



# **Pre-course: Mathematical Biochemistry in a Nutshell**

**Lecture 1**

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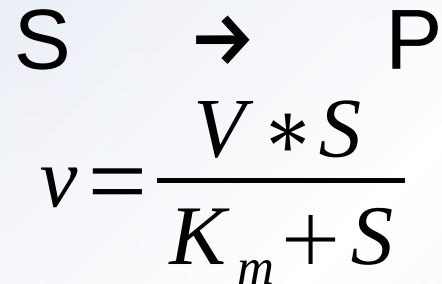
ALCSysBio 2014

## Course Outline

- Functions, parameters and variables; parameter fitting
- Calculating time courses of biological models
- Mathematical representation of cellular systems
- Sensitivity analysis – metabolic control analysis
- Course materials are available for downloading at:  
<http://mudshark.brookes.ac.uk/Meetings/MathBio14>

## Functions

Consider the Michaelis-Menten equation for the rate,  $v$ , of the catalysed reaction:



$v$  and  $S$  are **variables** (dependent and independent respectively);

$K_m$  and  $V$  are **parameters**.

If  $K_m = 2$  mM and  $V = 10$ U, what is  $v$  at  $S = 1, 2, 8$  and  $18$  mM?

## Generalised and dimensionless formats

If we don't want to specify a particular equation or functional form, we might abbreviate this to:

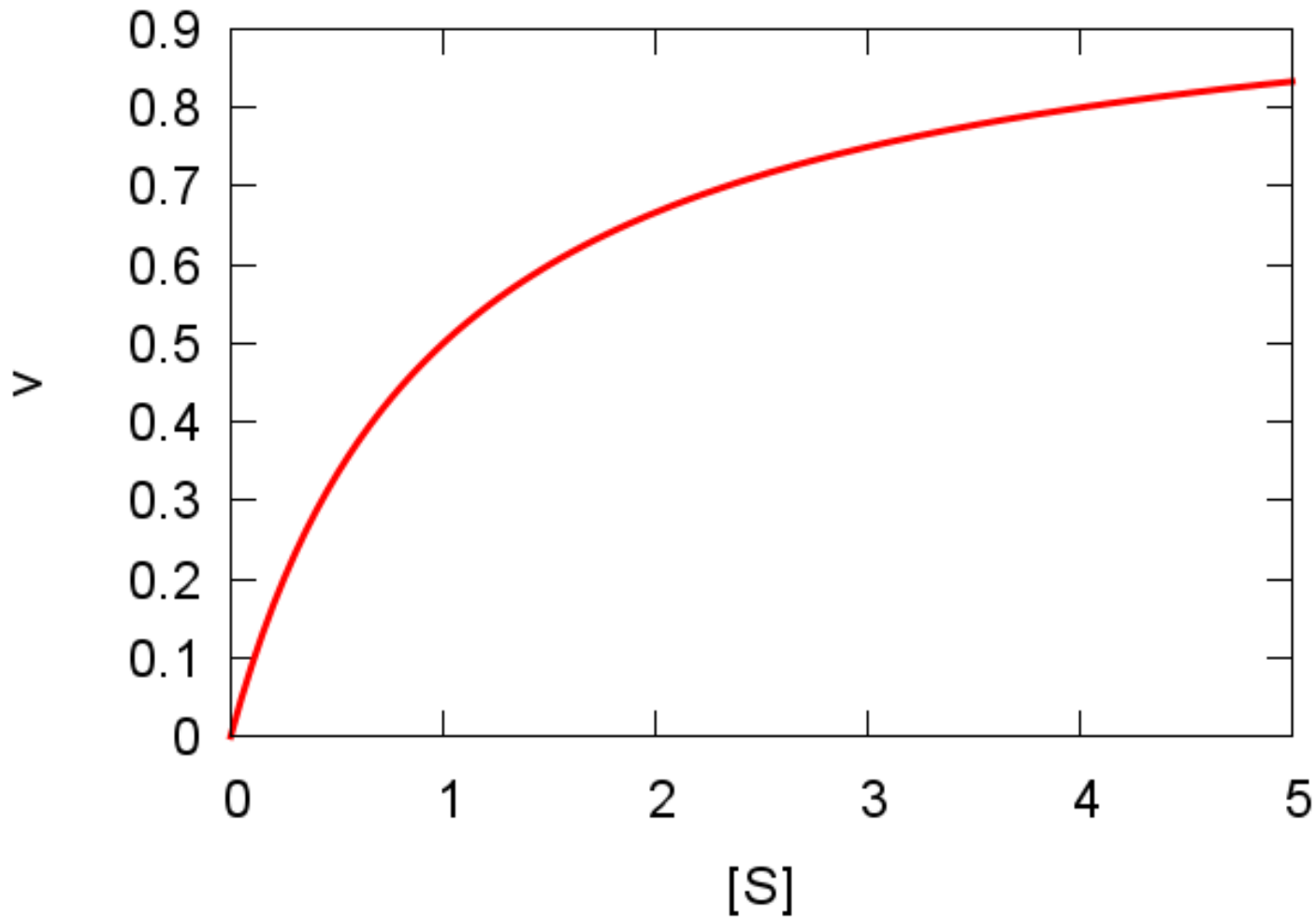
$$v = f(S, K)$$

where  $S$  is the variable and  $K$  is the set of parameters  $\{V, K_m\}$

If we're interested in the general form of the relationship without consideration of the units we use to measure  $v$  and  $S$ , then we can rearrange the function as:

$$\frac{v}{V} = \frac{\frac{S}{K_m}}{1 + \frac{S}{K_m}} \quad \text{or} \quad y = \frac{x}{1 + x}$$

# Illustration



Plotted for  $V = 1$ ,  $K_m = 1$  - another way of making the curve dimensionless.

# Plotting the function

- Use Gnuplot (Download and install from <http://www.gnuplot.info/> - Windows, Mac and Linux versions available)
- Use a spreadsheet (Excel, Gnumeric, Calc ...)
- Use Matlab/Octave
  
- Try plotting the Michaelis-Menten function yourself for different  $K_m$  and  $V$  values. Your substrate concentration range ( $S$ ) should be  $5 - 10 \times K_m$ .



## Solving equations graphically

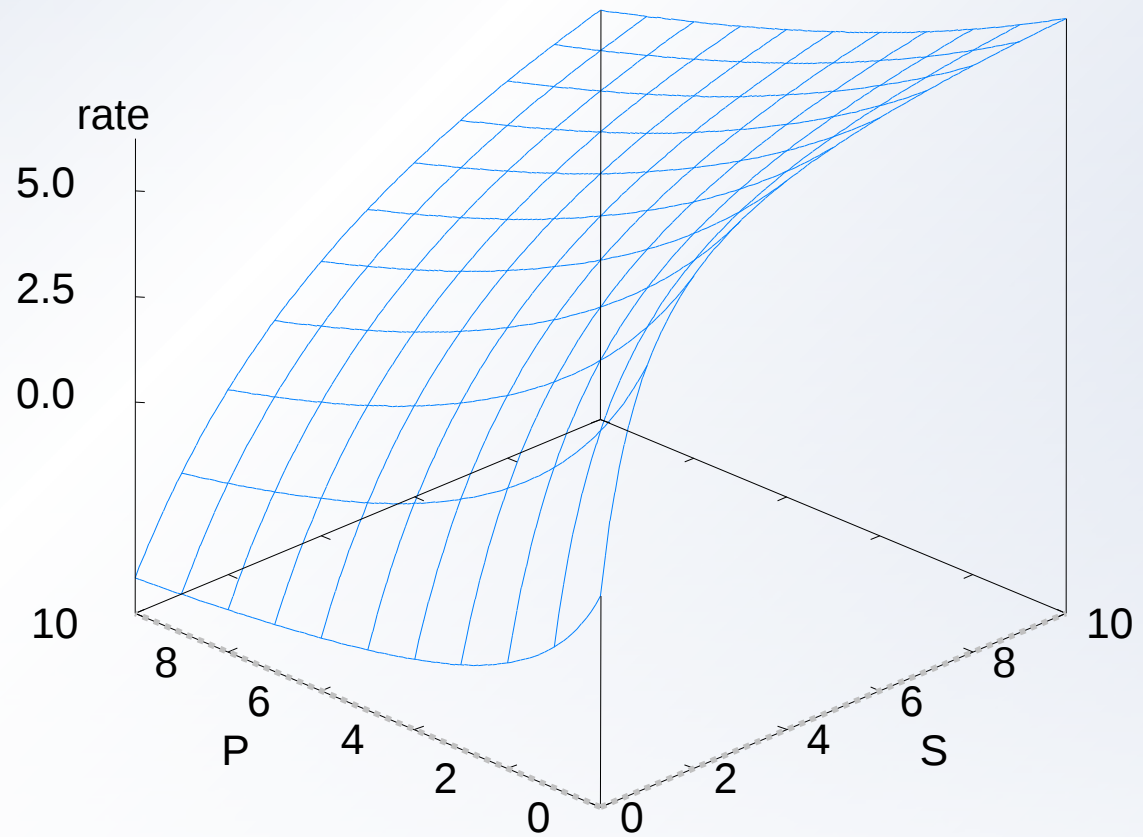
- Suppose we have a two-enzyme ( $E_1$  and  $E_2$ ) metabolism, fed from an inexhaustible reservoir of nutrient  $S$  and producing a product  $Z$  that is removed from the system without accumulating, i.e.:



- $E_2$  obeys Michaelis-Menten kinetics with respect to its substrate  $P$ ;  $E_1$  is inhibited if its product  $P$  accumulates (see next slide).
- If we know the parameter values for the enzyme equations, can we tell how the system behaves?

# Reversible Michaelis-Menten equation

$$v = \frac{V \left( S - \frac{P}{K_{eq}} \right)}{K_m \left( 1 + \frac{P}{K_P} \right) + S}$$



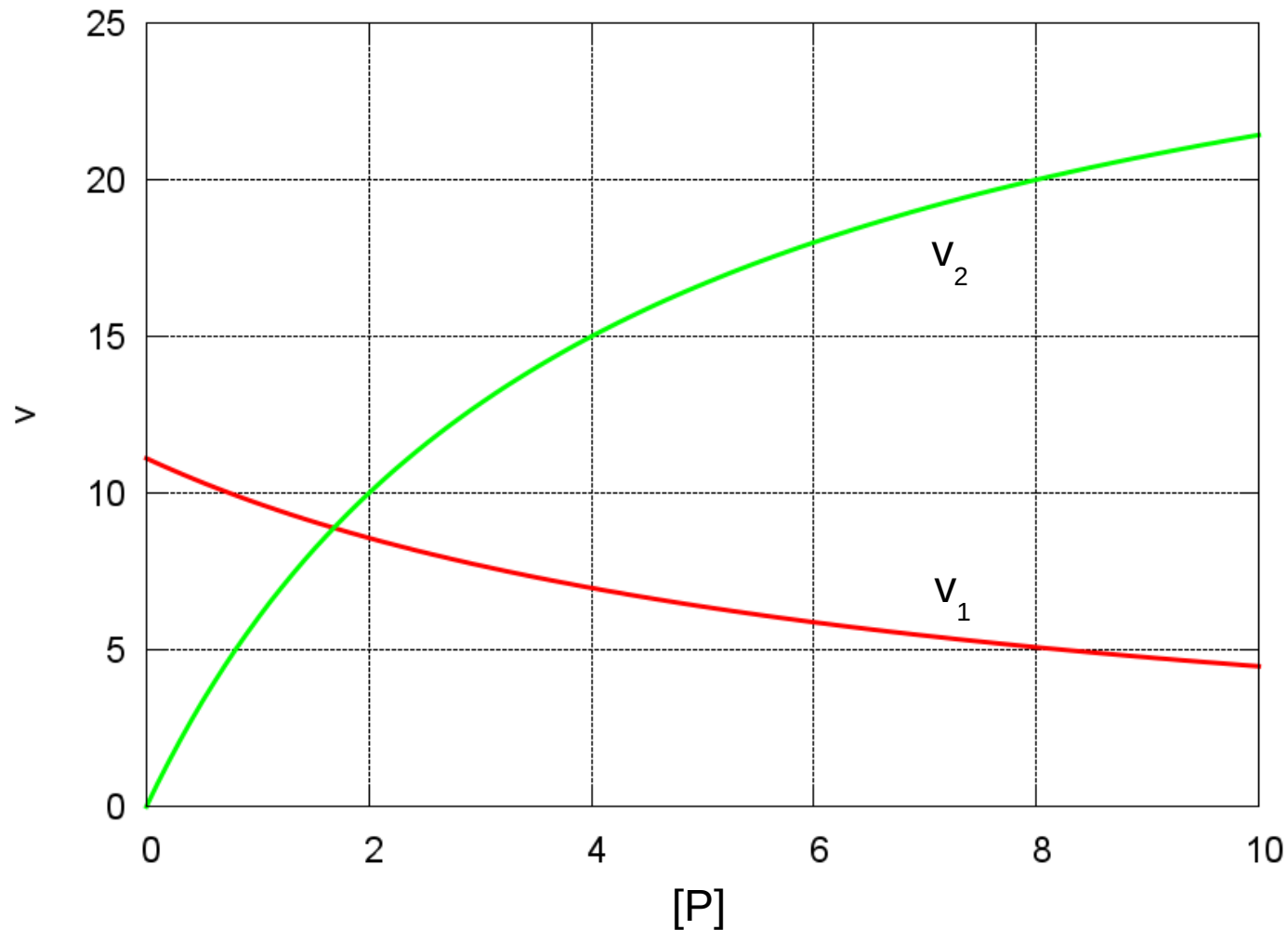


## Two enzyme system properties

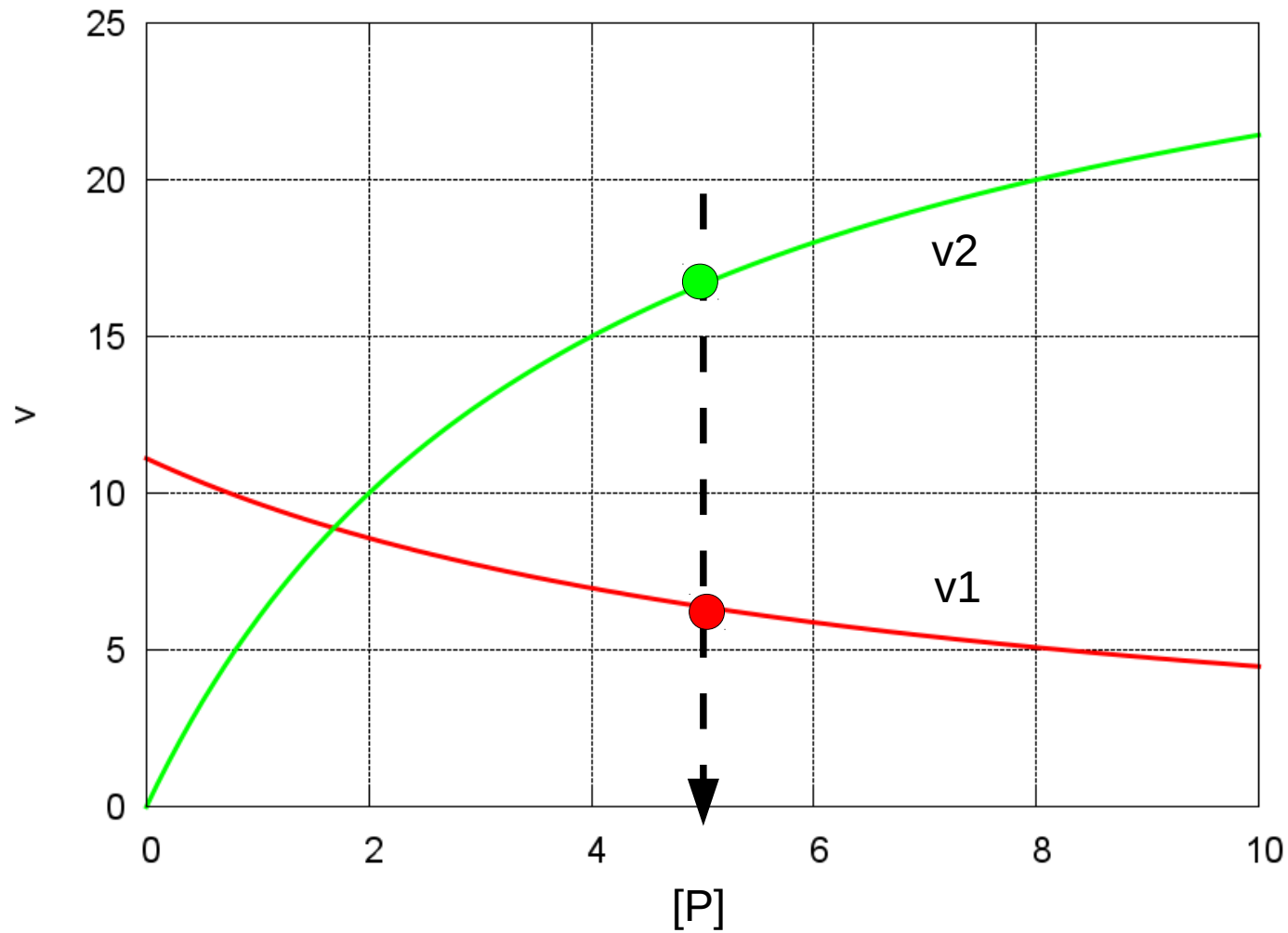
- Assume  $S = 5$  mM.
- For  $E_1$ , assume  $V_1 = 20U$ ,  $K_{eq} = \infty$ ,  $K_p = 3$ ,  $K_{m1} = 4$ .
- For  $E_2$ , assume  $V_2 = 30U$ ,  $K_{m2} = 4$ .
  
- How can we tackle this?

## Two enzyme system: behaviour

- Plot the rates of the two enzymes on the same graph as a function of  $S$ .

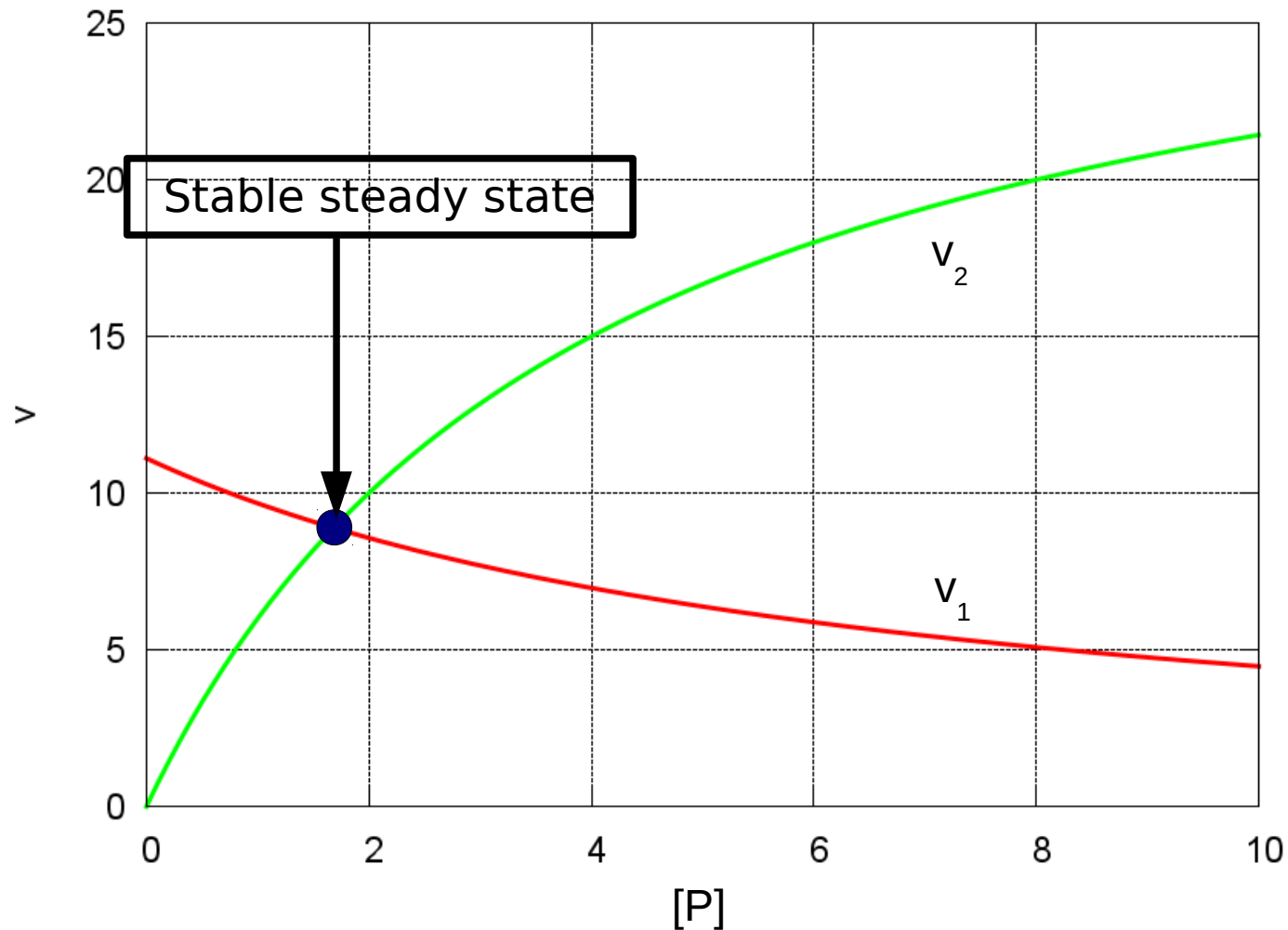


What happens next if P is added to 5 mM?



## Two enzyme system: behaviour

- Any perturbation of  $P$  will die away and the system will return to its stable steady state value.

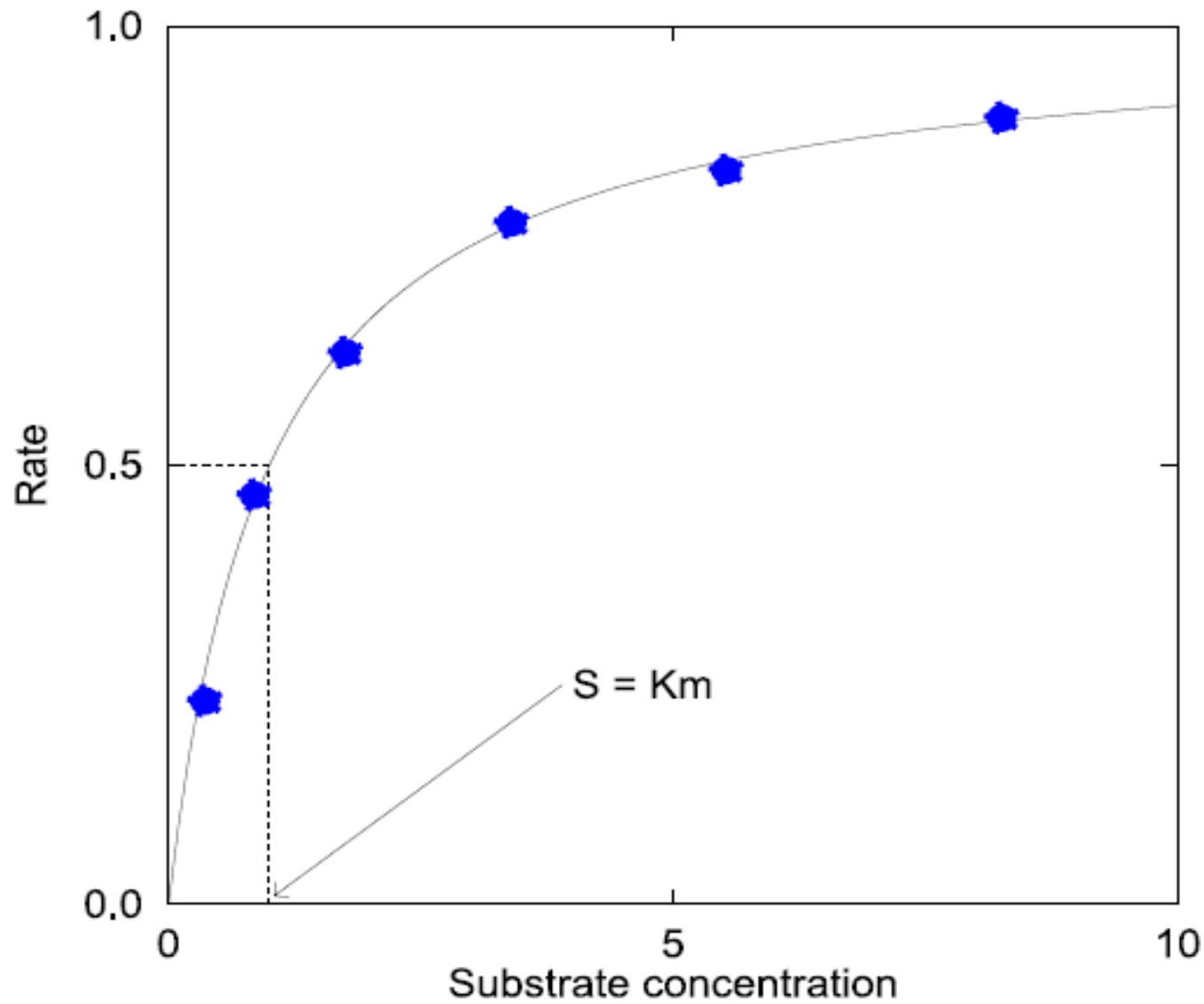


# Parameter estimation from experimental data

Using determination of  $K_m$  and  $V$  values from experimental data as an example:

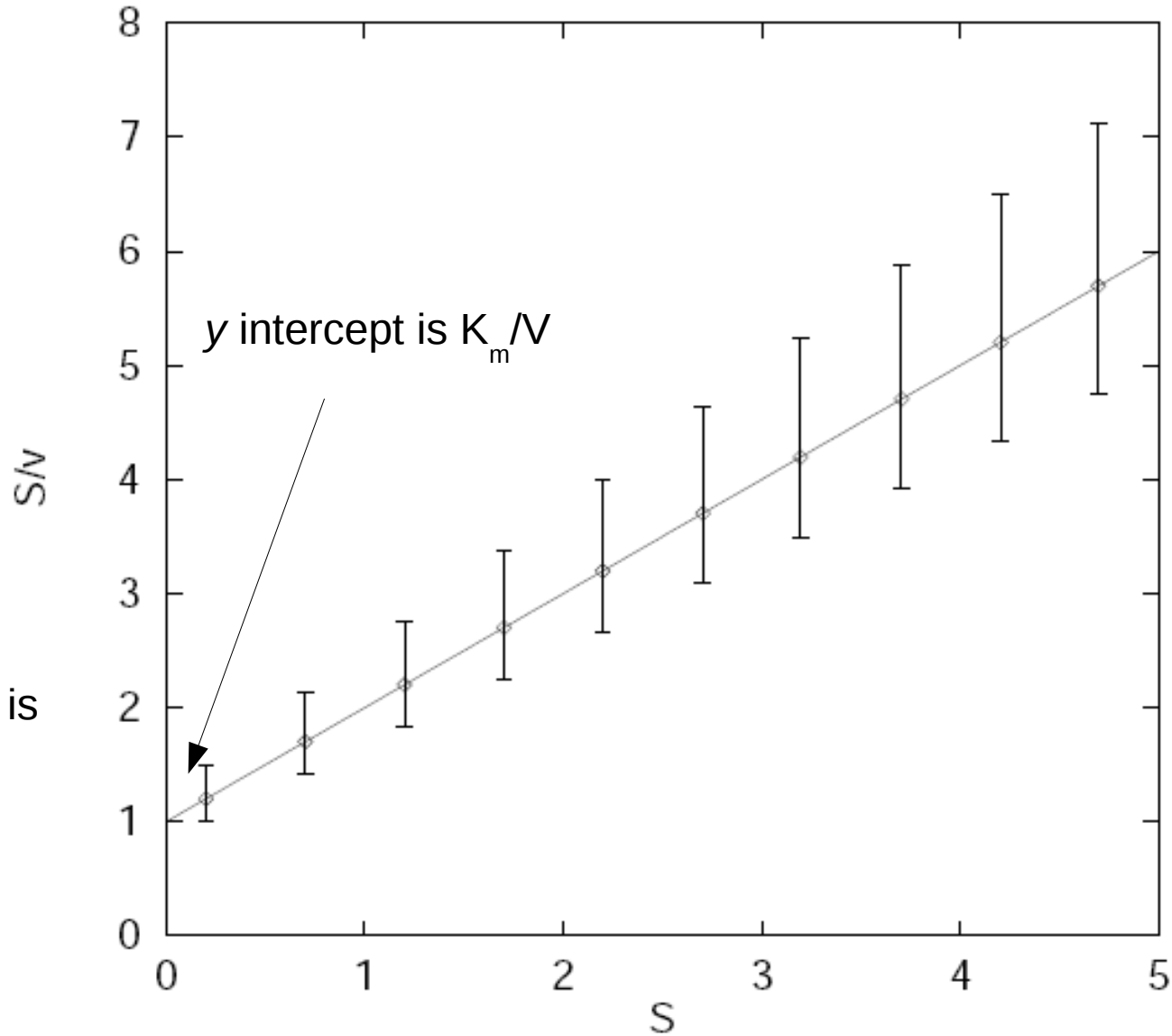
- Old approach – linear transformations
- New approach – non-linear fitting

# An enzyme kinetics experiment

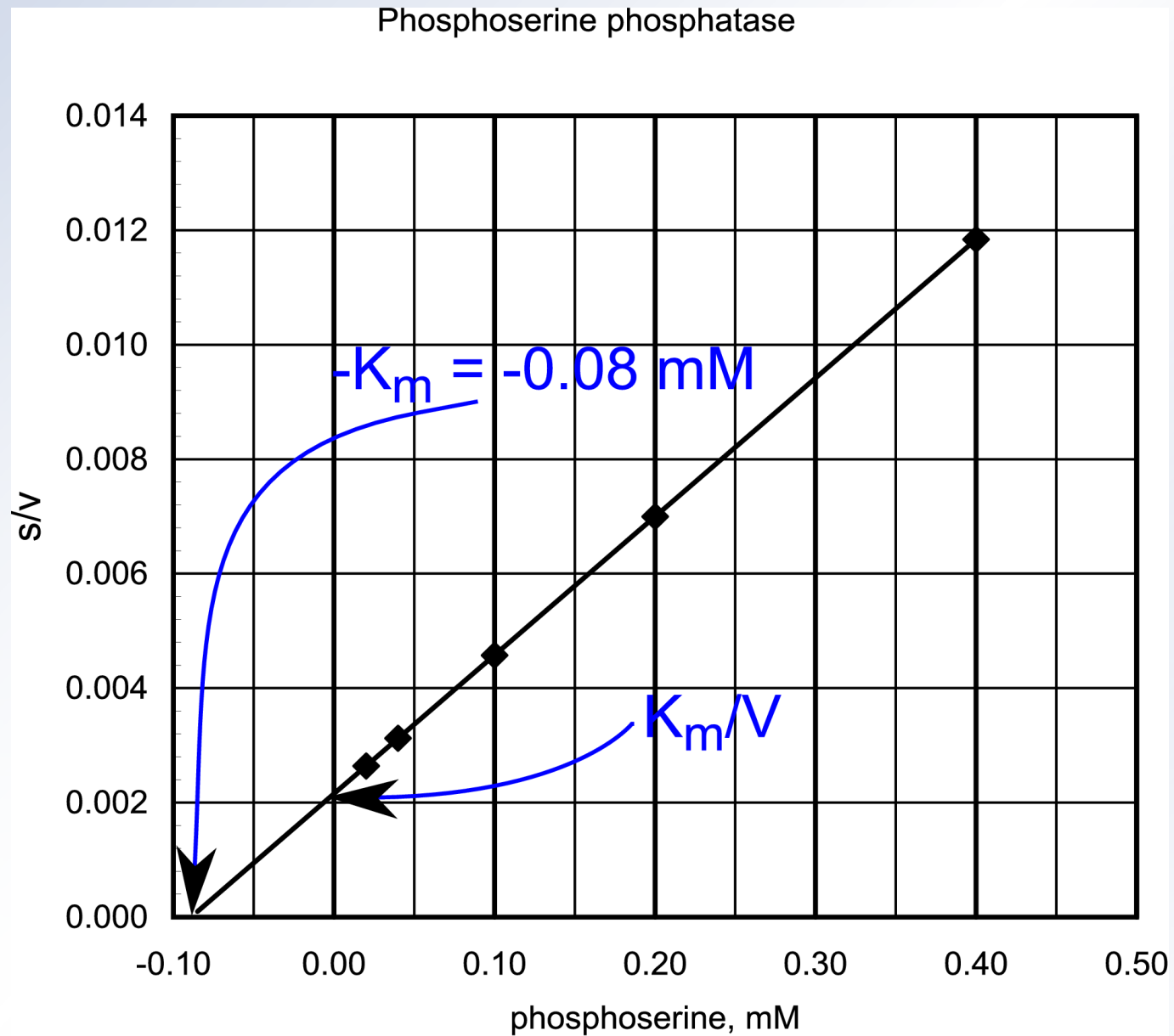




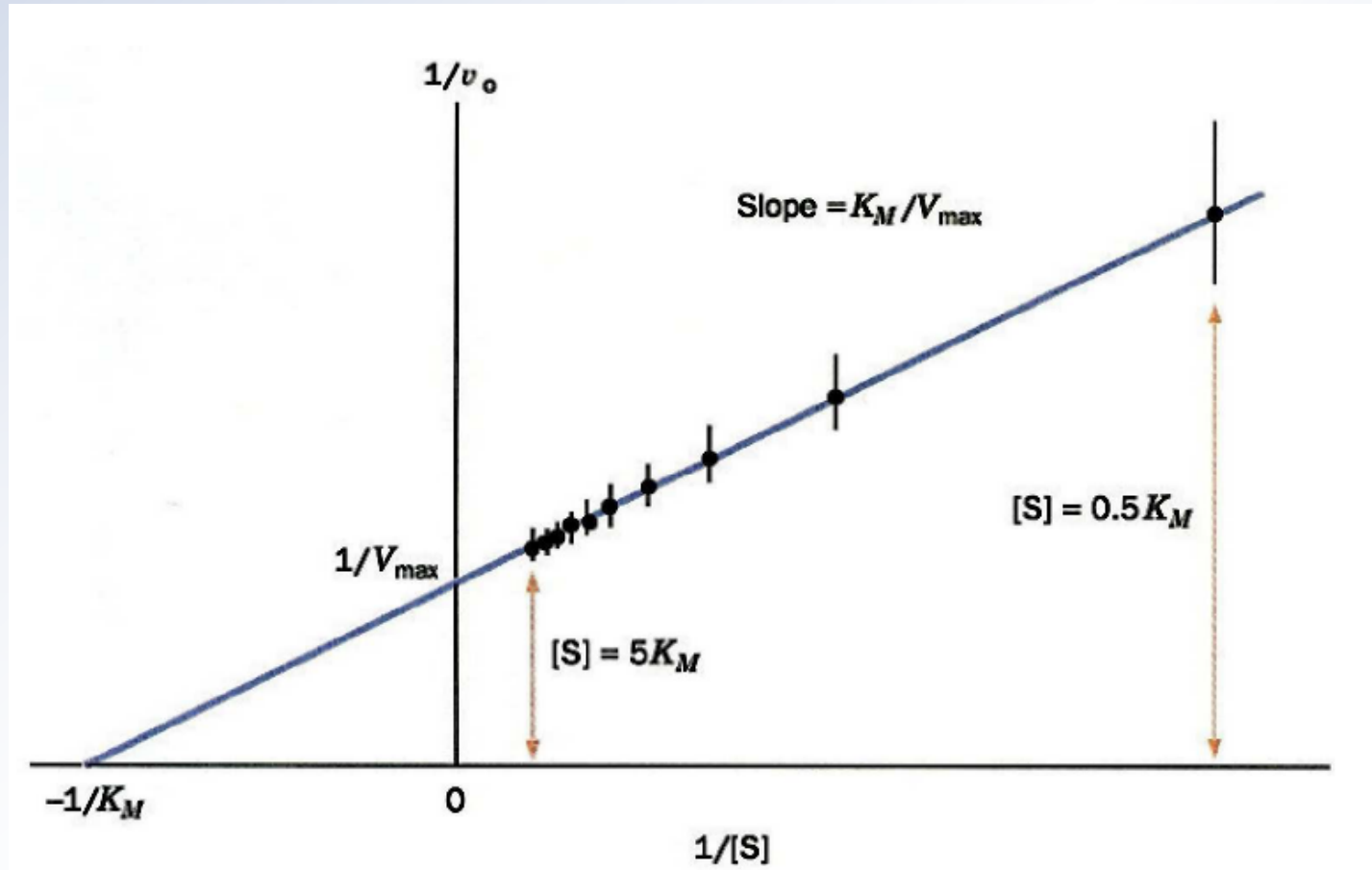
# Hanes plot



# Hanes plot showing intercepts



# Lineweaver-Burk plot



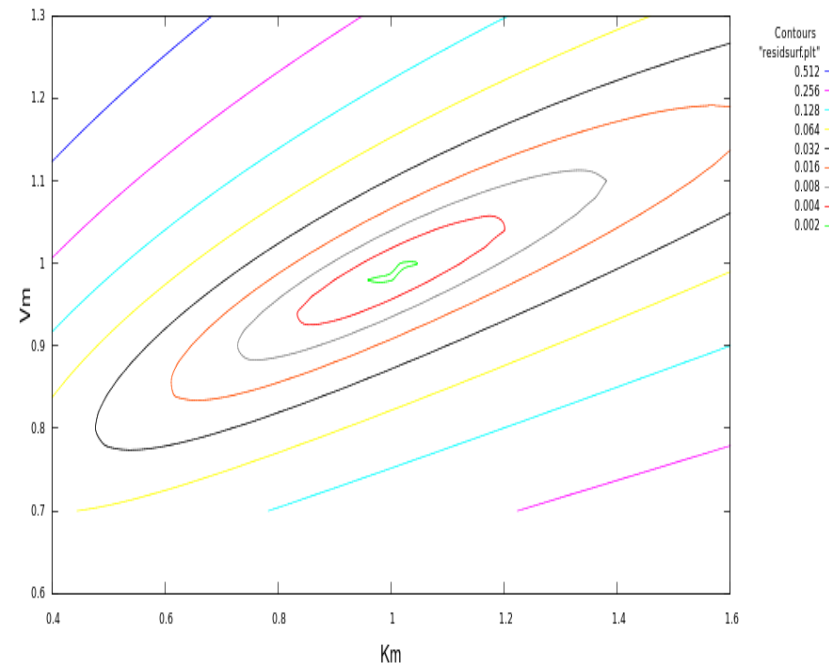
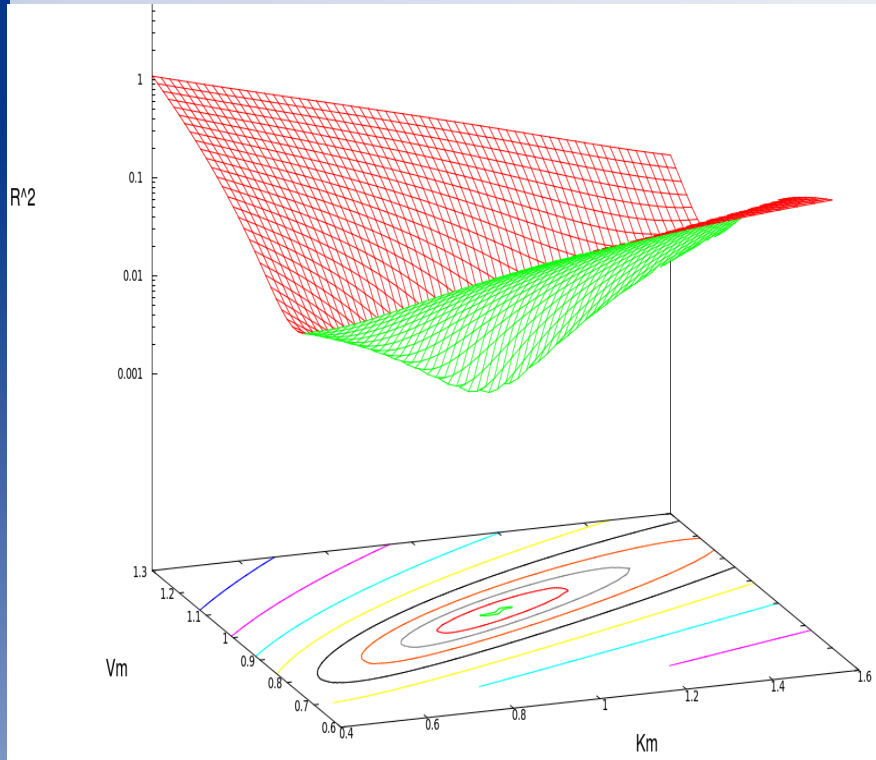
# Demonstration of non-linear fitting

- Finding the 'best-fit' hyperbola through a set of points.

(./runviewlogcont; ./runviewcontmap; gnuplot / load 'enzfitter.gnu')

- The aim is to minimise the sum of the squares of the deviations of the experimental points from a hyperbola by iteratively adjusting  $K_m$  and  $V$ .

# Dependence of the sum of squares on $K_m$ and $V$



## Finding the optimum

- For non-linear problems, solutions are obtained iteratively starting with initial estimates.
- Nonlinear solvers use various strategies to move 'downhill' to the solution.
- If there is more than one dip in the surface, and initial estimates are poor, the solver may find the nearest minimum and miss the best solution. Try using different initial estimates.
- There may be no satisfactory solution if:
  - Two or more parameters are strongly correlated so that changing one parameter can be compensated by changing one or more others.
  - The experimental points contain no information about one or more parameters.



# Problem

- The file `enz2.dat` contains data for the kinetics of phosphoserine phosphatase.
- Determine the  $K_m$  and  $V$  for the enzyme with respect to its substrate phosphoserine, either by:
  - Examining file `'enzfitter.gnu'` and performing the equivalent analysis on this data, or
  - Using the Excel file `'enzfitter.xls'` and modifying it with this data. You need to have the solver add-in activated in your copy of Excel.

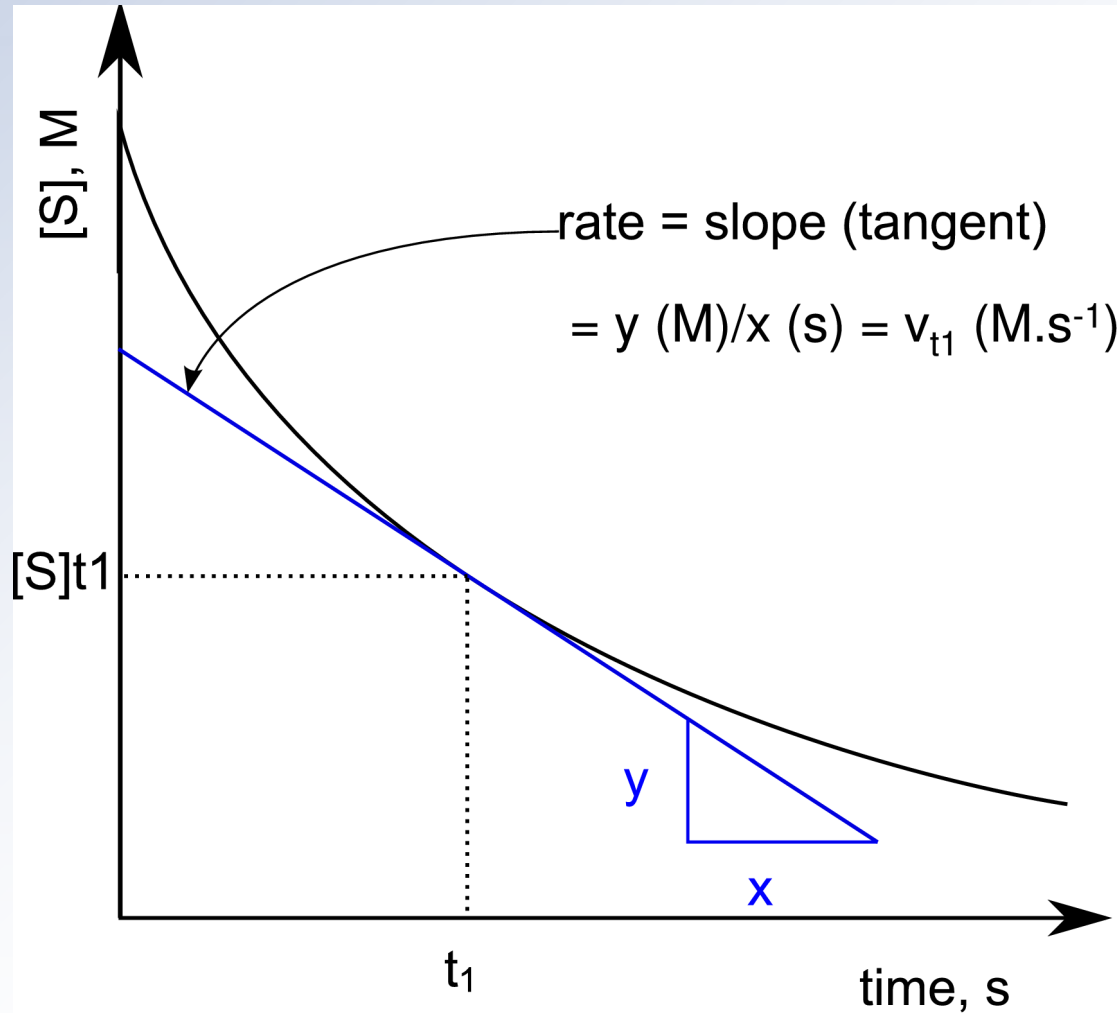
## Calculating Time Courses

Consider the Michaelis-Menten equation for the rate,  $v$ , of the catalysed reaction:

$$S \rightarrow P$$
$$v = \frac{V * S}{K_m + S}$$

Usually, the rate is measured as an initial rate at the start of the reaction ( $t = 0$ ) when  $P = 0$  and  $S$  is the initial concentration  $S_0$ .

## Rate at time $t_1$



$$v = \frac{dS}{dt} = f(S)$$

This is an Ordinary Differential Equation (ODE) because the derivative is with respect to one independent variable,  $t$ .

# Derivatives, finite changes and integration

$$\frac{dS}{dt} \approx \frac{\delta S}{\delta t}$$

where  $\delta S$  means a small, but not infinitesimal, change in  $S$ .

Therefore, for a small interval of time,  $\delta t$ , we can calculate the change in  $\delta S$  as:

$$\delta S \approx \frac{dS}{dt} \delta t$$

Hence if we know that at  $t_1$ ,  $S = S_{t_1}$ , then  $S$  at  $t_2 = t_1 + \delta t$  is:

$$S_{t_2} = S_{t_1} + \frac{dS}{dt} \delta t$$

# Euler integration

- Repeated application of the previous equation at a series of time points  $t_0, t_1 \dots t_n$  allows us to estimate  $S_{t_n}$  given a starting value  $S_0$ .
- This is the Euler integration method. It is easy to calculate even with a spreadsheet ...  
Try it ...for the MM equation with  $S_0 = 8$ ,  $K_m = 3.0$ ,  $V = 2.0$
- The issue is how to choose a suitable value for  $\delta t$ .
- (See 'euler.xls')



## But don't use Euler integration!

- The Euler method illustrates the basic principles, but is not advisable in general. When the function has very variable curvature (in kinetics, fast and slow processes), no single step length can be chosen to ensure accuracy.
- Improvements act in a number of ways:
  - 'Higher order' methods, e.g. Runge-Kutta, use information from more than a single point to make estimates, and correct for, the local curvature.
  - Variable step length methods continually adapt the step size according to how rapidly the function is changing.
  - Methods for solving 'stiff' ODEs combine both the previous features (e.g. Gear-type methods) and may also switch between methods during the course of the solution.

# Representing cellular systems

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Matrices and vectors