Pharmacology of the main drug targets

Find a Druggable Target

Druggable

- Ligands can get into the binding site. (Considering the hydrophilicity balance, neither too hydrophilic nor too hydrophobic).
- Containing a binding site, with high drug binding affinities (affinity means the degree of firmness the ligand binds to the protein).
- The binding must elicit a response. (efficacy)

Common Drug Targets

Ion Channels

This kind of drug targets are often located in the CNS and participate in pain controls.

The change of potential serves as an easy means to evaluate a single receptor's activity.

Voltage sensitive dyes can be used.

GPCR

G protein-coupled receptors (GPCRs), are a series of transmembrane receptors coupling with G proteins[1].

Quick Facts

- **Location**: on the plasma membrane.
- **Ligands** including peptides, hormones, photons, ions, and small molecules.
- GPCRs are **targets of 30% drugs**.

Signaling

Two features of GPCRs are expected to be focused:

1. Desensitisation: when signals are too high, cells internalise and recycle GPCRs to desensitise itself.
2. Biased agonism: The activation of a certain kind of G protein by one agonist may generate signals participating in many downstream pathways, which further account for **on-target** side effect.
3. Some GPCRs display intrinsic activities, which means they can be activated even there is no exogenous agonist.

Enzymes

Quick Facts

- Location: Soluble or membrane bound
- The most common type transfers phosphate and trigger cascade signalling.
- Ligands can interact the plasma-side of enzymes located on cell membrane, and create reactions inside of the cytosol.

Protein-Protein Interactions for Signalling

Protein-Protein interaction can transduct signals

> Protein-protein interactions occur for signal transduction & some enzymes are only active as multimeric complexes.

Drugs Targeting Protein-Protein Interactions

Since interacting proteins may have very large scales, drugs designed to involve (inhibit or stablise) in this process may also have a very large size.

This obviously does not comply Lipinsky rule-of-five. Drugs can be hard to be absorbed. Hence, some focus on designing drugs in reasonable sizes (such as some cyclic peptides) or take peptic compounds in terms of small molecules.

In another aspect, drugs are also designed to **increase binding** affinities between proteins through allosteric effect or acting as the interface.

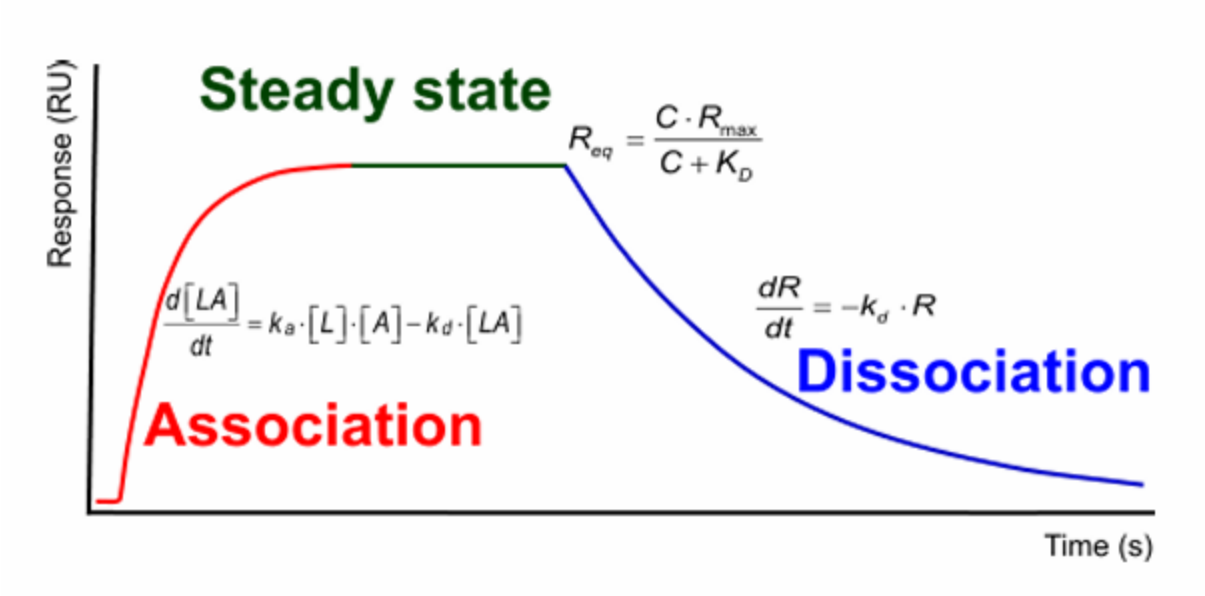
Receptor Pharmacology

Affinity

Affinity describes the process how drugs bind to their receptors:

$$Drug + Receptor \rightleftharpoons_{K_{off}}^{K_{on}} Drug - ReceptorComplex$$

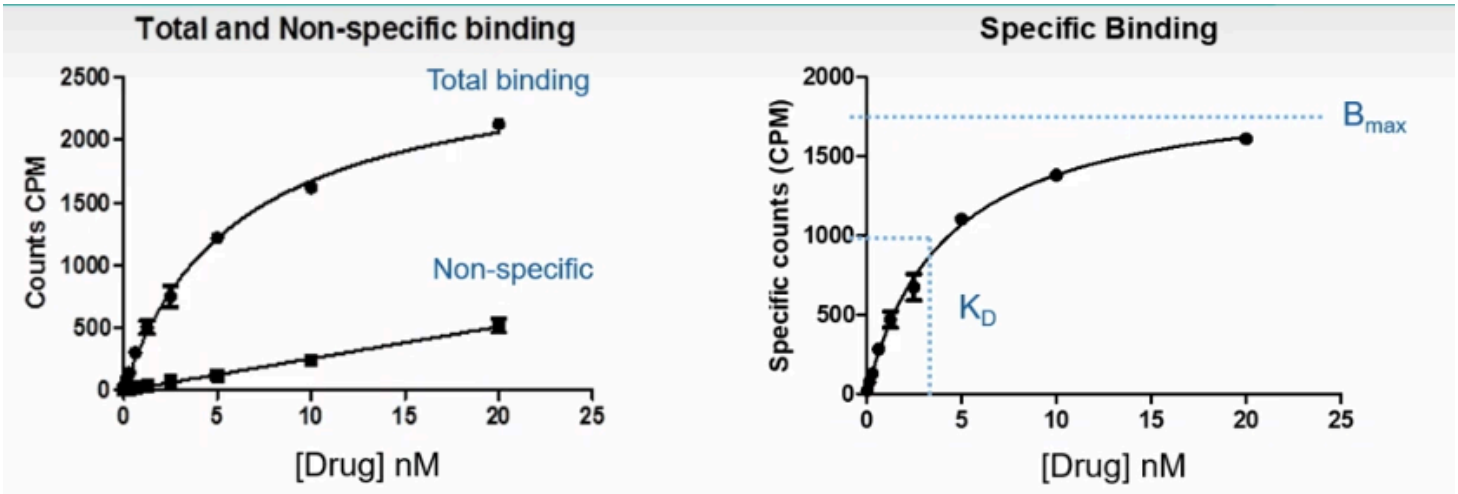
More specifically, the whole process can be described as **Association**, **Steady state**, and **Dissociation**. **Among these different stages, dissociation stage represents affinities between drugs and receptors.**



Meaning of Affinity

Higher affinity also means that a lower dosage and administration frequency are needed. These outcomes benifit in terms of decreasing off-target effects, achieving long-term actions, and improving patient compliances.

Quantitative Description: Dissociation Constant K_D



$$K_D = \frac{k_{off}}{k_{on}}$$

K_D is also defined as the half of 'max binding' B_{max}.

Efficacy

Efficacy describes to what extent responses are elicited after the binding occurs.

There are two concepts deescribing drugs' efficacies: **Full agonist and Partial agonist**

Binding does not mean response. But if a drug shows efficacy, it must bind somewhere.

Sometimes we do not want a very high efficacy aiming to get rid of side effects.

Potency

Potency is a measure of necessary amount of the drug to produce an effect of a given magnitude.

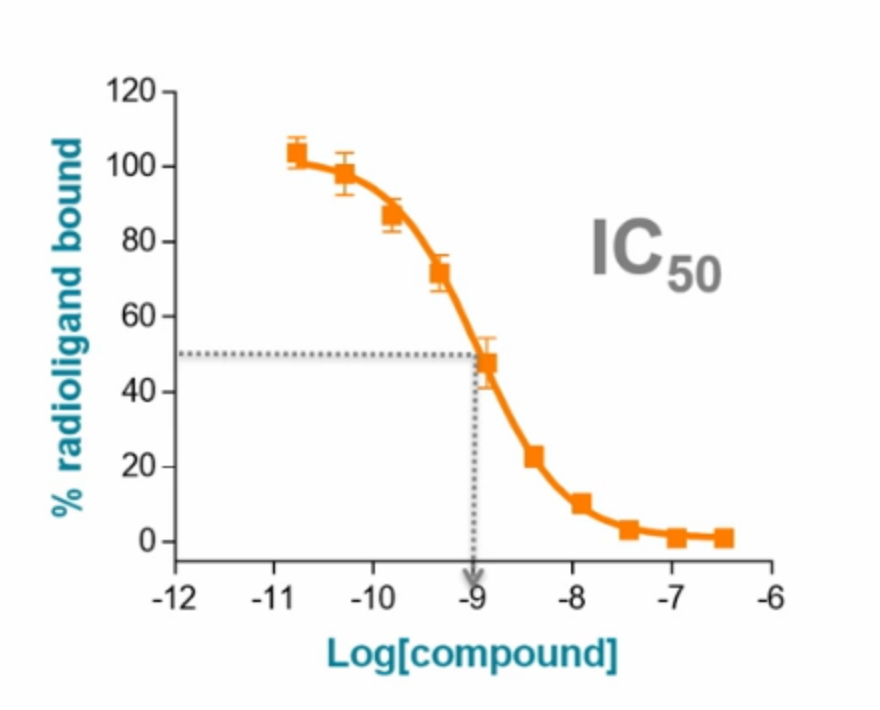
Potency depends on both **affinity** and **efficacy**

In general cases, **potency** lines up with **affinity**. If they are a long way different, it suggests there's a problem somewhere in one of the sites is not correct.

Quantitative Description: IC_{50} and EC_{50}

EC_{50} is the concentration of drug required to elicit a 50% response and reflects the potency of the given drug.

Smaller EC_{50} values indicate stronger potency

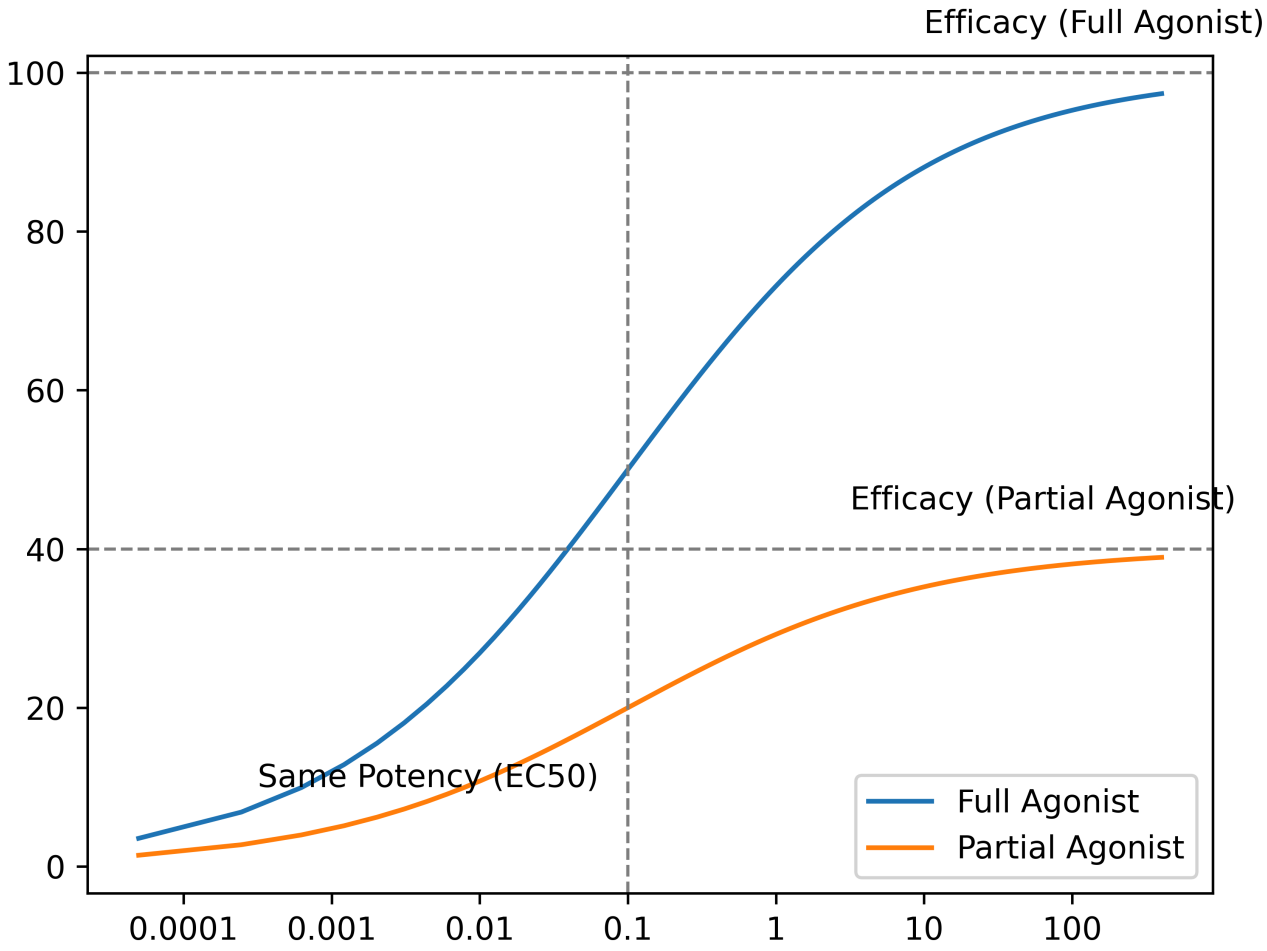


Plot bound percentage against $\log[Compound]$ and end up with a -1 slope (usually $k \in \{0.5, 2\}$) sigmoidal curve.

To get the maximum inhibitory concentration, you need to know what your inhibited enzyme is looking like, which might be challenging.

Ligand View

Full and Partial Agonist



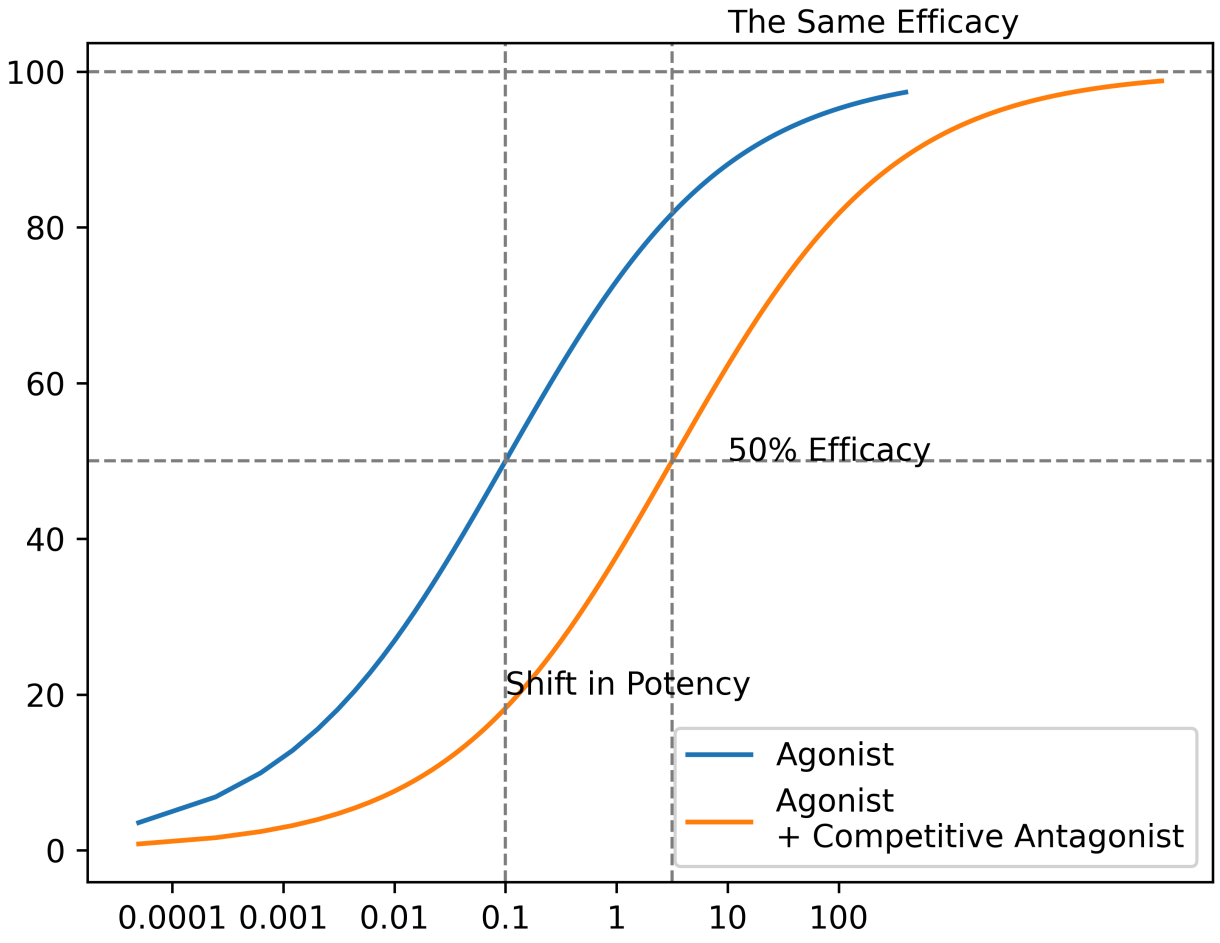
Full and partial agonist are two concepts describing the efficacy properties of drugs.

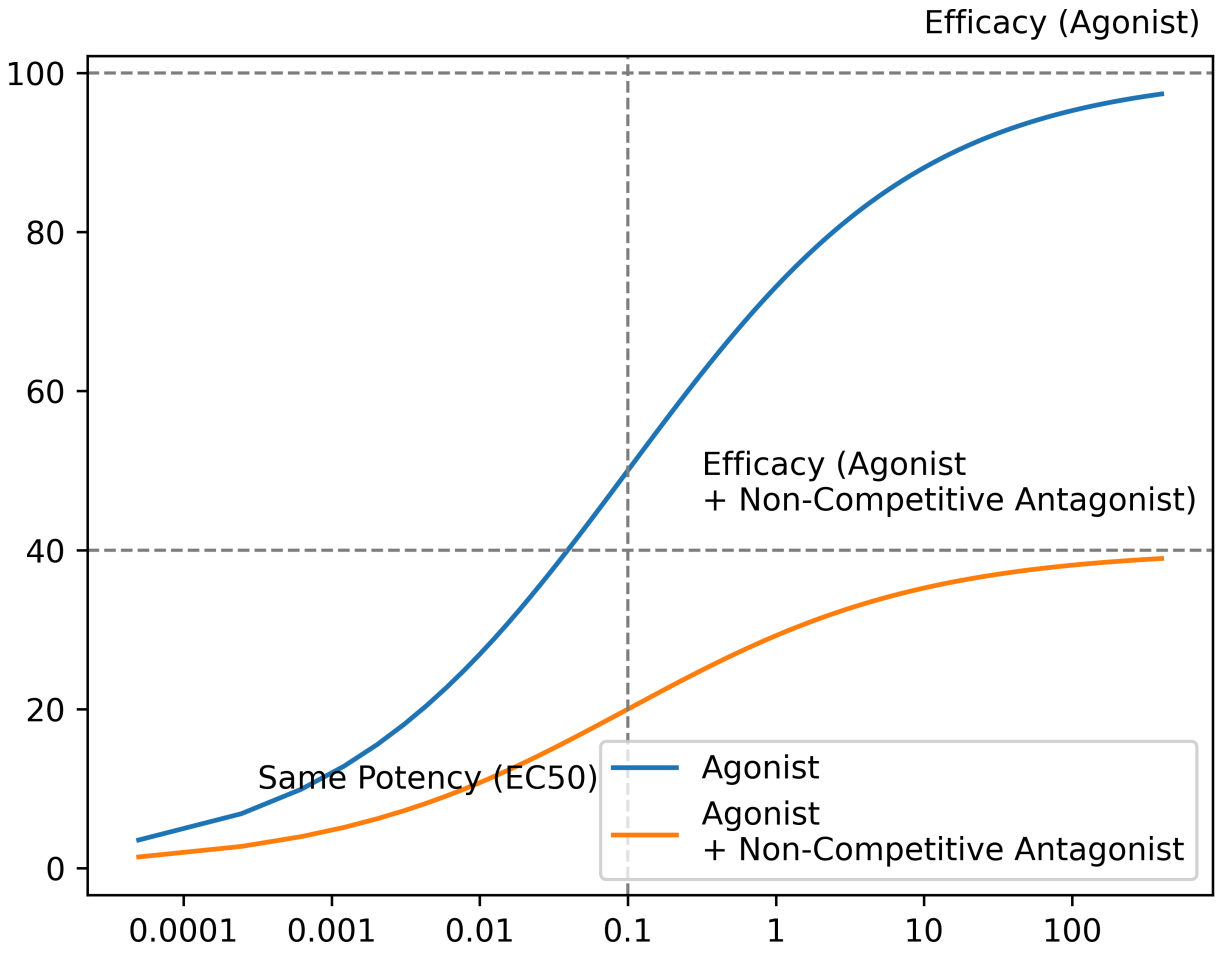
Full agonists elicit 100% efficacy, while partial agonists are weaker in terms of efficacy. (There is nothing to do with potency)

Antagonist

Antagonists do not trigger **any** effect.

Competitive vs Non-Competitive Antagonist





The situation in `Agonist + Non-Competitive Antagonist` is a little bit similar to that in `Full Agonist vs Partial Agonist`, in which efficacy is reduced and potency remains the same.

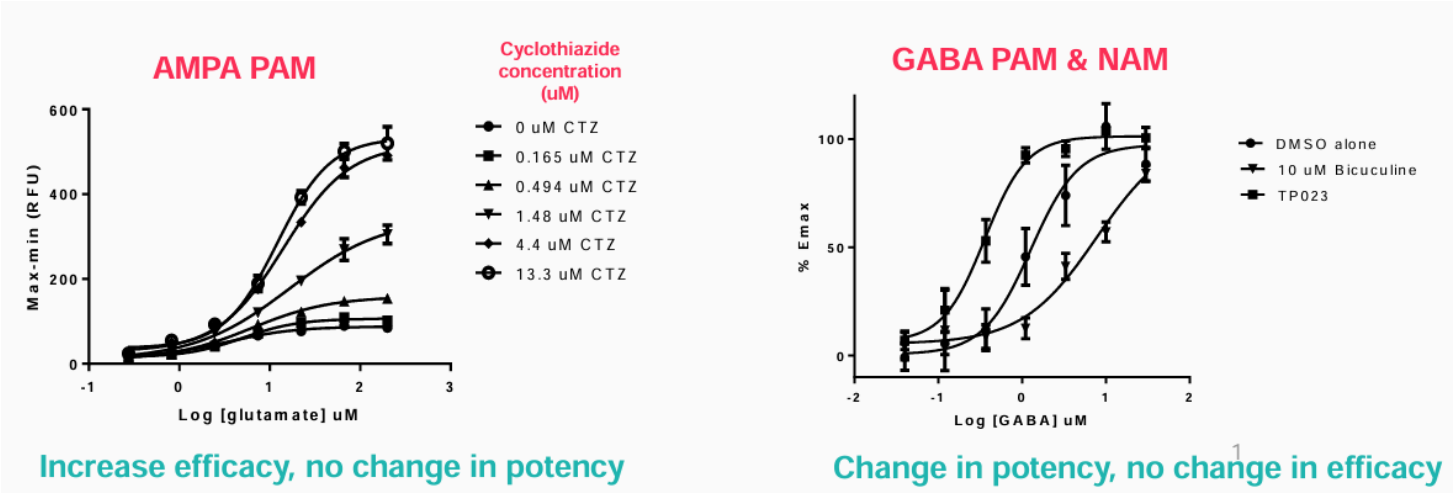
Inverse Agonist

Sometimes, there are might be some receptors (GPCRs or mutation-induced) are *instrinsic active*, which means they exhibit activities even there is no agonist. In this case, **inverse agonists** bind to inhibit the baseline activity.

Positive/ Negative Allosteric Modulaters

- Allosteric modulators have no effect on their own, but enhance or reduce the effect of agonists.
- PAMs enhance effect of agonist
- NAMs reduce effect of agonist
- Allosteric binding sites will not be distant away from original binding sites.

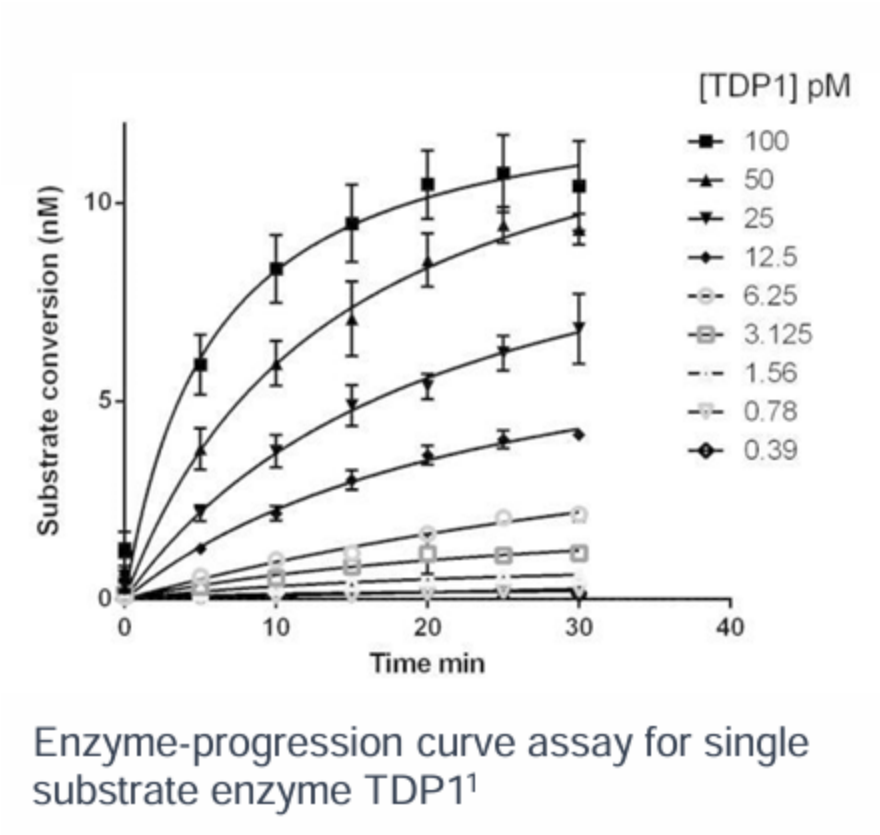
The results of efficacy and potency shifts can be diverse:



Enzyme Pharmacology

Reaction Rate

The measurement of reaction rate assuming the experimente system neither runs out of substrates nor has an abundance of substrate. (Measurements start at the point where 10% substrate is depleted.) This ensures the accuracy and sensitivity of assay.



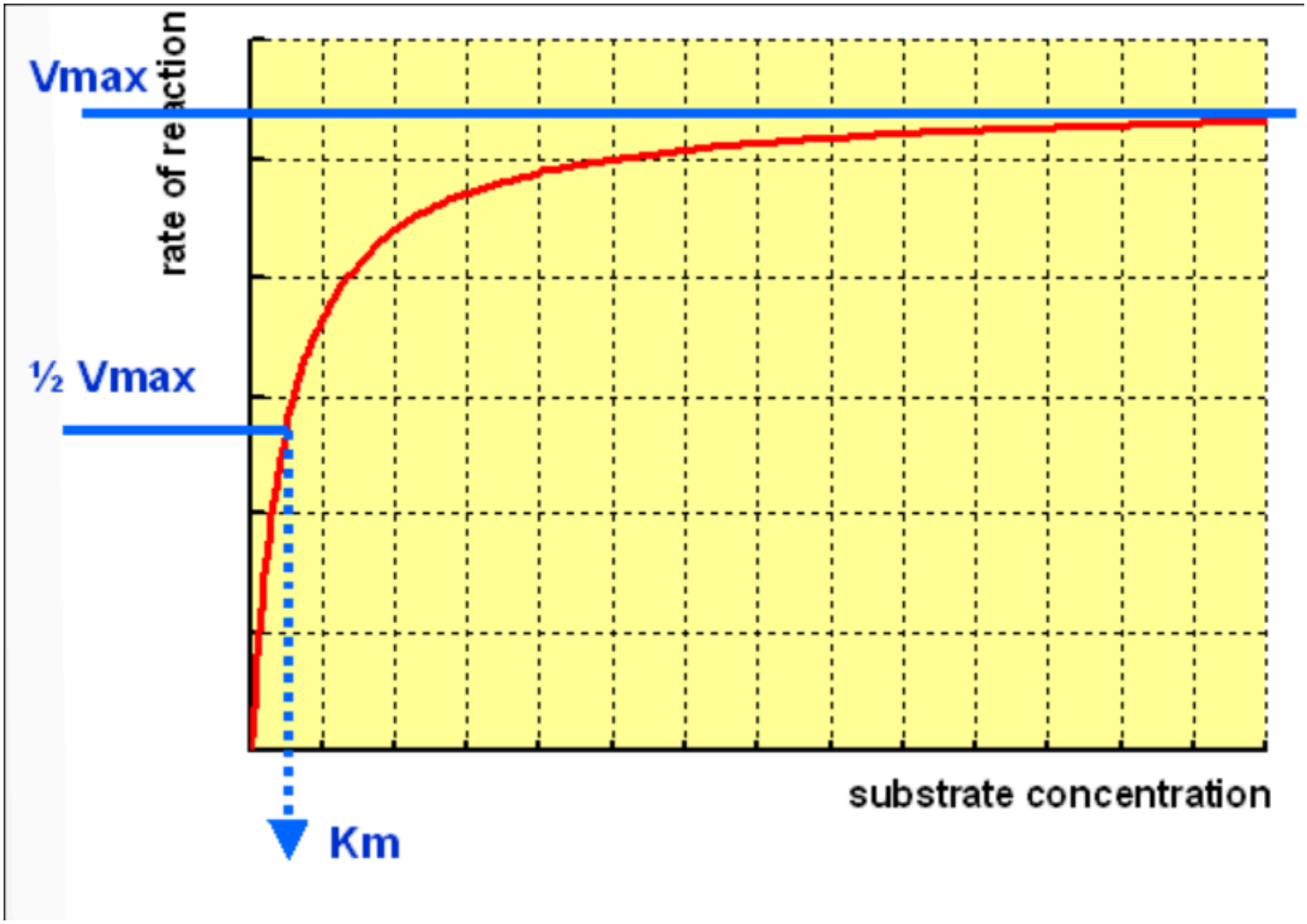
Michaelis-Menten Equation

Reaction rate and some constants can be figured out through this equation and experiment data.

$$v = \frac{V_{max} \cdot [S]}{[S] + K_m}$$

where v denotes the reaction rate, v_{max} denotes the maximal reaction rate, and K_m denotes the concentration of the substrate when reaction reaches half of v_{max} (**Michaelis constant**).

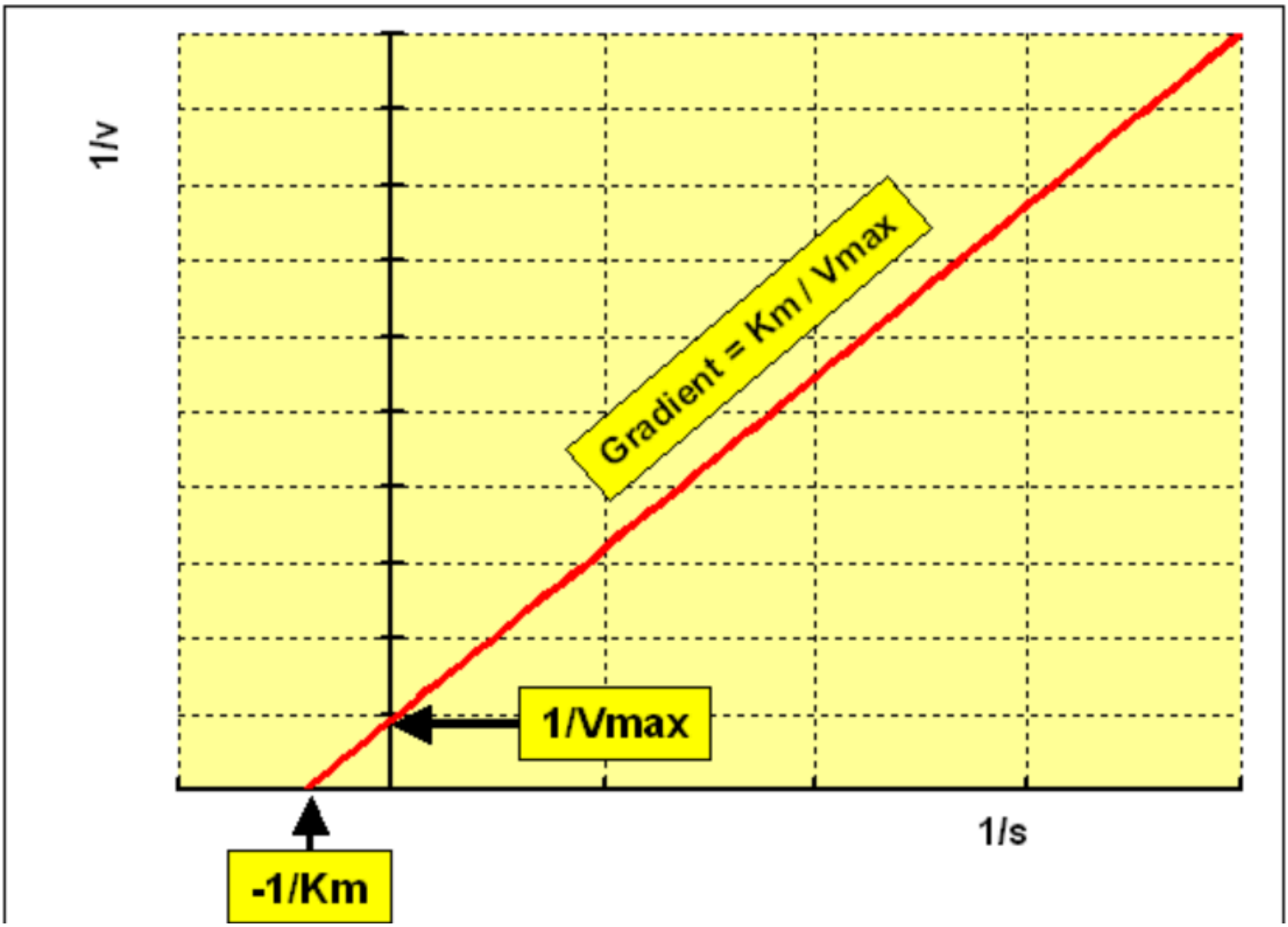
A typical Michaelis-Menten curve, which plots $rate$ against $[S]$, is shown below:



$$v_0 = \begin{cases} \lim\limits_{[S]\to 0} v_0 = \dfrac{V_{max}}{K_m} \times [S] \\ v_0 = \dfrac{1}{2} V_{max} \quad when \quad [S] = K_M \\ \lim\limits_{[S]\to \infty} v_0 = V_{max} \end{cases}$$

A tradition means to process the Michaelis-Menten equation is using Lineweaver-Burk double reciprocal plot:

$$\frac{1}{v} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \times \frac{1}{[S]}$$



Linearisation of Michaelis-Menten curve is based on some prerequisites, which may incur error. Only linearise data when checking its quality.

Inhibitors from the View of the Michaelis-Menten Equation

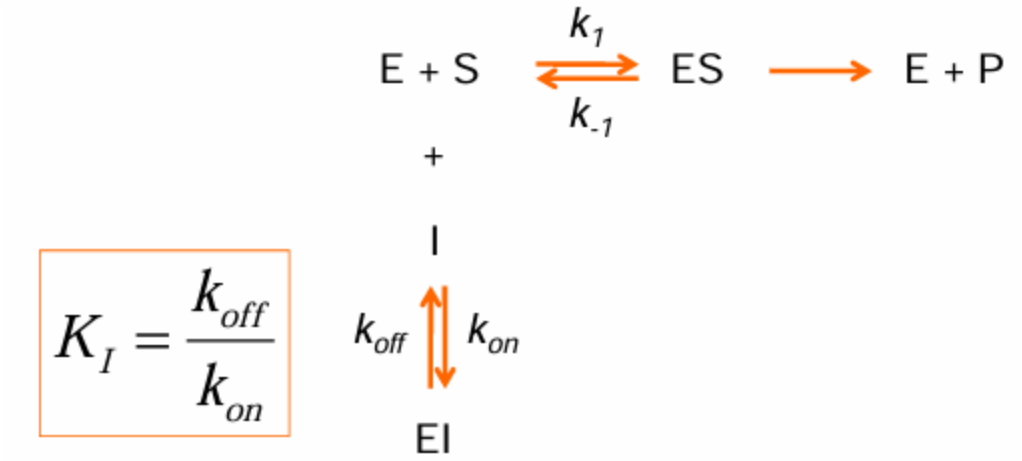
Inhibitor Type	V_{max}	K_m	Binding Site
Competitive Inhibitor	/	↑	Enzyme E only
Non-competitive Inhibitor	↓	/	ES and E
Uncompetitive Inhibitor*	↓	↓	ES complex only

*: Uncompetitive inhibitors interact with the $E-S$ complexes, which both hinders productions of product and dissociations.

Persumably, the interaction site is known, and drugable. A competitive inhibitor might be a good choice. Whereas in the condition that the interaction site is not drugable, having poor hydrophilic property for example. Allosteric (Non-competitive) inhibitors are good alternatives.

The choose of inhibitors also depends on the purpose: if the ultimate aim is reduce the product accumulation, noncompetitive inhibitors are the only option, while with a purpose of increasing the substrate concentration, competitive and noncompetitive inhibitors are both optional choices.

In terms of competitive inhibition:



smaller off-rate k_{off} indicates a longer effect.

Some exceptions where conventional methods are not valid

Time-dependent Inhibition

The k_{off} values of time-dependent inhibitors change with respect to time. According to the equation $K_f = \frac{k_{off}}{k_{on}}$, their inhibition capabilities increase, which further reflect changes in their potency.

In this case, the original metrics like IC_{50} and k_D are meaningless.

Bivalent Compounds

Bivalent compounds form trimer with two proteins instead of Drug-Protein dimer.

Bivalent compounds (such as PROTAC) might bind to proteins firmly when in pairs, but strongly repulse each other when forming trimer.

Hence, measuring the affinity using K_D only is not enough. Co-operativity, an odds ratio between K_D^{binary} and $K_D^{ternary}$, is leveraged in this situation:

$$\alpha = \frac{K_D^{binary}}{K_D^{ternary}} = \begin{cases} > 1 & positive \quad cooperative \\ = 1 & non \quad cooperative \\ < 1 & negative \quad cooperative \end{cases}$$

Degradation kinetic is another parameter for degraders

Discriminate from dissociation

1. G proteins contain 3 subunits, G_α, G_β, and G_γ. Among these subunits, G_α is the most investigated and in charge of cascade signal transduction. G_β and G_γ can signal as a dimer. ↩