A Thermodynamic Study of Insulin and DNA Binding
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This summer, my research investigated the binding interaction between insulin and a short segment of DNA called the insulin-linked polymorphic region (ILPR). The ILPR is located in a section of the human genome upstream of the insulin gene, which codes for the production of insulin, a protein hormone used to regulate blood sugar. In addition to its specific location, the ILPR is known for having guanine-rich regions that can form unusual DNA structures called G-quadruplexes. G-quadruplexes can form when a DNA strand contains consecutive guanine bases that can hydrogen bond with each other in the presence of a cation. Four guanines can form a single quartet, and multiple stacked quartets form a G-quadruplex. It has been previously found that G-quadruplexes form in the ILPR, and past work in the Sinniah lab suggests that insulin can bind to G-quadruplexes.

The ILPR contains repeats of fourteen different sequences of DNA. These sequences have nucleotide variations, but all possess a large number of guanine tracts. The most common sequence, also called the consensus sequence or ILPR a, has been found to have a high transcriptional activity. However, less common sequences, such as ILPR b and ILPR c, have lower transcriptional activity. When specific nucleotide bases are changed in these less common sequences, creating ILPR b variant and ILPR c variant, a high percentage of transcriptional activity is restored. In addition to these variations in sequences, the number of repeats in the ILPR also varies from individual to individual. There is literature evidence of a correlation between type 1 diabetes and ILPR class I alleles (which are shorter ILPR regions). Recent work suggests that insulin may play a role in regulation of its own transcription by binding to the ILPR, which points to the biomedical significance of this minisatellite region.

By characterizing the binding between the insulin and the ILPR repeat sequences –b, c, b variant, and c-variant—this project sought to confirm that the variants show higher transcriptional activity than unaltered ILPR b or ILPR c sequences, as suggested by previous literature (Amy Lew et al. PNAS 2000, 97, 12508–12512). This project evaluated the hypothesis that the differences in transcriptional activity were due to structural changes between the variants, which impacts the binding interaction between the DNA and insulin.

To investigate the binding interaction between the ILPR DNA and insulin, we used an instrument called the Isothermal Titration Calorimeter (ITC). The ITC measures the heat evolved from the introduction of small amounts of ILPR DNA to insulin. Modeling these heats allows us to gather valuable thermodynamic parameters, such as the dissociation constant, stoichiometric ratio, change in enthalpy, and change in entropy in a single experiment. Through ITC experiments we determined the binding strength of insulin with ILPR b, c, b variant and c variant DNA at four different temperatures: 20 °C, 25 °C, 30 °C and 37 °C. MUCH of this summer was spent characterizing the binding for all the repeats at the experimental temperatures. Through careful analysis we were able identify the formation of G-Quadruplexes, classify tight binding trends in ILPR b variant- and ILPR c variant-insulin interaction, categorize the enthalpy component of the ILPR b-insulin interaction and ILPR c-insulin interaction, and deconvolute the large, negative heat capacities of the ILPR b- and ILPR c-insulin interactions into their hydrophobic and vibrational components. Through analysis of the data, we are getting closer to submission of a publication to a high-impact journal.

Working in the Sinniah lab was a powerful experience not only because I was involved in an important project, but also because I got to work with brilliant students who taught me much. I became aware of the large amount of collaboration that makes good science possible and I found myself grateful to many people who were able to help me collect, analyze and organize data.