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Preparation of (Inorganic/Organic) Hybrid Hydrogel from Peptide Oligomer and Tubular Aluminosilicate Nanofiber

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1. Materials and methods

Materials. Imogolite was synthesized by previous reported methods.[1] All reagents and solvents for peptide synthesis were used without further purification. Dichloromethane (DCM), *N*,*N*-dimethylformamide (DMF), *N*,*N*-diisopropylethylamine (DIPEA), 1-[bis(dimethylamino)methyliumyl]-1H-benzotriazole-3-oxide hexafluorophosphate (HBTU), 1-hydroxy-1H-benzotriazole hydrate (HOBt•H_2O), piperdine (PPD), trifluoroacetic acid (TFA) were purchased from Watanabe Chemical Industry (Hiroshima, Japan). CHCA (α-cyano-4-hydroxycinnamic acid), 9-fluorenylmethyloxycarbonyl (Fmoc)-Arg(Pbf)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ser(PO(OBzl)OH)-OH and Rink Amide AM resin (100-200 mesh) were purchased from Merck Millipore (Burlington, MA). Fmoc-Asp(OtBu)-OH, Fmoc-Phe-OH was purchased from Peptide Institute (Osaka. Japan). Ethanol, methanol and triisopropylsilane (TIS) were purchased from Wako (Osaka, Japan). Ninhydrin was purchased from Tokyo Chemical Industry (Tokyo, Japan). Acetonitrile (ACN) and diethyl ether were purchased from Kanto Chemical (Tokyo, Japan).

Peptide Synthesis. All peptides (**Figure S1**) were synthesized by manual Fmoc solid-phase peptide synthesis. Rink Amide AM resin (0.1 mmol) was first loaded on a PD-10 empty column (GE healthcare, Little Chalfont, UK), and was pre-swollen in DCM for 60 min. The N-terminal Fmoc protecting group of the resin was deprotected by soaking 20% PPD in DMF for 10 min. The resin bearing NH_2-groups was washed with DMF (2 mL×5). The Fmoc deprotection was confirmed by a positive ninhydrin test. A Fmoc-protected amino acid (0.3 mmol, 3 equiv. to the loaded resin) was first activated in a coupling cocktail containing HBTU (3 equiv.), HOBt (3 equiv.), and DIPEA (6 equiv.) in DMF, and subjected to the resin for a 60-min coupling. The resin was washed with DMF (2 mL×5) and DCM (2 mL). The completion of the reaction was confirmed by a negative ninhydrin test. The process of Fmoc-group deprotection and subsequent Fmoc-protected amino acids coupling was repeated to the last amino acid residue. The resin was washed with DMF (2 mL×5), DCM (2 mL×5), DMF/DCM (1:1, 2 mL×5), methanol (2 mL×5), and soaked in methanol for 15 min. The shrunk resin was dried under reduced pressure for 60 min.

The cleavage of peptides from a resin was conducted soaking the resin in a solution of TFA/H_2O/TIS (95/2.5/2.5) for 120 min. The filtrate was concentrated by evaporation under reduced pressure and precipitated with cold diethyl ether (15 mL×3). The obtained crude peptides were dried under room temperature.

The crude peptides were dissolved in an ACN/water (30/70, 0.1% TFA). The filtered crude peptides were injected into a SunFireC_{18} column 19×50 mm (Waters Corporation, Milford, MA, USA) for reverse phase (RP)-HPLC purification. The collected fractions were lyophilized.

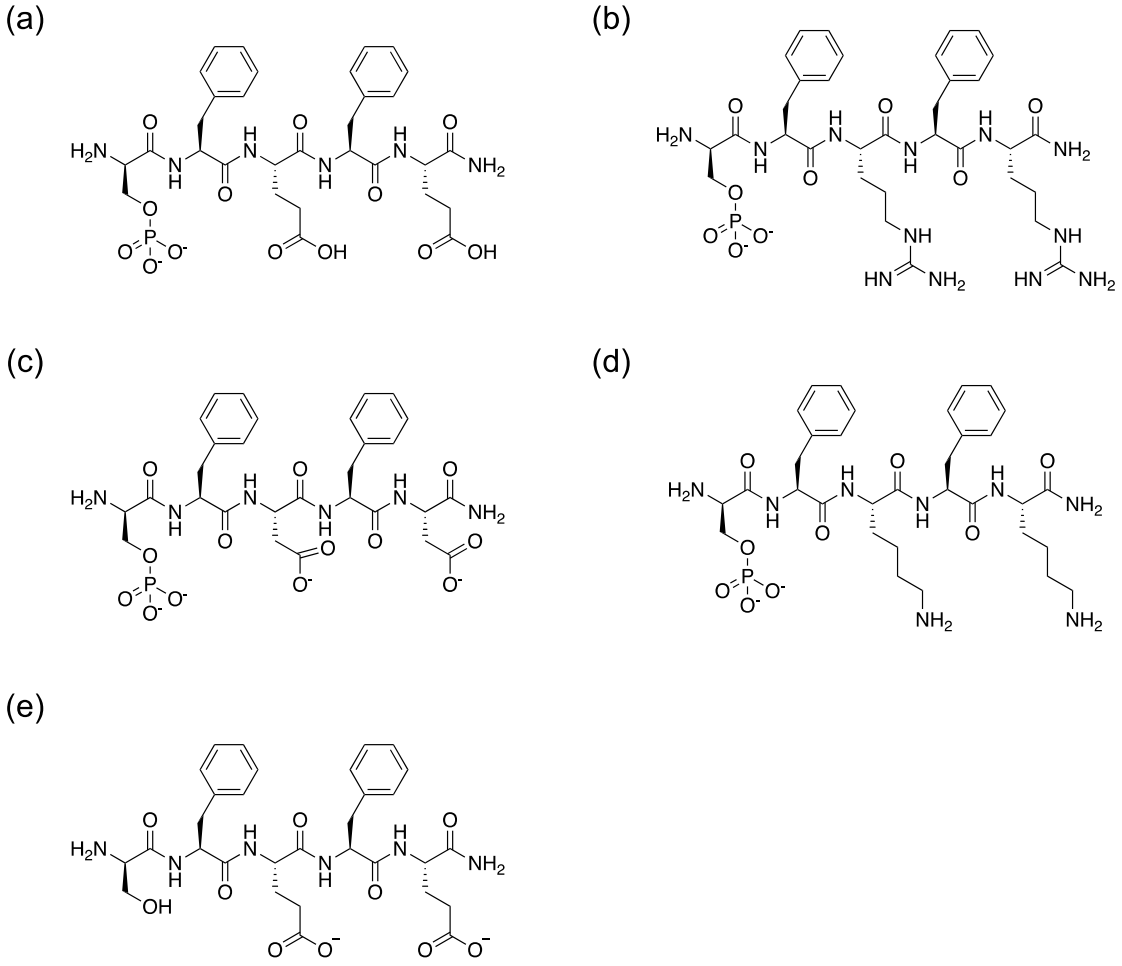

Fig. S1 Chemical structures of peptide oligomer. (a) NH_2-pSFEFE-NH_2, (b) NH_2-pSFRFR-NH_2, (c) NH_2-pSFDFD-NH_2, (d) NH_2-pSFKFK-NH_2, (e) NH_2-SFEFE-NH_2.

Characterization. NMR spectra of the synthesized peptides were measured with AVANCE III HD 400 MHz (Bruker Co. Ltd., Massachusetts, U.S.A.) using deuterium oxide as the solvents at RT. Mass spectrometry was performed with a MStation (JEOL Ltd., Tokyo, Japan). MS samples were prepared by dissolving the synthesized compounds in water and mixing with 3-nitrobenzyl alcohol (NBA) on the FAB probe tip. Calculated masses were obtained with the program ChemDraw Professional 16.0 (PerkinElmer, Waltham, MA, USA).

The topography of imogolite and NH_2-pSFEFE-NH_2/imogolite hybrid were visualized by scanning force microscopy (SFM, SII, SPA400). SFM samples were prepared by dip-coated on a Si wafer from 0.01 mg/mL NH_2-pSFEFE-NH_2/imogolite and pristine imogolite solutions. Thermogravimetric analysis (TGA) was carried out on imogolite or NH_2-pSFEFE-NH_2/imogolite hybrid placed in an aluminum pan and heated from 25 °C to 60 °C at 10 °C/min by TG/DTA6200 (Seiko Instruments Inc., Chiba, Japan). Dynamic light scattering of hydrogel was performed using ELS-Z2 (Otsuka Electronics Co., Ltd., Tokyo, Japan) with a standard cell at room temperature. A rheological test was carried out by a Physica MCR 101 rheometer (Anton Parr, Graz, Austria) with parallel-plate geometry (diameter 50 mm; gap length 1 mm) at 25 °C. X-ray photoelectron spectroscopy (XPS) was carried out with APEX (ULVAC-PHI Inc., Kanagawa, Japan) using a monochromatic Al-Kα X-ray source (1486.6 eV). The samples were fixed on the sample holder with carbon tape and measured (take-off angle of 45°). Isoelectric points of peptide oligomers were estimated from peptide sequence using Prot pi|Peptide Tool.[1] Persistence lengths were measured from SFM image of NH_2-pSFEFE-NH_2/imogolite and neat imogolite using ImageJ software.

2. Supplementary results

Assignment of NH_2-pSFEFE-NH_2

^{1}H-NMR (400 MHz, D_2O): δ (ppm): 7.37-713 (m, 10H), 4.58 (t, J= 7.7 Hz, 1H), 4.46 (t, J =7.7 Hz, 1H), 4.30-4.04 (m, 5H), 3.03 (d, J=7.7 Hz, 2H), 2.97 (d, J=7.7 Hz, 2H), 2.38-2.14 (m, 4H), 2.13-1.73 (m, 4H).

MS (*m*/*z*):[M+H]$^+$. Calculated for $C_{31}H_{41}N_6O_{13}P+H^+$, 737.3 Obs. 737.3.

FT-IR (KBr disk): ν (cm^{-1}): 3281 (amide I (N-H st.)), 2930 (CH_2 asym.), 169 3(amide I (C=O st.)), 1657 (aromatic ring st.), 1546 (amide II (N-H bend)), 1430 (amide III (C-N st.), 1200 (P=O st.)

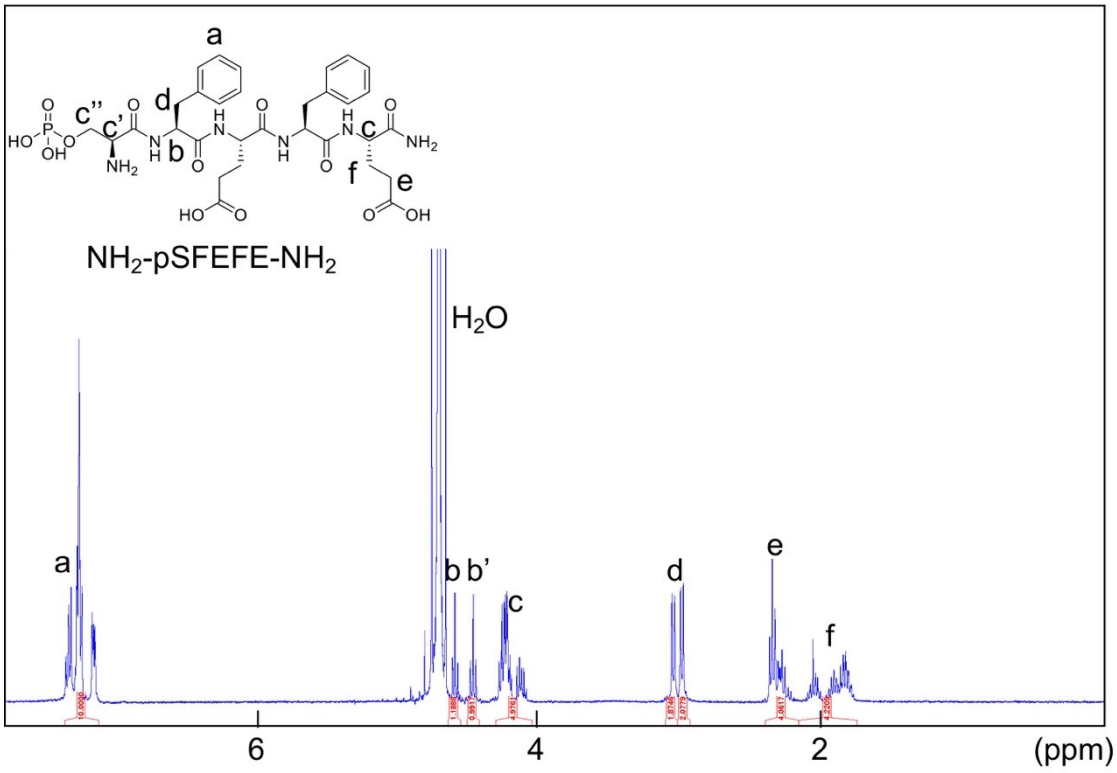

Fig. S2 ^{1}H-NMR spectrum of the NH_2-pSFEFE-NH_2.

Assignment of NH_2-SFEFE-NH_2

^{1}H-NMR (400 MHz, D_2O): δ (ppm): 7.32-7.08 (m, 10H), 4.54 (t, J= 7.4 Hz, 1H), 4.42 (t, J =7.4 Hz, 1H), 4.21-4.13 (m, 3H), 3.99 (t, J = 4.8 Hz, 1H), 3.86-3.81 (m,2H), 3.01-2.87 (m, 4H), 2.31-2.11 (m, 4H), 2.00-1.70 (m, 4H).

MS (*m*/*z*):$[M+H]^+$. Calculated for $C_{31}H_{40}N_6O_{10}+H^+$, 657.3 Obs. 657.4.

FT-IR (KBr disk): ν (cm^{-1}): 3266 (amide I (N-H st.)), 2932 (CH asym.), 1709 (amide I (C=O st.)), 1639 (aromatic ring st.),1536 (amide II (N-H bend)) 1430 (amide III C-N st.).

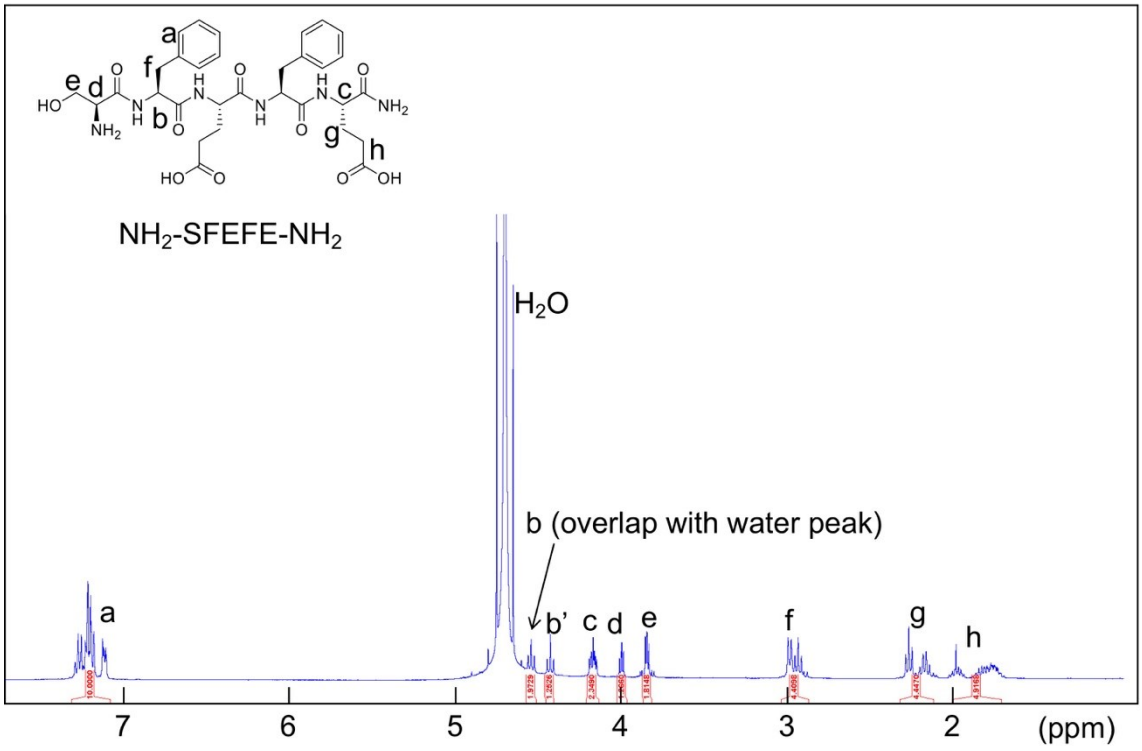

Fig. S3 ^{1}H-NMR spectrum of the NH_2-SFEFE-NH_2.

Assignment of NH_2-pSFKFK-NH_2

^{1}H-NMR (400 MHz, D_2O): δ (ppm): 7.38-7.04 (m, 10H), 4.55 (t, J= 7.6 Hz, 1H), 4.40 (t, J =7.6 Hz, 1H), 4.24-4.00 (m, 3H), 3.03-2.89 (m, 4H), 2.89-2.76 (m,4H), 1.81-1.45 (m, 8H), 1.34-1.12 (m, 4H)

MS (*m*/z):$[M+H]^+$. Calculated for $C_{33}H_{51}N_8O_9P$+H+, 735.4 Obs. 735.4

FT-IR (KBr disk): ν (cm^{-1}): 3278 (amide I (N-H st.)), 2946 (CH_2 asym.), 1693 (amide I (C=O st.)), 1633 (aromatic ring st.),1547 (amide II (N-H bend)) 1437 (amide III (C-N st.)), 1204 (P=O st.).

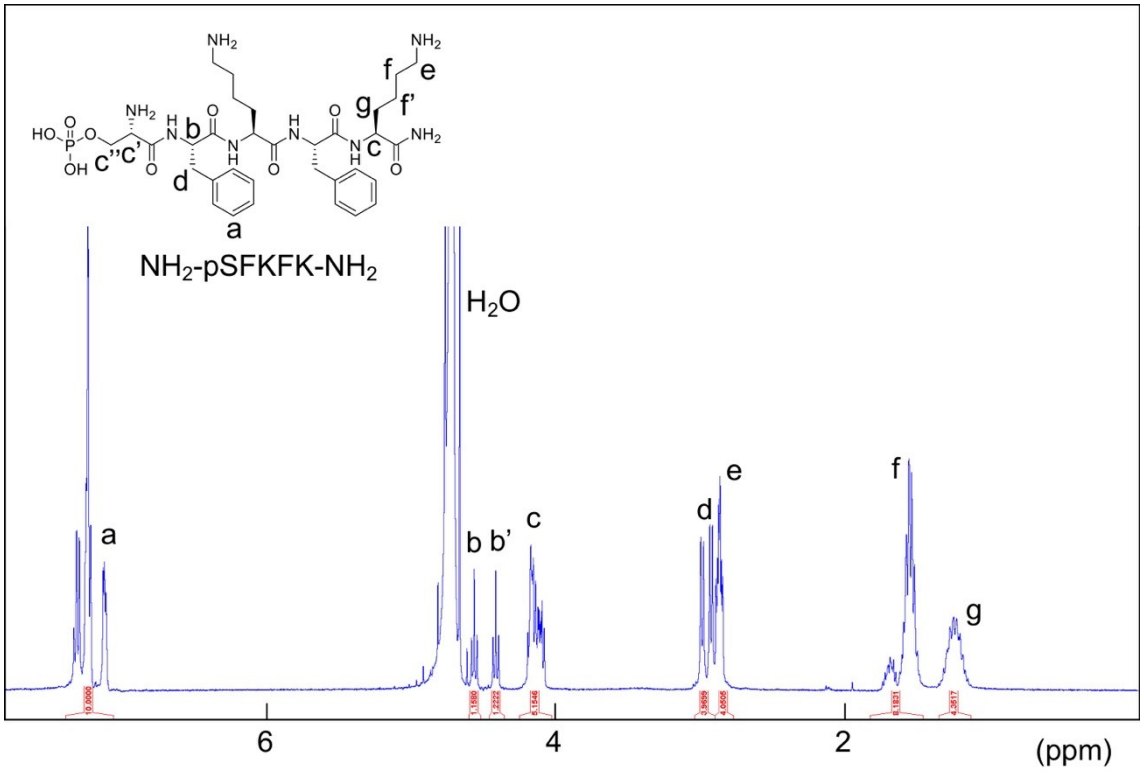

Fig. S4 ^{1}H-NMR spectrum of NH_2-SpFKFK-NH_2.

Assignment of NH_2-pSFRFR-NH_2

^{1}H-NMR (400 MHz, D_2O): δ (ppm): 7.39-7.04 (m, 10H), 4.59(t, J= 7.3 Hz, 1H), 4.42 (t, J =7.3 Hz, 1H), 4.23-4.06 (m, 5H), 3.13-2.88 (m, 8H), 1.82-1.27 (m, 8H).

MS (*m*/*z*): :$[M+H]^+$. Calculated for $C_{33}H_{51}N_{12}O_9P+H^+$, 791.4 Obs. 791.3

FT-IR (KBr disk): ν(cm^{-1}): 2941 (CH_2 asym), 1687 (amide I (C=O st.), 1650 (aromatic ring st.),1541 (amide II (N-H bend)) 1453 (amide III C-N st.), 1206 (P=O st.)

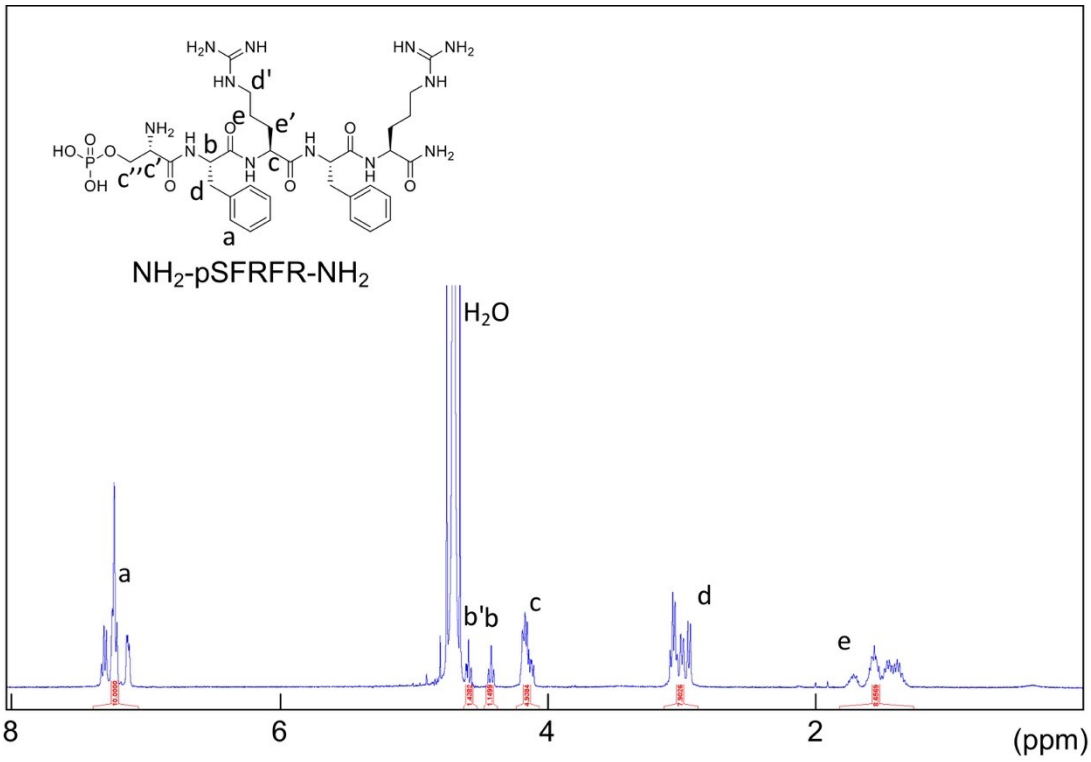

Fig. S5 ^{1}H-NMR spectrum of NH_2-pSFRFR-NH_2.

Assignment of NH_2-SpFDFD-NH_2

^{1}H-NMR (400 MHz, D_2O): δ (ppm): 7.50-7.01 (m, 10H), 4.50 (t, J= 7.6 Hz, 1H), 4.41(t, J =7.6 Hz, 1H), 4.19-3.92 (m, 5H), 3.17-2.84 (m, 4H), 2.72-2.45 (m, 4H).

MS (*m*/*z*): $[M+H]^+$. Calculated for $C_{29}H_{37}N_6O_{13}P+H^+$, 709.2 Obs. 709.3

FT-IR (KBr disk): ν (cm^{-1}): 3332 (amide I (N-H st.)), 2957 (CH_2 asym.), 1666 (amide I (C=O st.)), 1641 (aromatic ring st.), 1554 (amide II (N-H bend)), 1404(amide III (C-N st.)), 1192 (P=O, st)

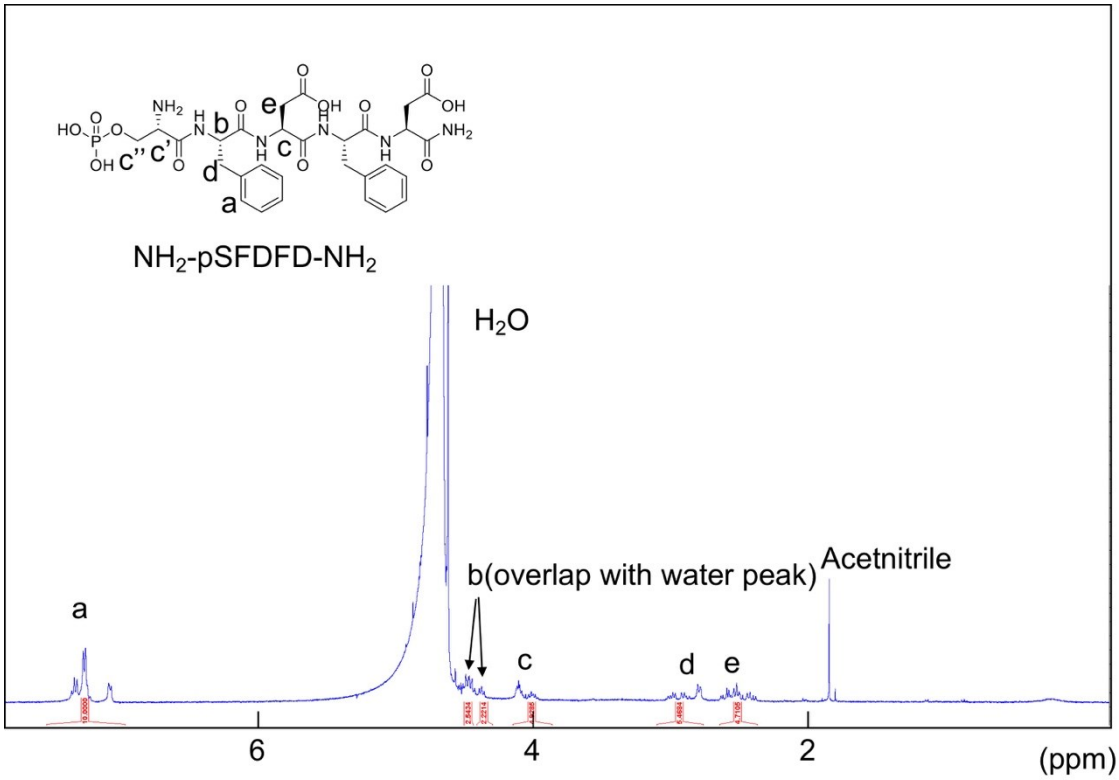

Fig. S6 ^{1}H-NMR spectrum of NH_2-SpFDFD-NH_2.

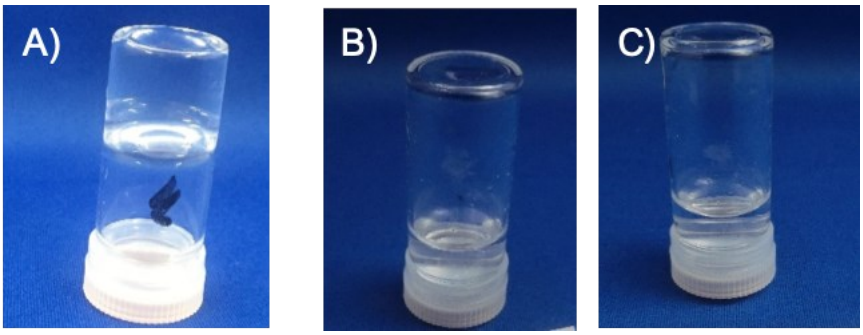

Fig. S7 Vial invertion test for NH_2-pSFEFE-NH_2/imogolite hybrid with various concentrations A) 0.30 g/mL, B) 0.15 g/mL, C) 0.10 mg/L imogolite in water.

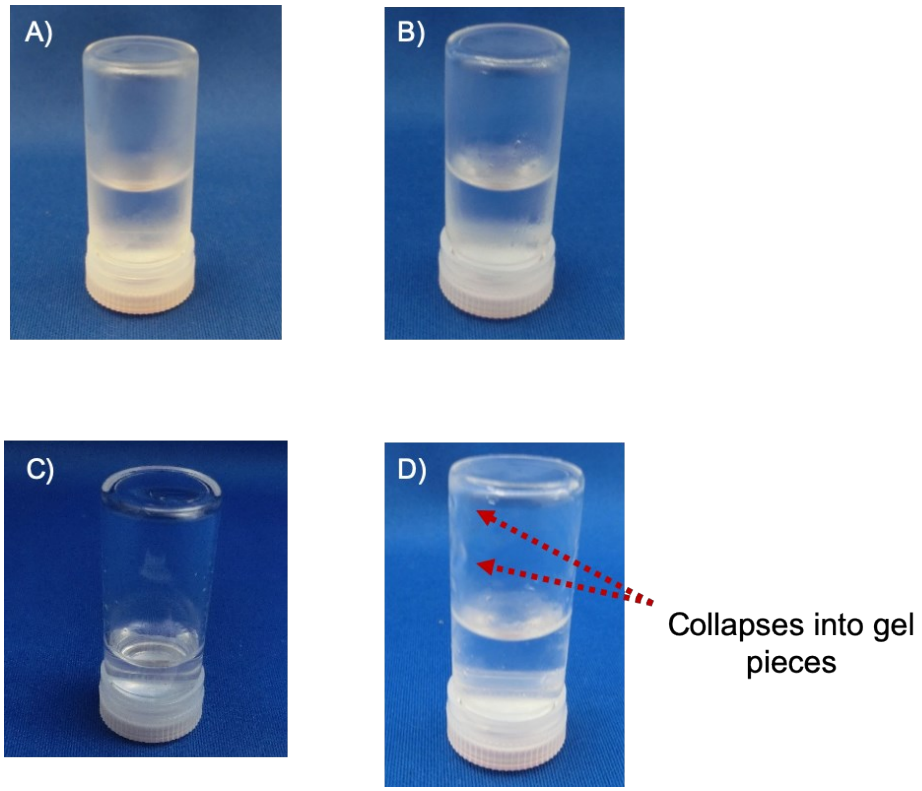

Fig. S8 Images of vial invertion test for imogolites modififed with peptides having four differet seaquences. A) NH_2-SFEFE-NH_2, B) NH_2-pSFKFK-NH_2, C) NH_2-pSFRFR-NH_2, D) NH_2-pSFKFK-NH_2.

TGA results. TGA was used to evaluate the interaction between peptide and imogolite. **Table S1** shows weight loss (wt%) of peptide-imogolite. Amount of peptide adsorbed on imogolite ($W_{peptide}$) was calculated from the following equation (1).

$$W_{Peptide} = (W'_{total} - W'_{water}) - (W_{total} - W_{water}) \quad (1)$$

Where W'_{total} and W'_{water} are the weight loss of neat imogolite at 873 K and 423 K, respectively. W_{total} and W_{water} are the weight loss of peptide-imogolite at 873 K and 423 K, respectively. The amount of peptide adsorption is calculated from the difference in the weight loss of imogolite before and after the adsorption of peptide excluding adsorbed water because the amounts of absorbed water in imogolite and peptide-adsorbed imogolite were different.

Table S1. Evaluation of peptide oligomer adsorption on imogolite by TGA.

	Weight loss (wt%)		
	W'_{total}	W'_{water}	-
Neat imogolite	65.5	83.1	-
	W_{total}	W_{water}	$W_{Peptide}$
NH_2-pSFEFE-NH_2-imogolite	66.7	82.5	1.8
NH_2-SFEFE-NH_2-imogolite	65.9	82.1	1.4
NH_2-pSFKFK-NH_2-imogolite	66.3	82.9	1.0
NH_2-pSFRFR-NH_2-imogolite	62.8	79.9	0.5
NH_2-SpFDFD-NH_2-imogolite	66.1	82.3	1.4

Deconvolution of CD spectra. The percentage of secondary structures in free NH_2-pSFFEFE-NH_2 and NH_2-pSFEFE-NH_2/imogolite hybrid were shown in **Table S2**.

Table S2. Estimated secondary structure content (%) of free NH_2-pSFFEFE-NH_2 and NH_2-pSFEFE-NH_2/imogolite hybrid.

	α-helix	Antiparallel β-sheet	Parallelβ-sheet	β-Turn	Others
free NH_2-pSFEFE-NH_2	0.00	26	0	25.1	48.9
NH_2-pSFEFE-NH_2/ Imogollite hybrid	5.7	1.8	22.4	16.3	53.9

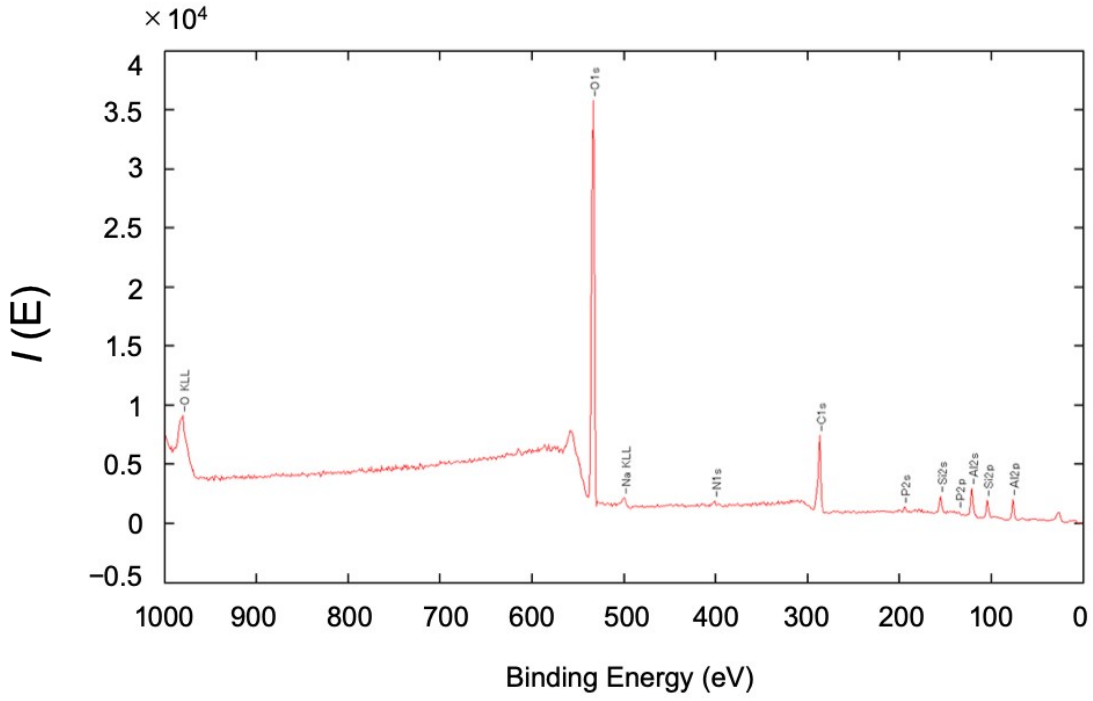

Fig. S9 X-ray photoelectron spectroscopy spectrum of NH_2-pSFEFE-NH_2/imogolite hybrid.
Si and Al peaks were assignment to imogolite. N and P peaks were assignment to peptide oligomer.

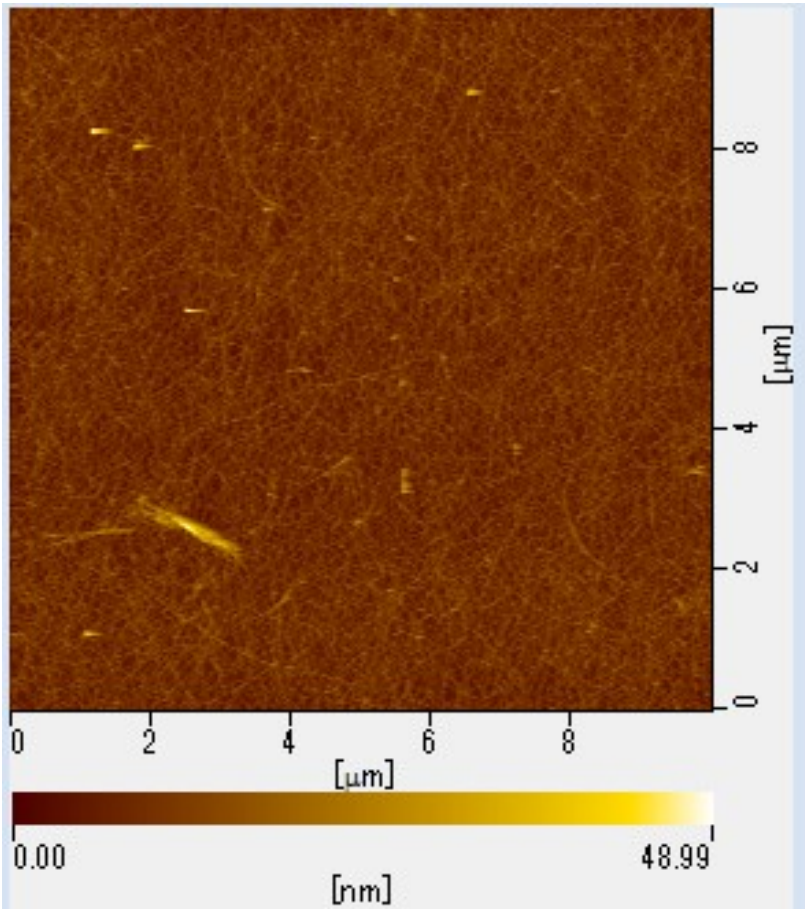

Fig. S10. SFM image of peptide/imogolite hybrid for large scanning area (10 µm x10 µm).

Reference

1. https://www.protpi.ch/Caluculator/ProteinTool/