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Nanoscopic Characterization of DNA within Hydrophobic Pores: Thermodynamics and Kinetics

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ABSTRACT

The energetic and transport properties of a double-stranded DNA dodecamer encapsulated in hydrophobic carbon nanotubes are probed employing two limiting nanotube diameters, $D = 4$ nm and $D = 3$ nm, corresponding to (51,0) and (40,0) zig–zag topologies, respectively. It is observed that the thermodynamically spontaneous encapsulation in the 4 nm nanopore ($\Delta G \approx -40$ kJ/mol) is annihilated when the solid diameter narrows down to 3 nm, and that the confined DNA *termini* directly contact the hydrophobic walls with no solvent slab in-between. During the initial moments after confinement ($t \leq 2$–3 ns), the biomolecule translocates along the nanopore's inner volume according to Fick's law ($\sim t$) with a self-diffusion coefficient $D = 1.713 \times 10^{-9}$ m^2/s, after which molecular diffusion assumes a single-file type mechanism ($\sim t^{1/2}$). As expected, diffusion is anisotropic, with the pore main axis as the preferred direction, but an in-depth analysis shows that the instantaneous velocity probabilities are essentially identical along the x, y and z directions. The 3D velocity histogram shows a maximum probability located at $\langle v \rangle = 30.8$ m/s, twice the observed velocity for a single-stranded three nucleotide DNA encapsulated in comparable armchair geometries ($\langle v \rangle = 16.7$ m/s, $D = 1.36$–1.89 nm). Because precise physiological conditions (310 K and [NaCl] = 134 mM) are employed throughout, the present study establishes a landmark for the development of next generation *in vivo* drug delivery technologies based on carbon nanotubes as encapsulation agents.

1. Introduction

A plethora of applications currently envisage carbon nanotubes (CNTs) as next-generation encapsulation media for biological polymers, such as proteins and nucleic acids [1,2]. Owing to several appealing features, such as large surface areas, well-defined physico-chemical properties and the hydrophobic nature of their pristine structure, CNTs are considered ideal candidates to be used as nanopores for biomolecular confinement. Present day potential applications span different purposes and objectives, such as intracellular penetration *via* endocytosis and delivery of biological cargoes [3–5], ultrafast nucleotide sequencing [6–8] and gene and DNA delivery to cells [5,9,10]. The remarkable experimental work by Geng et al. [5,11] has shown that carbon nanotubes can spontaneously penetrate the lipid bilayer of a liposome, and the corresponding hybrid incorporated into live mammalian cells to act as a nanopore through which water, ions and DNA are delivered to the cellular interior. For an efficient and cost-effective industrial fabrication of SWCNT-based technology for DNA encapsulation/delivery, the interactions between the solid and the biomolecule need to be thoroughly understood in order to render the DNA/SWCNT device able to be used under physiological conditions, $T = 310$ K and [NaCl] = 134 mM. Nonetheless, the energetics and dynamics of single- (ssDNA) and double-stranded DNA (dsDNA) encapsulation onto single-walled carbon nanotubes (SWCNTs) are virtually unexplored and the corresponding molecular level details remain rather obscure. Previous theoretical and experimental work with DNA and SWCNTs has fundamentally been focused on the solids' external volume, overlooking the possibility of molecular encapsulation [12–14]; nevertheless, it is well known that the conformational properties of biopolymers under confinement are of crucial relevance in living organisms (*e.g.*, DNA packaging in eukaryotic chromosomes, viral capsids). Unrealistic high temperature (400 K) studies revealed that depending on pore diameter, a small 8 nucleobase-long ssDNA strand can be spontaneously confined [15]. However, there is a critical diameter of 1.08 nm [16], below which molecular confinement is inhibited by an energy barrier of *ca.* 130 kJ/mol, arising essentially from strong

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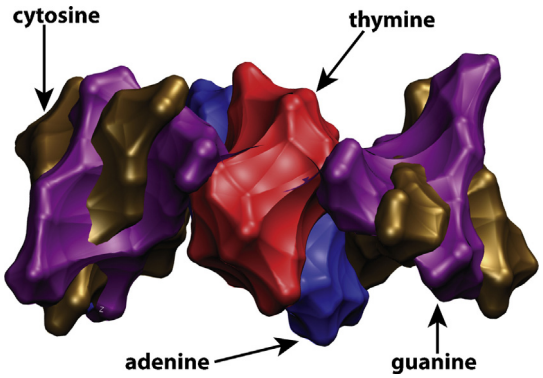

Fig. 1. Dickerson dsDNA dodecamer. Isovolumetric representation of the B–DNA Dickerson dodecamer [24] with sequence 5′-D(*CP*GP*CP*GP*AP*AP*TP*TP*CP*GP*CP*G)-3′, length $L = 3.8$ nm and skeletal diameter $D = 2$ nm, highlighting the changing chemical nature along the double strand; nucleobase residues are coloured according to their chemical nature, namely blue (A), red (T), purple (G) and brown (C). Note that the whole DNA molecule is atomistically detailed in the calculations, and thus each individual atom has a corresponding partial electrostatic charge. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

van der Waals repulsions [17]. These findings were extended for intratubular confinement of a 2 nm long ssDNA onto a SWCNT mimic of the constriction region of an α-hemolysin channel [18].

dsDNA confinement in carbon nanotubes remains utterly uncharted, for most of the earlier work has focused on temperatures remarkably distinct from the physiological value, thus preventing extrapolation of results to *in vivo* conditions. The pioneer work of Lau et al. [19] showed that a small dsDNA molecule (8 base pairs long), initially confined onto a $D = 4$ nm diameter nanotube, exhibits a root-mean squared displacement similar to the unconfined molecule, but that behaviour is drastically reduced as the nanotube narrows to $D = 3$ nm; Cruz et al. [20,21] have already demonstrated that diffusion inside SWCNTs can exhibit deviations from the classical Fickian behaviour. The insertion of dsDNA onto multi-walled carbon nanotubes has been experimentally observed by STM/STS, TEM and Rahman techniques [22,23]. However, it seems to be a competing mechanism with the wrapping of the biomolecule around the nanotube external walls; Iijima's reported data failed to identify the relevant conditions upon which the confinement process is favoured, such as ionic strength of the media and temperature [22]. In order to probe the thermodynamical spontaneity of encapsulation, we have adopted the well-known Dickerson dodecamer [24] as dsDNA model (Fig. 1) and conducted a series of well-tempered metadynamics calculations [25] involving two distinct nanotubes, namely (51,0) and (40,0) with skeletal diameters of $D = 4$ nm and $D = 3$ nm, respectively; very importantly, the results were obtained under precise physiological conditions, and the media ionic strength maintained at 134 mM by employing a NaCl buffer.

Our work indeed shows that the dsDNA molecule, initially in a bulk solution, can become encapsulated onto a $D = 4$ nm SWCNT leading to a pronounced decrease of the whole system's Gibbs free-energy [26]. The de Gennes blob theory for polymers has been extended by Jun et al. [27] to include the effect of cylindrical confinement upon the biopolymer free energy, and the latter decreases with an increase of nanotube diameter; because the blob description breaks down once the diameter approaches the DNA persistence length (strong confinement), Dai et al. [28] recently extended Jun's formalism to dsDNA strongly confined in slit-pore geometries. In order to address some of these encapsulation issues, the present work provides a full thermodynamical mapping of the associated Gibbs surface as well as a structural and kinetic analysis of dsDNA within cylindrical nanopores. The remainder of the manuscript is organized as follows: molecular models and methods are described in Section 2, followed by a discussion of the main results obtained (Section 3) and finally highlighting some conclusions and future lines of work (Section 4).

2. Methodology and algorithms

2.1. Molecular models

Molecules are described using atomistically detailed force fields, including electrostatic charges in each atom. The dispersive interactions are calculated with the Lennard-Jones (12,6) potential, cross parameters between unlike particles determined by the classical Lorentz–Berthelot mixing rules, and electrostatic energies described by Coulomb's law. DNA is treated as a completely flexible entity within the framework of the AMBER99sb-ildn force-field [29,30], the corresponding potential energies associated with bond stretching, $U(r)$, and angle bending, $U(\theta)$, are calculated with harmonic potentials, whilst the dihedral energies are computed using Ryckaert–Bellemans functions, $U(\varphi) = \sum_{\text{dihedrals}} \sum_{n=0}^{5} C_n[\cos(\varphi - 180^\circ)]$. To retain computational tractability, we have chosen the double-stranded B–DNA Dickerson dodecamer [24], exhibiting a pitch [31] of $P \sim 3.4$ nm obtained from an average of 10–10.5 base-pairs per turn over the entire helix [32], and with a double-strand end-to-end length of $L \sim 3.8$ nm measured between terminal (GC) base pairs (Fig. 1); the A–DNA form has $P \sim 2.6$ nm corresponding to an average of 11 base-pairs per turn [32]. Considering that the B–DNA backbone P atoms lie on a cylindrical surface, the diameter of the double-strand corresponds to $D \sim 2$ nm [31]. In spite of smaller in length than genomic DNA, the Dickerson dodecamer main structural features resemble those of genomic λ-bacteriophage DNA [33], namely in the radius of gyration and double-strand backbone diameter, $R_g \approx 0.7$–1 nm and $D \approx 2$ nm. The Na^+ and Cl^- ions are described with the parameterization of Aqvist and Dang [34] and the H_2O molecules by the TIP3P force field of Jorgensen et al. [35].

Large diameter ($D \approx 4$ nm) SWCNTs have been recently prepared by Kobayashi et al. [36]. In order to examine DNA confinement into such large, hollow nanostructures, two different diameter SWCNTs were adopted, both with zig–zag symmetry and length $L = 8$ nm; skeletal diameters, measured between carbon atoms on opposite sides of the wall, are $D = 4$ nm (51,0) and $D = 3$ nm (40,0). The solid walls are built up of hexagonally-arranged sp^2 graphitic carbon atoms, with a C—C bond length [21,37] of 1.42 Å, whose Lennard-Jones potential is given by Steele's parameterization ($\sigma = 0.34$ nm, $\epsilon = 28$ K) [38].

2.2. Molecular dynamics and metadynamics

Molecular dynamics (MD) simulations in the isothermal–isobaric ensemble (*NpT*) were performed using the Gromacs set of routines [39]. Newton's equations of motion were integrated with a time step of 1 fs and using a Nosé–Hoover thermostat [40,41] and a Parrinello–Rahman barostat [42] to maintain temperature and pressure at 310 K and 1 bar. A potential cut-off of 1.5 nm was employed for both the van der Waals and Coulombic interactions, and the long-range electrostatics were calculated with the particle-mesh Ewald method [43,44] using cubic interpolation and a maximum Fourier grid spacing of 0.12 nm. Three-dimensional periodic boundary conditions were applied throughout the systems.

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