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Proton relaxometry in a wide frequency range to study protein rotational diffusion under crowding conditions

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NMR relaxometry is a standard widely used experimental tool for studying protein dynamics in solution. The main emphasis for the last decades was placed on internal conformational dynamics paying much less attention to the overall Brownian protein tumbling. In most studies, it was assumed that the tumbling correlation function is a simple exponential with a single correlation time or a bit more complicated function due to the non-spherical shape of a protein. This assumption looks reasonable for the case of highly diluted protein solutions, however at high protein concentrations, which are more closely related to conditions in a living cell, the inter-protein distances are similar to the size of proteins, hence inter-protein interactions of different nature come into play and start affecting the overall dynamics. We have undertaken an extensive study of the overall Brownian dynamics of three different proteins (alpha-crystallin, bovine serum albumin and lysozyme) using ^{1}H NMR-relaxometry in a frequency range from 20 kHz to 20 MHz. The study includes the measurements of the relaxation times T_2, $T_{1\rho}$ and T_1 of the integral protein proton signal at different frequencies, temperatures and protein concentration. We used high (Bruker, 400 MHz), low (Minispec, 20 MHz) field NMR spectrometers as well as field-cycling machine (Stellar); the overall number of experiments amounts to several hundreds. The important issue of the work is the correct quantitative analysis of the multi-exponential relaxation decays. Such a shape of the decays arises due to the wide distribution of the dipolar couplings and motional parameters of different protons in a protein. This non-exponentiality is however dependent on the size of a protein, temperature and resonance frequency. We analyse this phenomenon in detail and show that the only correct quantitative treatment of such decays is a determination of a mean relaxation rate of all protein protons which corresponds to the initial slope of the decays.

The relaxation times then were quantitatively analyzed by means of a global fit of all the data for each protein on the basis of the model-free approach. The large number of experimental points enables confident and reliable extraction of the form of the rotational correlation function of a protein. The analysis has clearly shown that high protein concentration not only makes the rotation slower due to the viscosity increase, it changes the form of the correlation function: inter-protein interactions induce small local anisotropy of Brownian rotation which leads to an appearance of the additional slow component of the correlation function.

The concentration dependence of the rotational diffusion rate was also compared to that of the macroscopic viscosity that was measured for the same samples independently. The relative change of the rotational diffusion in respect to the viscosity at different concentrations appears to be protein-specific. We argue that this effect is due to the anisotropic nature of inter-protein interactions, which should be taken into account in studies of protein dynamics under crowding conditions.