**Using Molecular Dynamics Simulations to Analyze the change in the Free Energy of Binding of Glucose to GGBP caused by Mutation of Residue 213 from Ala to Trp**

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The Glucose/Galactose Binding Protein (GGBP) is crucial to bacterial chemotaxis in *E. coli* and other bacteria, binding either to glucose or galactose. GGBP has an open conformational state when it is not bound to a sugar molecule and a closed conformational state when it is bound to a sugar molecule. It was hypothesized that the magnitude of the binding free energy of GGBP would decrease (lower affinity) when a residue at the binding site, Ala-213, was mutated to Trp. The thermodynamic cycle (Figure 1) used to calculate the relative change in binding energy caused by the mutation can be evaluated by two different pathways using molecular dynamics: 1) the alchemical transformation (mutation) of Ala to Trp in open and closed conformations of GGBP, 2) the binding of glucose to GGBP and to mutated Trp-213 GGBP. The change in binding energy ($\Delta\Delta G_{binding}$) due to the mutation of wild-type GGBP (Ala-213) is calculated by finding the free energies for mutating Ala-213 to Trp-213, with and without bound glucose (Eq. 1). This method is more computationally efficient than determining the separate binding free energies for wild-type and mutated GGBP.

$$\text{Eq. 1} \quad \Delta\Delta G_{binding} = \Delta G^{Ala}_{binding} - \Delta G^{Trp}_{binding} = \Delta G^{bound}_{Ala\rightarrow Trp} - \Delta G^{unbound}_{Ala\rightarrow Trp}$$

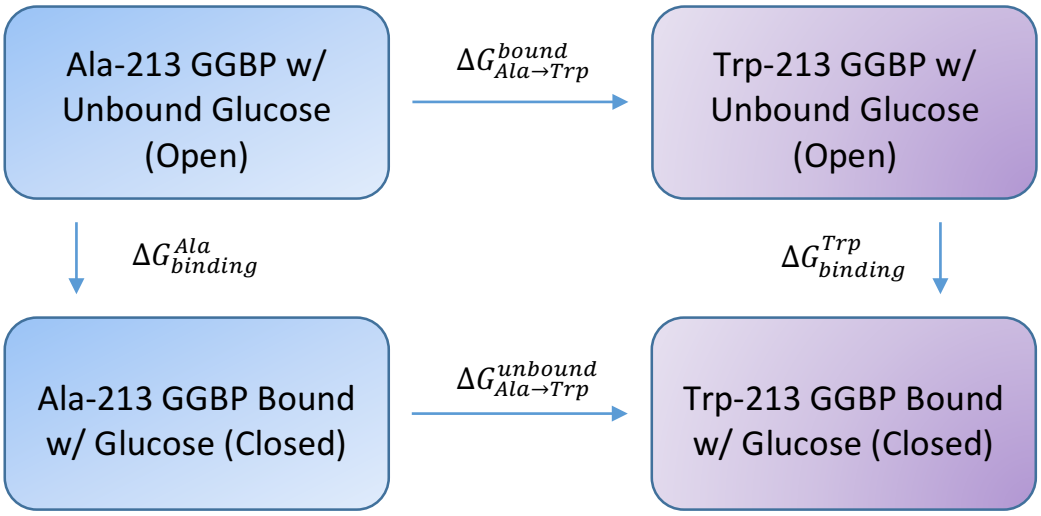


***Figure 1**. This thermodynamic cycle of GGBP shows the changes in free energy for the wild-type (blue) binding of glucose and the free energy of binding for the mutated GGBP (purple).*