DNA Origami for a Nanoscale Electrochemical Positioner Model

Introduction: When a virus enters a cell, it replicates to survive. This replication allows for the infected cell to "hide" so that the immune system will "lose track" the virus. Detection of viruses is key in disease diagnosis, but it has been proven difficult, as their lengths are typically measured in nanometers.[1] A way to successfully detect and remove viruses can involve binding a virus' exterior with multiple recognition cites to another structure in vivo for transport and analysis. This structure would need to be customizable, durable, and able to change conformation. One way to make such a structure is to use DNA Origami.

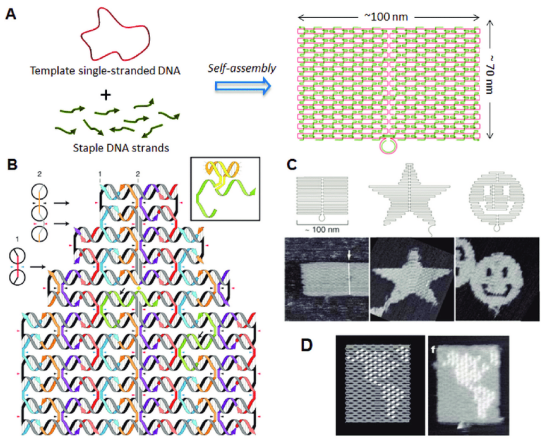

Figure 1- The process of making DNA Origami.[3]

Background: My current research involves synthesizing, analyzing, and taking microscopic images of nanoscale DNA origami. DNA origami is a technique used to fold many DNA strands to form two and three-dimensional shapes (Figure 1). It requires taking a long strand of plasmid, single-stranded DNA, and folding it into your desired shape with complimentary staple strands. This technique was first proposed by Paul Rothemund in 2006.[2] **Aim 1. To synthesize a DNA origami structure that, when bound to a virus, underwent a specific conformational change that could be transduced into a change in an analytical signal**. We chose a triangular origami shape because it was proven to bind to a VLP, (virus like-particle) using complementary binding sites and undergo a conformational change. Methylene Blue, MB, dye acts as redox reporters on the edges of the triangles. After synthesizing the triangles, we attached the origami to a gold electrode using thiol strands to perform electrochemistry, more specifically, Square Wave Voltammetry (SQW) to allow for the measurement of redox potentials. We analyze the potential where the redox reporter was reduced and its corresponding current. The paper for this research is under review for Angewandte Chemie. **My lab was able to successfully synthesize origami structures that detected mesoscale targets and produce binding-induced signaling, a first of its kind. I want to continue doing this research to find the optimal conditions for a virus to bind to our structure.**

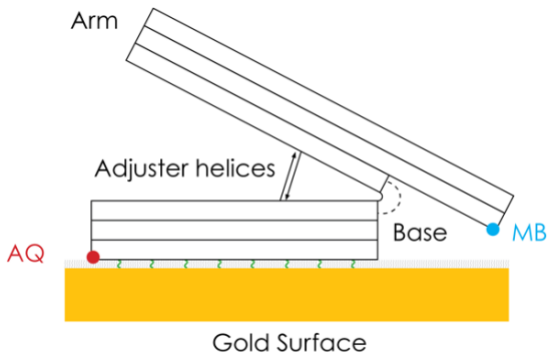

Figure 2- The positioner structure with labeled

Aim 2. The short-term goal of our project this past summer was to further investigate the electrochemical aspect of DNA and the distance dependence of redox reporters to an electrode. My research involved a new structure called the positioner (Figure 2). The new structure is in the shape of a hinge, having two origami beams linked by four DNA duplexes called adjusters. We call the beam touching the gold electrode the base and the beam farther away connected by the duplex the arm. The dye Anthraquinone (AQ) is a "reference" redox reporter and it is in a fixed location on the base of the positioner; AQ serves as an internal standard for each experiment. Methylene Blue (MB) is located on the edge of the arm of the hinge and serves as the measuring redox reporter. It varies

in distance from the electrode with various lengths of the adjuster helices. When the adjuster helices are longer, the MB is closer to the gold surface. Conversely, when shorter, the MB moves farther away from the gold surface. We want to have complete control of the angles created by the positioners to get distance-dependent measurements that will give us an electrochemical output.

Aim 3. The long-term goal of this project is to synthesize a structure that can be used as a sensor that enables macromolecular detection in biofluids. My focus in the project was in synthesis, gel electrophoresis, electrochemistry, and I handled the imaging of the structures. I checked that positioners were made correctly by pouring and running agarose gels to see where positioners showed on the gel compared to ladder and plasmid. When imaging positioners, I first tried Atomic Force Microscopy, but I realized that we needed higher quality images of the positioners to fully see the structures, so I sent the samples to a collaborator to successfully perform TEM. The gel and TEM images alike confirmed the existence of dimers. This issue is still being resolved, the culprit could stem from our annealing protocol, TEM conditions, or flaws in the structure design itself, which we are currently working on optimizing. As for electrochemistry, we were only able to see the MB reduction potential of SWV, as the AQ peak is hidden in the baseline. It may be due to the thiol monolayer not being completely filled, allowing oxygen on the gold surface.

We used VLPs instead of viruses because the undergraduate lab I am currently working in is not cleared for biochemical research. Funding for further research will be key in continuing this project in graduate school in a biochemical lab. This will help in understanding the process of this proposed mechanism in a living organism. This project will also benefit with the use of computational approaches to design more ideal DNA origami structures. I already use a program called caDNAno to design and order the DNA sequences we use to make our structures. I can create a molecular model of the origami structure and perform energy minimizations to generate an idealized 3D model. Molecular dynamic simulations can further be used to see the origami's atomic structure and mechanical qualities.

Intellectual Merit: We have proven that DNA origami system has the capability of undergoing a conformational change and that can bind to a VLP. My two years of laboratory experience has allowed me to develop a high skill in general biomolecular lab techniques, as well as in imaging and data analysis. I also have a working knowledge of computational techniques that can be used in the optimization of the structures.

Broader Impacts: This research project has potential for great scientific advancements in the detection of virus in vivo. Detection and successful transport of virus in living organisms will lower illnesses and death rates, as well as inspire other research in the usefulness of DNA origami. I will include undergraduate students in my research, and continue to attend international conferences as I have, such as BIOMOD, and hopefully garner a publication for this work in the near future.

References [1] Jones, M.G., Superfine, R. and Taylor, R., (1999). Virtual viruses. *Science Teacher*. *66*(7), 48-50. [2] Rothemund, P. W. K (2006). Folding DNA to Create Nanoscale Shapes and Patterns. *Nature*. 461, 74-77. [3] Endo M., Yangyang, Y., and Sugiyama, H (2013). DNA Origami Technology for Biomaterials Applications. *Biomaterials Science*. 1, 347-360. [4] Jabbari, H., Aminpour, M., and Montemagno, C (2015). *ACS Combinatorial Science*. *17* (10), 535-547.