Electronic Supplementary Information of

Mapping the Emitting Sites within a Single Conjugated Polymer Molecule

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Sample preparation

A diluted toluene solution of poly[2-methoxy-5-(2'-ethyl-hexyloxy)-1,4-phenylenevinylene] (MEH-PPV, Aldrich, M_n = 200,000, polydispersity index 5) was mixed with a 1.5 wt% toluene solution of polystyrene (PS, Polymer Standards, M_n = 9,580, polydispersity index 1.03, T_g = 368 K). A final concentration of MEH-PPV in the solution was 1×10^{-13} M (calculated based on M_n). The solution was spin-coated on a cleaned microscope cover slip. The concentration of the MEH-PPV molecule in the spin-coated polymer film was calculated to be 6.7×10^{-12} M, taking into account the concentration of polystyrene in toluene. The thickness of the film was 120 nm. An aqueous solution of fluorescent microspheres (1×10^{-13} M, Bangs laboratories, 60 nm in diameter) was spin-coated on top of the PS/MEH-PPV sample. The sample was vacuum-dried at 60 °C for 2 hours and used immediately for experiments.

Experimental setup

Fluorescence images were recorded using an inverted microscope (Olympus, IX71) equipped with a high N.A. objective lens (Olympus, ×100, N.A. = 1.3). A 488 nm line from an Ar ion laser (Coherent, Innova 70) was used for excitation. A circularly polarized light was obtained using a quarter-wave plate (Thorlabs), and introduced into the microscope through the objective. An illumination area was about 50 μm in diameter. The excitation power of the 488 nm light was 13.4 Wcm^{-2}. The fluorescence signal was corrected by the same objective, passed through a dichroic mirror (Omega optical, 500DRLP), an emission filter (Omega optical, 530ALP), magnified by an additional lenses (×1.6), and was focused on an EM-CCD camera (Andor technology, iXon^{EM}+). The fluorescence images were recorded with 100 millisecond exposure time. The pixel size of the images was 100 nm.

Analysis of single-molecule fluorescence images

Figure S1 shows fluorescence images of individual MEH-PPV molecules. Figure S1A and S1B are the same fluorescence image shown in different intensity scales. A brightest spot appeared in the top-right of the image is a fluorescent bead. The bead is distinguished easily from the MEH-PPV molecules by intensity (Fig. S1B). Figure S1A inset and S1B inset are typical images obtained from a

single MEH-PPV molecule and a fluorescent bead, respectively. Centroid positions of the spots were determined by using 2D Gaussian fitting[1] (equation 1) using routines written in Matlab,

$$z = z_0 + A\exp\left(-\frac{(x-x_c)^2}{2w_x^2}\right)\cdot\exp\left(-\frac{(y-y_c)^2}{2w_y^2}\right) \qquad \text{(eq. 1)}$$

where x_c and y_c are the centroid position along x and y axis, respectively. A and z_0 are a Gaussian height and an offset, respectively. w_x and w_y are the width of the Gaussian along x and y axis, respectively.

A drift of the microscope stage was corrected by fluorescent beads embedded in the thin film sample. Figure S2A shows centroid positions obtained from the bead. We recorded 500 images (exposure time 100 ms), which were typical experimental conditions for MEH-PPV molecules. The stage drift of ten to fifteen nanometers were observed (Fig. S2A) in the XY plane. A stage drift along Z-axis on the order of ten nanometers does not affect the analysis in the XY plane. The centroid positions displayed in FigS2A were corrected using another fluorescent bead in the same images. The corrected centroid positions are shown in Figure S2B. The standard deviation calculated from the image is about 2.5 nm, which is close to a theoretically calculated value based on the number of photon collected. The result demonstrates that the stage drift is corrected within nanometer accuracy.

The centroid positions obtained by 2D Gaussian fitting of the single-molecule fluorescence images were filtered using an edge preserving Chung-Kennedy (C-K) algorithm to enhance the spatial resolution of the experiment. The C-K detector is derived for trajectories with Gaussian noise and sharp edges, and serves as an edge detector[2-4]. The algorithm consists of two adjacent running windows whose output was the mean from the window possessing the smallest variance. The input trajectory x_i is defined at time points $t_i=ih$ spaced by h. Construct moving averages $X_{i\pm}$ and the variances $S_{i\pm}^2$ from these averages over two windows of W points, forward and backward of the ith time point.

$$X_{i\pm} = \frac{1}{W}\sum_{k=1}^{W} x_{i\pm k} \qquad \text{(eq. 2)}$$

$$s_{i\pm}^2 = \frac{1}{W}\sum_{k=1}^{W}\left(x_{i\pm k} - X_{i\pm}\right)^2 \qquad \text{(eq. 3)}$$

Weighting functions are simultaneously calculated as follow.

$$g_{i+} = \frac{s_{i-}^{2r}}{s_{i+}^{2r} + s_{i-}^{2r}} \qquad \text{(eq. 4)}$$

$$g_{i-} = \frac{s_{i+}^{2r}}{s_{i+}^{2r} + s_{i-}^{2r}} \qquad \text{(eq. 5)}$$

where the sensitivity factor r satisfies $1<<r<100$. Therefore, the filtered output function consists of

$$X_{\text{out}}(t_i) = g_{i+}X_{i+} + g_{i-}X_{i-} \qquad \text{(eq. 6)}$$

Figure S3A shows a fluorescence intensity trajectory of a bead, and centroid positions obtained from the bead is shown in Figure S3C. The obtained centroid positions along x and y axis are shown in

Figure S3B (open circle). Solid lines in Figure S3B are filtered trajectories using C-K algorithm (W = 10, r = 50). A 2D plot of the filtered data is displayed in Figure S3D. The standard deviation of the filtered data is about 0.90 nm, which is much smaller than the unfiltered data (standard deviation about 2.5 nm). It should be mentioned that the filtered data does not show detectable jumps/steps.

Spatial resolution of the experiment

The localization error of the 2D Gaussian fitting of the fluorescence images is described by

$$\sigma^2 = \frac{r_0^2 + q^2/12}{N} + \frac{8\pi r_0^4 b^2}{q^2 N^2} \qquad \text{(eq. 7)}$$

where r_0, q, N, and b represent standard deviation of the point spread function of the microscope (127 nm in our experimental setup), size of an image pixel (100 nm in our experimental setup), total number of photons collected, and background noise per pixel (8 photons), respectively. In our experiments, total number of photons collected from individual MEH-PPV molecules was in the range of 500 to 3000 per image, which corresponded to the localization error of 3 to 13 nm (N = 3000, 3 nm; N = 2000, 4 nm, N = 1000, 7 nm; N = 500, 13 nm).

We recorded fluorescence images of the beads embedded in a polyvinyl alcohol thin film (Fig. S4, S5). The samples were moved along the Y axis (5 nm (Fig. S4) or 10 nm steps(Fig. S5)) every three seconds by a piezoelectric stage. Figure S4A and S5A show fluorescence trajectories of the beads whose intensity are comparable to that of a single MEH-PPV molecule. We determined centroid positions of the images using 2D Gaussian fitting, and filtered the trajectory using C-K algorithm (W = 10, r = 50). Figure S4B (dots) and S5B (dots) show centroid positions along the Y axis obtained from the analysis. The 5 nm- or 10 nm-steps can be detected in the unfiltered trajectories. We calculated the centers and standard deviations of the centroid positions of the each steps (see Fig. S4C and S5C). The center positions in the circles in Figure S4C bottom and S5C bottom correspond to the center positions of the each steps, and the radius of the circles correspond to the standard deviations of the each steps. The radiuses of the circles are in the range of 3 to 4 nm, which agree with the theoretically calculated localization errors for the detected photon number (N = 3000, 3 nm). While the 5 – 10 nm steps can be detected in the unfiltered trajectories, those steps are much clear in the filtered trajectories (Fig. S4B line, S5B line, S4D, and S5D). Importantly, the steps are accurately detected in the filtered trajectory (Fig. S4B line, S5B line, S4D dots, S5D dots). Furthermore, the standard deviations of the centroid positions after the filtration (Fig. S4D bottom, S5D bottom, the radiuses of the circles) are in the range of 1 to 2 nm that are much smaller than those in the unfiltered trajectories. Those results clearly demonstrate that the C-K algorithm improves the localization accuracy of the centroid positions without losing small steps (5 nm-sizes) in the trajectories. The trajectories with different photon numbers demonstrated that the localization error obtained by the C-K filtration was 1 – 3 nm, which was smaller than those obtained by 2D Gaussian fitting without filtering (3 – 14 nm).

Analysis of the centroid positions obtained from a single MEH-PPV molecule

Figure S6A show an intensity trajectory obtained from a single MEH-PPV molecule. Centroid positions obtained from the molecule are shown in Figure S6B and S6C (open circle). Red and blue data points represent the centroid positions obtained from the red and blue intensity levels in Figure S6A. Solid lines in Figure S6B are filtered trajectories. While the spatial distributions of the red and blue data points in Figure S6C are different, the difference is more obvious in the filtered data (Fig. S6D). More importantly, such the spatial jump in the centroid positions is not detected in the filtered data of the fluorescent bead. This result confirms that nanometer-scale spatial jumps can be detected clearly with the C-K filter.

1. R. E. Thompson, D. R. Larson and W. W. Webb, *Biophysical Journal*, 2002, **82**, 2775-2783.
2. S. H. Chung and R. A. Kennedy, *Mathematical Biosciences*, 1996, **133**, 111-137.
3. M. C. Leake, D. Wilson, M. Gautel and R. M. Simmons, *Biophysical Journal*, 2004, **87**, 1112-1135.
4. D. A. Smith, *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, 1998, **353**, 1969-1981.
5. Y. Ebihara and M. Vacha, *Journal of Physical Chemistry B*, 2008, **112**, 12575-12578.

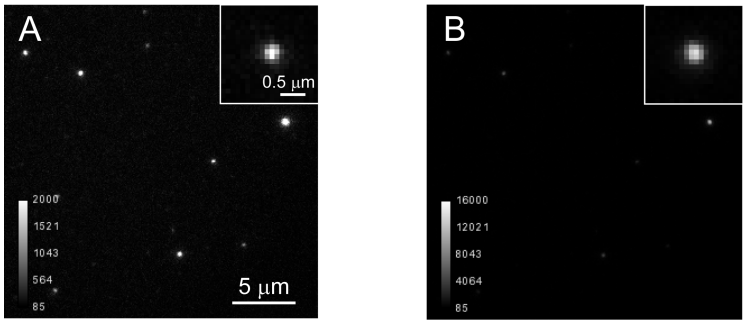

Figure S1. Fluorescence images of individual MEH-PPV molecules. Fig. S1A and S1B are the same image shown in different intensity scales. A bright spot located at top-right is a fluorescent bead. Fig. S1A inset and S1B inset are expanded fluorescence images of a single MEM-PPV molecule and a fluorescent bead.

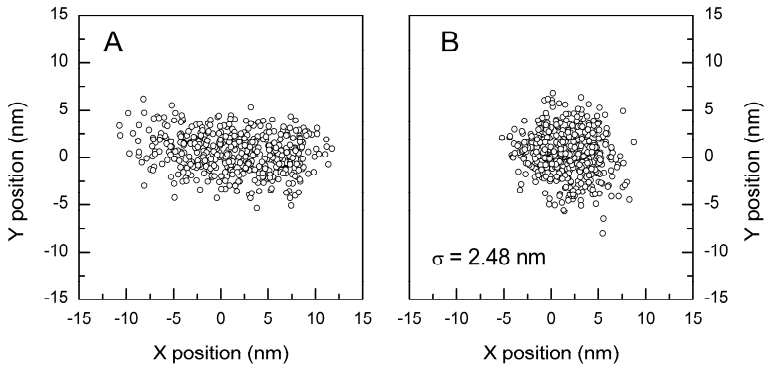

Figure S2. Calibration of stage drifting using a reference fluorescent bead. Centroid positions of a fluorescent bead determined by 2D Gaussian fitting (A) and the positions of the same particle which are corrected by another fluorescent bead (B).

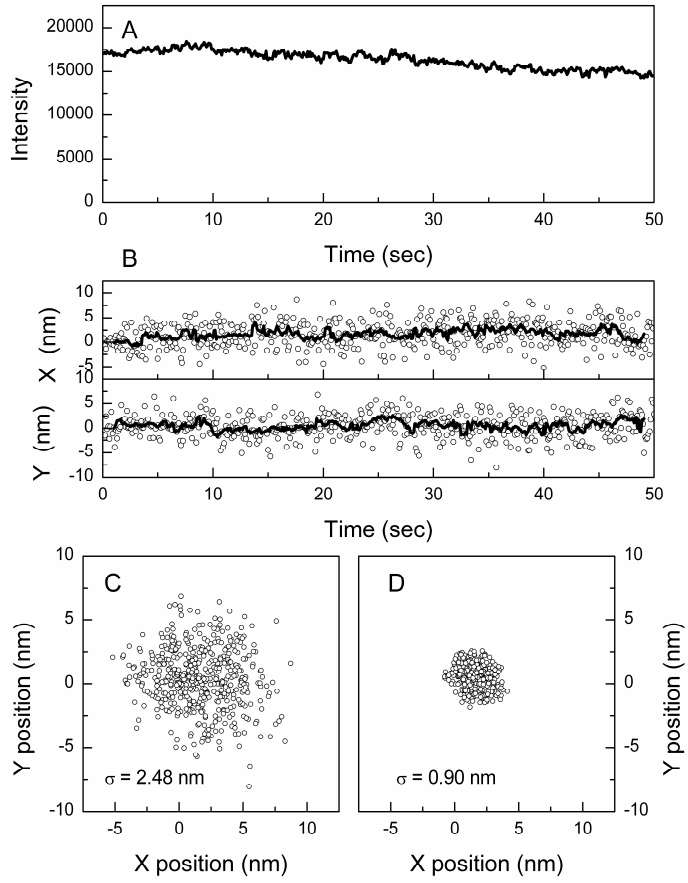

Figure S3. Analysis of the centroid positions using Chung-Kennedy algorithm. (A) Fluorescence intensity trajectory of a fluorescent bead. (B) Centroid positions of the particle along X (top) and Y (bottom) axis (open circle), and C-K filtered trajectories (solid lines). (C) 2D plot of the centroid positions. (D) 2D plot of the C-K filtered centroid positions.

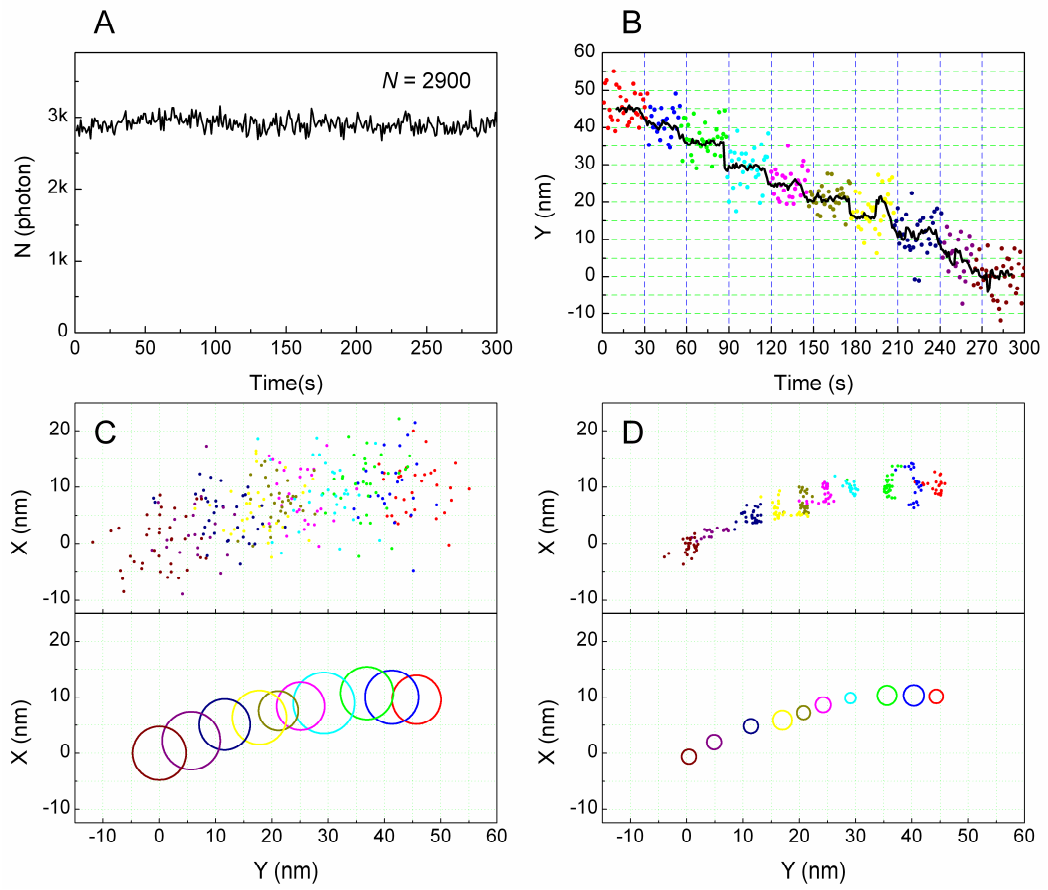

Figure S4. Detecting 5-nm steps of piezo-stage shift by fluorescent beads embedded in a polyvinyl alcohol thin film using the 2D Gaussian fitting and the C-K filter. (A) Fluorescence intensity trajectory of a single fluorescent bead. *N* is the average photon number detected in each images. (B) Centroid positions determined by 2D Gaussian fitting (dots). The solid line shows the filtered trajectory using the C-K algorithm. (C) 2D plot of the centroid positions obtained by the 2D Gaussian fitting (top). The center positions of the circles in the bottom panel correspond to the center positions of the each steps, and the radius of the circles correspond to the standard deviations of the each steps. (D) 2D plot of the centroid positions obtained by the 2D Gaussian fitting and the C-K filter (top). The center positions of the circles in the bottom panel correspond to the center positions of the each steps, and the radius of the circles correspond to the standard deviations of the each steps. Since a sample drifting was not calibrated in this analysis, the centroid positions moved about 10 nm along the X axis during the experiment.

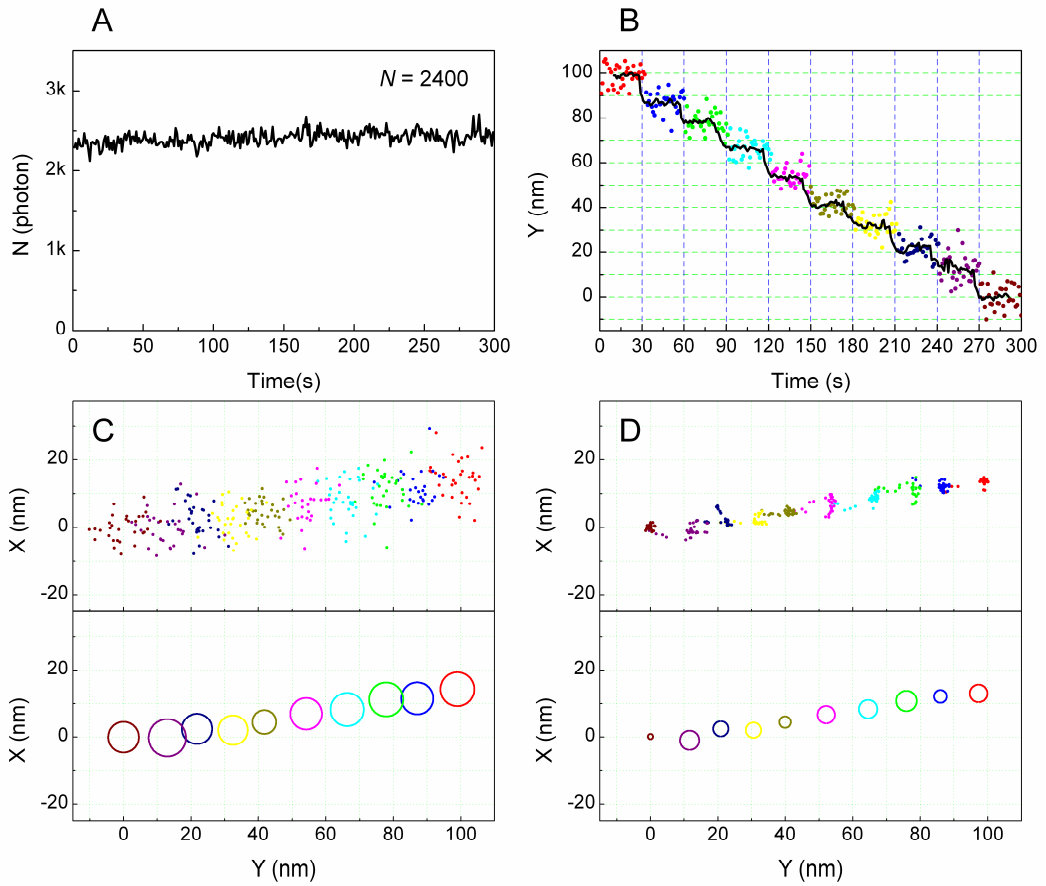

Figure S5. Detecting 10-nm steps of piezo-stage shift by fluorescent beads embedded in a polyvinyl alcohol thin film using the 2D Gaussian fitting and the C-K filter. (A) Fluorescence intensity trajectory of a single fluorescent bead. *N* is the average photon number detected in each images. (B) Centroid positions determined by 2D Gaussian fitting (dots). The solid line shows the filtered trajectory using the C-K algorithm. (C) 2D plot of the centroid positions obtained by the 2D Gaussian fitting (top). The center positions of the circles in the bottom panel correspond to the center positions of the each steps, and the radius of the circles correspond to the standard deviations of the each steps. (D) 2D plot of the centroid positions obtained by the 2D Gaussian fitting and the C-K filter (top). The center positions of the circles in the bottom panel correspond to the center positions of the each steps, and the radius of the circles correspond to the standard deviations of the each steps. Since a sample drifting was not calibrated in this analysis, the centroid positions moved about 10 nm along the X axis during the experiment.

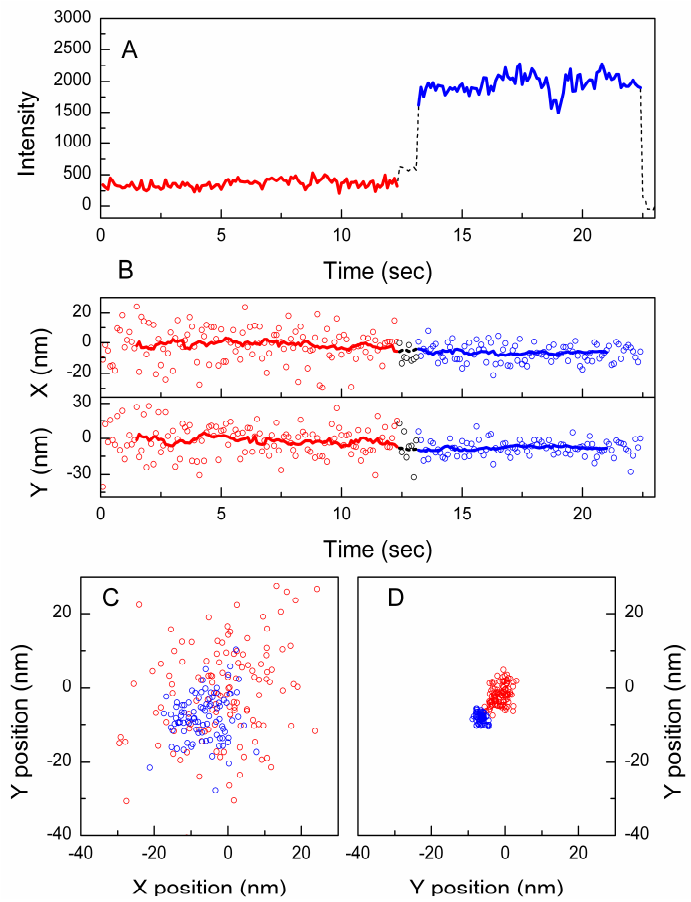

Figure S6. Analysis of the centroid positions determined for a single MEH-PPV molecule. (A) Emission intensity trajectory of a MEH-PPV molecule. (B) Centroid positions of the molecule along X (top) and Y (bottom) axis (open circle), and C-K filtered trajectories (solid lines). (C) 2D plot of the centroid positions. (D) 2D plot of the C-K filtered centroid positions.

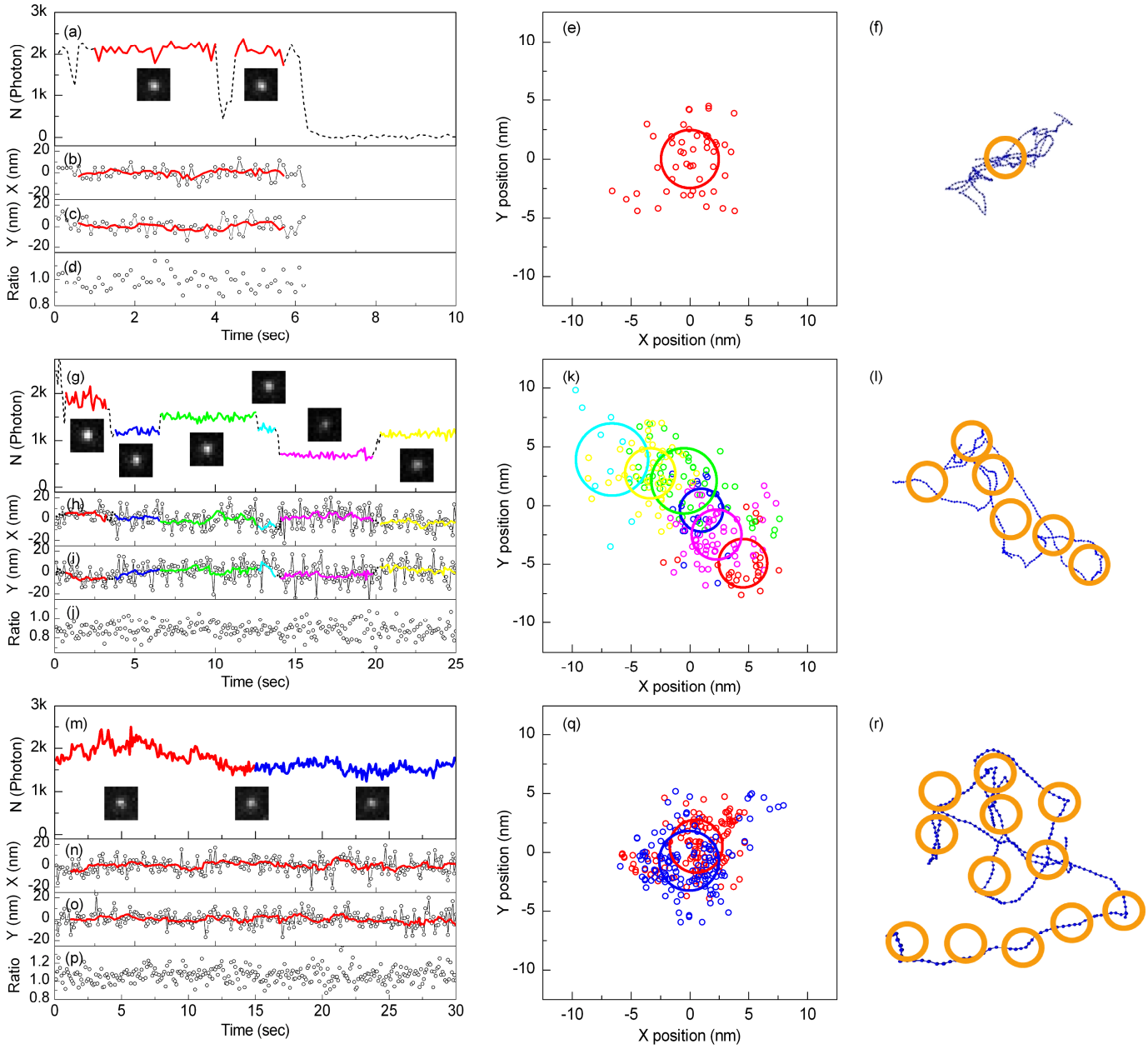

Figure S7. Relationship between the structure and photophysics of individual MEH-PPV molecules. (a, g, m) Fluorescence intensity trajectories of individual molecules. (Inset) Single-molecule fluorescence images of the MEH-PPV molecules. (b, c, h, i, n, o) Centroid positions in (b, h, n) X and (c, i, o) Y axis obtained from the intensity trajectories. The solid lines show filtered traces using the C-K filter. (e, k, q) Centroid positions obtained from (e) trajectory (a), (k) trajectory (g), and (q) trajectory (m). Large circles show positions of emitting sites. The radiuses of the circles correspond to standard deviations of the centroid positions. (d, j, p) Ratio of 2D Gaussian width along X and Y axis. (f, l, r) Schematic structures of the molecules. Emitting site within the molecules are indicated by orange circles.

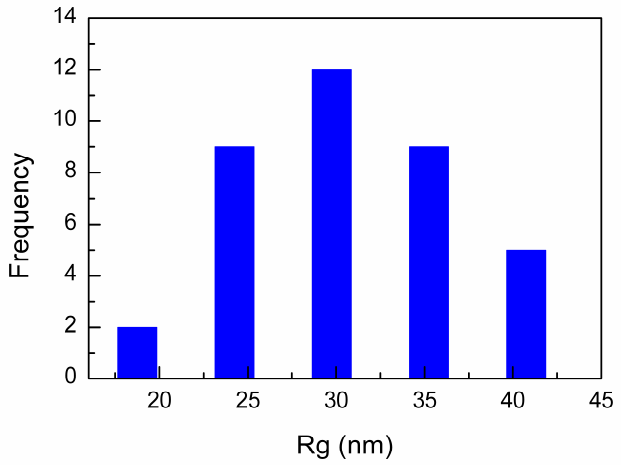

Figure S8. Frequency histogram of simulated gyration radius of MEH-PPV molecules. Details of the simulations were previously published[5].