Progress Report for DOE:

Dynamics of Biomolecules on Surfaces: 2006

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Summary:

During this year we performed two major projects:

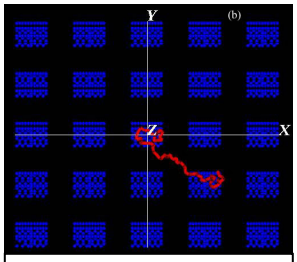

Fig. 1 DNA chain moving on a patterned surface.

I. We developed a detailed theoretical model which complements our experiments on surface DNA electrophoresis. We found that it was possible to enhance the separation of DNA chains by imposing a chemical nanoscale pattern on the surface. This approach utilized the surface interaction effect of the DNA chains with the substrate and is a refinement to our previous method in which DNA chains were separated on homogeneous flat surfaces. By introducing the nano-patterns on the surface, the conformational changes of DNA chains of different lengths can be amplified, which results in the different friction strengths with the substrate surface. Our results also show that, when compared to the DNA electrophoresis performed on homogeneous flat surfaces, nanopatterned surfaces offer a larger window in choosing different surface interactions to achieve separation. (See manuscript below which is being submitted to the journal Electrophoresis for publication.)

II. In collaboration with a large international manufacturer of skin care products we also embarked on a project involving photo toxicity of titanium dioxide nanoparticles, which are a key ingredient in sunscreen and cosmetic lotions. The results clearly implicated the nanoparticles in catalyzing damage to chromosomal DNA. We then used this knowledge to develop a polymer/anti-oxidant coating which prevented the photocatalytic reaction on DNA while still retaining the UV absorptive properties of the nanoparticles. The standard gel electrophoresis was not sufficient

in determining the extent of the DNA damage. The conclusions of this study were based predominantly on analysis obtained with the surface electrophoresis method. These results are being submitted for publication to JACS (see attached).

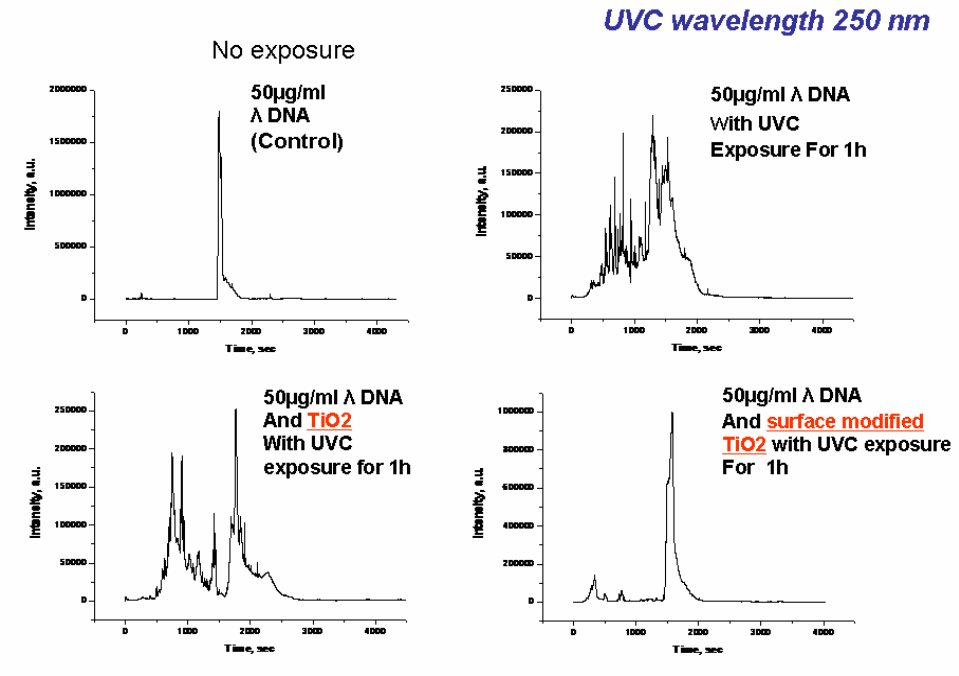

Figure 2: Surface electrophoresis of Lambda DNA (a) without exposure (b) exposed to UVC (c) exposed with TiO2 and with (d) coated TiO2.

Personnel:

Two graduate students were supported by this grant; Binquan Li and Xiaohua Fang. Both have obtained their PhD this year and are employed as postdoctoral associated at Columbia University.

One undergraduate student was supported; Eric Petersen who is now a sophomore at Harvard University.

One Research Associate Young Soo Seo, was also supported. He is now employed by LG Electronics.

One faculty member; Dilip Gersappe received summer (2 months) salary.

Other sources of funding:

NSF-MRSEC: Funds were used for equipment purchases and support of high school students participating in this work.

Petroleum Research Foundation: Haobin Luo, a postdoctoral associate was partially supported by a grant from the PRF.

Publications:

1. Li BQ, Fang XH, Luo HB, Petersen E, Seo YS, Samuilov V, Rafailovich M, Sokolov J, Gersappe D, Chu B. Influence of electric field intensity, ionic strength, and migration distance on the mobility and diffusion in DNA surface electrophoresis ***ELECTROPHORESIS*** 27 (7): 1312-1321 APR 2006

2**.** Fang XH, Li BQ, Petersen E, Seo YS, Samuilov VA, Chen Y, Sokolov JC, Shew CY, Rafailovich MH. Drying of DNA droplets ***LANGMUIR*** *22* (14): 6308 2006

3. BQ, Fang XH, Luo HB, Seo YS, Petersen E, Ji Y, Rafailovich M, Sokolov J, Gersappe D, Chu B. Separation of DNA with different configurations on flat and nanopatterned surfaces ***ANALYTICAL CHEMISTRY 78*** (14): 4743-2006

4. Haobin Luo, Dilip Gersappe and Miriam Rafailovich, DNA Separation on Nanoscaled Patterned Surfaces, to be submitted to **Electrophoresis.**

5. Wilson A. Lee, Nadine Pernodet, Bingquan Li, Chien H Lin, Miriam H. Rafailovich "The efficacy of surface modified nano titanium dioxide against UV damage to DNA" to be submitted to **JACS**.

Appendix: Manuscript for publication in Electrophoresis

DNA Separation on Nanoscaled Patterned Surfaces

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Summary

By using molecular dynamics simulation, we demonstrate that it is possible to achieve separation of DNA chains on nano-patterned surfaces with a chemical pattern. This approach utilizes the surface interaction effect of the DNA chains with the substrate and is a refinement to our previous method in which DNA chains were separated on homogeneous flat surfaces. By introducing the nano-patterns on the surface, the conformational changes of DNA chains of different lengths can be amplified, which results in the different friction strengths with the substrate surface. Our results also show that, when compared to the DNA electrophoresis performed on homogeneous flat surfaces, nanopatterned surfaces offer a larger window in choosing different surface interactions to achieve separation.

1 Introduction

The separation of DNA molecules is typically accomplished by electrophoretic methods which utilize the concept of entropic trapping. A long polymer molecule can be trapped entropically in a restrictive environment such as a gel, or obstacles on a surface [1-3]. This trapping effect becomes prominent when the size of the pores is comparable to the radius of gyration of the polymer. When a DNA molecule is migrating under the influence of an applied electric field, the collisions with the environment result in the separation of DNA by length inside the topologically restrictive matrix. While these methods have been successfully used to separate DNA fragments that differ by a single base, their read length is quite limited (e.g., the efficiency of gel electrophoresis deteriorates seriously for DNA molecules longer than 40,000 base pairs (40 kbps)). This is mainly due to the field-induced molecular orientation which minimizes the difference of interaction with restrictive matrix among long DNA molecules.

Several approaches can be applied to overcome this limitation. For example, by introducing pulsed fields, the long DNA can relax to a certain degree before being stretched again along the applied electric field. This relaxation allows the length-dependent interaction with the surrounding matrix and therefore separation of long DNA molecules can be achieved. Efficient separation can be achieved by pulsed-field capillary gel electrophoresis (PFCGE) [4, 5], but reports have been made about the DNA aggregation in PFCGE caused by an electrohydrodynamic instability that sometimes results in spurious and irreproducible peaks [6]. Recently a novel approach has been utilized successfully by Han et al. [7, 8] to sequence long DNA

fragments, using the microfabricated entropic trap array. In their approach, a nanofluidic channel comprising of alternately narrow constrictions and wider regions caused size-dependent trapping of DNA molecules at the onset of a restriction. This process creates electrophoretic mobility differences, thus enabling efficient separation without the use of gel matrix or pulsed electric field. Another interesting approach was reported by the Cox group [9, 10] in which an array of micron-scale posts was used as the sieving matrix. When asymmetric pulsed field was applied, chains of different lengths spent different times in elongating and backtracking and speedy continuous fractionation of large DNA molecules can then be achieved. In a more recent report, nanopillars have also been used for the separation of long DNA molecules under a direct current electric field [11].

So far, these approaches utilize only the effect of entropic trapping. A possible new approach to performing electrophoresis is to utilize the effect of surface interactions. In our recent work [12-14] it has been shown that the local friction between a fully absorbed DNA molecule and a flat homogeneous surface can result in the separation of long DNA fragment. In this approach the friction between the adsorbed segments and the surface is controlled by coating the surface with well controlled silane monolayer films. By the process of changing the surface energy of a flat surface, it is possible to change the size range of DNA that can be separated by this method. Molecular dynamics (MD) simulation [12] confirms the separation of DNA molecules by modifying the interaction between the DNA and substrate surface.

The success of this approach is based on the conformational characteristics of DNA molecules adsorbed on a flat surface. The balance between the loss of entropy due to the localization of DNA at the surface and the energetic gain on adsorption of the molecule results in the classic picture of DNA segments being present as either loops (that extend into the solution) or trains (that are contiguous segments adsorbed on the surface). Thus, for a fixed surface attraction, shorter chains maximize their entropy and have a larger number of loops or unadsorbed segments, while longer chains exploit the energetic gains on adsorption and have more trains or adsorbed segments [15]. Consequently, if an electric field is applied in the plane of the surface, the response of the molecule to the field should be a function of its conformation on the surface, and therefore there should be, in theory, a length dependent mobility.

Electrophoresis on a uniform surface, however, has some limitations. For example, as we have shown in our previous work, to achieve separation on the uniform surface, the interaction between the DNA molecules and the surface cannot be too strong or too weak. Under both extremes, the mobility of DNA approaches the free-draining limit. This limitation in the potential window of interaction strength could restrict the choices of candidate materials which can be used to build separation devices. Another limitation of uniform surface is that since it relies on a length dependent conformation to separate chains, chains of different lengths require significantly different conformation. Conformational fluctuations limit the sequencing capability of DNA on a uniform surface to distinguish between chain lengths much larger than the persistence length of the DNA. To overcome these limitations, an approach is to increase the complexity of the media to amplify the

small changes in conformational structures. One way to achieve this is to introduce a substrate surface which has a variable surface energy. For the adsorbed DNA molecules, the friction coefficient is then position dependent. By tuning the correlation length of different surface energy sites, it should be possible to achieve the size separation of DNA molecules.

2 Methods

Molecular dynamics (MD) simulations are carried out to probe the applicability of patchy substrate surface on the sequencing of DNA molecules. In the simulation the DNA is modeled as a linear polymer chain with N segments. Monomers of mass m and an effective charge q interact through a truncated Lennard-Jones (L-J) potential of the form $V(r) = 4\varepsilon[(\sigma/r)^{12} - (\sigma/r)^{6}]$ for $r < r^c = 2.2\sigma$. Here r is the distance between two monomers, ε and σ are the characteristic energy and length scales. The potential is zero for $r > r^c$. The adjacent monomers along the chain are coupled by an additional FENE potential of the form of $V_{CH}(r) = -0.5KR_0^2 \ln(1-(r/R_0)^2)$ with $K = 30\varepsilon$ and $R_0 = 1.5\sigma$. This FENE potential can prevent chain breaking and yield realistic dynamics for polymers [16].

The substrate surface consists of atoms forming two (111) planes of an fcc lattice. An L-J potential with modified parameters εs, $\sigma s = \sigma$, and $r^c_s = 2.2\sigma s$ is used to model the interactions between the monomers and the surface atoms. The temperature T is kept constant by coupling the polymer to a heat bath [16]. Periodic boundary conditions are applied within the plane of the walls to eliminate the edge effects. The equations of motion are integrated using a fifth-order predictor-corrector

algorithm with a time step of $\Delta t = 0.005\tau$ where $\tau = (m\sigma^2/\varepsilon)^{1/2}$. In all runs the T is fixed at $4.0\varepsilon/k_B$. A dimensionless electric field $Es = (q\sigma E)/(k_B T)$ is used, in which q = 1 is the charge per monomer.

The patchy surface is introduced by making two types of wall atoms which are differentiated by their interaction with polymer atoms. Certain parts of wall surface are made to be more "sticky" to polymer atoms which have a higher surface-polymer coupling εs, while the other parts of the wall atoms have a lower values of εs. Of these two types of wall atoms, the ones with a lower surface coverage will be referred to as patch atoms with the other atoms will be referred to as bare wall atoms. By varying the εs of patch atoms and bare wall atoms, different polymer-surface interactions can be modeled.

We design two types of patterned surface which are shown in Figure 1. The first one is made of patches which form a hexagonal structure along the electric field. In the second one the patches form square arrays along the same direction. The first type of patch pattern is denoted as hexagonal pattern and the second as square pattern. The percentage of surface area for patches is 25% in both types. The reason why we choose these two patch patterns is that the production of these surfaces is not hard under normal laboratory conditions. For example, the hexagonal structure can be produced by utilizing diblock polymer on silicon surfaces while the square pattern can be made through micro-contact printing. In the simulation, we vary the electric field strength and the interaction strength between polymer and wall surface. For convenience, the different set of parameters are put in the form of $\varepsilon_{sp}_\varepsilon_{sw}_$field, in which ε_{sp} stands for the epsilon between polymer and patches, ε_{sw} the epsilon

between polymer and bare wall, field the strength of electric field. For example, 2.25_2.0_0.02 stands for the set of parameters with ε_{sp} to be 2.25, ε_{sw} to be 2.0 and field to be 0.02.

Before we apply the electric field, the DNA molecules are equilibrated for long enough times to eliminate the correlation between successive runs. The simulation results are averaged over 80 runs for each set of surface interactions.

3 Results and discussion

The separation of DNA molecules on the patchy surfaces can be seen in Figure 2, in which the time evolution of the center of mass movement along the electric field direction are shown for DNA molecules with different chain length. Short chains move faster than longer chains. Over long enough time the complete separation for four chain lengths can be achieved on both types of patchy surface. Note that in Figure 2a the patch surface is less sticky than bare wall surface while in Figure 2b the situation is reversed.

We next study the effect of varying the surface interaction strength. Shown in Figure 3 is the mobility of DNA as a function of ε_{sp} and ε_{sw} on these two types of patchy surfaces. These results clearly show that if the interaction strength is too large (e.g. with an epsilon value of 3.0) we can see no separation of chain lengths. This is due to the fact that almost all the DNA segments are adsorbed on the strongly attractive sites and take the train conformations, no matter whether the chain length is short or long. Only with an intermediate surface interaction can we expect the DNAs with different chain lengths to exploit the different train-loop conformations

to get the fractionation. From Figure 3 we see that with an interaction strength of 2.25 or 2.5, reasonable separation can be achieved.

The density profile for a DNA chain adsorbed on the square patchy surface with a strong interaction is shown in Figure 4. The patch pattern is clearly reproduced in the density profiles for both the short and long chains, which means the DNA chains are strongly confined on top of the patches and spend most of the time on the patches. This can explain the poor separation of DNA chains for this set of parameters. For a surface with less sticky variable energy sites, the situation is different. Shown in Figure 5 are the density profiles for the same two chain lengths, but on a hexagonal patchy surface where the patches are less sticky than bare wall surface. We cannot see the patch pattern from the density profile for chain 60 while the patch pattern shows up for chain120. The possible explanation is as follows: as we know that surface interaction is weak or intermediate with an epsilon of 2.0 or 2.25, for this range of interaction strength, short chains (e.g., chain 60) tends to take the "loop" conformation and the density profiles are averaged out over the whole surface, regardless whether it is on top of the patch or bare wall surface. The patch pattern then cannot show up. While for longer chains (e.g., chain 120) "train" conformations are preferred and it is likely that the chains stay more on top of the bare wall surface because the higher attraction can lower the free energy more. So we can see the patch pattern clearly. When applied to electrophoresis, the difference in chain conformations for different chain lengths then leads to the separation of DNA chains.

The above mechanisms which control the electrophoresis of DNA can be further clarified by visualizing the movement of DNA chains after we apply the electric field. Shown in Figure 6 are the snapshots for two chain lengths on a strongly sticky square patch surface. They are taken from the systems with the same setting of parameters as those in Figure 4. We can see the "train" configuration dominates and most DNA segments are confined on top of the patch surface. For those segments which span between neighboring patches (which means they are on top of the less sticky bare wall surface), the strongly anchored points on top of the patches tend to "pull" them from both ends. This reduces the probability for them to form "loops".

With an intermediate interaction, the effect of chain length on "train-and-loop" configuration shows up. As shown in Figure 5, different chain lengths result in different density profiles. The snapshots in Figure 7 further complement this scenario. We can see short chains tend to form "loops" and loops span randomly on top of the surface. This explains why we cannot see clearly the patch patterns in the density profile. For longer chains, "trains" tend to lie on top of the stickier surface and the patch patterns can now been seen.

By showing separation of DNAs on different setting of surface interaction parameters and on different patch patterns, the electrophoresis on patch surface offers more choices when compared to the electrophoresis on bare surface. For bare surface only an intermediate surface attraction can make the separations [12], which restricts the choice of materials. The patch surfaces then show a larger window in choosing different surface interactions. This is invaluable to the real experimental designs since we can have more candidate materials available.

The effectiveness of patchy patterned surface was tested experimentally by Seo et al. using a hexagonal pattern of Ni patches superimposed upon a Si matrix [17]. In order for the chains to sense the pattern, the dimensions of the patches were chosen to be roughly 2 or 3 DNA persistence lengths (similar to the simulations), or approximately 150 to 200 nm. The electrophoresis was performed using a series of commercial double stranded DNA standards: 1 kbp ladder, λ-Hind III Digest, λ-DNA-Mono cut mix, λ-DNA and T2 DNA. The molecular weights of this mix ranged over three decades from 125 bp to 169K bp. Chromosomal S. Pombe DNA consisnting of three components at 3.5Mb, 4.7Mb, and 5.7Mb was also tested in order to probe whether a limiting size existed for our method.

The motilities of the DNA chains which span nearly five decades, can be plotted with the same power law dependence $\mu \propto N^{\alpha}$ where $\alpha = -0.36 \pm 0.05$. This exponent is intermediate between −0.25 and −0.87 obtained on homogenous bare and functionalized Si surfaces respectively [12, 14]. As shown in the previous simulations, the homogenous surfaces with either very strong or weak interactions, were applicable to either short or long DNA chains, respectively [12]. These results show that imposing a chemical pattern can increase the exponent without compromising effects at the shorter end of the length scale. Since both the Ni and Si could be further functionalized, we believe that the interactions as well as the length scale of the pattern could be further adjusted in order to increase the exponent and optimize the dispersion.

Another advantage of this technique is that the fractional resolution does not decrease with increasing base pair number and the fractional resolution of the peaks

is constant, for all DNA molecules studied, up to chromosomal length. Hence even though the resolution of this method is comparable to other separation techniques for low molecular weights, it is much better for the higher molecular weights. To the best of our knowledge this is one of the first methods that, without any optimization, have shown the ability to simultaneously separate such a broad range of DNA lengths on the same substrate and using a single set of experimental conditions.

In conclusion, DNA chains of different chain lengths can be seperated with the introduction of variable energetic patches onto the flat surface. For DNA chains absorbed on substrate surface, the chain segments can take train or loop conformations, which have different friction strength with the substrate. On a patterned surface, in response to the different interaction strength with the underneath patch sites, DNA chains with different length adopt different conformations, which, in turn, leads to the seperation. For the pattern surface, the energy difference between patches and the bare surface cannot be very large in order to achieve good separation. Our results have been tested experimentally and have been shown to separate chromosonal size DNA molecules.

We gratefully acknowledge funding from the DOE-BES.

4 References

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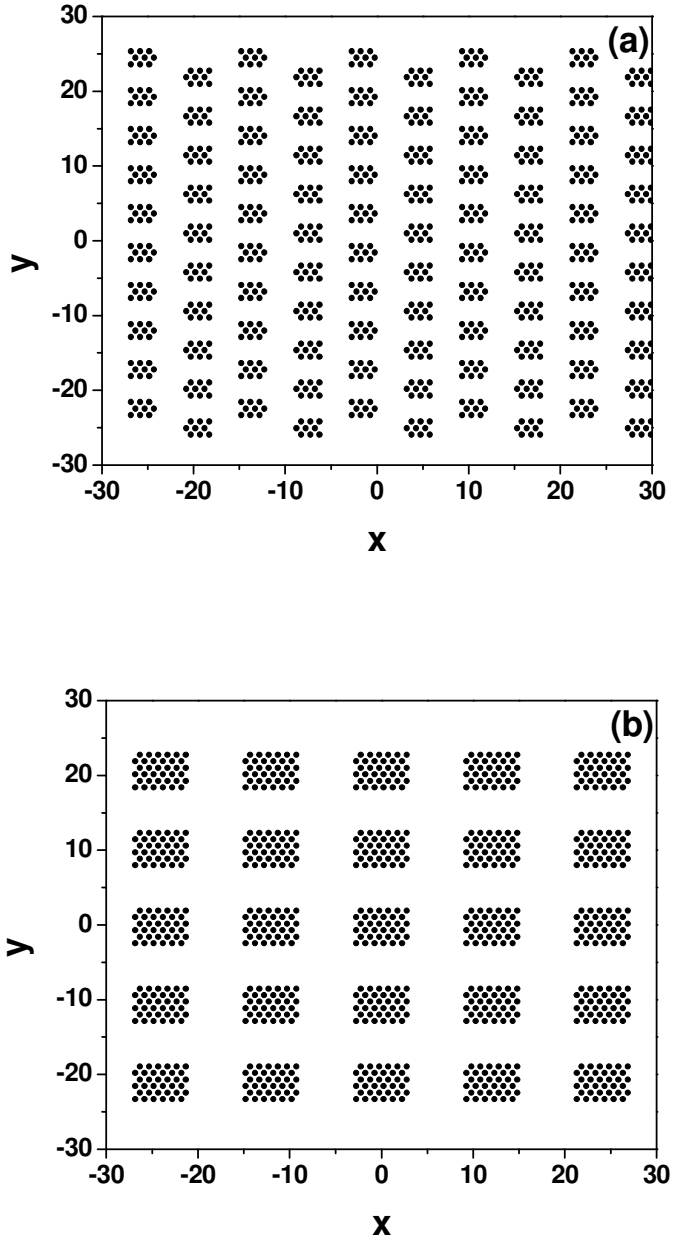


Figure 1. The pattern of patchy surfaces. The dark color stands for patches while the bare wall surface is shown as the white background. a): hexagonal patch surface; b): square patch surface.

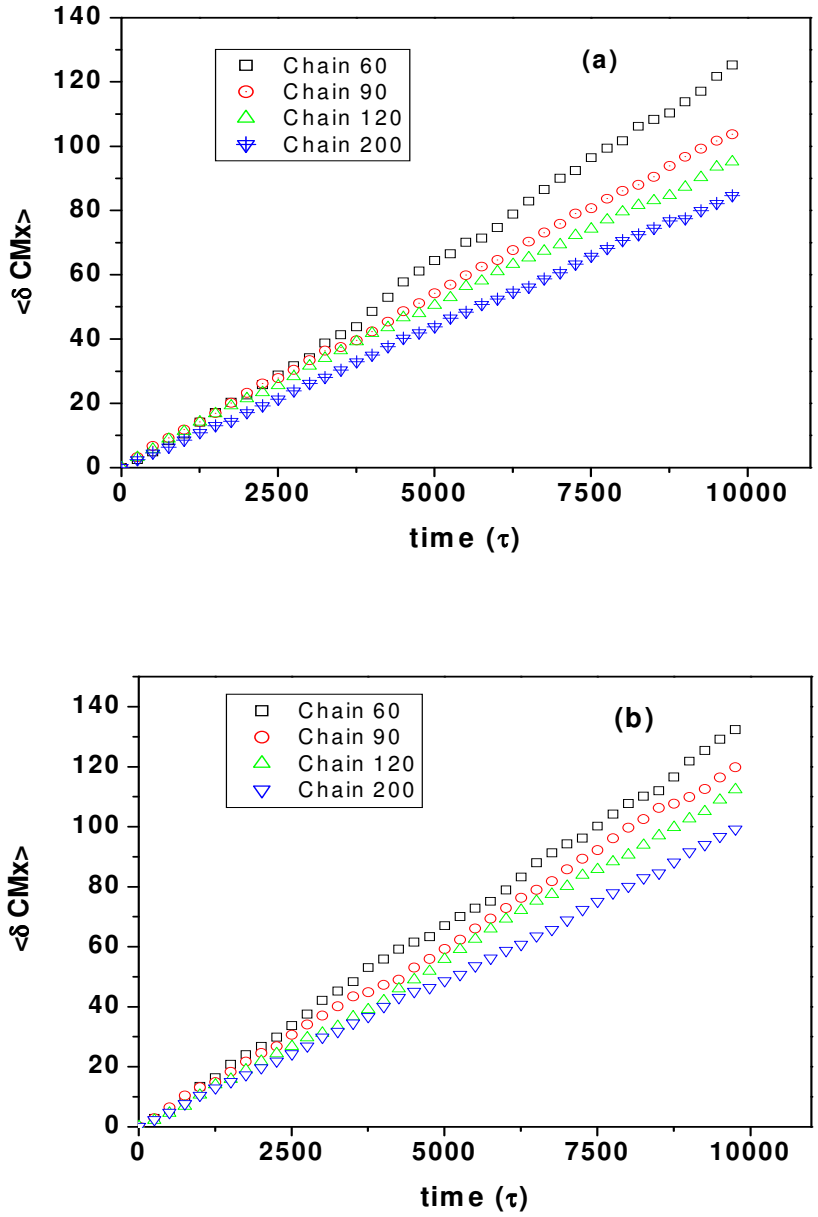


Figure 2. The dependence of center of mass movement ($<\delta cm_x>$) along the electric field on time. The setting of parameters are a): 2.0_2.25_0.02 on hexagonal surface; b): 2.5_2.0_0.025 on square surface.

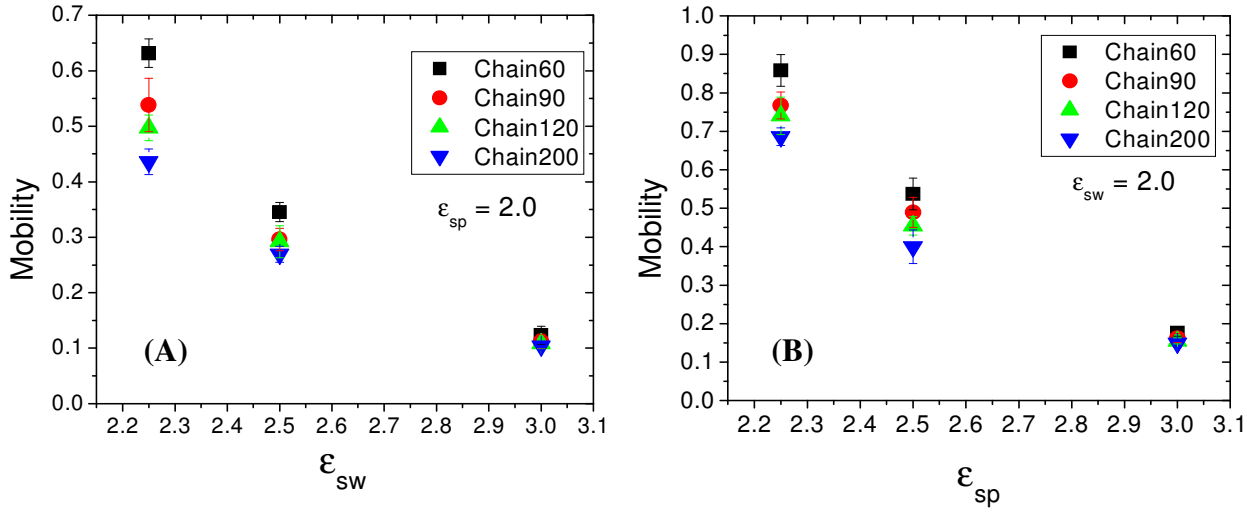


Figure 3. Mobility of DNA calculated by MD simulations **a**) Plot of the mobility of DNA on a hexagonal patch surface where the patches are less attractive than the bare wall. **b**) Plot of the mobility of DNA on a square patch surface where the patches are more attractive than the bare wall. In **(a)** the dimensionless electric field was fixed at E = 0.02 and in **(b)** E = 0.25.

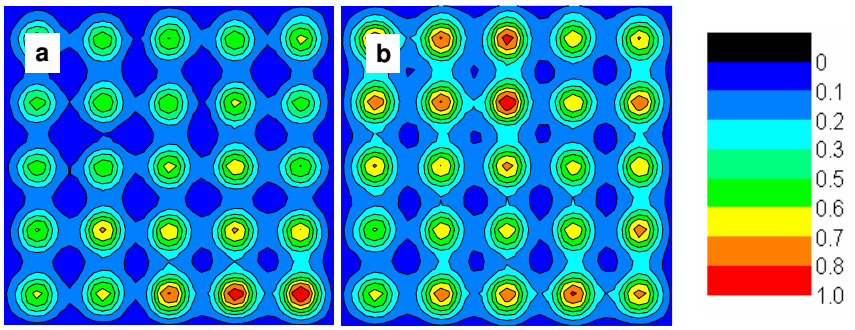


Figure 4. Normalized time averaged segment density for square pattern. The patch sites are strongly attractive with $\varepsilon_{sp} = 3.0$ and the bare wall sties have an $\varepsilon_{sw} = 2.0$. a): chain 60; b): chain 120.

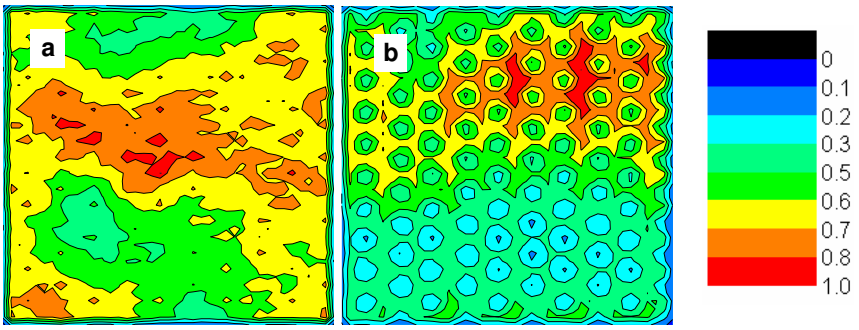
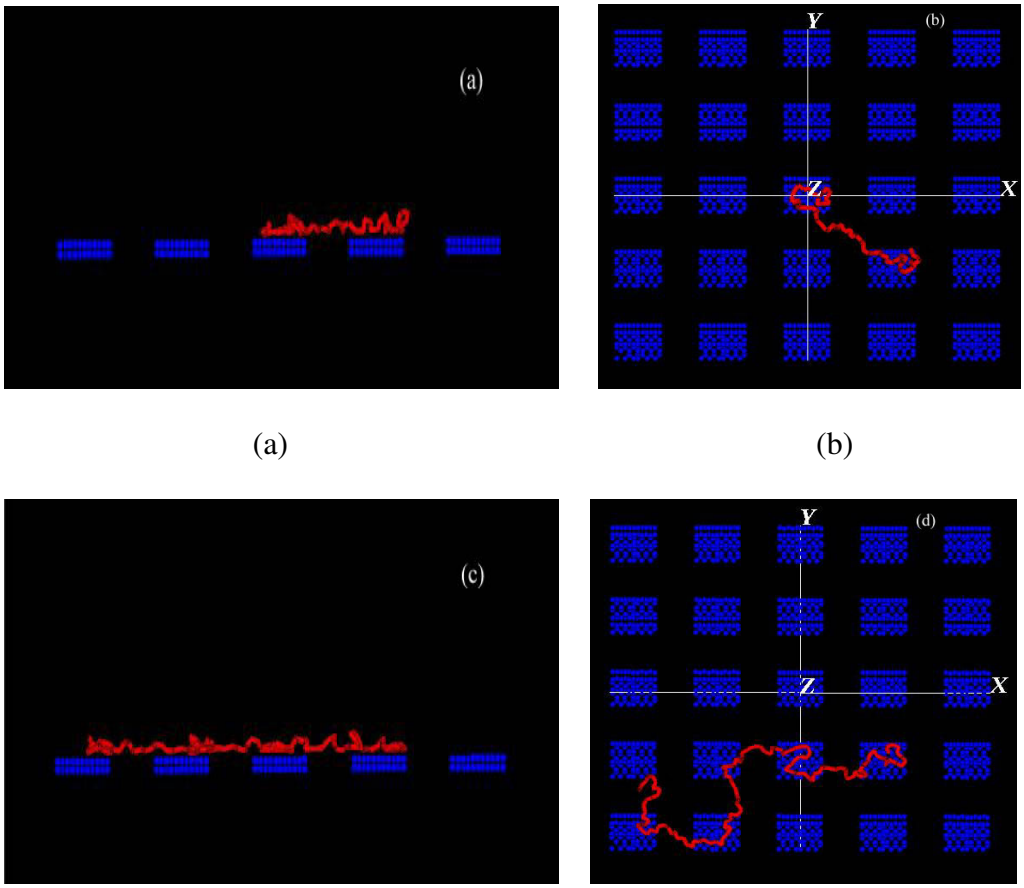


Figure 5. Normalized time averaged segment density for hexagonal pattern. The energy difference between patches and bare wall sites is not as pronounced as the one in Figure 4. The setting of parameters is 2.0_2.25_0.02. a): Chain 60; b): Chain 120.



(a) (b)

(c) (d)

Figure 6. Snapshots of DNAs on the sticky patch surface. The setting of parameters is the same as Figure 4. a): chain 60, x-z projection; b): chain 60, x-y projection; c): chain 120, x-z projection; d): chain 120, x-y projection.

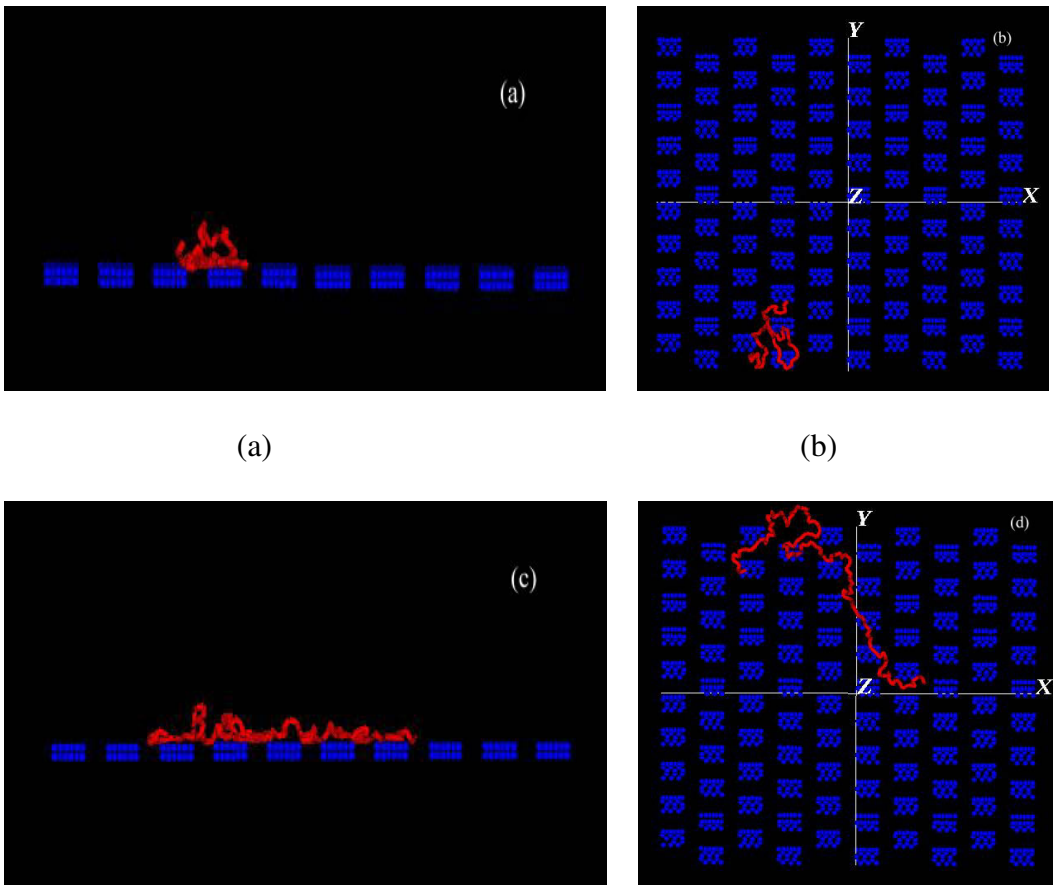


Figure 7. Snapshots of DNAs on the patch surface with an intermediate interaction. The setting of parameters is the same as Figure 5. a): chain 60, x-z projection; b): chain 60, x-y projection; c): chain 120, x-z projection; d): chain 120, x-y projection.