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Single-particle and ensemble diffusivities – Test of ergodicity

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Diffusion is the irregular, omnipresent motion of the elementary constituents of matter. It is prerequisite for life quite in general and key to innumerable processes in nature and technology. After one and a half centuries of diffusion measurements with large ensembles of diffusing particles [1], the option of single-particle tracking (SPT) with single molecule sensitivity [2] has recently provided us with a totally new view of diffusion. With this in mind, a central problem of matter dynamics can now be addressed by direct experimental evidence – the proof of the ergodic theorem indicating that the average value of the squared displacement $r^2(t)$ of a diffusing particle during a time interval t, if taken over many subsequent time intervals ("time average"), agrees with the average taken over many different particles ("ensemble average") during one and the same time interval t.

So far, the mutually contradicting measuring conditions have prohibited the application of ensemble and single-particle techniques to one and the same system: The trajectory of a diffusing single molecule is constructed by fitting the position of the molecule over time with SPT. Therefore the fluorescence signals of the molecules have to be clearly separated from each other, which requires very low concentrations. Additionally the measurements are limited by the signal-to-noise ratio, which is influenced by the brightness of the dye molecules as well as the integration time. Consequently there is an upper limit for the detectable diffusivity in SPT. Exactly the opposite conditions, namely high concentrations (for generating sufficiently strong signal intensities) and high diffusivities (for giving rise to observable displacements) must be fulfilled for the application of the pulsed field gradient (PFG) technique of NMR, representing the most powerful ensemble technique for diffusion studies.

By applying nanoporous glass as a host system and Atto532 as guest molecules, we found a system where the diffusivity can be controlled by adjusting the pore diameter. We were therefore able to bring the guest diffusivities into a range where both single-particle tracking and PFG NMR were applicable. The diffusivity of guest molecules in a mesoporous glass depends on their concentration. For high concentrations it is governed by guest-guest and guest-solvent interactions, whereas for low concentrations the host-guest interactions dominate. While single-molecule experiments are performed exclusively in the low-concentration regime, we managed to reduce the concentration in the PFG-NMR experiments to reach this regime.

For the first time, single-molecule and ensemble diffusion measurements are thus found to experimentally confirm the hypothesis of ergodicity, since (within the limits of accuracy) both techniques provide the same result [3]. With these experiments, the two so-far separated worlds of diffusion measurements have been brought together. As a prerequisite of this "marriage" we have considered a situation where the rules of normal diffusion are obeyed. However, single particle observations of e.g. biological systems [4] often seem to contradict ergodicity. Now, with the combined potentials of single-particle and ensemble measurements, the underlying reasons for such deviations of ergodicity can be revealed.

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