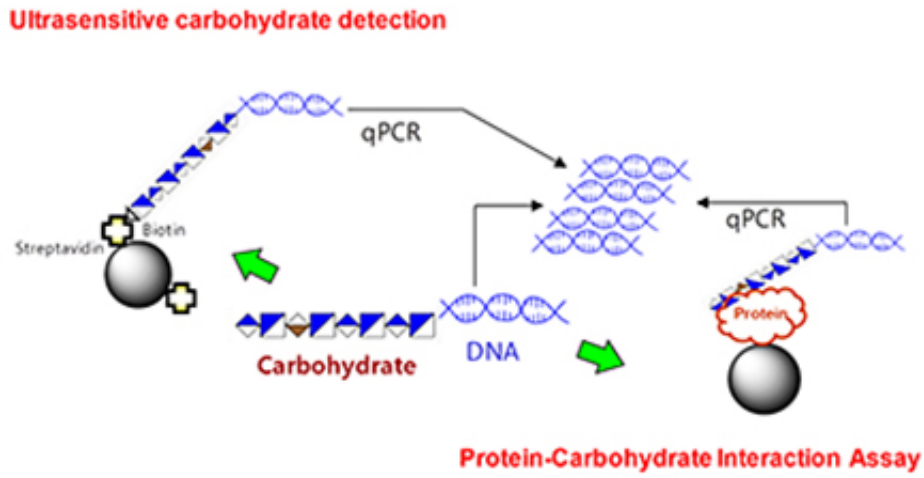
New technique enables high-sensitivity view of cellular functions

November 1 2012



Tiny amounts of carbohydrates (1 zmol, correspnding to a few hundred molecules) can be detected quantitatively by a real-time method based on the conjugation of carbohydrates with DNA marker. The method called glyco-qPCR uses amplification to provide uniform, ultrasensitive detection of carbohydrates, which can be applied to glycobiology, as well as carbohydrate-based drug discovery.

(Phys.org)—Researchers at Rensselaer Polytechnic Institute have developed an ultrasensitive method for detecting sugar molecules – or glycans – coming from living organisms, a breakthrough that will make possible a more detailed understanding of cellular functions than either genetic or proteomic (the study of proteins) information can provide. The researchers hope the new technique will revolutionize the study of

glycans, which has been hampered by an inability to easily detect and identify minute quantities of these molecules.

"The glycome is richer in information than the genome or the proteome. A cancer cell, for example, might have the same genome as a non-cancer cell, but it produces different sugars," said Robert Linhardt, the Ann and John H. Broadbent Jr. '59 Senior Constellation Professor of Biocatalysis and Metabolic Engineering at Rensselaer, and an author of the study. "Until now, the stumbling block in glycomics has been rapid and sensitive determination of the glycans present in a biological sample, and up to now we were very limited by how much we could detect. With this technique that we've developed, Glyco-qPCR, we can detect a very small number of molecules and that should accelerate the growth of the field."

The new technique is discussed in a paper titled "Signal Amplification by Glyco-qPCR for Ultrasensitive Detection of Carbohydrates: Applications in Glycobiology," which was published in the Oct. 16 online edition of Angewandte Chemie International Edition. Linhardt and Jonathan Dordick, director of the Rensselaer Center for Biotechnology and Interdisciplinary Studies (CBIS), vice president for research, and the Howard P. Isermann '42 Professor of Chemical and Biological Engineering, were joined in the research by Seok Joon Kwon, Kyung Bok Lee, Kemal Solakyildirim, Sayaka Masuko, Mellisa Ly, Fuming Zhang, and Lingyn Li.

Linhardt used the analogy of a house to explain the importance of glycans in biology and the promise of glycomics in medicine and biotechnology: If genes are the blueprints, and proteins are the structure, than sugars—glycans—are the decoration of all living matter. Just as dozens of houses in a development—despite a shared blueprint and identical external appearance—can have a unique interior identity based on wall colors and furnishings, so can two cells share the same genome, and similar proteome, but function very differently from one another,

Linhardt explained.

"You can look at a blueprint of a house and it can tell you something about the house, but it certainly can't tell you the colors of the walls," Linhardt said. "We've developed a method to start to detect what the decorations will look like, and that will give us an insight into what the house will ultimately become."

Linhardt said the technique is likely to find applications in the study of all complex multisystem diseases, such as cancer and diabetes.

"This gives us a new tool to study fundamental biology and chemistry," Linhardt said. "It allows us a higher resolution view into the functions of a cell than the genome or proteome. With this tool we can go inside a cell, poke around, and understand how to predict the behavior of that cell and ultimately control it."

As the name of the new technique suggests, Glyco-qPCR is built on Polymerase Chain Reaction (PCR), a technique, which enabled fast and cost-effective sequencing of genetic information, fueling a rapid expansion in genetics starting in the mid-1980s.

PCR allows researchers to produce mass copies of a particular sequence of DNA, or "amplify" the sequence, turning one precious sample into a nearly limitless supply of a particular sequence. The large sample makes it possible to perform other techniques that determine the identity of the particular sequence.

Glycans, the sugar molecules present in living cells, are even smaller and more complex than DNA sequences, and therefore, even more difficult to identify, Linhardt said. Moreover, unlike DNA, they have proven resistant to "amplification." So the Linhardt team took another approach.

The team has developed a technique for chemically attaching a specific DNA sequence to a specific sugar molecule. The team has built a catalogue of molecules that can be "tagged," each with a specific DNA sequence.

Once tagged, the team uses PCR to amplify the DNA tags, allowing them to identify the tags – and therefore the glycans – that are present, and the proportions in which they are present, in a given sample.

"We don't really detect the molecule, we detect the DNA that's attached to it," Linhardt said. "The DNA tags are cleverly designed so that they only attach to certain molecules. We can then amplify the DNA, see what kind of DNA it is, and then infer the molecule that it's attached to."

None of the currently used methods of glycan analysis, such as mass spectrometry or high-performance liquid chromatography, amplify the amount of sample that is present so they are much less sensitive, Linhardt said. While these current methods are capable of detecting a few billion glycan molecules, Glyco-qPCR can detect a few hundred glycan molecules.

The development of PCR in 1983 put the study of genes within reach of research labs around the world, unlocking the potential for knowledge about how genes work and treatments build on that knowledge. Linhardt hopes Glyco-qPCR will effect a similar transformation.

"Although it is an indirect method that piggy-backs on PCR, amplification technology like our Glyco-qPCR holds the same promise for glycomics research," Linhardt said. "I believe that it is revolutionary for the fields of glycomics and glycobiology."

More information: dx.doi.org/10.1002/anie.201205112

Provided by Rensselaer Polytechnic Institute