Electrostatic Interactions Regulate the Release of Small Molecules from Supramolecular Hydrogels

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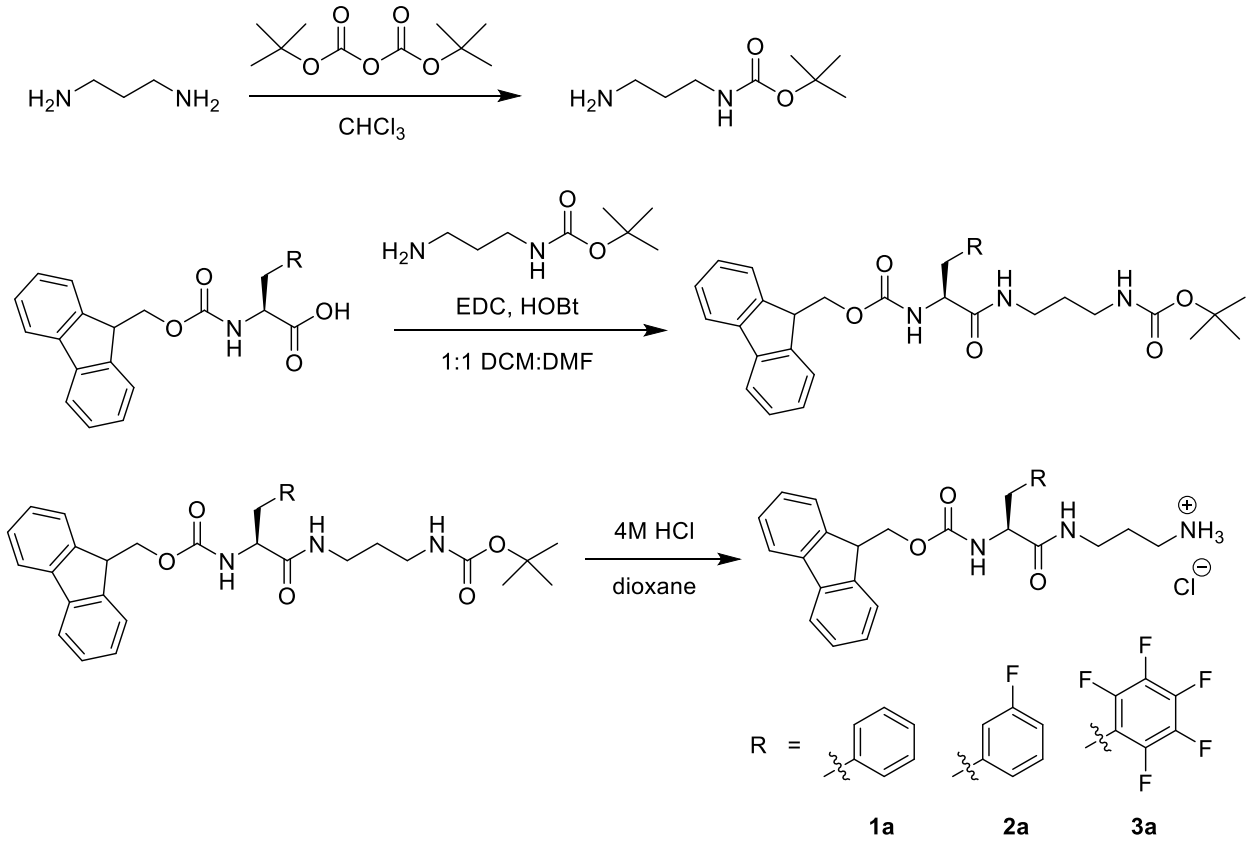
SUPPORTING INFORMATION

Contents:

Synthesis of Fmoc-Phe-DAP derivatives 1a–3a



The synthesis of Fmoc-Phe-DAP derivatives **1a**–**3a** was performed using the strategy outlined in the scheme above, a modified version of our previously reported scheme.[1] Details for the preparation of compound **1a** are provided as a representative example.

1,3-diaminopropane (15 mL, 179.69 mmol) was dissolved in 300 mL of chloroform and cooled to 0 ºC in an ice bath while stirring. Di-*tert*-butyl dicarbonate (3.92 g, 17.97 mmol) was dissolved in 125 mL of chloroform and added dropwise to the reaction mixture at a rate of approximately 1 drop per second. After all the solution was added, the reaction mixture was removed from the ice bath and left to stir at room temperature overnight. The mixture was then washed five times with 500 mL of deionized water. The organic solution was dried over anhydrous magnesium sulfate, which was removed by filtration, and the resulting solvent was removed by rotary evaporation to yield a clear oil (1.42g, 8.15 mmol, 45.4% yield).

Fmoc-Phe (2.82 g, 7.28 mmol) and hydroxybenzotriazole (HOBt) (1.42 g, 10.51 mmol) were dissolved in 20 mL of dichloromethane/dimethylformamide (1:1 DCM/DMF) and cooled to 0 ºC in an ice bath while stirring. The monoprotected 1,3-diaminopropane (1.41 g, 8.09 mmol) was dissolved in 30 mL of 1:1 DCM/DMF and added to the reaction mixture. 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) (1.40 g, 7.28 mmol) was dissolved in 30 mL of 1:1 DCM/DMF and added dropwise to the reaction mixture at a rate of approximately 1 drop every 3–5 seconds, for over approximately 2–3 hours. The mixture was concentrated by

rotary evaporation to remove DCM, then diluted with 100 mL of ethyl acetate and washed twice with each of the following: 100 mL of 5% aqueous acetic acid, 10% aqueous sodium bicarbonate, and brine. The organic layer was dried over anhydrous magnesium sulfate, which was removed by filtration and the resulting solution was concentrated by rotary evaporation to yield the product. The crude product was purified using column chromatography (silica gel, 3:2 ethyl acetate/hexanes (*v/v*)) to yield a white solid (2.584 g, 4.75 mmol, 65.3%).

The product obtained from purification (2.584 g, 4.75 mmol) was dissolved in 30 mL of dichloromethane/methanol (3:1 DCM/MeOH). To this was added 60 mL of 4 M HCl in dioxane and the reaction mixture was stirred for 30 minutes. The reaction mixture was divided between four 50 mL centrifuge tubes and concentrated by rotary evaporation until there was 5 mL or less of solution in each tube. Cold diethyl ether (20 mL) was added to each tube to precipitate the product, the tube was centrifuged for 10 minutes, and the supernatant was discarded. The pellet was resuspended in 20 mL cold diethyl ether, centrifuged, and decanted twice more for a total of three washes. The pellet was dried under vacuum to yield **1a** as a white solid (2.140 g, 4.46 mmol, 93.8% yield; overall yield for all step was 61.3%).

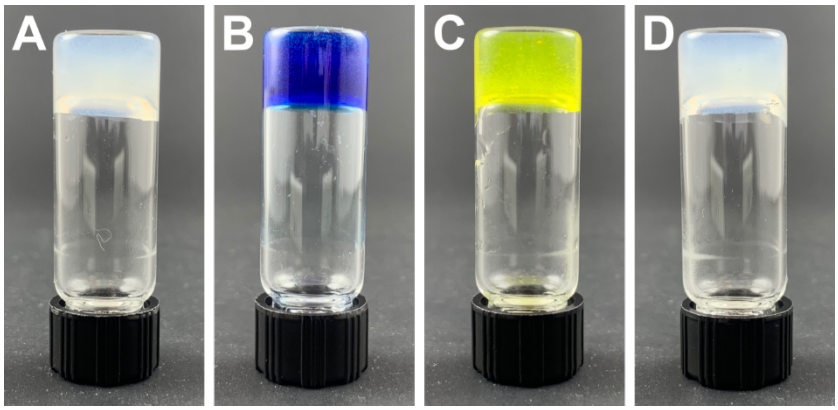


Figure S1. Digital images of hydrogels of **1a** one day after triggering gelation. (A) Unloaded hydrogel. (B) Hydrogel loaded with methylene blue. (C) Hydrogel loaded with naphthol yellow S. (D) Hydrogel loaded with caffeine.

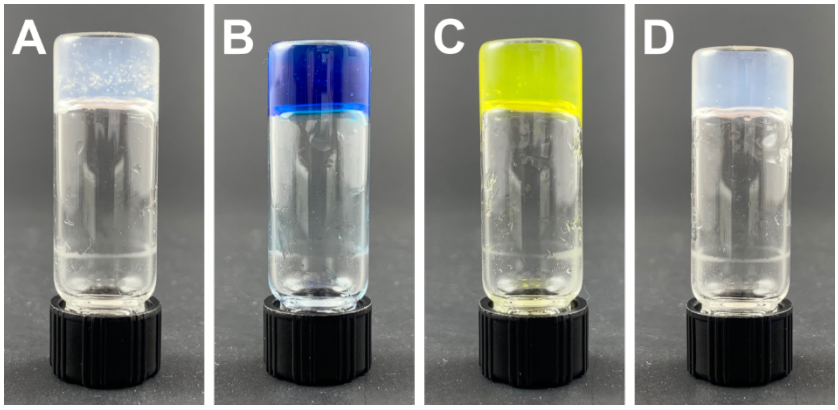


Figure S2. Digital images of hydrogels of **2a** one day after triggering gelation. (A) Unloaded hydrogel. (B) Hydrogel loaded with methylene blue. (C) Hydrogel loaded with naphthol yellow S. (D) Hydrogel loaded with caffeine.

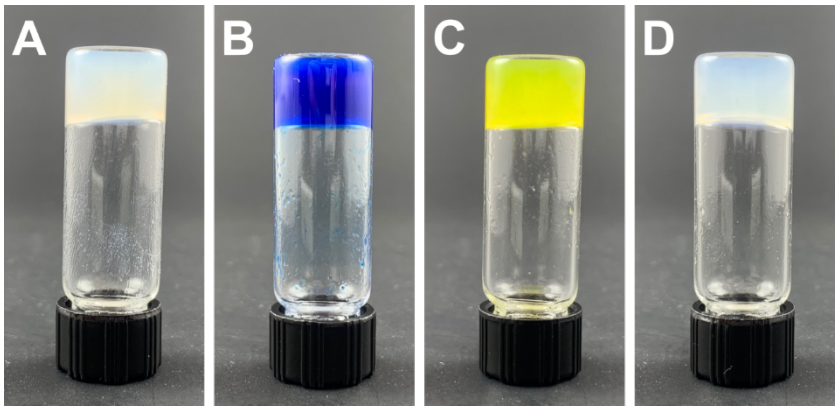


Figure S3. Digital images of hydrogels of **3a** one day after triggering gelation. (A) Unloaded hydrogel. (B) Hydrogel loaded with methylene blue. (C) Hydrogel loaded with naphthol yellow S. (D) Hydrogel loaded with caffeine.

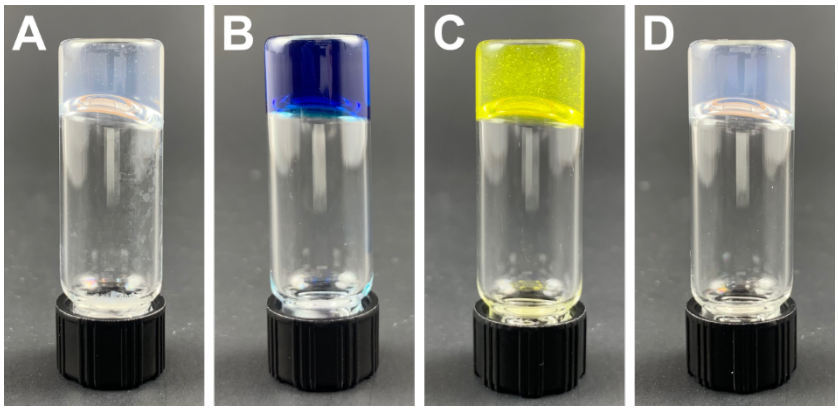


Figure S4. Digital images of hydrogels of **1b** one day after triggering gelation. (A) Unloaded hydrogel. (B) Hydrogel loaded with methylene blue. (C) Hydrogel loaded with naphthol yellow S. (D) Hydrogel loaded with caffeine.

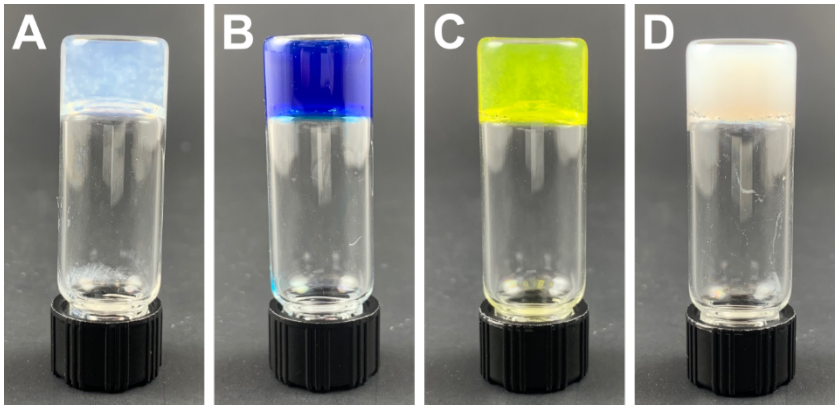


Figure S5. Digital images of hydrogels of **2b** one day after triggering gelation. (A) Unloaded hydrogel. (B) Hydrogel loaded with methylene blue. (C) Hydrogel loaded with naphthol yellow S. (D) Hydrogel loaded with caffeine.

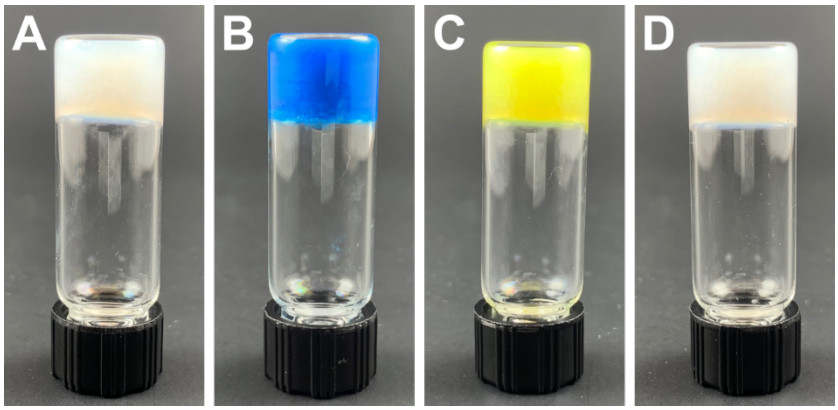


Figure S6. Digital images of hydrogels of **3b** one day after triggering gelation. (A) Unloaded hydrogel. (B) Hydrogel loaded with methylene blue. (C) Hydrogel loaded with naphthol yellow S. (D) Hydrogel loaded with caffeine.

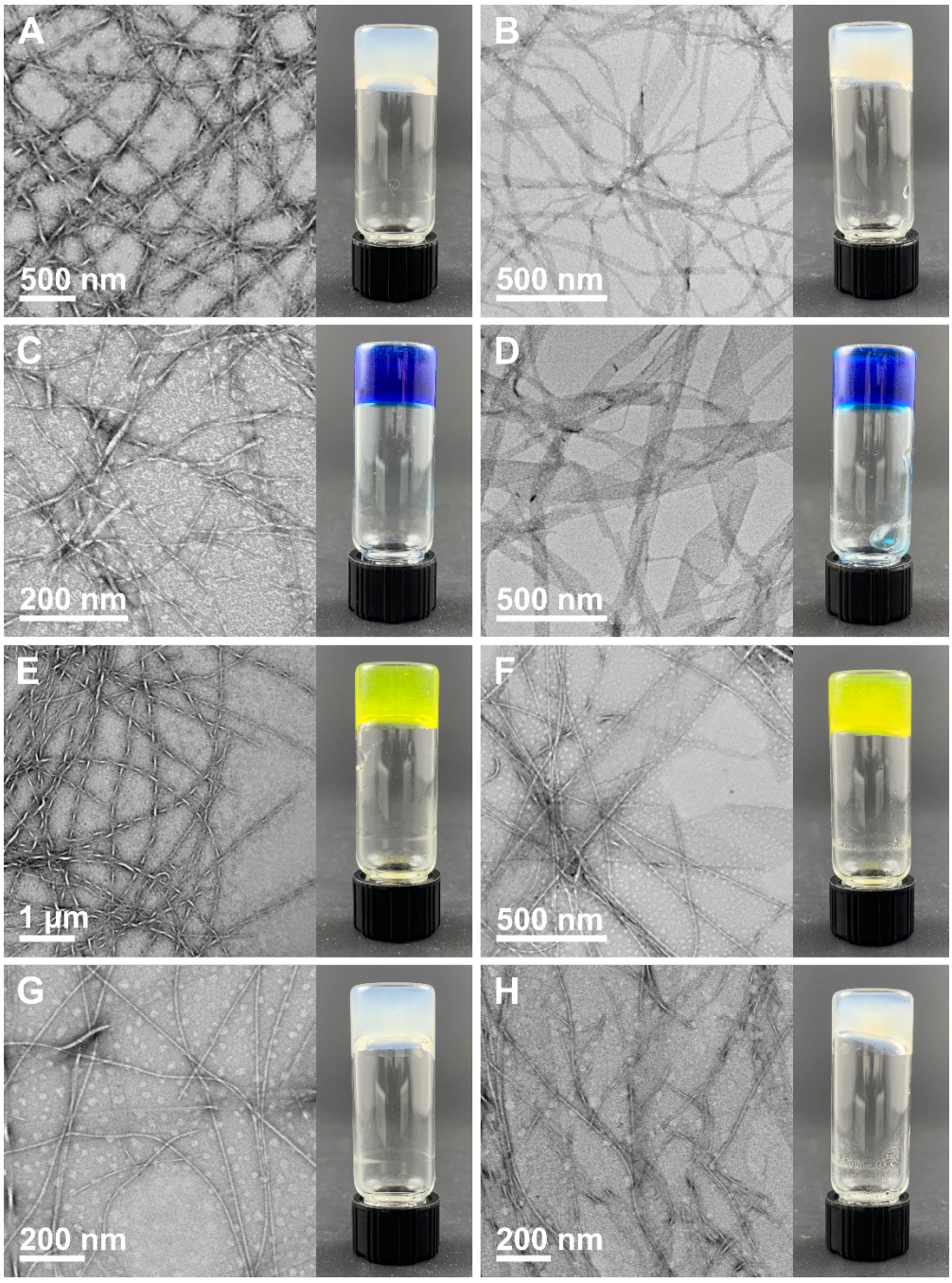


Figure S7. TEM and digital images of hydrogels of **1a** one and five days after triggering gelation. (A–B) Unloaded hydrogel after (A) one day and (B) five days. (C–D) Hydrogel loaded with methylene blue after (A) one day and (B) five days. (E–F) Hydrogel loaded with naphthol yellow S after (E) one day and (F) five days. (G–H) Hydrogel loaded with caffeine after (G) one day and (H) five days.

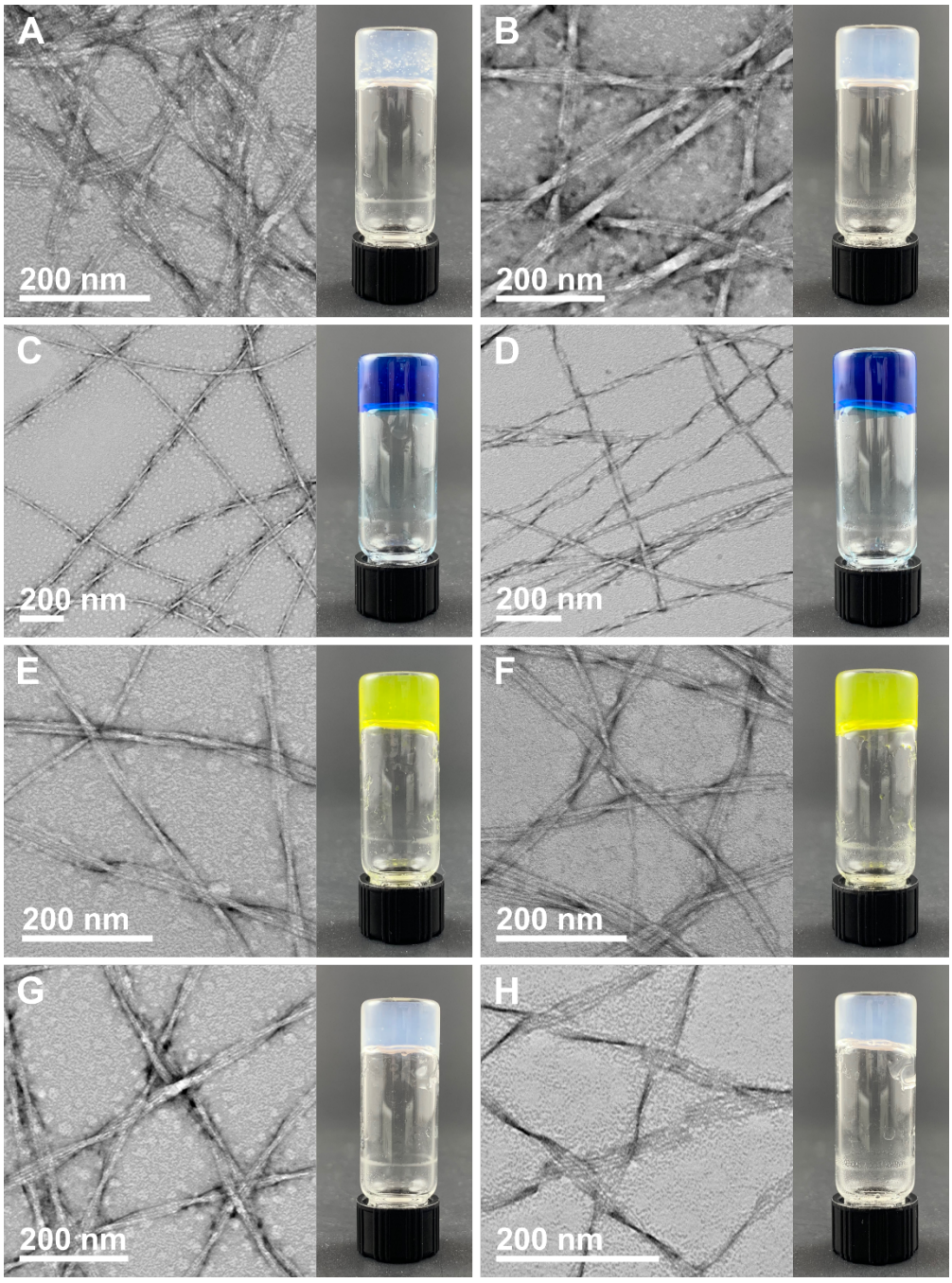


Figure S8. TEM and digital images of hydrogels of **2a** one and five days after triggering gelation. (A–B) Unloaded hydrogel after (A) one day and (B) five days. (C–D) Hydrogel loaded with methylene blue after (A) one day and (B) five days. (E–F) Hydrogel loaded with naphthol yellow S after (E) one day and (F) five days. (G–H) Hydrogel loaded with caffeine after (G) one day and (H) five days.

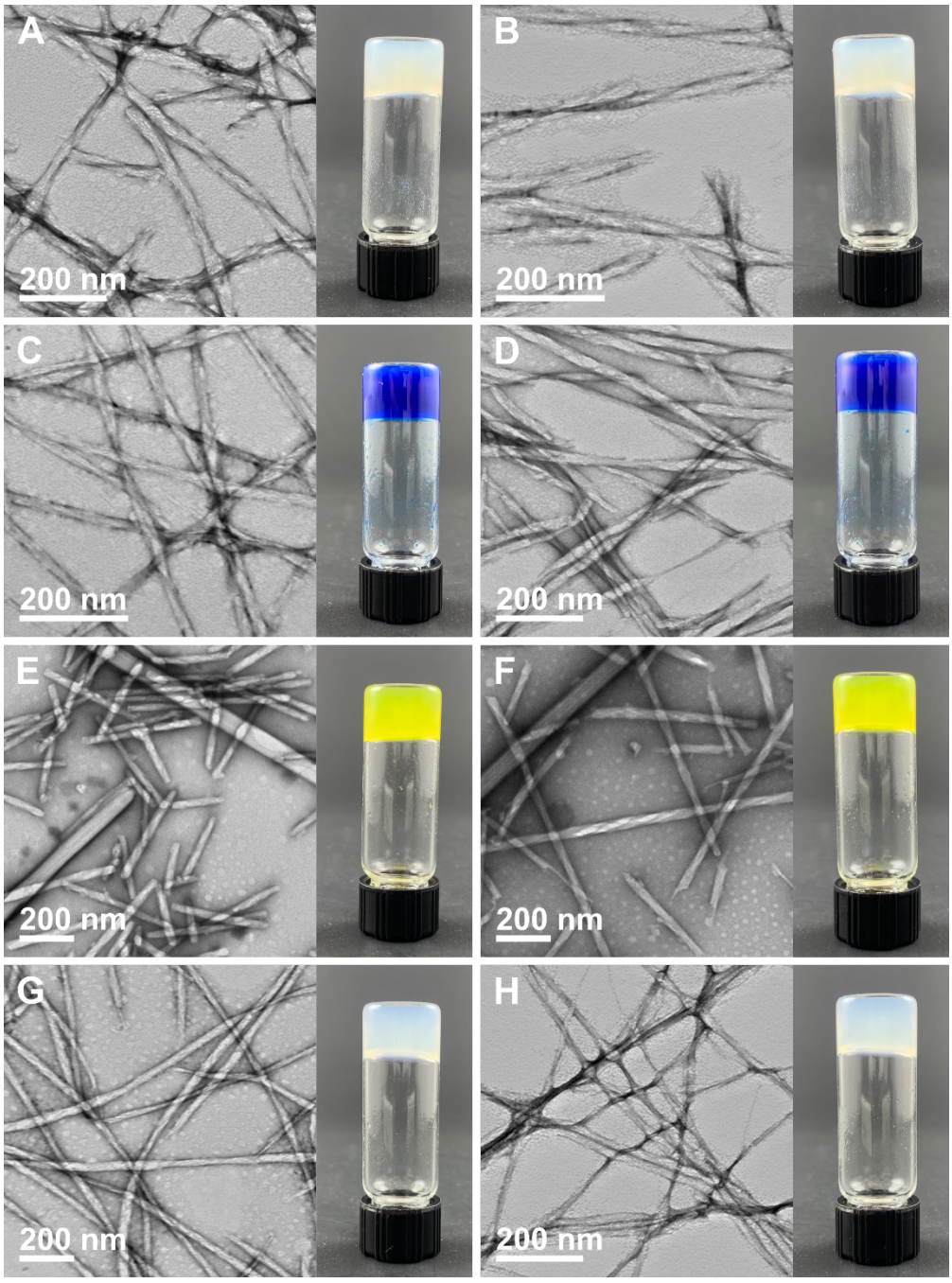


Figure S9. TEM and digital images of hydrogels of **3a** one and five days after triggering gelation. (A–B) Unloaded hydrogel after (A) one day and (B) five days. (C–D) Hydrogel loaded with methylene blue after (A) one day and (B) five days. (E–F) Hydrogel loaded with naphthol yellow S after (E) one day and (F) five days. (G–H) Hydrogel loaded with caffeine after (G) one day and (H) five days.

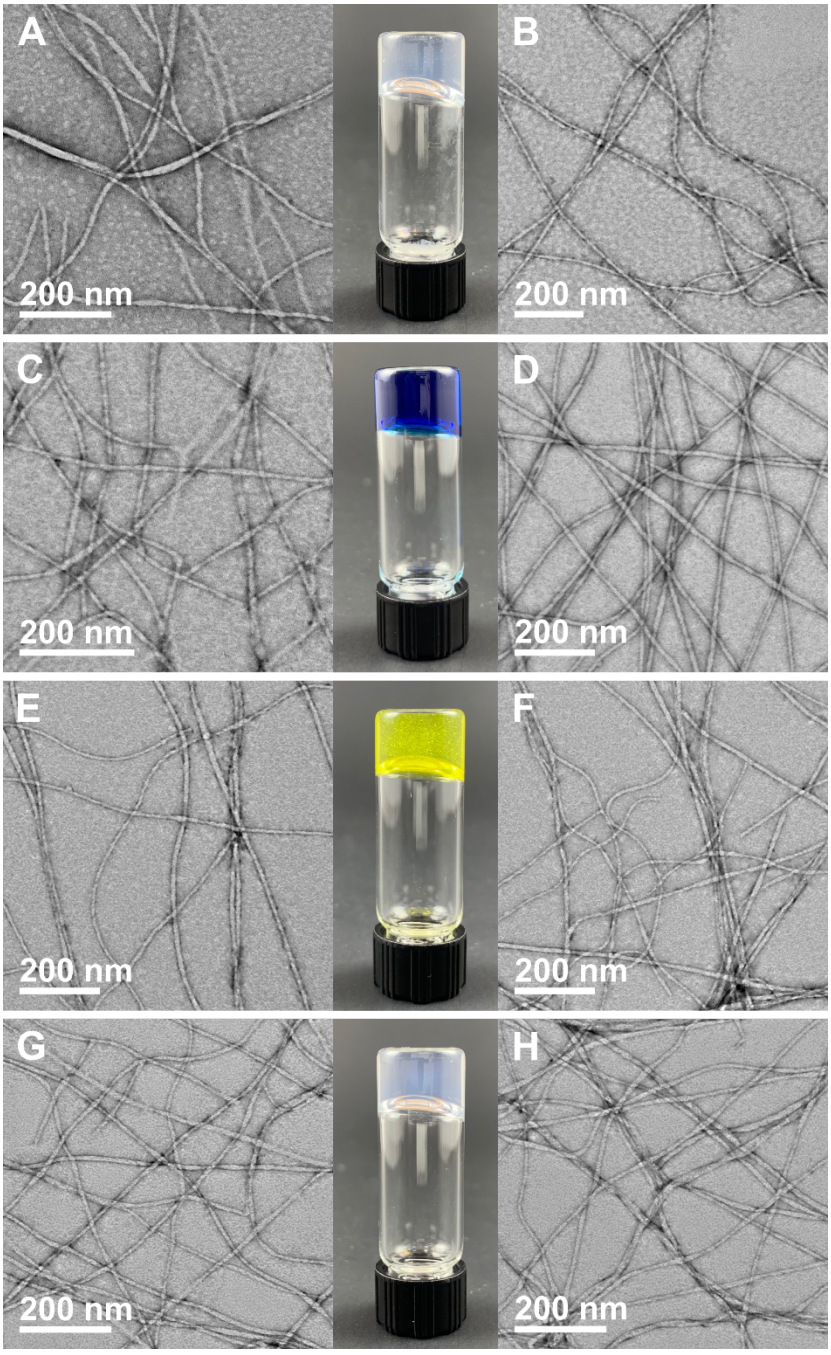


Figure S10. TEM images of hydrogels of **1b** one and five days after triggering gelation, and digital image of hydrogels after one day (no change was observed after five days). (A–B) Unloaded hydrogel after (A) one day and (B) five days. (C–D) Hydrogel loaded with methylene blue after (A) one day and (B) five days. (E–F) Hydrogel loaded with naphthol yellow S after (E) one day and (F) five days. (G–H) Hydrogel loaded with caffeine after (G) one day and (H) five days.

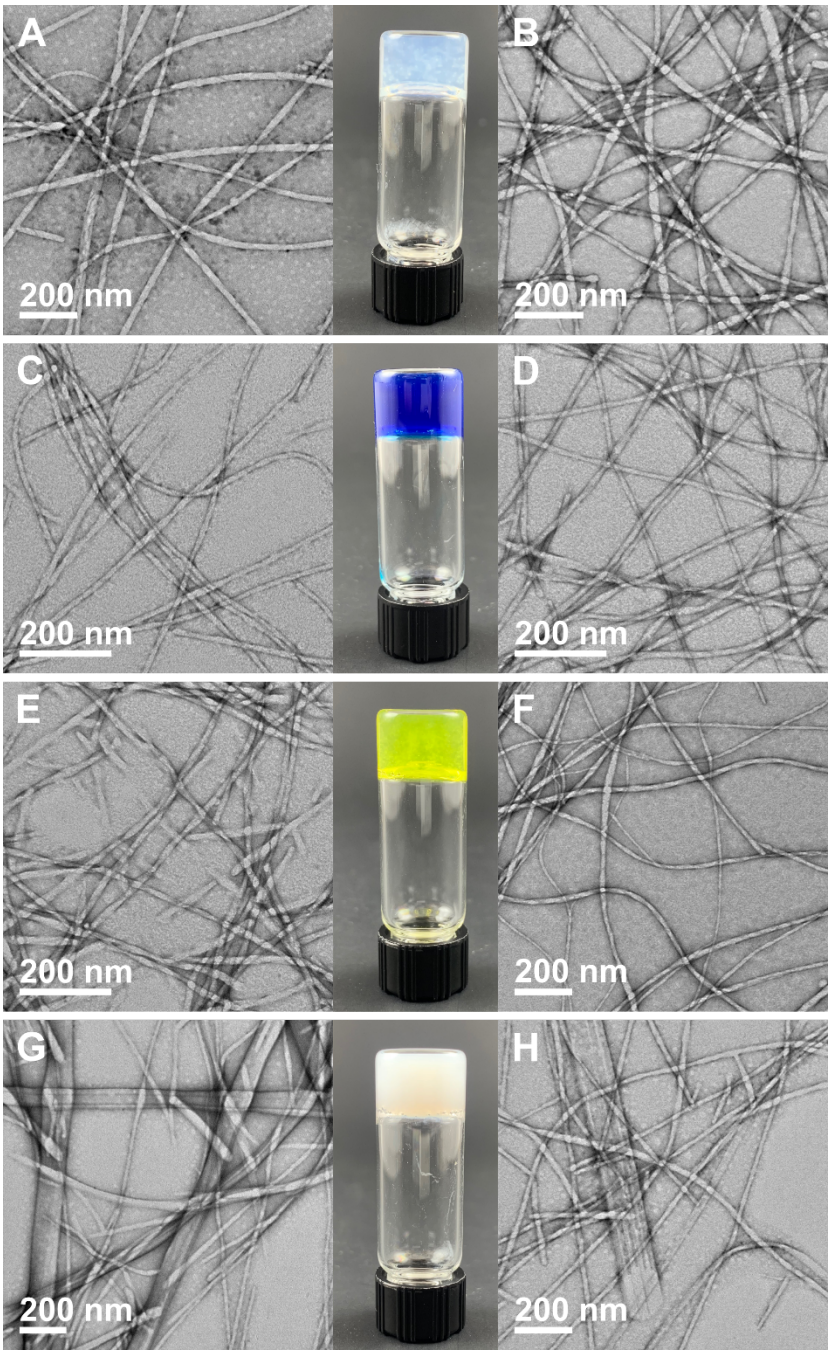


Figure S11. TEM images of hydrogels of **2b** one and five days after triggering gelation, and digital image of hydrogels after one day (no change was observed after five days). (A–B) Unloaded hydrogel after (A) one day and (B) five days. (C–D) Hydrogel loaded with methylene blue after (A) one day and (B) five days. (E–F) Hydrogel loaded with naphthol yellow S after (E) one day and (F) five days. (G–H) Hydrogel loaded with caffeine after (G) one day and (H) five days.

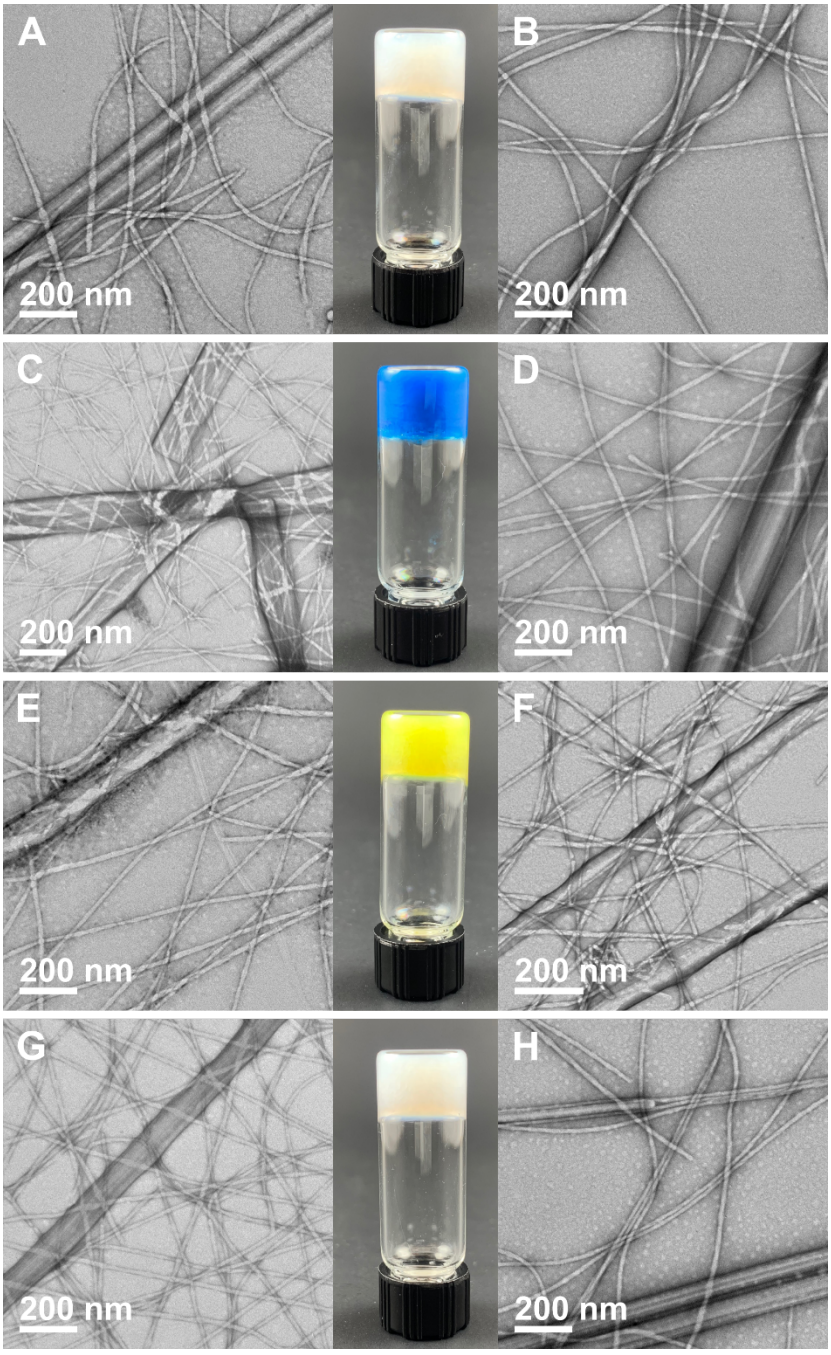


Figure S12. TEM images of hydrogels of **3b** one and five days after triggering gelation, and digital image of hydrogels after one day (no change was observed after five days). (A–B) Unloaded hydrogel after (A) one day and (B) five days. (C–D) Hydrogel loaded with methylene blue after (A) one day and (B) five days. (E–F) Hydrogel loaded with naphthol yellow S after (E) one day and (F) five days. (G–H) Hydrogel loaded with caffeine after (G) one day and (H) five days.

Table S1. Measurements of the width of nanostructures in hydrogels of **1a–3a**. F = fibril width (nm); error reported as the standard deviation about the mean of at least 100 measurements. L: width range of larger observed structures (nm). For gels of **2a**, fibril measurements are reported but the main motif was 2–6 fibrils laminated into twisting horizonal tapes.

		Cargo			
Gel	**Time**	**Unloaded**	**MB**	**NY**	**Caffeine**
1a	1 day	F: 11.0 ± 2.5 nm L: 30–59 nm	F: 9.1 ± 3.3 nm	F: 7.4 ± 1.3 nm	F: 9.8 ± 1.4 nm L: 28–291 nm
	5 day	F: 8.6 ± 1.3 nm L: 27–380 nm	F: 10.2 ± 1.3 nm L: 35–223 nm	F: 12.1 ± 2.1 nm L: 35–425 nm	F: 11.2 ± 2.1 nm L: 42–392 nm
2a	1 day	F: 7.4 ± 0.6 nm	F: 7.9 ± 0.8 nm	F: 6.9 ± 0.7 nm	F: 6.8 ± 0.6 nm
	5 day	F: 6.6 ± 0.7 nm	F: 7.6 ± 0.8 nm	F: 7.7 ± 0.9 nm	F: 7.2 ± 0.7 nm
3a	1 day	F: 33.0 ± 3.1 nm	F: 25.8 ± 3.5 nm	F: 32.5 ± 3.4 nm	F: 29.9 ± 4.2 nm
	5 day	F: 30.0 ± 4.3 nm	F: 26.4 ± 3.4 nm	F: 32.6 ± 2.9 nm	F: 26.1 ± 3.2 nm

Table S2. Measurements of the width of nanostructures in hydrogels of **1b–3b**. F = fibril width (nm); error reported as the standard deviation about the mean of at least 100 measurements. L: width range of larger observed structures (nm).

		Cargo			
Gel	**Time**	**Unloaded**	**MB**	**NY**	**Caffeine**
1b	1 day	F: 11.4 ± 1.3	F: 9.6 ± 1.4 nm	F: 9.9 ± 1.5 nm	F: 9.6 ± 1.6 nm
	5 day	F: 12.0 ± 2.0	F: 12.1 ± 1.4 nm	F: 11.8 ± 1.9 nm	F: 10.0 ± 1.3 nm
2b	1 day	F: 22.7 ± 3.9 nm	F: 18.2 ± 3.3 nm	F: 18.0 ± 2.4 nm	F: 19.7 ± 3.1 nm L: 91–397 nm
	5 day	F: 20.2 ± 3.3 nm	F: 15.3 ± 1.8 nm	F: 19.8 ± 3.9 nm	F: 17.7 ± 2.5 nm L: 46–416 nm
3b	1 day	F: 17.4 ± 2.4 nm L: 71–122 nm	F: 17.8 ± 3.1 nm L: 72–190 nm	F: 20.0 ± 3.3 nm L: 50–119 nm	F: 16.1 ± 2.0 nm L: 33–202 nm
	5 day	F: 15.5 ± 2.2 nm L: 46–135 nm	F: 17.2 ± 2.5 nm L: 77–203 nm	F: 14.8 ± 1.8 nm L: 35–139 nm	F: 17.7 ± 2.7 nm L: 42–146 nm

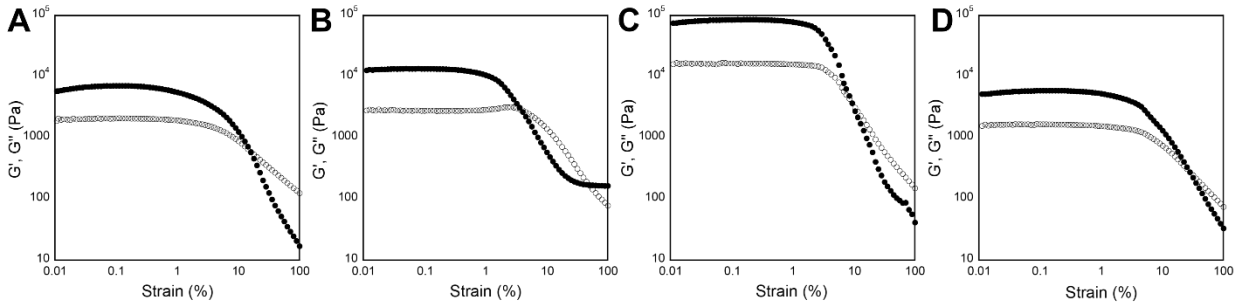


Figure S13. Strain sweep data collected via oscillatory rheology for 20 mM hydrogels of gelator **1a**. G′ and G″ values (Pa) are represented by closed circles and open circles, respectively. (A) Unloaded hydrogel. (B) Hydrogel loaded with methylene blue. (C) Hydrogel loaded with naphthol yellow S. (D) Hydrogel loaded with caffeine.

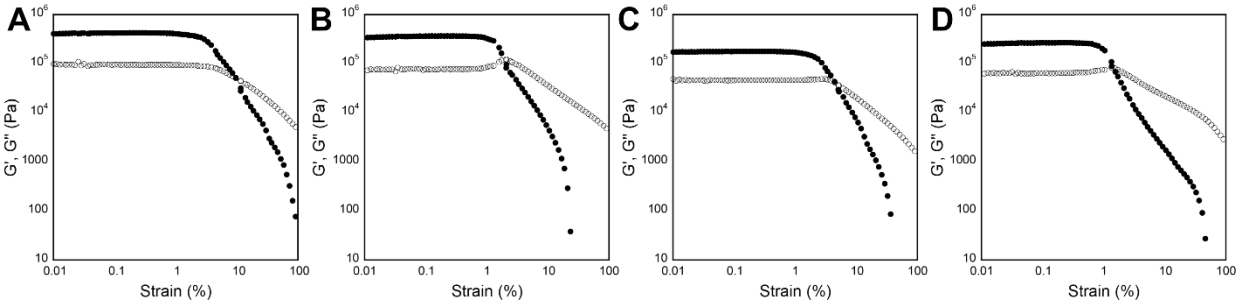


Figure S14. Strain sweep data collected via oscillatory rheology for 20 mM hydrogels of gelator **2a**. G′ and G″ values (Pa) are represented by closed circles and open circles, respectively. (A) Unloaded hydrogel. (B) Hydrogel loaded with methylene blue. (C) Hydrogel loaded with naphthol yellow S. (D) Hydrogel loaded with caffeine.

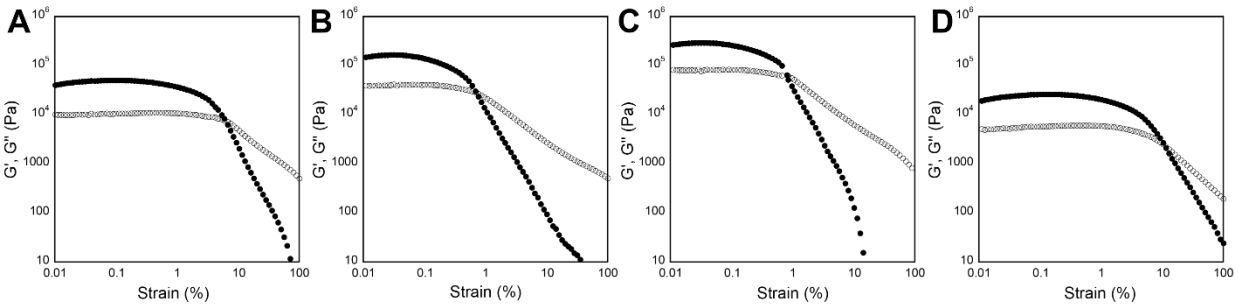


Figure S15. Strain sweep data collected via oscillatory rheology for 20 mM hydrogels of gelator **3a**. G′ and G″ values (Pa) are represented by closed circles and open circles, respectively. (A) Unloaded hydrogel. (B) Hydrogel loaded with methylene blue. (C) Hydrogel loaded with naphthol yellow S. (D) Hydrogel loaded with caffeine.

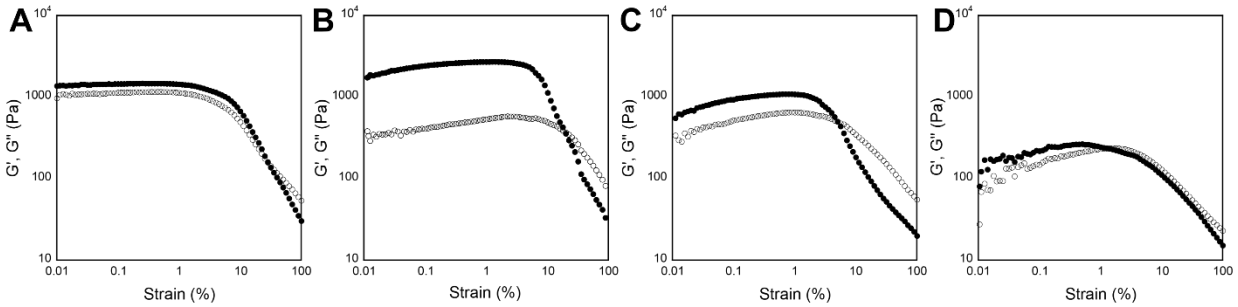


Figure S16. Strain sweep data collected via oscillatory rheology for 20 mM hydrogels of gelator **1b**. G′ and G″ values (Pa) are represented by closed circles and open circles, respectively. (A) Unloaded hydrogel. (B) Hydrogel loaded with methylene blue. (C) Hydrogel loaded with naphthol yellow S. (D) Hydrogel loaded with caffeine.

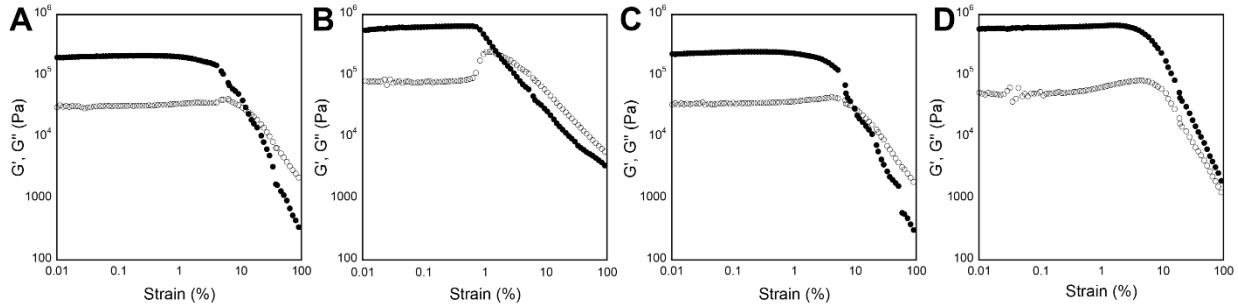


Figure S17. Strain sweep data collected via oscillatory rheology for 20 mM hydrogels of gelator **2b**. G′ and G″ values (Pa) are represented by closed circles and open circles, respectively. (A) Unloaded hydrogel. (B) Hydrogel loaded with methylene blue. (C) Hydrogel loaded with naphthol yellow S. (D) Hydrogel loaded with caffeine.

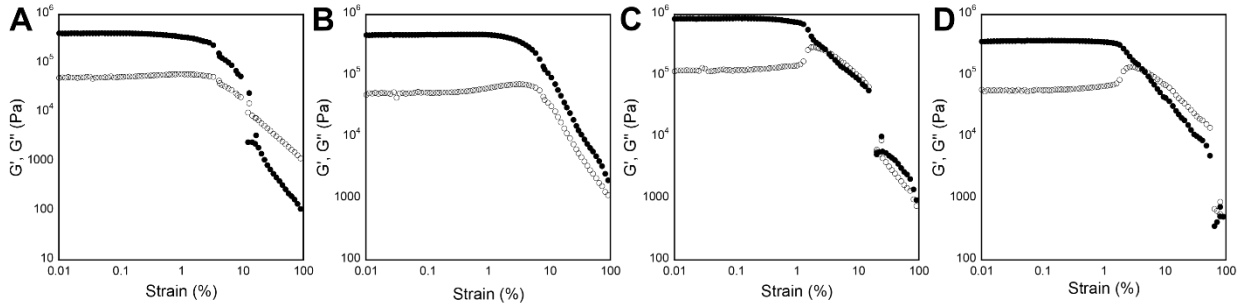


Figure S18. Strain sweep data collected via oscillatory rheology for 20 mM hydrogels of gelator **3b**. G′ and G″ values (Pa) are represented by closed circles and open circles, respectively. (A) Unloaded hydrogel. (B) Hydrogel loaded with methylene blue. (C) Hydrogel loaded with naphthol yellow S. (D) Hydrogel loaded with caffeine.

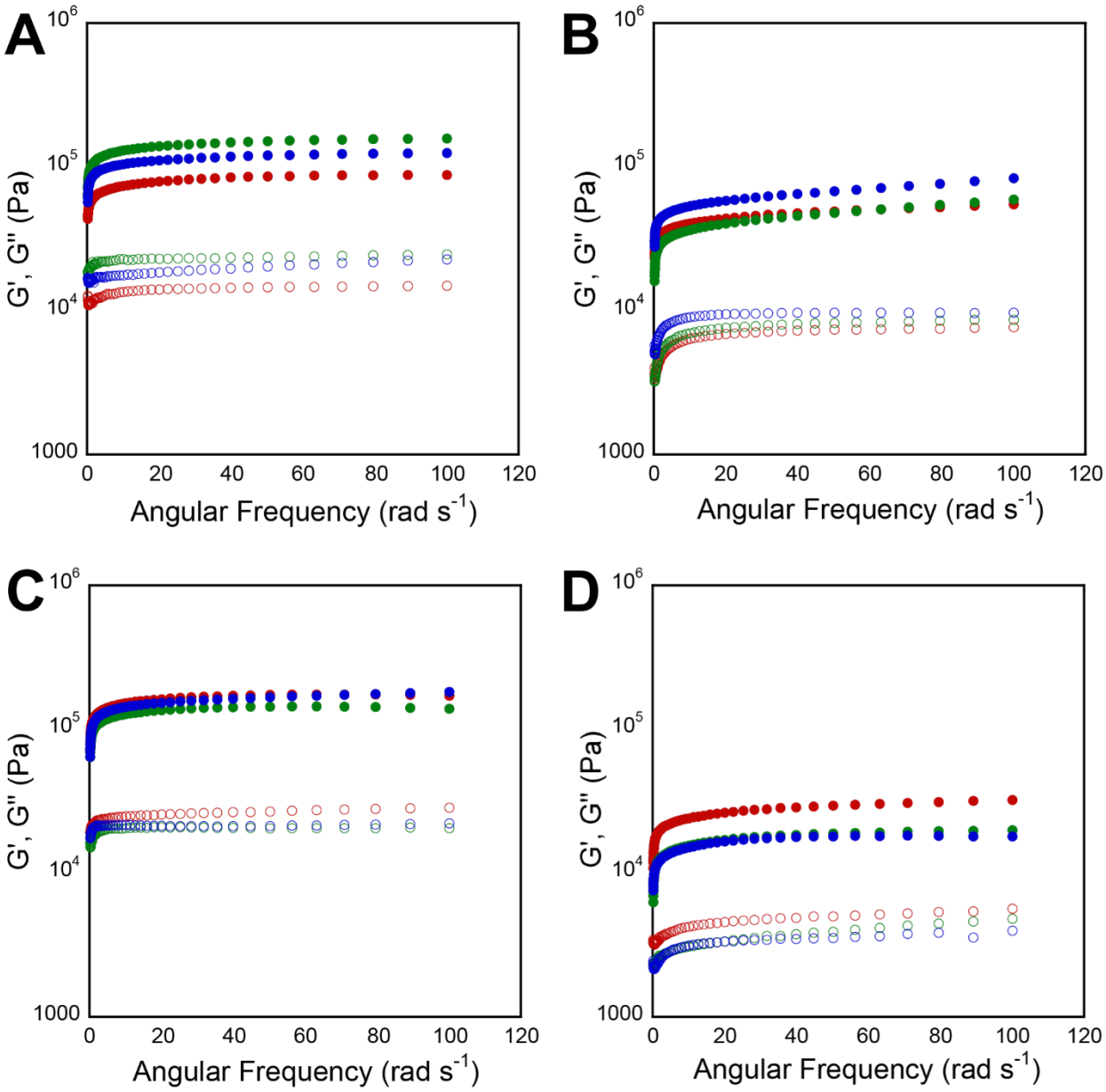


Figure S19. Frequency sweep data collected via oscillatory rheology for 20 mM hydrogels of gelator **1a**. G′ and G″ values (Pa) are represented by closed circles and open circles, respectively. Each plot contains three distinct measurements of three separate hydrogels. (A) Unloaded hydrogel. (B) Hydrogel loaded with methylene blue. (C) Hydrogel loaded with naphthol yellow S. (D) Hydrogel loaded with caffeine.

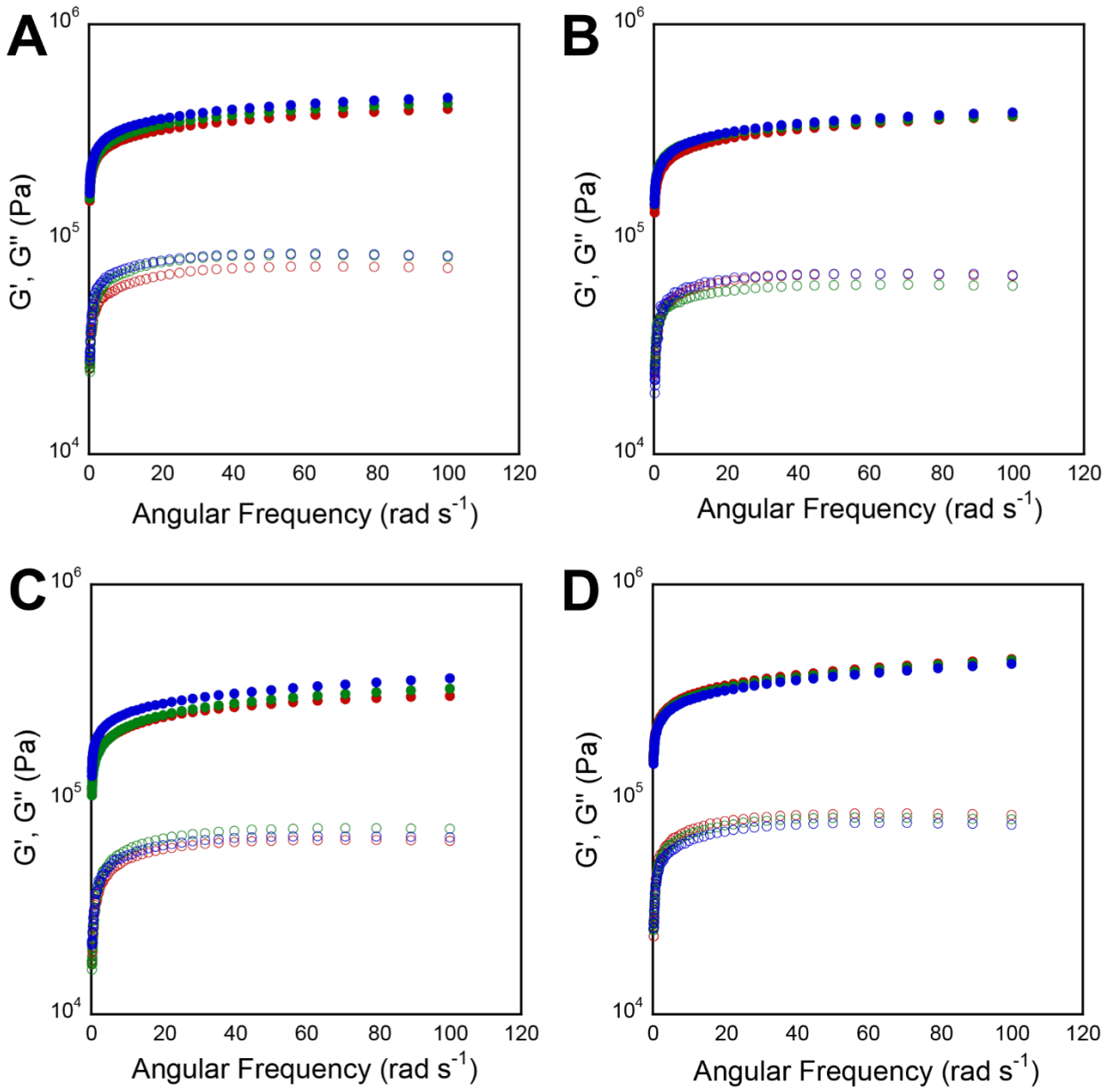


Figure S20. Frequency sweep data collected via oscillatory rheology for 20 mM hydrogels of gelator **2a**. G′ and G″ values (Pa) are represented by closed circles and open circles, respectively. Each plot contains three distinct measurements of three separate hydrogels. (A) Unloaded hydrogel. (B) Hydrogel loaded with methylene blue. (C) Hydrogel loaded with naphthol yellow S. (D) Hydrogel loaded with caffeine.

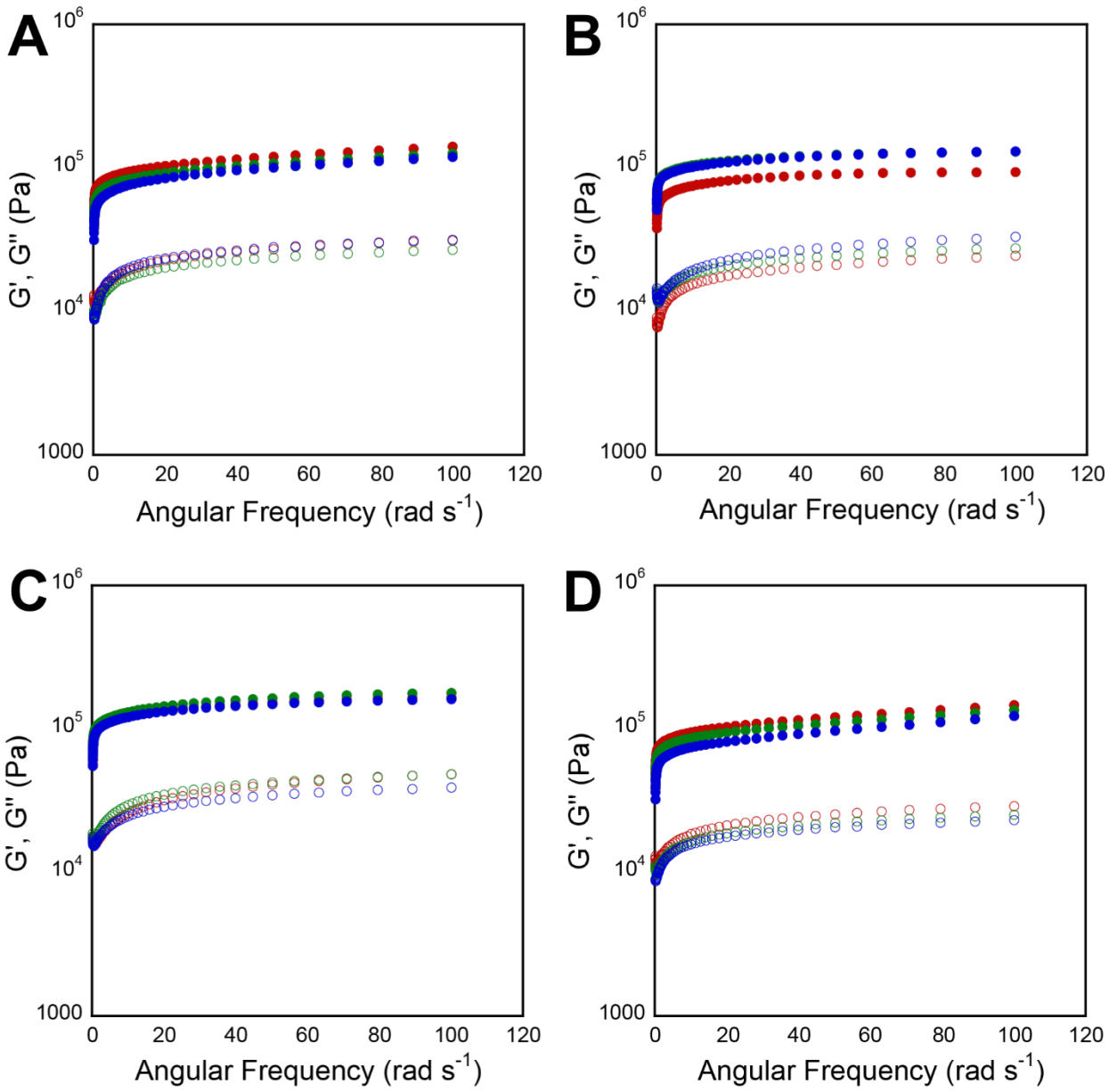


Figure S21. Frequency sweep data collected via oscillatory rheology for 20 mM hydrogels of gelator **3a**. G′ and G″ values (Pa) are represented by closed circles and open circles, respectively. Each plot contains three distinct measurements of three separate hydrogels. (A) Unloaded hydrogel. (B) Hydrogel loaded with methylene blue. (C) Hydrogel loaded with naphthol yellow S. (D) Hydrogel loaded with caffeine.

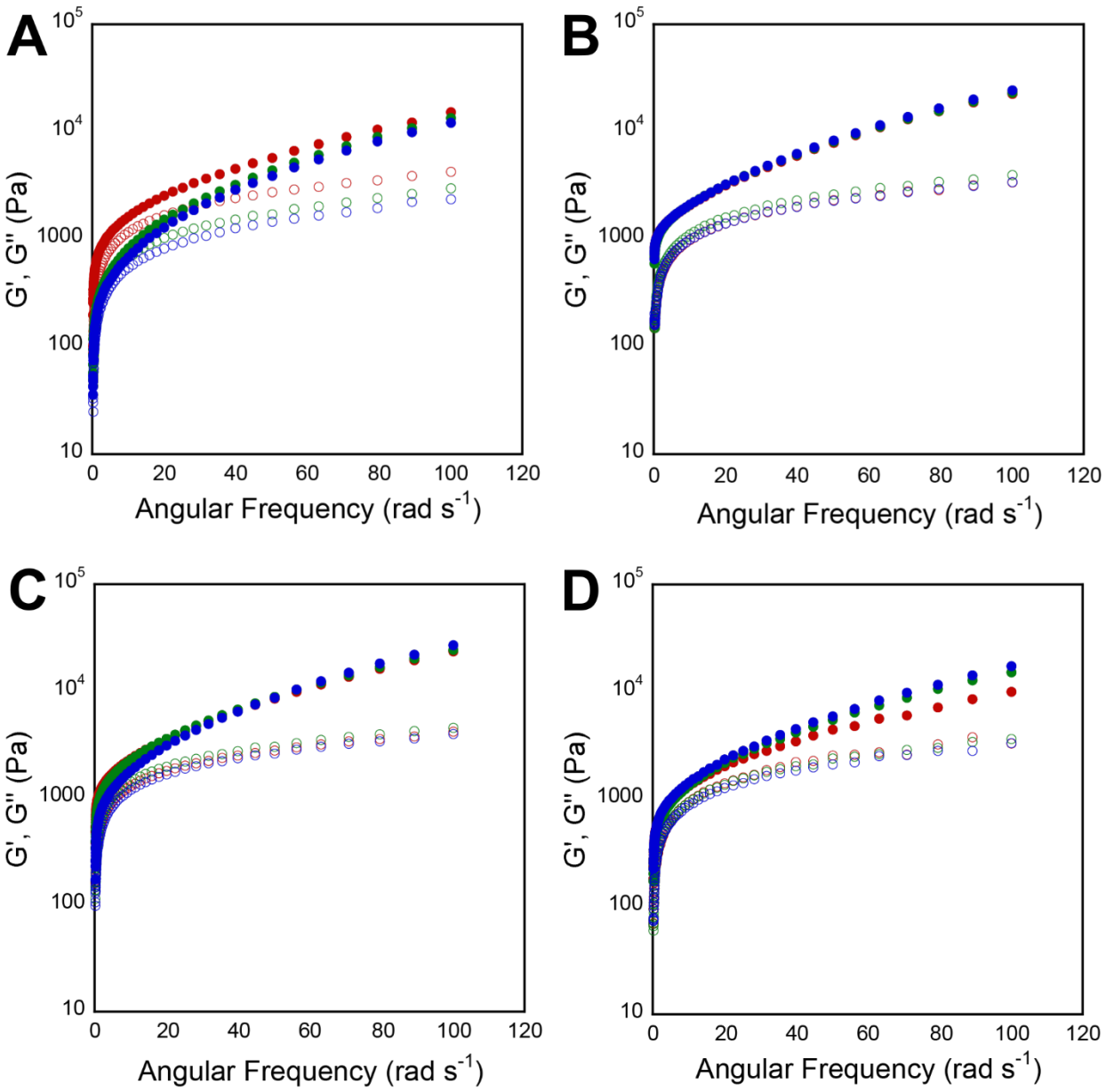


Figure S22. Frequency sweep data collected via oscillatory rheology for 20 mM hydrogels of gelator **1b**. G′ and G″ values (Pa) are represented by closed circles and open circles, respectively. Each plot contains three distinct measurements of three separate hydrogels. (A) Unloaded hydrogel. (B) Hydrogel loaded with methylene blue. (C) Hydrogel loaded with naphthol yellow S. (D) Hydrogel loaded with caffeine.

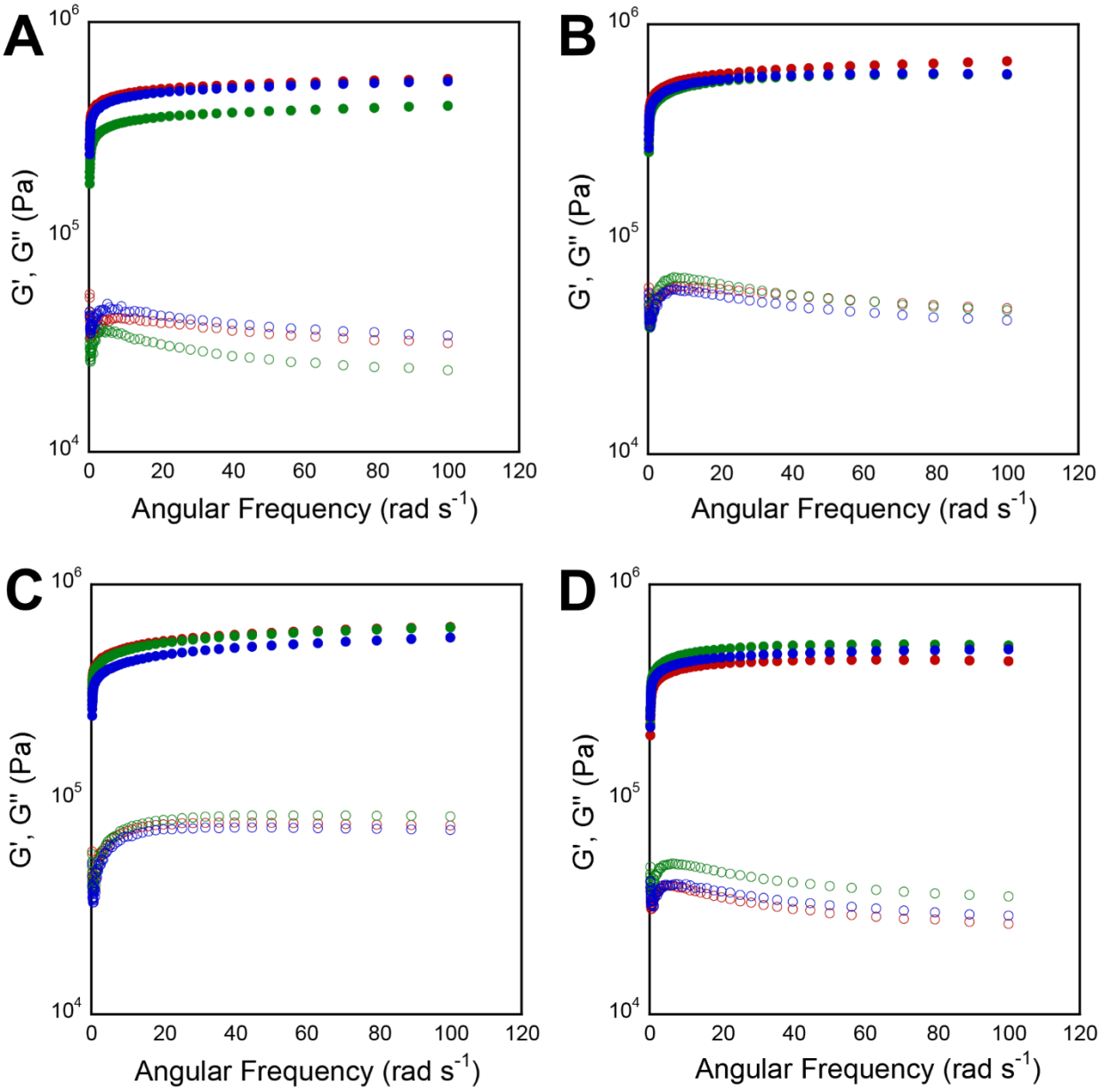


Figure S23. Frequency sweep data collected via oscillatory rheology for 20 mM hydrogels of gelator **2b**. G′ and G″ values (Pa) are represented by closed circles and open circles, respectively. Each plot contains three distinct measurements of three separate hydrogels. (A) Unloaded hydrogel. (B) Hydrogel loaded with methylene blue. (C) Hydrogel loaded with naphthol yellow S. (D) Hydrogel loaded with caffeine.

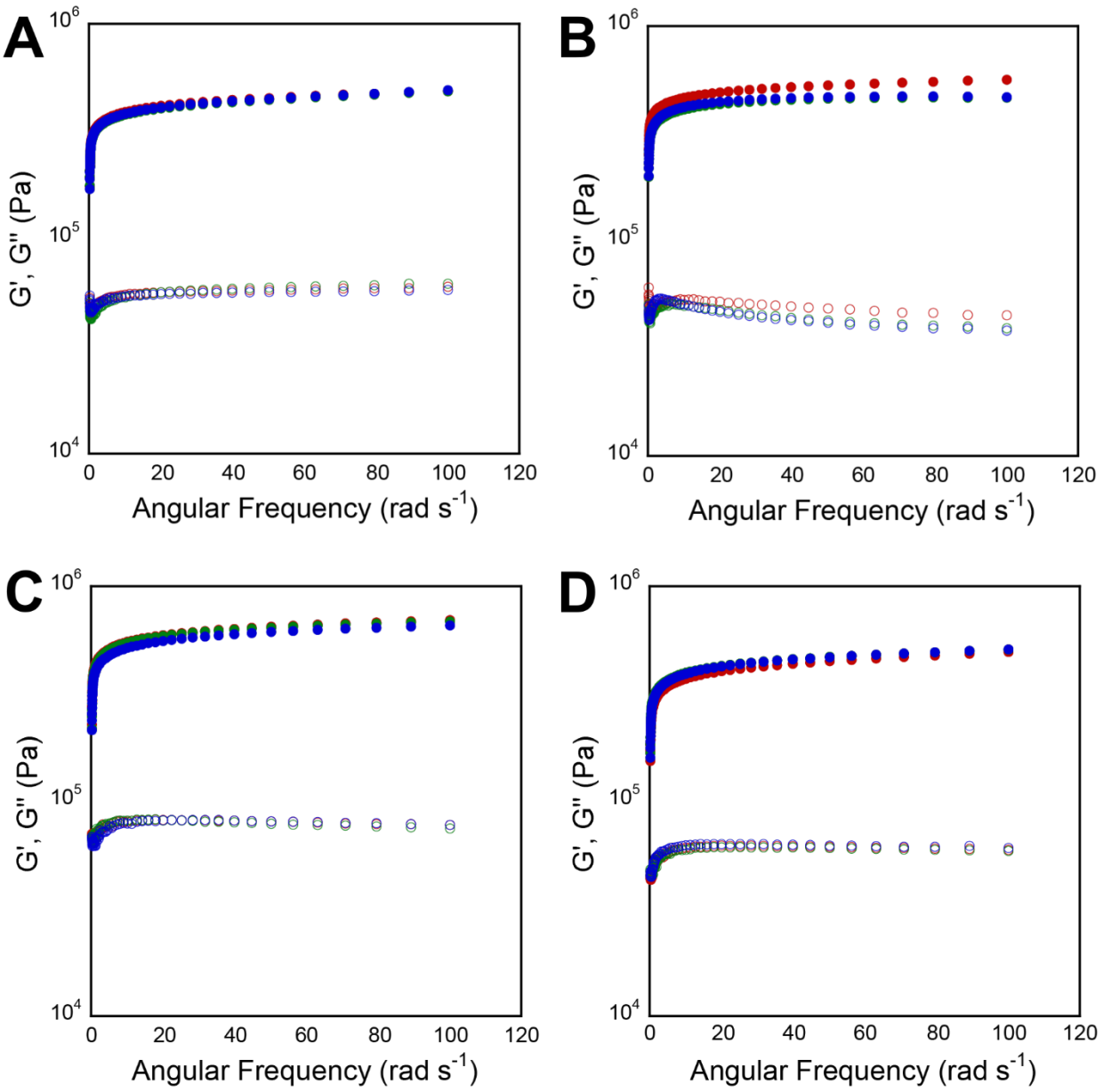


Figure S24. Frequency sweep data collected via oscillatory rheology for 20 mM hydrogels of gelator **3b**. G′ and G″ values (Pa) are represented by closed circles and open circles, respectively. Each plot contains three distinct measurements of three separate hydrogels. (A) Unloaded hydrogel. (B) Hydrogel loaded with methylene blue. (C) Hydrogel loaded with naphthol yellow S. (D) Hydrogel loaded with caffeine.

Table S3. Average G′ and G″ in kPa for each hydrogel calculated from frequency sweeps shown in **Figures S19–S24**. Error is reported as the standard deviation about the mean.

	Cargo			
Gelator	**Unloaded**	**MB**	**NY**	**Caffeine**
1a	G′: 124.3 ± 35.1 G″: 20.9 ± 5.2	G′: 66.4 ± 15.4 G″: 8.7 ± 1.0	G′: 164.9 ± 22.5 G″: 23.9 ± 4.2	G′: 23.5 ± 7.8 G″: 4.8 ± 0.8
2a	G′: 431.6 ± 26.0 G″: 80.4 ± 5.7	G′: 380.9 ± 8.9 G″: 65.8 ± 4.0	G′: 334.1 ± 31.8 G″: 68.2 ± 4.6	G′: 443.3 ± 11.3 G″: 81.0 ± 4.1
3a	G′: 129.5 ± 11.1 G″: 30.1 ± 2.6	G′: 118.6 ± 21.3 G″: 28.5 ± 4.4	G′: 169.6 ± 9.0 G″: 45.6 ± 5.4	G′: 136.2 ± 11.8 G″: 26.0 ± 3.0
1b	G′: 13.7 ± 1.6 G″: 3.2 ± 1.0	G′: 23.6 ± 0.9 G″: 3.6 ± 0.3	G′: 25.2 ± 1.8 G″: 4.3 ± 0.3	G′: 14.4 ± 3.8 G″: 3.5 ± 0.2
2b	G′: 497.0 ± 75.1 G″: 30.5 ± 5.6	G′: 618.3 ± 51.5 G″: 45.7 ± 3.1	G′: 614.4 ± 39.3 G″: 77.4 ± 5.9	G′: 489.4 ± 42.1 G″: 30.4 ± 4.7
3b	G′: 490.1 ± 3.0 G″: 60.1 ± 2.0	G′: 501.4 ± 57.1 G″: 41.1 ± 3.7	G′: 683.4 ± 20.5 G″: 76.9 ± 1.7	G′: 506.2 ± 8.1 G″: 59.9 ± 1.0

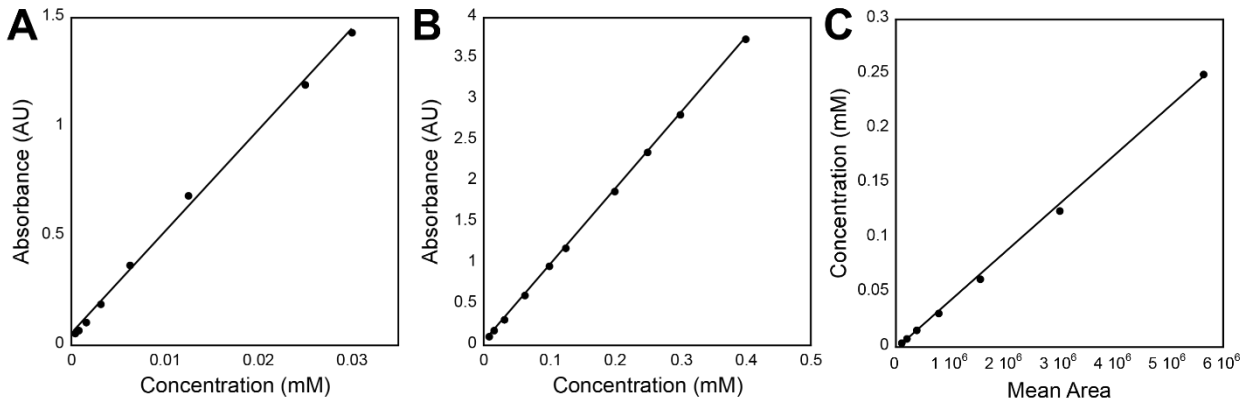


Figure S25. Concentration curves to determine the concentration of cargo in an aliquot removed from the layering solution above the hydrogels. (A) Methylene blue was measured by UV-Vis spectroscopy by absorbance at 666 nm. Equation of linear correlation: y = 46.483x + 0.0503. (B) Naphthol yellow S was measured by UV-Vis spectroscopy by absorbance at 428 nm. Equation of linear correlation: y = 9.2711x + 0.0357. (C) Caffeine was measured by HPLC by area under the curve of the caffeine peak. Equation of linear correlation: $y = 4.4182 \times 10^{-8}x - 0.0026$.

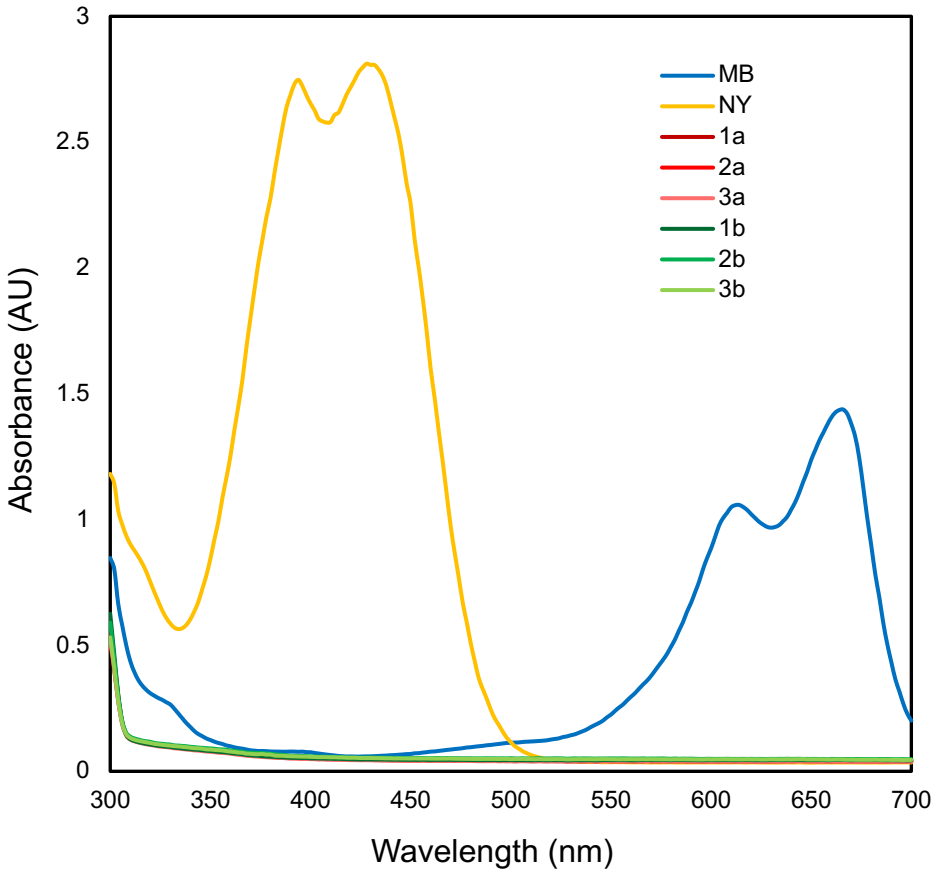


Figure S26. UV-vis spectra of methylene blue (blue, 0.03 mM) and naphthol yellow S (yellow, 0.3 mM) showcasing the λ_{max} at 666 nm and 428 nm, respectively. Spectra of gelators **1a–3b** at 1 mM concentration have been superimposed to show they have no appreciable absorbance in the visible region.

References

1. Rajbhandary, A.; Raymond, D. M.; Nilsson, B. L. Self-Assembly, Hydrogelation, and Nanotube Formation by Cation-Modified Phenylalanine Derivatives. *Langmuir* **2017,** *33*, 5803-5813.