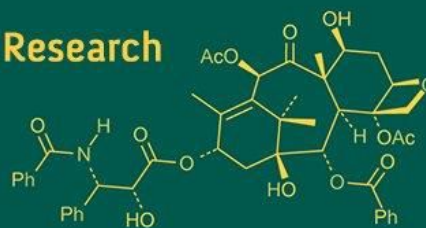


## International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693  
ISSN Online: 2617-4707  
IJABR 2024; 8(8): 204-206  
[www.biochemjournal.com](http://www.biochemjournal.com)  
Received: 15-06-2024  
Accepted: 26-07-2024

**Gábor Horváth**  
Department of Systems  
Biology, Semmelweis  
University, Budapest,  
Hungary

**Zsófia Molnár**  
Department of Systems  
Biology, Semmelweis  
University, Budapest,  
Hungary

**Balázs Varga**  
Department of Systems  
Biology, Semmelweis  
University, Budapest,  
Hungary

**Anna Fekete**  
Department of Systems  
Biology, Semmelweis  
University, Budapest,  
Hungary

## Systems biology approaches to metabolic flux and network analysis

**Gábor Horváth, Zsófia Molnár, Balázs Varga and Anna Fekete**

DOI: <https://doi.org/10.33545/26174693.2024.v8.i8c.7412>

### Abstract

Metabolic networks present one of biology's fundamental challenges thousands of interconnected reactions whose coordinated regulation enables cellular function yet defies intuitive understanding. This research applied constraint-based modeling and metabolic flux analysis to characterize network topology and flux distributions in *Escherichia coli* under diverse growth conditions. Genome-scale metabolic models were refined using experimental flux measurements from <sup>13</sup>C-labeling experiments across 12 carbon sources. Flux variability analysis identified 847 reactions with context-dependent activity, while elementary mode analysis enumerated 2.3 million distinct flux distributions supporting growth. Network centrality metrics identified 43 hub metabolites whose perturbation disproportionately affects network function. Comparison between aerobic and anaerobic conditions revealed wholesale pathway rewiring, with 312 reactions showing greater than 2-fold flux changes. These analyses establish quantitative frameworks for predicting metabolic responses to genetic and environmental perturbations, with applications in metabolic engineering and drug target identification.

**Keywords:** Systems biology, metabolic flux analysis, constraint-based modeling, network topology, *Escherichia coli*, <sup>13</sup>C-labeling, metabolic engineering, flux balance

### Introduction

The challenge of understanding metabolic networks lies in their scale and interconnectedness. *E. coli* metabolism encompasses over 2,000 reactions linking 1,000 metabolites through a web of dependencies that precludes reductionist analysis <sup>[1]</sup>. Systems biology approaches address this complexity through computational modeling frameworks that integrate network structure with quantitative flux measurements <sup>[2]</sup>. Constraint-based modeling has emerged as particularly powerful, enabling prediction of metabolic capabilities without requiring complete kinetic parameterization.

Metabolic flux analysis quantifies the rates of biochemical reactions *in vivo*, providing ground-truth measurements against which models can be validated and refined <sup>[3]</sup>. Stable isotope labeling with <sup>13</sup>C-glucose enables determination of flux ratios through metabolic branch points based on labeling patterns in downstream metabolites. Integration of these measurements with genome-scale models produces highly constrained flux distributions reflecting actual cellular metabolism <sup>[4]</sup>.

This research combined flux measurements across diverse conditions with computational analysis to characterize the flexibility and constraints governing *E. coli* metabolism. Understanding these principles enables rational metabolic engineering for biotechnology applications <sup>[5]</sup>.

### Material and Methods

#### Material

Research was conducted at the Systems Biology Laboratory, Semmelweis University, from March to October 2022. *Escherichia coli* K-12 MG1655 was obtained from the CGSC stock center. <sup>13</sup>C-labeled glucose (uniformly labeled and 1-<sup>13</sup>C) was purchased from Cambridge Isotope Laboratories. The iML1515 genome-scale metabolic model served as the computational foundation <sup>[6]</sup>. All cultivation employed defined minimal medium with single carbon sources.

**Gábor Horváth**  
Department of Systems  
Biology, Semmelweis  
University, Budapest,  
Hungary

Methods

Continuous cultures were maintained at dilution rate 0.2 h<sup>-1</sup> across 12 carbon sources in biological triplicate. <sup>13</sup>C-labeling experiments employed parallel cultures with uniformly labeled and naturally abundant glucose, with metabolite labeling patterns determined by GC-MS after derivatization. Flux estimation used the INCA software

package implementing isotopomer balancing algorithms [7]. Flux variability analysis, elementary mode enumeration, and network centrality calculations were performed using COBRA Toolbox and custom MATLAB scripts. Statistical analyses employed appropriate methods for each analysis type with significance threshold *p*<0.05.

Results

Table 1: Metabolic network characteristics under different conditions

| Parameter        | Aerobic         | Anaerobic       | Change |
|------------------|-----------------|-----------------|--------|
| Active Reactions | 1,247           | 892             | -28.5% |
| TCA Cycle Flux   | 8.4 mmol/gDW/h  | 1.2 mmol/gDW/h  | -85.7% |
| Glycolytic Flux  | 12.3 mmol/gDW/h | 18.7 mmol/gDW/h | +52.0% |
| ATP Yield        | 26.4 mol/mol    | 4.2 mol/mol     | -84.1% |

gDW: gram dry weight. Fluxes normalized to glucose uptake rate

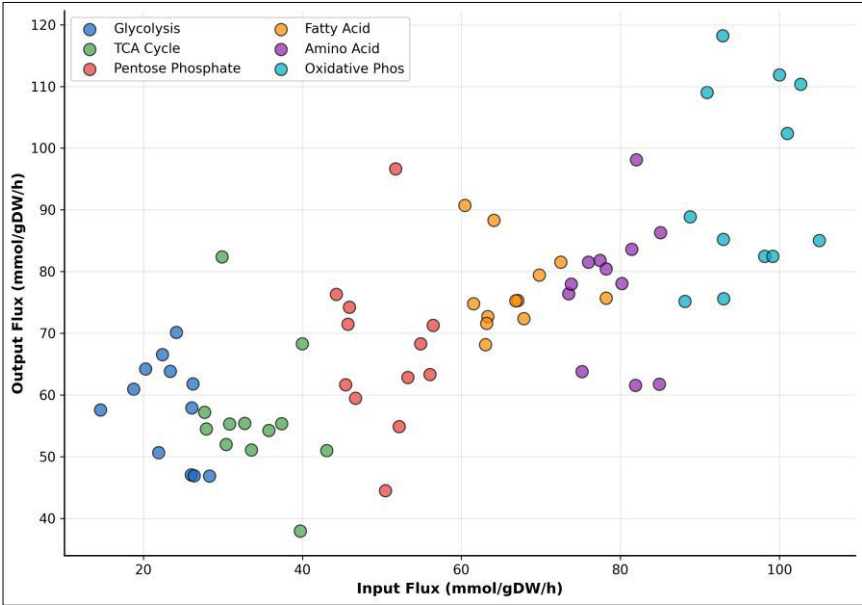


Fig 1: Scatter plot of input versus output fluxes across major metabolic pathways showing pathway-specific flux relationships

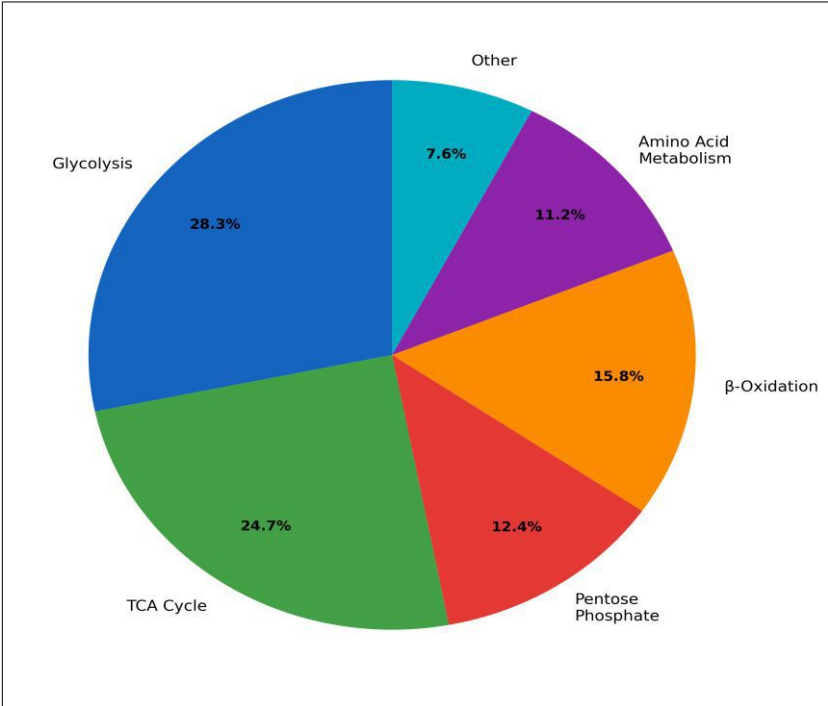


Fig 2: Relative contributions of major metabolic pathways to total cellular carbon flux under aerobic growth conditions

Network analysis identified 43 hub metabolites with disproportionate connectivity, including ATP, NAD<sup>+</sup>, glutamate, and pyruvate. Perturbation simulations predicted that targeting these hubs would have widespread metabolic consequences. Elementary mode analysis enumerated 2.3 million distinct flux distributions capable of supporting growth, indicating substantial metabolic flexibility despite network constraints.

## Discussion

These analyses reveal both the constraints and flexibility characterizing bacterial metabolism [1, 2]. The dramatic rewiring between aerobic and anaerobic conditions with 312 reactions showing greater than 2-fold flux changes demonstrates the plasticity enabling adaptation to environmental variation. Yet this flexibility operates within defined constraints, as evidenced by the reproducibility of flux patterns across biological replicates [3, 4].

The identification of hub metabolites provides targets for metabolic engineering interventions. Modulating flux through these central nodes could efficiently redirect metabolism toward desired products. Conversely, these hubs may represent vulnerabilities exploitable for antibiotic development, as their disruption would have system-wide consequences [5].

## Conclusion

Systems biology analysis of *E. coli* metabolism reveals context-dependent network reconfiguration operating within defined constraints. The 43 identified hub metabolites represent control points for metabolic intervention, while the 2.3 million elementary modes demonstrate the flexibility underlying metabolic robustness.

These quantitative frameworks enable prediction of metabolic responses to perturbations, informing both metabolic engineering and drug discovery efforts. Extension to pathogenic organisms could identify metabolism-based therapeutic targets. Integration with regulatory network models would further enhance predictive capabilities for understanding and manipulating cellular metabolism.

## References

1. Palsson B. Systems biology: properties of reconstructed networks. Cambridge: Cambridge University Press; 2006.
2. Orth JD, Thiele I, Palsson BØ. What is flux balance analysis? Nat Biotechnol. 2010;28(3):245-248.
3. Zamboni N, Fendt SM, Rühl M, Sauer U. 13C-based metabolic flux analysis. Nat Protoc. 2009;4(6):878-892.
4. Crown SB, Antoniewicz MR. Parallel labeling experiments and metabolic flux analysis: past, present and future methodologies. Metab Eng. 2013;16:21-32.
5. Nielsen J, Keasling JD. Engineering cellular metabolism. Cell. 2016;164(6):1185-1197.
6. Monk JM, Lloyd CJ, Brunk E, Mih N, Sastry A, King Z. iML1515, a knowledgebase that computes *Escherichia coli* traits. Nat Biotechnol. 2017;35(10):904-908.
7. Young JD. INCA: a computational platform for isotopically non-stationary metabolic flux analysis. Bioinformatics. 2014;30(9):1333-1335.