Biological Chemistry Laboratory
Biology 3515/Chemistry 3515
Spring 2018

Lecture 13:

Data Analysis for the V versus [S] Experiment and Interpretation of the Michaelis-Menten Parameters

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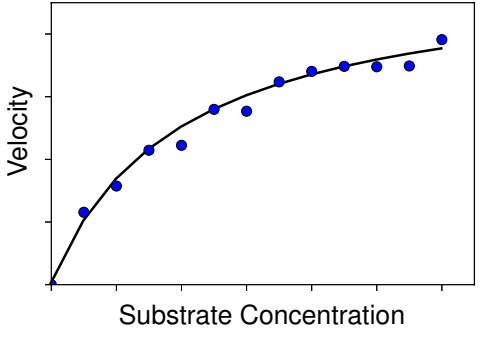
Analysis of Data from the V versus [S] Experiment



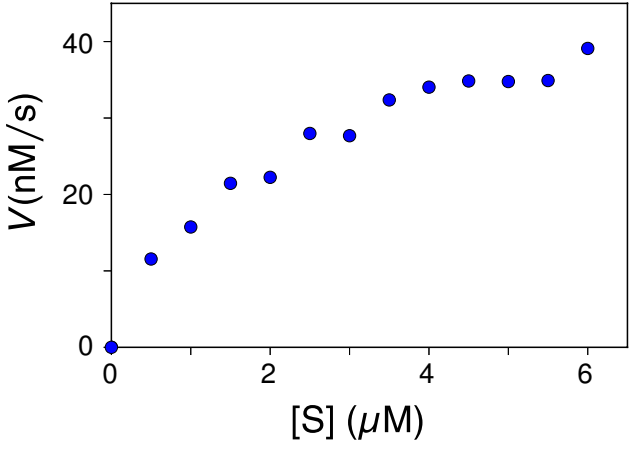
- We want to fit the experimental data to the Michaelis-Menten Equation:

$$V = \frac{[\mathrm{S}]\, V_{\mathrm{max}}}{[\mathrm{S}] + K_{\mathrm{m}}}$$

- From the fit, we obtain estimates of K_{m} and V_{max}.

Clicker Question #1

Estimate V_{max} from the graph:

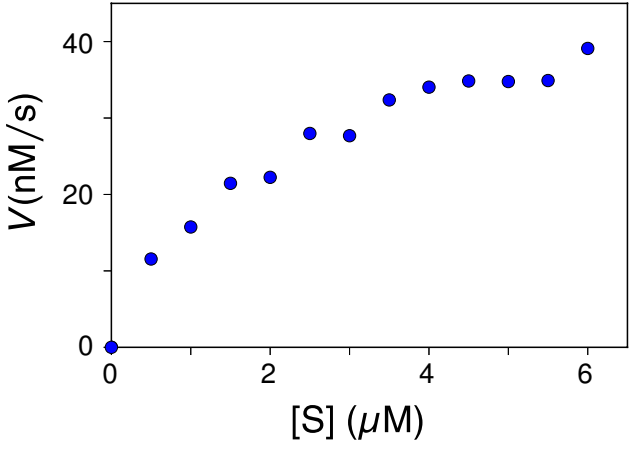


1. 30 nM/s
2. 40 nM/s *
3. 50 nM/s
4. 80 nM/s

*close enough for credit.

Clicker Question #2

Estimate K_m from the graph:



1. 1 μM
2. 2 μM
3. 5 μM
4. 10 μM

A Classic Method for Analyzing Enzyme Kinetics Data

- Rearrangement of the Michaelis-Menten Equation:

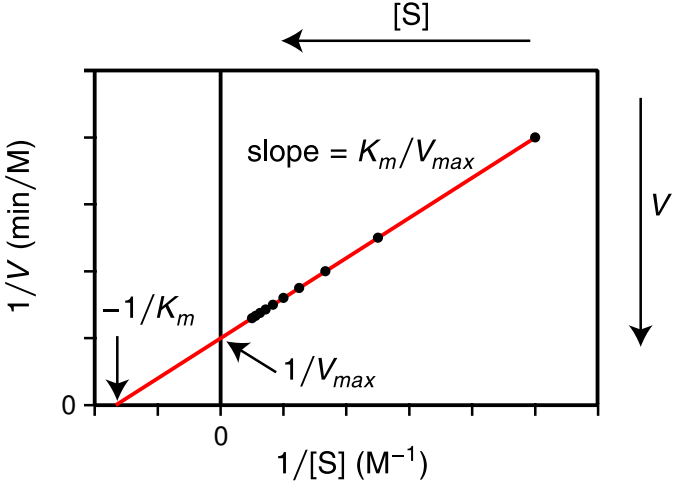
$$V = \frac{[\mathrm{S}]\,V_{\max}}{[\mathrm{S}] + K_{\mathrm{m}}}$$

$$\frac{1}{V} = \frac{[\mathrm{S}] + K_{\mathrm{m}}}{[\mathrm{S}]\,V_{\max}} = \frac{[\mathrm{S}]}{[\mathrm{S}]\,V_{\max}} + \frac{K_{\mathrm{m}}}{[\mathrm{S}]\,V_{\max}}$$

$$\frac{1}{V} = \frac{1}{[\mathrm{S}]} \cdot \frac{K_{\mathrm{m}}}{V_{\max}} + \frac{1}{V_{\max}}$$

- A plot of $1/V$ versus $1/[\mathrm{S}]$ should generate a straight line with a slope of $K_{\mathrm{m}}/V_{\max}$ and an intercept of $1/V_{\max}$ on the $1/V$ axis.

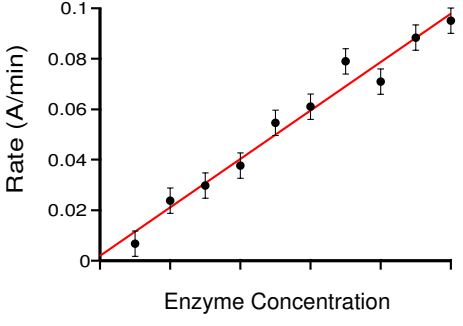
The Lineweaver-Burk Plot



- If the data are perfect, this plot gives good estimates of K_m and V_{max}.
- But, experimental error in V can lead to strange effects!

Experimental Error and Uncertainty

- Error bars for rate measurements are of approximately constant size (*e.g.,* ±0.005 A/min), rather than a constant percentage of the measurement.



- For 0.1 A/min, ±0.005 A/min = ±5%.
- For 0.01 A/min, ±0.005 A/min = ±50%.
- Least-squares fitting works well if the *absolute* uncertainties of all data points are approximately equal.

What Happens When We Take Reciprocals?

- $V = 0.1 \pm 0.005$

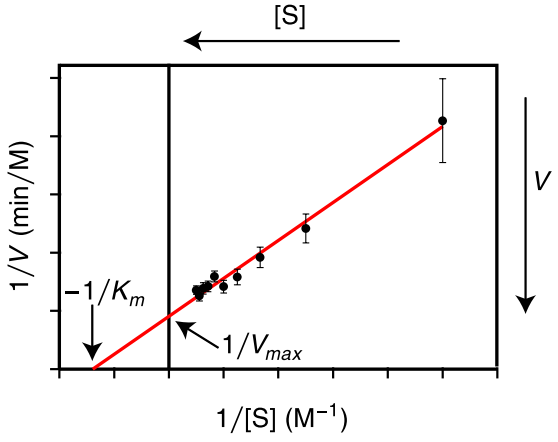
$$\frac{1}{0.105} = 9.52, \qquad \frac{1}{0.095} = 10.5, \qquad \frac{1}{V} = 10 \pm 0.5$$

- $V = 0.01 \pm 0.005$

$$\frac{1}{0.015} = 66.7, \qquad \frac{1}{0.005} = 200, \qquad \frac{1}{V} = 100 \pm 50$$

- The values of $1/V$ derived from small velocities can have huge *absolute* errors.

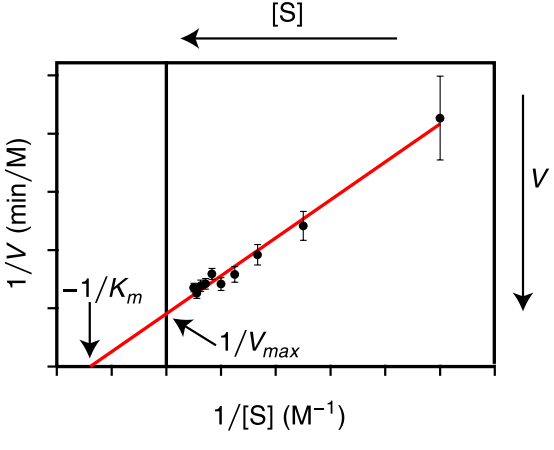
The Effects on a Lineweaver-Burk Plot



- Errors in the least precise measurements (low V) can cause large changes in the line fit to the Lineweaver-Burk plot.

Clicker Question #3

Which parameter is likely to be more sensitive to errors in a Lineweaver-Burk plot?



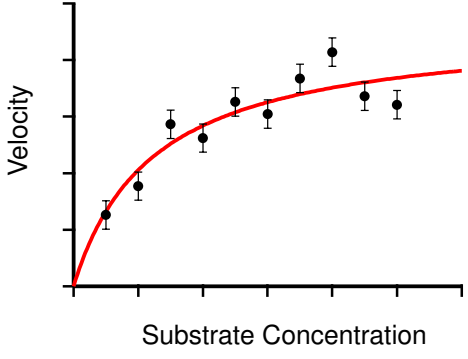
1 K_m *

2 V_{max}

* Provided that values of V approach V_{max}.

Two Ways to Deal with This Problem

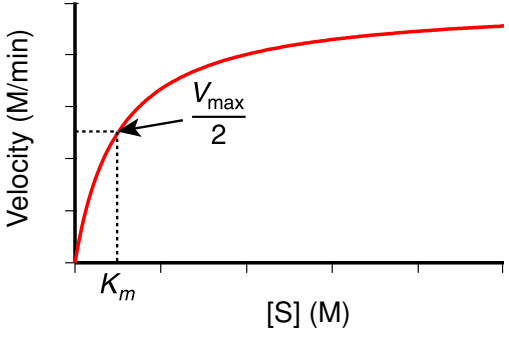
- Use Lineweaver-Burk, but weight data according to uncertainties in $1/V$.
- Fit velocity data directly to the Michaelis-Menten equation using non-linear least-squares method.



- Equal errors in V are weighted equally.

Interpreting K_m

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E{\cdot}S \xrightarrow{k_{cat}} E + P$$



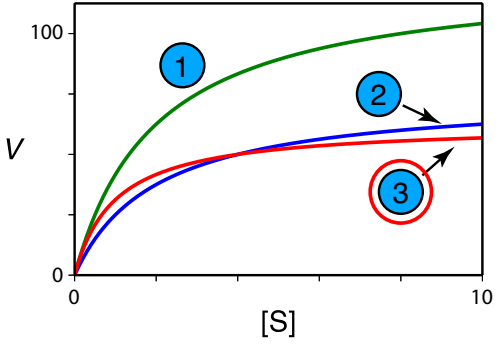
$$K_m = \frac{k_{-1} + k_{cat}}{k_1}$$

$$V = \frac{V_{max}}{2} \quad \text{when} \quad [S] = K_m$$

- When $[S] = K_m$, half of total enzyme has substrate bound.
- The larger K_m is, the more substrate is required to reach $V_{max}/2$, or any specified fraction of V_{max}.

Clicker Question #4:

Data for three substrates with the same enzyme.



Which substrate binds most tightly to the enzyme?

No wrong answers, for now.

A Closer Look at Binding and K_m: K_m versus K_d

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E{\cdot}S \xrightarrow{k_{cat}} E + P$$

- K_m is defined in terms of the rate constants:

$$K_m = \frac{k_{-1} + k_{cat}}{k_1}$$

- K_d is the equilibrium constant for dissociation.

$$K_d = \frac{[E][S]}{[E \cdot S]} = \frac{k_{-1}}{k_1}$$

A large K_d indicates weak binding.

K_m versus K_d

$$K_m = \frac{k_{-1} + k_{cat}}{k_1}$$

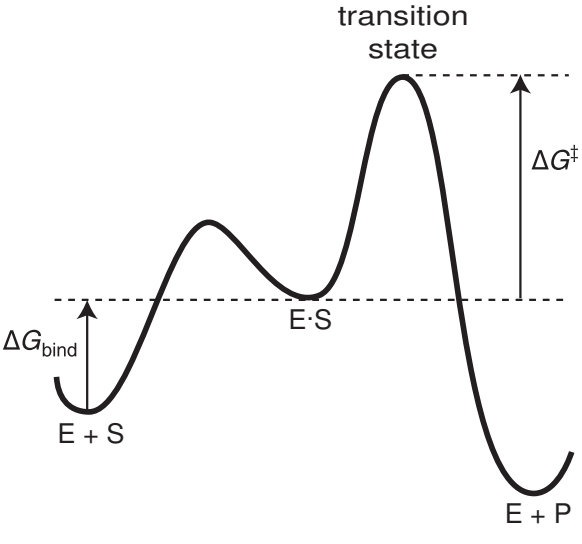
$$K_d = \frac{[E][S]}{[E \cdot S]} = \frac{k_{-1}}{k_1}$$

- If $k_{cat} \ll k_{-1}$, *i.e.,* the E·S complex is more likely to dissociate than undergo catalysis:

$$K_m \approx \frac{k_{-1}}{k_1} = K_d$$

- In general, $K_m \geq K_d$
- Strength of equilibrium binding may be greater than indicated by K_m.

Energy Profile for an Enzyme-Catalyzed Reaction



- Free-energy change for binding:

$$\Delta G_{\text{bind}} = RT \ln K_{\text{d}}$$

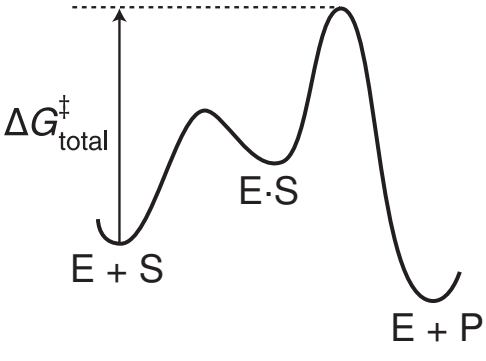
R = Gas constant
T = Temperature

- Free-energy change from E·S complex to transition state:

$$\Delta G^{\ddagger} = RT \ln \left(\frac{k_{\text{b}} T}{k_{\text{cat}} h} \right)$$

k_{b} = Boltzmann constant
h = Planck constant

The Significance of k_{cat}/K_m



- Free-energy difference between E + S and the transition state:

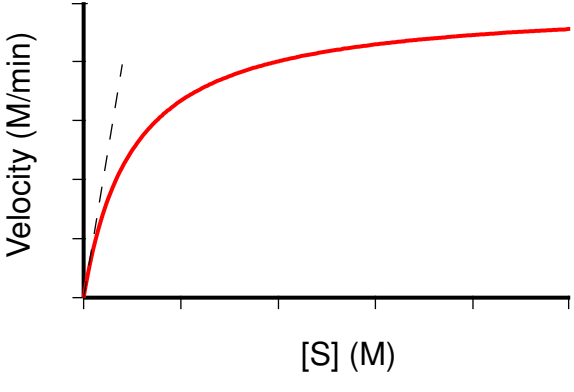
$$\Delta G^{\ddagger}_{total} = \underbrace{RT \ln\left(\frac{k_b T}{h}\right)}_{\text{Constant}} + RT \ln\left(\frac{K_d}{k_{cat}}\right)$$

- If $k_{-1} \gg k_{cat}$, $K_d \approx K_m$:

$$\Delta G^{\ddagger}_{total} = C - RT \ln\left(\frac{k_{cat}}{K_m}\right)$$

- The ratio k_{cat}/K_m reflects the free energy difference between E + S and the transition state. (Assuming $K_d \approx K_m$)
- k_{cat}/K_m is commonly interpreted as a measure of enzymatic efficiency.
- Catalytic efficiency is favored by a large value of k_{cat} and a small value of K_m.

k_{cat}/K_m Is Also the Apparent Second-Order Rate Constant at Low Substrate Concentrations



- If $[S] \ll K_m$:

$$V = \frac{[S][E]_T k_{cat}}{K_m + [S]}$$

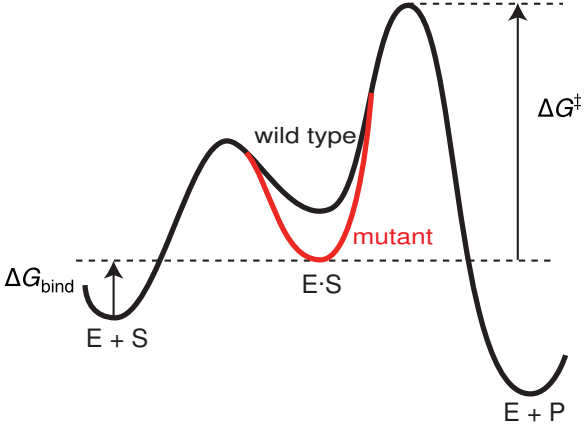
$$V \approx \frac{k_{cat}}{K_m}[S][E]_T$$

- Units of k_{cat}/K_m

$$\frac{s^{-1}}{M} = sec^{-1}M^{-1}$$

Is a Low K_m Always Good?

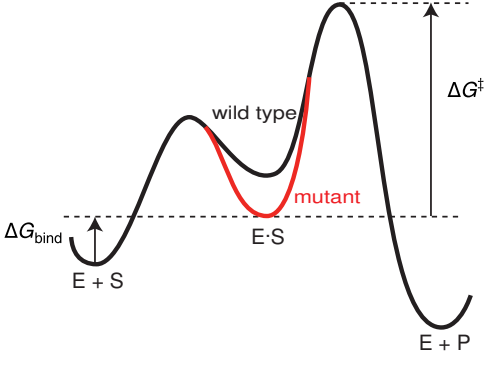
Suppose that we could design a mutant enzyme that forms a more stable complex with substrate.



This will lower K_m and k_{cat}, but leave k_{cat}/K_m the same.

Clicker Question #5

At **low** substrate concentration ([S] $\ll K_m$), will the velocity for the mutant enzyme be greater or less than that of the original enzyme?



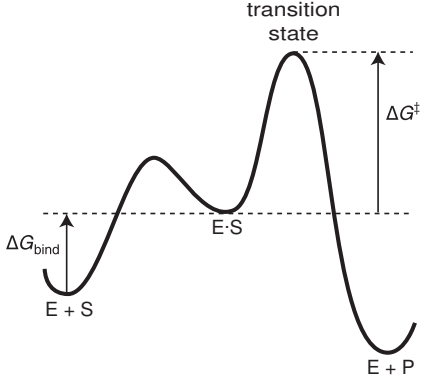
1. Greater than the original enzyme
2. Less than the original enzyme
3. The same as the original enzyme!

$$V = \frac{[S][E]_T k_{cat}}{K_m + [S]}$$

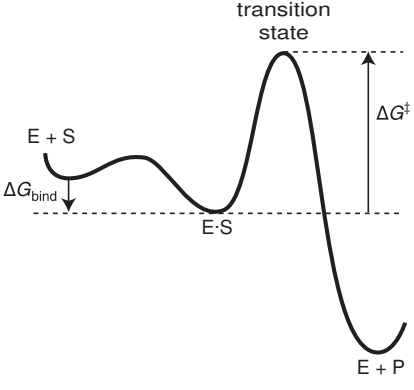
$$V \approx \frac{k_{cat}}{K_m}[S][E]_T$$

What About High Substrate Concentrations?

Low Substrate Concentration



High Substrate Concentration



- When [S] ≫ K_m, the enzyme-substrate complex is favored with respect to the free enzyme.