Biological Chemistry Laboratory
Biology 3515/Chemistry 3515
Spring 2022

Lecture 16:

Analyzing Reversible Inhibition Data

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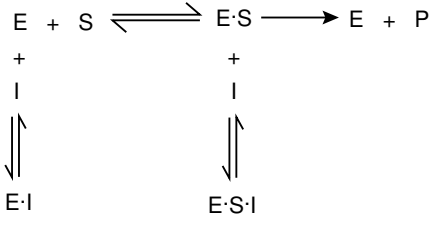
Significant Figures for this Class

- Rarely have precision greater than three significant figures.
- Can assume that concentrations of prepared solutions have precision of two significant figures.
- Spectrophotometric measurements can have precision of three significant figures.
- Reporting three rather than two significant figures isn't a major sin. (In this class!)
- Reporting more than three significant figures probably is!
- *Do* use extra decimal places for intermediate calculations, to avoid round-off errors.
- Use scientific notation when $|x| < 0.01$ or $|x| > 100$

Reversible Enzyme Inhibitors

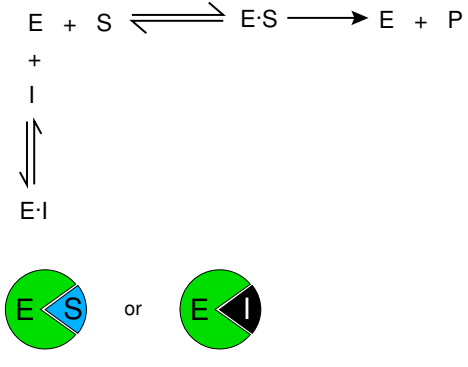
- Can bind to either free enzyme or enzyme-substrate complex



- Decrease the concentration of the enzyme-substrate complex.
- Classification of reversible inhibitors:
  - Competitive: Inhibitor binds only to free enzyme
  - Noncompetitive: Inhibitor binds equally well to E and $E \cdot S$
  - Uncompetitive: Inhibitor binds only to $E \cdot S$
  - Mixed inhibition: Inhibitor binds to E and $E \cdot S$, but with different affinities.
- Different classes have different kinetic properties.

Clicker Question #1:

How will a competitive inhibitor affect the Michaelis-Menten parameters?



A) Decrease V_{max}

B) Increase V_{max}

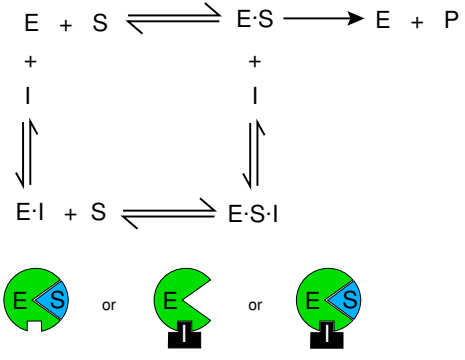
C) Decrease K_m

D) Increase K_m

E) Change V_{max} and K_m

Clicker Question #2:

How will a noncompetitive inhibitor affect the Michaelis-Menten parameters?



A) Decrease V_{max}

B) Increase V_{max}

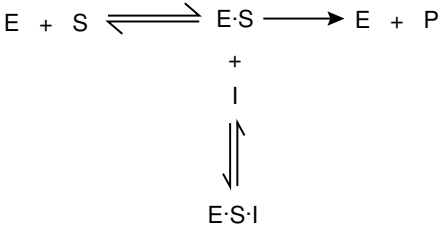
C) Decrease K_m

D) Increase K_m

E) Change V_{max} and K_m

Clicker Question #3:

How will an uncompetitive inhibitor affect the Michaelis-Menten parameters?



A) Decrease V_{max}

B) Increase V_{max}

C) Decrease K_m

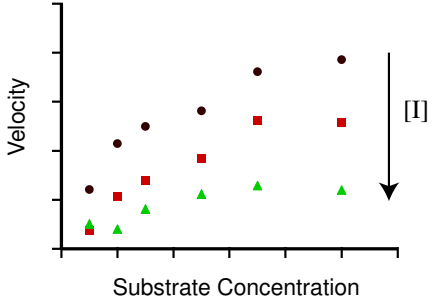
D) Increase K_m

E) Change V_{max} and K_m

Decreases both V_{max} and K_m

Strategy for Analyzing Reversible Inhibition

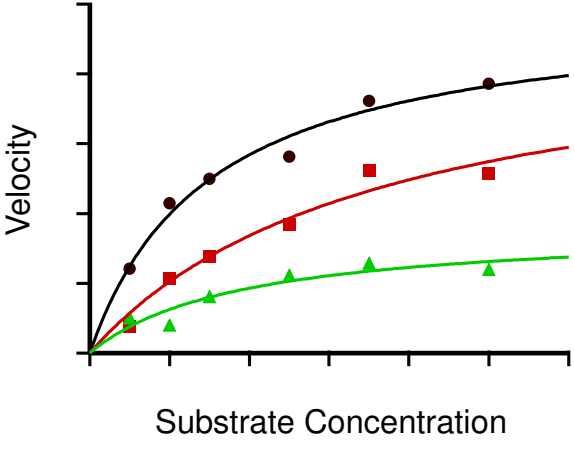
- Measure velocity versus [S] in the presence of different inhibitor concentrations



- Fit each data set to the Michaelis-Menten equation.
- Does the inhibitor change the apparent values of K_m, V_{max} or both?
- Experimental scatter can make it hard to tell!

Step 1

- Determine K'_m and V'_{max} from non-linear least squares fit to V vs [S] data



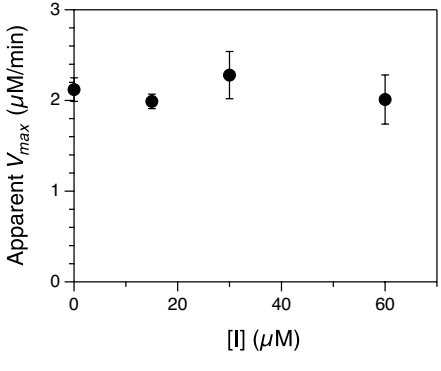
Step 2

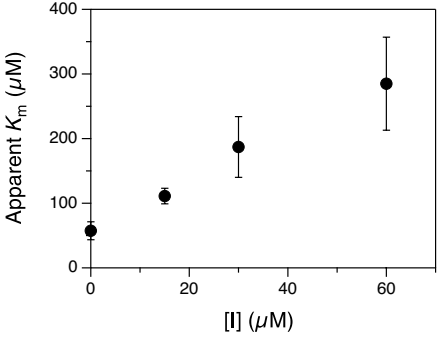
- Tabulate K'_m and V'_{max} data:

[I] (μM)	K'_m (μM)	V'_{max} (μM/min)
1.4		
16.4		
31.4		
61.4		

- Include small amount of benzamidine from the trypsin stock.
- Because of scatter in experimental data, patterns may not be obvious from inspection.

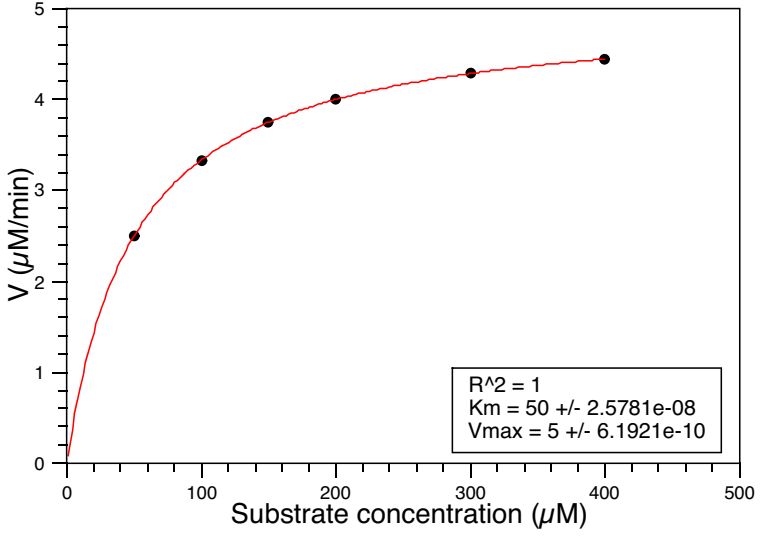
Step 3: Replot V'_{max} and K'_m versus Inhibitor Concentration



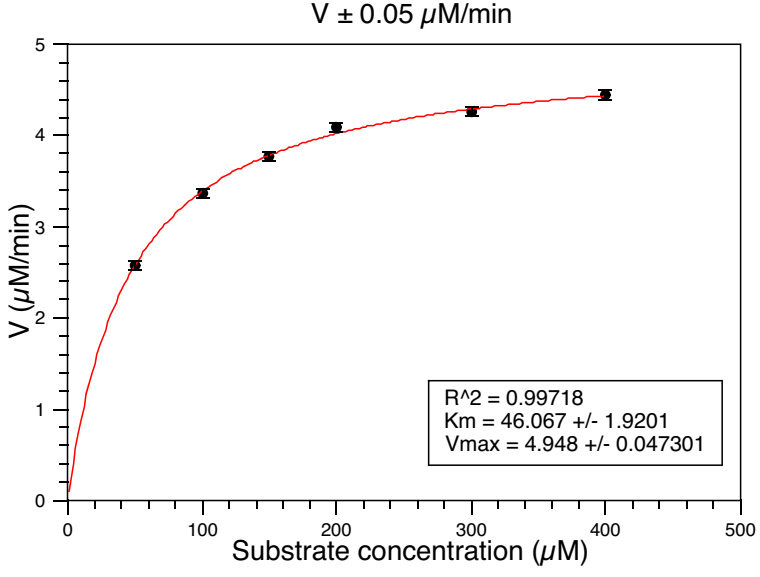


- Which parameter changes systematically?
- Error bars indicate uncertainty in the estimates of V'_{max} and K'_m.
- Error bars come from non-linear least-squares fit of V versus [S] data.
- Use uncertainties to weight the least-squares fits in the replots.

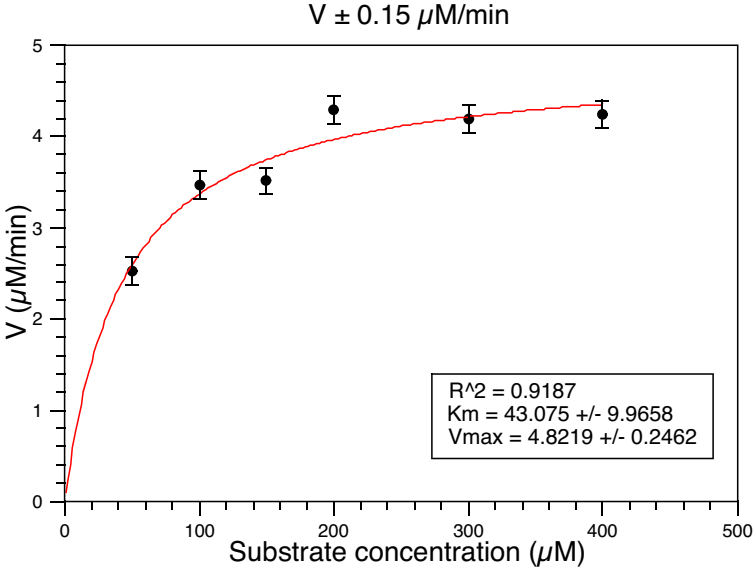
A Perfect Fit to Perfect Data



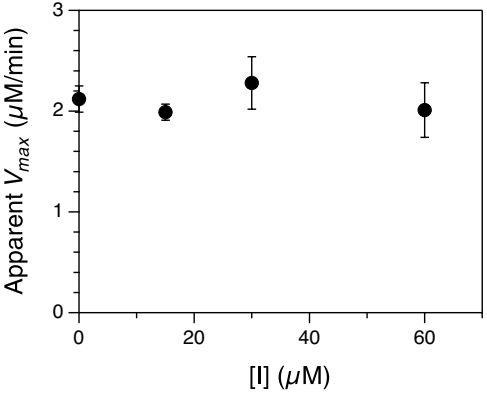
A Fit to Data with Simulated Errors



A Fit to Data with Simulated Errors

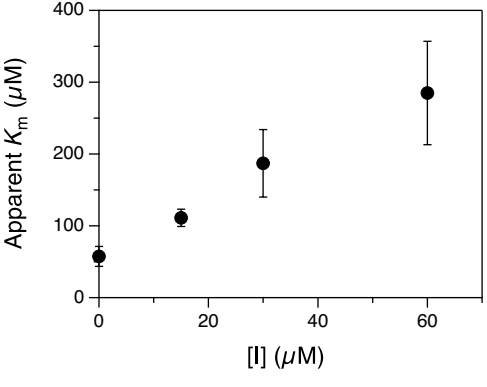


Replot #1: Apparent V_{max} versus Inhibitor Concentration



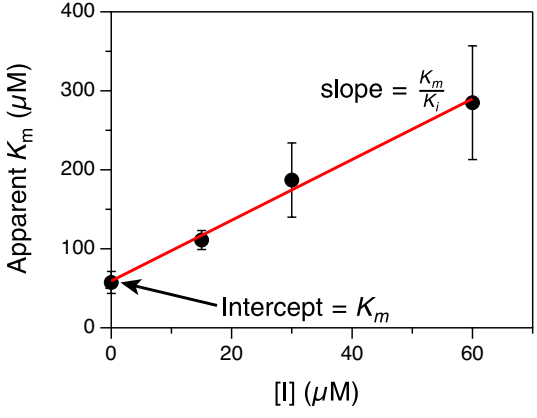
- Does V_{max} change consistently with the inhibitor concentration?
- Probably not in this case!

Replot #2: Apparent K_m versus Inhibitor Concentration



- Does K_m change consistently with the inhibitor concentration?
- Probably yes in this case!

For Competitive Inhibitor:



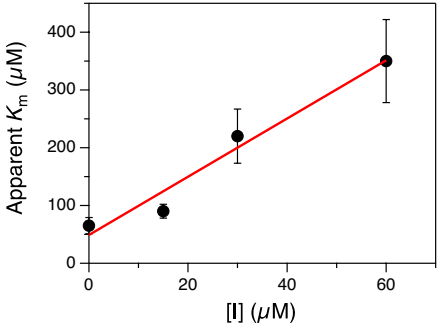
- Estimate K_m and K_i from fit of apparent K_m versus [I]

$$K'_m = K_m\,(1 + [\mathrm{I}]/K_i)$$
$$= \frac{K_m}{K_i}[\mathrm{I}] + K_m$$

- Use error estimates for K_m to weight data in fit.

Weighted vs. Un-weighted Least-Squares Fits

Data points weighted equally

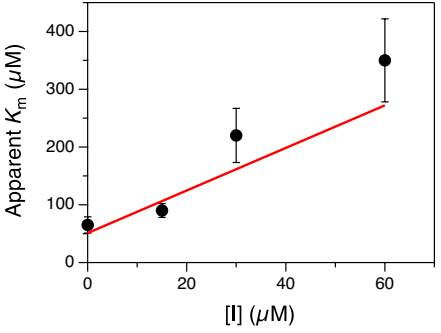


$R^2 = 0.96$

$K_m = 49 \pm 25\,\mu\text{M}$

$K_m/K_i = 5 \pm 0.7, \quad K_i = 10\,\mu\text{M}$

Data points weighted inversely by errors



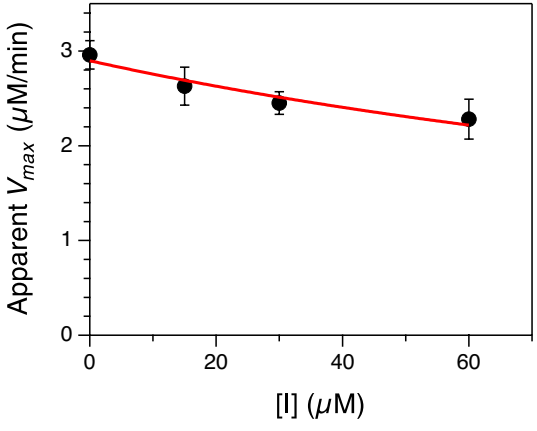
$R^2 = 0.47$

$K_m = 51 \pm 12\,\mu\text{M}$

$K_m/K_i = 3.7 \pm 0.9, \quad K_i = 14\,\mu\text{M}$

- Which fit gives the correct estimates for K_m and K_i?
- We don't know! But, the weighted fit is *more likely* to give better estimates.

If Apparent V_{max} Decreases with [I]



- For both noncompetitive and uncompetitive inhibition

$$V'_{max} = V_{max} / (1 + [I]/K_i)$$

- Is this real? Does K'_m:
  - Stay constant as [I] increases? (noncompetitive inhibitor)
  - Decrease as [I] increases? (uncompetitive inhibitor)
- If it looks real:
  - Estimate V_{max} and K_i from non-linear least-squares fit.

    Use error estimates for V_{max} to weight data in fit.

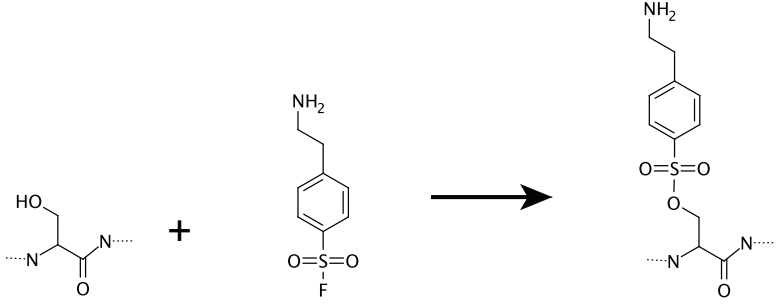
Warning!

Direction Change

Irreversible Enzyme Inhibitors

Irreversible Inhibition of Trypsin by AEBSF

4-(2-aminoethyl)-benzenesulfonyl fluoride

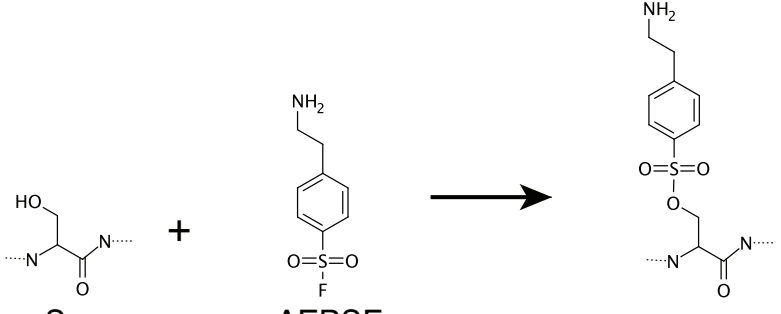


-Ser- AEBSF

- Reaction is specific for the catalytic Ser residue.
- Reaction is irreversible.

Clicker Question #4

Is this reagent likely to be good for you?



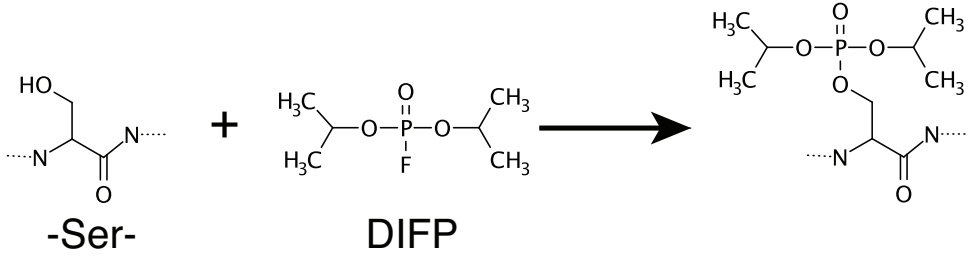
-Ser- AEBSF

A) Yes

B) No

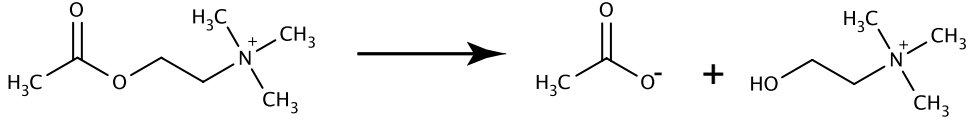
- But, it's not identified as a chemical hazard by the authorities.

An Earlier Irreversible Inhibitor of Serine Proteases: Diisopropyl Fluorophosphate



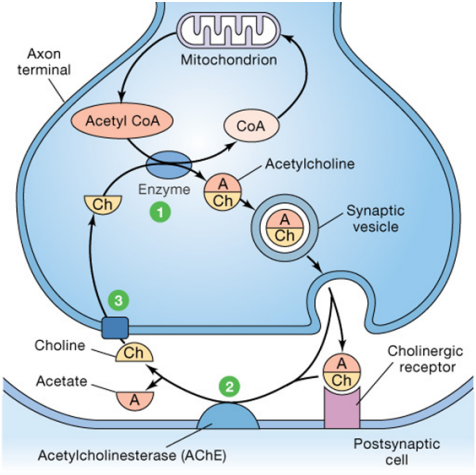
- First synthesized in the 1930's as a potential chemical weapon with neurotoxic effects.
- Found to inhibit esterases and proteases.
- Used as pesticide.
- Reported to be among chemical agents found in Syria in 2013.

Acetylcholine Esterase



- Acetylcholine is a major neurotransmitter in vertebrates, insects and other animals.
- Esterase reaction is very similar to peptide hydrolysis.
- Enzyme uses a catalytic triad (Ser-His-Glu).
- Enzyme is inhibited by DIFP and other serine-reactive agents.

A Cholinergic Synapse



- Irreversible inhibition of acetylcholine esterase is lethal.
- Mild reversible inhibition may be therapeutic.
- AEBSF is **much** less toxic than DIFP.

Figure from http://peaknootropics.com/acetylcholinesterase-memory-problems/