

Imaging of Tautomerism in a Single Molecule

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Tautomerization is a fundamental process in chemistry and biology, where it plays a major role in vision and enzymatic reactions. Usually, extensive spectroscopic ensemble studies are required to identify a tautomeric equilibrium. For instance, indirect evidence for the fast motion of the two inner hydrogen atoms between the nitrogen atoms has been deduced for porphycene,¹ a constitutional isomer of porphyrin, from complex NMR^{1,2} and fluorescence spectroscopy^{3,4} studies in a solid host for both ground and excited singlet states.

A fascinating aspect of single-molecule spectroscopy (SMS) is its capability to detect a chemical reaction in a single chromophore. This has been reported, for instance, for electron transfer,⁵ conformational changes,⁶ or photochromism.⁷ These studies recorded variations in fluorescence decay curves, fluorescence intensity trajectories, or fluorescence spectra. The fast exchange of the hydrogens in porphyrin-like molecules, however, leads to a dynamic equilibrium of chemically equivalent structures and hence cannot be inferred from lifetime or spectral measurements. On the other hand, the two structures differ in the orientation of the transition dipole moments of the S_0 – S_1 transition. As determined from bulk fluorescence anisotropy studies,⁴ the S_0 – S_1 transition moments in the two *trans* forms enclose an angle of about 70–80° (Figure 1A). We will exploit this information in the interpretation of the intensity patterns of fluorescence from single porphycene molecules.

Single-molecule transition dipole moments have been used previously to probe the electric field distribution in the focal spot of a confocal microscope.^{8–10} In this report, we show that the analysis of single-molecule fluorescence patterns in real space excited by an azimuthally polarized laser beam (Figure 1B) provides an important alternative tool for analyzing structural changes in the large class of compounds where fluorescence resonance energy transfer (FRET), fluorescence decay curves, or fluorescence spectra cannot be used. Porphyrins and porphycenes are important representatives of such a reaction. We show for the first time the direct visualization of the two tautomers resulting from double hydrogen transfer in a single molecule of porphycene. Samples of porphycene immobilized in a 10–20-nm-thick PMMA layer have been prepared by spin coating of a 10^{-9} M solution in toluene onto a quartz cover slide. Two-dimensional (2D) spatial images of the fluorescence intensities of individual molecules were obtained by raster scanning the sample in 20-nm steps per 5 ms. The fluorescence intensity is proportional to $|p \cdot E|^2$, the square of the projection of the electric field E on the transition dipole moment p . Hence, the observed patterns reveal the orientations of p ^{8,9} and can be compared with numerical simulations taking into account the experimental parameters of our microscope.

We observed a total of 66 spatially separated single-molecule patterns on one sample that revealed characteristic blinking and

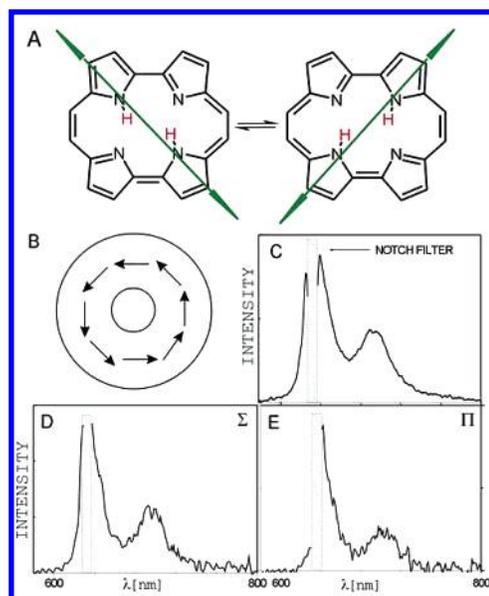


Figure 1. (A) Double proton transfer in porphycene, resulting in pseudo-rotation of transition moments p (thick double-headed arrows). (B) Electric field distribution in the focus of an azimuthally polarized laser beam (schematic). (C) Fluorescence spectrum of bulk porphycene in PMMA, excited at 632.8 nm; dashed areas indicate the part of the spectra cut out by the notch filter that is used to block scattered laser light. (D) Fluorescence spectrum obtained from molecule Σ . (E) Fluorescence spectrum from molecule Π .

sudden bleaching. Observation of single-molecule fluorescence from porphycene is not a trivial task in itself, since the quantum yield of triplet formation is about 40%.¹¹ However, our studies on the porphycene ensemble have shown that exposure of a thin polymer film sample to oxygen results in efficient triplet quenching (about 4-fold decrease of triplet lifetime from the value of about 200 μ s observed in the absence of oxygen) without acceleration of photobleaching. Using this procedure, a satisfactory count rate was achieved ($> 10^4$ s⁻¹), allowing the collection of a sufficient number of photons from a single molecule. The spectra of single porphycene molecules are similar but narrower than the bulk spectrum and reveal inhomogeneous broadening of the latter (Figure 1C–E). However, the spatial fluorescence patterns vary significantly for different molecules. Panels A and B in Figure 2 present the images obtained for two chromophores denoted Σ and Π . For the azimuthal polarization of the exciting light the double-lobe pattern observed for molecule Π is characteristic for a fixed transition moment oriented along the nodal line between the two intensity lobes. Crucial for our discussion is the observation of a ring shape for the other molecule, Σ . Such a pattern is not possible for any orientation of a single transition moment for the azimuthal polarization. For a molecule lying flat on the surface, it can only be reproduced with two transition moments of equal magnitude,

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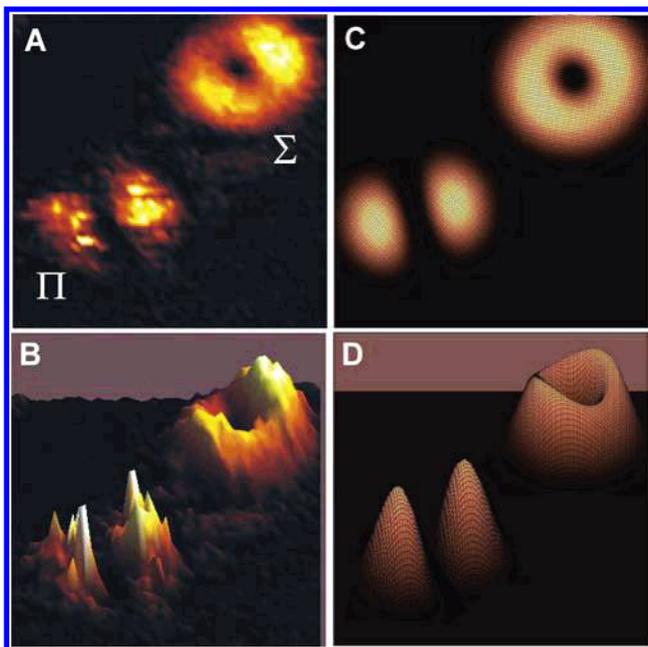


Figure 2. (A) Confocal image (scan range $2.5 \times 2.5 \mu\text{m}^2$) of single porphycene molecules showing two typical fluorescence patterns: a ring pattern for molecule Σ and a double-lobe pattern for molecule Π . (B) 3D plot of the same area as in (A). (C) Simulated fluorescence patterns for two differently oriented molecules with two transition dipole moments forming an angle of 72° (the same scale as (A)). In-plane orientation (tilt angle = 0°) was assumed for molecule Σ and 90° for molecule Π . (D) 3D plot of (C).

forming an angle of $72 \pm 3^\circ$. This result is in excellent agreement with the value of $71 \pm 5^\circ$ obtained from studies of bulk porphycene in polymeric films at 293 K. The error of $\pm 3^\circ$ is estimated for a fixed tilt angle. For out-of-plane orientations other angles between the transition moments can lead to a similar pattern, e.g., a 40° tilt yields an angle of 80° . Thus, the angle formed by S_0 – S_1 transition moments in the two tautomers can, in principle, be slightly different from 72° . However, the latter value seems most reliable, given the perfect match with the value obtained in an independent bulk measurement.

The double-lobe pattern observed for molecule Π can also be very well explained by such a molecule, where one of the transition moments is oriented perpendicular to the electric field polarization. In panels C and D of Figure 2 simulations of the fluorescence intensity patterns are shown for two such transition dipole moments. The observation of two transition moments represents the direct visualization of the two tautomers resulting from double hydrogen transfer in a single molecule.

The double-lobe pattern obtained for perpendicular orientation of the molecular plane with respect to the sample surface yields the same result as the case of a single transition moment, since now both transition moments are “seen” by the incoming beam as oscillating along the same direction. For an intermediate orientation, the observed pattern would contain both ring and double-lobe contributions. For a total of 66 patterns, 28 were rings and 10 were double-crescent shapes which have to be described by two transition

moments. The rest were double-lobe patterns which, in principle, could also be explained by a single transition moment of molecules where the hydrogen transfer is frozen. In this case, however, we should also have observed intermediate cases where the transition moment spontaneously changes the orientation which we actually never observed.

Two points should be noted: (1) Another source of a nonperfect ring shape could be due to the presence of a third transition moment. This is possible for porphycene, for which both *trans* and *cis* tautomers have been suggested,¹ the latter corresponding to the protons located on the adjacent nitrogen atoms. While we cannot at present exclude or confirm the presence of such forms, the results point to the dominance of *trans* tautomers. Simulations of the patterns expected for a different number of transition moments with different directions are under way. (2) Since our detection system does not discriminate between different polarizations in the emission, it cannot recognize a possible fluorescence depolarization due to the excited-state reaction. Most probably, the observed tautomerization contains contributions from both ground and excited-state processes. It should be noted that the time scale for tautomerization is orders of magnitudes shorter than the photon collection time. Various experimental arrangements are planned for separate detection of the reaction occurring in the lowest excited singlet and triplet states.

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Supporting Information Available: Details regarding the concept of azimuthally polarized light and the simulation procedure. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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