# Enhanced Mechanical Rigidity of Hydrogels Formed From Enantiomeric Peptide Assemblies.

Katelyn J. Nagy, Michael C. Giano, Albert Jin, Darrin J. Pochan, and Joel P. Schneider

## Supporting Information Table of Contents

**Materials**

PL-Rink amide resin was purchased from Polymer Laboratories. Fmoc-protected valine (L and D enantiomer) , threonine (L and D enantiomer), and proline (L and D enantiomer), and L-isoleucine were purchased through EMD Chemicals. Fmoc-protected L- and D- lysine were purchased from Bachem. Diisopropylethylamine, methanol, acetic anhydride, piperidine, 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU), and spectroscopic grade trifluoroacetic acid (TFA) were purchased through Sigma-Aldrich. 2-(6-Chloro-1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU) was purchased from Peptides International. N-methylpyrolidinone, acetonitrile, dimethylformamide (DMF), trifluoroacetic acid, thioanisole, ethanedithiol, anisole, BIS-TRIS propane (BTP), ethylenediaminetetraacetic acid (EDTA), sodium chloride (NaCl), diethyl ether, and hydrochloric acid were purchased through Fisher.

**Peptide Synthesis and Purification**

MAX1, DMAX1 and the control peptide were synthesized using standard Fmoc solid phase peptide synthesis with HCTU activation on an ABI 433A automated peptide synthesizer. Resin bound peptide was cleaved and side-chain deprotected using trifluoroacetic acid/thioanisole/ethanedithiol/anisole (90:5:3:2) for two hours under inert gas. Crude peptides were precipitated with cold diethyl ether after separation of resin by filtration. The resulting crude MAX1 and DMAX1 peptides were purified by RP-HPLC (Vydac C18 Column) at 40°C using an isocratic gradient from 0 – 2 minutes at 0% standard B, then utilizing a linear gradient from 0 – 15% standard B for 8 minutes followed by a gradient of 15 – 100% standard B over 149 minutes. Here, standard A is 0.1% TFA in water and standard B is 90% MeCN, 9.9% $H_2O$, and 0.1% TFA. Both

peptides elute at 36 minutes. Control peptide was also purified by RP-HPLC (Vydac C18 Column) at 40°C using an isocratic gradient from 0 – 2 minutes at 0% standard B, then utilizing a linear gradient from 0 – 17% standard B for 8 minutes followed by a gradient of 17 – 100% standard B over 174 minutes. Purified peptide solutions were lyophilized, resulting in pure peptide powders that were utilized in all assays. Purity of each peptide was confirmed by analytical HPLC and positive mode electrospray ionization - mass spectrometry.

**Circular Dichroism Spectroscopy**

The secondary structure of the peptides was analyzed on an Aviv 410 Circular Dichroism Spectrophotometer using a 0.1 mm quartz cell. Briefly, a 2 wt % peptide stock solution was prepared by dissolving 4 mg peptide in 200 μL chilled water. To this solution, an equal volume of chilled gelation buffer (100 mM BTP, 300 mM NaCl, pH 7.4) was added, yielding a 1 wt % peptide solution. For the enantiomeric mixtures, separate 2 wt % stock solutions were prepared for each of the L- and D-peptide. The stock solutions were then added volumetrically according to the desired ratio (either 3:1, 1:1, or 1:3 respectively) into a glass vial prior to the addition of the gelation buffer. Immediately following the addition of buffer, 150 μL of the 1 wt % peptide solution was placed into a 0.1 mm quartz cell and then placed into a 37 °C pre-equilibrated chamber. The evolution of β-sheet signal with respect to time was monitored until folding and assembly was complete (30 minutes). After this time, the elipticity was measured over a wavelength range of 205 – 260 nm. The resulting raw data was converted to mean residue ellipticty using the equation:

$$[\theta] = \theta_{obs}/(10*l*c*r)$$

where $\theta_{obs}$ is the measured ellipticity (mdeg), *l* is the path length (0.1cm), *c* is the concentration of peptide (M), and r corresponds to the number of amino acid residues in the peptide (20). Exact concentration of the 2 wt % stock solution was determined by UV spectroscopy at 220 nm using Beer's Law: $A_{220nm} = \varepsilon cl$. Here, A is absorbance at 220 nm, ε is the molar extinction coefficient (15750 $M^{-1}$ $cm^{-1}$), *c* is the concentration of peptide (M), and *l* is the pathlength of the cell (1 cm).

**Infrared Spectroscopy**

IR Spectra were collected on a JASCO-6100 Fourier Transform Infrared Spectrometer using a zinc-selenide flow cell. Samples were prepared by first dissolving the deuteriochloride salt of solid peptide in $D_2O$ followed by initiation of gelation by buffer (100 mM BTP 300 mM NaCl at pH 7.4), resulting in a 1 wt % gel. Deuteriochloride peptides were prepared by lyophilizing the TFA salt of the peptide once from 0.1 M HCl and twice from $D_2O$.

**Oscillatory Shear Rheology**

Rheological assessment was conducted on a Texas Instruments AR-G2 rheometer using a 25 mm stainless steel parallel geometry at a gap height of 0.5 mm. 4 mg of peptide was dissolved in 200 μL of chilled water yielding a 2 wt % peptide solution. To this solution, an equal volume of chilled gelation buffer composed of 100 mM BTP, 300 mM NaCl at pH 7.4 was added. For the enantiomeric mixtures, the 2 wt % stock solutions of each L- and D-peptides were prepared separately before mixing and subsequent addition of gelation buffer. Immediately following the addition of gelation buffer, 320 μL of the 1 wt % gel was added to the center of the plate and the upper geometry was lowered to a gap height of 0.5 mm. The temperature of the system was then increased from 5 $^{o}$C to 37

$^{o}$C over 100 seconds at a constant angular frequency of 6 rad/s and 0.2 % strain. The storage and loss moduli were then monitored for 2 hours at a constant angular frequency of 6 rad/s and 0.2 % strain at 37 $^{o}$C.

**Atomic Force Microscopy**

Fibrils imaged by AFM were prepared as follows. 2 wt % peptide stock solutions in water were prepared the night before each experiment. Gelation was then initiated with an equal volume of buffer composed of 100 mM BTP and 300 mM NaCl at pH 7.4. The resulting 1 wt % gel was incubated at 37 $^{o}$C overnight. After overnight incubation, an aliquot of gel was removed, diluted 40X with water and mixed thoroughly. 5 μL of the diluted gel was added to a freshly cleaved mica substrate and dried with nitrogen gas for imaging by AFM on a Digital Instruments Multi Mode Nanoscope IIA at a scan rate of 1.0 Hz and resonance frequency of 65 kHz. Antimony doped silicon tips (Veeco Probes, FESP7, force constant 3 N/m) were used for imaging. Analysis of fibril height was performed using the section feature that is part of the Nanoscope software.

**Transmission Electron Microscopy**

Images of diluted hydrogel samples were obtained using a Hitachi H-7650 transmission electron microscopy at a voltage of 80 kV. Fibrils imaged by TEM were prepared as follows. Samples were prepared the night before each TEM experiment was to occur. For each sample, 2 wt % peptide stock solutions in water were prepared. Gelation was initiated with an equal volume of buffer composed of 100 mM BTP and 300 mM NaCl at pH 7.4. The resulting 1 wt% gel was incubated at 37 $^{o}$C overnight. After overnight incubation, an aliquot of gel was removed, diluted 100X with water and mixed thoroughly. 5 μL of the diluted gel was added to a 400 mesh carbon coated copper grid

with excess liquid blotted away with filter paper. 1 % uranyl acetate solution was then added to the grid as a negative stain to enhance image contrast. Excess stain was blotted away and the grids were imaged immediately. All fiber width measurements were carried out using ImageJ.

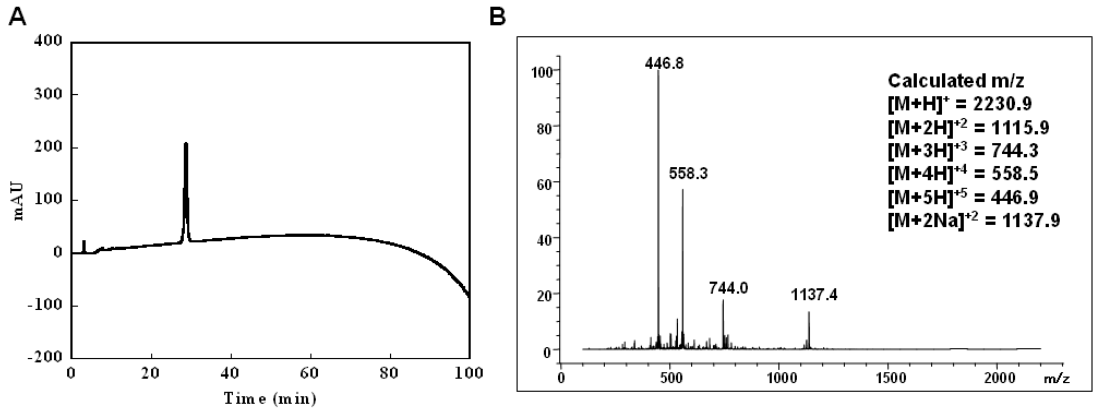


**Figure S1**. Analytical HPLC (A) and ESI-MS (B) of pure MAX1

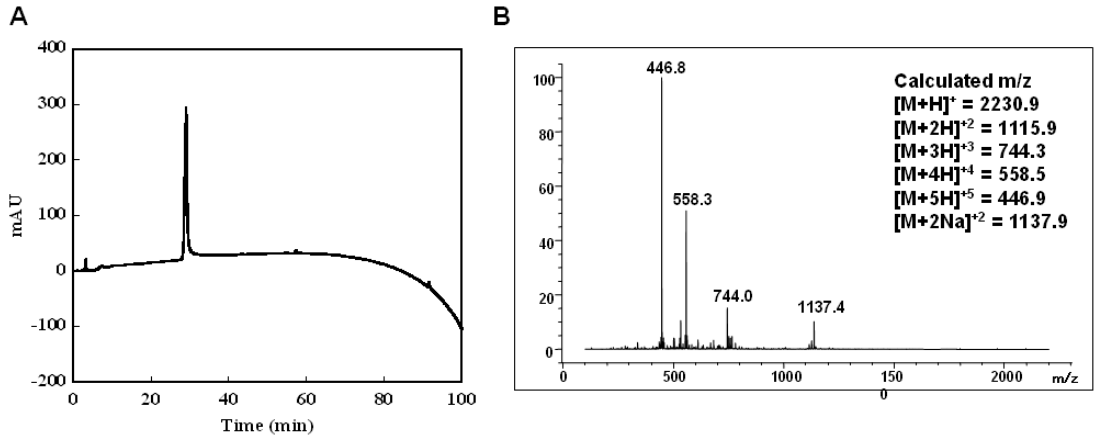


**Figure S2.** Analytical HPLC (A) and ESI-MS (B) of pure DMAX1

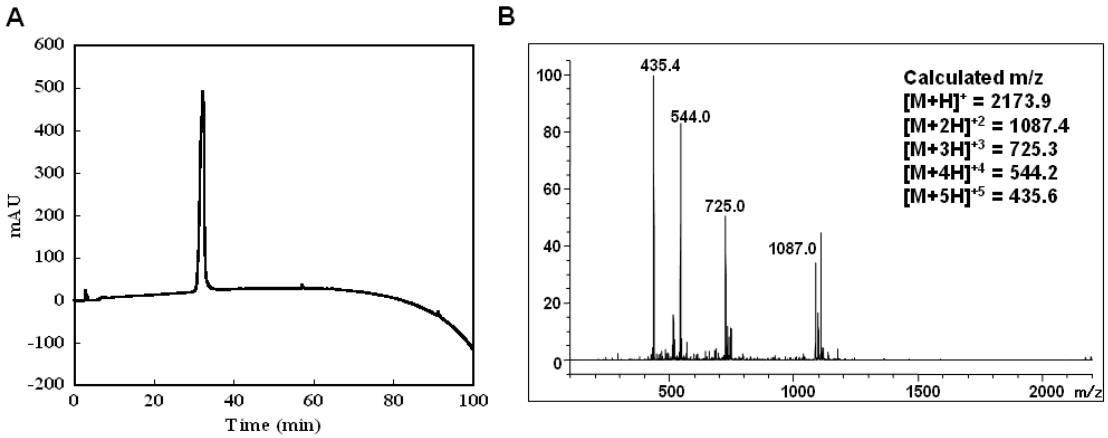


**Figure S3.** Analytical HPLC (A) and ESI-MS (B) of pure Control Peptide

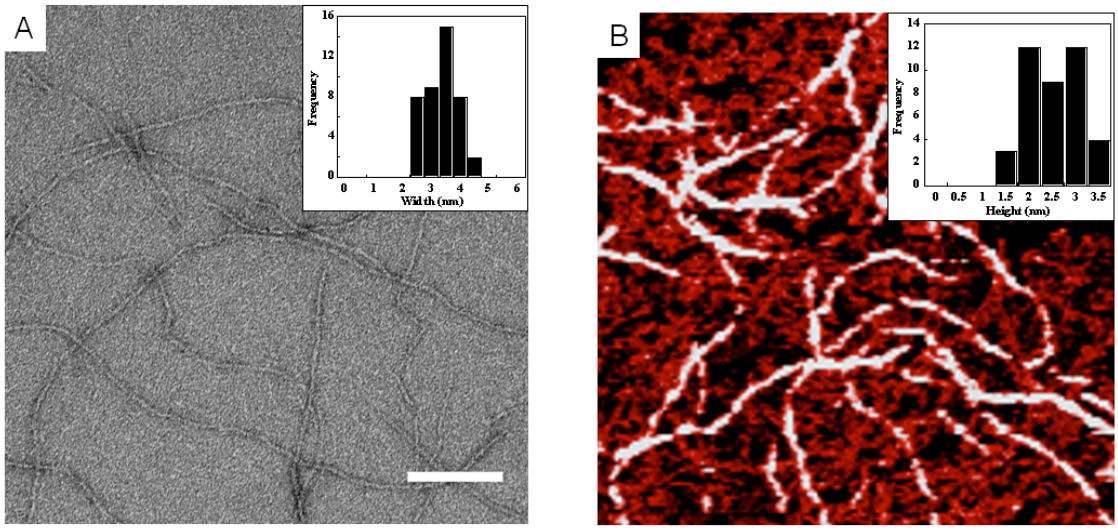


**Figure S4.** Analysis of MAX1 fibril morphology by TEM (A) and AFM (B). (A-inset) Multiple fibril width measurements (N=43) reveal average fiber widths of 3-3.5 nm. (B-inset) Fibril height measurements (N=40) provide an average fibril height of 2.5 nm at the indicated frequencies. (A) scale bar = 100 nm (B) 1 μm x 1 μm square is shown

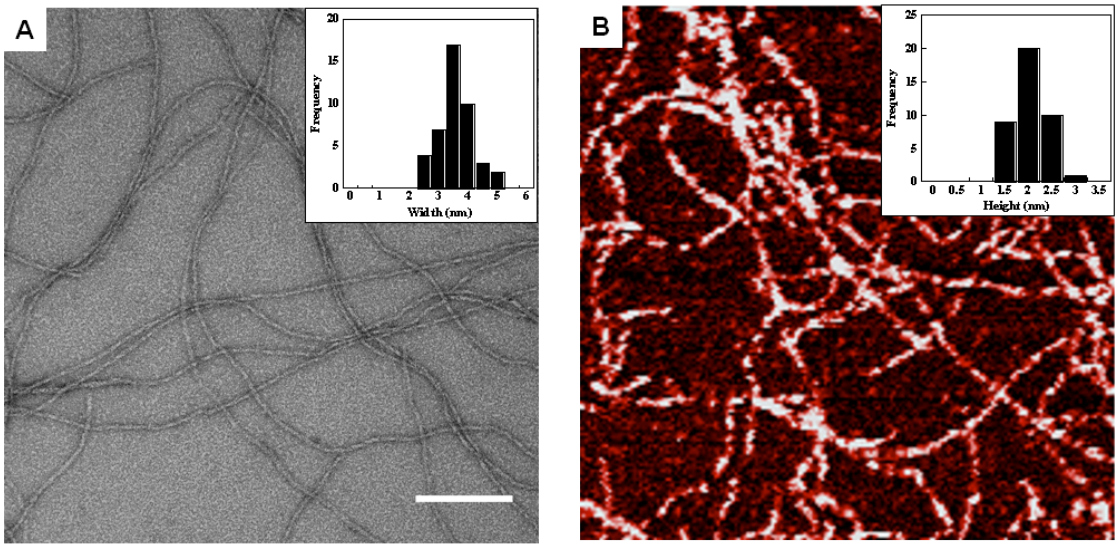


**Figure S5.** Analysis of DMAX1 fibril morphology by TEM (A) and AFM (B). (A-inset) Multiple fibril width measurements (N=43) reveal average fiber widths of 3.5 (B-inset) Fibril height measurements (N=40) provide an average fibril height of 2 nm at the frequencies shown. (A) scale bar = 100 nm (B) 1 μm x 1 μm square is shown

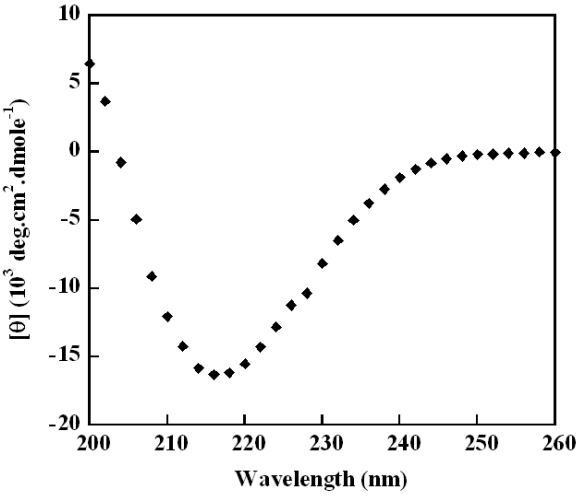


**Figure S6.** Circular dichroism spectrum of 1 wt % Control Peptide

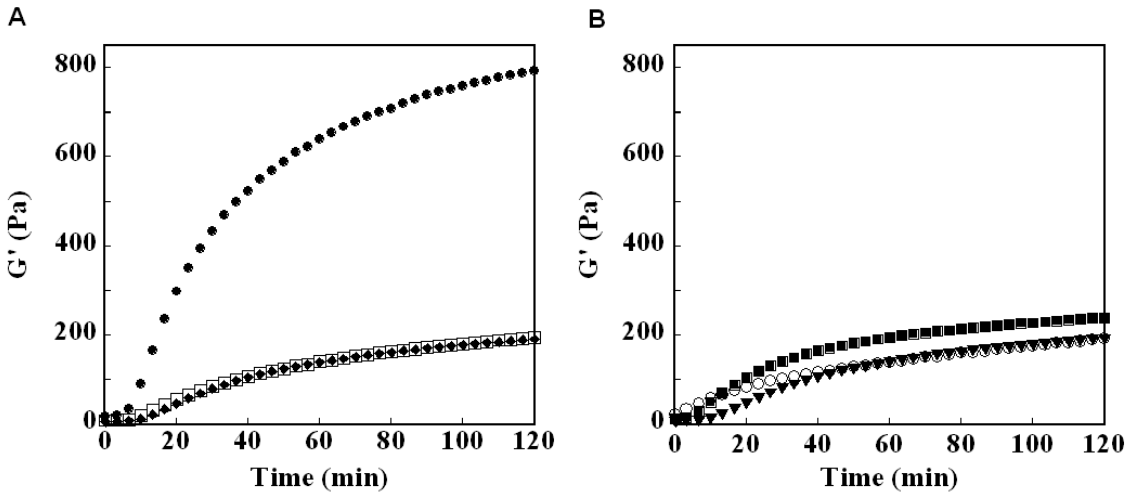


**Figure S7.** Oscillatory rheology time sweep data of 1wt % hydrogels depicting the enhancement in rigidity gained from enantiomeric pairs. The MAX1/DMAX1 system (A) shows a large increase in mechanical rigidity for the racemic hydrogel (●) that is four-fold greater than hydrogels composed of either pure enantiomer, MAX1 (□) or DMAX1 (♦). The Control /DMAX1 system shows no large enhancement in material rigidity in the hydrogel composed of a 1:1 Control:DMAX1 (■) when compared to either pure Control Peptide (○) or DMAX1 (▼) hydrogels.

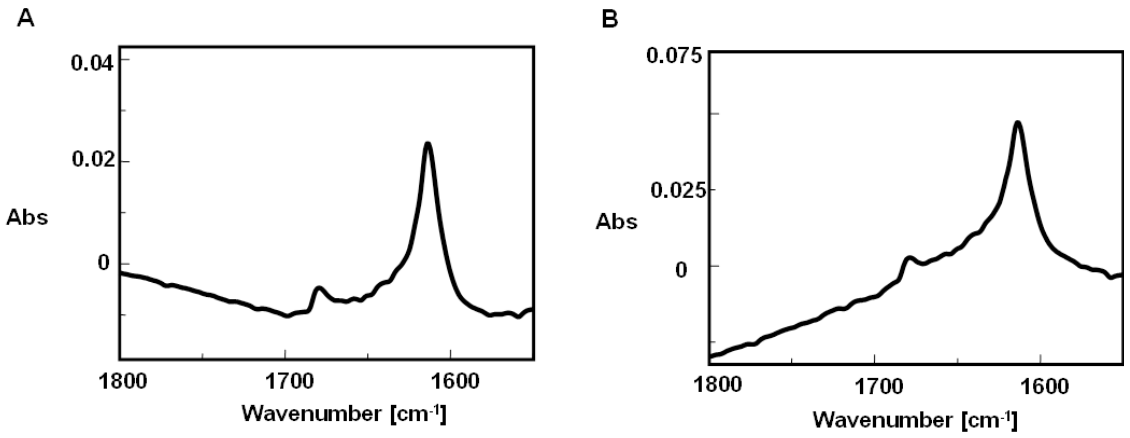


**Figure S8.** FTIR of 1 wt% (A) MAX1 and (B) DMAX1 hydrogels.