Supporting information for

Effect of Polyacrylamide Gel Elasticity on Collagen Type II Fibril Assembly

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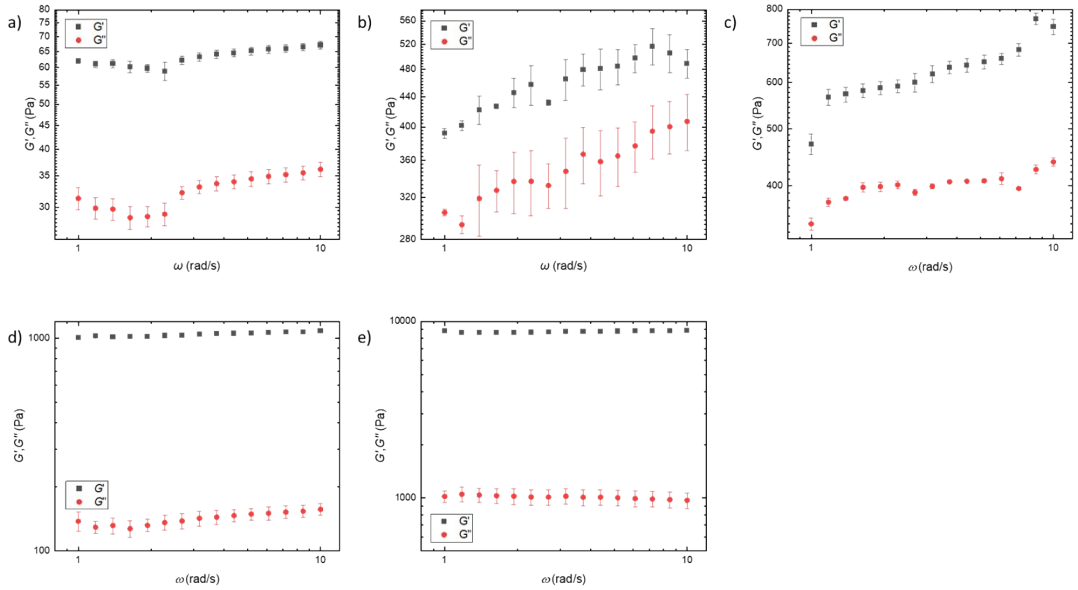


Figure S1. Small strain frequency sweeps of the polyacrylamide gels containing collagen type II fibrils used in the cryo-TEM study. a) 63 Pa gel, b) 460 Pa gel, c) 570 Pa gel, d) 1040 Pa gel, e) 8900 Pa gel.

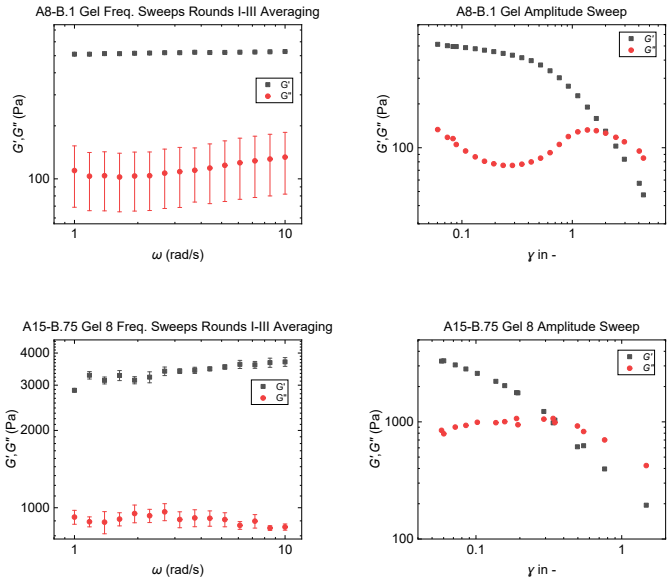


Figure S2: Small strain frequency, amplitude, and normalized amplitude sweeps of polyacrylamide gels containing collagen type II fibrils tagged with AZ488 used in the optical microscopy experiments.

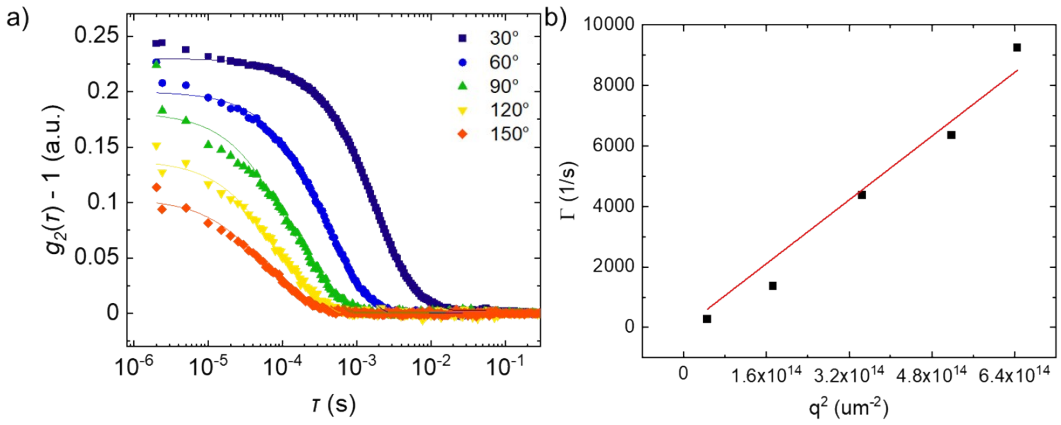


Figure S3: Diffusion of AZ488 Tagged collagen in pH 2 solutions. a) DLS Correlation plots curves of 0.1 g/L AZ488 Tagged collagen in 0.012M HCl. b) DLS relaxation rate vs scattering vector squared of 0.1 g/L AZ488 Tagged collagen in 0.012M HCl. D = 13.2 +/- 0.7 um^2/s

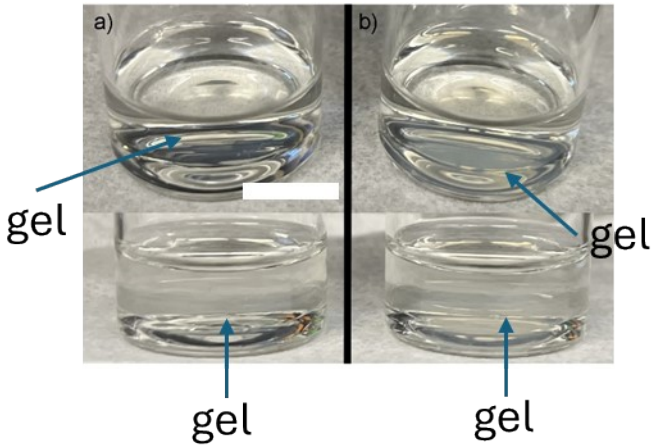


Figure S4: 0.1 g/L collagen in 0.6 kPa gels in a) 100 mM PBS and b) 0.012M HCl. The contrast is low because the gel is surrounded by the solvent. The scale bar is 14 mm.

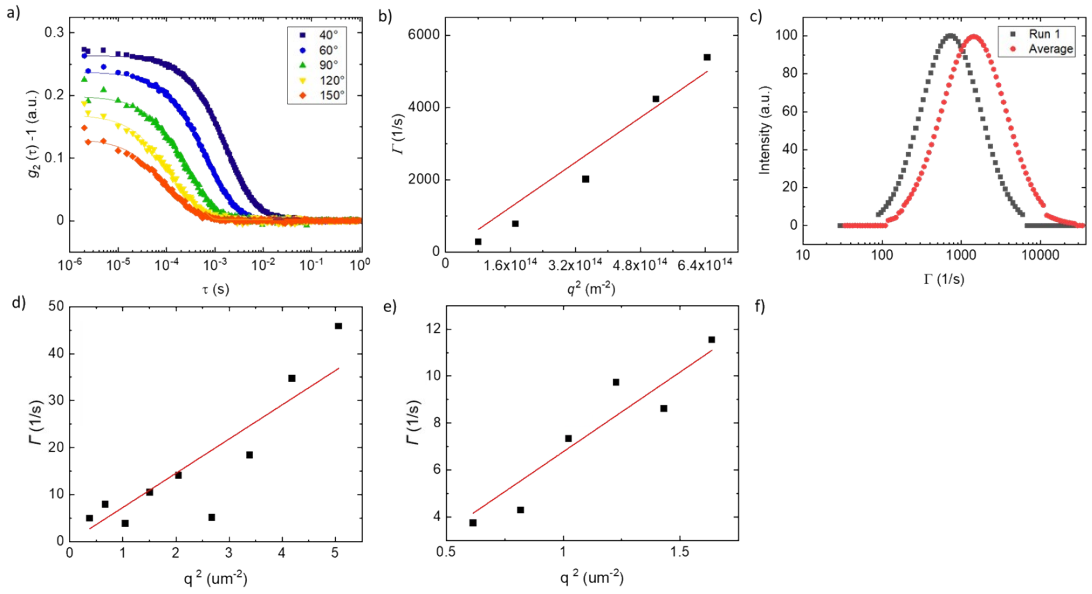


Figure S5: Preliminary study of diffusion of AZ488 Tagged collagen in pH 2 solutions. a) DLS Correlation plots curves of 0.1 g/L AZ488 Tagged collagen in 0.012M HCl. b) DLS relaxation rate vs scattering vector squared of 0.1 g/L AZ488 tagged collagen in 0.012M HCl. D = 7.8 +/- 0.6 um^2/s. (0.0625 g/L untagged collagen in 0.012 M HCl has a D = 9.2 +/- 0.6 um^2/s as determined by K.G. Wilcox, G.M. Kemerer, and S. Morozova, *J. Chem. Phys*. 158, 044903 (**2023**)). c) CONTIN distributions at 90° of 0.1 g/L AZ488 Tagged collagen in 0.012M HCl. d) Run 1 Results of Dynamic Differential Microscopy (DDM) relaxation rate vs scattering vector squared of 0.5 g/L AZ488 Tagged collagen in 0.012M HCl. (Experiments done by Bittany Roopnarine.) D = 7.3 +/- 0.6 um^2/s. e) Run 2 Results of DDM relaxation rate vs scattering vector squared of 0.5 g/L AZ488 Tagged collagen in 0.012M HCl. (Experiments done by Bittany Roopnarine.) D = 6.8 +/- 0.3 um^2/s. Overall, tagging the collagen triple helices has little effect on their diffusion.

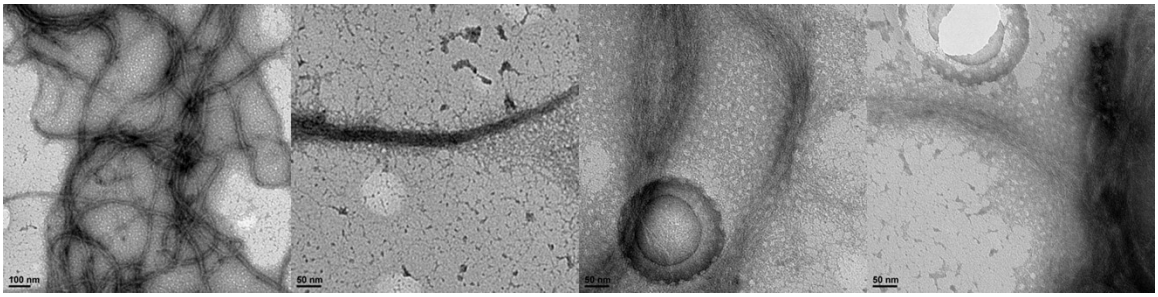


Figure S6: Representative TEM Images of 0.1g/L AZ488 Tagged collagen fibrils in 100 mM PBS solutions. Overall, the fibrils appear thinner than untagged collagen fibrils.

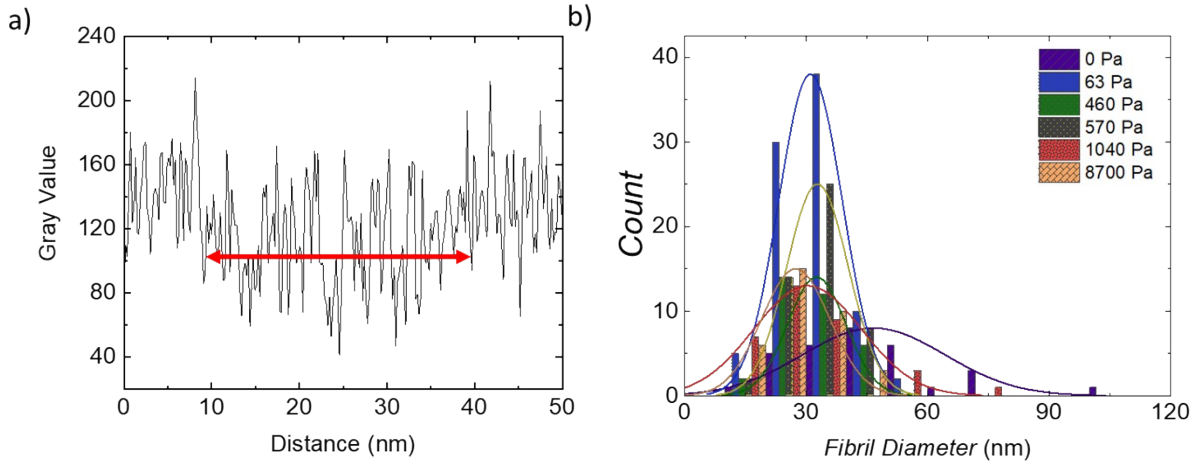


Figure S7: Single collagen fibril in solution and gels diameter analysis. a) Example of how diameter measurements are taken. The diameter of the half height of the gray values) is used as the diameter of the fibril in this study (as denoted by the red arrows). For example, the signal plateaus at 150 and has a minimum at 50 so the half height diameter is taken at 100. b) Histograms of all single fibril diameter measurements.

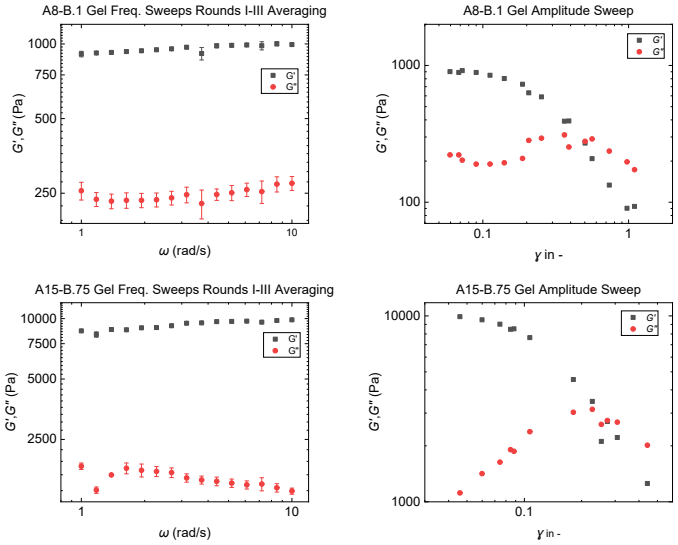
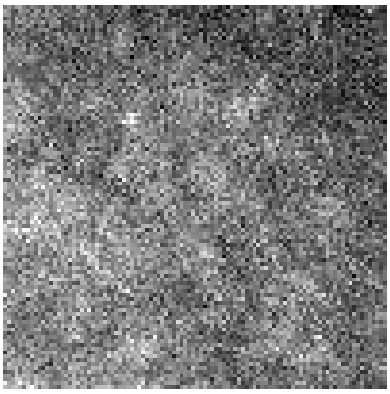


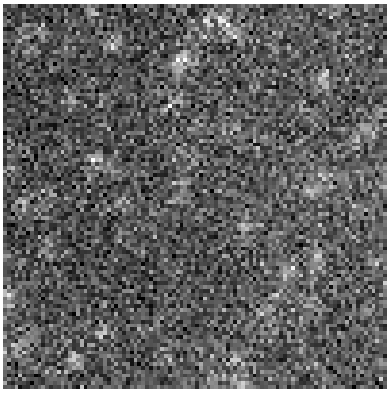
Figure S8: Frequency, amplitude, and normalized amplitude sweep graphs from the additional optical microscopy experiments on tagged collagen in gel to verify results.

Table S1: Average number of fibril bundles at each time point in soft gels, stiff gels, and solution from representative data in Figure 4.

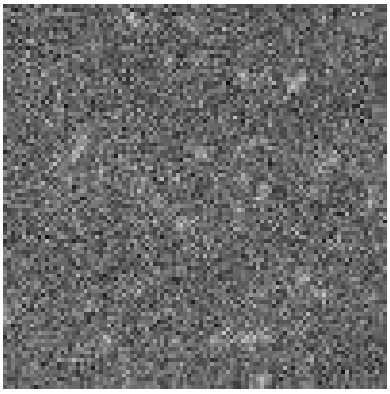
	HCl	PBS 0.5 hr	PBS 1 hr	PBS 3 hr	PBS 24 hr	PBS 48 hr
Soft Gel	90 ± 10	60 ± 20	80 ± 40	70 ± 50	70 ± 60	100 ± 40
Stiff Gel	130 ± 30	30 ± 30	10 ± 30	2 ± 6	8 ± 20	10 ± 30
Solution	40 ± 10	50 ± 30	30 ± 2	60 ± 10		



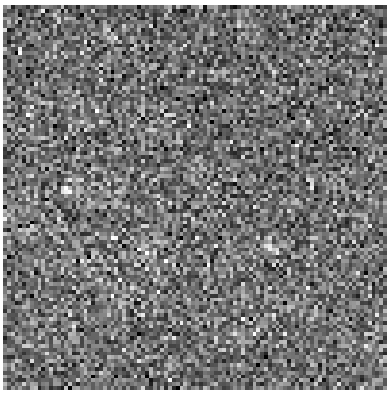
Video S1: Single frame of collagen diffusion in HCl collected at 200 fps.



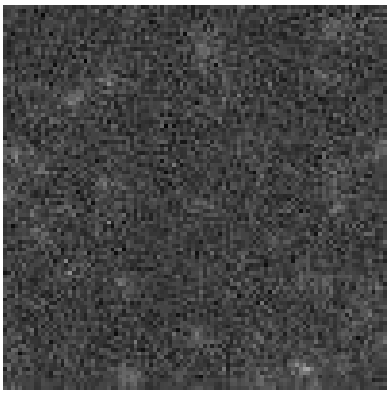
Video S2: Single frame of collagen diffusion at 15 min in PBS collected at 200 fps.



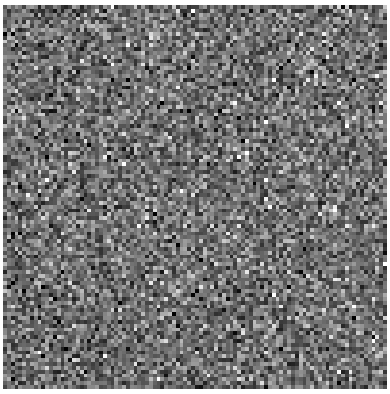
Video S3: Single frame of collagen diffusion within a soft gel soaked in HCl collected at 200 fps.



Video S4: Single frame of collagen diffusion within a soft gel soaked in PBS for 15 min collected at 200 fps.



Video S5: Single frame of collagen diffusion within a stiff gel soaked in HCl collected at 200 fps.



Video S6: Single frame of collagen diffusion within a stiff gel soaked in PBS for 15 min collected at 200 fps.

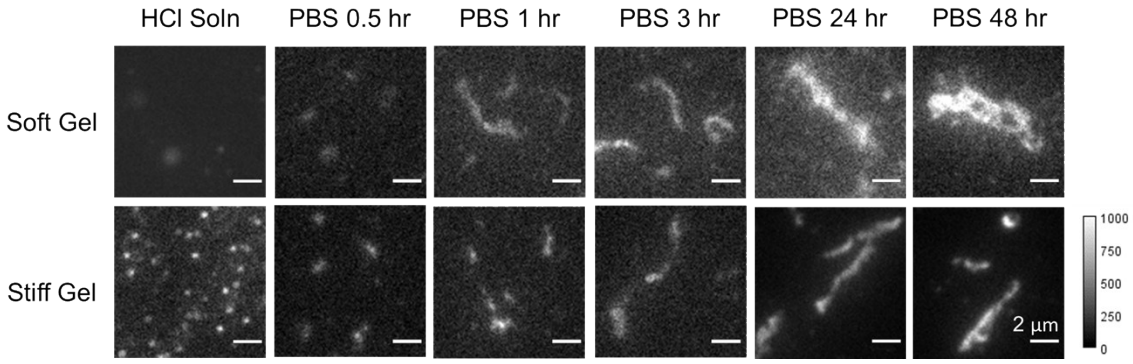
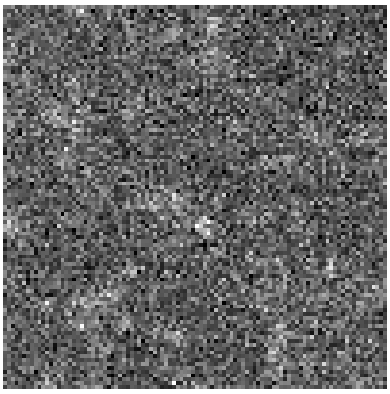
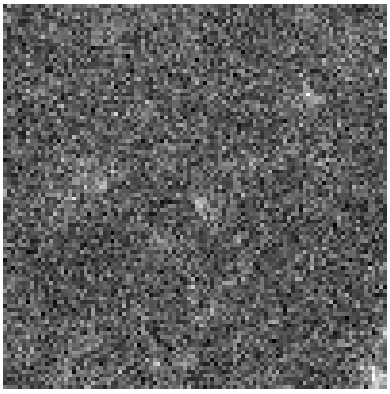


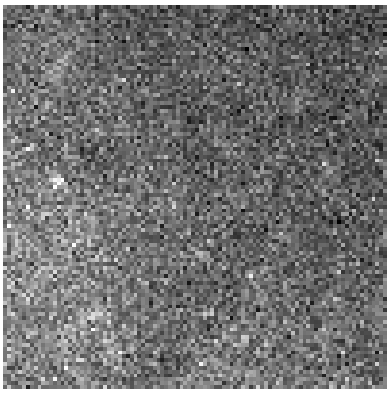
Figure S9: Optical images of tagged collagen in the soft (0.5 kPa), the stiff (3.4 kPa) gels and in solution at 0.11 g/L using TIRF and HILO. Scale bars are 2 µm.



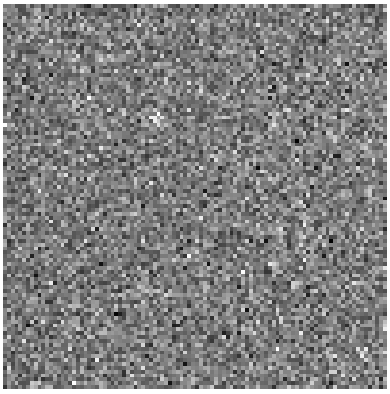
Video S7: Single frame of collagen diffusion within a soft gel soaked in HCl collected at 200 fps.



Video S8: Single frame of collagen diffusion within a soft gel soaked in PBS for 15 min collected at 200 fps.



Video S9: Single frame of collagen diffusion within a stiff gel soaked in HCl collected at 200 fps.



Video S10: Single frame of collagen diffusion within a stiff gel soaked in PBS for 15 min collected at 200 fps.

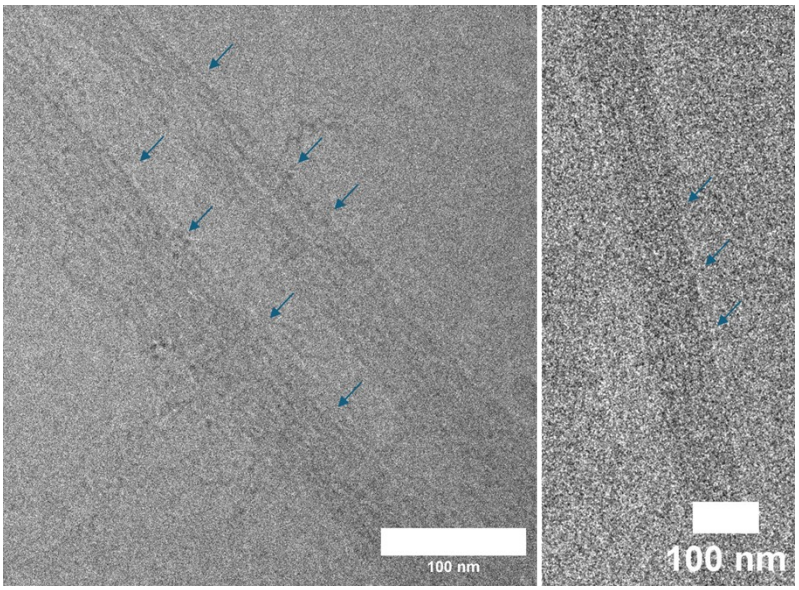


Figure S10. There is evidence of cross striations in the fibrillar assembly in both solutions (left) and gels (600 Pa, right)

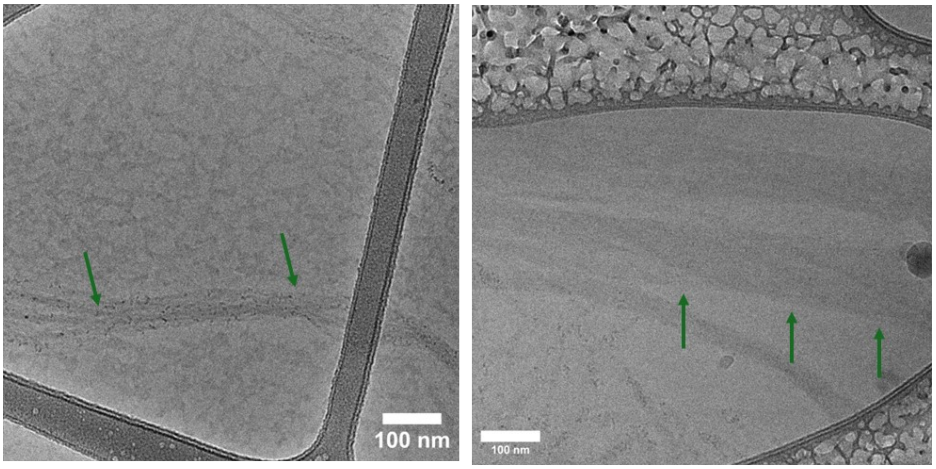


Figure S11. There is also evidence of twists in both fibrils in gels (60 Pa, left), and both fibrils and bundles in solution (right).

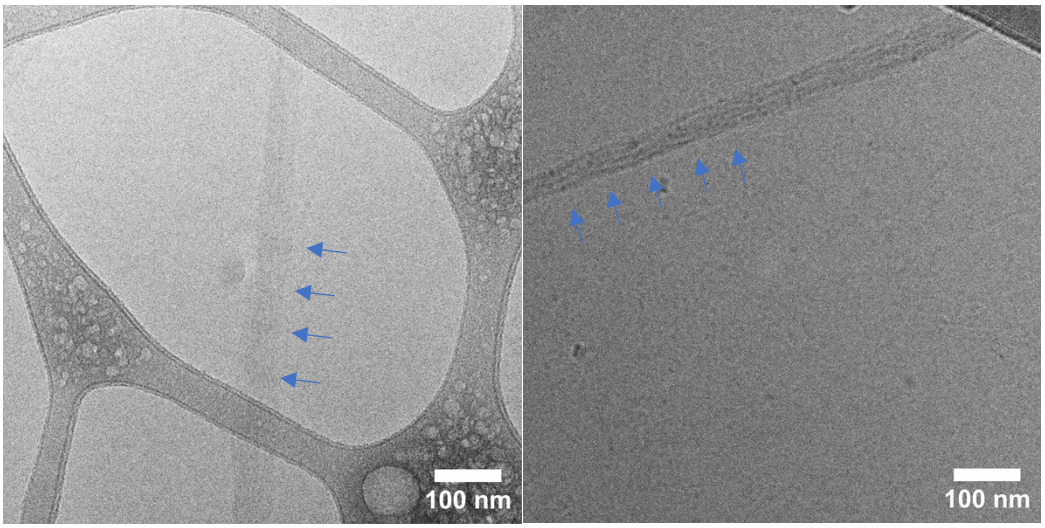


Figure S12. More evidence of cross striations in fibrils grown in gels (600 Pa)