Nanoscopic Visualization of Soft Matter Using Fluorescent Diarylethene Photoswitches

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Abstract: The in situ imaging of soft matter is of paramount importance for a detailed understanding of functionality on the nanoscopic scale. Although super-resolution fluorescence microscopy methods with their unprecedented imaging capabilities have revolutionized research in the life sciences, this potential has been far less exploited in materials science. One of the main obstacles for a more universal application of super-resolved fluorescence microscopy methods is the limitation of readily available suitable dyes to overcome the diffraction limit. Here, we report a novel diarylethene-based photoswitch with a highly fluorescent closed and a nonfluorescent open form. Its photophysical properties, switching behavior, and high photostability make the dye an ideal candidate for photoactivation localization microscopy (PALM). It is capable of resolving apolar structures with an accuracy far beyond the diffraction limit of optical light in cylindrical micelles formed by amphiphilic block copolymers.

The nanoscopic structure of soft-matter materials determines their properties.[1] Therefore, methods to directly visualize structures in the nanometer range are of paramount importance for the ongoing evolution of novel materials with specialized and adaptive properties for sophisticated applications. Scanning probe microscopy techniques give access to the nanometer range and determine surface properties such as topology and softness,[2,3] while modern electron microscopistions, such as scanning electron microscopy (SEM)[4,5] and transmission electron microscopy (TEM),[6–8] can yield structural information even in the subnanometer range when there is sufficient electron density contrast. Despite the success of these methods, they are technically demanding and time-consuming. Furthermore, many soft-matter samples possess poor electron contrast, and require non-invasive in situ imaging below the surface as well as the possibility to directly study dynamics. In recent years, super-resolved fluorescence microscopy has revolutionized optical imaging,[9–14] by utilizing the photophysical or photochemical switching of fluorescent dyes in a sophisticated manner in combination with modern optics. So far, the life sciences have benefited, in particular, from the new possibilities of resolving structures well beyond the diffraction limit of light. Only a few examples of the application of super-resolution microscopy to materials science have been reported,[15–18] since concepts that require, for example, the addition of (polar) switching buffers often fail for these systems. Therefore, the main bottleneck for more universal applications of super-resolution imaging are switchable dyes with suitable (photo-)physical and chemical properties, such as high photostability, adjustable switching rates, minimum interaction with the environment to be probed, and simple design, with the possibility of multiple and straightforward derivatization for the specific labeling of structures or compartments.[19] Diarylenes represent a powerful class of organic photoswitches that have various applications such as data storage and chemical sensing.[20–22] Despite their wide variability, the lack of derivatives in which the closed form has a strong fluorescence in the visible range has prevented their direct application in super-resolution imaging. Strategies to covalently link diarylethene switches with suitable fluorophores and to utilize the energy[23] or electron transfer[24] of the resulting multichromophoric systems have been successful, but require extended syntheses and result in rather large probes. Labeling structures with such extended probes can significantly alter the structures to be visualized.[23,25] As an alternative, suitable modification of the diarylethene photoswitch itself could give direct access to the envisioned properties. Uno et al.[26] and Gillanders et al.[27] have reported that the closed form of photochromic diarylethene derivatives bearing sulfone groups can fluoresce, which inspired our research.

Here, we present a novel photochromic diarylethene photoswitch for super-resolution microscopy that has a high photostability and strong fluorescence in its closed form. We investigate its photophysical and photochemical properties in solution and in polymers. The outstanding performance for photoactivated localization microscopy (PALM) on soft-matter structures is demonstrated by imaging the self-assembly of block copolymers. In a densely packed surrounding, the switching behavior can be used to probe free volume on a molecular basis.

The photoswitch (Figure 1a) was synthesized by a Suzuki–Miyaura coupling reaction using the 6,6-diiodo derivative of 1,2-bis(2-ethyl-1,1-dioxidobenzothiophene-3-yl)perfluorocyclopentene and naphthalene-2-yloboric acid, as described in the Supporting Information. In the following, we will demonstrate that the photoswitch meets all the requirements of an ideal dye for super-resolved fluorescence microscopy[28,29] spectrally well-separated on and off states, high absorption coefficients, high fluorescence quantum yield of
derivative is a significant advantage for super-resolved yield, in particular, of the naphthyl compared to the phenyl. As will be shown below, the lower off-switching quantum yield of the photoswitch dissolved in 1,4-dioxane upon excitation at 488 nm.

The on state, high photostability, a low quantum yield for the on—off switching, and a reasonable quantum yield for off—on switching with the possibility of tuning the off—on switching rate through the intensity of the activation laser. As will be shown below, the lower off-switching quantum yield, in particular, of the naphthyl compared to the phenyl derivative is a significant advantage for super-resolved localization microscopy.

The structure of our diarylethene photoswitch and its absorption spectra in the open and closed forms are presented in Figure 1. The open form does not absorb in the visible region and, therefore, appears colorless. In contrast, the absorption of the closed form ranges up to 550 nm, with a maximum at 475 nm. In apolar media, the closed form exhibits strong fluorescence, with a fluorescence quantum yield of 0.65 (determined in 1,4-dioxane) in the wavelength range between 500 and 650 nm (see Figure 1 b). In more polar solvents, the quantum yield drops significantly and the fluorescence of the closed form is bathochromically shifted (see Table S2 in the Supporting Information). The wavelength range of the fluorescence can be tuned by appropriate substitution.

The open and closed forms can be interconverted by irradiation at suitable wavelengths (Figure 2). Irradiation in the UV region mainly results in the formation of the closed form, whereas wavelengths between 400 and 550 nm recover the open form. After sufficient irradiation, a photostationary equilibrium is reached, which depends on the absorption coefficient of both species at the corresponding wavelength and their quantum yields. For super-resolution microscopy, a photoswitch should emit thousands of photons before switching back to its dark form, thus enabling the collection of single photoswitches before they turn dark. Similar quantum yields are also obtained on the single-molecule level for photoswitches embedded in thin polymer films (see below). In these measurements, we could additionally certify that the disappearance of molecules was mainly due to switching and not irreversible photodamage, because in most cases the fluorescence of the molecules could be reactivated with UV light. In the case of the photoswitch in polystyrene, on average 1.96 × 10^3 photons per cycle could be collected (see the Supporting Information). From this, the possible localization accuracy was estimated, according to Mortensen et al., to be 20 nm. More details on the evaluation of the photokinetics can be found in the Supporting Information.

The quantum yield of the cyclization was determined to be 0.045 in 1,4-dioxane (for the calculation see the Supporting Information). Thus, the fluorescent (closed) form of the photoswitch can be easily populated with low-intensity UV light. Correspondingly, by using a high power 488 nm LED, we determined a quantum yield of 6.9 × 10^{-4} for the cyclization. The extremely low quantum yield for the off-switching process is an essential prerequisite for PALM imaging, since it ensures there are enough photons to be collected for high-accuracy localization of the position of single photoswitches before they turn dark. Similar quantum yields are obtained on the single-molecule level for photoswitches embedded in thin polymer films (see below). In these measurements, we could additionally certify that the disappearance of molecules was mainly due to switching and not irreversible photodamage, because in most cases the fluorescence of the molecules could be reactivated with UV light. In the case of the photoswitch in polystyrene, on average 1.96 × 10^3 photons per cycle could be collected (see the Supporting Information). From this, the possible localization accuracy was estimated, according to Mortensen et al., to be 20 nm. More details on the evaluation of the photokinetics can be found in the Supporting Information.
The switching kinetics of the photoswitch depends on its environment. This dependency is especially pronounced in dense systems with a rather high viscosity. Therefore, choosing appropriate conditions, such as the timing and intensity of the illumination, is important to achieve good super-resolved images in such environments. On the other hand, this dependency can be used to obtain environmental information in addition to structure. We investigated the switching behavior of the photoswitch embedded in spin-coated polymer films of poly(ethyl acrylate) (PEA), poly(n-butyl methacrylate) (PBMA), polystyrene (PS), and poly(methyl methacrylate) (PMMA). Typical switching behavior in a PBMA film is shown in Figure 3 (for the other polymers, see the Supporting Information). We counted the number of fluorescent molecules within the activation cycles (see Figure S4 in the Supporting Information). As already mentioned above, some dyes do not switch in PS. They were photobleached with the 488 nm laser prior to the measurements. Analysis with ThunderSTORM yielded super-resolved PALM images, as shown by a representative example in Figure 4a. The super-resolved part of the image on the right shows structural details which are obscure in the diffraction-limited image on the left. Figure 4b shows a representative super-resolved Y-shaped structure and demonstrates the capability of resolving structures well beyond the diffraction limit. Intensity profiles along different vertical lines reveal that we can easily resolve cylinders with a separation of 84 nm. It should be noted that the resolution here is not limited by the properties of the dyes but by the width of the labeled cores. These exhibit a core diameter of about 50 nm, as estimated from the cylinder width of 62 nm determined by AFM (see Figure S6 in the Supporting Information). Taking into account that, as shown in Figure 4b, the cylinders were imaged with a full-width at half-maximum (FWHM) of about 70 nm and that this value is obtained by convolution of the real size of the labeled core with the accuracy of single-molecule localization, we can estimate the resolution to be approximately 50 nm with respect to the FWHM or 21 nm with respect to the standard deviation. This means that our photoswitch allows for PALM with a tenfold increase in resolution with respect to the diffraction limit.

In conclusion, we utilized for the first time a fluorescent photochromic diarylethene derivative for super-resolved photoactivation localization microscopy. We rationalized its suitability for super-resolved imaging by evaluation of the
photoswitching kinetics and found a sensitivity of photoswitching which depends on the surroundings and which can be used as an independent readout to learn about the local polymer density. We visualized the apolar core of self-assembled amphiphilic block copolymer nanostructures with a resolution far beyond the diffraction limit of light by using PALM imaging. The applicability of the photoswitch is, however, not restricted to PALM, but can also be used for reversible saturable optical fluorescence transitions (RESOLFT) microscopy and super-resolution optical fluctuation imaging (SOFI). The synthesis of these powerful photoswitches is straightforward, and a variety of substitutions by C–C coupling strategies is currently under way to specifically adapt their properties to the systems to be visualized. With suitable substitutions or embedding the chromophore into appropriate nanoparticles, we also envisage the possibility of expanding the applicability of the probe to imaging in polar media. We believe that our results lay the foundation for applying diarylethene derivatives to the super-resolution microscopy of systems which could not be accessed so far because of the lack of available dyes with suitable properties.

Acknowledgements

We thank Patrick Wünnemann for valuable discussions on atomic force microscopy measurements, Stefan Walta for his assistance in fluorescence lifetimes measurements, and Prof. Dr. Ulrich E. Steiner for proofreading the manuscript. Furthermore, we thank the German Research Foundation (DFG) for support within the SFB 985 “Functional Microgels and Microgel Systems” and by the Research Grant Hu 1682/3-1. Moreover, we appreciate financial support from the RWTH competence center FLAMENCO within the profile area MSE. A.O. acknowledges a PhD scholarship from the “Fonds der chemischen Industrie”.

Keywords: block copolymers · fluorescence microscopy · photochemistry · photoswitches · super-resolution

How to cite: Angew. Chem. Int. Ed. 2016, 55, 12698–12702
Angew. Chem. 2016, 128, 12890–12894