# Designing Biobetter Monoclonal Antibody Therapeutics By Glycoengineering

## Introduction

Monoclonal antibodies (MAbs) currently comprise the fastest growing class of protein therapeutics - primarily for the treatment of cancers and autoimmune, infectious and inflammatory diseases. Commercially, MAbs represent the most rapidly expanding segment of the pharmaceutical industry - by 2008 the US therapeutic antibody market was estimated to be around 40 billion USD with further significant increases predicted for the next decade. Furthermore, major advances in cell line development, bioreactor construction and operation, purification strategies and analytics (Sommerfield et al, 2005) have resulted in continual reductions in the manufacturing costs of MAbs which are now being produced in cell culture for 200 USD to 1,000 USD per gram. However, there is still a continual drive to produce more effective, safer and less expensive therapeutic antibodies. This drive has stimulated the development of 'biosimilars' (copies of original drugs) and 'biobetters' (improved versions of original drugs). Many regard this as an exciting prospect and of benefit to patients who could have access to a wider range of less expensive drugs - but it has prompted serious debates and initiatives relating to regulation, intellectual property (IP) and protection of commercial interests.

One area of great interest to developers, copiers and improvers of therapeutic antibodies is glycosylation, since it can significantly influence the safety and efficacy profiles of the drug (Arnold et al, 2007; Fernandes, 2009a; Beck et al, 2008; Jefferis, 2009). In this article, we show how both the original drug manufacturers and the designers of follow-on biologics could produce biobetter antibodies through glycoengineering. In particular, we examine strategies for optimising both Fab and Fc glycosylation to produce MAbs with improved clinical performance and better commercial profiles compared to existing drugs.

## The Coming of Biosimilars and Biobetters

### Biosimilars

The arrival of biosimilars and biobetters will have a tremendous impact on the therapeutic glycoproteins market. Some of the earliest biopharmaceuticals have already lost patent protection, and it is estimated that by 2013 about half of all products coming off patent will be biologics. The first biosimilars – Omnitrope and Valtropin (somatropin) – were approved in 2006, and the following year saw marketing authorisations for five epoetin products. In February 2008, the European Medicines Agency cleared four biosimilar filgrastims. Biosimilar antibodies will, no doubt, follow the same route. However, whilst the first biosimilars were relatively simple molecules, immunoglobulins are far more complicated in terms of size, structural complexity and their glycosylation patterns.

A key problem for developing biosimilar antibodies will be demonstrating comparability of their glycosylation to the original drug. This was highlighted for a non-antibody biopharmaceutical when Genzyme scaled up production of its drug Myozyme - an enzyme replacement therapy which was approved in 2006 for the treatment of Pompe disease, an inherited muscle disorder. The scale-up in 2000 litre reactors was carried out at a different plant (Allston, Massachusetts) from the one that produced the drug for the original approval (Framingham, MA). The glycosylation pattern was found to be significantly altered in the scaled-up drug, and the FDA considered the Myozyme coming out of the Boston plant to be a new product. Genzyme would need to file a new application (and thus provide more clinical data) for Myozyme if it wanted to sell it on the US market. To resolve this, the company decided to launch the same biologic under two different names in the US - Myozyme for the original product and Lumizyme for the scaled-up product. Conversely, Atryn (manufactured by GTC Biotherapeutics) which is a recombinant antithrombin produced in transgenic goats, was approved by the FDA earlier this year despite having a glycosylation profile different from plasma-derived antithrombin. In this case the different glycosylation pattern resulted in an increased heparin affinity but tests showed that the potency of the recombinant product is not different from that of the plasma-derived product.

The complexity of the molecules, and therefore the more clinical data that companies have to generate in order to obtain approval, may hold back the progress of biosimilars. Indeed, biosimilars have still not made a significant impact on the pharmaceutical market. This is in part due to the lack of a legal and regulatory framework for the production of biosimilars around the world; a regulatory pathway for approving biosimilars is established in the European Union but is still under consideration in the United States. Despite this, it is clear that the potential is there for the future and biosimilar MAbs are already under development. In 2008, GTC Biotherapeutics initiated the first of several research programmes to develop biosimilar versions of marketed MAbs that will begin to come off patent in the US from 2014 onwards. The products targeted had combined sales in 2008 of greater than $17 billion. GTC is seeking development partners to help commercialise its portfolio of biosimilar monoclonal antibodies. Well-designed comparability studies and attention to glycosylation patterns will be key to their success.

### Biobetters

We believe that 'biobetters' with improved glycosylation over the original drug are a more exciting proposition than biosimilars. Such drugs would offer medical advantages over biosimilars other than just lower price and increased choice. In the following sections we will focus on how to design glycovariant biobetter MAbs that share the same protein as the original drug

but which have modified glycosylation to enhance safety and efficacy profiles of the therapeutic.

## What is a Glycovariant Biobetter MAb?

The first step in designing a biobetter MAb is to define what exactly a biobetter is in relation to follow-ons and biosimilars. However, at present, in the biopharmaceutical industry, there seems to be a range of different interpretations of these terms. This confusion arises, in part, from the real difficulty in comparing variants of biopharmaceuticals that are heterogeneous mixtures of exceedingly complex structures with inherent batch-to-batch variability and for which we have only partial understanding of the mode of action and possible aberrant behaviour.

For example, during an expert panel session at a recent conference on biopharmaceutical comparability (Informa conference, Cologne, June 2009) there was a discussion on whether two therapeutics sharing the same protein structure but with different glycosylation should always be considered as the 'same' drug. Two opposing opinions, with far-reaching consequences, were expressed. The first was that the only consideration for 'sameness' should be amino acid sequence - so the variants should always be viewed as the same drug, no matter what the differences in glycosylation. This would strengthen the protection of a drug innovator from competition during the period of exclusivity, but subsequently would allow 'biosimilars' with vastly differing glycan profiles. The second opinion was that there could be conditions in which changes in glycosylation patterns would result in the biological behaviour altering so significantly that the variants should be considered as related but not the 'same'. This could allow glycoengineered biobetters to be produced during the period of exclusivity, but then narrow the field for biosimilars which would have to bear glycosylation that was substantially similar to that of the original drug.

Clearly, this fundamental disagreement on the definition of sameness needs to be resolved to clarify both the legal and regulatory positions for all drug developers and manufacturers. At Ludger, we propose to answer this by systematically dividing the problem of 'determining sameness' into smaller, independent comparison tasks and dealing with each of these in turn. So, to determine the degree of similarity of a glycosylation variant of an innovator's drug we compare it to the original with respect to a range of parameters. These include safety and efficacy profiles, biological behaviours (e.g. serum half-life and biological activities) and structural attributes (e.g. amino acid sequence and glycosylation patterns). The combination of comparison scores for the 'copy drug' indicates its similarity class. A simplified version of this procedure is given here, the first step being to define various parameters and comparison scores like so (each comparison is to the original drug):

S0, S-, S+ = safety profile: scores for same, poorer or better respectively

E0, E-, E+ = efficacy profile: scores for same, poorer or better respectively

Prot0 = same protein: same primary and secondary structures

ProtDiffs = different protein: differences in primary or secondary structures

Gly0 = same glycosylation: same glycosylation sites, glycan species and relative proportions

Gly1 = related glycosylation: same glycosylation sites and glycan species, different relative proportions

GlyDiffs = different glycosylation: different glycosyation sites and/or glycan species

The state of the copy drug compared to the original can be indicated by making a list of these parameters and classifying it according to the following scheme (note that this is a partial list of comparison classes and does not including 'bioworse' variants with S- or E- profiles or biobetters with different protein structures):

Type 1 Copy Drugs - Biosimilars
Type 1A: [S0 E0 Prot0 Gly0]
Type 1B: [S0 E0 Prot0 Gly1] (where glycosylation has little impact on safety or efficacy)
Type 1C: [S0 E0 Prot0 GlyDiffs] (where glycosylation does not impact on safety or efficacy)

Type 2 Copy Drugs – Biobetters with Related Glycosylation
Type 2SE: [S+ E+ Prot0 Gly1] (the most desirable Type 2 profile)
Type 2S: [S+ E0 Prot0 Gly1]
Type 2E: [S0 E+ Prot0 Gly1]
Type 2SEm: [S+ E- Prot0 Gly1] (improved safety at expense of efficacy)

Type 3 Copy Drugs – Biobetters with Different Glycosylation
Type 3SE: [S+ E+ Prot0 GlyDiffs] (the most desirable Type 3 profile)
Type 3S: [S+ E0 Prot0 GlyDiffs]
Type 3E: [S0 E+ Prot0 GlyDiffs]
Type 3SEm: [S+ E- Prot0 GlyDiffs] (improved safety at expense of efficacy)

Figure 1 illustrates how these variants relate to one another in safety-efficacy space.

Such classification allows different types of copy drug to be treated appropriately with respect to regulation, intellectual property and commercial positioning. Provided the rules for the different treatments were clear, consistent, and agreed within the industry this would overcome many of the problems of using crude definitions of 'sameness'.

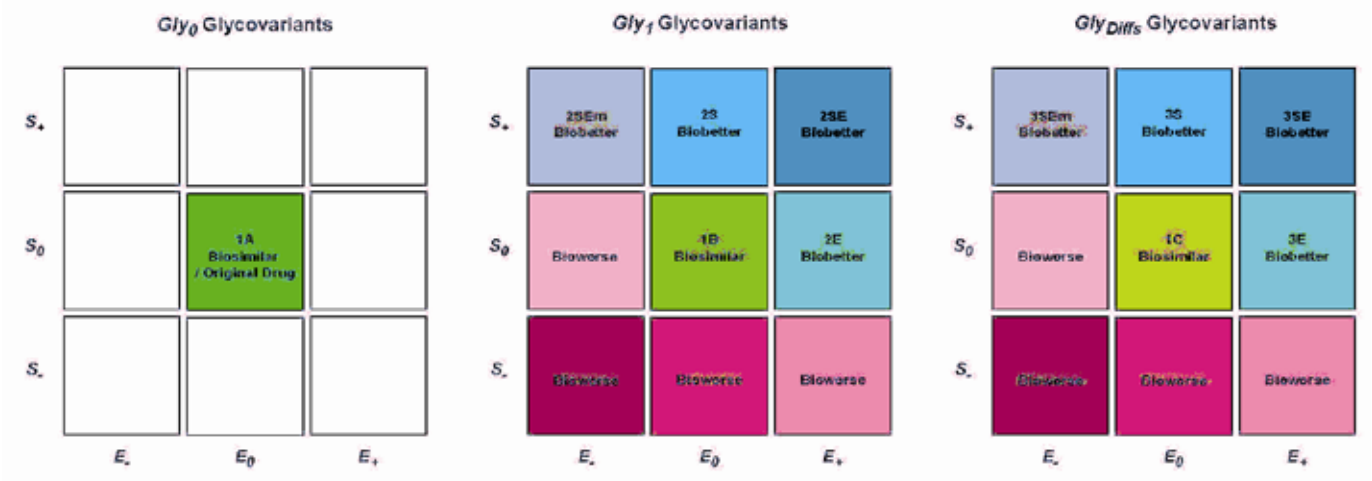


Figure 1: Classification of glycovariants of copy drugs with different safety and efficacy profiles relative to the original drug

This classification system is part of a theoretical framework called GTO-QbD currently being developed at Ludger to simplify the design, determination of comparability and analysis of complex biopharmaceuticals (Fernandes, 2009a). GTO-QbD is built on the principles of QbD (Quality by Design) introduced in ICH guidelines Q8 and Q8 annex with extensions, to cope with the complexity of biopharmaceutical PTMs (post-translational modifications) – glycosylation being one of the most noticeable PTMs of most MAbs. The system borrows ideas from mathematical graph theory and ontology (hence 'GTO') to map relationships (ontology is the study of relationships) between safety, efficacy, biology, structural attributes, material attributes and process parameters for glycoprotein therapeutics, and is being used to design biobetter antibodies (Fernandes, 2009b).

## When is a Glycoengineered BioBetter Commercially Better than a Biosimilar?

Our knowledge of structural-activity relationships for MAb glycosylation is now sufficiently advanced that improving existing drugs by glycoengineering is relatively straightforward. Given this, the choice between producing a biosimilar or a glycoengineered biobetter MAb is essentially a marketing one. The following illustrates how we can make such a choice based on commercial considerations:

First, we define the following commercial comparison parameters:

C0 C- C+ = manufacturing cost per therapeutic dose: similar to, or lower or higher than original drug

P0 P- P+ = selling price per therapeutic dose: similar to, or lower or higher than the original drug

For a biosimilar, you would pick a successful original drug coming out of its exclusive period, aim to make as close a copy as possible (e.g. a Type 1A biosimilar), and reduce both manufacturing costs and selling price - so the ideal drug copy profile would be [S0 E0 Prot0 Gly0 C- P- ]. In this case, your main advantages over the originator is that you have a lighter R&D effort than they had (you build on their efforts) and you can gain benefit from advances in manufacturing know-how to reduce your production costs (these are also reasons why some innovators object to the principle of biosimilars). However, your problems include the following:

a. You may experience technical difficulties in reproducing the glycosylation pattern of the original drug – you would be constrained to use a similar or identical cell expression system and would more likely achieve a Gly1 profile than a Gly0.

b. If your drug does have a Gly1 glycosylation pattern then the regulatory authorities may classify it as a different but related drug - so you could lose the ability for physicians to prescribe your product as a generic substitute, weakening your commercial position.

c. You are likely to have difficulties in proving complete equivalence of safety and efficacy profiles (it is easier to prove differences than equivalence in the clinic).

d. Your drug is likely to be one of several biosimilars, all of which compete with the original drug on price but without the innovator's brand recognition.

The case for developing biobetters is generally more compelling. The main problems for follow-on companies would be the high costs of developing a new drug, building brand recognition and gaining market share from the originator. However:

a. You have greater freedom in choosing your expression system (including non-mammalian cell lines or whole-organisms). This increases the likelihood of significantly lowering manufacturing costs.

b. If your drug has clear clinical benefits (e.g. it overcomes adverse reactions found with some patients treated with the original drug) then you can position it in the market as a 'new and improved' version, and may have less pressure to reduce the price per dose, so increasing your profitability.

c. You could build a stronger IP position if your product has clearly distinct glycosylation from the original drug or other copy drugs.

Of course, if you are the originator you could also make a biobetter as the next-generation of your own drug and enjoy even greater advantages over follow-on competitors.

## Strategy and Tools for Building Glycoengineered Biobetter MAbs

The design strategy we suggest is as follows:

a. Use a QbD-type paradigm for your drug development - its flexibility and power can simplify many tasks that would be difficult with older paradigms. In particular, ensure that you continually refine your models of the Design Space (DS) and Control Space (CS) both for your copy drug and the original drug - this is essential for demonstrating comparability or otherwise of drug variants. The GTO-QbD schema is an example of a QbD-based tool that you could use when glycoengineering MAbs (Fernandes, 2009).

b. Investigate the mode of action, clinical behaviour and glycan structure-function relationships for the original drug.

c. Identify GCQAs (glycosylation critical quality attributes) for the original drug. These are glycosylation features that significantly influence the safety or efficacy profiles of the therapeutic. GTO-QbD has methods for reliably determining and prioritising GCQAs.

d. Use the GCQA list to work out what non-human or other glycosylation features should be eliminated to produce a drug with an S+ profile.

e. Similarly, work out which glycosylation features need to be modified or designed in order to improve the in vivo efficacy in your target patients and achieve an E+ profile. Be aware that some glycan features may cause an increase in biological activities in vitro or in vivo animal models but the same effects may not be reflected in the clinic.

f. Draw up a new GCQA list for your hypothetical, ideally glycosylated drug.

g. Select glycoprofiling methods that allow reliable measurement of the GCQAs of your candidate drug and that are suitable for your analytical labs. Ensure that your glycoanalysis scheme complies with current regulatory guidelines – e.g. the 2008 Revised Guideline on Monoclonal Antibodies by the EMEA's Biologics Working Party (BWP).

h. Test a range of glycoprotein expression systems that could deliver your target glycosylation profile and select the best ones. Generally, if your target glycosylation is Gly1 then choose an expression system related to the one used for the original drug. This may reduce the possibility of producing a [C-] drug so it may affect profitability. However, if you are after a GlyDiffs pattern then you could use a totally different expression system with possible cost benefits. A bank of cell lines or other expression systems with diverse glycosylation machinery would be invaluable for this exercise.

## Designing Specific Glycosylation for Biobetter MAbs

The following are the first glycosylation features to consider modifying when designing biobetter MAbs. These include changes to the N-glycans in the Cγ2 domain of the Fc region (see Figure 2) as well as modifications to Fab glycans if they exist in the therapeutic. In general, major changes can be achieved by switching cell lines, and smaller changes by modifying cell culture conditions. Note that the modification of one glycosylation feature will generally affect others, so when

glycoengineering the glycosylation should be viewed as a whole, rather than the sum of independent components.

Figure 2: The Fc region of an IgG showing the two N-glycans in the Cγ2 domain

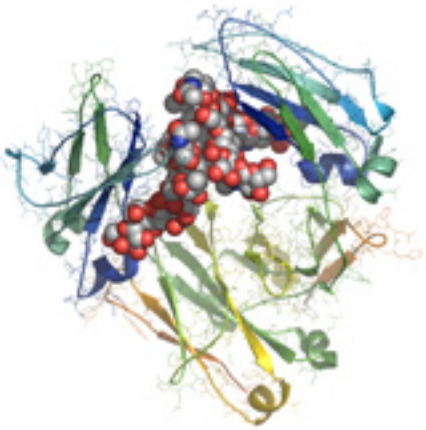

Figure 2: The Fc region of an IgG showing the two N-glycans in the Cγ2 domain
The antibody hinge region and pair of Fab arms are situated above this unit. The Fc glycans stabilise the configuration of the domain in which they sit and can confer functional diversity to the antibody. In particular, different monosaccharide residues on these glycans can alter the conformations of various parts of the peptide backbone that are involved in distinct Fc effector functions of the therapeutic.

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## Design Out Gal-α(1,3)-Gal

Gal-α(1,3)-Gal is an undesirable non-human disaccharide found on the glycans of some MAbs, particularly those expressed in mouse-derived cell lines. Immune reactions to Gal-α(1,3)-Gal are responsible for tissue rejection in xenotransplantation and the disaccharide has been shown to be directly recognised by NK cells (Lin et al, 2000; Inverardi et al 1997). All humans have IgG antibodies specific to the oligosaccharide Gal-α(1,3)-Gal, which is closely related to substances in the ABO blood group (Galili, 2005). Anti-Gal-α(1,3)-Gal IgE antibodies are found in high levels in some individuals who can show severe hypersensitivity reactions if treated with MAbs containing Gal-α(1,3)-Gal units on their glycans. Such anaphylactic reactions have been found in some patients treated with a form of the anti-cancer drug Cetuximab that was produced in a mouse cell and which contained high levels of Gal-α(1,3)-Gal in the glycans on Asn-88 of the Fab portion of the antibody heavy chain (Chung et al, 2008). Gal-α(1,3)-Gal can be designed out by switching to a non-mouse cell line, but it is advisable to still check for low level presence of the disaccharide after the switch. Eliminating this disaccharide should give a biobetter with an [S+ ] or [S+ E+] profile.

## Design Out NeuGc

The two main types of sialic acid residues found in MAbs produced in mammalian expression systems are N-acetyl-neuraminic acid (NeuAc) and N-glycolylneuraminic acid (NeuGc). NeuAc is the desired, normal human-type sialylation, while NeuGc is found in non-human glycoproteins and is considered an undesired, aberrant form of sialylation for therapeutic glycoproteins. Human cells are known to lack the enzyme CMP-NeuAc hydroxylase required for the synthesis of NeuGc, so any present will have become incorporated into a therapeutic via cell culture medium or from non-human cell lines producing the MAbs (Furukawa et al, 1988; Bardor et al, 2005). This can result in neutralisation by anti-NeuGC Abs (Nguyen et al, 2005). Controlling the ratio of NeuAc to NeuGc is important for biomanufacturers as (a) NeuGc may reduce drug efficacy through neutralisation by anti-NeuGc antibodies found in the serum of some patients and (b) NeuGc is thought to be linked to chronic inflammation in some individuals (Pader-Karavani et al, 2009). NeuGc can be designed out by switching to cell lines that produce just NeuAc and eliminating animal serum from cell culture medium. If sialylation is not important for your MAb, consider using an expression system that lacks sialyltransferases (e.g. plant or yeast). Elimination or reduction of NeuGc should move your drug towards an [E+] or possibly [S+ E+] profile.

## Design Out Fab Glycosylation

Some MAbs have potential N-glycosylation sites in their Fab region which may have full or partial glycan occupancy. These Fab glycans can reduce efficacy by interfering with antigen binding and increase product heterogeneity. In such cases, aim to reduce the Fab glycosylation via cell line switching. Note that for a [Prot0] biobetter you cannot remove glycosylation sites by modifying protein primary structure.

## Design Out Core Fc Fucosylation

ADCC activity of therapeutic IgG1 type Mabs can be greatly increased by reducing the levels of fucosylation (Shields et al, 2002; Shinkawa et al, 2003; Niwa et al, 2005). The mechanism is improved binding to FcγRIIIa of the low fucose MAb glycoforms (Okazaki et al, 2004; Yamane-Ohnuki et al, 2004). Engineered antibodies with low fucose are now being produced; their improved binding to FcγRIIIa allows them to evade the inhibitory effect on ADCC of plasma IgG (which is fucosylated and binds to FcγRIIIa with lower strength) (Iida et al, 2006; Natsume et al, 2008). Design out core Fc fucosylation of MAbs relying on ADCC by switching to a cell line producing low-fucose complex N-glycans (which now include commercially available galactosyltransferase knockout mammalian cells). This should give you an E+ biobetter. As an alternative, consider a high-titre non-mammalian expression system (e.g. producing oligomannose glycans) that could give you a drug with a C- profile. However, be aware of other effects (e.g. changes to pharmacokinetics).

## Modify β-Galactosylation Levels

If your MAb relies on CDC (complement-dependent cytotoxicity) for its mode of action then consider increasing the levels of terminal Gal-β(1,4) residues on the Fc glycans. The positive correlation between CDC activity and galactose has been found in the anti-CD20 antibody Rituximab where C1q binding to the Fc region increases with the percentage of galactosylation (Raju, 2008) and similar effects have been found in other CDC-dependent therapeutic antibodies (Jefferis, 2009; Kanda et al, 2007). Levels of terminal β-galactosylation can be modified by switching mammalian cell lines and changing cell culture conditions including dissolved oxygen (Kunkel et al, 1998).

## Conclusions

The use of glycoengineering to significantly improve the safety and efficacy profiles of existing therapeutic antibodies is now very straightforward. This is due to (a) advances in our knowledge of structure-function relationships between glycosylation and the in vivo bioactivity of antibodies, (b) the availability of new expression systems with useful glycosylation machinery and (c) improvements in glycoprofiling methods. In this article we have explained the advantages to making glycoengineered biobetter antibodies for both follow-on companies and the manufacturers of the original drugs. We have highlighted possible barriers to commercial exploitation of these glycosylation variant biobetters and proposed simple ways to overcome some of these barriers. Finally, we have outlined a strategy for intelligent design of such therapeutics based on the QbD (Quality by Design) paradigm with extensions to cope with the complexity of biopharmaceutical glycosylation. The next few years will see many changes in the commercial and regulatory landscapes for

therapeutic antibodies, and we believe that the development of glycovariant biobetters will play a positive part in shaping these for the advantage of both follow-on and originator companies. And most importantly, we look forward to affordable biobetter MAbs becoming available to the patients who need them.

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DR. CLAIRE MORGAN, BUSINESS DEVELOPMENT MANAGER AT LUDGER LTD.

Claire is responsible for liaising with biopharmaceutical clients prior to setup of glycoprofiling and method development projects, as well as general business development. Claire has a background in Immunology. She gained her PhD at The Royal London Hospital and followed that with post doctoral research positions at the Anthony Nolan Bone Marrow Trust (the Royal Free Hospital, London) then St. Bartholomew's Hospital, London. Before joining Ludger years Claire was in charge of Marketing and PR for the scientific events company EuroSciCon.

DR. DARYL FERNANDES, FOUNDER AND CEO OF LUDGER LTD, OXFORD, UK

Daryl has over 25 years' experience in developing and using glycoanalysis technology. He gained his doctorate at the Glycobiology Institute, University of Oxford in the 1980s, was a consultant on biopharmaceutical glycoprofiling to Monsanto and G.D. Searle, and helped spin out Oxford GlycoSciences (OGS) from the University. He joined OGS as Process Development Manager and then as the Head of Analytical Services. Daryl left OGS to set up Ludger in 1999. Ludger has laboratories at the Culham Science Centre near Oxford, UK and develops technology to measure and control biopharmaceutical glycosylation throughout the drug life-cycle. Daryl's research interests include the development of graph theoretic systems for analysis of complex glycosylation in biologics and high throughput glycoprofiling methods to support new drug development.