Electronic Supplementary Information (ESI)

for

Asymmetric Michael Addition Catalyzed by Copper–Amyloid Complexes

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Experimental Procedure

Peptide Synthesis: The peptides were synthesized by manual fuorenylmethyloxycarbonyl (Fmoc) solid-phase synthesis according to conventional procedures. Rink amide resin was swelled in *N*, *N*-dimethylformamide (DMF) overnight and then treated with 20 % piperidine for Fmoc group detachment. The first Fmoc-protected amino acid was coupled to the amino groups on the resins using 1-[bis(dimethylamino)methylene]-1H-benzotriazolium 3-oxide hexafluorophosphate (HBTU), 1-hydroxybenzotriazole (HOBt) and diisopropylethylamine (DIEA) as the coupling reagent in DMF. After reacting for 30 min, the mixture was filtered and the resins were washed by dichloromethane and DMF for three times. Next, 20 % piperidine in DMF was used for Fmoc removal, and then amino acid couplings were performed by using HBTU, HOBt and DIEA as the coupling reagent as described above. After the last coupling step, cleavage of the peptides from the resin and sidechain deprotection were simultaneously performed by the treatment with a mixture of trifluoroacetic acid (TFA)/H_2O/triisopropylsilane (TIS) (95:2.5:2.5, vol/vol) for 2 h at room temperature. The peptides were precipitated and washed with ice-cold diethyl ether. Purity of peptides was confirmed by reverse-phase high performance liquid chromatography (RP-HPLC) system (Agilent 1100 series HPLC system) with a C18 preparative column, using a linear gradient of solvent A (0.1 % TFA in Millipore H_2O) and solvent B (0.1 % TFA in CH_3CN). The peptides were identified by matrix-assisted laser desorption ionization time-of-flight mass spectroscopy (MALDI-TOF/MS) on Autoflex III smartbeam MALDI-TOF mass spectrometer (Bruker Co.). P01 (H_2N-HKLVFFAV-NH_2) TOF-MS: MW calcd 959.56, found 959.56. P02 (H_2N-HKLVFFAL-NH_2) TOF-MS: MW calcd 973.57, found 973.94. P03 (H_2N-HKLVFFAI-NH_2) TOF-MS: MW calcd 973.57, found 973.65. P04 (H_2N-HKLVFFAM-NH_2) TOF-MS: MW calcd 991.53, found 992.22. P05 (H_2N-HKLVFFAF-NH_2) TOF-MS: MW calcd 1007.56, found 1007.59. P06 (H_2N-HKLVFFAY-NH_2) TOF-MS: MW calcd 1023.55, found 1023.66. P07 (H_2N-HKLVFFAW-NH_2) TOF-MS: MW calcd 1046.57, found 1046.62. P08 (H_2N-HKLVFFAT-NH_2) TOF-MS: MW calcd 961.54, found 961.74. P09 (H_2N-HKLVFFAE-NH_2) TOF-MS: MW calcd 989.53, found 989.73. P10 (H_2N-HKLVFFAH-NH_2) TOF-MS: MW calcd 997.55, found 997.65. P11 (H_2N-HKLVFFAA-NH_2) TOF-MS: MW calcd 931.53, found 931.55. P12 (H_2N-HKLVFFAG-NH_2) TOF-MS: MW calcd 917.51, found 916.95. P13 (H_2N-AKLVFFAV-NH_2) TOF-MS: MW calcd 893.54, found 893.58. P14 (Ac-AKLVFFAV-NH_2) TOF-MS: MW calcd 935.55, found 935.53. P15 (H_2N-$^{D}H^{D}K^{D}L^{D}V^{D}F^{D}F^{D}A^{D}V$-$NH_2$) TOF-MS: MW calcd 959.56, found 959.65. P16 (H_2N-HKLVFFA^{t}Bu-NH_2) TOF-MS: MW calcd 973.59, found 973.63. P17 (H_2N-HKLVFFA^{c}P-NH_2) TOF-MS: MW calcd 985.59, found 985.57. P18 (H_2N-HKLVFFAPh-NH_2) TOF-MS: MW calcd 993.56, found 993.60. P19 (H_2N-HKLVFFAInd-NH_2) TOF-MS: MW calcd 1033.59, found 1033.74.

Preparation of Self-assembly of Peptide (Amyloid-like Fibril): A 1.0 μmol of peptide was dissolved in 60 μL of 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP). 1940 μL of 20 mM 3-(*N*-morpholino) propanesulfonic acid (MOPS) buffer (pH 6.5) containing 100 mM NaCl was added in the peptide solution.[1] After stirred, the solution was centrifuged (25 ℃, 20,630×g, 10 min) using micro refrigerated centrifuge (3700, KUBOTA Corp.) to gather the resulting amyloids. Supernatant was removed and the precipitant of amyloid fibril was collected.

Thioflavin T (ThT) Assay: Fluorescence spectra were obtained on a FP-6300 spectrofluorometer (JASCO Co.) with emission band pass set to 5.0 mm and excitation band pass set to 2.5 mm. The measurements were taken using a quartz cuvette with 1.0 cm excitation and emission path lengths. Samples were prepared by dissolving 1.0 μmol of peptide in 60 μL of HFIP and adding 1940 μL of 20 mM MOPS buffer (pH 6.5) containing 10 μL ThT and 100 mM NaCl. Measurement conditions were as follows; excitation bandwidth: 5.0 nm, emission bandwidth: 2.5 nm, fluorescence wavelength: 440 nm, emission wavelength: 480 nm.[1]

Circular Dichroism (CD) spectroscopy: The CD spectra were collected on a CD spectrometer, J-820AC, JASCO Co., using a step scan mode averaging four runs using a quartz cuvette with a 1 mm path length. Peptide samples were prepared at 500 μM in 20 mM MOPS buffer (pH 6.5). The secondary structure contents were analyzed from CD spectrum using the calculation software SELCON3.[2,3]

Fourier transform infrared (FT-IR) Spectroscopy: FT-IR spectroscopy measurement was acquired using a FT/IR-4100 Fourier transform infrared spectrometer (JASCO Co.). Samples (2 mL) were freeze-dried into powder. The powder and potassium bromide were compressed into a thin pellet.

Transmission Electron Microscopy (TEM): Peptide stocks were diluted 5-fold to an approximate concentration of 100 μM using a MOPS buffer (20 mM, pH 6.5). Sample aliquots of 10 μL were absorbed for 1 min onto formvar/carbon-coated, 200-mesh copper grids (Ted Pella, Redding, CA; glow-discharged prior to use). Samples were negatively-stained with 10 μL drops of freshly filtered 2 % (w/v) uranyl acetate. After drying, samples were viewed with Hitachi 7500 electron microscope (Hitachi, Tokyo, Japan) at an acceleration voltage of 80 kV.

ESR Experiments: ESR spectra were acquired by using a highly purified quartz tubes (I.D. 4.0 mm, Radical Research Inc.) with a rubber septum under an N_2 saturated conditions at 100 K on an EXMplus ESR spectrometer (Bruker Co.) equipped with a cryostat. MOPS buffer (20 mM, 200 μL), prepared self-assembly of peptide (0.5

μmol), and $CuSO_4$ solution in water (50 mM, 2 μL) were mixed to make a solution with 500 μM $CuSO_4$ and 2.5 mM peptide (200 μL final volume). The mixtures were transferred into an ESR tube and N_2 gas was flowed to the mixture at room temperature for 5 min. The mixture was frozen in liquid nitrogen. g//, g⊥ and A// values were determined based on the spectra.

Structural Model: Based on the X-ray crystal structure of an amyloid forming peptide (PDB: 3OW9), the structure model of copper-loaded catalytic amyloid was manually built with Coot software.[4] The amyloid molecular model of HKLVFFAV peptides was constructed by adding one histidine residue and one valine residue to N- and C-terminals, respectively, retaining β-strand structure. The copper-N-terminal histidine complex was built by employing the coordinate of histidine brace in pMMO (PDB: 5IJU).

Michael Addition Assay: An aqueous solution of $CuSO_4$ (24 μL, 1 mM) was added to prepared amyloid fibril (500 μM, 250 μL) in 20 mM MOPS buffer (pH 6.5) at 0 ℃ according to the literature.[5] A fresh stock solution (5 μL) of substrate in CH_3CN was added. After addition of dimethyl malonate (3 μL) at 0 ℃, the reaction mixture was stirred (600 rpm) in the closed vial at 0 ℃ for 1 day. After the reaction, 4-methoxy-1-naphthol as internal standard in hexane/iPrOH (v/v = 95/5) was added to the mixture. The products were isolated by extraction with 500 μL of hexane/iPrOH (95/5) mixture. The sample was analyzed with a HPLC system of an EXTREMA series (JASCO Co.) equipped with a normal-phase chiral column [CHIRAL ART Amylose-SA (5 μm, YMC Co., Ltd.)]. The products were identified from HPLC retention time and ^{1}H NMR spectra by comparing with those of the authentic samples.[6] The yields were calculated from a calibration curve: a plot of mole ratio (moles of organic compound/mol of internal standard) versus area ratio (area of organic compound/area of internal standard).

Synthesis of Substrates: The substrates, 1,2-unsaturated ketones **1a-e**, were readily prepared from aldol condensation reaction according to modified reported procedures as described below.[7–9]

(E)-3-phenyl-1-(pyridin-2-yl)prop-2-en-1-one (1a) A 17 mmol of 2-acetylpyridine and 16.5 mmol of benzaldehyde were introduced into 100 mL of water at temperatures below 5 ℃. The mixture was shaken thoroughly in order to obtained a finely dispersed emulsion. 10 mL of a 10 % sodium hydroxide solution was added. The mixture was again shaken and left overnight undisturbed at 4 ℃. The product separated as oil was solidified upon shaking, which was isolated by filtration and washed with water to give almost pure product in 25 %. The compounds were characterized by ^{1}H NMR and MS. ^{1}H NMR (400 MHz, $CDCl_3$), δ = 7.40-7.51 (m, 4H), 7.72-7.78 (m, 2H), 7.86-7.90 (m, 1H), 7.95 (d, J = 16.0 Hz, 1H), 8.19-8.21 (m, 1H), 8.32 (d, J = 16.0 Hz, 1H), 8.75-8.76 (m, 1H). HRMS [CI MS], calcd for $C_{14}H_{11}NO$ m/z = 210.0919 ($[M+H]^+$), found 210.0916; consistent with the NMR, and mass spectroscopic data previously reported for this compound.[7]

(*E*)-1-(pyridin-2-yl)-3-(*p*-tolyl)prop-2-en-1-one (1b) was synthesized by the same procedure described for the synthesis of **1a** in 11 % yeild: ^{1}H NMR (400 MHz, $CDCl_3$), δ = 2.40 (s, 3H), 7.23 (d, J = 8.0 Hz, 2H), 7.43-7.53 (m, 1H), 7.62-7.67 (m, 2H), 7.82-7.97 (m, 2H), 8.10-8.22 (m, 1H), 8.26 (d, J = 16.4 Hz, 1H), 8.68-8.77 (m, 1H). HRMS [CI MS], calcd for $C_{15}H_{13}NO$ *m/z* = 224.1075 ($[M+H]^+$), found 224.1072; consistent with the NMR, and mass spectroscopic data previously reported for this compound.[7]

(*E*)-3-(4-nitrophenyl)-1-(pyridin-2-yl)prop-2-en-1-one (1c) To a stirred ethanol solution (10 mL) containing 10 % aqueous sodium hydroxide (0.5 mL) and 8.25 mmol of 4-nitrobenzaldehyde, 8.25 mmol of 2-acetylpyridine was added dropwise over 2-3 h. The temperature was kept at 0 ℃. After being stirred for another 2 h, the reaction mixture was filtered to obtain solid material of **1c** in 36 %. The compounds were characterized by ^{1}H NMR and MS. ^{1}H NMR (400 MHz, $CDCl_3$), δ = 7.52-7.56 (m, 1H), 7.87 (d, J = 9.2 Hz, 2H), 7.89-7.96 (m, 2H), 8.21 (d, J = 8.4 Hz, 1H), 8.26-8.31 (m, 2H), 8.44 (d, J = 16.0 Hz, 1H), 8.74-8.79 (m, 1H). HRMS [CI MS], calcd for $C_{14}H_{10}N_2O_3$ *m/z* = 255.0770 ($[M+H]^+$), found 255.0772; consistent with the NMR, and mass spectroscopic data previously reported for this compound.[7]

(*E*)-3-(4-bromophenyl)-1-(pyridin-2-yl)prop-2-en-1-one (1d) was synthesized by the same procedure described for the synthesis of **1c** in 18 %: ^{1}H NMR (400 MHz, $CDCl_3$), δ = 7.48-7.53 (m, 1H), 7.53-7.57 (m, 2H), 7.58-7.62 (m, 2H), 7.84-7.92 (m, 2H), 8.18-8.21 (m, 1H), 8.31 (d, J = 16.4 Hz, 1H), 8.73-8.76 (m, 1H). HRMS [CI MS], calcd for $C_{14}H_{10}BrNO$ *m/z* = 288.0024 ($[M+H]^+$), found 288.0020; consistent with the NMR, and mass spectroscopic data previously reported for this compound.[8]

(*E*)-3-(naphthalen-2-yl)-1-(pyridin-2-yl)prop-2-en-1-one (1e) was synthesized by the same manner described for the synthesis of **1c** in 41 %: H NMR and MS. ^{1}H NMR (400 MHz, $CDCl_3$), δ = 7.49-7.56 (m, 3H), 7.83-7.95 (m, 5H), 8.12 (t, J = 8.0 Hz, 2H), 8.23 (d, J = 8.0 Hz, 1H), 8.43 (d, J = 16.4 Hz, 1H), 8.77-8.80 (m, 1H). HR-MS [CI MS], calcd for $C_{18}H_{13}NO$ *m/z* = 260.1075 ($[M+H]^+$), found 260.1073; consistent with the NMR, and mass spectroscopic data previously reported for this compound.[9]

Synthesis of Racemic Products: $CuSO_4 \cdot 5H_2O$ (12 mg, 0.048 mmol) was added to water (100 mL) at 60 ℃. Then, 0.32 mmol of substrate **1a** was dissolved in CH_3CN (10 mL), which was added to the stirred aqueous solution. After dimethyl malonate (6 mL, 52.4 mmol) was added to the solution, the mixture was stirred for 1 day at 60 ℃. The reaction mixture was filtered to give purple solid sample of **2a** in 27 % yield, which was characterized by ^{1}H NMR and MS.[6] The compound was mixture of (*R*)- and (*S*)-stereoisomer.

Dimethyl 2-(3-oxo-1-phenyl-3-(pyridin-2-yl)propyl)malonate (2a): ^{1}H NMR (400 MHz, CD_3CN), δ = 3.40-3.50 (m, 4H), 3.66 (s, 3H), 3.87-3.99 (m, 2H), 4.02-4.10 (m, 1H), 7.13-7.19 (m, 1H), 7.20-7.26 (m, 2H), 7.26-7.31 (m, 2H), 7.50-7.56 (m, 1H), 7.79-7.89 (m, 2H), 8.63-8.68 (m, 1H). HRMS [CI MS], calcd for $C_{19}H_{19}NO_5$ *m/z* = 341.1263 ($[M]^+$), found 341.1272.

Dimethyl 2-(3-oxo-3-(pyridin-2-yl)-1-(*p*-tolyl)propyl)malonate (2b): was synthesized by the same manner described above in 32 % yield: ^{1}H NMR (400 MHz, $CDCl_3$), δ = 2.25 (s, 3H), 3.49 (s, 3H), 3.55-3.62 (m, 1H), 3.72 (s, 3H), 3.78-3.85 (m, 1H), 3.86-3.95 (m, 1H), 4.15-4.23 (m, 1H), 7.02-7.06 (m, 2H), 7.17-7.21 (m, 2H), 7.41-7.43 (m, 1H), 7.74-7.79 (m, 1H), 7.89-7.93 (m, 1H), 8.63-8.67 (m, 1H). HRMS [CI MS], calcd for $C_{20}H_{21}NO_5$ *m/z* = 355.1420 ($[M]^+$), found 355.1418.

Dimethyl 2-(1-(4-nitrophenyl)-3-oxo-3-(pyridin-2-yl)propyl)malonate (2c): was synthesized by the same manner described above in 9 % yield: ^{1}H NMR (400 MHz, $CDCl_3$), δ = 3.52 (s, 3H), 3.60-3.68 (m, 1H), 3.76 (s, 3H), 3.89 (d, *J* = 10.0 Hz, 1H), 3.98 (q, *J* = 9.2 Hz, 1H), 4.29-4.37 (m, 1H), 7.44-7.49 (m, 1H), 7.53 (d, *J* = 8.8 Hz, 2H), 7.76-7.83 (m, 1H), 7.91 (d, *J* = 7.6 Hz, 1H), 8.13 (d, *J* = 8.8 Hz, 2H), 8.64-8.67 (m, 1H). HRMS [CI MS], calcd for $C_{19}H_{18}N_2O_7$ *m/z* = 386.1114 ($[M]^+$), found 386.1107.

Dimethyl 2-(1-(4-bromophenyl)-3-oxo-3-(pyridin-2-yl)propyl)malonate (2d): was synthesized by the same manner described above in a 10 % yield: ^{1}H NMR (400 MHz, $CDCl_3$), δ = 3.51 (s, 3H), 3.53-3.60 (m, 1H), 3.73 (s, 3H), 3.80-3.86 (m, 1H), 3.87-3.96 (m, 1H), 4.15-4.23 (m, 1H), 7.19-7.23 (m, 2H), 7.35-7.39 (m, 2H), 7.43-7.47 (m, 1H), 7.75-7.81 (m, 1H), 7.90-7.94 (m, 1H), 8.63-8.67 (m, 1H). HRMS [CI MS], calcd for $C_{19}H_{18}BrNO$ *m/z* = 419.0368 ($[M]^+$), found 419.0369.

Dimethyl 2-(1-(naphthalen-2-yl)-3-oxo-3-(pyridin-2-yl)propyl)malonate (2e): was synthesized by the same manner described above in 10 % yield: ^{1}H NMR (400 MHz, $CDCl_3$), δ = 3.43 (s, 3H), 3.63-3.71 (m, 1H), 3.74 (s, 3H), 3.98 (d, *J* = 10.8 Hz, 1H), 4.05 (q, *J* = 9.2 Hz, 1H), 4.36-4.44 (m, 1H), 7.39-7.45 (m, 3H), 7.45-7.50 (m, 1H), 7.71-7.78 (m, 5H), 7.87-7.90 (m, 1H), 8.64-8.67 (m, 1H). HRMS [CI MS], calcd for $C_{23}H_{21}NO_5$ *m/z* = 391.1420 ($[M]^+$), found 391.1415.

Supporting References

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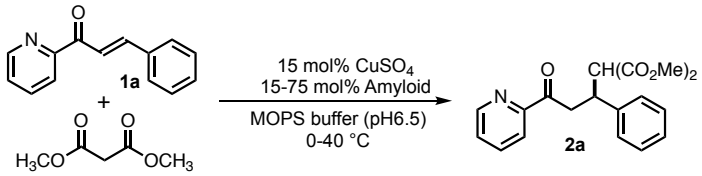
Table S1. Optimization of reaction temperature and catalyst amount in the Michael addition.



Entry	Catalyst	Temperature (C°)	Amyloid (mol%)	Yield (%)[c]	ee (%)[c]
1		40	75	61	18 (*S*)
2		20	75	77	21 (*S*)
3		0	75	78	38 (*S*)
4	P01	0	60	61	36 (*S*)
5		0	45	54	24 (*S*)
6		0	30	51	9 (*S*)
7		0	15	44	5(*S*)

Table S2. Secondary structure contents calculated by SELCON3

Peptide	α-Helix	β-sheet	Turn	Random coil
P01	9 %	46 %	11 %	34 %
P03	9 %	46 %	11 %	34 %
P05	9 %	46 %	11 %	34 %
P06	8 %	47 %	11 %	34 %
P11	8 %	47 %	11 %	34 %
P12	8 %	48 %	10 %	34 %

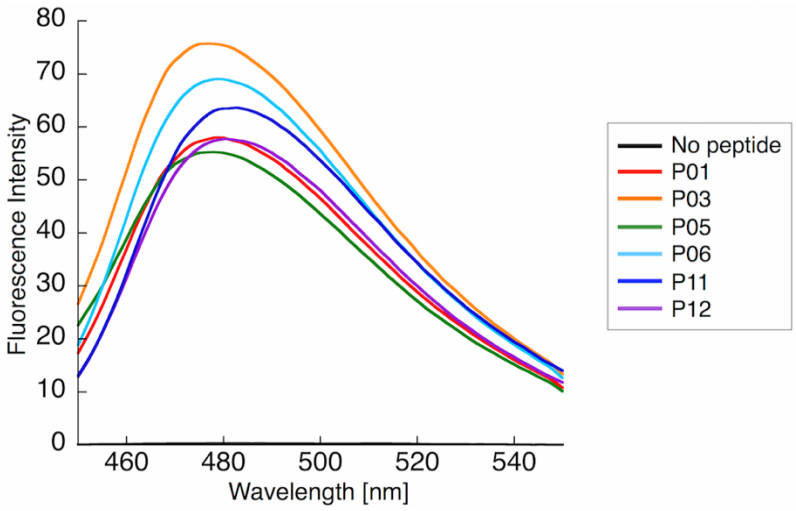


Figure S1. Fluorescence intensity of ThT (10 μM) in the absence and presence of the self-assembled peptides.

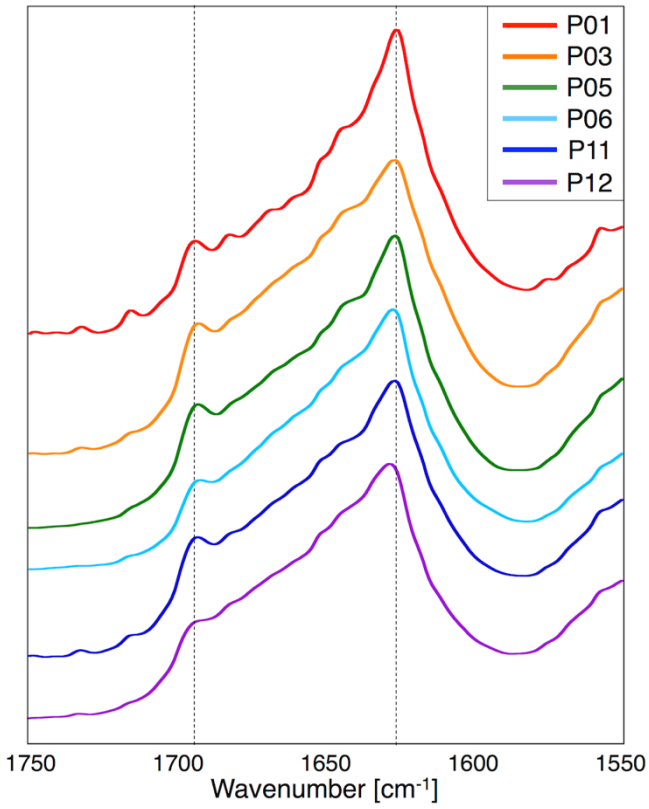


Figure S2. FT-IR spectra of the self-assembled peptides.

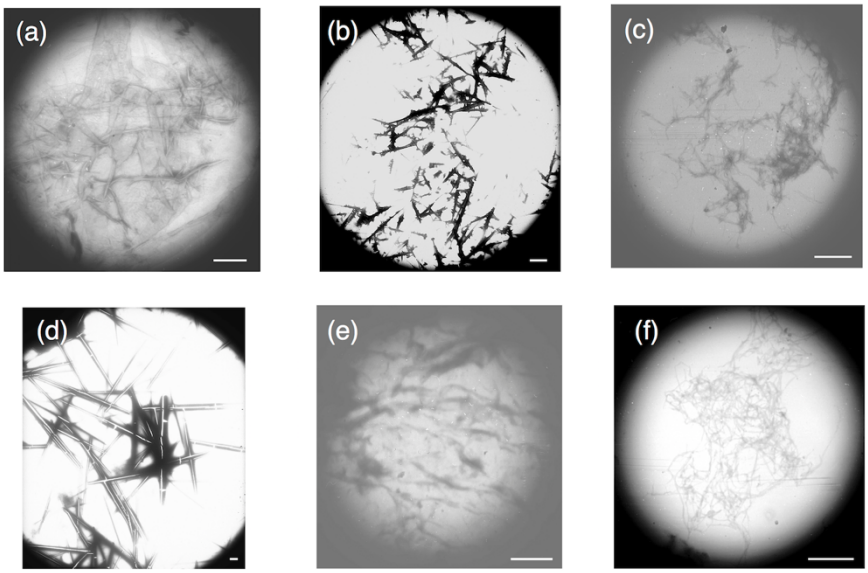
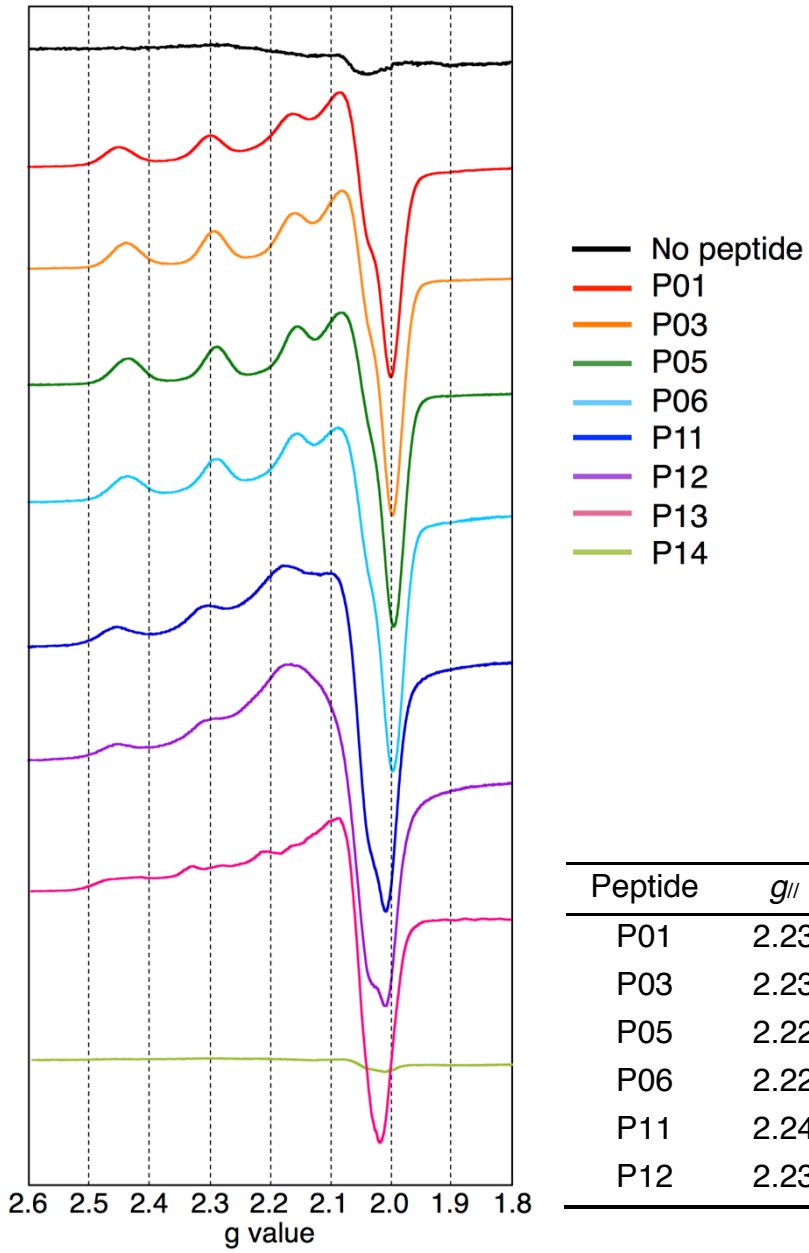


Figure S3. TEM image of the self-assembly of each peptide of P01 (a), P03 (b), P05 (c), P06 (d), P11 (e), P12 (f) (Scale bar: 1 μm) All samples gave fibrous or needle-like structures of a variety of molecular size. Even the peptide P12, which seems to be difficult to form defined fibrous structure due to flexibility of Gly, could form fibrous structure in TEM image.



Peptide	$g_{//}$	$g_{\perp}$	$A_{//}$ / G
P01	2.23	2.06	188
P03	2.23	2.05	188
P05	2.22	2.05	191
P06	2.22	2.05	190
P11	2.24	2.06	192
P12	2.23	2.07	196

Figure S4. X band ESR spectra of Cu^{2+} in the absence and presence of the self-assembled peptides. ESR spectra of the peptides (P03, 05, 06, 11 and 12) were measured. The $g_{//}$, $g_{\perp}$ and $A_{//}$ values were almost identical to that of P01, indicating that C-terminal residues have little effect on the coordination geometries of Cu^{2+} ion. Control experiments with ligand-lacking variants P13 and 14 were also performed. The spectrum of copper–amyloid complexes of P13 was more complicated than those of the other peptides, indicating that the Cu^{2+} ion binds to several positions of P13 because of the absence of the well-defined coordination unit. Furthermore, copper–amyloid complexes of P14 did not exhibit clear signals, which demonstrates that the self-assembly of peptides did not strongly coordinate to Cu^{2+} ion. These results suggested that N-terminal residue has an important role for the formation of the discrete coordination site in the amyloid-like fibrils.