The interplay between metabolic network topology and the kinetic parameters of enzymes, from autocatalytic cycles and beyond

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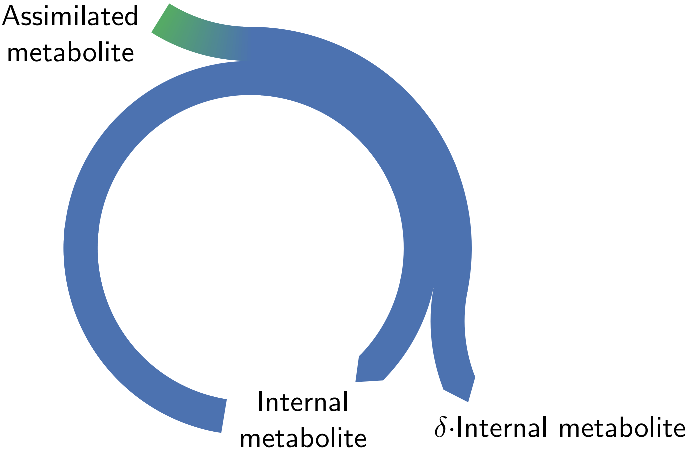
An autocatalytic cycle requires its internal metabolite to produce it

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



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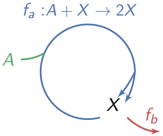
Why do we care about autocatalytic cycles?

- 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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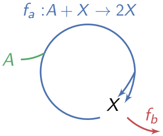

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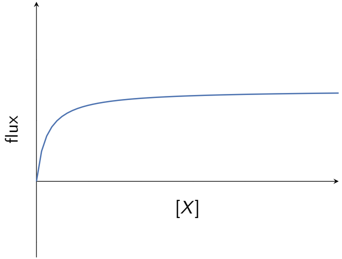

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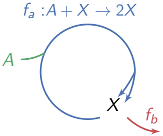

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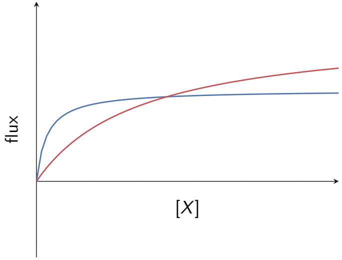

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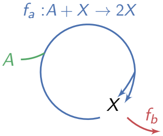

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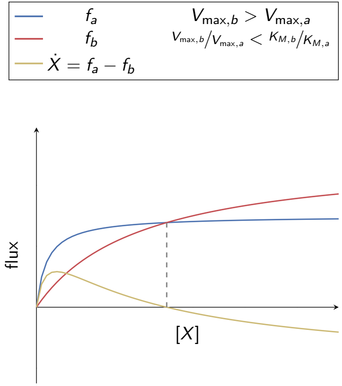

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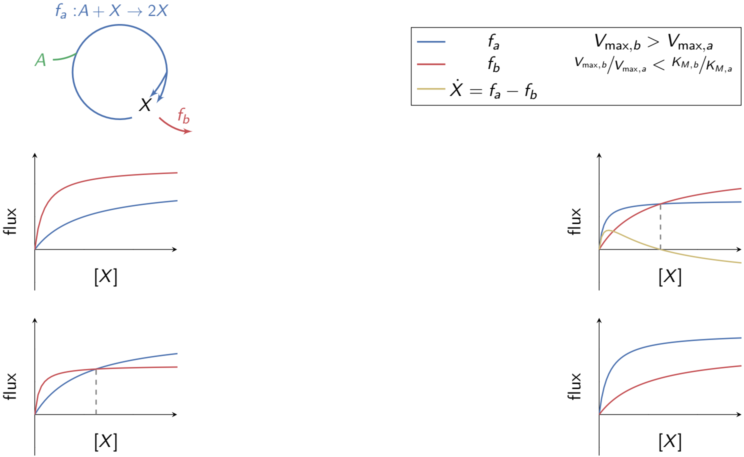

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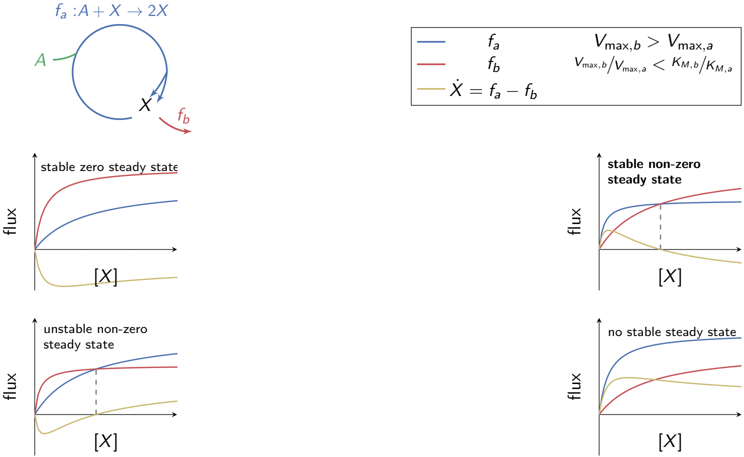

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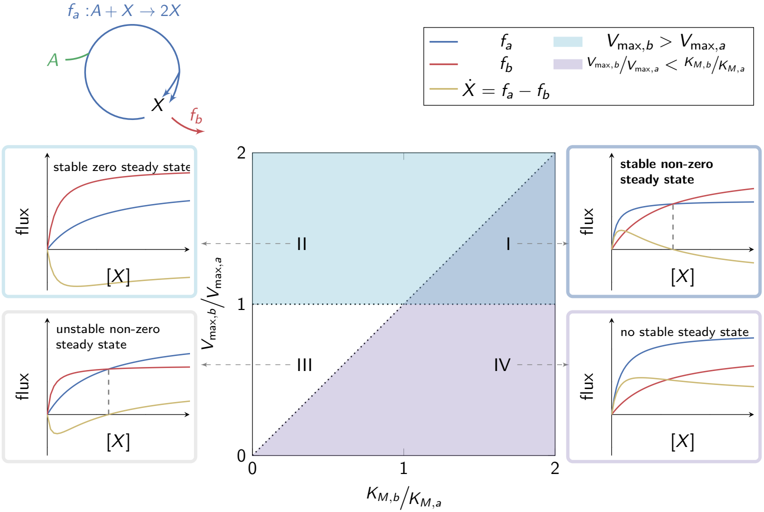

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Conclusions drawn from the simple model apply under various extensions

- Using bisubstrate reaction schemes for the autocatalytic reaction
  - Critical lower concentration of the assimilated metabolite exists
  - Upper bound on the affinity of the branch reaction remains in most schemes

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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

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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

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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

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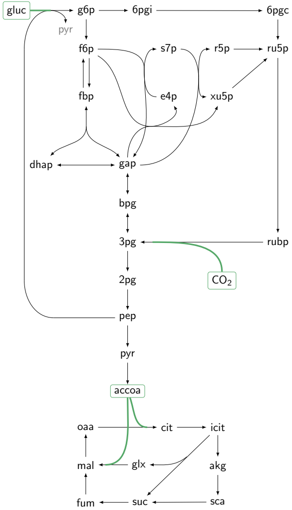
Autocatalytic cycles are abundant in central carbon metabolism



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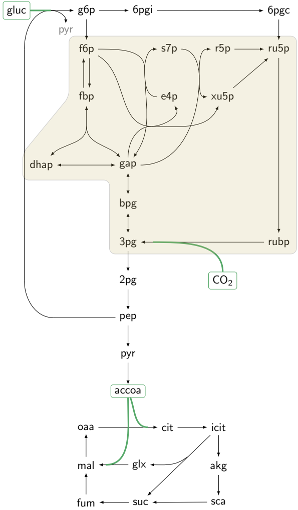

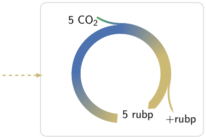

5 rubp + 5 CO_2 → 6 rubp

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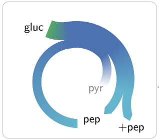
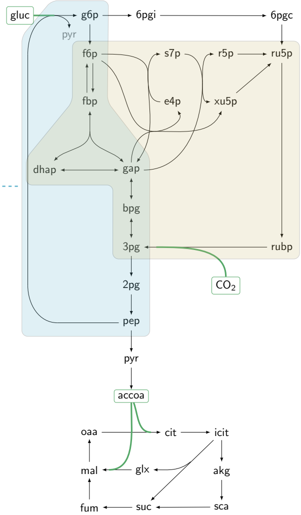
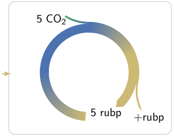


pep + gluc → 2 pep + pyr





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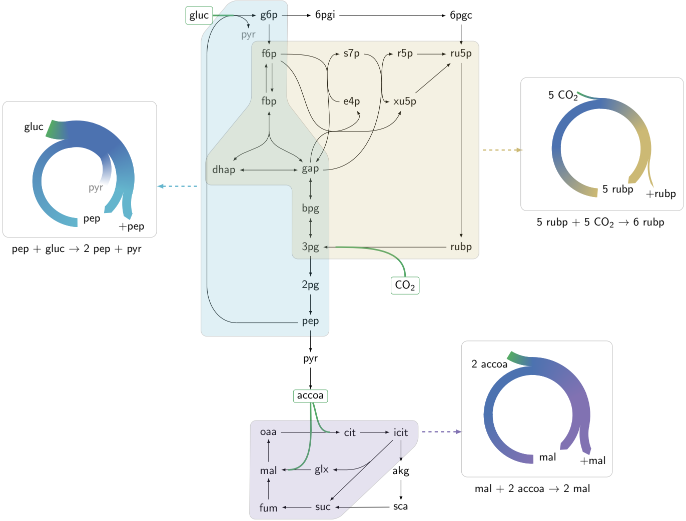

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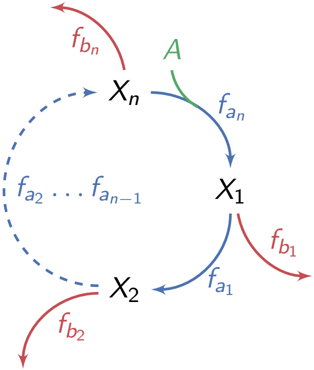
Stability criteria of the simple model can be extended for complex cycles



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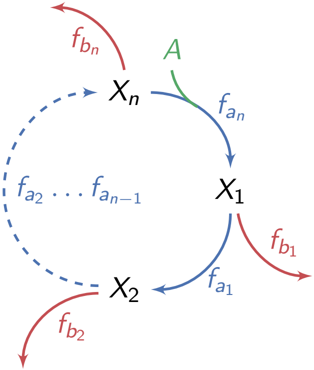

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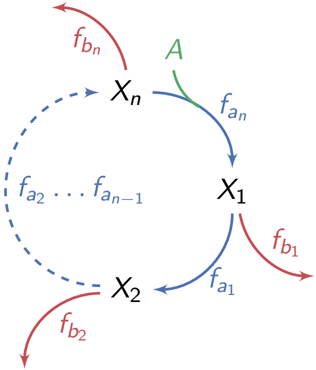

- At steady state: $\sum f_{b_i} = f_{a_n}$
- Sufficient condition for stability is: $\exists_i \quad \beta_i \geq \alpha_i$
  where $\beta_i = \frac{df_{b_i}}{dX_i}\Big|_{X_i^*}$ and $\alpha_i = \frac{df_{a_i}}{dX_i}\Big|_{X_i^*}$

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

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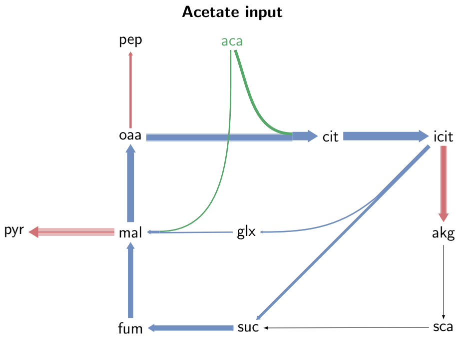
- Reaction saturation is the ratio of the actual flux to the potential flux, given expression level and catalytic rate
- For monotonically increasing, bounded, concave functions: saturation and derivative are inversely correlated
- Therefore, $\beta_i \geq \alpha_i$ imply that branch reaction is less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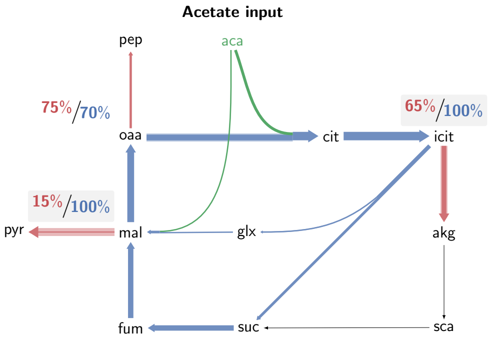

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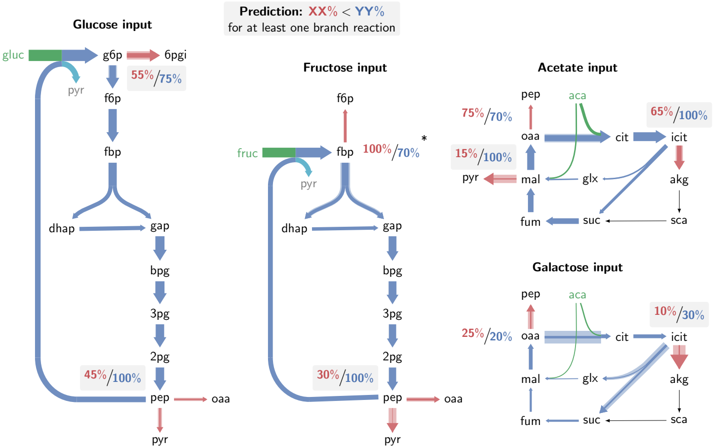

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Conclusions

- 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

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Future research questions

- What is the physical limit for lowering the activation energy barrier of a given reaction

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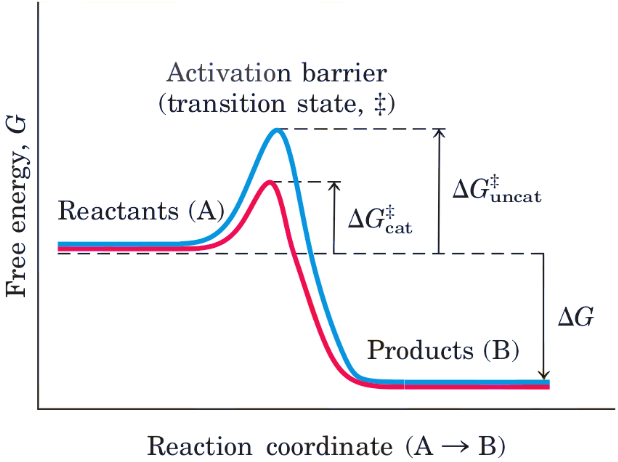
- What is the physical limit for lowering the activation energy barrier of a given reaction
- How is the affinity of an enzyme affected by the requirement to be selective

Textbook illustration



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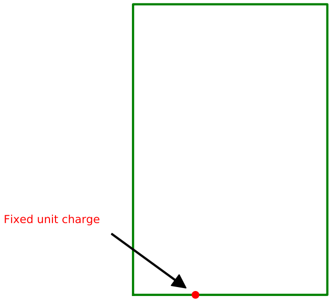
Modeling energy landscape modification in a classical system



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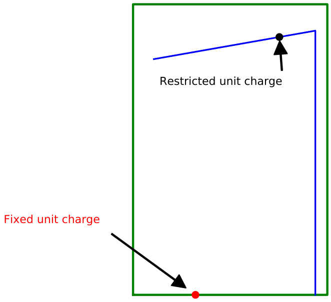

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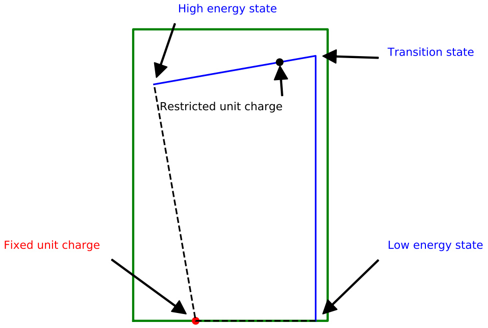

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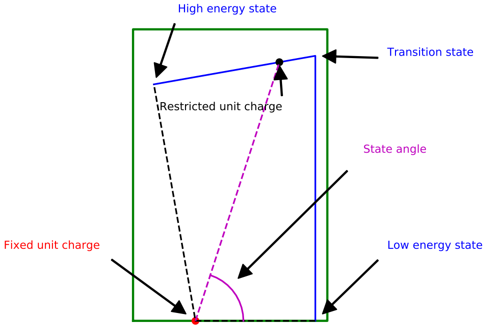

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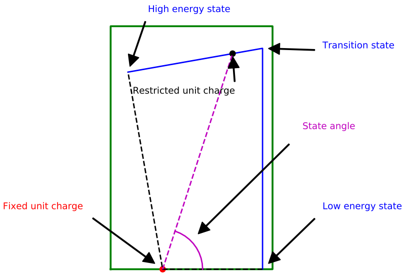
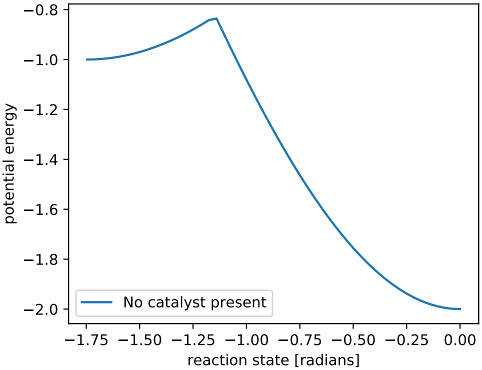
Reaction energy landscape of model substrate





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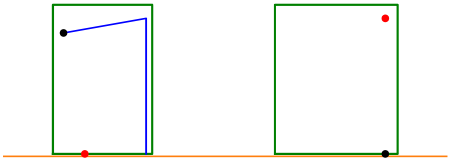
Introducing a model catalyst



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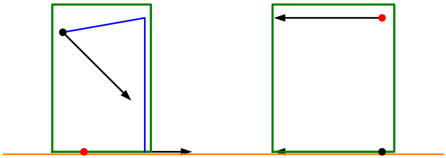

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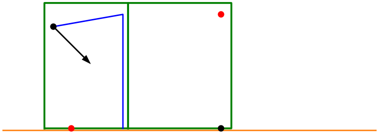

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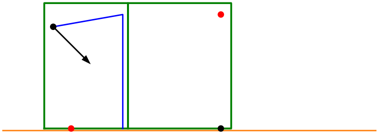

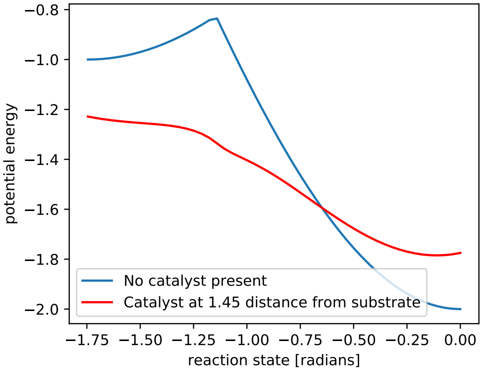

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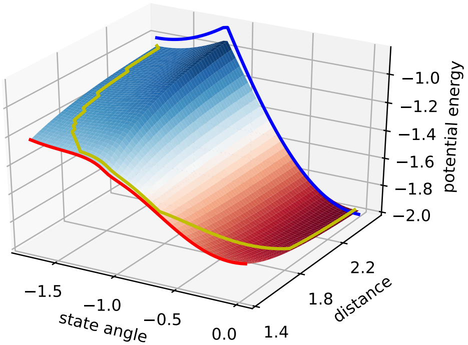
The catalyst creates a bypass to the energy barrier at the transition state



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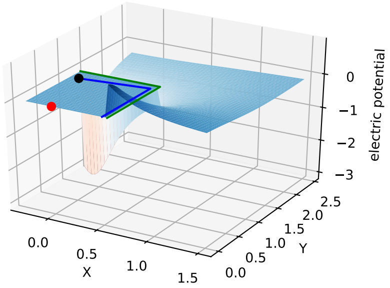
Subtracting the potential field at the transition state from the initial state produces an energy barrier reduction landscape



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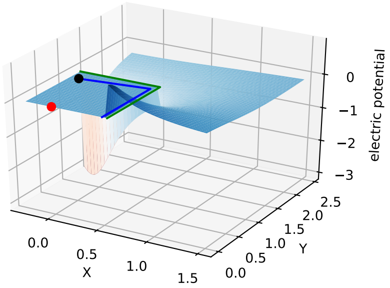

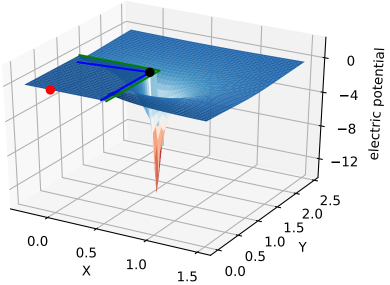

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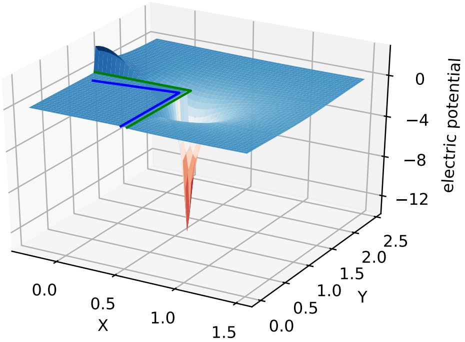

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- The resulting function quantifies the barrier reduction when positioning a positive point charge at any coordinate in space
- Placing charges at extremum points of this function achieves maximal barrier reduction

Methodological approach for investigating catalytic constraints

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- Apply theoretical framework to molecular domain
- Investigate metabolic network design implications
  - Synthetic biology applications
  - Origins of life metabolism

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Acknowledgments

SAERI Sustainability And Energy Research Initiative

erc European Research Council

Summary

- Basic challenges of biological systems are rarely investigated theoretically
- Transforming key problems to simplified models in accessible platforms can leverage innovation of wider audience and reveal novel principles
- Recently available datasets allow evaluation of hypotheses
- Mapping metabolic networks into the chemical space can highlight metabolic network motifs

References (autocatalysis):

- 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_{cat} from proteomics data: Davidi et. al., PNAS 2016
- This work: Barenholz et. al., eLife 2017

Thank you!

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Supplementary figures and data

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

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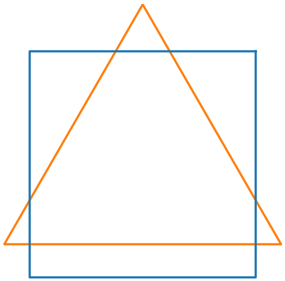
- Most enzymes are substrate-specific
- Structural similarity is used for drug discovery and promiscuous activity tests
- Metabolic networks must contain structurally similar metabolites
  - But can potentially reduce similarities at critical points
- Numerous examples for specificity tradeoffs in the literature

Why do we expect selectivity to decrease affinity?



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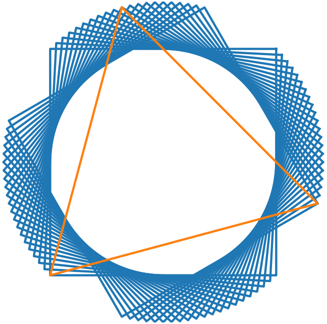

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Examples of specificity-affinity challenges

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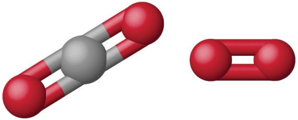
  - CO_2 versus O_2



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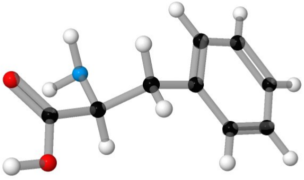
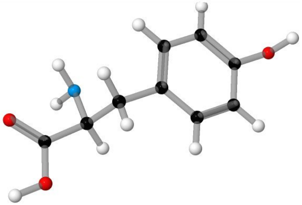
- Tyrosine ammonia lyase
  - Tyr versus Phe





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Examples of specificity-affinity challenges

- RuBisCo
  - CO_2 versus O_2
- Tyrosine ammonia lyase
  - Tyr versus Phe
- Bacterial DNA methyltransferase
  - Relaxing sequence specificity accelerates rate
- Bacterial hexose phosphate transporter

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Can we formulate a quantitative evaluation of the selectivity challenge?

- Given metabolites concentration data
  - Identify challenging reactions
  - Quantify expected cost

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Can we formulate a quantitative evaluation of the selectivity challenge?

- Given metabolites concentration data
  - Identify challenging reactions
  - Quantify expected cost
- Given reaction possibilities
  - Find biases in metabolic network structure maximizing structural differences

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Methodological approach for investigating selectivity tradeoffs

- Impact on metabolites concentrations and enzymes
  - BRENDA - identifying weak affinity enzymes
  - Promiscuous activity data from Sauer lab
  - Structural similarity metrics comparison with measured metabolites concentrations

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Methodological approach for investigating selectivity tradeoffs

- Impact on metabolites concentrations and enzymes
  - BRENDA - identifying weak affinity enzymes
  - Promiscuous activity data from Sauer lab
  - Structural similarity metrics comparison with measured metabolites concentrations
- Impact on network structure
  - Project metabolic networks to chemical space
  - Implement selectivity in constraint based modeling of metabolic networks

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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

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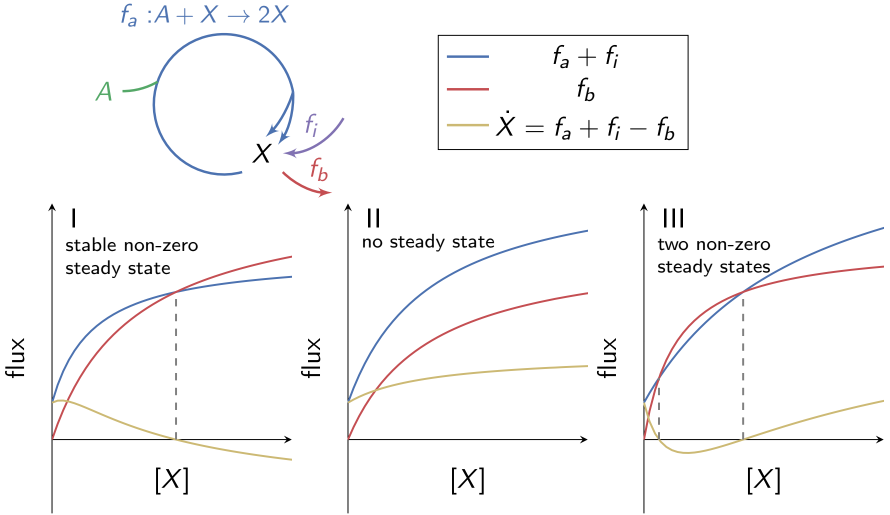
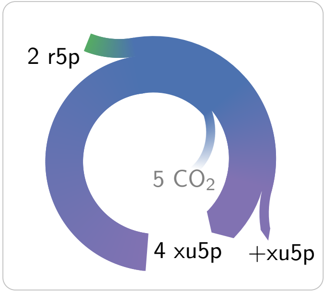
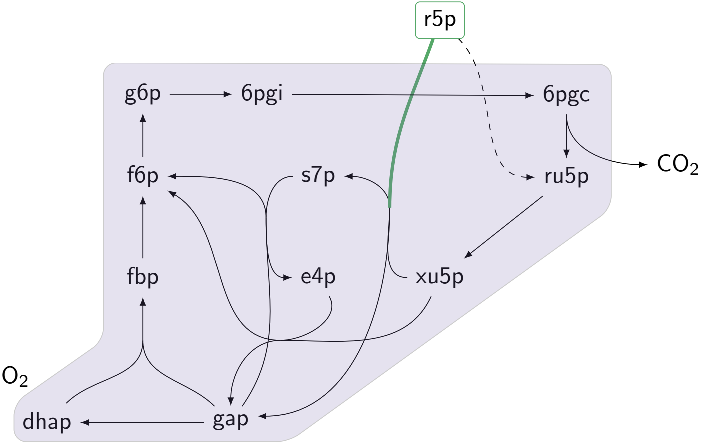
Input flux increases the range of parameters for which stable fluxes exist



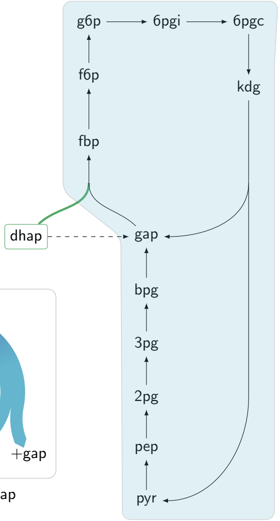
Additional autocatalytic cycles in central carbon metabolism

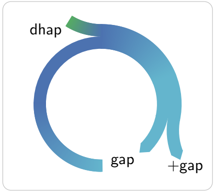


$$4 \text{ xu5p} + 2 \text{ r5p} \rightarrow 5 \text{ xu5p} + 5 \text{ CO}_2$$



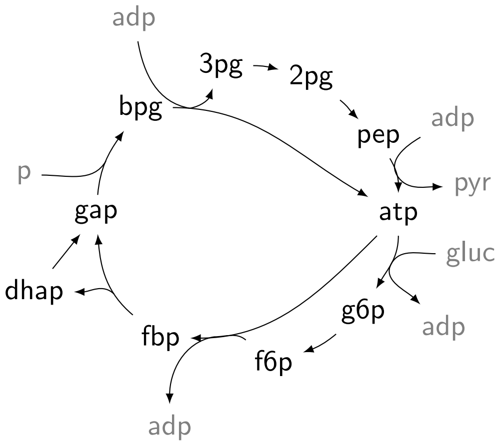
Additional autocatalytic cycles in central carbon metabolism





gap + dhap → 2 gap

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

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

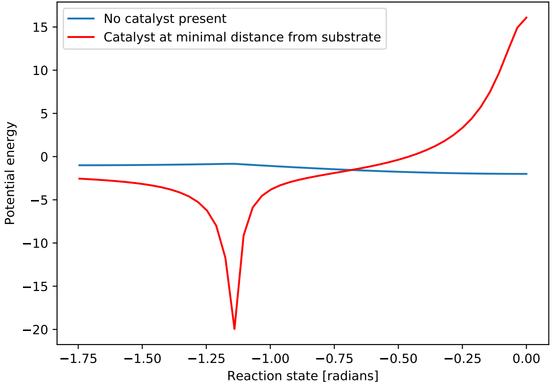
Reversible reaction equation

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

Work plan

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1. Analysis of the potential for catalysis of chemical reactions and its implication on network structure and function																								
1.1 design and production of sample hand-held models (1000 euro)																								
1.2 Theoretical framework for classical systems																								
1.3 crowd-source platform development (20,000 euro)																								
1.6 deployment of crowd-source platform and collection of leading designs (20,000 euro)																								
1.7 production of leading crowd-sourced designs (5000 euro)																								
1.4 Theoretical framework for molecular systems																								
1.5 analysis of metabolic networks in light of predictions																								
2. Analysis of affinity versus selectivity challenge of enzymes and implications on metabolic network function																								
2.1 application of similarity metrics to metabolites																								
2.2 identification of enzymes and reactions subject to selectivity challenges based on existing data leading to milestone 3 (below)																								
2.3 analysis of existing data/external collaboration to collect new data																								
2.4 Independent collection of novel data on promiscuous activities of relevant enzymes (20,000 euro)																								
2.5 theoretical analysis of selectivity from thermodynamic considerations																								
2.6 Chemoinformatic analysis of metabolic networks																								
2.7 Analysis of design principles in metabolic networks to overcome selectivity challenges																								
3. Assessment if and what experimental methods are needed to quantify selectivity tradeoffs					2.2																			
4. dissemination and exploitation			1.1	1.2				2.3		1.4	2.4		1.4			1.5			1.5				1.6	2.5
5. communication			1.1							1.3												1.6		1.7
color code	ongoing work			Completion – paper submission			Conference presentation			Completion – non-academic communication			Completion – milestone			Completion – deadline			Popular blogs/other media publication			Task intended for PhD student		

Catalyst design must track the entire reaction pathway



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