DNA Origami
How DNA structures can be used to transport Interleukin-2

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Outline

Our research is evaluating the usage of modelling DNA to help deliver drugs around the body. Specifically, we will be assessing the use of this for interleukin-2 (IL-2), a type of cytokine (protein). IL-2 is released by T-cells which can detect cancerous cells antigens and signals to the immune system that they are a threat. Additionally, we shall model our own structure (a star shape) out of DNA using a software called scaDNAno to design such a shape. Then, we shall synthesise a shape of DNA at a later date.

Hypothesis

Can “folding DNA” help the distribution of single IL-2 molecules around the body? To test this, we shall consolidate research completed by other sources and evaluate this question. Due to the complexity, we will not directly test this hypothesis as we can not design 3D structures. However, we will be producing a 2D shape to learn how to use designer DNA.

DNA origami

DNA origami is a project that focuses on folding long chains of DNA into useful and interesting shapes. Biologically, it contains the instructions for cells to grow and reproduce. Because DNA comes in pairs of nucleotides that are attracted to each other, we can program them to bond with other strands. Due to the “codable” nature of DNA, material scientists select it for a number of projects where small but precise molecules are required: novel data storage in systems, treating diseases, logic components in computer chips and, the focus of our research, in the transport of drugs around the body [1]. We will explore how this is done (designing a 2D structure) and, more importantly, its real life applications (in drug transportation). It is important to note that in this context DNA is not a double helix but single strand DNA (ssDNA).

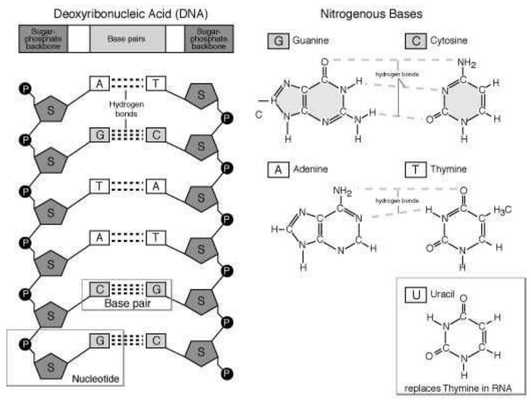

Figure 1: Base pairs of DNA [2]

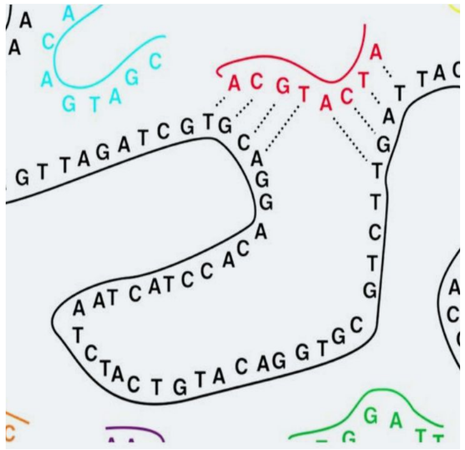

Figure 2: A diagram depicting how DNA folds in on itself [1]

DNA and how it folds

Deoxyribonucleic acid (DNA) has “base pairs” of chemicals called nueclotides. These combine with the backbone to form the strand of DNA. Adenine (A) bonds with thymine (T) to make one of these base pairs and cytosine (C) bonds with guanine (G) to make the other [3]. A-T pairs form two hydrogen bonds, whereas C-G pairs form three hydrogen bonds. This means that A-T rich strands will be slightly weaker but more flexible than C-G rich strands of DNA. However hydrogen bonds are weak so don’t make much difference to the overall stability of the structure. The attraction of these bonds is useful when we model DNA: we can put corresponding nucleotides on matching small and long strands of ssDNA to fold and lock the shape into more rigid positions. Therefore, these bonds can give DNA different shapes. There are only certain places the structure can bond (matching AT and CG pairs) so when it is mixed, it will self assemble under the correct conditions [4].

DNA cages

DNA is used in designing small structures which is particularly useful for transportation of chemicals. This is because they are very small. For example, interleukin-2 molecule is 2.0 nanometers (converted from kDa to nanometers) [5] whereas a human double helix DNA is 2.0 - 2.5 nanometers [6] in diameter. Additionally, they are able to be “coded” due to the AT CG bonds - a unique feature for something so microscopic. Scientists select DNA for “cages” to carry drugs around the body. This can be to regulate extraneous variables or to stop the molecule interacting with the wrong part of the body. Additionally, DNA cages can be designed to release IL-2 towards targeted places in the body. If we too were to design a cage to do this, it would be a cube (this shape would be very rough as it is so small) with the medicine in the centre.

Interleukin-2 (IL-2)

Interleukin-2 was discovered in 1976 by Morgan et al [7]. It works by stimulating the immune system, encouraging the growth and division of white blood cells. It works by activating other immune cells which in turn destroy the cancerous cells by interfering with the growth and multiplication of cells. The chemical is used to treat advanced kidney cancer where it has proliferated to other parts of the body [8].

Il-2 (or aldesleukin) has a short half life [9] which causes a need for higher dosages every few hours in blood transfusions. If DNA is used to encapsulate the cytokine, it could be more efficient instead of searching the body at random for tumours. One danger of IL-2 is that it can induce a further problem in the form of “cytokine release syndrome” - where the immune system responds to the immunostimulant aggressively. This is why we suggest using DNA cages for it should theoretically protect the IL-2 from interfering with other parts of the body than the tumour. Meaning a lower chance of a patient developing cytokine release syndrome.

Interleukin-2 does have side effects. These include flu-like symptoms such as chills, fever, fatigue, confusion, nausea, vomiting or diarrhoea. However in rare cases, there are some more serious side effects. These include an abnormal heartbeat, chest pain as well as other heart problems. On top of this, due to IL-2 having a short half-life, it must be taken in high doses and often, meaning that not only does IL-2 increase the likelihood of the side effects listed above but also must be done in a hospital [10]. We believe that by using DNA cages it could be targeted towards cancerous cells. Limiting these side effects and reducing the dosage due to it being further targeted towards the and therefore more efficient.

Designing a 2D DNA structure

Originally, we were planning on designing and then synthesising the cage that would house the IL-2 molecule. However, due to complexity in design, and the fact that scaDNAno cannot create 3D shapes we chose to only make a 2D shape. Therefore, we concluded that we should continue with the DNA origami project guidelines and form a star.

When designing a DNA structure on scaDNAno, we are aiming to make the shape as strong as possible. To do this, we create an outline of the star shape with long strands of ssDNA called scaffolds (in blue) and root it in place with shorter strands of DNA called staples (in red) which will weave throughout the structure. Generally, the edges of the scaffold will be the weakest. When designing the star, we followed the guidelines given by IRIS to make our staples strong eg. keeping each strand of DNA shorter than 32 base.

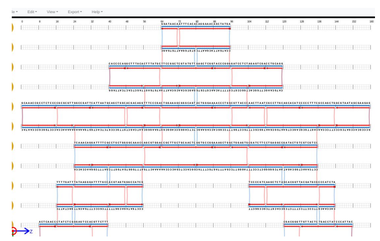

Figure 3: Our design of a DNA structure, made on scaDNAno

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Start,End,Sequence,Length,Color
4[15),5[15),GGGAGAGGCGGTTTGCGTATTGGGCGCCAGGG,32,#ff0000
10[15),11[31),GGTTGAGTGTTGTTCCAGTTTGGAACAAGAGT,32,#ff0000
9[24),11[39),AACGTCAAGGGCGAAAAGAACGCGCACTATT,32,#ff0000
8[31),7[39),CCCTTATATGGTGGTTCCGAAATC,24,#ff0000
10[31),8[16),TGGACTCCCGAGATAGGAATAGCCAATCAAAA,32,#ff0000
5[32),4[40),CAGTGAGACGGGCAACGCCAGCTG,24,#ff0000
4[39),5[23),CATTAATGAATCGGCCAACGCGCGTGGTTTTT,32,#ff0000
7[40),9[55),GAACCATCACCCAAATCGATGGCCTATCAGGG,32,#ff0000
9[40),8[32),AAACCGTCCACTACGTGGCAAAAT,24,#ff0000
6[47),5[31),CCAGCAGGCGAAAATCCTGTTTGACTTTTCAC,32,#ff0000
2[55),3[55),TGTAAAGCCTGGGGTGCCTAATGAGTGAGCTA,32,#ff0000
4[55),6[48),CCTGTCGTAGCTGATTGGTTTGCC,24,#ff0000
5[56),2[56),GCCCTTCATCGGGAAAACTCACATGCATAAAG,32,#ff0000
6[63),7[79),TCCACGCTCAAGTTTTTGGGGTCGAGGTGCC,32,#ff0000
2[71),0[64),AGCCGGAAGCTCACAATGTTATCC,24,#ff0000
3[72),4[88),TTGCGCTCCACGACGTTGTAAAACCCAGGGTT,32,#ff0000
5[72),6[88),CCCTGAGAGCTTCTGGTGCCGGAACCAGCTTT,32,#ff0000
1[80),2[72),GAATTCGTAATCATGGCCCGGGTACCGAGCTCAACATACG,40,#ff0000
7[80),6[96),GTAAATCACTAAATCCGGAACCCTACACTCCAG,32,#ff0000
4[87),3[71),TTCCCAGTACTGCCCGCTTTCCAGTAATTGCG,32,#ff0000
6[87),5[71),CCGGCACCGAGTTGCAGCAAGCGGCCGCCTGG,32,#ff0000
0[95),1[79),TCATAGCTGTTTCCTGTGTGAAATTTCCACAC,32,#ff0000
2[103),5[103),AGAGGATCGACGGCCAGGGTAACGACCAGGCA,32,#ff0000
3[104),2[104),GTGCCAAGCTTGCATGCCTGCAGGTCGACTCT,32,#ff0000
9[104),7[119),AAGCCGGCGACGGGGAAAGGGAGCCCCCGATT,32,#ff0000
6[111),4[104),GAAGATCGAAGCGCCAATTAAGTT,24,#ff0000
4[119),5[127),GCAAGGCGTTCGCCATTCAGGCTG,24,#ff0000
7[120),8[128),ATCTGCCAGTTTGAGGCGCATCGT,24,#ff0000
11[120),9[135),GATTCTCCACCCGTCGCGAGAAGGAAGGGAA,32,#ff0000
8[127),8[119),AACCGTGCTAGAGCTTGACGTGG,24,#ff0000
5[128),6[112),CGCAACTGGGACGACGACAGTATCGGCCTCAG,32,#ff0000
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Figure 4: The “code” of the DNA sequences, depicting how long it is, the order of base pairs and the colour assigned in scaDNAno

Analysis & conclusions

After sending the code of our sequences off to IRIS, we have had our model analysed. This has shown us how inherently stable the shape is. The parts in blue (in figure 4 & 5) are fully stable but the parts in red are less stable. Weaker parts of the structure will curve due to the twisted nature of DNA. These are quite clearly along the edges of the design. This would be because the routing is less secure as we cannot connect the scaffold to other parts of the structure. Particularly, we can see that the the edges of the design will twist slightly.

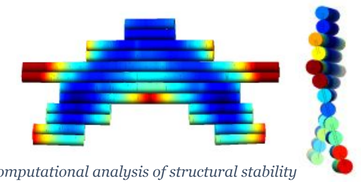

Figure 5 & 6: Computational analysis of structural stability

Overall, our structure is generally stable enough to be synthesised. At a later date we will do this to our DNA structure. If it were possible in future we would create a DNA cage to see if it this hypothesis would be able to have real world applications

References

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