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Two-dimensional patterning of bacterial light-harvesting 2 complexes on lipid-modified gold surface

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In a photosynthetic membrane, nano-scale patterns of light-harvesting (LH) pigment-protein complexes play an essential role in capturing photons and ensuring efficient excitation energy transfer. LH complexes 1 and 2 have drawn attention as building blocks of a nano-scale photoelectric device. For obtaining a device with efficiency comparable to that of the natural photosynthesis, a method has to be established for forming a two-dimensional assembly of LH complexes around a metal electrode. In this study, LH2 complexes isolated from *Rb. sphaeroides* were immobilized on a patterned gold surface. Quenching of photo-excitation energy by gold was prevented through the placement of a self-assembled phospholipid monolayer between the LH2 complexes and the gold surface. © *2012 American Institute of Physics*. [http://dx.doi.org/10.1063/1.4726105]

In the photosynthetic membrane, pigment-protein complexes carry out photoelectric conversion in an efficient manner.[1] In purple bacteria, light-harvesting (LH) 1 and 2 complexes perform photon absorption, whereas complexes called reaction center (RC) perform charge separation. For understanding the high efficiency of excitation energy transfer, the structure and function of LH complexes have been studied extensively over the years.[2,3] X-ray crystallography was used to determine the crystal structures of LH2 complexes in photosynthetic bacteria such as *Rhodopseudomonas acidophila* strain 10050, *Rhodospirillum molischianum*, and *Rhodopseudomonas palustris* with precisions of 2.0, 2.4, 7.5 Å, respectively.[3–6] Atomic force microscopy was used to reveal the two-dimensional arrangement of the LH complexes in an *in situ* photosynthetic membrane.[7] The LH2 complexes surround LH1-RC complex in the typical native membrane. Such contacting structure of LH1-RC and LH2 complexes in the native membrane guarantees excitation energy transfer within several picoseconds from LH2 to LH1-RC.[8]

For constructing a model system to study photon absorption and charge separation in natural photosynthesis, the pigment-protein complexes should be organized into a two-dimensional pattern. We chose LH1-RC and LH2 from *Rb. sphaeroides* as building blocks, because their structures are simpler than those complexes from plants and because site-directed mutation of amino acids would be easier.[3,9,10] Thus for, only a couple of attempts have been made to arrange LH1-RC or LH2 complexes into a two-dimensional pattern. In a past study, we have immobilized the LH1-RC complex on an indium tin oxide (ITO) electrode by using self-assembled-monolayer (SAM)-assisted 3-aminopropyltriethoxysilane.[11] A 100-μm-wide stripe pattern was fabricated, but smaller patterns were difficult to fabricate due to the surface roughness of ITO. Gold electrodes have the advantages of higher electric conductivity, optically flat surface, and chemical modification capability. The immobilization of LH1-RC on a gold electrode verified throughout the detection of photocurrent has been reported by several groups.[10,12,13]

In this study, we immobilized LH2 complexes on 5-μm-wide gold stripes. The LH2 patterns were characterized by absorption and fluorescence spectroscopy of bacteriochlorophyll (BChl) *a* bound to LH2. Quenching of the excitation energy of BChl *a* was suppressed using a SAM-assisted lipid-modified gold substrate. Substrate modification creates a space between the LH2 complexes and the gold surface. As a result, quenching becomes much slower than fluorescence, thus it becomes an inefficient process.

Figure 1 schematically shows the structure and fabrication of the LH2-immobilized gold substrate used in the present study. A 20-nm-thick layer of gold was deposited on a glass surface. For substrate A, LH2 with a thiol C-terminus (LH2-SH) was chemically bonded directly to the uniform layer of gold. For substrates B, C, and D, the gold layer was patterned into 5-μm-wide stripes with a gap of 5 μm between consecutive stripes. The pattern was made by positive photolithography using a 100 lines/mm chrome mask (Edmund Optics) and ion-beam etching. The patterned substrate was rinsed with methanol, treated by ultrasound sonication in chloroform for 5 min, and cleaned for 25 min using a UV-ozone cleaner (254 nm). For substrate B, LH2-SH was chemically bonded directly onto the patterned gold layer. All solvents used in this study were at least of spectroscopic grade.

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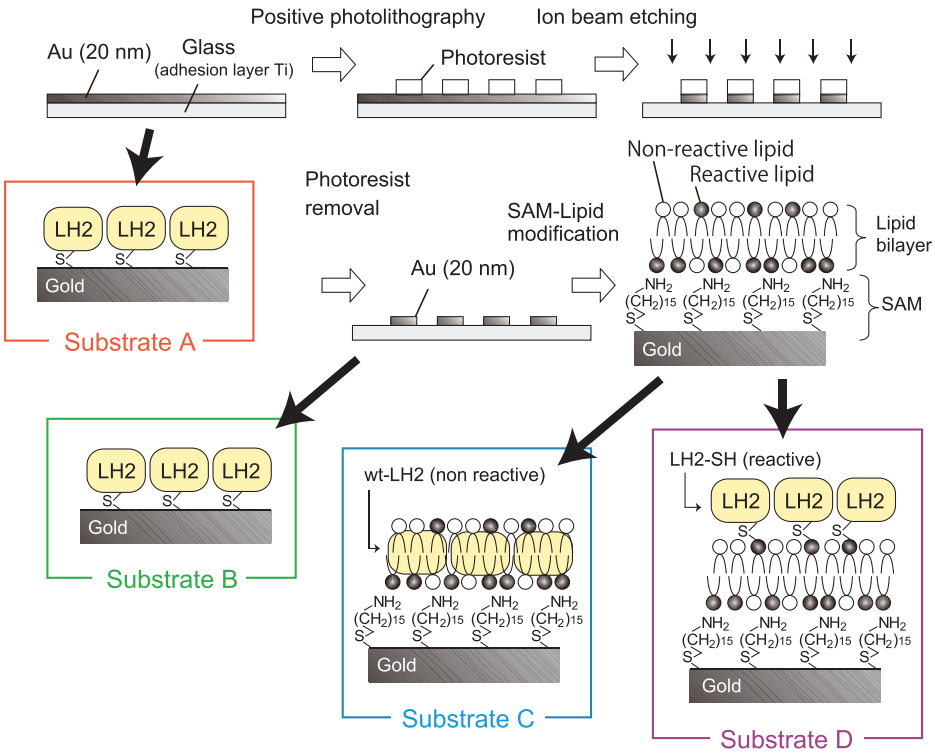

FIG. 1. Schematic presentation of the structure of substrates A to D and that of their preparation. As for the 20-nm-thick gold layer, it covers the substrate surface uniformly in substrate A and is patterned into 5-μm-wide stripes with a pitch of 10 μm in substrates B, C, and D. The pattern was fabricated by using positive photolithography and ion-beam etching. In substrates A and B, the thiol C-terminus of LH2-SH is chemically bonded to the gold surface directly. In substrates C and D, the gold surface is modified with SAM-assisted lipid bilayer. In substrate C, wt-LH2 is incorporated into the lipid bilayer, whereas in substrate D LH2-SH is chemically bonded with a reactive lipid of DOPE on top of the lipid bilayer.

For substrates C and D, the patterned gold layer was modified with SAM by immersing the substrate in a solution of 16-amino-1-hexadecanethiol and hydrochloride in ethanol for 7 h. The SAM-modified substrate was first rinsed with ethanol and then with deionized water. The SAM-modified surface was first immersed in a solution of small unilamellar vesicles (SUVs) for 2 min to allow the formation of a lipid bilayer on top of the SAM.

The SUVs consist by two lipids, maleimide-functinalized 1,2-Dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) and 1,2-Dioleoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol) (DOPG). The maleimide group of DOPE is reactive to LH2-SH specifically; however, DOPG is not, and instead binds the lipid bilayer to the SAM end group.

The maleimide-DOPE was synthesized as follows. Chloroform was distilled and stored with molecular sieves. N-(4-maleimidobutyryloxy) succinimide (GMBS, 0.07 M) was added to a solution of DOPE (0.05 M) in dry chloroform (0.6 ml). Then, triethilamine (0.01 ml) was dropped to the solution and stirred for 4 h in the dark at room temperature. After solvent evaporation, the product was eluted with chloroform and purified using a silica column. The yield point and constant were 27 mg and 86.3%, respectively.

The SUV solution was prepared as follows. A lipid film was casted from a chloroform solution of the maleimide-DOPE (0.38 mg) and DOPG (0.12 mg) and hydrated with Milli-Q water (0.5 ml) at 65 for 1 h. After vortex-mixing and freeze-thaw cycling, the liposomal solution was treated using a probe-type sonicator.

As for the LH2 complexes, substrates C and D employ wild-type LH2 (wt-LH2) from *Rb. sphaeroides* and LH2-SH, respectively. The processes for preparation and immobilization of LH2 are described in Refs. 10 and 11. In substrate C, wt-LH2 is physically trapped inside the lipid bilayer, because the C-terminus of wt-LH2 is not reactive. However, in substrate D the thiol C-terminus of LH2-SH binds to the reactive lipid of DOPE via the maleimide-thiol coupling reaction. All substrates were rinsed with a phosphate buffer (0.1% lauryldimethylamine-N-oxide, *p*H 7.2) before spectroscopic measurements.

Absorption spectrum of the sample was measured using a UV-visible spectrophotometer (UV-1800, Shimadzu). The fluorescence image was observed with an objective-type fluorescent microscope (TE2000-U, Nikon) equipped with an oil-immersion objective lens (Plan Fluor 40X, numerical aperture 1.45, Nikon), a 575-nm dichroic mirror, 590-nm long-pass, and 765-855-nm band-pass filters, and a cooled CCD camera (ORCA-ER, HAMAMATSU). The samples were excited with light of wavelength 535 nm using a 100 W Hg light source. All samples were measured at 1-s exposure time.

The upper part of Fig. 2 is the absorption spectra of substrates A-D and LH2-SH dissolved in phosphate buffer. The measurement was done on 0.3 cm^2 spot area. Fluorescence images of the corresponding substrates are also shown in the figure. The observed spectral coincidence indicates that a fair amount of LH2 is immobilized in substrates A through D.

A comparison of the absorption intensity shows that absorption of substrate A is the double of that of substrate B. This is because the area of the gold surface on substrate A is double of that on substrate B; both substrates were prepared using the same method. Substrates C and D have the same gold area as substrate B. A comparison of their absorption intensities shows that the immobilization of wt-LH2 with non-reactive C terminus is less effective than the immobilization of LH2-SH, and that the immobilization of LH2-SH on the lipid bilayer is more effective than on the gold surface.

The absorption spectrum of the LH2-SH dissolved phosphate buffer is used as a reference for the least damaged LH2. LH2 on substrate C is considered to have negligible

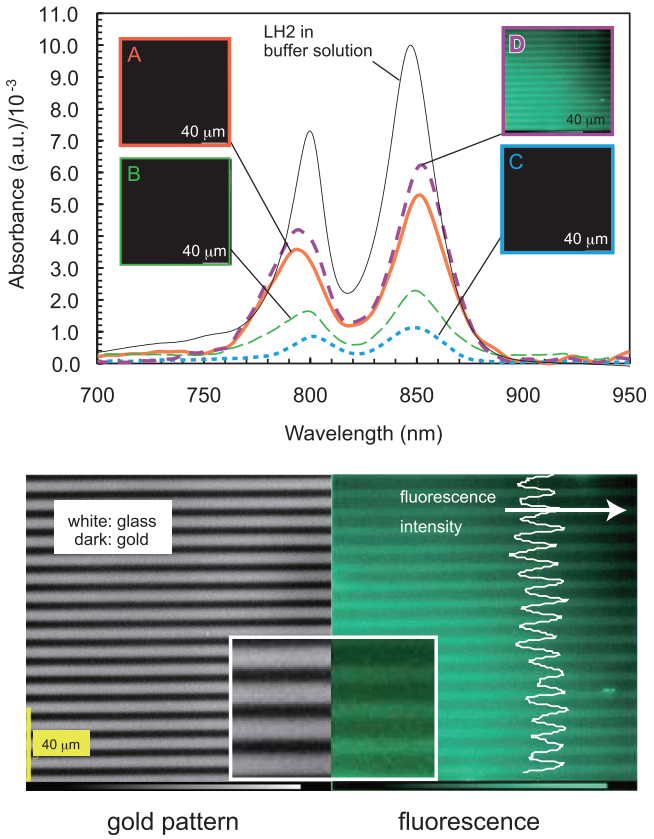

FIG. 2. (top) Absorption spectra and fluorescence images of substrates A-D. (bottom) Transmission image (left) and fluorescence image (right) of the same region of substrate D using 765-855 nm bandpass filter. All fluorescence images were measured at 1-s exposure time.

denaturation, because it shows similar B800 and B850 peak wavelengths as those of the reference. In addition, its B800/B850 intensity ratio (75%) is similar to that of the reference (73%).

In contrast, the B800 band in the spectra from substrates A, B, and D show peak shift and broadening towards shorter wavelengths. In addition, the B850 peak is redshifted in the spectra of substrates A and D. The B800/B850 peak ratios of A and D are more unbalanced than the reference; these are 68% and 67%, respectively. The absorption of substrate B shows B800 peak wavelength and intensity ratio (72%) similar to those of the reference.

These absorption spectra indicate that the LH2-SH chemically bonded to Au surface tends to lose the original structure of the B800-binding sites; however, the spectrum of the sample using wt-LH2 did not show such indication of deformation. The broadened B800 peaks observed in the spectra of substrates A and D suggest the presence of independent BChl *a* that fell off from the B800-binding site.

As for the fluorescence, only substrate D gives off fluorescence that is sufficient for imaging. Substrates A, B, and C do not give off detectable fluorescence, and their emissions correspond to less than $1/10^{-2}$ of the fluorescence intensity of substrate D. The absorption spectrum indicates that the LH2 complexes are present on all the four substrates; therefore, the low fluorescence from substrates A, B, and C is attributed to quenching by the gold. The quenching is considered to be most effective in substrate A, because it has the shortest LH2-gold distance. The fluorescence intensities of substrates B and C indicate that the thickness of SAM alone does not sufficiently suppress the quenching, and the quenching is still much faster than fluorescence with a lifetime of around 900 ps to 1.2 ns. The space created by the SAM and the lipid bilayer is necessary to suppress the quenching.

The absorption analysis suggested that the LH2-SH bound to substrate D was somewhat damaged, which indicates the presence of BChl *a* that fell off the B800-binding site. Those independent BChl *a* has fluorescence peaked around 800 nm at room temperature, which pass through the 765-855-nm bandpass filter used in the fluorescence imaging. The LH2 emits fluorescence peaked around 865 nm,[14] and the short wavelength region of the fluorescence can be observed using this bandpass filter. Hence, the observed image is considered to contain fluorescence from independent BChl *a* that could not transfer the excitation energy in LH2 as well as from the successfully excited LH2.

The bottom of Fig. 2 is the comparison of the transmission image (left) and the fluorescence image (right) from the same region of substrate D. The dark stripes in the former correspond to the gold pattern and the bright stripes in the latter to the fluorescing LH2. The spatial coincidence of the two stripes demonstrates the selective immobilization of LH2-SH to the top of the gold pattern. In addition, the image suggests that the BChl *a* that contributed to the broadening of the B800 absorption band is physically attached to the protein, but does not take part in excitation energy transfer in LH2.

In conclusion, a method for immobilizing LH2 on a 5-μm-scale gold pattern was established by modifying a gold surface with a SAM-assisted lipid bilayer. LH2 can be immobilized within, as well as onto, the bilayer, but fluorescence quenching by gold is suppressed only for LH2 immobilized on the bilayer. Although it has been suggested that the chemical bond between the thiol group in LH2 and Au would cause partial denaturation of LH2, fluorescing LH2 immobilized onto a gold pattern will be a useful model structure for studying the excitation energy transfer mechanism of the LH complexes.

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