Supporting Material

Thermal Memory in Self-assembled Collagen Fibril Networks

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Note S1. Determination of collagen fiber diameter and mass-length ratio by turbidimetry

The wavelength dependence of the turbidity of a solution of rigid fibers contains information about the fiber diameter, d, and their mass-length ratio, μ. The exact functional dependence is model-dependent. Assuming that the fibers are randomly oriented, rigid rod-like, and monodisperse in size, the turbidity scales with wavelength as (1, 2):

$$\tau\lambda^5 = A\mu(\lambda^2 - Bd^2) \qquad (1)$$

This relation is valid when the rods are longer than ~800 nm and have a diameter less than ~200 nm (2). According to Eq. (1), d and μ can be simply determined by plotting $\tau\lambda^5$ versus λ^2. The mass-length ratio, μ, follows from the slope of this linear relation, using the definition $A = (88/15)\pi^3 c n_s (dn/dc) 21/N_A$, where c is the protein concentration expressed in g ml^{-1}, n_s is the solvent refractive index (equal to 1.33), dn/dc is the specific refractive index increment (dn/dc = 0.186 cm^3 g^{-1} for collagen(3)), and N_A is Avogadro's constant. The fiber diameter d can be calculated by combining the measured intercept with the y-axis ($-A\mu Bd^2$) with the measured slope, using the definition $B = (92/426)\,\pi^2 n_s^2$.

We measured d and μ for collagen solutions during polymerization at 37°C by measuring the wavelength dependence of the turbidity at 8 evenly spaced wavelengths between 370 and 440 nm, using time intervals of 2 minutes between consecutive wavelength scans. For all wavelengths, the turbidity remained essentially zero during the first 15 minutes of assembly, indicative of a lag phase during which fibrils are nucleated. During this nucleation phase, we were thus unable to reliably track the fiber diameter and mass. After 15 minutes, the turbidity at each wavelength increased sigmoidally with time, indicating fiber growth. The wavelength dependence of the turbidity obeyed the linear scaling predicted by Eq. (1), consistent with the presence of rigid, high aspect ratio fibrils. The slope $A\mu$ increased over time, indicating that the fibrils grow laterally (Fig. S1). After 2 hours incubation at 37°C, we suddenly lowered the temperature to a value between 4 and 32°C in order to induce fibril disassembly. In response, both the magnitude of the turbidity and the slope $A\mu$ decreased, indicating that fibrils loose monomers from their sides (Fig. S1, solid red circles).

As shown in Fig. S2a (left hand side), the apparent mass length ratio of the fibrils increased in a sigmoidal fashion during the growth phase. At the end of the lag phase, the mass-length ratio was about $1.3.10^{12}$ Da.cm. Given a quarter-staggered axial packing of collagen molecules with a period of D = 67.2 nm, the number of molecules per fibril cross-section is $N = \mu/(M^*4.6D)$, where M is the molecular mass of collagen (290 kDa(4)). At the end of the turbidimetric growth phase, the fibrils thus have ~130 monomers per cross-section, consistent with prior reports (5, 6). The final mass length ratio of "mature collagen fibrils" formed after 2 hours at 37°C was $1.03.10^{13}$ Da/cm with a standard deviation of 7% (N

= 6), corresponding to an average of 1070 monomers per cross-section. These values are closely comparable to those measured for collagen fibrils formed at 37°C from purified rat tail collagen I (7).

Upon cooling, the mass-length ratio decreased in a bi-phasic manner, showing an initial fast decay followed by a slow decay that was incomplete even after 2 hours (Fig. S2a, right hand side). The loss of mass from the sides was strongly temperature dependent. At the lowest temperature (4°C), the final mass length ratio was $2.2.10^{12}$, corresponding to 229 monomers per fibril cross-section.

The apparent fibril diameter started at a value of about 20 nm at the end of the lag phase, and increased to values ranging between 42 and 70 nm after 2 hours assembly at 37°C (Fig. S2b). The average diameter of "mature fibrils" was 61 nm with a standard deviation of 22%. Upon cooling, the apparent diameter, surprisingly, jumped up to higher values (80-100 nm) and afterwards showed a biphasic decay which was often rather noisy (see for instance the 4°C trace in Fig. S2b). We currently have no definitive interpretation of this behavior, but we strongly suspect that the diameter measurements are prone to artifacts, since the sudden jump upon cooling is clearly unphysical. We suspect that the diameter measurements are fundamentally inaccurate because the diameters are about 10-fold smaller than the wavelengths used; as a result, the extrapolation required to measure the y-intercept of $\tau\lambda^5$ versus λ^2 curves becomes very inaccurate. We nevertheless expect that the measured values of μ are reasonable, since light scattering theories predict that μ can still be determined from the slope in the limit of fibrils much thinner than λ (1).

We can compare the reduction of μ as a function of disassembly temperature (μ_{dis}/μ_{37}) with the reduction of turbidity (τ_{dis}/τ_{37}). The latter quantity provides a model-independent measure of the overall loss in fibril mass. As shown in Fig. S4, μ_{dis}/μ_{37} is nearly identical to τ_{dis}/τ_{37} over the entire range of disassembly temperatures, strongly suggesting that fibrils mainly loose mass from their sides. We observe that μ_{dis}/μ_{37} is consistently somewhat larger than τ_{dis}/τ_{37}, which is consistent with mass being lost also from the fibril ends. This in turn is consistent with confocal microscopy data, showing that cooling promotes the formation of dangling ends.

Supporting References (References also appear in the Main Text)

1) Carr, M., and J. Hermans. 1978. Size and density of fibrin fibers from turbidity. *Macromolecules*. 11: 46-50.
2) Yeramonahos, C., B. Polack, and F. Caton. 2010. Nanostructure of the fibrin clot. *Biophys. J.* 99: 2018-2027.
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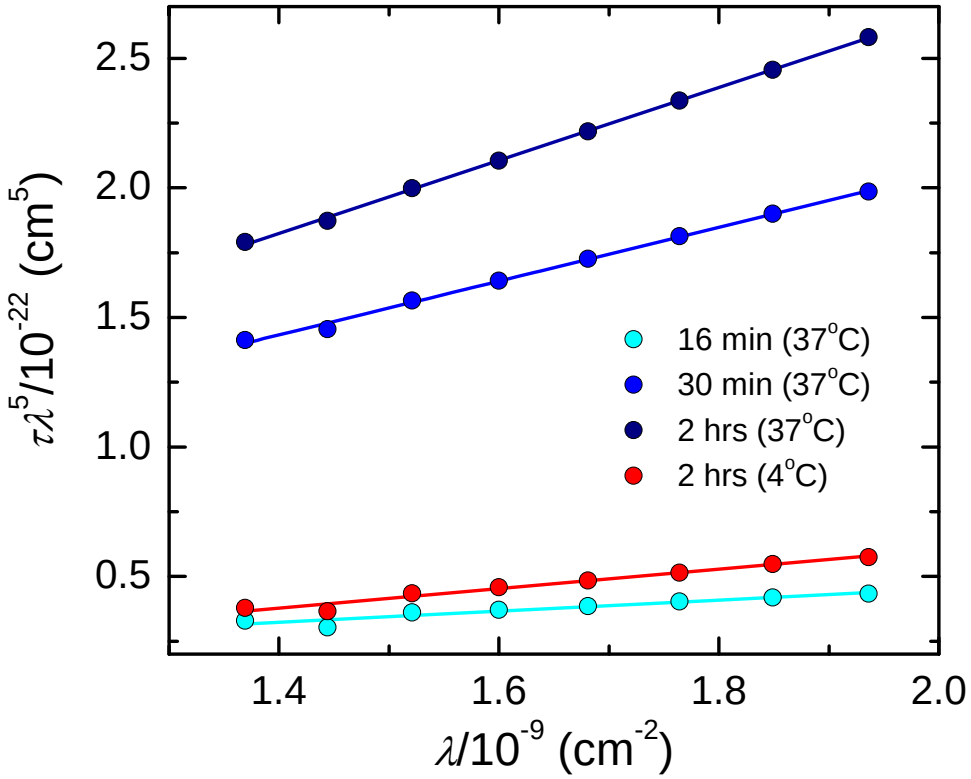
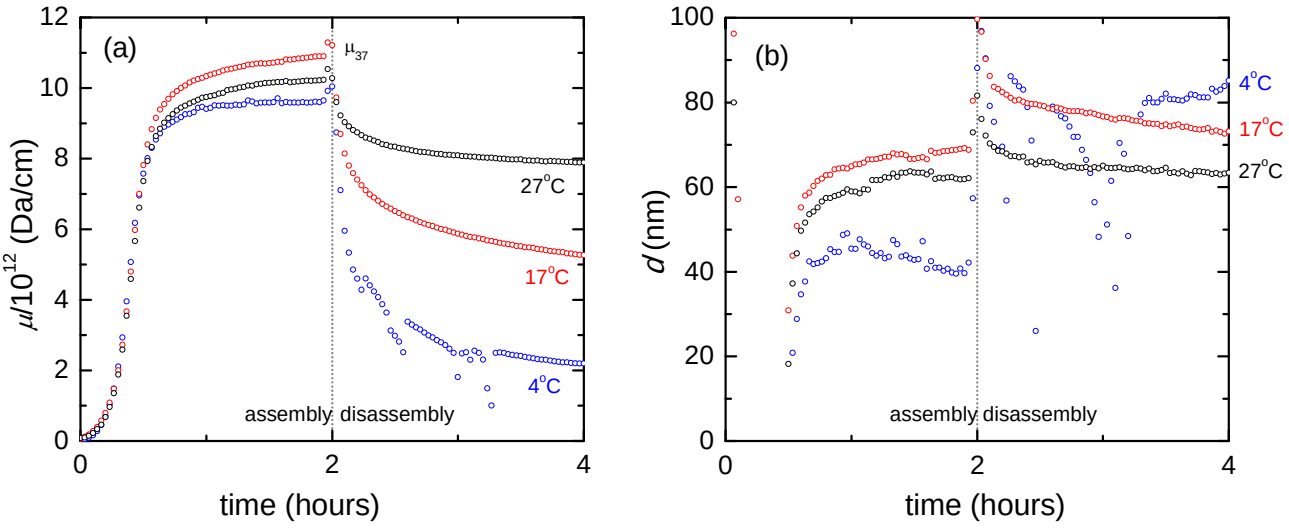
4) Wess, T. 2005. Collagen fibril form and function. *Adv. Protein Chem.* 2005: 341 - 374.
5) Bard, J., and J. Chapman. 1973. Diameters of collagen fibrils grown in vitro. *Nat. New Biol.* 246: 83 - 84.
6) Silver, F., and D. Birk. 1983. Kinetic analysis of collagen fibrillogenesis: I. Use of turbidity--time data. *Coll. Relat. Res.* 3: 393 - 405
7) Piechocka, I., A. VanOosten, R. Breuls, and G. Koenderink. 2011. Rheology of heterotypic collagen networks. *Biomacromolecules*. 12: 2797-2805.

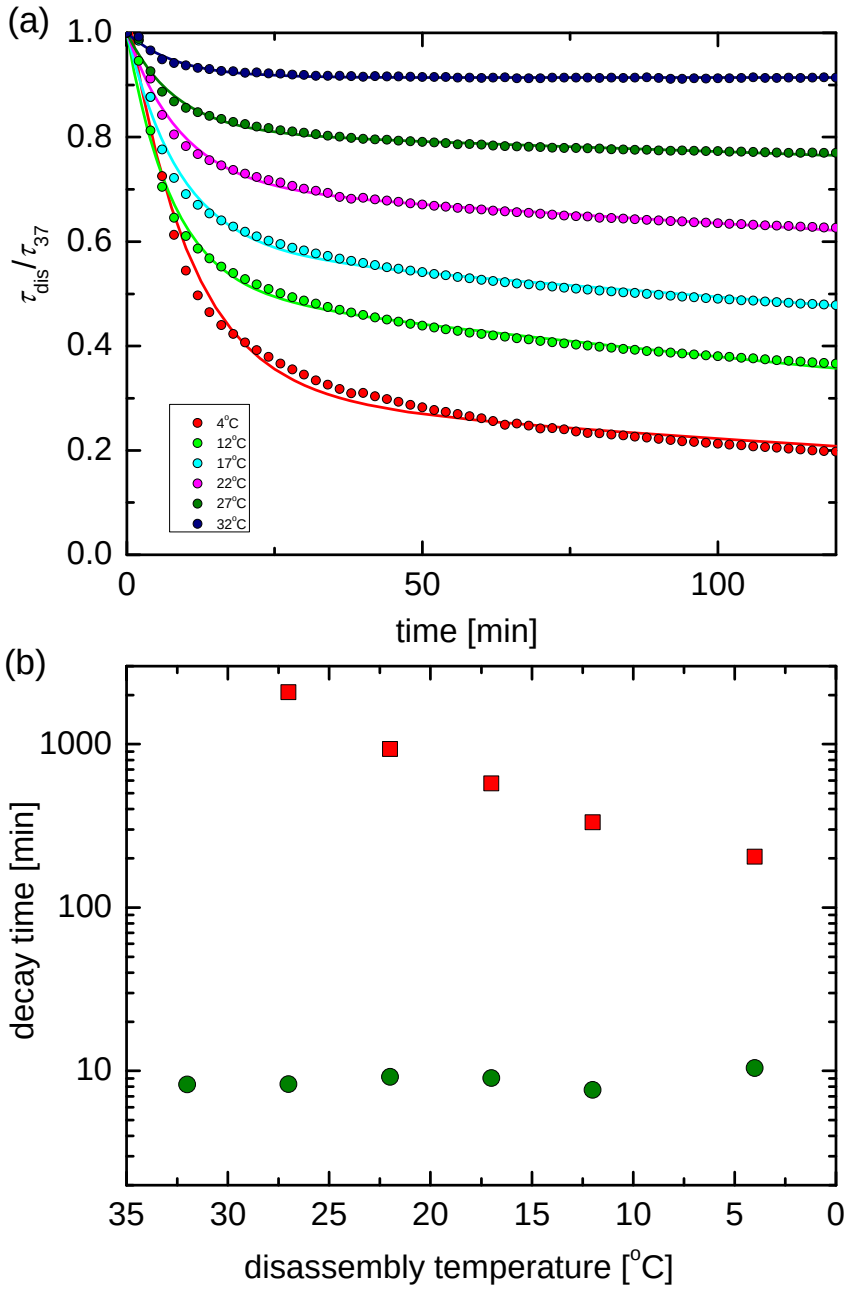
Supplemental Figures



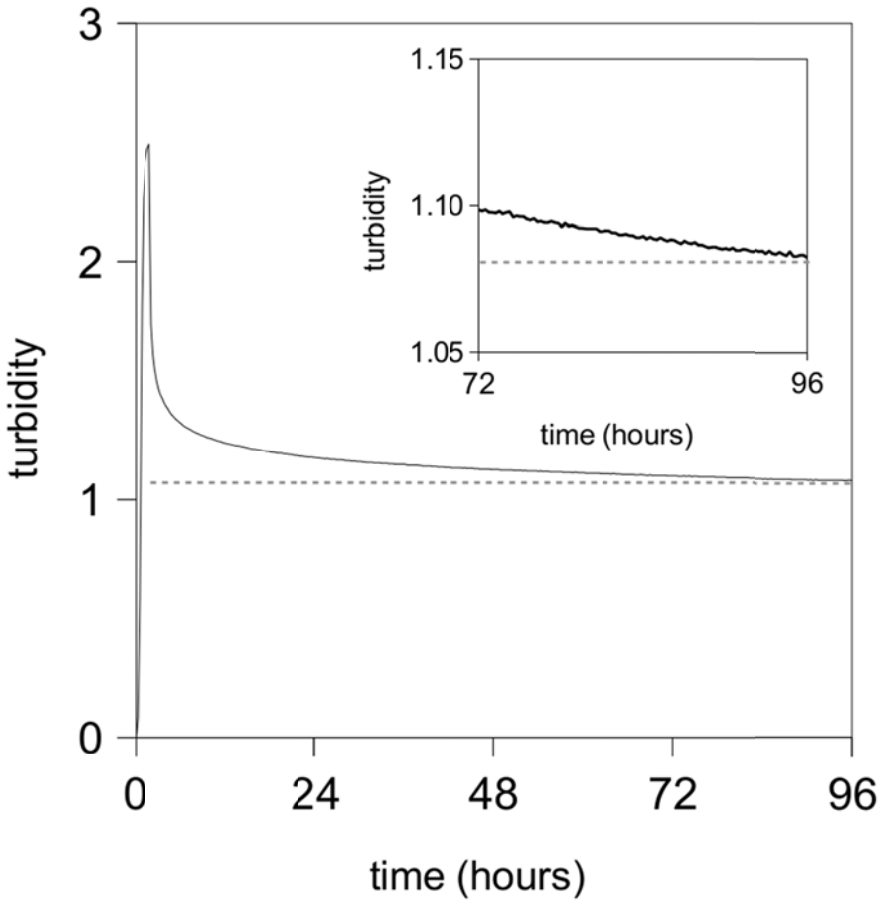
Supplemental Figure S1. **Wavelength dependence of the turbidity of a 1 mg/ml collagen gel, plotted according to Eq. 1 in Supporting Note S1.** The plots are linear, consistent with the presence of long, thin fibers (lines are best fits to the data). Three of the measurements shown were obtained at different times during fibril assembly at 37°C (16, 30, 120 minutes after induction of polymerization by warming from 4 to 37°C); fibril polymerization is evident from the increase in the magnitude of the turbidity and in the increase of the slope. One measurements was obtained after disassembly of the gel for 2 hours at 4°C (see figure legend); disassembly is evident from the decrease of the turbidity and the decrease of the slope.



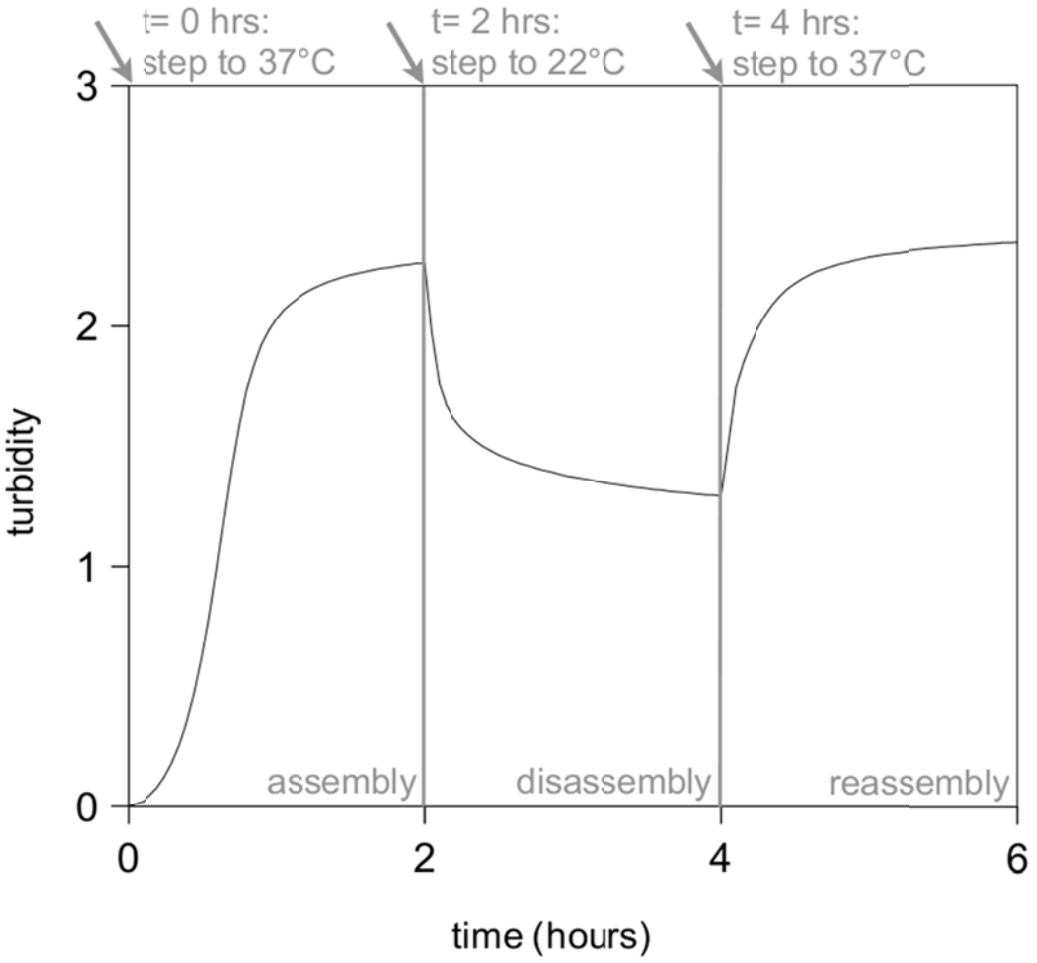
Supplemental Figure S2. **Structural properties of collagen fibrils during assembly and disassembly, as determined from the wavelength dependence of the turbidity.** (a) Fibril mass-length ratio and (b) fibril diameter, shown as a function of time during fibril assembly at 37°C for 2 hours (left of dashed vertical lines) and during fibril disassembly triggered by stepwise cooling to 4, 17, or 27°C**.** The analysis of the data assumes that the fibrils are long, thin, rigid, monodisperse, and randomly oriented, as explained in Supporting Note S1.



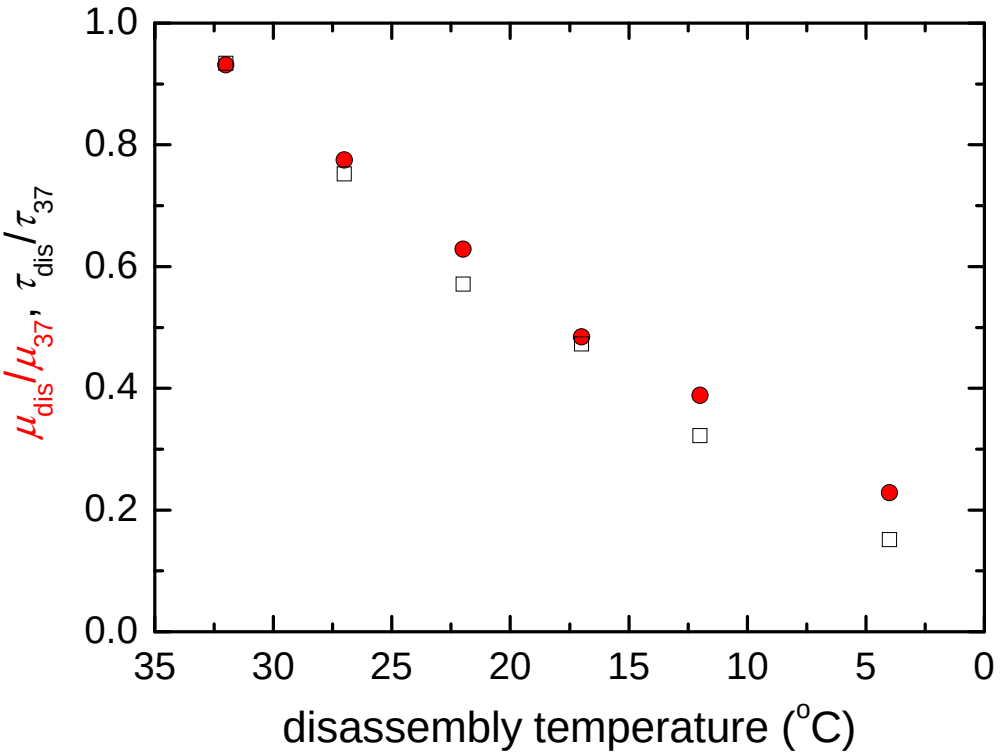
Supplemental Figure S3. **Empirical double-exponential fits to the time-dependent decay of the turbidity measured upon sudden cooling of collagen gels formed for 2 hours at 37°C.** The turbidity is normalized by the turbidity of the "mature collagen fibrils" (formed after 2 hours incubation at 37°C), according to τ_{dis}/τ_{37}. The fitting formula was: $\tau_{dis}/\tau_{37} = P_{fast} \exp(-t/ t_{fast}) + P_{slow} \exp(-t/t_{slow})$ with the constraint that $P_{fast} + P_{slow} = 1$. (a) Example data sets (symbols) with double-exponential fits (lines). (b) Dependence of the two decay times, t_{fast} and t_{slow}, on disassembly temperature. The fast decay time is temperature-independent. The slow time scale increases strongly with increasing disassembly temperature; at 32°C, t_{slow} diverges. The fraction of the fast-decaying component is 65% at 4°C, 49% at 12°C, 41% at 17°C, 29% at 22°C, 19% at 27°C, and 10% at 32°C.



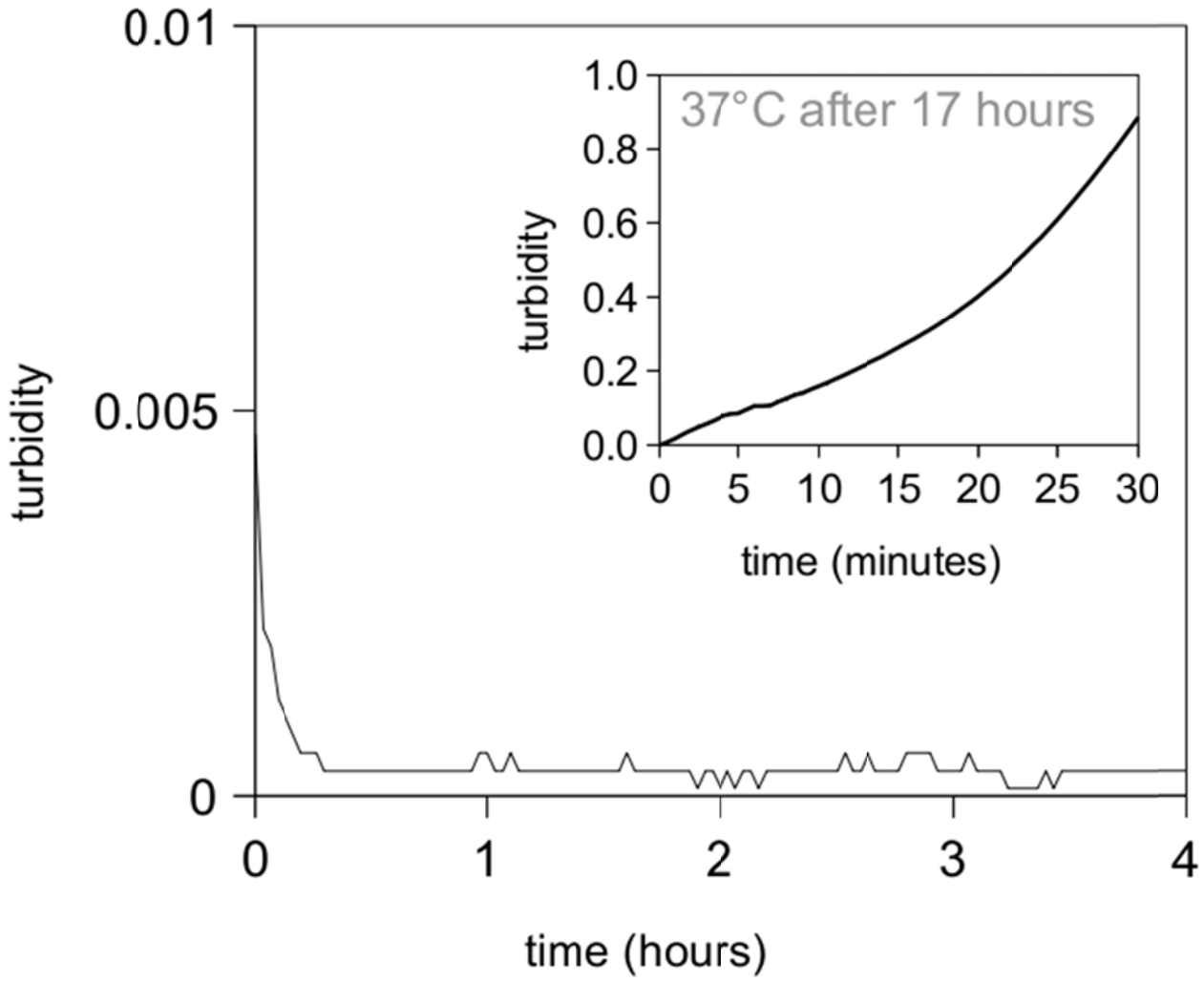
Supplemental Figure S4. **The turbidity of a 1 mg/ml collagen gel as a function of time.** After assembly at 37°C for 2 hours, the temperature is abruptly lowered to 22°C. Even after 96 hours, the turbidity is still slowly decreasing with time, which is more obvious in the *Inset*, which shows the same data, but zoomed in on the last 24 hours. The horizontal dotted lines are added to guide the eye.



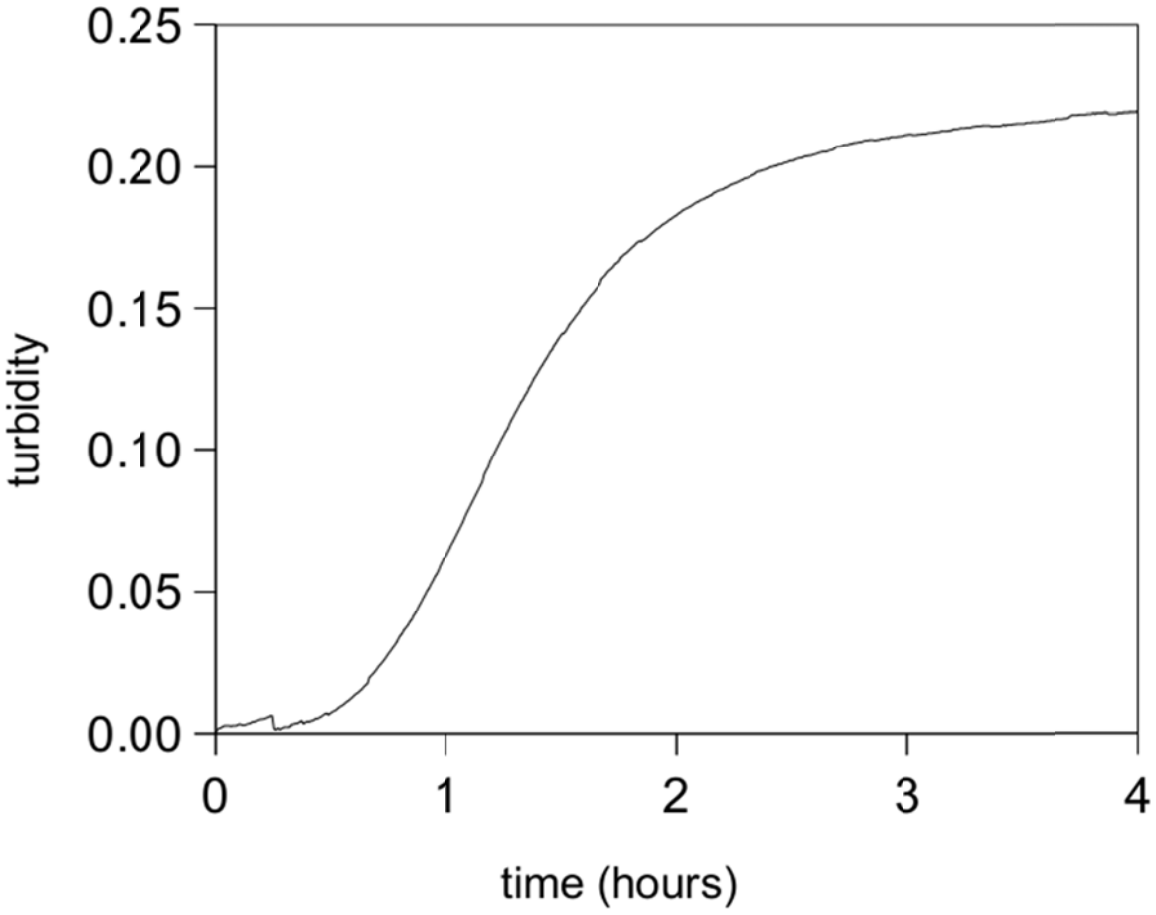
Supplemental Figure S5. **The turbidity of a 1 mg/ml collagen gel as a function of time.** After the 2 hours needed for assembly at 37°C, the temperature is lowered to 22°C, stepwise. After 2 hours of disassembly, it is raised again to 37°C, stepwise.



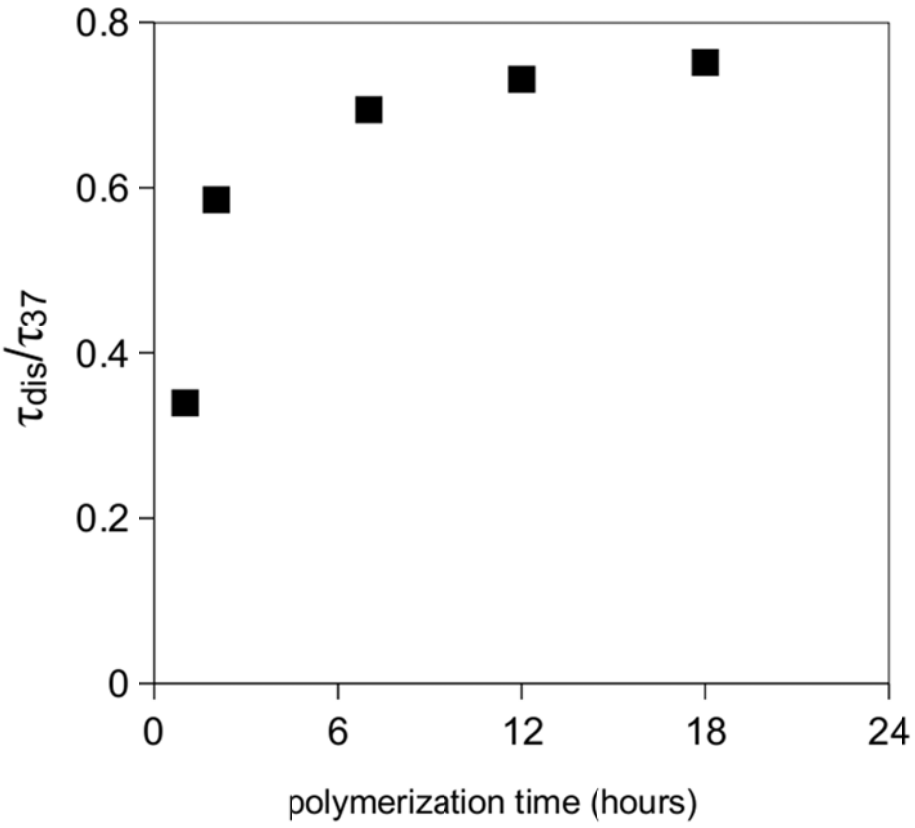
Supplemental Figure S6. **Comparison between the temperature dependence of the loss in fibril mass-length ratio (μ_{dis}/μ_{37}, solid red circles) determined by analyzing the wavelength dependence of the turbidity (which is model-dependent, see Supporting Note S1), with the loss in fibril mass** as quantified in a model-independent manner from the relative change in turbidity measured at a single wavelength of 370 nm (τ_{dis}/τ_{37}, open black squares)**.** The two measures are comparable, which suggests that mass is predominantly lost from the sides.



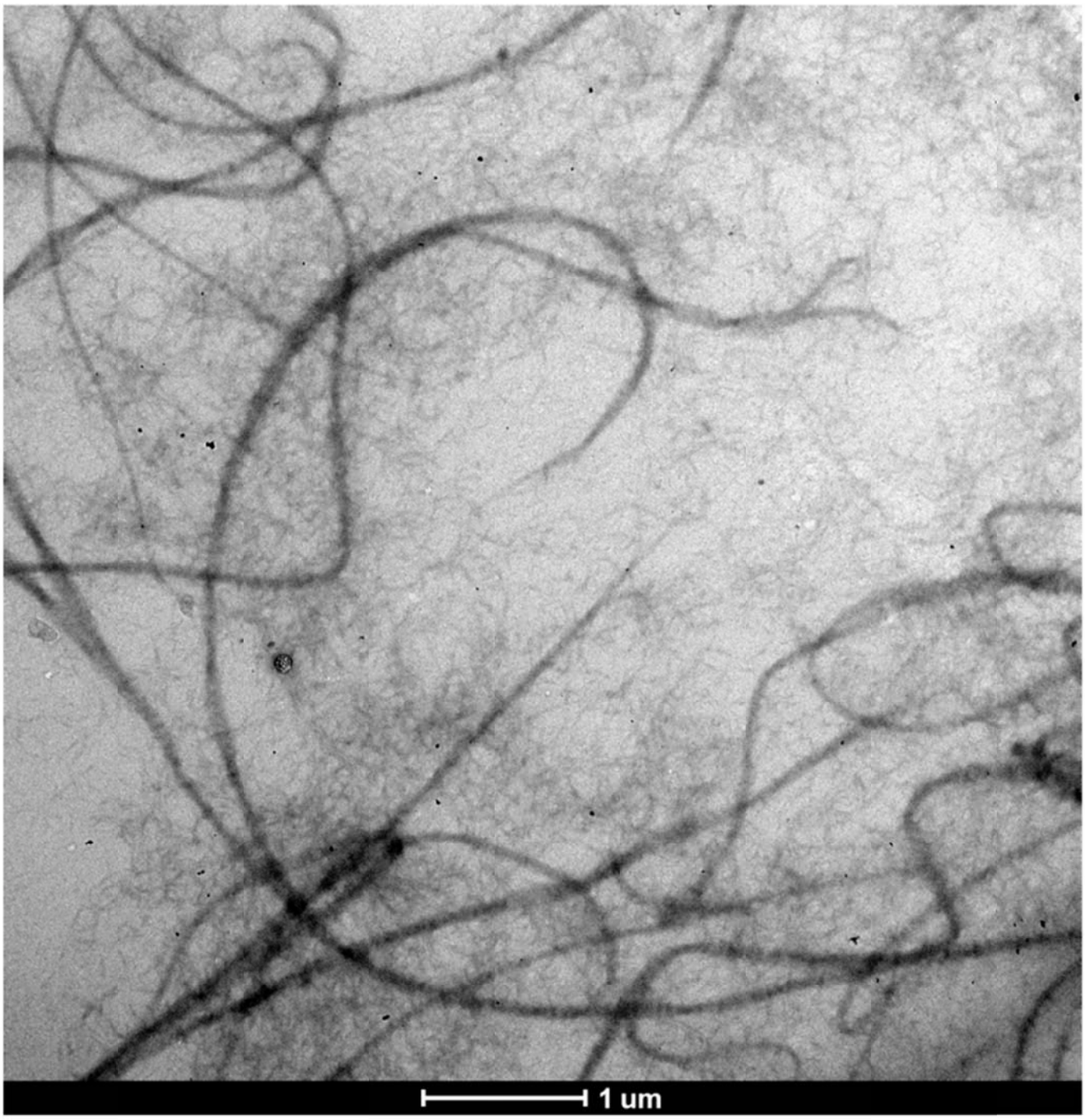
Supplemental Figure S7. **The turbidity of a 1 mg/ml collagen solution, kept at 4°C, as a function of time.** No appreciable turbidity develops, indicating that no fibril assembly takes place. The slightly higher value around $t = 0$ is an artefact common to these experiments. *Inset*: after 17 hours at 4°C, the temperature is increased stepwise to 37°C, causing an immediate increase in turbidity.



Supplemental Figure S8. **Turbidity as a function of time of a 0.1 mg/ml collagen gel.** The collagen used in this mixture is prepared by assembling a 1.0 mg/ml gel, and then redissolving it in 0.01M HCl at 4°C. There is a lag phase of approximately 30 minutes, indicating that the redissolved collagen solution contains no nuclei or other supramolecular aggregates. The experiment was performed by assembling fibrils inside a dialysis cartridge (Slide-a-lyzer, 30,000 MWCO, 0.5-3ml capacity, Thermo Scientific) pre-soaked in assembly buffer. After 2 hours of assembly, the gel was dialysed against 3 changes of 150 ml 0.01M HCl at 4°C. The collagen solution was then re-assembled by adding assembly buffer, adjusting the pH to 7.2, and raising the temperature to 37°C. Assembly was monitored by turbidimetry.



Supplemental Figure S9. **Age-dependence of fibril disassembly.** τ_{dis}/τ_{37} measured 2 hours after a temperature step to 22°C, as a function of the polymerization time.



Supplemental Figure S10: **Transmission electron microscopy image of collagen fibrils from a 0.25 mg/ml gel.** The fibrils were formed at 37°C for 2 hours, and then disassembled at 22°C for 2 hours.