High Performance Computing Studies of RNA Nanotubes

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Abstract. Based on the previous studies of RNA nanorings, in this contribution we computationally analyze the structure and properties of RNA nanotubes, where we focus on nanotubes consisting of up to five nanorings of around 20nm in diameter. We have developed a molecular dynamics (MD) method and implemented it by using the NAMD and VMD packages in a high-performance computing environment to study the structural and thermal properties of the nanotube in physiological solutions. In particular, we have analyzed such characteristics as the Root Mean Square Deviation (RMSD), the radius of gyration, the number of hydrogen bonds per base pairs, and the radial distribution function for the different nanoclusters in nanotubes of various sizes. Due to flexibility of RNA molecules, we can build various motifs which are essential in bio-nanotechnological applications such as scaffolding and drug delivery systems.

1 Introduction

DNA and RNA are biopolymers made of nucleotides which consist of a sugar molecule (ribose), a phosphate group and nitrogen-containing base. These biopolymers can be used as building blocks to construct various biopolymer nanostructures for medical and bioengineering applications. Studying their properties is a great challenge, in particular when they are embedded in a physiological solution or water in such applications. This is the case even when we study simplified biopolymer nanostructures, e.g. RNA nanorings [10]. RNA is a biopolymer with the ability to self-replicate that could both store information and catalyse chemical reactions, and as a nano-engineering material it brings additional advantages, as well as challenging features, such as much larger diversity in tertiary structural building blocks (compared, e.g., to DNA).

In this paper we focus on RNA nanotubes. Ribo Nucleic Acid (RNA) is a strand build of the nucleobases Cytosine, Guanine, Adenine and Uracil. These nucleobases are connected to the sager ring and then to the phosphate backbone to construct the complete strand. In the field of bio-nanotechnology, the RNA has many potential applications due to its relatively high thermal stability, varieties of functions and flexible structure. Among other applications, we emphasize that the particular structures of the

RNA assembles are very feasible in delivering drugs. RNA can produce a number of structural motifs, that is why it plays a vital role in designing the nanoparticle and nanoclusters. One of the most common structures that can be modeled using RNA as a building block is the RNA nanotube [3, 2]. By now, they can be assembled in vivo [1]. For applications in drug delivery, a proper way of assembling should be used in building the RNA nanotubes from the RNA building blocks [14, 6].

The delivery of therapeutic drugs can be done in two ways. One is by directly including it into the RNA building blocks and the second is by attaching the drug at the some particular ends of the RNA. Having these applications in mind, we study here properties of RNA nanotubes. In our earlier works, we focused on RNA nanorings [10]. Certain combinations of nanorings in piles would form RNA nanotubes. During MD simulations the base pairing between the two loops has been maintained and the angular variation between the loops has been traced. RNAI and RNAII are two kind of loops of RNA and they can be combined in different ways. Firstly, after some modifications in these RNAs, their combinations can be used as the building block for nanorings. Furthermore, the building blocks of the nanorings are engineered in such a way that the RNAI and RNAII ends are complementary to each other which eventually produces the sticky ends. This is an important feature of the rings to form the nanotubes. In short, by using six helical building blocks either one or two types (RNAI/RNAII) the nanorings are formed by self assembling them in a non-covalent way. The stability of the nanorings depends on the RNAI/RNAII interactions. The design of the sticking ends helped us to assemble the nanorings to build the nanotubes. The starting structures of RNAIi/RNAIIi complexes were taken from the protein data bank with the pdb code (2bj2.pdb) [7].

The rest of this paper is organized as follows. In section 2 we describe computational details of our developed models and procedures pertinent to full molecular dynamics simulations. Section 3 provides highlights of coarse-graining modelling algorithms developed here. The results and discussion are presented in Section 4, followed by concluding remarks in Section 5.

2 Computational Details

We have developed a molecular dynamics (MD) method and implemented it by using the NAMD and VMD packages in a high-performance computing environment to study the structural and thermal properties of the nanotubes in physiological solutions. We have performed the all-atom molecular dynamics simulation of RNA nanotubes using CHARMM27 force field [12] implemented in the NAMD package [9]. The modeling of the nanotubes, visualization and the analysis of the simulation outputs have been performed in the software VMD and gnuplot. The RNA nanotubes were solvated by the water in a water box. The size of the box has been taken in such a way that the distance wall of the water box is at a distance a bit larger than the cut off radius used in the MD simulation. In order to make the system neutral we have added 594, 924, 1254 and 1584 $^{23}Na^{+}$ for two ring, three ring, four ring and five ring nanotubes, respectively. Furthermore, to make the solution equivalent to the physiological solution we have added extra the $^{23}Na^{+}$ and $^{35}Cl^{-}$ ions. This system has been simulated at constant temperature and

pressure using NAMD software. The temperature in the system has been controlled by using the Langevin method with damping $\eta = 5\ \mathrm{ps}^{-1}$. Next, the coarse graining molecular dynamics simulations and calculation of radial distribution functions have also been carried out. We provide main highlights of the developed procedure in the next section. We have used software DLPOLY for the actual implementation.

In practice, the molecular dynamics simulations should be long enough to achieve suitable conclusions from the results which can be achieved from the CGMD simulations. In real life applications, however, such simulations should be supplemented by coarse-graining modelling. In this paper, one of the parameter that we will be studying is the Root Mean square Deviation (RMSD) which characterizes the equilibration of a molecular dynamics simulation and can be expressed as

$$RMSD(t_1,t_2) = \left[\frac{1}{N}\sum_{i-1}^{N}\|x_1(t_2) - x_i(t_1)\|^2\right]^{1/2}, \tag{1}$$

where $x_i(t)$ is the position of ith atom at time t , N is the number of atoms in the molecule. In traditional calculations the position at t_1 is taken as a reference point and then the $RMSD(t_1,t_2)$ is calculated for $t_2 > t_1$.

Another parameter under study is the radius of gyration which also describes the structure change during molecular dynamics simulation. Radius of gyration of the system can be defined as a weighted scalar length of each atom from the center-of-mass:

$$R_g = \sqrt{\frac{\sum r^2 m_i}{\sum m_i}}, \tag{2}$$

where m_i is the mass of the ith atom in the molecule.

3 Coarse-Graining Procedure

Coarse-graining (CG) models are designed in attempts to explain the behavior of the system at larger scales based on the information from smaller scales (starting from the atomistic classical model). The CG models should also be easier to use in simulations, compared to the full MD, and accurate enough to describe the physical characteristics of the system. In the CG modeling we represent a sum of atoms as a pseudoatom, and then define an effective energy function U_{CG} which is responsible for the thermodynamical properties.

Recently, the modeling of coarse-graining structures of RNA and RNA-Protein, using the Fluctuation Matching technique, has been performed by the authors of [5] where analogous assumptions to those originally proposed in [11] have been applied.

In paper [11] a series of coarse grained models have been developed to study the molecular dynamics of RNA nanorings. In these models one to three beads per nucleotide have been used. The aim of these approximations is to extend the length (up to microsecond) of the simulation time and the size of the cluster. Based on the results of [11], in our modeling of RNA nanotubes we have been using the 3B model. In the 3B approximation phosphate backbone (P), sugar ring (S) and the Nucleobase (B) are taken as first, second and third bead respectively.

The Boltzmann inversion method [13] has been used to fit the CG parameters. This method, originally developed for simple liquid systems, has proved to be efficient for organic polymers and RNA nanoclusters. In this technique the coarse graining potential is calculated from the pairwise potential (e.g., [8]):

$$U_{CG}(R) = u_{CG}(R_{ij}). \tag{3}$$

This idea is based on the uniqueness theorem [4] which states that for a given pair of radial distribution function there exists a pairwise potential. Our realization of this idea has been as follows.
1. Do all atom MD simulation.
2. Convert all atom simulation trajectory into the corresponding coarse-graining model and then calculate the radial distribution function for this CG pseudoatom.
3. Using these pseudoatomic radial distribution functions, calculate the pairwise potential for each pair of the CG pseudoatoms by applying the following formalism:

$$u_{CG}(R) = -k_B T ln g_A(R) \tag{4}$$

4. Then do the NVT MD simulation of the CG system using the computed pairwise potentials; then measure the radial distribution function for the pseudoatoms in the CG ensemble.
5. Now update the potential using the relation:

$$u_{CG}(R) = u_{CG}(R) - k_B T ln(g_R/g_{CG}). \tag{5}$$

6. Repeat the CG simulation as in step 4 until the value of u_{CG} converges.

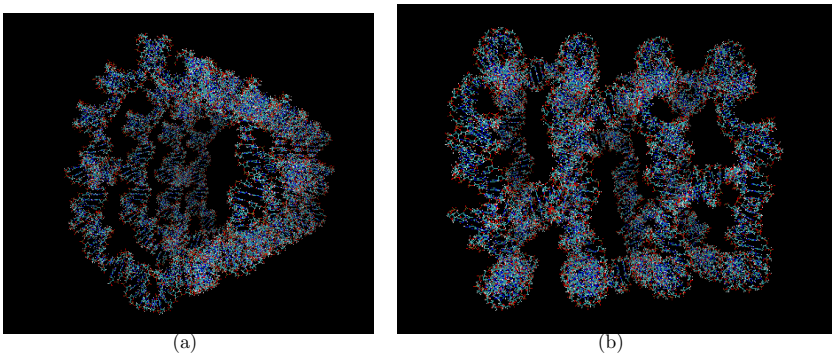
In the next section, we present some representative examples obtained from the application of this algorithm.

4 Results and Discussion

Starting from the previously modeled nanoring structures, we have modeled the RNA nanotubes with two, three, four and five nanorings. As an example, the nanotube with four nanorings is shown in figure 1. The RNA nanotubes were solvated in the salt solution, as explained in the previous section. Taking this solvated system we carried out the MD simulation at NVT for all of the nanotube systems. We started from the two ring nanotube and used it as a benchmark. The temperature and the energy variation have been analyzed in detail. From the curve for energy it was clear that the energy of the system is varying until the run goes up to around 500ps.

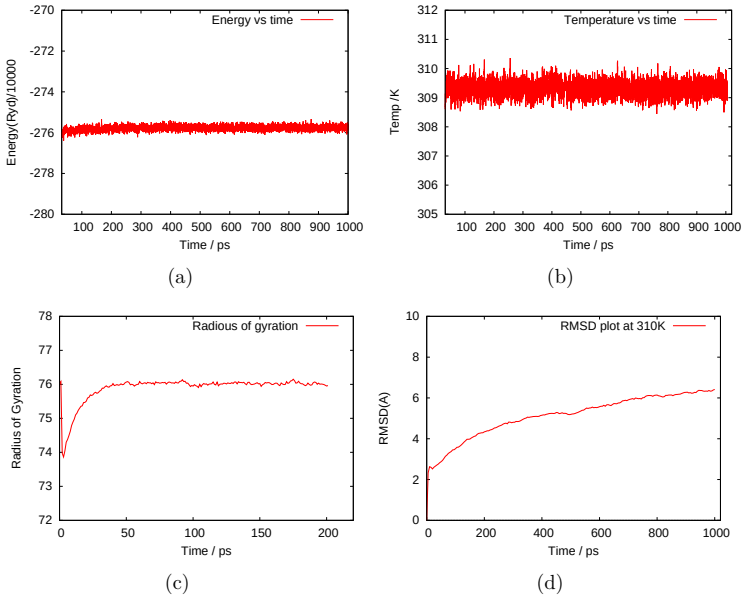
The results for the radius of gyration and root mean square deviation for the nanotubes under consideration have also been analyzed. From the curve for the radius of gyration it was clear that the value of the radius of gyration first decreases, while reaching saturation at the end of simulation. A similar tendency was also observed for the root mean square deviation.

In a similar way, we have performed the NVT simulation for the nanotubes with three, four and five RNA nanorings in physiological solutions. As an example, the results for energy, temperature variation, root mean square deviation and the radius of



(a) (b)

Fig. 1. RNA nanotube modeled from four nanorings using VMD (a) front view (b) side view



(a) (b) (c) (d)

Fig. 2. (a) Energy, (b) Temperature, (c) Radius of gyrations and (d)RMSD as a function of time for the four ring RNA nanotube from all-atom MD simulation in salt solution.

gyration for the RNA nanotube with four rings are shown in Fig. 2. The trend is similar to what was observed for the RNA with two rings.

Another important factor defining the stability of the model is the number of hydrogen bonds per base pairs. Hence, we have also analyzed the number of hydrogen bonds per base pairs for all the modeled nanotubes. We confirmed that this number has been maintained during MD simulations for all of RNA nanotube systems analyzed here.

Using the optimized structures of RNA nanotubes, obtained with VMD and NAMD, we have developed the coarse graining model for multiple-ring RNA nanotubes. As an

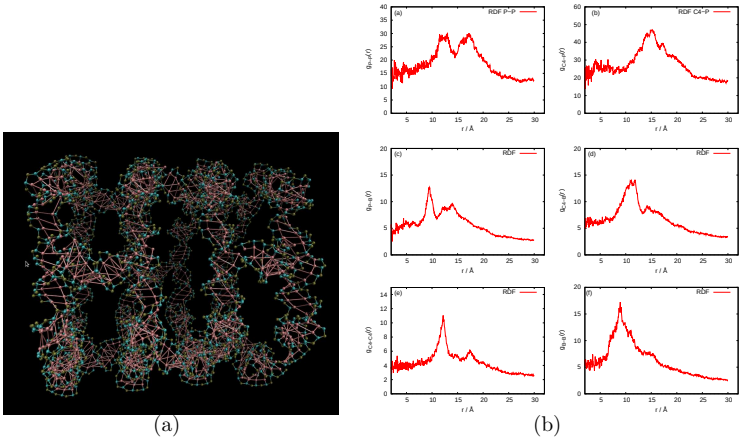


Fig. 3. (a) Coarse-graining model and(b) Radial distribution plots for four ring RNA nanotube

example, we present the structural model and the calculated radial distribution functions in Fig. 3. Finally, we would like to mention that the number of Na ions around the nanotubes within the distance of 5A at two different temperatures has also been studied. It has been found that the number of ions accumulated around the nanotubes within the specified distance is growing with increase in temperature from 310K to 510K. The final configurations of the systems simulated at 510K have been considered as the starting point for further MD simulations at 310K. We confirmed the process of ion evaporation with temperature decrease. This is due to the phenomenon of self-stabilization, first reported in [10].

5 Conclusions and Outlook

Starting from a previously modeled RNA nanoring, we developed models for studying the properties of multiple-ring RNA nanotubes. Such properties as root mean square deviation, the radius of gyration and the number of hydrogen bonds per base pairs were analyzed based on the molecular dynamics methodology. We also studied the energy and temperature variation with time during MD simulations in physiological solutions. Finally, we modeled the coarse grained structures of multiple-ring RNA nanotubes and calculated the corresponding radial distribution functions. The results obtained for these large complex systems are important in bio-nanotechnological applications of RNA nanotubes, in particular for scaffolding and drug delivery systems.

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