Supplementary Information: Silk molecular weight influences the kinetics of enzymatically crosslinked silk hydrogel formation

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6 figures, 4 pages

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Kinetic rheological measurements of the storage modulus ($G'(t)$) and loss modulus ($G''(t)$) are done at a fixed frequency of 1 Hz and strain amplitude 1%. Although we cannot directly show that these parameters correspond to a point in the linear viscoelastic (LVE) regime of the polymerizing gel, we perform strain amplitude and frequency sweeps to confirm that $G'(t)$ is measured in the LVE regime of the fully polymerized gels [Figure SS1].

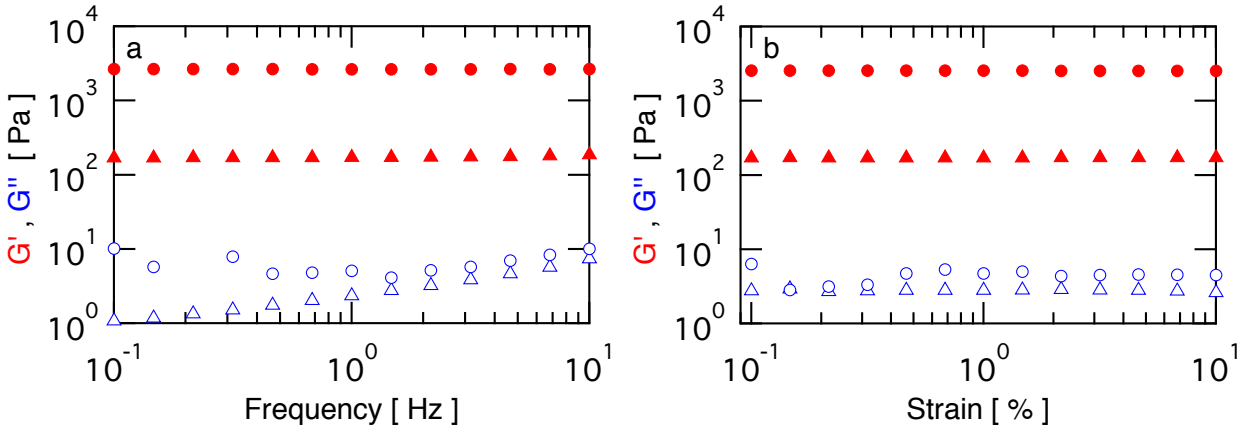

Figure S1: G' (solid symbols) and G'' (open symbols) as a function of frequency (a) and strain amplitude (b) of fully polymerized 340 kDa (circles) and 110 kDa (triangles) protein gels at $c = 25$. Note: the absolute values of G' and G'' vary amongst different silk batches.

Inflection points of $G'(t)$ are used to define the timescales t_c, t_p, and t_f. The critical time at which the modulus starts to grow is $t = t_c$ and is determined by the local maximum of d^2G'/dt^2. When t_c is small, this method of defining t_c picks out the first point. $t = t_p$ is the time at which percolation ends and is defined by the local minimum in d^2G'/dt^2. G' completes growth at $t = t_f$.

The raw polymerization curves for 340 kDa [Figure SS2] and 110 kDa [Figure SS3] gels for different concentrations of silk c show the relationships amongst G', G'', and the normal force. At short times, either $G' \sim G''$ or $G' > G''$ depending on interfacial effects. At the end of polymerization, $G' \sim 100 \times G''$ signifying that the hydrogel is elastic.

The growth of $G'(t)$ transitions from α_1 to α_2 growth at $t = t_p$ for the 340 kDa gels. A consistent feature of the normal force evolution also occurs at $t = t_p$; the normal force hits a local minimum [Figure SS2].

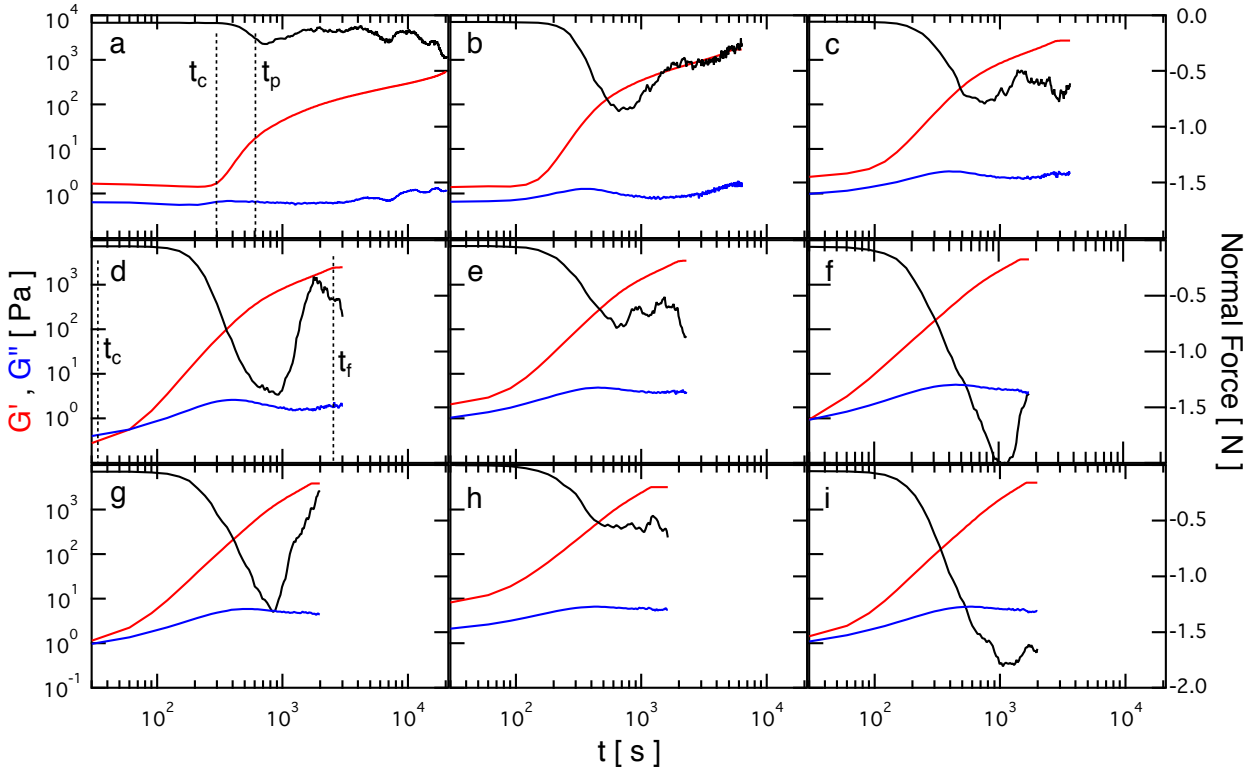

Figure S2: G' (red), G'' (blue), and NF (black) as a function of time during polymerization of 340 kDa gels. (a-i) Concentrations of silk corresponding to $c = 5, 10, 15, 20, 25, 30, 32, 34, \&36$ mg mL^{-1}.

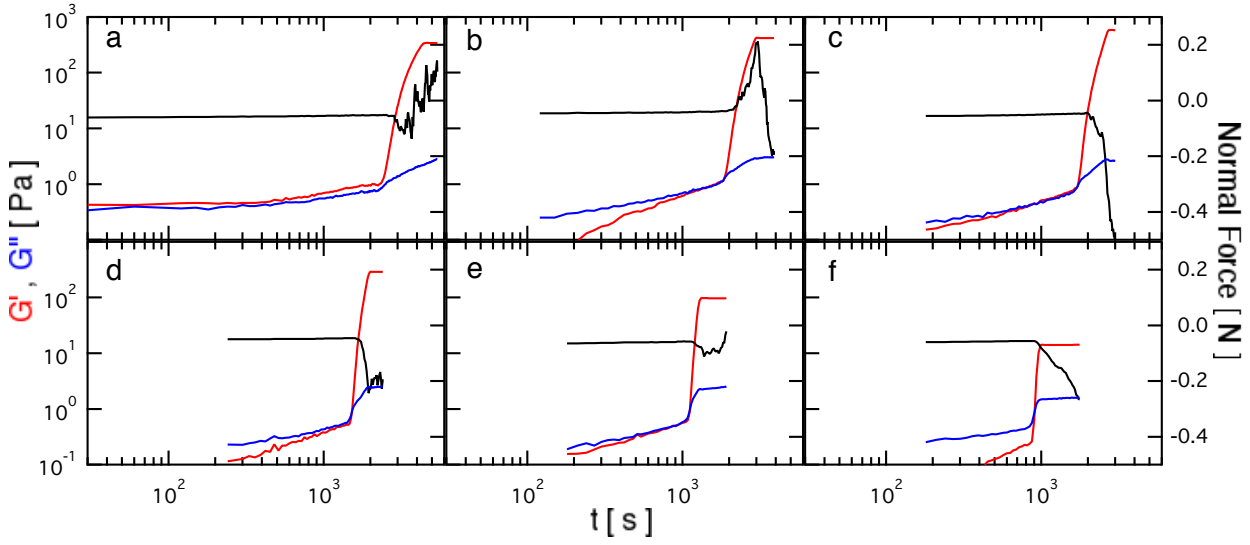

Figure S3: G' (red), G'' (blue), and NF (black) as a function of time during polymerization of 110 kDa gels. (a-f) Concentrations of silk corresponding to $c = 15, 20, 30, 40, 50, \& 55$ mg mL^{-1}.

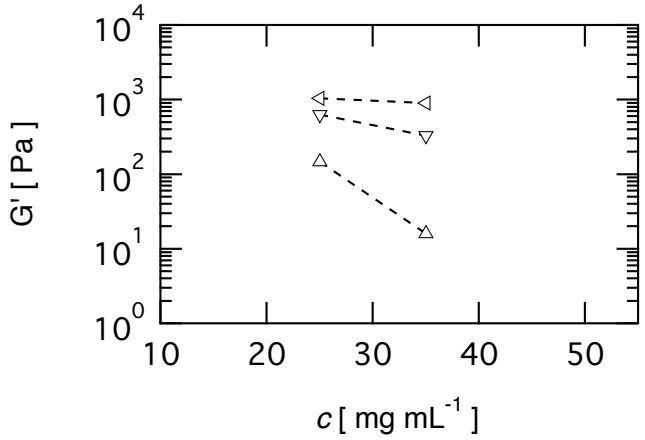

Figure S4: $G'(t_f)$ for 110 kDa gels made with H_2O_2 concentrations of 0.005% ($\triangle$), 0.0075% ($\triangledown$), and 0.01% ($\triangleleft$). Note: slight differences exist in $G'(t_f)$ between these data and Figure 1 due to protein batch differences.

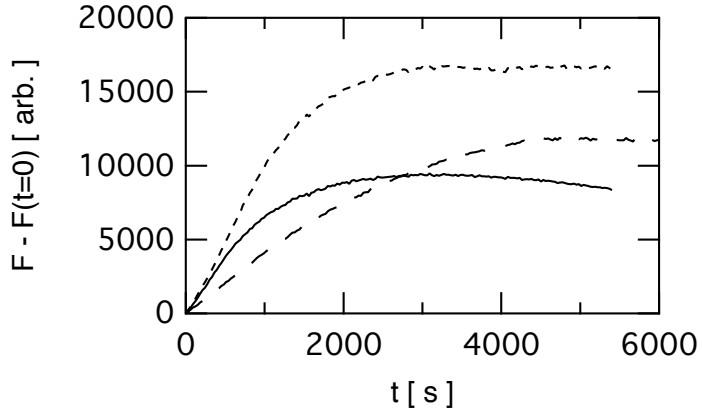

Figure S5: $c = 5$ for 340 kDa (solid line), $c = 10$ mg mL^{-1} for 340 kDa (short dashed line) and $c = 15$ mg mL^{-1} for 110 kDa (long dashed line) gels

The fluorescence intensity $F(t)$ represented on a linear scale shows that the lowest concentrations for 340 kDa gels have a slight decrease in F after the maximum, whereas the 110 kDa gels plateau at the maximum [Figure SS5].

Circular dichroism is done to monitor changes in secondary structure during polymerization [Figure SS6]. For both 340 kDa and 110 kDa gels, the ellipticity is unchanged during polymerization.

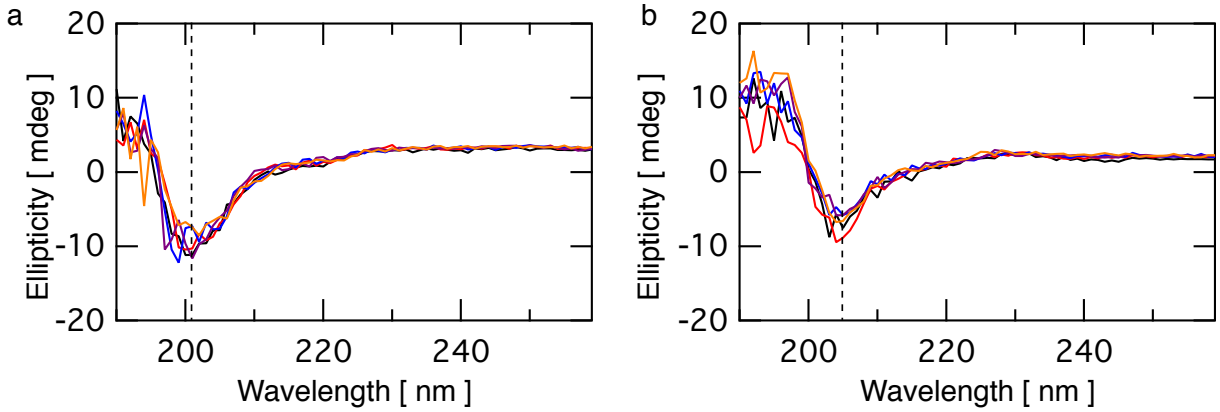

Figure S6: Ellipticity as a function of time from 0 min to 120 min (various colored lines) during polymerization for 340 kDa (a) and 110 kDa (b) gels as measured by circular dichroism is most closely aligned with a random coil configuration. Dashed lines are a guide to the eye. The ellipticity does not evolve during polymerization. The shift in the minimum between 340 kDa and 110 kDa, that exists from $t = 0$ onwards, is likely due to the difference in possible configurations due to differing molecular weight.