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A Sweet View of Biology in Action

Howard Hughes Medical Institute researchers have used some clever chemistry to visualize cells' ever-changing "sugar fingerprints." By chemically modifying glycans—sugar molecules that stipple cells' outer surfaces—the researchers can use fluorescence-based imaging to generate videos of biological processes that depend on those molecules.

Glycans are essential in many processes, including embryonic development, inflammation, and the progression of cancer and other diseases. New ways of imaging these molecules are creating a vibrant new biological frontier, says Carolyn R. Bertozzi, a Howard Hughes Medical Institute investigator at the University of California, Berkeley.

Bertozzi and her colleagues are developing new tools that they are now using to monitor glycans in living cells and organisms. With these tools, scientists can track changes in the number, type, and location of glycans, providing insight into a cell's physiological status. In the future, it may even be possible to determine an organism's complete "glycome," providing a valuable complement to its genome and proteome.

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- Carolyn R. Bertozzi

In a research article published the week of December 15, 2008, in *Proceedings of the National Academy of Sciences*, Bertozzi and Berkeley chemist Scott T. Laughlin unveil their new techniques, which they have used to track changes in glycans over time. This allows researchers to create videos of dynamic biological processes. "A picture gives you a snapshot in time," she says. "But a video shows you an object in action. It's like the difference between seeing a picture of a bird flying and a video of a bird flying. If a picture is worth a thousand words, a video is worth a million."

In the past, biologists labeled glycans using antibodies and proteins known as lectins, but both techniques have drawbacks. Antibodies tend not to bond

only weakly to glycans and do not pass easily through tissues. Lectins have both of these flaws and also are toxic, meaning that cells were often killed while being studied.

Researchers using these older approaches to study glycans got an incomplete picture of their function because glycans were not being viewed in their natural environment or in their normal biological context. “If you take an animal out of its habitat, its behavior can change, which limits what you can learn,” says Bertozzi. “The same goes for molecules. If you want to learn how a molecule behaves in its natural environment, you have to study it in a cell, and ideally in a living organism.”

Bertozzi's lab, which has been a leader in the multidisciplinary field of chemical biology, has developed a new way to label glycans by taking advantage of what she calls “bioorthogonality.” Cells are “fed” chemically modified sugars, which are taken up and incorporated into glycans. The modifications do not affect the sugars' biological function. The modifications include the addition of chemical functional groups that are inert with respect to the cells, a property termed “bioorthogonal.”

After the glycans are incorporated into the cell, researchers then expose that cell to a second compound—an imaging probe adorned with its own bioorthogonal functional group designed to react with the modified glycan. After that reaction takes place, either on cells or in living organisms, the glycan becomes visible using imaging techniques.

Bertozzi said this step is roughly akin to giving some of the people in a stadium pagers that respond to a particular signal. When the signal is broadcast into the stadium, the pagers would light up.

Bertozzi's lab has been using the technique to study changes in glycosylation in cancer cells and in cells that are differentiating. She and her colleagues also have developed a way to observe glycans in developing zebrafish embryos, which are transparent. They observed increases in glycan biosynthesis in the jaw region, pectoral fins, and olfactory organs 60 hours after fertilization, suggesting that the glycans can serve as markers of certain developmental stages.

Bertozzi's ultimate goal is to develop the technique for use in humans. “One of my long-term aspirations has been to develop tools that can be used in clinical diagnosis, and especially in cancer detection.” One other possibility is to monitor the glycosylation of cells removed during biopsies. A more ambitious goal is to inject the modified sugars and imaging probes directly into humans.

“We're not ready to start sprinkling these sugars on your Wheaties quite yet,” she says. Her lab continues to study the stability and metabolic processing of glycan components, subjects that “are of intense interest to the

pharmaceutical industry.” But she notes that modified sugars are already used in humans prior to positron emission tomography scans. The idea of using bioorthogonal molecules to monitor glycosylation “is not that far-fetched,” she says.

The use of glycans in imaging biological processes is still in the early stages, but Bertozzi and her colleagues are hoping to broaden the range of molecules they can target. They also are looking for chemical modifications that are orthogonal to existing modifications, so that different kinds of glycans can be monitored simultaneously. “We have barely scratched the surface of this field,” she says.