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Interactions of TUG-UBL1 and Insulin and Implications for Glucose Uptake

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Introduction

Diabetes is one of the most prevalent diseases in the US with prediabetes and diabetes affecting over 50% of the population.[1] The focus of this study is on type two diabetes as the disfunction of glucose uptake is due to insensitivity to insulin. TUG-UBL1 is a protein in the insulin signaling cascade that regulates the translocation of GLUT4 transport vesicles to the cellular membrane to facilitate glucose uptake into the cell.[2] The uptake of glucose is predicted to be initiated by the binding of insulin to the insulin receptor subunit (IRS), as shown in figure 1 below, causes conformational change which initiates a pathway of second messenger phosphorylation to activate the GAP (Rab GTPase Activating Protein) mediator protein. GAP releases PIST (syntaxin-6-interacting protein) which initiates the cleavage of TUG and allows translocation of GLUT4 from a GLUT storage vesicle (GSV) to the membrane to allow for cellular uptake.[3] If insulin is shown to bind to TUG, this mechanism must be altered to show the direct interaction of TUG to insulin. This research focuses on predicted binding between TUG and insulin to invalidate the proposed mechanism.

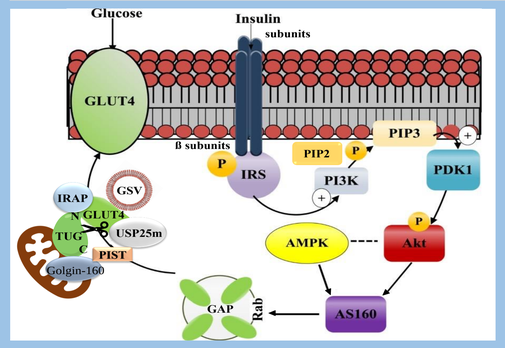


Figure 1. IRS: insulin receptor subunit, P13K: phosphoinositide-3-kinase, PIP_2: Phosphatidylinositol-4,5-bisphosphate, PIP_3: phosphatidylinositol-3,4,5-triphosphate, PDK1: phosphoinositidine dependent kinase 1, AMPK: AMP protein kinase, GAP: Rab GTPase Activating Protein, PIST: syntaxin-6-interacting protein, USP25m: TUG cleavage protease, TUG: Tether containing UBX domain for GLUT4, IRAP: insulin responsive aminopeptidase, GSV: glucose storage vesicle.[3,4]

Materials and Methods

Tertiary Structure → Refinement → Binding Analysis

- TUG-UBL1 and insulin amino acid sequences were obtained from the protein data bank: PDB 2AL3 for TUG-UBL1 and 2HIU for human insulin. The tertiary structures of the full-length TUG-UBL1 protein, the GLUT4 binding region of TUG-UBL1 of amino acid 83-377, and the insulin heterodimer was predicted using Phyre2.[5]
- Resulting models were refined using DeepRefiner followed by analysis by GalaxyWeb to provide ten models.[6,7] Further analysis was run by Molprobity to determine the most likely folding as shown in figures 2, 3 and 6, and raw and percentile score shown in table 1 and figure 7.[8]
- The models with least steric interactions of full-length TUG-UBL1, GLUT4 binding region of TUG-UBL1 and the insulin heterodimer were analyzed by HawkDock to analyze the predicted binding affinities of TUG-UBL1 full length or GLUT 4 binding region to the insulin heterodimer as shown in figure 8.[9]

Results

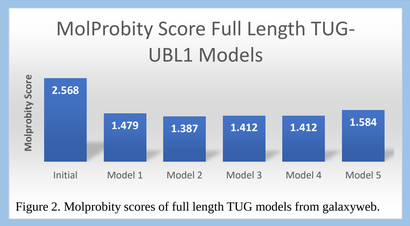


Figure 2. Molprobity scores of full length TUG models from galaxyweb.

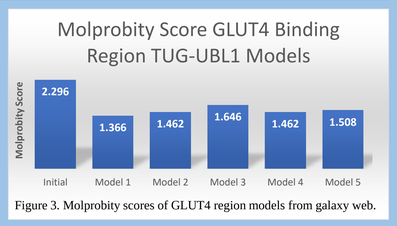


Figure 3. Molprobity scores of GLUT4 region models from galaxy web.

Figure 4. Full Length TUG-UBL1 generated by PyMOL

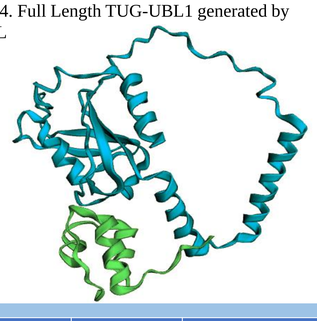


Figure 5. TUG-UBL1 GLUT4 Binding domain generated by PyMOL

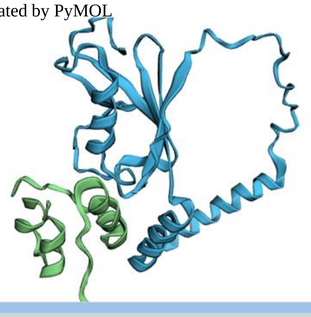


Table 1. Models used for binding analysis	Model 2, full length TUG	Model 1, GLUT4 binding domain
GalaxyWeb ClashScore Percentile	2.05 99th	3.48 97th
Molprobity Score Percentile	1.19 99th	1.45 96th

TUG-UBL1 models were refined by DeepRefiner followed by analysis by Molprobity for clash and Molprobity score to determine the most likely conformation with lowest steric strain based on all atom contact clashing, Ramachandran angles, and side chain rotamers (lower scores indicate more likely conformation)

Figure 6. Insulin Heterodimer for Binding Analysis generated by PyMOL

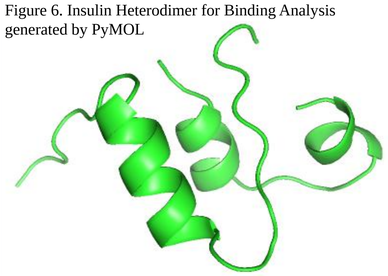


Figure 7. Molprobity and Clashscore percentile of insulin models from DeepRefiner

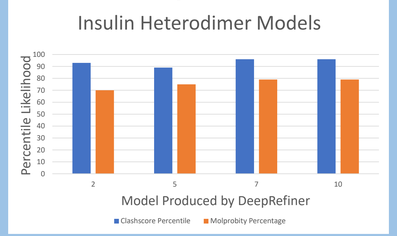
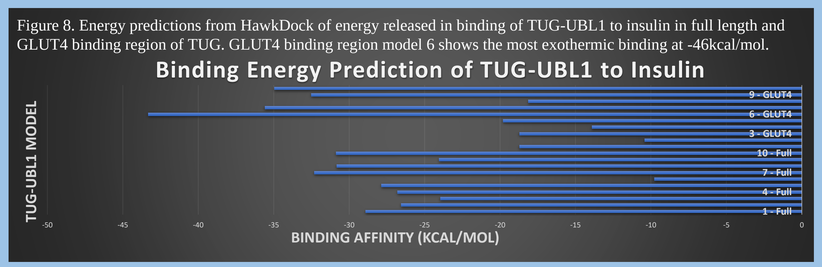


Figure 8. Energy predictions from HawkDock of energy released in binding of TUG-UBL1 to insulin in full length and GLUT4 binding region of TUG. GLUT4 binding region model 6 shows the most exothermic binding at -46kcal/mol.



Conclusions and Future Work

Figure 9. TUG-UBL1 Full-length bound to insulin by HawkDock

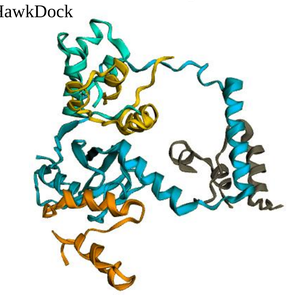
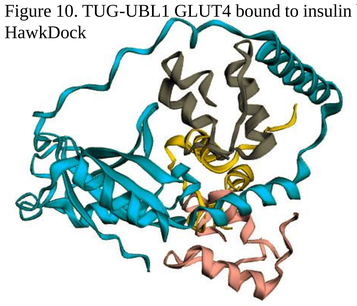


Figure 10. TUG-UBL1 GLUT4 bound to insulin by HawkDock



HawkDock predicted binding of insulin to TUG-UBL1 in both full length and GLUT4 binding region, with favor to the GLUT4 binding region as seen in figure 8, where the current model of second messenger pathways for insulin signaling does not predict this as seen in figure 1. Physical validation is needed in binding to locate a binding domain as predicted in figures 9 and 10.

TUG Over-expression → Purification → Fluorescent Binding

A plasmid containing the TUG-UBL1 GLUT4 binding domain has been obtained from Bogan *et. al.* and transformed into *E. coli* and overexpressed. This protein has been purified utilizing a Q-sephadex anion exchange column to elute the 32kDa protein. Future studies involve probing its binding to insulin via fluorescence spectroscopy. Binding of insulin and TUG in support of computational models would alter the understanding of glucose uptake in diabetic patients and may increase therapeutic options for diabetic treatments.

References

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Acknowledgements

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