SCIENCE CHINA
Chemistry

•MINI REVIEWS•
SPECIAL ISSUE: Celebrating the 130th Anniversary of Wuhan University

https://doi.org/10.1007/s11426-023-1660-x

Recent advances of microbial metabolism analysis: from metabolic molecules to environments

Na Chen, Xiaoming Zhang, Jing Xi, Yanbing Yang & Quan Yuan*

College of Chemistry and Molecular Sciences, Institute of Molecular Medicine, Renmin Hospital of Wuhan University, Wuhan University, Wuhan 430072, China

Received March 25, 2023; accepted June 5, 2023; published online September 18, 2023

Microbial metabolism analysis is of great significance to the biosynthesis industries and the diagnosis and treatment of infectious diseases caused by microorganisms. However, it remains a grand challenge to offer deep insight into microbial metabolism due to the particularly complex and dynamic processes. Analytical methods are recognized as the critical bottleneck that constrains the in-depth understanding of microbial metabolism. Specific and real-time analytical tools for microbial metabolic processes have currently drawn increasing attention. Here, we summarized the major advances over the past 5 years focusing on the following three aspects including real-time and dynamic analysis of metabolic molecules, selective analysis of specific metabolic pathways and quantitative analysis of the microbial metabolism. Finally, the remaining challenges and perspectives on microbial analysis are discussed and outlooked.

microorganism, metabolites, metabolic pathway, real-time, quantification

Citation: Chen N, Zhang X, Xi J, Yang Y, Yuan Q. Recent advances of microbial metabolism analysis: from metabolic molecules to environments. *Sci China Chem*, 2023, 66, https://doi.org/10.1007/s11426-023-1660-x

1 Introduction

Microbes refer to creatures that are usually invisible to the naked eye and are recognized as the dominant life on earth through billions of years [1]. Microbial communities and humans have co-existed and interacted with each other since the dawn of civilization and the microbes are found closely related to human health [2]. On one hand, microbes and hosts establish mutualistic relationships, which play critical roles in maintaining human health [3]. For example, normal microbial colonization in the human body is capable of providing resistance against colonization and invasion by pathogens through different ways including competition, digestion and secretion of various metabolites such as bacteriocins [4]. The intestinal microbiota can produce metabolite of short-chain fatty acid and thus help facilitate the growth and differentiation of intestinal epithelium by providing nutritional support [5]. On the other hand, microbial infections bring serious effects on human health. Across human civilization, microorganisms have caused multiple grievous public health and security incidents, which have made significant impacts on the course of human history [6]. For instance, the Black Death caused by the bacterium *Yersinia pestis* in Europe in the 14th century, claims an estimated 30%–50% of the European population in only a five-year period [7]. Developing chemical analytical tools for microorganisms is critical to meet human health needs and to reveal the disease mechanisms, providing strategic technical reserves to deal with major public health security events.

However, it remains a grand challenge to offer insight into microorganisms due to the particularly complex and dynamic processes. Firstly, various metabolites are involved in microorganisms such as nucleic acids, proteins, and chemical molecules [8]. The numerous metabolites are prone to

*Corresponding author (email: yuanquan@whu.edu.cn)

give rise to background signal interference, which limits the high sensitivity and specificity of real-time dynamic analysis of molecules in complex microbial metabolism. Secondly, the microorganism involves abundant and diverse metabolic pathways. The interconnected interactions between and within intricate metabolic pathways prominently increased the complexity of microbial metabolism [9]. Therefore, it is of great difficulty to realize the specific and precise analysis for the target metabolic pathway. Thirdly, both the complex internal and external environment, such as pH, temperature and host, have influences on microbial metabolism [10]. How to achieve specific and real-time analysis of complex microbial metabolic processes is of great significance to revealing the disease mechanism related to microbial metabolism as well as disease diagnosis and treatment.

In this mini-review, we summarized recent advances focusing on how to achieve specific and real-time analysis of complex microbial metabolic processes. Specifically, this mini-review elaborated on three important aspects: real-time and dynamic analysis of metabolic molecules, selective analysis of specific metabolic pathways and quantitative analysis of the microbial metabolism (Scheme 1). Looking forward, we discussed the remaining challenges and the perspectives on microbial analysis.

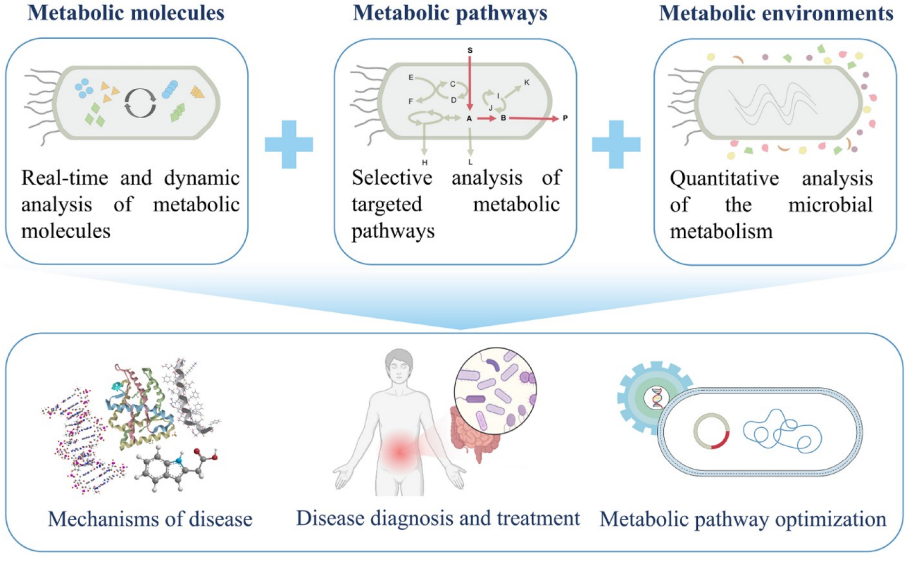
2 Microbial metabolism analysis

2.1 Real-time and dynamic analysis of metabolic molecules

Microbial metabolism is a dynamic process involving a series of biochemical reactions that include numerous decomposed and synthesized chemical molecules [8]. Having a real-time and dynamic view of the varied molecules in microbial metabolism enables to offer deep insight into microbial metabolic mechanisms in the pathological study and to guide the metabolic pathway optimization. Currently, microbial metabolic analysis mainly relies on the classical culture-based method [11]. This method allows viable bacteria to be detected and isolated, facilitating the observation of metabolic activity. However, this method is quite time-consuming and actually can not capture real-time information. Currently, the developed molecular detection techniques based on traditional analytical methods such as chromatography and mass spectrometry have greatly shortened the detection time. However, pretreatment/extraction protocols are needed in these methods to obtain the metabolic molecules. The "static" methods are also unable to realize the dynamic monitoring or tracing of metabolic molecules in microbial metabolism [12]. To achieve the real-time and dynamic monitoring of the metabolic molecules in microbial metabolism, researchers have recently developed a series of optical and electrical biosensing technologies with high sensitivity and specificity.

2.1.1 *Optical biosensing technologies*

Optical biosensors with high spatial and temporal resolution are preferred with the advantage of offering convenient ways to visualize multiple dynamic biological events at the molecular level in living organisms [13,14]. Organic fluorescent probes, with intrinsic advantages of low cost, high sensitivity, good biological compatibility, *etc*., have played critical roles in real-time detection in living cells [15]. Vendrell and co-workers [16–18] have performed excellent work on organic fluorescent probes for the real-time monitoring of the metabolites. Minimal chemical reporters that can retain the



Scheme 1 Schematic illustration of the microbial metabolism analysis from the three aspects: real-time and dynamic analysis of metabolic molecules, selective analysis of specific metabolic pathways and quantitative analysis of the microbial metabolism (color online).

molecular recognition properties and activity profiles of biomolecules have been designed successively. To be specific, new fluorogenic amino acids based on the phenylalanine core were designed to generate fluorescent antimicrobial peptides for rapid labeling of *Candida*, thus achieving *in situ* and real-time monitoring of the *Candida* infection (Figure 1a, b) [17]. In parallel with this study, the research team developed photoactivatable organic probes by introducing amino-substituted benzoselenadiazoles which can be covalently incorporated into the peptidoglycan cell wall and thus realized the real-time imaging of bacteria. It is worth mentioning that the designed organic fluorophores enable to kill pathogenic cells selectively after exposure to visible light, providing an integrated platform for bacteria diagnosis and treatment [18]. Gut microbiota is generally recognized to play crucial roles in maintaining host health and metabolism. Currently, tremendous organic probes have been proposed to offer real-time and dynamic views into gut microbiota *in vivo*, aiming to reveal the interactions between gut microbiota and host. In one of these studies, metabolic D-propargylglycine and azide-containing NIR-II probes were designed [19]. As shown in Figure 1c, gut microbiota was strongly labeled with NIR-II fluorescence on the peptidoglycan through the click reaction, achieving real-time visualization in deep tissue with high spatial resolution [19]. By coupling the metabolic labeling and CUBIC strategies, the same group further achieved quantitative 3D visualization of gut microbiota *in situ* with single-bacteria resolution throughout millimeter-thick tissues. It was found that gut microbiota biogeography changes with gastrointestinal motility according to the quantitative monitoring results. In parallel with this study, sequential tagging with D-amino acid-based metabolic probes was subsequently proposed to visualize transplanted microbiotas and evaluate their viabilities in the recipients, providing versatile tools for deciphering the complex biology of fecal microbiota transplantation, and potentially improving its treatment efficacy [20].

The microbial metabolism process involves different kinds of luminescent substances such as riboflavin and pigment molecules which bring about autofluorescence interference [8]. Luminescent lanthanide complex with the intrinsic advantages of long-lived emissions allows for time-gate detection to avoid background fluorescence and has been widely adopted for real-time monitoring of metabolites in the complex microbial metabolism [21]. For example, a turn-on lanthanide luminescent probe was designed for real-time and time-gated detection of nitroreductases (NTRs) in live bacteria. Specifically, the probe is activated through NTR-induced formation of the sensitizing carbostyrilantenna and consequently results in energy transfer to the lanthanide center, leading to the luminescent signal increase (Figure 1d) [22]. Overall, the turn-on lanthanide luminescent probe is an attractive option for future real-time analytical applications.

Compared with organic probes, gene-coding probes introduced into living cells or organisms in the format of DNA, possess many unique advantages, such as superior intracellular retention and simple manipulation for specific cell targeting [23]. It is worth mentioning that the gene-coding probes are expressed independently in single cells and therefore hold broad application prospects in real-time and dynamic metabolic molecule analysis at the single-cell level. Typically, fluorescent protein functions as the reporter module in the gene-coding probes [24]. Yang and co-workers [25] established highly responsive genetically encoded fluorescent protein indicators for real-time monitoring of NAD^+ dynamics in living microorganisms (Figure 1e). Another example is the highly sensitive redox-sensitive fluorescent proteins for real-time monitoring of redox metabolites including oxidized glutathione, reactive oxygen species, NADH and NADPH in *Saccharomyces cerevisiae* (Figure 1f) [26]. The constructed sensitive genetically encoded redox biosensors were further adopted to reveal the distribution and spatiotemporal dynamics of redox metabolites in yeast under various stress conditions, providing a reliable platform for the in-depth understanding of microbial metabolism. In another work, a toolbox consisting of multiple genetically encoded fluorescent probes was reported to sense the yeast intracellular environment including pH, ATP levels, oxidative stress, glycolytic flux, and ribosome production dynamically in a single cell. The toolbox based on genetically encoded fluorescent probes allows the exploration of microbial diversity and physiological responses as well as the possibility to monitor multiple metabolism processes in real-time [27].

Apart from the above-mentioned biosensing technology, label-free strategies such as Raman sensors have also raised great attention owing to the advantages of non-invasive and minimal interference towards the activity profiles of biomolecules [16]. For instance, rhabduscin at the periphery of wild-type *X. nematophila* and *E. coli* heterologously was visualized in real-time according to the characteristic Raman resonance of rhabduscin with the isonitrile functional group, which occurs rarely in naturally produced molecules [28]. Spectrochemical tools based on attenuated total reflection Fourier transform infrared spectroscopy were also proposed for real-time monitoring of antibiotic-resistance gene dynamics, offering new insights for the potential role of spectrochemical analysis in investigating the existence and trends of antibiotic resistance in microbes [29].

To sum up, various kinds of optical methods have been developed for real-time and dynamic microbial metabolism monitoring and can be used in different applications. Typically, metabolic optical probes such as fluorescent D-amino acid are suitable for *in situ* microbial metabolic imaging and are promising in providing real-time information for un-

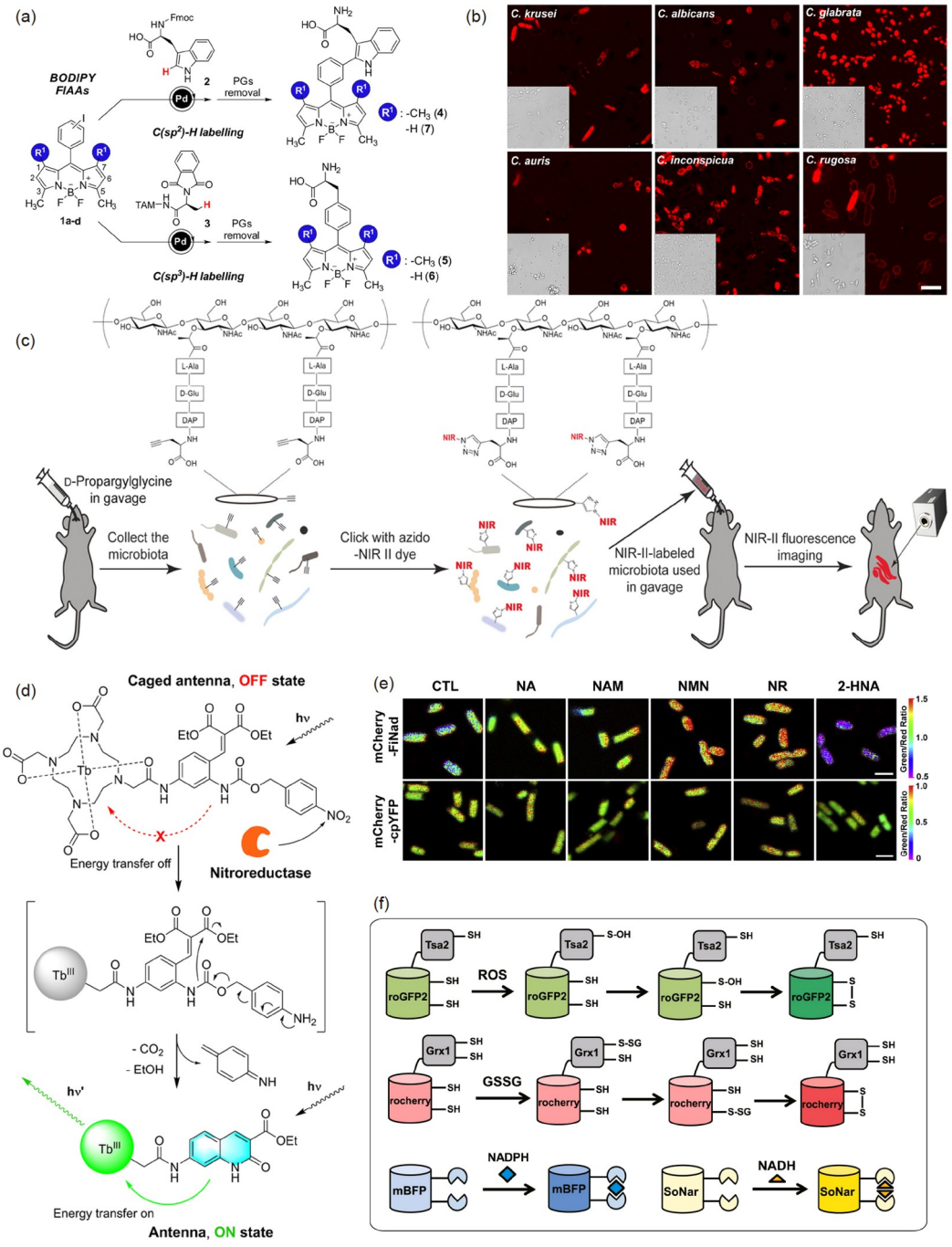


Figure 1 (a) Synthetic scheme for the preparation of fluorogenic amino acids. (b) Fluorescence live-cell confocal microscopy of different *Candida* strains. (c) Scheme of the NIR-II-based real-time fluorescence imaging of gut microbiota. (d) Design rationale and mode of action of the carbostyril-based nitroreductases activatable luminescent lanthanide probe. (e) Fluorescence images of *E. coli* with genetically encoded fluorescent protein responded to $NADP^+$. (f) Working mechanisms of the real-time monitoring redox biosensor system (color online).

raveling the mechanistic basis of many physiological and pathological processes. Persistent luminescent probes, whose luminescence remains after excitation ceases, have drawn increasing attention and have been widely adopted for real-time biosensing in complex samples with interfering substances owing to their special ability in eliminating autofluorescence. Currently, with the significant progress in biological technology, gene-coding probes are gaining wide use and are proving to be indispensable for unraveling the complex biological functions of individual molecules, opening new doors for the structural and molecular underpinnings of microbial behavior. The development of optical biosensors will facilitate our understanding of microbial metabolism mechanisms, providing guidance for disease

diagnosis and treatment.

2.1.2 *Electrochemical biosensing technologies*

Currently, electrochemical biosensors have gained tremendous attention as an attractive alternative to optical biosensors in the real-time detection of microbial metabolites. Electrochemical biosensors, allowing the detection of an analyte by converting biological information like analyte concentration into electronic signals, have been widely applied in microbial metabolism monitoring with advantageous features such as appreciable detection limit, miniaturization and low cost [30]. For example, Bentley and co-workers [31] realized the relayed information monitoring in real-time by electronically measuring response through the expression of β-galactosidase and its cleavage of 4-aminophenyl-β-D-galactopyranoside to the electronically detected p-aminophenol. Simoska and co-workers [32] developed electrochemical sensors based on transparent carbon ultramicroelectrode arrays for the real-time monitoring of the phenazine metabolites including pyocyanin, 5-methylphenazine-1-carboxylic acid and 1-hydroxyphenazine from wild-type *P. aeruginosa* liquid cultures. Although electrochemical biosensors are the promising candidate technologies due to their simplicity, high sensitivity and specificity, their applications were limited in microbial metabolism *in vivo* owing to the invasive electrode sizes. Currently, electrochemical biosensors are mainly used for metabolites biosensing *in vitro*.

2.1.3 *Other biosensing technologies*

In addition to optical and electrical bio-sensing, researchers have developed many other technologies such as magnetic and photoacoustic sensors [33,34]. One good example is the ^{19}F magnetic resonance probe, which was developed by Gao and co-workers [33] for real-time monitoring of the dynamic gut microbiota imaging. By using a panel of peptidoglycan-targeting metabolic fluorine labeling probes with ^{19}F atoms of different chemical shifts, *in situ* labeling of different microbiota subgroups was thus achieved. The magnetic labeling strategy holds great potential for noninvasive and real-time monitoring of metabolic activities and locations of gut microbe *in vivo*. Another strategy, proposed by Shapiro and co-workers [34], adopted ultrasonic signals for real-time monitoring of microorganisms *in vivo*. Specifically, by introducing acoustic reporter genes into *E. coli* and *Salmonella typhimurium*, a unique class of gas-filled protein capable to scatter sound waves and thereby produce ultrasound contrast was expressed. Therefore, *E. coli* and *Salmonella typhimurium* can be visualized and traced in real time by monitoring the gas-filled protein molecules. This technology equips microbial cells with a means to be monitored deep inside mammalian hosts.

2.2 Selective analysis of specific metabolic pathways

Generally, traditional metabolic analysis methods such as mass spectrum can detect and quantify microbial metabolism information. However, the metabolism information actually comes from various pathways in microorganisms and it is difficult to distinguish which pathway actually functions [9]. Selective and accurate analysis for targeted metabolic pathways is vital for a comprehensive understanding of the kinetic and thermodynamics process of microbial metabolism, thus providing guidance for revealing disease mechanisms, biosynthesis pathways optimization.

2.2.1 *In vitro reconstitution*

In vitro pathway reconstitution provides a reliable way to simplify the specific pathway with the advantages of controllable reaction conditions and negligible bio-molecular interference, and can be used for the selective and accurate analysis of the targeted pathway. Key kinetic and thermodynamic information of the specific microbial pathway can be obtained accurately with the simplified *in vitro* reconstituted pathway, offering insight for rate-limiting step prediction and enzyme optimization of the target microbial pathway (Figure 2a) [35]. The strategy of *in vitro* reconstitution plays a critical role in guiding disease treatment and microbial pathway optimization and has been widely adopted in biomanufacturing. For instance, by simplifying the complex pathway through *in vitro* reconstitution, Ryan and co-workers [36] accurately deduced the critical enzymatic steps in linking L-arginine to azomycin in azomycin biosynthesis pathway. The selective and accurate analysis of the azomycin biomanufacturing pathway sets the stage for the development of biocatalytic approaches to generate azomycin and related nitroimidazoles. Similarly, Yoon and co-workers [37] revealed the gentamic B biosynthesis pathway through *in vitro* reconstitution. Seven hitherto-unknown gentamicin biosynthetic intermediates and three enigmatic pathways were discovered for gentamic B biosynthesis. Based on the *in vitro* reconstitution strategy, multiple pathways such as fatty acid synthesis and mevalonate pathway were also optimized by obtaining the dynamic information of key compounds including substrates, cofactors and proteins [9]. Besides, our group demonstrates that long-lived electrons are capable of enhancing the efficiency of bio-reaction in the reconstructing enzyme-catalyzed process *in vitro*, offering a platform to deeply understand the enzymatic catalytic processes [38]. Overall, the simplified *in vitro* reconstitution method provides a possibility for the accurate and selective analysis of specific metabolic pathways, shedding new light on specific metabolic pathways in complex microbial systems. Meanwhile, it should be noted that *in vitro* reconstitution is not suitable for a pathway containing proteins with certain attributes, such as poor solubility,

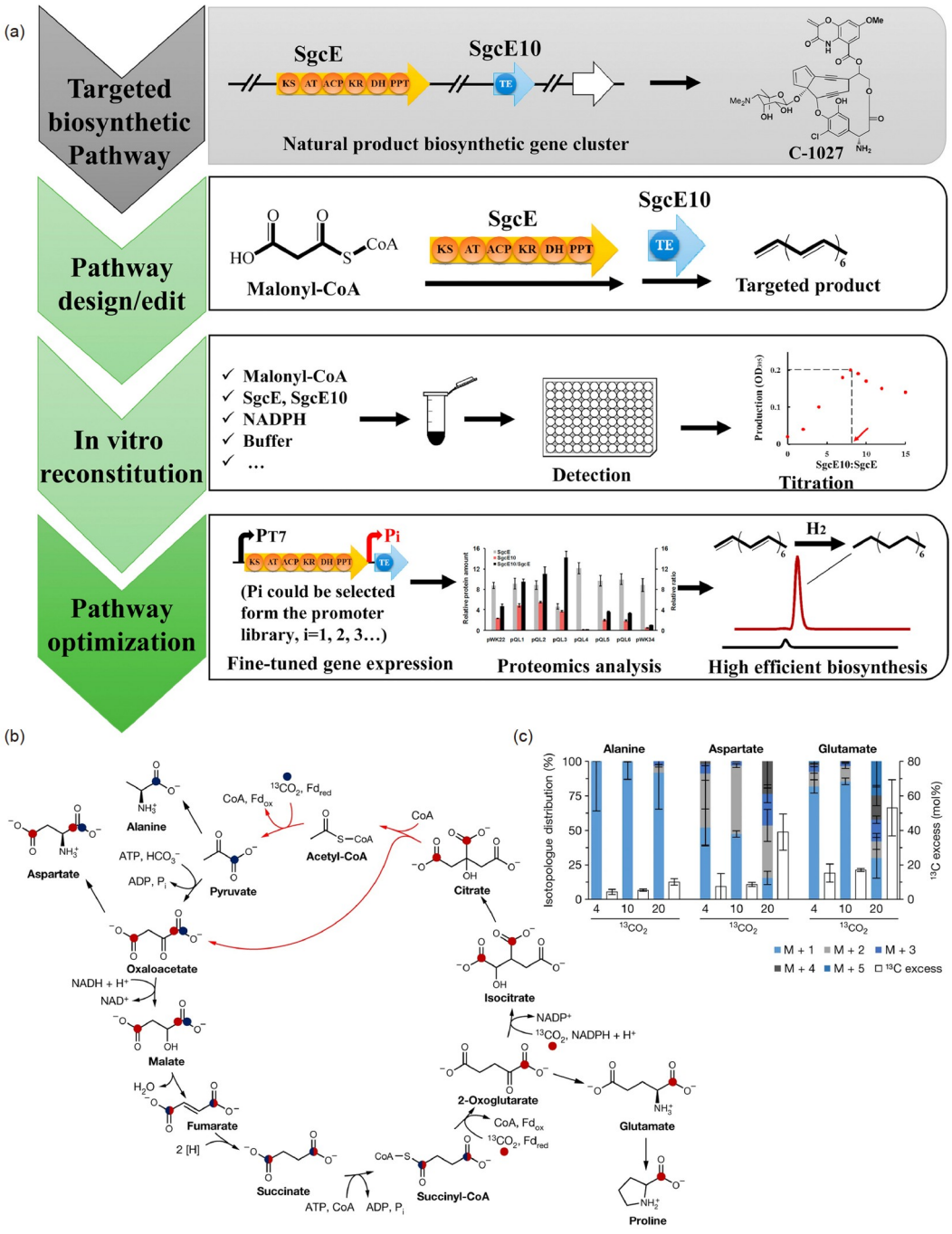


Figure 2 (a) Scheme for *in vitro* reconstitution-guided synthetic pathway refactoring for targeted product biosynthesis. (b) The cycle and incorporation of $^{13}CO_2$. (c) ^{13}C-isotopologue fractions analysis (color online).

purification difficulty, and susceptibility to activity loss *in vitro* [9].

2.2.2 *Isotopic tracing*

Isotope tracing is a technique that allows the tracking of labeled atoms within metabolites such as ^{13}C-labeled glucose and ^{15}N-labeled glutamine through biochemical reactions [39]. The specific metabolic pathway can be thus determined according to the labeled intermediate metabolites transformed from the originally labeled substrate. In this way, isotope tracing is capable of revealing metabolic activities specific to a substrate according to the isotopic peak distribution, thus realizing the selective analysis for the specific pathways. Isotope tracer mainly includes the stable and radioactive tracer. Both radioactive (*e.g.*, ^{18}F, ^{3}H, ^{14}C) and stable (*e.g.*, ^{2}H, ^{13}C, ^{15}N) isotope tracers have been widely adopted in microbial metabolism [40]. Traditionally, radioactive tracers were usually adopted in interrogating the ac-

tivity of single, predefined pathways due to their high sensitivity and specificity. For example, the specific pathways of acetate, propionate and butyrate formation by the human fecal microbial flora were elucidated with radioisotope analysis. The labeling strategy successfully proved that acetate and propionate were formed from CO_2 by the Wood-Ljungdahl pathway and butyrate was formed by classical routes of acetyl-S coenzyme A condensation [41]. By contrast, stable-isotope tracers allow for the interrogation of multiple pathways at once at the expense of sensitivity and have become increasingly popular *in vivo* studies, where the number of experimental subjects is necessarily limited [42]. Mass spectrometry (MS) has been widely adopted in tracking metabolite labeling from stable isotope tracers due to its unmatched capability for detecting low-abundance metabolites without interference from closely related species. For isotope tracing, high resolution can distinguish species labeled with different heavy nuclei. For example, M+1 palmitate isotopologues with one ^{2}H *versus* one ^{13}C can be separated at 100,000 mass resolution defined as the ratio $m/\Delta m$ [39]. Currently, by combing isotope tracing and MS technology, researchers have achieved selective and accurate analysis of targeted metabolic pathways. By growing the co-culture with labeled ^{13}C-$MnCO_3$ and ^{15}N-nitrate to aid, Leadbetter and co-workers [43] tracked the synthesis process of new biomass. Combined with the fluorescence *in situ* hybridization and nanometre-scale secondary-ion mass spectrometry technology, the stable isotope probe revealed that $^{13}CO_2$ fixation into cellular biomass was dependent upon Mn(II) oxidation. With the isotopic labeling and mass spectrometry technology, an additional mode of methanogenesis in *Ca*. *Methanoliparum* was discovered, adding to CO_2 reduction, methylotrophy, methyl reduction, acetate fermentation and the recently reported methoxydotrophy [44]. Similarly, by adopting the ^{13}C carbon isotope labeling method to trace and record the accumulation of ^{13}C intermediate molecules, it was validated that *Hippea maritima* could achieve CO_2 fixation through reverse tricarboxylic acid cycle under high CO_2 concentration (Figure 2b, c) [45]. The interesting discovery provides new insights into microbial ecology and the origin of life. Experimentally, Bar-Even and co-workers [46] revealed that *E*. *coli* grew on formate *via* the reductive glycine pathway by feeding the cultures with ^{13}C-formate/$^{12}CO_2$, ^{12}C-formate/$^{13}CO_2$ and ^{13}C-formate/$^{13}CO_2$ and measured the labeling pattern of proteinogenic amino acids. The engineered *E*. *coli* is hence capable of metabolizing methanol to formate, thus supporting growth on this C1 carbon source.

In general, stable-isotope tracer-based methods enable the interrogation of intracellular metabolism from a pathway-centric perspective. By administering isotopically enriched nutrients (or tracers) to a biological system, researchers are capable of assessing the contributions of different enzymatic reactions to the production or consumption of specific metabolites, playing a critical role in unraveling novel regulatory principles at both the cellular and whole-organism level.

2.3 Quantitative analysis of the microbial metabolism

Detailed external environmental niches, such as temperature, humidity, elevation and oxygen, shape microbial diversity. It is worth mentioning that microbes could dynamically regulate the metabolic pathways to adapt to the changing external environment. Additionally, upon dynamically sensing and dealing with the released signal information, the microbial community behaves differently. However, recapitulating the exact behaviors of microbial communities can be challenging due to the complex and dynamic interactions between the external environment and microbe [10]. Developing quantitative and analytical tools is of great significance to facilitating a deeper and broader understanding of collective microbial behavior. The quantitative analysis of microbial metabolism might open new doors for external environment response mechanisms, offering new insight into understanding microbial communication and life evolution.

In a previous study, we designed an electron transfer-triggered persistent luminescent probe for quantitative analysis of Fe(III) respiration metabolism in *S*. *putrefaciens* [8]. To be specific, the ZGO:Mn@Fe^{3+} probe with quenched persistent luminescence can be "lighted up" when Fe^{3+} accepted electrons from the dynamic Fe(III) respiration metabolism processes of *S*. *putrefaciens*, thus enabling the quantitative monitoring of Fe(III) respiration metabolism. Furthermore, we explored and tracked the metabolic response of *S*. *putrefaciens* to environmental stress such as temperature and oxygen (Figure 3a, b), and the interaction between *S*. *putrefaciens* and *R*. *palustris* was also quantified, guiding for microbial co-culture biosynthesis conditions optimization (Figure 3c–e) [8]. Similarly, Yang and co-workers [47] performed a quantitative analysis of the indigenous metabolic status of gut bacteria by proposing a metabolic D-amino acid-based labeling and *in situ* hybridization-facilitated strategy. With this quantitative analysis method, researchers found that the metabolic activities of *Gram-negative* and *Gram-positive* genera in the mouse microbiota are different during the daytime and night. In another work, by combining amplicon sequencing and flow cytometry, quantitative microbiota profiling was achieved to assess fecal microbiota variation in terms of absolute taxon abundances rather than proportions. The quantitative analysis further allows differentiating inflammation-associated taxa from genera specifically linked to biliary obstruction severity beyond stool moisture variation and revealed that makers for similar phenotypes presented patterns of mutual near-exclusion [48].

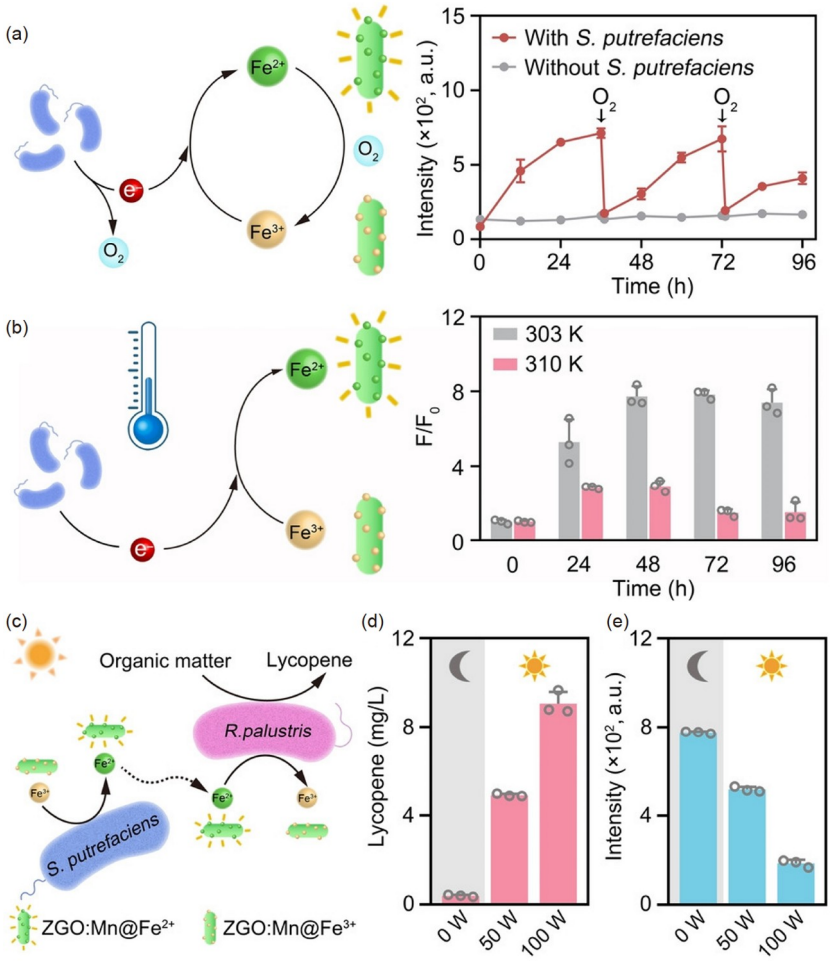


Figure 3 (a) Oxygen-stress and (b) temperature-stress Fe(III) respiration metabolism quantitative analysis with ZGO:Mn@Fe^{3+} probe. (c) Schematic illustration of the quantitative monitoring of lycopene biosynthesis process in microbial co-culture with ZGO:Mn@Fe^{3+} probe. (d) Lycopene biosynthesis efficiency in microbial co-culture. (e) The quantitative persistent luminescence intensity of ZGO:Mn@Fe^{3+} probe in microbial co-culture (color online).

Currently, MS has gained increasing attention in quantifying the target metabolites and studying the whole metabolome from the microbes. Actually, numerous workers have performed excellent work in microbial metabolism analysis with the aid of MS to reveal the metabolism responses towards the complex external environment [49]. For example, Jia and co-workers [50] reported an automated high-throughput quantitative method based on gas chromatography/time-of-flight MS platform for the analysis of microbial metabolites related to host-gut microbiota cometabolism, providing a powerful tool for quantitative microbiome metabolomics studies. Sonnenburg and co-workers [51] obtained the metabolic profiles of 178 gut microorganism strains using the library of 833 metabolites constructed with LC-MS technology. With the metabolomics resource, deviations in the relationships between phylogeny and metabolism were established. In their work, a previously undescribed type of metabolism in *Bacteroides* was discovered and candidate biochemical pathways were revealed.

Based on our discussion above, we can conclude that various methods have been proposed for microbial metabolism analysis. However, the application of the microbial metabolism analysis method discussed here is by no means exhaustive (Table 1). In the future, different methods with high spatial and temporal resolution will offer deep insights into microbial metabolism, enabling it to meet human health needs and reveal disease mechanisms.

3 Summary and outlook

In summary, we briefly reviewed the selective and real-time analysis methods for microbial metabolism. Research progress focusing on real-time and dynamic analysis of metabolic molecules, selective analysis of targeted metabolic pathways and quantitative analysis of the microbial metabolism was elaborated comprehensively. With the development of sensitive and selective analysis techniques, the microbial metabolic molecules, transformation process of substances and interaction between microorganisms and

Table 1 Representative summary of advances in microbial metabolism analysis

Method	Microorganism	Target	Application	Ref.
Phe-BODIPY Amino Acids	*Candida*	Peptide	Urinary tract infections	[17]
Benzoselenadiazole D-amino acids	*E. coli*	Peptidoglycan	Ablation of malignant cells	[18]
NIR-II probe	Gut Microbiota	Peptidoglycan	*In vivo* imaging	[19]
fluorescent D-amino acid	Gut Microbiota	Peptidoglycan	Assessing the viability of transplanted gut microbiota	[20]
Lanthanide luminescent Probe	*E. coli*	Nitroreductase	Bacterial infections	[22]
Fluorescent sensor	*E. coli*	NAD^+	Real-time monitoring	[25]
Fluorescent sensor	*E. coli*	Redox metabolites,	Real-time monitoring	[26]
Fluorescent sensor	*Saccharomyces*	PH, ATP, glycolytic	Real-time monitoring	[27]
Carbon ultramicroelectrode	*P. aeruginosa*,	Phenazine Metabolites	Real-time monitoring	[32]
Metabolic fluorine labeling probe	Gut microbiota	Peptidoglycan	*In vivo* imaging	[33]
Acoustic probe	*E. coli*	Gas vesicles	*In vivo* imaging	[34]
Isotopic tracing	*H. maritima*	CO_2	Metabolism mechanism	[45]
Isotopic tracing	*E. coli*	CO_2	Metabolism mechanism	[46]
GC/TOFMS	Microbes	microbial metabolites	host-gut microbiota cometabolism	[50]
LC–MS	Microbes	microbial metabolites	host-gut microbiota cometabolism	[51]

external environment can be real-time monitored. Consequently, it becomes possible for us to have a well-rounded understanding of the microbial metabolic mechanism, shedding new light on the disease mechanism as well as disease diagnosis and treatment.

Although many successful studies have been achieved in microbial metabolism analysis, challenges still remain in this field [52]. A wealth of exciting research opportunities are waiting in this rapidly emerging field. Questions that should be addressed in the future include: (1) how to real-time and dynamically monitor the microbial metabolism with high spatial and temporal resolution at the single cell level? (2) How to chemically identify and characterize metabolites with high throughput? (3) What is the interaction mechanism among microbial communities or between microorganisms and hosts?

Recent studies have demonstrated that cell-to-cell heterogeneities exist at both cellular and molecular levels. Single live-bacterial cell assay with high spatial and temporal resolution is capable of providing detailed information instead of the average one, overcoming the cell-to-cell heterogeneities. Combing the advantages of different methods may provide exciting opportunities [53]. For example, by combing optical analysis methods and super-resolution technology, it might offer efficient tools to image living microorganisms on a single-cell level and simultaneously visualize the distribution of metabolites with a high spatial resolution. For research and actual clinical applications, it is necessary to analyze a large number of individual cells simultaneously. Therefore, developing a high-throughput single-cell analysis method is required to obtain biologically meaningful data [54]. Based on the advanced microbial metabolism analysis methodologies, it is possible to pave new avenues to understand the interaction mechanism among microbial communities or between microorganisms and hosts, opening new doors for revealing the disease mechanism related to microbial metabolism. Moreover, computational advances in simulating microbial metabolic processes hold the potential in predicting the interaction mechanism based on high-throughput molecular information.

Acknowledgements This work was supported by the National Natural Science Foundation of China (21925401, 21904033), the National Key R&D Program of China (2017YFA0208000, 2021YFA1202400), the Fundamental Research Funds for the Central Universities (2042022rc0004), the New Cornerstone Science Foundation through the XPLORER PRIZE and the interdisciplinary innovative talents foundation from Renmin Hospital of Wuhan University.

Conflict of interest The authors declare no conflict of interest.

1 Salazar G, Sunagawa S. *Curr Biol*, 2017, 27: R489–R494
2 Niehaus L, Boland I, Liu M, Chen K, Fu D, Henckel C, Chaung K, Miranda SE, Dyckman S, Crum M, Dedrick S, Shou W, Momeni B. *Nat Commun*, 2019, 10: 2052
3 Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. *Science*, 2009, 326: 1694–1697
4 Pamer EG. *Science*, 2016, 352: 535–538
5 Belkaid Y, Hand TW. *Cell*, 2014, 157: 121–141
6 Tabish SA. *J Public Health Res*, 2020, 9: jphr.2020.1786
7 Bos KI, Schuenemann VJ, Golding GB, Burbano HA, Waglechner N, Coombes BK, McPhee JB, DeWitte SN, Meyer M, Schmedes S, Wood J, Earn DJD, Herring DA, Bauer P, Poinar HN, Krause J. *Nature*,

2011, 478: 506–510
8 Chen N, Du N, Wang W, Liu T, Yuan Q, Yang Y. *Angew Chem Int Ed*, 2022, 61: e202115572
9 Tan GY, Liu T. *Metab Eng*, 2017, 39: 228–236
10 Liu Y, Xu P. *Curr Opin Biotechnol*, 2022, 76: 102754
11 Gill A. *Front Microbiol*, 2017, 8: 777
12 Valeix N, Costa D, Basmaciyan L, Valot S, Vincent A, Razakandrainibe R, Robert-Gangneux F, Nourrisson C, Pereira B, Fréalle E, Poirier P, Favennec L, Dalle F. *Microorganisms*, 2020, 8: 1450
13 Huang J, Pu K. *Angew Chem Int Ed*, 2020, 59: 11717–11731
14 Shi H, Wang Y, Zheng J, Ning L, Huang Y, Sheng A, Chen T, Xiang Y, Zhu X, Li G. *ACS Nano*, 2019, 13: 12840–12850
15 Gonçalves MST. *Chem Rev*, 2009, 109: 190–212
16 Benson S, de Moliner F, Tipping W, Vendrell M. *Angew Chem Int Ed*, 2022, 61: e202204788
17 Mendive-Tapia L, Mendive-Tapia D, Zhao C, Gordon D, Benson S, Bromley MJ, Wang W, Wu J, Kopp A, Ackermann L, Vendrell M. *Angew Chem Int Ed*, 2022, 61: e202117218
18 Benson S, de Moliner F, Fernandez A, Kuru E, Asiimwe NL, Lee JS, Hamilton L, Sieger D, Bravo IR, Elliot AM, Feng Y, Vendrell M. *Nat Commun*, 2021, 12: 2369
19 Wang W, Yang Q, Du Y, Zhou X, Du X, Wu Q, Lin L, Song Y, Li F, Yang C, Tan W. *Angew Chem Int Ed*, 2020, 59: 2628–2633
20 Wang W, Lin L, Du Y, Song Y, Peng X, Chen X, Yang CJ. *Nat Commun*, 2019, 10: 1317
21 Luo Y, Li L, Feng Y, Li R, Yang J, Peijnenburg WJGM, Tu C. *Nat Nanotechnol*, 2022, 17: 424–431
22 Brennecke B, Wang Q, Zhang Q, Hu H-, Nazaré M. *Angew Chem Int Ed*, 2020, 59: 8512–8516
23 Ni Q, Mehta S, Zhang J. *FEBS J*, 2018, 285: 203–219
24 Wang M, Da Y, Tian Y. *Chem Soc Rev*, 2023, 52: 1189–1214
25 Zou Y, Wang A, Huang L, Zhu X, Hu Q, Zhang Y, Chen X, Li F, Wang Q, Wang H, Liu R, Zuo F, Li T, Yao J, Qian Y, Shi M, Yue X, Chen W, Zhang Z, Wang C, Zhou Y, Zhu L, Ju Z, Loscalzo J, Yang Y, Zhao Y. *Dev Cell*, 2020, 53: 240–252.e7
26 Liu X, Qin L, Yu J, Sun W, Xu J, Li C. *Biosens Bioelectron*, 2023, 222: 114988
27 Torello Pianale L, Rugbjerg P, Olsson L. *Front Microbiol*, 2021, 12: 802169
28 Crawford JM, Portmann C, Zhang X, Roeffaers MBJ, Clardy J. *Proc Natl Acad Sci USA*, 2012, 109: 10821–10826
29 Jin N, Paraskevaidi M, Semple KT, Martin FL, Zhang D. *Anal Chem*, 2017, 89: 9814–9821
30 Uniyal S, Sharma RK. *Biosens Bioelectron*, 2018, 116: 37–50
31 Terrell JL, Tschirhart T, Jahnke JP, Stephens K, Liu Y, Dong H, Hurley MM, Pozo M, McKay R, Tsao CY, Wu HC, Vora G, Payne GF, Stratis-Cullum DN, Bentley WE. *Nat Nanotechnol*, 2021, 16: 688–697
32 Simoska O, Sans M, Fitzpatrick MD, Crittenden CM, Eberlin LS, Shear JB, Stevenson KJ. *ACS Sens*, 2019, 4: 170–179
33 Chen D, Guo J, Li A, Sun C, Lin H, Lin H, Yang C, Wang W, Gao J. *Sci Adv*, 2023, 9: eabg6808
34 Bourdeau RW, Lee-Gosselin A, Lakshmanan A, Farhadi A, Kumar SR, Nety SP, Shapiro MG. *Nature*, 2018, 553: 86–90
35 Chen X, Gao C, Guo L, Hu G, Luo Q, Liu J, Nielsen J, Chen J, Liu L. *Chem Rev*, 2018, 118: 4–72
36 Hedges JB, Ryan KS. *Angew Chem Int Ed*, 2019, 58: 11647–11651
37 Ban YH, Song MC, Hwang J, Shin H, Kim HJ, Hong SK, Lee NJ, Park JW, Cha SS, Liu H, Yoon YJ. *Nat Chem Biol*, 2019, 15: 295–303
38 Wang J, Chen N, Wang W, Li Z, Huang B, Yang Y, Yuan Q. *CCS Chem*, 2023, 5: 164–175
39 Jang C, Chen L, Rabinowitz JD. *Cell*, 2018, 173: 822–837
40 Dong W, Rawat ES, Stephanopoulos G, Abu-Remaileh M. *Curr Opin Biotechnol*, 2022, 76: 102739
41 Miller TL, Wolin MJ. *Appl Environ Microbiol*, 1996, 62: 1589–1592
42 Fernández-García J, Altea-Manzano P, Pranzini E, Fendt SM. *Trends Biochem Sci*, 2020, 45: 185–201
43 Yu H, Leadbetter JR. *Nature*, 2020, 583: 453–458
44 Zhou Z, Zhang C, Liu P, Fu L, Laso-Pérez R, Yang L, Bai L, Li J, Yang M, Lin J, Wang W, Wegener G, Li M, Cheng L. *Nature*, 2022, 601: 257–262
45 Steffens L, Pettinato E, Steiner TM, Mall A, König S, Eisenreich W, Berg IA. *Nature*, 2021, 592: 784–788
46 Kim S, Lindner SN, Aslan S, Yishai O, Wenk S, Schann K, Bar-Even A. *Nat Chem Biol*, 2020, 16: 538–545
47 Lin L, Song J, Du Y, Wu Q, Gao J, Song Y, Yang C, Wang W. *Angew Chem Int Ed*, 2020, 59: 11923–11926
48 Vieira-Silva S, Sabino J, Valles-Colomer M, Falony G, Kathagen G, Caenepeel C, Cleynen I, van der Merwe S, Vermeire S, Raes J. *Nat Microbiol*, 2019, 4: 1826–1831
49 Gao P, Xu G. *Anal Bioanal Chem*, 2015, 407: 669–680
50 Zhao L, Ni Y, Su M, Li H, Dong F, Chen W, Wei R, Zhang L, Guiraud SP, Martin FP, Rajani C, Xie G, Jia W. *Anal Chem*, 2017, 89: 5565–5577
51 Han S, Van Treuren W, Fischer CR, Merrill BD, DeFelice BC, Sanchez JM, Higginbottom SK, Guthrie L, Fall LA, Dodd D, Fischbach MA, Sonnenburg JL. *Nature*, 2020, 595: 415–420
52 Chen N, Cheng D, He T, Yuan Q. *Chin J Chem*, 2023, 41: 1836–1840
53 D'Souza G, Shitut S, Preussger D, Yousif G, Waschina S, Kost C. *Nat Prod Rep*, 2018, 35: 455–488
54 Feng D, Li H, Xu T, Zheng F, Hu C, Shi X, Xu G. *Anal Chim Acta*, 2022, 1221: 340116