Supporting information for:

Experimental Evidence for a Cluster Glass Transition in Concentrated Lysozyme Solutions

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Verification of functionalization

Particles are dispersed in a 2 M NaCl solution that will effectively screen any electrostatics present. This will lead to aggregation in the case of particles not being sterically stabilized due to failure during the functionalization step. Verification of stable particles is performed by continuously recording the apparent particle size using dynamic light scattering. An example is shown in Figure S1 compared to untreated particles.

![Figure S1: Evolution of apparent hydrodynamic radius of functionalized (red diamonds) and untreated (blue triangles) tracer particles suspended in 2 M NaCl verifying the steric stabilization of the functionalized particles. The data is normalized with the hydrodynamic size measured in salt-free water, where neither particle type shows signs of aggregation.](image)

S2
Full dataset of lysozyme samples

For the sake of clarity, only a subset of the investigated range of lysozyme samples is shown in the main text. In this section we present the same information as shown in Figures 1 and 2 of the main text, but with the full set of data. It became apparent that it is very difficult to prepare samples with $\phi > 0.3$ using the aforementioned method, still a rather large variety in viscosity (Figure S2) and particle mobility (Figure S3) was found in this region since a small change in concentration will have a significant impact on parameters such as viscosity. This was directly observed in sample $\phi = 0.35$ where unwanted evaporation of the small sample volume for MPT was sufficient to induce a concentration change that significantly alters the results between the two techniques. Of special note is the sample with $\phi = 0.29$ which displays a clear signature of the arrested state using MPT. This was due to a preparation error, which led to a considerable delay (approximately 2 min) between deposition of the droplet onto the glass slide and sealing of the sticker cell with a cover slip which was sufficient to completely change the sample conditions.

Figure S2 also demonstrates that the value of $\phi_{\text{max}}$ obtained from the concentration dependence of the relative viscosity is quite insensitive to the choice of the relationship used to fit the data, as it also includes an analysis using the Krieger-Dougherty relation (KD), leading to $\phi_{\text{max}} = 0.35$. We see that the experimental data is indeed quantitatively reproduced by the KD model, which takes the form:

$$\eta_r = \left(1 - \frac{\phi}{\phi_{\text{max}}}\right)^{-[\eta]_{\phi_{\text{max}}}}$$  \hspace{1cm} (1)

where the two fitting parameters are the intrinsic viscosity $[\eta]$ and the volume fraction $\phi_{\text{max}}$ where the zero shear viscosity is found to diverge.
Figure S2: Relative sample viscosities for the MSDs displayed in Figure S3 obtained from CLSM-based MPT (squares) and DLS (circles) agree well below $\phi \approx 0.30$. At a higher concentration there is enough evaporation before the CLSM-sample can be sealed to induce a clear deviation between the two techniques. The relative viscosity from MPT of the two least concentrated samples in the evaporation series (blue squares, see also Figure 3 in the main text) agrees well with the non-evaporated samples. Two reference samples with non-fluorescent tracer particles measured with DLS are also included (white circles). The Quemada fit (black line) to the data yields a volume fraction for the liquid-solid transition of approximately 0.34, while a fit of the Krieger-Dougherty relationship (red line) gives a volume fraction of 0.35 for the transition. Inset: magnification of volume fraction range $0.26 < \phi < 0.34$ on linear axes.
Figure S3: Comparison of MSDs for the viscosities displayed in Figure S2 mirroring the agreement below $\phi \approx 0.30$ while clearly demonstrating the deviation for the sample with $\phi = 0.35$ as described in the text.
DLS-MSD

Figure S4 shows the MSD extracted from DLS for the sample with the highest concentration (\(\phi = 0.35 \pm 0.03\)) versus the time in a double logarithmic representation. The figure demonstrates that the MSD is completely linear in this representation, with a slope of 1, thus verifying that the tracer particles exhibit purely diffusive behavior over the entire accessible time window. This validates our approach to extract the zero shear viscosity from DLS-microrheology using the Stokes-Einstein relation.

Figure S4: MSD obtained from DLS experiments for the most concentrated sample at \(\phi = 0.35 \pm 0.03\) versus lag time \(\tau\). Perfect overlap is seen between the experimental data (circles) and predictions for purely diffusive motion (red line).
Evaporation experiment

For the evaporation experiment, a double-sided adhesive spacer cell (Secure-Seal Spacer, 9 mm diameter, 0.12 mm deep) was stuck on a clean glass slide. The glass slide and cover slip were placed on a scale with 0.01 mg accuracy and weighed just before and after the addition of 5 µl sample. The glass slide was carefully left undisturbed for the appropriate time after which the cover slip was pressed on top of the adhesive spacer, thus sealing the cell. The glass slide was then weighed again and the final average concentration was determined from the change in mass. Figure S5 shows the complete concentration series with squares denoting the samples further characterized with MPT in the main text.

Figure S5: Final sample concentrations after controlled evaporation of a 5 µl stock sample volume with concentration 369 mg/ml (ϕ = 0.27) placed in an open sample cell. The change in concentration was carefully monitored gravimetrically and subsequently terminated by sealing the cell. Concentrations and volume fractions were calculated assuming a homogenous sample. A reference sample immediately sealed was also monitored over several hours to establish that evaporation is absent after sealing. Square symbols represent samples further investigated using MPT as described in the main text. Inset: The change in reduced weight over time for both reference and the evaporation samples.
Comparison with literature

Comparison with the data presented by Godfrin\textsuperscript{S1} is shown in Figure S6 and appears rather consistent with our results, especially when taking the differences in sample preparation under consideration. A possibility is that the concentrations achieved in literature were simply not sufficiently high to completely reach the cluster glass transition.

![Figure S6: The data presented in Figure S2 together with the data from Godfrin\textsuperscript{S1} agrees remarkably well. All the data was obtained at 20°C but there are other significant differences with comparison to literature, such as sample preparation, solvent condition and measurement technique.](image)

References

(S1) Godfrin, P. D. Thermodynamic and Material Properties of Reversible Cluster Formation - Application to Concentrated Protein Solutions. Ph.D. thesis, University of Delaware, 2015.