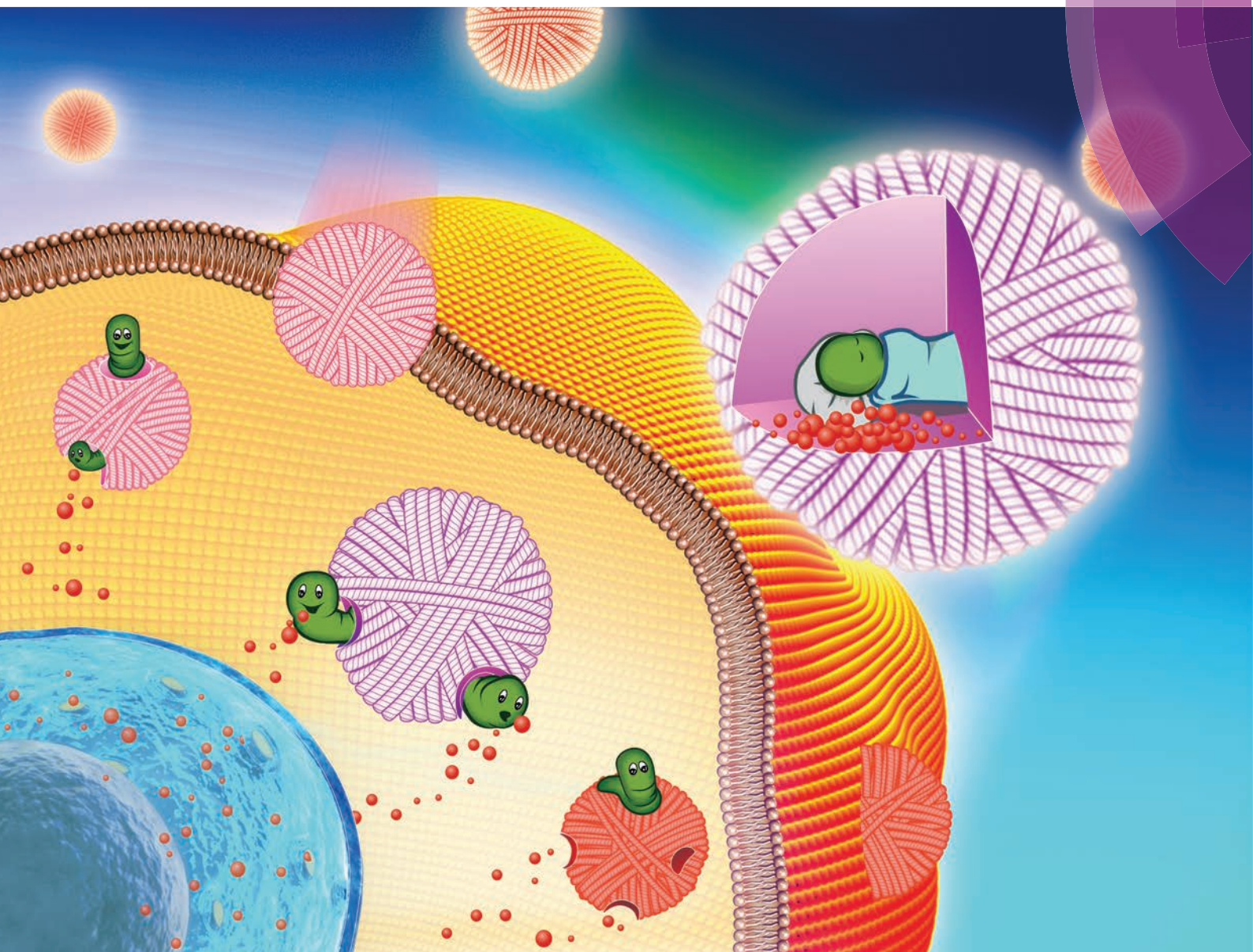


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## Engineering DNA scaffolds for delivery of anticancer therapeutics

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Engineering DNA nanostructures with programmability in size, shape and surface chemistry holds tremendous promise in biomedical applications. As an emerging platform for drug delivery, DNA nanostructures have been extensively studied for delivering anticancer therapeutics, including small-molecule drug, nucleic acids and proteins. In this mini-review, current advances in utilizing DNA scaffolds as drug carriers for cancer treatment were summarized and future challenges were also discussed.

### 1. Introduction

As an important tool in pharmaceuticals, versatile drug delivery systems have been developed to shield the drugs from detrimental physiological environments as well as reduce their side effects by enhancing their targeting efficiency.<sup>1,2</sup> With these carriers, numerous therapeutic cargos, such as small molecules drugs, nucleic acids, peptides and proteins were success-

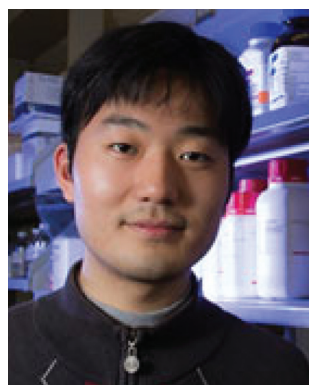
fully delivered to their intracellular or extracellular destinations.<sup>3</sup> Despite the extensively efforts of exploring different materials for anti-cancer drug delivery, only a few formulations based on liposome, polymer and protein carriers were approved by FDA.<sup>4,5</sup> To develop a reliable drug delivery system, the designing criteria often incorporate uniform structure, high stability, biocompatibility, targeting abilities, transduction abilities and stimuli-responsiveness.<sup>6</sup>

DNA is a polymer chosen by nature to store and transmit genetic information. The intrinsic programmability renders DNA a promising material in designing uniform nanostructures for drug delivery.<sup>7–11</sup> In addition, the prevalent existence of DNase in tissues endows DNA with superb biodegradability,<sup>12</sup> which could even be incorporated into metallic drug delivery carriers for improved biocompatibility.<sup>13</sup> Although transfection reagents, such as positively charged polymers or lipids,<sup>14</sup> were generally used for transgenic studies

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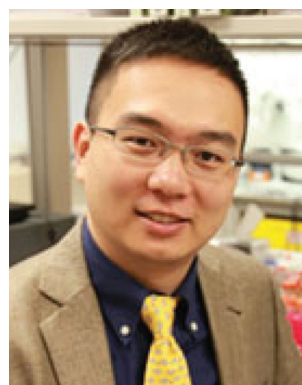
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due to the cell membrane impermeability of free DNA, DNA nanostructure can readily enter the cell membrane *via* endocytosis in an agent-free way.<sup>15,16</sup> Furthermore, DNA aptamers,<sup>17</sup> which are single stranded DNAs with antibody-like binding affinity to their target molecule, can interact with environmental signals, such as ATP and pH;<sup>18–20</sup> or cellular signal, such as extracellular receptors and intracellular mRNA,<sup>21,22</sup> have greatly expanded the toolbox for building DNA-based drug delivery carriers.

Besides hybrid drug delivery carriers that incorporate DNA as a functional moiety, pristine DNA assemblies have been extensively studied over the last three decades since the first demonstration of assembling an immobile nucleic acid junction based on simple Watson–Crick base pairing by Seeman *et al.* in the 1980s.<sup>23,24</sup>

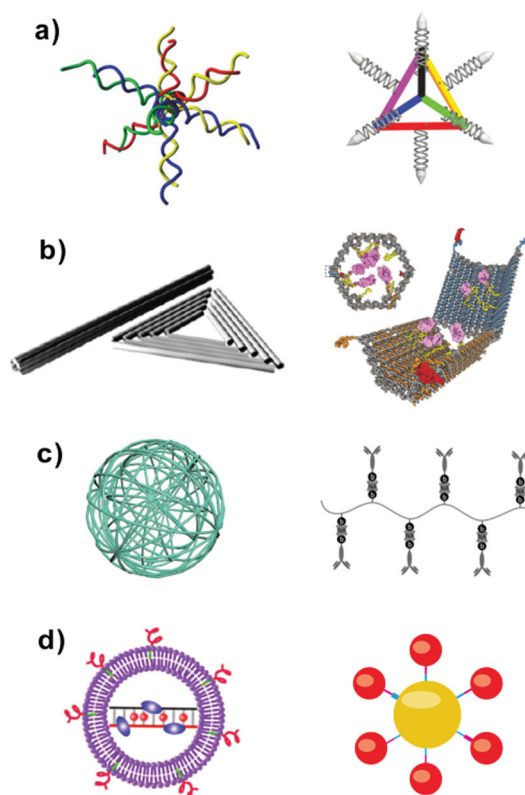
Various DNA nanostructures were assembled, such as cubes,<sup>25</sup> networks,<sup>26</sup> arrays or arbitrary shapes on a DNA canvas,<sup>27,28</sup> from customized DNA tiles with “sticky ends”. In contrast with the “bottom-up” approach of assembling a complex DNA structure from numerous tiles, an alternative “top-down” approach, “DNA origami”, was developed by Rothemund *et al.* in 2006 by folding a long single stranded DNA with numerous “DNA staples” into arbitrary shapes.<sup>8</sup> Since then, larger and more complex DNA nanostructures, such as logic-gated robot or a DNA box with controlled lid,<sup>29,30</sup> were developed. Thanks to the development of user-friendly software for predicting the structures of DNA assembly, customizing DNA nanostructures has become a routine work.<sup>31–34</sup> More recently, rolling circle amplification (RCA),<sup>35–37</sup> a polymerase chain reaction based method to amplify periodically repeated single stranded DNA from a circular single strand template, has also attracted considerable attention in assembling DNA nanostructures, such as sponges and tubes.<sup>38–43</sup>

The intrinsic multivalency makes DNA a superb carrier for easy drug loading and functionalization,<sup>44,45</sup> which can be achieved through covalent conjugation, inter-molecular hybridization or intra-molecular intercalation.<sup>10</sup> In this mini-review, recent advances using DNA scaffolds for anticancer drug delivery were summarized (Fig. 1) and the challenges for future developments were discussed.

## 2. Various DNA scaffolds for different anticancer drug delivery

### 2.1. Small-molecule drugs

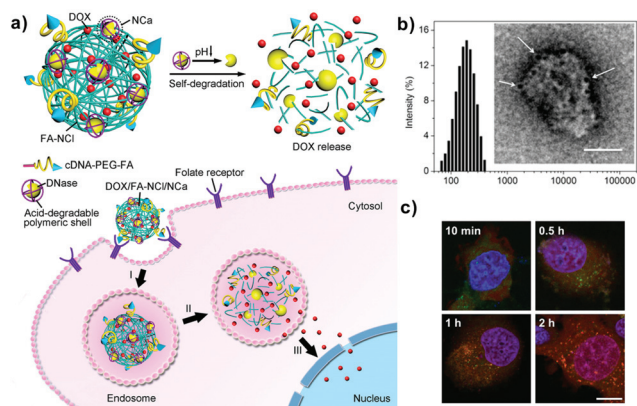
Doxorubicin (DOX) is an anthracycline antibiotic that works by intercalating into double stranded DNA, especially at the GC rich regions.<sup>46</sup> However, free DOX suffers from poor solubility, low selectivity and adverse side effects. The interaction between DOX and DNA makes DNA nanostructure a natural carrier for DOX and DNA nanostructures assembled *via* different methods were explored for intracellular delivery of DOX. Chang *et al.* developed a 3-D DNA polyhedra by assembling 5-star and 6-star “DNA tiles” and they were among the



**Fig. 1** Example DNA nanoscaffolds for anticancer drug delivery. (a) DNA nanostructures assembled from multiple “DNA tiles”.<sup>55,60</sup> (b) DNA nanocarriers based on “DNA origami”.<sup>29,47</sup> (c) DNA nanoassemblies based on rolling circle amplification.<sup>40,67</sup> (d) Hybrid drug delivery carriers with DNA scaffold as functional moiety.<sup>13,46</sup> Reproduced with permission.

first to demonstrate that structured DNA particles were readily uptaken by the cells.<sup>16</sup> By utilizing the DNA origami method, Jiang *et al.* folded the long viral single stranded DNA M13mp18 into triangular and tubular structures with hundreds of short DNA staples.<sup>47</sup> Using regular breast cancer cell line MCF7 and its DOX resistant subline, they demonstrated that the DNA origami carrier could circumvent drug resistance and kill DOX-resistant MCF7 cells. In addition, using a DNA origami based nanotube for DOX delivery, Zhao *et al.* tuned the intra-molecular twist of the nanostructure and found that higher twist of the structure lead to higher DOX loading capacity and lower intracellular elimination rates.<sup>48</sup> *In vivo* DOX delivery studies in nude mice xenografted with MDA-MB-231 tumors using a triangular DNA origami showed excellent passive targeting to the tumor tissue without observable systemic side effects.<sup>49</sup>

Instead of using “DNA tiles” or “DNA origami”, our group recently developed a “DNA nanococoon” for controlled DOX delivery using RCA (Fig. 2).<sup>40</sup> After cyclizing a linear ssDNA (~75 bp) into a circular template, long chain ssDNA products with repeated sequences complementary to the DNA template were obtained by RCA. Multiple GC pairs were encoded into the template for enhanced DOX loading and a palindromic



**Fig. 2** Intracellular delivery of DOX using a DNA nanococoon synthesized by rolling circle amplification. (a) Main components of the DNA nanococoon and mechanism for intracellular delivery of DOX. (b) Hydrodynamic size of DNA nanococoon and TEM image of DNA nanococoon complexed with gold nanoparticle stained DNaseI nanocapsule. Scale bar is 100 nm (c) confocal laser scanning microscopy images of DNA nanococoon mediated DOX delivery in MCF-7 cells. Red for DOX, green for endolysosome and blue for nucleus. Scale bar is 10  $\mu$ m. Reproduced with permission from ref. 40.

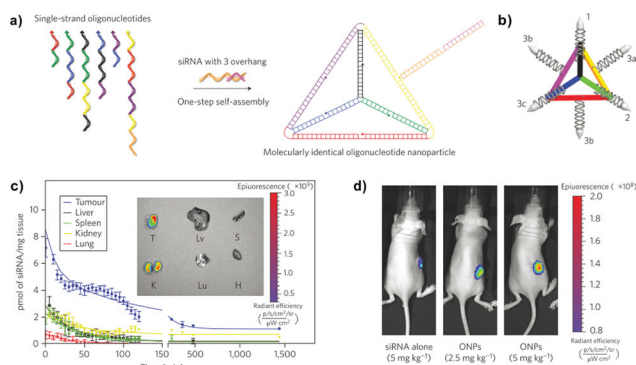
sequence was also incorporated to promote the self-assembly of the ssDNA product into a nanoclew-like structure (NCl). After incubating the DOX with NCl, a high DOX loading capacity (66.7%) was achieved using the NCl as carrier. In order to control the DOX release profile from DOX/NCl, DNase I was chosen as a trigger to promote the degradation of NCl. Instead of using free DNase I, an acid degradable polymeric shell was coated onto DNase I to cage the activity of DNase. Also, the positive charge of the polymeric shell converted the zeta potential of DNase I from negative to positive, making it possible to assemble the DNase nanocapsule (NcA) with NCl into a stable complex. The NCl/NcA complex showed increased size ( $\sim$ 180 nm) as compared with NCl alone ( $\sim$ 150 nm). To further enhance the targeting ability of DOX loaded NCl/NcA, folic acid (FA) was conjugate to a short ssDNA oligo with sequence complementary to NCl. The overall DOX/NCl-FA/NcA complex showed a pH responsive DOX release profile and a very fast intracellular delivery of DOX into the nucleus of MCF7 cell line could be remarkably observed even within 0.5 h after treatment.

In addition to DOX, other small molecules with anticancer effects, such as photosensitizer that can convert light energy into reactive oxygen species, can also be delivered intracellularly using DNA scaffolds. Unlike the preference for Watson-Crick pairing based GC pairs by DOX, photosensitizer TMPyP4 can intercalate into a special DNA assembly – G quadruplex,<sup>50,51</sup> which was built from stacks of G-quartet arising from the cyclic hydrogen-bonding between four guanines in physiological buffer conditions.<sup>52</sup> To demonstrate the delivery of photosensitizer TMPyP4 into CEM and Ramos cells, Wang *et al.* conjugated a DNA aptamer with targeting abilities with a G-quadruplex and the toxicity of TMPyP4 to target cells were doubled using the aptamer-G-quadruplex as a carrier.<sup>50</sup>

## 2.2. Nucleic acids

Besides small molecule drugs, nucleic acids, such as cytosine-phosphate-guanine (CpG),<sup>53</sup> small interfering RNA (siRNA)<sup>54</sup> and antisense nucleotides, can also work as anticancer therapeutics by interacting with their cellular targets.

**2.2.1 siRNA.** siRNA is a major player in the process of RNA interference inside eukaryotic cells. The long chain double stranded RNA is first processed by the enzyme Dicer into short siRNA ( $\sim$ 22 bp), which can be subsequently recruited into a protein complex RISC (RNA-induced silencing complex). The RISC then cleaves the sense strand of siRNA and uses the remaining antisense strand to find and degrade the target mRNA.<sup>54</sup> The prospects that synthetic siRNA can nearly silence any gene in the tissue lead to considerable efforts for its therapeutic applications. Lee *et al.* used a DNA tetrahedral nanoassembly for siRNA delivery by taking advantage of its homogeneous size and controllable ligand orientation (Fig. 3).<sup>55</sup> The tetrahedron was assembled from 6 DNA oligos with sticky ends and obtained a final length of 10 nm along each edge. The siRNA was pre-modified with 2'-OME for enhanced stability and reduced immunogenicity before incorporating into the DNA tetrahedral using its 3' sticky overhangs. Reporter proteins, such as luciferase and green fluorescent protein (GFP), were chosen as targets. *In vitro* study about the effect of targeting ligands on gene silencing efficiency revealed that a minimum of 3 folic acid targeting ligands were needed for GFP silencing. *In vivo* study in nude mice model xenografted with KB tumors capable of expressing firefly luciferase showed  $\sim$ 60% reduction in bioluminescence by tail-vein as well as intratumour injections. A tumor-specific accumulation of the tetrahedral nanoparticle was also observed after systemic injection and no significant immune response was detected. To simplify the process of loading siRNA into carriers, Hammond and coworkers adopt an approach similar to RCA that transcribed a long single strand RNA containing



**Fig. 3** Delivery of siRNA using a DNA tetrahedron assembled from DNA tiles. (a) Schematic for the DNA tetrahedron formation. (b) Structure of the DNA tetrahedron with ligands. (c) Pharmacokinetic profile of the siRNA loaded DNA tetrahedron in mice bearing KB tumor and distribution of the nanoparticle in major organs after 12 h of injection. (d) Representative fluorescent image of dose-dependent accumulation of the DNA nanoparticle in KB tumors. Reproduced with permission from ref. 55.

periodic repeats of siRNA from a circular DNA template by RNA polymerase.<sup>38</sup> Hairpin structures were programmed into the template to maintain the double strand structure of siRNA as well as to induce the assembly of the product into a sponge like structure. The RNA microsponge worked as a structural scaffold as well as a pro-drug that could be processed into functional siRNA intracellularly. With the help of polyethylenimine (PEI) as condensing as well as endosome escape agent, expression of firefly luciferase was inhibited by 57.6% in T22-Luc cells.

**2.2.2 Antisense nucleotides.** Similar to siRNA, antisense nucleotide can also block the expression of a target gene. Instead of using a RISC mediated mRNA degradation mechanism, antisense nucleotides can sequence-specifically bind to its target mRNA and recruit RNase H to degrade the RNA strand from the DNA/RNA complex.<sup>56</sup> Other mechanisms involved in antisense nucleotide regulation might be its ability to arrest transcription and alter mRNA splicing patterns.<sup>57</sup> Due to the similar gene regulation mechanisms of siRNA and antisense nucleotides, carriers developed for siRNA delivery could also be applied for delivering antisense nucleotides.

Keum *et al.* applied the DNA tetrahedron assembly for displaying antisense nucleotides.<sup>58</sup> Five out of the six edges of the tetrahedron were designed as dsDNA while the left one was designed as ssDNA for antisense nucleotide binding. The antisense nucleotide was designed as a floating loop to maximize its interaction with cytosolic mRNA. The displayed antisense nucleotide showed enhanced uptake efficiency than linear DNA and exhibited efficient gene silencing capabilities *in vitro*. In another report, Roh *et al.* adopted the RCA approach to generate a long chain ssDNA encoding periodic antisense nucleotides, which could self-assemble into a DNA sponge for intracellular delivery.<sup>39</sup> Charged polymers were coated onto the DNA in a layer-by-layer (LBL) method to condense the size of the microsponge from  $\sim 1.8 \mu\text{m}$  to  $\sim 200 \text{ nm}$ . The microsponge has a very high antisense nucleotide loading capacity and the LBL condensed microsponge achieved  $\sim 50$  fold higher luciferase knockdown efficiency than the same amount of free antisense nucleotide delivered by lipofectamine in a SKOV3-LUC/SKOV3 cell line. The formulation also showed superb stability and *in vivo* biodistribution in nude mice model *via* tail vein injection that primarily accumulated in tumor and kidney.

**2.2.3 CpG.** The pathogen derived CpG motifs contained an unmethylated cytosine while the CG dinucleotide in mammalian cells often contained methylated cytosine at its C5 position.<sup>53</sup> Toll-like receptor 9 (TLR9) in the endosome of immune cells, such as dendritic cells and macrophages, can recognize the CpG motif as a “danger signal” and activate the innate immune systems, generating cytokines, chemokines or antibodies.<sup>53</sup> The immunostimulatory capability makes CpG an effective therapeutic agent against cancer.<sup>59</sup>

Takakura and coworkers used three or more ssDNA oligos with CpG motif encoded to assemble various polypod-like structures containing different numbers of pod for CpG delivery.<sup>60,61</sup> The assemblies showed an average size of 10 nm

and the assemblies with six or more pods induced efficient secretion of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-6 (IL-6) from macrophage-like RAW264.7 cells.<sup>60</sup> The DNA polypod mediated CpG delivery can also be applied to other cell lines, such as splenic macrophages, murine and bone marrow-derived dendritic cells as well as human peripheral blood mononuclear but not for macrophages lacking TLR9.<sup>60</sup> Although CpG uptake in RAW264.7 cells was enhanced with increasing pod numbers, the structural integrity of the assemblies was diminished. To obtain a rigid and stable DNA carrier for CpG delivery, Li *et al.* assembled a cage-like tetrahedral DNA nanoassembly from four pre-designed DNA oligos. The tetrahedral structure was resistant to nuclease degradation and remained stable inside RAW254.7 cells, which was observed from the colocalization of two fluorescent dyes labeled on different vertexes on the tetrahedral after 8 hours.<sup>44</sup> Using the “DNA origami” method, Schüller *et al.* folded a 8634-bp ssDNA scaffold with 227 staples into a hollow DNA tube ( $\sim 80 \text{ nm}$  in length and  $\sim 20 \text{ nm}$  in diameter) with 62 binding sites for CpG oligo anchoring.<sup>62</sup> A degradation-resistant phosphorothioate backbone was incorporated into the CpG oligo for enhanced stability and this CpG decorated DNA nanotube achieved higher immunostimulation as well as lower cytotoxicity in isolated spleen cells compared with lipofectamine mediated CpG delivery. To simplify the DNA origami folding process, which usually required hundreds of specifically designed staples, Ouyang *et al.* replaced the commonly used bacteriophage genome with a long ssDNA scaffold prepared by RCA.<sup>63</sup> Due to the periodic nature of RCA products, only several staples were need to fold the RCA product into a DNA nanoribbon with tunable width and length by controlling the RCA template sequence as well the RCA reaction time. By artificially programming a region of the RCA template for anchoring CpG containing nucleotide, one CpG could be incorporated into each periodic unit of the RCA product and this strategy successfully delivered CpG into RAW 264.7 cells and induced the secretion of TNF- $\alpha$ .

### 2.3. Proteins

Proteins with anticancer activities, such as antibodies, cytokines, transcription factors and enzymes, were discovered capable of activating the apoptosis pathways or blocking growth signals in cancer cells.<sup>2,64</sup> In addition to directly interacting with cancer cells, proteins can also function as antigens to activate leukocytes, such as T cells, after being phagocytized and presented by antigen presenting cells.<sup>65</sup> The highly specific set of activities makes protein a very diverse and promising class of anticancer therapeutics.

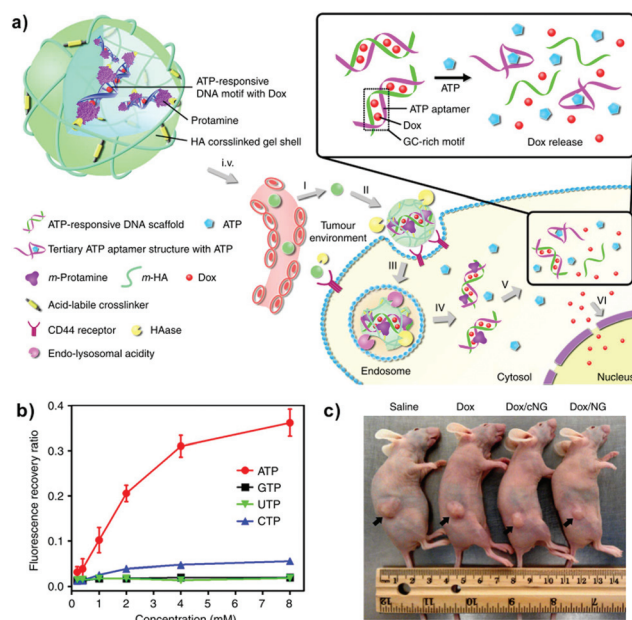
To design a virus-like vaccine for cancer, Liu *et al.* used the DNA tetrahedron structure to deliver a model antigen, streptavidin, into immune cells together with a CpG motif as antigen adjuvant.<sup>66</sup> Localization of the antigen inside the lysosome of RAW 264.7 was observed after 2 h of incubation due to the DNA tetrahedron assisted uptake of the antigen. The DNA tetrahedron delivered vaccine induced much higher anti-streptavidin antibody production in BALB/c mice than free

streptavidin and CpG over a period of 70 days and the safety of the DNA tetrahedron carrier was confirmed by the absence of any anti-dsDNA antibody. Using a “DNA origami” approach, Douglas *et al.* assembled a barrel-like DNA robot (35 nm × 35 nm × 45 nm) with the ability to sense cell surface signals to control its configuration for drug delivery.<sup>29</sup> The nanobarrel was locked with DNA aptamers, which can be opened by the specific antigen keys presented on cell surfaces. Antibody cargoes were loaded by conjugating to ssDNAs complementary to anchors inside the barrel. The nanorobot successfully delivered CD33 and CDw238 Fab' fragment antibodies to natural killer leukemia cell and arrested its growth and it also delivered CD3e Fab' and flagellin Fab' antibodies to T cells for T cell activation. Besides the “DNA tile” and “DNA origami” based structures, RCA based nanoassembly was also utilized for delivering anti-CD20 clusters to CD20 positive Ramos cells.<sup>67</sup>

### 3. DNA scaffolds-based stimuli-responsive drug delivery

Designing hybrid carriers by incorporating nanomaterials, such as polymeric gel,<sup>19</sup> liposome<sup>46</sup> and silica<sup>68</sup> with DNA aptamers is an efficient strategy in developing smart carriers with the ability to release the anticancer therapeutics in response to distinct environmental triggers.<sup>69</sup>

Using an ATP-binding aptamer, our group devised DNA functionalized polymeric drug delivery carriers, which can utilize the ATP concentration difference between intracellular and extracellular environments for controlled drug delivery.<sup>19,46,70</sup> The ATP-binding aptamer is hybridized with its complementary DNA, which forms a stable double stranded DNA encoding a GC-pair for DOX loading (Fig. 4).<sup>19</sup> The high level of intracellular ATP competitively binds the ATP aptamer and dissociated the double stranded DNA into single strands, leading to the release of DOX. The DNA scaffold was condensed with a positively charged protein to form a positively charged core, which was further coated by a layer of negatively charged polymeric hyaluronic acid (HA). In addition to forming a protective shell for the DNA scaffold, the HA also works as a targeting ligand to receptors like CD44 and RHAMM on the surface of several cancer cells. The hyaluronidase rich in tumor microenvironment can degrade the HA shell and facilitate the intracellular delivery of the DNA scaffold. *In vitro* DOX release study showed an ATP-specific response compared with other types of nucleotides. Intravenous injection of the nanogel into MDA-MD-231 tumour-bearing mice showed longer circulation time of DOX and a 4.19-fold higher DOX accumulation the tumor than DOX solution. Besides utilizing the intracellular ATP level, extrinsic ATP delivered by a liposomal carrier can also be used to trigger DOX release from a hybrid carrier composed of the ATP responsive DNA scaffold and a fusogenic liposome.<sup>46</sup>



**Fig. 4** ATP responsive delivery of DOX using polymeric nanoparticles functionalized by an ATP-binding DNA scaffold. (a) Schematic of the ATP responsive drug delivery system. (b) DOX release from the DNA-aptamer duplex as response to different nucleotides. (c) Representative image of MDA-MB-231 tumor bearing mice treated with different formulations. Reproduced with permission from ref. 19.

### 4. Conclusion and outlook

Integrating with the novel “self-assembly” technology as well as interaction between DNA and target drugs, many drug delivery carriers have been recently developed with promising biocompatibility, drug loading capacity and uptake efficiency. The degradability of DNA can be harnessed to improve the biocompatibility of other non-degradable systems. As demonstrated by Chan and coworkers,<sup>13</sup> DNA scaffolds were utilized to assemble small gold nanoparticles with size of 3 nm, 5 nm and 13 nm into larger colloid structures for enhanced tumor accumulation, which could also be degraded into individual components for facilitated elimination from the body. DNA is a highly programmable molecule characterized by its polyvalency. Instead of delivering one single kind of drug, DNA nanostructures could be programmed for co-delivering different drugs or synchronizing the activities of different proteins. For example, Liu *et al.* demonstrated the use of a branched DNA nanostructure in assembling three enzymes with complementary activities into a single nanocomplex, leading to greatly enhanced catalytic efficiencies.<sup>71</sup> To further enhance the efficacy of DNA drug carriers, elements capable of sensing the environmental signal should be incorporated to build smart drug delivery carriers. As demonstrated by the DNA robot for cell specific antibody delivery,<sup>29</sup> DNA devices capable of sensing inputs from the cell or environment and perform logic calculations for controlled drug release provides a guideline for developing the next-generation drug delivery systems.

Despite these advantages, as a new arrival to the drug delivery field, DNA nanostructures need to be further investigated for meeting the criteria of clinical potency. Complex interactions between DNA scaffold and living cells, such as DNA degradation<sup>12</sup> and immunogenicity,<sup>72</sup> posed huge challenges to DNA nanomedicine applications. Although chemical modifications improved DNA stability, specificity of DNA target recognition could be compromised occasionally.<sup>73</sup> The fact that numerous DNA therapeutics, including ssDNA oligos and dsDNA plasmids,<sup>72,74</sup> were developed for immunotherapy highlighted the immunogenic nature of DNA, which often raises a concern. In addition, even though the development of commercial DNA synthesis made customized DNA oligo readily available,<sup>75</sup> using DNA as a generic material instead of genetic materials is still limited to small scales. Besides improving chemical DNA synthesis techniques for reduced cost, utilizing the DNA synthesis capability of cells could be a natural solution to address this concern.<sup>76</sup>

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## References

- 1 D. Peer, J. M. Karp, S. Hong, O. C. Farokhzad, R. Margalit and R. Langer, *Nat. Nanotechnol.*, 2007, **2**, 751–760.
- 2 W. Sun, Y. Lu and Z. Gu, *Part. Part. Syst. Charact.*, 2014, **31**, 1204–1222.
- 3 J.-W. Yoo, D. J. Irvine, D. E. Discher and S. Mitragotri, *Nat. Rev. Drug Discovery*, 2011, **10**, 521–535.
- 4 Y. Zhang, H. F. Chan and K. W. Leong, *Adv. Drug Delivery Rev.*, 2013, **65**, 104–120.
- 5 V. J. Venditto and F. C. Szoka Jr., *Adv. Drug Delivery Rev.*, 2013, **65**, 80–88.
- 6 Z. Gu, A. Biswas, M. Zhao and Y. Tang, *Chem. Soc. Rev.*, 2011, **40**, 3638–3655.
- 7 F. Zhang, J. Nangreave, Y. Liu and H. Yan, *J. Am. Chem. Soc.*, 2014, **136**, 11198–11211.
- 8 P. W. K. Rothmund, *Nature*, 2006, **440**, 297–302.
- 9 S. J. Tan, M. J. Campolongo, D. Luo and W. Cheng, *Nat. Nanotechnol.*, 2011, **6**, 268–276.
- 10 J. Li, C. Fan, H. Pei, J. Shi and Q. Huang, *Adv. Mater.*, 2013, **25**, 4386–4396.
- 11 C. H. Lu, B. Willner and I. Willner, *ACS Nano*, 2013, **7**, 8320–8332.
- 12 K. Samejima and W. C. Earnshaw, *Nat. Rev. Mol. Cell Biol.*, 2005, **6**, 677–688.
- 13 L. Y. T. Chou, K. Zagorovsky and W. C. W. Chan, *Nat. Nanotechnol.*, 2014, **9**, 148–155.
- 14 H. Yin, R. L. Kanasty, A. A. Eltoukhy, A. J. Vegas, J. R. Dorkin and D. G. Anderson, *Nat. Rev. Genet.*, 2014, **15**, 541–555.
- 15 L. Liang, J. Li, Q. Li, Q. Huang, J. Shi, H. Yan and C. Fan, *Angew. Chem., Int. Ed.*, 2014, **53**, 7745–7750.
- 16 M. Chang, C.-S. Yang and D.-M. Huang, *ACS Nano*, 2011, **5**, 6156–6163.
- 17 A. D. Keefe, S. Pai and A. Ellington, *Nat. Rev. Drug Discovery*, 2010, **9**, 537–550.
- 18 R. Mo, T. Jiang and Z. Gu, *Angew. Chem., Int. Ed.*, 2014, **126**, 5925–5930.
- 19 R. Mo, T. Jiang, R. DiSanto, W. Tai and Z. Gu, *Nat. Commun.*, 2014, **5**, 3364.
- 20 L. Song, V. H. B. Ho, C. Chen, Z. Yang, D. Liu, R. Chen and D. Zhou, *Adv. Healthcare Mater.*, 2013, **2**, 275–280.
- 21 J. W. Kotula, E. D. Pratico, X. Ming, O. Nakagawa, R. L. Juliano and B. A. Sullenger, *Nucleic Acid Ther.*, 2012, **22**, 187–195.
- 22 W. Pan, H. Yang, T. Zhang, Y. Li, N. Li and B. Tang, *Anal. Chem.*, 2013, **85**, 6930–6935.
- 23 N. R. Kallenbach, R.-I. Ma and N. C. Seeman, *Nature*, 1983, **305**, 829–831.
- 24 J. W. de Vries, F. Zhang and A. Herrmann, *J. Controlled Release*, 2013, **172**, 467–483.
- 25 J. Chen and N. C. Seeman, *Nature*, 1991, **350**, 631–633.
- 26 Y. Wang, J. E. Mueller, B. Kemper and N. C. Seeman, *Biochemistry*, 1991, **30**, 5667–5674.
- 27 J. Zheng, P. E. Constantinou, C. Micheel, A. P. Alivisatos, R. A. Kiehl and N. C. Seeman, *Nano Lett.*, 2006, **6**, 1502–1504.
- 28 B. Wei, M. Dai and P. Yin, *Nature*, 2012, **485**, 623–626.
- 29 S. M. Douglas, I. Bachelet and G. M. Church, *Science*, 2012, **335**, 831–834.
- 30 E. S. Andersen, M. Dong, M. M. Nielsen, K. Jahn, R. Subramani, W. Mamdouh, M. M. Golas, B. Sander, H. Stark, C. L. P. Oliveira, J. S. Pedersen, V. Birkedal, F. Besenbacher, K. V. Gothelf and J. Kjems, *Nature*, 2009, **459**, 73–76.
- 31 E. S. Andersen, M. Dong, M. M. Nielsen, K. Jahn, A. Lind-Thomsen, W. Mamdouh, K. V. Gothelf, F. Besenbacher and J. Kjems, *ACS Nano*, 2008, **2**, 1213–1218.
- 32 S. M. Douglas, A. H. Marblestone, S. Teerapittayanon, A. Vazquez, G. M. Church and W. M. Shih, *Nucleic Acids Res.*, 2009, **37**, 5001–5006.
- 33 D.-N. Kim, F. Kilchherr, H. Dietz and M. Bathe, *Nucleic Acids Res.*, 2012, **40**, 2862–2868.
- 34 A. Rajendran, M. Endo and H. Sugiyama, *Angew. Chem., Int. Ed.*, 2012, **51**, 874–890.
- 35 H. Qi, M. Ghodousi, Y. Du, C. Grun, H. Bae, P. Yin and A. Khademhosseini, *Nat. Commun.*, 2013, **4**.
- 36 J. B. Lee, S. Peng, D. Yang, Y. H. Roh, H. Funabashi, N. Park, E. J. Rice, L. Chen, R. Long, M. Wu and D. Luo, *Nat. Nanotechnol.*, 2012, **7**, 816–820.
- 37 M. M. Ali, F. Li, Z. Zhang, K. Zhang, D.-K. Kang, J. A. Ankrum, X. C. Le and W. Zhao, *Chem. Soc. Rev.*, 2014, **43**, 3324–3341.

- 38 J. B. Lee, J. Hong, D. K. Bonner, Z. Poon and P. T. Hammond, *Nat. Mater.*, 2012, **11**, 316–322.
- 39 Y. H. Roh, J. B. Lee, K. E. Shopsowitz, E. C. Dreaden, S. W. Morton, Z. Poon, J. Hong, I. Yamin, D. K. Bonner and P. T. Hammond, *ACS Nano*, 2014, **8**, 9767–9780.
- 40 W. Sun, T. Jiang, Y. Lu, M. Reiff, R. Mo and Z. Gu, *J. Am. Chem. Soc.*, 2014, **136**, 14722–14725.
- 41 R. Hu, X. Zhang, Z. Zhao, G. Zhu, T. Chen, T. Fu and W. Tan, *Angew. Chem., Int. Ed.*, 2014, **53**, 5821–5826.
- 42 G. D. Hamblin, K. M. M. Carneiro, J. F. Fakhoury, K. E. Bujold and H. F. Sleiman, *J. Am. Chem. Soc.*, 2012, **134**, 2888–2891.
- 43 P. K. Lo, P. Karam, F. A. Aldaye, C. K. McLaughlin, G. D. Hamblin, G. Cosa and H. F. Sleiman, *Nat. Chem.*, 2010, **2**, 319–328.
- 44 J. Li, H. Pei, B. Zhu, L. Liang, M. Wei, Y. He, N. Chen, D. Li, Q. Huang and C. Fan, *ACS Nano*, 2011, **5**, 8783–8789.
- 45 W. Sheng, T. Chen, W. Tan and Z. H. Fan, *ACS Nano*, 2013, **7**, 7067–7076.
- 46 R. Mo, T. Jiang and Z. Gu, *Angew. Chem., Int. Ed.*, 2014, **53**, 5815–5820.
- 47 Q. Jiang, C. Song, J. Nangreave, X. Liu, L. Lin, D. Qiu, Z.-G. Wang, G. Zou, X. Liang, H. Yan and B. Ding, *J. Am. Chem. Soc.*, 2012, **134**, 13396–13403.
- 48 Y.-X. Zhao, A. Shaw, X. Zeng, E. Benson, A. M. Nyström and B. Högberg, *ACS Nano*, 2012, **6**, 8684–8691.
- 49 Q. Zhang, Q. Jiang, N. Li, L. Dai, Q. Liu, L. Song, J. Wang, Y. Li, J. Tian, B. Ding and Y. Du, *ACS Nano*, 2014, **8**, 6633–6643.
- 50 K. Wang, M. You, Y. Chen, D. Han, Z. Zhu, J. Huang, K. Williams, C. J. Yang and W. Tan, *Angew. Chem., Int. Ed.*, 2011, **50**, 6098–6101.
- 51 C. Chen, L. Zhou, J. Geng, J. Ren and X. Qu, *Small*, 2013, **9**, 2793–2800.
- 52 H. J. Lipps and D. Rhodes, *Trends Cell Biol.*, 2009, **19**, 414–422.
- 53 D. M. Klinman, *Nat. Rev. Immunol.*, 2004, **4**, 249–259.
- 54 K. A. Whitehead, R. Langer and D. G. Anderson, *Nat. Rev. Drug Discovery*, 2009, **8**, 129–138.
- 55 H. Lee, A. K. R. Lytton-Jean, Y. Chen, K. T. Love, A. I. Park, E. D. Karagiannis, A. Sehgal, W. Querbes, C. S. Zurenko, M. Jayaraman, C. G. Peng, K. Charisse, A. Borodovsky, M. Manoharan, J. S. Donahoe, J. Truelove, M. Nahrendorf, R. Langer and D. G. Anderson, *Nat. Nanotechnol.*, 2012, **7**, 389–393.
- 56 N. Dias and C. A. Stein, *Mol. Cancer Ther.*, 2002, **1**, 347–355.
- 57 N. M. Dean and C. F. Bennett, *Oncogene*, 2003, **22**, 9087–9096.
- 58 J.-W. Keum, J.-H. Ahn and H. Bermudez, *Small*, 2011, **7**, 3529–3535.
- 59 K. Mohri, M. Nishikawa, Y. Takahashi and Y. Takakura, *Eur. J. Pharm. Sci.*, 2014, **58**, 26–33.
- 60 K. Mohri, M. Nishikawa, N. Takahashi, T. Shiomi, N. Matsuoka, K. Ogawa, M. Endo, K. Hidaka, H. Sugiyama, Y. Takahashi and Y. Takakura, *ACS Nano*, 2012, **6**, 5931–5940.
- 61 S. Uno, M. Nishikawa, K. Mohri, Y. Umeki, N. Matsuzaki, Y. Takahashi, H. Fujita, N. Kadowaki and Y. Takakura, *Nanomed. Nanotechnol. Biol. Med.*, 2014, **10**, 765–774.
- 62 V. J. Schüller, S. Heidegger, N. Sandholzer, P. C. Nickels, N. A. Suhartha, S. Endres, C. Bourquin and T. Liedl, *ACS Nano*, 2011, **5**, 9696–9702.
- 63 X. Ouyang, J. Li, H. Liu, B. Zhao, J. Yan, Y. Ma, S. Xiao, S. Song, Q. Huang, J. Chao and C. Fan, *Small*, 2013, **9**, 3082–3087.
- 64 A. M. Scott, J. D. Wolchok and L. J. Old, *Nat. Rev. Cancer*, 2012, **12**, 278–287.
- 65 S. A. Rosenberg, J. C. Yang and N. P. Restifo, *Nat. Med.*, 2004, **10**, 909–915.
- 66 X. Liu, Y. Xu, T. Yu, C. Clifford, Y. Liu, H. Yan and Y. Chang, *Nano Lett.*, 2012, **12**, 4254–4259.
- 67 Z. Zhang, M. A. Eckert, M. M. Ali, L. Liu, D.-K. Kang, E. Chang, E. J. Pone, L. S. Sender, D. A. Fruman and W. Zhao, *ChemBioChem*, 2014, **15**, 1268–1273.
- 68 C.-L. Zhu, C.-H. Lu, X.-Y. Song, H.-H. Yang and X.-R. Wang, *J. Am. Chem. Soc.*, 2011, **133**, 1278–1281.
- 69 H. Xing, N. Y. Wong, Y. Xiang and Y. Lu, *Curr. Opin. Chem. Biol.*, 2012, **16**, 429–435.
- 70 R. Mo, T. Jiang, W. Sun and Z. Gu, *Biomaterials*, 2015, **50**, 67–74.
- 71 Y. Liu, J. Du, M. Yan, M. Y. Lau, J. Hu, H. Han, O. O. Yang, S. Liang, W. Wei, H. Wang, J. Li, X. Zhu, L. Shi, W. Chen, C. Ji and Y. Lu, *Nat. Nanotechnol.*, 2013, **8**, 187–192.
- 72 J. J. Donnelly, B. Wahren and M. A. Liu, *J. Immunol.*, 2005, **175**, 633–639.
- 73 D. A. Brown, S. H. Kang, S. M. Gryaznov, L. DeDionisio, O. Heidenreich, S. Sullivan, X. Xu and M. I. Nerenberg, *J. Biol. Chem.*, 1994, **269**, 26801–26805.
- 74 S. J. McConkey, W. H. H. Reece, V. S. Moorthy, D. Webster, S. Dunachie, G. Butcher, J. M. Vuola, T. J. Blanchard, P. Gothard, K. Watkins, C. M. Hannan, S. Everaere, K. Brown, K. E. Kester, J. Cummings, J. Williams, D. G. Heppner, A. Pathan, K. Flanagan, N. Arulanantham, M. T. M. Roberts, M. Roy, G. L. Smith, J. Schneider, T. Peto, R. E. Sinden, S. C. Gilbert and A. V. S. Hill, *Nat. Med.*, 2003, **9**, 729–735.
- 75 H. Bugl, J. P. Danner, R. J. Molinari, J. T. Mulligan, H.-O. Park, B. Reichert, D. A. Roth, R. Wagner, B. Budowle, R. M. Scripp, J. A. L. Smith, S. J. Steele, G. Church and D. Endy, *Nat. Biotechnol.*, 2007, **25**, 627–629.
- 76 C. Lin, S. Rinker, X. Wang, Y. Liu, N. C. Seeman and H. Yan, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 17626–17631.