Confocal Imaging of Tautomerism in a Single Molecule by Using Higher-Order Laser Modes

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Abstract

Single molecule scanning fluorescence microscopy is largely equivalent to the spatial mapping of molecular transition dipole moments at high resolution. In a typical experiment, the probability that a molecule with a fixed absorption dipole moment direction will absorb a given photon (and finally emit fluorescence) is proportional to cosθ, where θ is the angle between the transition dipole moment of the molecule and the polarization of the light beam. It has been demonstrated that the analysis of fluorescence patterns is especially useful in studying parent single porphyrin molecules [1] and their alkyl derivatives [2], generated by raster-scaning a sample through the focus of an azimuthally and radially polarized laser beam, as can be used to visualize simultaneously the effects of spatial orientation and tautomeration in a single molecule.

Tautomeration in porphyrin molecules involves the movement of two hydrogen atoms between the four nitrogen atoms in the inner cavity of the molecule, which leads to the rotation of transition dipole moments in the molecular frame [3–4]. As long as the reaction is not frozen on the timescale of the experiment, and the molecular plane is at the right angle to the detector, the observation of fluorescence, the observed excitation pattern must reflect the presence of two transition moments: one corresponding to a tautomeristic species, which leads to a ring-shaped pattern. For some molecules not a ring, but a double-tube emission pattern was observed, which is possible to be explained by a single transition moment of molecules for which the hydrogen transfer is frozen or by specific orientation of the emitting chromophores with respect to the detector, such that both transition moments are seen as oscillating along the same direction. In the study on single 2,7,12,17-tetraphenylporphyrine molecules embedded in a PMMA matrix, we have observed double-tube patterns, which in conclusive images show spontaneously their orientation to nearly orthogonal. More surprisingly, some changes in the orientation direction occurred through an intermediate case with a doughnut-shaped pattern.

"Breakfast in the Nano-World"

Typical results of confocal imaging of single quantum emitters using higher-order laser modes: Coffee Beans, Doughnuts, and Bonbons

Molecule as a classical dipole antenna

Calculated excitation patterns for a molecule with a single dipole moment

Calculated images, IEF, for different orientations of a single dipole emitted affected by radially polarized beam. The dipole is scanned in the x-y plane.

Fluorescence images of single Nile Blue dye molecules with radially polarized excitation focused with a 1.25 NA immersion objective. The field of view is 2.9 microns. The molecule labeled 2 has a transition dipole moment oriented perpendicular to the sample plane. The molecule labeled 3 has a transition dipole moment oriented in the sample plane.

"Molecular Roulette in a Nano-Casino"

Double hydrogen transfer in porphyrine resulting in pseudorotation of S,–S, transition dipole moment

 Orientation and tautomeration on a single molecule level

Fluorescence images observed for 2,7,12,17-tetra-t-butylporphyrine, excited either by an azimuthally (top) or a radially (bottom) polarized beam, showing three different orientations of the chromophore. A molecule lying flat on the surface has a dipole moment perpendicular to the molecular plane and perpendicular to the sample plane. In (b), two dipoles form the same angle with the surface plane (molecules stand edge-on), whereas in (c) one dipole is perpendicular to the surface and the other is almost parallel to it (molecule stands on one vertex). Left: Experimental pattern; right: simulated pattern

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References