Stability of autocatalytic cycles imposes constraints on kinetic parameters of enzymes

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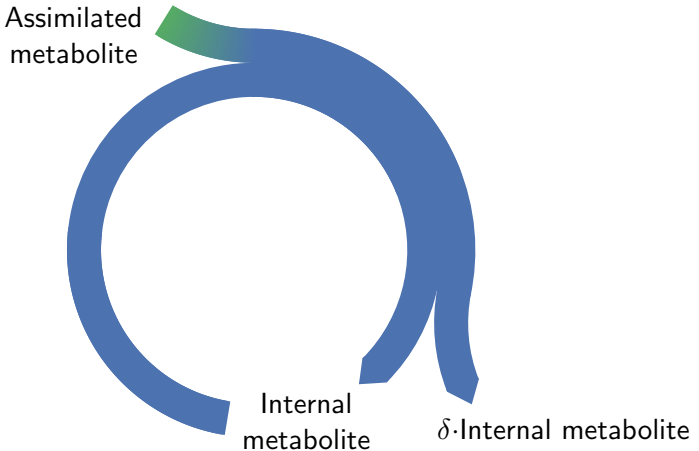
An autocatalytic cycle requires its internal metabolite to produce it

Internal metabolite + Assimilated metabolite $\rightarrow (1 + \delta)$ Internal metabolite

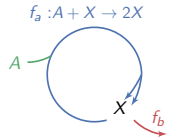


Why do we care about autocatalytic cycles?

- The lab implements the Calvin-Benson-Bassham cycle in *E.coli*[1]
- Two enzymes were introduced
- It didn't work
- Can we understand why?

[1] Antonovsky et. al., Cell 2016

Stable flux through an autocatalytic cycle constrains the kinetic parameters of its enzymes



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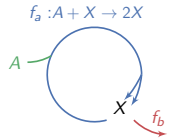
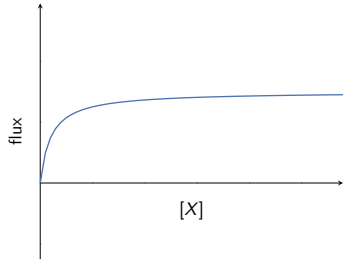


$$f_a = \frac{V_{\max,a} X}{K_{M,a} + X}$$



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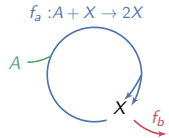

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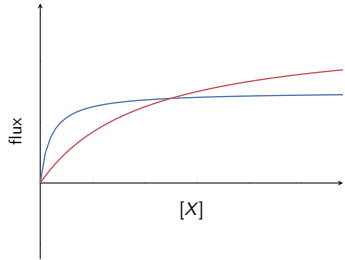

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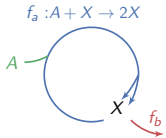

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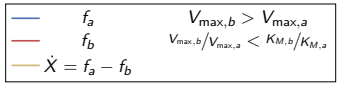

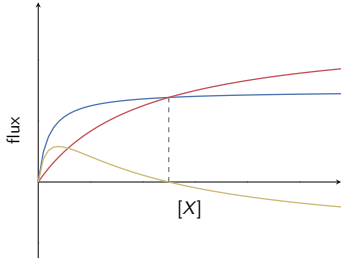

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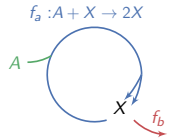

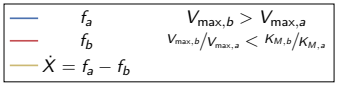

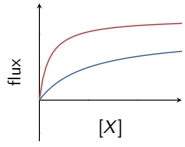

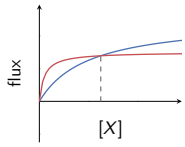

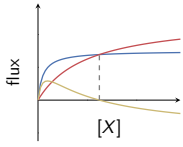

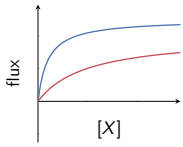

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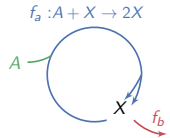
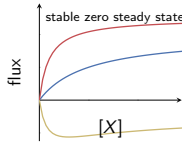
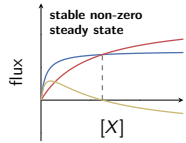


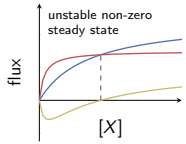
f_a $V_{\max,b} > V_{\max,a}$

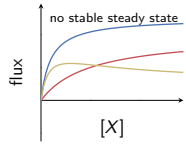
f_b $V_{\max,b}/V_{\max,a} < K_{M,b}/K_{M,a}$

$\dot{X} = f_a - f_b$









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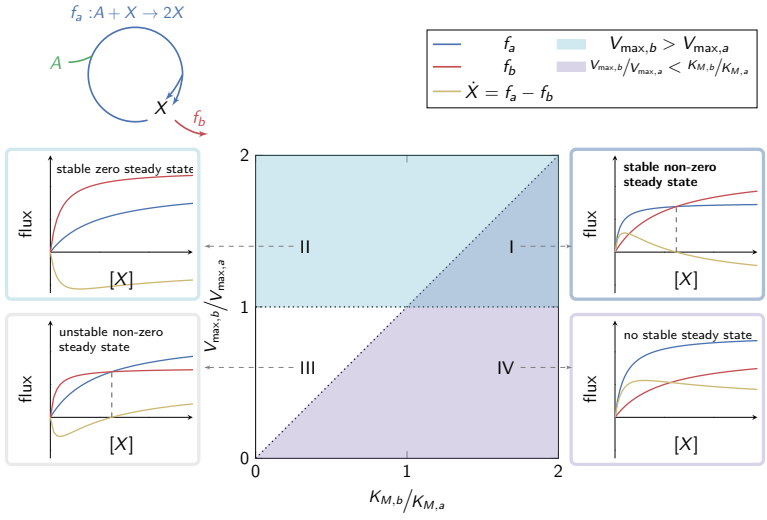

Conclusions drawn from the simple model apply under various extensions

- Using bisubstrate reaction schemes for the autocatalytic reaction
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- Assuming the autocatalytic reaction is reversible
  - Relaxes the constraint on the ratio of maximal fluxes between the autocatalytic and the branch reaction
- Assuming the branch reaction is reversible
  - Depending on the consumption of the branch reaction product, either the branch reaction, or the reaction downstream of it must have limited affinity

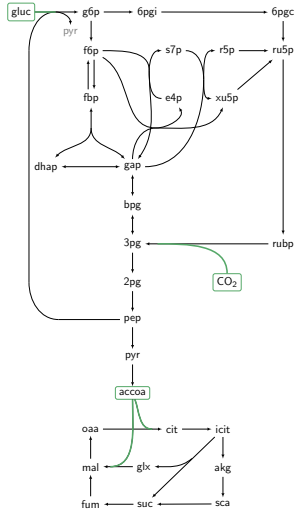
Directed evolution towards function of the CBB cycle required changes in kinetic parameters of main branch reactions

- 3 Directed evolution repeats evolved functioning CBB cycle
- Single common mutation: The major branch reaction gene, PRS
  - With other, different mutations in each strain
- In all cases K_{cat}/K_M of PRS decreased
- Minimal changes required for CBB function include mutations in other major branch reactions

Why should you care about autocatalytic cycles?

- Key metabolic processes are autocatalytic
  - In glycolysis ATP investment is required for the production of ATP
- Systematic search reveals autocatalytic cycles are abundant in central carbon metabolism

Autocatalytic cycles are abundant in central carbon metabolism



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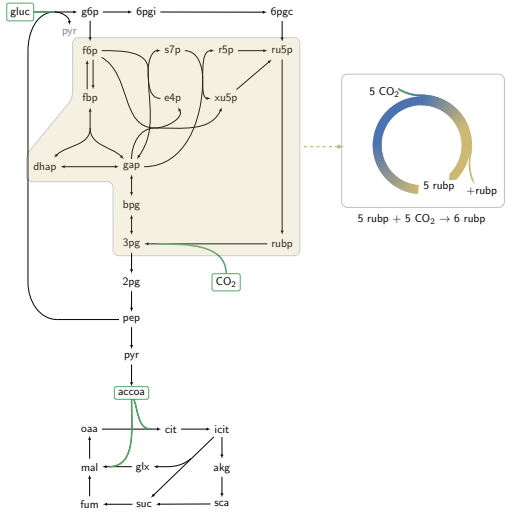

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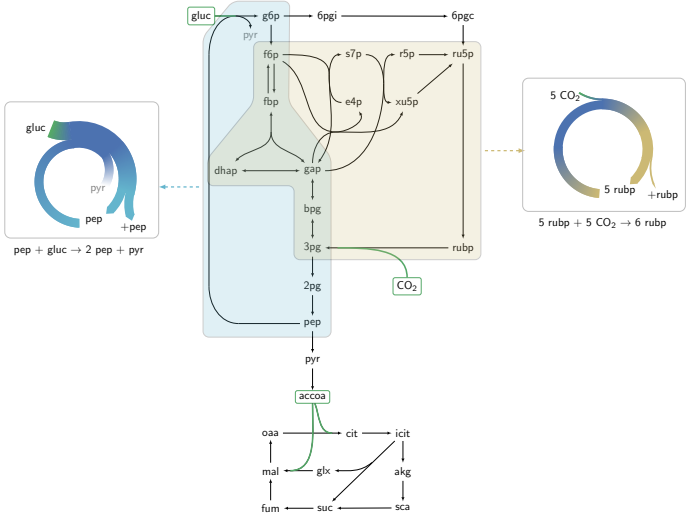

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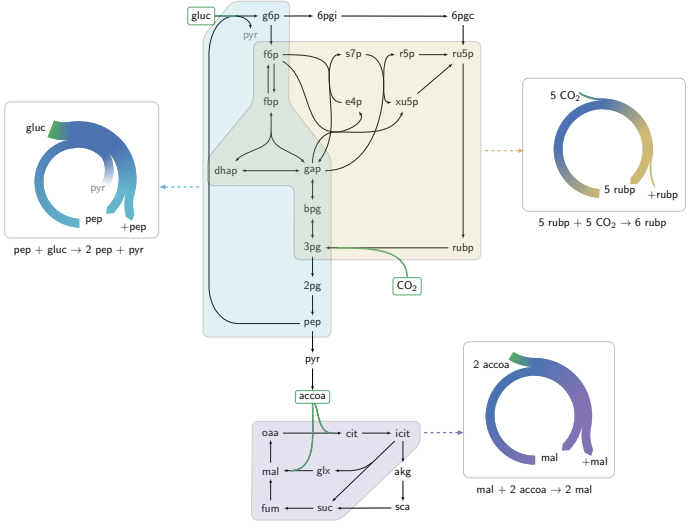
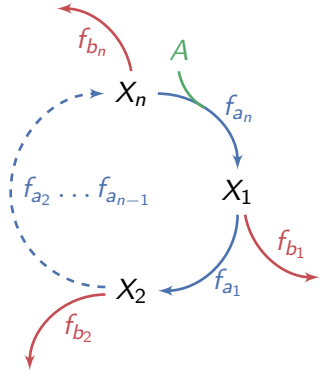


Stability criteria of the simple model can be extended for complex cycles



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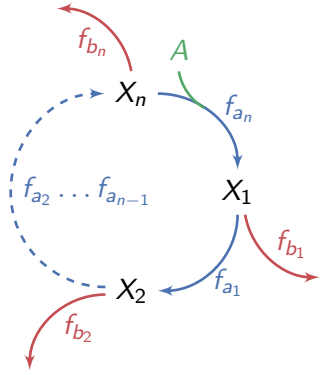

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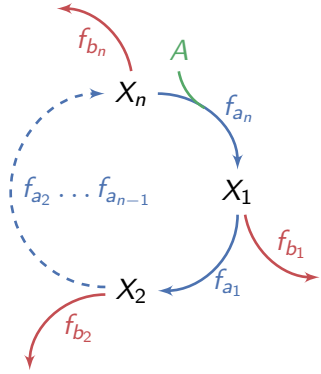

- At steady state: $\sum f_{b_i} = f_{a_n}$
- Sufficient condition for stability is: $\exists_i \quad \beta_i \geq \alpha_i$
  where $\beta_i = \left.\frac{df_{b_i}}{dX_i}\right|_{X_i^*}$ and $\alpha_i = \left.\frac{df_{a_i}}{dX_i}\right|_{X_i^*}$

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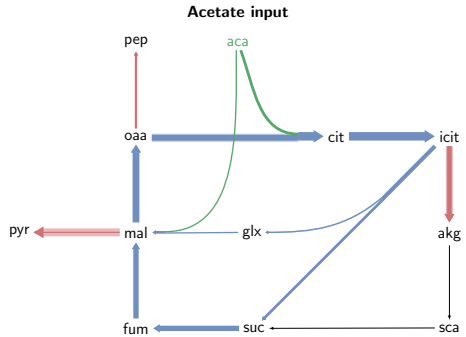
- Reaction saturation is the ratio of the actual flux to the potential flux, given expression level and catalytic rate
- For monotonically increasing, bounded, concave functions: saturation and derivative are inversely correlated
- Therefore, $\beta_i \geq \alpha_i$ imply that branch reaction is less saturated than autocatalytic reaction

Analysis of experimental fluxomics data[2] and proteomics data[3] shows branch reactions are consistently less saturated than autocatalytic reactions

Prediction: XX% < YY%
for at least one branch reaction



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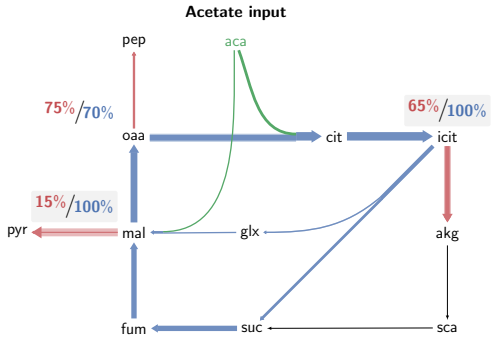

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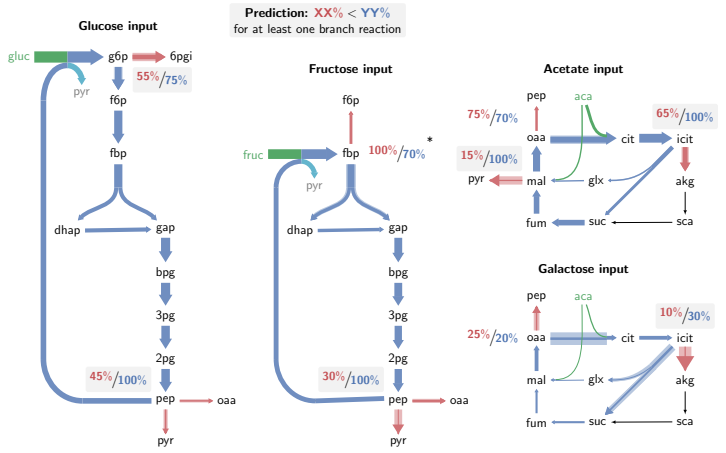

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Fructose PTS disagreement results from missing data on alternative transport pathways

- All fructose was assumed to be transported as fbp
- Experimental evidence shows other transport pathways are functioning[4]

[4] Kornberg, 1990

Allosteric regulation can accelerate convergence to steady state and increase robustness in fluctuating environment

- Convergence to steady state is faster when the differences between the cycle flux and the branch flux are larger
  - Intermediate metabolites activate branch reactions and inhibit cycle reactions

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- Adaptation of steady state fluxes to demand of cycle products is achieved by allosteric regulation of the branch products
  - Branch products should activate branch reactions and inhibit cycle reactions
- For the PTS using cycle, 11 out of 12 allosteric interactions agree with these predictions

Conclusions

- Autocatalytic cycles play a major role in central carbon metabolism
- Proper function of autocatalytic cycles depends on kinetic parameters of enzymes
  - Limits affinity of branch reactions
- In metabolic engineering of autocatalytic cycles, native kinetic parameters can prohibit function
- Stability of autocatalytic cycles depends on under-saturation of branch reactions
  - Excess expression of branch reactions enzymes is required
- Fluxomics data approves sub-optimality constraints are maintained in-vivo

Acknowledgments

Sustainability And Energy Research Initiative

European Research Council

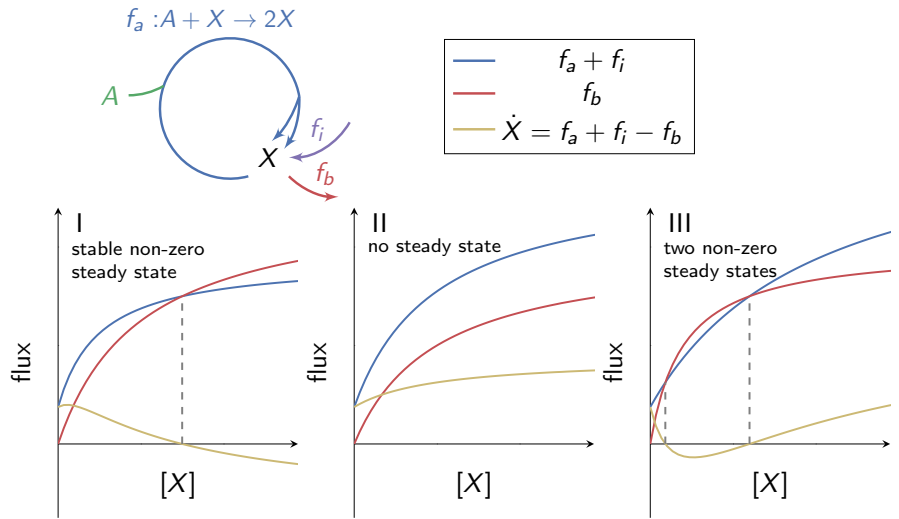
Outlook

- Efficient algorithm for identification of autocatalytic cycles in large metabolic networks
- Experimental exploration of different autocatalytic cycles function in-vivo
- Explore possible other uses of passive control of metabolic fluxes due to kinetic parameters

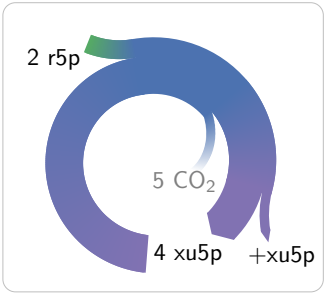
Thank You!

Supplementary figures

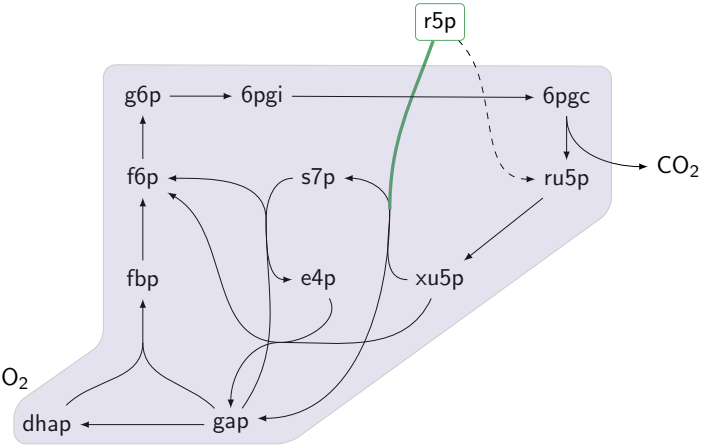
Input flux increases the range of parameters for which stable fluxes exist



Additional autocatalytic cycles in central carbon metabolism



4 xu5p + 2 r5p → 5 xu5p + 5 CO_2



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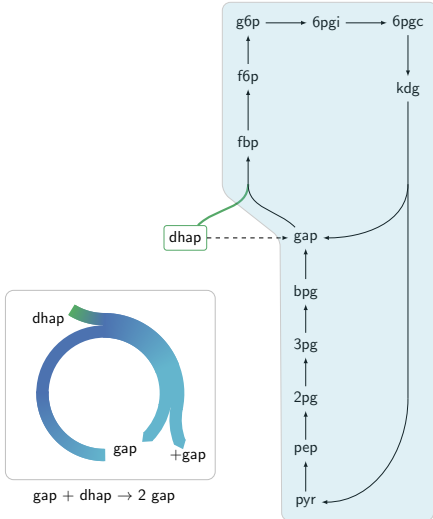
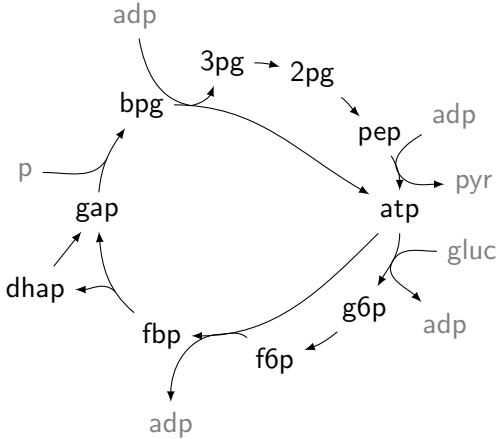


ATP autocatalysis in glycolysis



Supplementary equations

Bisubstrate reaction equations

- Substituted enzyme

$$f = \frac{V_{max} AX}{K_X A + K_A X + AX}$$

- Random binding ternary complex

$$f = \frac{V_{max} AX}{K_{i,A} K_X + K_X A + K_A X + AX}$$

- Ordered binding ternary complex, assimilated metabolite binding first

$$f = \frac{V_{max} AX}{K_{i,A} K_X + K_X A + AX}$$

- Ordered binding ternary complex, internal metabolite binding first

$$f = \frac{V_{max} AX}{K_{i,X} K_A + K_A X + AX}$$

Reversible reaction equation

$$f_b = \frac{V_{\max,b}(X - Y)}{K_X + X + \frac{K_X}{K_Y} Y}$$