

July 3rd, 2008

**The Final Research Report of
Memorandum of Understanding (MOU) of AOARD 074013**

Title of Proposed Research:

**Photoenergy Harvesting Organic PV Cells Using Modified Photosynthetic
Light-Harvesting Complex for Energy Harvesting Materials**

Key Researcher involved in the Proposed Project:

Mamoru Nango

Affiliation of Researcher:

Nagoya Institute of Technology

Address of Researcher:

Department of Life and Materials Engineering, Nagoya Institute of Technology
Gokiso-cho, Showa-ku, Nagoya 466-8555, Japan

<Duration of the proposed project> From September 2007 To August 2008

Report Documentation Page				Form Approved OMB No. 0704-0188	
Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.					
1. REPORT DATE 24 JUL 2008		2. REPORT TYPE Final		3. DATES COVERED 01-08-2007 to 01-08-2008	
4. TITLE AND SUBTITLE Photoenergy Harvesting organic PV Cells Using Modified Photosynthetic Light-harvesting complex for Energy Harvesting Materials				5a. CONTRACT NUMBER FA48690610131	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Nango Mamoru				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Nagoya Institute of Technology,Gokiso-cho, Showa-ku,Nagoya 466-8555,Japan,NA,466-8555				8. PERFORMING ORGANIZATION REPORT NUMBER N/A	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) AOARD, UNIT 45002, APO, AP, 96337-5002				10. SPONSOR/MONITOR'S ACRONYM(S) AOARD	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S) AOARD-074013	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT The purpose of this research is to use modified photosynthetic light-harvesting (LH) complexes from modern biosynthetic manufacturing methods of purple photosynthetic bacteria in order to control the direction and orientation of the complex on electrodes for developing dye-sensitized solar PV cells. The advantage of the LH complex is its high efficiency of light-energy conversion throughout the near UV to near IR region and much higher durability using these methods than ordinary LH complex isolated from photosynthetic bacteria. The present generations of PV solar cells are twenty to thirty times too expensive to be cost-effective compared to other existing energy technologies.					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Same as Report (SAR)	18. NUMBER OF PAGES 62	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			

Contents

I . Abstract of the project results	3 p
II . Result of the project.....	3 p
1. Introduction	3 p
2. Results & Discussion	4 p
3. List of Research Results	59 p
A. Conference presentations	59 p
B. Journal paper publications.....	61 p

I . Abstract of the project results

The purpose of this proposal is to use modified photosynthetic light-harvesting (LH) complexes from modern biosynthetic manufacturing methods of purple photosynthetic bacteria in order to control the direction and orientation of the complex on electrodes for developing dye-sensitized solar PV cells. The advantage of the LH complex is its high efficiency of light-energy conversion throughout the near UV to near IR region and much higher durability using these methods than ordinary LH complex isolated from photosynthetic bacteria. The present generations of PV solar cells are twenty to thirty times too expensive to be cost-effective compared to other existing energy technologies. Expanding existing PV technologies by incorporation of modified photosynthetic protein/pigments complexes or their protein-mimic materials to perform tasks of light-harvesting and charge separation, is currently explored as a novel concept, which makes use of natural protein environments to create a directional flow of light energy and electronic charge separation, meanwhile reducing the cost aspect by the use of bio-materials and their synthetic protein-mimic materials. The majority of the aim is construction of artificial photosynthetic antenna system and building solar batteries using modified photosynthetic protein materials for energy harvesting materials.

II . Result of the project.

Introduction

The past 10 years have seen tremendous progress in our understanding of the structure and function of the pigment-protein complexes involved in the primary reactions of bacterial photosynthesis. The structure of the reaction center (RC) revealed the nearly C_2 symmetrical arrangement of the redox centers and this system has now been extensively studied by ultrafast laser spectroscopy. The structures of the LH2 complexes has revealed the nanomeric and octameric arrangement of repeating units consisting of two apoproteins and one or two carotenoids and three BChls (EU white paper artificial photosynthesis, 2006).

The light-harvesting mechanisms in these light-harvesting complexes have been studied both spectroscopically and theoretically. These advances put us in a unique position of being able to exploit this information to design artificial antenna systems based on 'biological blueprint'. Our aim is to see if we could produce an antenna module, which acts as a 'sensitizer', and a light-induced redox component for solar batteries. As well as using LH2 complexes this summary also propose to use LH1-RC complexes. One of its unique features is that it works over a large dynamic range of incident light intensities. It has a remarkable ability to capture efficiently photons even at very low light fluxes, yet at the same time to withstand very high light fluxes by efficiently dissipating the excess photons, thereby protecting itself against the potential harmful effects of over-excitation.

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 is 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 solar-cells. Based on the experiments using the native antenna complexes, a variety of modified complexes will be synthesized and tested for their usefulness in artificial solar-cells. 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 Car and BChl as well as the supporting peptides. These modified photosynthetic protein-mimic complexes will be assembled on electrodes as a light-induced redox component, and the antenna complexes will be attached to a solar cell as a UV and Vis light harvester modules to produce a new type of PV cells. These approaches will provide a foundation for the use of the artificial core-antenna and antenna complexes and the development of new methods of dye-sensitized PV solar cells.

Approach

Integration of photosynthetic proteins or protein-mimics with PV devices for tasks of light-harvesting and charge separation will expand current PV technology with novel and inexpensive bio-components. Design principles of natural photosynthetic units will form the guideposts for the design and development of native light-harvesting and photoconversion matrix modules as described in the section of a plan of work bellow. A critical step is creating functional supramolecular assembly of small organic building blocks that co-operate to create a directional flow of energy using the operational principles of the natural systems. Properties of the building-block molecules intrinsically have the capacities to direct their co-operative assembly into structures with specific orientation and alignment. The advantages of the large scale of modern biosynthetic manufacturing methods offers a promising route to economically viable devices.

Our goal is to use modified photosynthetic light-harvesting complex 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 the development of energy harvesting materials. The advantage of the light-harvesting complex is its high efficiency of light-energy conversion 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 solar cells using modified photosynthetic light-harvesting materials .

Results and Discussion

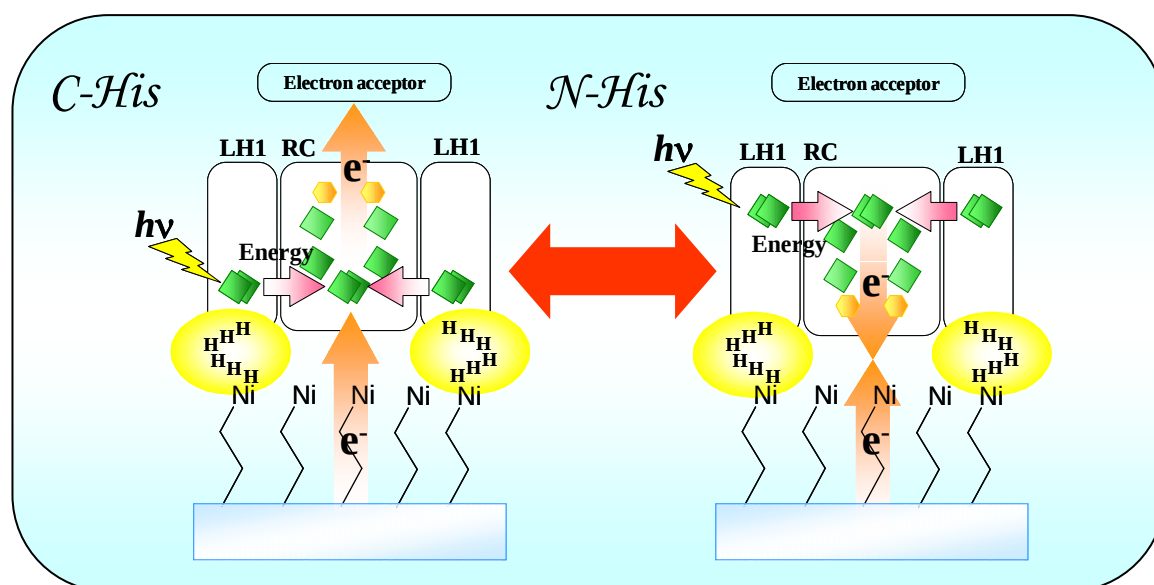
Please see Time Table 1 below.

1. Molecular assembly of modified photosynthetic antenna core (LH1-RC) complex with His-tag to control the orientation and direction of the complex on electrodes for the development of antenna-mimics PV cells

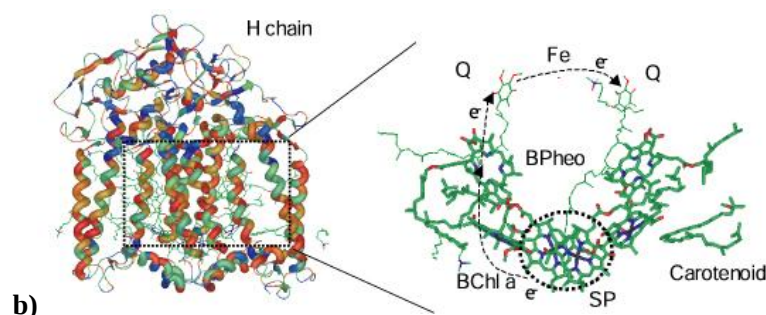
Molecular self-assembly of LH1-RC core complexes as shown in Scheme 1 and its model complexes onto various electrodes was used to develop new types of antenna-mimics PV cells. In the

current of our previous study, we used modified photosynthetic antenna complex with His-tag or modifiers at the LH polypeptide using molecular biological methods to control the orientation and direction of the complex onto electrodes as shown in Scheme 1 (T. Dewa, M. Nango et.al., *Biochemistry*, 44, 5129-5139 (2005))

The pigment-protein complexes of the modified LH1-RC complex were laid down onto functionalized electrodes, such as ITO or Au electrode. Upon illumination photocurrents could be successfully measured. Excitation spectra confirmed that these photocurrents were produced by light absorbed by the pigment-protein complexes as shown in our previous data (M. Ogawa, M. Nango, et.al., *Chem. Lett.*, 772-773 (2004); M. Nagata, M. Nango, et.al., *Trans. MRS-J*, **30**, 655-658 (2005); Y. Suemori, M. Nango, et.al., *Colloid Surf. B*, **56**, 182-187 (2007); M. Kondo, M. Nango, et.al., *Biomacromolecules* **8** 2457-2463 (2007)).



a)



b)

Scheme 1. Schematic model of the assembly of LH1-RC complex with His-tag on an electrode (a) and electron transfer pathway of RC (b).

(a) The C- terminal of LH1-RC complex and SP side of RC is oriented to hydrophilic SAMs on the electrode and the H-chain is oriented to aqueous phase (C-His), and in contrast, the H-chain is oriented to the electrode(N-His).

(b)Electrons are transferred along the pigments associated with the L-subunit of the RC i.e. from SP to BChl *a* (0.47 nm transfer distance), to Bphea (0.38 nm transfer distance) and finally to Q (0.9 nm transfer distance).

It proves critical in this study to capitalize on our knowledge of the behavior of the complex to select those that are the most stable and well organized. The best results were only obtained with the subset of the most stable complexes which the orientation and direction were controlled. These studies were examined to correlate the supramolecular organization of the complexes on the electrodes with the efficiency of photocurrent.

AFM study resolved the organization of antenna complexes in native photosynthetic membranes as shown in the manuscript attached (p.45-p.58) (Mikayama, Nango, et. al., J .Nanosci. and Nanotech. in press). These techniques were now being applied to investigate the organization of the antenna complexes and their synthetic model complexes on the electrodes. This work requires very careful attention to detail and the current pictures were very exciting.

These results were presented several international or national meetings as described bellow;

- 1) M. Nango "Construction of Bio-inspired artificial solar energy conversion system" Asia Optical Fiber Communication & Optoelectronic Exposition & Conference (Shanghai, China, 16th-19th October 2007)
- 2) M. Nango "Self-assembly of light-harvesting polypeptide / pigment complexes for construction of an artificial photoenergy conversion system" Okazaki Conference (Okazaki, Japan, 10th-12 November 2007.11.10-12)
- 3) M. Nango "Self-assembly of light-harvesting polypeptide / pigment complexes for construction of an artificial photoenergy conversion system" 7th International Conference on Tetrapyrrole Photoreceptors in photosynthetic Organisms (Kyoto, Japan, 9th-14 December 2007)
- 4) T. Sakurai, Y. Suemori, M. Hatasa, O. Goto, K. Iida, T. Dewa, K. Yamashita, H. Tanaka, T. Ogawa, H. Hashimoto, and M. Nango "Assembly of the light-harvesting / reaction center complexes of photosynthetic bacteria onto a gold substrate" 7th International Congress on Tetrapyrrole Photoreceptors in Photosynthetic Organisms (Kyoto, Japan, 9th-14th December 2007)
- 5) K. Nakagawa, S. Suzuki, A. Mizuno, R. Fujii, A. T. Gardiner, R. J. Cogdell, H. Hashimoto, M. Nango "Reconstitution of Light-harvesting 1 complex of photosynthetic bacterium *Rhodospirillum rubrum* with the LH1 subunit-type complex and carotenoids and its characterization" 2nd International Workshop of Photosynthetic Antennae and Coherent Phenomena (Osaka, Japan, 14th -16th December 2007)
- 6) M. Nango "Self-assembly of light-harvesting polypeptide / pigment complexes for construction of an artificial photoenergy conversion system" 14th International SPACC-CSJ Symposium (Taipei, Taiwan, 20-22nd December 2007)
- 7) K. Nakagawa, N. Fukui, T. Nakano, T. Horibe, S. Suzuki, R. Fujii, A.T. Gardiner, R. J. Cogdell, H. Hashimoto, and M. Nango "Probing the Effect of the Binding Site on the Electrostatic Behavior of a

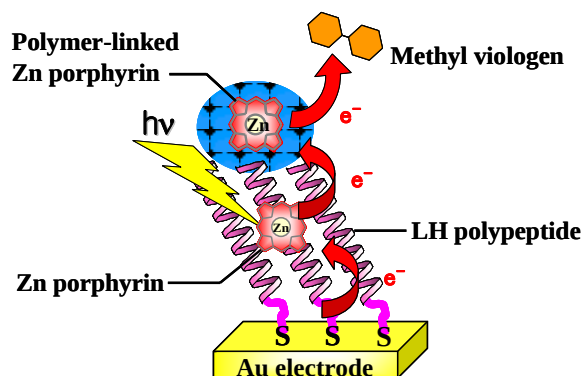
Series of Carotenoids Reconstituted into the Light-Harvesting 1 Complex from Purple Photosynthetic Bacterium *Rhodospirillum Rubrum* Detected by Stark Spectroscopy” 15th International Symposium on Carotenoids (Okinawa, Japan, 22-27th June, 2008)

- 8) N. Fukui, A. Mizuno, T. Nakano, K. Nakagawa, A. T. Gardiner, R. J. Cogdell, R. Fujii, H. Hashimoto, and M. Nango “Reconstitution of Antenna Light-Harvesting 1 Complexes from the Purple Photosynthetic Bacterium *Rhodobacter sphaeroides* with LH1-Polypeptides, Bacteriochlorophyll *a*, and Carotenoids: Carotenoid Specificity” 15th International Symposium on Carotenoids (Okinawa, Japan, 22-27th June, 2008)
- 9) T. Nakano, N. Fukui, K. Nakagawa, A. T. Gardiner, R. J. Cogdell, R. Fujii, H. Hashimoto, and M. Nango “Reconstitution of Light-Harvesting 1 Complexes from the Purple Photosynthetic Bacterium *Rhodospirillum rubrum* with Separately Isolated Polypeptides (LH1- α and LH1- β), Bacteriochlorophyll *a*, and Carotenoid” 15th International Symposium on Carotenoids (Okinawa, Japan, 22-27th June, 2008)
- 10) T. Joke, Y. Takeuchi, S. Ishigure, S. Osaka, T. Dewa, Y. Amao, H. Hashimoto, and M. Nango “Molecular Assembly of Chlorophyll Complexes on Electrodes for Construction of an Artificial Photoenergy Conversion System” 17th International Conference on Photochemical Conversion and Storage of Solar Energy (Sydney, Australia, 27 July - 1 August, 2008)
- 11) M. Hatasa, O. Goto, T. Sakurai, K. Fujii, T. Dewa, K. Iida, H. Hashimoto, and M. Nango “Self-Assembly of Light-Harvesting Complexes on Various Electrodes for Construction of an Artificial Photosynthetic System” 17th International Conference on Photochemical Conversion and Storage of Solar Energy (Sydney, Australia, 27 July - 1 August, 2008)

Please see the abstracts of meetings attached in this report (p.14- p.29).

2. Molecular assembly of synthetic LH model polypeptides with pigments on Au electrode

In the current of our previous study, LH1 synthetic model polypeptides with His-tag at the C- or N-teiminal, analogous to the native LH polypeptide were assembled on Au electrode. Then pigments such as native and synthetic porphyrin or carotenoid derivatives were further selected and assembled on the specific site of the LH1 synthetic model polypeptides to control the organization of the LH1-RC model complex on electrodes as shown in Scheme 2. The structural effects of the pigments and the synthetic polypeptides on the stable production of the efficient photocurrent were further examined.



Scheme 2. Schematic model of the assembly of synthetic LH model polypeptides with pigments on Au electrode

i) Molecular Assembly of BChl *a* Complexes onto ITO Electrode using Synthetic Light-harvesting Model Polypeptides Bearing Spermine Derivative

1 α -helix hydrophobic polypeptide bearing spermine, which has similar amino acid sequences to the hydrophobic core in the native photosynthetic light-harvesting (LH) 1- α polypeptide from *Rhodospirillum (Rsp.) rubrum*, was synthesized. Interestingly, an enhanced photoelectric current was observed when BChl *a* complexes together with the LH 1- α model polypeptide were assembled onto an ITO electrode.

This result was published in *Chem. Lett.*, **37** 98-99 (2008). Please see the manuscript attached in this report (p.18)

ii) Molecular Assembly of Zn Porphyrin Complexes onto a Gold Electrode using Synthetic Light-harvesting Model Polypeptides

Molecular assembly of Zn porphyrin pigments on a gold electrode using synthetic 1 α -helix hydrophobic polypeptides which have similar amino acid sequences to the hydrophobic core in the native photosynthetic light-harvesting (LH) 1- β polypeptide from *Rhodobacter sphaeroides*, has been achieved: this process is dependent on the structures of pigments and polypeptides. Interestingly, an enhanced photoelectric current was observed when Zn mesoporphyrin monomer with the LH 1 model polypeptide in an α -helical configuration was assembled onto the electrode. This result was published in *Tetrahedron Lett.* **48** 8468-8471 (2007). Please see the manuscript attached in this report (p.20)

iii) Molecular Assembly of Zn Porphyrin Complexes Using Synthetic Light -harvesting Model Polypeptides

Synthetic 1 α -helix hydrophobic polypeptides, which have similar amino acid sequences to the hydrophobic core in the native light-harvesting 1 β polypeptide from *Rhodobacter sphaeroides*, formed Zn porphyrin complexes on a gold electrode, as well as in octyl-glucoside micelles: this process is dependent on the structure of the pigments and the polypeptides. Interestingly, an enhanced photoelectric current was

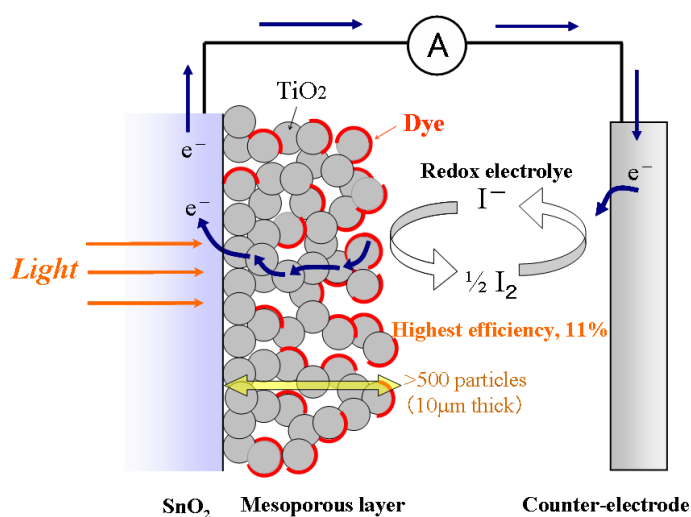
observed when Zn mesoporphyrin monomer complexed with the synthetic light-harvesting model polypeptide in an α -helical configuration was assembled with a defined orientation onto the electrode. Analogues of these light-harvesting model complexes are also useful in providing insights into the effect of polypeptide structure on the formation of light-harvesting complexes on and off electrodes. This result was published in *Photosynth. Res.* **95** 353-361 (2008). Please see the manuscript attached in this report (p.24)

3. Building Bioinspired Organic Solar PV Cells.

Development of Dye-Sensitized Solar PV Cells Using Photosynthetic Pigments

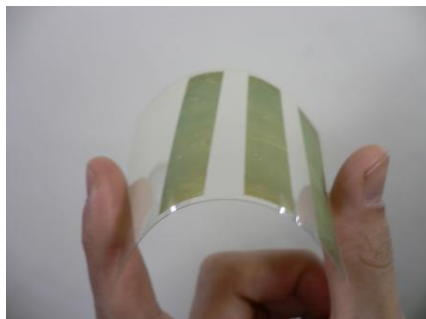
A dye-sensitized solar cell was fabricated using photosynthetic pigments such as chlorophyll derivatives (Chl) (or their mimic pigments) and carotenoids as described above. Their photosynthetic pigments were be examined to see electron transfer from the carotenoid to the radical cation of the dye sensitizer (Chl^+) blocks the reverse electron transfer from TiO_2^- and stabilizes the resultant charge-separated state between TiO_2^- and carotenoid $^{\bullet+}$. No dye-based PV solar cells have built-in photoprotection mechanisms. However, in protein-mimicking devices photoprotection could easily be achieved by incorporating carotenoid or carotenoid-mimicking components, through co-assembly with the chlorophyll/protein components. For effective energy triplet-triplet energy transfer, their absorption band should have spectral overlap and the co-assemblies should be tightly connected for strong excitonic coupling.

We are now ready to fabricate several types of dye-sensitized solar PV cell by combining three different kinds of pigments on various electrodes such as TiO_2 glass or plastic films (Scheme 3), collaborated with Prof. Minoru Taya, University of Washinton in Seattle,US (see Time table 2).

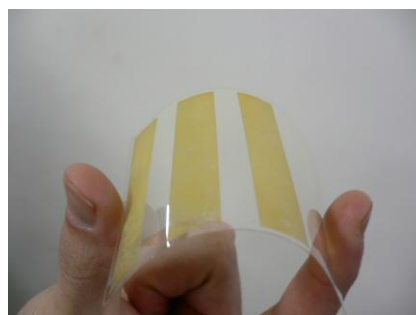


Scheme 3. Schemetic model of dye sensitized solar cell (DSSC)

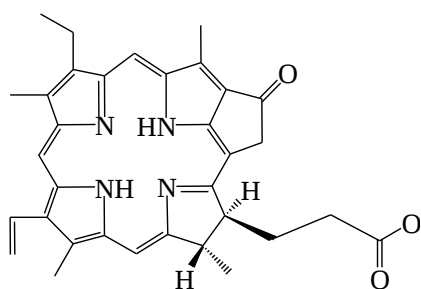
One testable example for plastic films (10 cm x 10 cm) using mesoporphyrin derivative (MPMME), chlorophyll derivative (PyOH, right) and carotenoid (Croctin, left) was prepared as shown bellow.



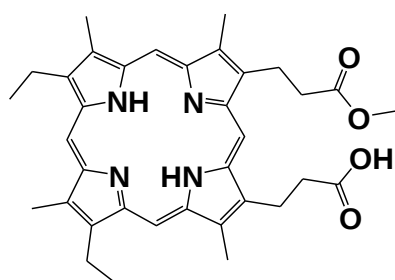
PyOH



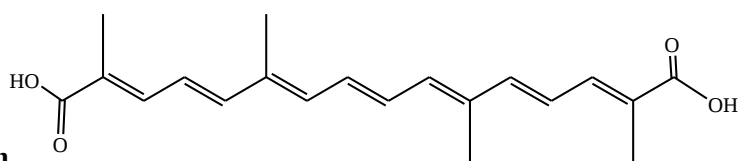
Crocetin



PyOH



MPMME



Crocetin

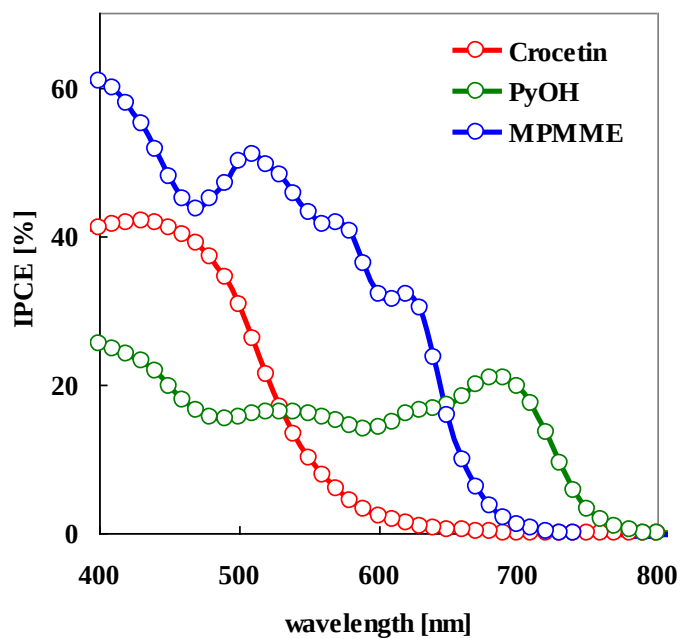


Figure 1. Photocurrent action spectra of TiO₂ electrodes sensitized with PyOH, MPMME, and Crocetin.

Table 1 Performance of the solar cell including short-circuit photocurrent densities (J_{sc}), open-circuit Photovoltage (V_{oc}), fill factors (FF) and the conversion efficiencies (η).

Dye	J_{sc} (mA/cm ²)	V_{oc} (V)	FF (-)	η (%)
Crocetin	3.82	0.554	0.68	1.4
PyOH	6.12	0.595	0.71	2.6
MPMME	6.87	0.662	0.71	3.2

Titania based solar cells were fabricated by the use of porphyrins, PyOH and carotenoid, Crocetin. Interestingly, an enhanced solar energy-to-electricity conversion efficiency was observed for MPMME (η = 3.00 %) in comparison to PyOH (η =2.48 %) and Crocetin (η =1.32 %) (see, in Table 1). The increase in photocurrent was explained in terms of high absorption intensity from 500 nm to 700 nm as shown in the action spectra (see, Figure 1). The co-assembly of these pigments onto TiO₂ will cover the wide rang wavelength from these action spectra, indicating the increase in solar energy-to- electricity conversion efficiency.

Time table 1: Photoenergy harvesting Organic PV Cells Using Modified Photosynthetic Light-Harvesting Complex for Energy Harvesting Materials inspired design of photoenergy harvesting organic PV cells for energy harvesting materials.

Subtasks	Project period		
	4 months	8 months	12 months
Task 1 : Molecular assembly of modified photosynthetic antenna core complex (LH1-RC) with His-tag to control the orientation and direction of the complex on electrodes			
Task 2 : Molecular assembly of the LH1-RC and LH2 complexes on electrodes			
ask 3 : Molecular assembly of synthetic LH model polypeptides with pigments on Au electrode			
Task 4 : Working electrode of TiO ₂ plastic film			
Task 5 : Design of Dye			

Time table 2: Bio-inspired Design of DSSC for energy harvesting material for MURI , collaborated with Prof. Minoru Taya group, University of Washington, Seattle, US.

Subtasks	Project period				
	1 st year	2 nd year	3 rd year	4 th year	5 th year
Task 1 TiO ₂ nano particles nano tube					
Task 2 TiO ₂ coated plastic Film					
Task 3 The design of Dye					
Task 4 Counter electrode C60					
Task 5 Electrolytes and Ionic electrolytes					

Pay-off

Effects of dissemination of research results are as follows,

1) Incorporation of photosynthetic antennas and their protein-mimic complexes on electrodes and into solar cells. This proposal aims to incorporate modified core complex (LH1-RC) and the antenna (LH2) complexes, and their model complexes onto Au, ITO or TiO₂ and polymer matrix as well as Grätzel and silicon solar cells. If this trial becomes successful, it can trigger the development of a new information technology, IT industry as well as a new evolution battery industry.

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 photo-electronic materials.

3) The wisdom of the nature. Photosynthetic organisms have been refining the photosynthetic systems in order to survive in their long history of life, *i.e.*, under the pressure of 'natural selection'. Therefore, the photosynthetic systems now on this planet must realize the principle of light-energy conversion, which has been developed as a result of trial-and-error. This principle, which is now being revealed, can be well beyond our imagination.

4) 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 a novel battery for the next generation using the solar energy which is exhaustible, clear and free of pollutant.

Summary

The present generations of PV solar cells are twenty to thirty times too expensive to be cost-effective compared to other existing energy technologies. Expanding existing PV technologies by incorporation of modified photosynthetic proteins or protein-mimics to perform tasks of light-harvesting and charge separation, is currently explored as a novel concept, which makes use of natural protein environments to create a directional flow of light energy and electronic charge separation, meanwhile reducing the cost aspect by the use of bio-materials. Based on biological design principles, future biology-based solar cells or its model synthetic organic PV cells could form clean and inexpensive future alternatives for electricity production. We propose a scenario where construction of artificial photosynthetic systems is expected to start from molecular and supramolecular entities in a variety of smart matrices that collect light energy and separate charge, leading to an electrochemical potential that can be used to produce current for developing new types of photoenergy harvesting organic PV cells for energy harvesting materials.

Abstract No.1

Asia Optical Fiber Communication & Optoelectronic Exposition & Conference
(Shanghai, China, 16th-19th October 2007)

Construction of Bio-inspired artificial solar energy conversion system

Mamoru Nango

Department of Applied Chemistry, Nagoya Institute of Technology,
Gokiso-cho, showa-ku, Nagoya 466-8555, Japan E-mail: nango@nitech.ac.jp

Abstract

Photocurrent response of photosynthetic light harvesting core complex (LH1-RC) and its model complex on electrodes were performed to attempt the construction of an artificial photosynthetic antenna complex toward developing useful nanodevices of solar-energy conversion.

1.Introduction

At the early stages of purple bacterial light-harvesting complexes, light-harvesting complexes, called LH1 and LH2, absorb solar energy and transfer it to the reaction center (RC), where upon the absorbed energy is efficiently converted into electrochemical energy (Figure 1) [1]. These reactions take place within a 'core complex' consisting of a RC located inside the LH1 complex, where pigment complexes play important roles on these reactions. We are interested in the rapid and efficient energy transfer between pigments in these complexes, photosynthetic units (PSUs) [2-4]. These pigment complexes have been aiming to construct an artificial solar energy device based on a natural solar energy conversion system such as the core complex. Recently, the X-ray crystal structure of the LH1-RC core complex has been reported and

revealed that it is oval rather than circular as shown in Figure 1.

Ingration of photosynthetic proteins or protein-mimics with solar energy devices for tasks of light-harvesting and charge separation will expand current solar energy device technology with novel and inexpensive bio-components. Our goal is to use modified photosynthetic light-harvesting complex 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 the development of energy conversion materials. The advantage of the light-harvesting complex is its high efficiency of light-energy conversion throughout the near UV to near IR region and much higher durability than ordinary isolated pigments supported by its inherent photo-protective function Thus, the results of the above grounds can be directly applied to the development of solar cells using modified

photosynthetic light-harvesting materials [2-7]

We have recently reported that LH1-RC core complexes isolated from *Rs. rubrum* can be assembled a cationically-modified transparent indium tin oxide (ITO) electrode, which exhibits photoinduced current generation [6]. Our current understanding of energy transfer and charge separation reactions in the LH2 and LH1-RC complexes has enabled the first step to be taken towards generating artificial systems from them that convert light energy into usable electrical current. Previous attempts to produce an artificial, energy-converting electrode system used either the LH1 complexes. Until now, there have only been a few attempts to immobilize intact core complexes, consisting of both the LH1 complex and the RC components together, onto an electrode [6,7]. We have recently developed a procedure to create a self-assembled monolayer (SAM) of reconstituted LH1 complexes on a transparent indium tin oxide (ITO) electrode modified with 3-aminopropyltriethoxysilane (APS-ITO) between

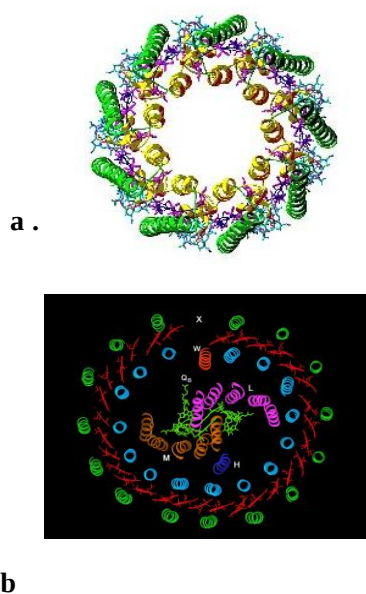


Figure 1. Structure of light-harvesting antenna complex (a) LH2 complex of *Rps. acidophilla* 10050LH2 and (b) LH1-RC complex of *R. palustris*.

the electrode surface and the anionic LH1 polypeptides at pH 8.0 [5,6]. The NIR absorption spectrum showed that the LH1 complex was stable when immobilized onto these electrodes. This study was extended using native LH1-RC complexes [6]. LH1-RC complexes isolated from *Rb. sphaeroides* were successfully assembled on APS-ITO. Efficient energy transfer and photocurrent responses could be observed upon illumination at 880 nm.

Further, we assembled PSUs, LH1-RC core complex, LH1, RC, and admixed complex of LH1 and RC on a modified Au electrode to investigate assembling manner and to develop photocurrent generation system for these assemblies to attempt the construction of an artificial antenna complex toward developing useful nanodevices.

2. Self-Assembled Monolayer of Light-harvesting Core Complexes of Photosynthetic Bacteria on an Amino-Terminated ITO Electrode

Figure 2 shows the NIR absorption spectra of the isolated *Rb. sphaeroides* core complexes in 20 mM Tris HCl buffer pH 8.0 OG micelle (dotted line) and assembled onto an APS-ITO electrode (solid line), respectively. These spectra show that these core complexes have the absorption maximum at 880 nm with two smaller peaks at 800 nm and 760 nm. The former peak is attributable to the overlap of bacteriochlorophyll *a* (BChl *a*) in the LH1 complex (880 nm) and the reaction center BChl *a* dimer ‘special pair’ (870 nm) and the latter two peaks to the BChl *a* called ‘accessory’ (800 nm) and bacteriopheophytin (760 nm) in the RC, respectively.

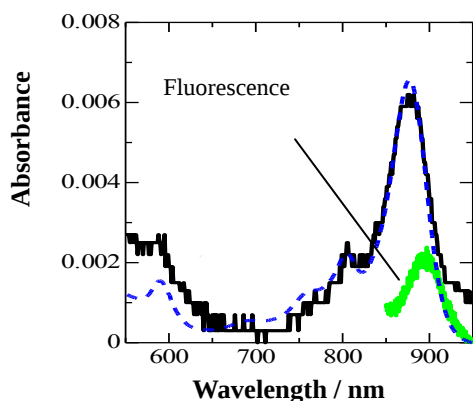


Figure 2. NIR absorption spectra of isolated *Rb. sphaeroides* in 20 mM Tris HCl buffer pH 8.0 OG micelle (dotted line) and assembled onto an APS-ITO electrode (solid line)

The NIR absorption spectra of these core complexes on the electrode indicate that these complexes are stable when assembled onto an APS-ITO. In the previous study it was apparent that when the RC of *Rb. sphaeroides* was assembled, by itself, on the electrode it was relatively labile. Whereas in present study the complete core complex, when assembled onto the electrode, provided to be quite stable. The enhanced stability of the RC surrounded by the LH1 complex probably results from supportive interactions between the two complexes. Interestingly, when illuminating at 880 nm the fluorescence emission of BChl *a* molecules in the LH complex of *Rb. sphaeroides* on the APS-ITO was strongly quenched, due to the presence of the RC of *Rb. Sphaeroides*. This indicates that an efficient energy transfer from BChl *a* in the LH1 complex to the RC in the core complex is still occurring on the electrode (data not shown). FT-IR spectra of the LH complex and the LH1-RC core complexes assembled on the APS-ITO show the absorptions at 1650 cm^{-1} and 1550 cm^{-1} . These bands can be assigned to the amide I and amide II

bands, respectively. These results indicate that the LH polypeptides are in the same α helical configurations on the ITO electrode as in OG micelles.

The time course of the photocurrent generated from the core complex or the RC of *Rb. sphaeroides* assembled onto an APS-ITO showed that an enhanced photocurrent was observed for the core complex when the electrode was illuminated with a pulse of light at 880 nm. In contrast no photocurrent was observed for either LH complexes or the RC. Under our experimental conditions a cathodic photocurrent was observed, implying that one-way electron transfer from pigments in the core complex (special pair of BChl *a*, SP in RC) to methyl viologen was occurring as shown in Figure 3.

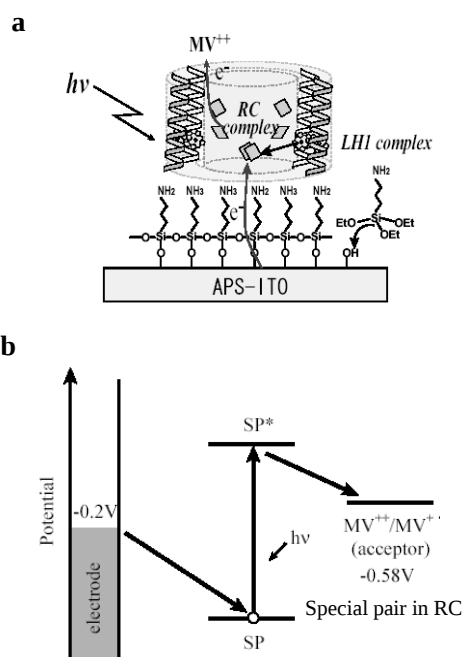


Figure 3. (a) Schematic drawing of LH1-RC core complexes on an APS-ITO electrode, which shows the electron flow from the complex to methyl viologen. (b) Energy diagram for cathodic photocurrent generation by the LH1-RC core complex.

Thus, the enhanced photocurrent observed at 880 nm in the assembled core complex can be ascribed to energy transfer from the LH1 to the RC and then electron transfer from the RC to the electrode as shown in Figure 4. This data indicates that the core complex was well organized on the ITO and the photocurrents were driven by light that was initially absorbed by the LH components.

3. Self-assembled Monolayer of Light-harvesting Core Complexes from Photosynthetic Bacteria on a Gold Electrode Modified with Alkanethiols.

LH1-RC complexes isolated from *Rb. sphaeroides* were self-assembled on a gold electrode modified with self-assembled monolayers (SAMs) of alkanethiols, $\text{NH}_2-(\text{CH}_2)_n\text{-SH}$; $n = 2, 6, 8, 11$, $\text{HOOC}-(\text{CH}_2)_7\text{-SH}$, and $\text{CH}_3-(\text{CH}_2)_7\text{-SH}$, respectively to attempt the construction of an artificial antenna core complex towards developing useful nanodevices as shown in Figure 4. The adsorption on a gold electrode modified with SAMs of $\text{NH}_2-(\text{CH}_2)_n\text{-SH}$, $n = 2, 6, 8, 11$ depended on the methylene chain length, where the adsorption increased with increasing the methylene chain length. The clear presence of the well

known LH and RC peaks NIR spectra of the LH1-RC complexes indicates that these complexes were only fully stable on the SAM gold electrodes modified with the amino group. In the case of modification with the carboxyl group the complexes were partially stable while in the presence of the terminal methyl group the complexes were extensively denatured. An efficient photocurrent response of these complexes on the SAMs of $\text{NH}_2-(\text{CH}_2)_n\text{-SH}$; $n = 2, 6, 8, 11$ was observed upon illumination at 880 nm when $n = 6$ as shown in Figure 4. This corresponds to a distance between the amino terminal group in $\text{NH}_2-(\text{CH}_2)_6\text{-SH}$ and the gold surface of 1.0 nm.

In conclusion, the SAM method is clearly successful in allowing assembly of functional core complexes on the electrode. This has been confirmed by NIR absorption spectroscopy, demonstrating that the photocurrent response, which is derived from electron transfer between the RC and the electrode, is enhanced by illumination at 880 nm. These results provide useful methodology to better understand the suprastructure of LH1-RC complex as well as to gain knowledge about building an artificial fabrication of LH1-RC complex on solid substrates toward useful nanodevices. Various combinations of these complexes are being tested for their usefulness in constructing artificial solar energy conversion devices.

ACKNOWLEDGMENT

The present work was partially supported by AOARD.

REFERENCES

- [1] A.W. Roszak, T.D.Howard, J.Southhall, A. Gardiner, C.J. Law, N.W.Issacs, R.J. Cogdell,

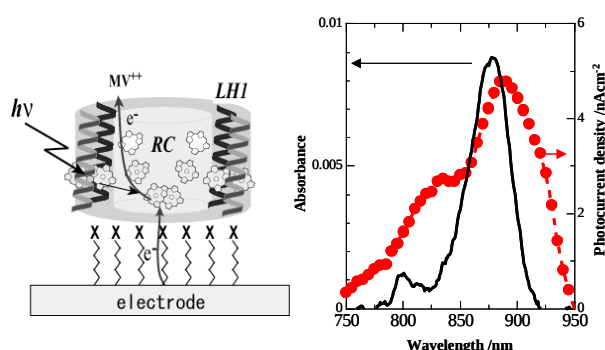


Figure 4. Schematic drawing of LH1-RC core complex on a gold electrode, absorption and action spectra of *Rb. sphaeroides* LH1-RC assembled onto a gold electrode modified with $\text{NH}_2-(\text{CH}_2)_6\text{-SH}$.

- Science* 302 (2003) 1969.
- [2] M. Nango, *Chlorophylls and Bacteriochlorophylls, Biochemistry, Biophysics and Biological Functions of Chlorophylls*, Chapter **28** Editors: B. Grimm, R. Porra, W. Rüdiger, H. Scheer, Springer, 2006.
- [3] T. Dewa, T. Yamada, M. Ogawa, K. Yoshida, Y. Nakao, M. Kondo, K. Iida, K. Yamashita, T. Tanaka, M. Nango, *Biochemistry*, **44**, (2005), 5129-5139.
- [4] K. Iida, J. Inagaki, K. Shinohara, Y. Suemori, M. Ogawa, T. Dewa, and M. Nango, *Langmuir*, **21**, (2005), 3069-3075.
- [5] Ogawa M, Shinohara K, Nakamura Y, Suemori Y, Nagata M, Iida K, Gardiner AT, Cogdell RJ and Nango M, *Chem Lett*, **33**, (2004) 772-773.
- [6] Y.Suemori, M.Nagata, Y. Nakamura, K.Nakagawa, A.Okuda, J. Inagaki, K.Shinohara, M.Ogawa, K.Iida, T.Dewa, K.Yamashita, A. Gardiner, R.J.Cogdell, M.Nango, *Photosynthesis Res.* **90**, (2006),17-21.
- [7] Das R, Kiley PJ, Segal M, Norville J, Yu AA, Wang L, Trammell SA, Reddick LE, Kumar R, Stellacci F, Lebedev N, Schnur J, Bruce BD, Zhang S and Baldo M, *Nano Lett* **4**, (2004) 1079-1083.

Self-assembly of light-harvesting polypeptide / pigment complexes for construction of an artificial photoenergy conversion system

Mamoru Nango

Professor
Nagoya Institute of Technology
Gokiso-cho, Showa-ku, Nagoya, 466-8555, Japan

Purple bacterial light-harvesting complexes, called LH1 and LH2, absorb solar energy and transfer it to the reaction center (RC) at the beginning of the photosynthetic process. The RC converts the absorbed energy into electrochemical energy. These reactions take place within a 'core complex' consisting of a RC located inside the LH1 complex. We are interested in understanding the rapid and efficient energy transfer between LH1 and RC, and have been aiming to construct an artificial solar energy device based on a natural solar energy conversion system such as the LH or the core complex. For example, the core complex, isolated from the photosynthetic bacterium, *Rps. palustris*, was successfully assembled onto an Au electrode modified with various terminated. Efficient energy transfer and photocurrent responses of the complexes were observed upon illumination at 880 nm. Interestingly, the fluorescence of bacteriochlorophyll *a* (BChl *a*) molecules in the LH1 complex was strongly quenched due to the presence of RC implying that an efficient energy transfer occurs from LH1 to the RC in the core complex assembled onto the electrode. Further, the photocurrent generated from the core complex on the Au electrode depended on the wavelength of the exciting light. An action spectrum of this photocurrent showed a maximum at the wavelength corresponding to the Q_y absorption band of LH1.

Self-assembly of light-harvesting polypeptide / pigment complexes for construction of an artificial photoenergy conversion system

M. Nango

Department of Applied Chemistry, Nagoya Institute of Technology, Nagoya, Japan

Purple bacterial light-harvesting complexes, called LH1 and LH2, absorb solar energy and transfer it to the reaction center (RC) at the beginning of the photosynthetic process. The RC converts the absorbed energy into electrochemical energy. These reactions take place within a 'core complex' consisting of a RC located inside the LH1 complex. Recently, the X-ray crystal structure of the LH1-RC core complex has been reported and revealed that it is oval rather than circular. We are interested in understanding the rapid and efficient energy transfer between LH1 and RC, and have been aiming to construct an artificial solar energy device based on a natural solar energy conversion system such as the LH or the core complex. For example, the core complex, isolated from the photosynthetic bacterium, *Rps. palustris*, was successfully assembled onto an Au electrode modified with various terminated (Figure 1). Efficient energy transfer and photocurrent responses of the complexes were observed upon illumination at 880 nm. Interestingly, the fluorescence of bacteriochlorophyll *a* (BChl *a*) molecules in the LH1 complex was strongly quenched due to the presence of RC implying that an efficient energy transfer occurs from LH1 to the RC in the core complex assembled onto the electrode. Further, the photocurrent generated from the core complex on the Au electrode depended on the wavelength of the exciting light. An action spectrum of this photocurrent showed a maximum at the wavelength corresponding to the Q_y absorption band of LH1.

An enhanced photocurrent was also observed in the case of LH 1 model polypeptides assembled with porphyrin derivatives onto an Au electrode (Figure 2). In this case the photocurrent response depended on the structure of the model complex. Various combinations of these complexes are being tested for their usefulness in constructing artificial solar energy conversion devices.

Abstract No.4

**7th International Congress on Tetrapyrrole Photoreceptors in Photosynthetic Organisms
(Kyoto, Japan, 9th-14th December 2007)**

**Assembly of the light-harvesting / reaction center complexes of
photosynthetic bacteria onto a gold substrate.**

Tomohiko Sakurai¹, Yoshiharu Suemori¹, Mikio Hatasa¹, Osamu Goto¹, Kouji Iida², Takehisa Dewa¹,
Keiji Yamashita¹, Hirofumi Tanaka³, Takuji Ogawa³, Hideki Hashimoto⁴, and Mamoru Nango¹

¹*Department of Applied Chemistry, Nagoya Institute of Technology, Nagoya 466-8555, Japan*

²*Nagoya Municipal Industrial Research Institute, Nagoya 456-0058, Japan*

³*Research Center for Molecular-Scale Nanoscience, Institute for Molecular Science, 5-1 Higashiyama,
Myodaiji, Okazaki 444-8787, Japan*

⁴*Department of Physics, Osaka City University, 3-3-138 Sugimoto, Sumiyoshi-ku, Osaka 558-8585,
Japan*

The light reactions occur in closely coupled pigment systems of the light-harvesting (LH) polypeptides, called LH1 and LH2 and reaction center (RC) complexes in photosynthetic bacteria; light energy is absorbed by excitonically coupled antenna pigments bound to the LH polypeptides, and the excitation energy is efficiently transported to RC. Artificial assembly of photosynthetic membrane can be useful for studying photo-excitation of electron and the subsequent electron transfer in biological process as well as for development of nanodevices. Recently, We have developed procedures to create a self-assembled monolayer (SAM) of LH1-RC complexes onto a gold electrode modified with alkanethiol¹⁾ and also to provide insights into electronic behaviors of RC complex on chemically modified Au(111) substrate by conductive atomic force microscopy(CAFM).^{1), 2)} In this study, these procedures have been further used to estimate the control of LH1-RC complexes assembled on the electrode as well as to immobilize LH1-RC complexes onto a gold electrode. The aim of this is to gain insights into a defined distance and orientation for assembly of LH1-RC complexes on solid substrates.

Abstract No.5

2nd International Workshop of Photosynthetic Antennae and Coherent Phenomena (Osaka, Japan, 14th -16th December 2007)

Reconstitution of Light-harvesting 1 complex of photosynthetic bacterium *Rhodospirillum rubrum* with the LH1 subunit-type complex and carotenoids and its characterization

Katsumori Nakagawa^{1,4}, Satoru Suzuki², Ayumi Mizuno¹, Ritsuko Fujii^{2,4},
Alastair T. Gardiner³, Richard J. Cogdell³, Hideki Hashimoto^{2,4}, and Mamoru Nango^{1,4}

1- Nagoya Institute of Technology, Japan; 2- Osaka City University, Japan;
3- University of Glasgow, Scotland, UK; 4- CREST/JST, Japan

Energy transfer from light-harvesting 1 (LH1) complexes to reaction centers (RC) is efficient because the distance and orientation of the co-factors involved (bacteriochlorophyll (BChl) and carotenoid) are precisely controlled at the nano-scale level. Recently, reconstitutions of carotenoids into the LH1 complex from *Rhodospirillum* (*Rs.*) *rubrum* were reported [1] – [3]. This study assumed that the reconstituted carotenoids were bound into the same binding sites as in the native LH1 complex *in vivo*. The aim of this present study was to use electroabsorption (Stark) spectroscopy to test this assumption.

The absorption spectrum of the LH1 complex (Figure b) reconstituted with spirilloxanthin was similar to that of the native LH1 complex isolated from *Rs. rubrum* S1 (Figure a). The nonlinear optical parameters, *i.e.* the change of polarizability $\text{Tr}(\Delta\alpha)$ and static dipole-moment $|\Delta\mu|$ upon photoexcitation, of carotenoids in reconstituted LH1 complex were somewhat different from those of the native LH1 complex, suggesting that the electrostatic environment around carotenoid molecules is slightly different in the reconstituted LH1 complex compared to the native complex. This raises some questions about previous functional studies on carotenoids reconstituted into the LH1 complex from *Rs. rubrum*. It is apparent that Stark spectroscopy can be used as a much more stringent test of the quality of reconstitution than simple absorption, fluorescence, or CD spectroscopy.

Similar Stark spectroscopy was applied to LH1 complexes reconstituted with spheroidene (Figure c).

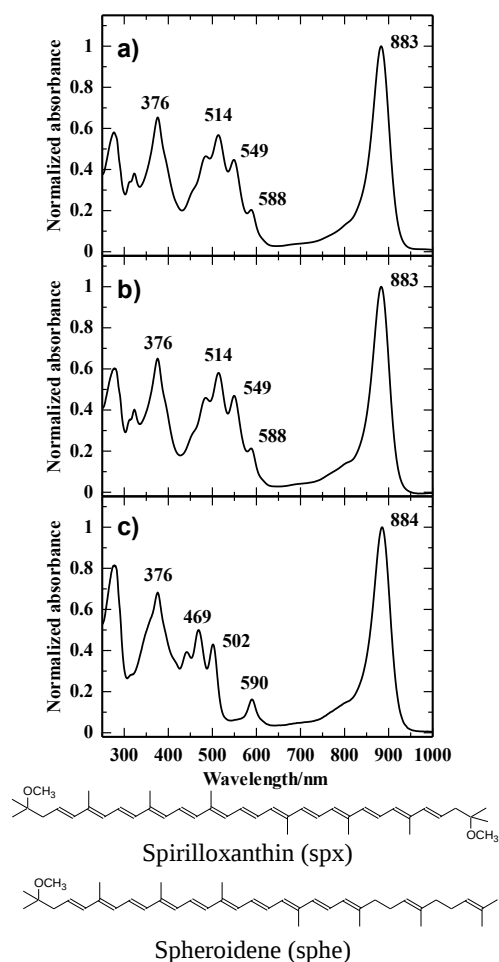


Figure. Normalized absorption spectra of native LH1 complex from *Rs. rubrum* S1 (a) and reconstituted LH1 complexes with spirilloxanthin (b) or spheroidene (c) in 0.01 % LDAO. Chemical structures of spirilloxanthin and spheroidene were also illustrated.

Self-assembly of light-harvesting polypeptide / pigment complexes for construction of an artificial photoenergy conversion system

Mamoru Nango

Department of Applied Chemistry, Nagoya Institute of Technology, Gokiso-cho, showa-ku, Nagoya
466-8555, Japan.

E-mail: nango@nitech.ac.jp

Purple bacterial light-harvesting complexes, called LH1 and LH2, absorb solar energy and transfer it to the reaction center (RC) at the beginning of the photosynthetic process. The RC converts the absorbed energy into electrochemical energy. These reactions take place within a 'core complex' consisting of a RC located inside the LH1 complex. Recently, the X-ray crystal structure of the LH1-RC core complex has been reported and revealed that it is oval rather than circular. We are interested in understanding the rapid and efficient energy transfer between LH1 and RC, and have been aiming to construct an artificial solar energy device based on a natural solar energy conversion system such as the LH or the core complex. For example, the core complex, isolated from the photosynthetic bacterium, *Rps. palustris*, was successfully assembled onto an Au electrode modified with various terminated (Figure 1). Efficient energy transfer and photocurrent responses of the complexes were observed upon illumination at 880 nm. Interestingly, the fluorescence of bacteriochlorophyll *a* (BChl *a*) molecules in the LH1 complex was strongly quenched due to the presence of RC implying that an efficient energy transfer occurs from LH1 to the RC in the core complex assembled onto the electrode. Further, the photocurrent generated from the core complex on the Au electrode depended on the wavelength of the exciting light. An action spectrum of this photocurrent showed a maximum at the wavelength corresponding to the Q_y absorption band of LH1.

An enhanced photocurrent was also observed in the case of LH 1 model polypeptides assembled with porphyrin derivatives onto an Au electrode (Figure 2). In this case the photocurrent response depended on the structure of the model complex. Various combinations of these complexes are being tested for their usefulness in constructing artificial solar energy conversion devices.

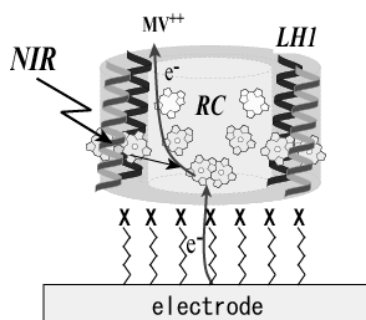


Figure 1. Schematic drawing of a LH1-RC core complex on an electrode.

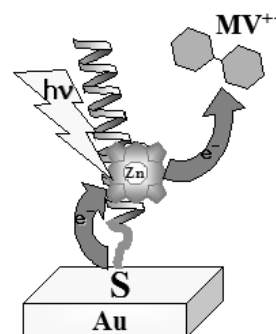


Figure 2. Schematic drawing of a LH1 model polypeptide with Zn porphyrin on an electrode.

Probing the Effect of the Binding Site on the Electrostatic Behavior of a Series of Carotenoids Reconstituted into the Light - Harvesting 1 Complex from Purple Photosynthetic Bacterium *Rhodospirillum Rubrum* Detected by Stark Spectroscopy

^{a,d} Katsunori Nakagawa, ^a Naomi Fukui, ^a Tsubasa Nakano, ^b Tomoko Horibe, ^b Satoru Suzuki, ^{b,d} Ritsuko Fujii, ^c Alastair T. Gardiner, ^c Richard J. Cogdell, ^{b,d} Hideki Hashimoto, and ^{a,d} Mamoru Nango
^a Department of Life Science and Materials, Nagoya Institute of Technology (e-mail: nango@nitech.ac.jp)

^b Department of Physics, Osaka City University

^c Glasgow Biomedical Research Centre, University of Glasgow, ^d CREST/JST

In the light-harvesting 1 (LH1) complex from purple photosynthetic bacteria, carotenoids capture the solar energy and transfer it to the bacteriochlorophyll (BChl) pigments. The distance and orientation of these pigments are cleverly controlled by transmembrane polypeptides. However, the precise interaction of the carotenoids with their binding sites in the LH1 complex is still unclear even in the crystal structure of RC-LH1 core complex from *Rhodopseudomonas (Rps.) palustris* [1].

In this study, we use a reconstitution methodology with LH1 complexes to investigate the interaction and conformation of the carotenoids in their binding sites. The series of carotenoids (Figure 1) were extracted from the purple photosynthetic bacteria. The LH1 complexes were reconstituted by the addition of each of these carotenoids to the LH1 subunit-type complex from *Rhodospirillum (Rs.) rubrum*. Stark spectroscopy was used to provide insight into the electrostatic properties of the binding sites around the different carotenoids and BChl *a* pigments.

Since, in our previous report [2], some aggregated carotenoids, in micellar solution, remained with the reconstituted LH1 complexes, a further purification step using a sucrose density gradient centrifugation was introduced to improve purity of the final reconstituted samples. The measured absorption, fluorescence-excitation and Stark spectra of the LH1 complex reconstituted with spirilloxanthin were identical to those of the native, spirilloxanthin- containing, LH1 complexes from *Rs. rubrum* S1. These results suggest that the electrostatic environments around carotenoids and BChl *a* pigments were essentially same in both preparations. Based on the values of the nonlinear optical parameters, i.e. the polarizability change $\text{Tr}(\Delta\alpha)$ and the static dipole-moment change $|\Delta\mu|$ upon photoexcitation,

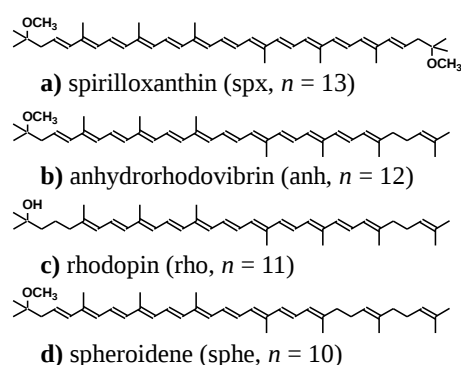


Figure 1. Chemical structures of the carotenoids used in this study, a) all-*trans*-spirilloxanthin (spx), b) all-*trans*-anhydrorhodovibrin (anh), c) all-*trans*-rhodopin (rho), and d) all-*trans*-spheroidene (spe). The numbers of C=C conjugated bonds (n) are also shown.

determined by Stark spectroscopy, we could calculate the conformations of carotenoids, anhydrorhodovibrin and spheroidene, in the LH1 complex using semiempirical molecular orbital calculations.

Acknowledgements

This work was supported by the Grant-in-aid from the Japanese Ministry of Education, Culture, Sports, Science and Technology (Grants No. 172004026, No. 17654083) and AOARD-07-4013. HH, RF, MN, ATG, and RJC thank SICP/JST for financial support. RJC and ATG thank the BBSRC for financial support.

[1] A.W. Roszak, T.D. Howard, J. Southall, A.T. Gardiner, C.J. Caw, N.W. Isaacs, and R.J. Cogdell, *Science*, **302**, 1969-1972 (2003).

[2] K. Nakagawa, S. Suzuki, R. Fujii, A.T. Gardiner, R.J. Cogdell, M. Nango, and H. Hashimoto, *Photosynth. Res.*, **95**, 345-351 (2008).

Reconstitution of Antenna Light-Harvesting 1 Complexes from the Purple Photosynthetic Bacterium *Rhodobacter sphaeroides* with LH1-Polypeptides, Bacteriochlorophyll *a*, and Carotenoids: Carotenoid Specificity

^a Naomi Fukui, ^a Ayumi Mizuno, ^a Tsubasa Nakano, ^{a,d} Katsunori Nakagawa, ^b Alastair T. Gardiner,

^b Richard J. Cogdell, ^{c,d} Ritsuko Fujii, ^{c,d} Hideki Hashimoto, and ^{a,d} Mamoru Nango

^a Department of Life science and Materials, Nagoya Institute of Technology (e-mail: nango@nitech.ac.jp),

^b Glasgow Biomedical Research Centre, University of Glasgow,

^c Department of Physics, Osaka City University, ^d CREST/JST

Recently, our group reported the reconstitution of LH1 complexes from a purple photosynthetic bacterium *Rhodospirillum* (*Rs.*) *rubrum* with a series of carotenoids [1-3]. In this study, however, we have focused our reconstitution methodology on the LH1 complexes from the purple bacterium *Rhodobacter* (*Rb.*) *sphaeroides*, using the carotenoids spirilloxanthin and spheroidene (Figure 1). The LH1 complexes were reconstituted with these carotenoids, BChl *a*, and isolated LH1-polypeptides (LH1- α and LH1- β) from *Rb. sphaeroides*.

The wavelength maximum of the BChl *a* Q_y absorption band is 870 nm in the reconstituted LH1 complex in the absence of carotenoids at 4 °C. A red shift of the wavelength maximum is observed in the complex reconstituted with spheroidene, analogous with that of native, spheroidene-containing LH1 complex in chromatophores from *Rb. sphaeroides*. Spirilloxanthin could not be reconstituted. In the reconstituted *Rhodospirillum* (*Rs.*) *rubrum* LH1 complex, spirilloxanthin as well as spheroidene could be reconstituted with isolated LH1- α and LH1- β polypeptides from *Rs. rubrum*. The LH1 polypeptides from *Rb. sphaeroides* are more selective with respect to which type of carotenoid they accept.

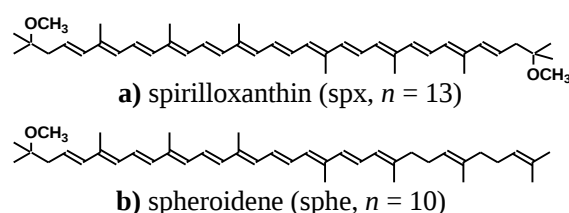


Figure 1. Chemical structures of carotenoids, a) all-trans-spirilloxanthin (spx) and b) all-trans-spheroidene (sphe), isolated from *Rs. rubrum* and *Rb. sphaeroides*, respectively. The numbers of C=C conjugated bonds (*n*) are also shown.

Acknowledgements

This work was supported by the Grant-in-aid from the Japanese Ministry of Education, Culture, Sports, Science and Technology (Grants No. 172004026, No. 17654083) and AOARD-07-4013. HH, RF, MN, ATG, and RJC thank SICP/JST for financial support. RJC and ATG thank the BBSRC for financial support.

Reconstitution of Light-Harvesting 1 Complexes from the Purple Photosynthetic Bacterium *Rhodospirillum rubrum* with Separately Isolated Polypeptides (LH1- α and LH1- β), Bacteriochlorophyll *a*, and Carotenoid

^a Tsubasa Nakano, ^a Naomi Fukui, ^{a,d} Katsunori Nakagawa, ^b Alastair T. Gardiner,

^b Richard J. Cogdell, ^{c,d} Ritsuko Fujii, ^{c,d} Hideki Hashimoto, and ^{a,d} Mamoru Nango

^aDepartment of Life Science and Materials, Nagoya Institute of Technology (e-mail: nango@nitech.ac.jp),

^bGlasgow Biomedical Research Centre, University of Glasgow,

^cDepartment of Physics, Osaka City University, ^dCREST/JST

In core light-harvesting 1 (LH1) complexes from purple photosynthetic bacteria, carotenoids capture the solar energy and transfer it to the bacteriochlorophyll (BChl) pigments.

Reconstitution of the LH1 complexes with carotenoids is powerful method with which to provide insights into the function of carotenoids in the LH1 complex [1]. In this study, all-*trans*-spirilloxanthin and spheroidene were

extracted from purple photosynthetic bacteria, *Rhodospirillum* (*Rs.*) *rubrum* and *Rhodobacter* (*Rb.*) *sphaeroides*, respectively. The chemical structures of these carotenoids were shown in Figure 1. The LH1 complexes were reconstituted with each of these carotenoids and isolated LH1-polypeptides (LH1- α and LH- β) and BChl *a* from *Rs. rubrum*.

In the reconstituted *Rs. rubrum* LH1 complex without carotenoid, the wavelength maximum of the Q_y absorption band of BChl *a* was measured to be at 870 nm at 4 °C. In the reconstituted LH1 complexes containing carotenoids (spirilloxanthin or spheroidene), however, the wavelength maximum was red-shifted to about 880 nm. This wavelength maximum was similar to that of native, spirilloxanthin-containing LH1 complex in chromatophores from *Rs. rubrum* S1 [2-4]. This result indicated that we could successfully reconstitute the LH1 complex from its constituent components.

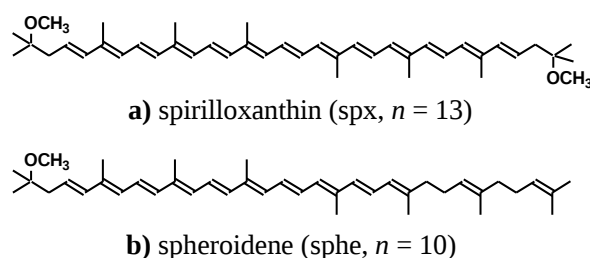


Figure 1. Chemical structures of carotenoids used in this study, a) all-*trans*-spirilloxanthin (spx) and b) all-*trans*-spheroidene (sphe). The numbers of C=C conjugated bonds (n) are also shown.

Acknowledgements

This work was supported by the Grant-in-aid from the Japanese Ministry of Education, Culture, Sports, Science and Technology (Grants No. 172004026, No. 17654083) and AOARD-07-4013. HH, RF, MN, ATG, and RJC thank SICP/JST for financial support. RJC and ATG thank the BBSRC for financial support.

Molecular Assembly of Chlorophyll Complexes on Electrodes for Construction of an Artificial Photoenergy Conversion System

Takashi Joke¹, Yoshito Takeuchi¹, Syuichi Ishigure¹, Shinichiro Osaka¹, Takehisa Dewa¹,
Yutaka Amao², Hideki Hashimoto³ and Mamoru Nango¹

¹*Department of Applied Chemistry, Nagoya Institute of Technology, Gokiso-cho, showa-ku, Nagoya
466-8555, Japan, JST/CREST, E-mail: nango@nitech.ac.jp*

²*Department of Applied Chemistry, Oita University, 700 Dannoharu, Oita, Oita, 870-1192*

³*Department of Physics, Graduate School of Science, Osaka City University, 3-3-138 Sugimoto,
Sumiyoshi-ku, Osaka 558-8585, Japan, JST/CREST*

Chlorophyll complexes in light-harvesting complexes absorb solar energy and transfer it to the reaction center (RC) at the beginning of the photosynthetic process. The RC converts the absorbed energy into electrochemical energy. We are interested in understanding the rapid and efficient energy transfer between chlorophyll complexes, and have been aiming to construct an artificial photoenergy conversion material based on a natural solar energy conversion system. The chlorophyll complexes, isolated from native bacterium or plant and their synthetic model complexes were successfully assembled onto various electrodes modified with various terminated. Efficient energy transfer and photocurrent responses of the complexes were observed upon illumination.

An enhanced photocurrent was also observed in the case of LH model polypeptides assembled with chlorophyll derivatives onto an electrode. In this case the photocurrent response depended on the structure of the model complex. Various combinations of these complexes are being tested for their usefulness in constructing artificial solar energy conversion materials.

Acknowledgements

This work was supported by the Grant-in-aid from the Japanese Ministry of Education, Culture, Sports, Science and Technology (Grants No. 172004026, No. 17654083) and AOARD-07-4013.

Abstract No.11

**17th International Conference on Photochemical Conversion and Storage of Solar Energy
(Sydney, Australia, 27th July - 1st August 2008)**

Self-assembly of light-harvesting complexes on various electrodes for construction of an artificial photosynthetic system

Mikio Hatasa¹, Osamu Goto¹, Tomohiko Sakurai¹, Kaoru Fujii¹, Takehisa Dewa¹, Kouji Iida²,
Hideki Hashimoto³ and Mamoru Nango¹

¹*Department of Applied Chemistry, Nagoya Institute of Technology, Gokiso-cho, showa-ku, Nagoya 466-8555, Japan, and JST/CREST, E-mail: nango@nitech.ac.jp*

²*Nagoya Municipal Industrial Research Institute, Rokuban 3-4-41, Atsuta-ku, Nagoya, 456-0058*

³*Department of Physics, Osaka City University 3-3-138 Sugimoto, Sumiyoshi-ku, Osaka 558-8585, Japan, and JST/CREST*

Light-harvesting complexes absorb solar energy and transfer it to the reaction center at the beginning of the photosynthetic process. The RC converts the absorbed energy into electrochemical energy. These reactions take place within a 'core complex' consisting of a RC located inside the LH complex. We are interested in understanding the rapid and efficient energy transfer between LH and RC, and have been aiming to construct an artificial solar energy device based on a natural solar energy conversion system such as the core complex. The core complex, isolated from the photosynthetic bacterium or plant was successfully assembled onto various electrodes modified with various terminated. Efficient energy transfer and photocurrent responses of the complexes were observed upon illumination. Further, the photocurrent generated from the core complexes on the electrode depended on the wavelength of the exciting light. An action spectrum of this photocurrent showed a maximum at the wavelength corresponding to the absorption band of the LH core complexes.

Acknowledgements

This work was supported by the Grant-in-aid from the Japanese Ministry of Education, Culture, Sports, Science and Technology (Grants No. 172004026, No. 17654083) and AOARD-07-4013.

Molecular Assembly of BChl *a* Complexes onto ITO Electrode Using Synthetic Light-harvesting Model Polypeptides Bearing Spermine Derivative

Tsuyoshi Ochiai,¹ Mitsuhiro Ota,¹ Takahide Asaoka,¹ Tomoya Kato,¹ Shinichiro Osaka,¹ Takehisa Dewa,¹

Keiji Yamashita,¹ Hideki Hashimoto,² and Mamoru Nango^{*1}

¹Materials Science and Engineering, Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya 466-8555

²Department of Physics, Graduate School of Science, Osaka City University, 3-3-138 Sugimoto, Sumiyoshi-ku, Osaka 558-8585

(Received August 28, 2007; CL-070926; E-mail: nango@nitech.ac.jp)

1 α -Helix hydrophobic polypeptide bearing spermine, which has similar amino acid sequences to the hydrophobic core in the native photosynthetic light-harvesting (LH) 1- α polypeptide from *Rhodospirillum (Rsp.) rubrum*, was synthesized. Interestingly, an enhanced photoelectric current was observed when BChl *a* complexes together with the LH1- α model polypeptide were assembled onto an ITO electrode.

Self-assembling of polypeptides and pigments have emerged as powerful techniques in the de novo design of native protein-like structures.¹ Recently, models for the LH complex of photosynthetic bacteria have been investigated to clarify or apply of their efficient energy and electron transfer.^{2,3} To perform an efficient energy and electron transfer, one should assemble the artificial LH complex with a defined orientation onto an electrodes. In our previous papers, immobilizing of artificial LH complexes on a gold electrode using *Rhodobacter (Rb.) sphaeroides* LH1- β model polypeptides bearing cysteine group and the photocurrent activity of the complexes was studied.⁴ However, these model polypeptides could not use BChl *a* derivatives as an electron donor because of their self-assembling properties.⁵

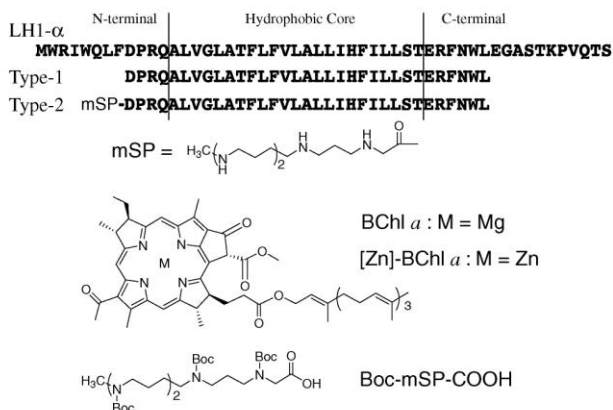
We now report the molecular assembly of BChl *a* derivatives on an ITO electrode with a defined orientation, using synthetic LH model polypeptides (Type-1 and Type-2 in Scheme 1) which have similar amino acid sequences to the hydrophobic core in the native LH1- α polypeptide from *Rsp. rubrum*. The aim of this is to gain insights into the structural requirements for assembly of the LH1 complexes with a defined orientation

on a solid substrate. Synthetic model polypeptides with spermine derivative at N-terminal, which can be easily bound with an ITO surface, were used. Further, we selected the LH1- α type polypeptide which either forms a stable LH1-like complex with [Zn]-BChl *a* or BChl *a* monomer complex in OG micelles.⁶ The monomer complex is expected to perform enhanced photocurrent using NIR light.⁴

BChl *a* was isolated from the carotenoidless mutant G9 of *Rsp. rubrum* and purified by HPLC as described elsewhere.⁵ [Zn]-BChl *a* was prepared from BChl *a* via transmetalation as previously reported.⁷ The native LH1- α and - β polypeptides, separately isolated from the LH1 complex of *Rsp. rubrum*, were prepared as previously described.⁸ LH1- α model polypeptides, Type-1 and Type-2 polypeptides, were prepared by solid-phase peptide synthesis using a Wang resin, N $^{\alpha}$ -Fmoc amino acids, HBTU and HOBt. Type-2 polypeptide was synthesized by condensation of Boc-mSP-COOH, prepared from spermine tetrahydrochloride as previously reported on N-terminal of Type-1. The cleavage reaction was performed as described previously⁹ and the purified product was confirmed by TOFMS spectrometry (Type-1: 3828.3 Da, Type-2: 4085.1 Da). The molecular assembly of pigments together with the synthetic polypeptides was carried out as previously reported.⁵ Complexes were immobilized on self-assembled monolayers (SAMs) by immersing of the ITO electrode in sample solution ([polypeptides] = 3.45 μ M, [pigments] = 2.41 μ M, [OG] = 26.7 mM) at 4 $^{\circ}$ C for 12 h. Then, the ITO electrode was washed by MilliQ and dried by N₂ flow. Photocurrents were measured at -0.2 V (vs. Ag/AgCl) in a homemade cell that contained three electrodes as described previously.¹⁰

The Qy band of [Zn]-BChl *a*-monomer in OG micelles (770 nm) is red-shifted to 849 nm in the presence of Type-1 or -2 polypeptide at 4 $^{\circ}$ C (Supporting Information Table S1).¹¹ This is analogous to LH1-type complex that forms with either LH1- α and LH1- β or LH1- α alone. This result again shows that the [Zn]-BChl *a* complex can be organized by this synthetic hydrophobic 1 α -helix polypeptide in OG micelles.¹² This result suggests that both the amino acid sequences both at N- and C-terminal segments are not crucial effect on formation of the LH1-type complex (Table S1).^{11,12} In contrast, similar red shift of the Qy band is not observed for BChl *a* in the presence of Type-1 or -2 polypeptide. Instead, in this case the Qy band was only red-shifted to 782 nm and aggregation peak of BChl *a* was not detected, indicating the formation of monomer complex of Type-2/BChl *a*.

FT-IR spectra of Type-2/[Zn]-BChl *a* complex immobilized on the electrode show at 1665 and 1544 cm⁻¹, Type-2/BChl *a* complex immobilized on the electrode showed absorptions 1665 and 1544 cm⁻¹. These bands can be assigned to the



Scheme 1. The amino acid sequences of *Rsp. rubrum* LH1- α and its synthetic model polypeptides and the structure of BChl *a* derivatives and Boc-mSP-COOH.

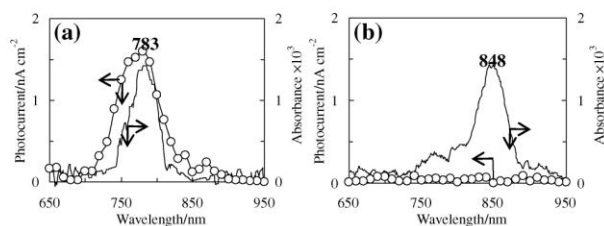


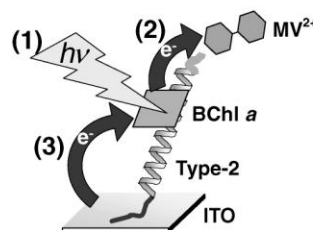
Figure 1. Action spectra (open circle and solid line) and absorption spectra (solid line) of Type-2/BChl *a* (a) and Type-2/[Zn]-BChl *a* (b) complexes assembled on an ITO electrode.

amide I and amide II band, respectively. It is also possible as previously described to use these bands to determine the tilt angle of the helices relative to the surface of the gold electrode.¹³ The tilt angle of the helix in Type-2/[Zn]-BChl *a* complex is 40°. For Type-2/BChl *a* complex, the angle is 38° for the complex formed between the Type-1 polypeptide and BChl *a*. Taken together, these results show that the LH1 model polypeptide is in an α -helical conformation when assembled together with these pigments onto an ITO electrode.

Figure 1 shows the NIR absorption and photocurrent action spectra of either BChl *a* or [Zn]-BChl *a* complexes assembled with Type-2 polypeptide onto an ITO electrode at room temperature. The Qy band of the BChl *a* and [Zn]-BChl *a* complexes showed an absorption maximum at 783 and 848 nm on the ITO electrode, respectively. This absorption maximum is analogous to the Qy band for BChl *a* or [Zn]-BChl *a* complexes assembled with Type-2 polypeptide in OG micelles at 4 °C (Table S1).¹¹ However, no absorption band was observed for both BChl *a* and [Zn]-BChl *a* complexes with Type-1 polypeptide on the electrode (data not shown). This result indicates that mSP moiety of Type-2 polypeptide is necessary for immobilization of complexes on an ITO electrode.

Further, it is clear from Figure 1a that an enhanced photocurrent was observed for Type-2/BChl *a* complex when the electrode was illuminated with a pulse of light at the Qy band of BChl *a* (783 nm). The photocurrent responses showed maxima at wavelengths corresponding to the maxima of the main absorption bands of the complexes. In contrast, no photocurrent was observed for Type-2/[Zn]-BChl *a* complex (Figure 1b), analogous to [Zn]-BChl *a* complex with the native LH1- α / β polypeptides immobilized on an ITO electrode modified with lipid bilayers.¹⁰ Under the present experimental condition, a cathodic photocurrent was observed (data not shown), implying that one-way electron transfer from BChl *a* in the LH model polypeptide complex to methyl viologen occurred as shown in Scheme 2 and Supporting Information Scheme S1.¹¹ This result indicates that the BChl *a* complex was well immobilized by the synthetic LH model polypeptide in an α -helical configuration on the ITO electrode and the photocurrents were driven by light that was initially absorbed by the BChl *a* complex. Furthermore, the NIR absorption and FT-IR spectra of the complexes immobilized on the ITO electrode was not changed after photocurrent measurement, implying that the complexes were stable during the measurement.

In conclusion, the amphiphilic compound, mSP conjugated to LH model polypeptide was successfully synthesized. The



Scheme 2. Schematic view of light irradiation (1), electron transfer from BChl *a* to MV²⁺ (2) and electron transfer from ITO to BChl *a* (3) in Type-2/BChl *a* complex immobilized on the ITO electrode.

mSP moiety of the model polypeptide can immobilize with BChl *a* and [Zn]-BChl *a* complexes onto the ITO electrode. This method will be useful for the self-assembly of these complexes in order to study the energy-transfer and electron-transfer reactions between individual pigments in the photosynthetic LH complexes on the electrode.

M. N. and H. H. are grateful to JST, CREST for financial support. The present work was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japanese Government and AOARD.

References and Notes

- 1 D. E. Robertson, R. S. Farid, C. C. Moser, J. L. Urbauer, S. E. Mulholland, R. Pidikiti, J. D. Lear, A. J. Wand, W. F. Degrad, P. L. Dutton, *Nature* **1994**, 368, 425.
- 2 D. Noy, P. L. Dutton, *Biochemistry* **2006**, 45, 2103.
- 3 L. G. Kwa, A. García-Martín, A. P. Végh, B. Strohmman, B. Robert, P. Braun, *J. Biol. Chem.* **2004**, 279, 15067.
- 4 T. Ochiai, T. Asaoka, T. Kato, S. Osaka, T. Dewa, K. Yamashita, H. Hashimoto, M. Nango, *Tetrahedron Lett.* **2007**, 48, 8468.
- 5 K. A. Meadows, K. Iida, K. Tsuda, P. A. Recchia, B. A. Heller, B. Antonio, M. Nango, P. A. Loach, *Biochemistry* **1995**, 34, 1559.
- 6 M. Nagata, M. Nango, A. Kashiwada, S. Yamada, S. Ito, N. Sawa, M. Ogawa, K. Iida, Y. Kurono, T. Ohtsuka, *Chem. Lett.* **2003**, 32, 216.
- 7 T. Dewa, T. Yamada, M. Ogawa, M. Sugimoto, T. Mizuno, K. Yoshida, Y. Nakao, M. Kondo, K. Iida, K. Yamashita, T. Tanaka, M. Nango, *Biochemistry* **2005**, 44, 5129.
- 8 P. S. Parkes-Loach, J. R. Sprinkle, P. A. Loach, *Biochemistry* **1988**, 27, 2718.
- 9 A. Kashiwada, H. Hiroaki, D. Kohda, M. Nango, T. Tanaka, *J. Am. Chem. Soc.* **2000**, 122, 212.
- 10 M. Nagata, Y. Yoshimura, J. Inagaki, Y. Suemori, K. Iida, T. Ohtsuka, M. Nango, *Chem. Lett.* **2003**, 32, 852.
- 11 Supporting Information is available electronically on the CSJ-Journal web site; <http://www.csj.jp/journals/chem-lett/>.
- 12 A. Kashiwada, H. Watanabe, T. Tanaka, M. Nango, *Chem. Lett.* **2000**, 24.
- 13 Y. Miura, S. Kimura, Y. Imanishi, J. Umemura, *Langmuir* **1998**, 14, 6935.



Available online at www.sciencedirect.com

Tetrahedron
Letters

Tetrahedron Letters 48 (2007) 8468–8471

Molecular assembly of Zn porphyrin complexes onto a gold electrode using synthetic light-harvesting model polypeptides

Tsuyoshi Ochiai,^a Takahide Asaoka,^a Tomoya Kato,^a Shinichiro Osaka,^a Takehisa Dewa,^a Keiji Yamashita,^a Hideki Hashimoto^b and Mamoru Nango^{a,*}

^aMaterials Science and Engineering, Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya 466-8555, Japan

^bDepartment of Physics, Graduate School of Science, Osaka City University, 3-3-138 Sugimoto, Sumiyoshi-ku, Osaka 558-8585, Japan

Received 27 August 2007; revised 24 September 2007; accepted 27 September 2007

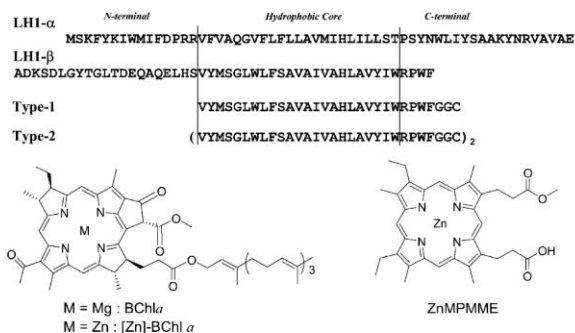
Available online 29 September 2007

Abstract—Molecular assembly of Zn porphyrin pigments, Zn substituted bacteriochlorophyll *a* ([Zn]-BChl *a*) and Zn mesoporphyrin monomer (ZnMPMME) on a gold electrode using synthetic 1 α -helix hydrophobic polypeptides which have similar amino acid sequences to the hydrophobic core in the native photosynthetic light-harvesting (LH) 1- β polypeptide from *Rhodobacter sphaeroides*, has been achieved: this process is dependent on the structures of pigments and polypeptides. Interestingly, an enhanced photoelectric current was observed when ZnMPMME with the LH1 model polypeptide in an α -helical configuration was assembled onto the electrode.

© 2007 Elsevier Ltd. All rights reserved.

LH polypeptides organize BChl *a* complexes so that an efficient energy transfer between BChls can occur in photosynthetic bacterial membranes.^{1,2} It is interesting that purified LH polypeptides and chemically synthesized polypeptides can be used to study both the assembly and the structure of LH1 complexes. Works from several laboratories have demonstrated that porphyrins can be assembled together with synthetic polypeptides to produce artificial hemoproteins and LH1 complex models.^{3,4} In our previous Letters, both native and artificial LH complexes were assembled onto an electrode modified with either aminopropyl silane or lipid bilayers using electrostatic interaction or supported lipid membrane.^{5,6} However, no study is observed on the assembly of the artificial LH complex with a defined orientation onto a gold electrode using synthetic polypeptides with cysteine group.

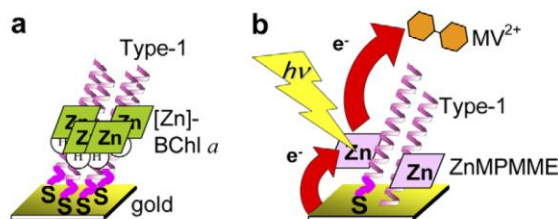
In this Letter, Zn porphyrin pigments, [Zn]-BChl *a* and ZnMPMME (see Scheme 1) were first assembled together with synthetic LH model polypeptides that have similar amino acid sequences to the hydrophobic core of the native photosynthetic LH1- β polypeptide from *Rhodobacter sphaeroides* (see Scheme 1) onto gold



Scheme 1. The structure of porphyrin pigments and the amino acid sequences of *R. sphaeroides* LH1- α and - β and synthetic LH1- β model polypeptides.

electrodes.^{7–9} The aim of this is to gain insights into the structural requirements for the assembly of LH1 complexes with a defined orientation on solid substrates (see Scheme 2). Synthetic model LH polypeptides with a cysteine group at C-terminal, which can be easily bound onto a gold surface, were used. These polypeptides were synthesized as described previously.¹⁰ Further, we selected synthetic model polypeptides (Scheme 1) that have similar amino acid sequences to the hydrophobic core of the native LH1- β polypeptide from *R. sphaeroides*. Zn porphyrin pigments were

*Corresponding author. Tel./fax: +81 52 735 5226; e-mail: nango@nitech.ac.jp



Scheme 2. Schematic view of Type-1/[Zn]-BChl *a* (a) and Type-1/ZnMPMME (b) complexes assembled on a gold electrode.

used because of its stability.^{8,11} [Zn]-BChl *a*, obtained synthetically by the transmetalation of BChl *a*,¹² is more stable than BChl *a*, and Zn porphyrin derivatives, therefore, have been widely used in model studies of light-harvesting. The native LH1- α and LH1- β polypeptides were separately isolated from *R. sphaeroides*. [Zn]-BChl *a* and ZnMPMME (see Scheme 1) were obtained as previously described.^{1,12–14} The molecular assembly of pigments together with the synthetic polypeptides was carried out as previously reported.¹⁴ Complexes were immobilized on self-assembly monolayers (SAMs) by immersing the gold electrode (0.9 $\times$ 2.0 cm, 50 nm²) in the sample solution at 4 °C for 12 h. Then the gold electrode was washed by MilliQ and dried by N₂ flow. Photocurrents were measured at –0.2 V (versus Ag/AgCl) in a homemade cell that contained three electrodes as described previously.⁶

The Qy band of [Zn]-BChl *a*-monomer in OG micelles (770 nm) is red-shifted to 809 nm in the presence of Type-1 polypeptide at 25 °C (supplementary data Table S1). This is analogous to the subunit complex that forms with either LH1- α and LH1- β or LH1- β alone. Cooling to 4 °C results in a further red-shift to 821 nm. This is similar to that seen using BChl *a* (Table S1). These results again show that the [Zn]-BChl *a* complex can be organized by this synthetic hydrophobic 1 α helix polypeptide in OG micelles.⁸ It is interesting that a similar red-shift of the Qy band is not observed in the presence of Type-2 polypeptide. Instead, in this case, the Qy band was only red-shifted to 807 nm and dramatically sharpened. This result suggests that the disulfide-linkage in the C-terminal segment obstructs the formation of a LH1-type complex as described previously.⁸ However, little difference in the intensities of the CD signals of reconstituted complexes involving the Type-1 and -2 was observed (Table S1).

The effect of the structure of the pigments on the LH1-complex formation, the reconstitution of the complexes was repeated using ZnMPMME. The Soret band of ZnMPMME (404 nm) in OG micelles was red-shifted to 407 nm at 25 °C in the presence of either Type-1 or -2 polypeptide. Upon cooling to 4 °C a further red-shift to 415 and 418 nm was observed (Table S1). In the absence of the polypeptides the Soret bands of ZnMPMME broadened. Interestingly, a split-CD signal located at the red-shifted Soret band for ZnMPMME was observed in the presence of the Type-2 polypeptide (Table S1). This split-CD signal was not observed in the

presence of the Type-1 polypeptide. This may indicate that ZnMPMME has a monomer-like structure in the presence of the Type-1 polypeptide but aggregates randomly in the presence of the Type-2 polypeptide. The fluorescence of ZnMPMME is significantly quenched in the presence of Type-2 polypeptide than Type-1 (Table S2). These results suggest that the ZnMPMME monomer complex is more stably organized in the presence of Type-1 polypeptide.

Figures 1 and 2 show the electronic absorption spectra of Zn porphyrin complexes assembled by the synthetic LH1 model polypeptides, using Type-1 polypeptide in an OG micellar solution at 4 °C or onto a gold electrode at room temperature. The Qy band of the [Zn]-BChl *a* complex formed with the Type-1 polypeptide showed an absorption maximum at 821 or 814 nm in an OG micellar solution or on the gold electrode, respectively. These absorption maxima indicate that, in both cases, these are of the subunit type complex, such as seen with LH1- β alone.¹⁴ The Soret bands of the complexes formed with ZnMPMME together with Type-1 polypeptide assembled in an OG micellar solution or onto the gold electrode showed maxima at 415 nm.

FT-IR spectra of the complex of Type-1 polypeptide and [Zn]-BChl *a* assembled on the electrode show at 1652 and 1543 cm^{–1}, the complex of Type-1 polypeptide and ZnMPMME showed absorptions 1662 and 1549 cm^{–1}. These bands can be assigned to the amide I and amide II bands, respectively. It is also possible as previously described to use these bands to determine the tilt angle of the helices relative to the surface of the gold electrode.¹⁵ The tilt angle of the helix in the complex of the Type-1 polypeptide and [Zn]-BChl *a* is 43°. This angle is 47° for the complex formed between the Type-1 polypeptide and ZnMPMME. Taken together

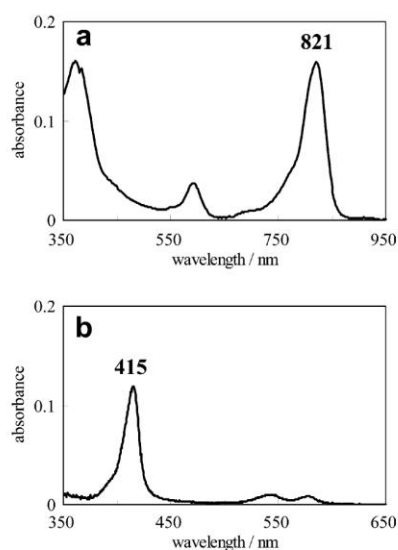


Figure 1. Absorption spectra of Type-1/[Zn]-BChl *a* (a) and Type-1/ZnMPMME (b) complexes assembled in 0.78% OG at 4 °C.

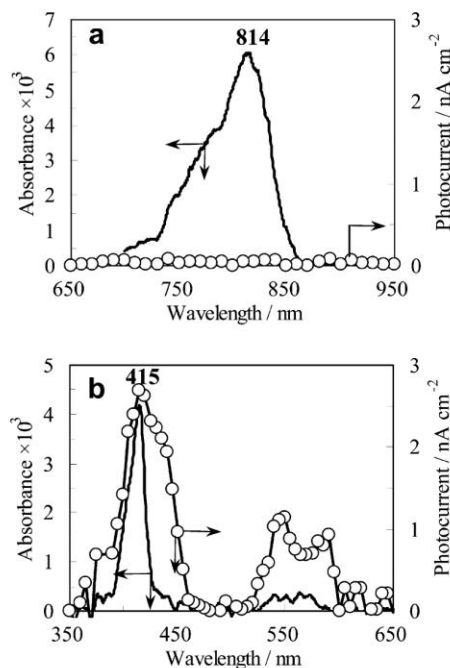


Figure 2. Action spectra (open circle) and absorption spectra (solid line) on a gold electrode modified with Type-1/[Zn]-BChl *a* (a) and Type-1/ZnMPMME (b) complexes in an aqueous methyl viologen (5 mM) solution.

these results show that the LH1 model polypeptides are in an α -helical conformation when assembled together with these pigments onto a gold electrode.

Figure 2 shows the excitation spectra of the photocurrent density obtained from the complexes formed from the Type-1 polypeptide and Zn porphyrin pigments and their electronic absorption spectra of Zn porphyrin complexes assembled onto a gold electrode. Complexes on the electrode were illuminated with a pulse of light that was either absorbed in the Qy band of [Zn]-BChl *a* or the Soret band of the ZnMPMME. It is clear from Figure 2 that an enhanced photocurrent was observed in the presence of the complex of ZnMPMME with Type-1 polypeptide (Fig. 2b). No photocurrent was observed with the complex formed from [Zn]-BChl *a* and Type-1 polypeptide (Fig. 2a). In this case, a cathodic photocurrent was observed, implying that one-way electron transfer from pigments in these complexes to methyl viologen was occurred. The excitation spectrum of the photocurrent density obtained from the complexes of Type-1 polypeptide with ZnMPMME indicated that the photocurrent responses showed maxima at wavelengths corresponding to the maxima of the main absorption bands of the complexes. Thus, the enhanced photocurrent observed in the assembled complex upon illumination at 415 nm can be ascribed to electron transfer from Au electrode to the ZnMPMME and then electron transfer from the ZnMPMME to methyl viologen as shown in Schemes 2b and S1. This data demonstrates that the ZnMPMME complex was well organized by

the synthetic LH1 model polypeptide that has its α -helix orientated with respect to the surface of the gold electrode and that the observed photocurrents were driven by light that was initially absorbed by ZnMPMME.

In conclusion, the SAM method is clearly successful in allowing the assembly of LH1 type functional complexes on the electrode. In this case, the photocurrent response depended on the structure of the pigments and synthetic LH1 model polypeptides. This method will be useful for the self-assembly of these complexes in order to study the energy transfer and electron transfer reactions between individual pigments in the supramolecular complexes on the electrode. Various combinations of these complexes are being tested for their usefulness in constructing artificial solar energy conversion devices.

Acknowledgements

M.N. and H.H. are grateful to the international joint grant of a Japan–UK Partnering Award of JST and JST, Crest for financial support. The present work was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japanese Government and AOARD.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.09.158.

References and notes

- Parkes-Loach, P. S.; Loach, P. A. In *Anoxygenic Photosynthetic Bacteria*; Blankenship, R. E., Madigan, T. M., Bauer, C. D., Eds.; Kluwer: Dordrecht, 1995; pp 437–471.
- Iida, K.; Parkes-Loach, P. S.; Loach, P. A.; Nango, M. In *Photosynthesis: From Light to Biosphere*; Mathis, P., Ed.; Kluwer: Dordrecht, 1996; Vol. 1, pp 235–238.
- Rabanal, F.; Degrado, W. F.; Dutton, P. L. *J. Am. Chem. Soc.* **1996**, *118*, 473–474.
- Arnold, P. A.; Shelton, W. R.; Benson, D. R. *J. Am. Chem. Soc.* **1997**, *119*, 3181–3182.
- Ogawa, M.; Shinohara, K.; Nakamura, Y.; Suemori, Y.; Nagata, M.; Iida, K.; Gardiner, A. T.; Cogdell, R. J.; Nango, M. *Chem. Lett.* **2004**, *33*, 772–773.
- Nagata, M.; Yoshimura, Y.; Inagaki, J.; Suemori, Y.; Iida, K.; Ohtsuka, T.; Nango, M. *Chem. Lett.* **2003**, *32*, 852–853.
- Kashiwada, A.; Nishino, N.; Wang, Z.-Y.; Nozawa, T.; Kobayashi, M.; Nango, M. *Chem. Lett.* **1999**, *28*, 1301–1302.
- Kashiwada, A.; Watanabe, H.; Tanaka, T.; Nango, M. *Chem. Lett.* **2000**, *29*, 24–25.
- Nango, M.; Kashiwada, A.; Watanabe, H.; Yamada, S.; Yamada, T.; Ogawa, M.; Tanaka, T.; Iida, K. *Chem. Lett.* **2002**, *31*, 312–313.

10. Kashiwada, A.; Hiroaki, H.; Kohda, D.; Nango, M.; Tanaka, T. *J. Am. Chem. Soc.* **2000**, *122*, 212–215.
11. Nagata, M.; Nango, M.; Kashiwada, A.; Yamada, S.; Ito, S.; Sawa, N.; Ogawa, M.; Iida, K.; Kurono, Y.; Ohtsuka, T. *Chem. Lett.* **2003**, *32*, 216–217.
12. Dewa, T.; Yamada, T.; Ogawa, M.; Sugimoto, M.; Mizuno, T.; Yoshida, K.; Nakao, Y.; Kondo, M.; Iida, K.; Yamashita, K.; Tanaka, T.; Nango, M. *Biochemistry* **2005**, *44*, 5129–5139.
13. Kashiwada, A.; Takeuchi, Y.; Watanabe, H.; Mizuno, T.; Yasue, H.; Kitagawa, K.; Iida, K.; Wang, Z.-Y.; Nozawa, T.; Kawai, H.; Nagamura, T.; Kurono, Y.; Nango, M. *Tetrahedron Lett.* **2000**, *41*, 2115–2119.
14. Meadows, K. A.; Iida, K.; Tsuda, K.; Recchia, P. A.; Heller, B. A.; Antonio, B.; Nango, M.; Loach, P. A. *Biochemistry* **1995**, *34*, 1559–1574.
15. Miura, Y.; Kimura, S.; Imanishi, Y.; Umemura, J. *Langmuir* **1998**, *14*, 6935–6940.

Molecular assembly of Zn porphyrin complexes using synthetic light-harvesting model polypeptides

Tsuyoshi Ochiai · Takahide Asaoka · Tomoya Kato · Shinichiro Osaka · Takehisa Dewa · Keiji Yamashita · Alastair T. Gardiner · Hideki Hashimoto · Mamoru Nango

Received: 16 September 2007 / Accepted: 15 November 2007 / Published online: 30 November 2007
© Springer Science+Business Media B.V. 2007

Abstract Synthetic single α -helix hydrophobic polypeptides, which have similar amino acid sequences to the hydrophobic core in the native light-harvesting 1- β polypeptide from *Rhodobacter sphaeroides*, formed Zn porphyrin complexes on a gold electrode, as well as in *n*-octyl- β -glucoside micelles: this process is dependent on the structure of the pigments and the polypeptides. Interestingly, an enhanced photoelectric current was observed when Zn mesoporphyrin monomer complexed with the synthetic light-harvesting model polypeptide in an α -helical configuration was assembled with a defined orientation onto the electrode. Analog of these light-harvesting model complexes are also useful in providing insights into the effect of polypeptide structure on the formation of light-harvesting complexes on and off electrodes.

Keywords Zn porphyrin complex · Light-harvesting model polypeptide · Reconstitution · Self-assembling Monolayer (SAM) · Photocurrent

Abbreviations

AFM	Atomic force microscopy
BChl	Bacteriochlorophyll
CD	Circular dichroism
Fmoc	9-fluorenylmethyloxycarbonyl
HBTU	<i>O</i> -(benzotriazol-1-yl)- <i>N,N,N'</i> -tetramethyluronium hexafluorophosphate
HOBt	1-hydroxybenzotriazole
ITO	Indium tin oxide
LH1	Core light-harvesting complex
LH2	Peripheral light-harvesting complex
LH1-RC	Light-harvesting antenna core complexes
MPMME	Mesoporphyrin monomethyl ester
MP-L-Lys(MP)	Mesoporphyrin-L-lysine-mesoporphyrin (covalently bridged mesoporphyrin dimer with an L-lysine)
MV	Methyl viologen
OG	<i>n</i> -octyl- β -D-glucopyranoside
<i>Rb.</i>	<i>Rhodobacter</i>
<i>Rs.</i>	<i>Rhodospirillum</i>
SAMs	Self-assembled monolayers
TFA	Trifluoroacetic acid
ZnMPMME	Zinc-substituted mesoporphyrin monomethyl ester
ZnMP-L-Lys(ZnMP)	Zinc-substituted mesoporphyrin-L-lysine-mesoporphyrin

Electronic supplementary material The online version of this article (doi:10.1007/s11120-007-9280-z) contains supplementary material, which is available to authorized users.

T. Ochiai · T. Asaoka · T. Kato · S. Osaka · T. Dewa · K. Yamashita · M. Nango (✉)
Materials Science and Engineering, Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya 466-8555, Japan
e-mail: nango@nitech.ac.jp

A. T. Gardiner
Glasgow Biomedical Research Centre, Institute of Biomedical and Life Sciences, University of Glasgow, University Avenue, Glasgow G12 8QQ, UK

H. Hashimoto
Department of Physics, Graduate School of Science, Osaka City University, 3-3-138 Sugimoto, Sumiyoshi-ku, Osaka 558-8585, Japan

Introduction

Light-harvesting (LH) polypeptides organize bacteriochlorophyll *a* (BChl *a*) so that efficient energy transfer between BChls can occur (Olsen et al. 1994; Karrasch et al. 1995; Parkes-Loach et al. 1995; McDermott et al. 1995; Pullerits and Sundström 1996; Sturgis et al. 1997; Cogdell and Lindsay 1998). The first X-ray crystal structure of the LH2 complex of *Rhodospseudomonas acidophila* strain 10050 was described at 2.5 Å resolution (McDermott et al. 1995). It consists of a ring of nine heterodimeric subunits, where each dimer consists of one α/β -apoprotein pair together with three molecules of BChl and one molecule of carotenoid. However, no such high resolution structure has yet been determined for an LH1 complex, although low-resolution structures have been determined from two-dimensional crystals of LH1 complexes (Karrasch et al. 1995; Stuart et al. 2002), X-ray crystallography of three dimensional crystals of LH1-RC complexes (Roszak et al. 2003) and atomic force microscopy (AFM) analyses of LH1-RC complexes (Bahatyrova et al. 2004). The LH1 complex forms a larger ring and it is probable that BChl *a* binding-sites in the LH1 complex are very similar to those associated with the LH2 complex.

The LH1 complex contains LH1- α and LH1- β polypeptides that have molecular weights of about 6 kDa and contain approximately 65% α -helical character based on far-UV and circular dichroism (CD) data (Parkes-Loach et al. 1995). Each polypeptide has a central, transmembrane hydrophobic core. Olsen et al. (1994) proposed a model for the structure of the minimal unit of the LH1 complex from *Rhodobacter* (*Rb.*) *sphaeroides*. This model was tested by site-directed mutagenesis (Sturgis et al. 1997). The results of this study indicated that a hydrogen bond between a tryptophan at the C-terminal region of the LH1 polypeptides and BChl *a* plays an important role in the organization of BChl *a*. Loach and coworkers reported that the LH1 complex of purple photosynthetic bacteria can be reconstituted from separately isolated LH1- α and - β polypeptides, or from chemically synthesized polypeptides, when added to BChl *a* either in lipid bilayers or n-octyl- β -D-glucopyranoside (OG) micelles (Bustamante and Loach 1994; Parkes-Loach and Loach 1995; Meadows et al. 1995, 1998; Kehoe et al. 1998; Noy and Dutton 2006). Upon reconstitution the Q_y band of BChl *a* is shifted from 777 nm (monomeric form) to near 820 nm in the subunit form of the B820 complex and to 870 nm in the holocomplex. These results indicate that both purified LH polypeptides and chemically synthesized polypeptides can be used to study both the assembly and the structure of LH1 complexes.

Further reports from several other laboratories have demonstrated that porphyrins can be assembled together with synthetic polypeptides to produce artificial hemoproteins or LH1 complex models (Pasternack et al. 1991;

Choma et al. 1994; Benson et al. 1995; Rabanal et al. 1996; Arnold et al. 1997). Moreover other studies have shown that it is possible to organize and assemble both native and artificial light-harvesting complexes on to electrodes (Bustamante and Loach 1994; Iida et al. 1998, 2000; Kehoe et al. 1998; Meadows et al. 1998; Kashiwada et al. 2000a, b; Nango et al. 2002; Ogawa et al. 2002).

Recently, the techniques of self-assembled monolayers (SAMs) of organic molecules on an indium tin oxide (ITO) or Au electrode have been developed (Nagata et al. 2003a; Suemori et al. 2006; Kondo et al. 2007). In this article, these techniques have been used to assemble Zn porphyrin pigments, [Zn]-BChl *a*, mesoporphyrin monomer, ZnMPME and Zn mesoporphyrin dimer, ZnMP-L-Lys-(ZnMP) (see Fig. 1) together with synthetic LH model polypeptides, Type-1 and 2, that have similar amino acid sequence to the hydrophobic core of the native photosynthetic LH1- β polypeptide from *Rb. sphaeroides*, see Fig. 1, (Kashiwada et al. 1999, 2000c; Nango et al. 2002) on to gold electrodes and into OG micelles. The aim of this is to gain insights into the structural requirements for assembly of LH1 complexes on solid substrates.

Synthetic model LH polypeptides with cysteine group at C-terminal, which can be easily bound onto a gold surface were used (see Fig. 2). These polypeptides were synthesized as described previously (Kashiwada et al. 2000d). Further, we selected synthetic model polypeptides (see Fig. 1) that have similar amino acid sequences to the hydrophobic core of the native LH1- β polypeptide from *Rb. sphaeroides*. Zn-substituted porphyrin complexes were used because of their stability (Kashiwada et al. 2000c; Nagata et al. 2003b). [Zn]-BChl *a*, obtained synthetically by the transmetalation of BChl *a* (Hartwich et al. 1998), is more stable than BChl *a*, and Zn porphyrin derivatives, therefore, have been widely used in model studies of light-harvesting.

Materials and methods

Materials

Unless stated otherwise, all chemicals and reagents were obtained commercially and used without further purification. A BChl *a* bearing geranylgeraniol ester side chain was isolated from the carotenoidless mutant G9 of *Rs. rubrum* and purified by HPLC as described elsewhere (Meadows et al. 1995). A [Zn]-BChl *a* was prepared from BChl *a* via transmetalation by following procedures previously reported (Dewa et al. 2005).

Zn Mesoporphyrin-IX Monomethyl Ester, ZnMPME, L-Lysine-linked Zn Mesoporphyrin dimer, ZnMP-L-Lys (ZnMP) were obtained as previously described. (Kashiwada et al. 2000a).

Fig. 1 The amino acid sequences of *Rb. sphaeroides* LH1- β (and its synthetic model polypeptides) and the structures of BChl *a* and Zn mesoporphyrin derivatives

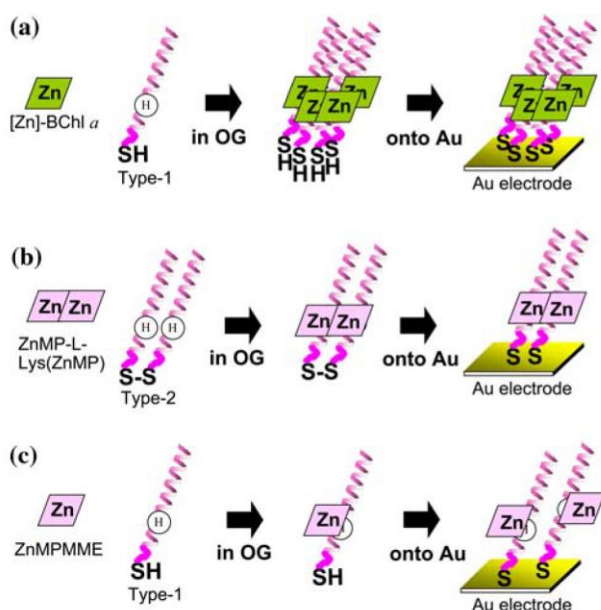
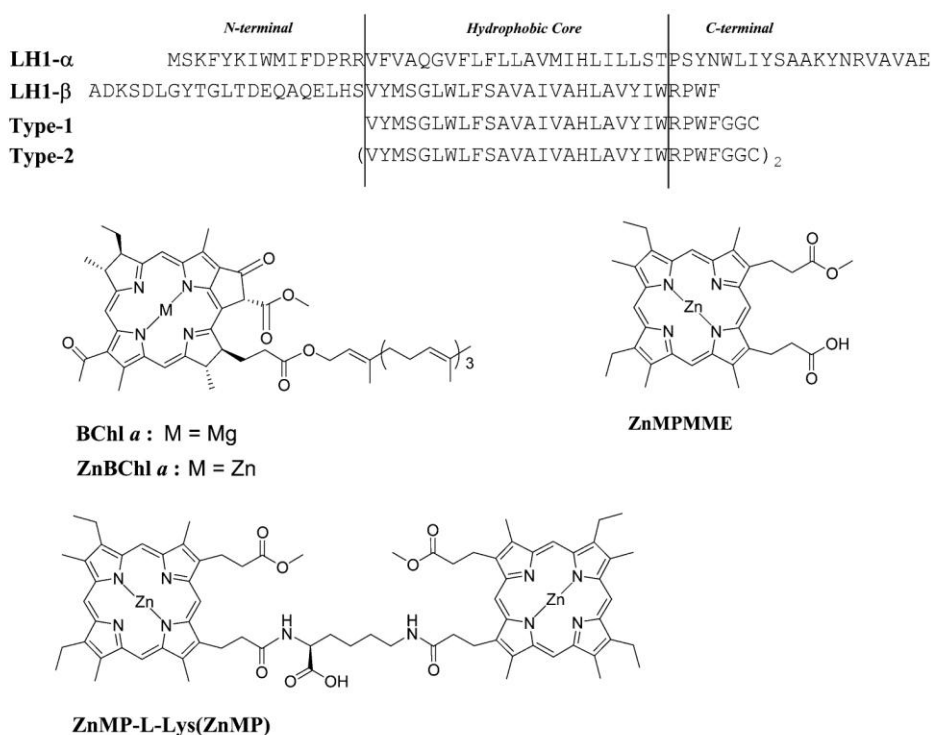


Fig. 2 Schematic view of Type-1 polypeptide/[Zn]-BChl *a* (a), Type-2 polypeptide/ZnMP-L-Lys(ZnMP) (b) and Type-1 polypeptide/ZnMPMME (c) complexes assembled on a gold electrode

LH1- β model polypeptides were prepared by solid-phase peptide synthesis using a Rink amide resin, *N*^α-9-fluorenylmethyloxycarbonyl (Fmoc) amino acids, *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU) and 1-hydroxybenzotriazole (HOBt). The cleavage reaction was performed with phenol in 90% trifluoroacetic acid (TFA), 5% thioanisole, and 5% H₂O solution for 90 min at room temperature. The product was filtered from the resin and precipitated in cold ethyl-ether. The ether solution was centrifuged for 5 min at medium speed and the solid product dried in vacuo. The crude product was further purified by reverse phase HPLC (YMC-Pack Protein-RP: 250 × 4.6 mm, 5 μm) and analyzed by TOF-Mass spectrometry. Solvent A was 0.1% TFA in H₂O. A 25-min gradient of 50–100% solvent B (0.1% TFA in 67% acetonitrile and 33% 2-propanol) was used. Absorbance was monitored at 280 nm. The desired product was confirmed by TOF-Mass spectrometry. Type-2 was synthesized from Type-1 by oxidation in 30% DMSO solution (Tam et al. 1991). The crude Type-2 product was further purified by reverse phase HPLC and analyzed by TOF-Mass spectrometry.

Preparation of native and LH1 model polypeptides

The native LH1- α and - β polypeptides were separately isolated from the LH1 complex of *Rb. sphaeroides* as previously described (Parkes-Loach and Loach 1995).

Reconstitution of LH1 model complexes

The molecular assembly of pigments together with the synthetic polypeptides was carried out as previously reported (Meadows et al. 1995). Ten nmol of the

polypeptides were dissolved in 4.5% OG in 50 mM phosphate buffer, pH 7.5, diluted to 0.90% OG with detergent-free buffer. Seven nmol of pigments, dissolved in organic solvents (e.g., acetone), were then added to this mixture. This solution was further diluted to 0.78% OG (the condition for subunit-type complex formation) and chilled at 4°C overnight (for LH1-type complex formation), after which the concentration of OG (26.7 mM) is approximately equal to its CMC (Ito et al. 2000).

Self-assembly of LH1 model complexes onto a gold electrode

The substrate for the assembly was a 20 nm gold thin film, which had been evaporated in a vacuum at room temperature onto a glass substrate. To improve the adhesion of the gold film to the glass, 10 nm of a Ti film was sputtered on to it prior to the Au deposition. The gold films were flame-annealed immediately before use. The evaporation rate of Au was 0.2 Å s^{-1} at a base pressure of 7×10^{-4} Pa.

Complexes were immobilized on the gold electrode as SAMs by immersion of the gold electrode in the sample solution at 4°C for 12 h. Successful self-assembly was detected by UV-Vis. and FT-IR spectroscopy.

Near IR, CD, FT IR, and fluorescence spectra

Near IR spectra were recorded with Hitachi U-2000 (spectral bandwidth of 2 nm) and U-3500 (spectral bandwidth of 1 nm) spectrophotometers. CD spectra were recorded with a Jasco J-600 or J-820 spectropolarimeter. FT-IR spectra were recorded with PerkinElmer Spectrum 2000. Steady-state fluorescence spectra were recorded using a fluorimeter composed of a CCD detector (Spec-10: 100BR/LN; Roper Scientific), two monochromators (SP-150M for excitation and SP-306 for emission; Acton Research Co.) and a tungsten-halogen light source (TS-428DC; Acton Research Co.). The slits were set at 0.50 mm for the LH1 complexes in OG and at 1.00 mm for the LH1 complex on the electrode.

All the spectroscopic measurements used sample solutions that were placed in a temperature-controlled chamber.

Photocurrent measurements

Photocurrent measurements were carried out in 100 mM phosphate buffer (pH 7.5) containing 100 mM sodium perchlorate, 5 mM methyl viologen (MV) in a home made cell that contained three electrodes (a gold electrode modified by

Table 1 Absorption and CD spectral data of the pigments in the presence of LH1 polypeptides from *Rb. sphaeroides* and the synthetic model polypeptides in 0.78% OG micellar solutions at 25°C or 4°C

Pigments	Polypeptides	λ_{max} (nm) ^a		CD λ_{max} (nm) (10^{-4} θ)
		25°C	4°C	
BChl <i>a</i>	None	777, 850 ^b	777, 850 ^b	No clear signal
	LH1- α and - β	821	873	888 (8.2), 855 (−6.1)
	LH1- β	821	823	828 (−4.5)
	Type-1	818	843	854 (6.0), 813 (−7.0)
	Type-2	814	814	823 (10), 802 (−20)
[Zn]-BChl <i>a</i>	None	770	770, 841 ^b	No clear signal
	LH1- β	815	815	820 (−12)
	Type-1	809	821	842 (24), 810 (−17)
	Type-2	807	807	822 (6.1), 792 (−21)
ZnMP-L-Lys(ZnMP)	None	386	391 ^b	401 (5.2), 379 (−7.4)
	LH1- β	386, 410 ^b	415	403 (5.8), 379 (−7.0)
	Type-1	386, 412 ^b	417	422 (−9.4), 413 (4.8), 399 (4.7), 377 (−4.7)
	Type-2	386, 412 ^b	418	425 (−0.3), 410 (1.7)
ZnMPME	None	404	396 ^b	408 (2.0), 385 (−3.0)
	LH1- β	407	414	419 (−3.3), 410 (2.2)
	Type-1	407	415	No signal
	Type-2	404	418	421 (−3.6), 413 (1.8)

^a Qy band for BChl *a* and [Zn]-BChl *a*, and Soret band for Zn mesoporphyrin derivatives

^b Broad peak was observed

addition of a SAM of the complex as the working electrode, a Pt electrode as a counter electrode and an Ag/AgCl electrode as the reference electrode). The working electrode was illuminated with a halogen lamp unit, AT-100HG, through a monochromator, SPG-120S (Shimadzu) for 30 seconds, and the response of photocurrent was monitored.

Results and discussion

Molecular assembly of a Zn porphyrin complex using synthetic light-harvesting model polypeptides in OG micellar solutions

To compare the effect of the structure of the polypeptides on the formation of the LH1 complex, we examined the molecular assembly of [Zn]-BChl *a* with Type-1 and 2 polypeptides in OG micellar solutions. Table 1 shows the position of the Q_y absorption band or the Soret band and the CD signals of the pigments in the presence of the synthetic hydrophobic polypeptides. It is clear from Table 1 that the Q_y band of [Zn]-BChl *a* monomer in OG micelles (770 nm) is red-shifted to 809 nm in the presence of Type-1 polypeptides at 25°C. This is analogous to the subunit complex that forms with either LH1- β and LH1- α together or with LH1- β alone. A further red-shift to 821 nm occurs when the sample is cooled to 4°C. This is qualitatively similar to that seen using BChl *a* (see Table 1). These data again shows that the [Zn]-BChl *a* complex can be organized by this synthetic hydrophobic single α helix polypeptide in OG micelles (Kashiwada et al. 2000c). It is interesting that a similar red-shift of the Q_y band is not observed in the presence of the Type-2 polypeptide. Instead in this case, the Q_y band was only red-shifted to 807 nm and dramatically sharpened. This result suggests that the disulfide-linkage in the C-terminal segment obstructs formation of a LH1-type complex as described previously (Table 1) (Kashiwada et al. 2000c). Very little difference between the intensities of CD signals of reconstituted complexes involving the Type-1 and Type-2 polypeptides was observed (Table 1).

To test the effect of the structure of the pigments on the LH1-complex formation, the reconstitution of the complexes was repeated using ZnMPMME and ZnMP-L-Lys(ZnMP). The Soret band of ZnMPMME (404 nm) in OG micelles was red-shifted to 407 nm at 25°C in the presence of either Type-1 or 2 polypeptides. Upon cooling to 4°C a further red-shift to 415 or 418 nm was observed (Table 1). Interestingly, the Soret band of ZnMP-L-Lys(ZnMP) in the presence of Type-1 or Type-2, in 0.78% OG at 25°C is split with peaks at 388 and 412 nm. Upon cooling either solution to 4°C, the Soret band at 388 nm almost disappears and the band at 412 shifts to 417 or

419 nm in the presence of Type-1 or 2 polypeptides, respectively. These changes in the Soret band in the presence of the two polypeptides were reversible with temperature. In the absence of the polypeptides the Soret bands of ZnMPMME and ZnMP-L-Lys(ZnMP) remained broad (Table 1). Interestingly, a split-CD signal located at

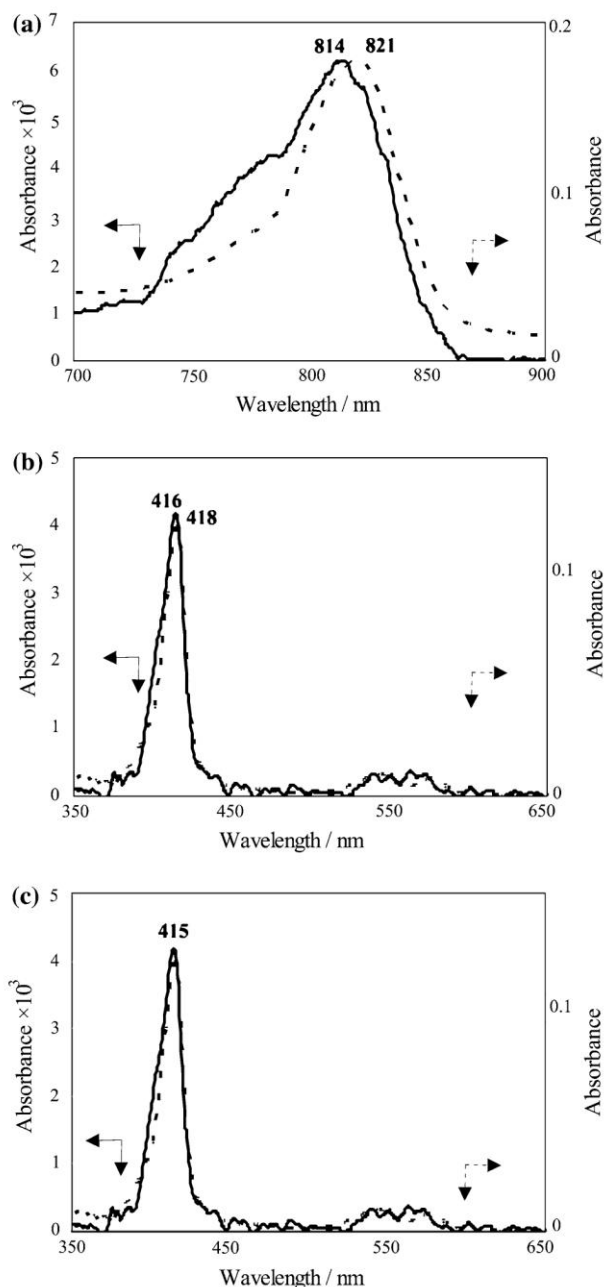


Fig. 3 Absorption spectra of Type-1 polypeptide/[Zn]-BChl *a* (a), Type-2 polypeptide/ZnMP-L-Lys(ZnMP) (b) and Type-1 polypeptide/ZnMPMME (c) complexes assembled on a gold electrode (dashed) and in 0.78% OG at 4°C (black). [Type-1] = 3.45 μ M, [Type-2] = 1.72 μ M in 0.78% OG

the red-shifted Soret band for ZnMPMME was observed in the presence of the Type-2 polypeptide. This split-CD signal was not observed in the presence of the Type-1 polypeptide. This may indicate that ZnMPMME has a monomer-like structure in the presence of the Type-1 polypeptide but becomes aggregated in the presence of the Type-2 polypeptide. CD signals at the red-shifted Soret band of ZnMP-L-Lys(ZnMP) were also observed in the presence of either the Type-1 or 2 polypeptides and again a split signal was observed only in the presence of Type-2 polypeptides. The fluorescence of ZnMPMME is more quenched in the presence of Type-2 polypeptides than Type-1 (Table S1). In contrast, the fluorescence of ZnMP-L-Lys(ZnMP) is more quenched in the presence of a Type-1 polypeptide (Table S1). These data suggest that the ZnMPMME monomer complex is more stably organized in the presence of Type-1 polypeptides, while the ZnMP-L-Lys(ZnMP) complex is more stably assembled in the presence of Type-2 polypeptides.

Molecular assembly of Zn porphyrin complexes onto a gold electrode using synthetic light-harvesting model polypeptides

Figure 3 shows the NIR absorption spectra of Zn-porphyrin complexes assembled by the synthetic LH1 model polypeptides, both using Type-1 or Type-2 polypeptides either in an OG micellar solution at 4°C or onto a gold electrode at room temperature. The Q_y band of the [Zn]-BChl *a* complex formed with the Type-1 polypeptide showed an

absorption maximum at 814 nm on the gold electrode and at 821 nm in an OG micellar solution. These absorption maxima indicate that both cases represent a subunit type complex, as seen with LH1- β alone (Meadows et al. 1995). The Soret bands of the complexes formed with either ZnMPMME or ZnMP-L-Lys(ZnMP) together with either Type-1 or 2 polypeptides assembled onto the gold electrode showed maxima at 415 (ZnMPMME) or 418 nm (ZnMP-L-Lys(ZnMP)).

The FT-IR spectra of the complex of Type-1 polypeptide and [Zn]-BChl *a* showed absorptions at 1652 and 1543 cm^{-1} the complex of Type-1 polypeptides and ZnMPMME showed absorptions 1662 and 1549 cm^{-1} and the complex of Type-2 polypeptide and ZnMP-L-Lys(ZnMP) showed absorptions at 1658 and 1543 cm^{-1} . These bands can be assigned to the amide I and amide II bands, respectively, and clearly indicate that the polypeptides are largely helical. It is also possible as previously described (Gremlich et al. 1983; Miura et al. 1998) to use these bands to

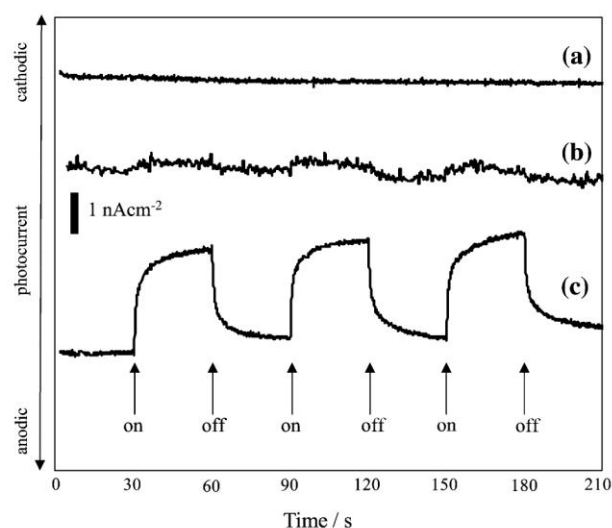


Fig. 4 Time course of the photocurrent on a gold electrode modified with Type-1 polypeptide/[Zn]-BChl *a* (a), Type-2 polypeptide/ZnMP-L-Lys(ZnMP) (b) and Type-1 polypeptide/ZnMPMME (c) complexes in an aqueous MV^{2+} (5 mM) solution upon illumination at the Soret or the Q_y absorption band of the pigment complex

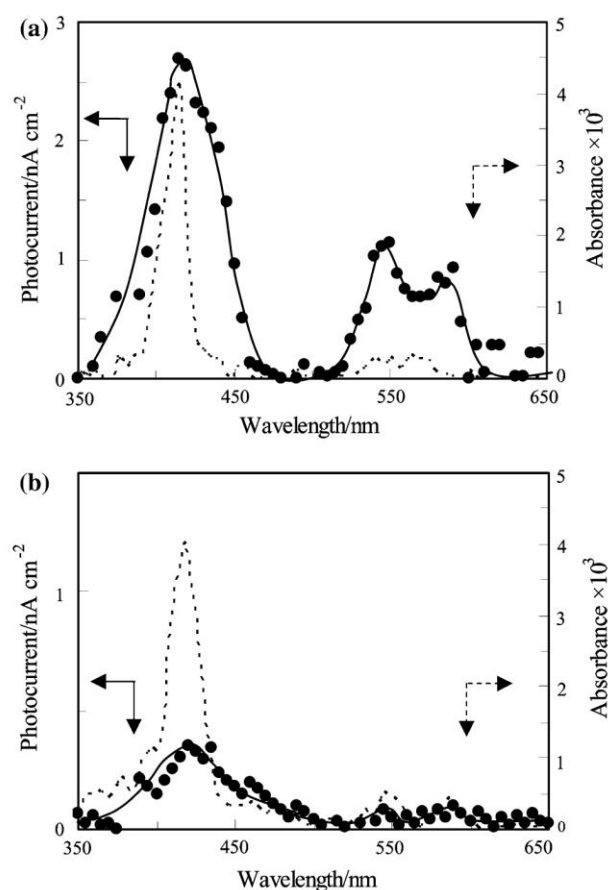


Fig. 5 Photocurrent excitation (solid circle and solid line) and absorption (dashed line) spectra on a gold electrode modified with Type-1 polypeptide/ZnMPMME (a) and Type-2 polypeptide/ZnMP-L-Lys(ZnMP) (b) complexes in an aqueous MV (5 mM) solution upon illumination at the Soret absorption band of the pigment complex

determine the tilt angle of the helices relative to the surface of the gold electrode. The tilt angle of the helix in the complex of the Type-1 polypeptide and [Zn]-BChl *a* is 43°. This angle is 47° for both the complex formed between the Type-1 polypeptide and ZnMPMME and for the one formed between the Type-2 polypeptide and ZnMP-L-Lys(ZnMP). Taken together these results show that the LH1 model polypeptides are in an α -helical conformation when assembled together with these pigments onto a gold electrode.

Photocurrent induced by illumination of Zn porphyrin complexes assembled onto a gold electrode using synthetic light-harvesting model polypeptides

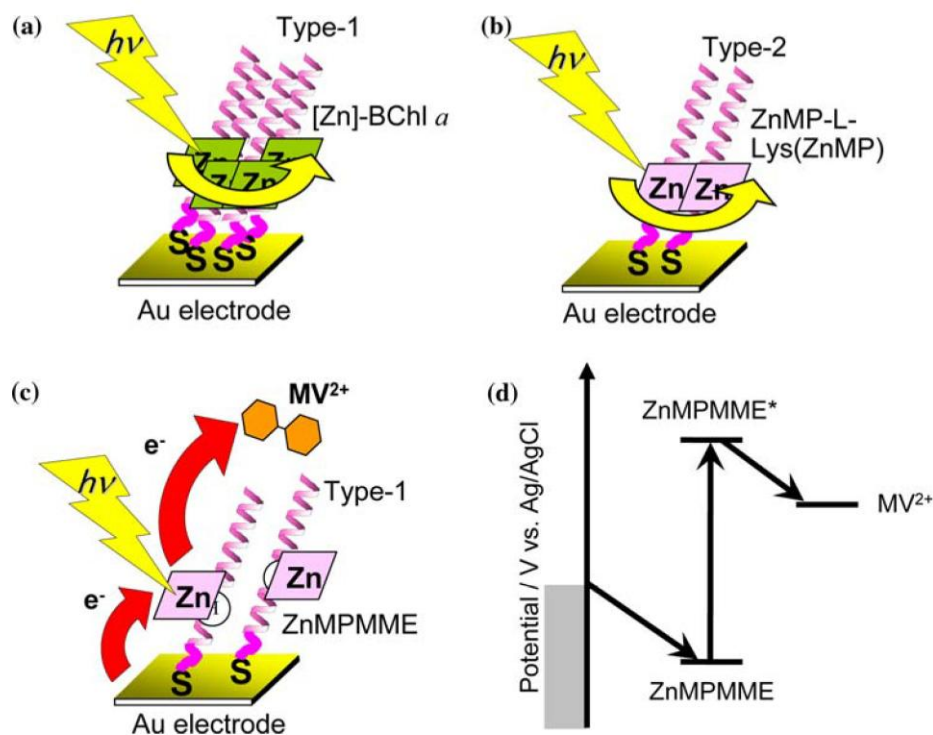
Figure 4 shows the time course of the photocurrent generated by complexes formed from either Type-1 or Type-2 polypeptides and Zn-porphyrin pigments assembled onto a gold electrode. The complex on the electrode was illuminated with a pulse of light that was either absorbed in the Q_y band or the Soret band of the Zn porphyrin. The irradiation was applied to the electrode periodically. It is clear from Fig. 4 that the strongest photocurrent was observed in the presence of the complex of ZnMPMME with Type-1 polypeptides. Less photocurrent was observed with the other complexes involving ZnMPMME. No photocurrent was observed with the complex formed from [Zn]-BChl *a*

and Type-1 polypeptides. In each case, a cathodic photocurrent was observed, implying that one-way electron transfer from pigments in these complexes to MV was occurring as described below.

Figure 5 shows excitation spectrum of the photocurrent density obtained from complexes of Type-1 polypeptides with ZnMPMME (a) and Type-2 polypeptides with ZnMP-L-Lys(ZnMP) (b). These photocurrent responses showed maxima at wavelengths corresponding to the maxima of the main absorption bands of the complexes. Interestingly, an especially enhanced photocurrent was observed upon illumination of the complex of Type-1 polypeptides with ZnMPMME at 415 nm.

This data suggests that electron transfer between the pigments in the assembled complexes occurs as shown in Fig. 6. This is analogous to the electron transfer that has been seen with LH1-type complexes, using [Zn]-BChl *a*, assembled onto an ITO electrode (Ogawa et al. 2002). Thus, the enhanced photocurrent observed in the assembled complex upon illumination at 415 nm can be ascribed to electron transfer from the Au electrode to the ZnMPMME and then electron transfer from the ZnMPMME to MV as shown in Fig. 6. This data demonstrates that the ZnMPMME complex was organized by the synthetic LH1 model polypeptide that has its α -helix orientated with respect to the surface of the gold electrode, and that the observed photocurrents were driven by light that was initially absorbed by the ZnMPMME.

Fig. 6 Schematic view of light energy transfer between pigments in (a) Type-1 polypeptide/[Zn]-BChl *a* and (b) Type-2 polypeptide/ZnMP-L-Lys(ZnMP) complexes assembled on a gold electrode and (c) electron transfers in Type-1 polypeptide/ZnMPMME complex assembled on a gold electrode, and (d) an energy diagram of the cathodic photocurrent



In conclusion, the SAM method is clearly successful in allowing assembly of LH1-type functional complexes on the electrode. In this case the photocurrent response depended on the structure of the pigments and synthetic LH1 model polypeptides. Various combinations of these complexes are being tested for their usefulness in constructing artificial solar energy conversion devices.

Acknowledgments M. N., H. H. and A.T.G. are grateful to the international joint grant of NEDO, BBSRC and a Japan–UK Partnering Award of JST for financial support. The present work was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japanese Government, JST, Crest and AOARD.

References

- Arnold PA, Shelton WR, Benson DR (1997) Peptide helix induction in a self-assembling hemoprotein model. *J Am Chem Soc* 119:3181–3182
- Bahatyrova S, Frese RN, Siebert CA, Olsen JD, van der Werf K, van Grondelle R, Niederman RA, Bulloah PA, Otto C, Hunter CN (2004) The native architecture of a photosynthetic membrane. *Nature* 430:1058–1062
- Benson DR, Hart BR, Zhu X, Doughty MB (1995) Design, synthesis, and circular dichroism investigation of a peptide sandwiched mesoheme. *J Am Chem Soc* 117:8502–8510
- Bustamante PL, Loach PA (1994) Reconstitution of a functional photosynthetic receptor complex with isolated subunits of core light-harvesting complex and reaction centers. *Biochemistry* 33:13329–13339
- Choma CT, Lear JD, Nelson MK, Dutton PL, Robertoson DE, Degradó WF (1994) Design of a heme-binding four-helix bundle. *J Am Chem Soc* 116:856–865
- Cogdell RJ, Lindsay JG (1998) Can photosynthesis provide a 'biological blueprint' for the design of novel solar cells. *Trends Biotechnol* 16:521–527
- Dewa T, Yamada T, Ogawa M, Sugimoto M, Mizuno T, Yoshida K, Nakao Y, Kondo M, Iida K, Yamashita K, Tanaka T, Nango M (2005) Design and expression of cysteine-bearing hydrophobic polypeptides and their self-assembling properties with bacteriochlorophyll *a* derivatives as a mimic of bacterial photosynthetic antenna complexes. Effect of steric confinement and orientation of the polypeptides on the pigment/polypeptide assembly process. *Biochemistry* 44:5129–5139
- Gremlich HU, Fringeli UP, Schwyzer R (1983) Conformational changes of adrenocorticotropin peptides upon interaction with lipid membranes revealed by infrared attenuated total reflection spectroscopy. *Biochemistry* 22:4257–4264
- Hartwich G, Fiedor L, Simonin I, Cmiel E, Schafer W, Noy D, Scherz A, Scheer H (1998) Metal-substituted bacteriochlorophylls 1 preparation and influence of metal and coordination on spectra. *J Am Chem Soc* 120:3675–3683
- Iida K, Nango M, Yasue H, Okuda K, Okita M, Kashiwada A, Takada N, Maekawa M, Kurono Y (1998) The effect of denaturants on the stability of light-harvesting complex. *Colloid Polym Sci* 276:152–159
- Iida K, Ohya N, Kashiwada A, Mimuro M, Nango M (2000) Characterization of the light-harvesting polypeptide/ bacteriochlorophyll *a* complex isolated from photosynthetic bacteria by the linear dichroism spectra. *Bull Chem Soc Jpn* 73:221–229
- Ito H, Ishido S, Nomura M, Hayakawa T, Mitaku S (2000) Estimation of the hydrophobicity in microenvironments by pyrene fluorescence measurements: *n*-octyl- β -D-octylglucoside micelles. *J Am Chem Soc* 122:212–215
- Karrasch S, Bullough PA, Ghosh R (1995) The 85 Å projection map of the light-harvesting complex I from *Rhodospirillum rubrum* reveals a ring composed of 16 subunits. *EMBO J* 14:631–638
- Kashiwada A, Nishino N, Wang Z-Y, Nozawa T, Kobayashi M, Nango M (1999) Molecular assembly of bacteriochlorophyll *a* and its analogues by synthetic 4 α -helix polypeptides. *Chem Lett* 28:1301–1302
- Kashiwada A, Takeuchi Y, Watanabe H, Mizuno T, Yasue H, Kitagawa K, Iida K, Wang Z-Y, Nozawa T, Kawai H, Nagamura T, Kurono Y, Nango M (2000a) Molecular assembly of covalently-linked mesoporphyrin dimers with light-harvesting polypeptides. *Tetrahedron Lett* 41:2115–2119
- Kashiwada A, Watanabe H, Mizuno T, Iida K, Miyatake T, Tamiaki H, Kobayashi M, Nango M (2000b) Structural requirements of zinc porphyrin derivatives on the complex-forming with light-harvesting polypeptides. *Chem Lett* 29:158–159
- Kashiwada A, Watanabe H, Tanaka T, Nango M (2000c) Molecular assembly of zinc bacteriochlorophyll *a* by synthetic hydrophobic 1 α -helix polypeptides. *Chem Lett* 29:24–25
- Kashiwada A, Hiroaki H, Kohda D, Nango M, Tanaka T (2000d) Design of heterotrimeric α -helical bundle by hydrophobic core engineering. *J Am Chem Soc* 122:212–215
- Kehoe JW, Meadows KA, Parkes-Loach PS, Loach PA (1998) Reconstitution of core light-harvesting complexes of photosynthetic bacteria using chemically synthesized polypeptides 2 determination of structural features that stabilize complex formation and their implications for the structure of the subunit complex. *Biochemistry* 37:3418–3428
- Kondo M, Nakamura Y, Fujii K, Nagata M, Suemori Y, Dewa T, Iida K, Gardiner AT, Cogdell RJ, Nango M (2007) Self-assembled monolayer of light-harvesting core complexes from photosynthetic bacteria on a gold electrode modified with alkanethiols. *Biomacromolecules* 8:2457–2463
- McDermott G, Prince SM, Freer AA, Hawthornthwaite-Lawless AM, Papiz MZ, Cogdell RJ, Isaacs NW (1995) Crystal structure of an integral membrane light-harvesting complex from photosynthetic bacteria. *Nature* 374:517–521
- Meadows KA, Iida K, Tsuda K, Recchia PA, Heller BA, Antonio B, Nango M, Loach PA (1995) Enzymatic and chemical cleavage of the core light-harvesting polypeptides of photosynthetic bacteria. Determination of the minimal polypeptide size and structure required for subunit and light-harvesting complex-formation. *Biochemistry* 34:1559–1574
- Meadows KA, Parkes-Loach PS, Kehoe JW, Loach PA (1998) Reconstitution of core light-harvesting complexes of photosynthetic bacteria using chemically synthesized polypeptides 1 minimal requirements for subunit formation. *Biochemistry* 37:3411–3417
- Miura Y, Kimura S, Imanishi Y, Umemura J (1998) Formation of oriented helical peptide layers on a gold surface due to the self-assembling properties of peptides. *Langmuir* 14:6935–6940
- Nagata M, Yoshimura Y, Inagaki J, Suemori Y, Iida K, Ohtsuka T, Nango M (2003a) Construction and photocurrent of light-harvesting polypeptides/zinc bacteriochlorophyll *a* complex in lipid bilayers. *Chem Lett* 32:852–853
- Nagata M, Nango M, Kashiwada A, Yamada S, Ito S, Sawa N, Ogawa M, Iida K, Kurono Y, Ohtsuka T (2003b) Construction of photosynthetic antenna complex using light-harvesting polypeptide- α from photosynthetic bacteria, *R rubrum* with zinc substituted bacteriochlorophyll *a*. *Chem Lett* 32:216–217
- Nango M, Kashiwada A, Watanabe H, Yamada S, Yamada T, Ogawa M, Tanaka T, Iida K (2002) Molecular assembly of

- bacteriochlorophyll a using light-harvesting model 1 α -helix polypeptides and 2 α -helix polypeptide with disulfide-linkage. Chem Lett 31:312–313
- Noy D, Dutton PL (2006) Design of a minimal polypeptide unit for bacteriochlorophyll binding and self-assembly based on photosynthetic bacterial light-harvesting proteins. Biochemistry 45:2103–2113
- Ogawa M, Kanda R, Dewa T, Iida K, Nango M (2002) Molecular assembly of light-harvesting antenna complex on ITO electrode. Chem Lett 31:466–467
- Olsen JD, Sockalingum GD, Robert B, Hunter CN (1994) Modification of a hydrogen bond to a bacteriochlorophyll a molecule in the light-harvesting 1 antenna of *Rhodobacter sphaeroides*. Proc Natl Acad Sci USA 91:7124–7128
- Parkes-Loach PS, Loach PA (1995) Structure–function relationships in core light-harvesting complex (LH1) as determined by characterization of the structural subunit and by reconstitution experiments. In: Blankenship RE, Madigan TM, Bauer CD (eds) Anoxygenic photosynthetic bacteria. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 437–471
- Pasternack RF, Giannetto A, Pagano P, Gibbs EJ (1991) Self-assembly of porphyrins on nucleic acids and polypeptides. J Am Chem Soc 113:7799–7800
- Pullerits T, Sundström V (1996) Photosynthetic light-harvesting pigment–protein complexes: toward understanding how and why. Acc Chem Res 29:381–389
- Rabanal F, Degrado WF, Dutton PL (1996) Toward the synthesis of a photosynthetic reaction center maquette: a cofacial porphyrin pair assembled between two subunits of a synthetic four-helix bundle multiheme protein. J Am Chem Soc 118:473–474
- Roszak AW, Howard TD, Southall J, Gardiner A, Law CJ, Isaacs NW, Cogdell RJ (2003) Crystal structure of the RC-LH1 core complex from *Rhodospseudomonas palustris*. Science 302:1969–1972
- Stuart J, Wang P, Qian P, Kirkland Y, Conroy MJ, Hunter CN, Bullough PA (2002) Projection structure of the photosynthetic reaction centre-antenna complex of *Rhodospirillum rubrum* at 85 Å resolution. EMBO J 21:3927–3935
- Sturgis JN, Olsen JD, Robert B, Hunter CN (1997) Functions of conserved tryptophan residues of the core light-harvesting complex of *Rhodobacter sphaeroides*. Biochemistry 36:2772–2778
- Suemori Y, Nagata M, Nakamura Y, Nakagawa K, Okuda A, Inagaki J, Shinohara K, Ogawa M, Iida K, Dewa T, Yamashita K, Gardiner A, Cogdell R, Nango M (2006) Self-assembled monolayer of light-harvesting core complexes of photosynthetic bacteria on an amino-terminated ITO electrode. Photosynth Res 90:17–21
- Tam JP, Wu CR, Liu W, Zhang JW (1991) Disulfide bond formation in peptides by dimethyl sulfoxide. Scope and applications. J Am Chem Soc 113:6657–6662

The Electronic behavior of a Photosynthetic Reaction Center Monitored by Conductive Atomic Force Microscopy

Takeshi Mikayama,^a Kouji Iida,^b Yoshiharu Suemori,^c Takehisa Dewa,^c Tokuji Miyashita,^{a,*}

Mamoru Nango,^{c,*} Alastair T. Gardiner^d and Richard J. Cogdell^d

^a Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, Japan

^b Nagoya Municipal Industrial Research Institute, Rokuban 3- 4-41, Atsuta-ku, Nagoya 456-0058,
Japan

^c Department of Applied Chemistry, Nagoya Institute of Technology, Japan

^d Division of Biochemistry and Molecular Biology, Biomedical Research Building, IBLS, University
of Glasgow, Glasgow G12 8TA, UK

Mailing Address: Department of Applied Chemistry, Nagoya Institute of Technology, Showa-ku,
Nagoya 466-8555, Japan

Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, Katahira 2-1-1,
Aoba-ku, Sendai 980-8577, Japan

Mamoru Nango, TEL&FAX :+81-52-735-5226, nango@nitech.ac.jp

Tokuji Miyashita, TEL:+81-22-217-5637, FAX:+81-22-217-5642, miya@tagen.tohoku.ac.jp,

Abstract

The conductivity of a photosynthetic reaction center (RC) from *Rhodobacter sphaeroides* was measured with conductive atomic force microscopy (CAFM) on SAM-modified Au (111) substrates. 2-mercaptoethanol (2ME), 2-mercaptoacetic acid (MAC), 2-mercaptopyridine (2MP) and 4-mercaptopyridine (4MP) were prepared as SAM materials to investigate the stability and morphology of RCs on the substrate by using near-IR absorption spectroscopy and AFM, respectively. The clear presence of the three well known RC near-IR absorption peaks indicates that the RCs were native on the SAM-modified Au(111). Dense grains with various diameters of 5-20nm, which corresponded to mixtures of single RCs up to aggregates of 10, were observed in topographs of RCs adsorbed on all the

different SAM-modified Au (111) substrates. The size of currents obtained from the RC using a bare conductive cantilever were produced in the following order for SAM molecules: 2MP>2ME>4MP>MAC. A clear rectification of this current was observed for the modification of the Au(111) substrate with the π -conjugated thiol, 2MP, indicating that 2MP was effective in both promoting the specific orientation of the RCs on the electrode and electron injection into the RC. Cyclic voltammetry measurements indicate that the 2MP is better mediator for the electron transfer between a quinone and substrate. The current with 2MP-modified cantilever was twice as high as that obtained with the Au-coated one alone, indicating that 2MP has an important role in lowering the electron injection barrier between special pair side of RC and gold electrode.

Keyword: photosynthetic reaction center, atomic force microscopy, conductive AFM, *Rhodobacter sphaeroides*.

1. Introduction

Photosynthetic organisms use well-defined pigment protein complexes to convert solar energy into a useful form of chemical energy. The nano-scale apparatus present in photosynthetic membranes has been optimized for low-power output and high-quantum efficiency operation. Advances from X-ray crystal structures and time-resolved optical spectroscopy have provided detailed insights into the structure and function of these complexes particularly in the case of the simplest photosynthetic apparatus from purple bacteria.¹⁻³ The primary light reactions of purple bacterial photosynthesis occur in transmembrane protein-pigment complexes that together form the so-called photosynthetic unit (PSU). The PSU consists of light harvesting (LH) complexes and reaction centers (RCs). In the case of purple photosynthetic bacteria, photons are usually initially absorbed by the LH 2 complex. The excitation energy is then funneled to the RC, via the LH 1 complex, where charge separation takes place across the photosynthetic membrane.^{4, 5}

In molecular electronics, great advances have been made in the integration and miniaturization of organic devices. Recently an organic device using nanoassembled films has been demonstrated.⁶⁻¹⁰ Similar advances in nanobiotechnology require systems for using proteins, such as those found in the bacterial photosynthetic membrane, in both nanoassembled films and on electrodes.¹¹⁻¹⁹ Recently, atomic force microscopy (AFM) has been used to visualize membrane proteins, such as the LH 2 complexes.²⁰⁻²⁶ Scheuring et al. observed the LH 1 complex as minor component together with the major LH 2 complex from *Rubrivivax geratinosus*²⁰ and the LH 1/RC complex in native photosynthetic membranes (chromatophore) from

*Rhodopseudomonas viridis*²¹ and *Rhodospirillum photometricum*²². Fotiadis et al. observed the LH 1/RC complex from *R. rubrum* in lipids from *E. coli*.²³ Bahatyrova et al. showed that the LH 1 complexes of a mutant lacking the RC form circular, elliptical, and even polygonal ring shapes as well as arcs and open rings²⁴, and that the LH 1 complexes are positioned to function as an energy collection hub from the LH 2 complex to RC in native membranes.²⁵ In our previous study, AFM of the LH 1 complexes showed a ring-like structure interpreted as an individual molecule of the LH 1 complex.¹² Scanning probe microscopy, including AFM, possesses the potential of not only imaging but also measuring a single biomolecule. RCs are an ideal membrane protein to use for these types of studies because of their stability and because the spectroscopic properties of their pigments can be used to check that the RCs remain in a native state when assembled into nanofilms or onto electrodes. Currents produced by RC films have already been reported.²⁷⁻²⁹ Recently scanning probe analyses, including STM (Scanning Tunneling Microscopy) and conductive atomic force microscopy (CAFM), have been shown to be very useful in these types of studies because they allow the study of the electronic properties of individual molecules in nanoassembled film on conductive metal substrates without the requirement for averaging inherent in most other methods. In particular, CAFM allows the contact resistance between the sample and tip to be controlled during current-voltage (*I-V*) measurements. In a previous publication, Stamouli et al.²⁹ reported the electron conduction of RCs in lipid bilayers on High Orientated Pyrolytic Graphite (HOPG) using CAFM with a Pt/Ir coated probe in air.

In this area of research there are two fundamental issues. Firstly, how to order proteins on the electrodes with respect to their sidedness, and secondly how to make a good electrical contact between the protein

and the electrode. Biotechnology allow the mutation of RC, the orientation is determined by His-tagged RC.³⁰ However there are rather few reports of studies looking for better chemical modifiers with good electric contact between metalloprotein and metal electrode.^{27, 31-32} We found that the adsorption of the LH1-RC on the SAMs depend on the termination group and methylene length of the alkanthiols.¹⁷ This issue is addressed in the present study.

Here, we report the use of AFM to obtain topographs in combination with electrical measurements with RCs from *Rhodobacter (Rb.) sphaeroides* laid down on a chemically modified Au(111) electrode as shown in Figure 1. We have tested several different types of chemical modifiers (Figure 1a) of the Au(111) electrodes and investigated how these affect the stability and the current-voltage characteristics of RCs using near-IR spectroscopy and scanning probe analyses. In our experiments Au(111) was used as coating metal for both the substrate and the probe to try to obtain electrodes with very similar work functions. If the metals used as top and bottom electrodes have different work functions this will, by itself, lead to an asymmetric *I-V* curve. Electrodes with similar work functions, therefore, allowed the detailed investigation of the electron injection barrier between the RCs and the Au electrode. Our aim was to try and derive the fundamental physical parameters that relate the electric functionality of the RCs to the properties of their redox carriers, requiring higher currents at single molecular level.³³ And we have examined chemically modified Au-coated probe to increase the current of RCs on the electrode using CAFM.

2. Materials and Methods

2.1. Preparation of the RCs

The RCs were isolated from the purple bacterium *Rb. sphaeroides* strain

PUC705BA as previously described.³⁴ The purified RCs were detergent exchanged into n-octyl β -D glucopyranoside in Tris-HCl buffer (10mM, pH 8), and purified further by Sephadex G-100 gel filtration. The final concentration of the RCs was about 1 μ M.

2.2. Preparation of chemical modified Au(111)

Cleaved mica was baked in a vacuum chamber at 2×10^{-4} Pa at 300 °C for 2 h. A 100 nm thickness of gold was epitaxially deposited onto this prebaked mica with an evaporation rate of 0.3-0.4 nm/sec.³⁵ Annealing was then carried out at 530 °C for 10 hours in a vacuum chamber. The presence of a wide, atomic flat terrace over an area of 200 nm x 200 nm area was confirmed using STM. After hydrogen flame annealing and immediate quenching into pure water, the Au(111) substrates were rinsed in ethanol and dried with N₂ gas. The Au(111) substrates were then immersed in a 1 mM solutions (in distilled ethanol) of one of the following, mercaptoacetic acid (HSCH₂COOH, MAC), 2-mercaptoethanol (HSCH₂CH₂OH, 2ME), 4-mercaptopyridine (C₅H₅NS, 4MP), and 2-mercaptopyridine (C₅H₅NS, 2MP) (Figure 1a), for 2 h. All the SAM materials were purchased from Tokyo Kasei Kogyo and used without further purification. After chemical surface modification, the electrodes were rinsed thoroughly with ethanol and dried with nitrogen.

2.3. Preparation of RCs on chemical modified Au(111)

A 100 μ l of a 1 μ M RC solution in 0.8% OG (10mM Tris-HCl buffer, pH 8) was cast by syringe onto each SAM-modified Au substrate (2cm x 1cm) for the near-IR absorption measurements. After casting, the samples without rinsing by water were placed in vacuum to dry for 4 hours in the dark. The chemically-modified Au(111)

substrates for the AFM measurements were incubated with a 1 μ M RC solution in 5 mL for 10 min in the dark. After rinsing thoroughly with distilled water to remove detergent and extra RCs for about 10 min, the substrates were again dried under nitrogen.

2.4. Near IR Absorption spectra of RC

For the near-IR absorption measurements transparent Au-deposited glass substrates

were used. 100 μ l of 1 μ M RCs were cast onto the SAM-modified gold substrate. Near-IR spectra of these RCs in the presence of a reducing agent, 20mM L-ascorbic acid in 1 mM Tris, pH 7.5, were recorded with a UV-3500 spectrophotometer (Hitachi, Japan).

2.5. Cyclic voltammogram

CV of ferricyanides for 2ME, MAC, 2MP and 4MP self-assembled onto a gold

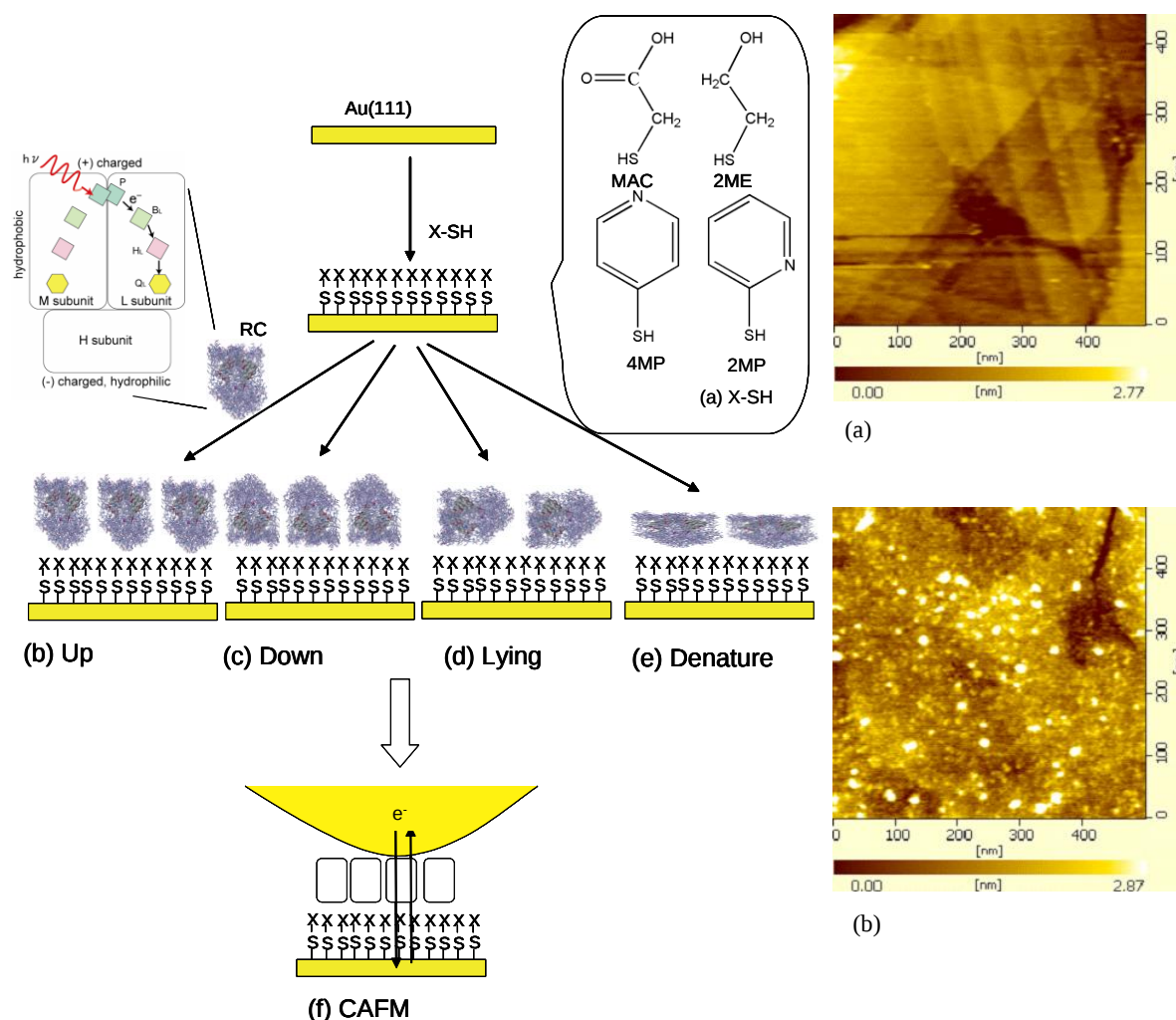


Figure 1. (a) The chemical structures of MAC, 2ME, 4MP and 2MP. (b) CAFM applied with RCs sandwiched between two chemically modified Au electrodes. (c) Illustration of the structure and the light-induced electron transfer pathway in RCs from *Rb. sphaeroides*.

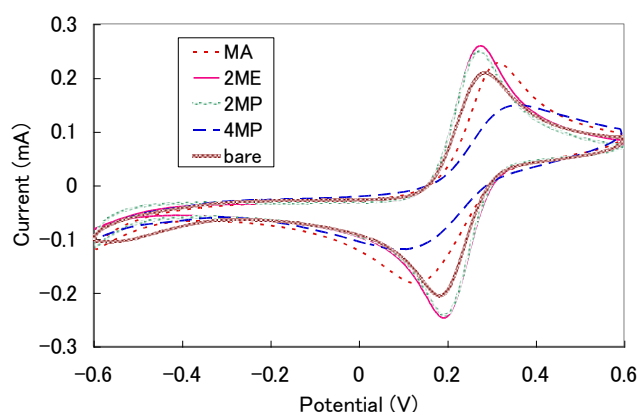
Figure 2. (a) AFM topograph of a 2MP-modified Au(111) substrate with gold coated cantilever. (b) AFM topograph (500nm scan) of RCs on a 2MP-modified Au(111) substrate with gold coated cantilever. Insert shows a high-resolution topograph with a 50nm scan.

electrode and a bare gold electrode in 0.1 M phosphate buffer containing 1 mM $K_3[Fe(CN)_6]$ and 0.1 M $NaClO_4$. The gold electrodes (purchase from Furuya Metal Co. Ltd. Roughness = 10-20 nm / 10 x 10 μm^2) were cleaned by Ozone.

2.6. AFM Imaging and CAFM *I-V* Measurements

AFM images were recorded with an SPI-3800 controller and SPA-300HV unit (SII Nanotechnology, Japan). SiN cantilevers, with a spring constant of 0.09 N/m, were used. The Au/Cr-coated SiN cantilevers (OLYMPUS) had a spring constant of 0.025 N/m and typical tip radius of 40 nm. The 2MP-modified Au-coated probes were obtained by immersion in SAM solution (distilled ethanol) for 2h, followed by rinsing in ethanol and drying under N_2 gas. Current was monitored with a highly sensitive current-voltage amplifier connected to the conductive tip.

The AFM imaging and CAFM *I-V* measurements were carried out under vacuum (5×10^{-5} Pa) at room temperature. We looked for areas of densely packed single RCs by searching for 5-20 nm diameter grains by AFM imaging. When such an area was found, the each conductivity of grains was measured. A ramp bias voltage, with a typical sweep time of 100 ms, was applied between the conductive tip and the lower Au(111) substrate. A typical applied cantilever force was 1 nN. Each CAFM *I-V* curve was obtained by averaging 40 individual scans.



2.7. Au nanoparticle array for checking radius of 2MP-modified probe

A Au nanoparticle array on silicon was prepared by adsorption of Au nanoparticles onto a poly(N-dodecyl acrylamide-co-4-vinyl pyridine, p(DDA/VPy)) Langmuir-Blodgett (LB) film.³⁶

3. Results and discussion

3.1. Chemical modification of Au(111) by SAM and RC adsorption on this substrate

Figure 2a shows AFM topographs, taken in vacuo (5×10^{-4} Pa), of a 2MP-modified Au(111) substrate, obtained with a bare Si probe. The SAM with 2MP has the tendency to cause aggregation with long dipping times (>6h). It was shown that this aggregation is not observed on the Au(111) substrate with shorter dipping times of ~ 2 h. This aggregation affects the surface roughness of the chemically-modified Au(111) substrate. Similar results were observed for MAC, 2ME and 4MP.

Figure 3 shows CV of ferricyanide using a gold electrode modified with these SAM molecules under 2h dipping condition. Good conductivity for ferricyanide was observed with Au electrode modified by 2MP as well as by 2ME and with bare Au using CV measurement (Figure 5), implying that the 2MP little packs onto the gold electrode for electron transfer.

Figure 2b shows AFM topographs,

Figure 3. Cyclic voltammograms of ferricyanides for 2ME, MAC, 2MP and 4MP self-assembled onto a gold electrode and a bare gold electrode in 0.1 M phosphate buffer containing 1 mM $K_3[Fe(CN)_6]$ and 0.1 M $NaClO_4$. The gold electrodes (purchase from Furuya Metal Co. Ltd. Roughness=10-20 nm/10x10 mm²) were cleaned by Ozone. The substrates were soaked in 1mM 2ME, MA, 2MP and 4MP.

Table 1. Current and rectification ratio of RCs at ± 1 V on various SAM modified Au(111) substrates

	Bpheo		Accessory BChl <i>a</i>		SP	
	Peak	FWHM	Peak	FWHM	Peak	FWHM
RC solution	756.6	41.7	802.4	26.3	863.3	65.6
RC solution (ox.)	757.0	39.7	802.6	27.9	860.8	77.3
RC on MAC	757.7	40	802.7	28.7	860.3	51
RC on 2ME	756.9	41.5	802.6	26.7	857.2	52.6
RC on 4MP	757.1	38.3	802.5	27.5	862.5	60.9
RC on 2MP	756.8	38.4	802.6	25.3	862.3	53.1

recorded in vacuo (5×10^{-4} Pa), of RCs adsorbed on a 2MP-modified Au(111) substrate, obtained with an Au-coated Si probe. The surface roughness of the RC films was 2.87 nm, which was smaller than the size of a single RC complex.^{37, 38} Dense grains with various diameters of 5-20 nm, which corresponded to aggregates of up to 10 RCs, were observed in all the topographs of the RCs adsorbed onto all the different SAM-modified Au(111) substrates.

We measured AFM morphology by time course of incubation of RC, 1 min, 2 min, 5 min, 10 min, 2 h, 6 h, 12 h and 24 h to obtain better AFM imaging quality. During 1 - 5 min, the morphology was unstable by scratching of AFM tip. When the incubation time was 10 min, the densely grains of RC was clearly observed as shown in Figure 2. After 2 h, the roughness was over 12 nm, indicating that the multilayer was made. Thus the suitable incubation time was 10 min.

Figure 4 shows the near IR absorption spectra of the RCs in solution and on the chemically-modified Au substrates in the presence of the mild reducing agent, 20 mM L-ascorbic acid. The RC on the substrate was in vacuo after adsorption on the substrate and then IR absorption spectra was measured. The well known RC absorption peaks at $\lambda=760$ nm and $\lambda=800$ nm were observed for the RC's cast on the 2ME-,

2MP-, and 4MP-modified Au (111) substrates, just as is seen with the RCs in solution. These absorption bands come from the bacteriopheophytin (H) and accessory bacteriochlorophyll (BChl *a*) molecules in the RCs, respectively.³⁹ Table 1 summarizes peak top wavelength (λ nm) and full width at half maximum (FWHM). As is apparent from Table 1, the peaks were not broader for using 2MP, 4MP and 2ME, respectively, indicating that the pigments were not decoupling in the RC on the SAM-Au substrate. Interestingly, the $\lambda=860$ nm peak, which is due to the BChl special pair (P) in the RC, was not observed in near-IR spectra of RCs on the SAM modified Au(111) substrates before they were dipped in the reducing agent. Clearly placing the RCs onto the gold substrate oxidizes them. The presence of these three near-IR absorption peaks clearly indicates that the RCs were native on the 2MP, 4MP, and 2ME-modified gold substrates after in vacuo (Figure 1b-d). While, RC on the MAC-modified Au might be slightly denatured (Figure 1e). In the absence of the SAM material on the Au(111) surface the RCs were not adsorbed.

3.2. I-V curves of RCs on the chemically modified Au(111)

Current-voltage measurements with Au-coated probes were determined in vacuo at room temperature on grains with diameters of 5-20 nm i.e. single RCs (Figure 2b inset). Gold was used as coating metal for both the substrate and the probe to try to obtain electrodes with very similar work functions. If the metals used as top and bottom electrodes have different work functions this will, by itself, lead to an asymmetric *I-V* curve. Electrodes with similar work functions, therefore, allowed the detailed investigation of the electron injection barrier between the RCs and the Au electrode. The *I-V* curves were obtained by averaging 40 individual *I-V* curves taken from different grains selected at random. Before the acquisition of every *I-V* curve the applied force of the AFM probe was increased so as to be able to measure a detectable current. It was noted, however, that increasing this applied force from 1nN to 5nN, between the tip and the RCs, resulted in an unsteady current. The suitable incubation time for *I-V* measurements were 10 min as well as imaging condition. When the incubation time was over 2h, the current was not observed for *I-V* curve due to the low conductivity. This also suggests that the

multilayer was made. Semiconductor like *I-V* for SAM of 2MP without the RCs was obtained and the current was 0 between ± 0.2 V and approximately 80 nA at 1 V using Au cantilever, indicating that the rectification comes from RC. The effective current path mediated by the 4 redox components (RSs) in the RCs may be, therefore, sensitive to protein deformation. Zhao et. al.⁴⁰ have observed such a pressure dependent current with a metalloprotein that includes azurin as an RS.

Figure 5 shows the measured current as a function of the applied voltage between the substrate and tip. The adsorbed RCs displayed different *I-V* characteristics, depending on the type chemical modifier. Table 2 summarizes this data with measured currents at ± 1 V and gives the rectification ratios. The current and the rectification ratio were the lowest at using MAC, SAM using MAC forms a negatively charged surface and this results in the denaturation of the RCs, as shown by their at Near-IR spectra (Figure 4). In this case the *I-V* curve was symmetric at about $V = 0$. The current of 570pA at 1V from RCs mediated by 2MP was the best and about twenty times higher than with the RCs mediated by 4MP.

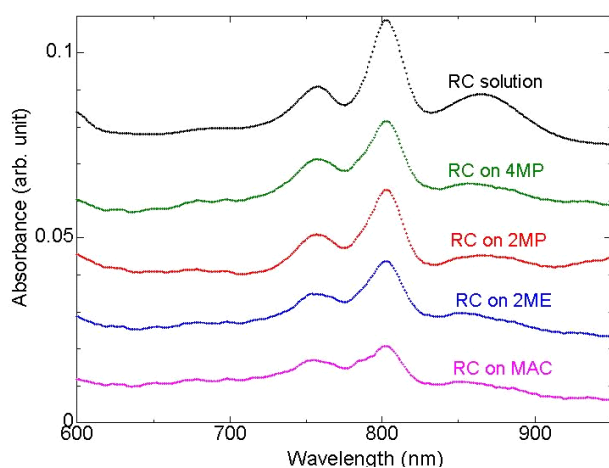


Figure 4. The near-IR spectra of RCs in solution and immobilized on MAC, 2ME, 4MP and 2MP-modified Au substrates. Each spectrum is shown with an offset. The spectrum of RC s in solution is shown at a scale of one-fiftieth.

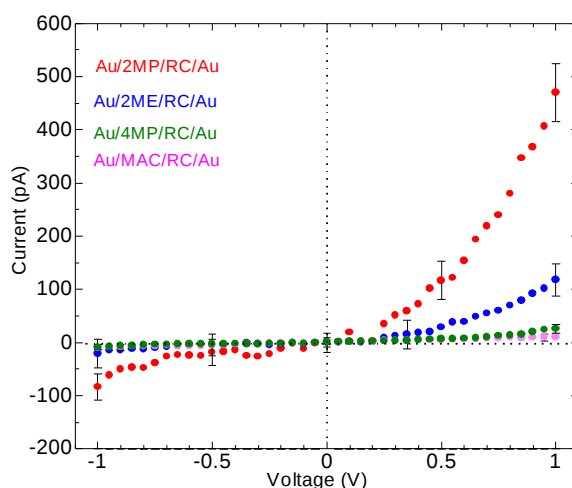


Figure 5. Four *I-V* graphs of RCs each with differently chemically-modified surfaces; substrate/SAM/RC/probe: Au/2ME/RC/Au, Au/MAC/RC/Au, Au/2MP/RC/Au and Au/4MP/RC/Au. Error bars indicate the variability of the 40 *I-V* curves.

I-V graphs of films with oriented RCs on 2ME, 2MP, and 4MP-modified Au(111) substrates showed an asymmetric conductance between the positive and negative bias voltages. This contrasts with the symmetric *I-V* graphs measured for the RCs on a MAC-modified substrate. In the former cases the current increases on reverse bias and remains small in forward bias. Current rectification by organic molecules has been reported in the literature for zwitterionic molecules,⁴¹ phythalocyanine molecules⁴² and photosynthetic RCs from plants.²⁸ In the case of photosystem I (PS I) from plants the current-rectifying *I-V* curve, obtained with STM, has been attributed to the 80% orientation of the PSI complexes on the surface using 2ME as SAM molecule²⁸ and has, therefore, been used to indicate the orientation with which the complex preferentially adsorbs on the surface.²⁸ The results were similar to our results. However, the current is not comparable because the PS I is measured by STM. The tip of STM is quite different, radius and shape with those of CAFM. Stamouli et al. note that the proteoliposome of RC immobilized on HOPG and the current was measured by CAFM. The rectification by *I-V* measurements is also similar (They represent the applied voltage for tips.). Lukins demonstrates high resolution of PSII by STM.⁴³ However, the rectification of PSII is not observed by STM/scanning tunneling spectroscopy (STS). Generally, STM can not control the distance between the tip and the sample during STS measurement and thus, the discussion of results becomes complicated because of the low conductivity and heterogeneity of these proteins surface.²⁹

The current-voltage curves of RCs sandwiched between two electrodes measured here clearly demonstrate that these proteins do conduct electrons. There are various models to account for electron transfer in organic systems. Firstly, direct tunneling from one electrode (tip) to the other. This source of charge propagation can

be simply ruled out due to the excessively large tunneling distances that would be involved in this case⁴⁴ e.g. 7.3 nm across the RC. An alternative type of electron transfer is via electronic states existing in the molecule sandwiched between the two electrodes. These could be localized or delocalized over the entire molecule. Since the X-ray crystal structure of the RC has been described, this information can be used to suggest a possible molecular electron transfer pathway (Figure 6).^{37, 38} In this study, the direction of the rectification in the cases of the 2ME, 2MP, and 4MP- modified Au(111) electrode SAM, suggests that the RCs from *Rb. sphaeroides* are preferentially adsorbed at H subunit (Figures 1b and 6a) and that the electron from gold coated probe transfers to the chemically modified Au(111) substrate by the pathway shown in

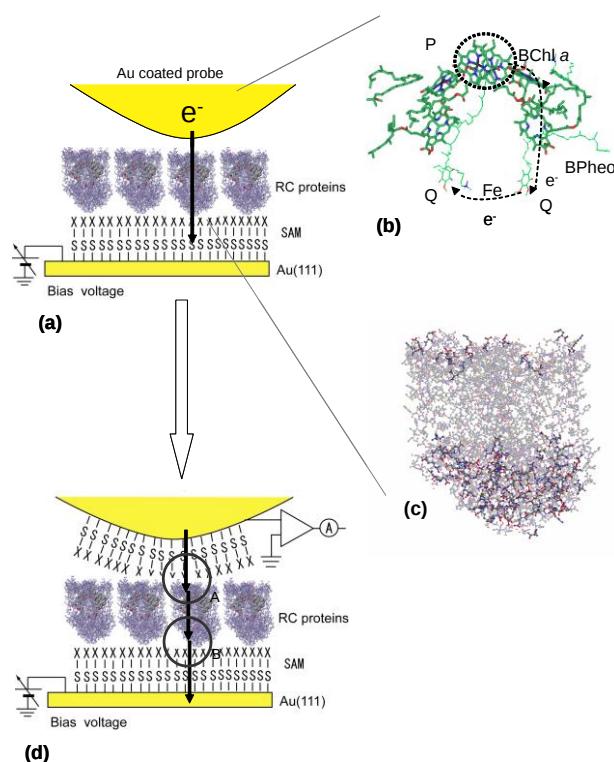


Figure 6. Schematic illustration of C-AFM and electron transfer mechanism. (a) bare Au cantilever for C-AFM. (b) electron pathway of pigments in RC (c) hydrophilic amino acid residues of RC are high-lighted. (d) SAM modified Au cantilever for C-AFM. Circle A: electron injection from cantilever to P in RC. Circle B: electron injection from RC to Au(111).

Table 2. Current and rectification ratio of RCs at ± 1 V on various SAM modified Au(111) substrates
a. A RC anchored perpendicular down and up (up as in Figure 1b) to Au(111).
b. Using a 2MP-modified Au-coated Si probe.

SAM molecules	I (pA)		Rectification ratio, %
	-1 (V)	+1 (V)	
	down ^a	up ^a	$ up /(down + up)^a$
MAC	-10	10	50.0
2ME	-20	120	85.7
2MP	-80	570	87.7
4MP	-8.0	25	75.8
2MP-2MP ^b	-150	1000	87.0

Figure 6a-b (and see below). Figure 6c illustrate the highlight of hydrophilic amino acid residues in the RC and many hydrophilic amino acids were found in H subunit. This implies that the RCs are physisorbed at H subunit on the substrate.

To estimate a possibility of the electron transfer mediated by quinone, the CV were measured in the presence of dichlorobenzoquinone (DCBQ) such a stable quinone in stead of native quinone. 2MP modified Au electrode could have the redox response of dichlorobenzoquinone (DCBQ) reversibly, however, the other electrodes could not have the redox response (Figure 7). This implies that the 2MP plays a role as a mediator between the quinone and the Au substrate.

Other possible reasons for the enhanced current with 2MP are (a) better orientation of the RCs and (b) lowering of the electron injection barrier. For efficient electron injection into proteins, the work function of the electrode has to match the energy level of the lowest unoccupied molecular orbital (LUMO) or highest occupied molecular orbital (HOMO) of the conducting species. Tuning of the metal work function with SAM enables the current density in organic solar cells to be increased,⁴⁵ as demonstrated by several studies using of π -conjugated

thiols to decrease the injection barrier. If the I - V measurements are performed on RCs sandwiched between a 2MP-modified Au(111) substrate and a 2MP-modified Au-coated probe (Figure 6d), then in principle the effect of lowering the carrier injection barrier can be distinguished from the effect of orientation of the RCs.

3.3. I - V curves measured with a 2MP-modified probe

The tip radius of the chemically-modified probe was evaluated by the surface observation of an Au nanoparticle array that had an uniform size (30nm-diameter as determined by Transmission Electron Microscope (TEM)) (Figure 8).³⁶ If the tip radius becomes too large this will increase the contact area between tip and RCs, which will cause an apparent enhancement of the measured current.

Figure 9a shows the AFM topograph of this Au nanoparticle array using a chemically-modified probe that was made with a dipping time of 2h. A clear image of the 30nm-diameter particles was obtained. Similar images were measured with the Au-coated probe before chemical modification.

Figure 8a shows AFM topographs, measured in vacuo (5×10^{-4} Pa), of RC complexes adsorbed on a 2MP-modified Au(111) substrate, obtained with a 2MP-modified Au-coated Si probe. The resolution is the same as using an unmodified Au-coated Si Probe. These results indicate that the tip radius is about 30 nm. There was, therefore, no increase in the contact area between probe and sample surface due to the chemical modification. Au-coated probes with tip radii of 10-20 nm have often been used in previous CAFM measurements of monolayers.^{40, 46} The tip radius of the chemically-modified probes used in our study are, therefore, only a little larger than those used by other groups.

Stable *I-V* curves were obtained using a 2MP coated Au-coated probe prepared with a dipping time of 2h. Using chemically-modified probes prepared with shorter dipping times (<1 h) the AFM images were similar but the CAFM current though RCs became quite unstable. The *I-V* curve of RCs sandwiched between a 2MP-modified Au(111) substrate and a 2MP-modified Au-coated probe is shown in Figure 9b and compared with the *I-V* curve obtained using an Au-coated probe alone. The current value of over 1 nA at 1 V with the 2MP-modified probe was twice as high than with the Au-coated one. This is a striking enhancement of the current, where the current rectification behavior was very similar to that seen using the unmodified Au-coated probe and the 2MP-modified Au(111) surface, implies that 2MP has an important role in lowering the electron injection barrier between RCs and the Au-coated electrode at both the probe (Figure 6d circle A) and the substrate (Figure 6d circle B) interface.

In nature, electron transfer takes place in photosynthetic RCs.⁴⁷ In Figure 6b the 3D structure of the RCs and their electron transfer pathway are shown. The BChl dimer P, a monomeric BChl, a bacteriopheophytin (H) and a quinone (Q) are the necessary electron transfer

components. Direct excitation with light or, indirectly, by resonance energy transfer from an antenna complex first promotes an electron on P to an excited singlet state. Electrons are then transferred along the chain of pigments associated with the L-subunit of the RC i.e. from P to BChl_L (0.47 nm transfer distance), to H_L (0.38 nm transfer distance) and finally to Q_L (0.9 nm transfer distance).

Higher currents were only seen with applied positive voltages to Au(111) substrate (negative ones to the AFM tip). This would be consistent with the RCs having their cytoplasmic side (H chain) facing the substrate. This observed preferential orientation of RCs probably results from the surface character of SAM-modified Au(111) substrate. SAM modified Au(111) substrates, modified with 2ME, 4MP or 2MP form a hydrophilic surface. Adsorption of the RCs onto these SAM-modified substrates would favor an interaction via the main hydrophilic surface of RCs that is the H chain (Figure 6c). This would then present the top of the L and M subunits to the AFM tip.²⁸ It is tempting to propose that the electron pathway mentioned

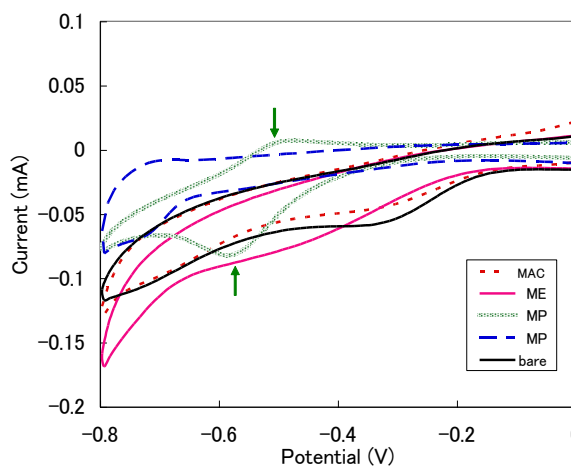


Figure 7. Cyclic voltammograms of dichlorobenzoquinone (DCBQ) for 2ME, MA, 2MP and 4MP self-assembled onto a gold electrode and a bare gold electrode in 0.1 M phosphate buffer containing DCBQ and 0.1 M NaClO₄. The gold electrodes were cleaned by Ozone. The substrates were soaked in 1mM 2ME, MA, 2MP and 4MP.

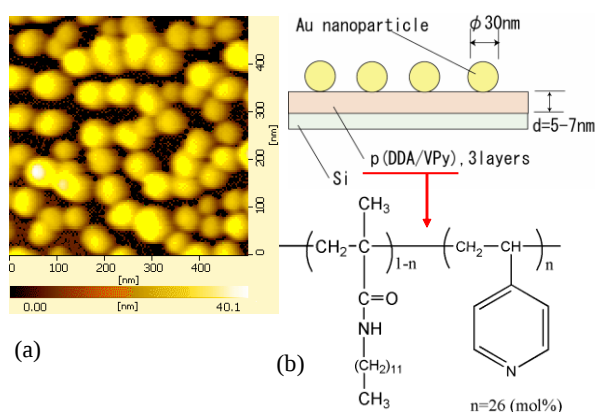


Figure 8. (a) AFM topograph of the Au nanoparticle array using a 2MP-modified probe produced by dipping in a SAM solution for 2h. (b) The structure of the Au nanoparticle array.³³

above also operates in this study. Electrons are injected into P via the AFM tip at positive voltage and electrons are then transferred along the pigments associated with the L-subunit of the RC from P to BChl_L, to H_L and finally to Q_L (Figure 6b). The reduced Q_L could then be directly oxidized by the substrate under the influence of the applied voltage. In this study, modification by 2MP is useful for the electron transfer due to not orientation of RC but lowering electron injection barrier. The rate constants for electron transfer in the case of *Rb. sphaeroides* have been determined. The rates for back electron transfer (Q_L to H_L to B_L to P) are significantly lower than the forward ones.⁴⁸ This could then explain the difference that lies at the root of the observed asymmetry in the current output between the applied positive and negative voltages.⁴⁹ Cyt-c or azurin show step like *I-V* curves due to a double tunneling junction (DTJ) of the RS in the protein sandwiched by probe and metal substrate, via an insulator.⁴⁶ This stepwise behavior was not observed here in *I-V* curves of the RCs.

The Fermi level of both electrodes (AFM tip and substrate) is the same, therefore, the rectifying behavior is most probably either an intrinsic property of the RCs themselves or due to an interfacial property between RCs and the metal

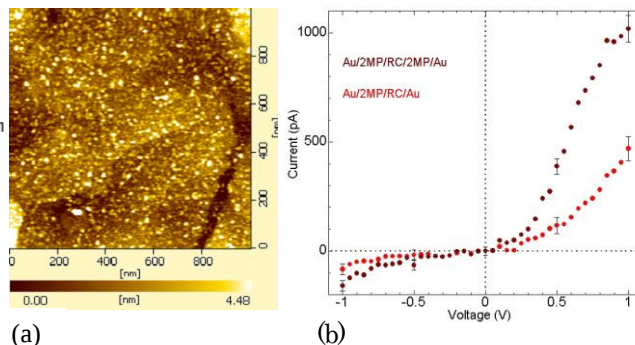


Figure 9. (a) AFM topograph of the Au nanoparticle array using a 2MP-modified probe produced by dipping in a SAM solution for 2h. (b) The structure of the Au nanoparticle array.³³

electrode. The RCs were oxidized on the substrate, observed from the peak of the absorption spectra, indicating that the Au substrate is electron withdrawing for RCs. However, in this case, Fermi level is very near among the substrate and the tip and thus, we conclude the charge transport is not much different compared with the reduced form of RC because the cantilever is also coated with gold. The possibility cannot be discounted, however, that the observed asymmetry in the measured *I-V* curves is due to the formation of a Schottky barrier.⁵⁰ Schottky barriers are formed when a metal is brought in physical contact with a semiconductor. At the interface, the Fermi level of the semiconductor is pinned by defects, or interfacial states, and the overall electrical properties of the device are then dominated by the resulting Schottky barrier height. There is still considerable controversy about the interface-density distribution at metal/protein interfaces, especially in terms of essential factors that determine the barrier height.⁴⁶ If the direction of current rectification of the RCs could be controlled by their orientation, while keeping the same electrode geometry, then the proposed presence of an inherent rectification by the RCs would be verified.⁵¹ This, however, is a challenging problem as well as single molecular level imaging.

Our goal is measurement of the step-like *I-V* curve expected by theory for the possible future prospects of the results.³³ RC has 4 redox species eg. P, accessory BChl *a*, bacteriopheophytin and quinone. The redox state should be observed by *I-V* measurement method. For this goal, the resolution for these proteins should be up to 1 molecule level and the observation of current should be up to 10 times. Our finding of efficient electron transfer of 2MP is very useful for the orientation and lower electric barrier between the solid substrate and RC for both P side and Q side interface of RC. Synchronous of electron transfer between input and output of RC is the most difficult one,⁵² since the excessive electron and hole may destroy RC. Thus, further explore of SAM molecules is important in constructing an artificial device using RC. The trial device of RC is already made by SAM method with C60²⁷ or cytochrome *c*⁵². We recently use LH1-RC for building devices since the LH1 enhances the stability of RC as well as the photosensitizer effect of the LH complex.¹⁴⁻¹⁷

In conclusion, using CAFM we have measured the electrical conduction properties of RCs sandwiched between a Au-coated probe and a Au(111) substrate in vacuo. The modification of the Au(111) substrate with the π -conjugated thiol, 2MP, was effective in both promoting the specific orientation of the RCs on the electrode and enhancing of the current. Higher currents at positive voltages applied to the Au(111) substrate and negative ones applied to the AFM tip imply that RCs have their cytoplasmic side (H chain) facing the substrate as described above. CV data indicate that the 2MP play as mediator between quinone in RC and the gold substrate. We found also that 2MP modification of both the Au-coated probe and the Au(111) substrate was even more effective in promoting electron injection into the RC, in the presence of an appropriately directed external bias voltage between the upper probe and the substrate. These

properties were strongly supported by CV. 2MP is therefore, a better chemical modifier for Au(111) with which to measure CAFM of single RCs.

ACKNOWLEDGMENT: This study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, the BBSRC (RJC) and AOARD-07-4013.

REFERENCES and NOTE

1. J. Deisenhofer, O. Epp, K. Miki, R. Huber and H. Michel, *Nature* 318, 618 (1985).
2. M. H. Vos, F. Rappaport, *Nature* 363, 320 (1993).
3. G. R. Fleming, J. L. Martin, and J. Breton, *Nature*, 333, 190 (1988).
4. G. McDermott, S. M. Prince, A. A. Freer, A. M. Hawthornthwaite-Lawless, M. Z. Papiz, R. J. Cogdell, N. W. Isaacs, *Nature*, 374, 517 (2002).
5. A. W. Roszak, T. D. Howard, J. Southall, A. T. Gardiner, C. J. Law, N. W. Isaacs and R. J. Cogdell, *Science* 302, 1969 (2003).
6. J. Matsui, M. Mitsuishi, A. Aoki, T. Miyashita, *Angew. Chem. Int. Ed.* 42, 2272 (2003).
7. M. Nagata, M. Nango, A. Kashiwada, S. Yamada, S. Ito, N. Sawa, M. Ogawa, K. Iida, Y. Kurono and T. Ohtsuka, *Chem. Lett.* 32, 216 (2003).
8. M. Nagata, Y. Nakamura, E. Nishimura, K. Nakagawa, Y. Suemori, K. Iida and M. Nango, *Trans. MRS-J* 30, 655 (2005).
9. J. Matsui, S. Yoshida, T. Mikayama, A. Aoki and T. Miyashita, *Langmuir* 21, 5343 (2005).
10. J. Matsui, K. Abe, M. Mitsuishi and T. Miyashita, *Mol. Cryst. Liq. Cryst.* 424, 187 (2004).
11. M. Ogawa, R. Kanda, T. Dewa, K. Iida and M. Nango, *Chem. Lett.* 31, 466 (2002).

12. K. Iida, J. Inagaki, K. Shinohara, Y. Suemori, M. Ogawa, T. Dewa and M. Nango, *Langmuir* 21, 3069 (2005).
13. K. Iida, A. Kashiwada and M. Nango, *Colloids Surf. A* 169, 199 (2000).
14. M. Ogawa, K. Shinohara, Y. Nakamura, Y. Suemori, M. Nagata, K. Iida, A. T. Gardiner, R. J. Cogdell and M. Nango, *Chem. Lett.* 33, 772 (2004).
15. Y. Suemori, M. Nagata, Y. Nakamura, K. Nakagawa, A. Okuda, J. Inagaki, K. Shinohara, M. Ogawa, K. Iida, T. Dewa, K. Yamashita, A. T. Gardiner, R. J. Cogdell, M. Nango, *Photosynth Res.* 90, 17 (2006).
16. Y. Suemori, K. Fujii, M. Ogawa, Y. Nakamura, K. Shinohara, K. Nakagawa, M. Nagata, K. Iida, T. Dewa, K. Yamashita, M. Nango, *Colloids Surf. B* 56, 182 (2007).
17. M. Kondo, Y. Nakamura, K. Fujii, M. Nagata, Y. Suemori, T. Dewa, K. Iida, A. T. Gardiner, R. J. Cogdell and M. Nango, *Biomacromolecules* 8, 2457 (2007).
18. T. Dewa, T. Yamada, M. Ogawa, M. Sugimoto, T. Mizuno, K. Yoshida, Y. Nakao, M. Kondo, K. Iida, K. Yamashita, T. Tanaka and M. Nango, *Biochemistry* 44, 5129 (2005).
19. T. Dewa, R. Sugiura, Y. Suemori, M. Sugimoto, T. Takeuchi, A. Hiro, K. Iida, A. T. Gardiner, R. J. Cogdell and M. Nango, *Langmuir* 22, 5412 (2006).
20. S. Scheuring, F. Reiss-Husson, A. Engel, J.-L. Rigaud, and J.-L. Ranck, *EMBO J.* 20, 3029 (2001).
21. S. Scheuring, J. Seguin, S. Marco, D. Levy, R. Bruno and J.-L. Rigaud, *Proc. Natl. Acad. Sci. USA* 100, 1690 (2003).
22. S. Scheuring, J. N. Sturgis, V. Prima, A. Bernadac, D. Lèvy and J.-L. Rigaud, *Proc. Natl. Acad. Sci. USA* 101, 11293 (2004).
23. D. Fotiadis, P. Quian, A. Philippsen, P. A. Bullough, A. Engel and C. N. Hunter, *J. Bio. Chem.* 279, 2063 (2004).
24. S. Bahatyrova, R. N. Frese, K. O. van der Werf, C. Otto, C. N. Hunter, J. D. Olsen, *J. Bio. Chem.* 279, 21327 (2004).
25. S. Bahatyrova, R. N. Frese, C. Siebert, J. D. Olsen, K. van der Werf, R. van Grondelle, R. A. Niederman, P. A. Bulloch, C. Otto and C. N. Hunter, *Nature (London)* 430, 1058 (2004).
26. A. Stamouli, S. Kafi, D. C. G. Klein, T. H. Oosterkamp, J. W. M. Frenken, R. J. Cogdell and T. J. Aartsma, *Biophysical Journal* 84, 2483-2491 (2003).
27. R. Das, P. J. Kiley, M. Segal, J. Norville, A. A. Yu, L. Wang, S. A. Trammell, L. E. Reddick, R. Kumar, F. Stellacci, N. Lebedev, J. Schnur, B. D. Bruce, S. Zhang and M. Baldo, *Nano Lett.* 4, 1079 (2004).
28. I. Lee, J. W. Lee and E. Greenbaum, *Phys. Rev. Lett.* 79, 3294 (1997).
29. A. Stamouli, J. W. N. Frenken, T. H. Oosterkamp, R. J. Cogdell and T. J. Aartsma, *FEBS Letters* 560, 109 (2004).
30. B. D. Reiss, D. K. Hanson, M. A. Firestone, *Biotechnol. Prog.* 23, 985 (2007).
31. I. Taniguchi, H. Ishimoto, K. Miyagawa, M. Iwai, H. Nagai, H. Hanazono, K. Taira, A. Kubo, A. Nishikawa, K. Nishiyama, Z. Dursun, G. P.-J. Hareau and M. Tazaki, *Electrochem. Commun.* 5, 857 (2003).
32. T. Mikayama, K. Iida, Y. Suemori, T. Miyashita and M. Nango, *Mol. Cryst. Liq. Cryst.*, 445, 291 (2006).
33. H. Sumi, *Chem. Phys.* 222, 269 (1997).
34. M. Y. Okamura, L. A. Steiner and G. Feher, *Biochemistry* 13, 1394 (1974).
35. M. Levlin, A. Laakso, H. E.-M. Niemi, and P. Hautojarvi, *App. Sur. Sci.* 115, 31 (1997).
36. H. Tanaka, M. Mitsuishi, T. Miyashita, *Langmuir* 19, 3103 (2003).
37. C.-H. Chang, O. El-Kabbani, D. Tiede, J. Norris, M. Schiffer, *Biochemistry* 30, 5352 (1991).
38. G. Katona, U. Andreasson, E. M. Landau, L.-E. Adreasson, R. Neutze, *J. Mol. Biol.* 331, 681 (2003).
39. R. E. Blankenship, M. T. Madigan, C. E. Bauer, *Anoxygenic Photosynthetic Bacteria.* ed.; Kluwer Academic Publishers (1995).
40. J. Zhao, J. J. Davis, M. S. P. Sansom, A. Hung, *J. Am. Chem. Soc.* 126, 5601 (2004).
41. A. S. Martin, J. R. Sambles, G. Ashwell, *Phys. Rev. Lett.* 70, 218 (1993).

42. C. C. Page, X. Chen, C. C. Moser, P. L. Dutton, *Nature* 402, 47 (1999).
43. P. B. Lukins, *Chem. Phys. Lett.* 321, 13 (2000).
44. M. Pomerantz, M. R. A. Aviram, L. Li, A. G. Schrott, *Science* 255, 1115 (1992).
45. B. D. Boer, A. Hadipour, M. M. Mandoc, T. v. Woudenberg, P. W. M. Blom, *Adv. Mater.* 17, 621 (2005).
46. J. J. Davis, C. L. Wrathmell, J. Fletcher, *J. Mol. Recognit.* 17, 167 (2004).
47. B. Ke, *Photosynthesis*; Govinjee; Kluwer Academic Publishers (2001).
48. V. P. Shinkarev, C. A. Wraigt, *The Photosynthetic Reaction Center*. ed.; Academic Press: New York, 11, 193 (1993).
49. The apparent conductance could be calculated under literature of Adams et al.⁵³ That shows estimation of relations between electron transfer rate and conductance. The electron transfer rate $k=k(\text{tunneling})$ should be calculated by electrochemical method (CV). Unfortunately, we could not measure the redox potential of RC (data not shown). We can just calculate the apparent conductivity from *I-V* measurements (10^{-9} W^{-1}). The apparent conductance (*g*) was calculated by following:
 $g \sim 5 \times 10^{-19} k_{\text{et}}/\text{DOS} = 1.5 \times 10^{-13} (\text{DOS} = \text{FCWD from eq. 2.23 by Adams et al.}^{53})$.
The electron transfer rate, $k = 10 \text{ s}^{-1}$ was by Kong et al.,⁵⁴ where *k* was obtained when RC was immobilized by poly(ethyleneimine). However, RCs have 4 redox sites among tip and substrate, and 2-MP SAMs were not well characterized as well as alkan thiol, so far. And this theoretical calculation is under 0 bias volt.
50. H. Ishii, K. Sugiyama, E. Ito, K. Seki, *Adv. Mater.* 11, 605 (1999).
51. R. Akiyama, T. Matsumoto, T. Kawai, *Phys. Rev. B* 62, 2034 (2000).
52. N. Lebedev, S. A. Trammell, A. Spano, E. Lukashev, I. Griva, J. Schnur, *J. Am. Chem. Soc.* 128, 12044 (2006).
53. D. M. Adams, L. Brus, C. E. D. Childsey, S. Creager, C. Creutz, C. R. Kagan, P. V. Kamat, M. Lieberman, S. Lindsey, R. A. Marcus, R. M. Metzger, M. E. Michel-Beyerle, J. R. Miller, M. D. Newton, D. R. Rolison, O. Sankey, K. S. Schanze, J. Yadley, X. Zhu, *J. Phys. Chem. B* 107, 6668 (2003).
54. J. Kong, W. Sun, X. Wu, J. Deng, Z. Lu, Y. Lvov, R. Z. B. Desamero, H. A. Frank, Rusling, J. F. *Bioelectrochemistry and Bioenergetics* 48, 101 (1999)

List of Research Results

Conference presentations

Invited.

1. M. Nango "Construction of Bio-inspired artificial solar energy conversion system" Asia Optical Fiber Communication & Optoelectronic Exposition & Conference (Shanghai, China, October16-19, 2007)
2. M. Nango "Self-assembly of light-harvesting polypeptide / pigment complexes for construction of an artificial photoenergy conversion system" Okazaki Conference (Okazaki, Japan, November10-12, 2007)
3. M. Nango "Self-assembly of light-harvesting polypeptide / pigment complexes for construction of an artificial photoenergy conversion system" 7th International Conference on Tetrapyrrole Photoreceptors in photosynthetic Organisms (Kyoto, Japan, December9-14, 2007)

International Conference

1. T. Sakurai, Y. Suemori, M. Hatasa, O. Goto, K. Iida, T. Dewa, K. Yamashita, H. Tanaka, T. Ogawa, H. Hashimoto, and M. Nango "Assembly of the light-harvesting / reaction center complexes of photosynthetic bacteria onto a gold substrate" 7th International Congress on Tetrapyrrole Photoreceptors in Photosynthetic Organisms (Kyoto, Japan, December9-14, 2007)
2. K. Nakagawa, S. Suzuki, A. Mizuno, R. Fujii, A. T. Gardiner, R. J. Cogdell, H. Hashimoto, M. Nango "Reconstitution of Light-harvesting 1 complex of photosynthetic bacterium *Rhodospirillum rubrum* with the LH1 subunit-type complex and carotenoids and its characterization" 2nd International Workshop of Photosynthetic Antennae and Coherent Phenomena (Osaka, Japan, December14-16, 2007)
3. M. Nango "Self-assembly of light-harvesting polypeptide / pigment complexes for construction of an artificial photoenergy conversion system" 14th International SPACC-CSJ Symposium (Taipei, Taiwan, December20-22, 2007)
4. K. Nakagawa, N. Fukui, T. Nakano, T. Horibe, S. Suzuki, R. Fujii, A.T. Gardiner, R. J. Cogdell, H. Hashimoto, and M. Nango "Probing the Effect of the Binding Site on the Electrostatic Behavior of a Series of Carotenoids Reconstituted into the Light-Harvesting 1 Complex from Purple Photosynthetic Bacterium *Rhodospirillum Rubrum* Detected by Stark Spectroscopy" 15th International Symposium on Carotenoids (Okinawa, Japan, June 22-27th, 2008)
5. N. Fukui, A. Mizuno, T. Nakano, K. Nakagawa, A. T. Gardiner, R. J. Cogdell, R. Fujii, H. Hashimoto, and M. Nango "Reconstitution of Antenna Light-Harvesting 1 Complexes from the Purple Photosynthetic Bacterium *Rhodobacter Sphaeroides* with LH1-Polypeptides, Bacteriochlorophyll *a*, and Carotenoids: Carotenoid Specificity" 15th International Symposium on Carotenoids (Okinawa, Japan, June 22-27th, 2008)
6. T. Nakano, N. Fukui, K. Nakagawa, A. T. Gardiner, R. J. Cogdell, R. Fujii, H. Hashimoto, and M. Nango "Reconstitution of Light-Harvesting 1 Complexes from the Purple Photosynthetic Bacterium *Rhodospirillum rubrum* with Separately Isolated Polypeptides (LH1- α and LH1- β), Bacteriochlorophyll *a*, and Carotenoid" 15th International Symposium on Carotenoids (Okinawa, Japan, June 22-27th, 2008)
7. T. Joke, Y. Takeuchi, S. Ishigure, S. Osaka, T. Dewa, Y. Amao, H. Hashimoto, and M. Nango "Molecular

Assembly of Chlorophyll Complexes on Electrodes for Construction of an Artificial Photoenergy Conversion System” 17th International Conference on Photochemical Conversion and Storage of Solar Energy (Sydney, Australia, July 27-August 1, 2008)

8. M. Hatasa, O. Goto, T. Sakurai, K. Fujii, T. Dewa, K. Iida, H. Hashimoto, and M. Nango “Self-Assembly of Light-Harvesting Complexes on Various Electrodes for Construction of an Artificial Photosynthetic System” 17th International Conference on Photochemical Conversion and Storage of Solar Energy (Sydney, Australia, July 27-August 1, 2008)

Domestic conferences

1. 21th Japan Carotenoid Conference (Osaka, Japan, September 6-7, 2007)
Katsunori NAKAGAWA, Satoshi SUZUKI, Ayumi MIZUNO, Naomi FUKUI, Tsubasa NAKANO, Ritsuko FUJII, Takehisa DEWA, Hideki HASHIMOTO, and Mamoru NANGO “Reconstitution of antenna LH1 complex with carotenoids from purple photosynthetic bacterium and its characterization”
2. 56th SPSJ Symposium on Macromolecules (Nagoya, Japan, September 19-21, 2007)
 - 1) Takashi JOKE, Syuichi ISHIGURE, Yuji KONDO, Yoshito TAKEUCHI, Takehisa DEWA, Keiji YAMASHITA, Yutaka AMAO, and Mamoru NANGO, “Functional Evaluation of Chlorin Derivatives on Dye-Sensitized Solar Cells.”
 - 2) Tsubasa NAKANO, Naomi FUKUI, Katsunori NAKAGAWA, Ayumi MIZUNO, Kouji IIDA, Takehisa DEWA, and Mamoru NANGO, “Reconstitution of bacterial antenna light-harvesting 1 complex with carotenoid.”
 - 3) Mikio HATASA, Yoshiharu SUEMORI, Tomohiko SAKURAI, Osamu GOTO, Takehisa DEWA, Keiji YAMASHITA, and Mamoru NANGO, Kouji IIDA, “Assembly of photosynthetic bacterial light-harvesting protein complex on a substrate and its functional analysis”
 - 4) Toshikazu TAKEUCHI, Ryuta SUGIURA, Akito HIRO, Yoshiharu SUEMORI, Takehisa DEWA, Keiji YAMASHITA, Ryugo TERO, Tsuneo URISU, and Mamoru NANGO “Organization of bacterial photosynthetic light-harvesting complexes in tethered lipid bilayers.”
 - 5) Tomoya KATO, Tsuyoshi OCHIAI, Takahide ASAOKA, Shinichiro OSAKA, Kosuke Shimoyama, Takehisa DEWA, Keiji YAMASHITA, Mamoru NANGO, “Synthesis of photosynthetic light thesis light-harvesting model polypeptides and its assembly with porphyrin derivative.”
 - 6) Akito HIRO, Toshikazu TAKEUCHI, Tsubasa NAKANO, Takehisa DEWA, Keiji YAMASHITA, and Mamoru NANGO, “Self-assembly of maltose-binding protein-based polypeptides with a photosynthetic pigment.”
 - 7) Kaoru FUJII, Ayumi OKUDA, Shuichi ISHIGURE, Yutaka AMAO, and Mamoru NANGO, “Functional assessment of photosynthetic complexes onto an electrode.”
 - 8) Yuji KONDO, Tatsurou MITSUI, Shingo ITO, Takashi JOKE, Yoshito TAKEUCHI, Shuichi ISHIGURE, Masaharu KONDO, Takehisa DEWA, Keiji YAMASHITA, and Mamoru NANGO, “Peroxidation Catalyzed by Chlorophyll derivatives in Lipid Bilayer.”
 - 9) Takahiro OTA, Takuya SUZUKI, Takehisa DEWA, Mamoru NANGO, Keiji YAMASHITA, and

- Yasushi INAGAKI, "Preparation of recycle system by functionalizing waste rubbers."
3. 22th Biorelated Functional Chemistry symposium (Sendai, Japan, September 28-29, 2007)
Ayumi MIZUNO, Katsunori NAKAGAWA, Naomi FUKUI, Tsubasa NAKANO, Kouji IIDA, Takehisa DEWA, Hideki HASHIMOTO, and Mamoru NANGO, "Reconstitution of carotenoids with the LH1 complexes from photosynthetic bacteria; Influence of carotenoids on the assembling property of LH1 complexes."
 4. 88th CSJ Spring Meeting (Tokyo, Japan, March 26-30, 2008)
 - 1) Yoshito TAKEUCHI, Yuji KONDO, Takashi JOKE, Syuichi ISHIGURE, Takehisa DEWA, Keiji YAMASHITA, Mamoru NANGO, "Electron Transfer by Lipid Binding Chlorin Derivatives in Lipid Bilayer."
 - 2) Tsubasa NAKANO, Naomi FUKUI, Katsunori NAKAGAWA, Ayumi MIZUNO, Takehisa DEWA, Keiji YAMASHITA, Mamoru NANGO "Reconstitution of photosynthetic antenna complex using carotenoid pigments effect."
 - 3) Ayumi SUMINO, Akito HIRO, Toshikazu TAKEUCHI, Yuki GOTO, Yoshiharu SUEMORI, Takehisa DEWA, Keiji YAMASHITA, Mamoru NANGO "Incorporation of Membrane Proteins into Supported Lipid Bilayer via Vesicle Fusion and its Direct Observation."
 - 4) Shinichiro OSAKA, Tsuyoshi OCHIAI, Tomoya KATO, SHIMOYAMA, Takehisa DEWA, Keiji YAMASHITA and Mamoru NANGO "Electron transfer of Light-Harvesting Model Polypeptide / Pigment Complexes on Au Substrates."
 - 5) Katsunori NAKAGAWA, Ayumi MIZUNO, Tsubasa NAKANO, Naomi FUKUI, Takehisa DEWA, Ritsuko FUJII, Hideki HASHIMOTO and Mamoru NANGO "Reconstitution of antenna LH1 complex from purple photosynthetic bacterium and its functional analysis of carotenoids."
 - 6) Osamu GOTO, Tomohiko SAKURAI, Mikio HATASA, Takehisa DEWA, Keiji YAMASHITA, Kouji IIDA and Mamoru NANGO "Organizing on substrate of photosynthesis antenna system Protein / pigment complex"

Journal paper publications

1. T. Ochiai, T. Asaoka, T. Kato, S. Osaka, T. Dewa, K. Yamashita, A.T. Gardiner, R. J. Cogdell, H. Hashimoto, and M. Nango "Molecular Assembly of Zn Porphyrin Complexes Using Synthetic Light-harvesting Model Polypeptides" *Photosynth. Res.* **95** 353-361 (2008)
2. T. Ochiai, M. Ota, T. Kato, S. Osaka, T. Dewa, K. Yamashita, H. Hashimoto, and M. Nango "Molecular Assembly of Bchl *a* Complexes onto ITO Electrode Using Synthetic Light-harvesting Model Polypeptides Bearing Spermine Derivative" *Chem. Lett.*, **37** 98-99 (2008)
3. K. Nakagawa, S. Suzuki, R. Fujii, A. Gardiner, R. Cogdell, M. Nango, and H. Hashimoto "Electrostatic effect of surfactant molecules on bacteriochlorophyll *a* and carotenoid binding site in the LH1 complex isolated from *Rhodospirillum rubrum* S1 probed by Stark spectroscopy" *Photosynth. Res.* **95** 345-351 (2008)
4. K. Nakagawa, S. Suzuki, R. Fujii, A. Gardiner, R. Cogdell, M. Nango, and H. Hashimoto "Probing binding site of bacteriochlorophyll *a* and carotenoid in the reconstituted LH1 complex from *Rhodospirillum rubrum*

- S1 by Stark spectroscopy” *Photosynth. Res.* **95** 339-344 (2008)
5. Y. Suemori, M. Nagata, M. Kondo, S. Ishigure, T. Dewa, T. Ohtsuka, and M. Nango “Phospholipid-linked Quinones-mediated Electron Transfer on an Electrode Modified with Lipid Bilayers” *Colloid Surf. B*, **61** 106-112 (2008)
 6. T. Ochiai, T. Asaoka, T. Kato, S. Osaka, T. Dewa, K. Yamashita, H. Hashimoto, and M. Nango “Molecular Assembly of Zn Porphyrin Complexes onto a Gold Electrode using Synthetic Light-harvesting Model Polypeptides” *Tetrahedron Lett.* **48** 8468-8471 (2007)
 7. T. Mikayama, K. Iida, Y. Suemori, T. Dewa, T. Miyashita, M. Nango, A. T. Gardiner, and R. J. Cogdell “The Electronic behavior of a Photosynthetic Reaction Center Monitored by Conductive Atomic Force Microscopy” *J. Nanosci. Nanotechnol.*, (2008) in press
 8. K. Nakagawa, S. Suzuki, R. Fujii, A. T. Gardiner, R. J. Cogdell, M. Nango, and H. Hashimoto “Probing the Effect of the Binding Site on the Electrostatic Behavior of a Series of Carotenoids Reconstituted into the Light-harvesting 1 Complex from Purple Photosynthetic Bacterium *Rhodospirillum rubrum* Detected by Stark Spectroscopy” *J. Phys. Chem. B*, (2008) in press