

Watching Macromolecules Diffuse at Surfaces and Under Confinement

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Summary: At the single-molecule level, this laboratory has been making direct measurements of polymer diffusion at and near surfaces. Parameters of special interest have been to understand (a) the role of molecular weight, N ; (b) the role of surface coverage, which may range from dilute to saturated, and (c) the role of the surface, which may range from hard (a silicon wafer) to soft (a supported lipid bilayer), and (d) the use of external fields to direct the transport of small molecules through narrow surface channels that are one macromolecule thick.

Keywords: correlation spectroscopy, diffusion, fluorescence, lipid bilayer, surfaces

Introduction

One of the ironies of polymer science is that the intense interest in the dynamics of flexible and semiflexible macromolecules in the bulk is not yet of major enough concern regarding polymer surfaces, the preoccupation being with equilibrated conformations. Polymer science does not yet have enough understanding of this question – the connection between rates, and surface-adsorbed states.

This laboratory has used two main experimental approaches, infrared spectroscopy in attenuated total reflection (FTIR-ATR), and fluorescence correlation spectroscopy (FCS), using which the center-of-mass diffusion can be inferred from the autocorrelation function of intensity-intensity fluorescence fluctuation as molecules diffuse into, and out of, the diffraction-limited focus of a femtosecond laser beam where intensity is high enough to induce fluorescence by two-photon excitation. Two-photon excitation has the additional advantages of spatial resolution combined with the ability to reject background signals. FCS measures the mutual diffusion coefficient (D_M) of fluorescing species. In systems where the fluorescent label is dilute, $D_M \approx D_{CM}$ (D_{CM} is the center-of-mass diffusion coefficient).

Self-Diffusion of Polymer Chains on a Solid Surface

The physical situation is known as the de Gennes “pancake”. Flexible chains are expected to flatten in the dilute adsorbed state because the enthalpic cost of flattening outweighs the entropic cost provided that the sticking energy per segments exceeds a computable small number. To test this idea, experiments were performed in which a flexible synthetic polymer, polyethylene glycol (PEG), was allowed to adsorb from aqueous solution onto a hydrophobic solid surface (quartz coated with a methyl-terminated self-assembled organic monolayer). The rate of self-diffusion, measured by fluorescence fluctuation spectroscopy, was found to scale as $D \sim N^{-3/2}$.^[1,2] This nonlinear dependence when the surface is solid contrasts strongly with linear dependence observed on a fluid membrane.^[3]

Definitive explanation is still speculative^[2] but reptation may explain the observed stronger dependence on N . In the reptation model, the terminal relaxation time scales as $\tau_{\text{rept}} \sim N^3$. Knowing that the radius of gyration (R_G) scales as $R_G \sim N^{3/4}$ in a good solvent in two dimensions and arguing that $D \sim R_G^2/\tau_{\text{rept}}$, it follows that $D \sim N^{-3/2}$ for chains with excluded volume statistics. This is suggestively like the data. It may seem paradoxical to imagine reptation – the diffusion of a chain, snakelike, along its own length – in this dilute system, where the physical origin of static constraints suppressing lateral motion is unclear. But if there were some slack between sticking points, loops of an isolated flexible chain might propagate with high probability along its length in caterpillar-like fashion, so that mathematics of the reptation model would apply despite the unconventional physical situation.

Phospholipid Membranes as Substrates for Polymer Adsorption

In the field of polymer science, adsorption is considered traditionally to occur onto surfaces whose structure is frozen. In contrast, in the study of phospholipid membranes, drug delivery and gene therapy, interactions with polymers are known phenomenologically to have the capacity to make membranes leaky, for example for the outflow of drugs from vesicles or the inflow of encapsulated DNA into cells, so in these cases membrane structure is clearly disrupted. Bacteriocidal action has even been demonstrated. It is interesting that these communities that have developed with little cross-talk. The polymer science and drug delivery communities have focused on extreme limits – the polymer science community focusing on the polymer side of the interface, the biologically-minded community focusing on the practical consequences when membranes are disrupted. It is also interesting to consider the middle

ground – a membrane surface that responds to the adsorbate but without being penetrated or destroyed. The data in Fig. 1 is discussed below.

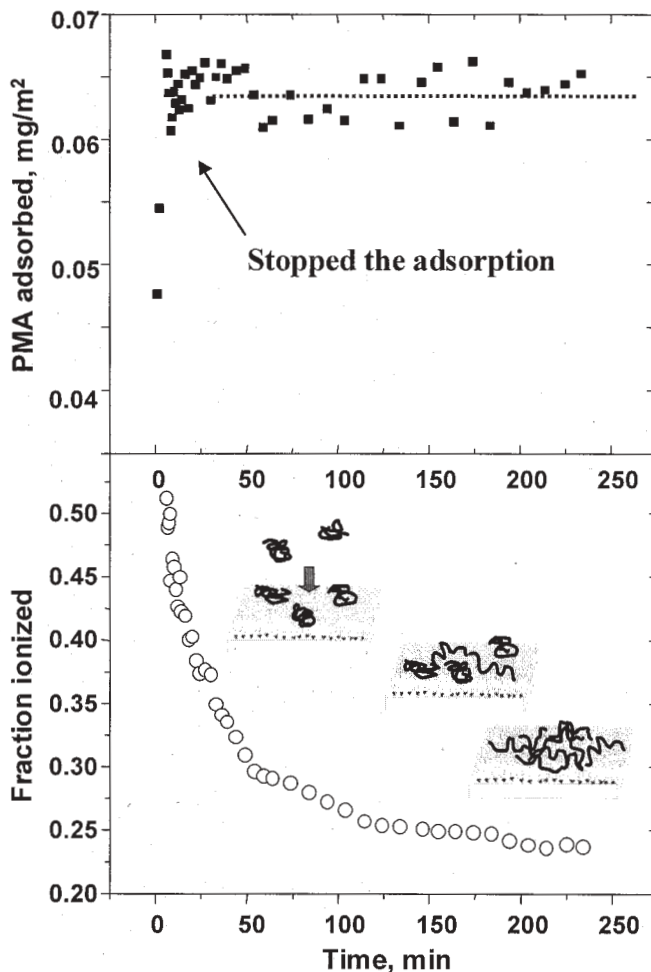


Fig. 1. Ionization of the carbonyl group plotted against elapsed time for a "starved surface" of polymethacrylic acid (PMA), $M_w = 40,000 \text{ g}\cdot\text{mol}^{-1}$ and $M_w/M_n = 1.02$. The polymer was allowed to adsorb, from $0.1 \text{ mg}\cdot\text{ml}^{-1}$ in aqueous D_2O solution buffered at $\text{pH} = 6.0$, onto a supported lipid bilayer of DMPC at 15°C such that the bilayer was in the gel phase. **Top panel** (squares) shows the time evolution of the mass of adsorbed PMA. Adsorption was allowed to proceed for 4 min, to $\approx 5\%$ of equilibrated total surface coverage, at which point the solution was replaced with the same buffered solution not containing polymer. The dotted line indicates that total mass adsorbed remained constant. **Bottom panel** (circles) shows fractional ionization of PMA plotted against time elapsed. The ionization fell over $>2 \text{ h}$. The ionization in bulk solution was 0.58. A schematic picture of the hypothetical spreading process is included.

Supported lipid bilayers were prepared of the phospholipid DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine using the method of vesicle fusion.^[3] These surfaces were “starved” of polymer by depositing a small amount, too small to saturate them. Fig. 1 shows an experiment in which, after polymethacrylic acid (PMA) was allowed to adsorb to $\approx 5\%$ of the saturated amount adsorbed, the solution of polymer was replaced by pure buffer solution. Thereafter the total mass adsorbed was constant (top panel) but the intensity ratio of the charged and uncharged carboxylic groups adsorbed continued to decrease (bottom panel). In this circumstance where surface coverage was limited to much less than a monolayer, one expects the polymer to adopt a flat “pancake” conformation at equilibrium. We suppose that the spreading of adsorbed chains, i.e. the increase of segment-surface contacts with the dipolar headgroups, produced the observed changing ratio of charged to uncharged ionized groups. The puzzle was to understand why the rate of spreading is so slow, as local diffusion times of lipids within DMPC bilayers are far more rapid than this.

The molecular weight of the spreading chains was varied over a wide range (a factor of 30) and this kinetic process was found to show no dependence on molecular weight. This is reminiscent of the N-independent spreading rate recently discovered when liquid droplets spread on a viscoelastic surface. The usual strong viscosity dependence of the spreading rate, for cases where the surface is a hard solid, is lost. The rate-limiting step is observed instead to be viscoelastic response of the underlying material. In this present system of supported phospholipid bilayer, measurements of the infrared dichroism of the headgroup gave evidence of direct coupling between the two processes, adsorption and surface reconstruction. Extrapolating from the theoretical prediction by others that polymer adsorption stiffens a membrane locally, we conjecture that the surface reorganization process involved changes of local curvature and local stiffness while the polymer chains unwound from the three-dimensional solution conformation towards the two-dimensional pancake conformation.^[3]

Diffusion of Small Molecules Embedded Within Adsorbed Polyelectrolytes

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. It is also attractive to consider the potential for directed flow of molecules contained within molecularly-thin layers.

The scheme we have selected to prepare molecularly-thin layers consists in using adsorbed polymers, which has the advantage of simplicity. Whereas for initial studies we have used polymers that coated a solid surface uniformly, it is easy to envision methods to 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 or temperature gradients.

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 but becomes problematical and laborious when it is intended that the surfaces be parallel over linear dimensions of millimeters. 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.

Initial studies focused 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 QPVP layer was tuned and the mobility of the probe molecules was measured accordingly using fluorescence correlation spectroscopy (FCS).^[4]

Such features of small (probe) molecules diffusing within surface channels one macromolecule thick raise a number of interesting questions that require further study. For example, how will the diffusion of the probe depend on the ionic strength of the solution? By studying this, the relationship between the mobility of the probe and the strength of the electrostatic interaction with the QPVP segment can be discovered. Another important factor is that the probe molecule can not only probe the structure and dynamics of the polyelectrolyte layer, but also can represent the mobility of the solvent molecules in the monolayer. By choosing dye molecules with different size as well as different chemical structures such as polarity and hydrophobicity, the dynamics of the solvent inside such a surface modification structure will be revealed. In addition to these questions that are science-motivated, the direction of flow by application of external fields may find technological application.

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