Lecture 19: Allosteric Inhibitors

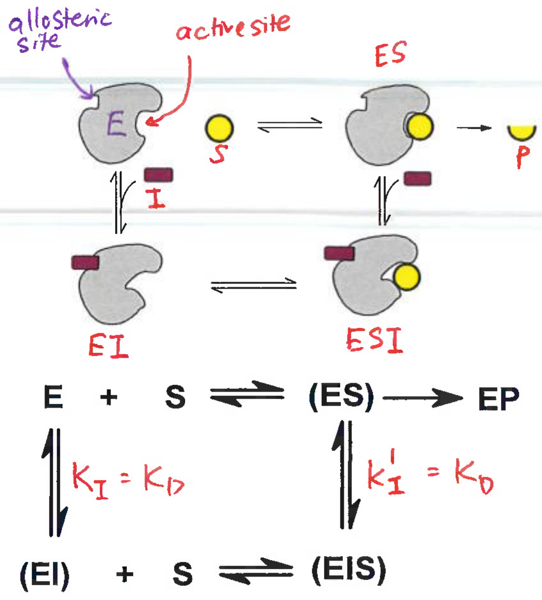
The binding site of the inhibitor is **not** at the active site. The inhibitor does not look like the substrate.

- *The binding is reversible.*
- The inhibitor can bind to both [E] and [ES].
- The inhibitor binding causes a change in the conformation of the protein that affects either substrate binding, the chemical step, or both.

There are two K_D values that describe the binding.

$$K_I = \frac{[E][I]}{[EI]} = K_D$$

$$K_I' = \frac{[ES][I]}{[ESI]} = K_D$$



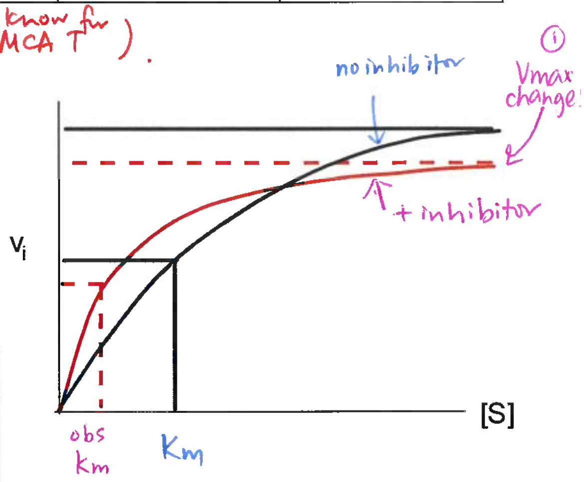
Special Cases of Mixed-type Inhibition: (Unrealistic simplifications).

	Binds to (E)	Binds to (ES)
Mixed type	yes	yes
Uncompetitive	no	yes
Noncompetitive	Same affinity	Same affinity

general case; special cases (know for MCA T)

- Both V_{MAX} and K_M can be altered by mixed inhibitors since the precise geometry of the active site is altered when the inhibitor is bound, potentially affecting binding and catalysis.
- The change in V_{MAX} and K_m can be used to find K_I and K_I'.

example if the Ser in Trypsin moved ~0.5 Å the enzyme would be inactive.



α = ratio of slopes (+I/no inh)

K_I = [I]/(α-1)

α' = ratio of y-intercept (+I/no inh)

K_I' = [I]/(α'-1)

No Inhibitor Present	Mixed Inhibitor
	$\alpha = 1 + \frac{[I]}{K_I}$ $\alpha' = 1 + \frac{[I]}{K_I'}$
$v = V_{MAX}\frac{[S]}{K_M + [S]}$	+inh. $v = \frac{\frac{V_{MAX}}{\alpha'}[S]}{\frac{\alpha}{\alpha'}K_M + [S]}$
$\frac{1}{v} = \frac{K_M}{V_{MAX}}\frac{1}{[S]} + \frac{1}{V_{MAX}}$	$\frac{1}{v} = \frac{\alpha K_M}{V_{MAX}}\frac{1}{[S]} + \frac{\alpha'}{V_{MAX}}$

change in Vmax; change in Km

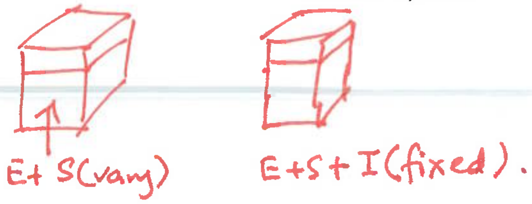
slope; y intercept; slope; y intercept.

change in slope gives α, as previously.

change in y-intercept give α'

Can get K_I & K_I' from changes in Km and Vmax

Obtaining K_I and K_I' for Mixed Inhibitors:



A. Data Collection.

1. Obtain v versus [S] in the *absence* of inhibitor.
2. Obtain v versus [S] in the *presence* of a *fixed* and *known* concentration of inhibitor.

B1. Direct Fitting to Data:

1. Predicted velocities: $v_{Pred} = \frac{\frac{V_{MAX}}{\alpha'}[S]}{\frac{\alpha}{\alpha'}K_M + [S]}$
2. Determine difference between observed and predicted:

   $\chi^2 = \sum |(v_{obs} - v_{pred})|_{I=0} + |(v_{obs} - v_{pred})|_{I>0}$
3. Use Solver to find best fit to data by varying K_M, V_{MAX}, α, and α'.
4. $K_I = \frac{[I]}{(\alpha - 1)}$ and $K_I' = \frac{[I]}{(\alpha' - 1)}$

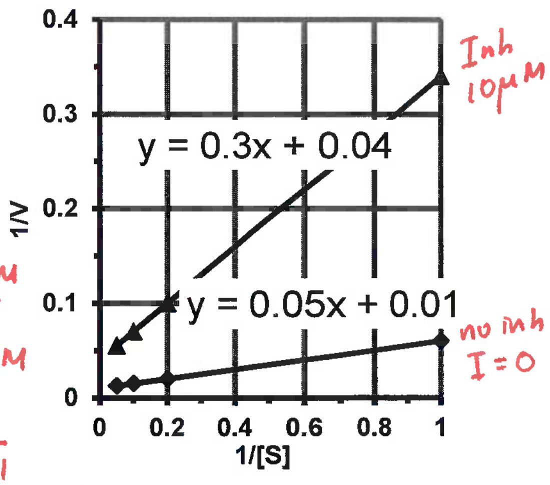
recitation.

B2. Double Reciprocal Plot:

1. Plot both data sets on a double reciprocal plot
2. **α = ratio of the slopes.**
3. **α' = ratio of Y-intercepts.**
4. $K_I = \frac{[I]}{(\alpha - 1)}$ and $K_I' = \frac{[I]}{(\alpha' - 1)}$

Example:

[S] mM	v ([I]=0)	v I=10μM	1/S (x)	1/V ([I]=0) (y)	1/V I=10μM
1	16.7	2.9	1.0	0.060	0.340
5	50.0	10.0	0.2	0.020	0.100
10	66.7	14.3	0.1	0.015	0.070
20	80.0	18.2	0.05	0.0125	0.055



K_I - Obtain α - ratio of slopes.

Slope ([I]=0): 0.05 Slope ([I]>0): 0.3

α =slope([I]>0)/slope([I]=0): 6 K_I: $\frac{10\mu M}{6-1} = 2\mu M$

K_I' Obtain α' – ratio of y-intercepts:

0.01 ← y-int([I]=0): ~~0.04~~ 0.01 y-int([I]>0): 0.04

α' = y-int([I]>0)/y-int([I]=0): $\frac{0.04}{0.01} = 4$ K'_I: $\frac{10}{4-1} = 3.3\mu M$

Use of double reciprocal plots to distinguish between inhibitor types.

i) Draw a line representing the un-inhibited reaction, label the intersection with the y-axis.

ii) Draw a line representing a competitive inhibitor. (Vmax unchanged, Km increased)

iii) Draw a line representing an allosteric inhibitor (Vmax & Km change)