**Supporting Information**

# Beyond temperature: Controlling collagen fibrillogenesis under physiological conditions *via* interaction with cucurbit[7]uril

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## 1. Experimental

### *1.1 Materials*

Cucurbit[7]uril (CB[7]) was purchased from Sigma-Aldrich (St. Louis, MO), while adamantanamine hydrochloride (≥99%) was obtained from Aladdin Bio-Chem Technology Co. Ltd. (Shanghai, China). L-phenylalanine (L-Phe, ≥99%) and L-tyrosine (L-Tyr, ≥99%) were supplied by Kelong Chemical Engineering Co. Ltd. (Chengdu, China). Ultrapure water (resistivity=18.2 MΩ cm at 25 °C) was obtained from a Millipore synergy water purification system. All reagents were used without further purification unless otherwise stated.

### *1.2 Preparation of type I collagen*

Following a previously disclosed procedure[1], type I collagen was extracted from fresh calf skins kindly donated by a local tannery. All the purification steps were performed at 4 °C. Briefly, the calf skin was first washed with cold ultrapure water to remove debris, and then shaved with a surgical scalpel to remove both fat and the epidermal layer. The resulting corium

layer was rinsed several times, first with 1% NaCl aqueous solution and then with ultrapure water. After being cut into 1-in. squares, it was extracted by slow stirring in 10 L of 0.5 M acetic acid for 24 h. Subsequently, the solution was filtered and centrifuged at 10000 g for 20 min to remove insoluble materials. From the acidic solvent, collagen was salted out by slowly adding solid NaCl to 3 M under constant stirring. The protein was collected by centrifugation at 10000 g for 20 min, followed by dispersion in 4 L of 0.05 M $NaP_i$ and 1 M NaCl, pH 7.0, buffer. The resulting solution was stirred for 16 h, and the insoluble matter was removed by centrifugation at 10000 g for 1 h. From this neutral solvent, collagen was again salted out by slowly adding solid NaCl to 3 M under constant stirring. The protein was collected by centrifugation at 10000 g for 20 min, dispersed in 2 L of 0.5 M acetic acid, and stirred for another 16 h to dissolve. This solution was then centrifuged at 37000 g for 1 h. From the supernatant, the salting-out procedure was repeated by adding solid NaCl to 3 M, and collecting the protein by centrifugation. The pellets were dissolved in 0.5 M acetic acid and then dialyzed against 1 mM acetic acid for three days using a Spectra/Por 7 regenerated cellulose dialysis membrane with a cut-off molecular weight of 1000 (Sigma-Aldrich, St. Louis, MO). The final collagen stock solution with a concentration of 10 mg/mL was completely transparent and stored at -20 °C before use.

*1.3 Characterization*

Upon heating to 37 ºC, kinetics of type I tropocollagen self-assembly in PS buffer (0.03 M $NaP_i$ and 0.1 M NaCl, pH 7.0) was monitored by measuring its turbidity at 350 nm, using a Lambda25 spectrophotometer (PerkinElmer, U.S.) equipped with an external thermostat (Neslab RTE digital circulating water bath). Excitation–emission fluorescence spectra were collected using a fluorimeter (Hitachi F-4010, Tokyo, Japan) equipped with a 1.0 cm quartz cell. Isothermal titration calorimetry (ITC) experiment was performed in PS buffer at 20 ºC on a Microcal ITC 200 (Malvern, U.K.) to generate the plot of power *vs.* time. The exothermic peaks in this plot were integrated to yield the Wiseman isotherm, which was fit to a binary equilibrium model to derive apparent binding constant, stoichiometry, and thermodynamic data. Zeta (ζ) potentials were determined by a zeta potential analyzer (Powepac Basic, Malvern, U.K.) and calculated using the Smoluchowski equation. All samples were analyzed in triplicate, and

means±standard deviation were reported. Dynamic light scattering (DLS) data were collected on a BI-200SM wide angle laser light scattering system (Brookhaven Instruments Co., U.S.) equipped with an MGL-III model 100 mv He–Ne laser (λ=532 nm), a computer-controlled BI-200SM goniometer, a BI-9000AT digital correlator, and an external thermostat. Polarizing optical microscopy (POM) images were obtained on a Nikon Eclipse LV100POL (NIKON, Japan) equipped with a temperature-controlled hot stage. Fourier transform-infrared (FT-IR) spectra were collected at 25 ℃ on a Nicolet iS10 FT-IR spectrometer (Thermo Scientific, U.S.) over a wavenumber range from 1000 to 2000 $cm^{-1}$ after 32 scans at 2 $cm^{-1}$ resolution. Circular dichroism (CD) analysis was conducted on a research-grade circular dichroism spectrometer (AVIV model 400) at a scanning rate of 20 nm/min and a bandwidth of 1 nm. The morphology of collagen fibrils was visualized by atomic force microscope (AFM, Shimadzu, SPM-9600, Japan) equipped with a Dimension 3100 Nanoscope IV controller using tapping mode. Images were obtained at a scanning rate of 1 Hz using ultrasharp AFM tips with a force constant of 12 N/m and a resonance frequency of 240 kHz (TipsNano, NSG10).

**2. Results**

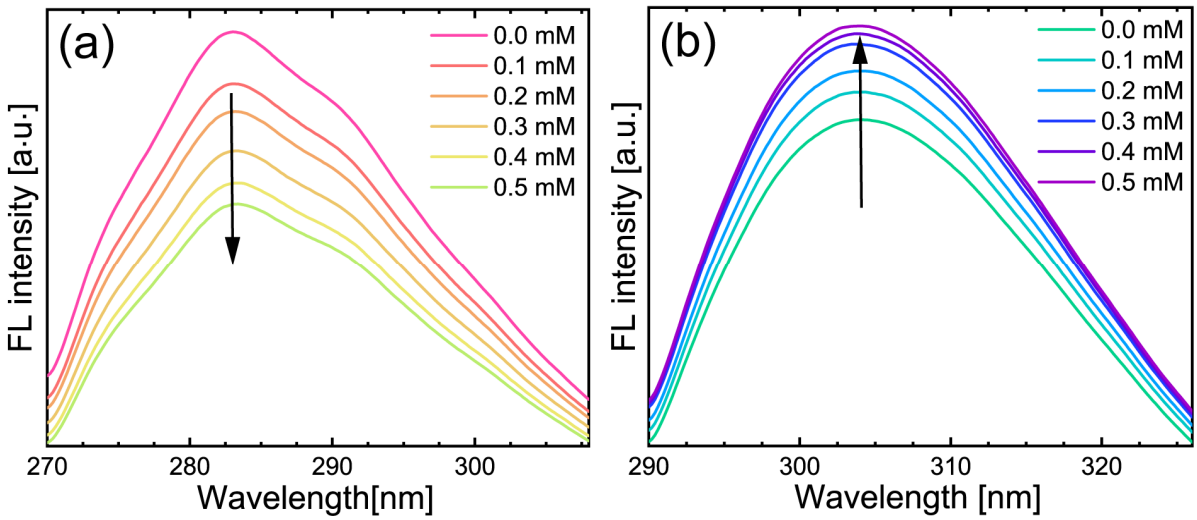


**Figure S1.** Fluorescence emission spectra ($\lambda_{ext}$=253nm) of (a) L-phenylalanine and (b) L-tyrosine dissolved in PS buffer containing CB[7] of varying concentration up to 0.5 mM. Note that even under identical experimental conditions, the characteristic emission peaks of both L-phenylalanine and L-tyrosine differed from those when they were joined *via* amide bonds in tropocollagen.

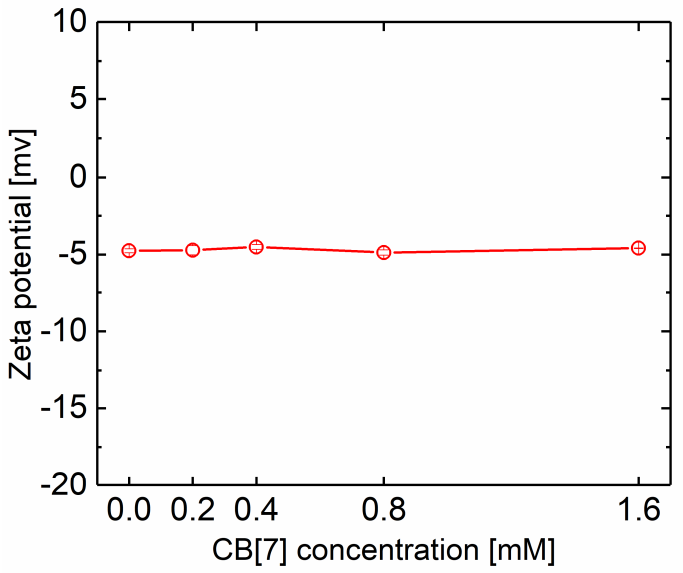


**Figure S2.** Zeta (ζ) potential of monomeric tropocollagen (0.5 mg/mL) dissolved in PS buffer as a function of CB[7] concentration up to 1.6 mM.

## References

1. George C. Na, Linda J. Butz, David G. Bailey, Robert J. Carroll. In vitro collagen fibril assembly in glycerol solution: Evidence for a helical cooperative mechanism involving microfibrils. Biochemistry, 1986, 25: 958-966.