Lecture 18: Steady State Kinetics

Assigned reading in Campbell: Chapter 6.6

18.1 Review of Steady State Enzyme Kinetics:

$$v = k_{cat}[E_T]\frac{[S]}{\frac{k_{-1}+k_{cat}}{k_1}+[S]} = V_{MAX}\frac{[S]}{K_m+[S]}$$

$V_{max} = k_{cat}$ [E_T] is the highest rate of product production possible. It is obtained at high substrate levels ([S]>>K_m). Under these conditions, *all* of the enzyme is in the [ES] form (i.e. [ES] = [E_T]).

$K_m = (k_{-1}+k_{cat})/k_1$. This is *almost* the same as the K_D (= K_{-1}/K_1). Therefore it is related to the affinity or strength of binding of a substrate to the enzyme. K_m is equal to the substrate concentration that gives 1/2 of the maximal velocity.

Turnover number, $k_{cat} = V_{max}$ / [E_T]. This is the number of reactions performed by a single enzyme molecule in a certain period of time *when the enzyme is saturated with substrate*.

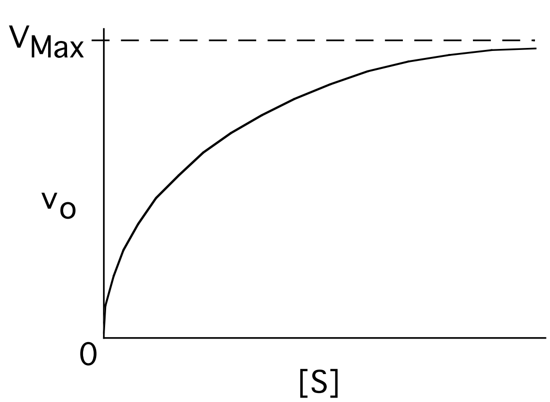
18.2 Throughput, or Efficiency, of Enzyme Systems:

High Substrate: At high substrate concentrations ([S]>>K_m) the rate becomes independent of [S] since all of the enzyme molecules are saturated with [S]. Therefore the intrinsic efficiency of an enzyme is given by k_{cat}. The overall rate depends only on k_{cat} and the amount of enzyme [E_T].

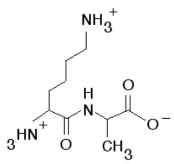
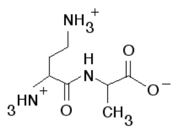
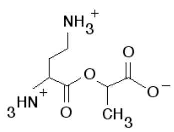
$$v = k_{cat}[E_T]$$

Low Substrate: At low substrate concentrations ([S]<<K_m) the efficiency of an enzyme will depend both on how efficiently it can bind substrate as well as how well it can perform the chemical step (K_{cat}). In other words, the intrinsic efficiency of an enzyme at low substrate levels is given by k_{cat}/K_m. The overall rate depends on the total amount of enzyme [E_T] as well as [S]:

$$v = \frac{k_{cat}}{K_m}[E_T][S]$$



18.3 Interpretation of K_m and K_{cat}: The following three substrates were presented to trypsin. Explain the differences in K_m of A versus B. Explain the differences in K_{cat} between B versus C.

Substrate	K_m	K_{cat}
A 	10 μM	1000 s^{-1}
B 	100 μM	1000 s^{-1}
C 	100 μM	100 s^{-1}

An example of how a change in K_m and hence the catalytic efficiency of an enzyme can cause disease:

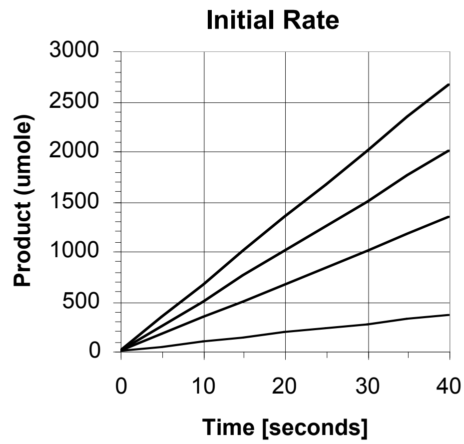
Sulfite Oxidase defiency is a rare genetic disorder that results from a single amino acid substitution in the enzyme sulfite oxidase. Silfite oxidase catalyzes the last step in the degradation of sulfur-containing amino acids: $SO_3^{-2} \rightarrow SO_4^{-2}$. The inability to catalyze this step causes severe neurological abnormalities and sometimes death.

Enzyme	$K_m^{sulfite}$ (μM)	K_{cat} (sec-1)	K_{cat}/K_m (10^6 $M^{-1}sec^{-1}$)
Wild type	17	18	1.1
R160Q	1900	3	0.0016

18.4 Measuring Km and Kcat (Vmax):

4. Measuring K_M and k_{CAT} (V_{MAX}):

Step 1: Measure the *initial* velocity at different substrate concentrations, keeping the enzyme concentration *constant*.



Step 2: Analyze data

A: [S] not limiting - Velocity Curve: $v = \frac{V_{MAX}[S]}{K_M + [S]}$

i) Plot *v* versus [S].
ii) Obtain V_{MAX} from v at very high [S].
iii) K_M is the substrate concentration at gives $v=V_{MAX}/2$

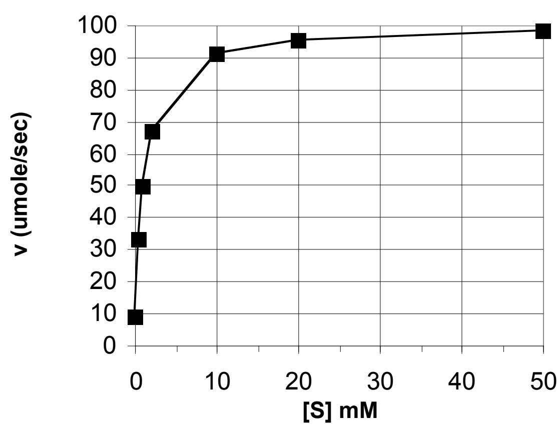
B: [S] Limiting - Double reciprocal plot:

i) Plot 1/v versus 1/[S]
ii) Slope = K_M/V_{MAX}
iii) y-Intercept = $1/V_{MAX}$
iv) x-intercept = $1/K_M$

Example Data:

The following velocity data was obtained for a number of substrate concentrations ($[E]_{Tot}$=2 nM).

Exp. #	[S] (mM)	v (umoles/sec)
1	0.1	9.0
2	0.5	33.4
3	1.0	50.0
4	2.0	66.6
5	10.0	91.1
6	20.0	95.2
7	50.0	99.0



ii) What is V_{MAX}?

i) What is K_M for this substrate?

iii) What is k_{CAT} for this substrate (E_T=2 nM):

$$v = \frac{V_{MAX}[S]}{K_M + [S]}$$

$$\frac{1}{v} = \frac{K_M + [S]}{V_{MAX}[S]}$$

$$\frac{1}{v} = \frac{K_M}{V_{MAX}}\frac{1}{[S]} + \frac{1}{V_{MAX}}$$