Molecular dynamics study of bio-manipulation in aqueous media

Mohsen Kheirodin[1], *Hossein Nejat Pishkenari*[1], *Seyed Hanif Mahboobi*[2]

[1]*Nano Robotics Laboratory, School of Mechanical Engineering, Sharif University of Technology, Tehran, Iran*
[2]*Department of Bioengineering, University of California at Berkeley, California, USA*
E-mail: nejat@sharif.edu

Published in Micro & Nano Letters; Received on 8th March 2015; Revised on 16th October 2015; Accepted on 20th October 2015

Nano-manipulation is one of the most important aspects of nano-robotics and nano-assembly. The positioning process is considered by many researches to be one of the most important parts of nano-assembly, but has been poorly investigated, particularly for biologic samples. This Letter is devoted to modelling the process of positioning a biomolecule with atomic force microscopy (AFM) in an aqueous media using molecular dynamics simulations. Carbon nanotube (CNT) and graphite sheet are selected as AFM tip and substrate, respectively. To consider the effects of the medium on the manipulation, several models for decreasing the calculations including implicit, coarse grained, and all-atom methods have been investigated. They examined several parameters which may affect the quality of the manipulation process such as the CNT initial position with respect to the sample and substrate, its diameter and positioning strategies (pushing or pulling). The results demonstrate that despite of the implicit solvent methods, coarse-grained model can simulate the aqueous media accurately with lower computational cost. Furthermore, pulling method with a CNT which has a larger diameter and a smaller gap with respect to the substrate is the most appropriate setting for manipulation.

1. Introduction: The bottom-up approach is growing rapidly in the construction of complex nano-entities from smaller blocks. In this approach, direct positioning and manipulation of different components are used to create a whole complex nano-system and can provide more precise and reliable results than self-assembly methods. Atomic force microscopy (AFM) has been evolved into a popular and powerful device in nano-robotics applications due to its capability in imaging and identification of biomolecules characteristics or manipulating them precisely.

There are several experimental studies and have been performed in the field of manipulation of a biomolecule with AFM tip. Pakes *et al.* [1] manipulated a single horse-spleen ferritin (protein that piles up iron for metabolism) by applying scanned-probe methods with mechanical pushing of an AFM tip which showed that mechanical pushing with the tip of an AFM could provide precise positioning. An important issue in manipulation is that the precise control of the tip–sample distance and interaction force can dramatically improve the final position and structure of the sample [2]. For example, Fotiadis *et al.* utilised AFM to cut up the chromosomes at some chosen points, and the complicated movement of biosample made the process hard [3]. Thus, some new techniques for simultaneous detection and manipulation of biomolecules have been investigated by Bustamante *et al.* [4, 5]. They have done experiments on DNA-binding molecular motors as a case study. Another investigation is the combined atomic force and far-field fluorescence microscopic tests which simultaneously facilitates the atomic force control and the optical monitoring of DNA molecules which was reported by Hards *et al.* [6]. This investigation clearly showed the benefit of the manipulation control with the help of a concurrent optical monitoring. In another work, He *et al.* [7] have developed a single-molecule AFM-fluorescence resonance energy transfer (FRET) nanoscopy method which is capable of manipulating a targeted dye-labelled single protein and simultaneously monitoring the structural changes of the targeted protein.

Beside these works on biomolecules, there are several theoretical and experimental studies on manipulating and imaging of nano-particles or nano-clusters. For example, Sitti *et al.* [8, 9] evaluated sticking, sliding, rolling, and rotation (spinning), which are the likely modes of motion in one dimensional pushing/pulling applications.

However, there are other important issues which the experimental devices and techniques cannot capture. On the other hand, it seems necessary before any practical effort to evaluate the mechanical behaviour of the system and modify the experimental setup based on the results of these molecular simulations. Thus, the computational modelling with its great capability to model and predict the molecular features of nano-structures and biosamples attracts the interest of many researchers in biotechnology.

Mechanical modelling of nano-manipulation has been widely researched. The first approaches used are classical models for simulation of tip–sample and substrate–sample interactions. Some of these famous analytical models which have been used in several works are the long-range attractive van der Waals force in addition to Johnson–Kendal–Roberts or Derjaguin–Muller–Toporov for modelling the contact force between particles [10]. However, these models are not precise for nano-metric systems and many researchers have used various other numerical simulations methods such as molecular dynamics (MD) for modelling the interaction between two nano-particles.

For example, Mahboobi *et al.* [11–16] worked on modelling the manipulation process of a metallic nano-particle by an AFM tip. They investigated the effect of many different parameters such as tip and substrate dimensions, materials, positions, and releasing mode on manipulation quality (precise positioning while not damaging the nano-particle) by the MD method. Zhu *et al.* [17, 18] and Zhang *et al.* [19, 20] simulated the scratching process of a substrate by an AFM tip through MD. In another works Pishkenari *et al.* simulated the imaging process of a nano-particle by MD and evaluated the effect of tip-mass, force hysteresis, temperature, higher oscillation modes of cantilever, and setup components flexibility on imaging process quality [21–27].

Furthermore, the flexible complex structures of biosamples calls for using MD for modelling their mechanical behaviour. Thus, many researches have been devoted to modelling different manipulation types on biomolecules with MD. For example, the extension of a biomolecule by an AFM tip [28–32], or applying an indentation force on a HBV virus using the coarse-grained (CG) MD method [33].

Although a considerable progress have been made in the manipulation of biological samples, there are still many topics under debate which are beyond the capability of the present experimental methods and devices. MD, with its significant analysis capability, can reveal the molecular-level features of biological samples in different environments and under variety of conditions. Despite

important role of MD simulations in study of nano-scale phenomena, modelling, and simulation in the field of bio-manipulation is not being addressed in a satisfactory manner. Here we try to evaluate the positioning process of a biomolecule in aqueous media. Here, the AFM tip is considered to be carbon nanotube (CNT) because of its unique properties such as high aspect ratio, low diameter, and high mechanical resistance [34].

One of the most important issues about biomolecule modelling is that they exist in solvent; therefore, we should simulate the system in aqueous media. Since considering aqueous media as all-atom model, makes MD simulations too computationally expensive, we try to evaluate some other methods such as CG or implicit solvent models to simulate the aqueous media with lower computational cost. Our results show that implicit solvent model [generalised born implicit solvent (GBIS)] and solvent accessible surface area (SASA)-based implicit method cannot model the aqueous media correctly, while CG model simulate the whole system properly with a considerable lower computational cost. Then we prepare variety of simulation setups to investigate the effect of some parameters on the manipulation process such as CNT geometry and position, solvent media, and manipulation mode (pulling or pushing).

The rest of this Letter is organised as follows. Section 2 introduces the setup's component and Section 3 presents the setup configuration, the MD parameter of simulations. Section 4 describes the different way of solvent media modelling and evaluates each method's authenticity. Section 5 addresses the results of the simulation and discusses that which parameters of CNT or manipulating mode leads to a successful positioning process and effect of each parameter on sample's deformation. Finally, Section 6 investigates the CNT detaching process from the biomolecule.

2. Setup component: The aim of this research is to study a biomolecule manipulation by a mechanical probe considering aqueous media. We have selected ubiquitin as biomolecule in our simulations. This protein is composed of 76 residues. The structure of ubiquitin is remarkably dense. About 87% of the polypeptide chain is integrated in a hydrogen-bonded secondary structure. Important secondary structural features include a mixed β-sheet that contains five strands, three and one-half turns of α-helix, a short piece of 3(10)-helix, and seven converse turns. Between the α-helix and β-sheet, there is a discernible hydrophobic core formed [35]. Before doing any simulation, minimisation and equilibration procedures must be done on the proteins. For this purpose, ubiquitin is placed in a periodic water box and the system is minimised for 1000 steps by the conjugate gradient method and then equilibrated for 50,000 steps. Here we use CHARMM27 force field to model the biomolecule.

As mentioned before, we use CNTs as the AFM tip. Recently, they have been introduced as effective AFM nano-probes for imaging and manipulating due to their very small diameters, high aspect ratios, and large mechanical strength [34, 36]. Here, we assume three zigzag single walled CNTs with $L_1 = 50$ Å and $D_1 = 7.8$ Å, $D_2 = 15.64$ Å and $D_3 = 23.45$ Å.

The third main component of our system is a graphite sheet as substrate. To achieve a successful positioning, the friction force between the protein and the substrate should be minimum. Graphene has low adhesion to protein unlike silicon and silicon nitride. It also has a tensile strength of 130 Gpa with an ultimate strength 200 times greater than steel. Its Young's modulus is about 0.5 Tpa and its spring constant is approximately in the range of 0.1–0.5 nN/Å [37]. Graphene also has an antibacterial property, which makes it suitable for using it as a substrate in bio-manipulation. Here, we use a two-layer zigzag graphite which its dimension in the *X*-direction is 100 Å and in the *Y*-direction is 200 Å and the lower layer is set to be fixed in the simulations.

3. Configuration of simulation: After merging these components with visual molecular dynamics (VMD) software, we use NAMD for performing the MD simulations in the bio-manipulation process [38]. Fig. 1 shows the whole setup for the pushing mode. To move the CNT atoms at the desired velocity, we use the constant velocity mode of the steered MD (SMD) method, a feature of NAMD software in which atoms are moved at a determined velocity [38]. Here, the atoms located at top 1 nm of CNT are considered to be the SMD atoms. To avoid escaping of water molecules, we apply periodic boundary condition on setup atoms. Other MD simulation parameters are presented in Table 1.

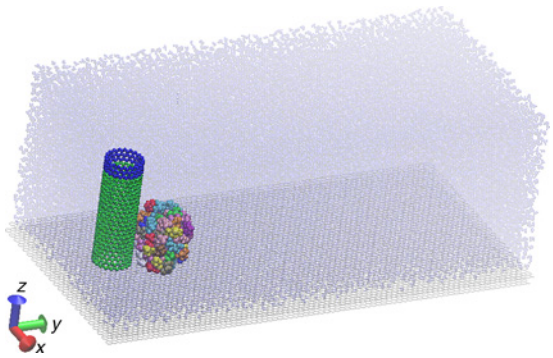

Fig. 1 *Simulation setup. The top 1 nm of CNT atoms (blue atoms) are SMD which are moved with an approximate constant velocity of 2 m/s in the Y-direction*

There are two possible modes of positioning including pulling and pushing modes. In pushing mode, the tip is located behind the sample and pushes it (see Fig. 1). On the other hand, in pulling mode the tip is located in front of the sample, and due to the attractive van der Waals force between the tip and sample, the sample follows the tip's movement.

It should be noted that like all MD simulations, modelling the tip movement with its real speed (about 10^{-5} m/s) is almost impossible. Hence we try to find an optimised speed which is slow enough to predict the qualitative behaviour of the system correctly. Therefore, we have decreased the tip speed from 100 to 0.5 m/s and found that the speed of 2 m/s has an acceptable accuracy.

4. Modelling aqueous media: On the basis of the aforementioned discussion, the most straightforward and accurate method for modelling aqueous media is to consider all molecules explicitly. However, this process is too time consuming. There are several other ways to model the system with lower computational cost. Here, we examine the efficacy of three popular approaches to model the whole setup for our setup, implicit solvent models (GBIS), SASA-based method, and coarse-graining (CG) method and compare the results with all-atom model.

The implicit solvent method tries to take into account the water media effects without considering any water molecules explicitly. Here, we use the GBIS method to model the aqueous media implicitly. Generally, the equation used for systems with electrostatic fields is the Poisson Boltzmann equation (PBE). The GBIS method is an approximate solution for the PBE equation. The electrostatic forces decrease in solvents compared with the vacuum

Table 1 Simulation setup. The top 1 nm of CNT atoms (blue atoms) are SMD which are moved with an approximate constant velocity of 2 m/s in the *Y*-direction

Time step	2 fs
temperature, thermostat	310 K, Langevin
cut-off and pair list distance	12 Å, 13.5 Å
SMD atoms velocity	[0 2 m/s 0]
SMD spring constant	10,000 kcal/mol/Å

doi: 10.1049/mnl.2015.0070

media, and the GBIS method tries to calculate this reduction. Briefly, this method assumes a parameter called the born radius, which is a criterion of how much an atom is exposed to solvent molecules.

Moreover, there has been a new improvement in implicit solvent modelling by adding some terms as SASA terms, which considers a surface tension for modelling the repulsive force of water molecules on setup elements [39]. Thus for more precise modelling we consider both GBIS and SASA terms and hereafter call this model as SASA-based method. However, this method is too time consuming for our simulation, even more than explicit modelling.

The third approach for modelling the system with lower computational effort is to use the CG model for the setup. Here, we use MARTINI force field, which considers each of the four water molecules and each amino-acid strand as a bead. For example, the mass of each water bead is 72 amu and its Lennard-Jones (LJ) parameters are $\varepsilon = 1.195$ kcal/mol and $\sigma = 5.275$ Å [40]. The main problem of this method is neglecting the electrostatics effect. However as we will show later, this estimation does not make great change in the simulation results.

We examined three approaches besides all-atom method to find the optimal method for modelling the system. It is clear that the all-atom method will be the criterion for evaluating the authenticity of each method. The time required for running an MD simulation for one step, on the same computer, is recorded for different methods of modelling. The results show that CG model is the fastest method, while implicit and SASA-based models are the slowest methods. To evaluate the capability of the aforementioned in modelling of bio-manipulation process, the displacement of the protein for a single positioning process is compared. According to the results in GBIS and SASA methods, the biosample follows the CNT displacement while the tip cannot pull the protein in all atom and CG methods. Thus we have concluded that contrary to the implicit and SASA models, the CG model was able to predict the outcome of a specific pulling setup properly. The interesting point is that the GBIS model damages the protein's structure completely, but with SASA method the biosample preserves its structure due to the surface tension terms of SASA model.

However, to deduce a general conclusion about CG method's authenticity, we should examine this model in the greater number of manipulation setups. To achieve a general conclusion about the reliability of the CG model, we examined outcomes of several different simulation setups for both all-atom and CG models and compared their results for a smaller period of time. The results show that the qualitative results of two methods are completely coincident in all of the considered simulation setups. This means if the all-atom model predicts a successful (or unsuccessful) outcome for a simulation setup, the CG model also correctly predicts the same consequence for this setup. However, it is worthy noting that the detailed response of two models slightly differs so that the final position and orientation of the protein slightly differs in two methods. Totally, due to computational efficiency, we use the CG model instead of all-atoms model to consider the solvent media. This choice makes longer periods of time possible, and we can check a single simulation several times (3–5 times) to make sure that the random effect of MD simulations in the initial condition does not affect the accuracy of our results. In the next sections, we evaluate the effect of CNT diameter, the gap between CNT and substrate, and positioning strategy on the manipulation.

Table 2 Assigned parameters for different simulation setups

Setups	Diameter, Å	Gap, Å	Strategy
$CG_{1/2}$	7.8	6	pull/push
$CG_{3/4}$	7.8	12	pull/push
$CG_{5/6}$	15.6	6	pull/push
$CG_{7/8}$	15.6	12	pull/push
$CG_{9/10}$	23.5	6	pull/push
$CG_{11/12}$	23.5	12	pull/push

5. Results: To achieve a successful bio-manipulation process, some of the simulation parameters should be suitably selected. We have investigated influence of some parameters on the success of manipulation process. These parameters are as follows:

- Three tip diameters ($d_1 = 0.78$ nm, $d_2 = 1.56$ nm, and $d_3 = 2.35$ nm).

- Two values for the gap between the tip and substrate ($g_1 = 0.6$ nm and $g_2 = 1.2$ nm).

- Two strategies for the manipulation of the protein by the CNT labelled as 'pulling' and 'pushing'. In the pulling strategy, the CNT is on the front side of the biomolecule, and when the CNT moves forward it attracts and pulls the protein, while in the pushing strategy, the CNT is on the back side of the protein (see Fig. 1), and when the CNT moves forward it repels and pushes the protein forwardly.

Therefore we have 12 different simulation setups. Table 2 shows the tip diameter, tip gap, and manipulation mode for each simulation setup. CG stands for the CG model.

Generally, there are four possible outcomes for the positioning process including successful pulling, unsuccessful pulling, semi-successful pushing, and unsuccessful pushing which will be described in the following section.

In a pulling mode manipulation, the interaction force between the tip and the biomolecule is attractive, and hence the biomolecule is pulled in the manipulation direction. This type of motion leads to a stable handling with a little rotational deviation of the protein. If the attractive interaction force between tip and biosample is strong enough to keep their bonding, manipulation will be successful; otherwise, the bonding will be ruptured leading to an unsuccessful manipulation. As an instance of a successful manipulation, Fig. 2 shows four representative snapshots of the simulation setup CG_9. In this simulation setup, the tip diameter is d_3 and the gap between the tip and substrate is g_1. In the pulling scenario, the interaction force between tip and biosample is mostly attractive meaning that the tip is pulling the biosample along the manipulation direction. On the other hand, water medium applies a negative force on the protein and tries to impede it from an effective handling. Analysis of the interaction force between protein and substrate reveals that the graphene applies a negligible force on the protein. This justifies using a graphene sheet as a suitable substrate for manipulation of biosamples.

When the gap between tip and substrate increases, the applied force on the biosample decreases. This is because only a small part of the tip has a significant interaction with protein. On the other hand, the strength of the van der Waals force between the CNT and biosample decreases for tips with smaller diameters.

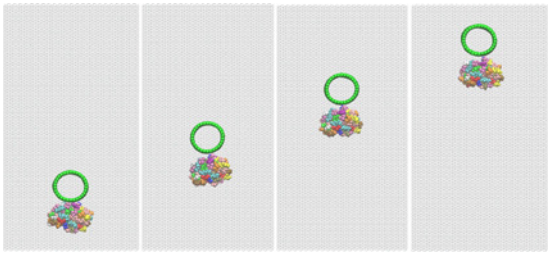

Fig. 2 *Top view snapshots of simulation setup CG_9 at the different times. The protein successfully pursues the tip motion*

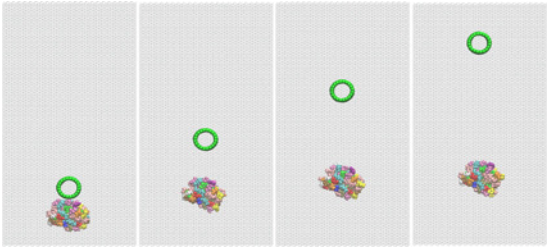

Fig. 3 *Top view snapshots of simulation setup CG_7 at the different times. The protein cannot pursue the CNT (unsuccessful pulling) because of the CNT low diameter and presence of solvent molecules*

Failure to consider some of the attractive contributions between tip and sample may lead to an unsuccessful handling. As an example for this failure, Fig. 3 depicts four representative snapshots of the simulation setup CG_7 showing that the protein does not appropriately follow the tip motion. The connection between tip and protein is broken approximately at $t = 1.2$ ns and hence the tip–sample interaction drops to zeros after this time. In this case, the protein stays behind the tip and does not pursue the CNT motion. In this simulation setup, the tip diameter is d_2 and the gap between the tip and substrate is g_2. As it will be presented later, for simulation setup CG_5 with the same tip diameter and a lower gap with respect to CG_7, we will have a successful manipulation.

The third and fourth possible scenarios are positioning the protein using pushing strategy. The conducted simulations illustrate that the pushing handling mode is converted to a pulling mode which may be successful or unsuccessful. Hereafter, we name the successful pushing mode as 'semi-successful' manipulation because the protein does not track exactly the tip trajectory, and in fact after rotation of protein around tip, it can pursue the tip motion. In this case, when the sample goes from front of the tip to its backside, an error $(D + W)$ will be present compared with the desired motion where D is the CNT diameter and W is the sample width.

Fig. 4 represents four representative snapshots of the simulation setup CG_6 at different time steps, showing that the protein rotates during manipulation and gradually goes to the back side of the tip and hence the pushing mode is transformed to the pulling mode. In this simulation setup, the tip diameter is d_2, the gap between the tip and substrate is g_1.

Analysis of the interaction forces for simulation setup CG_6 shows that at initial moments of manipulation ($t < 4.1$ ns) CNT applies a negative force on the protein. This means that the tip–sample interaction is in attractive region. This result might seem strange; however, our analysis shows that due to the presence of water molecules between the CNT and sample, the distance between tip and protein is relatively large preventing them from development of a repulsive force. Therefore, the tip–sample interaction is attractive such as the pulling modes. Totally, the CNT pushes that intervening water molecules and they push the biomolecule forward. The

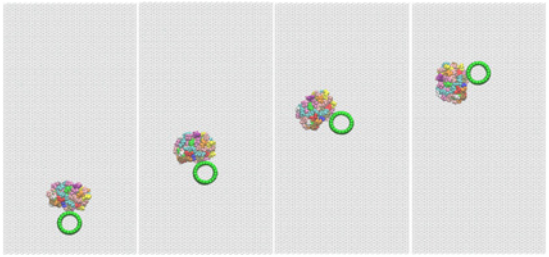

Fig. 4 *Top view snapshots of simulation setup CG_6 at the different times. The protein rotates around the tip and the manipulations change from pushing mode to pulling mode (semi-successful pushing)*

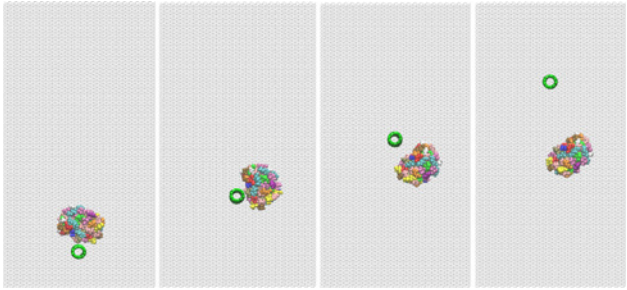

Fig. 5 *Top view snapshots of simulation setup CG_4 at the different times. The protein rotates around the tip and the biomolecule cannot pursue the CNT (unsuccessful pushing) because of the low CNT diameter, large gap between CNT and substrate, and presence of solvent molecules*

significant positive sample–water interaction force approves this point. Further force analysis shows that for $t > 4.2$ ns, CNT applies a positive force on the biosample. This positive interaction force rises from the fact the biomolecule has rotated around the CNT and the manipulation mode is converted to pulling.

Fig. 5 shows an unsuccessful pushing setup where the tip cannot carry the sample because CNT diameter is small (d_1) and the gap between tip and substrate is large (g_2). As it can be seen, this pushing setup is converted to the pulling mode and then the protein loses its connection with CNT and cannot pursue the CNT motion, just such as the second scenario. This setup was expected to be the worst case because of the lowest tip diameter and highest gap. This issue can be confirmed by studying the tip–sample interaction force which in this case is the lowest among all cases. Thus the CNT loses its connection with the sample immediately after mode conversion.

Results of all 12 simulation cases are summarised in Table 3. In this table, the displacement error in x and y directions, angle of rotation of protein in the Z-direction, the average of the absolute interaction force between tip and protein, mean of root-mean-square deviation (RMSD) of protein during manipulation, and change of protein radius of gyration (R_g) are listed for the different simulation setups. A simulation setup is appropriate if the displacement errors in y and x directions in addition to protein rotation are small, the average value of the absolute interaction force between tip and sample is large (while the small interaction forces between protein and substrate are desired), the mean value of RMSD is low, and the change of R_g is small. It can be seen in Table 3 that the CNT diameter and position have a great influence in the manipulation process. Generally as we increase the CNT diameter or decrease its gap, the chance of a successful manipulation increases. In pulling strategy, simulation setups CG_5, CG_9, and CG_{11} are successful due to their larger tip diameters and lower gaps.

As mentioned before, all of the cases with the pushing strategy are converted to pulling-based manipulation because of the stable nature of the pulling mode and the unstable nature of the pushing mode. As the CNT diameter increases or its gap decreases this mode conversion occurs sooner. In most cases of pushing setups (all cases except CG_2), after mode conversion the results are similar to the pulling mode results. Some part of the displacement error depicted in Table 3 rises from the fact that the pushing mode has been changed to the pulling mode. As mentioned previously, this virtual error is more notable for larger CNTs. The chance of successful positioning might be a little more in pushing strategy because the sample has gained a velocity in the direction of tip motion during mode conversion.

Table 3 shows that increasing the tip diameter and decreasing the gap, increases the tip–sample interaction forces leading to a stable and successful manipulation. On the other hand, the small interaction forces between the biosample and the graphite sheet facilitate the manipulation process due to small resistant force on the

doi: 10.1049/mnl.2015.0070

Table 3 Summary of simulation results

Setup	X error, Ang	Y error, Ang	Sample rot, deg	Mean of $f_{\text{UBQ-CNT}}$, nN	RMSD, Å	Change of R_g, Å
CG1	0.51	−92.34	34	0.00381	1.85	−0.15
CG2	14.04	−39.95	76	0.0594	2.89	0.09
CG3	1.36	−85.60	33	0.2451	2.33	−0.15
CG4	12.33	−71.57	113	0.0862	2.03	0.11
CG5	−2.44	−1.32	10	0.2756	1.97	−0.08
CG6	−19.7	−29.48	−106	0.1693	1.82	0.14
CG7	1.76	−89.46	12	0.0227	2.34	0.15
CG8	9.00	−58.73	95	0.0618	2.03	−0.02
CG9	1.74	−2.58	−14	0.3648	2.08	−0.01
CG10	−12.5	−6.50	−42	0.4424	2.38	0.1
CG11	−1.79	2.99	19	0.0727	2.72	−0.11
CG12	−12.8	−10.51	−102	0.2331	1.98	−0.06

biomolecule. According to the results, the negligible interaction forces between sample and surface justifies using graphene sheet as an appropriate substrate for manipulation of biological samples.

As mentioned earlier, one important criterion for evaluation of success of a manipulation setup is the sample deformation. Owing to their flexible structure, the biomolecules may be easily deformed. Here we have measured the biosample's deformation via two criteria: RMSD and radius of gyration. As it can be seen in Table 3, RMSD and R_g change are low, demonstrating small deformations of the protein. This is probably due to the low interaction force between the substrate and the biomolecule. The exerted force from the CNT to the biomolecule causes a low tension on the sample, because there is a negligible force from the substrate from the opposite direction. It is worthy noting that in our manipulation scheme, due to small spatial conformations of the protein during displacement, for evaluation of success of a manipulation setup, these two criteria are less important than the other criteria.

6. Conclusions: One of the most important issues in nano-robotics is nano-assembling and positioning particles to the desired locations. Recently, AFM has been evolved to a versatile tool for imaging, stretching, and handling of biomolecules. Despite considerable progress in the manipulation of the biomolecules, positioning the nanoscale biomolecule on the surface has not been addressed in a satisfactory manner. Since the word of 'manipulation' has different uses, it may seem there are a lot of works in the field of the current Letter. However, most of the previous works discussing the manipulation of biosamples exert a force on the sample to identify its different mechanical and morphological characteristics. In contrast, in this Letter, manipulation means applying external force for positioning the biomolecule in a controllable manner.

Owing to restrictions in capability and scope of the experimental techniques, there are certainly many questions needed to be answered. On the other hand, owing to the smallness, incomprehensive, and expensive experiments at the nanoscale, reveal the need to the molecular behaviour simulation before each kind of study. Owing to the complex dynamics of tip–sample interactions, in this research, MD simulations are used to investigate the manipulation of biomolecules by AFM tip. Here we examined a biomolecule as the sample, a CNT as the AFM tip, and a graphite sheet as the substrate. Some of the main conclusions of the current study are as follows.

Owing to negligible interaction force between the biosample and the graphene sheet, the biosample can easily follow the CNT movements, even in pulling mode (because of tip–sample attractive force). Thus, we examined the positioning process for pulling and pushing modes and deduced that due to instability nature of the pushing mode, this type of manipulation is always converted to a pulling mode, and after mode conversion the success of positioning is similar to its corresponding pulling mode.

In a real-world application, the biosample exists in an aqueous media. Therefore, it seems necessary to evaluate the effect of solvent media on the manipulation process. Thus we modelled the manipulation in an aqueous media. On the other hand, because of the high computational cost of MD simulations we tried to find a faster solution to model the manipulation correctly. Therefore, we examined the implicit solvent (GBIS-SASA) and CG (CGMD) approaches and compared them with the results of all-atoms (explicit) modelling. Results show that both implicit models, meaning GBIS and GBIS + SASA, are not capable to correctly predict the biomolecule displacement, but SASA terms at least can preserve the structure of the biomolecule properly. Contrary to the mentioned implicit methods, the CGMD modelling has the same qualitative results with respect to all-atom model in different cases. Thus, here we used the CG model.

As we expected, the success of a case was directly pertinent to the CNT diameter and its gap with respect to the substrate. Generally, as we increase the CNT diameter or decrease its gap, the chance of a successful handling increases and the mode conversion from pushing to pulling occurs sooner.

The interaction force between the graphene substrate and biosample is negligible in comparison with the CNT–sample forces. The CNT–sample interaction force for pulling mode is attractive, so the CNT can pull the sample. In pushing modes, the tip–sample distance is relatively large and hence their interaction is in the attractive region of van der Waals force. This large distance is due to the intervening water molecules which are located between the CNT and sample. However, as the tip pushes the water molecules, they push the biomolecule. Thus there is a significant water–sample repulsive interaction force which pushes the sample forward even in the existence of attractive tip–sample interaction force.

These findings can be used for manipulation and positioning of biomolecules in real media.

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doi: 10.1049/mnl.2015.0070