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Supplementary Information for

Liquid to Solid Transition of Elastin Condensates

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This PDF file includes:

Supplementary Information Text

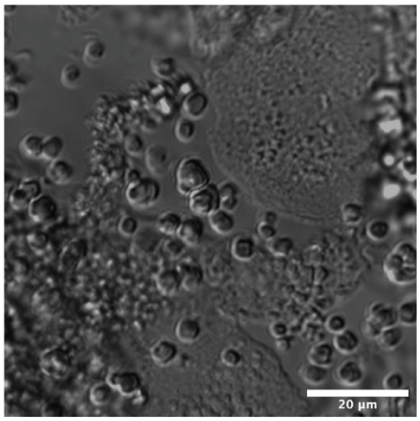

Fig. S1. DIC images of mature minielastin coacervates incubated overnight in solution containing 2%SDS/4M Urea. Sample was heated to 40 °C for 1 h before being left at room temperature overnight. Minielastin maturation was induced by concentrating protein to 230 µM and incubated overnight at 37 °C and stored at 4 °C before adding denaturant.

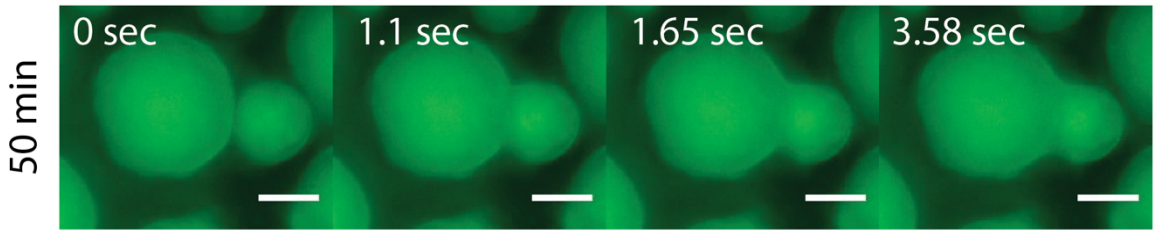

Fig. S2. Arrested fusion event of minielastin coacervate at 50 minutes of incubation time, scale bar 3 μm. Buffer conditions in all images 1.5M NaCl, 1 mM $CaCl_2$, 50 mM Tris, pH 7.4. Minielastin protein fluorescently labeled with Alexa Fluor 488. Protein concentration of 130 μM.

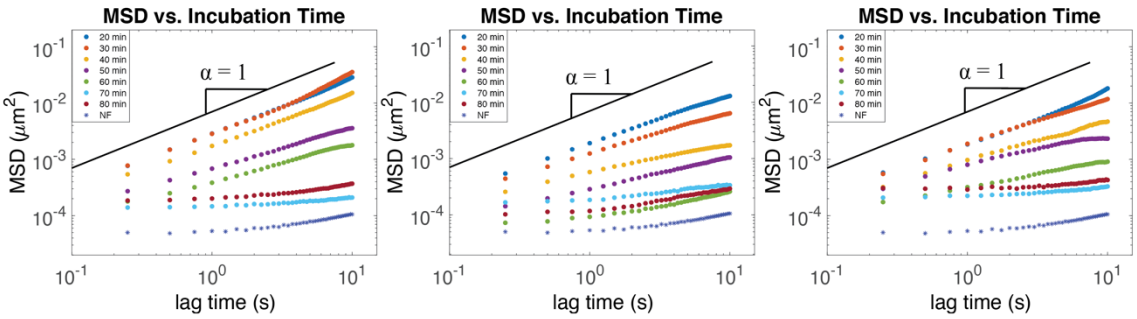

Fig. S3. Three independent experiments representing the decline of average mean square displacement (MSD) against lag time (A-C) of 100 nm beads within minielastin coacervates at increasing incubation times. Noise floor (NF) of confocal microscope represented with blue asterisk. Alpha (α) value represents diffusive exponent, black solid line represents slope of 1.

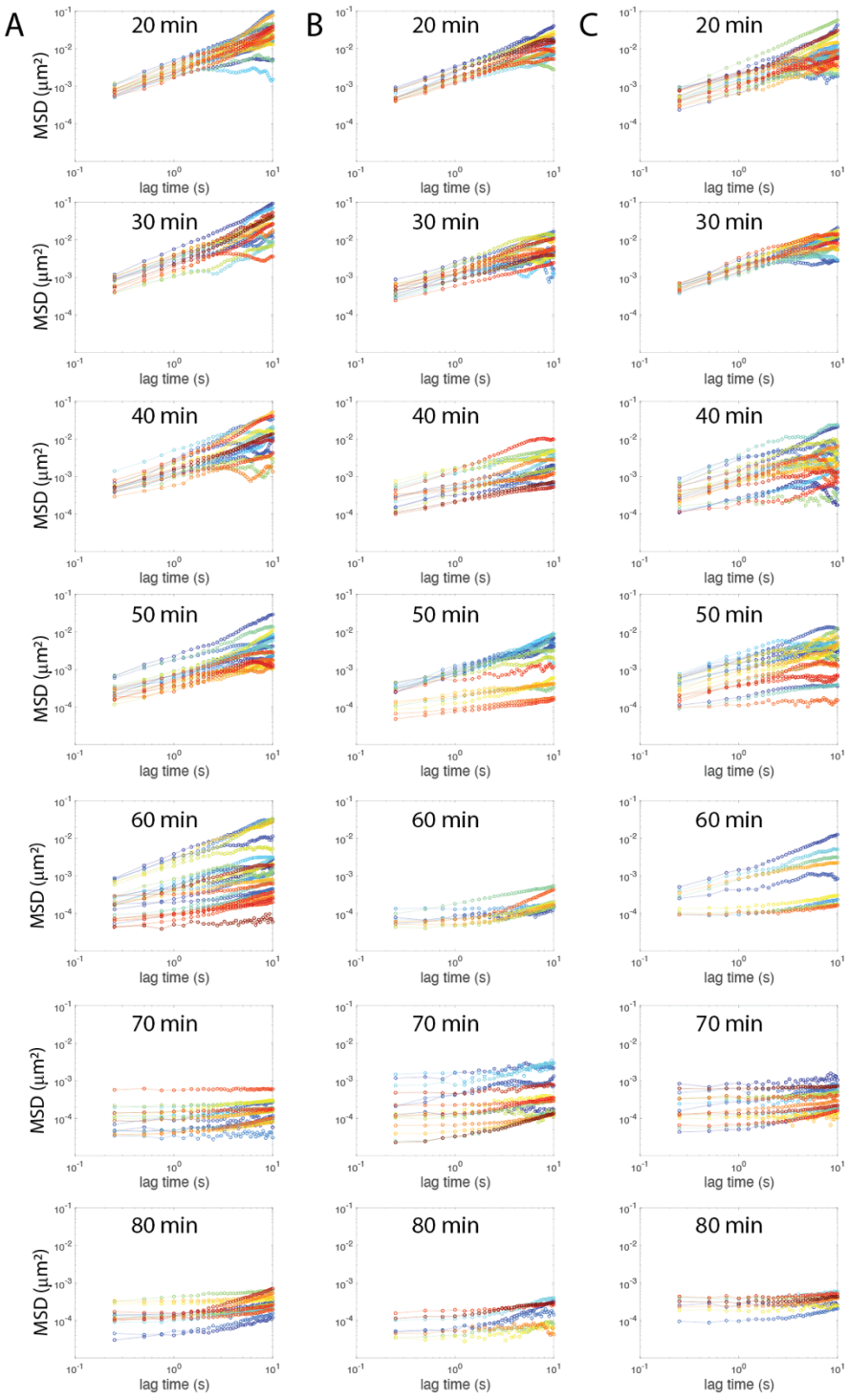

Fig. S4. Individual bead trajectories against lag time for experiments A-C (Fig. S3.) at increasing incubation times of 20, 30, 40, 50, 60, 70 and 80 minutes.

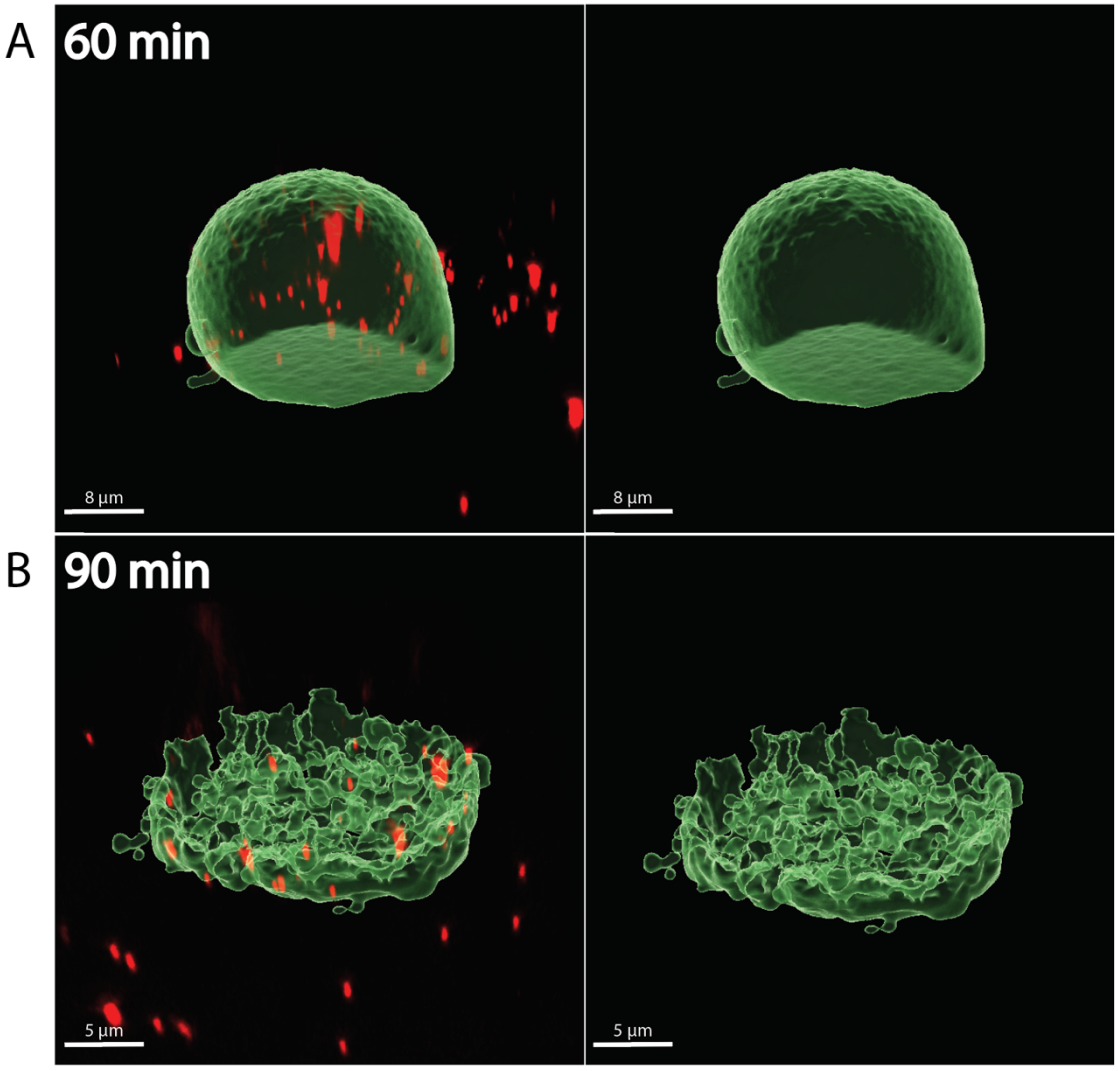

Fig S5: Z-stacks rendered in Imaris Software depicting 3D volumed of the same minielastin condensate before and after maturation takes place. A) 3D image of minielastin droplet at 60 minutes of incubation time, scale bar 8 µm. B) 3D image of minielastin droplet at 90 minutes of incubation time, scale bar 5 µm. Red particles are 100 nm fluorescent beads imbedded within the droplet. Minielastin concentration was of 130 µM and maintained at room temperature throughout the duration of the experiment.

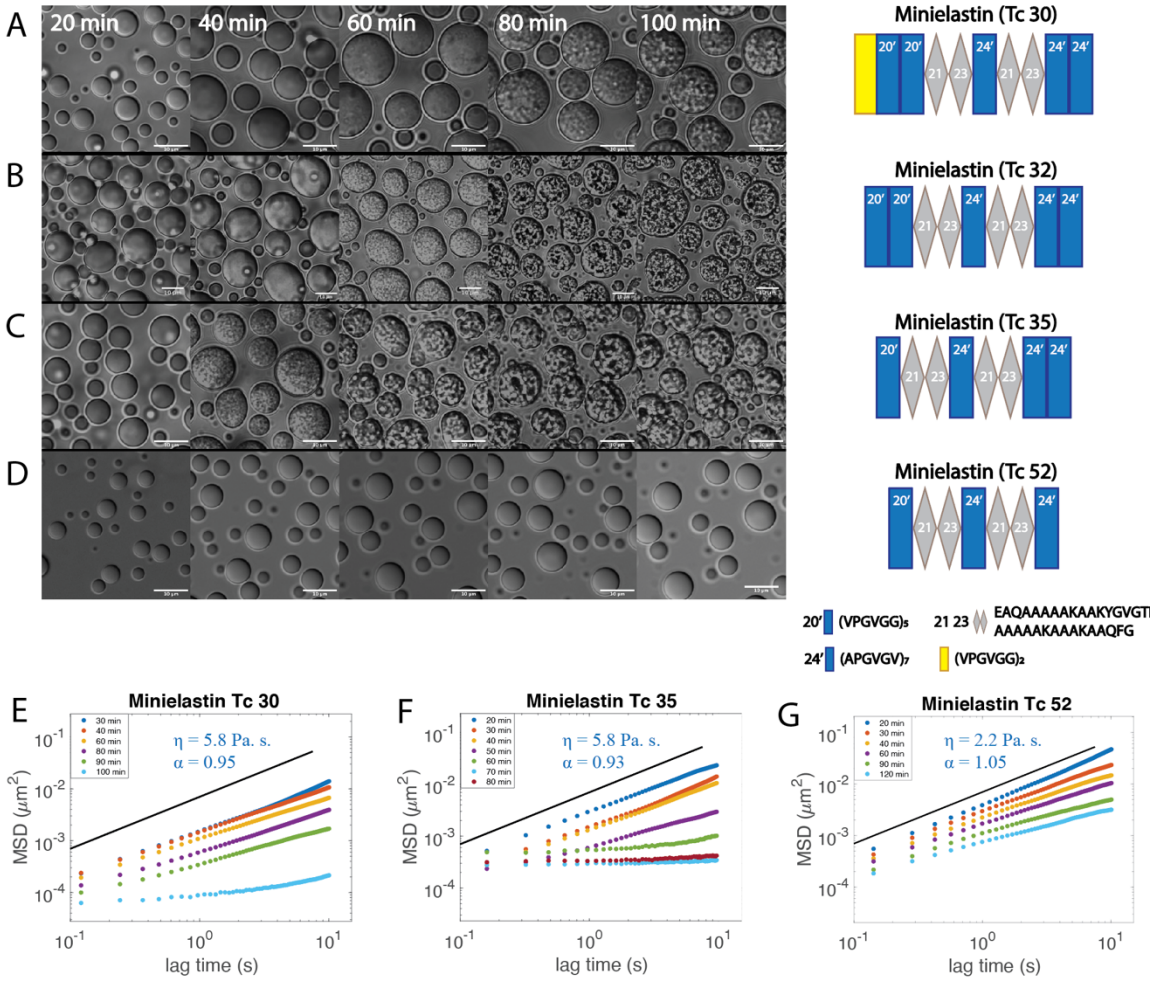

Fig S6: Maturation of minielastin liquid droplets. (A-D) DIC images depicting maturation of distinct minielastin droplets construct as a function of time. Specific minielastin sequences depicted in diagrams (Right). Tc refers to the specific coacervation temperature of the construct as determined in turbidity assays (440 nm), as described earlier (1) with the following specifications: 25 μM protein concentration dissolved in 50 mM Tris, 1.5 M NaCl, 1 mM $CaCl_2$ pH 7. Droplets in panel A-C were induced at a protein concentration of 130 μM, droplets in panel D were induced at a protein concentration of 270 μM. (E-G) Average MSD of bead particle against lag time within liquid droplets at increasing incubation times. η and α refer to the calculated viscosity and diffusive exponent respectively at early incubation times (20 and 30 minutes of incubation time) MSD of E-F were measured with a protein concentration of 130 μM, G was induced at a protein concentration of 300 μM. Protein was dissolved in 1.5 M NaCl, 1 mM $CaCl_2$, 50 mM Tris, pH 7.4 buffer for both DIC and microrheology experiments.

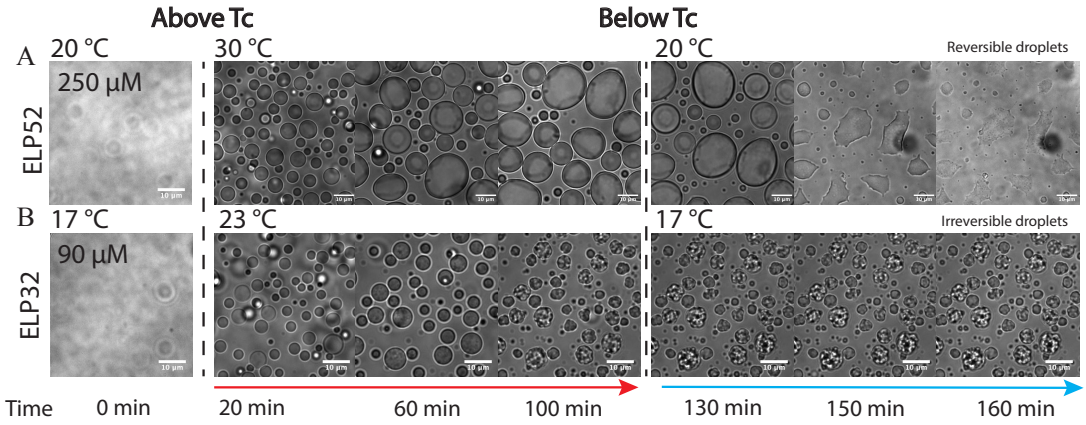

Fig S7: Bright field images of minielastin and minielastin52 condensates after incubation above respective transition temperatures (Tc) as a function of incubation time. A) Temperature ramping of minielastin 52 at 250 µM concentration. B) Temperature ramping of minielastin at 90 µM concentration. Sample buffer made of 1.5 M NaCl, 1 mM $CaCl_2$, 50 mM Tris, pH 7.4.

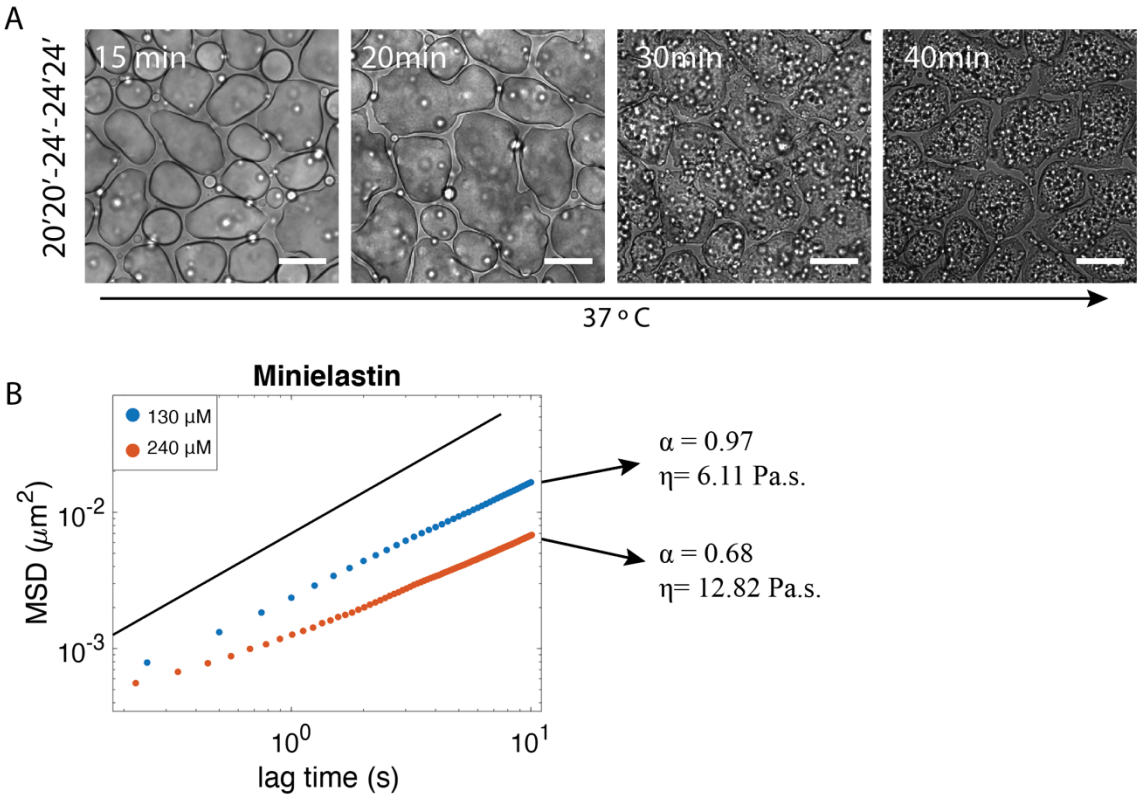

Fig S8: Temperature and concentration affects maturation of minielastin droplets. (A) Brightfield images of minielastin at 37 ºC at increasing incubation time. Protein concentrated to 130 µM. Scale bar 20 µm (B) Average mean square displacement (MSD) of minielastin at 130 µM (Blue) and 240 µM (Orange) protein concentration, η and α refer to the calculated viscosity and diffusive exponent respectively. All experiments done in buffer containing 1.5 M NaCl, 1 mM $CaCl_2$, 50 mM Tris, pH 7.4

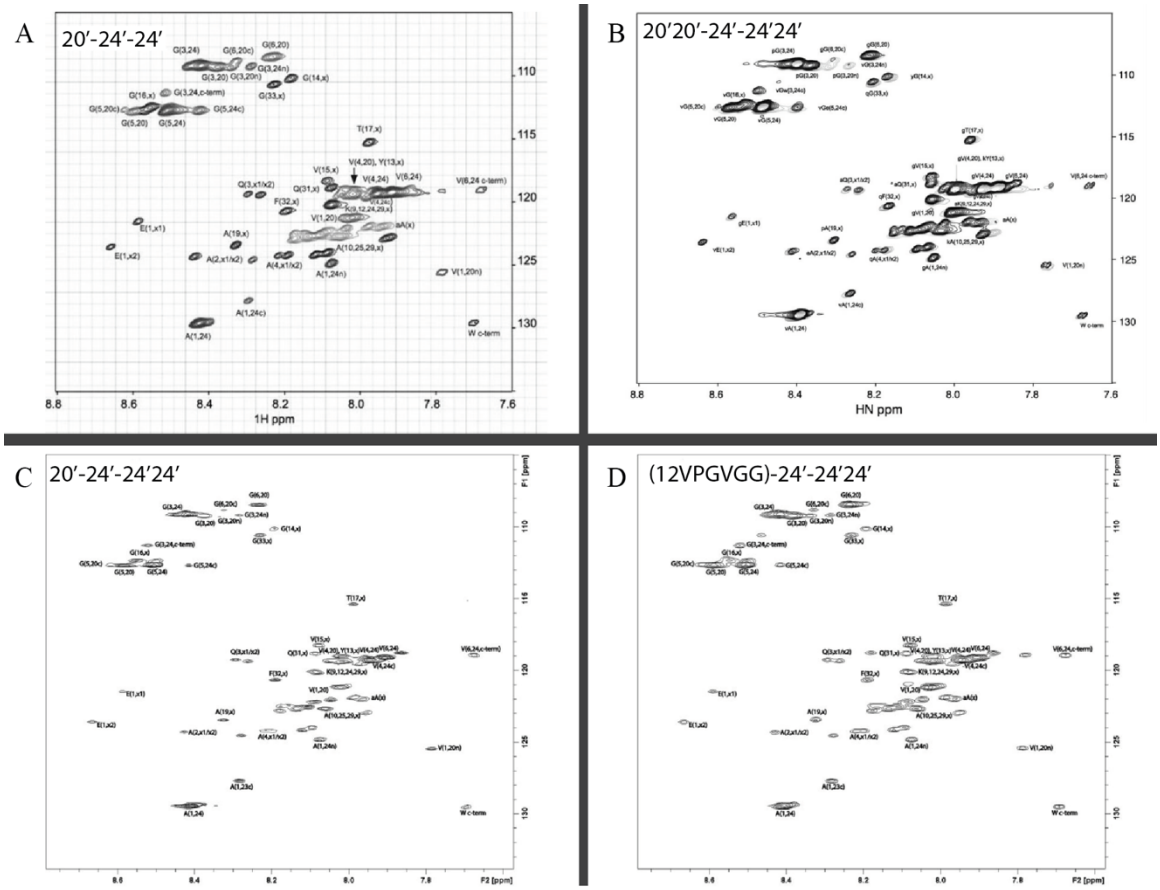

Fig S9: NMR spectral assignment of minielastin models dissolved in solution. Lyophilized protein was dissolved and diluted to 200 μM in buffer containing 45 mM phosphate buffer at pH 6.0.

Dropbox Link to SI Movies
https://www.dropbox.com/sh/31u6yjufotjb4fk/AAD_P1N5WjXfh1iRK82w4lXCa?dl=0

Movie 1: Brightfield microscopy videos depicting minielastin condensates flowing in capillary chamber at early incubation times (0 – 90 min). Protein concentrated to ~200 μM in buffer containing 1.5 M NaCl, 1 mM $CaCl_2$, 50 mM Tris, pH 7.4. Temperature stabilized to 27 ^oC. Time intervals in minutes.

Movie 2: Brightfield microscopy videos depicting minielastin condensates flowing in capillary chamber at late incubation times (90 – 180 min). Protein concentrated to ~200 μM in buffer containing 1.5 M NaCl, 1 mM $CaCl_2$, 50 mM Tris, pH 7.4. Temperature stabilized to 27 ^oC. Time intervals in minutes.

Movie 3: Confocal microscopy time lapse video of minielastin droplets depicting the maturation process at intermediate incubation times (60 – 78 min). Protein concentrated to 130 μM in buffer containing 1.5 M NaCl, 1 mM $CaCl_2$, 50 mM Tris, pH 7.4. Temperature stabilized to 23 ^oC. Time intervals in minutes.

Movie 4: Confocal microscopy time lapse video of minielastin droplets depicting the maturation process at late incubation times (80 – 90 min). Protein concentrated to 130 μM in buffer containing 1.5 M NaCl, 1 mM $CaCl_2$, 50 mM Tris, pH 7.4. Temperature stabilized to 23 ^oC. Time intervals in minutes.

Movie 5: Confocal microscopy time lapse video of minielastin droplets depicting the maturation process when maturation time is complet and no more change is observed (90 – 113 min). Protein concentrated to 130 μM in buffer containing 1.5 M NaCl, 1 mM $CaCl_2$, 50 mM Tris, pH 7.4. Temperature stabilized to 23 ^oC. Time intervals in minutes.

1. K. N. Greenland *et al.*, Order, Disorder, and Temperature-Driven Compaction in a Designed Elastin Protein. *J Phys Chem B* **122**, 2725-2736 (2018).