Development of DNA Origami Nanostructures for Application in Medicine

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Overview

DNA Origami in Medicine

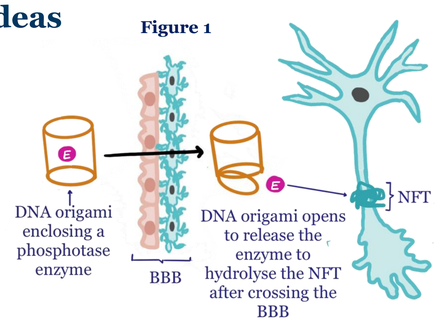
DNA origami has medical applications in targeted drug delivery, improving drug accuracy, and reducing toxicity. It can create containers for drug delivery to specific cells or tissues, improving drug pharmacokinetics, and enhancing efficacy. Additionally, it can be used for tumour imaging, monitoring cancer treatment efficacy, and as an alternative to traditional vaccines. In diagnostics, it can create probes for disease markers and point-of-care diagnostic devices.

Preliminary Ideas

Alzheimer's

DNA origami may bypass drug delivery challenges to the brain and restore neuronal function, with potential for treating cerebral issues. We proposed using DNA origami to carry enzymes that can break down NFTs across the BBB, based on previous research [1][4]. Figure 1 illustrates the enzyme containing DNA nanostructure. monomers.

Figure 1



Dialysis

DNA origami technology could eliminate the need for dialysis or organ transplants by creating the filtration system for a biomechanical kidney implant (see figure 2). This implant would use nano-scale hexagonal plates (see figure 3) to tessellate to form a partially permeable membrane to filter the blood and would reduce infections and damage caused by current treatments.

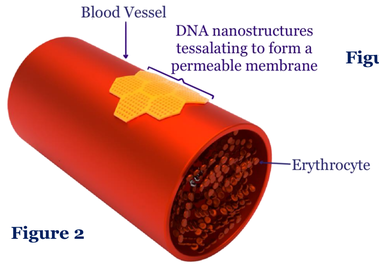
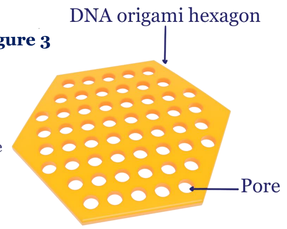


Figure 2

Figure 3



Cancer & Chemotherapy

Using DNA Origami, we could improve drug effectiveness by delivering it only to the tumour cells. The DNA origami mimics an antibody and has a complementary shape for antigens on tumour cells. The nanostructure could also be altered by an enzyme to potentially increase drug binding to the tumour. Figure 5 shows the catalytic action of the DNA nanostructure and enzyme as the removal of staples moves the drug closer to tumour cells.

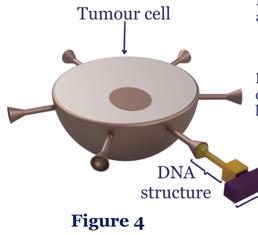


Figure 4

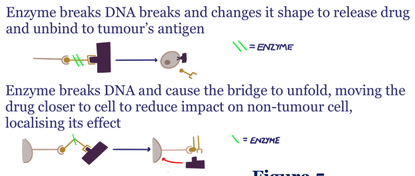


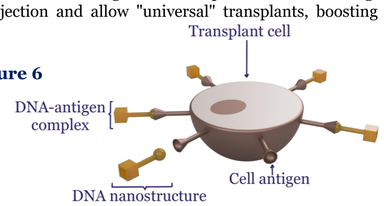
Figure 5

M015

Transplant + Rejection

DNA origami could solve compatibility issues by re-expressing matching antigenic surface glycoproteins and avoiding immune response as shown in Figure 6. This could reduce immunorejection and allow "universal" transplants, boosting transplantation rates and success.

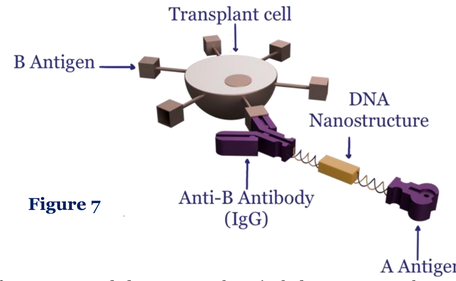
Figure 6



Final Design – RCAG

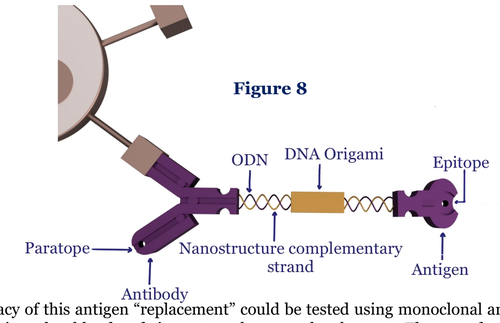
From the preliminary ideas, we decided to continue researching into transplantation and how we could apply DNA origami problems, focusing on primarily on how we could universalise donatory transplants, by re-expressing cell-surface antigenic glycoproteins (RCAG) to prevent rejection. However, it was determined that perfectly mimicking the binding site would not be possible with current design limitations, but it could be a potential area for future research.

Figure 7



Therefore, we expanded our research to include a structure that would allow for an attachment site for the antigen/antibody. A published paper had done this research and stated that a protein could allow for the intermediary binding of antibodies to DNA nanostructures that is site-specific. It uses a protein that has a single-strand of DNA exposed - an oligodeoxynucleotide (ODN) exposed, enabling the nanostructure to bind to the protein through complementary base pairing of DNA. This gives us control over where the protein attaches to the nanostructure, and thus where the antibody binds. However, a different protein would have to be research for the attachment to the antigen due to its different chemical composition. It would also required an exposed ODN. This allows us to position the antigen and antibody geometrically opposite from each other, which facilitates our concept of re-expression of antigens.

Figure 8



The efficacy of this antigen "replacement" could be tested using monoclonal antibodies to determine what blood and tissue type the organ has become. The use of antibodies for the antigen concealment could be replaced by aptamers, single stranded oligonucleotides that fold into defined structures and bind to targets similarly to antibodies. The aptamer would not require the conjugatory strategy as there is an immediate compatibility whereby aptamers may be attached to ODNs.

Integration of Software

Designing a Smiley Face in Scadnano

Our first major project on caDNAno single loop of scaffold and implement stapling for a complicated design, which posed a challenge due to the irregular shape requiring holes for the eyes and mouth. Issues with our stapling technique highlighted displacement between helices. We tried an alternative stapling method, which reduced fluctuations but did not eliminate distortion when viewed from the side.

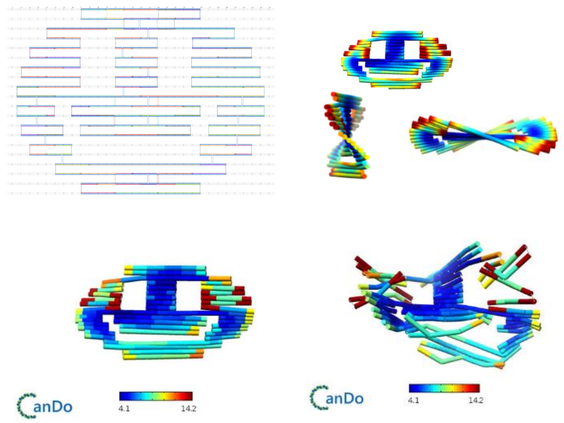


Figure 9

Designing a Simple Cube in caDNAno

For our first project in the migration to caDNAno, we began with a simple cube to get familiar with the software. We started with a row of helices spanning left to right, then alternating rows throughout the structure. This made adding crossovers easier on the 2D scaffold. We used CanDo to visualize helix strength, but its limited views were not sufficient. Switching to ChimeraX allowed us to produce a 3D model, but lacked strength testing. By combining the utilities of both the software, generating an analysis on CanDo and using a BILD file in ChimeraX, we were able to view the molecule's strength from all angles with the free view interface.

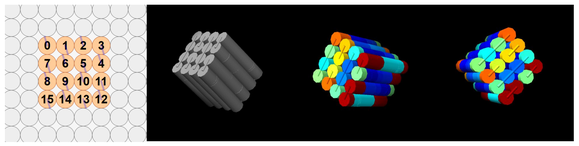


Figure 10

Final Design

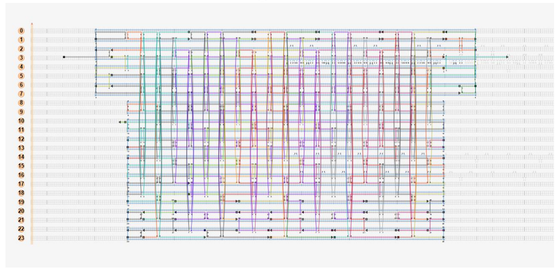
The mechanism we designed to deliver our concept, for the re-expression of antigens on a cell, simply needed to connect an antigen on the cell surface to a different antigen of our choice, so there was little constraint in the structure we had to create. We settled on generating a tube-like structure to act as the connection piece between the antigens, as this provided simplicity and meant our molecule was sufficiently strong to prevent fluctuations, and theoretically when mass producing DNA origami to put our concept into practice a tube would be relatively quick and cost effective to produce.

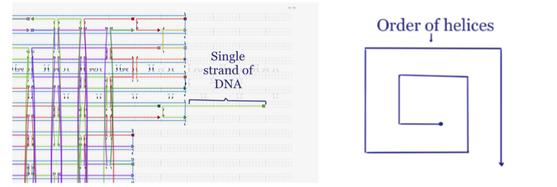
Although it was not able to viewed on ChimeraX, our molecule had to contain single strands sticking out on either end as these provided connection points to the proteins, which in turn were bound to the antibody and antigen on either end; these are highlighted below on the 2D map of our design.

Final Design (Continued)

Our structure is essentially made by rolling the sheet up and wrapping it around itself to form two layers of DNA helices – as indicated by the order of the helices. The regular implementation of crossovers and stapling within the helices was achieved by using the auto-staple feature available within caDNAno, and then tweaking slightly to ensure length of staples were the correct length to bind the structure effectively. The inner ring of helices are also longer than the outer layer, as this provides a lot of central stability in the molecule while also providing almost a chamfered finish at the ends which allows greater range of access to the single strand connection point.

Figure 11





Visualisation of the Final Design

Once our structure had been created, we used CanDo to convert our JSON file to a BILD file, and viewed the results on ChimeraX:

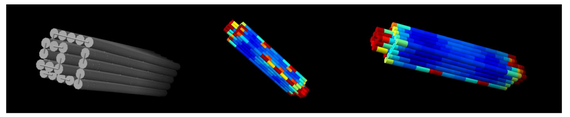


Figure 12

The molecule's length is substantial compared to its width, maintaining structural integrity especially in the central region, while weak spots were found at the ends, allow for flexibility in binding to the connection points. The molecule is slightly distorted down its length but this does not affect its function significantly.

Conclusion

The solution has potential however delivery inaccuracy requires excess DNA origami, which also need precise design to avoid warping, leading to a failure of re-expression and in succession, rejection. Further questions could arise in the aspect of cell growth and regeneration. The organ would be transplanted presenting, in theory, only compatible antigens but what happens when the cells that have the DNA origami bound to the antigens die? With more research, we could find potential solutions to these problems and relieve the tension on the donor system.

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