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# Synthesis and characterization of doxorubicin hydrochloride drug molecule-intercalated DNA nanostructures

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ABSTRACT

In this paper, we demonstrate the feasibility of constructing DNA nanostructures (*i.e.* DNA rings and double-crossover (DX) DNA lattices) with appropriate doxorubicin hydrochloride (DOX) concentration and reveal significant characteristics for specific applications, especially in the fields of biophysics, biochemistry and medicine. DOX-intercalated DNA rings and DX DNA lattices are fabricated on a given substrate using the substrate-assisted growth method. For both DNA rings and DX DNA lattices, phase transitions from crystalline to amorphous, observed using atomic force microscopy (AFM) occurred above a certain concentration of DOX (at a critical concentration of DOX, 30 μM of $[DOX]_C$) at a fixed DNA concentration. Additionally, the coverage percentage of DNA nanostructures on a given substrate is discussed in order to understand the crystal growth mechanism during the course of annealing. Lastly, we address the significance of optical absorption and photoluminescence characteristics for determining the appropriate DOX binding to DNA molecules and the energy transfer between DOX and DNA, respectively. Both measurements provide evidence of DOX doping and $[DOX]_C$ in DNA nanostructures.

## 1. Introduction

For last three decades, DNA nanotechnology has been considered as a very promising research topic in the fields of nanoscience and bio-nanotechnology. With the advantages of exceptional base-sequence programmability and efficient bottom-up self-assembly following the Watson-Crick's complementary rule, structural DNA nanotechnology can provide an effective platform to fabricate DNA structures with distinct dimensionalities and shapes with nanometer scale accuracy [1–4]. Additionally, due to the intrinsic physical properties and the simple chemical modifications, DNA can serve as an efficient template for decorating with various functionalized nanomaterials such as ions, nanoparticles, organic/inorganic molecules, dyes, proteins, and drugs [5–11].

Although nano-sized drug carriers such as micelles, nanoparticles, polymer drug conjugates and liposomes have been introduced, experimental results suggest a low release rate of drugs, significant accumulation of drugs in the body and reduced bio-compatibility [12–15]. Consequently, developing a safe and effective drug delivery system is very challenging. Recently, size controllable, relatively rigid and encapsulation-capable DNA structures with various dimensions and sizes have started to be used as carriers to deliver functionalized nanomaterials because of their molecular recognition and easy modification of strands [16–19].

The important advantage of DNA is its biodegradability; DNA molecules therefore show very little cytotoxicity or immunogenicity, both of which are important features in a drug delivery system [20,21]. In order to use DNA nanostructures as drug delivery vehicles, it is important to understand the particular binding mechanism among the selected drug and DNA nanostructures and the critical doping concentration of drug molecules at a given DNA concentration. Representative binding modes of drug molecules are available with DNA duplexes such as steric preference in major and minor grooves, intercalation between base-pairs, and electrostatic interaction with phosphate backbones [10]. Among the anticancer drugs, doxorubicin hydrochloride (DOX) is one of the most widely used in chemotherapy with fewer side effects. The DOX drug molecules bind through intercalation into a carrier made of DNA and inhibit the macromolecular biosynthesis of cancer cells [22].

In this article, we explore the construction and characterization of

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the artificially designed synthetic DNA rings and double crossover (DX) DNA lattices (grown using the substrate assisted growth (SAG) method) intercalated with various concentrations of DOX ([DOX]) to evaluate the critical doping concentration of DOX ($[DOX]_C$) at a given [DNA] and study the optical characteristics. Phase transitions of DNA nanostructures from crystalline to amorphous phases were observed above $[DOX]_C$ (*i.e.*, 30 μM in both DNA rings and DX lattices) and the intensities of optical measurements of the DNA nanostructures at $[DOX]_C$ showed extremum values. Additionally, the coverage percentages of DNA nanostructures on mica are determined and discussed in order to understand the crystal growth behaviour during the course of annealing. The phase change, absorbance, and photoluminescence (PL) characteristics of DOX-intercalated DNA nanostructures were studied using atomic force microscopy (AFM), ultraviolet–visible (UV–Vis.) spectrophotometry and PL spectroscopy, respectively, in order to show the feasibility of applications in the fields of drug delivery system and biosensors.

## 2. Experimental section

### 2.1. Fabrication of DOX-intercalated DNA nanostructures using the substrate assisted growth (SAG) method

The synthetic DNA oligonucleotides purified with high performance liquid chromatography are obtained from Bioneer (Daejeon, Korea). For the substrate assisted growth (SAG) method, individual 2 ring strands (or 8 DX DNA strands), freshly cleaved mica (size of 5 × 5 $mm^2$, for AFM) or $O_2$ plasma-treated quartz (5 × 5 $mm^2$, for optical) as well as the appropriate amount of DOX (*i.e.* 10, 20, 30, 40 and 50 μM) (Sigma Aldrich, USA) are added. The total sample volume of 250 μL in a 1 × TAE/$Mg^{2+}$ buffer (40 mM Tris, 20 mM Acetic acid, 1 mM EDTA and 12.5 mM magnesium acetate) is achieved. The sample test tube is placed in a Styrofoam box with 2 L of boiling water, and then cooled down slowly from 95 °C to 25 °C for 24 h. During the annealing process, the individual building blocks of a ring (or DX DNA tiles) are bound onto a given substrate through electrostatic interaction followed by the formation of complete rings (or lattices). Finally, DNA rings (or DX DNA lattices) with 50 nM of DNA concentration are positioned to fully cover the substrate. We called this annealing procedure the before-annealing method (*i.e.*, individual DNA strands and DOX with the substrate are mixed before annealing), which is adopted to obtain a critical concentration of DOX at a given DNA concentration.

We also use the after-annealing method (*i.e.*, DNA nanostructures on the substrate are formed before adding DOX), which is adopted for constructing the DNA nanostructures without deformation, even with slightly higher DOX concentration than the critical concentration of DOX. DOX-intercalated DNA nanostructures for ultraviolet–visible spectroscope and photoluminescence measurements are prepared using this method. (Fig. 1, Fig. S1, Fig. S2 and Table S1 ~ Table S4 in Online Supplementary Data (OSD))

### 2.2. Atomic force microscopy (AFM) imaging

For atomic force microscopy (AFM) imaging, the mica-assisted grown DNA rings or DX DNA lattices are placed on a metal puck and fixed with instant glue. A 30 μL of 1 × TAE/$Mg^{2+}$ buffer is added to the substrate and another 20 μL of 1 × TAE/$Mg^{2+}$ buffer is dispensed into the AFM tip (NP-S10, Veeco Inc., USA). The AFM images are obtained using a Multimode Nanoscope (Veeco Inc., USA) in the fluid tapping mode (Fig. 2).

### 2.3. Ultraviolet–visible (UV–Vis) spectroscope measurement

A spectrophotometer (Cary 5G, Varian, USA) is used to determine the optical absorbance of the DOX-intercalated DX DNA lattices on quartz in the visible and UV regions (wavelength between 800 and 190 nm). The spectrophotometer is equipped with two light sources: a deuterium arc lamp and a quartz W-halogen lamp. The instrument also has two detectors: a cooled PbS detector and a photomultiplier tube. The spectrophotometer measures the frequency-dependent light intensity passing either through a vacuum or through the sample (Fig. 3).

### 2.4. Photoluminescence (PL) measurement

The photoluminescence (PL) characteristics of the DOX-intercalated DNA rings and DX lattices grown on quartz are measured at room temperature using the Xe-arc lamp equipped fluorometer (FS-2, Scinco, Seoul, Korea) with 25 W. The excitation spectra are obtained at a fixed emission wavelength of 598 nm, while the emission spectra are measured by exciting the samples at 490 nm (Fig. 4).

## 3. Results and discussion

Fig. 1 shows the fabrication procedure of the doxorubicin hydrochloride (DOX)-intercalated DNA rings and DX lattices constructed using the SAG method. Two different DNA structures (*i.e. t*-tile based DNA rings and double-crossover (DX)-tile based DNA lattices) were designed, onto which the implementation of drug molecules was demonstrated. A DNA ring was comprised of 12 t-shaped unit building blocks. Each building block contained two single-stranded bulged loops in the middle of duplex and single-stranded segments at both ends of the helix. Bulged loops and segments were complementary to each other and acted as sticky-end base pairs. The designed ring dimensions of the inner- and the outer-diameters were 13 and 29 nm, respectively (Fig. 1(a), Fig. S1, Table S1 and Table S2 in the OSD) [23–25]. In addition, the DNA lattices were constructed of two different DX tiles (*i.e.*, DX1 and DX2 shown in Fig. S2, OSD) [26–29]. Two parallel duplexes in a DX tile (with the dimensions of 12.6 × 4 $nm^2$) were connected by the two crossover junctions (Fig. 1(b), Fig. S2, Table S3 and Table S4 in OSD). In order to grow DNA structures on a given substrate during the course of annealing, a substrate was added to the test tube containing individual DNA strands and DOX. To understand $[DOX]_C$ and coverage percentage of DNA structures, the annealing time of 24 h, substrate size of 5 × 5 $mm^2$ and DNA sample volume of 250 μL were fixed while varying [DOX] and [DNA], respectively.

Fig. 2(a–h) show the AFM images of DOX-intercalated DNA rings and DX DNA lattices with varying DOX concentrations ([DOX]). We performed the AFM measurement not only to verify the proper formation of DNA structures (*i.e.*, rings and lattices) but also to estimate the $[DOX]_C$ at a given [DNA] of 50 nM. Above $[DOX]_C$, topological changes occurred that transformed the DNA rings (DX lattices) to deformed rings (lattices), *i.e.*, crystalline to amorphous. The typical AFM images of DOX-intercalated DNA rings with 0, 10, 30 and 40 μM of [DOX] labelled as DOX 0, DOX 10, DOX 30 and DOX 40, respectively, are shown in Fig. 2(a–d). The inset image in Fig. 2(a) shows the magnified AFM image of DNA rings (scan size of 150 × 150 $nm^2$). Similarly, Fig. 2(e–h) depict the characteristic AFM images of the DOX-intercalated DX DNA lattices with 0, 10, 30 and 40 μM of [DOX]. The inset (scan size of 100 × 100 $nm^2$) in Fig. 2(g) shows the noise-filtered image reconstructed by fast Fourier transformation (FFT), which clearly revealed the periodicity of the DX tiles. AFM results revealed that the maximum doping concentrations of DOX in both DNA rings and DX lattices without structural deformation, *i.e.*, $[DOX]_C$, were unintentionally identical, which were 30 μM. The vertical green line in Fig. 2 indicates that the structures were either stable (left side of green line) or deformed or amorphous (right side). The DOXs were placed between layers of base-pairs of DNA duplexes through chemical bonds with nucleotides (Fig. 1). At a [DOX] higher than $[DOX]_C$, the excess DOXs that were non-specifically bound to DNA might produce unwanted stress and strain on the DNA duplexes, causing deformation of the DNA structures. The topological transitions (from crystalline to amorphous) of DNA structures were also observed with other functional