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Supporting Information

Tuning the Thermosensitive Properties of Hybrid Collagen Peptide-Polymer Hydrogels

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Experimental Section

Materials. General. H-Rink Amide-ChemMatrix® resin was purchased from BioMatrix Inc. (Quebec, Canada). Fmoc-Gly-OH and Fmoc-Pro-OH amino acids were purchased from Anaspec Inc. (Fremont, CA). Fmoc-Hyp(t-butyl)-OH amino acid and HBTU were purchased from Aapptec Inc. (Louisville, KY). Fmoc-Cys(Trt)-OH amino acid was purchased from SynPep Corp. (Dublin, CA). HOBT was purchased from Oakwood Products, Inc. (West Columbia, SC). TMP was purchased from Sigma Aldrich Chemical Co. (St. Louis, MI). 8-arm poly(ethylene glycol)maleimide (8-arm PEG-MAL: MW 40 kDa) was purchased from Creative PEGWorks Inc. (Salem, NC). TCEP was purchased from TCI America Inc. (Portland, OR). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MI).

Peptide Synthesis and Purification. Peptides were synthesized using a solid phase Fmoc-based approach on the ChemMatrix resin with HBTU as the coupling reagent. For cysteine coupling, a combination of HBTU and HOBT (1eq. each) were used for couplings. A peptide synthesis flask was charged with rink amide ChemMatrix resin (1g, 0.50 mmol). Fmoc-protected protected amino acids (6 equiv, 3.00 mmol) were treated with HBTU (6 equiv, 3.00 mmol), and DIEA (12 equiv, 6.00 mmol) and reacted for 3-4 hours. The peptide solution was drained and the resin washed with DMF, DCM, MeOH, DCM and DMF (3 x 5 mL each). The resin-bound amino acid was treated with piperidine (25% in DMF, 20 mL) and reacted for 20 mins, to Fmoc-deprotect the amino acid. The piperidine solution was drained and the resin washed with DMF, DCM, MeOH, DCM and DMF (3 x 5 mL each). The coupling and deprotection procedures were repeated until full peptide length was synthesized. After the final Fmoc-deprotection the resin was treated with acetic anhydride (5% acetic anhydride and 8% in 20 mL of DMF, for 30 mins) to acetylate the amino-terminus. The peptide was cleaved from the resin using a TFA cocktail solution (93% TFA, 1% triisopropylsilane (TIPS), 1% thioanisole, 2.5% ethanedithiol, 2.5% anisole). The resulting mixture was filtered (the resin was washed with additional TFA) and concentrated in vacuo. The residue was triturated with cold diethyl ether, and the precipitate was collected by centrifugation. For HPLC purification, the crude peptide was dissolved in water containing 10 mM TCEP (tris(2- carboxyethyl)phosphine) and purged with N_2 for 2 h for disulfide bond reduction. The peptide solution was then filtered and purified by reverse phase HPLC using a Phenomenex Luna C_{18} (50 x 21.20 mm, 100 Å, 5 micron) with an eluent consisting of solvent A (CH_3CN/0.1% TFA) and solvent B (H_2O/0.1% TFA) with different solvent A gradients over 60

mins and a flow rate of 12.00 mL/min (**λ**214nm and **λ**260nm). Purity of the peptides was verified by analytical reverse phase HPLC using a Phenomenex Luna C_{18} column (250 x 4.6 mm, 100 Å, 5 micron) with an eluent consisting of solvent A (CH_3CN/0.05% TFA) and solvent B (H_2O/0.05% TFA) with a 2-50% solvent A gradient over 30 mins and a flow rate of 1.20 mL/min (**λ**214nm). The structure of all the peptides was confirmed by mass spectrometry (MALDI-TOF).

Hydrogel Formation. In order to make **4% PSP-POG7, POG8** and **POG9** hydrogels (100 µL), the corresponding solid peptide stock (1.6, 1.8 and 2.0 mg, respectively) was dissolved in 50 µL of PBS buffer pH 7.4 and combined with 50 µL of a 5% w/v of the **8-arm PEG-MAL** polymer also prepared in PBS buffer pH 7.4. The solution was mixed with a pipette until gelation occurred.

Circular Dichroism. CD wavelength spectra scans were performed on a JASCO Model J810 circular dichroism spectropolarimeter (Easton, MD) equipped with a PFD-425S Peltier temperature control unit at 4°C using a 0.1 cm path length quartz cell. The spectra were averaged over three scans taken from 300 nm to 210 nm with a data pitch of 0.1 nm with a bandwidth of 1 nm. The scan rate of was 100 nm/min with a response time 1 second. The CD data obtained was processed from degrees of rotation to mean residue ellipticity. CD melting curves were determined by measuring the mean residue ellipticity at 225 nm, while running a temperature slope between 4-90°C at 6°C/hour with a data pitch of 0.2 °C, bandwidth of 4 nm and response time of 4 seconds. The peptide samples were prepared by making 250 µL solutions of 200 µM concentration of **POG7**, **POG8** and **POG9** in 10 mM phosphate buffer pH 7.4 and 5 mM of TCEP. The polymer-peptide sample was prepared by heating the **4% PSP-POG** hydrogels (approximately 5 mM in peptide, 100 µL) to 80 °C for 10 minutes and aliquoting the required volume (approximately 10 µL) to make 250 µL solutions of 200 µM in peptide of **PSP-POGX** in 10 mM phosphate buffer pH 7.4.

Rheology. All rheological analyses were performed on a TA instrument ARG2 rheometer (New Castle, DE) using a 20-mm cone and plate geometry with a 1° angle and a sample gap of 200 µm. For rheological experiments, an 8-arm PEG-MAL solution in phosphate buffer at pH 7.4 (5% w/v, 50 µL) was mixed with the desired peptide solution also in phosphate buffer at pH 7.4 (1.8 mg of peptide in 50 µL of PBS) on the rheometer plate to produce the **4% PSP-POGX** hydrogels (100 µL). To avoid evaporation, a solvent trap was placed around the sample. For each sample, three different measurements were performed. First the storage and loss moduli were monitored while applying an oscillation stress bewtween 1-500 Pa with a constant frequency of 5 Hz. Then, a frequency sweep was performed between 1-20 Hz with a constant oscillation stress of 5 Pa. The temperature sweep was monitored between 25-60 °C and 60-25ºC with a constant oscillation stress of 5 Pa and a frequency of 5 Hz with a gradient of 5°C/min. All measurements were taken in triplicate and the SEM was calculated.

Figure S1: Scheme of the collagen triple helix and the chemical structure of the **POGX** collagen peptides (X = 7, 8 or 9).

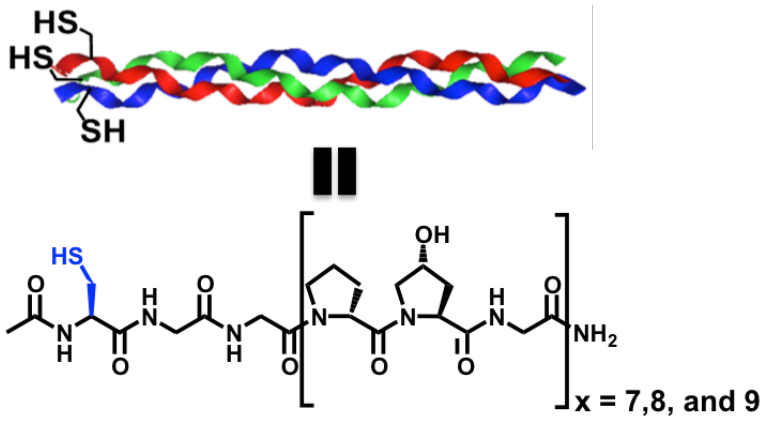

Figure S2: Analytical HPLC of (A) **POG7**, (B) **POG8** and (C) **POG9**.

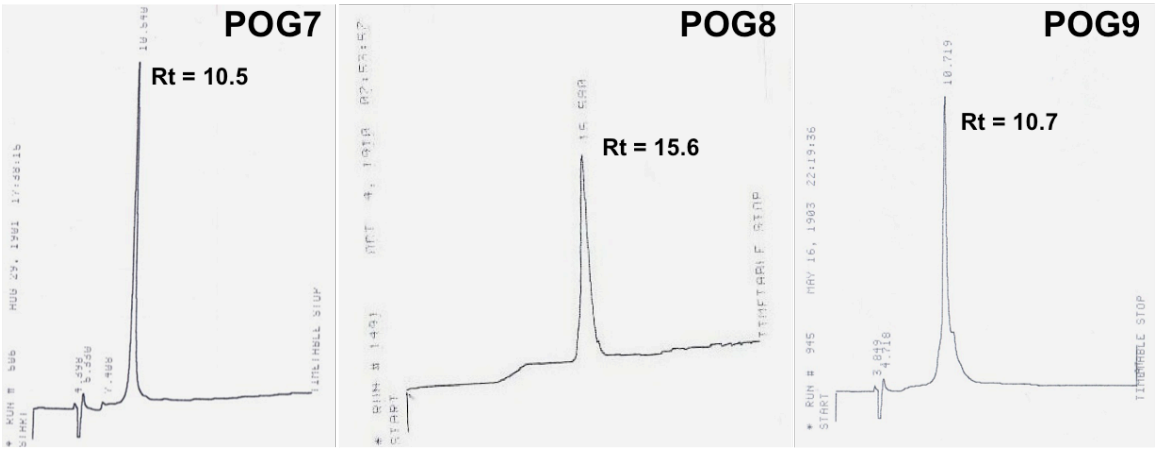

Table S1. Semi-prep HPLC conditions for the purification and MALDI-TOF analysis of **POG7**, **POG8** and **POG9**.

Semi-Prep HPLC conditions		
Peptide	**Gradient**	**Retention time**
POG7	**2 – 30% A[1] over 60 mins**	**21.3 min**
POG8	**2 – 30% A[1]over 60 mins**	**22.4 min**
POG9	**2 – 30% A[1] over 60 mins**	**19.8 min**

1: Solvent A: CH_3CN/0.1% TFA; Solvent B:H_2O/0.1% TFA

MALDI-MS results		
Peptide	**MW, calculated**	**MALDI-MS, observed**
POG7	**2145.94**	**2169.02 $(M+Na^+)^+$**
POG8	**2413.06**	**2458.56 $(M+K^+)^+$**
POG9	**2680.19**	**2702.65 $(M+Na^+)^+$**

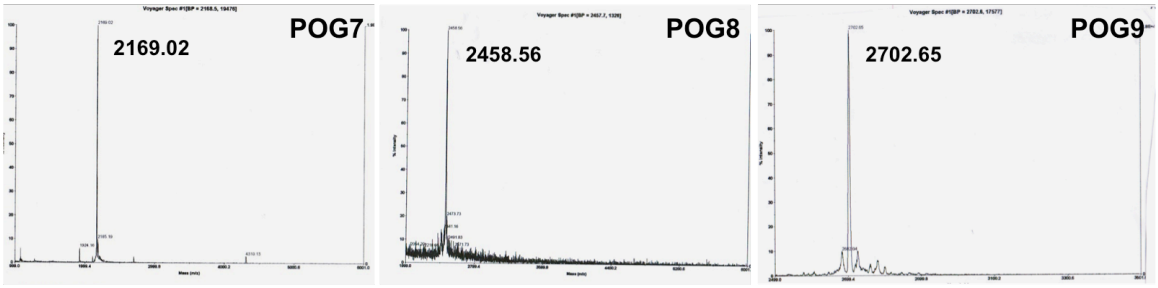

Figure S3: CD spectra summary of **POG7**, **POG8**, **POG9** and its corresponding PSP conjugate. Characteristic CD signal for the **free POGX** peptides (A) and the **PSP-POGX** conjugates (B). (C) And (D) shows the thermal denaturation of the collagen triple helix. (E) And (F) First derivative of the thermal denaturation curve. (X = 7, 8 or 9).

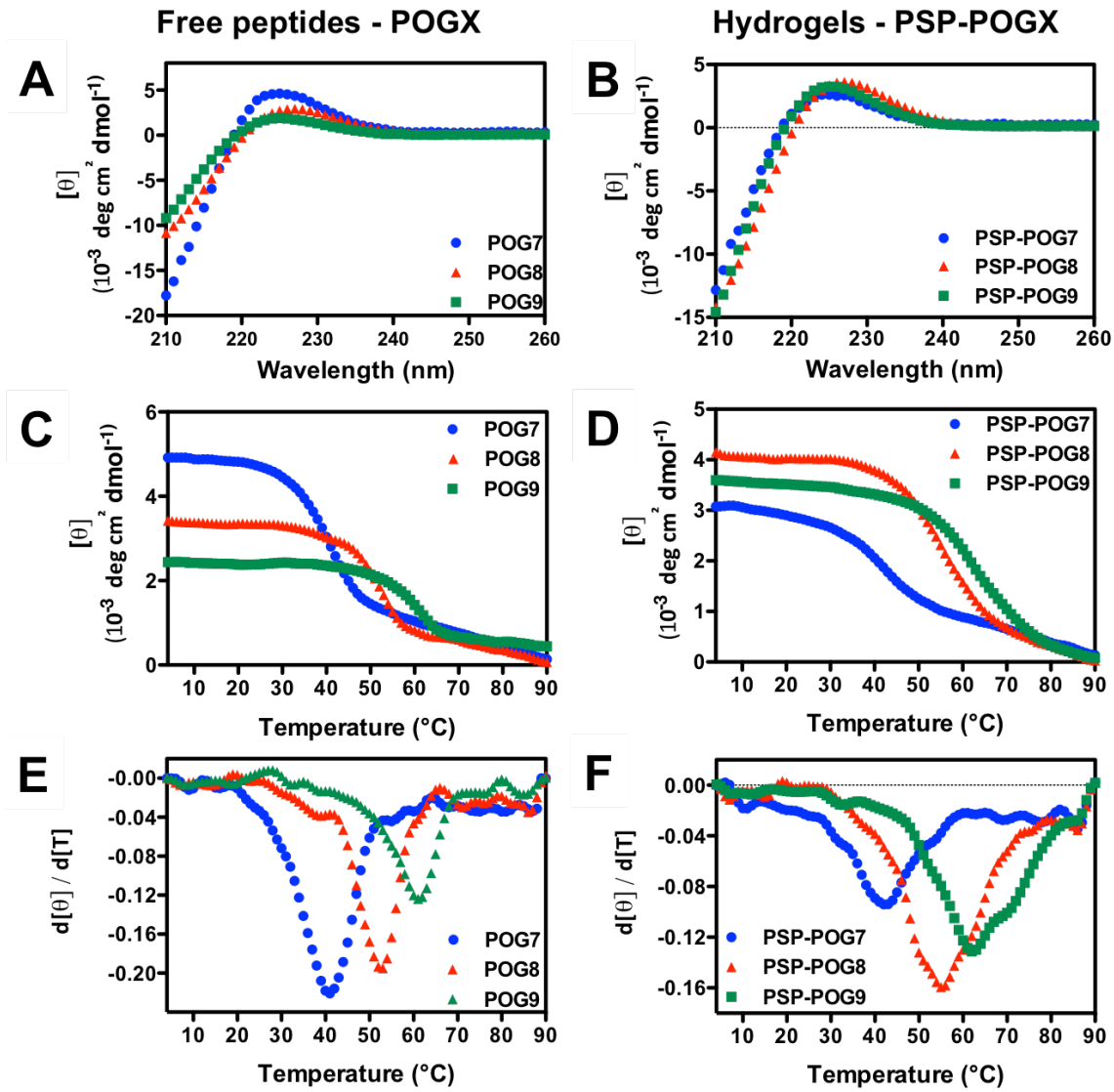

Figure S4: Graph summarizing the heating/cooling cycle on the rheometer for each of the **4% PSP-POGX** hydrogels. Only the mean value is shown at each temperature to show the potential correlation of thermodynamic refolding of the collagen triple helices and the increase of stiffness. The red arrow, closed symbols and red line represent the denaturation (heating) cycle and the blue arrow, open symbols and blue line represents the annealing (cooling) cycle.

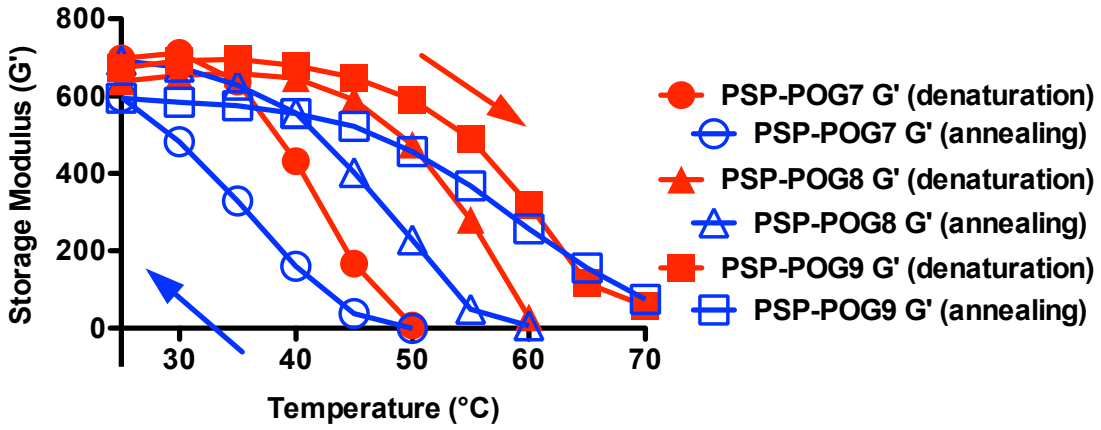

Figure S5. Cryo-SEM micrographs at high magnification of the **4%** (A) **PSP-POG7** and (B) **PSP-POG9** hydrogels.