Project Summary/Abstract

The ability to write long fragments of DNA with high accuracy and low cost would open up vast new possibilities in biological research. In stark contrast to DNA sequencing, which has undergone revolutionary technological advancements leading to cost reductions that outpace Moore's Law, the phosphoramidite coupling chemistry used to synthesize artificial DNA oligonucleotides has remained largely unchanged for 40 years. Furthermore, the single-stranded DNA oligonucleotides produced via phosphoramidite chemistry must be assembled into double-stranded DNAs via multi-step processes before they can be introduced into cells and used for synthetic biology applications. In contrast to chemical coupling methods, DNA polymerase enzymes can synthesize extremely long double-stranded DNA molecules with very high accuracy at almost no cost. However, researchers have not yet engineered biological enzymes to construct DNA strands in a programmable way.

Here, we propose to revolutionize DNA synthesis by engineering DNA polymerases that add one adenine (A), cytosine (C), guanine (G), or thymine (T) to the end of a growing DNA molecule in response to different wavelengths of light. In particular, we will use four spectrally distinct photoreceptor proteins to control four different engineered DNA polymerases, each of which adds a different non-templated nucleotide to the end of a DNA molecule. To enable repeated cycles of forward DNA polymerization, we will also engineer a reverse polymerase enzyme that regenerates a full double-stranded DNA molecule after each light-induced DNA polymerization event. Our enzyme engineering efforts are based upon established functions of known proteins, but require innovative approaches to substantially extend existing functionalities.

If successful, this technology could lead to dramatic reductions in the cost of DNA synthesis and allow the direct synthesis of long double-stranded DNAs without the need for chemical oligonucleotide synthesis or downstream assembly. Our work could enable the engineering of environmental microbes that seek and destroy chemical weapons, gut bacteria that allow warfighters to rapidly gain or lose brown fat to adapt to cold or hot climates, microorganisms that manufacture food, fuel, or materials from resources in austere environments, or DNA molecules that encrypt, store, and decode secure communications, among other biotechnologies that advance DoD capabilities. Ultimately, light-directed enzymatic DNA synthesis could provide researchers virtually unlimited access to artificial DNA sequences, fundamentally changing the way biological research is conducted.