A Tentative Route toward Nanofluidics: Directed Diffusion of Small Molecules Embedded within Adsorbed Polymers

Jiang Zhao and Steve Granick*

Departments of Materials Science and Engineering, Chemistry, and Physics, University of Illinois, Urbana, Illinois 61801

Received January 23, 2003
Revised Manuscript Received June 4, 2003

Introduction. The directed flow of fluids contained within small channels constitutes an emerging theme of modern chemistry and materials science. New applications are proliferating, for example, in the fields of microfluidics, chemical analysis, and protein crystallization. Most prior work has concerned channels whose cross-section dimensions are on the order of micrometers to hundreds of micrometers.[1] It is also attractive to consider the potential for directed flow of molecules within molecularly thin layers. In this communication, we present our initial findings directed to this end.

The scheme we have selected to prepare molecularly thin layers consists of using adsorbed polymers, which has the advantage of simplicity. Whereas for this initial study we have used polymers that coated the solid surface uniformly, it is easy to envision methods to generate patterned arrays of adsorbed polymers, for example by lithography or stamping a solid surface to render it selectively adsorbing. In this way we envision forming polymer wires or polymer ribbons, with size limited by the resolution available using lithography or stamping. Once such channels have been formed, we envision directing flow of molecules embedded within them under the stimulus of electric field[2] or temperature gradients.[3]

An alternative approach to produce molecularly thin channels might consist of placing two extended atomically smooth flat surfaces in close proximity, in a generalization of the surface forces technique. This is simple when dealing with curved surfaces[4] but becomes problematical and laborious when it is intended that the surfaces be parallel over linear dimensions of millimeters.[5] As another alternative one can envision more costly methods, such as fabricating nanometer-sized channels using advanced methods of silicon machining such as electron beams, which would have the advantage of higher spatial resolution.

With these considerations in mind, for investigation of the motion of solutes within nanometer-sized channels we have selected the system of polymers adsorbed at the solid−aqueous interface, and in this initial communication we consider adsorbed polyelectrolytes. Polyelectrolytes are polymers carrying permanent charges or ionizable groups. They can be made to adsorb on solid surfaces with opposite charge due to the electrostatic interaction between the polymer molecules and the surfaces.[6] There have been intensive studies of their adsorbed structures; this is found to depend on intrinsic and environmental factors such as charge density of the polyelectrolytes, ionic strength, and pH.[7,8] However, while there has been extensive research regarding the structure of polyelectrolytes at surfaces, studies of their dynamics are not yet so far advanced. Conventionally, nuclear magnetic resonance (NMR) spectroscopy and electron spin resonance (ESR) spectroscopy are applied to study the local mobility of the segments of adsorbed polymers. However, such experiments are conducted using colloidal suspensions, and therefore, the information obtained is rather averaged, lacking microscopic features. Furthermore, the issue of spatial heterogeneity cannot be addressed.[9−11] An alternative approach consists of using few-molecule fluorescence methods. The diffusion of fluorescent molecules can be measured using fluorescence correlation spectroscopy (FCS) and single-molecule trajectory imaging—techniques developed in the biological sciences.[12]

This study focuses on the dynamics of the complex system of a polycation, quaternized poly(4-vinylpyridine) (QPVP), which was allowed to adsorb to freshly cleaved mica, whose surface in aqueous environment is negatively charged. By changing the ionic strength as a variable in the adsorption condition, the structure of the QPVP layer was tuned from flat to swollen, and the resulting mobility of probe molecules within these layers was measured accordingly.

Previously, this laboratory studied, using the complementary methods of surface forces and nanorheology, this same aqueous QPVP−mica system.[15]

Experimental Section. a. Materials. The polymer quaternized poly(4-vinylpyridine) (QPVP) was prepared in this laboratory by quaternizing poly(4-vinylpyridine) (PVP) with ethyl bromide using the method described elsewhere.[7] The degree of quaternization, determined using infrared spectroscopy in D_2O, was 98%. The molecular weight of PVP (before quaternization) was 34 200 g mol^{-1} with a molecular weight dispersity of $M_w/M_n = 1.23$ (Polymer Source). Inorganic salts, sodium borate, and sodium chloride were used as received (General Storage). Deionized water was prepared using Milli-Q (Millipore) deionizing and filtration columns.

The experiments were performed at pH = 9.2. The QPVP was dissolved in 1.0 mM sodium borate solution at the concentration of 0.1 mg/mL. To change the ionic strength, QPVP was dissolved in 1.0 mM sodium borate solution with 1.0 M sodium chloride solution. The fluorescent dye, Alexa 488 (Molecular Probes), was selected as the probe molecule because of its exceptionally high brightness and photostability as well as the fact that its negative charge would discourage adsorption to the negatively charged mica surfaces. It was dissolved in deionized water at a concentration of ∼1.0 nM.

b. Sample Cell and Adsorption of QPVP. Adsorption of QPVP to mica was conducted in a sample cell made of Teflon (Figure 1). A plate of muscovite mica with one side freshly cleaved served as the adsorbing substrate as well as the optical window for FCS measurement. Its thickness was selected to be 100−200 μm in order to match the working distance of the objective lens for the fluorescence measurements. The QPVP solution was injected into the sample cell, and the adsorption time was controlled to be 1.0 h. Afterward, the sample cell was rinsed with more than 100 mL (more than 50 times larger than the volume of the cell) of sodium borate buffer solution. Then 400.0 μL dye solution was added into the sample cell. The negatively charged Alexa 488 dye segregated into the positively charged QPVP layer. After 5.0 min of adsorption, the

10.1021/ma034089f CCC: $25.00
Published on Web 07/03/2003

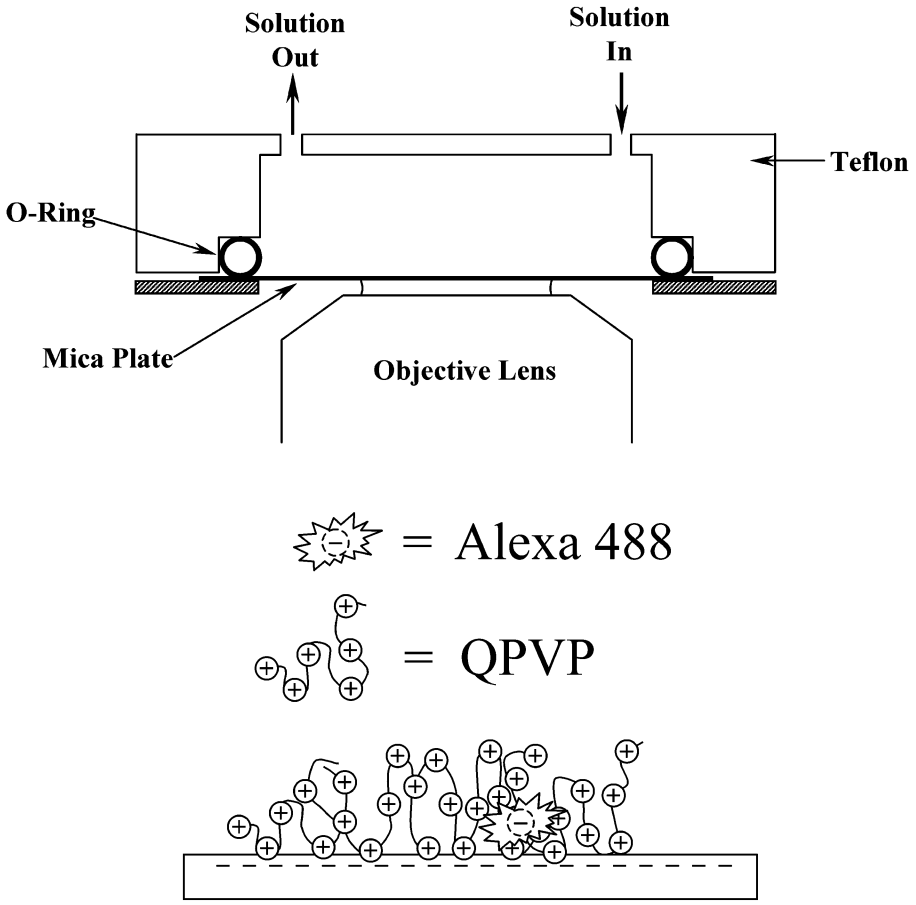

Figure 1. Experimental setup. (a) Sketch of the sample cell for QPVP adsorption and FCS measurement. A freshly cleaved mica plate of 100−200 μm thickness serves as the substrate for QPVP adsorption and as the optical window. (b) Schematic representation of QPVP (cationic) adsorbed onto mica, which in aqueous environment is negatively charged owing to dissolution from the mica lattice of K^+ groups.

sample cell was rinsed with >100 mL of buffer solution. Then the sample cell was mounted onto the microscope for measurements. It was verified that the dye remained within the polyelectrolyte layer and did not leak out into the surrounding dye-free solution.

c. Fluorescence Correlation Spectroscopy (FCS). The FCS measurements were performed using two-photon excitation of fluorescence using a home-built apparatus. A Zeiss Axiovert 135 TV microscope with an APOCHROMAT oil-immersion objective lens (63×, NA = 1.4)[13] formed the experimental platform. The output of a femtosecond laser (Tsunami, Spectra-Physics) was introduced and focused through the microscope. The wavelength of the laser was tuned at 800 nm, and its pulse width was ~50 fs at a repetition rate of 82 MHz. Through two-photon excitation, a tiny excitation volume (~10^{-15} L) was created. The dimensions of the Gaussian profile of this excitation, calibrated from the fluorescence induced in bulk solutions of known concentration of fluorescent dye, were found to be ~0.35 μm (beam diameter at the focus) and ~4 μm (depth of focus), but the latter was immaterial since in these experiments the fluorescence was localized to within the adsorbed polyelectrolyte layer. Note that this method of two-photon excitation of a small volume of fluid differs from single-photon confocal methods, which require using a pinhole.

The excited fluorescence was collected by the same objective lens, passed through a dichroic mirror and color glass filters, and was finally detected by a single-photon counting module (Hamamatsu). The fluctuation of the fluorescence photon counts was recorded by a commercially obtained FCS acquisition board (ISS, Champaign, IL), and data analysis was conducted using its software.

The principles and experimental technique of FCS have been reviewed extensively elsewhere.[12] As implemented here, the main point is that as the diffusion of dye molecules occurred in a plane on the surface; in the case of a single Fickian diffusion process one expects the autocorrelation function to be described by a two-dimensional diffusion model:

$$G(\tau) = \frac{1}{2\langle N\rangle}\left(1 + \frac{8D\tau}{{w_0}^2}\right)^{-1} \qquad (1)$$

where D is the diffusion coefficient, w_0 is the laser spot diameter at the focal point, $\langle N\rangle$ is the average number of molecules in the excitation area, and τ is time lag in computing the autocorrelation function. Only one parameter (D) determines the location of eq 1 on the time scale. In acquiring the data shown below, and in fitting it to this expectation, typically the data were acquired at 5 kHz and were averaged for 30 min.

d. Single-Molecule Video Microscopy. These experiments were conducted in a separate apparatus using a Zeiss Axiovert 200 microscope equipped with C-APOCHROMAT water-immersed objective lens (63×, NA = 1.2). An intensified CCD camera (I-PentaMax, Roper Scientific) was installed as detector in order to obtain single-molecule fluorescence images. The fluo-

rescence of the sample was excited by the frequency-doubled output of a diode-pumped Nd:YAG laser (CrystaLaser) in an ATR (attenuated total reflection) geometry.[14] In this experiment, the surface of fused silica slide was first soaked in 1.0 mM sodium borate buffer solution for 0.5 h, and QPVP solution was added to the surface. After 1.0 h of adsorption, the solution was rinsed by the buffer, and ~1.0 nM sulforhodamine G (another negatively charged dye from Molecular Probes) solution was introduced. After 5.0 min, the solution was exchanged with buffer solution, and the sample was mounted onto the microscope and measurements were conducted. Fluorescence images were collected at different rates, but typically they were collected at the speed of 10 frames/s.

Results. The main idea was that adsorbed polyelectrolyte could comprise a narrow channel within which aqueous solutes might be forced to flow. To test this idea, this initial study studied the rate of Brownian diffusion. In the results and discussion below, we quantify diffusion using FCS (fluorescence correlation spectroscopy) and validate, using single-molecule imaging, that translational diffusion was indeed the mechanism of fluorescence fluctuation. On the basis of comparing polyelectrolyte layers prepared in different ways, limitations of this nanofluidic approach are also discussed. The comparison to a complementary characterization of this same system using methods of surface forces measurement[15] is presented in the discussion section.

In the studies presented below, the final ionic strength during the measurements of dye mobility was always the same, 1.0 mM. However, to investigate the limits of these ideas, the polyelectrolyte layers were allowed to adsorb under either of two extremes of ionic strength, "low salt" (1 mM) and "high salt" (1 M), to produce the limits of relatively thin and swollen layers. Specifically, QPVP was dissolved either in 1.0 mM sodium borate buffer solution or in 1.0 mM sodium borate buffer containing 1.0 M NaCl. After adsorption was completed, the sample cell was rinsed thoroughly with 1.0 mM buffer solution. Figure 1 shows a schematic representation of the flow cell and of the adsorbed polyelectrolyte containing embedded dye.

Figure 2 shows the normalized autocorrelation functions of the negatively charged fluorescent dye (Alexa 488 dye) diffusing within the positively charged QPVP polyelectrolyte layers prepared with these two alternative histories. The normalization method is specified in the figure caption.

Curve 1 corresponds to the case of polylectrolyte adsorbed in "high salt" conditions, which produced a swollen layer. The solid line is the prediction using eq 1 using the single parameter, the translational diffusion coefficient (D). It is clear that the experimental data follow the prediction well using the diffusion coefficient of $D \sim 0.2\ \mu m^2/s$. By contrast, curve 2 in Figure 2 is the autocorrelation function of this same dye in QPVP layers formed in "low salt" conditions, which produced a relatively thin layer. This has a peculiar shape—empirically, a straight line on the linear−log scales. It cannot be fitted usefully by a single or dual species of Brownian motion model and qualitatively demonstrates less mobility than for the case of high ionic strength.

How to interpret these differences? For added information, dye mobility was also investigated using single-molecule imaging methods described in the Experimental Section. For these experiments, the polyelectrolyte was allowed to adsorb onto fused silica, which is negatively charged at the experimental pH of 9.2.[7] Under the microscope, the Brownian motion of the embedded sulforhodamine molecules was clearly observed. For layers formed from 1.0 M ionic strength, dye diffusion was relatively rapid and homogeneous; it appeared that every molecule that we imaged changed position with time elapsed. The situation was strikingly different for layers formed from 1.0 mM ionic strength. In this case it was evident to the eye that the dye molecules that we imaged possessed different mobilities and that some did not move at all. The typical trajectories of two molecules imaged under conditions corresponding to curve 1 and curve 2 in Figure 2 are illustrated in Figure 3.

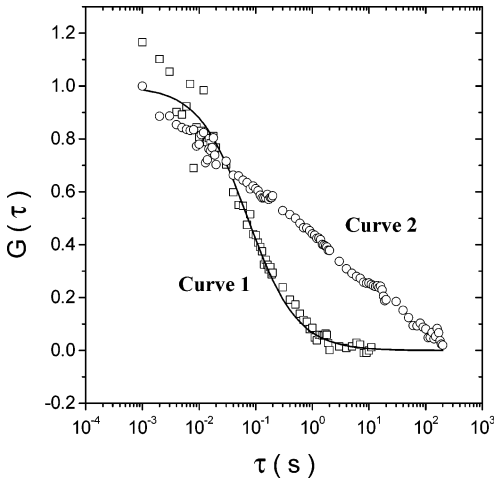

Figure 2. Normalized autocorrelation curves of intensity−intensity fluorescence fluctuation of Alexa 488 diffusing in adsorbed QPVP, plotted against logarithmic time elapsed. Curve 1: probe in QPVP layer adsorbed at 1.0 M ionic strength. Curve 2: probe in QPVP layer adsorbed at 1.0 mM ionic strength. These curves are normalized to unity at small times to account for different abundance of adsorbed dye in the two cases; before normalization, $G(0) \approx 0.7$ and 0.2 for curves 1 and 2, respectively. These two curves are typical data taken from more than 20 repeated measurements for each case. The solid line through curve 1 is a fit to eq 1, the prediction for a Fickian diffusion process and a single diffusion coefficient. The linear−log pattern traced by curve 2 is incapable of being fit so simply. Therefore, for potential nanofluidics applications, it would be preferable to employ as the channel a thick, swollen polymer layer.

While the single-molecule images in Figure 3 have the advantage that they demonstrate translational mobility, in practice we find it difficult at present to accumulate reliable statistics based on single-molecule trajectories and for this reason rely, for quantitative analysis, on the FCS autocorrelation curves.

Discussion and Conclusions. The structure of these polyelectrolyte layers adsorbed onto mica and onto silica at pH = 9.2 is expected to be similar, as in both instances the negative surface charge density is large. Quantitative evidence in support of this comes from integrated measurements obtained previously in this laboratory using using infrared spectroscopy in attenuated total reflection and surface forces measurements,[15] though it is true that the surface roughness of silica should be larger than for mica single crystals. In particular, from prior infrared[7] and surface forces[15] experiments in this laboratory on similar systems involving the same polyelectrolyte of similar molecular

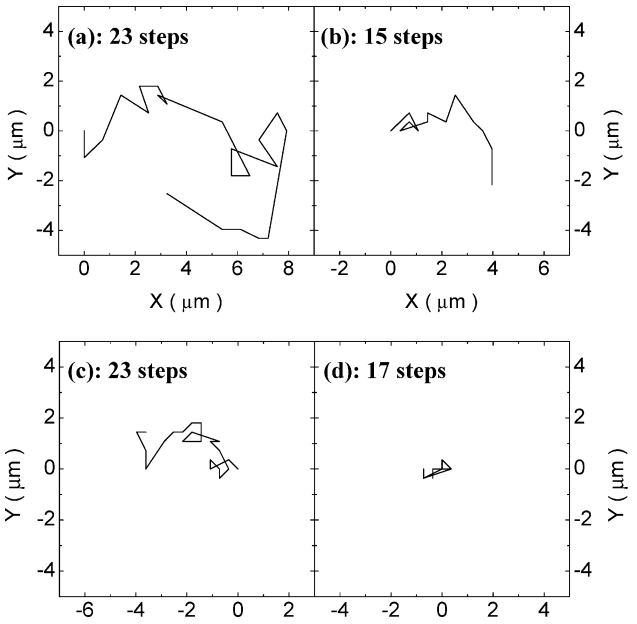

Figure 3. Typical trajectories in the $x-y$ plane obtained by single-molecule imaging for dyes diffusing under the same conditions as for the two situations analyzed using FCS in Figure 2. Panels a and b correspond to curve 1 in Figure 2; panels c and d correspond to curve 2 in Figure 2. The number of the steps of tracking is specified in each panel, and the time interval for each step was 0.1 s. The more rigorous quantification using FCS shows that trajectories described by curve 1 are described well as following a single diffusion coefficient, whereas for trajectories described by curve 2 the response is very heterogeneous.

weight at the same pH, it was found that the adsorbed amount was 3 times larger for the case of adsorption from 1.0 M than from 1.0 mM ionic strength. In addition, the thickness measured within a surface forces apparatus (SFA) were found to be larger for polyelectrolytes adsorbed in a "high salt" situation than in "low". For the current system, the thickness of QPVP layer was more than 7−8 nm for "high salt adsorption" and 1.6 nm for "low salt adsorption".[15]

From these observations, it is clear that the polyelectrolyte layers adsorbed from high ionic strength conditions possessed a relatively fluffy structure, with relatively many loops of the flexible chain dangling into the solution. In the case of adsorption from low ionic strength, the chains are stiffer and adopt a flatter conformation. There is much theoretical support for this.[16]

The dye studied in these experiments possessed opposite charge as the adsorbed polymer. Although explanation is speculative, it is reasonable to suppose that the dye segregated irreversibly within the adsorbed polyelectrolyte layers not only because of having opposite charge but also because of hydrophobic attraction to the backbone of the polyelectrolyte. It is not surprising to conclude that the dye underwent simple Brownian motion in the fluffy QPVP layer because in this case the environment was a thick layer of flexible segments of QPVP. In the alternative case of the flatter monolayer, the QPVP segments resided closer to the mica surface, and they should be much more immobilized. The slower diffusion of the dye within those polyelectrolyte layers adsorbed at the low ionic strength (which was confirmed by the single-molecule imaging) likely arises from heterogeneous hopping of dye molecules from site-to-site near the surface, although explanation is still speculative. The main point is the contrast to behavior obtained in the other case. Polyelectrolyte layers formed from solutions containing high salt concentration could potentially be used for nanofluidic flow. The opposite situation could not.

Looking to the future, we present this study as a proof of concept, showing that it is practical to follow the mobility of molecules embedded within single layers of adsorbed polyelectrolyte. Furthermore, it is also practical to tune this mobility. The path is now clear to explore more details of the physics of these processes, such as the dependence on the size, polarity, and hydrophobicity of the embedded dye, as well as finer details of the ionic strength of the surrounding aqueous solution. The path is also clear to attach fluorescent dye to larger molecules (oligomers, polymers, and proteins) and similarly to follow their diffusion based on this strategy. Furthermore, we anticipate that in the future it will become possible to direct this nanofluidic motion using gradients of electric field and temperature.[2,3]

Acknowledgment. For helpful discussions, we are indebted to S. A. Sukhishvili (Stevens Institute of Technology). This work was supported by the U.S. Department of Energy, Division of Materials Science, under Award DEFG02-91ER45439 through the Frederick Seitz Materials Research Laboratory at the University of Illinois at Urbana−Champaign.

References and Notes

(1) Whitesides, G. M.; Ostuni, E.; Takayama, S.; Jiang, X.; Ingber, D. E. *Annu. Rev. Biomed. Eng.* **2001**, *3*, 335.
(2) Olson, D. J.; Johnson, J. M.; Patel, P. D.; Shaqfeh, E. S. G.; Boxer, S. G.; Fuller, G. G. *Langmuir* **2001**, *17*, 7396.
(3) Kataoka, D. E.; Troian, S. M. *Nature (London)* **1999**, *402*, 794.
(4) Israelachvili, J. N.; Adams, G. E. *J. Chem. Soc. Faraday Trans. 1* **1978**, *74*, 975.
(5) Cho, Y.-K.; Watanabe, H.; Granick, S. *J. Chem. Phys.* **1999**, *110*, 9688.
(6) Dautzenberg, H.; Jaeger, W.; Kotz, J.; Philipp, B.; Seidel, Ch.; Stschherbina *Polyelectrolyte: Formation, Characterization, and Application*; Hanser: Munich, 1994.
(7) Sukhishvili, S. A.; Granick, S. *J. Chem. Phys.* **1998**, *109*, 6861.
(8) Sukhishvili, S. A.; Granick, S. *J. Chem. Phys.* **1998**, *109*, 6869.
(9) Kawaguchi, M.; Takahashi, A. *Adv. Colloid Interface Sci.* **1992**, *37*, 219.
(10) Cosgrove, T.; Griffiths, P. C. *Colloids Surf. A* **1994**, *84*, 249.
(11) Lin, W.-Y.; Blum, F. D. *Macromolecules* **1998**, *31*, 4135.
(12) Rigler, R., Elson, E., Eds. *Fluorescence Correlation Spectroscopy: Theory and Applications*; Springer-Verlag: Berlin, 2001.
(13) Zhao, J. J.; Xie, F.; Bae, S. C.; Granick, S. *Macromolecules* **2001**, *34*, 3123.
(14) Lacey, A. J., Ed. *Light Microscopy in Biology: A Practical Approach*; Oxford University Press: Oxford, 1999.
(15) Ruths, M.; Sukhishvili, S. A.; Granick, S. *J. Phys. Chem. B* **2001**, *105*, 6202.
(16) Fleer, G. J.; Cohen Stuart, M. A.; Scheutjens, J. M. H. M.; Cosgrove, T.; Vincent, B. *Polymer at Interfaces*; Chapman & Hall: London, 1993.

MA034089F