Spring 5-12-2016

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Amanda Lewis
Bristol-Myers Squibb Company, amanda.lewis@bms.com

Nelly Aranibar
Bristol-Myers Squibb Company

William Croughan
Bristol-Myers Squibb Company

Nicholas Abu-Absi
Bristol-Myers Squibb Company

Bethanne Warrack
Bristol-Myers Squibb Company

See next page for additional authors

Authors
Amanda Lewis, Nelly Aranibar, William Croughan, Nicholas Abu-Absi, Bethanne Warrack, Michael Borys, Michael Reily, and Zheng Jian Li

UNDERSTANDING AND CONTROLLING SIALYATION IN A CHO FUSION PROTEIN AT LAB AND MANUFACTURING SCALE USING TARGETED OMICS TECHNIQUES

Amanda M. Lewis, Bristol-Myers Squibb Company
amanda.lewis@bms.com
Nelly Aranibar, Bristol-Myers Squibb Company
William D. Croughan, Bristol-Myers Squibb Company
Nicholas R. Abu-Absi, Bristol-Myers Squibb Company
Bethanne Warrack, Bristol-Myers Squibb Company
Michael C. Borys, Bristol-Myers Squibb Company
Michael D. Reily, Bristol-Myers Squibb Company
Zheng Jian Li, Bristol-Myers Squibb Company

Key Words: glycosylation, N-acetylneuraminic acid, metabolomics, transcriptomics, CHO fusion protein

Biologics, including antibodies, hormones and cytokines, represent an increasingly important class of therapeutics, with 7 of the 10 top selling drugs from 2013 in this class. The glycosylation distribution of these proteins is an important characteristic that can impact biological activity, circulatory half-life, and immunogenicity. One property that affects glycoproteins is the terminal addition of N-acetylneuraminic acid (sialic acid) to glycosylation chains. Despite the importance of glycosylation in many therapeutic proteins, limited information is available to date linking process parameters to changes in glycosylation distribution. The majority of the work that has been done is limited to a small number of proteins (such as interferon gamma) and small scale systems (shake flasks and bench top bioreactors). Although this work represents a useful starting point, glycosylation is a parameter that is known to be influenced by production scale.

Here we examine a glycosylated CHO fusion protein for which sialyation level is known to impact protein quality. Variation in this parameter was observed across pilot and manufacturing scale batches. In order to better understand and control the biological source of the variation in the process, we employed metabolomic and transcriptomic methods, and successfully identified metabolic biomarkers, such as extracellular mannose, for sialylation level. Additional studies demonstrated that changes to sugar metabolism were contributing to a build-up of intermediates and inhibition of glycan sialyation, thereby identifying the biological source of variation in the process. As a result of these studies, we evaluated the impact of process modifications including feed composition and gassing to enable consistent control of sialyation profiles.

This work represents a novel contribution to the field. We examine sialyation control of a CHO fusion protein at laboratory and manufacturing scale. Furthermore, we combine ‘Omics techniques with bioprocess and analytical data to achieve a more detailed understanding of cell expression and metabolism [1], and leverage this understanding to refine the process and control a quality attribute. Finally, this approach can be generalized beyond this specific process and applied to additional cell lines where undesired process variation is observed.

1. Lewis, A.M., et al., *The Use of ‘Omics Technology to Rationally Improve Industrial Mammalian Cell Line Performance.* Biotechnology and Bioengineering, 2015: 10.1002/bit.25673