

# Stability of autocatalytic cycles imposes constraints on kinetic parameters of enzymes

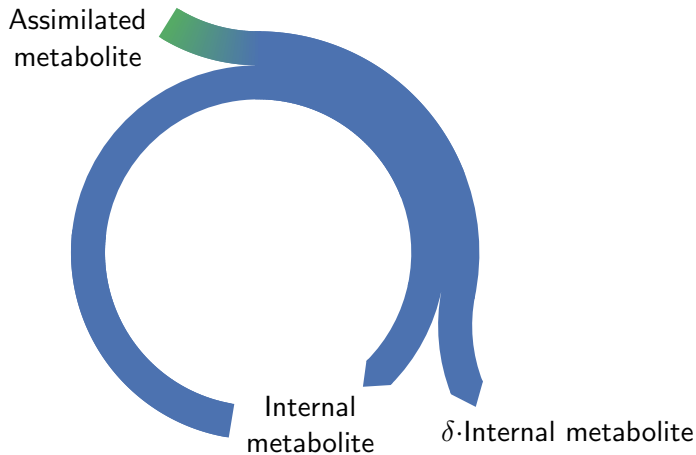
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Department of Plant & Environmental Sciences  
Weizmann Institute of Science, Rehovot, Israel

April 14, 2017

# An autocatalytic cycle requires its internal metabolite to produce it

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

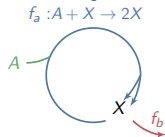


# Why do we care about autocatalytic cycles?

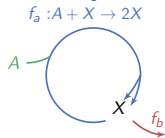
- ▶ The lab implements the Calvin-Benson-Bassham cycle in *E.coli*<sup>1</sup>
- ▶ Two enzymes were introduced
- ▶ It didn't work
- ▶ Can we understand why?

<sup>1</sup>Antonovsky et. al., Cell 2016

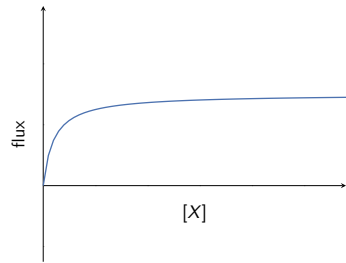
# Stable flux through an autocatalytic cycle constrains the kinetic parameters of its enzymes



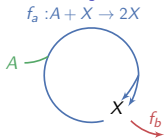
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$$f_a = \frac{V_{\max,a}X}{K_{M,a} + X}$$

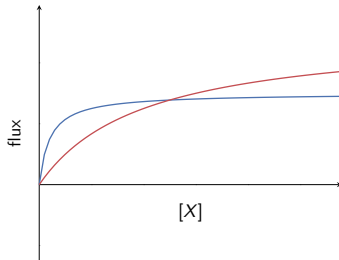


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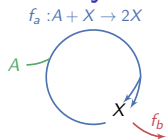


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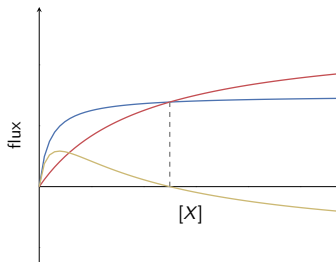
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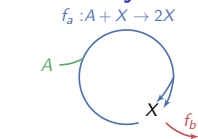
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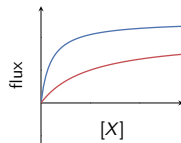
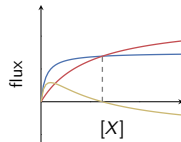
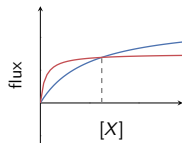
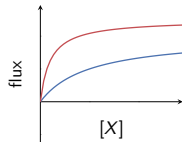
$f_a$	$V_{\max,b} > V_{\max,a}$
$f_b$	$V_{\max,b}/V_{\max,a} < K_{M,b}/K_{M,a}$
$\dot{X} = f_a - f_b$	



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

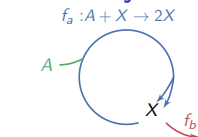


<span style="color: blue;">—</span>	$f_a$	$V_{\max, b} > V_{\max, a}$
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<span style="color: yellow;">—</span>	$\dot{X} = f_a - f_b$	

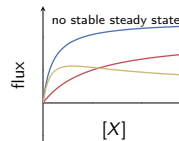
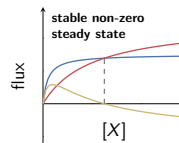
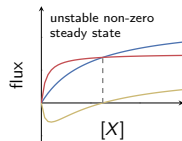
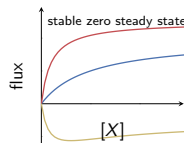




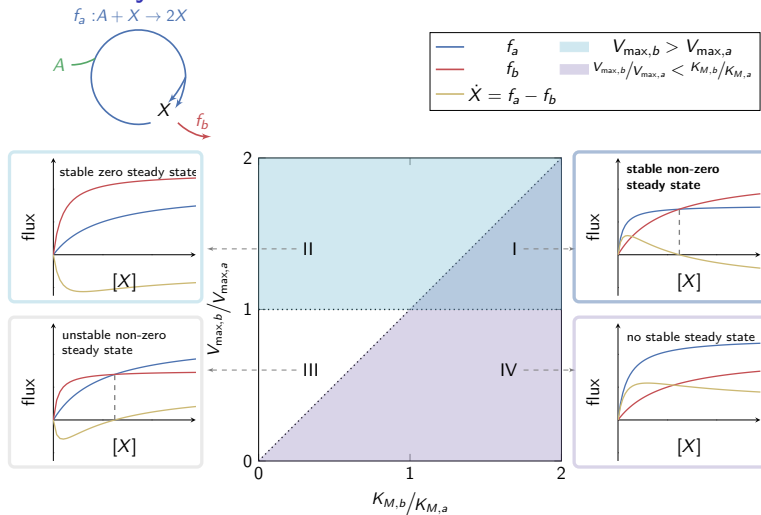
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# Stable flux through an autocatalytic cycle constrains the kinetic parameters of its enzymes



## Conclusions drawn from the simple model apply under various extensions

- ▶ Using bisubstrate reaction schemes for the autocatalytic reaction
  - ▶ Critical lower concentration of the assimilated metabolite exists
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  - ▶ 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

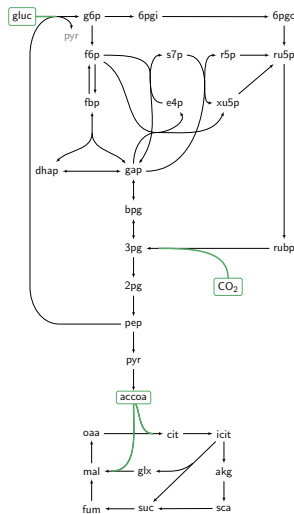
## Directed evolution towards function of the CBB cycle required changes in kinetic parameters of main branch reactions

- ▶ 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  $K_{\text{cat}}/K_M$  of PRS decreased
- ▶ Minimal changes required for CBB function include mutations in other major branch reactions

# Why should you care about autocatalytic cycles?

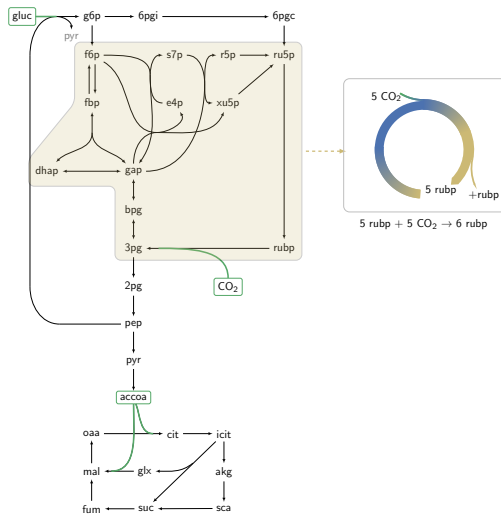
- ▶ Key metabolic processes are autocatalytic
  - ▶ In glycolysis ATP investment is required for the production of ATP
- ▶ Systematic search reveals autocatalytic cycles are abundant in central carbon metabolism

## Autocatalytic cycles are abundant in central carbon metabolism

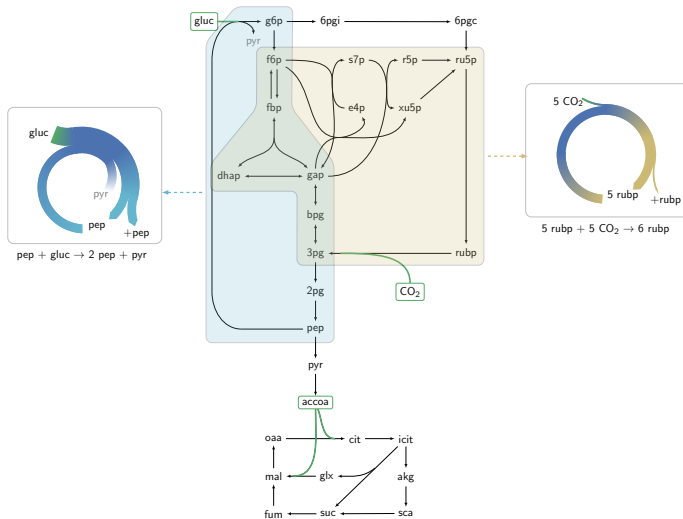




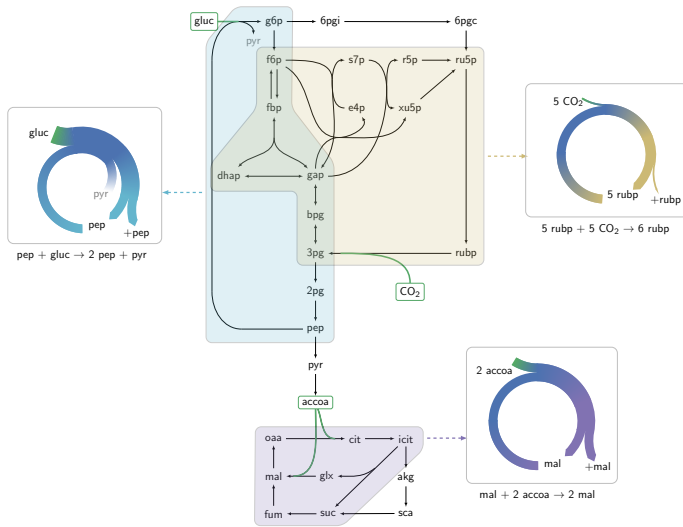
## Autocatalytic cycles are abundant in central carbon metabolism



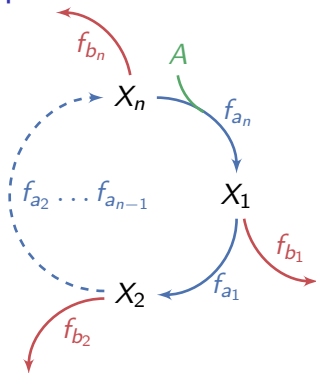
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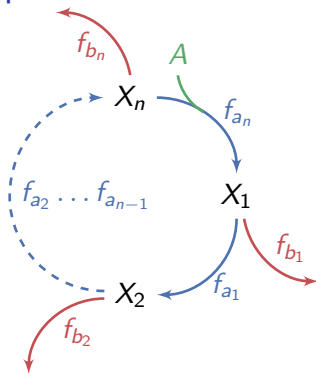
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Stability criteria of the simple model can be extended for complex cycles

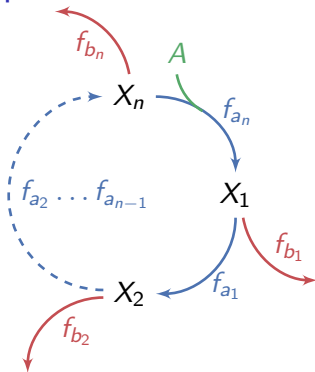


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- ▶ At steady state:  $\sum f_{b_i} = f_{a_n}$
- ▶ Sufficient condition for stability is:  $\exists_i \quad \beta_i \geq \alpha_i$   
where  $\beta_i = \left. \frac{df_{b_i}}{dX_i} \right|_{X_i^*}$  and  $\alpha_i = \left. \frac{df_{a_i}}{dX_i} \right|_{X_i^*}$

Theoretical  $\beta_i \geq \alpha_i$  constraint results in experimental prediction on reaction saturation level

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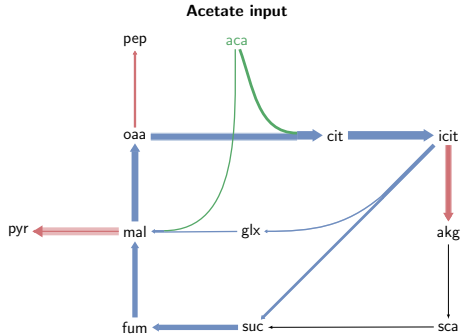


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- ▶ Reaction saturation is the ratio of the actual flux to the potential flux, given expression level and catalytic rate
- ▶ For monotonically increasing, bounded, concave functions: saturation and derivative are inversely correlated
- ▶ Therefore,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

Analysis of experimental fluxomics data<sup>2</sup> and proteomics data<sup>3</sup> shows branch reactions are consistently less saturated than autocatalytic reactions

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

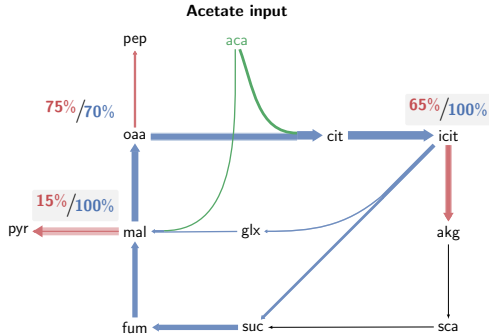


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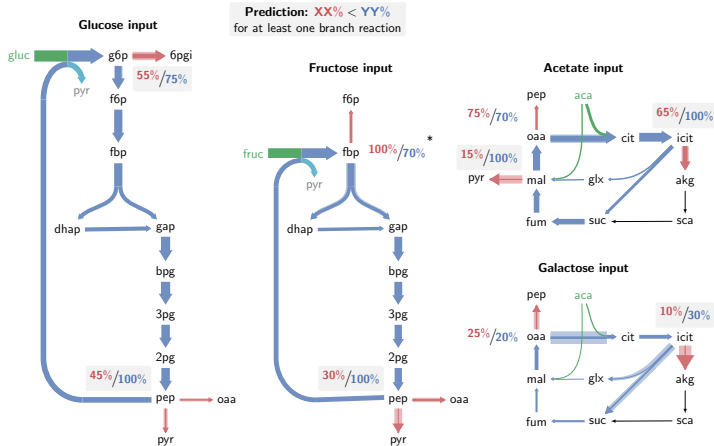
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## Fructose PTS disagreement results from missing data on alternative transport pathways

- ▶ All fructose was assumed to be transported as fbp
- ▶ Experimental evidence shows other transport pathways are functioning<sup>4</sup>

<sup>4</sup>Kornberg, 1990

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

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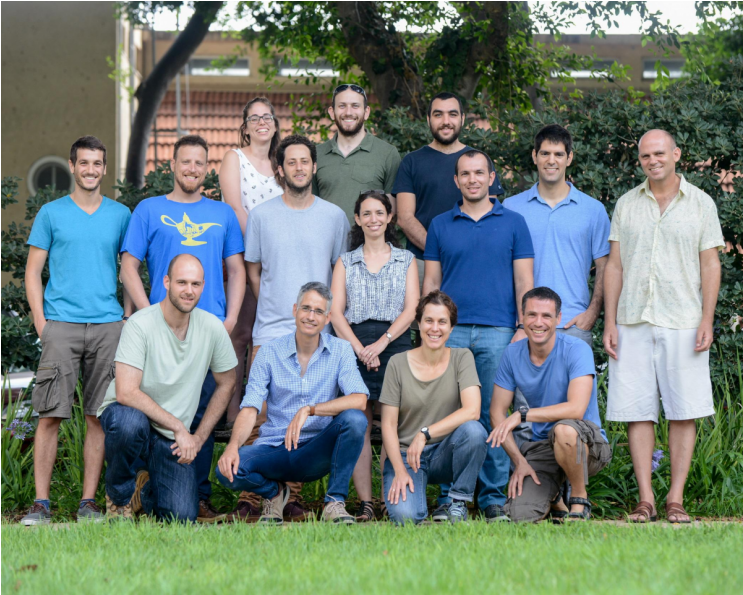
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- ▶ For the PTS using cycle, 11 out of 12 allosteric interactions agree with these predictions

# 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
- ▶ Possible other uses of passive control of metabolic fluxes due to kinetic parameters

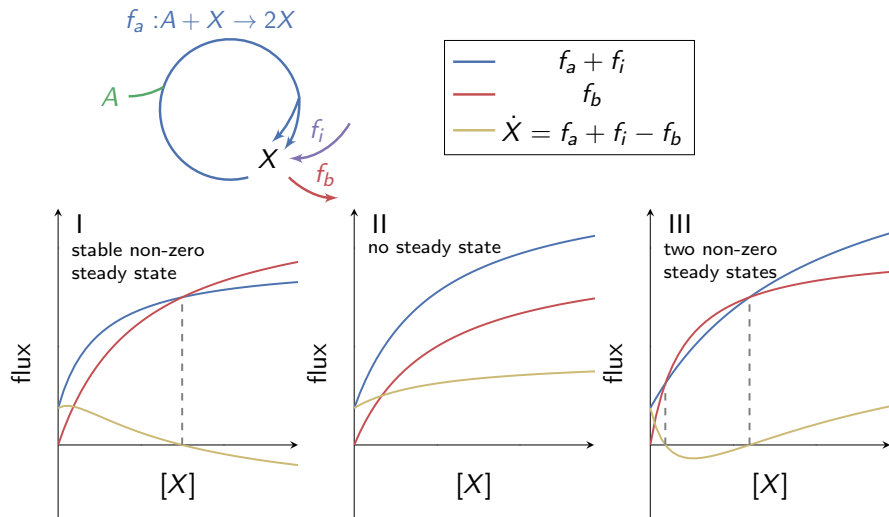
## References:

- ▶ Carbon fixation in *E.coli*: Antonovsky et. al., Cell 2016
- ▶ Emergence of autocatalysis in metabolic networks: Riehl et. al., PLoS CB 2010
- ▶ Algorithms for identifying autocatalytic cycles: Kun et. al., Genome Biology 2008
- ▶ Calculating  $k_{\text{cat}}$  from proteomics data: Davidi et. al., PNAS 2016
- ▶ This work: Barenholz et. al., eLife 2017

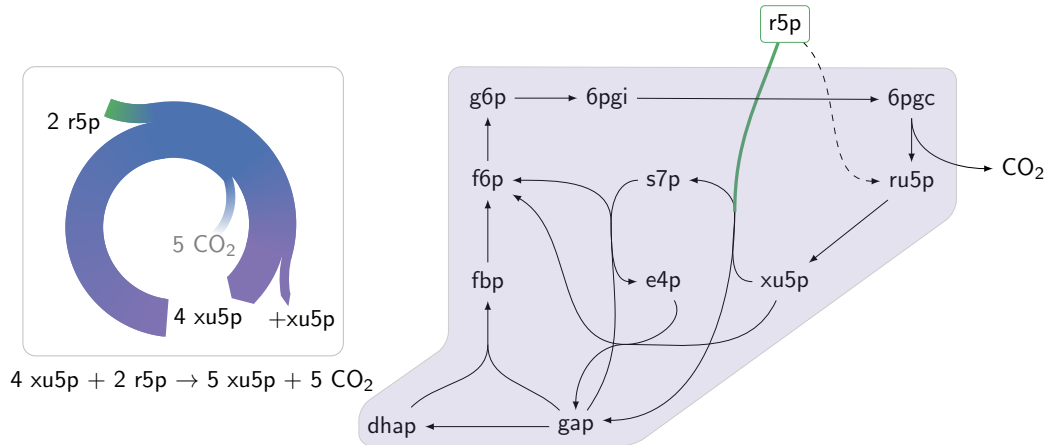
Thank You!

## Supplementary figures

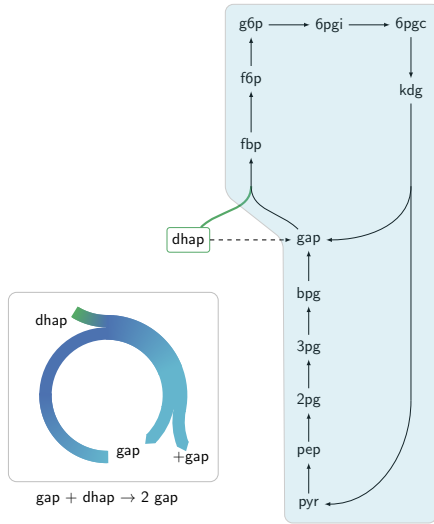
# Input flux increases the range of parameters for which stable fluxes exist



# Additional autocatalytic cycles in central carbon metabolism

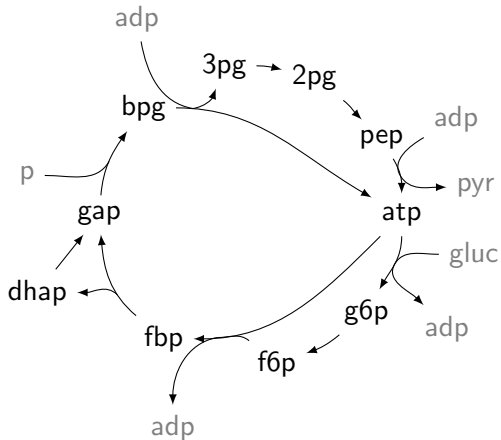


# Additional autocatalytic cycles in central carbon metabolism





# ATP autocatalysis in glycolysis



## Supplementary equations

## Bisubstrate reaction equations

- ▶ Substituted enzyme

$$f = \frac{V_{\max}AX}{K_XA + K_AX + AX}$$

- ▶ Random binding ternary complex

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

- ▶ Ordered binding ternary complex, assimilated metabolite binding first

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

- ▶ Ordered binding ternary complex, internal metabolite binding first

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

## Reversible reaction equation

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