



Single isolated macromolecules at surfaces

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Abstract

A new class of direct experiments has begun to address the structure, dynamics, and chemical responsiveness of isolated macromolecules at the solid–liquid interface. New fluorescence-based and atomic force microscopy-based methods provide the needed sensitivity, which formerly was unavailable. The initial findings have begun to pose a family of new questions that are practical as well as fundamental. Directions of research opportunity are surveyed. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

On the nanometre scale, a macromolecule at a surface is usually located close to other macromolecules. This is because if the first molecule adsorbs, many others do also – a small sticking energy per repeat unit of a macromolecule quickly adds up to a large sticking energy per macromolecule. The entropic gain of replacing many (small) solvent molecules by (a smaller number) of larger macromolecules further encourages the dense layers of adsorbed macromolecules that it is usual to encounter. On the characterization side, the experimental tools with the sensitivity required to probe dilute surface coverage – in situ, with temporal sensitivity, spatial sensitivity, or both – comprise another challenge. All of this helps to explain why study of isolated macromolecules at surfaces has consisted, till recently, mainly of theory or computer simulation. The interesting paradox results that experiments concerning macromolecules in three dimensions, and in two dimensions, have proceeded in diverging directions.

This helps to explain why what is probably the single most important foundation for understanding polymers in isotropic three dimensions – the single molecule in a sea of solvent or vacuum, without interactions with neighboring macromolecules – has not seen an experimental counterpart in surface studies. The isolated macromolecule is the situation that is conceptually most simple and pure. It leads to landmark predictions, among which we may count the Rouse and Zimm models of dynamics [1] and the scaling with molecular weight of the unperturbed radius of gyration [2]. Predictions concerning isolated macromole-

cules have had enormous influence on the development of polymer physics as concerns behavior in the bulk but direct experiments at surfaces have been largely nonexistent. Perhaps only thermodynamic measurements of the second virial coefficient of polymers in Langmuir monolayers at the air–water interface [3–5] constitute an exception to this statement.

Fortunately this has begun to change. A key building block in the evolution of this field has been that experimental techniques with the needed sensitivity – mainly fluorescence-based and atomic force microscopy-based techniques – no longer require, on the part of the user, a single-minded commitment. They have become more user-friendly and more widely accessible. Progress is now not so much a matter of the development of technique, as of applying working experimental tools to interesting physical problems.

2. Conformation and self-diffusion of chains in two dimensions

The physical situation is known as the de Gennes ‘pancake’ [6]. 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 segment exceeds a computable small number.

An elegant pioneering study by Maier and Rädler employed fluorescence microscopy to image the conformations and rates of self-diffusion of huge molecules of DNA when they were electrostatically bound to fluid cationic lipid bilayers [7,**8]. The molecules were so large that their shapes could be viewed inside the optical microscope. Their shapes were perfectly described as self-avoiding random walks and the long-predicted result for chain

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conformation in a 2D (two dimensional) environment with excluded volume interactions, $R_G \sim N^{3/4}$, was confirmed experimentally. (Here R_G denotes the radius of gyration and N the molecule's degree of polymerization.) The dynamics were also visualized optically. The self-diffusion coefficient (D) was found to obey Rouse dynamics, $D \sim N^{-1}$, indicating that the friction between different parts of the same chain was uncorrelated. This confirmation of the predicted static conformations was pleasing. However, why were the chain conformations so strongly affected by correlations between different segments, but the self-diffusion not correlated?

If the surface itself were mobile, in the sense that the rate-limiting event were determined by motion of sites on the surface itself, rather than by the polymer, uncorrelated Rouse dynamics of the binding sites that anchor a macromolecule flat onto the surface would obviously be expected.

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 $D \sim N^{-3/2}$ [9], as shown in Fig. 1. This nonlinear dependence when the surface is solid contrasted strongly with linear dependence observed on a fluid membrane.

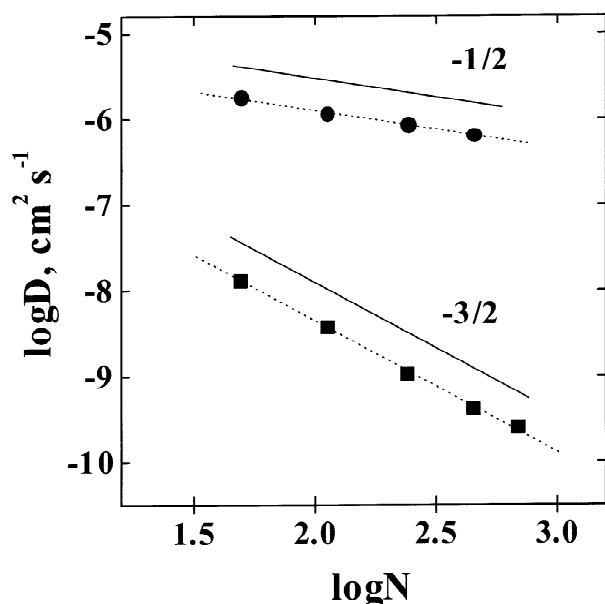


Fig. 1. Diffusion coefficients, D , are plotted on log–log scales against degree of polymerization of the diffusing chain, N , for polyethylene glycol (PEG) adsorbed from dilute aqueous solution onto a hydrocarbon methyl-terminated monolayer. The PEG molecules were end-labeled at one end with a bright, photostable fluorescent dye. The data shown refer to diffusion in solution (circles) and to surface diffusion (squares). Lines with slopes $-1/2$ and $-3/2$ are drawn for comparison. Adapted from Ref. [9].

Reptation may explain the observed stronger dependence on N . In this model [6], 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. In seeming agreement, a recent simulation gave $D \sim N^{-3/2}$ for a single self-avoiding chain diffusing among regularly-spaced obstacles in two dimensions [10]. Interpretation remains problematical when one considers that scaling exponents have also been reported from simulation to depend on obstacle density [11], so that if the monolayers included defects the scaling exponent might depend on the defect density. 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.

3. Varieties of surfaces

The studies summarized above illustrate that all surfaces are *not* created equal. A tentative division into a trinity of surfaces is illustrated in Fig. 2. We ignore here the very real issues of surface heterogeneity (chemical and topog-

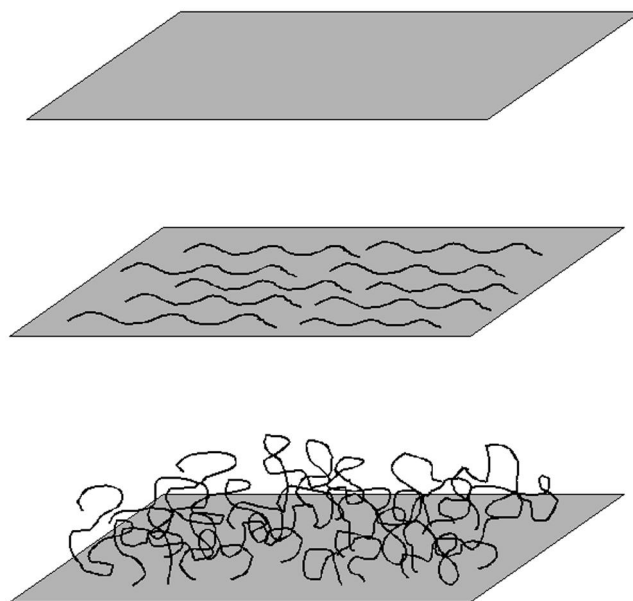


Fig. 2. Hypothetical representation of the distinction between (top panel) the 2D solid surface; (middle panel) the 2D fluid surface; and (bottom panel) the surface that has 3D depth.

raphical) that also play large roles in practice. Those practical complications are well-known and they are appreciated widely. Deserving of more recognition are the following additional distinctions.

3.1. The 2D solid surface

It is appealing to conceive a surface as a two-dimensional plane but, on the Angstrom scale, even a seemingly smooth crystalline surface is bumpy. Some bumpiness is spatial (the arrangement of atoms on the crystal). Some bumpiness concerns the energy landscape for adsorption: the sticking energy is higher at some atoms, lower at others. Therefore atoms in contact with a 2D crystal become pinned to particular surface sites.

This is exemplified by the history of computer simulations to explore consequences for diffusion and shear viscosity of confined fluids. The earliest simulations, employing solid surfaces that were modelled without structure in the plane, found minimal perturbations from bulk values of the diffusion coefficient [12]. Only by allowing for atomic structure in the plane of the surfaces and incorporating this into the simulation model, did these studies predict confinement-induced crystallization or vitrification [13]. The moral of the story is that the surface geometry and the sticking energy to it were essentially the same for the two computer models, but the implied dynamics were qualitatively different.

Even a solid is malleable – it changes in response to a change of environment. A single crystal can reconstruct *in response* to a macromolecule's adsorption. This was shown nicely this year by scanning tunnelling microscopy (STM) images of a metallic (copper) surface when a large organic molecule adsorbed [14]. The phenomenon is surely general.

3.2. The 2D fluid surface

This is the experimentalists' realization of a surface whose 2D energy landscape is smooth. While it is true that a fluid need not be geometrically smooth (the liquid has capillary waves), the energy with which adsorbates stick to it averages to be the same everywhere. There are two principal mechanisms by which this can happen. In some cases the potential that causes the adsorbate to stick is long-ranged (e.g. Coulombic) [7,**8]. In other cases the atoms or molecules that comprise the adsorbing surface rearrange dynamically more rapidly than the macromolecular adsorbate, as discussed in the previous section. In the class of '2D fluid surface', supported phospholipid bilayers comprise interesting and versatile instances [7,**8,15] because favorable systems switch from 'fluid' to 'gel' phase in the vicinity of room temperature without change of chemical composition.

3.3. The surface that has some 3D depth

Surfaces are commonly terminated with macromolecular chains. Therefore the active surface – by which it interacts with its environment – has the depth of the macromolecular chain. Common examples are polymer brushes (as concerns synthetic polymers) and glycolipids at a cell membrane (as concerns biologically-relevant polymers). The element in common is the difference whether an adsorbate sits on *top* of a 2D surface, or becomes *engulfed* within a surface with 3D depth.

The difference has major consequences for the structural stability of an adsorbate whose structure is complex structure (an enzyme, for example). An enzyme is less prone to denaturation when it is enveloped within a surface that carries 3D depth [16]. This may be a source of differences in the activity of surface-bound enzymes [17–19].

4. Local chain dynamics in the adsorbed state

DNA surface immobilization is an integral aspect of many routine DNA assays. The most common way of DNA immobilization is by direct chemisorption of thiol-labeled DNA onto a gold surface. However, this method of chemical attachment does not carry over to nonmetallic surfaces. It is difficult to adapt fluorescence measurements because nonradiative resonance energy transfer between the probe and the metal reduces the fluorescence signal [20].

Electrostatics provides a different method of surface immobilization. A cationic polyelectrolyte previously-adsorbed onto a solid surface can be used to immobilize DNA onto glass surfaces since DNA has a negatively-charged phosphate backbone. This approach has several advantages for working with isolated molecules. Firstly, it is so simple that any solid surface can be used. Secondly, the conformations of adsorbed DNA molecules can be modified easily by varying the ionic strength of the surrounding aqueous medium. Thirdly, it is easy to control DNA adsorption by changing the charge density on the polycation substrate (e.g. the fractional quaternization of polyvinylpyridine [21]); this is effective because the amount of DNA adsorbed depends on the surface charge of the substrate.

Local environment of adsorbed DNA can then be measured using time-resolved two-photon fluorescence anisotropy. As illustrated in Fig. 3, recently time-resolved fluorescence anisotropy measurements were used to investigate the local dynamical environment of surface-immobilized DNA oligomers by using two-photon excitation within an optical microscope and detecting the vertically- and horizontally-polarized responses using two detector channels simultaneously [21]. Results depend strongly on surface charge density and on salt concentration. Time-

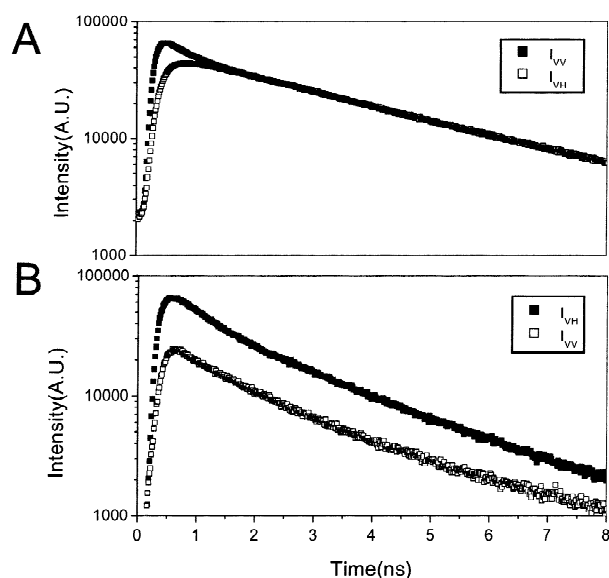


Fig. 3. Fluorescence intensity decay of fluorescent-labelled DNA oligomers (FAM, 6-fluorescein-6-carboxamido hexanoate) in bulk aqueous solution (panel A) and confined between quaternized polyvinylpyridine (QPVP) layers (panel B). The DNA oligomers had 15 base pairs and their sequence was 5'-FAM-GATGATGAGAAGAAC-3'. Polarized intensity is plotted against time on the nanosecond time scale. Fluorescence decays overlapped rapidly in bulk solution, indicating rapid rotational diffusion, but the anisotropy decay on the surface was slower. ■, Vertically polarized fluorescence; □, horizontally polarized fluorescence. All measurements were performed at room temperature and deionized water. Adapted from Ref. [20].

resolved fluorescence anisotropy measurement is a sensitive method to investigate subtle differences between complex local surface environments.

5. Spreading when flexible chains encounter a surface

When the conformations of adsorbed polymer chains are equilibrated, powerful methods of equilibrium statistical mechanics apply. Sophisticated theories describe this situation [22,23]. Experimenters, on the other hand, encounter the recurring problem in which polymer chains deposit decidedly more quickly than the surface structure equilibrates [23–25]. Conceptual understanding of this problem has been elusive because of the difficulty to make general statements about a problem where sample history necessarily plays a role [26].

A recent study undertook to determine if conformational distortions would be stable if adsorption were halted at points of incomplete surface coverage. We expected that if the surface were 'starved' of polymer by depositing an amount too small to saturate the surface, this would provide a driving force for chains to 'spread' and increase the number of segment-surface contacts [23] – to tend towards the pancake configuration of Fig. 1. In fact, direct measurements of the ionization of polymethacrylic acid as

it adsorbed onto a fluid-supported phospholipid bilayer [27] indicated a transition from ionization characteristic of the chain in bulk solution (early times) to considerably less ionization (several hours later) – though the experiment was designed so that the total mass adsorbed would remain constant (Fig. 4). These direct measurements of spreading rate *in* the adsorbed state highlight the shape flexibility of polymer chains. They quantify that conformational equilibration of flexible chains can be extremely slow to equilibrate, unlike the oft-analyzed case of adsorbed rigid objects.

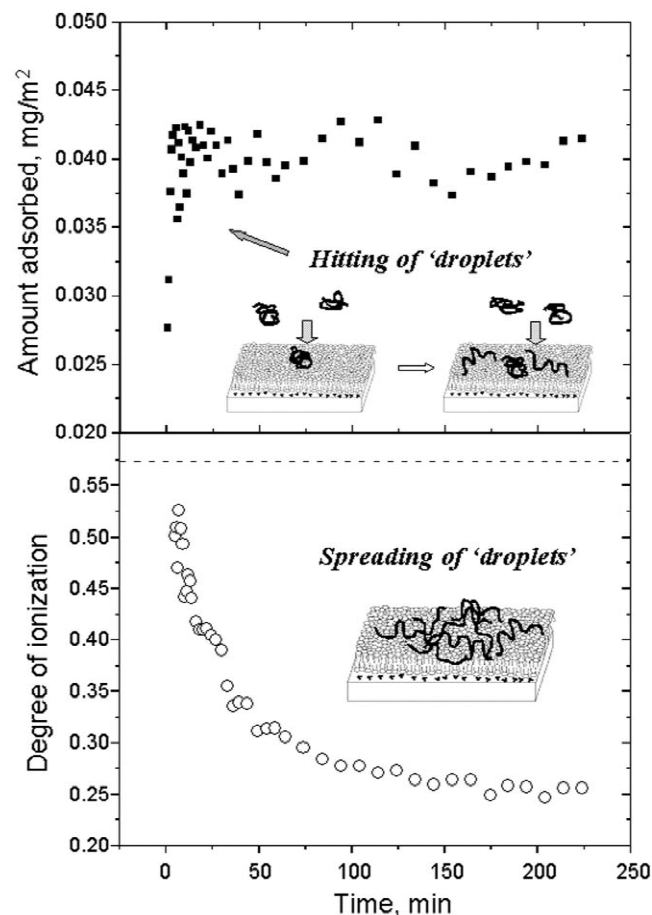


Fig. 4. Ionization of the carbonyl group plotted against elapsed time for a 'starved surface' of polymethacrylic acid (PMA), measured by infrared spectroscopy in the mode of attenuated total reflection [27]. PMA of narrow molecular weight distribution and number-average molecular weight $40,000 \text{ g mol}^{-1}$ was allowed to adsorb onto a supported lipid bilayer of DMPC (dimyristylphosphatidylcholine) at 16°C , a temperature in which the bilayer was in the gel phase. The adsorption condition was 0.01 mg ml^{-1} in aqueous D_2O solution buffered at $\text{pH}=6.0$. Top panel, adsorption was allowed to proceed for 10 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 mass adsorbed remained constant. Bottom panel, the ionization proceeded to fall over a period of hours without change of the total mass adsorbed, reflecting that the flexible chains 'spread' slowly from a random coil to a flattened conformation. The dotted horizontal line shows the ionization characteristic of this same polymer in dilute solution at the same pH. The time dependence of ionization decay follows single exponential kinetics.

The significance is both scientific and practical. On the practical side, a broad conformational distribution has evident bearing on situations where the usefulness of polymers hangs on the tenacity of their surface attachment – especially adhesion. The scientific significance is that this interpretation of surface structure differs decisively from the customary view that chain conformations are selected such that the free energy is minimized. However if surface impingement were comparable to or more rapid than the rate of spreading, as for concentrated solutions and melts, a different distribution should be expected.

6. Conclusions and prospects

The desirability of direct in situ dynamic spectroscopic measurements of macromolecules at a nonparticulate interface has long been clear, but has suffered from the great difficulty of limited signal-to-noise. The meager abundance of sample, buried between two bulk condensed phases with thickness $<1\text{--}2$ nm, sets harsh requirements on the experimental methods that would be sensitive to this small sample size. The mass present is, at complete surface coverage, not more than $\sim 100\text{--}200$ ng cm $^{-2}$. In order to elucidate the properties of isolated macromolecules it is desirable to work at quantities $<5\text{--}10\%$ of this.

This requirement of sensitivity is satisfied by the advent of sophisticated methods with single-molecule sensitivity, such as fluorescence spectroscopy [28–30], sum frequency generation [31,32] and atomic force microscopy to probe chain conformations [33] or dynamical rearrangements [34,35], whose instrumental development has reached the point that even nonspecialists in these techniques can employ them productively.

We are entering an exciting period when molecular quantities that were previously-inaccessible except by theoretical calculation or simulation can now be measured by direct experiment. These methods are expected to find wide application, in addition to the few examples presented above, by rational extension.

Acknowledgements

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