DNA Origami
# - DNA Origami for Targeted Drug Delivery Systems

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Introduction

DNA Origami refers to the process of constructing nanostructures out of DNA. The base concept is to take a natural loop of single stranded DNA (typically extracted from a Bacteriophage), and then designing and synthesising a number of short strands of DNA with base pairs complimentary to those of the DNA scaffold. When mixed together these staples will then bind to their specific sections of the scaffold, causing it to fold into a more rigid 3D design. When careful considerations are taken during the design of the staples, a number of complex 3D nanostructures can be produced, which can have applications all over material science and medicine.
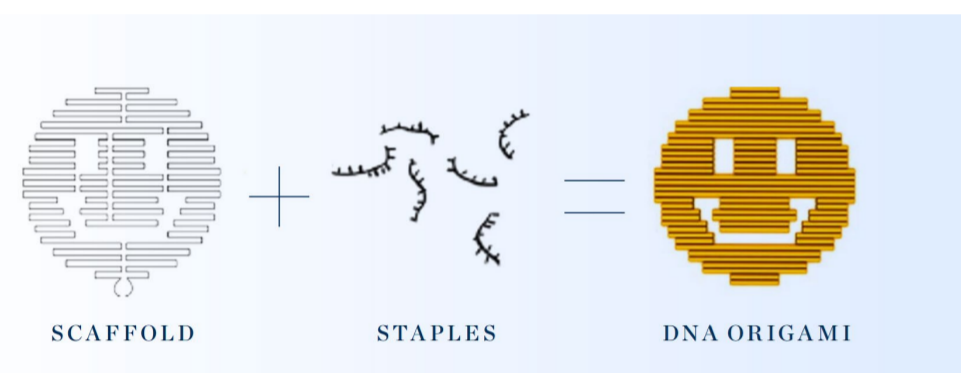
High level synthesis overview (1)

The process of designing these structures is made possible by an online software package call Scadnano (2). It allows for a number of DNA Helices to be created and arranged, and a scaffold shape drawn onto them, followed by a number of different staples designed to manipulate the shape of the DNA structure.
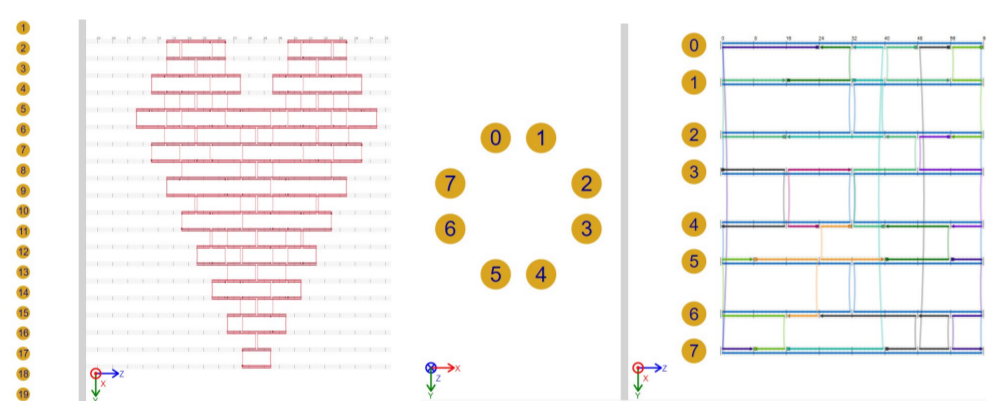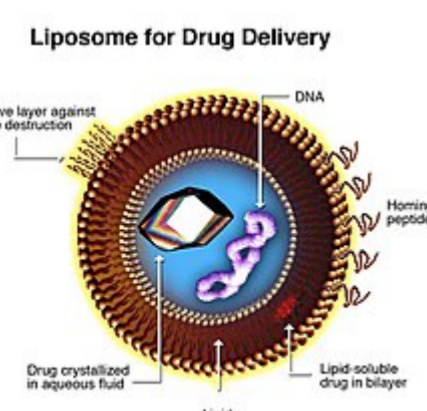

Scadnano design interface (2)

Once we have created the scadnano designs, we are then able to use another web based software suite called Cando to analyse our designs, by using two different exported files, containing information about the design structure, and the DNA sequences of the staples.

Project Overview

We viewed DNA origami as a perfect candidate for use in a drug delivery system within the human body, due to its organic roots, its strength, and the degree of control that we have over its structure thanks to DNA origami. We ultimately decided that a box like structure is likely to be the best design for an openable container, however did conduct some investigation into alternative shapes (one of which is shown to the right). The most exciting part of this branch of research is the possibility of adapting it to target particular antigens (e.g. for use in chemotherapy, or viral treatments).

Currently targeted drug delivery widely uses Liposomes (3), which are biocompatible lipid based nanocontainers which carry a payload in their hydrophilic interiors.

DNA Origami structures provide a greater degree of control over both the container size and shape, and the deployment of the payload, due to their programmable nature. Our box should be able to respond to environmental changes to deploy its contents exactly when desired.

**A Liposome for use in drug delivery (4)**

Initial Design Practice

In order to get to grips with the design process, we started off by creating a number of different, rather simplistic, 2D designs, some of which are shown below.
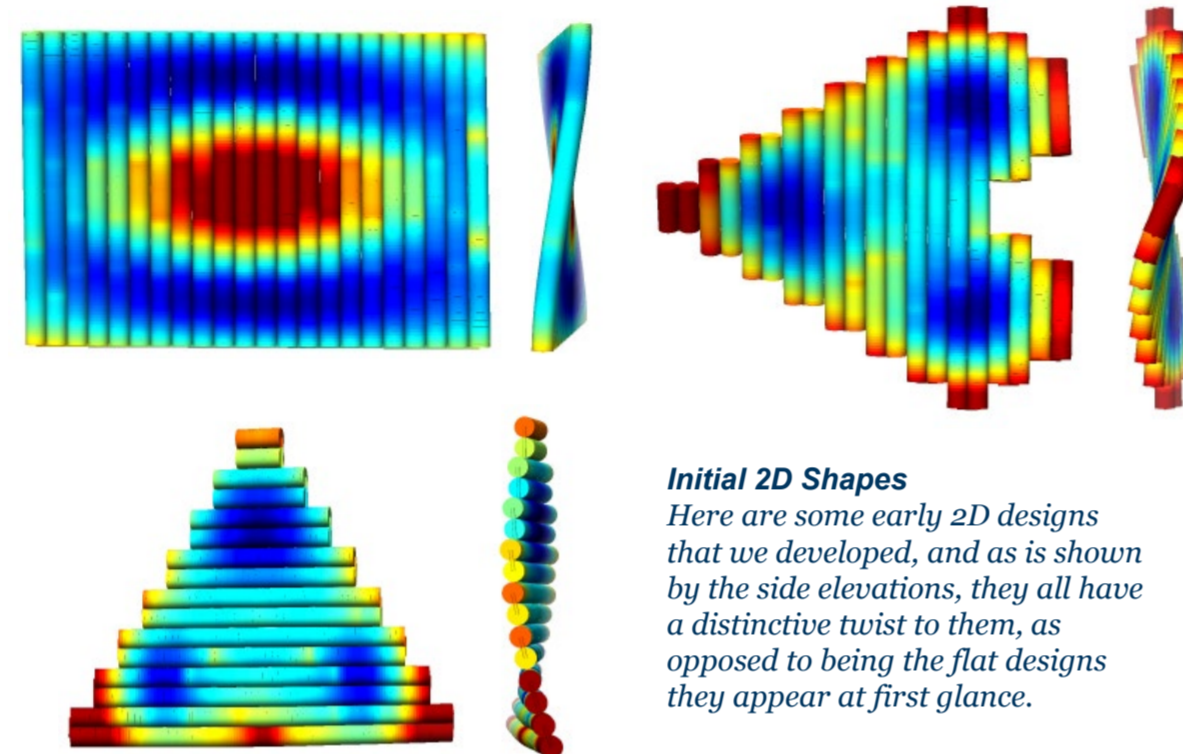
Initial 2D Shapes
Here are some early 2D designs that we developed, and as is shown by the side elevations, they all have a distinctive twist to them, as opposed to being the flat designs they appear at first glance.

Our ultimate goal of creating a drug delivery system requires the use of flat rectangular planes connected together, and, as shown above, we need to take special precautions to make sure that our designs are, in fact, flat, rather than twisted like the structures shown above. There are lots of different things that we can do to obtain a greater degree of control over our DNA designs, discussed to the right, however before we began to look more closely at making these flat planes, we decided to experiment with creating 3D structures from a single DNA scaffold, as shown below.
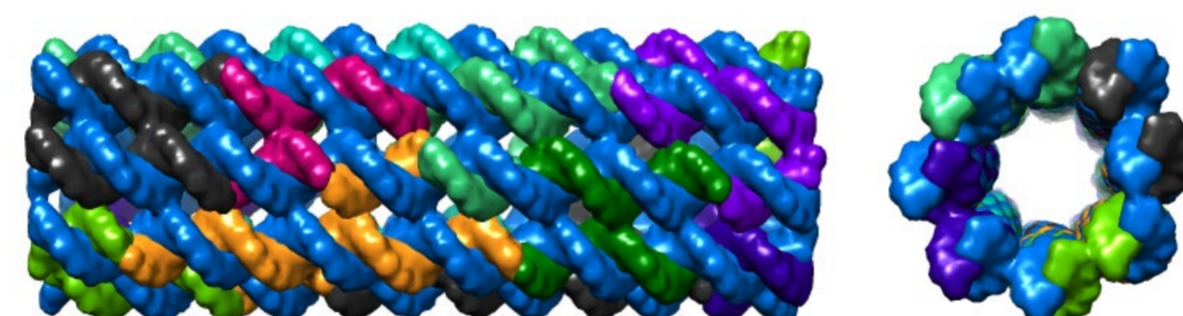

Early 3D Shapes
In order to practice creating 3D DNA designs we created a simple cylindrical shape. This has the added benefit of being a potential shape for use as a container for a payload.

Bridging Strands

DNA bridging strands are single strands of DNA, which work by binding to complementary single stranded extensions on two DNA origami structures in order to connect them, similarly to how staples hold parts of a scaffold together within a single structure.

They seen good candidates for use to connect planar 'container wall' structures together into a 3D box-like structure.

By designing one side of the bridging strand to (for example) fold up into a hairpin structure at certain pHs or temperatures, the connectivity provided by these strands can be controlled to a greater extent, allowing us to open and close our containers.
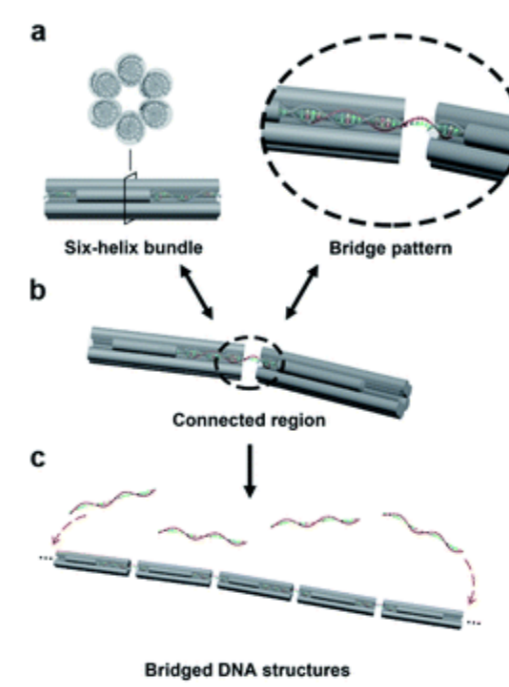

Bridging Strands in action (5)

Deletions Experiments

The term 'Deletion' refers to the (in this case artificial) removal of a nucleotide base pair from the scaffold, and is used to disrupt the helical structure of DNA, by effectively adding gaps, also known as 'notches', or 'grooves', into the scaffold structure, creating a structural disruption, and the subsequent asymmetry is resistant to bending and twisting. This allows for flat designs to be created, however if the deletions are added in such a way as to add symmetry, or are used almost randomly, a further twisting effect can be observed, as we learnt by examining the results of some of our below experimental designs.
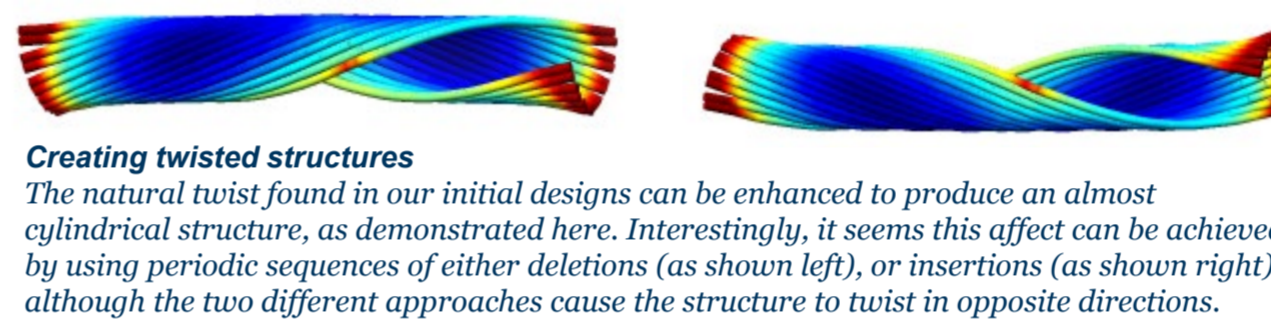
Creating twisted structures
The natural twist found in our initial designs can be enhanced to produce an almost cylindrical structure, as demonstrated here. Interestingly, it seems this affect can be achieved by using periodic sequences of either deletions (as shown left), or insertions (as shown right), although the two different approaches cause the structure to twist in opposite directions.
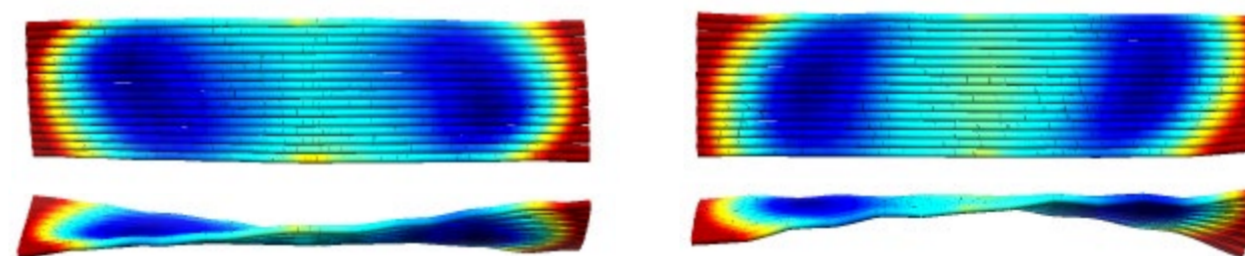
Modifying deletion regularity
Unlike the more regular deletions which caused an almost cylindrical design, the far less regular deletions used here (shown right) resulted in a much more subtle change from a design using no deletions at all (shown left). This use of deletions has in fact begun to reduce the twist seen in the design, almost limiting the deformation to one dimension, rather than the typical 2.

Mixing Insertions and Deletions
As shown by the unusual shape of the above design, insertions and deletions can be used to create some unusual shapes. This particular design shows a similar structure to a flat rectangle which has had extra material added to its middle, causing it to bulge outwards, and fold up at the edges, which is due to the use of insertions solely in the centre of the design.

Design Submissions

In order to analyse the 3D structures produced by the folding of our designs we submit two different files to a web based software suite called Cando (6). The CSV files containing information about the DNA sequences of the staples require a number of modifications before they are fit for processing, and making these changes by hand can take considerable time (especially for larger designs), and slow down our development process.

In order to overcome this problem, and speed up our development and research, we developed a simple python script to convert the files to the correct format, allowing us to prepare designs for analysis quickly, and remove the risk of potential human error in the translation, which has the potential to prevent the analysis working, or at least from working as desired.

```
# A program to convert scadnano CSV files to the format required for structure analysis

def readFile(name):
    file = []
    with open(name, "r") as f:
        for line in f:
            line = line.strip("\n")
            line = line.split(",")
            file.append(line)
    return file

def writeFile(name, list_):
    with open(name, "w") as f:
        for line in list_:
            f.write(",".join(line)+"\n")

def change(fileList):
    newFile = []
    newFile.append(["start", "end", "sequence", "length", "color"])
    for line in fileList:
        start, sequence = line
        start = start.strip("[]")
        start = start.split("][")[:-1]
        line = start
        line.append(sequence)
        line.append(str(len(sequence)))
        line.append("#0f0000")
        newFile.append(line)
    return newFile

directory = "CSV files/"
name = input("file name: ")
print("loading file...")
c = readFile(directory+name)
print("editing file...")
c = change(c)
print("saving file...")
newName = name.replace(".csv", "_new.csv")
writeFile(directory+newName, c)
```

Specification using Aptamers

In order for our drug delivery system to be able to target the chosen specific molecule, we investigated different ways of binding our DNA parcel to said molecule; one obvious method would be to use antibodies. Antibodies are proteins that are specific to a range of targets that are naturally produced by the body. There are however a few drawbacks to antibodies for example, they would be hard to incorporate into the DNA origami structure and they are laborious and expensive to produce. Because of this, we decided to use a ligand, more specifically we realised that using an aptamer would be the best method of binding our DNA cube to the chosen molecule.

Aptamers are oligonucleotides that have a high affinity and specificity in identifying target molecules (7, 8, 9). They can bind to small organic compounds, as well as proteins and nucleic acid. Aptamers naturally fold up into their unique structure and then bind to the recognised molecule by various mechanisms such as: induced fit, electrostatic attractions and hydrogen bonds.

Key features of aptamers:
- Non-immunogenic
- Nontoxic
- Can be selected in *vitro* and in *vivo*
- Have a high stability in *vivo*
- Good solubility
- Easy to synthesise
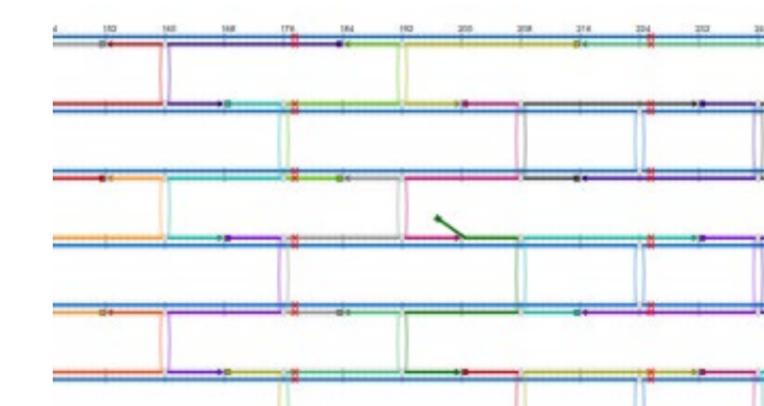
Adding Aptamers as Extensions
As shown left, scadnano supports the addition of single stranded DNA extensions, allowing us to add extensions with particular DNA sequences (for particular aptamers) to our designs.

Selecting Aptamers

The idea behind our system is that different users are able to alter the makeup of it depending on what it is they are targeting it to, to find the aptamer needed for the specific molecule, it can be selected using SELEX. SELEX (systematic Evolution of Ligands by EXponential enrichment) is used to isolate aptamers with high affinity and screen and evaluate aptamers against potential targets from combinational oligonucleotide libraries.

The required aptamers will then be incorporated into the DNA origami structure as an extension of a number of staples. We have tried to see if this method of integrating the aptamer would work by running the design in scadnano by adding it to a single staple on a flat rectangle; however due to software limitations we were unable to view the design in 3D to see if it would fold in the expected way to form the needed shape of the aptamer.

Conclusions & Next Steps

Our research into all of the separate components required for a drug delivery system has been very exciting and informative. Getting used to using the powerful tools provided by scadnano and cando to produce complex 3D structures has been a welcome challenge, and looking into the effects that deletions and insertions can have on the shapes of the designs has given us knowledge and experience which we can hopefully turn towards implementing our designs for a drug delivery system in the future. Researching bridging strands and aptamers as tools for turning our flat DNA planes into complex nano machines has also been exciting, and we look forward to focusing our efforts on unifying all of these parts into a singular design which is able to contribute to the amazing field of medical science in our own, small way.

References

1. https://resourcecentre.researchinschools.org/?projects=dna-origami
2. https://scadnano.org/
3. https://en.wikipedia.org/wiki/Targeted_drug_delivery/
4. https://en.wikipedia.org/wiki/File:Liposome.jpg
5. https://pubs.rsc.org/en/content/articlehtml/2022/sc/d1sc05060e
6. https://cando-dna-origami.org/
7. https://www.nature.com/
8. https://pubs.rsc.org/
9. https://www.biorxiv.org/
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