Single molecules in soft matter : a study of biomolecular conformation, heterogeneity and plasmon enhanced fluorescence

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7 Summary

In this thesis, we describe research on the dynamics of single molecules and individual nanoparticles in glycerol, a viscous liquid, at variable temperatures. Here, we summarize the main results and provide some perspectives on future work.

7.1 Temperature-cycle microscopy on single molecules

To access fast conformational dynamics of single molecules, we combine the temperature-cycle method and fluorescence resonance energy transfer (FRET) microscopy. Both polyproline molecules and double-stranded DNA (dsDNA) molecules possess rigid structures and are often used as model systems for FRET. Therefore, we first demonstrate our temperature-cycle method on these molecules. Chapter 2 and chapter 3 report on temperature-cycle microscopy of FRET-labeled polyproline and dsDNA molecules.

In chapter 2, we study FRET-labeled polyproline molecules of two different interdye distances, both in steady state and under temperature cycles. Single 6-residue polyproline constructs in steady state show a FRET efficiency distribution different from that expected by Förster theory. Instead of showing a major population in the high-FRET region, the steady-state FRET efficiencies show broad distributions. Moreover, on a single polyproline-6 construct, we observe transitions between a low-FRET state and a high-FRET state during 2600 temperature cycles. The resulting FRET efficiency distribution closely resembles that of the steady state. The origin of the FRET transitions was not determined. The results demonstrate that the temperature-cycle method is able to probe fast conformational dynamics.

In chapter 3, we further apply the temperature-cycle method on FRET-labeled single dsDNA molecules and compare the results with those for polyproline molecules of similar interdye distance. Using the assembly of a solid immersion lens and a single-component reflecting objective, we are able to improve the collection efficiency and spatial resolution of our low-temperature microscope setup and to access both fluorescence lifetimes and the polarization of the emitted light. In steady state, we observe different FRET efficiency

distributions on FRET-labeled polyproline and dsDNA molecules. Both distributions are broad. Polyproline constructs show a major population in the low-FRET region, while dsDNA constructs show a major population in the high-FRET region. Moreover, polyproline constructs and dsDNA constructs show different FRET behavior under the same temperature cycles. On polyproline constructs, we observe active transitions between three FRET states, a low-FRET state, a high-FRET state and a dark state. On dsDNA constructs, we find occasional transitions from a high-FRET state to a short-lived low-FRET state. We assign these transitions in FRET to the dye-dye interactions at short interdye distances, as suggested in molecular dynamics simulations [118] and other room temperature experiments [117, 124].

The results presented in chapter 2 and chapter 3 have demonstrated that the temperature-cycle FRET method provides new insight into fast conformational dynamics of single molecules. However, two challenges remain. On the one hand, the temperature-cycle method has been limited to glycerol so far. Glycerol, although often used as a cryoprotectant for biological molecules [88], shows complex dynamics at temperatures close to the glass transition [58,65,66,148]. On the other hand, the dye orientation cannot average out since we work below the glass transition (T_g). Therefore, besides the interdye distance, the dye orientation is another factor that has to be considered in temperature-cycle FRET measurements. Further work may require optimizing the temperature-cycle parameters to avoid complexity in solvent dynamics and allowing the dye orientation to average out during each measurement. For instance, by setting the low temperature of a temperature cycle above the crossover temperature (T_c). Besides using FRET labels, dyes and quenchers can also be used to probe conformational changes. Two-state dynamical systems such as DNA hairpins and Holliday-junctions are good candidates for further studies using the temperature-cycle method.

7.2 Heterogeneity in supercooled liquids

Although heterogeneity has been reported in many supercooled liquids by a variety of experimental methods, many questions remain. Previous work carried out in our group has indicated solid-like structures to appear at temperatures close to, but above the glass transition temperature (T_g) in glycerol [65,66,148]. However, the nature of such solid-like structures and their influence on the observed dynamical heterogeneity was not clear. In chapter 4, we report on a neutron scattering/diffraction study on supercooled glycerol with different thermal histories. We find that crystallite nucleation takes place at tempera-

tures close to T_g, but crystallization can only take place after a long time (30 hours) aging at 195 K (5 K above T_g). Our results emphasize the importance of controlling the thermal history for studying heterogeneity in supercooled liquids.

Without averaging over ensembles of molecules, heterogeneity is best examined at the single-molecule level [57–59]. Watching one molecule at a time, single-molecule studies report heterogeneity not only in space but also in time [33]. However, different spatial and temporal scales of heterogeneity have been reported in different experiments. Without an agreement on these physical measures, deeper mechanistic questions will remain open. In chapter 5, we demonstrate individual gold nanorods as new probes for studying heterogeneity in supercooled glycerol. Taking into account their volumes, tumbling rates of gold nanorods reflect the local viscosities. Following the rotational dynamics of 19 individual nanorods, we observe deviations of local viscosity from the bulk viscosity of glycerol at temperatures below 226 K. Our results indicate that heterogeneity at length scales comparable to the sizes of nanorods (30 nm) start to appear upon cooling below 226 K. Moreover, heterogeneity is not averaged over 50 times the rods' rotational time. However, the large volumes of our nanorods make it difficult to follow them at even lower temperatures. There remains a 10 K gap in temperature between our single-particle measurements and single-molecule measurements.

To explore the 10 K temperature gap between our single-particle study and the single-molecule studies, an obvious way is to use even smaller nanorods. In addition, fluorescence correlation spectroscopy (FCS) of dye molecules in the vicinity of gold nanorods can also be applied to report local viscosities, which is verified in the following section.

7.3 Plasmon enhanced fluorescence

Plasmonic nanostructures can strongly enhance the fluorescence of molecules nearby. The enhancement comes from two factors. On the one hand, the molecules in the vicinity of a plasmonic nanostructure feel an enhanced excitation owing to the concentrated optical field. On the other hand, both radiative and non-radiative decay rates of the dye can be enhanced by the antenna effect. Using a simple plasmonic nanostructure, a gold nanorod, we report large fluorescence enhancements of more than a thousand times for single dye molecules (crystal violet) in chapter 6. Allowing the dyes with a low quantum yield (0.019) to freely diffuse in glycerol, we observe strong fluorescence bursts in luminescence timetraces recorded on individual gold nanorods. From

the intensities of bursts, we calculate the fluorescence enhancement factors. Moreover, we demonstrate that the enhancement is strongly polarization dependent. Exciting the same gold nanorod at different wavelengths, stronger fluorescence enhancements are found when the excitation wavelength is close to the nanorods' surface plasmon resonance. In addition, we find that the fluorescence lifetimes during individual bursts correlate with their enhancement factors. A significant shortening in fluorescence lifetime is observed during bursts of strong intensities. This result clearly demonstrates changes in decay rates of the dye molecules. Fluorescence correlation spectroscopy (FCS) on the timetraces shows almost two orders of magnitude longer correlation time than what would be expected for free diffusion. It is likely due to sticking of dye molecules to the glass substrate.

Large fluorescence enhancement by individual gold nanorods demonstrated in this study provides a new tool for detecting weakly fluorescent molecules and for performing FCS at higher dye concentrations. However, much is to be learned in order to optimize this method for further applications. First, the spectral dependence of the fluorescence enhancement needs to be investigated. Understanding the spectral dependence will help to better design the experimental system, such as choosing proper dyes and nanostructures. It can potentially lead to spectrally selective detection of weakly fluorescent molecules. Second, the fluorescence enhancement strongly depends on the position of the dye relative to the nanostructures, which makes it useful for super-resolution imaging. This requires more experimental and theoretical work. Furthermore, FCS in the vicinity of a gold nanorod can extend the current FCS studies to higher concentrations and smaller volumes, which can be useful for studies of heterogeneity in supercooled liquids. Allowing dyes of low quantum yields to freely diffuse through the vicinity of a gold nanrod, FCS on the luminescence timetraces reflects the local viscosities. In this way, the detection volume is about $10^3\,\mathrm{nm}^3$. Comparing FCS in space and in time, dynamical heterogeneities can be studied. Moreover, since this method makes use of translational diffusion of dye molecules, it can be applied in a broad range of temperatures. Therefore, we hope that the 10 K temperature gap between single-molecule and single-particle measurements can be filled by applying FCS on single gold nanorods in the presence of freely diffusing dye molecules with low quantum yields.