Electronic Supplementary Material (ESI) for Soft Matter.
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Characterizing rheological properties and microstructure of thioester networks during degradation

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Supporting Information

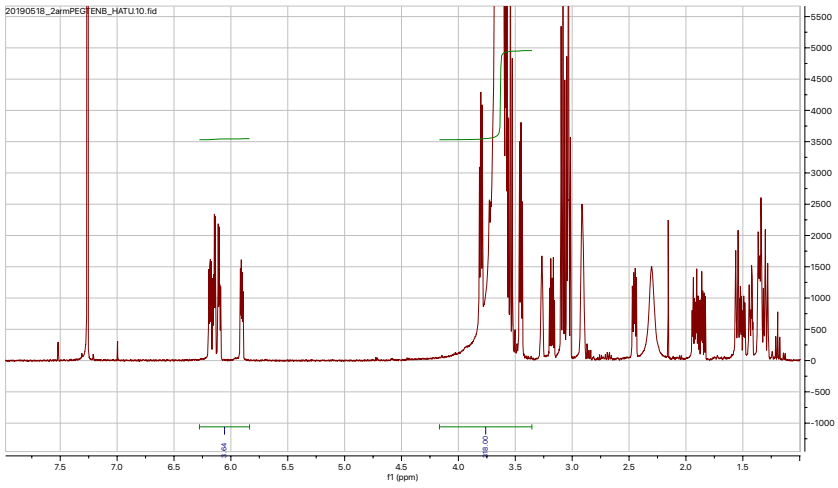

Fig. S1: Nuclear magnetic resonance spectrum of 3.5 kDa Poly(ethylene glycol)-thioester norbornene in $CDCl_3$ (400 Hz) δ 6.29–5.82 (m, 2H), 3.99–3.42 (m, 1818H)

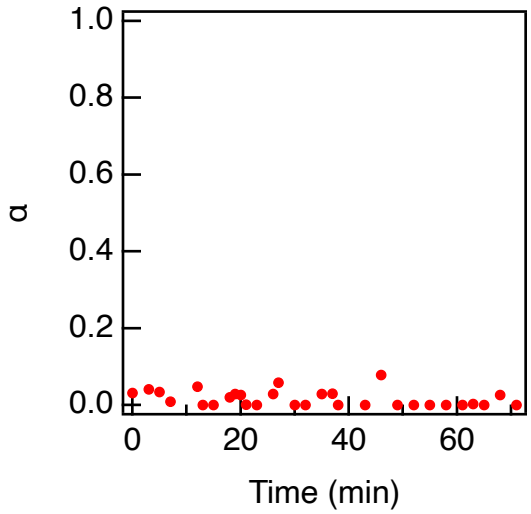

Fig. S2: Networks with 50% excess thiol degraded with 0.1 M L-cysteine. This network does not degrade in this concentration of L-cysteine solution.

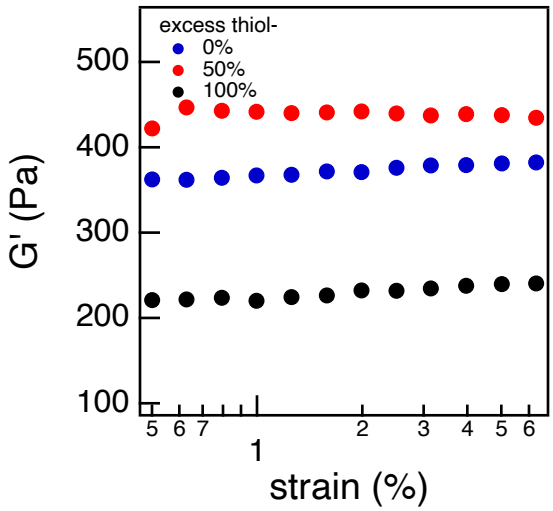

Fig. S3: Strain sweeps of PEG-thioester norbornene CANs. Strain sweeps are used to identify the linear viscoelastic regime. A strain sweep is used to measure all scaffolds between 0.5% and 10% strain at a frequency of 1 Hz. From these measurements, 1% strain is chosen for frequency sweep measurements in the linear viscoelastic regime.

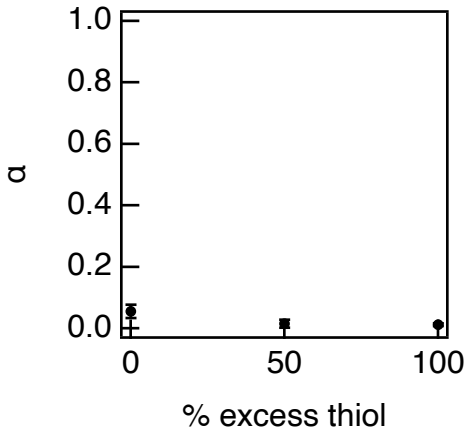

Fig. S4: The values of the logarithmic slope of the MSD, $\alpha = \frac{d \log \langle \Delta r^2(\tau) \rangle}{d \log \tau}$, for each network composition before incubating in L-cysteine solution. $\alpha \approx 0$ for all network compositions which is a measure of particles completely arrested in a gel network.

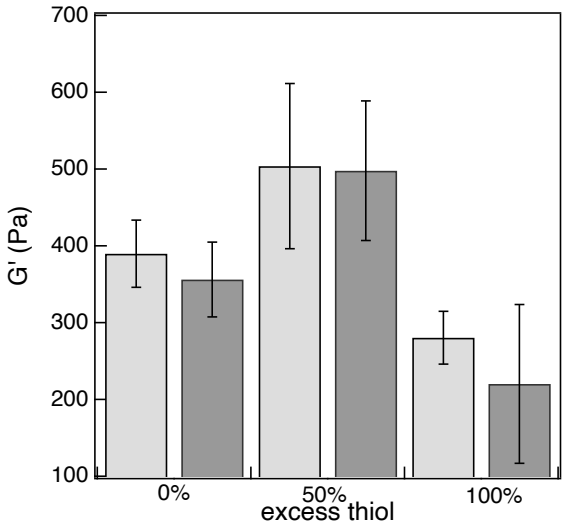

Fig. S5: Measurements of each CAN composition before and after incubation in 1× PBS for 3 *hours*. Light gray bars are the elastic moduli of unswollen hydrogels and dark gray bars are the elastic moduli of thioester networks after incubating for 3 *hrs* in 1× PBS. The elastic modulus, G', of thioester networks does not change.

Time-cure superposition

Networks with 0% excess thiol

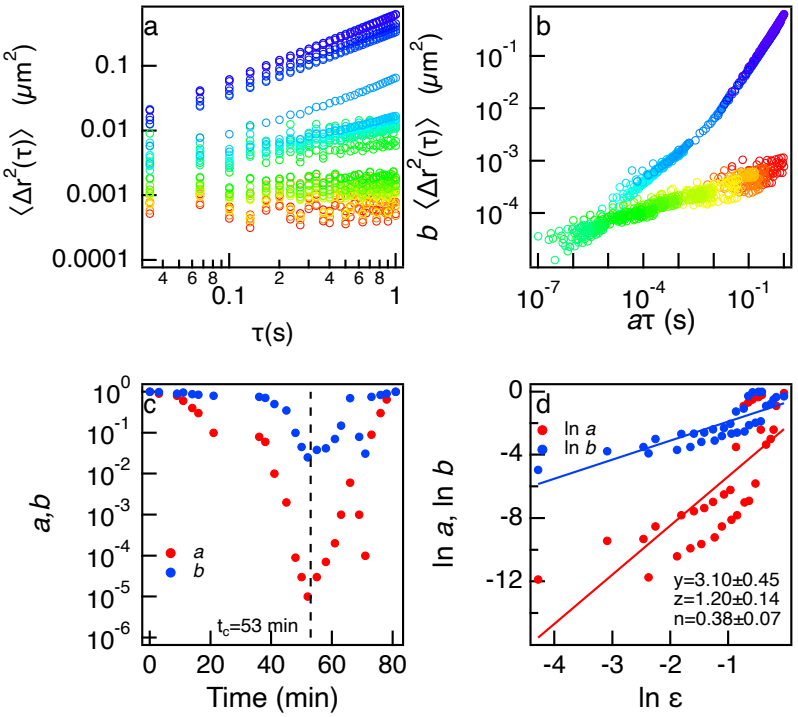

Fig. S6: Time-cure superposition of PEG-thioester networks with 0% excess thiol (second replicate experiment). (a) MSDs are measured during degradation of networks with 0% excess thiol. (b) MSDs are shifted into sol and gel master curves using shift factors a and b. (c) Shift factors a and b approach zero at $t_c = 53\ mins$. (d) y and z are calculated by plotting the logarithm of shift factors a and b against the logarithm of distance away from critical degradation time, $\epsilon = \frac{|t-t_c|}{t_c}$. The critical relaxation exponent, n, is calculated from scaling exponents y and z.

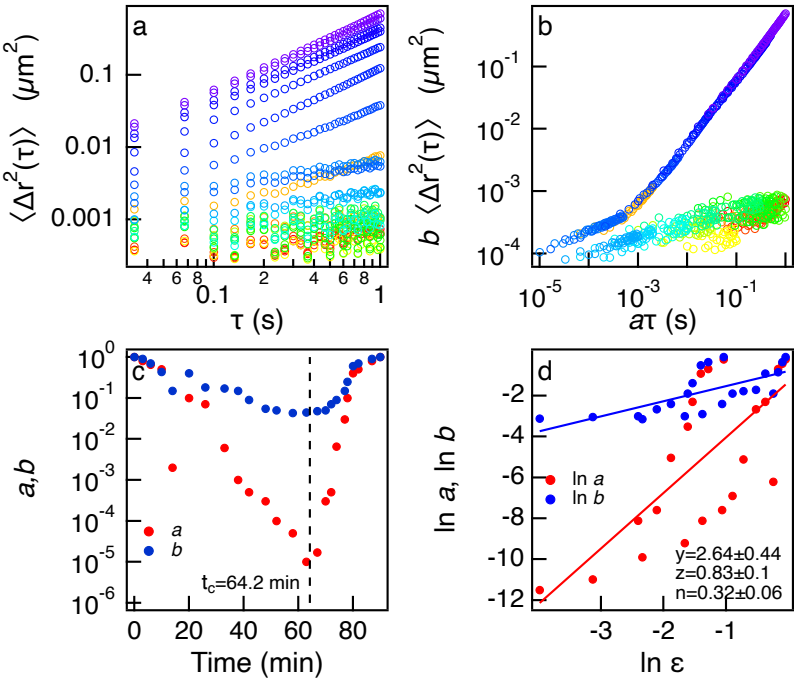

Fig. S7: Time-cure superposition of PEG-thioester networks with 0% excess thiol (third replicate experiment). (a) MSDs are measured during degradation of networks with 0% excess thiol. (b) MSDs are shifted into sol and gel master curves using shift factors a and b. (c) Shift factors a and b approach zero at $t_c = 64.2$ *mins*. (d) y and z are calculated by plotting the logarithm of shift factors a and b against the logarithm of distance away from critical degradation time, ϵ. The critical relaxation exponent, n, is calculated from scaling exponents y and z.

Networks with 50% excess thiol

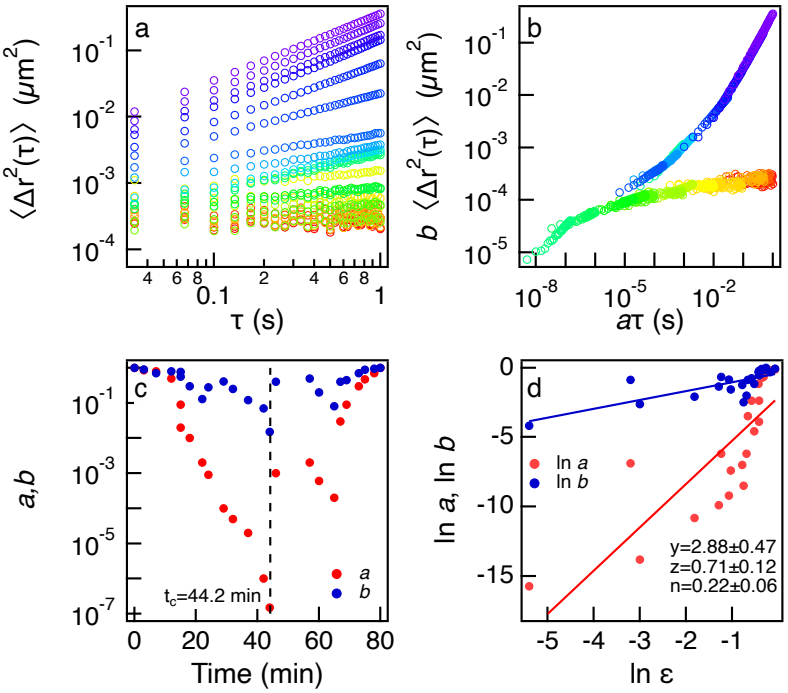

Fig. S8: Time-cure superposition of PEG-thioester networks with 50% excess thiol (first replicate experiment). (a) MSDs are measured during degradation of networks with 50% excess thiol with particles coated in BSA. (b) MSDs are shifted into sol and gel master curves using shift factors a and b. (c) Shift factors a and b approach zero at $t_c = 44.2$ *mins*. (d) y and z are calculated by plotting the logarithm of shift factors a and b against the logarithm of distance away from critical degradation time, ϵ. The critical relaxation exponent, n, is calculated from scaling exponents y and z.

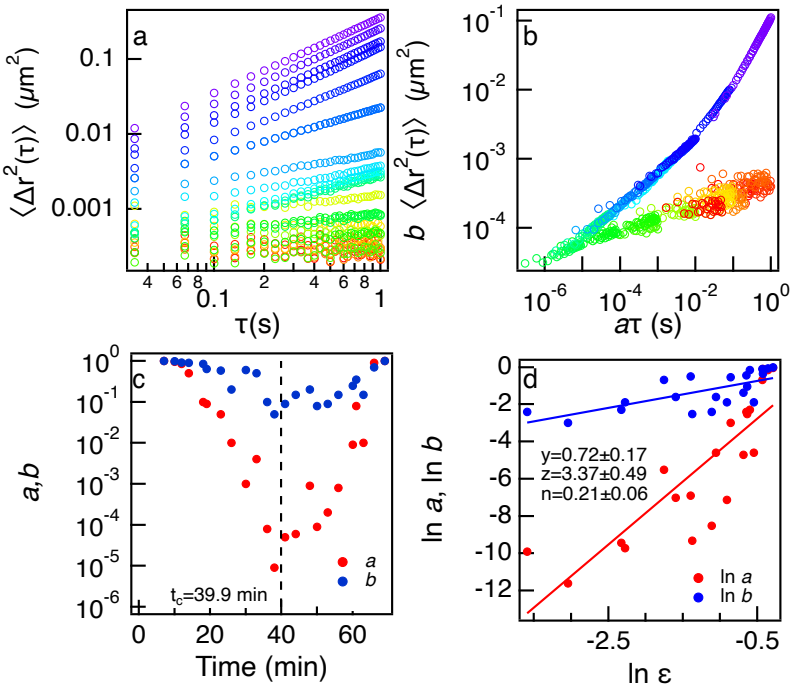

Fig. S9: Time-cure superposition of PEG-thioester networks with 50% excess thiol (second replicate experiment). (a) MSDs are measured during degradation of networks with 50% excess thiol with particles coated in BSA. (b) MSDs are shifted into sol and gel master curves using shift factors a and b. (c) Shift factors a and b approach zero at $t_c = 39.9$ $mins$. (d) y and z are calculated by plotting the logarithm of shift factors a and b against the logarithm of distance away from critical degradation time, ϵ. The critical relaxation exponent, n, is calculated from scaling exponents y and z.

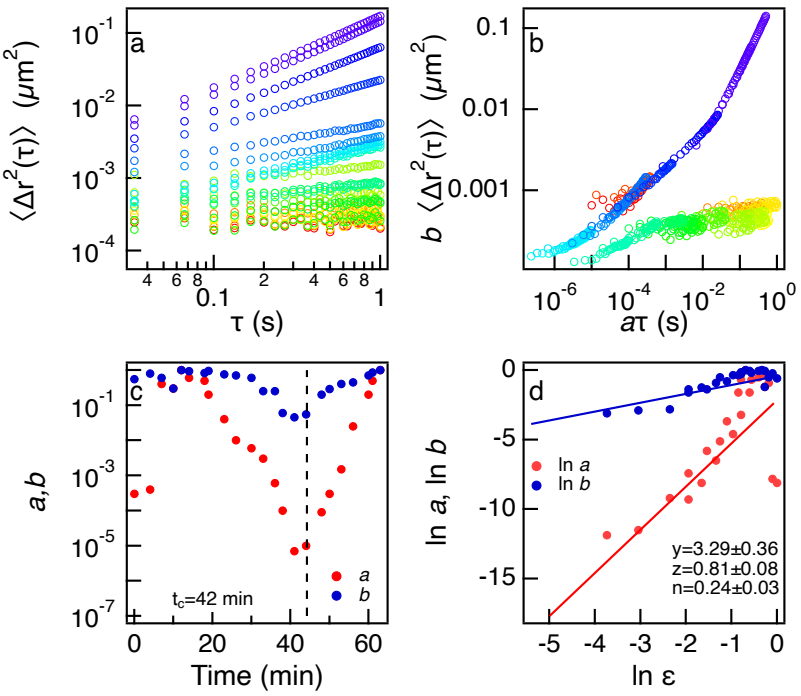

Fig. S10: Time-cure superposition of PEG-thioester networks with 50% excess thiol (third replicate experiment). (a) MSDs are measured during degradation of networks with 50% excess thiol with PEGylated particles. (b) MSDs are shifted into sol and gel master curves using shift factors a and b. (c) Shift factors a and b approach zero at $t_c = 42\ mins$. (d) y and z are calculated by plotting the logarithm of shift factors a and b against the logarithm of distance away from critical degradation time, ϵ. The critical relaxation exponent, n, is calculated from scaling exponents y and z.

Networks with 100% excess thiol

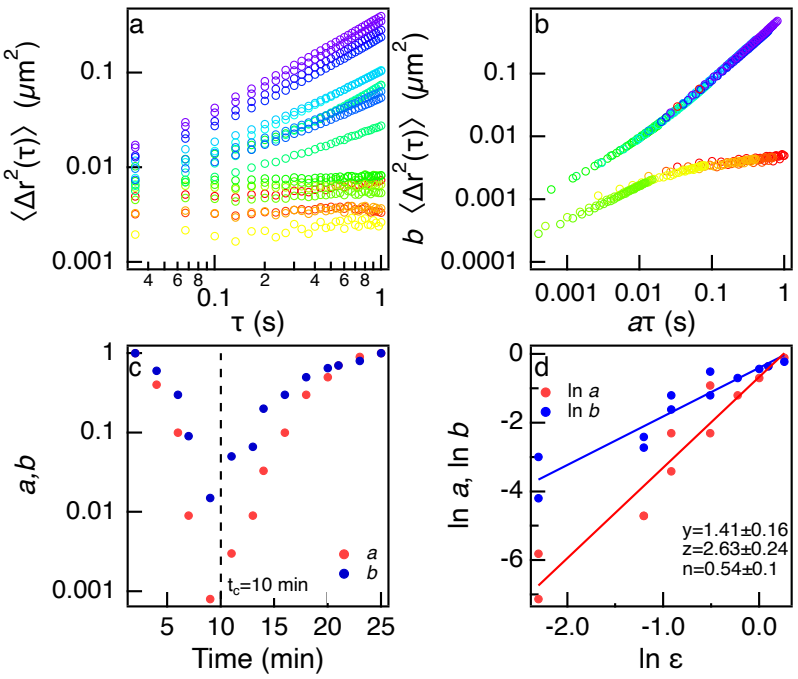

Fig. S11: Time-cure superposition of PEG-thioester networks with 100% excess thiol (first replicate experiment). (a) MSDs are measured during degradation of networks with 100% excess thiol. (b) MSDs are shifted into sol and gel master curves using shift factors a and b. (c) Shift factors a and b approach zero at $t_c = 10\ mins$. (d) y and z are calculated by plotting the logarithm of shift factors a and b against the logarithm of distance away from critical degradation time, ϵ. The critical relaxation exponent, n, is calculated from scaling exponents y and z.

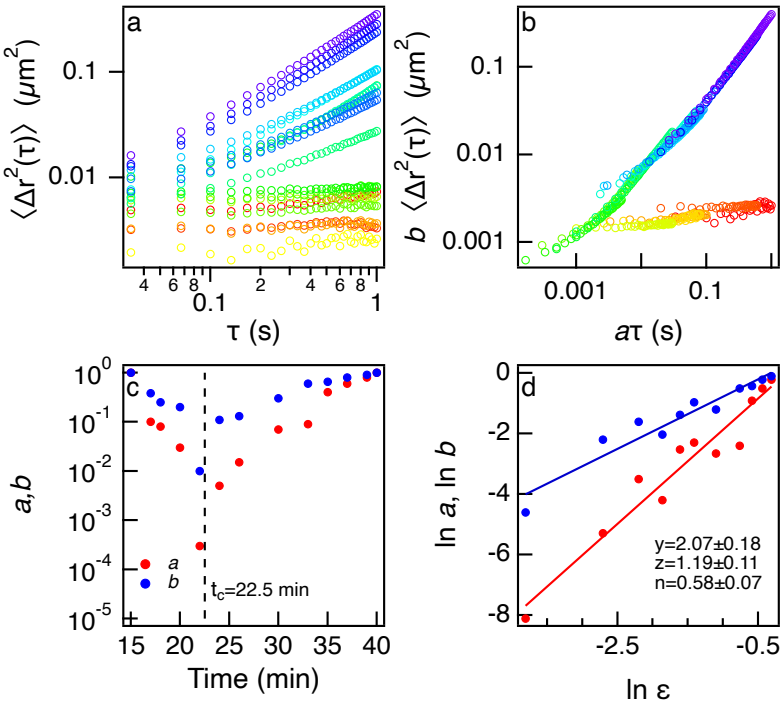

Fig. S12: Time-cure superposition of PEG-thioester networks with 100% excess thiol (second replicate experiment). (a) MSDs are measured during degradation of networks with 100% excess thiol. (b) MSDs are shifted into sol and gel master curves using shift factors a and b. (c) Shift factors a and b approach zero at $t_c = 22.5\ mins$. (d) y and z are calculated by plotting the logarithm of shift factors a and b against the logarithm of distance away from critical degradation time, ϵ. The critical relaxation exponent, n, is calculated from scaling exponents y and z.

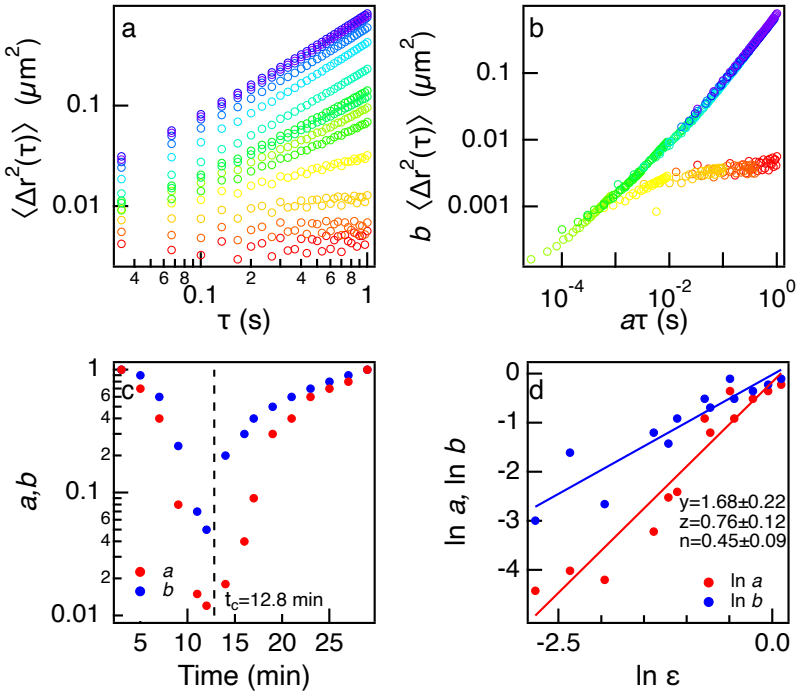

Fig. S13: Time-cure superposition of PEG-thioester networks with 100% excess thiol (third replicate experiment). (a) MSDs are measured during the degradation of networks with 100% excess thiol. (b) MSDs are shifted into sol and gel master curves using shift factors a and b. (c) Shift factors a and b approach zero at $t_c = 12.8\ mins$. (d) y and z are calculated by plotting the logarithm of shift factors a and b against the logarithm of distance away from critical degradation time, ϵ. The critical relaxation exponent, n, is calculated from scaling exponents y and z.

Networks with 0% excess thiol degraded with a second concentration of L-cysteine

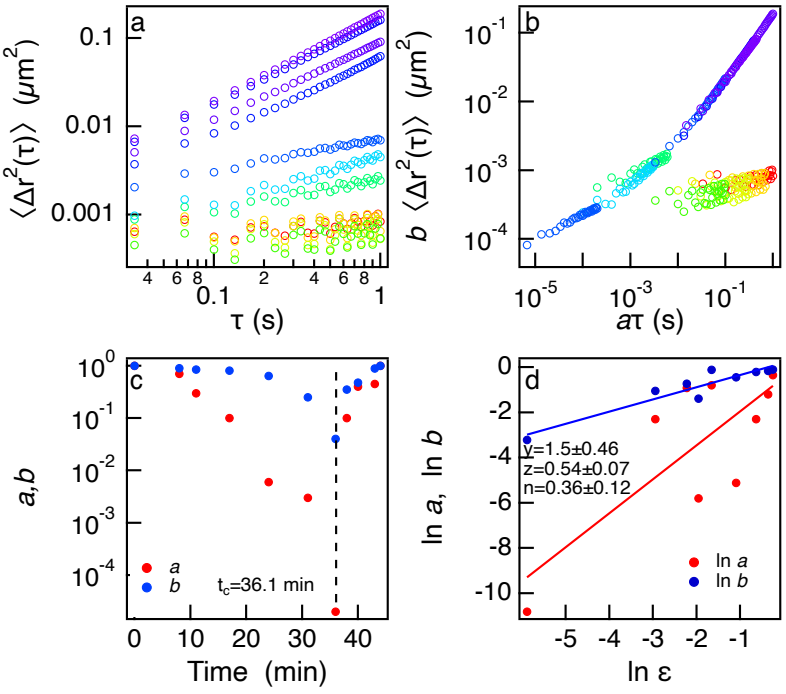

Fig. S14: TCS analysis of 0% excess thiol networks degraded with 0.2 M L-cysteine shows the value of n does not change when the concentration of L-cysteine is changed. (a) MSDs are measured during the degradation of networks with 0% excess thiol. (b) MSDs are shifted into sol and gel master curves using shift factors a and b. (c) Shift factors a and b approach zero at $t_c = 36.1$ $mins$. (d) y and z are calculated by plotting the logarithm of shift factors a and b against the logarithm of distance away from critical degradation time. The critical relaxation exponent, n, is calculated from the scaling exponents y and z.

MPT characterization of all three network compositions

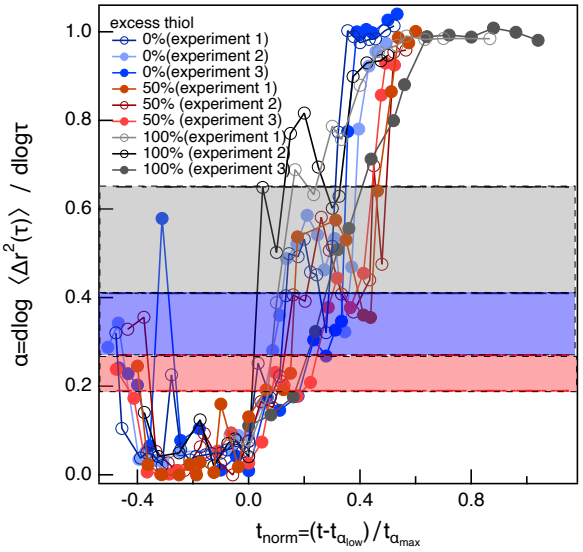

Fig. S15: Thioester networks with 0%, 50% and 100% excess thiol undergo rearrangement during degradation with L-cysteine. Network rearrangement in all three networks is compared by plotting the values of the logarithmic slope of the MSD, α, during degradation against normalized time, $t_{norm} = \frac{t - t_{\alpha_{low}}}{t_{\alpha_{max}}}$ where t is the time data are collected, $t_{\alpha_{low}}$ is the time when the value of α starts to increase and $t_{\alpha_{max}}$ is the time when α is a maximum.

Rheological and spatial heterogeneity analysis of MPT data of degradation of all three network compositions

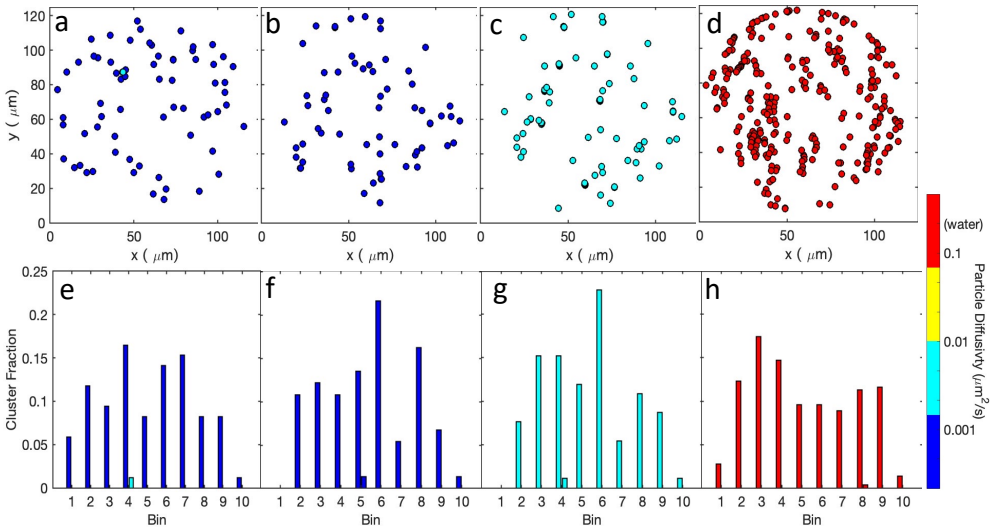

Fig. S16: Fig. S16 (a-d) Rheological and (e-h) spatial heterogeneity in the microenvironments particles are probing during degradation of 0% excess thiol. (a-d) Particle diffusivity is plotted at the starting position of the probe particle, color indicates the diffusivity. (e-h) Histograms of the diffusivity of probe particles along the x-axis, measuring spatial heterogeneity. Each bin is 12.5 μm in size. Each column of probe diffusivity and spatial heterogeneity is measured at a different time relative to the critical degradation time. This relative time, t_r, is calculated using $t_r = \frac{t-t_c}{t_c}$ where t is the time data are collected and t_c is the critical degradation time. (a, e) Data collected at $t_r = -0.32$, which is before degradation. Initial probe diffusivity is low and the system is rheologically and spatially homogeneous. (b, d) Data measured at $t_r = 0.23$, which is after t_c, where network rearrangement takes place. Since this is during rearrangement when a sample-spanning network is reformed, a lower value of probe diffusivity is measured. Both rheological and spatial heterogeneity are low. (c, g) Data collected after network rearrangement and before complete degradation at $t_r = 0.28$. There are a few particles in this data that have lower diffusivities and this is likely due to polymeric clusters that have not yet degraded. This sample has a small amount of rheological heterogeneity and is spatially homogeneous. (e, h) At $t_r = 0.45$, the sample has degraded completely. This sample is spatially and rheologically homogeneous.

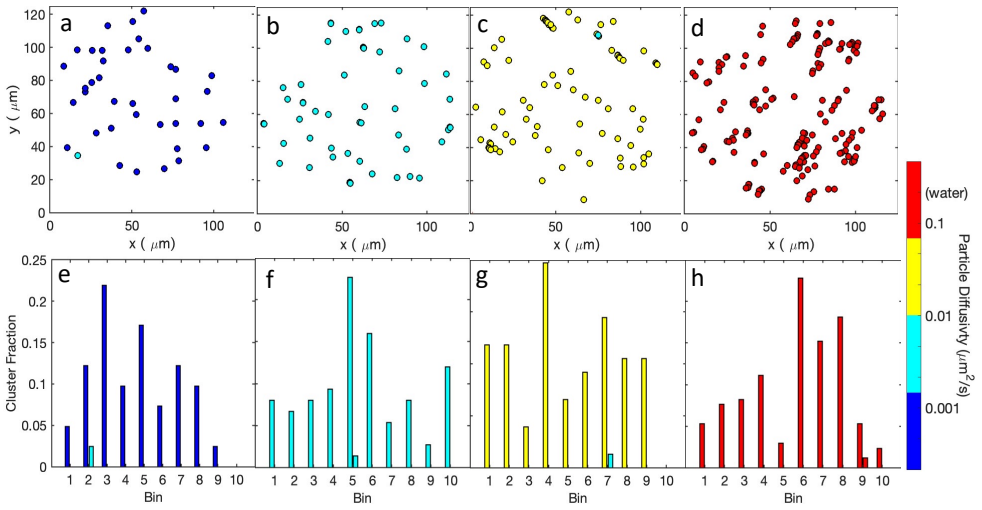

Fig. S17: (a-d) Rheological and (e-h) spatial heterogeneity in the microenvironments particles are probing during degradation of 50% excess thiol. (a-d) Particle diffusivity is plotted at the starting position of the probe particle, color indicates the diffusivity. (e-h) Histograms of the diffusivity of probe particles along the x-axis, measuring spatial heterogeneity. Each bin is 12.5 μm in size. Each column of probe diffusivity and spatial heterogeneity is measured at a different time relative to the critical degradation time. This relative time, t_r, is calculated using $t_r = \frac{t-t_c}{t_c}$ where t is the time data are collected and t_c is the critical degradation time. (a, e) Data collected at $t_r = -0.38$, which is before degradation. Initial probe diffusivity is low and the system is rheologically and spatially homogeneous. (b, d) Data measured at $t_r = 0.26$, which is after t_c, where network rearrangement takes place. Since this is during rearrangement when the material can reform polymer clusters but a sample-spanning network does not form, a moderate value of probe diffusivity is measured because these networks form sample-spanning networks. Both rheological and spatial heterogeneity is low. (c, g) Data collected after network rearrangement and before complete degradation at $t_r = -0.33$. There are a few particles in this data that have lower diffusivities and this is likely due to polymeric clusters that have not yet degraded. This sample has a small amount of rheological heterogeneity and is spatially homogeneous. (e, h) At $t_r = 0.5$, the sample has degraded completely. This sample is spatially and rheologically homogeneous.

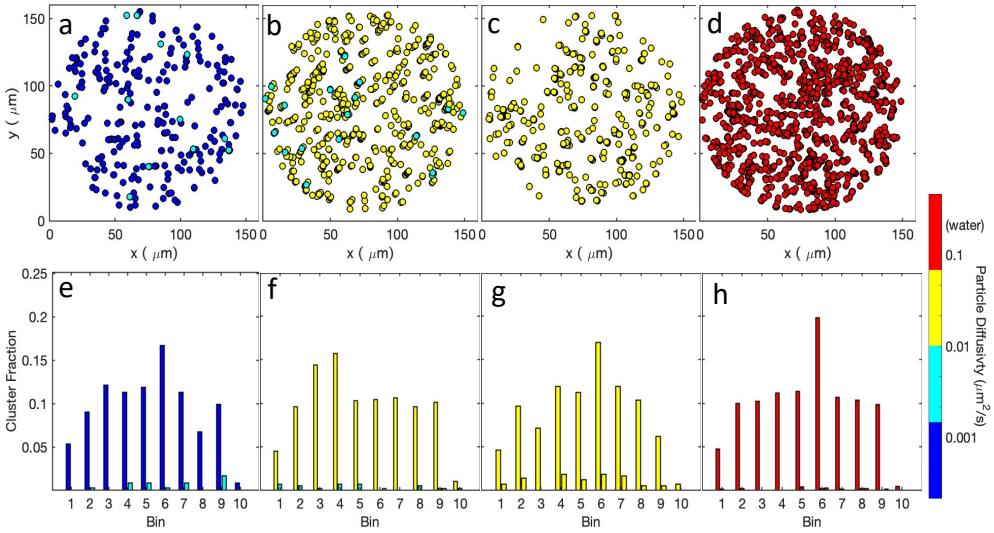

Fig. S18: (a-d) Rheological and (e-h) spatial heterogeneity in the microenvironments particles are probing during degradation of 100% excess thiol. (a-d) Particle diffusivity is plotted at the starting position of the probe particle, color indicates the diffusivity. (e-h) Histograms of the diffusivity of probe particles along the x-axis, measuring spatial heterogeneity. Each bin is 16 μm in size. Each column of probe diffusivity and spatial heterogeneity is measured at a different time relative to the critical degradation time. This relative time, t_r, is calculated using $t_r = \frac{t-t_c}{t_c}$ where t is the time data are collected and t_c is the critical degradation time. (a, e) Data collected at $t_r = -0.68$, which is before the degradation. Initial probe diffusivity is low. We do measure a small amount of rheological and spatial heterogeneity. This is likely due to the large amount of exchange reactions taking place. (b, d) Data measured at $t_r = 0.33$, which is after t_c, where network rearrangement takes place. Both rheological and spatial heterogeneity is measured but is relatively low. (c, g) Data collected after network rearrangement and before complete degradation at $t_r = 0.42$. This sample has no rheological or spatial heterogeneity. (e, h) At $t_r = 0.73$, the sample has degraded completely. This sample is spatially and rheologically homogeneous.

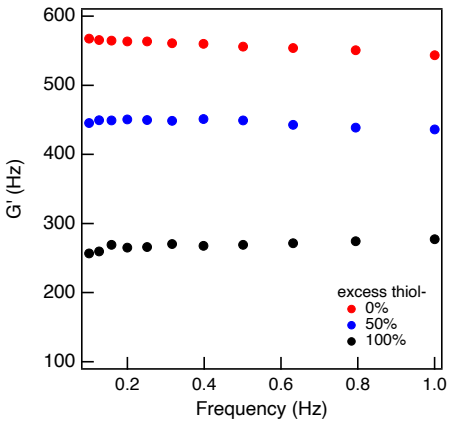

Fig. S19: Storage modulus, G', of thioester networks is measured using frequency sweep measurements at 1% strain.