Supporting information for: Real-Time Observation of Nonclassical Protein Crystallization Kinetics

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Supporting Information

Video on crystallization

The crystal growth and the number of crystals observed by optical microscopy in a time series can also be followed in the video movie.mpg attached as separate file.

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FTIR and Zeta-potential data on protein stability

Fourier transform infrared spectroscopy (FTIR) measurements in D_2O and circular dichroism (CD) measurements in H_2O were performed to prove that BLG stays in its native state when increasing concentrations of $CdCl_2$ were added (main manuscript). Here, in Figure S1a, we show FTIR measurements over a time span of 85 min for the mainly used sample condition in this work (20 mg/ml BLG, 15 mM $CdCl_2$) to demonstrate that there are also no time-dependent changes in protein stability. Circular dichroism requires low protein concentrations at which no turbidity in regime II is visible. To confirm that the complete charge inversion takes place in the investigated $CdCl_2$ range, zeta potential measurements were performed (Figure S1b).

Additional SAXS measurements and analysis

In Figure S2, the position of the minimum intensity and the exponent of the intensity as a function of q, $I(q) \propto q^{\alpha}$, in the low q region are plotted as a function of time. The q values at the minimum intensity decrease slightly before crystallization, followed by an increase after 30 min, which is also the moment at which Bragg peaks become detectable as illustrated by the dashed black line in Figure S2. The slope of the scattering intensity in low q changes from $q^{-2.0}$ to $q^{-2.7}$, indicating the formation of more compact structures.

Figures S3-S5 show additional real-time SAXS data and analysis for a different sample within the region of *pseudo-c*** (33 mg/ml BLG with 17 mM $CdCl_2$).

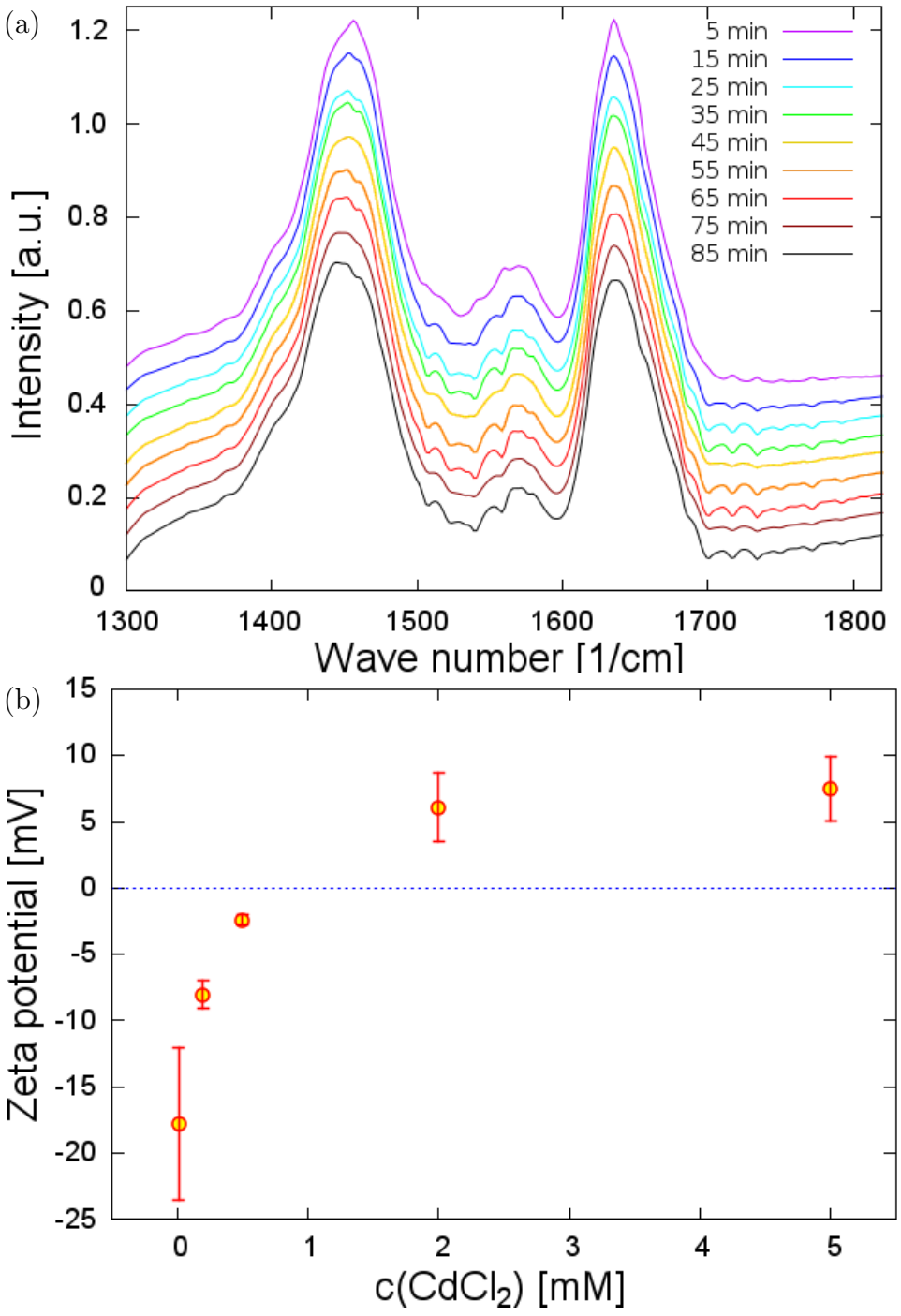

Figure S1: (a) Time-resolved FTIR spectra for a BLG concentration of 20 mg/ml and a $CdCl_2$ concentrations of 15 mM covering the time span of the SAXS experiments in time steps of 5 min. Curves are shifted in intensity for better visibility. (b) Zeta potential measurements for samples used for the CD experiments.

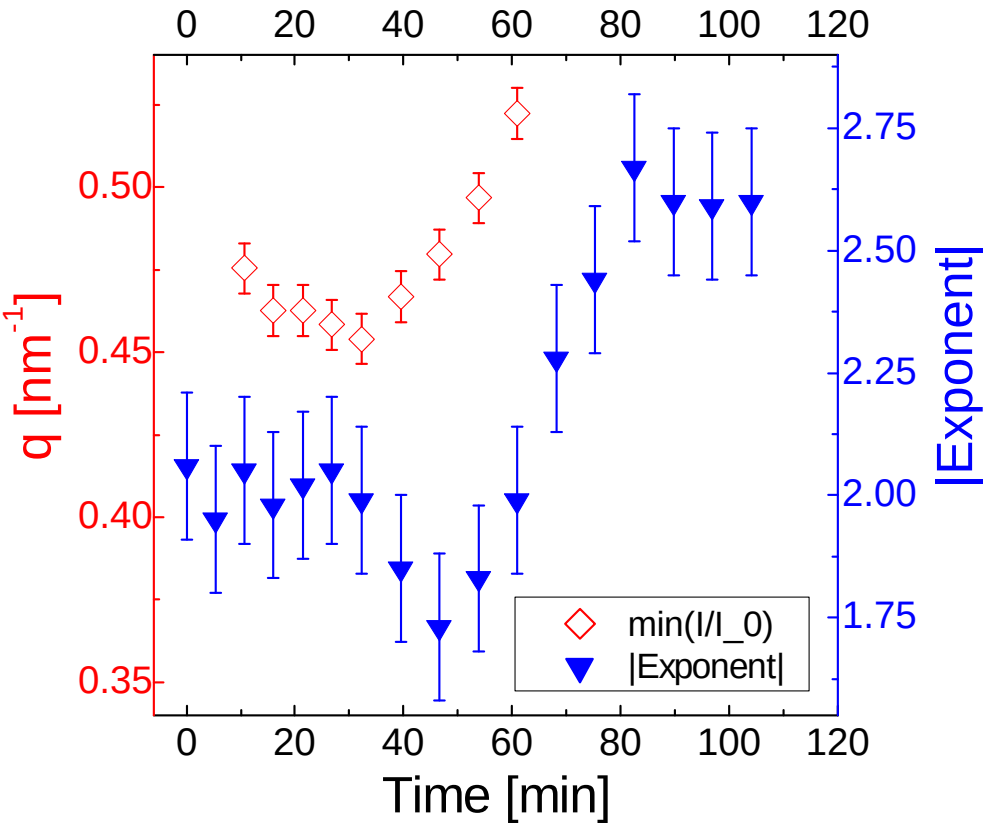

Figure S2: Position of scattering minimum of intermediate structure in $I(q)/I_0(q)$ and absolute value of exponent at low q for 20 mg/ml BLG with 15 mM $CdCl_2$.

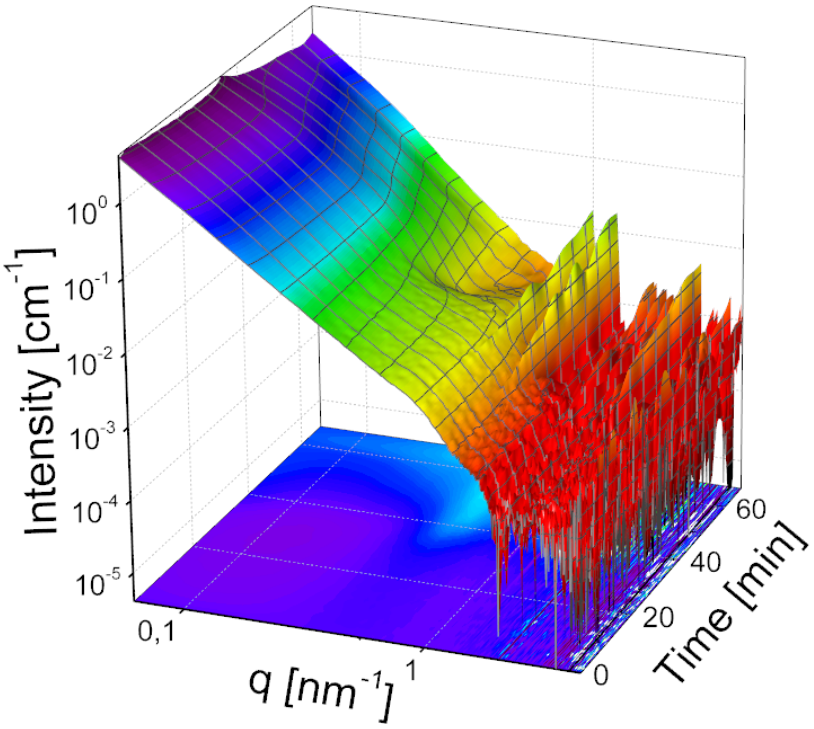

Figure S3: 3D-plot 33 mg/ml BLG + 17 mM $CdCl_2$.

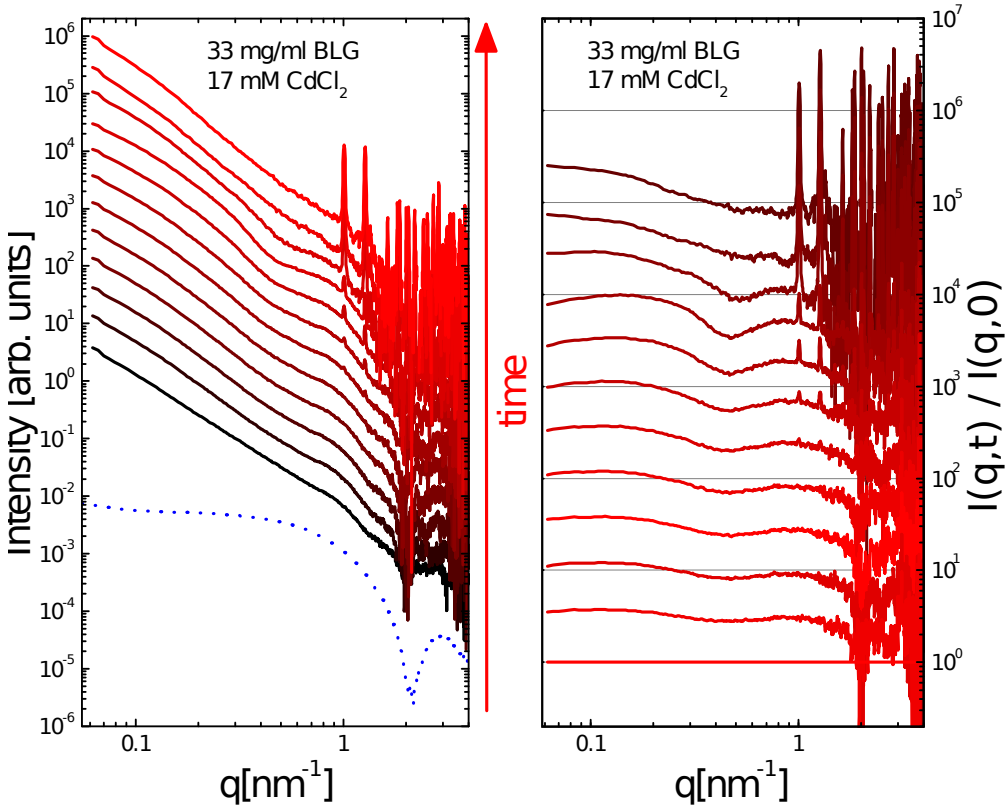

Figure S4: Overview: 33 mg/ml BLG, 17 mM $CdCl_2$.

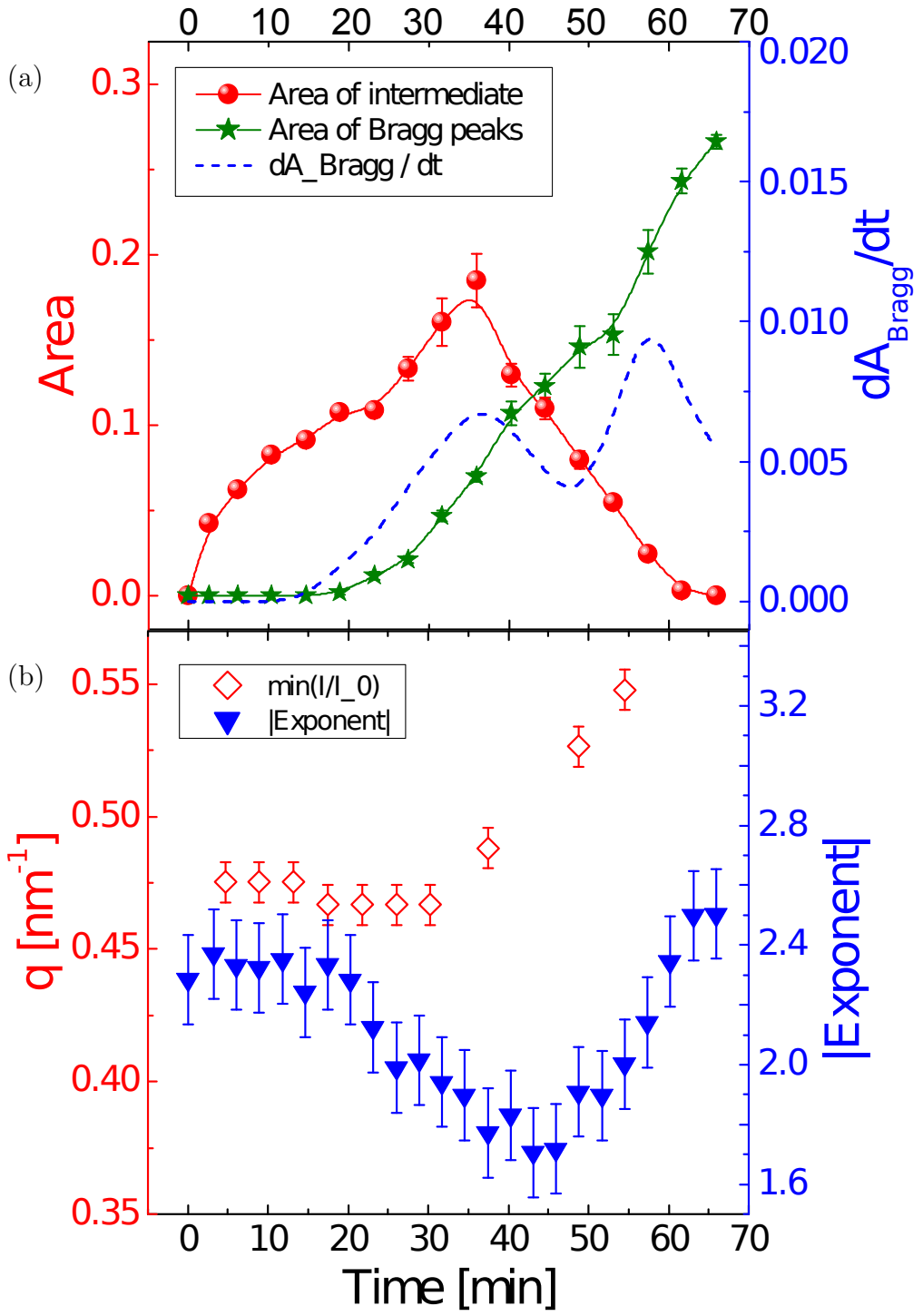

Figure S5: Time-dependent development of $I(q)/I_0(q)$ in 33 mg/ml BLG with 17 mM $CdCl_2$. (a) Red spheres (left axis) show $A_{\mathrm{interm}}(t)$, the integral of the broad Gaussian function connected to the intermediate. Green stars (left axis) show $A_{\mathrm{Bragg}}(t)$, the integral of the two Bragg peaks in this area. For clarity and better statistics, always three data points of $A_{\mathrm{interm}}(t)$ and $A_{\mathrm{Bragg}}(t)$ were merged into one. The blue dashed line (right axis) shows the time derivative of $A_{\mathrm{Bragg}}(t)$, the crystallization rate. (b) Position of minimum of intermediate structure and absolute value of exponent at low q. For clarity, only every third (minimum position) or second (exponent) data point is shown.