Modelling Biochemical Reaction Networks

Lecture 5: Passive transport

Marc R. Roussel

Department of Chemistry and Biochemistry

University of Lethbridge

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:

$$T_{out} \underset{k_{io}}{\overset{k_{oi}}{\rightleftharpoons}} T_{in}$$

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

$$T_{out} + G_{(out)} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C_{out} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} C_{in} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} T_{in} + G_{(in)}$$

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 $\mathsf{T}_{\mathsf{in}} + \mathsf{G}_{(\mathsf{in})} \xrightarrow{k_{-3}} \mathsf{C}_{\mathsf{in}}$ 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:

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

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

$$k_{oi}[\mathrm{T_{out}}] \approx k_{io}[\mathrm{T_{in}}]$$
$$k_1[\mathrm{T_{out}}][\mathrm{G_{(out)}}] \approx k_{-1}[\mathrm{C_{out}}]$$
$$k_2[\mathrm{C_{out}}] \approx k_{-2}[\mathrm{C_{in}}]$$

Simplification using equilibrium approximations

3. Before doing any algebra, remind yourself of the objective. We want the rate of glucose transport, $v = d[\mathrm{G}_{(\mathrm{in})}]/dt = k_3[\mathrm{C}_{\mathrm{in}}]$. Mathematically, it will be easier to leave $[\mathrm{C}_{\mathrm{in}}]$ 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:

$$
\begin{aligned}
[\mathrm{C}_{\mathrm{out}}] &= K_2[\mathrm{C}_{\mathrm{in}}] \\
[\mathrm{T}_{\mathrm{out}}] &= K_1\frac{[\mathrm{C}_{\mathrm{out}}]}{[\mathrm{G}_{(\mathrm{out})}]} = K_1K_2\frac{[\mathrm{C}_{\mathrm{in}}]}{[\mathrm{G}_{(\mathrm{out})}]} \\
[\mathrm{T}_{\mathrm{in}}] &= K_{oi}[\mathrm{T}_{\mathrm{out}}] \;\; = K_1K_2K_{oi}\frac{[\mathrm{C}_{\mathrm{in}}]}{[\mathrm{G}_{(\mathrm{out})}]}
\end{aligned}
$$

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 $[\mathrm{C_{in}}]$, then multiply by k_3 to get v:

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

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 $[G_{(out)}]$ 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

$$\mathsf{T}_{\mathsf{out}} \underset{k_{io}}{\overset{k_{oi}}{\rightleftharpoons}} \mathsf{T}_{\mathsf{in}}$$

$$\mathsf{T}_{\mathsf{out}} + \mathsf{G}_{(\mathsf{out})} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathsf{C}_{\mathsf{out}} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathsf{C}_{\mathsf{in}} \xrightarrow{k_3} \mathsf{T}_{\mathsf{in}} + \mathsf{G}_{(\mathsf{in})}$$

- 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

$$\mathsf{T}_{\text{out}} \underset{k_{io}}{\overset{k_{oi}}{\rightleftharpoons}} \mathsf{T}_{\text{in}}$$

$$\mathsf{T}_{\text{out}} + \mathsf{G}_{(\text{out})} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathsf{C}_{\text{out}} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathsf{C}_{\text{in}} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \mathsf{T}_{\text{in}} + \mathsf{G}_{(\text{in})}$$

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

$$v_{\max} = \frac{1}{2} k_3 T_0 \qquad K_M = K_1$$

- 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 $\mathrm{G}_{(\mathrm{out})} \rightarrow \mathrm{G}_{(\mathrm{in})}$:

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

- We cannot write $\frac{d[\mathrm{G}_{(\mathrm{in})}]}{dt} = -\frac{d[\mathrm{G}_{(\mathrm{out})}]}{dt} = v$ 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 $\frac{d\mathcal{N}(\mathrm{G}_{(\mathrm{in})})}{dt} = -\frac{d\mathcal{N}(\mathrm{G}_{(\mathrm{out})})}{dt}$ 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 $\frac{d[\mathrm{G}_{(\mathrm{in})}]}{dt} = v/V_{\mathrm{cells}}$ and $\frac{d[\mathrm{G}_{(\mathrm{out})}]}{dt} = -v/V_{\mathrm{medium}}$.
  - 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!