Supplementary Materials for “Examining dynamics in a polymer matrix by single molecule fluorescence probes of different sizes” by Hao Zhang et al,

1. Defocused single molecule fluorescence microscopy videos

Videos are provided for Probe-M recorded at 34.2 and 43.6°C. The dimension of the images is 27.4×27.4 μm.

2. Assemblies of 50 typical power spectra of the probes adopted

![Figure S1](image)

**Figure S1** Assembly of 50 power spectra of Probe-S doped in the 96 nm-thick PVAc film measured at 43.6°C. The red line denotes the average of these 50 power spectra.
Figure S2  Assembly of 50 power spectra of Probe-M doped in the 96 nm-thick PVAc film measured at 43.6°C. The red line denotes the average of these 50 power spectra.

Figure S3  Assembly of 50 power spectra of Probe-L doped in the 96 nm-thick PVAc film measured at 43.6°C. The red line denotes the average of these 50 power spectra.

3. Dielectric loss spectra of PVAc film
For the impedance measurement presented here, the samples, which are 0.5 mm in thickness and 10.0 mm in diameter, are sandwiched between two gold electrodes. The sample is housed inside a home-made sample cell, equipped with heating device and proper thermo-insulation. The sample’s temperature is precisely controlled using LakeShore 335 controller. The impedance analysis is conducted by Solartron SI-1260 Impedance/Gain-Phase Analyzer with the SI-1296 Dielectric Interface. The measurements were performed in the frequency range 0.01 Hz – 1.0 MHz at temperatures between 313 and 353 K. The typical loss spectra are displayed in Figure S4. It is noted that no reliable measurement can be conducted below frequency of $10^{-1}$ Hz.

![Figure S4](image_url)

**Figure S4** Dielectric loss spectra of PVAc film measured under different temperatures. The value of sample’s temperature is displayed in the figure.

4. *Comparison of temperature dependence of* $f_r$ *and power spectral intensity*

The integrated area of the power spectra density data (therefore the spectral intensity) was calculated as the indication of energy taken by the rotating fluorescent probes,
and it is plotted together with the data of temperature dependence of $f_r$ (Figure S5).

It is observed that the spectral intensity keeps increasing even after the $f_r$ value reaches unity. For example, for Probe-S, its power spectral intensity is still increasing way beyond its $T_u$ temperature. This is also true for Probe-M but with a larger slope compared with Probe-S. For Probe-L, the increase in spectral intensity is much lower at lower temperature (<9.8°C) where the increase of $f_r$ value is also small, while the increase in spectral intensity is much sharper at higher temperature (>9.8°C) where the increase of $f_r$ value is also bigger. This is a clear indication that the temperature for $f_r$ value to reach unity ($T_u$) is the starting of significant occurrence of glass transition, i.e. the starting of vast increase of heat flow in the DSC measurements.

**Figure S5** The $f_r$ value as a function of temperature is plotted together with power spectral intensity for comparison. For Probe-S, even after the $f_r$ value reaches unity, its power spectral intensity is still increasing at the same rate.

5. *Distribution of fluorescence probes inside the PVAc film*
Secondary ion Mass Spectroscopy (SIMS) was employed to investigate the
distribution of fluorescence probes along the direction normal to the film’s plane.
SIMS measurement was performed on a standard commercial TOF-SIMS
v(ION-TOF GmbH, Münster, Germany). The experimental condition is: Ion beam:
Ar⁺, incident angle: 45°. The depth profiling experiment was performed with an
analyzed area of 100 × 100 µm inside an etching area of 300 × 300 µm. All analyses
were carried out at 256 × 256 pixels. Figure S6 shows the results, in which the
intensity of CN group representing the fluorescence probes (perylendiimide) exhibits
a slight and gradual increase in intensity near polymer-air and polymer-substrate
interfaces – the intensity at polymer-air is about 10% higher than the middle of the
film while that at polymer-substrate interface is about 60% higher. The results
indicate segregation of the probes does exist but is not severe.

![Figure S6](image)

**Figure S6**  Intensity of signal from CN group representing the fluorescence probe
(perylendiimide) and C₂H₃O₂ group representing the polymer (PVAc) as a function
of experimental time.

6. **Surface morphology characterization by atomic force microscopy**

The surface morphology of the film is characterized by an atomic force microscope
(AFM, Multimode 8, Digital Instruments). The scan size is 10×10 µm. The typical
result is shown in Figure S7. No dewetting was observed at elevated temperatures.
Control experiments were conducted to compare the dynamics of fluorescence probes located at polymer-air or polymer-substrate interfaces. The probes are allocated to these interfaces by depositing them using selective solvent. Cyclohexane was chosen to dissolve the probes because it is a poor solvent for the polymer (PVAc). To allocate the probes at polymer-air interface, the probes were spin-casted onto the pre-deposited PVAc film. To allocate the probes at polymer-substrate interface, the probes were spin-casted on the substrate at first, and the PVAc films were spin-casted on top of it afterwards. The power spectra of rotational motion of Probe-S and Probe-M at these two interfaces at 39.8°C are displayed in Figure S8, together with the data for the same probe doped in the film. The data show that the power spectra of Probe-S and Probe-M at polymer-air interface show no significant difference to those doped in the films while much slower and less activated dynamics is found at polymer-substrate interface.
Figure S8  Averaged power spectra of Probe-M and Probe-S deposited at the polymer-air and polymer-substrate interfaces recorded at 39.8°C. The data for probes doped in the film as a whole is displayed together as a comparison. The film thickness is 96.0 nm.