Abstracts

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O1
Glycobiology – leukocyte trafficking into sites of inflammations

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A review of our recent work includes that we have:
- cloned new glycorelated enzymes and characterized their acceptor and site-specificities,
- generated a lethal glycosyltransferase KO mouse,
- characterized glycan zip codes for organ specific leukocyte traffic
- set up methods to analyze granulocyte and lymphocyte trafficking in vivo in patients.
- The most recent work deals with the fate of glucose within the cell, and we have asked how does the cell decide to use incoming glucose as energy (via glycolysis, TCA and ox phosphorylation) or as building blocks (GDP-Mannose and related sugar nucleotides). Conditional tetracylin-inducible knock-out S. cerevisiae strains were generated and cultured in a well controlled bioreactor environment in chemostate.

After turning down specifically gene after gene on the pathway from fructose-6-P to GDP-mannose we collected time-series data (100 specimens with time intervals of 1-60 min each), and analyzed a wealth of parameters including metabolites, enzyme activities, quantitative protein levels and locations, protein-protein complexes, and transcriptomes. With these data we try to understand the regulation of this pathway.

O2
Down-regulation of α-hydroxylated galactosyl-ceramide and unstable myelin in transgenic mice overexpressing UDP-galactose:ceramide galactosyl-transferase

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Galactosylceramide (GalC) and its sulfated derivative sulfatide are major lipid constituents of the myelin membrane making up about 30% of its lipid content. They fulfill essential functions in the formation and stabilization of myelin. We have generated transgenic mice overexpressing the galactocerebroside synthesizing enzyme UDP-galactose:ceramide galactosyltransferase (CGT) in oligodendrocytes under the control of the proteolipid protein (PLP) promoter. Elevated CGT activity led to a significant increase in non-hydroxy fatty acid (NFA)-galactosylceramide.
In contrast, however, a massive decrease in the normally predominant α-hydroxy fatty acid (HFA) form of GalC was observed. As a consequence total GalC levels were not clearly elevated in the transgenic mice. These mice exhibit deficits in motoric behavior and develop a progressive hindlimb paralysis. Ultrastrucural analysis revealed severe impairment in the formation of compact myelin and a progressive demyelination in the central nervous system, accompanied by axonal degeneration, vacuolation and a massive astrogliosis. The composition of myelin proteins was unchanged with the exception of a significant upregulation of the raft-associated MAL protein and mRNA.
Taken together these results strongly suggest that the HFA- to NFA-GalC ratio is an important factor in the formation and maintenance of compact myelin.

O3
Comparison of the polysialylation status in ST8SiaII and ST8SiaIV knock-out mice during postnatal brain development

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Polysialic acid (polySia) attached to N-glycans of the neural cell adhesion molecule (NCAM) promotes changes in cell interactions and plays a critical role in neural development. Two closely related polysialyl-transferases, ST8SiaII and ST8SiaIV, are able to synthesize this unique carbohydrate structure composed of α2,8-linked sialic acid residues.
To investigate the polysialylation competence of the individual polysialyl-transferases in vivo, we analyzed total polySia levels, polySia chain length, and degree of NCAM polysialy-lation in mice lacking either ST8SiaII or ST8SiaIV. In both genotypes a dramatic decrease in polySia levels were observed between postnatal day 1 and day 21. While in ST8SiaIV knock-out mice the time course of polySia down-regulation and the total polySia levels were similar to those observed in wild-type mice [1], clear differences were observed in animals lacking ST8SiaII. Already at day 1 total polySia levels were decreased by 36% compared to ST8SiaIV null mice. In addition, a substantial amount of NCAM was found nonpolysialy-lated, while in ST8SiaIV knock-out mice all expressed NCAM was found fully polysialylated up to day 5.
In contrast to marked differences observed in total polySia levels and amount of polysialylated NCAM, only minor differences were detected in the maximal chain length synthesized by either ST8SiaII or ST8SiaIV.
In summary, ST8SiaII seems to be the predominant polysialyl-transferase during postnatal brain development of the mouse and lack of this enzyme can not be fully compensated by ST8SiaIV.
[1] Galuska et al. (2004), accompanying abstract

O4

A combined defect in the biosynthesis of N- and O-glycans in patients with cutis laxa

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Based on our preliminary observation of abnormal glycosylation in a cutis laxa patient, five patients with autosomal recessive cutis laxa and neurological involvement were analyzed for congenital defects of glycosylation (CDG). Isoelectric focusing of plasma transferrin and apolipoproteinC-III were used to screen for defects in the biosynthesis of N-glycans and core 1 mucin type O-glycans, respectively. Three out of five cutis laxa patients had abnormal isoelectric focusing profiles for both plasma transferrin and apolipoproteinC-III. Mass spectrometric N-glycan analyses were performed to gain insight into the structural basis of the abnormal isoelectric focusing profiles. This revealed a relative increase of N-glycans lacking sialic acid and glycans lacking sialic acid and galactose residues. The patients' DNA was analyzed for mutations in the fibulin5-gene (FBLN5), as these have been reported in cases of autosomal recessive cutis laxa. No mutations were detected in this gene for these three patients.
The conclusions in this study were that the clinical phenotype of autosomal recessive cutis laxa seen in the three patients with abnormal transferrin and apolipoprotein-C-III isoelectric focusing profiles is not caused by mutations in FBLN5. These patients have a combined defect in the biosynthesis of N- and core 1 mucin type O-glycans. Our findings define a novel form of CDG with cutis laxa and neurological involvement due to a defect in sialylation and/or galactosylation of N- and O-glycans.

O5

Roles for glycosylation in the production, loading and presentation of class I and class II viral antigens

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Viruses exploit the glycosylation machinery of their hosts thus minimising the detection of their envelope glycoproteins by the immune system. Nevertheless some viral epitopes are detected by serum immunoglobulins, including the broadly neutralising domain swapped anti-HIV-1 antibody, 2G12, that recognises an unusual cluster of alpha 1-2 mannose residues in the outer face of gp120 [1,2]. This cluster may represent a potential therapeutic target. Viral antigens can be presented through both the MHC Class I and Class II pathways. Soluble calreticulin and the surface bound homologue, calnexin, both recognise ER glycoproteins containing $Man_{9\text{-}6}GlcNAc_2$ sugars and MHCI folds using the calnexin pathway. A sequence of events which accompanies the loading of antigenic peptide to MHC class I involves the assembly of complexes of MHCI with TAP, tapasin, ERp57 and the lectin folding chaperone, calreticulin. The loading mechanism uses an adaptation of the calnexin/calreticulin protein folding and quality control pathway [3,4]. MHC class II viral antigens have been described and the effect of protein glycosylation in the processing of antigenic peptides explored (Mimura, Elliot, Rudd and Dwek unpublished data). Recognition of peptide loaded MHC at the T cell/antigen presenting cell synapse involves many glycosylated proteins [5]. Virus envelope glycoproteins often depend on the calnexin/calreticulin protein folding and quality control pathway to achieve a properly folded state. The possibility of interfering with the folding pathway provides a therapeutic target [6].

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[2] Calarese DA, Scanlan CN, Zwick MB, Deechongkit S, Mimura Y, Kunert R, Zhu P, Wormald MR, Stanfield RL, Roux KH, Kelly JW, Rudd PM, Dwek RA, Katinger H, Burton DR, Wilson IA (2003) *Science* **300:** 2065-2071.
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O6

Glycoproteomics of schistosomes: affinity purified immunodiagnostic egg antigens are specifically fucosylated with multiple Fucα1-2Fuc elements.

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Schistosomiasis, the second most prevalent human parasitic disease after malaria, occurs mainly in developing countries and is caused by digenetic trematodes of the genus Schistosoma. Schistosomes produce a variety of glycoconjugates, which induce characteristic humoral and cellular immune responses. From a library of monoclonal antibodies (mAbs) derived from schistosome-infected or immunised mice, mAbs were selected that bind specifically to several different fucosylated glycan epitopes. These mAbs were used to survey the developmental expression of these glycan motifs in different Schistosoma mansoni life-cycle stages;

cercariae, adult worms and eggs. Glycolipid and glycoprotein preparations were analysed by immunostaining on HPTLC and Western blots and combined with immunolocalisation studies in immunofluorescence assays.
Two particular mAbs, 114-4D12 and 114-5B1, that are applicable in the immunodiagnostic detection of schistosome egg antigens in serum and urine of infected individuals, were used for affinity purification of their target glycoproteins. The glycosylation and protein backbones of the specific set of purified egg antigens were studied using multiple mass spectrometric techniques. It was determined that O-glycan structures with terminal (Fucα1-2Fucα1-3)GalNAc-(Fucα1-2Fucα1-3)GlcNAc-element are responsible for binding to mAb 114-4D12. Interestingly, this type of structure has been shown to be a potent inducer of innate immune responses and raises strong antibody response in infected individuals.

O7

Carbohydrate-based cross-reactivity of haemolymph glycoproteins from *Biomphalaria glabrata* with *Schistosoma mansoni*

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Schistosomiasis is one of the most important parasitic diseases of humans caused by trematodes of the genus Schistosoma. Within the *Schistosoma mansoni* life-cycle the miracidium invades the freshwater snail *Biomphalaria glabrata* in which the development to the human-pathogenic cercariae takes place. Carbohydrate analyses of glycolipids from *S. mansoni* eggs revealed the presence of a Fuc(α1-3)GalNAc-motif [1] which is recognised by the monoclonal antibody M2D3H [2]. This antibody has also been shown by Western blot analysis to bind to distinct proteins of total haemolymph from *B. glabrata* thus indicating the occurrence of shared carbohydrate epitopes within both species.
In this study we have analysed cross-reactive N-glycans from haemolymph proteins of *B. glabrata*. Glycans were released from tryptic glycopeptides by PNGase F treatment and labelled with 2-aminopyridine by reductive amination. Cross-reactive glycan species were isolated by immuno-affinity chromatography using a column with immobilised rabbit antibodies raised against *S. mansoni* soluble egg antigens (SEA). N-glycans expressing carbohydrate units related to the M2D3H epitope were recovered by subsequent chromatography using fucose-specific lectin column. Analyses by MALDI-TOF-MS and MS/MS techniques revealed two classes of oligosaccharide species with monosaccharide compositions of $Hex_{2-3}MeHex_{0-1}HexNAc_2Fuc_1Xyl_1$ and $Hex_{3-4}MeHex_{0-1}HexNAc_4Fuc_{1-3}Xyl_{0-1}$. The precise structures of these fucosylated N-glycans are under present investigation.
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O8

Molecular basis of the differences in binding properties of the highly related C-type lectins DC-SIGN and L-SIGN to Lewis X trisaccharide and *Schistosoma mansoni* egg antigens.

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The dendritic cell specific C-type lectin DC-SIGN functions as a pathogen receptor that recognizes *Schistosoma mansoni* egg antigens (SEA) through its major glycan epitope Galβ1,4(Fucα1,3)GlcNAc (Le^x) [1]. L-SIGN, a highly related homologue of DC-SIGN found on liver sinusoidal endothelial cells, binds to SEA but not to Le^x. L-SIGN does bind the Lewis antigens Le^a, Le^b and Le^y, similar as DC-SIGN. A specific mutation in the carbohydrate recognition domain (CRD) of DC-SIGN (V351G) abrogates binding to all Lewis antigens. In L-SIGN Ser^{363} is present at the corresponding position of Val^{351} in DC-SIGN. Replacement of this Ser into Val resulted in a "gain of function" L-SIGN mutant that binds Le^x, and shows increased binding to the other Lewis antigens. These data indicate that Val^{351} is important for the fucose-specificity of DC-SIGN. Molecular modeling, and docking of Lewis antigens in the CRDs of L-SIGN, DC-SIGN, and their mutants, demonstrate that Val^{351} in DC-SIGN creates a hydrophobic pocket that strongly interacts with the Fucα1,3/4-GlcNAc moiety of the Lewis antigens. The equivalent amino acid residue Ser^{363} in L-SIGN creates a hydrophilic pocket that prevents interaction with Fucα1,3-GlcNAc in Le^x but supports interactions with the Fucα1,4-GlcNAc moiety in Le^a and Le^b antigens. These data demonstrate that DC-SIGN and L-SIGN differ in their carbohydrate binding profiles, and will contribute to our understanding of the functional roles of these C-type lectins, both in recognition of pathogen and self glycan antigens. Part of this work was supported by a Neose Glycoscience award to IvD.
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O9

Characterisation of recombinant Xylosyltransferase I and Cys mutant enzymes

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We have recently established a system for the expression of an enzymatically active rXT-I protein in insect cells. In order to analyse the role of the cysteine residues on nucleotide sugar and heparin binding, we generated mutants individually lacking the conserved cysteine residues of the protein.

UDP and heparin were tested for their effects on enzyme activity using the alanine substituted mutants. Most of the mutations did not have any influence, however the inhibition of the XT-I activity by 0.25 mM UDP was significantly reduced in the C561A mutant. In addition, we tested the binding of rXT-I and mutants to immobilized UDP. We found no significantly different binding properties even using our enzymatically inactive mutants. As expected, the inhibitory effect of heparin to rXT-I was confirmed. However, the experiments revealed that all mutants were efficiently bound to immobilized heparin indicating that the conserved cysteine residues are not directly involved in heparin binding, nor do the alterations induce a misfolding of the heparin binding site.In addition, the role of the cysteine residues was investigated by modification with N-phenylmaleimide. Treatment with N-phenylmaleimide showed no effect on wild type XT-I but strongly inactivated the Cys mutants in a dose-dependant manner, indicating that seven intramolecular disulfide bridges are formed in rXT-I.

O10
The chaperones Hsc70 and Hsp70 specifically bind *O*-linked N-acetylglucosamine residues. Modulation of their lectinic activity.

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O-linked N-acetylglucosamine (*O*-GlcNAc) is the major glycosylation type found within the cytosol and the nucleus of eukaryotes. Despite the intensive study of this glycosylation, the functions played by the monosaccharide remain elusive. However, recent reports seem to demonstrate that the glycosylation of proteins with *O*-GlcNAc might inhibit their targeting to the proteasome (both by modifying the target proteins and the proteasome itself). Taking into account that the 70 kDa-Heat shock protein (Hsp70) and its constitutive form (Hsc70) play a role in the quality control of proteins conformation, we consider a putative lectinic activity of these HSP70 towards the *O*-GlcNAc moiety. If actually *O*-GlcNAc is a protective signal against the proteasomal degradation, it is conceivable that heat shock proteins could bind this monosaccharide. In this way we demonstrate that Hsp70 and Hsc70 bound to GlcNAc beads in a specific manner in HepG2 cultured cells and that these two chaperones are themselves affected by *O*-GlcNAc. When a thermic stress was associated to glucose starvation, the HSP70 glycosylation dramatically decreased while their lectinic properties towards the *O*-GlcNAc moiety markedly increased. The profile of *O*-GlcNAc modified proteins co-immunoprecipitating with Hsp70 was similar for cells submitted to the thermic and to nutrient stresses. These results support our suggestion of a new type of lectinic interaction between *O*-GlcNAc modified proteins and the 70 kDa-heat shock proteins members, and reinforce the new paradigm of *O*-GlcNAc influence on protein life and death.

O11
Influence of O-glycosylation on proteasomal processing of MUC1 glycopeptide

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MUC1 is a transmembrane glycoprotein localised on luminal surfaces of epithelial cells. On cancer cells, it shows pericellular membrane localization and expresses distinctively altered glycosylation patterns. In pregnant women and in cancer patients, poorly efficient cellular and humoral immune responses directed against MUC1 have been detected, indicating a break of self-tolerance to this antigen. In order to stimulate the immune response and increase probability of defeating cancer, the ways of processing and MHC presentation of glycosylated forms of MUC1 should be understood. Previously we have analysed the processing of MUC1 derived glycopeptides, by Catepsin L in the MHC class II pathway.
The aim of this study was to identify proteolytic fragments from proteasomal processing of MUC1 glycopeptides.
The MUC1 glycopeptides were processed *in vitro* by immunoproteasomes under various conditions, and processing products were analysed by MALDI-TOF mass spectrometry or reversed phase HPLC, and sequenced by nanoflow electrospray MS/MS. Analyses revealed the presence of glycopeptides of relevant length (9-11 amino acids), with the potential for forming the MHC class I/peptide complex. Statistical analysis of processing products has revealed considerably defined processing patterns, which are qualitatively and quantitatively controlled by O-glycosylation. Processing efficiency of glycosylated MUC1 peptides is dependent on the glycosylation type and position, as well as on the reaction conditions. The final processing products of non-glycosylated MUC1 are considerably different from the final processing products of the respective glycoforms.

O12
Benzyl mannose : a tool to discriminate lumenal and cytosolic sites of deglycosylation in ERAD.

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Recent studies demonstrated that deglycosylation step is a prerequisite for endoplasmic reticulum associated degradation (ERAD) of misfolded glycoproteins. We report the benefit of the use of benzyl-mannose during pulse-chase experiments to study the subcellular location of the deglycosylation step in CHO cell lines. Benzyl-mannose inhibited *in vivo* both, the ER-to-cytosol transport of oligomannosides and the trimming of cytosolic labeled oligomannosides by the cytosolic mannosidase. We pointed out the occurrence of two subcellular sites of deglycosylation. The first one is

located in the ER lumen and led to the formation of Man8GlcNAc2 (isomer B) in wild type CHO cell line and Man4GlcNAc2 in Man-P-Dol deficient cell line. The second one was revealed in CHO mutant cell lines for which a high rate of glycoprotein degradation was required. It occured in the cytosol and led to the liberation of oligosaccharides species with one GlcNAc residue and with a pattern similar to the one bound onto glycoproteins. The cytosolic deglycosylation site was not specific of CHO mutant cell lines since we demonstrated the occurrence of the cytosolic pathway when the formation of truncated glycans was induced in wild type cells.

O13
N-glycans: how to analyse and how to modify?

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Protein linked N-glycans have a wide range of functions and changes in their structure has been linked several times to human disease. Especially remarkable is their complexity and their heterogeneity, two aspects that are poorly understood. To develop approaches to answers these complexity and heterogneity questions, we have developed the powerfull DSA-FACE technique for analysis of N-linked glycans and engineered lower eukaryotes for synthesis of homogeneous humanised N-glycans.

Our DSA-FACE technology (Callewaert et al., 2001) is very sensitive, high resolution and high-througput. It was applied, among several other applications, for diagnostic purpose in CDG children and for diagnosis of liver disorders. CDG types I and II have been detected in the total serum N-glycome. For liver failure, high sensitivity and specificity markers for both detection of cirrhosis (79 and 86 respectively; both are 100% for decompensated cirrhosis) and follow-up of fibrosis were characterized.

Lower eukaryotes only synthesise N-glycans of the high-mannose type, whereas human glycoproteins have a very divers, complex type of N-glycans. Redirection of the fungal pathway, even to a simple hybrid or complex mammalian type requires several genetic interventions such as gene knock-outs and heterologous expression of mammalian glycosyl transferases. Furthermore, additional in vitro enzymatic manipulations may be required. Lower eukaryotes are exellent hosts for this engineering work as they don't have competing complex glycosyl tranferases coded for in their genome

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O14
Activating effect of replacing *N*-acetylglucosamine by *N*-propionylglucosamine in synthetic β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→O)$(CH_2)_7CH_3$ oligosaccharidic analogues on the acceptor specificities of α-2,6- and α-2,3-sialyltransferases

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The acceptor specificities of rat ST3Gal III, human ST3Gal IV, human ST6Gal I and human ST6Gal II were investigated using a panel of β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→O)$(CH_2)_7CH_3$ analogues. Modifications introduced at either C2, C3, C4, C5 or C6 of the terminal D-Gal residue, as well as *N*-propionylation instead of *N*-acetylation of the subterminal D-GlcNAc unit were tested for their influence on the α-2,3- and α-2,6-sialyltransferase acceptor activities. Both ST3Gal enzymes displayed the same narrow acceptor specificity and only accept reduction of the Gal C2 hydroxyl function. The ST6Gal enzymes, however, do not have the same acceptor specificity. ST6Gal II seems less tolerant towards modifications at Gal C3 and C4 than ST6Gal I. A particularly striking feature of all tested sialyltransferases is the activating effect of replacing the *N*-acetyl function of subterminal GlcNAc by an *N*-propionyl function.

O15
Characterisation of binding specificities of *Helicobacter pylori* adhesins by glycoconjugate arrays

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The microaerophilic bacterium *Helicobacter pylori* plays a substantial role in the development of different gastric diseases. Bacterial adhesins and corresponding binding sites on the epithelial surface allow *H. pylori* to colonize the gastric tissue. In the present investigation, the adhesion of *H. pylori* to dot-blot arrays of natural glycoproteins and neoglycoproteins was studied. Adhesion was detected by overlay with fluorescence labelled bacteria on immobilized (neo-)glycoproteins. The results confirmed the interaction between the adhesin BabA and H-, Lewis b and related fucose-containing antigens. In addition, *H. pylori* bound to terminal α2,3-linked sialic acids as previously described. The use of *sabA* KO mutants and neuraminidase treatment of the

glycoconjugate arrays showed that the adherence of *H. pylori* to laminin is mediated by the sialyl-Lewis x binding SabA adhesin. The adhesion to salivary mucin MUC5B is mainly associated with the BabA adhesin, and to a lesser extent with the SabA adhesin. This agrees with the fact, that MUC5B carries both fucosylated blood group antigens and α2,3-linked sialic acids. These glycoconjugate arrays will be useful for exploration and characterization of unknown adhesin specificities of *H. pylori* as well as of other bacterial adhesins.

O16

A novel class of high-affinity inhibitors of type 1 fimbrial adherence of *Escherichia coli* disclosed in the crystal structure of the FimH lectin domain.

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Mannose-binding type 1 pili are important virulence factors for the establishment of *Escherichia coli* urinary tract infections. These infections are initiated by adhesion of uropathogenic *E. coli* to uroplakin receptors in the uroepithelium via the FimH adhesin located at the tips of type 1 pili. Blocking of bacterial adhesion is able to prevent infection. We provide binding data of the molecular events underlying type 1 fimbrial adherence, by crystallographic analyses of the FimH receptor binding domains from a uropathogenic strain, and affinity measurements with mannose, common mono- and disaccharides, and a series of alkyl and aryl mannosides. The crystal structure illustrates that the lectin domain of the FimH adhesin is a stable and functional entity and that an exogenous butyl α-D-mannoside, bound in the crystal structure, exhibits a significantly better affinity for FimH (K_d = 0.15 μM) than mannose (K_d = 2.3 μM). Exploration of the binding affinities of α-D-mannosides with longer alkyl tails revealed affinities up to 5 nM. Aryl mannosides and fructose can also bind with high affinities to the FimH lectin domain, with respectively a 100-times improvement and 15-fold reduction compared to mannose. These relative FimH affinities correlate exceptionally well with the relative concentrations of the same glycans required for the inhibition of adherence of type 1 piliated *E. coli*.

O17

Implementation of chip electrospray mass spectrometry in structural glycobiology

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Mass spectrometry (MS) has the potential to revolutionize carbohydrate research and help in understanding of how post-translational events such as glycosylation affect protein activities. In the past decade, capillary nanoelectrospray (nanoESI) MS developed as an effective means in glycomics. However, the disadvantages of the method include low sample throughput, potential sample carryover, and poor reproducibility due to the variable shape of the spray tip. The recent introduction of chip-based ESI in biological MS is driven by the high performance, sensitivity and reduced analysis time. Automated chip-based ESI systems significantly increase the analysis throughput and efficiency.

Here we report upon the first implementation of chip ESI MS in structural glycobiology. Two different chip ESI systems: a fully automated chip-based nanoESI robot and a thin chip microsprayer have been coupled each to both a hybrid quadrupole time-of-flight (QTOF) MS and a Fourier transform ion cyclotron resonance (FTICR) MS at 9.4 T. The feasibility of the chip MS approaches was tested for structural elucidation of glycoconjugates originating from human body fluids and tissues such as urine glycopeptides and human brain and brain tumor gangliosides.

The high sensitivity and ionization efficiency provided at nano- and microscale level by the chip MS make this new technology ideal for applications that require identification of unknown, minor components in complex glycomixtures. We also show here that nano- and microchip ESI MS methods are capable of structural elucidation of glycoforms indicative of pathological states, which gives this technology perspectives for use in biomedical research and clinical diagnostics.

O18

Cell specific glycoproteome profiling in Arabidopsis

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Systematic protein profiling is used to unravel the complexity of signal transduction and to identify putative protein markers. However, established proteomics techniques are limited by cellular resolution, resolving power and throughput. We develop a novel approach to proteomics, allowing for cell specific profiling and high throughput sample handling. We use the Arabidopsis *cgl* mutant, which is defective in the enzyme Gnt1 which activity is essential for the initiation of complex N-glycan biosynthesis. Consequently, the *cgl* mutant has no

N-glycoproteins containing complex N-glycans. In this mutant plant, we restore normal N-glycan processing by expression of the wild type Gnt1 gene under control of different cell specific promoters. In this way, formation of complex N-glycans on glycoproteins will only be restored in the corresponding specified cell types. Because the complex glycans from plant glycoproteins contain core fucosylations and xylosylations that are recognized by specific antibodies, the proteins containing complex-glycans can be separated from other proteins in a whole tissue extract with affinity chromatography. Purified glycoproteins/peptides, derived from specified cell types, are deglycosylated and analysed by 2D-LCMS. *In silico* analysis of the Arabidopsis proteome predicts that approximately ten percent of the Arabidopsis proteome contains both ER targeting signals and one or more potential N-glycosylation sites. The *cgl1* plants with cell type specific complementation will also allow for the analysis of cell type specific glycan biosynthesis in Arabidopsis.

O19
A combined approach for the characterization of Glycoproteins by Mass Spectrometry: CE-ESI-TOF-MS and LC-ESI-IT-MS^n

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Most glycosylated proteins show a broad distribution of isoforms, depending on species and environment. Beside the investigation of released sugars, characterization of glycopeptides after proteolytic degradation is the common glycoprotein characterization approach. It provides a detailed analysis of the different glycosylation sites, however, lacking of comprehensive and quantitative information of an isoform. This requires the characterization on the level of intact proteins.
Here we describe a combined approach for the characterization of glycoproteins, based on detailed MS^n characterization of glycopeptides and the exact mass characterization of the corresponding intact protein. Ribonuclease B and bovine fetuin have been chosen as model compounds.
After proteolytic degradation the glycopeptides were analyzed with ESI-IT-MS^n following LC or CZE separation. The MS^n experiments were performed by means of data dependent precursor selection. Thus, the carbohydrate sequence and the peptide sequence could be unambiguously determined.
Integral proteins were analyzed by coupling CZE to a bench-top orthogonal time-of-flight mass spectrometer. Due to the separation of the glycoforms with the CZE and the high resolution of the ESI-oTOF-MS 75 isoforms of fetuin were distinguished. For ribonuclease B isotopical mass-resolution is possible enabling the determination of not only glycoforms, but also the disulfide bond state.
The analysis of the intact protein gives the different isoforms and their quantity, whereas the IT-MS approach with its MS^n capability allows the characterization of the glycan (MS^2) and the peptide structure (MS^3) site specific.

P1
Analysis of the O-linked N-acetylglucosamine modification of lens crystallins

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N-acetyl-glucosamine (O-GlcNAc) linked O-glycosidically to serine and threonine residues of proteins is a cytosolic protein modification which can interfere with phosphorylation in a reciprocal fashion and which may also be implicated in protein-protein interactions. There is also evidence that O-GlcNAc is implicated in type II diabetes.
The lens is the only vertebrate organ which grows throughout life but has no turnover of its structural proteins, named crystallins. These crystallins slowly aggregate to form large complexes during ageing. However, they keep their optical function over many decades. Diabetes has become one of the major blinding causes in industrialized countries due to its effect on lens transparency and retinal function.
We investigated the O-GlcNAc glycosylation of crystallins with respect to 3 parameters associated with diabetes: (a) ageing (b) stress and (c) increasing glucose concentration. Using 2D gel electrophoresis and Western blotting we demonstrate that O-GlcNAc increases with age. Incubation of lense sections in the absence or presence of glucose results in increased O-GlcNAc of some crystallins whereas other crystallins are either not O-GlcNAc modified or O-GlcNAc modified in a glucose-concentration independent manner.
These observations indicate that O-GlcNAc may be implicated in the increased non-functional crosslinking of crystallins leading to early loss of the optical quality of the lens, which is the main causative factor of diabetic cataract.

P2
Expression and Purification of the human UDP-Xylose/UDP-GlcNAc transporter

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Nucleotide sugar transporters (NSTs) supply activated substrates to glycosyltransferases in Golgi and ER and thus play an important role in the glycosylation process. NSTs are type III membrane proteins with probably ten trans-membrane domains. The protein structure is highly conserved in evolution and thus allowed the identification of many putative nucleotide

sugar transporter sequences in the existing databases. We screened human BLASTP database for candidate NSTs and after cloning and expression in *S. cerevisiae*, preparation of microsomal fraction, in vitro transport assay was perform. We found a new human transporter localized in the Golgi apparatus which has specificity for UDP-Xylose and UDP-GlcNAc. The *S. cerevisiae* Golgi and ER vesicles transport GDP-Mannose, UDP-glucose and UDP-GlcNAc due to the presence of endogenous transporters. Therefore, expression, purification and reconstitution in artificial lipoproteosomes could give us a more accurate system to assay NSTs. The human UDP-Xylose/UDP-GlcNAc transporter was expressed in *S. cerevisiae* as a His-tag fusion protein. The protein is very hydrophobic and initial studies show that 100% of the protein is insoluble and associate with microsomal fractions collected in 10 000 g and 100 000 g. Several detergent and different buffers were tested. Best results we obtain using sodium phosphate buffer and β-octyl-glucopiranoside. Only 1-2% of total protein is soluble. Soluble protein was purification with a Ni-agarose column after which the pure transporter could be reconstituted in liposomes by slowly removing of the detergent with "BioBeads".

P3

Differential transfer of *Toxoplasma gondii* glycosylphosphatidylinositols precursors

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Toxoplasma gondii is an ubiquitous parasite that infects nearly all warm-blooded animals. Toxoplasmosis, which is usually asymptomatic in healthy human adults can be a severe disease in immunosuppressed individuals and can cause severe damage to the fetus during congenital toxoplasmosis. The surface of the *T. gondii* tachyzoite is dominated by glycosylphosphatidylinositol (GPI)-anchored surface antigens (SAGs) and free GPIs. Two precursors were described to be attached to GPI-proteins of *T. gondii* grown in Vero cells. Both forms have a GalNAc residue bound to the first mannose of the conserved three-mannosyl core. One of these two forms was shown to contain an additional terminal Glc linked to the GalNAc residue. *T. gondii* GPIs elicit an early IgM immune response after primary infection. The immunogenicity of *T. gondii* GPIs is structure related: sera from infected humans have been found to be reactive only with glycolipids containing the Glc residue. Therefore, we investigated the structure of free and bound GPIs in human fibroblast cultures infected with *T. gondii*. The spectrum of glycolipids observed in human fibroblast cells lacks the GalNAc containing precursor obtained in infected Vero cell cultures. Analyzing the GPI anchor of SAGs we found that in contrast to infected Vero cells cultures only the precursor lacking terminal Glc is used to modify *T. gondii* SAGs. Further, our results indicate that Glc residues are found exclusively in the free GPIs of *T. gondii* grown in human fibroblasts.

P4

Thin Polymer Chip Coupled to Fourier Transform Ion Cyclotron Resonance Mass Spectrometry for Glycopeptide Screening

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The introduction of microfluidic devices in mass spectrometry, in particular ESI-MS, greatly revolutionized the bioanalysis by addressing the fundamental issue of MS investigation of samples originating from biological sources: sensitivity. The areas of implementation and applications of the chip technologies are predominantly proteomics, genomics, and drugs discovery, while their applications in glycomics is poorly represented.

We describe here the coupling of the thin polymer microchip with Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS) at 9.4 T via in-laboratory constructed interface. The methodology was optimized in negative ion mode and applied for screening of urine fractions from healthy individual and patient diagnosed with *Schindler's* disease.

The (-)microchipESI/FT-ICR MS has been optimized to promote high ionic yield under very constant and stable spray conditions, a fair sensitivity and capability to detect with a very good *signal-to-noise* ratio intact sialylated glycopeptides. The particular attribute of the polymer chip coupled to FT-ICR MS to combine ionization efficiency, sensitivity with high mass accuracy of detection is highlighted by the large number of major and minor glycopeptide structures detected and identified in these mixtures. Thus, glycoforms expressing various saccharide chain lengths ranging from tri- to dodecasaccharide bearing up to three sialic acid moieties could be detected and straight-forward assigned with an average mass deviation below 6 ppm.

The method is envisaged to represent a reliable platform for further structural elucidation of glycopeptides by sequencing techniques and an additional valuable and versatile tool for functional glycomic-studies in diseases.

P5

High-level expression in Pichia pastoris and characterisation of biologically active ^{15}N-labeled human chorionic gonadotropin

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Human chorionic gonadotropin (hCG) is a heterodimeric glycoprotein hormone involved in the maintenance of the corpus luteum during the first trimester of pregnancy. Usually, hCG is isolated from urine of pregnant women. Biologically active hCG has been successfully expressed in the methylotrophic yeast *Pichia pastoris* (phCG) (Gadkari et al., 2003) whereby the glycosylation pattern comprises $Man_8GlcNAc_2$ to $Man_{11}GlcNAc_2$ N-glycans, which are eventually phosphorylated (Blanchard et al., 2004). Being interested in the structure of hCG in solution, applying NMR spectroscopy, we have focused here on the expression in P. pastoris and characterisation of biologically active ^{15}N-labeled phCG.
The fermentation of phCG was performed in a 3-liter bioreactor maintained at 28 °C, in which 5 to 10 g/l of $^{15}NH_4Cl$ were fed every 24 h. At the end of the batch phase, just before methanol induction, the wet cell weight reached 109 g/l. Such a high cell density has not been reported earlier for recombinant glycoprotein hormones. Finally, about 30 mg of ^{15}N-phCG could be purified from the 3-liter culture supernatant. The protein was characterised by NMR spectroscopy, MALDI-TOF mass spectrometry, Circular Dichroism, RIA and RRA.

P6
Regulation of protein O-glycosylation *in vivo*

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O-glycan biosynthesis proceeds by sequential addition of monosaccharides. Hence a competition betweeen the glycosyltransferases involved in initiation and elongation of the glycan chains can be anticipated. The aim of this project was to get insight into the regulation of *in vivo* O-glycosylation by using a recombinant MUC1 probe expressed in ldlD-cells which lack UDP-GlcNAc/UDP-GalNAc epimerase. The O-glycan formation in ldlD cells can be controlled by medium supplementation with GalNAc (initiation of O-glycan synthesis) or GalNAc and Gal (elongation of the glycans). In order to elucidate a competition of the core 2 GnT3 with the core1-specific α3 or α6 STs we cotransfected ldlD cells, which are unable to form core 2 glycans, with the respective glycosyltransferase gene. The secretory MUC1 fusion protein was isolated by affinity chromatography, by rpHPLC and analysed for alterations of the O-glycosylation profiles. The glycans were liberated by hydrazinolysis, labelled with 2-aminobenzamide and separated on polymeric amino-phase column. The profiles of fusion protein from non- cotransfected and cotransfected ldlD cells differed significantly with respect to qualitative and quantitative aspects. MUC1 probes from co-transfectants displayed *de novo* core 2 formation and a considerable decrease of Gal1-3(NeuAc2-6)GalNAc, in accordance with the expected competition between C2GnT3 and α6ST for the same substrate position. No significant decrease of NeuAc2-3Gal1-3GalNAc was registrated in the HPLC profiles indicating that C1α3ST does not directly compete with C2GnT3 *in vivo*.

P7
Impact of the absence of osmoregulated periplasmic glucans on the proteome of *Erwinia chrysanthemi*

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Erwinia chrysanthemi is a phytopathogenic entero-bacterium (gram-negative bacteria). It causes soft-rot disease in a wide range of economically important crops. The soft rot symptoms are associated with the synthesis and secretion of pectinases, cellulases and proteases degrading plant cell wall components. Nevertheless, beside these activities easily detectable and well characterized, the virulence can only be achieved after the coordinately regulated expression of a large number of genes. Recognition and interaction with the host require signaling across the envelope. These are keys steps for infection success. Thus, bacterial cell envelope plays an important role in the infection process.
An outer membrane and a cytoplasmic membrane delimiting the periplasm constitute this envelope. It contains several molecule of importance such as proteins, peptidoglycan and osmoregulated periplasmic glucans (OPGs). *E. chrysanthemi*, like other gram-negative bacteria, accumulates large amounts of OPGs in response to low osmolarity of the growth medium. In *E. chrysanthemi*, OPGs consist of 5 to 12 glucose units arranged in a linear β-1,2-linked backbone to which β-1,6-linked branches are added. Moreover, this glucan backbone is substituted by *O*-acetyl and *O*-succinyl ester-linked residues.
Mutants of *E. chrysanthemi*, unable to synthezise OPGs present a pleitropic phenotype: complete loss of virulence on potato tubers and on chicory leaves, bile salt hypersensitivity, enhanced exopolysaccharides production, reduced motility and exo-enzymes synthesis and secretion. Thus, OPGs appear to be essential for the integrity of the cell envelope and for virulence.
Our objective is a better understanding of the complex interactions between components within envelope. Proteomic analysis revealed that the absence of OPGs induces a large modification of protein expression both for envelope and cytoplasmic proteins.

P8
Analysis of oligosaccharides by capillary-scale high-performance anion-exchange chromatography with pulsed amperometric detection (CHPAEC-PAD) and on-line electrospray-ionization ion-trap mass spectrometry (CHPAEC-ITMS).

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High-pH anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) is an established technique for the selective separation and analysis of underivatized carbohydrates. The miniaturization of chromatographic techniques by means of capillary columns, and on-line coupling to mass spectrometry are critical to the further development of glycan analysis methods which are compatible with the current requirements in clinical settings.
A prototype system has been developed based on a Dionex BioLC equipped with a microbore gradient pump and a PEEK flow splitter, a FAMOS micro autosampler, a modified electrochemical cell for on-line capillary PAD and a capillary column (380 μm i.d.) packed with a new type of anion-exchange resin. This system operates with a sensitivity in the low fmol range. In addition, an on-line capillary desalter has been developed to allow direct coupling to a Bruker Esquire 3000 ion-trap mass spectrometer with an electrospray ionisation interface (ESI-IT-MS). Both systems have been evaluated using standard oligosaccharides as well as urine from children with various lysosomal oligosaccharide storage diseases. Our data indicate that the robust and selective anion-exchange system in combination with ESI-IT-MS for structure confirmation and analysis provides a powerful platform which is complementary to existing nano-/capillary LC-MS methods for analytical investigations of oligosaccharides from biological samples.

P9

Titration calorimetry and conformational modelling of oligomannoside binding by the *Pterocarpus angolensis* seed lectin

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The seed lectin from the mukwa tree (*Pterocarpus angolensis*) is a dimeric leguminous lectin with a primary mannose/glucose binding site. Around the primary site there are a number of secondary sites which confer increased affinity for a number of oligosaccharides. Crystal structures of complexes of the lectin with five linear oligomannoses have been determined. One of the sugars exhibits a binding mode different from all the others. Thermodynamic parameters of binding for the different sugars were determined by means of isothermal titration calorimetry. The conformational properties of the sugars were studied by molecular modelling techniques using force fields with additional parametrization for carbohydrates. The change in conformational entropy upon binding was calculated to estimate the contribution of this factor to the total entropy. The structural, thermodynamic and modelling approaches are combined to analyze the structure/function relationship.

P10

Determination of specificity of monoclonal antibodies against *Schistosoma mansoni* antigen using neoglycoconjugates related to the CAA antigen

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The gut-associated circulating anodic antigen (CAA) is one of the major excretory antigens produced by the parasite *Schistosoma mansoni*. The immunoreactive part of CAA is a threonine-linked polysaccharide composed of long stretches of the unique →6)-[β-D-Glc*p*A-(1→3)]-β-D-Gal*p*NAc-(1→ repeating disaccharide. Two series of small oligosaccharides (di- to tetrasaccharides) related to the CAA polysaccharide disaccharide epitope were synthesized, and coupled to bovine serum albumin to evaluate the importance of different functional groups on MAb binding to the CAA epitope. The first three analogues, with the native β-D-Gal*p*NAc moiety replaced by β-D-Glc*p*NAc, were synthesized to investigate the specificity of the MAbs to the carbohydrate backbone of CAA. The second series of analogues, with the native β-D-Glc*p*A moiety replaced by β-D-Glc*p*6*S*, was synthesized to evaluate the importance of the type/nature of the charge of the CAA epitope for the MAb recognition. The binding of both neoglycoconjugate series to a selected panel of MAbs against *S. mansoni* was monitored by Surface Plasmon Resonance and ELISA detection.

P11

Gold glyconanoparticles as a model system to study the carbohydrate mediated self-recognition of marine sponge cells

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Sponges represent the most primitive extant multicellular animals. Nevertheless, they present a complex system responsible for cell motility and adhesion that suggests this system is common to all multicellular animals. Species-specific cell adhesion of the marine sponge *Microciona prolifera* is an event involving the interactions of extracellular proteoglycan-like aggregation

factors (MAF) via two highly polyvalent functional domains, a cell-binding and a self-interaction domain. The interaction of MAF proteoglycan with itself has been characterized as a calcium-dependent carbohydrate-carbohydrate interaction of multiple low affinity carbohydrate epitopes. Partial acid hydrolysis of MAF glycan showed that two carbohydrate epitopes, a sulfated disaccharide [GlcNAc3*S*(β1-3)Fuc] and a pyruvylated trisaccharide [GalPyr4,6(β1-4)GlcNAc(β1-3)Fuc], are implicated in the calcium-dependent self-aggregation phenomenon. SPR studies have demonstrated that the self-recognition of the sulfated disaccharide is a major force behind the proteoglycan aggregation phenomenon. Here, gold nanoparticles were coated with synthetic carbohydrates related to MAF sulfated disaccharide in order to investigate the self-recognition phenomenon. Gold glyconanoparticle aggregation, in the presence of Ca^{2+} ions, was observed by transmission electron microscopy. The aggregation experiments indicate that the combined occurrence of the sulfate and *N*-acetyl groups together with the methyl group of α-L-fucose are essential to the self-recognition phenomenon.

P12

Structural characterization of N-glycans present on gp28 from *Mucuna pruriens* seeds

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Mucuna pruriens seeds have been widely used against snake venom by traditional healers in Africa (Guerranti *et al.*, 2002, *J Biol Chem*, 277, 17072-17078).

Recent studies have shown that the anti-venom activity is mainly due to the presence of a protein which is present as three isomers migrating on SDS-PAGE in a very narrow area around 28 kDa, and thus named gp28. Being positive against ConA, gp28 was indicated to be an N-glycoprotein.

Using a combination of mass spectrometry, ^{1}H NMR spectroscopy and several analytical techniques, the complete characterization of the N-glycosylation pattern of gp28 could be achieved. The identified N-glycans belong to the oligomannose type, whereby the smaller compounds can be β(1-2)-xylosylated and/or α(1-3)-fucosylated, typical for plant glycoproteins.

The full elucidation of the glycan structures of gp28 will help to understand whether the N-glycans are involved at any level in the protection mechanism against the snake venom.

P13

Sulfatide storage in neurons of arylsulfatase A deficient mice overexpressing UDP-galactose:ceramide galactosyltransferase: A mouse model to study the neuronal phenotype of metachromatic leukodystrophy

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Metachromatic leukodystrophy (MLD) is a severe lysosomal storage disorder caused by deficiency in arylsulfatase A (ASA). Inability to degrade sulfatide causes a progressive demyelination and various neurological symptoms in human patients. ASA deficient (-/-) mice have been generated as a mouse model for MLD. A neuronal pathology has been observed in several leukodystrophies. In order to examine the consequences of sulfatide storage in neurons, we generated ASA(-/-) mice over-expressing UDP-galactose:ceramide galactosyltransferase (CGT) in postmitotic neurons under the control of the murine Thy1.2 promoter (Thy1-CGT). These mice showed a progressive C18:0 fatty acid containing sulfatide accumulation in the cortex and histochemically storage material could be detected in neurons of the cortex, hippocampus, and spinal cord. Sulfatide storage could be further increased by introducing a second transgene, cerebroside sulfotransferase (CST), which catalyse the final step in sulfatide synthesis. Starting at 2 months of age transgenic Thy1-CGT/ASA(-/-) mice develop severe motor behavior deficits and they rapidly loose the ability to stay on a rotating rod. Life span was reduced to 5 (line tg4743) and 10 months (line tg4747), respectively. Transgenic mice on an ASA(+/-) background behave normally and had a normal life span.

Ultrastructural analysis revealed evidence for axonal degeneration in the spinal cord of transgenic ASA(-/-) mice. These observations clearly suggests that sulfatide accumulation in neurons might contribute to the motor coordination deficits in ASA(-/-) mice and possibly in MLD patients.

P14

Hypomyelination in transgenic mice overexpressing polysialyltransferase ST8SiaIV in oligodendrocytes

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Polysialic acid (PSA) has been implicated as a negative regulator of myelination. PSA is expressed by oligodendrocyte precursor cells but is down-regulated when these cells differentiate into mature myelinating oligodendrocytes. Transgenic mice were generated in which the PLP promoter drives the expression of the polysialyltransferase ST8SiaIV (PST-1) in oligo-dendrocytes and thereby prevents the downregulation of PSA expression in mature oligodendrocytes. Oligo-

dendrocyte specific expression of the transgene was confirmed by *in situ* hybridization.
Immunofluorescence studies indicated co-localization of PSA and MBP in the white matter suggesting presence of PSA in myelin. This could be confirmed by Western blotting of purified myelin. *In vitro* differentiation of purified oligo-dendrocyte precursor cells was not significantly affected by the transgenic expression of PSA. Myelin content and MBP protein levels were, however, significantly reduced during the period of active myelination in transgenic mice compared to wild-type littermates.
These data suggest that presence of PSA on oligodendrocytes does not inhibit their differentiation *per se* but affects signaling from/interaction with other cells (neurons or astrocytes) in the myelinating brain.

P15
Coupling of fully automated ChipESI to FT-ICR mass spectrometry for high-performance glyco-screening and sequencing

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Modern achievements of microfabrication technology and robotics are currently intensively introduced in the mass spectrometry field, tending to replace gradually the classical ESI or MALDI systems. The latest achievements in chip-based ESI MS methods were mostly implemented in proteomics and genomics, while the applications in the glycomics field are rather poor represented.
Here we report upon the first introduction of fully automated chipESI/FT-ICR MS for high performance mapping and sequencing of carbohydrates. The NanoMate system has been coupled with FT-ICR MS and the approach probed for screening, sequencing by SORI-CID MS^2 and identification of O-glycosylated sialylated peptides from urine of patients suffering from Schindler's disease. Schindler's disease is a rare inherited metabolic disorder belonging to lysosomal storage diseases (LSDs), which leads to an abnormal accumulation of sialylated and asialo-glycopeptides and oligosaccharides of about 100 times higher concentration than in healthy controls. The novel automated chipESI/FT-ICR MS and SORI-CID MS^2 methodology is shown here to provide increased sensitivity, ionization efficiency, high resolution and mass accuracy of detection. Moreover, it was possible to detect a number of glycoforms previously undetectable and to determine their accurate mass as a tool for identification. This construction was shown to allow fragmentation experiments for more detailed structural-data to be obtained by SORI-CID MS^2.

P16
Changes in NCAM polysialylation during postnatal brain development of the mouse

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Polysialic acid (polySia), a posttranslational modification of the neural cell adhesion molecule (NCAM), plays a critical role in the neuronal development of vertebrates. It forms a linear homopolymer of α2,8-linked sialic acid which acts as a negative regulator of cell-cell adhesion due to its highly negative charge and large hydration volume. Polysialylated NCAM is involved in neural cell migration, neurite outgrowth, neural pathfinding and synaptic plasticity. PolySia-chains are synthesized by two polysialyltransferases, ST8SiaII and ST8SiaIV, which are both expressed during development. Earlier studies showed that polysialylated NCAM is abundant in embryonic brain, while most of the NCAM in adult brains does not contain polySia [1].
The aim of our study was to elucidate the time course of polySia down-regulation during postnatal mouse brain development. Brains were homogenized and polySia-NCAM was analyzed by DMB derivatization and HPLC [2] as well as by Western Blot analysis. Up to day 9 the total amount of polySia increased in parallel with the brain mass resulting in approximately constant polySia concentrations. By contrast, a dramatic decrease of the polySia level was observed between day 9 and 15. Since NCAM expression levels were not decreased, polySia down-regulation is caused by a drastic drop in polysialyltransferase activity starting at postnatal day 9. The length of polySia chains exhibited only slight differences from day 1 to day 21, indicating that reduction of polySia is mainly due to degradation of complete polySia chains rather than to constant shortening of existing chains.
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P17
TNFα-induced changes in the glycosylation of human endothelial cells.

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The privileged position of the endothelium, at the interface between blood and tissue, provides it with an important task in regulating the intercellular contacts that are necessary for the extravasation of immune cells in situations like trauma or inflammation. The initiation of these intercellular contacts involves the formation of low-affinity interactions through the binding of selectins, with their carbohydrate counter-ligands expressed at either the

endothelial or the leukocyte side. However, the carbohydrate-dependent interactions at the endothelium are not restricted to selectins, as it has been shown for other C-type lectins, galectins or siglecs, which can play an important role in situations like the trafficking of dendritic cells, neutrophils, B cells, etc. In order to gain insight in the glycosylation changes associated with inflammation, the effects of a prototype inflammatory cytokine (TNFα) were analysed in two different endothelial cell types, umbilical vein endothelial cells (post-capillary venule model), and foreskin endothelial cells (microvascular endothelial cell model), by studying the glycosyltransferases gene expression levels in combination with a panel of lectins and antibodies (flow cytometry). The gene expression results point towards an increase in the expression of galactosyltransferases and sialyltransferases, which could be confirmed by flow cytometry using several lectins. Furthermore, there are tissue-specific differences which suggest distinct patterns of glycosylation-dependent interactions in microvascular and postcapilary endothelium.

P18

Regulation of FUT-I expression controls binding of DC-SIGN to ICAM-2 on human umbilical vein endothelial cells

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C-type lectins constitute a large family of Ca^2-dependent carbohydrate-binding proteins that play important roles within the immune system. DC-SIGN, a dendritic cell-specific C-type lectin, functions both as pathogen-recognition receptor and as cell-adhesion molecule.

As a cell-adhesion molecule, DC-SIGN controls trans-endothelial migration of DC by interaction with ICAM-2 on endothelial cells. The glycans on ICAM-2 that interact with DC-SIGN have not yet been identified. Since DC-SIGN can interact with a variety of mannose- and/or fucose containing carbohydrates, it is expected that differential recognition of carbohydrate ligands by DC-SIGN may be pivotal in regulating DC effector functions. To get more insight in the factors that determine how differential glycosylation of ICAM-2 controls trans-endothelial migration of DC to specific sites in the body, we set out to identify the glycan ligands on ICAM-2 that mediate binding of DC-SIGN, and to investigate their regulation of expression.

Our data demonstrate that DC-SIGN binds to human umbilical vein endothelial cells (HUVECs) through Lewis-y (Le^y) glycan antigens on ICAM-2. By immunofluorescence analysis, we indeed could show co-expression of Le^y and ICAM-2 on HUVECs.

In addition, DC-SIGN binding is enhanced when HUVECs are cultured in the presence of IL-4 and IFNγ. Analysis of the expression of a panel of glycosyltransferase genes in HUVECs by quantitative real-time PCR showed that both IL-4 and IFNγ induce an upregulation of the expression of FUT1 by 60%. By contrast, no differences in the expression of ICAM-2 could be observed. These results indicate that both IL-4 and IFNγ upregulate FUT1 expression in HUVECS, which may consequently result in a higher expression of Le^y antigens on ICAM-2, and enhanced binding of DC-SIGN. This work was supported by a grant from the Mizutani Foundation to IvD.

P19

O-glycan analysis for the detection of congenital defects in mucin type O-glycosylation

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The congenital disorders of glycosylation (CDG) have demonstrated the importance of glycosylation in health and disease. This group of genetic disorders is characterised by a broad spectrum of clinical symptoms, such as psychomotor retardation, dysmorphy, and liver disease. Until now, 17 defects have been found in the biosynthesis of N-linked glycans, and 4 in the biosynthesis of O-linked glycans.
The biochemical mechanisms of N-glycan biosynthesis have been well studied. Disorders that result from defects in the biosynthesis of dolichol-linked oligosaccharides are known as CDG-I, disorders arising from defects in the processing of glycans in the endoplasmatic reticulum (ER) and Golgi are called CDG-II. As a screening method for defects associated with N-linked glycosylation, isoelectric focusing of serum transferrin is commonly used. For identification of the defective enzyme, analysis of the protein-linked glycans has proven useful. This is achieved by detailed analysis of enzymatically released N-glycans using HPLC and MALDI-TOF mass spectrometry. In contrast, the biosynthesis of O-glycans is less well understood, and has appeared to be more complex due to the large variety of core structures and the redundancy of the glycosyltransferases involved. As a screening test for defects in core I mucin type O-glycosylation, we introduced apolipoproteinC-III isoelectric focusing. Here, we present methods to gain insight into the O-glycan structures for the elucidation of genetic defects in mucin type O-glycosylation.

P20

Carbohydrates of keyhole limpet hemocyanin (KLH) mediating cross-reactivity with *Schistosoma mansoni*

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Keyhole limpet hemocyanin (KLH), the oxygen-carrying molecule of the marine snail *Megathura crenulata*, is often used as an adjuvant or as a carrier for low molecular mass haptens like peptides or oligosaccharides. Furthermore, it is known to serologically cross-react with *Schistosoma mansoni* glycoconjugates [1]. In order to provide a structural basis for this cross-reactivity, glycans

of KLH were released, fluorescently labeled, multiply fractionated and analyzed by linkage analysis, exoglycosidase treatment as well as by different mass spectrometric techniques.
The results revealed that all cross-reacting species represent novel types of N-glycans with a Fuc(α1-3)GalNAc(ß1-4)[Fuc(α1-3)]GlcNAc-motif which is known to occur also in schistosomal glycoconjugates. The tetrasaccharide unit is attached to a trimannosyl core, which is further decorated by galactosyl residues and may carry in addition a xylose residue in 2-position of the innermost mannose. This study provides for the first time detailed structural data on the KLH carbohydrate entities responsible for cross-reactivity with glycoconjugates from *S. mansoni*.
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P21
Dissecting posttranslational modifications in *Caenorhabditis elegans*

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Caenorhabditis elegans has been found to be a good model system for parasitic nematodes, drug screening and developmental studies. Structural analyses of glycoconjugates derived from this organism revealed the presence of nematode specific glycosphingolipids of the arthro-series, carrying, in part, phosphoryl-choline (PC) substituents [1]. At the protein level, structural data on the glycome of *C. elegans* revealed a multitude of interesting new carbohydrate structures in this nematode [2-5].
A further remarkable posttranslational modification of proteins is the substitution with PC [6] which plays an important role for the development of the nematode and, in the case of parasitic nematodes, for the modulation of the host´s immune system [1].
So far, PC has been reported to be exclusively bound via N-glycans to the protein backbone [2]. Recent studies performed in our laboratory provided, however, first evidence for other types of PC-substitutions (Lochnit, unpublished observations). Here we present methods and first results for dissecting these two important posttranslational modifications of *C. elegans* proteins, i.e. glycosylation and decoration with PC.
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P22
Convergent actions of IKKβ and PKCδ induced by TNFα modulate β1,4-galactosyltransferase I mRNA stability through phosphorylation of 14-3-3β complexed with TTP

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The pro-inflammatory cytokine TNFα upregulates the expression and enzyme activity of the glycosyl-transferase β1,4-galactosyltransferase I (β4GalT1) in primary human umbilical cord endothelial cells (HUVEC), a process that is controlled at the post-transcriptional level via mRNA stabilization. The 3'untranslated region (3'UTR) of β4GalT1 mRNA contains 4 sites that are potential binding sites for AU-rich elements (ARE)-binding proteins (AUBP), however we found that the TNFα-inducible stabilization of β4GalT1 mRNA was mediated solely through the second ARE (AU2). In resting HUVECs, AU2 was bound by a destabilizing complex of TTP and 14-3-3β, resulting in rapid mRNA turnover. We observed that TNFα induced two distinct signaling pathways, one mediated by inhibitor κB kinase β (IKKβ), the other by protein kinase C δ (PKCδ) that resulted in the release of the TTP/14-3-3β complex from AU2 and the nuclear sequestration of TTP, which was paralleled by an increase in the β4GalT1 mRNA half-life. We provide a mechanism for these observations through the phosphorylation of 14-3-3β by IKKβ and PKCδ on serine residues Ser132 and Ser60, respectively, which interferes with its binding to TTP and hence the retainment of TTP in the cytoplasm.

P23
Identification of glycans on ICAM-3 recognized by the Ctype lectin DC-SIGN

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Dendritic cell-specific ICAM-3-grabbing nonintegrin (DC-SIGN) is an adhesion receptor present as a cell-surface protein on dendritic cells that has been shown to induce T cell proliferation [1].
DC-SIGN is a C-type lectin containing a single carbohydrate-recognition domain with affinity for both high-mannose and Lewis-type glycans [2]. Interaction of DC-SIGN with T cells occurs via ICAM-3, which is a highly glycosylated adhesion molecule on T cells. Although the interaction of DC-SIGN with ICAM-3 has been investigated in detail, the specific carbohydrate structure on ICAM-3 that is recognized by DC-SIGN has yet to be identified.

In the present study we use recombinant ICAM-3 as a model for studying the glycans that are involved in DC-SIGN binding. ICAM-3 was enzymatically hydrolyzed and the glycopeptides were isolated by lectin-affinity chromatography. To obtain complete peptide and glycan sequences, glycans were enzymatically released and both deglycosylated peptides and glycans were analyzed by HPLC and nano-electrospray mass spectrometry (nanoES-MS). We identified a single glycopeptide containing high-mannose glycan structures that may be a target for DC-SIGN. We will use various binding assays to investigate whether the isolated glycopeptide interacts with DC-SIGN.

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P24

Possibilities for high-throughput SPR screening by the attachment of a Pt-pincer to low-molecular-mass carbohydrates

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Carbohydrate-protein interactions play key roles in a plethora of biological and pathological processes such as cell-cell interactions, immune response and cancer progression.

Interaction studies to elucidate these processes are hampered by the low availability of native glycans. Therefore, these studies are often performed with small glycan epitopes that can mimic the native glycan in the biological interaction. Nowadays, surface plasmon resonance (SPR) can be considered as one of the most powerful techniques to evaluate biological interactions in real time. The SPR response is related to the change in surface mass concentration of analyte, in relation to the number of ligand sites on the gold surface. This imposes a lower limit to the molecular mass of the glycan epitope that can be detected in SPR experiments.

Here, it is shown that the attachment of a Pt-pincer to a low-molecular-mass carbohydrate (mono- or disaccharide) enhances the resulting SPR signal upon binding to the immobilized receptor/lectin. The developed method of signal enhancement allows easy and reliable glycan epitope mapping and small-molecule library screening.

P25

Defects in protein N-glycosylation cause apoptosis in *Saccharomyces cerevisiae*

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N-glycosylation in the endoplasmic reticulum is an essential protein modification and highly conserved in evolution from yeast to man. The key step of this pathway is the transfer of the unique core-oligosaccharide $GlcNAc_2Man_9Glc_3$ from its dolichyl-linked precursor form to the nascent polypeptide chain, catalyzed by the hetero-oligomeric membrane complex oligosaccharyltransferase (OST). One of the nine OST subunits in yeast, Ost2, has homology to *DAD1*, originally characterized in hamster cells as a defender against apototic death. We have found that a temperature sensitive *ost2* and other *ost* mutants of *Saccharomyces cerevisiae* display at the restrictive temperature morphological and biochemical features of apoptosis. We observe nuclear condensation and DNA fragmentation as well as translocation of phosphatidylserine as revealed by DAPI, TUNEL and annexin staining, respectively. We also find induction of caspase-like activity, determined by flow cytometry and in cell free extracts, that was sensitive to specific caspase inhibitors. However, disruption of the only known metacaspase *YCA1* does not seem to abrogate apoptosis, as observed in the case of H_2O_2-induced stress. On the other hand heterologous expression of anti-apoptotic hBcl2p suppresses the temperature sensitive growth defect and caspase activation of *ost* mutants. We also observe release of cytochrome c from mitochondria and ROS accumulation in response to defective glycosylation. Altogether the results suggest that aberrant N-glycosylation induces a programmed cell death with apoptotic hallmarks.

P26

Biochemical engineering of the *N*-acyl side chain of sialic acid leads to increased calcium influx from intracellular compartments and promotes adhesion and differentiation of HL60 cells.

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Sialylation of glycoconjugates is essential for mammalian cells. Sialic acid is synthesized in the cytosol from N-acetylmannosamine by several consecutive steps. Using N-propanoylmannosamine, a novel precursor of sialic acid, we are able to incorporate unnatural sialic acids with a prolonged *N*-acyl side chain (e.g. N-propanoylneuraminic acid) into glycoconjugates taking advance of the cellular sialylation machinery. Here we report that unnatural sialylation of HL60-cells leads to an

increased release of intracellular calcium after application of thapsigargin, an inhibitor of SERCA Ca^{2+}-ATPases. Furthermore, this increased intracellular calcium concentration leads to an increased integrin-dependend adhesion to fibronectin.

P27

N-glycans of recombinant human acid α-glucosidase expressed in the milk of transgenic rabbits

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Pompe disease is a glycogen storage disease characterized by the lack of or a major deficiency in acid α-glucosidase (1,4-α-D-glucan glucohydrolase; EC 3.2.1.3/20). So far, several mutations in the gene encoding the acid α-glucosidase have been observed. Treatment of the disease is performed by intravenous injection of recombinant forms of the enzyme. Focusing on recombinant approaches to produce the enzyme means that specific attention has to be paid to the generated glycosylation patterns. Here, human acid α-glucosidase was expressed in the mammary gland of transgenic rabbits. The N-linked glycans of recombinant human acid α-glucosidase (rhAGLU) isolated from the rabbit milk were released by peptide-N^4-(*N*-acetyl-β-glucosaminyl)-asparagine amidase F. The glycan pool was fractionated and purified into individual components by a combination of anion-exchange, normal-phase and SNA lectin-affinity chromatography. The structures of the components were analyzed by 500 MHz one-dimensional and two-dimensional (TOCSY) ^{1}H-NMR spectroscopy, combined with 125.759 MHz two-dimensional ^{31}P-NMR RESED spectroscopy, and MALDI-TOF mass spectrometry. The recombinant rabbit glycoprotein contained a broad array of different N-glycans, comprising oligomannose-, hybrid- and complex-type structures. A fraction of the oligomannose-type glycans contained phospho-diesterbridged *N*-acetylglucosamine.

P28

Sialyl-Tn expression enhances the *in vivo* tumor growth of MDA-MB-231 breast cancer cells.

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Sialyl-Tn is a carbohydrate antigen over-expressed in several epithelial cancers including breast cancer, and usually associated with poor prognosis. Sialyl-Tn is synthesized by a CMP-Neu5Ac: GalNAc α2,6-sialyltransferase: ST6GalNAc I, which catalyzes the transfer of a sialic acid residue in α2,6-linkage to the GalNAcα1-*O*-Ser/Thr structure. However, usual breast cancer cell lines express neither ST6GalNAc I nor Sialyl-Tn antigen. We have previously shown that stable transfection of MDA-MB-231 cells with the hST6GalNAc I cDNA induces the Sialyl-Tn antigen expression at the cell surface. Here, we report that sialyl-Tn expression does not modify the cell growth of monolayer culture of MDA-MB-231 transfected clones, but induces a decrease of their cell growth in three dimensional culture conditions. These observations show that the modification of the *O*-glycans pattern is sufficient to modify the biological features of cancer cells. Finally, although their growth rate is decreased *in vitro,* the sialyl-Tn positive clones are found to grow faster in SCID mice than in the control cells. This suggest that the change of O-linked glycan structures may play role in the cancer / host cells interactions that favor the tumor growth. The study of such interactions is a strong field of investigations, for which the sialyl-Tn positive MDA-MB-231 cells constitute an useful cellular tool.

P29

Glycosidases – bizarre substrates and products

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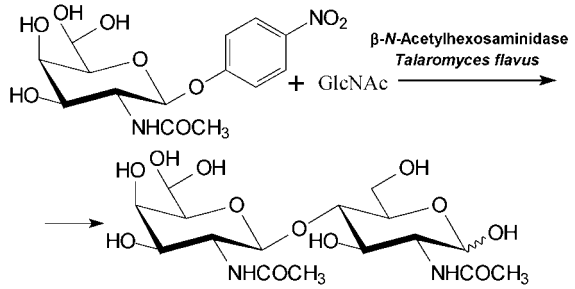
Contrary to glycosyltransferases glycosidases were only scarcely tested for their hydrolytic and synthetic activity with non-natural substrates.

We have prepared large set of modified substrates for β-*N*-acetylhexosaminidases bearing substitutions at C-2 and C-6 and tested as substrates for the hydrolysis and for transglycosylation with a panel of fungal enzymes. Modifications at C-2 acylamido group were studied with *p*-nitrophenyl 2-amino-2-deoxy-β-D-glucopyranoside and its four *N*-acyl derivatives (acyl = CH=O, $COCH_2OH$, $COCH_2CH_3$, $COCF_3$). Besides cleavage, β-*N*-acetylhexos-aminidases catalyse transglycosylations with *N*-acyl modified substrates in good yields (up to 78 %). They do not accept highly electronegative acyls, e.g. trifluoroacetyl, nor the NH_2 group.

Modifications at C-6 included substitutions with the CH_2OAc, CN, CHO, COOH and COOMe groups. Nitriles were partly accepted by the enzyme. Carboxy-group completely blocked enzymatic hydrolysis. C-6 carbaldehyde has been accepted in comparable rate as the natural substrate. Molecular modelling of the substrates

and the docking into the model of the active site of β-*N*-acetylhexosaminidase from *Aspergillus oryzae* demonstrated that aldehyde exists in solution in its hydrated form and this form interacts with the enzyme. This methodology substantially broadens methodology of enzymatic synthesis of glyco-structures.



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P30

_O_-GlcNAc glycosylation of the tumor suppressor HIC1 –Role of the glycosylation in the DNA-binding

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In this study we demonstrate that the tumor suppressor HIC1 (Hypermethylated in Cancer 1) is modified with *O*-GlcNAc, a highly dynamic glycosylation found within the cytosolic and the nuclear compartments of eukaryotes. This glycosylation is not directly involved in the interaction of HIC1 with the co-repressor CtBP as demonstrated by mammalian two-hybrid assay. We have shown by C-terminal deletion mutants that the *O*-GlcNAc modification of HIC1 proteins occurred preferentially in the DNA-binding domain. Using EMSA experiments, unglycosylated and glycosylated forms of the full-length HIC1 proteins, beforehand separated by WGA-affinity purification, displayed the same specific DNA-binding activity. However, N-terminal truncated forms corresponding to BTB-POZ-deleted (the BTB-POZ is an N-terminal dimerization and autonomous repression domain) proteins exhibited a strikingly differential activity, since the glycosylated truncated forms are unable to bind DNA. EMSA performed with separated pools of non-glycosylated and glycosylated forms of a construct exhibiting only the DNA-binding domain and the C-terminal tail of HIC1 (residues 399-714) and supershift experiments with WGA or anti-*O*-GlcNAc antibodies, fully corroborated these results. Nevertheless, these truncated proteins are not *O*-GlcNAc modified in the DNA-binding domain as for the full-length proteins but in their C-terminal tail. Thus oppositely to the truncated forms, the *O*-GlcNAc modification of the full-length HIC1 proteins does not affect the specific DNA-binding activity and the occurrence that it is not the same domain that is glycosylated in the two HIC1 proteic forms demonstrated that *O*-GlcNAc is highly sensitive to conformational effects, notably through the dimerization of the BTB-POZ domain and through its interaction with the *O*-GlcNAc transferase.

P31

Recognition of lead compounds from O-, S- and N-linked glycopeptide libraries by *Ricinus communis* agglutinin

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Interactions between glycoproteins decorating the cell membrane and carbohydrate binding proteins (CBP's) are important in mediating intercellular recognition processes. Only a few residues at the non-reducing end of the oligosaccharide are required for tight interaction with a CBP. Therefore, simplified molecules that can be rapidly prepared and mimic the natural ligand can be used for interaction studies. Here, glycopeptides were used as mimics of glycans, as they are readily obtained in large numbers by generating them in a library format.

O-linked glycopeptides are susceptible towards degradation by glycan-degrading enzymes or acidic conditions. Replacement of the O-linkage with an S- or N-linkage enhances the stability of the glycosidic bond towards biodegradation and acidic conditions. In addition, the increased rigidity of amide-linked galactose may enhance the affinity for the receptor.

O-, N- or S-linked glycopeptide libraries containing galactose were prepared and screened against *Ricinus communis* agglutinin and the effect of different types of glycosidic linkages on the affinity towards the lectin was studied with Surface Plasmon Resonance.

P32

Tumor-associated CD75s-gangliosides and CD75s-bearing glycoproteins with Neu5Acα2-6Galβ1-4GlcNAc-residues are receptors for the anticancer drug rViscumin

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The anticancer drug rViscumin, currently under clinical development, has been shown in previous studies to be a sialic acid specific ribosome inactivating protein (RIP). Comparative binding assays with the CD75s-specific monoclonal antibodies HB6 and J3-89 revealed rViscumin to be a CD75s-specific RIP due to identical binding characteristics toward CD75s-gangliosides. The receptor gangliosides are IV^{6}nLc4Cer, VI^{6}nLc6Cer, and the newly characterized ganglioside VIII^{6}nLc8Cer, all three carrying the Neu5Acα2-6Galβ1-4GlcNAc-motif. In order to elucidate the clinical potential of the rViscumin targets, CD75s-gangliosides were determined in several randomly collected gastrointestinal tumors. The majority of the tumors showed an enhanced expression of CD75s-gangliosides compared to the unaffected tissues. The rViscumin binding specificity was further investigated with reference glycoproteins carrying sialylated and desialylated type II N-glycans. Comparative Western blots of rViscumin and ricin, an rViscumin homologous but galactoside-specific RIP, revealed specific recognition of type II N-glycans with CD75s-determinants by rViscumin, whereas ricin failed to react with terminally sialylated oligosaccharides such as CD75s-motifs and others.This strict binding specificity of rViscumin and the increased expression of CD75s-gangliosides in various tumors suggest this anticancer drug as a promising candidate for an individualised adjuvant therapy of human tumors.

P33

Interaction of glycolipids from *Schistosoma mansoni* cercariae with DC-SIGN

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The recognition of pathogens by dendritic cells is a crucial step in the induction of protective immunity. Dendritic cells express various pathogen-recognition receptors such as Toll-like receptors and C-type lectins. The latter molecules bind sugars in a calcium-dependent manner via highly conserved carbohydrate-recognition domains [1].

Carbohydrates of the parasitic trematode *Schistosoma mansoni*, the causative agent of schistosomiasis, play an important role in immunological processes of this chronic disease [2]. In this context it has been shown recently that the C-type lectin DC-SIGN (Dendritic Cell-Specific Intercellular adhesion molecule (ICAM)-3 Grabbing Nonintegrin) binds soluble egg glycoproteins of *S. mansoni* via the Lewis X-epitope (Galβ1-4)[Fucα1-3)]GlcNAcβ1-) and that this binding is fucose-dependent [3]. Since glycolipids containing the Lewis X-epitope are also dominant antigens in cercariae [4], we proposed that these glycolipids may be recognized by DC-SIGN.

Therefore, isolated cercarial glycolipids were fractionated by HPLC and analysed by ELISA for potential interaction with DC-SIGN. Fractions that bound DC-SIGN contained glycolipids carrying a Lewis X-epitope as well as glycolipids carrying a pseudo-Lewis Y (Fucα1-3)Galß1-4)[Fucα1-3)]GlcNAcß1-) unit.

Hence, it could be demonstrated for the first time that authentic cercarial glycolipids interact with DC-SIGN. The question whether in addition to Lewis X also the pseudo-Lewis Y type of structure is recognised by this lectin is presently under investigation. The binding of DC-SIGN to cercarial glycolipids implies that dendritic cells may interact with the schistosomes very early in the infection process, stressing the importance to investigate the role of this interaction in the immunological response of the host.

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P34

Structural analysis of permethylated oligosaccharides using ESI QTOF tandem MS and deutero-reduction

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Deutero-reduced permethylated oligosaccharides were analyzed by electrospray mass spectrometry and tandem mass spectrometry (MS/MS) using a hybrid quadrupole orthogonal acceleration time-of-flight mass spectrometer, fitted with a nanoflow electrospray ion source. Under these ionization conditions such derivatives form preferentially sodiated molecular species in addition to protonated molecular species. Under collision-induced dissociation, protonated and sodiated molecular species yield simple and predictable mass spectra. A systematic study was conducted on a series of deutero-reduced permethylated glycans to allow rationalization of the fragmentation processes. MS/MS spectra were characterized by fragments resulting from the cleavage of glycosidic bonds. These fragments originating from both the reducing and the non-reducing ends of the glycan yield information on sequence and branching. Furthermore, the substituent 3-linked to a HexNAc unit was readily eliminated. Special attention was devoted to a systematic study of fucosylated glycans. The fucosylated deuteroreduced permethylated glycans were submitted to an acidic hydrolysis, releasing specifically the fucosyl residues. The nascent free hydroxyl groups were subsequently CD_3-labelled in order to determine the positions initially bearing the fucosyl residues along the oligosaccharide backbone. This methodology was finally applied to characterize a glycan pool enzymatically released from glycoproteins. In addition, the present data

show that structural elucidation can be achieved at the 50 fmol level.

P35
ECD of glycoconjugate ions

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Glycosylation of proteins represents the most abundant modification of proteins playing a major role in functional modulation. The structural elucidation of protein glycosylation remains an elusive goal due to the high diversity and microheterogeneity on single glycosylation sites and dynamic changes during the transport in the cell. The comprehensive characterization of a glycoprotein comprises the amino acid sequence, the glycan structure and the attachment site of the carbohydrate.
The interaction of electrons with multiply charged (bio-) macromolecules, electron capture dissociation (ECD) represents a powerful tool for the characterisation of such species. It has been shown that ECD of glycopeptides provides valuable information on both the amino acid sequence as well as on the glycosylation site.
Hypervalent radical cationic mucin-type *O*-glycopeptides formed upon EC additionally fragment by radical site induced fragmentation of the glycosidic bonds giving rise to further information on the glycan itself. These processes involve hydrogen atom migrations from the glycan to the peptide backbone and *vice versa*. To further investigate the fragmentation characteristics especially of oligosaccharides and their derivatives we have submitted such analytes to ECD. Besides cleavage of the glycosidic bonds giving rise to mainly to B-type ions also ring cleavages can be observed. Analytical and mechanistic aspects of this fragmentation processes will be presented and discussed.

P36
The *Drosophila melanogaster* homologue of the human histo-blood group P^k gene encodes a glycolipid-modifying α1,4-*N*-acetylgalactosaminyl-transferase [1]

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Insects express arthro-series glycosphingolipids which contain an α1,4-linked GalNAc residue. In order to determine the genetic basis for this linkage, we cloned a cDNA (CG17223) from *Drosophila melanogaster* encoding a protein with homology to mammalian α1,4-glycosyltransferases and expressed it in the yeast *Pichia pastoris*. Culture supernatants from the transformed yeast were found to display a novel UDP-GalNAc:GalNAc α1,4GlcNAcα1-R α1,4-*N*-acetylgalactosaminyltransferase activity when using either a glycolipid, *p*-nitrophenylglycoside or an N-glycan carrying one or two terminal *N*-acetylgalactosamine residues as acceptors. NMR and mass spectrometry in combination with glycosidase digestion and methylation analysis indicates that the cloned cDNA encodes an α1,4-*N*-acetylgalactosaminyltransferase. We hypothesise that this enzyme and its orthologues in other insects are required for the biosynthesis of the N5a-type [2] and subsequent members of the *arthro*-series of glycolipids as well as of N-glycan receptors for *Bacillus thuringiensis* crystal toxin Cry1Ac.
[1] Mucha J *et al.* (2004), *Biochem J,* **382:** 67-74
[2] Dennis R *et al.* (1985), *J Biol Chem,* **260:**5370-5375

P37
Evidence for O-N-acetylglucosaminylation in the protozoan parasite *Toxoplasma gondii*

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Toxoplasma gondii is an Apicomplexan parasite responsible of toxoplasmosis, a common infection which is asymptomatic for immunocompetent persons. When immunity of host is compromised, toxoplasmosis can cause an important and major disease if it is not treated. Indeed, the latent encysted bradyzoite form of the parasite that persist in brain and muscles, can be reactivated into the virulent tachyzoite form. This reactivation can damage host tissues. For example, in brain, the reactivation can lead to encephalitis toxoplasmosis. The molecular mechanisms involved in the tachyzoite-bradyzoite interconversion are not yet elucidated. However, the stage conversion is accompanied with morphological, physiological and metabolical changes. We have shown the existence of two forms of enolases, glycolytic enzymes, which are specifically expressed according to the parasitic stage. These enzymes are named ENO1 for the bradyzoite stage, and ENO2 for the tachyzoite form. We have recently reported that enolases were targeted in the nucleus during the active replication of the parasite. Using bio-informatic searches, we show that these two isoforms of enolases have putative sites of O-N-acetylglucosaminylation. More importantly, we demonstrate that these two enolases possess one or more O-GlcNAC moieties using the Wheat Germ Agglutinin lectin and a monoclonal antibody specific to O-GlcNAc residues (RL2). In addition, we have identified the putative O-GlcNAc transferase (OGT) in the genome database of *Toxoplasma gondii*. This work reports for the first time the presence of O-GlcNAc in the protozoan parasite *Toxoplasma gondii*.

P38

Molecular characterisation of tissue-engineered human articular chondrocyte transplants

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During the chondrogenic differentiation of mesenchymal stem cells structural changes of the ECM play an important role in the morphogenesis. In order to detect changes in the ECM homeostasis we quantified the mRNA levels of collagen type I, II and III, perlecan, decorin, xylosyltransferase I (XT-I), xylosyltransferase II (XT-II), galactosyltransferase I (GALT-I) and galactosyltransferase II (GALT-II).
After induction of chondrogenesis, bone marrow derived mesenchymal stem cells were cultured for four weeks. We observed an early increase of XT-I and GALT-I mRNA one day after induction. According to GALT-II only marginal changes were detected. Maximum mRNA levels of XT-II were observed one week after the start of chondrogenic differentiation whereas mRNA expression of collagen type I and type III reached a minimum after one week and maximum after four weeks. Expression of perlecan, decorin and collagen type II mRNA increased constantly up to four weeks.
The differential mRNA expression of target genes involved in the ECM metabolism is shown to be an additional method for the characterisation of the chondrogenic development. XT-I and GALT-I are potential markers for early chondrogenic stages whereas XT-II, perlecan, decorin, collagen type I, type II and type III are markers useful for the determination of differentiated chondrocytes.

P39

Xylosyltransferase I: a new marker for the fibrotic degeneration of the heart in dilated cardiomyopathy

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In dilated cardiomyopathy (DCM) remodeling of the failing myocardium is associated with a complex reorganisation of the extracellular matrix (ECM). Xylosyltransferase I (XT-I) is the key enzyme for the biosynthesis of proteoglycans, which are an important fraction of the ECM.
Using real-time RT-PCR we analysed XT-I mRNA levels in left and right ventricular samples of 18 patients with DCM and 6 non-failing donor hearts. Our data show increased amounts of XT-I mRNA in both ventricles in patients with DCM compared to non-failing controls. Raised XT-I levels were paralleled with an elevated content of proteoglycans detected by histological staining in failing hearts. Furthermore, XT-I and collagen type I mRNA levels in mechanically stretched human cardiac fibroblasts (HCF) were both significantly increased in the same range compared to untreated controls (XT-I, 1.28-fold; collagen type I, 1.36-fold; $p<0.02$ each).
XT-I, an important enzyme in fibrotic processes, has pathophysiological impact for ECM-remodeling in chronic heart failure. Myocardial remodeling correlates with increased XT-I mRNA expression and enhanced proteoglycan deposition. Mechanical stress induces XT-I expression in HCF and might be one important inductor in the dilated heart.

P40

Introduction of bisecting N-acetylglucosamine residues in tobacco N-glycans by overexpression of a cDNA encoding human ß1,4-N-acetylglucosaminyl-transferase III (GnT-III)

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In earlier studies aimed at humanization of plant N-glycosylation for the purpose of biopharmaceutical protein production, we showed that ß1,4-linked galactose residues could be introduced into plant N-glycans and that a strong reduction in core-bound xylose and fucose could be attained. In another effort to humanize plant N-glycosylation, transgenic tobacco plants were produced that overexpress the human GnT-III gene driven by the CaMV 35S promoter, which leads to ubiquitous expression of an enzyme activity that is completely lacking from wild-type plants. GnT-III catalyzes the attachment of a bisecting N-acetylglucosamine (GlcNAc) residue in β1,4-conformation to the ß-linked mannose of the N-glycan core. Normally, the enzyme is completely lacking from plants. Western blot analysis of leaf proteins of wild-type and transgenic plants, with a polyclonal antibody that is used to detect core-bound xylose and fucose residues, revealed a significant signal reduction with proteins from GnT-III plants compared to those from wild-type plants. Comparison of the released N-glycans from total leaf proteins of wild type and GnT-III plants by MALDI-TOF-MS and normal-phase nano-LC-MS/MS revealed that: a) the major wild-type N-glycan, $MMXF^3$ (xylosylated, core α1-3 fucosylated trimannosyl N-glycan) is absent from the GnT-III plant. b) the GnT-III plant exhibits $GnGnXF^3$bi as the major N-glycan species, carrying additional GlcNAc residues on both antennae and a bisecting GlcNAc.

The MS-data confirm the efficient expression of GnT-III in the transgenic plants and suggest that bisected N-glycans may be less prone to degradation by endogenous hexosaminidase activity.

P41

Expression and characterization of cerebroside sulfotransferase in baculovirus infected insect cells

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Cerebroside sulfotransferase (CST) synthesize sulfatide by the transfer of PAPS to galactosylceramide. In patients suffering from metachromatic leukodystrophy (MLD) sulfatide accumulates in oligodendrocytes and neurons leading to severe neurological symptoms and a progressive demyelination due to deficiency in arylsulfatase A.

Substrate deprivation therapy is an option for treatment of lysosomal storage disorders [1]. Therefore we try to produce larger amounts of the CST enzyme for structural analysis. We recently showed that CST can be expressed as a soluble active enzyme in mammalian cell lines [2]. Here we used the baculovirus system to express soluble hexahistidine-tagged CST in Sf21 cells. The protein could be purified by affinity chromatography. Data on the biochemical characterization of the enzyme will be presented.

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P42

Mannosamine can replace glucosamine in glycosylphosphatidylinositols of *Plasmodium falciparum*

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Neither parasite development nor GPI biosynthesis were blocked by mannosamine (2-deoxy-2-amino-D-mannose) treatment in *P. falciparum* cultures. It was shown by metabolic labeling with [^{3}H]mannosamine and subsequent monosaccharide analysis by high pH anion exchange chromatography (HPAEC) that mannosamine is converted at a high rate into glucosamine. Both mannosamine and glucosamine are incorporated into *P. falciparum* glycolipids, but the characterization of mannosamine-labeled glycolipids synthesized *in vivo* proved difficult. Therefore, a cell free system was developed to investigate the incorporation of [^{3}H]mannosamine into glycolipids in *P. falciparum*. It was observed that mannosamine is incorporated *in vitro* into very hydrophobic glycolipids, which possess a phosphate group. Chemical (nitrous acid deamination, mild acid hydrolysis and alkaline hydrolysis) and enzymatic (PI-PLC) treatments of [^{3}H]mannosamine-labeled glycolipids synthesized *in vitro* showed the presence of GPIs. Further analyses by Bio-Gel P4 size-exclusion chromatography and HPAEC demonstrated the presence of a mannosamine-containing GPI-like structure, where mannosamine is incorporated instead of glucosamine, i.e. Man_3–ManN-PI. This utilization of mannosamine is novel and has not been described for any other cellular or parasitic system.

P43

Novel vectors for the expression of glycosyltransferases in microbial hosts

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Effective expression systems for glycosyltransferases are important tools for carbohydrate engineering. The present abstract introduces two novel vector systems for the expression of mammalian glycosyltransferases in *E. coli* or *S. cerevisiae*.

The first expression vector pTS05 yields soluble expression of different mammalian glycosyltransferases in *E. coli*. The constructs are fusion proteins of an N-terminal His_6-tagged pro-peptide of a lipase from *Staphylococcus hyicus* with the corresponding catalytic glycosyltransferase domains. The expression is under the control of the *T7*-promoter. The fusion protein containing the catalytic domain of the human β-1,4-galactosyltransferase was expressed with a specific activity of up to 50 mU mg^{-1}. To our knowledge this is the best result for the expression of a mammalian galactosyltransferase in *E. coli*. Work is in progress for the soluble expression of the human polypeptide-*N*-actylgalactosaminyltransferase 2 and of mouse β-1,3-*N*-acteyl-glucosaminyltransferase 2.

The second expression vector pDR195 yields a constitutive protein expression in yeast. The cloned genes are under the control of the plasma-membrane-ATPase-promoter (*PMA1*). Cultivation of yeast cells under aerobic and glucose limited growth conditions gives protein expression with high productivity. The production of the plant Leloir glycosyltransferase sucrose synthase 1 from potato in a 30-L-bioreactor resulted in 12 000 U enzyme with a specific activity of 280 mU mg^{-1} and productivity rate of 150 U L^{-1} h^{-1} [1]

[1] Römer U, Schrader H, Günther N, Nettelstroth N, Frommer WB, Elling L (2004) *J Biotechnol* **107**:135-49

P44

Broadening the biocatalytic properties of recombinant Sucrose Synthase 1 by expression in *S. cerevisiae* and *E. coli*

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The present paper describes the different biochemical and biocatalytic characteristics of the recombinant glycosyltransferase sucrose synthase 1 (SuSy1, EC 2.4.1.13) from potato by the expression in *S. cerevisiae* and *E. coli*. In our previous work SuSy1 was produced in *S. cerevisiae* as a biocatalyst with a broad substrate spectrum leading to a variety of sucrose analogues [1]. Based on inhibitor studies SuSy1 produced in yeast prefers the $^1C_4$-conformation of β-D-fructopyranose. The kinetic data for recombinant SuSy1 expressed in yeast were in accordance with data from literature describing the effect of phosphorylation on the affinity of SuSy.
In order to obtain the non-phosphorylated enzyme the *sus1* gene was expressed in *E. coli*. Most strikingly, differences in the oligomeric organization of the SuSy1 subunits were elucidated by gel filtration studies. Inhibitor studies revealed a preferred acceptance of β-D-fructofuranose. This difference was further pronounced by the acceptance of different acceptor substrates, e.g. L-ribulose, D-tagatose, L-glucose, L-rhamnose, and 1,6-anhydroglucose, are new substrates only accepted by recombinant SuSy1 from *E. coli*.
In summary, our studies demonstrate that expression of SuSy1 in different hosts does have an influence on the enzyme's biochemical and biocatalytic properties which may be related to protein phosphorylation. Work is in progress to study the effect of phosphorylation on the biocatalytic properties of SuSy1 by mutagenesis of the conserved phosphorylation site at Ser11.

[1] Römer U, Schrader H, Günther N, Nettelstroth N, Frommer WB, Elling L (2004) *J Biotechnol* **107**: 135-49

P45

UDP-Galactopyranose Mutase in *Aspergillus fumigatus*

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Galactofuranose (Gal_f) has been found in the cell wall of many parasitic organisms ranging from bacteria to fungi and protozoans. In *Aspergillus fumigatus*, Gal_f is located in the extracellular galactomannan that forms the host-parasite interface. UDP-Galactopyranose mutase (UDP-Gal mutase) converts galactopyranose into the furanose form and is therefore the key enzyme in Gal_f biosynthesis. Humans lack galactofuranosyl residues, which makes enzymes in the Gal_f biosynthesis pathway attractive drug targets. It was previously shown that UDP-Gal mutase activity is essential for mycobacterial growth and viability, but for eukaryotic parasites such as *Aspergillus fumigatus* this remains to be proven.
We identified the UDP-Gal mutase gene in the *Aspergillus fumigatus* genome by sequence homology to bacterial mutases. The gene was amplified from cDNA and cloned into a prokaryotic expression vector. The cloned cDNA was shown to complement a UDP-Gal mutase deficient *E. coli* strain and, in addition, the encoded protein was active in an *in vitro* assay. We are now generating an *Aspergillus fumigatus* knock out mutant by substituting the UDP-Gal mutase gene with a phleomycin-resistance cassette via homologous recombination. The resulting phenotype should reveal the importance of the UDP-Gal mutase gene for *Aspergillus fumigatus*.

P46

Analysis of the association between diabetic nephropathy and genetic variations in the xylosyltransferase genes XT-I and XT-II in type 1 diabetic patients

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Epidemiological and family studies indicate that genetic predispositions for diabetic nephropathy (DN) exist, but as yet the causative genes remain unknown. The glomerular basement membrane (GBM) contains heparan-sulfate proteoglycans (HSPGs) which are responsible for the size- and charge-dependent passage of macromolecules. DN leads to reduction of HSPG content in the GBM. Xylosyltransferase I (XT-I) is the key enzyme in HSPG biosynthesis. It catalyses the transfer of xylose from UDP-xylose to serine residues in the core protein. The XT-II gene encodes a protein highly homologous to XT-I.
To investigate if these genes associated with the development of DN we screened all exons for variations using denaturing HPLC. In total we detected 34 variations. We genotyped 26 of these SNPs in type 1 diabetes patients (48 with and 48 without DN) and a nondiabetic control group.
SNP c.359G>A (p.R120H) in exon 2 of XT-II occured with a significantly different frequency in type 1 diabetes patients versus the nondiabetic control subjects ($p=0.0341$). A comparison of the SNPs genotypes and clinical characteristics of DN or nonDN patients revealed significant associations for 10 SNPs.
Our results suggest that XT-genes are not involved in the pathogenesis of DN in type 1 diabetic patients.

P47

Heart transplantation and ventricular assist device implantation and their effect on xylosyltransferase I activity

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Xylosyltransferase I (XT-I) initiates the biosynthesis of glycosaminoglycan chains in proteoglycans, which are involved in many biological processes.

In this study we monitored the XT-I activity in serum samples of patients after heart transplantation (HTX) and patients with a ventricular assist device (VAD) over a period of 6 to 40 days. The XT-I activity of patients after HTX droped during the first hours after transplantation (mean value 0.59 mU/L, SD 0.19) and reached a plateau within the normal range after approximately 5 days (mean value 0.84 mU/L, SD 0.27). Children were found to have high XT-I activities at the upper normal level (mean value 1.05 mU/L, SD 0.22). Patients with a ventricular assist device showed a slowly decreasing XT-I activity below the normal range within a few days after implantation (mean value 0.55 mU/L, SD 0.18). Several days later the enzyme activity stabilized at low normal levels (mean value 0.63 mU/L, SD 0.20).
Time-course monitoring of the XT-I activities in HTX and VAD patients revealed substantial individual differences, probably due to varieties in medication and operation techniques. The slow decrease of the XT-I activity in blood of VAD patients is probably due to an improved organ regeneration after VAD-mediated compensation of congestive heart failures.

P48
Identification and enzymatic characterisation of hST8SiaVI.

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Similarity searches in dbHTG and dbEST divisions of the GenBank database using the previously described sialyltransferases cDNAs as probes have revealed the existence of an additional open reading frame located on human chromosome 10 as yet undescribed. This nucleotide sequence encodes a protein of 398 amino acids with the conserved motifs of sialyltransferases (sialylmotifs L, S and VS) and the specific motif of the α2,8-sialyltransferases (I/L)(F/Y)GFWPF. Furthermore, sequences alignment shows 35% amino acid identity with the human α2,8-sialyltransferases hST8Sia I and hST8Sia V. We thus named this new sialyltransferase hST8Sia VI.
Expression of the gene of hST8SiaVI was analysed in human cultured cells by RT-PCR. It appears to be ubiquitously expressed at different levels depending on the cell type. A cDNA clone encoding the full-length hST8SiaVI was obtained from the breast cancer line MCF-7. The recombinant soluble form of hST8SiaVI expressed transiently in COS-7 cells was shown to transfer sialic acid residue onto O-glycans of fetuin. Furthermore, the unique incorporated sialic acid residue was α2,8-linked onto the sialic acid residues α2,3-linked of the trisaccharide Neu5Acα2-3Galβ1-4GalNAc. In order to determined precisely the substrate specificity of hST8Sia VI, we are producing this enzyme in Sf9 insect cells line. Other putative substrates such O-glycosylproteins or glycolipids will be used to refine the acceptor substrate specificity of hST8Sia VI.
Until now, the physiological relevance of this new sialyltransferase is unknown but could be implicated in the biosynthesis of the newly disialylated O-glycosylproteins described.

P49
LacNAc and LacdiNAc-containing glycoconjugates induce granulomas in an *in vivo* model for hepatic granuloma formation in schistosomiasis

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Periovular granulomogenesis leads to pathology in schistosomiasis. Using a mouse model based on hepatic implantation of antigen-coated beads, the glycans of schistosome egg glycoproteins have been identified as the primary driver of granuloma formation [1]. To determine which particular glycans induce granulomas, beads were prepared that carry defined synthetic fucosylated and non-fucosylated GlcNAc, LacdiNAc and LeX-derivates representative for schistosome eggs. In addition, RNase A and B and (modified) fetuin were included as controls. Of the tested beads, only those carrying glycoconjugates with terminal GalNAcβ1-4GlcNAc or Galβ1-4GlcNAc elements gave rise to granulomas, with macrophages, lymphocytes and eosinophil levels comparable to the granulomatous lesions caused by schistosome eggs in a natural infection. Uncoated beads and conjugates lacking terminally exposed Gal or GalNAc only attracted a monolayer of macrophages. The granulomas express galectin-3 that has been described in activated macrophages, fibroblasts and eosinophils as a regulatory molecule. Recently it was found that galectine-3 binds to LacNAc and LacdiNAc and is a receptor for schistosome glycoconjugates [2]. Our results indicate that LacNAc and LacdiNAc are triggers of granuloma formation and suggest that hepatic lectins such as galectin-3 play a role in this process.
[1] van de Vijver KK *et al.*, (2004) *Int J Parasitol* **34**: 951-61.
[2] van den Berg TK *et al.* (2004) *J Immunol* **173**:1902-7.

P50
A bioinformatics pipeline for genome-wide analysis of glyco-proteins.

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Although many different software packages are available for protein analysis and peptide Mass prediction, the coupling of these programs is not always easy due to differences in input/output format and lack of compatibility with bulk data input (e.g. full proteome sequence sets). We have developed a pipeline, which couples the bioinformatics analysis programs TargetP, SignalP, TMHMM and ProtGest. The pipeline enables *in silico* processing and analysis of full genome sequence-data input sets. The pipelines integrates (1) selection of

proteins with a SP, (2) selection of proteins with putative N-glycosylation, (3) *in silico* removal of the SP, (4) *in-silico* trypsin digestion, (5) *in-silico* deglycosylation by PNGases and finally, (6) peptide mass prediction. The pipeline can be used for selection of putative N-glyco-proteins from large sequence data sets and prediction of masses from putative glyco-proteins. Such data is used as inclusion lists in MS analysis of glyco-proteomes using in wet experiments. Using the pipeline the putative N-glyco-proteome of some model (plant) species were analyzed and compared.

P51
Structural analysis of anti-corrosive polysaccharides

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In previous studies it was shown that polysaccharides in marine bio-films have an effect on corrosion of ship hulls. Although literature is ambiguous about whether the effect is beneficial or adverse, the findings are reason enough to study polysaccharides as anti-corrosives more closely. In a pilot study it was found that two homopolysaccharides secreted by lactic acid bacteria had promising anti-corrosive properties. Both exopoly-saccharides (EPSs) are large, highly branched polysaccharides that contain only glucose units. In electrochemical studies it was shown that EPS A offered better protection against corrosion than EPS B. Comparing the structural differences between the two polysaccharides may provide information on the structural elements that provide best protection against corrosion. From linkage analysis it was found that EPS A is a dextran, containing mostly $\alpha(1\rightarrow6)$ linkages with $\alpha(1\rightarrow3)$ branches and EPS B is a structure with mainly $\alpha(1\rightarrow4)$ linkages and $\alpha(1\rightarrow6)$ branches. In both polysaccharides approximately one in eight residues is a branching residue. To identify the structural features of the two polysaccharides that may be involved in the anti-corrosive activity, the polysaccharides were subjected to partial acid hydrolysis. The fragments were fractionated by size-exclusion chromatography and HPAEC-PAD. Purified oligosaccharide fragments were analysed with MALDI-TOF/MS and NMR spectroscopy.

P52
The dendritic cell expressed C-type lectin MGL specifically interacts with terminal GalNAc residues

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The C-type lectin MGL is expressed by immature dendritic cells (DC) and macrophages in the human skin. The MGL protein contains one Carbohydrate Recognition Domain (CRD). Little is known about the actual carbohydrate specificity of MGL for self-glycoproteins or pathogens and how this recognition influences the immune system. We generated a MGL-Fc chimeric protein to facilitate efficient screening of potential membrane and soluble ligands or carbohydrates. Using a glycan micro-array system we identified a terminal GalNAc-specifity for MGL. Based on the results from the array, we identified helminth specific glycans in the soluble egg antigens of *Schistosoma mansoni* that interact with MGL. Furthermore, terminal GalNAc residues are associated with tumor pathogenesis. Indeed, tumorcells, either from T cell, melanoma or adenocarcinoma origin, were bound with high affinity by MGL. We identified MUC1 as the MGL ligand in the adenocarcinoma cell line ZR75-1. MGL recognition of MUC1 was specific for tumor-derived MUC1 as normal MUC1 expressed by mature DC did not interact with MGL. These results implicate a role for MGL in the pattern recognition of helminths and tumors by DC.

P53
Endogenous *N*-glycolylneuraminate biosynthesis in human mammary carcinoma cells

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N-glycolylneuraminate (Neu5Gc) is an abundant sialic acid in animal cells. In normal human cells, the CMP-*N*-acetylneuraminate monooxygenase involved in the formation of CMP-Neu5Gc in animal cells is not functional. Contrary to this, Neu5Gc has been detected in human mammary carcinoma cells by chemical and immunochemical assays, but these findings were ascribed to uptake of Neu5Gc from exogenous dietary sources.
The aim of our work is to confirm the endogenous formation of Neu5Gc in human tumor cells and to elucidate the biosynthetic pathway. To confirm the endogenous origin of NeuGc, we grew mammary carcinoma cell lines under serum-free conditions for several months. Neu5Gc detection was performed by GC/MS analysis after sialic acid hydrolysis from tumor cell glycoproteins. In contradiction to a previous report [1] we could demonstrate for several breast cancer cell lines that detection of Neu5Gc is independent from external uptake by the cells.
To establish the route of endogenous Neu5Gc formation, we currently perform tracer experiments with ^{14}C-labelled precursors. Mannosamines carrying nonphysiological groups at their *N*-positions (e.g., propanoyl or levulinoyl groups) are easily metabolized to the corresponding sialic acids. Accordingly, glycolyl-CoA might be the main source of the glycolyl group of glycolylmannosamine and finally of Neu5Gc.
To elucidate altered enzymatic activities yielding a metabolic pool of glycolate, a gene expression analysis is currently performed by DNA chip technology.

The status of Neu5Gc as a tumor marker is currently under investigation.

[1] Tangvoranuntakul P *et al* (2003), *PNAS* **100**: 12045-50

P54

Glycopeptide fragment ion analysis by MALDI-TOF/TOF-MS and nano LC-ion trap mass spectrometry

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Structural studies of protein glycosylation based on the analysis of glycopeptides rather than released glycans have intrinsic advantages, including being easier to integrate in existing proteomics approaches and more informative on site-specific glycosylation and heterogeneity. We have established different mass spectrometric (MS) techniques for the characterization of glycopeptides by fragment-ion analysis. MALDI tandem time-of-flight (TOF/TOF) MS of (tryptic) N-glycosylated peptides derived from horseradish peroxidase (HRP) [1] and asialofetuine gives rise to fragmentation of peptide bonds with retention of the intact glycan moiety. In addition, in the same spectra, a second group of readily interpretable signals arises from the cleavage of glycosidic linkages with retention of the intact peptide moiety. Thus, MALDI-TOF/TOF-MS provided detailed information on the peptide and glycan sequences as well as the glycosylation site in a single fragmentation spectrum. Furthermore, glycopeptides from HRP and ribonuclease B were analyzed by nano-liquid chromatography (LC)-MS/MS on an ion-trap instrument allowing multiple selection/fragmentation cycles. The MS/MS spectra of glycopeptides derived from HRP and ribonuclease B were dominated by cleavages of glycosidic linkages, with major fragments retaining a single *N*-acetylglucosamine on the peptide. A further isolation/fragmentation cycle applied to these ions yielded peptide fragments with the GlcNAc retained, allowing identification of the peptide and the glycosylation site. Taken together, the two outlined methods provided unparalleled insight in the glycosylation of HRP.

[1] Wuhrer M *et al.* (2004) *Rapid Commun Mass Spectrom* **18**: 1741

P55

Mass spectrometric identification of mannoproteins covalently bound to the skeletal glycan layer in the cell wall of *Saccharomyces cerevisiae*

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Fungi are covered with a layer of glycoproteins emanating from the underlying skeletal layer of the cell wall. In this study, we have used *Saccharomyces cerevisiae* as a model organism to develop a mass spectrometric method to directly identify covalently bound fungal cell wall proteins (CWPs). By harvesting cells at mid-log growth phase and at stationary phase, we have identified in total 19 CWPs, including 12 known or predicted GPI-CWPs. The same GPI-CWPs could be extracted with HF-pyridine, which preferentially cleaves phosphodiester bonds, supporting the notion that GPI-CWPs are linked to the skeletal layer of the cell wall through GPI modification. Extraction with NaOH revealed besides the four known Pir-CWPs and Cwp1p three new members of the class of alkali-sensitive proteins, *i.e.*, Tos1p and the β1,3-glucanases Scw4p and Scw10p. GPI-protein Cwp1p is the only protein that was present in both fractions, confirming earlier results that it can be linked in both ways to the cell wall glycan network. Interestingly, seven of the identified proteins are carbohydrate-active enzymes. Conceivably, they are involved in cell wall remodeling and biofilm formation. We will use this method to monitor the dynamics of cell wall protein composition in response to changes in environmental conditions. Also, since the method is independent of the nature of the linkages between the proteins and carbohydrates in the cell wall, it can also be applied to other fungi, e.g. the human pathogen *C. albicans*.

P56

Chinese Hamster Ovary cell deficient in UDP-Xylose Synthase (USX) , a Key Enzyme in Proteoglycan Synthesis

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Xylose is the first sugar residue within the core of all proteoglycans. Beside there, Xylose is found in other O-linked glycans. These proteins are very diverse in structure and function and the individual role of xylose in these complexes is still unknown. This problem can be addressed by producing O-linked glycans without xylose and subsequently studying the effect on the molecular function of the protein. Here we describe a method for the screening of CHO cells selected for defects in the proteoglycan biosynthetic pathway that are not able to incorporate xylose in other glycans. Such mutants should have a defect in UDP-Glucuronic Acid Decarboxylase (now known as UDP-Xylose Synthase and designated as UXS). This enzyme catalyzed conversion of UDP-Glucuronic acid into UDP-Xylose. UDP-Xylose is used as substrate by different xylosyltransferase which conjugates xylose, either directly to the Ser/Thr of the core protein or in growing sugar chain on different other proteins. Uniquely, plants contain an additional β(1,2)xylosyltransferase not found in mammals. It recognises $GlcNAcMan_3GlcNAc_2$ of N-glycans and

transfers a xylose residue on the β(1,4) linked Mannose. This xylose residue on core N-glycan is recognized by rabbit antisera produced against horseradish peroxidase (HRP). Using this information, we transiently co-transfected these mutant cells with cDNAs encoding *Arabidopsis* β(1,2)xylosyltransferase (AtXylT) and human UXS. While wild type cells are stained with anti-HRP antibody after transfection with AtXylT alone, the mutant only becomes positive when both AtXylT and human UXS are transfected. Therefore, these cells most likely are deficient in UXS.

P57

Transgenic rescue of UDP-galactose:ceramide galactosyltransferase deficient mice

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Galactosylceramide (GalC) and its sulfated derivative sulfatide are important constituents of the myelin sheath. GalC is synthesized in the ER by UDP-galactose:ceramide galactosyl-transferase (CGT). Mice lacking CGT form unstable myelin with disorganized paranodal regions. They have a reduced nerve conduction velocity and die at 1-3 months of age [1]. In the mammalian brain CGT is not only expressed in oligodendrocytes but also in some neurons. Furthermore, sulfatide has been detected in neurons and astrocytes. We therefore asked whether all phenotypic alterations observed in CGT(-/-) mice can be attributed to the lack of GalC in oligodendrocytes or whether lack of GalC in other cell types, e.g. neurons, contributes to the pathology and early death of CGT(-/-) mice. Therefore, we generated CGT(-/-) mice mice expressing CGT under the control of the oligodendrocyte specific proteolipid protein (PLP) promoter (PLP-CGT). Myelination in these mice was normal and lipid analysis revealed synthesis of GalC and sulfatide. Transgenic PLP-CGT/CGT(-/-) mice did not show obvious deficits in motor coordination. The phenotypic rescue was not possible by transgenic overexpression of CGT in neurons using the Thy1.2 promoter. These results show that loss of GalC and sulfatide in oligodendrocytes is mainly responsible for the phenotype and early death of CGT(-/-) mice. Transgenic PLP-CGT/CGT(-/-) are therefore a useful model to study the function of GalC and sulfatide in neurons and astrocytes, and other cell types in which the PLP promoter is not active.

[1] Coetzee T *et al* (1998) *Trends Neurosci* **21**: 126-130

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