2) Efficient usage of light energy. Photosynthetic antennas can collect light energy in the entire region from ultraviolet to near infrared. It has a unique property to harvest a small number of photons from all the different directions and to concentrate them for usage. This mechanism to enable high sensitivity in a wide spectral region can be used as a guiding principle in designing **the artificial leaf**.

3) Key to solve the energy and environmental crisis. Development of a safe and economical system for conversion of light energy into electricity is crucial in order to solve the energy and environmental crisis. The photosynthetic system is a best refined material in harmony with the global environment, and the present project aims to create “**Solar to Fuel**” as well as the reduction of carbon dioxide for the next generation using the solar energy which is exhaustible, clear and free of pollutant.

4. List of Publications:

A. Journal Paper publication

1) M. Nango, M. Sugiura ed., “Photosynthesis and artificial photosynthesis research”,

Res Chem Intermed, **40**, 9, Springer (2014).

2) “Structure–function relationships of the supramolecular assembly of the bacterial photosynthetic antennacomplexes in lipid membranes” *Res Chem Intermed*, **40**, 3243–3256 (2014)

3)“Immobilization of Photosystem I or II Complexes on Electrodes for Photoenergy-ConversionDevices”, *Res Chem Intermed*, **40**, 3287-3293 (2014)

4) “Self-assembly of the light-harvesting complex of photosystem II (LHCII) on alkanethiol-modified gold electrodes”, *Res Chem Intermed*, **40**, 3277–3285 (2014)

5) “Durability of oxygen evolution of photosystem II incorporated into lipid bilayers”

Res Chem Intermed, **40**, 3231–3241 (2014)

6) “Light-Driven Hydrogen Production by Hydrogenases and a Ru- Complex inside a Nanoporous Glass Plate under Aerobic External Conditions”

J. Phys. Chem. Lett., 5, 2403-2407(2014).

7) "Electric Device Approach Using Photosynthesis Assembly for the Development of Nanobiodevices, *Springer Series in Material Science, Lecture Note in Energy* 32, 437-454(2016); "Solar to Chemical Energy Conversion"; "Theory and Application", M. Sugiyama, K. Fujii, S. Nakamura Ed..

8) "Extension of Light-Harvesting Ability of Photosynthetic Light-Harvesting Complex 2 (LH2) through Ultrafast Energy Transfer from Covalently Attached Artificial Chromophores", *J. Am. Chem. Soc.* 137, 13121-13129 (2015).

9) "Photoinduced hydrogen production with photosensitization of Zn chlorophyll analog dimer as a photosynthetic special pair model "J. hydrogen energy, 40, 5313-5318 (2015)

10) "Light-Induced Hydrogen Production by Photosystem I-Pt Nanoparticle Conjugates Immobilized in Porous Glass Plate Nanopores", Springer, *Res. Chem. Intermed.* 42: 7731(2016)

11) "Light-Energy Conversion Systems for Hydrogen Production and Photocurrent Generation using Zinc Chlorin Derivatives", Springer, *Res. Chem. Intermed.*, 42, 7743 (2016)

12) "Oxygen-Evolving Porous Glass Plates Containing the Photosynthetic Photosystem Pigment-Protein Complex", *Langmuir*, 32, 7796-7805 (2016)

13) "Light-Driven CO₂ Reduction to Formic Acid by Formate Dehydrogenase with a Ru-Complex inside a Nanoporous Glass Plate", *ACS Applied Materials & Interfaces, ACS Appl. Mater. Interfaces*, 9, 3260-3265(2017).

14) "Assembly of Photosynthetic Antenna Complexes for the Development of Nanobiodevices" 2015 MRS Spring Meeting & Exhibit, April 6-10, 2015, San Francisco, California, US A

15) "Molecular Assembly of Photosynthetic Complexes for Development of Nanobiodevices", Proceeding: Invited Lecture IL-24. The 7th East Asia Symposium on Functional Dyes and Advanced Materials, Sept. 2-5, 2015, Osaka Prefecture University (B. Conference presentations: the abstract below).

16) The 17th International Congress on Photosynthesis Research, Photosynthesis in a Changing World AUGUST 7-12 2016, MECC, MAASTRICHT, THE NETHERLANDS, Abstract 2D.6, 6D.7, 6D.12 (the abstract below)

B. Conference presentations (International conference): Proceeding, Abstract

1. 2015 MRS Spring Meeting & Exhibit, April 6-10, 2015, San Francisco, California, US A Assembly of Photosynthetic Antenna Complexes for the Development of Nanobiodevices (Ref. 14))

The purpose of this study was to use a photosynthetic antenna complex (LH) assembled on substrates for the development of nanobiodevices from solar to fuel. We use the LH to construct an efficient energy transfer system for nanobiodevices and nanobiomaterial applications.

In this study, we primarily aimed to construct an array of photosynthetic antennae on substrates using a modified photosynthetic antenna complex prepared from modern biosynthetic manufacturing methods and in lipid membranes.

C- or N- terminal His-tagged RC-LH1s from *Rhodobacter (Rb.) sphaeroides* were engineered and attached to Ni-NTA-assembled substrates to assess their orientation and electric contact with the substrates. Point-contact current imaging AFM was used to determine their topography and current/voltage characteristics. The photocurrent responses of the C- or N-His RC-LH1 complexes on the gold electrodes modified with SAM (Ni-NTA and alkanethiol (1-decanethiol, C₁₀SH)) were measured when these electrodes were illuminated. These results indicate that one-way electron transfer from pigments in the RC-LH1 complex to methyl viologen (MV) occurred. Furthermore, the photocurrent density, normalized by RC-LH1 adsorbed, was greater for C-His RC-LH1 than N-His RC-LH1. The magnitude of the photocurrent was, therefore, very sensitive to the orientation of RC-LH1 complexes on the modified gold electrode. An AFM topography image was recorded under ambient (N₂) conditions of C-His LH1-RCs adsorbed onto the Ni-NTA substrate using an Au/Ir-coated Si probe. Current-voltage measurements showed a semiconducting *I-V* curve that shows a reversed rectification direction relative to those of C-His RC-LH1s. These results show that His tags fused to the C or N termini of the RC-LH1s can control the orientation of the transmembrane protein complexes assembled on the substrate.

Further, the electron conductivity and photocurrent of the RC-LH1 complex embedded in a lipid membrane were measured using C-AFM and photoelectrochemical analyses. AFM topography showed that RC-LH1 molecules were well oriented with their H-subunits toward the membrane surface. RC-LH1 embedded in a membrane-generated photocurrent upon irradiation when assembled on an electrode. The observed action spectrum was consistent with the absorption spectrum of RC-LH1. The control of the orientation of RC-LH1 by lipid membranes provided well-defined conductivity and photocurrent.

These methods of using self-assembly of photosynthetic protein complexes to study electron transfer reactions of LH on electrodes are promising for the development of nanobiodevices and nanobiomaterials from solar to fuel. Based on biological design principles, future biology-based or synthetic organic photonics could potentially provide clean and inexpensive energy alternatives.

2. The 7th East Asia Symposium on Functional Dyes and Advanced Materials

Sept. 2-5, 2015, Osaka Prefecture University, Osaka (Ref. 15)). Invited Lecture IL-24

IL-24. Molecular Assembly of Photosynthetic Complexes for Development of Nanobiodevices

The purpose of this study is to use a photosynthetic light-harvesting complexes (LH) assembled on substrates for development of nanobiodevices from solar to fuel. We use the LH to construct an efficient energy transfer system for nanobiomaterial applications.

In this study, we primarily aimed to construct an array of the LH on substrates using a modified photosynthetic complexes prepared from modern biosynthetic manufacturing methods and in lipid membranes.

C- or N- terminal His-tagged RC-LH1s from *Rhodobacter (Rb.) sphaeroides* were engineered and attached to Ni-NTA-assembled substrates to assess their orientation and electric contact with the

substrates. The photocurrent responses of the C- or N-His RC-LH1 complexes on the gold electrodes modified with SAM (Ni-NTA and C₁₀SH) were measured when these electrodes were illuminated. The photocurrent density, normalized by RC-LH1 adsorbed, was greater for C-His RC-LH1 than N-His RC-LH1. The magnitude of the photocurrent was, therefore, very sensitive to the orientation of RC-LH1 complexes on the modified gold electrode. Current-voltage measurements (C-AFM) showed a semiconducting *I-V* curve that shows a reversed rectification direction relative to those of C-His RC-LH1s. These results show that His tags fused to the C or N termini of the RC-LH1s can control the orientation of the LH protein complexes assembled on the substrate. Further, the electron conductivity and photocurrent of the RC-LH1 complex embedded in a lipid membrane were measured using C-AFM and photoelectrochemical analyses. AFM topography showed that RC-LH1 molecules were well oriented with their H-subunits toward the membrane surface. RC-LH1 embedded in a membrane-generated photocurrent upon irradiation when assembled on an electrode. The control of the orientation of RC-LH1 by lipid membranes provided well-defined conductivity and photocurrent.

These methods of using self-assembly of photosynthetic protein complexes to study electron transfer reactions of the LH on electrodes are promising for the development of nanobiodevices from solar to fuel. Based on biological design principles, future biology-based or synthetic organic photonics could potentially provide clean and inexpensive energy alternatives (1).

(1): *J. Am. Chem. Soc.* 137, 13121 (2015)

3. The 17th International Congress on Photosynthesis Research, Photosynthesis in a Changing World AUGUST 7-12 2016, MECC, MAASTRICHT, THE NETHERLANDS (Ref. 16). Abstract 2D.6, 6D.7, 6D.12 (the abstract below)

2D.6

Stabilization of Charge-Separated State and Photocurrent Generation Activity of LH1-RC in Lipid Environments

Purple bacterial light-harvesting/reaction center complex (LH1-RC) possesses a high potential for photovoltaic devices, however, it has been still challenge to bring out the potential ability of LH1-RC invitro. In this study, we examined effects of phospholipids on the stability of charge-separated state of LH1-RC, which may influence photocurrent generation activity of LH1-RC assembled onto an electrode. LH1-RC was isolated from *Rhodospseudomonas palustris* and dissolved in a Tris-HCl solution (20 mM pH8.6) containing 0.02wt% n-dodecyl- β -D-maltoside (DDM). Kinetics of charge recombination (from P⁺-QA⁻ and P⁺-QB⁻ to P-QA,B, t₁ and t₂, respectively) was measured by transient absorption spectrometry. In the presence of phosphatidylglycerol (DOPG) in the micellar solution, the third time constant t₃ appeared with significantly slow time range ~3 s, indicating that DOPG stabilizes the charge-separated states. Photocurrent generation of LH1-RC incorporated in planar lipid membranes composed of either DOPG or phosphatidylcholine (DOPC) that were assembled onto amino-functionalized ITO electrodes was

observed in the presence of ubiquinone (UQ0) and cytochrome c as electron mediators. Significant photocurrent generation was observed for LH1-RC in DOPG membrane with a prominent quantum efficiency (26%) compared to that in DOPC (10%) and without a lipid system (~2%). These results suggest that the LH1-RC with more stable charge-separated state tuned by phospholipids functions as an efficient photoelectric conversion device. We show a novel approach to exerting potential of LH1-RC.

6D.7

Oxygen-evolving Porous Glass Plates Containing Photosynthetic Photosystem II

Pigment-Protein Complex

The development of artificial photosynthetic systems has been largely focused on the coupling of a photo-anode and cathode, wherein the production of hydrogen (or energy carriers) is coupled to the electrons derived from water-splitting reactions. Here we used a hybrid approach to construct an artificial water-splitting system in which, a new photoanodic device was constructed incorporating stable photosystem II (PSII) purified from *Thermosynechococcus vulcanus* through immobilization within 20- or 50-nm nanopores contained in porous glass plates (PGPs). The PSII complex has a diameter of 20 nm, and can split water efficiently. It was shown that PSII in the nanopores retained its native structure and high photo-induced water splitting activity. The photocatalytic rate (turnover frequency) of PSII in PGP was enhanced 11-fold compared to that in solution, yielding a rate of 50–300 mol e⁻/(mol PSII•s) with 2,6-dichloroindophenol (DCIP) as an electron acceptor. The PGP system realized high local concentrations of PSII and DCIP and enhanced the collisional reactions in nanopores with a low disturbance of light penetration. The system allows a direct visualization/determination of the reaction inside the nanopores, which contributed to the optimization of local reaction conditions. The PSII/PGP hybrid device may significantly contribute to the construction of artificial photosynthesis using water as the ultimate electron source.

6D.12

Photosensitization of the Light-Harvesting complex of Photosystem II Immobilized in Nanoporous Glass Plates

In the photosynthetic membranes of higher plants, membrane protein-pigment complexes convert solar energy into electrical and chemical energy with very high efficiency. Recently, membrane protein-pigment complexes were found to exhibit photo-energy conversion activities from photocurrent generation, and photo-induced hydrogen evolution in vitro. We are interested in studying the light-harvesting complex of photosystem II (LHCII) in nanoporous glass plates (PGP) for the conversion of solar energy into electricity or fuel. Previously, we reported that photo-induced hydrogen production composed of photosensitizer (Ru complex), electron mediator (methylviologen; MV), and hydrogenase works efficiently in PGP under aerobic conditions. In this study, LHCII, isolated from spinach, and MV were immobilized in PGP to perform a photo-induced reduction of MV by LHCII. The reduction rates of MV upon light irradiation under various conditions were quantified to investigate rate limiting factors. Fluorescence lifetimes of LHCII immobilized in PGP were evaluated by time-resolve fluorescence spectroscopy to characterize the state of LHCII. These results indicated that a detergent,

n-dodecyl- β -Dmaltoside, promoted the reduction rate of MV and inhibited the quenching state of LHCII.
We also discuss the conditions that lead to efficient hydrogen production in this presentation.