Molecular, local, and network-level basis for the enhanced stiffness of hydrogel networks formed from co-assembled racemic peptides: Predictions from Pauling and Corey

Katelyn Nagy-Smith1,2, Peter J. Beltramo3,4, Eric Moore5,6, Robert Tycko5, Eric M. Furst3, and Joel P. Schneider1*

1Chemical Biology Laboratory, National Cancer Institute, Frederick, MD 21702
2Department of Chemistry and Biochemistry, University of Delaware, Newark, DE 19716
3Department of Chemical and Biomolecular Engineering, University of Delaware, Newark, DE 19716
4Department of Materials, ETH Zürich, Vladimir-Prelog-Weg 5, HCI H 515, 8093 Zürich, Switzerland (Current Address)
5Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-0520
6Bruker BioSpin Corporation, Billerica, MA 01821-3991 (Current Address)

Supporting Information Table of Contents

I. Experimental Information
   a. Materials S3
   b. Peptide Synthesis and Purification S3
   c. Oscillatory Rheology S4
   d. Transmission Electron Microscopy S5
   e. Fluorescence Spectroscopy S6
   f. Small Angle Neutron Scattering S6
   g. Diffusing Wave Spectroscopy S7
   h. Solid State NMR S10
   i. Infrared Spectroscopy S11
   j. Synthesis of Fmoc-Lys(Boc)-OH 13C =18O and Fmoc-Val-OH 13C =18O S11

II. Circular Dichroism Spectroscopy of MAX1 (Figure S1) S12

III. Analytical HPLC and ESI-mass spectra of pure peptides
   a. MAX1 (Figure S2) S13
   b. DMAX1 (Figure S3) S13
   c. MAX1-Azide (Figure S4) S14
   d. DMAX1-Biotin (Figure S5) S14
   e. MAX1-EDANS (Figure S6) S15
   f. DMAX1-Dabcyl (Figure S7) S15
   g. MAX1-IR1 (Figure S8) S16
   h. MAX1-IR2 (Figure S9) S16
   i. DMAX1-NMR1 (Figure S10) S17

Supporting Information Table of Contents
j. MAX1-NMR1 (Figure S11) S17
k. MAX1-NMR2 (Figure S12) S18
l. MAX35 (Figure S13) S18
m. DMA35 (Figure S14) S19
n. MARK4 (Figure S15) S19
o. DMARK4 (Figure S16) S20

IV. Oscillatory Rheology of Hydrogels Composed of MAX1-Azide, DMA1-Biotin, and 1:1 MAX1-Azide:DMA1-Biotin (Figure S17) S20

V. Characterization of Nanoparticle Binding Specificity (Figure S18)
And original micrograph used to construct Figure 3A S21

VI. Oscillatory Rheology of Hydrogels Composed MAX1-EDANS, DMA1-Dabcyl, and 1:1 MAX1-EDANS:DMA1-Dabcyl (Figure S19) S22

VII. 2D RAD NMR $^{13}$C-$^{13}$C spectra of 1:1 MAX1-NMR2:DMA with a 25 ms mixing period (Figure S20) S22

VIII. Oscillatory Rheology of MAX1 and 1:1 MAX1-NMR2:DMA Hydrogels Under Conditions Used in ssNMR Figure S21) S23

IX. Characterization of Isotopically-labeled Amino Acids for IE-FTIR
a. HPLC and ESI-mass spectra
i. Fmoc-Val-$^{18}$OH (Figure S22) S23
ii. Fmoc-Lys(boc)-$^{18}$OH (Figure 23) S23
b. NMR
i. $^1$H-NMR Fmoc-Val-$^{16}$OH & Fmoc-Val-$^{18}$OH S24
ii. $^{13}$C-NMR Fmoc-Val-$^{16}$OH & Fmoc-Val-$^{18}$OH S25
iii. $^1$H-NMR Fmoc-Lys(boc)-$^{16}$OH & Fmoc-Lys(boc)-$^{18}$OH S26
iv. $^{13}$C-NMR Fmoc-Lys(boc)-$^{16}$OH & Fmoc-Lys(boc)-$^{18}$OH S27

X. References S28
Experimental Methods

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-Glu-EDANS were purchased through EMD Chemicals. Fmoc-protected L- and D-lysine (boc & MTT protected amino acids) and azide functionalized lysine were purchased from Bachem. NHS-Biotin was purchased from Fisher. Dabcyl-SE was purchased from Sigma. Gold nanoparticles were purchased from Nanopartz. DBCO-amine was purchased from click chemistry tools. All isotopically labeled amino acids and heavy water were purchased from Cambridge Isotope Laboratories. 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-tetramethylammonium hexafluorophosphate (HCTU) was purchased from Peptides International. N-methylpyrrolidinone, 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

All peptides 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. Crude MAX1, MAX1-IR1, MAX1-IR2, 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% H₂O, and 0.1% TFA. Peptides elute at 36 minutes. Peptides sued for ssNNMR were purified utilizing the same gradient, but at 60°C. DMAX1-Dabcyl, MAX1-Azide and DMAX1-Biotin were purified with the same gradient and elute at 38 minutes, 41 minutes, and 42 minutes respectively. MAX1-EDANS was 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 – 24% standard B for 4 minutes followed by a gradient of 24 – 100% standard B over 153 minutes. MAX1-EDANS elutes at 26 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.

**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. For all experiments, separate stock solutions of MAX1 and DMAX1 were prepared by dissolving 4 mg of peptide in 200 μL of chilled water yielding 2 wt % peptide stock solutions. For the frequency sweep measurements in Figure 2A of pure MAX1 or DMAX1, an equal volume of chilled gelation buffer composed of 100 mM BTP, 300 mM NaCl at pH 7.4 was added to a desired volume of each peptide stock solution separately affording 1 wt % solutions that were allowed to gel in the rheometer. For the racemic sample, equal volumes of each 2 wt % L- and D-peptide stock solution were mixed resulting in a racemic stock that is 2 wt% in total peptide. Then, an equal volume of chilled gelation buffer was added to a desired volume of the racemic stock solution affording a 1 wt %
racemic solution that was allowed to gel in the rheometer. For each sample, immediately following the addition of gelation buffer, 300 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 °C to 37 °C over 100 seconds at a constant angular frequency of 6 rad/s and 0.2 % strain. A time-sweep was initially performed where the storage and loss moduli were monitored for 2 hours at a constant angular frequency of 6 rad/s and 0.2 % strain at 37 °C. After which, a dynamic frequency sweep was performed varying the frequency between 0.1 and 10 rad/sec at constant strain (0.2%). The time-sweep data for Figure 2B was collected in a similar manner, but using pre-assembled or co-assembled equimolar racemic mixtures of MAX1 and DMAX1, as well as temperature variations as outlined in the main text.

**Transmission Electron Microscopy**

Micrographs 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 °C overnight. After overnight incubation, an aliquot of gel was removed, diluted 40X with water and mixed thoroughly. 2 µL of the diluted gel was added to a 200 mesh carbon coated copper grid with excess liquid blotted away with filter paper. 5 µL of 10 nm dibenzylecyclooctyne-conjugated gold nanoparticles was added to the grid and allowed to bind for 5 minutes. Excess liquid was blotted away with filter paper and the grid was rinsed with a solution of 1 % TWEEN to remove non-specifically bound nanoparticles. The grid was rinsed with water then 5 µL 5 nm streptavidin-labeled gold nanoparticles were added to the grid for 5
minutes. Again, excess solution was blotted away and the grids were rinsed with 1 % TWEEN followed by water. 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.

**Fluorescence Spectroscopy**

Fluorescence spectra of diluted hydrogels were obtained on a Photon Technologies 400 Fluorometer. 1 wt % hydrogels were prepared the night before each experiment was to occur as described above. For each sample, hydrogels were diluted to obtain 4 μM MAX1-EDANS for fluorescence analysis. The samples designed to mimic enantiomer self-sorting were prepared by mixing equal volumes of 4 μM MAX1-EDANS and 4 μM DMAX1-Dabcyl solutions that were diluted from 1 wt % MAX1-EDANS and DMAX1-Dabcyl hydrogels respectively. 1 wt % 1:1 MAX1-EDANS:DMAX1-Dabcyl hydrogels were diluted to 8 μM total peptide where the quenched fluorescence of 4 μM MAX1-EDANS was analyzed. 175 μL of the diluted gel was added to a quartz microcuvette with a path length of 3 mm and placed in the sample chamber at room temperature. EDANS fluorescence was monitored over 400 – 600 nm with a $\lambda_{max}$ at 494 nm.

**Small Angle Neutron Scattering (SANS)**

Samples were prepared as described for the oscillatory rheology experiments described above, except in D$_2$O. Hydrogel samples were prepared within the sample cell the night prior to the scattering experiment. After initiation of gelation, peptide solutions were introduced into the sample cell and incubated at 37ºC for 2 hours. Cells were then removed from the incubator and maintained at room temperature overnight. The experiments were performed at the NG-3 beamline at the NCNR at NIST in Gaithersburg, MD, with $\lambda = 6$ Å and sample-to-detector
distances of 1, 4, and 13 m. Established protocols for SANS data acquisition and analysis were followed.

**Diffusing Wave Spectroscopy (DWS)**

Samples for the DWS experiments were prepared in a similar fashion as described for the oscillatory rheology experiments. Here, however, 2 wt % and 1.5 wt % peptide stock solutions were prepared with a 2 % solution of 1 \( \mu \)m polystyrene microspheres. After initiation of gelation by an equal volume of aqueous buffer (100 mM BTP 300 mM NaCl at pH 7.4), 1 mL peptide/microsphere solution was introduced to plastic cuvettes with a pathlength of 4 mm and incubated at 37°C for 2 hours. Cuvettes were removed from the incubator and maintained at room temperature overnight.

A multispeckle detection scheme based on the design of Scheffold et al.\(^1\) is implemented to measure non-ergodic systems such as the peptide hydrogels under study. A vertically polarized argon ion laser (\( \lambda = 514.5 \) nm, Coherent Innova 90-C-5) is expanded and incident on the sample cuvette with path length \( L = 4 \) mm. The transmitted scattering from the sample is collimated using a lens (focal length = \( 4 \) cm) and imaged onto a gritted disk (Thorlabs, Inc. DG20-1500). The disk is rotated by a stepper motor at approximately 0.5°/hr to generate a reference autocorrelation function that decorrelates over seconds. A horizontally polarized plate ensures the signal collected by the fiber optic cable is exclusively multiply scattered light. The autocorrelation function is obtained by splitting the signal into two photomultipliers (Brookhaven, BI-CCDS) and taking their cross-correlation with an autocorrelator board (Brookhaven, BI-9000AT). Using this method, delay times ranging from 25 ns to 10 s are probed.
To extract the ergodic intensity correlation function of the sample of interest, the composite correlation function of sample and disk is divided by the disk correlation function\(^1\),

\[
g_{\text{sample}}^{(2)}(\tau) - 1 = \frac{g_{\text{sample+disk}}^{(2)}(\tau) - 1}{g_{\text{disk}}^{(2)} - 1}
\]  

(1)

The normalized field correlation function is then found using the Siegert relation, \(g^{(2)}(t) = 1 + \beta |g^{(1)}(t)|^2\), where \(\beta\) is an experimental parameter that accounts for the optical properties of our setup.

We numerically calculate the mean-squared displacement of the probe particles from its relation to the field autocorrelation function in plane-wave transmission geometry, given by

\[
\frac{g^{(2)}(\tau) - 1}{\beta} = g^{(1)}(\tau) \approx \frac{\left(\frac{L}{l^*} + \frac{4}{3}\bar{R}(\tau)\right)}{\left(1 + \frac{4}{27}R^2(\tau)\right)\sinh\left[\left(\frac{L}{l^*}\right)R(\tau)\right] + \frac{4}{3}R(\tau) \cosh\left[\left(\frac{L}{l^*}\right)R(\tau)\right]}
\]

(2)

where \(L\) is the sample path length, \(l^*\) is the photon mean-free scattering path of the sample, and \(R(\tau) \equiv \sqrt{k_0^2 \langle \Delta r^2(\tau) \rangle}\) with \(k_0 = 2\pi n/\lambda\). The photon mean free path for each sample is determined by relating the transmitted light intensity of the sample, \(T\), to the transmittance, \(T_{\text{ref}}\), of a sample with known \(l^*\) in the same path length range using\(^2\)\(^3\)

\[
l^* = \frac{T}{4l^*_{\text{ref}} - 3L(T_{\text{ref}} - T)}
\]

(3)

The same 1 \(\mu\)m diameter spherical particles are suspended in water at a volume fraction of 1 % and used as a reference to find \(T_{\text{ref}}\) and \(l^*_{\text{ref}}\). For spheres freely diffusing in water, the Einstein equation gives the mean squared displacement as

\[
\langle \Delta r^2(\tau) \rangle = 6D\tau = \frac{kT\tau}{\pi a} \eta
\]

(4)
where $kT$ is the thermal energy, $a$ is the particle radius and $\eta$ is the medium viscosity. Equation S2 is fit to the data using Equation S4 for the MSD with $\beta$ and $l^*$ as the unknown parameters. The mean free scattering path for 1 $\mu$m diameter spherical particles in water determined in this manner is $l^*_{\text{ref}} = 303$ $\mu$m, in good agreement with theory.

We use an approximate form of the Generalized Stokes-Einstein Relation (GSER) to relate the mean-squared displacement of the probe particles to the peptide hydrogel viscoelastic properties

$$G^*(\omega) = \frac{k_BT}{\pi a \langle \Delta r^2(\tau) \rangle \Gamma(\alpha(\tau)+1)}$$

Where $\omega = 1/\tau$ and $\Gamma(\alpha)$ is the Gamma function. In this procedure, a power-law form of the MSD is assumed and $\alpha(\tau)$ is the logarithmic slope of the MSD evaluated at each lag time,

$$\alpha(\tau) = \frac{d \ln \langle \Delta r^2(\tau) \rangle}{d \ln \tau}$$

The storage and loss moduli follow as:

$$G^*(\omega) = G'(\omega) + iG''(\omega)$$

In order to bring the microrheological modulus in concert with that determined by mechanical rheology, we use an effective particle radius of $a = 1$ $\mu$m for the gels. This is necessary due to the slight particle aggregation that occurs during gelation, and treatments of this nature are often observed but heretofore unexplained.

The data shown in Fig 5 are the average of three independent samples. Unless otherwise noted, the error in fitted results is that from multiple samples, which is always greater than or equal to the error in the fit of an individual sample. Results are reproducible between peptide
batches and measurements taken of the course of weeks on the same sample show no signs of aging.

**Solid State Nuclear Magnetic Resonance**

1 wt% hydrogels of 1:1 MAX1-NMR1:DMAX1-NMR1 and 1:1 MAX1-NMR2: DMAX were prepared as described previously, here using 250 mM BTP 20 mM NaCl at pH 9 to initiate gelation. The ssNMR experiment require low salt concentrations, thus a higher pH buffer was necessary to trigger gelation. Under these buffering conditions at room temperature, the enhancement in mechanical rigidity for the racemic hydrogel is retained as assessed by oscillatory rheology as shown in Figure S21. Gels were maintained at room temperature for 2 hours and then frozen in liquid nitrogen followed by lyophilization. The resulting freeze-dried hydrogel was packed into the MAS rotor and rehydrated prior to solid state NMR experiments.

Solid state NMR data were acquired on a Bruker Avance III spectrometer operating at 100.8 MHz $^{13}$C NMR frequency (400.9 MHz $^1$H NMR frequency) and a Varian InfinityPlus spectrometer operating at 100.4 MHz $^{13}$C NMR frequency (399.2 MHz $^1$H NMR frequency). A triple resonance Varian MAS probe with 3.2 mm rotors was used. Mixing periods in 2D $^{13}$C-$^{13}$C spectra used radio-frequency-assisted diffusion/dipolar-assisted rotational resonance (RAD/DARR)\(^7\)-\(^8\) with 9.00 kHz MAS, 105 kHz $^1$H decoupling, a maximum $t_1$ period of 5.08 ms, 25 ms or 500 ms mixing periods, a 0.5 s recycle delay and a total measurement time of 36 h or 54 h. $^{13}$C chemical shifts are relative to 2,2-dimethyl-2-silapentane-5-sulfonic acid (DSS). NMR data were processed with NMRpipe\(^9\); contour levels in contour plots increases by successive factors of 1.3. PITHIRDS-CT data were acquired as previously described.\(^10\)-\(^11\) Experimental data in Figure 8B are corrected for an 18% contribution from natural-abundance carbonyl $^{13}$C signals, which is assumed to be independent of the dipolar recoupling time.
**Isotope-Edited Fourier Transform 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$_2$O followed by initiation of gelation by buffer (100 mM BTP 300 mM NaCl at pH 7.4), resulting in a 1 wt % gel. The labeled valine within MAX1-IR1 and MAX1-IR2 is $^{13}$C and $^{18}$O labeled at the amide carbonyl only. The labeled lysine within MAX1-IR1 is $^{13}$C labeled at all carbons and $^{18}$O labeled at the carbonyl. Deuteriochloride peptides were prepared by lyophilizing the TFA salt of the peptide once from 0.1 M HCl and twice from D$_2$O.

**Synthesis of Fmoc-Lys(Boc)-$^{13}$C$^{18}$O$_2$H and Fmoc-Val-$^{13}$C$^{18}$O$_2$H**

Synthesis of isotopically labeled amino acids was performed as in Seyfriend et al$^{12}$. All starting materials and reagents were extensively dried by azeotropic removal of water under reduced pressure using acetonitrile. Briefly, dried Fmoc-Val-$^{13}$CO$_2$H or Fmoc-Lys(Boc)-$^{13}$CO$_2$H (Lys also $^{13}$C labeled at side chain carbons) was dissolved in dry DMF and added via syringe to a solution containing 20 eq. 3,5-dimethylpyridine hydrobromide, 10 eq. EDC•HCl and 50 eq. $^{18}$OH$_2$ in DMF. The mixture was stirred at room temperature for 20 hr under N$_2$. Two subsequent 10 eq. of EDC•HCl were added stirred for 8 and 15 hrs. The product was extracted by ethyl acetate, dried over MgSO$_4$, filtered, and solvent was removed by rotavap and high vacuum. The isolated amino acids were purified by RP-HPLC (Vydac C18 Column). Fmoc-Lys(Boc)-$^{13}$C$^{18}$O$_2$H (also $^{13}$C labeled at side chain carbons) and Fmoc-Val-$^{13}$C$^{18}$O$_2$H were purified using a gradient of 50 -100 % Standard B over 50 minutes. Purity of each compound was confirmed by analytical HPLC, ESI-MS, and NMR (Figures S22-S27).
Figure S1. Temperature dependent evolution of β-sheet secondary structure for 1 wt % MAX1 hydrogel (A). At 5°C (▲), MAX1 is unfolded. Increasing the temperature to 25°C (★) drives MAX1 folding and self-assembly (B).
Figure S2. Analytical HPLC (A) and ESI-MS (B) of pure MAX1. [M+H+Na]$^{2+}$ and [M+2Na]$^{2+}$ adducts are also observed.

Figure S3. Analytical HPLC (A) and ESI-MS (B) of pure DMAX1. [M+H+Na]$^{2+}$ and [M+2Na]$^{2+}$ adducts are also observed.
Figure S4. Analytical HPLC (A) and ESI-MS (B) of pure MAX1-Azide. \([\text{M+H+Na}]^{2+}\) and \([\text{M+2Na}]^{2+}\) adducts are also observed.

Figure S5. Analytical HPLC (A) and ESI-MS (B) of pure DMAX1-Biotin. \([\text{M+H+Na}]^{3+}\) and \([\text{M+2Na}]^{2+}\) adducts are also observed.
Figure S6. Analytical HPLC (A) and ESI-MS (B) of pure MAX1-EDANS. [M+H+Na]^{2+} and [M+2Na]^{2+} adducts are also observed.

Figure S7. Analytical HPLC (A) and ESI-MS (B) of pure DMAX1-Dabcyl. [M+H+Na]^{2+} and [M+2Na]^{2+} adducts are also observed.
Figure S8. Analytical HPLC (A) and ESI-MS (B) of pure MAX1-IR1. \([\text{M+H+Na}]^{2+}\) and \([\text{M+2Na}]^{2+}\) adducts are also observed. The labeled valine within MAX1-IR1 is $^{13}$C and $^{18}$O labeled at the amide carbonyl only. The labeled lysine within MAX1-IR1 is $^{13}$C labeled at all carbons and $^{18}$O labeled at the carbonyl.

Figure S9. Analytical HPLC (A) and ESI-MS (B) of pure MAX1-IR2. \([\text{M+H+Na}]^{2+}\) and \([\text{M+2Na}]^{2+}\) adducts are also observed. The labeled valine within MAX1-IR2 is $^{13}$C and $^{18}$O labeled at the amide carbonyl only.
Figure S10. Analytical HPLC (A) and ESI-MS (B) of pure DMAX1-NMR1. The labeled proline within DMAX1-NMR1 is $^{13}$C labeled at the amide carbonyl only.

Figure S11. Analytical HPLC (A) and ESI-MS (B) of pure MAX1-NMR1. Note: This peptide is $^{13}$C labeled at the amide carbonyl of V1 and $^{15}$N labeled at V20 (not indicated in Table 1). The $^{15}$N label is inconsequential to the PTHIRDS experiments performed.
Figure S12. Analytical HPLC (A) and ESI-MS (B) of pure MAX1-NMR2. [M+H+Na]^{2+} and [M+2Na]^{2+} adducts are also observed.

Figure S13. Analytical HPLC (A) and ESI-MS (B) of pure MAX35. [M+H+Na]^{2+} and [M+2Na]^{2+} adducts are also observed.
**Figure S14.** Analytical HPLC (A) and ESI-MS (B) of pure DMAX35. \([\text{M}+\text{H}+\text{Na}]^{2+}\) and \([\text{M}+2\text{Na}]^{2+}\) adducts are also observed.

**Figure S15.** Analytical HPLC (A) and ESI-MS (B) of pure MARK4. \([\text{M}+\text{H}+\text{Na}]^{2+}\) and \([\text{M}+2\text{Na}]^{2+}\) adducts are also observed.
**Figure S16.** Analytical HPLC (A) and ESI-MS (B) of pure DMARK4. [M+H+Na]^{2+} and [M+2Na]^{2+} adducts are also observed.

**Figure S17.** Dynamic time sweep oscillatory rheology measuring storage moduli of 1 wt % hydrogels composed of MAX1-Azide (◆), DMAX1-Biotin (●) or 1:1 MAX1-Azide:DMAX1-Biotin (■) indicating the rheological phenomenon is retained for the functionalized peptides compared to unfunctionalized peptides.
**Figure S18.** Nanoparticles specifically bind to intended target peptide. Streptavidin labeled 5 nm gold nanoparticles do not bind to MAX1-Azide network, only DMAX1-Biotin network (A,C). DBCO-conjugated 10 nm gold nanoparticles react with MAX1-Azide fibrils (B) and show minimal non-specific interactions with the TEM grid (D). Panel E shows the original non-colored micrograph used to construct Figure 3A in the main text.
Figure S19. Dynamic time sweep oscillatory rheology measuring storage moduli of 1 wt % hydrogels composed of MAX1-EDANS (●), DMAX1-Dabcyl (●) or 1:1 MAX1-EDANS:DMAX1-Dabcyl (■) indicating the rheological phenomenon is retained for these functionalized peptides compared to the unfunctionalized ones.

Figure S20. 2D RAD NMR $^{13}$C-$^{13}$C spectra of 1:1:1 MAX1-NMR2:DMAX with a 25 ms mixing period (A) where green and blue lines correspond to 1D slices at the Cα and Cβ chemical shifts of V20 respectively which are shown in panel B. Panel C shows spectrum with a 500 ms mixing time.
Figure S21. Dynamic time sweep oscillatory rheology measuring storage moduli of 1 wt % hydrogels composed of MAX1 (◆), and 1:1 MAX1:DMAX1 (■) indicating the rheological enhancement is retained under conditions used in solid state NMR experiments (125 mM BTP 10 mM NaCl pH 9, 25°C).

Figure S22. Analytical HPLC (A) and ESI-MS (B) of pure Fmoc-Val\textsuperscript{13}C\textsubscript{18}O\textsubscript{2}H

Figure S23. Analytical HPLC (A) and ESI-MS (B) of pure Fmoc-Lys(boc)-\textsuperscript{13}C\textsubscript{18}O\textsubscript{2}H (also \textsuperscript{13}C label at side chain)
Figure S24. $^1$H-NMR Fmoc-Val-$^{16}$OH (A) and Fmoc-Val-$^{18}$OH (B)
Figure S25. $^{13}$C-NMR Fmoc-Val-$^{16}$OH (A) and Fmoc-Val-$^{18}$OH (B)
Figure S26. $^1$H-NMR Fmoc-Lys(boc)-$^{16}$OH (A) and Fmoc-Lys(boc)-$^{18}$OH (B)
Figure S27. $^{13}$C-NMR Fmoc-Lys(boc)-$^{16}$OH (A) and Fmoc-Lys(boc)-$^{18}$OH (B)

A

Fmoc-Lys(Boc)$^{16}$-OH

B

Fmoc-Lys(Boc)$^{15}$-OH
References

1. Scheffold, F.; Skipetrov, S. E.; Romer, S.; Schurtenberger, P. Diffusing-wave spectroscopy of nonergodic media. *Phys. Rev. E* **2001**, *63*, 061404-1 - 061404-11.
2. Kaplan, P. D.; Dinsmore, A. D.; Yodh, A. G.; Pine, D. J. Diffuse transmission spectroscopy - a structural probe of opaque colloidal mixtures. *Phys. Rev. E* **1994**, *50*, 4827-4835.
3. Dasgupta, B. R.; Weitz, D. A. Microrheology of cross-linked polyacrylamide networks. *Phys. Rev. E* **2005**, *71*, 021504-1 - 021504-9.
4. Lu, Q.; Solomon, M. J. Probe size effects on the microrheology of associating polymer solutions. *Phys. Rev. E* **2002**, *66*, 061504-1 - 061504-11.
5. Mason, T. G. Estimating the viscoelastic moduli of complex fluids using the generalized Stokes-Einstein equation. *Rheol. Acta* **2000**, *39*, 371-378.
6. Mason, T. G.; Ganesan, K.; vanZanten, J. H.; Wirtz, D.; Kuo, S. C. Particle tracking microrheology of complex fluids. *Phys. Rev. Lett.* **1997**, *79*, 3282-3285.
7. Morcombe, C. R.; Gaponenko, V.; Byrd, R. A.; Zilm, K. W. Diluting abundant spins by isotope edited radio frequency field assisted diffusion. *J. Am. Chem. Soc.* **2004**, *126*, 7196-7197.
8. Takegoshi, K.; Nakamura, S.; Terao, T., C-13-H-1 dipolar-assisted rotational resonance in magic-angle spinning NMR. *Chem. Phys. Lett.* **2001**, *344*, 631-637.
9. Delaglio, F.; Grzesiek, S.; Vuister, G. W.; Zhu, G.; Pfeifer, J.; Bax, A. NMRPIPE - A multidimensional spectral processing system based on UNIX pipes. *J. Biomol. NMR* **1995**, *6*, 277-293.
10. Nagy-Smith, K.; Moore, E.; Schneider, J.; Tycko, R. Molecular structure of monomorphic peptide fibrils within a kinetically trapped hydrogel network. *Proc. Nat. Acad. Sci. of the United States of America* **2015**, *112* (32), 9816-9821.
11. Tycko, R. Symmetry-based constant-time homonuclear dipolar recoupling in solid state NMR. *J. Chem. Phys.* **2007**, *126*, 064506-1 - 064506-1.
12. Seyfried, M. S.; Lauber, B. S.; Luedtke, N. W., Multiple-Turnover Isotopic Labeling of Fmoc- and Boc-Protected Amino Acids with Oxygen Isotopes. *Org. Lett.* **2010**, *12*, 104-106.