Electronic Supplementary Information

Photoinduced growth system of peptide nanofibers addressed by DNA hybridization

Masahiro Furutani,[a,b] Akihito Uemura,[a] Akira Shigenaga,[c] Chiaki Komiya,[c] Akira Otaka[c] and Kazunori Matsuura*[a]

[a] Graduate School of Engineering, Tottori University, 4-101 Koyama-Minami, Tottori 680-8552, Japan.
[b] Department of Pure and Applied Chemistry, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan (Current address).
[c] Graduate School of Pharmaceutical Sciences, The University of Tokushima, 1-78-1 Shomachi, Tokushima 770-8505, Japan.

Contents:

1. Equipment and materials.

High performance liquid chromatography (HPLC) was performed using a Shimadzu LC-6AD liquid chromatograph with GL Science Inertsil WP300 C18 columns (4.6 × 250 mm for analysis and 20 × 250 mm for purification). Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectra were taken using a Bruker Daltonics Autoflex II with 3-hydroxypicolinic acid as a matrix. Electrospray ionization (ESI) mass spectrum of the conjugate was taken using a Thermo Scientific Exactive ESI/DART FTMS.

UV-vis spectra were obtained using a Jasco V-630 with a quartz cell (S10-UV-1, GL Sciences Inc., Tokyo, Japan). Fluorescense measurements were performed using a Jasco FP-8200. ATR-FT-IR spectra were measured using a PerkinElmer Spectrum 65 FT-IR.

The aggregated and self-assembled nanostructures were observed using a Jeol JEM 1400 Plus transmission electron microscope (TEM) with a grid (C-SMART Hydrophilic TEM grid, ALLIANCE Biosystems Inc., Osaka, Japan), and a Hitachi SU8020 scanning electron microscope (SEM). Vapor deposition was performed for the specimen of SEM observation using a Hitachi E-1045. A DNA hybridizing behaviors were observed using an OLYMPUS FLUOVIEW FV 10i-O with a fluorochrome.

UV irradiation was performed using an LED lump, ULEDN 102-CT (365 nm, 4 W/cm^2, NS Lighting Co., Ltd.).

The reagents used were purchased from Watanabe Chemical Ind., Ltd. (Hiroshima, Japan), Tokyo Chemical Industry Co. (Tokyo, Japan), Dojindo Laboratories Co., Ltd. (Kumamoto, Japan) and Wako Pure Chemical Industries (Osaka, Japan). Chemically modified DNAs (dA_{20}-5'aminoC6, dT_{20}-5'mercaptoC6, and dA_{20}-5'mercaptoC6) were purchased from Gene Design Inc. (Osaka, Japan). All the chemicals were used without further purification. In all experiments, ion exchanged water was used.

2. Synthesis of DNA conjugated peptide **1**.

2.1. Synthesis of Fmoc-protected photoresponsive amino acid.

An Fmoc-protected photoresponsive amino acid, (*S*)-3,3-dimethyl-3-[2,4-dimethyl-6-(2-nitrobenzyloxy)phenyl]-2-(9-fluorenylmethoxycarbonylamino)propionic acid, was synthesized according to the literature.[S1]

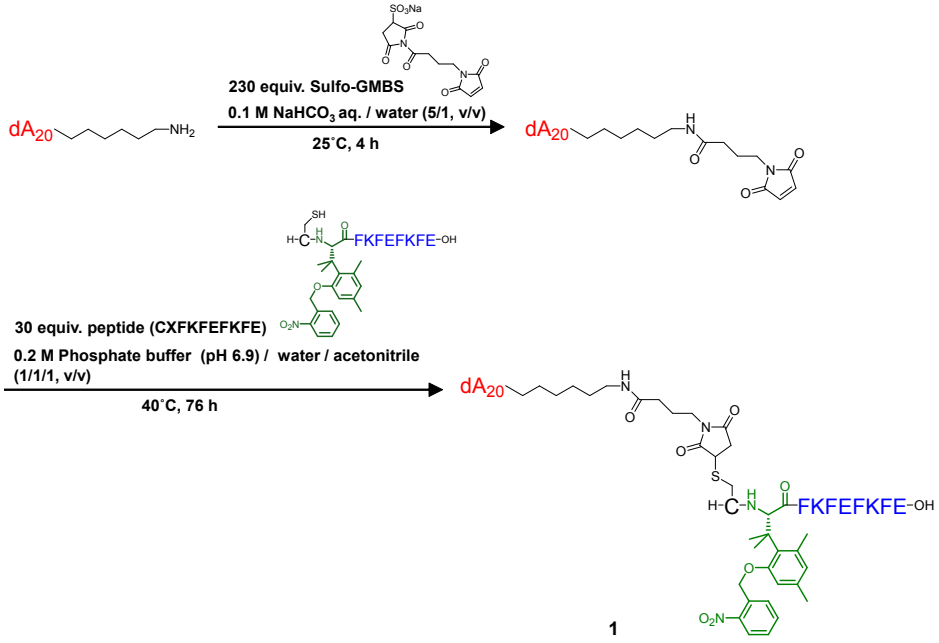
2.2. Photoresponsive peptide moiety.

The 10-mer photoresponsive peptide, CXFKFEFKFE (C: cysteine, X: photoresponsive amino acid residue, F: phenylalanine, K: lysine, and E: glutamic acid), was synthesized by Fmoc solid phase peptide synthesis. The amino group of each amino acid was protected with 9-fluorenylmethyloxycarbonyl (Fmoc) group. The mercapto, amino, and carboxy groups of C, K, and E were protected with trityl, *tert*-butoxycarbonyl, and *tert*-butyl groups, respectively.

To the resin having free amino groups as reaction sites (0.81 mmol/g, 25 mg) were added Fmoc amino acid (4 equiv.), 1-[(1-(Cyano-2-ethoxy-2-oxoethylideneaminooxy)-dimethylamino-morpholinomethylene)] methanaminium hexafluorophosphate (COMU, 4 equiv.), and diisopropylethylamine (DIPEA, 8 equiv.) in *N*-methylpyrrolidone (NMP). Each condensation reaction was performed at room temperature for 1 h. Removal of Fmoc groups from the resin was performed using a 20 % piperidine *N*,*N*-dimethylformamide (DMF) solution. Both introduction of Fmoc amino acids and removal of Fmoc groups were checked by a 2,4,6-trinitrobenzene sulfonic acid (TNBS) test kit (Tokyo Chemical Industry Co., Ltd.) after washing the resin with NMP (2 mL × 5).

Cleavage of the 10-mer peptide and deprotection at side chains were performed with a cleavage cocktail (trifluoroacetic acid (TFA)/thioanisole/water/ethanedithiol/ triisopropylsilane = 81.5/5/5/1/1, v/v/v/v/v). The mixture was stirred at room temperature for 3 h. After filtration, the solution was evaporated, followed by trituration with *tert*-butylmethylether (25 mL × 3) to give the product in a 79% yield (crude) as white solid. Purification was performed by reverse phase (RP) HPLC with water/acetonitrile (both containing 0.1 % TFA, 75/25 to 40/60, v/v for 70 min, linear gradient, 10 mL/min, detected at 220 nm) to give the purified product in a 39 % yield (3.9 mg). ESI-MS: *m/z* 1578.73 ($[M+H]^+$), 790.87 ($[M+2H]^{2+}$).

2.3. DNA conjugated peptide **1**.



Scheme S1 Synthesis of DNA conjugated peptide **1**.

At first, a maleimide group was introduced to the 5' end of a dA_{20}. To a 1.05 mM aqueous solution of dA_{20}-5'aminoC6 (20 mer of deoxyadenosine monophosphate having an amino group via a hexamethylene chain at the 5' end, denoted as dA_{20}, 32 nmol, 30 μL) were added *N*-(4-maleimidobutyryloxy)-sulfosuccinimide sodium salt (Sulfo-GMBS, 230 equiv., 2.8 mg, 7.3 μmol) and 0.1 M $NaHCO_3$ aqueous solution (150 μL). The mixture was incubated at 25˚C for 4 h, monitored by RP-HPLC with 0.1 M ammonium formate aqueous solution/acetonitrile (95/5 to 0/100, v/v for 95 min, linear gradient, 1 mL/min, detected at 260 nm). Dialysis was performed using a dialysis membrane (Spectra/por7, cutoff M_w: 1000, Spectrum Laboratories, Inc.) with water for 20 h. After evaporation, the residue was used for the next reaction without further purification. MALDI-TOF-MS: *m/z* found: 6570 ($[M+Na]^+$), calcd. for $C_{214}H_{262}N_{102}O_{104}P_{20}Na$: 6570.

To CXFKFEFKFE peptide (1.5 mg, 0.95 μmol) in water/acetonitrile (1/1, v/v, 600 μL) was added 0.2 M phosphate buffer (300 μL, pH 6.9). The peptide solution was then added to the residue. The mixture was incubated at 40˚C for 76 h, monitored by RP-HPLC with 0.1 M ammonium formate aqueous solution/acetonitrile (95/5 to 0/100, v/v for 95 min, linear gradient, 1 mL/min, detected at 260 nm). Purification was performed by RP-HPLC with 0.1 M ammonium formate aqueous solution/acetonitrile (95/5 to

0/100, v/v for 95 min, linear gradient, 10 mL/min, detected at 260 nm) to give the purified product. MALDI-TOF-MS: *m/z* found: 8137 ($[M+Na]^+$, broard peark), calcd. for $C_{295}H_{365}N_{115}O_{122}P_{20}SNa$: 8148. The product was kept at -20˚C in a freezer, and the concentration of sample solutions was defined using UV-vis spectroscopy, just before the measurement.

3. Analysis of photocleavage reaction of DNA conjugated peptide **1**.

UV irradiation was performed at 365 nm to 1 μM of DNA conjugated peptide **1** solution (20 μL). The solution was then subjected to RP-HPLC analysis (0.1 M of ammonium formate aqueous solution/acetonitrile (100/0 to 0/100, v/v for 400 min, linear gradient, 1 mL/min, detected at 260 nm).

After UV irradiation, as shown in Figure S1, the peak of **1** disappeared, while new two peaks of **2** and **3** appeared. By MALDI-TOF-MS and HPLC analyses, compounds **2** and **3** were found to be the photocleavaged DNA fragment and the peptide fragment, FKFEFKFE (Scheme S2). MALDI-TOF-MS for **2**: *m/z* found: 6892 ($[M+Na]^+$), calcd. for $C_{230}H_{283}N_{104}O_{107}P_{20}SNa$: 6891. MALDI-TOF-MS for **3**: *m/z* found: 1143 ($[M+Na]^+$), calcd. for $C_{58}H_{76}N_{10}O_{13}Na$: 1143.

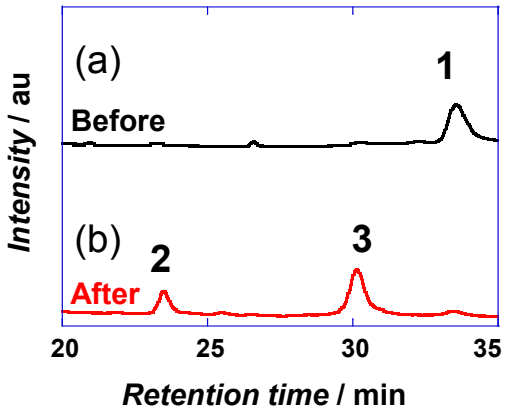
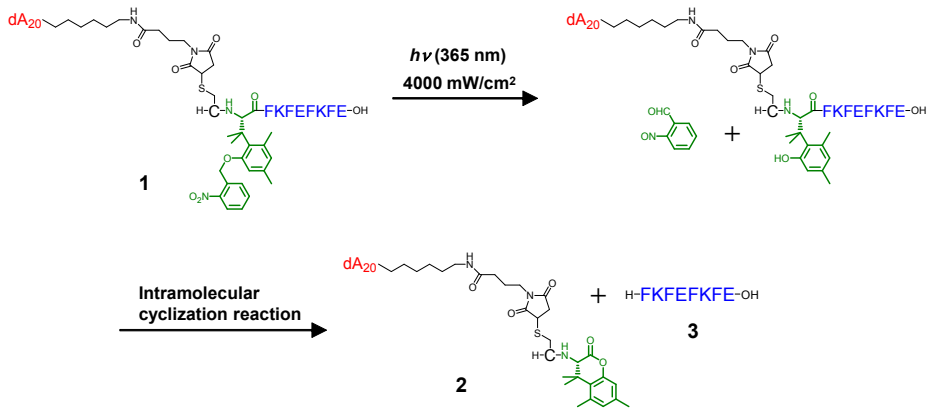


Figure S1 HPLC charts of DNA conjugated peptide **1** aqueous solutions before (a) and after (b) UV irradiation for 90 s.



Scheme S2 Photodecomposition reaction of DNA conjugated peptide **1**.

4. Analyses of photoinduced growth of peptide fibers.

4.1. TEM.

UV irradiation was performed at 365 nm to 10 μM of DNA conjugated peptide **1** solution (water/acetonitrile (9/1, v/v), 5 μL). The solution was put on a TEM grid, allowed to stand for 1 min, and then removed. The grid was exposed to 2% phosphotungstic acid ($Na_3(PW_{12}O_{40})$ $(H_2O)_n$) aqueous solution (5 μL), which was allowed to stand for 1 min, and then removed. The resulting grid was dried in vacuo.

4.2. Turbidity measurement.

Optical density at 400 nm was monitored by an UV-vis spectrometer. Before UV irradiation, 10 μM of DNA conjugated peptide **1** solution (water/acetonitrile (9/1, v/v), 50 μL) was subjected to the measurement. The sample in a cuvette was irradiated at 365 nm for total 12 min (20 s × 36). The measurement was continued at 30 s intervals.

4.3. ATR-FT-IR measurement.

UV irradiation was performed to 60 μM of DNA conjugated peptide **1** solution (water/acetonitrile (9/1, v/v), 3 μL) for 4 min, and the solution was allowed to stand for 16 h. On the ATR prism, the solution (1 μL) was dropped and dried by an air blower for

the measurement.

5. Analyses of photoinduced growth of peptide nanofibers addressed by DNA hybridization.

5.1. Preparation of DNA-immobilized glass substrates.[S2]

Glass substrates (cover glasses, Matsunami 18 × 18 mm, No. 1) were subjected to an ultrasonic treatment in chloroform/methanol (1/1, v/v) for 5 min. After air drying, the substrates were treated with a Piranha solution (conc. H_2SO_4/30% H_2O_2 aqueous solution, 7/3, v/v) for 3 h, followed by washing with water and subsequent drying in vacuo. DNA molecules were then linked as shown in Figure S2.

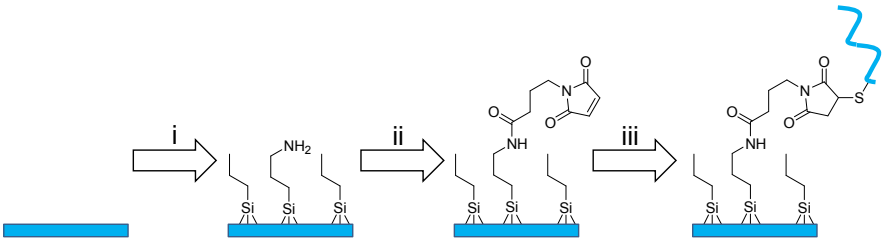


Figure S2 Illustration of the procedure for immobilization of DNA molecules on glass substrates.

(Step i: introduction of amino groups) A mixture of 3-aminopropyltriethoxysilane and triethoxy(propyl)silane (1/10, v/v, the appearance molecular weight: 207.72) was prepared, and diluted to 50 mM with toluene. The solution was put on a washed glass substrate, and allowed to stand at room temperature for 3 h. The substrate was washed with toluene, followed by heating at 120˚C for 1 h.

(Step ii: introduction of maleimide groups) To 20 mM of Sulfo-GMBS DMF solution (20 μL) was added 50 mM of $NaHCO_3$ aqueous solution (pH 8.4, 180 μL). A droplet of this solution (50 μL) was put on the substrate having amino groups, and allowed to stand at room temperature for 3 h. The substrate was washed with water and dried in vacuo.

(Step iii: introduction of DNA moieties) A buffer solution (pH 7.0) containing 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, 10 mM), ethylene-diaminetetraacetic acid (EDTA, 5 mM), dithiothreitol (DTT, 65 mM), and a single strand DNA (dT_{20} (or dA_{20})-5'mercaptoC6, 20 mer of deoxyadenosine (or thymidine) monophosphate having an mercapto group via a hexamethylene chain at the 5' end, denoted as dT_{20} (or dA_{20}), 200 μM) was prepared. The solution was incubated at 37˚C for 2 h, followed by washing ethyl acetate (200 μL × 5) for removal of excess DTT. The buffer was mixed with dimethylsulfoxide (DMSO) (buffer/DMSO = 6/4, v/v), which was put on the substrate having maleimide groups, and allowed to stand at room

temperature for 2 h. The substrate was washed with water (50 μL × 2) and dried in vacuo.

5.2. SEM.

To a DNA-immobilized substrate was dropped 10 μM of DNA conjugated peptide **1** solution (water/acetonitrile = 9/1, v/v, 10 μL). The sample was allowed to stand at room temperature for 8 h in dark, followed by washing with water (20 μL × 3). UV irradiation was performed for 150 s. Each sample was then dried in vacuo for 3 h in dark, and vapor deposition was performed with Pt-Pd.

The combinations of DNAs and UV irradiation are as follows,

(a) solution-dA_{20}, substrate-dT_{20}, with UV irradiation,

(b) solution-dA_{20}, substrate-dT_{20}, without UV irradiation,

(c) solution-dA_{20}, substrate-dA_{20}, with UV irradiation,

where DNA conjugated peptide **1** has a dA_{20} moiety.

5.3. Fluorescence microscopy.

Hybridization between dA_{20} and dT_{20} would be effective under the condition, which was further examined by a fluorescence microscopic observation using 4',6-diamidino-2-phenylindole (DAPI, Fig. S4). DAPI is a fluorophore that has an emission wavelength at 460 nm, and it is specifically bound by a minor groove consisted of adenines and thymines.[S3]

To a dT_{20}-immobilized glass substrate was dropped 10 μM DNA conjugated peptide **1** solution (water/acetonitrile = 9/1, v/v, 5 μL). The sample was allowed to stand at room temperature for 8 h in dark, followed by washing with water. After that, 2.5 μM 4',6-diamidino-2-phenylindole (DAPI) aqueous solution (10 μL) was dropped, allowed to stand for 30 min, and followed by washing with water.

A dT_{20} immobilized glass substrate was treated with 10 μM solution of **1**, obtaining a stronger fluorescence intensity than that of the case without such treatment (Figure S3).

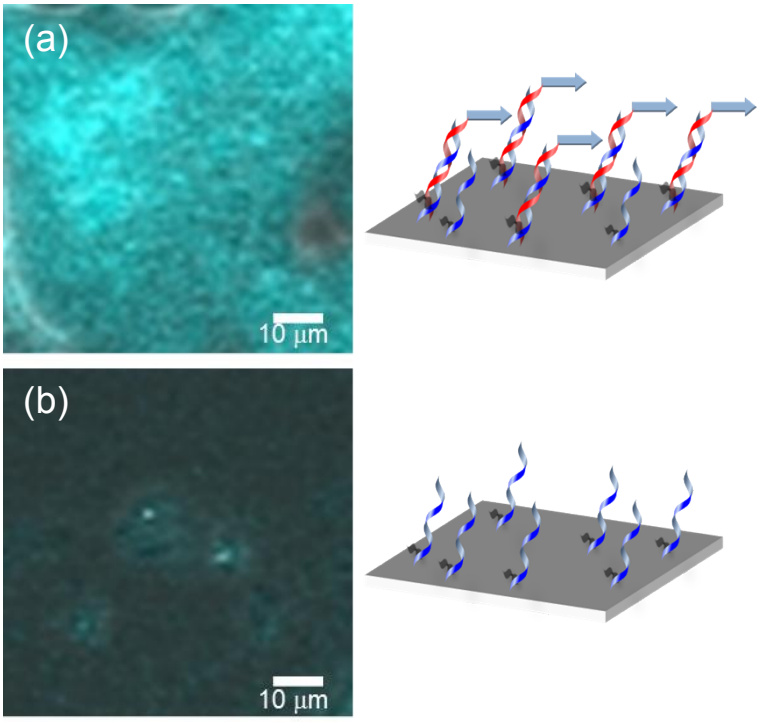


Figure S3 Fluorescence microscopic images with DAPI (excitation wavelength: 365 nm, emission wavelength: 460 nm) reflecting a DNA hybridization between dA_{20} and dT_{20}. (a) Immobilized dT_{20} with DNA (dA_{20}) conjugated peptide **1**, and (b) immobilized dT_{20} only.

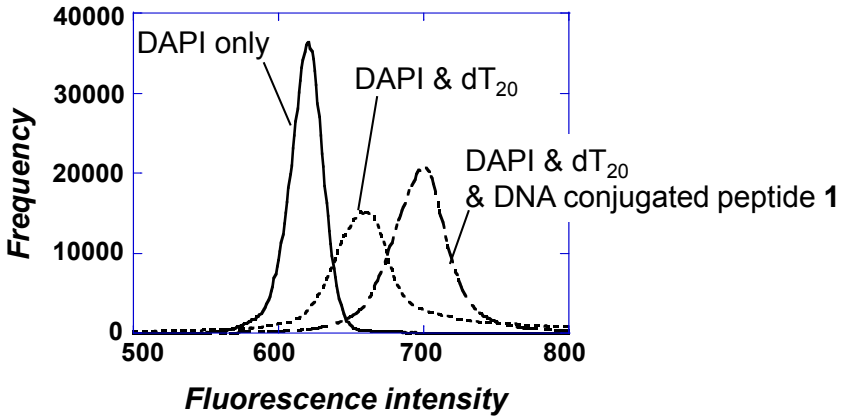


Figure S4 Frequency of fluorescence intensity of the samples consisted of DAPI, dT_{20} (on glass substrate), and/or DNA conjugated peptide **1**.

On the fluorescence microscopic observation (excitation wavelength: 365 nm), frequency of the fluorescence intensity of samples is shown in Figure S4. This would also indicate that the hybridization between dA_{20} and dT_{20} contributes to enhancement of the fluorescence intensity.

6. Quantitative analysis of hybridization.

To a dT_{20}-immobilized glass substrate was dropped dA_{20} solution (water/acetonitrile = 9/1, v/v, 5 μL). The concentrations of the solutions were 0.1, 1.0, 2.5, 5.0, and 10 μM. Each sample was allowed to stand at room temperature for 8 h, followed by washing with water. After that, 2.5 μM DAPI aqueous solution (10 μL) was dropped, allowed to stand for 10 min, and followed by washing with water. Fluorescence intensity at 460 nm (excitation wavelength: 365 nm) for each concentration was plotted as shown in Figure S5.

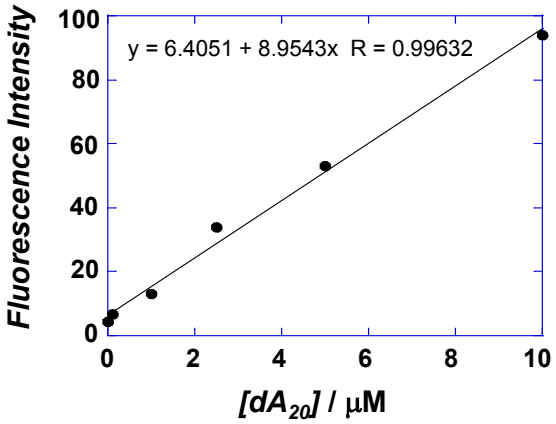


Figure S5 Calibration curve of fluorescence intensity at 460 nm (emission wavelength of DAPI) of dT_{20} solutions (water/acetonitrile = 9/1, v/v), titrated with dA_{20}.

To another dT_{20}-immobilized glass substrate was dropped 10 μM of DNA conjugated peptide **1** solution (water/acetonitrile = 9/1, v/v, 5 μL). The sample was allowed to stand at room temperature for 8 h, followed by washing with water. After that, 2.5 μM of DAPI aqueous solution (10 μL) was dropped, allowed to stand for 10 min, and followed by washing with water. Fluorescence intensity at 460 nm (excitation wavelength: 365 nm) was then measured to estimate the amount of hybridized **1** on the substrate. The hybridization rate between dT_{20} moieties and **1** was estimated at ca. 50% using the calibration curve. A bulky peptide moiety could disturb the hybridization, resulting in halving of the amount of hybridized **1**.

7. Calculation of peptide densities for self-assembling.

From our experimental result (TEM observation in Fig. 2b), DNA-conjugated peptide **1** could self-assemble to form fibrous structures after UV irradiation, with a 10 μM solution. The number of peptides per unit volume is calculated as follows using the Avogadro's number (6.0×10^{23} molecules/mol),

$$10^{-5}\ [\text{mol/L}] \times (6.0 \times 10^{23})\ [\text{molecules/mol}] = 6.0 \times 10^{18}\ [\text{molecules/L}]$$
$$= 6.0 \times 10^{-6}\ [\text{molecules/nm}^3]$$

where 1 L = 10^{24} nm^3. Given a space having a height corresponding to the maximum length of the FKFEFKFE peptide (5.6 nm, the extended state in anti-parallel β-sheet structures[S4]) where peptides can move only in a transverse direction, the density of peptides is,

$$6.0 \times 10^{-6}\ [\text{molecules/nm}^3] \times 5.6\ [\text{nm}] = \mathbf{3.4 \times 10^{-5}}\ [\text{molecules/nm}^2].$$

On the other hand, the DNA-immobilized glass substrate was fabricated by following the procedure in the literature,[S2] and the molecular density is estimated as 0.3 molecules/nm^2. From the result at section 6 in this ESI, the hybridization rate between immobilized DNAs and conjugates was estimated at ca. 50% using the calibration curve. The net density is then calculated as,

$$0.3\ [\text{molecules/nm}^2] \times 0.5 = \mathbf{1.5 \times 10^{-1}}\ [\text{molecules/nm}^2].$$

Above calculated results indicates that the peptide density at the hybridized surface could be enough for the liberated peptides to form peptide fibrous structures.

Reference

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