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REVIEW

30th ASMS Asilomar Conference on Advances in Glycomics and Glycoproteomics: Methods and Applications

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The 30th ASMS Asilomar Conference (October 10–14, 2014) was focused on the topic "Advances in Glycomics and Glycoproteomics: Methods and Applications," and was co-organized by Yehia Mechref (Texas Tech University) and Carlito Lebrilla (University of California, Davis). The Conference highlighted new advances in mass spectrometry (MS) methods and instrumentation facilitating unequivocal characterization of glycans and glycoproteins.

Glycosylation is one of the most common but structurally diverse modifications of proteins and lipids. Glycosylation is a non-template driven process, involving the attachment of glycan moieties to proteins and lipids produced by the combination of consecutive cleavages and additions of monosaccharides. The processes are dependent on the concerted expression and activities of several transferases and exoglycosidases to yield the glycome, which refers to the oligosaccharides associated with cells, tissues, and organisms. Therefore, the structural complexity of the glycome makes it a significant analytical challenge. Nonetheless, the glycosylation of proteins and lipids is responsible for many critical biological functions and has also been implicated in many mammalian diseases. Additionally, the comprehensive characterization of protein glycosylation at high sensitivity is an important step in the development of protein therapeutic targets. The majority of current and emerging recombinant protein drugs, particularly monoclonal antibodies, are glycoproteins, the biological activities of which are directly influenced by glycosylation. Glycosylation also plays an important role in the rapidly emerging areas of biosimilars (biogenerics). Thus, sensitive and reliable methods capable of addressing glycan structural complexity with high sensitivity are clearly needed. MS and separation science continue to play an integral part in these methods.

The conference consisted of two keynote presentations, 25 invited talks, and 38 posters. The opportunity to provide 5-min "lightning talks" was also offered to the poster presenters. The conference was attended by 125 attendees from six countries. Yehia Mechref gave the introductory remarks and indicated that this conference is the second Asilomar Conference dedicated to glycomics and glycoproteomics. The first was the 18_{th} ASMS Asilomar Conference, co-organized by Carlito Lebrilla and Julie Leary (both currently at the University of California, Davis). Yehia also briefly presented the history of the Asilomar Conference and reintroduced a consistent attendee, namely the monkey with cymbal that is used to indicate to the speakers that their time is up. The return of the monkey was made possible this year by the efforts of Sharon Pitteri.

The first keynote lecture was given by Gerald Hart of the Institute for Basic Biomedical Sciences at John Hopkins School of Medicine, Baltimore, MD. The talk highlighted the importance of glycosylation in many biological processes. Dr. Hart also discussed the biological importance of protein modification with *O*-acetylglucosamine (O-GlcNAc) and the biological roles of cross-talk between different post-translational modifications of proteins. The second keynote lecture was given by Richard Cummings of the Department of Biochemistry at Emory University School of Medicine, Atlanta, GA. Dr. Cummings highlighted the importance of tandem mass spectrometry (MS/MS) in characterizing structures that are employed in glycan microarrays. He also discussed the use of bioinformatic tools in conjunction with lectin microarray that facilitate effective characterization of glycan structures associated with glycoproteins and their biological importance.

The first oral session of the conference was entitled "New Methods for Structure Analysis of Glycans." Catherine Costello of the Department of Biochemistry at Boston University School of Medicine, described several MS-based techniques and methods that enabled efficient characterization of glycoproteins. Dr. Costello indicated that MS is offering structural insight that was not possible otherwise. She also highlighted the importance of hydrophilic interaction liquid chromatography (HILIC) in enriching glycopeptides, and introduced new results on the use ion-mobility spectrometry (IMS)-MS to characterize glycan isomers in a rapid manner. Vernon Reinhold of the Department of Molecular, Cellular, and Biomedical Sciences at the University of New Hampshire, highlighted the importance of multi-stage MS/MS (i.e., MS^n) to characterize multiple glycan structures present within a single *m/z* range. This approach is information rich and enables the characterization of isomeric mixtures. He showed that the behavior of ions when subjected to fragmentation is structurally distinct and that much information can be derived from their

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respective dissociation reactions. The last talk of this oral session was given by Michiko Tajiri of Osaka Medical Center and Research Institute for Maternal and Child Health (Osaka, Japan). She discussed the use of IMS for the separation of glycan structures and monitoring changes in peptide cross sections attributable to the addition of a single GlcNAc residue. IMS data were also utilized to model the interactions between glycoproteins and synthesized glycans. At the end of the first oral session, the new NIH Common Fund in Glycoscience was described by Karl Krueger, a program officer at the National Cancer Institute (NCI). He discussed the purpose of the request for application (RFA) in areas related to MS. He stressed the need for developing MS-centered methods that are accessible and transferrable. Karl Kruger also answered and addressed questions and concerns raised by the attendees.

The second oral session was entitled "LC and LC-MS of Oligosaccharides." The first talk in this session was given by Pauline Rudd of the National Institute for Bioprocessing Research and Training (Dublin, Ireland). She described the profiling of glycans using HILIC in conjunction with exoglycosidases. She also described an automated approach and miniaturization, which are enabling high-throughput analysis. This method was also coupled with MS and MS/MS. The high throughput method was used to characterize glycans in different human disorders. She presented data related to congenital disease and breast cancer, and indicated that environmental conditions directly influence glycosylation process. She further presented data suggesting that glycosylation is changing epigenetically. The second talk in this session was given by David Muddiman of the Department of Chemistry at North Carolina State University, Raleigh, NC. The talk discussed the understanding of ovarian cancer through glycomics and genomics using chicken as a model organism. An interesting component of this talk was the "Big Data" aspect and interpretation through pathway modeling. The results presented involved the analysis of over 500 samples collected from disease-free and diseased animals. This study was further enhanced by the development of new reagents and software for analysis. Ron Orlando of the Complex Carbohydrate Research Center at the University of Georgia (Athens, GA) described the selective reaction monitoring (SRM) LC-MS/MS approach, enabling sensitive quantitation of glycans. The approach is based on using oxonium ions as a transition for SRM quantitation. Isomeric separation of sialylated glycans was attained using amine-based HILIC columns. These columns were described in greater detail in the next talk, given by Barry Boyes of Advanced Materials Technology (Wilmington, DE). The talk discussed the ability to attain partial glycoform separations of glycoproteins such as ribonuclease B, but with limited chromatographic resolution. Carlito Lebrilla of the Department of Chemistry at the University of California, Davis, described a method involving the use of porous graphitic column separation in conjunction with MS to rapidly profile *N*-glycan structures. MS, MS/MS, and exoglycosidases were used to create a database that was effectively employed to monitor glycomic changes associated with many human diseases. Dr. Lebrilla also discussed the use of a similar method for characterizing the oligosaccharides of human milk. This method enabled the analysis of monoclonal antibody drugs in biologics and biosimilars. The last talk of this session was given by Yehia Mechref of the Department of Chemistry and Biochemistry at Texas Tech University (Lubbock, TX). Dr. Mechref presented results from LC-MS and LC-multiple reaction monitoring (MRM)-MS/MS of permethylated glycans derived from biological samples to characterize and quantify different glycan types according to their characteristic fragments employed as transitions.

The third session was entitled "Glycans/Glycopeptides as Disease Biomarkers" and included three talks. The first talk was given by Sharon Pitteri of Stanford School of Medicine, (Palo Alto, CA) where glycoproteomic approaches were used for the detection of cancer. The method is based on the separation of intact proteins after depletion of the most abundant proteins. Quantitation was based on heavy and light labeling of cystine residues. Lectin enrichment was also included in the method with emphasis on using lectin materials specific for enriching fucosylated glycoproteins. The method was employed for characterizing the glycoproteins derived from blood serum, cancer cells, cancer tissues, and adipose tissue. Manfred Wuhrer of the Department of Chemistry and Pharmaceutical Sciences at VU University Amsterdam (Amsterdam, The Netherland) then described glycomic profiling of IgG employing automated sample purification, thus enabling a fast and reliable quantitative method for the analysis of glycans. The importance of glycosylation in pregnancy and inflammation was described. Dr. Wuhrer also described new reagents that enable the distinction between sialic acid isomers originating from linkage differences. The last talk in this session was

given by Habtom Ressom of the School of Medicine at Georgetown University (Washington, DC) in which glycomic and glycoproteomic analyses enabled the differentiation between hepatocellular carcinoma and cirrhosis.

The next oral session took place Sunday morning and was entitled "Glycans in Biosimilars/pharmaceuticals." Stephen Jacobson of the Department of Chemistry at Indiana University (Bloomington, IN), described a microfluidic capillary electrophoresis approach that was used to assess glycan changes prompted by the development and progression of different cancers, including breast, ovarian, and esophagus. Morten Thaysen-Andersen of Macquarie University (Sydney, Australia) described the detection of truncated glycans (paucimannose) in pathogen infections. Truncated glycans are also present in humans and increase with infection. Hyun Joo An of Chungnam National University (Seoul, Korea), discussed the importance of eliminating sample matrix for effective and efficient characterization of glycans. The importance of glycosylation in drug development was also discussed. Dr. An also discussed the variation in glycosylation observed in different biosimilars. Maria Lorna De Leoz of the National Institute of Standards and Technology (Washington, DC) described the different glycan and glycoproteins that are available at NIST. She also described the current and future capabilities of NIST towards a comprehensive MS/MS library of structures and in providing standards for the glyco-community.

Sunday afternoon was free and attendees participated in different leisure activities, including biking, hiking, kayaking, and wine tasting. Other attendees elected to take it easy and relax by the beach.

Two talks were given Sunday evening in the oral session entitled "New Methods for the analysis of Glycoproteins/glycopeptides." Heather Desaire of the Department of Chemistry at the University of Kansas (Lawrence, KS) described the use of MS and MS/MS to characterize the microheterogeneity of glycoproteins. She also described new computer algorithms that were developed to interpret and score both CID and electron transfer (ETD) data associated with glycopeptides. These methods were employed by Dr. Desaire to characterize the glycosylation sites of gp 120 on the HIV envelope protein. Katalin F. Medzihradszky of the University of California (San Francisco, CA) then described and discussed the use of Byonic software to characterize the glycosylation sites of proteome samples, along with the advantages and disadvantages associated with the use of such automated software.

The Monday morning oral session was dedicated to glycosaminoglycans and related compounds. Julie Leary of the Department of Molecular and Cellular Biology at the University of California, Davis, gave the first talk. Dr. Leary provided an excellent introduction of glycosaminoglycans and paved the road for other speakers of this session. She described a sample preparation method that entailed several chromatographic steps, as well as the use of IMS for the separation of different isomers. The described methods were employed to assess rheumatoid arthritis where 6-O-sulfation increased. Jon Amster of the Department of Chemistry at the University of Georgia (Athens, GA) discussed MS/MS of glycosaminoglycans using different fragmentation methods, including EDD and NETD. He also described software that enables reliable assignment of tandem MS data. The last talk of this oral session was given by Joe Zaia of the Department of Biochemistry at Boston University School of Medicine (Boston, MA). He described a method involving the enzymatic digestion of glycosaminoglycans on the surface of tissue slices. The use of different enzymes and different deposition of such enzymes was described and discussed. Dr. Zaia also described laser microdissection capture methods for analyzing proteins on the surface of tissues.

The last oral session was held on Monday afternoon, and was entitled "Bioinformatics and Analysis of Glycoconjugates." Haixu Tang of the School of Informatics and Computing, at Indiana University (Bloomington, IN) described several algorithms that were developed to facilitate automated interpretation and annotation of glycomic and glycoproteomic data. He indicated that there is still a need for more rigid scoring algorithms that account for the different sources of errors. He then described an orthogonal scoring function that accounts for HCD, CID, and ETD glycopeptide data. Combining the scoring function prompts a reduced false discovery rate and permits more reliable annotation. Marshall Bern of Protein Metrics Inc. (Palo Alto, CA) described the different features of his company's Byonic software and discussed the different capabilities and scoring functions permitted by this software. Julian Saba of Thermo Fisher Scientific (San Jose, CA) described the analysis of prostate specific antigen employing both bottom-up and top-down approaches. The use of Lys C generated large peptides that were then

subjected to LC-MS/MS. HCD, PD, and ETD were utilized to characterize the large peptides. Carol Nilsson of the Department of Pharmacology and Toxicology at the University of Texas Medical Branch (Galveston, TX), gave the last talk, and described the glycomic, glycoproteomic, and genomic analyses of glioma cancer stem cells.

The banquet dinner was held on Monday evening where Pauline Rudd of the National Institute for Bioprocessing Research and Training gave an engaging talk entitled "The challenges of integrated biology and other things." The full transcript of this talk is available at http://www.asms.org/publications/journal-of-the-american-society-for-mass-spectrometry-group/news-and-views. Placed within a historical context, the talk took us through the challenges and triumphs associated with glycan analysis and identification. Dr. Rudd concluded her talk by saying, "However hard it is, let's keep searching for the end of the rainbow!" Although the importance of glycans in biological systems has been long known, advancement in this challenging area of research has been limited for years by the lack of reliable and sensitive methods. This situation is rapidly changing because of new advancements in MS instrumentation and methods, which will soon facilitate the much-needed comprehensive characterization of glycans and glycoproteins with high sensitivities.