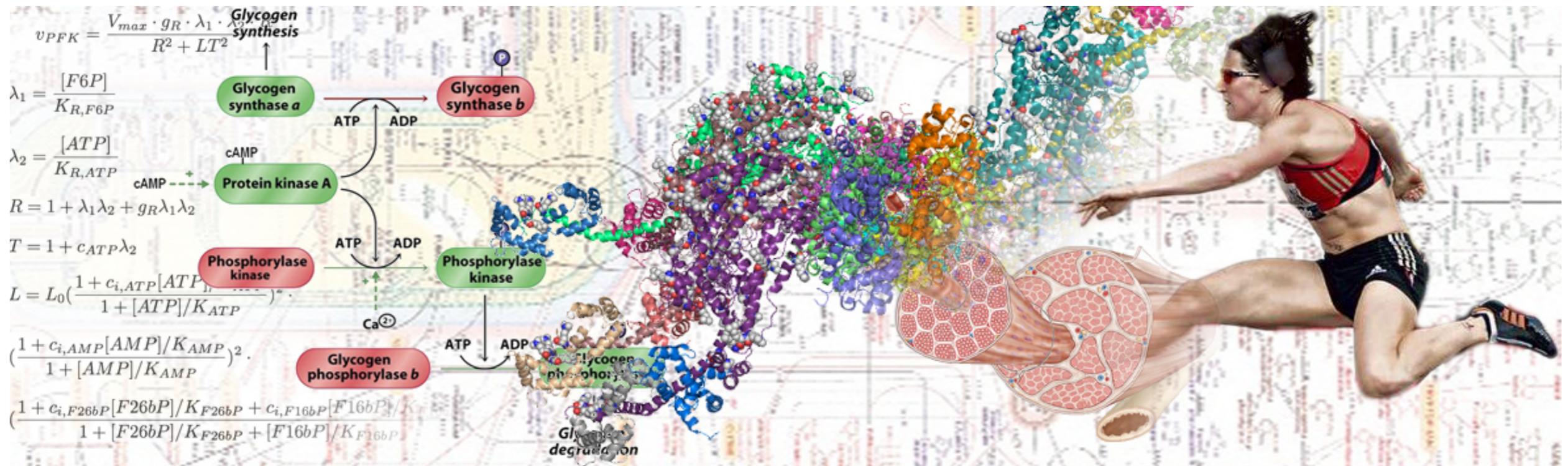


Biochemistry 714 Mini-course: Molecular Systems Biology



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

March – April 2025

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

Today

- Parameter estimation
- Coupled reactions
- Kinetic model in steady state

Parameter estimation

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

Enzymology

2 Phosphoglucoisomerase

$$v_{3PGI} = \frac{g_{6p} \left(1 - \frac{f_{6p}}{g_{6p} K_{v3eq}}\right) V_{3PGI}}{\left(1 + \frac{f_{6p}}{K_{v3f6p}} + \frac{g_{6p}}{K_{v3g6p}}\right) K_{v3g6p}}$$

	Estimate	Standard Error	t-Statistic	P-Value
K_{v3f6p}	0.096651	0.00499949	19.3322	2.12596×10^{-25}
K_{v3g6p}	0.974424	0.101138	9.63463	1.04986×10^{-12}

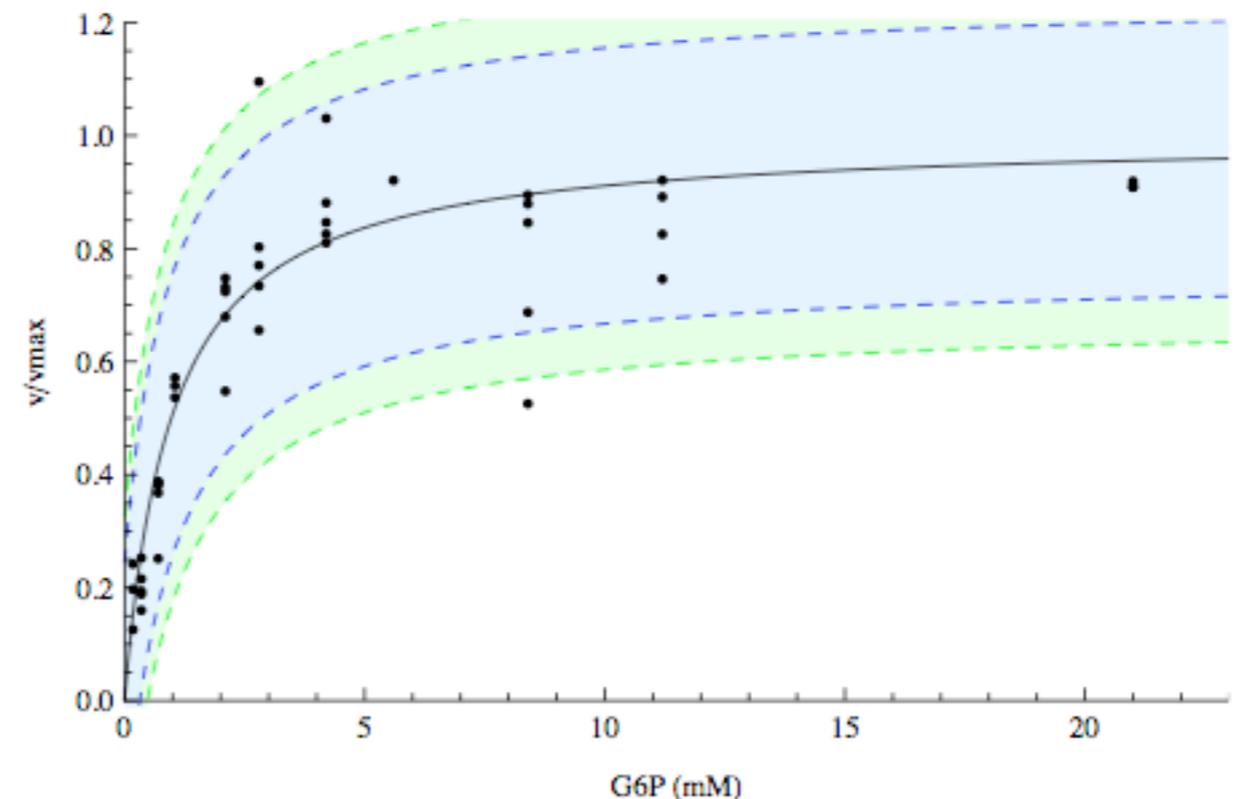
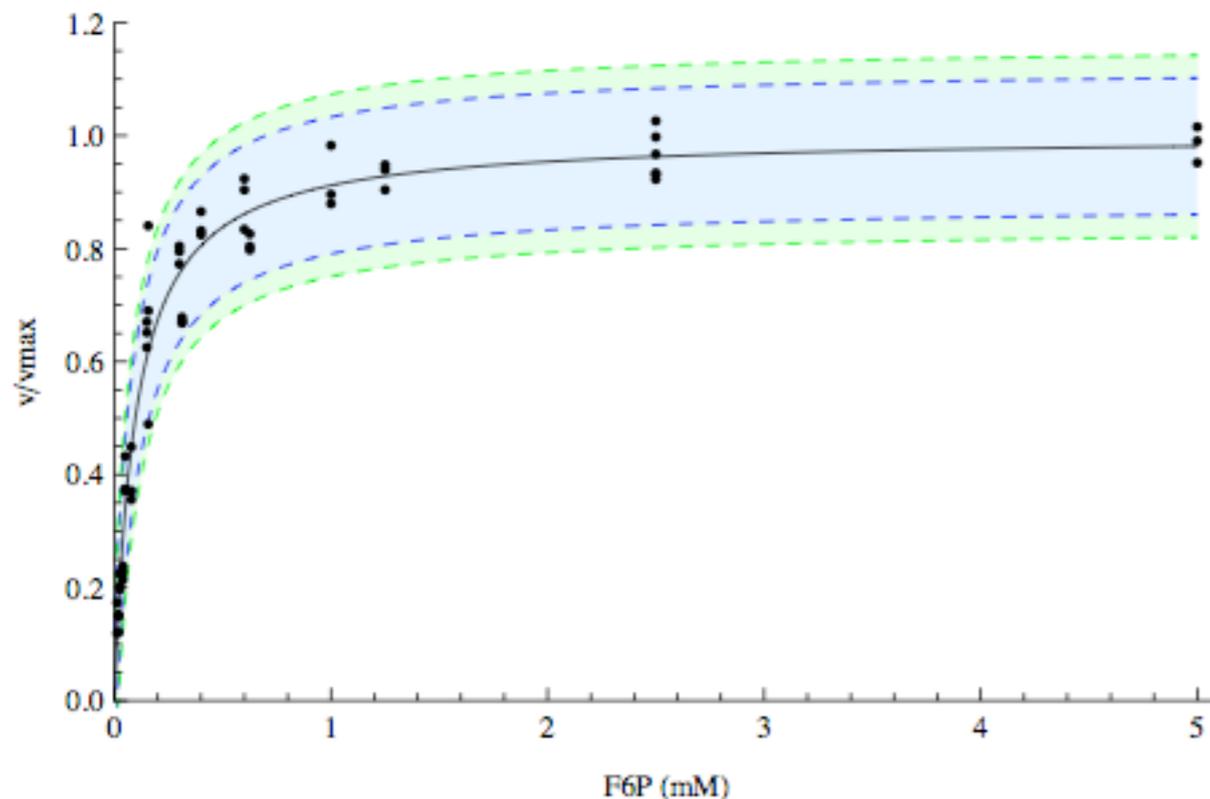


Figure 2: Characterization of Plasmodium falciparum phosphoglucoisomerase

Enzymology

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

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

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

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

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

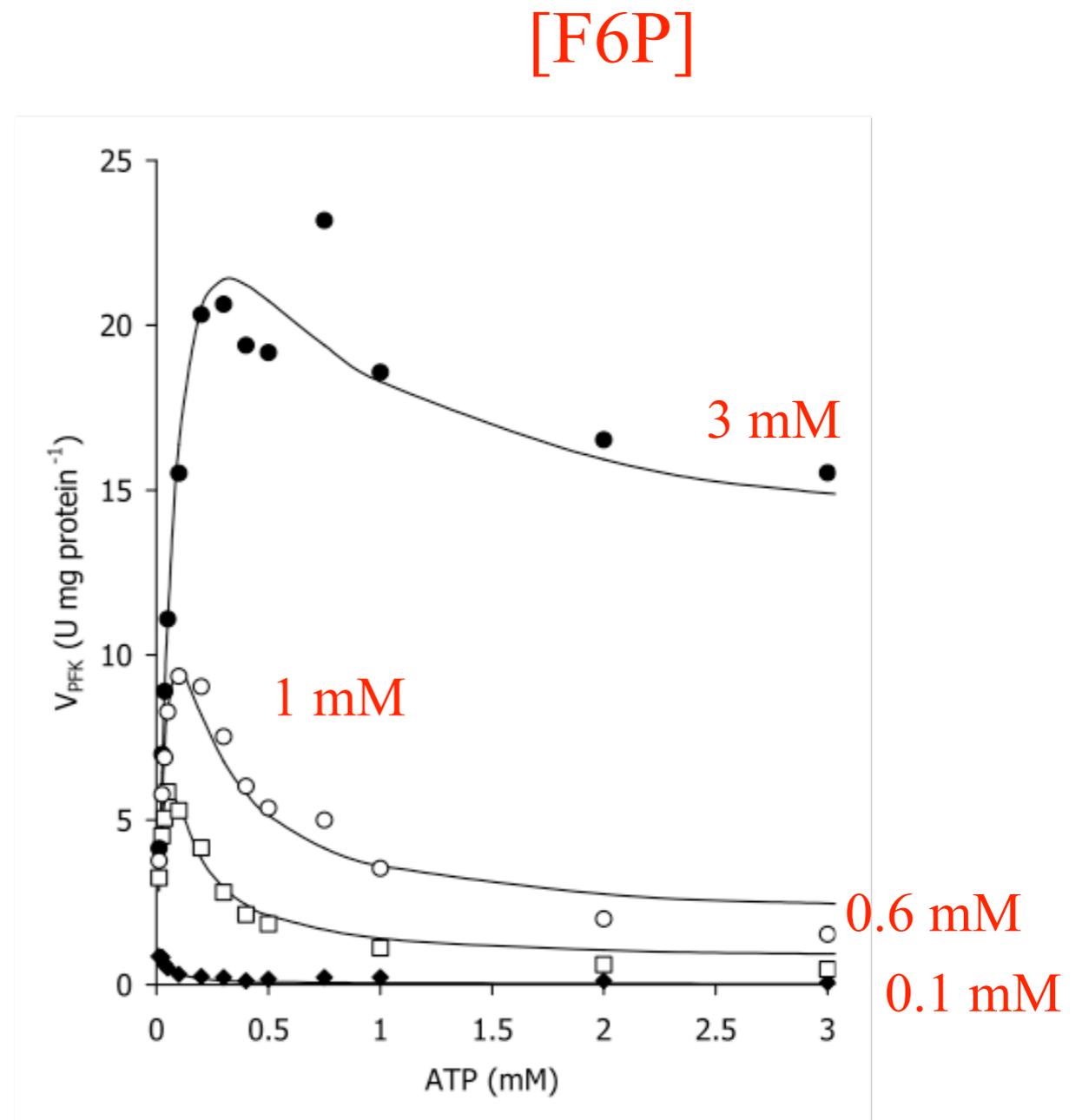
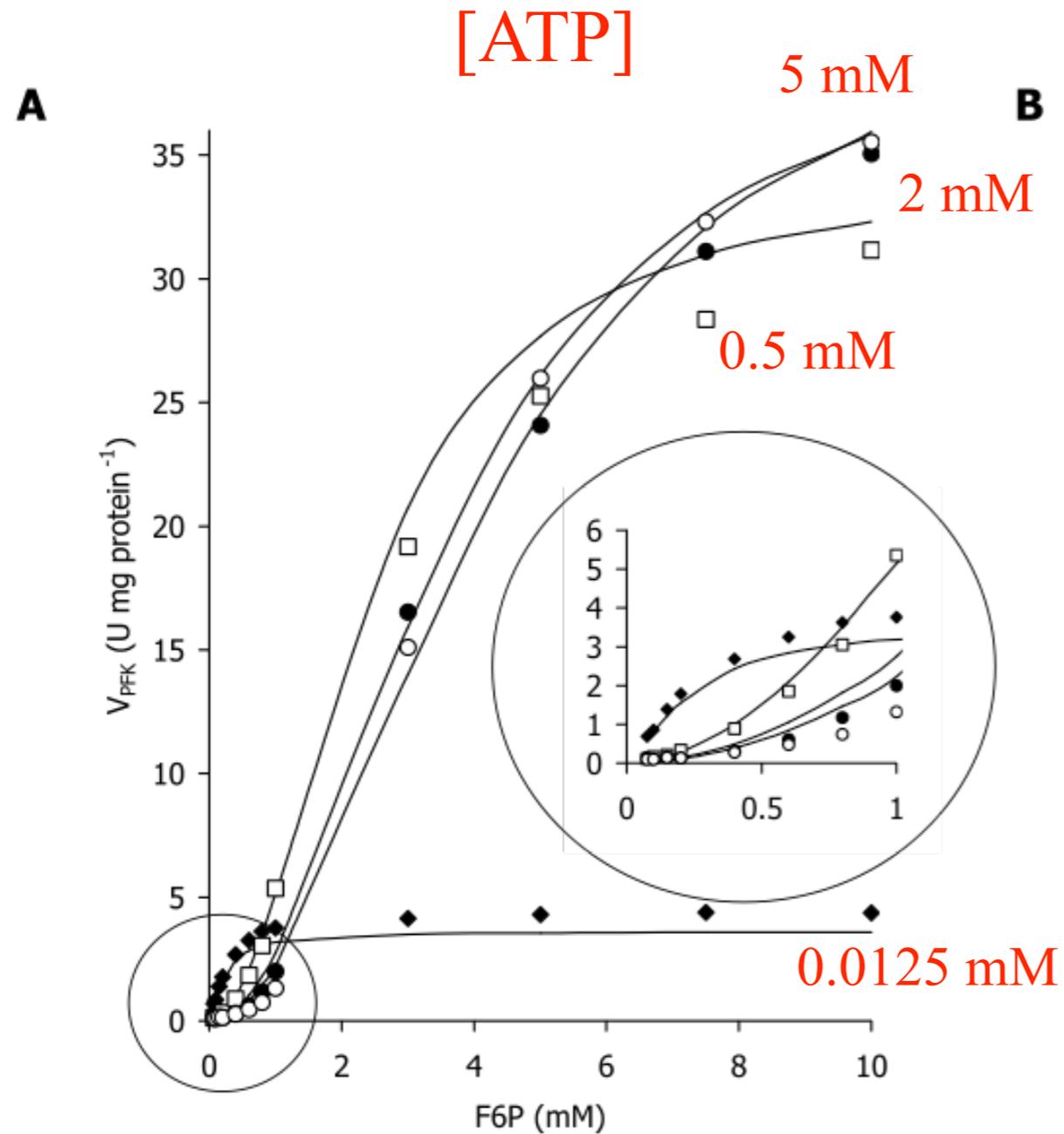
$$T = 1 + c_{ATP} \cdot \lambda_2$$

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

$$\left(\frac{1 + C_{i,AMP} \cdot [AMP]/K_{AMP}}{1 + [AMP]/K_{AMP}} \right)^2 \cdot$$

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

In vitro experimental data



Coupled reactions

Consider the following set of coupled reactions:



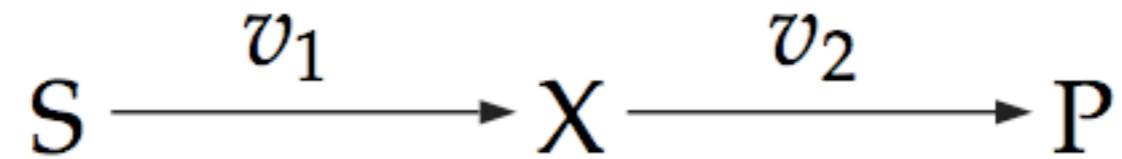
Assume a K_{eq} value of 10 for the first reaction and a value of 2 for the second reaction.

- 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 $A = 10 \text{ mM}$. Calculate 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

$$v_1 = k_1 s$$

$$v_2 = k_2 x$$

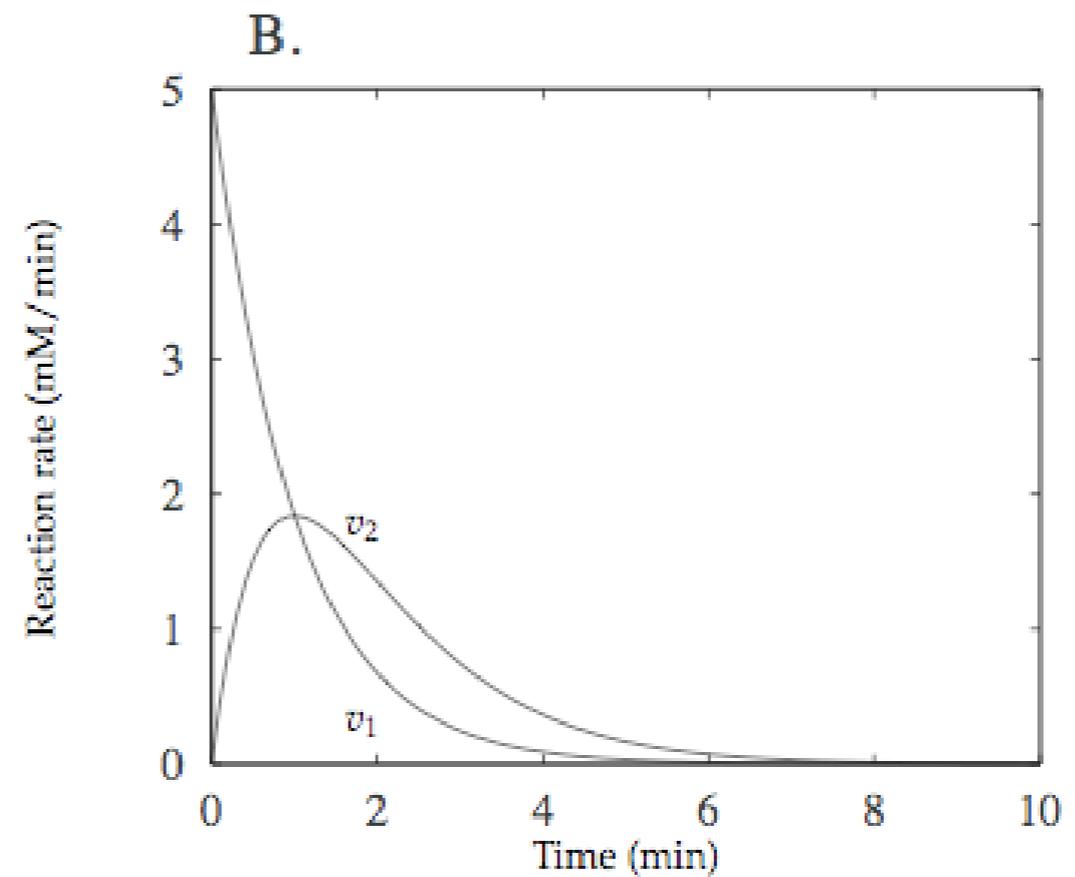
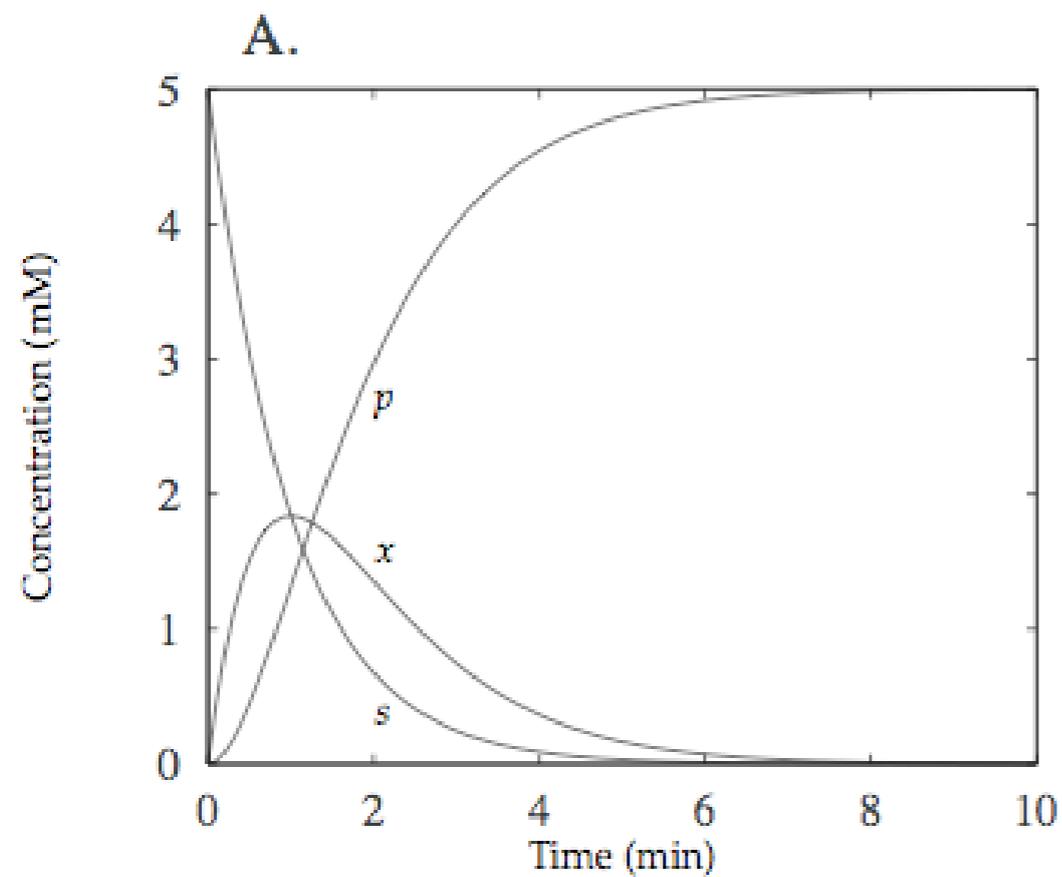
balance equations

$$\frac{ds}{dt} = -v_1 = -k_1 s$$

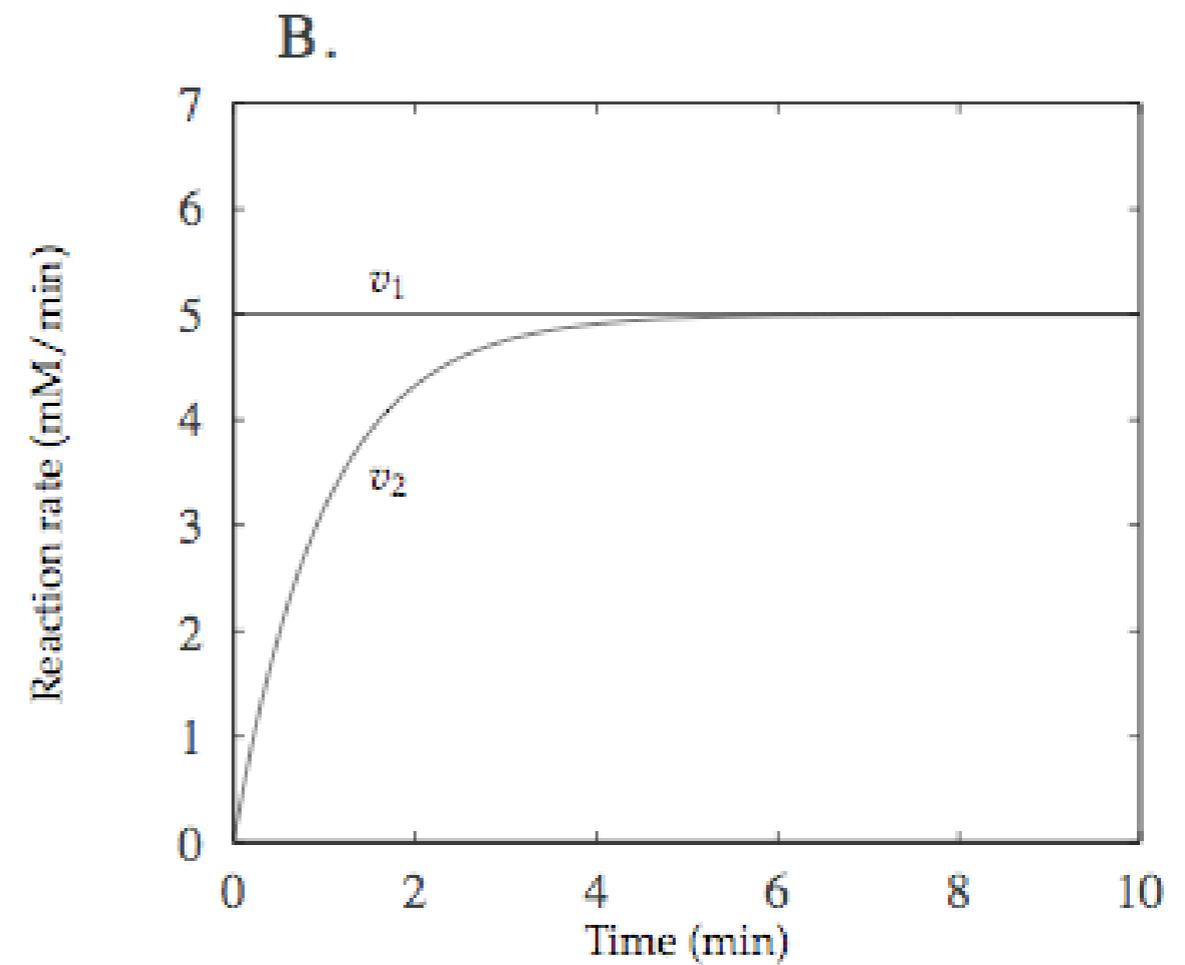
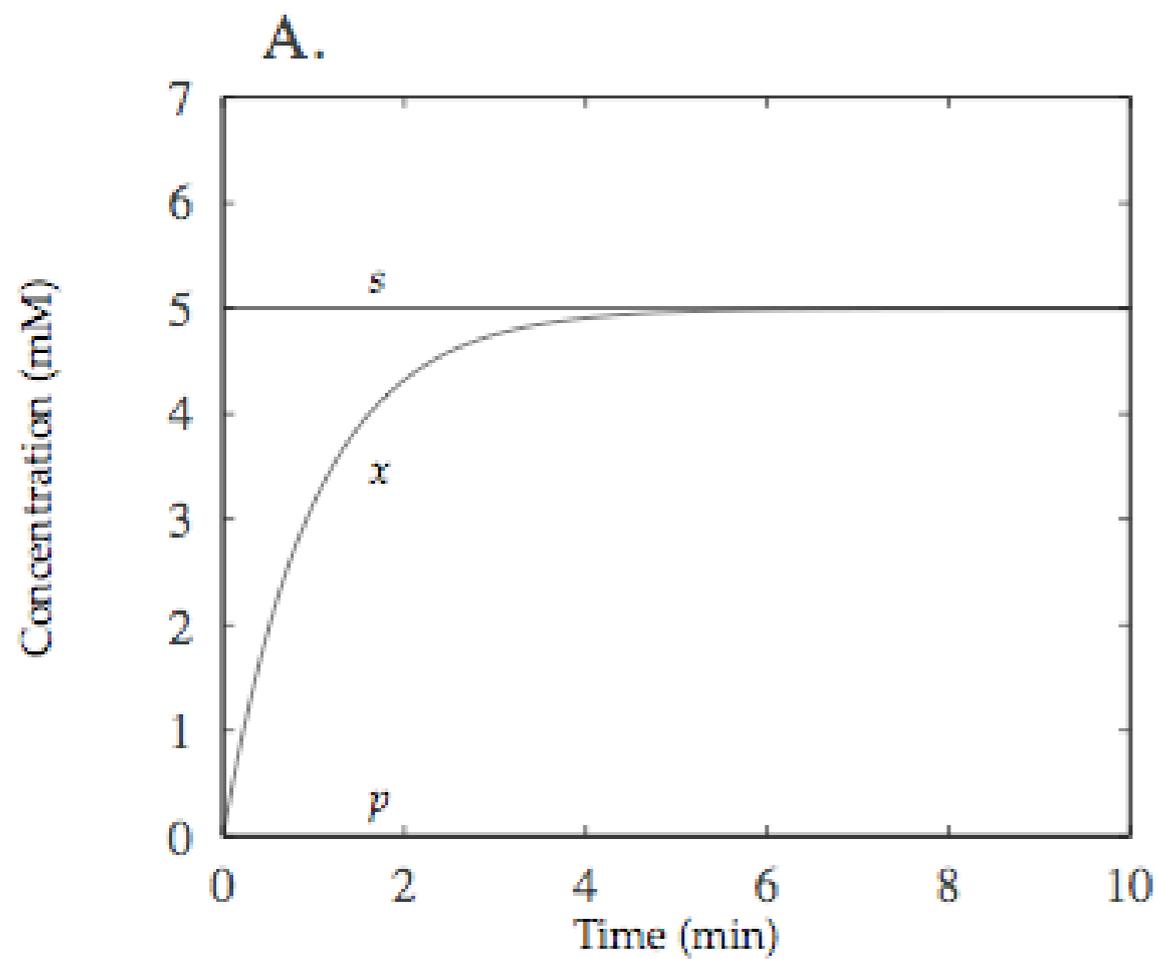
$$\frac{dx}{dt} = v_1 - v_2 = k_1 s - k_2 x$$

$$\frac{dp}{dt} = v_2 = k_2 x$$

Progress curves: closed system



Progress curves: open system



Steady state

$$\frac{dx}{dt} = \bar{v}_1 - \bar{v}_2 = k_1s - k_2\bar{x} = 0$$

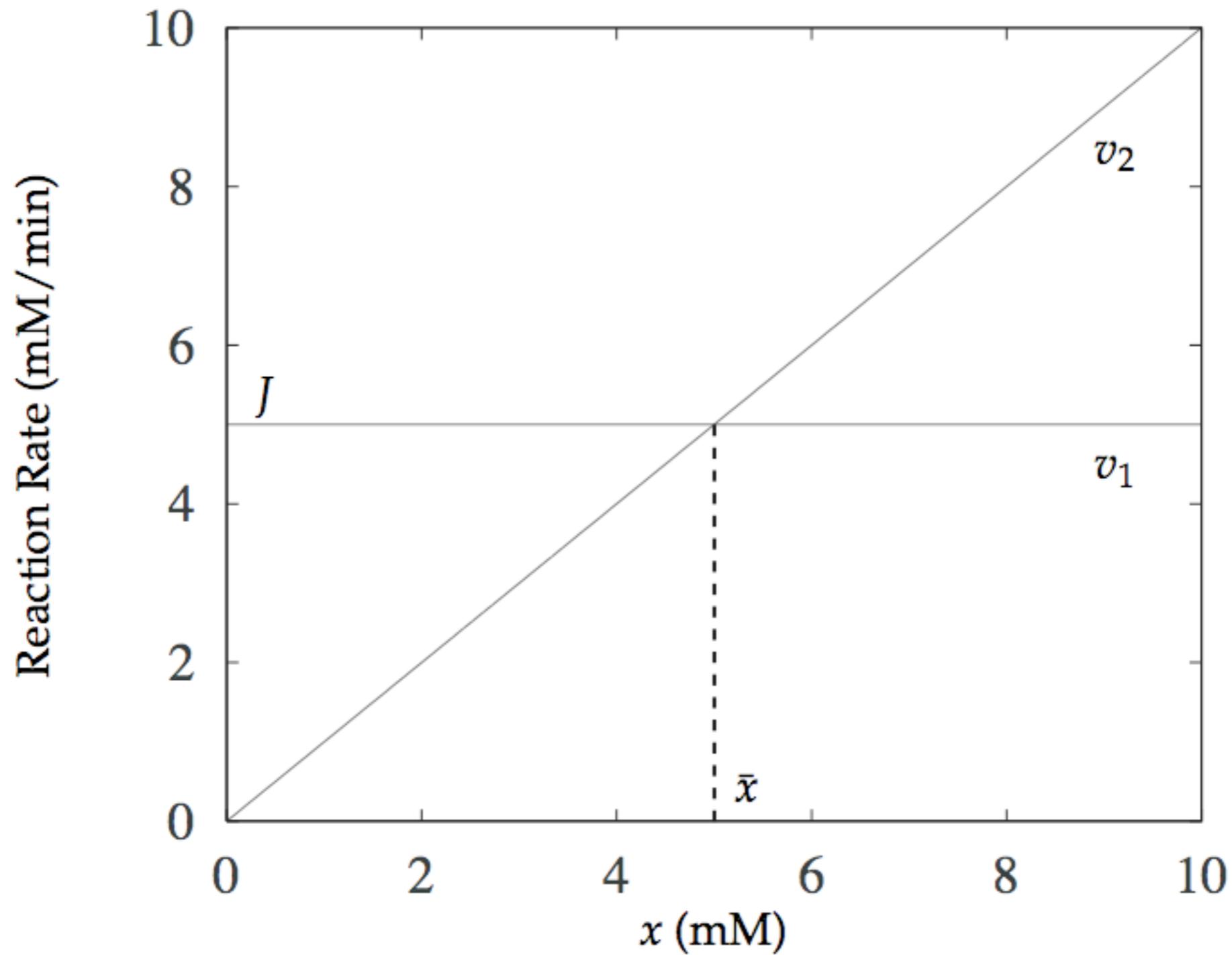
Steady state flux:

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

Steady state concentration:

$$\bar{x} = \frac{k_1s}{k_2}$$

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:

$$V_{mf} = 1 \text{ mM/s}, K_{eq} = 10, K_{m,substrate} = 1 \text{ mM},$$

$$K_{m,product} = 10 \text{ mM}$$

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



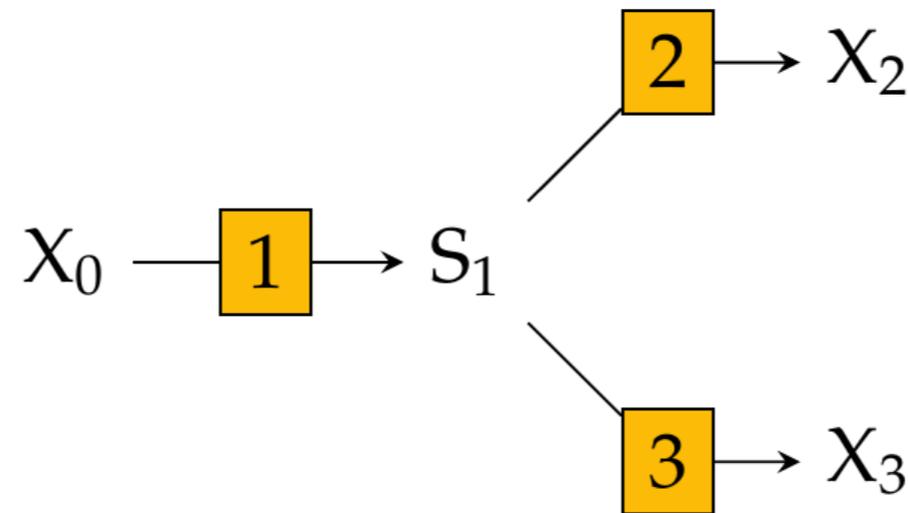
$$ds_1/dt = J_1 - J_2 = 0$$

$$ds_2/dt = J_2 - J_3 = 0$$

Flux relationships

$$J_1 = J_2 = J_3$$

Example: Branched system in steady state

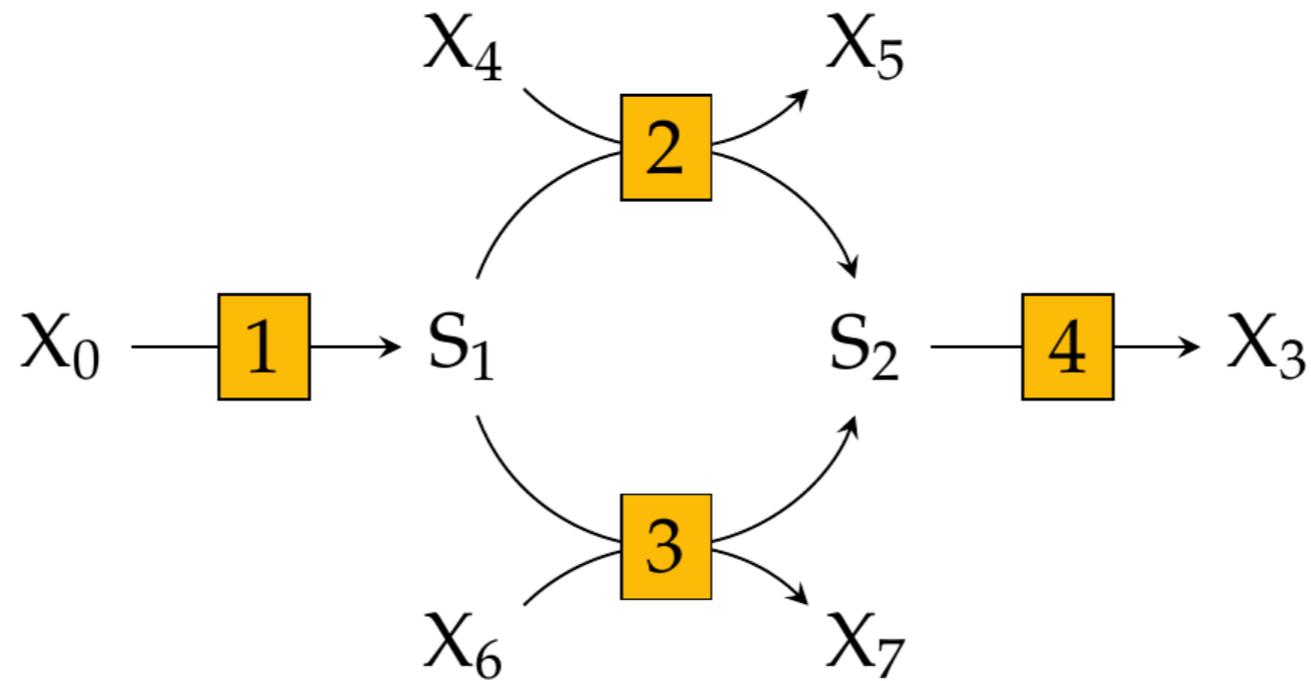


$$ds_1/dt = J_1 - J_2 - J_3 = 0$$

Flux relationships

$$J_1 = J_2 + J_3$$

Example: Parallel looped system in steady state



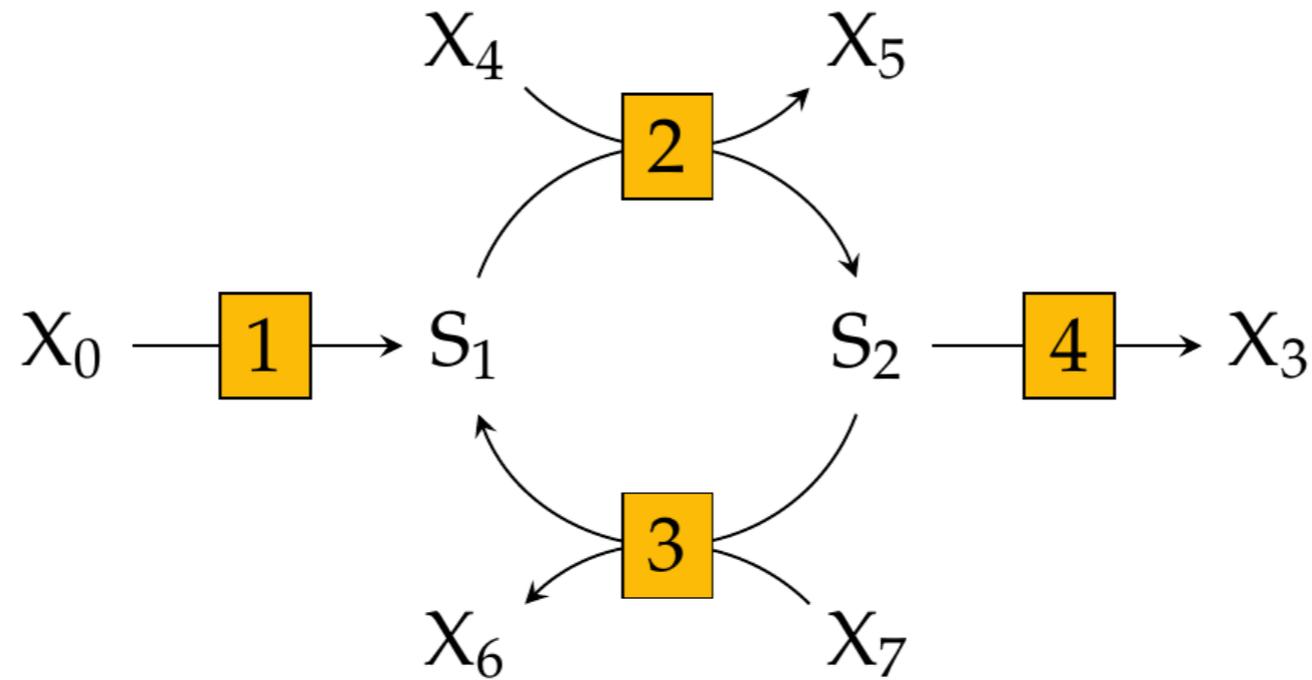
$$ds_1/dt = J_1 - J_2 - J_3 = 0$$

$$ds_2/dt = J_2 + J_3 - J_4 = 0$$

Flux relationships

$$J_1 = J_4 = J_2 + J_3$$

Example: Anti-parallel looped system in steady state



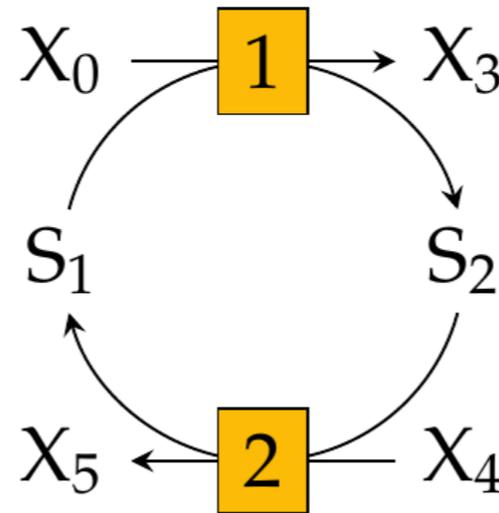
$$ds_1/dt = J_1 - J_2 + J_3 = 0$$

$$ds_2/dt = J_2 - J_3 - J_4 = 0$$

Flux relationships

$$J_1 = J_4 = J_2 - J_3$$

Example: Moiety-conserved system in steady state



$$ds_1/dt = J_2 - J_1 = 0$$

$$ds_2/dt = J_1 - J_2 = 0$$

Flux relationships

$$J_1 = J_2$$

Conservation relationships

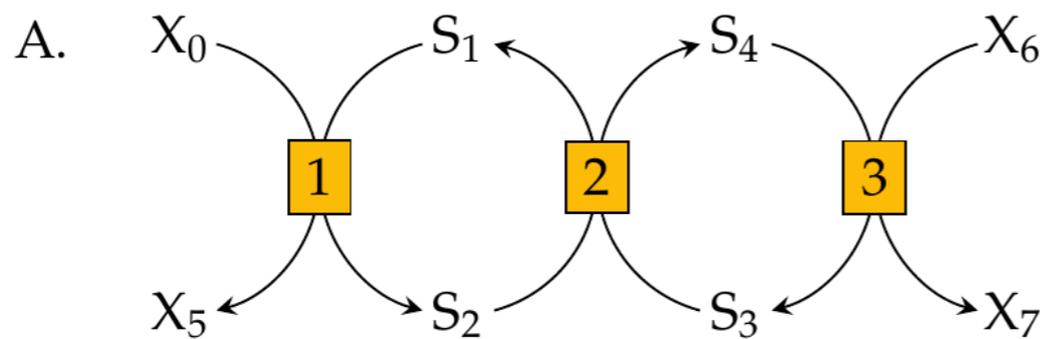
$$ds_1/dt + ds_2/dt = d/dt(s_1 + s_2) = 0$$

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

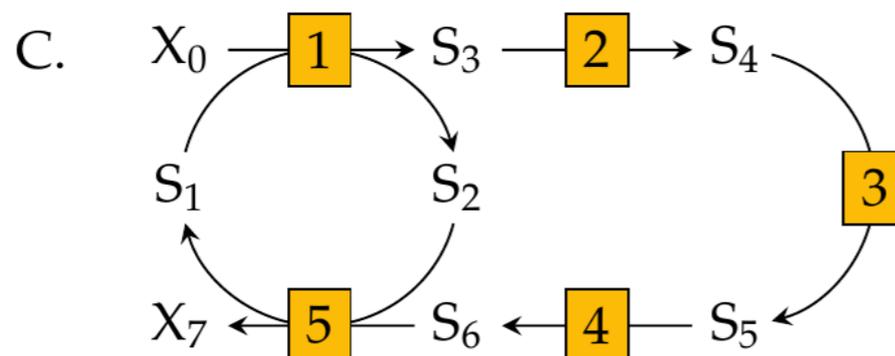
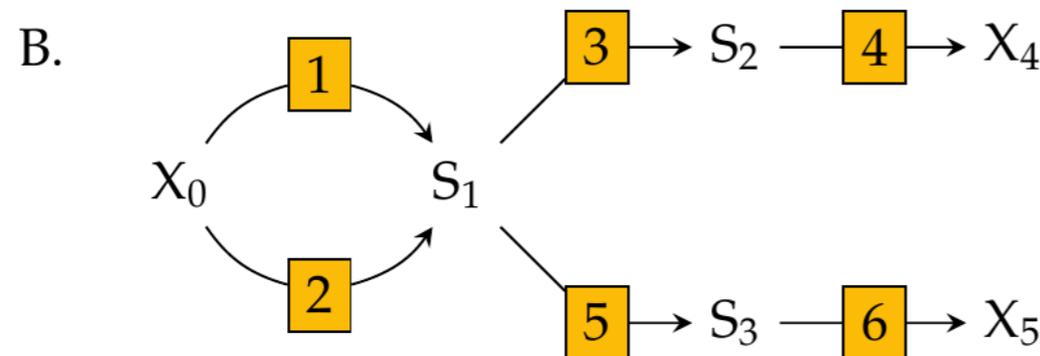
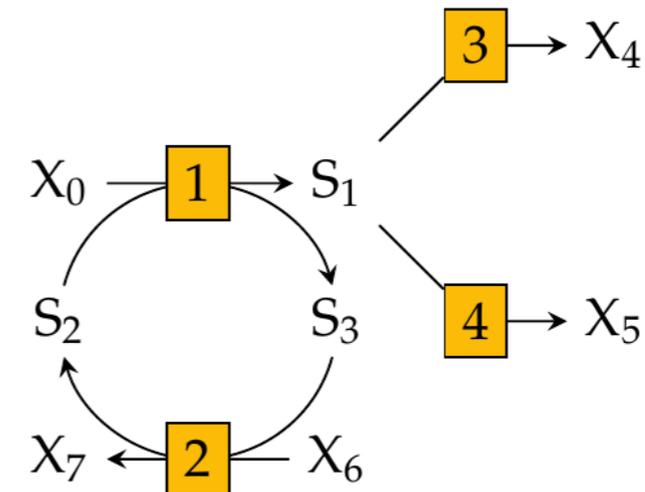
Exercise 2

For each of the following pathways, write down the

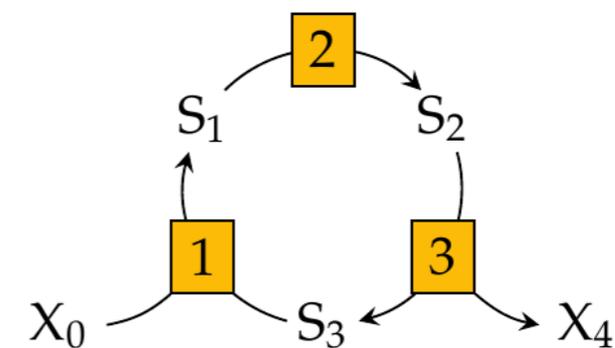
- Balance equations
- Steady-state flux relationships
- Moiety-conservation relationships (if present)



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 $10\times$ the value k , what K_m would result in a substrate concentration of 1 at steady state?