Article:
Turnbull, WB orcid.org/0000-0002-7352-0360, Imberty, A and Blixt, O (2019) Synthetic glycobiology. Interface Focus, 9 (2). 20190004. ISSN 2042-8898

https://doi.org/10.1098/rsfs.2019.0004

AUTHOR ACCEPTED MANUSCRIPT

Synthetic Glycobiology

W. Bruce Turnbull[1], Anne Imberty[2] and Ola Blixt[3]

[1]School of Chemistry and Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds, UK

[2]Univ. Grenoble Alpes, CNRS, CERMAV, 38000 Grenoble, France

[3]Department of Chemistry, University of Copenhagen, Frederiksberg, Denmark

ORCID: WBT, 0000-0002-7352-0360; AI, 0000-0001-6825-9527; OB, 0000-0003-4143-6276

Email: w.b.turnbull@leeds.ac.uk; anne.imberty@cermav.cnrs.fr; olablixt@chem.ku.dk

Abstract: Re-engineering carbohydrates and carbohydrate-binding proteins for novel applications was the topic of a Royal Society Theo Murphy meeting at the Kavli Royal Society Centre, Chichelely Hall in Buckinghamshire, UK, 8–9 October 2018.

All biological cells are covered with a forest-like coating of carbohydrates called the glycocalyx. Specialised carbohydrate-binding proteins, known as lectins, mediate interactions between the cell and the extra-cellular world. For example, carbohydrate-mediated cell adhesion enables diverse biological processes from fertilisation to inflammation. Protein-carbohydrate interactions also provide mechanisms for viruses, bacteria and their toxins to target and enter cells. In plants, the glycocalyx becomes the cell wall that provides the mechanical properties necessary to support the largest living organisms on land. However, glycobiology (i.e. the biology of carbohydrates) is a challenging field of study: carbohydrates are structurally much more complex than nucleic acids and proteins, and glycosylation is not a templated process that can be manipulated directly using classical molecular biology techniques. Many talented glycoscientists have risen to meet this challenge through the development of innovative chemical biology and biophysical methods to manipulate glycosylation. Advances in analytical and structural biology methods have also revealed a huge amount of detail of the glycocalyx and its interactions relevant to both health and disease.

Our rapidly growing understanding of glycobiology coincides with the rise of synthetic biology: the precise (re)design of genes and proteins to create novel biological systems that are revolutionising both medicine and materials science. Together, these advances present exciting opportunities for embedding the principles of glycoscience into synthetic biology, and the methodologies of synthetic biology into glycoscience. A new field of “Synthetic Glycobiology” is thus emerging, in which diverse cell surface processes may be controlled through reengineering the glycocalyx and the proteins with which it interacts. This topic was the subject of a recent Royal Society Theo Murphy meeting at the Kavli Royal Society Centre, Chichelely Hall, UK, 8–9 October 2018. A diverse panel of chemists, biochemists, biophysicists and cell biologists came together to review the state of the art in synthetic glycobiology and how it may have impact, both on our understanding of glycobiology and also in diagnostic and therapeutic applications. The articles and reviews in this issue of *Royal Society Interface Focus* have been contributed by scientists who spoke at the synthetic glycobiology meeting.

The lectins that interact with the glycocalyx comprise a widespread group of sugar-binding proteins that adopt a large variety of scaffolds. Hirabayashi reviews the different methods of lectin engineering by which the protein structure, stability or binding specificity can be finely tuned [1]. A novel concept of conferring sugar-binding properties to synthetic peptides or non-lectin proteins is also proposed.

Chemical modification provides another approach for re-engineering lectins. Wiltschi presents an alternative to the classical conjugation methods that are widely used for functionalization or immobilization of proteins [2]. Site-specific introduction of non-canonical amino acids carrying bioorthogonal reactive groups resulted in variants of three different lectins. These reactive handles were then used for conjugation of small molecules and for artificial oligomerization to create new architectures of lectins.

Engineering novel glycocalyxes requires access to complex oligosaccharides and biocatalysis is a tool of choice for their synthesis. André reviews the recent progress in engineering of glycoenzymes based on structural knowledge and computer-aided design [3]. Engineering now allows for improving the catalytic efficiency of the reactions, but also for extending the repertoire of synthetic oligosaccharides and glycoconjugates.

The complex cell surface polysaccharides that form biofilms, cell walls or capsules, often need to cross biological membranes as part of their life cycle of biosynthesis and degradation. Zimmer's review covers recent structural data on the various systems for translocating polysaccharides across membranes of prokaryotes and eukaryotes [4].

Several strategies have been developed for the creation of artificial glycocalyxes. A review by Godula highlights recent advances in glycocalyx precision engineering in living cells using synthetic nanoscale glycomaterials [5]. Synthetic glycoconjugates with tunable architectures and functionality hold a prominent position as a particularly powerful tool to approximate the complexity and nanoscale organization of the native glycocalyx and to decipher how the cellular boundary regulates the exchange of information between the cell and its surroundings.

Understanding their mechanical properties of artificial hyaluronan (HA)-rich matrices and surface coatings is essential for their applications in bioengineering research. An article by Richter describes a systematic analysis of the variations in HA thickness based on $Ca2^{+}$ concentrations and pH towards the design of stimuli-responsive surface coatings with tailored properties [6].

Another form of glycocalyx that can be re-engineered *in vitro* is the crystalline bacterial S-layer. Schäffer reviews the current knowledge of Gram-negative anaerobe *Tannerella forsythia* [7], which naturally forms S-layer glycocalyxes, and summarises its capacity to form Periodontitis, a polymicrobial biofilm-caused inflammatory disease affecting the tooth-supporting tissues. Specific attention towards protein O-glycosylation involving nonulosonic acids are discussed which the authors suggest to be a valuable target for the design of novel anti-infective strategies.

Lipid vesicles have been widely used as protocells, i.e. very simple models for cells. Römer reviews the recent research to integrate glycoconjugates into these lipid membrane mimics of natural cell surfaces. Interactions between such protocells and lectins can be used to recreate complex processes including endocytosis or even to form proto-tissues tethered to solid supports.

Lectin-glycocalyx interactions can also be dissected through chemical intervention in glycan biosynthesis as reported by Kohler [9]. Here specific inhibition of glycan biosynthesis leads to cells with differing glycocalyxes that allow assessment of the importance of different glycans for cholera

toxin binding. Furthermore, metabolic incorporation of photoaffinity groups on glycans enables crosslinking of the lectin to its glycoprotein ligands.

The composition of the glycocalyx also changes when cells become cancerous. Dwek presents a recent study that shows that the IgA1 in the serum of BCa patients has variations of their O-glycosylation repertoire and that these alterations could be useful as cancer biomarkers [10]. Their study utilized several validated commercial lectins with detailed analysis of serum IgA1 glycosylation in breast cancer and illustrates the potential utility of IgA1 glycosylation as a biomarker for breast cancer prognostication.

The comprehensive review by Tkac provides important information that prostate-specific antigen (PSA) glycoprofiling can also be implemented as a diagnostic prostate cancer biomarker in serum [11]. Specific glycoforms can be detected with already validated commercial lectins and provides significant advantage over PSA currently used in the clinics.

This edition of *Royal Society Interface Focus* captures a snapshot of the diverse science that underpins the development of synthetic glycobiology and points towards some potential applications in cancer diagnostics. Engineering novel lectins with higher specificity for target glycans has huge potential for establishing simple diagnostics that do not rely on expensive instrumentation which may not be routinely available in non-specialist clinics. Lectins that could bind selectively to cell surface biomarkers also have potential for targeted drug delivery. Such tools are now also being developed for quality control of glycosylated biopharmaceuticals. Glycan engineering also has potential to provide biopharmaceuticals with more uniform glycosylation patterns. Once that concept is extended to engineering the whole glycocalyx, not only is it a powerful tool for helping us to understand glycobiology, but it also provides opportunities to modulate and control biological processes that are dependent on the glycocalyx such as immune recognition and stem cell differentiation. Furthermore the ability to engineer novel polysaccharides and to organise them into supramolecular arrays offers the prospect of creating novel biomaterials with defined mechanical properties. Learning to manipulate and re-engineer the glycocalyx and its associated lectins provides us with an opportunity to move the glycoscience narrative beyond understanding glycobiology and into the realm of “synthetic glycobiology”. This nascent field has the potential to develop into an innovative and inherently creative strategic direction for glycoscience research.

Data accessibility. This article has no additional data.

Competing interests. We declare we have no competing interests.

Funding. We thank the Royal Society for funding the Synthetic Glycobiology Theo Murphy meeting.

References

1. Hirabayashi J, Arai R. 2019 Lectin engineering: the possible and the actual. Interface Focus 9, 20180068. (doi:10.1098/rsfs.2018.0068)

2. Tobola F, Sylvander E, Gafko C, Wiltschi B. 2019 ‘Clickable lectins’: Bioorthogonal reactive handles facilitate the directed conjugation of lectins in a modular fashion. Interface Focus 9, 20180072. (doi:10.1098/rsfs.2018.0072)

3. Benkoulouche M, Faure´ R, Remaud-Sime´on M, Moulis C, Andre´ I. 2019 Harnessing royalsocietypublishing.org/journal/rsfs Interface Focus 20190004 glycoenzyme engineering for synthesis of bioactive oligosaccharides. Interface Focus 9, 20180069. (doi:10.1098/rsfs.2018.0069)

4. Zimmer J. 2019 Structural features underlying recognition and translocation of extracellular

polysaccharides. Interface Focus 9, 20180060. (doi:10.1098/rsfs.2018.0060)

5. Purcell SC, Godula K. 2019 Synthetic glycoscapes: addressing the structural and functional complexity of the glycocalyx. Interface Focus 9, 20180080. (doi:10.1098/rsfs.2018.0080)

6. Chen X, Richter RP. 2019 Effect of calcium ions and pH on the morphology and mechanical properties of hyaluronan brushes. Interface Focus 9, 20180061. (doi:10.1098/rsfs.2018.0061)

7. Bloch S, Tomek MB, Friedrich V, Messner P, Schäffer C. 2019 Nonulosonic acids contribute to the pathogenicity of the oral bacterium Tannerella forsythia. Interface Focus 9, 20180064. (doi:10.1098/rsfs.2018.0064)

8. Omidvar R, Römer W. 2019 Glycan-decorated protocells - novel features for rebuilding cellular processes. Interface Focus 9, 20180084. (doi:10.1098/rsfs.2018.0084)

9. Sethi A, Wands AM, Mettlen M, Krishnamurthy S, Wu H, Kohler J. 2019 Cell type and receptor identity regulate cholera toxin subunit B (CTB) internalization. Interface Focus 9, 20180076. (doi:10.1098/rsfs.2018.0076)

10. Lomax-Browne HJ, Robertson C, Antonopoulos A, Leathem AJC, Haslam SM, Dell A, Dwek M. 2019 Serum IgA1 shows increased levels of a2,6 linked sialic acid in breast cancer. Interface Focus 9, 20180079. (doi:10.1098/rsfs.2018.0079)

11. Tkac J, Gajdosˇova´ V, Hroncˇekova´ Sˇ, Bertok T, Hires M, Jane E, Lorencova L, Kasak P. 2019 Prostatespecific antigen glycoprofiling as a diagnostics and prognostic biomarker of prostate cancer. Interface Focus 9, 20180077. (doi:10.1098/rsfs.2018.0077)