Forecasting the Reaction of DNA Modifying Enzymes on DNA Nanostructures by Coarse Grained Model for Stimuli-Responsive Drug Delivery

Yingnan Deng

Beijing University of Chemical Technology

Yuanhang Tan

Beijing University of Chemical Technology

Linghao Zhang

Beijing University of Chemical Technology

Chunyi Zhang

Beijing University of Chemical Technology

Xin Su (✉ pkusuxin@gmail.com)

Beijing University of Chemical Technology <https://orcid.org/0000-0002-6629-9856>

Article

Keywords: DNA modifying enzymes, DNA nanotubes, doxorubicin, nanomedicine

Posted Date: November 3rd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1038517/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

The reactivity of DNA modifying enzymes on their natural nucleic acid substrates has been fully understood. However, their reactivity on self-assembled nanostructures of nucleic acid is complicated and unpredictable. Here, we employed the molecular dynamic simulation to forecast the reactivity of tumor biomarker enzymes on DNA nanotubes by coarse grained model. It is found that the enzyme accessibility and the potential energy of the reaction products co-determine the structural change of DNA nanotubes. The reactivity can be regulated by the position of enzyme recognition site. According to the simulation results, stimuli-responsive drug nanocarrier with superior sensitivity and selectivity was developed. Drug payloads released in cancer cells is 3.7~5.5-fold higher than that in normal cells. The DNA nanocarrier equipped with cancer-specific aptamer AS1411 is used to deliver doxorubicin (DOX) to tumor-bearing mice not only effectively inhibiting tumor growth but also protecting major organs from drug-caused damage. This work provides new insights into the enzymatic reactivity of DNA nanostructures enriching the library of DNA-based reactions and heralding broad applications in nanomedicine.

Introduction

DNA nanotechnology offers controllable self-assembly on the nanoscale, allowing for the design of static structures, dynamic machines and computational architectures. Such nanotechnology provides unprecedented opportunities for nanomedicine applications including biosensing, cell modulation, bioimaging and drug delivery^{1,2}. Owing to the excellent modularity and biocompatibility of DNA nanostructures, stimuli-responsive drug nanocarriers were established. A variety of drug molecules including chemotherapeutic agents³, small interfering RNA (siRNA)⁴, immunostimulatory sequences⁵, can be released in response to external and internal stimuli. The interaction of DNA nanostructure and enzymes particularly those DNA modifying enzymes or nucleases have drawn increasing attention because enzymes or nucleases are capable of enriching the applications in nanomedicine.

Nuclease activity is desired to be inhibited because nucleases present in the physiological environment can cause the undesired degradation of DNA nanostructures in real-life applications^{3,6}. On the contrary, nuclease activity is desired to be maintained on DNA nanostructures for developing molecular probes⁷, responsive systems⁸, and complicated networks^{9,10}. However, unlike the reaction between enzymes and natural substrates, the reactivity of enzymes on DNA nanostructure is complicated and unpredictable because the environment of enzyme recognition sites on nanostructures and the internal properties of nanostructures can be different from that of natural substrates.

Coarse-grained models provide a level of resolution between fully atomistic treatments and secondary-structure descriptions like the nearest-neighbor model¹¹. Coarse-grained models represent individual nucleotides using a small number of interaction sites, which interact through effective potentials. If well parameterized, they can capture the known thermodynamic, structural and mechanical properties of DNA in a simple and naturally dynamical representation¹². oxDNA a simulation code which implements the

coarse-grained DNA model has been utilized to optimize DNA nanodevices including DNA tweezer¹³, origami¹⁴, and strand displacement reaction¹⁵. Therefore, this platform holds great potential to investigate the interaction of DNA modifying enzymes and DNA nanostructures.

Here, we forecast the reaction of DNA nanotubes and the enzyme, glycosylase (uracil degradation glycosylase) and endonuclease (Human apurinic/apyrimidinic (AP) endonuclease, APE1) which involves in cellular base excision repair (BER) pathway by coarse-grained model. These enzymes are significantly active in the cytoplasm of cancer cell line rather than that of the normal cell line, have emerged as promising biomarkers for cancer diagnostics and treatment. The simulation discloses that the orientation of enzyme recognition site and the potential energy of the product of enzymatic reaction exhibit synergistic effects on the responsiveness of DNA nanotubes. The nanotube was used to carry the anticancer drug doxorubicin (DOX). The simulation-guided location choice of enzyme recognition site permits highly sensitive and specific drug release in cancer cells. DNA nanotubes equipped with tumor-specific aptamers effectively inhibited tumors growth in tumor-bearing mice, and effectively protected major organs from damage. We anticipate that this work provides new insights into the design of functional DNA nanostructures and breaks through the bottleneck of stimuli-responsive nanocarriers in sensitivity and specificity.

Result And Discussion

Simulation of uracil-contained DNA nanotubes by coarse grained model

BER pathway is significantly active in cancer cells¹⁷. The critical enzymes UDG and APE1 in this pathway are overexpressed in the cytoplasm of various cancer cells¹⁸⁻²⁰. These two enzymes process uracil-containing DNA. UDG as a glycosylase catalyzes the hydrolysis of the *N*-glycosidic bond from deoxyuridine to release uracil thereby generating AP site²¹. APE1 as an endonuclease cleaves DNA phosphodiester backbone at AP sites via hydrolysis leaving a one nucleotide gap with 3'-hydroxyl and 5' deoxyribose phosphate termini (Scheme 1)²². We implemented uracil-containing DNA nanotubes in response to these enzymes. Figure 1A shows the principle structure of DNA nanotube. The DNA tile consisting of five single-stranded DNA is the building block of DNA nanotube. Sticky end hybridization allows for the nanotube formation. To make the DNA nanotubes responsive to UDG and APE1, thymine (T) needs to be substituted by uracil (U). Enzyme accessibility and the stability of enzymatic reaction product determine the degradation degree of DNA nanotubes in the presence of enzymes. Accordingly, the T to U substitution position is crucial for the reaction, which can be evaluated by coarse grained simulation. With the advance of *in silico* design tools such as caDNAno, Tiamat, CanDo, and DAEDALUS, the design of such DNA nanostructures is now a relatively fast and well-developed process²³. To start simulation, we use caDNAno to design DNA nanotubes as the input structure for coarse grained model simulation on oxDNA platform²⁴. According to previous study, such DNA nanotube has the diameter with an average of 13.5 nm, the circumference contains 14 DNA tiles (Figure 1, Figure S1)^{24,25}. DNA

nanotubes is usually stabilized by an appropriate Mg concentration (e.g. 12.5 mM). Under this ionic strength (12.5 mM Mg equals to 0.09397 M monovalent cation), 87% hydrogen bond strength was used as oxDNA simulation input because of the small root mean squared fluctuations (RMSF) and its narrow distribution (Figure S2). RMSF can observe how various traps modify the flexibility of individual nucleotides, it can use single value decomposition alignment to calculate the mean position of every nucleotide, then again analyzes the trajectory to get RMSF of each particle from its mean position²⁶. The RMSF can reveal the dynamics of each area of the nanotube^{16,27}. A small and narrowly distributed RMSF reflects a stable structure.

First, we simulated the conformation of DNA nanotube to study the enzyme accessibility which depends on base orientation. We focused on fourteen T to U substitution sites on S1 and S5 strands which are hybridized with the bases of S3 strand (highlighted in Figure 1A). Under the simulation condition, the detailed environment of the fourteen positions are shown in Figure 1B and Figure S3. Briefly, S1-P3, S1-P1, and S5-P6 bases are outward, the others are inward. These results imply the U to T substitution at S1-P3, S1-P1, and S5-P6 may exhibit high enzyme accessibility.

Second, we investigated the stability of enzymatic reaction products by simulation. According to the enzyme properties, one-base gap can be generated after enzymatic cleavage. Figure 1C shows the enzymatic reaction product if the uracil is located at S1-P1 position. According to the simulation, fourteen reaction products of nanotubes whose tiles have one-base gap exhibit different potential energy (Figure 1D). The control structure (no enzyme-caused break) with highest stability yields the lowest energy. S1-P5 shows the highest potential energy among all the candidates indicating that it has lowest stability. We analyzed the average structure and the distribution of RMSF of each nucleotide for the nanotubes. As shown in Figure 1E, the control structure (no enzyme-caused break) exhibits a small RMSF and a narrow distribution. At a glance of the positions on S1 strand, RMSF of S1-P5 is significantly higher than that of the other structures implying that S1-P5 can yield a high degradation degree. S5-P1 in S5 strand shows the similar feature as S1-P5 (Figure S4). Hydrogen bonding is an important parameter that defines the geometric structure of DNA nanotechnology¹⁶. Since the stable final structure is designed as the theoretical minimum global free energy, the hydrogen bond is maximized. The change in the hydrogen bond occupancy rate shows that the current structure deviates from the designed structure and points to other important topological strain regions. Therefore, we calculated the occupancy of hydrogen bonds with a strength of less than 10% hydrogen bond strength to the total hydrogen bonds. The occupancy rate of hydrogen bonds indicates the degree of dissociation of the entire DNA nanotubes structure (Figure 1F). Consistent with the results of potential energy and RMSF, S1-P5 yields the lowest occupancy of hydrogen bonds indicating a high degree of degradation.

By comprehensively analyzing the simulation results, we categorized the fourteen candidates into two classes: 'high quality', and 'low quality', which reflect the potential degradation degree of the nanotubes after enzymatic reaction. 'High quality' class has the following characteristics: outward base orientation or low stability of the cleaved tile. In contrast, the candidates in 'low quality' class have inward base

orientation and high stability of the cleaved tile. Accordingly, for S1 strand, S1-P5, S1-P4, and S1-P3 belongs to 'high quality' class, S1-P1 and S1-P2 belongs to 'low quality' class.

Characterization and Responsiveness of DNA nanotubes

All candidates on S1 strand were tested to validate the simulation results. The nanotubes were assembled by the reported protocol^{25,28}. Gel electrophoresis reveals that the assembled nanotubes have high purity, and T to U substitution has no influence on the nanotube assembly (Figure S5). Further characterization was performed by using total internal reflection fluorescence (TIRF) microscopy which provides high S/N for near field excitation²⁹. As shown in Figure S6A, short strip was found, and the length of nanotube was estimated as ~400 nm which is consistent with the measurement by dynamic light scattering (DLS) (Figure S6B). The reaction of uracil-containing nanotube and enzymes were first characterized by gel electrophoresis (Figure 2A). Only when both enzymes are present can the nanotubes be degraded. The degradation degree of S1-P3, S1-P4, S1-P5 is significantly higher than the that of S1-P1 and S1-P2. This result is perfectly matched with the simulation results. S1-P3 and S1-P5 were chosen as the nanocarriers for drug delivery. Detailed characterization was carried out for S1-P3 and S1-P5. Fluorophore and quencher were labeled on their S3 and S4 strands for the measurement of reaction kinetics (Figure S7). As shown in Figure 2B, the fluorescence of S1-P3 and S1-P5 rapidly increases in the presence of UDG and APE1. In contrast, the control nanotube (no uracil) shows no fluorescence enhancement. These two nanotubes can respond the low concentration of enzyme down to 200 pM of APE1 and 1 nM of UDG. The nanotubes show the same level of enzymatic reactivity as uracil-containing double-stranded substrate, which subverts traditional cognition that the reactivity of DNA nanostructures is weaker than that of natural substrates (Figure S8). By the assistance of simulation, the reaction sensitivity of nucleic acid nanostructure can be modulated as that of natural substrate. Specificity is another important parameter for these nanotubes. Owing to the nature of nucleic acids, DNA nanostructures are vulnerable to non-specific nucleases³⁰. In contrast, our results show that the nanotubes are resistant to a variety of nucleases (Figure S9). Tubular structure may make these nanotubes resistant to endonucleases, and the terminal structure of DNA tiles reduces the accessibility of exonucleases. The resistance of nanotubes to nonspecific nuclease is superior than double-stranded DNA³¹. This result guarantees the specificity of the nanotube. Furthermore, TIRF imaging was performed to directly visualize the UDG/APE1-caused degradation of nanotubes (Figure 2C). After enzymatic reaction, the uracil-containing nanotubes disappear, and the control nanotube keeps intact. Collectively, the simulation-guided nanotubes exhibit high sensitivity and specificity towards UDG and APE1, which makes them as excellent stimuli-responsive nanocarriers.

Next, we explored enzyme-stimulated drug release of the nanotubes. DOX is commonly used to as model drug to test the drug delivery system owing to its excellent antitumor effect and fluorescence property³². DOX is suitable for DNA-based nanocarriers because it intercalates into the minor groove of double-stranded DNA³³. The low pH in tumor microenvironment (TME) is shared by healthy joints³⁴, intracellular milieu³⁵, and the stomach. pH-responsive drug release lacks tumor specificity. As shown in Figure 2D, the nanotube is not sensitive to pH, the disassembly of DNA nanotubes is slow at pH 7.4 (physiological) and

5.6 (endo-lysosome), and the drug release is less than 10% within 24h. In contrast, in the presence of BER enzymes, the DNA nanotubes are rapidly disintegrated, and more than 50% of the drug is released within 4h, the release can reach 77% within 24h. The loading efficiency of DNA nanotubes of 78%, which can be comparable with currently used nanomaterials^{36,37}. These results imply that the nanotubes are robust and not sensitive to pH and can effectively release drugs under the environment containing highly expressed BER enzymes.

Cellular uptake of DNA nanotubes and drug delivery

The drug nanocarriers S1-P3 and S1-P5 were used to demonstrate the stimuli-responsive release in different cell lines. Highly inclined and laminated optical sheet (HILO) fluorescence microscopy which provides high S/N for cellular fluorescence excitation was used to cellular the cellular behaviors of the DNA nanotubes. The size and shape of DNA nanostructures has influence on their cellular uptake efficiency³⁸. We first explored the cellular internalization of the nanotubes with different length which were assembled with different annealing speed, 18h for long nanotube and 1.5h for short nanotube (Figure S10). As a result, the short nanotube exhibits significantly higher efficiency of internalization than the long one (Figure 3A). The fluorescence of long nanotube mainly accumulates on the cell membrane. The long one-dimensional size results in the adsorption of lipid and DNA nanotube and the poor cellular uptake efficiency. Thus, short uracil-containing nanotube was used for drug delivery in cell.

The overexpression of BER enzymes UDG and APE1 have been verified in the cytoplasm of a variety of cancer cell lines¹⁸. We used fluorophore and quencher labeled uracil-containing nanotubes to demonstrate the differential expression level of these enzymes in cancer and normal cell lines (Figure 3B). The nanotubes exhibit significantly higher Cy5 fluorescence in cancer lines HeLa, MCF7, and A549 than in normal cell line HEK-293 (Figure 3B, S11, and S12). For S1-P5 nanotube, the mean fluorescence intensity (MFI) is 7, 19, and, 4-fold higher in HeLa, MCF-7, and A549 cell lines than in HEK-293 cell line (Figure 3B, S11, and S12). The control nanotube (no uracil) shows weak fluorescence in all cell lines indicating the reaction specificity. APE1 inhibitor 7-nitroindole-2-carboxylic acid (NCA) was used to further confirm the specificity³⁹. As expected, negligible fluorescence was found in HeLa cell for S1-P5 nanotube in the presence of NCA (Figure S13). All these results suggest that the uracil-containing nanotubes are capable of reacting with cellular UDG and APE1 accordingly.

DOX could fight against various cancers by embedding in genomic DNA, covalently binding to proteins involved in DNA replication and transcription, or inhibiting topoisomerase II, inducing apoptosis of tumor cells and the main target of DOX is nucleus DNA⁴⁰. According to previous study, DNA nanostructures cannot enter nucleus⁷. Moreover, our results also show that the nanotubes mainly distribute in cytoplasm rather than nucleus (Figure 3B, S11, and S12). Therefore, only when the nanotube drug carrier disintegrates in cytoplasm can the drug enter the nucleus. As expected, strong DOX fluorescence can be observed in the nucleus of all the cancer cell lines when uracil-containing nanotubes are used as DOX carriers (Figure 3C, S14, and S15). According to the MIF in nucleus, nanotube drug carriers release DOX

3.7~5.5-fold higher in cancer cell lines than in normal cell line (Figure 3C, S14, and S15). In contrast, free DOX without using any carriers can undifferentiated enter the nucleus of cancer and normal cells (Figure 3C, S14, and S15). We further evaluated the cell toxicity of this system. As shown in Figure S16, all the drug-free nanotubes (uracil and non-uracil) do not show any observable toxicity on cancer and normal cell lines. Uracil-containing nanotubes exhibit high toxicity on cancer cell but not on normal cell when DOX was loaded. The DOX loaded control nanotube (no uracil) have relatively weak toxicity to all cells. Free DOX exhibit high toxicity to all types of cell. In this way, the drug effect can be precisely administrated by the BER enzyme-responsive DNA nanotubes allowing for selectively inhibiting cancer cells and protecting normal cells.

In vivo therapeutic efficacy

Inspired by the excellent performance at the cellular level, we further evaluated the *in vivo* therapeutic efficacy. All animal experiments were approved by the ethics committee of our institute and were performed in accordance with the guidelines of the institution animal care and use. DNA nanotubes and DOX were applied to the tumor-bearing mice (HeLa cell) which were continuously treated for 12 days by tail vein injection of saline, free DOX, control-DOX, S1-P5-DOX (DOX equivalent 2 mg/kg), the administration was shown in Figure 4A. DNA nanotubes were equipped with AS1411 aptamer to enhance tumor targeting (Figure S17)⁴¹. First, the *in vivo* enzymatic reaction was investigated by using the fluorophore and quencher labeled nanotubes (no DOX). From the fluorescence imaging, we found that the uracil-containing nanotube (S1-P5) shows stronger fluorescence signal at the tumor site than the non-uracil nanotube within 4h (Figure 4B). This implies that the aptamer equipped nanotubes can accumulate at the tumor, and BER enzymes are sufficiently active to stimulate DNA nanotubes in the implanted tumor cells.

DOX-loaded nanotubes were used to show the anticancer effect. During 12 days of the therapeutic period, there is no obvious change of the body weights for all groups implying no significant toxicity for the DNA nanotubes (Figure S18). The tumor volumes of all groups were recorded in different days. As shown in Figure 4C and 4D, the tumor volumes in the groups of PBS, free DOX, and control-DOX gradually increased by 15.5, 6.0, and 11.7 fold, respectively, while S1-P5-DOX could obviously inhibit the growth of tumor. Furthermore, the survival rate of HeLa-tumor-bearing mice was evaluated. Mice treated with S1-P5-DOX achieved a 100% survival rate after 50 days (Figure 4E). Hematoxylineosin (H&E) and Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining revealed that the therapeutic delivered by S1-P5-DOX induced a higher level of nucleus necrosis and apoptosis of tumor tissue than the other groups (Figure 4F). H&E staining analysis of major organs reveals the high biocompatibility of all the groups except for the free DOX group (Figure 4G and S19). Owing to the side effect of DOX, focal necrotic cell death (blue arrow) heart and liver can be observed (Figure 4G)⁴². The above results indicate that the BER stimuli-responsive DNA nanotubes effectively reduce the side effects of chemotherapy drugs on the major organs, and massively accumulated at the tumor site then specifically release the drug on demand to achieve the promising therapeutic effect.

Discussion

Targeted drug delivery remains effective approach for cancer therapeutics⁴³. Stimuli-responsive drug carriers that undergo degradation or conformational change in response to external or endogenous stimuli have been engineered to control drug release in the tissue of interest effectively enhancing targetability and mitigating side effects^{2,44,45}. The abnormal pH in microenvironment of tumor, cancer molecular biomarkers (e.g. nucleic acids, proteins), and even external energy sources (e.g. light, sound) have been employed as stimuli to design and engineer drug carriers^{46–48}.

The sensitivity and specificity of drug carriers are the limiting factors for the efficacy of stimuli responsive drug delivery. Base excision repair (BER) pathway is a cellular mechanism that repairs damaged DNA induced by eliminating damaged (oxidized or alkylated) or inappropriate bases that are generated endogenously or induced by genotoxins throughout the cell cycle⁴⁹. BER recruits multiple enzymes including DNA glycosylase, endonuclease, polymerase, and ligase²². BER has been proven to be more active in tumor cells and the enzymes were significantly overexpressed than normal cells¹⁷. It is advantageous in the specificity and sensitivity to exploit the enzymes in BER pathway as the stimuli to develop responsive drug delivery system. Despite the rapid progress made in the field of DNA based nanocarriers, it remains challenging to accurately predict the enzyme responsiveness at the design stage by using current design procedures because the interaction of complicated DNA nanostructure-enzyme is distinct from that of natural nucleic acid substrate-enzyme.

In this work, molecular dynamic (MD) simulation based on coarse grained model is employed to predict the reaction of BER enzymes which are DNA modifying enzymes and DNA nanotubes. With the guidance of MD simulation, uracil-containing DNA nanotubes can respond to the BER enzymes in cancer cells with high sensitivity and specificity. The nanotubes hold potential to program the drug release kinetics by altering T to U substitution position. Owing to superior release selectivity, DNA nanotubes can release drugs in a variety of tumor cells (HeLa cells, MCF7 cells, and A549 cells) but not in normal cells (HEK 293 cells). DNA nanotubes equipped with AS1411 aptamers effectively inhibit tumor growth in HeLa-bearing mice, and protect major organs from damage. The simulation procedure provides new insights into the interaction of DNA nanostructures and DNA modifying enzymes which enriches the library of DNA-based reactions. This work is anticipated to find broad applications in DNA chemistry and biomaterials.

Declarations

Acknowledgement

This work was supported by National Natural Science Foundation of China (31971361), State Key Research Development Program of China (2019YFC1200500, 2019YFC1200502), the Natural Science Foundation of Beijing Municipality (5212013), the Capital Health Research and Development of Special under Grant (021-1G-4302), the Fundamental Research Funds for the Central Universities and Research projects on biomedical transformation of China-Japan Friendship Hospital (XK-2020-08).

Author Contribution

X.S. conceived the ideas and designed the study. Y.D., C.Z., and X.S. designed, performed and analysed the experiments. Y.T., L.Z. completed the molecular simulation. Y.D. wrote the manuscript, with critical revision by X.S. X.S. provided technical support and advice. All the authors read and approved the final version of the manuscript.

Competing interests

There is no conflict to declare.

Data availability

The data that supports the plots within this paper and other finding of this study are available from the corresponding author upon reasonable request.

Supplementary information

Supplementary Figure S1-19, Supplementary Table 1-2.

References

1. Wu, N. & Zhao, Y. DNA Nanostructures as Drug Carriers for Cellular Delivery. *Chem. Res. Chin. Univ.* **36**, 177–184 (2020).
2. Duangrat, R., Udomprasert, A. & Kangsamaksin, T. Tetrahedral DNA nanostructures as drug delivery and bioimaging platforms in cancer therapy. *Cancer Sci* **111**, 3164–3173 (2020).
3. Wang, C. *et al.* Rational Design of DNA Framework-Based Hybrid Nanomaterials for Anticancer Drug Delivery. *Small* **16**, 2002578 (2020).
4. Zhao, D. *et al.* A photo-triggerable aptamer nanoswitch for spatiotemporal controllable siRNA delivery. *Nanoscale* **12**, 10939–10943 (2020).
5. Li, J. *et al.* Self-Assembled Multivalent DNA Nanostructures for Noninvasive Intracellular Delivery of Immunostimulatory CpG Oligonucleotides. *ACS Nano* **5**, 8783–8789 (2011).
6. Chandrasekaran, A. R. Nuclease resistance of DNA nanostructures. *Nat Rev Chem* **5**, 225–239 (2021).
7. Zhang, Y. *et al.* Probing and regulating the activity of cellular enzymes by using DNA tetrahedron nanostructures. *Chem. Sci.* **10**, 5959–5966 (2019).
8. Li, L. *et al.* Base excision repair-inspired DNA motor powered by intracellular apurinic/apyrimidinic endonuclease. *Nanoscale* **11**, 1343–1350 (2019).
9. Zhou, Z., Ouyang, Y., Wang, J. & Willner, I. Dissipative Gated and Cascaded DNA Networks. *J. Am. Chem. Soc.* **143**, 5071–5079 (2021).

10. Wang, S., Yue, L. & Willner, I. Enzyme-Guided Selection and Cascaded Emergence of Nanostructured Constitutional Dynamic Networks. *Nano Lett* **20**, 5451–5457 (2020).
11. Ouldridge, T. E. *et al.* Optimizing DNA Nanotechnology through Coarse-Grained Modeling: A Two-Footed DNA Walker. *ACS Nano* **7**, 2479–2490 (2013).
12. Ouldridge, T. E., Louis, A. A. & Doye, J. P. K. Structural, mechanical, and thermodynamic properties of a coarse-grained DNA model. *The Journal of Chemical Physics* **134**, 085101 (2011).
13. Ouldridge, T. E., Louis, A. A. & Doye, J. P. K. DNA Nanotweezers Studied with a Coarse-Grained Model of DNA. *Phys. Rev. Lett.* **104**, 178101 (2010).
14. Kauert, D. J., Kurth, T., Liedl, T. & Seidel, R. Direct Mechanical Measurements Reveal the Material Properties of Three-Dimensional DNA Origami. *Nano Lett.* **11**, 5558–5563 (2011).
15. Irmisch, P., Ouldridge, T. E. & Seidel, R. Modeling DNA-Strand Displacement Reactions in the Presence of Base-Pair Mismatches. *J. Am. Chem. Soc.* **142**, 11451–11463 (2020).
16. Poppleton, E. *et al.* Design, optimization and analysis of large DNA and RNA nanostructures through interactive visualization, editing and molecular simulation. *Nucleic Acids Research* **48**, e72–e72 (2020).
17. Visnes, T. *et al.* Targeting BER enzymes in cancer therapy. *DNA Repair* **71**, 118–126 (2018).
18. Jiang, Y. *et al.* DNA Nanodevices for Base Excision Repair Regulates ATP In Situ Imaging and Tumor Therapy. *ACS Appl. Bio Mater.* **3**, 8507–8514 (2020).
19. Li, C., Chen, H., Hu, J. & Zhang, C. Rolling circle amplification-driven encoding of different fluorescent molecules for simultaneous detection of multiple DNA repair enzymes at the single-molecule level. *Chemical Science* **11**, 5724–5734 (2020).
20. Wang, L., Liang, L., Liu, B., Jiang, B. & Zhang, C. A controlled T7 transcription-driven symmetric amplification cascade machinery for single-molecule detection of multiple repair glycosylases. *Chemical Science* **12**, 5544–5554 (2021).
21. Parsons, Z. D., Bland, J. M., Mullins, E. A. & Eichman, B. F. A Catalytic Role for C–H/ π Interactions in Base Excision Repair by *Bacillus cereus* DNA Glycosylase AlkD. *J. Am. Chem. Soc.* **138**, 11485–11488 (2016).
22. Hegde, M. L., Hazra, T. K. & Mitra, S. Early steps in the DNA base excision/single-strand interruption repair pathway in mammalian cells. *Cell Res* **18**, 27–47 (2008).
23. Shi, Z., Castro, C. E. & Arya, G. Conformational Dynamics of Mechanically Compliant DNA Nanostructures from Coarse-Grained Molecular Dynamics Simulations. *ACS Nano* **11**, 4617–4630 (2017).
24. Kekic, T. & Barisic, I. In silico modelling of DNA nanostructures. *Comput Struct Biotechnol J* **18**, 1191–1201 (2020).
25. Green, L. N. *et al.* Autonomous dynamic control of DNA nanostructure self-assembly. *Nat. Chem.* **11**, 510–520 (2019).

26. Poppleton, E., Romero, R., Mallya, A., Rovigatti, L. & Šulc, P. OxDNA.org: a public webserver for coarse-grained simulations of DNA and RNA nanostructures. *Nucleic Acids Research* **49**, W491–W498 (2021).
27. Suma, A., Stopar, A., Nicholson, A. W., Castronovo, M. & Carnevale, V. Global and local mechanical properties control endonuclease reactivity of a DNA origami nanostructure. *Nucleic Acids Research* **48**, 4672–4680 (2020).
28. Rothemund, P. W. K. *et al.* Design and Characterization of Programmable DNA Nanotubes. *J. Am. Chem. Soc.* **126**, 16344–16352 (2004).
29. Johnson-Buck, A. *et al.* Kinetic fingerprinting to identify and count single nucleic acids. *Nat Biotechnol* **33**, 730–732 (2015).
30. Keum, J.-W. & Bermudez, H. Enhanced resistance of DNA nanostructures to enzymatic digestion. *Chem. Commun.* 7036 (2009) doi:10.1039/b917661f.
31. Su, X. *et al.* Simultaneous Fluorescence Imaging of the Activities of DNases and 3' Exonucleases in Living Cells with Chimeric Oligonucleotide Probes. *Anal. Chem.* **85**, 9939–9946 (2013).
32. Gigli, M., Doglia, S. M., Millot, J. M., Valentini, L. & Manfait, M. Quantitative study of doxorubicin in living cell nuclei by microspectrofluorometry. *Biochimica et Biophysica Acta (BBA) - Gene Structure and Expression* **950**, 13–20 (1988).
33. Hassani Moghadam, F., Taher, M. A. & Karimi-Maleh, H. Doxorubicin Anticancer Drug Monitoring by ds-DNA-Based Electrochemical Biosensor in Clinical Samples. *Micromachines* **12**, 808 (2021).
34. McCawley, L. J. & Matrisian, L. M. Matrix metalloproteinases: multifunctional contributors to tumor progression. *Molecular Medicine Today* **6**, 149–156 (2000).
35. Mura, S., Nicolas, J. & Couvreur, P. Stimuli-responsive nanocarriers for drug delivery. *Nature Mater* **12**, 991–1003 (2013).
36. Wang, J. *et al.* Nonviolent Self-Catabolic DNAzyme Nanosponges for Smart Anticancer Drug Delivery. *ACS Nano* **13**, 5852–5863 (2019).
37. Zhang, P. *et al.* pH- and miRNA-Responsive DNA-Tetrahedra/Metal–Organic Framework Conjugates: Functional Sense-and-Treat Carriers. *ACS Nano* **15**, 6645–6657 (2021).
38. Wang, P. *et al.* Visualization of the Cellular Uptake and Trafficking of DNA Origami Nanostructures in Cancer Cells. *J. Am. Chem. Soc.* **140**, 2478–2484 (2018).
39. Madhusudan, S. *et al.* Isolation of a small molecule inhibitor of DNA base excision repair. *Nucleic Acids Res* **33**, 4711–4724 (2005).
40. Mizutani, H., Tada-Oikawa, S., Hiraku, Y., Kojima, M. & Kawanishi, S. Mechanism of apoptosis induced by doxorubicin through the generation of hydrogen peroxide. *Life Sciences* **76**, 1439–1453 (2005).
41. Soundararajan, S., Chen, W., Spicer, E. K., Courtenay-Luck, N. & Fernandes, D. J. The Nucleolin Targeting Aptamer AS1411 Destabilizes *Bcl-2* Messenger RNA in Human Breast Cancer Cells. *Cancer Res* **68**, 2358–2365 (2008).

42. Lamas, Dj. *et al.* Selective cytoprotective effect of histamine on doxorubicin-induced hepatic and cardiac toxicity in animal models. *Cell Death Discovery* **1**, 15059 (2015).
43. Wang, H., Luo, D., Wang, H., Wang, F. & Liu, X. Construction of Smart Stimuli-Responsive DNA Nanostructures for Biomedical Applications. *Chem. Eur. J.* **27**, 3929–3943 (2021).
44. Jia, R. *et al.* Advances in Multiple Stimuli-Responsive Drug-Delivery Systems for Cancer Therapy. *IJN Volume* **16**, 1525–1551 (2021).
45. Rosenblum, D., Joshi, N., Tao, W., Karp, J. M. & Peer, D. Progress and challenges towards targeted delivery of cancer therapeutics. *Nat Commun* **9**, 1410 (2018).
46. Zhang, P. *et al.* A programmable polymer library that enables the construction of stimuli-responsive nanocarriers containing logic gates. *Nat. Chem.* **12**, 381–390 (2020).
47. Gong, X. *et al.* A smart multiantenna gene theranostic system based on the programmed assembly of hypoxia-related siRNAs. *Nat Commun* **12**, 3953 (2021).
48. Wang, J., Li, Y. & Nie, G. Multifunctional biomolecule nanostructures for cancer therapy. *Nat Rev Mater* **6**, 766–783 (2021).
49. Wallace, S. S., Murphy, D. L. & Sweasy, J. B. Base excision repair and cancer. *Cancer Letters* **327**, 73–89 (2012).
50. Methods
51. **Reagents and materials.** All DNAs were synthesized by Sangon Biotech (Shanghai) Co., Ltd, modified oligonucleotides were purified by HPLC, while unmodified oligonucleotides were purified by PAGE. The sequences are listed **Table S1**. Nucleases and their corresponding buffers were obtained from NEB (Ipswich, MA). Doxorubicin was obtained from Beijing Solarbio Science & Technology Co., Ltd. All chemicals were used as received without additional purification. DNase/RNase-free deionized water (ddH₂O) from Tiangen Biotech Co. (Beijing, China) was used in all experiments. Hoechst 33342 was obtained from Solarbio Co., LTD (Beijing, China).
52. **oxDNA simulation.** Structural analysis of the different types of the DNA nanotubes were achieved by using oxDNA which is a coarse-grained molecular dynamics (MD) simulation software. We used caDNAno to draw the DNA nanotubes, and export the file formats to “xx.top” and “xx.oxdna” through TacoxDNA. The position and orientation of each base and the overall DNA structure are intuitively presented in a visual image. The image is visualized by the oxDNA generated file in the oxView (<https://sulcgroup.github.io/oxdna-viewer/>), then we used “RELAX” option to oxDNA interaction, we can modify parameters in the input file, such as the number of steps, temperature and other conditions, and adjust hydrogen bond strength in the file of “oxDNA2_sequence_dependent_parameters” for simulation. The detailed simulation parameters and conditions are noted in Table S2. We used Python files to run the obtained trajectory files and extracted and analyzed the data to obtain the results of RMSF and hydrogen bond occupancy. Then we plotted the data and compared the data of different DNA nanotubes.
53. **Preparation and characterization of DNA nanotubes.** All sequences were dissolved and diluted in DNase/RNase-free deionized water. To synthesize tubular DNA, 2.5 µL of 20 µM of DNA sequences

(S1-P1, S1-P2, S1-P3, S1-P4, and S1-P5, for sequences see Table S1) were mixed in TM buffer (40mM Tris, 1 mM EDTA- Na_2 , 12.5 mM Mg^{2+} , pH=8). The DNA solutions were heated to 90°C for 5 min and slowly cooled down to room temperature (1.5h or 18h). The products were characterized by 8% native polyacrylamide gel electrophoresis which was operated at 4°C for 1 h at a constant voltage of 120 V. The gel was subsequently stained with SYBR Gold dye. Cy5-labelled DNA nanotubes were imaged using TIRF microscope with a TIRF objective (100× magnification, 1.49 NA, Nikon). The lenses, reflection mirrors, and dichroic mirrors were from Semrock (USA). For TIRF illumination, a solid laser of 520 nm was coupled into single-mode fiber cable (Solamere Technologies). Samples containing nanotubes were imaged at 100 nM tile concentration in TM buffer.

54. **Reaction kinetics of the nanotubes to nucleases.** 1 μM Cy5 labeled strand (S3-Cy5), 1 μM BHQ3 labeled strand (S4-BHQ3) and 1 μM other strands (S1-P1, S1-P2, and S1-P5) were mixed and annealed in TM buffer to prepare dual-labeled nanotubes. In a typical fluorescence dequenching assay, 250 nM nanotubes, and enzymes were incubated in TM buffer. Once the enzymes were added, fluorescence was recorded immediately in the Cy5 channel (ex: 625 nm, em: 660 nm) of a real-time PCR cycler (Rotor-Gene Q, QIAGEN, Germany) at 37°C with a time interval of 5 s.
55. **DOX loading and in vitro release.** For DOX loading, 500 nM as-prepared nanotubes and 1 mM DOX was mixed with nanotubes and incubated for 24 h at 37°C and incubated for different time in TM buffer with different pH value. Ultrafiltration (14,000 rpm for 10 min) was applied to remove free DOX. The bound DOX was estimated according to the concentration of free DOX. The drug loading efficiency and loading content were calculated as follow:
 56. Loading efficiency (%) = loaded DOX (mg)/initial DOX (mg) $\times$ 100% (1)
 57. Loading content (wt%) = loaded DOX (mg)/nanostructure (mg) $\times$ 100% (2)
58. To obtain DOX release curve, the drug containing nanostructures was prepared in TM buffer at pH 5.6 (endosomal pH) or at pH 7.4 (physiological pH). The solution was shaken at 50 rpm, followed by ultracentrifugation at different time intervals. The absorbance of supernatant at 492 nm was measured on a UV-visible spectrometer (AMR-100, Hangzhou Allsheng Instrument CO., Ltd).
59. **Cell Culture.** The HeLa cells, A549 cells and MCF 7 cells were cultured in a RPMI 1640 medium with 10% fetal bovine serum and 1% penicillin/streptomycin under standard conditions (5% CO_2 , 37°C). The HEK293T cells were cultured in a DMEM medium with 10% fetal bovine serum and 1% penicillin/streptomycin under standard conditions (5% CO_2 , 37°C). The medium was replaced every 24 h, and the cells were digested with trypsin and resuspended in fresh complete medium before plating.
60. **In vitro cellular uptake.** The cellular uptake behavior of nanotubes and DOX delivery in cultured cell lines were investigated by using the HILO fluorescence microscopy. According to the cell thickness and the S/N of single-molecule fluorescence, the micrometer-driven optical rail for Z adjustment was adjusted to achieve HILO illumination. The cells were seeded into confocal dishes with a density of 4×10^5 cells/well and incubated with RPMI 1640 Medium in a humidified atmosphere containing 5%

CO₂ at 37°C for 24 h. Then the culture medium was replaced by RPMI 1640 Medium with nanotubes in TM buffer. After 2h of incubation, the mixture in each well was dumped, and washed three times with 1 × PBS. The cell nucleus was stained by Hoechst 33342. The Cy5 fluorescence of nanotubes (ex: 650 nm, em: 670 nm) and DOX fluorescence (ex: 480 nm, em: 550 nm) were recorded by HILO fluorescence microscopy. The Hoechst 33342 (ex: 350 nm, em: 460 nm) was used to stain nucleus.

61. **In vivo tumor therapeutics.** BALB/c nude mice were obtained from Servicebio Co., Ltd. (Wuhan, China), and raised in a specific pathogen free animal facility and had access to water and food. The animal experiment protocols were approved by the Animal Ethics Committee of the Beijing University of Chemical Technology. To set up xenograft tumor models, HeLa tumor cells (2×10^6 cells per 100 μ L in 1:1 (v/v) PBS and Matrigel, BD bioscience) were inoculated under the armpits of mice until the tumor volume reached 5 mm in diameter. The tumor-bearing nude mice were randomly divided into five groups (n = 5, each group). The mice were then treated three times on 0 day, 3 day, 6 day and 9 day by tail vein injection of PBS, free DOX, control (no uracil)-DOX and S1-P5-DOX (nanotubes equivalent 17.4 mg kg⁻¹, DOX equivalent 2 mg kg⁻¹). After two-week treatment, the tumor was harvested and analyzed by histological section. The body weight and tumor size (length × width²/2) were measured at 2 days intervals after treatment. After all the experiment were completed, the tumors were collected and weighed. The tumors and major organs of mice were harvested and fixed in 4% paraformaldehyde. After embedded in paraffin, sectioned, and stained with Hematoxylin and Eosin (H&E) staining and TdT-mediated dUTP Nick-End Labeling (TUNEL) was performed for histological examination and to assess apoptosis levels in the tumor.

Methods

Reagents and materials. All DNAs were synthesized by Sangon Biotech (Shanghai) Co., Ltd, modified oligonucleotides were purified by HPLC, while unmodified oligonucleotides were purified by PAGE. The sequences are listed **Table S1**. Nucleases and their corresponding buffers were obtained from NEB (Ipswich, MA). Doxorubicin was obtained from Beijing Solarbio Science & Technology Co., Ltd. All chemicals were used as received without additional purification. DNase/RNase-free deionized water (ddH₂O) from Tiangen Biotech Co. (Beijing, China) was used in all experiments. Hoechst 33342 was obtained from Solarbio Co., LTD (Beijing, China).

oxDNA simulation. Structural analysis of the different types of the DNA nanotubes were achieved by using oxDNA which is a coarse-grained molecular dynamics (MD) simulation software. We used caDNAno to draw the DNA nanotubes, and export the file formats to “xx.top” and “xx.oxdna” through TacoxDNA. The position and orientation of each base and the overall DNA structure are intuitively presented in a visual image. The image is visualized by the oxDNA generated file in the oxView (<https://sulcgroup.github.io/oxdna-viewer/>), then we used “RELAX” option to oxDNA interaction, we can modify parameters in the input file, such as the number of steps, temperature and other conditions, and adjust hydrogen bond strength in the file of “oxDNA2_sequence_dependent_parameters” for simulation. The detailed simulation parameters and conditions are noted in Table S2. We used Python files to run the

obtained trajectory files and extracted and analyzed the data to obtain the results of RMSF and hydrogen bond occupancy. Then we plotted the data and compared the data of different DNA nanotubes.

Preparation and characterization of DNA nanotubes. All sequences were dissolved and diluted in DNase/RNase-free deionized water. To synthesize tubular DNA, 2.5 μL of 20 μM of DNA sequences (S1-P1, S1-P2, S1-P3, S1-P4, and S1-P5, for sequences see Table S1) were mixed in TM buffer (40mM Tris, 1 mM EDTA- Na_2 , 12.5 mM Mg^{2+} , pH=8). The DNA solutions were heated to 90 $^{\circ}\text{C}$ for 5 min and slowly cooled down to room temperature (1.5h or 18h). The products were characterized by 8% native polyacrylamide gel electrophoresis which was operated at 4 $^{\circ}\text{C}$ for 1 h at a constant voltage of 120 V. The gel was subsequently stained with SYBR Gold dye. Cy5-labelled DNA nanotubes were imaged using TIRF microscope with a TIRF objective (100 $\times$ magnification, 1.49 NA, Nikon). The lenses, reflection mirrors, and dichroic mirrors were from Semrock (USA). For TIRF illumination, a solid laser of 520 nm was coupled into single-mode fiber cable (Solamere Technologies). Samples containing nanotubes were imaged at 100 nM tile concentration in TM buffer.

Reaction kinetics of the nanotubes to nucleases. 1 μM Cy5 labeled strand (S3-Cy5), 1 μM BHQ3 labeled strand (S4-BHQ3) and 1 μM other strands (S1-P1, S1-P2, and S1-P5) were mixed and annealed in TM buffer to prepare dual-labeled nanotubes. In a typical fluorescence dequenching assay, 250 nM nanotubes, and enzymes were incubated in TM buffer. Once the enzymes were added, fluorescence was recorded immediately in the Cy5 channel (ex: 625 nm, em: 660 nm) of a real-time PCR cycler (Rotor-Gene Q, QIAGEN, Germany) at 37 $^{\circ}\text{C}$ with a time interval of 5 s.

DOX loading and in vitro release. For DOX loading, 500 nM as-prepared nanotubes and 1 mM DOX was mixed with nanotubes and incubated for 24 h at 37 $^{\circ}\text{C}$ and incubated for different time in TM buffer with different pH value. Ultrafiltration (14,000 rpm for 10 min) was applied to remove free DOX. The bound DOX was estimated according to the concentration of free DOX. The drug loading efficiency and loading content were calculated as follow:

Loading efficiency (%) = loaded DOX (mg)/initial DOX (mg) $\times$ 100% (1)

Loading content (wt%) = loaded DOX (mg)/nanostructure (mg) $\times$ 100% (2)

To obtain DOX release curve, the drug containing nanostructures was prepared in TM buffer at pH 5.6 (endosomal pH) or at pH 7.4 (physiological pH). The solution was shaken at 50 rpm, followed by ultracentrifugation at different time intervals. The absorbance of supernatant at 492 nm was measured on a UV-visible spectrometer (AMR-100, Hangzhou Allsheng Instrument CO., Ltd).

Cell Culture. The HeLa cells, A549 cells and MCF 7 cells were cultured in a RPMI 1640 medium with 10% fetal bovine serum and 1% penicillin/streptomycin under standard conditions (5% CO_2 , 37 $^{\circ}\text{C}$). The HEK293T cells were cultured in a DMEM medium with 10% fetal bovine serum and 1% penicillin/streptomycin under standard conditions (5% CO_2 , 37 $^{\circ}\text{C}$). The medium was replaced every 24 h, and the cells were digested with trypsin and resuspended in fresh complete medium before plating.

In vitro cellular uptake. The cellular uptake behavior of nanotubes and DOX delivery in cultured cell lines were investigated by using the HILO fluorescence microscopy. According to the cell thickness and the S/N of single-molecule fluorescence, the micrometer-driven optical rail for Z adjustment was adjusted to achieve HILO illumination. The cells were seeded into confocal dishes with a density of 4×10^5 cells/well and incubated with RPMI 1640 Medium in a humidified atmosphere containing 5% CO₂ at 37 °C for 24 h. Then the culture medium was replaced by RPMI 1640 Medium with nanotubes in TM buffer. After 2h of incubation, the mixture in each well was dumped, and washed three times with 1 × PBS. The cell nucleus was stained by Hoechst 33342. The Cy5 fluorescence of nanotubes (ex: 650 nm, em: 670 nm) and DOX fluorescence (ex: 480 nm, em: 550 nm) were recorded by HILO fluorescence microscopy. The Hoechst 33342 (ex: 350 nm, em: 460 nm) was used to stain nucleus.

In vivo tumor therapeutics. BALB/cnude mice were obtained from Servicebio Co., Ltd. (Wuhan, China), and raised in a specific pathogen free animal facility and had access to water and food. The animal experiment protocols were approved by the Animal Ethics Committee of the Beijing University of Chemical Technology. To set up xenograft tumor models, HeLa tumor cells (2×10^6 cells per 100 μ L in 1:1 (v/v) PBS and Matrigel, BD bioscience) were inoculated under the armpits of mice until the tumor volume reached 5 mm in diameter. The tumor-bearing nude mice were randomly divided into five groups (n = 5, each group). The mice were then treated three times on 0 day, 3 day, 6 day and 9 day by tail vein injection of PBS, free DOX, control (no uracil)-DOX and S1-P5-DOX (nanotubes equivalent 17.4 mg kg^{-1} , DOX equivalent 2 mg kg^{-1}). After two-week treatment, the tumor was harvested and analyzed by histological section. The body weight and tumor size ($\text{length} \times \text{width}^2/2$) were measured at 2 days intervals after treatment. After all the experiment were completed, the tumors were collected and weighed. The tumors and major organs of mice were harvested and fixed in 4% paraformaldehyde. After embedded in paraffin, sectioned, and stained with Hematoxylin and Eosin (H&E) staining and TdT-mediated dUTP Nick-End Labeling (TUNEL) was performed for histological examination and to assess apoptosis levels in the tumor.

Figures

Figure 1

Design and simulation of uracil-containing DNA nanotube in response to the critical enzymes UDG and APE1 in BER pathway. (A) The formation of DNA nanotube relies on the hybridization of the sticky ends of DNA tiles. T to U substitution on the nanotube can activate UDG and APE1. The nanotube has diameter of 13.5 nm, and its circumference contains 14 tiles. (B) Base orientation of potential T to U substitution positions (P1-P5) on S1 strand simulated by oxDNA. (C) Cleaved DNA tile by enzymes if the T to U substitution is located at S1-P1 position. T is removed at the position and one-base gap forms. (D) Potential energy of the nanotubes with one-base gap at the positions of S1 strand shown in Panel A. (E)

Mean structures and RMSF of the nanotubes with one-base gap. (F) Hydrogen bond occupancy of the nanotubes with one-base gap.

Figure 2

Characterization and function test of the DNA nanotubes. (A) Characterization of the reaction of enzymes (UDG and APE1) and uracil-containing nanotubes by gel electrophoresis. (B) Reaction kinetics measurement of the nanotubes by fluorescence dequenching assay (for labelling position see Figure S7). (C) TIRF images of nanotubes and the corresponding enzymatic cleavage products. (D) Drug release curve of the nanotube S1-P5 under different conditions. The concentration of nanotubes and DOX are 100nM and 4 μ M, UDG and APE1 in all assays are 20 nM and 4 nM, respectively.

Figure 3

Exploring cellular enzymatic reaction and drug release by HILO fluorescence microscopy. (A) HILO images of A549 cell incubated with long and short DNA nanotubes. Scale bar: 10 μ m (B) Characterization of the enzymatic reaction of nanotubes and enzymes in living cells. MFI is shown for each group. Scale bar: 5 μ m. (C) Exploring the cellular distribution of DOX (4 μ M) which was carried by different vehicles. MFI is shown for each group. The nucleus is stained by Hoechst33324 in all experiments. Scale bar: 5 μ m. ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

Figure 4

Evaluation of the therapeutic efficacy in vivo. (A) Schematic illustration of HeLa tumor xenograft establishment, saline and the therapeutic agent administration modalities. (B) In vivo fluorescence imaging at different times after tail vein injection of DNA nanotubes. (C) Images of the tumors that harvested from the euthanized mice. Scale bar: 1 cm. (D) Relative tumor size after exposure to different treatments, $n = 5$, $**P < 0.01$. (E) Survival rates of different groups. (F) H&E and TUNEL staining of the tumors after treatment. Scale bar: 100 μ m. (G) Histological examination of H&E stained slices of heart and liver after different treatments. Heart and liver of Dox-treated animals displaying focal necrotic cell death (blue arrow). Scale bar: 200 μ m.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [DengetalsimulationguidedDNAnanotubeSI.pdf](#)
- [floatimage1.png](#)