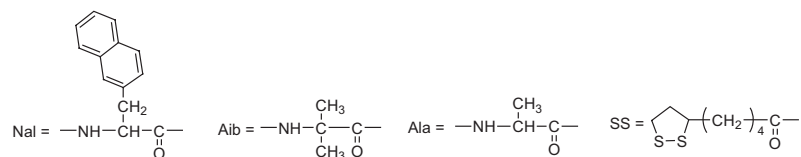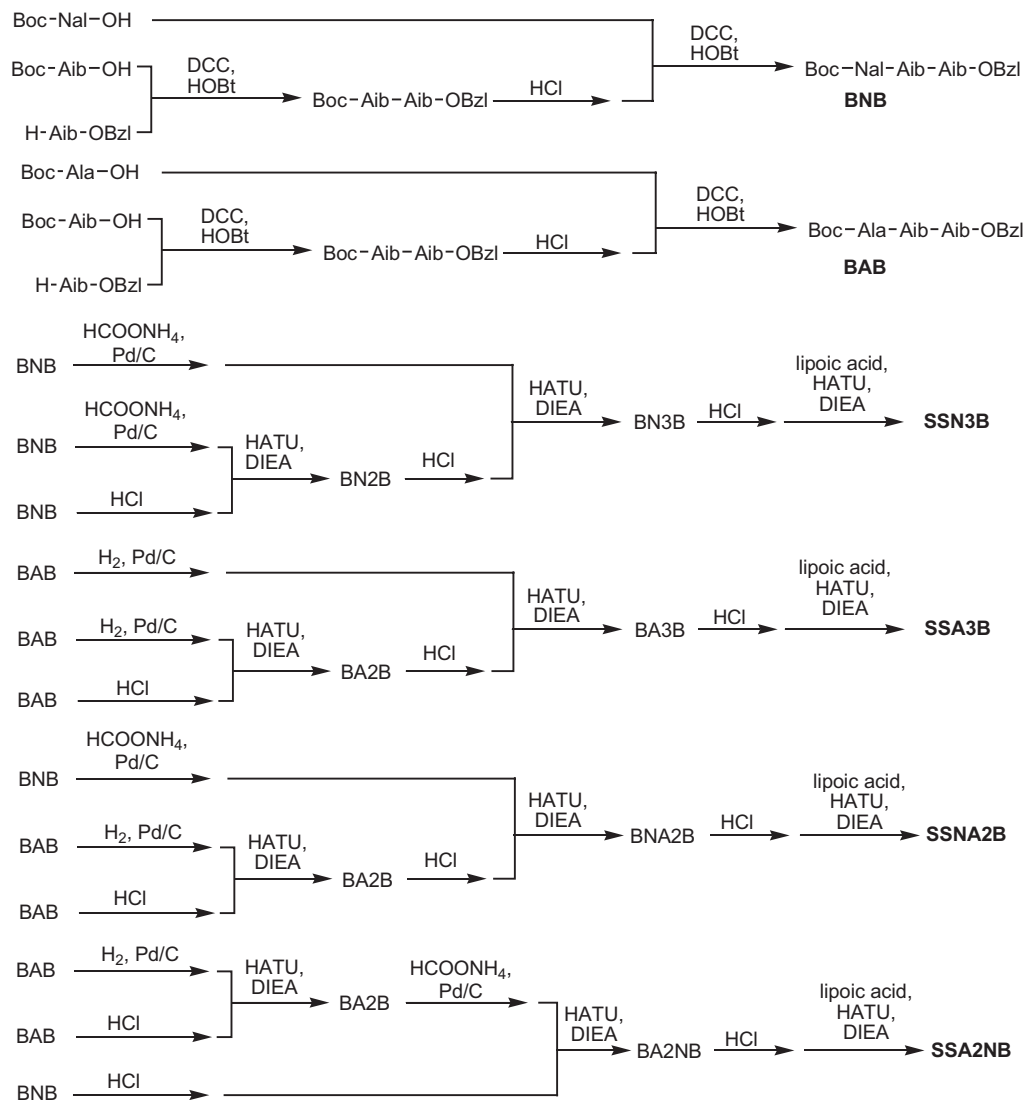**Efficient Photocurrent Generation by Self-Assembled Monolayer Composed of**

3_{10}-Helical Peptide Carrying Linearly-Spaced Naphthyl Groups at Side Chains

Kazuyuki Yanagisawa, Tomoyuki Morita and Shunsaku Kimura*

Experimental Details:

Synthesis of helical peptides. SSN3B, SSA3B, SSNA2B and SSA2NB were synthesized according to Scheme S-1. All the peptides were synthesized by the conventional liquid-phase method. The starting materials, L-alanine (Ala) was purchased from PEPTIDE Institute Inc., Japan, 3-(2-naphthyl)-L-alanine (Nal) was purchased from Wako Co., Ltd., Japan, and 2-aminoisobutyric acid (Aib) was purchased from Tokyo Kasei Co., Ltd., Japan. These reagents were used without further purification. Four kinds of intermediate nonapeptides, Boc-(Nal-Aib-Aib)$_3$-OBzl (BN3B), Boc-(Ala-Aib-Aib)$_3$-OBzl (BA3B), Boc-(Nal-Aib-Aib)$_2$(Ala-Aib-Aib)-OBzl (BNA2B) and Boc-(Ala-Aib-Aib)$_2$(Nal-Aib-Aib)-OBzl (BA2NB) were synthesized from tripeptides of Boc-Nal-Aib-Aib-OBzl and/or Boc-Ala-Aib-Aib-OBzl, where Boc and OBzl represent *tert*-butyloxycarbonyl for N-terminal protection and benzyl ester for C-terminal protection, respectively. The tripeptides were synthesized from Ala or Nal and Aib using *N,N'*-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) as coupling reagent. Protection of N- or C-terminal of the amino acids was performed by (Boc)$_2$O or esterification using benzylalcohol, respectively, and Boc and OBzl group were removed prior to the coupling respectively by treatment with a 4 N HCl dioxane solution, and ammonium formate (HCOONH$_4$) or hydrogenation with using paradium carbon. The hexapeptides and nonapeptides were obtained by fragment coupling by using *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) as a coupling reagent. Finally, after removal of Boc group, lipoic acid was introduced to the N-terminals of these nonapeptides by HATU in the presence of *N,N*-diisopropylethylamine (DIEA) to afford final products. All intermediates and final products were identified by ^{1}H NMR (300 MHz or 400 MHz), and their purity was checked by thin layer chromatography (TLC). The final products were further confirmed by mass spectroscopy. The identification data are described in page S-3.

Scheme S-1. Synthetic scheme of SSN3B, SSNA2B, SSA2NB and SSA2NB.

Boc‒Nal‒OH ——————————————————————┐
                                                    DCC, HOBt
Boc‒Aib‒OH ┐ DCC, HOBt   ——→ Boc‒Nal‒Aib‒Aib‒OBzl
           ├——————→ Boc‒Aib‒Aib‒OBzl ——HCl——→   **BNB**
H‒Aib‒OBzl ┘

Boc‒Ala‒OH ——————————————————————┐
                                                    DCC, HOBt
Boc‒Aib‒OH ┐ DCC, HOBt   ——→ Boc‒Ala‒Aib‒Aib‒OBzl
           ├——————→ Boc‒Aib‒Aib‒OBzl ——HCl——→   **BAB**
H‒Aib‒OBzl ┘

BNB —HCOONH₄, Pd/C——————————┐
                                        HATU, DIEA   lipoic acid, HATU, DIEA
BNB —HCOONH₄, Pd/C— HATU,DIEA   ——→ BN3B —HCl→ ——→ **SSN3B**
BNB —HCl—   ——→ BN2B —HCl——┘

BAB —H₂, Pd/C——————————————┐
                                        HATU, DIEA   lipoic acid, HATU, DIEA
BAB —H₂, Pd/C— HATU, DIEA   ——→ BA3B —HCl→ ——→ **SSA3B**
BAB —HCl—   ——→ BA2B —HCl——┘

BNB —HCOONH₄, Pd/C——————————┐
                                        HATU, DIEA   lipoic acid, HATU, DIEA
BAB —H₂, Pd/C— HATU, DIEA   ——→ BNA2B —HCl→ ——→ **SSNA2B**
BAB —HCl—   ——→ BA2B —HCl——┘

BAB —H₂, Pd/C— HATU, DIEA   HCOONH₄, Pd/C
BAB —HCl—   ——→ BA2B ——————————→   HATU, DIEA   lipoic acid, HATU, DIEA
BNB —HCl————————————————————┘   ——→ BA2NB —HCl→ ——→ **SSA2NB**

Nal = —NH‒CH‒C—
              ‖
              O   (CH₂ / naphthalene)

Aib = —NH‒C‒C—
        | ‖
       CH₃ O
        CH₃

Ala = —NH‒CH‒C—
         | ‖
        CH₃ O

SS = (dithiolane)‒(CH₂)₄‒C—
                          ‖
                          O

Boc = *tert*-butyloxycarbonyl   OBzl = benzyl ester   DCC = *N,N'*-dicyclohexylcarbodiimide   HOBt = 1-hydroxybenzotriazole

HATU = *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate

DIEA = *N,N*-diisopropylethylamine

SSN3B. ^{1}H NMR (CDCl$_3$, 300 MHz): δ(ppm) 1.26-1.67 (42H, m, AibCH_3, SSCH$_2$CH$_2$CH (CH_2)$_3$CH$_2$CO), 1.76, 2.32 (2H, m, SSCH$_2$CH_2CH (CH$_2$)$_3$CH$_2$CO), 1.92 (2H, brs, SSCH$_2$CH$_2$CH (CH$_2$)$_3$CH_2CO), 3.08 (2H, m, SSCH_2CH$_2$CH (CH$_2$)$_3$CH$_2$CO), 3.28 (6H, m, NalC$^\beta$$H_2$), 3.52 (1H, m, SSCH$_2CH_2CH$ (CH$_2$)$_3$CH$_2$CO), 4.16 (3H, brm, NalC$^\alpha$$H$), 5.11 (2H, s, OC$H_2C_6H_5$), 6.28, 6.60, 7.08-7.92 (35H, brm, naphthyl-H, OCH$_2$C$_6$$H_5$, NalN$H$, AibN$H$). MS (FAB, matrix; nitrobenzylalcohol): m/z 1420 (calcd for C$_{78}$H$_{95}$N$_9$NaO$_{11}$S$_2$ [(M Na)$^+$] m/z 1420). TLC: R$_f$ (chloroform/methanol/pyridine = 95/5/3 v/v/v) = 0.46, R$_f$ (ethyl acetate) = 0.54.

SSA3B. ^{1}H NMR (CDCl$_3$, 400 MHz): δ(ppm) 1.18-1.80 (51H, m, AlaC$^\beta$$H_3$, AibC$H_3$, SSCH$_2CH_2$CH (C$H_2$)$_3CH_2$CO), 1.92, 2.46 (2H, m, SSCH$_2$CH_2CH (CH$_2$)$_3$CH$_2$CO), 2.35 (2H, t, SSCH$_2$CH$_2$CH (CH$_2$)$_3$CH_2CO), 3.16 (2H, m, SSCH_2CH$_2$CH (CH$_2$)$_3$CH$_2$CO), 3.60 (1H, m, SSCH$_2$CH$_2$CH (CH$_2$)$_3$CH$_2$CO), 3.96, 4.10 (3H, brm, AlaC$^\alpha$$H$), 5.13 (2H, s, OC$H_2C_6H_5$), 6.68-6.90, 7.19, 7.30-7.41, 7.64-7.83 (14H, OCH$_2$C$_6$$H_5$, AlaN$H$, AibN$H$). MS (FAB, matrix; nitrobenzylalcohol): m/z 1042 (calcd for C$_{48}$H$_{77}$N$_9$NaO$_{11}$S$_2$ [(M+Na)$^+$] 1042). TLC: R$_f$ (chloroform/methanol = 10/1 v/v) = 0.44, R$_f$ (chloroform/methanol/ammonia water = 13/5/1 v/v/v) = 0.81.

SSNA2B. ^{1}H NMR (CDCl$_3$, 400 MHz): δ(ppm) 1.18-1.70 (48H, m, AlaC$^\beta$$H_3$, AibC$H_3$, SSCH$_2CH_2$CH (C$H_2$)$_3CH_2$CO), 1.87, 2.40 (2H, m, SSCH$_2$CH_2CH (CH$_2$)$_3$CH$_2$CO), 2.22 (2H, t, SSCH$_2$CH$_2$CH (CH$_2$)$_3$CH_2CO), 3.12, 3.40 (5H, m, SSCH_2CH$_2$CH (CH$_2$)$_3$CH$_2$CO, NalC$^\beta$$H_2$), 3.95, 4.14 (2H, m, AlaC$^\alpha$$H$), 4.42 (1H, brm, NalC$^\alpha$$H$), 5.12 (2H, s, OC$H_2C_6H_5$), 6.10, 6.54, 7.01, 7.17, 7.25-7.39, 7.40, 7.45-7.57, 7.59-7.72, 7.78-7.90 (21H, naphthyl-H, OCH$_2$C$_6$$H_5$, AlaN$H$, AibN$H$, NalN$H$). MS (FAB, matrix; nitrobenzylalcohol): m/z 1168 (calcd for C$_{58}$H$_{83}$N$_9$NaO$_{11}$S$_2$ [(M+Na)$^+$] 1168). TLC: R$_f$ (chloroform/methanol = 10/1 v/v) = 0.35, R$_f$ (chloroform/methanol/ammonia water = 13/5/1 v/v/v) = 0.87.

SSA2NB. ^{1}H NMR (CDCl$_3$, 300 MHz): δ(ppm) 1.41-1.64 (48H, m, AlaC$^\beta$$H_3$, AibC$H_3$, SSCH$_2CH_2$CH (C$H_2$)$_3CH_2$CO), 1.88, 2.40 (2H, m, SSCH$_2$CH_2CH (CH$_2$)$_3$CH$_2$CO), 2.32 (2H, t, SSCH$_2$CH$_2$CH (CH$_2$)$_3$CH_2CO), 3.12 (2H, m, SSCH_2CH$_2$CH (CH$_2$)$_3$CH$_2$CO), 3.24, 3.48 (2H, d, NalC$^\beta$$H_2$), 3.50 (1H, m, SSCH$_2CH_2CH$ (CH$_2$)$_3$CH$_2$CO), 3.96 (2H, brm, AlaC$^\alpha$$H$), 4.36 (1H, brm, NalC$^\alpha$$H$), 5.13 (2H, s, OC$H_2C_6H_5$), 6.88, 7.08, 7.20-7.40, 7.48, 7.68-7.84 (21H, OCH$_2$C$_6$$H_5$, AlaN$H$, AibN$H$, NalN$H$). MS (FAB, matrix; nitrobenzylalcohol): m/z 1168 (calcd for C$_{58}$H$_{83}$N$_9$NaO$_{11}$S$_2$ [(M+Na)$^+$] 1168). TLC: R$_f$ (chloroform/methanol = 5/1 v/v) = 0.59, R$_f$ (chloroform/methanol/acetic acid = 95/5/3 v/v/v) = 0.28.

Solvent effect on the chemical shifts in ^{1}H NMR measurement.   To examine the conformation of SSN3B and SSA2NB in solution, their derivatives BN3B and BA2NB was investigated by ^{1}H NMR spectroscopy in a $(CD_3)_2SO$ / $CDCl_3$ mixed solvent with changing the ratio.   It is well known that a chemical shift of peptide NH proton, which is exposed to solvent, shifts to lower magnetic field with the addition of a hydrogen-bond accepting solvent in a nonpolar solvent.   When the peptide takes α-helical conformation, three NH protons are exposed to solvent, but in the case of 3_{10}-helical conformation, two NH protons are exposed to solvent.[S-1]   BN3B and BA2NB, where lipoic carbonyl group in SSN3B or SSA2NB is replaced with a *tert*-butyloxycarbonyl group, are suitable for the measurement because one of the NH protons can be easily assigned due to the urethane amide proton.   The results are shown in Figure S-1.   NH protons, which are not overlapped by the peaks of aromatic protons, are plotted against the amount of $(CD_3)_2SO$.   In both peptides, only urethane NH proton and one Aib-NH singlet proton shifted significantly to lower magnetic field with the addition of $(CD_3)_2SO$.   These results indicate that these peptides take 3_{10}-helical conformation in chloroform, suggesting that SSN3B and SSA2NB also take 3_{10}-helical conformation.   The other peptides, SSA3B and SSNA2B showed the similar results (data not shown).
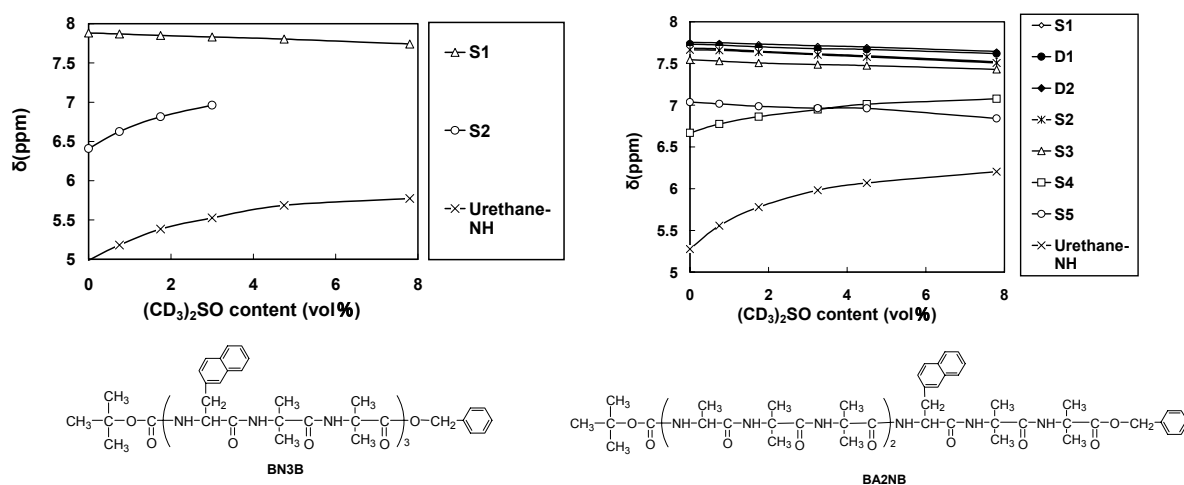

Figure S-1.   Chemical shift changes of NH protons of BN3B and BA2NB in $CDCl_3$ with the addition of $(CD_3)_2SO$. The letters S and D represent singlet NH protons of Aib residues and doublet NH protons of Nal or Ala residues, respectively.

Molecular modeling. In order to predict the optimized geometry of the peptide, MM2 calculation of SSN3B was carried out by using the software of Chem3D pro (ver. 4.0, CambridgeSoft Corporation, MA). The energy minimization was started from a standard right-handed 3_{10}-helix ($\phi = -60^{o}$, $\varphi = -30^{o}$). The result is shown in Figure S-2. The calculation showed that the 3_{10}-helical conformation of the peptide is stable, where three naphthyl groups are spaced in a linear manner along the helix axis and take a plane-to-plane orientation.


Side view   **Top view**
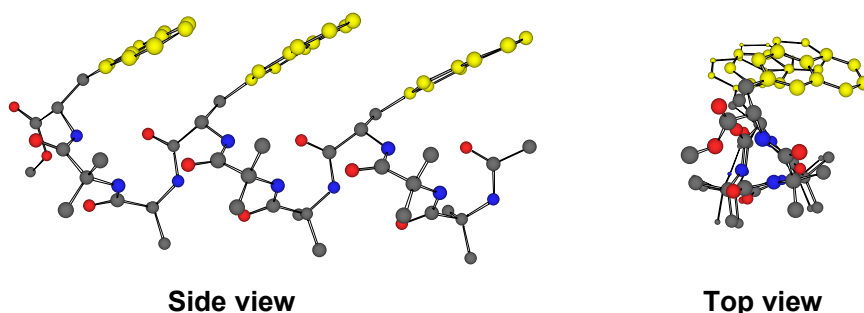
Figure S-2. The energy-minimized conformation of SSN3B based on MM2 calculation. The hydrogen atoms are omitted for simplicity. Red, blue and yellow colors express oxygen atoms, nitrogen atoms and naphthyl carbon atoms, respectively.

Circular dichroism (CD) spectroscopy. CD spectra of SSN3B and the other peptides were measured in ethanol at 20 ^{o}C on a CD spectropolarimeter (J-600, JASCO Co., Ltd., Japan) using an optical cell of 0.1 cm optical path length. The CD spectrum of SSN3B in ethanol is shown in Figure S-3.
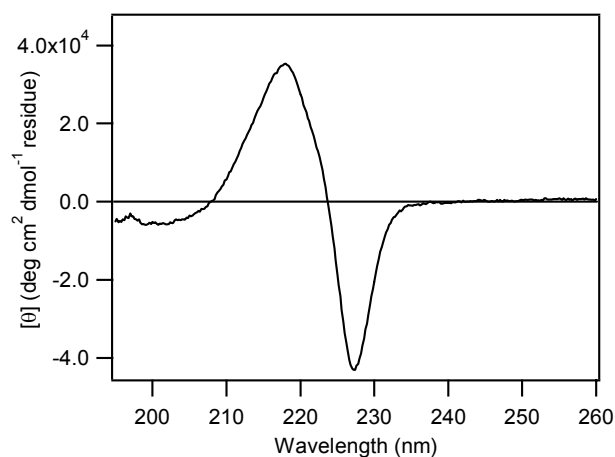
Figure S-3. CD spectra of SSN3B in ethanol.

Absorption and fluorescence spectroscopy.   Absorption and fluorescence spectra of SSN3B and SSA2NB in ethanol were recorded on a Ubest-50 spectrometer (Jasco Co., Ltd., Japan) and an F4010 fluorometer (Hitachi Co., Ltd., Japan), respectively.   The spectra of SSN3B and SSA2NB are shown in Figure S-4.
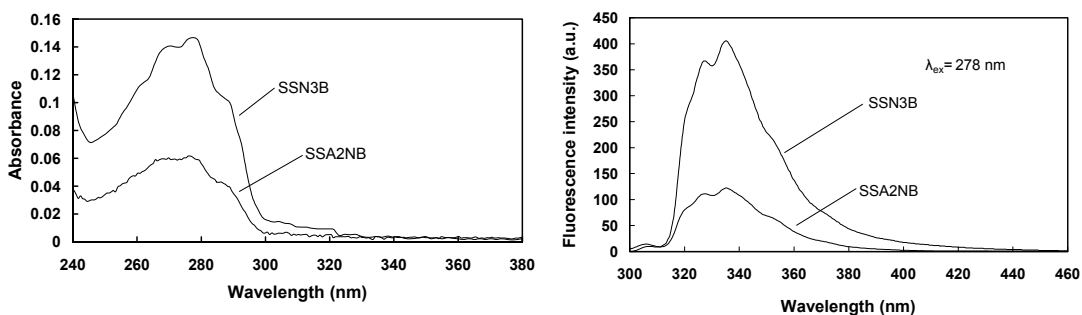

Figure S-4.   Absorption spectra (left) and fluorescence spectra (right) of SSN3B and SSA2NB in ethanol.

Preparation of self-assembled monolayers.   A slide glass was treated with sulfonic acid for 3 h followed by thoroughly washing with methanol and distilled water. A gold substrate was prepared by vapor deposition of chromium and then gold (99.99   ) onto the slide glass.   The thicknesses of the chromium and gold layers, monitored by a quartz oscillator, were approximately 500 and 2000 Å, respectively.   The gold substrate was used for a self-assembling experiment immediately after preparation.   The gold substrate was incubated in an ethanol solution of the helical peptide (ca. 0.1 mM) for 24 h.   Then the substrate was rinsed rigorously with ethanol, dried in a steam of dry nitrogen gas, and finally dried under vacuum for 10-15 min.   The size of substrates used for infrared reflection-absorption spectroscopy was 76 mm x 26 mm, while that for electrochemical measurements was 76 mm x 5 mm.

Infrared reflection-absorption spectroscopy (IRRAS). The infrared spectra were recorded on a Fourier transform infrared spectrometer (Magna 850, Nicolet Japan Co., Ltd., Japan) at room temperature. For IRRAS measurements, a reflection attachment (model RMA-1DG/VRA, Harrick Co,. Ltd., NY) was used. The incident angle was set at 85° from the surface normal. The number of interferogram accumulations was 1000. Molecular orientation of the peptide on the surface was determined on the basis of the amide I / amide II absorbance ratio in the IRRAS spectrum according to eq. S-1 under the assumption of uniform orientation of the helix axis around the surface normal.[S-2]

$$\frac{I_1}{I_2} = 1.5 \times \frac{(3\cos^2\gamma - 1)(3\cos^2\theta_1 - 1)\quad 2}{(3\cos^2\gamma - 1)(3\cos^2\theta_2 - 1)\quad 2} \qquad (S-1)$$

I_i, γ, θ_i (i=1 or 2 corresponding to amide I or amide II) represent the observed absorbance, the tilt angle of helix axis from the surface normal, and the angle between each transition moment and the helix axis, respectively. The values of the θ_1 and θ_2 are taken to be 39° and 58°, respectively. The IRRAS spectra of the helical peptide SAMs are shown in Figure S-5.
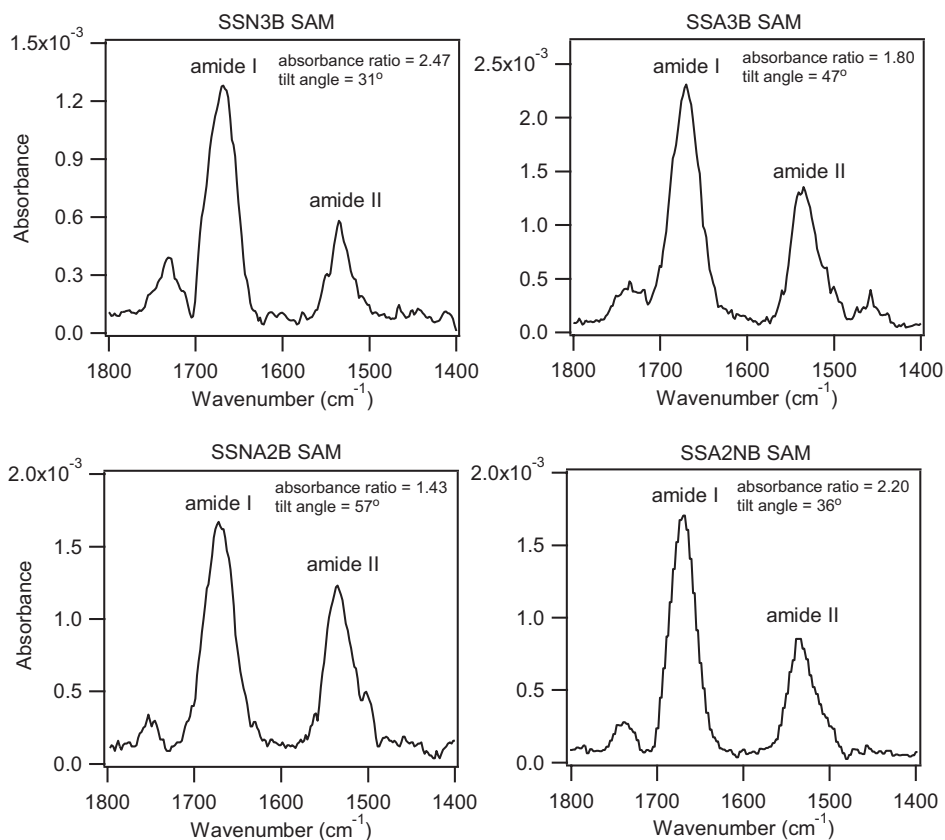
**Figure S-5.** IRRAS spectra of the helical peptide SAMs on gold.

Cyclic voltammetry. Cyclic voltammograms were obtained using a voltammetric analyzer (model 604, BAS Co., Ltd., Japan) at room temperature. A standard three-electrode setup was used with the SAM-modified substrate as the working electrode, Ag/AgCl/NaCl as the reference electrode, and a platinum wire as the auxiliary electrode in a glass vessel. All of the electric potentials reported in this paper were measured with respect to the reference electrode. The redox reagent used for blocking experiments was a 1 mM $K_4[Fe(CN)_6]$ in 1 M KCl aqueous solution. The sweep rate was set at 50 mV s^{-1} for all measurements. The formation of the well-packed peptide SAMs are identified as shown in Figure S-6.
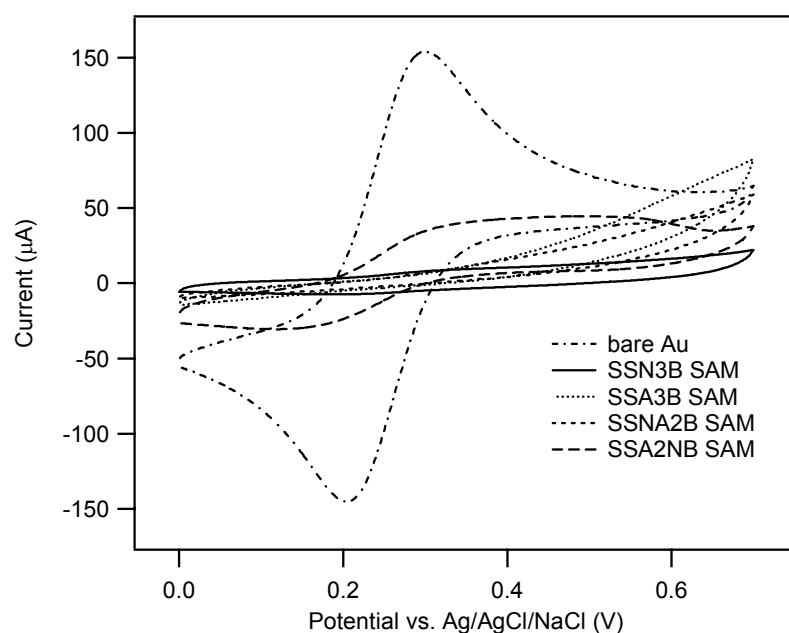
Figure S-6. Cyclic voltammograms of a bare gold substrate and the SAM-modified substrates in a 1 mM $K_4[Fe(CN)_6]$ and 1 M KCl aqueous solution at a scan rate of 50 mV s^{-1}.

Photocurrent generation experiments. Photocurrent measurements were carried out at applied potential of 0 V on gold with respect to the reference electrode at room temperature using the three-electrode setup described above. The supporting electrolyte was a 0.1 M Na_2SO_4 aqueous solution. The SAM-modified substrates were photoirradiated with a Xe lamp (500W, JASCO Co., Ltd., Japan) equipped with an MZ0280 band pass filter (fwhm 17 nm, Asahi Spectra Co., Ltd., Japan). The intensity of irradiating light at 280 nm was evaluated to be 1.2×10^{14} photons/s by a potassium ferrioxalate actinometry (S-3). The irradiated area of electrode was set at 0.2 cm^2. The current from the substrate was detected by the voltammetric analyzer describe above. The concentration of an electron donor, triethanolamine, was 50 mM. The action spectrum of the bare gold substrate and SSN3B SAM for anodic photocurrent generation is shown in Figure S-7 together with absorption spectrum of SSN3B in ethanol. The action spectrum was recorded by a light through a monochrometer (fwhm 10 nm) and normalized to the photon intensity at each wavelength. The action spectrum of the SSN3B SAM deviates from the absorption spectrum at the shorter wavelength region (250-270 nm) because of the absorption by gold. At the longer wavelength region (280-330 nm), however, the action spectrum agrees well with the absorption spectrum. Quantum yield for photocurrent generation by the SSN3B was calculated by dividing the photocurrent by the number of photons absorbed by the naphthyl groups per unit time. The latter value was calculated on the basis of molar extinction coefficient and surface density of the naphthyl group, and light intensity. The surface density of the naphthyl groups was obtained according to the molecular cross-sectional area of 1.30 nm^2 and the tilt angle determined by the IRRAS measurement.
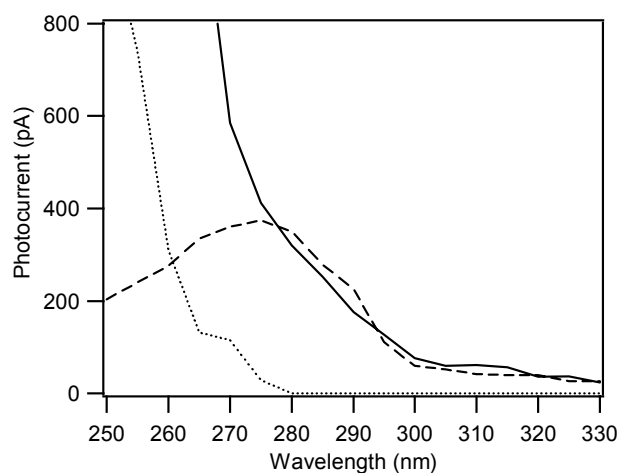
Figure S-7. Photocurrent action spectra of the bare gold substrate (dotted line) and SSN3B SAM (solid line) for anodic photocurrent generation with triethanolamine and the absorption spectrum of SSN3B in ethanol (dashed line).

Reference

(S-1) Inai, I.; Hirabayashi, T. *Biopolymers* **2001**, *59*, 356-369.

(S-2) Miura, Y.; Kimura, S.; Imanishi, Y.; Umemura, J. *Langmuir* **1998**, *14*, 2761-2767.

(S-3) Hatchard, C. G.; Parker, C. A. *Proc. R. Soc., Ser. A* **1956**, *235*, 518-536.