Bionanobots for Drug-Delivery

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ABSTRACT

In this work we report a simulation of a bionanobots, for treatment of tuberculosis. Across molecular dynamics we simulate dendrimers of three generations with different pH conditions. We describe the specifics of the complexation and encapsulation of rifampicin inside dendrimer. We also developed an algorithm for coupling molecules inside dendrimers in optimal positions. These results were validated across experimental results of our group not showed here.

Keywords: Drug delivery, molecular modeling, dendrimers, nanotechnology, Tuberculosis.

1 INTRODUCTION

Tuberculosis is an infection, primarily in the lungs, caused by bacteria called ***Mycobacterium tuberculosis.*** This disease has 9,2 million new cases and 1.7 million deaths per year [1]. In recent years the control of tuberculosis has become a global challenge due the emergence of multi-resistant tuberculosis and your association with infection by HIV. The fact that some patients have HIV infection or the possibility of later coinfection with HIV has the potential to make this global HIV/Tuberculosis epidemic untreatable with current therapy. The tuberculosis infection with bacteria occurs when patients inhale minute particles of infected sputum from the air. The bacteria gets into the air when someone who has a tuberculosis infection coughs or sneezes making this disease of easy transmission.

Tuberculosis is treated with a cocktail of three drugs: isoniazid, rifampicin and pirazinamide. The rifampicin is a drug of primary line for tuberculosis treatment. The mechanism of action of rifampicin is to inhibit ***Mycobacterium*** transcription by targeting DNA-dependent RNA polymerase [7]. During the infection with tuberculosis, if the patients have rifampicin resistance, then the probability of resistance of other drugs is great. With the advent of nanotechnology is now possible to build drug nanocarriers. These systems deliver the drug directly into an organ of interest thereby reducing side effects arising from the toxicity of the drug. The most promising of these nanocarries is called dendrimers[2]. These molecules can form noncovalent complexes with pharmaceutical compounds and act as vehicles for controlled-release[11]. A particular class of such molecules namely poly (amidoamine) dendrimers (PAMAM) that has been widely considered for biomedical applications[9][10]. Properties such as biocompatibility, water solubility and versatility in modify your functional groups render these molecules appropriate for such uses. PAMAM dendrimers have primary amine groups at each branch end and tertiary amine groups at each branching point. When the generation of dendrimer increases, the number of primary amine increases exponentially (Table 1).

Generation	Atoms	Primary Amines	Tertiary Amines
0	84	4	2
1	228	16	6
2	516	32	14
3	1092	64	30
4	2244	128	62

Table 1: Number of amines for each generation.

In this work we use a generation four (G4) of PAMAM dendrimers. This work has three major goals. First: investigate systematically the behavior of PAMAM dendrimer at various protonations levels in a good solvent. Second an algorithm for coupling molecules inside dendrimers in best positions and investigate the complexation of twenty molecules of rifampicin inside dendrimers. After the constructions of structures, PAMAM G4, we construct an algorithm for coupling molecules inside dendrimers and perform atomistic molecular dynamics for investigate the conformational structure of dendrimers at various pH conditions. Due the high flexibility of these structures, we made a molecular dynamics of large-scale during 44 nanoseconds. This type of molecular simulations describes satisfactorily the interactions between dendrimers and solvent and interactions with drugs inside cavities. The electrostatics interactions, for example, hydrogen bound, van der Waals forces, are responsible for low toxicity and formation of

complex. All systems were simulated with a periodic box with solvent and couterions (Cl^-). The gromacs package [8] is used for simulations.

2 METHODOLOGY

We have employed fully atomistic molecular dynamics simulations to examine the structure of 3 states of protonation: High pH, Neutral pH and Low pH, for G4 PAMAM dendrimer. At High pH (pH > 10) PAMAM fully deprotonated (uncharged dendrimer), at neutral pH with all primary amines protonated (pH ~7), but tertiary amines deprotonated, and low pH with primary and tertiary amines fully protonated (pH < 7)[5][6]. The Hyperchem software was used for construct the initial structures of G4 PAMAM. We started the simulation with a box of water. This box has 10 angstroms solvation shell around the dendrimer structure, which was copied and repeated in all three spatial directions to create a box sufficiently large to contain the dendrimer[4]. To maintain charge neutrally, addition of an appropriate number of Cl^-couterions in neutral and low pH was made. MD simulations used OPLS-AA all-atom force field which has previously demonstrated reasonable accuracy for diverse chemical systems, including dendrimers. Majority of the OPLS-AA force field parameters were used without modification. However we derived additional parameters.

Prot	Atoms	Charges	Ions	Water
High	2244	0	0	106458
Neutral	2308	64	64	106088
Low	2370	126	126	100527

Table 2: Models for the dendrimers.

After these procedures, the systems were subjected to energy minimization by at least 100 000 steepest descent and conjugated gradient cycles to correct bad contacts geometry. Following energy minimizations, we divided the molecular dynamics in two phases. First, the dendrimer was maintained at your starting position by a constant force in their heavy atoms, while the water molecules and couterions were relaxed around the structure. 4ns of molecular dynamics were performed in this phase at 300 K at a pressure 1atm. Second, we performed 44 nanoseconds of dynamics at a temperature of 300K with a pressure of 1 atm for collection of data. It was used the fast LINCS algorithm to constrain the bond lengths within the dendrimers and to constrain the water geometry respectively. For electrostatics interactions, the PME method was used. After these simulations the radius of gyration, total energy, kinetic energy and RMSD were measured to check the balance of the system.

The algorithm for coupling of molecules inside dendrimer has the following procedure: The molecules are generated randomly in the search space, and these molecules are represented by a vector. The first three positions of vector represent the Cartesian coordinates (X,Y,Z). The next three positions of the vector represent the angles of rotation of the rigid body of the molecule. The final positions of vector represent the rotation of bonds. These final positions depend on the number of rotational bonds of each molecule. The next step is an evaluation of energy of complex for a force field. The algorithm runs 250 times, for each round the algorithm calculates (dendrimers and twenty molecules) the potential energy. At the end of these 250 rounds, the complex with minimum energy is stored. These positions (in complex with minimal energy) of the molecules that represent places of minimum energy at the surface are now sites where the molecules are generated by over 250 rounds. These molecules are generating in these positions with a Cauchy distribution. This procedure improves the search because the molecules are generated only in places close to the minimum energy exploiting more efficiently the surface of energy. At the end, the complex of minimum energy is given as input for molecular dynamics.[12,13]

3 RESULTS AND DISCUSSION

3.1 Dendrimers Conformations

We calculate radius of gyration through the equation (1) to measure the shape and size of dendrimer.

$$Rg = \left(\frac{\sum \| r_i \|^2 m_i}{\sum_i m_i} \right)^{\frac{1}{2}} \quad (1)$$

At high pH, when there is no protonation, just water increases the size of dendrimer by almost 12,3% compared vacuum simulations. At neutral pH, when all primary amines are protonated, dendrimers increase 22,4% compared with vacuum simulations. As the pH lowered further, where all amines are protonated (primary and tertiary), the dendrimer increases your shape in 28,6% compared to the simulations in vacuum. The Coulombic repulsions between tertiary amines protonated inside the dendrimerand primary amines protonated at periphery give rise to dramatic increase in radius of gyration. The presence

of counterions also increases the swelling. The results are showed in Table 3.

pH	Radius (G)	Deviation
Vacuum	1.52	±0.03
High	1.73	±0.02
Neutral	1.96	±0.03
Low	2.13	±0.03

Table 3: Radius of gyration for three states of protonation.

With presence of good solvents like water, the branches are more stretched giving rise uniformity in the spatial distribution of various branches. With the protonation of all amines (primary and tertiary), the hydrogen bonding with solvent is more than when amines are deprotonated. This fact indicates a structure more soluble. The table 4 shows the average of hydrogen bonding with water molecules during the eight final seconds of simulation.

pH	H.Bond Average	Deviation
High	416.619	±10.536
Neutral	450.850	± 9.312
Low	471.251	±10.428

Table 4: Hydrogen Bond for three states of protonation. Where H,Bond average represents the average over eight nano seconds of molecular dynamics and deviation is the standard deviation

The interaction between dendrimer and molecules attached in your structure is due to interactions between primary amines of the surface and these molecules. For each pH condition we observed a different distribution of surface amines. We plotted the radial distribution in relation of center of mass of dendrimer.

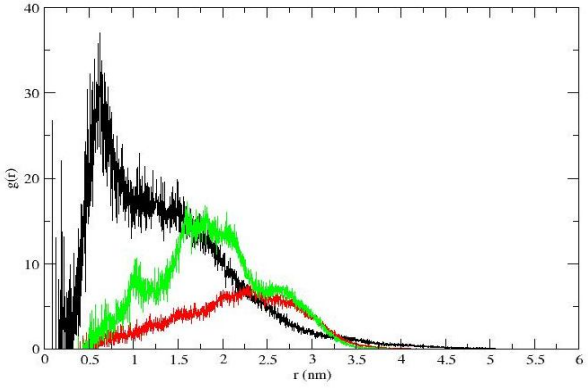

Figure 1: Radial distribution of surface amines around center of mass of dendrimer.

The black curve (Figure 1) represents the high pH where the major distribution of amines is about 0.5 nanometros of center of dendrimer. This curve indicates a distribution more closed with amines directed inside dendrimer. At neutral pH (green curve) the major distribution of surface amines are in range [1.5,2] nanometers. This distribution indicates a structure more open with amines outside the center of dendrimer. For low pH we have a maximum distribution about 2.5 angstroms of center of dendrimer. This distribution indicates a structure more open due the repulsions between amines groups protonated. The Figure 2 shows the conformational changes in different pH conditions.

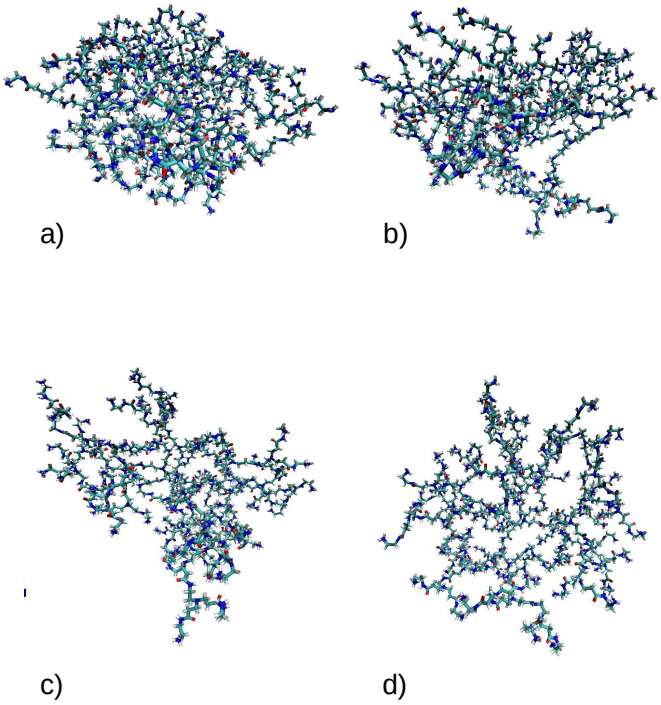

Figure 2: Snapshots of structures of dendrimer for three pH conditions. a) the structure without solvent, b) At high ph, c) the structure of neutral pH and d) at low pH. The solvente was excluded here for better visualization.

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3.2 Molecules Inside Dendrimer

We implemented a metaheuristic for distribution the molecules inside dendrimers. This procedure decreases the time of molecular dynamics by putting molecules inside dendrimers in optimal positions. For a radius of distribution the algorithm insert molecules in cavities of dendrimer. The maximum radius of distribution of molecules encountered by algorithm was 15 angstroms of center of mass. The rifampicin is a good molecule for testing the algorithm because this molecule has 117 atoms and many degrees of

freedom. The success rate for insert molecules inside cavities is shown in Figure 3. The algorithm showed that the maximum of molecules inside of the dendrimer is twenty for neutral pH. This pH is important since this is the physiological pH, responsible for transport of molecules in body.

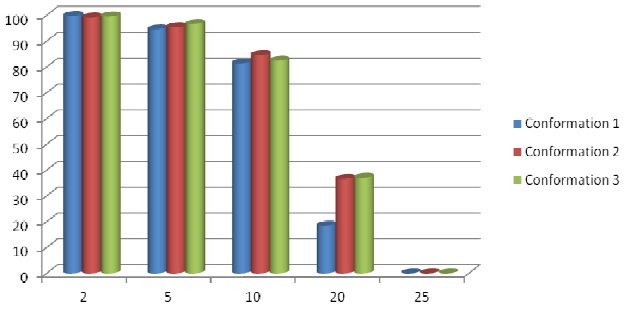

Figure 3: Success for found molecules inside cavities at neutral pH. The x axis represents the number of molecules and y axis success rate.

The structure with minimal energy (more stable) encountered by the algorithm is the initial complex for molecular dynamics. We perform 30 nanoseconds of molecular dynamics with complex (rifampicin-dendrimer). After these simulations the total energy, kinetic energy and radius of gyration were measured. The results of molecular dynamics indicate the preference of rifampicin molecules for the periphery. When the complex is formed, the solvent molecules are around dendrimer indicating a hydrophobic core. The average of hydrogen bond between dendrimer and molecules of rifampicin is 38,056 for each step of simulation. The electrostatic interactions among rifamícin and carboxil and hidroxil groups keep the formation of complex. The figure 4 is a snapshot of average structure during molecular dynamics.

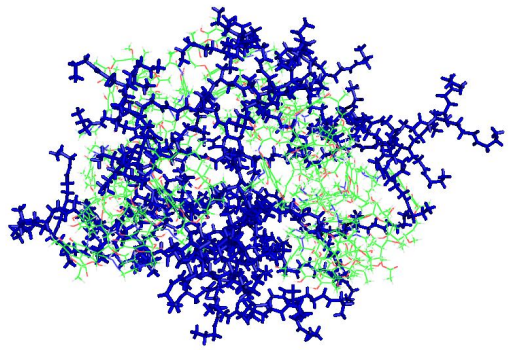

Figure 4: Snapshot of complex with twenty molecules of rifampicin inside dendrimer and dendrimer in blue.

ACKNOWLEDGMENT

The simulations of this work were provided through ICA laboratory (applied computational intelligence) at PUC/RIO Brazil and this research has support of CAPES Brazil.

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