Single-molecule diffusion in a periodic potential at a solid–liquid interface†

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We used single-molecule tracking experiments to observe the motion of small hydrophobic fluorescent molecules at the interface between water and a solid surface that exhibited periodic chemical patterns. The dynamics were characterized by non-ergodic, continuous time random walk statistics. The step-size distributions displayed enhanced probability of steps to periodic distances, consistent with theoretical predictions for diffusion in an atomic/molecular scale periodic potential. Surprisingly, this general behavior was observed here for surfaces exhibiting characteristic length scales three orders of magnitude larger than atomic/molecular dimensions, and may provide a new way to understand and control solid–liquid interfacial diffusion for molecular targeting applications.

1. Introduction

Surface diffusion is a key step in any surface process that involves the encounter of two entities. A classic example is heterogeneous catalysis1–3 where in some bimolecular interactions, diffusion at a surface can significantly enhance reaction rates over the homogenous case.4,5 Periods of surface diffusion govern many biomolecular recognition events5–7 which are critical to biomaterial surface design8,9 and biosensor and microarray technologies.10,11 Surface diffusion also controls the growth of organic,12 inorganic13 and supramolecular14 thin films. For all of these processes, the simplest assumption is surface diffusion governed by normal Gaussian statistics; however, this is rarely found to be valid in reality. Anomalous diffusion, when it is present, can significantly alter reaction kinetics or encounter probabilities.15–17 It is therefore important to understand the specific mechanisms of surface diffusion.

Surface diffusion at solid–gas interfaces has been extensively studied,18,19 using scanning tunneling microscopy and other techniques to observe atomic motion on crystalline surfaces. The process is conventionally described as activated hopping between atomic-scale minima in a periodic energy landscape. Theoretical descriptions have advanced in parallel to describe the range of diffusive behavior expected on solid surfaces, from subdiffusion to superdiffusion.18–20 In contrast, there is no simple description for diffusion at solid–liquid interfaces, possibly because there are fewer experimental tools that can reveal the full statistical distribution of behavior. Fluorescence correlation spectroscopy has been used to investigate surface diffusion in chromatography systems21,22 and polymer surface diffusion,23,24 and both have exhibited behavior inconsistent with simple assumptions. To provide a more detailed and complete picture, single-molecule tracking has recently been developed to study the behavior of surfactants25 and proteins,26,27 revealing bulk-mediated surface diffusion28,29 that was previously predicted theoretically.30 Interestingly, all studies of surface diffusion at a solid–liquid interface to date have discovered behavior more complicated than a single Brownian (i.e. random walk) diffusive mode.

Here, we present the results of single-molecule tracking experiments where we followed the motion of a small organic probe molecule at both chemically uniform and mesoscopically patterned solid–liquid interfaces. The results demonstrate that the dynamics of the fluorescent probe were consistent with continuous time random walk statistics (CTRW)31 subject to a broad distribution of waiting times. The broad distribution of waiting times gave rise to dynamic heterogeneity like that observed in disordered environments.32–34 Additionally, by using a chemically patterned surface, we were able to generate behavior analogous to a particle in a periodic potential. This type of behavior has previously been observed only at solid–gas interfaces and at atomic length scales.18,19 In contrast, our observations were at a solid–liquid interface and over mesoscopic length scales. By...
demonstrating consistency between our data and models of dynamic heterogeneity and diffusion in a 2D potential, we hope to provide a framework to better understand and possibly control solid–liquid interfacial diffusion.

2. Materials and methods

2.1 Surface preparation

We prepared patterned surfaces that presented a triangular array of hydrophobic rings (i.e., annuli), to which the probe molecule had high affinity. The chemical patterns were created using particle lithography, in which monodisperse polystyrene particles were assembled into a close-packed array on a surface to serve as a mask to the vapor deposition of an organosilane. The organosilane that we used was octadecyltrithoxysilane (OTES), which because of its moderate reactivity, selectively deposited around the base of each colloidal particle where liquid water had condensed. After removal of the colloidal particle mask, the result was an array of hydrophobic OTES rings on an otherwise hydrophilic silicon or fused silica surface. The distance, d, between ring centers was equal to the mask particle diameter and so was easily controlled by using different sized polystyrene particles. We prepared three different patterns with d = 0.50, 0.75, and 1.00 μm respectively. The ring diameter was only minimally dependent on the particle size and was approximately 0.15 μm for all three patterns. For comparison to the patterned surfaces, we prepared two chemically uniform surfaces. The first was a uniform monolayer of octadecyltrichlorosilane (OTS). In this case we used a trichlorosilane rather than the triethoxysilane OTES, but the resulting self-assembled monolayer presented the same 18 carbon surface as the OTES used in the ring patterns. The other uniform surface was unmodified fused silica.

2.2 Single-molecule tracking

We exposed each of the surfaces to a very low concentration solution (10 fm–100 pm) of the fluorescent probe, Atto Rho6G (AttoG, ATTO-TECH GmbH, Germany), in ultrapure water (Millipore Milli-Q, >18 MΩ cm). The sample was illuminated by a 532 nm laser and imaged with a prism-based total internal reflection fluorescence microscope (TIRFM). Single molecule trajectories of adsorption, diffusion and desorption of the probe molecule were recorded with an exposure time of Δt = 20–60 ms and analyzed as previously described. Approximately 104–105 trajectories were recorded on each of the surfaces.

2.3 Simulations

Simulations were performed by integrating the dynamic equations for overdamped Brownian particles in a two-dimensional space where the traps appear as a landscape with a modulated potential. The equations of motion are

\[ \gamma \ddot{r}_i = \vec{F}(\dot{r}_i) + \xi(t, r_i) = (x_i, y_i) \]  

where the noise \( \xi(t) \) is a delta-correlated stochastic process accounting for the thermal fluctuations at temperature \( T \) (i.e., the fluctuation–dissipation relation is satisfied),

\[ \langle \xi_i(t)\xi_j(t') \rangle = 2\delta_{ij}\delta(t-t') \]  

The indices \( i, j \) refer to particles and the indices \( k, l \) to the \( x, y \) coordinates. The force \( \vec{F} = -\nabla V \) arises from a two-dimensional potential \( V(x, y) \) which is modulated in space and has been constructed by placing wells on a triangular lattice. The total potential acting on the particles is constructed as the sum of Lorentzian terms, one for each trap, of the form

\[ V_n(r) = -V_0 + \delta V_0 \alpha_n \frac{1}{1 + (r/a)^2} \]  

where \( r_i = \sqrt{(x - x_n)^2 + (y - y_n)^2} \) indicates the distance to the center of the corresponding well at position \( (x_n, y_n) \). The total potential is then \( V(x, y) = \sum_n V_n(r) \). The parameter \( V_0 \) is the mean depth of the wells, \( \delta V_0 \) is the standard deviation of the depths, and the \( \alpha_n \) are Gaussian random numbers of unit variance. The parameter \( a \) is the length scale associated with the interaction with a well. In the simulations we have used the parameter values given in Table 1, which have been chosen to best adjust to the experimental scales of time, space, and energy.

Note that the tails of the interaction potentials reach distances of the order of the separation between traps. Thus, particles moving among wells simultaneously experience interactions with several wells. The net effect of the superposition of all these potentials is that the space between traps acts as a potential barrier, confining particles to wells for a long time until a large fluctuation permits any given particle to escape and reach another well. Moreover, the values of the potential in the regions between the wells depend on the local aggregates of the superposition of the interactions with different wells. Hence the net effect is that the effective transport properties of the medium depend in a rather complex manner on the distances between traps, as can be observed in Fig. 1. In this figure we see cross-sections of potentials for two different values of the parameter \( d \). We see how the well depth is highly dependent on the distance \( d \). Closer wells lead to shallower minima. We also observe a small randomness in the potential depths that results from the dispersion \( \delta V_0 \alpha_n \) introduced in eqn (3). Without this term all the wells would have the same depth. While this would not have changed the results by much, inclusion of this term seems appropriate in view of the variation in the peak heights in the experimental results.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Parameters used in the simulations</th>
</tr>
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<tbody>
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<td>Parameter</td>
<td>Value</td>
</tr>
<tr>
<td>( k_bT )</td>
<td>4.2 \times 10^{-3} \text{ pN , \mu m}</td>
</tr>
<tr>
<td>( \gamma )</td>
<td>0.5 \text{ pN , ms , \mu m}^{-1}</td>
</tr>
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<td>( V_0 )</td>
<td>12 ( k_bT )</td>
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<td>( \delta V_0 )</td>
<td>1.2 ( k_bT )</td>
</tr>
<tr>
<td>( a )</td>
<td>0.2 \mu m</td>
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</table>
3. Results and discussion

3.1 Random walk in a periodic potential

We used total internal reflection fluorescence microscopy to track the motion of single molecules at the interface between a solid surface, either chemically patterned or uniform, and water. The patterned surfaces displayed a triangular array of hydrophobic rings on a hydrophilic fused silica substrate (Fig. 2). We investigated surfaces with three different periodic spacings, \( d = 0.50, 0.75, \) and 1.00 \( \mu m \). The uniform surfaces were bare fused silica and fused silica coated with a hydrophobic self-assembled monolayer (OTS). The fluorescent probe molecule was moderately hydrophobic, so it had a higher affinity for hydrophobic surfaces and regions. In experiments on uniform silica and OTS surfaces, the measured adsorption rate constant of Atto6G onto fused silica, \( 1.75 \times 10^8 \mu m^{-2} s^{-1} M^{-1} \), was approximately four orders of magnitude lower than the adsorption rate constant onto the hydrophobic OTS surface, \( 5 \times 10^{12} \mu m^{-2} s^{-1} M^{-1} \).

On all of the surfaces, we observed intermittent dynamics, where each molecular trajectory switched between periods of immobilization and mobility. In this way, the dynamics were qualitatively similar to what was found for other molecules at a solid–liquid interface.\(^m\) The distributions of surface displacements, \( P(r, \Delta t) \), possessed a large, approximately Gaussian peak at small displacements and an extended non-Gaussian tail at large displacements (Fig. 3a). This was markedly different than

![Fig. 1 Potential profiles for \( d = 1 \mu m \) (top) and \( d = 0.75 \mu m \) (bottom). Thin lines are single well potentials (eqn (3)). The thick line is the total potential.](image1)

![Fig. 2 AFM height images of the hydrophobic ring patterns consisting of OTES rings on a silicon surface. The inter-ring spacing, \( d \), was controlled by the latex particle diameter and was equal to 0.50, 0.75 or 1.00 \( \mu m \). Each image shows a 3 \( \mu m \times 3 \mu m \) area. Overlaid on each image is a height profile along the path indicated by the straight line. (The height scale bars are 5 nm.)](image2)

![Fig. 3 Step-size distributions for the probe diffusion on (a) the three patterned surfaces, a uniform hydrophobic surface and (b) in the simulations. For all of the data, \( \Delta t = 60 \) ms. \( d \) is the inter-ring spacing (see Fig. 2). The inset in (a) presents the same data as in panel a, but using logarithmic binning to highlight the enhancement of steps to the periodic distances, \( d \). The single and double vertical bars indicate nearest and next-nearest neighbor distances respectively.](image3)
the simple Gaussian distribution expected for normal Brownian diffusion (the nature of the peak at small displacements will be discussed below). We also observed an enhancement of displacements for distances related to the periodicity of the patterned surfaces. In particular, Fig. 3a shows an increased probability of displacements to nearest neighbor rings at \( r = d \) in the step-size distributions \( P(r, \Delta t) \) for all three patterned surfaces \( (d = 1.00, 0.75 \text{ and } 0.50 \mu m) \). The \( d = 0.50 \mu m \) surface also exhibited an enhanced probability of steps to next-nearest neighbor rings at \( r = 2d \cos(\pi/6) = 0.87 \mu m \). The possibility that the observed displacements are the result of falsely linking molecules that simultaneously adsorbed and desorbed, could be ruled out based on previous work that showed that false trajectories lead to rapid decay of the displacement distribution.\(^9\) We could also rule out the possibility that the trajectories reflected random blinking of pattern-associated defects for two reasons. First, the probability of such an artifact would not depend on feature spacing, yet we observed a clear decrease in peak height with increasing periodic spacing. Second, in a control experiment without the probe molecule, the few impurities identified did not display enhanced displacements to periodic distances.

Interestingly, the enhancement of displacements to periodic distances was previously observed in the atomic-scale dynamics of highly damped particles on the periodic potential of a crystal surface.\(^{20,26–28}\) Here, we observed conceptually similar behavior, but at enormously greater length scales and at a solid–liquid interface. To model the experimental data, we performed simulations of Langevin dynamics in a two-dimensional (2D) periodic potential.\(^{24}\) As an initial approach, we chose this model because it captured the essential features of the experimental data, and avoided unwarranted assumptions about the detailed mechanisms in the experiments. So although the simulations lacked many of the molecular details of the experiments, they provided a basic framework for interpreting the data and connected the experimental results with what is known about dynamics in periodic potentials. We found qualitative agreement between the simulations and experiments. Like the experimental data, the simulated displacement distributions displayed clear peaks at periodic distances and the peak heights systematically decreased with increasing \( r \) (Fig. 3b). The peak heights also decreased in magnitude with increasing lattice spacing, \( d \). To capture this feature of the experimental data, the simulations used a potential well depth that depended on the spacing, \( d \). This suggests that the effective in-plane potential seen by the molecules in the experiments was modified by changes in the mesoscale surface pattern. We should emphasize that in the experiments, the molecules probably did not experience such a simplified 2D potential, but that diffusion in a 2D potential provides a useful analogy for understanding the resulting surface transport. It also provides a framework for designing surfaces with more complex surface patterns to control surface transport. For example, a gradient in the spacing \( d \), which could be achieved with standard lithography techniques, would create a gradient in the effective barrier heights, akin to a tilted washboard, and thus biased motion.

The primary discrepancy between the experimental and simulated results is that the peak heights were systematically lower in the experimental displacement distributions (Fig. 3). We suspect that the underlying reason for this difference is that the experimental surface displacements occurred through a three-dimensional (3D) mechanism involving desorption, bulk diffusion through solution, and reabsorption to a neighboring ring (Fig. 4). The roughly power-law decay of the displacement distributions (Fig. 3a) was consistent with a desorption-mediated transport mechanism that was shown to be common at a solid–liquid interface.\(^{29}\) The alternative explanation, that the molecule diffused in 2D across the silica surface between rings, is unlikely because there was very little mobility of the molecule on bare fused silica (Fig. S2\(^\dagger\)) and the displacements we observed appeared to be instantaneous. We note that low mobility on the fused silica does not necessarily imply a strong probe-surface interaction; in the context of desorption-mediated diffusion, low effective mobility may reflect a low probability of readesorption. For example, previous work investigating protein dynamics on hydrophobic and hydrophilic surfaces observed a coexistence of low mobility and low surface residence times on hydrophilic fused silica surfaces.\(^{27}\)

The probability of taking nearest or next-nearest neighbor steps was likely governed by the probability that a bulk-mediated step reached a certain distance. Bychuk et al. showed that a single bulk mediated step follows a distribution with power-law tails \( (r^{-3}) \).\(^{30}\) Our experimental system was more complex, with an adsorption rate that varied across the surface and an additional \( d^{-1} \) factor coming from the decreasing probability of finding another ring at increasing distances. However, these differences did not change the fact that the probability for taking large bulk-mediated steps rapidly decayed at increasing distance. As a result, the peaks in the displacement distributions decreased with increasing spacing, \( d \). Another feature of the 3D mechanism is that during each bulk excursion there was a chance that the molecule would never return to the surface. In the simulations, particles never escaped from the surface; this is one reason that the experimental peak heights were suppressed compared to the simulations. The experimental peaks might also be partially obscured because of the small, but finite

![Fig. 4 Schematic representation of the proposed mechanism of molecule displacements across the patterned surfaces. The mechanism combines periods of immobilization at the surface (most likely on a hydrophobic ring) and periods of diffusion through the bulk water above the surface.](image-url)
probability for the molecule to adsorb between rings on the fused silica. Although the molecule had much higher affinity for the hydrophobic rings, there may have been other small hydrophobic regions not identified in the AFM images that would have allowed the probe molecule to adsorb at non-periodic distances. In fact, there are imperfections in the surface pattern evident in Fig. 2 that may be the result of isolated chains or small patches of silane coating. We estimate, based on analysis of multiple AFM images, that these imperfections cover less than 0.5% of the surface area. Adsorption of the probe onto these random imperfections would have produced non-periodic displacements that further obscured the peaks in the displacement distributions. To better match the experimental data, future simulations could test the influence of the full 3D mechanism in the presence of a heterogeneous adsorption rate, but the simplified 2D model that we used satisfactorily captured the main qualitative features of the experimental data.

### 3.2 Continuous-time random walk dynamics

The peak at small displacements in the simulated distributions (Fig. 3b), is due to localized vibrations within potential minima. Similarly, the small displacement peak in the experimental distributions was due to imperfect experimental localization during temporary immobilization of the probe molecule. Temporary immobilization is further illustrated by the time ($\Delta t$) evolution of $P(r, \Delta t)$ as shown in Fig. 5 for the $d = 0.50 \mu m$ surface. In Fig. 5, the low displacement peak does not broaden with increasing $\Delta t$, indicating that these small apparent steps are not associated with transport. The non-zero width of the peak ($\sigma^2 = 0.008 \mu m^2$) arises because of experimental uncertainty in determining the position of the immobile molecule. This width is consistent with an apparent diffusion coefficient ($\sigma^2 = 4D\Delta t$, $D = 0.034 \mu m^2 s^{-1}$), very similar to that observed with another probe and a very different set of surfaces.\textsuperscript{29} In contrast, the extended non-Gaussian tails of $P(r, \Delta t)$ are the result of actual probe movement on the surface and broaden with increasing $\Delta t$. We could not rule out the possibility of confined diffusion on a single ring contributing to the apparent immobilization because the size of the hydrophobic rings ($R \sim 150 nm$, Fig. 2) was not significantly larger than our localization precision.

The intermittent immobilization we observed on all of the surfaces was consistent with a continuous time random walk (CTRW) process.\textsuperscript{31} In contrast to the constant time interval between steps in a normal random walk, a CTRW exhibits a distribution of waiting times $\psi(t)$ between steps. The waiting times give rise to intermittent dynamics and the coexistence of immobility and mobility, like we observed. The shape of $\psi(t)$ depends on the physical process leading to immobilization. For example, if molecules are temporarily immobilized by binding within a trap of energy $E_0$, the characteristic escape time is $\tau \propto \exp(E_0/kT)$ and the waiting time distribution becomes $\psi(t) \propto \exp(-t/\tau)$. In this case, the dynamics are intermittent on timescales $t < \tau$. Where a spectrum of binding energies is present, $\psi(t)$ is more broad than a single exponential and in some cases, $\psi(t)$ can fail to have a mean, and diffusion becomes anomalous at all times. Such behavior has been observed for a variety of systems involving diffusion within disordered environments, including glasses and particles near jamming,\textsuperscript{32,33} and biological systems.\textsuperscript{34,40}

To characterize the waiting times between surface displacements in our experimental data, we defined a distance threshold of $R = 0.2 \mu m$ to distinguish between periods of immobilization and true surface displacements. (The threshold was selected based on the displacement distributions (Fig. 3a) and possible error associated with selecting different thresholds was previously discussed.\textsuperscript{29}) All of the waiting-time distributions were non-exponential, suggesting a spectrum of binding energies, and were approximately power-law distributed, $\psi(t) \sim t^{-1-\alpha}$, where $0.8 < \alpha < 1.3$ (Fig. S1†). Notably, CTRWs only exhibit normal Gaussian diffusion when $\alpha > 2$, and in cases where $\alpha < 1$, they exhibit weak ergodicity breaking.\textsuperscript{41} Because of the difficulty of extracting meaningful power-law exponents from experimental data,\textsuperscript{42} we did not pursue physical interpretations based on the precise value of the waiting-time power-law exponents. The important point is that the experimental waiting-time distributions were broader than exponential, suggesting a distribution of binding energies, and possibly non-ergodic dynamics in some cases.

Possible non-ergodic dynamics is further supported by analysis of the mean squared displacement behavior. Fig. 6 presents the mean squared displacement analysis for the $d = 0.50 \mu m$ surface. The mean can be calculated in several different ways. The temporal average was calculated for each trajectory longer than 1.2 s as:

$$\overline{r^2(\Delta t)} = \frac{1}{T - \Delta t} \int_0^{T-\Delta t} (r(t + \Delta t) - r(t))^2 dt \quad (4)$$

where $T$ is the total length of the trajectory. The MSDs that lie horizontal near the bottom are trajectories that were trapped...
and never left their initial positions. The ensemble average quantity is given by

\[ \langle r^2(\Delta t) \rangle = \frac{1}{N_T} \int_0^{N_T} \langle r(\Delta t) - r(0) \rangle^2 \, dt \]  

(5)

where \( N_T \) is the number of trajectories. To calculate the full ensemble-temporal average \( \langle r^2(\Delta t) \rangle \), both means were applied.

Overall, there is dramatic scatter in the trajectories between those with relatively high mobility and those that are immobile. Some of the scatter is due to the limited length of the trajectories, but the pronounced scatter, even at low \( \Delta t \), is characteristic of the ergodicity breaking of a CTRW.\(^\dagger\) The inset of Fig. 6 presents the distribution of \( \xi = \langle r^2(\Delta t) \rangle / \langle r^2(\Delta t) \rangle \) for \( \Delta t = 0.06 \) s, illustrating the asymmetric scatter of the temporally averaged MSDs.\(^\dagger\) The gray line illustrates the Gaussian scatter centered at \( \xi = 1 \) expected for an ergodic process.

Fig. 6 Mean squared displacement (MSD) behavior for the \( d = 0.50 \) \( \mu m \) surface for \( 0.06 < \Delta t < 1.2 \) s. Solid black lines are the temporal average, \( \langle r^2(\Delta t) \rangle \) (eqn (4)), for each of 300 different trajectories. The lines with symbols are the ensemble average MSD over all trajectories (eqn (5)), and the full ensemble-temporally averaged MSD. Inset is the distribution of \( \xi = \langle r^2(\Delta t) \rangle / \langle r^2(\Delta t) \rangle \) for \( \Delta t = 0.06 \) s, illustrating the asymmetric scatter of the temporally averaged MSDs.\(^\dagger\) The gray line illustrates the Gaussian scatter centered at \( \xi = 1 \) expected for an ergodic process.

4. Conclusion

Using uniform and chemically patterned surfaces, we observed molecular surface diffusion characterized by continuous time random walk dynamics and the enhancement of displacements to periodic distances. Simulations of particle dynamics in a 2-dimensional periodic potential demonstrated the analogy between the mesoscale dynamics we observed and atomic scale surface diffusion. An advantage of the system we studied, compared to an atomic system, is that the periodicity could be easily changed, which allowed us to control the relative number of surface displacements to particular distances. The next step is to use more sophisticated surface patterning to direct and control surface transport. For example, a gradient in the periodic spacing might produce a directional bias in the motion analogous to a tilted washboard potential. A surface pattern could also be designed to enhance displacement to, and immobilization at, surface reactive sites. Such an arrangement could lead to advancements in targeting and reactions at surfaces.\(^\dagger\)\(^\ddagger\)\(^\dagger\) Future work in this direction should take advantage of the growing body of work investigating the connection between diffusive processes and reactivity at surfaces.\(^\dagger\)\(^\ddagger\)\(^\dagger\) Combined with the knowledge about dynamics in a periodic potential gained from the study of atomic systems, and common surface patterning techniques, this could reveal new strategies for manipulating surface transport to enhance surface processes.

Acknowledgements


References


