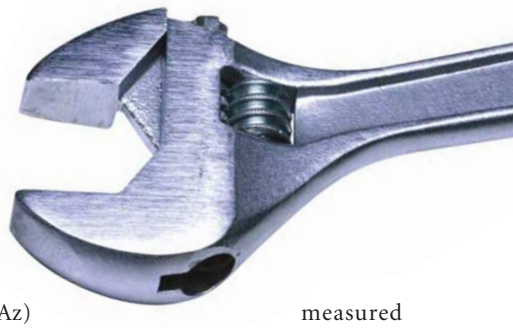
CHEMISTRY

Chemical engineering *in vivo*



A strategy to chemically modify cell-surface oligosaccharides has been adapted for use *in vivo* and could prove to be a valuable tool for non-invasive imaging and therapeutic targeting. The process, called metabolic oligosaccharide engineering, incorporates unnatural sugars into cell-surface glycans, and has been previously demonstrated *in vitro*; now Carolyn Bertozzi and colleagues report in *Nature* that this technique can also be used *in vivo*.

Oligosaccharide engineering requires a pair of reactive groups: one that is delivered to the cell-surface glycan by the metabolism of an unnatural sugar precursor; the other, an exogenous reagent. These functional groups must possess two qualities: they must exhibit exquisite chemical selectivity and must be mutually reactive under physiological conditions.

The authors developed the Staudinger ligation reaction between azides (which are metabolized and presented on the cell surface) and a specific class of phosphines (the exogenous reagent) to meet these criteria. The properties of these reagents have already been shown to be amenable to use *in vivo* — the azide is a component of the HIV drug azidothymidine, and phosphine–metal conjugates are established diagnostic agents.

Certain carbohydrate biosynthetic pathways have a high tolerance for unnatural substrates, which can be exploited when delivering azides to the cell surface for the Staudinger reaction. A peracetylated version of the unnatural sugar *N*-α-azidoacetylmannosamine (Ac_4ManNAz) was used in this study, because it is efficiently converted into *N*-α-azidoacetyl sialic acid (SiaNAz) in cell-based assays; in addition, analogues of the natural substrate *N*-acetylmannosamine (ManNAc) are converted into the corresponding sialic acids in rats, establishing that the precursors are successfully metabolized *in vivo*.

The initial step in adapting the Staudinger reaction to animals was to demonstrate the conversion of the unnatural precursor Ac_4ManNAz to SiaNAz *in vivo*. This was done by exposing mice to Ac_4ManNAz, harvesting the splenocytes (which are rich in sialosides) and measuring the levels of cell-surface azides using a phosphine probe incorporating a 'Flag' peptide (Phos-Flag) and a fluorescein-labelled anti-Flag antibody. Splenocytes treated with Ac_4ManNAz produced a robust dose- and azide-dependent fluorescent signal compared with splenocytes from mice treated with free ManNAz or vehicle.

Having established the conversion of Ac_4ManNAz to SiaNAz, the authors then attempted the actual Staudinger reaction *in vivo*. Mice were given intraperitoneal injections of Ac_4ManNAz daily for seven days followed by a single injection of Phos-Flag by the same route, after which the splenocytes were harvested and the level of fluorescence was measured using flow cytometry. An increase in fluorescence was only observed in splenocytes from mice treated with both Ac_4ManNAz and Phos-Flag, proving that the Staudinger reaction had proceeded successfully *in vivo*. Then, to determine the extent of the reaction after 90 minutes, harvested splenocytes were subjected to further reaction with Phos-Flag. Splenocytes previously treated with both reagents showed a less pronounced increase in fluorescence, indicating that a significant proportion of the available cell-surface azides had been modified.

The ability to change the functionality of a cell surface has numerous applications, from studying the role of glycans in health and disease to targeting overexpression of these molecules as a therapeutic strategy for cancer. This study provides a basis from which to identify new reactions in addition to the Staudinger ligation so that the full potential of this technique can be realized.

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References and links

ORIGINAL RESEARCH PAPER Prescher, J. A., Dube, D. H. & Bertozzi, C. R. Chemical remodelling of cell surfaces in living animals. *Nature* **430**, 873–877 (2004)

WEB SITE
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