Multi-Scale Simulation of an Immobilizing F$_1$-ATPase Molecular Motor

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Immobilization, including specific attachment, positioning and orientation, is essential for the integration of F$_1$-ATPase molecular motors into nanoelectromechanical systems (NEMS). The stability of the immobilization is a very critical technology to prolong the functional lifetime of the molecular motor. Hybrid quantum mechanics and molecular mechanics (QM/MM) and classical molecular dynamics (MD) simulations were separately implemented to investigate the immobilization of the F$_1$-ATPase molecular motor on different substrates. The QM/MM simulation was employed to give insight into the interaction between the (Histidine)$_6$-tagged peptide and the nickel-nitrilotriacetate (Ni-NTA). The analysis of the charge redistribution revealed that the Ni ion increased the negative charge on the nitrogen atoms of imidazoles, resulting in a strong chemical bonding. The maximum torque that the His-tagged peptide could apply to the rotary molecular motor was estimated from the results of the MD simulation. The immobilization stability was analyzed and is discussed.

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I. INTRODUCTION

The immobilization of biomolecules on substrates is widespread in nanotechnology and is becoming a potentially important prerequisite for the application of molecular devices, such as molecular motors, biosensors and bioelectronics [1]. The bio-adhesion issues in the nanoelectromechanical systems (NEMS) rely on immobilization of biomolecules. In order to integrate molecular devices into NEMS, functional biomolecules are used extensively in specific attachment, selective linkage, precise positioning and orientation [2–6].

Many immobilization methods are based on the ability of consecutive histidine (His) residues chelating to metal ions. Hochuli et al. [2] applied His tags in selective binding proteins when they discovered that proteins containing isolated His led to complexes that were less stable than those arising from proteins having two consecutive His tags at one terminus ((His)$_2$-tag). On this basis, a stretch of six consecutive His tags ((His)$_6$-tag) was commonly tagged to the primary sequence of proteins, making it possible to immobilize molecular motor or biosensors for biomolecular interaction analyses [3]. Because of the very high affinity for Ni-NTA, poly-His tags were linked to the N-terminus of the $\beta$-subunit to permit tight binding of the F$_1$-ATPase molecular motor to Ni-NTA [4]. They found that the poly-His tags were bound so tightly to the Ni-NTA-coated coverslip that the $\alpha_3\beta_3\gamma$ subunit was immobilized even under the high torque (~40 pN·nm) produced by the rotating actin filaments. In order to specifically immobilize F$_1$-ATPase molecular motors (Figure 1) to selectively interface with nanofabricated structures, Montemagno et al. [5, 6] used His-tags combined with a $\beta$-subunit cod-
ing sequence attached to a nanofabricated Ni cap with a precision greater than 30 nm. 
Agarwal et al. [7] demonstrated that poly-His-tagged peptides and proteins could be chelated to ionized regions on a metallic Ni surface by applying an electric potential to the atomic force microscope (AFM) tip in the dip-pen nanolithography (DPN) process while no deposition of the His-tagged peptide was observed on the Ni surface with zero potential. They verified the (His)$_6$ tags could only be deposited on the ionized region of the Ni surface. The bond rupture forces of (His)$_6$ on a number of different surfaces were measured as a function of the loading rate [8,9]. Their results showed the attachment of (His)$_6$ on a metallic Ni planer surface could be applied for immobilization and specific orientation in hybrid NEMS. There was a slight disagreement between the above two groups in the experiments. The molecular forces of the coordination bonds of the Ni$^{2+}$-NTA/His-tag complex at a constant loading rate of 0.5 μm/s were obtained by Schmitt et al. [10]. The Ni-NTA protein separation process was simulated by reproducing the approach: binding, stretching and unbinding under external forces between individual His tags and individual Ni-NTA chelating groups [11]. The chelator lipid concept with NTA functionalized lipids [12] gave insights into the functional aspects of immobilized proteins [13]. Multivalent interactions between the (His)$_6$ tags and the NiNTA groups were demonstrated in experiments, which involved immobilization of (His)$_6$-tagged proteins on chelating lipid membranes at different surface concentrations [14]. High affinity chelator thiols for switchable and oriented immobilization of His-tagged proteins were developed [15]. The immobilized His-tagged proteins are uniformly oriented and can be removed from the chip surface under mild conditions (switchability). These experiments indicate that nitrogen donor atoms of imidazoles chelate to Ni ions and form strong bonds (Figure 2). The polypeptides can specifically bind to inorganic materials, which could provide new opportunities for motor-driven nanoscale assemblies of different materials [16]. Applications of such technologies in single molecule operation and bio-NEMS integration are increasing gradually and have an optimistic prospect [16,17].

In this paper, a multiscale simulation method, including quantum mechanics and molecular mechanics (QM/MM), was employed to give insight into the (His)$_6$-tagged synthetic peptide chelating to Ni-NTA. Based on our previous work [18], the integrated molecular orbital/molecular mechanics (IMOMM) [19] scheme was used to deal with the partition in the multi-scale simulation. With this method, the active part of the system was treated with molecular orbital (MO) methods and the rest with MM. The high affinity was analyzed according to the Mulliken charge population. Classical MD simulations were also implemented to study the (His)$_6$ tags adsorption on the neutral Ni surface and on the charged Ni surface separately for comparison. The maximum torque that the His-tagged peptide can apply for the immobilization was estimated to be ~10$^4$ pN nm, which was far more than the torque induced by ATP hydrolysis.

II. SIMULATION

The His-tagged peptide studied in this paper consists of fourteen amino acid residues, including six glycines (Gly), two lysines (Lys) and six histidines (His) with the following sequences: Gly-Gly-Lys-Gly-Gly-Lys-Gly-(His)$_6$ [5]. The linkage between His tags and Ni-NTA involves a coordination bond with a chemical reaction. The QM/MM method was employed to partition the peptide-Ni-NTA system into a QM subsystem and a MM subsystem (Figure 3(a)). The Ni$^{2+}$ ion and the surrounding atoms (Figure 3(b)) are selected as QM atoms, the rest as MM atoms. Then, the boundary problem between the QM and the MM subsystems was treated with the IMOMM approach, which was embedded into the GAMESS [20]/TINKER [21] package. With the prepared systems, full geometry optimizations and energy calculations were performed at the B3LYP (6-311+G$^*$)/MM level. For the QM subsystem, the criteria used for geometry optimizations follow the GAMESS
Fig. 3. (a) His-tagged peptide chelates to Ni-NTA. The highlighted atoms are QM atoms and the rest are MM atoms. (b) QM atoms of the QM/MM simulation.

Table 1. The Mulliken charge population and valence on the main QM atoms. The nitrogen atoms N2 and N3 belong to His-tags. O1, O2, O3 and N1 are the nearest atoms to the Ni$^{2+}$ that belong to NTA molecule.

<table>
<thead>
<tr>
<th>Mulliken charge</th>
<th>Total valence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Isolated</td>
</tr>
<tr>
<td>Ni ion</td>
<td>0.608</td>
</tr>
<tr>
<td>O1</td>
<td>−0.880</td>
</tr>
<tr>
<td>O2</td>
<td>−0.867</td>
</tr>
<tr>
<td>O3</td>
<td>−0.836</td>
</tr>
<tr>
<td>N1</td>
<td>−0.934</td>
</tr>
<tr>
<td>N2</td>
<td>−0.230</td>
</tr>
<tr>
<td>N3</td>
<td>−0.230</td>
</tr>
</tbody>
</table>

default. For the MM minimization, the convergence criterion used is that the root-mean-square (RMS) energy gradient is less than 0.001 kcal/mol Å$^{-1}$. The MM3 all-atom force field in TINKER was used in the MM calculation with no cut-off for nonbonded interactions, which incorporated a second generation force field technology and was the most accurate force field known for generating molecular geometries and energies.

To understand the interaction between the His tags and the metallic Ni planar surface, we carried out classical MD simulations to simulate the (His)$_6$ tags adhesion on the substrate. The difference in adhesion between the neutral surface and charged surface was compared. For the peptide, the standard amino acid residue topology and the parameters based on the CHARMM all-atom parameters were used. The Lennard-Jones parameters of Ni atoms were selected as $\varepsilon = 6030 K_B$ deg., $\sigma = 2.282$ Å [22]; here, $K_B$ is the Boltzmann constant. The MD simulations were done by employing the code CHARMM 33b2 [23] and the leapfrog algorithm [24] was used for integrating the Newtonian equations of motion for each atom, with a time step of 1 fs. The periodic boundary condition using image conventions was applied in calculating the nonbonded interactions. With a nonbond cutoff of 12 Å, the nonbonded pair list was updated every 10 steps and the nonbonded interaction energies and forces were smoothly shifted to zero at 10 Å. For the initial configuration, the peptide was placed on the metal surface with a mean distance of about 6 Å. Then, the system was equilibrated with a 200-ps dynamics simulation at a temperature about 300 K. The temperature was checked every 100 steps and was adjusted by scaling the velocities only if the average temperature of the system was outside the range 300 \( \pm 10 \) K. Thus, the average temperature was maintained at 300 K with a NVT ensemble.

To mimic the loading in molecular rotary motion, we pulled the N-terminal of the peptide to the height of about 2.3 nm above the substrate with the His-tags still being adsorbed on the substrate and with an additional 30-ps equilibration. The F$_1$ molecular motor rotates counter-clockwise (Figure 4). In the following simulation, the N-terminal of the (His)$_6$-tagged peptide was controlled to hold this height, which could be considered as the linkage with the rotary molecular motor. The external force $F$ was applied at the point A in the tangential direction. $F$ was considered as the force the molecular motor applied to the His-tagged peptide. During the loading simulation, the system was equilibrated for 1 ps after a 1-ps loading, which could decrease the loading rate effect. The average value of $F$ was recorded every 1 ps in the data collection process. The images of the MD...
results were processed by using the VMD software [25].

III. RESULTS AND DISCUSSION

The binding energy between the His-tagged peptide and Ni-NTA was defined as

\[ \Delta E = E_{\text{tot}} - E_{\text{pep}} - E_{\text{Ni-NTA}} \]

where \( E_{\text{tot}} \) is the total energy of the complex and \( E_{\text{pep}} \) and \( E_{\text{Ni-NTA}} \) are the energy of the isolated peptide and Ni-NTA, respectively. The binding energy of His-tagged peptide interaction with Ni-NTA is obtained to be -286.810 KJ/mol, which is lower than the binding energy of peptide-Ni\(^{2+}\) systems (-247.913 KJ/mol) [18]. This result shows that the peptide-Ni-NTA system is more stable than the peptide-Ni\(^{2+}\) system. The Mulliken population analysis (MPA) in Table 1 shows that the nitrogen atoms N1 and N2 in the imidazoles receive more negative charges after chelating with the Ni-NTA. The nitrogen atom in the imidazole of histidine has one pair of electrons, which can be donated to a metal ion, especially a transitional metal ion (Figure 2). It can be deduced that the negative charges transfer to the nitrogen atoms in the chelation process and form strong coordination bonds with the Ni\(^{2+}\) ion. The valence results also show that the QM atoms (Figure 3(b)) become more saturated in valence in the chelation, which also verifies the binding strength.

The biomolecular attachment to the solid substrates has been investigated by using MD simulations [26-28], in which the DNA or protein molecules were pulled off from the substrates to obtain the adhesion force. In this paper, we implemented the MD simulation to explore the immobilization region of the rotary molecule motor. Figures 5 and 6 show the removed processes on different surfaces for comparison. We define the sequence Gly-Gly-Lys-Gly-Gly-Lys-Gly-Gly as S1 and (His)\(_6\) as S2 (Figure 4). On the neutral surface, the angle between the S1 section of the peptide and the substrate is about 41.5° and changes to about 38.0° on the charged surface. It is shown the S2 section kept the status of attachment in the removed processes. The relationship of the critical shear forces to the time to remove the His-tagged peptides from the surface is obtained and illustrated in Figure 7. The force for the charged Ni surface is about 4
times the force for neutral surface. The maximum torque that one His-tagged peptide can apply to the molecular motor is defined as

$$M = F \times R,$$

where $R$ is the radius of the molecular motor, as shown in Figure 4 and $F$ is the minimum shear force to remove the His-tagged peptide from the substrate. For the F$_1$-ATPase molecular motor, $R$ is about 10 nm [29,30]. $F_{\text{neutral}}$, the force $F$ for the neutral surface, is about $2 \times 10^3$ pN and $F_{\text{charged}}$, the circumferential force $F$ for the charged surface, is about $8 \times 10^3$ pN (Figure 7). Thus, the maximum torque the His-tagged peptide can apply for the rotary molecular motor can be estimated to be $M_{\text{neutral}} \approx 2 \times 10^4$ pN-nm and $M_{\text{charged}} \approx 8 \times 10^4$ pN-nm while, for the F$_1$-ATPase molecular motor, the total torque $M$ of the propellers’ rotation can be calculated by using the following formula [6]:

$$M = \frac{4\pi \mu \omega L^3}{3\cosh^{-1}(h/r)},$$

where $\mu$ is the viscosity of the medium ($10^{-3}$ N-m$^{-2}$-s), $h$ is the height of the cylinder’s axis relative to the surface (200 nm), $r$ is half the width of the propeller (75 nm), $\omega$ is the angular velocity and $L$ is the length of the propeller extending from the rotational axis. The experimental results show that the torque of the propeller is $\sim$40 pN-nm [4,6,31], which is far less than the results estimated by the MD simulation.

The electrochemical DPN (E-DPN) experiment [7] suggested that attachment of His-tagged peptides/proteins to a metallic Ni surface requires ionization of the nickel surface. The calculation results also show that the attachment of the His-tagged peptide to the charged Ni surface is more reliable than to the neutral surface. The bond rupture forces of (His)$_6$-Ni on a number of different surfaces have been measured as functions of the loading rate [8]. Their data indicate that the bond length of (His)$_6$-Ni is greater than that of (His)$_6$-Ni-NTA. It can be inferred that bonds to nickel are weaker.

Based on our simulations, it is the charge redistribution in (His)$_6$-Ni-NTA interaction that leads to the strong chemical linkage. A charged surface improves the adhesion strength, which is expected to result in stronger devices with longer functional lifetimes.

IV. CONCLUSION

In summary, the hybrid QM/MM method was employed to study the His-Ni-NTA system, which could specifically immobilize a F$_1$-ATPase molecular motor on a substrate. The Mulliken charge population shows the nitrogen atoms in the imidazole of histidines obtain more negative charges after interaction with Ni-NTA, which results in a strong coordination bond. It is interesting to note that cations such as Ca$^{2+}$ and Mg$^{2+}$ do not influence the ability of the His-tag to bind to the NTA complex [10]. Because of these advantages, the His-Ni-NTA system has been used to immobilize fusion proteins at different interfaces. It can be expected to play crucial role in the development of hybrid nanomechanical machines and devices.

The maximum torque that the His-tagged peptide can apply to the rotary F$_1$-ATPase motor was estimated by using classic MD simulations. The calculation results also show that the attachment of His-tagged peptide to the charged Ni surface is more reliable than that to the neutral surface. Experiments [9] have verified that three metal substrates, including Ni, are suitable for attachment of biomolecules and are compatible with current nanofabrication techniques. Based on previous experiments [7,30] and our simulation, we suggest that the ionized Ni surface is more suitable and reliable for immobilization in hybrid NEMS devices integration. With the prerequisites of molecular biology and nanofabrication technology, the ability to create hybrid organic/inorganic NEMS will open the door for seamless integration of NEMS with living systems.

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