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A multi-omic trip through the human gut

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Most of human microbiome research has focused on analysing faecal samples, which represent the final stop of the digestive journey. Two recent articles use a novel sampling approach to capture luminal content at different points during digestion and reveal that the analysis of faecal samples tells only a fraction of the story.

Changes in the microbiology and physiology of the upper gastrointestinal tract have historically been underappreciated. The vast majority of human microbiome studies use faecal samples, because of their ready availability and because sampling other regions often requires surgical procedures. Consequently, little is known about changes in microbiome and metabolome composition that occur along the human gastrointestinal tract. Companion papers by Shalon et al.[1] and Folz et al.[2] take us on a wild ride through the human gastrointestinal tract using innovatively engineered sampling capsules (Fig. 1). Our journey starts in the mouth, with the swallowing of four capsules that are designed to collect samples on the basis of the changing pH of the environments they encounter. As we travel through the gut via peristalsis, samples are collected at different locations from proximal to distal. Deep in the bowels of our trip, we take literal twists and turns through the small intestine on our way to the colon — all the while learning about shifting gut microbiology and biochemistry. The end of our intestinal 'magic school bus ride' is a bit messy; we wind up in the faeces of our human volunteers, but the data collected reveal some long-hidden mysteries about the physiology of human digestion and the role of the microbiome.

The sampling capsule used in these studies can collect small amounts of luminal content through the action of its one-way valve, which only opens when the capsule's coating dissolves (dependent on pH). Although it can vary depending on the individual, the pH of the human gut is lower in the duodenum, increases towards the ileum and then drops again upon the transition to the colon[3]. The authors of both papers take advantage of this pH transition by having each of 15 healthy volunteers ingest four separate capsules designed to sample three locations in the small intestine and one in the ascending colon. The luminal contents contained in these four devices were then analysed using multi-omics with the aim of comparing differences between the four devices, as well as with the faecal sample that marks the end of each capsular adventure. The article by Shalon et al., published in *Nature*, provides a more general overview of the sampling approach and a broad analysis of the metagenome, metabolome, virome and proteome in the sampled material[1], whereas the Folz et al. paper, published in *Nature Metabolism*, takes a deeper dive into the diverse metabolomic data in which thousands of biomolecules were detected throughout the gastrointestinal tract (many of which remain unknown)[2], making both articles nicely complementary.

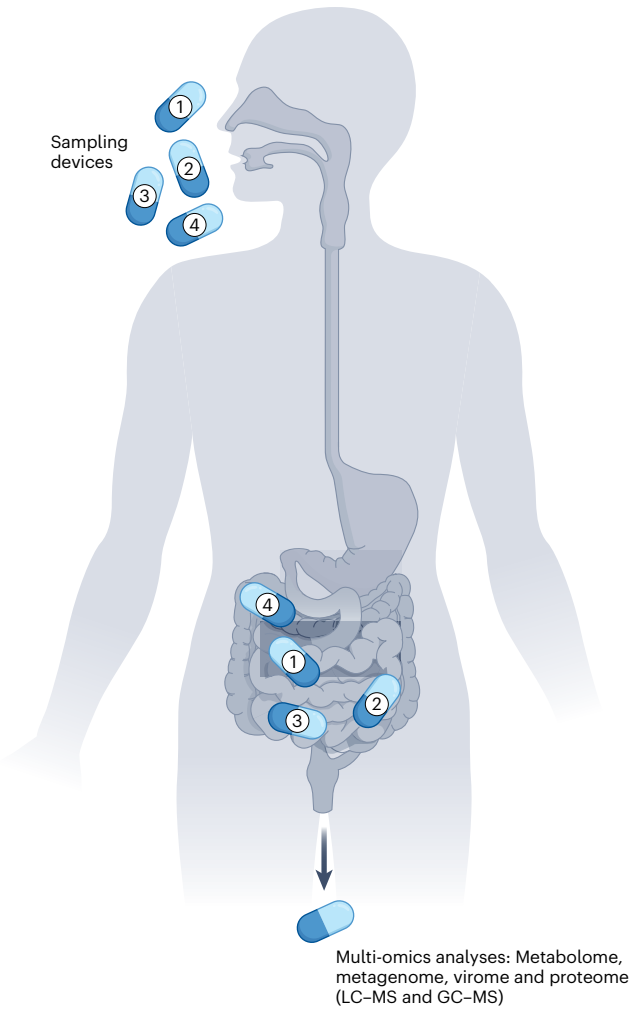

Fig. 1 | Sampling approach. Healthy volunteers ingested four different sampling capsules that collect luminal contents at different locations along the digestive tract on the basis of the changing pH of the environment.

Getting back to our intestinal journey, we find spatially distinct microbial communities along the way. As we traverse the intestines we encounter *Bilophila wadsworthia*, a bile-resistant bug with an important role in human digestion[1,4], and other Proteobacteria that are more abundant here than in faeces. Twenty-three percent of the bacteria detected by 16S rRNA amplicon sequencing in the intestines were not found in stool. This points to a missing microbiome — the bacteria living in the upper gastrointestinal tract are not accounted for in studies of human faecal microbiota. The capsules may be able to capture these missing microorganisms alive, as the authors were able to culture different bacterial isolates from the devices after collection.

By contrast, bacteriophages identified from metagenomic sequencing of the capsule contents showed more overlap between the upper gastrointestinal and stool, indicating these tiny microbes complete the whole gastrointestinal journey with us. Analysis of prophages alone, however, showed evidence for more bacteriophage induction in the intestine as compared to stool.

Our molecular trip through the gastrointestinal tract is even more complex, as it includes thousands of proteins (the proteome) and metabolites (the metabolome) — the source of which can be either human or microbial. By combining different omics approaches, the authors describe a shifting proteome and metabolome from the proximal to the distal gut that is quite variable across individuals, indicating that our journey could be very different depending on who has ingested the capsules. We see high concentrations of peptides as we pass through the upper gastrointestinal tract, and as we enter the colon we are met by more abundant short-chain fatty acids, secondary bile acids and phospholipids. Fifty-four percent of the most abundant molecules we encountered on our journey were more abundant in the upper gastrointestinal tract, as compared to the lower. The relative proportions of these metabolites varied among participants, which the authors attribute to differences in diet. As if the complete lack of light does not make this journey dark enough, the matter gets even darker in their metabolomic dataset, where 9,317 of the commonly detected molecules are completely unknown[2,5].

The authors focused much of their analysis on bile acids, which are highly abundant steroid detergents produced by our liver that help to digest fat. Primary bile acids are host-derived and include those conjugated with the amino acids glycine or taurine. These molecules decreased from the proximal to distal intestine, probably owing to the action of the microbial enzyme bile salt hydrolase (which hydrolyses amino acids). Microbially produced secondary bile acids were most abundant in faeces, changing little throughout our journey until its end. Most of the bile acid transitions observed along our way were ones that we expected, as the physiology of primary and secondary bile acids is quite well understood[6]. What was surprising, however, was variation in the recently discovered microbially conjugated bile acids (MCBAs). Because we brought recent reading material with us on our capsular journey, we know that MCBAs are newly discovered bile acids that are uniquely conjugated with different amino acids by gut bacteria[7,8]. Little is known about them, but the authors found they were more abundant in the intestines than in stool and the abundances of each changed through the gastrointestinal tract depending on the amino acid conjugated. MCBAs conjugated with cysteine, serine and alanine correlated with liver conjugates, whereas other amino acids trended with deconjugated molecules. How these unique microbial molecules affect the host and other microorganisms along the gastrointestinal tract remains mostly unknown, but the authors show that, regardless of what they are doing, location matters.

Although this time we travel only through the guts of healthy people, these two articles set a platform for future studies of individuals with gastrointestinal diseases. For example, the study of inflammatory bowel disease is an exciting potential application because subtypes of the disease are location-specific. Future work with these sampling capsules could lead to better treatment approaches that are targeted at different gastrointestinal locations, and we could envision future developments in which the capsules themselves could be engineered to serve as the delivery vehicle. The noninvasive sampling approach developed by the authors greatly enhances our ability to sample contents from different gut regions from many human volunteers, although dietary variation across individuals can substantially affect the results. Overall, the most important observation along our journey is that the microbiome, virome, proteome and metabolome vary at different locations through the gut, and faeces provide only a snapshot taken at the rear end of that trip.

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Competing interests

The authors declare no competing interests.