**Quantitative Physiology I / Molecular and Cellular Systems, BMEN E4001x**

**Notes 02 - Enzyme kinetics**

**Chapter 1 of Keener & Sneyd**

# Life is based on regulation of processes (see B&B Chapt. 58)

Glycolysis is a key step in carbohydrate metabolism. This is the breakdown of glucose into pyruvate, which goes into the citric acid cycle, yielding two energy stores, ATP and NADH. Here are the first few steps, for which enzyme regulation plays a key role.

This process takes some ATP, converting it to the lower energy ADP, but ATP is replenished later in metabolism. In this scheme, consider phosphofructokinase (PFK1)

* adds a second phosphate group (from ATP) to fructose, leading later to splitting of this molecule
* ATP is a substrate for this enzyme
* ATP is also an inhibitor of PFK1, leading to feedback regulation of this enzyme and metabolism

# Enzyme Kinetics

In an enzymatic relation, something happens after the binding. So, our equations need to include this conversion. However, measured kinetics show a slightly different kinetics than would be alluded to using our bimolecular model.

Note that for the initial A+B->C reaction, the initial rate reaction, assuming [C](t=0)=0 is:



In contrast, enzymatic systems often show saturation.

A simple mechanism to express simple enzyme kinetics, Michaelis Menten (1913):



This system allows regeneration of E.

It is also recognized that P+E-> C can conceptually happen, but it is assumed here that P gets removed immediately.



## Equilibrium Approximation

In this *original* form, Michaelis and Menten assumed fast equilibrium between the S,E and C. Thus:

k1se=k-1c

and by using e+c=e0, with a similar chain of algebra as for biomolecular binding.

k1s(e0-c)=k-1c

se0-sc=(k-1/k1)c

c[(k-1/k1)+s]=se0

c=e0\*s/((k-1/k1)+s)

At the end, they got:



Quite reasonable, when it is recognized that this is the same form as bimolecular binding. Put another way, this is bimolecular binding leading to a slow evolution of C into P + E. As the next step is



we can say that the rate of reaction is proportional to [C], or



## Quasi-steady-state approximation

Briggs and Haldane (1925) proposed a refinement, one in that the rate constants associated with production and use of intermediary species are equal. That is, for an intermediary species such as [C], d[C]/dt is small during the bulk of the reaction following an initial, high excursion period.

Formally, make the following non-dimensionalization substitutions:



In non-dimensionalization, these values are plugged back into the original system of equations. Here, the important one is to look at the dc/dt equation.



Now, enzymes are very effective, so a little acts on a lot of enzyme with high efficiency. Thus, ε is typically small. Thus, dχ/d is very responsive to changes in the other variables, adjusting to them. Moreover, this acts to restore χ towards some equilibrium value. So, we can approximate dχ/d ~ 0.

Working this through dc/dt=0 leads to:



Equations of these forms are generally referred to as Michaelis-Menton forms; the switch from KS to KM is reflective of this change.

# Regulation of Enzyme Kinetics

## Competitive Inhibition



with system of equations



Use the quasi-steady state approximation for C1 and C2

C1:

C2:



plug expression for C2 back into eqn. for C1



Multiply by (1+Ki/i), change signs



expand, group c1’s on left, e0’s on right



canceling out symbols in bold, solving for c1



rearrange into



This gives us



## Non-competitive Allosteric Inhibition

Allosteric site binds to E or ES, preventing conversion of ES to P+E



Following as Keener & Sneyd work through the equilibrium case, which was derived from rapid equilibrium over the four double-headed arrows, with the convention of e=e0-x-y-z, where

X=ES Y=EI Z=EIS

1. 
2. 

This is a system of four equilibria. Note that by going around a box and using transitivity, one of these equations is not needed.

The full derivation is included below. Let’s skip this for class, and jump to the result, indicated a few lines below.

Solve eqn 1 for x



Use eqn 3 (y=zk-1/(k1s))



Use eqn 4 (z=xik3/k­3)



Mult. both sides by k-3/k3 and also divide both sides by k1



Now, using

Ki=k-3­/k3 and Ks=k-1/k1

we get



rewrite as



**AND FINALLY**

; Ki=k-3­/k3 and Ks=k-1/k1

and in terms of rates



## Uncompetitive Allosteric Inhibition

Allosteric site binds to ES (but not E), preventing conversion of ES to P+E



X=ES Y=EIS

Equilibrium approach, system equations become:

1. 
2. 
3. 

Rearrange 2 into



Combine equations 1 & 3



Substitute for y and rearrange



divide everything by k1, then rearrange



And thus, the reaction rate V = k2x=

; Ki=k-3­/k3 and Ks=k-1/k1

# Comparing inhibitor behavior:

These were generated with [I]/KI = 1, Ks=10. Things to look at: maximum velocity, which is approached for each situation, and will be either 1 or 0.5. Also substrate concentration at which velocity is half of the value approached in that situation. Compare against forms presented above, and justify.

## Lineweaver-Burk plots

Okay, given a set of reaction data, how do we get rate constants? Sounds simple to find the substrate concentration that gives maximum reaction velocity. However, that velocity is never reached.

Take the reciprocal of both sides:

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becomes

Chart, box and whisker chart

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From this Lineweaver-Burk plot, we can get Vmax and KM from the slope and intercept.

A similar approach can be used to measure Keqfor the biomolecular binding systems discussed earlier.

However, note that measurements at small [S] and V are prone to large errors and have a large input on these curves. One can go through sensitivity analysis of this system, but it’s not pretty. In my opinion, using contemporary curve fitting software provides much more reliable results.

This brings the fair point of why to study this traditional treatment at all. Why not use programs for solving differential equations? Here are two good reasons:

* The concept of KM is very useful for comparing and characterizing reactions
* The statements about the wide range of rate constants are true, and pose difficult challenges in numerical solutions; uniting timescales that vary over several orders of magnitude is tricky

Chart, diagram

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