DNA Origami
Biotech Blueprint

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INTRODUCTION

There is art in science. Few innovations embody this axiom as well as DNA origami. Nearly three decades after Watson, Crick, and Franklin's groundbreaking research established a set of base-pairing rules for DNA strands, research published in the Journal of Theoretical Biology suggested the idea of turning DNA into building blocks for nanostructures.[1] Today, the use of DNA as a structural material has become more prevalent. These advances use the specific complementary base pairing properties of DNA for applications in drug delivery, biosensors, and enzyme-cascades.[2] This article will review the DNA origami design process and its promising applications.

DESIGNING THE STRUCTURE

DNA origami involves the directed folding of a long single-stranded DNA (ssDNA), called the scaffold, through the binding of hundreds of specifically designed shorter ssDNA, called staples. The scaffold is usually sourced from viral DNA (e.g. M13 bacteriophages) and is typically 7,000 nucleotides long.[3] The staples are capable of base pairing to different regions of the scaffold, thereby bringing physically distant regions of the long ssDNA together. The design of the DNA origami structure is dependent on the staple sequences.[3] Designing a DNA origami structure requires translating the desired structure into a series of folds followed by synthesizing the appropriate staples to perform them. Creating a DNA origami structure is simplified through computer-assisted design. Currently, three generations of DNA origami design tools exist. While the first-generation tools require manually routing the scaffolds and generating the crossovers where staples are needed, second and third-generation tools, such as ATHENA and Adenita, are more user-friendly and demand less technical knowledge.[3]

Designing a DNA origami nanostructure begins with the manual generation of a block diagram, which consists of rectangular blocks representing the width of one DNA turn (~3.6 nm) and the height of roughly two helical widths (~4 nm).[4] Next, a folding path is manually designed using a raster filling pattern to fit the block diagram. This process mimics how DNA strands would line and fill the block diagram.[4] A "first pass" design is then generated by computer software. This design is displayed in the form of a series of numerical coordinates that map out the sequence of the scaffold and the positions at which staples are annealed to the scaffold. This software output constitutes a rudimentary DNA filling design for the structure.[4] To minimize torsional strain on the DNA in the first pass design, the computer modifies the crossover patterns, which are produced by the staple positioning. This ensures a more secure design.[4] This process is aided by DNA staples, which use complementary base pairing to secure a stable antiparallel position at the crossover regions.[4] Upon completion of the software-assisted design, the complementary strands of the staples and scaffolds are annealed and DNA nicks are placed in the scaffold backbone of the DNA to balance strain in the overall structure.[4]

APPLICATIONS OF DNA ORIGAMI: CONTROLLED DRUG DELIVERY

DNA origami nanostructures (DONs) hold potential for use in drug delivery systems. The structural versatility of DONs allows them to be programmed to bind to different therapeutic agents, facilitating the delivery of these agents to their targets.[5] In addition, the charge of DONs may be altered by surface modifications (capsid proteins, cationic polymers, etc.) to improve uptake by certain organs or intracellular organelles. Overall, the performance of these nanostructures are dependent on factors such as size, geometry, charge, stability, degradation, and drug capacity.[5]

The efficacy of conventional cancer therapy is limited due to low solubility, low stability, and cytotoxicity of conventional chemotherapeutic agents. DONs can help overcome these drawbacks by acting as a targeted drug delivery system that can deliver the agents specifically to tumour cells.[6] DONs have notably been used to deliver the anticancer drug doxorubicin in chemotherapy treatments.[5,6] Doxorubicin (Adriamycin®) is used to treat solid tumours by inhibiting tumour DNA synthesis and causing cell death in areas such as the breast,

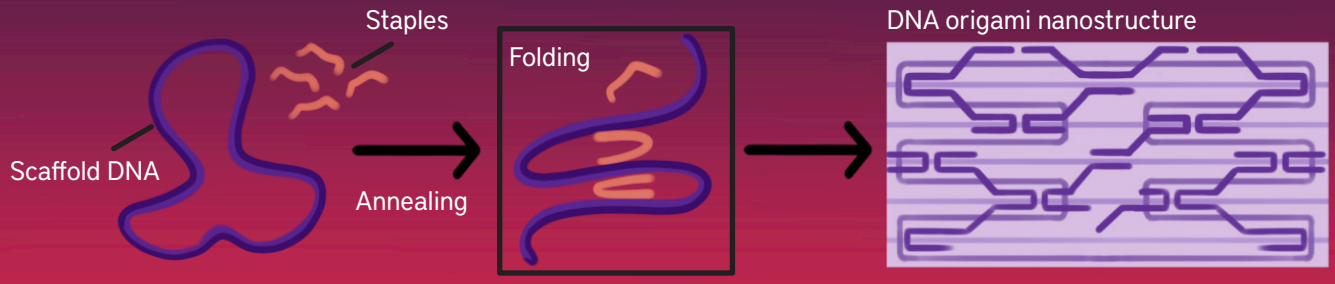

Figure 1. Illustration of structural design for the DNA origami nanostructure.[3]

bile ducts, and endometrial tissue.[7] DONs readily incorporate doxorubicin in their DNA structure through intercalation between G-C base pairs to form DON–doxorubicin (DON-dox) complexes.[8] By increasing the stability of the drug, DON–dox complexes increase delivering efficiency of doxorubicin to target tumour cells. Upon uptake by the cell, the DON is degraded by the endolysosomal pathway and releases doxorubicin for translocation to the nucleus, where doxorubicin can inhibit tumour DNA synthesis.[5] DON–dox complexes may also hold additional cargo or modifications to further inhibit tumour growth. For example, Liu et al. used DONs to simultaneously deliver the anticancer p53 gene (which would then be expressed as protein in the cell) and doxorubicin in vitro in tumour-bearing mice.[9] They discovered that the co-delivery of p53 and doxorubicin in mice displayed effective inhibition of tumour growth without apparent systemic toxicity.[9] However, the delivery performance of the DON–dox complexes depends on environmental factors such as pH.[10] Currently, in vitro studies on pH dependence have only focused on the release of dox between pH 4.5–5.5, which cannot be generalized to intracellular conditions with an approximate pH of 7.4.[5,11,12] Zeng et al. found that the accumulated release of the DON–dox complexes at pH 4.5 was double the accumulated release at pH 7.4 and 6.6.[11]

The potential of DONs as drug delivery systems is enhanced by their biocompatibility. Cell line studies of DONs have revealed a lack of cytotoxicity.[11] Studies involving hematological indices (e.g. measurements of kidney function and liver enzymes in blood) and histological examinations (e.g. spleen, kidney, lungs, liver, and skin) following administration of DONs showed no toxicity.[5,13-15] Overall, these studies highlight the versatility and biocompatibility of DONs as a drug carrier and show DONs' potential as a drug-delivery mechanism.

FUTURE DIRECTIONS

The future direction of DNA origami is spearheaded by the user-base's drive for the discovery of new applications and development of new technologies and software. For instance, there are efforts to increase the user-friendliness of DNA origami synthesis through automating an even greater portion using computer aids. A better understanding of the folding process can provide better rulesets for software to design, automatically synthesize, and assemble DNA origami structures.[3] Another branch of improvements aims to implement DNA origami procedures for in vivo applications. Namely, researchers are exploring the effectiveness of in vivo RNA origami: folding of single-stranded nascent RNA as it is being transcribed. This process is preferable to DNA origami, as it can occur in conditions isothermal to living cells. The design would also incorporate RNA-binding proteins to help with the stability of the structure in an intracellular setting.[3]

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