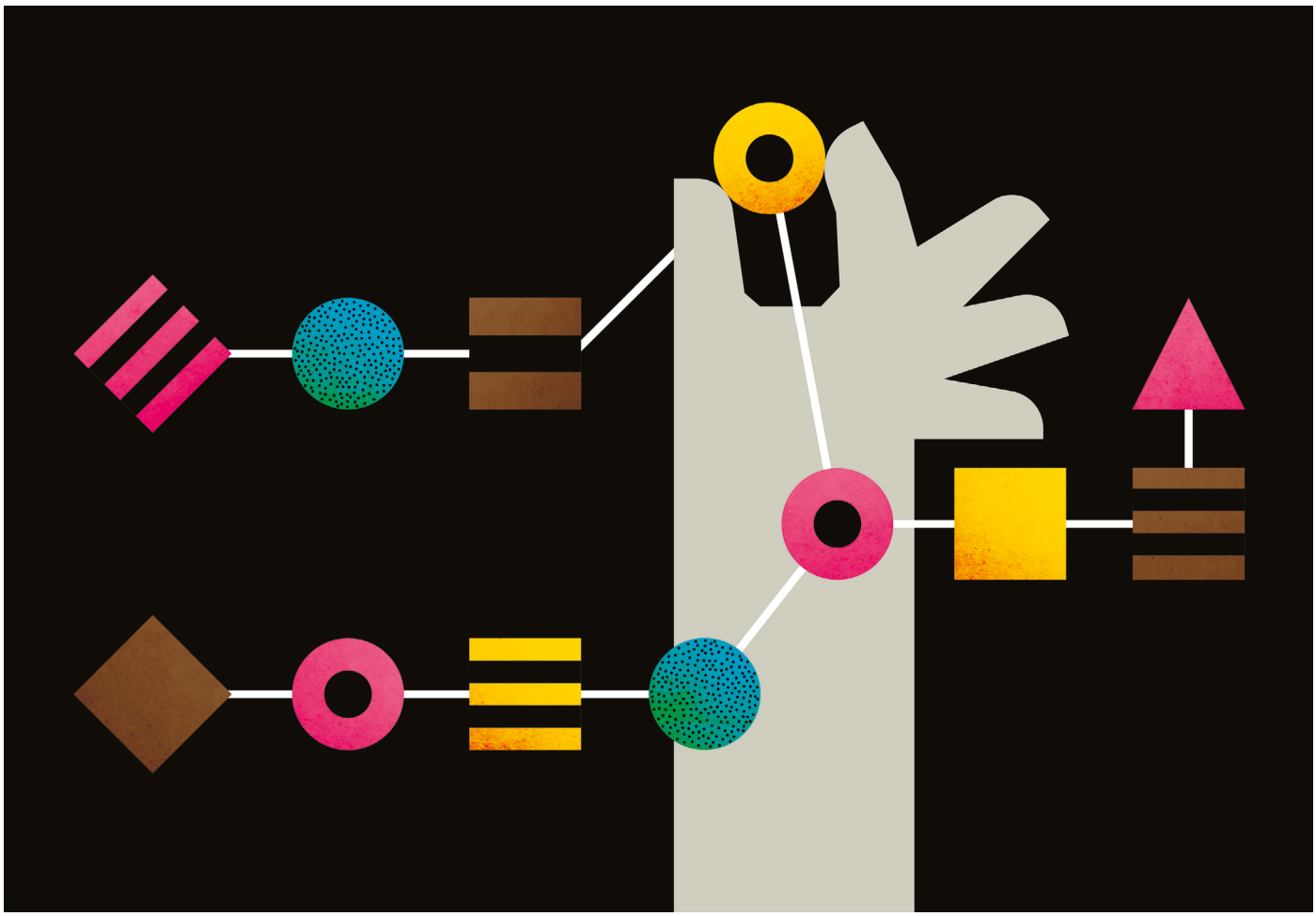

ILLUSTRATION BY PROJECT TWINS

AN AFFINITY FOR SWEETS

Antibodies and other reagents for sugar biomolecules have lagged behind those for proteins and nucleic acids, but the field is catching up. **By Amber Dance**

Sugars are everywhere in biology. They decorate cells, proteins and lipids. According to a surprise finding, published in June, they even adorn RNAs[1]. Yet these carbohydrates, also called glycans, have garnered little attention in recent decades.

"Glycans form an essential biological language, the glycome," says Mark von Itzstein, director of the Institute for Glycomics at Griffith University in Brisbane, Australia. They facilitate cell–cell communication and immune responses; are altered in cancer cells; and are exploited by pathogens to get a foothold in host cells. It is slight differences in the glycans on red blood cells that define human blood as being type A, B, AB or O. And it is glycans that both shield SARS-CoV-2 from the immune system and shape the spike protein as the coronavirus attempts to enter our cells[2]. "The spike needs the glycan shield to open," says Elisa Fadda, a computational glycoscientist at Maynooth University in Ireland and a member of the team that made the discovery.

Most biologists, however, have focused on DNA, RNA and proteins. Part of the reason is that, compared with those neatly encoded biomolecules, glycans are incredibly complex. A small number of simple sugars can link up in a variety of ways, producing variably branching structures with a final form that depends on the presence or absence of dozens of sugar-adding and -removing enzymes. "The sheer complexity, it's mind-boggling," says Ajit Varki, a physician and glycobiologist at the University of California, San Diego (UCSD).

But there's also a dearth of tools with which to study glycans, says Douglas Sheeley, co-leader of the US National Institutes of Health (NIH) Glycoscience programme in Bethesda, Maryland. The programme aims to build up the toolbox and has, for example, funded its own answer to biomolecule databases such as GenBank, namely GlyGen.org. Researchers can enter a favourite protein on the website and discover what glycans are known to decorate it, or start with a specific glycan structure to find proteins that carry it.

In the laboratory, glycobiologists would like to be able to perform the same assays, western blots, cell stainings and other experiments

that antibodies enable for protein studies. But although a handful of glycan-binding 'affinity reagents' do exist, most are suboptimal. Some scientists are attempting to improve them; others are seeking fresh options in environments such as the microbiome of the human mouth or the bloodstream of sea lampreys. These new reagents promise to make glycobiology experiments straightforward even for non-specialists.

Upgrading nature

Historically, the leading affinity reagents for glycans have been lectins, natural carbohydrate-binding proteins from diverse organisms. But most researchers in the field have a love–hate relationship with lectins, says glycobiologist Lance Wells at the University of Georgia in Athens. Their prominence comes not so much from superior performance as from the fact that, as he puts it, "they exist" and are easily obtainable. For example, Vector Laboratories in Burlingame, California, sells lectin-based screening kits to identify diverse pieces of glycans.

The downside is that lectins are not particularly specific for individual glycans. Their interface with glycan targets is shallow, less lock-and-key than cup-and-saucer. "Lectins are great for an initial screen, to say, 'OK, do you have glycoproteins present?'" says Iain Wilson, a glycobiologist at the University of Natural Resources and Life Sciences in Vienna. But "the absence of binding, or the presence of binding, doesn't prove the structure".

One exciting approach, says von Itzstein, is to engineer lectins so they fit desired targets better. This was the route that scientists at New England BioLabs (NEB) in Ipswich, Massachusetts, took to find a lectin that would bind to all glycans that attach to asparagine residues in a protein (called *N*-glycans), but not to ones that link to serine and threonine residues (*O*-glycans). The researchers started with Fbs1, a protein normally involved in degrading malformed glycoproteins, and used random mutagenesis and screening to create the reagent that they were after[3]. The team is happy to share a research-grade version of it, and a commercial-grade version should be available in early 2022, says co-inventor Chris Taron, scientific director of protein expression and modification research at the company. The latter will be part of NEB's 'Enzymes for Innovation' collection, products from which typically cost US$70–$300.

Lectenz Bio in Athens, Georgia, is another company using protein engineering to convert highly specific enzymes that act on carbohydrates into refined affinity reagents.

Riches from the sea

It is possible to generate conventional antibodies for use as affinity reagents to glycans, but they rarely bind tightly to their target antigens and can cross-react with other molecules. That's led to a belief that generating antibodies just doesn't work well for carbohydrates, but that's a myth, says glycoimmunologist Rick Cummings, co-director of the Harvard Medical School Center for Glycoscience in Boston, Massachusetts. If you look in the right place, he says, you can find an abundance of specific, high-affinity antibodies to all kinds of carbohydrates.

Around 2000, immunologist Max Cooper at Emory University in Atlanta, Georgia, found one such place: jawless sea lampreys (*Petromyzon marinus*), snakelike creatures with toothy, round mouths. Cooper was comparing their immune systems with those of jawed creatures, and discovered that lamprey antibodies take a different form, with a crescent-shaped binding site[4]. This turns out to be more rigid than the binding site in mammalian antibodies, so it's less likely to flex to fit antigens that are similar to, but not the same as, the intended targets – a characteristic that confers high specificity. "It can distinguish many glycans that, to an antibody, look the same," says Thomas Boehm, an evolutionary immunologist at the Max Planck Institute of Immunobiology and Epigenetics in Freiburg, Germany.

> **"It can distinguish many glycans that, to an antibody, look the same."**

"You can make antibodies with specificities that have never been seen before," says Cooper. Cummings, a collaborator on the project, was stunned to identify lamprey antibodies for glycans he didn't even know existed.

In Atlanta, Cooper co-founded a company, NovAb, to produce lamprey antibodies for targets that are difficult to tackle using conventional antibodies. One of its offerings, says co-founder and chief executive Ed Cannon, will be a sort of 'antibody Velcro' with multiple copies of the binding domain for a given target, to ensure firm, irreversible binding.

Cummings, meanwhile, has created a diverse array of antibodies, all encoded in yeast libraries, by immunizing lampreys with complex immunogens, such as cells or human milk. His centre can work with collaborators to investigate any given glycan using a microarray, to see whether anything in the library will bind to it. If not, the centre can immunize lampreys to generate new antibodies.

To make the lamprey molecules more convenient for laboratory and, eventually, therapeutic applications, Cooper and Cummings designed hybrid molecules that meld the 'base' of mammalian antibodies (the vertical section of their Y-shaped structure) with lamprey binding domains[5]. These 'smart anti-glycan reagents' can be detected using the same secondary antibodies that researchers already use to identify more-conventional antibodies.

The reagents right under your nose

Most glycan subunits common in humans have backbones with six carbon atoms, but another class of sugars, called sialic acids, has nine. "If you think glycans are scary to people, sialic acid is scary to glycobiologists, because [these acids are] so much more complex," says Varki. Sialic acids – of which there are more than 50 types – often cap glycan branches. They're negatively charged, which allows glycoproteins that contain them to slide past each other and create slippery surfaces such as the interior of the mouth; without them, our mouths would be perpetually dry, says Stefan Ruhl, an oral biologist at the University at Buffalo in New York.

Sialic-acid-tipped glycoproteins also make great handles that enable bacteria to grab on to our teeth, providing a foothold for them to form cavities, says Ruhl, who used to be a practising dentist. He reasoned that those microbes must have evolved to produce excellent sialic-acid-binding molecules, and he has discovered some in streptococci bacteria, the details of which he hopes to publish soon. Reagents based on these molecules will be freely available from Ruhl's lab as part of the NIH Glycoscience programme.

Varki's team also used microbes as a source of sialic-acid affinity reagents. The team has assembled a panel of nine reagents, including bacterial lectins and toxins; viral proteins and enzymes; a plant lectin and a chicken antibody[6]. The set includes a general detector for mammalian sialic acids, as well as several for more-specific structures or linkages common in mammals. For tissue staining, the panel's reagents are easier to use than some lectins that require special buffer conditions, says pathologist Nissi Varki, director of the Histopathology/Mouse Phenotyping Division of UCSD's Comparative Phenotyping Core. Ajit Varki, her husband, has used them in unpublished work to study the role of sialic acids in a mouse model of the inflammatory disease colitis.

With glycans being so little understood, there's plenty to learn. "We are like kids in a candy shop," says Ajit Varki. "The bad part is, there aren't many kids in the shop." The new reagents should make it easier for other biologists to get a taste.

Amber Dance is a freelance science journalist in the Los Angeles, California, area.

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