Supplementary Information

Trigonal Tryptophane-Zipper as a Novel Building Block for pH-Responsive Peptide Nano-assemblies

Kazunori Matsuura,* Hiroki Hayashi, Kazuya Murasato, and Nobuo Kimizuka

Experimental

General. Reagents were obtained from commercial source and used without further purification. Deionized water of high resistivity (> 18 MΩ cm) purified with a Millipore Purification System (Milli-Q water) was used as a solvent of trigonal-peptide conjugates. ^{1}H-NMR spectra were recorded on Bruker AV300M spectrometer. Reversed-phase HPLC was performed at ambient temperature with a Simadzu LC-6AD liquid chromatograph equipped with a UV/Vis detector (220 nm, Shimadzu SPD-10AVvp) using Inertsil ODS-3 (GL Science) or COSMOSIL Protein-R (Nakarai Tesque) columns (250 × 4.6 mm or 250 × 20 mm). MALDI-TOF mass spectra were obtained on Autoflex III (Bruker Daltonics) under the linear/positive mode with α-cyano-4-hydroxy cinnamic acid (α-CHCA) as matrix. CD spectra were taken at 25°C in a 1.0 mm quartz cell with a JASCO J-820 spectrophotometer equipped with a Peltier-type thermostatic cell holder.

C_3-Symmetric iodoacetoamidated core molecule (**1**) was synthesized from trimesoyl chloride according to the our previous report.[2a]

Solid phase synthesis of peptide H-CKTWTWTE-OH. Peptide H-Cys(Trt)-Lys(Boc)-Thr(tBu)-Trp(Boc)-Thr(tBu)-Trp(Boc)-Thr(tBu)-Glu(OtBu)-Alko resin was synthesized on α-*p*-alkoxybenzyl alcohol resin (Alko resin, Watanabe Chemical Ind. Ltd, 0.60 mmol / g) using standard Fmoc-based FastMoc coupling chemistry (3 eq. Fmoc-amino acids) with an ABI 433A synthesizer (Applied Biosystems). DMF solution of 2-(*1H*-benzotriazole-1-yl)-1, 1, 3, 3-tetramethyluronium hexafluorophosphate (HBTU, 0.5 M) and 1-hydroxybenzotriazole hydrate (HOBt·H_2O, 0.5 M) was used as a coupling reagent. 2.0 M diisopropylamine in NMP and 20 % piperidine in NMP were used for neutralization and for Fmoc deprotection, respectively. The peptidyl-resin was washed with NMP, dichloromethane and methanol then dried under vacuum. The peptide was deprotected and cleaved from the resin by treatment with a cocktail of TFA / 1, 2-ethanedithiol / water / triisopropylsilane = 94 / 2.5 / 2.5 / 1 in volume at room temperature for 2 h. The reaction mixture was filtered to remove the resin and the filtrate was concentrated under vacuum. The peptide was precipitated by

adding ice-cooled methyl-*tert*-butyl ether (MTBE) to the residue and the supernatent was decanted. After repeating the MTBA washing 6 times, the precipitated peptide was dried under vacuum. The crude product was purified by reversed-phase HPLC (Inertsil ODS-3) eluting with a liner gradient of CH_3CN / water (18 / 82 to 30 / 70 over 60 min) containing 0.1 % TFA. The elution fraction containing the desired peptide was lyophilized to give a flocculent solid. The isolated yield was 24 %. MALDI-TOF-MS (matrix: α-CHCA): m/z = 1054.00 $[M + H]^+$. The peptide sequence was confirmed by MS / MS analysis. ^{1}H-NMR (D_2O, δ / ppm, J / Hz): 7.42-7.47 (2 H, m, indole of Trp), 7.23-7.32 (2H, m, indole of Trp), 6.94-7.08 (6H, m, indole of Trp), 4.51 (1H, t, J = 7.2, α-C*H* of Trp), 4.45 (1H, t, J = 7.2, α-C*H* of Trp), 4.20 (1H, t, J = 7.2, α-C*H* of Lys), 4.15 (1H, d, J = 5.1, α-C*H* of Thr5), 4.05-4.10 (3H, m, α-C*H* of Thr3, Thr7, and Cys), 3.85-4.01 (4H, m, β-C*H* of Thr and α-C*H* of Glu), 3.10 (2H, t, J = 6.3, β-CH_2 of Trp), 2.89-2.97 (4H, m, β-CH_2 of Trp and Cys), 2.67 (2H, t, J = 7.8, ε-CH_2 of Lys), 2.22 (2H, t, J = 7.8, γ-CH_2 of Glu), 1.84-1.99 (1H, m, β-CH_2 of Glu), 1.68-1.84 (1H, m, β-CH_2 of Glu), 1.48-1.58 (2H, m, β-CH_2 of Lys), 1.37-1.48 (2H, m, δ-CH_2 of Lys), 1.07-1.20 (2H, m, γ-CH_2 of Lys), 0.98 (3H, d, J = 6.3, CH_3 of Thr5), 0.91 (3H, d, J = 6.3, CH_3 of Thr7 or Thr3), 0.88 (3H, d, J = 6.3, CH_3 of Thr7 or Thr3). The purity of peptide is determined by reversed-phase HPLC to be 98.2 %.

Solid phase synthesis of peptide H-CKTFTFTE-OH. Peptide H-CKTFTFTE-OH was prepared by the almost same procedure described above**.** The crude product was purified by reversed-phase HPLC eluting with a liner gradient of CH_3CN / water (20 / 80 to 29 / 71 over 100 min) containing 0.1 % TFA. The isolated yield was 38 %. MALDI-TOF-MS (matrix: α-CHCA): m/z = 976.84 $[M + H]^+$. The peptide sequence was confirmed by MS / MS analysis. The purity of peptide is determined by reversed-phase HPLC to be 98.7 %.

Synthesis of Trigonal-WTW (Scheme S1)**.** Peptide H-CKTWTWTE-OH (5.0 mg, 4.7 μmol) was dissolved in dry DMF (1.0 mL, degassed by freeze-and-thaw method) under nitrogen at 0°C. To the mixture were added a solution of C_3-Symmetric iodoacetoamidated core molecule **1** (0.82 mg, 0.98 μmol) in degassed dry DMF (0.5 mL) and then diisopropylethylamine (3.4 μL, 19.5 μmol) at the same temperature in the dark. The mixture was stirred for 1.5 h under the same condition. After removal of DMF under reduced pressure, the residue was purified by reversed-phase HPLC eluting with a linear gradient of CH_3CN / water (28 / 72 to 28 / 62 over 100 min) containing 0.1 % TFA. The isolated yield was 1.5 mg (43 %). MALDI-TOF-MS (matrix: α-CHCA): m/z = 3619.25 $[M + H]^+$. ^{1}H-NMR (D_2O, δ / ppm, J / Hz): 7.71 (3H, s, Ar-*H* of core benzene), 7.07-7.27 (12H, m, indole of Trp), 6.80-6.95 (12H, m, indole of Trp),

6.68-6.77 (6H, m, C^2-*H* of indole of Trp), 4.44 (6H, m, α-C*H* of Trp), 4.22 (3H, d, α-C*H* of Lys), 4.09-4.13 (12H, m, α-C*H* of Thr and Cys), 3.80-4.00 (12H, m, β-C*H* of Thr and α-C*H* of Glu), 3.15-3.30 (12H, m, β-CH_2 of Trp and Ar-CONH-CH_2CH_2NHCO), 3.10 (6H, s, NHCO-CH_2-S), 2.83-3.05 (12H, m, β-CH_2 of Trp and Cys), 2.78 (6H, br, Ar-CONH-CH_2CH_2NHCO), 2.65 (2H, t, J = 7.5, ε-CH_2 of Lys), 2.19 (2H, t, J = 7.5, γ-CH_2 of Glu), 1.82-1.98 (3H, m, β-CH_2 of Glu), 1.63-1.80 (3H, m, β-CH_2 of Glu), 1.46-1.60 (6H, br, β-CH_2 of Lys), 1.33-1.46 (6H, m, δ-CH_2 of Lys), 1.10-1.21 (6H, br, γ-CH_2 of Lys), 0.92 (9H, d, J = 6.0, CH_3 of Thr), 0.89 (9H, d, J = 6.0, CH_3 of Thr), 0.83 (9H, d, J = 6.0, CH_3 of Thr). The purity of **Trigonal-WTW** is determined by reversed-phase HPLC to be 99.8 %.

Synthesis of Trigonal-FTF. Trigonal-FTF was also prepared by similar procedure described above. The residue was purified by reversed-phase HPLC (COSMOSIL Protein-R) eluting with a linear gradient of CH_3CN / water (22 / 78 to 32 / 68 over 100 min) containing 0.1 % TFA. The isolated yield was 1.0 mg (21 %). MALDI-TOF-MS (matrix: α-CHCA): m/z = 3384.09 $[M + H]^+$. ^{1}H-NMR (D_2O containing 0.1% TFA, δ / ppm, J / Hz): 8.01 (3H, s, Ar-*H* of core benzene), 6.90-7.20 (30H, m, Ar-*H* of Phe), 4.43-4.54 (6H, m, α-C*H* of Phe), 4.20-4.29 (6H, m, α-C*H* of Lys and Glu), 4.08-4.16 (12H, m, α-C*H* of Thr and Cys), 3.83-4.00 (9H, m, β-C*H* of Thr), 3.34-3.41 (6H, br, Ar-CONH-CH_2CH_2-NHCO), 3.28-3.34 (6H, br, Ar-CONH-CH_2CH_2NHCO), 3.17 (6H, s, NHCO-CH_2-S), 2.63-3.03 (24H, m, ε-CH_2 of Lys, β-CH_2 of Phe and Cys), 2.32 (6H, t, J = 7.5, γ-CH_2 of Glu), 1.96-2.11 (3H, m, β-CH_2 of Glu), 1.76-191 (3H, m, β-CH_2 of Glu), 1.42-1.67 (12H, m, β-CH_2 and δ-CH_2 of Lys), 1.12-1.30 (6H, m, γ-CH_2 of Lys), 1.01 (9H, d, J = 6.0, CH_3 of Thr), 0.96 (9H, d, J = 6.0, CH_3 of Thr), 0.92 (9H, d, J = 6.0, CH_3 of Thr). The purity of **Trigonal-FTF** is determined by reversed-phase HPLC to be 99.2 %.

Scanning Electron Microscopy (SEM). The stock solutions of trigonal-peptide conjugates (0.13 mM) were prepared by dissolving in 4.8 mM aqueous NaOH, and then diluted in water, 50 mM phosphate buffer (pH 7), and 50 mM citrate buffer (pH3), respectively. An aliquot (10 μL) of sample solutions was mounted on a fleshly cleaved mica substrate. The substrate was incubated for 30 min, rinsed with Milli-Q water, and dried under reduced pressure. The substrate was coated with platinum (ca. 7-9 nm: Hitachi E-1030 ion sputter, 10 mA, 10 Pa, 50 s) and subjected to SEM observation (Hitachi S-5000 instrument) with an accelerating voltage of 25 kV.

Dynamic Light Scattering (DLS). The SEM sample solutions were also used for DLS. DLS was measured with a Zetasizer NanoZS (MALVERN) instrument at 25 ºC using incident He-Ne laser (633 nm). The correlation time of scattered light intensity

$G(\tau)$ was measured several times and their averaged data were fitted to the equation 1, where B is a baseline, A is amplitude, q is scattering vector, τ is delay time, and D is the diffusion coefficient.

$$G(\tau) = B + A \exp(-2q^2 D\tau) \qquad (1)$$

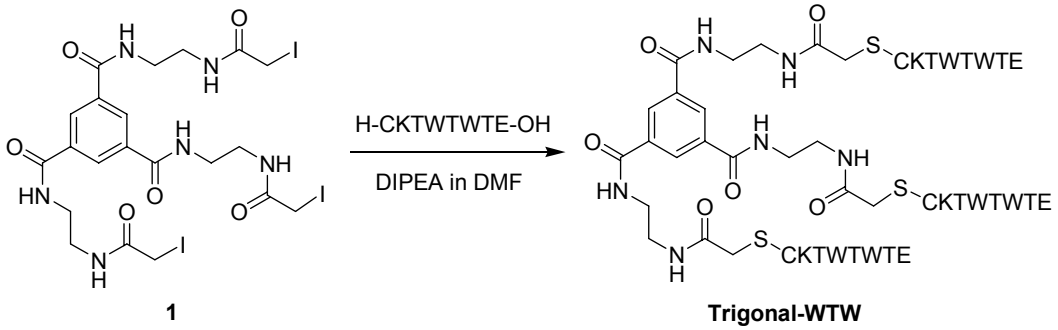
The hydrodynamic radius (R_H) of the scattering particles was calculated by the Stokes-Einstein equation (eq. 2), where η is solvent viscosity, k_B is Boltzmann's constant and T denotes the absolute temperature.

$$R_H = \frac{k_B T}{6\pi\eta D} \qquad (2)$$

Molecular Mechanics Calculation. Molecular mechanics calculations were carried out by using OPLS2005 force field in MacroModel 9.1 program (SCHRÖDINGER). Total energy of peptides and their dimers with parallel and anit-parallel arrangements in water was calculated with the GB/SA solvation model of the program, assuming the charge of peptides at pH 7. All geometric parameters (bond lengths, bond angles, and dihedral angles) were optimized without any assumption by using low mode / torsional sampling (MCMM) method (step number: 3000) to search the global minimum conformation (Figure S2).

The formation energy (ΔE) of peptide dimer with anit-parallel arrangements in water at pH 7 was calculated from the difference between the most stable energy of the peptide dimer (E_{dimer}) and the double of the most stable energy of the peptide monomer ($E_{monomer}$) (eq. 3, Figure S2).

$$\Delta E = E_{dimer} - 2\, E_{monomer} \qquad (3)$$



1 **Trigonal-WTW**

Scheme S1. Synthesis of **Trigonal-WTW**.

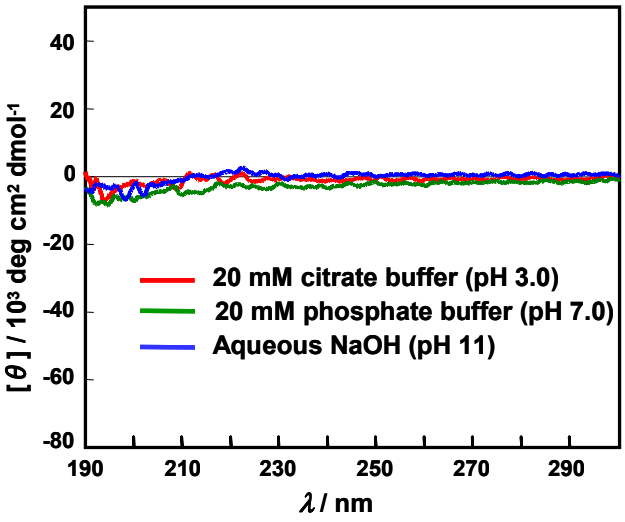


Figure S1. CD spectra of aqueous solutions of **Trigonal-FTF** (10 mM) at 25 ^{0}C in 20 mM citrate buffer (pH 3.0, red line), 20 mM phosphate buffer (pH 7.0, green line), and aqueous NaOH solution (pH 11, blue line).

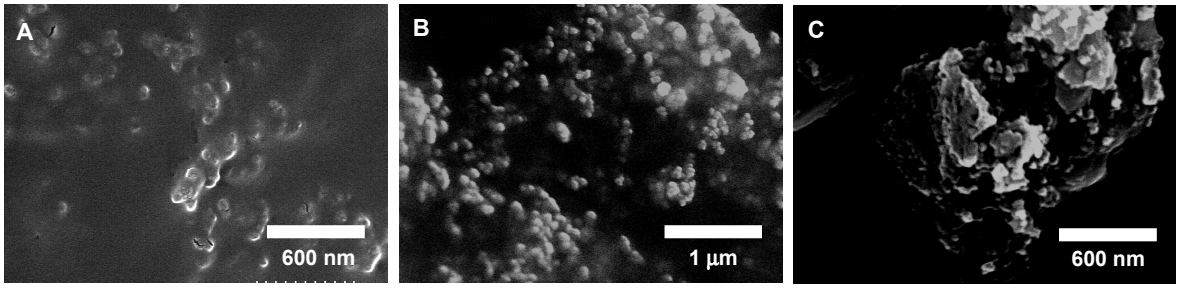


Figure S2. SEM images of **Trigonal-FTF** (10 mM) in (A) 20 mM citrate buffer (pH 3.0), (B) 20 mM phosphate buffer (pH 7.0), and (C) aqueous NaOH solution (pH 11) on mica substrate. SEM samples on mica were coated with 7-9 nm Pt.