

Phys Chem Lett. Author manuscript; available in PMC 2013 December 23.

Published in final edited form as:

J Phys Chem Lett. 2011 March 17; 2(6): . doi:10.1021/jz200065f.

Transient 3-Dimensional Orientation of Molecular Ions in an Ordered Polyelectrolyte Membrane

Carmen Reznik[‡], Rosalie Berg[‡], Ed Foster[†], Rigoberto Advincula[†], and Christy F. Landes^{‡,*}
[‡]Rice University, Houston, Texas 77005

[†]University of Houston, Houston, Texas 77204

Abstract

Single-molecule fluorescence spectroscopy is employed to reveal 3-dimensional details of the mechanisms underpinning ion transport in a polyelectrolyte thin film possessing polymer-brush nanoscale order. The ability to resolve fluorescence emission over three discrete polarization angles reveals that these ordered materials impart 3-dimensional orientation to charged, diffusing molecules. The experiments, supported by simulations, report global orientation parameters for molecular transport, track dipole angle progressions over time, and identify a unique transport mechanism: translational diffusion with restricted rotation. Generally, realization of this experimental method for translational diffusion in systems exhibiting basic orientation should lend itself to evaluation of transport in a variety of important, ordered, functional materials.

Keywords

Diffusion; polymer brush; reorientation; spectroscopy; single molecule

The ability to sequester and transfer charge and matter via functionally responsive materials promises to transform the field of electrochemical storage and conversion. 1-3 Movement of small molecules and charge through materials is dependent on the molecular and nanoscale architectures extant in these materials, with the presence of accessible active surfaces, molecular sized channels, and general orientation vectors all contributing to transport characteristics. 1,4-6 Recent examples in which divergent diffusion behavior in constrained systems is observed highlight the importance of nanoscale architectures with respect to transport behaviors.^{5,7} These and other recent observations inspire the rational design of functional materials to exploit structurally mediated sequestration, transport, and release. 1,3-4,8 Combined with growing understanding of stimuli-responsive, switchable functional properties demonstrated by certain soft surfaces, especially polymer brushes,³ a powerful toolkit for building functional and tunable materials is being assembled. Strong polyelectrolyte thin films are likely to be part of the stimulus-response toolkit. Characterized by complete proton dissociation even at low pH (1 and lower), these materials serve as proton-exchange membranes in fuel cells. 9 It is well established that polymer brush architecture introduces oriented nano-scale structure in membranes, with an orientation vector normal to the grafting surface, and that this structural orientation leads to orders of magnitude increase in charge transport for polyelectrolyte membranes. 10–11 What is not yet known are the specifics of transport mechanisms within these thin films. By observing

^{*}Corresponding author: cflandes@rice.edu.

transport of positively charged R6G fluorescent molecules as they transit a sub-femtoliter focal volume, and employing a novel 3-angle polarization resolved detection scheme, we are able to provide a 3-dimensional description of transport within a model system of well-characterized poly(styrene sulfonate) (PSS) brushes. 12–13

Fourkas first proposed a three component polarized-light detection system in 2001 as a way to exploit the angle collection properties of high numerical aperture (NA) objectives used in emerging confocal single-molecule techniques. ¹⁴ The strength of this technique, in contrast to 3-D orientation imaging methods, is improved time resolution, making the method ideal for evaluating oriented modes of molecules that experience significant translational diffusion. That it is only now being applied to a translating experimental system, as discussed in the Supplementary Information (SI), is likely because the system under analysis must have sufficiently ordered motion to be distinguished by the analytical algorithms. In this work, we achieve millisecond time resolution, enabling discernment of orientation during transport within the polyelectrolyte brush. Because model experimental systems with known orientation, diffusion rates, and time scales do not exist, 15 demonstrating the inherent information available and limits of this method requires use of simulations to model the physical dynamics of the system. To this end, we developed a Monte Carlo rotation/ translation random walk simulation of diffusion, incorporating kinetic state transitions and the ability to define a preferred molecular orientation, to fully describe the experimental milieu and generate model data.

The detection scheme, molecular environment, and spherical coordinate scheme is illustrated in Figure 1. Note from the representation of the focal volume intersection with the polymer brush in 1.b that there is a heterogeneous environment encompassed by the focal volume. The focal volume is ~1 μ m in half height, while the polymer brushes in the experiments here are between 5 and 6 nm thick, with even coverage of \pm 0.5 nm as verified by ellipsometry (see SI). However, as discussed in previous work and in more detail below, because of the strong interaction between the brush and dye, observed translational diffusion in this system consists almost exclusively of brush:dye interactions. Here, for a molecule with an absorption dipole oriented along z (the observation axis of the microscope), Θ is equal to 0. The use of high NA optics allows determination of the spherical coordinates associated with the molecular transition dipole. He intensity measured at three detectors (I_0 , I_{90} , I_{45}) for a molecule in the detection volume is related to calculated polar (θ) and azimuthal (φ) angles as shown in equations 1–3:14

$$\phi = \frac{1}{2} \tan^{-1} \left\{ \left[I_{45}(\Theta, \Phi) - \frac{I_o(\Theta, \Phi) + I_{90}(\Theta, \Phi)}{2} \right] \left[\frac{I_o(\Theta, \Phi) - I_{90}(\Theta, \Phi)}{2} \right]^{-1} \right\}$$
(1)
$$I_{tot}(t, t + \tau) = \frac{1}{2A} \left[\left(1 - \frac{B}{C \cos 2\Phi} \right) I_o + \left(1 + \frac{B}{C \cos 2\Phi} \right) I_{90} \right]$$
(2)
$$\theta = \sin^{-1} \left\{ \left[\frac{I_o(\Theta, \Phi) - I_{90}(\Theta, \Phi)}{2I_{tot}(t, t + \tau) C \cos 2\Phi} \right]^{1/2} \right\}$$
(3)

Because the direction of the molecular transition dipole is invariant with respect to the molecular axes, polarization techniques allow reporting of molecular orientations. For our discussion here, capital Θ and Φ represent *actual* angles adopted by a dipole is sample space, and lower case θ and φ represent the *calculated* values. The factors A-C in the equations are NA dependent (see the SI). ¹⁴ There is 8-fold degeneracy for the experimental environment

with respect to these trigonometric functions ^{15,18}; angles explored by a dipole are mapped to 90° quadrants in the calculated polar and azimuthal angle planes. Detailed descriptions of the method, the analytical function space, and simulations are found in the SI.

Experiments for this system are conducted with the concentration of R6G adjusted so that there is on average less than one molecule on the focal volume at a time (~ 100 pM). At this concentration and with the given focal volume size, the likelihood of a diffusion event arising from more than one molecule in the focal volume is < 3%. Analysis via equations 1–3 rests on the ability to accurately identify diffusion events. Adequate counts are required to identify an event with statistical accuracy, and to produce meaningful analytical results. In this case, we selected as events instances in which photon counts were at least 20 counts/bin-time, and greater than 3 times background levels (see SI for further discussion of event identification). To achieve adequate counts for the polarization resolved analysis, then, excitation power was adjusted to ~ 800 W/cm², and data was collected at a 1 ms bin-time. As shown explicitly in previous work, at this power, photobleaching of single molecules becomes evident, where intensity levels are observed to drop suddenly to background levels. For determination of translation diffusion rates using Fluorescence Correlation Spectroscopy (FCS), excitation power was reduced to ~ 80 W/cm², where the effects of photobleaching on measured diffusion rates are not evident.

Examination of molecular transport in PSS brushes from previous work reveals both heterogeneous diffusion mechanisms as well as strong local interactions with PSS. 13 Fluorescence correlation spectroscopy (FCS) yields translational diffusion constants for cationic R6G (with dimensions of <1.5 nm) in these brushes at neutral pH of $\sim\!10^{-14}$ m²/s. $^{12-13}$ FCS studies for this strongly charged dye:brush system reveal significantly slowed translational diffusion indicative of dye diffusion in the brush, without a significant component of fast translational diffusion indicative of diffusion in bulk water or near surfaces. $^{12-13}$ Single molecule blip analysis of the translational diffusion occurring in the brush furthermore reveals periods of very slow rotation/reorientation, as well as periods of faster molecular rotation that occurs on a time scale faster than the data collection. 13

Several single molecule diffusion events within the polymer brush are shown in the trajectory in Figure 2.a), where data acquired from the 3-detector analysis is plotted. In 2.b) an expanded single event is shown. For the discussion here, fast rotation/reorientation which is evidenced by equal signal on all three channels is termed 'Type 1,' as signified in 2.b). Oriented periods with restricted rotation, marked by significant inequality among the three channels, is termed 'Type 2.' As discussed in the previous work, Type 2 events consist of both moving and adsorbed molecules, and are strongly suggestive of Coulombic interactions between the cationic probe and the polyanionic PSS.¹³ Within this 3-detector method, Type 1 traces arise from molecules that are rotationally diffusing on a time scale faster than the time scale of the data collection, and also result from molecules that are *oriented*, but with Θ close to 0° . An analysis of lifetimes for these two populations in the PSS:R6G system detailed in previous work¹³ yields lifetimes of 1.7 ms \pm 0.3 ms for Type 1 events and 3.7 ms \pm 0.7 ms for Type 2 events.

Using automated algorithms (see SI) to isolate Types 1 and 2 diffusion, we report the distribution of angles occupied during these two distinctive transport regimes occurring in the brushes. Figure 2.c shows histograms of the calculated spherical coordinates for these two populations over a representative 5 minute diffusion trace, with the purple and red histograms indicating the distribution of Type 1 orientation and Type 2 orientation respectively. The azimuthal angle distribution (left histogram) for Type 1 diffusion shows a relatively smooth profile with peaks at $\pm 45^{\circ}$, and a polar angle distribution centered around low angles peaking at $\sim 23^{\circ}$ (right histogram). These values are typical of free diffusional/

rotational transport, reflecting angles that correspond to dipole orientations near 0° Θ : the distribution arises from actual occupation of orientations near $\Theta = 0^{\circ}$, and also from fast rotation presenting the same intensity profile.

In contrast, the distributions obtained for oriented, Type 2 diffusion show a distinct, nonuniform profile, with azimuthal distribution centered at 12° for this brush sample, and a shifted polar angle distribution centered at 39°. This clustering of angle values around specific values as measured over all oriented events suggests a strong preferential orientation for R6G dye molecules that are interacting strongly with the PSS brush. In an isotropic environment, this kind of preferential orientation would not be expected, however an orientation preference for this particular system can be understood by considering the anisotropic shape of R6G (see Figure 1), and the oriented nature of the polymer brush. Both steric and Coulomb interactions between the brush and dye become potential contributors to alignment, on average, of R6G with the brush orientation vector. These can give rise to confined diffusion within naturally formed aqueous channels, ^{13,19} or strong Coulomb driven alignment of the dye with the polymer strands exhibiting net orientation normal to the surface. Coulomb alignment may be especially important during Type 2, strong interaction with the brush, with a possible hopping diffusion mechanism. It is a strength of this single molecule technique that even in the presence of heterogeneous interaction mechanisms and sample environment, preferences in orientation can be resolved.

To investigate the hypothesis that these results arise from a preferred orientation of the molecules in the brush, random walk simulations were conducted. Results from random walk simulation in which there is Type 2 slow/restricted rotational diffusion are shown in Figure 2.d and e. Shown in Figure 2.d are results for a simulated system that exhibits restricted rotational diffusion but no *preferred* orientation. In this case, Type 2 azimuthal histograms present a flat distribution across all angles, as would be expected, with a population identified by the automatic algorithm as Type 1 diffusion as well. The polar angle distribution also shows identification of approximately equal occupation of the central angles, in this case with the occupation split between the two types of diffusion. The distributions here reveal that the analysis algorithms tend to undercount angles outside of $\theta = 0^{\circ} \pm 35^{\circ}$, due to partial limitation in the function space and shot noise. ^{15,18} Analysis of the effects of shot-noise on angles calculated calculated from the 3-detector intensity profiles has been evaluated previously, ^{15,18} and the effect is discussed at more length in the SI.

In 2.e, data is shown for a simulated experiment in which a preferred dipole orientation of 12° Φ and 65° Θ is introduced. In this case, we observe clear resolution of these orientation preferences for both azimuthal ($\varphi=12^{\circ}$), and polar angles ($\theta=60^{\circ}$), with increased occurrences of these angles. The results of these simulations support a hypothesis that Type 2 transport in the brush occurs in the presence of an orienting potential. Azimuthal angle identification is accurate, with the polar angle distribution shifted by 5° , again due to limitations in the function space notes earlier.

Table 1 summarizes the statistics and angle preferences measured for 2 brush syntheses, over multiple sampling periods/locations on the brush surfaces. The data demonstrate the ability of the method to identify significant variance in transport characteristics in the two brushes.

In further analysis, an evaluation of time-dependent behaviors allows resolution of the reorientation characteristics of translating molecules in comparison to stationary molecules. In this analysis, periods of slow oscillation during single molecule diffusion events are revealed. Figure 3 shows time progressions for several events in the PSS brush, as well as events for stationary molecules embedded in spin-cast PMMA. Molecules in the PMMA

film are translationally stationary at room temperature and exhibit only very slow rotation. Traces for PMMA (left) and PSS (right) are shown in Figure 3 (a-d). The histograms, (b-e), show polar and azimuthal angle distributions measured over each event. As expected, the angle distributions are narrower for the highly rotationally-restricted individual molecules in PMMA, than for diffusing molecules in the PSS brush. The PMMA control experiments demonstrate optical system resolution of azimuthal angles over the entire 90° range. The range of polar angles returned, evaluated over 28 PMMA single molecules (not shown) extends across a range of $\sim 10 < \theta < \sim 75$.

Figure 3.c-f, shows time progressions of molecular orientation for the top panel traces. In the PMMA time progression in c we observe a 56 ms span in which the probe is stationary (highlighted region), and during which θ and φ fluctuate by ~5% (mean values: $<\theta>=6.2$ °, $<\varphi>=56.9$ °). Stationary regions identified across several events allows estimation of experimental noise limits for angle resolution, which we calculate to be ± 5 ° for angles from -45°< φ < <45° and <23°< <45°.

Regions of dipole movement that extend beyond the noise-limited fluctuations can be attributed to dipole reorientation. In the case of the PSS measurements, multiple examples were observed in which there is transient oscillation of dipole orientation in the θ/ϕ space during strong interactions. This is highlighted in f), with a span of 56 ms over which oscillations occur with an approximate period of 9 ms. The SI provides further illustration of this phenomenon. A possible origin for these observed transient oscillations may be transient occupation of local energy 'metabasins' in the structure. $^{21-22}$

Detection over three polarization angles also allows clarification of the mechanisms underpinning the Type 2 transport of oriented molecules. This information arises from evaluation of the total detected fluorescence intensity at each time bin, $I_{tl,em}(t) = I_0 + I_{90} + I_{45}$. $I_{tl,em}(t)$ is a function both of a dipole location within the Gaussian intensity profile of the excitation volume, and the dipole orientation with respect to the propagation direction of excitation light. These dependencies and the equations expressing them are illustrated in Figure 4.a-b. Time dependent changes in intensity can thus arise from either translation or slow rotation within the excitation volume. As we demonstrate below, there is a limit to the possible magnitude of change in total intensity due to rotation only, and a careful evaluation of the concurrent time dependence of polar angle and total intensity reveals oriented translational transport.

The limit to the magnitude of change in $I_{tl,em}(t)$ that can be attributed to rotation only can be demonstrated with simulated traces. Plotted in Figure 4.c are simulated intensity traces (center panel), polar angle (bottom) and a total intensity (top) for a dipole rotating smoothly in 0.5 degree steps from $\Theta = 0^{\circ}$ to $\Theta = 90^{\circ}$, with shot-noise added. This trace is generated with $\Phi = 45^{\circ}$ and intensity at the detectors matched to experimental conditions. A linear fit to the highlighted region of the trace yields dI/dt = 2.3 counts/s and $d\theta/dt = 1.1^{\circ}$ /s. Taking the ratio of the two derivatives yields change in intensity with respect to changing polar angle: $dI/d\theta = 2.1$ counts/°. This is the maximum achievable change in intensity due to rotation. Note, that Figure 4.c (top panel) illustrates a feature of the high NA optics, namely, that even for $\Theta = 0^{\circ}$, significant excitation still occurs (see SI), $^{14,16-17}$ resulting in reduced modulation depth and limiting the maximum value of $dI/d\theta$.

Figure 4.d shows representative results for an R6G diffusion event in polymer brush. In this example we identify a region in which there is a largely continuous drop in total intensity (top panel) accompanied by significant orientation (center). The $dI/d\theta$ for this region is -56 counts/°, significantly higher than the maximum $dI/d\theta$ that can be attributed to rotation only.

This measured change in intensity in excess of that arising from rotation can be attributed to translational motion within the focal volume during this strong, oriented interaction.

In this case, we witness enhanced translational diffusion with respect to rotational diffusion within a single event, which is a strong indicator of dynamic heterogeneity $^{21,23-24}$ or spatially confined diffusion. $^{5,25-26}$ Data evaluating multiple oriented single molecule events reveals that $\sim \frac{1}{4}$ exhibit translation without significant reorientation. (See SI for table of data).

In summary, the use of three angle polarization resolved fluorescence detection, combined with simulations of the oriented polymer brush experimental milieu makes it possible to identify and quantify oriented translational diffusion mechanisms occurring in strongly interacting systems with nanoscale orientation characteristics. The analysis presented here has revealed oriented translational movement within a PSS polymer brush, and has identified a global orientation parameter defining preferred orientation for guest molecules in this thin-film. Having demonstrated that it is possible to quantify decoupled rotation/ translation in ordered polyelectrolyte membranes, future efforts will focus on understanding the relationship between this decoupling and local order within the brush system.

The details of thin film synthesis and experimental procedures can be found in the supporting information.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

C. Landes thanks the Norman Hackerman Welch Young Investigator Program at Rice University. Acknowledgment is made to the Donors of the American Chemical Society Petroleum Research Fund for partial support of this research. C. Reznik thanks the NIH for funding through Molecular Biophysics Training Grant (T32 GM008280). We also thank the following individuals for providing insight and productive conversation with respect to this work: Dr. Stephan Link, Dr. Anatoly Kolomeisky, Dr. Wei-Shun Chang, and Alexei Tcherniak.

References

- Simon P, Gogotsi Y. Materials for Electrochemical Capacitors. Nat. Mater. 2008; 7:845–854.
 [PubMed: 18956000]
- 2. Slota J, He X, Huck WTS. Controlling Nanoscale Morphology in Polymer Photovoltaic Devices. nanoToday. 2010; 5:231–242.
- 3. Stuart MAC, Huck WTS, Genzer J, Muller M, Ober CK, Stamm M, Sukhorukov GB, Szleifer I, Tsurkruk VV, Urban M, et al. Emerging Application of Stimuli-Responsive Polymer Materials. Nat. Mater. 2010; 9:101–113. [PubMed: 20094081]
- Ho PKH, Kim J, Burroughes JH, Becker H, Sam FYL, Brown TM, Cacialli F, Friend RH. Molecular-Scale Interface Engineering for Polymer Light-Emitting Diodes. Nature. 2000; 404:481–484. [PubMed: 10761912]
- 5. Wang B, Anthony SM, Bae SC, Granick S. Anomalous Yet Brownian. Proc. Natl. Acad. Sci. 2009; 106:15160–16154. [PubMed: 19666495]
- Norsten TB, Guiver MD, Murphy J, Astill T, Navessin T, Holdcroft S, Frankamp BL, Rotello VM, Ding J. Highly Fluorinated Comb-Shaped Copolymers as Proton Exchange Membranes (PEMs): Improving PEM Properties Through Rational Design. Adv. Funct. Mater. 2006; 16:1814–1822.
- Kirstein J, Platschek B, Jung C, Brown R, Bein T, Brauchle C. Exploration Of Nanostructured Channel Systems With Single-Molecule Probes. Nat. Mater. 2007; 6:303–310. [PubMed: 17351614]

 Argun AA, Ashcraft NJ, Hammond PT. Highly Conductive, Methanol Resistant Polyelectrolyte Multilayers. Adv. Mater. 2008; 20:1539–1543.

- Li Q, He R, Jensen JO, Bjerrum NJ. Approaches and Recent Development of Polymer Electrolyte Membranes for Fuel Cells Operating Above 100 Degrees C. Chem. Mater. 2003; 15:4896–4915.
- Whiting GL, Snaith HJ, Khodabakhsh S, Andreasen JW, Breiby DW, Nielsen MM, Greenham NC, Friend RH, Huck WTS. Enhancement of Charge-Transport Characteristics in Polymeric Films Using Polymer Brushes. Nano Lett. 2006; 6:573–578. [PubMed: 16522065]
- 11. Advincula, RC.; Brittain, WJ.; Baster, KC.; Ruhe, J. Polymer Brushes: Synthesis, Characterization, Applications. Wiley; 2004.
- Reznik C, Darugar Q, Wheat A, Fulghum T, Advincula RC, Landes C. Single Ion Diffusive Transport within a Poly(Styrene Sulfonate) Polymer Brush Matrix Probed by Fluorescence Correlation Spectroscopy. J. Phys. Chem. B. 2008; 112:10890–10897. [PubMed: 18630854]
- 13. Reznik C, Estillore N, Advincula RC, Landes CF. Single Molecule Spectroscopy Reveals Heterogeneous Transport Mechanisms for Molecular Ions in a Polyelectrolyte Polymer Brush. J. Phys. Chem. B. 2009; 113:14611–14618. [PubMed: 19813742]
- 14. Fourkas JT. Rapid Determination of the Three-Dimensional Orientation of Single Molecules. Opt. Lett. 2001; 26:211–213. [PubMed: 18033550]
- 15. Hohlbein J, Hubner CG. Three-Dimensional Orientation Determination of the Emission Dipoles of Single Molecules: The Shot-Noise Limit. J. Chem. Phys. 2008; 129 094703-094701-094706.
- 16. Axelrod D. Carbocyanine Dye Orientation In Red Cell Membrane Studied by Microscopic Fluorescence Polarization. Biophys. J. 1979; 78:557–574. [PubMed: 263688]
- 17. Forkey JN, Quinlan ME, Goldman YE. Protein Structural Dynamics by Single-Molecule Fluorescence Polarization. Prog. Biophys. Mol. Biol. 2000; 74:1–35. [PubMed: 11106805]
- Lu C-Y, Vanden Bout DA. Analysis of Orientational Dynamics of Single Fluorophore Trajectories from Three-Angle Polarization Experiments. J. Chem. Phys. 2008; 128 244501-244501 -244501-244510.
- 19. Daniels C, Reznik C, Landes CF. Dye Diffusion at Surfaces: Charge Matters. Langmuir. 2010; 26:4807–4812. [PubMed: 20163084]
- Bartko A, Xu K, Dickson RM. Three-Dimensional Single Molecule Rotational Diffusion in Glassy State Polymer Films. Phys. Rev. Lett. 2002; 89 026101-026101.
- Adhikari AN, Capurso NA, Bingemann D. Heterogeneous Dynamics and Dynamic Heterogeneities at the Glass Transition Probed with Single Molecule Spectroscopy. J. Chem. Phys. 2007; 127 114508 - 114501 - 114508 - 114509.
- 22. Doliwa B, Heuer A. Hopping in a Supercooled Lennard-Jones Liquid: Metabasins, Waiting Time Distribution, and Diffusion. Phys. Rev. E. 2003; 67 030501-030501 -030501-030504.
- 23. Blackburn FR, Wang C-Y, Ediger MD. Translational and Rotational Motion of Probes in Supercooled 1,3,5-Tris(Naphthyl)Benzene. J. Phys. Chem. 1996; 100:18249–18257.
- Jagannathan K, Sung BJ, Yethiraj A. Dynamics of Probes in Model Glassy Matrices. Phys. Rev. Lett. 2006; 97 145503-145501 -145503-145504.
- 25. Jung C, Hellriegel C, Platschek B, Wohrle D, Bein T, Michaelis J, Brauchle C. Simultaneous Measurement of Orientational and Spectral Dynamics of Single Molecules in Nanostructured Host-Guest Materials. J. Am. Chem. Soc. 2007; 129:5570–5579. [PubMed: 17408267]
- Schmidt-Rohr K, Chen Q. Parallel Cylindrical Water Nanochannels in Nafion Fuel-Cell Membranes. Nat. Mater. 2008; 7:75–83. [PubMed: 18066069]

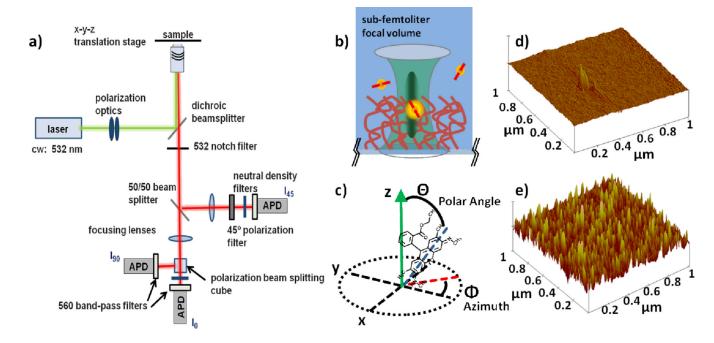


Figure 1. a) Schematic of epiflourescence microscope with three angle polarization detection. b) Cartoon showing the intersection of the laser focal volume with a polymer brush, and diffusing molecules with emission dipoles depicted (not to scale). c) Coordinate scheme, with R6G and emission dipole (in blue), Capital Θ and Φ , as shown, are the *actual* polar and azimuthal angles, respectively, adopted by a dipole. d) and e) AFM images of the surface of a clean coverslip (d), and a polymer brush modified coverslip (e). The scale in z is 10 nm for the AFM images, and in×and y is 1 μ m.

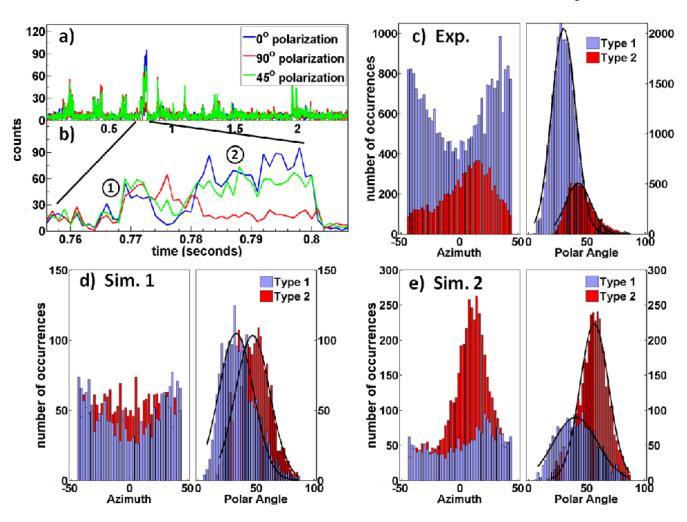


Figure 2. a) A fluorescence trace with several single molecule diffusion events for R6G in a polymer brush. b) A close-up of a single molecule transition through the laser focal volume, showing the intensity traces acquired from the three detectors. Region labeled Type 1: Nonoriented diffusion with rotation. Region labeled Type 2: Oriented diffusion with restricted rotation. c) Distribution of R6G azimuthal and polar angles, respectively, sampled in the polymer brush, measured over all diffusion events during a 5 minute period. The two types of diffusion, non-oriented, signified as Type 1, and oriented, Type 2, are shown in the separate purple and red histograms respectively. The polar angle distributions are well fit by a Gaussian. The azimuthal angle distributions hint at a number of subpopulations and fits with up to 4 Gaussians do not converge. d) Distribution of angles calculated for a random walk simulation in which oriented transport occurs without a preferred orientation vector. e) distribution of angles calculated for a simulation in which preferred orientations of $12^{\circ} \Phi$ and $65^{\circ} \Theta$ are introduced. Amplitudes of the histograms are a measure of the number of oriented/non-oriented events in a trace.

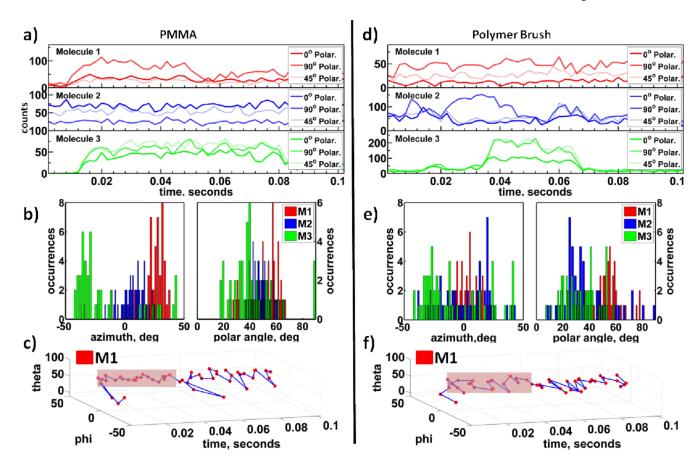


Figure 3. (left) Data for 3 different single molecule events for R6G embedded in PMMA is shown, a) intensity traces, b) distributions of sampled angles for the three events, c) a scatter plot for the top trace in a) over the time course shown. (right) Data for R6G events in polymer brush, with intensity traces, angle distributions and scatter plots in d-f. The highlighted region in part c shows a portion of the trajectory from which the maximum standard deviation due to instrument noise can be estimated. The highlighted region in part f) shows a region of repeating oscillation. See the Supporting Information for further discussion.

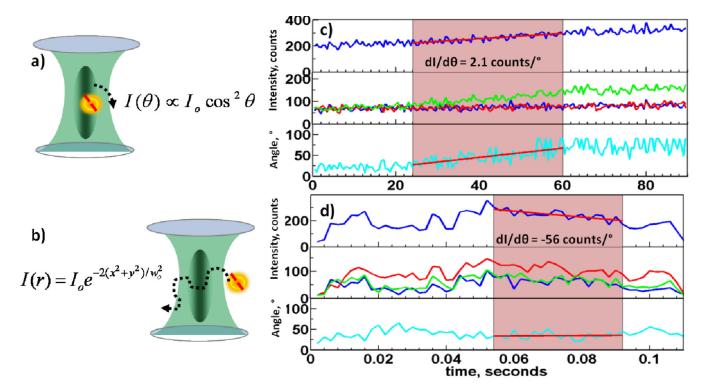


Figure 4. Evaluation of change in intensity with respect to changes in polar angle. a) and b) are representations of the separate processes upon which intensity is dependent: a) rotational diffusion within the focal volume, b) translational diffusion through the focal volume. c) analytically generated traces for a rotating dipole moving in 0.5 degree steps from $\Theta = 0$ to $\Theta = 90$. The top panel shows the summed intensity measured over the three detectors. The center panel shows the individual detector signals with shot-noise added (blue: I_0 , red: I_{90} , green: I_{45}), and the bottom panel shows the θ calculated from the traces shown in the center panel. d) Intensity profiles and calculated θ for experimental data. For both the simulated and experimental data, regions of \sim continuous intensity change over which calculations were performed are highlighted.

Table 1

are 6° (azimuth), and 37° (polar angle). See SI for representative angle histograms from the two brush syntheses. Locations across the brush surfaces were hour monolayer), fewer than 10% of events were oriented. The mean angle locations in Brush 1 are 14° (azimuth), and 39° (polar angle), and for Brush 2, Oriented diffusion statistics over two brush syntheses. For Brush 1 (48 hour monolayer), on average, 24% of events were oriented, while for Brush 2 (32 selected from within a 10 µ area.

Reznik et al.

Sample trial	trial	# event bins	% Oriented	# event bins % Oriented Num oriented bins	Prefe	rred o	Preferred φ Preferred θ
Brush 1	1	68221	73%	15691	12		40
	2	51887	24%	12453	15		39
	3	49948	25%	12487	12		38
	4	62013	22%	13643	15		37
Brush 2	1	163191	%7	6528	9		39
	2	132168	%8	10573	8		35
	3	275139	%8	22011	3	14.6	36

Page 12