

Plasmon-stimulated Biophotovoltaic Cells based on Thylakoid-AuNR Conjugate

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Characterization of HS-PEG-NHS ester on Au film surface

Au film on silicon substrate was used to demonstrate the attachment of HS-PEG-NHS on the Au surface (**Figure S2a-e**). As shown in a spectrum in **Figure S2b**, clean and sharp Au peaks were detected from both bare Au film and Au film-PEG-NHS ester samples but noticeably suppressed in tethering molecule-coated sample. Detection of Au trace from the coated Au film was due to a deeper penetration depth of X-ray (~ 10 nm) compared to the length of tethering molecules (~ 3 nm). On the other hand, C, O peaks dramatically increased after coating the tethering molecules which indicated attached chemical molecules on the surface of Au film (**Figure S2 c,d**). Although the HS-PEG-NHS ester molecule had one nitrogen atom, however, it was easily dissolved while preparation for XPS analysis. Therefore, no N peak was observed in either sample (**Figure S2e**).

Use of tethering molecules

Using a tethering molecule is a popular technique to wire biological material to other nanoparticles or substrates. Recently, wiring photosynthetic apparatuses such as PS II or TM to electrode surfaces have been investigated.^{1, 2} These approaches mainly contributed in constructing an electron pathway to enhance charge transfer between PS II and an electrode. Recently, Miyachi, *et al.* utilized PS II reconstituted with platinum nanoparticles (PtNPs) with a short tethering molecule,³ however, the only purpose of PtNPs was wiring PS II with an Au electrode to maintain close distance for enhanced electron conductivity. Meanwhile, it is noteworthy that Zhou, *et al.* conjugated TM with poly(fluorene-co-phenylene) (PFP), a cationic conducting polymer, to construct electron pathway and also to absorb UV light energy into TM using FRET.⁴ Since LHC of PS II absorbs both blue (~450 nm) and red (~680 nm) light during

photosynthesis, additional absorption of UV light resulted in 200% increase of water oxidation to oxygen. However, P680 utilizes photons only after converting any other light to 680 nm photons by releasing surplus energy as heat or fluorescence, therefore, additional injection of blue-range wavelength is prone to induce photon-induced damage to the photosynthetic pigments and may negatively affect the TM viability.^{5,6}

Experimental Methods

Materials

AuNRs for localized surface plasmon resonance generation was purchased from NanoComposix (peak absorbance at 660 nm, product No. GRCH660-5M). The tethering molecule, HS-PEG-NHS ester, was purchased from Biochempeg (Cat. No. SH-PEG-SC, 200). A PBS solution which contains 10 mM phosphate, 2.7 mM KCl and 137 mM NaCl were produced using PBS tablets (product No. P4417, Sigma-Aldrich). Saccharose (product No. 57-50-1, Duksan) and magnesium chloride ($MgCl_2$) (product No. 7791-18-6, Duksan) were used to make PBS density derivatives and osmotic shock agent during TM isolation. An Ag/AgCl electrode (product No. 6211-M, San-Xin Instrumentation Inc.) was used as a reference electrode and a platinum mesh electrode (product No. 002250, ALS Co., Ltd.) was used as counter electrode in all electrochemical analysis. A 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) (product No. D2425, Sigma-Aldrich) was used as an inhibitor which blocks electron flows between PS II and PQ.

TM isolation

TMs were isolated based on the protocols from the previous work,⁷ using spinach leaves which were purchased in local supermarket. First, stalks and veins were carefully torn out from spinach from a local market. Leaves were vigorously blended using a laboratory blender (Product No. 8011EG, Waning) with a solution of PBS and 0.3 M saccharose at a 22,000 rpm for 20 seconds. This was filtered using a 25 μm nylon mesh (Product No. NMO-P2-25, CS Technofil Co., Ltd.) and the filtered solution was centrifuged at 4,750 rpm for 10 minutes using a centrifuge (Product No. Allegra X-15R, Beckman Coulter). The supernatant was removed, and the pellet was resuspended using a PBS solution with 0.3 M saccharose and 5 mM MgCl_2 followed by ultrasonication (Product No. Q125, Qsonica) for decomposition of chloroplasts. Then, the solution was centrifuged at 4,000 rpm for 10 minutes and supernatant was discarded as well. The pellet was resuspended using a PBS with 0.3 M saccharose and was centrifuged at 20,000 rpm for 20 minutes using high speed ultracentrifuge (Optima XPN-100, Beckman Coulter). Afterwards, the supernatant was scrapped and the pellet was carefully homogenized in a PBS with 1.8 M saccharose solution using a glass tissue grinder (7727-15, Corning Life Sciences). Subsequently, a PBS solution with 1.3 M saccharose was deliberately stacked on the homogenized solution to maintain the two solutions as separate layers. After centrifugation at 38,000 rpm for an hour, TM pellets were floated between the two separate layers. The pellets were thoroughly collected and resuspended in a pure PBS solution. The obtained TMs were washed at 20,000 rpm for 20 minutes and stored in a PBS for further use. All centrifugation steps were processed at 4 $^\circ\text{C}$.

Preparation of Au-TM

First, 10 μL of an AuNR solution with 1.74 mg/mL mass concentration was diluted with distilled water to have 390 μL volume. Then, 10 μL of a tethering molecule, HS-PEG-NHS

ester (3.8 M), was added to the diluted AuNR solution followed by vigorous shaking to form chemical or electrostatic bonds between thiol (-SH) group and AuNR surfaces. After that, 500 μ L of TM which was suspended in $\times 2$ PBS solution was added and agitated so that NHS ester of the tethering molecule and the amine (-NH₂) groups of TMs could form chemical bonds. A final solution contained 174 μ g/mL of AuNRs, 38 mM of the tethering molecule and 0.1 mg Chl/mL of TMs and was rest for a few hours in a refrigerator under a dark condition before electrochemical measurements.

Spectral and optical characterization of conjugates

Products of each conjugation step was characterized by optical and spectral methods. The shapes of AuNR, AuNR-PEG-NHS ester and Au-TM were observed using TEM (JEM-F200, JEOL, Ltd.) and SEM (JSM-7610F Plus, JEOL, Ltd.). Each sample solution was spread on a rigid substrate and dried overnight. The zeta potentials of pure AuNR and AuNR-NHS conjugates in the solution forms were measured using a zeta-potential analyzer (ELS-100ZS, Otsuka Electronics Co., Ltd.). The surface analysis of pure AuNR and AuNR-PEG-NHS ester were performed by XPS (K-Alpha, Thermo Fisher Scientific) to confirm whether chemical molecules were coated on AuNR.

Electrochemical analysis

All electrochemical measurements including CA and EIS were using potentiostat. The potentiostat device (CompactStat, Ivium Technologies) was employed to induce a required potential on a working electrode and to monitor electrical signal with high sensitivity. All measurements were made in a standard three-electrode configuration. A metal-deposited glass (Borosilicate-33) specimen of 5 mm² size was used as a working electrode on which

pure TM or Au-TM were deposited. A Pt mesh electrode and an Ag/AgCl electrode were used as a counter and reference electrode, respectively. For production of PEs, a white light source (LG-PS2, Olympus), a near-UV light source (Dr's light AT, Swish Dental) and a red-light source (LV-SP 3W R12, LV Vision) were used. All measurements were processed in a custom-built faraday cage to prevent any unwanted electrical noise.

Numerical analysis

Electric field enhancement induced by plasmonic AuNRs on TM was calculated using a 3D FDTD method. AuNR was assumed to be a cylinder with hemispherical sides. The length of AuNR was 45 nm with the diameter of 17 nm. TM was approximated as a 5 nm-thick lipid bilayer with refractive index $n = 1.41$ in an aqueous environment.⁸ AuNR was located to be 3 nm above a lipid bilayer, considering 3 nm-long tethering molecules coated on the AuNR surface. Refractive indices of gold and water were taken as $1.608 + j3.659$ and 1.33 for 680 nm light wavelength.⁹ Two orthogonal polarization modes, transverse electric and transverse magnetic, were simulated to calculate the responses to unpolarized light incidence by incoherent superposition of the electric field enhancement. Under normal plane-wave incidence, field enhancement was measured in two configurations: (1) single AuNR and (2) double AuNRs aligned in parallel by varying the distance between AuNR and the FDTD monitor from 0 to 10 nm with 0.5 nm step along the direction orthogonal to the TM. For the case of double AuNR configurations, the gap between the two AuNRs was also varied from 2.5 to 21.25 nm with a 1.25 nm step.

Construction of BPV cell

The BPV cell module assembly was customized by JinIl Co. to fit with the size of anode substrate, $1 \times 2 \text{ cm}^2$. The assembly included cathode holder, anode holder and anode cap. PBS saline was selected as anolyte to electrically connect anode and cathode and anolyte of 1.5 mL was filled in anolyte chamber of anode holder. A cathode (0.5 mg/cm^2 60% Platinum on Vulcan -Cloth, SLGDE, FuelCellsEtc) was employed to consume generated PEs and construct an intact electrochemical circuit. One side of the cathode was coated with Nafion, therefore the anolyte directly contacted to Nafion-coated side of the cathode. Also, the other side of cathode was coated with Teflon to prevent from flooding which occurred by ORR. An array of BPV cells was constructed by connecting several modules in serial or parallel. A power management system for PEs accumulation consisted of DC rectifier and capacitor. Since the BPV cell did not produce alternative current, a capacitor (E/C 16V 220 μF , Samyoung Electronics Co., Ltd.) and a switch were simply connected to collect PEs and amplify output power.

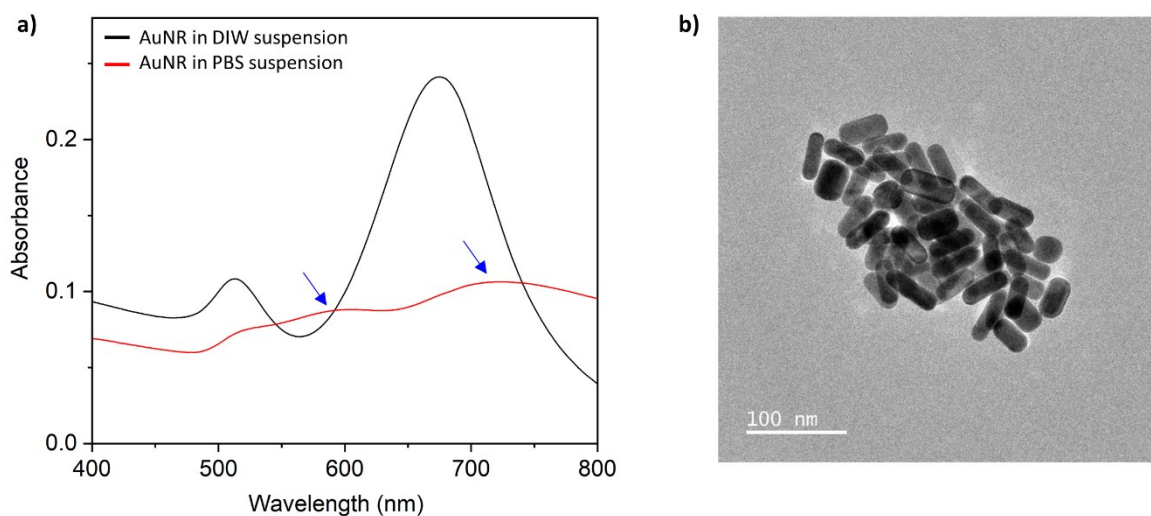


Figure S1. AuNR aggregation tendency in a suspension with high ionic strength. (a) Absorption spectrum of AuNRs in DIW and PBS suspension. Notice that basically pure AuNRs have plasmon resonance peak at 520, 675 nm because of their asymmetric shape. Blue arrows indicate broadened and red-shifted plasmon resonance peak of AuNRs aggregates in which they are suspended in high-ionic-strength-fluid such as PBS. (b) TEM image of AuNR aggregate in PBS suspension.

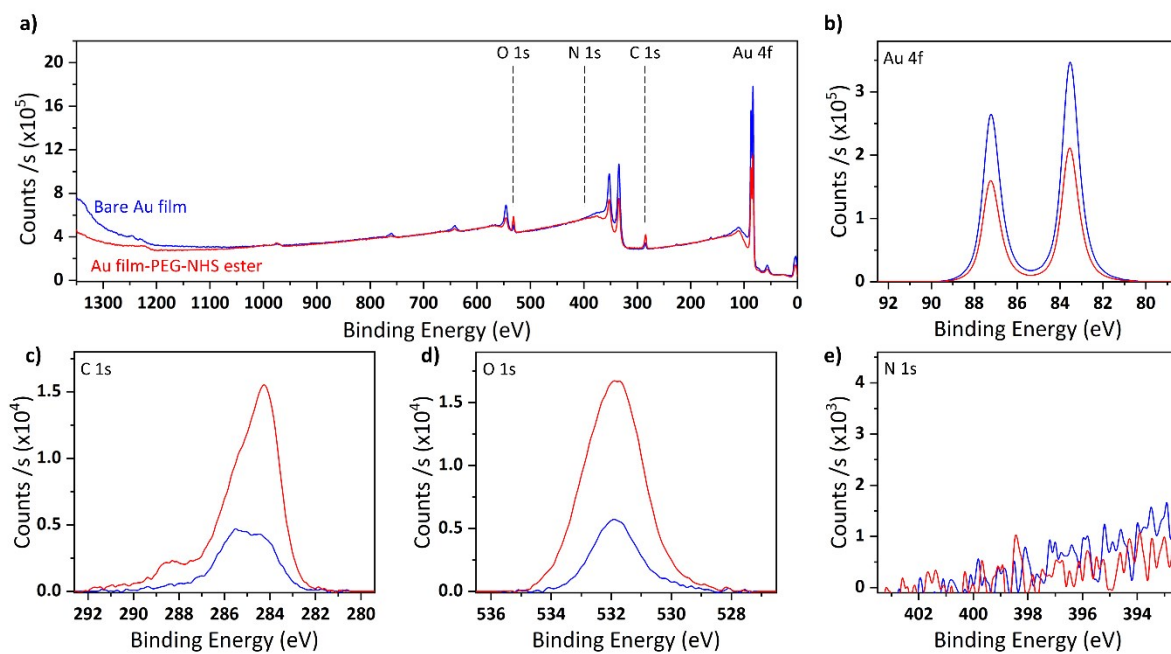


Figure S2. Characterization of tethering molecule coated on Au film using XPS analysis. (a) Overall XPS spectrum of bare Au film and Au film-PEG-NHS ester. (b-e) XPS spectra of (b) Au, (c) C, (d) O and (e) N in bare Au film and Au film-PEG-NHS ester sample.

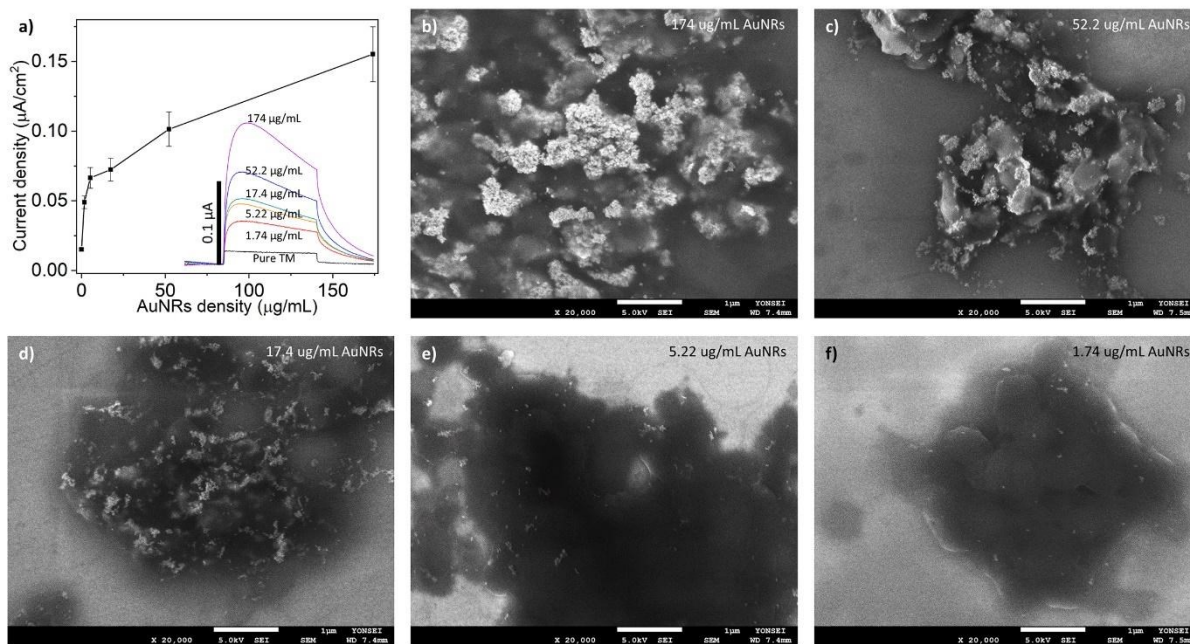


Figure S3. PE current dependency on AuNR concentrations of Au-TM. AuNRs are plasmonic energy source so that essential in PE current enhancements. (a) PE current of each conjugate with different AuNR density. Actual measured PE currents under illumination are plotted as inset image. AuNR density of each samples is observed using SEM images in case of (b) 174, (c) 52.2, (d) 17.4, (e) 5.22, and (f) 1.74 μg/mL of AuNR, respectively.

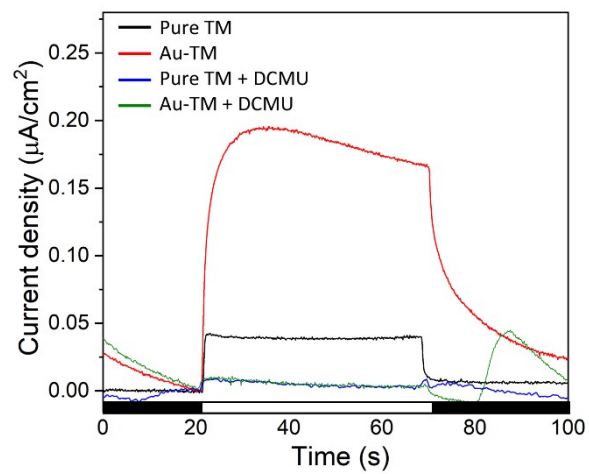


Figure S4. Result of DCMU treatment test. DCMU is an herbicide which block PE transfer chain so that PEs cannot be extracted.

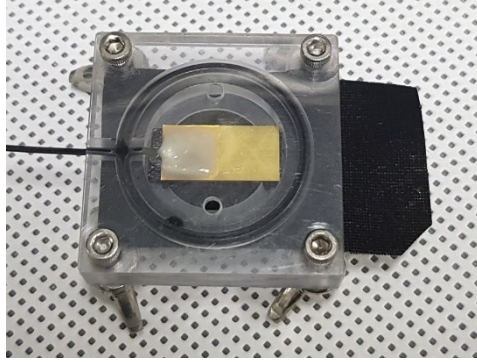


Figure S5. Photographs of the assembled BPV cell module.

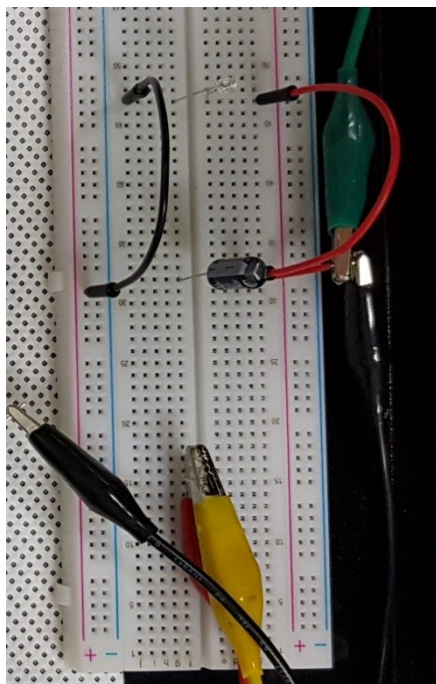


Figure S6. Photographs of the PEs accumulation circuit. Charges from BPV cells were accumulated in the 220 μF capacitor and they were discharged through the LED after reached enough potential.

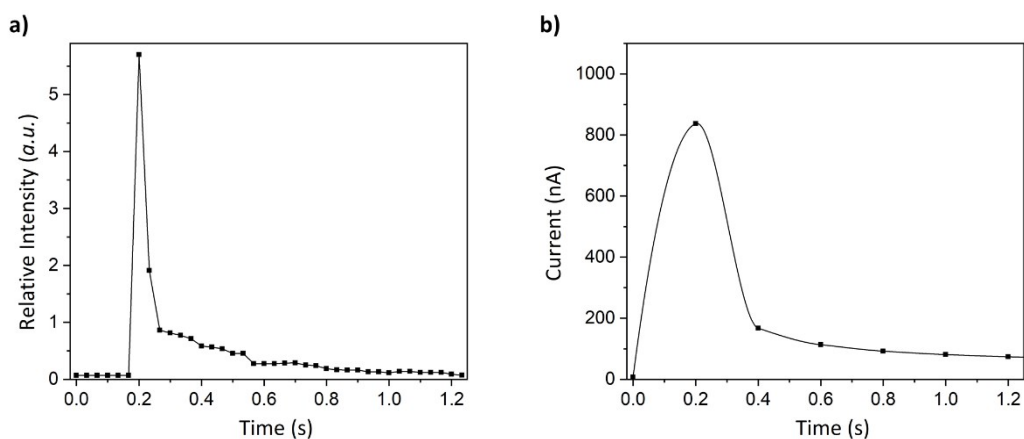


Figure S7. Transient performance of periodic operation. (a) Relative light intensity of the LED during discharging. The LED was lighted at 0.2 sec and the brightness rapidly diminished. (b) Measured current during discharging. Due to a hardware issue, time interval of the current plot was not enough to be precisely analyzed, however, note that the current reached its peak at 0.2 sec and rapidly decreased.

Table S1. Parameters of each component in equivalent circuit from Nyquist plot of bare electrode, AuNRs, pure TMs and Au-TM under illumination.

	Bare electrode		AuNRs		Pure TM		Au-TM conjugates		Units
	Value	Error (%)	Value	Error (%)	Value	Error (%)	Value	Error (%)	
R1	93.78	1.75	92.21	1346	80.11	1.61	66.61	1.73	Ω
C1	350	1.35	330	1.03	425	1.3	687	1.87	nF
C2	2.23	4.06	2.54	3.31	2.45	3.82	2.99	4.04	nF
Zw	11.9	5.06	9.91	3.42	11.7	3.94	17.1	5.12	$10^{-5}/\Omega \cdot \sqrt{\text{Hz}}$

Table S2. Parameters of each component in equivalent circuit from Nyquist plot of bare electrode, pure TM and Au-TM under light illumination conditions.

	Bare electrode (light off)		Bare electrode (light on)		Pure TM (light off)		Pure TM (light on)		Au-TM (light off)		Au-TM (light on)		Units
	Value	Error (%)	Value	Error (%)	Value	Error (%)	Value	Error (%)	Value	Error (%)	Value	Error (%)	
R1	130.9	2.63	126.7	2.88	95.55	4.24	80.56	3.15	123.1	2.77	66.61	1.73	Ω
C1	118	1.02	105	0.96	272	2.79	228	1.47	475	2.04	687	1.87	nF
C2	8.96	6.73	0.92	7.35	1.14	10.97	1.29	8.21	0.89	7.94	2.99	4.04	nF
Zw	6.95	6.50	5.65	5.59	3.21	3.55	5.68	3.94	7.82	5.23	17.1	5.12	$10^{-5}/\Omega \cdot \sqrt{\text{Hz}}$

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