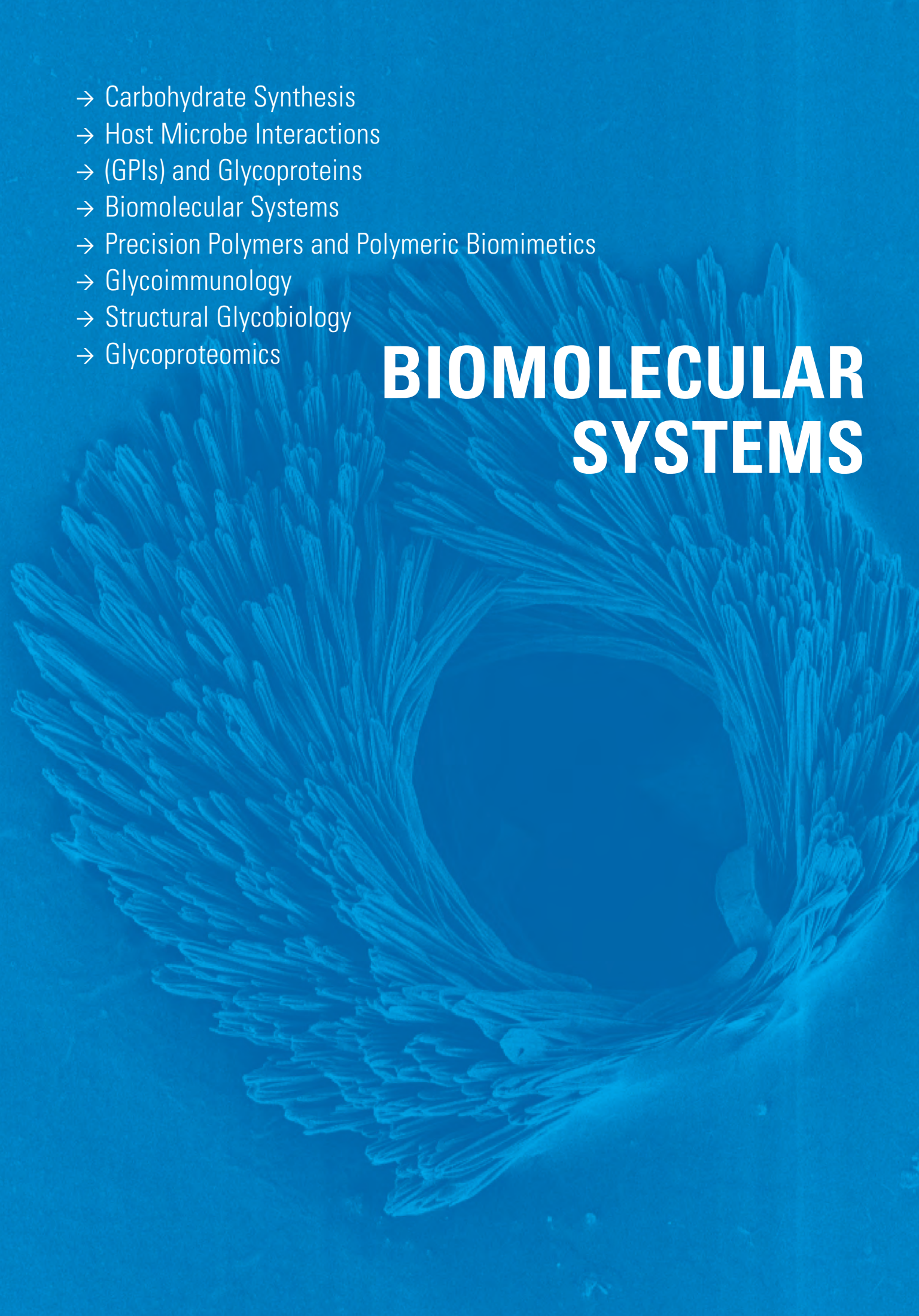

→ Carbohydrate Synthesis
→ Host Microbe Interactions
→ (GPIs) and Glycoproteins
→ Biomolecular Systems
→ Precision Polymers and Polymeric Biomimetics
→ Glycoimmunology
→ Structural Glycobiology
→ Glycoproteomics

BIOMOLECULAR SYSTEMS

Research in the Department of Biomolecular Systems

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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 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 infectious disease epidemiology. **Vaccine development** of several glycoconjugate vaccine candidates is rapidly advancing towards clinical trials. In the past four years the Department has developed by the addition of several new groups following the move from ETH Zurich in 2009. Glycan sequencing and glycomics (Dr. Kolarich) helps to identify glycans of biological importance particularly on interfaces of the human body – skin and intestine. The role of glycans is assed (Dr. Lepenies, glycoimmunology) using particularly animal models of infectious diseases (glycobiology and vaccinology (Dr. Anish). We are actively pursuing different questions in the glycosciences 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 are continuing to be pursued in the department. The Emmy-Noether group of Dr. Hartmann is producing well-defined polymers and collaborates closely with the glycoimmunologists to assess the *in vivo* activity of complex synthetic molecules. Our increased interest in establishing structure-function correlations of glycans is expressed by the addition of the Emmy-Noether group of Dr. C. Rademacher in 2012. This group is concerned with questions relating to structural immunology and as such forms a bridge between the synthetic chemists, glycobiologists and glycoimmunologists. The increasing importance of continuous-flow synthesis has been addressed by adding a new member of the Department, Prof. Dr. T. McQuade an expert in this area who joined us in May 2012.

The department is engaged in collaborations with the Colloid Department concerning the synthesis of colloidal polymers and supported catalysts. Many other applications of the flow paradigm from organic to nanoparticle synthesis and polymer chemistry are currently progressing rapidly.

Automated Synthesis of Carbohydrates

Since our arrival at the institute, we have expanded the scope of our core technology – the automated oligosaccharide synthesizer. After streamlining the process and inventing new linkers as well as a set of "approved" building blocks, the first demonstrator model of a new synthesizer has been completed. This new synthesizer is entering service in early 2013. Thus, the Department is closing 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.

Automated synthesis has allowed us to set a new world record by assembling a 30-mer oligosaccharide. After more than ten years of work, the automated synthesis of glycosaminoglycans has become possible. Currently, different members of this class of biologically extremely important oligosaccharides such as chondroitin sulfate heparin open completely new areas for biology but also material sciences.

Synthetic Tools for Glycobiology

Access to synthetic oligosaccharides has given rise to tools such as glycan microarrays, glycan nanoparticles, glycan 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

We have established a comprehensive program targeting the development of fully synthetic carbohydrate vaccines. Our commitment is evidenced by the addition of an additional group leader (Dr. C. Pereira) who is in charge of vaccine chemistry. This team is currently producing a host of antigens found on the surface of pathogenic bacteria. Conjugation and analysis of the antigens is now routinely performed as are immunological and functional studies in several disease models in experimental animals. Our integrated in-house approach has accelerated the development of important immunological tools as well as vaccine development.

Carbohydrate-based Nanotechnology

The attachment of carbohydrates to the surface to nanoparticles has been expanded across many platforms. Glycosylated-fullerenols have surprising activity against neurological damage in a stroke model in rats. Further preclinical studies are currently underway. The past two years have seen new projects focused at *in vivo* imaging using a new tridentate ligand system we have developed for carbohydrate labeling. With this approach we have monitored the distribution of specific oligosaccharides in animla models of disease.

Glycoimmunology

Carbohydrate recognition by C-type lectin receptors influences key functions of dendritic cells such as antigen presentation, cytokine release, and the expression of co-stimulatory molecules. Since all of these processes impact T cell priming and differentiation, CLR targeting is a means to orchestrate an initiated immune response. Recently, in collaboration with the MPI-DKTS in Magdeburg we investigated the impact of hemagglutinin (HA) *N*-glycosylation on influenza virus immunogenicity. Virus deglycosylation dramatically decreased cytokine production by spleen cells and reduced HA-specific antibody responses upon immunization of mice indicating a crucial role of HA *N*-glycosylation for immunogenicity.

To identify immune stimulatory and immune modulatory CLR ligands, a screening platform has been developed, followed by *in vitro* and *in vivo* assays. The extracellular domains of different CLRs were expressed as fusion proteins and used in conjunction with the glycan array technology for high-throughput screening of lectin/carbohydrate interactions. Novel binding partners of CLRs were identified and interactions with known ligands confirmed. Carbohydrate-protein interactions were further characterized by surface plasmon resonance (SPR) measurements. This platform brings together CLR ligand identification and their immunologic evaluation. Hence, it is a highly useful tool for the functional analysis of CLR ligands.

Carbohydrate-carbohydrate interactions

As part of the Collaborative Research Centre (SFB) 765 ("Multivalency as chemical organization and action principle"), we focus on the characterization of carbohydrate-carbohydrate interactions. Interactions between carbohydrates are even weaker than carbohydrate/lectin interactions, thus are often hardly measureable. In this context we focus on biophysical and biological analyses of interactions between the tumor-specific carbohydrate antigens GM3 and Gg3 as well as GB4 and GalGB4. Currently, we investigate whether these multivalent carbohydrate interactions are suitable for cell-specific targeting and imaging.

Continous Flow Microreactors as Tools for Organic Chemists

After pioneering the use of continuous flow microreactors for use by synthetic organic chemists more than ten years ago, the department is now utilizing continuous flow to address different chemical problems. In 2012 the most high profile project was the continuous synthesis of the anti-malarial compound artemisinin using photochemistry. His project has resulted in the formation of a spin-off company "ArtemiFlow" that is scaling the synthesis to ton scale and is working with international organizations to bring the scientific advance to bear on the production of the drug.

With the addition of Prof. Tyler McQuade to the department an additional focus is placed on the production of generic drugs using the benefits of flow synthesis. In addition, different chemistries ranging from gold-catalyzed glycosylations to transition mediated photochemistry to polymerizations are pursued.

Peter H. Seeberger
Director of the Department of Biomelecular Systems

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Automated Solid Phase Oligosaccharide Synthesis

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Sequencing and synthesis of peptides and oligonucleotides have been successfully automated. Given the structural diversity and complexity of carbohydrates access to glycans has ben very time consuming to date. In order to delineate the structure–function relationship of carbohydrates usable quantities of defined, pure glycans is required.

The ultimate goal of our group is the development of an generally solid phase automated synthesis method for glycan synthesis. In this context we analyzed the bacterial glycospace using bioinformatics tools. Interestingly, this analysis revealed that relatively few monosaccharide building blocks are required to access most bacterial oligosaccharide structures **[1]**. Following on fundamental work regarding all aspects of automated oligosaccharide synthesis **[2]** we developed an efficient and reliable production of structurally defined oligosaccharides based on a standardized, automated synthesis procedure that will provide an essential foundation for the field of glycobiology **[3]**. To be of use in glycobiology studies, these oligosaccharides are usually immobilized or conjugated through a functional linker (**Fig. 1**).

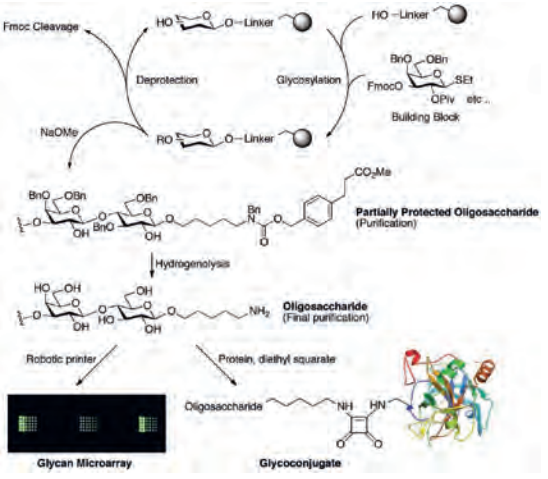

Fig 1. Overview of the fully-automated solid-phase oligosaccharide synthesis work flow [3].

Sialylated glycans are particularly important for interactions of complex glycans with proteins relevant for viral infections. For the first time we have been able to extend the automated solid phase synthesis paradigm to the incorporation of sialic acid residues into oligosaccharides of biological relevance **[4]**.

Presentation of Carbohydrates on DNA Duplexes

Multivalent interactions occur throughout biology in which two biological entities interact with each other through simultaneous, specific association of two or more ligands and receptors. Many different polymeric backbones, including peptides, have been used for the presentation of carbohydrate antigens as vaccine candidates. However, most of these approaches result in constructs that require long synthetic routes and spatially undefined structures.

A new method **[5]** for the spatially defined alignment of carbohydrates on a duplex DNA scaffold is presented (**Fig. 2**). The use of a NHS-ester phosphoramidite along with carbohydrates containing an alkylamine linker allows for on-column labeling during solid-phase oligonucleotide synthesis. This modification method during solid-phase synthesis only requires the use of minimal amounts of complex carbohydrates. The covalently attached carbohydrates are presented in the major groove of the B-form duplex DNA as potential substrates for type II C-type lectin receptors mMGL1 and mMGL2. CD spectroscopy and thermal melting revealed only minimal disturbance of the overall helical structure.

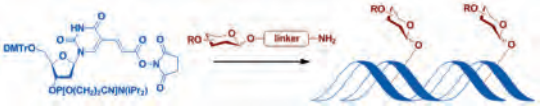

Fig 2. Conjugation of carbohydrates synthesized by automation or in solution is achieved during the DNA synthetic cycle to produce DNA-carbohydrate conjugates.

Automated Solid Phase Synthesis of Alginates.
For the first time the automated platform was utilized by other groups in 2012. In collaboration with a group at Leiden Uniersity, mannuronic acid alginate oligomers, featuring up to twelve 1,2-cis-mannosidic linkages were constructed using the second-generation automated oligosaccharide synthesizer The stereoselective formation of the β-mannosidic linkages was secured through the use of novel mannuronic acid building blocks. The use of the synthesizer allowed us to rapidly access target structures, without intermediate purifications and in quantities that are not only sufficient to cater for biological experiments but also to facilitate verification of the structural integrity of the compounds **[6]**.

Automated Solid-Phase Synthesis of Glycosaminoglycans
Glycosaminoglycans (GAGs) are important sulfated carbohydrates prevalent in the extracellular matrix. The synthesis of structurally defined GAGs requires laborious procedures, and incorporating defined sulfation patterns is challenging. Novel orthogonal linkers are key to this very challenging project and a new acylsulfonamide safety-catch linker was developed **[7]**.

Automated Synthesis of Sialylated Oligosaccharides.
Sialic acid-containing glycans play a major role in cell-surface interactions with external partners such as cells and viruses.

Straightforward access to sialosides is required in order to study their biological functions on a molecular level. An automated oligosaccharide synthesis was used to facilitate the preparation of this class of biomolecules **[4]**. Our strategy relies on novel sialyl α-(2→3) and α-(2→6) galactosyl imidates, which, used in combination with the automated platform, provided rapid access to a small library of conjugation-ready sialosides of biological relevance.

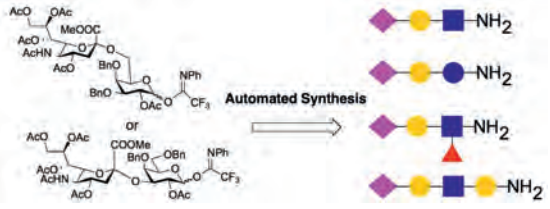

Fig 3. Fully Automated synthesis of sialosides starting from common disaccharide building blocks **[4]**.

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Synthetic Carbohydrate Antigens

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Carbohydrates and glycoconjugates are playing important role in human health and the fight against harmful pathogens. This burgeoning field requires fundamental insights into the role specific oligosaccharides play in human immunity. Bacteria are display different types of carbohydrates that are crucial in our quest to protect humans from pathogens.

The bacterial surface is covered by capsular polysaccharides (CPS), cell-wall polysaccharides (CWPS), exopolysaccharides (EPS), secondary cell-wall polysaccharides (SCWPS), lipopolysaccharides (LPS) and others. While the analysis of cell-surface glycans has progressed, access to synthetic oligosaccharides as tools for biological evaluations are still limited. The vaccine subgroup is heavily invested in the synthesis of cell surface glycans of a host of different pathogens based on the development of protocols for the synthesis of rare sugars and new synthetic approaches. The synthetic glycans serve to evaluate biological function and to develop novel diagnostics and vaccines. Novel immunoassay methods, new vaccine carrier concepts and delivery systems are currently being explored.

In 2012, the group has focused on the CPS of Streptococcus pneumoniae and Haemophilus influenzae, LPS of Neisseria meningitidis, Yersinia pestis, and other gram negative bacteria, Lipophosphoglycans (LPG) of Leishmania species, CWPS of Clostridium difficile, rare sugars in Escherichia coli O111 and Legionella.

Streptococcus Pneumonia

The group is currently pursuing several serotypes and has finished the synthesis of a number of the targets **[1]**. Immunological evaluations have been followed up by challenge studies that are ongoing.

Haemophilus Influenza

The syntheses of several synthetic oligosaccharides of different length have been achieved and have been subjected to immunological evaluation **[2]**. The synthesis of further oligosaccharides is underway (**Fig. 1**). Hib oligosaccharides serve to evaluate a new carrier system for vaccine development in an effort to obviate the need to maintain a cold chain and thus reduce cost of vaccines. This class of oligosaccharides helps us address fundamental questions of glycococonjugate vaccinology.

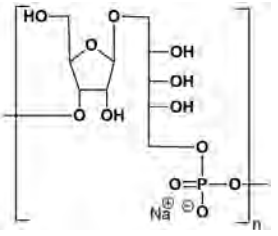

Fig. 1: Structure of H. influenzae CPS repeating unit.

Lipopolysaccharides

The tetrasaccharide antigen of Neisseria meningitidis has been subjected to immunological studies and functional evaluation (**Fig. 2**) **[3]**. Monoclonal antibodies against Yersinia pestis were raised against a synthetic antigen and are now being developed for a point of care diagnostic test. (**Fig. 2**) **[4]**. Various other LPS structures have been synthesized and will give rise to a unique glycan microarray **[5]**.

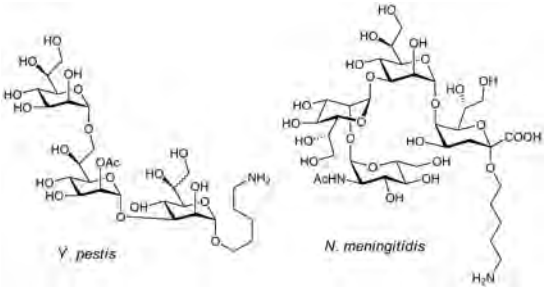

Fig. 2: Synthetic LPS structures synthesized of different pathogens

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Clostridium Difficile

Following immunological studies, the C. diff. PS-I antigen is entering challenge studies (**Fig. 3**). In addition, the PS-II antigen is explored in conjunction with PS-I and the toxins A and B **[6]**.

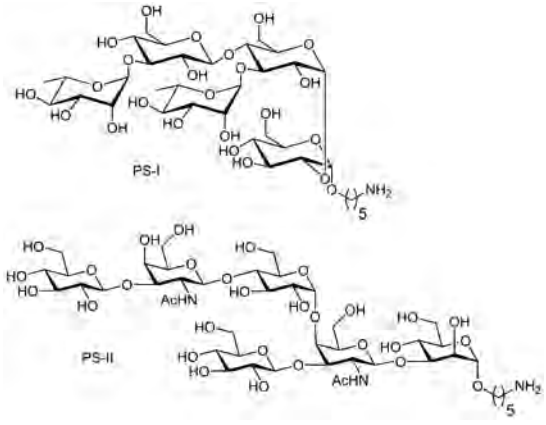

Fig. 3: PS-I and PS-II structure of C. difficile

Leishmania

Various LPG capping oligosaccharides have been synthesized and immunologically evaluated (**Fig. 4**). Sera from humans and dogs have been screened using glycan microarrays in efforts to develop novel diagnostic tests for this parasitic infection **[7]**.

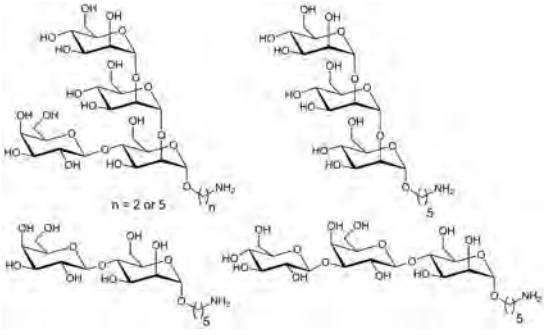

Fig. 4: Structure of capping oligosaccharides of LPG

Rare Sugars

Synthesis of rare sugar L-colitose that is present on the surface of many bacteria has been achieved (**Fig. 5**) **[8]**. Using the building block, the O-antigen repeating unit of E. coli O111 responsible for major health outbreak has been synthesized **[9]**. Other rare sugars have been synthesized using de novo approaches **[10]**.

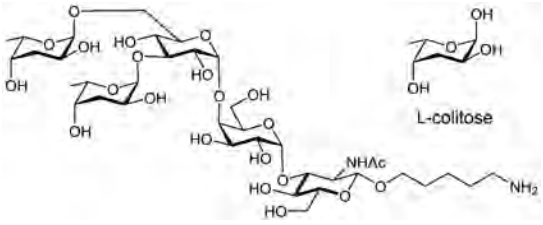

Fig. 5: Structure of the O-antigen of E. coli type O111 that contains the rare sugar colitose.

Vaccine Chemistry:

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Glycobiology of Cell Surface Glycans

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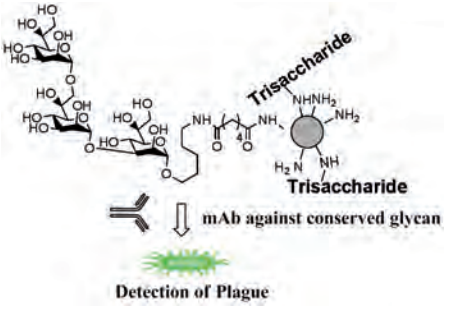
Pathogens invading humans often express glycan structures on the cell surface that interact with the host receptors and cell surface targets. As with other major classes of biomolecules, cell surface glycans have an important biological role that span the spectrum from relatively subtle, to those that are crucial for the survival of the organism that makes them. This group is interested in the structural and functional aspects of cell surface glycans. These glycans play important roles in biological recognition processes such as immune surveillance, inflammatory reactions and infection. Understanding the interactions of glycans with its binding partners helps to define the basic processes involved in invasion and infection. Differences in cell surface glycan composition and its organization can be exploited to develop glycan based prevention and detection strategies. We investigate four different topics: 1) Cell surface glycan based Pathogen detection and diagnostic tests; 2) Bacterial Glycans as vaccine candidates (collaborations with N. Suttorp, L. Sander, M. Witzenrath at the Charite-Berlin; and U. Vogel University of Wurzburg); 3) Cell surface Glycans at the interface of host-parasite interface; 4) Glycan binding proteins and their role in host-microbe interactions. All four topics involve the basic flow of work starting from identification, characterization and functional evaluation of glycan binding to its interaction partners.

Cell Surface Glycan Based Pathogen Detection and Diagnostic Tests

Many pathogens decorate themselves with unique glycan residues that are distinct from the host. Immunological tools like antibodies or anti-glycan sera that recognize these unique differences can serve to detect the pathogen. The presence of antibodies in the serum of an infected host is a good biomarker for the diagnosis and prognosis of the infection. Two projects are currently being pursued towards these goals:

- Detection of plague (*Yersinia pestis*) based on a unique triheptose that is conserved in the lipopolysaccharide **[1]**.
- Detection of *Leishmania chagasi* based on capping oligosaccharide residues in the lipophosphoglycan **[2, 3]**.

Plague pathogen expresses a unique triheptose residue in the lipopolysaccharide. Synthetic triheptose oligosaccharide was conjugated to a carrier protein. The resulting glycoconjugates were immunized in mice to generate monoclonal antibodies (mAbs) specifically recognizing the lipopolysaccharides. Compared to other gram negative bacteria, generated antibodies specifically recognized plague pathogen (**Fig. 1**).



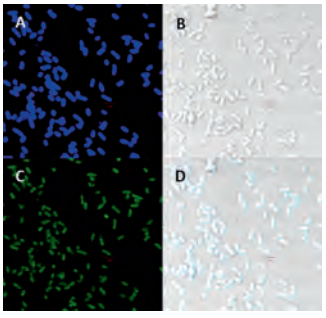


Fig. 1: Indirect immunofluorescent staining of Y. pestis by anti- LPS mAbs. CLSM images of immunostained Y. pestis; A: counter staining of bacterial DNA with DAPI B: DIC images showing unstained bacteria C: FITC specific fluorescence indicating binding of secondary antibody and D: overlay of all three layers.

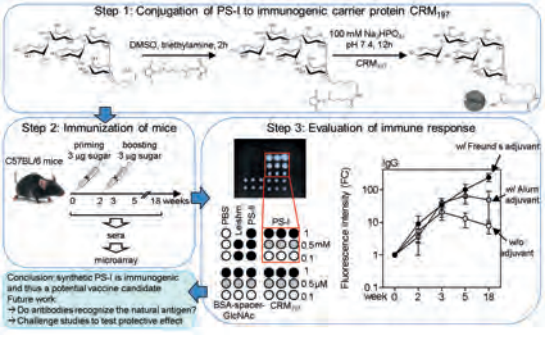


Fig. 2: Major steps involved in the immunological evaluation of glycoconjugates based on PS-1 glycan from C.difficile

Bacterial Glycans as Vaccine Candidates

Antibodies against bacterial cell surface glycans have been proven to be immunoprotective and three commercial vaccines against bacterial capsular polysaccharides are available in the clinic. In close collaboration with the vaccine chemistry subgroup we are evaluating the potential of synthetic oligosaccharides as vaccine candidates for bacterial infections. Our focus are the following aspects of the vaccine development programs:

- Immunological evaluation of synthetic oligosaccharides based on clostridium difficile exo-polysaccharide (PS-1) **[4,5]**
- Synthetic Lipopolysaccharide (LPS) -based vaccine candidate for meningococcal disease **[6,7]**
- Evaluation of vaccine potential of synthetic oligosaccharides based on bacterial capsular polysaccharides **[8]**

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[3] C. Anish, C. E. Martin, D. C. K. Rathwell, F. Matsumura, M. Leddermann, C. L. Pereira and P. H. Seeberger, Immunogenicity of spacer moiety limits the antigen specific antibody response against synthetic oligosaccharides (Manuscript under preparation)

Synthetic oligosaccharides, based on bacterial cell surface glycans, are prepared by the vaccine chemistry group. We prepare glycoconjugates of these oligosaccharides and carry out immunization experiments in animal models. The immune response is evaluated using glycan microarrays, ELISA and surface plasmon resonance (SPR). The functional evaluation of the immune response in preventing infection is assessed by *in vitro* surrogate assays and experimental infection models. Clostridium difficile exo-polysaccharide-based oligosaccharides are potential candidates for vaccine development. The antigens were immunogenic in mice and monoclonal antibodies recognizing the structures were developed. Glycan microarray and SPR studies identified a minimal disaccharide epitope that retains immunogenicity (**Fig. 2**). Studies on clinical specimens from patients infected with C. difficile demonstrated the presence of antibodies recognizing these structures **[4]**.

The synthetic oligosaccharide vaccine candidates based on meningococcal LPS and pneumococcal capsular polysaccharides are ongoing. Immunization with glycoconjugates based on these structures elicited significant antibody responses in mice. Challenge studies, bacterial immunofluorescence studies and opsonophagocytic assays to evaluate the immunoprotective effects of the elicited responses are in progress.

Cell Surface Glycans at the Interface of Host-parasite Interface

Numerous parasites elicit immune responses directed to glycan determinants within cell surface and secreted glycoconjugates in animals and humans. Parasite glycans are also important in host–parasite interactions. This realization prompted renewed interest in defining parasite-derived glycans to develop conjugate vaccines and new diagnostics for parasitic infections. In addition, we aim to understand the biochemical role of these glycans. Within this theme the group works on three projects:

- Role of extra-cellular vesicles in the infection biology of the apicomplexan parasite *Toxoplasma gondii* **[9]**
- Structure-function correlation studies on malarial glycosylphosphatidylinositols (GPIs) using anti-GPI antibodies
- Evaluation of *Toxoplasma gondii* GPIs as a vaccine and diagnostic marker for toxoplasmosis.

Apicomplexan parasites like Plasmodium and Toxoplasma are known to actively invade their respective hosts by hijacking and modulating the host cell responses. While the secretory proteins of these parasites have been studied in depth, no reports on the release of exosomes or microvesicles and the effector mechanisms they may mediate with the cells of the host system exist. Using *T. gondii* as a model organism we have isolated membrane vesicles released by parasites in the range of 40-150 nm. Biochemical characterization revealed the presence of certain immunodominant proteins and glycolipids like Glycosylphosphatidylinositols (GPI). We are characterizing the vesicles released by virulent and non-virulent strains of Toxoplasma and the possible roles they play in modulating the host cells in the context of invasion or innate responses against these parasites.

Monoclonal antibodies against malarial GPIs have been studied using glycan microarrays and STD NMR for epitope mapping. The mAbs recognize both natural as well as synthetic GPIs. Further studies on parasite binding and the potential of anti-GPI antibodies in limiting the invasion of parasite to RBCs are in progress.

We have investigated the potential of T. gondii GPIs using synthetic glycan based arrays for the diagnosis of toxoplasmosis **[10]**. Screening of clinical specimens from patients infected with T.gondii showed significantly higher levels of anti-GPI antibodies indicating the biomarker potential of GPIs. We are further investigating the immunogenicity of T. gondii GPI glycoconjugates to evaluate their potential as vaccine candidates.

Glycan Binding Proteins and their Role in Host-microbe Interactions

Identifying and characterising glycan binding proteins on host cell surface is important to understand their role in the host-pathogen interactions. To meet this aim, we use the glycan array platform to screen cell lysates; clinical specimens as well purified recombinant proteins.

· Role of parasite GPIs during host cell activation **[11, 12]**

GPIs present on the cell surface of the Apicomplexan parasites participate actively in the stimulation of immune host cell system and/or in the host cell invasion process. Using the synthetic glycan array platform we identified a cell surface isoform of moesin that interacts specifically with GPIs (**Fig. 3**). The moesin-GPI interaction is essential for macrophage activation in vitro mediating a pro-inflammatory response. Due to increasing drug resistance, targeting the GPI-moesin recognition process should enable novel modes of therapeutic intervention against Apicomplexan infection.

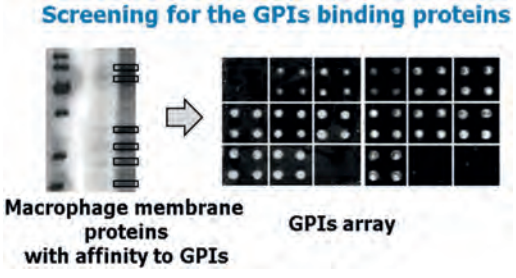

Fig. 3: Screening of malarial parasite GPI binding proteins to macrophage membrane proteins

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Synthesis and Properties of GPI-Anchors

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Glycosylphosphatidylinositols (GPIs) are complex glycolipids that are found in eukaryotic cells either attached to the C-terminus of proteins or in free form. GPIs contain a phosphoethanolamine unit connecting the C-terminus of the protein to the glycan, a conserved pseudo-pentasaccharide core and a lipid attached to the core glycan via a phosphodiester **[1]**. The conserved GPI structure can be further decorated by various substituents including additional phosphoethanolamine units, an additional fatty acid ester and oligosaccharide branches. The lipid subunit is variable and may include diacylglycerol, alkylacylglycerol or a ceramide, with chains of different length and varying degrees of unsaturation (**Fig. 1**).

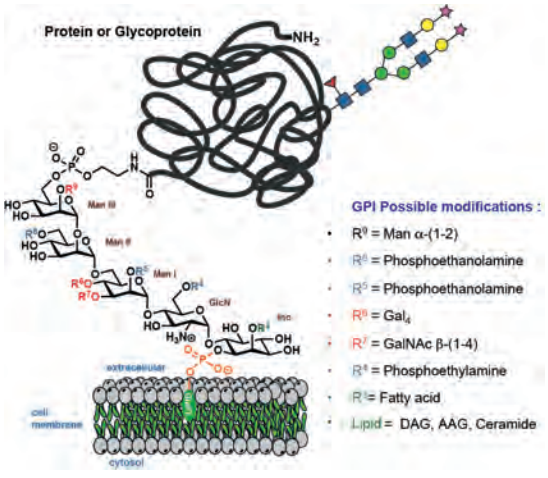

Fig. 1: Structure and possible modifications of GPI anchors. (DAG Diacylglycerol, AAG: Alkylacylglycerol)

The primary biological role of GPIs is to localize the attached protein to the outer surface of the plasma membrane bilayer **[2]**. However, it is suggested that GPIs play a role in the association of anchored proteins with lipid rafts and are, thereby, involved in diverse processes such as regulation of innate immunity, protein trafficking, and antigen presentation **[3]**.

Development of a General Strategy to obtain GPIs

Studies that would link a specific function to a structurally unique GPI rely on availability of homogeneous material of these glycolipids. To address this need we have developed a general synthetic route to obtain well-defined GPI glycolipids **[4]**. Our strategy is based on modular assembly of common building blocks and relies on a fully orthogonal set of protecting groups that enables the regioselective introduction of phosphodiesters and efficient assembly of the GPI glycans (**Fig. 2**). This general strategy has been applied to the syntheses of different branched and structurally distinct GPIs: the GPI of T. gondii, the low molecular weight antigen of T. gondii (**Fig. 2**), the GPI anchor of T. congolense VSG and the GPI of T. brucei VSG 117 **[5]**.

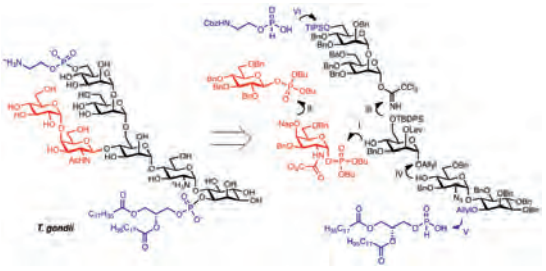

Fig. 2: Assembly sequence of the low molecular weight antigen from T. gondii using the general strategy

The assembly of the glycan is dictated by the positioning of the temporary protecting groups, which is kept constant across the set of common building blocks. The glycosylations required for the assembly of different GPI glycans are performed between similar coupling partners making the reactions conditions broadly transferable between different GPI syntheses. Both the late stage phosphorylations and glycosylations that form glycosidic bonds around the central mannose have been optimized with respect to yield and stereoselectivity, and shown to be competent in syntheses of diverse GPI targets. This work constitutes the first general approach to the synthesis of diverse GPI structures and the first synthetic route capable of producing GPIs with various substitution patterns including: monosaccharide and complex oligosaccharide branches with synthetically challenging glycosidic bonds, branches at both C3 or C4 position of Man I, di- and triphosphorylated structures, and generally diverse GPIs isolated from different organisms. With the ability to produce homogeneous native GPI structures and the flexibility that can be used to accommodate further modifications and produce unnatural analogues, this general strategy for the synthesis of GPI structures will enable extensive investigation into the biological roles of these glycolipids.

Biophysical Studies with GPI-Fragments

Insights into the behavior of GPIs and GPI-anchored proteins (GPI-APs) in cell membranes could contribute to the understanding of the roles GPIs play in biological processes. In this context, we have synthesized different lipidated GPI-core fragments and have evaluated in collaboration with the interfaces department the structural characteristics and conformational behavior of GPIs in well-defined membrane models. This biophysical study revealed the unprecedented crystalline two-dimensional structure of GlcNα1-6*myo*-Ino-1-phosphodistearoylglycerol monolayers. These monolayers are characterized by two commensurate lattices: the oblique lattice of the alkyl chains and the molecular lattice formed owing to highly ordered head groups (**Fig. 3**). The head-group ordering is observed regardless of incubation period probably because a hydrogen-bond network rigidifies the monolayer structure and can be disrupted on highly concentrated urea subphases **[6]**.

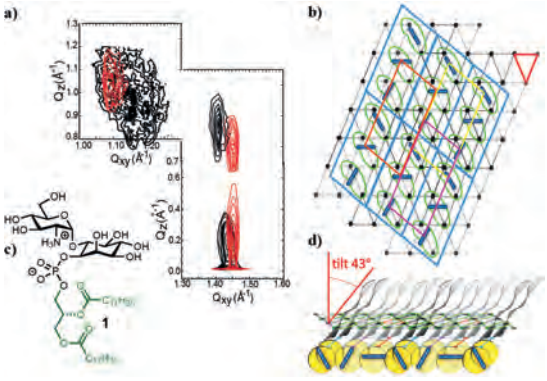

Fig. 3: a) Contour plots of corrected X-ray intensities as a function of in-plane (Qxy) and out-of-plane (Qz) scattering vector components of monolayers of 1 on PBS (2 mNm^{-1} black, and 30 mNm^{-1} red). b) Representation of the commensurable lattices describing the lateral order of the alkyl chains and of the molecules. Repeating unit cells (red, yellow, and magenta) c) Structure of the GPI-Core fragment 1. d) Representation of the molecules at the air/water interface: lateral view.

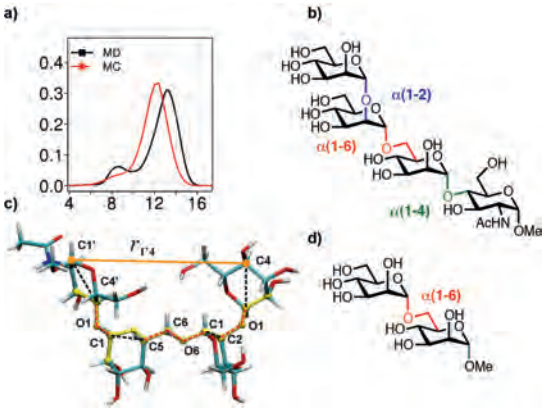

Fig. 4: a) Distribution p($r_{1\text{-}4}$) for the end-to-end distance from MD data (black) and the MC result (red). b) Structure of the GPI tetrasaccharide GPI fragment. c) Translation of an all-atom tetrasaccharide backbone into a reduced topology. d) Critical α(1→6) dimannoside.

Studies on mixed monolayers of the GPI-fragments and POPC demonstrate that above a certain concentration of the fragment 1, phase-separation occurs owing to the strong headgroup interactions. Below this concentration, the fragment mixes with the liquid disordered POPC and induces order in a highly cooperative way. Thus, the GPI fragment 1 tends to create ordered phases as it either forms a highly crystalline structure or induces liquid ordered domains (rafts). This ability could have important implications for the interactions of GPI-APs and GPIs in cell membranes.

Additional to high purity compounds, and the studies on membranes, to disclose structure-activity relationship (SAR) of GPIs, it's necessary to obtain structural information of the GPI-glycans. In collaboration with theory department we have performed a thorough conformational analysis and NMR characterization of GPI glycan fragments **[7]**. A spreadsheet compilation of data from regular MD trajectories covering a complete collection of substructures provides a survey of the overall conformational character of the fragments. We have performed biased MD simulations on a selected set of substructures. The biased dynamics permit us to explore free energy landscapes of glycosidic angles. The analysis clearly identifies the dimannoside α1→6 linkage as critical with respect to sampling efficiency and accuracy. Corresponding data sets from regular MD runs were then used, in combination with the results on disaccharides, to verify that the tetrasaccharide can be viewed as a sequence of independent glycosidic linkages, the conformational preferences of which are essentially inherited from disaccharide substructures (**Fig. 4**).

GPI-Anchors and Infection Diseases

Certain pathogenic parasites express non-protein-linked, free GPIs, which have been suggested to regulate the host immune response during parasitic infections **[8]**. However, in most cases, the heterogeneity and difficult isolation of pure GPIs have limited the evaluation of their function and the relationship with the GPI structure.

The parasite T. gondii, causing toxoplasmosis, expresses two different GPIs; one of them is a free GPI and is known as the low-molecular weight antigen. This GPI was synthesized using the general strategy and immobilized on glass slides. Recognition studies with anti-GPI monoclonal antibodies showed a specific recognition of this GPI structure, implying a structure-immunogenicity relationship and suggesting their applicability in parasitic disease research. Furthermore, T. gondii GPIs bearing a thiol linker have been prepared and used to obtain GPI-conjugates that are valuable tools in toxoplasmosis research and are currently under evaluation. Other synthetic GPIs have also been printed in micro-arrays and used to evaluate the presence of antibodies anti-GPI in other infections. Similarly to T gondii, during the infection of T. congolense specific immunological responses anti-GPI has been probed and the obtained antibodies did not show cross reactivity with other parasitic GPIs, demonstrating the importance of having well-defined molecules to disclose biological functions. Further studies with these and other GPI are in progress.

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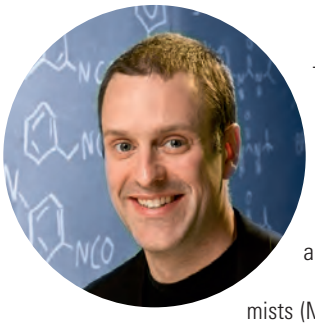
Microreactors as Tools for Organic Chemists



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2001-2007: Assistant Professor of Chemistry and Chemical Biology (Cornell University, Ithaca, NY, USA)
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The efficient synthesis of molecules requires control over chemical reactivity and reaction conditions. Advances in reaction condition control have accelerated new method development and discovery. Recent tools include automated synthesizers, microwave reactors, and flow/microreactors **[1,2]** reactors.

In the Microreactors as Tools for Organic Chemists (MTOC) group, flow reactors are used for three purposes:

- To develop continuous chemical processes. Specifically, MTOC is creating cost effective routes to medicines critical for the developing world (with C. Correia, K. Gilmore, D. Kopetzki and Prof. Seidel-Morgenstern - Max-Planck-Institut für Dynamik komplexer technischer Systeme in Magdeburg).
- To create novel chemical methods. Microreactors provide unique environments that enable new chemistry. Flow reactors are being used by the MTOC to synthesize bifunctional reactive intermediates and multi-functional fluorophores, for example (D.T. McQuade, F. Bou-Hamdan, A. O'Brien and M. Plutschack; with Prof. Dr. Meggers).
- To perform oligosaccharide synthesis. Microreactors enable optimization of glycosylations, creation of novel glycosylation methods and production of novel saccharide-based materials.

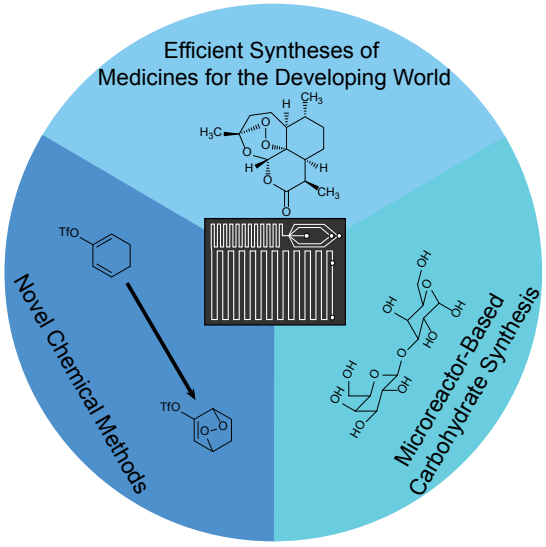


Fig. 1: The three working groups within the Microreactors team (Biomolecular Systems Department)

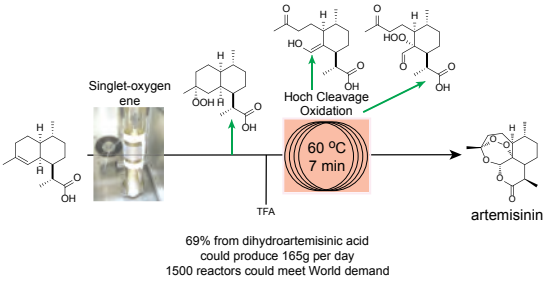
Efficient Syntheses of Medicines for the Developing World

Continuous synthesis enables the low-cost manufacturing of medicines. Millions of people in the developing world cannot afford life-saving medicines. Access to medicines such as anti-malarial and anti-viral agents would be increased if production costs could be decreased. The MTOC is actively developing advanced chemical syntheses of key active pharmaceutical ingredients used to treat malaria and HIV.

The team has recently synthesized the anti-malaria drug artemisinin using a continuous photochemical process. The process begins from dihydroartemisinic acid (DHA) **[3]**, a starting material now available on large scale via fermentation (**Scheme 1**).

The initial configuration used a mercury arc lamp and provided 39% yield of artemisinin from DHA.

Recently, the process was optimized to 69% yield from DHA. The increased yield leads to a throughput of 165g of artemisinin per day using visible light LED illumination. The MTOC is currently synthesizing the artemisinin-based APIs artemether, artemotil and artesunate. In addition, we are developing novel continuous routes into other medicines where decreased cost could increase access to those in great need of these agents.



Scheme 1: Recently reported continuous synthesis of artemisinin starting from dihydroartemisinic acid.

Novel Chemical Methods: Leveraging Continuous Reactors

Continuous reactors have been used by chemical engineers for over a century, but micro- and meso-reactors (flow reactors, collectively) have only recently become broadly available to the synthetic organic chemist. These reactors offer a number of significant advantages including:

(1) controlled heat transfer;
(2) controlled mixing, both fast and slow;
(3) increased photon-flux in photochemical reactions;
(4) increased electrode surface-to-reactor volume ratio (electrochemistry);
(5) increased solution-solid phase interactions;
(6) controlled use of highly reactive/toxic materials; and
(7) increased capacity to run serial reactions.

Over the past 8 years, the MTOC team has developed a wide range of flow reactor-based chemistries.**[4]** Most recently, the team has focused on transformations facilitated by light.

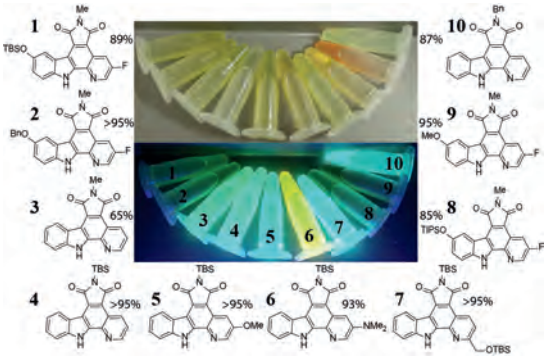


Fig. 2: The fluorescent products resulting from an in-flow photoelectrocyclization.

Fig. 2 illustrates ten fluorophores produced in a recent collaboration with Prof. Dr. Meggers group where a photoelectrocyclization was performed in flow, resulting in substantially better yields and product quality relative to batch methods.**[5]**

Homogeneous catalysts are expensive and methods enabling their continuous re-use while still retaining their active/selectivity represents an unmet challenge. The MTOC team has recently reported two new approaches to supporting catalysts specifically for use in flow. The first method uses a monolith-based approach.**[6]** Monoliths are highly porous solids and are typically prepared using polymers such as acrylates that do not exhibit wide chemical compatibility. The MOTC prepared polystyrene monoliths using a photoinitiated-radical polymerization (K. Krüger and K. Tauer). These monoliths were readily functionalized with catalysts and demonstrated wide chemical compatibility.

The MTOC has also created a carbon-nanotube-based catch and release strategy for supporting catalysts (**Fig. 3**).**[7]** Catalysts were appended to pyrene and captured using a column of carbon nanotubes. When the nanotube bed was heated, the catalysts were released and could be used in a flow reactor. At the exit of the flow reactor, a cooled carbon-nanotube packed bed then captured the catalyst, allowing product to pass freely. This capture and release strategy was used for multiple rounds of reactions without significant loss of catalytic activity.

Microreactor-Based Oligosaccharide Synthesis

Complex oligosaccharides play a fundamental role in cell-cell, bacteria-cell and virus-cell interactions. While the importance of these recognition interactions is becoming increasingly clear, the chemical synthesis of these biopolymers remains a significant challenge.

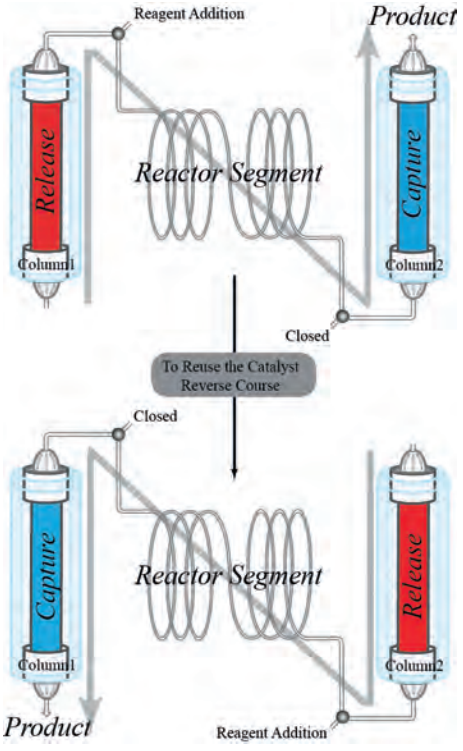


Figure 3: A carbon-nanotube-based capture and release catalyst system. Captured catalysts are released by heating and used immediately within the reactor segment. Once exiting the reactor segment, the catalysts are then recaptured on cooled nanotubes. The system is then reversed to recycle the catalyst.

The MTOC group is contributing to this area by:

· developing new strategies to optimize glycosylations;
· creating new flow-based glycosylation reactions;
· synthesizing sugar monomers continuously and
· creating new sugar-based materials in flow.

Fig. 4 shows a recent example from the MTOC where a flow glycosylation is achieved using mild gold-based catalysis. This method will enable the coupling of glycosyl-monomers that contain acid-sensitive functional groups (S. Bhunia).

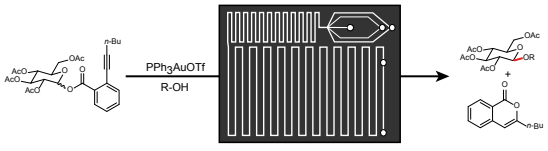


Fig. 4: Flow-based glycosylations using gold-catalysis

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Precision Glycopolymers for Receptor Targeting and Drug Development

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Precision Polymers

Over the past decades, polymer-based materials have evolved as a powerful tool in biomedical and pharmacological applications e.g. as carriers in drug and gene delivery, macromolecular therapeutics, polymeric diagnostics or 3D scaffolds for tissue engineering. Our aim is the development of the next generation of polymer based biomimetics by combining chemical precision (monodisperse molecules) and high degrees of functionality obtaining a new class of polymers, the so-called precision polymers.**[1]** In contrast to classical polymeric systems, these precision polymers do not exhibit any molecular weight or size distribution but are monodisperse. Furthermore they are multifunctional systems with the functionalities being positioned along or within the polymer backbone with a specific order or sequence.

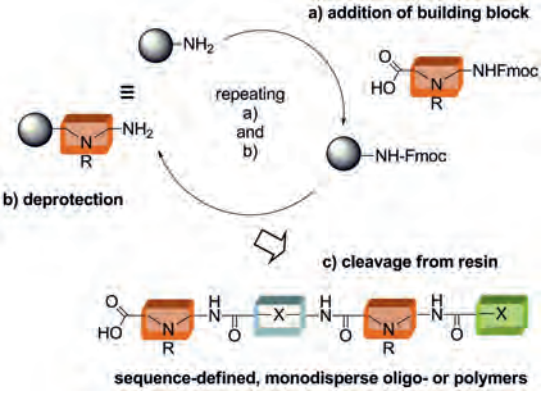

Fig. 1: General scheme of solid phase polymer synthesis: dimer building blocks are assembled on solid-support by stepwise activation, addition and deprotection. The final sequence-defined, monodisperse scaffolds are obtained directly after cleavage from the support. The whole process is automated by using a standard peptide synthesizer.

In order to develop a straightforward synthetic route to such sequence-defined, monodisperse polymer segments, solid phase-supported synthesis is applied (Solid Phase Polymer Synthesis, SPPoS) (**Fig. 1**).**[1,2]** Tailor-made dimer building blocks are coupled sequentially offering different spacer units as well as natural and non-natural functionalities within the main or side chain (**Fig. 2**).**[2-5]** Since for every addition a different building block can be used, different functionalities can be introduced and positioned within the chain as well as different architectures e.g. branched or ring structures can be realized depending on the choice of the monomer sequence.

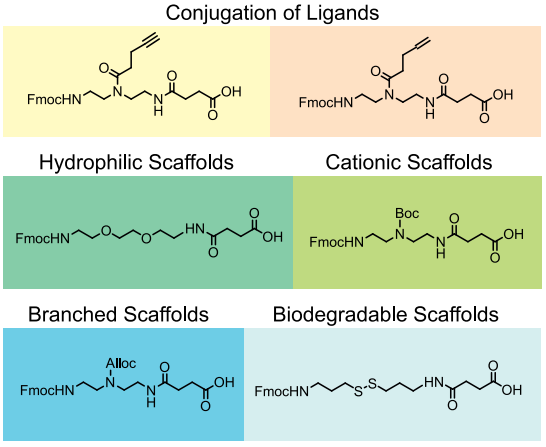

Fig. 2: Examples of functional dimer building blocks suitable for the solid phase synthesis of precision polymers.[2-5]

With this new synthetic platform in hand, we now focus on exploring two major advantages of precision polymer based biomimetics: On the one hand the control over their chemical composition allows for deeper insights into the structure-property relations of polymers for biomedical applications, so far mainly obtained by purely empirical studies. On the other hand, they allow for the straightforward synthesis of highly complex, multifunctional systems e.g. combining natural and non-natural ligands or functionalities and therefore have a great potential for the development of multicomponent therapeutics and drug delivery vehicles.

Glycopolymers for Receptor Targeting

Special focus is devoted to the combination of our precision polymers with sugar ligands. Such carbohydrate ligands take part in many biological processes like intercellular recognition and pathogen identification, often involved with multivalent presentation of the sugar ligands on a protein scaffold. Replacing the scaffold with a polymer therefore is a straightforward approach leading to more easily accessible, more stable and multifunctional sugar and sugar-protein mimetics. Precision glycopolymers now allow for the total control over the number, density and distancing of different sugar ligands along the scaffold and thus for a systematic structure-property relation study.

In a first set of glycopolymers consisting of a hydrophilic, flexible backbone of the same length, we varied the number and distancing of sugar ligands (mannose) (**Fig. 3**) and determined the binding affinity towards the lectin receptor Concavalin A (ConA) via surface plasmon resonance.**[3]** We found a strong dependence of the binding affinity on the number as well as the distancing of the sugar ligands. To our surprise, even the monovalent system (just one sugar attached to the polymer scaffold) already shows an increase in affinity by 100 fold while the scaffold itself does not show any unspecific interactions. We conclude that some of the hydration water from the scaffold is released upon binding resulting in an entropic gain (the effect of water as 'molecular mortar'). If we go up to a trivalent system, we even see an increase in affinity of 260 fold per sugar compared to the monosaccharide ligand, one of the highest values for comparable systems reported in literature so far. **[3]** Altogether these results show the importance of tailor-made polymer scaffolds for the design of novel glycomimetics.

Another advantage of our synthetic approach is the straightforward access to so-called heteromultivalent systems, presenting different sugar ligands at different positions along the polymer backbone (**Table 1**).**[4,6]**

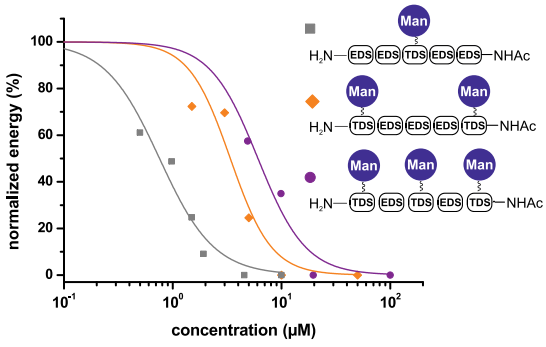

Fig. 3: A first set of precision glycopolymers shows different inhibitory concentrations depending on the number and distancing of sugar ligands along the polymer backbone (measured via SCP-RICM) [6, 8].

In a first experiment we found that the trivalent all-mannose system exhibits the same IC50 value as the trivalent heteromultivalent (Man, Gal, Man) system. So far we attribute this to a possible divalent binding mode of the two mannose ligands that is not altered by the nature of any additional ligands.**[6]**

Man and Gal	Man and Glu
Gal Man Gal H_2N—TDS EDS TDS EDS TDS—NHAc	Glu Man Glu H_2N—TDS EDS TDS EDS TDS—NHAc
Man Gal Man H_2N—TDS EDS TDS EDS TDS—NHAc	Man Glu Man H_2N—TDS EDS TDS EDS TDS—NHAc

Table 1: Heteromultivalent Glycopolymers obtained via SPPoS

Ongoing studies expand the library of glycopolymer ligands towards more complex architectures including hydrogels as well as take a closer look at the molecular interactions of glycopolymer ligands and the targeted protein receptor.**[7,8]** Currently our precision glycopolymers are tested in various biological applications such as targeted gene delivery, vaccine development and as antibacterial agents.

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C-type Lectin Receptors: From Glycan Arrays to Murine Studies

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Innate immunity is of crucial importance as a first line of defense against invading pathogens. Particularly, dendritic cells (DCs) play a pivotal role in antigen presentation and the initiation of a protective immune response. DCs sense pathogens via pattern recognition receptors (PRRs) that bind to conserved pathogen-associated molecular patterns. C-type lectin receptors (CLRs) represent a major PRR family predominantly expressed by cells of the innate immune system. CLRs recognize carbohydrate structures in a Ca^{2+}-dependent manner and orchestrate innate responses to a number of pathogens including bacteria, viruses, fungi, and helminths (**Fig. 1**). Since ligands have yet been identified for only a limited number of CLRs, one major goal of the Glycoimmunology group is to screen for novel carbohydrate ligands of CLRs. As carbohydrate/lectin interactions usually display low affinities, multivalent ligand presentation is often required. Thus, we use different scaffolds for multivalent display of CLR ligands to allow for a specific targeting of DCs. Finally, we explore the function of CLRs *in vivo* in mouse models of infection and inflammation. CLR-deficient mice are used to investigate the role of a single CLR in inflammatory processes (**Fig. 1**).

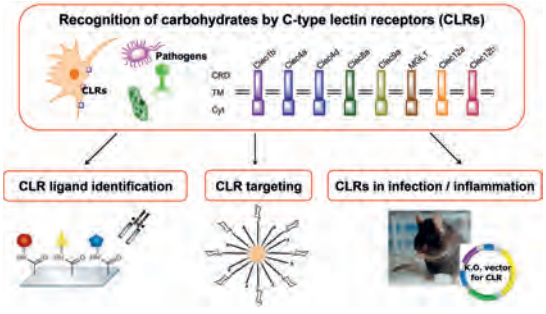

Fig.1: Main research goals of the Glycoimmunology group. The main focus is on carbohydrate recognition by C-type lectin receptors (CLRs, top panel). In innate immunity, CLRs are predominantly expressed by antigen-presenting cells such as dendritic cells. They recognize conserved carbohydrate structures of pathogens such as bacteria, viruses, or fungi. CLRs consist of an extracellular part containing one or more carbohydrate-recognition domains (CRD), a transmembrane region (TM), and a cytoplasmic part (Cyt). Specific research topics of the Glycoimmunology group are: 1) the identification of novel CLR ligands using the glycan array platform (bottom left), 2) cell-specific drug delivery by CLR targeting with multivalent ligands (bottom middle), and 3) elucidating the role of CLRs in vivo using mouse models of infection and inflammation (bottom right).

Glycan Recognition by Antigen-Presenting Cells Impacts Immunogenicity

Carbohydrate recognition by CLRs influences key DC functions such as antigen presentation, cytokine release, and the expression of co-stimulatory molecules. Since all of these processes impact T cell priming and differentiation, CLR targeting is a means to orchestrate an initiated immune response (**Fig. 2**).

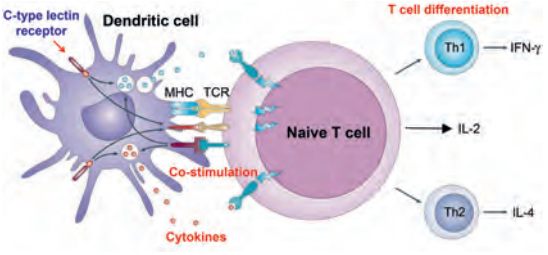

Fig. 2: C-type lectin receptor targeting to modulate immune responses. The engagement of CLRs expressed by dendritic cells (DCs) influences DC functions such as co-stimulation, and cytokine production. Naïve T cells recognize peptides presented by MHC molecules on the DC membrane through their T cell receptor (TCR). To proliferate and differentiate into T effector cells, a second signal is required that is provided by co-stimulatory molecules expressed on the DC surface. Since CLR engagement impacts this process, CLR targeting might be a means to shape an initiated immune response.

Carbohydrate recognition by lectin receptors is not only important for pathogen sensing but also for binding of glycosylated vaccine antigens. As a consequence, the differential glycosylation of vaccine antigens can affect their recognition by CLRs, thus may influence immunogenicity. In recent work performed in collaboration with the MPI-DKTS in Magdeburg (E. Rapp & U. Reichl), we investigated the impact of hemagglutinin (HA) *N*-glycosylation on influenza virus immunogenicity **[1]**. HA is the most abundant protein in the virus particle membrane, thus it is an essential component of most influenza vaccines. While the importance of HA glycosylation for influenza virus entry into host cells is well-known, we were interested in the impact of HA *N*-glycosylation on the recognition by antigen-presenting cells and subsequent T cell priming (**Fig. 2**). Influenza virus HA *N*-glycosylation was dependent on the host cell line used for virus production. Moreover, two cell line-produced influenza A virus variants with diverse HA N-glycosylation patterns markedly differed in their immunogenicity. Namely, T cell activation and cytokine production *in vitro* and humoral immune responses *in vivo* were affected by the differential HA glycosylation. Virus deglycosylation dramatically decreased cytokine production by spleen cells and reduced HA-specific antibody responses upon immunization of mice indicating a crucial role of HA *N*-glycosylation for immunogenicity. Our findings have implications for cell line-based influenza vaccine design: appropriate host cell lines can be selected for virus propagation or may even be glyco-engineered to enhance immunogenicity.

In another study, we determined structure-activity relationships of fucoidans with regard to activation of antigen-presenting cells **[2]**. Fucoidans are sulfated polysaccharides mainly consisting of sulfated α-L-fucopyranose. They are found in various species of brown algae and brown seaweed and were reported to exhibit a wide range of biological activities including anticoagulant and antitumor effects. Native fucoidan from *Fucus evanescens* as well as hyposulfated, deacetylated, and both, hyposulfated and deacetylated derivatives of fucoidan were prepared and used to stimulate pri-

mary DCs and macrophages. Hyposulfation and deacetylation led to markedly reduced cytokine secretion by DCs and macrophages. Both, hyposulfation and deacetylation almost completely abolished cytokine production thus indicating a crucial role of sulfate/acetyl groups for the immune stimulatory activities of fucoidan.

A Platform towards Carbohydrate-Based Adjuvants and Immune Modulators

To identify immune stimulatory and immune modulatory CLR ligands, we have developed a screening platform, followed by *in vitro* and *in vivo* assays. The extracellular domains of different CLRs were expressed as fusion proteins with the F_C part of human IgG_1 molecules. The CLR-F_C fusion proteins were used as tools to screen for carbohydrate ligands of CLRs. We employed the glycan array technology that allows for high-throughput screening of lectin/carbohydrate interactions (shown in **Fig. 3**). Indeed, novel binding partners of CLRs were identified and interactions with already known ligands could be confirmed. Carbohydrate-protein interactions were further characterized by surface plasmon resonance (SPR) measurements. Next, CLR-recognizing carbohydrates were covalently coupled to the model antigen ovalbumin (OVA). The OVA-glycan conjugates were used in co-cultivation assays of DCs and T cells to stimulate transgenic T cells *in vitro*. In addition, mice were immunized with these conjugates to identify immune modulatory CLR ligands *in vivo*. This platform brings together CLR ligand identification and their immunologic evaluation. Hence, it is a highly useful tool for the functional analysis of CLR ligands (Eriksson, Maglinao et al., manuscript in preparation). Identified carbohydrate/CLR interactions will be investigated in detail to elucidate their role in immunity **[3]**.

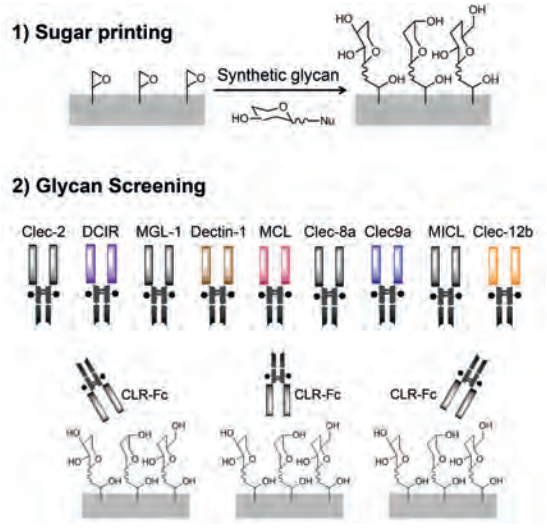

Fig.3: The glycan array platform to identify novel carbohydrate ligands of C-type lectin receptors. 1) Sugar printing: Synthetic glycans (each containing a linker with a terminal nucleophile) are covalently immobilized on epoxide-activated glass slides. 2) Glycan screening: To identify glycan ligands of CLRs, the array is incubated with the respective CLR-F_C fusion protein (some examples of CLR-F_C library members are given). Detection is then performed using a fluorescently labeled secondary antibody.

Multivalent Targeting of C-type Lectin Receptors

Due to the generally low affinities of single carbohydrate/lectin interactions multivalent ligand display is usually a prerequisite for specific CLR targeting. Recent studies indicate that multivalency is indeed a means to overcome these low affinities and exert biological effect **[4, 5]**. Carbohydrate ligands of the CLR macrophage galactose-type lectin (MGL) were covalently attached to a DNA backbone and presented in the major groove of the B-form duplex DNA. MGL binding was analyzed by SPR measurements and uptake studies in primary macrophages and DCs. Specific ligand binding to MGL was detected indicating the suitability of multivalent carbohydrate ligand presentation for CLR targeting **[5]**. Multivalent targeting approaches can also be employed to increase the targeting specificity of drugs for tumor cells. We used reversible addition-fragmentation chain transfer polymerization (RAFT) to covalently and site-specifically append a defined HPMA polymer to the cancer drug SN-38 (collaboration with J. Tsanaktsidis, CSIRO Melbourne, Australia) **[6]**. The poly-HPMA-SN-38 conjugates displayed excellent aqueous solubility and stability and retained the cytotoxic activity of the parent drug SN-38. *In vitro* assays using cancer and non-cancer cell lines showed the specificity of the RAFT-derived poly-HPMA-SN-38 conjugates for cancerous cells. Further specific tumor targeting might be achieved by covalent attachment of small molecules (e.g. carbohydrates) to the polymer-drug conjugates.

Carbohydrate-carbohydrate Interactions

In a recently started project as part of the Collaborative Research Centre (SFB) 765 ("Multivalency as chemical organization and action principle"), we focus on the characterization of carbohydrate interactions. Interactions between carbohydrates are even weaker than carbohydrate/lectin interactions, thus are often hardly measureable. The SFB project deals with the biophysical and biological analysis of interactions between the tumor-specific carbohydrate antigens GM3 and Gg3 as well as GB4 and GalGB4. Since multivalent presentation is essential to measure these low affinity-interactions, the relevant carbohydrate antigens are functionalized on the surface of nanoparticles. Currently, we investigate whether these multivalent carbohydrate interactions are suitable for cell-specific targeting and imaging.

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Specific C-type Lectin Receptor Ligands

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Carbohydrate structures represent a fundamental class of biopolymers and have been identified as important mediators of many recognition processes in health and disease. Their function is determined by the context of their presentation, as glycoproteins or glycolipids, and the structure and dynamics of their receptors. Myeloid C-type lectin receptors (CTLRs) are one subfamily of carbohydrate binding proteins of particular interest. This receptor family is defined by it consensus protein fold and for most members, the calcium-mediated recognition of self- as well as non-self carbohydrates structures. This protein/carbohydrate interaction then shapes for example the cellular response to necrotic cells or determines the immune response to invading pathogens. The details of this molecular interaction and mechanisms coupling it to immunological outcomes are not well understood. Fundamental insights are expected from these investigations into this exciting field of molecular immunology and will provide potential for the development of immune modulatory therapeutics. Therefore, state of the art biophysical techniques such as nuclear magnetic resonance and fluorescence spectroscopy, together with computational modelling are applied to address questions of high relevance for immunology **[1]**. In particular, these insights are utilized to design high affinity, specific and chemically defined probes to investigate the biology of C-type lectin receptors.

Computer-Aided Design and Synthesis of Ligands for CTLRs

Compared to the number of members of the C-type lectin fold family, the structural information is rather sparse. Still, a few members of the subfamily of myeloid CTRLs have been characterized by X-ray crystallography and NMR spectroscopy. The resolution of these structures is of sufficiently high resolution allowing in silico methods to be used to aid the search for small molecular probes for these lectins. CTLRs that obey a calcium-mediated recognition of glycans share a shallow binding site (**Fig. 1**). This not only explains the intrinsically low affinity of these cell surface receptors for their glycan ligands, but also imposes a challenge to any rational design of high affinity ligands for these proteins.

Available X-ray structures are chosen as a starting point, which display the respective CTLR in complex with one or more glycan ligands. The design process therefore starts from an already existing ligand pose and hence develops this into a high affinity lead structure **[2]**. This has advantages compared to de novo design strategies and builds on the expertise of the department in the field of carbohydrate chemistry. The chemistry of carbohydrate derivatization implies certain restrictions onto the possible lead candidates and is therefore directly incorporated into the design process, allowing a rapid advancement of computational models into synthetic molecules.

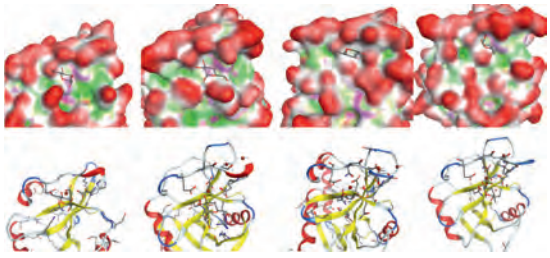

Fig. 1: C-type lectin receptor carbohydrate recognition sites. A selection of CTLR binding sites is show, highlighting the shallow, calcium-mediated carbohydrate interaction. Upper panel: glycan in stick presentation, receptor depicted in Connolly surface representation (red: solvent exposed, green: hydrophobic, magenta: hydrophilic). Lower panel: Ribbon representation of the corresponding receptor/ligand complexes (red: α-helix, yellow: β-sheet, blue: turn).

Fragment-Based Drug Design

The pipeline of many pharmaceutical companies has experienced a decline of preclinical candidates during the last decade, raising a serious demand on novel strategies for rapid hit finding and lead progression. Since Fesik and coworker's key contribution to the field by establishing SAR-by-NMR **[3]**, fragment-based approaches in drug discovery have developed many facets. These approaches are no longer limited to nuclear magnetic resonance and make use of other sensitive techniques such a surface plasmon resonance (SPR) and X-ray crystallography of ligand cocktails for screening. With respect to the screening methodology, a sensitive detection is mandatory, as molecular fragments of the size of 250 Da or less are intrinsically of low affinity. What renders them interesting starting points for ligand design is their limited chemical complexity, allowing to fit many structural requirements imposed by their potential receptors. Hence, these fragment libraries have high hit-rates. These hits can then be developed into lead structures by processes such as fragment-linking or -growth **[4]**, giving rise to an impressive list of clinical drug candidates against challenging candidates **[5]**.

Since Fesik's first description of this procedure, many academic groups build on these findings and developed related techniques **[6]**. While the academic environment favours the development of preclinical candidates, many applications focus on the development of chemical probes for chemical biology. In particular, undruggable, challenging binding sites have been explored using fragment-based approaches, discovering that the static nature of structures determined by X-ray determined can be misleading **[7]**. Here, we use fragment-based design strategies to identify CTLR ligands from pools of small molecules. To this end, a fragment library was rationally designed using chemoinformatics tools. The pool bearing over 1000 diverse compounds was assembled from commercial vendors and academic collaborations (**Fig. 2**). The library is now available for screening and will benefit from its diversity.

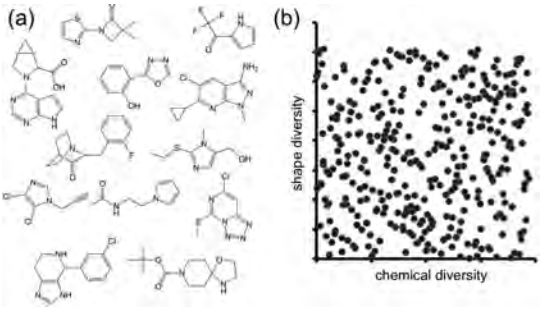

Fig. 2: Diverse fragment screening library. (a) A selection of small heterocyclic compounds is shown. (b) A scatterplot of the chemical versus shape diversity highlights the coverage of chemical space of the library. Chemical diversity is assessed using MACCS chemical fingerprint combined with the Tanimoto similarity coefficient and plotted against the diversity as determined using the 3Deigenshape function of MOE (Chemical Computing Group).

Glycan Fingerprints

CTLRs interact with a diverse set of glycan structures of self- as well as non-self origin. To understand these lectin receptor interactions with their natural glycan ligands, a unifying picture of glycan diversity has to be developed that is able to cope with this complex pattern. To rationally address this problem and to approach related problems in glycobiology, we developed a formal guide to quantify glycan diversity **[8]**. In contrast to other biopolymers such as peptides and oligonucleotides, the branched structure of carbohydrates imposes challenging demands on the comparison of glycan structures. This rational can then guide the construction of diverse glycan libraries, a central question in the field of glycomics **[8]**.

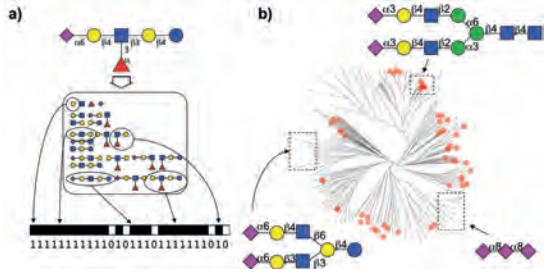

Fig. 3: Glycan Fingerprints. (a) The CFG representation of a complex glycan is decomposed into all possible fragments. The existence of all unique fragments is stored into a bit string, transferring a branched structure into a linear representation. (b) Based on the string representation of glycans, the similarity between carbohydrates can be assessed quantitatively and a non-rooted tree depiction of a glycan library is derived. Clusters of high density as well as non-represented clusters are highlighted to guide the chemistry to fill these gaps [8].

To date, the selection of glycans for carbohydrate libraries such as microarray studies was done empirically, drawing on experience. Therefore, an algorithm was developed for the analysis of glycan library diversity based upon an analogy between a simplified glycan representation of monosaccharides and glycosidic bonds, as found in the symbol notation of the Consortium for Functional Glycomics (CFG), and small molecule graphs as used in chemoinformatics. This novel and powerful approach allowed us to derive a linear representation of complex glycan structures, which was then used to calculate pairwise similarities. These similarities expressed in numerical values are fundamental to finally derive diversities of groups of glycans and by that answering key questions for future development of glycobiology tools such as glycan microarrays.

Furthermore, this tool will find its application in other fields of glycobiology such as the analysis of glycosylation patterns. The glycosylation machinery is a very important component of the cell/cell communication system. It provides and stores information on the cell surface in defined recognition patterns and is dynamically adjusted. Means to quantify this diversity have been missing. The introduction of Glycan Fingerprints opens a door towards the systematic understanding of the dynamic interplay between cell physiology and glycosylation in a quantitative way. Transforming the overwhelming diversity of glycosylation into numbers will unravel new paradigms and thereby integrating today's data from e.g. mass spectrometry into a system wide framework.

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Deciphering the Glycocode for Understanding Intercellular Communication

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Numerous so-called glycoconjugates are crucial key players of intercellular communication. Information between cells is often mediated by secreted or membrane bound glycoconjugates such as glycoproteins. Both, the protein and the glycan moiety of such a glycoconjugate are important information carriers and in order to understand the language cells are using for communication in health and disease scientists require robust and solid techniques that allow monitoring and deciphering of communication events on cellular level. In the context of protein glycosylation and its role in bio-messaging the human immune system is one of the best understood cellular mechanisms that has been shown to be significantly influenced by the type and action of glycans **[1]**. Immune cell glycan alterations play a critical role in e.g. regulating effector functions such as dendritic cell or T cell activation **[reviewed in 1]**. Glycan structures on immune cells interact with lectins such as C-type lectins, S-type lectins (e.g. galectins), or I-type lectins (e.g. siglecs), thus deeper insights into the cellular glycome promise to deepen our understanding of intercellular communication mechanisms **[2]**.

In order to decipher the language of complex multicellular systems such as the human immune system detailed knowledge on both, the individual proteins and their particular post-translational modifications such as glycosylation is vital. In the last two decades the study of biomolecules has been greatly facilitated by novel developments in mass spectrometric techniques and instruments, nevertheless further advancements in technologies and approaches are required for the robust and accurate identification and characterisation of glycoconjugates derived from biological specimens.

LC-ESI MS is a Powerful Tool for Glycoconjugate Analysis

Combining nano-sale liquid chromatography (LC) online with state of the art mass spectrometric (MS) detection techniques provides us with powerful opportunities to separate, isolate and characterise femto- to picomol amounts of biomolecules derived from biological samples. Nano-scale LC separation is an important additional dimension increasing significantly the amount of information that can be obtained from a single sample simply by supplying the compounds of interest to the mass spectrometer over the entire time frame of the LC separation, resulting in an increased depth of analysis and number of ions detected from a single sample. Without this prior separation molecules present in lower concentrations might not be detected and thus information on these components would be lost. In addition, the choice of separation medium linked to the mass spectrometer provides us with the opportunity to specifically target the respective compounds of interest based on their molecular properties. Subsequently, reversed phase separation media are usually chosen for the analysis of glycopeptides and peptides **[3]**, whereas far better data can be obtained for oligosaccharides released from glycoproteins when subjected to LC separation using porous graphitized carbon (PGC, **Fig. 1, 3**) **[4]**.

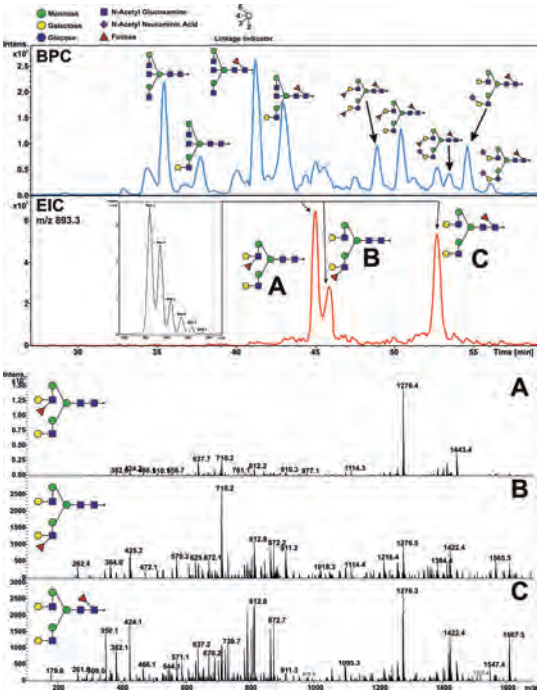

Fig. 1: Glycan structure isomers can be separated using PGC LC and signature ESI-MS/MS spectra enable unambiguous glycan identification Figure taken from [1].

Defined Standard Compounds Help us in Developing Novel Glycoproteomics Techniques

The easy availability of well-defined biomolecules has always been a key for scientific advancements. Simple access to custom-made nucleotide or peptide sequences has turned out to be vital for any molecular biology and biochemistry research. Recent advances in automated oligosaccharide synthesis are very promising to bride this gap in glycomics research **[5]**. However, glycoproteomics research requires access to molecules that can be tailored on both, the peptide and the glycan side of the molecule.

Current strategies based on total synthesis of both, the glycan and peptide moiety are suffering from limitations of establishing a native peptide-glycan bond under synthetic conditions as well as easy diversification of the glyco-moiety into larger structures. Making use of nature's glycosylation potential, the combination of controlled proteolytic digestion

with state of the art separation technologies enables us to obtain glycosylated amino acids that can further be modified to be used in standard solid phase peptide synthesis for the production of tailor made glycopeptides.

Using these synthetically produced glycopeptides and peptides we now have the opportunity to establish quantitative correlations of the different compounds that are frequently detected in a glycoproteomics experiment. The fact that these natural biomolecules exhibit significantly different chemical properties makes it impossible to extract quantitative information from MS data without having appropriate standards in hand. These well-defined standards enable determination of quantitative relationships from the detected signals and thus make label free quantitation of glycopeptides a reality (**Fig. 2**) **[6]**.

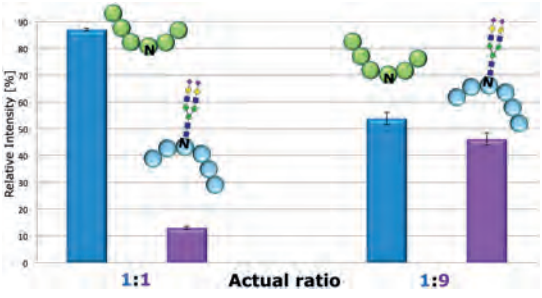

Fig. 2: The application of exactly defined and quantified peptides (green circles) and glycopeptides (blue circles) produced by solid phase peptide synthesis uncovered that glycopeptides differ significantly in their ionisation efficiency. Injection of equal amounts of the target molecules resulted in significant differences in detected signal intensities. The ability to determine and quantify these differences is an important step towards obtaining useful quantitative information for glycopeptides from MS-experiments [6].

Disease Glycoproteomics – Deciphering Intercellular Communication Signals in Inflammatory Bowel Disease and Skin Neoplasia

The glycoproteomics group is using its tools and developments to understand how the intracellular glycoprotein communication network is changing in the course of diseases. With this information in hand it is possible to gain a better understanding on disease onset and progression. In cooperation with medical institutions and international networks we are focusing on identifying and characterising glycoproteins and glycans associated with inflammatory bowel diseases, which have a prevalence of 0.8% and are associated with high morbidity, definite mortality and an increasing economic burden in particular in western countries. We are partners in the IBD-BIOM consortium, an EU-funded project joining cutting edge epigenomic, glycomic, glycoproteomic and activo - mic approaches to elucidate particular IBD associated pathways and disturbances to the immune system.

Another major focus is the determination of skin neoplasia glycoprotein signatures. With around 2-3 million cases per year skin cancer is one of the most prevalent cancer types worldwide. Malignant melanoma, one of the most dangerous types of cancer if detected late, represents around 5% of the cases. The majority of reported cases comprise the so-called non-melanoma skin cancers such as basal cell carcinoma or squamous cell carcinoma, which are seldom lethal but can be disfiguring and a psychological burden to patients if detected late or left untreated. First results obtained on basal cell carcinoma specimens provided promising data indicating that substantial changes in the glycome and glycoproteome are occurring. This information will be used to gain a deeper understanding on the onset and progression of skin neoplasia.

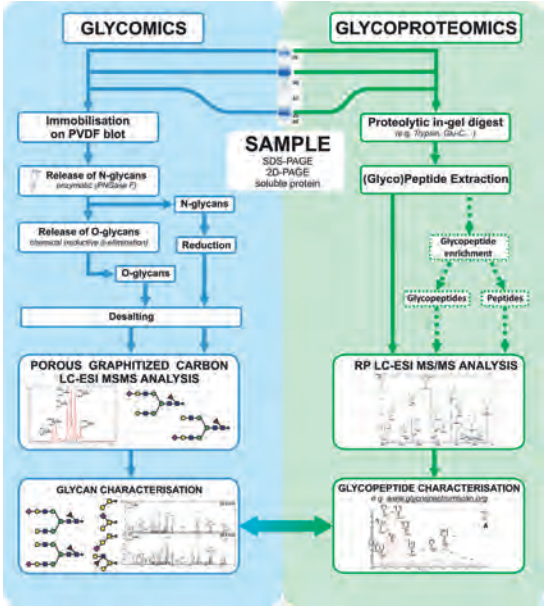

Fig. 3: Using Glycomics and Glycoproteomics detailed information can be determined from µg amounts of initial protein. LC-ESI-MS/MS techniques can be successfully applied to identify proteins and their post translational modifications to understand their role in health and disease. [3,4]. Figure taken from [3].

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