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Editorial overview: Probing environmental processes and microbiome functions using stable isotopes as smart tracers in analytical biotechnology

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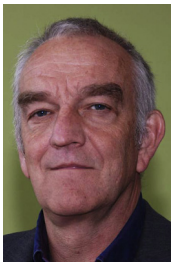

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Hans H. Richnow is head of the Department of Isotope Biogeochemistry at the Helmholtz Centre for Environmental Research. He received a Diploma in Geology, PhD in Geochemistry and Habilitation in the field of Biogeochemistry at the University of Hamburg, Faculty of Geosciences. He was a pioneer in using isotope fractionation for the assessment of *in situ* biodegradation. Particularly, he is developing concepts applying isotope techniques for environmental monitoring, remediation studies and for the behaviour of chemicals in the environment. His interdisciplinary research examines topics in hydrology, geology, microbiology and environmental chemistry.

Stable isotope probing (SIP) approaches have come of age and are capable of providing researchers with answers to questions clearly beyond the original concepts of 'who is doing what' and proving biodegradation. While SIP was originally conceived as the application of stable isotope tracers for analyzing fluxes of elements (C, N, O, S) in biological systems, recent analytical developments have boosted its application to a wide range of distinct biomarkers (proteins, lipids, nucleic acids, metabolites) using natural isotope composition or isotopically labelled substrates for process studies, and with a level of resolution ranging from single cells to multi-organismic interactions. Key organisms in given processes can be identified via labelling of their genomic inventories as well as expressed transcripts or proteins. Active metabolic pathways and transformation reactions can be studied by the isotopic footprint of biochemical bond cleavage mechanisms. Concepts from geochemistry have been adopted for the tracing of transformation reactions in the environment: compound specific stable isotope analysis (CSIA) has emerged from fundamental studies to environmental applications for monitoring the fate of organic compounds at the ecosystem level.

More than a decade after the last focal issue of Current Opinion in Biotechnology on SIP in 2006, this issue boasts some of the most exciting and recent advances in its use. In times of rampant analytical developments and widespread efforts to generally measure more biological molecules and chemicals (genes, proteins, metabolites, solutes, organics) in current research, SIP lives up to the promise of providing functional context to increasingly complex multi-omics and mass spectrometric data sets. Thus, SIP continues to enable deeper insights into the controls of environmental processes and microbiome functioning, important prerequisites for the development of better solutions in biotechnology, environmental engineering and health care.

Stable isotope probing of cellular biomarkers

In the first section of this thematic issue, Wegener *et al.* highlight the most powerful recent developments in membrane lipid-SIP, such as combinatorial labelling with heavy carbon and hydrogen isotopes. In addition to the well-established quantitative nature of phospholipid fatty acid SIP (PLFA-SIP), this adds a new conceptual level of interpretation to the approach as it allows, for the first time, to differentiate between autotrophy and heterotrophy in complex microbiomes. The authors illustrate the appeal of this strategy with a case study on anaerobic methane cycling in hydrothermal marine sediments.

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Next, Coyotzi *et al*. give an excellent overview of the ongoing development of DNA-SIP towards a prime approach for targeted metagenomics. In contrast to often non-directed and mostly technology-driven high-throughput sequencing approaches, this provides selective access to the genomes of microorganisms involved in processes of interest or with a desired metabolic feature. The article summarizes the enormous prospects of this approach for biotechnology applications, including biodegradation, biotransformation, and biosynthesis.

The selection of biomarker-related SIP strategies is then continued by Lueders *et al*. Besides a comprehensive overview of the most recent advances in the application of rRNA-SIP to improve our understanding of carbon flow, ecophysiologies and interactions in complex microbiomes, the roadmap for the ongoing development of total RNA-SIP towards a targeted approach in transcriptomics is discussed. Undisputedly, this is where the technology will develop its greatest appeal for applications in environmental, engineering and host microbiome research in the coming years.

Advances in metaproteomics coupled to stable isotope probing (Protein-SIP) are summarized by Jehmlich *et al*. The exploitation of multiple isotope labelling approaches (i.e. ^{13}C, ^{15}N, ^{18}O, $^{34/36}S$) offers perspectives to study the metabolization of complex organic substrates containing these elements in microbial communities at molecular level. The combination of labelling techniques enables to detect and monitor the anabolism of individual species, their growth rates and metabolic activity with respect to fluxes of major elements needed for the biosynthesis of biomass. Protein-SIP has developed to a tool with large potential to elucidate microbial interactions in biomes.

Biochemical pathways and enzymatic functions of microorganisms in mixed cultures can also be explored by tracing their central metabolism in labelling studies. Isotopologues of central metabolites and peptides/amino acids are identified for metabolic flux analysis needed in bio(geo)chemistry as well as biotechnology as summarized by Adrian and Marco-Urrea.

Single-cell stable isotope probing

In the second section of this issue we summarize contributions on isotope probing of single cells for understanding physiology, bio(geo)chemistry and ecology of microbiomes and their interactions with their living or non-living surrounding. The analysis of single cells in microbial communities with respect to structure, function, activity and regulation is a major advance with considerable prospects for SIP at cellular level.

Wang *et al*. summarize recent advances in confocal Raman microspectroscopy for stable isotope probing of individual cells in microbial communities. Raman spectra can provide intrinsic biochemical profiles of single cells. Cells labelled with stable isotopes such as ^{13}C, ^{15}N and ^{2}H can be identified for tackling metabolic interactions in aquatic habitats and revealing intra-population functional heterogeneities. Another major advantage of confocal Raman microspectroscopy is the structural analysis of biological components in the water phase at the scale of a microbe under natural habitat conditions. In combination with fluorescence *in situ* hybridization (FISH), the isotope-induced Raman band shifts can link metabolic activity to cellular identity offering unique opportunities in microbial ecology.

Nanoscale secondary ion mass spectrometry (NanoSIMS) and correlative microscopic analyses summarized by Jiang *et al*. have opened new avenues