

## Week 12

# Experimental Kinetics

### 12.1 Kinetics of Catalytic Reactions

11/19:

- Last week's lectures.
  - Very simple kinetic scenarios.
  - Linearizations allows us to extract essential kinetic parameters for reactions.
  - Kinetic descriptions for multistep processes.
  - Steady-state approximation, quasi-equilibrium approximation.
- Today: Kinetics of Catalytic Reactions.
- Catalysts speed up the rate of reaction without altering the thermodynamics.
- Consider the following balanced chemical reaction.



- Since the catalyst appears on both sides of the reaction, we typically write it over the arrow.
- This notational simplification alides a great deal of multistep complexity.
- Consider a hypothetical potential energy surface.

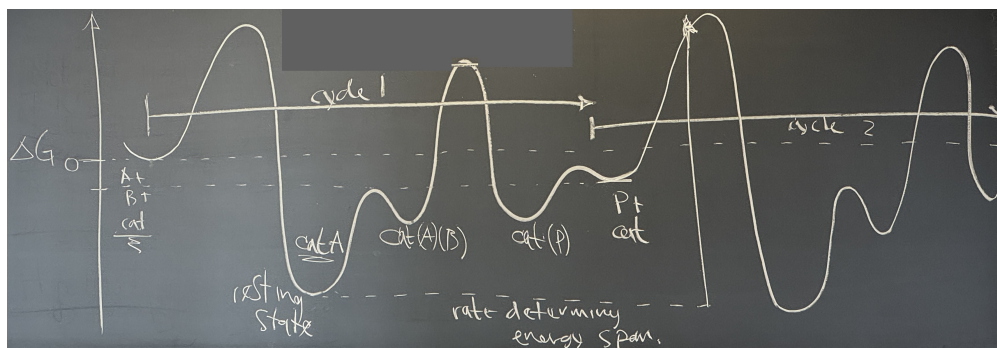


Figure 12.1: Model catalytic potential energy surface.

- Define a reference energy as zero; suppose the starting materials ( $A + B + \text{cat}$ ) begin here.
- Suppose, then, that they proceed through a multistep potential energy surface along our reaction coordinate.

- If this were a stoichiometric reaction, the first step would be rate-determining (highest energy barrier), and the first intermediate would be the product (lowest energy species).
  - But we're catalytic, so we have to consider cycles 2, 3, ...
  - These cycles are driven forward by the ever-so-slight difference in energy  $\Delta G$  between starting materials and products.
- The “first intermediate” is actually the catalyst **resting state**.
  - Indeed, the thing that we throw in may not be the dominant species in solution!
  - It could be  $\text{cat} \cdot (\text{A})$ ,  $\text{cat} \cdot (\text{A})(\text{B})$ , or  $\text{cat} \cdot (\text{P})$ !
- Similarly, the difference in energy between the lowest valley and highest peak is the **rate-determining energy span**.
- Reference (good description of rate-determining energy span): Kozuch and Martin (2011).
- Because continuous potential energy surfaces are not great representations, we typically view catalysts as acting in **catalytic cycles**.

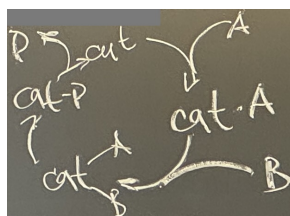


Figure 12.2: Model catalytic cycle.

- This is not that uncommon a catalytic cycle to find!
- Let's now do a kinetic analysis of this model catalytic cycle, using some of the tools developed last lecture.

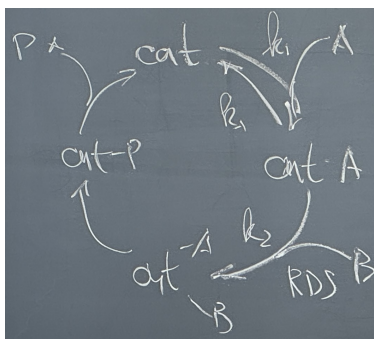


Figure 12.3: Model catalytic cycle (kinetic analysis).

- Assume that the first step is a reversible binding to A.
- Recall that the rate-determining step follows the resting state. Thus,

$$\text{rate} = \frac{d[\text{P}]}{dt} = -\frac{d[\text{A}]}{dt} = k_2[\text{cat} \cdot \text{A}][\text{B}]$$

- We can then apply our rule of thumb for the steady-state approximation.

$$\text{rate} = \frac{k_1 k_2 [\text{A}][\text{B}][\text{cat}]}{k_{-1} + k_2 [\text{B}]}$$

- Going forward, it will be useful to define the total concentration of catalyst

$$[\text{cat}]_{\text{T}} := [\text{cat}] + [\text{cat} \cdot \text{A}] + [\text{cat} \cdot \text{AB}] + [\text{cat} \cdot \text{P}]$$

- To derive the rate law for Figure 12.3 in terms of  $[\text{cat}]_{\text{T}}$  — an observable — we'd need a system of equations.
- Let's now consider a different (simpler) catalytic cycle to develop some core ideas.

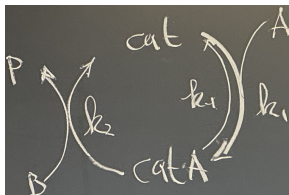
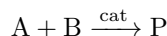


Figure 12.4: Model two-step catalytic cycle (kinetic analysis).

- This cycle pertains to a reaction



- If the second step is rate-determining, then

$$\text{rate} = k_2[\text{cat} \cdot \text{A}][\text{B}]$$

- It will also be useful to have the assumption

$$[\text{cat}]_{\text{T}} = [\text{cat}] + [\text{cat} \cdot \text{A}]$$

- Let's now evaluate this catalytic cycle using the quasi-equilibrium assumption.

- Warning: Lots of algebra coming up!

■ Squiggly lines on the board mean abbreviations.

- The quasi-equilibrium assumption tells us that

$$K = \frac{k_1}{k_{-1}} = \frac{[\text{cat} \cdot \text{A}]}{[\text{cat}][\text{A}]}$$

- We first solve for the concentration of the catalyst.

$$[\text{cat}] = \frac{[\text{cat} \cdot \text{A}]}{K[\text{A}]}$$

- We can drop this into our expression for the total catalyst.

$$[\text{cat}]_{\text{T}} = \frac{[\text{cat} \cdot \text{A}]}{K[\text{A}]} + [\text{cat} \cdot \text{A}]$$

- We can factor out the  $[\text{cat} \cdot \text{A}]$ 's.

$$[\text{cat}]_{\text{T}} = [\text{cat} \cdot \text{A}] \left( \frac{1}{K[\text{A}]} + 1 \right)$$

- Rearrange this to solve for  $[\text{cat} \cdot \text{A}]$ .

$$[\text{cat} \cdot \text{A}] = \frac{[\text{cat}]_{\text{T}}}{1/K[\text{A}] + 1}$$

- Remove this fractional denominator via multiplication by a clever form of 1 (namely,  $K[A]/K[A]$ ).

$$[\text{cat} \cdot \text{A}] = \frac{K[A][\text{cat}]_{\text{T}}}{1 + K[A]}$$

- We can now drop this back into the rate expression to get an expression for the rate in terms of the overall catalyst concentration, which is more useful because that's an observable (we know how much we put in!).

$$\text{rate} = \frac{k_2 K[A][B][\text{cat}]_{\text{T}}}{1 + K[A]}$$

- Note that we could also write the  $K$ 's above as  $K_{\text{eq}}$ 's.

- Let's now derive an analogous expression for the catalytic cycle in Figure 12.4, but this time under the steady-state approximation.

- We initially obtain

$$[\text{cat} \cdot \text{A}] = \frac{k_1[A][\text{cat}]}{k_{-1} + k_2[B]}$$

- Rearranging yields

$$[\text{cat}] = \frac{[\text{cat} \cdot \text{A}](k_{-1} + k_2[B])}{k_1[A]}$$

- Using the fact that  $[\text{cat}] = [\text{cat}]_{\text{T}} - [\text{cat} \cdot \text{A}]$ , we obtain

$$\frac{[\text{cat} \cdot \text{A}](k_{-1} + k_2[B])}{k_1[A]} = [\text{cat}]_{\text{T}} - [\text{cat} \cdot \text{A}]$$

- We get rid of the denominator by multiplying both sides by  $k_1[A]$ , collect a couple of terms, and rearrange into

$$[\text{cat} \cdot \text{A}](k_1[A] + k_{-1} + k_2[B]) = k_1[A][\text{cat}]_{\text{T}}$$

- Then divide both sides by the term in parentheses on the left.

$$[\text{cat} \cdot \text{A}] = \frac{k_1[A][\text{cat}]_{\text{T}}}{k_1[A] + k_{-1} + k_2[B]}$$

- This substitution can now be dropped back into our rate law.

$$\begin{aligned} \text{rate} &= k_2[\text{cat} \cdot \text{A}][B] \\ &= \frac{k_1 k_2 [A][B][\text{cat}]_{\text{T}}}{k_{-1} + k_2[B] + k_1[A]} \end{aligned}$$

- We now multiply by another clever form of 1 (namely the inverse of  $k_{-1}$  on both top and bottom).

$$\text{rate} = \frac{\frac{k_1}{k_{-1}} k_2 [A][B][\text{cat}]_{\text{T}}}{1 + \frac{k_2}{k_{-1}} [B] + \frac{k_1}{k_{-1}} [A]}$$

- This is known as the **one plus rate form** of the rate law because of the “1+” in the denominator.

- We can now compare the two rate laws we've derived.

- We do this by assuming that  $k_{-1} \gg k_2$ , which is exactly the scenario in which the quasi-equilibrium assumption would apply!
- We approach a limit where we can ignore the  $k_2/k_{-1}$  term in the denominator, and  $k_1/k_{-1} = K_{\text{eq}}$  (as established previously).

- Let's consider some limiting scenarios.
  - This will help us partially eliminate complexity.
  - First, let's consider the scenario in which

$$1 \gg \frac{k_2}{k_{-1}}[B] \approx \frac{k_1}{k_{-1}}[A]$$

- In this case, the denominator vanishes and the simplified rate law is

$$\text{rate} = \frac{k_1}{k_{-1}}[A][B][\text{cat}]_T$$

- Since this constraint implies that  $k_{-1} \gg k_2$ , the second step must be rate-determining.
- It also follows that  $[\text{cat}]_T \approx [\text{cat}]$ , and hence the resting state of the catalyst is the unbound catalyst!
- We can also say that the second step is **turnover-limiting**.
- Second, let's consider the scenario in which

$$\frac{k_2}{k_{-1}}[B] \gg \text{others}$$

- By “others,” we mean the other two terms in the denominator.
  - In this case, the simplified rate law is
- $$\text{rate} = k_1[A][\text{cat}]_T$$
- This constraint implies that the first step is rate-determining.
  - Hence, the reaction with B (zero-order) is post-rate limiting.
  - It follows additionally that once again, the resting state of the catalyst is the unbound catalyst!
  - Third, let's consider the scenario in which

$$\frac{k_1}{k_{-1}}[A] \gg \text{others}$$

- In this case, the simplified rate law is
- $$\text{rate} = k_2[B][\text{cat}]_T$$
- Zero-order dependence on [A] implies that the catalyst is fully saturated with [A].
  - Hence, the second step is rate-determining and the resting state is the bound catalyst.
  - Fourth, everything matters.
    - This scenario is not limiting but is, unfortunately, common.
    - This implies a kinetic pathway in which all species are at roughly similar energies with roughly similar transition structures.
    - This is often a good thing for catalysis, but we'll get there.

- Aside: The one plus rate form.

$$\text{rate} = \frac{c_1[A][B][\text{cat}]_T}{1 + c_2[A] + c_3[A][B] + c_4[P]}$$

- The rate law takes on the above general structure.
- We have a constant (the “kinetic term”)  $c$  modified by the concentrations of the inputs in the numerator, collectively referred to as the potential terms (because they reflect something about the TST with respect to the ground state).

- The denominator — the **adsorption term** — consists of all the forms that the catalyst can take.
- What do the constants tell us?
  - $c_1$  tells us about the naked catalyst.
  - $c_2[A]$  can tell us about the  $\text{cat} \cdot \text{A}$  complex.
  - $c_3[A][B]$  can tell us about the  $\text{cat} \cdot \text{AB}$  complex.
  - $c_4[P]$  can tell us about the  $\text{cat} \cdot \text{P}$  complex.
- Goal of this exercise: Gain intuition for the algebra.
  - The best kineticists can easily see the chemistry in rate laws the way that most organic chemists can see it in Lewis structures.
    - Donna Blackmond at Scripps is one of Alex's favorite kineticists.
  - Similarly, spectroscopists can see chemistry in derivative waveforms; "what a power to have!"
- How do we increase the rate, i.e., max out the rate law?
  - We want to get the denominator to go away.
  - This gives Scenario 3, in which all of the substrate is bound to the catalyst and it's doing its thing as fast as it can.<sup>[1]</sup>

$$\text{rate}_{\max} = k_2[B][\text{cat}]_{\text{T}}$$

- Let's plop  $\text{rate}_{\max}$  into our steady-state approximation rate law, and multiply it by  $1 = k_{-1}/k_{-1}$ .

$$\text{rate} = \frac{\text{rate}_{\max} k_1[A]}{k_{-1} + k_2[B] + k_1[A]}$$

- Now define

$$\frac{1}{K_m} = \frac{k_1}{k_{-1} + k_2[B]}$$

- It follows that

$$\text{rate} = \frac{\text{rate}_{\max}[A]}{K_m + [A]}$$

- Under the quasi-equilibrium assumption, we can assume something else.

$$\text{rate} = \frac{\text{rate}_{\max} K_{\text{eq}}[A]}{1 + K_{\text{eq}}[A]}$$

- Now define

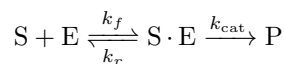
$$K_D = \frac{1}{K_{\text{eq}}}$$

as the dissociation constant for the  $\text{cat} \cdot \text{A}$  complex.

- It follows that

$$\text{rate} = \frac{\text{rate}_{\max}[A]}{K_D + [A]}$$

- $K_m$  takes us into **Michaelis-Menten kinetics**.
  - Defined in the early twentieth century to guide our emerging understanding of biochemical kinetics.
  - Using the historical nomenclature (substrate and enzyme), we have




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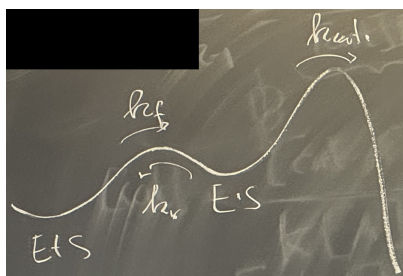
<sup>1</sup>Why should this scenario give the fastest rate??

- There is a relationship here to saturation kinetics, defined in the limiting scenarios with Figure 12.4.
- Michaelis and Menten talked about a kinetic velocity  $v_{\max}$  where we go from asymptotic speed down to a first-order dependence on  $[S]$ .
- Other relevant expressions:

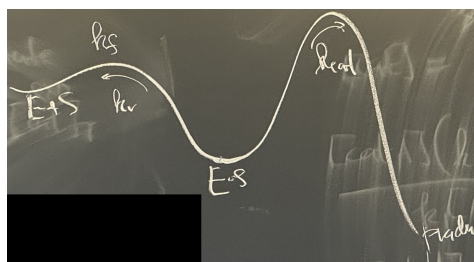
$$K_m = \frac{k_r + k_{\text{cat}}}{k_f} \qquad v = \frac{v_{\max}[S]}{K_m + [S]}$$

- If  $K_m = [S]$ , then  $v = v_{\max}/2$ .
- We can experimentally determine the Michaelis constant by assaying a bunch of different initial rates at different concentrations.

- What's the point of Michaelis-Menten kinetics?



(a) Zeroth-order.



(b) First-order.

Figure 12.5: Michaelis-Menten kinetic regimes.

- Enzymes can be characterized according the Michaelis constant  $K_m$  and the intrinsic rate constant  $k_{\text{cat}}$ .
- When we're zeroth order in the substrate, we have one kinetic regime.
- When we're first-order in the substrate, we have a kinetic regime that's more like quasi-equilibrium!
- How well the enzyme binds the substrate is a measure of how efficient the catalyst is.

- When  $k_r \gg k_{\text{cat}}$ ,

$$K_m \approx \frac{k_r}{k_f} = K_D$$

- This ratio measures how well the enzyme binds to the substrate.

- When  $k_{\text{cat}} \gg k_r$ ,

$$K_m \approx \frac{k_{\text{cat}}}{K_m} =: \text{specificity constant}$$

- These ratios can be analyzed as the specificity constant for a specific enzyme.
- Describes an enzyme's preference (both in terms of binding and reactivity) for one substrate over another.

- Example: Fumarase (responsible for redox transport).

- $K_m = 5 \times 10^{-6}$ , and  $k_{\text{cat}} = 8 \times 10^2$ , so SC is  $10^7$ .

- Example: Carbonic anhydrase.

- $K_m = 2.6 \times 10^{-2}$ , and  $k_{\text{cat}} = 4 \times 10^5$ , so SC is  $10^8$ .

- So for great catalysis, you want the catalyst to find substrate quickly and immediately turn it over.
  - These two catalysts operate near the diffusion limit (which is ideal); they are near-perfect.
- References.
  - Knowles (1991).
  - A beautiful piece of literature; the touchstone for biological catalysis, per Alex.