Supplementary Material

for

Tandem-Homodimer of a β-Sheet-Forming Short Peptide Inhibits Random-to-β Structural Transition of Its Original Monomer

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(1) Analytical HPLC profile for RU003.



Figure S1. Analytical HPLC profile for RU003. Conditions: acetonitrile content: 28% (t = 0 min) \rightarrow 32% (t = 30 min); UV Detection: 220 nm.

(2) MALDI-TOF-MS for RU003.



Figure S2. MALDI-TOF-MS for RU003. Calculated molecular weight: 1081.6; observed molecular weight: 1082.3 $[M + H]^+$, 1104.2 $[M + Na]^+$, 1119.4 $[M + K]^+$.

(3) Analytical HPLC profile for Abu4-TD.



Figure S3. Analytical HPLC profile for Abu4-TD. Conditions: acetonitrile content: 40% (t = 0 min) $\rightarrow 44\%$ (t = 30 min); UV Detection: 220 nm.

(4) MALDI-TOF-MS for Abu4-TD.



Figure S4. MALDI-TOF-MS for Abu4-TD. Calculated molecular weight: 2189.2; observed molecular weight: 2192.4 $[M + H]^+$.

(5) Analytical HPLC profile for Ape5-TD.



Figure S5 Analytical HPLC profile for Ape5-TD. Conditions: acetonitrile content: 26% (t = 0 min) $\rightarrow 28\%$ (t = 30 min); UV Detection: 220 nm.

(6) MALDI-TOF-MS for Ape5-TD.



Figure S6. MALDI-TOF-MS for Ape5-TD. Calculated molecular weight: 2202.5; observed molecular weight: 2205.0 $[M + H]^+$.

(7) Analytical HPLC profile for Ahx6-TD.



Figure S7. Analytical HPLC profile for Ahx6-TD. Conditions: acetonitrile content: 37% (t = 0 min) $\rightarrow 41\%$ (t = 30 min); UV Detection: 220 nm.

(8) MALDI-TOF-MS for Ahx6-TD.



Figure S8. MALDI-TOF-MS for Ahx6-TD. Calculated molecular weight: 2217.2; observed molecular weight: 2219.3 $[M + H]^+$.

(9) Analytical HPLC profile for Ahp7-TD.



Figure S9. Analytical HPLC profile for Ahp7-TD. Conditions: acetonitrile content: 37% (t = 0 min) \rightarrow 50% (t = 30 min); UV Detection: 220 nm.

(10) MALDI-TOF-MS for Ahp7-TD.



Figure S10. MALDI-TOF-MS for Ahp7-TD. Calculated molecular weight: 2230.6; observed molecular weight: 2231.5 $[M + H]^+$, 2253.0 $[M + Na]^+$.

(11) Analytical HPLC profile for Aoc8-TD.



Figure S11. Analytical HPLC profile for Aoc8-TD. Conditions: acetonitrile content: 36% (t = 0 min) $\rightarrow 50\%$ (t = 30 min); UV Detection: 220 nm.

(12) MALDI-TOF-MS for Aoc8-TD.



Figure S12. MALDI-TOF-MS for Aoc8-TD. Calculated molecular weight: 2244.6; observed molecular weight: 2246.7 $[M + H]^+$, 2268.6 $[M + Na]^+$, 2284.9 $[M + K]^+$.

(13) Thioflavin T assay for RU003 and Ape5-TD alone



Figure S13. Thioflavin T (ThT) assay for the self-assembling processes of (A) RU003 and (B) Ape5-TD alone (conditions: the RU003 aqueous solution (100 μ M) and the Ape5-TD aqueous solution (10 μ M) were separately sonicated at 50 °C for 2 minutes, incubated at 40 °C for 1 day, then at 25 °C for 2 days. Aliquot (30 μ L) was removed from the reaction mixture at 0, 1, and 3 day(s) after the start of incubation, transferred into a microtube, and mixed with ThT solution (10 μ M, 270 μ L). Fluorescence spectra were acquired on Fluorescence Spectrophotometer F-4500 (Hitachi, Tokyo, Japan), $\lambda_{ex} = 440$ nm). Incubation period '0 day' means '5 minutes after the reaction started'.



Figure S14. Typical CD spectra of RU003 in the presence of Abu4-TD (conditions: [RU003] = 100μ M and [Abu4-TD] = (A) 5.0, (B) 10, and (C) 20 μ M in water at 40 °C). *y*-Axis indicates the mean residual ellipticity. Incubation period '0 day' means '5 minutes after the reaction started'.



Figure S15. Typical CD spectra of RU003 in the presence of Ape5-TD (conditions: [RU003] = $100 \,\mu\text{M}$ and [Ape5-TD] = (A) 5.0, (B) 10, and (C) 20 μM in water at 40 °C). *y*-Axis indicates the mean residual ellipticity. Incubation period '0 day' means '5 minutes after the reaction started'.



Figure S16. Typical CD spectra of RU003 in the presence of Ahx6-TD (conditions: [RU003] = $100 \,\mu\text{M}$ and [Ahx6-TD] = (A) 5.0, (B) 10, and (C) $20 \,\mu\text{M}$ in water at 40 °C). *y*-Axis indicates the mean residual ellipticity. Incubation period '0 day' means '5 minutes after the reaction started'.



Figure S17. Typical CD spectra of RU003 in the presence of Ahp7-TD (conditions: [RU003] = 100μ M and [Ahp7-TD] = (A) 5.0, (B) 10, and (C) 20 μ M in water at 40 °C). *y*-Axis indicates the mean residual ellipticity. Incubation period '0 day' means '5 minutes after the reaction started'.



Figure S18. Typical CD spectra of RU003 in the presence of Aoc8-TD (conditions: [RU003] = $100 \,\mu\text{M}$ and [Aoc8-TD] = (A) 5.0, (B) 10, and (C) 20 μM in water at 40 °C). *y*-Axis indicates the mean residual ellipticity. Incubation period '0 day' means '5 minutes after the reaction started'.



(19) TEM images of the mixtures of RU003 and RU003 tandem-homodimers

Figure S19. TEM images of the mixtures of RU003 (100 μ M) in the (A) absence and presence of RU003 tandem-homodimers (10 μ M), (B) Abu4-TD, (C) Ape5-TD, (D) Ahx6-TD, (E) Ahp7-TD, and (F) Aoc8-TD incubated in water at 40 °C for 5 days. Samples were negatively stained with 2% phosphotungstic acid.