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PRELIMINARY STUDIES OF INTERACTION BETWEEN NANOTUBES AND TOLL-LIKE RECEPTORS

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Abstract: Toll-like receptors (TLRs) are a group of proteins which play a crucial role in the innate immune system. The main function of TLRs is to recognize structurally conserved molecules, which are inserted to the organism of the host by microbes, and then to activate the immune response.

Current development of drugs is often connected not only with the drug itself, but also with the way it is delivered into the human body to interact directly with the source of the problem. Carbon nanostructures, particularly nanotubes, are one of the carrier molecules of the future. However, there is still no knowledge about the exact mechanisms of toxicity and possible interactions with macromolecules, such as proteins. In our study we tried to determine, if the nanotubes could interfere with the innate immune system by interacting with TLRs. For this purpose, we used the following TLR structures downloaded from the RCSB Protein Data Bank: TLR2 (3A7C), TLR4/MD (3FXI), TLR5 (3V47), TLR3 (2A0Z), and the complexes of TLR1/TLR2 (2Z7X) and TLR2/TLR6 (3A79). The preliminary results of our Steered Molecular Dynamics (SMD) simulations have shown that nanotubes interact very strongly with the binding pockets of some receptors (*e.g.* TLR2), which results in their binding to these sites without substantial use of the external force.

Keywords: CNTs, toll-like receptors, molecular dynamics

1. Introduction

Toll-like receptors (TLRs) (Figure 1 a) are a group of proteins which play a crucial role in the innate immune system. TLRs are monomeric receptors placed in a lipid bilayer. Usually they are expressed in sentinel cells, such as macrophages

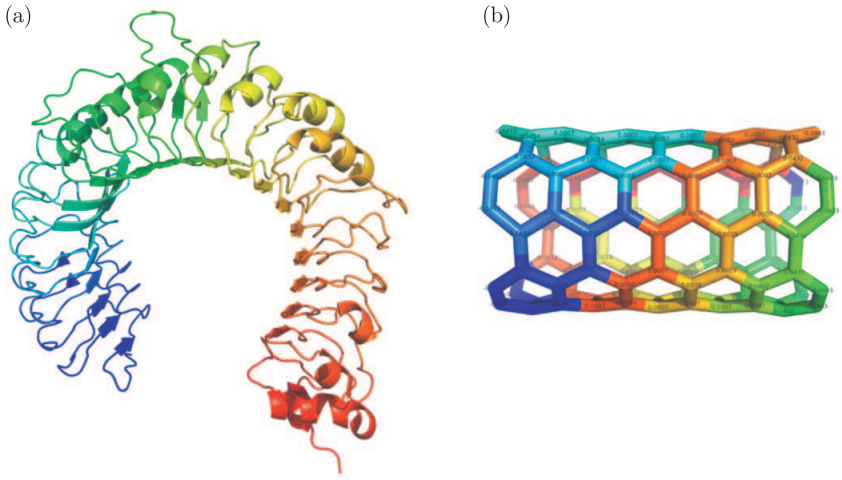

Figure 1. (a) Example of Toll Like Receptor: TLR2; (b) CNT after short MD simulation (partial charges shown in the pictures)

and dendritic cells. TLRs recognize structurally conserved molecules, which are inserted by microbes [1]. When pathogens cross the barrier (*e.g.* skin), they are recognized by TLRs, which activate the immune response of the body [2].

One of the goals of modern medicine is to create better drugs and drug carriers. A recent idea has been to use modern materials, such as carbon nanotubes (CNTs), as carriers (Figure 1 b). However, as CNTs can interact with some proteins in organisms, there is concern about the immunotoxicity of nanoparticles. There are many studies claiming that CNTs can be toxic [3], but in most cases, it is only the mechanisms of cell membrane penetration or lung diseases that are considered. Although CNTs are rather low-reactive compounds, the edges (ends) can be highly reactive in the open-end form [4], and can be spontaneously passivated by hydrogen atoms in proper conditions.

The goal of this study was to provide data assessing the safety of CNTs as drug carriers. In this study the armchair type of Single-Wall Nanotube (SWNT) was parameterized using the *ab-initio* methods implemented in the R.E.D. server. Several series of Molecular Dynamics (MD) and Steered Molecular Dynamics (SMD) simulations with different pulling velocities were performed to investigate the affinity of CNTs to TLRs. We used the AMBER12 force field to investigate the interaction of TLRs and CNTs [5]. During the binding of a CNT to TLR2 it could be observed that some structural changes in the binding region were required to fit a CNT in the binding pocket of the receptor.

2. Methods

We used the AMBER12 program package with the latest, ff12SB, force field to investigate the interactions of TLRs with CNTs. At the beginning, we performed MD simulations of the TLR2 protein (PDB: 3A7C) to relax the structure and equilibrate the TIP3P water molecules (500,000 steps, each of 2 fs – 1 ns in total).

In the next step we parameterized the CNT by using the *ab-initio* methods [6] implemented in the R.E.D. server [7]. In particular, the partial charges were determined from the molecular electrostatic potential calculated with the HF/6-31G* function basis set. The stability of the CNT was assessed by MD simulations (Figure 1 b).

In the next step, the CNT was docked to the TLR2 receptor by carrying out several consecutive SMD simulations (two trajectories for each set of conditions) with different velocities (~ 34 nm/ns and ~ 7 nm/ns), models of water (explicit TIP3P water model or implicit water model) and different pulling forces (1, 2, 5, 5000 kcal/mol*Å^2). The simulation time varied from 1 to 5 ns. The SMD simulations enabled us to reduce the long real time required for the process to occur and to examine the putative pathways in reasonable computational time. Simulations in different conditions enabled us to observe the occurrence of quasi-stable conformations during the simulations.

3. Results and Discussion

The MD simulations of CNT using optimized parameters from the R.E.D. server (optimal torsional angles were changed to the theoretical values) showed that new parameters did not cause any damage to the structure during the simulation and that the CNT was stable.

After performing the SMD simulations of TLR2 with CNT we preliminary analyzed them and we were able to observe that some structural changes in the binding region were required to fit a CNT in the binding pocket of the receptor (Figure 2). We found that the CNT interacted strongly with the binding pocket

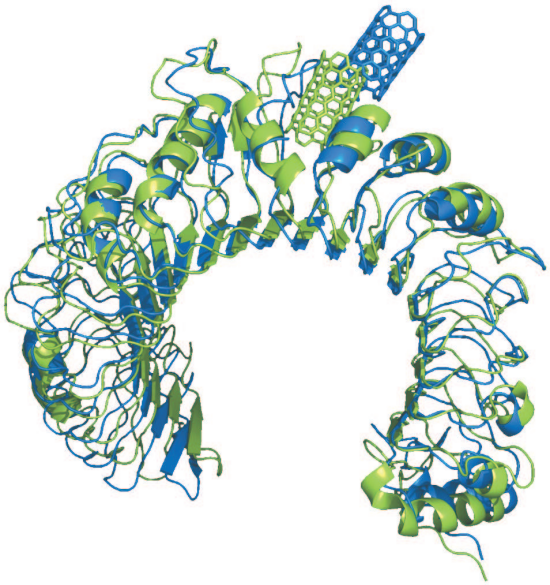

Figure 2. TLR2 receptor with CNT before simulation (blue) and after 1 ns of SMD simulation (green)

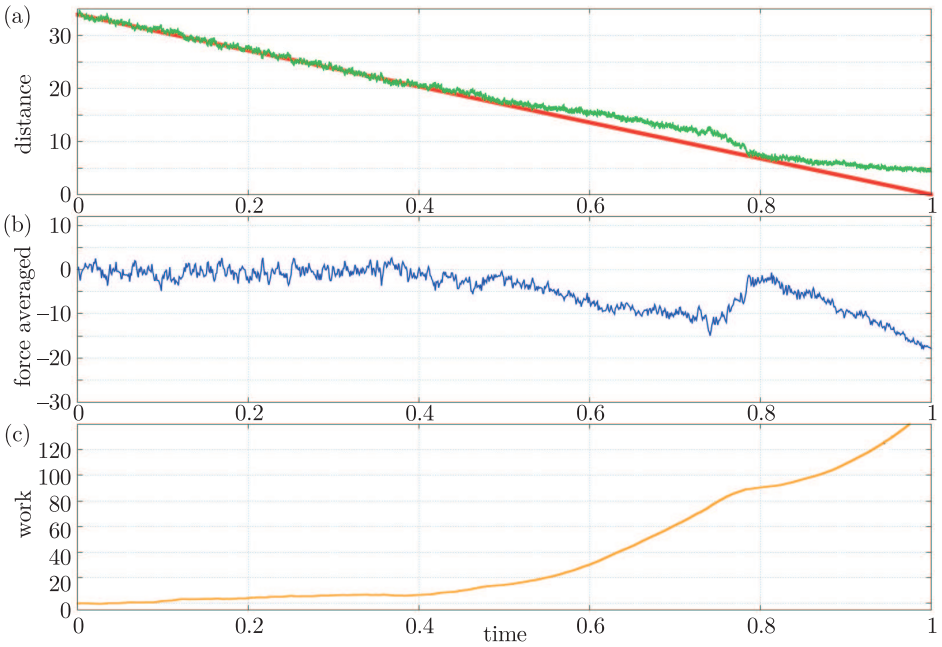

Figure 3. Example plots from SMD simulation of TLR2 and CNT (a) plot of distances between CNT and pocket binding [Å] vs time [ns], red line represent the theoretical distance (for restrained simulation) and the green line represents the real changes of distances during simulation; (b) plot of used force [kcal/mol·Å^2] vs time [ns], where force is equal to $-kx$; (c) plot of work [kcal/mol·Å] vs. time [ns]

of the receptor, which resulted in their binding to these sites. During the binding of a CNT to TLR2, it could be observed that there was an optimal position of the CNT in the pocket around 7 Å from the bottom of the pocket (Figures 2 and 3).

4. Future plans

We plan to simulate the TLR1/TLR2 complex (PDB: 2Z7X) with CNTs of different size and hydrogen passivation at edges. We will use the same procedure as for the monomeric protein (MD simulations to obtain the relaxed structure, then docking, using SMD simulations in different conditions; the initial conformation of the TLR1/TLR2 complex with CNT is shown in Figure 4).

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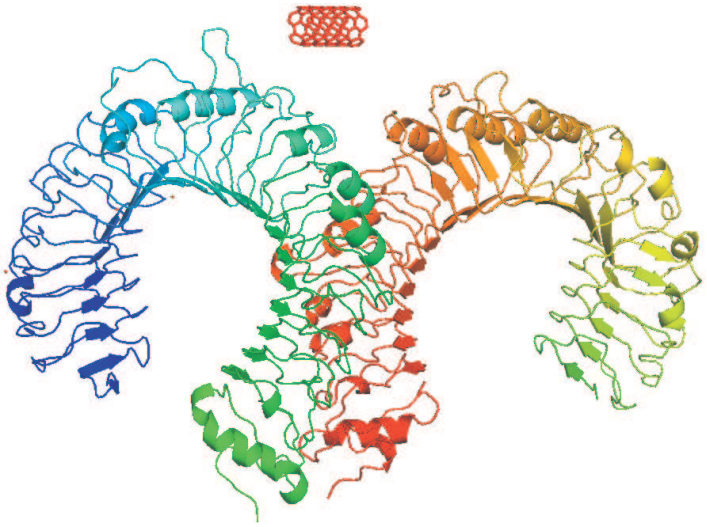

Figure 4. Initial structure of TLR1/TLR2 receptor complex with CNT (before SMD simulations)

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