

SINGLE MOLECULE ROTATIONAL AND TRANSLATIONAL DIFFUSION OBSERVED BY NEAR-FIELD SCANNING OPTICAL MICROSCOPY

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Recent advances in single molecule detection and spectroscopy have opened the possibility to investigate the physical and chemical environment at the molecular level. Initial experiments reported on the spectral selection of single impurities in a solid matrix [1] by tuning the excitation wavelength to the absorption band of a single molecule. Information on the specific local environment of the selected molecule could thus be extracted. During recent years, improvement in the efficiency of photodetectors and the introduction of scanning (probe) microscopes have created the possibility to observe single molecules at ambient conditions. The first true imaging of immobilized single dye molecules by optical means at room temperature was achieved by near-field scanning optical microscopy (NSOM) [2]. Several exciting single molecule experiments have been performed since then. The emission spectrum of a single molecule at various conditions was detected, revealing single molecule spectral jumps [3]. Single molecular fluorescence lifetimes were determined showing the local photophysical heterogeneity [4] and the influence of non-radiative quenching in the presence of an aluminum coated probe [5].

We have observed rotational and translational diffusion of single molecules under ambient conditions using a near-field scanning optical microscope with two polarization detection channels [6]. In successive images the fluorescence of single molecules was followed over about one hour, with 10 ms integration time, until photo-dissociation. The position of single molecular fluorescence could be located with an accuracy of 1 nm. From the lateral diffusion of Rhodamine-6G molecules on glass during successive images a diffusion constant of $(6.7 \pm 4.5) \cdot 10^{-15} \text{ cm}^2/\text{s}$ was determined. The orientation of the in-plane emission dipole of all molecules in one image could be directly determined with an accuracy of a few degrees, by simultaneous detection in two perpendicular polarization directions. By rotating the excitation polarization we could selectively excite different sets of molecules and compare their in-plane absorption and emission dipole orientation. Monitoring DiI molecules in PMMA over one hour we found rotation of less than 10 degrees for the majority of molecules, while incidental fast rotation and transition to a dark state occurs. The fluorescence intensity was observed to be molecule dependent, which is an indication for out-of-plane orientation and different local photophysical environment. In conclusion, single molecule experiments indeed provide a sensitive tool to study the local environment of a single molecule at ambient conditions.

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