New tool identifies therapeutic proteins in a 'snap'

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(Phys.org) —In human and bacterial cells, glycosylation – the chemical process of attaching complex sugar molecules to proteins – is as fundamental as it gets, affecting every biological mechanism from cell signaling to immunity to inflammation. It's also relatively understudied, and experiments are often limited to studying just one protein at a time.

Cornell researchers led by Matthew DeLisa, the William L. Lewis Professor of Engineering, offer a powerful new tool for direct study and subsequent engineering of enzymes involved in glycosylation. Their efforts are described in *Nature Chemical Biology*, published online Aug. 17, and could be a big step forward in using bacteria to engineer therapeutic proteins, such as monoclonal antibodies, that require glycosylation for proper function.

Their new screening technology, which they call glycoSNAP (glycosylation of secreted N-linked acceptor proteins), quickly pinpoints individual bacterial cells carrying out the process of protein glycosylation and distinguishes these from a large background of non-glycosylating cells.

Glycoproteins made by such glycosylating cells are easily identified as spots on blotting paper. This strategy is faster and less tedious than traditional methods, such as detecting one protein at a time using Western blot technology.

"Our method allows you to interrogate hundreds or even millions of cells

and find that needle in a haystack – a cell performing a specific glycosylation reaction on a protein of interest to us, such as a monoclonal antibody," DeLisa said.

The paper also demonstrates the utility of glycoSNAP. The researchers used it to identify enzyme mutations that radically change the mechanism by which the ever-important sugar-to-protein attachment function is performed. These enzymes, called oligosaccharyltransferases, were engineered to efficiently attach key sugars to human therapeutic proteins, which exist on the market today for treatment of cancer and other diseases.

The work builds on a previous *Nature Chemical Biology* study in which DeLisa and colleagues introduced the machinery of human glycosylation into E. coli cells. In that study, they showed that their modified E. coli could assemble complex sugar structures (glycans) of human origin and transfer these to specific proteins. That breakthrough was limited by the fact that their oligosaccharyltransferase enzymes were picky about which proteins they would glycosylate, requiring a very specific "acceptor site" for glycan attachment.

In the current paper, they fixed the problem by using glycoSNAP to quickly identify oligosaccharyltransferases that could transfer sugars to a wider array of protein acceptor sites – essentially loosening the enzyme's acceptor site requirements for performing glycosylation.

"So now we can make human glycans and attach them, in theory, to virtually any protein – especially those with high clinical relevance – without having to change the sequence of the protein," DeLisa said.

Glycosylation is a key process in biopharmaceutical manufacturing, and DeLisa's startup company, Glycobia, harnesses this process in E. coli for specific medical applications. Monoclonal antibodies, for example, are

extremely popular biopharmaceuticals, and require glycosylation for engaging cells like macrophages and neutrophils, which ingest antibody-coated pathogens and kill them. Other genetically engineered glycoproteins can bind to receptor sites in, for example, cancer cells to keep them from multiplying, or immune cells to block them from attacking the body's own tissues in autoimmune disorders.

So crucial to understanding human biology is glycoscience that the National Institutes of Health has highlighted the need to learn more about it.

More information: "Engineered oligosaccharyltransferases with greatly relaxed acceptor-site specificity." Anne A Ollis, et al. *Nature Chemical Biology* (2014) DOI: 10.1038/nchembio.1609. Received 31 March 2014 Accepted 12 June 2014 Published online 17 August 2014

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