DNA Origami – Applications in Therapeutics

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Summary

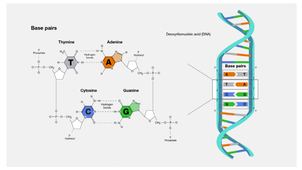
DNA is a ***stable and biocompatible molecule*** due to its chemical and physical properties. Recent advancements in DNA nanotechnology allow the application of DNA origami nanostructures in therapeutics, as delivery vehicles for chemotherapeutic drugs, as well as applications in photothermal and photodynamic therapy.

Research Aim

Previous studies have mainly focused on the applications of DNA origami in ***cancer therapeutics***. The aim of the present research is to generalise its therapeutic functions and discuss a ***wider range of mechanisms***.

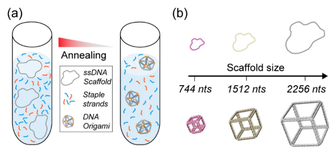
DNA Origami – Design and Synthesis

The concept of DNA origami allows the formation of DNA nanostructures using a ***ssDNA scaffold*** (typically Bacteriophage genome M13mp18). The scaffold has seams, and its shape is manipulated using ***staple strands***, which bind to specific sections on the scaffold due to ***complementary base pairing***. These crossovers are often considered to have negligible lengths and their positions largely affect the nanostructure's stability.



Several software have been developed to allow the computational design of DNA origami, including caDNAno, scaDNAno, oxDNA, etc. The designs can be inputted into CanDo, which can be used to conduct ***FEA***, testing the ***stability*** of the nanostructures.

DNA origami is usually ***synthesised in a buffer*** containing 5mM Tris, 1mM EDTA, 5mM NaCl, 12.5mM $MgClCl_2$, 20nM bacteriophage genome M13mp18, and ddH_2O. The results can then be ***analysed using TEM*** or ***AFM imaging***.



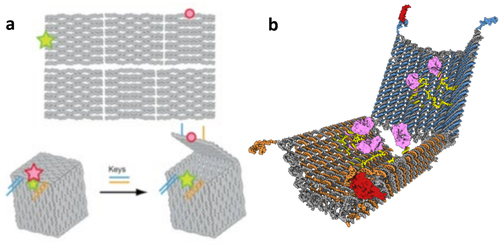
DNA Origami Boxes and Nanorobots

Origami Boxes

- 3D DNA Origami boxes have been created with programmable lids. The boxes use a ***dual lock-key system***, requiring two externally supplied key strand that fit into the toehold binding site due to the ***complementary strands*** on the box's front face being longer than the temporary closing strands on the lid. The box is then opened irreversibly via ***TMSD***.
- This process was made ***reversible***, allowing more control over when the drug is released, reducing the severity of side effects.

Nanorobots

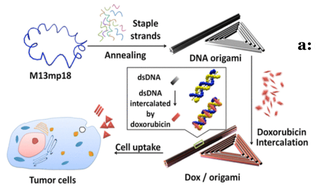
- DNA nanorobots were also designed and are able to ***detect single cells from mixed populations***.
- They consist of two sections with ssDNA entropic springs on one side and two locks on the other. They are loaded with ***antibodies*** to human ***CD33*** and human ***CDw328 Fab' fragments***, inhibiting growth of ***NK cells***. Meanwhile, nanorobots loaded with antibodies to human ***CD3ε Fab'*** and ***flagellin Fab'*** were mixed with T cells and found to activate these cells, implying that the nanorobots are capable of ***manipulating cell behaviour***.



a: DNA origami box
b: DNA nanorobot

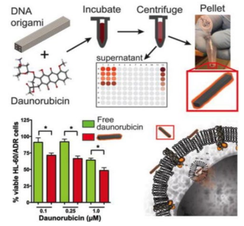
Anthracyclines – Doxorubicin and Daunorubicin

DNA origami nanostructures have also been used in ***chemotherapeutics***, as vehicles for doxorubicin. ***Doxorubicin*** is an anthracycline used in ***chemotherapy***, which inhibits the replication and transcription of functional genes in cancer cells. This drug ***lacks specificity*** and as a result can cause a variety of ***side effects***.



a: doxorubicin delivery vehicle

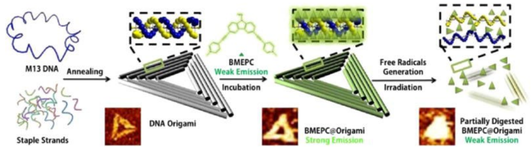
Similar methods can also be employed using another chemotherapeutic drug, ***daunorubicin***. Recently, daunorubicin-loaded trojan horse DNA nanostructures were formed for ***daunorubicin delivery***. These were ***rod-like*** and were successfully taken in by HL-60 cells. They have also been found to ***reduce the effect of MDR***, which therefore ***reduces the need for prescribing patients with higher doses of chemotherapeutic drugs***.



b: daunorubicin-loaded trojan horse

Photothermal and Photodynamic Therapy

DNA origami nanostructures can also be utilised in ***photodynamic and photothermal therapy***. There was an attempt to ***inhibit the growth*** of MCF cancer cells using a DO-AuNR complex, which was tested in vivo using MCF-7 xenograft tumour bearing mice. This technology was further improved through the development of an optoacoustic imaging agent and use of DO-AuNR complexes to ***enhance the imaging resolution*** of the mouse tumour tissues. DNA origami also has applications in photodynamic therapy as a ***photosensitive agent***.

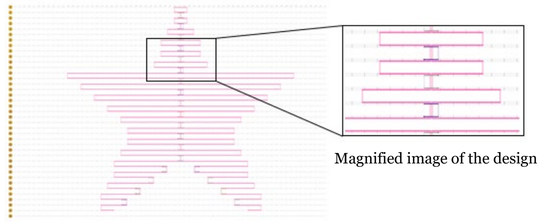


Our Designs

Oriongami

The Oriongami is a DNA nanostructure with a concave decagon shape. It consists of one scaffold strand and 22 staple strands. Its order 5 rotational symmetry makes it a great drug delivery vehicle as it has multiple binding sites. Its simple yet symmetrical structure also gives it a relatively large surface area, allowing it to bind to substances efficiently, giving it a high potential in therapeutics. The following are a few examples of the Oriongami's most probable applications in therapeutics:

- **<u>Anthracycline delivery vehicle:</u>** 5 vertices which can act as intercalation sites
  - *Doxorubicin:* When doxorubicin is adsorbed to Oriongami, the drug's ability to induce cell death in doxorubicin-resistant MCF-7 adenocarcinoma cells is boosted.
  - *Daunorubicin: Daunorubicin* can be successfully taken in by HL-60 cells and will efficiently circumvent drug resistance mediated by efflux pumps in leukemia cells. This is because P-gp, which is overexpressed in many drug-resistant tumour cells, can get downregulated through the use of Oriongami, reducing the effect of MDR.
- **<u>Photothermal and photodynamic therapy:</u>**
  - Heat generated from NIR of the DO-AuNR complex can be used to inhibit tumour growth and BMEPC can also be intercalated to Oriongami, triggering the apoptosis of tumour cells through free radical production.



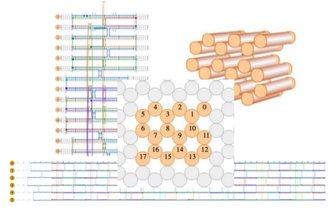
Magnified image of the design

Nanotube Drug Delivery

- A nanotube is a cylindrical structure made of DNA, with a hollow core that can be used for drug delivery or as a template for the synthesis of nanowires.
- When creating the nanotube, we had to start by designing a long scaffold strand of 100-200 nucleotides. Then, adding staple strands perpendicular to the scaffold strand to form a rectangular shape with a width of approximately 25-50 nm. Once a rectangular shape is formed, we can add more staple strands to close the ends and create a tube.
- Then, when folding using DNA origami techniques we can mix the scaffold and staple strands together in a buffer solution and slowly cool the solution to allow the DNA strands to self-assemble into the desired nanotube structure, using complementary base pairing.
- We can verify the structure of the DNA nanotube using imaging techniques such as atomic force microscopy (AFM) or transmission electron microscopy (TEM).
- With the correct number of crossover points, DNA nanotubes are highly stable structures that can withstand high temperatures, extreme pH conditions, and other harsh environments. This makes them useful for applications in biotechnology and materials science.

Potential applications of DNA nanotubes include:

- templates for the synthesis of nanowires and other nanomaterials.
- delivery vehicles for drugs or other molecules.
- sensors for detecting biological molecules or other analytes.
- scaffolds for tissue engineering and regenerative medicine.



Conclusion and Recommendations

DNA origami nanostructures have been shown to be successful drug delivery vehicles as they can survive long enough to arrive at the target site. They have also been used in cancer treatment through photothermal and photodynamic therapy.

However, the potential use of DNA origami for the treatment of many diseases is often neglected. Also, more sophisticated computational methods for the design of DNA origami should also be developed for the design of complex shapes.