Metabolic networks and flux balance analysis

1. **The Krebs cycle.**

   The Krebs cycle provides the energy for oxidative phosphorylation. Derivatives of pyruvate enter the cycle, in which a series of enzyme-catalysed reactions produce NADH and free protons, as well as several other chemical species; at the end of the cycle, the product is combined with fresh pyruvate derivative to allow its continuation. We will use the Krebs cycle to illustrate the process of data collection for metabolic analyses.

   (a) Find the set of Krebs cycle reactions, reactants and products. Where do the initial reactants come from? How do the products feed into later parts of oxidative phosphorylation? How is energy transferred and used to produce ATP?

   (b) Write down a stochiometry matrix for the Krebs cycle as a whole (starting from acetyl-CoA). Ensure that reactions exist to balance the system (this may involve explicitly writing down input and output processes).

   (c) Imagine we wish to maximise the system's output of NADH. Why would flux balance analysis on this system be rather trivial?

2. **Flux balance analysis.**

   We will now explore an artificial metabolic network that is less trivial that the cycle and small networks we met previously. The model network is pictured in Fig. 1.

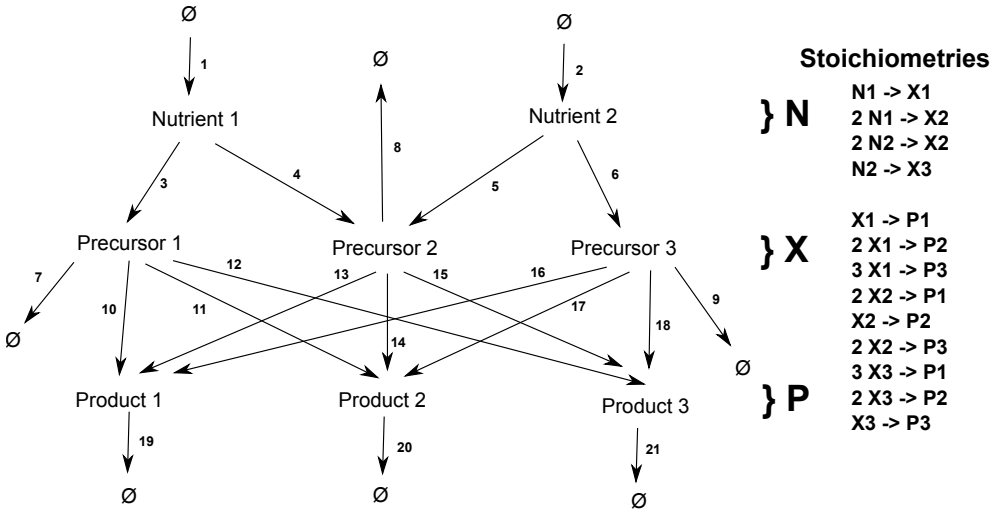

Figure 1: A model metabolic network for FBA.

   (a) The file `FBAprac.m` contains the stochiometry matrix for this network. *Note that this is stored as $\underline{\underline{S}}^T$ in the code to aid interpretation – you will need to use its transpose in linear programming.* Use flux balance analysis to determine an optimal set of fluxes for the production of Product 3. (Use Matlab's `linprog` command. The appropriate task is to find $\underline{v}$ to maximise $\underline{c} \cdot \underline{v}$ subject to $\underline{\underline{S}}\underline{v} = \underline{0}$ and $\underline{0} \leqslant \underline{v} \leqslant \underline{v}_{max}$, where $\underline{\underline{S}}$ is the stochiometry matrix, $\underline{c}$ is your objective function, and $\underline{v}_{max}$ is some maximal flux constraint.)

   (b) `PlotFBABiograph.m` visualises the flux through this network, taking as an argument $\underline{v}$, the vector of fluxes. Use this code to produce a plot of the network demonstrating the fluxes that solve this problem. Does the result match how you would maximise production?

   (c) Our system is now placed in an environment where Nutrient 2 is present at only 1% the level of Nutrient 1 – so the maximum uptake of Nutrient 2 is 0.01 of maximal Nutrient 1 uptake. Adapt your FBA model to incorporate this. How does the optimal flux change?

   (d) In the same environment, the gene encoding the enzyme that produces Product 3 from Precursor 3 is knocked out. Adapt your FBA model appropriately. Visualise the changing flux.

   (e) It is vital for the system to produce a certain amount (say 5% of Nutrient 1 uptake) of Product 2 to stay alive. How can we maximise the adapted model for Product 3 output while meeting this condition?