Supplementary Information for
Restricted Diffusion of Guest Molecules in Polymer Thin Films on Solid Substrates as Revealed by Three-Dimensional Single-Molecule Tracking

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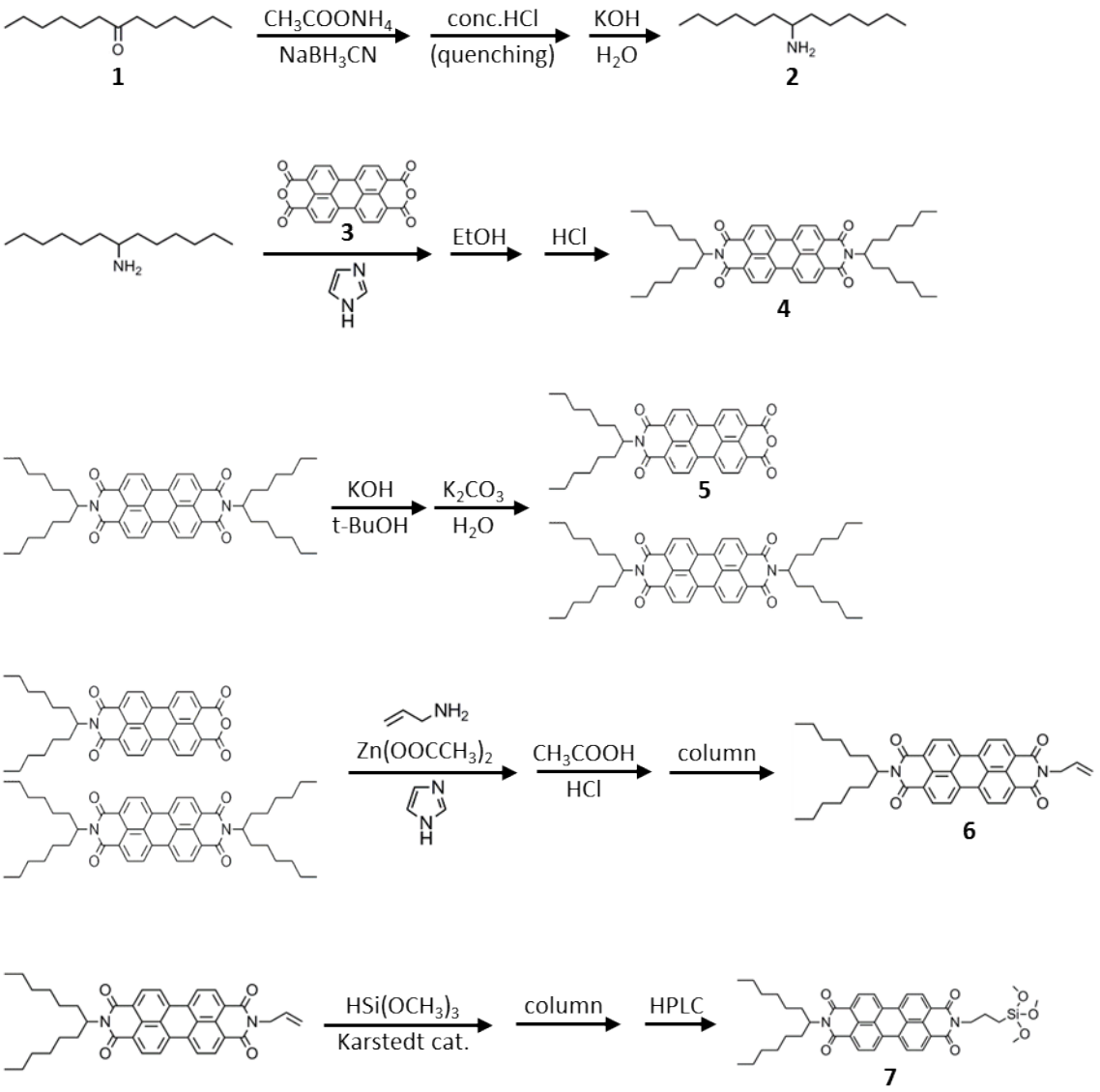
1. Sample preparation

The astigmatism imaging method can provide the relative Z-position of a single dye, and hence a standard of Z-position is required in order to determine the distance between a guest dye and the surface of a glass substrate in 3D-SMT. In the present study we immobilized perylenediimide (PDI) derivatives onto the glass surface via covalent bonds, and measured distance between the immobilized dyes and guest dyes moving freely in the polymer films.

A PDI derivative with a trimethoxysilylpropyl group, 2-(1-hexylheptyl)-9-(3-trimethoxysilylpropyl)anthra[2,1,9-*def*;6,5,10-d'e'f']diisoquino- line-1,3,8,10-tetraone (msPDI), was synthesized according to literatures.[1,2] Scheme S1 shows the synthetic pathway of msPDI. A drop of ethyl lactate solution of msPDI (1.0×10^{-9} M) was dropped onto well-cleaned cover slips, followed by stationary preservation for 1 hour at a room temperature (295K). The cover slips were then well rinsed with pure ethyl lactate and placed into an ultrasonic bath filled with pure ethyl lactate to remove physically adsorbed msPDIs. After the ultrasonic cleaning, the coverslips were dried by a flow of nitrogen. This process was conducted immediately before the preparation of polyHEA films by spin-casting in which 75 μL of ethyl lactate solution including polyHEA and BP-PDI was dropped onto the surface-modified coverslips. By changing the

concentration of polyHEA and the rotating speed, 100-nm, 500-nm, and 1000-nm thick polyHEA films were prepared. The thickness of the prepared films was evaluated by using an interference thickness meter (F20-UV, Filmetrics). The polyHEA films were preserved at 323K for at least 12 h in a constant-temperature oven before the wide-field single-molecule imaging.



Scheme S1. Synthesis of **7**.

2. 3D single-molecule tracking: setup and accuracy of 3D localization

For the 3D SMT of the guest dyes, we modified a wide-field fluorescence imaging system consisting of an inverted optical microscope (IX-70, Olympus) and a 532-nm CW DPSS laser (Exelsior532, Spectra Physics) as an excitation light source. The details of the imaging system were reported previously.[3] Figure S1(a) shows the astigmatism imaging system used in the present study. The CW 532-nm laser beam was focused into the back aperture of the objective plane (UPlanApo Oil Iris, x100, NA1.35, Olympus) for wide-field illumination. The illumination area of the 532-nm light at the sample plane was adjusted by using two external lenses and a beam expander. The fluorescence from individual molecules in the host polymer films was detected by an electron-multiplying charge-coupled device (EM-CCD) camera (C9100-13, Hamamatsu Photonics) as digital videos. A cylindrical lens with a focal length of 300 mm inserted into the imaging path of the wide-field microscope modified fluorescence spots of single molecules depending on their Z-positions. In the present study, the temperature of an experimental room was stabilized at 295 ± 0.5 K. All measurements were conducted after the thermal equilibrium was attained.

Fig. S1(b) shows typical fluorescence spots of single BP-PDI at different Z positions. Fig. S1(c) shows X- and Y-lengths of elongated fluorescence spots as a function of the Z-position, from which the Z-position of the guest dyes was deduced. To evaluate localization accuracy of the 3D SMT with the astigmatism wide-field microscope, we tracked a single BP-PDI immobilized in a PMMA film. The 3D position of the single guest dye fluctuated as typically shown in Fig. S1(d). The full width at half maximum (FWHM) values of the positional fluctuations along the X-, Y-, and Z-axes were 31, 28, and 120 nm, respectively.

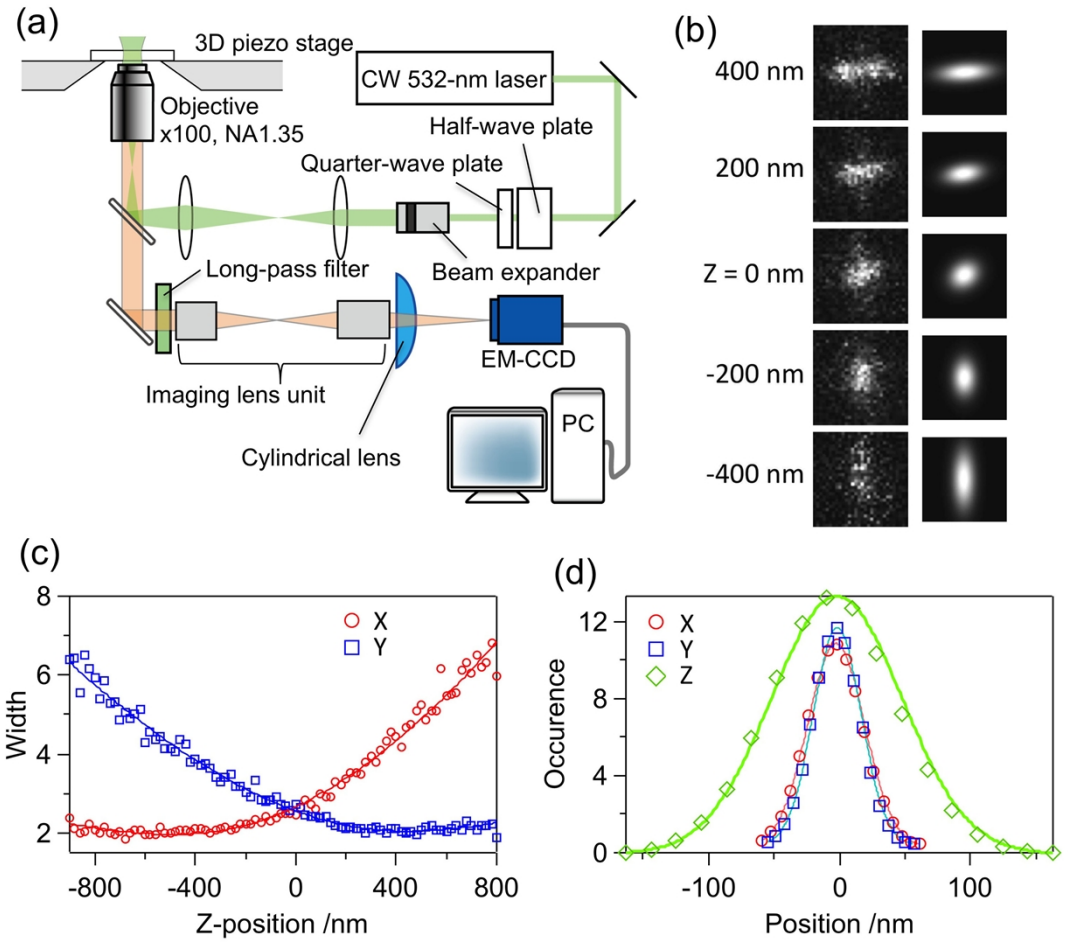


Fig. S1. (a) The schematic illustration of the astigmatism wide-field imaging system used in the present work. (b) (left panels) Fluorescence images a single BP-PDI embedded in a PMMA film at different Z-positions and (right panels) corresponding 2D fitting results. (c) Plots of the X- and Y-widths of a single fluorescence spot at different Z-position. (d) Distributions of the positional fluctuations of single BP-PDI embedded in a PMMA film along X (red circle), Y (blue square) and Z (green square).

3. Experimental setup for TIR fluorescence microscopy

To obtain fluorescence images of the samples under total internal reflection (TIR) illumination, we attached a TIR illumination module (IX2-RFAEVA-2, Olympus) to the inverted microscope. 488-nm CW light from a solid-state laser (Exelsior488, Spectra Physics) was coupled to an objective (UAPON100XOTIRF) with a NA = 1.49 via the TIR illumination module, resulted in TIR at the interface between the polyHEA with a refractive index of 1.47 and the cover slip with a refractive index of 1.515.

References

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