Basic Concepts for Modelling Enzyme Kinetics

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- Fundamentals of Enzyme Kinetics
- Simulating an enzyme time course
- Accounting for the Product
- Enzyme kinetics and pathway steady states
- Stochastic Modelling
- Reading

- Michaelis–Menten enzyme kinetics
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- Making Initial Rate Measurements
- Chemical and Enzyme Reactions Differ
- Michaelis–Menten Enzyme Kinetics
- The Meaning of K_{m}

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Initial Rate Measurements

The *rate of an enzyme reaction*, v, is now defined as the change in *concentration* of product in unit time. Formerly it was defined as the *amount*, in moles or μmoles, formed per unit time (which is now termed the *rate of conversion* and used as a basis for the unit of enzyme catalytic activity).

Since there is usually 100% conversion of substrates to products, rates can usually also be measured by use of substrate.

Unless otherwise stated, rates refer to *initial rates*, the instantaneous rate for known concentrations of substrates *in the absence of products*.

Making Initial Rate Measurements

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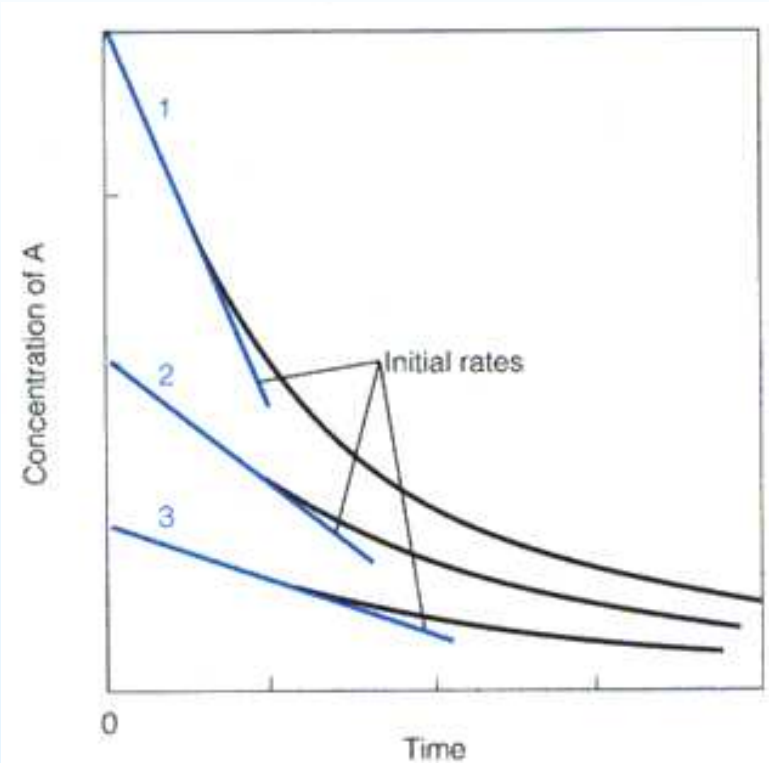
For a reaction $S \longrightarrow P$, started at different concentrations of S and zero P:



Initial rate measurement is easier with continuous rather than intermittent or spot measurement.

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Chemical and Enzyme Reactions Differ

For a simple chemical reaction S $\longrightarrow$ P, the uncatalysed *rate* of reaction in the absence of P would normally be directly proportional to S, i.e.

$$v = k.S$$

where v is the rate and k is a rate constant. (This is known as first–order kinetics.)

Enzyme-catalysed reactions are different: the rate does not depend linearly on substrate concentration.

Michaelis–Menten Enzyme Kinetics

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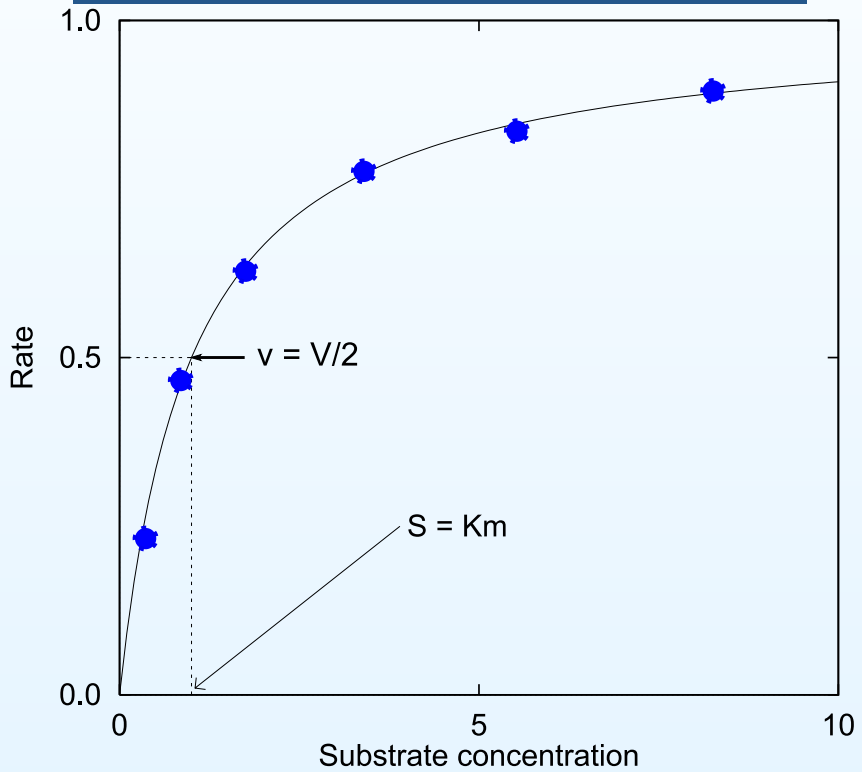
$$v = \frac{SV}{S + K_{\mathrm{m}}} \quad \text{or} \quad v = f(S)$$



The K_{m} and V have arbitarily been set to 1, where V is the *limiting rate* (or maximum velocity, V_{m}) and K_{m} is the *Michaelis constant*.

The Meaning of K_{m}

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$$v = \frac{SV}{S + K_{\mathrm{m}}}$$

Consider the case where $K_{\mathrm{m}} = S$. Then:

$$v = \frac{SV}{S + S} = \frac{SV}{2S} = \frac{V}{2}$$

That is, K_{m} is the *substrate concentration* at which the initial rate of reaction is half V.

Its usual unit is mol·dm^{-3}, i.e. M, or a derivative (e.g. mM).

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- But Don't Use Euler Integration

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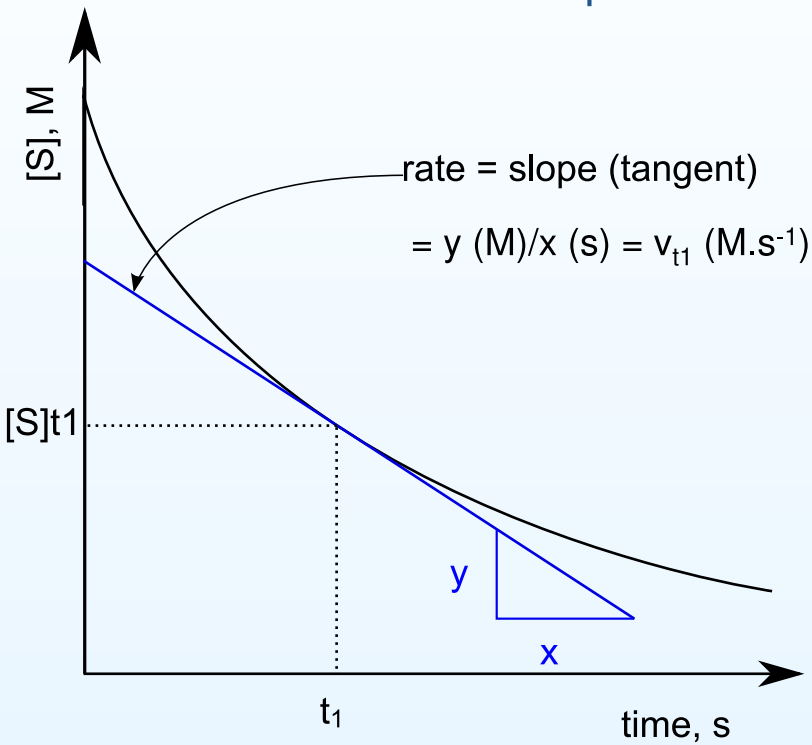
How do we link the rate equation and the time course?



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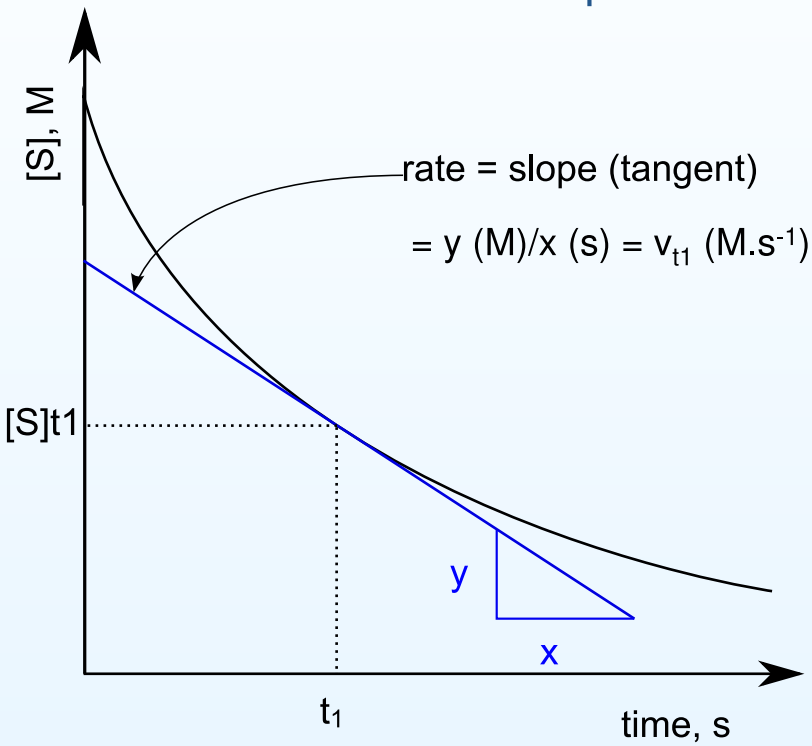

$$v = -\frac{dS}{dt} = f(S)$$

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

Derivatives, finite changes and integration

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

where δS means a small, but not infinitesimal, change in S.

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where δS means a small, but not infinitesimal, change in S. Therefore, for a small interval of time, δt, we can calculate the change in δS as:

$$\delta S \simeq \frac{dS}{dt}.\delta t$$

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$$\delta S \simeq \frac{dS}{dt}.\delta t$$

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

$$\boxed{S_{t_2} \simeq S_{t_1} + \frac{dS}{dt}.\delta t}$$

Euler Integration

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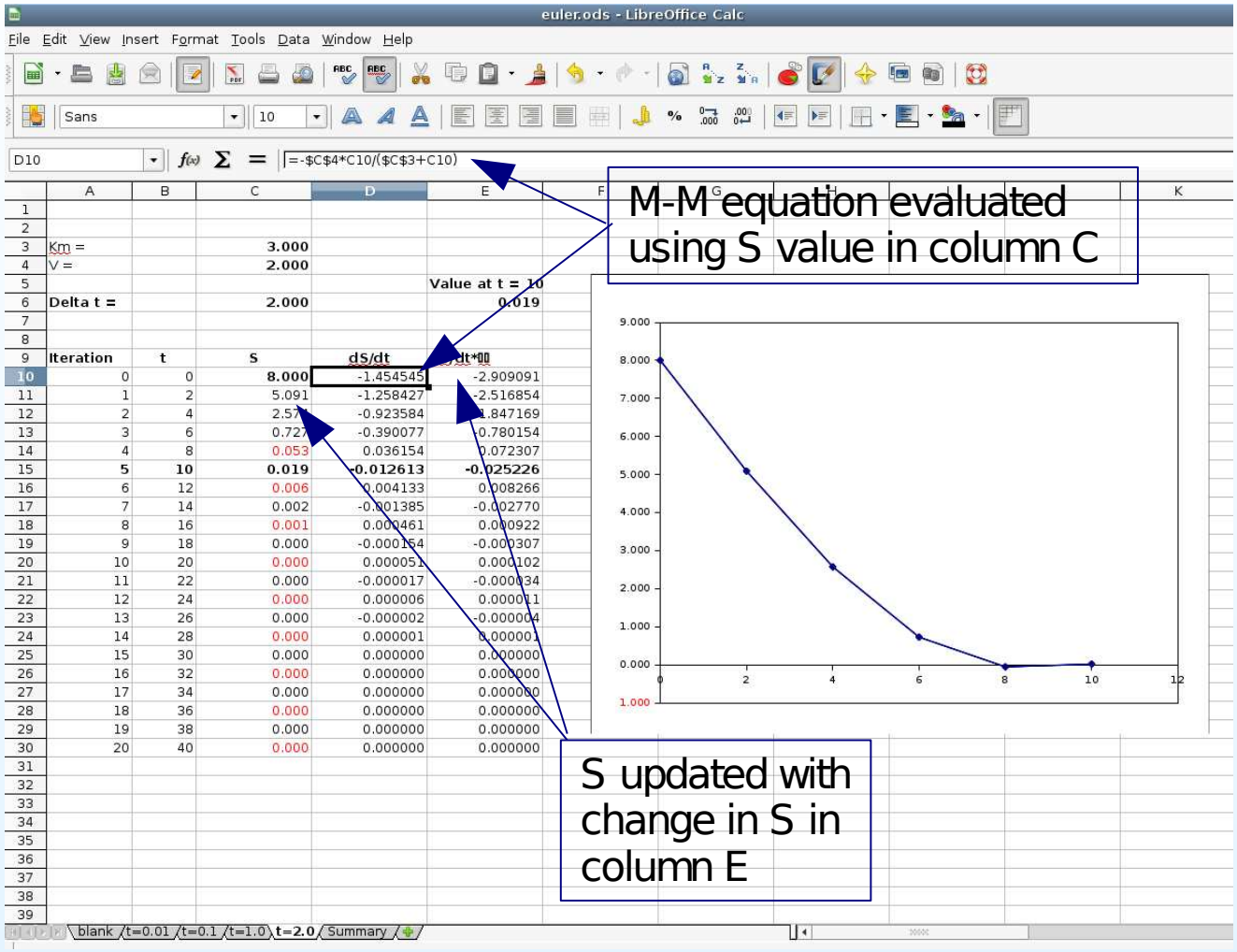
- Repeated application of the previous equation at a series of time points $t_0, t_1 \ldots t_n$ allows us to estimate S_{t_n} given a starting value S_{t_0}.
- This is the Euler integration method. It is easy to calculate even with a spreadsheet ...
- The difficult issue is how to choose a suitable value for δt

Euler by Spreadsheet



	A	B	C	D	E
3	Km =		3.000		
4	V =		2.000		
5					Value at t = 10
6	Delta t =		2.000		0.019
9	Iteration	t	S	dS/dt	dt*dS/dt
10	0	0	8.000	-1.454545	-2.909091
11	1	2	5.091	-1.258427	-2.516854
12	2	4	2.571	-0.923584	-1.847169
13	3	6	0.727	-0.390077	-0.780154
14	4	8	0.053	0.036154	0.072307
15	**5**	**10**	**0.019**	**-0.012613**	**-0.025226**
16	6	12	0.006	0.004133	0.008266
17	7	14	0.002	-0.001385	-0.002770
18	8	16	0.001	0.000461	0.000922
19	9	18	0.000	-0.000154	-0.000307
20	10	20	0.000	0.000051	0.000102
21	11	22	0.000	-0.000017	-0.000034
22	12	24	0.000	0.000006	0.000011
23	13	26	0.000	-0.000002	-0.000004
24	14	28	0.000	0.000001	0.000001
25	15	30	0.000	0.000000	0.000000
26	16	32	0.000	0.000000	0.000000
27	17	34	0.000	0.000000	0.000000
28	18	36	0.000	0.000000	0.000000
29	19	38	0.000	0.000000	0.000000
30	20	40	0.000	0.000000	0.000000

But Don't Use Euler Integration

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- 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.

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- The Reversible M–M Eqn.
- Taking the equation apart: 1
- Taking the equation apart: 2
- Taking the equation apart: 3

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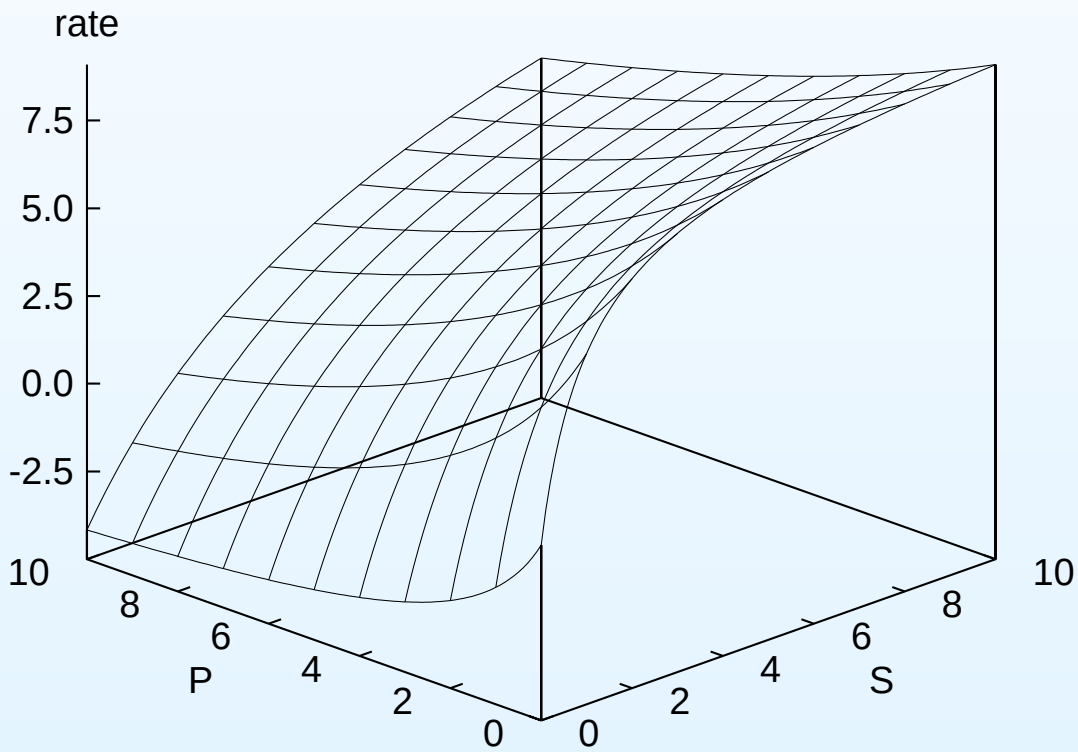
The Reversible M–M Eqn.

$$v_{net} = \frac{(V_f/K_{m,S})\,(S - P/K_{eq})}{1 + S/K_{m,S} + P/K_{m,P}} \quad \text{or} \quad v = f(S, P)$$



Simultaneous dependence of enzyme rate on both substrate and product. The parameters have been set to: $K_{m,S}$ =1;

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Taking the equation apart: 1

The equation is actually composed of two parts:

$$v_f = \frac{(V_{\mathrm{f}}/K_{\mathrm{m,S}})\,(S))}{1 + S/K_{\mathrm{m,S}} + P/K_{\mathrm{m,P}}}$$

and

$$v_r = \frac{(V_{\mathrm{f}}/K_{\mathrm{m,S}})\,(-P/K_{\mathrm{eq}})}{1 + S/K_{\mathrm{m,S}} + P/K_{\mathrm{m,P}}}$$

and

$$v_{net} = v_f + v_r$$

so it is the numerator term that contains the effect of the reverse reaction, whilst the denominator is common.

Taking the equation apart: 2

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Looking at the forward reaction only:

$$v_f = \frac{(V_\mathrm{f}/K_\mathrm{m,S})\,(S))}{1 + S/K_\mathrm{m,S} + P/K_\mathrm{m,P}}$$

the equation still contains a term in the product concentration P.

This reflects the *product inhibition* that exists because of its binding at the active site, even when the K_eq is so large that the reverse reaction rate v_r is very small.

Taking the equation apart: 3

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Considering the reverse reaction:

$$v_r = \frac{(V_\mathrm{f}/K_\mathrm{m,S})\,(-P/K_\mathrm{eq})}{1 + S/K_\mathrm{m,S} + P/K_\mathrm{m,P}}$$

This could also be written as the forward component of the equation written for $P \longrightarrow S$:

$$v_r = -\frac{(V_\mathrm{r}/K_\mathrm{m,P})\,(P)}{1 + S/K_\mathrm{m,S} + P/K_\mathrm{m,P}}$$

which shows that:

$$K_\mathrm{eq} = \frac{V_\mathrm{f}}{K_\mathrm{m,S}} . \frac{K_\mathrm{m,P}}{V_\mathrm{r}}$$

This is the *Haldane relationship*, showing that it suffices to know three of the four parameters provided the K_eq is known.

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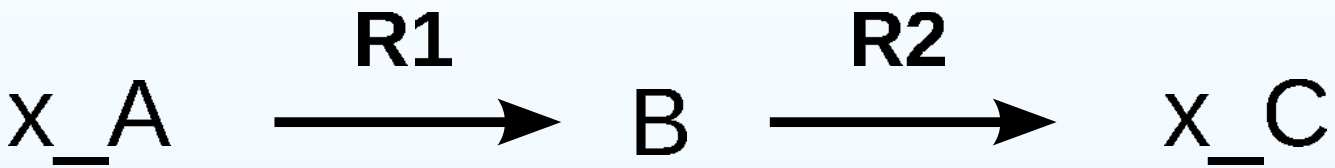
Steady State of a Pathway

Consider the case where an enzyme is in a pathway, where its product is present as the substrate of the next enzyme, e.g.:



If both reactions R1 and R2 are Michaelis–Menten enzymes, how will their rates depend on metabolite B?

Graphical Solution

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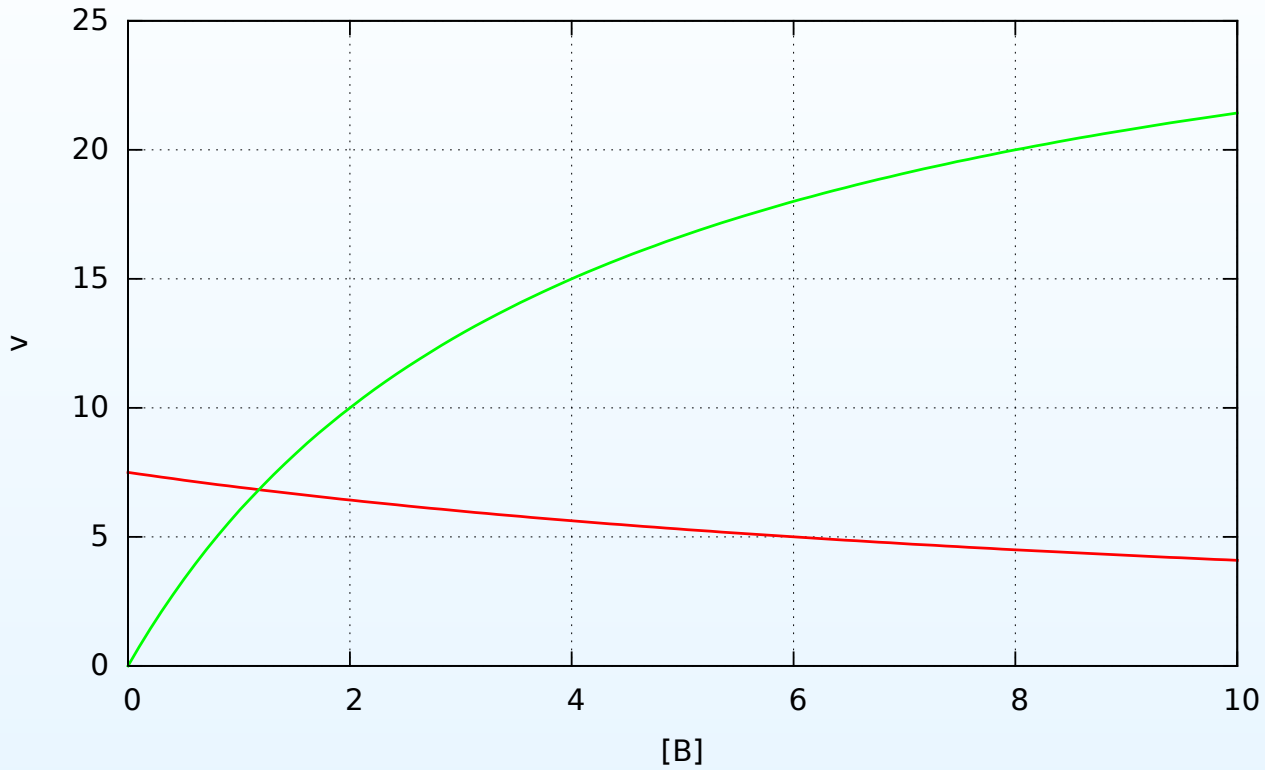

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Plotting with Gnuplot

The example was obtained using gnuplot as follows:

```
~$ gnuplot
gnuplot> mm(S) = Vm*S/(Km + S)
gnuplot> rmm(S,P) = Vm1*(S -P/Keq1)/
(Km1*(1+P/Kp1) + S)
gnuplot> Vm = 30
gnuplot> Km = 4
gnuplot> Vm1 = 15
gnuplot> Keq1 = 1000000
gnuplot> Km1 = 5
gnuplot> Kp1 = 6
gnuplot> A = 5
gnuplot> plot [B=0:10] rmm(A,B) wi li lw 3,
 mm(B) wi li lw 3
```

Interpreting the Graph — 1

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What happens next if B is currently 3.0 mM?

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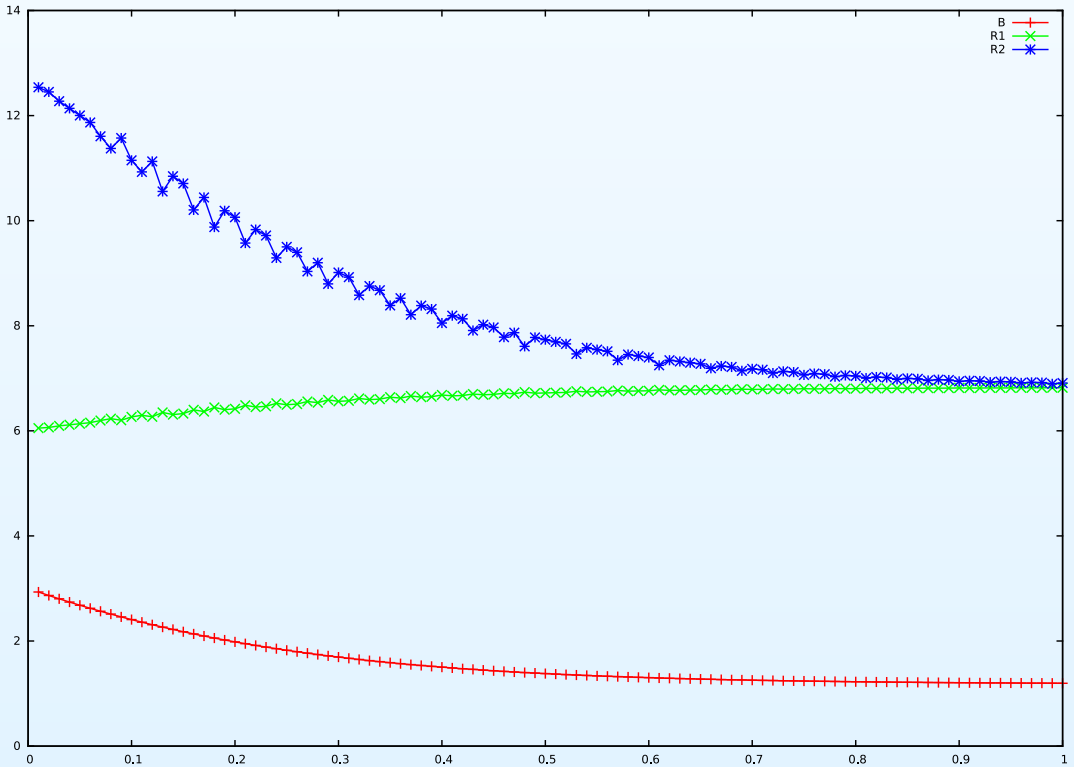

Interpreting the Graph — 2

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What happens next if B is currently 0.5 mM?

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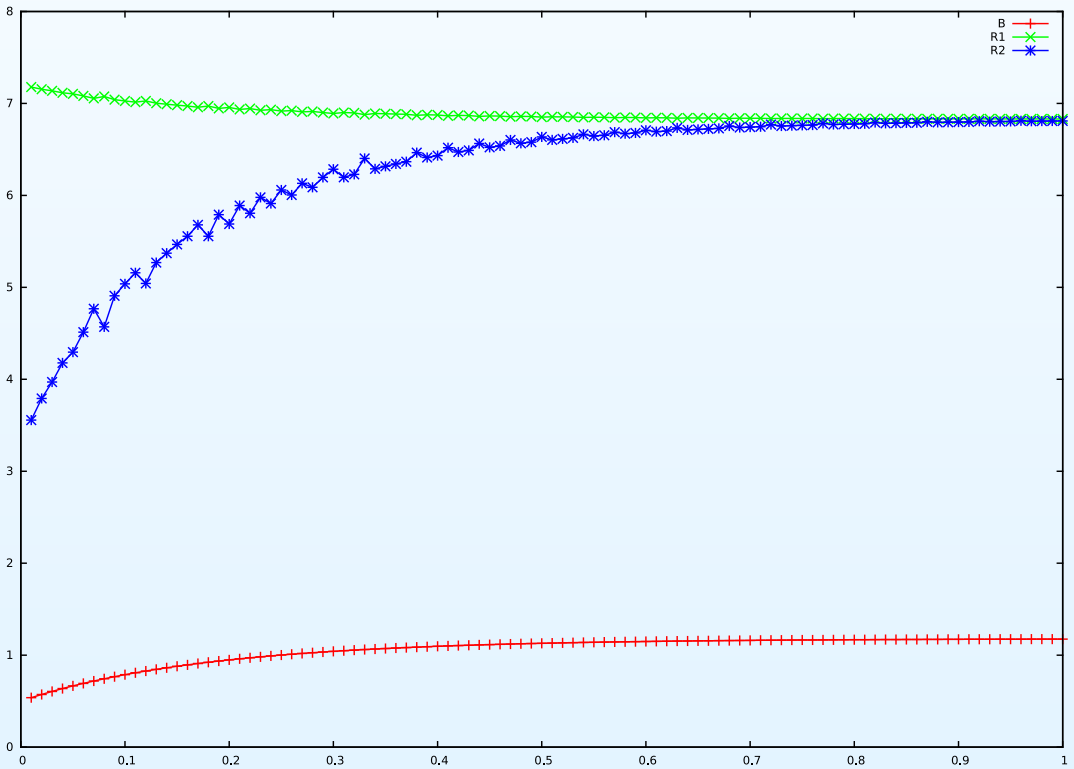
What happens next if B is currently 0.5 mM?



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Stochastic Dynamic Modelling

- Individual molecules/particles of the species are represented — with or without spatial information — and the fate of each particle is followed. Track particle numbers, not concentrations.
- At each small time step, a molecule may move, react or remain unchanged with a probability related to the diffusion and rate constants.
- Computationally demanding; only feasible for modelling small volumes, and outcome is different every time.
- However, represents the intrinsic variability in systems with small numbers (< 1000) of reacting particles (e.g. DNA molecules, some transcription factors etc).

Reading List

- Schuster, S. and Fell, D. A. (2007) Modelling and simulating metabolic networks. In Lengauer, T. (ed.), *Bioinformatics: From Genomes to Therapies*, vol. 2, pp. 755–806. Wiley–VCH, Weinheim
- Fell, D. A. (2008) Metabolic networks. In Képès, F. (ed.), Biological Networks, vol. 3 of *Complex Systems and Interdisciplinary Science*. World Scientific Publishing Co, Singapore