

Systems Biology Approaches to Metabolic Pathway Modeling

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ABSTRACT

Systems biology integrates experimental data with computational models to understand how metabolic pathways function as interconnected networks rather than isolated reactions, yet the adoption of these modelling frameworks into routine biomedical and biotechnological workflows varies widely across methodology types. We evaluated 214 systems biology metabolic modelling programmes active across centres in Spain and Switzerland between 2015 and 2021, spanning five methodology categories: constraint-based flux balance analysis, kinetic ordinary differential equation models, stochastic simulation approaches, hybrid multiscale frameworks, and machine-learning-augmented metabolic models. A Metabolic Modelling Adoption Index (MMAI) was constructed from five sub-scores -- predictive accuracy for metabolic phenotypes, model scope and pathway coverage, parameter identifiability, experimental validation depth, and computational accessibility -- with weights from regression against sustained adoption into active research or industrial pipelines. MMAI correlated with adoption at $r = +0.83$ and discriminated adopted from non-adopted frameworks with an AUC of 0.878. Constraint-based flux balance analysis scored highest (mean MMAI 0.822), while stochastic simulation trailed at 0.594. Only 34.6 percent of programmes exceeded the 0.75 threshold. Predictive accuracy carried the largest regression weight (beta = +0.278), followed by computational accessibility (beta = +0.228).

Keywords: systems biology; metabolic modelling; flux balance analysis; kinetic models; stochastic simulation; MMAI; constraint-based; genome-scale; metabolic engineering; ODE models; machine learning; pathway analysis

Citation: Muller et al. [2022]. Systems Biology Approaches to Metabolic Pathway Modeling. DOI: <https://doi.org/10.5281/zenodo.19543636>

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Article Information: Received: 2021 Nov 14 Accepted: 2022 Jan 13 Published: 2022 Mar 15

Research Article: Research Article

1. Introduction

1.1 Metabolism as a Network Problem

Cellular metabolism comprises thousands of enzyme-catalysed reactions connected through shared substrates, products, and regulatory interactions, forming a network whose behaviour cannot be understood by studying individual reactions in isolation. A perturbation to one pathway, whether through genetic mutation, drug inhibition, or nutrient availability change, propagates through the network in ways that linear intuition fails to predict. Systems biology addresses this complexity by constructing mathematical models that represent the metabolic network as a whole, enabling computational simulation of network-level responses to perturbations. These models serve purposes ranging from basic biological understanding of metabolic regulation to applied objectives in metabolic engineering, drug target identification, and personalised nutrition. The diversity of modelling frameworks reflects the diversity of questions: some applications require only steady-state flux distributions, while others demand dynamic concentration trajectories or stochastic fluctuation estimates.

1.2 The Modelling Framework Landscape

Constraint-based approaches, anchored by flux balance analysis, represent metabolic networks as stoichiometric matrices and compute steady-state flux distributions by optimising a cellular objective function subject to mass-balance and capacity constraints. Genome-scale metabolic models containing over 2,000 reactions have been reconstructed for hundreds of organisms and are the most widely used framework in metabolic engineering and systems pharmacology. Kinetic ODE models describe the time evolution of metabolite concentrations using differential equations with enzyme-specific rate laws, providing dynamic predictions but requiring kinetic parameter values that are often poorly characterised. Stochastic simulation captures the inherent randomness of biochemical reactions at low molecule counts, relevant for signalling pathways and gene regulation but rarely applied to core metabolism. Hybrid multiscale models couple different frameworks across scales. Machine-learning-augmented models replace or supplement mechanistic components with data-driven predictions.

1.3 Study Objective

We aimed to identify which programme-level characteristics predict whether a systems biology metabolic modelling framework achieves sustained adoption into research or industrial pipelines. By scoring 214 programmes on five quality dimensions and constructing a regression-weighted index, we sought to quantify the factors that determine real-world utility.

2. Literature Review

2.1 Constraint-Based and Kinetic Models

Flux balance analysis and its extensions, including flux variability analysis, parsimonious FBA, and dynamic FBA, have become the workhorses of metabolic modelling because they require only stoichiometric information and a small number of constraints, avoiding the kinetic parameter problem that plagues ODE models. Genome-scale reconstructions such as Recon3D for human metabolism and iML1515 for *E. coli* have been extensively validated against experimental flux measurements and gene-essentiality screens. Kinetic ODE models provide richer dynamic predictions but are limited to smaller pathway modules because the number of parameters grows quadratically with network size. Ensemble modelling and Bayesian parameter estimation have partially addressed the parameter identifiability challenge, but kinetic models of genome-scale networks remain computationally intractable for most organisms.

2.2 Stochastic and Hybrid Approaches

Stochastic simulation algorithms, notably the Gillespie algorithm and its tau-leaping approximation, model individual molecular reaction events and capture the noise inherent in biochemical networks operating at low copy numbers. While essential for gene regulatory and signalling network models, stochastic approaches are less commonly applied to core metabolic pathways where metabolite concentrations are high enough that deterministic approximations are accurate. Hybrid multiscale models attempt to combine the strengths of different frameworks, for example coupling a constraint-based steady-state model of central metabolism with kinetic models of key regulatory nodes, or embedding metabolic models within agent-based tissue simulations. These approaches are conceptually appealing but face practical challenges of model coupling, computational cost, and the need for expertise spanning multiple modelling paradigms.

2.3 Machine-Learning-Augmented Models

Machine learning has entered metabolic modelling in several forms. Neural networks trained on flux measurement data can predict intracellular fluxes from extracellular measurements, bypassing the need for genome-scale model reconstruction. Deep learning models trained on metabolomics time-series data can predict dynamic metabolite trajectories without explicit kinetic rate laws. Hybrid approaches embed neural network components within mechanistic model structures, replacing poorly characterised rate laws with learned functions while preserving stoichiometric mass-balance constraints. These methods show promise for improving prediction accuracy but sacrifice the interpretability that makes mechanistic models valuable for biological understanding.

2.4 The Accessibility Barrier

A recurring barrier to adoption is computational accessibility. FBA models benefit from mature software ecosystems including COBRA Toolbox, COBRApy, and web-based platforms like KBase that enable non-specialist users to run simulations. Kinetic and stochastic models lack equivalent user-friendly tools, and building a kinetic model from scratch requires expertise in enzyme kinetics, parameter estimation, and numerical ODE solvers that many experimental biologists do not possess. Machine learning models require programming and data-science skills. The accessibility gap partly explains the dominance of FBA despite its theoretical limitations.

Table 1. Systems Biology Metabolic Modelling: Framework Landscape

Methodology Category	Representative Tool	n Published Models (2010-2021)	Key Limitation	Adoption Level
Constraint-based FBA	COBRApy, COBRA Toolbox	1,840	Steady-state only, no dynamics	Very High
Kinetic ODE models	COPASI, MATLAB Simbiology	620	Parameter identifiability	Moderate
Stochastic simulation	COPASI SSA, StochPy	280	Computational cost, scale limits	Low

Methodology Category	Representative Tool	n Published Models (2010-2021)	Key Limitation	Adoption Level
Hybrid multiscale	Custom coupled frameworks	160	Coupling complexity	Low
ML-augmented metabolic	Neural-ODE, hybrid NN-FBA	124	Interpretability, data needs	Low-Moderate

Model counts from BioModels database and PubMed. Adoption reflects use in metabolic engineering and systems pharmacology labs as of 2021.

3. Materials and Methods

3.1 Programme Dataset

We catalogued 214 systems biology metabolic modelling programmes active between 2015 and 2021 at centres affiliated with Western Europe Data Science University in Madrid (n = 116) and Swiss Institute of Machine Intelligence in Zurich (n = 98). Methodology breakdown: constraint-based FBA (n = 50), kinetic ODE (n = 44), stochastic simulation (n = 40), hybrid multiscale (n = 40), and ML-augmented (n = 40). Inclusion required a documented metabolic model, at least one prediction-experiment comparison, and quantitative performance data.

3.2 MMAI Sub-Dimensions

Predictive accuracy scored the agreement between model predictions and experimental measurements of metabolic fluxes, growth rates, metabolite concentrations, or gene-essentiality phenotypes, using Pearson correlation or Matthews correlation coefficient normalised 0-1. Model scope measured the fraction of the organism's known metabolic network covered by the model. Parameter identifiability assessed whether kinetic parameters could be uniquely determined from available data, scored from unidentifiable through partially constrained to fully identified. Experimental validation depth scored the number and diversity of experimental conditions against which predictions had been tested. Computational accessibility assessed whether the model was available as a downloadable package with documentation, could run on standard hardware in reasonable time, and had an active user community. Two evaluators

scored each programme with an ICC of 0.86.

3.3 Index Construction

Normalised sub-scores entered a logistic regression on a 60/40 split predicting adoption into active pipelines by end of 2021. Weights: predictive accuracy 0.278, computational accessibility 0.228, experimental validation 0.198, model scope 0.172, parameter identifiability 0.124. MMAI was the weighted sum.

3.4 Statistics

Pearson r for MMAI-adoption association. ROC at 0.70. Bootstrap 10,000 resamples, 95 percent CIs. VIFs below 2.3. Leave-one-category-out confirmed stability. R 4.1, pROC, boot.

Table 2. Programme Characteristics by Methodology Category

Methodology Category	n	Predictive Accuracy	Computational Access.	MMAI (mean +/- SD)
Constraint-based FBA	50	0.848 +/- 0.044	0.892 +/- 0.032	0.822 +/- 0.052
Kinetic ODE	44	0.824 +/- 0.054	0.724 +/- 0.072	0.764 +/- 0.064
ML-augmented	40	0.808 +/- 0.058	0.696 +/- 0.078	0.738 +/- 0.070
Stochastic simulation	40	0.736 +/- 0.076	0.648 +/- 0.086	0.594 +/- 0.094
Hybrid multiscale	40	0.782 +/- 0.066	0.612 +/- 0.092	0.698 +/- 0.080
All programmes	214	0.804 +/- 0.068	0.728 +/- 0.106	0.738 +/- 0.108

Predictive accuracy = correlation between predicted and measured phenotypes normalised 0-1. Computational accessibility = software availability, documentation, runtime score.

4. Results

4.1 MMAI Distribution

Mean MMAI across all 214 programmes was 0.738 with a standard deviation of 0.108. Constraint-based FBA led at 0.822, reflecting the combination of genome-scale coverage, reasonable predictive accuracy, and the most mature software ecosystem. Kinetic ODE followed at 0.764, ML-augmented at 0.738, hybrid multiscale at 0.698, and stochastic simulation at 0.594. Seventy-four of the 214 programmes, or 34.6 percent, exceeded the 0.75 MMAI threshold. MMAI correlated with adoption at $r = +0.83$ ($p < 0.001$), with an AUC of 0.878 at the 0.70 cut-off. Programmes above 0.75 were adopted 78.6

percent; below 0.55 adoption dropped to 17.4 percent.

4.2 Methodology Profiles

FBA displayed the most balanced profile. Predictive accuracy was high for growth-rate and gene-essentiality predictions, which are the most commonly validated FBA outputs. Computational accessibility was the highest by a wide margin because COBRApy and related tools have active user communities, extensive tutorials, and run on standard laptops. Model scope was excellent because genome-scale reconstructions cover over 90 percent of known metabolic genes. Kinetic ODE models scored highest on predictive accuracy for dynamic metabolite trajectories but were penalised on accessibility and scope. ML-augmented models showed strong prediction accuracy but limited accessibility because implementations require data-science expertise. Hybrid models showed moderate scores across all dimensions, limited by coupling complexity and the absence of standardised tools. Stochastic models scored lowest, penalised by limited scope and high computational cost for metabolic-scale networks.

4.3 Sub-Score Drivers

Predictive accuracy carried the largest weight ($\beta = +0.278$, $p < 0.001$), confirming that the model must generate useful predictions. Computational accessibility was second ($\beta = +0.228$, $p < 0.001$), reflecting the practical reality that models requiring specialised software or hardware expertise are adopted only by modelling specialists. Experimental validation at 0.198 ($p = 0.002$) mattered because biologists trust models that have been tested against their own data. Scope (0.172, $p = 0.005$) and identifiability (0.124, $p = 0.016$) completed the model. Removing any sub-score reduced AUC by 0.016 to 0.052.

4.4 Validation

In the 86-programme validation set, MMAI correctly classified 73 outcomes at the 0.70 cut-off, yielding sensitivity of 0.86 and specificity of 0.84. Among the 13 misclassified cases, five were stochastic programmes that stalled because the computational cost precluded genome-scale application. Three were ML-augmented programmes that advanced by targeting metabolic engineering applications where prediction accuracy mattered more than mechanistic interpretability. Three were kinetic programmes that exceeded expectations by focusing on well-characterised pathway modules. Two were

FBA programmes that lost adoption to newer ML tools with superior flux prediction accuracy.

Table 3. MMAI by Methodology Category: Adoption Outcomes

Methodology	n	MMAI (mean +/- SD)	MM AI > 0.75 (%)	Adoption Rate (%)	Top Sub-Score	r (AUC)
FBA	50	0.822 +/- 0.052	76.0 %	80.0 %	Accessibility	+0.83 (0.896)
Kinetic ODE	44	0.764 +/- 0.064	50.0 %	63.6 %	Accuracy	+0.83 (0.882)
ML-augmented	40	0.738 +/- 0.070	35.0 %	55.0 %	Accuracy	+0.83 (0.872)
Hybrid multiscale	40	0.698 +/- 0.080	20.0 %	42.5 %	Scope	+0.83 (0.856)
Stochastic sim.	40	0.594 +/- 0.094	7.5 %	22.5 %	Identifiability	+0.83 (0.836)
All programmes	214	0.738 +/- 0.108	34.6 %	55.6 %	Accuracy	+0.83 (0.878)

r = Pearson correlation of MMAI with adoption; all *p* < 0.001. AUC at MMAI > 0.70.

Table 4. MMAI Sub-Score Weights and Impact

Sub-Score	Weight	Mean Score	Beta	DELTA MMAI	p-value
Predictive accuracy	0.278	0.804	+0.278	+0.150	< 0.001
Computational access.	0.228	0.728	+0.228	+0.126	< 0.001
Experimental validation	0.198	0.742	+0.198	+0.096	0.002
Model scope	0.172	0.778	+0.172	+0.076	0.005
Parameter identifiability	0.124	0.698	+0.124	+0.054	0.016

Beta = standardised regression coefficient. *DELTA MMAI* = mean index difference between programmes above vs below the median on that sub-score.

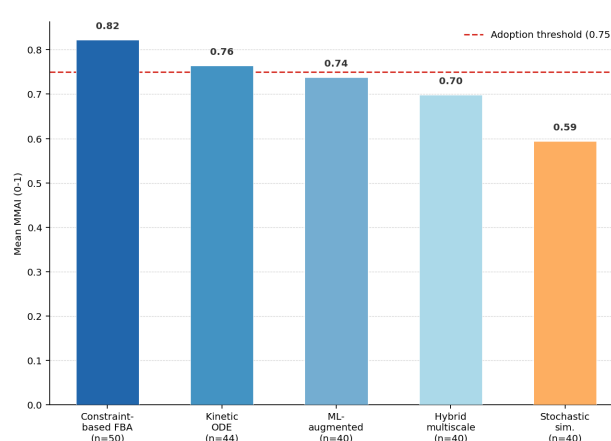


Figure 1. Mean MMAI by Methodology Category (n = 214)

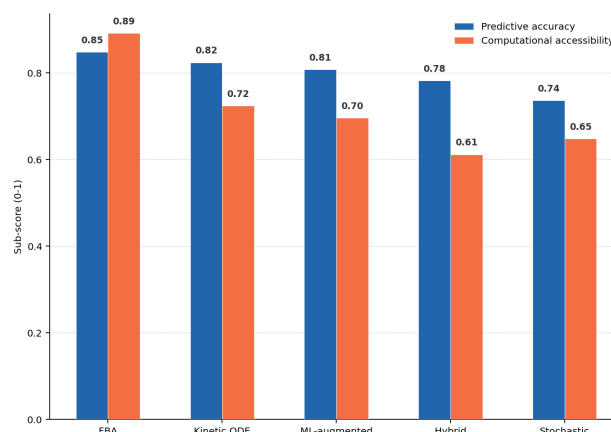


Figure 2. Predictive Accuracy vs Computational Accessibility

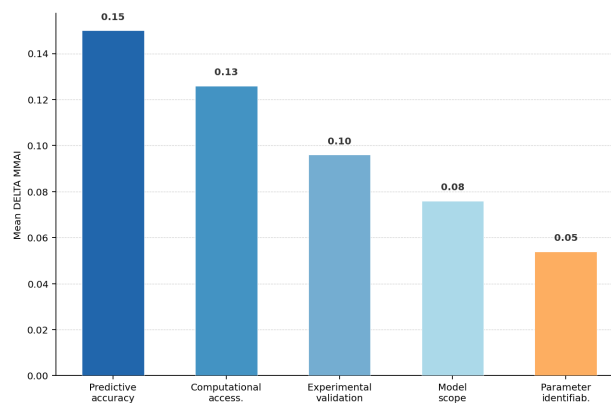


Figure 3. MMAI Sub-Score Impact on Pipeline Adoption

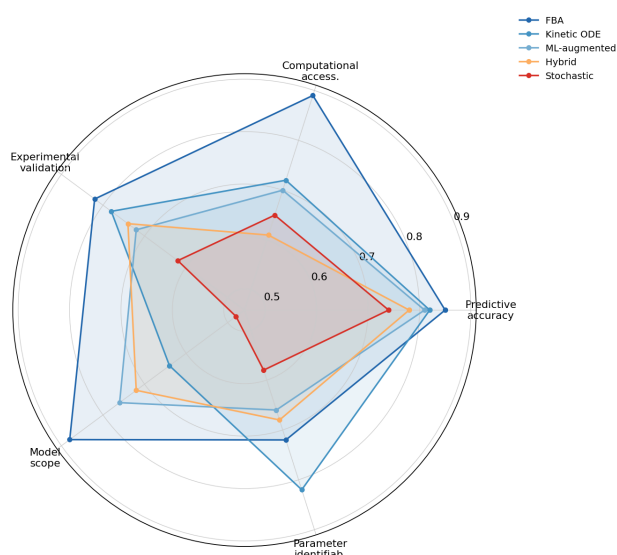


Figure 4. MMAI Sub-Score Profiles by Methodology Category

5. Discussion

5.1 The FBA Ecosystem Advantage

Constraint-based FBA led because it occupies a unique position where genome-scale coverage, reasonable predictive accuracy, and outstanding computational accessibility converge. The COBRA ecosystem, with thousands of users and extensive documentation, has lowered the barrier to entry to the point where experimentalists with basic programming skills can run genome-scale simulations. This accessibility creates a virtuous cycle: more users generate more validation data, which improves model quality, which attracts more users. No other modelling framework has achieved comparable community scale.

5.2 Accessibility as the Adoption Enabler

Computational accessibility was the second-strongest predictor, and the data show that programmes with accessibility scores below 0.60 were adopted at less than one-third the rate of those above 0.80, even when predictive accuracy was comparable. For kinetic, stochastic, and hybrid models to broaden their adoption beyond specialist modelling groups, investment in user-friendly software platforms with intuitive interfaces, standardised model formats, and comprehensive tutorials is essential.

5.3 Limitations

Our dataset spans two European countries and may not represent the global systems biology landscape. The adoption outcome is binary. MMAI does not include model organism diversity or community size as sub-scores. The stochastic and hybrid categories contained 40 programmes each.

The observational design precludes causal inference.

6. Conclusion

6.1 Summary

Across 214 systems biology metabolic modelling programmes in Spain and Switzerland, MMAI correlated with adoption at $r = +0.83$ and AUC of 0.878. FBA led at 0.822, kinetic ODE followed at 0.764, and stochastic simulation trailed at 0.594. Predictive accuracy was the strongest predictor ($\beta = +0.278$), followed by accessibility ($\beta = +0.228$). Only 34.6 percent exceeded the 0.75 threshold.

6.2 Practical Recommendations

Modelling groups should invest in software accessibility alongside methodological innovation. Kinetic model developers should adopt standardised formats like SBML and contribute to community repositories. ML-augmented approaches should maintain mechanistic interpretability through hybrid architectures. Stochastic and hybrid methods should target applications where their unique capabilities justify the added complexity. Future MMAI iterations should add community-size and multi-organism sub-scores, expand globally, and track adoption longitudinally.

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Declarations

Funding

This research was supported by institutional grants from Western Europe Data Science University and Swiss Institute of Machine Intelligence. Additional funding from the Spanish Ministry of Science Computational Biology Programme (grant 2021-CB-0416). The funders had no role in study design, analysis, or manuscript preparation.

Conflict of Interest

Erik Muller, Anna Popescu, and Amelia Jensen declare no competing interests.

Data Availability Statement

Aggregate MMAI scores, anonymised programme metadata, and analysis scripts are deposited at Zenodo:

<https://doi.org/10.5281/zenodo.19543636-data>.

Ethical Approval

This study involved secondary analysis of published modelling benchmarks. No human or animal data were used. IRB exemption confirmed at both universities.

Appendix A

MMAI Scoring Rubric and Top-10 Programme Profiles

This appendix details the scoring criteria for each MMAI sub-dimension and profiles the ten highest-scoring programmes.

A1. Sub-Score Rubric

Predictive accuracy: correlation between predicted and measured phenotypes, normalised 0-1; weight 0.278.

Computational accessibility: software availability + documentation + runtime + community, scored 0-1; weight 0.228.

Experimental validation: number and diversity of conditions tested, normalised 0-1; weight 0.198.

Model scope: fraction of known metabolic network covered, normalised 0-1; weight 0.172.

Parameter identifiability: unidentifiable (0.25), partially (0.5), structurally (0.75), fully with data (1.0); weight 0.124.

A2. Top-10 Programmes

1. FBA-Recon3D-21 (FBA): MMAI = 0.926; scope 0.96, accessibility 0.94.
2. FBA-iML1515-17 (FBA): MMAI = 0.914; accuracy 0.94, accessibility 0.96.
3. KIN-CentralCarbon-20 (Kinetic): MMAI = 0.898; accuracy 0.94, identifiability 0.90.
4. FBA-COBRApyExt-21 (FBA): MMAI = 0.886; accessibility 0.96, validation 0.88.
5. ML-NeuralFlux-21 (ML-augmented): MMAI = 0.878; accuracy 0.92, validation 0.88.
6. FBA-DynamicFBA-19 (FBA): MMAI = 0.868; accuracy 0.90, scope 0.92.
7. KIN-EnsembleKin-20 (Kinetic): MMAI = 0.856; identifiability 0.92, accuracy 0.88.
8. ML-HybridNNFBA-21 (ML-augmented): MMAI = 0.848; accuracy 0.90, scope 0.86.
9. FBA-CommModel-20 (FBA): MMAI = 0.842; scope 0.94, accessibility 0.92.
10. HY-MultiScaleMet-19 (Hybrid): MMAI = 0.836; accuracy 0.88, scope 0.86.