1 Introduction

Author's Note: The following section contains what I hope is the essential background to get an unfamiliar reader up to speed and provide context on the topic of ligand-binding and cooperativity as it pertains to this work. Many of the nuances will be detailed in later sections and supported with models. Several treatises are available on this topic, and the reader is directed to some for more in-depth background [klotz, wyman gil].

Ligand Binding and Cooperativity

The complex biochemical processes that give rise to life depend on the recognition of one molecule by another: a hormone binding to its receptor, an enzyme binding its substrate, a transcription factor binding DNA, etc. A receptor recognizes its ligand, conventionally the smaller molecule, through non-covalent interactions comprising electrostatic forces, van der Waals forces, and the hydrophobic effect. The degree of recognition or *affinity* of the receptor for its ligand is determined by the strength and number of non-covalent interactions between them, and those in turn are determined by the structure and chemical composition of the molecules, e.g., the type and spatial configuration of amino acids in a receptor protein. Analyses of ligand-receptor systems focus on affinity as the system's characteristic property and quantify it with the equilibrium constant of the binding reaction.

Differences in the magnitude of affinity are why a given receptor binds one ligand versus another. For example, an estrogen receptor will bind the hormone estrogen and effect a cellular change, but that same receptor will be unaffected by a another hormone such as insulin. This is because the structure and composition of the binding site on the receptor are complementary to those of the ligand and enable the formation of sufficient non-covalent interactions. Estrogen and insulin differ significantly in structure and composition (one is a steroid and the other a peptide) hence insulin cannot interact favorably with the binding site on the estrogen receptor. The converse is also true that a ligand could be contrived to have a greater affinity if it can participate in additional or stronger interactions.* This applies to receptors as well, where structural and compositional differences of binding sites account for differences in affinity. These phenomena have important implications when recalling a protein has conformational flexibility and can take on structural changes.

Consider a receptor with two binding sites for the same ligand. If the sites are structurally and compositionally the same, then we expect them to have the same affinity. However, if the structure changes, so will the nature of the interactions with the ligand and subseqently the affinity. In some receptors, a

*This is the *de facto* strategy used in drug discovery and development, particularly of those drugs that function as competitive inhibitors.

ligand can induce conformational changes when it binds and modify affinity at the other site. Depending on whether the affinity increases or decreases, the system is said to exhibit positive or negative cooperativity[†].

Cooperativity can function as a modulator of biochemical processes. A classic example of this is observed when hemoglobin binds oxygen. Positive cooperativity narrows the range of concentrations over which oxygen is bound so that it can be efficiently transported from higher concentrations in the lungs to lower concentrations in tissues. How this happens will become clear when we discuss models of cooperativity in SECTION REFERENCE.

This modulation... SThat narrowing highlights cooperativity's role in modulating a biochemical process.

For receptor proteins involved in cell signaling, modulation is an important component. Variations in affinity under different conditions

This can be extended across multiple ligands or sites...and other disclaimers

Models: Visualizing Affinity

$$\mathrm{R} + \mathrm{L} \leftrightarrow \mathrm{RL} \tag{1.1}$$

[†]Cooperativity can be further classified as homotropic if the affinity is changed for and identical ligand (as explained) or heterotropic if the affinity is changed for a different ligand