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A genome scale model of *Cupriavidus necator* for 3-HP production

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Synthetic Biology Research Centre

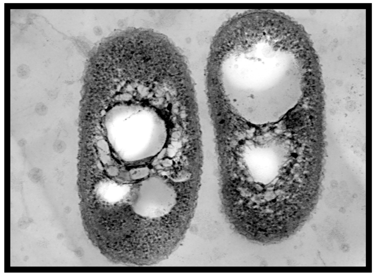
26th January, 2017

Gas fermentation for platform chemical production

Industrial waste

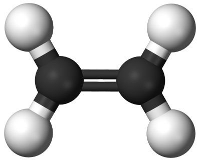
$CO_2 + H_2$

Microbial Fermentation

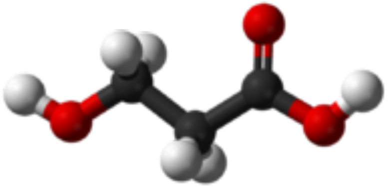


Cupriavidus necator

Platform chemicals



ETHYLENE



3-HYDROXY-PROPRIONIC ACID

Gas fermentation for platform chemical production

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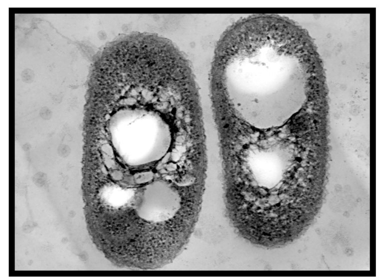

Cupriavidus necator

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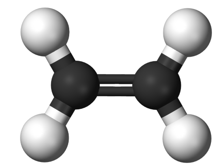

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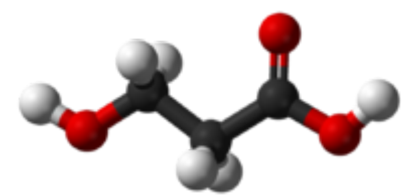
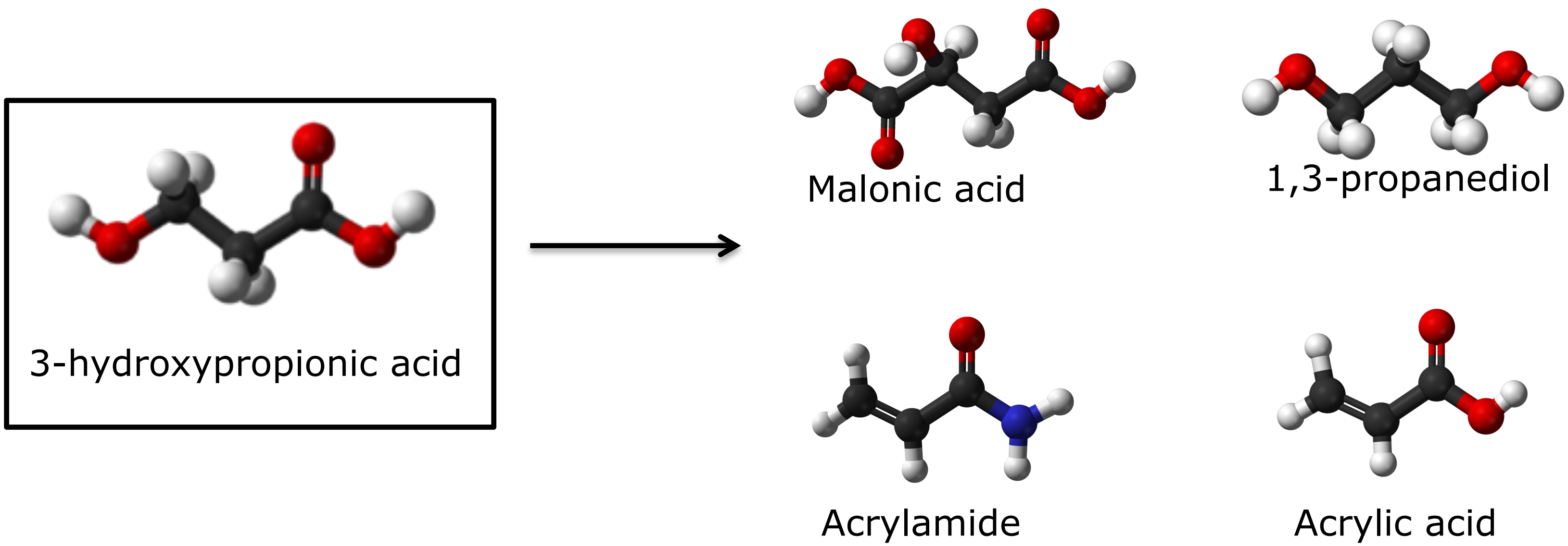


3-HYDROXY-PROPIONIC ACID

3-HP production – first intermediate chemical



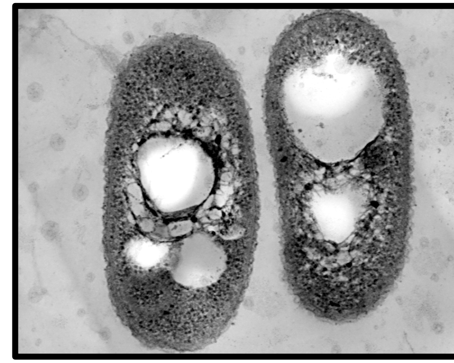
- Important building block for **biorenewable** polymers
- Chemical synthesis - is not commercially feasible due to low yield and high production cost

C. necator an ideal chassis for biotechnology

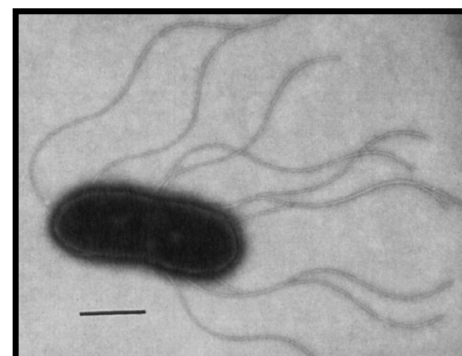
- Facultatively **chemolithoautotrophic** bacteria - grow with organic substrates or $\mathbf{H_2}$ and $\mathbf{CO_2}$ under aerobic conditions

- Grow to high-cell densities under lithoautotrophic or heterotrophic conditions

- Produces large amounts of a biodegradable polymer polyhydroxybutyrate (PHB)



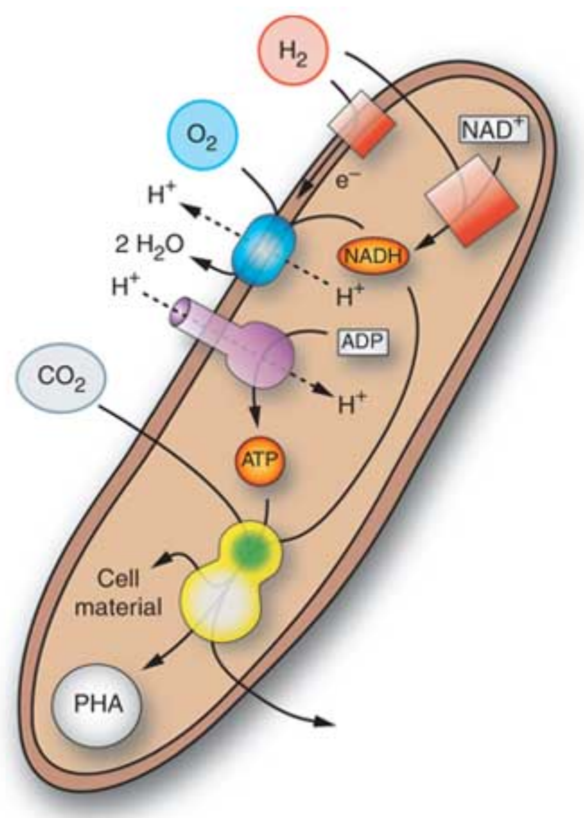
Flagellation of strain N-1. Bar, 1.0 µm



STEM picture of *Cupriavidus necator* harbouring PHB granules

C. necator lithoautotrophic metabolism

- Carbon dioxide is fixed via the Calvin cycle
- Membrane bound hydrogenase directly connected to the electron transport chain (ETC) for generating ATP
- Soluble hydrogenase that is coupled to NADH synthesis that is required for the Calvin cycle or ETC
- Oxygen final electron acceptor (under anaerobic conditions nitrate is used)



Lithoautotrophic metabolism

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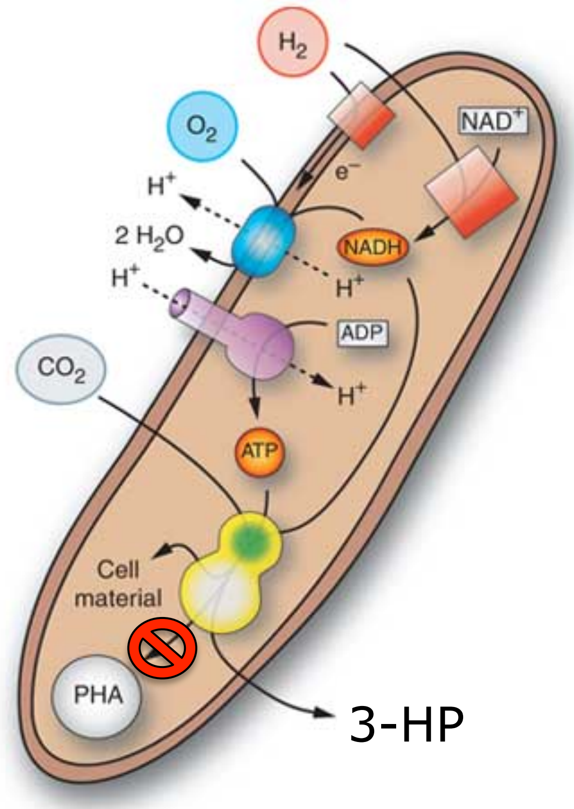
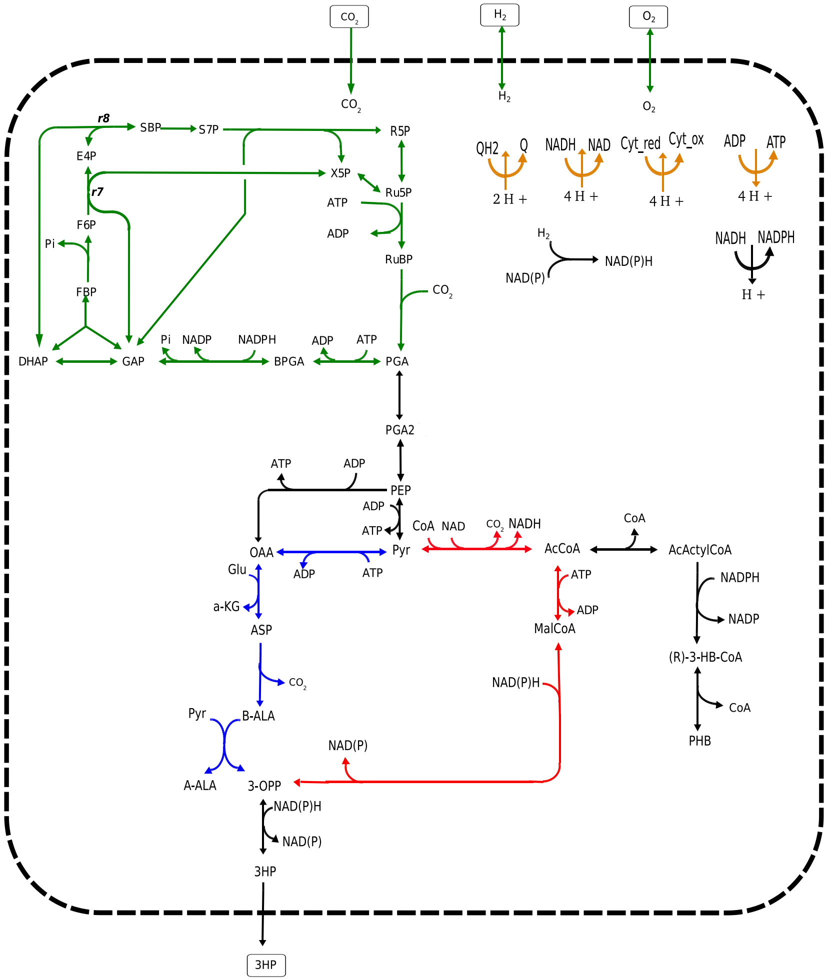


Lithoautotrophic metabolism

Metabolic modelling of *C. necator* for 3-HP production

- Assess 3-HP pathways
  - Product yield
  - Oxygen requirements
  - ATP yield
- Simulate behaviour of bacteria for
  - insertion/knockouts of reactions
  - varying feeding ratios
- Predict metabolic interventions for increasing 3-HP production

Assessing 3-HP pathways using EMA



Computed **elementary modes** of a small model of *C. necator* to assess two **3-HP pathways**

- Calvin cycle (13)
- Electron transport chain (6)
- 3-HP pathways (13)
- PHB pathway (3)

Assessing 3-HP pathways using EMA

Pathway	Theoretical max. Yield (mol/mol co$_2$)	O_2 requirement (mol/mol product)	H_2 requirement (mol/mol product)	No. of gene additions
PHB	1.0	[5.57, 6.5]	[20.14, 22]	0
3-HP I. BAPAT	1.0	[3.0, 3.66]	[12.0, 13.33]	0
3-HP II. MCR	1.0	[3.27, 3.83]	[12.28, 13.66]	1

Net stoichiometry of example elementary mode:

$$3\ CO_2 + 12\ H_2 + 3\ O_2 \longrightarrow 1\ \text{3-HP} + 1\ H_2O + H^+$$

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Net stoichiometry:

$$3\ CO_2 + 12\ H_2 + 3\ O_2 \longrightarrow 1\ \text{3-HP} + 1\ H_2O + H^+$$

- Deletion of **membrane hydrogenase** removes all non-optimal modes in terms of oxygen requirements

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Genome scale model of *C. necator*

- GSM construction based on BioCyc database for *C. necator* H16

- Model constructed as set of modules:
  - BioCyc reactions (1101)
  - Transport reactions (58)
  - Electron transport chain (10)
  - Additional reactions (152)

- Minimal aerobic media: fructose, oxygen, sulfate, phosphate and ammonium

- Final model consists of 1301 reactions and 1200 metabolites

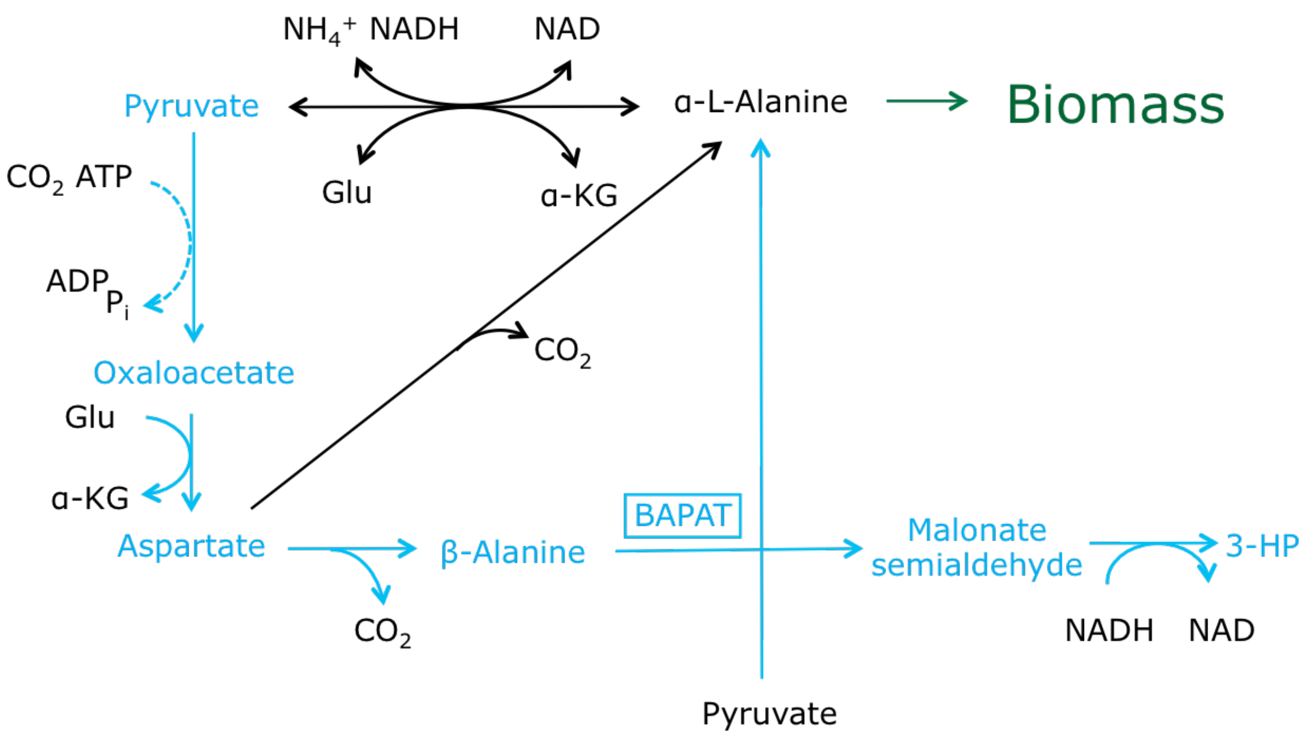
Simulating reaction knockouts

$$\text{minimise}: \ |\mathbf{v}|$$ ← Minimise the sum of reaction fluxes

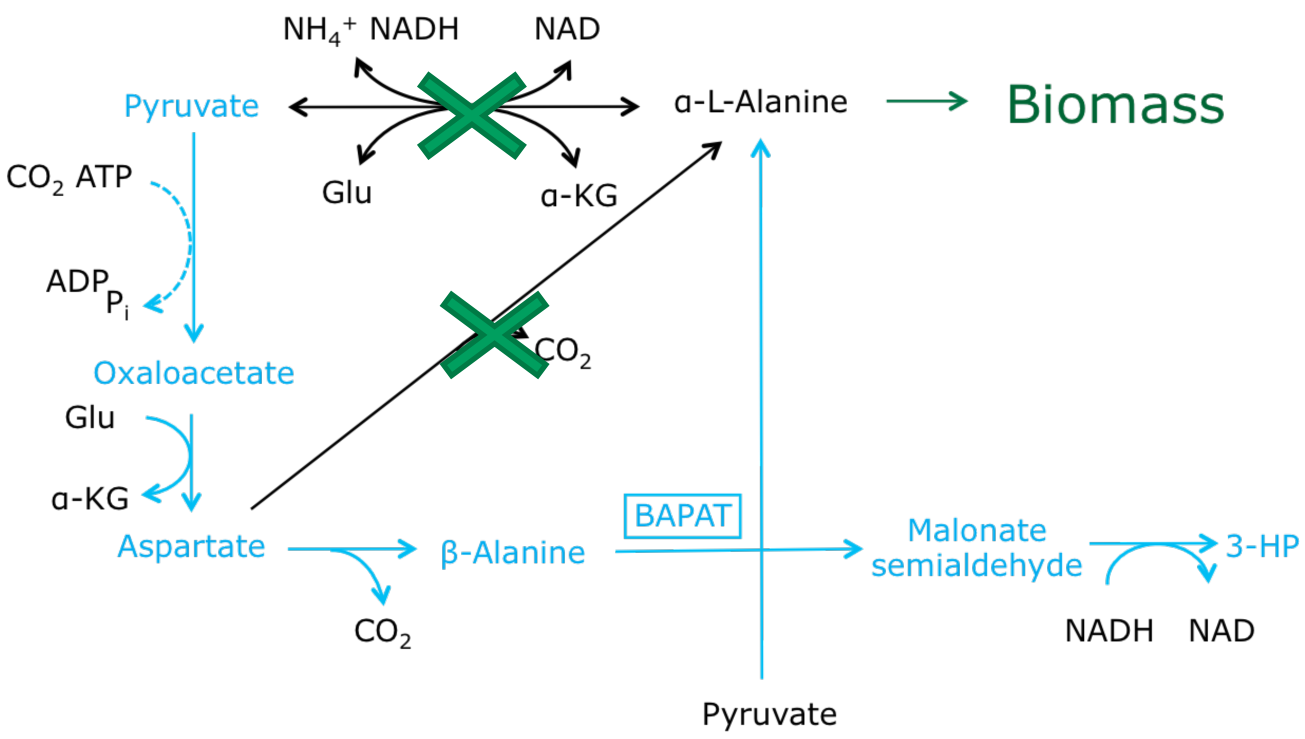
$$\text{subject to}: \begin{cases} Nv = 0 \\ v_j = t_j; \\ v_{\text{ATPase}} = J_{\text{ATPase}} \\ v_{\text{ko}} = 0 \end{cases}$$

- $Nv = 0$ ← Steady state assumption
- $v_j = t_j;$ ← Biomass transporters
- $v_{\text{ATPase}} = J_{\text{ATPase}}$ ← ATP maintenance
- $v_{\text{ko}} = 0$ ← Reaction knockouts

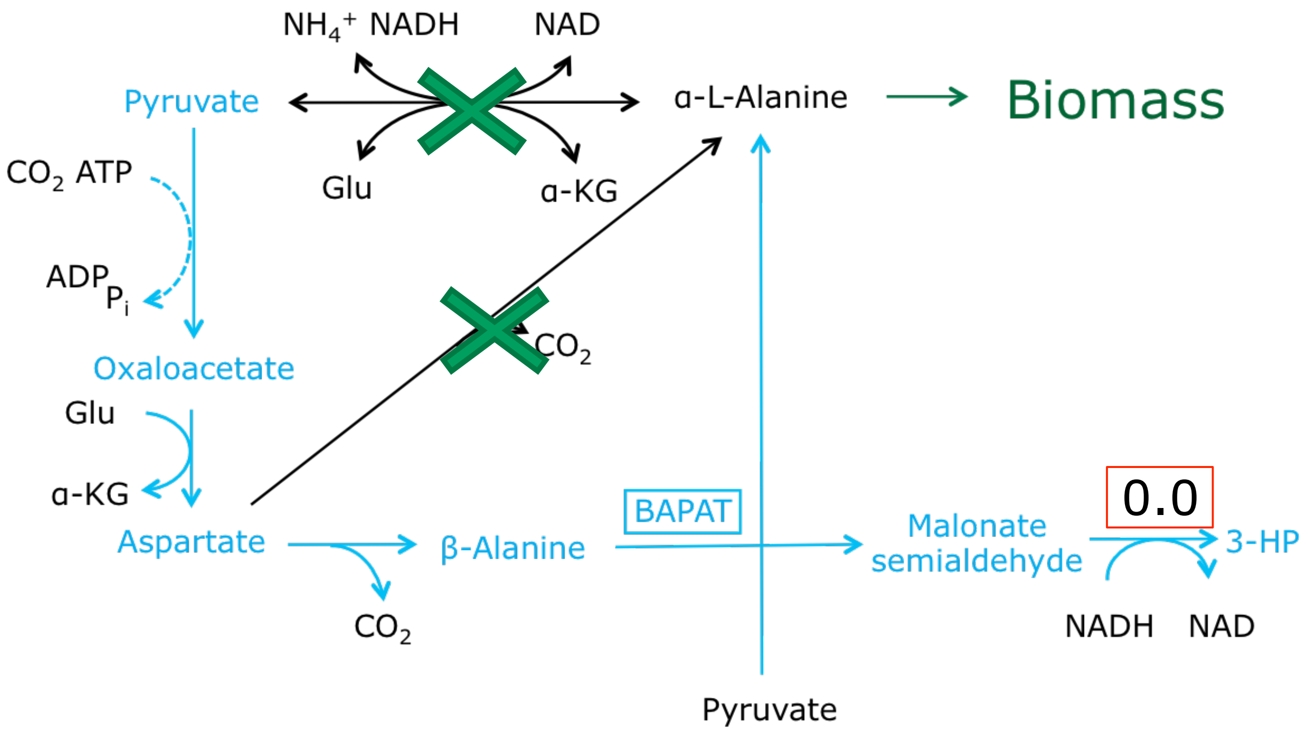
3-HP growth coupling strategy 1



3-HP growth coupling strategy 1:

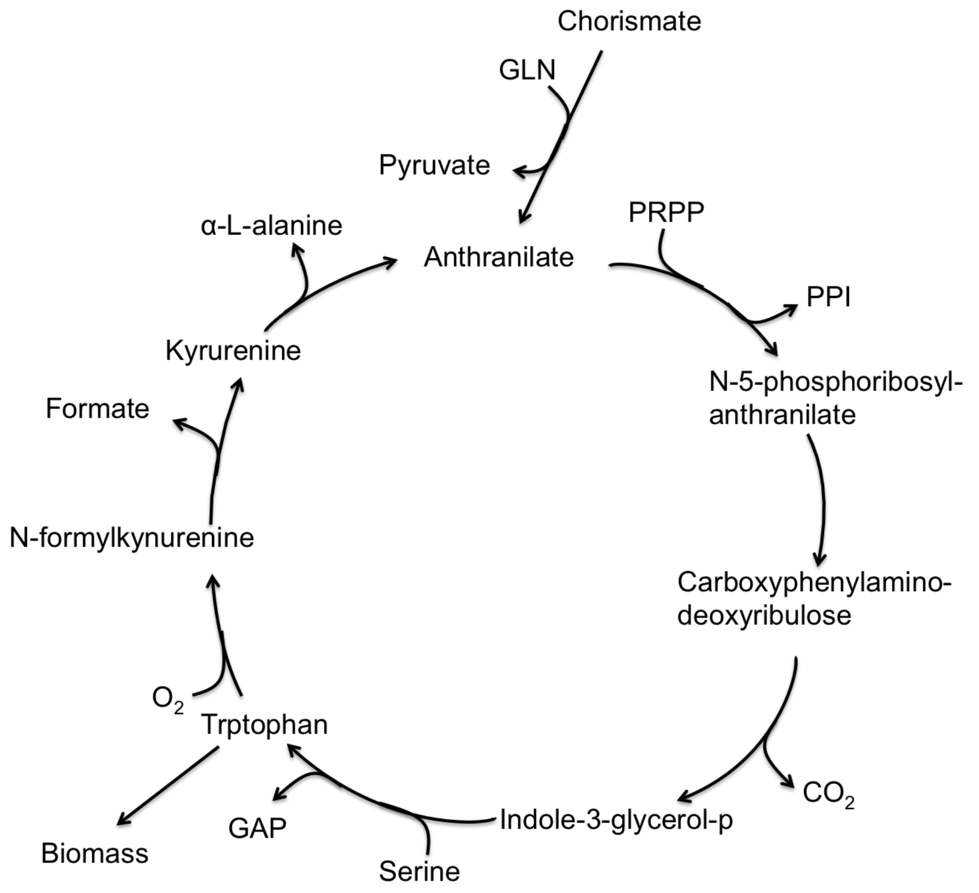


3-HP growth coupling strategy 1: results



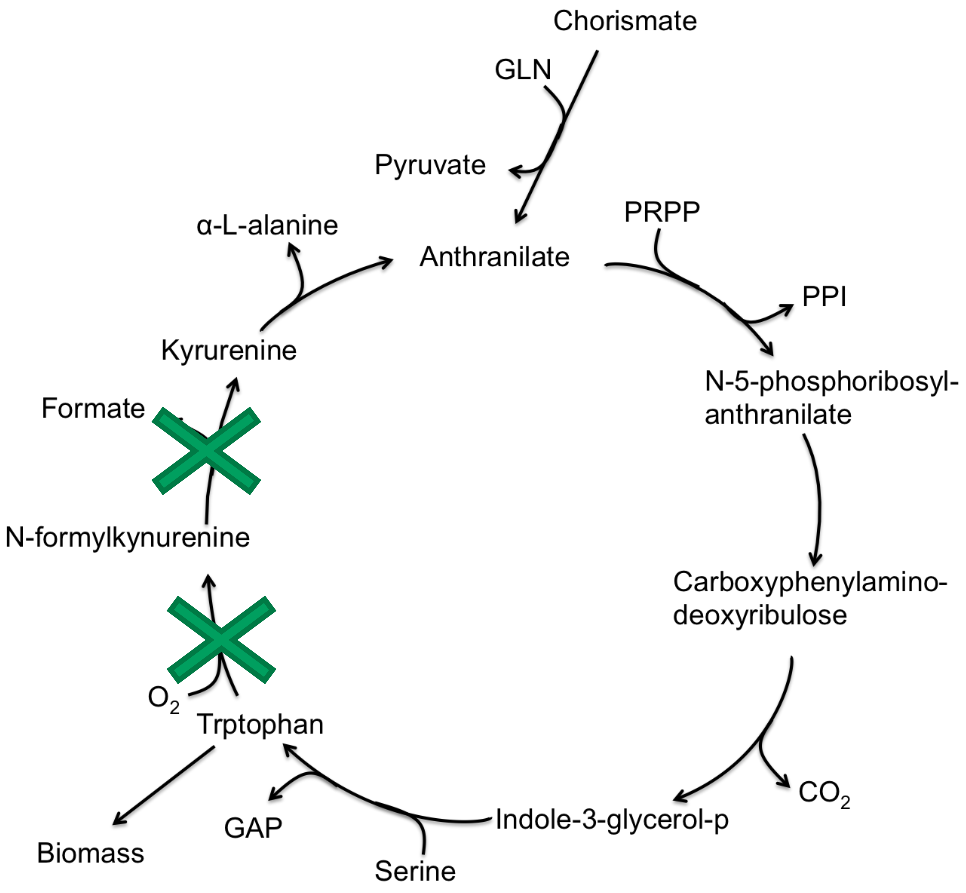
3-HP growth coupling strategy 1: results

- Simulated **single knockouts** on each reaction in model
- Identified **7 candidate reactions** whose deletion resulted in 3-HP production

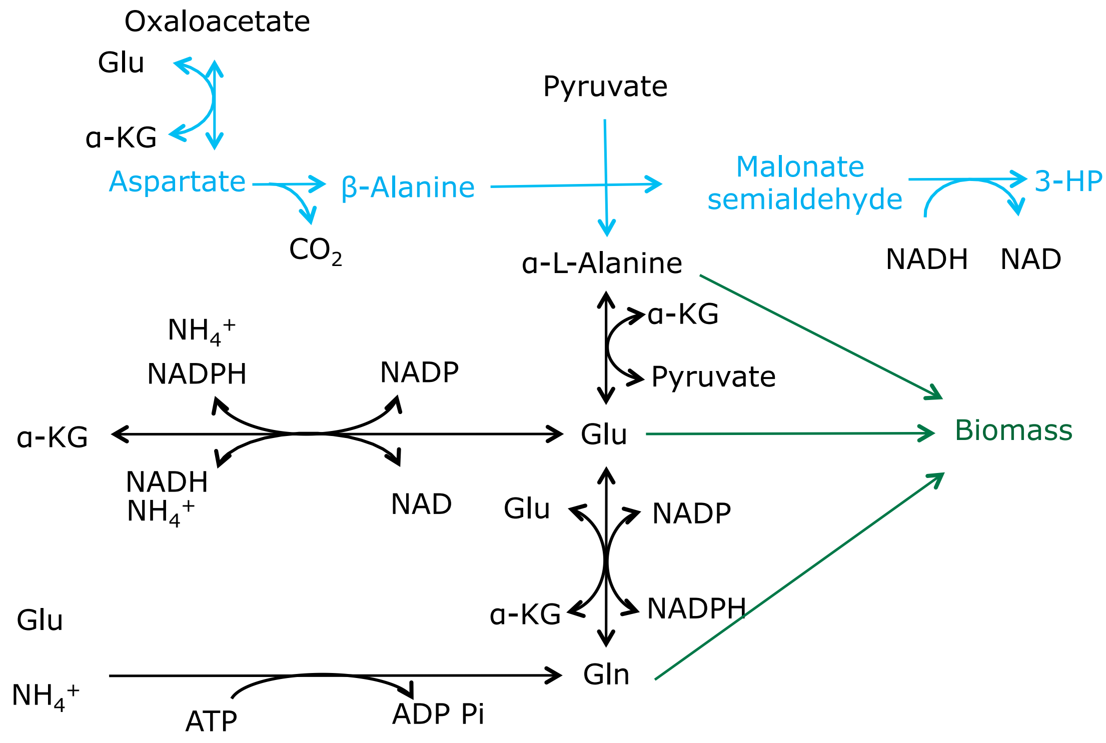


3-HP growth coupling strategy 1: results

- Simulated **single knockouts** on each reaction in model
- Identified **7 candidate reactions** whose deletion resulted in 3-HP production
- Highest 3-HP flux was **0.98**, with **0.05 carbon yield**



3-HP growth coupling strategy 2



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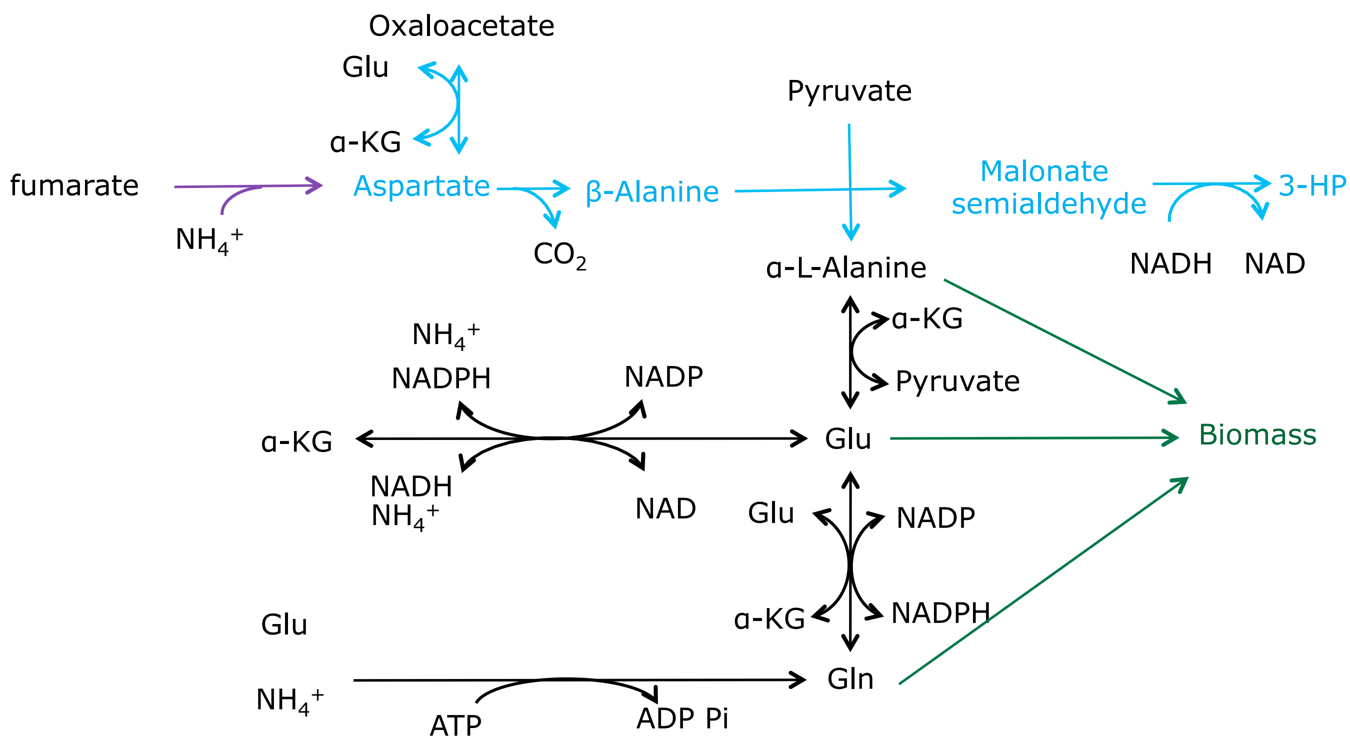

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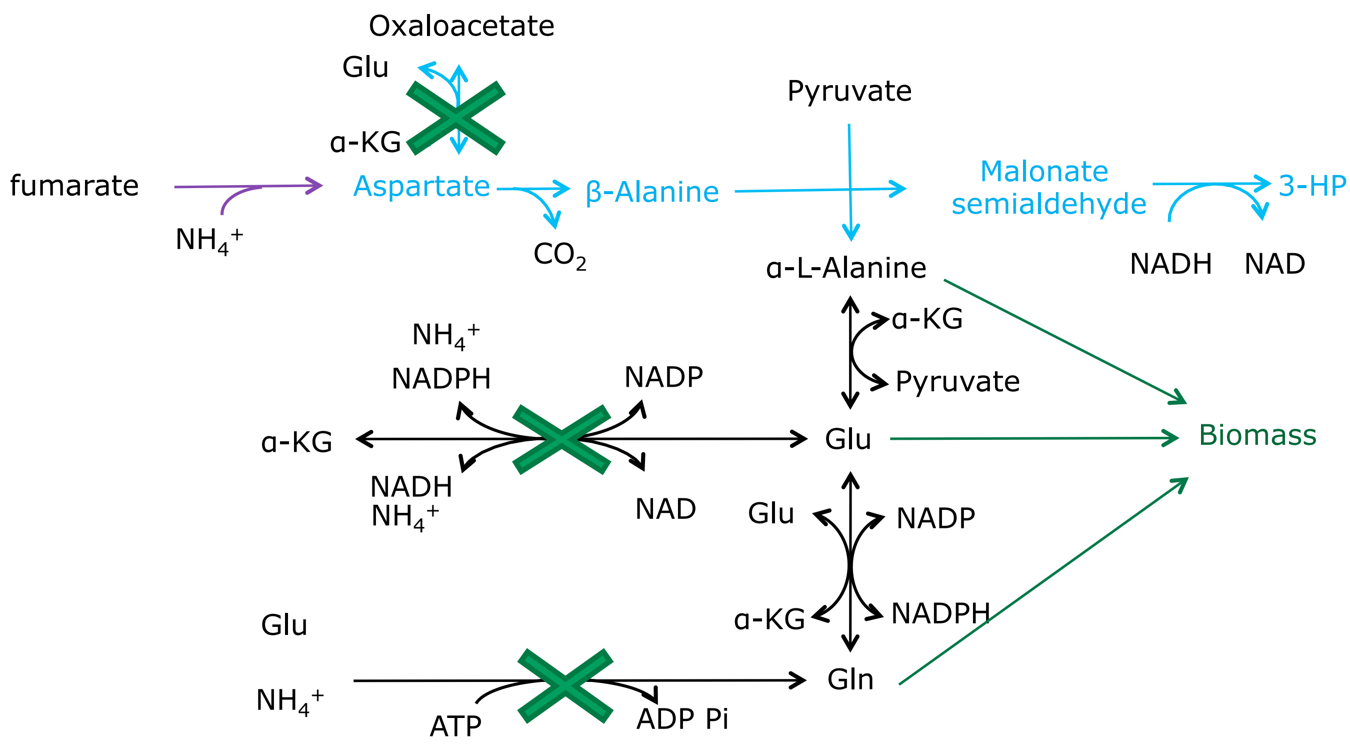

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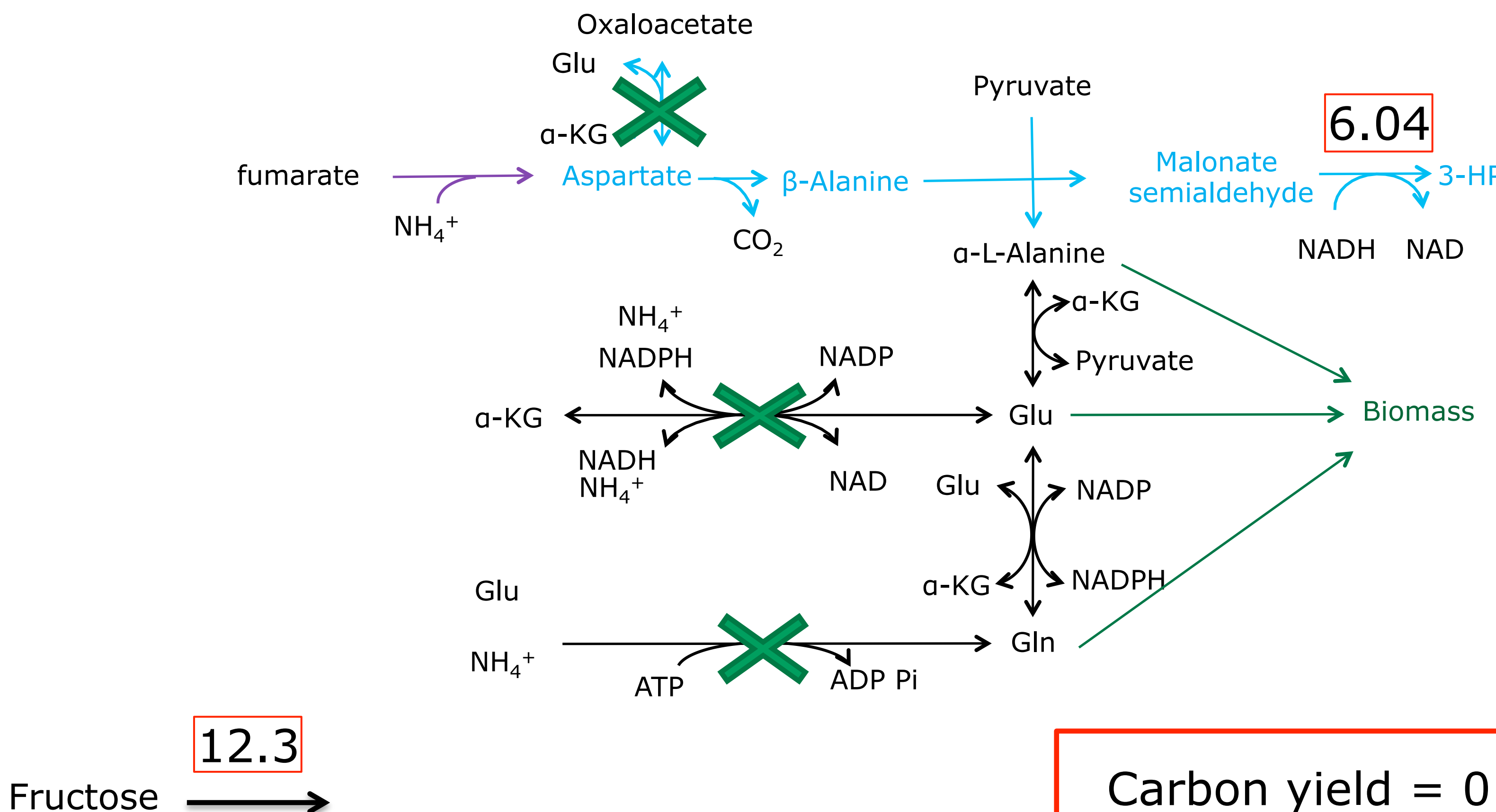


Carbon yield = 0.24

Production envelopes to assess growth coupling

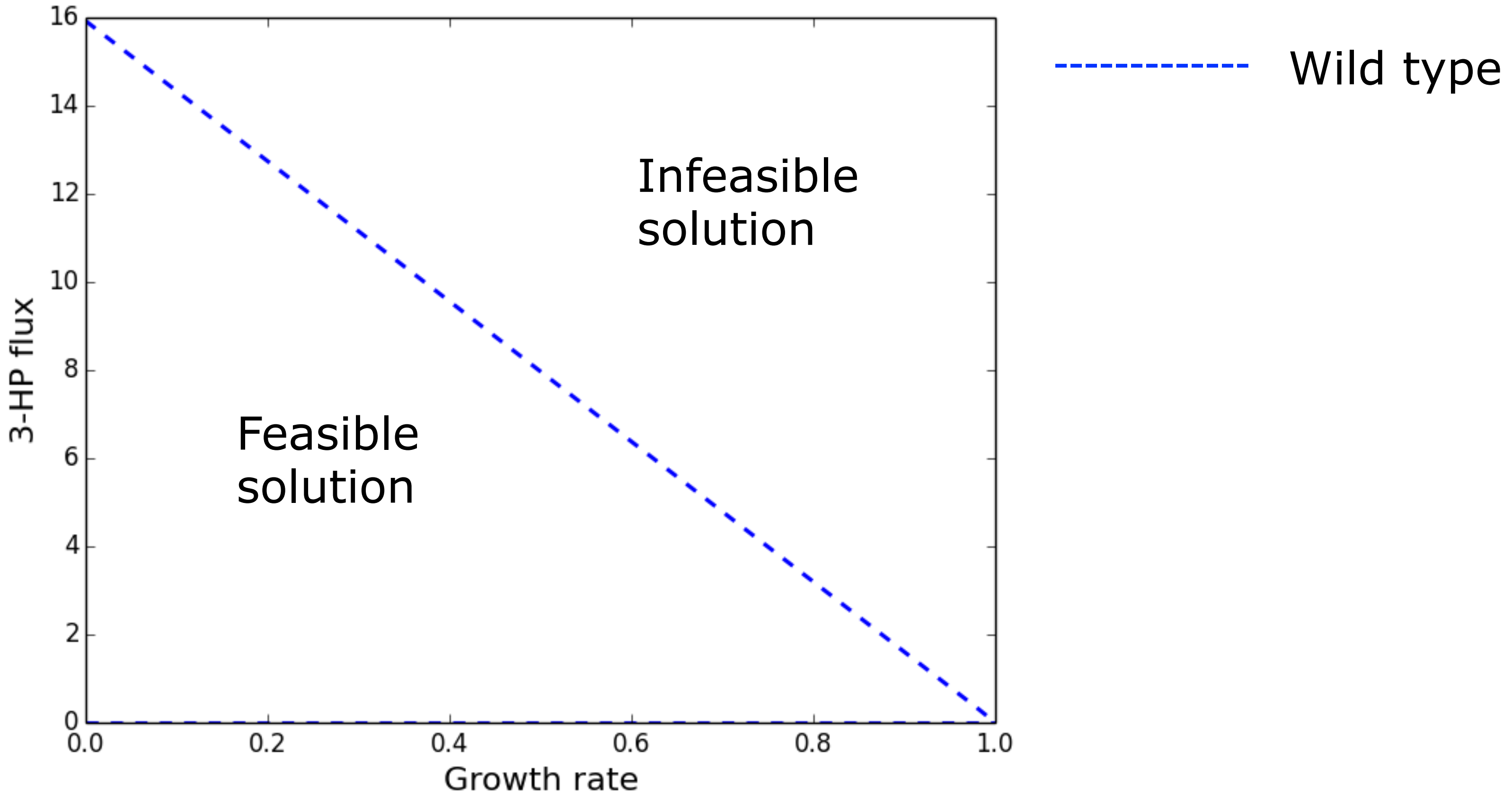
$$\text{maximise or minimise } v_{3-\mathrm{HP}}$$

$$\text{subject to} : \begin{cases} N\mathbf{v} = 0 \\ v_j = t_j \\ v_{\mathrm{ATPase}} = J_{\mathrm{ATPase}} \\ v_{\mathrm{ko}} = 0 \\ 0 \leq v_{\mathrm{fru}} \leq 8.8 \end{cases}$$

← Constrain maximum fructose uptake

- LP resolved for increasing growth rates for both minimising and maximising 3-HP flux

Production envelopes to assess growth coupling



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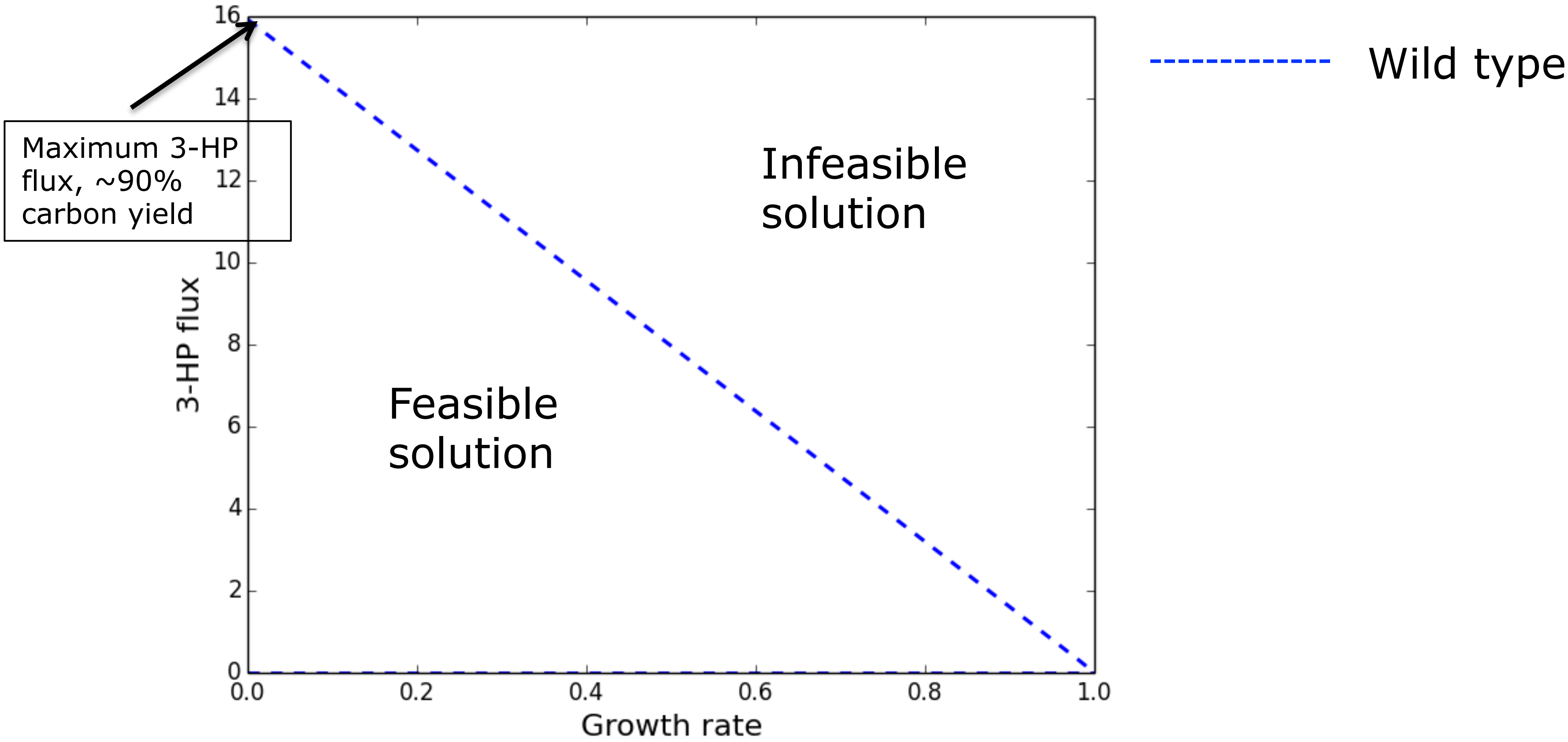
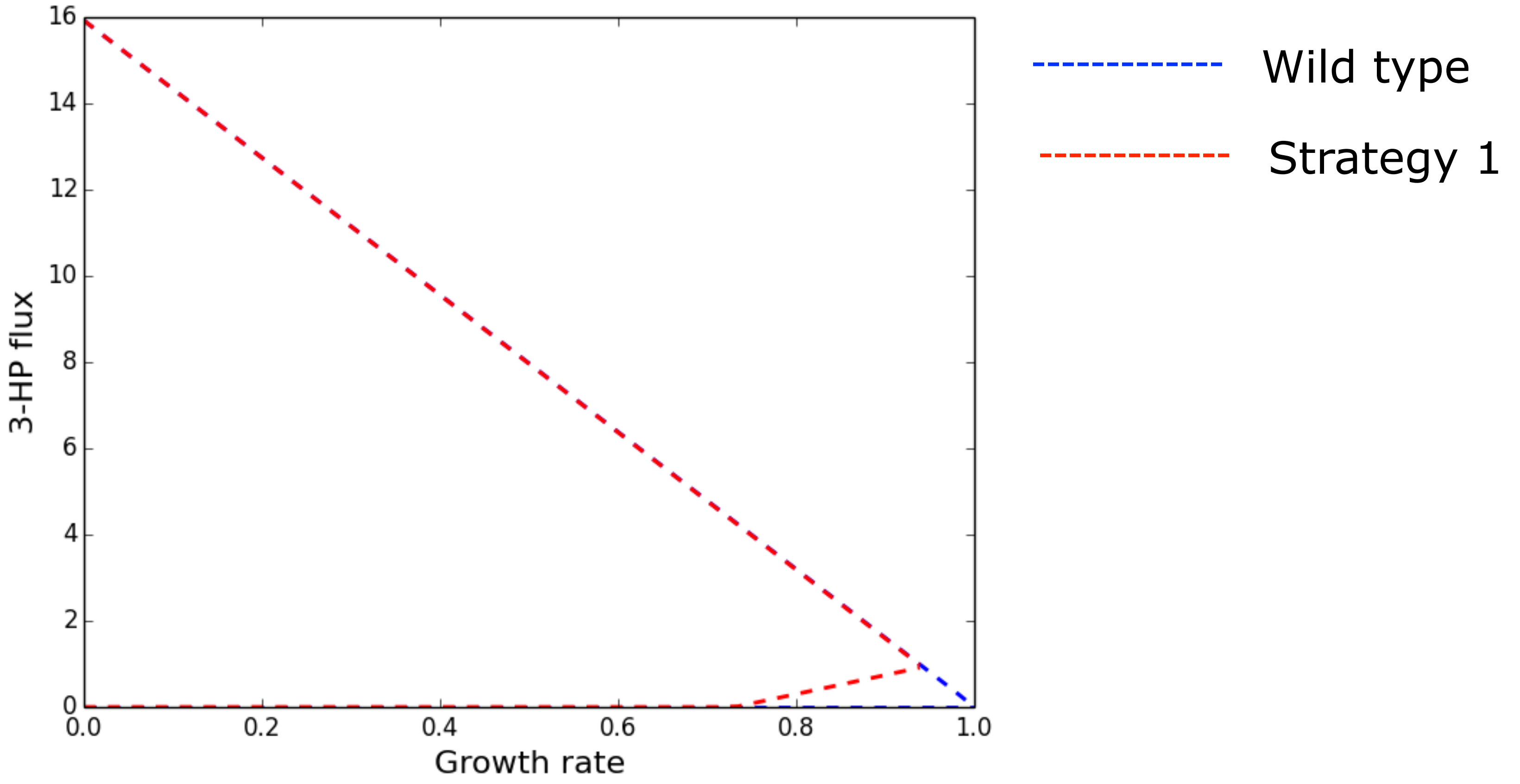


Flux varied analysis to assess growth coupling



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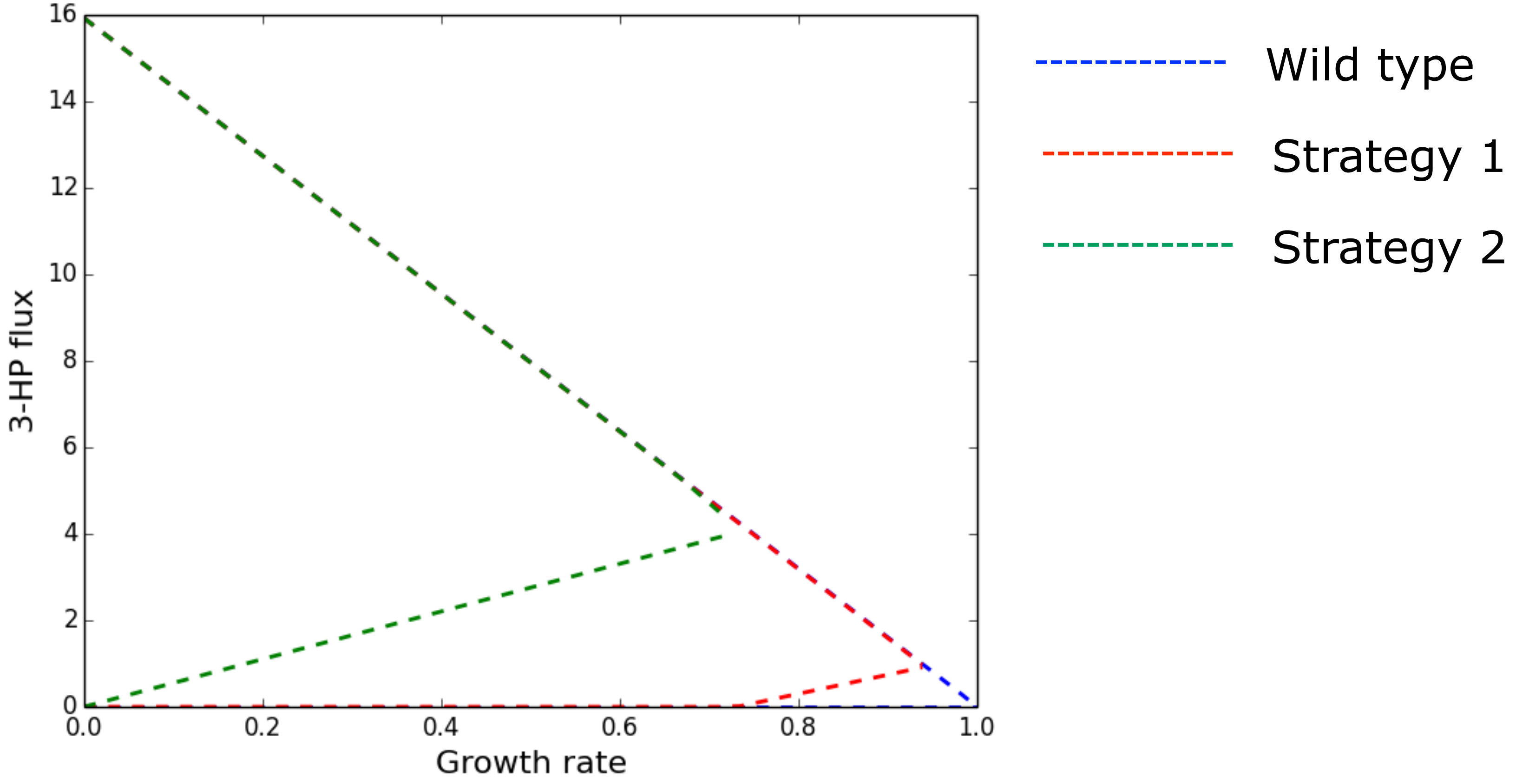

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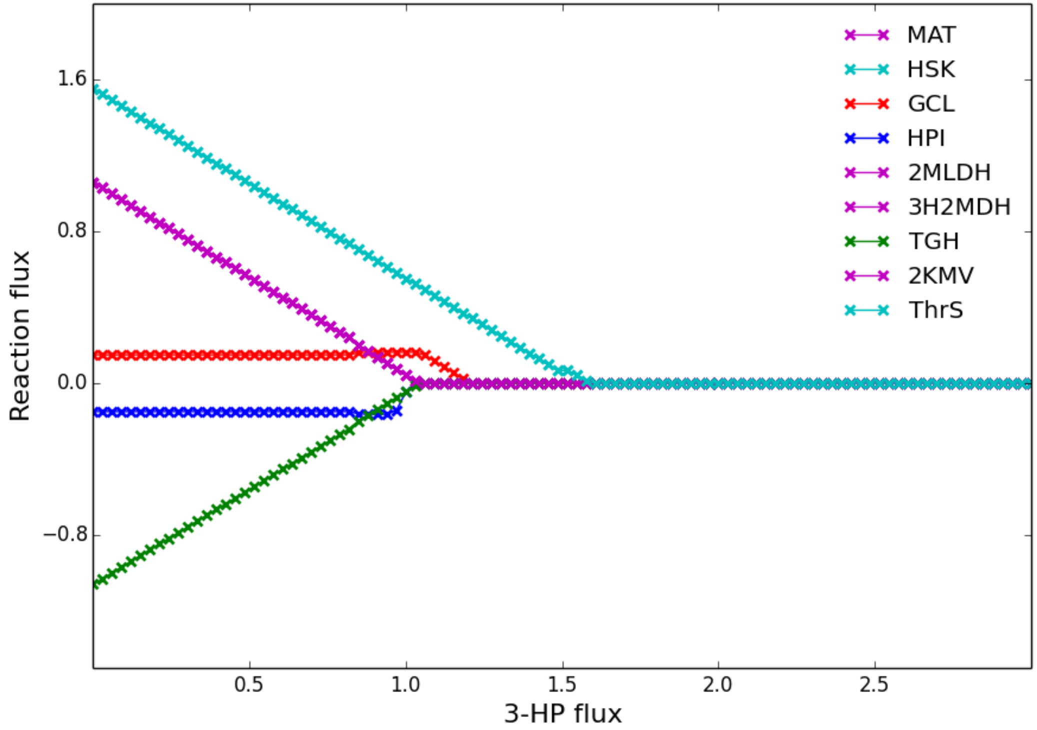
3-HP scan analysis

$$\text{minimise}: \ |\mathbf{v}|$$

$$\text{subject to}: \begin{cases} Nv = 0 \\ v_j = t_j; \\ v_{\text{ATPase}} = J_{\text{ATPase}} \\ v_{\text{ko}} = 0 \\ v_{\text{3HP}} = k \end{cases}$$

- LP resolved for increasing values of 3-HP flux
- Identify reactions that respond to increase 3-HP, fluxes that decrease are candidate knockouts

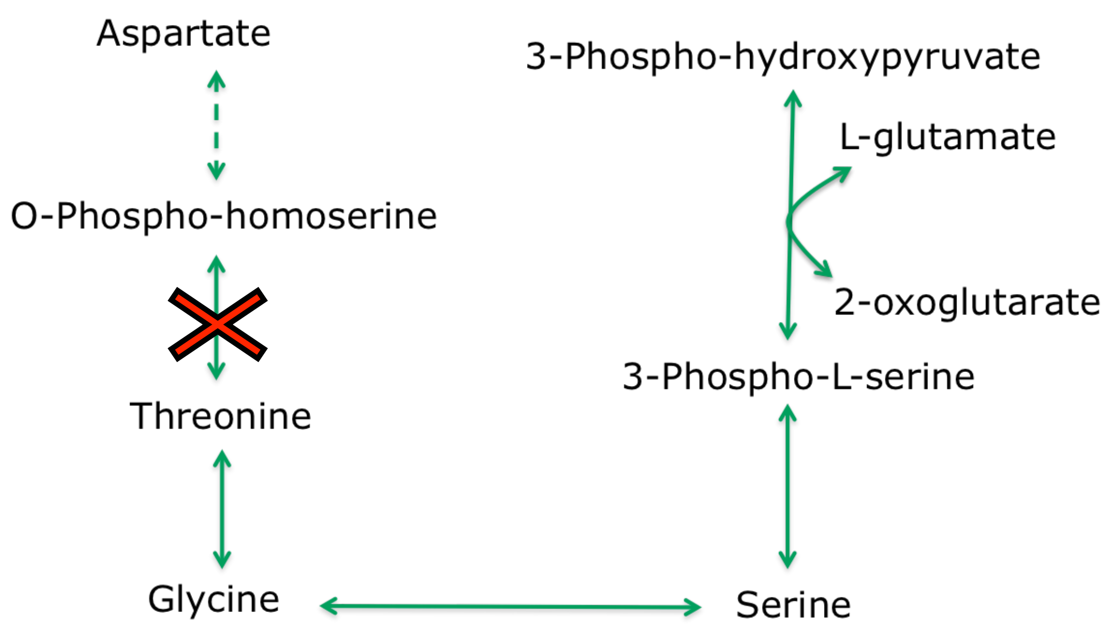
3-HP scan analysis



- Simulate knockout combinations of the reactions that decreased as 3-HP increased

3-HP scan analysis: results

- We identified 1 reaction that **increased 3-HP carbon yield** from **0.24** to **0.30**



Conclusions & Outlook

- Example of biology and modelling working together:
  - Using model to assess knock out strategy, and then to identify further knock outs that coupled 3-HP to essential biomass precursors
  - Using experimental data to refine model – test the predictions in the lab

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  - Using model to assess knock out strategy, and then to identify further knock outs that coupled 3-HP to essential biomass precursors
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- Future work:
  - Implement MOMA (minimisation of metabolic adjustments) for simulating knockouts
  - Implement techniques such as OptGene, for predicting knockouts

Acknowledgements

Cell Systems Modelling Group

- Mark Poolman
- David Fell
- Hassan Hartman

SBRC group

- Jamie Twycross
- Frederik Walter