

Dr. Greg Springsteen- Design and Evolution of Molecular Binding and Detection Agents

- I. Stemloop Aptamers:** The goal of this project is to develop a therapeutically-effective nucleic acid-based aptamer against the HIV gp120 entry protein. Our lab seeks to evolve DNA aptamers through in vitro evolution using a unique hair-pin strategy (Figure 1). In Vitro Evolution is a technique where DNA strands are selected based on ability to bind a target protein with high affinity and specificity. This procedure requires two phases: (1) separation of the aptamers from non-binding sequences based on affinity and (2) amplification of the binding aptamers using the polymerase chain reaction. A randomized DNA region (sequence space where the aptamer will evolve) is sandwiched between two complementary primer binding sites in a stem-loop configuration. The annealing of the two primer binding site regions may: 1) provide greater stability to the evolving aptamer due to an enforced region of self-complementarity, 2) allow cleavage of large portions of the primer binding sites after aptamer evolution without affecting aptamer geometry; potentially reducing the final aptamer size by half, and 3) facilitate automation due to the lack of a requirement for complementary strand removal at each step; a particularly difficult process to automate.

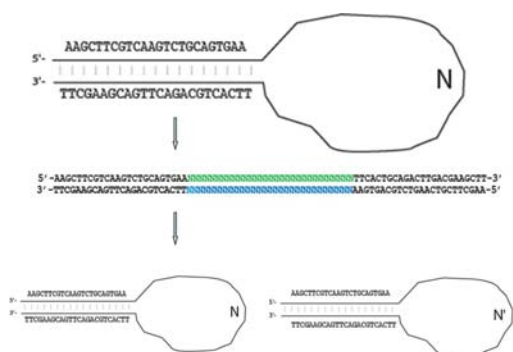
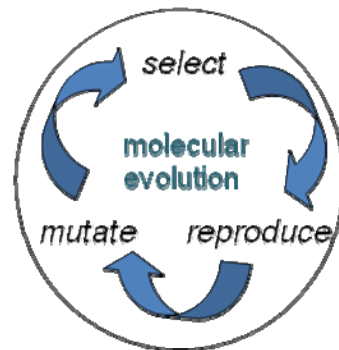
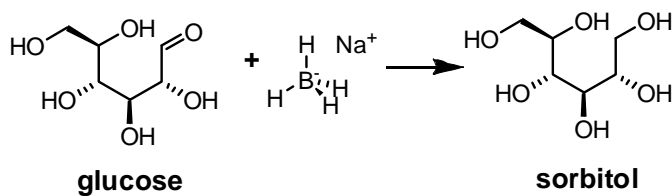


Figure 1. Amplification and spontaneous regeneration of single-stranded stem-loop nucleic acids

Researchers working on this project will run molecular biology experiments such as gel electrophoresis, and PCR amplification. They will also be given the option to design and build automated reactors.

- II. Boronic Acid-Based Glucose Chemosensors:** In contrast to the current method of blood-glucose testing, which employs enzyme-laden strips and potentiometers to determine glucose concentrations, this project aims to develop a small-molecule based chemical system that reports concentrations optically. Enzyme-dependent glucose meters are effective, however due to their recurring consumable costs of ~\$10/day, they are not available to a significant proportion of the world's diabetic population. In addition, the enzyme strips require careful environmental control to remain effective. The aim of our project is to develop an affordable glucose test based on the interaction of boronic acids with the glucose reduction-product sorbitol.

Although glucose interacts with boronic acids poorly, sorbitol has been shown to bind well. This interaction is stable and detectable through optically-active aryl groups on the boronic acid. The reduction reaction is compatible with aqueous conditions. It is hoped that such a system will replace the expensive and fragile enzyme components of current meters.



Researchers working on this project will use traditional organic and analytical techniques to synthesize and test potential boronic acids for their ability to detect glucose.