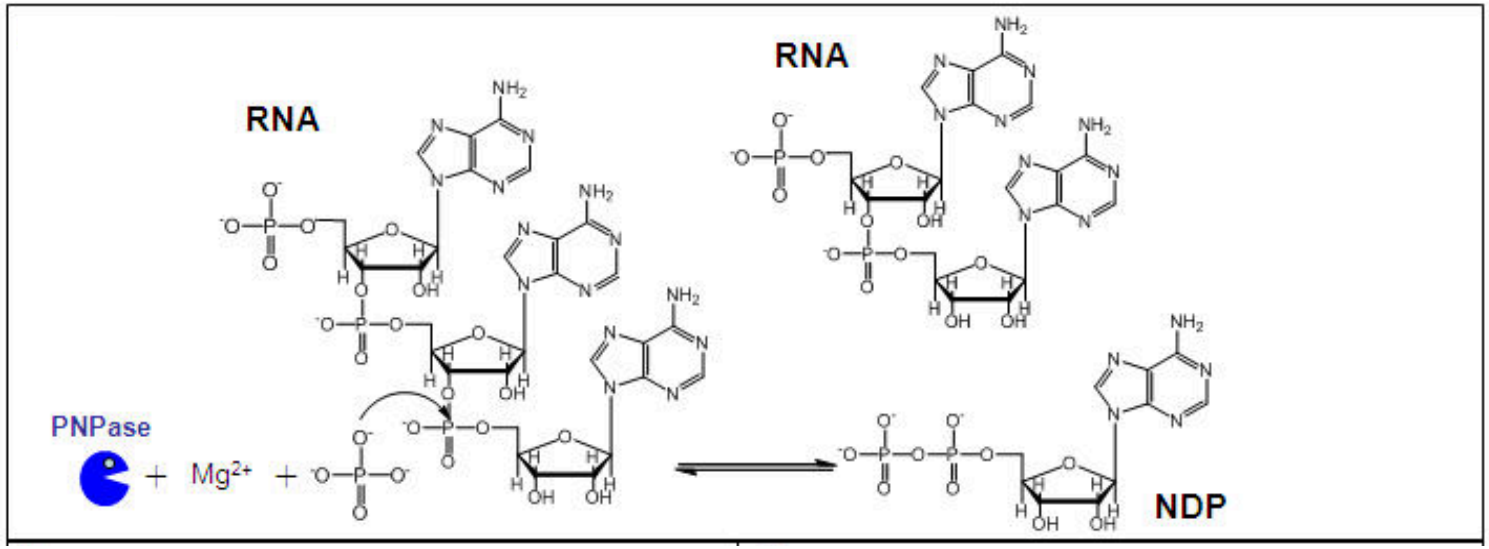
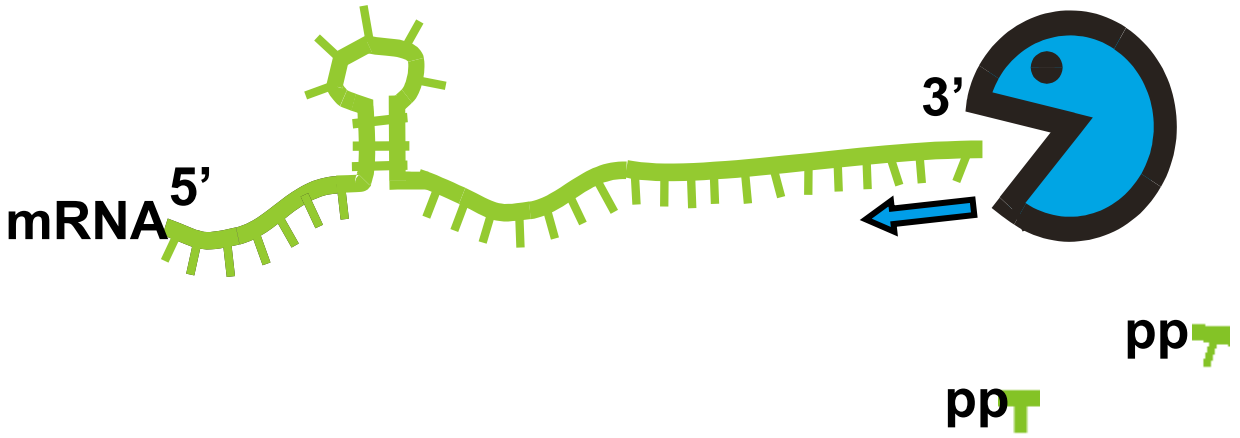
Reviews in Computational Biology

A Curiosity from Structural Biology

Ben Luisi

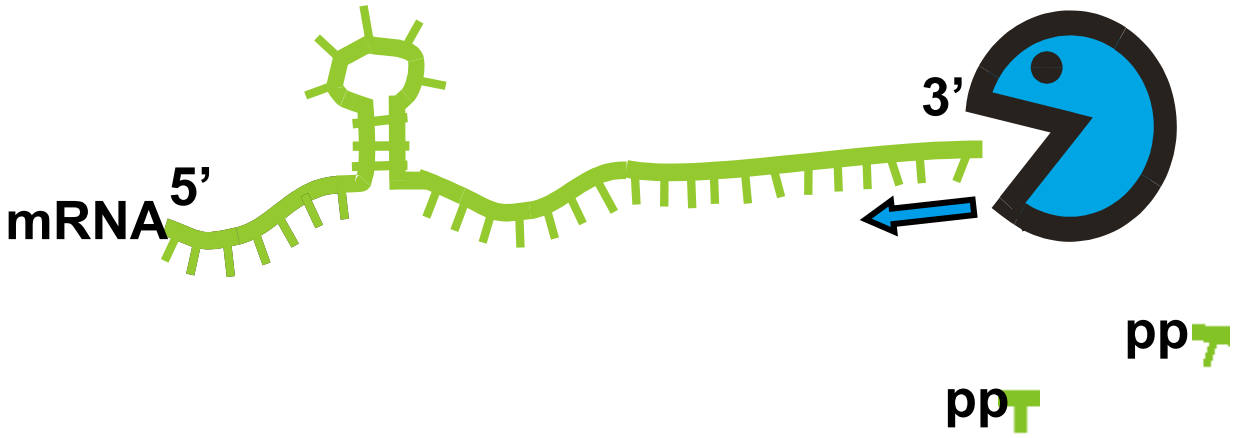
January 2012



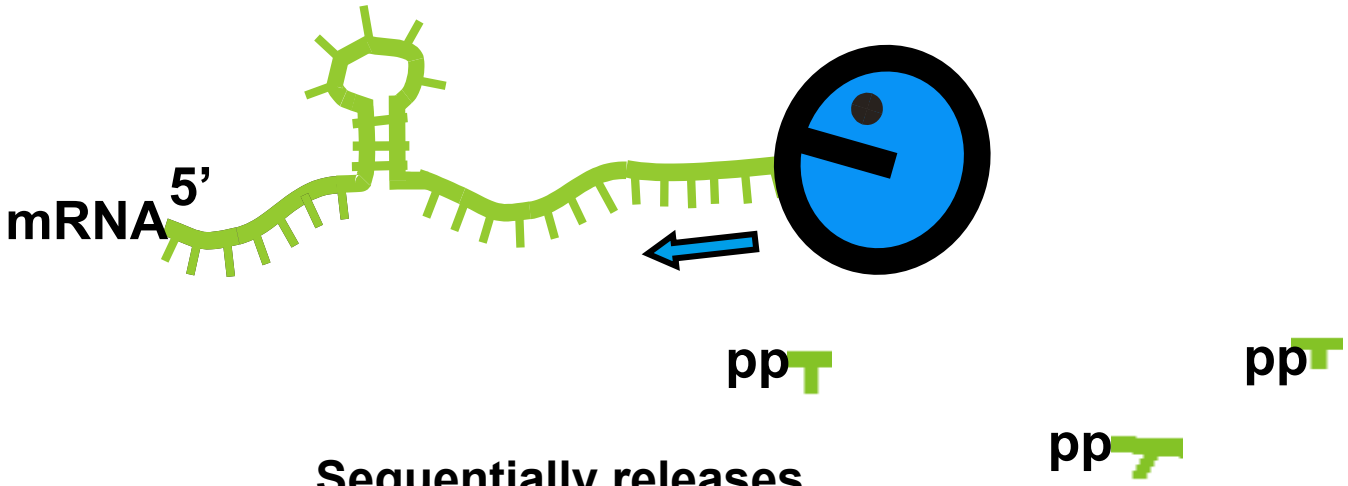


PNPase : Phosphorolytic cleavage mechanism

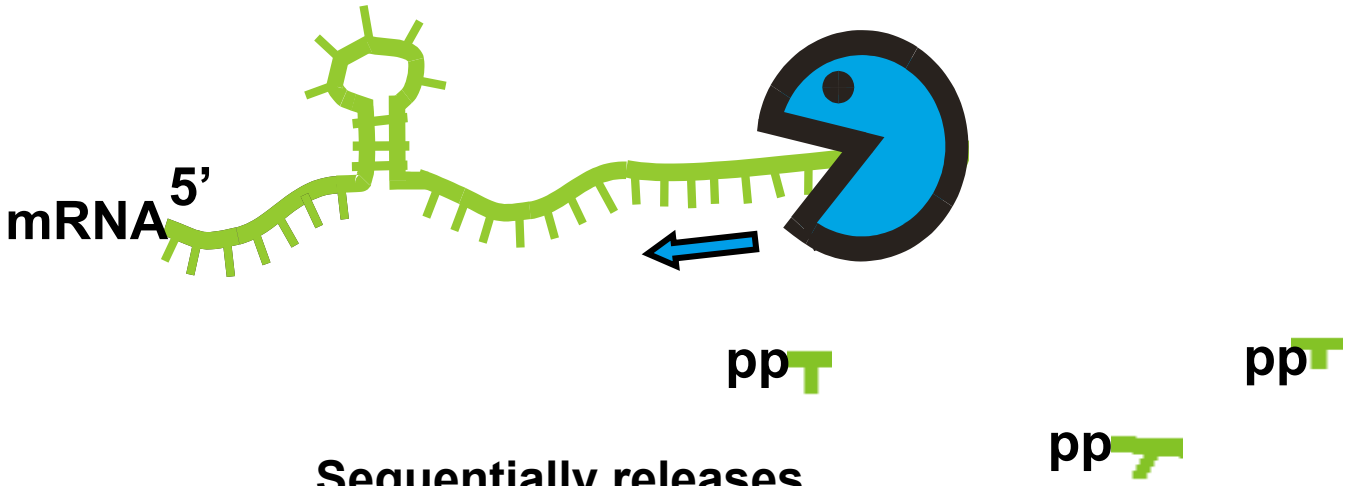
220kDa homo-trimeric exoribonuclease



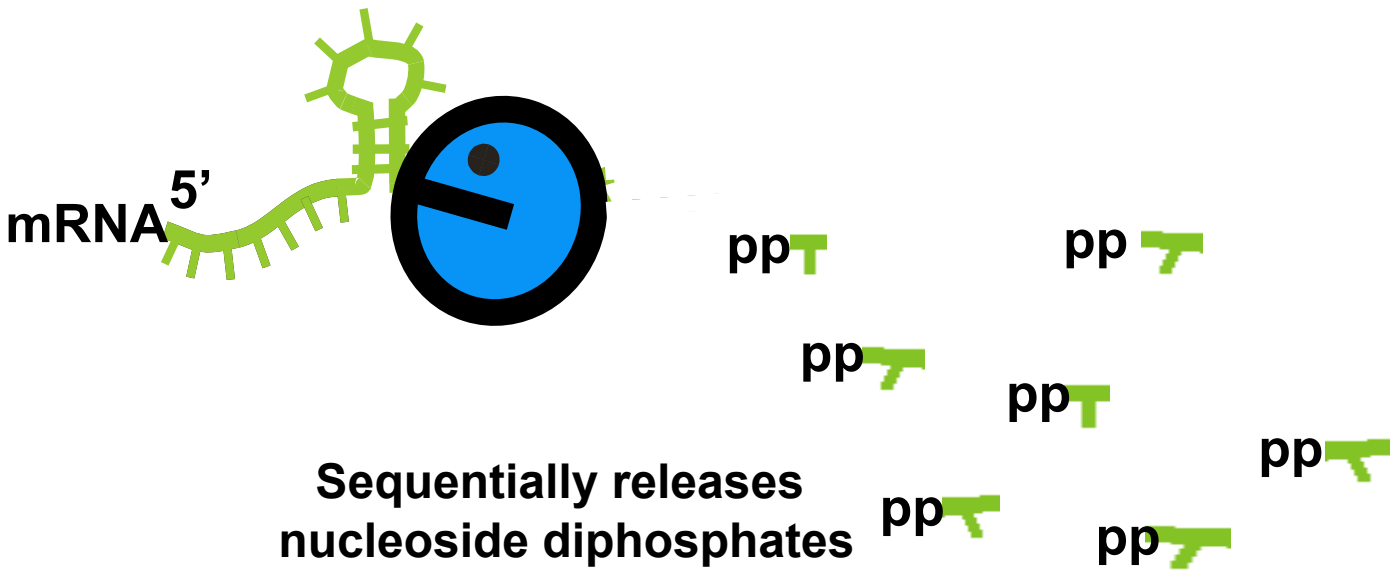
Sequentially releases nucleoside diphosphates

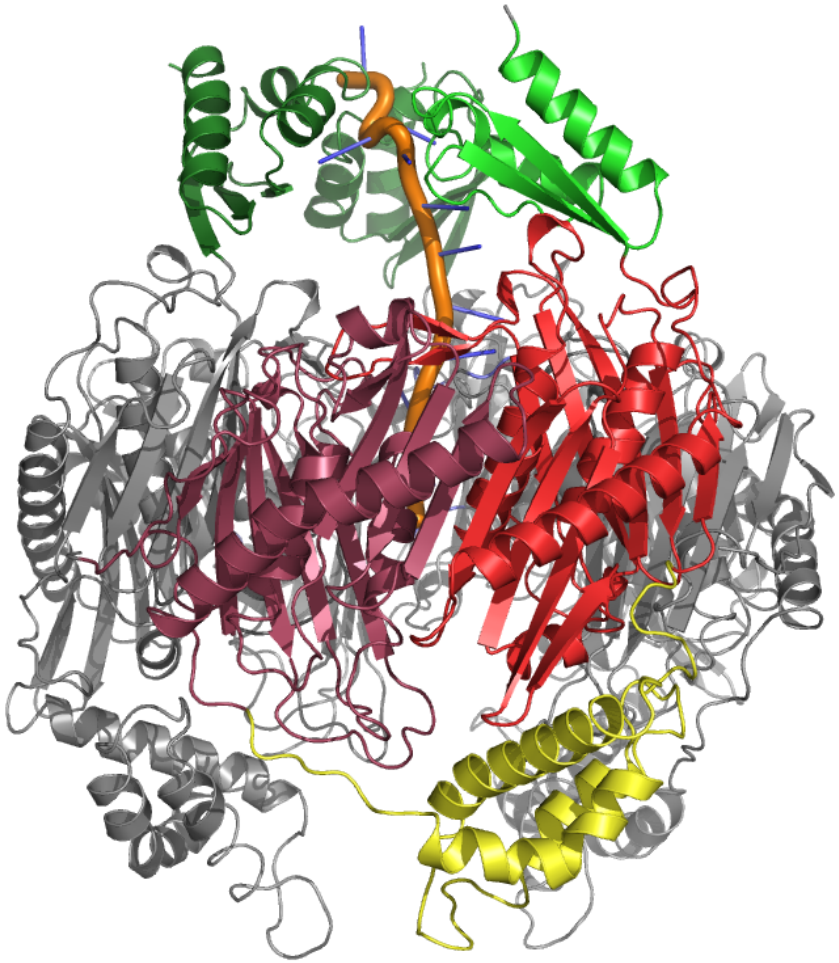


Sequentially releases nucleoside diphosphates



Sequentially releases nucleoside diphosphates

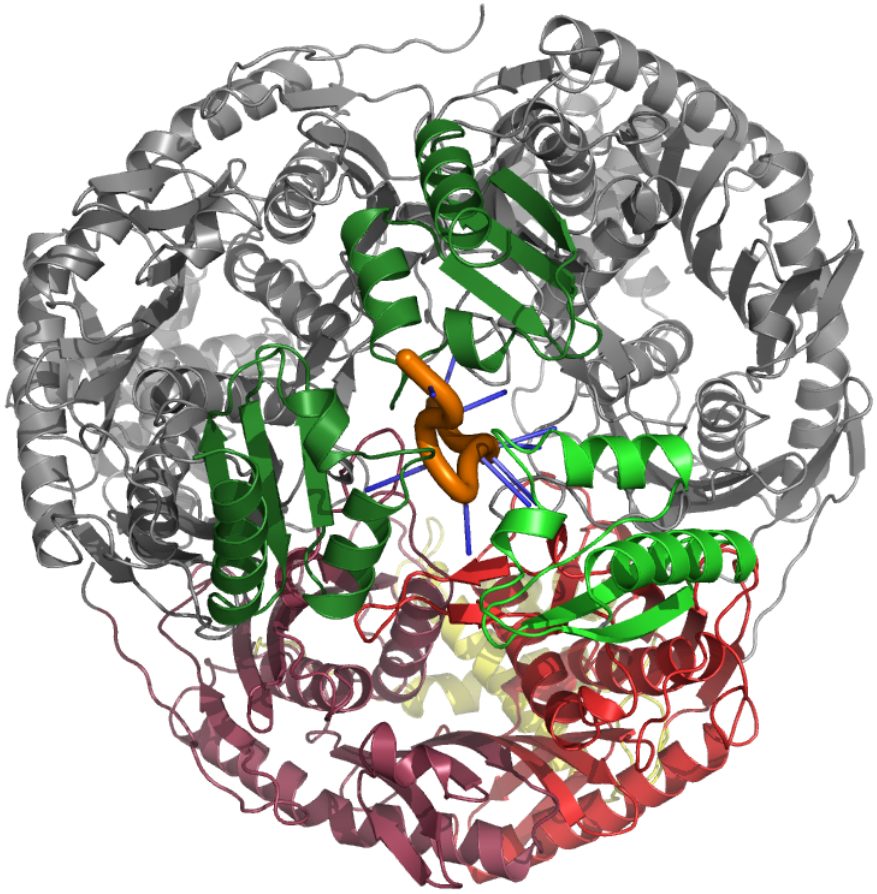

mRNA
5’
pp
pp
pp
pp
pp
pp
Sequentially releases
nucleoside diphosphates

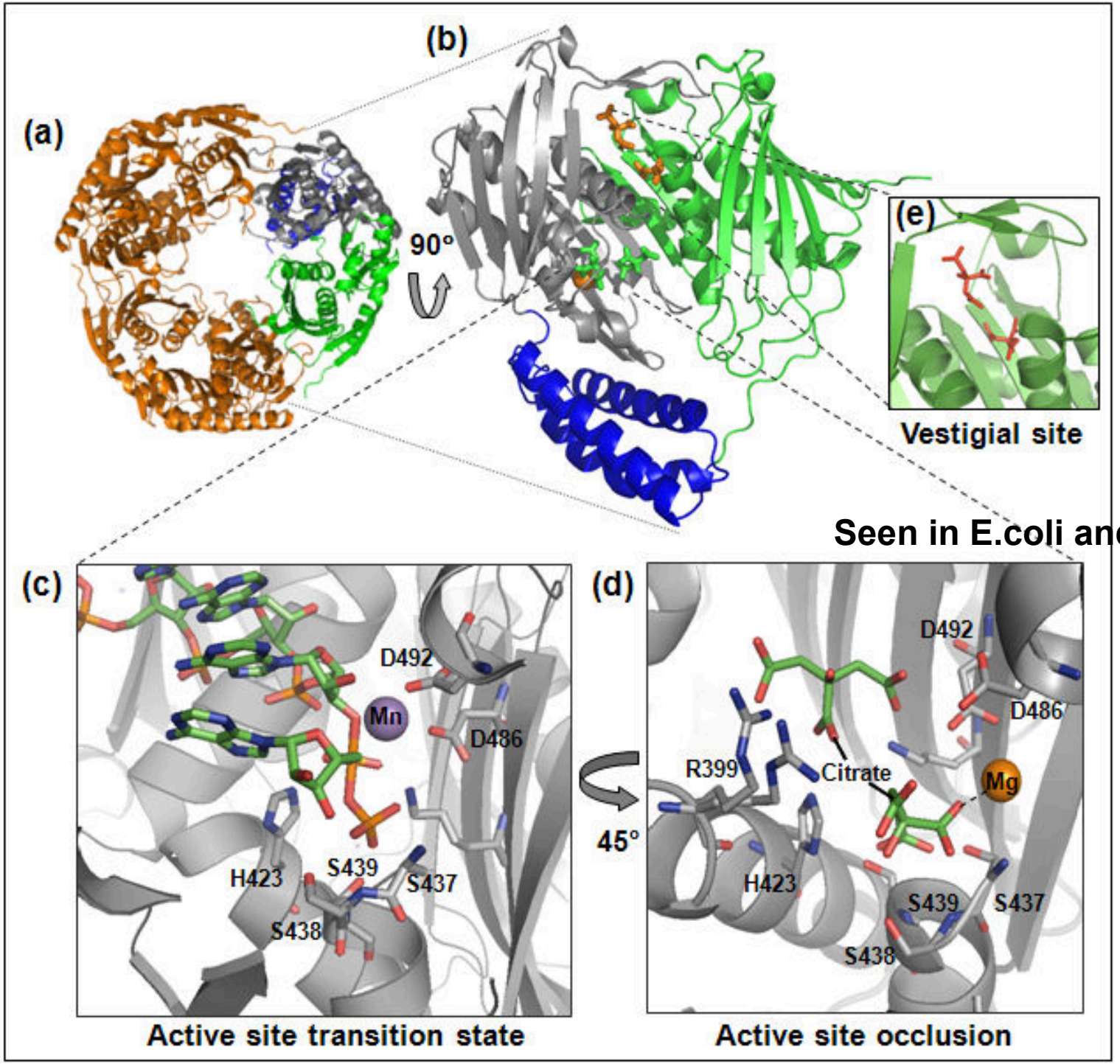


RNA remains bound during purification (~20mer)

Space group $P2_12_12$

3.0 Å resolution





Seen in E.coli and human PNPase

K_d ~ 1mM
Is the interaction 'real' and meaningful?

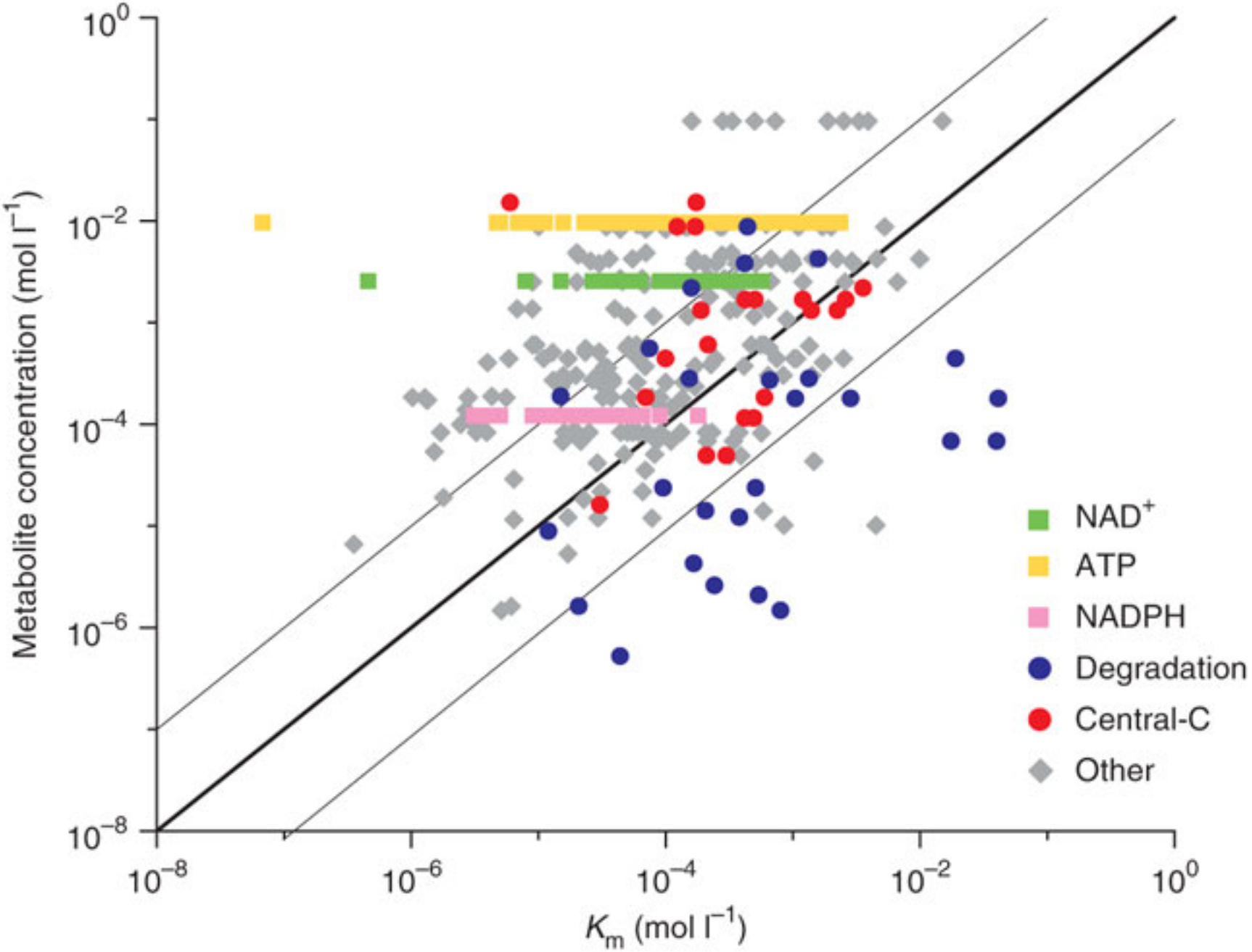
nature
chemical biology

Absolute metabolite concentrations and implied enzyme active site occupancy in *Escherichia coli*

Bryson D Bennett[1], Elizabeth H Kimball[1], Melissa Gao[1], Robin Osterhout[2], Stephen J Van Dien[2] & Joshua D Rabinowitz[1]

Absolute metabolite concentrations are critical to a quantitative understanding of cellular metabolism, as concentrations impact both the free energies and rates of metabolic reactions. Here we use LC-MS/MS to quantify more than 100 metabolite concentrations in aerobic, exponentially growing *Escherichia coli* with glucose, glycerol or acetate as the carbon source. The total observed intracellular metabolite pool was approximately 300 mM. A small number of metabolites dominate the metabolome on a molar basis, with glutamate being the most abundant. Metabolite concentration exceeds K_m for most substrate-enzyme pairs. An exception is lower glycolysis, where concentrations of intermediates are near the K_m of their consuming enzymes and all reactions are near equilibrium. This may facilitate efficient flux reversibility given thermodynamic and osmotic constraints. The data and analyses presented here highlight the ability to identify organizing metabolic principles from systems-level absolute metabolite concentration data.

In E. coli many metabolites are at concentrations > Km for enzymes

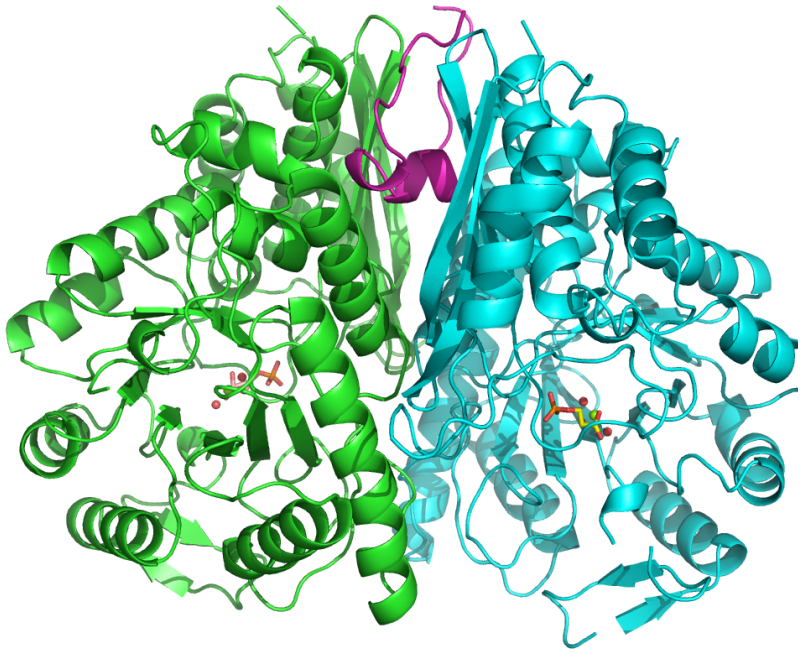


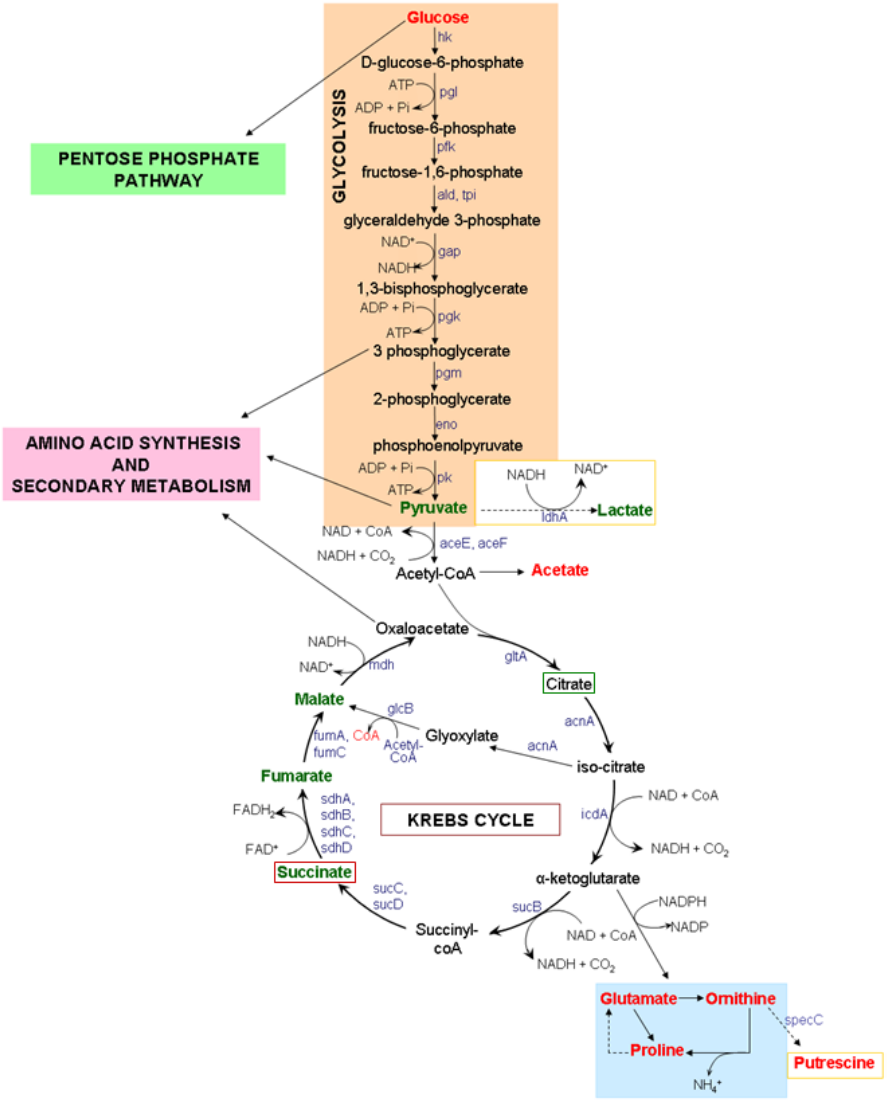
Bennett et al. (2009) Nature Chem Biol.

“Organisms, like pinatas, were there to be burst open in order to get at the (biochemical) goodies within—a view of microorganisms that, with justification, persists today among some subfields of microbiology.”

Woese, C. R., and Goldenfeld, N. (2009) Microbiol & Mol Biol Rev 73, 14-21

Crystal structure of enolase in complex with peptide C and 3-phosphoglycerate





2.0 Å resolution crystal structure
Dijun Du, Biochemisty

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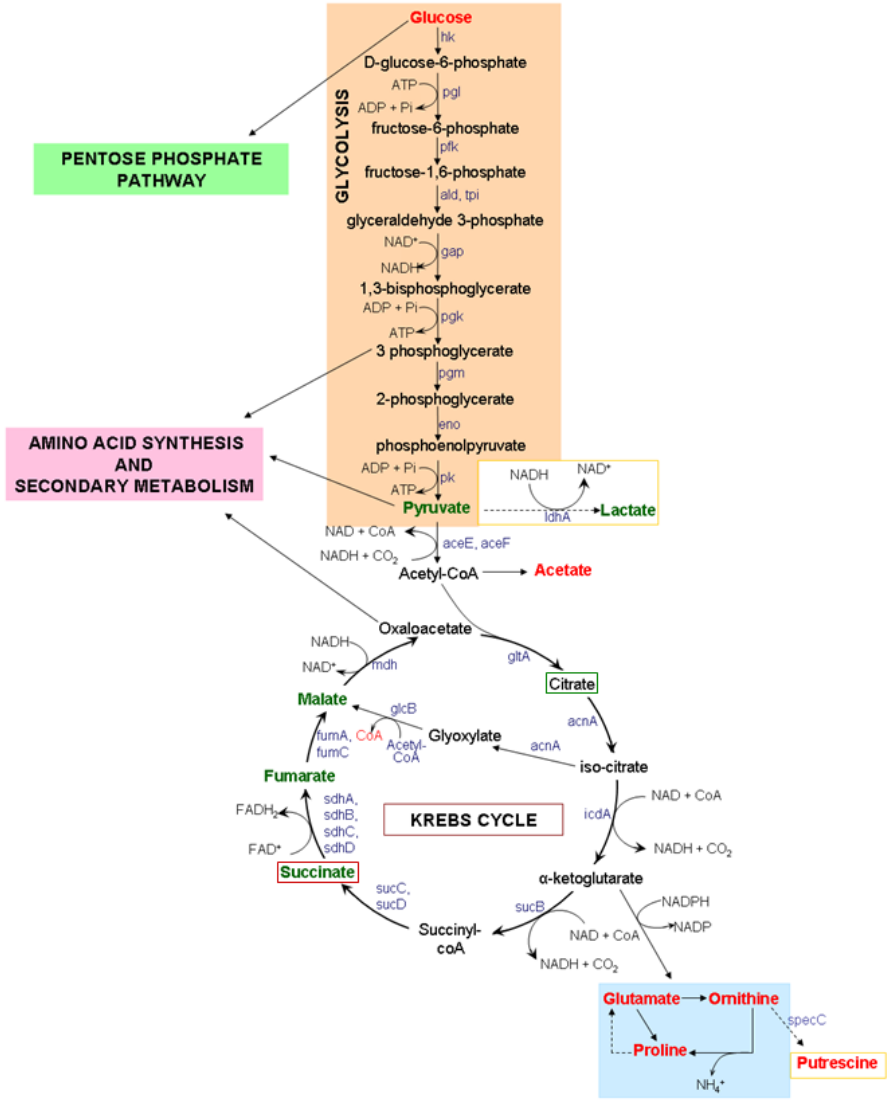
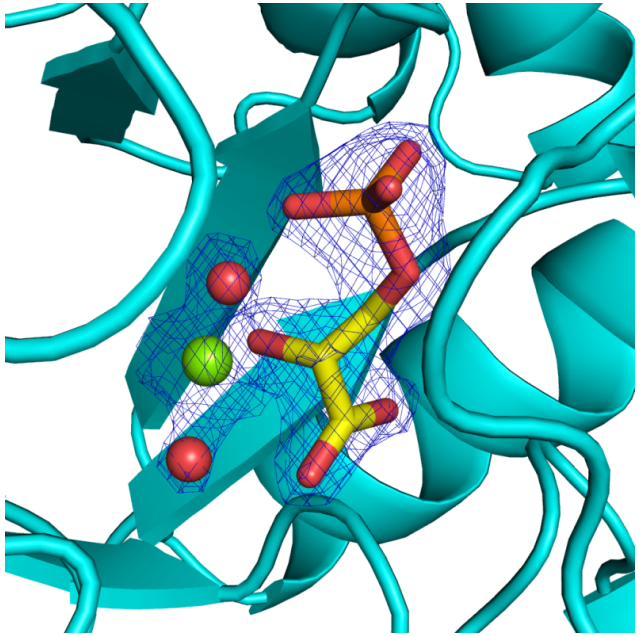
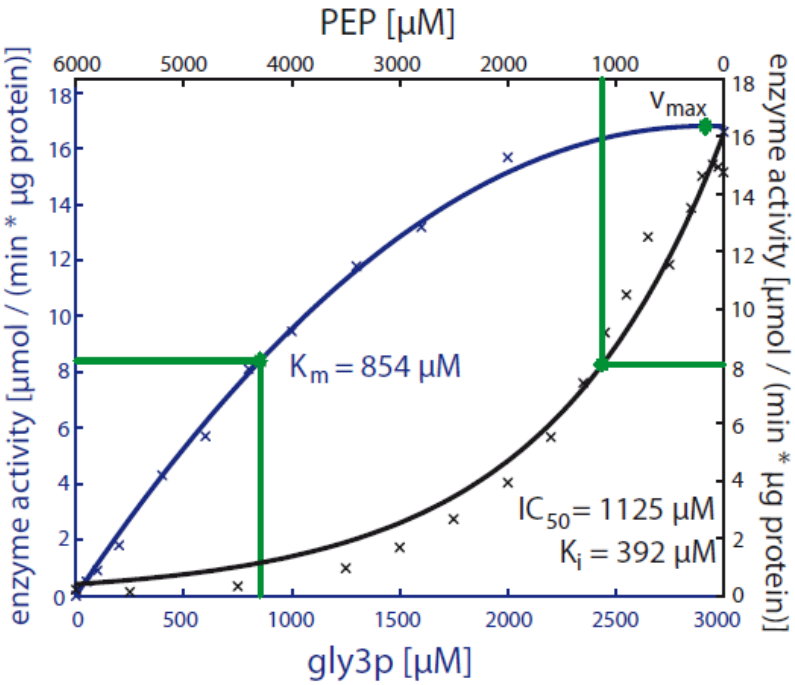
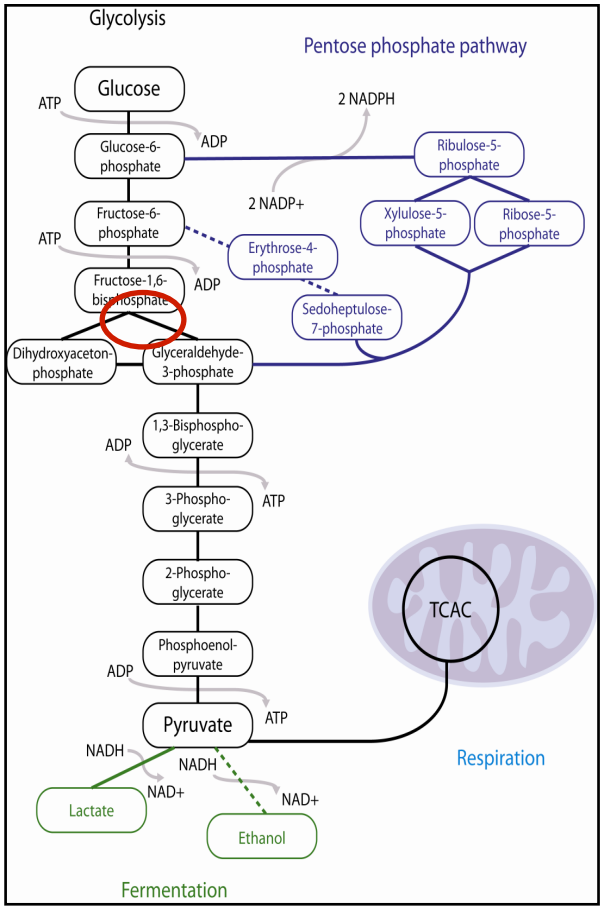




3-phosphoglycerate in active site

PEP inhibits triosephosphate isomerase, an upstream enzyme





Marcus Ralser, Biochemistry

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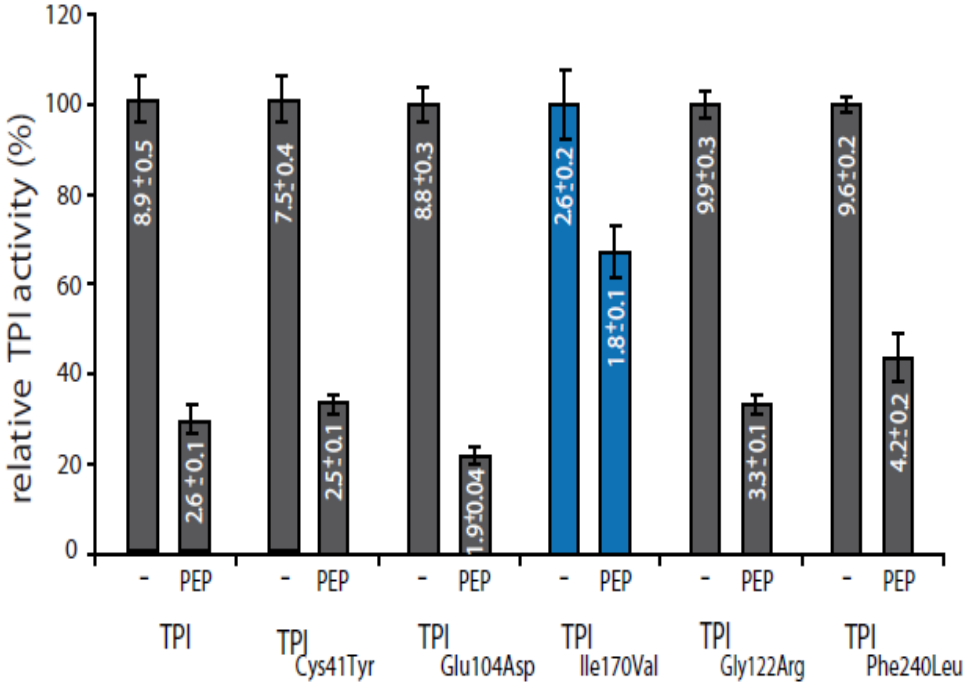

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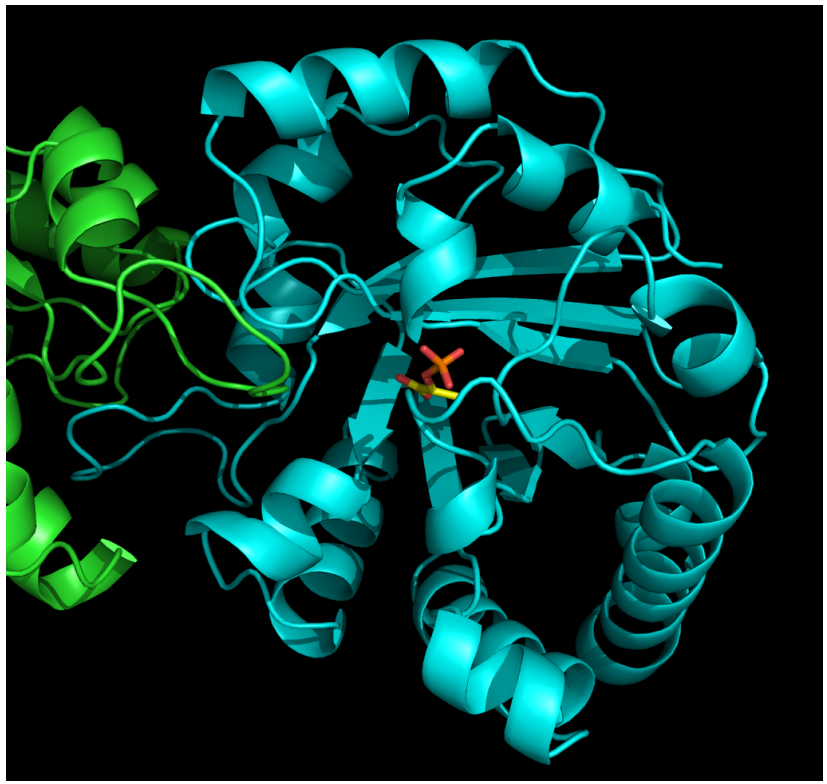
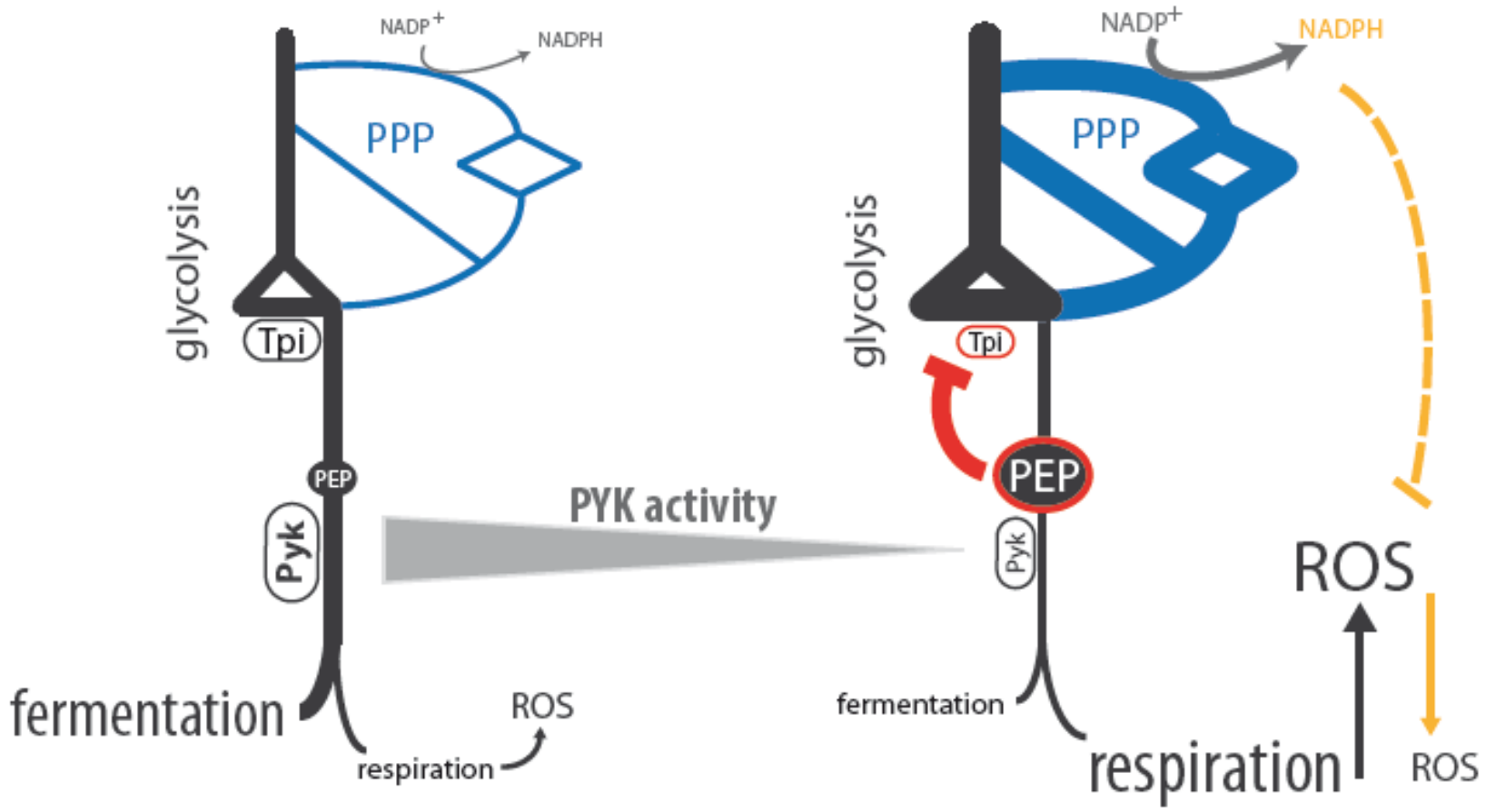


Dijun Du, Biochemistry

PK in regulating redox metabolism



Marcus Ralser, Biochemistry

Summary and conjecture, part 1:

Given the high concentrations (and ratios, ~40 metabolites for every protein molecule in E. coli), metabolites may be competitive inhibitors and allosteric modulators (activating or inhibiting).

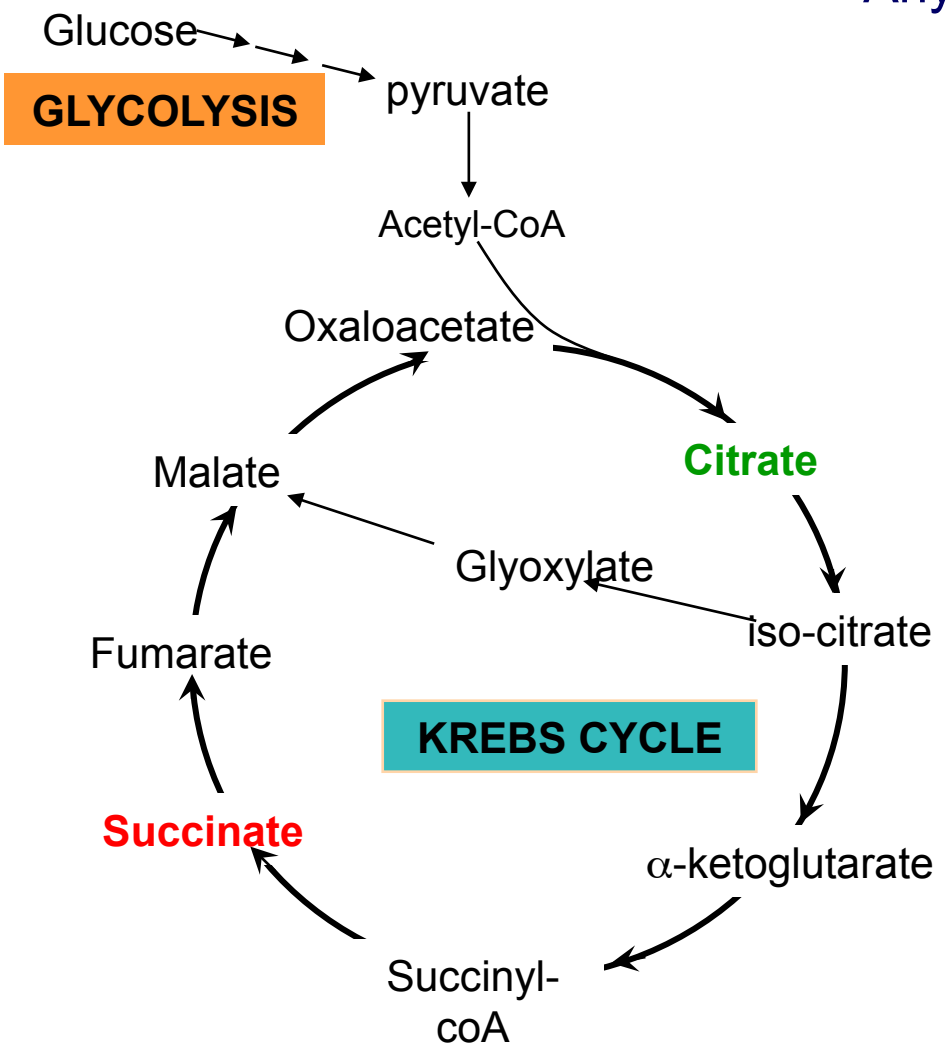
Competitive inhibitor mode for metabolites that change by perhaps more than 3-fold with growth conditions

Allosteric modulators for metabolites that change by less than 3-fold, depending on cooperative behaviour of the putative targets

Regulation likely to be at points highly favored thermodynamically (effectively irreversible). May be needed to avoid Swings in metabolite levels (since concentrations > enzyme Km's)

Bennett et al. (2009) Nature Chem Biol.

Any bottlenecks?



Kacser/Burns Heinrich/Rapoport

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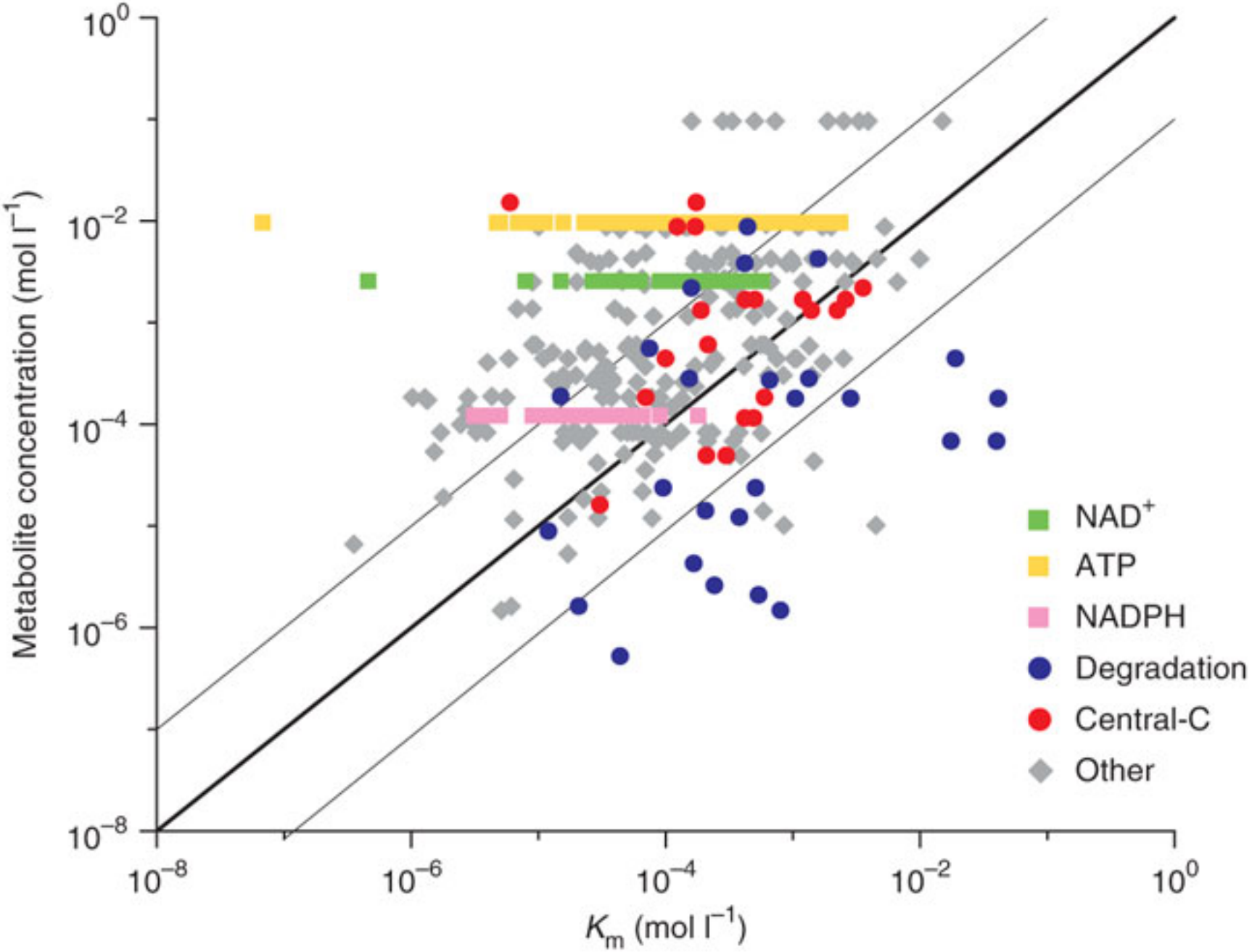

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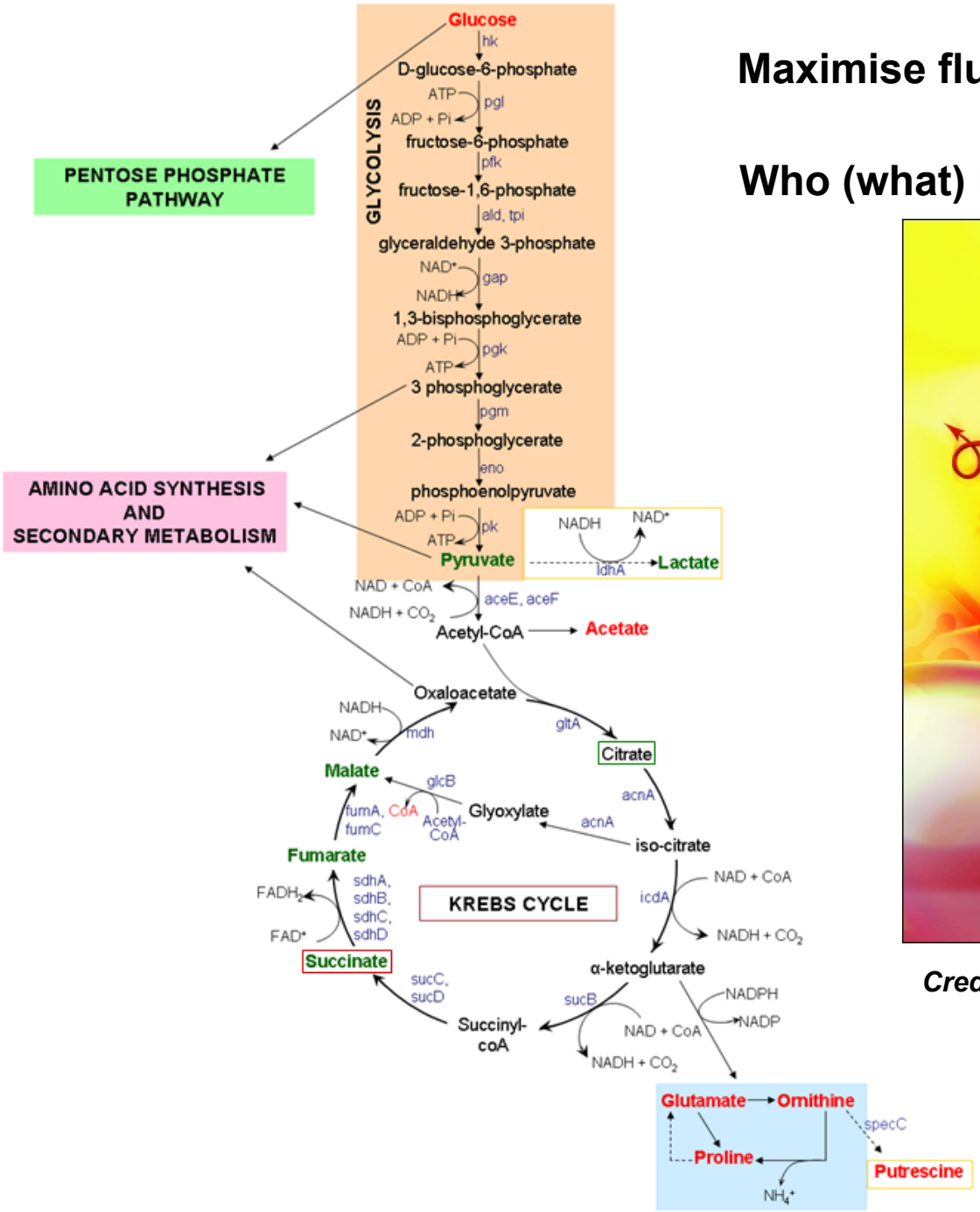


Maximise flux/enzyme

Who (what) is organising the network?

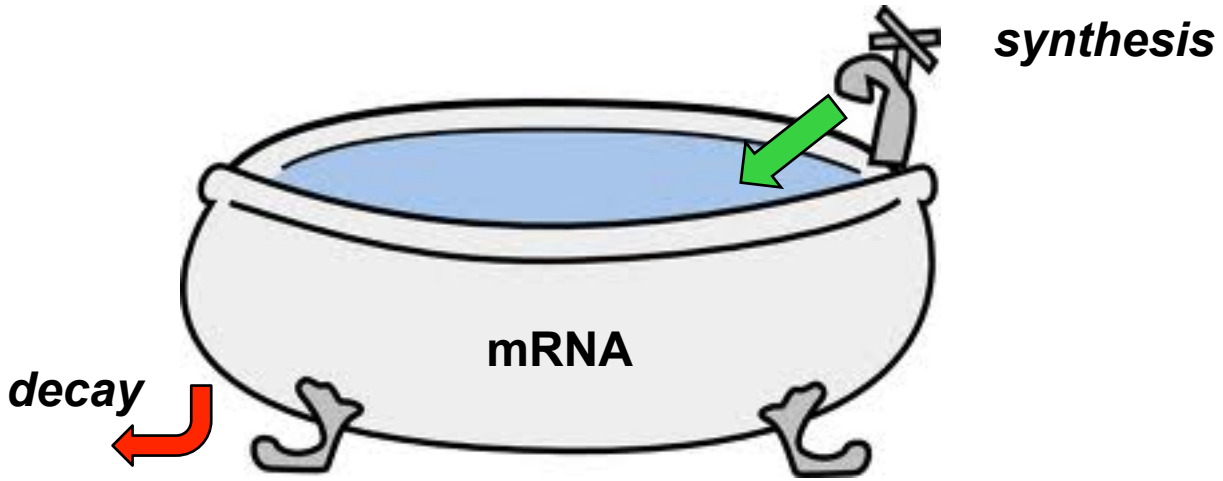
Credit: Peter MacDonald, Edmonds UK.

Control of gene expression in mammals

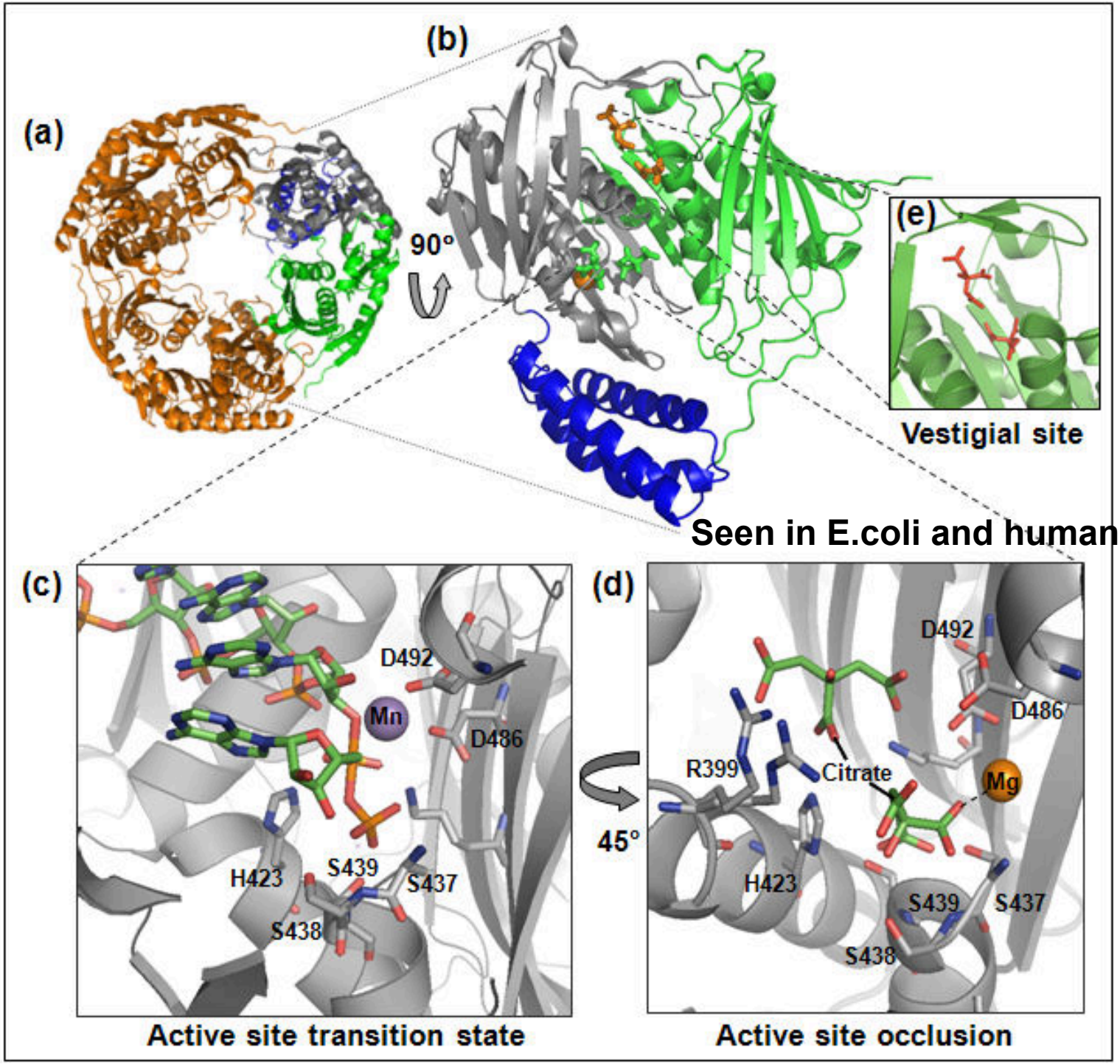
Quantitative study: Protein level proportional to transcript level

Protein level poorly correlated with transcript half-life

Schwanhausser et al. (2011) Global quantification of mammalian gene expression control. Nature 473:337-342.



Possibly, transcription rates and degradation rates are matched and adjusted to control levels of individual transcripts


(a)
(b)
90°
(e)
Vestigial site
Seen in E.coli and human PNPase
(c)
D492
Mn
D486
H423
S439
S437
S438
Active site transition state
45°
(d)
D492
D486
R399
Citrate
Mg
H423
S439
S437
S438
Active site occlusion

In vivo requirements for PNPase and two other exoribonucleases (RNase II & RNase R)

- PNPase is not essential for survival, but …
- PNPase$^-$ RNase II$^-$ cells are inviable
- PNPase$^-$ RNase R$^-$ cells are inviable

In a RNase II/RNase R double null, inactivation of PNPase will impede growth

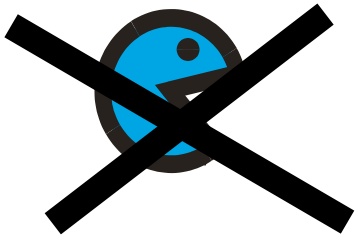
Helen Vincent
Portsmouth University

Growth rates in the presence of Mg Citrate

	Doubling Time (minutes)		
Strain			Wild type
- Mg citrate			48.7 ±2.6
+ Mg citrate			49.2 ±1.9

- **PNPase is partly inhibited by Mg citrate *in vivo***
- **PNPase is responsible for a significant proportion of Mg citrate-mediated effects**

PNPase activity affects the ‘metabolome’



Strains cultured to stationary phase

↓

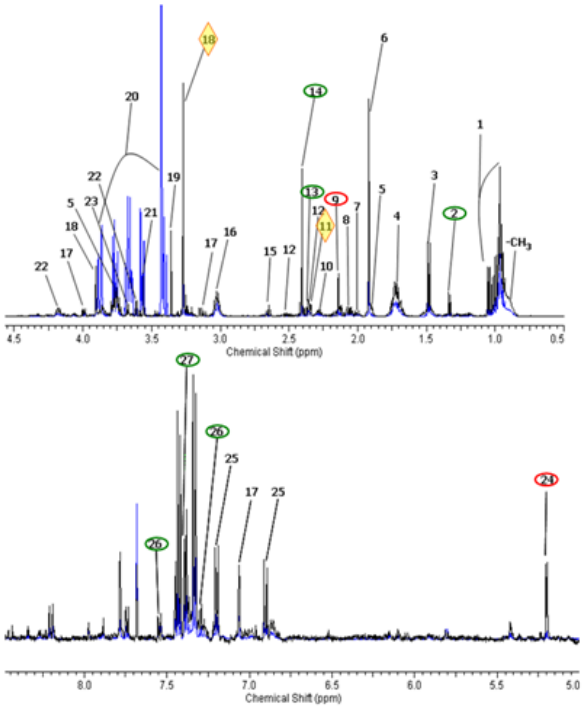
Metabolic activity quenched

↓

Samples extracted

↓

Analysis of metabolites by GC-MS and NMR



Characteristic Changes: Mutant vs. Wildtype

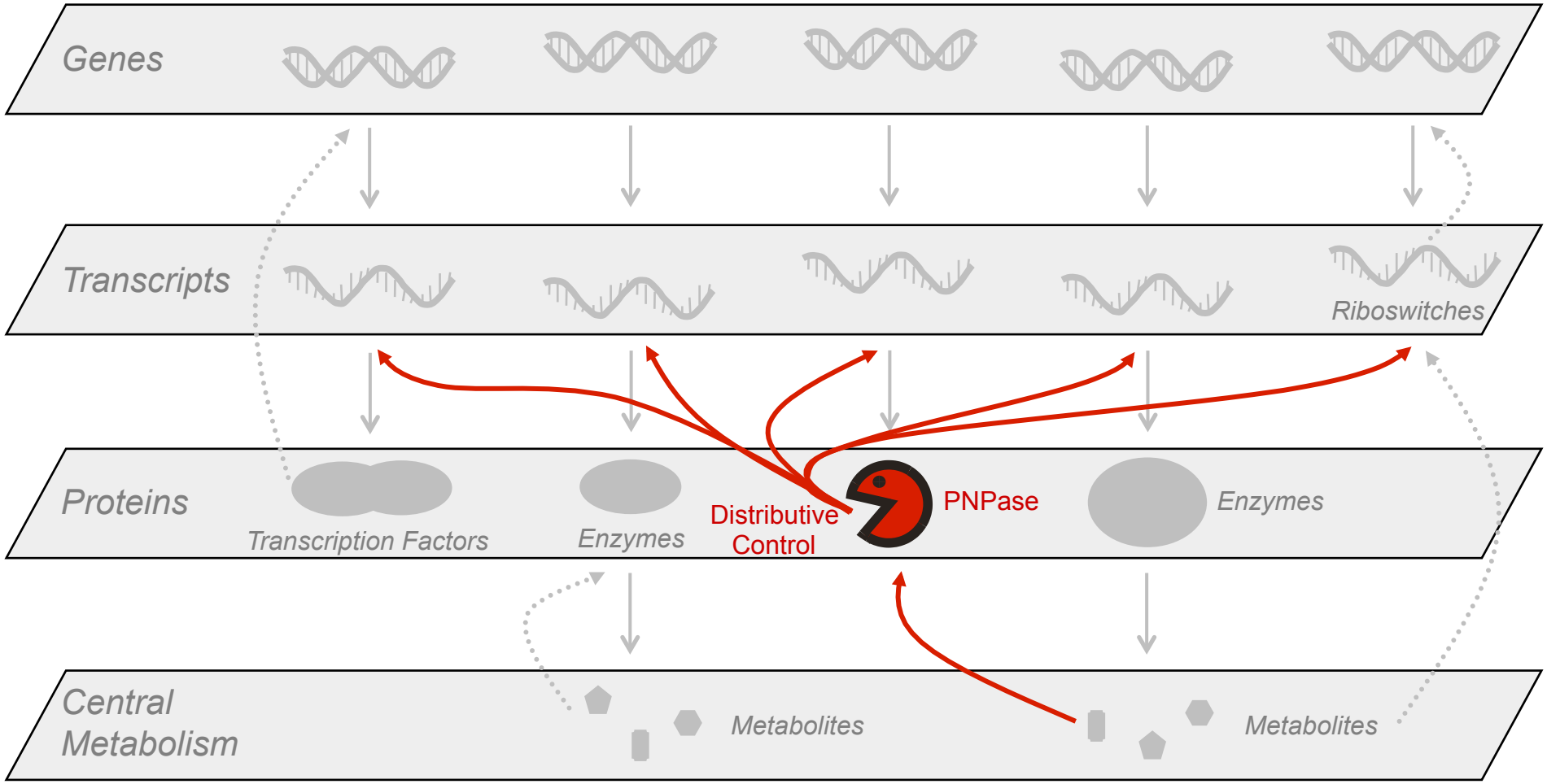
Unstressed condition

Increase:	Decrease:
18) Betaine	24) α –glucose
27) Phenylalanine	9) Methionine
14) Succinate	11) Proline
26) Tryptophan	
13) Pyruvate	
2) Lactate	

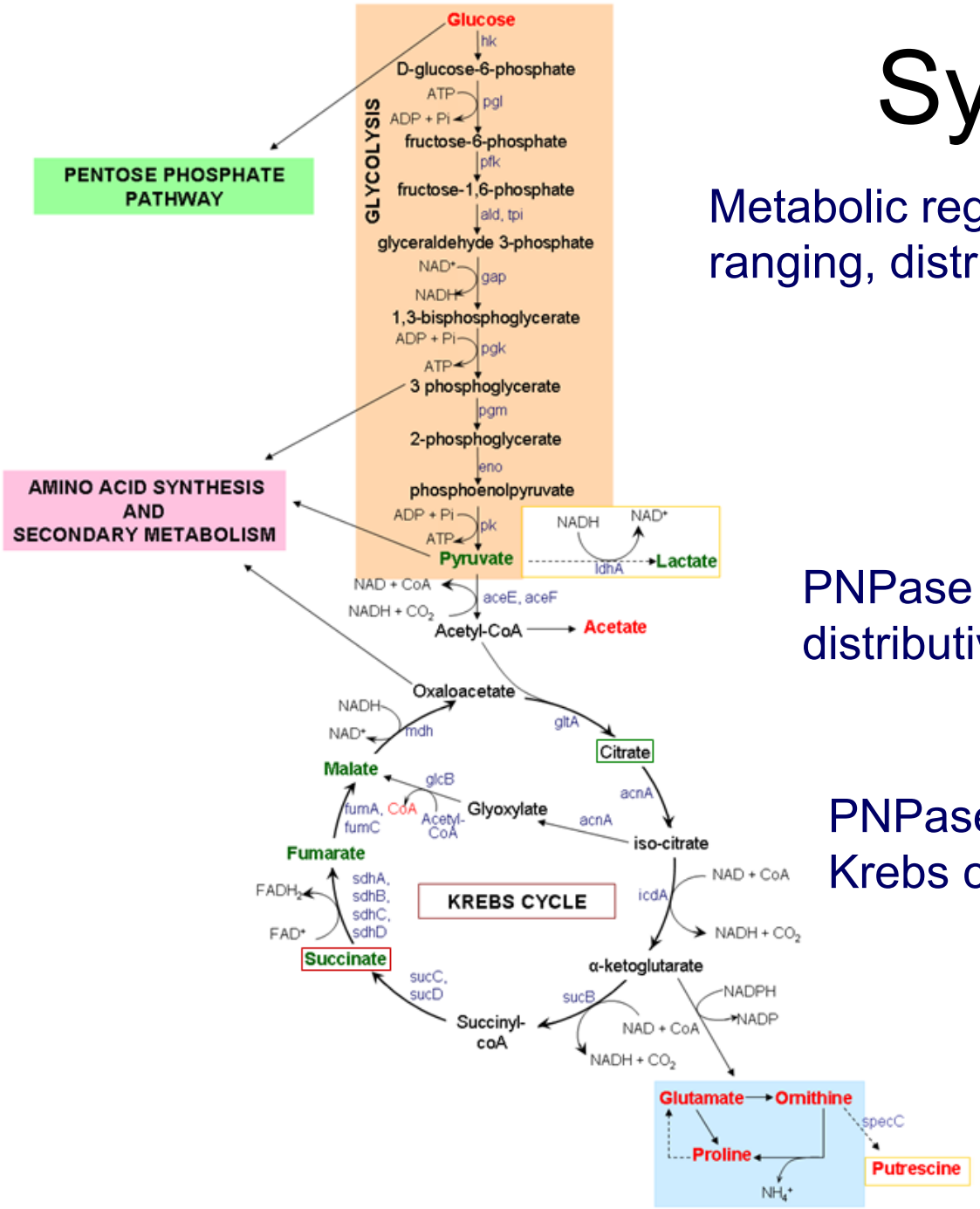
Phosphosugar Stress

Increase:	Decrease:
11) Proline	18) Betaine

RNA degradosome: towards a systems biology



Schematic illustration of the cellular mechanisms of control (grey) highlighting the link between metabolic status and RNA turnover (red). The activity of PNPase (red pacman) can be impacted by the Krebs cycle metabolite, citrate. Wide ranging, distributive control can consequently be mediated (red arrows).



Synopsis

Metabolic regulation likely requires wide ranging, distributive control

PNPase affects *E. coli* metabolism distributively

PNPase may be regulated Krebs cycle metabolites

Summary and conjecture, part 2:

To account for the steady-state levels of some metabolites, there must be some global coordination of enzyme levels. This is somehow optimised to balance between wasteful synthesis of enzyme and having insufficient enzyme activity

Somehow there is feedback of metabolic flux or derivatives at two or more points to transcription and post-transcription

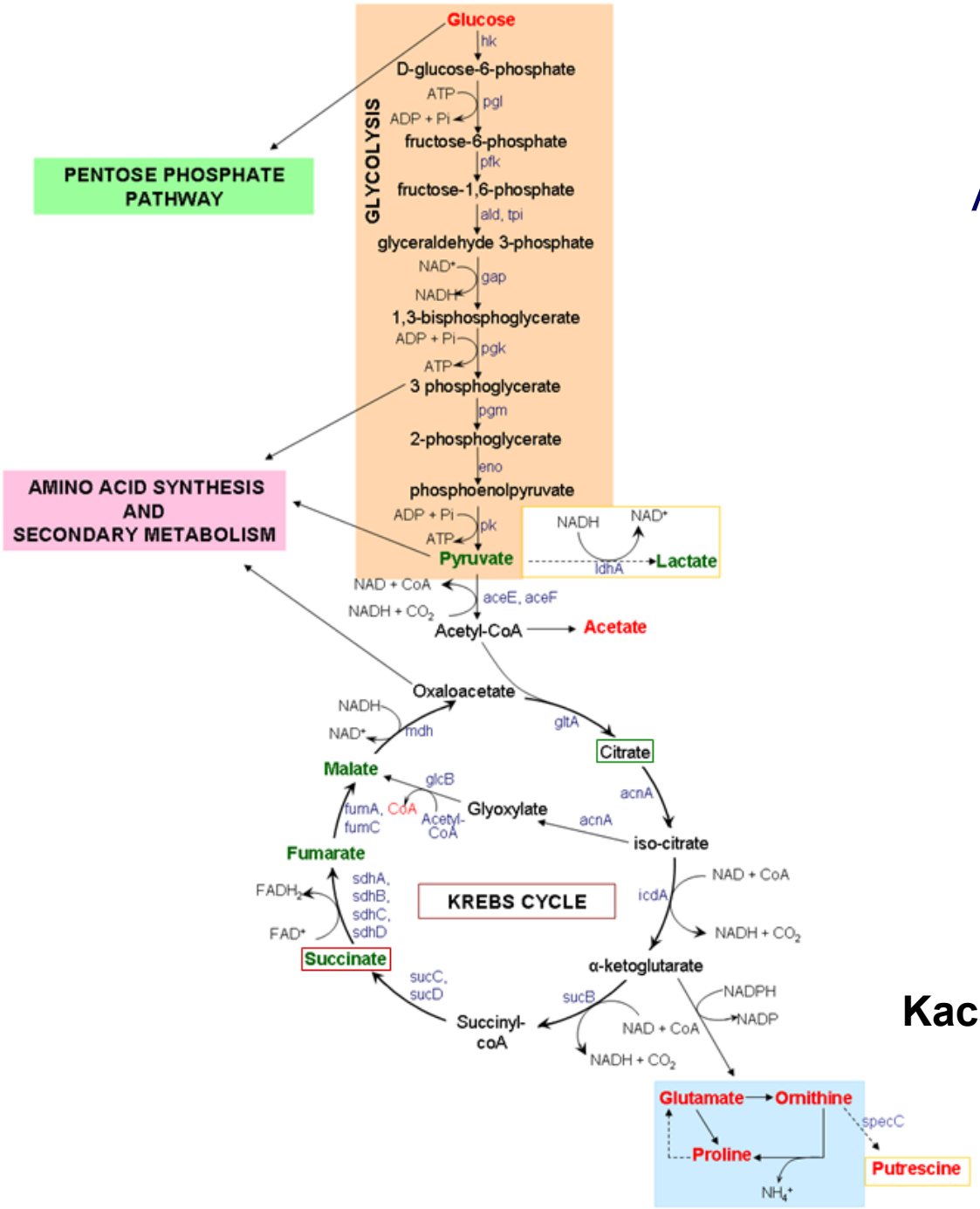
?

Questions to explore

To what extent are metabolites used for communication between distant points in metabolic pathways, and what impact does this have?

What organises metabolic systems so that metabolites accumulate?

Is there a meaningful link between metabolism and post-transcriptional regulation?



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