Glycans for the greater good

Jessica Lloyd (University of Manchester, UK)

Carbohydrates are ubiquitous in nature and present across all kingdoms of life – bacteria, fungi, viruses, yeast, plants, animals and humans. They are essential to many biological processes. However, due to their complexity and heterogeneous nature they are often neglected, sometimes referred to as the 'dark matter' of biology. Nevertheless, due to their extensive biological impact on health and disease, glycans and the field of glycobiology have become increasingly popular in recent years, giving rise to glycan-based drug development and therapeutics. Forecasting of communicable diseases predicts that we will see an increase in pandemics of humans and livestock due to global loss of biodiversity from changes to land use, intensification of agriculture, climate change and disruption of ecosystems. As such, the development of point-of-care devices to detect pathogens is vital to prevent the transmission of infectious disease, as we have seen with the COVID-19 pandemic. So, can glycans be exploited to detect COVID-19 and other infectious diseases? And is this technology sensitive and accurate? Here, I discuss the structure and function of glycans, the current glycan-based therapeutics and how glycan binding can be exploited for detection of infectious disease, like COVID-19.

What are glycans?

Carbohydrates, otherwise known as sugars or glycans, are essential biomolecules in nature. They exist as simple monosaccharides from which longer linear and branched structures can be formed – oligosaccharides or polysaccharides. Organisms incorporate glycans into biomaterials known as glycoconjugates, where sugar moieties are attached to proteins (glycoproteins) or lipids (glycolipids; see Figure 1b). Different organisms synthesize a unique complement of glycan structures and utilize different, and sometimes, unusual monosaccharides.

So, how are glycans biosynthesized? Well, unlike proteins, where the template-driven translation from DNA results in a hierarchical structure, this is not the case for glycans. The glycome is defined as the complete set of glycan structures expressed at a particular time and spatial position in specific cells, tissues or organisms. Enzymatic biosynthesis of glycans gives rise to heterogeneous and, often, complex structures.

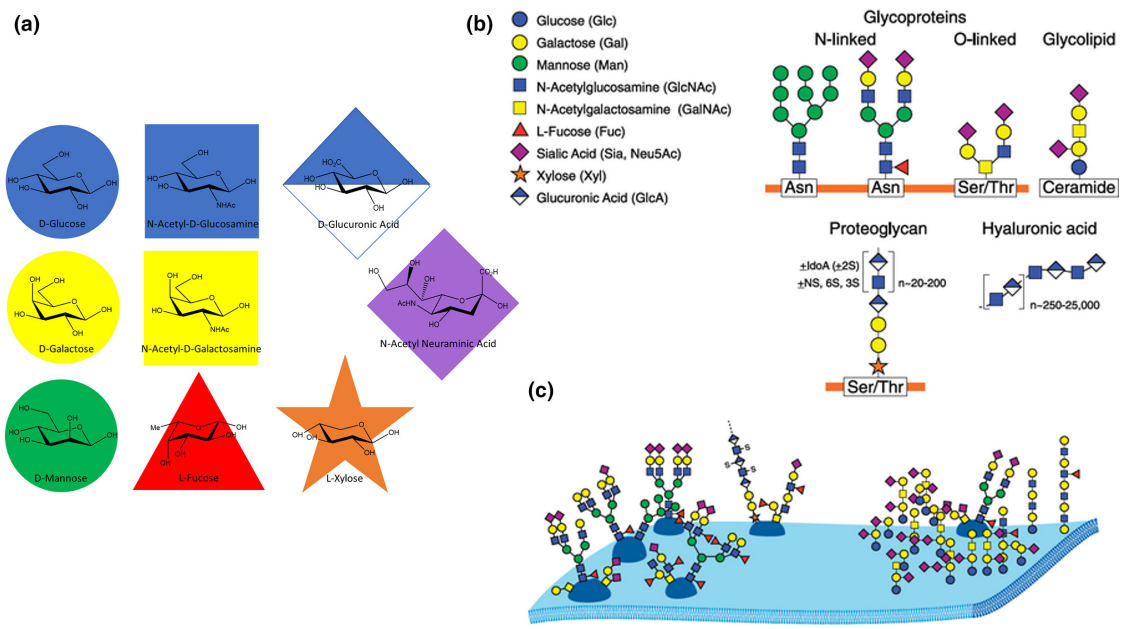

Figure 1. (a) Schematic and chemical representation of the nine most common monosaccharides found in the human glycome. (b) Major classes of human glycans. Representative asparagine (N-linked) and serine or threonine (*O*-linked) glycoprotein structures, a glycosphingolipid (ceramide-linked), a proteoglycan (most frequently *O*-linked) and hyaluronic acid (unlinked) are shown. (c) A schematic representation of glycans on a cell surface. (Revised from Schnaar R. L., 2016).

The human glycome is built from nine monosaccharide building blocks: D-glucose, D-galactose, D-mannose, L-xylose, *N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine, sialic acid (*N*-acetyl neuraminic acid) and D-glucuronic acid (see Figure 1a). These monosaccharides consist of either 5- or 6-carbon atoms arranged in a ring conformation, which has unique stereochemical configuration (such as epimers and α/β anomeric configuration), leading to different biochemical properties and occurrence in nature. This, along with changeable sequence, functional groups and linkage positions, means an impressive diversity of distinct structures can be created from just a few building blocks. Therefore, the structural diversity and potential chemical information of the glycome far exceeds that of the genome or proteome.

Why are glycans important in biology?

The biological functions of glycans span the spectrum from relatively subtle to crucial for development, growth, maintenance or survival of the organism that synthesizes them. And for many glycans, a function is not yet evident. The surrounding cells are a dense network of glycoconjugates, which bear resemblance to hair-like projections, termed the glycocalyx. The glycocalyx, which covers all eukaryotic cells and the polysaccharide coats of various microorganisms, represents a substantial physical barrier, often consisting of thousands of monosaccharide units. It is the identity and three-dimensional features of glycans of the glycocalyx that direct the biological effects.

So, what are the main biological effects of glycans? We can divide these functions into four broad categories: (1) structural and modulatory properties, (2) extrinsic (interspecies) recognition, (3) intrinsic (intraspecies)

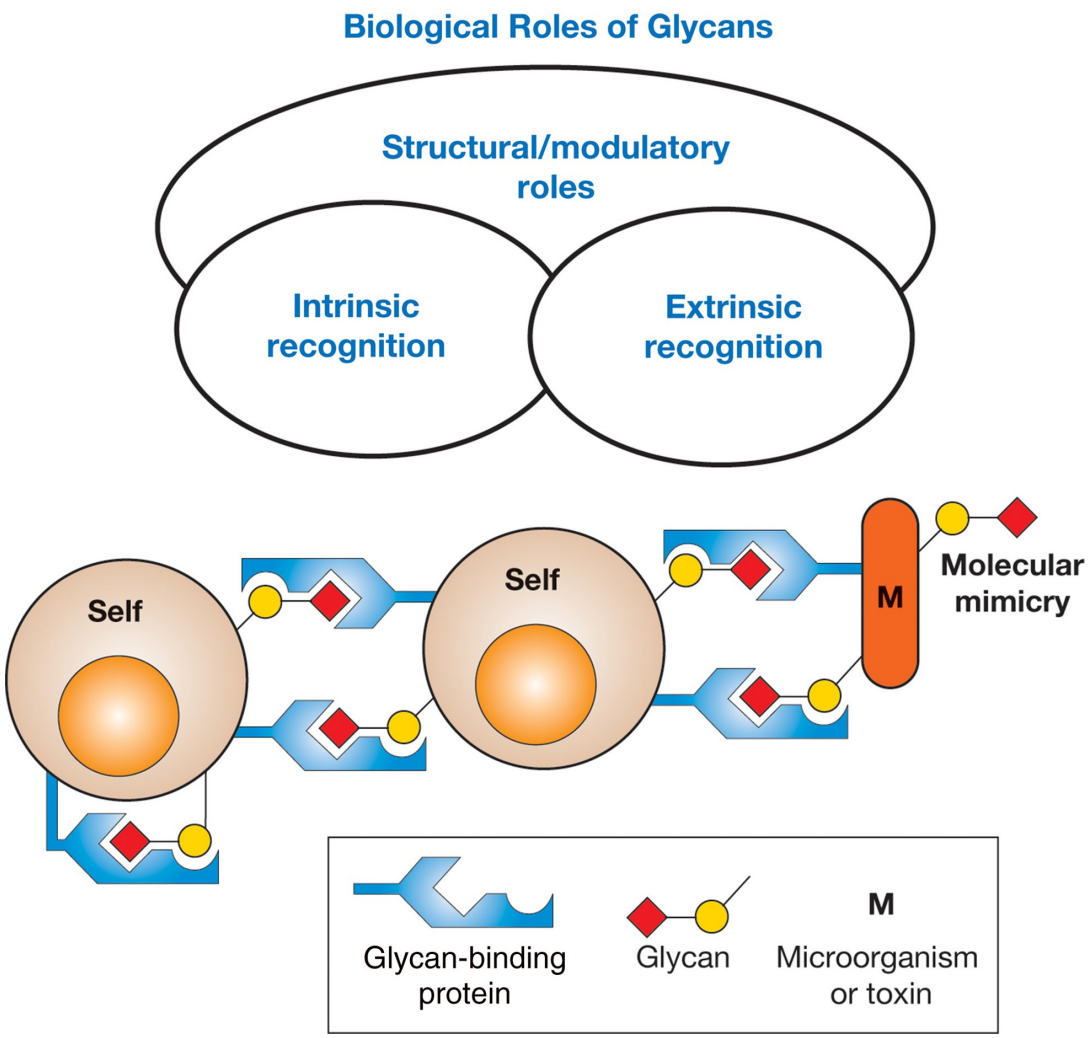

Figure 2. General classification of the role of glycans. Binding shown on the left of the central 'self' cell represents intrinsic recognition, and extrinsic recognition is represented by binding shown to the right of that cell (Taken from Varki, A. 2017).

Table 1. Biological function of glycans (Taken from Varki, A. 2017)

Structural and Modulatory Properties	Extrinsic (interspecies) recognition of glycans	Intrinsic (intraspecies) recognition of glycans	Molecular mimicry of host glycans
Physical structure	Bacterial, fungal and parasite adhesins	Intercellular signalling	Convergent evolution of host-like glycans
Water solubility of macromolecules	Viral agglutinins	Intercellular adhesion	Appropriation of host glycans
Diffusion Barriers	Bacterial and plant toxins	Triggering of endocytosis and phagocytosis	
Glycoprotein Folding	Pathogen-associated molecular patterns (PAMPs)	Self-associated molecular patterns	
Modulation of membrane receptor signalling	Antigen recognition, uptake and processing	Antigenic epitopes	
Nutritional Storage	Host decoys	Xeno-autoantigens	
Protection from immune recognition	Soluble host proteins that recognise pathogens	Intracellular glycoprotein folding and degradation	

recognition and (4) molecular mimicry of host (see Figure 2 for conceptual organization and Table 1 for examples). It is important to note that abnormalities in the glycan 'coat' of cells or misregulation of the glycoenzymes that synthesize them gives rise to a number of diseases and these can also act as biomarkers.

In essence, glycans are part of an elaborate communication system vital for cellular recognition, cell–cell interactions, protein transport, immune defence and more. They are traditionally more difficult to study than their protein counterparts but, due to their ubiquitous nature, glycans make useful drug targets and therapeutics. Advances in analytical techniques have driven progression in the field of glycobiology over the past decade or more giving rise to new hope for glycan-based technology.

Glycans: a therapeutic perspective

Despite their enormous potential as therapeutics, glycans remain a widely untapped drug class – overlooked as a consequence of being poorly understood and difficult to design. New insights into the structure and function of the glycome can now be applied to therapy development and could improve our ability to fine-tune immunological responses and inflammation, optimize the performance of therapeutic antibodies and boost immune responses to cancer. These examples illustrate the potential of the emerging field of 'glycomedicine'.

Small molecule drugs

The most widely known glycan therapeutic is heparin, which has been in clinical use as an anticoagulant since the early 1900s and is still one of the most highly prescribed drugs today. Heparin binds and activates antithrombin, a protease inhibitor of the coagulation cascade. Antithrombin activation leads to rapid inhibition of thrombin and factor Xa, shutting down the production of fibrin clots. Multiple variations of heparin have since been commercialized, such as Fondaparinux, a synthetic heparin.

However, the therapeutic potential of glycan-based drugs is vast and could be used for the treatment of fibrosis, infectious diseases, inflammatory diseases and cancer. Examples include zanamavir and oseltamivir phosphate (Tamiflu), which are both neuraminidase inhibitors which are used to prevent and treat influenza infections, and miglustat which is a treatment for type I Gaucher disease. Topiramate is an antiepilepsy drug that has been approved for use in weight loss treatments. Voglibose is an α-glucosidase inhibitor used for lowering blood glucose levels in diabetes patients. Miglitol and acarbose are oral drugs used to control diabetes mellitus type 2 (see Figure 3).

Cancer

Cancers often exhibit specific phenotypes that are reflected in the nature of their glycoconjugates. These changes in glycosylation drive metastatic properties, inhibition of apoptosis and resistance to chemotherapy. Glycoconjugates frequently overexpressed by cancer cells are the sialylated Lewis-type blood group antigens such as sialyl-Lewis a (SLe^a) and its isomer sialyl-Lewis x (SLe^x) as terminal epitopes of *O*-glycans, *N*-glycans and glycolipids. Over- or *de novo*-expression of tumour-associated O-linked antigen, STn, is also common due to alternations in the regulation of *O*-GalNAc glycosylation. The prevalence of these distinct carbohydrate epitopes holds great potential for developing targeted therapeutics, including selective drug delivery, precise inhibition

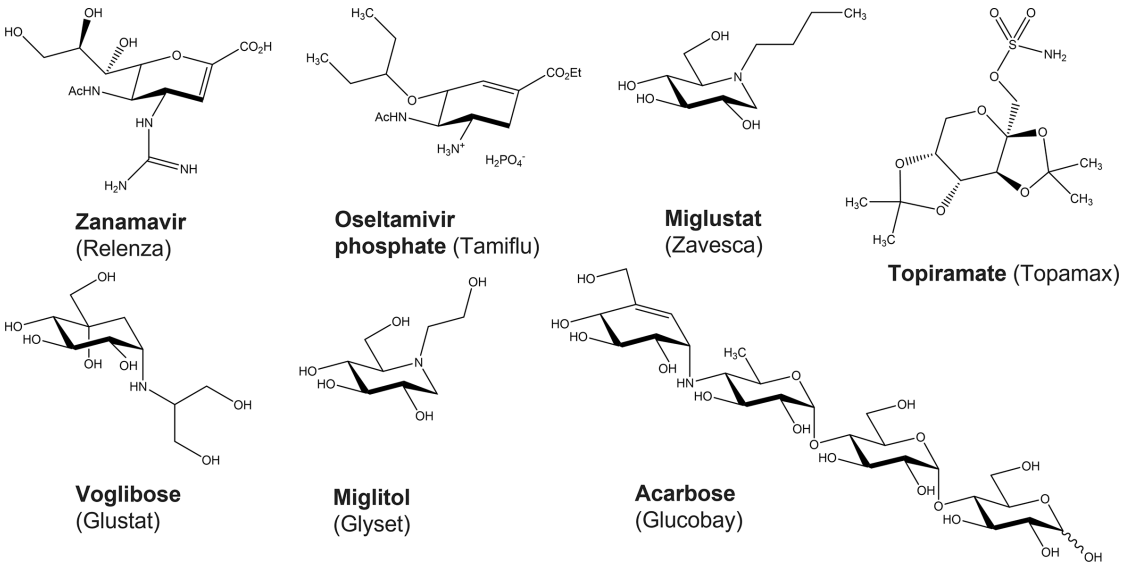

Figure 3. Examples of carbohydrate-based drugs (Revised from Seeberger, P. H. and Cummings, R. D., 2017, pp. 57).

of key oncogenic pathways and immunotherapy (see Figure 4).

Vaccination using short-chain cancer-associated glycans is a concept that is being explored in clinical trials. One such example is a pentavalent carbohydrate-based vaccine bearing several carbohydrate antigens, including STn (Figure 4A). Another emerging approach relates to exploiting chimeric antigen receptor (CAR) T cells engineered to target glycosylated moieties in cancer cells, promoting selective cell death (Figure 4B). There are also several monoclonal antibodies capable of targeting abnormally glycosylated cells, which promote antibody-dependent cellular cytotoxicity (ADCC) or block oncogenic receptors on the cell surface leading to cell death (Figure 4C).

What if we could selectively inhibit glycan–receptor interactions or abrogate glycan biosynthesis pathways? This is another appealing approach to glycan therapeutics and one currently being exploited in clinical trials for various types of cancer. One such example is a known fucosylation inhibitor, 2-fluorofucose, to inhibit the fucosyltransferase responsible for the addition of fucose to glycans, to consequently prevent tumour metastasis (Figure 4D). Nanotechnology also provides promising opportunities to target therapeutic small molecule drugs (chemotherapy, siRNA) to specific glycan structures, e.g., cancer oncofoetal phenotypes. These approaches may be pivotal for reducing off-target side effects of conventional chemotherapy.

Glycans and SARS-COV-2

As we are all aware, we are in the grips of a global pandemic, the likes of which many of us have not experienced in our lifetime and one which rivals the

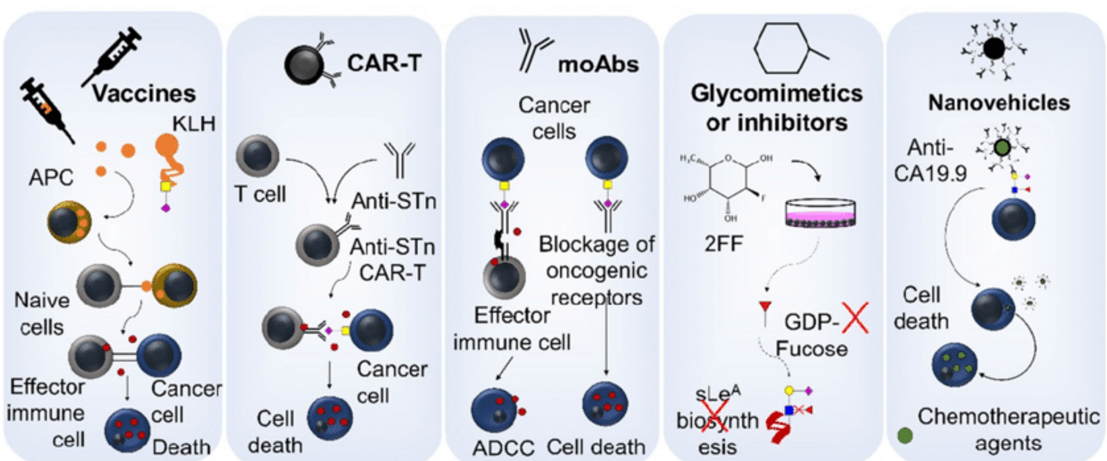

Figure 4. Glycan-based therapeutics to treat cancer (Taken from Fernandes, E., et al., 2020).

death toll of the Great Plagues of the 17th century. Since the official declaration of the respiratory disease COVID-19 as a pandemic during a WHO media briefing on 11 March 2020, our lives have drastically changed.

Paramount to our success in defeating the SARS-COV-2 virus is our ability to quickly and effectively test for positive cases to prevent spread of the virus and drive down the reproduction (R) value. Since the successful genome sequencing of SARS-COV-2, reverse transcription polymerase chain reaction (RT-PCR)-based diagnostics were rapidly developed, later followed by the introduction of an alternative diagnostic device, the lateral flow device (LFD). Traditional LFDs, such as the home pregnancy test, are immunoassay based. This means that antigens from a biological sample bind to immobilized antibodies on the test strip to produce a photochemical reaction. It's this colour change that indicates the result of the test, e.g., in the pregnancy test example, two red lines indicate a pregnancy. But could glycans offer an alternative?

The surface of enveloped viruses can be extensively glycosylated. As we have already seen, glycans can be used to prevent infection by influenza virus. Influenza virus has two major surface proteins, hemagglutinin and neuraminidase, and it is the hemagglutinin that initiates infection by binding to cell-surface sialic acids. Analysis of the 2009 swine influenza outbreak showed that porcine viral hemagglutinins, which normally bind α2,3′-linked sialic acids, switched to binding α2,6′-linked sialic acids found in human respiratory tracts. In short, glycans are important mediators of viral infection and SARS-COV-2 utilizes a similar mode of infection to the flu. All coronaviruses display homotrimers of spike glycoproteins on their surface. Sialic acid binding by the S1 spike protein subunits is crucial for coronavirus to engage host cells, while the S2 domain initiates viral fusion. Clearly, as with many other pathogens, glycans are vital for infection to occur and by exploiting this essential interaction, researchers have created a novel glycan-based method of detecting COVID-19.

The evidence shows that sialic acid binding is crucial in coronavirus infection and new technology has exploited the glycan 'anchoring' of coronavirus to develop a novel glycan-based LFD for point-of-access diagnostics. A glycan-based lateral flow detection system has been developed that can detect the spike glycoprotein from the SARS-COV-2 virus in under 30 minutes. The device consists of polymer-stabilized, multivalent gold nanoparticles bearing sialic acid derivatives which interact with the spike glycoprotein from SARS-COV-2 (see Figure 5). One of the benefits of utilizing glycans is the ability to detect intact viruses. For SARS-COV-2, viral RNA (e.g., from a positive RT-PCR result) is detected past the point where patients are no longer infectious, resulting in extended hospital stays and false-positive test results. The work provides proof that glycan binding can be exploited to create rapid point-of-care diagnostics in a format which requires no infrastructure and limited training. The cost-effectiveness of these inexpensive devices has been demonstrated by various

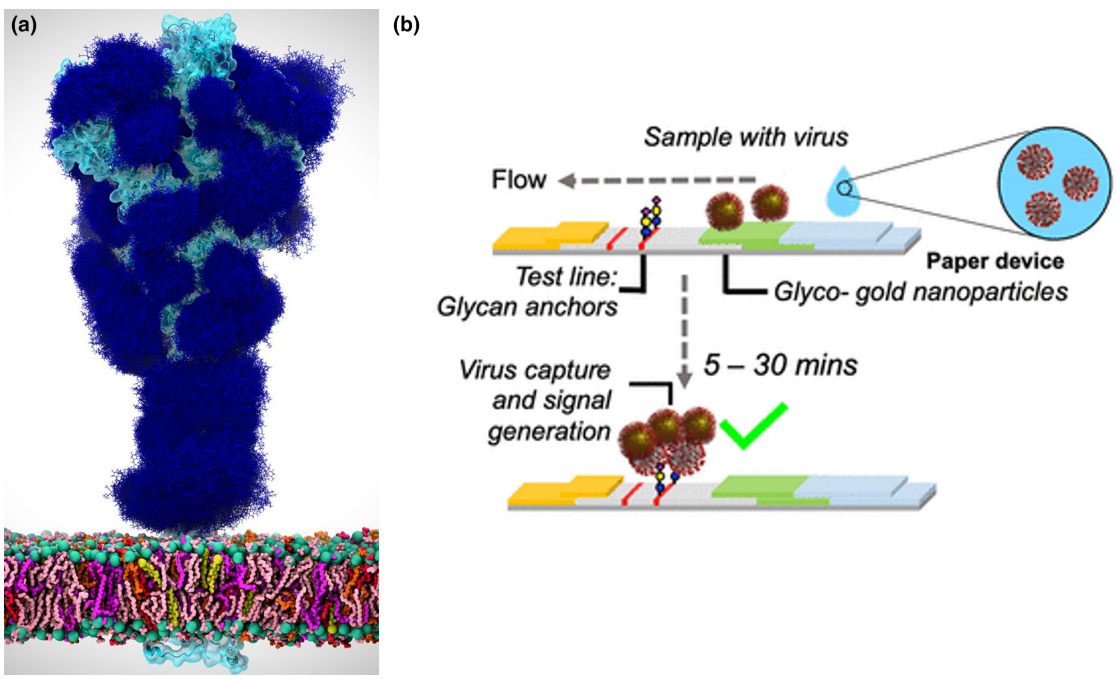

Figure 5. (a) Image of glycans (dark blue) of the SARS-COV-2 spike protein (cyan) interacting with the cell membrane. (b) Novel glycan-based lateral flow test (Revised from Baker, A. N., *et al,* 2020 and Casalino, L., *et al*., 2020).

studies of malaria rapid diagnostic tests and similar glycan-based devices are in development for the treatment of other zoonotic and parasitic infections including schistosomiasis.

To summarize, glycans present interesting opportunities to target, treat or diagnose disease; and their use is only expected to expand as research develops. Although we have only discussed implications for human health here, natural and synthetically derived carbohydrates are also used extensively in other industries. Sugars are increasingly making their way into areas of biotechnology, the food industry and material science for the development of smart materials, e.g., to promote wound repair and tissue engineering in biomedical devices. Research is also uncovering the impact of carbohydrates on human nutrition, especially with respect to the hugely complex gut microbiome and infant nutrition, including the bioactive effects of human milk oligosaccharides. In short, the possibilities are endless and we're only just starting to glimpse the true potential of sugar. ■

Further reading

- TEDx Talks, Carolyn Bertozzi: www.youtube.com/watch?v=5HeZaYtfDf8
- Varki, A. (2017) Biological roles of glycans. *Glycobiology* **27**, 3–49. DOI: 10.1093/glycob/cww086
- Dedola, S., Rugen, M.D., Young, R.J., and Field, R.A. (2020) Revisiting the language of glycoscience: readers, writers and erasers in carbohydrate biochemistry. *Chembiochem* **21**, 423–427. DOI: 10.1002/cbic.201900377
- Schnaar, R.L., (2016) Glycobiology simplified: diverse roles of glycan recognition in inflammation. *J. Leukoc. Biol*. **99**, 825–838. DOI: 10.1189/jlb.3RI0116-021R
- Paderi, J., Prestwich, G. D., Panitch, A. et al. (2018) Glycan therapeutics: resurrecting an almost pharma-forgotten drug class. *Adv. Therap*. **1**, 1800082. DOI: 10.1002/adtp.201800082
- Fernandes, E., Sores, J., Cotton, S., et al. (2020) Esophageal, gastric and colorectal cancers: Looking beyond classical serological biomarkers towards glycoproteomics-assisted precision oncology. *Theranostics* **10**, 4903–4928. DOI: 10.7150/thno.42480
- Baker, A.N., Richards, S.-J., Guy, C.S. et al. (2020) The SARS-COV-2 spike protein binds sialic acids and enables rapid detection in a lateral flow point of care diagnostic device. *ACS Cent. Sci*. **6**, 2046–2052. DOI: 10.1021/acscentsci.0c00855
- Casalino, L., Gaieb, Z., Goldsmith, J. . et al. (2020) Beyond shielding: The roles of glycans in the SARS-CoV-2 spike protein. *ACS Cent. Sci*. **6**, 1722–1734. DOI: 10.1021/acscentsci.0c01056
- Seeberger, P. H. and Cummings, R. D. (2017). Glycans in Biotechnology and the Pharmaceutical Industry. In: Varki, A., Cummings, R. D., Esko, J. D., et al., *Essentials of Glycobiology*, 3rd edition. [Online]. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp. 57.

Jessica Lloyd is a PhD candidate in Prof Rob Field's group at the University of Manchester (Manchester Institute of Biotechnology). The group embraces the development and exploitation of chemical principles and tools to address questions in molecular science, in the broadest sense, with a carbohydrate theme running throughout. Projects range from bacterial adhesion and infection, through plant and algal polysaccharide biochemistry and enzymology, to the development of small molecule inhibitor approaches to understand carbohydrate metabolism. Jess' PhD work focuses on the bioactive properties of the non-lactose carbohydrate component of human milk and how analogues of these compounds can be exploited to benefit colonization of commensal bacteria in the gut and affect pathogen adhesion. Email: Jessica.lloyd-3@postgrad.manchester.ac.uk. Twitter: https://twitter.com/JessLlo32291290