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A chemist's approach to biochemical complexity

In the past few years, research activity and innovation at the interface between chemistry and biology has grown by leaps and bounds. Chemical biology is an emerging discipline, involving the use of chemical and physical tools to investigate the structure and function of complex biological molecules, pathways and systems. *ChemComm* is delighted to have secured the services of Barbara Imperiali, who is the Ellen Swallow Richards Professor of Chemistry and Professor of Biology at the Massachusetts Institute of Technology (MIT), as the journal's first Associate Editor for Chemical Biology.

Introduction

Imperiali and her team at MIT are looking at a wide range of problems in chemical biology, and developing new tools to take the research forward. Glycosylation has long been the focus of Imperiali's work.[1] "We will use any method or tool we can to try to understand this very complex biological process, which involves the enzyme-catalysed modification of proteins with complex carbohydrates to alter their structure or function," she says.

The highly interdisciplinary approach adopted in Imperiali's lab has three major themes. First, there is the study of the key enzyme, oligosaccharyl transferase (OT), which catalyses N-linked glycosylation at asparagine residues in proteins (Fig. 1). OT is a complex enzyme,[2] being both multimeric and membrane-bound, so unravelling its architecture using protein biochemistry and molecular biology techniques is a real challenge.

Second, the group is using a range of chemistries to make small molecules that are used as probes to uncover how the enzyme works.[3] This allows the development of mechanistic models to provide insight into the enzyme function.

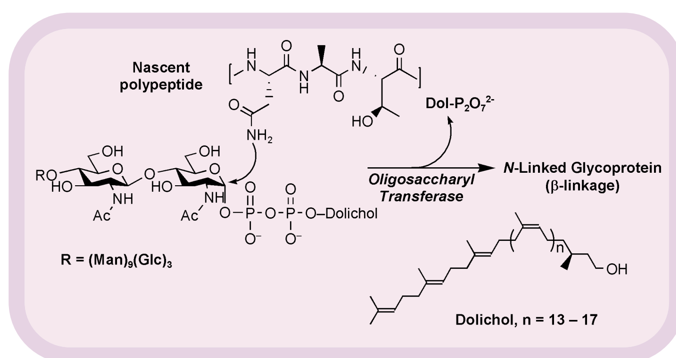

Fig. 1 Reaction catalyzed by oligosaccharyl transferase.

"The chemist's way of understanding an enzyme is to design specific molecules that can inhibit the enzyme. The biologist's way is to do a genetic 'knock out' experiment. I like to think of these as being complementary approaches," explains Imperiali. "In a sense, the chemical tool may have advantages, because the activity can be instantaneous, whereas the biological approach may need protein biosynthesis to be down-regulated for changes to be observed. We have made significant progress on the development of compounds that work very well *in vitro*. A major focus now is to get these compounds into cells and specifically to the target enzyme *in vivo*. That task needs chemical design, synthesis, and manipulation, and the development of high throughput cellular assays to see if we can get these compounds to work in the sub-cellular compartment where the enzyme is localised."

The third aspect of the glycosylation programme is to understand what attaching a carbohydrate does to protein structure. This aspect of the research involves biophysical studies with a range of

Barbara Imperiali took up her current post as Ellen Swallow Richards Professor of Chemistry at Massachusetts Institute of Technology (MIT) in 1999. She was educated at University College London and gained a Ph.D. in synthetic organic chemistry at MIT in 1983. Professor Imperiali has gained many honours and awards during her career, including a Kennedy Memorial Trust Fellowship in 1979, an Arthur C. Cope Scholar Award in 1996, the 5th Annual Richard P. Feynman Award for Excellence in Teaching in 1998 and, last year, the MIT School of Science Award for Excellence in Undergraduate Education. She was the co-chair and organizer of the first Gordon Research Conference on bio-organic chemistry in 1992 and serves as an Editorial Advisory Board Member on a number of academic journals. In 2001, Professor Imperiali was elected to the American Academy of Arts and Sciences. For more on research in the Imperiali group at MIT, go to: http://web.mit.edu/imperiali/

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spectroscopic tools, including nuclear magnetic resonance, circular dichroism, fluorescence and microscopy.

Imperiali adds that early glycosylation events may be key determinants for correct protein folding. Asparagine-linked glycosylation is co-translational, occurring as the protein is being synthesised on the ribosome and growing into the endoplasmic reticulum (ER). OT is the 'gatekeeper' to the secretory pathway, acting on partially folded proteins to attach the carbohydrate through a covalent linkage, and continuing to do this as the remainder of the protein is biosynthesised. When this is complete, the protein is released into the ER.

"Then, a lot of different events occur downstream of the initial glycosylation reaction, so we feel that we are looking at the gatekeeper to the secretory pathway," she says. "Proteins have to go through this gatekeeper—you can think of the translocation machinery as the gateway and OT as the gatekeeper".

In an ambitious new programme, Imperiali's team has begun to look at all the enzymes—14 or so in total—which are responsible for building up the saccharide donor which is the substrate for OT, in what is known as the 'dolichol pathway.' "We are using the expertise we have developed with the OT programme to look at what's around OT in that subcellular site. In this area, chemical biologists are trying to focus as much as possible on re-constructing assemblies of macromolecules. This is an important change in thinking—because for many many years we would de-construct complex systems into single components. But what we're really excited about now is understanding how all these processes work together in integrated and efficient processes."

Developing bioprobes

The 'bioprobes' group within the team is developing new probes and techniques—all of which involve some element of synthetic or semi-synthetic chemistry—which can be applied to a number of problems in chemical biology, including the analysis of protein complexes. Examples of new directions in the bioprobes subgroup include the development of lanthanide binding tags (LBTs) where short peptide sequences, optimised for terbium binding and luminescence, are incorporated into the gene of a target protein to form a 'tagged' fusion protein. This new initiative builds upon expertise in the Imperiali group including the design of peptide-based metal ion sensors and the assembly of stable mini-protein motifs.[4] In the LBT, energy transfer from a trytophan residue, within the tag, to the lanthanide ion ensures that the LBT acts as a site-specific fluorophore within the protein of interest (Fig. 2). Thus, this tag will enable the study of protein-protein interactions, and maybe even the observation of transient complexation and de-complexation in real time. While the LBT concept has been around for some time, it is only through the chemical methods of parallel synthesis and high throughput screening applied in the Imperiali group, that LBT sequences with optimal binding affinity and selectivity for applications in chemical biology have been generated.

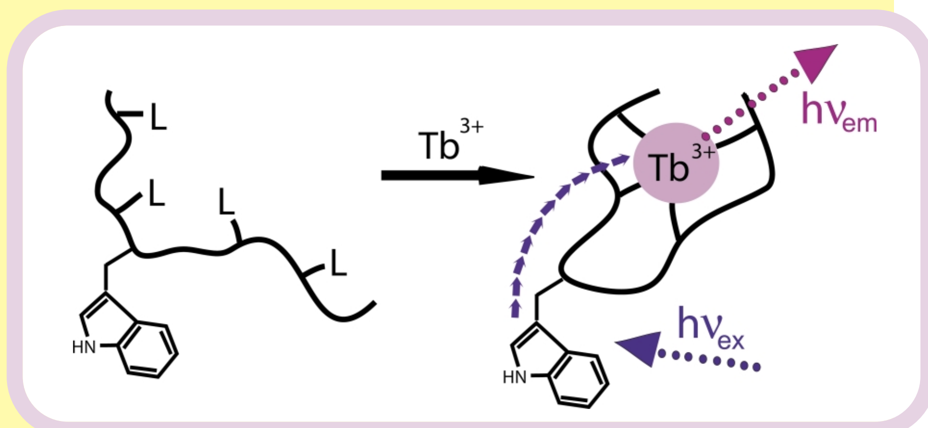

Fig. 2 Lanthanide binding tags.

Another bioprobe tool is based upon 'caged' phosphopeptides and phosphoproteins for the study of signal transduction events in processes such as cell migration and cell cycle control. These processes are heavily dependent upon phosphorylation and dephosphorylation by protein kinases and phosphatases. The approach that the Imperiali group is taking involves the development of robust synthetic procedures for synthesising phosphorylated peptides and proteins, where the phosphate is 'caged' by a 2-nitrophenylethyl group.[5] This moiety can be released in the cell by photolysis, so exposing the functional phosphopeptide or phosphoprotein that is then able to mediate specific effects within live cells (Fig. 3). In other words, the caging process allows one to intercept a biochemical pathway at a point of interest. When the molecule is 'uncaged' the chemical biologist and biologist are able to work together to study the specific response of the cell to the active agent – whether it moves, or divides, for instance.

"We are collaborating in what is known as a Glue grant, which is a broad-based multi-disciplinary initiative funded by the National Institutes of Health, that harnesses the capabilities of researchers from many disciplines in studying all aspects of cell migration. So the methods that we've developed for synthesizing caged phosphopeptides and caged phosphoproteins have now enabled us to make caged analogs of the focal adhesion kinase (FAK)—and we are working with the Glue grant team to see if we can intercept key steps in cell migration

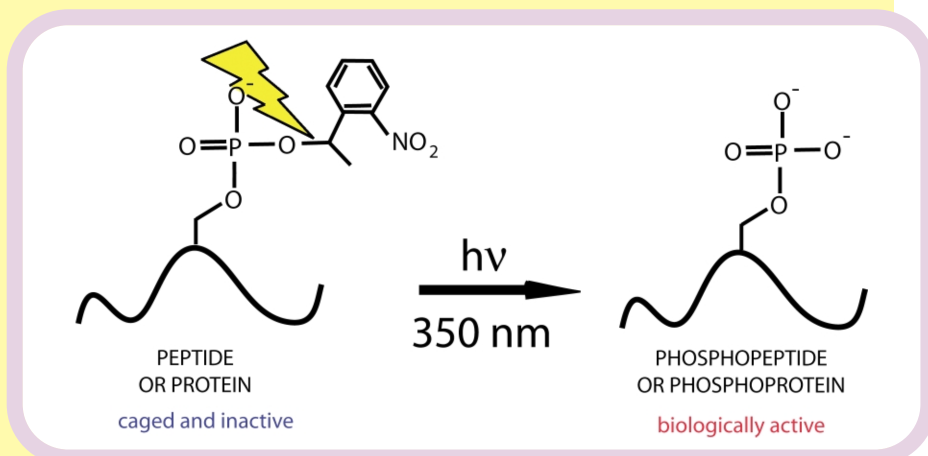

Fig. 3 Caged phosphopeptides and phosphoproteins.

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Chemical biology in *ChemComm*

Professor Imperiali's role at *ChemComm* is to encourage contributions from the American chemical biology community into the journal. She is especially keen to focus on the younger generation of researchers – a group that is particularly attracted to working at the interface between chemistry and biology—and make them aware of the publication."The breadth of communications in *ChemComm* is important, and I find that the journal is reaching a broader community all the time. The speedy process of paper reviewing is a real asset in the rapidly evolving field of chemical biology and having the journal cited on Medline has also been a significant development."

Professor Imperiali will be pleased to receive enquiries and manuscripts at: Professor Barbara Imperiali, Department of Chemistry, Building 18, Massachusetts Institute of Technology, Cambridge, MA 02139, USA. Tel: (+1) (617) 253 1838; Fax: (+1) 617 452 2419; E-mail: chemcomm@mit.edu

through, in a sense, 'hot wiring' the system," comments Imperiali. The caged phosphopeptides are also being used to study cell cycle control, in collaboration with Professor Michael Yaffe of the MIT Cancer Center.

The chemistry involved in development of the bioprobes is challenging. For the phosphopeptides, the group has taken inspiration from nucleic acid chemistry. There is also a great deal of challenging heteroatom chemistry and work with polar molecules, which can be notoriously difficult to handle and purify.

The future of chemical biology

Chemical biology has become highly multidisciplinary, attracting people who have expertise in more than one area, and are not afraid to try something new. "It can be very demanding," Imperiali admits. "You've got to know a lot about a lot of different things and you have to get to grips with both the chemistry and the biology as much as you can. There is no technique we won't try. Yes, it's very challenging, but it's also a very exciting place to be."

In the future, Imperiali is expecting to see more and more applications of bioprobes in complex biological systems. "It's simply not viable nowadays to develop tools that only look good in simple systems. We need to keep on evolving to get closer to the important biological questions. We are now being pushed forward and are responding by working side by side with the biologists, using chemistry. All of biology is chemistry and all of these biological molecules are beautiful structures. Understanding their intricate working is our job for the next 100 years, and the more tools we have to do this with, the better."

Barbara Imperiali was talking to Susan Aldridge.

Notes and references

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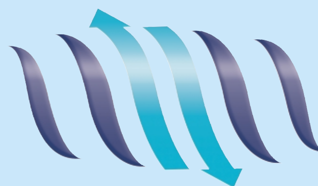

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