Subdiffraction-Resolution Optical Measurements of Molecular Transport in Thin Polymer Films

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ABSTRACT: The measurement of molecular transport within polymer films yields information about the internal structural organization of the films and is useful in applications such as the design of polymeric capsules for drug delivery. Layer-by-layer assembly of polyelectrolyte multilayer films has been widely used in such applications where the multilayer structure often exhibits anisotropic transport resulting in different diffusivities in the lateral (parallel to the film) and transverse (normal to the film) directions. Although lateral transport can be probed using techniques such as fluorescence recovery after photobleaching (FRAP), it cannot be applied to probing transverse diffusivity in polymer films smaller than the diffraction limit of light. Here we present a technique to probe the transport of molecules tagged with fluorophores in polymer films thinner than the optical diffraction limit using the modulation of fluorescence emission depending on the distance of the tagged molecules from a metal surface. We have used this technique to probe the diffusion of proteins biotin and bovine serum albumin (BSA) in polyelectrolyte multilayer films. We also studied the interdiffusion of chains in multilayer films using this technique. We observed a 3 order of magnitude increase in interdiffusion as a function of the ionic strength of the medium. This technique, along with FRAP, will be useful in studying anisotropic transport in polymer films, even those thinner than the diffraction limit, because the signal in this technique arises only from transverse and not lateral transport. Finally, this technique is also applicable to studying the diffusion of chromophore-labeled species within a polymer film. We demonstrate this aspect by measuring the transverse diffusion of methylene blue in the PAH−PAA multilayer system.

INTRODUCTION

Several emerging applications in multiagent targeted drug delivery require the use of polymer capsules. The loading and release behavior of drugs from such capsules strongly depends on the diffusivity of the drug molecules within these polymers whose thicknesses can range from tens of nanometers to micrometers. 1−4 For instance, the programmed release of a drug, with temporal control of the release rate, would require detailed knowledge of the diffusive behavior of the drug, which includes the depth of penetration, concentration profile, and so on. One might also be interested in the change in these parameters as a function of environmental variables. Layer-by-layer (LbL) deposition of polyelectrolytes is a very versatile technique for the fabrication of devices, functionalization of biomaterials, design of reservoirs for protein and drugs in tissue engineering, and drug delivery applications. 5−10 Polyelectrolyte multilayer (PEM) capsules have been extensively explored for targeted drug delivery and programmed release. 11,12 PEM films have also been used as scaffolds and reservoirs of growth agents in tissue engineering applications that also require a good understanding of diffusion through PEMs. In addition to the study of a diffusing species across PEM films, another interesting aspect of PEMs is the interdiffusion of their constituent PEs, the extent of which strongly influences the PEM growth regime, ranging from linear or exponential with respect to the number of layers. 13,14 These examples illustrate the importance of techniques to probe the diffusion of molecules in PEMs with thicknesses ranging from a few nanometers to a few micrometers.

The measurement of diffusion phenomena in PEM systems can be broadly classified into two types (optical techniques based on fluorescence from the diffusing species and nonoptical techniques such as X-ray photoelectron spectroscopy (XPS) and secondary ion mass spectrometry (SIMS)), which have been extensively used to measure diffusion in inorganic materials. For example, the role of interdiffusion in exponential layer buildup was recognized by the measurement of interdiffusion profiles in poly(l-lysine)/poly(l-glutamic acid) (PLL/PGA) using confocal microscopy. 15 However, optical measurements can be made only on films thicker than the optical diffraction limit. For example, the study of interdiffusion in PLL/PGA using confocal microscopy was possible because

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Figure 1. Distance-dependent modulation of fluorescence intensity near a metal surface calculated theoretically. (a) The intensity of fluorescence emitted from a fluorophore undergoes a periodic modulation depending on its distance from a metal surface. (b) The fluorescence from a polymer film is dependent on the diffusion profile as well as the thickness of the polymer layer. (c) Interference of back-reflected electric fields leads to a standing wave pattern of intensity that modulates the fluorescence signal emitted from a fluorophore depending on its distance from the substrate. In the case of a perfectly reflecting substrate \((r = \pm 1)\), the interference can lead to almost complete quenching of fluorescence.

the PEM films, in this case, are thicker than a micrometer, well above the diffraction limit. Optical techniques cannot be used to probe transverse diffusion in PEM films with thickness smaller than about 0.5 \(\mu m\) because of diffraction-limited optical resolution. Therefore, techniques such as X-ray photoelectron spectroscopy (XPS) and secondary ion mass spectrometry (SIMS) are required to probe diffusion in ultrathin PEM layers.\(^{16,17}\) However, the use of these techniques for studying diffusion in soft polymeric thin films suffers from several challenges. First, access to these sophisticated instruments is more limited than for optical microscopes, which are widely available. Second, XPS and SIMS can damage soft polymer layers, which can potentially result in erroneous conclusions drawn from these studies. For example, C60 cluster ions have recently been used in depth-profiling XPS to minimize the damage to soft materials.\(^{16,17}\) Although similar in their destructive nature, SIMS has an approximately 3 orders of magnitude better LoD compared to that of XPS.\(^{17,18}\) However, the identification of secondary ions unique to the diffusing species could be a problem in polymer systems that are much more heterogeneous than inorganic materials for which these techniques were initially developed.\(^{18}\) Therefore, it is of interest to consider alternate measurement techniques that do not suffer from the disadvantages of XPS and SIMS when probing diffusion in subdiffraction-limit thin films.

In this article, we describe an optical method capable of extracting the concentration profile of a fluorescently labeled diffusing species in PEM films as thin as 150 nm, well below the 500 nm axial resolution of confocal microscopes.\(^{19}\) The basis of this technique is the distance-dependent periodic modulation of the fluorescence intensity of a fluorophore from a metal surface.\(^{20}\) This phenomena is described in greater detail in the Theoretical Modeling section. Although the discussion presented there is centered on fluorescence emission, it is equally applicable in the case of emission from chromophores from a metal surface. We illustrate this aspect of the technique by probing the transverse diffusion of the methylene blue dye molecule in a PEM system. Henceforth, we will describe the technique in terms of fluorescence emission with the understanding that the entire discussion is equally applicable in the case of chromophores.

The fluorescence signal obtained from a sample as a function of time is a result of the cumulative fluorescence from fluorophores distributed different distances from the metal surface as shown in Figure 1a. By measuring the fluorescence as a function of time or the PEM thickness, we can extract the diffusion profile based on the theoretical model described subsequently. This technique can be applied to study the diffusion of any fluorophore or chromophore labeled molecule through a diffusing medium deposited on a metal surface, although in this article we primarily focus on fluoroscence labeled systems because of their wide applicability in biological studies.

### THEORETICAL MODELING

Calculation of Fluorophore Diffusion Profiles. The diffusion profile \(c(z, t)\) is the solution of the one-dimensional diffusion equation

\[
\frac{dc(z, t)}{dt} = D \frac{d^2 c(z, t)}{dz^2}
\]

where \(D\) is the diffusion coefficient of the diffusing species. The nature of the function \(c(z, t)\) depends on the initial and/or boundary condition and conditions appropriate for the system under consideration. The fluorophores shown in Figure 1a enter into the polymer film from the bulk fluid above. We consider two models of fluorophore incorporation and diffusion
into the PEM. In one model, the fluorophores continuously get incorporated into the PEM surface from the bulk fluid above and diffuse into the PEM layer. We refer to this model as the continuous diffusion model. The other possibility that we considered is that the incorporation of fluorophores on the surface presents an electrostatic barrier that prevents further incorporation beyond a threshold. Diffusion is then essentially a redistribution of the initially incorporated fluorophore population within the PEM. We refer to this model as the barrier redistribution of the initially incorporated condition for the continuous diffusion model can be written as

\[ c(0, t) = c_{\text{bulk}} \] for all values of \( t \). The metal substrate requires a no-flux boundary condition (because it is an impenetrable barrier) at \( z = d \), and the diffusion is confined between the upper and lower surfaces of the polymer film, i.e., \( z = 0 \) and \( d \), respectively. Then, the solution of the diffusion equation yields

\[ c(z, t) = c_{\text{bulk}} \left[ 2 - \text{erf}\left( \frac{z}{\sqrt{4Dt}} \right) - \text{erf}\left( \frac{2d - z}{\sqrt{4Dt}} \right) \right] \] (1)

The alternate picture of the barrier diffusion model can be considered to be a redistribution of an initial impulse loading of \( c_{\text{thresh}} \) molecules on the top surface. The boundary condition can then be written as

\[ c(z, 0) = c_{\text{thresh}} \delta(z) \] for the imposition of a no-flux condition at \( z = d \). With these boundary conditions, the solution can be written as

\[ c(z, t) = \sqrt{\pi Dt} \sum_{m=-\infty}^{m=\infty} \exp\left( -\frac{(z-2md)^2}{4Dt} \right) \] (2)

Figure 1b shows the distribution of fluorophores within the PEM thickness for various values of diffusion coefficient \( D \) calculated from our theoretical model.

**Modulation of Fluorescence Intensity.** The modulation of reflectance is the result of interference between the incident light field and the reflected light field, which can be written as

\[ \Gamma(z) = 1 + r(z) \gamma \] where \( r \) is the Fresnel reflection coefficient of the thin film structure consisting of the metal and the PEM above it with thickness \( z \). The total fluorescence intensity from the PEM layer is obtained by integrating the product of \( c(z) \) over the thickness of the PEM.

As shown in Figure 1c, the net electric field at point \( P \), which is a distance \( z \) above the substrate, is given by the interference of the incident and back-reflected wave components. The net electric field at point \( P \) can be written as

\[ E(z) = E_0 e^{i \phi_0} (1 + re^{i \phi}) \] (3)

where \( t \) is the transmission coefficient between the air and polymer film interface, \( r \) is the reflection coefficient for the film–substrate interface, \( E_0 \) is the amplitude of the incident field, \( \phi_0 \) is the phase acquired due to propagation in the polymer film from the interface to point \( P \), and \( \phi_t \) is the distance-dependent phase factor given by

\[ \phi_t = \frac{4\pi}{\lambda} n_2 z \] (4)

The interference between these two components creates a standing wave pattern of the electric field; consequently, the light field intensity as a function of distance from the substrate surface will be given by

\[ I(z) = |E(z)|^2 \]. From eq 3, a distance-dependent modulation factor, \( \gamma(z) \), corresponding to the standing wave created can be defined as

\[ \gamma(z) = |1 + re^{i \phi}|^2 = 1 + r^2 + 2r \cos\left( \frac{4\pi}{\lambda} n_2 z \right) \] (5)

The fluorescence excitation process is related to the number of photons available for excitation, which in turn is proportional to the light field intensity at the spatial location of the fluorophore. Therefore, the standing wave patterns of the intensity fields corresponding to excitation and emission wavelengths will modulate the fluorescence yield because of the modulation in the photon number densities corresponding to the spatially varying intensity fields. The combined modulation factor \( \Gamma(z) \) is the product of the modulation factors corresponding to excitation, \( \gamma_{\text{ex}}(z) \), and emission, \( \gamma_{\text{em}}(z) \):

\[ \Gamma(z) = \gamma_{\text{ex}}(z) \gamma_{\text{em}}(z) \] (6)

\( \gamma_{\text{ex}}(z) \) and \( \gamma_{\text{em}}(z) \) can be calculated by substituting the values of the excitation and emission wavelengths, respectively, into eq 4. The distance-dependent modulation of fluorescence from fluorophores diffusing through a porous polymer film is schematically shown in Figure 1a.

One can see from eq 4 that a strongly reflecting substrate with a high value of \( r \) can lead to significant modulation in the fluorescence intensity. For example, a perfect metal, with a reflection coefficient of \( r = -1 \), should lead to the complete quenching of fluorescence at the surface, (i.e., \( z = 0 \)). For weakly reflecting substrates such as glass, this effect is not prominent and may be below the resolution of ordinary CCD cameras with limited dynamic range. This modulation effect has previously been used to enhance the fluorescence signal by engineering substrates to have high reflectivity (e.g., multilayer dielectric mirrors with \( r = 1 \)). In the case of absorbing dyes such as methylene blue, the modulation term in eq 6 will contain only a single term equal to \( \gamma_{\text{abs}}(z) \) corresponding to the peak absorption wavelength of the dye.

The net fluorescence (or color output in the case of absorbing dyes) intensity \( I_{\text{FL}} \) from the PEM thickness will then be given by

\[ I_{\text{FL}}(d, t) \propto \int_{z=0}^{z=d} c(z, t) \Gamma(z) \, dz \] (7)

where \( c(z, t) \) is obtained as described in the previous section.

**EXPERIMENTAL SECTION**

**Materials.** Polymers poly(allylamine hydrochloride) (PAH, Mw \( \approx 15 \) kDa) and poly(acrylic acid) (PAA, Mw \( \approx 200 \) kDa), poly(ethyleneimine) (PEI, Mw \( \approx 25 \) kDa), poly-(l-glutamic acid) (PGA Mw \( \approx 50–100 \) kDa), poly-(l-histidine) (PLL, Mw \( \approx 30 \) kDa), fluorescein-labeled PLL (PLL-FITC, Mw \( \approx 30 \) kDa), fluorescein-labeled bovine serum albumin (FITC-BSA), fluorescein-conjugated biotin, 1-ethyl-3-(3-dimethylamino)propyl) carboxamide (EDC), and N-hydroxysuccinimide ( NHS) were purchased from Sigma-Aldrich. Sodium chloride (NaCl), sodium hydroxide (NaOH), and hydrochloric acid (HCl) were purchased from Merck. Methylene blue (MB, Mw \( \approx 319 \)) was purchased from Rankem Industries Limited. All solutions were prepared by using ultrapure water from Millipore with a resistivity of 18.2 MΩ. Aqueous solutions of polyelectrolytes PAH and PAA (0.01 M, based on the repeat unit molecular weight) were prepared by DI water of ionic strength 0.1 M NaCl. The solution pH was adjusted to 5.5 using 0.1 M NaOH and 0.1 M HCl. For interdiffusion experiments, PGA/PLL (1 mg/mL) polyelectrolyte solutions were made in water using 0.1 M NaCl at pH 4.4. FITC-BSA (100 μg/mL)
and FITC-biotin (100 μg/mL) were prepared with a phosphate-buffered saline solution (PBS), and an MB solution of 100 μg/mL was prepared in ultrapure water. All chemicals were used without any further purification.

**Multilayer Film Deposition.** For metal distance-dependent fluorescence measurements, a 100 nm aluminum layer was deposited on piranha-cleaned (3:1 H₂SO₄/H₂O₂) microscopic glass slides by a homemade thermal evaporator. The thickness was measured with a Bruker XT Stylus Dektak pro profiler. Then, polyelectrolyte multilayer films were deposited on Al-coated substrates by a dip-assisted layer-by-layer (LbL) self-assembly process where PEMs were constructed mainly as a result of electrostatic interactions by alternate deposition into solutions of polycations and polyanions. The dip time in each polyelectrolyte solution was 1 min, followed by three rinses with agitation in water and drying in pure nitrogen. The desired number of polyelectrolyte bilayer stacks ranging from 0.5 BL up to 20.5 BL at an interval of 2 BL has been made by repeating the LbL cycle with (PAH/PAA), and PEI(PGA/PLL),PGA polyelectrolytes. Thickness measurements of PEMs were carried out using a spectrosopic ellipsometer from J.A. Woollam Co. Inc. (section 2 and Figure S2 in the SI).

**Fluorescence Measurements for Transverse Diffusion.** For diffusion measurements, FITC-labeled BSA and FITC-biotin were allowed to adsorb on (PAH/PAA), multilayers for 35 min of incubation time, followed by three washing with PBS or water. A similar incubation procedure has been used to study the interdiffusion of FITC-PLL (1 mg/mL) in PEI(PGA/PLL),PGA films. For time-based saturation experiments at the fluorescence node and antinode, the incubation time is varied from 5 min to up to 75 min. To enhance the diffusion experiments, 1 M NaCl has been used instead of normal 0.1 M NaCl to prepare the FITC-PLL solution. To capture the fluorescence signatures in a nondiffusive environment, EDC-NHS cross-linking chemistry has been utilized to form the chemical cross-links between the PEI(PGA/PLL),PGA films. PEI(PGA/PLL),PGA films were kept in a freshly prepared solution of EDC(400 mM)-NHS(100 mM) dissolved in 0.15 M NaCl solution for 16 h, followed by rigorous washing with 0.15 M NaCl. Fluorescence imaging was carried out by using an Olympus BX51 M inverted microscope with a DP 71 CCD camera with a 5× objective and a 400 ms exposure time. The quantitative estimation of the temporal variation of fluorescence intensity was carried out by using ImageJ software to extract the transverse distribution of fluorophores in PEMs.

**RESULTS AND DISCUSSION**

We developed a mathematical model to calculate the fluorescence modulation as a function of PEM thickness for various values of the diffusion constants. The details of the model are already explained. Briefly, we used the analytical solution of the one-dimensional diffusion equation to calculate the distribution of fluorophores through the thickness of the PEM, referred to as $c(z)$ in Figure 1a. Then we calculated the modulation of fluorescence intensity, referred to as $\Gamma(z)$ in Figure 1a, and calculated the total intensity from the PEM layer by integrating the product of $c(z) G(z)$ over the thickness of the PEM. This theoretical model reveals a diffusion-dependent periodic modulation of the fluorescence from the PEM as a function of layer thickness. The origin of this periodic modulation is the interference of the incident light field with the one reflected from the metal surface. This interference leads to periodic constructive and destructive effects for the fluorescence emission as a function of the distance of the fluorophore from the metal surface. The presence of diffusion maintains this periodic nature but shifts the position of the minima laterally (different thickness compared to that in the zero diffusion case) and vertically (minima occurs at higher values compared to those in the zero diffusion case). Figure 1b shows the effect of the diffusion coefficient on the fluorescence intensity as a function of PEM thickness according to our theoretical model. We see from Figure 1b that very low values of the diffusion coefficient, e.g., $D = 1.6 \times 10^{-16}$ cm²/s (black curve in Figure 1b), exhibit nearly perfect destructive interference leading to zero intensity for certain PEM thicknesses. This happens because nearly all of the fluorophores are sitting at a thickness corresponding to destructive interference between incident light and the light reflected from the metal. However, when the diffusion coefficient is increased, e.g., $D = 5 \times 10^{-14}$ cm²/s (blue solid curve in), the distribution of the fluorophores within the PEM is broadened and there is a significant fluorophore population located apart from the complete destructive interference. This increases the minima and also produces a small lateral shift in the position of the minima. This is the physical basis for the observations related to fluorescence intensity. The thickness positions corresponding to constructive and destructive interference would be on the order of one-fourth of the wavelength used (~100 nm for FITC green fluorescence corresponding to an excitation and emission of 488 and 525 nm, respectively). Such a short spatial range corresponding to a large dynamic range in.
fluorescence intensity implies that very small values of $D$ can be measured easily with this technique. With diffusion-limited measurements such as FRAP, one would need to wait a long time $t$ for diffusion length $l_d$ which scales as $l_d \approx \sqrt{D t}$, to be measurable. In contrast, small measurement times would produce a sufficient optical contrast in our technique because the diffusion length, even if it is as small as 10 nm, would be a significant fraction of the spatial range ($\sim$100 nm as mentioned before) corresponding to the maximum dynamic range of our measurement. By measuring the fluorescence from PEMs with different thicknesses and fitting them to this model, the diffusion coefficient can be extracted, as we show in subsequent examples.

To experimentally confirm the theoretical calculations described above, we measured the fluorescence intensity of FITC-labeled BSA and biotin diffusing through PAH/PAA multilayers deposited over aluminum-coated glass slides (Figure 2b). We use a nomenclature scheme (PAH/PAA)$_n$, where $n$ refers to the number of bilayers. Periodic fluorescence intensity modulation was observed as predicted by the model. The periodic intensity modulation was also observed in the case of the MB dye according to our theoretical model (Figure 2a). Diffusion coefficients of $5 \times 10^{-15}$ and $1.3 \times 10^{-14}$ cm$^2$/s fit the experimentally observed behavior of BSA and biotin, respectively. The higher diffusion coefficient of biotin could be due to the smaller size of biotin (Mw = 500 Da) compared to that of BSA (66 kDa). Previous measurement of BSA diffusion has concluded a very small value of the diffusion coefficient below the measurement limit of the experimental system reported previously. $^{24}$ The diffusion coefficient of MB in the PAH/PAA multilayer system was estimated to be around $1 \times 10^{-15}$ cm$^2$/s, which is close to the previously reported value. $^{25}$

We then used this technique to probe the interdiffusion of PEs in a PEM. We used the poly(1-lysine)/poly(1-glutamic acid) (PLL/PGA) system to study interdiffusion phenomena because there are several reports in the literature describing this effect in the PLL/PGA system. $^{15,26}$ To probe interdiffusion, we examined the distance-dependent fluorescence intensity of FITC-labeled PLL as it diffused through the PEM matrix, which consisted of PEI(PGA/PLL)$_n$PGA deposited on aluminum. Here, poly(ethyleneimine) PEI is used to promote adhesion on aluminum-coated surfaces, and $n$ refers to the number of bilayers. We used various postassembly modifications of the PEM matrix to enhance or suppress the diffusivity of PLL as shown schematically in Figure 3a. Figure 3a represents the strategies we employed to control interlayer diffusion in PEMs, namely, enhancing diffusion by using high ionic strength and suppressing diffusion using cross-linked layers. By increasing the ionic strength of the solution containing FITC-PLL to 1 M of NaCl, we were able to demonstrate a higher diffusivity compared to an ionic strength of 0.1 M NaCl. From Figure 3b, we can see that the minimum intensity of the fluorescence modulation in the case of high salt (1 M, red curve) is much higher compared to the low salt case (0.1 M, black curve). The extracted $D$ values from these curves from best fits are $1.6 \times 10^{-14}$ and $1.6 \times 10^{-11}$ cm$^2$/s for the 0.1 and 1 M cases, respectively. There is a 3 order of magnitude increase in diffusivity in the presence of high salt, which we attribute to nearly total screening of electrostatic effects in the PEM. Previous reports on the effect of ionic strength on diffusion $^{27–34}$ have described an increase in diffusivity that could be due to the swelling of the films caused by extra salt.
ions. Although there are no reports to directly compare our data for the PGA/PLL system, an increase in diffusivity of between 1 and 4 orders of magnitude between 0.1 and 1 M ionic strength may be expected. The high salt condition favors diffusion by decreasing the density of polyanion–polycation complexes and by increasing the salt ion–polyion combinations. The effects of high salt concentration on film morphology and stability are provided in section 2 and Figures S2 and S3 of the SI.

To further probe the connection between diffusivity and the observed fluorescence intensity modulation, we cross-linked the PEI(PGA/PLL),PGA films using a well-characterized method involving 1-ethyl-3-(3-dimethyl amino propyl)-carbodiimide (EDC)/ N-hydroxysuccinimide (NHS) chemistry. Cross-linking is expected to make the PEM much denser, resulting in lower diffusivity. Specifically, we wanted to investigate to what extent the enhanced diffusivity under high salt conditions can be reversed by cross-linking. In the cross-linking method used, NHS aids the formation of amide bonds between the carboxylic groups of PGA and amine group of PLL. EDC catalyzes the formation of amide bonds between carboxylic groups of PGA and amine groups of PLL. The formation of amide bonds was revealed by FTIR spectroscopy (Figure S7) and refractive index measurements using an ellipsometer, which showed an increase in the RI value from 1.52 to 1.67 measured for 9 BL, presumably indicating the formation of cross-links between PGA and PLL that cause the PEM to stack more densely, consequently increasing the refractive index. The fluorescence modulation data from cross-linked PEMs shown in Figure 3b indicates that the enhanced diffusivity of PLL due to the high

Figure 4. (a) Theoretical model showing the continuous diffusion time evolution of fluorescence intensities at antinodal (AN) and nodal (N) points. (b) The same for the barrier diffusion model.

Figure 5. Temporal intensity modulation with different incubation time (a) for FITC-biotin on (PAH/PAA) at 8.5 BL and 12.5 BL, (b) FITC-BSA on (PAH/PAA) at 8.5 BL and 12.5 BL, (c) FITC-PLL (0.1 M NaCl) on PEI(PGA/PLL),PGA at 9 BL and 11 BL, and (d) FITC-PLL (1 M NaCl) on PEI(PGA/PLL),PGA at 9 BL and 11 BL, all (a–d) with representative fluorescence micrographs taken at 5, 30, 60, and 75 min.
salt condition could be suppressed to that of low salt values by
cross-linking. This observation implies that diffusion under the
cross-linked condition is dominated by the film morphology
and not by electrostatic interactions. We did not observe any
significant difference in diffusivity between cross-linked
and non-cross-linked PEMs for the low salt condition. This may be
because the diffusivity was already quite low for the low salt
case and further suppression due to cross-linking was not
significant in a relative sense.

In addition to enabling the extraction of diffusion
coefficients, this technique can also help us to develop a
physical picture of molecular diffusion in PEMs. To illustrate
this, we consider two models of molecular diffusion as
described in the Theoretical Modeling section (Figure 4).
One is referred to as a continuous diffusion model where the
molecules continuously diffuse into the PEM matrix from a
constant reservoir above (fluid containing the labeled
molecule), and the other referring to the initial adsorption of
charged molecules (such as proteins or PEI) near the PEM
surface presents an electrostatic barrier to the further
incorporation of molecules at the surface. The continuously
diffusing model predicted a monotonic increase in the FL
intensity at the nodal and antinodal points with respect to
incubation time (Figure 4a) whereas the barrier model
predicted an increase in the FL intensity at the nodal point
and a decrease at the antinodal point as shown in Figure 4b.
The nodal (N) and antinodal (AN) points refer to the bilayer
thicknesses producing the minimum and maximum FL
intensities, respectively. This lead us to conclude that the
measurement of temporal evolution of the fluorescence signal
will shed some light on the adsorption process.

We experimentally measured fluorescence intensity as a
function of time at the nodal and antinodal points of the
fluorescence modulation curves. The data is shown in Figure 5.
We observed that in the case of the diffusion of proteins
through the PAH/PEI system and the interdiffusion of FITC-
PLL in the PEI(PGA/PLL), PGA system under low ionic
strength (0.1 M, Figure 5a–c), the fluorescence intensity of the
antinodal samples decreased as a function of time as predicted
by the barrier diffusion model. The continuous diffusion model,
on the other hand, predicted an increase. Therefore, our
experimental data provides support for the barrier diffusion
model to operate under the regimes investigated in the present
work. The robustness of the experimental observation leading
to this conclusion was further confirmed by examining the
histograms of fluorescent images as shown in Figure S6. It was
found that not only the mean values but the entire distribution
of fluorescence intensities shifted to lower values as a function
of increasing incubation time for antinodal samples as predicted
by the barrier diffusion model. All of these experimental
observations show that the barrier diffusion model is the
dominant mechanism for the incorporation of charged species
and their diffusion into the PEM. Diffusion of FITC-PLL under
high salt conditions showed a flat temporal response (Figure 5d)
as predicted by either model.

## CONCLUSIONS

We have demonstrated a technique to probe the transverse
diffusive behavior of labeled molecules through ultrathin (100–
200 nm) polymer films. The data presented in this article
provide a good physical picture related to the incorporation of
charged molecules from solution into a PEM matrix and their
diffusion within the PEM stack. This knowledge will be useful
for developing architectures for applications ranging from the
design of biomaterial surfaces to programmed drug delivery,
where precise control of the chemical concentrations and their
distribution through the matrix are desired.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the
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Details of the model for the calculation of the fluorophore diffusion profile and calculation of the temporal intensity at nodal and antinodal points. Film growth analysis and effect of salt on film thickness monitored by spectroscopic ellipsometer. Data validating our experiments. (PDF)

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### Notes

The authors declare no competing financial interest.

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