Characterizing the Interactions Between G-Quadruplex DNA and Insulin
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My summer research focused on different aspects of the binding between insulin and G-quadruplex DNA. G-quadruplexes are a DNA structure that differs from the more common single strand or double stranded helix forms. This structure forms in guanine-rich DNA when hydrogen bonds form between the guanines to create a G-quartet, or a box of guanines, and then multiple quartets can stack on top of each other. G-quadruplexes have been found to form in many different regions of the human genome. The G-quadruplexes that we are interested in are formed in the insulin-linked polymorphic region (ILPR). The ILPR is composed of repeats of short sequences of DNA, and 363 base pairs away from the insulin gene, so the fact that insulin binds to the ILPR means that insulin could be influencing its own transcription. Insulin is a protein in organisms that causes the uptake of glucose from the blood, to be used as an energy source. The binding of insulin to the ILPR is also of interest because it has ties to type 1 diabetes. Individuals with 40-60 repeats are more susceptible to type 1 diabetes than individuals with the more common 120 repeats.

We investigated these binding interactions using an isothermal titration calorimeter (ITC); this is an instrument which injects our ligand, ILPR DNA, into the protein, insulin, and gives a full set of thermodynamic parameters for the interaction with just one experiment. The ITC measures the power to keep the temperature constant when the injections occur. This allows us to determine the heat of binding.

This summer we did experiments to investigate the number of protons that are transferred in the insulin-ILPR binding interaction. This can be done by testing the binding of insulin to ILPR in different buffers. Varying the buffer has an effect on the observed enthalpy of the system, and enthalpy of ionization is a constant property for any buffer. These two values can be plotted against each other on a graph, and the slope from the regression line is the expected number of protons transferred in the interaction. We tried many different buffers this summer but we encountered problems with control runs that were not reproducible and no binding in the actual experiment, and only the HEPES buffer and phosphate buffer provided successful results. Two points were enough for us to obtain a line of regression and determine that three protons are released and absorbed in the ILPR-insulin interactions. We would still like to find another buffer that works with our system to give further support for this finding.

This summer we were also looking to reproduce the experiments done with different repeats, and variant repeats of the ILPR. There are 14 different ILPR repeats in the human genome; we only work with repeats a, b, and c, as these repeats are the most common. Previous work has shown that the highest level of transcriptional activity occurs with the most common or consensus sequence: repeat a. The transcriptional activity for sequences b and c are 30% and 20%, respectively, compared to those of repeat a. It has been speculated that the increase in transcriptional activity is due to a more stable G-quadruplex. When a single nucleotide is changed in the sequences of repeats b and c to make the variant sequence the transcriptional activity goes back up to close to 100% for both variants. We are still doing experiments to characterize the binding between insulin and different ILPR repeat b, b variant, c, and c variant sequences.

Working in the Sinniah lab this summer has been a very valuable and enjoyable experience for me. I learned about the uncertainty of lab work, and that although we fail we can still learn from our mistakes and try again. I also learned a lot from the group I worked with; teamwork with the other students was important and working with them was always a pleasure. Working on summer research was a completely different way to experience science.