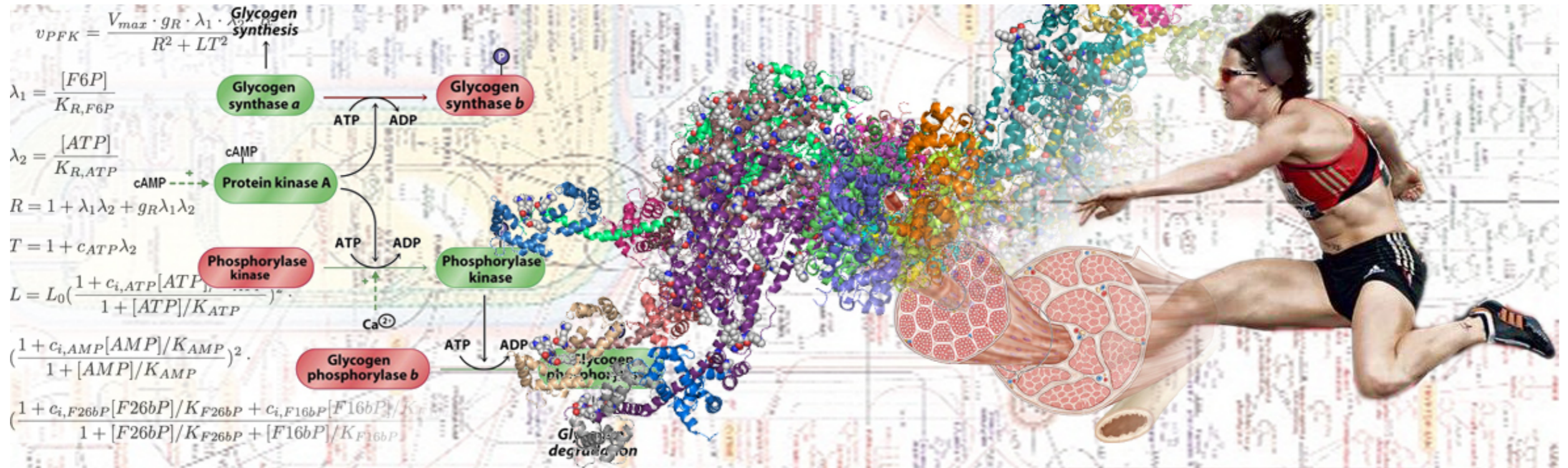


# Mini-course: Molecular Systems Biology



Profs Jacky Snoep and Johann Rohwer

March 2018

# Thus far

- First Lecture: Chemical kinetics
- Direction of reaction:  $dG$ ,  $\Delta G/Keq$
- How far:  $Keq$ ,  $dG^0$
- 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

$$v_{3PGI} = \frac{g6p \left(1 - \frac{f6p}{g6p K_{v3eq}}\right) V_{3PGI}}{\left(1 + \frac{f6p}{K_{v3f6p}} + \frac{g6p}{K_{v3g6p}}\right) K_{v3g6p}}$$

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

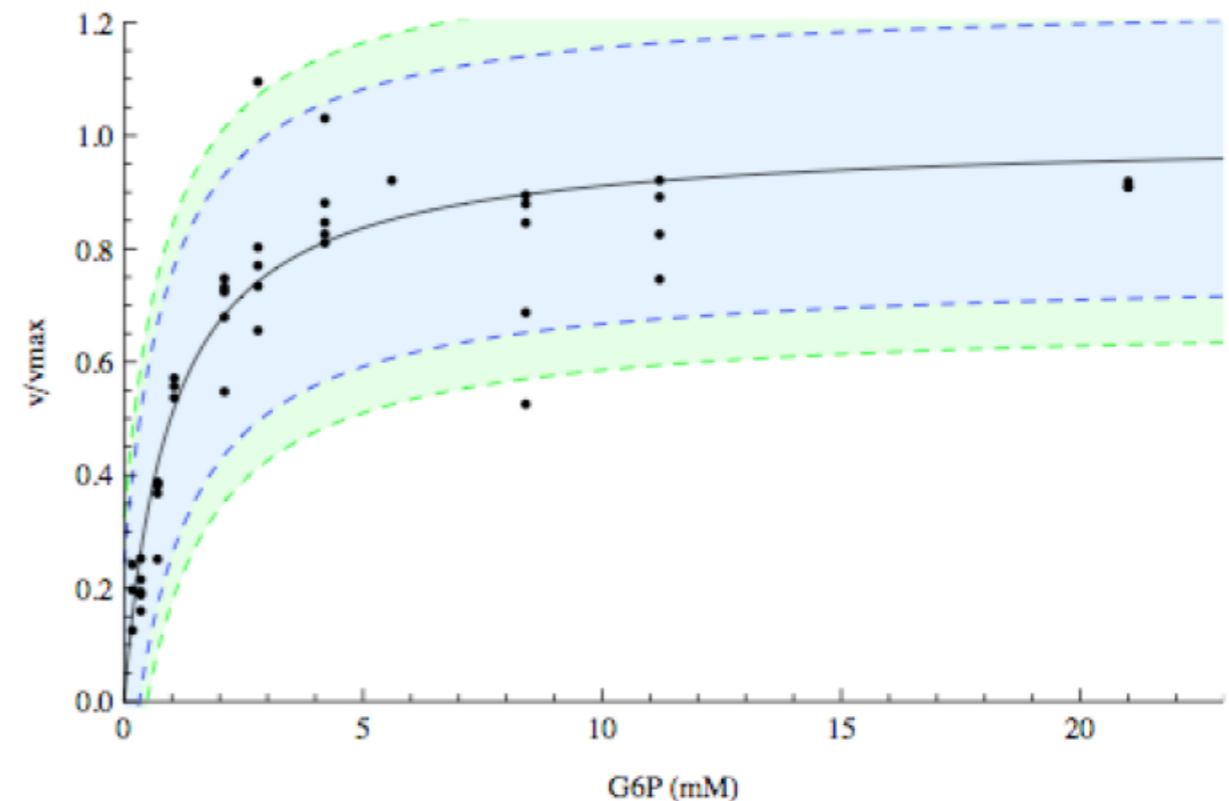
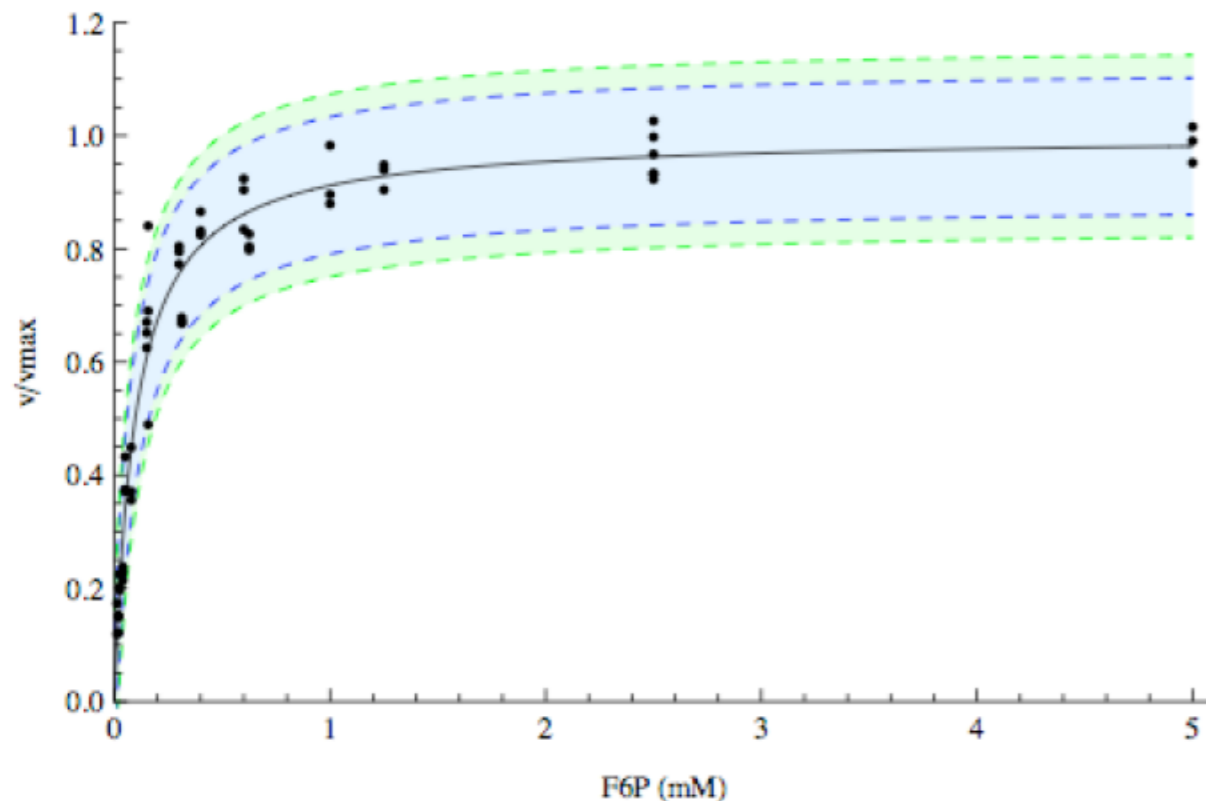


Figure 2: Characterization of Plasmodium falciparum phosphoglucoisomerase



# Enzymology

Phosphofructokinase : Monod, Wymann, Changeux model (physiological conditions,  $V_{max}$ )

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

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

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

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

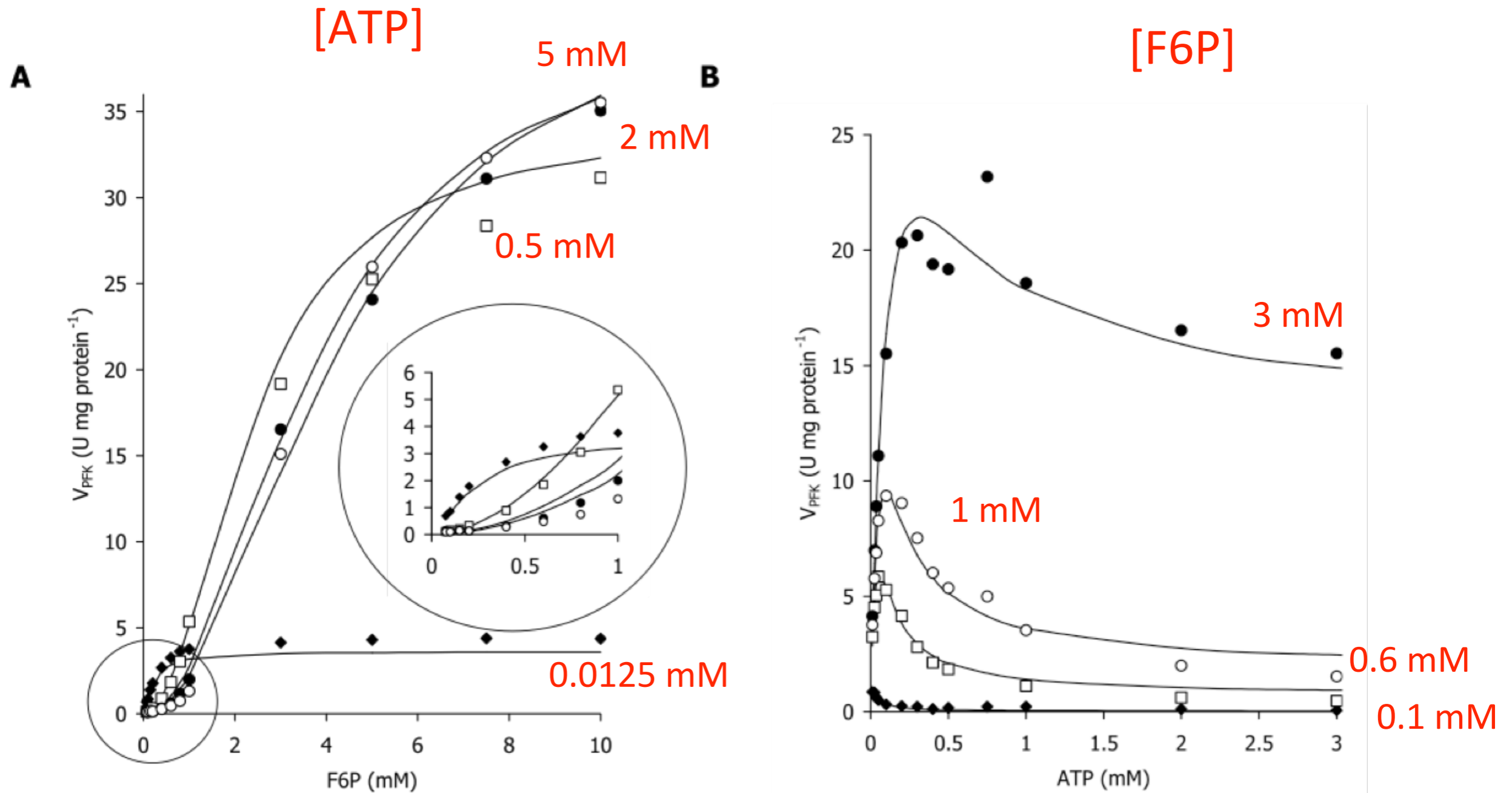
$$T = 1 + c_{ATP} \cdot \lambda_2$$

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

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

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

# In vitro experimental data





# Coupled reactions

Consider the following set of coupled reactions:



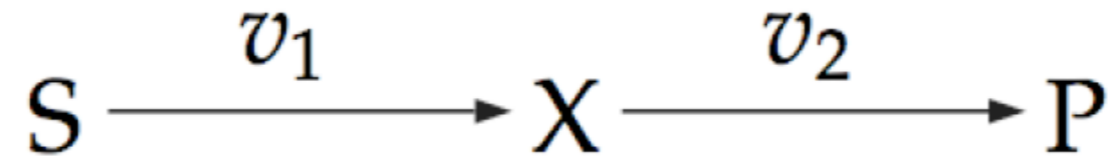
Assume a  $K_{eq}$  value of 10 for the first reaction and a value of 2 for the second reaction. What would the equilibrium ratio between C and A be? How would the  $dG$  values of the individual reactions relate to the  $dG$  of the overall system? Assume an initial concentration of  $A = 10$  mM and 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

$$v_1 = k_1 s$$

$$v_2 = k_2 x$$

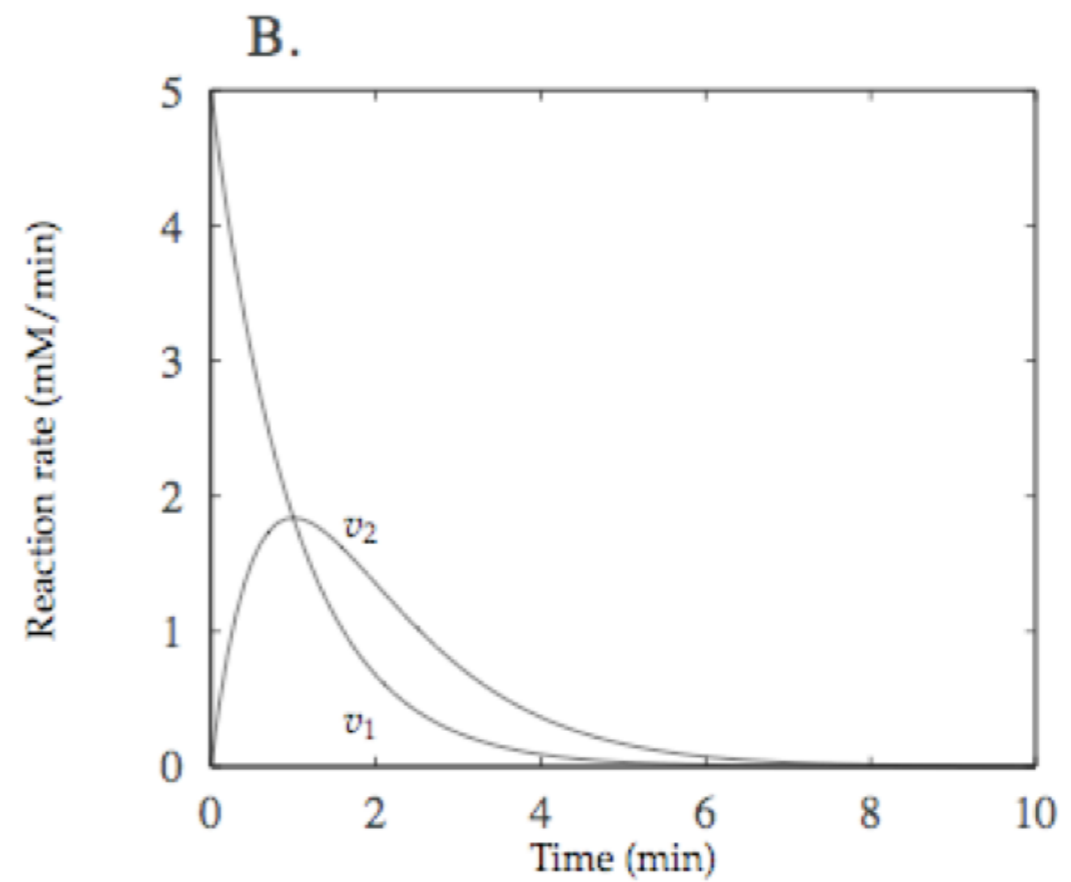
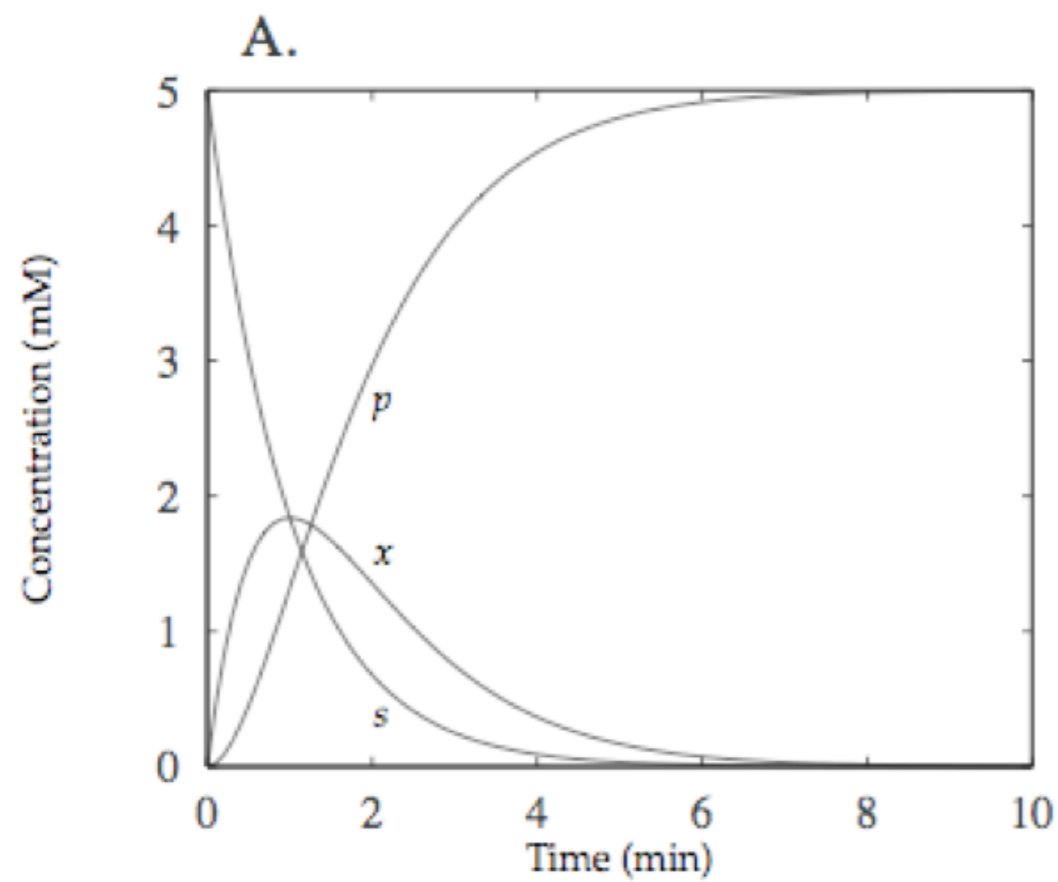
balance equations

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

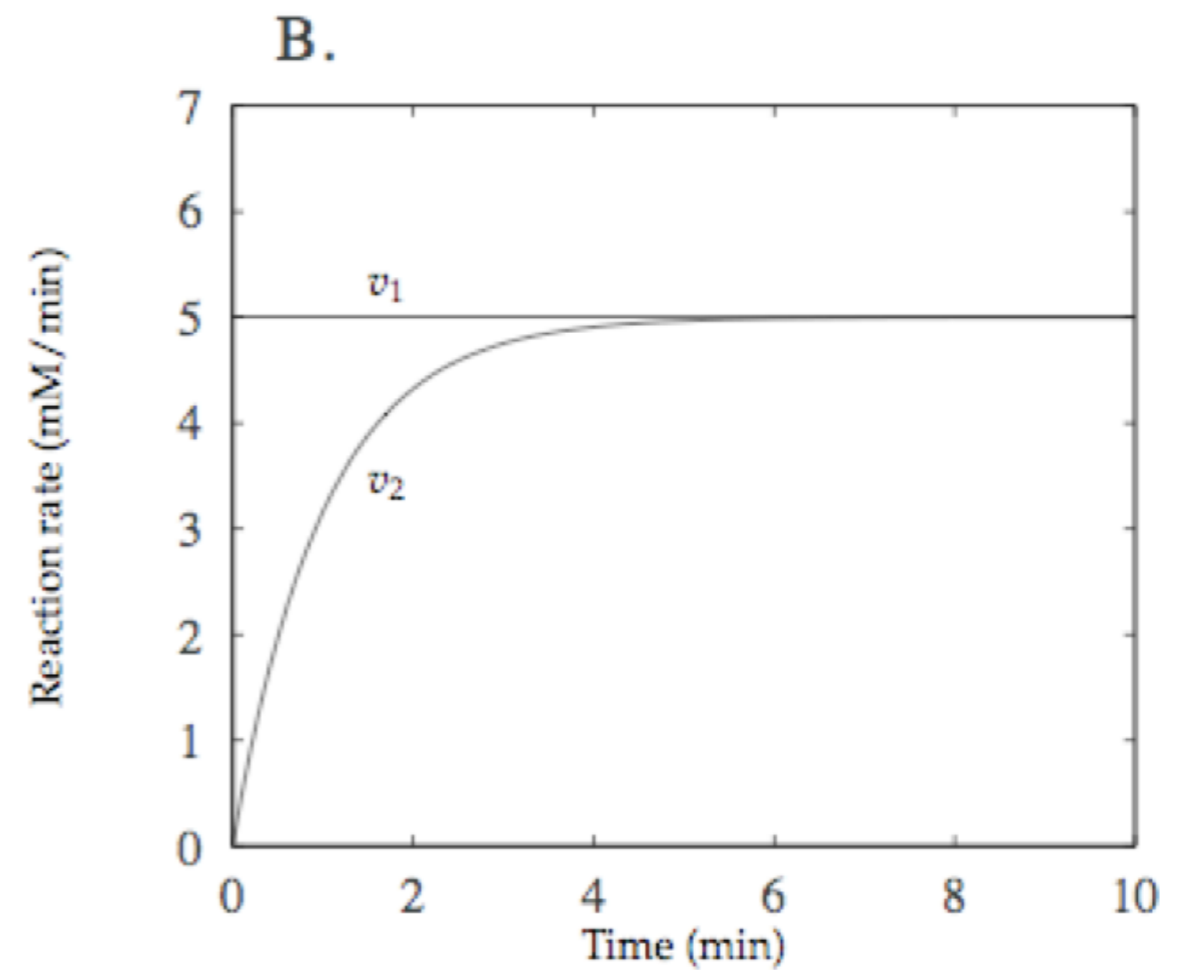
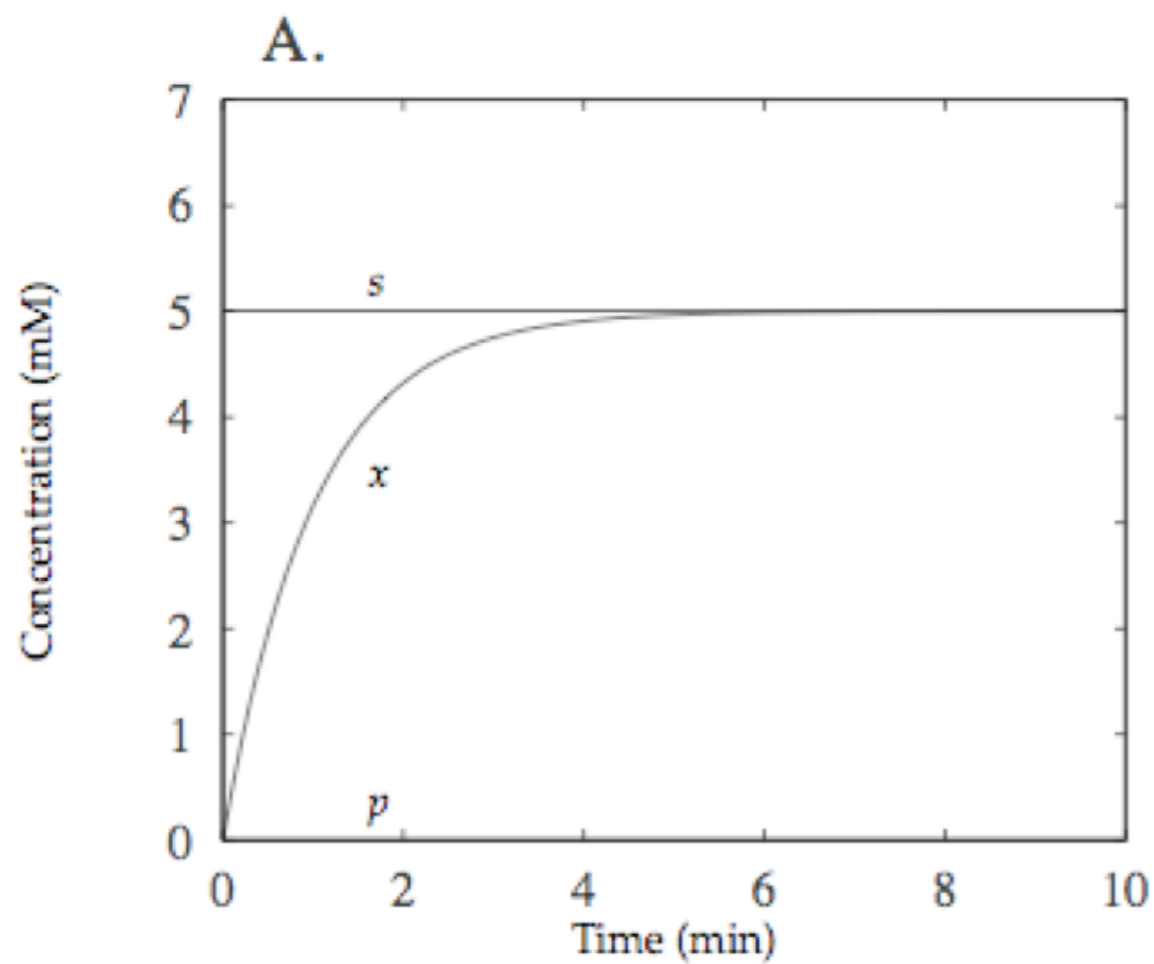
$$\frac{dx}{dt} = v_1 - v_2 = k_1 s - k_2 x$$

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

# Progression curves: closed system



# Progression curves: open system



# Steady state

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

Steady state flux:

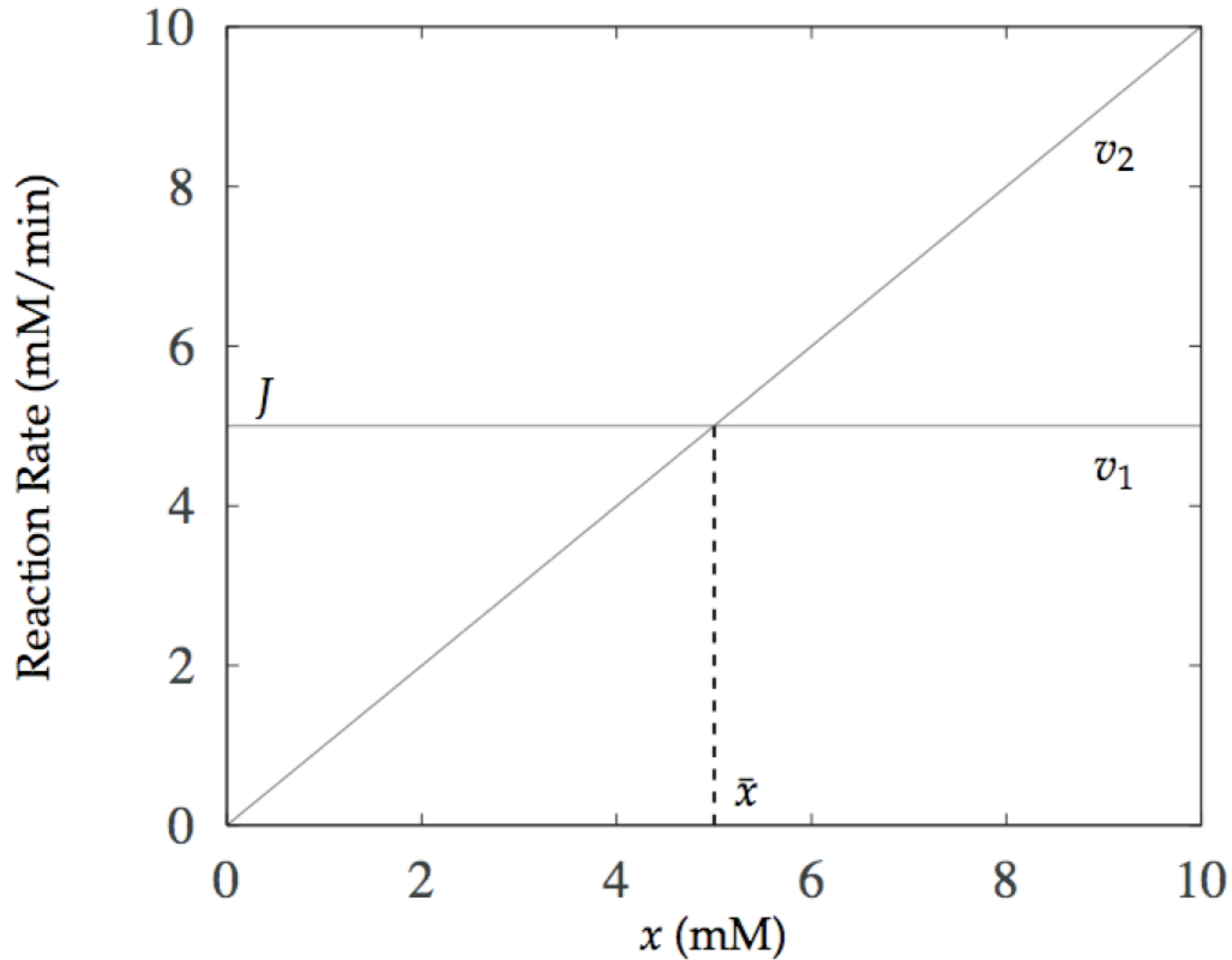
$$\bar{v}_1 = \bar{v}_2 = J$$

Steady state concentration:

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



# Rate characteristics



# Exercise

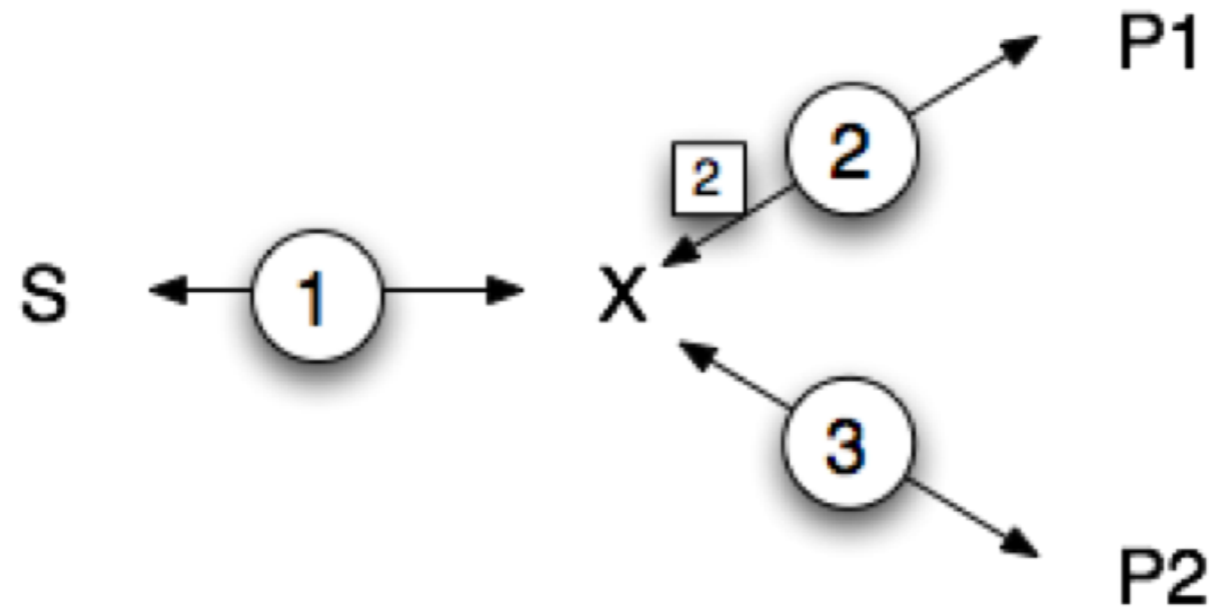
Given an open system consisting of two enzymes that catalyze the conversion of substrate S (fixed at 10 mM) to product P (fixed at 1 mM), with common intermediate X.

The enzymes obey rev MM kinetics with identical parameter values:

$V_m = 1 \text{ mM/s}$ ,  $K_{eq} = 10$ ,  $K_m \text{ substrate} = 1 \text{ mM}$ ,  $K_m \text{ product} = 10 \text{ mM}$

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

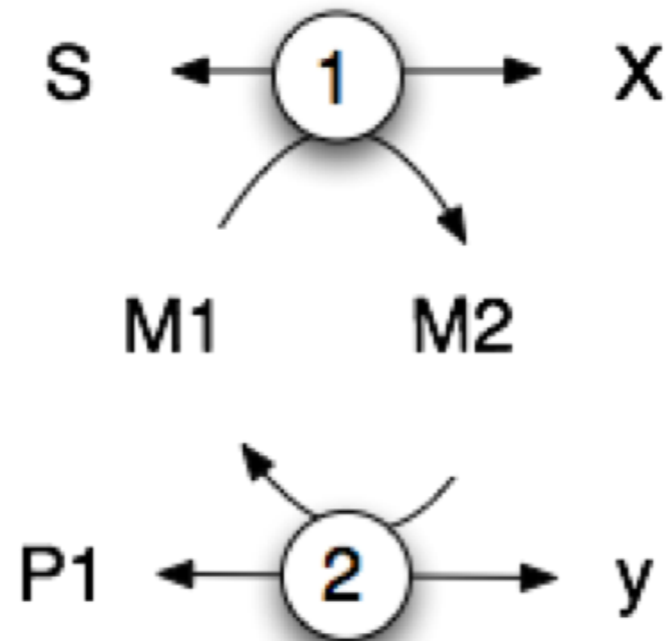
# Branched systems



Give the ODE for  $X$  in terms of  $v1$ ,  $v2$  and  $v3$

How many independent steady state fluxes does the system have?

# Conserved moieties



$$\frac{d(m1)}{dt} = -\frac{d(m2)}{dt}$$
$$m1 + m2 = cst$$
$$m2 = cst - m1$$

Use linear algebra methods to find flux dependencies and moiety conservations.

N matrix, L matrix and K matrix.

Examples on JWS Online