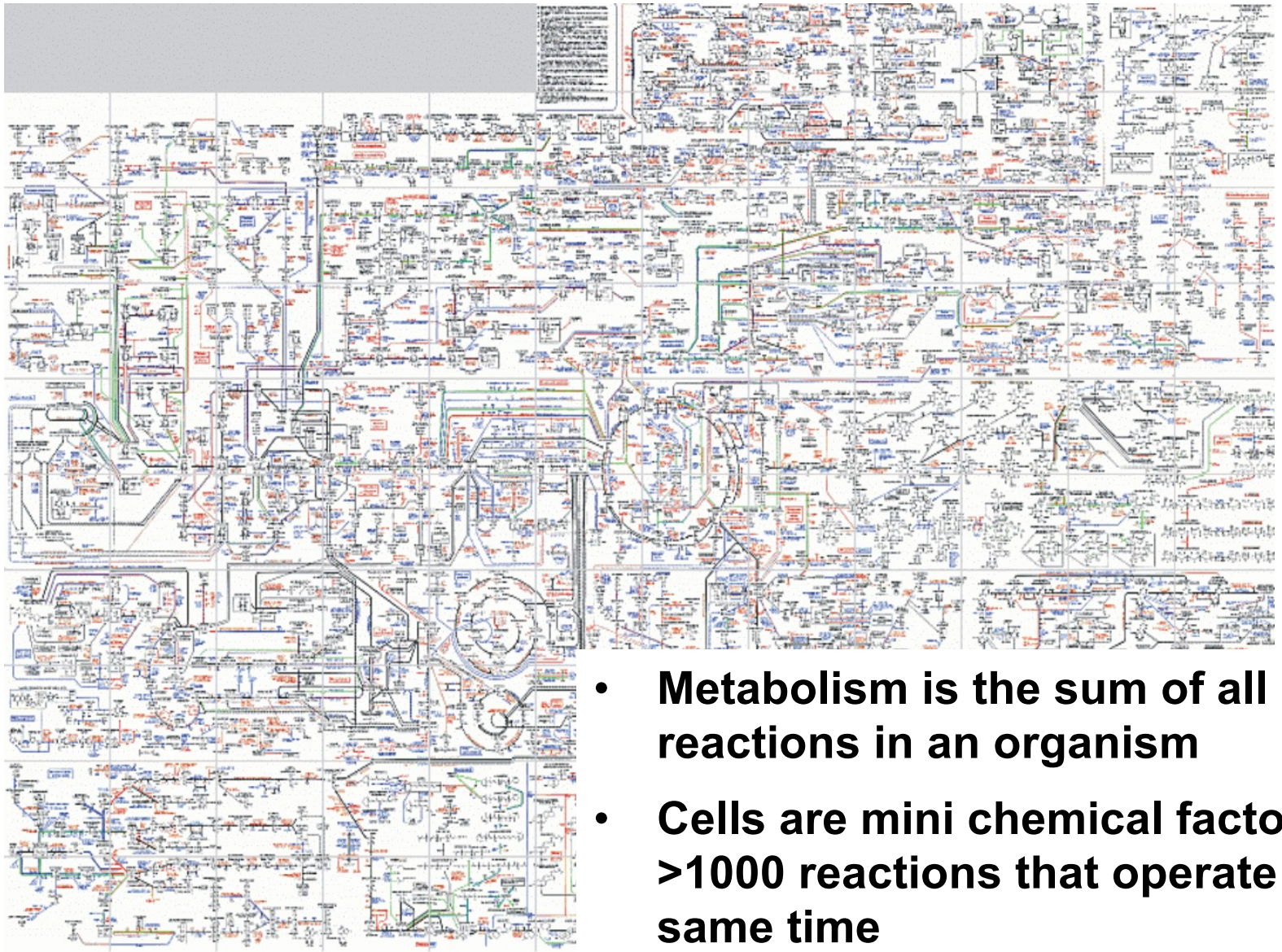
Introduction to Metabolism

Uwe Sauer, ETH Zurich

- Structure & principles of metabolic networks
- Quick glance at key pathways & principles of energy generation
- Mechanisms of regulating metabolic flux
- How to assess metabolic fluxes?



- **Metabolism is the sum of all chemical reactions in an organism**
- **Cells are mini chemical factories with >1000 reactions that operate at the same time**
- **Highly conserved from bacteria to humans**

Two Levels to Aid Understanding Metabolism

Reaction level: kinetics/thermodynamics

$$[E] \bullet k_{cat} \bullet \frac{[S]}{k_m + [S]} = \text{reaction rate (flux)}$$

Speed

$$\Delta G = \Delta G^{0\prime} + RT \ln [ATP]/[ADP]\cdot[P_i]$$

Direction

The standard free energy of ATP hydrolysis is 30.5 kJ/mol.
In vivo it is more like 50 kJ/mol.

Network level: structure

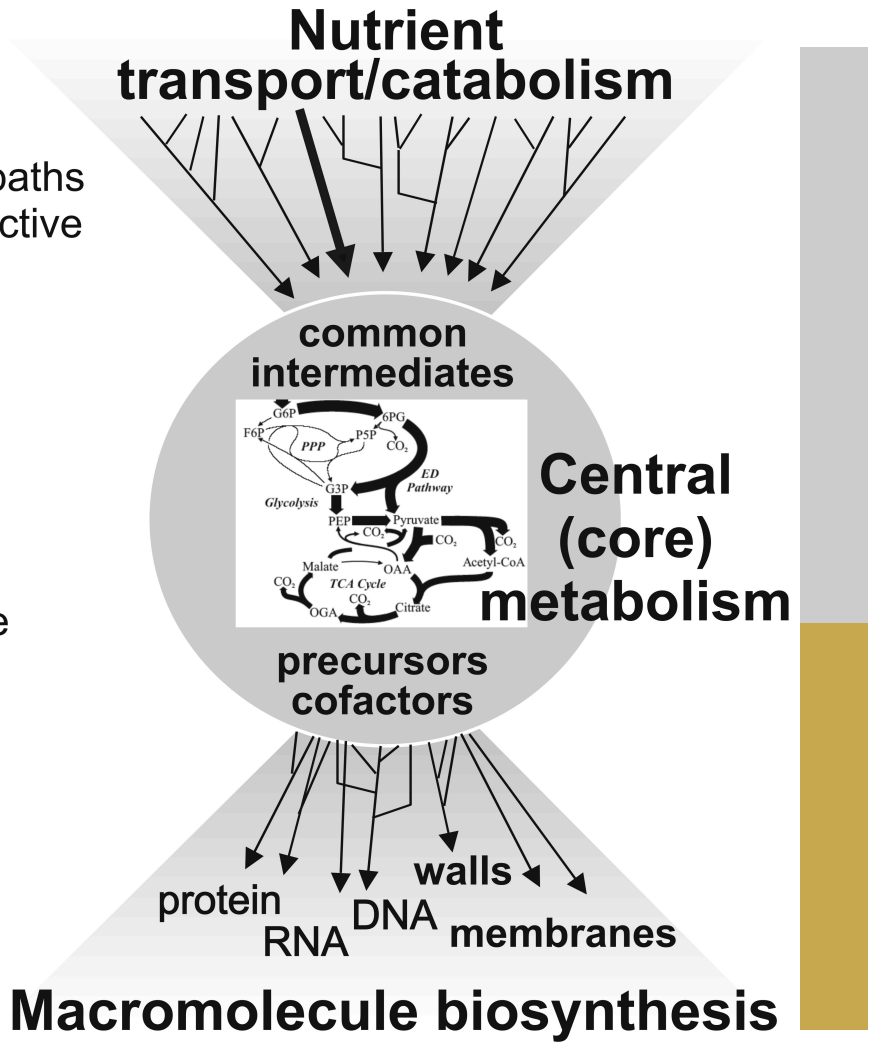
Simple structure from birds eye …..

Complicated but Structured

- linear
- convergent
- few connections
- typically one or few paths are simultaneously active
- one-way flux

- many cycles
- many connections
- redundant
- flux direction variable
- ubiquitous

- linear
- divergent
- few connections



Catabolism

- Catabolic pathways release energy by breaking down complex molecules into simpler compounds (eg respiration)
- Thermodynamically downhill (mostly!)

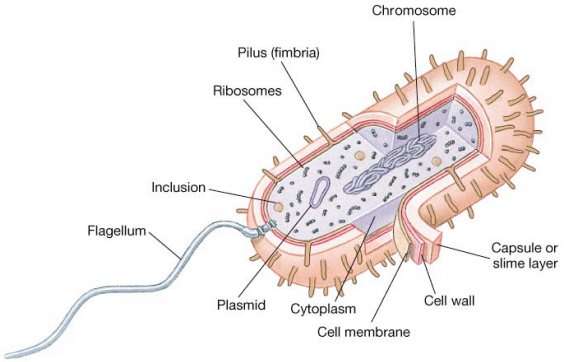
Anabolism

- Anabolic pathways consume energy to build complex molecules from simpler ones (eg protein synthesis from amino acids)
- Thermodynamically uphill (in general)

What is Needed to Make a Microbe

(as an example for cells that can make everything from one C source)

Typical Cell Composition



Minimal medium

- **5 g/L Glukose**
- 6 g/L Na_2HPO_4
- 3 g/L KH_2PO_4
- 0.5 g/L NaCl
- 1 g/L NH_4Cl
- 2 mM $MgSO_4$
- 25 µM $CaCl_2$
- Spurenelemente (Fe, Co, Cu, Mn, Zn, Mo)
- evtl. Vitamine & Aminosäuren

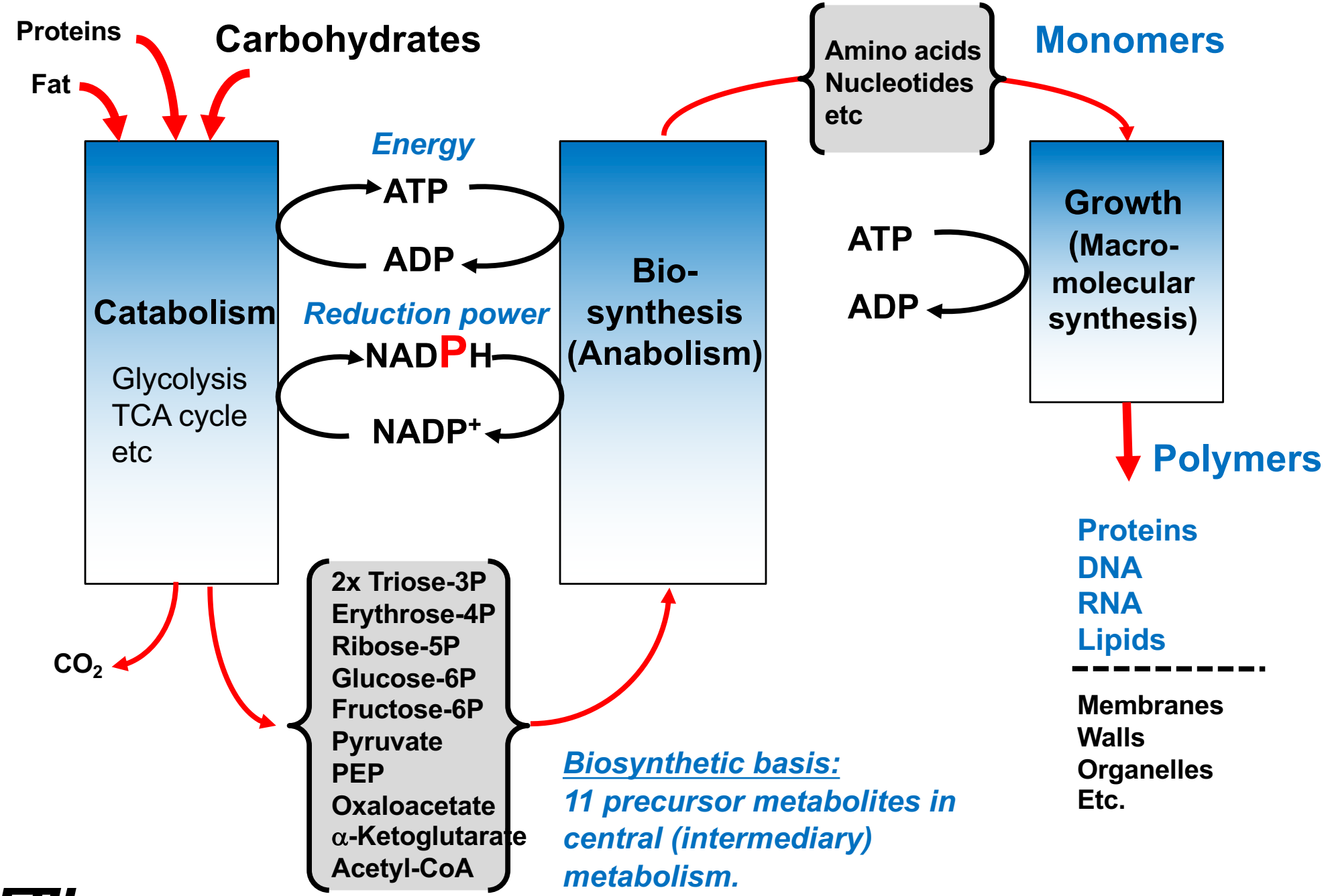
11 carbon precursor molecules and 3 cofactors

Chemical composition

- 46-52% C
- 20-30% O
- 10-14% N
- 8 % H
- 2-3% P
- 1-4<% K
- 0.2-1% S
- 0.01-1% Ca,Mg,Cl
- 0.02-0.2% Fe
- trace elements
- 4-10% ash

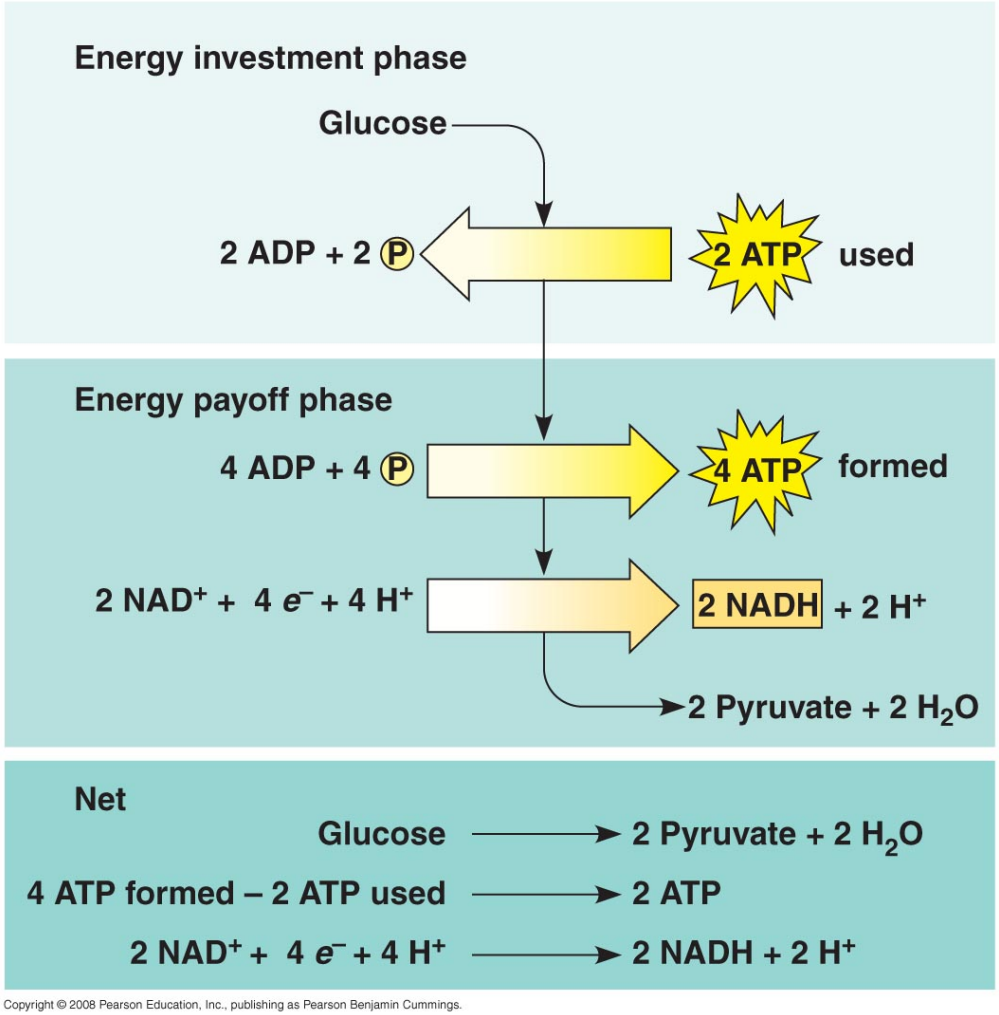
Macromolecular Composition

- 40-60% protein
- 15-30% RNA
- 3% DNA
- 10% lipids
- 0-5% lipopolysacch.
- 0-10% murein
- 0-5% glykogen
- 2-10% soluble metabolites
- 0.5-3% salts


Proteins
Fat
Carbohydrates
Catabolism
Glycolysis
TCA cycle
etc
Energy
ATP
ADP
Reduction power
NADPH
NADP+
Bio-
synthesis
(Anabolism)
Amino acids
Nucleotides
etc
Monomers
ATP
ADP
Growth
(Macro-
molecular
synthesis)
Polymers
Proteins
DNA
RNA
Lipids
Membranes
Walls
Organelles
Etc.
CO2
2x Triose-3P
Erythrose-4P
Ribose-5P
Glucose-6P
Fructose-6P
Pyruvate
PEP
Oxaloacetate
α-Ketoglutarate
Acetyl-CoA
Biosynthetic basis:
11 precursor metabolites in
central (intermediary)
metabolism.

Key Central Metabolic Pathways: Glycolysis

- Glycolysis ("splitting of sugar") breaks down glucose into two molecules of pyruvate
- Has two major phases:
  - Energy investment phase
  - Energy payoff phase
- The immediately generated ATP comes from substrate level phosphorylation !



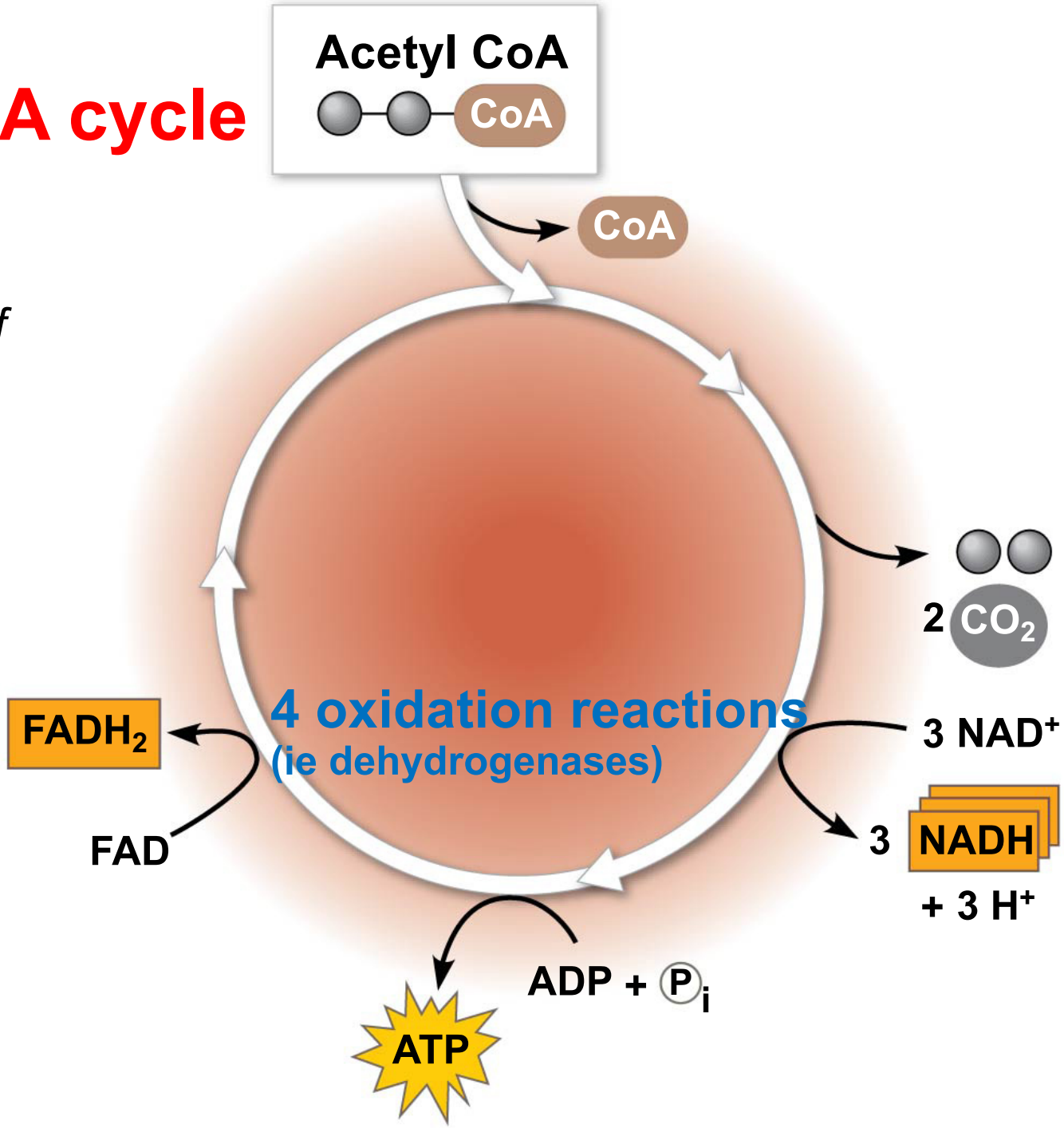
1 glucose ->

2 pyruvate
2 NADH
2 ATP

Key Central Pathways: TCA cycle

Complete oxidation to CO_2 and generation of reducing equivalents

Acetyl-CoA ->
- **2 CO_2**
- **1 ATP**
- **3 NADH**
- **1 $FADH_2$**



Complete Aerobic Degradation of Glucose

Glycolysis	*Pyruvat dehydrogenase*	*Tricarboxylic acid cycle*	
Glukose ⟶	2 Pyruvat **2 ATP** **2 NADH** ⟶	2 Acetyl-CoA **2 CO_2** **2 NADH** ⟶	**4 CO_2** **2 ATP** **6 NADH** **2 $FADH_2$**

Glucose -> 6 CO_2 $\Delta G^{0'}$ -2870 kJ/mol
ADP + P -> ATP $\Delta G^{0'}$ +30.5 kJ/mol

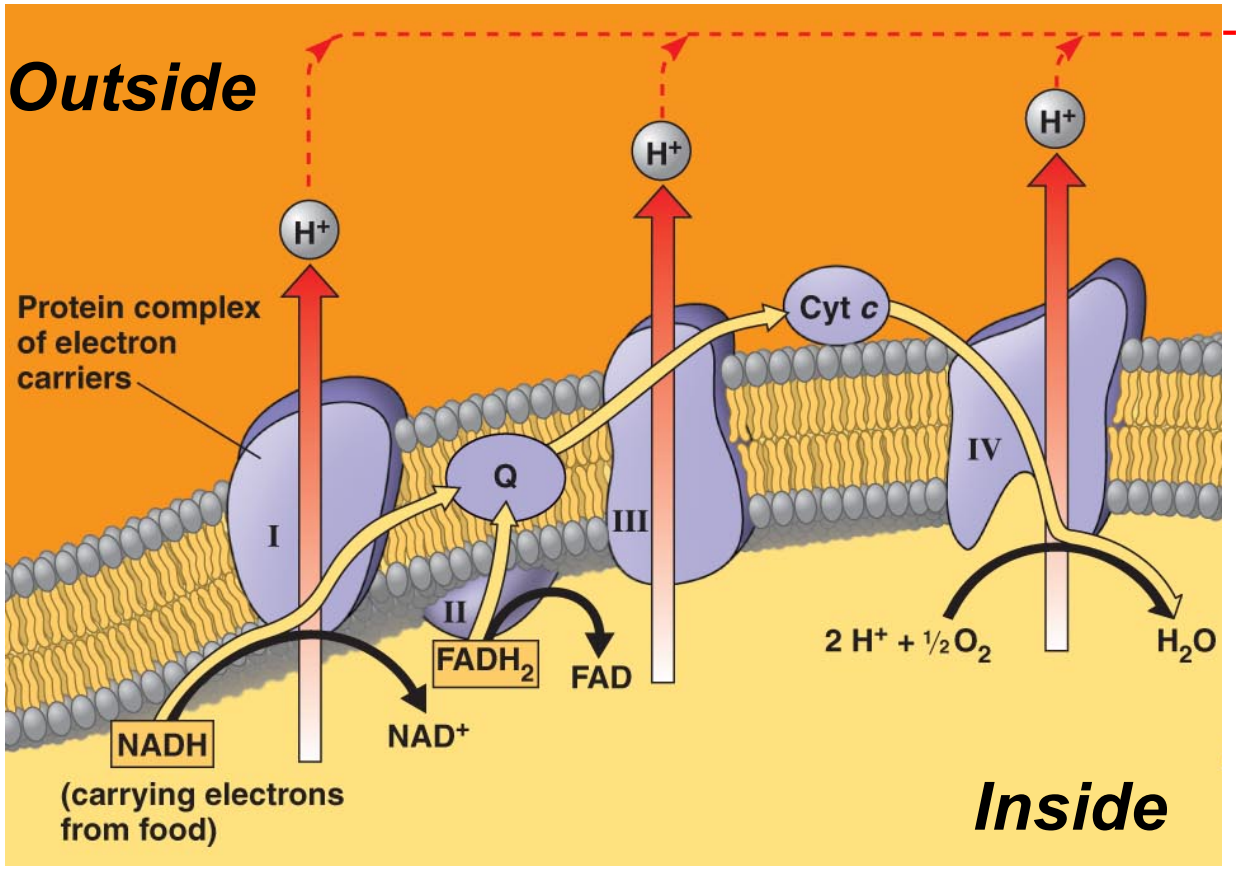
-2870 + 4x 30.5 -> -2748 kJ/mol still unexploited!

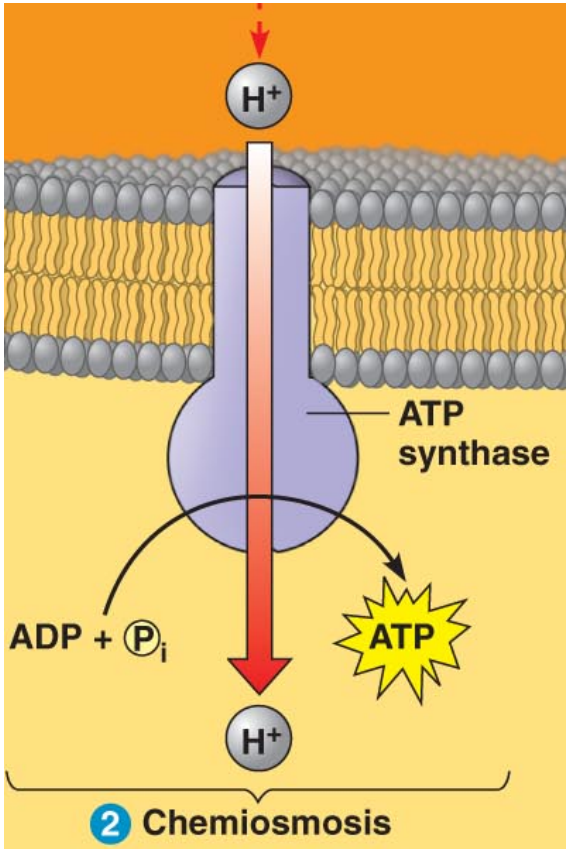
How many ATP can be made from one NADH?

Respiration (ie oxidative phosphorylation): Electron Transport Chain and ATP Synthase

Electron transport chain: electron transfer to oxygen and generation of proton gradient

Conversion of proton gradient into ATP formation





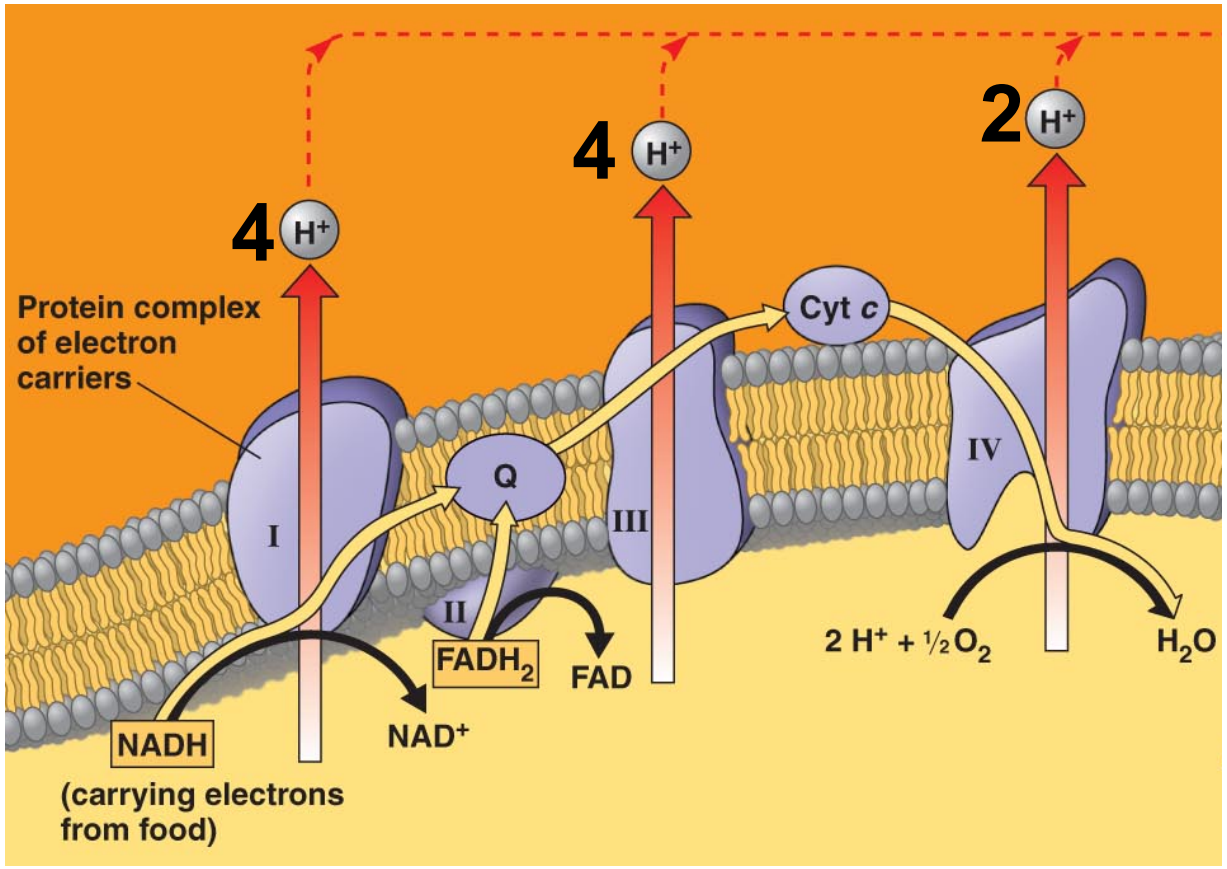
Stoichiometry of Oxidative Phosphorylation

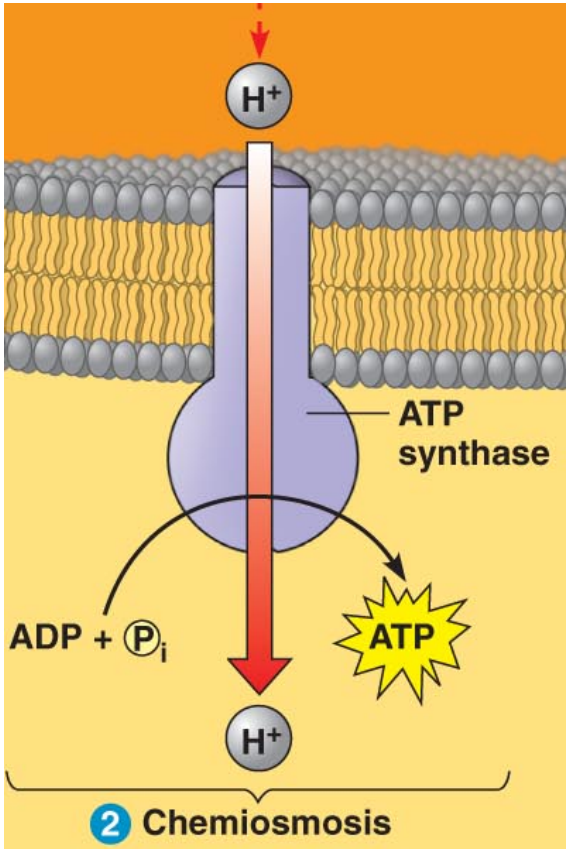
10 H^+ are maximally exported during respiration.

Bacteria have fewer coupling sites!

P-to-O ratio
ATP per Oxygen atom
Maximum 3

3-4 protons must be translocated to generate an ATP



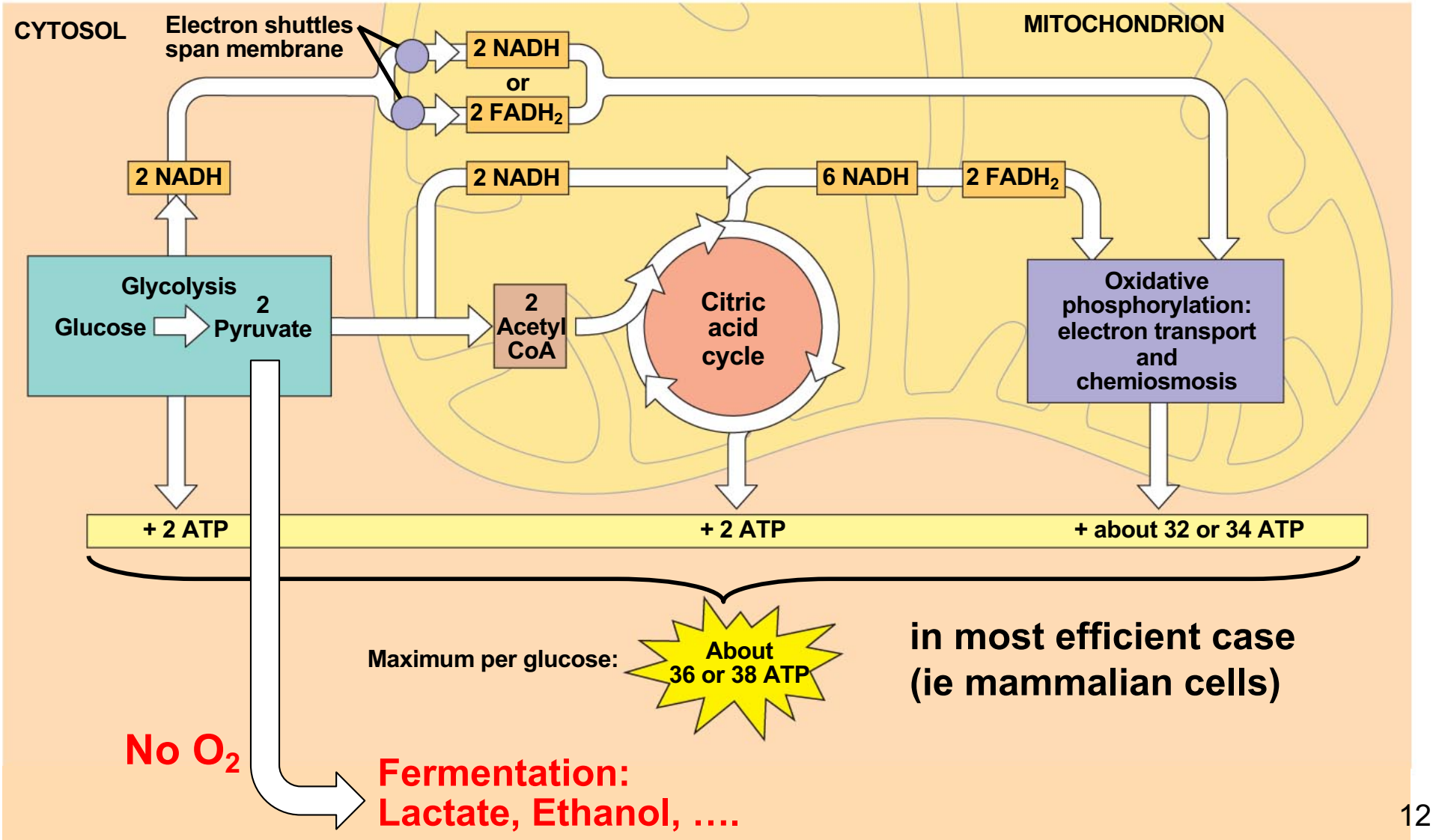


Accounting of ATP Production by Cellular Respiration

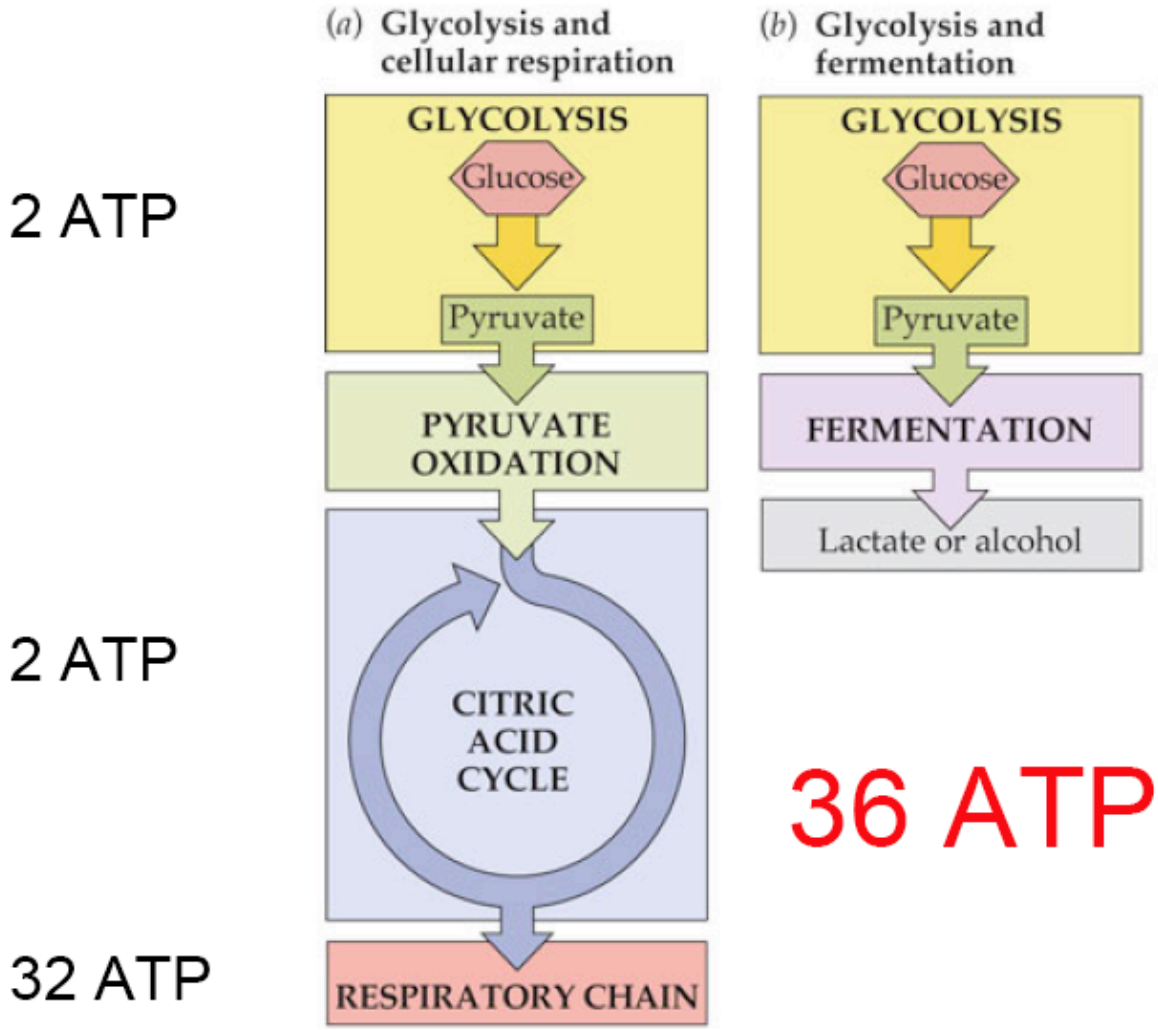
1 NADH → 10 H^+
1 FADH → 8 H^+

3-4 H^+ → 1 ATP

1 NADH → 3 ATP (2.5 – 3.3)
1 FADH → 2 ATP



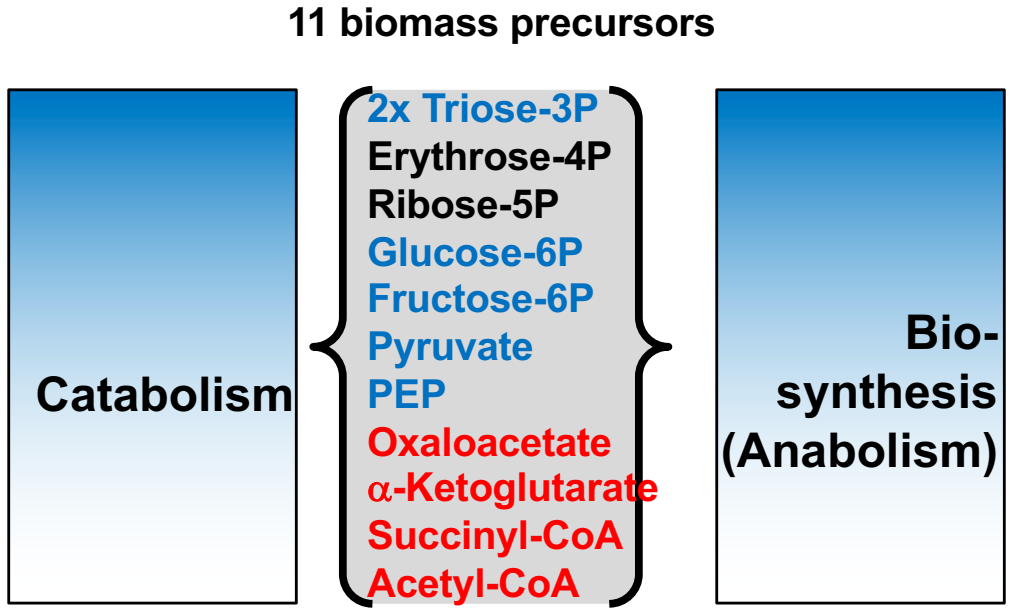
Energetic Consequence of Different Modes of Metabolism

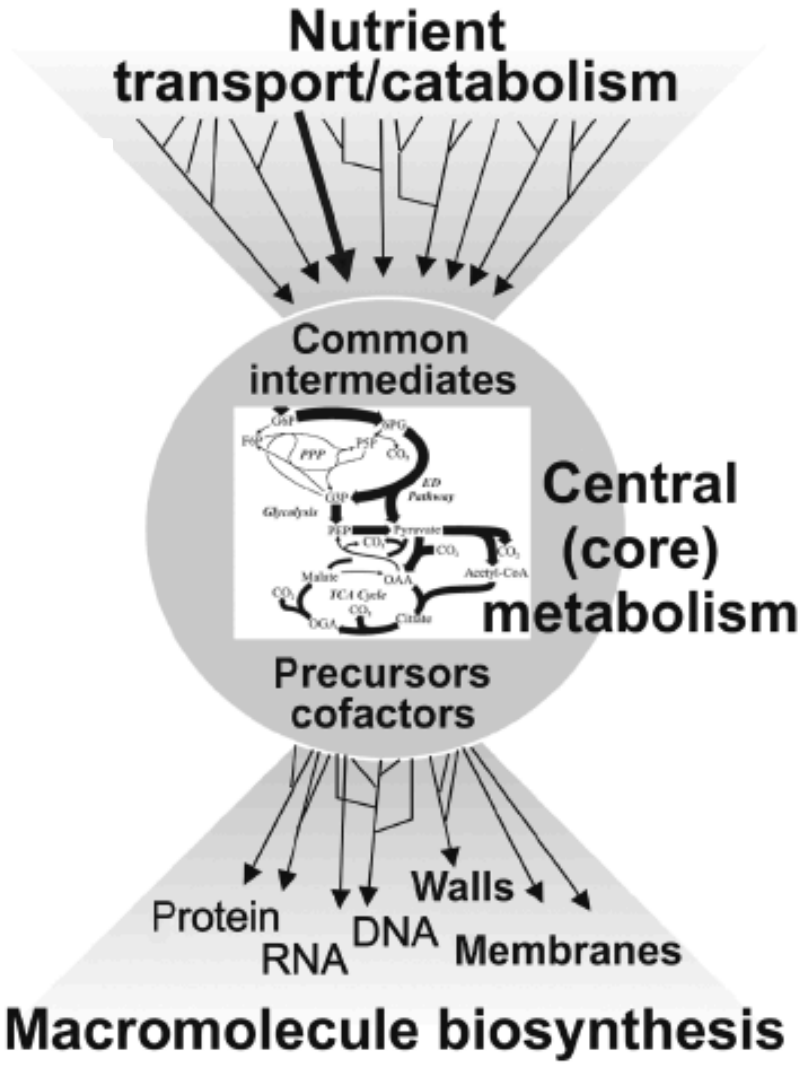


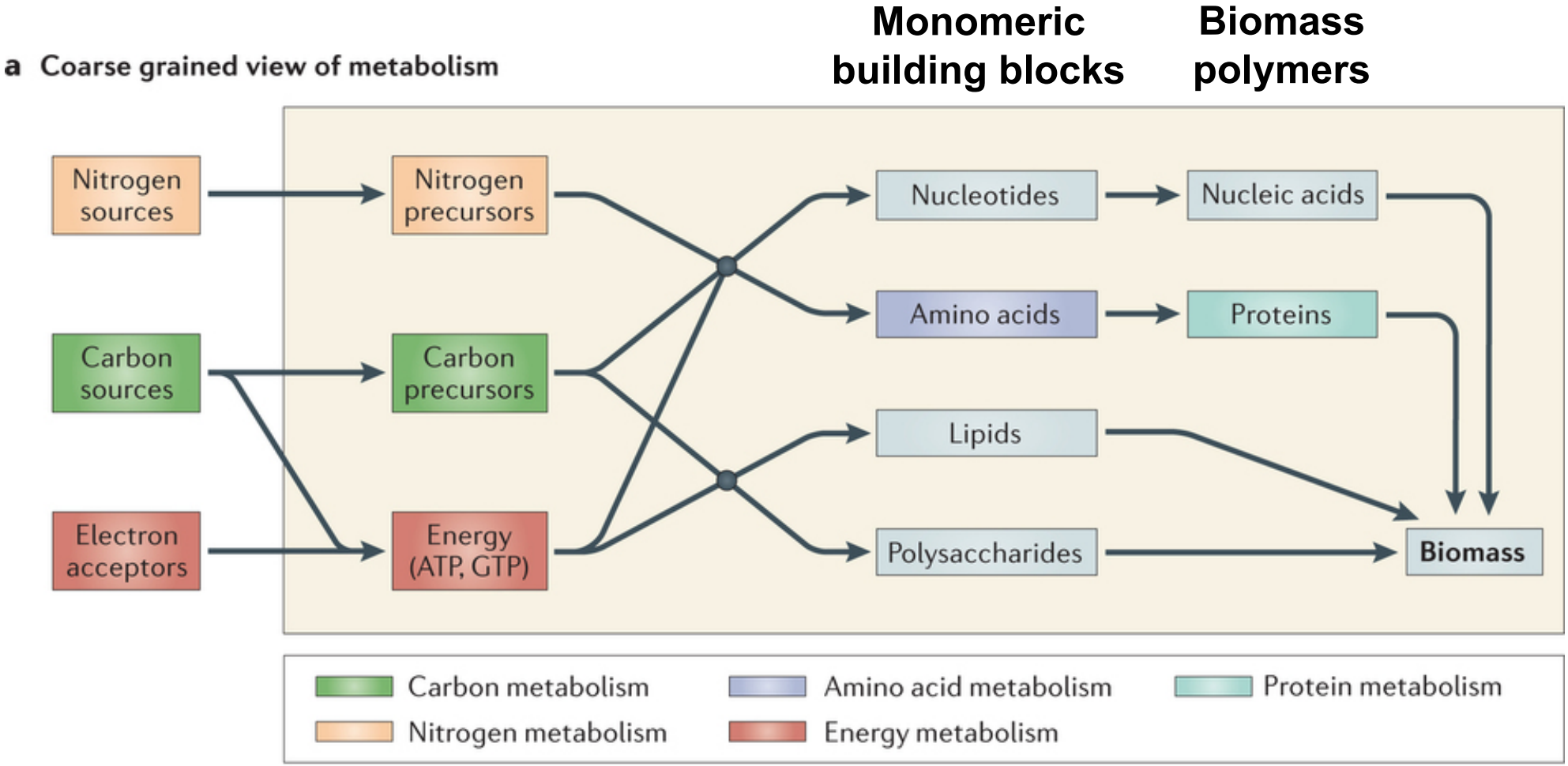
Even with O_2:
Tumors
Yeast

Glycolysis & TCA Cycle: At the Crossroads of Metabolism

- Glycolysis and the TCA cycle are **THE** key pathways of central (or intermediary) metabolism







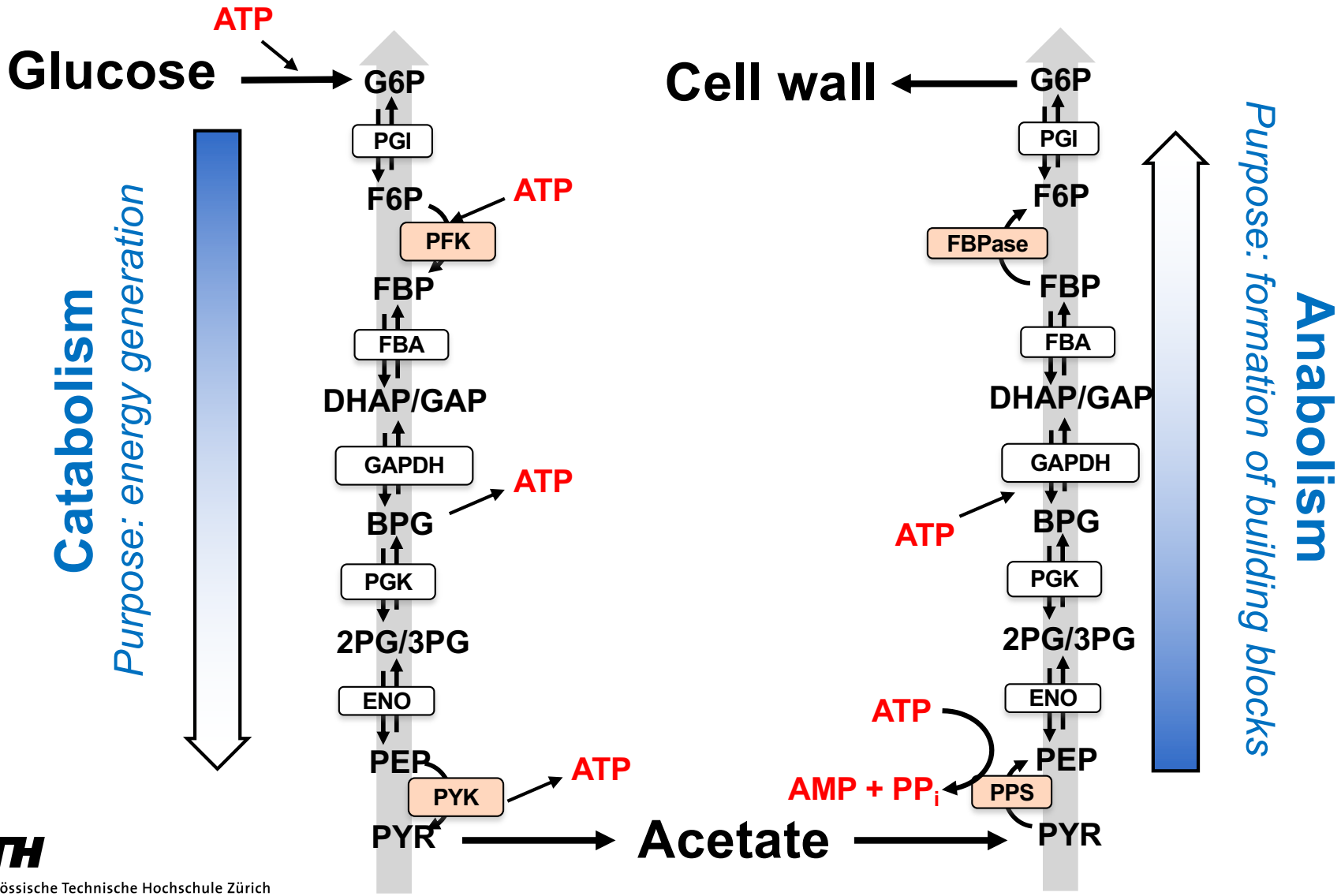
Chubukov et al, 2014 Nature Microbiology Reviews

Take Home Message: Structure of Metabolism is “Simple”

- Catabolic down hill reactions bring various nutrients into central metabolism.
- Anabolic uphill reactions synthesize cellular buildings blocks from 11 central intermediates *(driven by catabolically generated ATP)*.
- Central metabolism is the flexible core of it all, able to run into different directions depending on the enviromental conditions.

Central Metabolism Must be Flexible: Glycolysis vs Gluconeogenesis

Mostly the same enzymes, but uphill requires energy …..



How is all of this coordinated?

What Matters for a Reaction Flux?

$$[E] \bullet k_{cat} \bullet \frac{[S]}{k_m + [S]} = \text{reaction rate (=flux)}$$

Enzyme conc

Kinetic parameter

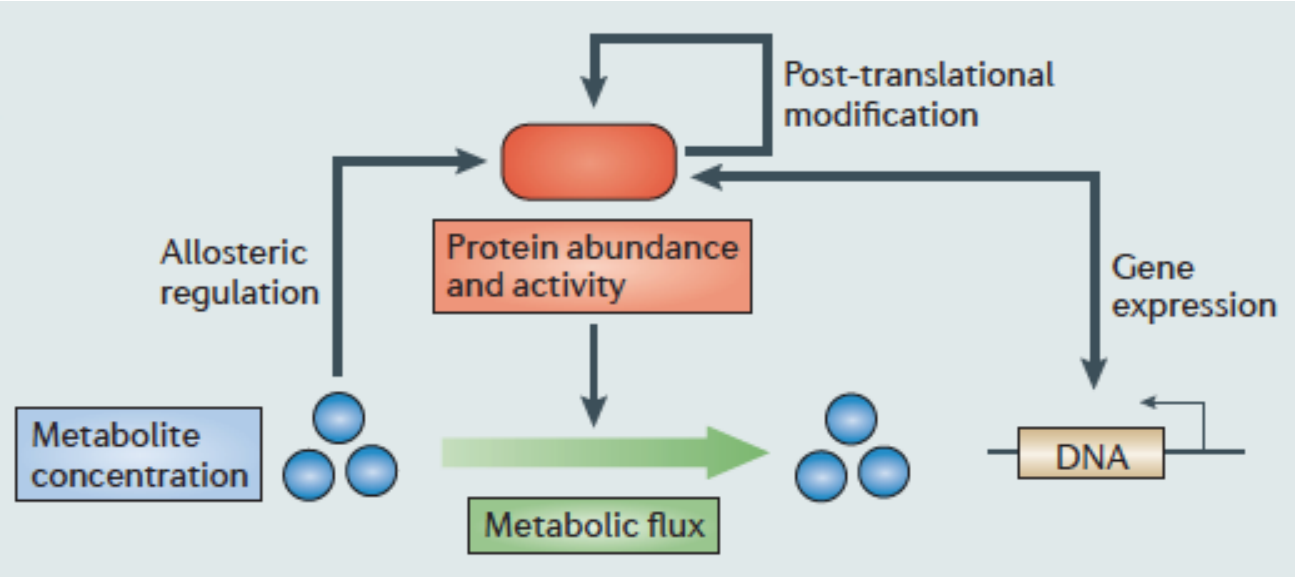
K_{cat} – turnover

k_m - affinity

Reactant concentration [S]

How can cells affect those variables and parameters?

How Cells Influence Reaction Fluxes ?



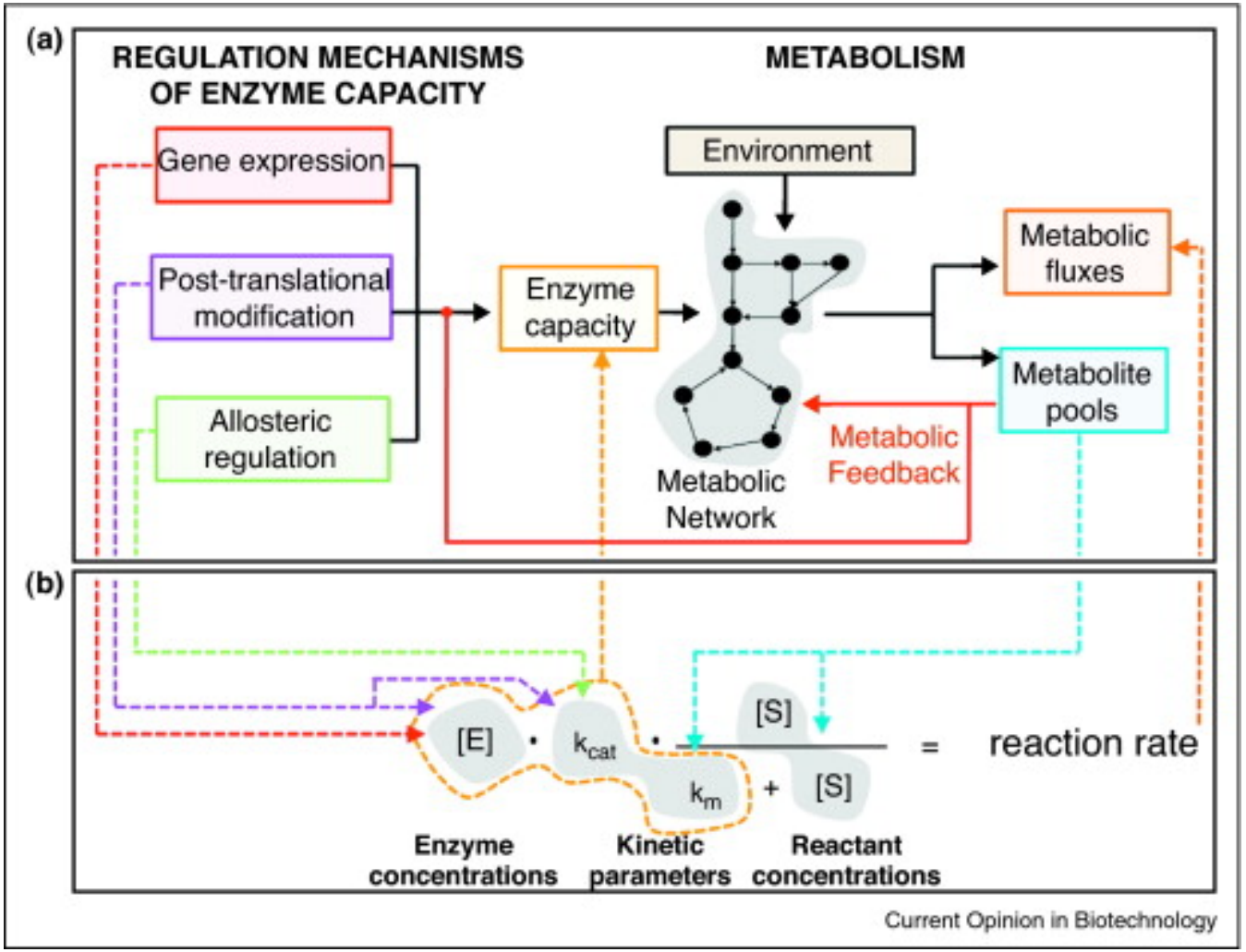
Chubukov et al, 2014
Nature Microbiology Reviews

Given the relatively slow response time and stochastic nature of transcription and translation events, expression of individual enzymes is not fine-tuned in accordance to changes in metabolic flux, but rather to set the scope of possible fluxes (Kochanowski, Sauer and Noor 2015 Curr Opin Microbiol)

Which fluxes are realized depends more on enzyme kinetics & regulatory small molecule-protein interactions: either through stable covalent binding (post-translational modification) or through fleeting non-covalent interactions (allostery).

In Networks All is Intertwined

Gerosa and Sauer, Curr Opin Biotechnol 2011



We still cannot understand network behavior from the individual (parameterized) reactions

Key Experimental Methods for Metabolic Research in Sys Bio

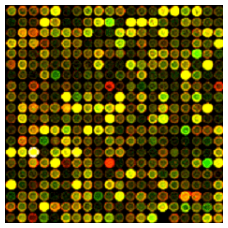
Classically it was enzymology;
ie in vitro *enzyme assays*

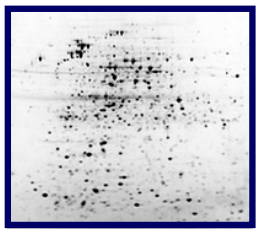
- **Proteomics**
  (or transcriptomics since easier)
- **Metabolomics**
- **Flux analysis**

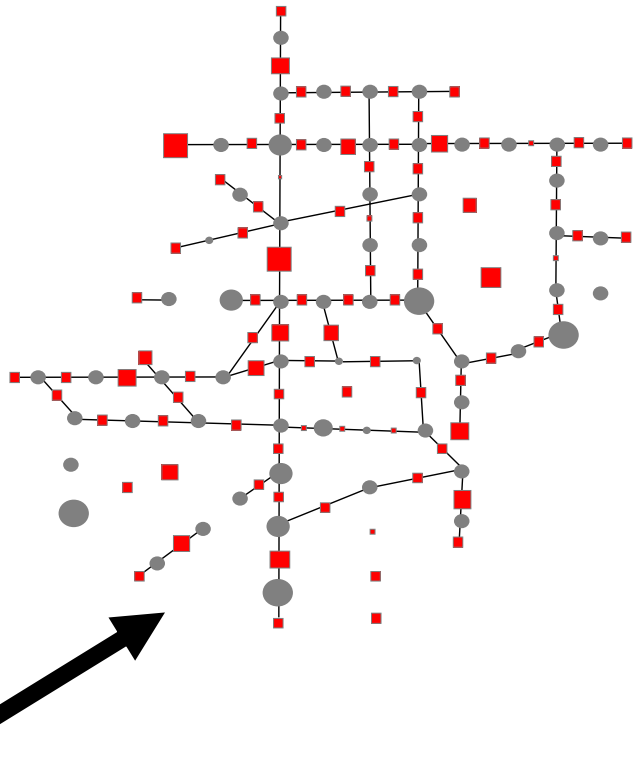
Mass spectrometry the key enabling technology!

Experimental Analysis of Metabolic Networks as Systems

Network composition [c]



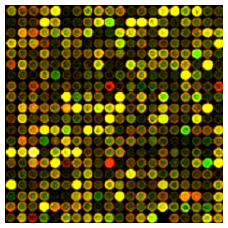


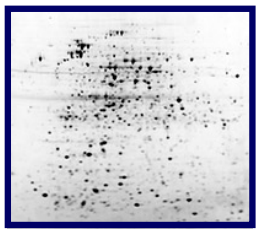


Transcriptome indirect
Proteome direct
Metabolome direct

Experimental Analysis of Metabolic Networks as Systems

Network composition [c]





Transcriptome indirect
Proteome direct
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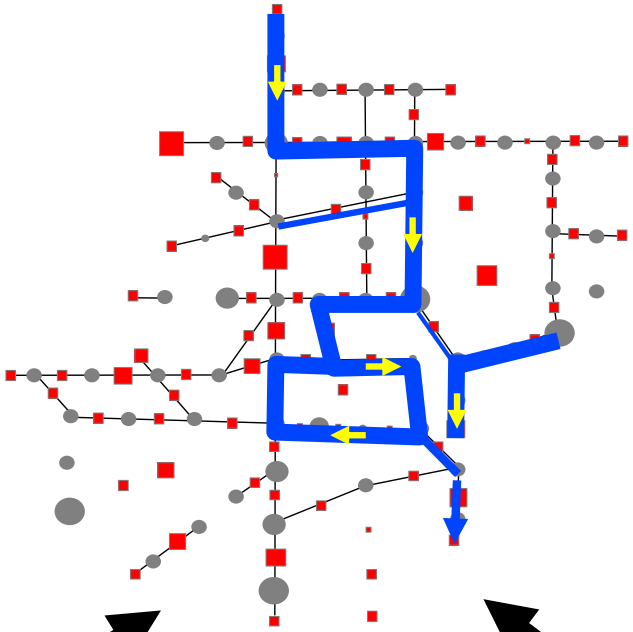


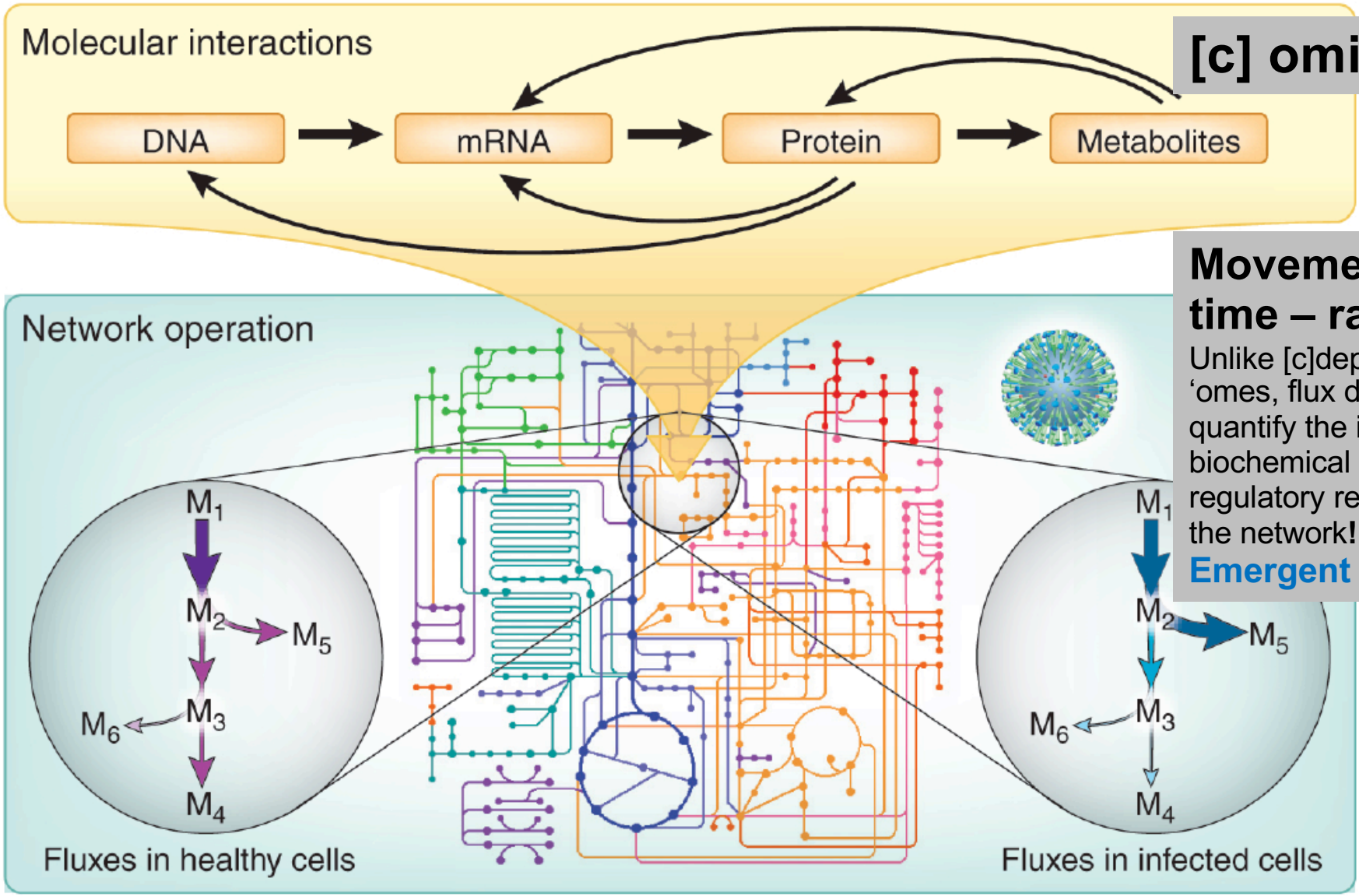
Network operation

[mmol / g_{cells} h]

‘Fluxes‘

Systems-level *in vivo* activity

From Components to the Functional State:
Metabolic Flux Analysis



[c] omics

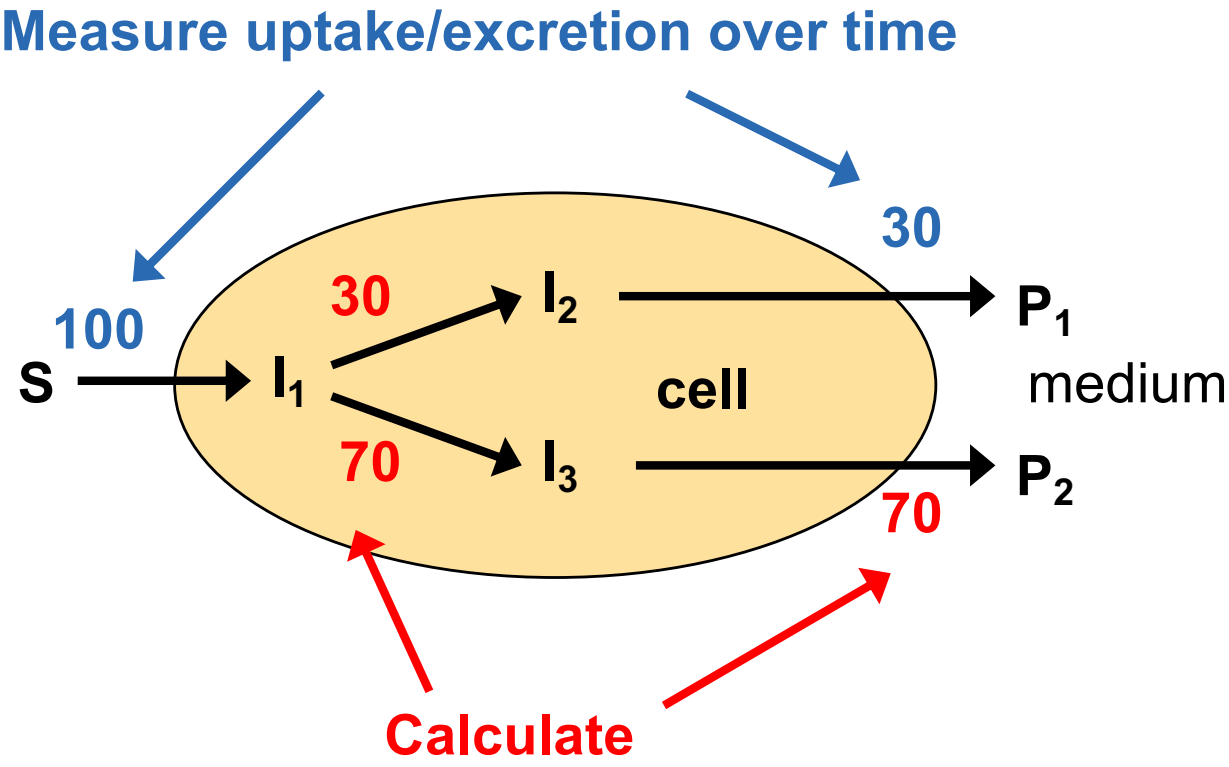
Movement in time – rates

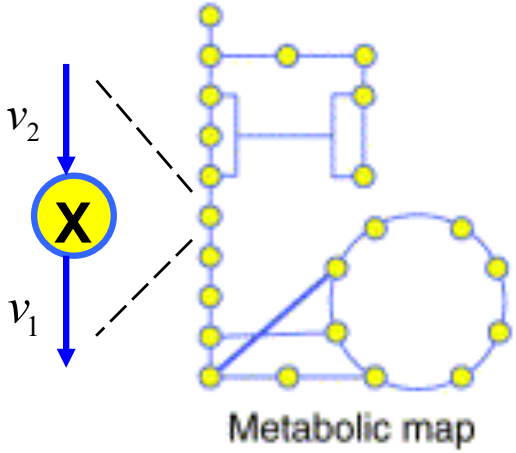
Unlike [c]dependent 'omes, flux data quantify the integrated biochemical & regulatory response of the network!

Emergent Property

Sauer & Zamboni 2008. Nature Biotech

Infer a Non-Measurable Quantity
Stoichiometric Flux Analysis





Mass balance around metabolite *X*

$$\frac{dX}{dt} = -1 \cdot v_1 + 1 \cdot v_2 = 0$$

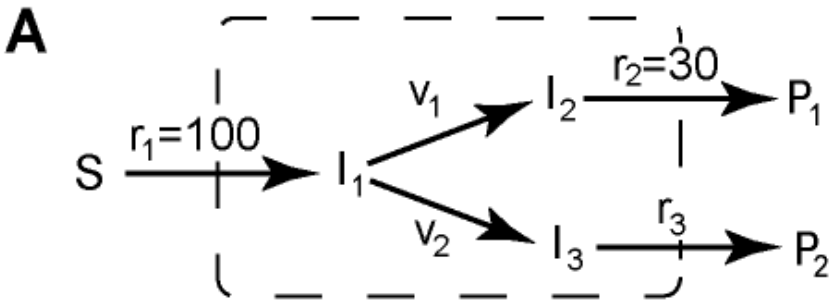
↑

Steady state assumption

What could happen if not in steady state?

Assumption: Steady state for intracellular metabolite concentrations

The Mathematical Framework



In pseudo steady-state:

$$\begin{pmatrix} S \\ R \end{pmatrix} \bullet v = \begin{pmatrix} 0 \\ r_m \end{pmatrix}$$

Where:

$$\begin{pmatrix} S \\ R \end{pmatrix} = \begin{array}{c} \begin{matrix} S & I_1 & I_2 & I_3 & P_1 & P_2 \end{matrix} \\ \begin{pmatrix} 0 & -1 & 1 & 0 & 0 & 0 \\ 0 & -1 & 0 & 1 & 0 & 0 \\ -1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & -1 & 0 & 1 & 0 \\ 0 & 0 & 0 & -1 & 0 & 1 \end{pmatrix} \end{array} \qquad v = \begin{pmatrix} v_1 \\ v_2 \\ r_1 \\ r_2 \\ r_3 \end{pmatrix}$$

Allowed residuals in reaction reactants

$$\begin{pmatrix} 0 \\ r_m \end{pmatrix} = \begin{pmatrix} 0 \\ 0 \\ 100 \\ 30 \\ ? \end{pmatrix}$$

v – intracellular flux

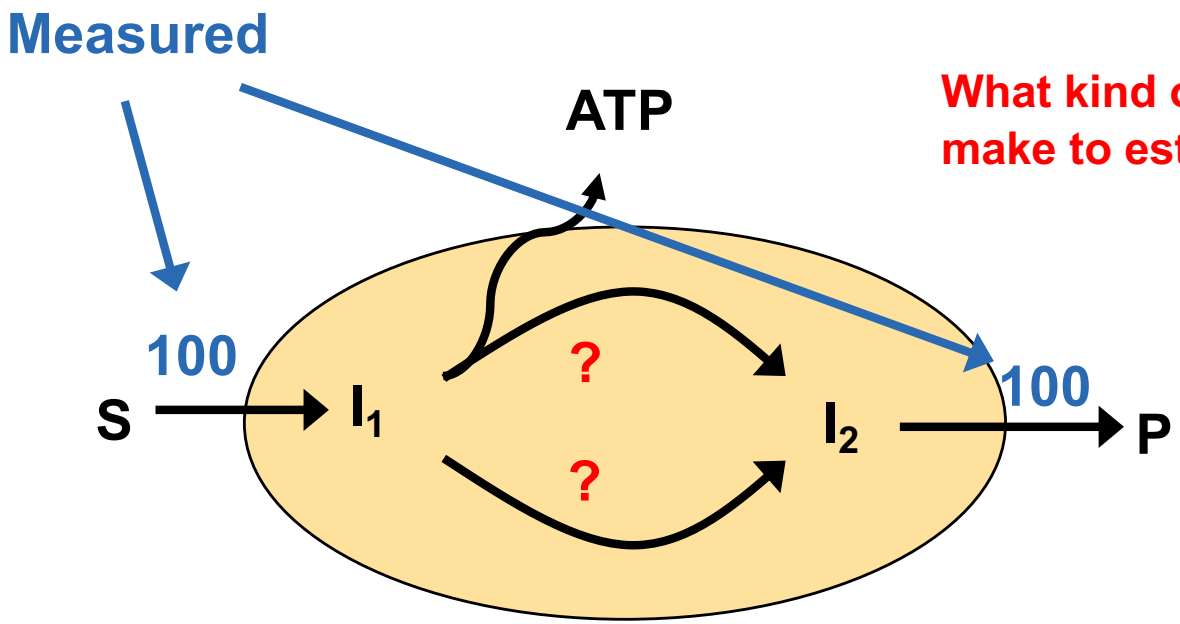
r – extracellular (exchange) flux

S – stoichiometric matrix

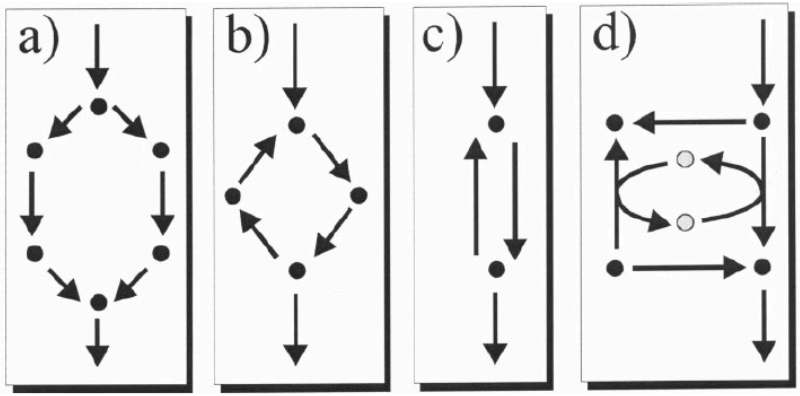
R – reactant residuals

$$v = \text{pinv}\begin{pmatrix} S \\ R \end{pmatrix} \bullet \begin{pmatrix} 0 \\ r_m \end{pmatrix} \Rightarrow \begin{pmatrix} v_1 \\ v_2 \\ r_1 \\ r_2 \\ r_3 \end{pmatrix} = \begin{pmatrix} 30 \\ 70 \\ 100 \\ 30 \\ 70 \end{pmatrix}$$

Why don't we just rely on this computational analysis?



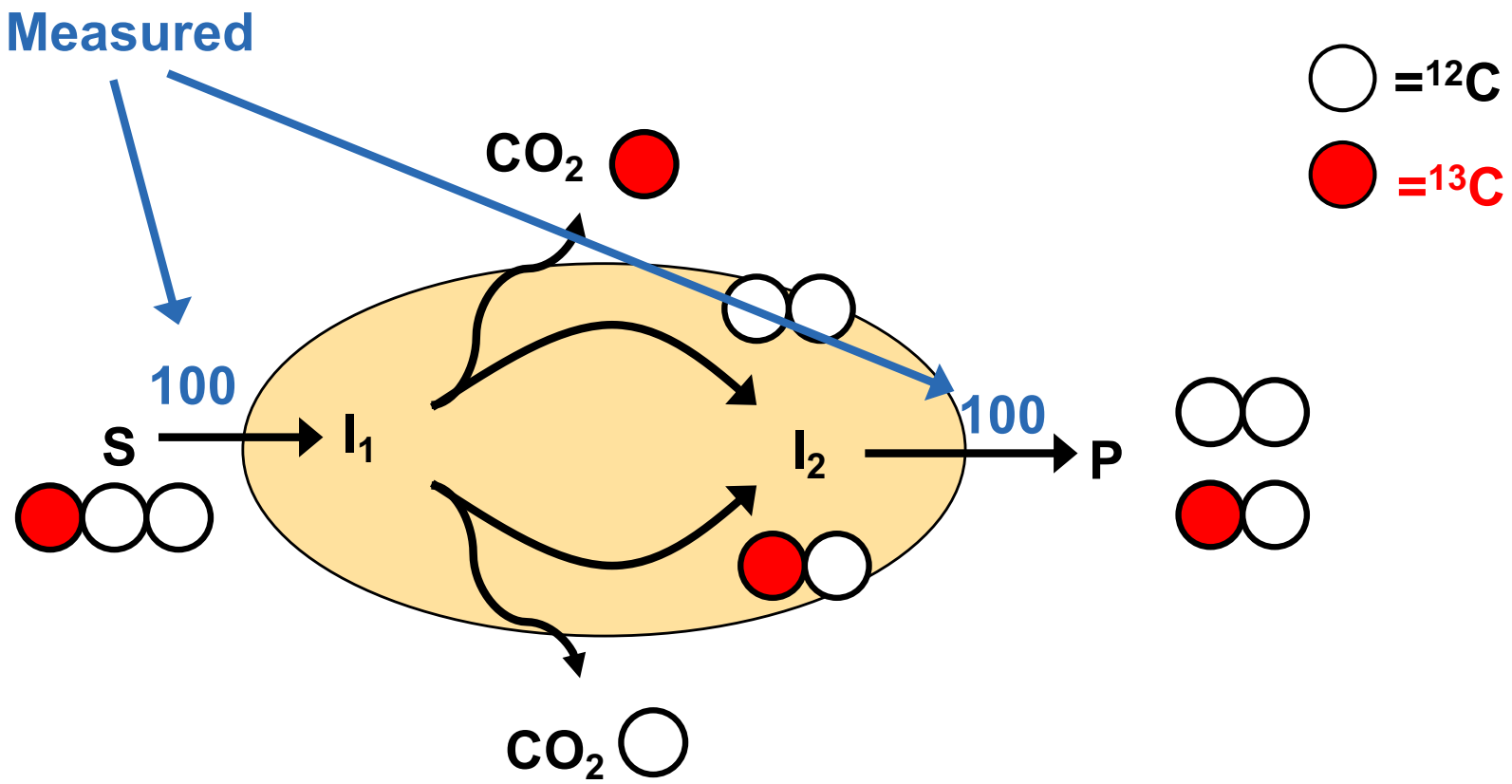
What kind of assumptions could we make to estimate the 2 reactions?



Typical situations where stoichiometric flux analysis fails: (a) parallel pathways without any related flux measurement, (b) certain metabolic (futile) cycles, (c) bidirectional reactions, and (d) split pathways when cofactors (gray circles) are not balanced.

To deal with such **underdetermined systems**, typically strong biological assumptions are made (e.g. on ATP production, cofactor balancing or cellular objective), which severely limit the value of obtained results.

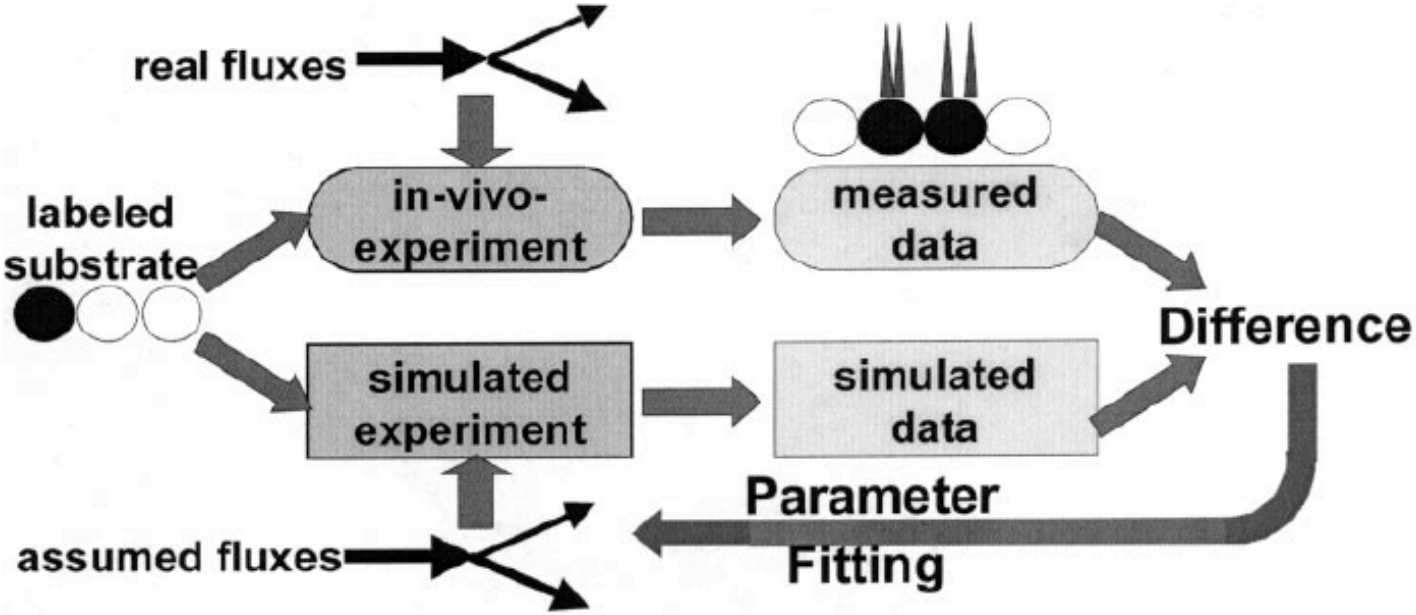
The Basics: ^{13}C-based MFA



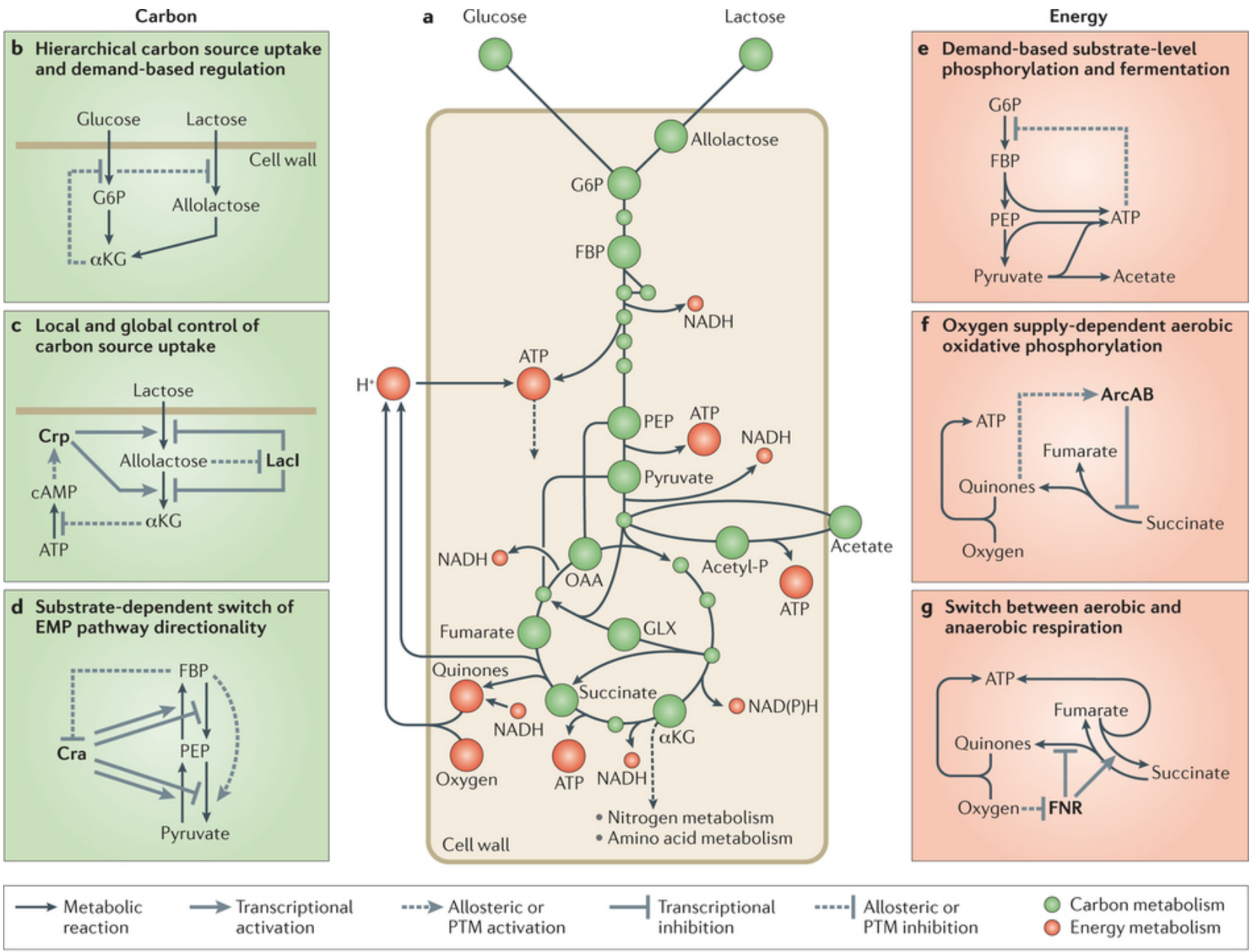
Iterative Isotopologue Balancing

How to fit fluxes to data ?

Using a reaction and atom resolved model of metabolism



- Global isotopologue balancing:
  - provides a single flux solution for the network
  - provides flux number for each reaction in the network
  - is computation intensive (non linear problem)



Chubukov et al, 2014 Nature Microbiology Reviews

Summary

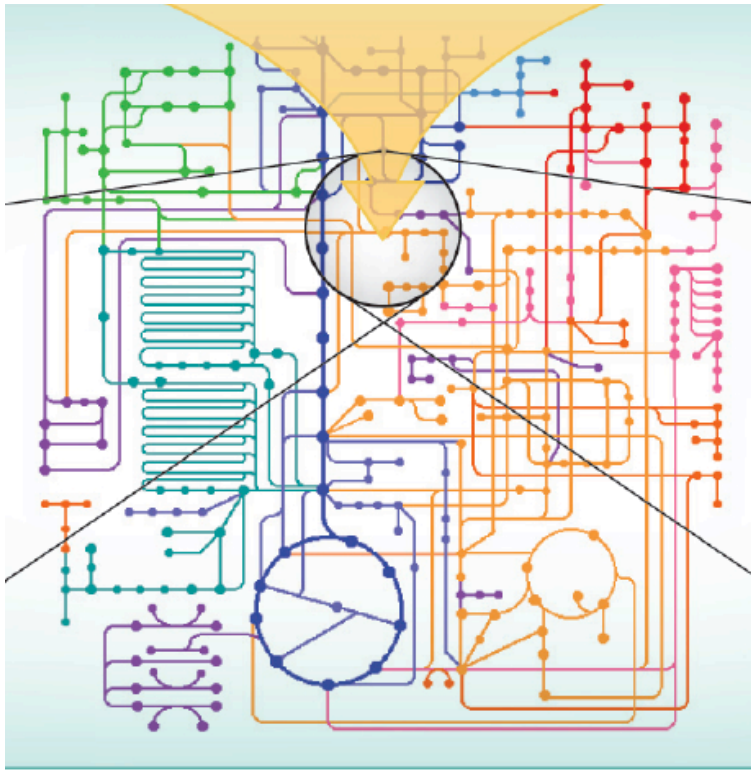
- Metabolic networks are structured into mostly linear anabolic (& catabolic) pathways and an interconnected and flexible central metabolism.
- Most energy is conserved in redox cofactors which respiration converts into ATP (with varying stoichiometries!).
- Flux regulation: gene expression sets more the scope, while PTMs and metabolite binding actually regulate the fluxes.
- Intracellular fluxes cannot be measured, but need to be inferred computationally. Some methods exploit isotopic tracing for higher confidence inference.

Central Metabolism and Growth-Lag Tradeoffs

Uwe Sauer, ETH Zurich

- **Allosteric regulation topology**
- **Growth-lag tradeoff**

Metabolism



After decades of discovery and detailed mechanisms: metabolism is the furthest in terms of knowledge.

Not only a workhorse network but also an internal ***signal generator*** *!*

Metabolite-protein interactions are the basis of these signals and drivers of network coordination

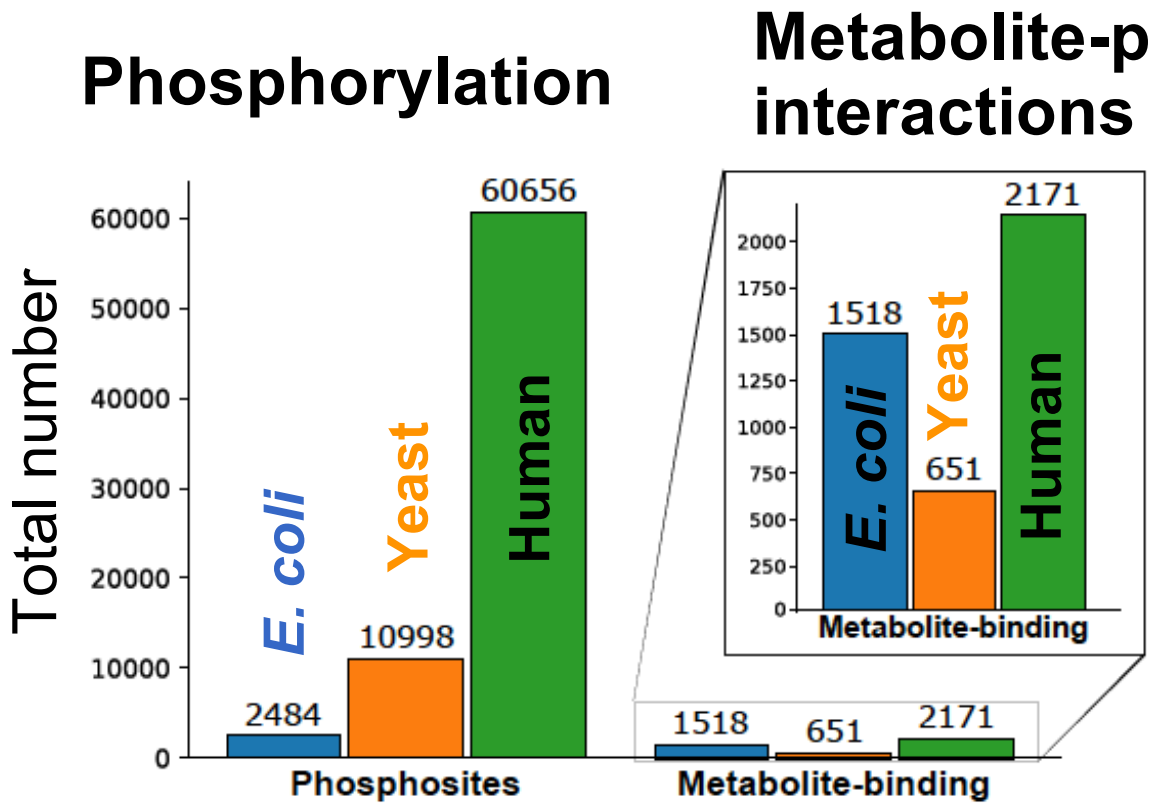
Non-Covalent vs Covalent (Post-Translational) Metabolite Regulation

- Both are fast and reversible
- But PTMs can achieve lasting changes and combinatorics, however, at a cost of additional, modifying enzymes

Hypothesis: non-covalent metabolite-protein interactions are are more ancient and mainly used to regulate **conserved biological processes that maintain cellular homeostasis**, for example metabolism and transcription - in Pro- and Eukaryotes alike.

Non-Covalent vs Covalent (Post-Translational) Metabolite Regulation

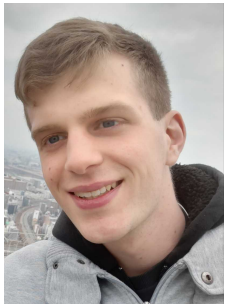
in Enzymes of Pro- and Eukaryotes



Non-covalent interactions are equally abundant across kingdoms

Average phosphorylation:
1.7 in E. coli and 36 in human reactions

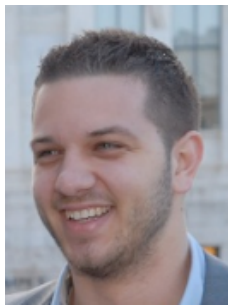
Chris Gruber



What Is Our Present Knowledge of Metabolite-Enzyme Regulation?

- In contrast to metabolic networks, there is no equivalent regulation network let alone a model .....
- Developed a framework leveraging the vast, but fragmented, biochemical literature to reconstruct metabolite-protein regulatory network
- Genome-scale architecture of *E. coli* small molecule regulatory network:
  - **1,669 unique regulatory interactions** between 321 metabolites and 364 enzymes
  - **> 83% interactions are inhibitory**
  - **≈ 50% of enzymes regulated**

Collaboration with Ed Reznik & Daniel Segre
Boston University

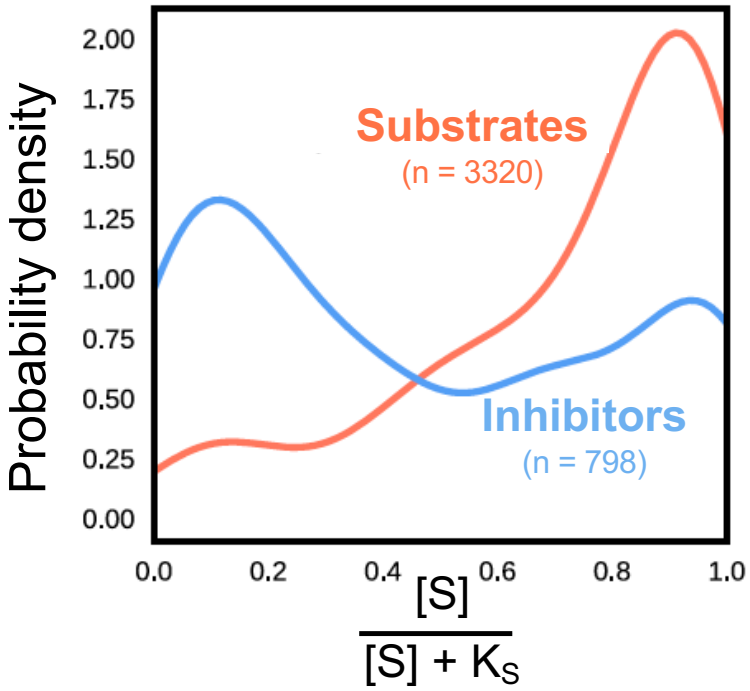


Dimitris Christodoulou

Elad Noor

Combining metabolomics data from many conditions with metabolic control analysis, we showed:

- fundamental trade-off between regulation and enzymatic activity,
- an inherent cost associated with metabolite regulation,
- **Under physiological conditions, most enzymes operate at or near saturation.**

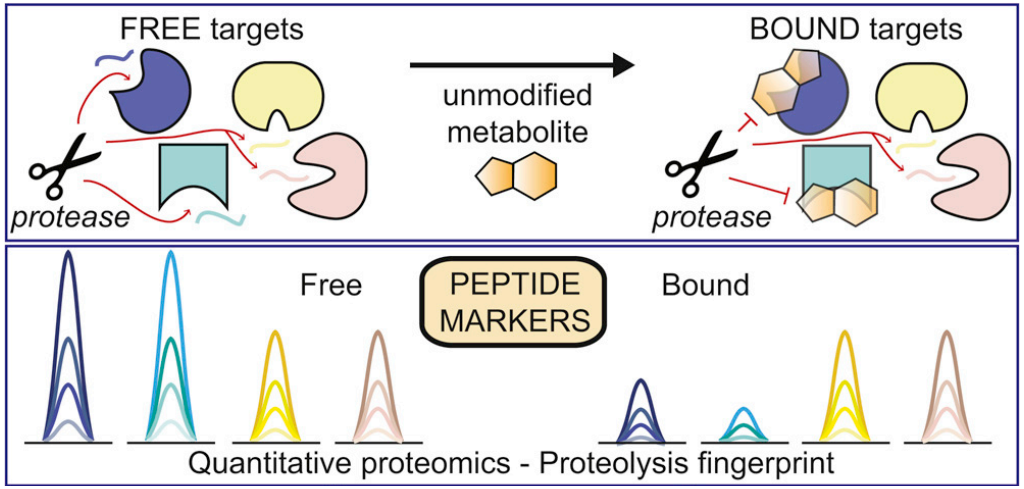


$K_S = K_M$ or K_I

A Chemoproteomic Map of Metabolite-Protein Interactions

A method developed by the Picotti Lab @ ETH

Limited proteolysis **w/o** metabolites



Limited proteolysis **with** metabolites

Captures different interactions:
- catalytic
- allosteric
- protein complex

E. coli interactome atlas for 20 metabolites
(CCM, 4 amino acids, 7 nucleotides, cAMP)

⇨ **1678 interactions; ¾ unknown (!)**

Chemoproteomic Mapping of Metabolite-Protein Interactions

collaboration with Picotti Lab @ ETH

Strength

- Proteome-wide discovery !!!
- All types of interactions (… but regulatory ones need to be inferred indirectly)

Weakness

- Metabolite centric: no comprehensive mapping of a specific subnetwork

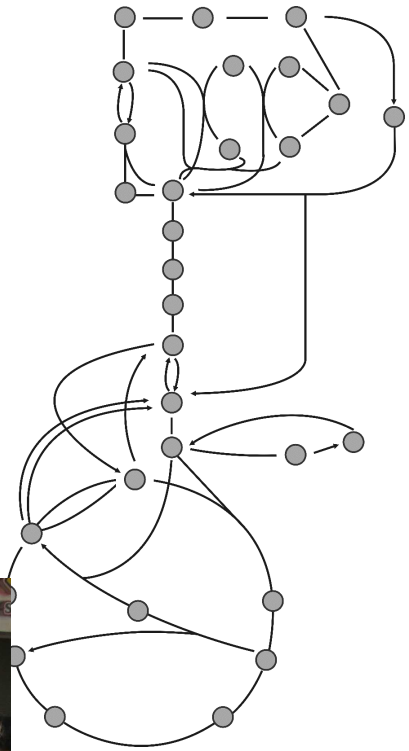
How deep is our knowledge of regulatory metabolite-protein interactions ?

How Many Met-Prot Interactions are in Central Metabolism?

collaboration with Allain Lab @ ETH

Purified 29 central enzymes of *E. coli*

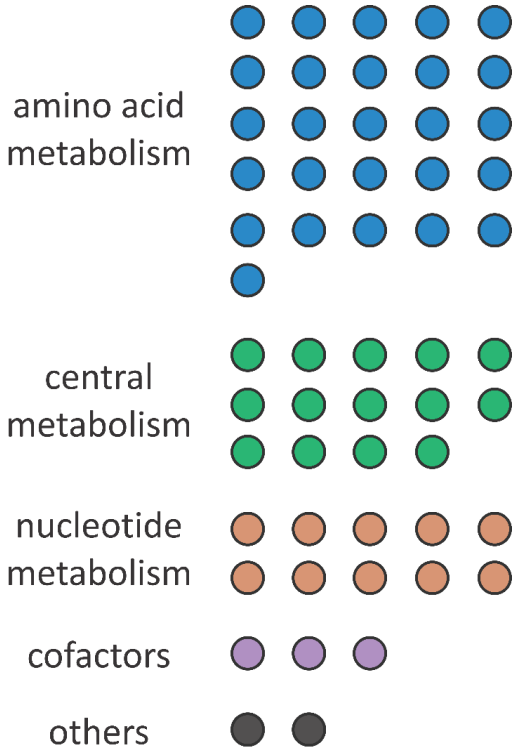
no isomerases, no hetero complexes



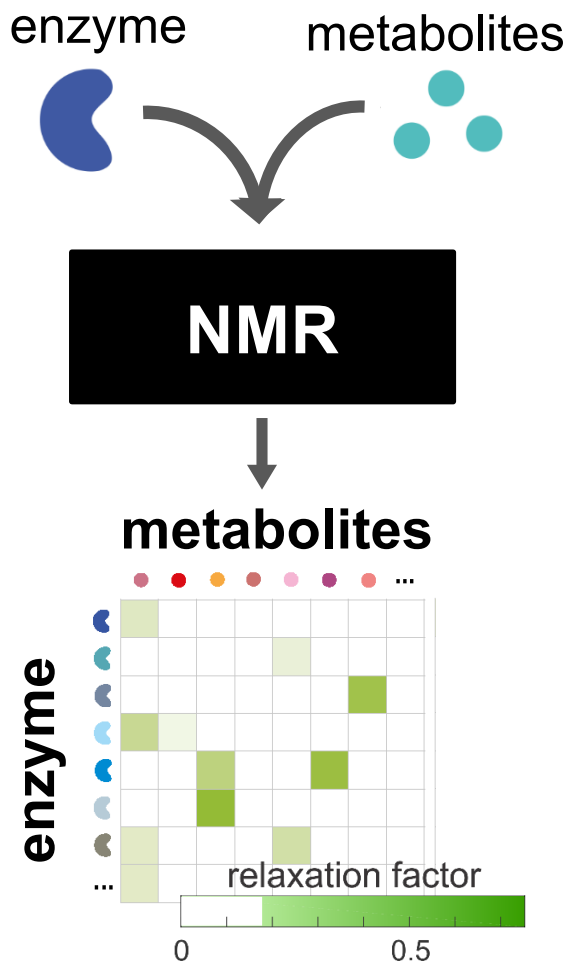
Maren Diether

55 metabolites

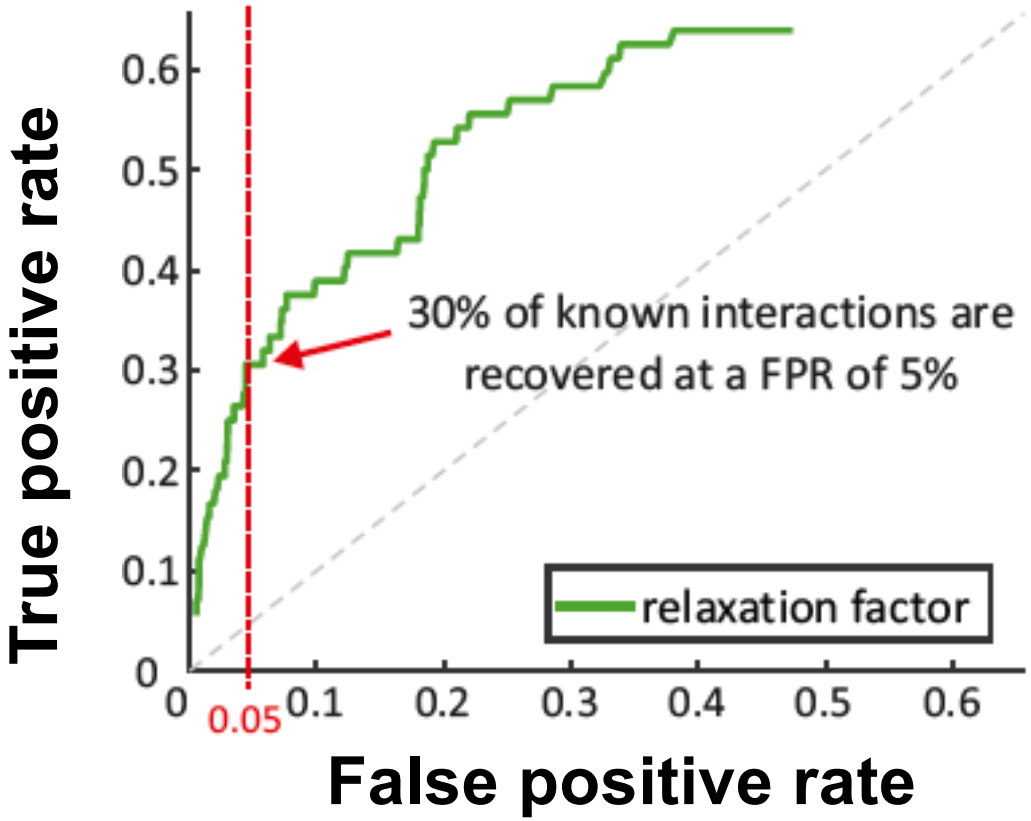
distributed in 4 mixes



1D ^{1}H NMR (T1rho) spectroscopy



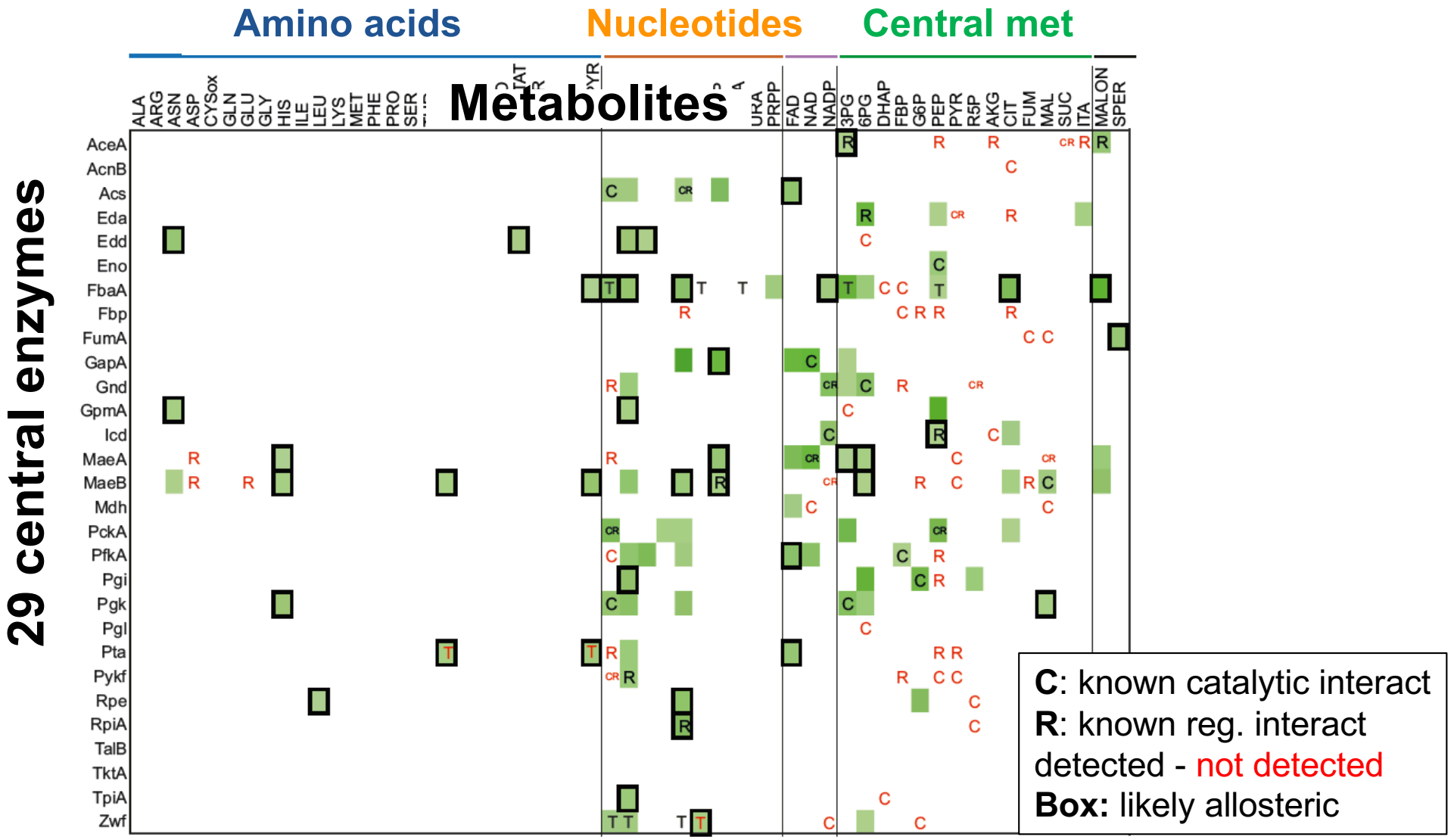
Subnetwork Focused Mapping of Metabolite-Protein Interactions



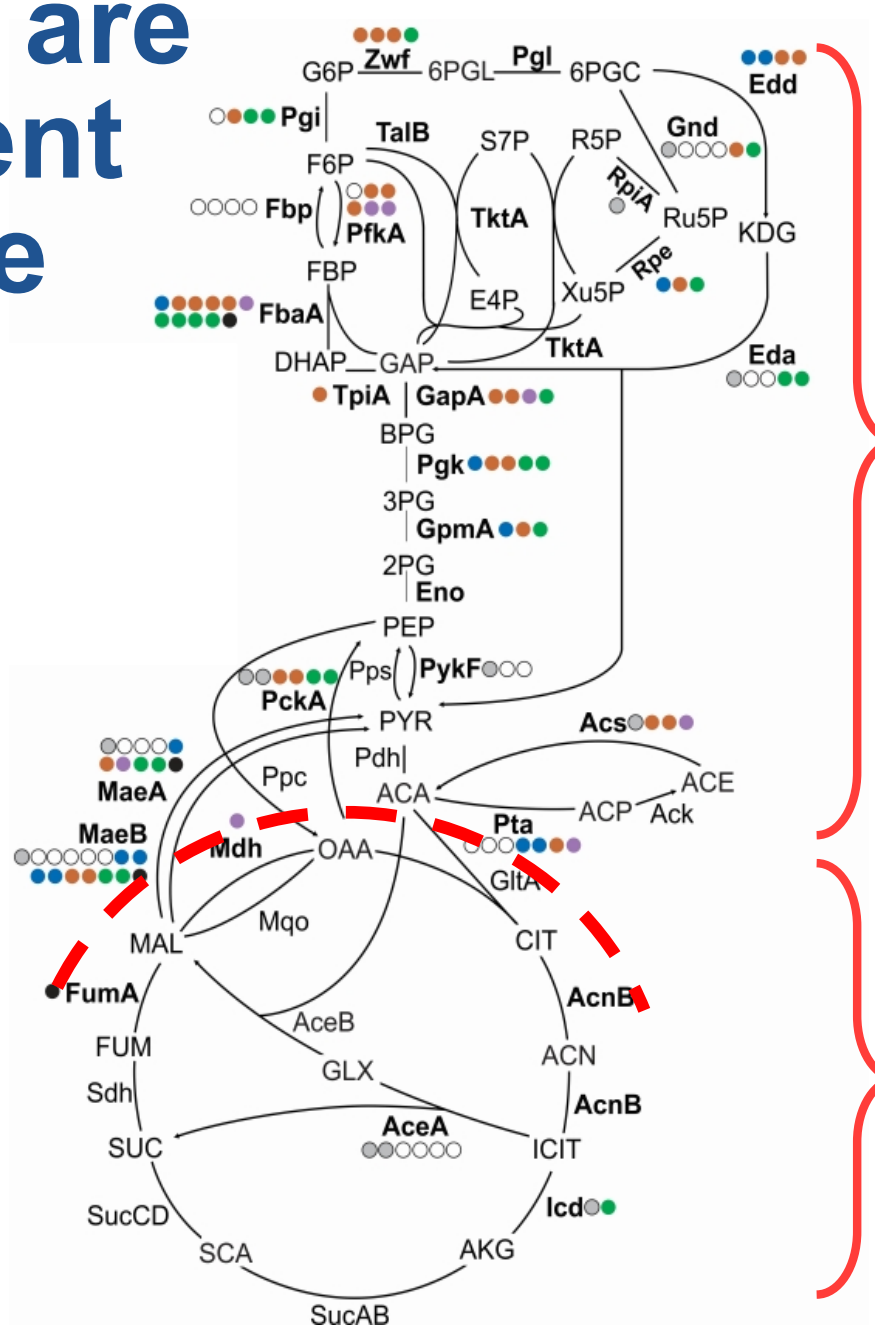
40 regulatory, 43 catalytic interactions were known already

Cutoffs set for low false positive rate (30% recovery)

Subnetwork Focused Mapping of Metabolite-Protein Interactions



Interactions are Less Frequent in TCA Cycle



known regulatory interactions
- detected
- not detected

new interactions
- amino acid biosynthesis
- nucleotide biosynthesis
- redox
- central metabolism
- other

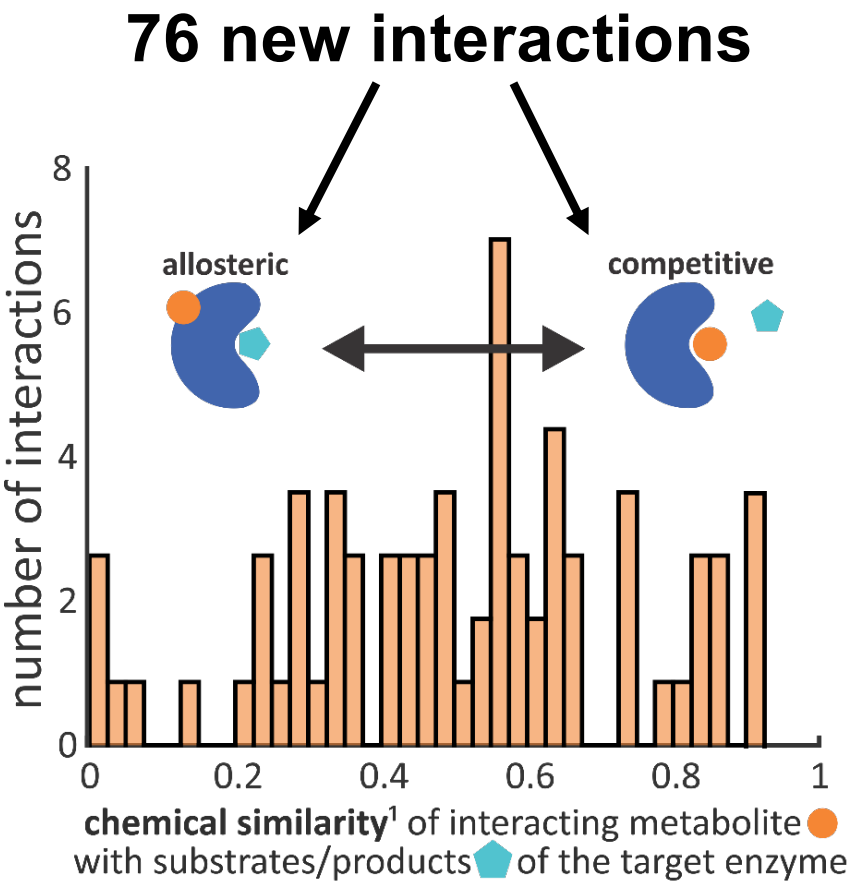
Average numbers

3.4 interactions per enzyme

1 interaction per enzyme

P-value: 0.004

NMR Detects ‘Likely’ Allosteric and Competitive Interactions



Equally distributed between ‘likely allosteric’ and ‘likely competitive’

Hattori et al. (2010) *Nucleic Acids Res.*

Conclusion

- ~ 100 interactions detected - 76 are novel (!)
- Doubled number of regulatory interactions in central metabolism **-> much larger interaction network than currently known**
- Based on chemical similarity ~ about 50% of novel interactions are allosteric!
- So far, 8 out of 11 tested interactions functionally validated

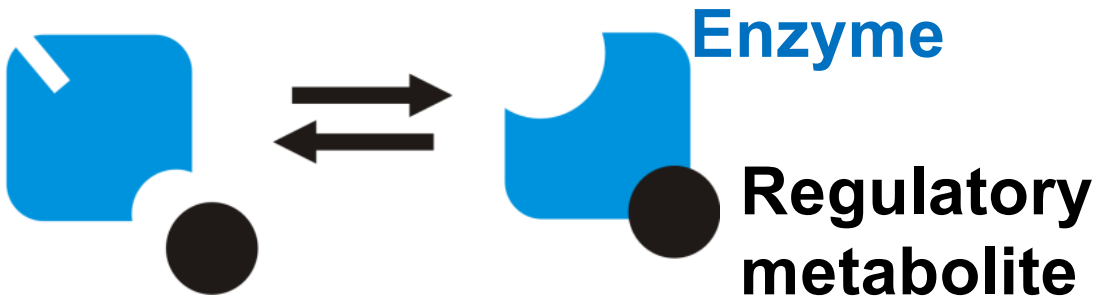
From to Topology to Function .....

Working towards a „comprehensive“ met-prot interaction map of central metabolism.

But a long way from network topology to understand *in vivo* function in cellular context .....

Which regulatory interactions govern a given adaption, and precisely how do they do it?

Avoiding Confounding (other) Regulation When Assessing Allosteric Metabolite-Protein Regulation



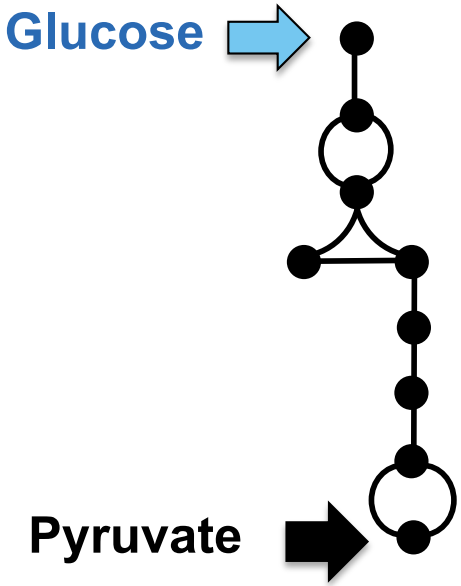
Assumption: allosteric effects dominate very rapid (!) responses

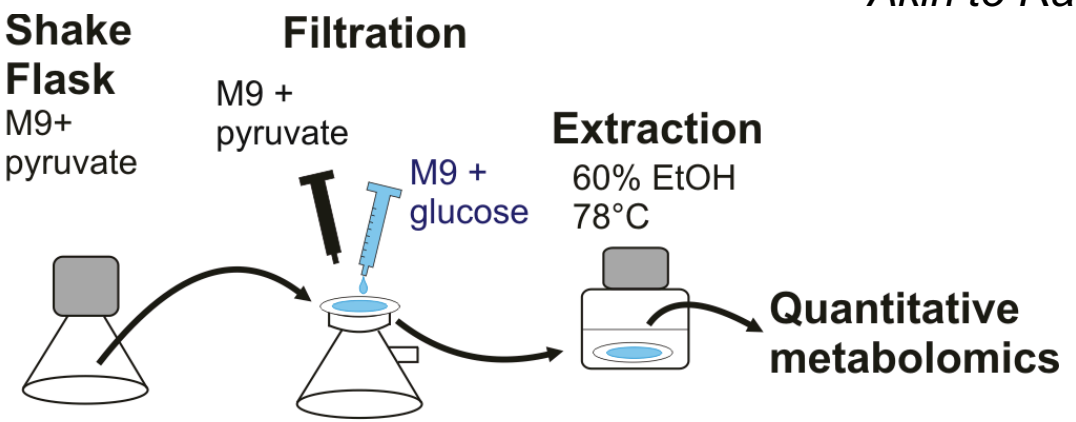
Hannes Link

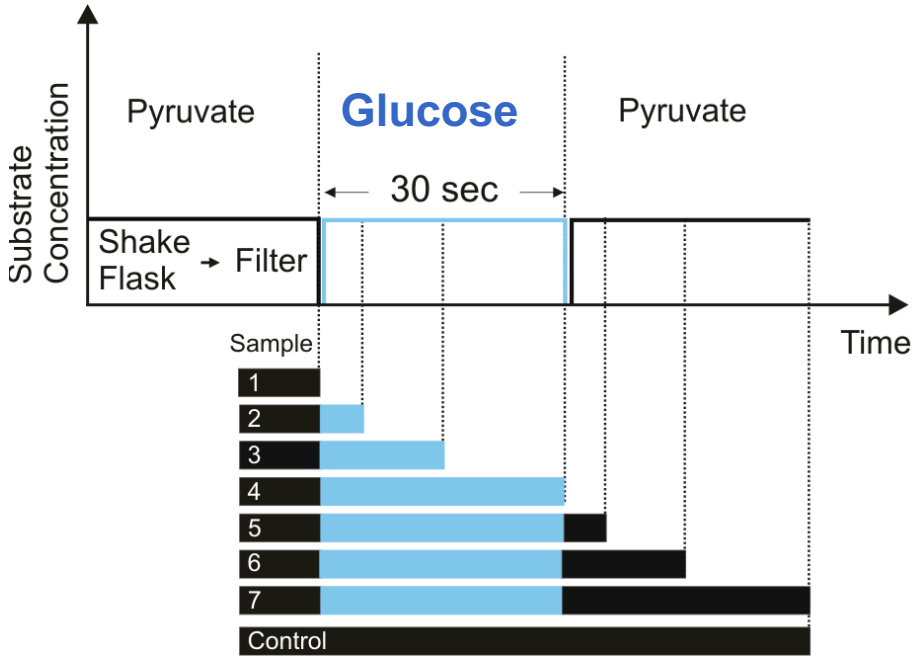
Karl Kochanowski

Gluconeogenesis to Glycolysis Switch of *E. coli* within 30 Seconds

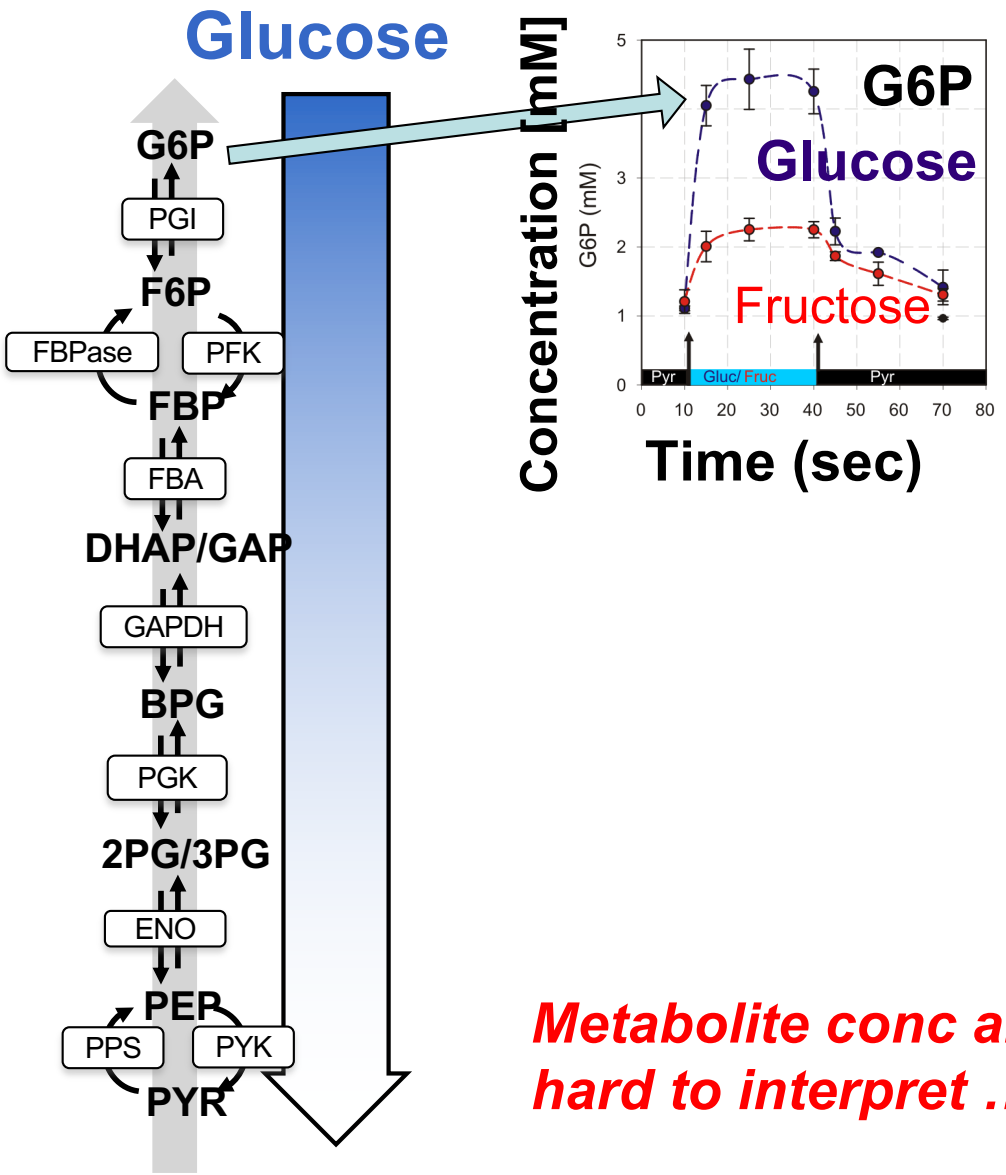
Akin to Rabinowitz method





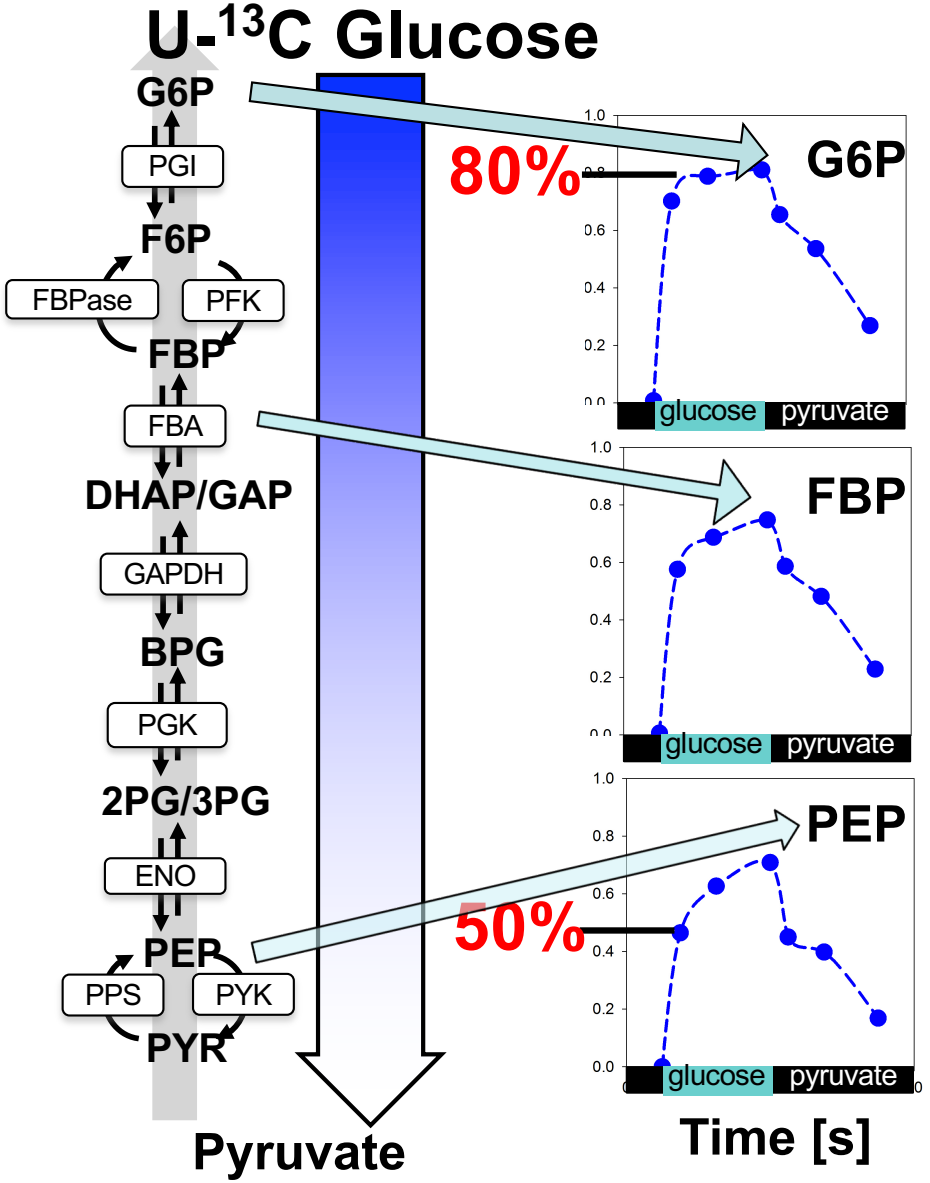


Dynamic [Metabolite] Response (30 s)



Metabolite conc are hard to interpret ….

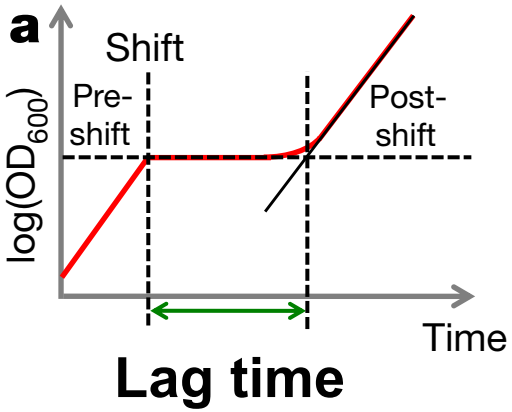
Dynamic ^{13}C-Experiments Demonstrate: Flux Reversion within 5 Seconds



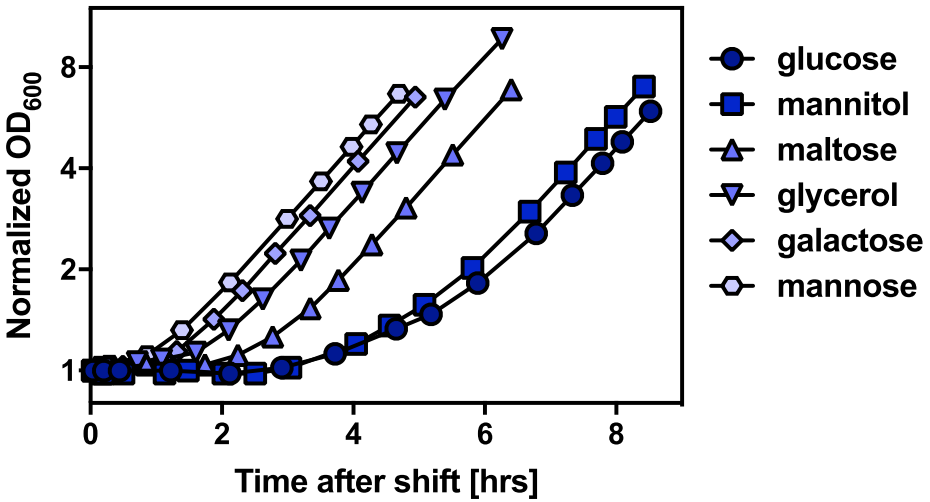
Fully ^{13}C-labelled fraction

- Combine short-term metabolite dynamics with ODE modeling
- Infer allosteric regulation relevant for a given adaptation
- Identify novel allostery

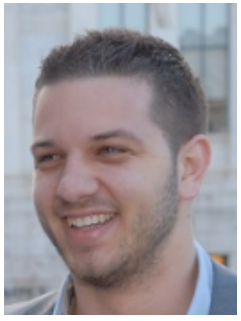
Can *E. coli* always shift rapidly between glycolytic & gluconeogenic C-sources?



Shift from sugars to acetate



In collaboration with Terry Hwa (UCSD) and Johan Paulsson (Harvard)

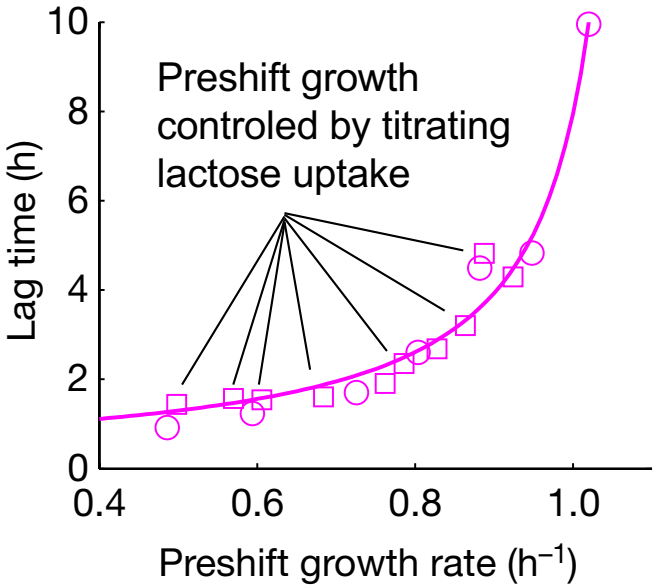


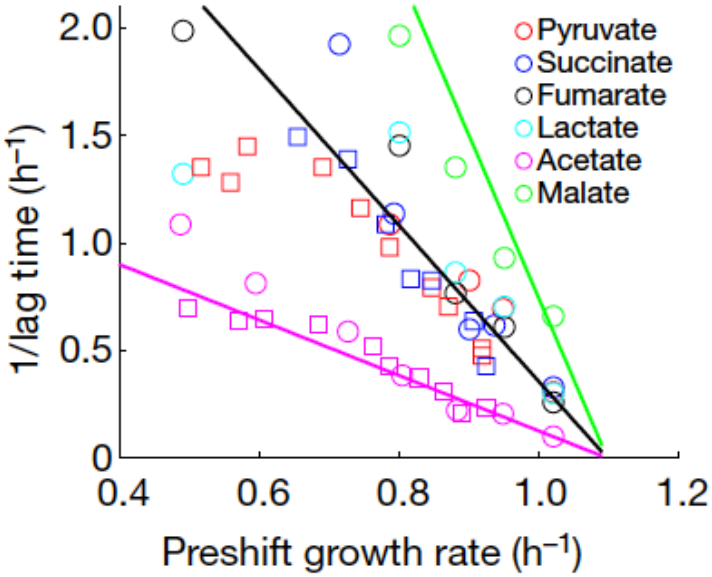
Markus Basan

Dimitris Christodoulou

Fast Growth Rate -> Long Lag Phase

Shift from various C-sources to acetate

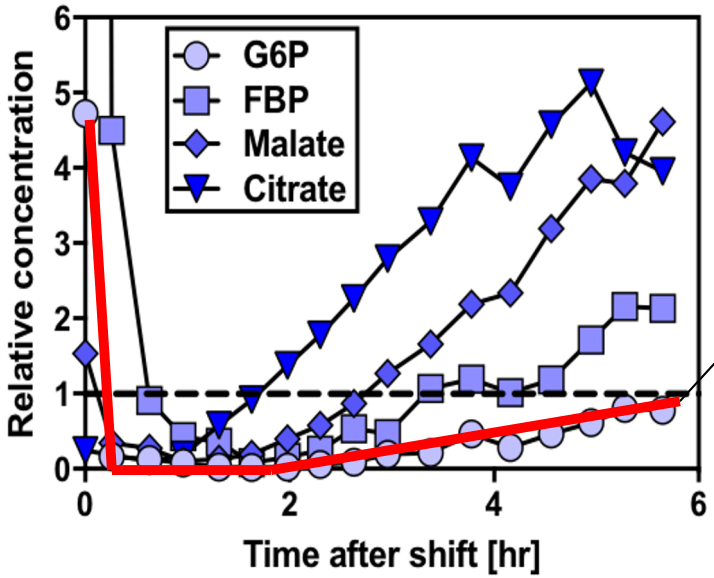




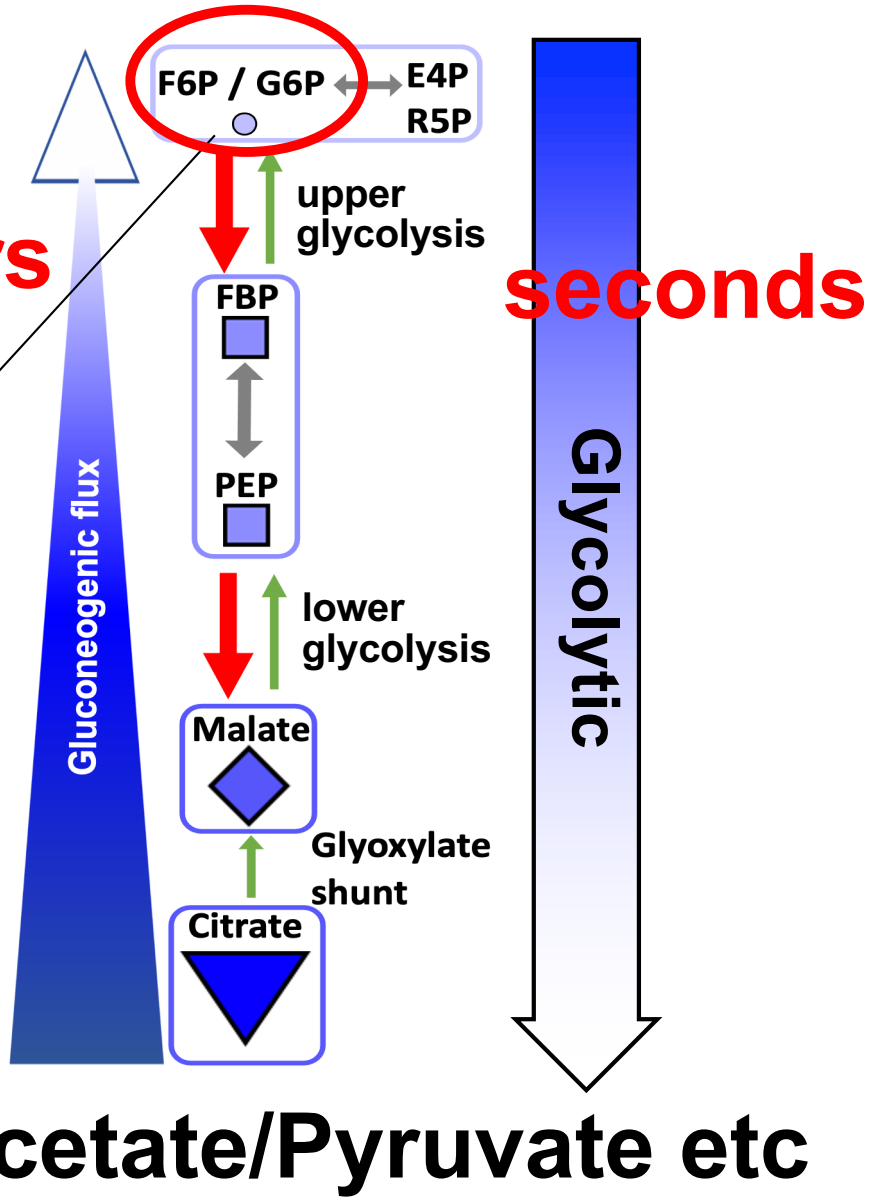
Growth-lag relationship depends on influx rather than specifics of C-source

- Linear relationship btw inverse lag time ($1/T_{lag}$) (**ie adaptability**) and preshift growth rate
- Found a general linear relationship for shifts from 6 glycolytic to 6 gluconeogenic C-sources

Why are the Shifts Rapid Towards Glycolysis But Slow in the Other Direction?



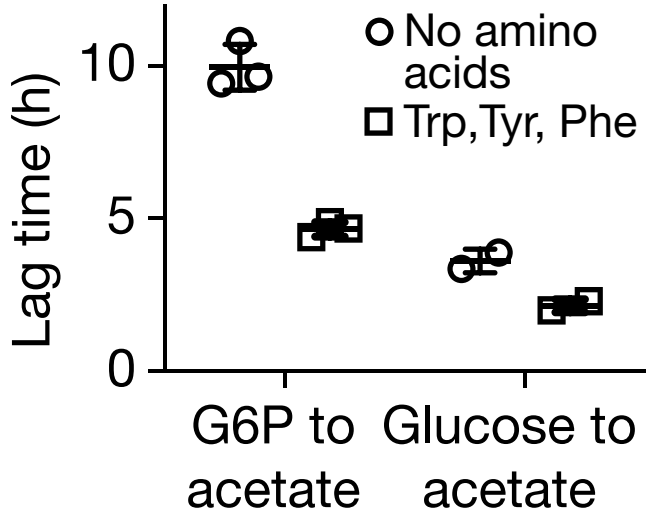
Temporal metabolite profiles throughout lag phase from glucose to acetate



Why are the Shifts Rapid Towards Glycolysis But Slow in the Other Direction?

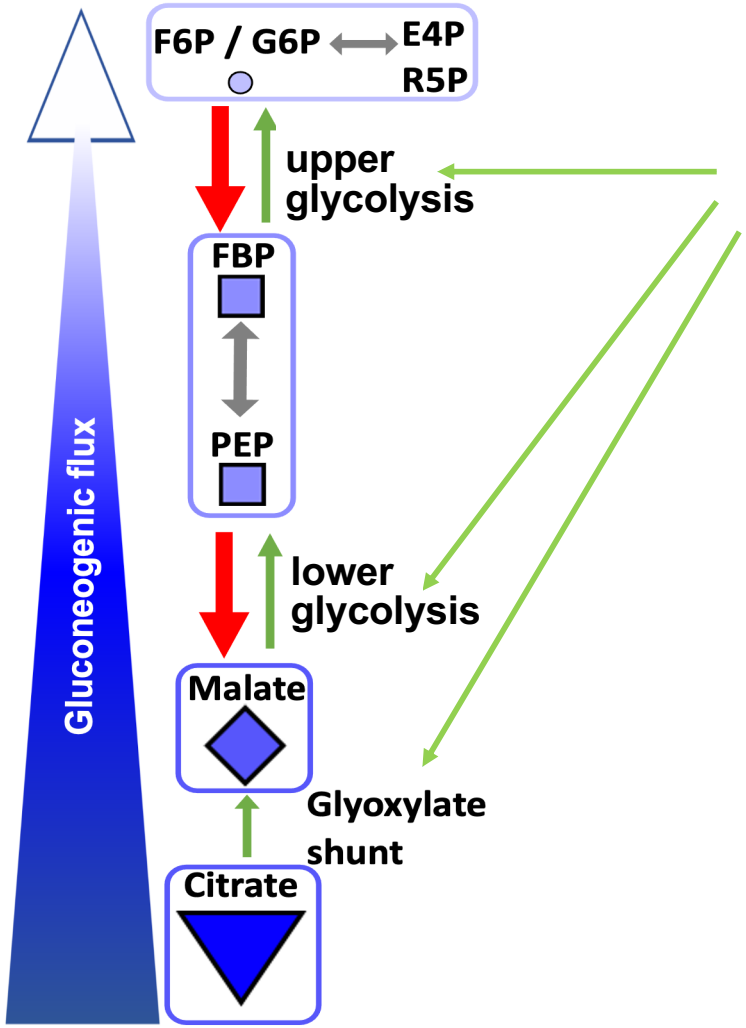
- Metabolites in upper glycolysis increase very late
- Typically when growth resumes …..
  (eg FBP drops 200x from growth on glucose in the lag phase)
- ^{13}C-flux tracing confirms almost no gluconeogenic flux, while TCA cycle flux is rapid during lag

Hypothesis: Gluconeogenic flux limits biosynthesis of biomass components derived from upper glycolysis.



Supplementing AA from upper glycolysis & PP pathway reduces lag phase!

Why do Cells Have Such a Hard Time to Revert Glycolysis?

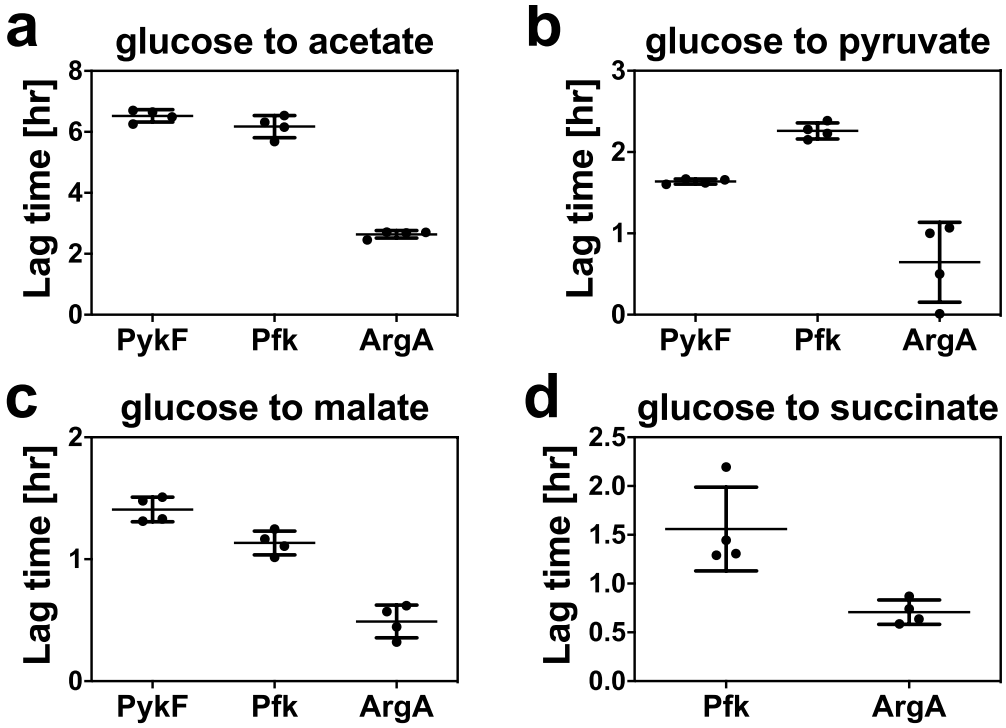


- Dedicated gluconeogenic enzymes must be synthesized in the lag phase
- But their glycolytic counterparts are still present – **the faster cells grew before the shift the more of them are present!!!!**
- The lingering huge glycolytic capacity counteracts the upward flux

After the shift, cells are trapped in state where gluconeogenesis limits synthesis of AA, which in turn limits production of the very enzymes needed to alleviate the bottleneck

Why can *E. coli* not Avoid Depletion of Gluconegenic Metabolites?

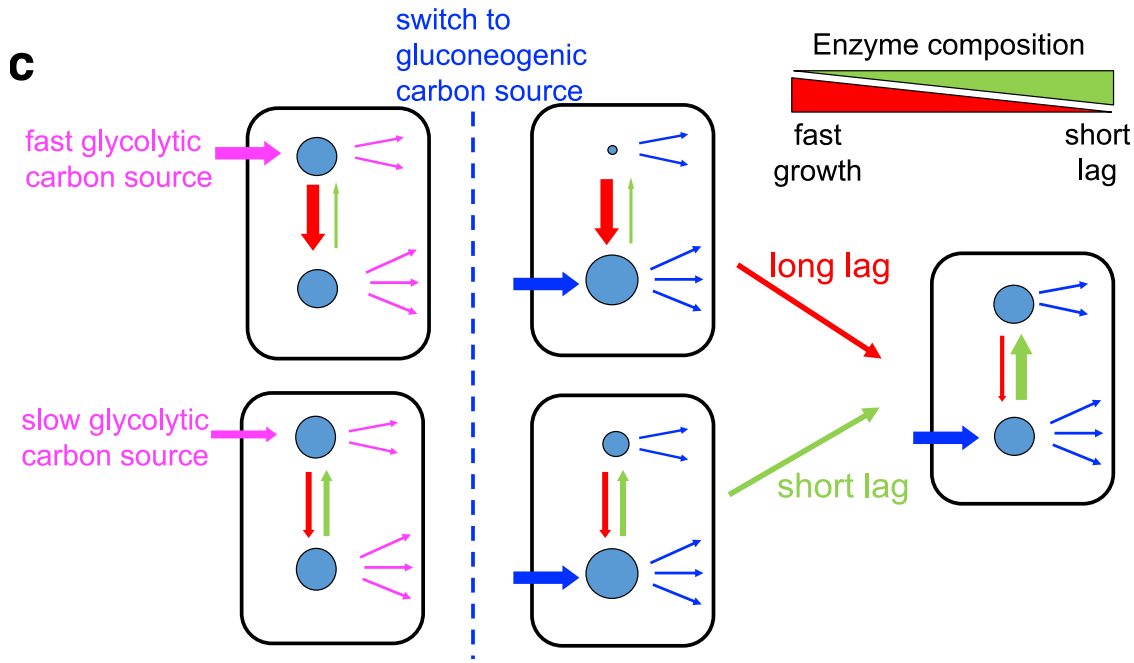
Hypothesis: Allosteric regulation of opposing glycolytic enzymes does not achieve complete inhibition



Consistently, overexpression of irreversible glycolytic enzymes increases lag phase over the control (ArgA)

Conclusion

- A series of low metabolite pools in upper gluconeogenesis causes long lag phases
- Because for fast glycolytic growth the distribution of enzymes strongly favors glycolysis of gluconeogenesis
- **Classical tradeoff between the fitness measures optimal growth and adaptability:** Maximum growth in one condition increases time to adapt to other conditions.



Tradeoff Between Maximum Growth and Adaptability

- A unique perspective on why cells grow at a certain rate in a given condition:
  Can be advantageous to grow slower for the benefit of shorter adaptation
- Growth rate is a reflection of ecological likelihood that conditions will change:
  - eg although chemically very similar, *E. coli* grows slow on fructose and mannose but fast on glucose. Deleting Cra – the TF that activates gluconeogenesis and represses glycolysis – increases the growth rate on fructose and mannose BUT prevents shift to many gluconeogenic substrates!
  - Thus Cra may be designed to slow growth on glycolytic substrates to enable adaptation to gluconeogenesis
- We found similar tradeoffs in other respiro-fermentative microbes, eg *B. subtilis* and *S. cerevisiae*
- In contrast, anaerobes – that do not grow on gluconeogenic C-sources - do not show this tradeoff; ie. the gut microbe *B. thetaiotaomicron* grew equally fast on all tested C-sources

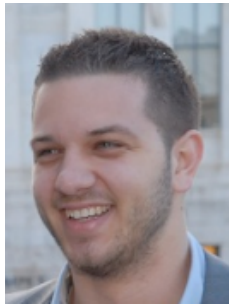
Take Home Messages .....

- Non-covalent met-prot interactions regulate **conserved homeostatic processes** (in pro- and eukaryotes).
- Still largely unknown met-prot interaction network. “Comprehensive” interaction map on horizon for *E. coli.*
- Understanding the function of allosteric regulation requires dynamic data and computer models.
- Allosteric regulation works not «perfectly», eg cannot entirely shut off an unnecessary activity.
- Consequently there is a fundamental tradeoff btw the fitness traits of maximum growth and adaptability.
- A series of low metabolite pools in upper gluconeogenesis causes long lag phases after shifts from sugars to organic acids

THANKS

Karl Kochanowski

Hannes Link



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