Spatial distribution of single guest molecules along thickness of thin films of poly(2-hydroxyethyl acrylate)

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Abstract

We have investigated three-dimensional distribution and diffusion behaviors of single guest dyes in 1- μm thick films of poly(2-hydroxyethyl acrylate) (PHEA) by using astigmatism imaging method. Perylene diimide derivative (BP-PDI) in the PHEA films localized along the Z-axis at ca. $Z = 600\text{--}700$ nm distant from the interface ($Z = 0$) between PHEA and glass substrate. This Z-localization was not observed in different polymer films of poly(methyl methacrylate) (PMMA), poly(methyl acrylate) (PMA), and polystyrene (PSt). Because the glass transition temperature of the PHEA is lower than the room temperature, BP-PDI in the PHEA films exhibited Brownian motion, normal diffusion on the XY plane and confined motion along the Z-direction. For elucidating the mechanism of the peculiar localization of the guest dyes along film thickness in the PHEA films, we measured diffusion behaviors of different dyes, R6G and Atto 488, in 1- μm thick PHEA films, obtaining result that the Z-distributions of the dyes were overall similar to that of BP-PDI. The result indicates that the Z-localization of the guest dyes should be ascribed not to the interaction between glass surface and guest dye but mainly to the Z-dependent property of the PHEA film. Indeed, the lateral diffusion coefficients of the guest dyes depended on their Z-positions.

Keywords 3D single-molecule tracking · Super-resolved fluorescence microscopy · Polymer thin film

1 Introduction

Reactive resins undergoing polymerization, network formation, and dissociation under exposure of quantum beams of photons or electrons are a class of main materials in

advanced nanofabrication techniques [1–5]. In these materials, guest chemical reagents with low molecular weights, such as photoinitiator, crosslinker, photoacid generator, and inhibitor, play significant roles in the nanofabrication processes [6, 7]. To achieve high processing accuracy, chemical reactions are required to uniformly proceed in the target exposure area with quantum beams. Accordingly, it is indispensable for guest reagents to be homogeneously distributed in host materials and thus the information on the spatial distribution of guest molecules with a nanometer accuracy is of great significance for rational design of advanced reactive resins.

It is, however, rather difficult to evaluate the sub 100 nm–resolved three-dimensional (3D) distribution of guest molecule in resin films thinner than 1 μm . Though scanning electron microscopy (SEM) enables us to image nanostructures with a very high spatial resolution (< 5 nm) [8–10], it cannot be generally applied to molecular imaging inside the solids. Scanning probe microscopy (SPM) such as scanning tunneling microscope (STM) [11, 12], atomic force microscope (AFM) [13], and scanning near-field microscope (SNOM) [14], also have very high spatial resolution, while

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Scheme 1 Chemical structures of guest dyes, BP-PDI, R6G, and Atto488, and msPDI, and polymers, PHEA, PMMA, PMA, and PSt

the imaging is limited to molecules mainly on the surface of solids. Although scanning (confocal) laser microscopy can provide three-dimensional information on solid systems [15–17], the spatial resolution is restricted by the diffraction-limit of light, ca. a half of wavelength.

Wide-field fluorescent microscopy is a powerful tool for the imaging of single molecules in transparent organic solids. Although a fluorescent image of each molecule detected by a CCD camera is a diffraction-limited spot as approximated as point spread function (PSF), the position of single molecule can be tracked at a very high accuracy, typically 10–20 nm in imaging (*XY*) plane by applying the localization method to the PSF [18–22]. The wide-field fluorescence microscopy and its related techniques have been applied to the investigation of a variety of polymer systems [23–28]. In addition, by using astigmatism imaging technique [29, 30] the position of single molecule can be tracked along the optical (*Z*) axis at an accuracy of several tens of nanometers. Actually, we have recently revealed by using the 3D single-molecule tracking (3D-SMT) that a perylene diimide derivative in 1- μm thick poly(2-hydroxyethyl acrylate) (PHEA) films localized ca. 500–700 nm away from the interface between glass substrate and the polymer [31]. Elucidation of this peculiar distribution of guest molecules can provide the information on the factors regulating the distribution of guest dyes along the thickness of polymer thin films, which

is important for the realization of homogeneous distribution of guest molecules leading to the fabrication with high accuracy. Along this line, we have studied the 3D position of several guest dyes, hydrophobic and hydrophilic ones, in thin films of PHEA. Also, the 3D position of guest dye in poly(methyl methacrylate) (PMMA), poly(methyl acrylate) (PMA), and polystyrene (PSt) was measured for the investigation of host polymer dependence.

2 Experimental section

2.1 Samples

Scheme 1 shows structures of guest fluorescent molecules and polymers used in the present work. As guest, we used a perylene diimide derivative, *N,N'*-dipropyl-1,6,7,12-tetrakis-(4-*tert*-butylphenoxy)-3,4,9,10-perylenetetracarboxydiimide (BP-PDI, Yamada Chemical), Atto 488 (ATTO-TEC), and Rhodamine 6G (R6G, Nippon Kankoshikiso). PMMA [$M_w = 15,000$, $T_g = 378$ K (105 °C), Sigma Aldrich], PMA [$M_w = 40,000$, $T_g = 282$ K (9 °C), Sigma Aldrich], PSt ($n \approx 2400$, $T_g = 383$ K (110 °C), Kishida Chemical), and PHEA [$M_w = 3550$, $T_g = 290$ K (17 °C)] were employed as host polymers. The dyes, BP-PDI, Atto 488, and R6G, and polymers, PMMA, PMA, and PS, were used as received without

further purification, while PHEA was synthesized (the supplementary information).

As samples, ca. 1- μm thick polymer films including small amount (ca. $1\text{--}2 \times 10^{-10}$ M) of guest fluorescent molecules were prepared on well cleaned microscope cover-slips (NEO 24 $\times$ 32, Matsunami) by spin-casting. The thickness of the polymer films was measured using an interferometric thickness gauge (F20UV, Filmetrics). As a marker to determine the Z-position of glass-polymer interface, a perylene diimide derivative with methoxysilyl groups (msPDI) was attached onto the surface of the cover slips by silane coupling [31]. Prior to fluorescence imaging, the polymer films were kept longer than 24 h in an electric oven at the temperature by 30 K higher than the glass transition temperature of each polymer.

2.2 Wide-field imaging system for 3D single-molecule tracking

Three-dimensional (3D) single-molecule tracking of the guest dyes was performed by using a home-built wide-field microscope with an astigmatism-imaging system. The details of the setup are shown in the supplementary information. Briefly, the wide-field imaging setup consists of an inverted optical microscope (IX-70, Olympus) and a 532-nm CW solid-state laser (Exelsior532, Spectra Physics) as an excitation light source. The CW green laser beam with circular polarization was focused into the back aperture of an objective lens (UPlanApo Oil Iris, 100 $\times$, NA1.35, Olympus) for wide-field illumination. The illumination area of the 532-nm light at the sample plane was adjusted by using a pair of plano-convex spherical lenses and a beam expander (LBED-5, SIGMA KOKI). Fluorescence images of individual molecules in host polymer films were obtained by using an electron-multiplying charge-coupled device (EM-CCD) camera (C9100-13, Hamamatsu Photonics). To introduce astigmatism to the imaging system, a cylindrical lens with a focal length of 300 mm was inserted into the imaging path of the wide-field microscope, leading to ellipsoidal fluorescence spots of single molecules whose aspect ratios are depending on their Z-positions.

2.3 Brownian dynamics simulation

Brownian dynamics simulation (BDS), of which bases were developed by Ermak and McCammon [32], was used for the numerical analyses of the 3D lateral diffusion of individual guest dyes. In this simulation, polymer matrix was regarded as continuum medium. In addition, motions of individual polymer chains or their interactions with guest dyes were not taken into account. The guest dye was approximated by a small sphere exhibiting Brownian motion. The detailed

simulation procedure has already described in a previous report [33].

3 Results and discussion

3.1 Dependence of the position of guest dyes on host polymers

Figure 1 shows the single-molecule astigmatism images of BP-PDIs in films of PHEA, PMMA, PMA, and PSt with ca. 1- μm thickness. Fluorescence spots in the PHEA film (Fig. 1a) are classified into two patterns: horizontally elongated fluorescence spots as major cases and a small number of vertically elongated spots. The former horizontally elongated patterns correspond to BP-PDIs in the PHEA film and the latter vertically elongated ones to msPDIs attached onto the surface of glass substrate as a positional reference of the Z-axis. Compared with the fluorescence image in PHEA in Fig. 1a, the shapes of the fluorescence spots in other polymer films (Fig. 1b–d) show a variety of aspect ratios, from vertically elongated ($d_x < d_y$) to horizontally elongated ($d_x > d_y$) ones. These images imply that the guest molecules more homogeneously distributed in the films both on the XY plane and along the film thickness (Z-direction) in PMMA, PMA and PSt films.

Fig. 1 Astigmatism fluorescent images of individual BP-PDIs in thin (1- μm thick) films of **a** PHEA, **b** PMMA, **c** PMA, and **d** PSt. In the images, fluorophores on the surface of cover slip (msPDI) show vertically elongated fluorescence spots, while those several 100 nm away from the glass surface (BP-PDI) show horizontally elongated spots as indicated in (a) for instance

To quantitatively obtain the information on the distribution along the Z-axis, we plotted the time-averaged (from the beginning of measurement to the permanent photobleaching, 7.9 s on average) Z-positions of individual BP-PDIs. Figure 2a clearly shows that the BP-PDIs in the PHEA film are localized at ca. 500–700 nm from the surface of the cover slip along the Z-axis and none of the dyes was detected in the region < 500 nm. On the other hand, much wider distribution along the Z-axis was observed for BP-PDI in other polymers, as shown in Fig. 2b–d.

The glass transition temperature, T_g , of PHEA is 290 K, which is lower than the temperature of the experimental room (296 K). Accordingly, translational diffusion of BP-PDI was detected during the fluorescence imaging. To elucidate the relation between the localization and the lateral diffusion, single molecule trajectories on the XY plane and along the Z-axis are plotted in Fig. 3a, b, as a representative example. The translational diffusion on the XY plane looks normal behaviors, while the motion along the Z-axis is apparently restricted. The mean square displacement (MSD) of the lateral diffusion on the XY plane is linearly dependent on the time with a slope of unity as shown in Fig. 3c,

Fig. 2 Distributions of the time-averaged Z-position of BP-PDI in 1- μ m thick films of **a** PHEA, **b** PMMA, **c** PMA, and **d** PSt

Fig. 3 Single-molecule trajectory of a BP-PDI in a PHEA film **(a)** on the XY plane and **b** along the Z-direction. The distribution of the Z-position is also shown in the right of **(b)**. **c** MSD vs. time plot of the BP-PDI of which trajectory is shown in **(a)** and **(b)**

indicating that the 2D diffusion on the XY plane can be regarded as normal diffusion (Brownian motion in homogeneous media). On the other hand, the relation between MSD and time for the 1D diffusion along the Z-axis shows almost constant value without any increase during the observation, directly reflecting the result that the lateral diffusion along the Z-axis is confined in a small area as observed in Fig. 3b.

3.2 Distribution and diffusion dependent on the property and structure of guest dyes

To explore factors leading to the localized distribution of the guest dye along the Z-axis in the PHEA film, we investigated the Z-axis distribution of different guest molecules in PHEA films. Figure 4 shows the spatial distributions of Z-position for Atto 488 and R6G. Here, the individual Z-positions in the distributions are time-averaged values as performed in Fig. 2. The medians, averages, and standard deviations of the distributions of the time-averaged Z-position of the three guest dyes are summarized in Table 1.

Fig. 4 Distributions of Z-position in 1- μ m PHEA films for **a** rhodamine 6G and **b** Atto 488

Table 1 Medians, averages, and standard deviations of the histograms of time-averaged Z-positions of guest dyes: BP-PDI, R6G, and Atto 488

Dye	Num. of dyes analyzed	Z-position of guest dyes (distance from glass surface)		
		Median (nm)	Average (nm)	Standard deviation (nm)
BP-PDI	114	613	631	82.4
R6G	102	596	592	54.9
Atto 488	103	669	674	50.8

These results indicate that these dyes are also localized along the Z-axis in regions ca. 500–700 nm from the surface of the cover slips as observed for BP-PDI in Fig. 2a, although the medians and standard deviations of the distributions are somewhat different from one another. Because R6G and Atto 488 are soluble in water, it was expected before measurement that these dyes may stay around the surface through the hydrophilic interaction with the surface of the cover slip bearing –OH groups. However, as shown in Fig. 4a, b, these dyes were also localized around at 600–700 nm from the glass surface. This result indicates that the direct hydrophilic/hydrophobic interaction between the dyes and the glass surface plays negligible role in the localized Z-distribution.

As shown above, although the property and structure of the guest dye slightly affects the averaged position of the distributions, localization was observed for all the guest dyes. To more clearly obtain the information on this dependence, we analyzed the dynamic diffusion process. Figure 5 shows the time profiles of MSD for single molecules of R6G and Atto 488. As observed for BP-PDI in Fig. 3c, both of the dyes exhibited normal 2D diffusion on the XY plane while their diffusion behaviors along the Z-axis were confined in thin layers with ca. 200-nm thickness. These diffusion behaviors and the localized Z-distributions are quite similar to those observed for BP-PDI. These results strongly

Fig. 5 MSD vs. time plots of 2D (red lines, on the XY plane) and 1D (blue lines, along the Z-direction) diffusion for **a** R6G and **b** Atto 488 in PHEA films with 1- μ m thickness

suggests that the localized distribution and the specific diffusion of guest dyes are mainly due to the property of the host PHEA material dependent on the Z-position.

3.3 Correlation between the Z-position and diffusion coefficient of the guest dyes

In the previous section, it was strongly suggested that the localized distribution and specific diffusion behaviors are mainly ascribable to the host polymer, PHEA. To more directly obtain the property of the host polymer dependent on the Z-axis, we plotted the time-averaged diffusion coefficient of 3D lateral motion of individual BP-PDIs, as a function of the Z-position. In Fig. 6, spatially averaged diffusion coefficients over every 150-nm bin (410–560, 560–710, and 710–860 nm) are also superimposed as blue rectangles. In this figure, the histogram of the time-average Z-position (as shown in Fig. 2a) is also indicated so as to clearly correlate the diffusion coefficients and the distribution. The diffusion coefficient of the guest dye almost monotonically increases with increasing Z-position from the middle part (ca. $Z=500$ nm) to the air/polymer interface. The profile of the distribution of diffusion coefficient cannot be directly associated with the probability distribution density of the existence. However, the spatially dependent diffusion coefficient strongly suggests that the property of the polymer gradually changes along the Z-axis in a range of hundreds of nm from the middle part to the surface (air/polymer interface) of the film. Similar behavior was also observed in the

Fig. 6 Z-distributions of (top) the averaged diffusion coefficient and (bottom) existing probability density of single BP-PDIs in PHEA films. Spatially averaged diffusion coefficients over every 150-nm bin are also plotted in the top as blue rectangles with error bars of standard deviation

PHEA film with different thickness as shown in the supplementary information S5.

As was discussed above, Fig. 6 implies that properties of the host polymer are dependent on the Z-position. To obtain more detailed information beyond the averaged values over many single-molecule trajectories of the guest dyes, we analyzed the correlation between Z-position and square displacement (SQD) of 3D diffusion in short exposure time (100 ms) for the three kinds of guest molecules (Fig. 7). Compared with Fig. 6, the Z-position is more widely distributed typically in the range from 300 to 800 nm. This is because individual Z-positions in a time trajectory (every 100 ms) are plotted and, the temporal behaviors are not averaged in the figure. Spatially averaged SQDs over 80-nm bin (blue solid circles) show that the dependence on guest dye is categorized into two tendencies. For the hydrophobic dyes, BP-PDI, the spatially averaged SQDs have a minimum around 500–600 nm and the values increase toward both interfaces of the polymer films, namely polymer-glass and polymer-air (Fig. 7a). Especially, the increase in averaged SQD toward the polymer-air interface is much prominent. On the other hand, for the hydrophilic dyes, R6G and Atto 488, the spatially averaged SQDs have blunt peaks at $Z = \text{ca. } 600 \text{ nm}$ and the values slightly decrease toward both of the interfaces (Fig. 7b, c). The results indicate that the viscosity of the PHEA film is not simply dependent on the Z-position and, the diffusion coefficient of guest dye is affected also by the solute-medium interaction. The viscosity of polymer is in general strongly related with T_g . The glass transition temperature at the interface of polymer has been intensively studied using experimental approaches and computational simulations. These studies reported that the T_g of the interfacial area between polymer and air is lower than that of

Fig. 7 Correlation between the Z-position and square displacement for individual diffusion steps within the exposure time (100 ms) for **a** BP-PDI, **b** R6G, and **c** Atto 488. Averaged values over every 80-nm bin are also plotted as blue circles with corresponding error bars

bulk polymer [34], while the T_g near the substrate-polymer interface is higher [35]. In the present case, the change of T_g may strongly affects the diffusion behaviors of guest molecule because the T_g of PHEA (290 K) is only slightly lower than the experimental temperature (296 K). Although the information of PHEA in the solid–solid interfacial area ($Z < 200 \text{ nm}$) was not obtained by the present method owing to almost no probability to find the guest dyes near the glass–polymer interface, the diffusion behavior of the hydrophobic guest dyes showing higher diffusion coefficient near the polymer-air interface is consistent with the low T_g at the polymer-air interface as previously reported. On the other hand, the hydrophilic dyes did not show such behavior near the polymer-air interface.

To obtain more detailed information on the translational motion depending on the Z-direction, we analyzed the diffusion behaviors in the component of the one-dimensional (1D) translation along Z-axis and that of the two-dimensional

(2D) diffusion on the *XY*-plane. Figure 8 shows the result of the analysis as SQDs for each exposure time (100 ms) and the averaged SQDs over every 80-nm bin. For the hydrophobic BP-PDI, averaged SQDs for the 2D and 1D diffusions have minimum values around $Z=600$ nm and they increase toward both interfaces of the PHEA films (Fig. 8a). The degree of the increase in averaged SQD is small for the 2D diffusion in the *XY* plane, while the increase is very large for the 1D diffusion along the *Z*-axis toward the polymer-air interface. On the other hand, the averaged SQD of the

2D diffusion of the hydrophilic dyes, R6G and Atto 488, show almost constant values and independent on the *Z*-axis, while the averaged SQD of the 1D diffusion along the *Z*-axis slightly decrease near interfaces. At the present stage of the investigation, it is difficult to derive a clear conclusion on these behaviors depending on the property of the guest dye. However, this result again indicates that the translational diffusion is not simply regulated by the property of the host polymer near the interfacial area. The interaction between the guest dyes and the host polymer also plays significant role and it induces this difference between hydrophobic and hydrophilic dyes.

3.4 Brownian dynamics simulation of guest dyes in PHEA films

As shown in Fig. 8 on the analysis of the SQD, the *Z*-position dependence of averaged SQD of the 3D diffusion is mainly ascribed to the difference of the 1D diffusion along the *Z*-axis. For hydrophobic dyes, 1D diffusion coefficient along *Z*-axis increases with approaching both of the interfaces and, especially, the increase near the polymer-air interface is prominent. On the other hand, the SQD of hydrophilic dyes slightly decreases toward the two interfaces. Although the *Z*-dependences of diffusion coefficient for the hydrophilic and hydrophobic dyes showed opposite behaviors each other, both dyes localized in common in the *Z*-range of 500–700 nm from the polymer-glass interface. For further figuring out the mechanism of the *Z*-localization, we conducted a series of computational simulation on the diffusion behavior of the guest dyes in the polymer film by using Brownian dynamics method, especially in order to elucidate the relation between the spatial distribution of diffusion dynamics and the localization of guest molecules along the *Z*-axis.

In this simulation we assumed that a guest dye undergoes elastic reflection at both the top and bottom interfaces. First, we simulated the Brownian motion of a guest dye in a 1- μm thick polymer film with homogeneous diffusion coefficient of $4 \times 10^{-14} \text{ m}^2 \text{ s}^{-1}$ ($= 4 \times 10^{-2} \mu\text{m}^2 \text{ s}^{-1}$). The value is determined from the lateral diffusion coefficient of BP-PDI shown in Fig. 6. Figure 9a shows a trajectory of the *Z*-position of a guest molecule as a function of time. The guest molecule uniformly explored in the film although the lateral diffusion in the *Z*-direction is confined in the 1- μm region. Under this condition, the *Z*-position of the guest molecule is almost homogeneously distributed as shown in Fig. 9b exhibiting the histogram of the *Z*-position during the random walk.

Next, we investigated the effect of the *Z*-position-dependent diffusion coefficient on the distribution of the averaged position of the guest dye in the polymer film. Figure 9c, e show typical trajectories of the *Z*-position of a guest dye in a 1- μm thick polymer film with an internal distribution

Fig. 8 *Z*-distributions of SQD for (a, c, and e) 2D diffusion in the *XY* plane and (b, d, and f) 1D motion along the *Z*-axis for individual diffusion steps within the exposure time (100 ms) for a, b BP-PDI, c, d R6G, and e, f Atto 488. Averaged SQDs of the 2D and 1D diffusions over every 80-nm bin are also plotted as blue circles with corresponding error bars. The magnified plots of the blue circles are shown in the supplementary information

Fig. 9 Results of BDS of a guest dye in a 1-μm thick polymer film. **a**, **c**, and **e** Time trajectories of the Z-position of a guest dye in a polymer film with **a** uniform and **c**, **e** Z-position dependent diffusion coefficient. The spatial distributions of diffusion coefficient for (c) and (e) are shown as blue lines in (d) and (f), respectively. **b**, **d**, and **f** The probability distributions of existence along the Z-axis (**b**) derived from the time trajectory of (a), (**d**) from (c), and (**f**) from (e)

of lateral diffusion coefficient mimicking the experimental results shown in Fig. 8. The distribution of diffusion coefficient along the Z-axis is shown in Fig. 9d, f as blue lines. The condition of Fig. 9d corresponds to the experimental results of Fig. 8a (hydrophobic dye), while that of Fig. 9f corresponds to those of Fig. 8b, c (hydrophilic dyes). Although the time trajectories in Fig. 9c, e look similar to that in Fig. 9a, histograms of the Z-position (Fig. 9d, f) show inhomogeneous distributions. Figure 9d with larger diffusion coefficients near the interface shows that the existing probability of guest molecule is higher in the middle part with a peak around $Z = 500$ nm. This result qualitatively reproduces the experimental results for the hydrophobic dyes as was shown in Fig. 8a. On the other hand, Fig. 9f under the condition with smaller diffusion coefficients near the interfaces, the existing probability near the interfaces is higher than that in the middle part. As shown in these results, the Z-dependence of the diffusion coefficient affects the averaged distribution of the guest dye along the Z-axis, but this effect does not account for the result of the hydrophilic dyes.

The above results imply that factors other than the Z-position dependent diffusion coefficient take important roles in the localization of the guest dye and, hence, we employed a qualitative model shown in Fig. 10. In this model, we

Fig. 10 **a** Qualitative model illustrating the internal Z-distribution of chemical potential, μ , of solute in a PHEA film. **b**, **d** Z-distributions of diffusion coefficient (blue line) and force potential (red line) in the polymer film used for BDS. Here, k_B and T are respectively the Boltzmann constant and Kelvin temperature. **c**, **e** Results of BDS of a guest dye in a 1-μm thick polymer film with internal distributions of diffusion coefficient and force potential shown in (b, d), respectively; (left) time trajectory of the Z-position of the guest dye and (right) corresponding probability distributions of existence along the Z-axis

consider the Z-dependent chemical potential of solute molecules, μ , in the polymer due to the low solubility of guest dyes near the interfaces (glass-PHEA and PHEA-air). In other words, the μ value near the interfaces is higher than that in the middle part along the Z-axis in the PHEA films. The μ value decreases with increasing distance from each of the interfaces and, at the crossing point $Z = Z_{\min}$, μ shows a minimum value as shown in Fig. 10a. The spatial gradient along the Z-axis of the chemical potential produces a flow (drift) of the guest dyes toward the minimum point, $Z_{\min}$. As a result, the guest dyes are trapped and localized around the potential minimum. The depth of the potential here should be much larger than the averaged thermal energy, $k_B T$, at the room temperature (294 K), for stable localization along the Z-axis. In the actual simulation, we employed a Gaussian trapping potential with a depth of $10 k_B T$ the bottom of which is located at $Z = 600$ nm as well as the distribution of diffusion coefficient along the Z-axis as shown in Fig. 10b (smaller near the interface) and Fig. 10d (larger near the

interface). The molecule in the simulation experienced a force derived by spatial gradient of the potential along the Z-axis and the Z-position dependent diffusion coefficient. Figure 10c, e show the simulated temporal profiles of Z-position and corresponding probability distributions of existence under the conditions respectively shown in Fig. 10b, d. Both of the simulations well reproduce the experimental result shown in Fig. 3b. In both cases the Z-position of guest molecule is localized in a layer with ca. 200-nm thickness centered at $Z=600$ nm. Although the Z-profiles of diffusion coefficient in Fig. 10b, d are quite different, such difference in Z-distribution of diffusion coefficient does not remarkably affect the diffusion behavior along the Z-axis under the potential with the depth of $10 k_B T$. The localization of guest dyes has been thus explained by the distribution of the chemical potential (solubility) of guest dyes along the Z-axis.

4 Summary

In the present study, we have tracked the 3D position of single guest dyes in 1- μm thick films of PHEA, PMA, PMMA, and PSt on glass substrate. The BP-PDIs in PHEA films localized along the Z-axis at ca. $Z=600$ nm on average from the polymer-glass interface; the BP-PDIs did not exist in the region up to $Z=400$ nm from the glass surface. On the other hand, BP-PDIs in other polymer films of PMA, PMMA, and PSt are much more widely distributed along the Z-axis. To elucidate the mechanism of the peculiar localization of guest dyes along the Z-axis in the PHEA films, we measured 3D diffusion behaviors of different dyes, R6G and Atto 488, in 1- μm thick PHEA films in addition to BP-PDI. The Z-distribution of these dyes are overall similar to that of BP-PDI although the median of the distance from the glass surface was slightly different among the dyes.

The detailed 3D single-molecule tracking revealed that all of the guest dyes in the PHEA films exhibited normal diffusion (Brownian motion) on the sample (XY) plane and confined motion along the Z-direction. The lateral diffusion coefficients were dependent on the Z-positions of each guest dye and, the distributions of diffusion coefficient along the Z-axis showed correlation to the existing probability of the guest dyes. The lateral diffusion coefficient of the hydrophobic dyes, BP-PDI, increased towards the polymer-air interface. While that of the hydrophilic dyes, R6G and Atto 488, showed slight decrease near the interface. The behavior of the hydrophobic dyes is consistent with the decrease in glass transition temperature near the polymer-air interface. On the other hand, the behavior of the hydrophilic dyes is peculiar and cannot be simply explained by the decrease in T_g . To figure out the behavior, we considered the difference of the solubility (chemical potential) of the solute with a spatial distribution along the Z-axis. At both of the interfaces,

PHEA-glass and PHEA-air, the chemical potential of the solute is higher and it decreases with distance from the interfaces. The chemical potential has a minimum at $Z=500$ – 700 in the PHEA films. The spatial gradient of the potential is the driving force for the solute to localize at the potential minimum in between the two interfaces. The series of BDS considering the Z-distributions of chemical potential and diffusion coefficient reproduced the experimental results.

The present study successfully provided detailed information of the single guest dyes on the Z-distributions of the averaged position and lateral diffusion coefficient in polymer thin films. The existing probability density and lateral diffusion coefficient were dependent on the Z-position as well as the property of guest molecule such as hydrophobicity. The behaviors of guests in interfacial regions are generally specific and they play significant roles in the performance of thin-layer polymeric materials. The present study revealed that the control of both the properties of host polymer and guest molecule are required for achieving desired behaviors of guest chemical reagents, providing a new significant insight into the design of polymeric thin-film materials. We foresee the approach is potentially applicable to the evaluation of a wide variety of polymeric materials in which interfacial regions play important roles, such as multilayered polymers, blended polymers with microscopic phase-separation structures, polymeric materials including nanomaterials, and polymeric liquid crystals.

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Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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