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Protein self-diffusion in crowded solutions

The motion of macromolecules inside a biological cell is strongly influenced by the presence of other macromolecules in the intracellular fluid. Therefore, a quantitative understanding of macromolecular motion driven by diffusion on a molecular scale contributes to fundamental biological insights. Biological macromolecules and in particular proteins are soft and in general inhomogeneous in shape and surface charge pattern. Nevertheless, recent simulation results on the protein diffusion in a hypothetical cellular environment have found reasonable agreement with predictions from colloid theory [1, 2]. In our study [3], we experimentally investigate the protein self-diffusion under the conditions of macromolecular crowding and compare the results with the existing colloid theories. In our model system, the protein Bovine Serum Albumin (BSA) in an aqueous (D_2O) solution serves as both crowding agent and tracer particle at the same time.

The nanosecond time window of neutron backscattering allowed for the first time to access the short-time limit of protein self-diffusion. In this limit, the diffusion is solely affected by hydrodynamic interactions, since the displacement is too small to considerably change interparticle interaction potentials. A large number of backscattering spectra have been recorded to obtain the diffusion constant D(φ) as a function of the protein volume fraction φ. The model used for the fits accounts for intramolecular dynamics, the instrumental resolution and, importantly, diffusion of the entire protein (an example data set with fit components is depicted in the inset of **figure 1**) [3]. The quadratic behavior of the linewidths γ of the narrow Lorentzian (green line), representing the diffusion of the entire protein, versus the scattering vector Q indicates simple diffusive behavior on the experimental time and length scales even in crowded solutions (main part of **figure 1**).

However, due to the experimental scales, the extracted diffusion coefficient D(φ) consists of both translational and rotational diffusion. In order to isolate the experimental translational diffusion coefficient D_t(φ), we developed a new framework for the full volume

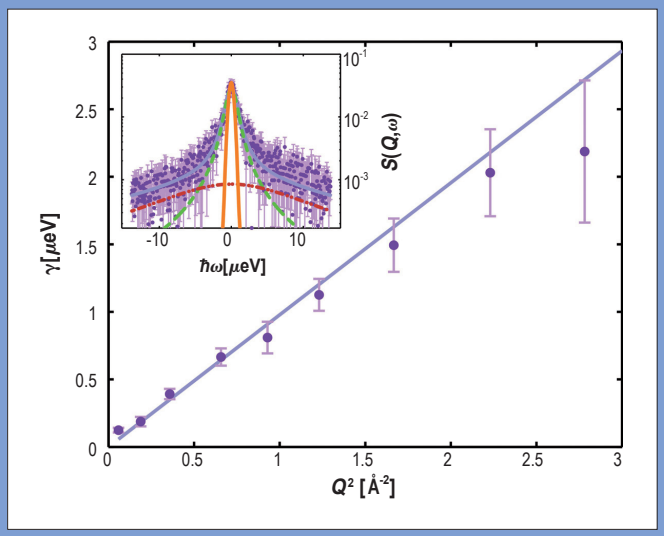

Figure 1:
Inset: *Example of a backscattering spectrum (symbols) recorded at IN16 for BSA in D_2O (concentration 500 mg/mL, φ = 28.5 %, T = 300 K, individual detector at Q= 0.81 Å^{-1}). The model (magenta solid line) accounts for diffusion (green dashed line), intramolecular dynamics (red dash-dotted line) and the resolution function (orange solid line). Main figure: Fitted linewidth γ (symbols) of the narrow Lorentzian vs. Q^2.*

Backscattering spectrometers IN10 and IN16
ESRF beamline ID02

Reaction kinetics, information exchange, and transport processes in biological systems are based on molecular motion. The interior of biological cells contains macromolecules at a high volume fraction typically in the range of 30 to 40% in the aqueous intracellular fluid. This macromolecular crowding influences the macromolecular motion, which governs intracellular transport processes and information exchange. Applying neutron backscattering, we study concentrated aqueous solutions of a globular protein as a model system. The resulting protein short-time self-diffusion as a function of the protein volume fraction on nanosecond time scales and nanometer length scales agrees quantitatively with predictions from colloid theory. This opens up a new path to a fundamental understanding of protein diffusion.

fraction range based on predictions [2] for diffusion of charged and uncharged hard spheres. To this end, the protein molecule was mapped to an effective sphere using Perrin factors and an approximate ellipsoid shape using data from small-angle X-ray scattering. By this means we can compare the theoretical predictions from colloid theory for effective hard spheres to the experimental system of non-spherical soft proteins.

Figure 2 displays the resulting experimental translational diffusion constants $D_t(\varphi)/D_0$ for two temperatures (symbols) in comparison with theoretical predictions for short-time self-diffusion of charged and uncharged hard spheres (blue dashed and blue solid line, respectively). D_0 is the diffusion constant in the dilute limit of the protein concentration obtained from dynamic light scattering. Within the experimental accuracy, the agreement is quantitative, suggesting that the slowing down of the self-diffusion is caused solely by hydrodynamic interactions. This agreement despite the rather simplistic spherical protein model is promising for future studies also on intramolecular dynamics of freely diffusing proteins is aqueous solution [4].

The experimental data (**figure 2**) show that $D_t(\varphi)/D_0$ decreases by more than a factor of 5 at biologically relevant volume fractions around 30% compared to the dilute limit. This considerable slowing down of the self-diffusion already on nanosecond time scales and nanometer length scales indicates a non-negligible role of hydrodynamic interactions also for longer time scales.

In conclusion, hydrodynamic interactions are an important ingredient for a full understanding of cellular processes and macromolecular crowding. Interestingly, diffusion can be understood at least to some level in terms of colloid theory also for the case of complex biomolecules. Future studies will address charge effects through a systematic charge tuning. This fundamental research can be assumed to be of interest in biomedical applications.

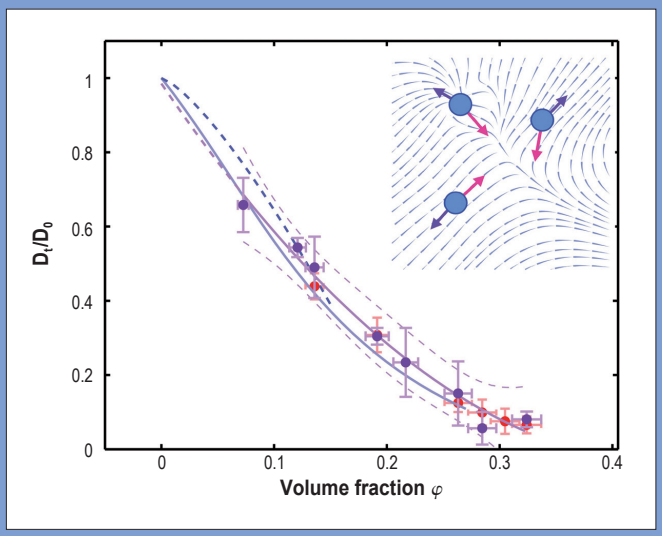

Figure 2:
Normalised translational self-diffusion coefficients $D_t(\varphi)/D_0$ (circles) vs protein volume fraction φ in aqueous solution for two temperatures (red: 280K, purple: 300K) indicating a temperature-independent master-curve (purple line as guide to the eye).
The data agree with predictions from colloid theory for short-time self-diffusion of hard spheres (light blue solid line) and charged spheres (dark blue dashed line).