CFG Annual Participating Investigators Meeting

The Consortium for Functional Glycomics held its seventh annual meeting of the Participating Investigators (PIs) on November 11, 2007, in Boston, MA. The PI meeting, titled “Glycans in Cell Communication,” was a satellite meeting of the annual conference of The Society for Glycobiology. Traditionally open to the public, the meeting gathered over 250 investigators, postdoctoral associates, and students representing laboratories from North and South America, Mexico, United Kingdom, Europe, Israel, Russia, India, Japan, Taiwan, Korea, and Australia. The meeting agenda at http://glycomics.scripps.edu/PI2007.html provides links to the abstracts of PIs presentations. The presentations encompassed a broad scope of topics involving the roles of glycans in cell communication—from host-pathogen interactions and functions of mammalian glycan binding proteins to glycomics and glycoinformatics.

Opening the meeting, the Consortium Principal Investigator Dr. James Paulson welcomed the participants, briefly summarized the CFG accomplishments of the last year, and discussed the plans for the second phase of the project. He also encouraged the PIs to provide feedback and suggestions to the CFG cores on any issues and new developments.

The CFG/Nature Functional Glycomics Gateway Editor Dr. Mirko von Elstermann spoke about the Gateway project highlighting the Functional Glycomics Update contributed each month by Nature and alerting the participants to the new networking resources and tools available through the Gateway. The Web site offers various ways to reach out to the other molecular biology disciplines and provides an easy access to the editorial synopses of the hot research topics.
Understanding the diversity of C-type lectins and their carbohydrate-specific recognition profile are important to understanding of the DC pathogen recognition in many pathogenic disorders.

Sandra van Vliet from Yvette van Kooyk’s laboratory at the VU University Medical Center presented an impressive study on C-type lectins in innate and adaptive immune responses. Dendritic cells (DCs), specialized in the recognition of pathogens, play an important role in the control of immunity. C-type lectins DC-SIGN and MGL facilitate antigen presentation to T cells. Modification of glycosylate d antigen can strongly affect the antigen uptake and presentation capacity of DCs. The researchers discovered that DC-SIGN has a unique signaling capacity by which it can modify TLR signaling processes. Both MGL and DC-SIGN can also mediate cellular interactions. MLG binds to a specific population of effector T cells through CD45 and affects TCR signaling by inducing apoptosis. DC-SIGN has been also shown to mediate neutrophil binding which affects DC maturation. The regulated glycan expression levels determine which cells interact with DCs. Understanding the diversity of C-type lectins expressed on DCs and their carbohydrate-specific recognition profile are key to understanding of the DC pathogen recognition in many pathogenic disorders and the regulation of cellular interactions of DCs that are essential in the control of immunity.

Christine Wells of the Griffith University, Australia, in her talk demonstrated that Mincle was a novel macrophage receptor for the yeast Candida albicans, a common pathogen causing chronic and systemic infection. The experiments to confirm Mincle’s role in the induction of inflammatory signaling in response to Candida albicans infection were conducted with the Mincle knockout mice from the Consortium. The researchers observed that Mincle is expressed in splenic macrophages and localizes around phagosomes. In the experiments, Mincle bound soluble yeast extracts mediating the inflammatory signaling but were not a phagocytic receptor. The researchers showed that Mincle had a role in the innate immune production of cytokines and was differentially expressed in immune tissues in a genetic model of Candida albicans resistance. And finally, it was proven that mice lacking Mincle were more susceptible to Candida albicans yeast, especially to systemic candidiasis.

A study on immune cell C-type lectins that recognize viruses, including DC-SIGN and endothelial cell receptors DCSIGNR and LSECtin, was presented by Kurt Drickamer of Imperial College, London. The viruses, such as HIV and Ebola, interact selectively with glycan-binding receptors on cells of the immune system. These C-type lectins bind viral glycoproteins such as Ebola glycoprotein, providing the viruses with access to cells. The experiments showed that DC-SIGN, DCSIGNR, and LSECtin interact with different types of glycans present on the viruses. The lectins screened on the glycan array bound to distinct though sometimes overlapping sets of glycans and viruses. High-mannose oligosaccharides on HIV and Hepatitis C viruses were targeted by DC-SIGN and DC-SIGNR but not by LSECtin. Ebola virus could interact with all three receptors. The two hypotheses dealing with the role of the C-type lectins interactions are based either on the receptors’ benevolent or malevolent binding of the glycoproteins on the viruses. According to one theory, the receptors are evolving to recognize and fight viruses. The other theory presents the opposite picture, in which the receptors are hijacked by the viruses to gain access to the cells. Along with many other investigators, Dr. Drickamer noted the importance of careful selection of the
Richard D. Cummings of Emory University was another investigator who drew the attention of the audience to the importance of concentration-dependent binding in the studies of the protein interactions with glycans on the printed microarray. At the meeting, Dr. Cummings presented his group’s research on the glycan binding properties of galectins, the most widely expressed class of lectins in all animals. Although galectins can weakly recognize lactose, they demonstrate high affinity in their recognition of complex glycans, and each galectin shows differences in glycan recognition. Recombinant human galectins, including galectins 1, 2, 3, 4, 7, and 8, were examined on the glycan array and showed high affinity interactions with specific structurally different glycans. The studies revealed that this binding on the glycan microarray could be related to the many signaling and binding activities of galectins toward human neutrophils and lymphocytes, including the potential contribution of galectins to regulated leukocyte turnover and death. Interestingly, all galectins showed higher binding to some sulfated glycans on the microarray, an area that needs to be further pursued in relation to the natural glycans of human leukocytes.

The galectin family was further unraveled in the talk on the role of Galectin-1 in the regulation of immune cell homeostasis by Gabriel Rabinovich of the Institute of Biology and Experimental Medicine, Argentina. Galectins bind to a wide array of glycoconjugates and, through binding to specific glycoconjugates, can deliver intracellular signals and mediate cell-cell and cell-matrix interactions. In his experiments with in vivo models, Dr. Rabinovich demonstrated that Gal-1 contributes to tumor cell evasion of immune responses in models of melanoma and Hodgkin’s lymphoma. Differential glycosylation of T helper cells can selectively regulate susceptibility to Gal-1. Gal-1-deficient mice showed increased susceptibility to Th1 and Th17-mediated autoimmune neuro-inflammation. The studies also discovered that Gal-1 plays a central role in the fetal-maternal tolerance in vivo by promoting the generation of tolerogenic DCs which in turn favor the expansion of regulatory T cells. Finishing the talk, Dr. Rabinovich noted that further experiments were needed to explain why different immune cell experiences different physiological outcomes.

The role of Siglec-G in B cell signaling was tackled upon by the group of Lars Nitschke of the University of Erlangen, Germany. The researcher demonstrated that Siglec-G had an inhibitory effect on BCR-induced Ca2+ signalling of B1 cells. It shows a B-cell restricted expression with high levels in B1 cells. Siglec-G mice had increased natural antibodies (IgM), but normal thymus immune responses. Siglec-G deficient mice demonstrated a highly expanded B1a cell population but did not develop high affinity IgG autoantibodies. Dr. Nitschke argued that the novel negative regulatory pathway in B1 cells might explain the naturally muted signaling response of B1 cells.

In the section devoted to pathogen host interactions, Dr. Gillian M. Air of the University of Oklahoma Health Sciences Center presented her research on the human parainfluenza virus (hPIV) receptors. hPIV binds receptors containing
Streptococcus mitis platelet aggregation factor (Sm-hPAF) is a novel member of the cholesterol-dependent cytolysin (CDC) family of toxins. Rodney K. Tweten of the University of Oklahoma Health Sciences Center gave a talk about Streptococcus mitis platelet aggregation factor (Sm-hPAF) as a novel member of the cholesterol-dependent cytolysin (CDC) family of toxins. Dr. Tweten and colleagues performed an analysis of the SM-PAF to determine if the CDC and fucolectin domains were functional. It was shown that individually the CDC and fucolectin domains were functional, but that in the intact Sm-PAF the glycan binding site of the fucolectin was apparently sterically occluded. The investigator proposed that the glycan-binding site was exposed only in the oligomeric membrane pore complex of Sm-hPAF. Preliminary studies showed that the Sm-hPAF gene was present in some isolates of S. mitis, S. pseudopneumoniae and S. pneumoniae.

Ronald L. Schnaar of the Johns Hopkins School of Medicine was presenting his study on myelin-associated glycoprotein (MAG or Siglec-4) in axon regeneration within the emerging topics section of the PI meeting. MAG is an “axon regeneration inhibitor” which binds to sialoglycans (gangliosides GD1a & GT1b). In his experiments with two spinal cord injury models, Dr. Schnaar demonstrated that reversing MAG binding to gangliosides with sialidase treatment enhances axon regeneration in vitro and in vivo. These data indicate that sialic acid-directed therapies might contribute to anatomical and functional recovery of the injured spinal cord.

Eric S. Bennett of the University of South Florida College of Medicine presented his group’s study of the changing glycome of the developing myocardium. In their studies the researchers aimed to determine whether cardiomyocyte glycosylation-associated gene expression and glycan structures are regulated and remodeled throughout the developing heart. The structural and gene microarray analyses demonstrated significant glycome remodeling among neonatal and adult atrial and ventricular myocytes. Over 40 percent of the glycosylation-associated genes were significantly differentially expressed among myocyte types. Further, marked differences in complex N-glycan structures were observed among myocyte types, with adult atrial myocytes producing the greatest relative density of bi-antennary complex structures and neonatal ventricular myocytes producing the least relative number of such structures. ST8Sia-II (STX) knockout mice were used to test whether the regulated expression of a polysialyltransferase was relevant to cardiac excitability. The data indicated that cardiac action potential waveform and voltage-gated sodium channel (Na+) gating were altered by the regulated expression of STX. Together, the data suggested that glycosylation-associated
gene expression throughout myocardial development leads to altered N-glycan structures, and this remodeled glycome has an apparent effect on Na, function and overall cardiac excitability.

Stuart M. Haslam, Director of Core C at Imperial College, London, Rahul Raman, Director of Core B at the Massachusetts Institute of Technology, and Martin Frank of the German Cancer Research Center presented in the analytical glycomics and bioinformatics section of the meeting.

The parallel approach to the submitted samples facilitates integrated data analysis of glycan profiles and glycol-gene expression.

Stuart Haslam spoke about the integrated glycomic analysis of pure populations of human and murine leukocytes which represents the current high priority area of analysis for Core C. He highlighted the application of new technology such as MALDI-TOF-TOF MS/MS analysis used at Core C to allow the determination of glycan structurally informative fragmentation information from very minor components in the complex mixture generated from the glycomic analysis of immune cells. It is also a key objective that whenever possible cells provided to Core C for glycomic profiling are also be provided to Core E for glyco-gene chip microarray analysis. This parallel approach by Cores C and E to the submitted samples facilitates integrated data analysis of glycan profiles and glycol-gene expression enabling the detailed comparison of glycome profiles of different cell populations. This method was highlighted in the presentation of data generated in collaboration with the van Kooyk group as part of her bridging grant detailing changes in glycan profiles as dendritic cells mature.

Rahul Raman presented the new accomplishments at the Consortium's Bioinformatics Core in building integrated databases and interfaces to glycomics databases. The primary components of the CFG glycomics databases include acquisition and dissemination interfaces for the CFG datasets, the specialized glycan structures, GT, and GBPs databases, and interfaces for tracking and uploading data generated from the CFG resources by the participating investigators.

Martin Frank concluded the meeting by talking about the world efforts for building unified glycan structure databases. Under the EUROCarbDB initiative, the group of late Claus-W. von der Lieth, led the international effort for creating centralized database with controlled vocabulary. interface to see data from different databases CarbBank notation has been converted into Glyde II format and an interface that allows to see data from different databases GlycanBuilder has been created. The prototype of the EuroCARB database will be available in Spring of 2008. The next step in establishing a unified Carbohydrate Structure Database would be solving the issues of data validation and curation. Dr. Frank welcomed any suggestions for making this an effective process and for acquiring essential funding for the continued effort.

The meeting also had a vibrant poster session during lunch and coffee breaks with over 25 posters displayed. Three posters were selected for short highlight talks in the mains session, and were presented by Michiko N. Fukuda of the Burnham Institute for Medical Research, Nicholas M. Stamatos of the University of Maryland, and Sachiko Sato of the Research Centre for Infectious Diseases, Quebec, presented their posters.
Michico Fukuda reported interesting findings on the identification of novel carbohydrate binding endothelial receptors by a carbohydrate-mimicking peptide. Pre-mRNA splicing factor and annexin 1 are novel endothelial surface receptors. The results of her study suggested a potential of l-peptide in therapies against cancer.

Nicholas M. Stamatos demonstrated that neuropilin-2 was a newly-recognized polysialylated protein expressed in human DCs that modulates DC-T lymphocyte interactions. The researcher is further exploring the molecular mechanisms by which PSA on neuropilin-2 affects the interaction of DCs with T lymphocytes and other cells of the immune system.

Sachiko Sato showed the involvement of galectin-3 in leukocyte recruitment in a murine model of lung infection by *Streptococcus pneumonia*. The study identified the adhesion molecules involved in neutrophil recruitment during lung infection by S. pneumonia. The research data suggested that galectin-3 acts as a soluble adhesion molecule in the recruitment of neutrophils to lungs infected by *S. pneumoniae*, which in turn induces β2-integrin-independent migration.

During the lunch break, J. Paulson addressed the CFG PIs with a request for feedback several areas of the Consortium’s activities. The PIs were asked to provide suggestions for and annotations of the GlycoGene chip version 4, synthesis targets for the glycan microarray, suggestions for monoclonal antibodies, and targets for knockout mice. Additionally, Dr. Paulson reminded the PIs about the responsibility to upload data from the CFG-related experiments as soon as it becomes available and referred them to the new data uploading interface on the CFG Web site. The PIs welcomed the idea of a larger involvement into the Consortium’s decisions and immediately made several suggestions, including additional knockout mice, glycolipid targets for the glycan microarray (are in the plans), and adding new capabilities for quantitative protein-carbohydrate interactions.

Core Directors collected more feedback at the CFG booth at the Society for Glycobiology annual meeting and in the direct communications of the PIs and the CFG core directors.

In summary, the meeting was praised by many attendees as very interesting and well-organized. The PI meeting highlighted progress in the field and was complimentary to the meeting of the Society that followed.
In Memoriam of Dr. Claus-W. von der Lieth

It is with great sadness that we have to announce that Dr. Claus-Willi von der Lieth, a CFG Participating Investigator and the head of EuroCarbDB, passed away on November 16, 2007.

Senior Scientist and Head of the Modeling Group at the Central Spectroscopy Department of the German Cancer Research Center, Dr. von der Lieth was emerging as the leader of the glycobiological community in the quest to establish a worldwide carbohydrate database. Despite his medical condition, he worked up to the last.

At the last Participating Investigator meeting, Martin Frank presented a carbohydrate structure meta-database created within the project and scheduled to be released in the Spring of 2008, which provides a structure-oriented access to all major worldwide glycan databases. It is a significant step in the direction of a unified, curated, and sustainable database for carbohydrate structures.

New Happenings at the CFG Scientific Cores

The Consortium has continued to develop infrastructure, unique reagents, novel tools, research teams, integrated and interactive databases, and alliances to facilitate progress towards the goals of the Consortium. The new happenings at the CFG are highlighted below.

New Interactive Interface for Analytical Glycomics Data

The CFG Bioinformatics Core successfully launched the first version of the interactive browser and completed the integration of the MALDI-MS profiling with CFG glycan structure database. Please visit the Glycan Profiling page of the Analytical Glycotechnology Core to explore the new interface. To browse the new interface at http://www.functionalglycomics.org/glycomics/publicdata/glycoprofiling.jsp, follow the instructions below.

1. Chose the image of a mouse
2. Chose the tissue type Brain
3. Click the icon depicting an annotated spectrum in the sub-column “APP” of the N-linked Glycans column for C57Black/6
   
   **Note:** Please make sure that your browser pop-up is not blocked.
4. Click the cartoon to get to the entry in the glycan structures database
   
   **Note:** The glycan topology captured by the cartoon (without explicit linkage assignment) can be loaded into a substructure search to get exact structures that have the same topology as the substructure.

The other new features coming soon to the CFG web site include a link to search for exact glycan structures based on the composition of the annotated cartoon and links to other Core C samples that have been annotated using the same cartoon.

This essential step towards the integration of the analytical glycomics data with the glycan structures database was made possible by the collaboration of all CFG cores and assistance from D. Goldberg’s bridging grant.
New PI Interface for Uploading Experimental Data

In an effort to enrich further the diverse datasets generated by the Cores, the Bioinformatics Core launched a new PI interface for uploading data related to a specific CFG resource request. From now on, after the investigators who receive resources from the CFG acquire any data from their use, they can upload it from the new interface.

To browse the new interface, log in to the CFG website as a CFG member (http://www.functionalglycomics.org/glycomics/common/jsp/CDBlogin.jsp). Once logged in, select Home/CFG Resources/Approved ResourceRequests from the left navigation menu and your name from "By Investigator" list. The link "Upload data" in the data column of your request would lead you to the data uploading form.

Quarterly Surveys of Principal Investigators

Over the six years of its existence, the Consortium and the resources generated by its Cores have become a prominent force in the glycobiological research. The body of the Participating Investigators, members of the Consortium, has grown extensively. By the end of year 6 of the project, the Consortium membership has increased to 370 scientists from all over the world. Additionally, over 200 non-members who have requested the CFG resources agreed to deposit data or acknowledge the Consortium in their publications.

This increase in the scientists using the CFG resources called for an automation of the quarterly surveys conducted by the Consortium in its efforts to document accomplishments and utilization of resources. The new interface allows pulling the resource requests pertaining to a given scientist and merging the quarterly request for publication citations and data submission. The first survey of year 7 conducted using this automatic survey interface demonstrated a dramatic cut in time spent on the task.

New Targets for the Mouse Transgenics Pipeline

The Mouse Transgenics Core (F) has adopted new transgenic targets for the knockout strains. The targets proposed by participating investigators for Core F pipeline were discussed and approved by the Mouse sub-committee and the Steering Committee. Seven targets were adopted including two for double KO strains.

- **ppGalNAc T12**: There is a hypothesis that partial loss of ppGalNAc T activity would lead to a less glycosylated Muc2 that would have altered properties, perhaps being more susceptible to proteolysis, with poor gel forming barrier functions. This gene is associated with carcinogenesis in rectal cancer.
- **MGL2**: This C-type lectin can be used for targeting purposes and antigen presentation, with OVA and tumor models. There is preliminary data with the glycan specificity of MGL-1 and -2 but no in-vivo work for MGL-2.
- **MGL1 and MGL2 Double Knockout**: The hypothesis is that these two MGL genes are redundant, and a double KO is required to see the most dramatic phenotype, which seems reasonable since there is only one MGL in humans. It was envisioned that the double KO would be made by mating the two single KOs. However, these two genes are on the same chromosome and only 30 kb away from each other. To produce double knockout mice by crossing two individual knockout mice, a BAC DNA construct (3 loxp sites flanking two genes) should be made.
- **GalNAc4ST-1**: 4-sulfotransferase that adds sulfate to N-acetylgalactosamine.
- **GalNAc4ST-2**: 4-sulfotransferase that adds sulfate to N-acetylgalactosamine.
- **clec-2**: This C-type lectin-like receptor from the Dectin-1 cluster has been shown to play a role in platelet activation and to recognize podoplanin through sialylated O-glycans.
• **Galectin-3 and -9**: The double null mice in the background of C57 might be helpful for various infectious diseases, such as parasitic diseases and pneumonia, for the researchers who have some limitations in the occupation of animal facility for reproduction. Since the current mice at the CFG were acquired from another facility with imposed restrictions on the distribution, Core F is re-deriving the CFG Galectin-9 knockout which is currently in the chimeric mouse stage. If successful, it will be used to make the double KO.

Two targets were placed on the CFG pending list pending additional evidence for the protein ability to recognize carbohydrates: clec-9, a C-type-like lectin from the Dectin-1 cluster with a single cytoplasmic tyrosine motif shown to function in cellular activation, and clec-16a, a new C-type lectin associated with diabetes type I in humans.

**New Version of Glycan Array (3.1)**

The most recent version of glycan array v3.1, containing 377 glycans, has just been printed in November 2007. The array contains most of the major terminal glycan classes, including sulfated glycans, glycolipids, and some with NeuGc compounds. The list of the structures can be found at http://www.functionalglycomics.org/static/consortium/resources/resourcecoreh8.shtml.

**Seeking Suggestions/Input from Participating Investigators**

Moving into the second phase of the project, the Consortium is making every effort to facilitate achieving the overarching goal of defining paradigms by which glycan binding proteins mediate cell communication. The CFG is exploring new ways of enhancing the utility of existing resources, developing the databases with increased integration and interfaces that allow intuitive access to CFG data, collecting the data generated within the project, documenting the impact of the CFG against the overall goal, and planning for evergreening novel resources to assure continued access to the community. The CFG has just launched a major initiative of integrating the participating investigators towards achieving the overall goal by soliciting their input on following:

• Suggestions for synthesis of compounds for glycan microarray library and N-linked and O-linked glycan standards for synthesis by the Glycan Array Synthesis Core (Core D)

• Pathogen glycans for the pathogen glycan microarray (Cores D and H)

• Suggestions for KO targets for the Mouse Transgenics Core (Core F)

• Mentors for KO mice phenotyped by the Mouse Phenotype Core (Core G)

• Pure human and murine cell populations for glycomics profiling (Core C) and gene expression analysis (Core E)

• Annotation of glycan binding protein molecule pages for the CFG database (Core B)

Further information about the specific requests can be found at the Updates on the Consortium page (http://www.functionalglycomics.org/static/consortium/whatsnew.shtml).

**Available Resources and Services**

The Consortium for Functional Glycomics is producing novel resources and services for Participating Investigators and interested scientists. Please visit our resources page (http://www.functionalglycomics.org/static/consortium/resources/resources.shtml) to view available carbohydrate compounds, mouse knockout strains, glycan array screening, glyco-gene microarray analysis, glycan analysis, and mouse phenotyping. Visitors can also find instructions for completing and submitting resource requests online. Request forms received online are forwarded to the Director and Coordinator of the appropriate Core for evaluation. All requests are given the same consideration regardless of Consortium member status; non-members proposing experiments within the scope of the Consortium agree to submit their data to the Consortium database prior to publication. Most requests, along with the Director’s recommendation, are reviewed at the following Steering Committee meeting. The website is updated with new offerings as additional resources become available.
**New Members**

The Consortium welcomes the following new members:

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<tr>
<th>Investigator</th>
<th>Institution</th>
<th>Subgroup</th>
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<tr>
<td>Blixt, Ola, Ph.D. Associate Professor</td>
<td>Department of Cellular and Molecular Medicine University of Copenhagen, Denmark</td>
<td>Other</td>
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<td>Dall'Olio, Fabio Associate Professor</td>
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<td>Hacioglu, Bilge, Ph.D. Senior Scientist</td>
<td>AlphaSniffer, LLC</td>
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<td>Havran, Wendy L., Ph.D. Professor</td>
<td>The Scripps Research Institute</td>
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<td>Huang, Xuefei, Ph. D. Associate Professor</td>
<td>The University of Toledo, Department of Chemistry</td>
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<td>Kawula, Thomas H. Associate Professor</td>
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<td>Le Doux, Joseph M., Ph.D. Associate Professor</td>
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<td>Lebrilla, Carlito B., Ph.D. Professor</td>
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