1. Particle Interactions

We observe a small but significant increase in scattering intensity at low $q$-values when we reproduce the pH 7 data in Figure 1 with a sample containing additional small amounts of NaCl (ionic strength of solutions between 5 and 10 mM; see Fig. S1). At low ionic strengths we observe the influence of repulsive interactions between the proteins, a typical sign of which is the suppression of the scattering at low angles (low values of $q$). It is therefore not correct to determine $R_G$ from the sample at pH 7 without added salt as the Guinier extrapolation is valid for non-interacting particles only. However, samples with 5 and 7.5 mM NaCl respectively give very similar scattering curves with a flat plateau at small angles. This result suggests that at these salt and protein concentrations the proteins can be viewed as non-interacting. Increasing the NaCl concentration to 10 mM leads to an upturn of the scattering intensities at small angles, indicating that the interaction potential already became effectively attractive (Fig. S1). Therefore, the sample with 5 mM NaCl was used for the evaluation. At pH values lower than 7 only a very little influence of the salt concentration on the scattering curve is observed (Fig. S2). The intrinsic ionic strength at these pH conditions is sufficient to screen the electrostatic repulsions.
**Figure S1:** Subtracted small angle x-ray scattering curves (sample – water) of native 1 wt% β-Lactoglobulin solution at pH 7 and 25°C with different concentrations of added salt (data are slit smeared).

**Figure S2:** Small angle x-ray scattering curves (sample – water) of native 1 wt% β-Lactoglobulin solution at pH 5.9 and 25°C in water and with 5 mM NaCl added (data are slit smeared).
2. Stability of native protein solution

Figure S3: Small angle x-ray scattering curves of native 1 wt% β-lactoglobulin solution at pH 5.9 as a function of storage time at 25°C. No measurable changes can be found within the observation time (5 days).

3. Polydispersity of Mβlg – centrifugation experiment

In order to characterize the internal structure of Mβlg the polydisperse sample was fractionated by mild centrifugation. After 6 hours of centrifugation at 450 g, a relatively narrow monomodal distribution was obtained. This can be seen from the extended linear range of the Guinier plot (Figure S4) and the weak angular dependence of the hydrodynamic radius (Figure S5). This sample now contains only one particular size distribution of Mβlg and can thus be used to study the internal structure avoiding the difficulties connected with polydisperse samples. The ratio of $R_G$ and $R_H$ (extrapolation to a scattering angle of zero) is 0.6 and thus much smaller than 0.775, which is typical for homogeneous spheres. From Cryo-TEM it is known that Mβlg are spherical in shape. Therefore we can conclude that the internal structure has to be inhomogeneous with a relatively dense core and a loose shell that contains more solvent.
**Figure S4**: Guinier plot of static light scattering on βlg heated at 85°C for 15 min and after gentle centrifugation (450 g) for different times. A Guinier extrapolation could be performed after centrifugation for 6 h. $R_G$ was 54 nm then while $R_H$ (from DLS) of the same was 90 nm. Homogeneous spheres are characterized by a value for $R_G/R_H = 0.775$. Here we now measured $R_G/R_H = 0.6$, which is characteristic for particles possessing a rather dense core surrounded by a rather loose shell.
Figure S5: In agreement with the static intensities shown in Figure S6 also the dependence of the hydrodynamic radius is strongly affected by the polydispersity of the original sample. The larger particles contribute much more to the overall signal at small angles compared to large ones. In the observed angular regime (20 – 150°) the detected hydrodynamic radius drops from more than 200 to less than 100nm. After centrifugation for 6 hours only the smallest particles are left in the supernatant and the polydispersity is very much reduced. The decay in hydrodynamic radius with q is only very small and appears to be linear. This allows for an extrapolation to a scattering vector of zero.


Light scattering is very sensitive to impurities of large particles. Especially at small scattering angles they dominate the signal. Therefore, the angular dependence of the two parameters of the Cumulant analysis (hydrodynamic radius and polydispersity parameter) contains valuable information about the sample polydispersity. In Tables S1 and S2 the values obtained for various samples are summarized. The strong increase of \( R_H \) at small angles is an indication for the presence of large aggregates. If these large particles were absent, \( R_H \) would increase less steeply. For a perfectly monodisperse sample, \( R_H \) is constant and no angular dependence is expected.
Table S1: Angular dependence of the hydrodynamic radii $R_H$ for all samples investigated by dynamic light scattering. For the interpretation mainly the value measured at 120° was used. However, the angular dependence contains valuable information about the sample polydispersity. Only for a perfectly monodisperse sample the measured $R_H$ is independent of the scattering angle. For polydisperse samples $R_H$ decreases with increasing scattering angle. Very large $R_H$ values at very small angles indicate traces of impurities consisting of larger species.

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<th>q (nm⁻¹)</th>
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<th>Insoluble fraction</th>
<th>Soluble fraction</th>
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Table S2: Angular dependence of the polydispersity parameter of the cumulant analysis (PDI) for all samples investigated by dynamic light scattering. It is evident that the PDI of Mblg decreases during heating. Finally, a polydispersity of approximately 20% is achieved.

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