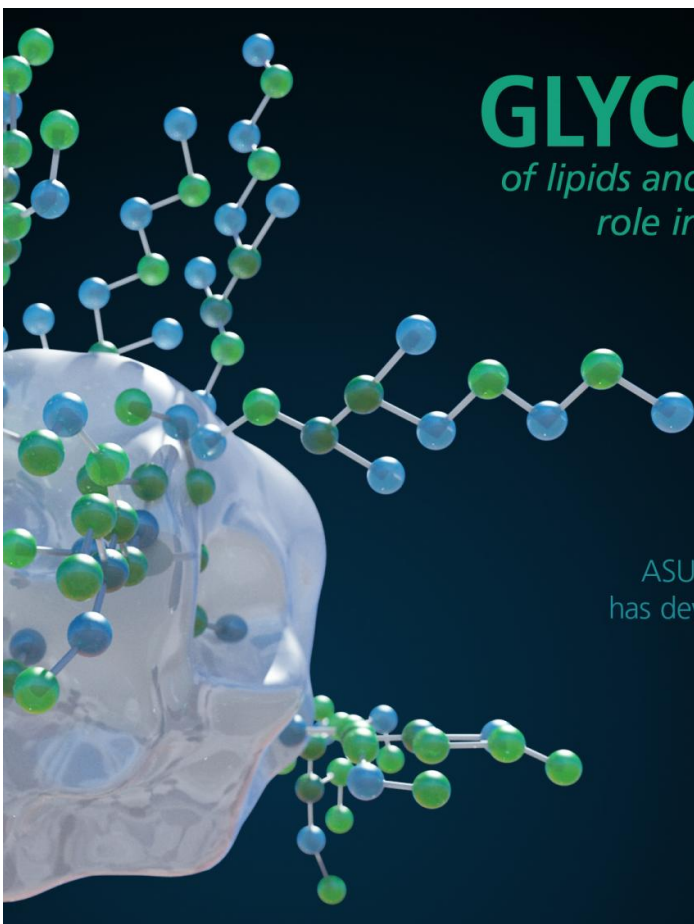


Novel technique helps ID elusive molecules

December 21 2016, by Richard Harth



GLYCOSYLATION
of lipids and proteins plays an essential role in these biological processes:

- protein folding
- cell adhesion
- signal transduction
- pathogen recognition
- immune responses

ASU Biodesign researcher Stuart Lindsay has developed a new technique to identify these glycosylating carbohydrates at the single-molecule level.

ASU Biodesign Institute
Arizona State University

Carbohydrates can attach themselves to other biomolecules, modifying their activities in vital ways. Credit: Jason Drees for The Biodesign Institute at Arizona State University

Among the most important molecules in the living world are sugars or

carbohydrates, which play a vital role in life processes. Sugars provide the main source of fuel for the body, protect muscles from damage and contribute to the immune response. They also act as the brain's key metabolite (in the form of glucose), powering basic function and influencing memory and mood. Dysregulation of carbohydrates can lead to a raft of major illnesses, including cancer.

Now, Stuart Lindsay, a researcher at Arizona State University's Biodesign Institute, has devised a clever means of identifying these molecules quickly and accurately. The results of his research, which appear in the current issue of *Nature Communications*, pave the way for a new generation of analytic tools capable of ferreting out carbohydrates for diagnosis and eventual treatment of many diseases.

Essential and mysterious

Although they represent one of the four fundamental building blocks of life, (along with proteins, lipids and nucleic acids), carbohydrates have received much less scrutiny from researchers, until now. One reason for this is that carbohydrates can occur in an astonishing variety of chemically similar forms, which are tricky to identify in biological samples.

Glycobiology—the study of sugars— is now seen as a critical area for improving human health as well as advancing materials science and energy research, as the National Academy of Sciences outlines in a roadmap for the next decade of serious inquiry.

"Glycobiology has been, relatively speaking, in a primitive state compared to proteomics (the study of proteins) and genomics (the study of DNA) for the simple reason that the structural information is not readily available," Lindsay says. Carbohydrates often form larger structures of great variety and complexity. "If you count up the number

of possible ways you can put six [sugar molecules](#) together, it turns out to be about a trillion."

Techniques like mass spectroscopy are limited when it comes to identifying carbohydrates, as multiple chemical variants or isomers of a given sugar have identical molecular weight. Another technique, known as nuclear magnetic resonance or NMR has sometimes been used for characterization, but the technique's accuracy and efficiency are limited, requiring exceptional sample purity and a sizable amount of carbohydrate for analysis. The situation has left researchers largely in the dark.

From noise comes clarity

The approach described in the new study involves delicately suspending a carbohydrate molecule between a pair of electrodes. When electricity is passed through the molecule, it releases a burst of current spikes, which can be measured and analyzed in order to identify the given carbohydrate. In the present study, a broad range of [carbohydrate molecules](#) are read using the new method, which Lindsay has earlier applied to rapid DNA sequencing and the identification of [amino acids](#).

On first inspection, the current spikes produced when current passes through the molecule appear to be random noise, yet these bursts of activity actually contain vital information pertaining to the unique characteristics of each molecule. The current fluctuations seen in the experiments represent instants of high and low conductivity as the molecule quivers within the gap between electrodes and its geometry is subtly altered. Lindsay and his team were able to decode the language of this electrical activity over many iterations, identifying 10 of the most common carbohydrate molecules with an accuracy of better than 90 percent.

Building on the technique, which Lindsay calls "sequencing by recognition," will require that linear chains of carbohydrate molecules be fed through a very narrow aperture, known as a nanopore. As each molecule pokes its head through the tiny opening, current passes through it and it is sequentially read and identified. The basics of this technique can be seen in the accompanying video. (While the method shown is used to sequence amino acids rather than carbohydrate molecules, the basic principle is the same.)

A molecule of many faces

Sugar molecules are deceptively simple in their construction, consisting of just oxygen, hydrogen and carbon. When a single sugar molecule is present, it is known as a monosaccharide. Simple carbohydrates contain just one or two sugars, such as fructose, which is found in fruits or galactose, present in milk. One of the most critical monosaccharides is glucose ($C_6H_{12}O_6$), a molecule created through photosynthesis and central for cellular respiration.

Glucose is transported through blood vessels to the brain, crossing the blood-brain barrier and producing adenosine triphosphate (ATP), the primary form of chemical energy within cells. When neurons transmit their electrical signals, local capillaries dilate, delivering more blood, along with extra glucose- and oxygen-laden blood. Making up just 2 percent of the body's weight, the brain nevertheless consumes about 20 percent of the body's overall energy budget, making the brain an exceptionally glucose-rich environment.

Other sugars are more complex. Those containing three or more sugars are known as polysaccharides. These branched chains of carbohydrates form starches and glycogen for storing energy as well as structural polysaccharides like cellulose and chitin.

Molecular managers

All living cells are adorned with a layer of sugars (also known as glycans), providing cell- and tissue-specific identity. While these glycans are important in and of themselves, their real power in living systems comes from the ways in which they interact with other biological molecules. Through a process known as glycosylation, glycans attach to and augment the function of proteins, lipids and nucleic acids in ways researchers have only begun to probe.

Glycans are found across the web of life, in archaea, bacteria and eukaryotic organisms. Their wide spectrum of activity is crucial for an organism's development, growth, functioning and survival. Among their numerous functions in cells, they contribute to physical and structural integrity, extracellular matrix formation, signal transduction, protein folding and information exchange between cells and pathogens. They are also frequent targets for the binding of microbial toxins.

In the human immune system, pathogen-specific glycan sequences found on bacteria, viruses, and fungi, trigger an [immune response](#). Glycans are important for cell adhesion and movement, guiding [white blood cells](#) to the site of injury or infection, for example. Glycans are therefore highly promising candidates for therapeutic drug discovery and as diagnostic markers of disease.

Agents of sickness and health

Over 50 percent of all human proteins undergo glycosylation, while aberrations in glycosylation are linked with the transformation of cells from a healthy to a cancerous state. Because recognition sequencing can finely discriminate between individual sugars at the single molecule level, the technique holds the promise of precise detection of cancer

biomarkers—early warning beacons of disease traceable through proteins found in the blood.

Aberrant glycan molecules have been clinically implicated in a range of deadly cancers including ovarian, prostate, pancreatic, liver, multiple myeloma, breast, lung, gastric, thyroid and colorectal. It is believed these abnormal glycans act to facilitate metastasis. The common presence of aberrant glycans in tumor cells makes them good candidates for disease biomarkers, if they can be accurately identified.

Additionally, glycans are involved in the discrimination of self vs non-self, which may play an important role in the pathology of various autoimmune diseases. Tumor cells, for example, produce abnormal glycans, which are recognized by a particular receptor found on the body's natural killer cells— a critical component in the innate immune system.

Lindsay is optimistic about scientific advances now opening a window onto these vital molecules. Nevertheless, the challenges for recognition sequencing can be formidable, particularly in terms of the extreme precision required in manufacturing electrode-nanopore devices. The next phase of the project will be to combine the sequencing technology for individual [carbohydrate](#) molecules with a nanopore, allowing linear threads of sugar molecules to be read out accurately.

Provided by Arizona State University

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