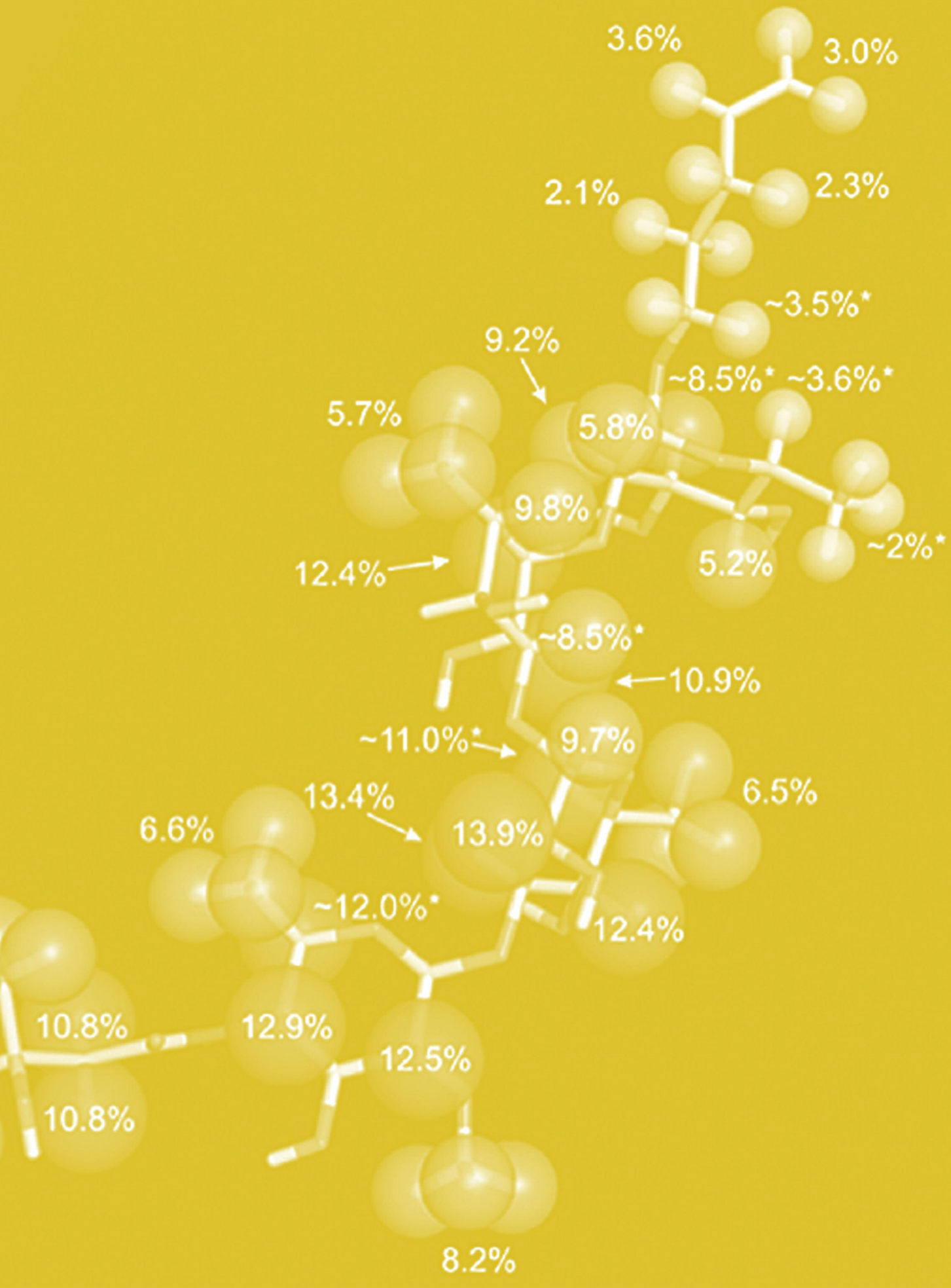
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→ Glycoimmunology
→ Carbohydrate Synthesis
→ Glycoproteomics
→ Precision Polymers and Polymeric Biomimetics
→ Biomolecular Systems
→ (GPIs) and Glycoproteins

BIOMOLECULAR SYSTEMS

Research in the Department of Biomolecular Systems

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1989: Vordiplom (Univ. Erlangen-Nürnberg)
1995: PhD, Biochemistry (University of Colorado, Boulder)
1995-1997: Research Fellow (Memorial Sloan-Kettering Cancer Center)
1998-2003: Assistant and Associate Professor (Massachusetts Institute of Technology, MIT, Cambridge, USA)
2003-2009: Professor (Swiss Federal Institute of Technology (ETH) Zurich, Switzerland)
Since 2003: Affiliate Professor (The Burnham Institute for Medical Research, La Jolla, USA)
Since 2009: Director, Department of Biomolecular Systems (Max Planck Institute of Colloids and Interfaces)

The Department for Biomolecular Systems, founded in 2009, conducts research at the **interface of chemistry, engineering, biology, immunology and medicine.** The approach is transdisciplinary and interactive between the groups in the department that cover different areas of expertise. The core focus is the development of **synthetic methods** for the chemical synthesis of defined oligosaccharides. The compounds are the basis for **chemical tools** that aided **biochemical investigations** into the fundamental roles complex carbohydrates play in biological processes that underlie disease. The findings helped create diagnostic carbohydrate arrays to begin to understand **immunological aspects** of malaria epidemiology. **Vaccine development** of several infectious disease carbohydrate vaccine candidates is becoming increasingly more important for the laboratory. In the past two years groups have been established to cover the other core technology, glycan sequencing and glycomics (Dr. Kolarich) in order to identify glycans of biological importance and to assess the role of glycans *in vivo* (Dr. Lepenies, glycoimmunology). We are actively pursuing different aspects of glycobiology including the structure, function and biological role of sugars found on the surface of mammalian and bacterial cells particularly in the areas of immunology, biochemistry and human disease.

Materials aspects related to carbohydrates have become increasingly important since our move. The group of Dr. Hartmann merges polymer synthesis with our biomolecule expertise and in close collaboration with the glycoimmunologists, the *in vivo* activity of the complex synthetic molecules is assessed. Continuous-flow synthesis has been drastically increasing in importance and is beginning to pervade all aspects of synthetic chemistry. Important collaborations for the synthesis of colloidal polymers have yielded exciting results and many other applications of the flow paradigm from organic to nanoparticle synthesis and polymer chemistry are currently progressing rapidly.

Automated Synthesis of Carbohydrates

The past two years, since our arrival in Potsdam the Department of Biomolecular Systems we have greatly expended on the first automated oligosaccharide synthesizer (Science 2001, 291, 1523). Now, the entire process is streamlined and based on a set of building blocks, a polymeric support and new linker as well as a new instrument, access to complex oligosaccahrides is not only fast but requires little technical expertise. The Department is beginning to close in on the ultimate goal of creating a commercially available instrument that uses a defined set of monosaccharide building blocks to assemble most oligosaccharides reliably.

The concept has been extended now to the automated synthesis of glycosaminoglycans, a class of biologically extremely important oligosacchrides (e.g. heparin). With the help of an ERC Advanced grant a new linker that is cleaved via continuous flow photochemistry, a new synthesis instrument and novel synthetic schemes were combined to create a process that speeds synthesis times from many months down to three days! This breakthrough will open completely new areas for biology but also material sciences involving growth factor interactions.

Synthetic Tools for Glycobiology
Using the synthetic oligosaccharides, we have expanded on the preparation of tools such as glycan microarrays, glycan nanoparticles, glacan dendrimers and glycans on polymers and fibers as well as inorganic materials such as quantum dots and zeolithes. These tools are now commonly used by the glycobiologists in the department to elucidate fundamental processes such as the entry mechanism of parasites into host cells.

Synthetic Carbohydrate Vaccines
Using synthetic oligosaccharides as basis, the department is now advancing a large number of carbohydrate-conjugate vaccine projects. While in the past the focus was almost exclusively on the synthesis, we are now conducting also conjugation and formulation as well as immunological assessment. With this integrative approach, vaccine development has accelerated in house and a number of molecules are rapidly advancing. Following earlier work on malaria (Nature 2002, 418, 785) a host of antigens against bacterial diseases are now at different stages of development.

Carbohydrate-based Nanotechnology
The attachment of carbohydrates to the surface to nanoparticles and surfaces has seen rapid progress in the department since the move to Potsdam. With the ability to characterize the products of our studies much more thoroughly and faster than previously, metalloglycodendrimers, glyco-quantum dots, glyco-gold islands and glyco-fullerenols have been prepared and are now beginning to see applications in biology and even in applications towards clinical use.

Glycoimmunology
The immunology group investigates the role of C-type lectin receptors (CLRs) in infections and autoimmune diseases. CLRs are carbohydrate-binding proteins of the innate immune system that share a conserved calcium-dependent carbohydrate recognition domain and include many endocytic receptors, collectins and selectins. CLRs belong to the innate immunity since they recognize conserved carbohydrate structures on pathogens and thus play a crucial role in the initiation of a protective immune response and for the maintenance of tolerance to autoantigens.

Animal models to analyze the function of CLRs during malaria infection and in autoimmune diseases such as colitis and encephalomyelitis have been established. The goal is to provide answers to the following questions: How is the expression pattern of CLRs altered during the course of infections and autoimmune diseases? Are CLRs involved in the induction of pathology during infection/inflammation? Do CLRs represent valuable drug targets to modulate ongoing immune responses *in vivo*?

Continous Flow Microreactors as Tools for Organic Chemists
Traditionally, organic chemists have performed chemical transformations in batch mode. Our department has pioneered the use of continuous flow microreactors for use by synthetic organic chemists. The department has utilized commercially available as well as internally developed microreactor systems to develop an automated reaction screening platform for organic chemists. Using these microreactor systems a host of chemical transformations has been rendered more efficient. In particular, dangerous, highly exothermic reactions as well as radical chemistry and photochemistry have benefited from the new way to run synthetic organic chemistry. Currently, these systems are being expanded to a host of applications in the area of total synthesis, methods development but most importantly, also to the preparation of organic and inorganic nanoparticles and colloids.

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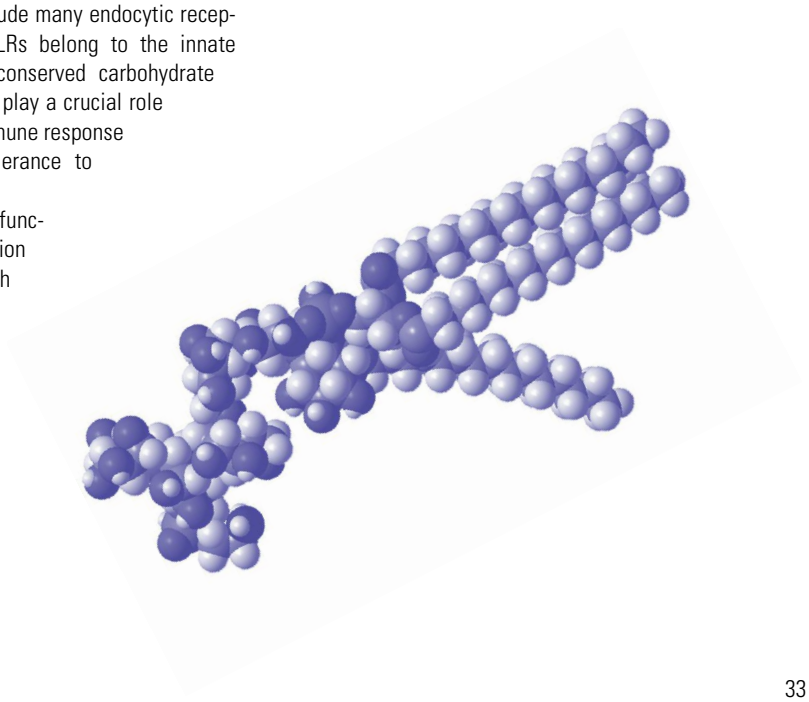

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Targeting C-type Lectins to Modulate Immune Responses

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2005-2007: PhD, Biology ("summa cum laude")
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Thesis: Role of the co-inhibitors CTLA-4 and BTLA in T cell regulation during malaria
2008: Postdoc, Chemical Biology (Swiss Federal Institute of Technology, ETH Zurich, Switzerland)
Since 2009: Group Leader, Glycoimmunology (MPI of Colloids and Interfaces, Potsdam)

Host defense relies on a concerted action of both innate immunity and adaptive immunity. In this interplay, innate immunity encompasses numerous rapid defense mechanisms to infections and other challenges. Cells of the innate immune system use a variety of so-called pattern recognition receptors to recognize molecular structures shared between pathogens. C-type lectin receptors (CLRs) are carbohydrate-binding receptors that recognize glycan structures on pathogens (as shown in **Fig. 1**).

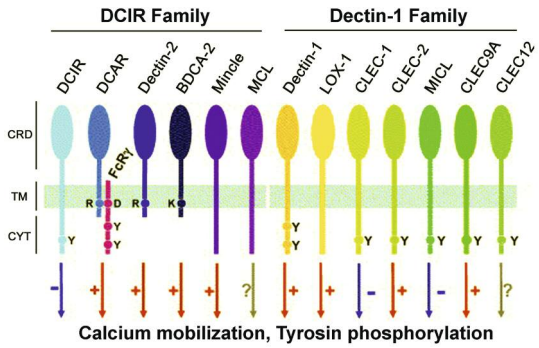

Fig. 1: C-type lectin receptors (CLRs) in the immune system are expressed by antigen-presenting cells, particularly dendritic cells. They are crucial for antigen uptake and cell activation. Dendritic cell immunoreceptor (DCIR) and DC-associated C-type lectin (Dectin-1) are the prototypes for CLRs on dendritic cells.

They are predominantly expressed on antigen-presenting cells such as dendritic cells and bind carbohydrate structures in a Ca^{2+}-dependent manner. Many endocytic receptors, collectins, and selectins belong to the CLR superfamily. In the immune system, CLRs are mainly involved in pathogen recognition. Additionally, they are important for the maintenance of tolerance to autoantigens **[1]**. The main goals of our group are, first, to understand in detail how CLRs influence inflammatory processes *in vivo* and, second, to exploit CLRs for cell-specific targeting and immunomodulation.

Targeting of C-type Lectin Receptors

Targeting glycan-binding receptors is an attractive approach to inhibit competitive binding of natural ligands or to deliver drugs specifically into cells expressing these receptors. However, since glycan-binding proteins exhibit only a low affinity for their ligands, multivalent interactions are often required to exhibit biological effects **[2]**. In proof-of-principle experiments, we showed that a specific targeting of the CLR asialoglycoprotein receptor (ASGP-R) can be achieved by using multivalent carbohydrate ligands **[3]**. ASGP-R is a glycoprotein that binds to desialylated (i.e. galactosyl-terminal) glycoproteins and is expressed exclusively in hepatic parenchymal cells. When hepatocytes were incubated with quantum dots (QDs) capped with D-galactose (D-Gal) or control sugars, preferential uptake of D-Gal QDs was observed *in vitro* caused by ASGP-R-mediated endocytosis. Similar results were obtained when galactose dendrimers and liposomes that displayed D-GalNAc-terminated lipids were employed to target ASGPR **[4]**. Thus, dendrimers and glycoliposomes displaying multiple D-Gal/D-GalNAc residues are suitable hepatocyte-specific targeting systems. Moreover, intravenous injection of QDs capped with terminal D-Gal or D-GalN residues into mice resulted in specific sequestration of those QDs in the liver (see **Fig. 2**).

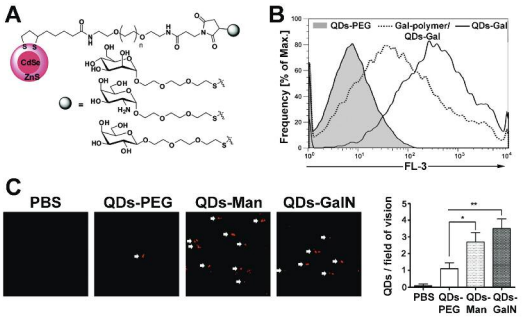

Fig. 2: Targeting of asialoglycoprotein receptor (ASGP-R) using carbohydrate-capped quantum dots. A, Quantum dots (QDs) and sugars used in this study (n = 45–50). B, Specific uptake of D-Gal capped QDs by HepG2 cells. For inhibition of Gal-QDs binding and uptake, HepG2 cells were preincubated with a PLL-Gal-polymer. C, Specific liver sequestration of D-mannose (D-Man) and D-galactosamine (D-GalN) capped QDs. Mice were i.v. injected with PBS, 2.5 nmol PEG-QDs or QDs capped with D-Man or D-GalN. 2 h after injection mice were sacrificed, paraffin sections of the livers were prepared, and QD sequestration was visualized by fluorescence microscopy [3].

In conclusion, carbohydrate-protein interactions exhibit specificity and, thus, glycan-binding receptors such as CLRs may be valuable targets for cell-specific drug and gene delivery *in vivo*.

Immunomodulation via C-type Lectin Receptors

Carbohydrate synthesis represents a useful tool to develop vaccines against infectious diseases or cancer and to synthesize ligands for glycan-binding proteins **[5]**. Additionally, synthetic carbohydrates may be used as "danger signals" for the immune system to provoke a pro-inflammatory immune response. This is of particular interest for the design of novel adjuvants. We aimed at targeting CLRs expressed by dendrit-

ic cells by using synthetic carbohydrates chemically coupled to model antigens. For this purpose, phosphatidylinositol mannoside (PIM) glycans, biologically important glycoconjugates present in the cell wall of *Mycobacterium tuberculosis*, were used. The synthetic PIM glycans were immobilized on microarray slides and were shown to bind to the dendritic cell specific intercellular adhesion molecule-grabbing non-integrin receptor (DC-SIGN). Interestingly, the PIM glycans served as efficient immune stimulators and increased the efficacy of vaccines. Immunization of mice with model antigens covalently coupled to the PIM glycans led to increased antibody levels and T cell effector functions such as cytokine release compared to well-established adjuvants (as shown in **Fig. 3**).

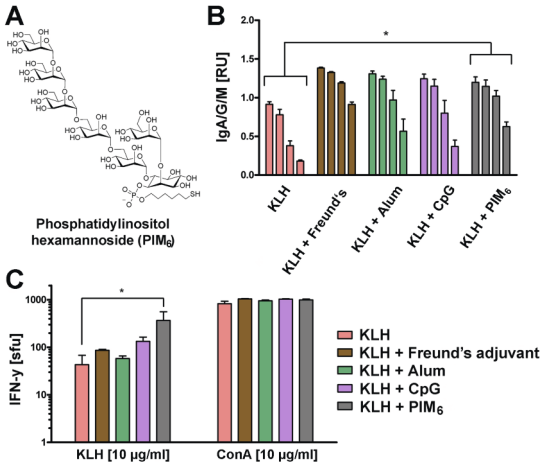

Fig. 3: CLR targeting with synthetic PIM glycan. A, Structure of the synthetic PIM6 glycan from Mycobacterium tuberculosis. B, C57BL/6 mice were s.c. immunized with keyhole limpet hemocyanin (KLH) in the presence of the indicated adjuvants or after covalent linkage to PIM6. On day 17 after immunization, levels of anti-KLH antibodies were measured by ELISA in serial dilutions of the sera. C, On day 20, splenocytes were stimulated with KLH or concanavalin A (ConA) and the frequency of IFN-producing cells was determined by ELISpot [6].

This finding indicates that synthetic glycan structures indeed have a great potential as adjuvant candidates **[6]**.

Functionalization of surfaces with glycans is another way to influence cellular functions such as proliferation or differentiation and to modulate inflammatory responses. In this respect, we could show that D-mannose-functionalized PCL/PPfpMA fiber meshes enhanced the cytokine production by murine macrophages upon lipopolysaccharide (LPS) stimulation whereas control fibers functionalized with galactose- or aminoethanol had no effect on cytokine production **[7]**.

Tools for Analyzing the Function of C-type Lectins

To investigate the role of C-type lectin receptors in inflammatory processes *in vivo*, we are currently establishing mouse models for infection and autoimmunity. To identify yet unknown carbohydrate ligands for CLRs, additional tools are needed. One such tool that we have already constructed is CLR-Ig fusion proteins consisting of the extracellular domain of the respective CLR and the constant fragment of antibody molecules (shown in **Fig. 4**).

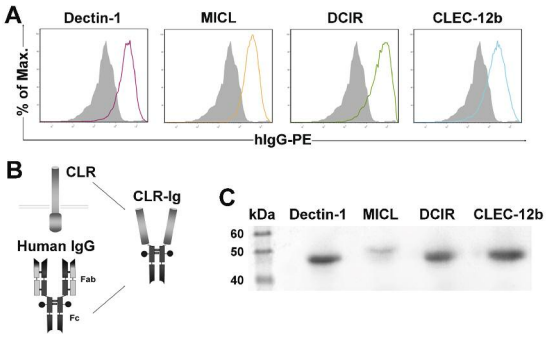

Fig. 4: A, Eukaryotic expression of different CLR-immunoglobulin (CLR-Ig) fusion proteins in CHO cells. B, Schematic representation of CLR-Ig construction. C, Western Blot to detect CLR-Ig protein levels in CHO cells. CLR-Ig fusion proteins were expressed for the following CLRs: CLEC-1, CLEC-2, DCIR, DCAR, MCL, Mincle, Dectin-2, Dectin-1, Lox-1, CLEC-9a, MICL, CLEC-12b, MGL.

These fusion proteins are then used to screen libraries of glycan structures by glycan array, a method that allows for testing carbohydrate-protein interactions in a high-throughput manner. Using the glycan array platform, we will identify new CLR ligands that are further characterized for their immunomodulatory properties in cell-based assays and *in vivo*. We are also producing monoclonal anti-CLR antibodies with either blocking or agonistic activity to be administered as CLR agonists or antagonists in mouse models of infection and autoimmunity.

Finally, the physiological function of a protein of interest can be best investigated in animals lacking the respective protein. Consequently, CLR-deficient mice are currently being generated in our group. In those mice, the functional copy of a CLR gene is swapped for the mutated (inactive) version in mouse embryonic stem cells. These "knockout mice" will provide a deeper insight in how CLRs act *in vivo* and which role CLRs play in infectious and autoimmune diseases.

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Automated Solid Phase Synthesis of Complex Carbohydrates

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Oligosaccharides are one of the most important classes of biomolecules. They are involved in a variety of biochemical processes, such as cell differentiation, proliferation and adhesion, inflammation and immune responses. **[1, 2]** In higher organisms, proteins are posttranslationally modified by the attachment of different oligosaccharides, influencing the physical and chemical properties of a glycoprotein such as folding, solubility, charge, and half-life. Due to their microheterogeneity the isolation of pure glycoproteins from natural sources is a tedious process and many functions of these complex carbohydrates are poorly understood. Another promising approach is the use of specific carbohydrates on the surface of parasites, bacteria or cancer cells for the creation of vaccines. However, it is often difficult or even impossible to cultivate those organisms as source for the desired carbohydrate structures. Chemical synthesis is a powerful tool in order to furnish sufficient amounts of pure oligosaccharides for biological evaluation or vaccine production. However, the classical solution phase synthesis of oligosaccharides is often a time-consuming task and usually requires a special strategy for each molecule.

Automated Solid Phase Synthesis

To overcome these problems solid phase synthesis is a powerful alternative approach. A solid support functionalized with a linker carries the growing oligosaccharide chain during the synthesis. During this process a building block is glycosylated to the linker followed by removal of a temporary protecting group for the next chain elongation step (see **Fig. 1**). By using a large excess of reagents complete conversion in glycosylation and deprotection reactions can be achieved. The number of chromatographic purification steps is reduced to a minimum because excess reagents can be removed simply by washing. After complete assembly of the oligosaccharide chain, the product is cleaved from solid support followed by global deprotection of permanent protecting groups. This approach could lead to carbohydrate structures of interest, that could be further used for the synthesis of glycoconjugates or glycoarrays (see **Fig. 1**).

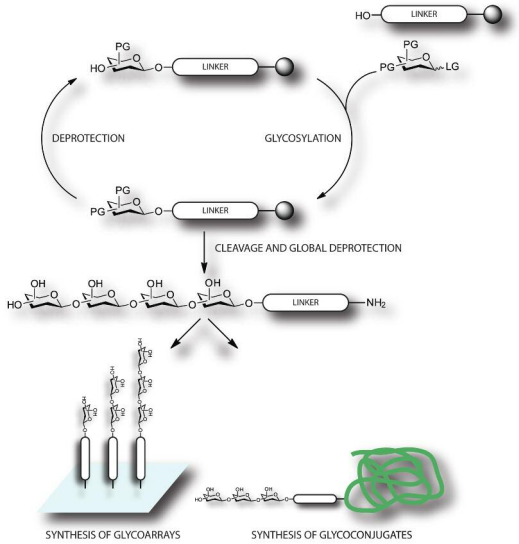

Fig. 1: Schematic Overview of Solid-Supported Oligosaccharide Synthesis: Connected via a linker the oligosaccharide chain is elongated on the solid support in alternating glycosylation and deprotection reactions. After cleavage from the resin and global deprotection the oligosaccharides can be used for the synthesis of glycoconjugates or glycoarrays.

Fully Automated Carbohydrate Synthesizer

The repetitive character of solid-supported oligosaccharide synthesis makes this process suitable for automation like in peptide or oligonucleotide synthesis. The first syntheses were performed successfully in a modified ABI peptide synthesizer, which features were adapted for carbohydrate chemistry. **[3]** With the help of this setup it was shown that automated synthesis reduces the expenditure of time dramatically **[4]** and an anti-malaria toxin was already obtained. An anti-malaria vaccine was obtained from this GPI anchor derivative and it is expected to enter clinical trials in 2011. **[5]** To meet the specific requirements of carbohydrate synthesis properly, a fully automated carbohydrate synthesizer was developed (see **Fig. 2**). **[6]**

Fig. 2: Automated Oligosaccharide Synthesizer: The reactions take place in the reaction vessel (5) and are completely controlled by a PC (1) software and a controller (9). The temperature can be adjusted by a cryostat (2) and the reagents (3) are delivered via syringe pumps (6). Solvents (8) to wash the resin are delivered and removed by Ar pressure (7) and solenoid valves (4) and reaction solutions can be collected in a fraction collector (10) for further analysis.

Reactions take place in a double-jacketed reaction vessel which allows for cooling and heating of the reaction partners. The solvents used for washing the resin between the individual reaction steps are delivered and removed by argon pressure via solenoid valves and reagents and building blocks are added with the help of syringe pumps, rotary valves and loops. The reaction solutions can be discarded or collected in the fraction collector for later analyses, such as Fmoc quantification for evaluating the coupling efficiency. The entire process is fully controlled using special programs, which are composed of various modules for the different chemical reactions and washing steps. A controller acts as an interface between the computer and all components of the synthesizer. This setup allows the complete synthesis of oligosaccharides without further intervention.

Automatically Obtained Oligosaccharides

In combination with the development of the new prototype of synthesizer, also a versatile linker was designed. **[6]** The linker enables the use of a greater variety of building blocks and was used for the automated solid phase synthesis of β-1,6-linked glucosamine oligosaccharides (see **Fig. 3**).

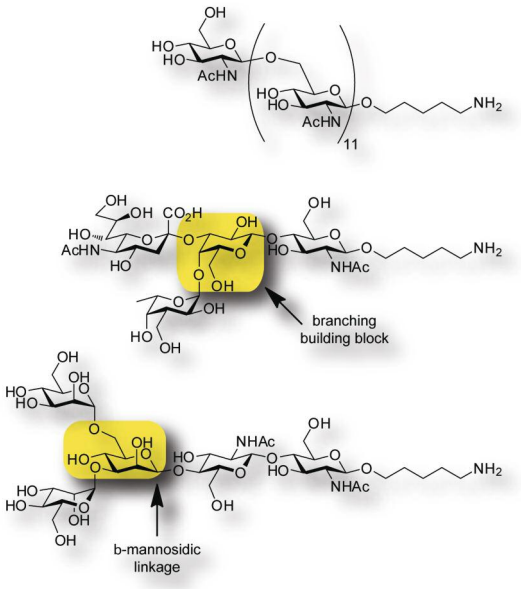

Fig. 3: Automatically Synthesized Oligosaccharides: The β-1,6-glucosamine was synthesized in different chain lengths with a miximum of 12 sugar residues. By using orthogonal protecting groups, a branched Sialyl Lewis X tetrasaccharide was obtained. For the synthesis of the core pentasaccharide of N-glycans the challenging β-mannosidic linkage was successfully glycosylated on solid support.

These carbohydrate structures are for instance involved in biofilm formation of *Staphylococcus* bacteria. Oligomers with up to twelve sugar residues were synthesized in 43% yield after cleavage from solid support. By using orthogonally removable protecting groups, a branched Sialyl Lewis X tetrasaccharide was obtained. Another automated synthesis furnished the common core pentasaccharide structure of *N*-linked protein glycosylations. This *N*-glycan contains a challenging β-mannosidic linkage, which was introduced in former approaches by the use of a disaccharide building block, already bearing this concerning linkage. Using an appropriate glycosylating agent **[7]** the *N*-glycan core pentasaccharide was synthesized for the first time on solid support from monosaccharide building blocks. All these syntheses proove, that the automated oligosaccharide synthesizer can be used to obtain complex and biologically relevant carbohydrate structures.

Automated Synthesis of Glycosaminoglycans

A current project is the automated synthesis of glycosaminoglycans that are linear oligosaccharides containing a disaccharide repeating unit. Glycosaminoglycans bear sulfate and carboxylic acid groups and thus are highly negatively charged molecules. Due to this fact, their synthesis belongs to one of the greatest challenges for carbohydrate chemists. In order to avoid many solution synthesis steps after the assembly of the oligosaccharide chain, sulfation and partial deprotection steps should be carried out on solid support. This leads to a high demand on linker and building blocks for the automated synthesis of glycosaminoglycans. As a proof of principle, the synthesis of different chondroitin sulfates (see **Fig. 4**)

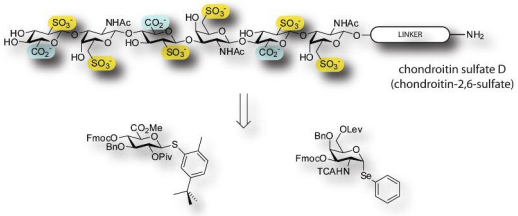

Fig. 4: Retrosynthetical Analysis of Chondroitn Sulfate D: After the assembly of the linear oligosaccharide chain the sulfate groups should be introduced on the solid support. Carboxylic esters should be hydrolyzed in an automated way followed by introduction of native N-acetates. This approach should be transferred to the synthesis of different glycosaminoglycans.

was chosen. In future reaction conditions could be transferred to the automated synthesis of different glycosaminoglycans by using a set of appropriate building blocks.

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Quantitative Glycomics and Glycoproteomics for Biomarker Discovery

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Lead Actors of Life: Glycoproteins

If the cell was a movie, DNA is the director, with proteins taking the lead roles. However, production of a blockbuster requires the perfectly organised actions of the whole film crew providing actors and director with all the requisites and conditions they require for their outstanding performance. In the last decades genomics (director) and proteomics (actors) have been rightly standing very much in the scientific spotlight, providing a tremendous boost in these areas that has resulted in ground breaking genomic toolsets enabling mankind to sequence genomes in a couple of weeks to months and proteomics applications and technology that allow scientists to identify even the tiniest amounts of proteins present in a cell. The information output of these experiments is without doubt invaluable and has set an indispensable basis for various biosciences. Nevertheless, it has become more evident that knowing who is directing and playing the lead does not result in knowing and understanding the plot. So looking at the bigger picture on how cells communicate, how cells interact and what distinguishes „good" cells from „bad" ones requires tools that provide us with the means that not only tell us who is acting and directing but what the costume, make up and script of the actors look like.

Protein Function Fine Tuning by PTMs

One of the cell's most important costume and makeup artists are the protein Post Translational Modifications (PTMs) such as phosphorylation and glycosylation. These PTMs are frequently used by nature to influence the biological activity of proteins and endow them with additional functions. Phosphorylation is mostly found on intracellular proteins, whereas various different types of glycosylation are the common modifications found outside the cell on secreted and membrane proteins. More than 50% of human proteins are predicted to be glycosylated **[1]**, however knowledge on the type of glycosylation and its impact on the biological activity of particular glycoproteins is still restricted due to the great heterogeneity of glycoproteins, their involvement in diverse biological events and the lack of adequate methods for high throughput glycoproteomics screening that simultaneously includes both parts of the glycoprotein – the peptide AND its glycosylation.

Glycoproteomics requires interdisciplinary interaction between glycomics and proteomics

Understanding the intra and inter-cell biological protein functions requires sophisticated and novel tools and means for identifying and characterising PTMs and their location on the proteins. In principle, glycoproteomics is subdivided in three categories (**Fig. 1**) **[2]**: glycoprotein focused glycoproteomics is targeting the concomitant identification of both aspects of glycoproteins, their protein backbone as well as their glycosylation and in the best case also providing site specific structural information on the glycans present on a particular site. This glycoprotein focused approach provides the maximum in information, however is currently more constrained to bottom up analyses rather than top down approaches due to the limitations of available software and almost exponentially increasing complexity of analysing peptides including their glycosylation.

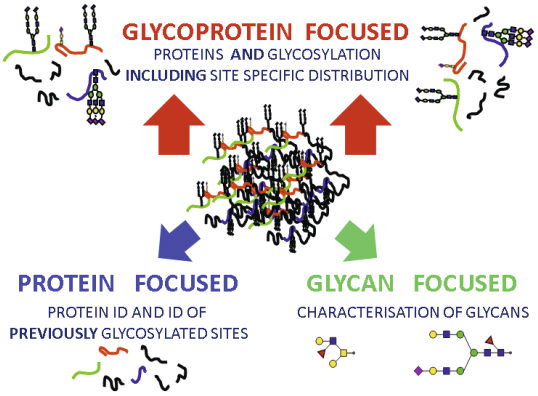

Fig. 1: Variations of Glycoproteomics. The glycoprotein focused approach targets at obtaining the most comprehensive information from glycoproteins and is supported by the protein and the glycan focused approaches.

PGC LC ESI MS/MS Glycomics

Porous Graphitized Carbon (PGC) LC ESI MS/MS provides the perfect basis that allows development of a ground breaking platform for simultaneous qualitative and quantitative glycomics. Current technologies are limited with regard to accurate absolute quantitation and/or appropriate glycan isomer identification and quantitation. The work in this project, however, will take high throughput PGC LC ESI MS/MS, to a completely novel and never before seen level of accuracy for both, qualitative and quantitative glycomics. One of several reasons making PGC LC ESI MS/MS the method of choice is its supreme power of separating so called isobaric glycan isoforms, structures that have the same mass but differ in the way the monosaccharide building blocks are linked together (**Fig. 2**) **[3]**. Exact and distinct differentiation of altered linkages is however crucial when dealing with glycoproteins since these differences are one of nature's opportunities for altering biomessaging pathways. Thus understanding glycoprotein functions and how e.g. cell-cell interactions are regulated requires efficient, sensitive and high throughput ways of differentiating exactly these structural features and is best done using released glycans.

Glycoproteins – Promising Biomarkers

Glycosylation has been shown to have tissue specific characteristics and reflect changes in various diseases particularly in cancer and inflammation **[4]**. Various proteomics studies on tissue and/or plasma often report differences between healthy and disease states at the protein level **[4, 5]**, however unless a tissue or disease specific protein is identified (e.g. prostate specific antigen [PSA], itself a glycoprotein **[6]**), single protein biomarkers alone often lack specificity and do not allow for unambiguous conclusions about a disease and its progression. Exploiting concomitantly both the glycan AND protein aspect of glycoproteins as diagnostic markers promises more specificity and sensitivity **[7, 8]** and will improve diagnosis and monitoring of diseases. Furthermore, an integrated approach investigating both aspects of glycoproteins will also lead to better understanding of biological processes and may result in identification of unique signatures for certain diseases.

Glycoproteins and Inflammatory Bowel Diseases

Crohn's disease and ulcerative colitis are chronic inflammatory diseases resulting from an inappropriate immune response to microbial antigens of commensal microorganisms. This inappropriate response is promoted by certain environmental factors, genetic predisposition, nutrition, environment and ethnicity. Both diseases manifest themselves primarily in the gastrointestinal tract yet can, in principle, affect all of the organ systems of the body. More than 300 000 people in Germany alone suffer from IBD. The incidence and prevalence of IBD have risen in the past 10 years, particularly for Crohn's disease with every fifth IBD patient being a child or adolescent. IBD is also associated with an increased risk of colorectal cancer, which itself is already the third most common cancer in developed countries. A cure of IBD is still not possible, yet the opportunities for diagnosis and treatment did show some improvement in recent years **[9]**. Early diagnosis is important so that patients can be referred onward for further diagnostic evaluation and appropriate treatment without delay. Nevertheless, the exact causes and triggers are still not well understood and efficient and specific biomarkers are still not well developed. There are several independent studies showing the connection between lectin and toll like receptors in autoimmune diseases **[10, 11]**. The finding of a variety of antibodies directed against different bacterial glycans is one of the latest approaches for diagnosing IBD **[9]**, however these approaches focus on the body's immunological reaction rather than the actual events occurring in the affected tissue. These previous findings strongly substantiate the significance of approaching IBDs and auto immune diseases from a glycoproteomics perspective. Understanding IBD initiation and development will help improving diagnosis, monitoring and treatment of IBDs as well as increase the understanding of factors leading to the progression to bowel cancer.

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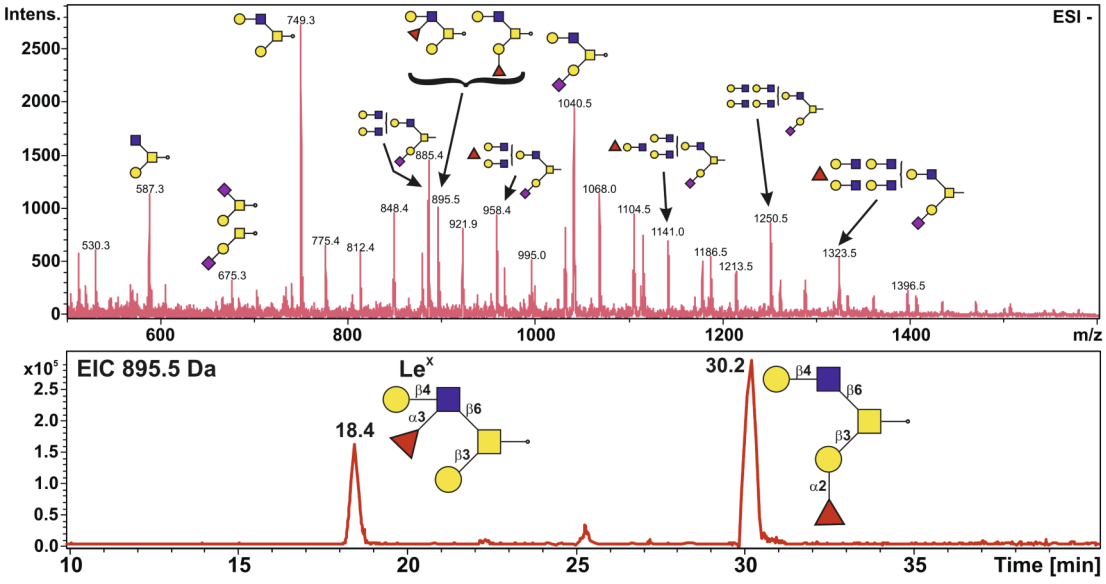

Fig. 2: Glycan isomer separation on PGC. Top: Mass spectrum summed from 16-35 min giving an overview of different glycan compositions present. Bottom: Extracted Ion Chromatogram (EIC) of m/z 895.5 corresponding to the singly charged O-glycan pentasaccharide. The difference of the fucose linkage and position results in significantly different retention times allowing separate analysis and thus clear distinction by MS of these isobaric glycan isomers. (modified from Kolarich D, Jensen PH, Cheah WY, Grinyer J, Packer NP, Protein glycosylation of human breast milk: an antimicrobial defense mechanism, manuscript in preparation)

Solid Phase Polymer Synthesis

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Introduction

Over the past decades, polymer-based materials have evolved as a powerful tool in biomedical and pharmacological applications. Designing a suitable polymer-based material for a specific biomedical application starts with the choice of monomer, but the crucial building block of material design is the homopolymer chain. The choice of chain length, geometry and the ability to couple different homopolymer chains influences the resulting morphology and chemical and biological properties, e.g. through the self-organization of block copolymers. In contrast to this approach, nature is able to control material properties using much smaller building blocks. The most dominant example is the primary sequence of proteins. Such molecular precision and advanced functionality is not yet possible for artificial polymer-based materials. Combining Nature's elegant approach with the existing possibilities of polymer nanotechnology, it seems feasible to achieve new generations of highly functional well defined polymeric materials. Therefore, the central focus of our work is the design and implementation of new synthetic strategies towards sequence-defined, monodisperse polymers using solid phase polymer synthesis.**[1, 2]**

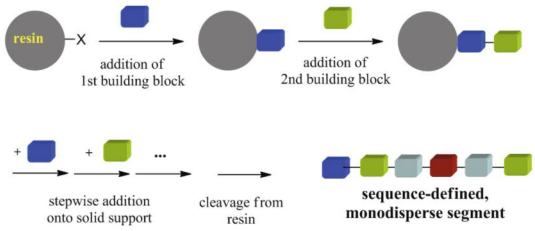

Fig. 1: General scheme for solid phase polymer synthesis. The stepwise addition of building blocks allows for the control over chain length and the positioning of different functionalities within the polymer chain.

In order to develop a straightforward synthetic route to sequence-defined, monodisperse polymer segments, a solid phase-supported synthesis strategy was applied (**Fig. 1**). The approach is based on the classical Merrifield solid phase peptide synthesis (SPPS). In theory, the carefully controlled stepwise addition of building blocks should provide polymer segments of a defined length with no molecular weight distribution. For every addition, a different building block can be used, introducing different functionalities within the chain depending on the choice of the monomer sequence.

Building Blocks for Solid Phase Polymer Synthesis

The choice of monomeric building blocks depends on a set of prerequisites: In order to maximize the flexibility and minimize the synthetic complexity for building block design, two different approaches are suitable (**Fig. 2**): 1.) The coupling of diacid and diamine building blocks allows for the formation of the polyamide backbone without the use of protecting groups.**[3]** 2.) Alternatively, a dimer building block can be used. This dimer is synthesized by condensing a diamine and diacid building block prior to solid phase coupling. In this case, an additional Fmoc-protecting group on the amine group must be introduced to avoid side reactions.

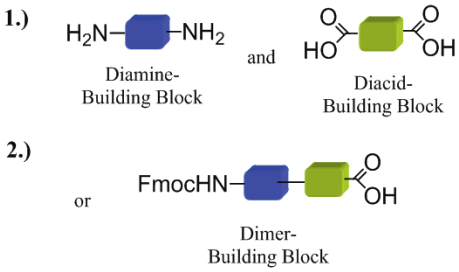

Fig. 2: Building blocks suitable for solid phase polymer synthesis can be either diamine and diacid building blocks (1.) or dimer-building blocks (2.) comprising a Fmoc-protected amine group on one side and a carboxylic functionality on the other side of the repeating unit.

In both cases, additional functionalities can be incorporated into the side chains for each monomer. Depending on the desired functionality, additional protecting groups may be required.**[4, 5]** In the ideal case each coupling will proceed with complete fidelity to avoid formation of side products and deletion sequences, thus eliminating the need for chromatographic separation after the final cleavage. Automated synthesis using a standard peptide synthesizer is of particular interest, since these systems have proven fast, efficient and reliable for generating defined polymeric structures. For this reason, the building blocks used on the synthesizer must be compatible with the reaction conditions suitable for automation such as chemical stability at room temperature, solubility in DMF or NMP and fast coupling reactions.

Introducing Functionality through Building Block Design

Taking the general concept of diamine and diacid or dimer building blocks, different functionalities can be introduced either in the polymer backbone or in the side chain. So far four different classes of building blocks have been established (**Fig. 3**).

The first class of building blocks (**Fig. 3**, A and B) are the functional building blocks. They introduce additional functionality for further modifications. Here, two examples are presented: The introduction of alloc-protected amines allows for modification of the building block following standard peptide coupling protocols (**Fig. 3 A**). Alternatively, the introduction of an alkyne group facilitates the use of click chemistry e.g. to attach azide-functionalized moieties (**Fig. 3 B**). These functionalities are of particular interest since they have been used to couple biologically active molecules such as monosaccharides. (**Fig. 3, C and D**). Another class of building blocks, known as charged building blocks (**Fig. 3, E and F**) focuses on the introduction of different charges such as cationic amine functionalities or anionic sulfate groups. Here, protecting groups are necessary to maintain solubility and avoid undesired side reactions during the solid phase coupling process. The fourth class of building blocks introduces chirality to the building block design (**Fig. 3, G and H**). Chiral building blocks will help to further control the structure of the polymer segment. For example, they can induce formation of secondary structures such as helices through stereo-controlled intramolecular interactions.

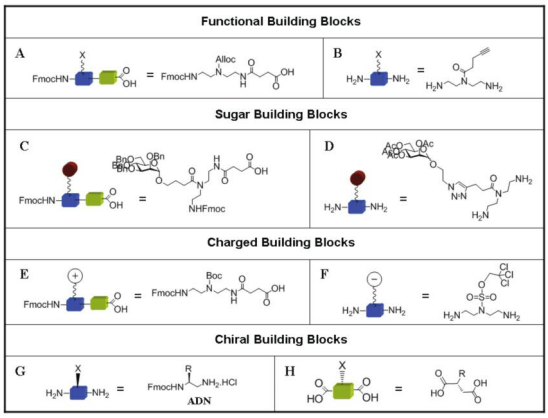

Fig. 3: Building Block Alphabet. Several building blocks suitable for solid phase polymer synthesis are shown. Functional building blocks allow for the modification through additional reactions steps e.g. by peptide chemistry or click chemistry (A and B). One important example thereof is the attachment of sugar moieties to the building blocks (C and D). Charged building blocks allow for the precise positioning of different charges within the polymer chain such as cationic amine groups or anionic sulfate groups (E and F). In order to control the structure of the polymeric backbone, chiral diamine and diacid building blocks have been introduced (G and H).[6]

An Example: Introducing Chirality to Monodisperse Poly(amidoamine)s

In order to obtain chiral building blocks suitable for solid phase synthesis, we introduced a synthetic strategy using a natural amino acid as starting material for the synthesis of a mono Fmoc-protected diamine building block. In a six-step synthesis starting from Boc-L-alanine, the chiral building block ADN was obtained in high yields and on a multi-gramm scale sufficient for solid phase synthesis.**[6]** Applying standard automated polymer synthesis protocols, the first polyamide oligomer was synthesized using succinic anhydride (Suc) as the diacid and ADN as the diamine building block. After addition of 4 repeating units, the final product NH_2-(Suc-ADN)$_4$-NHFmoc was cleaved off the resin and isolated by precipitation from Et_2O. Analysis by MS and HPLC confirmed the monodispersity of the final oligomer and the absence of any deletion sequence or undesired side products (**Fig. 4**).

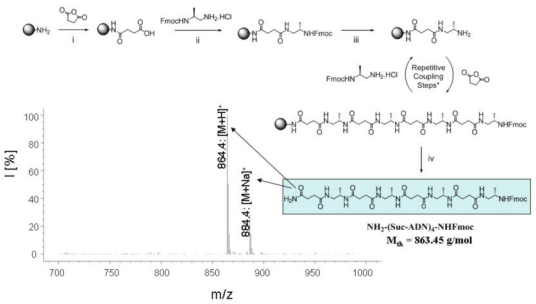

Fig. 4: Synthesis and analysis of the first poly(amidoamine) segment introducing chiral centers. The ESI-MS spectrum shows the signal for the monodisperse polymer segment and proves the absence of any side products or deletion sequences.

Outlook:

There is great potential for highly defined polymer systems with no molecular weight distribution and offering control over the monomer sequence. Our future work in this area will focus on expanding the repertoire of building blocks that can be used to generate polymers with different functional moieties and structural morphologies. These systems will be of particular interest for biomedical applications as they will serve as tools to study the direct correlation between the monomer sequence and the resulting chemical and biological properties. This will help to further understand the interactions of fully synthetic systems with biological systems and to use these interactions for the design of bioactive polymer-based materials.

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Synthetic Carbohydrate Vaccines

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Vaccines are efficient and cost-effective means for the global prevention of diseases. Carbohydrate antigens presented on the surface of disease causing pathogens have the potential to serve as vaccines. Candidates for carbohydrate based vaccines against cancer, viruses, bacteria and parasites form the focal point of research **[1, 2]**. Our department follows a comprehensive approach to synthetic carbohydrate vaccine development, starting from the synthesis of novel carbohydrate building blocks, to the generation of complex carbohydrate antigens, followed by conjugation to carrier-proteins and immunological evaluation including *in vivo* challenge studies.

Carbohydrate Antigens from Bacteria and Parasites

Carbohydrate structures of bacteria and parasites often differ significantly from mammalian structures and are recognized by the mammalian immune system. Carbohydrate-based vaccines against several bacterial pathogens such as *Neisseria meningitides, Streptococcus pneumoniae*, and *Salmonella typhi* are marketed. These vaccines are typically composed of purified carbohydrates isolated from the respective pathogen. The isolation of carbohydrates from cultured bacteria, however, often yields scarce amounts of heterogeneous oligosaccharide mixtures. Furthermore, only a small number of bacteria can be cultured in the laboratory. The synthesis of carbohydrates on the other hand gives access to large quantities of pure, well-defined carbohydrates.

Synthetic Carbohydrates

Automated oligosaccharide synthesis pioneered by our department is a powerful tool for the synthesis of carbohydrate antigens, as it allows for the rapid assembly of complex oligosaccharides. The targeted carbohydrate antigens are oligosaccharides composed of monosaccharide units. Most monosaccharide building blocks are readily accessed by modification of commercially available sugars. Some monosaccharides, specific to certain bacteria, are only accessible via *de novo* synthesis. One particular monosaccharide, 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose (AAT) is a component of zwitterionic polysaccharides (ZPSs) found on the cell surface of some pathogenic bacteria. ZPSs are of particular interest as they induce a T-cell-dependent immune response. AAT building block 2 (**Fig. 1**), synthesized in less than twelve steps from *N*-Cbz-L-threonine 1, was used to construct disaccharide 3, a fragment of the repeating unit of polysaccharide A1 (PS A1) 4 found in *Bacteroides fragilis* **[3]**. The first total synthesis of the PS A1 repeating unit 4 **[4]** was then performed starting from 3. PS A1 repeating unit 4 is currently being used to develop immunological probes for *B. fragilis*, which hopefully will help unravel the mechanism and action of zwitterionic PS A1.

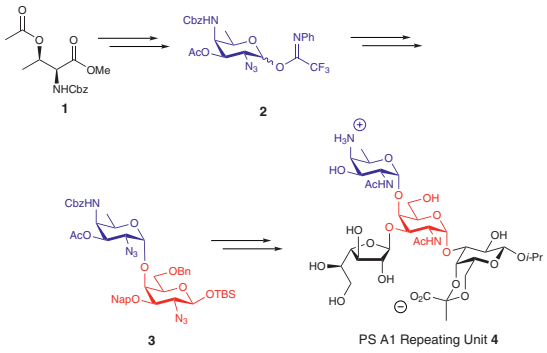

Fig. 1: Total synthesis of the repeating unit of the zwitterionic polysaccharide A1 4 from B. fragilis, via the AAT-containing disaccharide 3.

From Synthetic Carbohydrates to Vaccines

Carbohydrates are generally T-cell-independent antigens that neither promote immunoglobulin class switching from IgM to IgG nor memory responses, crucial for long-lasting protection. For this reason, carbohydrate antigens are covalently linked to carrier proteins. This leads to carbohydrate-specific antibody production and memory cells with carbohydrate based vaccines then eliciting long-lasting protection. The immune response can further be enhanced by the use of adjuvants.

Tools for Studying Carbohydrate Antigen-Antibody Interactions

A central element for understanding the mechanism of vaccines requires developing an understanding of the underlying antigen-antibody interactions. These are investigated by enzyme-linked immunosorbent assay (ELISA), glycan microarrays, surface plasmon resonance (SPR) and saturation transfer difference (STD) NMR.

Glycan microarrays consist of carbohydrates immobilized on surfaces in high density and spatially defined manner. They can be employed as diagnostic tools for the detection of anti-carbohydrate antibodies **[2]**. Glycosylphosphatidylinositol (GPI) microarrays were recently used to determine the specificity of anti-GPI antibodies for a synthetic GPI from *Plasmodium falciparum* in healthy and malaria diseased individuals **[5]**.

The synthetic tetrasaccharide component of the glycoprotein Bc1A of *Bacillus anthraxis* is immunogenic in mice, leading to the production of antibodies. Quantification of the tetrasaccharide-antibody interaction confirmed the tight binding previously observed with glycan microarrays. Furthermore, the tetrasaccharide-antibody pair was subjected to STD NMR experiments, which give insight in crucial binding elements of the antibody-binding surface of the carbohydrate antigen on atomic level (**Fig. 2**) **[6]**.

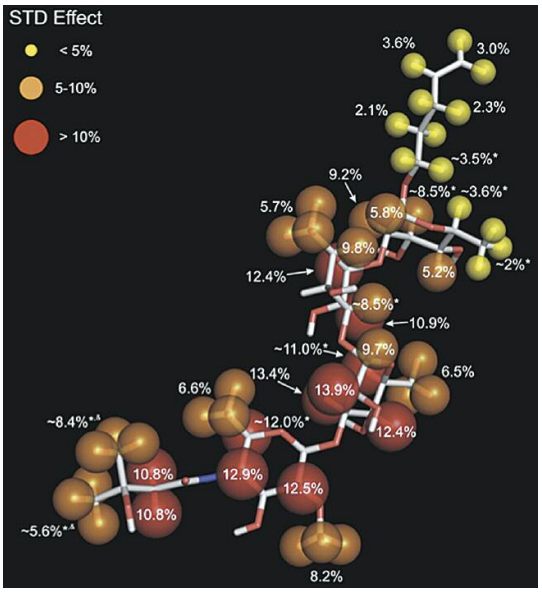

Fig. 2: Epitope mapping of the tetrasaccharide-antibody interaction by STD NMR. Percent STD effects are shown for individual protons of the tetrasaccharide.

The Future of Synthetic Carbohydrate Vaccines

Most carbohydrate-based vaccines on the market still contain purified carbohydrates isolated from biological material; this is due to the lack of accessibility to large amounts of synthetic carbohydrates. Automated oligosaccharide synthesis could help overcome this bottleneck, giving a significant boost to synthetic carbohydrate vaccines.

At the moment we are investigating a number of synthetic carbohydrate vaccine candidates, ranging from pathogens responsible for a large number of casualties in developing countries, to antibiotic resistant bacteria, typically found in hospitals of developed countries.

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Continuous Flow Reactors as Tools for Organic Chemists

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Our department has pioneered the use of continuous flow reactors by synthetic organic chemists. Continuous flow reactor systems offer significant advantages when performing reactions which are mixing controlled, or where heat- and mass-transfer are important. These properties derive from the exceptionally high surface-to-volume ratios accessible in continuous flow reactors. It is simple to scale-up microreactor processes either by numbering-up reactors or running the reaction for extended duration. We utilize both commercially available and internally developed systems (**Fig. 1**), ranging from microliter-volume etched silicon chips to milliliter-volume tubing reactors. Our work with these systems has exemplified the broad scope of their application, not only in traditional organic chemistry, but also in the preparation of polymers, nanoparticles and functionalized biomolecules.

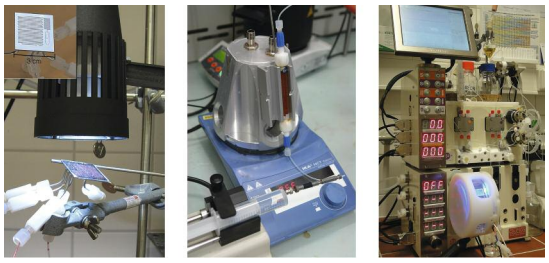

Fig. 1: Microreactor systems in our department (from left): 78.6 μL silicon chip under irradiation using an LED light and (inset) detailed view of the reactor chip; a packable column for the continuous flow use of solid reagents and catalysts; HPLC pump-driven Vapourtec R-Series system fitted with 10 mL cooled reactor.

Reactions of Hazardous and High-Energy Species

Continuous flow reactor systems provide ideal conditions for the detailed study of the formation and subsequent transformations of high-energy compounds. High temperature and pressure conditions can be achieved with improved safety and efficiency compared to batch processes; superheating of solvents is simple and hazardous reagents can be more safely handled by minimizing their concentration at the point of reaction. Additionally, the short residence times accessible in microreactors often reduce the potential for side reactions of highly reactive species. In addition to safe and efficient amidations of esters using pyrophoric $AlMe_3$ **[1]**, radical reductions **[2]** and fluorinations with the thermally-unstable fluorinating agent DAST **[3]**, we have studied the continuous flow generation of nitrenes *via* thermolysis of azides **[4]**. Continuous flow thermolysis of 3-aryl-2-azidoacrylates to give indole 2-carboxylates, previously requiring sealed tube conditions and extended heating of potentially explosive azides, has been successfully performed in our laboratory (**Fig. 2**). We applied this method to the synthesis of a variety of heterocycles, with exceptionally high productivity.

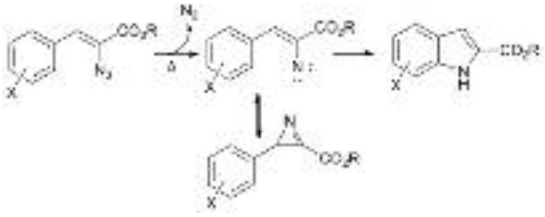

Fig. 2: Continuous flow thermolysis of azidoacrylates to give indole-2-carboxylates.

Polymers

Classical emulsion polymerization produces high molecular weight polymers at high rates of polymerization, rendering this process very attractive for industrial applications. Recent improvements in the regulation of reaction conditions, safety and quality control have prompted efforts toward miniaturization, embracing advances in microfluidics. In collaboration with the MPIKG Colloid Department and the ETH Zürich, we have developed a continuous flow emulsion polymerization process using phosphine oxide photoinitiators **[5]**. Polymer nanoparticles of very high molecular weights were formed by a novel mechanism (**Fig. 3**). Incorporation of phosphine oxide units into the polymer backbone induces repeated, snowballing radical generation upon irradiation where polymer-associated mono- and diradicals are created and do not terminate instantly. This process dramatically increases the radical polymerization rate and generates long polymer chains with ultrahigh molecular weights. The avalanche-like formation of radicals that occurs inside the latex particle also causes an enormous increase in the average number of growing polymer chains per particle. A stochastic model was used to simulate snowballing kinetics and quantitatively rationalize the polymerization process.

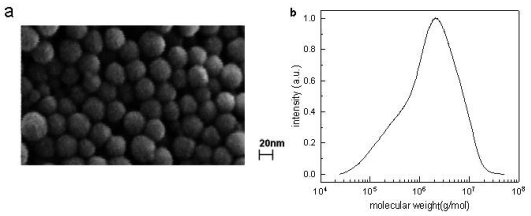

Fig. 3: a) SEM image of polystyrene, b) Molecular weight distribution of the polystyrene chains produced via photoinitiated emulsion polymerization.

Controlled free radical polymerizations (CRP) have evolved over the last 20 years into very useful and widely applied techniques for polymer synthesis, combining the excellent control of traditional ionic living polymerizations with robust conventional free radical polymerizations. Among these techniques, reversible addition fragmentation chain transfer (RAFT) represents the most versatile and facile method. In contrast to generally fast free radical polymerizations, the controlled living process requires longer reaction times.

Heating by microwave irradiation can considerably shorten the reaction times, but the scale-up of microwave reactions is difficult. We developed the first homogeneous RAFT polymerizations in a continuous flow reactor **[6]**. The polymerization is considerably faster when compared to batch reactions (**Fig. 4**). Thermoresponsive PNIPAM with apparent molecular weight of 20 kDa was obtained within minutes in flow, instead of hours in batch. The continuous flow polymerization exhibited similar kinetics as under microwave irradiation, but with the advantage of being readily scalable.

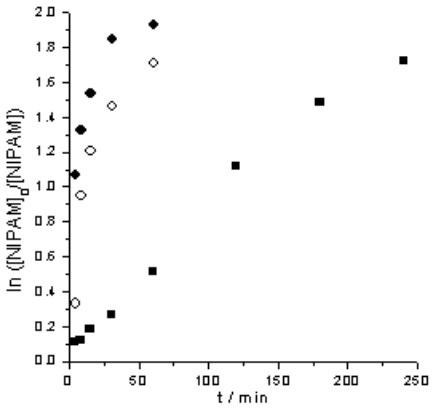

Fig. 4: Pseudo first-order plots of the polymerization at 90 °C and n = 200 in batch with conventional heating (■), microwave irradiation (●), and conventional heating in continuous flow (○).

Nanomaterials

The need for large quantities of monodisperse semiconductor nanocrystrals, (quantum dots – QDs), and the difficulty of their preparation via traditional batch techniques has prompted us to explore the use of continuous flow microreactors **[7]**. Taking advantage of the precise temperature control and efficient heat transfer of continuous flow microreactors allowed reduction of the reaction temperature from 300° C to 160° C. Lower temperature prevented the fast nucleation and generation of large non-homogeneous nanocrystals. By varying the residence time between 3 and 30 minutes, different sized CdSe and CdTe nanoparticles were obtained. The different size leads to different physical properties, especially the luminescence maxima (**Fig. 5**). Characterization of the different QDs by transmission electron microscopy (TEM) revealed highly crystalline, monodisperse, cubic nanoparticles. A microreactor was also used for the preparation of carbohydrate-functionalized QDs under mild liquid-phase conditions for the investigation of specific carbohydrate-lectin interactions.

Functionalization of Biomolecules

Dendronized polymers are multivalent, flexible systems that can bend to adapt to the environment of a pathogen surface and optimize binding to bacterial carbohydrate receptors. Functionalization of these polymers is challenging as the coupling reaction must be selective and high yielding, whilst not contaminating the end product. To address this challenge, we

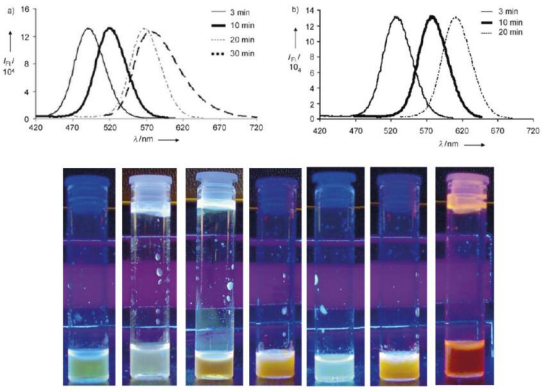

Fig. 5: Normalized luminescence spectra of a) CdSe nanoparticles in chloroform after 3, 10, 20, 30 min, and b) CdTe nanoparticles in chloroform after 3, 10, 20 min.

explored the usefulness of photochemical [2+2] cycloaddition, which can be carried out in water using inexpensive starting materials; it is pH independent and circumvented the use of heavy metals or other reagents that contaminate the polymer product **[8]**. Traditionally, photochemical reactions have been poorly scalable. Using a continuous flow photochemical reactor allowed us to develop an efficient, fast and readily scalable synthetic route to dendronized polymers. We are continuing to investigate the continuous flow conjugation of biomolecules with carbohydrates.

Reaction Optimization and System Development

In both industrial and academic settings, much of the effort spent by synthetic organic chemists is consumed searching for optimal reaction conditions to achieve a particular transformation. A key advantage to performing chemistry in microreactor systems is the speed with which mechanistic data can be obtained and conditions altered. Thus, only small quantities of reagent are required for the optimization process. We have developed screening platforms for the systematic study of glycosylation reactions: a transformation of critical importance for our department **[9]**. By combining automated screening with inline analysis and design-of-experiment algorithms, we are now developing completely automated optimization systems. We work closely with industrial partners in this area, using our experience in continuous flow microreactor technology for rapid process development.

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Glycosylphosphatidylinositols

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GPI-Anchored Proteins

Many proteins and glycoproteins are attached to membranes by means of a glycolipid called Glycosylphosphatidylinositol (GPI). In Malaria infection, specific GPIs were found to act as toxin, nevertheless the divers biological role(s) of these glycolipids is still under investigation. GPIs are becoming recently of high interest in carbohydrate based vaccines and glycobiology.**[1, 2]** The structure of the GPI molecule is variable and species depending, however a conserved common core pentasaccharide has been observed (**Fig. 1**). These GPI conserved core pentasaccharide have been reported containing different modifications like phosphorylations, acylations and additional glycosylations. **[3]**

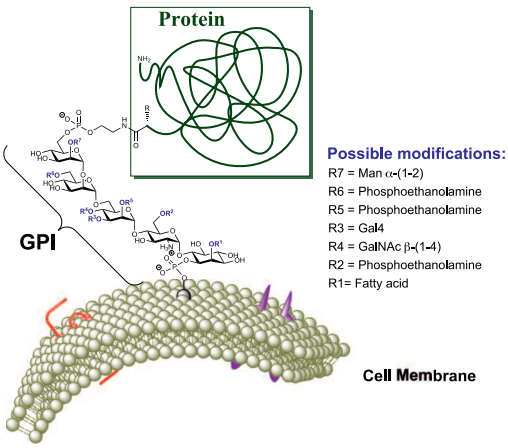

Fig. 1: Structure and modifications of the glycosylphosphatidylinositol (GPI) anchor.

An example of a GPI-anchored protein is the Prion protein (PrP), a protein known to be the infectious agent causing transmissible spongiform encephalopathies (TSEs). A secondary structural modification of the ubiquitous PrP to the infective form, called scrapie form (PrPsc) of the protein, increases the beta sheet conformation, which favored the aggregation of the protein and the generation of fibrils. The mechanism of this conformational transformation is still unclear; however recent studies have reported a faster spreading of this miss folding with the presence of the GPI-Anchor. **[4]**

In order to understand the role of the GPI in the conversion of PrP to PrPsc, in the pathogenesis of PrPsc and other different biological processes, the synthesis of uniform GPI units and GPI-anchored proteins is highly necessary.
Native chemical ligation (NCL) is actually the most extended method for the chemical synthesis of proteins, including proteins with posttranslational modifications.**[5]** This chemoselective reaction is based in a transthioesterification between a thioester and the thiol group of a cysteine residue following by an S→N acyl transfer to generate an irreversible new amide bond.

In a previous report of the Seeberger group **[6]**, an expressed PrP bound to a synthetic GPI unit with two phosphorylations was obtained by NCL. Although the GPI unit was not in its natural structure, the general feasibility of this strategy for obtaining GPI-anchored proteins was clearly demonstrated.

In order to obtain the natural GPI anchored PrP, the syntheses of the GPI unit have been improved and extended. The new general strategy allows now the incorporation of typical modifications found in mammalian GPI molecules as phosphorylation and branching in the Man 1 residue, lipidation and the cysteine residue required for the NCL (**Fig. 2**).

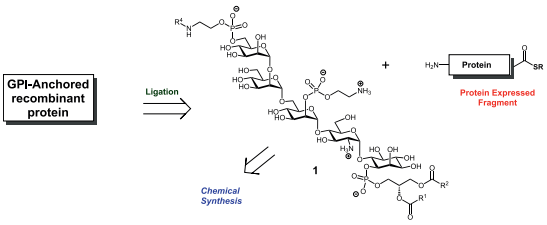

Fig. 2: General strategy for the synthesis of GPI-anchored proteins.

Synthesis of GPI Anchors

GPI molecules can been obtained by a modular strategy using different building blocks. The pseudo glycan part has been synthesized using a [2+1+2] glycosylation strategy with three building blocks (**Fig. 3**). After obtaining the building blocks, the efforts have been concentrated on two aspects of the synthesis: the introduction of three phosphorylations into the core glycan pentasaccharide using H-phosphonates and the synthesis of the branched glycan part (**Fig. 3**).

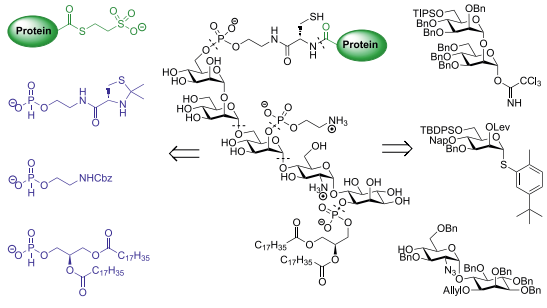

Fig. 3: Retrosynthetic analysis for the synthesis of GPI anchors.

A set of orthogonal protecting group was introduced to perform three phosphorylations sequentially. Three H-phosphonates were pre-synthesized from phosphonic acid under known conditions **[7]**, a diacyl glycerol, a phosphoethanolamine and a cysteine containing block. The activation of the H-phosphonates was performed with pivaloyl chloride and good yields were obtained.

Using the strategy described synthesis of the first fully phosphorylated Prion GPI has been achieved. Furthermore the strategy for incorporation of the galactosamine branching has been optimized. The synthesized molecules are now being used for NCL to obtain GPI-anchored proteins. These steps are currently performed and manuscripts summarizing these latest results are prepared for submission to well recognized journals.

The challenge of introducing the branching glycosylation found on the GPI of PrP and other mammalian GPIs was achieved by using the napthyl protecting group present in the strategy. This approach allowed us to obtain the core pseudopentasaccharide. The protecting group can be removed selectively using oxidative conditions. The pre-synthesized building blocks can be bound using different glycosylation strategies resulting in the desired GPI pseudo glycan structures before introducing the phosphorylations. This methodology enabled the elongation of the GPI glycan moiety with different branched structures up to a heptasaccharide. The availability of these synthetic GPIs builds the basis for the continuing with the next steps for synthesizing GPI anchored PrP and other proteins containing different carbohydrate structures. In future this will allow us to evaluate the biological role of GPI structures.

Synthesis of Homogeneous Glycoproteins

Glycoproteins are involved in diverse biological events like fertilization, neuronal development, hormonal regulation and immune and inflammatory responses. However, the influence of particular glycoprotein carbohydrate structures on these processes is still largely unknown.**[8]**

Glycoproteins are naturally present as a mixture of so-called glycoforms, (identic protein sequence with different glycosylation patterns), making the determination of the carbohydrate role in protein function challenging.

The synthesis of uniform glycoproteins has recently emerged as a promising alternative to overcome the essentially impossible isolation of single well-defined glycoforms. Native chemical ligation has developed as an appropriate method for chemical glycoprotein synthesis (**Fig. 4**). Chemically synthesized glycopeptides are coupled with recombinantly expressed protein fragments to result in a uniform glycoprotein. Although this strategy has allowed the synthesis of small proteins, new ligation methods and improvement of the fragment synthesis are required.

Using therapeutically used interferons (glycoproteins of the immune system) as a model, methods are currently developed to obtain uniform glycoproteins based on the chemical synthesis. Initial efforts are concentrated in glycopeptide solid phase synthesis followed by ligation coupling methods resulting in intact glycoproteins.

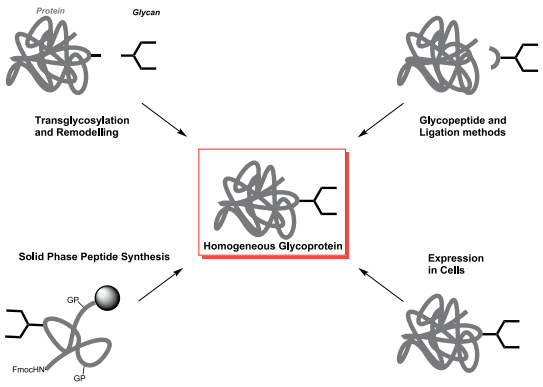

Fig. 4: Methods to obtain homogeneous glycoproteins.

First, a sialoglycopeptide undecasaccharide has been isolated from the delipidated fraction of egg yolk. The purification was performed by size exclusion chromatography and anion exchange chromatography. A glycosylated asparagines is obtained by enzymatic hydrolysis of the glycopeptide and can be selectively manipulated to obtain the protected building block for traditional Fmoc peptide synthesis.

In the second step, synthetic glycopeptides derived from the interferons alpha and beta will be converted to peptide thioesters or other active groups. These glycopeptides can then be submitted to ligation strategies with other peptides or coupled to expressed fragments resulting in well-defined glycoproteins.

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