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Anomalous, caged and obstructed diffusion as seen through the lens of inverted variable-lengthscale fluorescence correlation spectroscopy

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The diffusion of macromolecules in cells and in complex fluids is often found to deviate from simple Fickian diffusion, and to have a strong dependence on lengthscale. Yet protein diffusion measurements usually only probe a narrow range of lengthscales. To circumvent this issue, we use variable-lengthscale fluorescence correlation spectroscopy (VLS-FCS), where the size of the volume of observation is varied over several orders of magnitude. We combine it with a numerical inversion of the data allowing to retrieve the mean-squared displacement of the process over over up to five decades in time. We performed computer simulations to examine the signature of several biologically relevant diffusion processes (simple diffusion, continuous-time random walk, caged diffusion, obstructed diffusion, two-state diffusion and diffusing diffusivity) in inverted VLS-FCS. We compare the results of our simulations to the diffusion of probes in gels and crowded polymer solutions. Although in both cases the diffusion is anomalous, it has astonishingly different characteristics in these two systems. Our work shows that even for noisy diffusion processes such as the ones encountered in cells, an unbiased discrimination between different classes of diffusion models is possible.

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