

## Supporting Information

# Gelation, Phase Behavior and Dynamics of $\beta$ - Lactoglobulin Amyloid Fibrils at Varying Concentrations and Ionic Strengths

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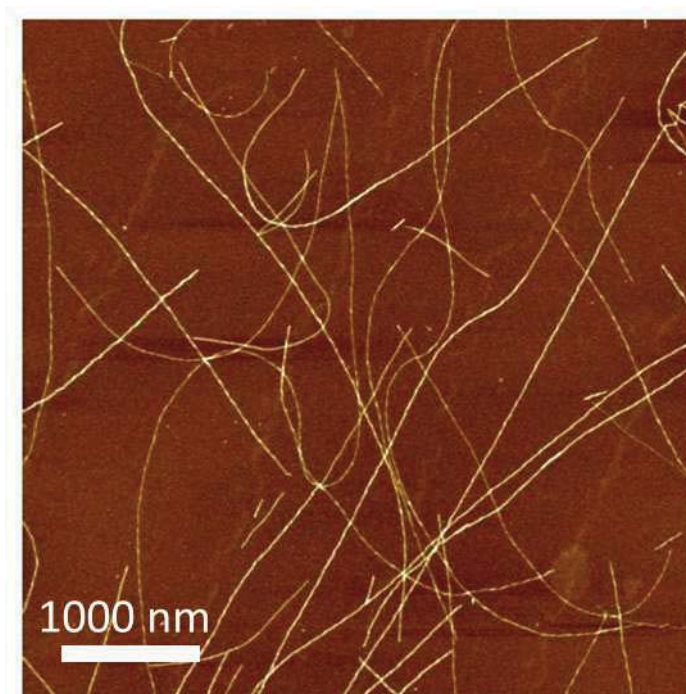
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### *Atomic Force Microscope*

Tapping mode atomic force microscope (AFM) was carried out on a Nanoscope VIII Multimode Scanning Force Microscope (Veeco). The 20  $\mu\text{l}$  of solutions were first deposited onto freshly cleaved mica sheets, incubated for 2 min, rinsed with Milli-Q water and dried by nitrogen. For all experiments, MPP-11100-10 tips for tapping mode in soft tapping conditions were used (Veeco, USA) at a vibrating frequency of 300 KHz. Images were simply flattened using the Nanoscope 8.1 software, and no further image processing was carried out.



*Figure S1. Atomic force microscope image of the  $\beta$ -lactoglobulin amyloid fibrils.*

### *Theoretical analysis of the SLS data by the PRISM model*

Scattering data collected at wide range of the magnitude of the scattering vector can be analyzed by polymer reference interaction site model (PRISM) to extract the intra- and intermolecular correlation functions. The background corrected scattering intensity  $I(\mathbf{q}, \rho)$  for a system consisting of monodisperse particles is given by PRISM as <sup>S1</sup>

$$I(\mathbf{q}, \rho) = \rho V_p^2 (\Delta k)^2 (P(\mathbf{q}) + \rho h(\mathbf{q}, \rho)) \quad (1)$$

Where  $V_p$  is the volume of a dissolved particle,  $\rho$  is the particle number density, and  $\Delta k$  is the contrast of the solute resulting from the difference of average scattering length density of the dissolved particles and the scattering length of the solvent.  $P(\mathbf{q})$  is a particle-averaged intramolecular correlation function which characterizes the geometric shape of the particles. Hence  $P(\mathbf{q})$  describes how the scattering intensity is modulated by interference effects between radiation scattered by different parts of the same particle. The intramolecular correlation function depends on the particle number density and follows from a statistical average over particle configurations. The particle-averaged total and direct correlation functions  $h(\mathbf{q}, \rho)$  and  $c(\mathbf{q}, \rho)$ , respectively, are related to mutual interactions between different particles. These correlation functions are calculated numerically by solving the generalized Ornstein-Zernike equation<sup>S2</sup>

$$h(\mathbf{q}, \rho) = P(\mathbf{q})c(\mathbf{q}, \rho)P(\mathbf{q}) + \rho c(\mathbf{q}, \rho)h(\mathbf{q}, \rho)P(\mathbf{q}) \quad (2)$$

The PRISM formalism has been used to determine the total correlation function in anisotropic orientationally ordered phases consisting of flexible polymers,<sup>S3</sup> rigid rods,<sup>S4</sup> and rigid platelets.<sup>S5</sup>

Here we analyze the scattering intensity of  $\beta$ -lactoglobulin amyloid fibrils on the basis of supplementary eqn 1 – 5 of the PRISM formalism, pursuing a two-pronged strategy: (i) We analyze SLS intensities both in the isotropic phase and in the nematic phase using analytically tractable forms of the form factor  $P(\mathbf{q})$ , the structure factor  $S(\mathbf{q})$ , the total correlation function  $h(\mathbf{q})$  and  $c(\mathbf{q}, \rho)$ . (ii) We use the numerically determined intramolecular correlation function of the multistranded fibrils<sup>S6</sup> together with the Laria-Wu-Chandler closure<sup>S7</sup> in order to study local order on small length scales accessible by SANS. Both approaches provide important

information because presently, available theoretical tools and computer simulations are far from being able to capture both intramolecular and intermolecular correlation functions of  $\beta$ -lactoglobulin amyloid fibrils for various concentrations and ionic strengths.

If the preferred orientation of  $\beta$ -lactoglobulin amyloid fibrils of length  $L$  is the  $z$ -direction, we consider the following form factor:<sup>S4</sup>

$$P(\mathbf{q}) = \left[ 1 + L^2 \left( (1 + 2S_2)q_z^2 - (1 - S_2)q_\perp^2 \right) / 18 \right]^{-1/2} \quad (3)$$

where the subscript  $\perp$  refers to the plane orthogonal to the  $z$ -axis, and  $S_2$  is the usual nematic order parameter (in dilute isotropic phase  $S_2 = 0$ ). Here we have adopted spherical coordinates for the scattering vector with  $|\mathbf{q}|^2 = q^2 = q_z^2 + q_\perp^2$ . The total correlation function  $h(\mathbf{q})$  is related to the form factor  $P(\mathbf{q})$  by:<sup>S4</sup>

$$h(q) = \frac{c_0 P^2(\mathbf{q})}{1 - \rho c_0 P(\mathbf{q})} \quad (4)$$

The nematic order-dependent structure factor is obtained by the usual PRISM formalism from the above supplementary eq. 3 and eq. 4 as discussed in the main manuscript and more extensively in references S3-S4:

$$S(\mathbf{q}, \rho) = 1 + \rho h(\mathbf{q}, \rho) / P(\mathbf{q}) \quad (5)$$

In the limit of very small particle number densities  $\rho$ , it is possible to obtain an analytic solution of the generalized Ornstein-Zernike equation with the closure  $c(\mathbf{q}, \rho) = c_0$  when  $q < \pi/d_{eff}$  and  $c(\mathbf{q}, \rho) = 0$  otherwise.<sup>S4</sup> The parameter:

$$C_0 \approx -L^2 d_{eff} \frac{\pi}{3\sqrt{3}} \frac{\sqrt{S_2(1-S_2)}}{\arctan \sqrt{3S_2/(1-S_2)}} \quad (6)$$

follows from the core condition:

$$-1 = \frac{1}{(2\pi)^2} \int_0^{\pi/d_{\text{eff}}} dq q^2 \int_{-1}^1 d(q_z/q) h(\mathbf{q}, \rho) \quad (7)$$

Here the electric double layers around the fibrils are taken into account in terms of the effective diameter:

$$d_{\text{eff}} = d + \kappa^{-1} \left[ \log(2\pi\sigma^2 \kappa^{-1} l_B e^{-\kappa d}) + C + \ln 2 - 1/2 \right] \quad (8)$$

Where  $\kappa$  is the inverse Debye screening length,  $\sigma$  is the linear charge density of the fibrils,  $l_B$  is the Bjerrum length,  $d$  is the diameter of the fibrils, and  $C \approx 0.577$  is Euler's constant. The effective diameter of  $\beta$ -lactoglobulin amyloid fibrils at various ionic strengths and pH values has been discussed in detail in Ref S8. Thermodynamic properties can be calculated from the compressibility relation leading to the following excess part of the free energy per interaction site:

$$f_{\text{exc}} = k_B T \frac{\rho d}{2L} |c_0| \quad (9)$$

Here each fibril is considered to be composed of  $L/d$  tangent interaction sites of diameter  $d$ . The excess part of the free energy is due to interactions between fibrils, while the loss of free energy due to anisotropic orientation of fibrils is given by:

$$f_{\text{ori}} = -k_B T \frac{d}{l_p} \log \left[ (1 - S_2) \sqrt{1 + 2S_2} \right] \quad (10)$$

where  $l_p$  is the persistence length of the fibrils. A small nematic order parameter expansion

$f_{\text{exc}} + f_{\text{ori}} = f_0 + f_2 S_2^2 + O(S_2^3)$  yields:

$$f_2 = k_B T \left( \frac{3d}{2l_p} - \frac{2\pi L d d_{eff} \rho}{45} \right) \quad (11)$$

The isotropic phase becomes linearly unstable to orientational fluctuations when  $f_2$  changes sign at the volume fraction:

$$\phi_c = V_p \rho_c = \frac{135d^2}{16l_p d_{eff}} \quad (12)$$

where  $V_p = \pi d^2 L / 4$ . Using the previously determined model parameters  $l_p = 1980$  nm,  $d = 4$  nm, and  $d_{eff} = 20$  nm leads to  $\phi_c = 0.0034$  which corresponds to the concentration 0.34 wt % because the fibril density is  $1.002$  g/cm<sup>3</sup>.<sup>S8</sup> This concentration is comparable to the experimentally determined concentration at the isotropic to nematic phase transition (see Figure 1 in the main manuscript and in reference<sup>S8</sup>).

Figure 5A in the main manuscript displays SLS intensities of  $\beta$ -lactoglobulin amyloid fibrils without added salt (symbols) together with the PRISM calculated radially averaged scattering intensities (lines), inclusive of the treatment of orientation anisotropic order in the nematic phase by:

$$I(q, \rho) = \int_{-1}^1 d(q_z / q) I(\mathbf{q}, \rho) \quad (13)$$

## Two dimensional SANS intensities

Now we turn our attention to the SANS data. The two-dimensional scattered intensities are shown in Figures S2 A - C for 0.3 wt %, 4 wt %, and 13.7 wt %  $\beta$ -lactoglobulin amyloid fibril suspensions without added salt. The scattering pattern is nearly isotropic for the 0.3 wt % sample, while it is anisotropic for the 4 wt % sample due to orientational ordering of the fibrils in

the nematic phase. In the case of the highly concentrated 13.7 wt % sample the scattering intensity distribution is rather isotropic except of two peaks at small scattering vectors. As mentioned before, at this high concentration, the system is in the gel phase: the isotropic nature of the gel is possibly the reason behind the decreased anisotropic scattering.

In order to obtain more information on the degree of anisotropy of the scattering intensity of the 4 wt % sample in the nematic phase, we have plotted in Figure S2 D the scattering intensity  $I(q_z, q_\perp, \rho)$  along the directions  $q_z = 0$  (red squares) and  $q_\perp = 0$  (blue circles). The difference between  $I(q_z, 0, \rho)$  and  $I(0, q_\perp, \rho)$  is rather small as compared to the pronounced differences found in computer simulations of short rigid spherocylinders.<sup>S9</sup>

In view of the rather small degree of anisotropy of the SANS data, Figure S3 A displays radially averaged scattering intensities of  $\beta$ -lactoglobulin amyloid fibrils (symbols) together with calculated scattering intensities (lines) for five fibril concentrations. Here we have used the interaction potential  $U(r)$  shown by the solid line in Figure S3 B together with the numerically determined intramolecular correlation function of multistranded twisted ribbon fibrils<sup>S6</sup> as input into the Laria-Wu-Chandler closure relation:<sup>S7</sup>

$$h(r, \rho) = \ln [h(r, \rho) + 1] + P(r) \star [c(r, \rho) + U(r)/(k_B T)] \star P(r) \quad (14)$$

where the star  $\star$  denotes a convolution product.

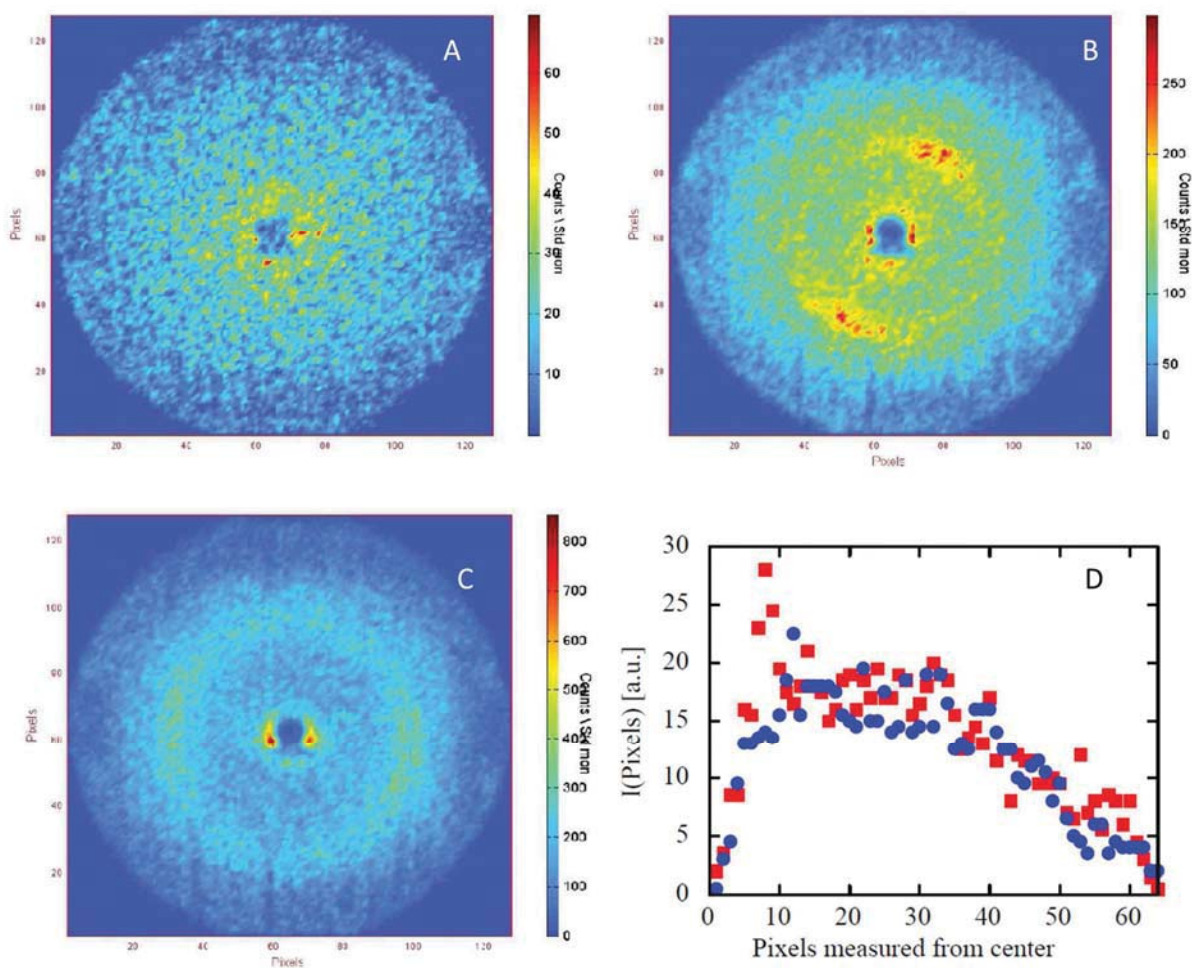


Figure S2 A), C) Two-dimensional small-angle neutron scattering intensity from  $\beta$ -lactoglobulin amyloid fibrils without added salt. From panel A to C, the concentrations of the samples are 0.3 wt %, 4 wt %, and 13.7 wt %. D) Scattering intensity along the direction of the outer peaks of the 4 wt % fibril suspension (red squares) together with the scattering intensity along the orthogonal direction (blue circles) plotted as a function the radial direction from the center of the figure in panel C.

Thereafter the direct and total correlation functions have been calculated numerically by solving the generalized Ornstein-Zernike equation together with the Laria-Wu-Chandler closure relation.



From Figure S3 A one may conclude that the density-independent interaction potential  $U(r)$  together with the integral equation theory yields shapes of the scattering intensities which are similar to the experimental data for fibril concentrations up to about 4 wt %. This interaction potential has to be considered as an effective interaction potential taking into account both steric and Coulomb interactions as well as many body effects. For the 13.7 wt % sample the calculated scattering intensities on the one hand and the experimental data on the other hand deviate, indicating a change of local order. The pronounced excess scattering at small scattering vectors can be due to correlations associated with a network structure. Moreover, the peak at  $q \approx 0.61 \text{ nm}^{-1}$  reflects small range order with a characteristic real space distance of  $2\pi/q \approx 10 \text{ nm}$  that is comparable with the diameter of the fibrils. Future theoretical work may focus on a detailed understanding of local order  $\beta$ -lactoglobulin amyloid fibrils at high concentrations.

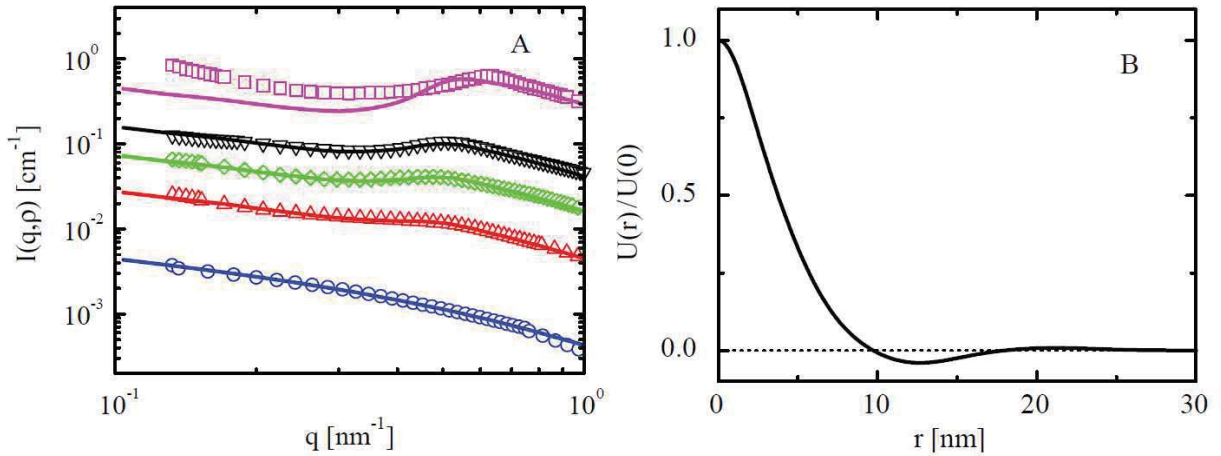
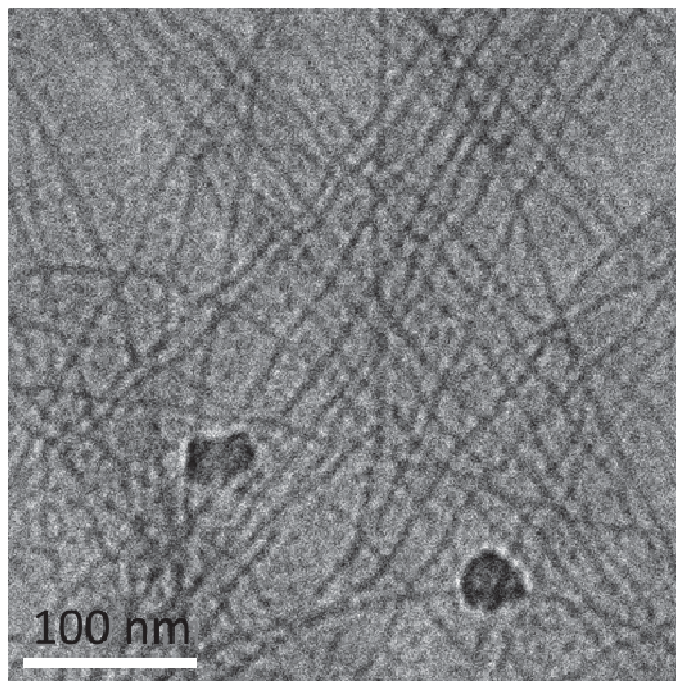


Figure S3 A) Measured small-angle neutron scattering intensity  $I(q, \rho)$  of  $\beta$ -lactoglobulin amyloid fibrils without added salt for five fibril concentrations: 0.3, 1.7, 3, 4, 13.7 wt % (from bottom to top). For clarity, the uppermost scattering densities have been shifted up by a factor 2, 4, 8, 16, respectively. The solid lines represent the results of the integral equation theory using the effective interaction potential  $U(r)$  shown by the solid line in panel B as input in eq 14.



*Figure S4: Cryo-TEM representative image of  $\beta$ -lactoglobulin protein fibrils at 0.5 wt % concentrations.*

Concentration of $\beta$ -lactoglobulin fibrils in wt %	Rotational diffusion coefficient ( $s^{-1}$ )	Translational diffusional coefficient ( $nm^2/s$ )
0.05	895	$4.6 \cdot 10^8$
0.1	858	$4.42 \cdot 10^8$
0.15	844	$4.14 \cdot 10^8$

*Table S1. Rotational & translational diffusion coefficient measured by depolarized dynamic light scattering at different concentration of protein fibrils.*

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