Acta Scientific Microbiology (ISSN: 2581-3226)

Volume 9 Issue 2 February 2026

Review Article

Ab Initio Whole Cell Kinetic Model of *Lacticaseibacillus paracasei* Subspecies paracasei NTU 101 (lcaKKNC26)

Cheryl Kai Ning Kang[1,2], Sragvi Verma[1,2], Diya Nanthakumarvani[1,2], Shafeeqa Abul-Hasan[1,2], Leesha Haarshiny Perumal[1,2], Atoshi Abirami RajKumar[1,2] and Maurice HT Ling[2,3,4]*

[1]*Department of Applied Sciences, Northumbria University, United Kingdom*

[2]*Management Development Institute of Singapore, Singapore*

[3]*Newcastle Australia Institute of Higher Education, University of Newcastle, Australia*

[4]*HOHY PTE LTD, Singapore*

***Corresponding Author:** Maurice HT Ling, Management Development Institute of Singapore, Singapore.

DOI: 10.31080/ASMI.2026.09.1574

Received: December 12, 2025

Published: January 31, 2026

Abstract

Lacticaseibacillus paracasei subspecies *paracasei* NTU 101 was isolated from the faecal matter of a healthy, breast-fed newborn infant in Taiwan. It has been shown to be potentially useful in gut health, and recognised by US FDA as "generally recognised as safe" leading to potential engineered probiotics applications. Mathematical kinetic models provide time-course profile of modelled metabolites, which can be used to guide metabolic engineering approaches. However, there is no kinetic model of *L. paracasei* to-date. In this study, we present a whole cell simulatable kinetic model of *L. paracasei* subspecies *paracasei* NTU 101, lcaKKNC26, constructed using *ab initio* approach by identifying enzymes from its published genome. The resulting model consists of 846 metabolites, 356 enzymes with corresponding transcriptions and translations, and 677 enzymatic reactions; which can be a baseline model for incorporating other cellular and growth processes, or as a system to examine cellular resource allocations necessary for engineering.

Keywords: Whole-Cell Model; Kinetic Model; GRAS; Differential Equations; AdvanceSyn Toolkit

Introduction

Lacticaseibacillus paracasei subspecies *paracasei* NTU 101, belonging to the lactic acid bacteria (LAB) family [1], was isolated from the faecal matter of a healthy, breast-fed newborn infant in Taiwan [1-3]. As such, it is considered "Generally Recognised As Safe (GRAS)" by US FDA under GRAS Notice 1232. *L. paracasei* NTU 101 demonstrating strong resistance to gastric acid and bile salts; thereby, allowing probiotic efficacy upon transit through the gastrointestinal (GI) tract [4]. Human trials demonstrated that *L. paracasei* NTU 101 consumption improves peristalsis and shortens defecation interval [4,5]; and potentially has anti-obesity effects by regulating the AMPK pathway, enhancing fatty acid oxidation, and promoting lipolysis [6]. At the same time, it demonstrates immunomodulatory action, specifically promoting maturation and function of Regulatory T (Treg) cells, rebalancing Th1/Th2/Th17 ratios, offering significant therapeutic potential in preventing and alleviating allergic pathologies such as atopic dermatitis [1,7]. Hence, metabolic engineering of *L. paracasei* NTU 101 has been considered [8].

Mathematical modelling is central to metabolic engineering, shaping how strategies are designed and refined [9,10]. Two major frameworks dominate the field [11,12]: genome-scale models (GSMs), also called constraint-based models, and kinetic models (KMs). Each offers distinct advantages, but KMs have a particular strength in handling in silico gene knock-ins with greater ease than GSMs [13]. More importantly, KMs provide predictions for both metabolic rates and end-product yields, whereas GSMs tend to focus mainly on flux distributions [14]. This dual predictive capability positions KMs as a stronger platform for screening metabolic engineering ideas before committing to laboratory work. Consequently, there is a growing push within the community to expand efforts in constructing more comprehensive kinetic models [15,16].

However, there is no KM of *L. paracasei* to-date. As such, this study aims to construct a KM of *L. paracasei* NTU 101 using *ab initio* approach by identifying enzymes from its published genome [17], and identifying the corresponding reaction from KEGG [18]. The result is a whole cell KM of *L. paracasei* NTU 101, named as lcaKKNC26 using the nomenclature proposed by Cho and Ling [19], which consists of 846 metabolites, 356 enzymes with corresponding transcriptions and translations, and 677 enzymatic reactions.

Materials and Methods

Identification of Reactome. The genome of *Lacticaseibacillus* paracasei subspecies *paracasei* NTU 101 (NCBI RefSeq assembly GCF_002901165.3; NCBI GenBank Accession NZ_CP167253.1) was used as source to identify enzymatic genes using the process previously described [13,20,21]. Briefly, each enzymatic gene was identified as a presence of complete Enzyme Commission (EC) number in the GenBank record and mapped into reaction IDs via KEGG Ligand Database for Enzyme Nomenclature [18]. For example, EC 1.1.1.23 (https://www.genome.jp/entry/1.1.1.23) catalyses reactions R01158, R01163, and R03012; where the substrates and products of each rection can be identified.

Model development

Enzyme productions and reactions were modelled using the ordinary differential equation (ODE) format described by Sim., *et al*. [22]. Using BioNumbers estimates, an *E. coli* cell contains roughly 3000 RNA polymerase molecules (BioNumbers 106199) [23], of which about a quarter are active at any moment (BioNumbers 111676) [24]. With a polymerization rate of 22 ribonucleotides per second (BioNumbers 104109) [25] and an average nucleotide mass of 339.5 Da, the total mRNA production rate comes to about 5600 kDa per second, or 9.3e-18 grams per second. Given the cellular volume of about 0.7 cubic micrometres [26] and 4225 protein-coding genes (BioNumbers 105443) [27], this yields an approximate synthesis rate of 2.92 micromolar per gene per second. The mean mRNA half-life is 107.56 seconds (BioNumbers 107666) [28], which corresponds to a decay of 0.93% per second. Hence, the resulting rate law for mRNA synthesis is d[mRNA]/dt = 0.00292 – 0.0093[mRNA]. For translation, mammalian data indicate a median of 1000 peptides per transcript per hour (BioNumbers 106382) [29] or 0.278 peptides per second. Protein degradation in *E. coli* occurs at roughly 1% per hour (BioNumbers 109924) [30]. Thus, d[peptide]/dt = (0.278[mRNA] – 0.00000278[peptide]) micromolar per second. The full reactome was modelled as ODEs [20,31], with kcat = 13.7 per second and Km = 1 millimolar, following the typical enzyme parameters reported by Bar-Even., *et al*. [32], and formatted according to the AdvanceSyn Model Specification [33].

Model simulation

The constructed model was tested for simulatability using AdvanceSyn Toolkit [33]. Initial concentrations of all mRNA and enzymes were set to 0 mM. Initial concentrations of all metabolites were set to 1 mM except the following which were set to 1000 mM: (I) C00001 (Water), (II) C00002 (ATP), (III) C00003 (NAD+), (IV) C00004 (NADH), (V) C00005 (NADPH), (VI) C00006 (NADP+), (VII) C00007 (Oxygen), (VIII) C00011 (Carbon Dioxide), (IX) C00014 (Ammonia), (X) C00025 (L-Glutamate), (XI) C00031 (D-Glucose), (XII) C00037 (Glycine), (XIII) C00041 (L-Alanine), (XIV) C00042 (Succinate), (XV) C00047 (L-Lysine), (XVI) C00049 (L-Aspartate), (XVII) C00059 (Sulfate), (XVIII) C00064 (L-Glutamine), (XIX) C00065 (L-Serine), (XX) C00067 (Formaldehyde), (XXI) C00073 (L-Methionine), (XXII) C00097 (L-Cysteine), (XXIII) C00124 (D-Galactose), (XXIV) C00133 (D-Alanine), (XXV) C00137 (myo-Inositol), (XXVI) C00145 (Thiol), (XXVII) C00148 (L-Proline), (XXVIII) C00159 (D-Mannose), (XXIX) C00178 (Thymine), (XXX) C00180 (Benzoate), (XXXI) C00208 (Maltose), (XXXII) C00221 (beta-D-Glucose), (XXXIII) C00243 (Lactose), (XXXIV) C00246 (Butanoic acid), (XXXV) C00275 (D-Mannose 6-phosphate), (XXXVI) C00279 (D-Erythrose 4-phosphate), (XXXVII) C00310

(D-Xylulose), (XXXVIII) C00327 (L-Citrulline), (XXXIX) C00363 (dTDP), (XL) C00380 (Cytosine), (XLI) C00390 (Ubiquinol), (XLII) C00392 (Mannitol), (XLIII) C00396 (Pyrimidine), (XLIV) C00399 (Ubiquinone), (XLV) C00404 (Polyphosphate), (XLVI) C00463 (Indole), (XLVII) C00511 (Acrylic acid), (XLVIII) C00536 (Triphosphate), (XLIX) C00577 (D-Glyceraldehyde), (L) C00620 (alpha-D-Ribose 1-phosphate), (LI) C00644 (D-Mannitol 1-phosphate), (LII) C00672 (2-Deoxy-D-ribose 1-phosphate), (LIII) C00689 (alpha,alpha'-Trehalose 6-phosphate), (LIV) C00704 (Superoxide), (LV) C00794 (D-Sorbitol), (LVI) C00810 ((R)-Acetoin), (LVII) C00812 (Alkyl thiol), (LVIII) C00850 (Aryl sulfate), (LIX) C00860 (L-Histidinol), (LX) C00900 (2-Acetolactate), (LXI) C00962 (beta-D-Galactose), (LXII) C00966 (2-Dehydropantoate), (LXIII) C01035 (4-Guanidinobutanoate), (LXIV) C01100 (L-Histidinol phosphate), (LXV) C01101 (L-Ribulose 5-phosphate), (LXVI) C01159 (2,3-Bisphospho-D-glycerate), (LXVII) C01165 (L-Glutamate 5-semialdehyde), (LXVIII) C01267 (3-(Imidazol-4-yl)-2-oxopropyl phosphate), (LXIX) C01335 (ROH), (LXX) C01336 (Aryl thiol), (LXXI) C01344 (dIDP), (LXXII) C01345 (dITP), (LXXIII) C01659 (Acrylamide), (LXXIV) C01929 (L-Histidinal), (LXXV) C01962 (Thiocysteine), (LXXVI) C02291 (L-Cystathionine), (LXXVII) C02505 (2-Phenylacetamide), (LXXVIII) C02527 (Butanoylphosphate), (LXXIX) C03078 (4-Guanidinobutanamide), (LXXX) C03169 (Pyrimidine nucleoside), (LXXXI) C03287 (L-Glutamyl 5-phosphate), (LXXXII) C03291 (L-Xylulose 5-phosphate), (LXXXIII) C03406 (N-(L-Arginino)succinate), (LXXXIV) C03620 (Monocarboxylic acid amide), (LXXXV) C03912 ((S)-1-Pyrroline-5-carboxylate), (LXXXVI) C04666 (D-erythro-1-(Imidazol-4-yl)glycerol 3-phosphate), (LXXXVII) C05167 (alpha-Amino acid), (LXXXVIII) C05394 (3-Keto-beta-D-galactose), (LXXXIX) C05399 (Melibiitol), (XC) C05400 (Epimelibiose), (XCI) C05403 (3-Ketolactose), (XCII) C05539 (N-Acetyl-L-2-amino-6-oxopimelate), (XCIII) C06006 ((S)-2-Aceto-2-hydroxybutanoate), (XCIV) C06010 ((S)-2-Acetolactate), (XCV) C06019 (D-arabino-Hex-3-ulose 6-phosphate), (XCVI) C06030 (Methyloxaloacetate), (XCVII) C06244 (Acetamide), (XCVIII) C06423 (Octanoic acid), (XCIX) C06697 (Arsenite), (C) C06892 (2-Deoxy-5-keto-D-gluconic acid), (CI) C06893 (2-Deoxy-5-keto-D-gluconic acid 6-phosphate), (CII) C07086 (Phenylacetic acid), (CIII) C09815 (Benzamide), (CIV) C11038 (2'-Deoxy-5-hydroxymethylcytidine-5'-diphosphate), (CV) C11215 (Arsenate ion), (CVI) C14899 (3-Dehydro-L-gulonate 6-phosphate), (CVII) C15498 (ROOH), (CVIII) C15584 (Phenol), (CIX) C16737 (5-Deoxy-D-glucuronate), (CX) C20904 (2-Iminopropanoate), (CXI) C21860 (3-Demethylubiquinol), (CXII) G00275 (Maltose), (CXIII) G09795 (Trehalose 6-phosphate), (CXIV) G10504 (Lactose), (CXV) G10529 (Epimelibiose), (CXVI) G10531 (3-Ketolactose). The model was simulated using the fourth-order Runge-Kutta method [34,35] from time zero to 3600 seconds with timestep of 0.1 second, and the concentrations of metabolites were bounded between 0 millimolar and 1000 millimolar. The simulation results were sampled every 2 seconds.

Results and Discussion

The annotated genome of *L. paracasei* NTU 101 consists of 2956 genes as of the latest annotation dated 20 August 2024, including 2780 protein coding sequences. 356 unique EC numbers consisting of 677 enzymatic reactions involving 846 metabolites were identified and developed into a model based on AdvanceSyn Model Specification [33]. In addition, 712 ODEs acting as placeholder for enzyme transcriptions and translations were added.

The constructed lcaKKNC26 model was executed using the AdvanceSyn Toolkit [33], and the successful generation of simulation trajectories (Figure 1) indicates that the model is syntactically correct and structurally coherent as argued in recent model constructions [13,21,36-40] – conversely, if the model has either syntax error(s) or is structurally incoherent, it cannot be simulated and resulting in no simulation results or trajectories. Although the simulated pool of AMP (adenosine monophosphate) appears to be cyclical, this outcome should not be interpreted biologically because all turnover numbers and Michaelis–Menten constants were fixed at their median values across enzymes [32]. These placeholder values inevitably distort flux distributions. Nevertheless, what we offer here is a functional and fully simulatable kinetic model template of *L. paracasei* NTU 101 that can serve as a flexible foundation for incorporating organism-specific parameters, additional pathways, or higher-level cellular processes such as growth, regulation, or resource allocation [41-43] or as a system to examine cellular resource allocations [44-47].

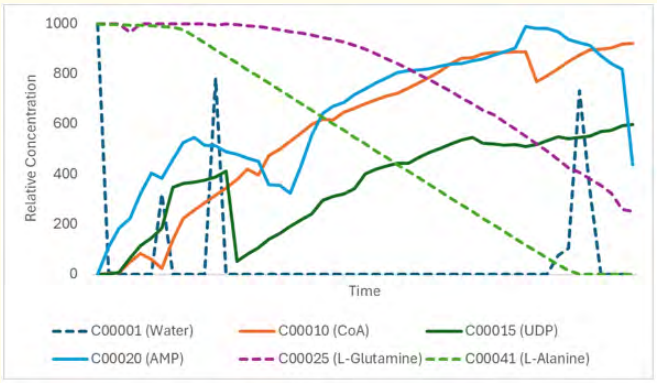

Figure 1: Selection of Simulation Results.

Conclusion

In this study, we present an *ab initio* whole cell kinetic model of *Lacticaseibacillus paracasei subspecies paracasei* built from the enzymes found in the genomic sequence of *Lacticaseibacillus paracasei* subspecies *paracasei* NTU 101. The resulting kinetic model, lcaKKNC26; comprising of 846 metabolites, 356 enzymes with corresponding transcriptions and translations, and 677 enzymatic reactions.

Supplementary Materials

Reaction descriptions and model can be download from https://bit.ly/lcaKKNC26.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

The authors wish to thank the institute, Management Development Institute of Singapore, for its support towards this work. The cost of publication fees was borne by the authors.

Bibliography

1. Chen C-T., *et al*. "Comprehensive Safety Assessment of Lacticaseibacillus paracasei subsp. paracasei NTU 101 Through Integrated Genotypic and Phenotypic Analysis". *Current Issues in Molecular Biology* 46.11 (2024): 12354-12374.
2. Chiang S-S and Pan T-M. "Beneficial Effects of Lactobacillus paracasei subsp. paracasei NTU 101 and its Fermented Products". *Applied Microbiology and Biotechnology* 93.3 (2012): 903-916.
3. Lin F-M., *et al*. "Fermentation of a Milk-Soymilk and Lycium Chinense Miller Mixture Using a New Isolate of Lactobacillus paracasei subsp. paracasei NTU101 and Bifidobacterium longum". *Journal of Industrial Microbiology & Biotechnology* 31.12 (2004): 559-564.
4. Chen C-L., *et al*. "Lactobacillus paracasei subsp. paracasei NTU 101 Lyophilized Powder Improves Loperamide-Induced Constipation in Rats". *Heliyon* 6.4 (2020): e03804.
5. Lin T., *et al*. "Effects of Lactocaseibacillus paracasei subsp. paracasei NTU 101 on Gut Microbiota: A Randomized, Double-Blind, Placebo-Controlled Clinical Study". *Journal of the Science of Food and Agriculture* 104.15 (2024): 9494-9505.
6. Kim S., *et al*. "Lactobacillus paracasei subsp. paracasei NTU 101 Prevents Obesity by Regulating AMPK Pathways and Gut Microbiota in Obese Rat". *Biochemical and Biophysical Research Communications* 731 (2024): 150279.
7. Liu C-F., *et al*. "The Beneficial Role of Lactobacillus paracasei subsp. paracasei NTU 101 in the Prevention of Atopic Dermatitis". *Current Issues in Molecular Biology* 46.3 (2024): 2236-2250.
8. Carolak E., *et al*. "Being a Better Version of Yourself: Genetically Engineered Probiotic Bacteria as Host Defense Enhancers in the Control of Intestinal Pathogens". *Gut Microbes* 17.1 (2025): 2519696.
9. Khanijou JK., *et al*. "Metabolomics and Modelling Approaches for Systems Metabolic Engineering". *Metabolic Engineering Communications* 15 (2022): e00209.
10. Gudmundsson S and Nogales J. "Recent Advances in Model-Assisted Metabolic Engineering". *Current Opinion in Systems Biology* 28 (2021): 100392.
11. Richelle A., *et al*. "Towards a Widespread Adoption of Metabolic Modeling Tools in Biopharmaceutical Industry: A Process Systems Biology Engineering Perspective". *npj Systems Biology and Applications* 6.1 (2020): 6.

12. Lee YQ., *et al*. "Genome-scale metabolic model-guided systematic framework for designing customized live biotherapeutic products". *NPJ Systems Biology and Applications* 11.1 (2025): 73.

13. Yeo KY., *et al*. "Ab Initio Whole Cell Kinetic Model of Yarrowia lipolytica CLIB122 (yliYKY24)". *Medicon Medical Sciences* 8.4 (2025): 01-06.

14. Prabhu S., *et al*. "Derivative-Free Domain-Informed Data-Driven Discovery of Sparse Kinetic Models. *Industrial & Engineering Chemistry Research* 64.5 (2025): 2601-2615.

15. Foster CJ., *et al*. "Building Kinetic Models for Metabolic Engineering. *Current Opinion in Biotechnology* 67 (2021): 35-41.

16. Lázaro J., *et al*. "Enhancing genome-scale metabolic models with kinetic data: resolving growth and citramalate production trade-offs in Escherichia coli. *Bioinformatics Advances* 5.1 (2025): vbaf166.

17. Yu DS., *et al*. "Complete Genome Sequence of the Probiotic Bacterium Bifidobacterium bifidum Strain BGN4". *Journal of Bacteriology* 194.17 (2012): 4757-4758.

18. Okuda S., *et al*. "KEGG Atlas mapping for global analysis of metabolic pathways". *Nucleic Acids Research* 36 (2008): W423-W426.

19. Cho JL., *et al*. "Adaptation of Whole Cell Kinetic Model Template, UniKin1, to Escherichia coli Whole Cell Kinetic Model, ecoJC20". *EC Microbiology* 17.2 (2021): 254-260.

20. Kwan ZJ., *et al*. "Ab Initio Whole Cell Kinetic Model of Stutzerimonas balearica DSM 6083 (pbmKZJ23). *Acta Scientific Microbiology* 7.2 (2024): 28-31.

21. Maiyappan S., *et al*. "Four Ab Initio Whole Cell Kinetic Models of Bacillus subtilis 168 (bsuLL25) 6051-HGW (bshSM25), N33 (bsuN33SS25), FUA2231 (bsuGR25)". *Journal of Clinical Immunology and Microbiology* 6.2 (2025): 1-6.

22. Sim BJH., *et al*. "Multilevel Metabolic Modelling Using Ordinary Differential Equations". *Encyclopedia of Bioinformatics and Computational Biology (Second Edition)*, eds Ranganathan S, Cannataro M, Khan AM (Elsevier, Oxford) (2025): 491-498.

23. Müller-Hill B . "The lac Operon: A Short History of a Genetic Paradigm". (Berlin, Germany) (1996).

24. Churchward G., *et al*. "Transcription in Bacteria at Different DNA Concentrations. *Journal of Bacteriology* 150.2 (1982): 572-581.

25. Gray WJ and Midgley JE. "The Control of Ribonucleic Acid Synthesis in Bacteria. The Synthesis and Stability of Ribonucleic Acid in Rifampicin-Inhibited Cultures of *Escherichia coli*". *The Biochemical Journal* 122.2 (1971): 161-169.

26. Kubitschek HE. "Cell Volume Increase in Escherichia coli After Shifts to Richer Media. *Journal of Bacteriology* 172.1 (1990): 94-101.

27. Hu P., *et al*. "Global Functional Atlas of Escherichia coli Encompassing Previously Uncharacterized Proteins". *PLoS Biology* 7.4 (2009): e96.

28. So L-H., *et al*. "General Properties of Transcriptional Time Series in Escherichia coli. *Nature Genetics* 43.6 (2011): 554-560.

29. Schwanhäusser B., *et al*. "Corrigendum: Global Quantification of Mammalian Gene Expression Control". *Nature* 495.7439 (2013): 126-127.

30. Maurizi MR. "Proteases and Protein Degradation in Escherichia coli". *Experientia* 48.2 (1992): 178-201.

31. Murthy MV., *et al*. "UniKin1: A Universal, Non-Species-Specific Whole Cell Kinetic Model". *Acta Scientific Microbiology* 3.10 (2020): 04-08.

32. Bar-Even A., *et al*. "The Moderately Efficient Enzyme: Evolutionary and Physicochemical Trends Shaping Enzyme Parameters". *Biochemistry* 50.21 (2011): 4402-4410.

33. Ling MH. "AdvanceSyn Toolkit: An Open Source Suite for Model Development and Analysis in Biological Engineering. *MOJ Proteomics & Bioinformatics* 9.4 (2020): 83–86.

34. Yong B. "The Comparison of Fourth Order Runge-Kutta and Homotopy Analysis Method for Solving Three Basic Epidemic Models". *Journal of Physics: Conference Series* 1317:012020.

35. Ling MH. "COPADS IV: Fixed Time-Step ODE Solvers for a System of Equations Implemented as a Set of Python Functions. *Advances in Computer Science: an International Journal* 5.3 (2016): 5-11.

36. Saisudhanbabu T., *et al*. "Ab Initio Whole Cell Kinetic Model of Limosilactobacillus fermentum EFEL6800 (lfeTS24)". *EC Clinical and Medical Case Reports* 8.4 (2025): 01-04.

37. Arivazhagan M., *et al*. "Ab Initio Whole Cell Kinetic Model of Bifidobacterium bifidum BGN4 (bbfMA24). *Acta Scientific Nutritional Health* 9.1 (2025): 42-45.

38. Senthilkumar A., *et al*. "Ab Initio Whole Cell Kinetic Model of Lactobacillus acidophilus NCFM (lacAS24)". *Journal of Clinical Immunology and Microbiology* 6.1 (2025): 1-5.

39. Wong TB., *et al*. "Ab Initio Whole Cell Kinetic Models of Escherichia coli BL21 (ebeTBSW25) and MG1655 (ecoMAL25). *Scholastic Medical Sciences* 3.2 (2025): 01-04.

40. Ambel WB., *et al*. "UniKin2 - A Universal, Pan-Reactome Kinetic Model". *International Journal of Research in Medical and Clinical Science* 3.2 (2025): 77-80.

41. Ahn-Horst TA., *et al*. "An Expanded Whole-Cell Model of E. coli Links Cellular Physiology with Mechanisms of Growth Rate Control". *npj Systems Biology and Applications* 8.1 (2022): 30.

42. Chagas M da S., *et al*. "Boolean Model of the Gene Regulatory Network of Pseudomonas aeruginosa CCBH4851". *Frontiers in Microbiology* 14 (2023): 1274740.

43. Hao T., *et al*. "Reconstruction of Metabolic-Protein Interaction Integrated Network of Eriocheir sinensis and Analysis of Ecdysone Synthesis". *Genes* 15.4 (2024): 410.

44. Thornburg ZR., *et al*. "Fundamental Behaviors Emerge From Simulations of a Living Minimal Cell". *Cell* 185.2 (2022): 345-360.e28.

45. Bianchi DM., *et al*. "Toward the Complete Functional Characterization of a Minimal Bacterial Proteome. *The Journal of Physical Chemistry B* 126.36 (2022): 6820-6834.

46. Sun G., *et al*. "Cross-Evaluation of E. coli's Operon Structures via a Whole-Cell Model Suggests Alternative Cellular Benefits for Low- Versus High-Expressing Operons". *Cell Systems* 15.3 (2024): 227-245.e7.

47. Choi H and Covert MW. "Whole-cell modeling of E. coli confirms that in vitro tRNA aminoacylation measurements are insufficient to support cell growth and predicts a positive feedback mechanism regulating arginine biosynthesis". *Nucleic Acids Research* 51.12 (2023): 5911-5930.