

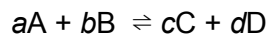
Key biochemistry equations

Here's an overview of what I feel are the key biochemistry equations to know (including thermodynamics, Henderson-Hasselbalch, and Michaelis-Menten). I recommend trying to get to the point where you don't need an equation sheet (or Google) for these, those should just be a failsafe in case you blank. I think it's more important to know the concepts the equations encompass, be able to see where these concepts come from, be able to interpret the equations' implications, and know how to use the equations as tools, than having memorized something you can easily Google. But of course, you will need to memorize them if your class requires it!

Bioenergetics and thermodynamics

Generic formula for an equilibrium constant (K_{eq} , or simply K) - this relates the concentrations of products & reactants at equilibrium

For the generic reaction



$$K_{eq} = \frac{[C]^c [D]^d}{[A]^a [B]^b}$$

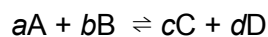
Where those are the concentrations at equilibrium

Why it's important to know

- Forms of it will show up everywhere! (K_a , K_d , etc.)
- It will allow you to recognize that the equilibrium constant will be >1 if products are thermodynamically favored, <1 if reactants are favored
- You'll need it for the Gibbs calculations

Generic formula for a mass-action ratio, aka reaction quotient (Q) - this relates the actual concentrations of products & reactants

For the generic reaction



$$Q = \frac{[C]^c [D]^d}{[A]^a [B]^b}$$

Sometimes this is abbreviated as $[\text{products}]/[\text{reactants}]$, but remember you need to take stoichiometry into account and multiply, not add, if you have multiple reactants and/or products

Why it's important: You'll need this for the Gibbs free energy calculations

Gibbs free energy change

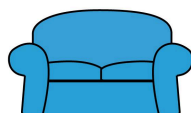
- if ΔG is positive, the reaction is thermodynamically unfavorable
- if ΔG is negative, the reaction is thermodynamically favorable
- if ΔG is 0, the reaction is at equilibrium

$$\Delta G = \Delta H - T\Delta S$$

Why it's important to know

- It will allow you to see how entropy, enthalpy, and temperature contribute to whether a reaction is thermodynamically favorable

How comfy is your couch?



how much room do you
have to spread out?
how cushy is it? do you
have to squirm a lot?

entropy, S

enthalpy, H

Is that one better?

depends on my mood... how hot is it? **temperature, T**
if it's hot I care more about spreading out

Is it worth moving? **is ΔG negative?**

Are you too lazy? do you have the activation
energy you need?

will the
reaction occur
spontaneously? **ΔG** = **ΔH** - **$T\Delta S$**
change in free energy change in enthalpy change in entropy

will it give
off heat? **temperature** do the products
have more
freedom than the
reactants?

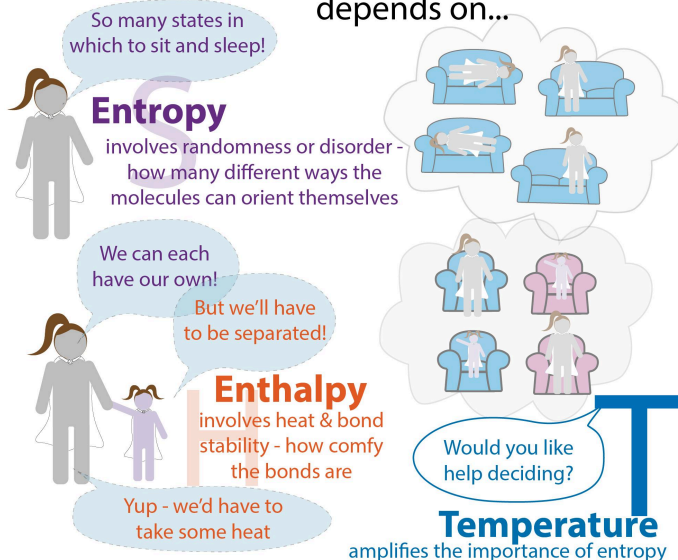
NEGATIVE ΔG : exergonic
(releases energy); **spontaneous**

NEGATIVE ΔH : exothermic
(releases heat)

POSITIVE ΔG : endergonic (takes
energy); **non-spontaneous**

POSITIVE ΔH : endothermic
(takes in heat)

**difference in
Gibb's free energy $\Delta G = \Delta H - T\Delta S$**
change in molecular happiness, & thus reaction favorability
depends on...



Standard free energy change

Technical note: In biochemistry, we often use the terms ΔG° & $\Delta G'^\circ$ (also can be written as $\Delta G''^\circ$) interchangeably, but there is a formal distinction (so apologies in advance if I slip up).

- **ΔG°** = standard free-energy change (physical constant of a reaction) - under chemistry definitions of standard state
 - the standard state in chemistry is defined as: all reactants and products at 1M concentrations (1 atm for gasses) & temperature = 298 K (25°C)
- **$\Delta G'^\circ$** = standard transformed free-energy change (physical constant of a reaction) - under biochemistry definitions of standard state
 - the standard state in biochemistry is defined as: pH 7.0 ($[H^+] = 10^{-7}$ M); $[H_2O]$ is 55.5 M; $[Mg^{2+}]$ is 1mM
 - Why? Biochemical reactions don't take place under chemistry-defined standard conditions, where pH = 0!!! Our reactions take place in pH-buffered aqueous solutions where pH is closer to 7 and water is everywhere! But even the biochemistry-defined "standard conditions" are much different from reality, hence, you will have to account for this in your calculations of ΔG as defined below
- **ΔG** = *actual* free energy change under the given conditions (a very important concept in biochemistry is that the concentrations of products and reactants (which can be controlled) greatly influences free energy change and thus favorability!)

$$\Delta G'^\circ = -RT \ln K'_{eq}$$

Why it's important to know

- It will help you recognize that if products are favored at equilibrium, $\Delta G'^\circ$ will be negative. And the more the products are favored (larger the K'_{eq}), the more negative it will be
- You'll need it below

Actual free energy change

$$\Delta G = \Delta G'^\circ + RT \ln Q$$

This is why I want you to know Q!

Sometimes this is written as

$$\Delta G = \Delta G'^\circ + RT \ln \left(\frac{[\text{products}]}{[\text{reactants}]} \right)$$

but remember the warning I gave when I introduced Q

If you know these, you can now rewrite this formula in various ways, including:

$$\Delta G = -RT \ln K'_{eq} + RT \ln Q$$

$$\Delta G = -RT (\ln K'_{eq} - \ln Q)$$

$$\Delta G = -RT \left(\ln \left(\frac{K'_{eq}}{Q} \right) \right)$$

Now, you can hopefully see that:

- If $Q > K'_{eq}$, ΔG will be positive $\rightarrow$ reaction will be driven towards reactants to reach equilibrium
- If $Q < K'_{eq}$, ΔG will be negative $\rightarrow$ reaction will be driven towards products to reach equilibrium
- If $Q = K'_{eq}$, ΔG will be 0 $\rightarrow$ reaction is at equilibrium $\rightarrow$ no net change in concentrations of products or reactants

Acids, bases, etc.

$$\text{pH} = \log \frac{1}{[\text{H}^+]} = -\log[\text{H}^+]$$

Recognize that if $[\text{H}^+]$ is high, pH is low and vice versa

$$\text{pK}_a = \log \frac{1}{[\text{K}_a]} = -\log \text{K}_a$$

Why this is important to know

- It lets you recognize that if K_a is high (deprotonation is favored), pK_a is low and vice versa -> the stronger the acid, the higher the K_a , and thus the lower the pK_a
- You will need it for the Henderson-Hasselbalch equation, which will allow you to do things like figure out what proportion of an acid will be protonated or deprotonated at a given pH, which will be super handy

Note: K_a is just the equilibrium constant for an acid, sometimes called the acid dissociation constant

Henderson-Hasselbalch equation

$$\text{pH} = \text{pK}_a + \log \frac{[\text{A}^-]}{[\text{HA}]}$$

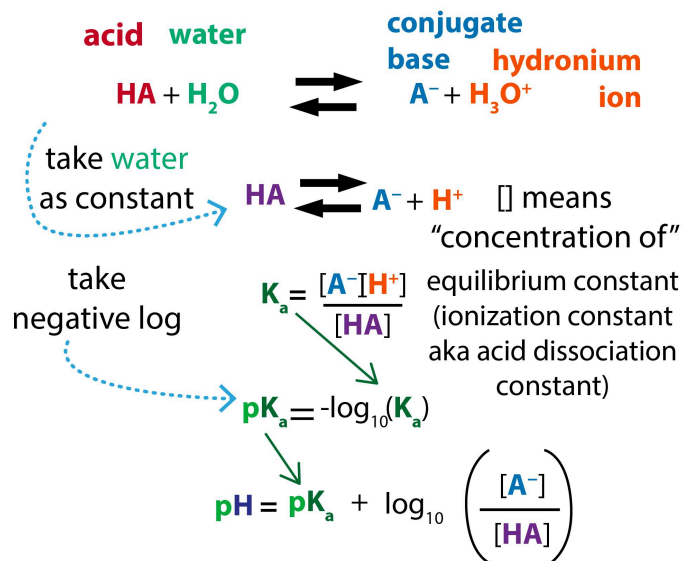
Why this is important to know

It allows you to go between pH and pK_a , doing things like:

- figuring out the pH of a solution given concentrations of acid and base
- figuring out how much of an acid is deprotonated at a given pH (super important for when thinking about what protonation state amino acids have &, therefore, how they might react!)

Henderson-Hasselbalch equation

gives a way to go back and forth between pH & pK_a showing you how a buffer can decide where the pH will stay!



Enzyme kinetics

Michaelis-Menten equation

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

Why this is important to know

It lets you see that...

- If [S] is very low compared to K_m , the [S] in the denominator becomes insignificant, so this simplifies to $v = V_{max}[S]/K_m$
- If [S] is very high compared to K_m , K_m becomes insignificant, so this simplifies to $v = V_{max}$
- When $v = \frac{1}{2} V_{max}$, $K_m = [S]$
- Since K_m is on the bottom, the higher K_m is, the lower v is and vice versa

It will allow you to do calculations to find rates of enzymatic reactions

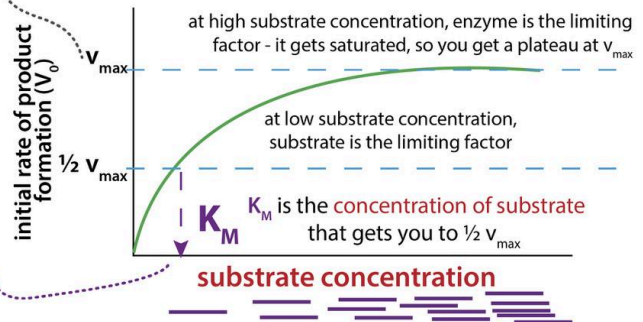
Note: the "v" we're talking about here is technically " v_o ", the initial velocity (before the enzyme runs out of substrate, etc.)

Michaelis-Menten equation on a graph

$v = \frac{V_{max}[S]}{K_m + [S]}$ Plot V_o 's found from the linear, steady state, parts of product vs. time graphs with different substrate concentrations

V_{max} is the the fastest that a *lot of copies* of an enzyme, working together can work - it *does* depend on enzyme concentration.

if you want to know how fast *one copy* of an enzyme can work, divide V_{max} by enzyme concentration to get k_{cat}



K_M tells you about the *affinity of the enzyme for the substrate* - and does NOT depend on enzyme concentration

Derivations at end

$$k_{cat} = V_{max}/[E_t]$$

k_{cat} is aka the turnover number and it corresponds to how many substrate molecules a single copy of an enzyme can convert to product in some given unit of time if it is saturated with substrate; it's the rate constant for the slowest step

So you can rewrite the MM equation as:

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

See that the faster the enzyme can work (higher the k_{cat}), the faster the reaction will be at any given total enzyme concentration $[E_t]$ & $[S]$

See that a "better" enzyme would have a high k_{cat} and a low K_m - BUT this doesn't tell you anything about specificity, ability to regulate it etc. which are often what makes a really great enzyme in vivo!

Derivations

getting to the

Michaelis-Menten equation

I couldn't deprive you of the derivation!

We start off with an **Enzyme** & a **Substrate**.

They can hang out on their own or together depending on how much they like each other & how much of each there is



The more they like to be together (*higher affinity*) the lower the K_M

and this changing occurs at a rate given by k_{cat} the better it is at turning substrate into product, the higher the k_{cat}

We can represent how much of each thing there is by sticking them in brackets to indicate "concentration of"

in Michaelis-Menten kinetics we use the **steady state assumption**, which assumes that substrate binding to the enzyme occurs at the same rate as it either unbinds or gets converted into product, so



k_{on} has units of $\text{concentration}^{-1}\text{time}^{-1}$

k_{off} has units of time^{-1}

k_{cat} has units of time^{-1}

$k_{on} = k_{off} + k_{cat}$ the rates are the same, but not the concentrations, so you could have



$$k_{on}[E_T][S] - k_{on}[ES][S] = (k_{off} + k_{cat})[ES]$$

shift things around to put the ES's on the same side

$$k_{on}[E_T][S] = (k_{off} + k_{cat})[ES] + k_{on}[ES][S]$$

then put them together

$$k_{on}[E_T][S] = [ES]((k_{off} + k_{cat}) + k_{on}[S])$$

then get them by themselves

$$\frac{k_{on}[E_T][S]}{((k_{off} + k_{cat}) + k_{on}[S])} = [ES]$$

now just change what's on what side of the = sign

$$[ES] = \frac{k_{on}[E_T][S]}{((k_{off} + k_{cat}) + k_{on}[S])}$$

separate out the S

$$[ES] = \frac{[E_T][S]}{\left(\frac{(k_{off} + k_{cat})}{k_{on}}\right) + [S]}$$

this thing is awkward, so let's just "lump it" and call it K_M

we get this, which looks nicer

$$[ES] = \frac{[E_T][S]}{K_M + [S]}$$

so if we define $K_M = \frac{(k_{off} + k_{cat})}{k_{on}}$

and we now have that $[ES]$ we need for our velocity equation

$$V = \frac{V_{max}[S]}{K_M + [S]}$$

rearrange for the **steady state assumption** that $[ES]$ is constant

mathematically, we can write this as

$$\text{change in } [ES] \text{ over time: } k_{on}[E][S] - (k_{off} + k_{cat})[ES] = 0$$

so rate of ES formation = rate of ES breakdown

$$\text{substrate binds } k_{on}[E][S] = (k_{off} + k_{cat})[ES] \quad \text{substrate falls off or gets converted into product}$$

what we're trying to find is the rate of product formation, and that requires change in $[ES]$ so we want to isolate that value

$$k_{on}[E][S] = (k_{off} + k_{cat})[ES]$$

we know the **total amount of E** we put in $[E_T]$, and we know that any E that's not bound to substrate has to be free, so

$$[E_T] = [ES] + [E]$$

rearrange

$$[E] = [E_T] - [ES]$$

and exchange

$$k_{on}([E_T] - [ES])[S] = (k_{off} + k_{cat})[ES]$$

$$\text{then } k_{on}([E_T][S] - [ES][S]) = (k_{off} + k_{cat})[ES]$$

factor it out

$$k_{on}[E_T][S] - k_{on}[ES][S] = (k_{off} + k_{cat})[ES]$$

change in $[P]$ over time: & we now know

put them together

$$V = k_{cat}[ES] \quad [ES] = \frac{[E_T][S]}{K_M + [S]} \quad V = k_{cat} \frac{[E_T][S]}{K_M + [S]}$$

this works for V at any $[S]$

something special happens when you have a **lot of S**

the maximum velocity (V_{max}) occurs when you have so much **substrate** that the **enzyme** is saturated (all the enzyme's bound), so $E_T = ES$, so you don't have to worry about all that other stuff

so we often measure kinetics with a great excess of substrate so we can do this...

$$V_{max} = k_{cat}[E_T]$$

rearrange...

$$[E_T] = \frac{V_{max}}{k_{cat}}$$

if we substitute E_T for ES , we can now define the velocity at any substrate concentration in terms of V_{max}

exchange...

$$V = k_{cat} \frac{[E_T][S]}{K_M + [S]} \quad \text{math it out... } V = k_{cat} \frac{\frac{V_{max}}{k_{cat}}[S]}{K_M + [S]} \quad V = \frac{V_{max}[S]}{K_M + [S]}$$

this is the Michaelis-Menten equation as you'll commonly see it

Michaelis-Menten equation

$$V = \frac{v_{\max} [S]}{K_M + [S]}$$

what happens at $\frac{1}{2} V_{\max}$?

these cancel out...

$$\frac{1}{2} V_{\max} = \frac{v_{\max} [S]}{K_M + [S]}$$

$$\frac{1}{2} V_{\max} = \frac{v_{\max} [S]}{K_M + [S]}$$

then just math it out

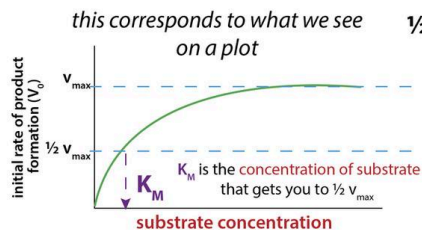
$$\frac{1}{2} = \frac{[S]}{K_M + [S]}$$

$$\frac{1}{2} K_M + \frac{1}{2} [S] = [S]$$

$$\frac{1}{2} K_M = \frac{1}{2} [S]$$

$$K_M = [S]$$

tada!



rate constants (e.g. k_{cat} , k_{on} , k_{off}) won't change depending on concentration

but the **rates** at which the concentrations of the various players are formed **will change** because those rates **depend on the concentrations**

usually we measure rate of product formation (increase in **P** over time), but how much product you can make depends on how much **ES** you make and how well that gets converted to **E + P**

rate of product formation

$$k_{\text{cat}} [\text{ES}]$$

rate of ES formation
only 1 way to form it

$$k_{\text{on}} [\text{E}] [\text{S}]$$

rate of ES breakdown
but 2 ways to "lose" it

$$(k_{\text{off}} + k_{\text{cat}}) [\text{ES}]$$

just let go turn it into product

$$\text{change in } [S] \text{ over time: } -k_{\text{on}} [\text{E}] [\text{S}] + k_{\text{off}} [\text{ES}]$$

$$\text{change in } [E] \text{ over time: } -k_{\text{on}} [\text{E}] [\text{S}] + (k_{\text{off}} + k_{\text{cat}}) [\text{ES}]$$

$$\text{change in } [\text{ES}] \text{ over time: } k_{\text{on}} [\text{E}] [\text{S}] - (k_{\text{off}} + k_{\text{cat}}) [\text{ES}]$$

$$\text{change in } [P] \text{ over time: } k_{\text{cat}} [\text{ES}]$$

How do we measure it experimentally?

What's happening to our stick-breaker & sticks when we measure kinetics?

you normally can only measure the **product formation (green line)**, but here's what's happening to everything

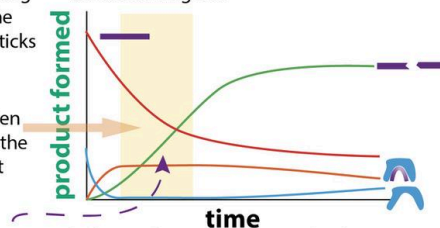
Throughout the process, the total enzyme concentration stays the same, but it can either be **bound to substrate (orange line)**, or **free (blue line)**

in the very very beginning, you're at "**pre-steady state**" where the enzyme grabs up the substrate - there's plenty to go around but there's not much breaking going on because the enzymes have to find sticks to break

you quickly reach the **steady state**, where enzymes are doing grab-break-grab-break without having to worry about running out

and then you enter **post-steady state** when you run out of sticks to break

for Michaelis-Menten kinetics, we look to the **steady state part**



the slope of this linear part before it plateaus is the **initial velocity (V_0)**

the plateau on this graph is NOT the plateau we use to find $V_{\max}$ - that's the next graph - first we have to find V_0 for a range of substrate concentrations - then we plot those & that'll give us another plateau & that will be our $V_{\max}$. $V_{\max}$ corresponds to running out of enzyme, this plateau's from running out of substrate



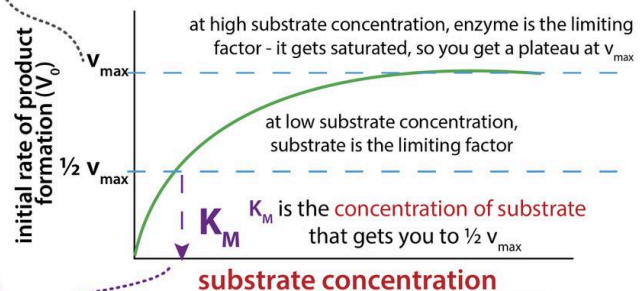
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