# Improved Cofactor $F_{420}$ production in *Escherichia coli*

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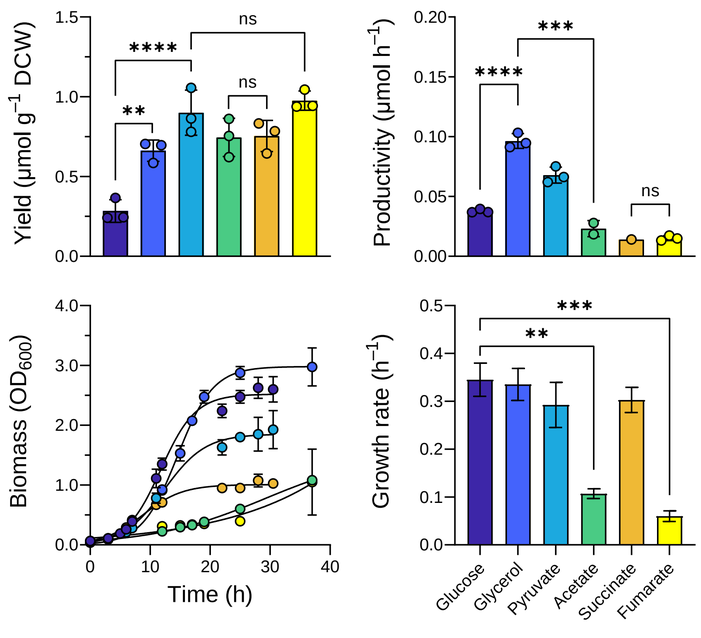
**The deazaflavin cofactor $F_{420}$ is a low-potential, two-electron redox cofactor produced by some Archaea and Eubacteria that is involved in methanogenesis and methanotrophy, antibiotic biosynthesis, and xenobiotic metabolism. However, it is not produced by bacterial strains commonly used for industrial biocatalysis or recombinant protein production, such as *Escherichia coli*, limiting our ability to exploit it as an enzymatic cofactor and produce it in high yield. Here, we have utilised a genome-scale metabolic model of *E. coli* and constraint-based metabolic modelling of cofactor $F_{420}$ biosynthesis to optimise $F_{420}$ production in *E. coli*. This analysis identified phosphoenolpyruvate (PEP) as a limiting precursor for $F_{420}$ biosynthesis, explaining carbon source-dependent differences in productivity. PEP availability was improved by using gluconeogenic carbon sources and overexpression of PEP synthase. By improving PEP availability, we were able to achieve a ~40-fold increase in the space-time yield of $F_{420}$ compared with the widely used recombinant *Mycobacterium smegmatis* expression system. This study establishes *E. coli* as an industrial $F_{420}$-production system and will allow the recombinant *in vivo* use of $F_{420}$-dependent enzymes for biocatalysis and protein engineering applications**

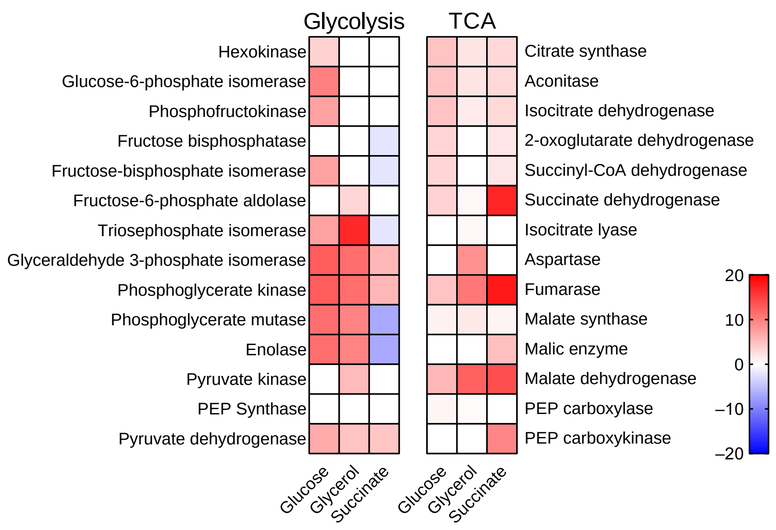
## Effect of carbon source

Compared with glucose, all tested carbon sources gave higher $F_{420}$ yields however this was at the expense of biomass for acetate, succinate and fumarate, as shown by the growth curves. While glycerol gave the highest productivity, pyruvate gave significantly higher yields with only a modest trade-off in biomass. The high yield and productivity achieved with pyruvate implies that pyruvate has a key impact on $F_{420}$ biosynthesis through phosphoenolpyruvate, a precursor to $F_{420}$.



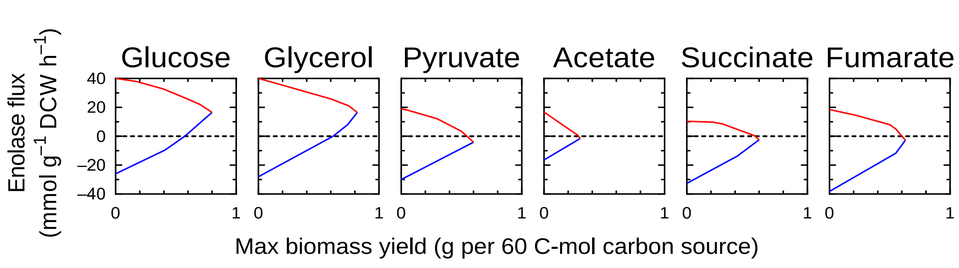

## Flux balance analysis

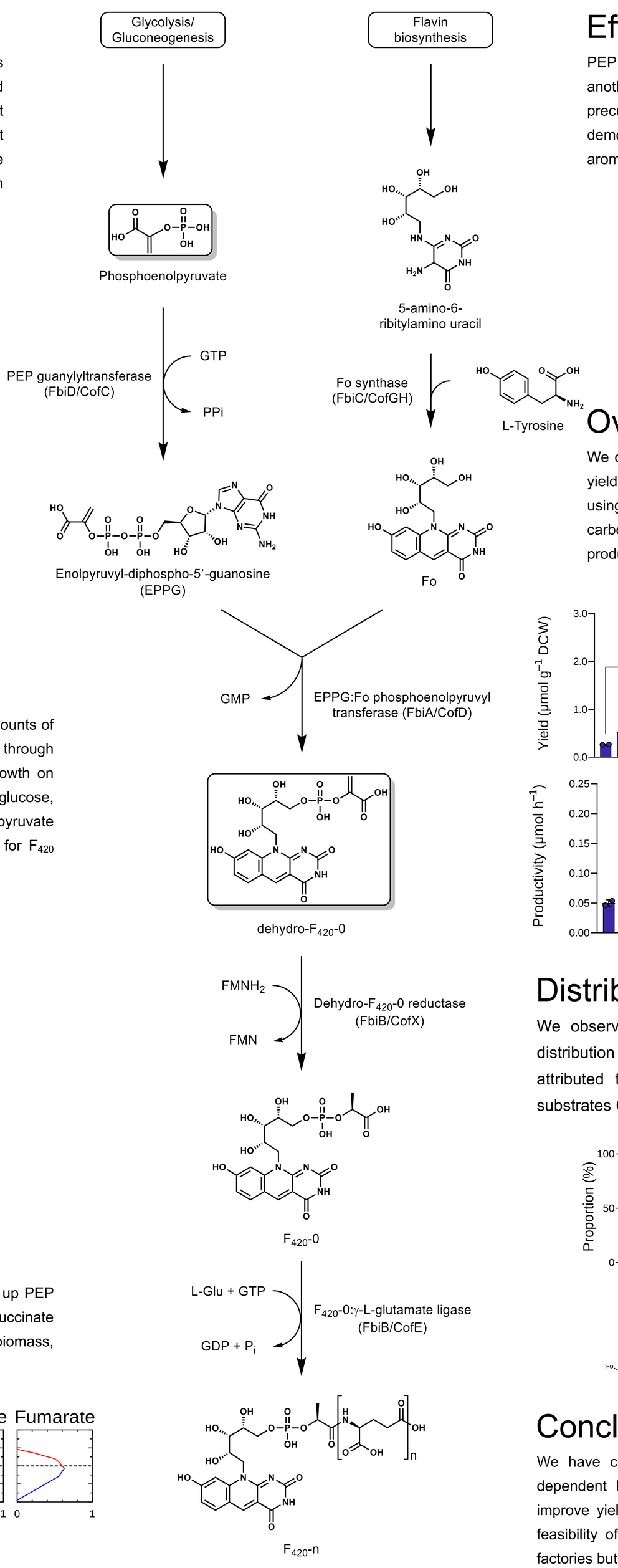
Flux balance analysis revealed that growth on glucose consumed large amounts of PEP *via* the glucose:PEP phosphotransferase system while having no flux through the PEP-producing enzymes PEP synthase and PEP carboxykinase. Growth on glycerol turned off upper glycolysis and produced 27% more ATP than glucose, while lowering flux through the TCA cycle. Growth on succinate lead to pyruvate production primarily via malate dehydrogenase, thereby freeing up PEP for $F_{420}$ biosynthesis.




## Flux variability analysis of enolase

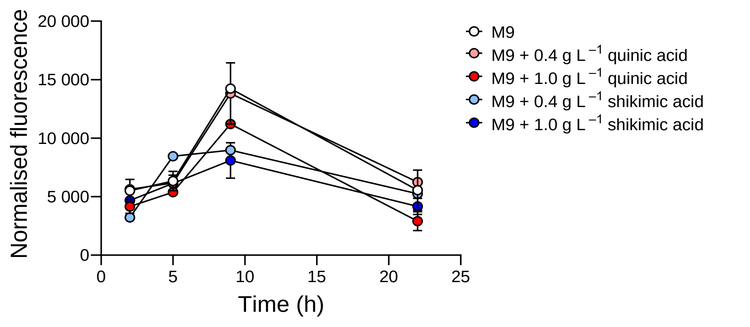
Flux variability analysis revealed that reducing biomass differentially frees up PEP for $F_{420}$ biosynthesis for different carbon sources. Growth on pyruvate, succinate and fumarate show negative minimum flux through enolate at maximum biomass, indicating that $F_{420}$ biosynthesis can proceed without affecting biomass.



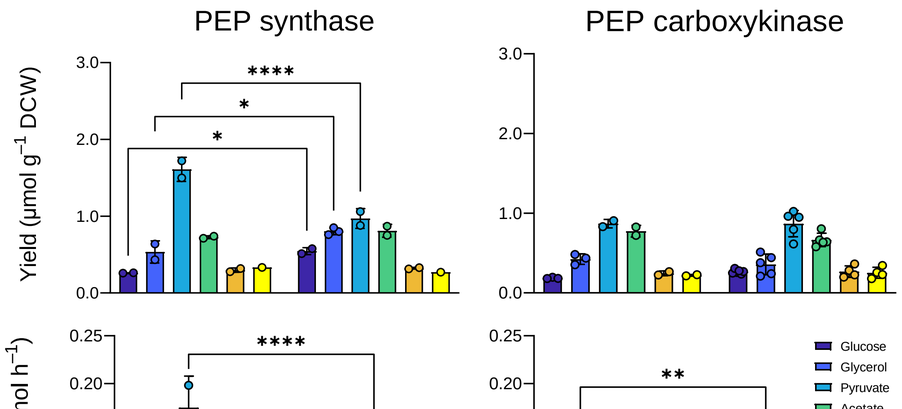

## Effect of tyrosine supplementation

PEP is incorporated directly into $F_{420}$ but is also a precursor to tyrosine, another substrate for $F_{420}$ biosynthesis. Supplementation of soluble tyrosine precursors quinic acid and shikimic acid did not improve $F_{420}$ production, demonstrating that the effect of PEP on $F_{420}$ yield is not mediated though aromatic amino acid biosynthesis.



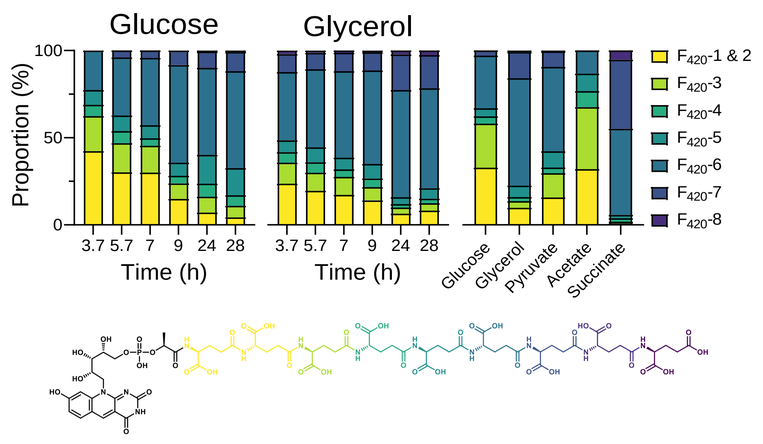

## Overexpression of PPS and PPCK

We overexpressed two PEP-producing enzymes to test their effect on $F_{420}$ yield and productivity. Overexpressing PEP synthase (PPS) improved yield using glucose and glycerol, and decreased yield using pyruvate. PEP carboxykinase conversely had no effect on yield while adversely affecting productivity using glycerol and pyruvate.




## Distribution of tail lengths

We observed both time and carbon source dependent changes in the distribution of polyglutamate chain lengths. Carbon source differences are attributed to deviations in the ratio of fluxes producing the two FbiB substrates GTP and L-glutamate.




## Conclusions and Future directions

We have constructed a genome-scale model of *E. coli* containing the PEP-dependent $F_{420}$ biosynthesis pathway and successfully used its predictions to improve yields by increasing the availability of PEP. This not only improves the feasibility of industrial-scale production of $F_{420}$ in well established microbial cell factories but provides a valuable model for future optimisations.

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See the full paper here: