

SUPPLEMENTARY MATERIAL TO: DYNAMIC LIGHT SCATTERING MICRORHEOLOGY FOR SOFT AND LIVING MATERIALS

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I. MATERIALS

Hyaluronic acid polymer solutions were prepared by dissolving 700 kDa sodium hyaluronate (Lifecore Biomedical # HA700K-1) in Milli-Q water at 0.5 wt%. Prior to DLS μ R measurements, polystyrene microspheres (Polysciences) functionalized with polyethylene glycol (100-nm and 2000-nm diameter sizes, PEGylation procedure detailed below) were added to a final concentration of 0.1% (w/v).

Polyethylene glycol thiolvinylsulfone gels were prepared by dissolving 20 kDa 8-arm polyethylene glycol vinylsulfone (JenKem Technology USA # A10034-1) at 3 wt%, 5 wt%, or 7 wt% and poly(ethylene) glycol dithiol (Millipore Sigma # 704369-1G) at 3 wt%, 5 wt%, or 7 wt% in Triethanolamine buffer solution (Sigma # T0449-120ML). The polyethylene glycol vinylsulfone and polyethylene glycol dithiol solutions were mixed at a stoichiometric ratio of functional groups of 1:1 (1 vinylsulfone: 1 thiol).

Polyethylene glycol gels formed by reacting maleimide and methylfuran were prepared by dissolving 20 kDa 4-arm PEG-maleimide and 20 kDa 4-arm PEG-methylfuran (both synthesized following the procedure outlined in [1]) in phosphate buffered saline (Corning # 21-031-CM) at 55.5 mg/mL and mixing the two components at a volume ratio of 1:1 and adding in 500-nm PEGylated polystyrene tracer particles to a final concentration of 0.1% (w/v).

To prepare collagen I solution, a 3.0 mg/mL solution of acid-solubilized porcine tendon collagen I (Nitta Gelatin Cellmatrix # 631-00651) was mixed on ice with 10 \times concentrated Dulbecco's Modified Essential Media (Sigma # D2429-100ML) and collagen neutralization buffer (26 mM sodium bicarbonate, 0.05 N NaOH, 200 mM HEPES) at a ratio of 8:1:1 to form a neutralized solution of 2.4 mg/mL collagen I.

MDA-MB-231 cells were from a gift from Dr. Michelle Vitolo (University of Maryland Baltimore).

Sputum samples from subjects with and without cystic fibrosis were collected by Dr. Carlos Milla and Dr. Elizabeth Burgener. All patient samples were collected following protocols approved by the Stanford Institutional Review Board (protocol #IRB-37232). Freshly collected sputum samples were divided into aliquots after collection and then snap frozen in liquid nitrogen before storing at -80°C . DLS μ R measurements were performed after freeze-thaw of the collected samples and PEGylated polystyrene microspheres were

added to a final concentration of 0.1% (w/v).

II. METHODS

A. Synthesis of 40 kDa Hyaluronic Acid terminated with alkyne group (HA-alkyne)

In a dry round-bottom flask, 40 kDa sodium hyaluronate (100 mg, Lifecore Biomedical # HA40K-1) was dissolved in 200 mM pH 4.5 MES buffer to a concentration of 1 wt%. Propargylamine (96.1 μ L, 6 eq, Sigma # P50900-5G) was added to the reaction mixture. N-hydroxysuccinimide (172.6 mg, 6 eq, Thermo Fisher # 24500) was added to the reaction mixture, followed by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (287.6 mg, 6 eq, Thermo Fisher # 22980). The reaction mixture was stirred overnight before being dialyzed against Milli-Q water (9×4 L) and lyophilized to give a fluffy white product. The degree of modification was quantified by ^1H NMR spectroscopy (Figure S3).

B. Synthesis of 40 kDa Hyaluronic Acid terminated with cucurbit[7]uril (HA-CB[7])

Azide-modified cucurbit[7]uril (CB[7]-N₃) was synthesized according to published methods [2], and the synthesized product was attached to HA-alkyne following previously established ‘click’ chemistry methodology [3]. Briefly, CB[7]-N₃ (25.0 mg) was combined with HA-alkyne synthesized as described above (78.0 mg, *20% of sugar units modified with alkyne*), copper(II) sulfate pentahydrate (CuSO₄·5H₂O, 1.0 mg, BDH, ACS grade), and N,N,N’,N’’,N’’-pentamethyldiethylenetriamine (PMDETA, 98%, 0.8 μ L, Acros) and dissolved in 10 mL DMF/water (1/4, v/v) in a Schlenk flask. The flask was degassed through three freeze-pump-thaw cycles. On the last cycle, the flask was opened to quickly add sodium ascorbate (10 mg) before re-capping the flask. The flask was vacuumed and backfilled with N₂ for 5 cycles before immersion in a 50°C oil bath to thaw the solution and initiate the ‘click’ reaction. After 96 h, the reaction was quenched by exposure to air. The reaction mixture was then transferred into dialysis tubing (MWCO = 3500, Thermo Scientific) and dialyzed against 3 L deionized water over 72 h with water changed every 6 h. The pure

product was obtained after lyophilization as yellow solid (96.0 mg).

C. Synthesis of 40 kDa Hyaluronic Acid terminated with azido group (HA-azide)

First, 40 kDa sodium hyaluronate (100 mg, Lifecore Biomedical # HA40K-1) was dissolved in 200 mM pH 4.5 MES buffer to a concentration of 1 wt%. 3-Azidopropylamine (147.2 μ L, 6 eq, Click Chemistry Tools # AZ115-1000) was added to the reaction mixture for synthesizing a final product of azide-functionalized hyaluronic acid, respectively. N-hydroxysuccinimide (172.6 mg, 6eq, Thermo Fisher # 24500) was added to the reaction mixture, followed by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (287.6 mg, 6 eq, Thermo Fisher # 22980). The reaction mixture was stirred overnight before being dialyzed against Milli-Q water (9×4 L) and lyophilized to give a fluffy white product. The degree of modification was quantified by ^1H NMR spectroscopy (Figure S5).

D. Synthesis of 40 kDa Hyaluronic Acid terminated with p-xylylenediamine (HA-diN-Xyl)

A small molecule guest, diN-Xyl-alkyne, was prepared according to previously published methods for conjugation via ‘click’ chemistry to HA-azide [3]. Briefly, HA-azide (82.0 mg, *30% of sugar units modified with azide*) was synthesized as described above and combined with diN-Xyl-alkyne (16.8 mg), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (1.5 mg), and PMDETA (1.2 μ L) and dissolved in 10 mL DMF/water (1/4, v/v) in a Schlenk flask. The flask was degassed through three freeze-pump-thaw cycles. On the last cycle, the flask was opened to quickly add sodium ascorbate (10 mg) before re-capping the flask. The flask was vacuumed and backfilled with N_2 for 5 cycles before immersion in a 50°C oil bath to thaw the solution and initiate the ‘click’ reaction. After 96 h, the reaction was quenched by exposure to air. The reaction mixture was then transferred into dialysis tubing (MWCO = 3500, Thermo Scientific) and dialyzed against 3 L water over 72 h with water change for every 6 h. The pure product was obtained after lyophilization as yellow solid (93.0 mg).

E. Preparation of poly(ethylene) glycol (PEG) functionalized polystyrene tracer particles

Polystyrene microspheres were functionalized with PEG by coupling carboxylated microspheres (Polysciences) to 750 Da methoxypolyethylene glycol amine (Millipore Sigma # 07964) by ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC)/N-hydroxysulfosuccinimide sodium salt (sulfo-NHS) ester chemistry. Microspheres were washed three times by centrifuging at $9000 \times g$ for 3 minutes (for 100-nm diameter microspheres, $16000 \times g$ for 10 minutes) with 50 mM pH 6.0 MES buffer to a final concentration of 2.6% (w/v). EDC (Thermo Fisher # 22980) and sulfo-NHS (Thermo Fisher # 24510) were added to the particle suspension to final concentrations of 2 mM and 5 mM, respectively. The suspension was gently rocked for 30 minutes at room temperature until an equal volume of 2 mM methoxypolyethylene glycol amine (dissolved in freshly prepared 100 mM sodium bicarbonate pH 8.0) was added to the suspension. The mixture was rocked gently for 30 minutes at room temperature. The reaction was quenched by the addition of an equal volume of 200 mM glycine, followed by rocking for 30 minutes at room temperature. Finally, the PEG-functionalized microspheres were washed 10 times by repeatedly centrifuging at $9000 \times g$ for 3 minutes (for 100-nm diameter particles, $16000 \times g$ for 10 minutes) and resuspending in Milli-Q water.

F. Preparation of poly(ethylene) glycol (PEG) functionalized gold tracer nanoparticles

PEG-functionalized gold nanoparticles (Sigma # 741973-25ML) were prepared by dissolving 2000 Da poly(ethylene) glycol methyl ether thiol (Millipore Sigma # 729140) in Milli-Q water at 100 mg/mL. Gold nanoparticles were centrifuged at $7000 \times g$ for 3 minutes, and the supernatant was extracted. Three times the volume of gold nanoparticles that was centrifuged of the poly(ethylene) glycol methyl ether thiol solution was added to the gold nanoparticles. The mixture was gently rocked at room temperature overnight. Finally, the mixture was centrifuged at $7000 \times g$ for 3 minutes, and the particles were resuspended in one-third the volume of the reaction mixture of Milli-Q water.

G. Encapsulation of MDA-MB-231 cells in Collagen I

Prior to encapsulation, MDA-MB-231 cells were propagated in 2D on tissue culture treated polystyrene. Cells were maintained in high glucose Dulbecco's Modified Eagle Medium (DMEM) containing phenol red, sodium pyruvate, and GlutaMAX (Thermo fisher #11995065) supplemented with 10% Fetal Bovine Serum (FBS) and 1% penicillin-streptomycin. Culture was performed in a humidified tissue culture incubator at 37°C and 5% CO₂. Media was changed every other day. Cells were passaged at 79-90% confluency using TrypLE Express (Thermo Fisher #12604013) following the manufacturer's protocol.

Immediately prior to encapsulation for 3D-embedded collagen I culture, a pH-neutralized collagen I precursor solution was prepared on ice. To prepare the collagen solution, a 3.0 mg/mL solution of acid-solubilized porcine tendon collagen I (Nitta Gelatin Cellmatrix #631-00651) was mixed on ice with 10× concentrated Dulbecco's Modified Essential Media (Sigma #D2429-100ML) and collagen neutralization buffer (26mM sodium bicarbonate, .05N NaOH, 200mM HEPES) at a ratio of 8:1:1 to form a neutralized solution of 2.4 mg/mL collagen I.

Prior to encapsulation in collagen I, cells were dissociated to single cells using TrypLE Express, resuspended in complete growth media, and counted manually using a hemocytometer. Cells were then pelleted and resuspended on ice in pH-neutralized collagen precursor solution at a final cell density of 5×10^5 cells/mL in a sufficient volume for 40 μ L per cuvette. The cell/collagen precursor solution was pipetted up and down 20 times to ensure a homogenous cell suspension. 40 μ L of the cell suspension was then cast into a disposable cuvette (pre-sterilized with 70% ethanol). The cuvette was incubated at 37°C for 30 minutes in a humidified 5% CO₂ tissue culture incubator to allow the collagen to gel. After 30 minutes, 300 μ L of pre-warmed complete cell culture media was added on top of the gel. Day 0 DLS μ R measurements were performed immediately after adding media onto the gels. DLS μ R measurements were performed at 37°C. The correlation function was collected for 30 minutes following a 5 minute pre-equilibration time in the instrument, and a sweep over measurement positions was performed to correct for broken ergodicity. The gels were maintained in the tissue culture incubator at 37°C between measurements and media was changed daily.

After 6 days of culture, cells were treated with 500 nM latrunculin A (Enzo Life Science

#BML-T119-0500) by diluting a 1000 \times concentrated DMSO stock solution into complete cell culture media.

III. RHEOLOGICAL PROCEDURES

A. Oscillatory Rheometer Measurements

Oscillatory rheology was performed on PEG-thiol-vinylsulfone gels using a stress-controlled rheometer (ARG2, TA Instruments) with a 20 mm 1 $^\circ$ cone and plate geometry with a geometry gap of 28 μ m. Frequency-dependent shear moduli were obtained from frequency sweeps collected at 1% strain. PEG-thiol-vinylsulfone gels were allowed to polymerize for 1 hour at a temperature of 23 $^\circ$ C prior to conducting measurements.

B. Setup of DLS Instrument for dynamic light scattering microrheology (DLS μ R)

DLS μ R was performed using a Malvern Zetasizer Nano ZS (633 nm laser) operated in 173 $^\circ$ non-invasive backscatter detection mode. For best results, we have isolated the instrument from mechanical vibrations on a benchtop mechanical vibration isolation system (Minus K Technology # 50BM-8). Disposable 40 μ L cuvettes (Malvern # ZEN0040) were used to perform all measurements. Zetasizer software version 7.13 was set up to run a standard operating procedure that measures the raw intensity autocorrelation function at a specified measurement position for 30 minutes. The translating lens of the Zetasizer Nano ZS enables measurement within the cuvette at a range of measurement positions, which was selected for producing a constant autocorrelation function for tracer particles diffusing in water at equal concentrations used in microrheology. The procedure is automated using the SOP player function of the Zetasizer Nano ZS, and populating the SOP playlist with the SOPs in the order in which they are listed in Table S1. Automatic attenuator selection is used, and the derived photon count rate is used to determine the scattering intensity of each position. The scattering measurement was taken assuming a solvent of water and scattering material of polystyrene. To ensure compatibility with the analysis code, selected data were exported in a particular order as a .csv file using a custom export template (Table S2).

C. Extraction and Analysis of DLS Data Output

Once the data collected by the DLS are exported, it can be analyzed using our custom software package (available on <https://dlsur.readthedocs.io/>) to extract the complex modulus. The documentation for the software package functions can be found on ReadtheDocs.org.

1. Extraction of Data

The first step of the software is to extract the data from the .csv file into a Pandas Dataframe. A decision is made by the software regarding the use of either the scattering intensity autocorrelation $g^{(2)}(\tau)$ or the intermediate scattering function $g^{(1)}(\tau)$, where $g^{(1)}(\tau)$ is preferred if available due to the additional precision provided (see Equation (2) in the main text). Lastly, the derived count rate is saved for each measurement position for any nonergodic samples.

2. Analysis of Data

The second step of the software is to fit the correlation function data to obtain the zero time intercept. The default option is to fit the data to the form:

$$g^{(2)} - 1 = x_0 \exp(-\alpha t^\beta) \tag{1}$$

where x_0 , α , and β are parameters to fit [4]. This correlation function fit is then used to obtain $g^{(1)}$ following the equations in the main text. In the case of nonergodic samples, an additional step is needed to isolate the component of the scattering intensity that is due to dynamic fluctuations. Using the derived count rate for the spectrum of measurement positions, an ensemble averaged scattering intensity $\langle I_e \rangle$ can be determined and used with the longer time-averaged measurement $\langle I_t \rangle$ to get a correct $g^{(1)}(\tau)$ (see Equation (8) in the main text).

Next, using $g^{(1)}(\tau)$, the mean-squared displacement $\langle \Delta r^2(t) \rangle$ can be determined. The mean-squared displacement is leveraged to calculate the frequency-dependent complex modulus in two ways: power-law and Laplace transform. In the power-law method, the mean-squared displacement is fitted to a power-law by performing a logarithmic linear regression

at each time t (see main text).

$$\alpha = \frac{d \log(\langle \Delta r^2(t) \rangle)}{d \log(t)} \Big|_{t=1/\omega} \quad (2)$$

where α is power-law scaling. The unilateral Fourier transform of the mean-squared displacement at that time or frequency is then approximated by the unilateral Fourier transform of this power-law fit, which is numerically evaluated using the relation

$$|G| = \frac{k_B T}{\pi a \langle \Delta r^2(t) \rangle \Gamma(1 + \alpha)} \quad (3)$$

where a is the radius of the particle and $\Gamma(1 + \alpha) = \int_0^\infty x^\alpha e^{-x} dx = \alpha!$. Subsequent determination of the elastic and viscous moduli uses Euler's equation [5].

$$G'(\omega) = |G| \cos(\pi\alpha/2) \quad (4)$$

$$G''(\omega) = |G| \sin(\pi\alpha/2) \quad (5)$$

For the unilateral Laplace transform, a direct Laplace transform of the mean-squared displacement $\langle \Delta r^2(s) \rangle$ is calculated numerically and an expression for $G(s)$ is found:

$$G(s) = \frac{k_B T}{\pi a s \langle \Delta r^2(s) \rangle} \quad (6)$$

Next, we perform a power-law analysis on $\log(G) = \gamma \log(s) + C$, where α is the scaling factor. Then, we use analytical continuation $s = i\omega$ to get the magnitude $|G| = G(s = \omega)$, and use the following equations to determine the frequency-dependent elastic and viscous moduli.

$$G'(\omega) = |G| \cos(\pi\gamma/2) \quad (7)$$

$$G''(\omega) = |G| \sin(\pi\gamma/2) \quad (8)$$

However, due to the methodology in evaluating the direct Laplace transform, the very low and very high frequencies of the moduli are inaccurate and asymptote to ∞ and $-\infty$. Thus, for the final output of the Laplace transform method used in the analysis code, the low and high frequencies are replaced by the calculated value of the complex modulus using the power-law method.

D. Simulation of intensity correlation function with Gaussian-distributed noise

The intensity correlation function was measured by DLS. For each time point t in the data set, a Gaussian distribution was applied with a mean of the measured correlation value and a standard deviation of

$$\sigma = \frac{0.03}{\sqrt{n}}$$

where n is the number of intervals of length t in the total time interval of measurement. In the case of the collagen I gels, the total time of measurement was 30 minutes, so for time point 60 seconds, $n = 30$. A random value was chosen from this Gaussian distribution for each of these time points to produce the noisy correlation function. This process was repeated two more times. For each noisy correlation function, the Laplace method and Fourier method were applied to evaluate the complex modulus.

IV. INSTRUCTIONS FOR CUSTOM ANALYSIS SCRIPT PACKAGE DOWNLOAD

Please visit <https://dlsur.readthedocs.io/> for documentation on the code and instructions on downloading the analysis script package.

Welcome to dlsmicro's documentation!

In this documentation, you will find a complete guide to using the analysis tools for analyzing exported data from the Zetasizer for microrheology.

Please visit [this package's Github page](#) to see more detail about the code in this package.

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V. SUPPLEMENTAL MATERIAL FIGURES

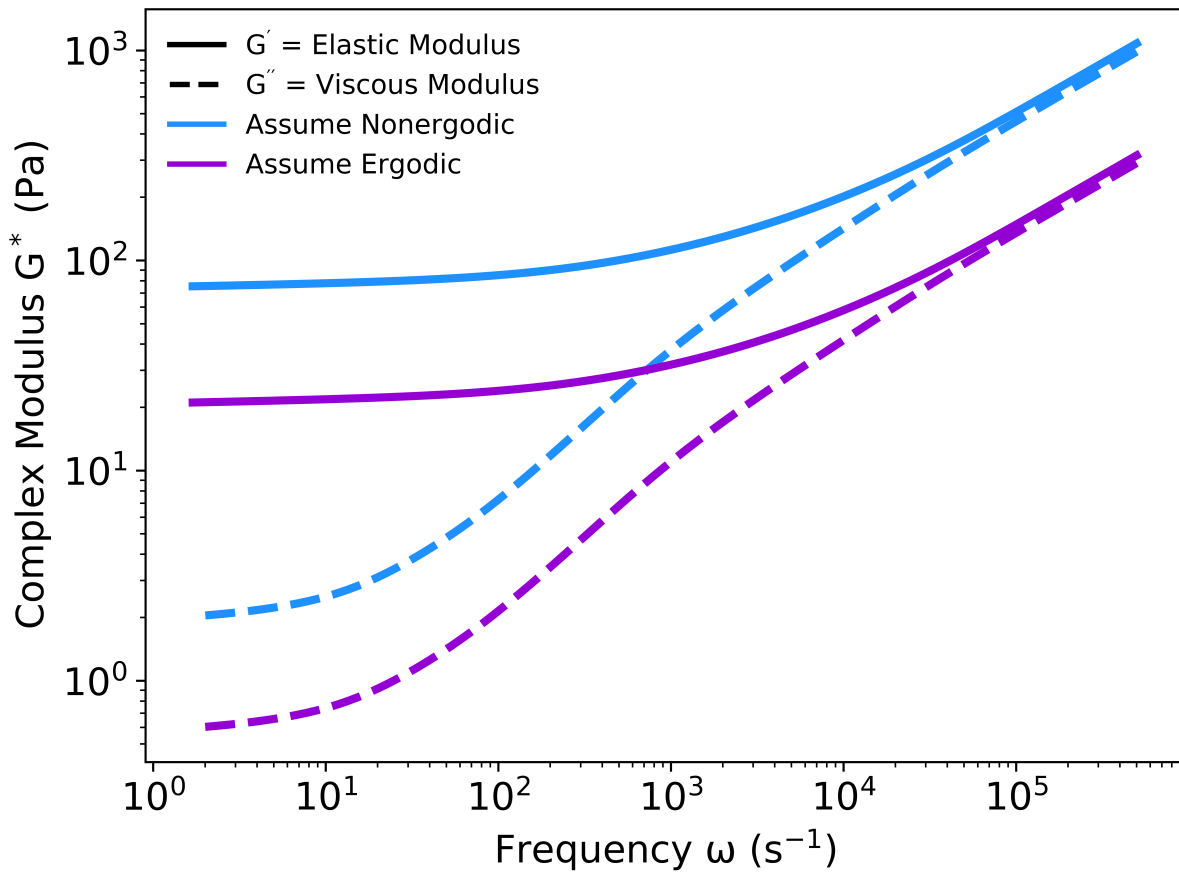


FIG. S1: Comparison of evaluating the complex modulus assuming the material is ergodic and nonergodic for a polymer gel (5 wt% PEG-thiolvinylsulfone)

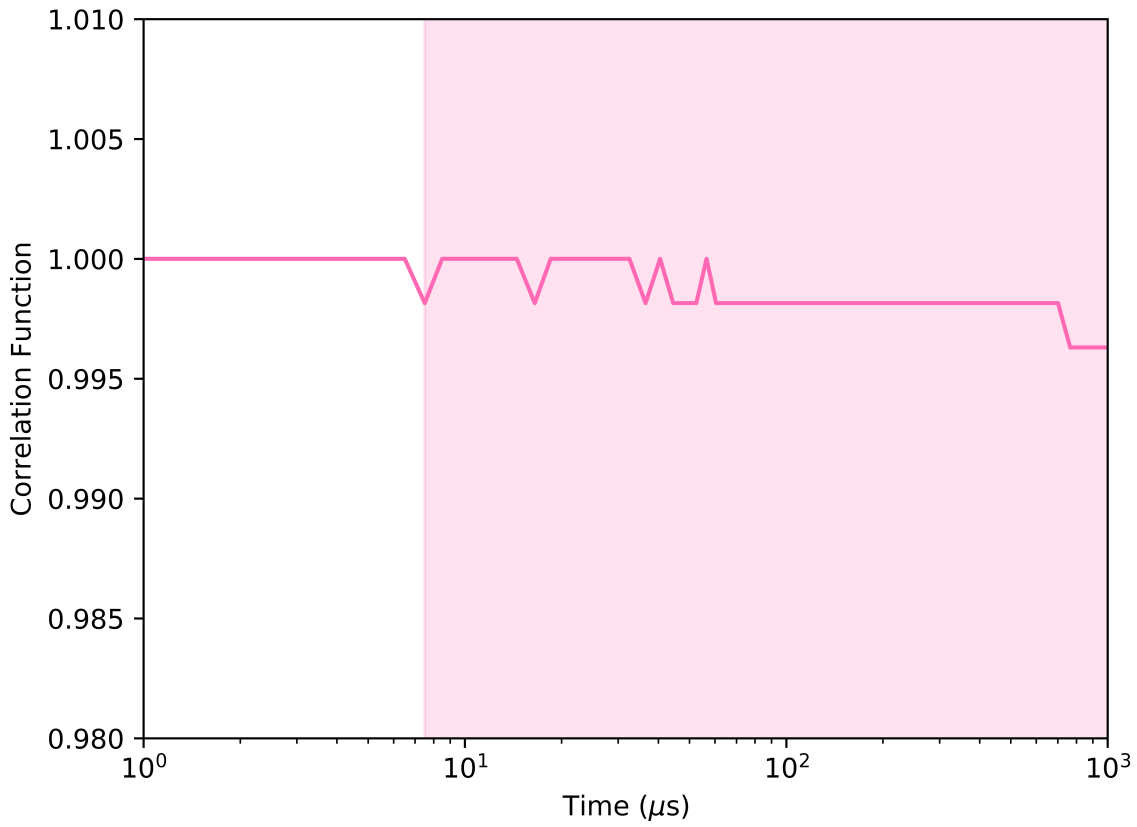


FIG. S2: Intensity autocorrelation function for a polymer gel (5 wt% PEG-thiolvinylsulfone) from time $t = 1\mu\text{s}$ to $t = 10^3\mu\text{s}$.

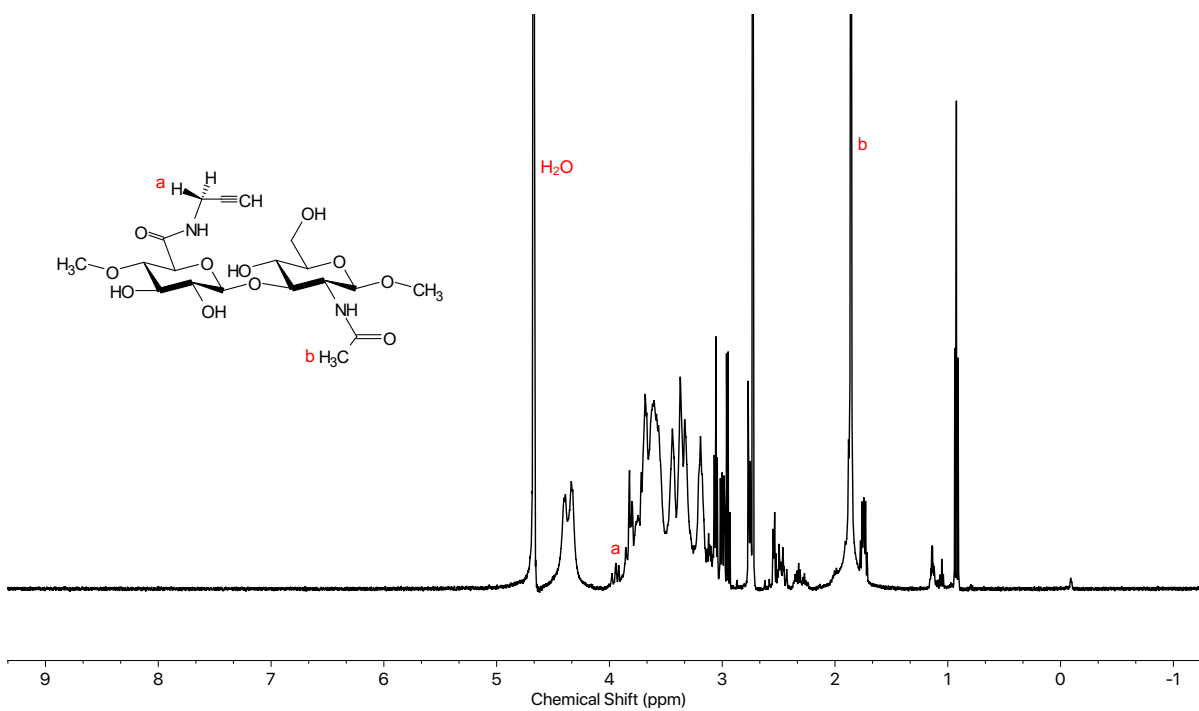


FIG. S3: ¹H NMR characterization of HA-alkyne (500 MHz, D₂O)

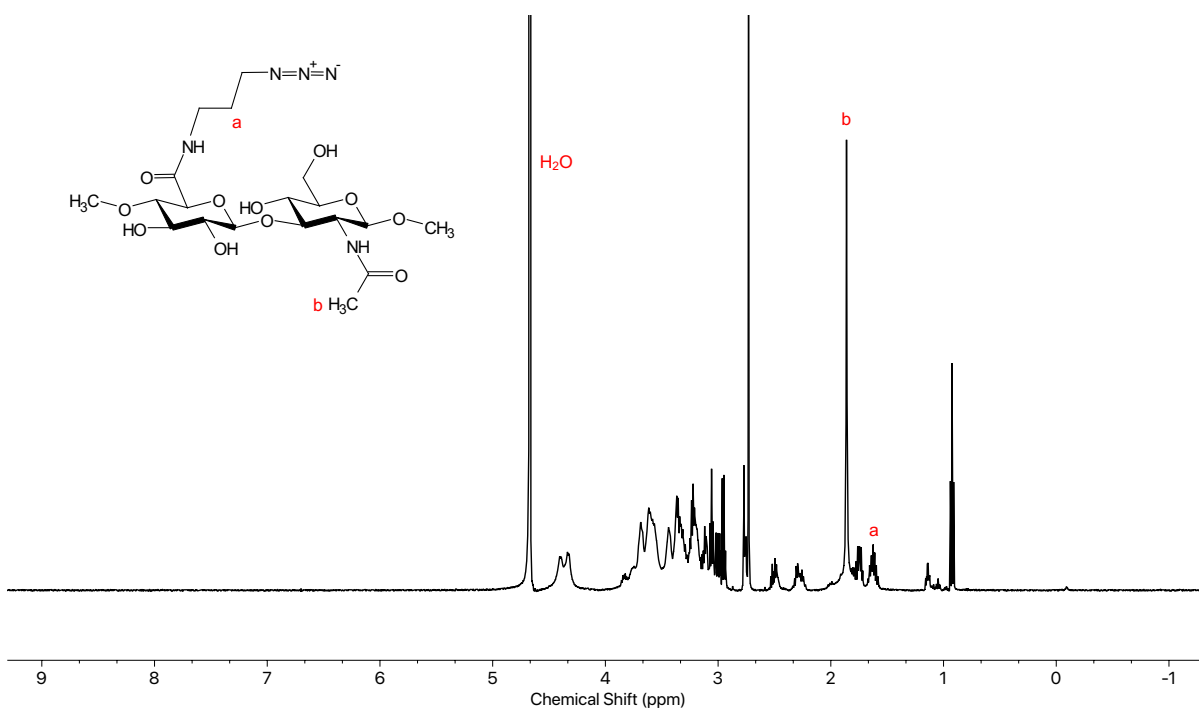


FIG. S4: ¹H NMR characterization of HA-azide (500 MHz, D₂O)

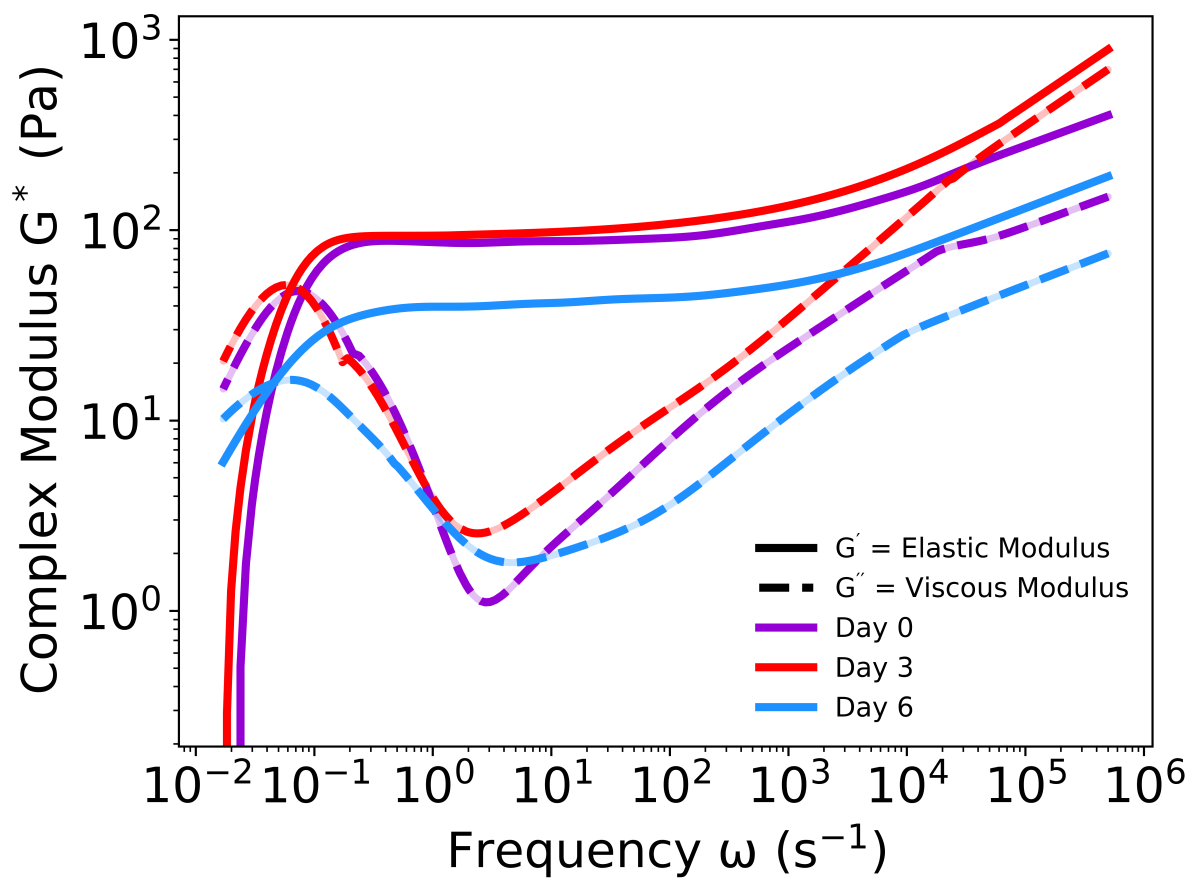


FIG. S5: DLS μ R of collagen I gels without cells over the course of 6 days

VI. SUPPLEMENTAL MATERIAL TABLES

TABLE S1: Order of Measurements for Typical Standard Operating Procedures (SOP) Player in
DLS μ R

Step	Duration	Description
1	5 min	Equilibration Period for Desired Temperature
2	30 min	Scattering Measurement (Measurement Position 3.0)
3	10 sec	Scattering Measurement (Measurement Position 2.0)
4	10 sec	Scattering Measurement (Measurement Position 2.1)
5	10 sec	Scattering Measurement (Measurement Position 2.2)
6	10 sec	Scattering Measurement (Measurement Position 2.3)
7	10 sec	Scattering Measurement (Measurement Position 2.4)
8	10 sec	Scattering Measurement (Measurement Position 2.5)
9	10 sec	Scattering Measurement (Measurement Position 2.6)
10	10 sec	Scattering Measurement (Measurement Position 2.7)
11	10 sec	Scattering Measurement (Measurement Position 2.8)
12	10 sec	Scattering Measurement (Measurement Position 2.9)
13	10 sec	Scattering Measurement (Measurement Position 3.0)
14	10 sec	Scattering Measurement (Measurement Position 3.1)
15	10 sec	Scattering Measurement (Measurement Position 3.2)
16	10 sec	Scattering Measurement (Measurement Position 3.3)
17	10 sec	Scattering Measurement (Measurement Position 3.4)
18	10 sec	Scattering Measurement (Measurement Position 3.5)
19	10 sec	Scattering Measurement (Measurement Position 3.6)
20	10 sec	Scattering Measurement (Measurement Position 3.7)
21	10 sec	Scattering Measurement (Measurement Position 3.8)
22	10 sec	Scattering Measurement (Measurement Position 3.9)
23	10 sec	Scattering Measurement (Measurement Position 4.0)

TABLE S2: Custom Data Export Template for Compatibility with Analysis Code

Step Data Collected (Column Title)	
1	Record Number
2	Sample Name
3	Measurement Position
4	Correlation Data
5	Correlation Delay Times
6	Distribution Fit Data
7	Distribution Fit Delay Times
8	Cumulants Fit
9	Cumulants Fit Delay Times
10	Derived Count Rate
11	Measured Intercept
12	Measured Size Baseline

- [1] C. M. Madl and S. C. Heilshorn, *Chemistry of Materials* **31**, 8035 (2019).
- [2] B. Vinciguerra, L. Cao, J. R. Cannon, P. Y. Zavalij, C. Fenselau, and L. Isaacs, *Journal of the American Chemical Society* **134**, 13133 (2012).
- [3] L. Zou, A. S. Braegelman, and M. J. Webber, *ACS Applied Materials & Interfaces* **11**, 5695 (2019).
- [4] B. A. Krajina, C. Tropini, A. Zhu, P. DiGiacomo, J. L. Sonnenburg, S. C. Heilshorn, and A. J. Spakowitz, *ACS Central Science* **3**, 1294 (2017).
- [5] T. G. Mason, *Rheologica Acta* **39**, 371 (2000).