Electronic Supplementary Information (ESI) for

Visualization of microstructure and position-dependent diffusion coefficient in blended polymer solid using photo-activation localization microscopy combined with single-molecule tracking based on one-color fluorescence-switching of diarylethene

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S1. Steady state absorption and fluorescence spectra of fDAE

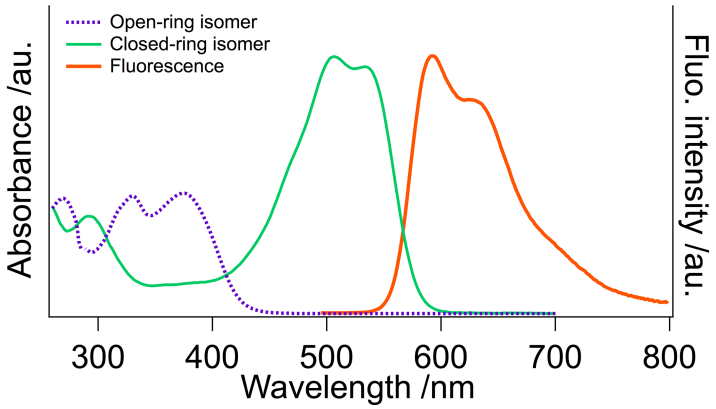

Fig. S1. Steady-state absorption and fluorescence spectra of the fDAE in 1,4-dioxane solution. The purple dotted line and green solid one are the absorption spectra of the open-ring and closed-ring isomers, respectively. The orange solid line shows the fluorescence spectrum of the closed-ring isomer.

S2. Sample and optical setup

Thin films of the blended polymer were prepared by spin casting of the solution of PHEA, HBSP and a small amount (10^{-9} – 10^{-10} M) of fDAE on a well-cleaned cover slip (NEO, 24×32 mm, MATSUNAMI). The cover slips used in the present study were washed in the following manner. They were first soaked in acetone for 60 min to remove most of contaminants, then rinsed with ultrapure water. Second, they were soaked in 5% aqueous solution of sodium hydrate for 60 min followed by rinsing in ultra-pure water several times. The cleaned coverslips were kept in ultrapure water. Just before spin casting, they were dried and further treated in a UV-ozone cleaner (SSP 16-110, SEN lights corporation) for 60 min.

In the report, if without any notice, the thin films of the PHEA-HBSP blend were prepared from 1-methoxy-2-propanol solution of PHEA (0.5 wt%) and HBSP (0.25 wt%) under the condition of spin casting: dropping volume 75 μL, spinning speed 2000 rpm and rotation time 120 s. Figure S2(a) shows a macroscopic appearance (picture) of a PHEA-HBSP film on a cover slip thus obtained. The film is transparent at the macro level because the thickness of the film is very thin (70-80 nm, as shown in Fig. S2(f)) and the light scattering strength of the film is negligibly small although sub-micrometer sized spherical domains of HBSP exist in the film as shown in Fig. S2(d).

Figure S2(b) shows an optical transmission image of a PHEA-HBSP film prepared by spin-casting at 600 rpm with the solution containing 1.0-wt% PHEA and 0.25-wt% HBSP. The light (whity) and dark regions in the image respectively correspond to PHEA-rich areas and HBSP-rich spherical domains, which was confirmed by the result that the area of the light region increased with an increase in the mixing ratio of PHEA as shown in Fig. S2(c). The surface morphology and position-dependent property of the PHEA-HBSP films were evaluated by using an AFM (SPM-9700, SHIMADZU) operated as dynamic (tapping) and phase mode. Fig. S2(d) shows an AFM height image of the blended polymer film, in which submicrometer sized spherical domains of HBSP are observed as bright areas, indicating that the sample has a profile as illustrated in Fig. S2(e). The thickness of the PHEA-HBSP films was in the range of 70 - 80 nm (74 nm on average), which were measured by using a stylus profiler (Dektak XT, Bruker, Fig. S2(f)).

A wide-field fluorescence microscope (WFM) was used to obtain fluorescent images of fDAEs. The detail of the setup was already described in our previous reports. [S. Ito, et al., *Chem. Commun.*, 2015, **51**, 13756; Y. Arai, et al., *Chem. Commun.*, 2017, **53**, 4066] In brief, the WFM consists of an inverted optical microscope (IX71, Olympus), a 532-nm continuous wave (CW) laser (Exelsior 532, Spectra-Physics), an objective lens (UPlanFLN Oil Iris[100x/NA1.30], Olympus), and an electron-multiplying charge-coupled device (EMCCD) camera (ImagEM, C9100-13, Hamamatsu Photonics). The illumination area of the 532-nm light at the sample plane was adjusted using a pair of plano-convex spherical lenses (f=300 and f=200) and a beam expander (LBED-10, SIGMA KOKI). Fluorescence images of single fDAEs in the blended polymer films were obtained by using the EMCCD camera. An optical long-pass filter (LP02-532RU-25, Semrock) was inserted into the optical path of the fluorescence imaging system to remove scattered 532-nm light propagating to the EMCCD camera. The very high (> 6) optical density of the long-pass filter ensured fluorescence imaging with negligible back-ground due to the excitation laser.

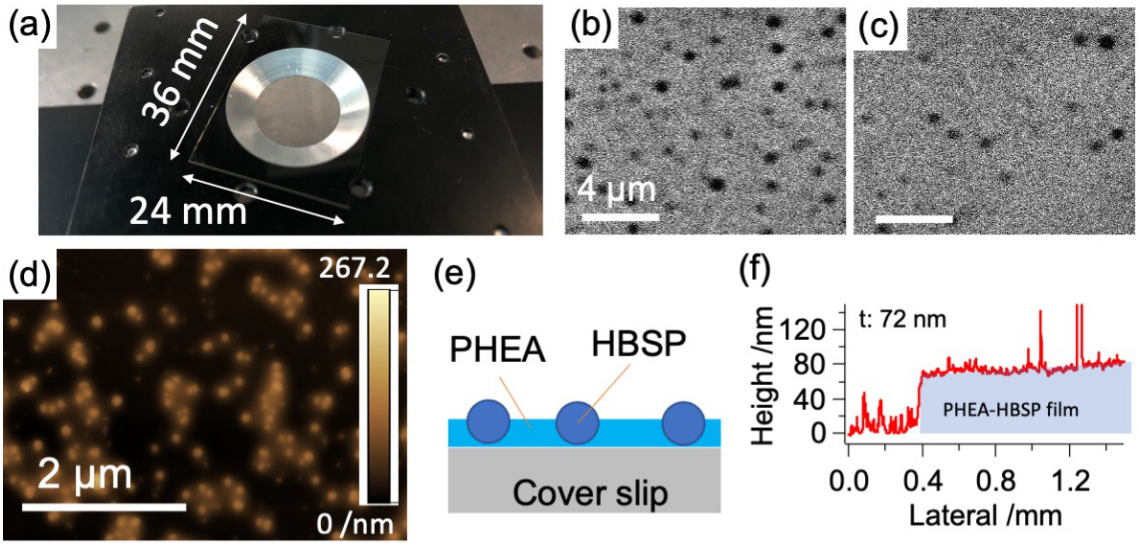

Fig. S2. (a) Photograph of a PHEA-HBSP film on a cleaned cover slip. (b, c) magnified optical transmission images of the same area in a PHEA-HBSP film prepared by spin-casting at 600 rpm with the solution containing (b) 1.0-wt% PHEA and 0.25-wt% HBSP and (c) 1.5-wt% PHEA and 0.25-wt% HBSP. (d) Dynamic (tapping) mode AFM image of a PHEA-HBSP film. (e) Illustration of the profile of the PHEA-HBSP film. (f) Measurement of film thickness with a stylus profiler. The film was prepared by spin-casting at 2000 rpm with the solution containing 0.5-wt% PHEA and 0.25-wt% HBSP.

S3. Long-time stability of the phase separation structure evaluated with optical transmission microscopy

To evaluate the stability of the phase-separation structure for the measurement time of this study (ca. 60 min.), we obtained a couple of optical transmission micrographs of the PHEA-HBSP film at the time interval of 60 min. Figure S2 shows thus obtained optical transmission images of the same area in a PHEA-HBSP film. No change in shape was observed for the sea-island structure in the polymer blend, ensuring the sample can be used for the present long-time measurement for 60 min.

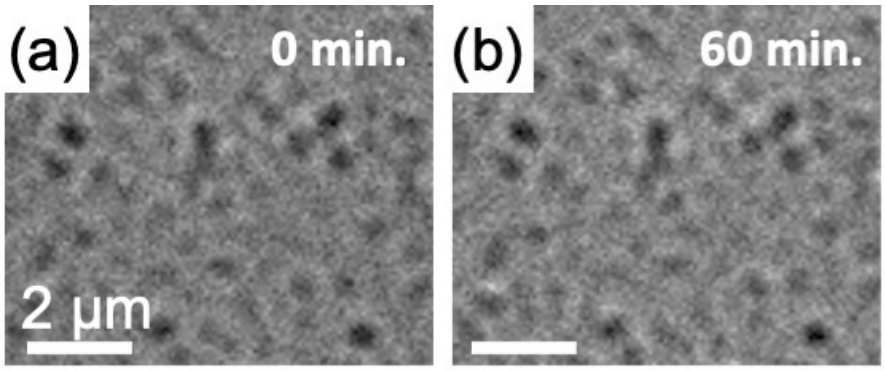

Fig. S3. magnified optical transmission images of the same area in a PHEA-HBSP film prepared by spin-casting at 2000 rpm with the solution containing 0.5-wt% PHEA and 0.25-wt% HBSP (a) at the beginning of the observation and (b) after 60 min.

S4. Time-evolution of the number of fDAEs in the ON state for 60 min.
Although after 60 min multiple fDAEs in the ON state were detected in fluorescence image, the number of fluorescence spots gradually decreases with observation time. This decrease is ascribable to the evolution to the photo-stationary state in the initial stage and photodegradation in the later stage. [Y. Arai, et al., *Chem. Commun.*, 2017, **53**, 4066] Figure S4(a) shows the time course of the number of fluorescence spots (fDAEs in the ON state) for different excitation intensities. The photodegradation of the fDAE was enhanced with increasing incident light intensity. However, at the light intensity of 33 W/cm^2 (1.9 mW), ca 20 fDAEs in the ON state were observed even after 60-min. This result indicates that long time measurement is possible in the present optical condition. Indeed, 7039 fDAEs were successfully tracked in 60 min. As shown in Fig. 4a and 4b in the main manuscript, the trajectories of the 7039 molecules can cover the whole imaging area and, nanostructure in the polymer blend due to the phase separation and 2D distribution of diffusion coefficient of fDAE are clearly visualized. This is consistent with the previous study on the SMT of fDAE in a neat PHEA film [Y. Arai, et al., *Chem. Commun.*, 2017, **53**, 4066], where all the imaging area was covered with the trajectories of fDAEs. The present result (Fig. 4a and 4b) consistent with the previous study on neat PHEA films ensures that tracking ca. 7000 fDAEs is sufficient for the evaluation of almost observation area.

The fDAEs under CW 532-nm photoexcitation underwent photo-isomerization between the open- and closed-ring isomers as the discussion on Fig. 1c in the main manuscript. Figure S4(b) shows the distribution of the ON-time of 7039 fDAEs under 532-nm photoexcitation at 33W/cm^2. The averaged ON-time and median of that are respectively 1.2 and 0.77 s.

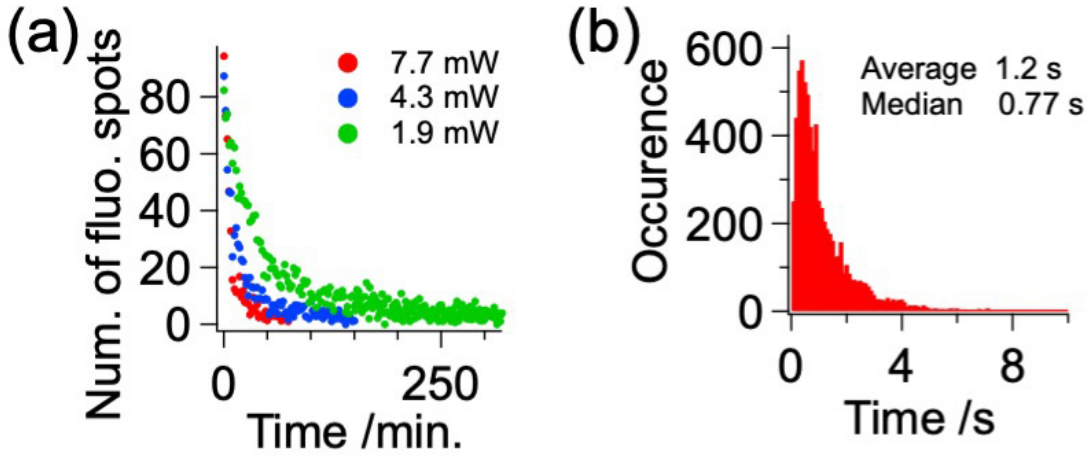

Fig. S4. (a) Plots of the number of fluorescent spots in PHEA-HBSP films under 532-nm irradiation for different incident laser intensities, 33 W/cm^2 (1.9 mW), 72 W/cm^2 (4.3 mW) and 132 W/cm^2 (7.7 mW). (b) Distribution of the fluorescence ON-time for 7039 fDAEs in a PHEA-HBSP film observed for 60 min at the excitation intensity 33 W/cm^2.

S5. Fluorescence and optical transmission image of PHEA-HBSP film including larger HBSP domains

Figure 2 in the main manuscript shows that the fDAEs do not enter the spherical HBSP domains. To further confirm this, we prepared much thicker neat HBSP films by drop casting and PHEA-HBSP films with larger HBSP domains and obtained fluorescence images of fDAEs in these films using WFM.

Figure S5(a) and (b) respectively shows fluorescence and optical transmission images of the PHEA-HBSP film with larger HBSP domains. The PHEA-HBSP film containing larger HBSP domains was prepared by changing mixing ratio between PHEA and HBSP (1-methoxy-2-propanol solution of PHEA (2 wt%) and HBSP (0.25 wt%), dropping volume 15 μL) and speed of spin casting (spinning speed 1200 rpm and rotation time 120 s). The concentration of fDAE in this sample is ca. 100 times larger than that in PHEA-HBSP polymer films the data of which is shown in Fig. 2b and 2c in the main manuscript.

In the fluorescence image (Fig. S5(a)) a micrometer sized dark area where no fluorescence spot exists is observed. The corresponding area can be seen in the optical transmission image (Fig. S5(b)), indicating that the refractive index of this area is different from the surroundings. The fDAEs did not enter the micrometer sized HBSP domains, which is consistent with the data shown in Fig. 2 and 4 in the main manuscript.

Figure S5(c) shows fluorescence image of fDAEs on the surface of HBSP film prepared by drop casting (thickness > 50 μm). The fDAEs existed only on the surface of the film; no fluorescent spot was observed inside the film as shown in Fig. S5(d).

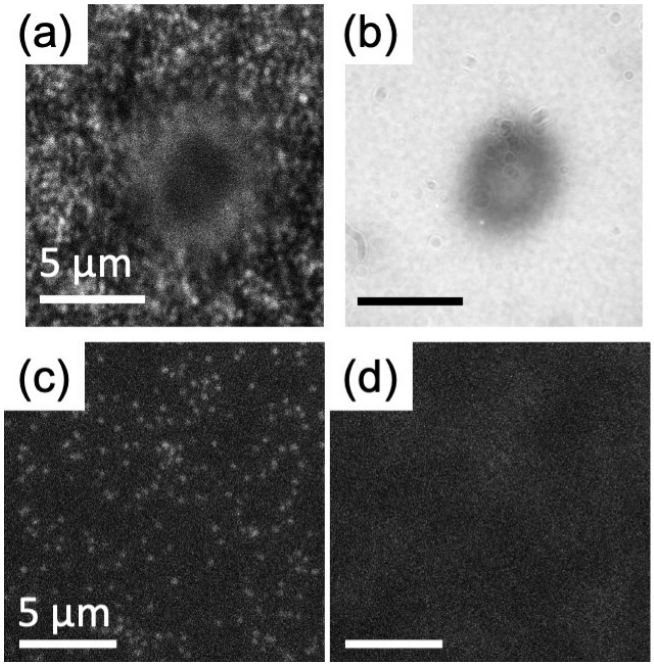

Fig. S5. (a) Fluorescence and (b) optical transmission images of the PHEA-HBSP film with larger HBSP domains. (c) Fluorescence image of fDAEs on the surface (polymer/air interface) of a neat HBSP film prepared by drop casting. (d) Fluorescence image of the neat HBSP film; the focus of the microscope was ca. 5 μm below the surface.

S6. Photo-degradation of normal dyes

To compare the dynamic PALM with fDAE and SMT with normal dyes, we obtained a time-evolution of fluorescence image of a perylenediimide derivative the structure of which is shown in Fig. S6 right. The excitation condition is almost same as the imaging with the fDAE, wavelength 532 nm and intensity 35 W/cm^2. The data in Fig. S6 shows that almost fluorescent spots of the perylenediimide derivative disappeared after 6-min photo-excitation owing to photodegradation.

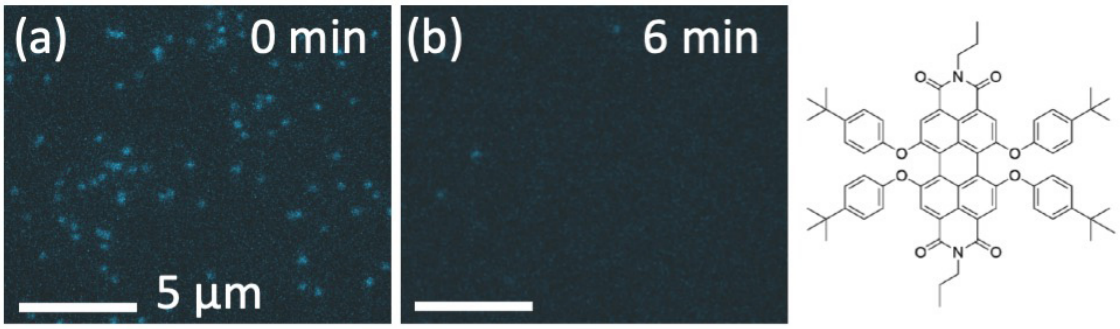

Fig. S6. Single-molecule fluorescence images of a perylenediimide derivative (shown in the right) in a PHEA film (a) at the beginning and (b) after 6-min photoexcitation at 532 nm, 35 W/cm2.

S7. Lateral diffusion coefficient of fDAE in neat PHEA film

The distribution of diffusion coefficient of fDAEs in a neat PHEA film is shown in Fig. S7. The peak is located at ca. 0.02 µm^{2}s^{-1}, which is similar to the blue distribution in Fig. 4(f) in the main manuscript.

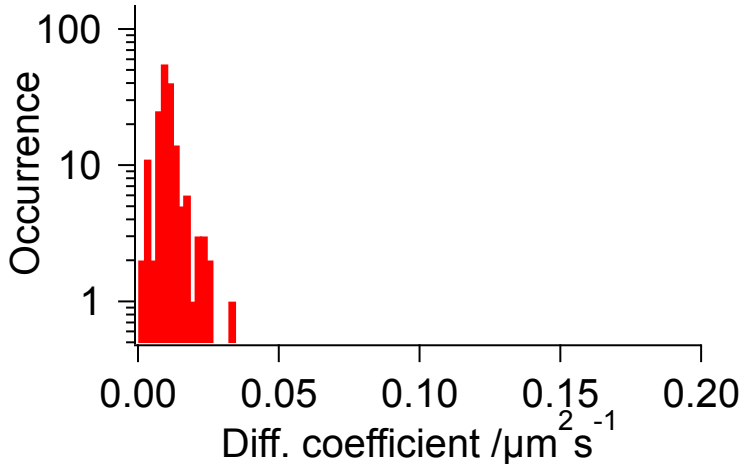

Fig. S7. Distribution of lateral diffusion coefficient of fDAEs in a neat PHEA film.

S8. Dynamic (tapping) and phase mode AFM images of the PHEA-HBSP film

Figure 4 in the main manuscript shows that the diffusion coefficient of the fDAE is larger in the vicinity of the interface between PHEA and HBSP domains, indicating that the viscoelastic property of the interfacial areas is different from that in the neat PHEA area. To experimentally confirm the difference in the viscoelastic property around the HBSP-rich domain, dynamic (tapping) and phase mode AFM images of the PHEA-HBSP blend film were measured. Figure S8(a) shows the dynamic mode image. The bright spots correspond to small particles of HBSP (HBSP-rich domains), while relatively dark flat areas mainly consist of PHEA (PHEA-rich areas). Figure S8(b) shows the phase-mode AFM image of the same area as the dynamic mode image shown in Fig. S8(a). Dark small spots surrounded by bright rings in Fig. S8(b) is attributable to the small spheres of HBSP, by comparing these two AFM images. The signal intensity in the phase-mode image corresponds the phase shift of the oscillation of a cantilever at each point and the contrast in a phase-mode image includes the change in both topological and viscoelastic properties of the sample. [J. Tamayo and R. Garćıa, *Appl. Phys. Lett.*, 1997, **71**, 2394.] Therefore, the difference in the phase-mode signal for two areas with the same height indicates the difference in viscoelastic property between the areas. In this case, the brightness in the phase-mode image increases with increasing softness of the polymer blend; hence the phase-mode image (Fig. S8(b)) indicates that the PHEA-rich areas surrounded by the HBSP-rich domains (that can be typically seen in areas marked by B and C) are softer than PHEA-rich areas (typically seen in an area marked by A). While the dynamic mode image (Fig. S8(a)) indicates that the PHEA-rich areas marked by A, B, and C have comparable heights. From the AFM observations we can conclude that the PHEA-rich areas around the HBSP-rich domains becomes softer compared with almost neat PHEA areas. This observation is consistent with the SMT showing different diffusion coefficients between the two areas as shown in Fig. 4.

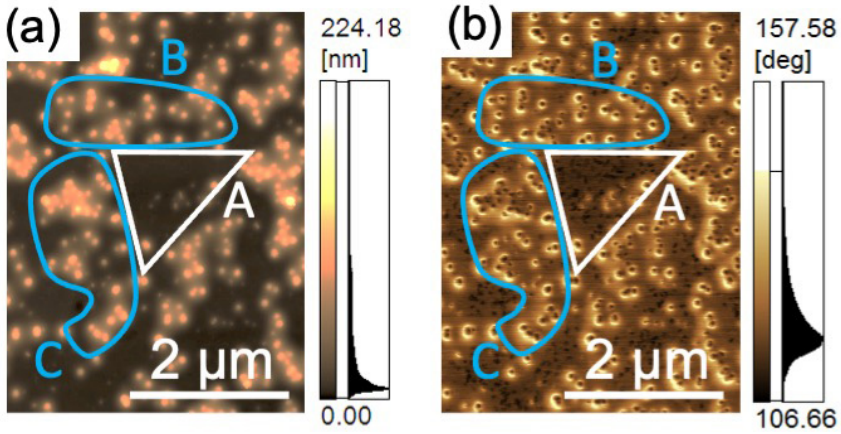

Fig. S8. (a) Dynamic and (b) phase mode AFM images of a PHEA-HBSP blend.

S9. Stability of the setup and localization accuracy over 60 min.

In the present study, we observed the Brownian motions of single fDAE molecules for longer than 60 min. using the home-built wide-field microscope. Within such long observation time, thermal drift of the microscope stage is expected to decrease localization accuracy of single molecules. Before the long-time observation, we evaluated practical localization error due to the drift of the stage during 60 min by tracking single quantum dot (CdSeS/ZnS alloyed quantum dot, diameter 6 nm, emission wavelength 665 nm, Sigma Aldrich) immobilized in a PVA film.

Figure S9a shows the two-dimensional plot of the localized position of a quantum dot observed for 60 min. Figures S9b and S9c respectively show the time courses of the X- and Y-positions of the corresponding quantum dot.

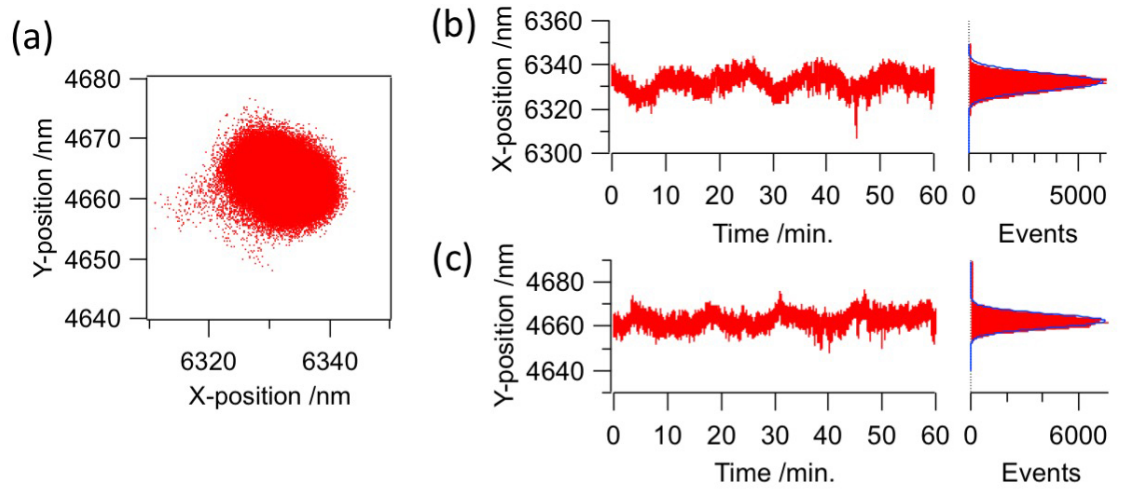

Fig. S9. (a) 2D (on XY plane) trajectory of the single quantum dot for 60 min and (b, c) corresponding time traces of the X and Y positions of the quantum dot.