

# The Scientific Report from the Charles Warren III Workshop

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## **SUMMARY**

The third Charles Warren Workshop in glycol analysis was held in Hindås/Gothenburg, Sweden on the 27<sup>th</sup>-30<sup>th</sup> of August 2010.

([http://www.biomedicine.gu.se/biomedicine/Charles\\_Warren\\_Workshop\\_III](http://www.biomedicine.gu.se/biomedicine/Charles_Warren_Workshop_III)) with a total of 83 national and international speakers and delegates present. A total of 39 scientific oral and 26 poster presentations were presented at the conference. The ambition of the conference was to update the field of recent progress in the area of glycoanalysis. In addition it also to provide an educational opportunity, where selected younger researchers in the field presented their work as part of the main sessions, as well as in a dedicated poster session. Senior speakers were encouraged to not only provide updates about their research, but also to cover the background of their field. This was also part of the strategy to allow new researchers in the field to get a broad understanding of the current issues and techniques in glycoanalysis. With the financial support from ESF, Swedish Research Council and Bruker Daltonics, the educational organizational board with Dr Karlsson (University of Gothenburg), Prof Rudolf Geyer (University Giessen, Germany) and Dr Stuart Haslam (Imperial College London, UK) distributed the financial support for younger researchers presenting at the workshop, covering accommodation and conference expenses as well as a small contribution to travel, based on their location. The scientific board identified five topics for the sessions in the workshop that was split into 4 sessions spread over the days of the conference; glycomic techniques, analysis of glycopeptides, analysis of proteoglycans, glycoarrays and glycobioinformatics. In order to emphasize the biological importance of glycosylation, the workshop was initiated by plenary talks from two invited glycobioinformaticians (Prof Thomas Boren and Prof Hakon Leffler). Also associated with the conference were two satellite meetings organized by Prof William York (University of Georgia, USA, Consortium of Functional Glycomics Bridging Grant – Informatics Development Workshop 2010, 23-27<sup>th</sup> of August, Gothenburg, 12 participants, and Unicarb-DB development meeting organized by Dr Niclas Karlsson, 8 participants).

## SCIENTIFIC CONTENT AND DISCUSSION

Analysis of glycoconjugates was the focus for the third Warren workshop held in a beautiful spot in a conference center located in Hindås in the Swedish forest just outside Gothenburg. The first two Warren workshops have previously been held at the University of New Hampshire and was initiated by Prof Vern Reinhold in commemoration of the glycobiochemist Dr. Charles E. Warren, who tragically died in a hang gliding accident in 2005.

### *Keynote program-session*

The workshop was commenced with two plenary talks from Prof Thomas Boren, Umeå University and Prof Hakon Leffler, Lund University. Both these talks provided excellent examples of how structural characterization of glycoconjugates will have to be incorporated into glycobiochemical research to solve complex biological questions. Prof Boren presented how the gastric pathogen *Helicobacter pylori*, responsible for peptic ulcer and gastric cancer, has developed a molecular mimicry coat of lipopolysaccharides expressing glyco-epitopes also present on the human gastric mucosa. Thus allowing the pathogen to escape host immune recognition. In addition, the pathogen expresses adhesins which bind to gastric sialylated and fucosylated glycoconjugates. This allows the bacteria to remain in the stomach environment causing chronic infection and inflammation. Dr Leffler described the involvement of galectins in cancer and inflammation and how the fine tuning of the galectin carbohydrate binding domain directs intracellular trafficking. The affinity of galectins allow them to be able to interact with specific glycoproteins in serum amongst other glycoproteins with similar glycosylation.

### *Glycomics techniques-session*

The glycomics session was initiated by Prof Veron Reinhold, University of New Hampshire emphasizing that we need to adopt methods for glycomics that provide the glycobiochemist with the detailed information required for solving their biological questions. This introduction was followed by Prof Kay-Hooi Khoo, Academia Sinica, Taiwan presenting latest technologies for mass spectrometry based analysis of sulfated oligosaccharides, including both sample preparation using permethylation and sample enrichment. Prof Elke Schweda, Karolinska Institute, Sweden, presented the analytical technologies using LC-ESI-MS<sup>n</sup> to explore *Haemophilus influenzae* lipopolysaccharides from clinical isolates. Dr Joseph Zaia, Boston University, US presented the technique using LC-MS and how proteomic software tools can

be used to interpret the data from glycosaminoglycan digest. Also showing the latest development and issues with fragmentation analysis of polysulfated oligosaccharides and how the sulfate groups can be stabilized by increasing charge states and derivatisations. Dr Stephen Levery presented an excellent overview of analysis of glycolipids and how it can be utilized together with novel glycosphingolipid array technology to explore binding specificity of glycosphingolipids. Prof Pauline Rudd (National Institute for Bioresearch and Training, Ireland) presented the use of HPLC based glycomics to investigate the biology of glycosylation and how it can be used in genome wide screening. Three short talks from selected younger researchers Dr Josephine Grass (University of Natural Resources and Applied Life Sciences, Austria), Dr Kazuhiro Aoki (Complex Carbohydrate Research Center, University of Georgia, US) and Dr Chunsheng Jin (University of Gothenburg, Sweden) of various aspects of glycomics of oligosaccharides and glycolipids (see program and abstract presentations on [http://www.biomedicine.gu.se/biomedicine/Charles\\_Warren\\_Workshop\\_III](http://www.biomedicine.gu.se/biomedicine/Charles_Warren_Workshop_III)) were also presented.

### ***Glycoproteomics/Proteoglycans- session***

The discussion facilitator Dr Nicolle Packer, MacQuarie University, Australia introduced the session by discussing how glycoproteomics is a combination of proteomic and glycomic analysis. Dr Manfred Wuhrer (Leiden University, The Netherlands) described the generic fragmentation pattern of glycopeptides and how it can be used to explore glycosylation related congenital diseases and *Schistosoma mansoni*-infection. Dr Jonas Nilsson (University of Gothenburg, Sweden) described a method for isolation of glycopeptides and how it can be used to identify novel N- and O-linked glycosylation sites in cerebrospinal fluid. Dr Martin Larsen (University of Southern Denmark, Odense), described the technique for simultaneous isolation of phosphopeptides and sialoglycopeptides followed by LC-MS<sup>n</sup>, Dr Daniel Kolarich (MacQuarie University, Sydney) described the various software and hardware analytical techniques to merge glycomic and proteomic data for glycoproteomic analysis. Prof Jeremy Turnbull (University of Liverpool, UK) described a set of techniques used to explore the biology of proteoglycans including saccharide libraries, microarrays, ESI-MS sequencing, purification, fluourescent labeling and bioinformatics. The session was ended with four short talks from selected younger researchers Dr. Giuseppe Palmisano (The University of Southern Denmark, Denmark),

Dr. Yao-Yun Fan (ETH Zürich, Switzerland), Dr. Hykollari Alba. (University of Natural Resources and Life Sciences, Vienna) and Dr. Tina Ravnsborg (University of Southern Denmark, Odense), of using quantitative and qualitative glycoproteomic analysis.

### ***Glycoarray session***

While the emphasis of the Warren workshop in the past has been of mass spectrometric based analysis, various glycoarrays are now utilized in glycomic analysis. Prof. Lara K. Mahal (New York University, USA) described the use and development of lectin arrays in glycoanalysis. Dr. Ola Blixt (Copenhagen University, Denmark), described various methods for immobilizing glycoconjugates (oligosaccharides and glycopeptides) to microarrays to explore their binding, and Prof Rob Woods (NUI Galway, Ireland and Complex carbohydrate Research Centre, Athens, Georgia, US) described how computer models of molecular docking can be used to identify binding motifs of oligosaccharides and to verify experimental binding data. Dr Bernd Lepenies (Max Planck Institute of Colloids and Interfaces, Berlin), described automated oligosaccharide synthesis, glycan arrays and nanoparticles as tools to understand infectious diseases and Prof Fredrik Höök (Chalmers school of Technology, Gothenburg, Sweden) described surface based methods for studies of protein and virus interactions with glycolipid containing cell-membrane mimics.

### **Glycodatabases, Glycobioinformatics and Glycosoftware session**

Dr Gavin Davey (Trinity University, Dublin, Ireland) initiated this session with a description of a computer model for N-linked glycosylation of CHO cells and how it can be used for glyco knock out models and glycoregulation and Dr Ulrike Schweiger-Hufnagel showed how Bruker's MS platform has adopted academic glycoanalysis software for glycopeptides analysis. Dr Stuart Haslam (Imperial College, London) described glycodatabases and glycobioinformatics for structural glycomics and how software developed in the consortium for functional glycomics and at Imperial College (EurocarbDB) can be used in mass spectrometric based glycomics. Prof Kiyoko F. Aoki-Kinoshita (Soka University, Japan) described the project to integrate Japanese glycoresources with the consortium for functional glycomics and the identification of pulmonary disease markers using glycodatabases. Dr Frederique Lisacek (Swiss Institute of Bioinformatics, Geneva, Switzerland) described how glycobioinformatic resources will be integrated with large European biological databases and

bioinformatic resources. The session finished with three short presentations from selected younger researchers Dr Jarkko Rabinä (Finnish Red Cross Blood Service, Research and Development, Helsinki, Finland), Dr Mindy Porterfield (Complex Carbohydrate Research Center, University of Georgia, Athens, GA) and Dr Catherine Hayes (University of Gothenburg, Department of Medical Biochemistry, Sweden) about glycobioinformatics applications to glycomics.

### ***Closing Panel session***

Dr Rene Ranzinger (CCRC, Athens Georgia) described the outcome of the Gothenburg Glycobioinformatic Hackathone 24<sup>th</sup> to 27<sup>th</sup> of August and how there had been progress in developing a glycobioinformatic platform resource to exchange information between global glycobioinformatics resources. Prof Nicolle Packer (MacQuarie University, Australia) described the vision of an integrated glycobioinformatics platform. Prof. Rudolf Geyer described the ESF Network Program EUROGLYCOFORUM, funding of glycoresearch and funding for conferences and training. The session was finished by a discussion about incorporation of various glycomic data into databases, and if current formats are supporting researchers in the field. It was felt that a lot of current emphasis is related to glycosylation of glycoproteins and that resources in proteoglycan research and glycolipids are insufficient.

### **ASSESSMENT OF THE RESULTS AND IMPACT OF THE EVENT ON THE FUTURE DIRECTION OF THE FIELD**

This Warren workshop and previous ones have been forums, where researchers present both the pros and cons about their research. Hence, the open discussion made it clear that the division between glycomics and glycoproteomics has to be bridged now, since it is obvious that each technique is providing complementary data, where neither of the techniques are providing the full picture of the biological map of glycoproteins. For analysis of glycoprotein glycosylation this also provides an excellent opportunity to build on current protein databases and expanding this research by incorporating software's for proteomics and adopting database concepts from the protein research. The glycobioinformatics development is now one issue that unites the research and public bioinformatic resources are one prerequisite for the field to move on, from where it has spent a lot of time for the analytical techniques to be developed. The bottle neck is still the data interpretation and the vacuum that will be created after current

international initiatives (eg EurCARBDB, CFG) come to an end will have to be filled. The awareness of the researchers is that this will have to be built on smaller funding from the national level, with current efforts collaborating with individual research groups or smaller constellation to bring the glycoinformatics development further, based on platforms and directions reiterated in the current Warren workshop. The action from the workshop is to continue providing educational modules (software's and hardware) for glycoanalysis for newcomers in the field both nationally and internationally, for example there will be both MS and bioinformatic workshops at the international Glyco21 conference in Vienna 2011. With the Swiss institute for Bioinformatic also committed to provide glycoresources, there are cross continental discussion about coordination of global glycoresources between Japan, Australia, US and European, where it is obvious from a historic point of view and the current strength of European glycoresearch, that Europe has an opportunity to take a lead on this, with Swiss institute for bioinformatics (GlycosuiteDB) and EBI (EuroCarbDB) currently hosting global glycoresources and are committed to expand their involvement.

The workshop was also well attended with good mixture of younger researchers (45 PhD students and post docs). The talks with an emphasis on overview worked really well with high educational value for the next generation of glycoresearchers. Several opportunities during coffee breaks, as well as during meals (breakfast, lunch and dinner) in the conference facility's communal halls allowed interaction between younger and established researchers and scientific discussions and collaboration to be established. The poster session was well attended and in a relaxed atmosphere were the younger researchers had the opportunity to present their work or expand on topics in the posters from their oral presentation.