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Emerging Topic

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# Real-Time Monitoring of Dynamic Chemical Processes in Microbial Metabolism with Optical Sensors

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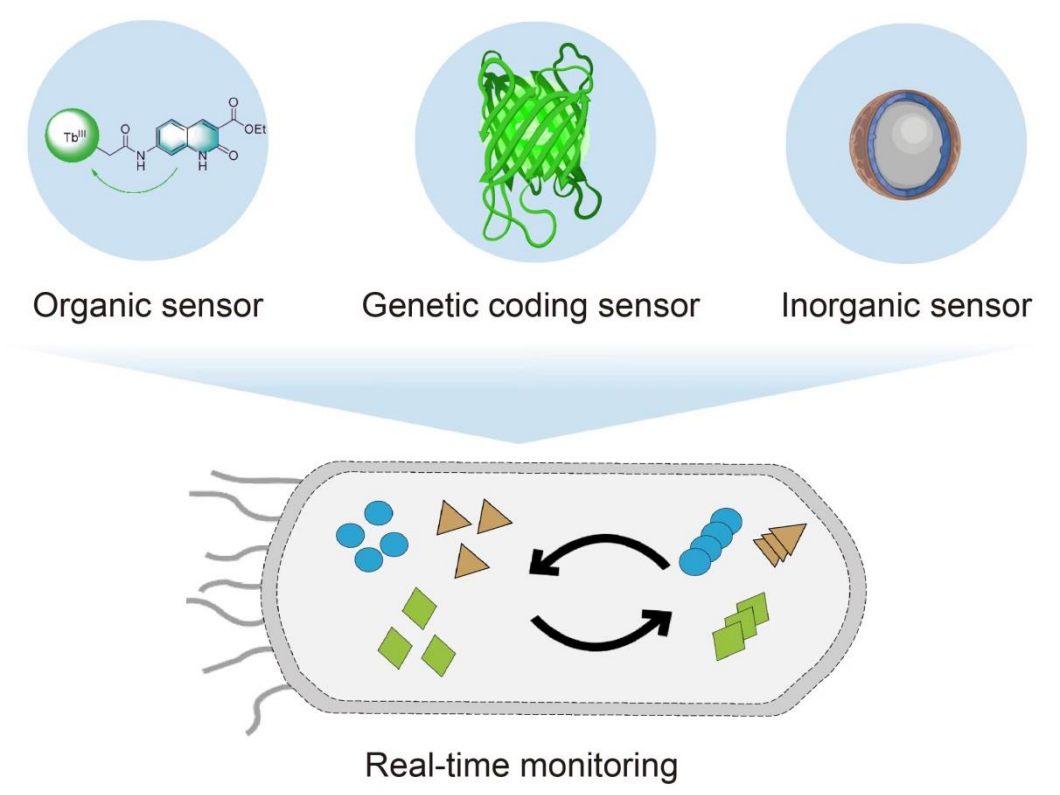
## Keywords

Real-time | Microbial metabolism | Dynamic | Monitoring | Optical biosensor

## Abstract




Monitoring microbial metabolism is vital for revealing the mechanism of disease related to microbial metabolism and providing guidance for biomanufacturing processes optimization. However, it remains a grand challenge to offer real-time insights into microbial metabolism owing to the complex and dynamic process. In this paper, the recent advances and prospects of optical biosensors including the organic, genetic coding and inorganic optical biosensors are briefly described for real-time monitoring of dynamic microbial metabolism. This paper points out that challenges remain in microbial heterogeneity. We believe that this work will inspire the application of developing new methods for single cell real-time analysis.

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## Background

Microbes have inhabited the earth widespreadly for billions of years and may be the earliest life forms on our planet. Microorganisms, living on and within human beings, are recognized to have significant impacts on human health. On one hand, microorganisms are capable to maintain the host healthy by impeding the colonization of exogenous microbe and synthesizing critical nutrients, *etc*., sharing a mutualistic relationship with host. On the other hand, microorganisms cause diseases such as bacterial infection. The latest statistical results of the World Health Organization (WHO) demonstrate that 13% of cancers are caused by microorganisms.[1] One of the pathogenic mechanisms is that toxic microbial metabolites interfere with the normal physiological function of host cells. For example, calibactin metabolites produced by *Escherichia coli* are believed to alkylate DNA on adenine residues and induce double-strand breaks in cells, thus leading to the occurrence of colorectal cancer.[2] Furthermore, there are significant differences in the microbial metabolic information among different disease stages. Hence, integrative analysis of metabolite species, concentrations and fluxes is vital for the deep understanding of the kinetic and thermodynamics process of microbial metabolism, thus providing guidance for revealing the mechanism of disease occurrence and progression related to microbial metabolism.

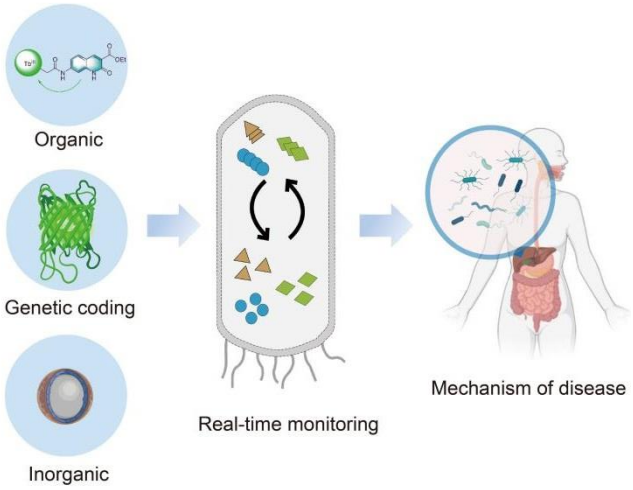
However, it remains a grand challenge to offer insights into microbial metabolism due to the particularly complex and dynamic processes.[3] Various substances such as nucleic acids, proteins and chemical molecules are involved in the microbial metabolism process. Additionally, the species and concentration of the metabolic molecules change in real-time owing to the dynamic microbial metabolism process. Currently, traditional analytical methods such as chromatography and mass spectrometry are widely adopted in bioanalysis. However, it is needed to extract the metabolic molecules from the microorganism for further bioanalysis by these methods. These "static" methods are quite time-consuming and actually cannot capture the real-time information of the dynamic microbial metabolism. Developing new analytical methods with high spatial and temporal resolution and high selectivity may provide robust tools for complex organism metabolism and pave new ways for understanding the disease mechanism associated with microbial metabolism.

Recently, various non-invasive molecular sensing and imaging technologies have been developed including magnetic, electrochemical and optical biosensors. Among these methods, optical biosensors with high spatial and temporal resolution have drawn increasing attention owing to the ability to realize real-time monitoring of the dynamic metabolic process in living organisms. In this Emerging Topic, we introduce recent advances involving the organic, genetic coding and inorganic optical probes for real-time monitoring of dynamic microbial metabolism, enabling to provide robust tools for revealing disease mechanism associated with microbial metabolism (Scheme 1). Meanwhile, we hope that this topic will open a new avenue for the real-time monitoring of dynamic chemical processes in microbial metabolism.

## Recent Advances

Currently, optical probes based on small organic molecules have obtained increasing attention in real-time analysis of living systems.[4-6] The organic probes have the intrinsic advantages of simplicity, high selectivity and sensitivity, whilst being non-invasive. Besides, small-molecule fluorescent probes are able to make use of selective, bioorthogonal chemistries to report on specific analytes in living systems with minimal perturbation. Different organic label tags including small fluorophores or Raman labels are emerging as potential approaches for real-time monitoring of cell metabolism. Yang and co-workers have done much work in real-time gut microbiota imaging *in vivo* with small-molecule luminescent probes (Figure 1a).[4] To be specific, a metabolic *D*-amino acid-labeled fluorescent dye was proposed for the quantitative analysis of the indigenous metabolic status of gut bacteria. Besides, Benson and co-workers have performed excellent work on small organic fluorophores design for real-time monitoring or tracking of essential metabolites in live cells and *in vivo* with high spatial and temporal resolution. Specifically, photoactivatable organic probes based on amino-substituted benzoselenadiazoles were constructed to mimic *D*-amino acids metabolites, allowing for the specific monitoring of the *D*-amino acids incorporation process into the peptidoglycan cell wall in real-time. It is worth to mention that the designed organic fluorophores can not only provide real-time tracking of the small metabolites in cell metabolism but also modulate microbial metabolism such as causing cell death upon light irradiation with high specificity (Figure 1b).[5] Luminescent lanthanide complexes which have several inherent advantages over conventional organic fluorophores have also attracted considerable attention in biosensing and bioimaging. Specifically, luminescent lanthanide complexes have long-lived emission allowing for time-gated detection without interfering background fluorescence and large Stokes shift avoiding spectral cross-talk. Marc Nazare and co-workers developed a turn-on lanthanide luminescent probe for time-gated real-time detection of nitroreductases (NTRs) in live bacteria (Figure 1c).[6] The designed NTR-responsive lanthanide probe can be activated upon interaction with the NTRs through forming the antenna enabling energy transfer to the lanthanide center, enabling to trace enzymatic activity in live bacteria as shown in the fluorescence lifetime imaging (Figure 1d). This Off-On probe concept is an attractive option for molecular mechanism study and medical diagnostics applications.

**Scheme 1** Schematic illustration of optical sensors for real-time monitoring of dynamic chemical processes in microbial metabolism




With the development of synthetic biology, genetically-encoded biosensors are currently leading a revolution in the real-time visualization and monitoring of various cellular events.[7-9] Compared with organic small molecule probes, gene encoded probes with the advantages of high selectivity, lasting labeling ability and excellent biocompatibility have also drawn great attentions in real-time monitoring of dynamic chemical processes in microbial metabolism. Genetically-encoded biosensors such as fluorescent protein-based biosensors, allosteric transcription factor-based biosensors and nucleic acid-based biosensors have been widely adopted in microbial metabolism study and strain screening through the real-time metabolic metabolism monitoring (Figure 2). Yang and co-workers have done much work in genetical-

ly-encoded fluorescent protein. For example, they developed a ratiometric genetically-encoded fluorescent protein FiNad that covers physiologically relevant $NAD^+$ concentrations and sensitively responds to increases and decreases in $NAD^+$ in living bacteria, realizing the real-time readout of $NAD^+$ metabolism.[7] The real-time monitoring genetically-encoded fluorescent protein will expand our mechanistic understanding of associated physiological and pathological processes and facilitate screening for drug or biomanufacturing process. Allosteric transcription factor-based biosensors can not only monitor the metabolites but also control downstream gene expression, and have been widely adopted in microbial metabolism analysis and regulation. Bennett developed two different allosteric transcription factor-based reporters in two strains and the reporters can be driven by promoters in response to positive and negative signal molecules, respectively. It is recognized that the signal molecules regulate microbial behaviors. Consequently, by reading the reporter information, the signal transduction and bacterial interactions can be monitored dynamically in real-time. The design concept based on gene encoded biosensor provides a universal platform for the dynamic and real-time detection of microbial metabolism and microbial interactions.[8] Compared to the transcription factor-based biosensors, nucleic acid-based biosensors generally consisted of an aptamer and a reporter, which have the advantages of high sensitivity and low metabolic burden on cells, and have also been widely adopted in microbial metabolism monitoring, regulation mechanisms and pathway optimization. For example, Jung and co-workers developed an artificial riboswitch that specifically responds to caprolactam by using SELEX and *in vivo* screening.[9] The developed biosensor based on the developed artificial riboswitch provides real-time and quantification views for the gene expression by measuring the fluorescence intensity from the superfolder green fluorescent protein with varying concentrations of caprolactam.

Actually, our group has also reported a real-time monitoring strategy for microbial metabolism.[3] To be specific, we proposed an electron transfer trigged persistent luminescent organic probe, achieving the real-time and dynamic monitoring of the Fe(III) respiration metabolism (Figure 3). As shown in Figure 3a, the quenched persistent luminescence of $Zn_2GeO_4$:Mn@$Fe^{3+}$ can be lighted up when $Fe^{3+}$ accepted electrons from the dynamic Fe(III) respiration metabolism, thus realizing the real-time monitoring of microbial metabolism. It is worth mentioning that the proposed probe in our work is capable to remain luminescent even after the

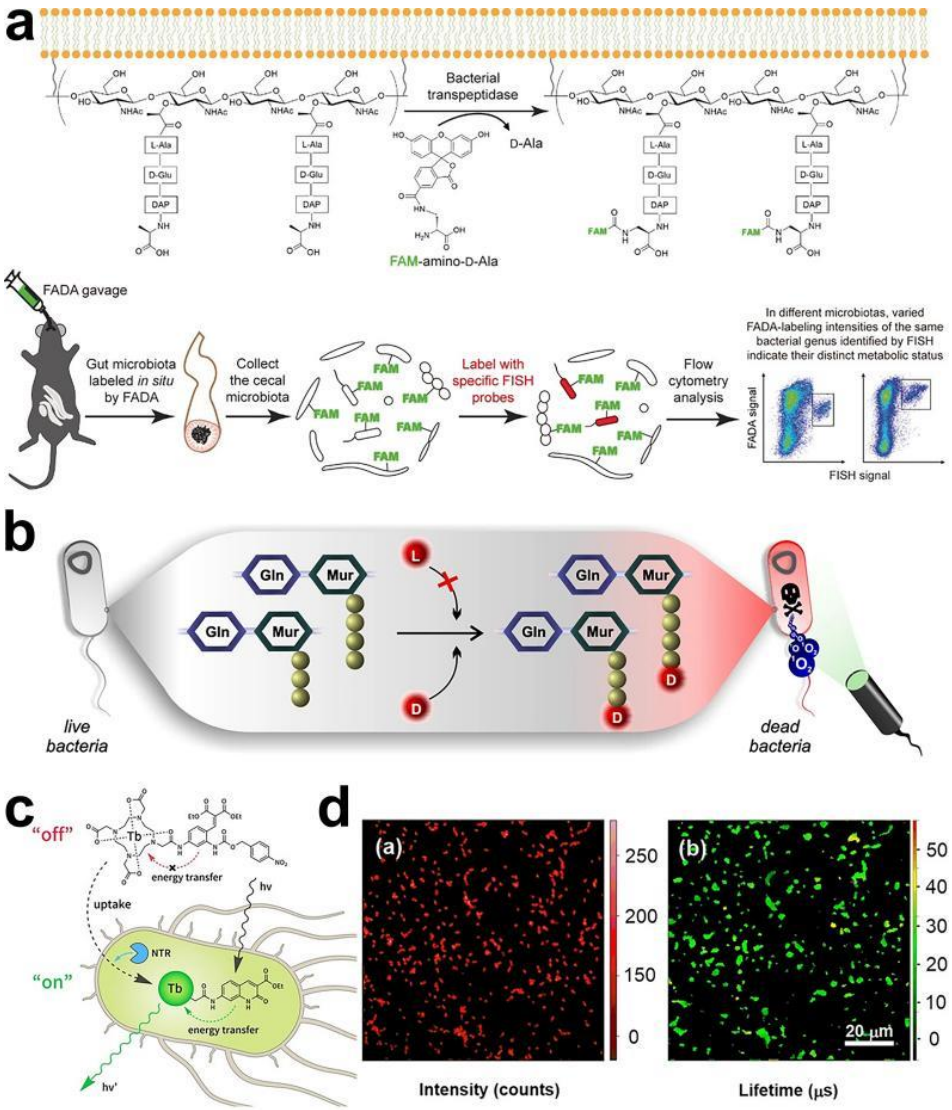



**Figure 1** a) Schematic illustration of the gut microbiota imaging. b) *D*-Alanine is incorporated into the peptidoglycan structures. Incorporation followed by illumination results in the production of singlet oxygen and concomitant cell death (Gln: *N*-acetylglucosamine, Mur: *N*-acetylmuramic acid). c) A terbium-based luminescent turn-on probe for tracing nitroreductase in live bacteria using fluorescence lifetime imaging. d) Fluorescence lifetime imaging of live *E.coli* bacteria incubated with the terbium-based luminescent turn-on probe.

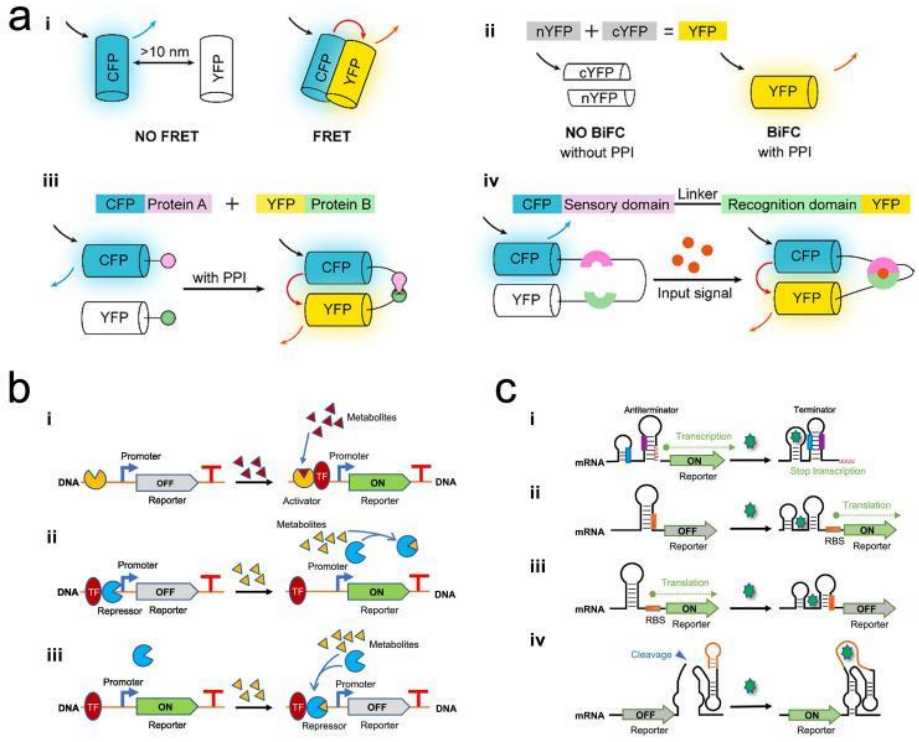



**Figure 2** a) Schematic representations of fluorescent protein-based biosensors. b) Transcription factor-based biosensors. c) Riboswitch-based biosensors.

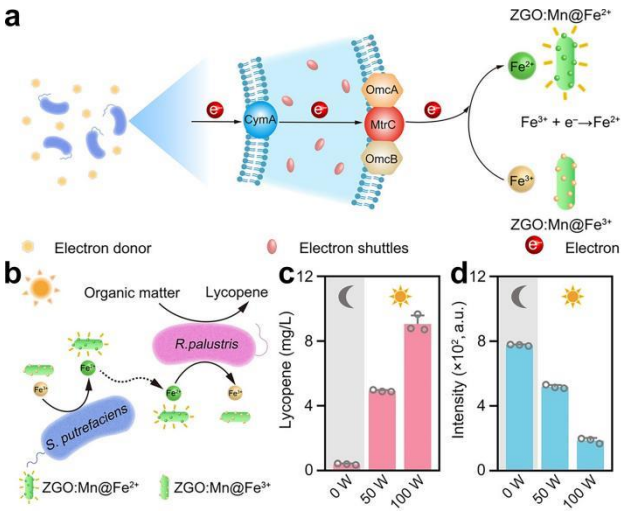



**Figure 3** a) Schematic illustration of the monitoring of microbial Fe(III) respiration metabolism with ZGO:Mn@$Fe^{3+}$ probe. b) Schematic illustration of the monitoring of lycopene biosynthesis process in microbial co-culture with ZGO:Mn@$Fe^{3+}$ probe. c) Lycopene biosynthesis efficiency in microbial co-culture under different light irradiations. d) Persistent luminescence intensity of ZGO:Mn@$Fe^{3+}$ probe in microbial co-culture under different light irradiations.

excitation ceases, enabling to eliminate autofluorescence interference. Furthermore, the probe was adopted to monitor the dynamic complex metabolism processes in nature including environmental stress response and microbial consortia metabolism. With excellent Fe redox cycling metabolism real-time monitoring ability for microbial consortia, the probe provides guidance for biosynthesis efficiency optimization (Figures 3b—d). This proposed electron transfer trigged optical probe might provide new ideas for complex microbial metabolism monitoring and represent a critical step to construct robust real-time metabolism monitoring platform for engineering microbial metabolic relevant activities.

## The potential and challenges

Although much work has been done in microbial metabolism analysis, challenges still remain in this field. Recent studies showed that the microorganisms carried substantial cell-to-cell heterogeneities at both cellular and molecular levels. Additionally, microbial metabolism can be affected with the external environment such as temperature, oxygen, pH, light molecule, *etc*. One major challenge for multispecies studies is that diversity and spatial organization often lead to a high degree of spatial and chemical heterogeneity. However, the current microbial analysis methods can only provide average results of population analysis, and the average ensemble measurement of millions of cells together is not enough to provide detailed information about cells.

Considering that microorganism has smaller size compared to cells, developing optical biosensors with high spatial and temporal revolution for real-time single cell analysis is challenge. For the organic optical probes, minimal chemical reporters that can retain the molecular recognition properties and activity profiles of biomolecules are needed. Genetically, encoded biosensors are recognized as a promising avenue for measuring metabolite levels in single cells. For the genetically encoded biosensors, how to merge the sensing domain with an actuator domain such as artificial riboswitches is of great challenges. Additionally, combing the sensitive optical biosensor with the super-resolution microscopy or other instruments is promising for the real-time monitoring of the dynamic chemical processes in microbial metabolism with high resolution.

The design and application of optical biosensors with high spatial and temporal resolution in microbial metabolism will accelerate the development of revealing the mechanism of disease occurrence and progression related to microbial metabolism and provide guidance for pathway optimization in biomanufacturing.

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