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Binding Affinity: The Physics of Interaction

Every drug is discovered or made it has a specific target (protein), the relation between protein and the ligand is not static relation but dynamic, and the dynamic equilibrium is a fundamental prerequisite for pharmacological efficacy is the interaction between a drug molecule (ligand) and its biological target (proteins), this equation shows the dynamic equilibrium ($P + L \leftrightarrow PL$) [1]. According to Clark's Occupancy Theory, the biological effect is proportional to the amount of complex (PL) formed, Therefore the goal is to shift the equilibrium right towards complex formation and here comes the role of binding affinity. Binding Affinity is the thermodynamic measure of the strength of the interaction between a protein (P) and a ligand (L) to form a stable complex (PL). It is quantified by the Gibbs Free Energy of Binding (ΔG), which represents the difference in energy between the bound state and the unbound state. And numerically represented by this equation ($\Delta G = R\ T\ \ln K^d$) as ΔG is Gibbs Free Energy (kcal/mol or kJ/mol), R is the Ideal Gas Constant ($1.987 cal.K^{-1}.mol^{-1}$), T is the Temperature in Kelvin (K) and K^d represents the molar concentration of ligand required to occupy 50% of the available protein sites. There is an inverse relationship between these metrics: a lower K^d corresponds to a more negative ΔG , signifying higher affinity and a tighter protein-ligand complex[2][3].

Optimizing the binding affinity of the drug:

To optimize a lead compound, medicinal chemists manipulate the components of the Gibbs equation: ($\Delta G = \Delta H - T\Delta S$) an effective way to shift the equilibrium to the right is by optimizing the lead's chemical structure to lower the dissociation constant(K^d). By Enthalpic Optimization ΔH: Involves improving the specific geometric fit between the drug and the binding pocket. This is achieved by adding functional groups that form specific hydrogen bonds or van der Waals interactions, releasing heat (negative ΔH), or by Entropic Optimization ΔS: Involves the "Hydrophobic Effect." When a hydrophobic drug enters the pocket, it displaces organized water molecules into the bulk solvent. This increase in disorder (positive ΔS) provides a significant energetic contribution to binding affinity,all of that lead to make the forward reaction ΔG more spontaneous, ensuring high occupancy even at low drug concentrations [4][5][6].

The Gold Standards:

The standards today of measuring binding affinities are ITC (Isothermal Titration Calorimetry) and SPR (Surface Plasma Resonance) for measuring protein-protein or protein-ligand based

interactions [7].ITC's is a biophysical technique that have been developed and is still being in use till this day, and ITC is used to measure the heat being adsorbed ore being released of which is in a process that changes the composition of the system [8].ITC requires no labeling and allows simultaneous determination of the affinity Ka , stoichiometry, change of free energy ΔG , in ΔH enthalpy changes, and as well in ΔS or entropy in one single experiment, and from these forces one can find the estimate driving forces that characterize the interaction of a protein with a ligand for example[9].While ITC measures thermodynamics, SPR is a developing techniques of which measure kinetics of ligand binding interactions with membrane proteins. Moreover, SPR is an optical method of which measures change in refractive index in close vicinity of a metal. By detecting these optical shifts, the technique can monitor how soluble molecules interact with receptors that are immobilized on the metal layer [10].

IC50 vs Ki:

Moreover, an important barrier to train accurate or robust ML models on public datasets is the the inherent inconsistency of the labeled data. A major contributor to data noise is merging two distinct metrics, the half-maximal inhibitory concentration (IC50) and the inhibition constant (Ki). Ic50 is the concentration required for 50% inhibition and depends on the assay conditions [9] while the binding strength of reversible inhibitor is quantified from the Ki obtained from enzymatic activity measurements [11]. This distinction is critical because IC50 and Ki values could be treated as equivalent using the Cheng-Prusoff equation, therefore without the use of this equation to standardize, it will be creating noise between those distinctions.

Data Noise and Curation Errors:

However, noise level arises when one compares experimental results from different laboratories and as well having differences for instance as in assay protocols and reagents in IC50. Furthermore, the identity and concentration of the substrate are critical variables for IC50 values derived from competition assays, as the specific substrate and its amount directly influence the experimental outcome. In contrast, Ki quantifies the intrinsic binding affinity of a ligand to an enzyme, therefore, its values are fundamentally independent of the substrate's type or concentration. Nevertheless, human error in database creation also degrades model performance. Large public datasets like ChEMBL often contain human errors like transcription mistakes or incorrect units as for example reporting μm instead of nm that are copied from the primary literature. To mitigate this, data compatibility should be assessed by analyzing the reference compounds shared between assays. If values for these common ligands differ by less than the standard experimental error (typically $\Delta pIC50 < 0.3$ or a twofold difference), the assays and datasets are considered compatible [12].

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