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# Deciphering the bacterial glycocode: recent advances in bacterial glycoproteomics

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Bacterial glycoproteins represent an attractive target for new antibacterial treatments, as they are frequently linked to pathogenesis and contain distinctive glycans that are absent in humans. Despite their potential therapeutic importance, many bacterial glycoproteins remain uncharacterized. This review focuses on recent advances in deciphering the bacterial glycocode, including metabolic glycan labeling to discover and characterize bacterial glycoproteins, lectin-based microarrays to monitor bacterial glycoprotein dynamics, crosslinking sugars to assess the roles of bacterial glycoproteins, and harnessing bacterial glycosylation systems for the efficient production of industrially important glycoproteins.

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## Introduction

Pathogenic bacterial strains that are resistant to existing antibiotics pose a widespread health risk. Thus, there is an urgent need to identify new pathogen-associated molecules that can be targeted for the selective eradication of bacterial infections. Bacterial glycoproteins – proteins that are post-translationally modified by the covalent addition of one or more monosaccharides – are an attractive class of untapped targets.

It was long believed that bacteria could not synthesize glycosylated proteins due to the simplicity of their cellular structure and concomitant lack of subcellular organelles. Despite this long-standing dogma, the synthesis of glycoproteins in select bacterial strains has been firmly established by a number of leaders in the field [1,2,3•]. Two important themes have surfaced since the discovery of bacterial glycoproteins. First, bacterial glycoproteins are frequently linked to pathogenesis (see review in [4•]). For example, in some pathogenic bacteria, protein glycosylation is essential for locomotion, and without the appropriate flagellar glycans these bacteria cannot colonize their hosts [5]. In other bacterial pathogens, the glycans on proteins mediate interactions with host cells, such as adhesion to host cells [6] or evasion of the host's immune system [7]. In several cases, bacterial strains with altered protein glycosylation display decreased fitness within the host [3•,5]. The link between bacterial glycoproteins and pathogenesis indicates that this class of biomolecules should be exploited for therapeutic intervention.

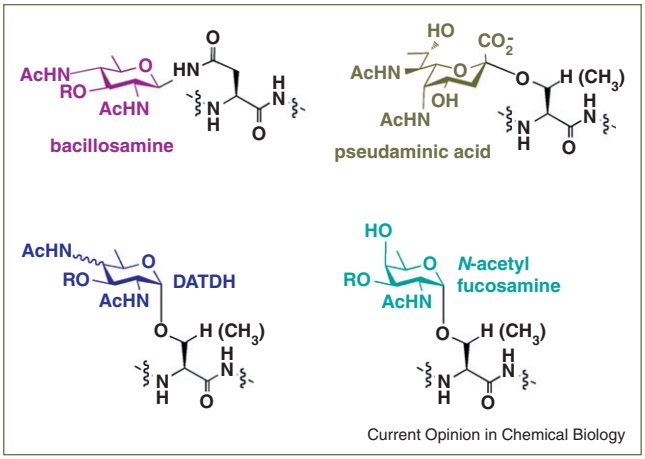
The second theme that has emerged is that bacterial glycoproteins contain unique glycans that are absent from humans [4•,8]. Although bacteria are capable of synthesizing the two major classes of glycoproteins that are found in eukaryotes – those that have asparagine-linked (N-linked) glycans and those that have serine/threonine (O-linked) glycans – their structures often contain sugars that are distinctly bacterial. For example, the amino- and deoxy-monosaccharides pseudaminic acid [5], bacillosamine [9], 2,4-diacetamido-2,4,6-trideoxyhexose (DATDH) [10], and *N*-acetyl fucosamine [11] are found in bacterial glycans but are absent from human glycans (Figure 1). Thus, in addition to their links to pathogenesis, bacterial glycoproteins distinguish bacterial cells from human cells. Together, these attributes make bacterial glycoproteins attractive targets for new antibacterial treatments.

Despite their potential therapeutic importance, many bacterial glycoproteins remain understudied. This relative paucity of information is due, partly, to the structural complexity of the bacterial glycoproteome [8,12]. Bacterial glycoproteins contain heterogeneous glycans composed of myriad monosaccharides that can be linked in a branched or linear manner; moreover, unlike in DNA and protein synthesis, no template directs glycan synthesis. This structural complexity poses analytical challenges, yet this very complexity encodes the biological information that provides the impetus for studying bacterial glycoproteins. In this review, we focus on the latest methods developed to decipher the bacterial glycocode and efforts to harness this information for biotechnological applications.

## Discovering bacterial glycoproteins

The discovery of bacterial glycoproteins is typically a three-step process that includes the detection, isolation, and characterization of these biomolecules. In this section, we discuss recent advances in chemistry that facilitate these steps.

**Figure 1**



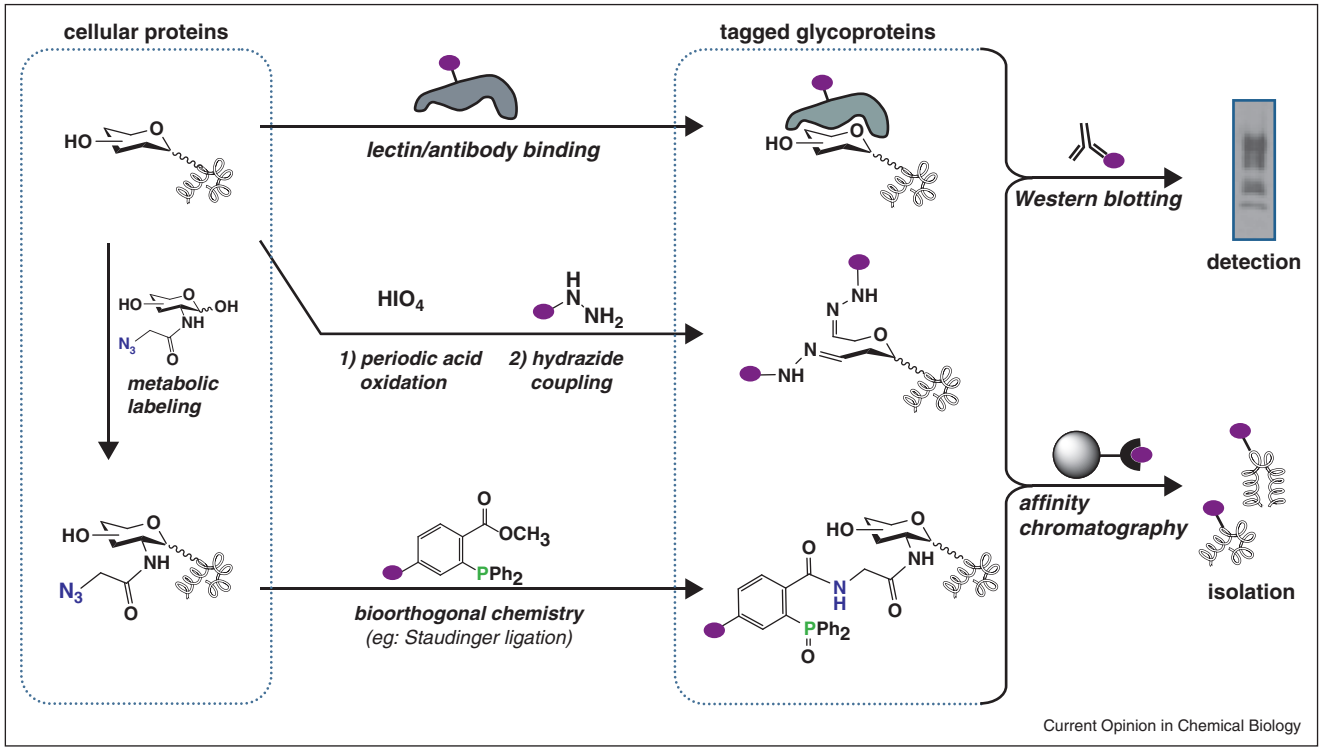

Examples of bacterial glycan structures. Deoxy- and amino-monosaccharides, such as bacillosamine, pseudaminic acid, 2,4-diacetamido-2,4,6-trideoxyhexose (DATDH), and *N*-acetyl fucosamine, are found in bacterial glycans but are absent from human glycans. These glycan structures can be elaborated with additional monosaccharides at the indicated positions (–OR).

## Detection of bacterial glycoproteins

The first step in discovering bacterial glycoproteins is to broadly detect their presence. Eukaryotes are expected to synthesize glycoproteins, but not all bacteria do; thus, the synthesis of glycoproteins by a given bacterial strain is not known in advance. Traditional biological methods to detect bacterial glycoproteins include using glycan-binding proteins such as lectins or antibodies that recognize and bind to particular glycan structures [8] (Figure 2). Conventional chemical approaches, such as periodic acid/hydrazide labeling or boronic diester modification of *cis* diols, employ reagents that covalently modify carbohydrate functionalities [8] (Figure 2). Both of these approaches have enabled the detection of glycoproteins in numerous species [3•,9,13] yet suffer from limitations. As an example of a limitation, biological approaches require *a priori* knowledge of glycan structure and the existence of a biological reagent that binds to bacterial glycans. By contrast, traditional chemical methods detect a wide array of glycan structures but their promiscuity leads to undesired interactions [14], resulting in low signal to noise and false positives.

To address these limitations, metabolic oligosaccharide engineering, a chemical method that was pioneered by

**Figure 2**




Methods to detect and isolate bacterial glycoproteins. Bacterial glycoproteins are tagged via (1) binding to glycan-binding reagents, such as lectins or antibodies (top arrow), (2) periodic acid-assisted oxidation followed by hydrazide attack (middle arrow), or (3) metabolic labeling with an unnatural sugar, such as an azide-containing sugar (shown on left), followed by bio-orthogonal chemistry (e.g. Staudinger ligation, bottom arrow). Once glycoproteins are tagged, they can be detected by Western blot or isolated via affinity chromatography from other cellular proteins.