Two Dimensional Ordering of DNA Using Stacking Bonds

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Abstract

Since the inception of the method of DNA origami in 2006 by Paul Rothemund [1], many advancements in the ability to create rationally designed DNA nanostructures have been seen[2-6]. One of the short term goals researchers are seeking is the ability to scale up the physical size of these DNA nanostructures. In an effort to reach this goal, we are working to study the directed stacking behavior exhibited by DNA structures. Scaling up has the potential to solve many problems facing nanotechnological advancements, such as the ability to efficiently control the organization of nanoparticles on a substrate. We have previously designed two DNA origami structures to take advantage of the stacking behavior. To better study this phenomenon we have modified one of our structures, which will afford us better atomic force microscopy (AFM) analysis.

Introduction

A key problem in nanotechnology is to develop a method to efficiently control the organization of nanoparticles on a substrate. By controlling the spatial and material makeup of nanostructures, one can utilize the magnetic, photonic, and electronic properties of nanoparticles. Advancements in many areas of science and technology, such as, electronics, sensors, and microscopy, rely on understanding the properties of nanomaterials [7-9]. One route towards this goal that shows a lot of promise, is the self-assembly of DNA into multi-dimensional structures incorporating nanoparticles [10]. A recent approach towards DNA self-assembly, called DNA Origami, introduced by Paul Rothemund, allows the use of computer-aided design principles to be applied to DNA self-assembly [1]. DNA Origami involves folding a long single “scaffold” strand of DNA into the geometric shape of your choosing. The folded DNA is complemented with hundreds of short DNA “staple” strands, held together by Watson-Crick base pairs. Although, any DNA strand whose sequence is known can be used as a scaffold, in practice, it is the DNA of bacteriophage M13 that is most often used since its base sequence is precisely known, readily available and long enough to create origami that is a few hundred nanometers in dimension (~7000 bases), and can be imaged by atomic force microscopy (AFM). This method has been used to create complex structures in two- and three- dimensions [2,11,12].

Naturally, one would hope to extend this method to construct much larger structures. Making use of a longer scaffold strand is one potential solution, but as the length of the scaffold increases, the number of staple strands needed for synthesis increases. Currently, the cost of a 2.5 nm long DNA oligomer is approximately $1.00/nmol of material. Using the entire length of an M13 scaffold strand costs about $100/nmol of material. Thus, the use of a longer scaffold strand does not provide a cost effective solution.

Researchers have attempted to construct larger structures by connecting preformed DNA origami structures [4-6,13,14]. There are essentially two classes their approaches fall into: the “sticky end” approach (Figure 1.), and the ”base stacking” approach (Figure 2). The sticky end approach involves creating single stranded DNA overhangs at specific sites on an origami structure. A second origami structure is created with a complementary overhang, and the two structures are brought together by Watson-Crick base pairs. The base stacking approach uses the non-covalent
interaction between adjacent base pairs. Structures that terminate with specific base pairs tend to stack together through this interaction [1,2,7,12]. Woo, and Rothemund have shown particular ordered arrangements of origami structures can form through stacking interactions [12].

Figure 1 Sticky Ends Sticky ends are depicted in green and red. The overhangs will bind at specific locations to their complementary bases.

Figure 2 Base Stacking (A) The base stacking approach utilizes the Van der Waals forces that exist between adjacent base pairs. (image created in Chimera) (B) The smiley faces above show the stacking interaction that occurs between DNA structures. These structures were not designed to do this, they formed this way due to more favorable energetic conditions. (Image courtesy P.W.K. Rothemond [1])

Design

We had previously designed a small rectangular structure that only utilized one third of M13 bacteriophage. This was done in order to minimize the cost of experiments. Our design focused on three key points: (A) planar structures, (B) specific base pairs at edges, and (C) rough edge geometry (Figure 3). By controlling the curvature of structures, [3], the creation of planar structures with close to zero curvature has been shown to produce higher yields of correctly formed bonds [12]. The stacking interaction that occurs between adjacent base pairs has been shown to be sequence dependent [15]. In an effort to control the strength of bonds, choosing specific base pairs at the edges will afford higher energetic conditions [12]. We implemented rough edge geometry, as we believe correctly formed bonds will provide the most energetically favorable conditions.

We experienced some difficulty in our first attempts to image our structures after formation. We attribute this to two possible problems with the original design: 1.) too small of a structure 2.) single stranded loop out interference. In regards to 1, we have only utilized one third of the chosen single strand of DNA. Typically researchers have used the entire M13 strand. This was done so as to limit the cost of synthesizing our structures. Paul Rothemund saw this problem in one of his experiments [1]. The problem lies in that DNA and mica are both negatively charged. A salt buffer solution is required to get the DNA origami structures to stick to the mica surface for viewing with the AFM. With a smaller surface area we believe it is difficult to get the DNA to stick firmly on the mica surface. The quality of
the AFM tips also effect the quality of imaging one can obtain [1]. In regards to 2 the single stranded DNA loop outs we have placed in our design may be interfering, prohibiting our structure to stick flatly on the mica surface.

In an attempt to rule out the first of these two problems, we have implemented a way to increase the size of our structures without redesigning it completely. On the flat edge of our structure, the side without rough edges, we replaced all the staples and made it so we could use the sticky end technique to double the surface area of each of our structures (Figure 3). This also affords the ability for formation of long chains along one direction, where we can better analyze the stacking behavior. We also do not have to lose any of our original design criteria. It should be noted that the sticky end technique here is only used to increase the surface area of our structure without having to redesign and purchase a complete new set of DNA staples. We can then analyze our design criteria that focuses on the stacking interaction to determine how well our technique works.

We optimized this new design by making use of the simulation software CanDo, http://cando-dna-origami.org/, provided by Dr. Mark Bathes group at MIT. The software predicts the structural stability and thermal fluctuation of DNA nanostructures. Comparisons were made between our original structures stability and our new design (Figure 4). We also looked at the effects of placement of our sticky ends. The quantity, length, and placement of sticky ends has been experimentally verified to play a significant role in structural stability, [14], and CanDo takes this into consideration to some degree. Our design is predicted to be the most stable of our possible placement choices.

**Figure 3 Raster Pattern Schematic of Our Structure** (A) Skips were strategically placed to make our structures as planar as possible. One helical turn of DNA is approximately 10.5 base pairs, but the program used, caDNAno, assumes 10.67 base pairs per turn. We placed skips, represented by x’s, every 32 base pairs, which gives an overall average of 10.5 base pairs per turn. (B) Single stranded loop outs were put in place to ensure the rough edges ended in a GC base pair. Adjacent GC base pairs were shown to have the highest stacking energy [14]. (C) The rough edges of our structure will only form correctly in one way. We believe the proper formation will be the most energetically favorable condition for our structure. (D) Placement of sticky ends in our new design.
**Figure 4 CanDo Simulations** (A) The DNA double helices are depicted as flexible rods, and the figure shows the predicted deformed shape of our new design. The color map indicates the root mean square thermal fluctuation (RMSF) range after formation. (B) This is our original design. By increasing the size our the RMSF predictions are roughly the same, and the planarity is similar.

**Future Work**

We are working to adjust our protocol in order to optimize our resources. We have purchased the DNA needed for our modified design, and are running experiments. We would like to extend the sticky end technique to allow formations of chains in both the x and y directions, provided this new design proves the single stranded loop outs are not a problem. This will allow further analysis of the stacking behavior, and the possibility of creating even larger two dimensional structures. In the event we still have trouble with imaging, we will have to address the problem of the single stranded loop outs by removing some or all of them.

**References**


**Budgetary Information**

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We plan to purchase more staples depending on the results of our new design once it is optimized.