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Design maps for cellular uptake of gene nanovectors by computer simulation

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ABSTRACT

Understanding how nanovectors transport DNA molecules through cell membranes is of great importance in gene therapy. In this paper, we systematically investigate the mechanism of cellular uptake of cationic polymeric nanovectors containing DNA molecules through dissipative particle dynamics simulations. Our results show that the property of polyelectrolyte chains grafted to nanovector and DNA molecules can have important impacts on the endocytosis. Interestingly, it is found that the nanovector can be fully taken up with proper number of DNA molecules on its surface. On the contrary, in the absence of DNA it may become harder to be totally engulfed. Since the adsorption number of DNA is related to external pH, the cellular uptake could exhibit pH-responsive behavior. Further, we also provide insights into the comparison of uptake behaviors between cancer and normal cells, and importantly, we find that the enhanced uptake of gene nanovectors may be an inherent property of cancer cells. The present study may give some significant suggestions on future nanovector design for gene delivery.

1. Introduction

Efficient delivery of nucleic acids (i.e., DNA or RNA) into target cell interiors is of great importance in gene therapy [1–3]. Though some viruses (i.e., viral vectors) can offer greater efficiency of gene delivery, now the viral vectors are not the optimal choice because of the limitation of their sizes and types of nucleic acids, and particularly they could lead to severe immune/inflammatory reactions in patients [1,4]. Non-viral vectors, typically based on cationic lipids or polymers [5–8], offer several advantages (e.g., low toxicity, facile fabrication) over their viral counterparts. However, the efficiency of non-viral ones now is still very low and cannot reach the demand by the clinical applications [9,10].

Therefore, it is a huge challenge to increase the transfection efficiency as well as decrease the potential toxicity: on one hand, we may make the best use of different nanovectors and combine them to design and develop the multifunctional nanovectors [1]; on the other hand, it is also of great importance to investigate the underline mechanism of nanovectors interacting with bio-systems at different transfection stages. Although some previous experimental studies have made some progress on these problems [4,11], it is rather difficult to systematically probe and visualize the full delivery process under various conditions, due to available experimental technologies. Computer simulations, on the contrary, may offer some useful information for the underline mechanism because it can provide the insights from the microscopic or mesoscopic view and the simulation conditions can be well controlled [12]. Actually, some previous simulation studies have given some insights into the self-assembly mechanism of DNA (or RNA) with cationic nanoparticles and macromolecules [13–17]. Nevertheless, little attention has been paid to the mechanism of cellular uptake of nanovectors containing DNA molecules, though some previous studies have shown that the physicochemical properties of nanoparticles can have important impacts on the cellular uptake of nanoparticles [18–23]. How will the DNA affect the cellular uptake of nanovectors, and what is the optimal strategy for designing nanovectors in the presence of DNA? Besides, since the external pH could affect the self-assembly behaviors of DNA with cationic vectors [15,16], does it have any effect on the cellular uptake?

In this paper, we undergo the first computational study to examine the molecular mechanism of cellular uptake of nanovectors containing DNA molecules by using dissipative particle dynamics (DPD) simulations [24,25]. As we will show below, the presence of DNA could have important impacts on the cellular uptake of nanovectors and in turn may affect the optimal design

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strategy of nanovectors. Further, the effect of external environments (i.e., pH values) as well as cell membrane property on the uptake will also be studied, and finally the comparison of uptake behaviors between cancer and normal cells is given.

2. Model and methods

Fig. 1a shows the coarse-grain models of different components in our simulations. The nanovector is composed of one inorganic rigid nanoparticle and some polymer chains [10]. The rigid particle is fabricated by arranging the hydrophilic DPD beads (P) on a fcc lattice with lattice constant $\alpha = 0.50$ nm into a desired geometry shape and volume, and all beads comprising a nanoparticle move as a rigid body [26]. The polymer chain decorated on the particle surface is composed of several connected beads, the first two beads of which are treated as ligand ones (L). The other beads (their number N can be varied) in the chain are pH-responsive polyelectrolyte (PE) ones (C), and some beads may carry a charge of $+e$, which depends on the system's pH value (i.e., protonation degree) and its intrinsic acid dissociation constant (pK_a), etc. [27,28]. The DNA molecules used here are double stranded ones, which are widely used in gene delivery [9]. In simulations, they are often treated as the rigid rods [14,29], because of the long persistent length of double stranded DNA [30,31]. Additionally, some surface beads carry the charge of $-e$ to ensure that the linear charge density is about $1e$/0.17 nm [29]. When placing the nanovector in the DNA solution, the nanovector-DNA complex (NDC) can be formed. The number of adsorbed DNA molecules will be dependent on the PE density, protonation degree, etc.

Each amphiphilic lipid consists of a headgroup containing four connected hydrophilic beads (H) and two tails with respective three hydrophobic beads (T) (Fig. 1b) [32–34]. The first head bead carries a charge of $+e$, while the second head bead carries a charge of $-e$; the remaining two beads are uncharged [35]. Particularly, when modeling the negatively charged lipids, non-charged hydrophilic bead is used to take place of the first positive charged bead in lipid molecule. The receptor molecule has the same conformation of lipid molecules [20,21,36], but its first two head beads (R) are uncharged and can interact with the ligand bead (L) *via* soft Lennard-Jones (LJ) potentials [26]. When lipids and receptors are immersed in the water, they can form a stable membrane. Here, the percent of the receptors in the membrane is set to be 50% [20,21,36], and the nanoparticle radius is fixed as 4 nm in the simulations. Unless otherwise stated, the DNA length and concentration (c) in the system are fixed as 3.5 nm and 240 μM, respectively. Additionally, the number of PE beads per chain will be 4 with half beads randomly carrying charge of $+e$, and PE-ligand chain density (σ) will be 1.50 nm^{-2}.

The DPD is a coarse-grained simulation technique with hydrodynamic interaction [25]. The dynamics of the elementary units which are so-called DPD beads, is governed by Newton's equation of motion $d\mathbf{v}_i/dt = \mathbf{f}_i/m$. Typically, in the DPD, there are three types of pairwise forces acting on bead i by bead j: the conservative force, dissipative force, and random force. In the present work, the electrostatic force is introduced to take into account the electrostatic interactions between charged beads. The conservative force $\mathbf{F}_{ij}^C = a_{ij}(1 - r_{ij}/r_c)\hat{\mathbf{e}}_{ij}$ is used to model the repulsive interaction of beads i and j, where $r_{ij} = |\mathbf{r}_{ij}|$ is the distance between beads i and j, $\hat{\mathbf{e}}_{ij} = \mathbf{r}_{ij}/r_{ij}$ is the unit vector, r_c is the cutoff radius of the force, and a_{ij} represents the maximum repulsion interaction of beads i and j. For any two beads of the same type, we take the repulsive parameter $a_{ii} = 25$, and for any two beads of different types, we set the interaction parameter $a_{WH} = a_{WP} = a_{WI} = a_{WC} = a_{HP} = a_{HI} = a_{HC} = a_{PI} = a_{PC} = a_{IC} = a_{LC} = a_{LH} = a_{LW} = a_{LI} = a_{LP} = 25$ and $a_{LT} = a_{WT} = a_{PT} = a_{HT} = a_{IT} = a_{CT} = 100$ (W stands for water bead, and I represents counterion bead which is introduced into the system to ensure the charge neutrality) to denote the hydrophilic/hydrophobic property of the beads [37,38]. The dissipative force $\mathbf{F}_{ij}^D = -\gamma(1 - r_{ij}/r_c)(\hat{\mathbf{e}}_{ij}\cdot\mathbf{v}_{ij})\hat{\mathbf{e}}_{ij}$ and random force $\mathbf{F}_{ij}^R = \sqrt{2\gamma k_B T}(1 - r_{ij}/r_c)\zeta_{ij}\Delta t^{-1/2}\hat{\mathbf{e}}_{ij}$ are for thermostat, where $\mathbf{v}_{ij} = \mathbf{v}_i - \mathbf{v}_j$ is relative velocity between beads i and j, γ is the strength of friction, ζ_{ij} is a symmetric random variable with zero mean and unit variance, and Δt is the time step of simulation [25]. The electrostatic interactions were introduced into DPD simulations by Groot [24]. The soft potential in the DPD allows for the overlap between DPD beads, therefore, when charged DPD beads are modeled, this can lead to the formation of artificial ion pairs and cause the divergence of the electrostatic potential. To avoid this problem, Groot chose to spread out the charges using the distribution [24]: $\rho_e(r) = 3/\pi r_e^3(1 - r/r_e)$ with $r < r_e$, where r_e is the electrostatic smearing radius, and is typically set as $1.6r_c$.

Besides, the harmonic bond $U_s = k_s(1 - r_{i,i+1}/l_0)^2$ (here we choose $k_s = 64$, $l_0 = 0.5r_c$) is applied between the neighboring beads in a single molecule to ensure the integrality of lipids and PE-ligand, where k_s is the spring constant and l_0 is the equilibrium bond length. A weaker bond is inserted ($k_s = 10$, $l_0 = 0.5r_c$) between the first hydrophobic beads on two tails of the lipid to keep the tails oriented in the same direction [37,38]. We also use a three-body bond angle potential $U_a = k_a(1 - \cos(\varphi - \varphi_0))$ to depict the rigidity of lipid tails ($k_a = 10$, $\varphi_0 = 180°$) and PE-ligand chains ($k_a = 10$, $\varphi_0 = 180°$), where ϕ is the angle formed by three adjacent beads in the same tail and φ_0 is the equilibrium value of the angle.

Further, we use a so-called "soft" LJ potential to mimic the receptor–ligand interaction [26]: $U_{ij}^{LJ} = 4\varepsilon[(\sigma/r_{ij})^{12} - (\sigma/r_{ij})^6] + 0.22\varepsilon$, where $r_{ij} \le r_{cut}$, $\sigma = 0.624r_c$, and ε represents the strength of the receptor–ligand interaction. We will fix ε as 5.0 k_BT, and the cutoff r_{cut} of the potential is the same as that in the DPD (i.e., r_c) unless otherwise stated. Additionally, the repulsive force is set to be $25k_BT/r_{cut}$ if it is larger than $25k_BT/r_{cut}$ to guarantee the proper running of the DPD technology.

In DPD simulations, we apply the velocity-Verlet integration algorithm and the integration time step $\Delta t = 0.015\tau$. Additionally, the cutoff radius r_c, bead mass m, energy k_BT are chosen as the simulation units. All simulations are performed in the NVT ensembles. The periodic boundary conditions are adopted in three directions. The initial size of the simulation box is $75r_c \times 75r_c \times 40r_c$ with the number density of $\rho = 3/r_c^3$. The area (A_0) per lipid when the membrane is under zero tension at the

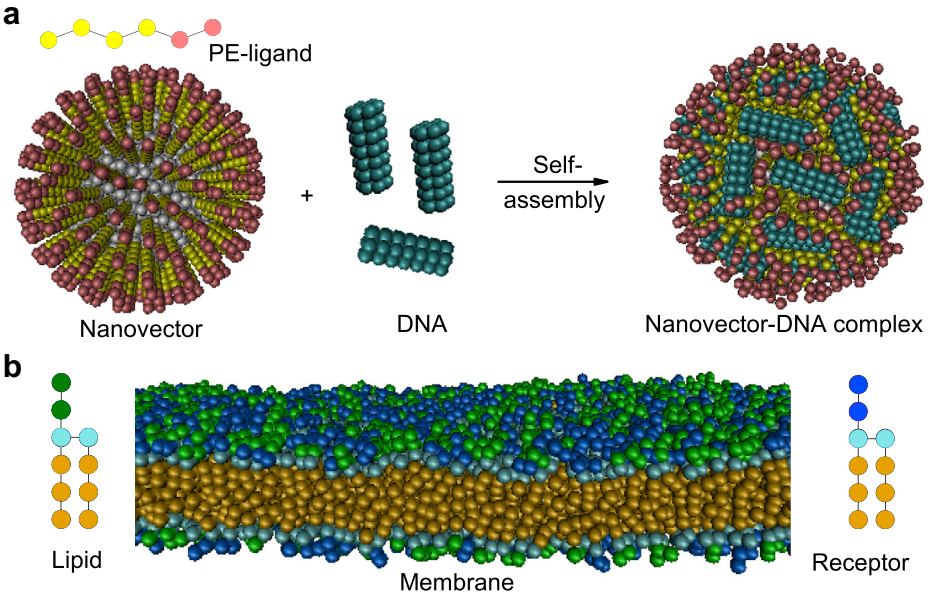

Fig. 1. Schematic illustration of the models in computer simulations. (a) Snapshots of the nanovector (rigid nanoparticle with polymer chains coating on its surface), DNA molecules, and their complexes in water; (b) Snapshot of membranes and architecture of lipids and receptors in DPD simulations. Green bead represents charged head in lipid molecule (the first green bead containing $+e$ and the second one containing $-e$), while lime bead stands for lipid head with no charge; the orange bead represents lipid tail, the blue bead stands for receptor head bead, and the cyan bead is DNA bead (half of the surface beads with charge $-e$); the white bead is nanoparticle bead, and the polymer chains are formed of ligand beads (pink) and PE beads (yellow) where some beads may carry charge $+e$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)