

Modelling Biochemical Reaction Networks

Lecture 5: Passive transport

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Recommended reading

- ▶ Fall, Marland, Wagner and Tyson, sections 3.1 and 3.2

Transport mechanisms

- ▶ Many substances are unable to pass through the cell membrane.
- ▶ Substances that must transit through the membrane are often assisted by specific transporters.
- ▶ Two types of transport through membranes:
 - Active:** pumping, sometimes against a chemical gradient;
 - ▶ Uses energy or a favorable chemical gradient of another substance
 - Passive:** travel of certain substances facilitated without expenditure of energy;
 - ▶ On average, material can only be transferred with a chemical gradient
 - ▶ Many mechanisms, ranging from simple pores to proteins with enzyme-like substrate recognition

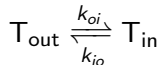
Passive glucose transport

- ▶ Hxt7 is a yeast passive glucose transporter expressed at low glucose concentrations.
- ▶ It presumably plays a role in maintaining uptake of glucose in conditions of falling glucose concentration.
- ▶ What kinetic features of this transporter make it particularly suitable at low glucose concentrations?

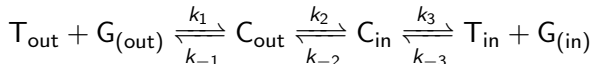
Ref.: Ye et al., Yeast **18**, 1257 (2001).

A simple model

- ▶ The transporter (T) has two conformations, one (T_{out}) in which a glucose binding site is exposed on the outside of the cell, and one (T_{in}) in which the binding site is exposed inside the cell. It makes random transitions between these two states:



- ▶ Once glucose is bound, the conformational change of T moves it across the membrane:



First simplification

- ▶ Yeast cells either use or sequester glucose very rapidly, so the intracellular concentration of free glucose is typically extremely low. Thus, the process $T_{in} + G_{(in)} \xrightarrow{k_{-3}} C_{in}$ is negligible.

Simplification using equilibrium approximations

- ▶ We probably can't observe the intermediate states of the transporter.
- ▶ We probably can't get all the rate constants.
- ▶ What we want to know is how the rate depends on the rate constants and the total number of transporters.
- ▶ Conformational changes can be very fast.
- ▶ Ideal opportunity to apply the equilibrium approximation!

Simplification using equilibrium approximations

It pays to do things in a disciplined (ordered) way in these problems.

- 1 The model only includes transporter interconversions, so total transporter concentration is a constant:

$$T_0 = [T_{\text{out}}] + [T_{\text{in}}] + [C_{\text{out}}] + [C_{\text{in}}]$$

- 2 Because conformational changes can be fast, apply the equilibrium approximation to each of the reversible steps:

$$k_{oi}[T_{\text{out}}] \approx k_{io}[T_{\text{in}}]$$

$$k_1[T_{\text{out}}][G_{(\text{out})}] \approx k_{-1}[C_{\text{out}}]$$

$$k_2[C_{\text{out}}] \approx k_{-2}[C_{\text{in}}]$$

Simplification using equilibrium approximations

- 3 Before doing any algebra, remind yourself of the objective. We want the rate of glucose transport, $v = d[G_{(\text{in})}]/dt = k_3[C_{\text{in}}]$. Mathematically, it will be easier to leave $[C_{\text{in}}]$ as the **last** variable you solve for.
- 4 Solve the equilibrium approximations for the other transporter concentrations starting from the **bottom**, back-substituting as you go:

$$[C_{\text{out}}] = K_2[C_{\text{in}}]$$

$$[T_{\text{out}}] = K_1 \frac{[C_{\text{out}}]}{[G_{(\text{out})}]} = K_1 K_2 \frac{[C_{\text{in}}]}{[G_{(\text{out})}]}$$

$$[T_{\text{in}}] = K_{oi}[T_{\text{out}}] = K_1 K_2 K_{oi} \frac{[C_{\text{in}}]}{[G_{(\text{out})}]}$$

with $K_{oi} = k_{oi}/k_{io}$, $K_1 = k_{-1}/k_1$, $K_2 = k_{-2}/k_2$

Simplification using equilibrium approximations

- 5 Substitute into conservation relation, and solve for $[C_{in}]$, then multiply by k_3 to get v :

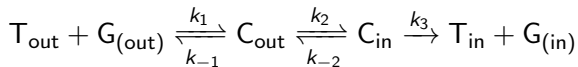
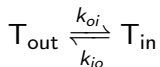
$$v = \frac{\frac{k_3 T_0}{1+K_2} [G_{(out)}]}{[G_{(out)}] + \frac{K_1 K_2 (1+K_{oi})}{1+K_2}}$$

This is in the Michaelis-Menten form with $v_{\max} = \frac{k_3 T_0}{1+K_2}$ and $K_M = \frac{K_1 K_2 (1+K_{oi})}{1+K_2}$.

Analysis of the result

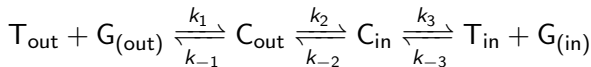
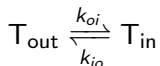
- ▶ For a given $v_{\max}$, we get the largest uptake rate when K_M is small.
Having a small K_M is particularly important when $[G_{(\text{out})}]$ is small.
- ▶ Experimental K_M in the low millimolar range (vs 50 mM for other yeast glucose transporters)
- ▶ The following factors will minimize K_M : small K_1 , small K_2 and small K_{oi} .
- ▶ Small K_2 also maximizes $v_{\max}$, so there may be particularly strong selective pressure on this parameter.

Analysis of the result



- ▶ Small $K_2 = k_{-2}/k_2$ implies a bias toward having the glucose-bound transporter in its conformation with the glucose on the cytoplasmic side of the membrane.
- ▶ Small $K_{oi} = k_{oi}/k_{io}$ implies a bias toward having the binding site of the unloaded transporter on the extracellular side. This may seem contradictory to the requirement for a small K_2 , except that the transporter is oriented in a membrane and so need not be symmetric. Binding glucose can cause conformational changes that change the bias.

Symmetric transporter



$$K_{oi} = 1 \qquad K_2 = 1 \qquad k_1 = k_{-3} \qquad k_{-1} = k_3$$

$$v_{\text{max}} = \frac{1}{2} k_3 T_0$$

$$K_M = K_1$$

- ▶ Maximizing k_3 alone increases v_{max} , but also increases $K_M = k_{-1}/k_1 = k_3/k_{-3}$, which is undesirable.
- ▶ $k_{-3} = k_1$ also has to be large.

Compartmentation and the rate laws

- ▶ Reduced rate law for the overall process $G_{(out)} \rightarrow G_{(in)}$:

$$v = \frac{v_{\max}[G_{(out)}]}{[G_{(out)}] + K_M}$$

- ▶ We **cannot** write $\frac{d[G_{(in)}]}{dt} = -\frac{d[G_{(out)}]}{dt} = v$ since the two compartments have different volumes, so equal changes in concentration correspond to different changes in number of moles, i.e. mass non-conservation.
- ▶ Note: This is not just a problem for the reduced model. In general we have to be very careful when a system has compartments of different volumes.

Compartmentation and the rate laws

- ▶ What is true is that $\frac{d\mathcal{N}(G_{(in)})}{dt} = -\frac{d\mathcal{N}(G_{(out)})}{dt}$ where $\mathcal{N}(\cdot)$ refers to the number of moles (or molecules) of a substance.
- ▶ Need to consider units carefully:
 - ▶ If we can get $v_{\max}$ (and thus v) in mol/s (or equivalent units), then $\frac{d[G_{(in)}]}{dt} = v/V_{\text{cells}}$ and $\frac{d[G_{(out)}]}{dt} = -v/V_{\text{medium}}$.
 - ▶ k_3 is in s^{-1} .
 - ▶ If we use the number of moles of T for T_0 , then we'll have what we want.

$$T_0 = \left\{ \begin{array}{c} \text{Moles of} \\ \text{transporters} \\ \text{per cell} \end{array} \right\} \times \left\{ \begin{array}{c} \text{Concentration} \\ \text{of cells} \end{array} \right\} \times \left\{ \begin{array}{c} \text{Volume of} \\ \text{culture} \end{array} \right\}$$

Next time

Coupling glucose uptake to growth
and our first numerical simulations!