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Scientific Report (*Final version*)

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Convener Name: Professor Lubbert Dijkhuizen, Groningen, NL

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1. Summary

The Summer Course Glycosciences is a bi-annual event that was organized for the 12th time this year in close collaboration between Wageningen University, University of Groningen, CERMAV-CNRS and Grenoble Universités / ESRF.

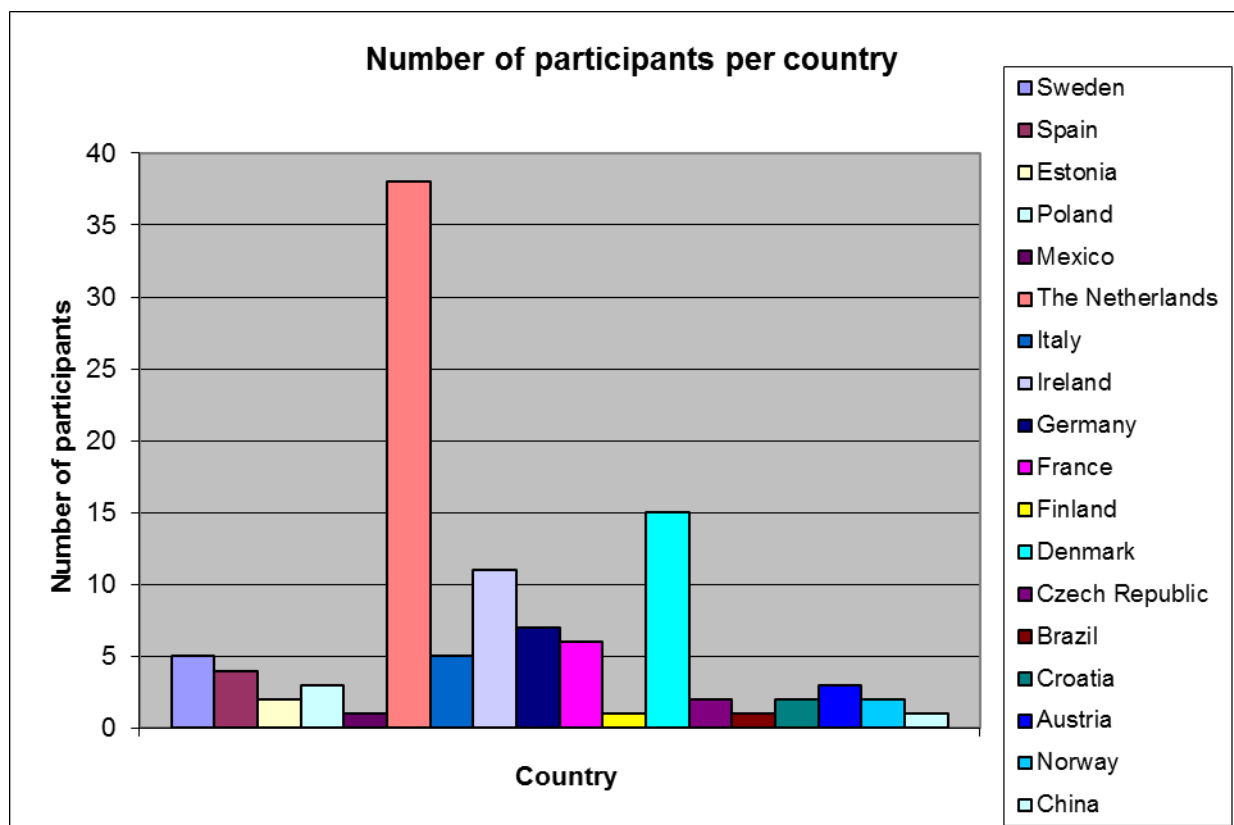
Distinctive for the Summer Course Glycosciences is that general introductions in the field of polysaccharides and glycoproteins are combined with in depth parallel sessions. This gives participants the possibility to focus on specific interests without losing a broad education.

Another feature of the Summer Course is that most lecturers will remain present during the course for further communication with participants. In addition, within the programme 20-25% of time was scheduled for discussions.

During this edition the participants were exposed to 19 plenary sessions and seven parallel sessions, altogether 33 lectures given by 23 senior scientists from eight European countries.

On average this Summer Course attracts about 100 participants. This year we welcomed 115 participants from 18 different European countries and 3 participants from other continents, namely South America (Brazil, Mexico), Asia (China)!

To encourage participation by researchers from Eastern Europe and from developing countries, a number of grants were made available. This resulted in a further increase in participants from the above mentioned target group, to 11% of total count, compared to 6.7% in 2010.



Prior to the start of the course, all participants and lecturers have been given access to our special Glycosciences website with course information and biographical sketches of the lecturers, and downloadable background literature. In addition, after the course all presentations have been made available as PDF-files. The website will remain open to all participants for at least one year. A course binder containing hardcopies of all presentations, a Book of Abstracts and a printed Book Chapter were handed out to all participants and lecturers during registration.

2. Scientific content and discussion

Introduction

The 2012 Summer Course Glycosciences (12th European Training Course on Carbohydrates), held from June 3 to June 7, 2012, in Groningen, The Netherlands, comprised a well-balanced educational programme for the 118 participants (master students, PhD students, post-docs, lecturers, industrial scientists), mostly from all over Europe. The topics were grouped in 6 topical research areas:

- **Medical and Health Aspects of Carbohydrates**
- **Polysaccharides and Functional Properties**
- **Carbohydrate Degrading Enzymes**
- **Carbohydrate Analysis**
- **Chemical and Enzymatic Synthesis of Carbohydrates**
- **Valorization of Carbohydrate-rich Biomass**

Medical and Health Aspects of Carbohydrates On the opening evening Professor Dr. J. Garssen (Danone Research and University of Utrecht) discussed health aspects of poly- and oligosaccharides, with emphasis on the benefits of non-digestible oligosaccharides on the immune system. He distinguished a non-specific first line defense, a non-specific innate immunity and finally a specific adaptive immunity. Their development, function, action and interaction as well as the consequences of its failure were described. Results of recent studies of prebiotics on the immune system were then discussed. Their indirect (through the composition of the gut flora) as well as direct effect on the immune system were demonstrated with results of studies. Prebiotic mixtures of scGOS/lcFOS were found to affect both DC/T cell interactions, macrophages as well as TLR9 stimulation in a direct way without any bacteria or bacterial products available. scGOS/lcFOS were also shown to lead to highly significant effects on infections as well as allergies.

Professor Dr. J.M.F.G. Aerts (AMC, University of Amsterdam) reported studies on Gaucher disease, the most common lysosomal storage disorder due to a deficiency in an enzyme, lysosomal acid beta-glucosidase (Glucocerebrosidase; GBA1), currently treated by enzyme supplementation therapy. He described the synthesis of activity-based probes allowing specific and sensitive visualization of GBA1, i.e. in cultured cells, and in different tissues of mice.

Dr. E. van Leusen (FrieslandCampina) reviewed the synthesis of galacto-oligosaccharides (GOS) from lactose with the enzyme beta-galactosidase, detailing the scaling up process from small scale laboratory experiments to commercial scale bulk production. Since their market introduction about 10 years ago, GOS are nowadays broadly applied in nutritional formulations.

Prof. Dr. O. Holst (Research Center Borstel, Germany) discussed the endotoxic properties of Gram-negative bacterial lipopolysaccharides (LPS), phase variation of LPS/oligosaccharides and the role of LPS in host cell invasion. Capsular polysaccharides present good vaccine candidates, exemplified by the *Haemophilus influenza* type b polysaccharide vaccine.

Dr. K. Venema (TNO) outlined current insights in polysaccharide conversion by bacteria resident in the human gut. His contribution focused on recent technical advances to study fermentation of prebiotics in the colon. Novel molecular tools to identify members of the microbiota that ferment these substrates were highlighted, as well as the use of stable-isotope (¹³C) labeled substrates. These novel technologies allow detailed analysis of such fermentations, including monitoring of changes in microbial populations, and in metabolites produced.

Polysaccharides and Functional Properties dealt with plant cell wall polysaccharides, bacterial polysaccharides, the storage polysaccharide starch, and cellulose. Dr. H.A. Schols (Wageningen University) gave a description of the most dominant polysaccharides as present in different plants (monocotyledonous versus dicotyledonous plants; primary versus secondary cell walls), the cross-links within the

cell wall between different (classes of) polysaccharides, their isolation and characterization and detailed information on the structural elements of pectin and the wide variety of variations possible within these structural elements depending on origin and localization within the plant. Examples were given of how to approach the elucidation of the chemical structure of such highly complex and highly variable plant polysaccharides by making use of chromatography and pure and well-defined carbohydrases in combination with identification of oligosaccharides released using state-of-the-art analytical techniques like e.g. mass spectrometry. The polysaccharide composition and chemical structure and architecture of cell wall polysaccharides were related to the functional properties of (isolated) cell wall polysaccharides and the quality of foods derived from plant produce.

Prof. Dr. O Holst (Research Center Borstel, Germany) gave an overview of the broad variety of polysaccharides found in bacteria, in particular with a focus on structures, functions, and biosynthesis of polysaccharides from Gram-negative and -positive bacteria, and from mycobacteria, e.g. lipopolysaccharides, capsular polysaccharides, lipoteichoic acids, arabinogalactan, and others. Together with this, the general architectures of the Gram-negative, -positive and mycobacterial cell walls were discussed.

Dr. B.E. Christensen (NTNU, Trondheim, Norway) presented an overview of the use of size exclusion chromatography for the characterization of physico-chemical properties of polysaccharides. This was illustrated with examples of various polysaccharides (e.g. dextrans, xanthans, alginates).

In a second lecture Dr. Christensen described the discovery, production and characterization of alginates, their bacterial biosynthesis and processive epimerization. Alginates are structurally diverse and examples of structure elucidation by H-NMR analysis were presented. Alginates find applications in food, as well as in pharmacy and biomedicine.

Dr. P. Buwalda (AVEBE) discussed the structures of starches from different botanical sources and explained how the complex architecture of starch granules correlated with starch functionality. The crystallinity of the amylopectin part of starch is influenced not only by natural variations like phosphate and lipid levels, but can be changed upon chemical modification of the starches. Also crosslinking and gelling of starches to improve their functionality in food and non-food applications were discussed.

Carbohydrate degrading enzymes were reviewed by Prof. Dr. M.O. Coutinho (CNRS, Marseille, France), presenting criteria for the systematic of classification and annotation of carbohydrate degrading enzymes. In an additional lecture he gave a state of the art overview of carbohydrate binding modules and enzyme modularity.

The structural and functional analysis of carbohydrate acting enzymes was discussed by Dr. T. Pijning (University of Groningen), with emphasis on glucanase and fructanase enzymes of *Lactobacilli*. X-ray crystallography, the availability of crystallized enzymes with bound substrates or products, were illustrated to be of key importance for the analysis of substrate and product specificity of these enzymes.

Dr. M. Kabel (Wageningen University) covered the plant cell wall polysaccharides (i.e. cellulose, pectin and hemicelluloses) degrading enzymes. Several aspects of plant polysaccharide degrading enzymes were presented, in particular their mode of action towards the complex cell wall polysaccharides in relation to their application in food and bio-based technologies.

Enzymes active on starch were discussed by Prof. Dr. M.J.E.C. van der Maarel (University of Groningen). Starch accounts for a large part of the dietary energy of man and animals. In plants starch is stored in a crystalline form in compact spherical granules. The chemical and physical properties of starches, and changes therein upon heating in aqueous media, were reviewed. Also the industrial processing of starch to maltodextrins, maltose and glucose syrups using starch degrading enzymes was discussed. Hydrolyzing enzymes that are able to modify starches by transglycosylation reactions were also covered, e.g. cyclodextrin glucanotransferase and amyloamylase.

Chemical and Enzymatic Synthesis of Carbohydrates Prof. Dr. S. Oscarson (UCD Dublin, Ireland) presented an Introduction to Carbohydrate Synthesis and an Advanced lecture on Chemical Synthesis of Oligosaccharides. He gave a general background and focused on three areas of major importance: protecting group manipulations, stereoselective glycosylation reactions and synthetic strategies, including examples of possible and often frequent pitfalls.

Prof. Dr. J.P. Kamerling (University of Groningen) gave an introduction in the Biosynthesis of glycoprotein N- and O-linked carbohydrate chains. This involves a multitude of enzyme reactions and may give rise to a population of glycoproteins, called glycoforms, with identical amino acid sequences but with glycan chains that differ in nature, location and incidence.

Dr. P. Messner (Universitat für Bodenkultur Wien, Austria) reviewed Glycoprotein synthesis in bacteria, an example of post-translational modification. His contribution dealt with new structural, biosynthetic and molecular findings related to prokaryotic protein glycosylation, exemplified with cell surface glycoproteins such as S-layer, flagella, pili.

Prof. Dr. H. Höfte (INRA Versailles, France) presented an overview of our current understanding of the Synthesis of cell wall polysaccharides using genomics of the model species *Arabidopsis thaliana* for the identification of key players in cell wall synthesis. Items discussed involved cell wall composition, glycosyl transferase enzymes, synthesis of (hemi)cellulose and pectins.

Prof. Dr. V. Kren (Academy of Sciences, Prague, Czech Republic) gave an overview of methods that can be employed for the chemo-enzymatic preparation of oligosaccharides. Strategies for the synthesis of a variety of natural oligosaccharides and analogues of natural structures were covered using e.g. glycosyltransferases, glycosidases, as well as engineered mutant enzymes.

Prof. Dr. L. Dijkhuizen (University of Groningen) reported the characterization and engineering of glucansucrase enzymes (GTFA, GTF180) converting sucrose into alpha-glucans with various glucosidic linkages. Rational engineering of these enzymes, involving mutational changes in amino acids in the glucansucrase active site, allowed conversion of GTFA from a mainly alpha-(1->4) into a mainly alpha-(1->6) synthesizing enzyme.

Carbohydrate Analysis Dr. H. Schols (Wageningen University) described the extraction, fractionation and characterization of polysaccharides from plant origin. The lecture focused on the extraction of different plant cell wall polysaccharides -pectins, (hemi)cellulose-, methods used for analysis of sugar linkage composition, and use of enzymatic fingerprinting techniques to determine the distribution of substituents over the polysaccharide backbone.

Prof. Dr. T. Peters provided basic knowledge about carbohydrate NMR methodologies and strategies in glycosciences. This included the fundamental properties of carbohydrate ^1H and ^{13}C NMR spectra, and how to derive conclusions about e.g. anomeric configurations or types of glycosidic linkages from such data. Also concepts that aim at sequence and conformational analyses were introduced e.g. the structural reporter group concept. Important novel NMR technological developments and their impact on the analysis of carbohydrates were explained. Such developments are the dramatic improvement in sensitivity and the availability of specialized pulse sequences that deconvolute crowded carbohydrate NMR spectra. In a second lecture Prof. Peters gave an overview of NMR spectroscopy of carbohydrate-protein interactions. Transfer NOE experiments and saturation transfer difference (STD) NMR experiments were explained in detail and applied to selected examples. It was shown that this methodology is very well suited for the application in biological systems since the targets can be e.g. whole cells or viruses.

Dr. S. Haslam (Imperial College London, UK) explained that mass spectrometry (MS) with its ultra-high sensitivity and ability to analyze complex mixtures of glycans, is the most powerful tool available for glycan structure analysis. His presentation reviewed MS strategies incorporating MALDI-MS/MS and nanoLC-ES-MS/MS for defining glycomes of cells, tissues and purified glycoconjugates as well as establishing glycoprotein site specific glycosylation.

In a second lecture, Dr. S. Haslam discussed the need and potential of accessible, curated and comprehensive data collections and software tools for Glycosciences. Glycobioinformatics is still in an

explorative stage, and the sparseness of glycan databases hampers large scale high throughput glycomics studies. This lecture provided an overview of the current status of the field and highlighted possible future developments.

Dr. N.C. Reichardt (CICbiomaGUNE, San Sebastian, Spain) gave an overview of the applications of microarray technology to glycoscience, with emphasis on the present state glycan and lectin array technology. Important glycan array applications include substrate screening of carbohydrate processing enzymes, affinity screening of newly discovered lectins. After an introduction of the different chemical approaches of preparing suitable glycan microarrays, mentioning advantages and disadvantages of the various protocols used, he focused on their high-through applications in glycobiology and glycomedicine. Several examples were presented of the usefulness of glycan micorarrays as a first step in tracing and understanding biological interactions in which carbohydrates play a major role.

Prof. Dr. S. Perez (CNRS Grenoble, France) reviewed the use of computational methods to the characterization of the three-dimensional conformation of complex carbohydrates, alone or in their interaction with proteins. He explained the basics of molecular modeling using molecular mechanics and molecular dynamics approaches, and gave a series of applications of molecular modeling, thereby demonstrating the importance of such approaches in understanding interaction phenomena in the glycofield. In a second lecture Prof. Perez outlined the neutron and synchrotron infrastructures in Europe, and their major contributions to the advancement of sciences and technology. The lecture focused on the possible use of these unique facilities in research on structural glycosciences.

Valorization of carbohydrate-rich biomass Dr. M. J. O'Donohue (INRA-CNRS-INSA Toulouse, France) lectures dealt with Carbohydrate biorefineries - concepts, technologies and process configurations and Industrial bio-transformation of sugars into value-added fuels and chemicals. In his first lecture the notion of biorefining was explained and some basic facts and figures were supplied to help the audience to get a basic understanding of the issues at stake. Starting with 1st generation biorefining, some basic process concepts were introduced followed by a discussion of 2nd generation biorefining. The hurdles and drawbacks of the different technologies were underlined and a view of some future prospects in this exciting area was presented The second lecture provided a general overview of Industrial Biotechnology with some basic figures which gave the audience a grasp of the current status and future potential of this technology. Further, modern technologies that underpin the creation or improvement of biocatalysts were outlined and several examples of existing biotechnologically-driven processes were discussed. The lecture was concluded by featuring future frontiers, especially concerning the prospect of harnessing microbial consortia. It is expected that Industrial Biotechnology will be a key driving force in a bio-based economy, which will use carbohydrates as the principal feedstock for fuels and chemicals.

Poster Sessions During the meeting 2 sessions of about 2 hours each were held where the participants showed posters of their own ongoing research work. These sessions were highly appreciated by all participants, with lively discussions between participants and between participants and lecturers. Participants were challenged to select the best posters which they did with a broad consensus. The award winning posters were named at the final dinner.

3. Assessment and impact:

All participants were asked to fill in an evaluation form and give an overall rate of the course varying from excellent to poor. The course was rated as excellent by 43% of the respondents, as good by 48% of the respondents, as moderate by 8% of the respondents and insufficient by 1% of the respondents. A total of 94% of the respondents was very satisfied with the amount of information that was provided by us (book of abstracts and handouts in course binder); 6% suggested to replace the course binder by a memory stick. A total of 90% of the respondents indicated that the course met up to their expectations or even more. Various participants (14 %) indicated that they had expected some more

focus on other topics, e.g. synthesis (3%) or biomedical topics (2%), applications (3%), glycobiology (2%), lectins (3%) or stem cell glycosylation (1%), but were otherwise satisfied. A total of 73% was very satisfied about the framework of the course. The other 27% would have appreciated some more breaks and free time. To reduce the information load per day, 17% of the respondents advised us to shorten the lectures to 45 minutes with 15 minutes breaks in between, 7% suggested having one lecture less a day and extending the course with one day and 13% recommended small breaks after each lecture. Although the poster presentations were rated as excellent in relation to interaction and networking, 31% of the responding audience would have appreciated even more interaction during the course. To achieve this, 11 % recommended organizing work or discussion groups, 9% advised us to include an excursion or field trip, 6% suggested to give participants the opportunity to present their work and 5% thought that more parallel sessions would be useful. Finally, 99% of the respondents found the special Glycosciences webpage very useful. 83% was very satisfied with the information provided on the website. The remaining 17 % had some suggestions for improvement, e.g. add a page to interact with participants and researchers (3%), make posters available (3%), add links to research groups of teachers (2%), add a link to Pubmed (2%), make it available earlier (3%).

The course level was overall rated as good. Starting scientist and participants from industry liked the broad range of topics addressed during the course. Occasionally they found the lectures outside their field too advanced whereas more experienced and senior scientists would have preferred even more in depth information next to the basic information provided. A suggestion for the next edition might be to start the course with a one day master class with all the basics for scientists relatively new to the field. The mix of lectures from academia and industry was very much appreciated although 5% of the audience would like to enhance the industrial input. The participants were very impressed that most lecturers were present during the entire course and also available for questions and discussions.

In conclusion, this course clearly meets the need of young glyco-scientists for a broader background in glycosciences and the need for more specific knowledge in specific areas of glycosciences.

The course also contributes to formation of networks and collaborations at several levels:

- Between young European scholars and the renowned senior scientists teaching the courses.
- Between young, starting European glyco-scientists
- Between European science institutions on glyco-sciences
- Between academic and industrial glyco-scientists

From the evaluation it could also be concluded that the existing course needs continuous updating. For future courses, the participants expressed a wish for more attention for bio-medical aspects, applications and glycobiology.

4. Final programme:

Sunday June 3, 2012			
15.30-16.20	Registration		
16.20-16.45		Opening Summer Course Glycosciences	Prof. S. Poppema and Prof. R. Rabbinge
16.45-17.45	Plenary session 1:	Health aspects of poly- and oligosaccharides	Prof. J. Garssen
17.45-20.30	<i>Drinks and Buffet Dinner</i>		

Monday June 4, 2012			
08.45-09.45	Plenary session 2:	Architecture and characterization of plant polysaccharides	Dr. H.A. Schols
09.45-10.45	Plenary session 3:	Biosynthesis of glycoprotein glycans	Prof. J.P. Kamerling
10.45-11.15	<i>Coffee/tea break</i>		
11.15-12.15	Plenary session 4:	Pitfalls in carbohydrate synthesis	Prof. S. Oscarson
12.15-13.15	<i>Lunch</i>		
13.15-14.15	Plenary session 5:	Carbohydrate-protein interactions – molecular modelling insights	Prof. S. Perez
14.15-15.15	Plenary session 6:	Characterizing physico-chemical properties of polysaccharides	Dr. B.E. Christensen
15.15-15.45	<i>Coffee/tea break</i>		
15.45-16.45	Plenary session 7:	Introduction to plant cell wall synthesis	Dr. H. Höfte
16.45-17.45	Plenary session 8:	Bacterial polysaccharides	Prof. O. Holst
18.30-20.00	<i>Dinner</i>		
20.00-21.30	<i>POSTERS and drinks</i>		

Tuesday June 5, 2012			
08.45-09.45	Plenary session 9:	Fractionation and characterization of polysaccharides	Dr. H.A. Schols
09.45 - 10.45	Plenary session 10:	Carbohydrate degrading enzymes: classification and annotation	Prof. P.M. Coutinho
10.45-11.15	<i>Coffee/tea break</i>		
11.15-12.15	Plenary session 11:	Studies on Gaucher disease: Ultrasensitive visualization of beta-glucosidases with activity-based probes	Prof. J.M.F.G. Aerts
12.15-13.15	<i>Lunch</i>		
13.15-14.15	Plenary session 12:	NMR methodologies and strategies in glycoscience	Prof. T. Peters
14.15-15.15	Plenary session 13:	The stories behind the prebiotic GOS	Dr. E. van Leusen
15.15-15.45	<i>Coffee/tea break</i>		
15.45-16.45	Parallel session I A:	Advanced carbohydrate synthesis	Prof. S. Oscarson
	Parallel session I B:	Neutron and synchrotron radiations for glycosciences	Prof. S. Perez
16.45-17.45	Parallel session II A:	Glycoproteins in bacteria	Dr. P. Messner
	Parallel session II B:	Applications of alginates	Dr. B.E. Christensen
18.30-20.00	<i>Dinner</i>		
20.00-21.30	<i>POSTERS and drinks</i>		

Wednesday June 6, 2012			
08.45-09.45	Plenary session 14:	Mass spectrometry approaches in glycoscience	Dr. S. Haslam
09.45 - 10.45	Plenary session 15:	Applications of microarray technology to glycosciences	Dr. N. Reichardt
10.45-11.15	<i>Coffee/tea break</i>		
11.15-12.15	Plenary session 16:	Structural and functional analysis of carbohydrate acting enzymes	T. Pijning (MSc)
12.15-13.15	<i>Lunch</i>		
13.15-14.15	Parallel session III A:	NMR spectroscopy of carbohydrate-protein interactions	Prof. T. Peters
	Parallel session III B:	Carbohydrate Binding Modules and enzyme modularity	Prof. P.M. Coutinho
14.15-15.15	Parallel session IV A:	Enzymatic synthesis of oligosaccharides	Prof. V. Kren
	Parallel session IV B:	Starch biochemical properties	Dr. P. Buwalda
15.15-15.45	<i>Coffee/tea break</i>		
15.45-16.45	Parallel session V A:	Biomedical aspects of polysaccharides	Prof. O. Holst
	Parallel session V B:	Properties and applications of starch converting enzymes	Prof. M.J.E.C. van der Maarel
16.45-17.45	Plenary session 17:	Carbohydrate bio refineries - concepts, technologies and process configurations	Dr. M. J. O'Donohue
18.30-23.00	<i>Course Dinner with Poster Prize awarding</i>		

Thursday June 7, 2012			
08.45-09.45	Plenary session 18:	Glycoinformatics - databases and software tools for glycosciences	Dr. S. Haslam
09.45-10.45	Parallel session VI A:	Plant cell wall polysaccharide degrading enzymes in food and non-food applications	Dr. Mirjam Kabel
	Parallel session VI B:	Polysaccharide conversion in the gut	Dr. K. Venema
10.45-11.15	<i>Coffee/tea break</i>		
11.15-12.15	Parallel session VII A:	Carbohydrate bioengineering: Tailoring glucosidic bond specificity in glucansucrase enzymes	Prof. L. Dijkhuizen
	Parallel session VII B:	Industrial biotransformation of sugars into value-added fuels and chemicals.	Dr. M. J. O'Donohue
12.15-13.15	Plenary session 19:	Trends in future	Dr. K. de Gooijer
13.15-13.30	Closing session		
13.30-14.30	<i>Farewell Lunch</i>		