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## Anomalous Protein Diffusion and Solvent-mediated interactions in Crowded Solutions with Coherent X-Ray Scattering Using XFELs

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Proteins play essential roles in life, for instance serving as carriers, participants in the immune response or for their structural role. In vivo, they exist within crowded environments with protein volume fractions typically ranging as high as 30%. When the environment becomes highly concentrated, the dynamics of proteins deviate significantly from those observed in a dilute system. However, the precise mechanisms influencing these dynamics across different time scales are not yet fully understood. Here we present our recent results [1], where we investigated the effect of self-crowding on protein diffusion in a ferritin solution with varying concentrations using X-ray Photon Correlation Spectroscopy. This technique allows simultaneous monitoring of both the structure, through small angle scattering, and the diffusion, through intensity-autocorrelation functions, of the protein solution, as demonstrated in our previous study [2]. By analyzing the scattering intensity, we observed that the ferritin particles become more densely packed with increasing protein concentration, indicated by a pronounced peak in the structure factor that shifts towards lower momentum transfer values. The protein diffusion, measured at all concentrations, follows a Brownian type of motion, but exhibits deviations at the peak position. This deviation can be attributed to the crowding effect caused by neighboring proteins, which act through hydrodynamic interactions. The hydrodynamic functions, which reflects these interactions, exhibit a peak which coincides with that of the structure factor indicating the connection of the crowding and the hydrodynamic interactions. To elucidate the underlying mechanism, we compare the hydrodynamic functions with estimations based on the  $\delta\gamma$ -theory, which considers the non-trivial interactions between particles. The model indicates that the protein diffusion is slower than that of non-interacting hard spheres due to the presence of solvent-mediated interactions and effective local friction between the particles.

[1] Girelli, Filianina et al., in preparation

[2] Reiser, Girelli et al., Nature Communications 2022,13 (1), 5528

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