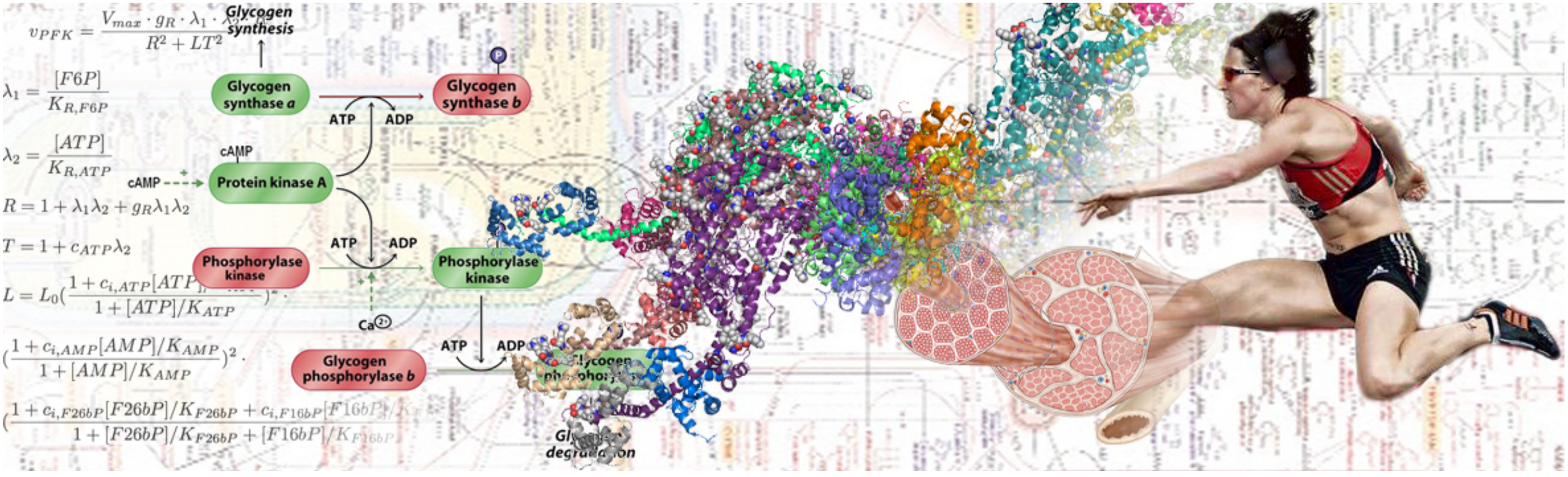
Biochemistry 714 Mini-course: Molecular Systems Biology



Prof Jacky Snoep (lectures), Prof Johann Rohwer (tutorials and data analysis), Dr Dawie van Niekerk (practical)

March - April 2024

Thus far

- First Lecture: Chemical kinetics
- Direction of reaction: ΔG, Γ / K_{eq}
- How far: K_{eq}, ΔG^0
- How fast: mass action kinetics

- Second Lecture: Enzyme kinetics
- Derivation of rate equations: equilibrium binding, steady-state approximation
- V_{max}, K_m, saturation, cooperativity, allostery, reversibility, product inhibition

Parameter estimation

- *in vitro* measurements on isolated components
- *in vivo*, system measurements

Enzymology

2 Phosphoglucoisomerase

$$v3PGI = \frac{\text{g6p}\left(1 - \frac{\text{f6p}}{\text{g6pKv3eq}}\right)\text{V3PGI}}{\left(1 + \frac{\text{f6p}}{\text{Kv3f6p}} + \frac{\text{g6p}}{\text{Kv3g6p}}\right)\text{Kv3g6p}}$$

	Estimate	Standard Error	t-Statistic	P-Value
$Kv3f6p$	0.096651	0.00499949	19.3322	2.12596×10^{-25}
$Kv3g6p$	0.974424	0.101138	9.63463	1.04986×10^{-12}

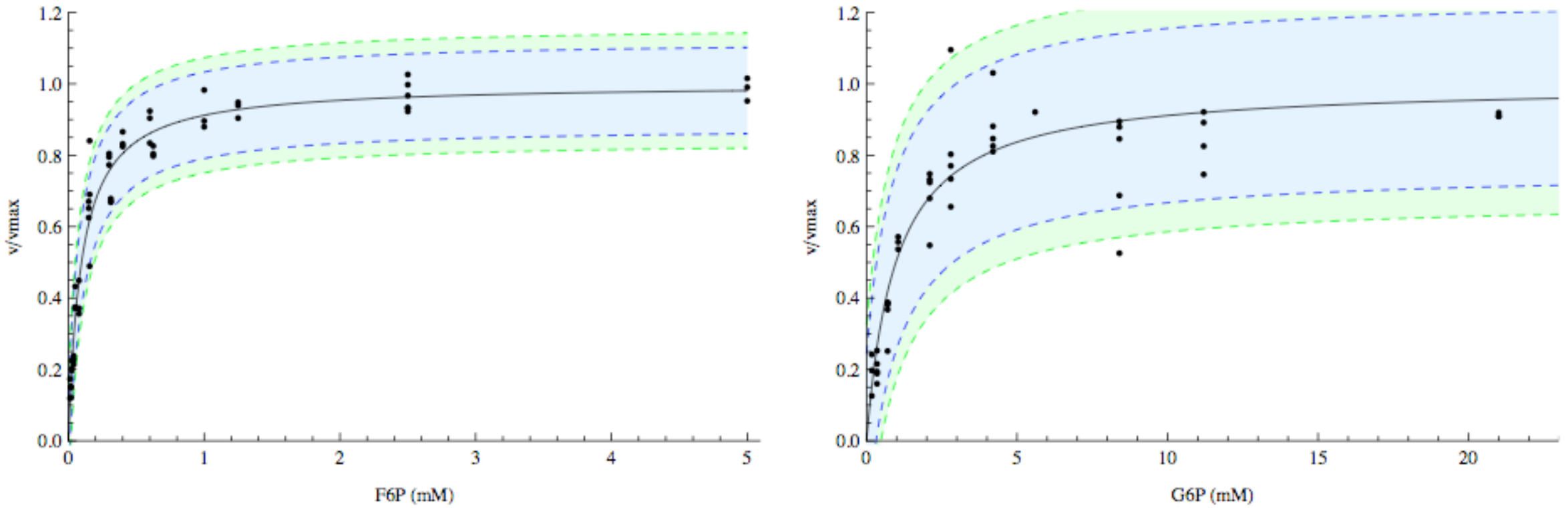


Figure 2: Characterization of Plasmodium falciparum phosphoglucoisomerase

Enzymology

Phosphofructokinase: Monod, Wyman, Changeux model; cooperative enzyme (physiological conditions, V_{max})

$$v_{PFK} = V_{max} \cdot \frac{g_R \cdot \lambda_1 \cdot \lambda_2 \cdot R}{R^2 + L \cdot T^2}$$

$$\lambda_1 = \frac{[F6P]}{K_{R,F6P}}$$

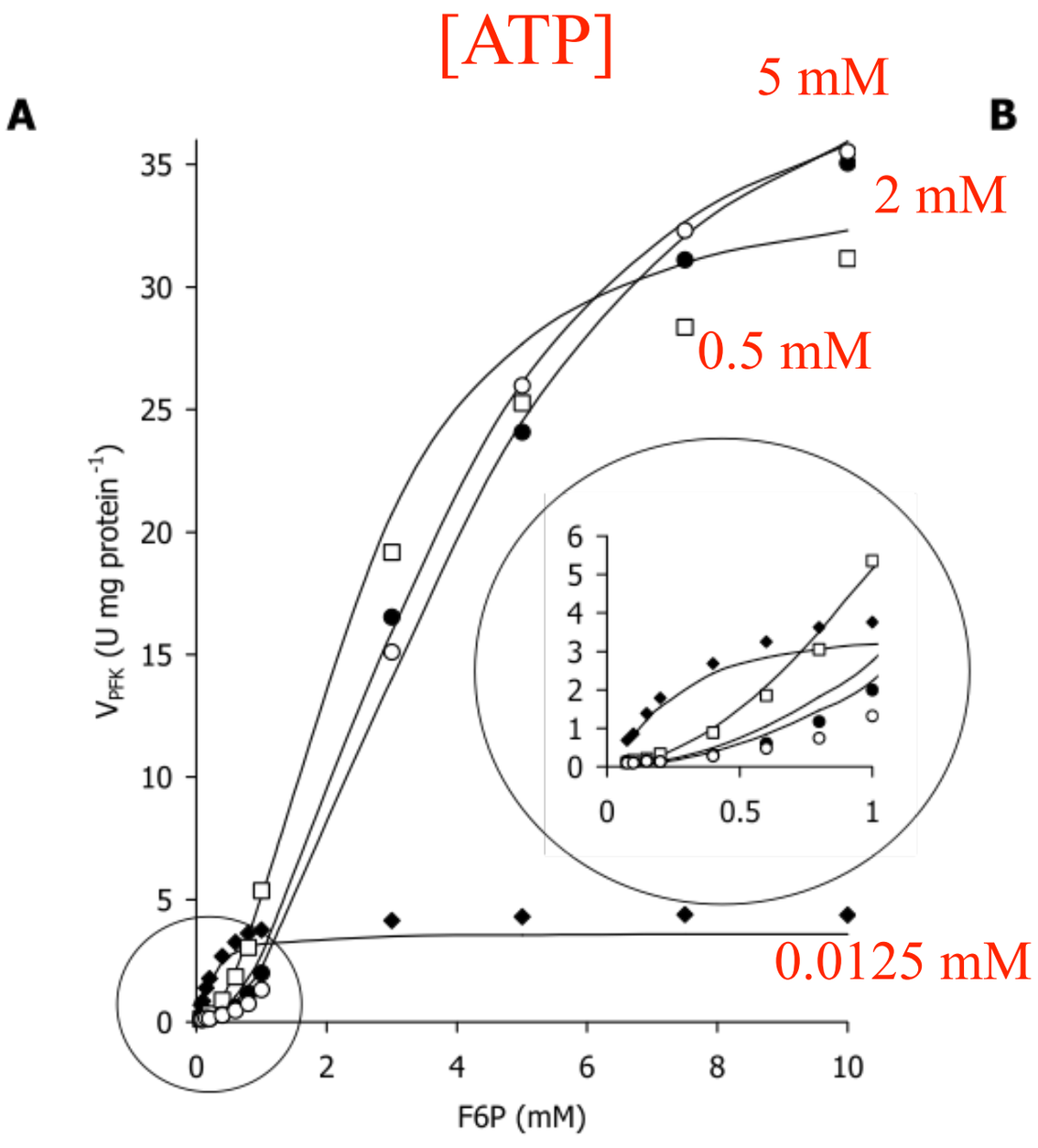
$$\lambda_2 = \frac{[ATP]}{K_{R,ATP}}$$

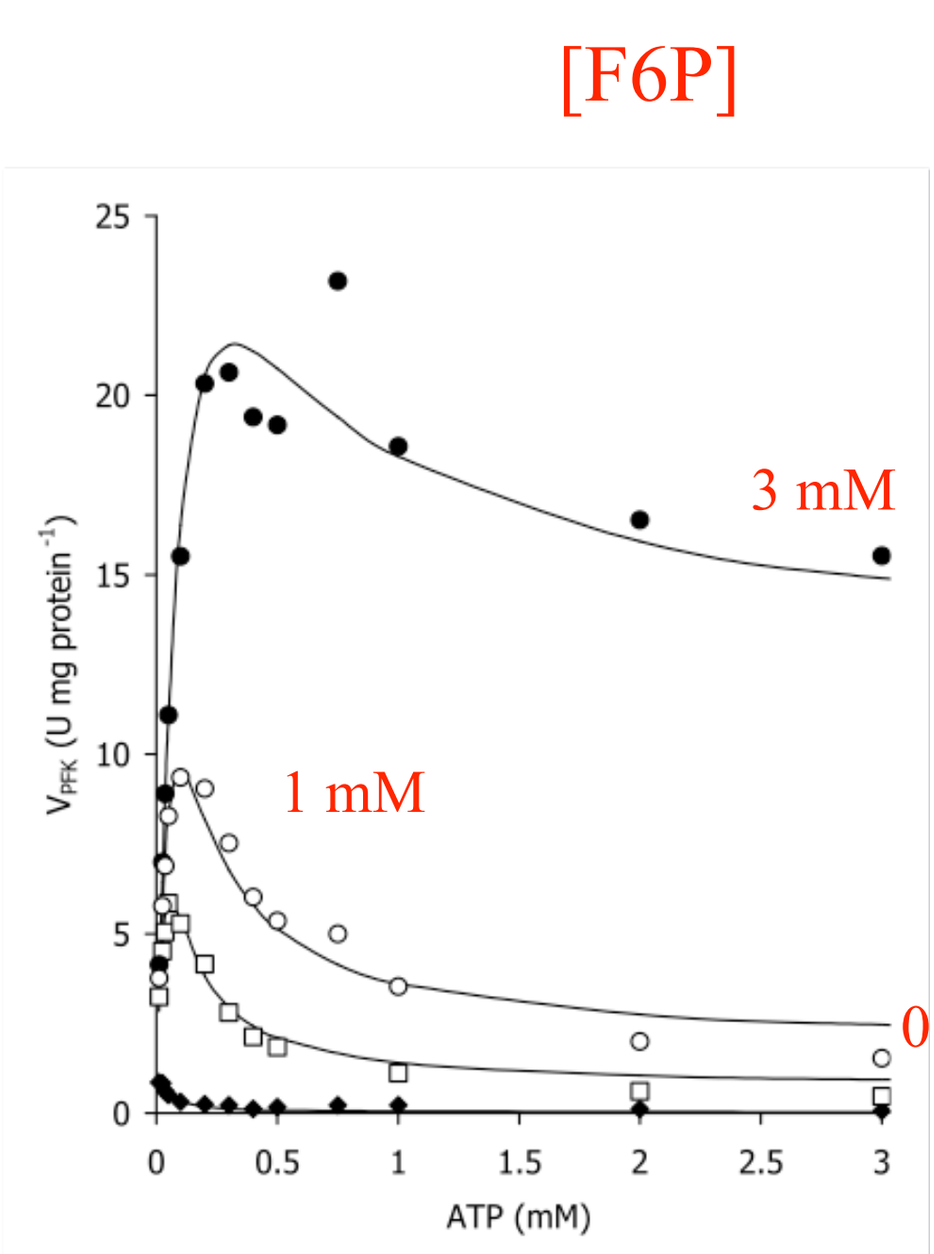
$$R = 1 + \lambda_1 \cdot \lambda_2 + g_R \cdot \lambda_1 \cdot \lambda_2$$

$$T = 1 + c_{ATP} \cdot \lambda_2$$

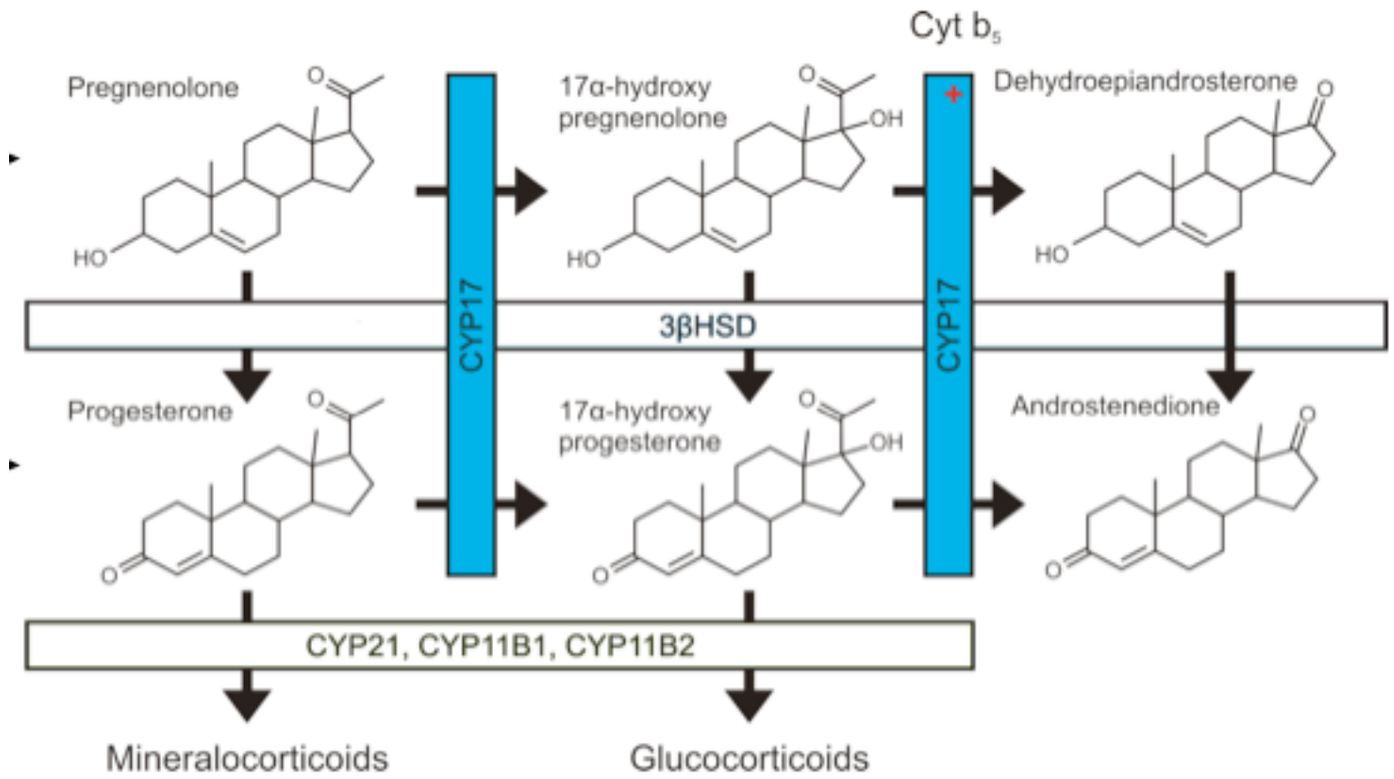
$$L = L_0 \cdot \left(\frac{1 + C_{i,ATP} \cdot [ATP]/K_{ATP}}{1 + [ATP]/K_{ATP}}\right)^2 \cdot \left(\frac{1 + C_{i,AMP} \cdot [AMP]/K_{AMP}}{1 + [AMP]/K_{AMP}}\right)^2 \cdot \left(\frac{1 + C_{i,F26bP} \cdot [F26bP]/K_{F26bP} + C_{i,F16bP} \cdot [F16bP]/K_{F16bP}}{1 + [F26bP]/K_{F26bP} + [F16bP]/K_{F16bP}}\right)^2$$

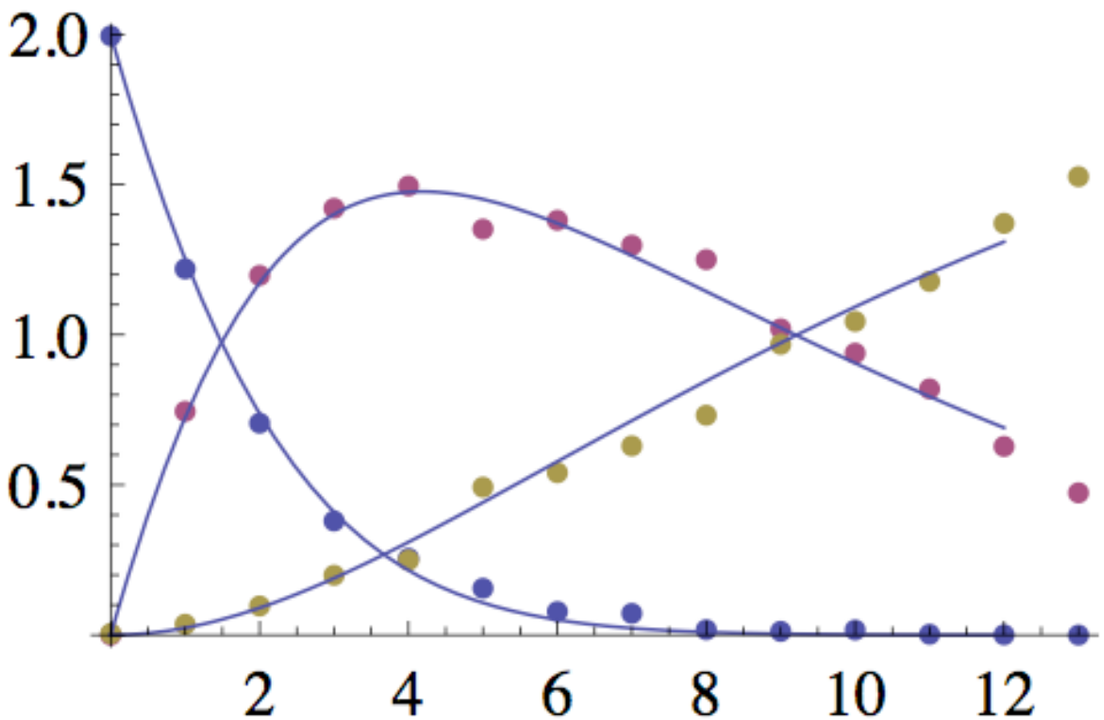
In vitro experimental data

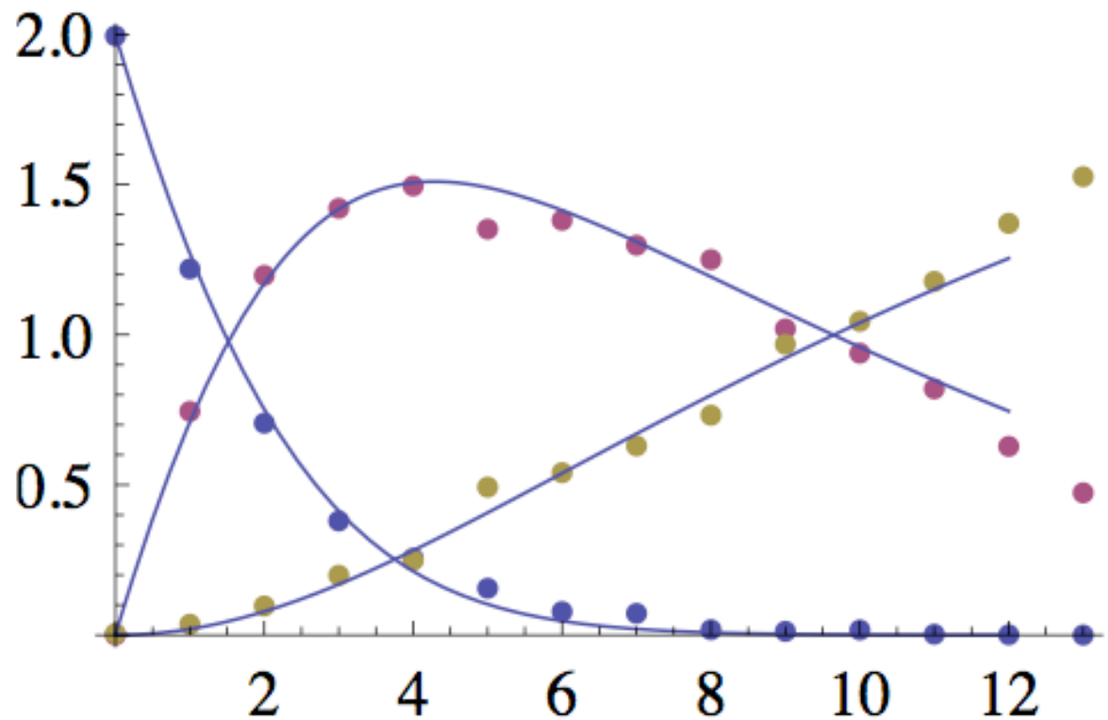




Progress curves – analysis of pathways







Coupled reactions

Consider the following set of coupled reactions:

$$A \rightleftharpoons B \rightleftharpoons C$$

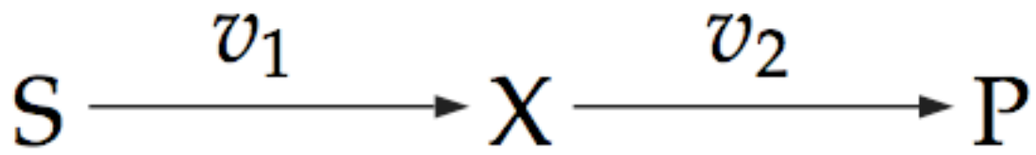
Assume a K_{eq} value of 10 for the first reaction and a value of 2 for the second reaction.

- What would the equilibrium ratio between C and A be?
- How would the ΔG values of the individual reactions relate to the ΔG of the overall system?
- Assume an initial concentration of A = 10 mM. Cal-culate the equilibrium concentrations for A, B, and C.
- Do the same if the K_{eq} values of the two reactions were exchanged.

Closed vs open systems

If left sufficiently long, all closed systems will eventually end up in an equilibrium state. Biological systems manage to stay away from equilibrium by a continuous uptake of substrates and excretion of products, i.e. they are open systems in terms of mass transport over the system boundary. Typically (but not always) such systems, when incubated under constant external conditions, end up in a steady state.

Two coupled irreversible reactions



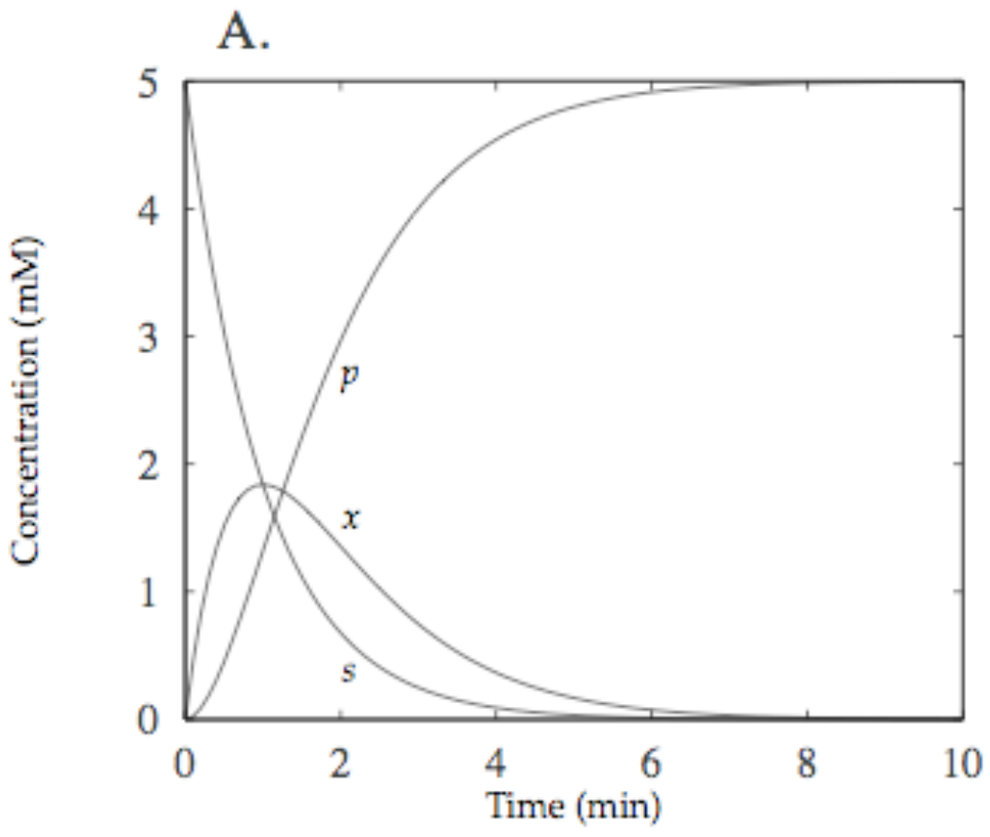
rate equations

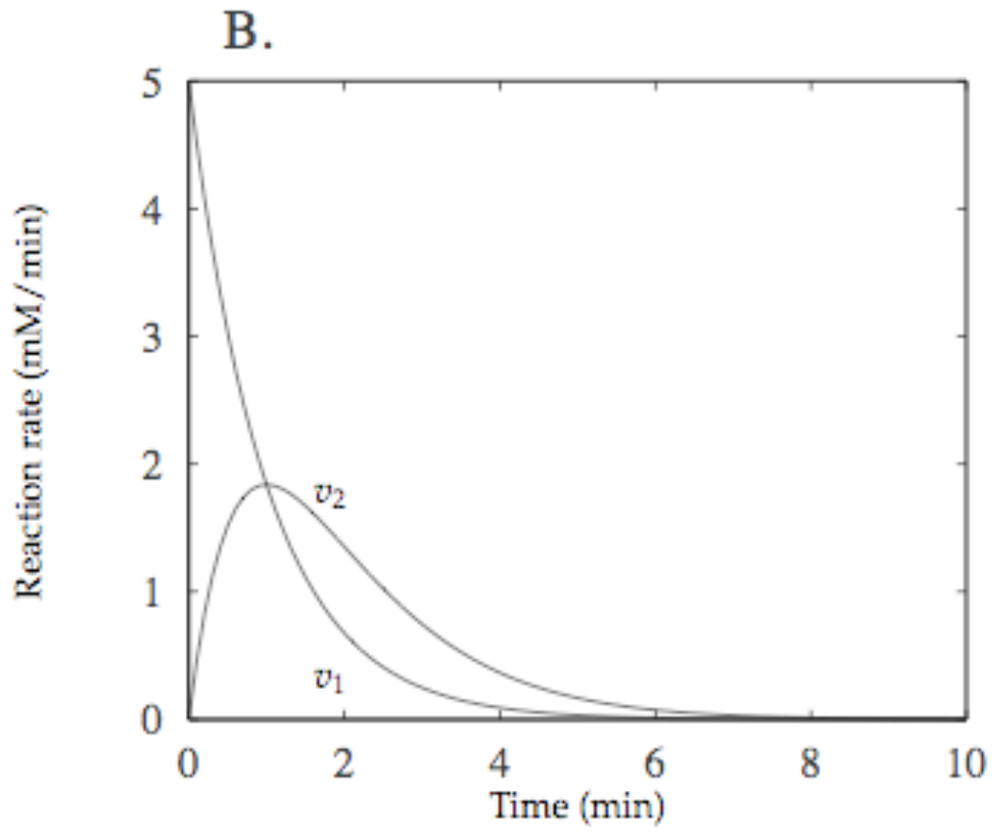
$$\begin{aligned} v_1 &= k_1 s \\ v_2 &= k_2 x \end{aligned}$$

balance equations

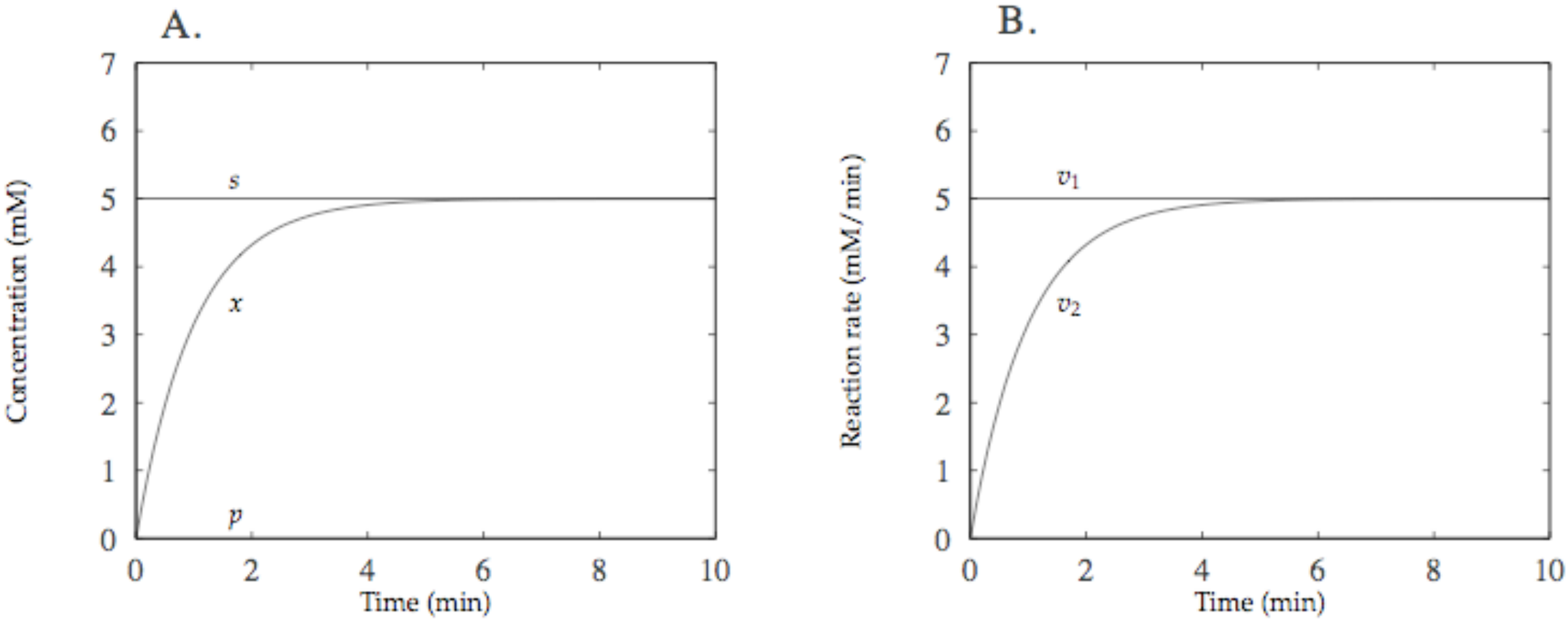
$$\begin{aligned} \frac{ds}{dt} &= -v_1 = -k_1 s \\ \frac{dx}{dt} &= v_1 - v_2 = k_1 s - k_2 x \\ \frac{dp}{dt} &= v_2 = k_2 x \end{aligned}$$

Progress curves: closed system





Progress curves: open system



Steady state

$$\frac{dx}{dt} = \bar{v}_1 - \bar{v}_2 = k_1 s - k_2 \bar{x} = 0$$

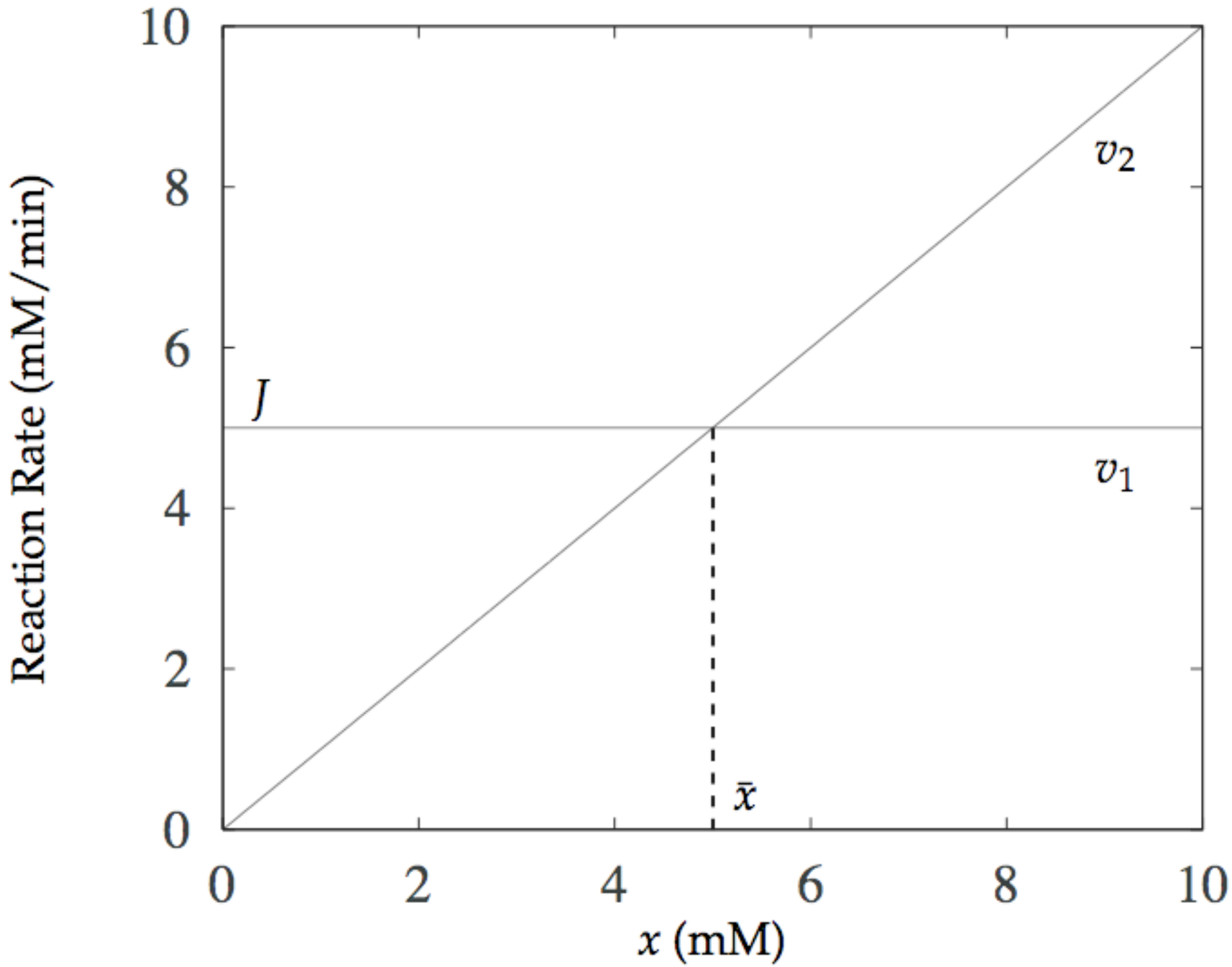
Steady state flux:

$$\bar{v}_1 = \bar{v}_2 = J$$

Steady state concentration:

$$\bar{x} = \frac{k_1 s}{k_2}$$

Rate characteristics



Exercise 1

Given an open system consisting of two enzymes that catalyze the conversion of substrate S (fixed at 10 mM) to product P (fixed at 1 mM), with common intermediate X.

The enzymes obey reversible Michaelis-Menten kinetics with identical parameter values:
V_{mf} = 1 mM/s, K_{eq} = 10, $K_{m,substrate}$ = 1mM, $K_{m,product}$ = 10 mM

Calculate the steady-state flux and the steady-state concentration of the intermediate X.

Analysis of example pathways of the kinetic model in steady state

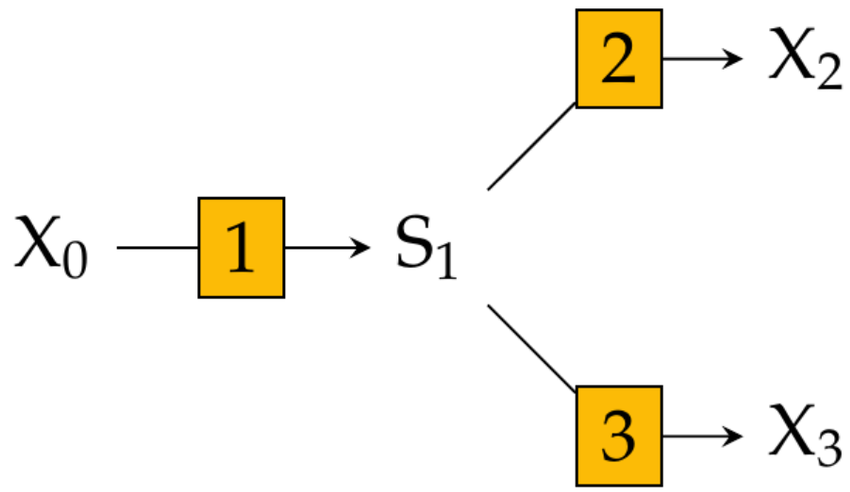
Example: Linear system in steady state

$$
\begin{aligned}
ds_1/dt &= J_1 - J_2 = 0 \\
ds_2/dt &= J_2 - J_3 = 0
\end{aligned}
$$

Flux relationships

$$J_1 = J_2 = J_3$$

Example: Branched system in steady state

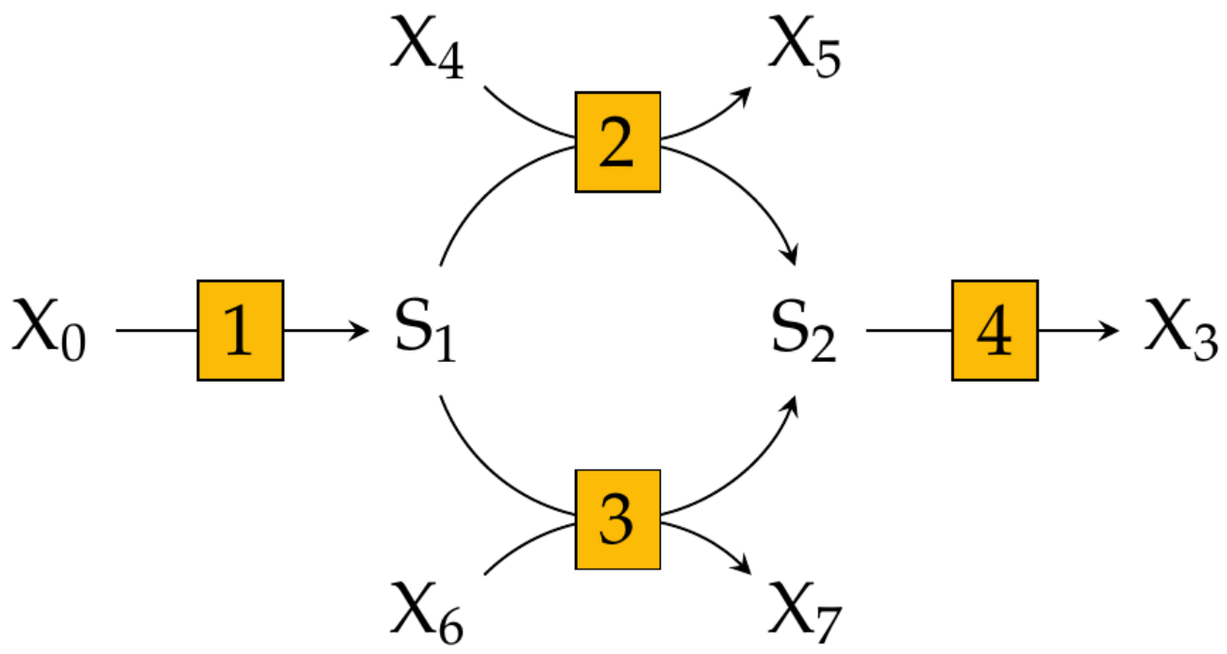


$$ds_1/dt \quad = \quad J_1 - J_2 - J_3 = 0$$

Flux relationships

$$J_1 = J_2 + J_3$$

Example: Parallel looped system in steady state

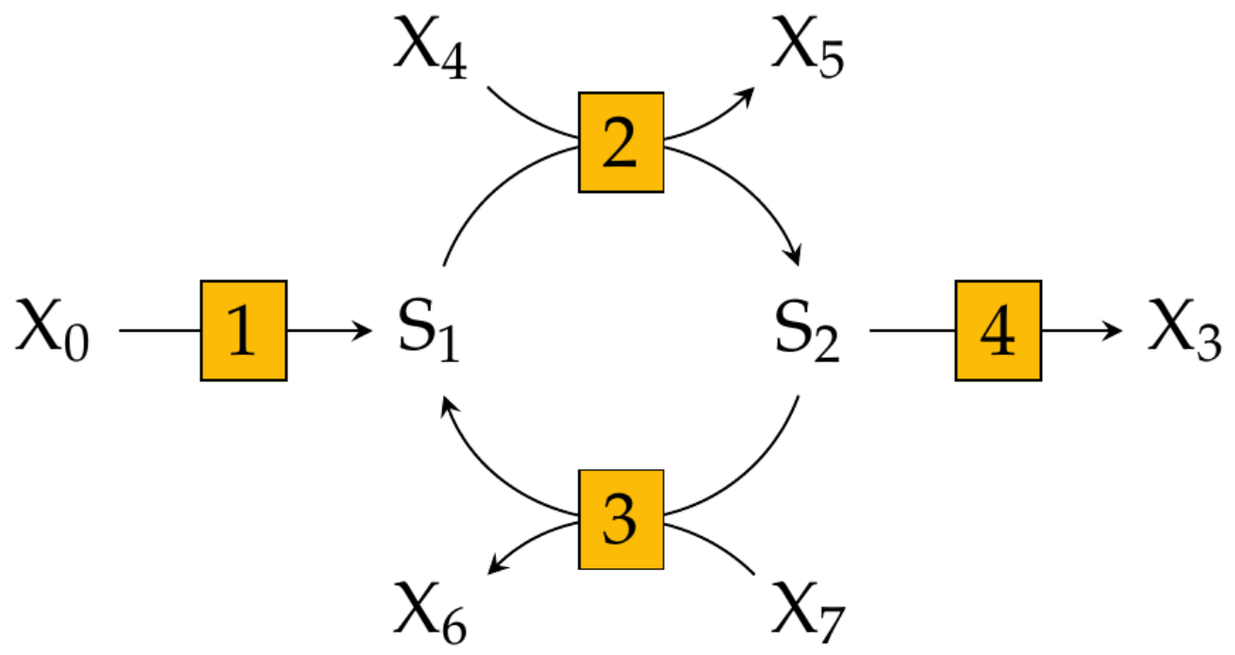


$$\begin{aligned} ds_1/dt &= J_1 - J_2 - J_3 = 0 \\ ds_2/dt &= J_2 + J_3 - J_4 = 0 \end{aligned}$$

Flux relationships

$$J_1 = J_4 = J_2 + J_3$$

Example: Anti-parallel looped system in steady state

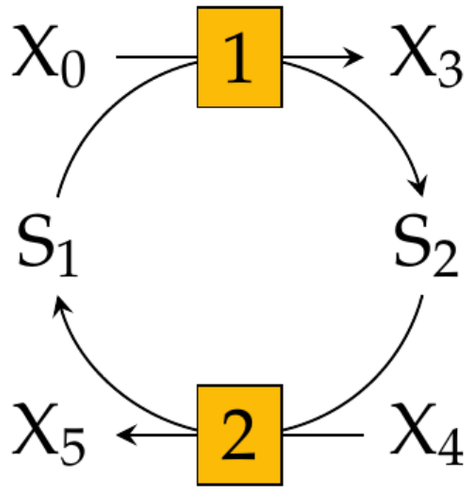


$$\begin{aligned} ds_1/dt &= J_1 - J_2 + J_3 = 0 \\ ds_2/dt &= J_2 - J_3 - J_4 = 0 \end{aligned}$$

Flux relationships

$$J_1 = J_4 = J_2 - J_3$$

Example: Moiety-conserved system in steady state



$$\begin{aligned} ds_1/dt &= J_2 - J_1 = 0 \\ ds_2/dt &= J_1 - J_2 = 0 \end{aligned}$$

Flux relationships

$$J_1 = J_2$$

Conservation relationships

$$ds_1/dt + ds_2/dt = d/dt(s_1 + s_2) = 0$$

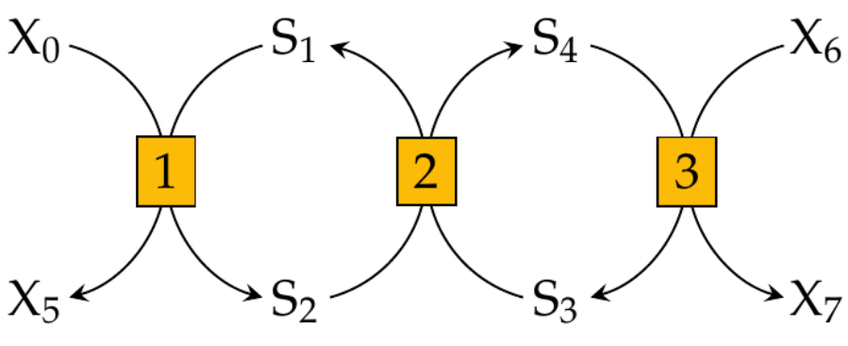
$$s_1 + s_2 = \text{constant}$$

Exercise 2

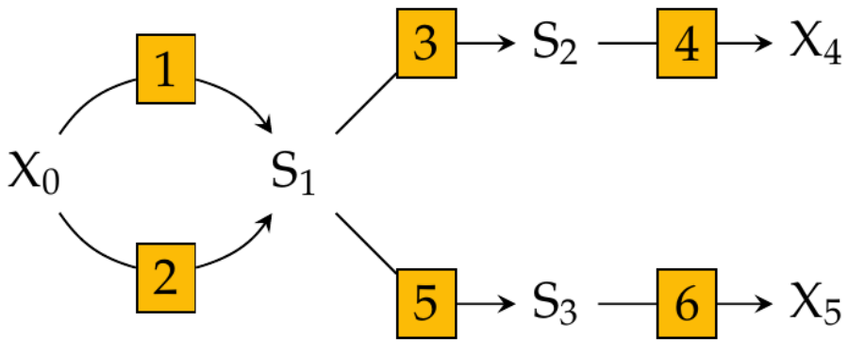
For each of the following pathways, write down the

- Balance equations
- Steady-state flux relationships
- Moiety-conservation relationships (if present)

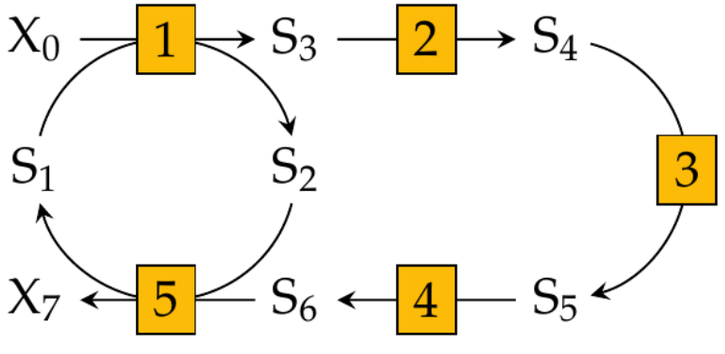
A.



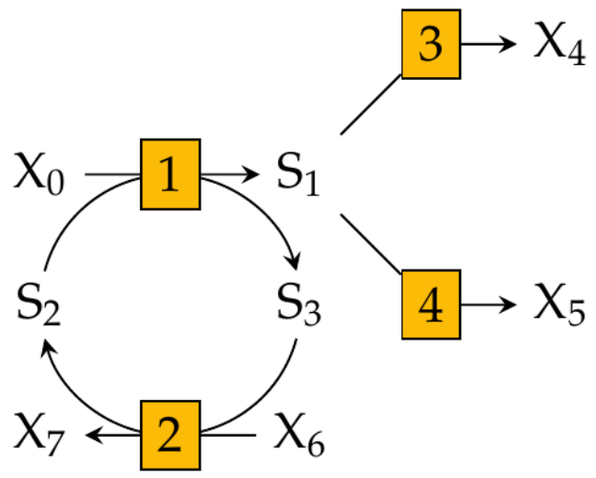
B.



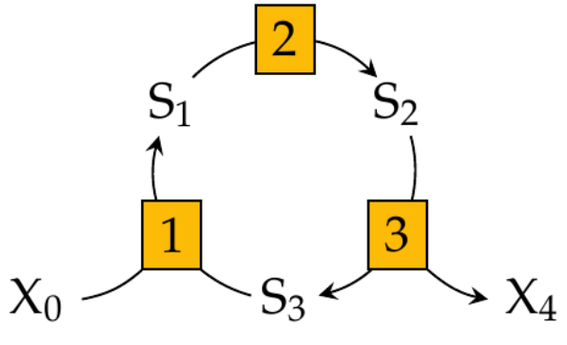
C.



D.



E.



Revision Exercise

A substrate is delivered at a constant rate, *k* and is consumed by an enzyme that follows classic Michaelis-Menten kinetics (i.e. irreversible, product insensitive). If the V_{max} of the enzyme is 10× the value *k*, what K_m would result in a substrate concentration of 1 at steady state?