FACT SHEET

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DNA Birdnests: A Novel Genetic & Generic Material

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ABSTRACT

The project I hope to pursue this coming summer involves capturing and binding proteins to the DNA template from which they originated, allowing for signal amplification and resolution between the products of individual mutant DNA sequences. The Luo lab uses nucleic acids to create various functional nanomaterials, and has recently developed a relatively large DNA-based structure, known as a DNA bird-nest, that contains thousands of copies of a single circular DNA template and is about the size of a small cell. While this structure is remarkable for the material properties it holds as a DNA gel, my previous work in the lab has focused on harnessing the genetic capabilities of the particles by optimizing the expression of fluorescent proteins in vitro, using individual bird-nests as the genetic material. I have also learned to use microfluidic technology to isolate single bird-nest particles in emulsion droplets that can be easily imaged via fluorescent microscopy to visualize the fluorescence emitted from individual particles. The challenge now is to be able to bind the proteins onto the DNA particle.

Protein-DNA binding can be accomplished by coupling chemical modification of the DNA in the bird-nests with the incorporation of amino acid “tags” on the proteins. DNA can be made to indirectly bind nickel ions, which in turn will specifically recognize and bind proteins that have been tagged with histidine residues. While we have some preliminary evidence to suggest that the particles can specifically bind tagged proteins, I will work this summer to couple the binding capabilities of the particles with my existing expression work, such that the particles will specifically and effectively bind the products of their own expression. I hope to eventually be able to develop the particles as a platform for in vitro directed evolution of proteins through screening libraries of DNA sequence mutants, particularly the optimization of fuel production from cellulose by screening mutant libraries of cellulase proteins.
Biographical Sketch

My parents both grew up in small Indian villages; after they married 28 years ago, they immigrated to America with a collective $96 in their pockets and no confirmed job prospects. Working multiple minimum-wage jobs, they put themselves through college in the hope of achieving a better life. My mother was the first woman in her family to ever attend college, and my father always told me that his focus on education was the only thing that allowed him to pursue a career outside of my grandfather’s grocery store in India. I was raised in a household with a profound respect for the sanctity and power of knowledge, and cultivated a thirst for learning in every subject as I was growing up.

It was not until junior year of high school that I happened upon my first love: chemistry. I was enthralled by the way that simple microscopic models could explain macroscopic phenomena that we encounter every day, be it the diffusion of tea into hot water or the neurochemistry underlying human behavior. I particularly enjoyed our unit on biochemistry, wherein I learned about the molecular basis of different nutrient deficiencies, the mechanisms by which different drugs affected the body, and basic molecular biology. I came to Cornell as a biology major, and quickly declared a second major in chemistry.

Being a people-person, I knew that I wanted to put my knowledge of science to practical use in order to effect macro-scale changes in society. I joined the Genetically Engineered Machines project team during my freshman year, and shortly thereafter joined the Luo lab in Biological Engineering, where I have worked ever since. My research experience has allowed me to translate classroom learning into meaningful solutions to real-world problems, and to combine my intellectual passions for chemistry and biology in novel ways. I plan to pursue a PhD and eventually a career in academic research on molecular biology.
STATEMENT OF PURPOSE

Directed evolution of enzymes \textit{in vivo} is challenging because the functionality of the enzyme and the viability of the cells may be in direct conflict with each other. This concern is particularly relevant in the growing field of biofuel development (Dien et al. 2003). As such, it is prudent to consider developing methods for enzyme optimization \textit{in vitro}. However, cells confer certain benefits that are much more difficult to achieve \textit{in vitro} – they effectively isolate individual mutants of any transformed gene to one cell line, and produce several copies of the same mutant, allowing for effective screening of mutant libraries. Isolating single mutants on a large enough scale to be able to distinguish the individual expression levels has proven to be a great challenge \textit{in vitro}.

To this end, we have created particles known as DNA birdnests that, coupled with \textit{in vitro} compartmentalization (IVC) techniques, could provide a convenient way to screen mutant libraries of genes (Stapleton & Swartz 2010). There is currently no robust system for performing directed evolution on enzymes \textit{in vitro}, and our novel methodology could be developed to fill that current void. We have also sought to make our methodology compatible with the directed evolution of gene sets rather than just individual genes, a feature that truly sets this work apart from previous directed evolution studies.

While the size and gene copy number of the DNA birdnests greatly enhances the viability of IVC as a method of isolating single mutants, there are other challenges. Our lab hopes to be able to visually distinguish and select individual mutants, represented by single droplets in IVC. To amplify the signal produced by each birdnest particle, we seek to chemically modify portions of
the DNA polymer to be able to selectively bind the proteins that are being produced using its genetic material. The DNA-protein binding process has been shown to successfully aggregate proteins around a large, controllable structure \textit{in vitro}; however, what I will be doing this summer is optimizing the conditions under which genetic expression and DNA-protein binding can be coupled. I believe that these DNA birdnest\-es represent a vast, untapped resource for molecular engineering applications. The one I hope to pursue is the directed evolution of a cellulase for the degradation of raw, cellulose-containing feedstocks into glucose for subsequent fermentation into fuel ethanol. This case is illustrative of the need for novel \textit{in vitro} methodologies, as researchers have been struggling for years to overcome the limitations on cell viability when overproducing fuel products.

The synthesis of DNA birdnest particles is accomplished through a two-step process, consisting of rolling-circle amplification followed by multi-primed chain amplification. In the first step, rolling-circle amplification (RCA), a single-stranded circular DNA template and a primer are exposed to Φ29 polymerase to create a long, linear, single-stranded DNA with many repeats of the template. Portions of this are then made double-stranded by introducing two more primer oligonucleotides in multi-primed chain amplification (MCA). This two-step process has previously been optimized by our lab to produced stable and durable DNA birdnest particles, and the necessary resources are all available in the lab. While we believe that each birdnest originates from a single circular template, part of what I will be doing this summer is proving that this is the case. In order to do so, we have chosen the templates for the DNA birdnest\-es to be eGFP and mCherry, which are green and red fluorescent proteins with minimal spectral overlap. Production
of birdnests from a mixed batch of these templates should produce particles that are distinctly either red or green in color.

However, these experiments cannot be conducted in bulk solutions, as we need to be able to isolate the fluorescent proteins expressed from each individual birdnest particle. This is accomplished by a technique known as in vitro compartmentalization. Using a CAD to create a streamlined microfluidic device in a PDMS mold, the birdnest particles in dilute solution are mixed with *E. coli* cell lysate (providing the cellular components necessary for in vitro protein expression) and encapsulated in oil, yielding a water-in-oil emulsion with individual hydrophilic droplets suspended in hydrophobic solution. The concentration of the birdnest solution, the width of the input channels, and the relative flow rates of the aqueous and oil phases are chosen such that a Poisson distribution yields either 0 or 1 birdnests per droplet (Edd et al., 2008).

I will simultaneously be working to amplify the signal produced within each droplet by implementing a chemical method for protein aggregation. The protein being expressed is converted to a fusion protein, linked to six histidine residues at the C-terminus, commonly known as a His-tag. One of the universal primers for MCA will be functionalized with a thiol group. *Tris*(2-carboxyethyl)phosphine, or TCEP, then reduces the disulfide bond. The key chemical is maleimido-C3-NTA (MC3N, for short), which contains an α,β-unsaturated carbonyl group that is prone to undergoing conjugate addition with the reduced thiol. MC3N also contains a dicarboxylate group at its other terminus that allows it to selectively bind divalent cations. Flushing the molecule with nickel salts saturates it with nickel, which in turn can very selectively
bind to His-tags on proteins. Through this series of covalent and non-covalent interactions, each primer of this sort present in the DNA birdnests captures multiple copies of the tagged protein. While we have evidence to suggest that the DNA functionalization and binding processes are both successful, we have not yet been able to express and capture proteins from the same birdnest particles, and this is what I will be doing this summer.

It is crucial to be able to specifically bind each birdnest’s unique protein products, as this amplifies the individual signal from each particle such that we can observe significant differences in expression levels. The ability to screen for such differences is the central feature of directed evolution studies, and this summer’s work is what will allow me to translate our novel DNA material into a useful platform for engineering biotechnological solutions.
