SUPPLEMENTARY INFORMATION

ULTRAFAST LASER-PROBING SPECTROSCOPY FOR STUDYING MOLECULAR STRUCTURE OF PROTEIN AGGREGATES

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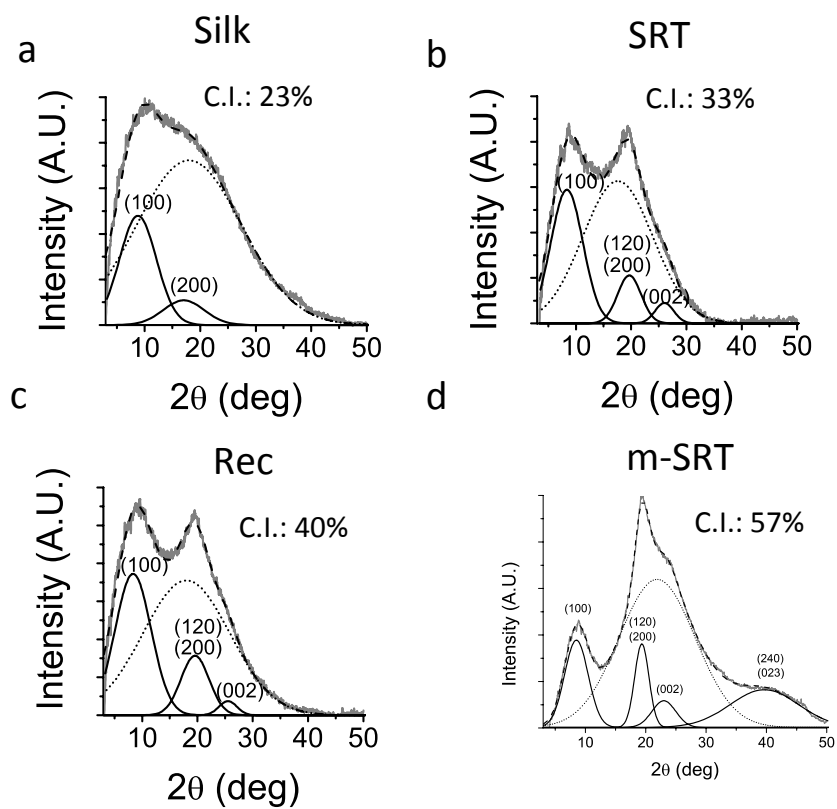[#] These authors contributed equally.

Figure S1. XRD (a, b, c, d) spectra for silk, native SRT, recombinant SRT (Rec) and methanol treated SRT (m-SRT) proteins show a semicrystalline profile where crystalline peaks (solid line) and an amorphous halo (dotted line) are fit.
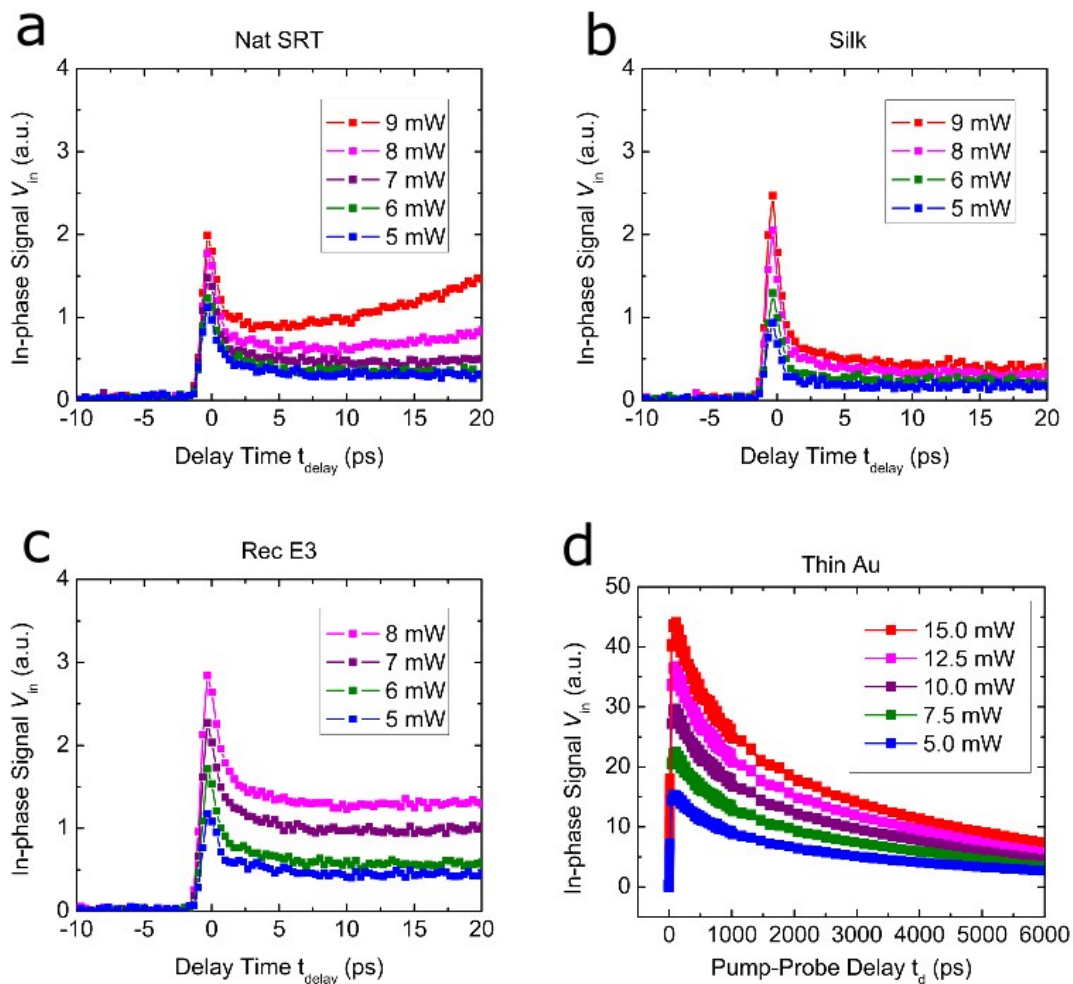
Figure S2. In-phase signal due to a heating event from a pump laser pulse as a function of the delay time between the pump and probe beams for native SRT (a), silk (b) and recombinant SRT (c) proteins. The measured change in transmissivity (dTr) at the different pump powers, which increases the per-pulse temperature rise, is representative of the change in refractive index due to a change in temperature (dn/dT). (d) Control experiment with Au thin film.
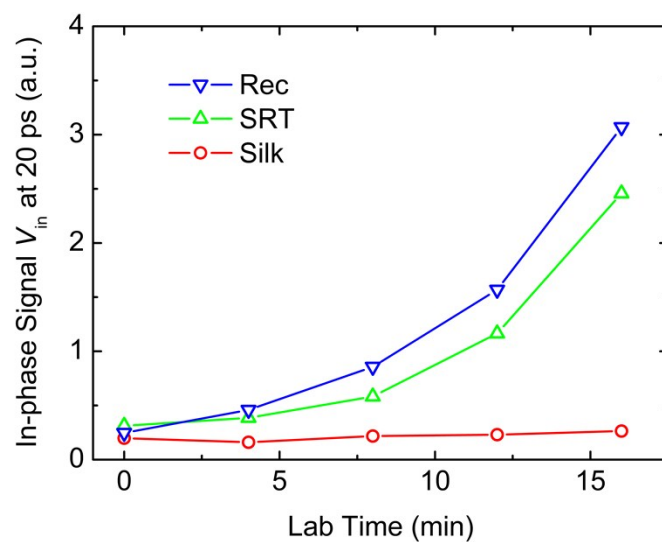
Figure S3. In-phase signal at 20 ps pump-probe delay time as a function of lab time. Measurements reveal thermal runaway effects after only 5 minutes in recombinant (green upwards triangles) and native SRT (blue downwards triangles) but not silk (red circles). All data reported in the manuscript were taken at less than 5 minutes exposure to the TDTT pump laser, ensuring minimal thermal runaway.
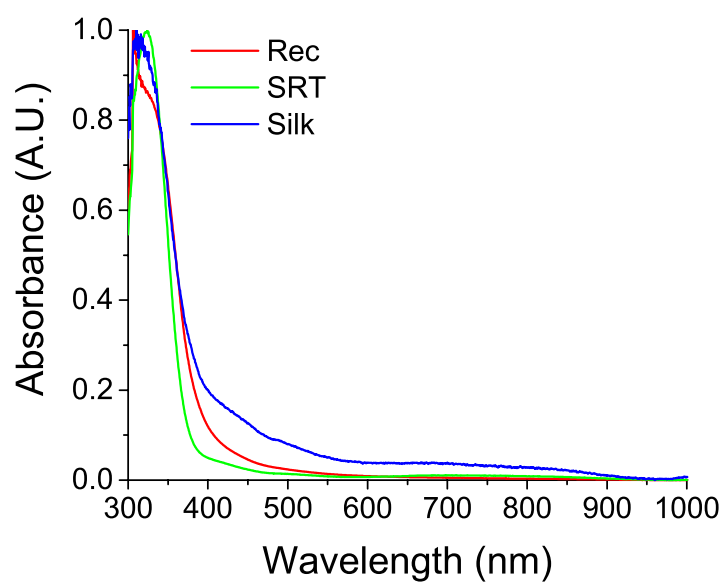
Figure S4. Optical absorbance of three proteins studied in this manuscript. High absorbance in 280-360nm regions is observed (as expected), whereas absorbance is low in 800 nm regions. We also note that typical laser stability of protein films are 1mW/mm^2.
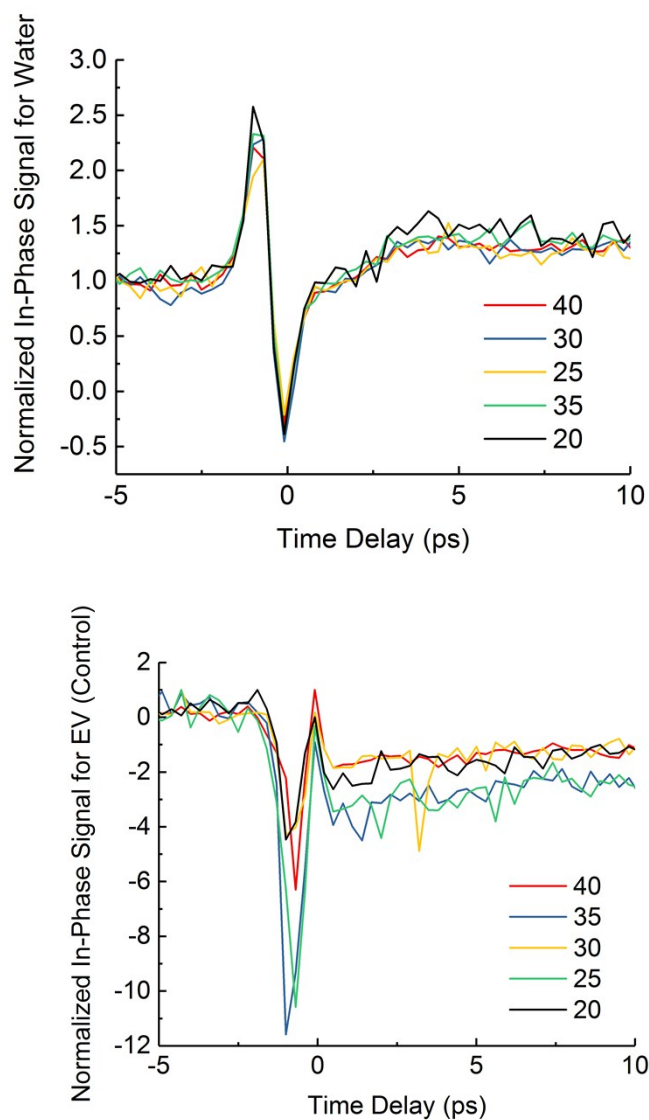
Figure S5. TDTT data on (top) DI water and (bottom) empty vector *E. coli* as control. The large sharp peaks near time delay of 0 ps are indicative of non-linear absorption effects that dominate the signal, more so than the signal from the media of interest (DI water or control *E. coli.*). This leads to strong interference effects that were not present in the TDTT data in the expressed *E. coli*.

Calculated temperature increase in protein thin film samples

Following Eq. 2 of Appendix, we measure the change in transmission due to a temperature induced by a short pulsed heating event as a function of time after the heating event. The raw data shows that the change in transmission due to the temperature change is positive. From the previous derivation, the change in transmissivity measured with TDTT is directly related to the change in refractive index (i.e. $dTr/dT \propto dn/dT$) and has the same sign, assuming $n > 1$. The refractive index n was measured to be ~1.55. Thus, the measured change in transmissivity is representative of the change in refractive index due to a change in temperature.

The induced temperature rise in the probed region of sample containing a thin film can be estimated:

$$C\frac{dT}{dt} = \frac{AF}{dt_p}\exp\left(-2.77\left(\frac{t-2t_p}{t_p}\right)^2\right)$$

where AF is the absorbed laser fluence, C is the heat capacity, d is the thickness of the thin film, and t_p is the pulse width. Solving this ODE yields

$$T(t) = \frac{AF}{Cd}\left(0.532 - 0.532\,\mathrm{erf}\left(3.33 - \frac{1.66\,t}{t_p}\right)\right)$$

where erf is the error function. The absorption of the sample at the pump or probe wavelength is shown in **Figure S4**.

We calculate the absorption by measuring the laser power of the pump and probe beams before and after the sample and accounting for reflection from a glass substrate at normal incidence. The temperature rise for our samples ranged from 0.8 mK to 1.3 mK depending on the pump and probe powers and spot sizes used in the measurements.

Even though transient temperature rises are quite small, we observe long time heating effects after laser exposure time as little as 5 minutes which is most likely due to the accumulation of pulse energy from our 80 MHz oscillator. This thermal runaway is characterized in **Figure S3** which shows the signal at a specified delay time as a function of lab time. Silk is insensitive to these effects while native and recombinant SRT increase substantially with lab time. Because of this, we only consider measurements taken within 5 minutes to be representative of the intrinsic material properties we are interested in and must rely on measuring multiple spots on the sample to gather reliable data.

TDTT on *E. coli.* suspensions

The transmission data on the over-expressed E. coli samples are obtained by focusing the probe beam approximately one millimeter into the solution. While the total path length of the sample is one centimeter, by focusing the beams towards the front of the solution we are able to avoid major losses from linear absorption of the medium. Though it is difficult to calculate temperature rises along the beam column at any point, the peak intensity, and thus largest modulation in transmission of the probe beam due to excitement of the sample from the pump, will occur at the focal point.

The average in-phase value used for Fig. 3d (main document) is calculated by averaging the normalized in-phase response of the transmission decay following pump excitation from pump-

probe time delays of 2 to 5 picoseconds; these values are also used for calculation of the error bars. These transmission measurements are also repeated on DI water and empty vector *E. coli.* as controls. We find that the DI water has nearly no change in in-phase signal for varying pump intensities and the non-expressing E. coli response is greatly reduced compared to that of the over-expressed *E. coli.* shown in Fig. 3d (main document). We show exemplary in-phase TDTT data on the DI water and empty vector *E. coli.* in **Figure S5**, which demonstrates the near negligible signal compared to the TDTT data on the over expressed *E coli.* (Fig. 3d, main manuscript).