Glucose is a vital source of the energy needed to fuel cellular metabolic processes. One way it enters the cell is by protein-mediated, equilibrative transport. The GluTs are one of the largest families of glucose transporters in mammalian cells. This study focuses specifically on GluT1 since it is the first transporter to be identified, purified and cloned. There is no single model that completely explains all aspects of its behavior, however previous observations suggest that GluT1 contains multiple ligand binding sites on both sides of the cell membrane that can modify its activity and the affinity for its substrate. This summer was dedicated to characterizing and modifying the behavior of GluT1 across multiple cell lines.

For my research, I worked primarily with the human kidney-2 (HK2) cell line, which has moderate glucose uptake activity and also expresses the facilitative Sodium-Glucose Linked Transporters (SGLTs) in addition to GluT1. All cells were cultured in specific growth media, incubated, and split according to individual replication rates until they were needed for glucose uptake assays or immunoblotting. Glucose uptake assays involve treatments with certain compounds and then counts of the amount of radioactive glucose taken up by the cells. Immunoblotting allows for the detection of the size and location of GluT1 within the cell.

The two main projects I was involved in were the characterization of GluT1 in the HK2 cells, and the investigation of the importance of lipid rafts in glucose transport. It was determined that HK2 cells have a similar uptake activity to the Human Corneal Limbal Epithelial (HCLE) cells, a line with high expression and activity of GluT1. Immunoblotting with a western blot showed that the HK2s do express GluT1. Our standard uptake assays showed GluT1 could be activated consistently by glucose starvation. Other compounds that consistently activated or inhibited glucose uptake in our other cell lines gave inconsistent results in the HK2 line, so more work needs to be done to determine its how these compounds are interacting with the transporter. There was a pH effect in the HK2s. Glucose uptake increased with increasing pH, with the greatest activation occurring from pH 7 to pH 8.

Lipid rafts are separate plasma membrane domains rich in cholesterol. Our hypothesis is that when GluT1 is found in lipid rafts, it will be more active. To confirm this, we ran experiments with methyl-cyclodextrin, a compound that destroys lipid rafts. Instead of seeing an inhibition in our uptake assays, we found an activation. We then used a western blot to confirm that GluT1 was indeed moving out of the lipid rafts after treatment. This contradiction to our hypothesis has brought up more questions and is a possible subject for future research.
This summer of research at Calvin has helped me to grow as a scientist and as a student. I have learned how to think critically, and have continued to develop my abilities to problem solve. I really enjoy manipulating and analyzing data to try and determine whether the results confirm or contradict our hypotheses. Working in a lab has enabled me to work with others and function as a team with a common goal. My lab techniques and efficiency have improved quite a bit, and I look forward to applying these skills toward doing more research in the future.