Supporting Information

Coiled-coil protein hydrogels engineered with minimized fiber diameters for sustained release of doxorubicin in triple negative breast cancer

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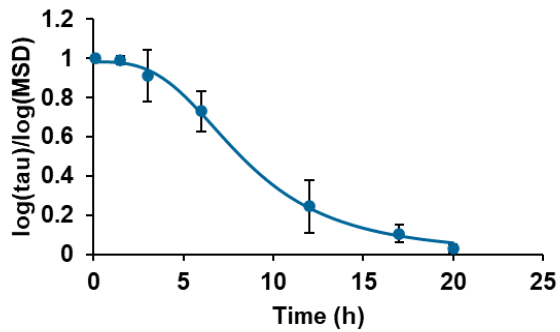
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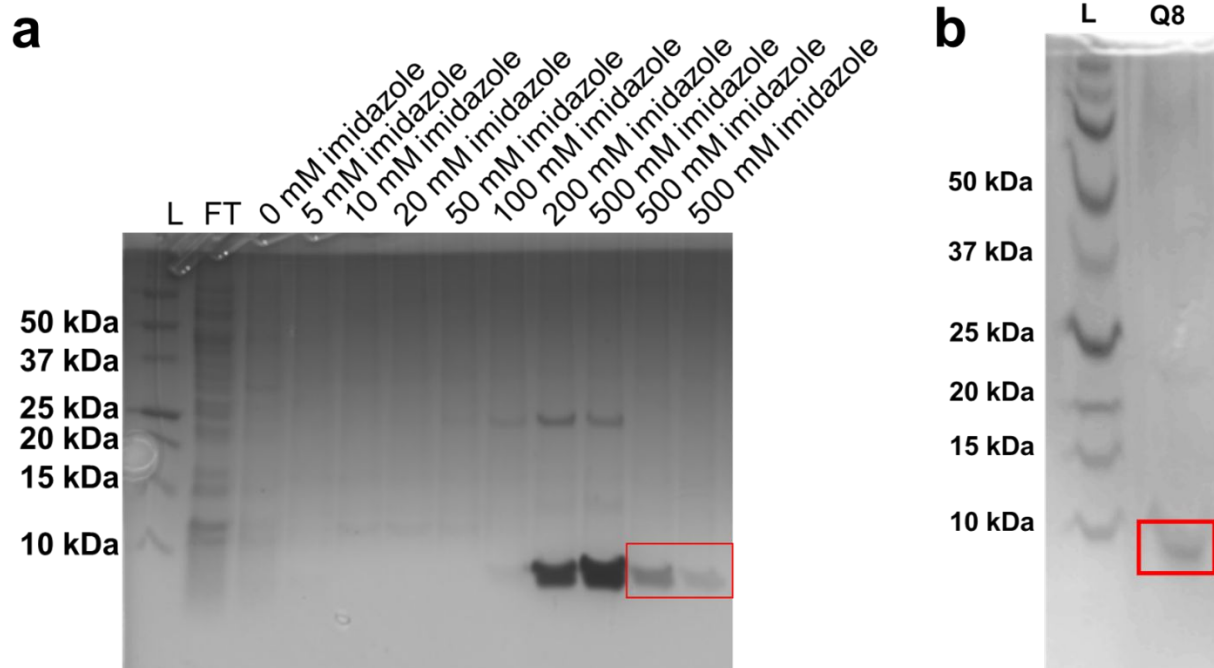
Figure S1 a. 12% SDS-PAGE of Q8 purification using cobalt-charged NiNTA affinity chromatography. L: Ladder, FT: flow-through. **b.** 12% SDS-PAGE of Q8 at 1 µM following dialysis and concentration.
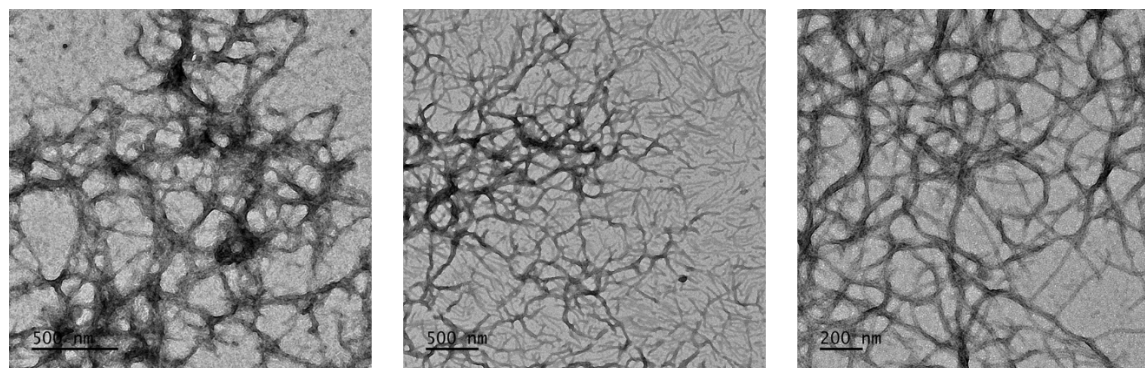
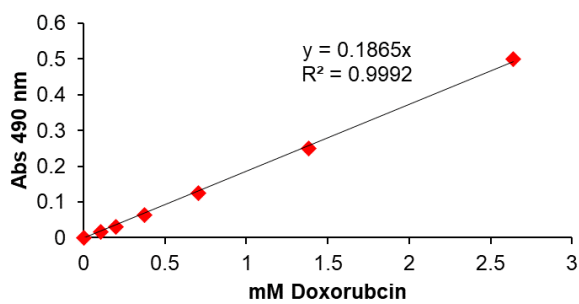**Figure S2** Various transmission electron micrographs of Q8.

Figure S3 Sigmoidal fit of exponents from MPT analysis of Q8 microrheological assay. Error bars represent the standard deviation of three independent trials.
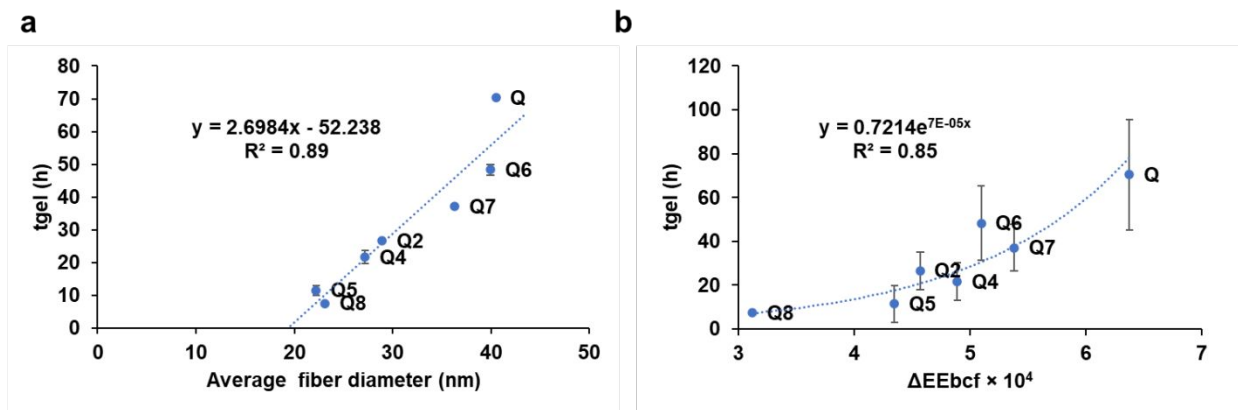

Figure S4 a. Updated linear regression model for relationship between average fiber diameter and critical gelation time (t_{gel}) for all gelling Q variants. Error represents the average of three independent trials. **b.** Exponential relationship between ΔEE_{bcf} and critical gelation time (t_{gel}) for *in silico* prediction. Error bars represent the standard deviation of three independent trials.
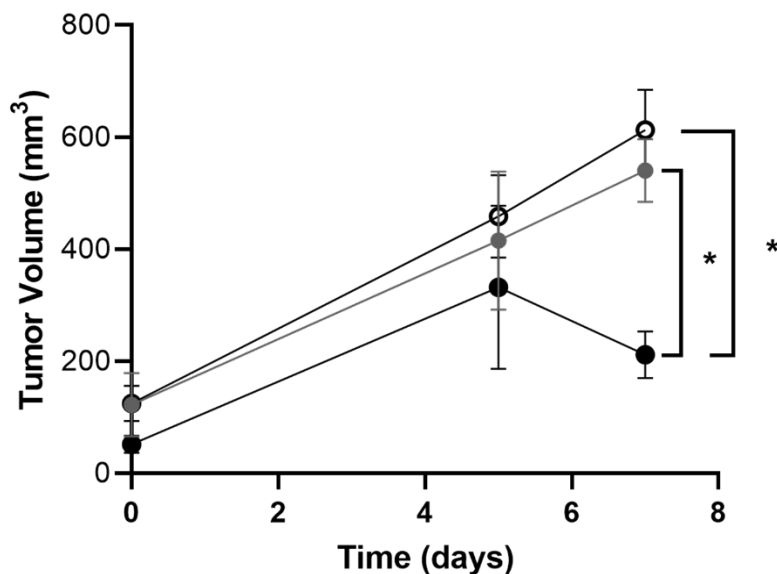
S-3

Figure S5 Representative image of Q in the gel state (2 mM) after loading onto parallel plate rheometer.

Figure S6 Standard curve of absorbance at 490 nm by a microplate reader (BioTek) for doxorubicin concentration.
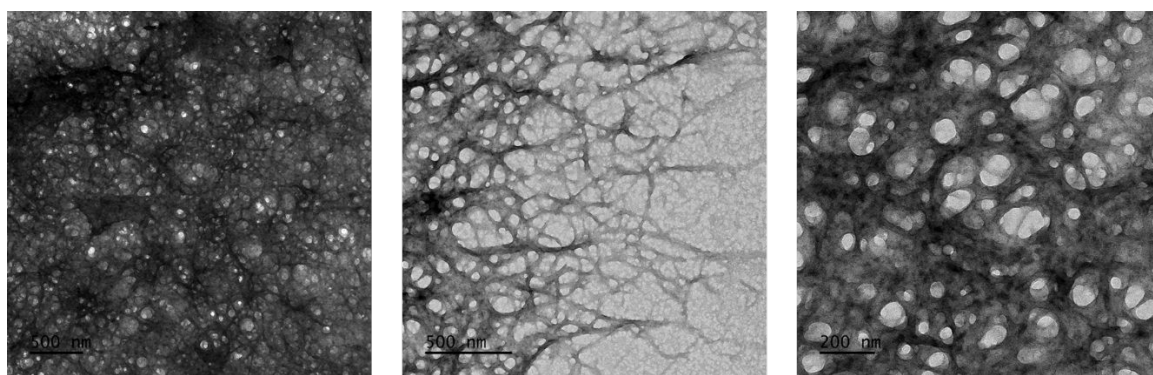
Figure S7 Various transmission electron micrographs of Q8•Dox.

Figure S8 Average tumor volume following treatment of Q8 (open circles), Dox (grey filled circles), and Q8•Dox (black filled circles) measured at baseline (0 days), 5 days, and 7 days post-treatment. Error bars represent the standard error of the mean: 5 mice for Q8•Dox and Dox, 4 mice for Q8. * represents p-value < 0.05 calculated by 2-way ANOVA.

Table S1 Average and standard deviation of MRE values of CD spectra α-helical minima and % secondary structure content assessed by peak deconvolution of ATR-FTIR spectra

Phase	Circular Dichroism Minima			FTIR Deconvolution (%)		
	$-\Theta_{208}$ (deg·cm²·dmol⁻¹)	$-\Theta_{222}$ (deg·cm²·dmol⁻¹)	Θ_{222} / Θ_{208}	α-helix	β-sheet	Random Coil
Solution	$27,000 \pm 2,000$	$33,000 \pm 3,000$	1.2 ± 0.1	28.8 ± 4.2	41.9 ± 5.8	25.9 ± 6.8
Gel	$8,000 \pm 2,000$	$9,000 \pm 1,000$	1.1 ± 0.0	34.7 ± 2.0	39.9 ± 6.7	26.5 ± 9.0

Table S2 Comparison of experimental and predicted values of α-helicity (gel state) and increase in structured content measured by FTIR using univariate linear regression models from Britton *et al.* [1]

Structure metric	Actual	Predicted from Model	Residual	RMSE from model
α-helicity (gel) – univariate with Rosetta Score	34.7	41.6	6.9	1.0
Increase in Structure (%) – univariate with Rosetta Score	5.9	5.6	0.3	1.0

Supporting Information References

(1) Britton, D.; Christians, L. F.; Liu, C.; Legocki, J.; Xiao, Y.; Meleties, M.; Yang, L.; Cammer, M.; Jia, S.; Zhang, Z.; et al. Computational Prediction of Coiled–Coil Protein Gelation Dynamics and Structure. *Biomacromolecules* **2024**, *25* (1), 258-271. DOI: 10.1021/acs.biomac.3c00968