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### Proteinase-mediated drastic morphological change of peptide-amphiphile to induce hydrogelation

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#### Experimental

Materials: Fmoc amino acids, MBHA-rink amide resin, amino acid-preloaded Barlos resin, 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium (HBTU), trifluoroacetic acid (TFA) and triisopropyl silane (TIPS) were purchased from Watanabe Chemical Industry (Hiroshima, Japan). Diisopropylethylamine (DIEA) was purchased from Tokyo Chemical Industry (Tokyo). Palmitic acid and dimethylformamide (DMF) were purchased from Kishida Chemical Industry (Osaka). Matrix metalloproteinase-7 (MMP-7) as matrilysin human recombinant, acetonitrile and diethylether were purchased from Wako Pure Chemicals Industries (Osaka). Kaiser reagents for ninhydrin tests were purchased from Kokusan Chemical (Tokyo). MMP inhibitor II (N-hydroxy-1,3-di-(4-methoxy-benzenesulfonyl)-5,5-dimethyl-[1,3]-piperazine-2-carboxamide)[10] was purchased from Merck. BSA (bovine serum albumin) and thrombin from bovine were purchased from Sigma.

Solid-phase syntheses of N-acylated peptides: Palmitylated peptides were synthesized by the standard fluorenylmethoxycarbonyl (Fmoc) solid-phase peptide synthesis protocols.<sup>1</sup> Fmoc protected amino acid (3 equiv) were coupled to 0.3 mmol amino acid-preloaded Barlos resin, using 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium (HBTU) and 1-hydroxybenzotriazole (HOBt) as the coupling agents in the presence of diisopropylethylamine (DIEA) in dimethylformamide (DMF). N-termini of the peptides synthesized on the resin were subjected to N-acylation with palmitic acid (3 equiv). Qualitative ninhydrin tests were used to confirm the completion of each coupling reaction. Deprotection and cleavage of the palmitylated peptides were performed in a mixture of trifluoroacetic acid (TFA), triisopropyl silane (TIPS) and water at a ratio of 95:2.5:2.5 for 2 h at room temperature. Palmitylated peptides in the cleavage mixture

were precipitated with water or diethylether, collected by filtration, washed with water and dried under vacuum. Resultant products were washed with acetonitrile and identified by a matrix-assisted laser desorption ionization–time of flight mass spectrometer (MALDI-TOF-MS) and <sup>1</sup>H NMR (supporting information).

Preparation of hydrogel: Palmitylated peptides were dissolved in water or buffer solution at given concentrations in a glass microtube by heating and then slowly cooling down to room temperature to form a hydrogel. Gelation was confirmed by inverting the glass microtube containing the solution.

Enzymatic reactions: After 1 mg of precursor was dissolved in 400 µl TBS buffer (50 mM Tris, NaCl 150 mM, CaCl<sub>2</sub> 2 mM, pH = 7.4), the solution was heated and cooled down to room temperature, followed by the addition of 100 µL of MMP-7 (2 µg/ml) solution. After incubation at room temperature, gelation was confirmed by inverting the glass vial containing the solution. For the inhibition study, MMP inhibitor 2 was added to the precursor solution before the addition of MMP-7. Enzymatic reaction solutions were analyzed by reverse phase HPLC equipped with an ODS column.

HPLC analysis: An inertsil ODS-3 (10×250 mm) column was used to quantify MMP-7-catalyzed hydrolysis. The gradient was 20% acetonitrile in water containing 0.1% TFA with a linear gradient of 20 to 50% acetonitrile in water containing 0.1% TFA over 10 min and then to 100% acetonitrile containing 0.1% TFA at 20 min. Isocratic 100 % acetonitrile was kept for 10 min. The eluted compounds were observed by detecting absorbance at 230 nm.

TEM observation: Hydrogels and precursor solutions were prepared for TEM observation. Carbon coated copper grids were dipped in the hydrogel samples, followed by staining with 2 % uranyl acetate and drying under vacuum to remove solvent. Solution samples were dropped onto the TEM grids, followed by the same process. TEM experiments were performed by using a Hitachi H-7000 TEM (acceleration voltage of 120 kV).

#### <sup>1</sup>H NMR assignments

*N*-Palmitoyl-GGGH: MS calc.  $M^+ = 565.4$  [m/z], obsvd.  $(M+1)^+ = 565.4$  [m/z]

$^1\text{H-NMR}$ , (DMSO- $d_6$ , 500MHz)  $\delta$  8.17(m, 1H), 7.18(s, 1H), 4.49(m, 1H), 3.71(m, 6H), 3.44-3.43(m, 2H), 3.07-2.95(m, 2H), 1.47(m, 2H), 1,23(m, 24H), 0.85(m, 3H)

*N*-Palmitoyl-GGHG: MS: calc.  $M^+ = 565.4$  [m/z], obsvd.  $(M+1)^+ = 565.4$  [m/z]

$^1\text{H-NMR}$ , (DMSO- $d_6$ , 500MHz)  $\delta$  8.26(m, 1H), 7.08(s, 1H), 4.34(m, 1H), 3.69(m, 6H), 3.44-3.40(m, 2H), 3.05-2.91(m, 2H), 1.47(m, 2H), 1,23(m, 24H), 0.85(m, 3H)

*N*-Palmitoyl-GHGG: MS [m/z]: calc.  $M^+ = 565.4$  [m/z], obsvd.  $(M+1)^+ = 565.5$  [m/z]

$^1\text{H-NMR}$ , (DMSO- $d_6$ , 500MHz)  $\delta$  8.16(m, 1H), 7.02(s, 1H), 4.35(m, 1H), 3.68(m, 6H), 3.43-3.40(m, 2H), 2.99-2.91(m, 2H), 1.46(m, 2H), 1,23(m, 24H), 0.85(m, 3H)

*N*-Palmitoyl-HGGG: MS calc.  $M^+ = 565.4$  [m/z], obsvd.  $(M+1)^+ = 565.4$  [m/z]

$^1\text{H-NMR}$ , (DMSO- $d_6$ , 500MHz)  $\delta$  8.26(m, 1H), 7.08(s, 1H), 4.34(m, 1H), 3.69(m, 6H), 3.44-3.40(m, 2H), 3.06-2.91(m, 2H), 1.47(m, 2H), 1,23(m, 24H), 0.85(m, 3H)

*N*-Myristoyl-GGGH: MS calc.  $M^+ = 537.3$  [m/z], obsvd.  $(M+1)^+ = 537.4$  [m/z]

$^1\text{H-NMR}$ , (DMSO- $d_6$ , 500MHz)  $\delta$  8.16(m, 1H), 7.11(s, 1H), 4.48(m, 1H), 3.71(m, 6H), 3.45-3.43(m, 2H), 3.02-2.94(m, 2H), 1.47(m, 2H), 1,23(m, 20H), 0.85(m, 3H)

*N*-Stearoyl-GGGH: MS calc.  $M^+ = 593.4$  [m/z], obsvd.  $(M+1)^+ = 593.4$  [m/z]

$^1\text{H-NMR}$ , (DMSO- $d_6$ , 500MHz)  $\delta$  8.16(m, 1H), 7.12(s, 1H), 4.48(m, 1H), 3.70(m, 6H), 3.44-3.43(m, 2H), 3.03-2.94(m, 2H), 1.47(m, 2H), 1,23(m, 28H), 0.86(m, 3H)

*N*-Palmitoyl-GGGHGPLGLARK-NH<sub>2</sub>: MS calc.  $M^+ = 1356.7$  [m/z], obsvd.  $(M+1)^+ = 1356.1$  [m/z]

$^1\text{H-NMR}$ , (DMSO- $d_6$ , 500MHz)  $\delta$  8.21(m, 1H), 7.06(s, 1H), 4.16(m, 1H), 4.04(m, 1H), 3.88-3.72(m, 13H), 3.57(m, 1H), 3.37-3.29(m, 7H), 3.08(m, 2H), 2.11(t, 2H), 1.91(m, 2H), 1.77(m, 1H), 1.63-1.47(m, 17H), 1.23(m, 24H), 0.86(m, 9H)

#### Results for the effect of the acyl chain length

We investigated three different types of acyl chains (myristoyl, palmitoyl and stearoyl peptides) and found that the palmitoyl peptide showed the highest gelation

ability (Table S1). The balance of hydrophilicity and hydrophobicity in a peptide-amphiphile would play a critical role in gelation.

**Table S1** Gelation properties of acylated GGGH having different fatty acids.

pH	Myristoyl-GGGH	Palmitoyl-GGGH	Stearoyl-GGGH
0	G (0.2) <sup>b</sup>	G (0.03)	PG
Deionized water	PG <sup>c</sup>	I <sup>c</sup>	VS
3.0	G (0.1)	I	I
7.5	PG	G (0.03)	G (0.2)
10.1	S	VS	VS

Solutions at various pH values were the same as those in Table 1.

Abbreviations are the same as those in Table 1.

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