CHEMICAL BIOLOGY

Creating a 'sweet spot' for *in vivo* cell-surface tagging

Using a highly specific chemical reaction, investigators at the University of California at Berkeley have developed an innovative system to covalently label cell-surface sugar molecules in live mice.

Four years ago, a *Science* article by Eliana Saxon and Carolyn Bertozzi resurrected a nearly century-old organic chemistry reaction as a mechanism for the targeted labeling of cell-surface sugar molecules. "We were thinking about what kinds of chemistries could be crafted using functional groups that can traverse a biosynthetic pathway in living cells," says Bertozzi, "and at the same time, have the ability to undergo a chemical reaction with no interference from the biological milieu." This organic chemist would find her inspiration in the Staudinger reaction (see box, "Chain Reaction").

They fed *N*-azidoacetylmannosamine (ManNAz), a modified sugar precursor, to Jurkat cells, where endogenous enzymes converted it to a cell-surface sialic acid molecule with an appended azide group. This azide enabled the covalent tagging of the sugar with a phosphine-containing substrate via their modified Staudinger reaction.

Since then, this technique has gained increasing interest from investigators looking for a straightforward and orthogonal reaction system for biochemical studies. Now, in a new article from *Nature* (Prescher *et al.*, 2004), the Bertozzi group demonstrate the use of this reaction to achieve *in vivo* modification of cell surfaces in living animals.

They began by injecting mice with ManNAz. Isolated splenocytes, a cell type known to express sialic acid on their surface, were readily labeled with a FLAG-tagged phosphine probe (Phos-Flag), indicating that *in vivo* modification had occurred. Labeling was predominant on tissues whose cells are sialylated and that are involved in first-pass metabolism, such as the heart, kidney and liver. Cells in other tissues remained unlabeled, owing either to lower levels of sialic acid biosynthesis or to their inaccessibility to the azido-sugar.

The entire labeling process could also be performed *in vivo*. Mice were treated with ManNAz for 1 week, and then given an injection of Phos-Flag; splenocytes isolated 90 minutes later showed strong labeling. This labeling diminishes over time, disappearing completely within a few days, an effect the authors attribute to the natural process of cycling and degradation of surface glycans.

Having demonstrated the general feasibility of this approach, the team is looking to design more specialized phosphine probes, conjugated to tags suitable for a variety of imaging modalities. Cancer cells have glycosylation patterns that are significantly different from those of normal tissues, and Bertozzi is excited by the possibility that clinicians could exploit these changes to visualize tumors noninvasively.

Bertozzi also makes it clear that their system could prove a valuable tool for basic research. "For example," she says, "people have shown that glycosylation changes take place during embryogenesis... and different stages of embryonic development in different organs have characteristic sugars. I think it would be really interesting to be able because... it's a really mild, very selective way of converting azides to amines," says Berkeley chemist Carolyn Bertozzi. Her group had been investigating methods to tag sugar molecules on cell surfaces, and a key consideration for developing such *in vivo* tags is the principle of bio-orthogonality — that functional groups being introduced neither interfere with endogenous cellular functions nor be inappropriately recognized by the host.

The Staudinger reaction relies on two synthetic functional groups — the azide and the phosphine — that do not seem to engage in any inappropriate interactions within the cell. However, the classical reaction produces separate molecules, so Bertozzi's team had to modify the reaction to generate a covalent linkage. They found that adding a methyl ester to the reacting phosphine produces a stable, covalent amide bond connecting the two reactants (**Fig. 1b**). This modified reaction, known as the Staudinger ligation, has since shown strong potential for cell modification and other biochemical tagging strategies (see above).

Bertozzi cites the *in vivo*–friendly reaction conditions as a major benefit to this technique, and adds, "there aren't too many other reactions that have that level of selectivity, where the reacting partners see only each other and ignore everybody else in the room, and this is one of those few."

Michael Eisenstein

CHAIN REACTION

In 1919, Hermann Staudinger, future Nobel Laureate and forefather of polymer chemistry, described a room-temperature reaction that could be performed in water to convert a phosphine and an azide to a corresponding primary amine and phosphine oxide (**Fig. 1a**). A useful reaction, but why has this German chemist's 90-year-old discovery gotten so much attention lately?

"It always fascinated me as a chemical transformation,

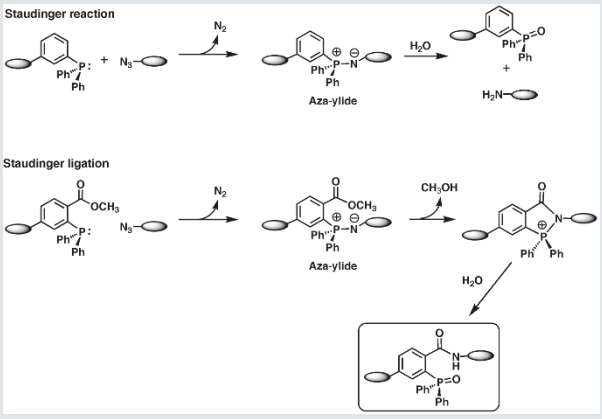

Figure 1 | Schematics of: (**a**) The classic Staudinger reaction, (**b**) the Bertozzi group's modified Staudinger ligation. Ph, phenyl.