Biological Chemistry Laboratory
Biology 3515/Chemistry 3515
Spring 2023

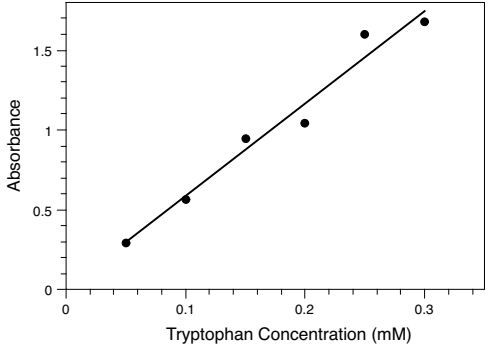
Lecture 13

Determination of K_m and V_{max}

21 February 2023

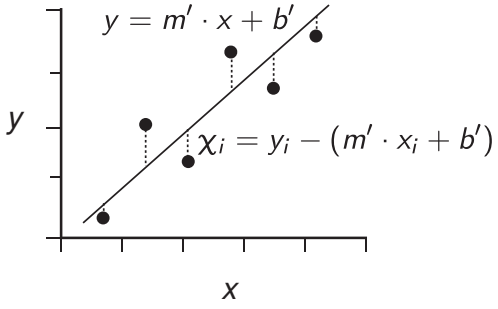
©David P. Goldenberg
University of Utah
goldenberg@biology.utah.edu

From Quiz 1, Problem 3a



- Should we fit a different function to the data?
  - Is there a systematic deviation of the data from the linear relationship?
  - Does the linear relationship (or a different function) represent a good model to account for the data?

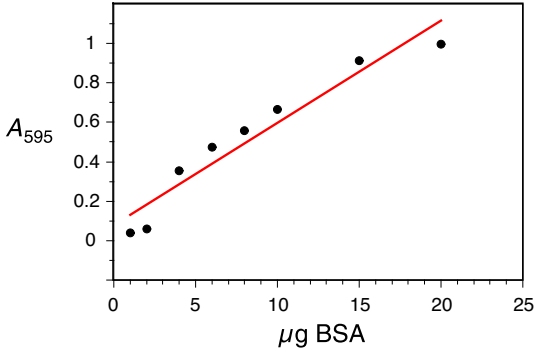
The Method of Least Squares: χ^2



$$\chi^2 = \sum \chi_i^2$$
$$= \sum \left(y_i - (m' \cdot x_i + b')\right)^2$$

- Adjust m' and b' to minimize the value of χ^2 for the particular values of x_i and y_i in the experimental data set.
- The method can be applied to other functions to fit paramaters.

The Coefficient of Determination, R^2



$R^2 = 0.93$

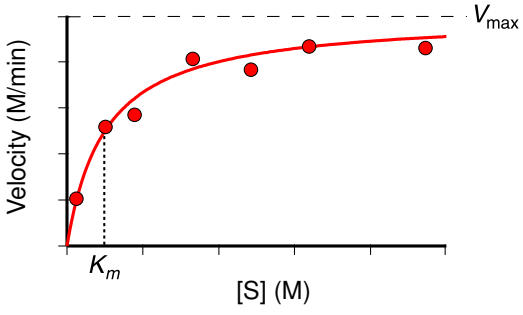
- R^2 represents the fraction of the variation that is accounted for by the fit function.
- R^2 usually lies between 0 and 1.
- R^2 can be negative for certain functions and data sets!

Warning!

Direction Change

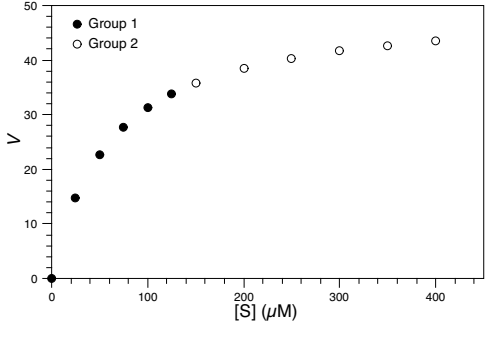
Determining K_m and V_{max}

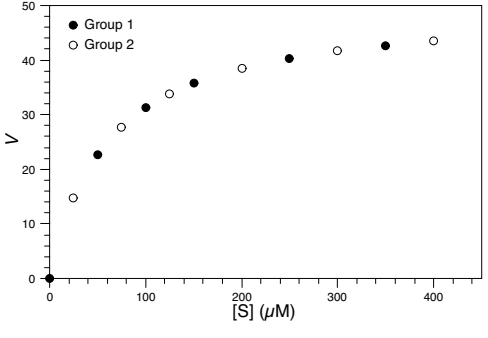
Experiment 3, Part D: Velocity as a Function of Substrate Concentration



- To reliably estimate both K_m and V_{max}, substrate concentrations must cover range both below and above K_m.
- We will use eleven substrate concentrations, plus a control without substrate.
- Two groups of six reactions.
- How should the reactions be grouped?

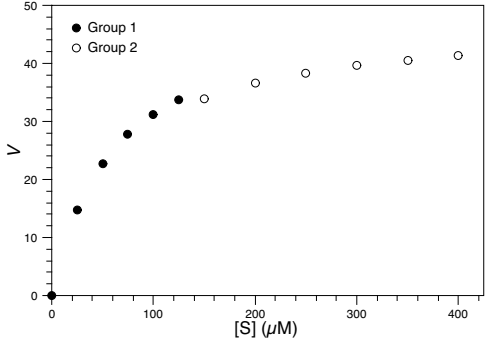
Two Ways to Group the Reactions

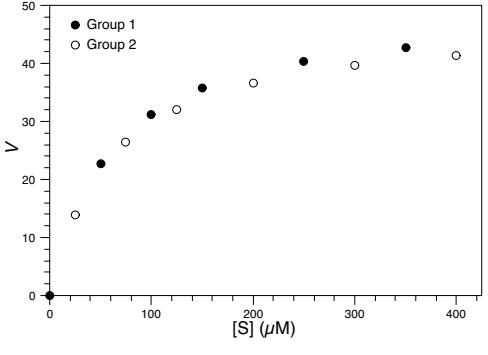




- Is one way better than the other?
- What if something changes between the reactions?

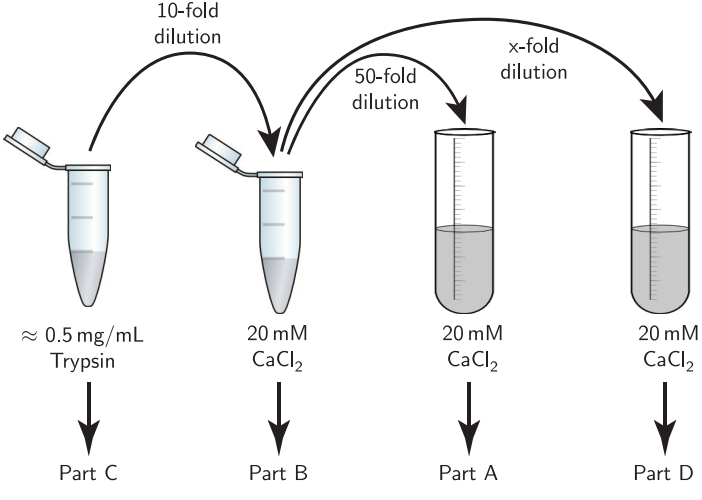
Two Ways to Group the Reactions



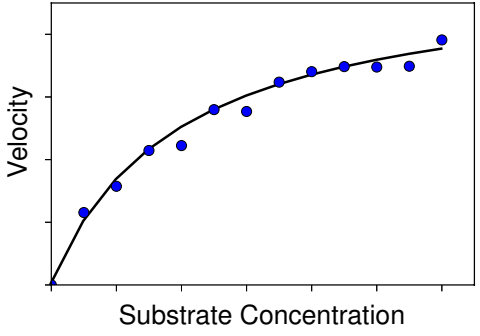


- 1–6, 7–12 grouping: changes between reaction groups may be hard to detect.
- Odd–even grouping: changes between reaction groups are easier to detect.
  Fitting data together averages effects more evenly.

Dilutions of Trypsin Solutions for Experiment 3



Analysis of Data from the V versus [S] Experiment



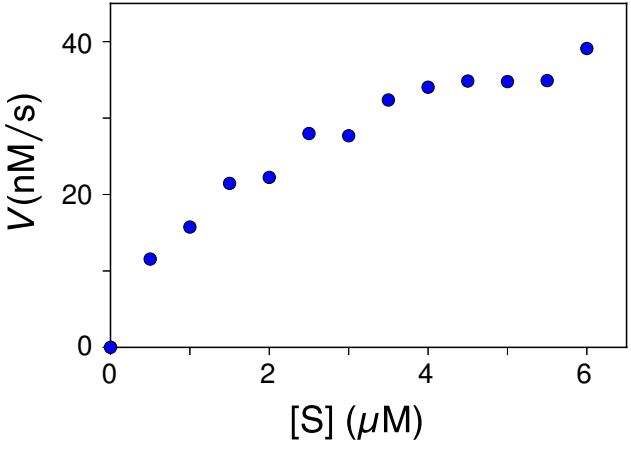
- We want to fit the experimental data to the Michaelis-Menten Equation:

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

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

Clicker Question #1

Estimate V_{max} from the graph:



A) 30 nM/s

B) 40 nM/s *

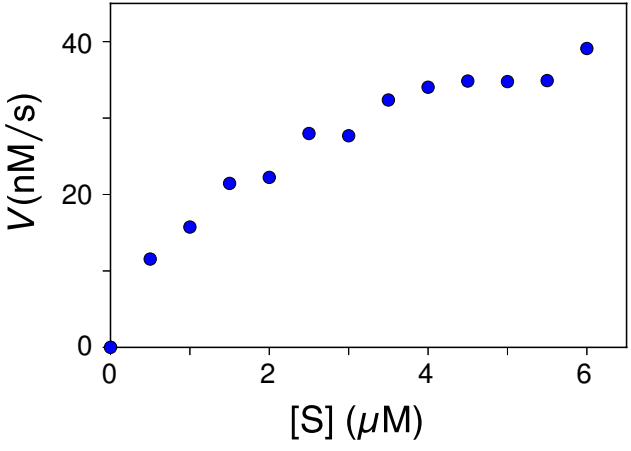
C) 50 nM/s

D) 80 nM/s

*close enough for credit.

Clicker Question #2

Estimate K_m from the graph:



A) 1 μM

B) 2 μM

C) 5 μM

D) 10 μM

A Classic Method for Analyzing Enzyme Kinetics Data

- Rearrangement of the Michaelis-Menten Equation:

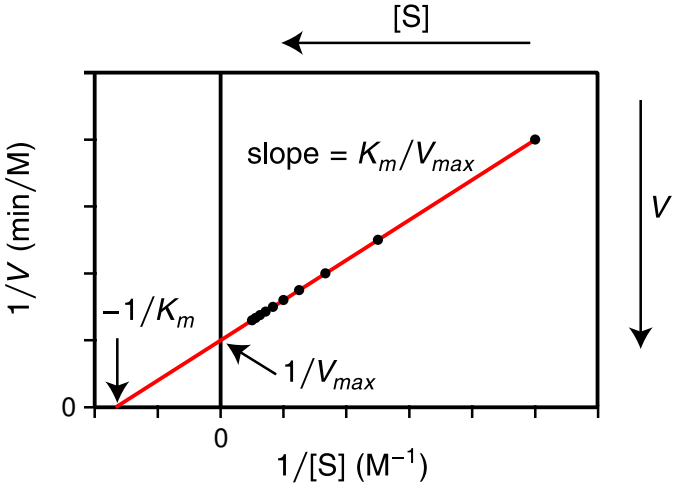
$$V = \frac{[\mathrm{S}]V_{\max}}{[\mathrm{S}] + K_{\mathrm{m}}}$$

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

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

- A “double-reciprocal” 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.

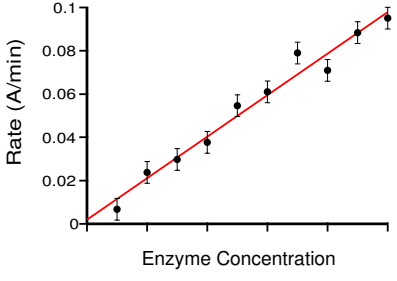
The Lineweaver-Burk Plot



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

What Happens When We Take Reciprocals?

- $V = 0.1 \pm 0.005$

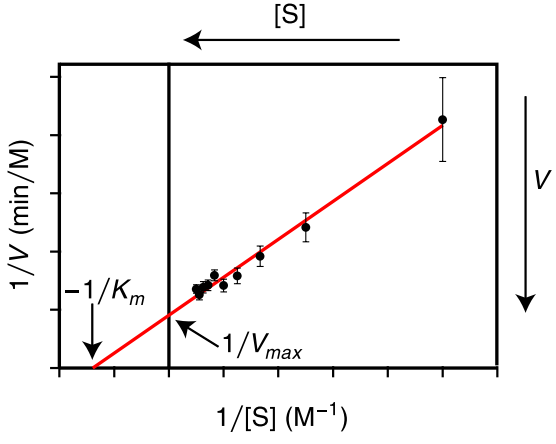
$$\frac{1}{0.105} = 9.52, \qquad \frac{1}{0.095} = 10.5, \qquad \frac{1}{V} = 10 \pm 0.5$$

- $V = 0.01 \pm 0.005$

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

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

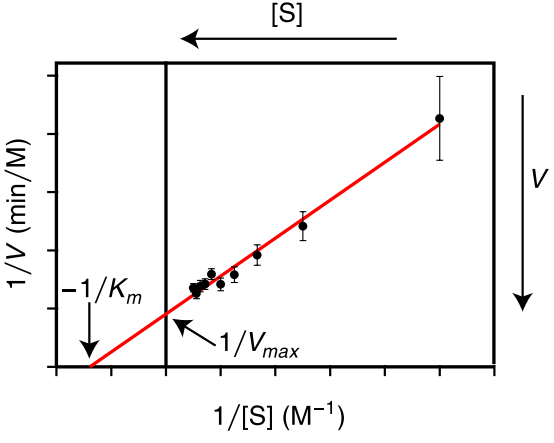
The Effects on a Lineweaver-Burk Plot



- 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?



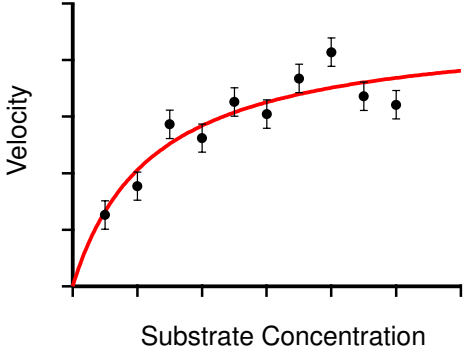
A) K_m *

B) V_{max}

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

Two Ways to Deal with This Problem

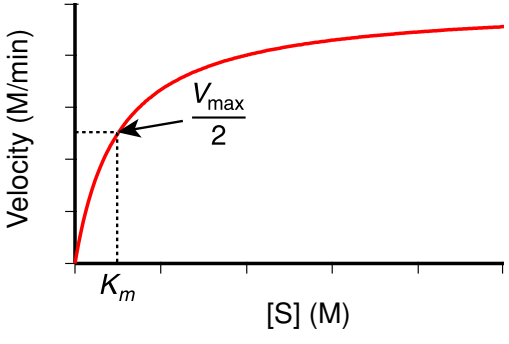
- Use Lineweaver-Burk, but weight data according to uncertainties in $1/V$.
- Fit velocity data directly to the Michaelis-Menten equation using non-linear least-squares method.



- Equal errors in V are weighted equally.

Interpreting K_m

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



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

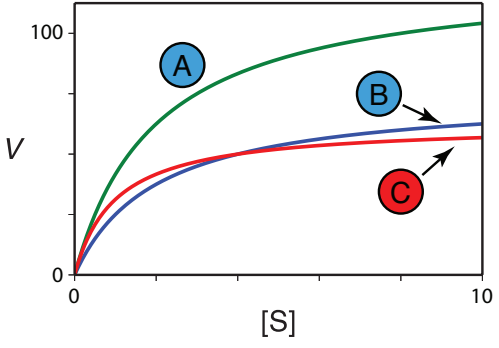
$$V = V_{max}\frac{[S]}{[S] + K_m}$$

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

- 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

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

- 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.

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

A large K_d indicates weak binding.

K_m versus K_d

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

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

- If $k_{cat} \ll k_{-1}$, 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.