

Systems biology of host–microbe metabolomics

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The human gut microbiota performs essential functions for host and well-being, but has also been linked to a variety of disease states, e.g., obesity and type 2 diabetes. The mammalian body fluid and tissue metabolomes are greatly influenced by the microbiota, with many health-relevant metabolites being considered ‘mammalian–microbial co-metabolites’. To systematically investigate this complex host–microbial co-metabolism, a systems biology approach integrating high-throughput data and computational network models is required. Here, we review established top-down and bottom-up systems biology approaches that have successfully elucidated relationships between gut microbiota-derived metabolites and host health and disease. We focus particularly on the constraint-based modeling and analysis approach, which enables the prediction of mechanisms behind metabolic host–microbe interactions on the molecular level. We illustrate that constraint-based models are a useful tool for the contextualization of metabolomic measurements and can further our insight into host–microbe interactions, yielding, e.g., in potential novel drugs and biomarkers. © 2015 The Authors. *WIREs Systems Biology and Medicine* published by Wiley Periodicals, Inc.

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INTRODUCTION

The Human Gut Microbiome

Humans harbor a complex ecosystem, the gut microbiota, in their intestines. The collective genome of the microbiota, the ‘microbiome’, contains 100–150 times as many genes as the human genome.¹ The gut microbiota performs many important functions for the host, such as digestion of nutrients,² maturation of the host immune system,³ maintenance of epithelial cell layer integrity,⁴ and protection against pathogens.⁵ Worldwide research efforts, including MetaHIT¹ and the Human Microbiome Project,⁶ have elucidated the genetic repertoire and the phylogenetic composition of the human gut microbiome. These efforts have revealed that the human gut microbiome

is dominated by two bacterial phyla, Bacteroidetes and Firmicutes, which account for more than 90% of the detected phylotypes.^{7,8} Other microbial phyla found in the human gut microbiome include Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia.⁹ Moreover, the concept of three enterotypes allowing stratification of human individuals according to their gut microbiome composition has been proposed.⁹

The gut microbiome is implicated in the etiology of many diseases. For instance, obesity has been directly linked to the gut microbiota.¹⁰ The obesity epidemic is partly caused by the high-sugar, high-fat diet consumed in developed countries; this diet is known to affect the composition of the gut microbiota.¹⁰ It has been proposed that there is a relationship between an increased ratio of Firmicutes to Bacteroidetes and an obese phenotype in humans and mice^{7,11}; however, this finding has not been consistently reproduced, and its importance remains unclear.¹⁰ Obesity-related diseases, such as metabolic syndrome, type 2 diabetes, and cardiovascular disease as well as inflammatory bowel diseases, have also

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been associated with changes in the gut microbiome composition.¹⁰ Furthermore, there is increasing evidence that an altered gut microbiota is associated with autism¹² and neurodegenerative diseases, such as Parkinson's disease.¹³ An underweight status and malnutrition in children have also been associated with altered gut microbiota development.¹⁴ It has been proposed that our 'Western' diet has resulted in a 'dysbiotic', less diverse microbiota, leading to the observed dramatic increase in lifestyle-associated diseases.¹⁵ For instance, lower microbial gene richness has been linked to adiposity, higher insulin resistance, and inflammation.¹⁶ The gut microbiota contains 'keystone' species (e.g., *Ruminococcus bromii*) that are low in abundance but specialize in important functions, such as resistant starch degradation,¹⁷ and these species may be partially eliminated in human societies consuming the Western diet.¹⁵ However, the mechanisms underlying the relationship between the gut microbiota and disease states are still not completely understood. While certain genera are considered to be either protective against inflammation (e.g., *Faecalibacterium*)¹⁸ or proinflammatory (e.g., *Escherichia*),¹⁹ the role of most genera present in the gut in human health and well-being remains unclear.

Mammalian–Microbial Co-Metabolism and Impact on Human Health

Recent research efforts have moved beyond studying the composition (what is there) to investigating the functionality (what are they doing) of the human gut microbiota.²⁰ While the same phyla are consistently found in the gut microbiomes of humans, inter-personal variation in type and abundance at the genus and species level is high.²¹ In fact, the gut microbiota varies greatly even between closely related individuals.²² In contrast, a human gut microbiome gene catalog assembled from 124 Europeans revealed a core set of approximately 300,000 redundant microbial genes present in at least 50% of individuals, which included genes involved in essential pathways, such as polysaccharide degradation, short-chain fatty acid production, and amino acid and vitamin biosynthesis.¹ Consequently, it has been proposed that there is a 'core microbiome' consisting of a set of universal metabolic functions, rather than common genera or species.^{20,21}

Metabolomic analysis has resulted in an emerging picture of the mammalian–microbial 'co-metabolome'.²³ Gut microbes secrete a variety of health-relevant metabolites that play a role in the etiology and prevention of complex diseases.^{24,25} For instance, gut microbial conversion of choline to trimethylamine increases the risk of cardiovascular

disease²⁶ and gut microbes also transform dietary polyphenols into metabolically active antioxidative compounds.²⁷ The microbiota strongly influences host metabolic phenotypes and treatment with antibiotics significantly alters the urine and fecal metabolome.²⁸ Metabolites synthesized by the microbiota include compounds that can directly regulate and modulate host metabolism, such as neurotransmitters and hormones. In fact, the gut microbiota can be considered as an additional endocrine organ.²⁹ The gut microbiota also metabolizes and transforms xenobiotics, including a variety of drugs, which must be taken into consideration in future drug development.³⁰ Hence, the gut microbiota affects a variety of health-relevant metabolic functions in human (Table 2).

Notably, despite the presence of a core set of metabolic functions, there is some interpersonal variability in the metabolic activity of the microbiota. For example, two distinct phenotypes in rats can be distinguished based on the gut microbial metabolism of phenylalanine, with a possible connection to biomarkers associated with autism.³¹ Moreover, changes in the gut microbial composition, e.g., through bariatric surgery, can alter the metabolic phenotype of the host.^{32,33} Such findings may lead to the development of personalized medicine²³ and personalized nutrition³⁴ tailored to patients' individual genetic phenotypes, lifestyles, and microbiota compositions. The numerous factors affecting human health (e.g., genetic traits, environment, nutrition, and the microbiota composition) require an integrated systems biology approach.²³ Such a systems biology framework could reveal nonintuitive relationships between these factors, identifying novel biomarkers.^{23,35}

SYSTEMS BIOLOGY APPROACHES FOR STUDYING HOST–MICROBE CO-METABOLISM

Top-Down and Bottom-Up Systems Biology

The gut microbial ecosystem that co-exists with the human host can be described as a superorganism whose metabolic potential far exceeds that of a human alone.³ High-throughput methods, such as metagenomic, metatranscriptomic, and metabolomic analyses, have greatly increased our knowledge about the diversity and functionality of the human gut microbiota³⁶ and the influence of the gut microbiota on metabolic phenotypes.³⁷ To achieve a comprehensive understanding of host–microbe interactions, such 'big data' must be contextualized. One established method for placing multi-omics data into context is overlaying these data with network reconstructions.³⁸

TABLE 1 | Summary of the Main Modeling Methods Discussed in This Review

Feature	Top-Down Metabonomics	Topological Network Modeling	Constraint-Based Modeling
Model system	Multivariate statistical model	Supra-organism network	Genome-scale reconstruction(s)
Scope	Metabolite profiles of the gut microbiota/host organs and biofluids	Microbiome-wide	One or more target organisms (host or microbes) on the genome scale
Main inputs	Metabolomic measurements from biofluids or tissue	Metagenomic data	Target organism's genome sequence Optional inputs: <ul style="list-style-type: none"> • Target organism's physiological and biochemical traits • High-throughput data
Types of predictions	<ul style="list-style-type: none"> • Statistical correlations between microbes and metabolites • Metabolite profiles discriminating study from control group • Enriched pathways in study versus control group 	<ul style="list-style-type: none"> • Topological structure of the microbiome's metabolic network • Microbiome-wide enzyme abundances in host health or disease states 	<ul style="list-style-type: none"> • Reaction fluxes under condition-specific constraints (high-throughput data, nutrient environment) • Multispecies interactions • Gene knockout phenotypes
Advantages	<ul style="list-style-type: none"> • Directly driven by precise quantitative metabolite profiles • Depicts host metabolism on the pan-organismal level • No extensive manual curation required 	<ul style="list-style-type: none"> • Large scale • Depicts global topological features of the microbiome's metabolic network • No extensive manual curation required 	<ul style="list-style-type: none"> • Mechanistic, includes reaction stoichiometry • Organism-resolved • Includes species origin of genes
Disadvantages	<ul style="list-style-type: none"> • Not accounting for species' abundance and genomic information • Not mechanistic 	<ul style="list-style-type: none"> • Does not include species–species boundaries and the species origin of genes • Not mechanistic 	<ul style="list-style-type: none"> • Laborious reconstruction • Computationally intensive on a multispecies or multiorgan scale • Does not directly represent metabolite concentrations

There are a variety of network modeling techniques for high-throughput data with different strengths and weaknesses, including constraint-based modeling, kinetic modeling, and Bayesian approaches.³⁹ The main methods discussed in this review include top-down systems biology, topological models, and constraint-based models, which are compared in Table 1.

A distinction can be made between top-down approaches, in which network structures and conclusions are inferred through statistical analysis, and bottom-up approaches that employ manually

constructed and validated networks.⁴⁰ In the top-down approach, genome-wide high-throughput data are the starting point. Top-down analysis can easily integrate metabolomic, transcriptomic, and proteomic data.⁴¹ From this view of the system as a whole, mechanisms closer to the bottom are inferred with the aim of biological discovery. Networks are inferred from experimental data. Rather than being knowledge-based, top-down systems biology aims to glean new knowledge from the correlations between data points.⁴¹ The strengths of top-down approaches are that they are broad in scope, provide complete,

genome-wide views of the modeled organism(s), and do not require extensive manual curation efforts. A disadvantage of these methods is that they lack accuracy and mechanistic insight because network structures are merely inferred and not based on structural and biochemical knowledge.⁴⁰ In contrast, the bottom-up systems biology approach aims to be well structured and accurate. Pathways are assembled based on detailed information obtained from experimental studies and integrated into a large-scale reconstruction.⁴² Bottom-up reconstructions are mechanism based⁴¹ and predictive. A main weakness of the bottom-up systems biology approach is the laborious network reconstruction involved. The genome-scale reconstruction of a well-studied microorganism can take up to 6 months.⁴³ Moreover, owing to the need for manual curation and computational power, bottom-up networks are limited in scope, which is especially true for network models of large microbial communities. However, automated reconstruction tools^{44–49} and efficient algorithms^{50,51} designed to partly overcome these weaknesses are available. The methods for bottom-up network reconstruction of genome-scale metabolic networks are well established,⁴³ but this methodology has also been applied to nonmetabolic cellular processes, such as macromolecular synthesis^{52,53} and signaling pathways.^{54–56}

Top-Down Host–Microbe Metabolomics

Top-down systems biology combined with high-throughput metabolomic data has provided valuable insight into the co-metabolism of mammals and their microbiota. The metabotype, or the metabolic phenotype of a target organism as measured from its biofluids (e.g., blood, plasma, and urine), is commonly measured using nuclear magnetic resonance (NMR) spectroscopy or mass spectrometry (MS).^{23,57} Metabolomic analyses are untargeted, resulting in the broad quantification of biofluid metabolites, or targeted, thus accurately quantifying selected metabolites of interest.⁵⁷ Untargeted metabolomics may result in the discovery of novel biomarkers.⁵⁷ The interpretation of NMR spectroscopy measurements via multivariate statistical analysis is deemed ‘metabonomics’.^{58,59} Common multivariate statistical methods are principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA), which result in a separation of data points into clusters according to different metabotypes.⁵⁷ Statistical analyses may result in the discovery of biomarkers that explain the differences between control groups and clinical states.⁵⁷ Metabolomic measurements can

also be mapped onto pathways, for example, through metabolite set enrichment analysis (MSEA).⁵⁷ MSEA compares metabolomic measurements with metabolic pathway maps or databases, which can identify upregulated pathways compared with the control condition.⁵⁷

Top-down systems biology approaches have been applied to a variety of human and animal studies. For example, the metabolic profiles of germfree mice colonized with human baby flora and conventional mice were compared.⁶⁰ This analysis revealed significant differences in bile acid and lipid metabolism in the host.⁶⁰ Another study investigated the effects of probiotic lactobacilli on germfree mice colonized with human baby flora.⁶¹ Probiotic supplement modulated bile acid and energy metabolism and increased the levels of the microbial co-metabolites indoleacetyl-glycine, phenylacetyl-glycine, and tryptamine.⁶¹ Probiotics, prebiotics, and synbiotics (the combined application of both) also modulated multiple host organs in another human baby flora mouse model.⁶² Zucker rats are established model organisms of obesity and type 2 diabetes. Different microbiome compositions and metabolic phenotypes have been found in lean and obese Zucker rats.⁶³ Several studies have compared the metabolomes of germfree and conventional mice, revealing that the microbiota has systemic effects on whole-body metabolism and on biofluid and tissue metabolite profiles.^{64–68} Similarly, antibiotic treatment of rats altered a wide range of urinary and fecal mammalian–microbial co-metabolites demonstrating that the gut microbiota strongly impacts a variety of metabolic subsystems.²⁸ Correlations between mammalian–microbial co-metabolites and certain microbial species have been inferred through multivariate statistical analysis of metabolomic data. For instance, 10 bacteria were shown to be correlated with urinary metabolites (e.g., lactate, citrate, phenylacetylglutamine, and 4-cresol sulfate) in humans.⁶⁹ However, the mechanisms underlying such links between microbes and host metabolism remain poorly understood.

Topological Network Models

Topological network models of the human gut microbiome are constructed based on the microbiome-wide metabolic gene content, typically with nodes representing metabolites and links representing reactions (Figure 1(a)). Metagenomic data serve as input for these models, which consider the gut microbiota as a single supra-organism, where species boundaries and the species origin of genes are ignored. The resulting network reflects the metabolic potential of the entire

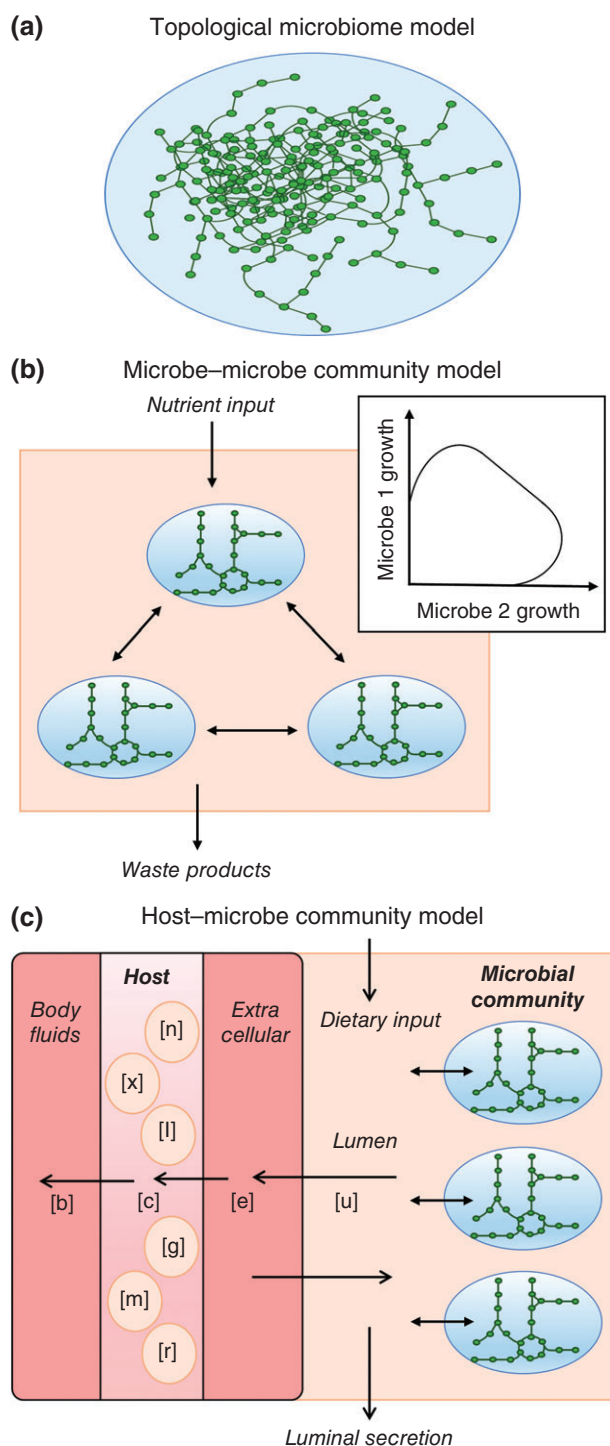


FIGURE 1 | Legend on the same page.

gut microbiome and thus represents the gut ecosystem on a systems level.

Greenblum et al. constructed one of the first topological network models by integrating shotgun metagenomic data from a human microbiome gene catalog assembled from 124 European subjects

by Qin et al.¹ into a metabolic network.⁷⁰ This ‘metagenomic systems biology’ approach revealed topological network differences associated with obesity and inflammatory bowel disease. For instance, the betweenness centrality, which was defined as the proportion of shortest paths passing through a given node, was determined for each enzyme. Based on this feature, each enzyme in the network was classified as peripheral, intermediate, or central. Enzymes associated with obesity and inflammatory bowel disease tended to have low centrality and to be peripheral rather than central.⁷⁰ Another approach based on metagenomic data (the HUMAnN method) was used to reconstruct microbial networks for seven body sites.⁷⁵ The HUMAnN pipeline is based on short DNA sequence reads, from which the presence, absence, and abundance of microbial gene families and pathways are computed. From the resulting pathway coverage and pathway abundance data for the seven body sites, metabolic pathways that are enriched in certain habitats could be identified. For example, glycosaminoglycan degradation was unique to the gut microbiota.⁷⁵ Yet another modeling framework used 5026 sequenced samples from 18 body sites and 239 individuals.⁷⁶ The resulting network of 3005 co-occurrence and co-exclusion relationships between 197 microbial clades has been constructed using generalized boosted linear models and correlation and similarity measures (e.g., Pearson correlation). The interactions participated in were clade-specific.

FIGURE 1 | Schematic representation of the major network modeling approaches utilized in systems biology analyses of host–microbe interactions. (a) Topological microbiome model. In this approach (e.g., Ref 70), the gut microbiota is treated as a single supra-organism without species–species boundaries, with nodes representing metabolites and links representing reactions. Topological features of the gut microbial metabolic network, e.g., betweenness centrality (defined as the proportion of shortest paths passing through a node) or neighborhood connectivity (average number of neighbors of a node’s neighbors)⁷⁰ can be elucidated. (b) Constraint-based microbe–microbe model. In a constraint-based multispecies model (e.g., Refs 71 and 72), metabolic reconstructions targeting two or more individual species are joined in an organism-resolved manner. Multispecies models allow the prediction of cross-feeding and mutualistic, commensal, or competitive interactions between microbial species. The tradeoff between two simultaneously growing microbes can be computed (e.g., see Ref 72). (c) Constraint-based host–microbe community interaction model. In a constraint-based host–microbe model, a reconstruction of host metabolism is joined with one⁷³ or more⁷⁴ metabolic networks of representative gut microbes. The setup enables a tractable exchange of host and microbial metabolites and provides outlets for luminal secretion and host secretion into body fluids (e.g., blood and urine). Hence, the host biofluid metabolome can be predicted (see also Figure 2).

For example, the known co-exclusion of Streptococci and Porphyromonaceae in the subgingival plaque was captured. The Actinobacteria and Bacilli only formed co-exclusion relationships with other clades, while pathogenic *Treponema* and *Prevotella* co-occurred in the oral microbiome.⁷⁶

To investigate the variable metabolic potential in human gut microbiomes, as well as links between gut microbes and human drug targets, another study mapped the gene catalog published by Qin et al.¹ to the MetaCyc⁷⁷ and the KEGG⁷⁸ databases.⁷⁹ The resulting metabolic network summarized the metabolic potential of the gut microbiota. Three distinct clusters of individuals with high, medium, or low metabolic potential were identified.⁷⁹ Moreover, the microbial network was overlaid with Recon1, a manually curated reconstruction of human metabolism that covers the reactions occurring in any human cell.⁸⁰ This strategy resulted in an interactome map of 'non-human' microbial metabolites and the human proteome. The chemical similarities between drug molecules in DrugBank⁸¹ and metabolites in the interactome map were compared, predicting that 603 existing drugs could perturb 515 metabolic microbial reactions. Moreover, 18 microbial metabolites overlapped with known experimental drugs indicating that the gut microbiota acts as a natural pharmacy.⁷⁹ Another supra-organismal network⁸² was recently constructed from the KEGG database⁷⁸ based on the genome annotations for selected bacterial strains reported to be present in the human gut.¹ The resulting network, accounting for 3449 reactions, has been used to predict 49 amino acid biotransformation products, of which 26 were confirmed to be present in the cecal contents of mice. The majority of the detected metabolites were microbe-derived and/or significantly reduced or absent in germfree mice,⁸² demonstrating the influence of the microbiota on shaping the luminal metabolome.

The seed set framework relies on genome-scale reconstructions of individual microbes⁸³ and a graph theory-based algorithm to compute an organism's metabolic potential to extract metabolites from the environment. This 'reverse ecology' approach assumes that selection pressure from the environment and the presence of other species is reflected in the metabolic network of an organism.⁸³ The seed set method was applied to 154 species from the human gut microbiota⁸⁴ and the metabolic profiles, competition, and complementarity indices for each pair have been computed. The predicted interactions have been compared with co-occurrence patterns for the 154 species based on their abundances from metagenomic data.¹ Metabolic competition correlated positively

with co-occurrence suggesting that habitat filtering drives microbiome assembly.⁸⁴

Taken together, these studies demonstrate the advantages of microbiome-wide topological networks. Because species boundaries are not accounted for in most of these studies, topological networks are extensive in scope and present a global view of the gut microbial ecosystem and its metabolic capabilities. Moreover, they can be easily linked to high-throughput data, such as those obtained through metagenomic or metabolomic analysis.⁸⁵ One shortcoming of not accounting for species boundaries is that diffusion (or facilitated transport) of almost any compound in the supra-organismal metabolic network is assumed, whereas, in reality, only a subset of metabolites can be exchanged between species, and their transport often requires energy, e.g., via ATP-binding cassette transporters.⁸⁶

Constraint-Based Modeling

At the heart of the constraint-based modeling and analysis (COBRA) approach lies well-structured mathematical models that were constructed in a bottom-up manner. Briefly, metabolic networks that describe the target organism on the genome scale are manually reconstructed based on the organism's genome sequence, biochemistry, and physiology. These genome-scale reconstructions (GENREs) can be converted into predictive mathematical models.⁸⁷ The process of generating GENREs has been divided into 96 steps.⁴³ To speed up parts of this reconstruction process, tools, such as Model SEED,⁴⁴ have been developed to generate automated draft reconstructions. Such draft reconstructions still require intensive manual curation and validation against the available literature to yield a high-quality reconstruction that captures the metabolic traits of the target organism.^{43,73,88} This bottom-up reconstruction process results in a biochemically, genetically, and genomically structured knowledge base for the target organism.⁴³ More than 140 manually curated GENREs are now available,⁸⁹ including many for microbes colonizing the human body.⁹⁰

Flux balance analysis is one of the most broadly used methods for the interrogation of constraint-based models.⁹¹ This method requires an objective function, such as the biomass objective function representing all molecular biomass precursors required to produce a new cell.⁹² The second key feature of flux balance analysis is the addition of constraints representing mass conservation, enzyme capacities, and environmental conditions (e.g., nutrient availability).⁹² It is assumed that the simulated biological system is

in a steady state. Solving the set of linear equations representing the biochemical network and imposed linear inequality constraints results in the prediction of a phenotype.⁹² A variety of constraint-based methods are available to contextualize high-throughput data, such as metabolomic measurements.^{91,93} For example, metabolomic measurements from physiologically normal and Leigh's syndrome fibroblasts were overlaid with a fibroblast reconstruction to study the metabolic differences between healthy and disease states.⁹⁴ Additionally, metabolomic and transcriptional data were integrated with Recon1 to construct condition-specific models for two cancer cell lines, which elucidated the distinct metabolism of the two cell types.⁹⁵ The integration of metabolomic data into the metabolic network of *Escherichia coli* resulted in accurate prediction of aerobic and anaerobic growth.⁹⁶ Moreover, tools have been developed to allow the simultaneous contextualization of quantitative metabolomic and proteomic measurements⁹⁷ or of transcriptomic and metabolomic datasets,^{95,98} yielding condition-specific models. Another established method for the contextualization of metabolomic data is ¹³C flux analysis, in which labeled carbon substrates are measured.⁹⁹ Because constraint-based modeling does not directly capture metabolite concentrations, fluxes are mathematically inferred from time-dependent changes in concentrations.⁹⁹ The integration of metabolomic data requires adequate information for the metabolites, e.g., InChI strings.^{100,101}

Constraint-Based Multispecies Interaction Models

A growing number of constraint-based modeling efforts have been devoted to developing multispecies models. Unlike the supra-organism approach employed in topological network models, constraint-based models combine multiple species by setting well-defined species boundaries.^{90,102} Another advantage is that they account for the genomic and biochemical traits of each included species. Typically, the species are reconstructed separately and then joined through an appropriate *in silico* scheme⁹⁰ (Figure 1(b) and (c)). Moreover, a community objective function must be defined to allow the optimization of multispecies growth. In a first effort to model microbe–microbe interactions, the interaction between a sulfate reducer and a methanogen was simulated using a small-scale model that allowed nutrient exchange through an additional shared compartment.¹⁰³ Simultaneous growth was simulated by fixing the ratios between the two microbes.¹⁰³ In a more complex modeling scheme, Klitgord and

Segre joined seven microbe reconstructions pairwise in a shared *in silico* environment, where each species retained its separate extracellular space.¹⁰⁴ To optimize simultaneous growth, minimal growth of each species was assumed. The interactions between the pairs were then systematically investigated by simulating different compositions of minimal media as well as genetic perturbations. Three types of synthetic interactions (mutualism, neutralism, and commensalism) were distinguished.¹⁰⁴ Taffs et al. developed three modeling approaches integrating microbial groups as guilds into a consortium.¹⁰⁵ This approach was applied for modeling thermophilic, phototrophic natural communities.¹⁰⁵ Using an approach combining metabolic modeling and *in vitro* culture, Wintermute and Silver showed that *E. coli* mutants that are auxotrophic for amino acids can complement each other's growth.¹⁰⁶ In yet another study, Freilich et al. predicted the metabolic interactions between 6903 bacterial pairs derived from 118 automated metabolic models.¹⁰⁷ Each pair was grown on an *in silico* competition-inducing medium. Based on the predicted growth rates, 'winners' that grew faster in co-culture and 'losers' that grew slower were identified. Furthermore, give–take interactions in samples from 59 ecological niches were predicted.¹⁰⁷ In a first effort to link high-throughput data with metabolic modeling of the gut microbiota, Shoaie et al. reconstructed three gut microbe species: *Eubacterium rectale* (Firmicutes), *Bacteroides thetaiotaomicron* (Bacteroidetes), and *Methanobrevibacter smithii* (Archaea).⁷¹ A co-growth model of the three species was then constructed and tailored to be condition specific based on transcriptomic data from *E. rectale* and *B. thetaiotaomicron* grown in ex-germfree mice.¹⁰⁸ Another study investigated the metabolic interactions between 11 gut microbes spanning three phyla.⁷² The pairwise tradeoffs between microbes (Figure 1(b)) were investigated on 12 varying nutrient environments. Anoxic conditions were predicted to induce mutualistic interactions between certain microbes, which were abolished in the presence of oxygen.⁷²

Various strategies have been applied to overcome the challenge of predicting realistic simultaneous growth in a microbial consortium. For instance, the dynamic multispecies metabolic modeling (DMMM) framework integrates existing metabolic reconstructions and uses dynamic flux balance analysis to predict time-dependent growth.¹⁰⁹ Multiple microbes can interact through transfer of metabolic products from one species through the other. However, DMMM cannot directly predict simultaneous community growth as the biomass objective functions of the included species are independent from each

other. Using this method, Zhuang et al. modeled the competition between two Fe(III)-reducing bacteria in a soil community.¹⁰⁹ Recently, dynamic flux balance analysis was also implemented into a lattice where a separate optimization problem is solved in each box.¹¹⁰ This approach, deemed COMETS (Computation of Microbial Ecosystems in Time and Space), enables the simulation of spatiotemporal dynamics of a multispecies microbial community.¹¹⁰ A community flux balance analysis approach solves a nonlinear optimization problem to simulate steady-state balanced growth of a microbial community, resulting in the prediction of the individual species abundances at optimal total community growth.¹¹¹ Yet another modeling framework (OptCom) optimizes multiple objective functions on the community and single species level,¹¹² thereby predicting community growth. Different types of interactions, such as commensalism, competition, parasitism, and mutualism, can be modeled with OptCom. As a downside, OptCom is computationally intensive compared with other frameworks, such as DMMM. OptCom was applied to predicting the interactions within a phototrophic microbial community.¹¹² An extension of this framework (dynamic OptCom) allows for dynamic modeling and the inclusion of substrate uptake kinetics.¹¹³ Recently, OptCom was also applied to modeling the interaction between two gut microbes, the Firmicutes representative *Faecalibacterium prausnitzii* and the Actinobacterium representative *Bifidobacterium adolescentis*.¹¹⁴ The existing studies demonstrate that constraint-based modeling accurately captures the behavior of individual species and natural interaction patterns of commensalism, mutualism, and competition. Moreover, unexpected, nonintuitive species–species interactions can be predicted. A disadvantage of constraint-based modeling is the intensive manual curation effort required to construct such models, which has caused most studies performed to date to be small in scope and to include only a limited number of species.

Constraint-Based Modeling of Host–Microbe Interactions

While microbe–microbe interaction models are well established, few constraint-based studies have been conducted to model host–microbe interactions. In a first effort, Bordbar et al. constructed a host–pathogen model simulating the infection of the human alveolar macrophage with *Mycobacterium tuberculosis*. The model was constructed by placing a reconstruction of *M. tuberculosis* into an *in silico* compartment simulating the intracellular phagosome.¹¹⁵ A

constraint-based model of a host and a commensal microbe⁷³ linked a published mouse reconstruction¹¹⁶ with a reconstruction of the prominent human gut symbiont *B. thetaiotaomicron*. The two species could consume simulated dietary inputs and exchange nutrients with each other through an *in silico* compartment simulating the intestinal lumen. The mouse could secrete into a separate outlet representing mouse biofluids, e.g., blood or urine. This setup was modeled after a gnotobiotic mouse mono-associated with *B. thetaiotaomicron*, which is a well-established animal model used in gut microbiome research.¹¹⁷ To enable simultaneous growth, the tradeoff between host and microbe biomass production was computed. The *in silico* model captured known traits of the *in vivo* model, including *B. thetaiotaomicron*'s foraging on dietary and host glycans as well as the production of short-chain fatty acids,¹¹⁷ which were then consumed by the host. Moreover, the model predicted that lethal mouse gene deletion phenotypes would be rescued by the presence of *B. thetaiotaomicron* and vice versa. Finally, the implementation of the separate host outlet enabled the prediction of the mouse biofluid metabolome, which was validated against experimental metabolomic data.⁷³

Moving from these initial efforts to a comprehensive, predictive model of human–microbe co-metabolism requires a well-curated, extensive network of human metabolism. The global human reconstruction Recon2 was curated through a community effort¹¹⁸ and is far more comprehensive than its predecessor, Recon1.⁸⁰ Importantly, it captures the majority of known exometabolites, which are extracellular metabolites found in biofluids, such as blood and plasma,¹¹⁸ making it an excellent tool for prediction of the human metabolome and the contextualization of metabolomic data. Most health-relevant metabolites and pathways known to be influenced by the gut microbiota are present in Recon2 (Table 2). Taking advantage of these characteristics, Recon2 was used to predict the global effects of a representative gut microbe community on the host's metabolome.⁷⁴ The community accounted for 11 published, manually curated gut microbe reconstructions spanning three phyla (Bacteroidetes, Firmicutes, and Proteobacteria), including commensal, probiotic, and pathogenic bacteria.⁹⁰ The microbial reconstructions were linked to Recon2 using a previously established framework,⁷³ which enables dietary input and metabolic exchange between host and microbes (Figure 1(c)). The human global metabolome was systematically predicted in the presence of the 11 microbes and in their absence ('germfree' human).

TABLE 2 | Metabolites Relevant for Human Health and Disease, and Associated References

Metabolite	Health Implications	References	HMDB ID	Recon2 ID
Short-chain fatty acids produced by gut microbiota	Acetate	Serves as carbon source in the liver, affects immune system by binding GPR receptors, may have a role in adipogenesis, and stimulates colonic function.	10,119 HMDB000042	ac
	Propionate	Serves as carbon source in the liver, has anti-inflammatory properties, lowers blood cholesterol, stimulates satiety, and alters brain function in rats.	10,119 HMDB000237	ppa
Microbial fermentation products	Butyrate	Serves as the main energy source for colonocytes, has anti-inflammatory properties, prevents oxidative stress, and may protect against colonic cancer.	120,119 HMDB000039	but
	Lactate	Certain circumstances in the gut may lead to toxic levels of D-lactate in blood, causing D-lactic acidosis. Positively correlated with <i>Faecalibacterium prausnitzii</i> .	69,119 HMDB01311	lac_D
	Formate	Decreased levels were found in urine of IBD patients. Formate is associated with blood pressure.	121,122 HMDB00142	for
Lipid metabolism	Ethanol	Produced by many gut bacteria, disrupts the intestinal epithelial barrier. Ethanol consumption can promote intestinal overgrowth.	123,124 HMDB00108	etoh
	Acetaldehyde	Produced from ethanol by gut microbiota, toxic and carcinogenic. Disrupts the intestinal epithelial barrier.	24,123 HMDB00090	acald
	Succinate	Decreased levels were found in urine of IBD patients and in a rat model after bariatric surgery.	32,121 HMDB00254	succ
	Choline	Choline deficiency and disrupted choline metabolism are associated with NAFLD. Choline has also been linked to CVD risk.	25,26,125 HMDB00097	chol
	Betaine	Phosphatidylcholine-derived betaine has been linked to CVD risk.	26 HMDB00043	glyb
Phosphatidylcholine	Ethanolamine	Host-derived ethanolamine can be exploited as a carbon source by pathogenic <i>Salmonella typhimurium</i> during inflammation.	126 HMDB00149	etha
	Phosphatidylcholine	Biomarker of disrupted fatty acid metabolism, related to obesity and resistance to insulin. Phosphatidylcholine metabolites are linked to CVD.	26,57 pchol_hs	pchol_hs

TABLE 2 | Continued

Metabolite	Health Implications	References	HMDB ID	Recon2 ID
Phosphatidyl-ethanolamine	Altered in plasma of individuals with type 2 diabetes.	127	HMDB060501	pe_hs
L-Carnitine	Higher L-carnitine levels were found in obese subjects. Dietary L-carnitine is converted to TMA by gut microbiota, linking it to CVD risk.	128,129	HMDB00062	crn
Acylcarnitines	Biomarkers of disrupted fatty acid metabolism after high-fat feeding. Acylcarnitine pools are altered in obese subjects.	57,128		e.g., acrn, c4crn, and c8crn
Dimethylamine	Increased urinary levels in NAFLD. Negatively correlated with <i>F. prausnitzii</i> .	69,125	HMDB00087	
Trimethylamine (TMA)	It is formed from choline and L-carnitine by gut bacteria, and has been linked to CVD. Increased urinary levels in NAFLD.	26,125,129	HMDB00906	
Bile acids	Gut microbes transform bile acids to secondary bile acids, permitting their reabsorption via the colonic epithelium. The gut microbiota may contribute to obesity, type 2 diabetes, inflammation, and cancer by controlling bile acid pools.	10,124,130		chololate C03990 gchola tchola C04483 dgchol tdchola
Cholate			HMDB00619	
Lithocholate			HMDB00761	
Glycocholate			HMDB00138	
Taurocholate			HMDB00036	
Deoxycholate			HMDB00626	
Glycochenodeoxycholate			HMDB00637	
Taurochenodeoxycholate			HMDB00951	
Amino acids	High levels of leucine, isoleucine, and valine are associated with obesity and type 2 diabetes.	128,131–133	HMDB00172	ile_L leu_L val_L
Branched-chain amino acids			HMDB00687	
Aromatic amino acids	High levels of tyrosine, phenylalanine, and tryptophan are associated with type 2 diabetes.	128,132,133	HMDB00883	phe_L tyr_L trp_L
Glutamine and glutamate	High glutamate/glutamine ratio is associated with type 2 diabetes.	132	HMDB00159	gln_L glu_L
Taurine	Elevated levels were found in fecal samples of UC patients. Associated with certain gut bacterial species.	69,134	HMDB00158 HMDB00929 HMDB00641 HMDB00148 HMDB00251	taur

TABLE 2 | Continued

Phenolic compounds	Metabolite	Health Implications	References	HMDB ID	Recon2 ID
	Phenylacetate	Increased production by gut bacteria on a high-protein diet, may give rise to toxic products. Elevated in colorectal cancer.	25,135	HMDB00209	pac
	Phenylpropionate	Can be converted to benzoic acid by gut bacteria and give rise to hippurate, which is implicated in a variety of disease states.	136	HMDB11743	
	3-Hydroxyphenylpropionate (3-HPPA)	Can be converted to benzoic acid by gut bacteria and give rise to hippurate, which is implicated in a variety of disease states.	136	HMDB00375	3hpa ¹
	Phenylacetylglutamine	Decreased excretion in autism. Lower levels in obese subjects. Positively associated with age in humans. Associated with certain gut bacterial species.	31,69,128,137	HMDB06344	pheaagl
	Benzoic acid	Converted into hippurate by the host, which is implicated in a variety of disease states.	136	HMDB01870	bz
	4-Hydroxyphenylacetate	Elevated in colorectal cancer. Negatively correlated with <i>Subdoligranulum variable</i> .	25,69	HMDB00020	4hphac
	Hippurate (N-benzoylglycinate)	Related to a variety of health and disease states. Hippurate levels in urine are decreased in CD patients due to altered gut microbiota. Diminished excretion was also found in obese individuals as well as patients with schizophrenia and autism. Associated with certain gut bacterial species.	31,69,136,138,139	HMDB00714	bgly
	p-Cresol	Produced by gut bacteria. May be a significant factor in autism, is elevated in colorectal cancer, and has been linked to inflammatory bowel disease. Also linked to cardiovascular disease in chronic kidney disease patients. Interferes with the sulfonation of acetaminophen (paracetamol).	25,31,140,141	HMDB01858	pcresol ¹
	p-Cresyl sulfate	Formed from gut bacteria-derived p-cresol, and is directly linked to progression of chronic kidney disease. Elevated in the urine of children with autism. Positively associated with age in humans. Associated with certain gut bacterial species.	25,69,137,142	HMDB11635	pcs ¹

TABLE 2 | Continued

Metabolite	Health Implications	References	HMDB ID	Recon2 ID
Indolic compounds				
Indole	Produced by gut bacteria, converted to uremic toxin indoxyl sulfate by the host.	143	HMDB00738	indole ¹
Indoxyl sulfate	Formed from bacteria-derived indole, and is directly linked to progression of chronic kidney disease.	142	HMDB00682	inds ¹
Polyamines				
Cadaverine	Produced by gut bacteria. Increased levels were found in fecal samples of UC patients.	134	HMDB02322	
Putrescine	Produced by gut bacteria and in human tissues. Polyamines are toxic at high concentrations and associated with cancer. Increased fecal putrescine was found in a rat model after bariatric surgery.	24,32	HMDB01414	ptrc
Spermine			HMDB01256	sprm
Spermidine			HMDB01257	spmd
Hormones/ Precursors				
GABA (γ -aminobutyric acid)	Directly produced by gut bacteria with implications for the gut-brain axis. Increased urinary levels were found in a rat model after bariatric surgery.	29,32	HMDB00112	4abut
Dopamine	Directly produced by gut bacteria with implications for the gut-brain axis.	29	HMDB00073	dopa
Norepinephrine	Directly produced by gut bacteria with implications for the gut-brain axis.	29	HMDB00216	npphr
Serotonin	Directly produced by gut bacteria. The microbiota also regulates tryptophan availability, affecting serotonin biosynthesis in the CNS and thus brain and behavior.	29	HMDB00259	srtn
Histamine	Histamine and <i>N</i> -methylhistamine levels are increased in IBD patients.	29,144	HMDB00870	hista
<i>N</i> -Methylhistamine	Histamine is also directly produced by gut bacteria.		HMDB00898	mhista
L-Dopa	L-Dopa is dehydroxylated by the gut microbiota. Manipulating the microbiota has been proposed as an approach to control L-Dopa bioavailability in PD treatment.	29,145,146	HMDB00181	34dhphe
Other				
Ammonia	Produced by many gut bacteria. Inhibits the mitochondrial oxygen condition and short-chain fatty acid oxidation in colonocytes.	147	HMDB00051	nh4
Hydrogen sulfide	Produced by some gut bacteria, e.g., <i>Escherichia coli</i> . Toxic to colonocytes, disturbs their metabolism. Found to be increased in stool samples of colonic cancer patients. Implicated in IBD.	24,147,148	HMDB00598	
Glutathione	Glutathione levels in the liver were lower in mice colonized with human baby flora than in conventional mice. Increased glutathione synthesis as stress response was also found in NAFLD.	60,149	HMDB00125	gthrd

CD, Crohn's disease; CNS, central nervous system; CVD, cardiovascular disease; IBD, inflammatory bowel disease; NAFLD, nonalcoholic fatty liver disease; PD, Parkinson's disease; UC, ulcerative colitis. If available, HMDB¹⁵⁰ (<http://www.hmdb.ca/>) and Recon2¹¹⁸ (<http://humanmetabolism.org>) IDs are shown.

¹ Will be included in subsequent releases of Recon2.

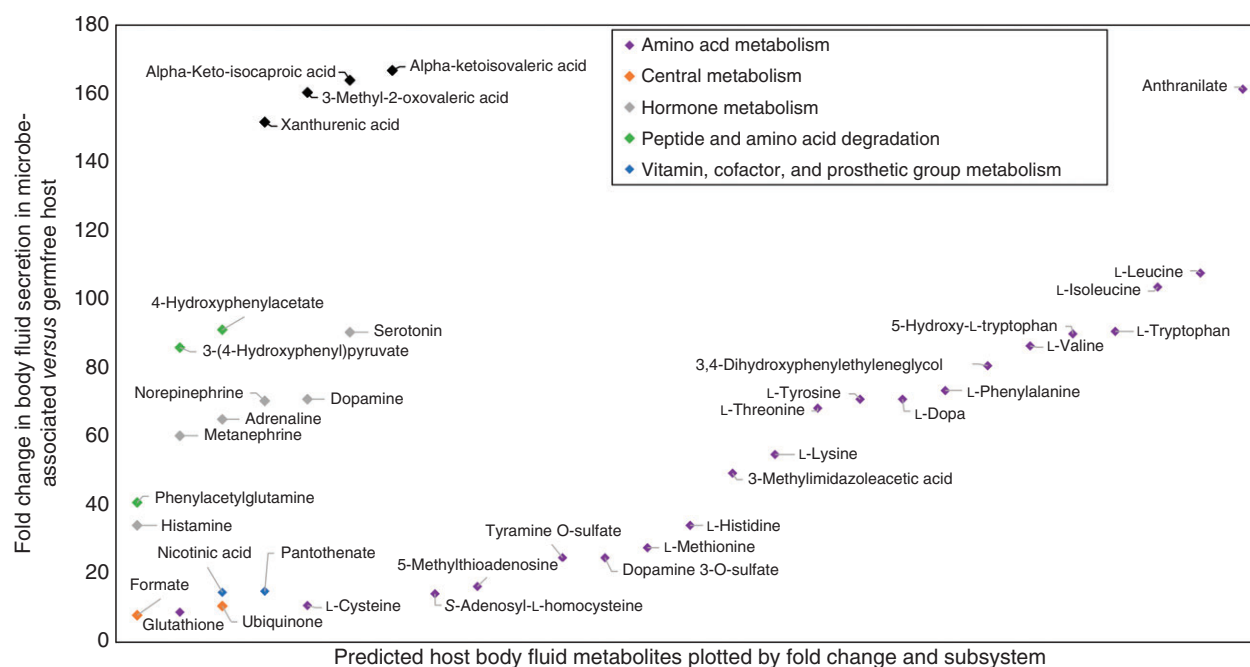


FIGURE 2 | Prediction of health-relevant host body fluid secretion using a constraint-based modeling framework. Using a constraint-based modeling framework (Figure 1(c)), the maximal quantitative metabolite secretion was predicted in the presence and in the absence of a model community of 11 microbes. A dietary regime approximating the amounts of protein, carbohydrate, and fat consumed by a typical Western citizen (<http://www.ars.usda.gov/>) was simulated. Shown are examples for metabolites for which the secretion flux was at least fivefold increased in the presence of the microbe community compared with the ‘germfree’ condition. The complete data analysis is available in Ref 74.

Recon2 secreted a total of 342 metabolites. Surprisingly, only 11 of these metabolites could not be secreted by the ‘germfree’ human, but the quantitative secretion flux of 52 metabolites was at least fivefold higher in the presence of the microbe community (Figure 2). Notably, the secretion of known mammalian–microbial co-metabolites, such as phenylacetylglutamine and 4-hydroxyphenylacetate,⁶⁹ as well as several hormones and neurotransmitters, was up to 160-fold higher in the presence of the 11 microbes (Figure 2). Moreover, the microbes’ potential to secrete luminal metabolites was systematically predicted, including a variety of phenolic compounds as well as short-chain fatty acids, lactate, formate, and ethanol.⁷⁴ These metabolites are known to have beneficial or detrimental effects on host health.¹²⁰ Thanks to the bottom-up systems biology approach, underlying mechanisms for the observed microbial effects on host metabolite secretion could be proposed. For instance, the microbes increased glutathione and leukotriene production by the host through secreting the synthesis-limiting precursor L-cysteine.⁷⁴ In summary, this systems-level framework predicted that the microbes profoundly affected host metabolism, in agreement with the emerging view of the microbiota as an additional organ.²⁹ In future efforts, more comprehensive gut microbial communities could be

considered, which would be a prerequisite for the integrative analysis of metagenomic and metatranscriptomic data. Such applications will be valuable for gaining mechanistic insight into human–microbe co-metabolism.

FUTURE PERSPECTIVES

Elucidating the Unknown Metabolic Potential of the Microbiota

Despite the recent advances in the field of human gut microbiome research, our understanding of the mechanisms through which the microbiota affects host health is still incomplete. Two worldwide research initiatives, the Human Microbiome Project and MetaHIT, have resulted in enormous amounts of data, including more than 2800 reference sequences for microbes from various sites in the body (<http://www.hmpdacc.org/>). However, many of these strains are uncultured and uncharacterized, and the genome sequence alone does not offer complete insight into the metabolic capabilities of specific bacteria.¹⁵¹ As a result, it is difficult to identify keystone species through metagenomic approaches alone.¹⁵¹ Ultimately, only cultivation can fully elucidate the biochemical and physiological traits of

a strain¹⁵¹; yet cultivating the mostly anaerobic gut microbiota is difficult, time-consuming, and impossible to be performed for thousands of species. Constraint-based modeling could bridge the gap between genome sequences and *in vitro* culture by predicting a species' metabolic potential based on its genome sequence.^{88,152,153} Such predictions could identify potential keystone species, whose metabolic properties (e.g., nutrient requirements) could be predicted and subsequently experimentally validated. For instance, a combined *in silico/in vitro* approach has yielded a chemically defined growth medium for the oxygen-sensitive gut symbiont *E. prausnitzii*.⁸⁸ Such an iterative approach could be readily applied to the other poorly characterized or uncharacterized species in the gut.

Predicting Mechanisms by Combining Top-Down and Bottom-Up Approaches

In addition to the characterization of individual species in the gut, it is of utmost importance to identify links between gut microbial metabolism and human phenotypes. Metagenomic datasets have provided valuable insights, for example, giving rise to the enterotype concept.⁹ However, many of these studies used top-down approaches, which identified patterns rather than mechanisms.¹⁵⁴ Moreover, the causality between phenotypes and the microbiome composition (i.e., whether the host's phenotype drives changes in the gut microbiome composition or vice versa) remains unclear.¹⁵⁴ The combination of top-down approaches revealing patterns and bottom-up methods elucidating the mechanisms underlying these patterns is a key future challenge.¹⁵⁴ Supporting experimental bottom-up approaches with computational methods will require well-structured, accurate networks. Constraint-based reconstructions are ideal tools to provide insight into phenotype-gut microbiome causality because they can predict mechanisms at the biochemical level.³⁹ Initial efforts could demonstrate that constraint-based modeling can provide mechanistic hypotheses on how microbes affect the host's potential to synthesize health-relevant metabolites.^{73,74} A host-microbe community model could also be combined with top-down methods to contextualize high-throughput data (Figure 3). For instance, in a second phase of the Human Microbiome Project, a multi-omic analysis of the microbiotas of mothers and neonates will be performed, including metagenomic, metatranscriptomic, metabolomic, and metaproteomic analyses.¹⁵⁵ In addition, host lipidomes and cytokines will be analyzed.¹⁵⁵ These datasets will be accessible through

public databases¹⁵⁵ and could be readily mapped onto the human metabolic reconstruction Recon2,¹¹⁸ which accounts for many lipid derivatives, or be used in a gut microbiota community model. Moreover, top-down statistical approaches have previously inferred associations between metabolites and specific microbes from metabolomic data. For instance, *E. prausnitzii* has been shown to correlate with the presence of eight human urinary metabolites.⁶⁹ Integrating top-down inference models with bottom-up reconstructions could elucidate the mechanisms underlying such patterns. A proposed pipeline for using constraint-based modeling to contextualize high-throughput data is shown in Figure 3.

Toward a Predictive Bottom-Up Host-Microbe Community Model: Challenges

Currently, the application of constraint-based modeling to gut microbiome research is limited by the availability of high-quality reconstructions. While the number of high-quality, manually curated metabolic reconstructions is steadily growing, the thousands of species inhabiting the human gut are still poorly represented. A number of bacteria colonizing the human gut have been reconstructed^{88,90,71,114}; however, they do not represent the gut microbiome composition well. While Proteobacteria are overrepresented, the important Bacteroides and Clostridium groups are only represented by one reconstructed species, and reconstructions for minor phyla, such as Verrucomicrobia, are lacking. Moreover, the currently available reconstructions lack standardization in terms of nomenclature and the reconstruction structure, which hampers their integration onto a community model.^{101,74,156} To overcome this challenge, MetaNetX, a tool that allows the mapping of metabolic reconstructions from different sources into a common namespace, has been developed.¹⁵⁷ Using automated reconstruction tools, such as Model SEED⁴⁴ or Pathway Tools,⁴⁷ one could easily construct a community model of hundreds of species from the genome sequences archived by the Human Microbiome Project.⁶ The resultant loss of accuracy would be compensated by a dramatic increase in scope. Considering that most gut microbes are both uncultivated and uncharacterized, automated draft reconstructions are a reasonable first approximation of their metabolism. A challenge in this context is that the quality of a draft reconstruction depends mostly on the genome sequence. Many published sequences are incomplete. Additionally, gene annotations are lacking or unspecific and/or the associated pathways are not accounted for by the automated

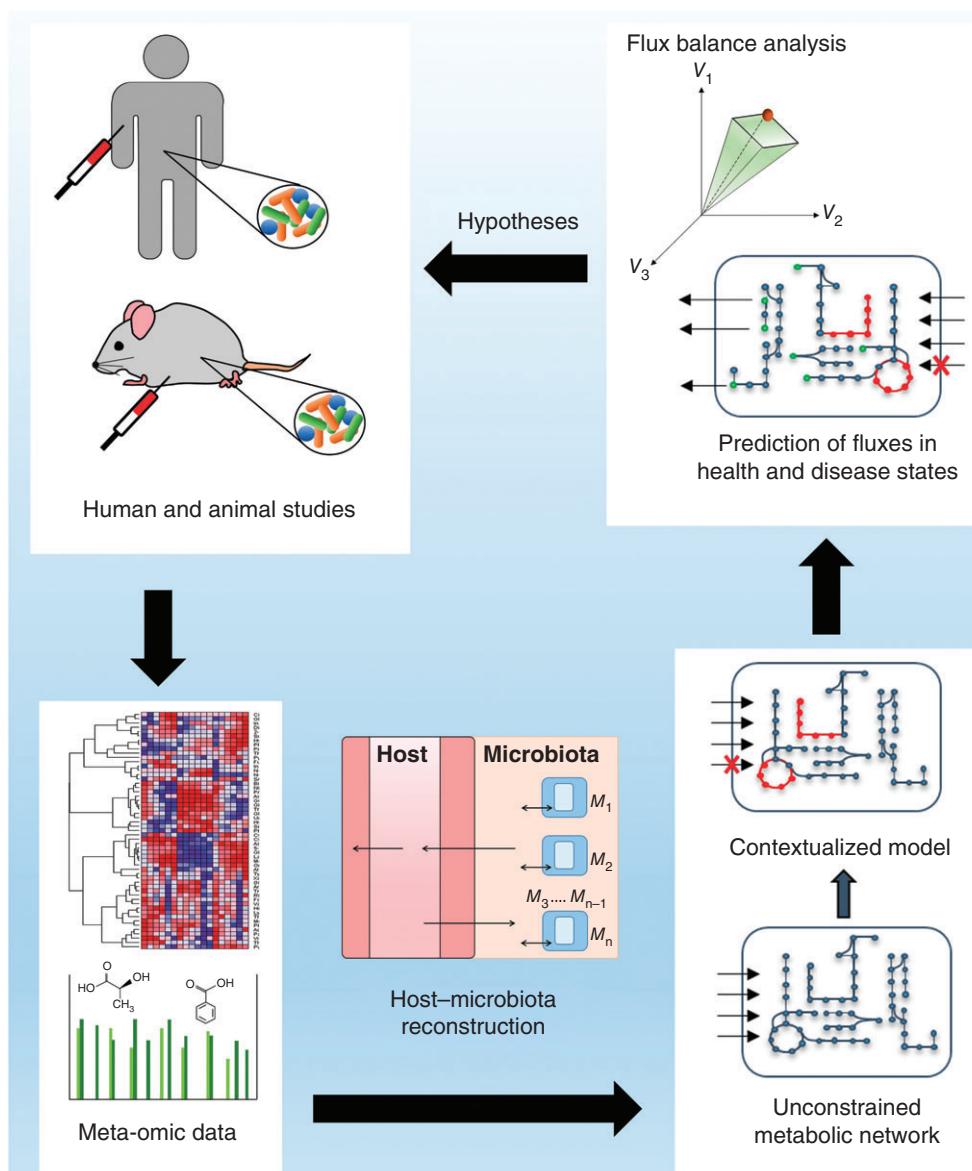


FIGURE 3 | Schematic overview of a pipeline for using constraint-based models to contextualize high-throughput metagenomic, metatranscriptomic, metaproteomic, and metabolomic data from human and animal studies.

reconstruction pipelines, resulting in a less predictive draft reconstruction. Moreover, even universal pathways vary in the enzymes carrying out individual steps.¹⁵⁸ As a result, annotation platforms often fail to correctly reconstruct these variant pathways.¹⁵⁸ Several recent comparative genomics studies have resulted in the improvement of genome annotations in gut microbial genomes.^{159–161} Such efforts will ultimately result in an improved predictive potential of automated reconstructions derived from the genome sequence.

Peripheral and species-specific pathways are generally lacking in automated reconstructions.⁴⁴

For instance, the dietary glycan degradation by gut microbes is highly specialized and species-specific¹⁶² and the inclusion of the associated pathways into metabolic reconstructions currently requires intensive manual curation.⁷³ Recently, a computational pipeline has been developed to predict the glycan degradation potential of gut bacterial species¹⁶³ and will facilitate the incorporation of species-specific carbohydrate degradation pathways into metabolic reconstructions. Xenobiotic transformations by gut microbes are currently not well captured in draft metabolic reconstructions. The ongoing advances in metagenomic analyses will further elucidate drug transformations

performed by gut bacteria,¹⁴⁵ which could then be included into reconstruction pipelines.

Constraint-Based Modeling of Host–Microbe Co-Metabolism: Future Applications

The field of human gut microbiome research has exploded during the last decade and will undoubtedly grow even more in the upcoming years. The prevalence of lifestyle diseases directly linked to the gut microbiota is estimated to increase exponentially in the next decades. For instance, worldwide obesity has nearly doubled since 1980; there are currently 1.4 billion overweight people, including 500 million obese individuals.¹⁶⁴ The prevalence of type 2 diabetes in developed and developing countries is predicted to double between 2000 and 2030.¹⁶⁵ Thus, the interest in the relationship between the human microbiota and host health is ever growing. In this section, we propose key contributions that constraint-based host–microbe modeling could make.

A key application area of systems biology research on host–microbe interactions is drug development. It is well established that the gut microbiota affects drug metabolism.³⁰ In fact, at least 30 drugs have been shown to be co-metabolized by the microbiota.³⁰ For instance, the well-studied drug acetaminophen (paracetamol) is differentially metabolized in individuals due to gut microbial activity.¹⁴⁰ Microbiota-derived *p*-cresol competes over human sulfo-transferase 1 with acetaminophen, resulting in lower acetaminophen sulfonation capacity in individuals with high bacterial *p*-cresol production. As, many xenobiotics are substrates for sulfo-transferase 1 and sulfonation alters the physical properties of molecules, this finding has implications for the metabolism and toxicity of various drugs.¹⁴⁰ The cardiac drug digoxin has been shown to be inactivated by the gut bacterium *Eggerthella lenta*.¹⁶⁶ Using a computational framework, the transformation of drugs and xenobiotics (e.g., antibiotics) can be predicted and linked to specific species. Recon2 accounts for 1290 drugs included in DrugBank,⁸¹ mapped to 308 enzymes and enzymatic complexes,¹¹⁸ making it an excellent tool for modeling drug metabolism. As constraint-based modeling can predict drug effects at a mechanistic level,³⁵ and its applications for drug discovery are promising (reviewed in more detail in Refs 35, 167, and 168). For instance, metabolic modeling has led to the prediction and subsequent validation of a novel cancer drug.^{169,170} Recently, Sahoo et al. expanded Recon2 with a manually curated drug metabolism module for the five most highly prescribed drug

groups, including acetaminophen.¹⁷¹ This expanded human network was then used to predict the effects of dietary intake and inherited metabolic disorders on drug metabolism. The flux through sulfo-transferase 1, which carries out the sulfonation of acetaminophen, has been predicted to be lower under a vegetarian diet compared with a Western or balanced diet.¹⁷¹ Interestingly, the same reaction was found to be affected by high gut microbial production of *p*-cresol, which competes with acetaminophen for sulfo-transferase 1.¹⁴⁰ Currently, such studies are using the human metabolic network as the sole modeling environment and are therefore not accounting for the well-known drug transformations performed by the microbiota. By using a host–microbe community framework,⁷⁴ drug co-metabolism by humans and microbiota depending on the diet could be investigated.

A logical next step after modeling the co-metabolism of drugs and xenobiotics is the prediction of individual-specific drug metabolism. The global human reconstruction can be tailored to be both cell-type and individual specific, e.g., by overlaying it with transcriptomic or metabolomic data,¹⁷² and it has been applied successfully to the contextualization of high-throughput data from pathological states, such as type 2 diabetes¹⁷³ and cancer.^{95,174} Recently, Agren et al. constructed personalized genome-scale models for carcinoma patients and used these models to predict cancer drug targets.¹⁷⁵ Moreover, Yizhak et al. built personalized cancer cell models for more than 700 breast and lung cancer patients.¹⁷⁶ Low growth rates *in silico* were found to be correlated with longer patient survival.¹⁷⁶ Personalized human cell models could also be constructed based on intestinal metabolomic measurements (e.g., condition-specific models based on measured drug degradation products). One could even envision a personalized gut microbiota model based on an individual's gut microbiome composition. Such knowledge-based, bottom-up network models would valuably complement or be combined with the existing top-down inference models, such as the pharmacometabonomics approach developed by Nicholson et al.¹⁷⁷

Biomarker discovery is another important application of systems biology. For instance, constraint-based modeling-based analyses have predicted biomarkers of inborn errors in human metabolism,^{118,178} with 77% accuracy in the case of Recon2.¹¹⁸ The human reconstruction was also applied to predicting novel biomarkers of type 2 diabetes^{173,179} and Alzheimer's disease.¹⁸⁰ Linking Recon2 with a microbial community⁷⁴ could be applied to predicting the effects of the microbes on disease-associated biomarkers in humans.

Yet another useful application is modeling the interplay between the diet, microbiota, and host energy metabolism. Several constraint-based approaches have been employed to model energy metabolism (reviewed in Ref 181). Obesity can generally be considered to be the result of an imbalance between energy uptake and consumed energy. The gut microbiota has long been known to contribute approximately 10% of the host energy intake¹⁸² and to directly modulate host energy metabolism.³ Systems biology models have been proposed as a tool for modeling the thermodynamic properties of the gut microbiota and its costs from the perspective of the host.¹⁸³ There are established methods for integrating thermodynamic constraints into genome-scale models,^{184–189} which could be used for this type of application. Multiple studies have investigated adipocyte metabolism using cell-type-specific models.^{190–192} Such genome-scale adipocyte models would be combined with a microbe community model to elucidate the effect of the presence of microbes on host fat storage and energy metabolism.

Finally, while this review focuses on human–gut microbiota interactions, the described systems biology approach is not limited to human hosts. For instance,

we⁷³ and others⁸² have shown that mouse–gut microbiota interactions can also be computationally modeled. Ex-germfree mice colonized with a defined or conventional microbiota are important animal models employed in gut microbiome research,¹⁹³ especially in metabolomics analyses.^{65,67} Computational models can be useful in complementing or possibly partially replacing such animal studies. Other mammals could be reconstructed using the human reconstruction as a template,¹⁷² and these reconstructions could subsequently be combined with a microbe community model. Moreover, host–microbe modeling can be carried out for organisms other than mammals. For example, the survival of the endangered honeybee may be improved by manipulating its gut microbiota,¹⁹⁴ which could be predicted *in silico*. Another potential application of constraint-based multispecies modeling is the investigation of plant–microbe interactions. There are several plant genome-scale reconstructions^{195,196} as well as tissue-specific reconstructions of *Arabidopsis thaliana*,¹⁹⁷ and multiorgan models of maize¹⁹⁸ and barley,¹⁹⁹ which could be applied to modeling the interactions between plants and their associated symbiotic or pathogenic microbes.

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