

MULTISCALE SPECTROSCOPY OF DIFFUSING MOLECULES IN CROWDED ENVIRONMENTS

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Living cells are known to be crowded with organelles, biomembranes, and macromolecules such as proteins, DNA, RNA, and actin filaments. It is believed that such macromolecular crowding affect biomolecular diffusion, protein-protein and protein-substrate interaction, and protein folding. In this contribution, I will discuss our recent results on rotational and translational diffusion of small and large molecules in crowded environments using time-resolved anisotropy and fluorescence correlation spectroscopy methods. In these studies, rhodamine green and enhanced green fluorescent protein are used as fluorescent probes diffusing in buffers enriched with biomimetic crowding agents such as Ficoll-70, bovine serum albumin (BSA), and ovalbumin. Controlled experiments on pure and glycerol-rich buffers were carried out as environments with variable, homogeneous viscosity. Our results indicate that the microviscosity differs from the corresponding bulk viscosity, depending on the nature of crowding agents (i.e., proteins versus polymers), the concentration of crowding agents and spatio-temporal scaling of our experimental approach. Our findings provide a foundation for fluorescence-based studies of diffusion and binding of biomolecules in the crowded milieu of living cells.