Supporting Information for:

A Molecular Chaperone for G4-Quartet Hydrogels

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General experimental: All ^{1}H solution-state NMR spectra were recorded on a Bruker DRX-400 operating at 400.13 MHz or a Bruker AVIII-600 operating at 600.13 MHz. Chemical shifts are reported in ppm relative to the residual solvent peak. Deuterated solvents were purchased from Cambridge Isotope Labs. Circular dichroism spectroscopy was performed on a Jasco J-810 spectropolarimeter. UV-visible spectroscopy measurements were made on a Varian Cary 100 spectrometer. Fluorescence spectroscopy measurements were recorded on a Hitachi F-4500 fluorescence spectrophotometer. All rheological data was collected using an AR2000 stress-controlled rheometer from TA instruments. Chemicals and solvents were purchased from Santa Cruz Biotechnology, Aldrich, Fisher, and Acros.

General Procedure for Gel Preparation: Guanosine **1** was weighed into a vial, and the appropriate amount of LiOH solution (and water if necessary) was added. The mixture was sonicated for approximately 30 s, and the appropriate amount of $B(OH)_3$ solution was added. The suspension was heated to 90 - 100 °C in a water bath until all material was dissolved, and the solution was clear. The solution was then removed from the heat bath and allowed to cool to room temperature. Unless otherwise noted, gels were formed using a 2:1 ratio of G **1**:$LiB(OH)_4$.

Rheology Procedure: Gels were prepared at 2.8 wt% (100 mM G **1**; 50 mM $LiB(OH)_4$) in the presence of the desired amount of ThT **2** following the general gel procedure. Rheological experiments were performed at 22 °C using parallel plate geometry (20 mm diameter). The gel samples were allowed to equilibrate on the plate for 10 min. Frequency sweeps were performed at 1% strain. Strain sweeps were performed at 10 rad/sec by varying the strain from 0.1 to 100%. Hysteresis loops were performed by alternating from low strain (5 %) to high strain (200 %) at a constant angular frequency of 10 rad/sec.

Procedure for Diffusion-Ordered Spectroscopy Measurements: A Li^+ GB hydrogel (50 mM G **1**; 25mM $LiB(OH)_4$) was prepared in D_2O according to the general gel preparation procedure. The warm gel (0.2 mL) was then transferred into a Shigemi tube (Shigemi, Inc., Allison Park, PA), and the gel was allowed to cool overnight. Diffusion experiments were performed on a Bruker AVIII-600, using a Stimulated Echo Pulse Gradient sequence in FT mode.[1–3] Experiments consisted of 32 points at 50 scans with a delay of 4 s, a gradient pulse length of 2.3 ms, and Δ value of 60.0 ms. The temperature was controlled at 25.0 °C, and the measurements were repeated at least 3 times. Diffusion coefficients were calculated by integrating of the peaks of interest and deriving a single exponential decay using the "Simfit (Bruker XWINNMR)" software.

Procedure for Powder X-Ray Diffraction: A 1 wt% Li^+ GB hydrogel (36 mM G **1**, 18 mM $LiB(OH)_4$) was prepared according to the general gel procedure and lyophilized to form a white powder. X-ray powder diffraction measurements were performed with a Cu radiation source at 20 °C using a Bruker D8 Advance Bragg-Brentano Diffractometer equipped with a LynxEye detector.

Procedure for Circular Dichroism Spectroscopy Measurements: CD spectroscopy was performed at room temperature with a 100 μM solutions of ThT **2** in H_2O, 50 mM $LiB(OH)_4$ and in a 100 mM Li^+ GB hydrogel prepared using general gel procedures. Samples were allowed to cool and set for at least 24 h. Measurements were made in a quartz cell with 10 mm optical path length. Spectra were obtained using a scanning speed of 500 nm/min, response time of 2 s, and bandwidth of 1 nm. At least three scans were accumulated from 600 to 350 nm for each trial.

Procedure for Thioflavin T 2 Assays: GB hydrogels were prepared at 50 mM G **1**: 25 mM $MB(OH)_4$ according to the general procedure. While warm, ThT **2** (5 μM) was added, and the samples were shaken to ensure the dye distributed throughout. Gels were then transferred to a quartz cuvette (10 mm path length) and allowed to cool for 2h. UV-visible and fluorescence spectra were obtained at 25 °C. The emission response was recorded from 455 to 600 nm after exciting at 450 nm. Fluorescence spectra were acquired at a scanning speed of 240 nm/min, a response time of 0.5 s, with slit widths of 2.5 nm. All photographic images were obtained using 100 μM ThT.

Dynamic Light Scattering Procedure: A 72 mM Li^+ GB hydrogel was prepared according to the general gel procedure. While warm, aliquots of this gel were transferred into vials containing the appropriate amounts of water and ThT **2**, if necessary, to dilute to 50 mM G **1**, and the gels were reheated to 95 ºC. The gels were then removed from the heat and allowed to cool for 2 h. Scattering measurements were performed on a Photocor-FC light scattering instrument with a 5 mW laser source at 633 nm at 25 ºC and a scattering angle of 90º. Radii were obtained from estimated diffusion coefficients using the Stokes-Einstein relationship. Each measurement was repeated at least three times.

Viscometry Procedure: All viscometry experiments were performed using Cannon Ubbelohde Semi-Micro Size 50 Viscometers. Gel solutions between 0.05-1.0 wt% in G **1** were prepared following the general gel procedure. The solutions were pipetted into the viscometer and allowed to equilibrate for the time indicated. Each efflux time measurement was repeated 3 times. The average of these efflux times was multiplied by the viscometer constant provided to obtain the kinematic viscosity (η). Agitation cycles were performed by sonicating the solution in the viscometer for the time indicated and repeating the viscometry procedure after allotted rest times.

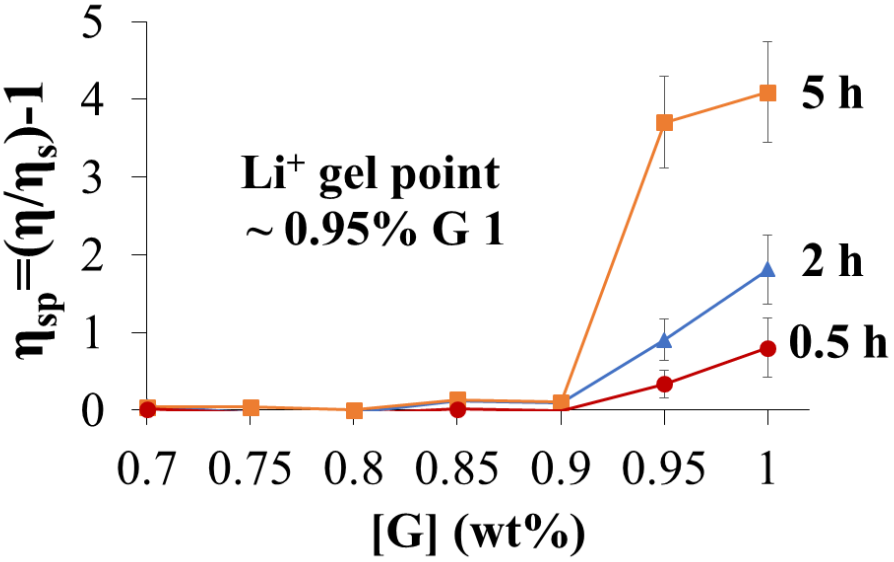

Figure S1. The critical gel concentration (CGC) of the Li^+ GB system (1.0 equiv of G **1** and 0.5 equiv of $LiB(OH)_4$) is between 0.90 and 0.95 wt% G **1** (32.4 - 34.2 mM). The apparent viscosity increases over time as the gel forms, but the CGC does not change.

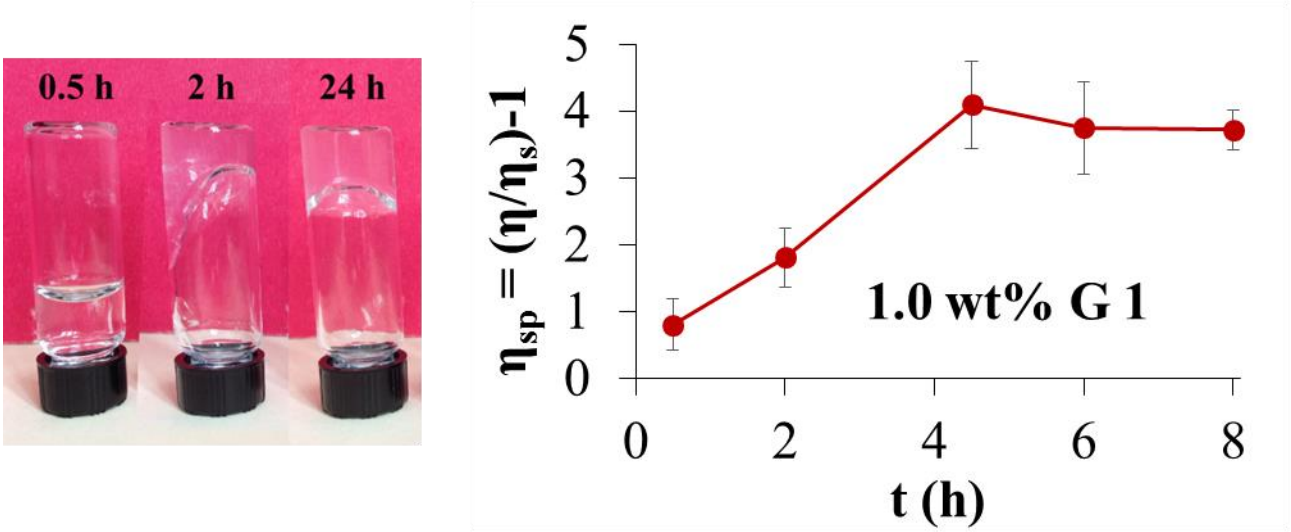

Figure S2. Left) The Li^+ GB hydrogel (2 wt %; 72 mM G **1**, 36 mM $LiB(OH)_4$) requires a longer time to form a non-flowing gel than does either the Na^+ or K^+ GB gel (see refs 7a and 7b in the paper). Right) This change in viscosity as a function of time was consistent for the kinematic viscosity, which was measured near the CGC at 1 wt %.

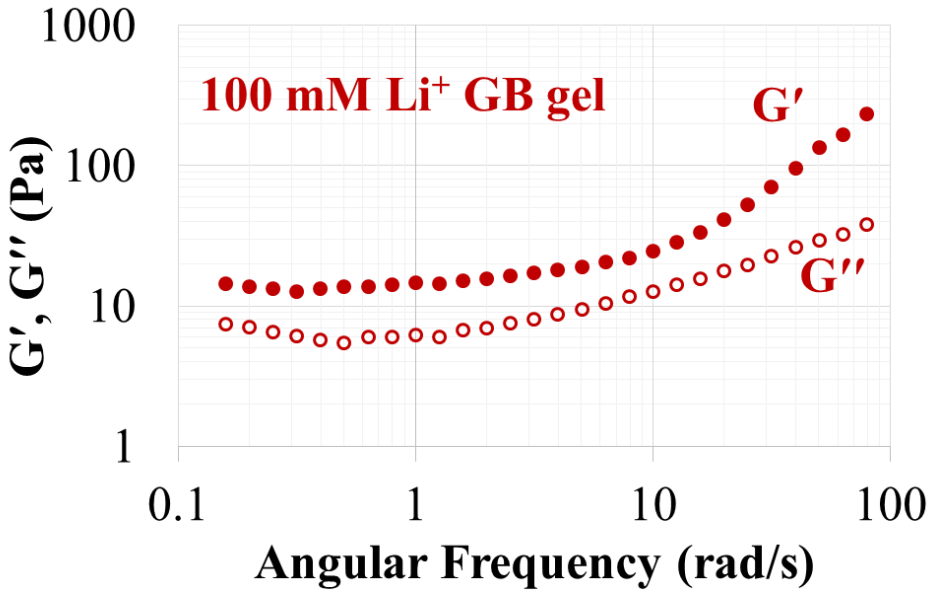

Figure S3. Frequency sweep at 1% strain for a Li^+ GB hydrogel (100 mM G **1**, 50 mM $LiB(OH)_4$) after it had been allowed to sit for 24 h at room temperature.

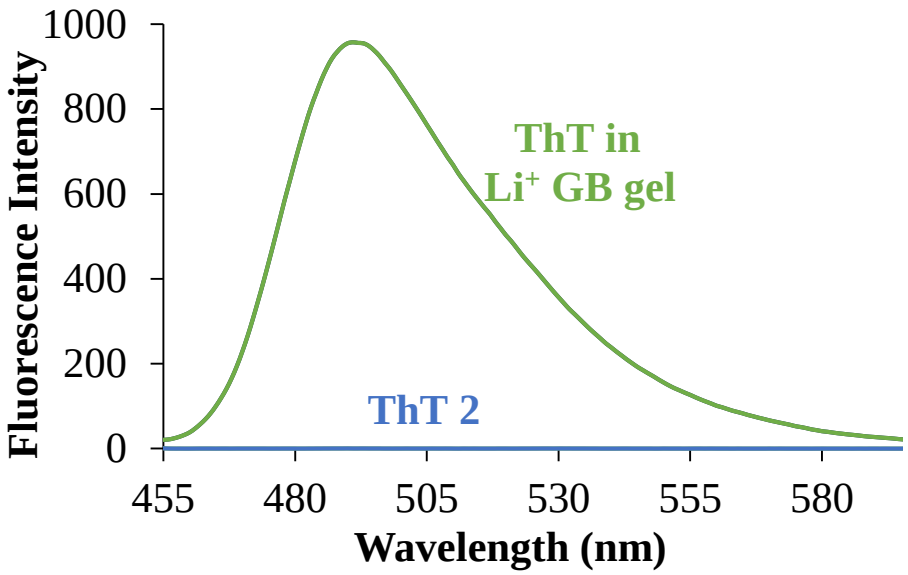

Figure S4. ThT **2** fluoresces in the Li^+ GB gels (50 mM G **1**, 25 mM $LiB(OH)_4$, 5 µM ThT **2**).

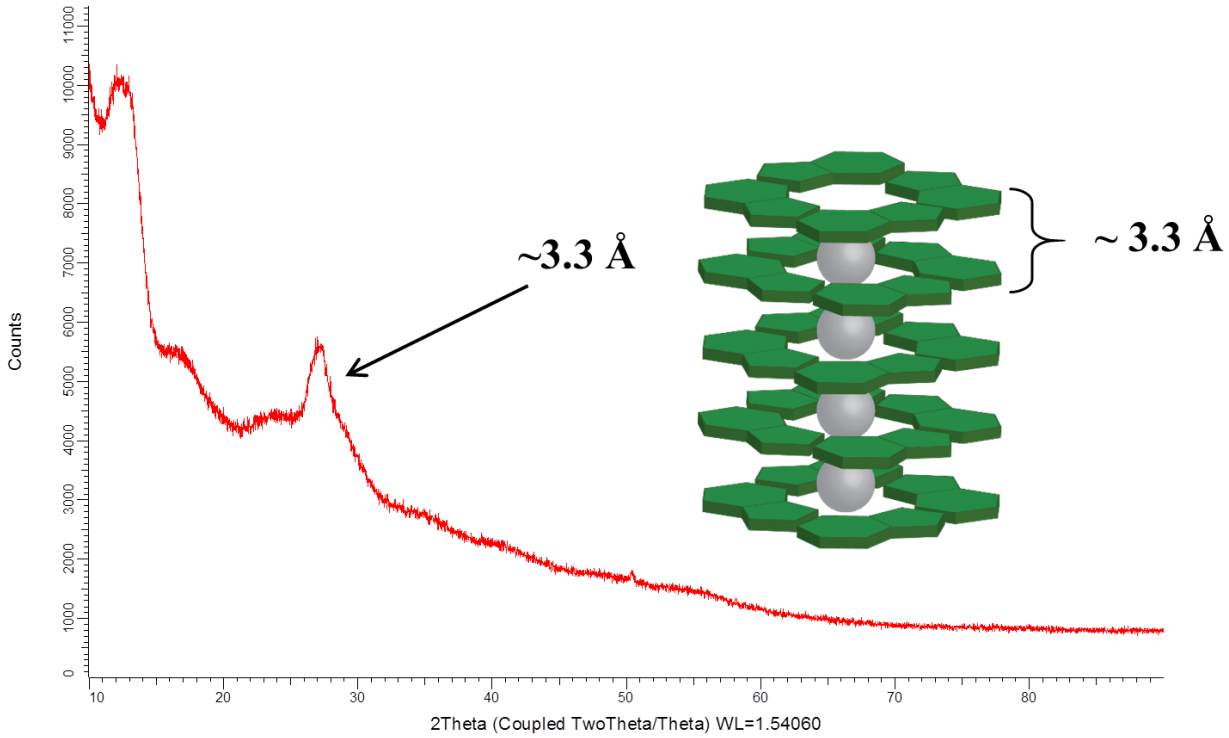

Figure S5. The powder X-ray diffraction pattern of a 1 wt% Li GB gel shows a signal at 2θ ≈ 27º, which corresponds to a distance of ~ 3.3 Å. This spacing is consistent with the distance between G4-quartets in a stacked assembly.

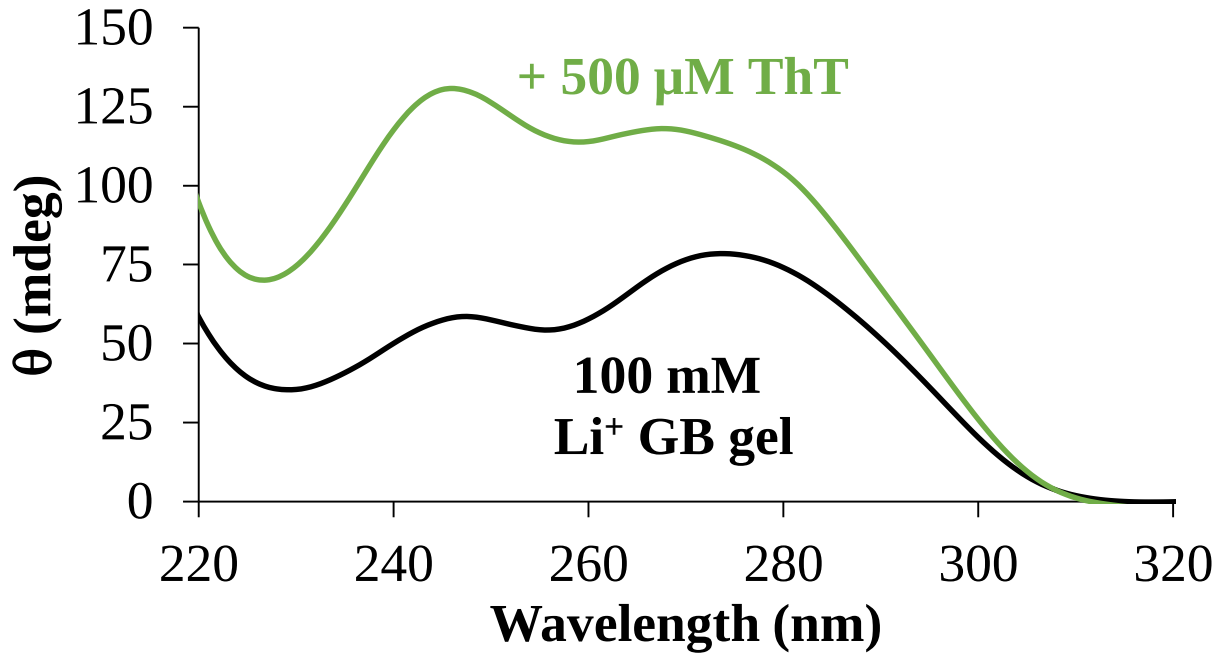

Figure S6. CD Spectra of a 100 mM Li^+ GB hydrogel, with and without added ThT **2** (0.5 mM).

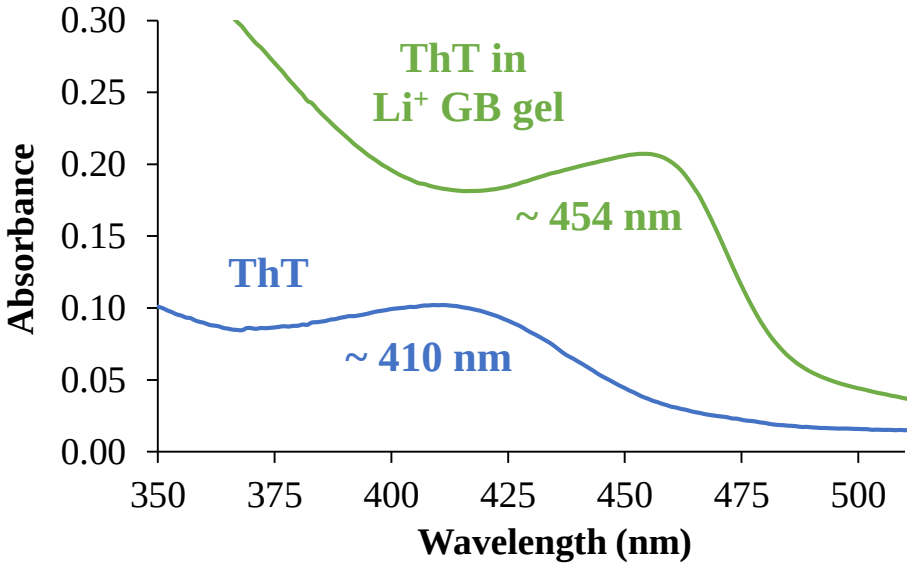

Figure S7. The absorbance maximum of ThT **2** shifted ~ 44 nm in the presence of the Li^+ GB hydrogel (50 mM G **1**, 25 mM $LiB(OH)_4$, 5 µM ThT **2**).

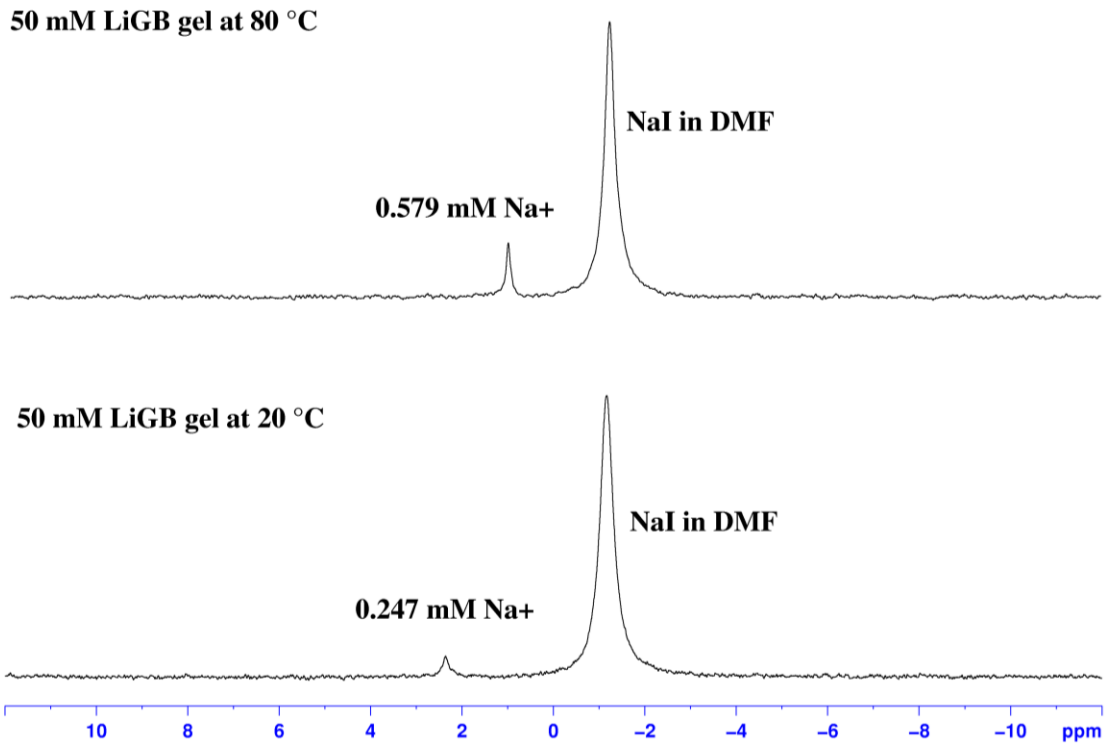

Figure S8. ^{23}Na NMR of the 50 mM Li^+ GB hydrogel at 20 ºC and 80 ºC with a NaI in DMF internal standard. The Li^+ GB hydrogel was prepared and allowed to cool in an NMR tube for 24 h. Spectra were obtained on a Bruker AV-400 operating at 105.8 MHz. The NaI internal standard was standardized with a 155 mM NaCl solution. These data suggest that ~332 µM Na^+ is present in the gel network.

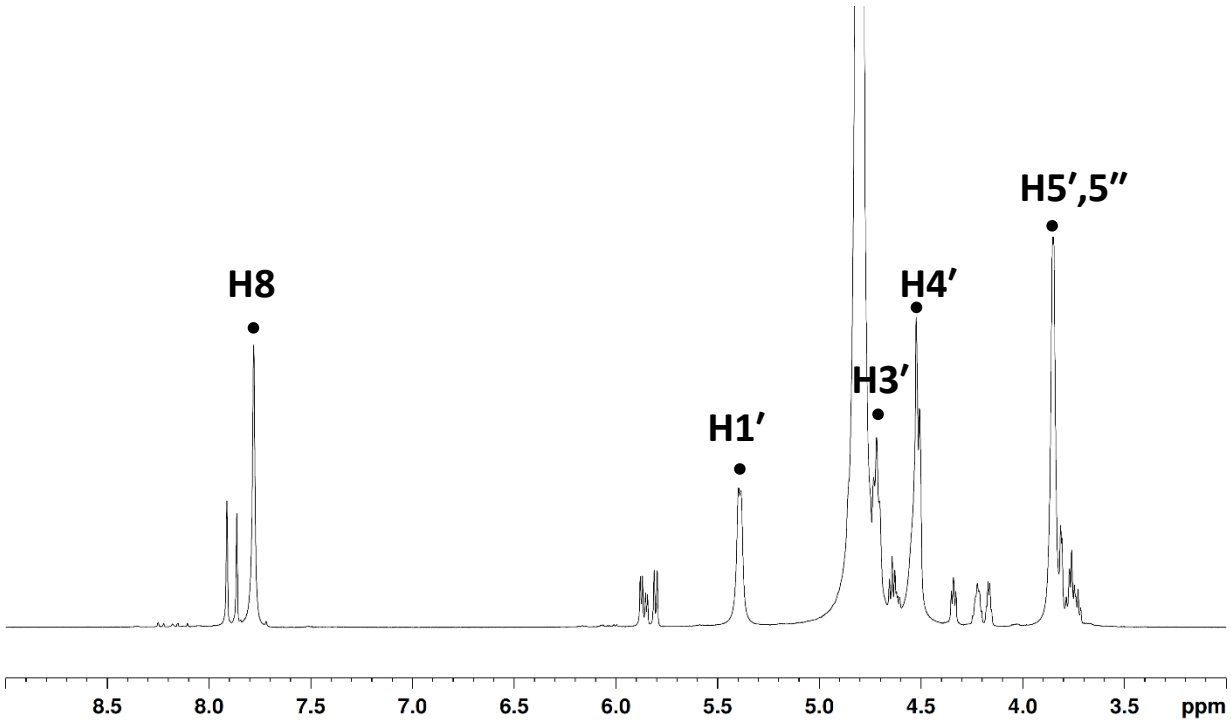

Figure S9. Full ^{1}H NMR spectrum of a 50 mM Li^{+} GB hydrogel. Signals for the proposed G_n-intermediate, which are broader than the signals for G **1** and its borate esters, are marked with a black dot. The signal for H2′ is under the residual HDO solvent peak. These signals were assigned from 2D-COSY and 2D-NOESY experiments. The set of broader signals marked by the black dot had smaller diffusion constants than the signals for G **1** and its borate esters (see Fig. S8).

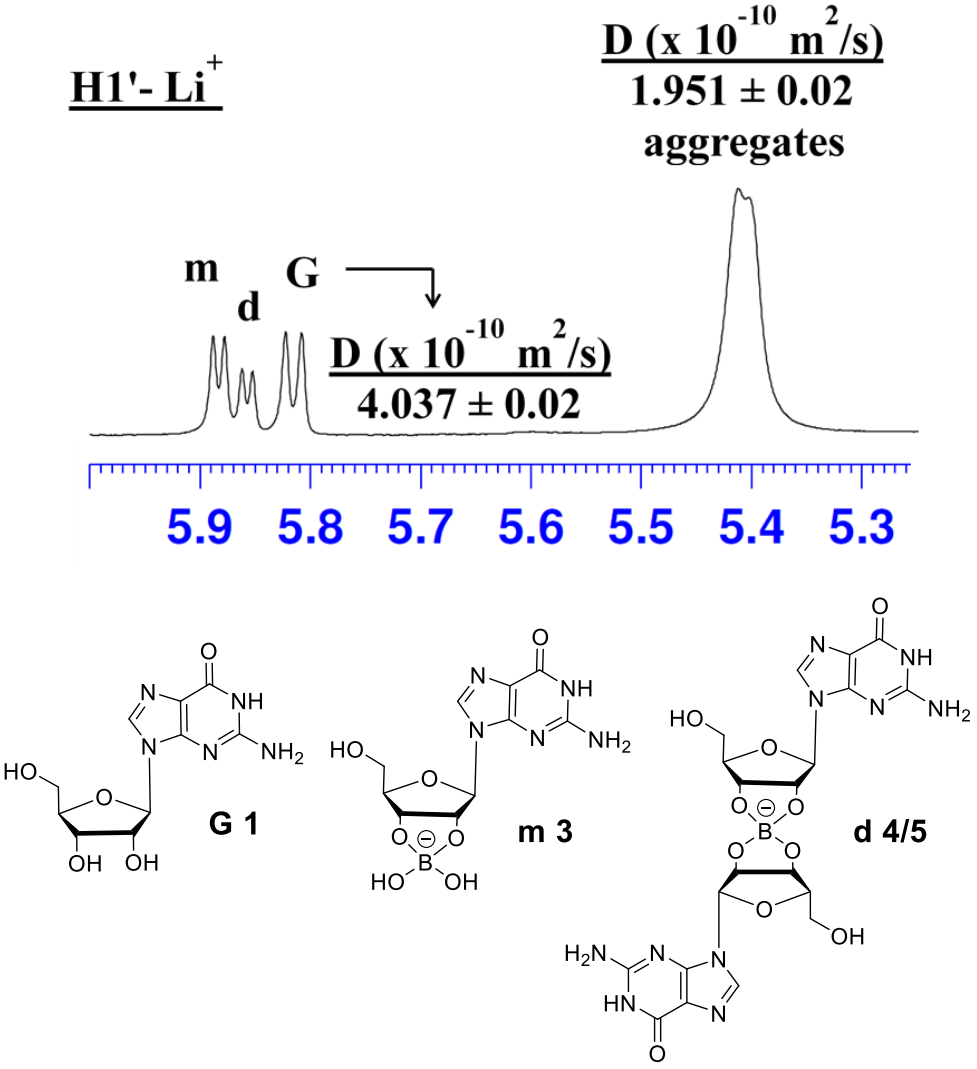

Figure S10. The H1′ region of the ^{1}H NMR of a Li^{+} GB gel (50 mM G **1**, 25 mM $LiB(OH)_4$) shows signals for each of the unassembled species, the GB monoester (m **3**), GB diesters (d **4**/**5**), and G **1**, as well as a broad signal at δ 5.4. Diffusion-ordered spectroscopy (DOSY) NMR experiments indicated that this species with the broader signals is significantly larger than compounds **1**-**5**. This intermediate does not show up in spectra of the Na^{+} or K^{+} GB gel. We assigned this signal to be an intermediate G_n-aggregate, which slowly disappears from solution with time (see Fig. 2 in the main paper).

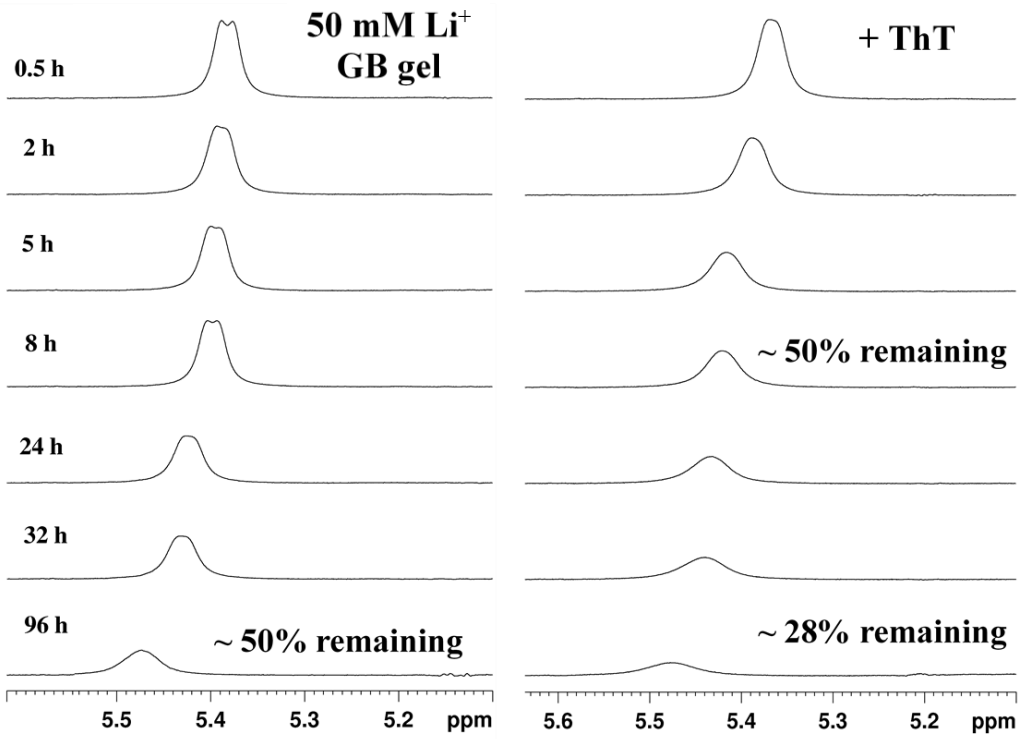

Figure S11. The signal for the H1′ of the G_n-assemblies decreases as a function of time. In the presence of 0.5 mM ThT **2** (right panel) this signal decreases faster than when ThT **2** is not present (left panel). Signals for the monoester **3**, diesters **4**/**5**, and G **1** did not undergo any notable changes in integration over this same time period.

Table S1. Hydrodynamic radii as a function of ThT 2 concentration.

[ThT **2**] (mM)	R (nm)
0	195 ± 6
0.1	397 ± 39
0.25	469 ± 35
0.5	537 ± 33
1.0	706 ± 54

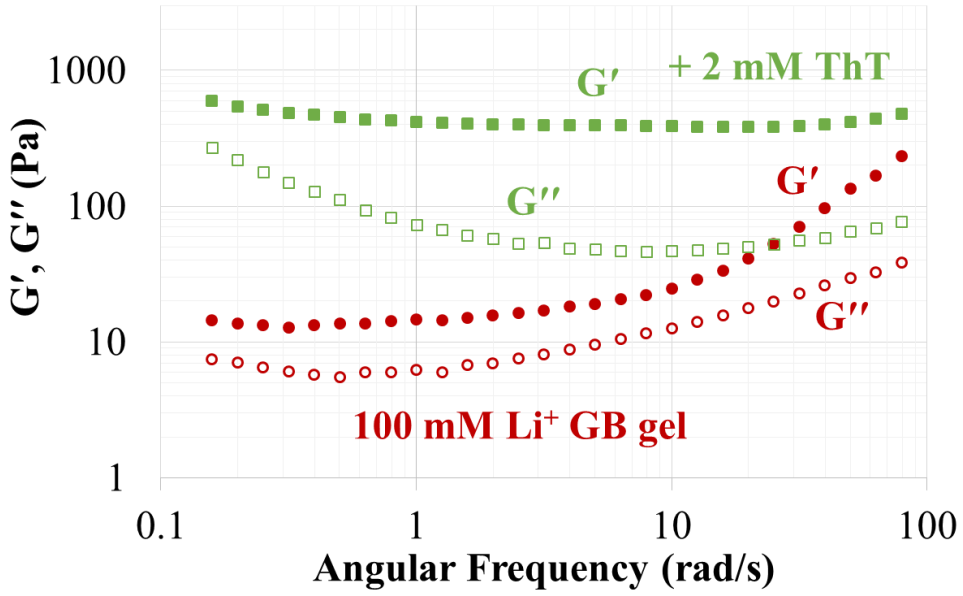

Figure S12. Frequency sweeps of a 100 mM Li^+ GB hydrogel with (green) and without (red) ThT **2** added to the system (2 mM). With ThT **2** present, the system is a notably stronger gel.

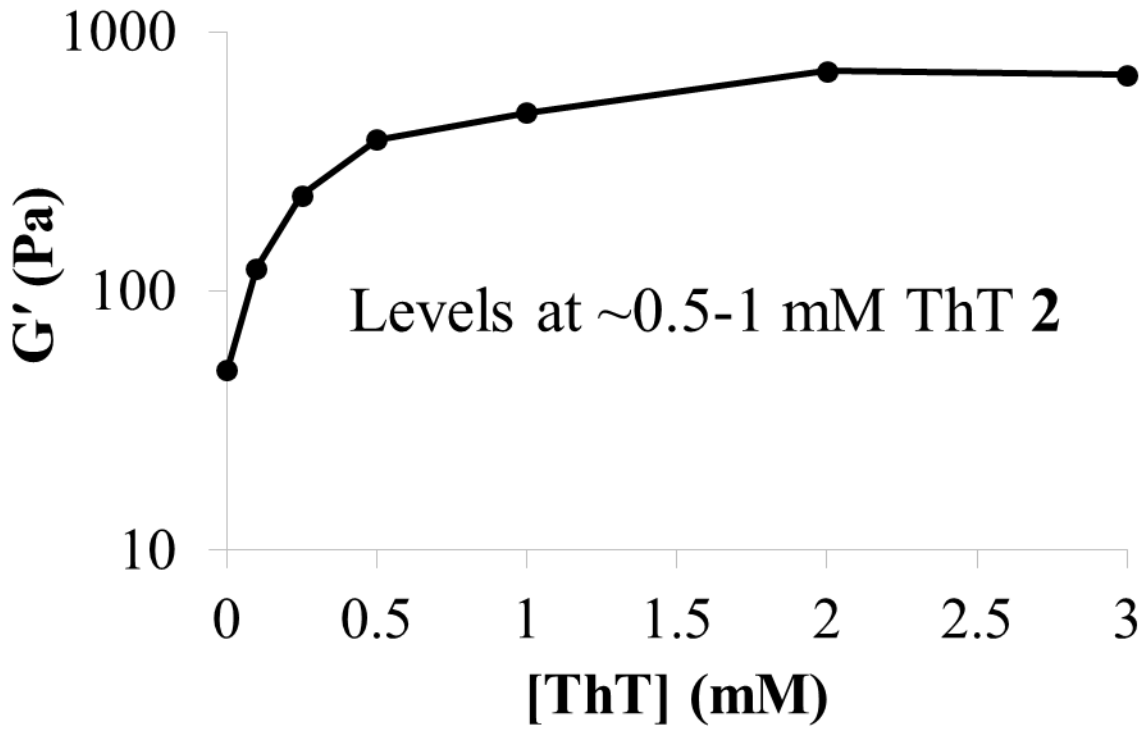

Figure S13. Plot of G′ (0.5% strain, 10 rad/s) as a function of ThT **2** concentration shows that the modulus increases significantly from 0 to 0.5 mM ThT **2**. At ThT **2** concentrations greater that 0.5 mM the G′ value levels off, suggesting the system has been saturated.

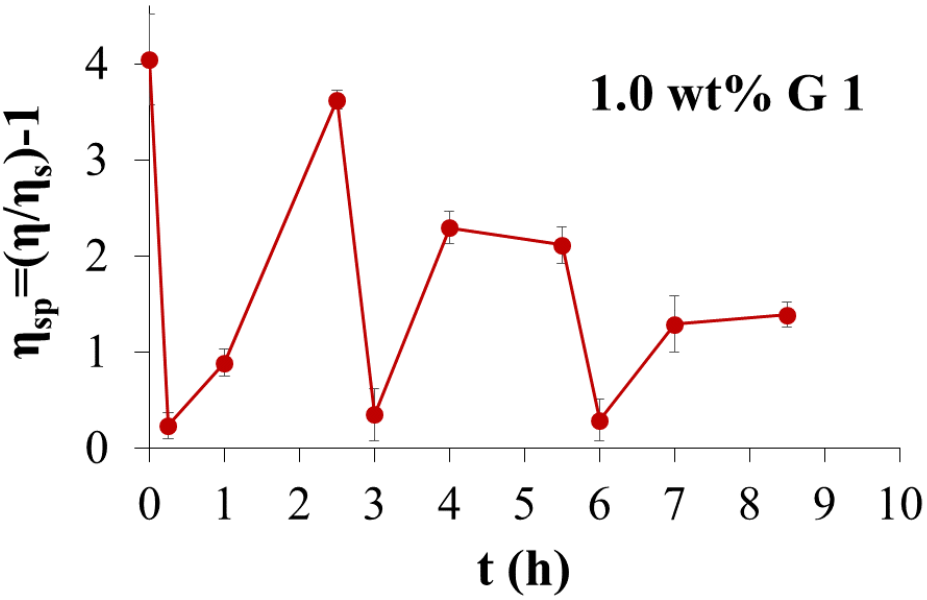

Figure S14. After sonicating a 1 wt% Li^+ GB hydrogel for 15 m, the apparent kinematic viscosity decreased to zero (i.e. a free-flowing water solution). Over time, the viscosity increased, but never returned to its initial maximum. Repeating these agitation cycles resulted in a progressive weakening of the Li^+ GB hydrogel.

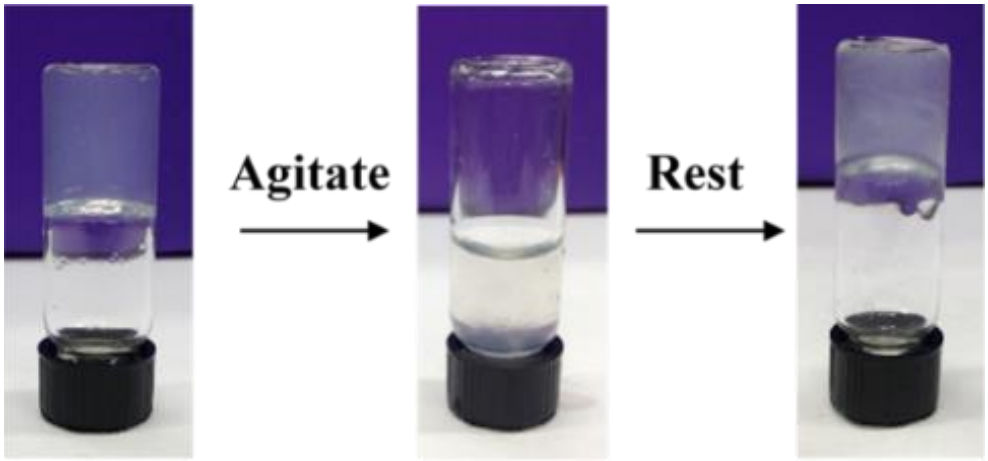

Figure S15. Upon physical agitation by sonication, a 2.8 wt% Li^+ GB hydrogel (100 mM G **1**, 50 mM $LiB(OH)_4$) became a free-flowing solution. Given time, a weaker, more opaque gel reformed.

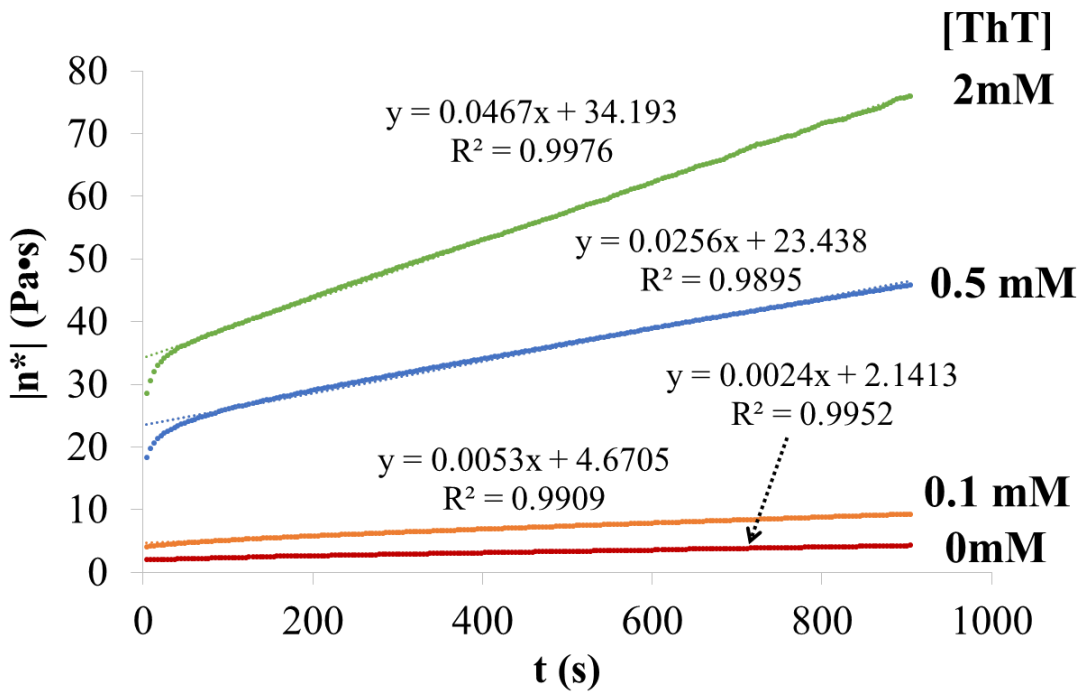

Figure S16. Time sweeps after the Li^+ GB hydrogel (100 mM G **1**, 50 mM $LiB(OH)_4$) was broken show that viscosity increases and the gel reforms faster when more ThT **2** is present.

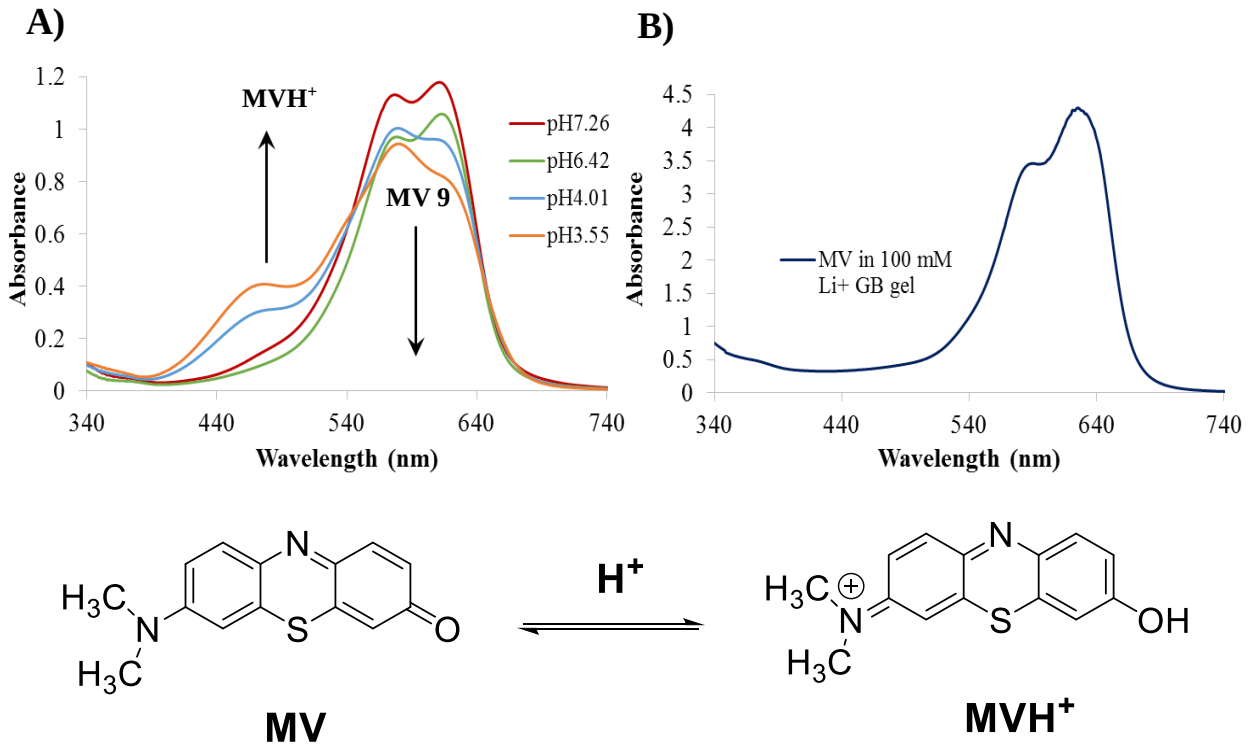

Figure S17. As the pH of a solution of MV (100 µM in 50 mM $LiB(OH)_4$) is lowered, the absorbance signal at 603 nm decreases in intensity. Simultaneously, a signal at 470 nm, attributed to a protonated MVH^+ species, grows in. (B) In the Li^+ GB hydrogel (100 mM G **1**, 50 mM $LiB(OH)_4$), the absorbance spectrum shows that MV is in a neutral form. For band assignments see reference 4.

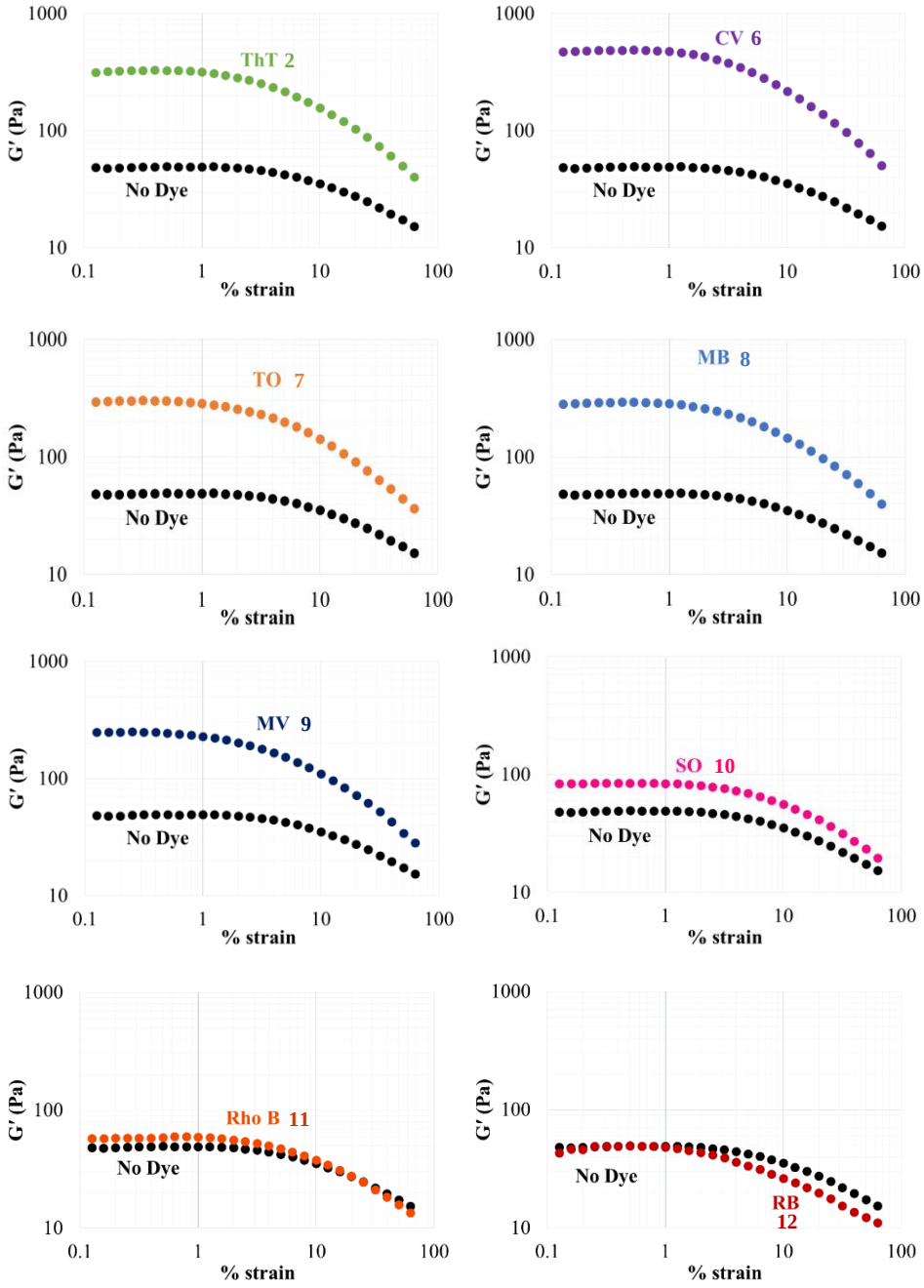

Figure S18. Strain sweeps of a Li^+ GB hydrogel (100 mM G **1**, 50 mM $LiB(OH)_4$) at a constant angular frequency of 10 rad/s with and without added dyes **2** or **6-12** (250 μM). The impact of 2 equiv. of NaCl (500 μM) was subtracted from the sweep with RB to obtain the graph shown. The ΔG′ values in Fig. 5 of the paper were obtained from these traces, using the values at 1% strain.

References:

(1) Tanner, J. E. *J. Chem. Phys.* **1970**, 2523.

(2) Cohen, Y.; Avram, L.; Frish, L. *Angew. Chem. Int. Ed.* **2005**, 520–554.

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