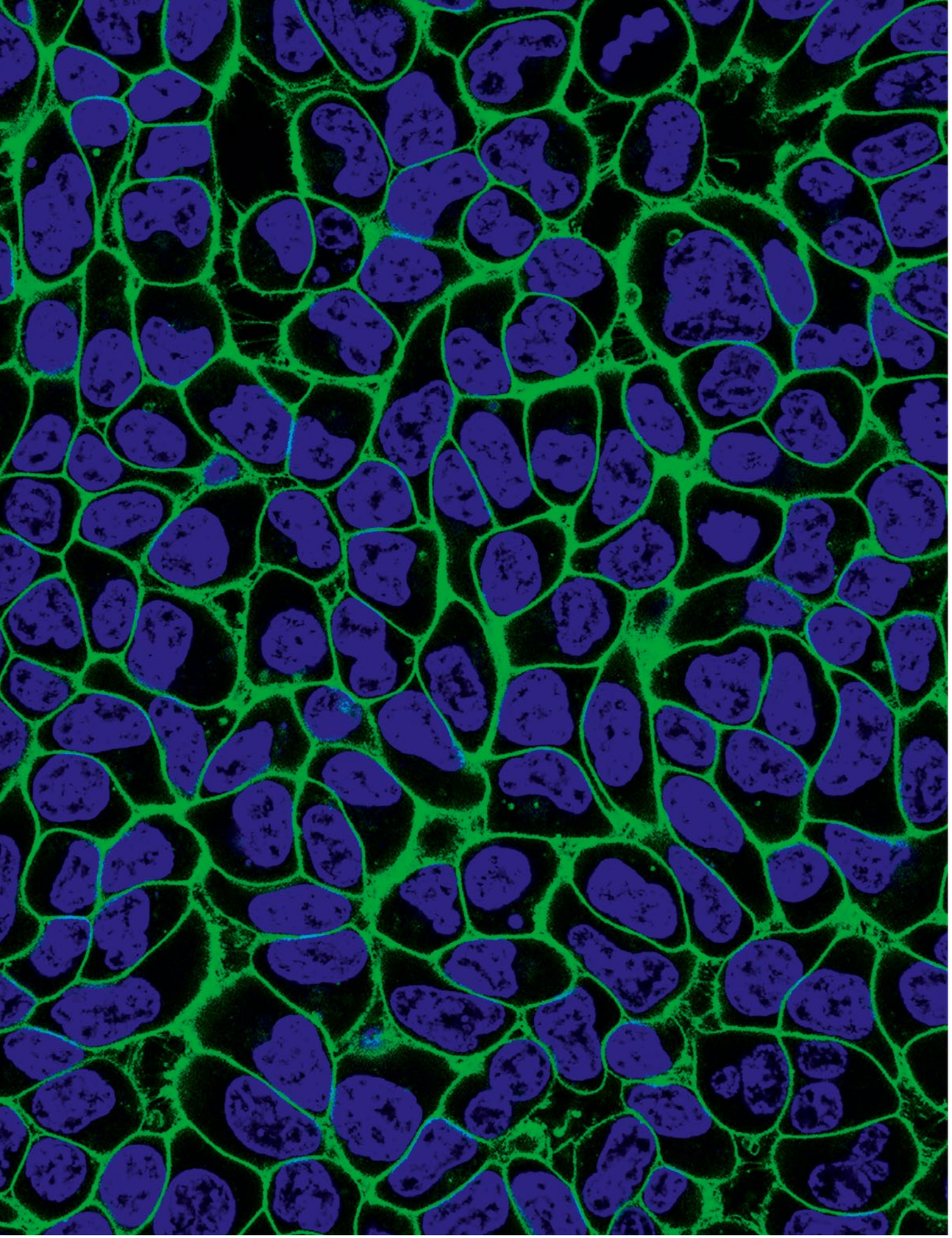


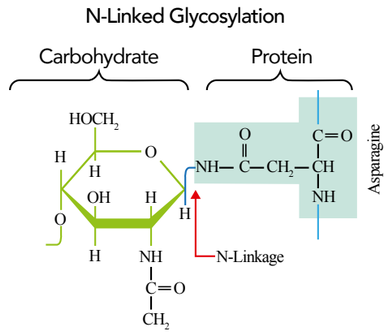
# How to trace glycoproteins in living cells? Metabolic glycoengineering provides the answer

Carbohydrates, through the process of glycoconjugation, play a vital role in a number of important eukaryotic cell signalling processes. **Professor Valentin Wittmann** and his team at the University of Konstanz focus their research on the mechanisms behind this, using metabolic glycoengineering techniques to enable the identification of particular interactions.

Carbohydrates are part of a variety of important biological signalling functions in eukaryotic cells. Through the process of glycosylation, carbohydrates are covalently bonded to macromolecules such as lipids or proteins. Such post-translational modification serves a variety of purposes, including facilitating the structural stability of proteins and correct folding patterns. Glycosylation also enables an immunological response via cell–cell adhesion, although research is only now beginning to understand the biological functions behind this.

## METABOLIC GLYCOENGINEERING TO VISUALISE CELL REACTIONS

Professor Valentin Wittmann and the Wittmann research group at the University of Konstanz are employing a variety of methods to identify such underlying functions. Having obtained his PhD from the Technical University of Munich, Professor Wittmann joined the University of Konstanz in 2003. Since 2016, he has been acting as the Head of the Department of Chemistry and Vice Coordinator of the Collaborative Research Center SFB 969. As part of his research, he examines carbohydrate–protein interactions and how glycosylation modifies a protein's function such as its structural stability and folding, enzymatic activity and localisation.




**The Wittmann Group uses metabolic glycoengineering techniques to accomplish the integration of functional groups into the carbohydrate portion of a glycoprotein**

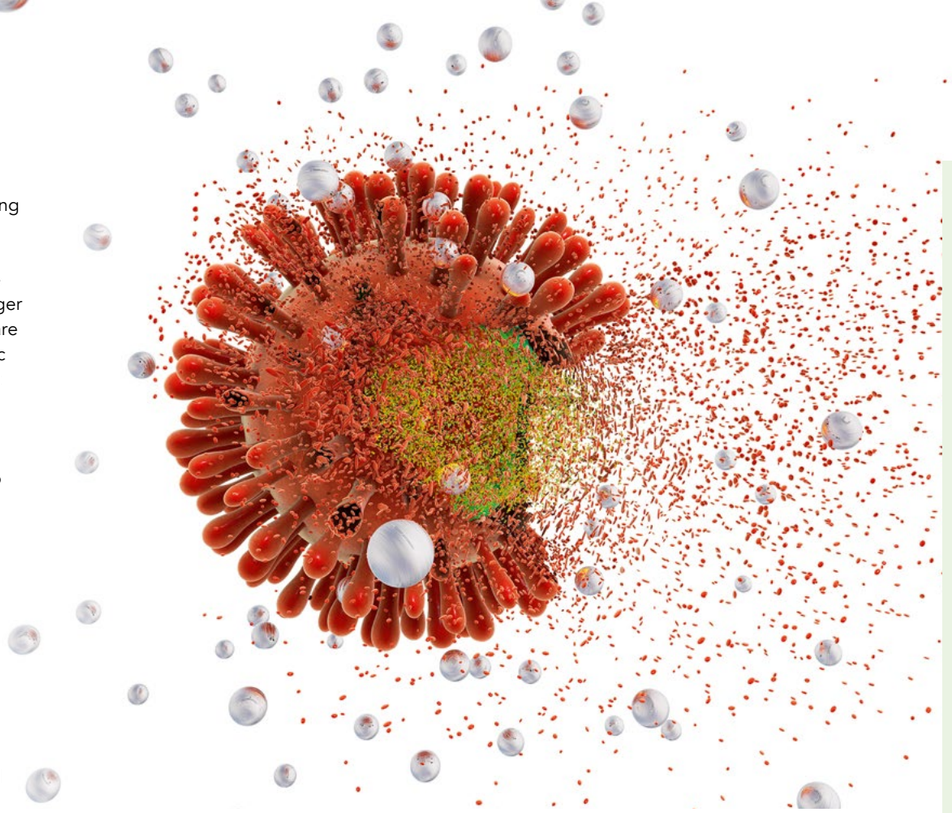
The Wittmann Group uses metabolic glycoengineering techniques to accomplish the integration of functional groups into the carbohydrate portion – glycan – of a glycoprotein. Successful incorporation allows them to fluorescently label the carbohydrates through bioorthogonal chemical ligation, which avoids disturbing the native biochemical reaction of a cell. This helps them to visualise the interactions inside a living cell. Ultimately, the fine-tuning of the glycosylation pathway modifies a protein's function without changing the underlying amino acid sequence.

## THE IMPORTANCE OF GLYCANS

Glycans are involved in many processes that are vital to eukaryotic cell functioning, including quality control, protein transport, immune and developmental responses. For example, N-linked glycans (which are glycans attached to asparagine side chain nitrogen) play an important role in the cancerous cell recognition process. This makes them a potential target in cancer therapeutics. In addition, glycoproteins of viruses such as the human immunodeficiency virus (HIV) contain various N-glycosylation sites, which may aid in shielding the virus from immune system recognition. Removal and modification of such glycans helps to understand viral functioning and develop suitable treatments.

## ON TO BETTER GLYCOPROTEIN DETECTION METHODS

The need to visualise protein glycosylation



within living cells has driven the development of metabolic glycoengineering over the past two decades. Professor Wittmann has spent much of his career dedicated to detecting glycoproteins. The initial detection methods, such as Staudinger ligation and azide-alkyne cycloadditions, are limited, in some cases even cytotoxic (toxic to cells), and do not allow for independent labelling of two different carbohydrate residues. The inverse-electron-demand Diels-Alder (DAinv) reaction introduced to bioconjugation in 2008 has been shown to be a more suitable bioorthogonal ligation reaction – it can occur inside the body without disrupting existing biochemical processes and in parallel to azide-alkyne cycloaddition.

**INVERSE-ELECTRON-DEMAND DIELS-ALDER REACTION**

During initial trials using the DAinv reaction, Professor Wittmann synthesised monosaccharides and found that terminal alkenes could be successfully metabolised and thus fused into glycoconjugates for subsequent labelling. Recently, Professor Wittmann examined protein-specific glycosylation of the intracellular proteins OGT, Foxo1, p53, and Akt1 in living cells. The DAinv approach provides several advantages. Reactions cannot only be performed in aqueous solutions but also without addition of toxic catalysts. In addition, the reaction is irreversible. DAinv has also been demonstrated to facilitate the transport of substances to target cells acting as a therapeutic carrier.

Termed click chemistry, such approaches involve synthesising drug-like molecules, which could potentially aid in discovering new drugs. Professor Wittmann has been part of experimental research showing that metabolic oligosaccharide engineering has been successfully employed to implement functional groups amenable to bioorthogonal labelling ('click groups') into the extracellular matrix of human dermal fibroblasts. This method also has potential for medical implant ingrowth.

**MONITORING INTERACTIONS WITH CARBOHYDRATE MICROARRAYS**

Carbohydrate microarrays have presented as a suitable tool to monitor interactions between carbohydrates and proteins. Microarrays have distinct advantages, which include multivalent binding to examine cell–cell interactions. In addition, only small amounts of ligands are necessary to facilitate a binding reaction. Experiments that aim to detect pathogens by use of carbohydrate microarrays further allow researchers to collect and examine such pathogens for additional analysis.

**MULTIVALENCY AS A NOVEL METHOD TO EXAMINE IMMUNE SYSTEM PROCESSES**

For a long time, the Wittmann Group has also been examining multivalency in biological recognition. Multivalency enables strong bonds by employing multiple weak binding sites of low-affinity ligands. This concept has been shown to be of importance in carbohydrate–lectin interactions and it further enhances binding specificity. Even small changes of ligand structure can have dramatic consequences on their ability to bind and the efficiency of this process. The development of multivalent carbohydrates further helps to understand how high-affinity lectin ligands may aid in the diagnosis of inflammatory disease processes, pathogen recognition, and the modification of immune processes. A variety of interactions are now known to take place between multivalent ligands and receptors.

Though multivalency approaches are gaining acceptability among researchers, in terms of their potential within therapeutic and diagnostic applications, the underlying mechanisms of how affinity is increased are not well understood. Indeed, additional insights into the structural aspects of such interactions are required, alongside innovative developments to further examine the multivalent interaction structure. The Wittmann group has utilised X-ray crystallography and EPR spectroscopy to gain a better mechanistic understanding of protein–ligand interactions. Though structural information of ligand-receptor complexes is rare, the researchers managed to unravel the structure of a ligand multiply bound to wheat germ agglutinin. The result provided the basis for the development of a new type of multivalent ligands currently under investigation.

**Recently, the Wittmann group achieved imaging of protein-specific glycosylation within living cells using the inverse-electron-demand Diels-Alder reaction**

## Q&A

***What sparked your interest in metabolic glycoengineering?***
When I first read about bioorthogonal chemistry, I was fascinated by the possibility of carrying out chemical reactions in living systems. These reactions are a good example of the fact that chemistry does not necessarily stand for harsh conditions as is often the perception by the public. Metabolic glycoengineering provides the opportunity for a true interdisciplinary collaboration between chemists and biologists and a way of using chemistry to answer biological questions.

***How versatile is this approach? Which other areas of research or medical investigation could it be applied to?***
Metabolic glycoengineering offers a number of applications in diagnosis, therapeutic treatment, and basic science. Many diseases are associated with changes in the glycosylation pattern on the cell surface. In cancerous tissues, for example, sialic acid levels are increased. This could be exploited to visualise such tissues by metabolic glycoengineering. In addition, the approach can be used to direct drugs to such tissue areas, allowing a selective treatment (drug targeting). In basic science, the approach has great value to study the impact of the glycosylation of specific proteins of interest on their biological function.

***What are the next steps for your research?***
We are constantly working on the development of new chemistries enabling improved bioorthogonal ligations reactions. This includes the search for smaller probes that react even faster than the established ones and their application to diverse biological systems (cells, zebrafish, and nematodes). The field provides a playground for talented and creative researchers, and I have many of them in my wonderful group of co-workers. Currently, we apply metabolic glycoengineering to investigate the role of protein glycosylation in proteostasis (the entirety of processes that control the activity of cellular proteins). This research is carried out within our collaborative research centre SFB 969 which is just one example of the close collaboration between our Chemistry and Biology Departments fostered by the University of Konstanz.

***Which future predictions could you make about metabolic glycoengineering?***
Carbohydrates are found everywhere, and the majority of all proteins are glycosylated. Nevertheless, the glycans are often neglected in protein research. I expect that metabolic glycoengineering will have a huge impact on glycobiology. It will not only help to identify, enrich, and isolate glycoproteins but will also be a tool to change properties of proteins or cells in a desired manner. Since different glycosyltransferases have different substrate specificities, it might be possible to learn more about the function of specific glycosyltransferases. The technique is also suited to introduce chemical functionalities in the components of the extracellular matrix with implications for biomaterial science, including tissue engineering.

***What are the prerequisites to carry out research at the chemistry–biology interface?***
In an ideal case you need an education in both chemistry and biology. Therefore, we started the study programme 'Life Science' in Konstanz 15 years ago. This programme, which is jointly offered by the two departments (Chemistry and Biology), provides training in both disciplines and was the foundation of a very successful cooperation of the departments leading to the joint collaborative research centre SFB 969 funded by the Deutsche Forschungsgemeinschaft (DFG) and the Konstanz Research School Chemical Biology (KoRS-CB) funded within the excellence initiative. Nowadays it is very common that researchers from one department go over to the other department, being in the same building, to discuss current research.

## Detail

**RESEARCH OBJECTIVES**

Professor Wittmann's research focuses on elucidating the biological functions of carbohydrates. He has carried out a whole host of research into metabolic glycoengineering over the years, and is also especially interested in the investigation of multivalent carbohydrate–protein interactions and RNA-targeting antibiotics.

**FUNDING**

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**COLLABORATORS**

University of Konstanz:
- Prof Andreas Zumbusch
- Prof Thomas U Mayer
- Prof Andreas Marx
- Prof Malte Drescher

Fraunhofer Institute Stuttgart:
- Prof Petra Kluger

University of Bonn:
- Prof Günter Mayer

University of Freiburg:
- Prof Maja Köhn

**BIO**

Professor Wittmann received his PhD from the Technical University of Munich before carrying out postdoctoral research at the Goethe University of Frankfurt and The Scripps Research Institute in La Jolla, California. Since 2003, he has been professor of organic/bioorganic chemistry at the University of Konstanz, and in 2016 became the head of the Department of Chemistry and the vice coordinator of the Collaborative Research Center SFB 969.

**CONTACT**

Prof Valentin Wittmann
University of Konstanz
Department of Chemistry
Universitätsstr. 10
78457 Konstanz
Germany

**E:** Valentin.Wittmann@uni-konstanz.de
**T:** +49 (7531) 88-4572
**W:** www.valentin-wittmann.de