SUPPORTING INFORMATION

High-throughput microrheology for the assessment of protein gelation kinetics

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Sigmoidal fitting criteria. Relaxation exponents were fit to sigmoidal curves (**Equation 1**) to determine the inflection point and time to gelation for each condition, with max_a and min_a being the maximum and minimum relaxation exponent during the gelation process, k being a kinetic gelation constant [h^{-1}], and t_{gel} being the time to gelation [h].

$$a = max_a - (max_a - min_a)\,\frac{1}{1+\exp\left(-k\left(t-t_{gel}\right)\right)} \qquad \textbf{Equation 1}$$

Constraints were set such that the fit parameters had physically relevant constants. The minimum relaxation exponent was restricted to the range of 0-0.2, to account for the final equilibrated relaxation exponent of the gel. Conversely, the maximum relaxation exponent was given an upper limit of 1.05 or 1.05x of the highest observed relaxation exponent, in order to account for any bulk motion/stage drift that may inflate the recorded relaxation exponents. The only restriction on k and t_{gel} was that they be positive values.

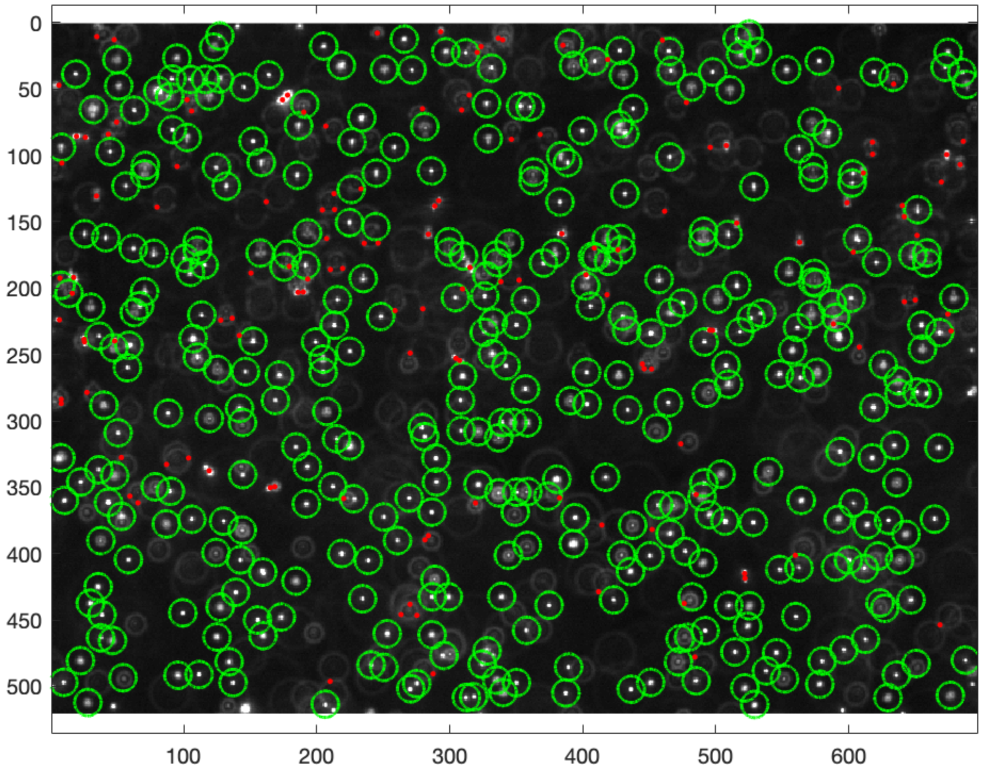

Figure S1. Confirmation of particles that meet user-defined thresholds for MPT analysis. Particles circled in green were considered in the tracking algorithm, while red particles were rejected for analysis. Axes indicate position in frame [pixels].

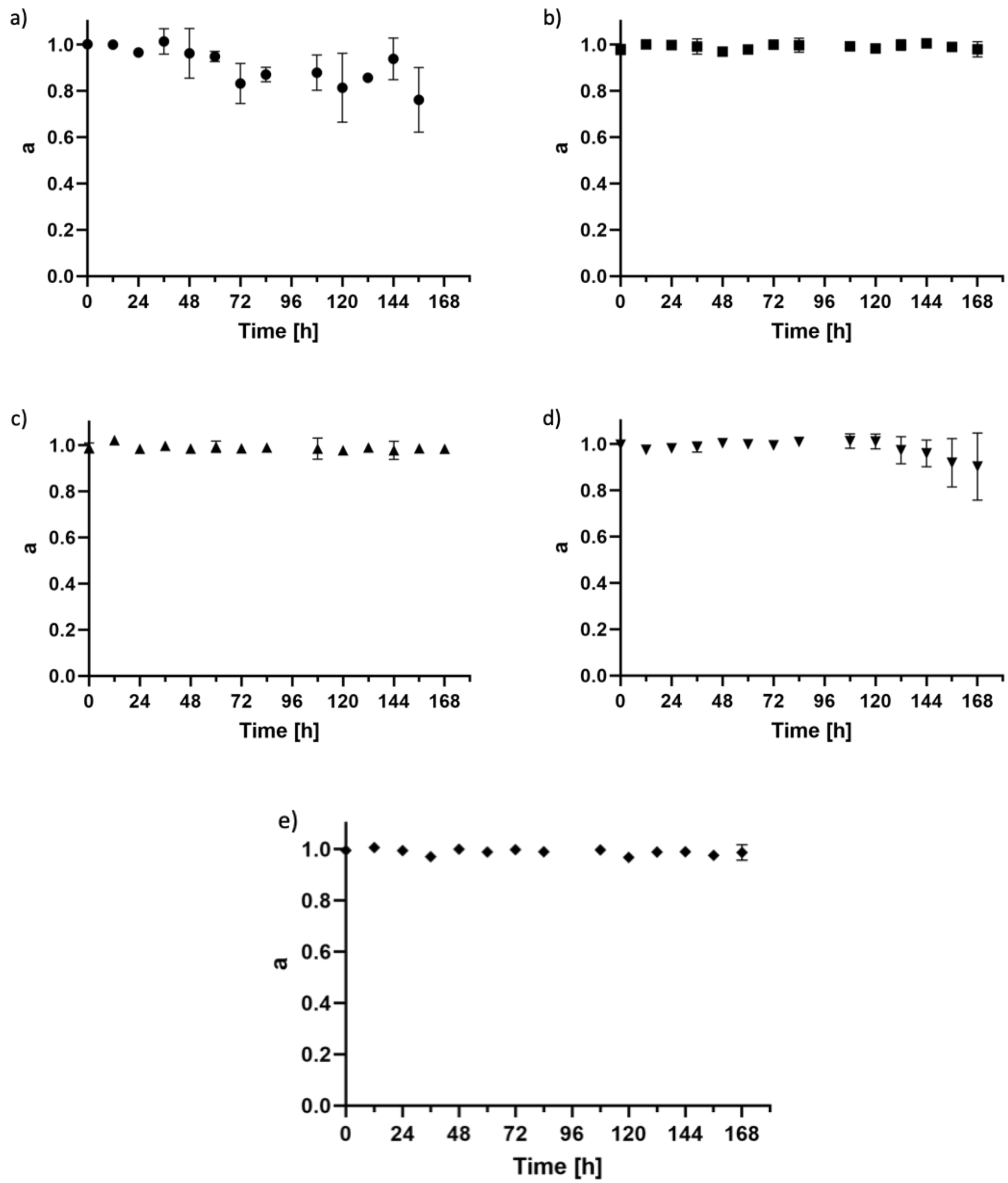

Figure S2. Relaxation exponents for Q at pH 6 over a one-week period for (a-e) 250, 375, 500, 750, and 1000 mM NaCl, respectively. Constant relaxation exponents of ~1 indicate that no gelation or change in viscoelastic behavior occurs for Q at pH 6. Error bars, which represent standard deviation, are shown for an average of three trials; points without error bars had standard deviations that were too small to be shown.

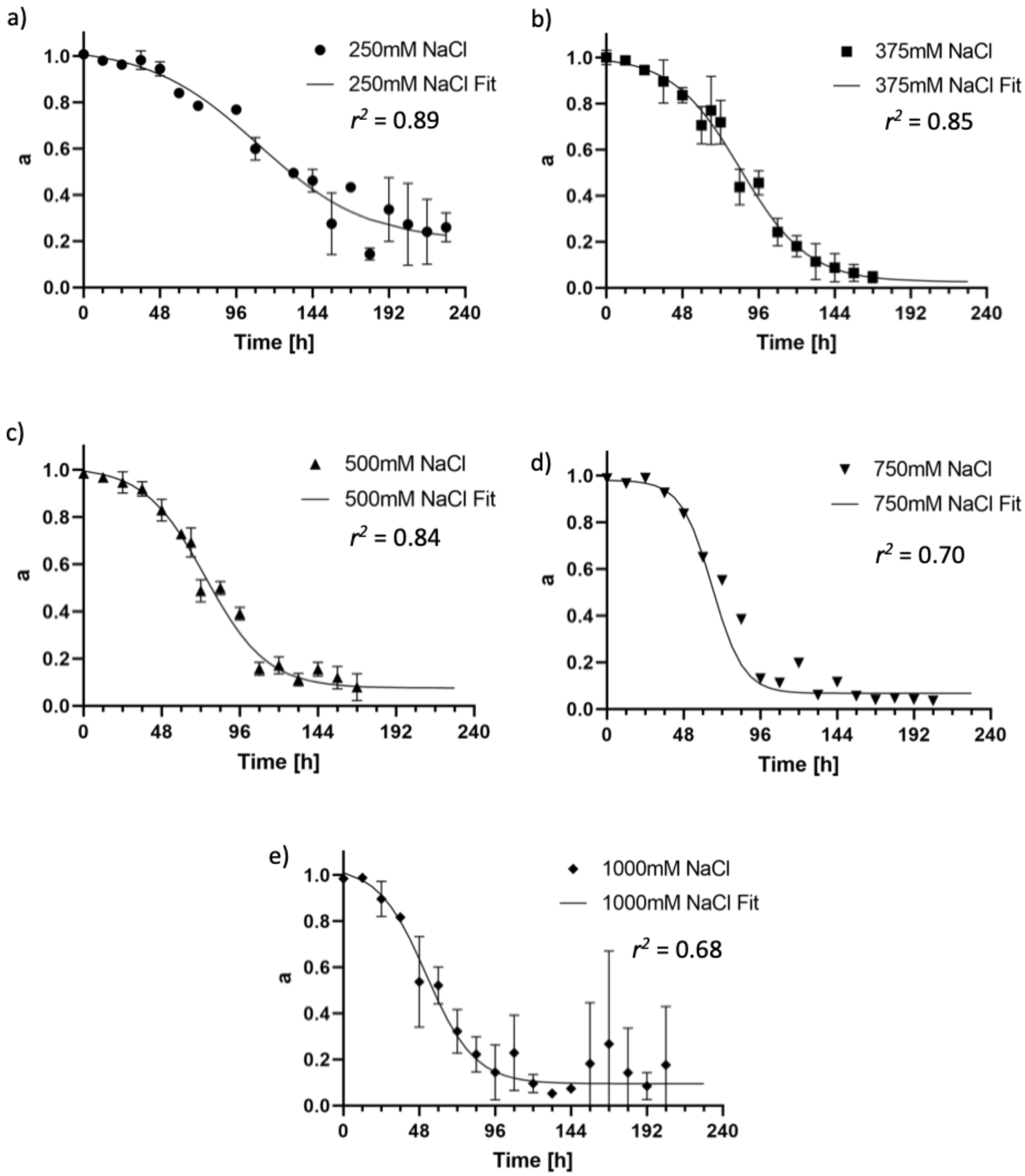

Figure S3. Relaxation exponents for Q at pH 7.4 over time for (a-e) 250, 375, 500, 750, and 1000 mM NaCl, respectively. Relaxation exponents were fit to sigmoidal curves, indicated by the solid line. Representative plots are shown for (d), while averages of three trials are shown for (a-c, e). Error bars, which represent standard deviation, are shown for an average of three trials; points without error bars had standard deviations that were too small to be shown.

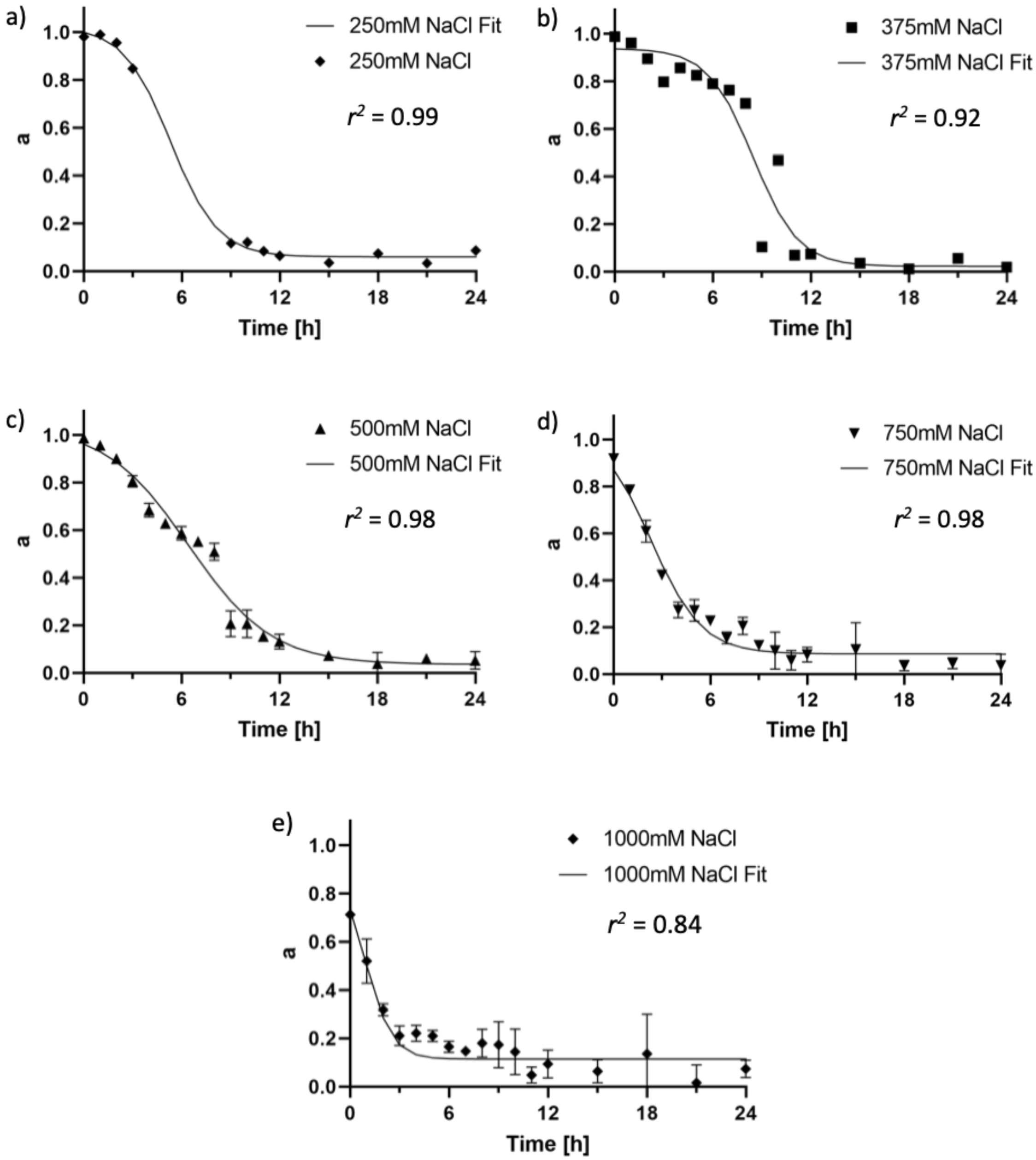

Figure S4. Relaxation exponents for Q at pH 10 over time for (a-e) 250, 375, 500, 750, and 1000 mM NaCl, respectively. Relaxation exponents were fit to sigmoidal curves, indicated by the solid line. Representative plots are shown for (a, b), while averages of three trials are shown for (c-e). Error bars, which represent standard deviation, are shown for an average of three trials; points without error bars had standard deviations that were too small to be shown.

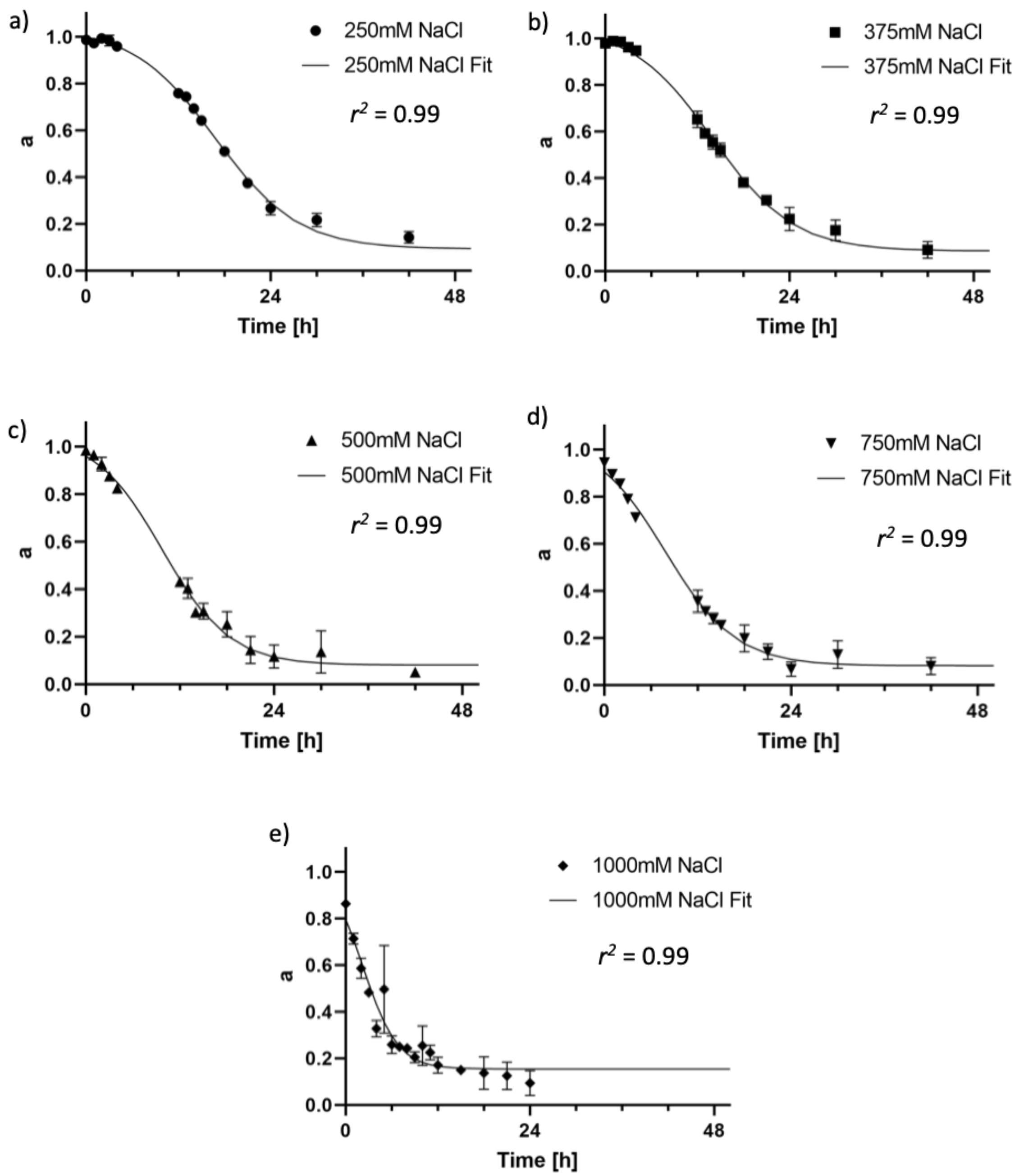

Figure S5. Relaxation exponents for Q at pH 11 over time for (a-e) 250, 375, 500, 750, and 1000 mM NaCl, respectively. Relaxation exponents were fit to sigmoidal curves, indicated by the solid line. Error bars, which represent standard deviation, are shown for an average of three trials; points without error bars had standard deviations that were too small to be shown.

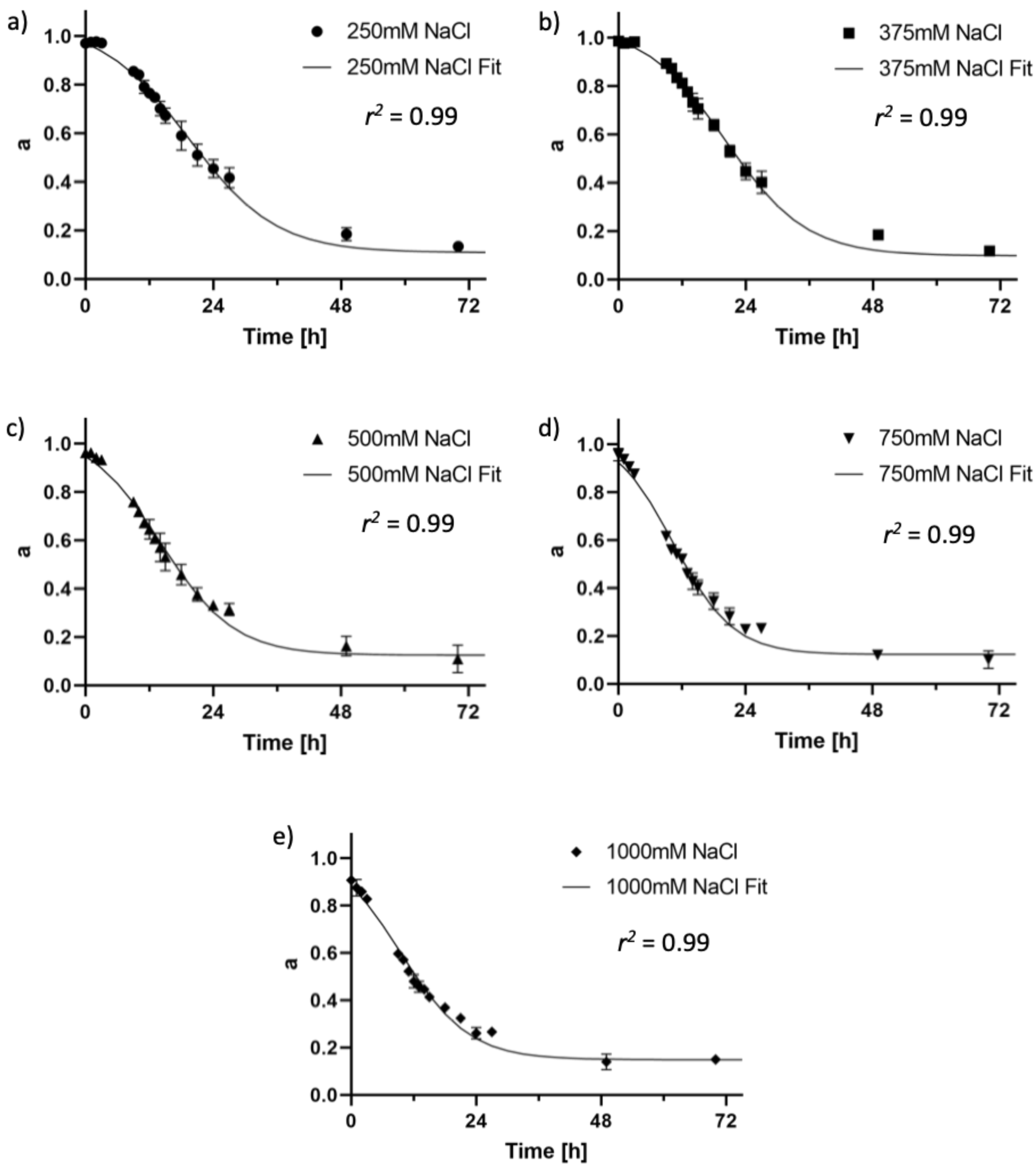

Figure S6. Relaxation exponents for Q at pH 12 over time for (a-e) 250, 375, 500, 750, and 1000 mM NaCl, respectively. Relaxation exponents were fit to sigmoidal curves, indicated by the solid line. Error bars, which represent standard deviation, are shown for an average of three trials; points without error bars had standard deviations that were too small to be shown.

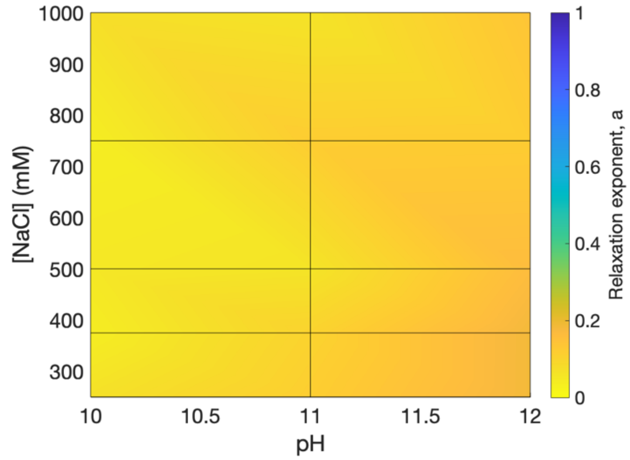

Figure S7. Surface plot of relaxation exponents at 48h for MSDs determined through MPT for all ionic strengths studied for Q at pH 10-12. Warm colors representing relaxation exponents < 0.25 indicate gelation of each condition shown.

Table S1. Critical relaxation exponents determined through time-cure superposition for Q at different combinations of pH and NaCl concentration.

pH	**[NaCl] (mM)**	n_c
7.4	250	0.69 ± 0.05
	375	0.61 ± 0.00
	500	0.62 ± 0.02
	750	0.66 ± 0.04
	1000	0.62 ± 0.11
10	250	0.44 ± 0.03
	375	0.44 ± 0.03
	500	0.52 ± 0.02
	750	0.49 ± 0.02
	1000	0.40 ± 0.03
11	250	0.54 ± 0.01
	375	0.54 ± 0.03
	500	0.51 ± 0.04
	750	0.51 ± 0.01
	1000	0.45 ± 0.02
12	250	0.61 ± 0.04
	375	0.63 ± 0.03
	500	0.54 ± 0.02
	750	0.55 ± 0.02
	1000	0.57 ± 0.00

Table S2. Summary of fitted parameters for relaxation exponents modeled with sigmoidal curves.

pH	[NaCl] (mM)	t_{gel} [h]	k [h^{-1}]	max_a	min_a	r^2
7.4	250	109.6 ± 3.9	0.029 ± 0.005	1.04 ± 0.01	0.20 ± 0.00	0.89 ± 0.05
	375	84.4 ± 4.3	0.047 ± 0.004	1.01 ± 0.03	0.03 ± 0.05	0.85 ± 0.10
	500	75.0 ± 4.7	0.053 ± 0.009	1.01 ± 0.01	0.08 ± 0.06	0.84 ± 0.08
	750	70.1 ± 3.8	4.060 ± 4.613	0.96 ± 0.02	0.16 ± 0.08	0.71 ± 0.05
	1000	52.4 ± 10.7	0.066 ± 0.023	1.04 ± 0.02	0.09 ± 0.09	0.68 ± 0.08
10	250	6.9 ± 1.8	4.624 ± 7.035	1.01 ± 0.04	0.09 ± 0.03	0.95 ± 0.07
	375	8.3 ± 0.1	2.950 ± 2.000	0.93 ± 0.01	0.06 ± 0.03	0.93 ± 0.02
	500	6.4 ± 0.2	0.393 ± 0.037	1.04 ± 0.02	0.04 ± 0.01	0.98 ± 0.00
	750	2.3 ± 0.0	0.636 ± 0.057	1.05 ± 0.00	0.09 ± 0.01	0.98 ± 0.02
	1000	0.9 ± 0.6	1.215 ± 0.959	0.94 ± 0.19	0.11 ± 0.04	0.84 ± 0.10
11	250	16.8 ± 0.3	0.187 ± 0.020	1.04 ± 0.02	0.09 ± 0.02	0.99 ± 0.00
	375	14.0 ± 0.3	0.191 ± 0.009	1.05 ± 0.00	0.09 ± 0.02	0.99 ± 0.01
	500	9.8 ± 0.3	0.232 ± 0.009	1.05 ± 0.00	0.08 ± 0.04	0.99 ± 0.01
	750	7.8 ± 0.3	0.221 ± 0.003	1.05 ± 0.00	0.08 ± 0.02	0.99 ± 0.01
	1000	2.1 ± 0.3	0.434 ± 0.070	1.05 ± 0.00	0.15 ± 0.03	0.99 ± 0.07
12	250	19.3 ± 1.8	0.122 ± 0.012	1.05 ± 0.00	0.11 ± 0.02	0.99 ± 0.00
	375	20.2 ± 0.8	0.131 ± 0.007	1.05 ± 0.00	0.10 ± 0.02	0.99 ± 0.00
	500	14.2 ± 0.5	0.146 ± 0.009	1.05 ± 0.00	0.13 ± 0.02	0.99 ± 0.00
	750	10.4 ± 0.3	0.178 ± 0.006	1.05 ± 0.00	0.12 ± 0.03	0.99 ± 0.00
	1000	9.5 ± 0.3	0.153 ± 0.006	1.05 ± 0.00	0.15 ± 0.02	0.99 ± 0.00

Table S3. Statistical analysis using two-way ANOVA of gelation times determined by MPT-sig, MPT-tc, and DDM. Conditions that were not shown to be statistically significantly different are indicated (n.s.) ($p > 0.050$).

		p-values		
pH	**[NaCl] (mM)**	**MPT-sig vs. MPT-tc**	**MPT-sig vs. DDM**	**MPT-tc vs. DDM**
7.4	250	> 0.999, n.s.	> 0.999, n.s.	> 0.999, n.s.
	375	> 0.999, n.s.	> 0.999, n.s.	> 0.999, n.s.
	500	> 0.999, n.s.	> 0.999, n.s.	> 0.999, n.s.
	750	> 0.999, n.s.	> 0.999, n.s.	> 0.999, n.s.
	1000	> 0.999, n.s.	> 0.999, n.s.	> 0.999, n.s.
10	250	> 0.999, n.s.	0.976, n.s.	> 0.999, n.s.
	375	> 0.999, n.s.	> 0.999, n.s.	0.712, n.s.
	500	> 0.999, n.s.	0.010	0.086, n.s.
	750	> 0.999, n.s.	> 0.999, n.s.	> 0.999, n.s.
	1000	> 0.999, n.s.	> 0.999, n.s.	0.990, n.s.
11	250	> 0.999, n.s.	> 0.999, n.s.	> 0.999, n.s.
	375	> 0.999, n.s.	> 0.999, n.s.	> 0.999, n.s.
	500	> 0.999, n.s.	> 0.999, n.s.	> 0.999, n.s.
	750	> 0.999, n.s.	> 0.999, n.s.	> 0.999, n.s.
	1000	> 0.999, n.s.	> 0.999, n.s.	> 0.999, n.s.
12	250	> 0.999, n.s.	> 0.999, n.s.	> 0.999, n.s.
	375	> 0.999, n.s.	> 0.999, n.s.	> 0.999, n.s.
	500	> 0.999, n.s.	> 0.999, n.s.	> 0.999, n.s.
	750	> 0.999, n.s.	0.329, n.s.	0.335, n.s.
	1000	> 0.999, n.s.	0.039	0.084, n.s.

Table S4. Non-Gaussian parameters (*N*) for Q at different pH and ionic strengths pre- (0 h) and post- (final timepoint) gelation for a lag time of 1.1 s. *For Q at pH 6, which did not form a hydrogel, initial (0 h) and final (168 h) non-Gaussian parameters are reported.

		***N* (τ = 1.1 s)**	
pH	**[NaCl] (mM)**	**Pre-Gelation (Initial)**	**Post-Gelation (Final)**
6*	250	0.022 ± 0.083	0.067 ± 0.096
	375	0.007 ± 0.035	0.001 ± 0.058
	500	0.053 ± 0.101	0.080 ± 0.010
	750	0.047 ± 0.021	0.022 ± 0.073
	1000	0.031 ± 0.024	0.040 ± 0.058
7.4	250	0.058 ± 0.012	0.376 ± 0.017
	375	0.116 ± 0.108	0.183 ± 0.027
	500	0.043 ± 0.037	0.299 ± 0.095
	750	0.039 ± 0.006	0.598 ± 0.189
	1000	0.040 ± 0.045	0.802 ± 0.232
10	250	0.024 ± 0.037	0.933 ± 0.078
	375	0.087 ± 0.014	0.893 ± 0.095
	500	0.046 ± 0.007	0.843 ± 0.080
	750	0.085 ± 0.090	0.800 ± 0.085
	1000	0.046 ± 0.078	0.896 ± 0.137
11	250	0.029 ± 0.076	0.459 ± 0.154
	375	0.061 ± 0.060	0.871 ± 0.317
	500	0.071 + 0.035	0.495 ± 0.146
	750	0.023 ± 0.055	0.606 ± 0.073
	1000	0.039 ± 0.033	0.600 ± 0.153
12	250	0.019 ± 0.088	0.276 ± 0.105
	375	0.104 ± 0.030	0.493 ± 0.148
	500	0.128 ± 0.072	0.557 ± 0.152
	750	0.012 ± 0.049	0.388 ± 0.037
	1000	0.077 ± 0.016	0.492 ± 0.052

Table S5. Times to gelation for Q at different pH and ionic strengths as determined through sigmoidal fitting of MPT-determined MSDs (MPT-sig), time-cure superposition of MPT-determined MSDs (MPT-tc), and DDM.

pH	[NaCl] (mM)	t_{gel} [h] (MPT-sig)	t_{gel} [h] (MPT-tc)	t_{gel} [h] (DDM)
7.4	250	109.6 ± 3.9	109.0 ± 4.2	102.0 ± 6.0
	375	84.4 ± 4.3	83.0 ± 4.2	78.0 ± 6.0
	500	75.0 ± 4.7	71.3 ± 6.1	66.0 ± 6.0
	750	70.1 ± 3.8	79.3 ± 13.7	96.0 ± 20.8
	1000	52.4 ± 10.7	50.7 ± 11.0	52.0 ± 6.9
10	250	6.9 ± 1.8	7.3 ± 1.4	8.0 ± 0.0
	375	8.3 ± 0.1	8.3 ± 0.2	8.0 ± 0.0
	500	6.4 ± 0.2	7.1 ± 0.2	9.7 ± 2.3
	750	2.3 ± 0.0	2.6 ± 0.2	3.0 ± 1.0
	1000	0.9 ± 0.6	1.3 ± 0.2	0.3 ± 0.6
11	250	16.8 ± 0.3	16.8 ± 0.3	20.0 ± 4.6
	375	14.0 ± 0.3	14.5 ± 0.0	14.0 ± 1.0
	500	9.8 ± 0.3	9.7 ± 0.3	11.7 ± 7.1
	750	7.8 ± 0.3	7.8 ± 0.3	4.0 ± 0.0
	1000	2.1 ± 0.3	3.9 ± 0.2	4.0 ± 1.7
12	250	19.3 ± 1.8	19.2 ± 1.0	21.0 ± 0.0
	375	20.2 ± 0.8	19.8 ± 0.6	22.0 ± 4.6
	500	14.2 ± 0.5	14.5 ± 0.0	14.0 ± 0.0
	750	10.4 ± 0.3	10.4 ± 0.2	17.0 ± 8.9
	1000	9.5 ± 0.3	10.3 ± 0.1	18.7 ± 5.0