

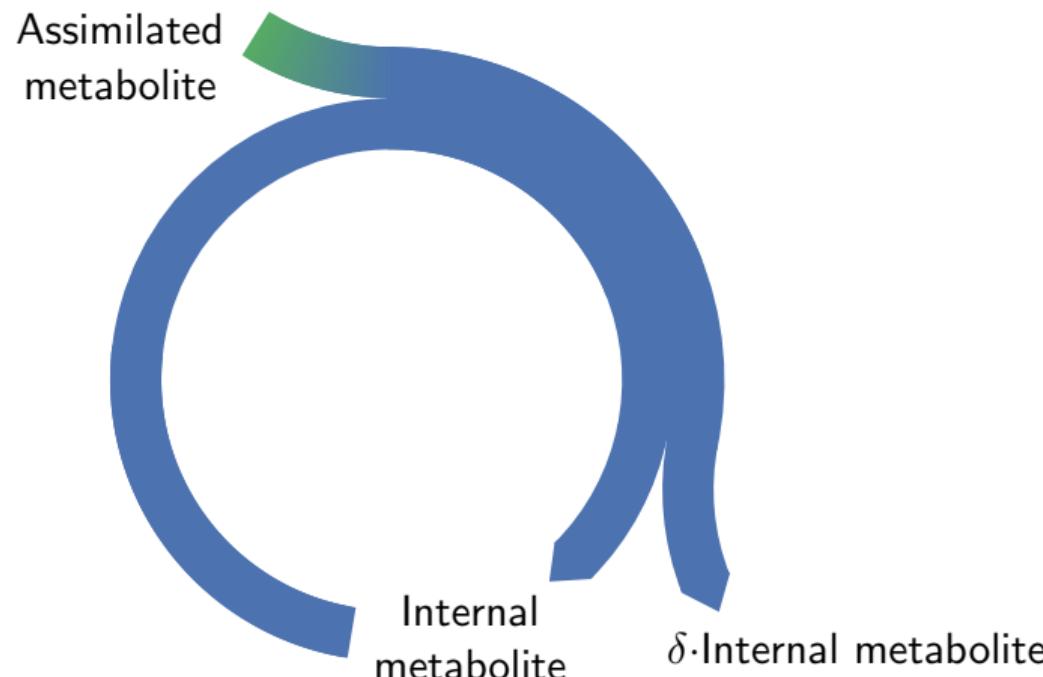
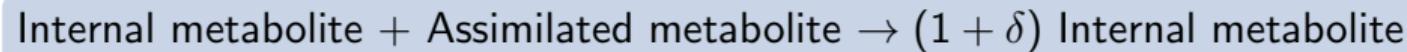
# The interplay between metabolic network topology and the kinetic parameters of enzymes, from autocatalytic cycles and beyond

Uri Barenholz

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

November 3, 2017

An autocatalytic cycle requires its internal metabolite to produce it

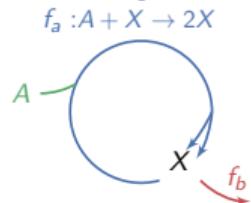


## Why do we care about autocatalytic cycles?

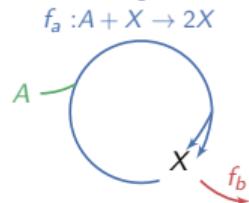
- ▶ The lab implements the Calvin-Benson-Bassham cycle in *E.coli*<sup>1</sup>
- ▶ Two enzymes were introduced
- ▶ It didn't work
- ▶ Can we understand why?

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

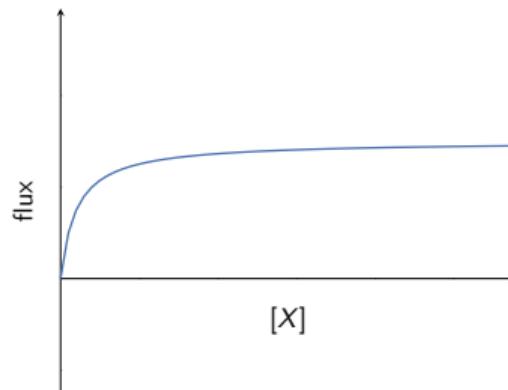
# Stable flux through an autocatalytic cycle constrains the kinetic parameters of its enzymes



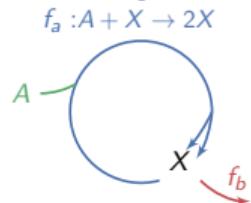
# Stable flux through an autocatalytic cycle constrains the kinetic parameters of its enzymes



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

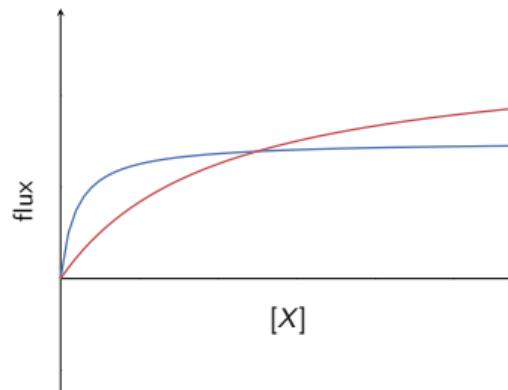


# Stable flux through an autocatalytic cycle constrains the kinetic parameters of its enzymes

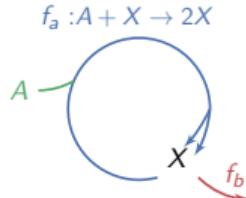


$$f_a = \frac{V_{\max,a}X}{K_{M,a}+X}$$

$$f_b = \frac{V_{\max,b}X}{K_{M,b}+X}$$



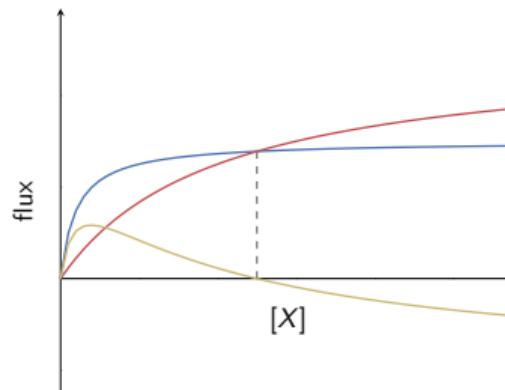
# Stable flux through an autocatalytic cycle constrains the kinetic parameters of its enzymes



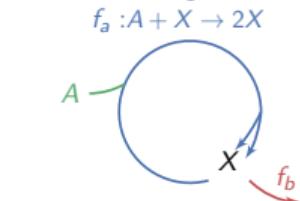
—	$f_a$	$V_{\max,b} > V_{\max,a}$
—	$f_b$	$V_{\max,b}/V_{\max,a} < K_{M,b}/K_{M,a}$
—	$\dot{X} = f_a - f_b$	

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

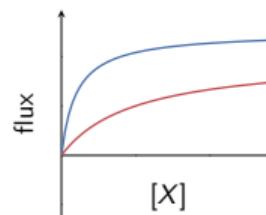
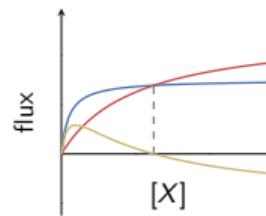
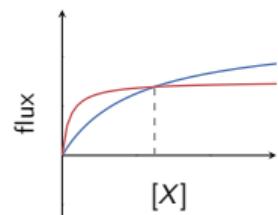
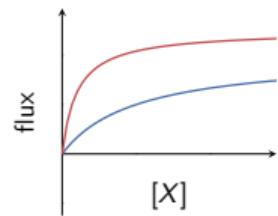
$$f_b = \frac{V_{\max,b}X}{K_{M,b}+X}$$



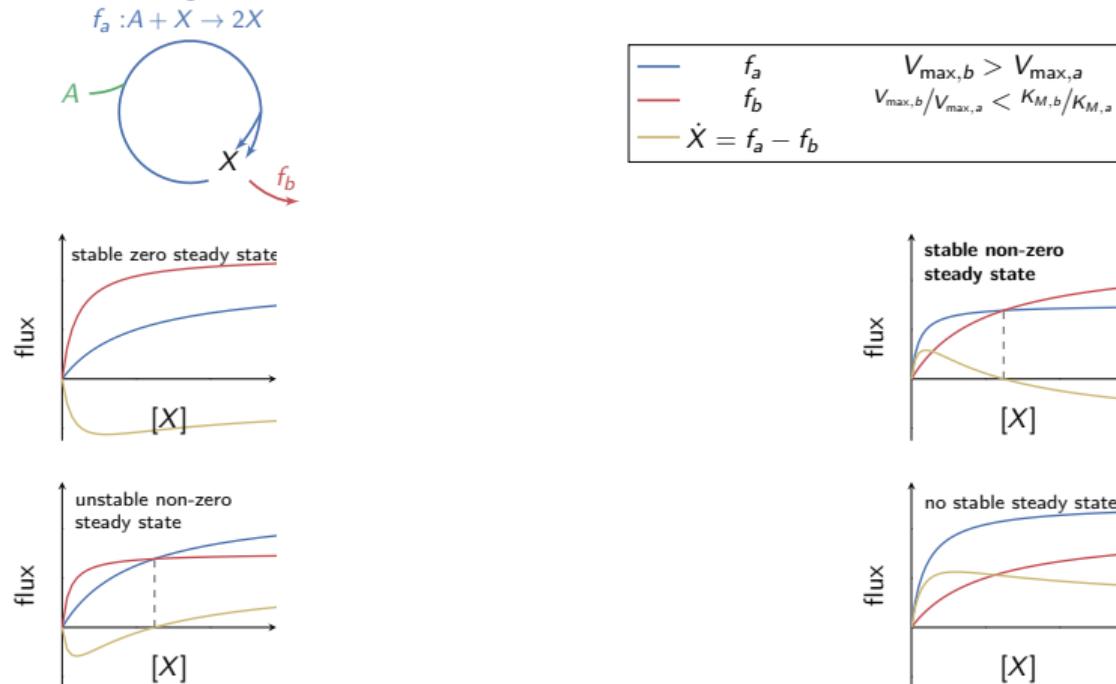
# Stable flux through an autocatalytic cycle constrains the kinetic parameters of its enzymes



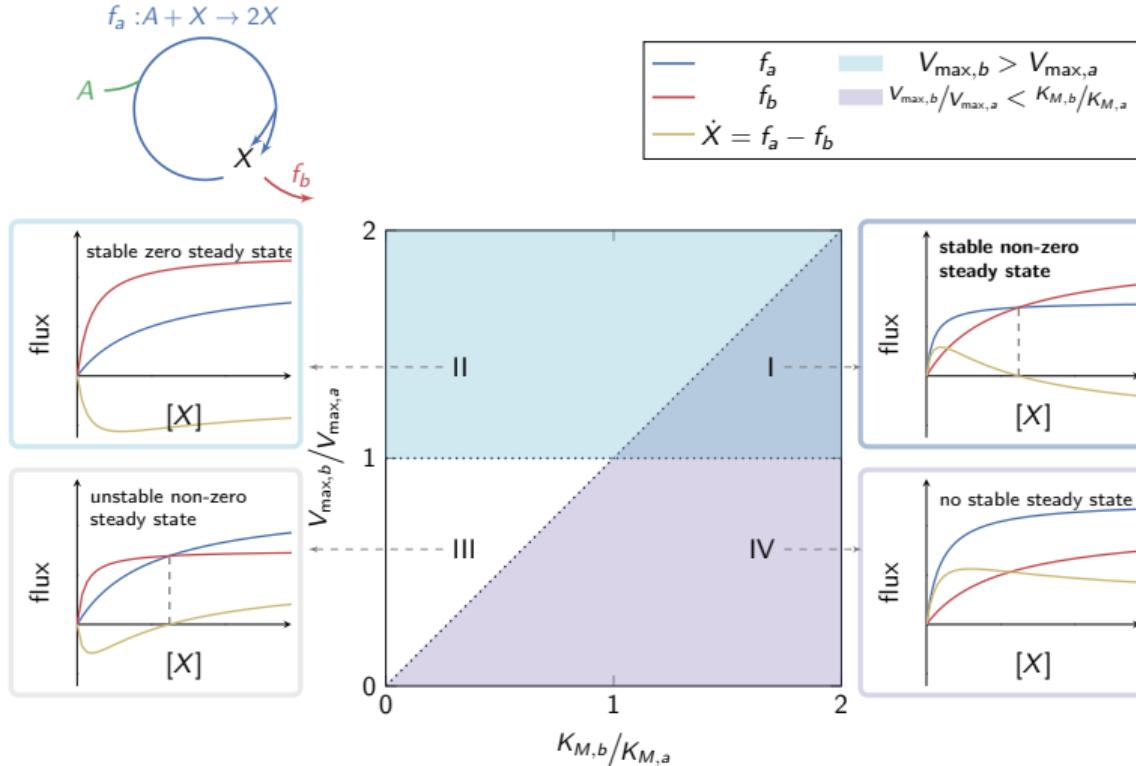
$f_a$	$V_{\max,b} > V_{\max,a}$
$f_b$	$V_{\max,b}/V_{\max,a} < K_M,b/K_M,a$
$\dot{X} = f_a - f_b$	



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



# 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
  - ▶ Upper bound on the affinity of the branch reaction remains in most schemes

## 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
- ▶ Assuming the autocatalytic reaction is reversible
  - ▶ Relaxes the constraint on the ratio of maximal fluxes between the autocatalytic and the branch reaction

## 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
- ▶ 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

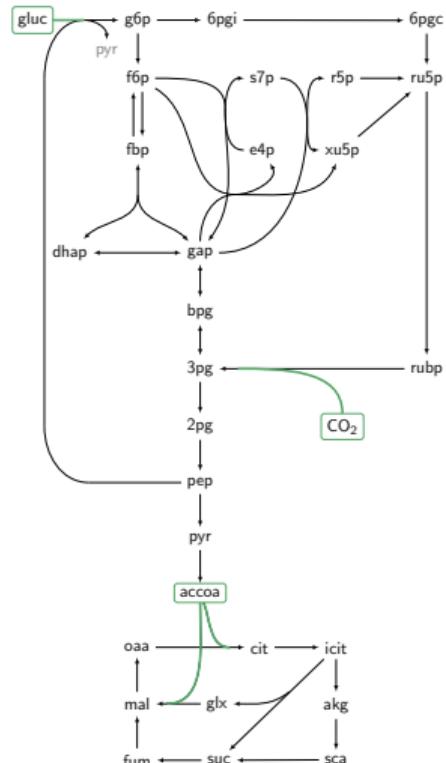
## Directed evolution towards function of the CBB cycle required changes in kinetic parameters of main branch reactions

- ▶ 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

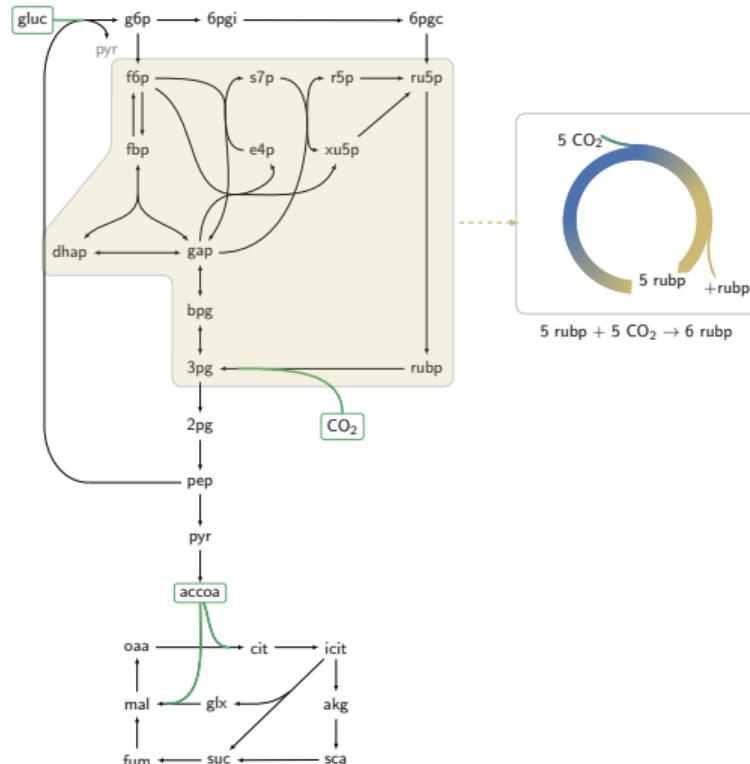
## Why should you care about autocatalytic cycles?

- ▶ 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

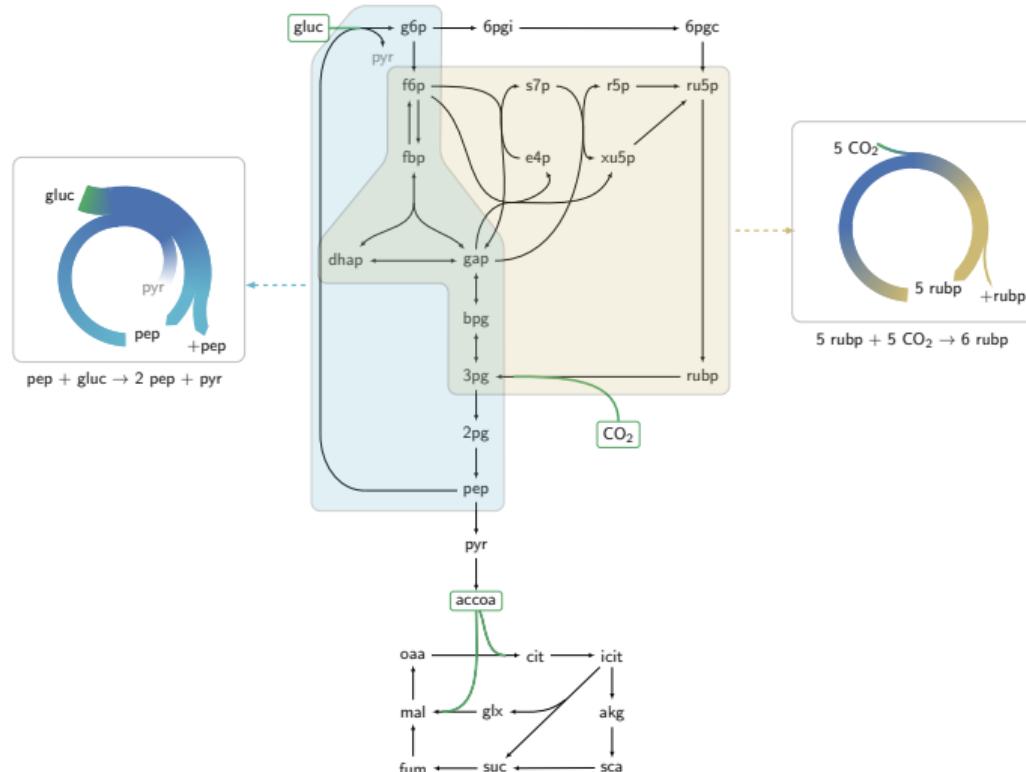
# Autocatalytic cycles are abundant in central carbon metabolism



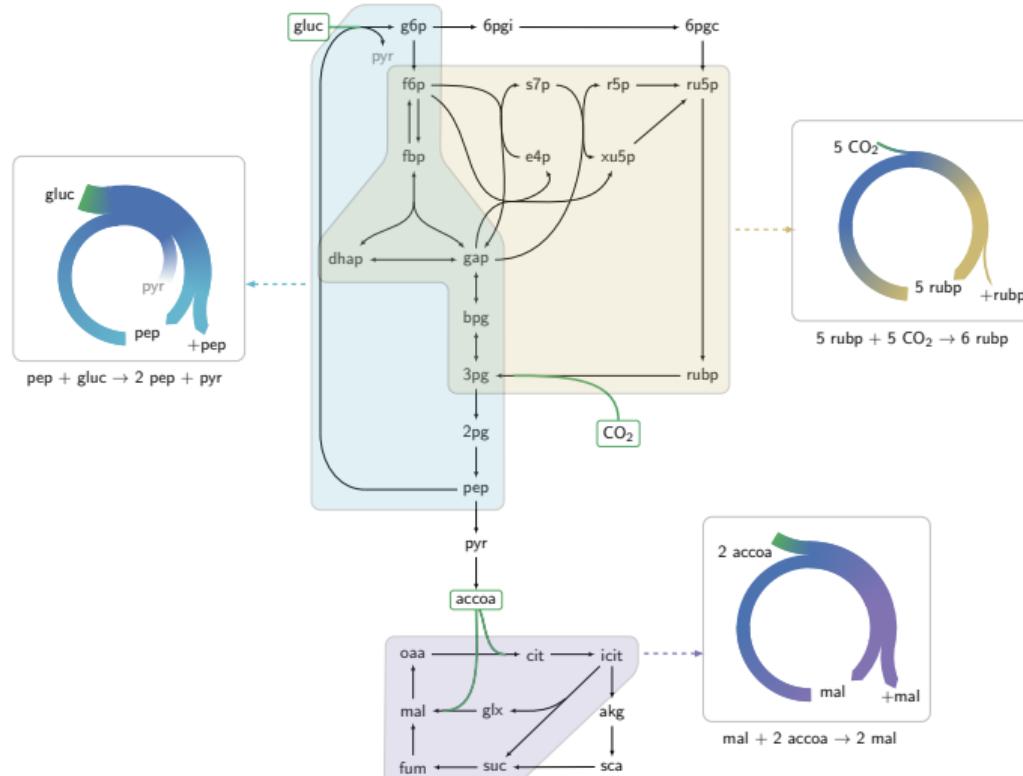
# Autocatalytic cycles are abundant in central carbon metabolism



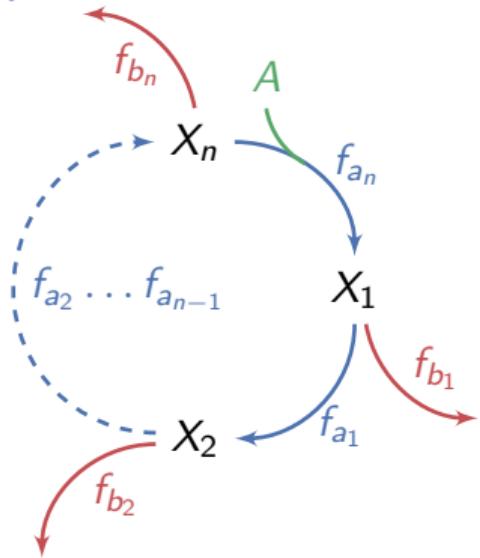
Autocatalytic cycles are abundant in central carbon metabolism



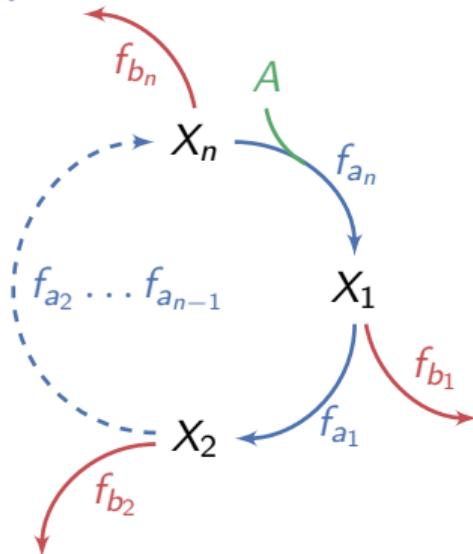
# Autocatalytic cycles are abundant in central carbon metabolism



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

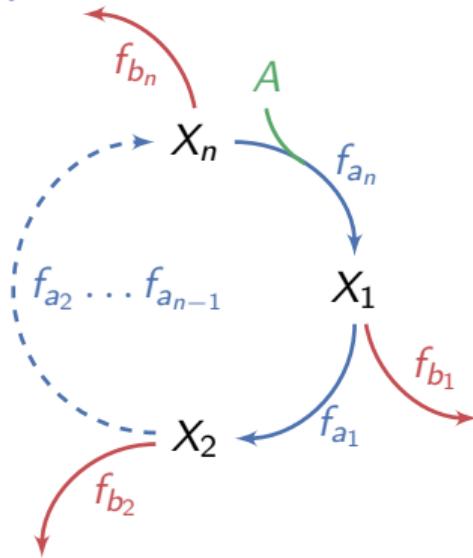


Stability criteria of the simple model can be extended for complex cycles



- At steady state:  $\sum f_{b_i} = f_{a_n}$

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



- ▶ 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  $\alpha_i = \frac{df_{a_i}}{dX_i}\Big|_{X_i^*}$

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

- ▶ Reaction saturation is the ratio of the actual flux to the potential flux, given expression level and catalytic rate

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

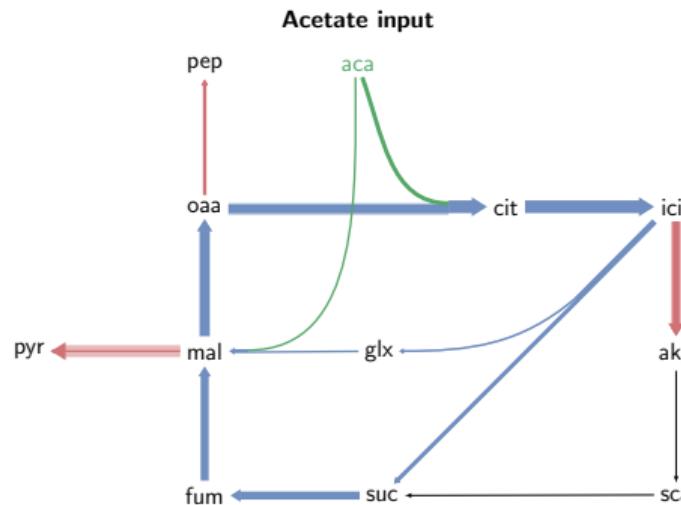
- ▶ Reaction saturation is the ratio of the actual flux to the potential flux, given expression level and catalytic rate
- ▶ For monotonically increasing, bounded, concave functions: saturation and derivative are inversely correlated

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

- ▶ 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

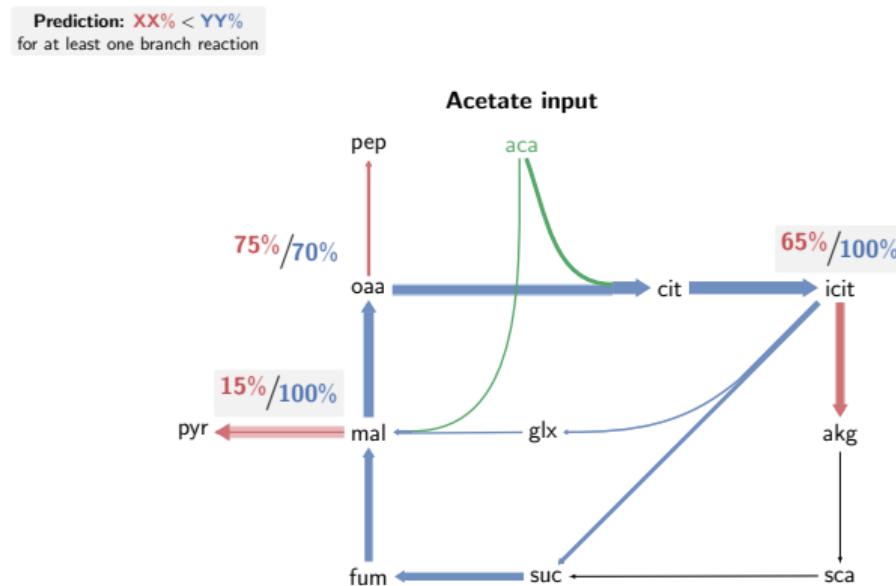
Prediction: XX% < YY%  
for at least one branch reaction



<sup>2</sup>Gerosa et. al., Cell Systems 2015

<sup>3</sup>Schmidt et. al., Nature Biotechnology 2016

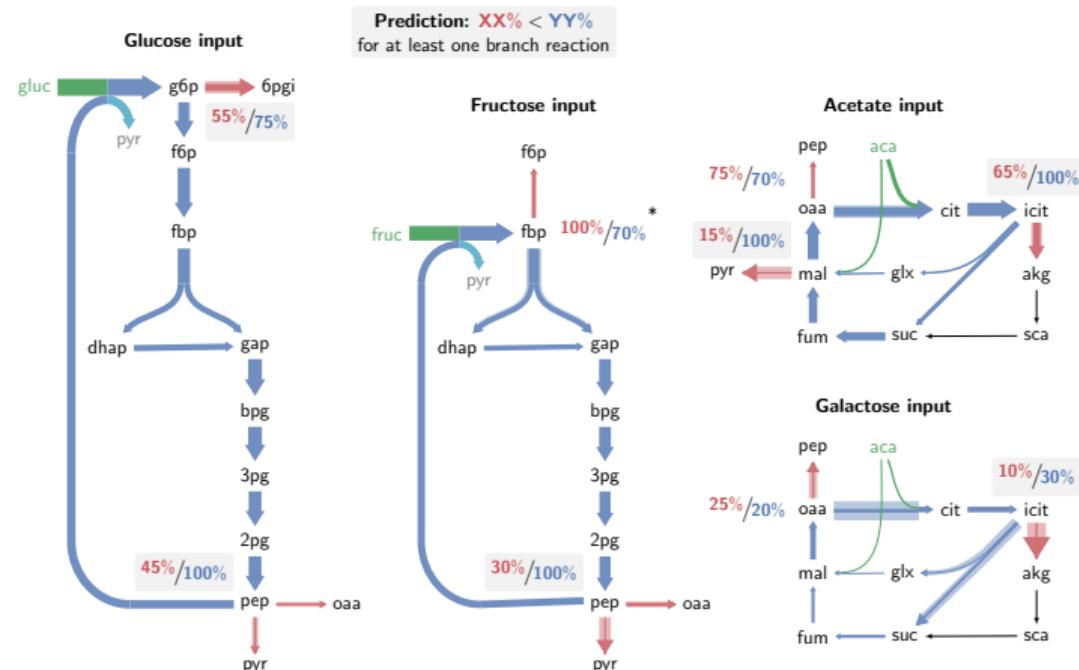
Analysis of experimental fluxomics data<sup>2</sup> and proteomics data<sup>3</sup> shows branch reactions are consistently less saturated than autocatalytic reactions



<sup>2</sup>Gerosa et. al., Cell Systems 2015

<sup>3</sup>Schmidt et. al., Nature Biotechnology 2016

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



<sup>2</sup>Gerosa et. al., Cell Systems 2015

<sup>3</sup>Schmidt et. al., Nature Biotechnology 2016

# 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

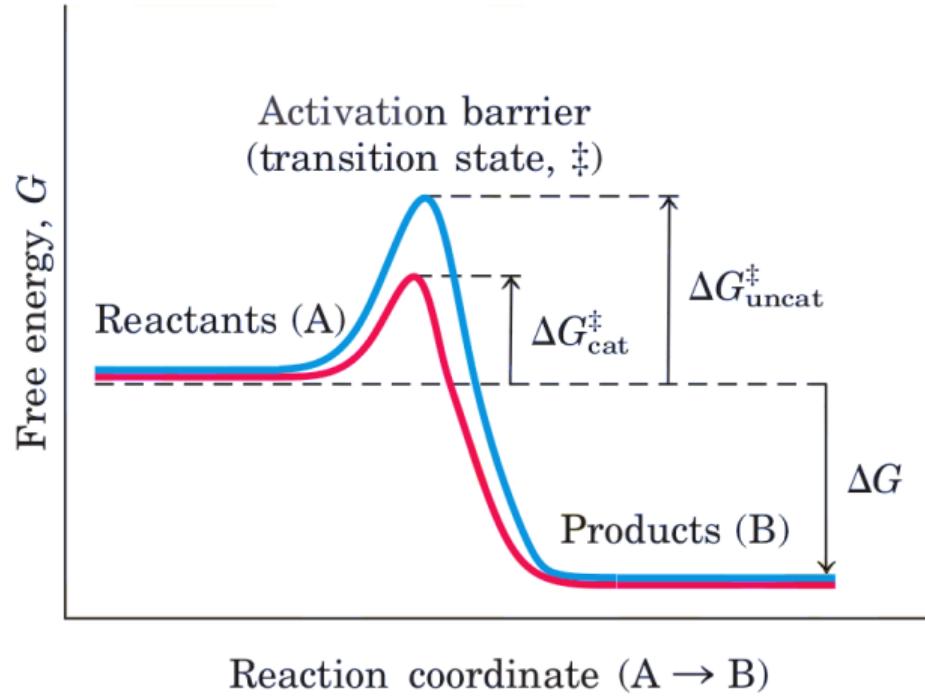
## Future research questions

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

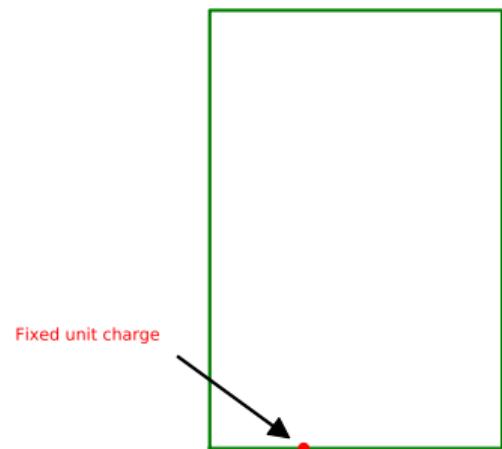
## Future research questions

- ▶ 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

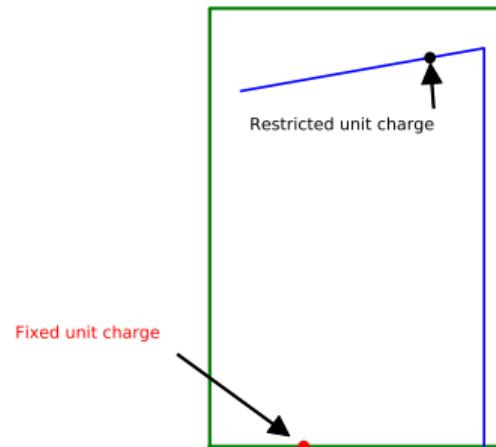
## Textbook illustration



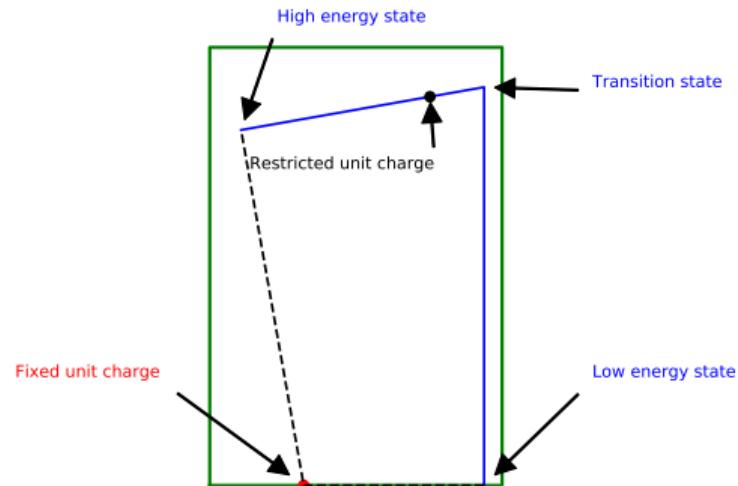
# Modeling energy landscape modification in a classical system



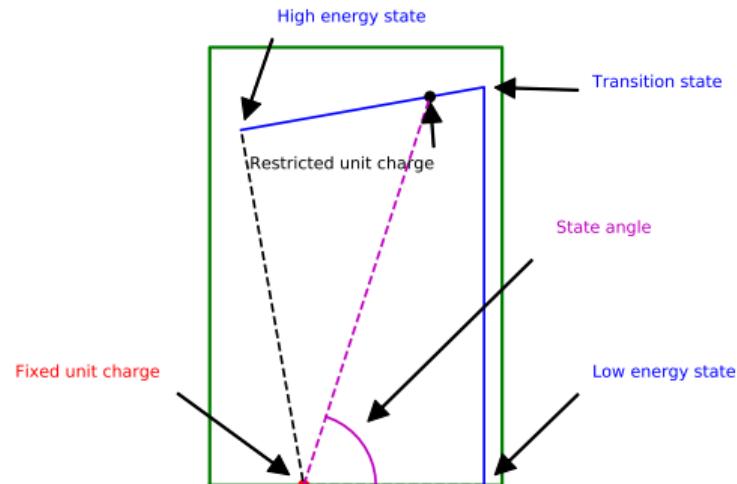
# Modeling energy landscape modification in a classical system



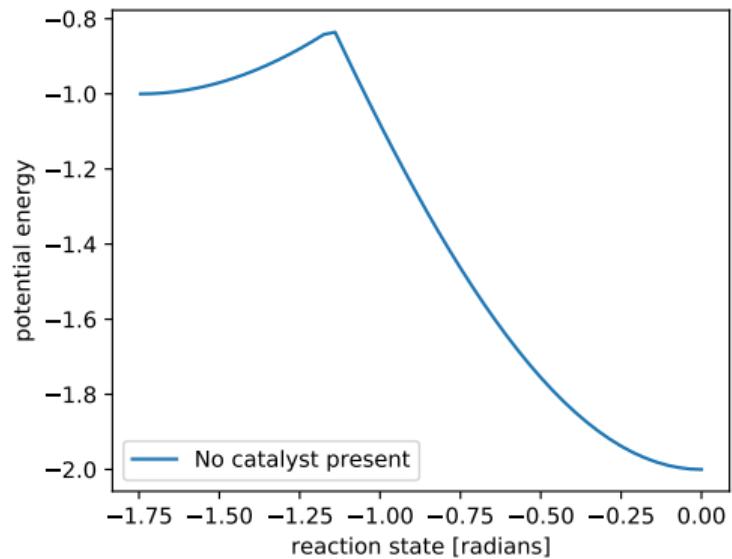
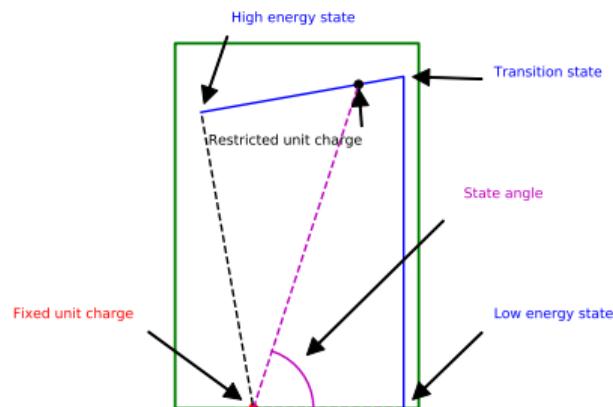
## Modeling energy landscape modification in a classical system



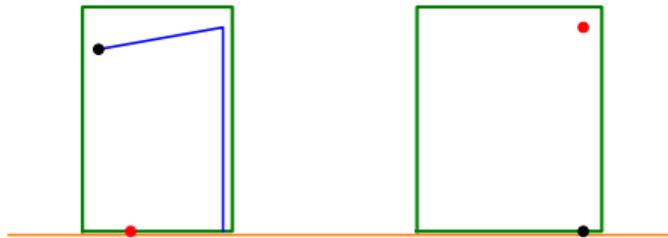
# Modeling energy landscape modification in a classical system



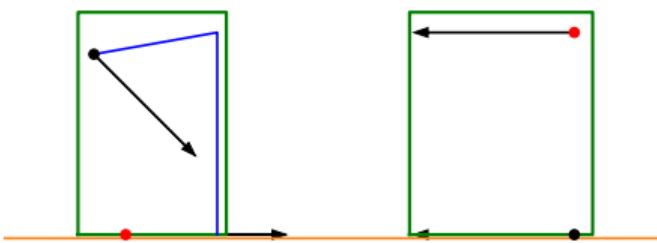
# Reaction energy landscape of model substrate



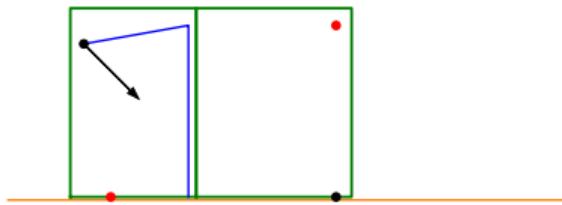
# Introducing a model catalyst



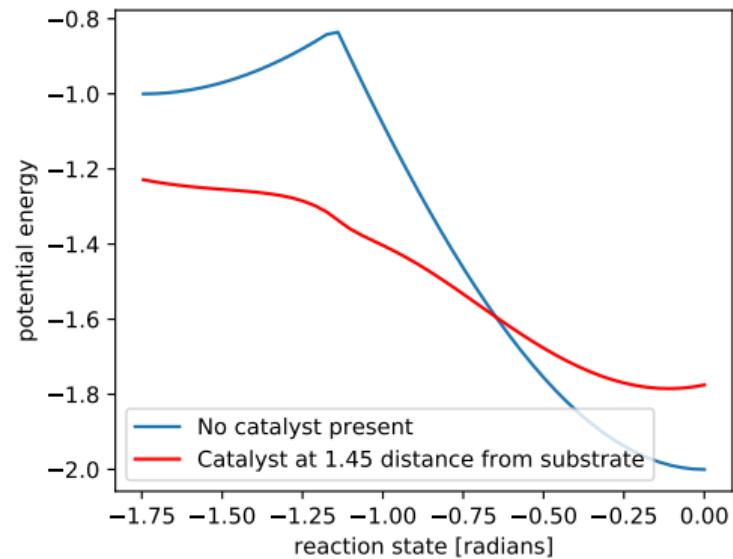
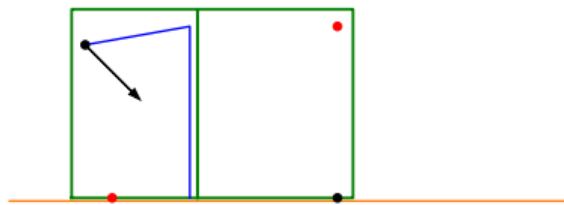
# Introducing a model catalyst



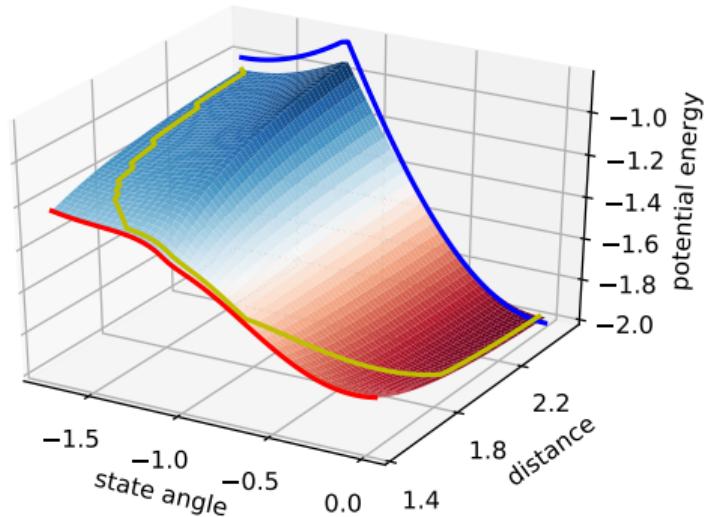
## Reaction energy landscape of bound substrate



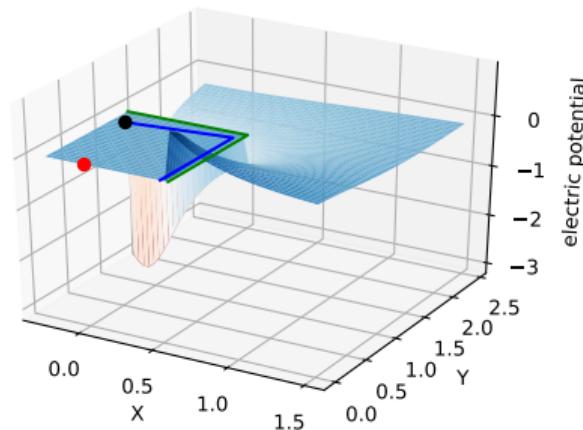
# Reaction energy landscape of bound substrate



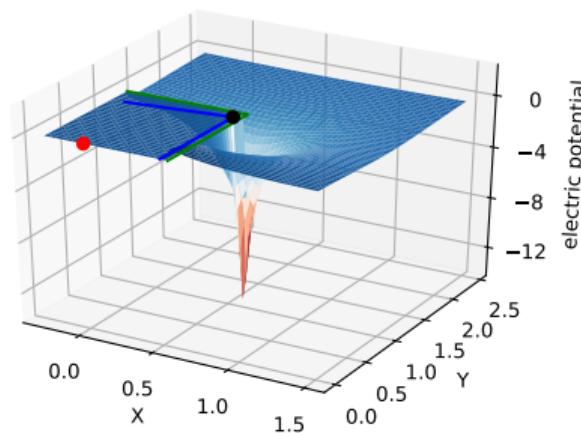
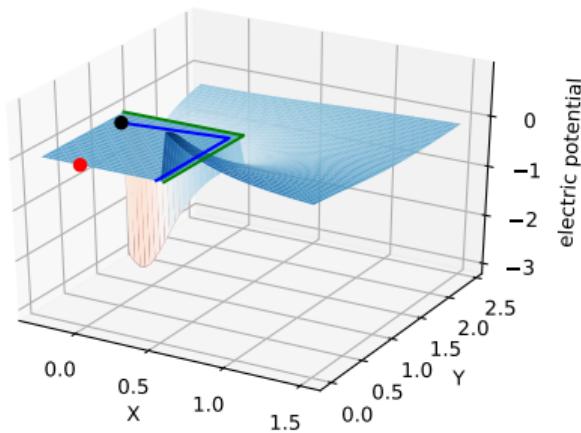
The catalyst creates a bypass to the energy barrier at the transition state



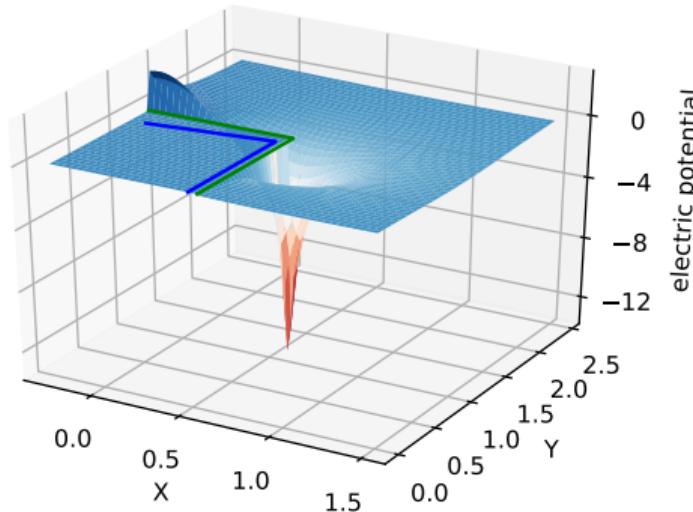
Subtracting the potential field at the transition state from the initial state produces an energy barrier reduction landscape



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



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



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

- ▶ The resulting function quantifies the barrier reduction when positioning a positive point charge at any coordinate in space

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

- ▶ 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

# Methodological approach for investigating catalytic constraints

- ▶ Crowd-sourcing platform
  - ▶ Challenge existing assumptions
  - ▶ Reveal potential catalytic mechanisms

# Methodological approach for investigating catalytic constraints

- ▶ Crowd-sourcing platform
  - ▶ Challenge existing assumptions
  - ▶ Reveal potential catalytic mechanisms
- ▶ Handheld, functional models
  - ▶ Demonstrate and communicate catalytic principles

# Methodological approach for investigating catalytic constraints

- ▶ Crowd-sourcing platform
  - ▶ Challenge existing assumptions
  - ▶ Reveal potential catalytic mechanisms
- ▶ Handheld, functional models
  - ▶ Demonstrate and communicate catalytic principles
- ▶ Apply theoretical framework to molecular domain

# Methodological approach for investigating catalytic constraints

- ▶ Crowd-sourcing platform
  - ▶ Challenge existing assumptions
  - ▶ Reveal potential catalytic mechanisms
- ▶ Handheld, functional models
  - ▶ Demonstrate and communicate catalytic principles
- ▶ Apply theoretical framework to molecular domain
- ▶ Investigate metabolic network design implications
  - ▶ Synthetic biology applications
  - ▶ Origins of life metabolism

# Acknowledgments



Sustainability  
And  
Energy  
Research  
Initiative



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  $k_{\text{cat}}$  from proteomics data: Davidi et. al., PNAS 2016
- ▶ This work: Barenholz et. al., eLife 2017

Thank you!

<https://git.io/vSodL>

## 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

### 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

# Does structural similarity limit affinity in metabolic networks?

# Does structural similarity limit affinity in metabolic networks?



# Does structural similarity limit affinity in metabolic networks?

- ▶ Most enzymes are substrate-specific

# Does structural similarity limit affinity in metabolic networks?

- ▶ Most enzymes are substrate-specific
- ▶ Structural similarity is used for drug discovery and promiscuous activity tests

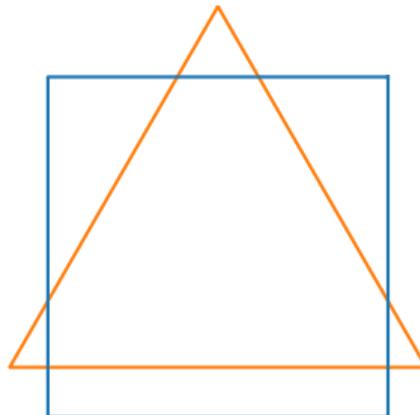
# Does structural similarity limit affinity in metabolic networks?

- ▶ 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

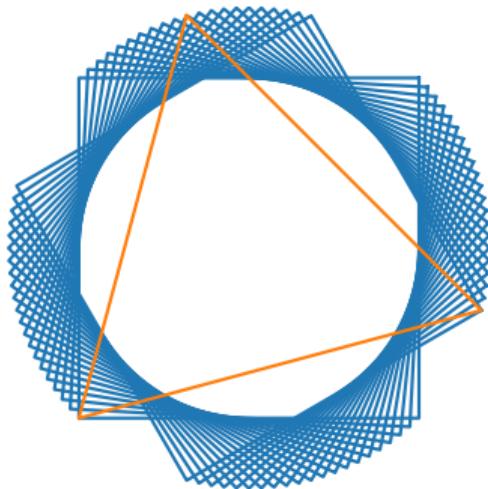
# Does structural similarity limit affinity in metabolic networks?

- ▶ 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?

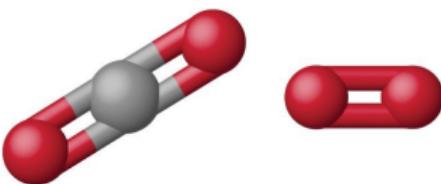


## Why do we expect selectivity to decrease affinity?



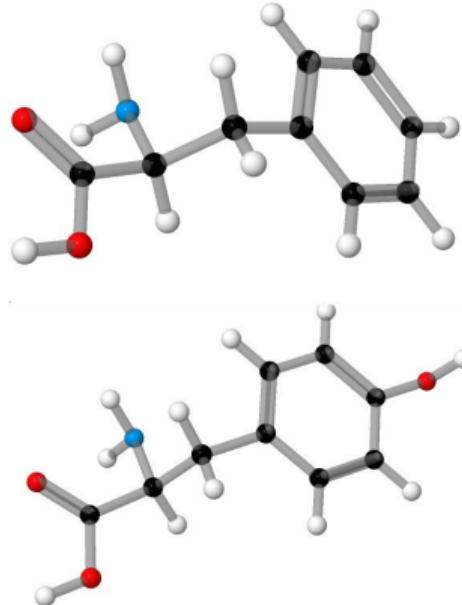
## Examples of specificity-affinity challenges

- ▶ RuBisCo
  - ▶ CO<sub>2</sub> versus O<sub>2</sub>



# Examples of specificity-affinity challenges

- ▶ RuBisCo
  - ▶ CO<sub>2</sub> versus O<sub>2</sub>
- ▶ Tyrosine ammonia lyase
  - ▶ Tyr versus Phe



## Examples of specificity-affinity challenges

- ▶ RuBisCo
  - ▶ CO<sub>2</sub> versus O<sub>2</sub>
- ▶ Tyrosine ammonia lyase
  - ▶ Tyr versus Phe
- ▶ Bacterial DNA methyltransferase
  - ▶ Relaxing sequence specificity accelerates rate
- ▶ Bacterial hexose phosphate transporter

# Can we formulate a quantitative evaluation of the selectivity challenge?

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

# 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

# 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

# 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

## 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

- ▶ 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

## 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
- ▶ Adaptation of steady state fluxes to nutrient availability is achieved by allosteric regulation of the assimilated metabolite
  - ▶ The assimilated metabolite should activate branch reactions and inhibit cycle reactions

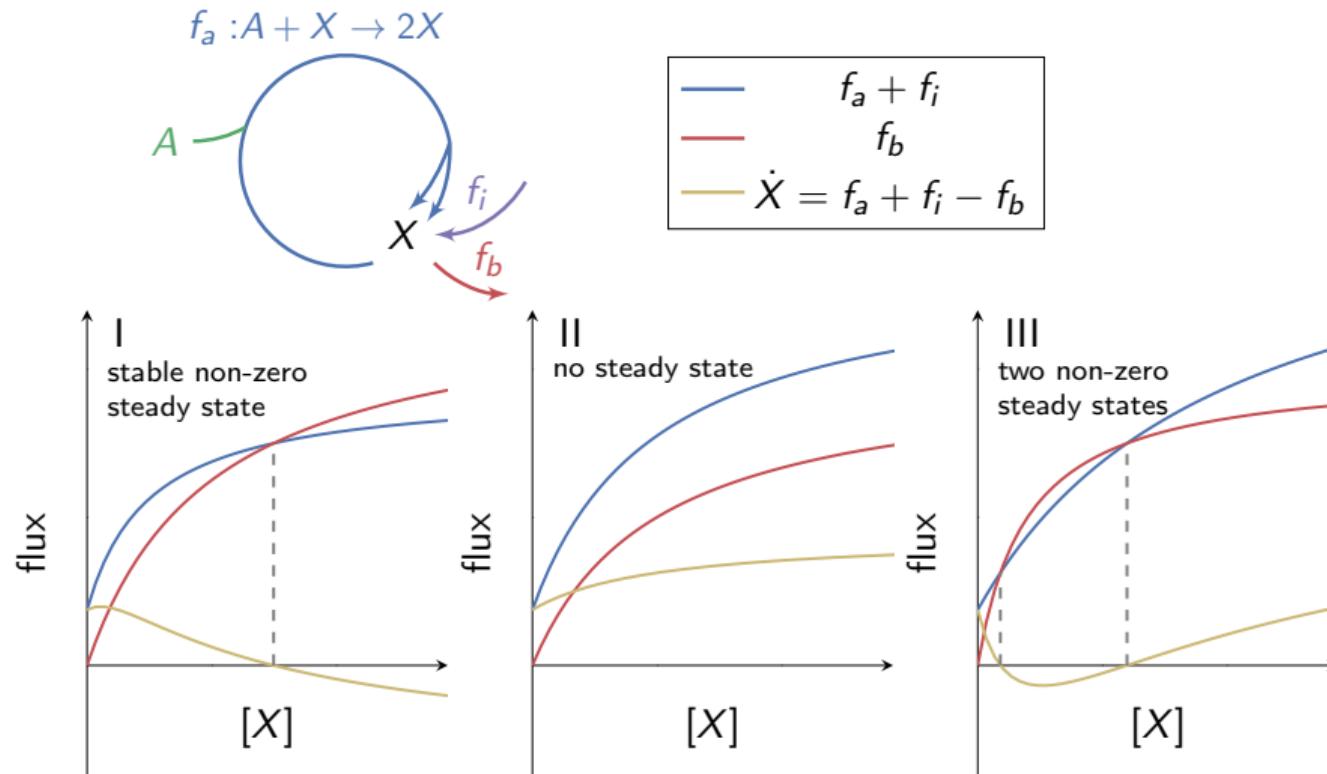
## Allosteric regulation can accelerate convergence to steady state and increase robustness in fluctuating environment

- ▶ 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
- ▶ Adaptation of steady state fluxes to nutrient availability is achieved by allosteric regulation of the assimilated metabolite
  - ▶ The assimilated metabolite should activate branch reactions and inhibit cycle reactions
- ▶ 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

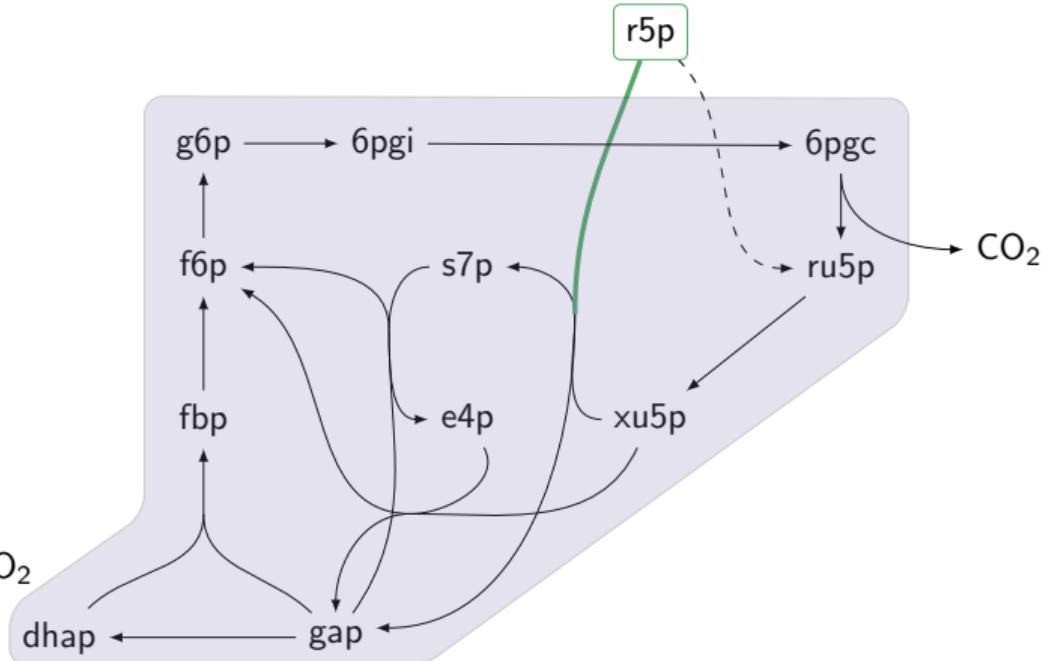
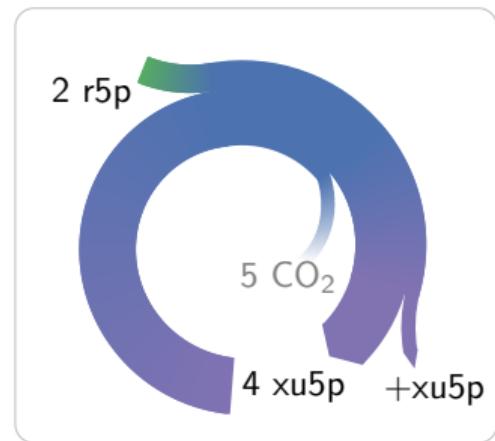
## Allosteric regulation can accelerate convergence to steady state and increase robustness in fluctuating environment

- ▶ 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
- ▶ Adaptation of steady state fluxes to nutrient availability is achieved by allosteric regulation of the assimilated metabolite
  - ▶ The assimilated metabolite should activate branch reactions and inhibit cycle reactions
- ▶ 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

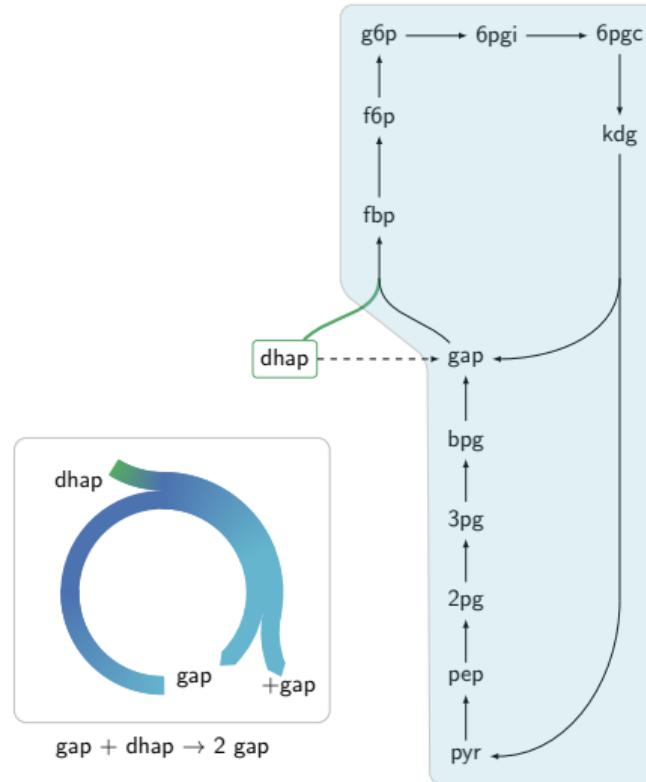
# Input flux increases the range of parameters for which stable fluxes exist



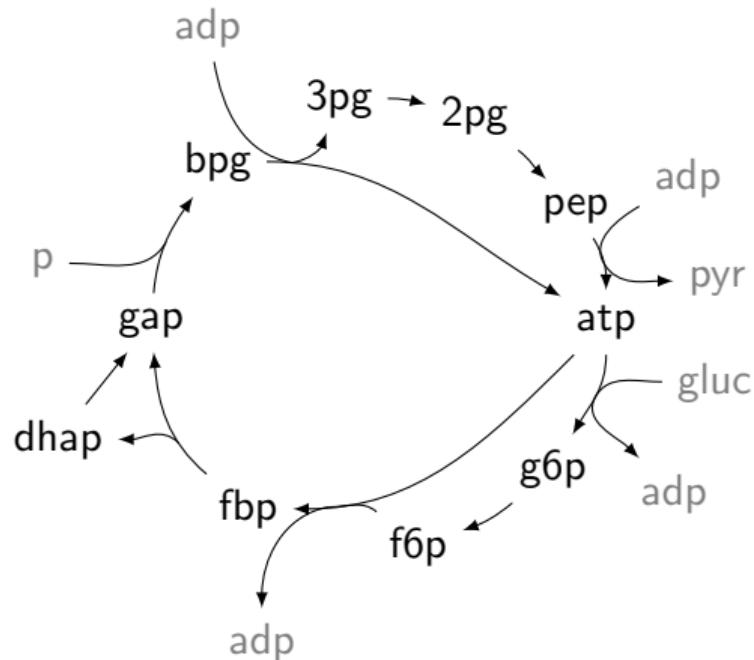
# Additional autocatalytic cycles in central carbon metabolism



# 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

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

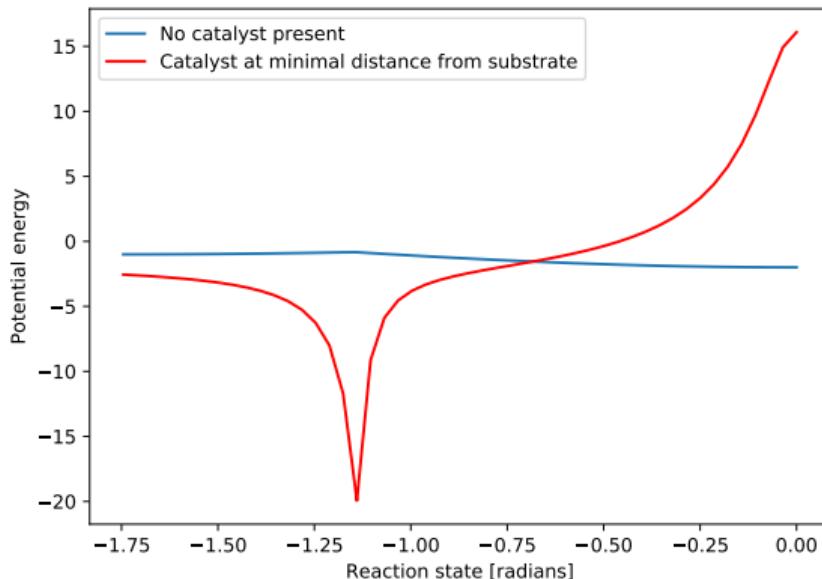
## Reversible reaction equation

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

# 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	
<b>1. Analysis of the potential for catalysis of chemical reactions and its implication on network structure and function</b>																									
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																									
<b>2. Analysis of affinity versus selectivity challenge of enzymes and implications on metabolic network function</b>																									
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																									
4. dissemination and exploitation			1.1	1.2					2.3		1.4	2.4		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



## References

1. Cooper S, et al. (2010) Predicting protein structures with a multiplayer online game. *Nature*
2. Cao Y, et al. (2008) ChemmineR: a compound mining framework for R. *Bioinformatics*
3. Reymond J-L (2015) The chemical space project. *Acc Chem Res*
4. Wang Y, et al. (2013) fmcsR: mismatch tolerant maximum common substructure searching in R. *Bioinformatics*
5. Bar-Even A, et al. (2015) The Moderately Efficient Enzyme: Futile Encounters and Enzyme Floppiness. *Biochemistry*
6. Alam MT, et al. (2017) The self-inhibitory nature of metabolic networks and its alleviation through compartmentalization. *Nat Commun*
7. Schomburg I, et al. (2004) BRENDA, the enzyme database: updates and major new developments. *Nucleic Acids Res*
8. Svin DC, et al. (2017) Nontargeted in vitro metabolomics for high-throughput identification of novel enzymes in *Escherichia coli*. *Nat Methods*
9. Savir Y, et al. (2010) Cross-species analysis traces adaptation of Rubisco toward optimality in a low-dimensional landscape. *PNAS*
10. Tcherkez GGB, et al. (2006) Despite slow catalysis and confused substrate specificity, all ribulose bisphosphate carboxylases may be nearly perfectly optimized. *PNAS*
11. Danos V, et al. (2015) Rigid Geometric Constraints for Kappa Models. *Electron Notes Theor Comput Sci*