AN AUDIENCE WITH...

Carolyn Bertozzi


Credit: American Chemical Society

The sugars that coat the surface of the cell have long fascinated Carolyn Bertozzi, a Professor of Chemistry at Stanford University. But her study of these complex carbohydrates has had much further reaching implications. Her pioneering work on bioorthogonal chemistry — reactions that can be run in living systems, without damaging them — has opened up basic drug discovery and therapeutic applications alike, for example. In her hands, these chemical tools have led to the creation of new therapeutic modalities including antibody–enzyme conjugates that can reshape the glycocalyx and lysosome-targeting chimaeras (LYTACs) that can degrade membrane-bound and extracellular targets. These, in turn, are helping her to further unravel the role of sugars in biology and in immuno-oncology. Bertozzi has already founded eight companies to advance these and other approaches. More are on the way. Speaking with **Asher Mullard**, she discussed her work and why drug developers have been so slow to see the sugars on the surface of the cell.

You developed bioorthogonal chemistry — an approach that is often listed as a Nobel Prize contender — more than 20 years ago to image sugars. These chemistries are now used as enabling drug discovery tools, and being developed as potential therapeutics. Did you anticipate such wide-reaching applications?

I think we knew that they would be very useful beyond the world of glycoscience, for sure. But I can't honestly say that I foresaw everything people were going to do with this. We thought it would be useful for protein engineering and to make medicines like antibody–drug conjugates. But, outside of my lab, others have found lots of interesting applications.

People in big pharmaceutical companies have published some beautiful work using bioorthogonal chemistries for target identification, for example. Or, they have a drug for which they know the primary target, but they're curious about the off-target interactions. So they'll arm an analogue of the drug with a functional group, and then click on an efficient probe in vivo to try and pull out or image the interactors to see where the drug is concentrated. And of course, in terms of chemoproteomics, groups are using these warheads to basically go fishing for small molecules that can bind targets across the proteome. It's become a discovery tool, a target validation tool, and an off-target identification tool. These chemistries are now baked into the fabric of biopharmaceutical research.

They are also now being used directly in therapeutics. There's an ongoing clinical trial of a radiotherapeutic approach, for example, where researchers are first targeting cancer cells with a functionalized antibody and then treating patients with a radiopharmaceutical that will bind to the antibody in patients. Their hope is that this will overcome some of the problems of dosing and the half-life of radioactive decay. There's a company called Shasqi that has just done its first human dose of a bioorthogonal chemistry-based cancer drug. They've made a hydrogel material that is functionalized with a bioorthogonal group that they inject into patient tumours, and then they administer a chemotherapy systemically. This chemotherapy is a caged prodrug that is harmless in circulation. But when it comes into contact with the injected polymer, the bioorthogonal reaction uncages the drug locally. With this, they hope to deliver a higher local dose of a chemotherapy than they could otherwise achieve.

How could bioorthogonal chemistry reagents be made better?

I think people feel pretty satisfied with what's out there, but you can always do better. We can always use another orthogonal chemistry, especially if it is orthogonal to the ones that are already out there.

What about faster-acting warheads?

That's always helpful. Right now, there are three 'bread and butter' bioorthogonal chemistries that stand out as being really very efficient. It's this tetrazine ligation that has the super kinetics that you need for some of these in vivo applications. In fact, the two examples I'm aware of where people are doing bioorthogonal chemistry in human patients, they both use that fast chemistry. This chemistry was discovered by Neal Devaraj, who first published on it as a postdoc. The kinetics of that reaction are quite enabling.

But although other chemistries might be slower, they can be much easier to work with. And sometimes people just want to be able to put reagents on a shelf, and grab them when they need them.

Are you still developing bioorthogonal chemistries?

We've moved on to other things. We certainly use them all the time, but we just buy the reagents from the catalogs now.

Some of your more recent work has focused on the role of glycosylation in cancer immunobiology. How did you get started here?

I started working in glycoscience when I was a grad student, and my bioorthogonal chemistry work really came from some big unmet needs in this space. Around 10 years ago, the cancer field really realized and discovered the potential of the checkpoint receptors, and the role that these proteins play in tumour immune evasion. As it happens, there's a big cluster of carbohydrate-binding receptors called SIGLECs on our immune cells that bind sialoglycans. And the signalling biochemistry of these receptors is almost exactly like that of PD1. These receptors are immunosuppressive receptors, but instead of binding PDL1 they bind sugars.

People had been thinking along these lines for decades, but I just happened to be one of the people who noticed it at a time when it mattered, I think. So, we started to form hypotheses around how glycans could engage with these immune-modulatory receptors, and how cancer cells could use them to promote immune evasion.

This work led you to develop an antibody–enzyme conjugate — an anti-HER2 antibody fused to a sialidase enzyme — that mows these sugars off the surface of cancer cells. This approach formed the basis of Palleon Pharmaceuticals. Why target the sialic acids rather than the receptors?

There are 14 SIGLECs, and at least 9 of them seem to have this immune-inhibitory signalling. They're often expressed in combinations, and multiple different sialic signals may contribute to suppression. But one thing that all the SIGLECs have in common is that they all require that their ligand is sialylated. And we thought, well, let's just go right after the sialic acid, because that's the heart of the beast.

Your recent Nature Chemical Biology paper on this work notes the potential benefits of fusing the sialidase enzyme to other anti-cancer antibodies. What about fusing antibodies to other types of enzymes?

Yeah, we're doing that. We have protease-based antibody–enzyme conjugates that cut off certain types of glycoprotein that are bad actors — mucins — in the cancer setting. We'll see how that goes. That's pretty new, but we've got it looking pretty good so far.

In general, I do think enzymes have been underutilized as therapeutics. There are so many different enzymes that do such interesting chemistries. And if you look at the approved biologic list, there are not as many enzymes in there as you might think. The ones that are in there are mostly enzyme replacement therapies, for rare diseases. I think there's a lot of room for creatively deploying these natural biocatalysts as therapeutics.

You've also recently published in Nature on lysosome-targeting chimaeras (LYTACs), targeted degraders for extracellular and cell-membrane proteins. How did you get interested in these?

For me, our antibody–enzyme conjugated sialidases are a form of targeted degradation for extracellular glycans. Basically we bring an enzyme to a target to cut it up with these. But what about the opposite, I wondered? Could we grab a target and pull it to the enzyme?

The lysosome is full of degradative enzymes, of course, and that's normally where membrane proteins and extracellular proteins go to be degraded. So, we thought, wouldn't it be great if we could grab the thing you want to degrade and just somehow yank it into the lysosome. And for anyone who has ever studied glycobiology, which I recommend everyone do, one of the first things you learn is the role of glycosylation in subcellular trafficking. There's this well-known mannose 6-phosphate receptor (M6PR) system that shuttles glycoproteins carrying a mannose 6-phosphate marker to the lysosome, offering a built in delivery system. We put two and two together, and took small molecules or antibodies against targets of interest and fused these to glycopeptide ligands that bind the M6PR. And I have formed a company called Lycia Therapeutics around this approach.

This approach is analogous to the PROTACs, which bridge the divide between a target and the degradation machinery of the proteome. That's exactly what we did for the lysosomes, and we've called these LYTACs as an homage to Craig Crews and the PROTACs.

How could LYTACs be improved?

One thing that I love about the PROTACs is that they're not themselves degraded rapidly, and so each compound has the opportunity to mediate the degradation of multiple targets. This means, in principle, that they can be dosed much lower than traditional drugs. For us to be able to play on that same field with PROTACs, we would need to engineer our LYTACs so that they are not degraded in the lysosome. We didn't put any special effort into this catalytic property for that first generation of LYTACs. But, since then, we have certainly thought about how to achieve that.

Since we've published on this, a couple of other groups have also gotten into the LYTAC game. We put a preprint up in July on a LYTAC that latches on to a different lysosomal trafficking receptor called the ASGPR. This is the same receptor that Alnylam and Ionis use to drive hepatocyte uptake of their oligonucleotide-based drugs. And within weeks, two other labs also released preprints on tri-GalNAc-based degraders.

Do you think industry pays enough attention to glycoscience?

No. Even just a few years ago, I'd give talks at biopharmaceutical companies — including the pantheons of immuno-oncology — about the SIGLECs, their parallels with PD1, and the potential role of hypersialylation and immune suppression. And afterwards people would ask me, "how come we've never heard of these SIGLECs before?" It was quite stunning, because if you take the sequence of PD1 and see what else looks like it, boom, all of these SIGLECs pop up.

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Hopefully, decades from now, when important medicines have come from the field of glycosciences, there will be some retrospective failure analysis to understand why it took so long for drug developers to take note of this science. But people have unconscious biases. If you ask people in biopharma companies or venture capital investors about building glycoscience groups or glycoscience companies, they'll say "glycoscience is messy. It's nonspecific. It's complicated". And if you try to get to the next level about why they think that, they're not sure. The truth is, people don't really know this science well enough to know why they have biases against it. It's just they heard somewhere that it was complicated.

Honestly, the best thing that has ever happened to glycosciences is epigenetics. If you think glycoscience is scary, then don't go near epigenetics. But, once people started to appreciate the complexity of genomic regulation, genomic organization, epigenetic modifications and the combinatorial use of information, they started to accept the fact that the central dogma that they had learned was a gross oversimplification of how things worked. Then their brains could adjust to the reality that biology is complicated.

I have found that now, much more so than 10 years ago, people are accepting of that complexity. One of the great things about the modern way of doing biology is that more and more people are working with unbiased screens, for instance using CRISPR to try and figure out the machinery that's important for a particular biological process. When they do these screens, inevitably their hit lists include all these glycogenes. In the past, when people had a hypothesis that they wanted to pursue, glycoscience didn't factor in and they would miss a lot of biology. Now when the top ten genes are glycogenes, what are they going to do? Either you learn some glycoscience, or you walk away.