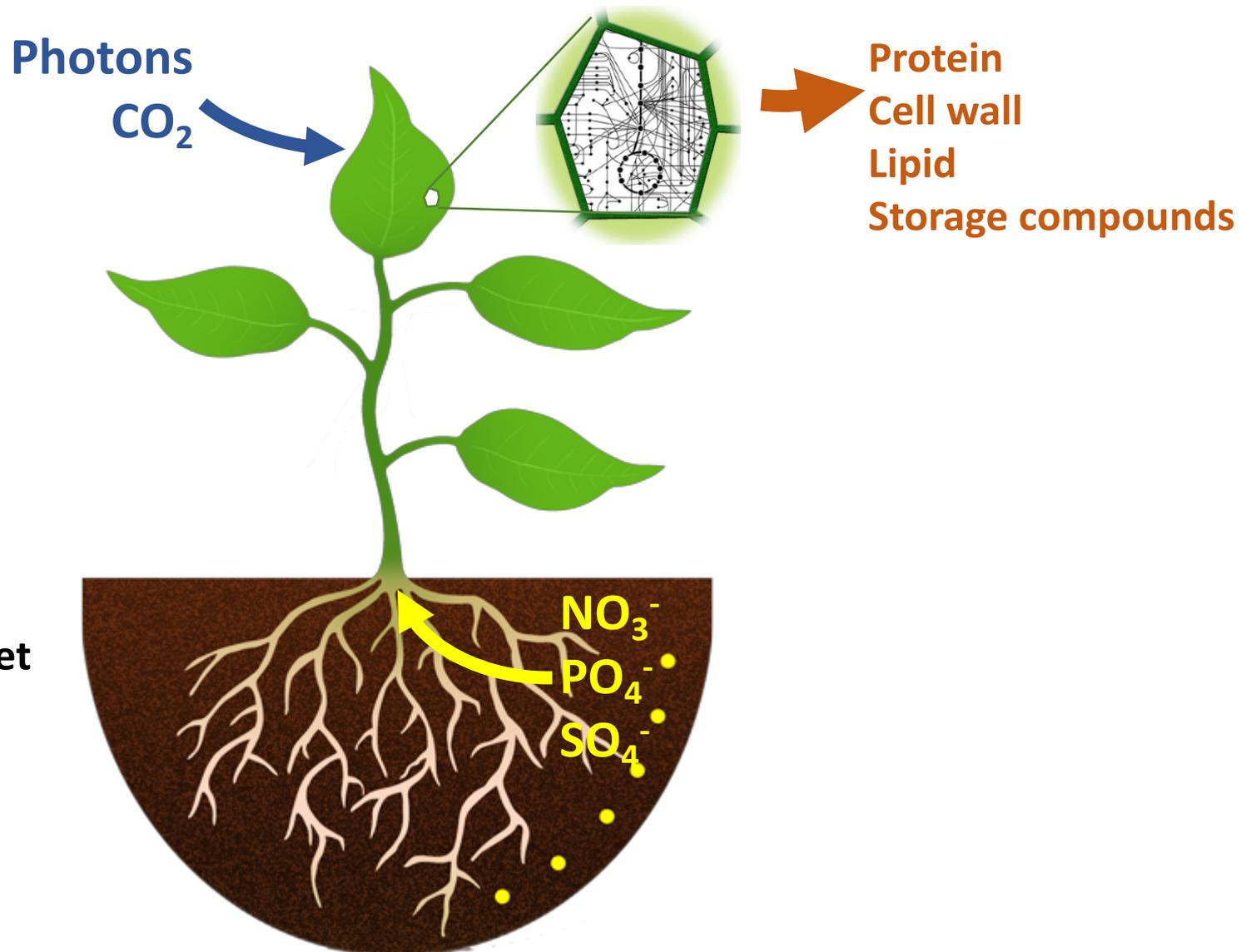


Using flux balance analysis to model plant metabolism



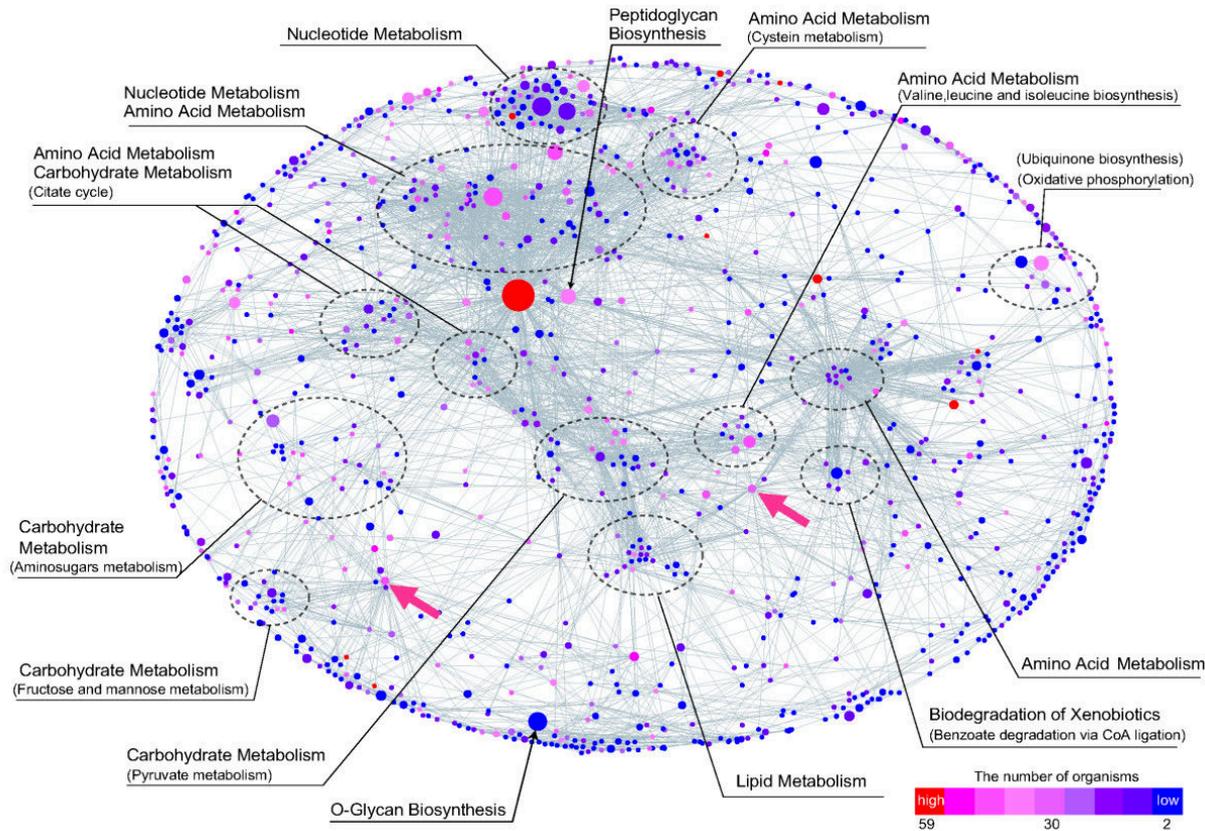
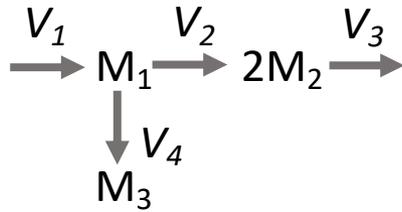
Lee Sweetlove
George Ratcliffe

Sanu Shameer
Nadine Töpfer
Mann Konuntakiet



Metabolism forms a connected network

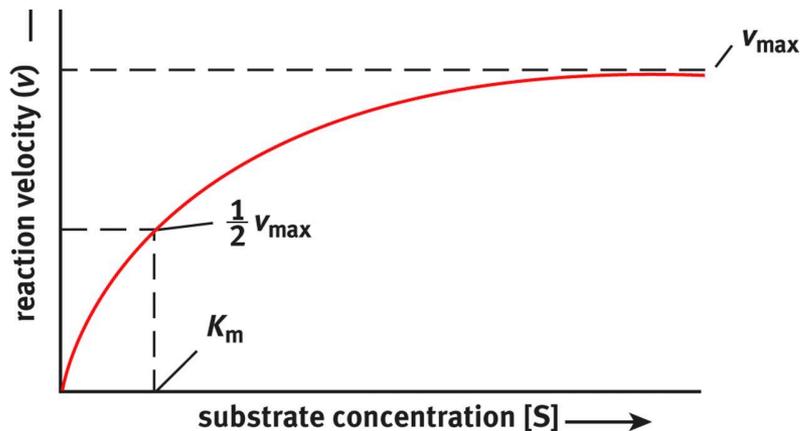
Metabolite steady state



Yamada, T., Kanehisa, M., & Goto, S. *BMC bioinformatics*, 7, 130

Modelling metabolism

Enzyme kinetic modelling



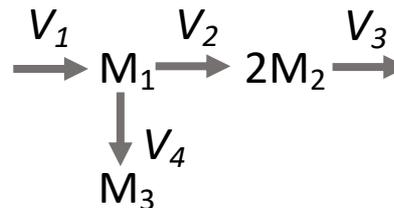
$$V_1 = \frac{V_{1\max} A}{K_{M1} + M_1}$$



$$\frac{dM_2}{dt} = V_1 - V_2$$

$$r_{PFK} = \frac{\frac{V_f^{PFK} C_{MgATP}^c C_{F6P}^c}{K_{F6P}^{PFK} K_{MgATP}^{PFK}} - \frac{V_r^{PFK} C_{MgADP}^c C_{F16BP}^c}{K_{F16BP}^{PFK} K_{MgADP}^{PFK}}}{\left(\left(1 + \frac{C_{F6P}^c}{K_{F6P}^{PFK}} \right) \left(1 + \frac{C_{MgATP}^c}{K_{MgATP}^{PFK}} \right) + \left(1 + \frac{C_{F16BP}^c}{K_{F16BP}^{PFK}} \right) \left(1 + \frac{C_{MgADP}^c}{K_{MgADP}^{PFK}} \right) - 1 \right)} N_{PFK}$$

Flux balance analysis (FBA)



Stoichiometry Matrix

Rates Vector

$$S = \begin{array}{c} \text{Metabolite} \\ \begin{pmatrix} 1 & -1 & 0 & -1 \\ 0 & 2 & -2 & 0 \\ 0 & 0 & 0 & 1 \end{pmatrix} \end{array} \begin{array}{c} \text{Reaction} \\ 1 \quad 2 \quad 3 \quad 4 \\ M_1 \\ M_2 \\ M_3 \end{array}$$

Note: In the original image, a dashed box highlights the second column of the matrix (reaction 2) and the second row (metabolite M2), with an arrow pointing to the reaction equation $M_1 \rightarrow 2M_2$.

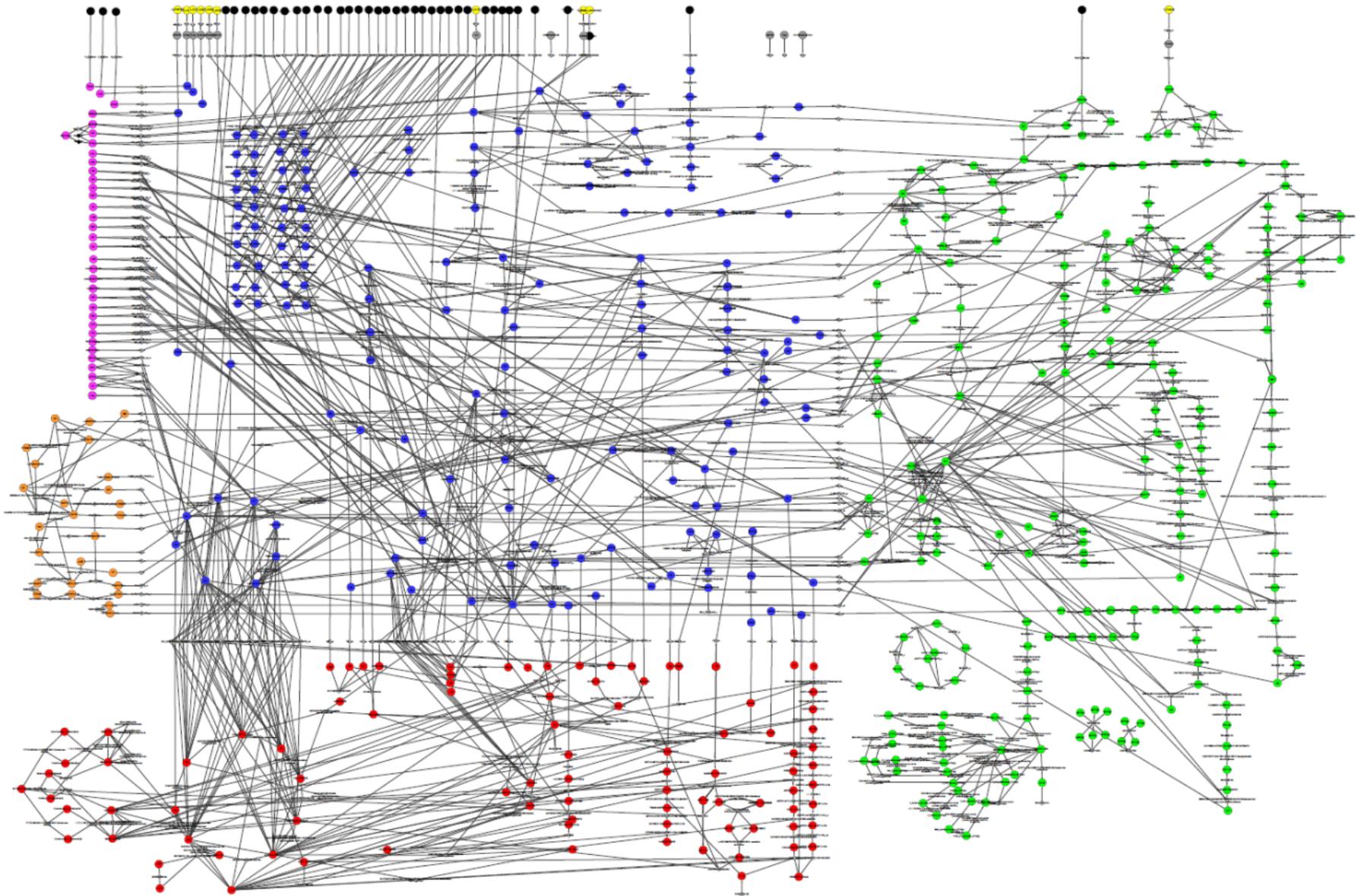
$$\vec{v} = \begin{pmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \end{pmatrix}$$

$$S \cdot v = 0$$

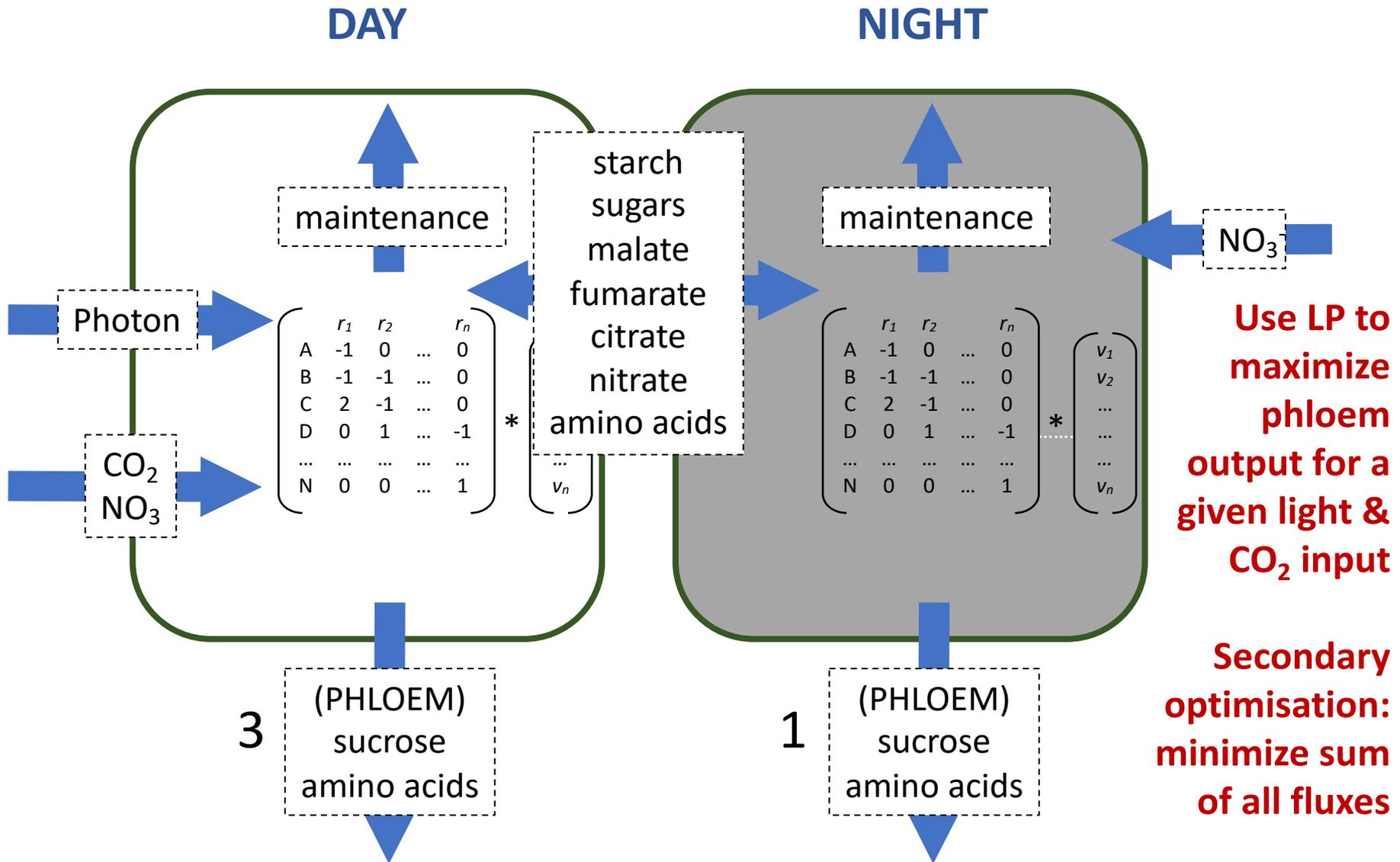
→
a system of
linear
equations:

$$\begin{aligned} v_1 - v_2 - v_4 &= 0 \\ 2v_2 - 2v_3 &= 0 \\ \text{etc} \end{aligned}$$

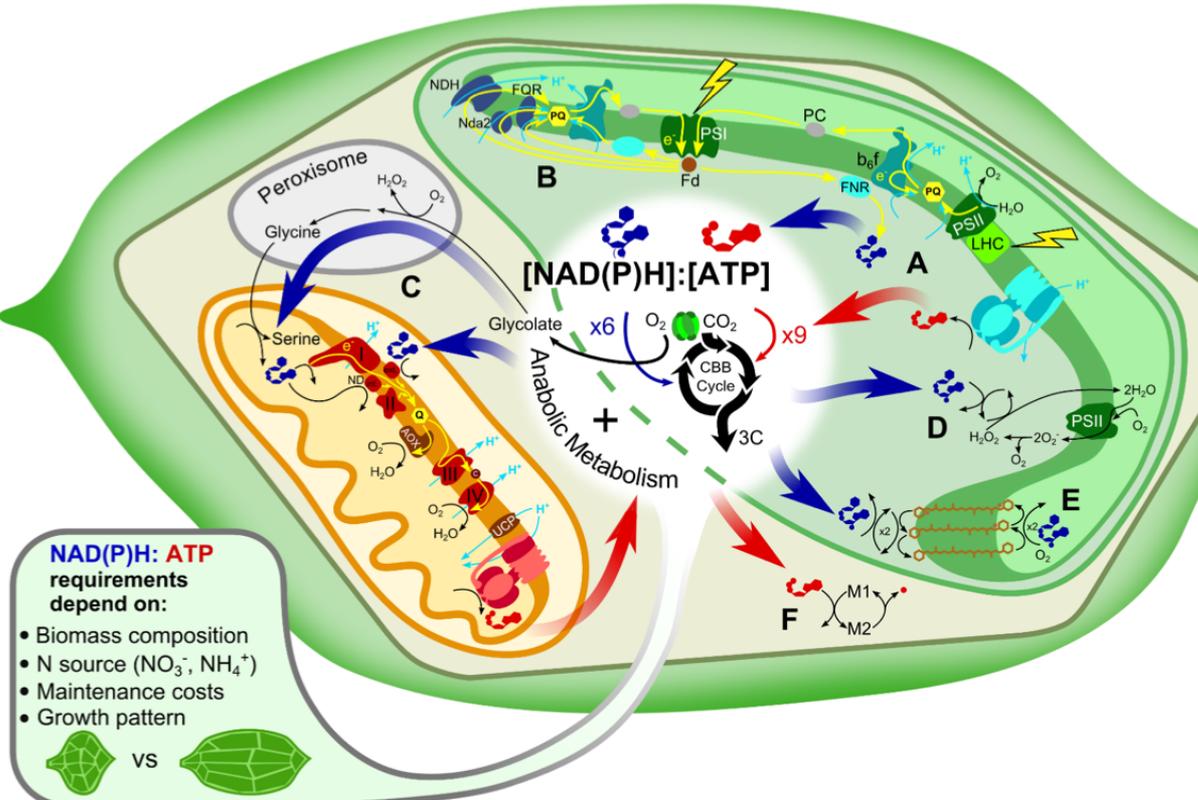
Stoichiometric model of central metabolism



Setting up an FBA model for a leaf



The model generates a remarkably accurate prediction of known C₃ leaf metabolism



- Carbon fixation by the Calvin-Benson cycle
- Recycling of phosphoglycolate by the photorespiratory cycle
- Daytime storage of starch and a smaller amount of malate
- Assimilation of nitrate during the day using nocturnally-generated citrate as a source of carbon skeletons
- Mitochondrial TCA cycle: cyclic at night but incomplete, bifurcated cycle during the day

NAD(P)H: ATP requirements depend on:

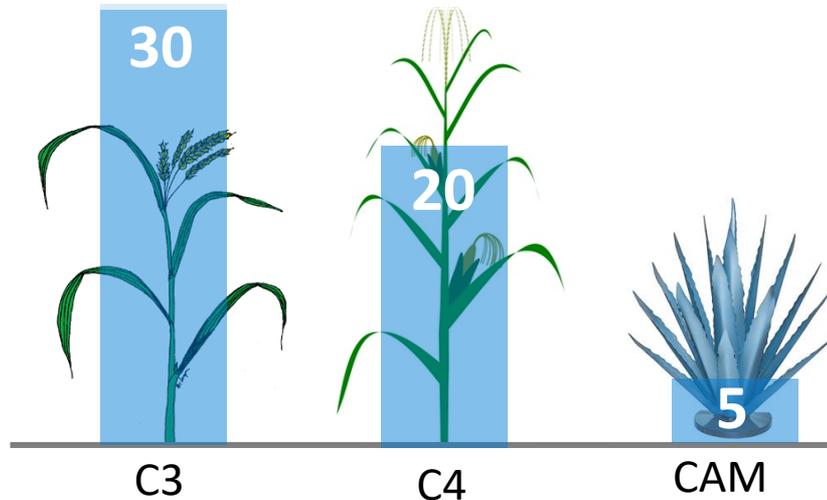
- Biomass composition
- N source (NO₃⁻, NH₄⁺)
- Maintenance costs
- Growth pattern

VS

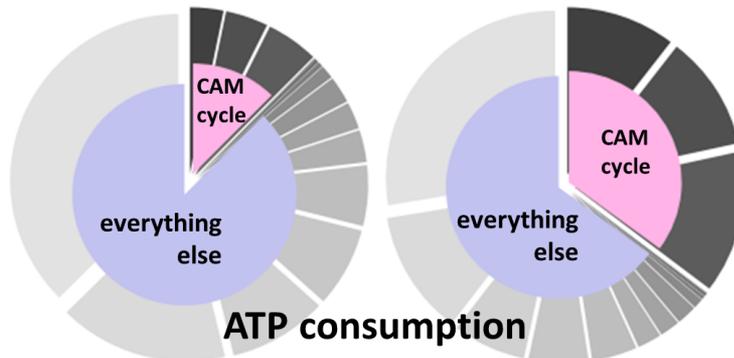
What can we do with this model?

1. Help guide crop engineering efforts

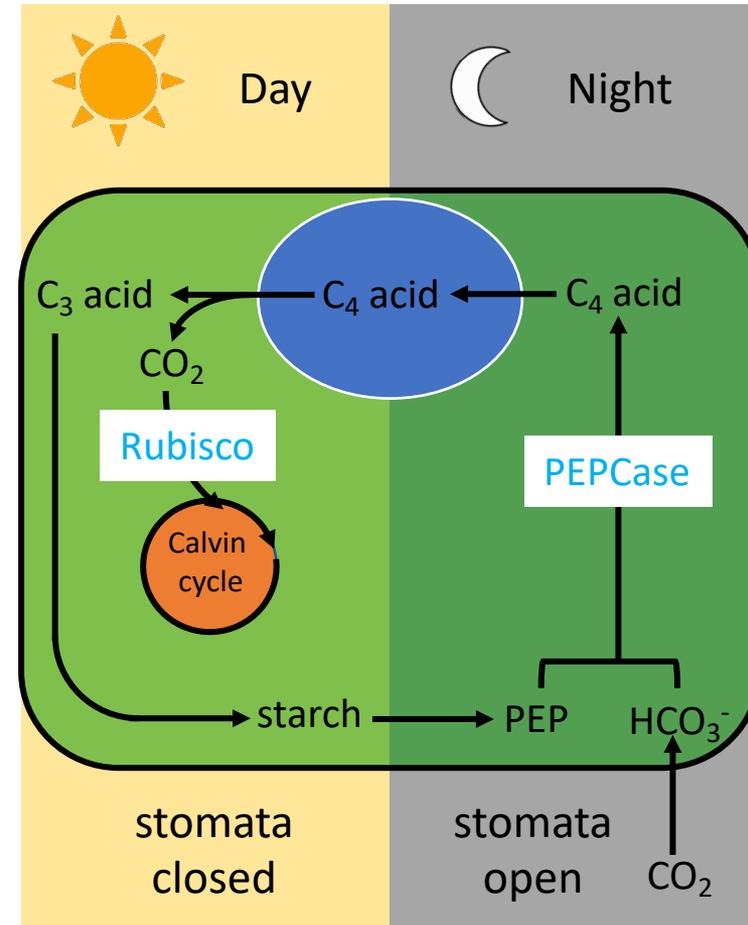
kg water ha⁻¹ yr⁻¹



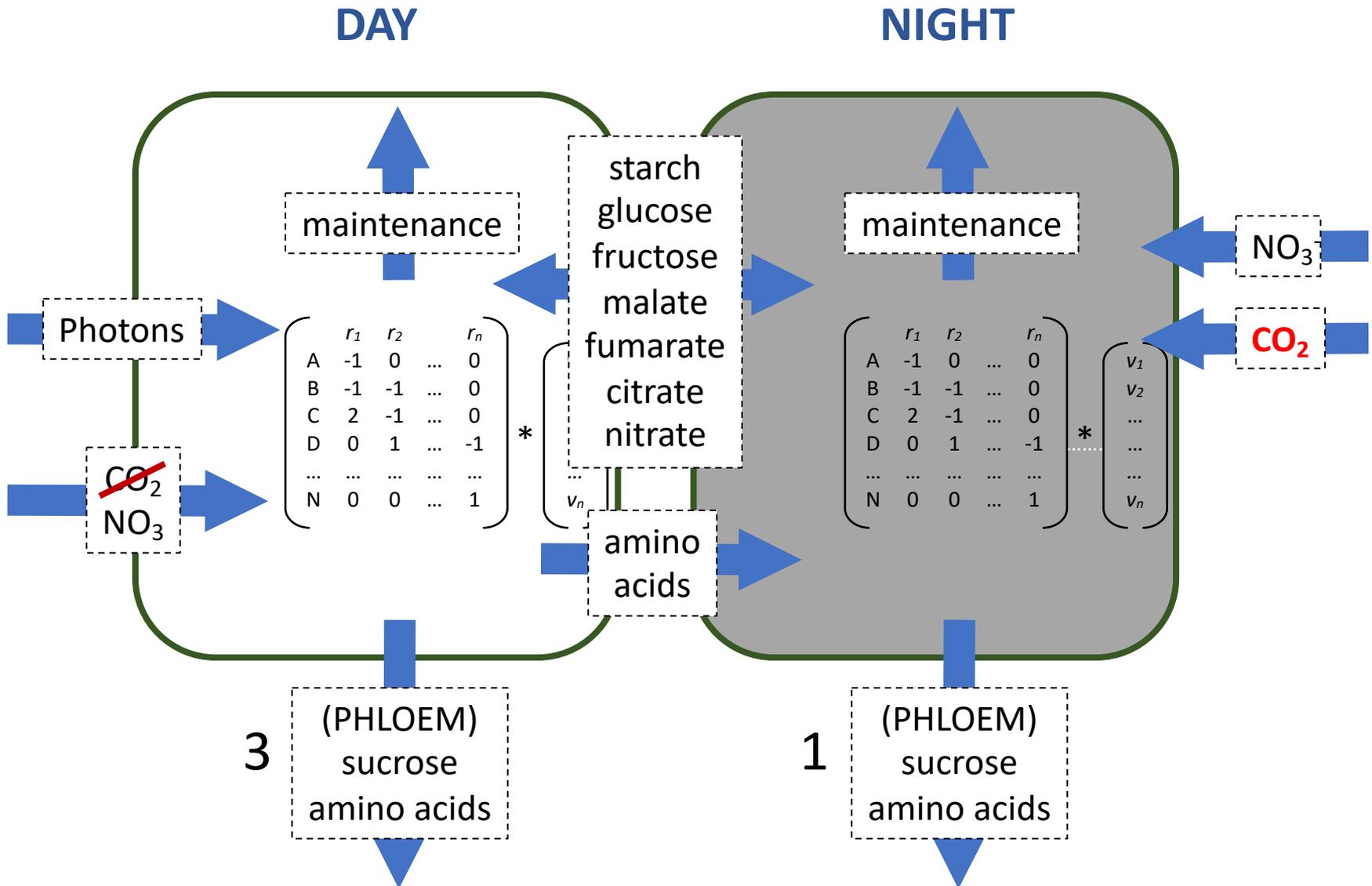
Type of photosynthesis



CAM PHOTOSYNTHESIS

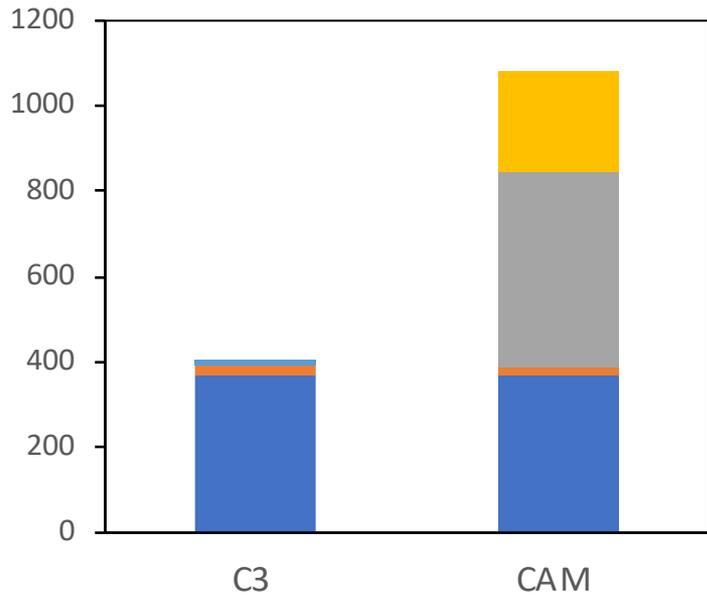


Constraining the model for CAM



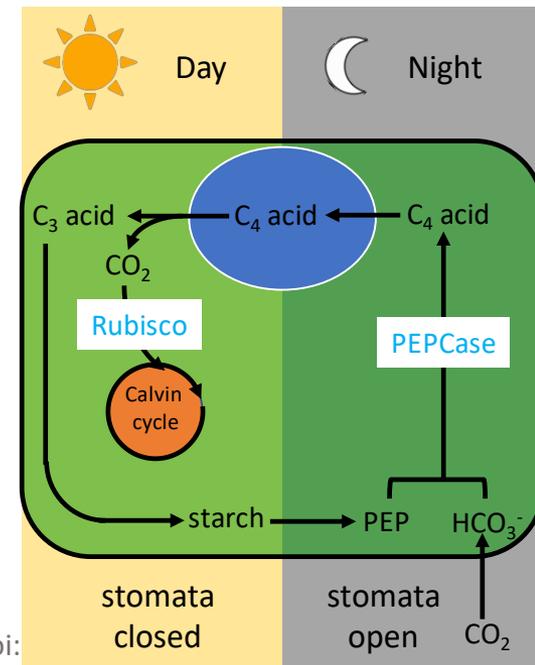
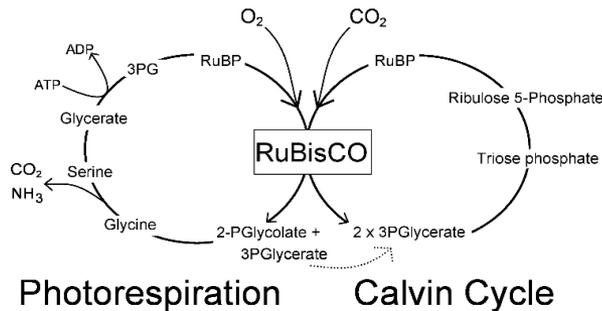
The cost of CAM

- During the day, the ATP demand of the CAM model is similar to that of the C₃ model
- At night, the CAM model requires a massive 270% more ATP than the C₃ model



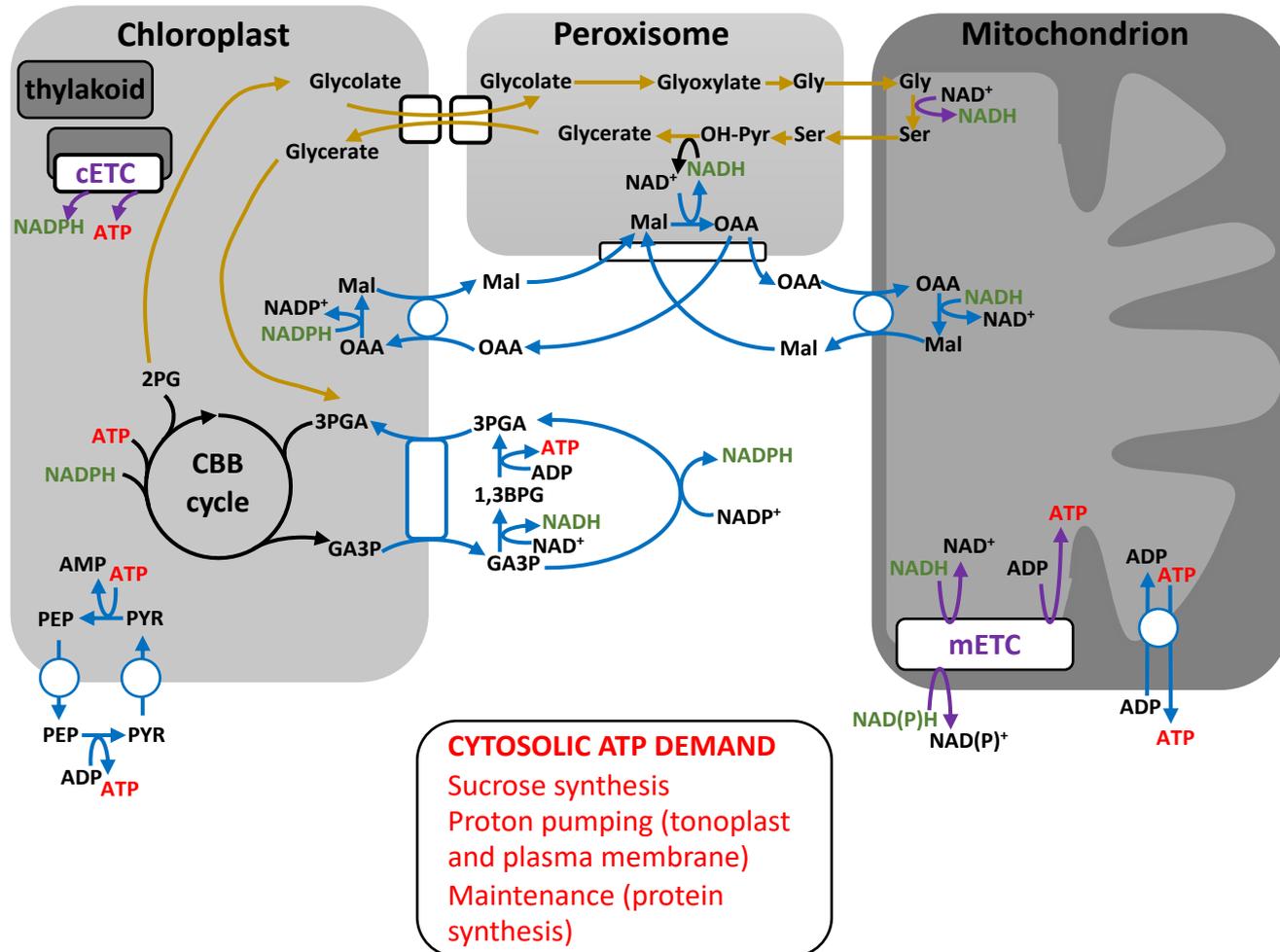
- Maintenance
- Output to phloem
- Tonoplast ATPase
- glycolysis (PFK)
- Starch breakdown

- Despite the extra cost, the CAM model can match the productivity of the C₃ model
- How?



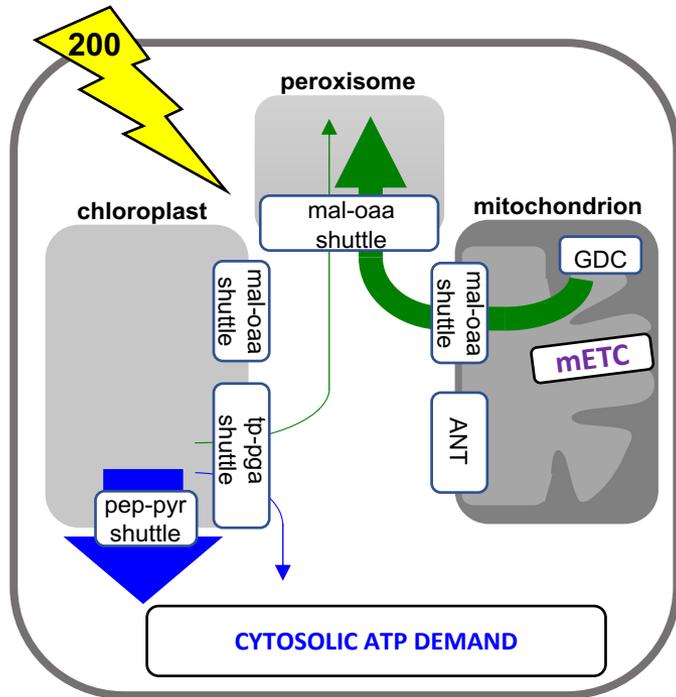
What else can we do with this model?

2. Address fundamental knowledge gaps



Even at low light intensities, the chloroplast can theoretically generate all the ATP the leaf needs

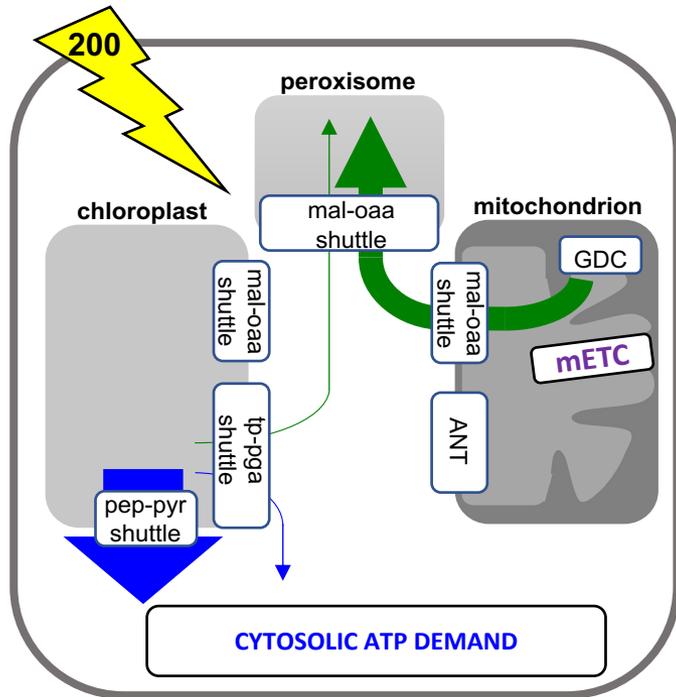
Subcellular ATP and NADH exchange fluxes predicted by the C₃ leaf model during the day



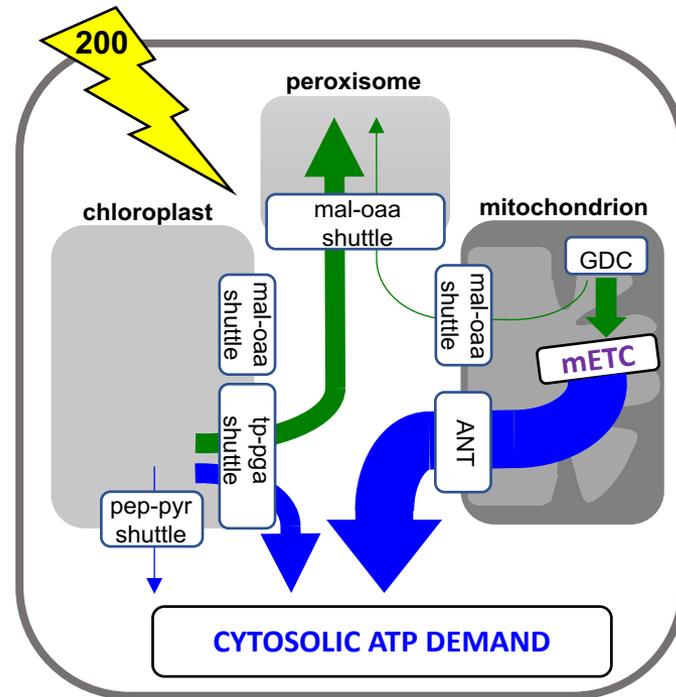
$2 \mu\text{mol m}^{-2} \text{s}^{-1}$ \rightarrow ATP $2 \mu\text{mol m}^{-2} \text{s}^{-1}$ \rightarrow NAD(P)H

...but capacity limits in the chloroplast ATP-export shuttles necessitate mitochondrial respiration

Subcellular ATP and NADH exchange fluxes predicted by the C₃ leaf model during the day



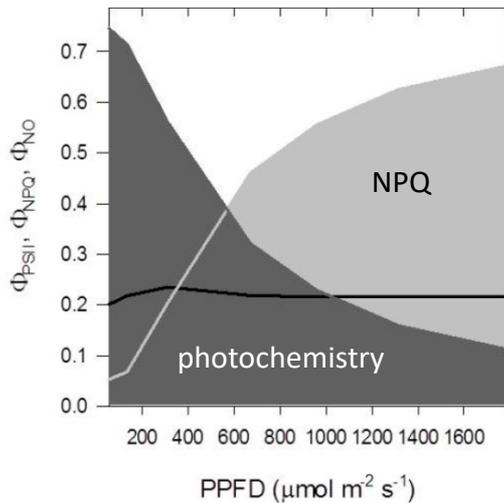
Predicted fluxes when the maximum capacity of the chloroplast ATP shuttles are constrained to experimental values



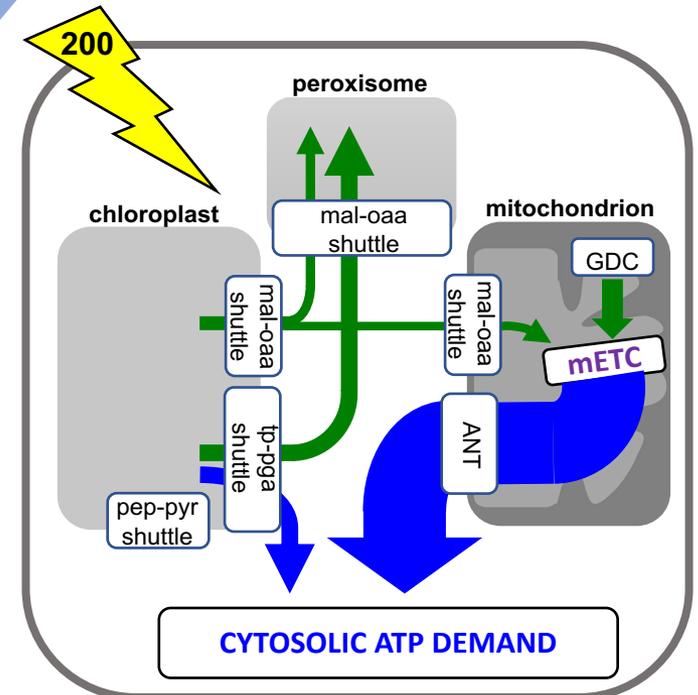
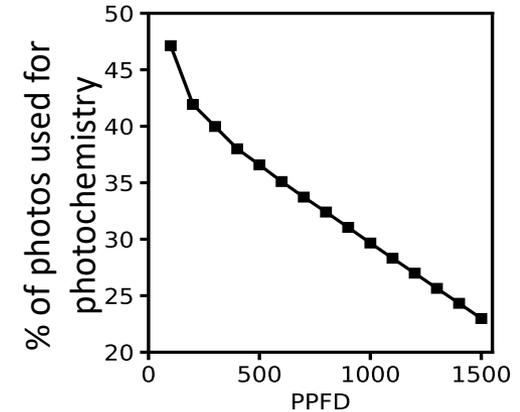
$2 \mu\text{mol m}^{-2} \text{s}^{-1}$ ATP $2 \mu\text{mol m}^{-2} \text{s}^{-1}$ NAD(P)H

Why don't plants invest in greater ATP shuttling capacity?

Khanal N, et al (2017) *Planta* **223**: 532-541

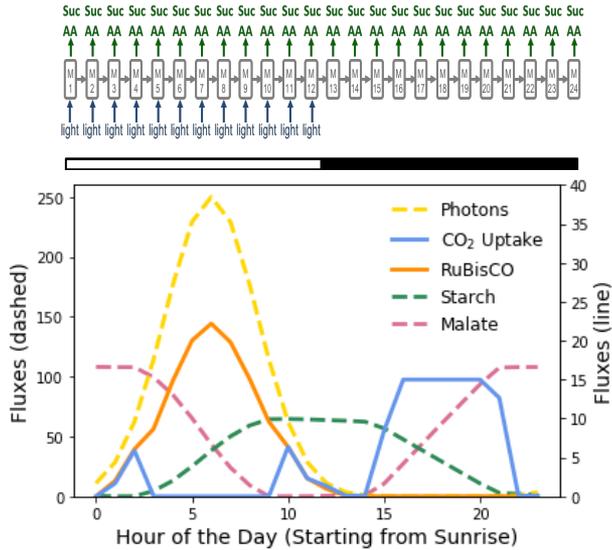


PREDICTIONS
WHEN MODEL IS
NOT FORCED TO
USE ALL THE
INCIDENT LIGHT

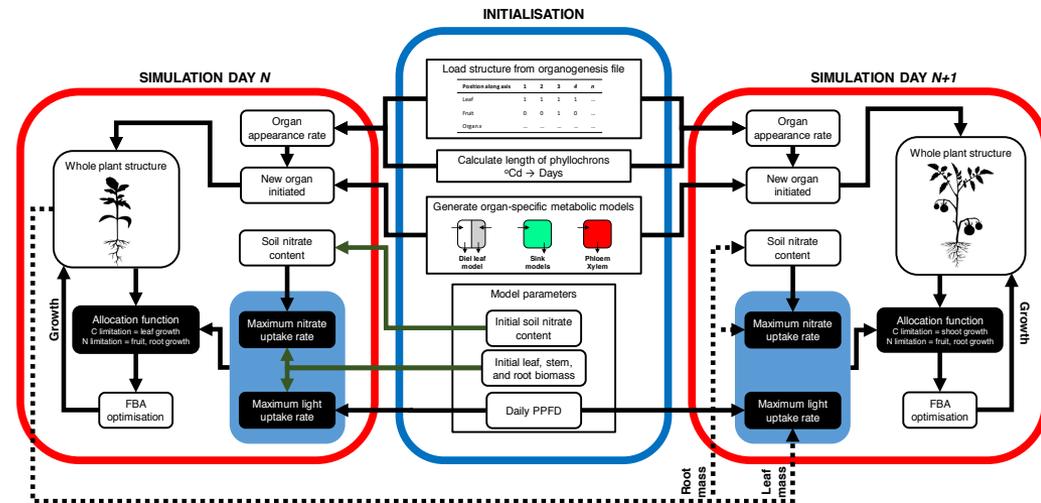


We're doing lots more with FBA

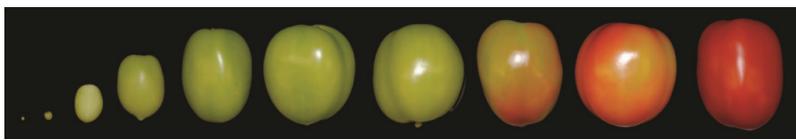
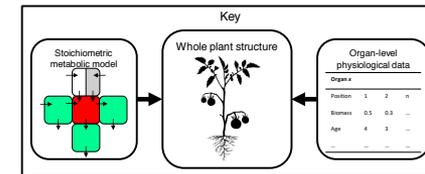
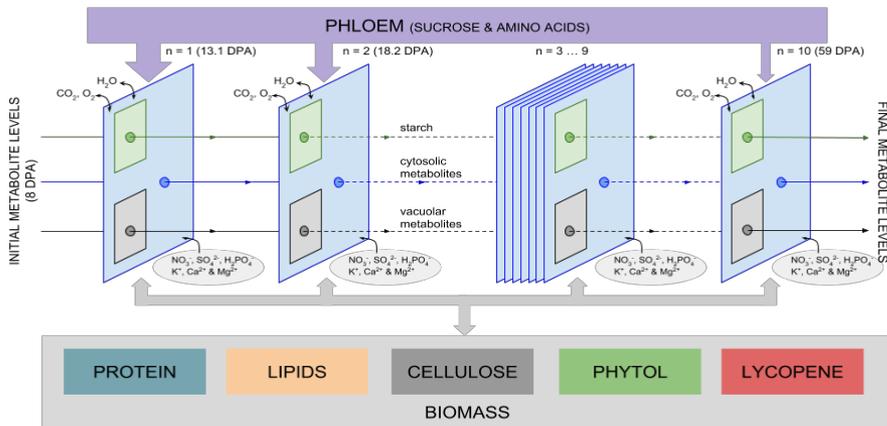
1. FBA for metabolic dynamics



3. Embedding FBA models into larger models of plant growth and form



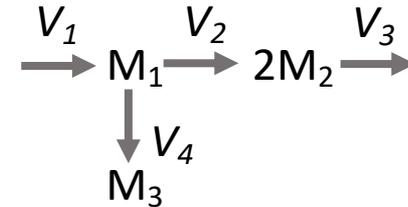
2. Osmotic-constraints for expanding cells



Take-home messages

- ❑ FBA is able to make accurate and useful predictions about metabolism despite lack of mechanistic features
- ❑ FBA can help understand system-level properties such as energy balancing
- ❑ FBA can be used to address knowledge gaps and guide metabolic engineering
- ❑ FBA can be used to model dynamic processes and different types of growth
- ❑ FBA can easily be embedded into larger models

Flux balance analysis (FBA)



Stoichiometry Matrix

Rates Vector

$$S = \begin{array}{c} \text{Metabolite} \\ \begin{pmatrix} 1 & -1 & 0 & -1 \\ 0 & 2 & -2 & 0 \\ 0 & 0 & 0 & 1 \end{pmatrix} \begin{array}{l} M_1 \\ M_2 \\ M_3 \end{array} \end{array}$$

$\begin{array}{cccc} & \text{Reaction} & & \\ & 1 & 2 & 3 & 4 \end{array}$

$$\vec{v} = \begin{pmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \end{pmatrix}$$

$$S \cdot v = 0$$

→
a system of
linear
equations:

$$\begin{array}{l}
 v_1 - v_2 - v_4 = 0 \\
 2v_2 - 2v_3 = 0 \\
 \text{etc}
 \end{array}$$