Two Dimensional Ordering of DNA Using Stacking Bonds

Suffolk University SPS Chapter, Boston MA 02114

Students: Lee Wizda, Yosuke Sugishita

Faculty Advisor: Prashant Sharma

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Lee Wizda

Yosuke Sugishita

Prashant Sharma
Abstract

A key problem in nanotechnology is the precise assembly of nanoparticles on a substrate. DNA self-assembly using Watson-Crick base pairing, termed DNA Origami, is regarded as a promising route towards achieving nanoscale precision. We propose to address the problem of scaling up DNA Origami, by designing Origami structures whose mutual interaction is spatially non-uniform and results in directed stacking of different origami tiles.
The self-assembling property of DNA makes it an excellent choice as a building material for bottom up fabrication of nanostructures. The method of DNA Origami recently introduced by Paul Rothemund [1], provides a simple yet highly efficient mechanism to construct complex structures with nanoscale precision. It involves folding a long single stranded DNA molecule into a desired geometric structure that is held together by Watson-Crick base pairing with several short DNA strands. Design of DNA Origami structure can be implemented on a computer by first raster-filling the desired two-dimensional shape using a single-stranded DNA molecule. A large number of shorter single stranded DNA oligomers are then designed to bind with the scaffold using complementary base pairing. The single stranded scaffold that is used is the viral DNA M13mp18, which is about 7000 base pairs long, and whose base sequence is precisely known. The DNA molecule is long enough to create structures on the order of 100 nm in length. The computer-aided designs using this scaffold generate base sequences for the shorter staples that can be synthetically designed. The designed structure is experimentally realized by thermal annealing of a mixture of the M13 DNA and the staple oligomers. This approach has thus far been used to create complex two and three-dimensional structures at a sub-micron scale [1, 2, 3].

One of the key factors limiting the size of DNA Origami is the cost of creating different staple strands that a larger structure would need. A DNA oligomer that is about 2.5 nm long costs about $1 per nmol; using the entire length of the M13 scaffold to create
an Origami would therefore cost around $100 per nmol. Scaling up the size of a DNA origami using the aforementioned technique would therefore not be cost effective.

A few methods to scale up the physical size of DNA origami structures by bringing together the identical copies of DNA Origami created in the experiment have been examined [4, 5, 6, 7]. These approaches fall into two classes: the “sticky end” approach and the “base stacking” approach. The “sticky end” approach uses extra single stranded DNA oligomers as overhangs from the smaller designed DNA Origami. The overhangs are arranged in complementary pairs on different portions of the smaller DNA Origami causing them to bind together using Watson-Crick base pairing. The “base stacking” approach utilizes the attractive interaction between adjacent base pairs of DNA. Structures that have edges that terminate with a base pair have a propensity to stack together due to this interaction [1]. Recently the stacking interactions of DNA origami were used to create a particular ordered arrangement of origami structures [4]. It was shown that the geometry and placement of binding sites plays an important role in creating stable binding of DNA Origami [4, 8].

Our method to scale up DNA Origami focuses on developing the base stacking approach further by identifying certain key features of design: (a) planar structures; (b) specific base pairs at edges, and (c) rough edge geometry. We have experimentally explored these features and presented our preliminary results recently at the 2011 BIOMOD competition (http://biomod.net/) at the Wyss Institute, Harvard University. We have designed rectangular, and triangular structures and shown that they can be stacked together to form larger structures. To limit the cost of the experiment to less than $1000, we chose to only use one third of the M13mp18 scaffold strand. We now seek
financial support to create similar but larger structures by using the entire M13mp18 scaffold. This will offer better visualization of both the Origami structure and its stacking, using conventional atomic force microscopy. It will also enable us to study the anisotropic nature of base stacking interaction that we have tried to design.

We have utilized the computer software caDNAno to construct these larger structures. This has provided us with a sequence of bases for the staple DNA oligomers that we will need to purchase. We will purchase these strands from Integrated DNA Technologies. Suffolk University’s Nanoscience lab is equipped with an atomic force microscope, which we will use to view our structures, and a PCR machine will be used to anneal our solutions. We have fine-tuned our experimental protocol to work with our new structures, by testing it on the existing smaller designs in which both the formation of Origami and and base stacking have been demonstrated. We will present this portion of our research during the March APS Meeting in Boston, MA.

In conclusion, our proposed study will build on work already done by us on the promising approach of directed self-assembly of DNA Origami in two-dimensions using base stacking. This work will allow us to compare our approach with the aforementioned alternative approaches that have been recently published.

**Bibliography:**


**Proposed Budget**

- DNA Staple Strands  Cost: $1600
- Veeco Inc. SNL AFM tips  Cost: $400

Total Cost: $2000.00