Thesis Abstract:

Despite ~100 years of literature on diabetes mellitus, and substantial reports on glucose-stimulated insulin secretion (GSIS) in pancreatic β-cells, cellular-level understanding of many fundamental aspects remains elusive. One such unknown aspect is the reason(s) behind the glucose specificity of GSIS, which was/is believed that ATP generated from glucose metabolism is primarily responsible for GSIS. However, this ignores two well-established facts: (i) ATP molecules produced from other metabolic sources are indistinguishable from those produced via glucose metabolism, while ATP production saturates beyond specific levels of extracellular glucose concentrations, and (ii) the rate of intracellular glucose transport by glucose transporter proteins (GLUTs) surpasses glucose phosphorylation rate by 100 times. Additionally, previous studies have primarily focused on the role of extracellular glucose towards inciting GSIS. Thus, considering this particularly fascinating yet unaddressed possibility, we wondered whether intracellular, but unphosphorylated glucose molecules somehow contribute towards the glucose specificity of GSIS. Through differential gene expression analyses of the well-established cell culture model system of Mouse Insulinoma 6 (MIN6) cells exposed to high extracellular glucose concentration (high EGC), our lab had recently identified the upregulation of 3 secretion-specific genes corresponding to the proteins KIF11, ATP6V0A4, and CACNB4, from whole transcriptome data. Using glucose as a ligand, we carried out rigorous computational investigations with the 3 test proteins, which will require further wet experimental validation. The glucose binding scores (in kcal/mol) obtained were also compared with binding scores for positive controls (GCK and GLUT2), along with negative controls (RPA1, KU70-80, POLA1, ACAA1A, POLR1A), respectively. Binding affinity scores of glucose molecules for the 3 proteins were found to be closer to positive controls. Therefore, we report glucose binding ability of 3 secretion-related proteins, and a possible direct role of intracellular glucose molecules in GSIS.

Molecular mechanisms leading to onset of Type 1 diabetes mellitus (T1DM) through viral infections are limited, although clinical correlations between the onset of T1DM and viral infections are frequently reported. Hence, another important aspect was to obtain mechanistic insights into virus-induced T1DM at a cellular level. Thus, we extended our investigations towards obtaining mechanistic interactions of a Chikungunya viral peptide (we have termed it as CV1) towards PAC1R (PAC1 receptor), the receptor of an established GSIS regulator,

Pituitary Adenylate Cyclase-Activating Polypeptide 38 (PACAP38 or P38). Using the recently reported Cryo-EM structure (PDB ID: 6M1I) of P38-bound PAC1R, as a positive control through AlphaFold 3 and through additional computational investigations, we found that CV1-PAC1R structure resembles P38-PAC1R, but with distinct binding in a nearby region. This observed conformational plasticity in binding explains and validates synergistic, but nonlinear modulations of GSIS by β-cells in presence of CV1 and P38, as obtained through previous wet experimental studies in our lab. We also calculated the equilibrium dissociation constant (K_d value) for the CV1-PAC1R complex using P38-PAC1R complex.

Having obtained mechanistic insights into 2 important aspects of GSIS through computational studies, we extended the obtained relationship of 'glucose-KIF11' towards understanding a cell biological aspect of GSIS, particularly the role of cell cycle in regulating GSIS, especially since KIF11 is an established cell cycle regulator. Using the cell culture model system of MIN6 cells, we found that cell cycle synchronization at the G2/M phase results in an enhanced GSIS, while total cellular RNA, cellular protein and supernatant protein remain constant, respectively. Interestingly, glucose interacts with the established residues located in an allosteric site of KIF11 which are known drug targets towards KIF11 inhibition. Thus, the binding of glucose to KIF11 might have potential functional implications towards KIF11 activity that remain to be explored, especially on the context of enhanced GSIS, underscoring the glucose specificity of GSIS. This also highlights the differential regulation of cellular secretory systems through the cell cycle. Additionally, the observed increase in GSIS during G2/M phase arrest might provide first-of-its-kind cellular-level link to the clinically observed 'honeymoon phase' in T1DM and its variants.