Using Thermodynamic Integration to calculate ΔG Binding of Glucose for
Asp → Cys-236 Mutated GGBP

Sabrina Davis '18, Natalie Nguyen '17, and Amil Anderson

Department of Chemistry, Wittenberg University, Springfield OH, 45501

The purpose of this research is to determine the free energy change of binding glucose for an Asp → Cys-236 mutation of Glucose/Galactose Binding Protein (GGBP). The thermodynamic cycle used for this calculation is pictured below (Figure 1). Measurements needed to calculate the free energy change of binding include: the free energy change associated with turning harmonic restraints on, removing the sugar from the protein, adding the sugar back into the solvent, and turning the harmonic restraints off. This is completed using thermodynamic integration, a free energy molecular dynamics simulation method.

The mutation of Asp → Cys-236, directly influences the binding site. Cysteine is less polar and slightly smaller than aspartic acid, which was hypothesized to make cysteine less effective at hydrogen bonding, resulting in a weaker binding of glucose to GGBP. Wild-type GGBP is highly saturated at the physiological glucose concentration and therefore will not work well as a biosensor[1]. By mutating Asp→Cys-236 the binding affinity of glucose is lowered, making GGBP a more effective biosensor.

$$-\Delta G_{\text{binding}} = \Delta G_{\text{restraint on}} + \Delta G_{\text{sugar annihilation}} + \Delta G_{\text{sugar solvation}} + \Delta G_{\text{restraint off}}$$

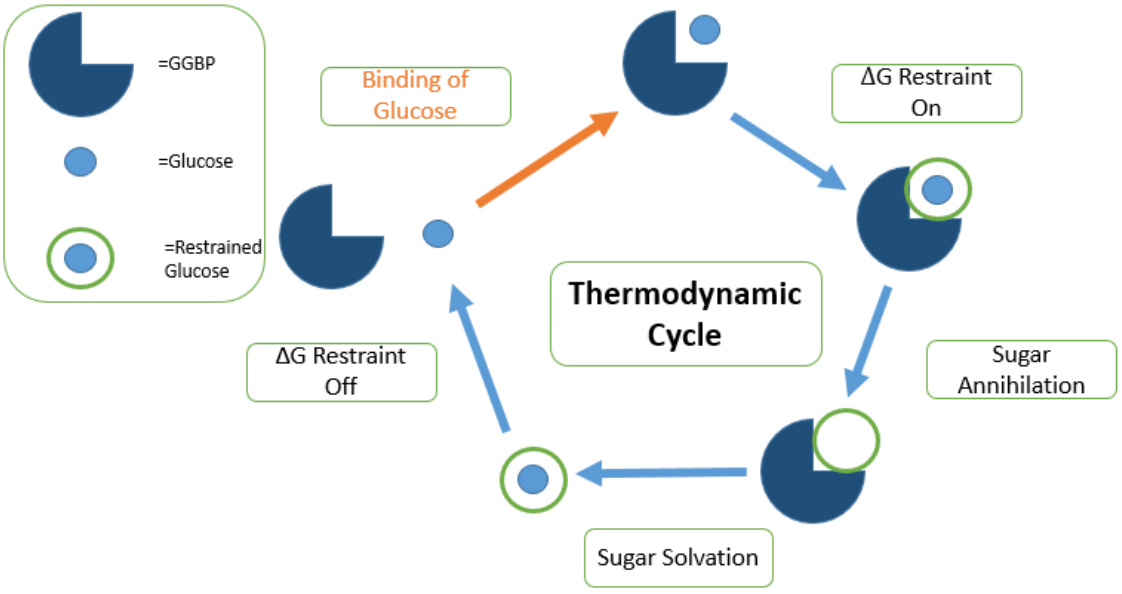

Figure 1: Thermodynamic cycle used to determine the binding of glucose. The process started with glucose bound to Cys-236 GGBP, applied positional restraints, annihilated the bound sugar, grew the sugar back into the solvent, and turned the positional restraints off.

---

[1] Pickup, J. C.; Hussain, F.; Evans, N.D.; Rolinski, O.J.; Birch, D.J. S. *Fluorescence-base glucose sensors*. *Biosensors and Bioelectronics.,* **2004**, 20, 2555-2565.