

PUBLIC PREVIEW / 公开预览版

本文尚未接收。为体现对在审项目与合作者成果的保密责任，公开版本仅对一处关键内容作集中遮挡，其余内容保持完整可读。

From Multi-omics to Systems Biology: New Insights in Microbiota-host Interactions in the Gut-Brain Axis

Ziqi Wen^{1,2,†}, Shiyun Peng^{1,2,†}, Feiyang Luo^{1,2,†}, Haitao Sun^{1,2,3,*}

¹Clinical Biobank Center, Microbiome Medicine Center, Department of Laboratory Medicine, Zhujiang Hospital and the Second Clinical Medical College, Southern Medical University, Guangzhou, China.

²Neurosurgery Center, The National Key Clinical Specialty, The Engineering Technology Research Center of Education Ministry of China on Diagnosis and Treatment of Cerebrovascular Disease, Guangdong Provincial Key Laboratory on Brain Function Repair and Regeneration, The Neurosurgery Institute of Guangdong Province, Zhujiang Hospital, Southern Medical University, Guangzhou, China.

³Key Laboratory of Mental Health of the Ministry of Education, Guangdong–Hong Kong–Macao Greater Bay Area Center for Brain Science and Brain-Inspired Intelligence, Southern Medical University, Guangzhou, China.

† Ziqi Wen, Shiyun Peng, and Feiyang Luo contributed equally to this work.

* Correspondence:

Dr. Haitao Sun

2009sht@smu.edu.cn

Abstract

Having co-evolved with humans over millennia, gut-resident microbiota and their derivatives modulate systemic physiological and pathological states—particularly within the nervous system—via distinct communication networks. Recent advances in high-throughput multi-omics have yielded massive high-dimensional datasets that delineate the multilayered complexity of the microbiota–gut–brain axis at unprecedented resolution. However, prevailing research paradigms frequently interpret these data as isolated features, neglecting systemic functional interplays and succumbing to a reductionist pitfall. To address these limitations, this review delineates the paradigm shift from multi-omics integration toward systems-biology modelling, proposing a conceptual framework to decode the systemic interactions of the microbiota–gut–brain axis. We discuss contemporary multi-omics integration strategies, scrutinizing the trade-offs between feature selection, cross-omics connectivity, and biological interpretability, while emphasizing the capacity of artificial-intelligence architectures to resolve high-dimensional heterogeneity. We explore how systems-biology frameworks—including network topology analysis, kinetic modelling, and constraint-based reconstruction—enable static association mapping, dynamic prediction, and multiscale mechanistic understanding of the microbiota–gut–brain axis. Crucially, we introduce an iterative systems biology framework—spanning mechanistic elucidation, model construction, and clinical application—to bridge the gap between multi-omics research and clinical practice. Finally, we discuss current challenges and outlined the future directions of interdisciplinary integration, including single-cell omics, organ-on-a-chip platforms, and organoid models.

Keywords

Multi-omics · systems biology · gut–brain axis · data integration · microbiome · neurological and psychotic disorders

1 Introduction

Originating over 3.5 billion years ago, microorganisms represent the oldest life forms on Earth, continuously

shaping the global environment and human health^{1, 2}. Predominantly colonizing the gastrointestinal tract, these microbial communities have co-evolved with humans as intrinsic regulators of host physiology, interacting with genetic and environmental factors to drive disease progression^{3, 4}. Beyond local gut dynamics, the microbiota-gut-brain axis also modulates the pathogenesis of neurological disorders—including Alzheimer's disease (AD)⁵, autism spectrum disorder⁶, and Parkinson's disease (PD)⁷—via integrated neural, immunological, and metabolic signaling. This systemic perspective necessitates multi-omics to delineate the interplay between microbial function, host-cell fate, and cross-organ microenvironments, offering transformative therapeutic insights^{8, 9, 10, 11, 12}. Consequently, diverse integrative computational methods have emerged to address the compositionality, sparsity, and high-dimensionality inherent in multi-modal microbiome datasets^{10, 13, 14}.

However, interpreting multi-omics data as isolated features while neglecting their functional interplays risks a reductionist pitfall. Conventional reductionist approaches deconstruct complex biological systems into discrete units, obscuring the holistic landscape of host-microbe interactions and limiting the generalizability of findings¹⁵. For example, a recent multi-omics analysis identified elevated serum levels of microbiota-derived deoxycholic acid as a key feature associated with blood-brain barrier dysfunction in Parkinson's disease¹. However, such association-based analyses provide limited insight into the dynamic thresholds of signaling activation or the nonlinear system-level feedback loops that may emerge following specific interventions. Consequently, they offer insufficient mechanistic guidance for subsequent experimental validation of the complex, nonlinear circuits through which microorganisms influence the host, including the brain. Similarly, another multi-omics study suggested that the coordinated action of *R. faecis* and *A. muciniphila* acts as an upstream factor influencing glioma growth². Nevertheless, conventional analytical approaches are not well suited to dissect how nonlinear microbial cooperation gives rise to global effects on disease phenotypes. Systems biology modeling can integrate high-dimensional data from multiple sources and support phenotype-driven, whole-system analyses of disease pathophysiology³. Moreover, quantitative modeling and biological network simulation enable *in silico* perturbation of human biological systems, allowing researchers to examine the global network-level consequences of targeting specific regulatory nodes. These approaches may therefore provide new insights into how microbial communities and microbiota-targeted interventions reshape disease-associated regulatory networks⁴.

Consequently, establishing a systemic framework—characterized by non-linearity, multidimensionality, and causality—is essential for mapping dynamic networks across molecular scales. Systems biology provides a holistic perspective, conceptualizing biological systems as complex networks in which the whole exceeds the sum of its parts^{16, 17}. This paradigm facilitates computational modeling and comprehensive analysis of systemic responses under perturbation^{17, 18}. By delineating these dynamic interplays, systems biology has already proven effective in addressing challenges related to microbial diversity¹⁹.

To this end, this review evaluates the utility of multi-omics integration and systems-level modeling within the microbiota-gut-brain axis, focusing on the synergistic analysis of microbiome and host-microbial datasets. By synthesizing current methodologies and applications, we propose a transformative framework to facilitate more systemic and comprehensive investigations of the gut-brain axis.

2 Integration Strategies for multi-omics data in the Gut-Brain Axis

The ability of gut microorganisms and their products to cross biological barriers and reshape brain ecology and function indicates that research on the gut-brain axis is no longer confined to the anatomical concept of vagal signaling^{5, 6, 7}. Importantly, the gut microbiota and its modulation have provided new insights into the interpretation and therapeutic targeting of brain disorders and brain aging.

Single-omics approaches, including microbiomics, transcriptomics, proteomics, and metabolomics, have revealed associations between microbial or molecular features and disease phenotypes, thereby facilitating the discovery of novel biomarkers^{8, 9, 10}. However, owing to the complexity of the intestinal ecosystem and the marked heterogeneity of the host, single-modality data alone are insufficient to explain or validate these cross-system mechanisms and biological processes.

Multi-omics integration can help identify key mediators linking the microbiota to brain diseases or brain function,

uncover biologically meaningful hub molecules, reduce interfering signals, and provide actionable clues for experimental validation. For example, integrated microbiome, transcriptome, and metabolome analyses have shown that gut microbiota can influence glioma progression through the key metabolite butyrate, which modulates the CASP3 pathway in microglia within the tumor microenvironment².

Moreover, compared with single-dimensional analyses, integrative network approaches can improve model complementarity, noise resistance, and robustness through cross-layer validation and signal refinement, thereby supporting the development of more accurate clinical models for early screening and risk prediction in brain disorders [Robust Integrative Analysis of Multi-omics Datasets via Nuclear-norm Maximization]^{11, 12} [Methodological Insights into Cancer Driver Gene Prediction: Comparing Graph-Based and Traditional ML Approaches]. Accordingly, selecting an appropriate multi-omics integration strategy according to the specific research question has become a central consideration, particularly in studies of the gut–brain axis.

Several taxonomies have been proposed for vertical multi-omics integration, including classifications based on mathematical principles (correlation, similarity, fusion, and multivariate)²⁸, on whether the approach is supervised or unsupervised²⁹, or on the stage of the analytical pipeline at which integration occurs^{14, 30}. Here, following the framework proposed by Picard et al., we focus on the integration process, aiming to summarize the multi-omics integration research in the field of the gut–brain axis and provide researchers with an intuitive and practical classification method. Since hierarchical integration often relies on prior biological knowledge, such as gene regulatory networks, we discuss those approaches under network modelling (section 4.1). Accordingly, the strategies considered here are divided into early, mixed, intermediate, and late integration (Figure 1).

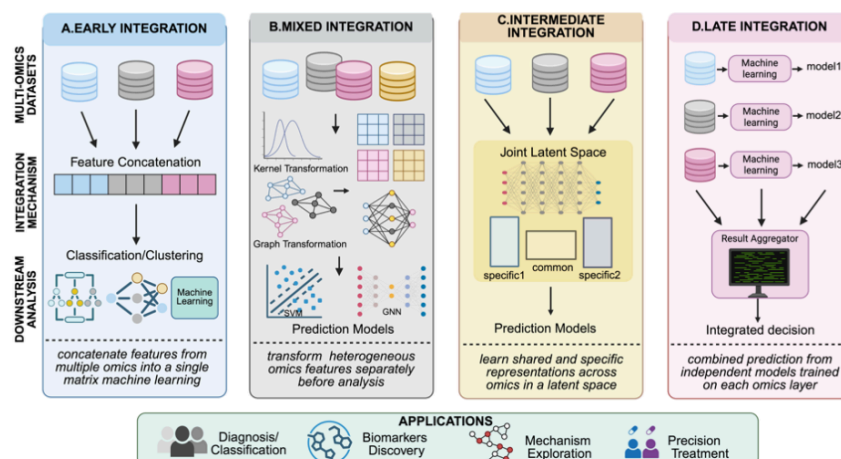


Figure 1. Different strategies for multi-omics data integration in the gut–brain axis. Multi-omics data integration strategies in gut–brain axis research can be broadly classified into early, mixed, intermediate and late integration. A) Early integration. Before data analysis, different omics datasets are directly combined into a single large matrix, which can then be used with machine-learning methods to construct predictive models for tasks such as disease classification. B) Mixed integration. Before data analysis, each omics dataset is first transformed into an intermediate representation using methods such as kernel- or graph-based approaches. The transformed data are then integrated with machine-learning methods, such as support vector machines or graph neural networks, to build models. C) Intermediate integration. During data analysis, different omics datasets are projected into a shared latent space to capture both common and modality-specific information, which is subsequently integrated to generate predictive models. D) Late integration. Each omics dataset is first analyzed or modelled

independently, and the resulting analytical outputs or predictive models are then further integrated to produce the result.

2.1 early integration

Early integration combines multiple omics datasets into a single large matrix before downstream analysis, allowing machine-learning models to directly explore potential interactions across different omics layers¹³. Commonly used approaches in this framework include least absolute shrinkage and selection operator (LASSO), random forest, and support vector machine (SVM). LASSO performs feature selection through regularization and helps reduce overfitting, thereby providing relatively high biological interpretability. By contrast, random forest and SVM generally offer stronger capacity for modeling nonlinear relationships.

For example, a random forest model integrating gut microbiome and metabolomic profiles was used to construct an early prediction model for subjective cognitive decline (SCD) in patients with Alzheimer's disease, achieving an AUC of 0.78¹⁴. In addition, random forest-based integration of microbiome and metabolomic data has provided insights into how gut microbes may influence brain function by modulating gene expression and metabolite activity, including mechanisms related to attention regulation and the pathophysiology of bipolar disorder^{15, 16}.

Nevertheless, in clinical research, models with strong predictive performance often rely on complex architectures that reduce transparency, whereas more interpretable models may have limited predictive capacity^{17, 18}. Accordingly, although random forest models can improve predictive performance, they remain limited in explaining how model predictions relate to disease heterogeneity. The incorporation of explainable artificial intelligence approaches, such as SHapley Additive exPlanations (SHAP), may help improve the interpretability of these models.

On the one hand, by concatenating different omics datasets into a unified feature matrix, early integration enables supervised learning models to directly use cross-omics information for prediction tasks. On the other hand, this strategy also increases noise and dimensionality, and algorithms may preferentially learn from omics layers with larger numbers of variables while overlooking biologically meaningful signals from smaller data blocks. Therefore, early integration may be more appropriate for datasets with sufficient sample size and relatively balanced feature scales across omics layers^{13, 19}. Otherwise, the substantial differences in feature numbers across omics layers may further increase the risk of overfitting, particularly in small-sample multi-omics studies.

2.2 Mixed integration

Mixed integration addresses some of these limitations by first transforming each omics dataset into an intermediate representation, such as a kernel matrix or graph, and then fusing these transformed data³⁰. This strategy can reduce dimensionality, dampen noise, and partially reconcile heterogeneity among data types. Kernel-based approaches represent each dataset through pairwise similarities among samples, after which the resulting kernel matrices can be used in downstream machine-learning models such as support vector machines (SVM)³⁸. For example, after separate feature selection for electroencephalography, metabolomics and gut microbiome data, an SVM-based multi-omics model achieved higher accuracy, precision, recall rate and AUC value than single-omics or dual-omics models, achieving better performance in distinguishing normal ageing from neurocognitive disorders³⁹. A more formal multiple-kernel learning framework goes further by combining modality-specific kernels under their respective weights β into a mixed kernel before classifier training. Using this strategy, Xi et al. built a multimodal SVM classifier based on gut microbial species, brain functional connectivity and grey matter volume to distinguish bipolar depression from controls and quetiapine responders from non-responders, and to identify the optimal weight combination that achieved the highest model accuracy. Graph-based mixed integration provides an alternative route. In this setting, each omics layer is first represented as a graph and then analyzed jointly³⁰. For example, Peng et al. fused functional brain networks and gut microbial networks that were constructed separately to build brain-gut microbiome networks. Using the connectivity matrix of fused networks as input, they constructed an optimal discriminant model for schizophrenia diagnosis, achieving an accuracy of 0.90 and an AUC of 0.97⁴⁰. Graph neural networks could be particularly attractive because they can learn directly from graph-structured data and are often more efficient than kernel-based methods for graph classification. Jiang

et al. constructed a deep learning framework named GCATCMDA to predict potential associations between microbes and diseases from known microbe-disease association datasets. In five-fold and ten-fold cross-validation experiments, the improvements in the model's AUC compared with the best predictive performance of other models were approximately 1.3% and 1.0%, respectively. Likewise, Wang et al. proposed a multi-omics graph convolutional network with attention mechanisms, demonstrating that models based on brain–gut combined graphs outperformed those based on brain or gut data alone in schizophrenia classification. For integrating highly heterogeneous omics modalities, multiple kernel learning within the mixed-integration framework can provide a flexible way to represent and fuse distinct data views. In contrast, when the data can be naturally organized as biological networks—such as protein–protein interaction (PPI) networks, gene regulatory networks (GRNs), or metabolic networks (MNs)—graph neural networks (GNNs) may offer greater advantages by directly modeling topological relationships and cross-layer biological dependencies^{20, 21}.

2.3 intermediate integration

Unlike mixed integration, which transforms each modality separately before fusion, intermediate integration typically projects different omics datasets into a shared latent space, followed by dimensionality reduction and further analysis³⁰. The principal advantage of this strategy is that it can uncover joint structures across datasets while highlighting complementary information contributed by each modality. Methods derived from non-negative matrix factorization as well as related approaches have already been used for subtype analysis in neurologic tumors and biomarker discovery in neurodegenerative disorders, illustrating their potential for non-linear feature extraction from complex multi-omics data^{30, 41, 42}. However, applications of these approaches to the gut–brain axis remain relatively limited, possibly due to the inherent properties of microbiome data, such as sparsity and compositionality, as well as the substantial heterogeneity between microbiome and host-derived omics layers⁴³. To address this, Song et al. developed a multi-loss disentangled generative–discriminative learning model based on a variational autoencoder and generative adversarial network⁴⁴. This model transformed host SNPs data and microbial 16S rRNA data into latent representations and further decomposed them into genetic-specific, microbiome-specific and shared gene–microbiome components, which were then used to enhance functional MRI features and improve schizophrenia diagnosis and cognitive score prediction. Another method often classified as intermediate integration is Canonical Correlation Analysis, although it differs conceptually in that it identifies correlated structures between datasets rather than constructing a shared latent space. By integrating metabolomics and microbiome data through multilayer network construction and Regularized Canonical Correlation Analysis, Dissanayaka et al. found that amyloid- β burden in preclinical AD was associated with elevated short-chain fatty acid concentrations linked to gut microbial function, suggesting that coordinated microbe–metabolite–host interactions may represent therapeutic targets for AD prevention⁴⁵. However, a key limitation of intermediate integration is that dimensionality reduction can obscure the contribution of the original features, thereby weakening biological interpretability. This trade-off remains one of the central challenges for latent-space-based methods. Intermediate integration is more suitable for multi-omics datasets with relatively low inter-modal heterogeneity, or for datasets that have undergone appropriate feature selection and preprocessing. In this context, methods such as DIABLO and MOFA offer particular advantages for identifying shared cross-omics patterns that may contribute to heterogeneity within the gut–brain axis^{13, 22, 23}.

2.4 Late integration

Late integration takes a different approach by modelling each omics dataset separately and then combining the resulting predictions or associations⁴⁶. Because each data layer is analysed independently, this strategy avoids interference introduced during joint normalization and can offer clearer modality-specific interpretability than some intermediate integration methods. In the gut–brain-axis literature, many late-integration studies rely on correlation-based approaches, especially Spearman correlation analysis, to build microbiota–metabolite–brain function networks. Such studies in AD, autism spectrum disorder and dietary intervention cohorts have highlighted associations between microbiota-derived metabolites and neurocognitive performance, thereby generating new hypotheses regarding disease mechanisms, therapeutic opportunities and diet-responsive microbial signatures^{32, 47}. Similar strategies have also been used to investigate drug mechanisms. For example, Gao et al. integrated

16S rRNA sequencing, brain metabolomics and transcriptomics in rats and suggested that ergosterol-loaded liposomes exert neuroprotective effects in ischemic stroke through coordinated modulation of a gut microbiota–metabolite–transcriptome regulatory axis⁴⁸. Although this strategy reduces interference among omics layers during preprocessing, its independent modelling structure limits its capacity to capture higher-order cross-omics interactions. More structured approaches, such as similarity network fusion or weighted co-expression network analysis, may therefore offer more informative representations of latent relationships among data layers^{49, 50}. When gut–brain axis studies seek to integrate multiple pre-existing single-omics analytical outputs, late integration may be a more appropriate strategy. By modeling each omics layer separately before aggregating the resulting outputs, this approach preserves the modality-specific contribution of individual omics layers while enabling a higher-level integrative interpretation^{13, 24, 25}.

Taken together, multi-omics integration has shifted gut–brain-axis research away from strictly hypothesis-driven or single-layer investigation towards more unbiased discovery of mechanisms and more precise identification of clinically relevant biomarkers. By linking features across multiple biological scales, these strategies provide new opportunities to clarify the mechanisms and biological significance of key factors operating within the gut–brain axis. At the same time, the emergence of systems biology has prompted increasing attention to the cooperative networks that maintain homeostasis or drive disease progression.

4 Systems biology of the Gut–Brain axis

Multi-omics integration in gut-brain axis research has begun to address several descriptive and analytical questions: which molecular features differ across gut-brain axis states, how these features can be linked through different integration strategies, and how they can be used to construct predictive models. Moving from description to mechanistic understanding, however, requires asking a more fundamental question: why do these multilayered alterations occur? This transition from association mapping to mechanistic explanation represents the core value of systems biology modeling²⁶. As a central methodology of systems biology, systems biology modeling uses mathematical and computational approaches to formally describe and quantitatively analyze biological systems, with the goal of understanding biological processes at the systems level (Introduction to systems biology).

Systems biology strategies can be broadly divided into bottom-up and top-down approaches²⁶. Bottom-up systems biology is mechanism-driven. It builds models from known biological mechanisms and uses parameter estimation to obtain biochemical, physiological, and kinetic information about system components, as well as response data for subsystems under environmental perturbations. Its major strength lies in mechanistic clarity and interpretability, although the construction of high-dimensional models is often challenging, time-consuming, and labor-intensive. By contrast, top-down systems biology starts from large-scale omics data and uses data-driven strategies to identify molecular association patterns, thereby generating a broad and comprehensive landscape of responses to specific perturbations, such as disease states²⁶ (Biology--Biomedical Modeling). Accordingly, the mathematical relationships inferred by top-down approaches do not necessarily map directly onto specific biological mechanisms, and their capacity for causal inference remains limited^{26, 27}. In this section, we discuss three commonly used systems biology modeling strategies and their applications in gut-brain axis research

(Figure).

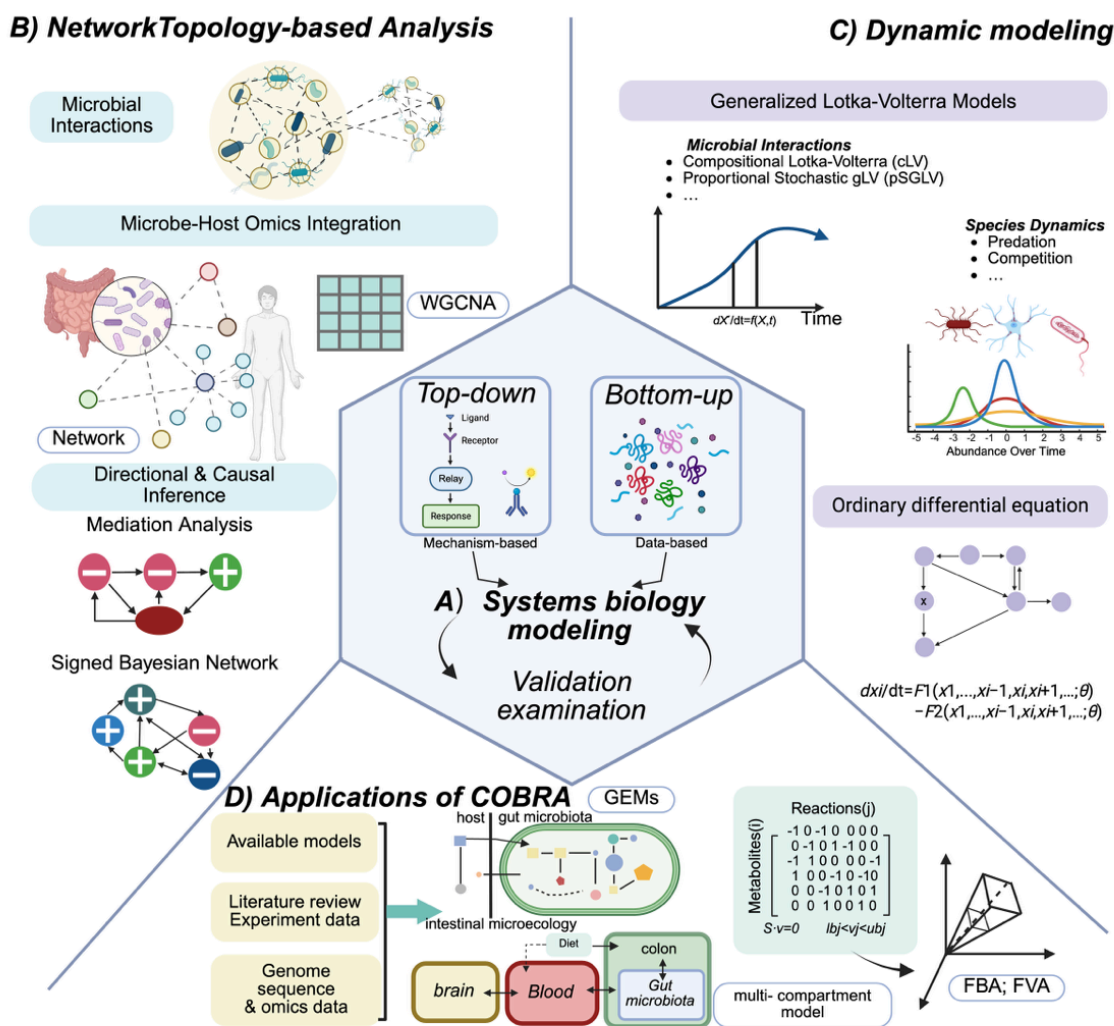


Figure 2. Systems biology analyses of the gut–brain axis. A) Top-down modelling approaches based on defined biological mechanisms and bottom-up modelling approaches based on multi-omics data in systems biology. After model construction, experimental validation is used iteratively to refine and improve the models. B) Network topology-based analysis of the gut–brain axis. This strategy includes constructing interaction networks at the microbial level, integrating multi-omics data to build microbe–host networks and identify co-expression modules, and conducting further causal inference analyses, such as mediation analysis and signed Bayesian networks. C) Dynamic modelling in the gut–brain axis. This includes the construction of ordinary differential equation models, as well as generalized Lotka–Volterra equations and their extensions, which can capture microbial community dynamics and interspecies relationships. D) Constraint-based reconstruction and analysis (COBRA) can be used for systems biology modelling of the gut–brain axis by integrating available models, literature-derived and experimental data, as well as genomic and multi-omics sequencing data. The resulting models may include local genome-scale metabolic models (GEMs) that represent microbial community metabolic activity and host–microbiota interactions, or whole-body metabolic models incorporating multiple host compartments. The core principle is to constrain metabolic reactions using flux balance analysis (FBA) or flux variability analysis (FVA)

4.1 Network Topology-Based Analysis: Mapping the Architecture of Gut-Brain Communication

Gut microorganisms are embedded in complex competitive, mutualistic, and symbiotic relationships, together with interactions between microbial metabolites and between metabolites and microorganisms²⁸. These interactions can be conceptualized as a network system composed of multiple subnetworks that collectively maintain the stability of the intestinal ecosystem. In the context of the gut-brain axis, information transfer between the gut and the brain is mediated through layered ecological, molecular, and neural networks, giving rise to a richer microbe-host network that ultimately shapes the phenotypic characteristics of central nervous system disorders⁷. Network topology analysis provides a suitable mathematical framework for organizing dispersed omics information into networks composed of nodes and edges. Nodes may represent microbial taxa, metabolites, genes, or brain regions, whereas edges represent associations or interactions. This approach has been widely applied in microbiome research and multi-omics integration^{29, 30 31}. Information within network modules can be quantified using topological metrics such as connectivity, average path length, clustering coefficient, and graph density^{32, 33}. In gut-brain axis studies, network-based methods can reveal global differences between disease and control states, identify core regions or hub nodes, and provide network-level biomarkers, interaction mechanisms, and potential intervention targets^{34, 35, 36}.

4.1.1 Microbial Ecological Networks: Revealing Disease-Associated Remodeling of Community Structure

A fundamental step in understanding the gut-brain axis is to characterize disease-associated changes in gut microbial abundance and inter-microbial interactions. Correlation and co-occurrence networks are among the most commonly used modeling approaches. Spearman correlation provides an intuitive statistical framework for representing positive and negative association networks^{37 38}. In patients with acute stroke, gut microbial networks show marked topological alterations compared with healthy controls, including reduced network connectivity and an increased number of isolated nodes, suggesting impaired microbial interactions after brain injury³⁴. A longitudinal study further showed that during Alzheimer's disease progression in mice, the gut microbial network shifts from a scale-free to a random topology, suggesting a progressive loss of community organization during disease development³⁵.

However, microbiome data are intrinsically compositional, meaning that species relative abundances are constrained by sequencing depth³⁹. Spearman-based approaches may therefore generate false-positive associations. To address this issue, SparCC infers correlations using log-ratio transformation and has been applied in Alzheimer's disease models and Parkinson's disease cohorts to identify hub taxa and co-occurrence clusters^{40, 41}. Another strategy for handling compositionality is to combine Gaussian graphical models with centered log-ratio transformation to infer conditional dependencies. In schizophrenia, weighted network analysis indicated that differential abundances of Lachnospiraceae and Ruminococcaceae taxa contributed to a reduction in stable competitive interactions within the gut microbiome⁴².

Beyond static differences in network structure, network modeling can also help identify key microorganisms that may drive network remodeling from a healthy to a disease-associated state. Random node-removal strategies can simulate ecological attacks on the network and highlight microorganisms that enhance network resistance to perturbation⁴³. NetShift quantifies changes in microbial association networks between healthy and disease states and calculates the neighborhood shift score and the centrality metric Bscaled for microbial network nodes, thereby predicting microbial components that may drive gut microbial network rewiring under central nervous system disease conditions⁴⁴. This approach identified *Terrisporobacter*, *Blautia*, *Dorea*, *Fusicatenibacter*, and *Anaerostipes* as potential driver taxa in autism spectrum disorder, and 16 driver species have also been identified in patients with schizophrenia⁴⁵. More recently, NetEnsa was developed to address the challenges faced by traditional methods such as NetShift when handling microbiome network nonlinearity and sparsity. By integrating multi-stage analyses, NetEnsa can identify disease-specific combinations of key nodes and may enable more

precise identification of key microbial species associated with specific neurological disorders in future gut-brain axis research (Gut microbiome network ensemble analysis for identifying disease-specific key species in targeted interventions). It should be noted, however, that these results are based on comparisons of static topological networks. Although the inferred driver taxa may contribute to disease, their causal directionality and temporal dynamics require further investigation.

4.1.2 Gut Microbiota-Host Networks

At the microbe-host interface, a central question for network analysis is which microbial metabolites may mediate communication between gut microorganisms and host physiological systems in central nervous system disorders. Weighted gene co-expression network analysis (WGCNA) identifies co-expression modules from omics data using unbiased hierarchical clustering and evaluates their associations with host phenotypes^{46, 47}. Xie et al. jointly analyzed the fecal microbiome, liver lipid metabolome, and hippocampal neurotransmitters in a chronic unpredictable mild stress mouse model of depression. WGCNA identified five differential metabolite modules mainly involved in glycerophospholipid metabolism, and integrative analysis suggested a potential Lachnospiraceae-glycerophospholipid metabolism-GABA pathway in depression⁴⁸. In multiple sclerosis, Ntranos et al. analyzed plasma and cerebrospinal fluid metabolomes in relapsing-remitting patients before and after dimethyl fumarate treatment. They found that bacterial phenolic and indolic metabolites, including p-cresol sulfate, indoxyl sulfate, and N-phenylacetylglutamine, were enriched in the cerebrospinal fluid before treatment. Weighted correlation network analysis associated these metabolites with cerebrospinal fluid neurotoxicity potential, suggesting that tryptophan- and phenylalanine-derived phenolic and indolic derivatives may participate in gut-brain communication in multiple sclerosis⁴⁹.

4.1.3 Integrating the Microbiome with Brain Networks

Incorporating brain networks into the network analysis framework provides an initial route for exploring whether changes in microbial communities map onto specific brain regions or neurological pathological mechanisms. To quantify changes in brain function within the gut-brain axis, graph-theoretical analysis can abstract fMRI signals into networks composed of brain regions and functional connections, which can then be linked to gut microbial features. Based on this strategy, increased abundance of unclassified Ruminococcaceae has been associated with a shift toward greater randomization of brain network topology, and functional gradient values were negatively correlated with depression and anxiety scores. Transcriptomic analysis further suggested that the GPCR-Rho/integrin-vesicular trafficking pathway may represent a key molecular mechanism⁵⁰. Peng et al. constructed individualized brain-gut microbiota networks for patients with schizophrenia and healthy controls. A major advantage of this approach is that it builds individualized cross-system connection networks, directly integrates brain and gut features into a unified network framework, and preserves specific connection information between the two systems⁵¹.

Network construction for microbial communities and microbe-host interactions has provided a systems-level perspective for understanding associations between microbial states and host conditions. Nevertheless, undirected networks do not provide information about interaction directionality or causality. Combining network analysis with causal inference represents a complementary strategy. Chen et al. applied bidirectional mediation analysis to fecal, serum, and cortical metabolites, revealing causal relationships between transgenic genotype and metabolite changes under Alzheimer's disease-associated stress⁴⁰. Bayesian networks, which are directed acyclic graph models based on conditional probabilities, can further be used to infer directional relationships within microbial communities⁵². These efforts represent an important shift in gut-brain axis network research from associative description toward mechanistic inference, providing theoretical support and candidate targets for the design of future network-guided intervention strategies, such as targeted microbial modulation or metabolite supplementation.

4.2 Dynamic Modeling in the Gut-Brain Axis

Network topology-based modeling addresses questions such as who is associated with whom, which nodes act as key hubs, and how networks are remodeled under disease conditions. However, topological network analysis is essentially a static snapshot of the system and is therefore limited in its ability to capture temporal dynamics⁵³. Changes in microbial community composition, metabolite production, vagal signaling, and neurotransmitter synthesis in the gut-brain axis are all multiscale, nonlinear, and time-dependent processes. Environmental changes, including diet, disease state, and fecal microbiota transplantation, can perturb these processes. Conversely, changes in the intestinal ecosystem may influence disease progression through the gut-brain axis^{7, 54}. Dynamic modeling addresses these temporal questions by representing biological processes as systems of equations that evolve over time, enabling researchers to simulate trajectories, predict responses to perturbations, and identify key transitions from health to disease.

4.2.1 Ordinary Differential Equation-Based Modeling

The most classical strategy for dynamic model construction converts signaling and interaction networks at molecular, cellular, tissue/organ, or organismal scales into ordinary differential equations (ODEs). By solving these equations, ODE-based models describe the continuous temporal dynamics of biological components. A central challenge of this approach is parameter identifiability, that is, the ability to uniquely determine model parameters from limited observational data^{55, 56, 57}. In gut-brain axis research, ODE frameworks have been used for mechanistic exploration across multiple levels. Travier et al. constructed a multicompartment linear dynamic model in which the mouse body was divided into six interconnected compartments and described using ODEs. This model clarified the microbiota-gut barrier-blood-brain barrier mechanism underlying neonatal susceptibility to late-onset group B streptococcal meningitis and identified the choroid plexus as the main route of group B streptococcal invasion⁵⁸. More recent studies have begun to integrate longitudinal metagenomic and metabolomic data into ODE frameworks, using Bayesian inference to estimate bacteria-metabolite interaction rates from time-series data and to quantitatively describe metabolite consumption and production⁵⁹. However, such applications remain relatively limited in gut-brain axis research.

4.2.2 Lotka-Volterra-Based Models

Generalized Lotka-Volterra equations are nonlinear dynamical systems that describe the rate of change in species abundance as the sum of species-specific growth rates and pairwise interaction effects. They are commonly used to model competitive, cooperative, and other interspecies interactions, as well as the temporal dynamics of microbial communities⁶⁰(Hofbauer J, Sigmund K (1998) Evolutionary games and population dynamics. Cambridge University Press; . The Lotka-Volterra framework can also be used to simulate dynamic interactions between the microbiome and neuronal populations. Microbial overgrowth, metabolite accumulation, and inflammation may perturb neuronal populations and promote the progression of neurodegenerative diseases (Agent-Based Simulation of Gut-Neuron Interactions and Inflammatory Pathways in Neurodegenerative Diseases). Mayalu et al. adapted Lotka-Volterra equations to construct a bacterial growth and competition model, which was further integrated into a cross-organ systems model. Simulations showed that under high-fat, low-carbohydrate dietary input, abnormal microbes became dominant, produced more metabolites, and, together with immune-cell activation and increased vagal stimulation frequency, reduced calcium influx in postsynaptic neurons and consequently inhibited hippocampal long-term potentiation (Systems Level Model of Dietary Effects on Cognition via the Microbiome-Gut-Brain Axis). This work demonstrates the quantitative capacity of a gLV-centered framework to translate dietary interventions into microbial community dynamics and, subsequently, into cognitive functional outcomes, providing a quantitative model for predicting multi-organ system responses from microbial ecological dynamics in the gut-brain axis.

Lotka-Volterra models are also being adapted to the specific properties of microbiome data. Classical gLV models require absolute abundance data, whereas microbiome sequencing data are intrinsically based on relative abundances. Compositional Lotka-Volterra models address compositionality and can infer parameters directly from relative abundance data⁶¹. Iterative Lotka-Volterra approaches improve the recovery accuracy of interaction coefficients and the prediction of species trajectories through iterative optimization, offering potential for dissecting

key microbial changes under disease conditions⁶². A newer proportional stochastic generalized Lotka-Volterra model introduces Brownian motion-driven stochastic perturbation terms, incorporating the random effects of environmental fluctuations on community dynamics. This may be particularly useful for analyzing how stochastic factors such as dietary fluctuations, circadian rhythms, and stress regulate microbial communities in the gut-brain axis (Proportional stochastic generalized Lotka-Volterra model with an application to learning microbial community structures). Nevertheless, these methods remain underused in gut-brain axis disease cohorts, partly because gLV parameter inference requires dense longitudinal time-series data, whereas most current gut-brain axis disease cohorts are cross-sectional and have insufficient temporal resolution (observed time-series data of the human gut microbiome is not informative enough for dynamic inference purpose).

4.2.3 Agent-Based Models

Unlike differential equation-based models, which describe average behavior at the population level, agent-based models (ABMs) treat each microorganism or cell as an individual agent with its own attributes and behavioral rules. ABMs are particularly well suited to capturing the spatial and temporal dynamics of the gut microbiota⁶³. In recent years, ABMs have been used to simulate the spatiotemporal metabolic dynamics of gut mucosal microbial communities across different intestinal regions, such as the proximal small intestine and cecum (MetaBiome: A metabolic agent-based model for simulating the spatiotemporal dynamics of gut mucosal microbiomes). A human colon ABM has also been developed to represent the epithelial layer, mucus bilayer, and intestinal lumen, using four cell types as agents to investigate mechanisms of colonization resistance against pathogens (An agent-based model of the human colon wall ecosystem). These studies indicate that ABMs have unique advantages for dissecting the spatial organization and local interactions of gut microbial communities.

Direct applications of ABMs in gut-brain axis research remain extremely limited. Weston et al. developed an ABM framework containing *Clostridium*, *Desulfovibrio*, and *Bifidobacterium* to evaluate autism spectrum disorder risk. However, the model included only three genera and therefore did not capture the true complexity of the gut microbial community. This gap reflects several potential limitations of ABMs in the gut-brain axis field, particularly the difficulties of parameterization and computational complexity under multiscale conditions. Parameterization requires the specification of numerous behavioral rules and interaction parameters for heterogeneous entities, including microorganisms, intestinal epithelial cells, immune cells, and neurons. Computational complexity increases sharply when the number of agents approaches the scale of a real intestinal community and can far exceed that of ODE-based models⁶⁴. Nevertheless, with the development of spatial transcriptomics, spatial metabolomics, and related technologies, the high-resolution spatial data required to construct and validate ABMs are expected to accumulate, which may help overcome some of these challenges⁶⁵.

4.3 Applications of COBRA for Modeling the Gut-Brain Axis

Network analysis and dynamic modeling reveal the topology and temporal evolution of the gut-brain axis, respectively, but neither can directly answer mechanistic questions about which metabolites gut microbial communities are capable of producing and how these metabolites influence host brain function through the gut-brain axis. System-level modeling of the gut-brain axis is further complicated by marked inter-individual variation in metabolite permeability across the intestinal barrier and blood-brain barrier, as well as by the current lack of systematic and standardized experimental measurement methods⁶⁶. Constraint-based reconstruction and analysis (COBRA) is a bottom-up systems biology approach based on genome-scale metabolic models (GEMs). By encoding metabolic reactions as mathematical matrices and using physicochemical and biological constraints to define the feasible flux space of metabolic networks, COBRA mechanistically connects genotype to phenotype^{67 68}. Based on a steady-state assumption, COBRA uses stoichiometric matrices and reaction bounds to predict flux distributions. Thus, it enables the study of cross-species and cross-organ metabolic communication without requiring prior determination of microbial and host kinetic parameters across different time scales, providing a feasible quantitative tool for studying such complex systems in the absence of kinetic parameters (What is flux balance analysis?). Flux balance analysis (FBA) and flux variability analysis (FVA) are fundamental

approaches for analyzing metabolic models. FBA commonly uses linear programming to identify optimal flux distributions satisfying all constraints, whereas FVA explores the minimum and maximum possible fluxes under the same constraints and optimality conditions, thereby evaluating flux flexibility and alternative pathways in metabolic networks⁶⁹ (What is flux balance analysis?; Flux variability analysis reveals a tragedy of commons in cancer cells;).

4.3.1 Community-Level Metabolic Modeling of the Gut Microbiome

In the gut-brain axis field, the COBRA framework has been combined with multi-omics analysis to functionally characterize the gut-brain axis and to discover novel biomarkers and intervention targets⁷⁰. In general, reconstruction of gut microbial GEMs can be achieved through three main strategies. First, automated or semi-automated tools such as KBase-ModelSEED⁷¹, CarveMe⁷², metaGEM⁷³, and DEMETER⁷⁴ can support de novo reconstruction from genomic data. Second, data can be mapped onto metabolic reconstruction resources such as AGORA⁷⁵, AGORA2⁷⁶, APOLLO⁷⁷, and the Virtual Metabolic Human (VMH) database⁷⁸. Third, individual GEMs can be compiled to construct community-level GEMs (Co-GEMs) that more comprehensively represent the complex metabolism of gut microbial communities^{79, 80}. Overall, de novo reconstruction is suitable for newly identified species lacking reference genomes, resource mapping enables rapid modeling in large-scale cohorts, and community-level integration is used to study overall metabolic interactions among species.

Genome-scale reconstructions can integrate multi-omics data, including transcriptomics, proteomics, and metabolomics, to generate condition- and/or sample-specific models, thereby enabling analyses that move beyond correlation^{81, 82}. In gut-brain axis research, the MICOM modeling framework has been used to compare metabolite flux rates in the guts of individuals with attention-deficit/hyperactivity disorder (ADHD) and healthy controls, revealing *Alistipes putredinis* as an important short-chain fatty acid producer in ADHD. This species not only produced higher amounts of acetate but also generated small quantities of isobutyrate and isovalerate, with metabolite fluxes influenced by diet⁸³.

The integration of multi-omics data with community metabolic modeling has also been applied in Parkinson's disease (PD). By integrating plasma metabolomics with metagenome-based gut microbial community modeling, metabolomic data can be used to validate model predictions, while the model provides community-level mechanistic interpretation for high-throughput metabolomic measurements. Computational modeling predicted sulfur metabolic alterations driven by *Akkermansia muciniphila* and *Bifidobacterium wadsworthia*, consistent with metabolomic changes and suggestive of PD-specific microbe-host sulfur co-metabolism⁸⁴. Personalized community modeling further indicated increased production of homocysteine, indole, and tryptophan in PD patients and identified specific species within the community that may participate in the production or consumption of distinct metabolites. These community-driven metabolic changes were subsequently confirmed by plasma metabolomic comparisons between PD and control participants from the same cohort⁸⁵. However, when model predictions cannot be replicated in serum metabolomics, discrepancies may reflect incomplete genome annotation used for model construction or confounding factors such as inter-individual variation in diet and medication use⁸⁶. Therefore, combining model predictions with experimental validation in independent cohorts remains a critical step for translating COBRA-based approaches into mechanistic understanding and predictive applications in gut-brain axis research.

4.3.2 Integrated Gut-Brain Metabolic Modeling

Since the introduction of personalized whole-body metabolism (WBM) models based on Recon3D, it has become possible to evaluate host-gut microbial co-metabolism in an organ- and individual-specific manner⁸². Compared with personalized germ-free WBM models, personalized microbiome-associated WBM models showed a marked increase in the capacity for brain neurotransmitter production. The substantial inter-individual variability in this capacity could not be explained solely by individual species or available metadata, suggesting personalized patterns of host-gut microbial co-metabolism. Extending this modeling strategy to the gut-brain axis can mechanistically link changes in gut microbiome composition to brain metabolic perturbations at the individual

level.

COBRA modeling integrated with multi-omics data has made progress in gut-brain axis studies of neurodegenerative disorders. Whole-body metabolic modeling revealed that formate metabolism in Alzheimer's disease is altered by genetic variation, whereas microbial metabolites promote urinary formate secretion flux through host-microbiome co-metabolism. Urinary formate has been proposed as an early biomarker of Alzheimer's disease⁸⁷. Similar modeling approaches in PD identified combinations of gut microbial contributors to different predicted plasma fluxes, showing that reduced blood butyrate levels in PD can be traced to coordinated changes in the abundances of multiple species rather than the loss of a single species⁸⁸.

To more comprehensively capture the systemic nature of the gut-brain axis, computational modeling frameworks must extend beyond microbial and molecular networks alone and represent, as far as possible, multi-compartment anatomical and physiological interactions. Tas et al. constructed a three-compartment gut-blood-brain model, in which gene-protein-reaction-based GEMs were used to simulate gut microbial metabolism, and ADHD-related metabolism was simulated by deleting NOS1 from the iBrain model. This framework allowed systematic evaluation of how parameters such as age, sex, and diet influence the production and consumption of brain metabolites by altering microbial metabolic outputs⁸⁹. Although this study likely oversimplified the mechanisms of the gut-brain axis and the polygenic nature of the disease, it highlighted the feasibility of applying perturbations, such as gene deletion, to simulate gut-brain axis metabolism under disease conditions. Best et al. used Fastcore to extract context-specific metabolic networks and constructed a context-specific meta-model representing the metabolic state of aging mice. In this model, three host tissues--colon, liver, and brain, connected through the blood--interacted with the microbial community model through the gut lumen. Among the interactions predicted by the meta-model, 42 (51%) were supported by previous experimental evidence, providing strong support for the ability of the meta-model to capture metabolic microbiome-host interactions⁹⁰.

Nevertheless, COBRA has several important limitations. First, its steady-state assumption means that it simulates flux distributions under steady-state conditions^{86, 91}. Second, derivative methods such as dynamic flux balance analysis (dFBA) still assume quasi-steady-state metabolism at each time point and do not directly account for spatial distribution or time-dependent dynamics, which may limit their ability to capture transient metabolic fluctuations⁹². Third, even when multiple metabolic network resources are integrated or manually curated to improve modeling coverage^{88, 93}, knowledge-based approaches may still overlook as yet undiscovered metabolic routes. To overcome these limitations, several derivative and integrated frameworks are being developed. Because COBRA lacks kinetic detail while kinetic models often face parameter-identifiability problems, COBRA-k integrates the flexibility of COBRA with kinetic precision and has been developed and initially applied to large-scale *Escherichia coli* models. In the future, it may support prediction and mechanistic interpretation of concentration changes in specific metabolites within the gut-brain axis⁹¹. In parallel, integrated COBRA-PBPK models combine constraint-based tissue-specific metabolic models with whole-body physiologically based pharmacokinetic modeling. This strategy has been applied in autism spectrum disorder and has shown potential for predicting therapeutic effects of dietary and probiotic interventions⁹². XomicsToModel further enables researchers to start from generic models and rapidly extract thermodynamically and stoichiometrically consistent context-specific metabolic models based on multi-omics data from specific disease cohorts or experimental conditions⁹⁴.

5. Challenges and Future Directions

In this review, we have described recent advances and translational potential in data-driven microbiome research on the gut-brain axis. We discussed multiple aspects of omics-related technologies, including data integration and model construction. However, several key challenges remain between the current state of the field and a complete mechanistic understanding of gut-brain axis involvement in central nervous system disorders, as well as its further clinical translation.

5.1 Data Heterogeneity and Standardization

Limited sample size and restricted geographic coverage may constrain the generalizability of some findings, highlighting the need for larger cohorts and samples from more diverse regions⁹⁵. However, differences across studies in sample collection and storage, DNA extraction methods, sequencing platforms, and bioinformatic pipelines can substantially affect the identification and abundance estimation of microbiome taxa, thereby impeding the reproducibility of host-microbiome association studies^{43, 93}. In addition, inference and analysis based on different subsets of the original cohort in multi-omics studies may introduce variability and limit the interpretability of associations across omics layers⁹⁶.

To address these challenges, standardization initiatives have been proposed in recent years. The STORMS (Strengthening the Organization and Reporting of Microbiome Studies) guideline provides a structured checklist for reporting human microbiome studies, covering study information, experimental design, and analytical methods. For methodological issues specific to gut-brain axis research, Ou et al. provided practical recommendations for selecting confounding factors, using integrative biostatistical methods, and moving from correlative findings toward causal conclusions. For cross-cohort modeling, Bayesian differential ranking algorithms have been used to integrate 10 cross-sectional microbiome datasets and 15 additional datasets to identify autism spectrum disorder-associated molecular and taxonomic features⁹⁶. Future work will require larger, multicenter, standardized cohort studies, systematic assessment of confounding factors, and transparent reporting practices to improve cross-study comparability and the reliability of meta-analyses.

5.2 Model Construction and Improvement

Computational models provide a route for generating new hypotheses in gut-brain axis research while reducing the cost of extensive experimental testing⁹⁷. With the emergence and development of the digital twin concept, personalized digital twin models constructed from individual multi-omics data, clinical information, and lifestyle factors may eventually be used to simulate disease progression, predict treatment responses, and optimize intervention strategies⁹⁸. However, current models still lack sufficient detail, and model interpretability and biological plausibility remain key bottlenecks limiting clinical application^{97, 98}. For multi-omics integration in the gut-brain axis, machine learning approaches in microbiome research face batch and cohort effects that can reduce transferability across studies (A comparative study of machine learning methods for predicting the effects of microbiota on human health). In contrast, mechanistic systems biology models, such as ODE-based and constraint-based reconstruction models, offer mechanistic interpretability but require extensive experimental data for parameterization and validation, which is particularly challenging given the multiscale and multi-tissue nature of the gut-brain axis. To bridge computational prediction and biological validation, several promising integrated frameworks have emerged. The GutMIND framework uses a two-stage validation strategy: models are first evaluated in high-quality discovery cohorts using nested cross-validation and then validated in independent cohorts, providing a model for systematic validation of computational gut-brain axis models (GutMIND: a framework for machine learning-based discovery of gut microbiome biomarkers).

To more accurately identify and simulate realistic intestinal environments in the gut-brain axis, future studies should also consider the roles of complex cellular components⁹⁹. Thiele et al. manually assembled a metabolic reconstruction of human small intestinal epithelial cells from the literature, enabling simulation of dietary digestion and absorption in the small intestine, as well as the effects of enzymopathies on enterocyte metabolic function¹⁰⁰. Jiang et al. developed iColonEpithelium, the first cell-type-specific genome-scale metabolic model of human colonic epithelium, based on existing transcriptomic data from human colonic epithelium. They further connected a MICOM-based microbial community metabolic model with iColonEpithelium to construct an integrated model for studying metabolite exchange between the gut microbiome and human colonic cells¹⁰¹. In addition, advances in single-cell omics technologies enable researchers to resolve heterogeneous responses of key intestinal epithelial cell subpopulations to microbial signals. These approaches can not only reveal a gene-intestinal epithelium-microbiota-behavior mechanistic axis in gut-brain axis multi-omics studies¹⁰², but also support the generation of

context-specific reconstructions¹⁰¹. Together, these technologies and advances provide new directions for future gut-brain axis research.

5.3 In Vitro Validation Platforms

Despite continuous advances in computational methods, the mechanisms inferred by computational models and their predictions still require in vitro validation, because computational models cannot fully reproduce the complexity of in vivo and ex vivo biological environments. In recent years, organ-on-a-chip and organoid technologies have provided transformative in vitro validation platforms for computational findings, with the potential to reduce cost, improve efficiency, and mitigate ethical concerns (Integrating organoids and organ-on-a-chip devices). Organ-on-a-chip systems integrate microfluidics and three-dimensional culture technologies to reproduce gut-brain axis environments in vitro, providing physiologically relevant alternatives to traditional models (Integrating organoids and organ-on-a-chip devices). Multiple gut-brain axis-on-a-chip systems have been designed and fabricated to study microbiota-brain interactions, toxin transport and inflammatory mechanisms, drug testing, and pharmacokinetics^{103, 104}. Human intestinal organoids derived from human pluripotent stem cells can now be enhanced by the intestinal stem-cell niche factor EPIREGULIN to promote differentiation with enteric glial populations and mesenchyme, and can form functional vasculature. These systems provide more complex models for simulating interactions between the gut organ and the whole body¹⁰⁵. In the future, these in vitro technologies can serve as preliminary validation platforms for computational model predictions, helping to prioritize the most promising hypotheses and intervention strategies before animal experiments and clinical trials.

6. Conclusion

In summary, the mission of gut-brain axis research is not simply to accumulate more data, but to transform large volumes of heterogeneous information into mechanistic understanding of multilevel biological networks. Future advances are likely to arise from the synergistic application of multiscale computational models that integrate spatial and temporal dimensions, more refined model construction, and controllable validation platforms such as organ-on-a-chip systems. Ultimately, a systems-level perspective on the gut-brain axis may provide new solutions for early intervention and personalized treatment of central nervous system disorders.

CONFIDENTIAL CONTENT REDACTED

稿件尚未接收。为保护在审项目，仅对这一处核心内容作集中遮挡，敬请谅解。

Protected scope: The original systems-biology framework figure

Acknowledgements

Author contributions

Z.W., S.P., and F.L. wrote the paper and revised the paper. Z.W. and S.P. drew pictures. H.S. designed the project and revised the paper. All authors contributed to the article and approved the submitted version.

Funding

None.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics and Consent to Participate

Not applicable.

Animal and human studies Experimental approval board

Not applicable.

Financial disclosure

None.

Competing interests

No pertinent conflicts of interest relevant to this manuscript.

References

1. Dodd MS, *et al.* Evidence for early life in Earth's oldest hydrothermal vent precipitates. *Nature* **543**, 60-64 (2017).
2. Fullerton KM, *et al.* Effect of tectonic processes on biosphere–geosphere feedbacks across a convergent margin. *Nature Geoscience* **14**, 301-306 (2021).
3. Sender R, Fuchs S, Milo R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLoS Biol* **14**, e1002533 (2016).
4. Origüela V, Lopez-Zaplana A. Gut Microbiota: An Immersion in Dysbiosis, Associated Pathologies, and Probiotics. *Microorganisms* **13**, (2025).
5. Chen Y, *et al.* Gut microbiota-driven metabolic alterations reveal gut-brain communication in Alzheimer's disease model mice. *Gut Microbes* **16**, 2302310 (2024).
6. Ji P, *et al.* Single-cell delineation of the microbiota-gut-brain axis: Probiotic intervention in Chd8 haploinsufficient mice. *Cell Genom* **5**, 100768 (2025).
7. Zhao Z, *et al.* Multi-omics identifies microbiota-derived deoxycholic acid as a key mediator of blood-brain barrier dysfunction in Parkinson's disease. *Imeta* **4**, e70076 (2025).
8. Chen C, Wang GQ, Li DD, Zhang F. Microbiota-gut-brain axis in neurodegenerative diseases: molecular mechanisms and therapeutic targets. *Mol Biomed* **6**, 64 (2025).
9. Ju Y, *et al.* GutMIND: A multi-cohort machine learning framework for integrative characteristics of the microbiota-gut-brain axis in neuropsychiatric disorders. *Gut Microbes* **18**, 2630563 (2026).
10. Duan D, *et al.* Advances in multi-omics integrated analysis methods based on the gut microbiome and their applications. *Front Microbiol* **15**, 1509117 (2024).
11. Zhang Y, *et al.* Predicting functions of uncharacterized gene products from microbial communities. *Nat Biotechnol*, (2025).
12. Kulkarni AS, *et al.* Mass spectrometry-based metabolomics approaches to interrogate host-microbiome interactions in mammalian systems. *Nat Prod Rep*, (2025).
13. Deek RA, Ma S, Lewis J, Li H. Statistical and computational methods for integrating microbiome, host genomics, and metabolomics data. *Elife* **13**, (2024).
14. Chetty A, Blekhman R. Multi-omic approaches for host-microbiome data integration. *Gut Microbes* **16**, 2297860 (2024).

15. Fomenko S, Rozera T, Elindy E, Ianiro G, Kolodny O. Integrating data and experimental models to clinical trials: iterative strategies in microbiome research. *Cell* **180**, 1175-1187 (2023).
16. Corti A, Colombo M, Migliavacca F, Rodriguez Matas JF, Casarin S, Chiastra C. Multiscale Computational Modeling of Vascular Adaptation: A Systems Biology Approach Using Agent-Based Models. *Front Bioeng Biotechnol* **9**, 744560 (2021).
17. Mardinoglu A, Nielsen J. Systems medicine and metabolic modelling. *J Intern Med* **271**, 142-154 (2012).
18. Hood L. Systems biology: integrating technology, biology, and computation. *Mech Ageing Dev* **124**, 9-16 (2003).
19. Daybog I, Kolodny O. A computational framework for resolving the microbiome diversity conundrum. *Nat Commun* **14**, 7977 (2023).
20. Yang SY, Han SM, Lee JY, Kim KS, Lee JE, Lee DW. Advancing Gut Microbiome Research: The Shift from Metagenomics to Multi-Omics and Future Perspectives. *J Microbiol Biotechnol* **35**, e2412001 (2025).
21. Hernández-Cacho A, et al. Multi-omics approach identifies gut microbiota variations associated with depression. *NPJ Biofilms Microbiomes* **11**, 68 (2025).
22. Zu X, et al. Characteristics of gut microbiota and metabolic phenotype in patients with major depressive disorder based on multi-omics analysis. *J Affect Disord* **344**, 563-576 (2024).
23. Jia L, et al. Metagenomic analysis characterizes stage-specific gut microbiota in Alzheimer's disease. *Mol Psychiatry* **30**, 3951-3962 (2025).
24. Li B, et al. Mild cognitive impairment has similar alterations as Alzheimer's disease in gut microbiota. *Alzheimers Dement* **15**, 1357-1366 (2019).
25. Osama A, et al. Integrative multi-omics analysis of autism spectrum disorder reveals unique microbial macromolecules interactions. *J Adv Res* **77**, 265-279 (2025).
26. Ni Y, Cai H, Shao Y, Le W. Multi-omics insights into GBA1-associated Parkinson's disease: interplay of genomics, transcriptomics, proteomics, and lipidomics. *Mol Neurodegener* **21**, 6 (2026).
27. Reel PS, Reel S, Pearson E, Trucco E, Jefferson E. Using machine learning approaches for multi-omics data analysis: A review. *Biotechnol Adv* **49**, 107739 (2021).
28. Subramanian I, Verma S, Kumar S, Jere A, Anamika K. Multi-omics Data Integration, Interpretation, and Its Application. *Bioinform Biol Insights* **14**, 1177932219899051 (2020).
29. Rozera T, Pasolli E, Segata N, Ianiro G. Machine Learning and Artificial Intelligence in the Multi-Omics Approach to Gut Microbiota. *Gastroenterology* **169**, 487-501 (2025).
30. Picard M, Scott-Boyer MP, Bodein A, Périn O, Droit A. Integration strategies of multi-omics data for machine learning analysis. *Comput Struct Biotechnol J* **19**, 3735-3746 (2021).
31. Han S, et al. Application of Sparse Representation in Bioinformatics. *Front Genet* **12**, 810875 (2021).
32. Liang X, et al. Gut microbiome, cognitive function and brain structure: a multi-omics integration analysis. *Transl Neurodegener* **11**, 49 (2022).
33. Xu H, et al. Multiple omics reveal link between the microbiota-gut-brain axis and intracranial aneurysm rupture. *iScience* **27**, 111184 (2024).
34. Lin Y, et al. Uncovering stage-specific neural and molecular progression in Alzheimer's disease: Implications for early screening. *Alzheimers Dement* **21**, e70182 (2025).
35. Castells-Nobau A, et al. Gut microbial modulation of 3-hydroxyanthranilic acid and dopaminergic signalling

36. Li Z, *et al.* Multi-omics analyses of serum metabolome, gut microbiome and brain function reveal dysregulated microbiota-gut-brain axis in bipolar depression. *Mol Psychiatry* **27**, 4123-4135 (2022).
37. Rodríguez-Pérez R, Bajorath J. Interpretation of machine learning models using shapley values: application to compound potency and multi-target activity predictions. *J Comput Aided Mol Des* **34**, 1013-1026 (2020).
38. Briscik M, Tazza G, Vidács L, Dillies MA, Déjean S. Supervised multiple kernel learning approaches for multi-omics data integration. *BioData Min* **17**, 53 (2024).
39. Han Y, *et al.* The fusion of multi-omics profile and multimodal EEG data contributes to the personalized diagnostic strategy for neurocognitive disorders. *Microbiome* **12**, 12 (2024).
40. Peng R, *et al.* The brain-gut microbiota network (BGMN) is correlated with symptom severity and neurocognition in patients with schizophrenia. *Neuroimage* **308**, 121052 (2025).
41. Clark C, Dayon L, Masoodi M, Bowman GL, Popp J. An integrative multi-omics approach reveals new central nervous system pathway alterations in Alzheimer's disease. *Alzheimers Res Ther* **13**, 71 (2021).
42. Chen Y, *et al.* An Improved Deep Semi-supervised JNMF Method for Biomarker Extraction of Alzheimer's Disease. *J Mol Neurosci* **75**, 159 (2025).
43. Lu J, *et al.* Multi-omics decodes host-specific and environmental microbiome interactions in sepsis. *Front Microbiol* **16**, 1618177 (2025).
44. Song P, Yuan X, Li X, Song X, Wang Y. Multi-Loss Disentangled Generative-Discriminative Learning for Multimodal Representation in Schizophrenia. *IEEE J Biomed Health Inform* **28**, 6395-6404 (2024).
45. Dissanayaka DMS, *et al.* Functional Pathways of the Gut Microbiome Associated with SCFA Profiles in Preclinical Alzheimer's Disease. *Aging Dis*, (2026).
46. Rappoport N, Shamir R. Multi-omic and multi-view clustering algorithms: review and cancer benchmark. *Nucleic Acids Res* **46**, 10546-10562 (2018).
47. Wang D, *et al.* Gut microbial GABA imbalance emerges as a metabolic signature in mild autism spectrum disorder linked to overrepresented Escherichia. *Cell Rep Med* **6**, 101919 (2025).
48. Gao M, *et al.* Ergosterol-loaded brain-targeted liposomes attenuate stroke via gut microbiota-metabolite-brain axis modulation. *J Nanobiotechnology* **24**, 28 (2025).
49. Wang B, *et al.* Similarity network fusion for aggregating data types on a genomic scale. *Nat Methods* **11**, 333-337 (2014).
50. Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics* **9**, 559 (2008).
51. Bruggeman FJ, Westerhoff HV. The nature of systems biology. *Trends Microbiol* **15**, 45-50 (2007).
52. Sobie EA, Lee YS, Jenkins SL, Iyengar R. Systems biology--biomedical modeling. *Sci Signal* **4**, tr2 (2011).
53. Noordijk B, Garcia Gomez ML, Ten Tusscher K, de Ridder D, van Dijk ADJ, Smith RW. The rise of scientific machine learning: a perspective on combining mechanistic modelling with machine learning for systems biology. *Front Syst Biol* **4**, 1407994 (2024).
54. Xiao Y, Angulo MT, Friedman J, Waldor MK, Weiss ST, Liu YY. Mapping the ecological networks of microbial communities. *Nat Commun* **8**, 2042 (2017).
55. Dugourd A, *et al.* Causal integration of multi-omics data with prior knowledge to generate mechanistic hypotheses. *Mol Syst Biol* **17**, e9730 (2021).

56. Wang Y, et al. A graph-based multi-omics clustering framework to analyze integrated multi-edge networks. *Cell Informatics* **23**, 100000 (2022).
57. Ma'ayan A. Introduction to network analysis in systems biology. *Sci Signal* **4**, tr5 (2011).
58. Barabasi AL, Oltvai ZN. Network biology: understanding the cell's functional organization. *Nat Rev Genet* **5**, 101-113 (2004).
59. Chen J, Wu X, Wang X, Yuan R, Zhu Q. Alterations in gut microbiota and metabolomic profiles in acute stroke: insights into brain-gut axis dysregulation. *Front Microbiol* **16**, 1580231 (2025).
60. Li Y, Chen Y, Fan Y, Chen Y, Chen Y. Dynamic network modeling of gut microbiota during Alzheimer's disease progression in mice. *Gut Microbes* **15**, 2172672 (2023).
61. Francis D, Sun F. A comparative analysis of mutual information methods for pairwise relationship detection in metagenomic data. *BMC Bioinformatics* **25**, 266 (2024).
62. Ma L, et al. A role of gut-brain axis on prophylactic actions of arketamine in male mice exposed to chronic restrain stress. *Pharmacol Biochem Behav* **238**, 173736 (2024).
63. Gloor GB, Macklaim JM, Pawlowsky-Glahn V, Egozcue JJ. Microbiome Datasets Are Compositional: And This Is Not Optional. *Front Microbiol* **8**, 2224 (2017).
64. Wallen ZD, et al. Metagenomics of Parkinson's disease implicates the gut microbiome in multiple disease mechanisms. *Nat Commun* **13**, 6958 (2022).
65. Protic D, et al. Microbiome modulation and behavioural improvements in children with fragile X syndrome following probiotic intake: A pilot study. *Sci Rep* **16**, 560 (2025).
66. Friedman J, Alm EJ. Inferring correlation networks from genomic survey data. *PLoS Comput Biol* **8**, e1002687 (2012).
67. Wang Y, et al. Multi-omics reveal microbial determinants impacting the treatment outcome of antidepressants in major depressive disorder. *Microbiome* **11**, 195 (2023).
68. Kuntal BK, Chandrakar P, Sadhu S, Mande SS. 'NetShift': a methodology for understanding 'driver microbes' from healthy and disease microbiome datasets. *ISME J* **13**, 442-454 (2019).
69. Luo M, et al. Gut microbiome network ensemble analysis for identifying disease-specific key species in targeted interventions. *Food Bioscience* **68**, (2025).
70. Nie L, et al. Correlation between symptoms and cognitive function changes in patients with primary insomnia and pathways in gut microbiota. *Biochem Biophys Res* **37**, 101629 (2024).
71. Xie J, Zhong Q, Wu WT, Chen JJ. Multi-omics data reveals the important role of glycerophospholipid metabolism in the crosstalk between gut and brain in depression. *J Transl Med* **21**, 93 (2023).
72. Ntranos A, et al. Bacterial neurotoxic metabolites in multiple sclerosis cerebrospinal fluid and plasma. *Brain* **145**, 569-583 (2022).
73. Thompson DS, et al. Differential co-expression networks of the gut microbiota are associated with depression and anxiety treatment resistance among psychiatric inpatients. *Prog Neuropsychopharmacol Biol Psychiatry* **120**, 110638 (2023).
74. Chen Y, Xu R. Context-sensitive network-based disease genetics prediction and its implications in drug discovery. *Bioinformatics* **33**, 1031-1039 (2017).
75. Wang Q, et al. Gut-microbiota-microglia-brain interactions in Alzheimer's disease: knowledge-based, multi-dimensional characterization. *Alzheimers Res Ther* **13**, 177 (2021).

76. [REDACTED] *communities using signed regulatory networks*. *PLoS Comput Biol* **21**, e1008333 (2025).
77. Mei H, Wang Z, Yang H, Li X, Xu Y. Network analysis of multivariate time series data in biological systems: methods and applications. *Brief Bioinform* **26**, (2025).
78. Savulescu-Fiedler I, Benea SN, Căruntu C, Nancoff AS, Homentcovschi C, Bucurica S. Rewiring the Brain Through the Gut: Insights into Microbiota-Nervous System Interactions. *Curr Issues Mol Biol* **47**, (2025).
79. Adrian MA, Ayati BP, Mangalam AK. A mathematical model of *Bacteroides thetaiotaomicron*, *Methanobrevibacter smithii*, and *Eubacterium rectale* interactions in the human gut. *Sci Rep* **13**, 21192 (2023).
80. Li G, *et al.* A mechanistic systems biology model of brain microvascular endothelial cell signaling reveals dynamic pathway-based therapeutic targets for brain ischemia. *Redox Biol* **78**, 103415 (2024).
81. Paredes-Vazquez A, Balsa-Canto E, Banga JR. Identification of dynamic models of microbial communities: A workflow addressing identifiability and modeling pitfalls. *PLoS Comput Biol* **21**, e1013204 (2025).
82. Travier L, *et al.* Neonatal susceptibility to meningitis results from the immaturity of epithelial barriers and gut microbiota. *Cell Rep* **35**, 109319 (2021).
83. Stein RR, *et al.* Ecological modeling from time-series inference: insight into dynamics and stability of intestinal microbiota. *PLoS Comput Biol* **9**, e1003388 (2013).
84. Jabeen T, Karimi F, Zomorodi AR, Khalilpour K. Agent-Based Simulation of Gut–Neuron Interactions and Inflammatory Pathways in Neurodegenerative Diseases. In: *2025 IEEE International conference on Medical Artificial Intelligence (MedAI)* (2025).
85. Mayalu MN, Sarma A, Xiao F, Doyle JC, Murray RM. Systems Level Model of Dietary Effects on Cognition via the Microbiome-Gut-Brain Axis. In: *2021 European Control Conference (ECC)* (2021).
86. Joseph TA, Shenhav L, Xavier JB, Halperin E, Pe'er I. Compositional Lotka-Volterra describes microbial dynamics in the simplex. *PLoS Comput Biol* **16**, e1007917 (2020).
87. Huang Y, Tang T, Dai X, Sun F. Quantifying microbial interactions based on compositional data using an iterative approach for solving generalized Lotka-Volterra equations. *PLoS Comput Biol* **21**, e1013691 (2025).
88. Xu L, Kong D, Wang L, Gu H, Kenney T, Xu X. Proportional stochastic generalized Lotka–Volterra model with an application to learning microbial community structures. *Applied mathematics and computation*, 448 (2023).
89. O'Brien EJ, Monk JM, Palsson BO. Using Genome-scale Models to Predict Biological Capabilities. *Cell* **161**, 971-987 (2015).
90. Heinken A, Hertel J, Thiele I. Metabolic modelling reveals broad changes in gut microbial metabolism in inflammatory bowel disease patients with dysbiosis. *NPJ Syst Biol Appl* **7**, 19 (2021).
91. Bhowmick R, Campit S, Katkam SK, Keshamouni VG, Chandrasekaran S. Genome-scale modeling identifies dynamic metabolic vulnerabilities during the epithelial to mesenchymal transition. *Commun Biol* **7**, 1704 (2024).
92. Arkin AP, *et al.* KBase: The United States Department of Energy Systems Biology Knowledgebase. *Nat Biotechnol* **36**, 566-569 (2018).
93. Machado D, Andrejev S, Tramontano M, Patil KR. Fast automated reconstruction of genome-scale metabolic models for microbial species and communities. *Nucleic Acids Res* **46**, 7542-7553 (2018).
94. Zorrilla F, Buric F, Patil KR, Zelezniak A. metaGEM: reconstruction of genome scale metabolic models directly from metagenomes. *Nucleic Acids Res* **49**, e126 (2021).
95. Heinken A, Magnusdottir S, Fleming RMT, Thiele I. DEMETER: efficient simultaneous curation of genome-

96. Magnúsdóttir S, *et al.* Generation of genome-scale metabolic reconstructions for 773 members of the human gut microbiota. *Nat Biotechnol* **35**, 81–89 (2017).
97. Heinken A, *et al.* Genome-scale metabolic reconstruction of 7,302 human microorganisms for personalized medicine. *Nat Biotechnol* **41**, 1320–1331 (2023).
98. Heinken A, *et al.* APOLLO: A genome-scale metabolic reconstruction resource of 247,092 diverse human microbes spanning multiple continents, age groups, and body sites. *bioRxiv*, (2023).
99. Noronha A, *et al.* The Virtual Metabolic Human database: integrating human and gut microbiome metabolism with nutrition and disease. *Nucleic Acids Res* **47**, D614–D624 (2019).
100. Beura S, Kundu P, Das AK, Ghosh A. Genome-scale community modelling elucidates the metabolic interaction in Indian type-2 diabetic gut microbiota. *Sci Rep* **14**, 17259 (2024).
101. Orth JD, Thiele I, Palsson BØ. What is flux balance analysis? *Nature Biotechnology* **28**, 245–248 (2010).
102. Ovalle A, Lopez E, Sierralta J, Paricio N, Garrido D. A rationally designed microbial consortium modulates neurodegeneration in a *Drosophila melanogaster* model of Parkinson's disease. *NPJ Biofilms Microbiomes* **11**, 185 (2025).
103. Martinelli F, *et al.* Whole-body metabolic modelling reveals microbiome and genomic interactions on reduced urine formate levels in Alzheimer's disease. *Sci Rep* **14**, 6095 (2024).
104. Paklao T, Suratanee A, Plaimas K. IGM: Integrated Gene-expression Modeling for multi-condition flux-preserving genome-scale metabolic models. *PLoS One* **21**, e0342294 (2026).
105. Heirendt L, *et al.* Creation and analysis of biochemical constraint-based models using the COBRA Toolbox v.3.0. *Nat Protoc* **14**, 639–702 (2019).
106. Thiele I, *et al.* Personalized whole-body models integrate metabolism, physiology, and the gut microbiome. *Mol Syst Biol* **16**, e8982 (2020).
107. Mohammad FK, Palukuri MV, Shivakumar S, Rengaswamy R, Sahoo S. A Computational Framework for Studying Gut-Brain Axis in Autism Spectrum Disorder. *Front Physiol* **13**, 760753 (2022).
108. Baldini F, *et al.* Parkinson's disease-associated alterations of the gut microbiome predict disease-relevant changes in metabolic functions. *BMC Biol* **18**, 62 (2020).
109. Tas E, Ulgen KO. Understanding the ADHD-Gut Axis by Metabolic Network Analysis. *Metabolites* **13**, (2023).
110. Rosario D, *et al.* Systematic analysis of gut microbiome reveals the role of bacterial folate and homocysteine metabolism in Parkinson's disease. *Cell Rep* **34**, 108807 (2021).
111. Hensen T, *et al.* In silico metabolic modelling links microbiome-derived metabolites to risk factors of Alzheimer's disease. *Gut Microbes Rep* **2**, 2443171 (2025).
112. Hensen T, Thiele I. Metabolic modeling links gut microbiota to metabolic markers of Parkinson's disease. *Gut Microbes* **17**, 2554195 (2025).
113. Tas E, Ulgen KO. Exploring the Integrated Gut-Brain Metabolic Model for ADHD. *Biochem Genet*, (2025).
114. Best L, *et al.* Metabolic modelling reveals the aging-associated decline of host-microbiome metabolic interactions in mice. *Nat Microbiol* **10**, 973–991 (2025).
115. Vlassis N, Pacheco MP, Sauter T. Fast reconstruction of compact context-specific metabolic network models. *PLoS Comput Biol* **10**, e1003424 (2014).

116. Walskova A, Rozman D, Rozman M, Kralj M, Meston M. Guided extraction of genome-scale metabolic model for the integration and analysis of omics data. *Comput Struct Biotechnol* **19**, 3521-3530 (2021).
117. Bekiaris PS, Klamt S. COBRA-k: A powerful framework bridging constraint-based and kinetic metabolic modeling. *Sci Adv* **12**, eaeb3022 (2026).
118. Moimenta AR, *et al.* An integrated multiphase dynamic genome-scale model explains batch fermentations led by species of the *Saccharomyces* genus. *mSystems* **10**, e0161524 (2025).
119. Aminian-Dehkordi J, Dickson A, Valiei A, Mofrad MRK. MetaBiome: a multiscale model integrating agent-based and metabolic networks to reveal spatial regulation in gut mucosal microbial communities. *mSystems* **10**, e0165224 (2025).
120. Preciat G, Wegrzyn AB, Luo X, Thiele I, Hankemeier T, Fleming RMT. XomicsToModel: omics data integration and generation of thermodynamically consistent metabolic models. *Nat Protoc*, (2025).
121. Kashyap PC, Chia N, Nelson H, Segal E, Elinav E. Microbiome at the Frontier of Personalized Medicine. *Mayo Clin Proc* **92**, 1855-1864 (2017).
122. Yuan X, *et al.* Depression and anxiety in patients with active ulcerative colitis: crosstalk of gut microbiota, metabolomics and proteomics. *Gut Microbes* **13**, 1987779 (2021).
123. Yang J, *et al.* Landscapes of bacterial and metabolic signatures and their interaction in major depressive disorders. *Sci Adv* **6**, (2020).
124. Baloni P, *et al.* Metabolic Network Analysis Reveals Altered Bile Acid Synthesis and Metabolism in Alzheimer's Disease. *Cell Rep Med* **1**, 100138 (2020).
125. Teng T, *et al.* Microbiota alterations leading to amino acid deficiency contribute to depression in children and adolescents. *Microbiome* **13**, 128 (2025).
126. Weston B, Fogal B, Cook D, Dhurjati P. An agent-based modeling framework for evaluating hypotheses on risks for developing autism: effects of the gut microbial environment. *Med Hypotheses* **84**, 395-401 (2015).
127. Saratkar SY, Langote M, Kumar P, Gote P, Weeraratna IN, Mishra GV. Digital twin for personalized medicine development. *Front Digit Health* **7**, 1583466 (2025).
128. Sittipo P, Choi J, Lee S, Lee YK. The function of gut microbiota in immune-related neurological disorders: a review. *J Neuroinflammation* **19**, 154 (2022).
129. Taj T, *et al.* Microbiota-brain interaction: The role of gut-derived proteins in addressing various neurological disorders including Parkinson's (PD) and Alzheimer's diseases (AD). *Biomed Pharmacother* **193**, 118861 (2025).
130. Sahoo S, Thiele I. Predicting the impact of diet and enzymopathies on human small intestinal epithelial cells. *Hum Mol Genet* **22**, 2705-2722 (2013).
131. Jiang B, *et al.* Understanding disease-associated metabolic changes in human colonic epithelial cells using the iColonEpithelium metabolic reconstruction. *PLoS Comput Biol* **21**, e1013253 (2025).
132. Zhong C, Wang S, Yan K, Zhao Y, Yang H, Yu J. Integrating organoids and organ-on-a-chip in dental research. *Biomaterials* **330**, 124065 (2026).
133. Mao S, Fonder C, Rubby MF, Phillips GJ, Sakaguchi DS, Que L. An integrated microfluidic chip for studying the effects of neurotransmitters on neurospheroids. *Lab Chip* **23**, 1649-1663 (2023).
134. Tran M, *et al.* A 3D gut-brain-vascular platform for bidirectional crosstalk in gut-neuropathogenesis. *Nat Commun* **17**, (2026).
135. Childs CJ, *et al.* Coordinated differentiation of human intestinal organoids with functional enteric neurons and

1. Zhao Z, *et al.* Multi-omics identifies microbiota-derived deoxycholic acid as a key mediator of blood-brain barrier dysfunction in Parkinson's disease. *Imeta* **4**, e70076 (2025).
2. Chen ML, *et al.* Ketogenic diet inhibits glioma progression by promoting gut microbiota-derived butyrate production. *Cancer Cell* **43**, 2119-2135.e2110 (2025).
3. Fernandes SQ, Kothare MV. A compartmental model for simulating the gut-brain axis in gastric function regulation. *Front Physiol* **17**, 1727491 (2026).
4. Schmidt BJ, Papin JA, Musante CJ. Mechanistic systems modeling to guide drug discovery and development. *Drug Discov Today* **18**, 116-127 (2013).
5. Interino N, *et al.* Microbiota-Gut-Brain Axis: Mass-Spectrometry-Based Metabolomics in the Study of Microbiome Mediators-Stress Relationship. *Biomolecules* **15**, (2025).
6. Park G, *et al.* A modified Mediterranean-style diet enhances brain function via specific gut-microbiome-brain mechanisms. *Gut Microbes* **16**, 2323752 (2024).
7. Chen C, Wang GQ, Li DD, Zhang F. Microbiota-gut-brain axis in neurodegenerative diseases: molecular mechanisms and therapeutic targets. *Mol Biomed* **6**, 64 (2025).
8. Li B, *et al.* Mild cognitive impairment has similar alterations as Alzheimer's disease in gut microbiota. *Alzheimers Dement* **15**, 1357-1366 (2019).
9. Jia L, *et al.* Metagenomic analysis characterizes stage-specific gut microbiota in Alzheimer's disease. *Mol Psychiatry* **30**, 3951-3962 (2025).
10. Zhao Y, *et al.* Time-restricted feeding mitigates Alzheimer's disease-associated cognitive impairments via a B. pseudolongum-propionic acid-FFAR3 axis. *Imeta* **4**, e70006 (2025).
11. Hemandhar Kumar S, Tapken I, Kuhn D, Claus P, Jung K. bootGSEA: a bootstrap and rank aggregation pipeline for multi-study and multi-omics enrichment analyses. *Front Bioinform* **4**, 1380928 (2024).
12. Ni Y, Cai H, Shao Y, Le W. Multi-omics insights into GBA1-associated Parkinson's disease: interplay of genomics, transcriptomics, proteomics, and lipidomics. *Mol Neurodegener* **21**, 6 (2026).
13. Picard M, Scott-Boyer MP, Bodein A, Périn O, Droit A. Integration strategies of multi-omics data for machine learning analysis. *Comput Struct Biotechnol J* **19**, 3735-3746 (2021).
14. Lin Y, *et al.* Uncovering stage-specific neural and molecular progression in Alzheimer's disease: Implications for early screening. *Alzheimers Dement* **21**, e70182 (2025).
15. Castells-Nobau A, *et al.* Gut microbial modulation of 3-hydroxyanthranilic acid and dopaminergic signalling influences attention in obesity. *Gut* **75**, 705-724 (2026).
16. Li Z, *et al.* Multi-omics analyses of serum metabolome, gut microbiome and brain function reveal dysregulated microbiota-gut-brain axis in bipolar depression. *Mol Psychiatry* **27**, 4123-4135 (2022).
17. Jaotombo F, Adorni L, Ghattas B, Boyer L. Finding the best trade-off between performance and interpretability in predicting hospital length of stay using structured and unstructured data. *PLoS One* **18**, e0289795 (2023).
18. Rudin C. Stop Explaining Black Box Machine Learning Models for High Stakes Decisions and Use Interpretable Models Instead. *Nat Mach Intell* **1**, 206-215 (2019).
19. Cai Z, Poulos RC, Liu J, Zhong Q. Machine learning for multi-omics data integration in cancer. *iScience* **25**, 103798 (2022).
20. Valous NA, Popp F, Zörnig I, Jäger D, Charoentong P. Graph machine learning for integrated multi-omics

21. Mariette J, Villa-Vialaneix N. Unsupervised multiple kernel learning for heterogeneous data integration. *Bioinformatics* **34**, 1009-1015 (2018).
22. Singh A, *et al.* DIABLO: an integrative approach for identifying key molecular drivers from multi-omics assays. *Bioinformatics* **35**, 3055-3062 (2019).
23. Argelaguet R, *et al.* Multi-Omics Factor Analysis-a framework for unsupervised integration of multi-omics data sets. *Mol Syst Biol* **14**, e8124 (2018).
24. Rappoport N, Shamir R. Multi-omic and multi-view clustering algorithms: review and cancer benchmark. *Nucleic Acids Res* **46**, 10546-10562 (2018).
25. Brière G, Darbo É, Thébault P, Uricaru R. Consensus clustering applied to multi-omics disease subtyping. *BMC Bioinformatics* **22**, 361 (2021).
26. Bruggeman FJ, Westerhoff HV. The nature of systems biology. *Trends Microbiol* **15**, 45-50 (2007).
27. Noordijk B, Garcia Gomez ML, Ten Tusscher K, de Ridder D, van Dijk ADJ, Smith RW. The rise of scientific machine learning: a perspective on combining mechanistic modelling with machine learning for systems biology. *Front Syst Biol* **4**, 1407994 (2024).
28. Daybog I, Kolodny O. A computational framework for resolving the microbiome diversity conundrum. *Nat Commun* **14**, 7977 (2023).
29. Xiao Y, Angulo MT, Friedman J, Waldor MK, Weiss ST, Liu YY. Mapping the ecological networks of microbial communities. *Nat Commun* **8**, 2042 (2017).
30. Dugourd A, *et al.* Causal integration of multi-omics data with prior knowledge to generate mechanistic hypotheses. *Mol Syst Biol* **17**, e9730 (2021).
31. Loers JU, Vermeirssen V. SUBATOMIC: a SUBgraph BAsed mulTi-OMics clustering framework to analyze integrated multi-edge networks. *BMC Bioinformatics* **23**, 363 (2022).
32. Ma'ayan A. Introduction to network analysis in systems biology. *Sci Signal* **4**, tr5 (2011).
33. Barabási AL, Oltvai ZN. Network biology: understanding the cell's functional organization. *Nat Rev Genet* **5**, 101-113 (2004).
34. Chen J, Wu X, Wang X, Yuan R, Zhu Q. Alterations in gut microbiota and metabolomic profiles in acute stroke: insights into brain-gut axis dysregulation. *Front Microbiol* **16**, 1580231 (2025).
35. Li Y, Chen Y, Fan Y, Chen Y, Chen Y. Dynamic network modeling of gut microbiota during Alzheimer's disease progression in mice. *Gut Microbes* **15**, 2172672 (2023).
36. Osama A, *et al.* Integrative multi-omics analysis of autism spectrum disorder reveals unique microbial macromolecules interactions. *J Adv Res* **77**, 265-279 (2025).
37. Francis D, Sun F. A comparative analysis of mutual information methods for pairwise relationship detection in metagenomic data. *BMC Bioinformatics* **25**, 266 (2024).
38. Ma L, *et al.* A role of gut-brain axis on prophylactic actions of arketamine in male mice exposed to chronic restrain stress. *Pharmacol Biochem Behav* **238**, 173736 (2024).
39. Gloor GB, Macklaim JM, Pawlowsky-Glahn V, Egozcue JJ. Microbiome Datasets Are Compositional: And This Is Not Optional. *Front Microbiol* **8**, 2224 (2017).
40. Chen Y, *et al.* Gut microbiota-driven metabolic alterations reveal gut-brain communication in Alzheimer's disease model mice. *Gut Microbes* **16**, 2302310 (2024).

41. Wallen ZD, et al. Metagenomics of Parkinson disease implicates the gut microbiome in multiple disease mechanisms. *Nat Commun* **13**, 3353 (2022).
42. Wang D, Russel WA, Sun Y, Belanger KD, Ay A. Machine learning and network analysis of the gut microbiome from patients with schizophrenia and non-psychiatric subject controls reveal behavioral risk factors and bacterial interactions. *Schizophr Res* **251**, 49-58 (2023).
43. Wang Y, et al. Multi-omics reveal microbial determinants impacting the treatment outcome of antidepressants in major depressive disorder. *Microbiome* **11**, 195 (2023).
44. Kuntal BK, Chandrakar P, Sadhu S, Mande SS. 'NetShift': a methodology for understanding 'driver microbes' from healthy and disease microbiome datasets. *Isme j* **13**, 442-454 (2019).
45. Xiang M, et al. Intestinal Microbes in Patients With Schizophrenia Undergoing Short-Term Treatment: Core Species Identification Based on Co-Occurrence Networks and Regression Analysis. *Front Microbiol* **13**, 909729 (2022).
46. Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics* **9**, 559 (2008).
47. Nie L, et al. Correlation between symptoms and cognitive function changes in patients with primary insomnia and pathways in gut microbiota. *Biochem Biophys Res* **37**, 101629 (2024).
48. Xie J, Zhong Q, Wu WT, Chen JJ. Multi-omics data reveals the important role of glycerophospholipid metabolism in the crosstalk between gut and brain in depression. *J Transl Med* **21**, 93 (2023).
49. Ntranos A, et al. Bacterial neurotoxic metabolites in multiple sclerosis cerebrospinal fluid and plasma. *Brain* **145**, 569-583 (2022).
50. Liu X, Huang Z, Liu L, Wang S, Huang Y, Liu X. Gut unclassified Ruminococcaceae reweights cortical functional gradients and small-world topology with links to mood and diet. *Neuroimage* **329**, 121830 (2026).
51. Peng R, et al. The brain-gut microbiota network (BGMN) is correlated with symptom severity and neurocognition in patients with schizophrenia. *Neuroimage* **308**, 121052 (2025).
52. Sazal M, Mathee K, Ruiz-Perez D, Cickovski T, Narasimhan G. Inferring directional relationships in microbial communities using signed Bayesian networks. *BMC Genomics* **21**, 663 (2020).
53. Mei H, Wang Z, Yang H, Li X, Xu Y. Network analysis of multivariate time series data in biological systems: methods and applications. *Brief Bioinform* **26**, (2025).
54. Savulescu-Fiedler I, Benea SN, Căruntu C, Nancoff AS, Homentcovschi C, Bucurica S. Rewiring the Brain Through the Gut: Insights into Microbiota-Nervous System Interactions. *Curr Issues Mol Biol* **47**, (2025).
55. Adrian MA, Ayati BP, Mangalam AK. A mathematical model of *Bacteroides thetaiotaomicron*, *Methanobrevibacter smithii*, and *Eubacterium rectale* interactions in the human gut. *Sci Rep* **13**, 21192 (2023).
56. Li G, et al. A mechanistic systems biology model of brain microvascular endothelial cell signaling reveals dynamic pathway-based therapeutic targets for brain ischemia. *Redox Biol* **78**, 103415 (2024).
57. Paredes-Vázquez A, Balsa-Canto E, Banga JR. Identification of dynamic models of microbial communities: A workflow addressing identifiability and modeling pitfalls. *PLoS Comput Biol* **21**, e1013204 (2025).
58. Travier L, et al. Neonatal susceptibility to meningitis results from the immaturity of epithelial barriers and gut microbiota. *Cell Rep* **35**, 109319 (2021).
59. Jansma J, Landi P, Hui C. Bayesian inference captures metabolite-bacteria interactions in a microbial community. *ISME Commun* **6**, ycag066 (2026).

60. Stein HA, et al. Ecological modeling from time-series inference: insight into dynamics and stability of microbial communities. *PLoS Comput Biol* **9**, e1003306 (2013).
61. Joseph TA, Shenhav L, Xavier JB, Halperin E, Pe'er I. Compositional Lotka-Volterra describes microbial dynamics in the simplex. *PLoS Comput Biol* **16**, e1007917 (2020).
62. Huang Y, Tang T, Dai X, Sun F. Quantifying microbial interactions based on compositional data using an iterative approach for solving generalized Lotka-Volterra equations. *PLoS Comput Biol* **21**, e1013691 (2025).
63. Nagarajan K, Ni C, Lu T. Agent-Based Modeling of Microbial Communities. *ACS Synth Biol* **11**, 3564-3574 (2022).
64. Dewan A, Mascellino MT. Computational and multi-omics systems biology for precision microbiome therapeutics. *Front Microbiomes* **5**, 1842701 (2026).
65. Ntekas I, et al. Spatial transcriptomics maps host-gut microbiome biogeography at high resolution. *Nat Microbiol* **11**, 1193-1204 (2026).
66. Yang C, et al. Explainable Artificial Intelligence to Decode the Blood-Brain Barrier Permeability of Gut Microbial Metabolites. *Biochemistry* **65**, 417-428 (2026).
67. Heirendt L, et al. Creation and analysis of biochemical constraint-based models using the COBRA Toolbox v.3.0. *Nat Protoc* **14**, 639-702 (2019).
68. O'Brien EJ, Monk JM, Palsson BO. Using Genome-scale Models to Predict Biological Capabilities. *Cell* **161**, 971-987 (2015).
69. Ovalle A, López E, Sierralta J, Paricio N, Garrido D. A rationally designed microbial consortium modulates neurodegeneration in a *Drosophila melanogaster* model of Parkinson's disease. *NPJ Biofilms Microbiomes* **11**, 185 (2025).
70. Boris V, Vanessa V. Molecular systems biology approaches to investigate mechanisms of gut-brain communication in neurological diseases. *Eur J Neurol* **30**, 3622-3632 (2023).
71. Arkin AP, et al. KBase: The United States Department of Energy Systems Biology Knowledgebase. *Nat Biotechnol* **36**, 566-569 (2018).
72. Machado D, Andrejev S, Tramontano M, Patil KR. Fast automated reconstruction of genome-scale metabolic models for microbial species and communities. *Nucleic Acids Res* **46**, 7542-7553 (2018).
73. Zorrilla F, Buric F, Patil KR, Zelezniak A. metaGEM: reconstruction of genome scale metabolic models directly from metagenomes. *Nucleic Acids Res* **49**, e126 (2021).
74. Heinken A, Magnúsdóttir S, Fleming RMT, Thiele I. DEMETER: efficient simultaneous curation of genome-scale reconstructions guided by experimental data and refined gene annotations. *Bioinformatics* **37**, 3974-3975 (2021).
75. Magnúsdóttir S, et al. Generation of genome-scale metabolic reconstructions for 773 members of the human gut microbiota. *Nat Biotechnol* **35**, 81-89 (2017).
76. Heinken A, et al. Genome-scale metabolic reconstruction of 7,302 human microorganisms for personalized medicine. *Nat Biotechnol* **41**, 1320-1331 (2023).
77. Heinken A, et al. APOLLO: A genome-scale metabolic reconstruction resource of 247,092 diverse human microbes spanning multiple continents, age groups, and body sites. *bioRxiv*, (2023).
78. Noronha A, et al. The Virtual Metabolic Human database: integrating human and gut microbiome metabolism with nutrition and disease. *Nucleic Acids Res* **47**, D614-d624 (2019).

79. Saha S, Kulkarni P, Das SK, Ghosh A. Genome-scale community modelling elucidates the metabolic interaction in Indian type 2 diabetic gut microbiota. *Sci Rep* **14**, 17255 (2024).
80. Diener C, Gibbons SM, Resendis-Antonio O. MICOM: Metagenome-Scale Modeling To Infer Metabolic Interactions in the Gut Microbiota. *mSystems* **5**, (2020).
81. Sen P, Orešič M. Integrating Omics Data in Genome-Scale Metabolic Modeling: A Methodological Perspective for Precision Medicine. *Metabolites* **13**, (2023).
82. Thiele I, *et al.* Personalized whole-body models integrate metabolism, physiology, and the gut microbiome. *Mol Syst Biol* **16**, e8982 (2020).
83. Taş E, Ülgen KO. Understanding the ADHD-Gut Axis by Metabolic Network Analysis. *Metabolites* **13**, (2023).
84. Hertel J, *et al.* Integrated Analyses of Microbiome and Longitudinal Metabolome Data Reveal Microbial-Host Interactions on Sulfur Metabolism in Parkinson's Disease. *Cell Rep* **29**, 1767-1777.e1768 (2019).
85. Rosario D, *et al.* Systematic analysis of gut microbiome reveals the role of bacterial folate and homocysteine metabolism in Parkinson's disease. *Cell Rep* **34**, 108807 (2021).
86. Hensen T, *et al.* In silico metabolic modelling links microbiome-derived metabolites to risk factors of Alzheimer's disease. *Gut Microbes Rep* **2**, 2443171 (2025).
87. Martinelli F, *et al.* Whole-body metabolic modelling reveals microbiome and genomic interactions on reduced urine formate levels in Alzheimer's disease. *Sci Rep* **14**, 6095 (2024).
88. Hensen T, Thiele I. Metabolic modeling links gut microbiota to metabolic markers of Parkinson's disease. *Gut Microbes* **17**, 2554195 (2025).
89. Tas E, Ulgen KO. Exploring the Integrated Gut-Brain Metabolic Model for ADHD. *Biochem Genet* **64**, 4091-4129 (2026).
90. Best L, *et al.* Metabolic modelling reveals the aging-associated decline of host-microbiome metabolic interactions in mice. *Nat Microbiol* **10**, 973-991 (2025).
91. Bekiaris PS, Klamt S. COBRA-k: A powerful framework bridging constraint-based and kinetic metabolic modeling. *Sci Adv* **12**, eaeb3022 (2026).
92. Mohammad FK, Palukuri MV, Shivakumar S, Rengaswamy R, Sahoo S. A Computational Framework for Studying Gut-Brain Axis in Autism Spectrum Disorder. *Front Physiol* **13**, 760753 (2022).
93. Baloni P, *et al.* Metabolic Network Analysis Reveals Altered Bile Acid Synthesis and Metabolism in Alzheimer's Disease. *Cell Rep Med* **1**, 100138 (2020).
94. Preciat G, Wegrzyn AB, Luo X, Thiele I, Hankemeier T, Fleming RMT. XomicsToModel: omics data integration and generation of thermodynamically consistent metabolic models. *Nat Protoc* **21**, 2665-2706 (2026).
95. Yang J, *et al.* Landscapes of bacterial and metabolic signatures and their interaction in major depressive disorders. *Sci Adv* **6**, (2020).
96. Teng T, *et al.* Microbiota alterations leading to amino acid deficiency contribute to depression in children and adolescents. *Microbiome* **13**, 128 (2025).
97. Weston B, Fogal B, Cook D, Dhurjati P. An agent-based modeling framework for evaluating hypotheses on risks for developing autism: effects of the gut microbial environment. *Med Hypotheses* **84**, 395-401 (2015).
98. Saratkar SY, Langote M, Kumar P, Gote P, Weerarathna IN, Mishra GV. Digital twin for personalized medicine development. *Front Digit Health* **7**, 1583466 (2025).
99. Sittipo P, Choi J, Lee S, Lee YK. The function of gut microbiota in immune-related neurological disorders: a

100. Sahoo S, Thiele I. Predicting the impact of diet and enzymopathies on human small intestinal epithelial cells. *Hum Mol Genet* **22**, 2705-2722 (2013).
101. Jiang B, *et al.* Understanding disease-associated metabolic changes in human colonic epithelial cells using the iColonEpithelium metabolic reconstruction. *PLoS Comput Biol* **21**, e1013253 (2025).
102. Ji P, *et al.* Single-cell delineation of the microbiota-gut-brain axis: Probiotic intervention in Chd8 haploinsufficient mice. *Cell Genom* **5**, 100768 (2025).
103. Mao S, Fonder C, Rubby MF, Phillips GJ, Sakaguchi DS, Que L. An integrated microfluidic chip for studying the effects of neurotransmitters on neurospheroids. *Lab Chip* **23**, 1649-1663 (2023).
104. Tran M, *et al.* A 3D gut-brain-vascular platform for bidirectional crosstalk in gut-neuropathogenesis. *Nat Commun* **17**, (2026).
105. Childs CJ, *et al.* Coordinated differentiation of human intestinal organoids with functional enteric neurons and vasculature. *Cell Stem Cell* **32**, 640-651.e649 (2025).