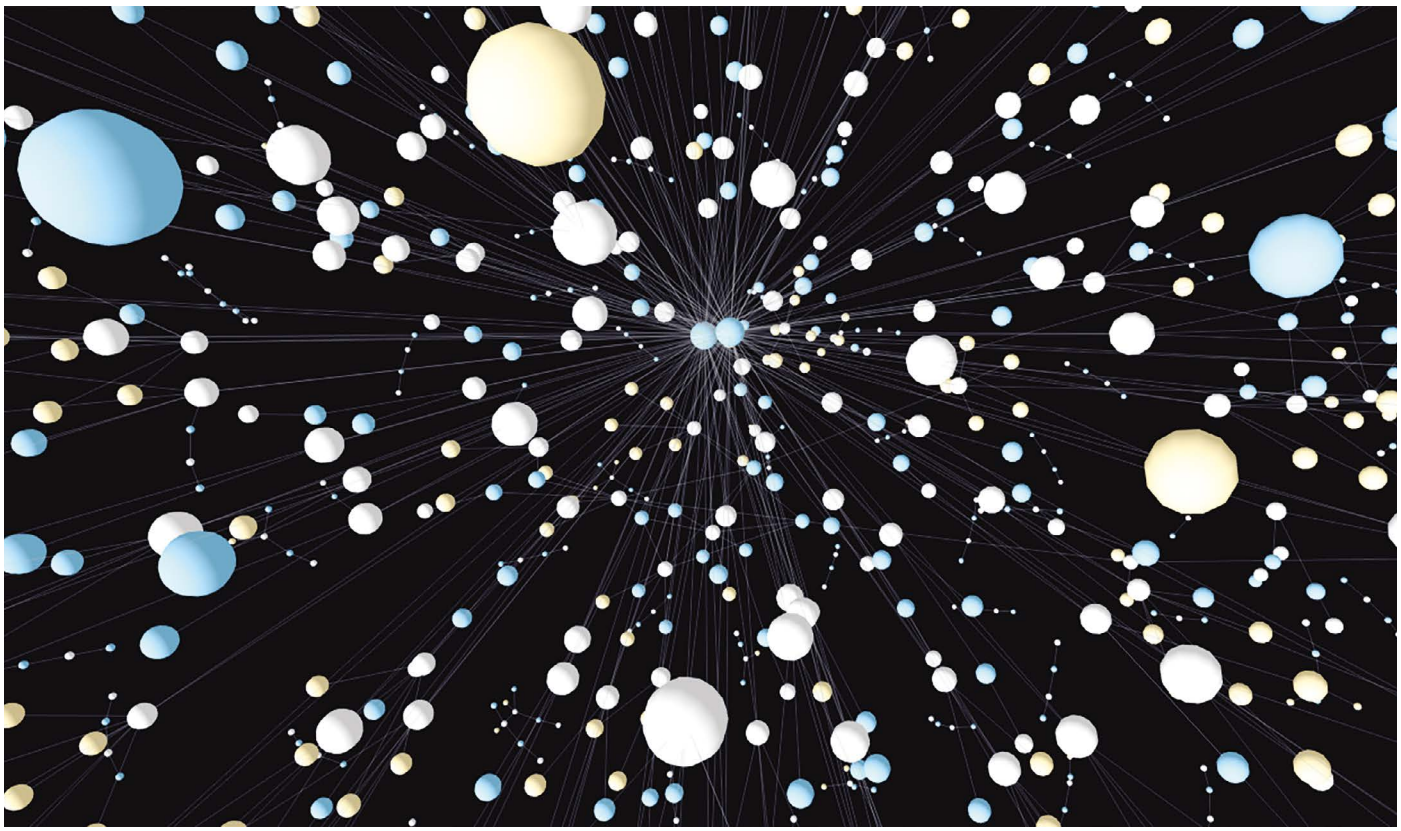


J. ROBINSON *ET AL. SCI. SIGNAL.* **13**, EAAZ1482; 2020 (CC BY SA 4.0)

A genome-scale metabolic model of yeast. Each coloured sphere represents a substance that the organism uses in metabolism.

THE MICROBIOME MODELLERS

Computational approaches can reconstruct the complex interactions between gut bacteria and their human hosts. **By Michael Eisenstein**

There is something comforting in the elegance of a chemical reaction. Inputs and conditions on one side of the reaction predictably yield a defined set of products on the other. But this predictability is quickly lost in complex biological systems, where thousands of reactions occur in parallel among vast numbers of interacting cells.

Consider the human gut microbiome, in which roughly 1,000 bacterial species compete and collaborate while communicating with their host. This crosstalk rapidly becomes too complex to capture in a diagram. But understanding these communities is crucial, because their biological activity directly affects our health and susceptibility to disease.

Now, systems biologists are building models to illuminate these black boxes. "The old-school way of looking at hundreds of thousands of reactions is just not feasible, and not desirable either," says Ines Thiele, a microbiome researcher at the National University of Ireland in Galway. "But we have techniques now that try to help us see what's happening much quicker and tackle the emergent complexity that arises."

Thiele and her colleagues are developing mathematical and statistical approaches that use a range of data, from areas such as genomics and biochemistry, to reconstruct highly diverse gut microbial communities computationally. They capture only a fraction of the complex biological reality of the microbiome, but can reveal interactions between microbes and their hosts that would be near-impossible to detect otherwise.

Polished GEMs

For many modelling efforts, the starting point is a genome-scale metabolic model (GEM). Constructed by scanning an organism's genome to determine the biochemical processes it can perform, GEMs are essentially blueprints of the enzymatic assembly lines in every microbe. "They're capturing all of the metabolic capabilities that the cell has," says Jens Nielsen, a systems biologist at Chalmers University of Technology in Gothenburg, Sweden. Researchers can then mathematically model how these inputs and outputs feed into one another.

This approach is particularly powerful for studying the gut, the microbes of which are often difficult to cultivate in the laboratory. Researchers can also draw on existing biological knowledge to extrapolate the possible function of a gene using sequence similarities with known enzymes, for instance. Even sparse information can be useful. Systems microbiologist Karsten Zengler at the University of California, San Diego, and his colleagues, for example, developed a GEM for a relatively under-studied species of marine diatom (a type of plankton), even though functions had been identified for only about one-tenth of its genome[1]. "Surprisingly, it worked," Zengler says. "We have enough information about metabolism to understand what they're doing." His team's modelling approach assigned functions to more than 1,000 diatom genes, which collectively perform nearly 4,500 interconnected biochemical reactions, and predicted where in the cells these reactions are likely to occur.

Currently, most microbiome samples are studied by isolating total DNA from a microbial community and breaking it into small pieces for sequencing, a strategy called shotgun metagenomics. The resulting data sets can provide an inventory of the species present in a sample without needing to culture or isolate them. Researchers can combine GEMs to flesh out larger microbial community models, and identify patterns of enzymatic activity on the basis of genes uncovered in the data. They can then thread these processes together to understand which chemicals the particular system is taking up, which products are being released, and which cells are interacting to drive these processes. "You get non-obvious relationships between the microbes that you would just not see by looking at genome sequences themselves," says Thiele.

In one study[2], Zengler's group modelled the dynamic interplay between the alga *Chlorella vulgaris* and the yeast *Saccharomyces cerevisiae*, uncovering unexpectedly high levels of exchange between the two species for certain amino acids. Experimental profiling of metabolites in the sample might have overlooked this exchange, says Zengler, who offers the analogy of trying to infer children's snack preferences by what's left on the table after a birthday party. "You might observe the table and say, 'Look at all those apples – that's what children must like to eat.' But that is not the case."

Not all interactions can be modelled: it's currently impractical to capture the full interplay between all reactions from every cell at the same time. As a result, these models are often best suited for testing existing hypotheses, says Joao Xavier, a systems biologist at the Memorial Sloan Kettering Cancer Center in New York City. "For example, we have several hypotheses about the role of fermentative bacteria that produce short-chain fatty-acid compounds, such as butyrate, that are associated with intestinal health," he says. "So we could try to look for genes that belong to those pathways." Xavier's approach draws on principles used in conventional ecological modelling – for example, equations used for studying predator–prey interactions that capture how changes in one population can affect another. His group then uses machine-learning techniques to model these effects in more complex microbial systems.

But even seemingly simple interactions can hide complexity. Consider the straightforward example of one microbe producing a carbohydrate that another microbe likes to eat. Changes in both the composition of a community and its environmental conditions can influence interactions between species, Zengler notes, citing his yeast–algae study. "In one condition, they loved each other and were best buddies and exchanged everything and grew happily together – better than they did by themselves," he says. But something as simple as changing ammonia levels could make them more adversarial. "This relationship just got worse and worse, and at one point they ended up having a 'divorce' and fighting over their belongings."

"Even seemingly simple interactions can hide complexity."

Just part of the picture

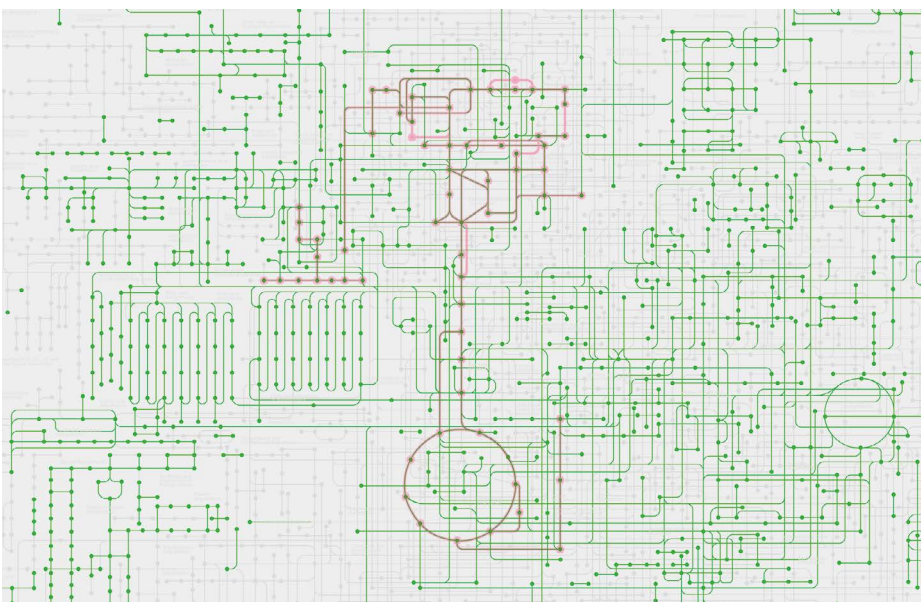
Models can reliably draw such inferences only if they have good underlying data. But those can be difficult to get. The gene databases that researchers use to deduce enzymatic function and other biological activities are still incomplete, and even well-characterized species can hold surprises. In an effort to improve his group's metabolic models, Nielsen tested how well *S. cerevisiae* grew on different carbon-based nutrients[3]. "We found it used many carbon sources that we were not taking into account even in our most state-of-the-art models," he says.

Models of the microbiome in the human gut might also overlook crucial aspects of the host environment, such as the physical structure of the large intestine – a lengthy organ with a bacterial composition that varies from one section to the next. "The geometry of the gut is quite complicated", as are the mechanics of faecal matter travelling through it, says Xavier. There is also extensive communication between the host and microbiome, both at the intestinal barrier and through chemical signals that the intestinal cells subsequently relay throughout the body.

Researchers can indirectly measure the impact of such interactions using samples of blood, urine or cerebrospinal fluid. Thiele has been developing a 'virtual metabolic human' that can integrate these and other data with microbiome models to create a more holistic picture of these interactions in various disease states. "We now have about 30 organs or tissues, arranged in an anatomically accurate manner," she says. Her group is using those models to search for microbial perturbations that might contribute to disorders such as Parkinson's disease.

Xavier's group is collaborating with clinicians to understand how antibiotics affect the gut microbiomes of bone-marrow-transplant recipients, whose immune systems are suppressed to minimize rejection of the transplant. They found that the resulting microbial disruptions can markedly affect the function of the immune system after the transplant, and that supplementing individuals with healthy microbial populations could improve their recovery[4].

Elhanan Borenstein, a systems biologist at Tel Aviv University in Israel, says, "People may underestimate the importance of modelling as a tool. It's not just to simulate a real environment, but also a way to understand first principles." Armed with these fundamentals, researchers might ultimately be able to identify targeted microbiome interventions that meaningfully change clinical outcomes, even if much of the system remains a black box.



M. KANEHISA & S. GOTO *NUCLEIC ACIDS RES.* **28**, 27–30 (2000)

A map of the complex pathways in human metabolism (carbohydrate pathway in red).

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