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Cell-selective metabolic labeling of biomolecules with bioorthogonal functionalities

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Metabolic labeling of biomolecules with bioorthogonal functionalities enables visualization, enrichment, and analysis of the biomolecules of interest in their physiological environments. This versatile strategy has found utility in probing various classes of biomolecules in a broad range of biological processes. On the other hand, metabolic labeling is nonselective with respect to cell type, which imposes limitations for studies performed in complex biological systems. Herein, we review the recent methodological developments aiming to endow metabolic labeling strategies with cell-type selectivity. The cell-selective metabolic labeling strategies have emerged from protein and glycan labeling. We envision that these strategies can be readily extended to labeling of other classes of biomolecules.

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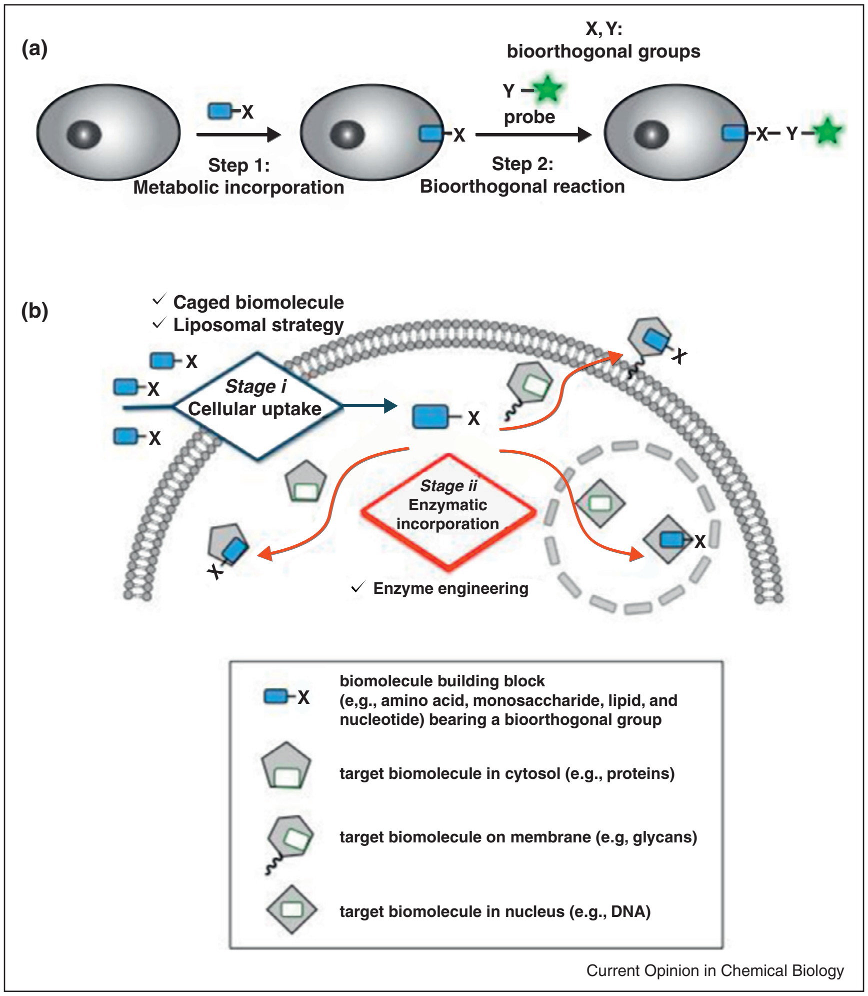
Introduction

Labeling of biomolecules with fluorescent probes or affinity tags is a crucial means to elucidate the molecular details of various biological processes. Monoclonal antibodies [1] and fluorescent protein fusions [2] are two classic and revolutionary tools for fulfilling this task, which are now routinely used by numerous laboratories. Nevertheless, the fascinating and sometimes frustrating complexity of biology involves lots of processes where the conventional tools are not applicable and therefore always calls for new technologies. The past 15 years have witnessed the emanation of bioorthogonal chemistry as a complementary and powerful tool for labeling biomolecules in living cells and whole organisms [3]. Bioorthogonal chemistry refers to a category of ligation reactions that proceed rapidly under the physiological environment without interfering with, or being interfered by, the surrounding molecules. The two participating reactants or bioorthogonal functional groups are non-native, and selectively reactive with each other. Development of bioorthogonal chemistry is a significant chemical challenge and is currently a very active research area in chemical biology [4].

In a common strategy that take advantage of bioorthogonal chemistry for biomolecular labeling, one of the two bioorthogonal functionalities is chemically installed onto the monomeric building blocks of biomolecules (e.g., amino acids and monosaccharides) and metabolically incorporated into the target biomolecules. In a second step, the incorporated bioorthogonal group is covalently ligated with a probe bearing the complementary bioorthogonal functionality (Figure 1a). The most popular bioorthogonal functional groups are azide and alkyne, which undergo copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC), also known as 'click chemistry', and its variants including copper-free click chemistry [5] and biocompatible ligand-assisted CuAAC [6]. Of note, bioorthogonal functionalities can also be introduced into proteins via genetic and enzymatic approaches [7], which are not within the focus of this review. The two-step metabolic labeling approach, also known as the chemical reporter strategy, has gained extensive attentions, due to its ease to use, high efficiency, and broad applicability. It doe not require genetic manipulation and has been employed to label four major classes of biomolecules including proteins [8], glycans [9], lipids [10], and nucleic acids [11–13]. In principle, the unnatural building blocks are metabolically incorporated into and globally tag all the biomolecules containing that building block. One attractive feature of this method is that it labels newly synthesized biomolecules and therefore it is well-suited for probing the dynamic changes of the proteome, glycome, lipidome, and genome. The metabolic labeling method is also compatible with *in vivo* studies. It has proved to be an invaluable tool for various applications that were not possible before, such as *in vivo* imaging of zebrafish glycome during development [14], visualization of dynamics of protein synthesis in neurons [15], probing functional roles of protein lipidation in immunity [16], and analysis of RNA transcription rates *in vivo* [12].

To probe biomolecules within their physiological environments, it is often desirable to selectively label the biomolecules in a specific cell type. For genetic approaches, cell-selectivity can be conveniently achieved by employing proper tissue-specific promoters [17]. For

Figure 1



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Schematic of cell-selective labeling of biomolecules with bioorthogonal functionalities. **(a)** The two-step metabolic labeling strategy. The building blocks (e.g., amino acids, monosaccharides, lipids, and nucleotides) functionalized with a bioorthogonal functional group are incorporated into the target biomolecule via cellular metabolism. In a second step, the bioorthogonal functional group is reacted with a probe containing a complementary bioorthogonal group. **(b)** General strategies for achieving cell-selectivity. The metabolic incorporation step can be further divided into two stages where cell-selective incorporation can be accomplished by: **(i)** cellular entry of the unnatural building blocks; **(ii)** enzymatic incorporation of the building blocks. Liposomal delivery and caging of building blocks are two strategies used at stage i for cell-selective metabolic glycan labeling. Engineering the metabolic enzymes at stage ii is developed for cell-selective metabolic protein labeling.

example, the expression of GFP tag in zebrafish can be confined to muscle cells using a muscle-specific actin promoter [18]. By contrast, metabolic labeling approach generally suffers from nonspecificity with respect to cell identity; biomolecules from all cell types are globally labeled. It is obvious that supplementing the metabolic labeling method with a mechanism of cell-selectivity will tremendously expand its utility. For example, it is interesting to label biomolecules in a specific cell type of interest when studying cell-cell interactions, so that the origin of the labeled biomolecules is not obscured by surrounding cells sharing a common genome. Furthermore, cell-selective labeling can facilitate the detection of low-abundance biomolecules, or biomolecules in low-abundance cells. Those attractive benefits have motivated recent efforts to endow the metabolic labeling techniques with cell-selectivity. In this review, we describe the recent methodological advances that have enabled the cell-selective metabolic labeling of biomolecules, mainly proteins and glycans.

Methodology for endowing metabolic labeling with selectivity

It is reasonable to tackle this challenge by intercepting the first step of metabolic labeling, that is, modulating the metabolic incorporation of the bioorthogonal group-bearing building blocks to be cell-selective. There are two promising stages where cell-selective incorporation can be accomplished: cellular entry of the unnatural building blocks and enzymatic incorporation of the building blocks