Redistribution of fluorescent molecules at the solid/liquid interface with total internal reflection illumination

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ABSTRACT

Many intriguing physical and chemical processes commonly take place at the solid/liquid interface. Total internal reflection illumination, together with single molecule spectroscopy, provides a robust platform for the selective exploration of kinetic processes close the interface. With these techniques, it was observed that the distribution of Rhodamine B molecules close to a solid/liquid interface could be regulated in a photo-induced route. The laser-induced repulsion force at this interface is enough to compromise the Brownian diffusion of single molecules in a range of several hundred nanometers normal to the solid/liquid interface. This observation is fundamentally and practically interesting because moderate laser intensity is enough to initiate this repulsion effect. Therefore, it might display extensive applications in the development of photo-modulation technique with high throughput capability.

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1. Introduction

Development of new techniques for the manipulation of nanoscopic objects has aroused ongoing interest in diverse areas [1–4]. Owing to the non-invasive remote manipulation feature as well as high tempo-spatial resolution capability, optical-based approaches enable previously inaccessible information to be extracted from chemo-physical systems and have become broadly useful [1,2,5,6]. For example, optical tweezers are excellent tools to manipulate nanoparticles ranging in size from several micrometers to tens of nanometers ($\sim$25 nm to 10 μm) [7,8]. With plasmonic dipole antennas, it is also possible to trap single gold nanoparticles with size down to 10 nm [9]. These techniques mainly take advantage of near field trapping forces in a tiny window to overcome the thermal motion. It is therefore very difficult to handle large number of objects simultaneously in a high throughput manner. In addition, strong laser intensity is commonly required (e.g., the required laser power for optical tweezers to trap a one hundred nanometer latex particle is normally as high as 5×10^6 W/cm^2) [7]. This requirement is a major challenge for applications in biological systems that are sensitive to laser-induced thermal effect.

The rapid development of nano-fabrication techniques enables integration of novel nanostructures and optical microfluidic elements into a single device [3,10,11]. It therefore provides a promising approach to manipulate target objects in a high throughput fashion. This achievement is significant because it affords new insights into the development of novel separation methodology with the capability of regulating the selection process in a controllable way [12,13]. However, some of those approaches are still dependent on the near field confinement effect and thereby, limits the strength of the trap and excludes the use of moderate laser power.

In many separation modalities, the dynamic adsorption and desorption processes of analytes on the matrix or channel wall constitute the basic selection mechanism during the separation process [14–21]. Controlling the dynamic behavior of target objects at the interface in a chemo-physical manner is thus fundamentally important. It not only affords insightful information for the accurate elucidation of the separation mechanism but also provides new methodologies to manipulate target molecules in complex surroundings.

In this work, we demonstrate that the dynamic behavior of single Rhodamine B molecules close to a solid/liquid interface can be manipulated in a photo-induced route. Laser-induced repulsion enables regulation of the distribution of Rhodamine B molecules normal to the solid/liquid interface in a range of several hundred nanometers. To understand the mechanism in detail, comprehensive control experiments were performed to confirm this scenario. The laser-induced desorption kinetic curve is also well

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consistent with the simplified model raised according to the assumption as noted above. This observation is fundamentally interesting because moderate laser intensity (143 W/cm^2) is sufficient to initiate this repulsion effect, potentially suitable for the development of photo-modulation technique with high throughput capability.

2. Experimental section

The single molecule imaging experiments were performed on a home-built prism type total internal reflection fluorescence imaging setup. In brief, the focused excitation light (with a final spot size on the sample around 2800 μm^2) from a 532 nm diode laser was reflected to the side face of an isosceles triangle prism (IB-21.6–60.6-SF10, 25 × 25 × 25 mm, CVI Melles Griot, U.S.A.). In order to selectively record the fluorescent molecules close to the liquid-solid interface, the entrance angle of the laser line on the glass/water interface was carefully adjusted to the value slightly larger than the critical angle (around 66.5 degree). The penetration depth of evanescent wave, which usually ranges between 30 and 300 nm, is independent of the incident light polarization direction, and decreases as the reflection angle grows larger. Generally, the characteristic penetration depth (d) at an excitation wavelength of λ is given by:

$$d = \frac{\lambda}{4\pi\sqrt{n_1^2 \sin^2\theta - n_2^2}}, \tag{1}$$

where n$_1$, n$_2$ and θ are the refractive index of fused silica, water and the incident angle of laser beam respectively. In this case, the estimated penetration depth is around 300 nm. A 100 × objective (N.A. 1.45, Plan. Apo. Nikon, Japan) was then used to collect fluorescence from single molecules after filtered by a band-pass filter (FF01–580/50–25, Semrock Inc., New York, U.S.A.). The diffusion dynamics of individual molecules close to the silica surface were recorded by an EMCCD (Andor Ultra 897) with a frame rate of 20 Hz. The images were processed with ImageJ (http://rsbweb.nih.gov/ij/) and the data were analyzed with Matlab.

3. Results and discussion

3.1. Redistribution of single molecules at the solid/liquid interface

In this study, clean cover slips (22 × 22 mm^2) were firstly treated in an oven for 3 h at 150 °C. After this treatment, the adsorbed thin water layer on the surface was effectively evaporated and the silica surface became relatively hydrophobic even after dipping in water for one day. 7 μL of Rhodamine B solution (1 nM in deionized water) was evenly spread out between two cover slips. The depth of the sample solution is around 14.5 μm (determined by dividing the volume of the sample solution by the area of the cover slip). The estimated time scale for a single molecule to travel through this gap is around 375 ms based on the diffusion coefficient of 2.8×10^{-6} cm^{2}s^{-1} [22,23]. The spatial distribution of fluorescent molecules between these two cover slips should therefore rapidly reach equilibrium provided no significant interactions occurred at the solid/liquid interface. Indeed, the counted number (262 ± 40) of individual molecules within the evanescent field layer (EFL, around 300 nm in depth [24]) is in agreement with the predicted result of 267 within a field-of-view of 512 × 113 (pixel-by-pixel) as shown in Fig. 1. The majority of dyes exhibited obvious adsorption and desorption events and a few of them were slowly sliding on the solid surface at the initial stage. Long-term continuous adsorption was not observed within

the time scale of observation (one min). These results indicate that the spatial distribution of single molecules in the whole chamber is basically controlled by Brownian diffusion. Surprisingly, when the laser was continuously directed at the focused spot with low to moderate power (from 71 to 572 W/cm^2 or 2–16 mW), a gradual decrease in dye number within EFL could be observed that finally reached an equilibrium state, Fig. 2.

Because Rhodamine is an organic molecule, complicated photochemical reactions might break the chemical bonds once a threshold dosage of light is introduced. This process would contribute to the laser-induced decrease in numbers close to the surface. To test this hypothesis, we measured the rate constant of the photobleaching process for Rhodamine dye at different laser intensities (from 71 to 500 W/cm^2 or 2–14 mW). As a strict comparison, the experiments were performed with the same setup except for making the incident angle of the laser smaller than the critical angle. This guarantees that molecules in the bulk solution can be evenly illuminated and the density of photons delivered to the sample is the same as that at the interface.

The measured photobleaching rate is almost negligible in comparison with the desorption rate at the interface, Fig. 3a. Since the diffusion of single molecules in the bulk solution is very fast, if the photobleaching process is slower than the time for a single molecule to travel through the field-of-view, the measured photobleaching rate might be an underestimation. In this regard, we further inhibited the Brownian diffusion of Rhodamine B by embedding the sample into 10% agarose gel. The porous structure of agarose gel restricts mass transport but still renders the same chemical environment as that in the previous experiments [25]. The measured rates agree well with that determined in free solution and are also noticeably slower than that for laser-induced depletion on a hydrophobic surface, Fig. 3a. According to these results, two significant conclusions can be drawn herein: firstly, the adopted laser intensity has a negligible effect on the photochemical destruction of Rhodamine dye; secondly, the laser-induced depletion is not caused by photobleaching in consideration of the large differences among the three sets of data. The single molecule imaging experiment in agarose gel further confirms these points, Fig. 3b.

An alternative picture that might account for the reduction of dye numbers would be laser-induced redistribution at the interface. Previous experimental results have demonstrated that many interesting physical and chemical processes can be initiated in the presence of moderate laser illumination and typically follows an intensity dependent relationship. For example, earlier work has demonstrated that illumination of a glass surface with visible light at low intensities leads to a release of alkali atoms, which have been adsorbed to the surface [26]. Our previous work has also found that the composition and spatial structure of silver clusters formed by several atoms could be modulated under total internal illumination [27]. On this account, one of the possible explanations of the above observation is that the dye depletion might be due to laser-assisted ionization of surface silanol groups, creating an electrostatic repulsion barrier at the interface.

3.2. Electrostatic interaction at the solid/liquid interface

To test whether there is a permanent photo-induced change to the surface, we performed a control experiment in which the cover slip was exposed to relatively strong laser power (572 W/cm^2 or 16 mW) for 1 min and then the light was blocked for 10 min Due to Brownian diffusion, a new equilibrium distribution of dye molecules in the solution should be established after 10 min If the surface is inert to laser illumination, the initial counted number of molecules at a relatively low laser power (143 W/cm^2 or 4 mW) should be the same as that without first illuminating with high