Accelerating the Research in Drug Delivery System; A Challenge of the Earth Simulator to Medical Innovation

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Objectivity of our project is to help the research of drug delivery system by means of modern high performance computers. Last year we carried out some large-scale simulations on the system of DNA and cationic polymers. However the results were not necessarily satisfactory since our computational resources were restricted. This year we put greater amount of resources into similar calculations and succeeded to observe behavior of DNA and polymers. The results showed that hydrogen and nitrogen atoms of amines in polymers moved around actively while the other parts of polymer remained relatively still. Considering the fact that amines form cationic bases in solution, our results agree to the well-known experimental fact that cationic property plays a significant role in DNA condensation.

Keywords: drug delivery system, DNA, poly-L-lysin, poly-ethylene-glycol, density functional theory

1. Introduction

Since the end of the last decade nanotechnology has been grown at rapid pace and spread over many different fields out of its birthplace, material science. Now in medical science, nanotechnology is expected to open up the door to the innovative methods of treatments no one can imagine twenty years ago. One of its novel application is drug delivery system (DDS). The main purpose of DDS is to target the seat of a disease and carry drugs there precisely.

Our project aims to accelerate the research of DDS by fully exploiting the amazing computational ability of the Earth Simulator. Last year we have conducted some large-scale simulations on the system of DNA and block copolymers which comprised of poly-ethylene-glycol (PEG) and poly-L-lysin (PLL). However the results were not necessarily satisfactory since our computational resources were restricted. All CPU times assigned to us were exhausted in preparatory calculations. This year we put greater amount of resources into calculations. As a result, we were able to calculate molecular dynamics and observe behavior of DNA and the polymers. The results agree to the experimental fact that cationic property plays a significant role in DNA condensation.

This report is organized as followings. The next section describes the physical aspects of DDS in detail. In the third section, we discuss computational methods. Especially, Density Functional Theory (DFT) is described as computational method adopted in this report. The forth section gives computational results. Final section summarizes this report.

2. Drug delivery system

Many kind of DDS technology has been proposed so far. Amongst them, our project pays much attention to the method using nano-sized particle called micelle mainly comprised of PEG-PLL block copolymers. This method was recently developed by Professor Kataoka of the University of Tokyo and is expected to be promising in near future gene delivery because of its relatively low impact on human body [1]. Although the whole process of PEG-motivated DDS is much complicated, we can recognize four characteristic stages in that. See Fig. 1.

In the first stage, relaxed DNA attached to a PEG-PLL copolymer starts to condense in solution. Shortly after, DNA is condensed to a small object like ball. Notice that PEG-PLL-DNA complex has a hydrophilic end in PEG and a hydrophobic end in DNA. In short, it has amphiphathic property. In the next stage, hundreds of those PEG-PLL-DNA complexes in the water meet together and spontaneously form sphere called micelle in which each condensed DNA heads for the center and PEG heads for the surface. The driving force of this self-organizing formation is amphipathic property mentioned above. In the third stage, micelles

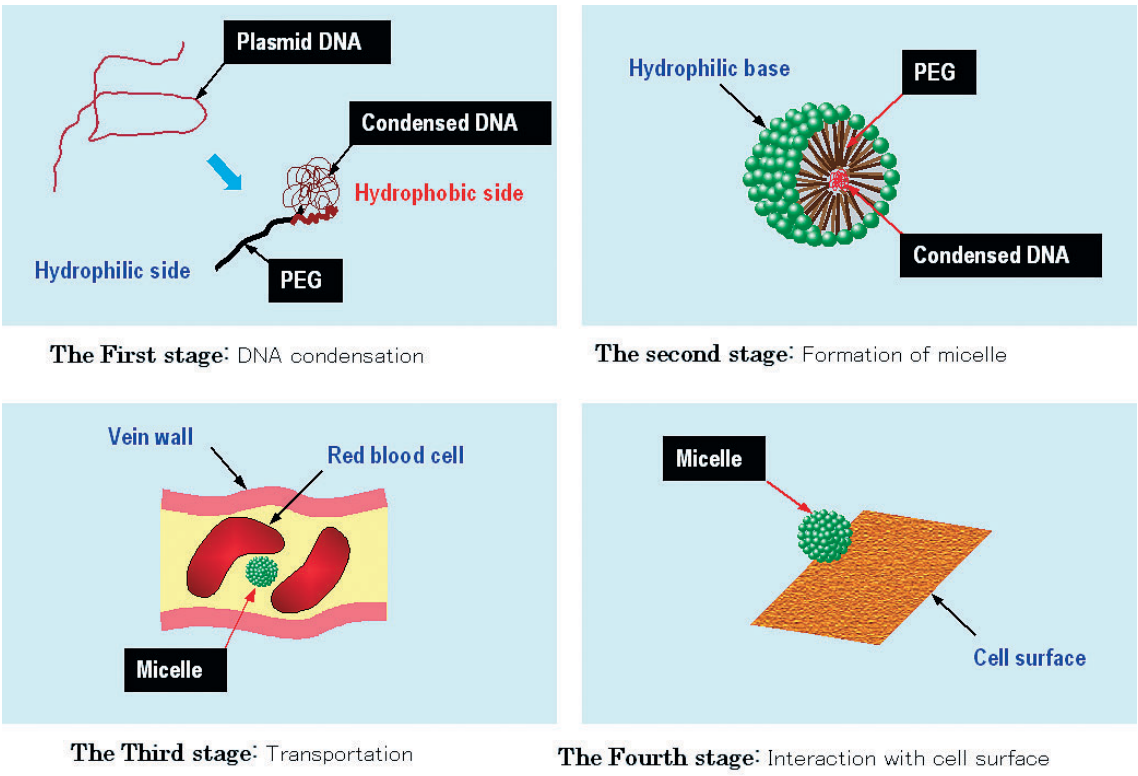

Fig. 1 Characteristic stages of drug delivery system.

are carried through vein and capillary tube slipping through the red blood cells. In the last stage, micelles reached the targeted portion of disease are attracted by the local gradient of ion concentration in the vicinity of the cell surface and then absorbed into it through the carrier or channel protein located at the membrane.

Those types of problem are recently paid attention in many fields and classified as multi-scale and multi-principle phenomena in contrast to single-scale and single-principle phenomena conventional science has been dealing with. To simulate such kind of complicated phenomena efficiently we must choose effective computational methods corresponding to each stage and combine them interactively. However, in this report, we should focus our interest on the first stage, i.e. DNA condensation, since sophisticated simulation techniques like that are not solidified yet.

3. Computational methods

In DNA condensation, the electronic structure inside DNA is thought to play an important role.

Therefore quantum mechanical consideration is significant to calculate such phenomena. Usually DFT is preferred for those kinds of problems. Although DFT can perform highly accurate calculation and give reliable results, its computational workload is immensely large, sometimes unacceptable. From that reason, DFT has been applied for relatively small systems up to few hundred of atoms even on the current top-rated supercomputers while at least few thousands of atoms are indispensable to simulate condensation of DNA in solution. On the other hand, there exist molecular dynamics (MD) methods work with thinner workloads by far. However, MD is based on heuristic potentials and is insufficient in accuracy. To mitigate such difficulties, we adopted DFT as calculation method for accuracy and ignore water molecules to reduce problem size. After all, in this paper, we considered a DNA short segment and a PLL in vacuum.

In what follows, we describe DFT method in short. The basic ideas of DFT originate in the papers written by Hohenberg, Kohn and Sham [2, 3]. At the beginning, they proved mathematically that total energy of ground state is a functional of electron density. In other word, total energy of ground state depends on electron density alone. This fact made many-electron problems extremely easy to deal with, since each wavefunction of electron is no longer needed to be specified. Combining the fact with variational principle, Kohn-Sham equations were obtained after some manipulations.

$$\left[\frac{-\hbar^2}{2m}\nabla^2 + V^{eff}(r, n(r))\right]\phi_i(r) = \varepsilon_i\,\phi_i(r)$$

Where $\hbar$ is Planck's constant divided by 2π, m is the mass of electron, V^{eff} is an effective potential, n is electron density, ϕ_i is wavefunction and ε_i is Lagrange multiplier.

Notice that Kohn-Sham equations are single electron equations. All many-electron effects are confined and hidden

in the effective potential through electron density. Consequently, Kohn-Sham equations can be treated as a simple eigen value problem just like hydrogen atom. However, in actual calculation, outer iteration loop is needed for electron density to satisfy following selfconsistent relation with wavefunctions in addition to solving Kohn-Sham equations.

$$n(r) = \sum_i \left| \phi_i(r) \right|^2$$

Effective potential can be written as follows.

$$V^{eff}(r, n(r)) = -\sum_I \frac{Z_I e^2}{|R_I - r|} + e^2 \int \frac{n(r')}{|r - r'|} dr' + \frac{\delta E^{XC}(n(r))}{\delta n(r)}$$

Where Z_I and R_I is charge and location of nuclei respectively. Arguments of long standing are the form of the last term called exchange-correlation potential. In the early days, Local Density Approximation (LDA) was used extensively. Recently, Generalized Gradient Approximation (GGA) is getting wider in use to incorporate nonlocal effects [4]. By the way, prominent advantage of DFT over Hartree-Fock (HF) method is its lean computational workload. HF requires workloads in proportion to the fourth power of the number of electrons to calculate exchange term, while DFT needs only square of them [5]. The difference grows immensely large in case of many electrons.

Another drawback of HF is that it has to take all electrons in the system into account to make wavefunctions fully antisymmetric. To the contrary, there are pseudopotentials of nuclei, which enables DFT to omit core-electrons and reduce calculations further. Their specific forms in polynomial expression are tabulated comprehensively in literatures [6, 7].

We adopted PWscf code as our main computational tool, which is developed as a part of program package called Quantum ESPRESSO at DEMOCRITOS National Simulation Center, Trieste, Italy, and is available freely under the GNU General Public License [8]. PWscf implements DFT with pseudopotentials on plane wave basis and is programmed off-the-shelf parallelization for multiprocessor computers. However, its parallel performance is not satisfactory especially on the Earth Simulator. To make the matter worse, it is not fast at all for vector pipeline facilities. To improve computational efficiency of Pwscf, we had vectorized the code intensively and modified further to realize aggressive parallelization last year. All calculations in this paper are carried out using that improved code on the Earth Simulator.

4. Results

Prior to DNA condensation, PEG-PLL cationic copolymers and DNA are thought to form an object called poly-ion complex. Consequently electronic structure of DNA is modified to some degree and then mechanical properties of DNA change locally. We expect understanding how those changes occur reveals the onset of DNA condensation. To clarify the course of poly-ion complex formation, we carried out numerical simulations for three typical configurations (PLL alone, DNA short segment alone, and DNA-PLL coexisting system). Reader should notice that in this paper PEG is omitted from the computational point of view, since PEG is expected to matters significantly in formation of micelles in solution, but less significant in formation of poly-ion complex. Each result is described in the following subsections.

4.1 PLL

PLL is poly-peptide of Lysins, which are one of twenty amino acids exist in nature. Lysin has amine at the end of its side chain. In general, amine is chemically active and positively charged in water. Therefore it is natural to think that portion takes part in the formation of complex. We calculated the behavior of PLL comprised of eight Lysins. Number of atoms amounts to 270. It took 1500 node-hours on Earth Simulator to calculate 272 simulation steps. Figure 2 is a snapshot of PLL in motion. From the movie of computational results, we can see that nitrogen and hydrogen atoms in amines are moving around actively while the other atoms remain relatively still. In addition, ionic charge density and its isoelectric surfaces are shown in Fig. 3. We can see the isoelectric surfaces exist at nitrogen atoms. From the results, we concludes that amines in PLL have strong tendency to be cationic and is mobile well. Our conclusions agree to the

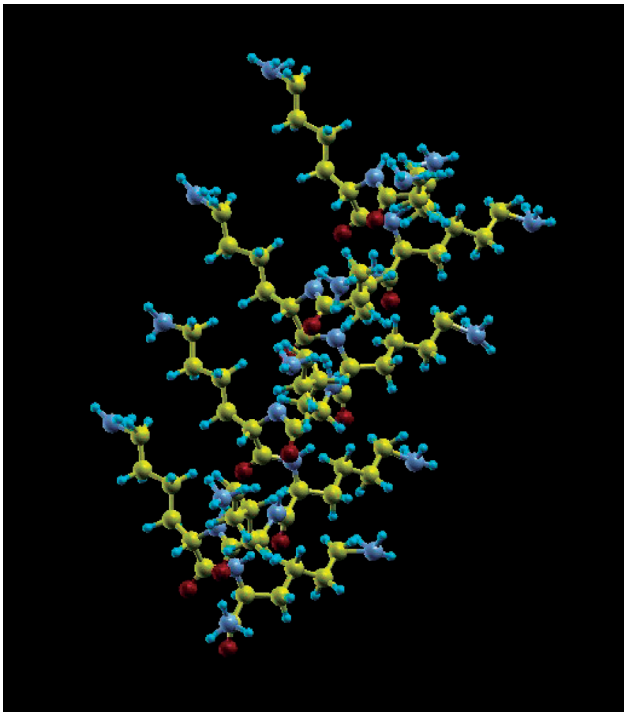

Fig. 2 Snapshot of PLL in motion.

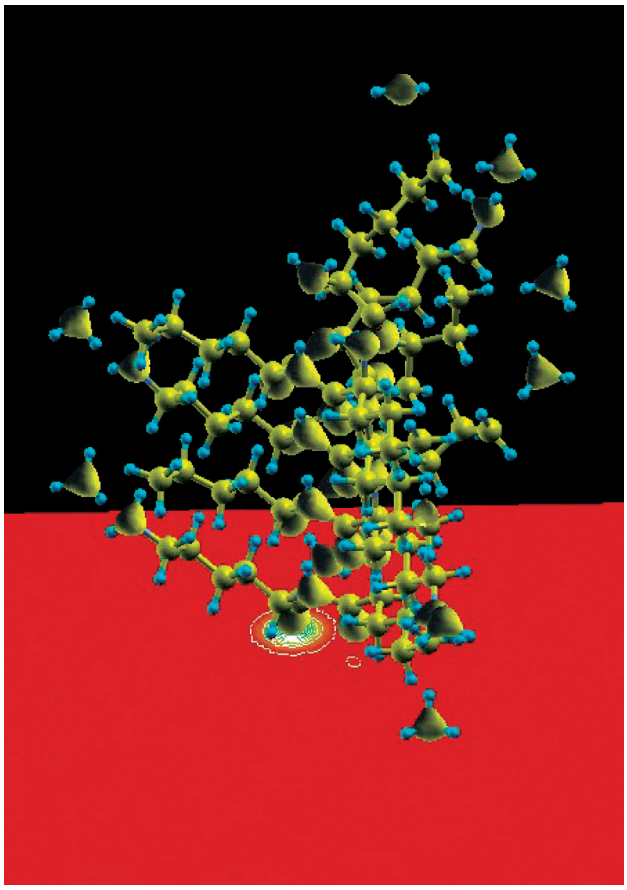

Fig. 3 Charge density contours and isoelectric surfaces.

well-known experimental fact that cationic property plays a significant role in DNA condensation.

4.2 DNA

It is well known that DNA consists of double strands. Dynamically it can be thought of as stiff thread. In addition, phosphoric portions of DNA are negatively charged in water. To investigate its behavior, we carried out calculation of DNA segment consists of 14 base pairs. Number of atoms amounts to 637. It took 1500 node-hours on Earth Simulator to calculate 12 simulation steps. Figure 4 is a snapshot of DNA in motion. From the movie of computational results, we can recognize hydrogen atoms move slightly, while the rest of atoms remain still. However the number of steps simulated is insufficient to bring out useful knowledge of dynamical properties.

4.3 DNA and PLL

Simulating the formation of poly-ion complex adequately under DFT approximation requires certainly more than current top-rated computers. But, we dared to try the calculation to asses its possibility. DNA segment consists of 14 base pairs and a PLL comprised of eight Lysins are considered. Number of atoms amounts to 907. It took 600 node-hours on Earth Simulator to calculate 3 simulation steps. Figure 5 is a snapshot of DNA and PLL coexisting system in motion. We can not find out any physical conclusion from the results. Nearly thousands times faster computer than Earth Simulator is expected to be available around 2011. Then few thousands of simulation steps are able to perform and seeing their dynamical behavior is also possible at that time.

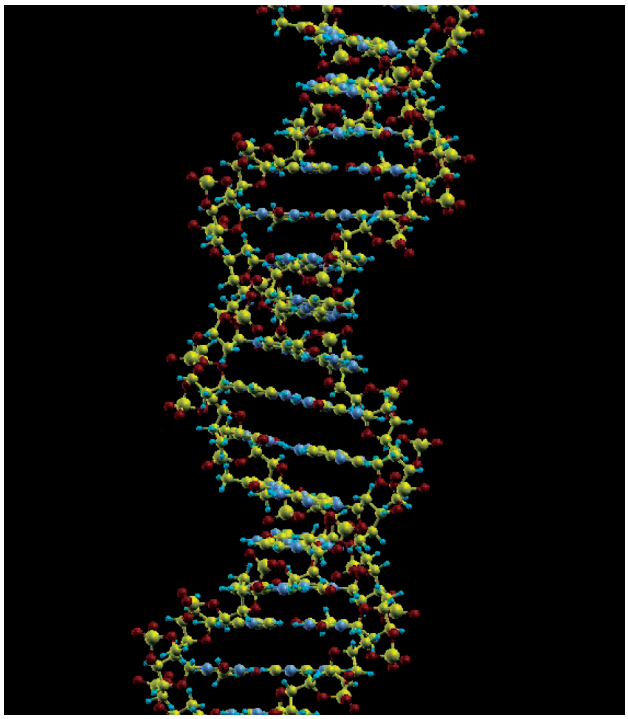

Fig. 4 Snapshot of DNA in motion.

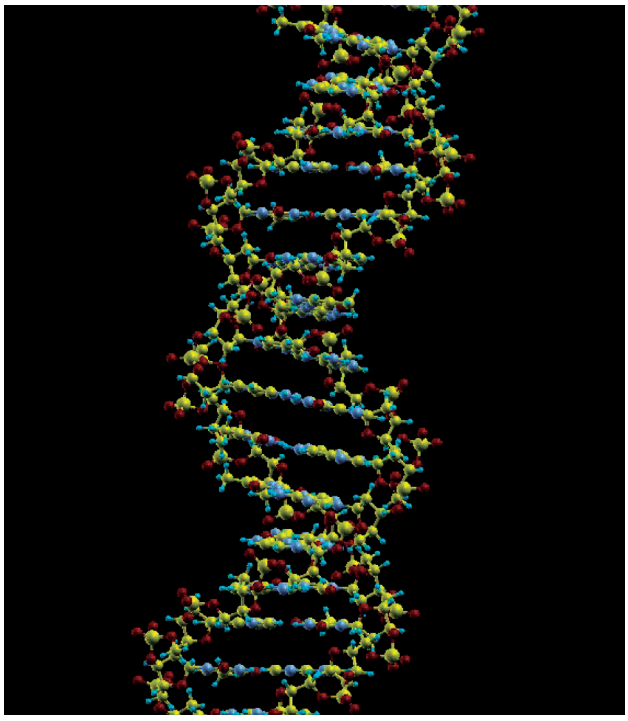

Fig. 5 Snapshot of PLL attaching to DNA.

5. Summary

To accelerate the research of drug delivery system by means of modern high performance computers, we carried out three large-scale simulations in terms of DNA and cationic polymers.

From the results of PLL calculations, we found out that amines in PLL had strong tendency to be cationic and is mobile well. Those facts agree to the well-known experimental fact that cationic property plays a significant role in DNA condensation. On the other hand, the results including DNA were quite unsatisfactory. From the calculations of DNA alone, we managed to recognize hydrogen atoms move slightly, while the rest of atoms remain still. But, any useful physical knowledge was not found out in DNA and PLL coexisting system from the resultant few computational steps. We expect coming Petaflops computer will be served as those calculations.

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地球シミュレータを利用したドラッグデリバリシステムの研究：革新的医療への挑戦

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本プロジェクトの目的は高性能計算機を用いることでドラッグデリバリシステムの研究を加速することにある。昨年度はDNAとカチオン性高分子からなる系の大規模シミュレーションを実施したが、計算資源の制約から満足な結果が得られなかった。本年度はより大きな計算資源を投入して再度計算を試み、動的振る舞いの計算に成功した。その結果、カチオン性を担う高分子中のアミノ基は活発に運動するが他は比較的落ち着いていることが解った。これはカチオン性がDNA凝縮において決定的な役割を果たすというよく知られた実験的事実に符合する。

キーワード：ドラッグデリバリシステム, DNA, ポリエチレングリコール, ポリリシン, 密度汎関数法