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Supporting information

Colorimetric and Fluorometric Monitoring of the Helix Composition of Collagen-like Peptides at nM level

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Materials and Methods

Sample Preparation

All peptides were synthesized by Chinese Peptide Company (Hangzhou, China), and their purities were confirmed by mass spectrometry. Fresh solution with a starting concentration of 300 μM was prepared for peptide FAM-G$(PRGPOG)_5$ in 20 mM PBS buffer at pH 7.4. The mixture of peptide FAM-G$(PRGPOG)_5$ (denoted as peptide A) and G$(POG)_{10}$ (denoted as peptide B) at a molar ratio of 1:2 (300 μM A and 600 μM B, denoted as AB) and the mixture of peptide FAM-G$(PRGPOG)_5$ and $(GPO)_6$GERSEQ$(GPO)_6$ (denoted as peptide C) at a molar ratio of 1:2 (300 μM A and 600 μM C, denoted as AC) were prepared in 20 mM PBS buffer at pH 7.4.

Fluorescence Assays

Fluorescence spectra were measured on a RF-5301PC fluorescence spectrometer by using a Xenon lamp as an excitation source (Shimadzu Instruments Ltd., Japan). The homotrimer peptide FAM-G$(PRGPOG)_5$ was prepared at a concentration of 1 μM in 20 mM PBS buffer at pH 7.4. The mixture AB and AC was each diluted 300 times to obtain a final concentration of peptide A as 1 μM. The emission spectra were recorded for peptide A, peptide mixture AB and AC, individually, under three conditions including incubation at 4 °C, heating at 70 °C for 20 min, and re-equilibration at 4 °C for > 24 hrs after the heating at 70 °C. Fluorescence measurements were conducted from 505 to 650 nm with a 1 nm increment per step at an excitation wavelength of 497 nm.

Thermal unfolding was determined by real-time monitoring the fluorescence intensity at 524 nm as a function of increasing temperature (15、20、25、30、33、35、38、40、45、48、50、55、58、60、65、70 °C). The fluorescence spectra were recorded

after incubation for 20 min at each temperature. The refolding process was monitored for 6 hrs at room temperature immediately after the incubation at 70 °C.

The peptide mxitures of A:B were prepared at different molar ratios, heated to 70 °C for 20 min and then annealed at 4 °C for > 24 hrs. The final concentration of A was obtained at 1 μM by dilution, while the concentration of B was varied (0、50、100、200、300、400、500、1000、2000、3000、4000 nM). Fluorescence spectra were recorded on the A:B mixture at different molar ratios and the fluorescence intensity at 524 nm was measured. All the measurements were repeated for 3 times.

Circular Dichroism Spectroscopy

CD spectra were recorded on an Aviv model 400 spectrophotometer (Applied Photophysics Ltd, England). Cells with a path length of 2 mm were used, and the temperature of the cells was controlled using a Peltier temperature controller. CD measurements were performed for peptide A (300 μM) and peptide mixture AB (300 μM A and 600 μM B) in 20 mM PBS buffer at pH 7.4. The peptide mixture AB was heated to 70 °C for 20 min and then annealed at 4 °C for > 24 hrs to allow the formation of heterotrimer. Wavelength scans were conducted from 215 to 260 nm with a 0.5 nm increment per step and a 0.5 s averaging time at 4 °C, and each scan was repeated three times. Thermal unfolding curves were measured by monitoring the amplitude of the characteristic CD peak at 225 nm as a function of increasing temperature with an average heating rate of 0.4 °C/min. The melting temperature (Tm) was determined from the first derivative of the thermal unfolding curves.

CD refolding experiments were performed at 4 °C immediately after the unfolding measurements. The ellipticity at 225 nm was monitored for ~8 h, with a time interval of 10 s. Fraction folded was calculated from the refolding curves as a ratio: $F(t)=(\theta_t-\theta_0)/(\theta_f-\theta_0)$, where θ_t, θ_0, and θ_f are the ellipticities at time t, at time 0 and at final time point when momomer:trimer equilibrium has been reached. The half time of refolding, $t_{1/2}$, was calculated as the time at which the fraction folded reached 0.5.

Colorimetric measurements

Photographs were recorded for peptide A, peptide mixture AB and AC, individually, under three conditions including incubation at 4 °C, heating at 70 °C for 20 min, and re-equilibration at 4 °C for > 24 hrs after the heating at 70 °C with a digital camera (Nikon D7000).

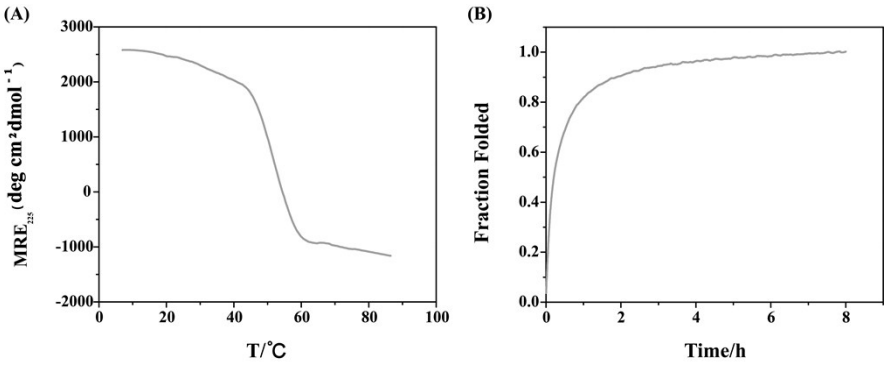

Figure S1. CD thermal unfolding (A) and refolding curves (B) of homotrimer peptide FAM-G(PRGPOG)$_5$. The peptide was prepared at a concentration of 300 μM in 20 mM PBS buffer at pH 7.4. The thermal unfolding and refolding process was monitored by CD spectroscopy at 225 nm.

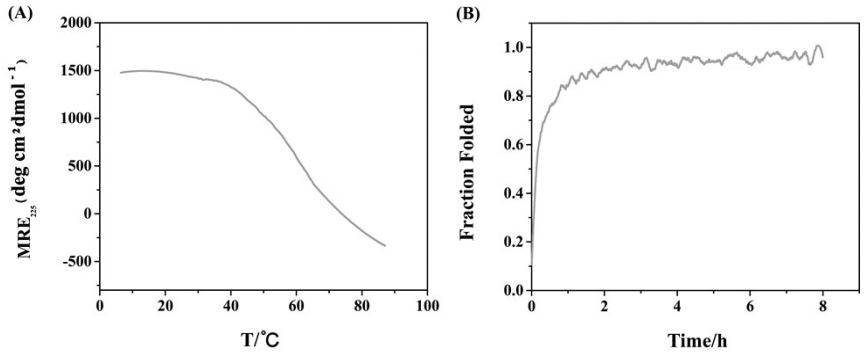

Figure S2. CD thermal unfolding (A) and refolding curves (B) of peptide mixture AB at a molar ratio of 1A:2B. The peptide mixture AB was prepared by incubation at 4 °C for > 24 hrs after the heating at 70 °C for 20 min to allow the heterotrimer formation. The thermal unfolding and refolding process was monitored by CD spectroscopy at 225 nm.

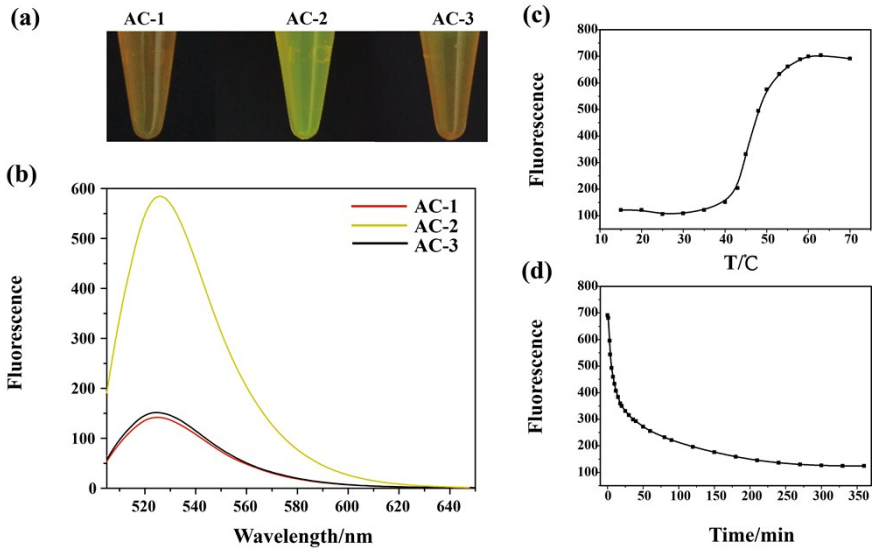

Figure S3. Colorimetric and fluorometric characterization of the mixture of peptide FAM-G(PRGPOG)$_5$ (denoted as peptide A) and (GPO)$_5$GERSEQ(GPO)$_5$ (denoted as peptide C) at a molar ratio of 1:2. Visual changes (a) and fluorescent profiles of the peptide mixture incubated at 4 °C (AC-1), heated to 70 °C for 20 min (AC-2), and re-equilibrated at 4 °C (AC-3). Fluorescence monitoring of the thermal transitions (c) and refolding of the peptide mixture AC (d) at the wavelength of 524 nm. The emission spectra were taken at an excitation wavelength of 497 nm.