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Current Opinion in Chemical Engineering

Computational tools for predicting and controlling the glycosylation of biopharmaceuticals

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Glycosylation is a critical quality attribute of biopharmaceuticals because it is a major source of structural variability that influences the *in vivo* safety and therapeutic efficacy of these products. Manufacturing process conditions are known to influence the monosaccharide composition and relative abundance of the complex carbohydrates bound to therapeutic proteins. Multiple computational tools have been developed to describe these process/product quality relationships in order to control and optimise the glycosylation of biopharmaceuticals. This review will provide a summary highlighting the strengths and weaknesses of each modelling strategy in their application towards cellular glycoengineering or bioprocess design and control. To conclude, potential unified glycosylation modelling approaches for biopharmaceutical quality assurance are proposed.

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Current Opinion in Chemical Engineering 2018, **22**:89–97

This review comes from a themed issue on **Biotechnology and bioprocess engineering: biomanufacturing**

Edited by **Michael Betenbaugh** and **Nigel Titchener-Hooker**

https://doi.org/10.1016/j.coche.2018.08.007

Introduction

Twenty of the fifty top-selling pharmaceuticals are glycosylated recombinant proteins that achieved worldwide revenues of over US$90 billion in 2017 [1]. Eighteen of these biopharmaceuticals are monoclonal antibodies (mAbs), which contain two consensus asparagine-linked (N-linked) complex carbohydrates (glycans) on their constant fragment, Fc (Figure 1a). The remaining two blockbuster products, Enbrel® and Eylea®, are heavily glycosylated Fc fusion proteins, which contain up to six N-linked and twenty-six Serine/Threonine-linked (O-linked) glycans [2] (Figure 1b). Many other therapeutic proteins are also glycosylated, with tissue plasminogen activator (tPA), interferon gamma (IFN-γ) and human recombinant erythropoietin (rHuEPO) (Figure 1c) being key examples [3].

The glycosylation of therapeutic glycoproteins (TGPs) is highly variable and heavily influences the safety and therapeutic efficacy of these products. Presence or absence of glycans (macroheterogeneity) on TGPs affects their serum half-life in patients [4,5], while the glycosidic linkages and monosaccharide composition (microheterogeneity) are widely reported to impact the safety, pharmacokinetics and pharmacodynamics of TGPs [6]. Microheterogeneity arises from varying degrees of mannosylation, antennarity, core fucosylation, galactosylation and sialylation (Figure 1d through g) [3].

All TGPs are produced through large-scale culture of mammalian cells, in particular of Chinese Hamster Ovary (CHO) cells, to ensure compatibility for administration in humans. Importantly, the conditions under which mammalian cells are cultured heavily influence the glycosylation profiles of biopharmaceuticals [3].

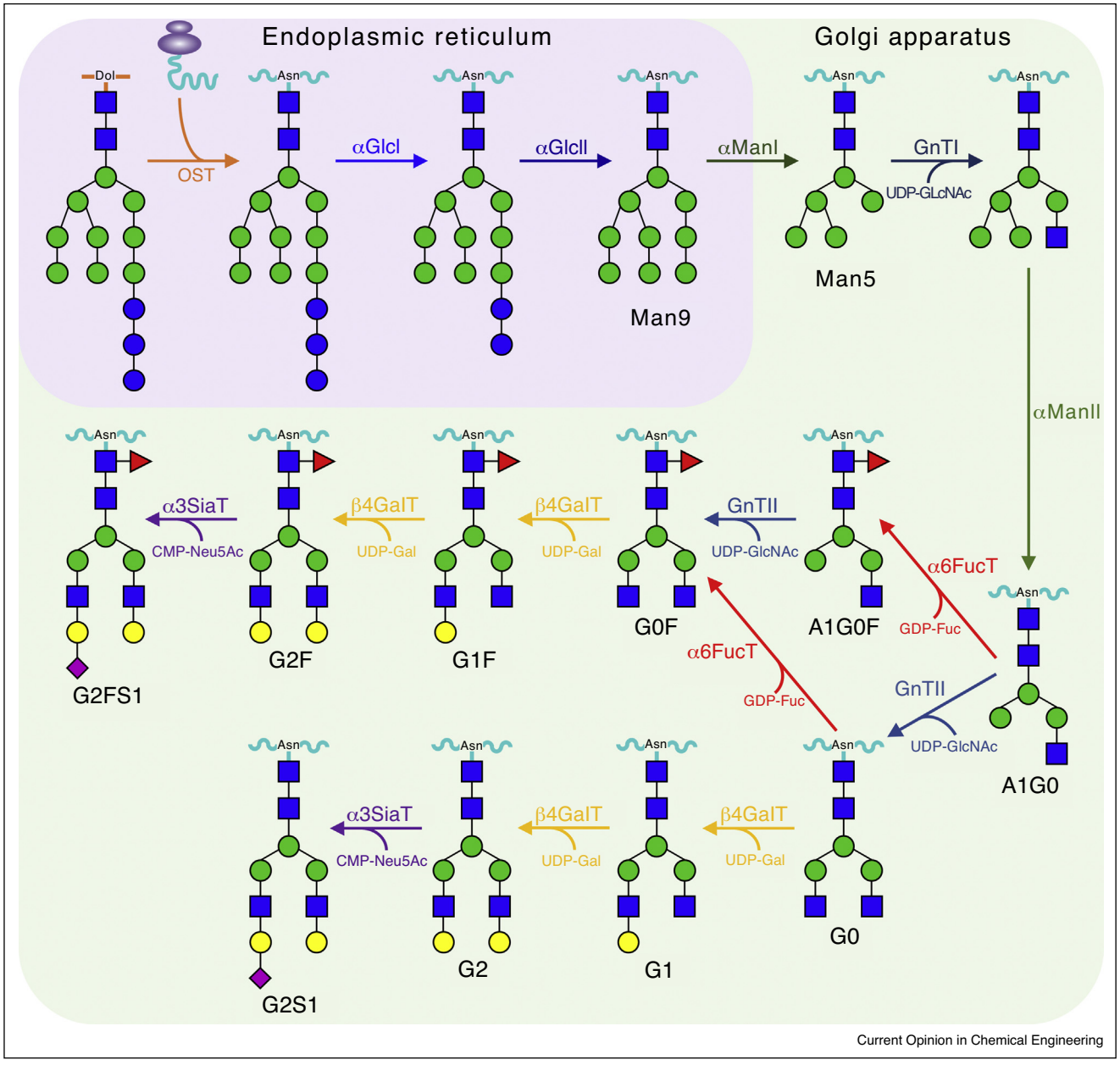
Herein, we provide an overview of recent glycosylation models in the context of glycoprotein quality assurance for biopharmaceutical manufacturing and discuss their advantages and disadvantages. The review concludes with perspectives for potential unified modelling strategies to control the manufacture of biopharmaceuticals with optimal and consistent glycosylation patterns.

Glycosylation as a critical quality attribute of biopharmaceuticals

Based on the definition of Critical Quality Attributes (CQAs) within the Quality by Design (QbD) framework [7], industry and regulatory agencies consider glycosylation a CQA of TGPs because it is a property that must be controlled within an appropriate range or distribution to ensure product safety and therapeutic efficacy [3]. The influence glycosylation has on the safety, pharmacokinetics and pharmacodynamics of TGPs is summarised in Table 1.

Glycosylation is widely acknowledged as a major source of variability and one of the most difficult to control CQAs because even modest changes in manufacturing process conditions can influence TGP glycan distributions [8]. Despite the available regulatory guidelines for the assessment and control of TGP glycosylation-associated quality

Figure 1



Common therapeutic glycoproteins and glycans produced by CHO cells [3].
Three common therapeutic glycoproteins, including N-linked and O-linked glycosylation sites, are schematically represented. **(a)** Is an IgG-based mAb, **(b)** is an Fc fusion protein (Etanercept®) and **(c)** is erythropoietin (rHuEPO). The monosaccharide composition and glycosidic bond linkages of oligomannose **(d)**, complex biantennary **(e)** and complex tetra-antennary **(f)** N-linked glycans, as well as O-linked glycans (**(g)** and **(h)**, respectively) are shown. The symbolic representation of each monosaccharide present in the glycans is outlined at the bottom.

[3], substantial variations have been reported across different production lots of marketed products [9]. Glycosylation remains a key challenge for manufacturers and regulators alike and highlights the need for strategies that mechanistically link bioprocess conditions with TGP glycan distributions.

Protein glycosylation in mammalian cells

Glycosylation is a non-template driven processes which is thought to have evolved to confer glycoconjugates with additional levels of variability and enhanced functional adaptability [10]. The mammalian N-linked glycosylation process, which is summarised in Figure 2 [11], begins in