The Consortium Holds Its Participating Investigators’ Meeting

The third annual meeting of the Participating Investigators of the Consortium for Functional Glycomics, coming at the conclusion of the second full year of funding for the grant, was held on December 7, 2003, at the Catamaran Resort Hotel in San Diego. The meeting followed the Annual Conference of The Society for Glycobiology, which was held on December 3 – 6. After breakfast, the Consortium’s Principle Investigator, Jim Paulson [PPT], welcomed attendees.

Accelerating members’ participation in the Consortium was seen as an important goal for this year, and this effort has been successful by several measures:

- The year saw a significant increase in the number of Participating Investigators from 56 to over 90.
- More than 45 online requests by investigators for resources from the Scientific Cores were approved by the Steering Committee (SC).
- Feedback solicited from Participating Investigators at several times during the year had substantial impact on strategic directions and Core objectives.

Attendees focus on morning presentations.

Core G Director Sally Orr, Jim Paulson and Participating Investigator Geoffrey Kansas chat between presentations.
Several important new partnerships for the Consortium have been forged during the last year:

- The National Center for Research Resources (NCRR) is supporting a bridging grant with the Palo Alto Research Center (PARC) for the development of a program for automatic annotation of mass spectrometry data.
- The Joint Center for Structural Genomics (JCSG) under the leadership of Ian Wilson has committed to determining structures of murine and human glycan-binding proteins submitted by Participating Investigators.

Following Paulson’s opening remarks, the Core Directors gave overviews of the progress of their Cores in the last year. Links to their PowerPoint presentations are available on page 9 of this newsletter, as well as being included in the following articles. Highlights from the presentations included:

- High-throughput glycan array screening
- Glycomics
- Glyco-gene microarray screening

These topics are discussed in the following articles in this newsletter.

In the afternoon, the Subgroups (C-type lectin, Galectin, Siglec, TCR/CD1/MHC and Other) met individually to discuss their specific needs, priorities and concerns. The Subgroup leaders Kurt Drickamer, Rick Cummings, Paul Crocker, Mitch Kronenberg and Jim Paulson subsequently reported the substance of these meetings back to the whole group. This collated feedback will influence a range of significant Steering Committee decisions in the coming year.
High-throughput Glycan Array Screening

The Protein-Carbohydrate Interaction Core (H) (at the University of Oklahoma Health Sciences Center, directed by Richard Alvarez [PPT] and Coordinator Richard Cummings) and the Carbohydrate Synthesis/Protein Expression Core (D) (led by Ola Blixt [PPT] at The Scripps Research Institute and Nicolai Bovin at the Shemyakin Institute of Bioorganic Chemistry in Moscow) are developing high-throughput screening platforms for identifying lectin-ligand interactions.

The first generation glycan array uses a streptavidin/biotin platform in microtiter plates. Version 2.2 of the array has 170 unique glycans (in triplicate), with approximately 250 anticipated to be present by August of 2004. The saccharide compounds for the array are synthetic compounds prepared using chemical and enzymatic synthesis, and compounds isolated from natural glycans and N- and O-linked glycopeptides. To date, 26 investigator-supplied lectins have been analyzed and an additional 10 lectins and 8 carbohydrate antibodies have been approved for screening. As an example of the kind of data that is being produced by Core H, Figure 1 compares the binding profiles of the extracellular domains of two C-type lectins, DC-SIGN and DC-SIGNR, provided by Participating Investigator Kurt Drickamer. Screening of these naturally tetrameric proteins on the version 1 array showed a difference in binding preference between the two species – DC-SIGN binds a spectrum of monovalent and polyvalent fucosylated glycans, whereas DC-SIGNR binds none of these.

![Figure 1. Binding of fluorescein-labelled DC-SIGN (left panel) and DC-SIGNR (right panel) to the streptavidin/biotin glycan array.](image)
Under the direction of Ola Blixt and Steve Head, Core D is developing a second generation glycan array in the format of covalent attachment of the glycan library printed with a robotic slide printer onto glass slides (Figure 2). The library of oligosaccharide compounds with a functional amino group reacts with the N-hydroxysuccinimide ester-activated slide surface to form stable amide linkages. The printed array has great potential for future analyses due to the small (microgram) quantity of glycan-binding protein needed for analysis, the large number of glycans that can be printed per slide in a short time, and the ability to re-use the slides.

Figure 2. The slide printer (left) at the Gene Microarray (Core E) facility (right) at The Scripps Research Institute is being used to print the glass slides.

Figure 3 shows the binding profile of human siglec CD22 obtained on the printed array.

Figure 3. Detection of FITC-conjugated lectin binding to oligosaccharide compounds on the printed array (left panel). Binding profile of human siglec CD22 on the printed array (right panel).
With two glycan array formats that appear promising, and that have both generated novel data, the Consortium must decide how to proceed with glycan array screening in the future. The Steering Committee will make this determination in the next few months based on a comprehensive comparison of the two formats that is currently being performed.

Core B Director Rahul Raman, Participating Investigator Fu-Tong Liu, and Bridging Grant Pls Paul Crocker, John Lowe and Mitch Kronenberg enjoy a lunchtime discussion.

**Glycomics**

The Analytical Glycotechnology Core (C) (under the leadership of Coordinator Anne Dell [PPT] and Director Stuart Haslam at the Imperial College London), has undertaken the comprehensive profiling of the most important N- and O-linked glycans of the mouse glycome. Chosen tissues from wild type and selected glycosyltransferase knockout strains are being examined, as well as glycans from mouse neutrophils and Wehi-3 cells, a murine leukemic cell line provided by Richard Cummings and Richard Alvarez at the University of Oklahoma Health Sciences Center. N- and O-linked glycans are extracted and derivatized and an initial MALDI screening is performed. After the spectra are interpreted and annotated, ES-MS/MS analysis is performed on selected mass peaks to identify glycan structures, and GC/MS linkage analyses are performed as indicated. Representative spectra can be seen in Figure 4, which shows the low molecular weight end of the MALDI spectra of N-glycans from mouse neutrophils and the murine leukemic cell line Wehi-3.

Participating Investigators Willi von der Lieth, Bridgitte Schmitz and Pradman Qasba chat between presentations.
Figure 4. The low molecular weight end (from 1500 to 3250 mass [m/z]) of the MALDI spectrum of N-glycans from murine neutrophils (top panel) and the mouse leukemic cell line WeHi 3 (lower panel).
To date, 97 tissue and cell samples have been analyzed. Other N-linked and O-linked glycan profiles from selected tissues from several strains of wild type and knockout mice are available for viewing on the Consortium website at: [http://web.mit.edu/glycomics/consortium/organization/sciCores/dataCoreC.shtml].

David Goldberg from the Palo Alto Research Center (PARC) has been awarded a Bridging Grant funded by the National Center for Research Resources (NCRR) to develop automatic annotation of mass spectrometry data in collaboration with Anne Dell. Core C sees this capability as being essential to the analysis of the large number of spectra being produced. A prototype program is being developed for the analysis of MALDI and MS/MS spectra of N-glycans. The first step in automated annotation is the generation of potential cartoon structures. Cartoons for monosaccharides are entered individually, and the program automatically generates a larger “dictionary” set of feasible structures using an algorithm based on possible biosynthetic pathways. The search algorithm will identify mass matches to this repertoire of structures, so it is desirable to generate a “superset” that is not limited just to known structures. A separate dictionary set will be generated for each organism and tissue.

Human glycomics will be an exciting initiative at Core C in the coming year; human tissues have just become available. The Consortium eagerly anticipates the study of tissue-specific glycosylation patterns in human tissues, and the comparison of glycan structures between mouse and human tissues.

Glyco-gene Microarray Screening

The Gene Microarray Core (E) (under the leadership of Steve Head [PPT] at The Scripps Research Institute), has developed a custom Affymetrix Glyco-gene Chip expression array that contains probe sets for 1800 human and murine genes relating to the function of GBPs, including the GBP genes themselves and genes for the glycosyltransferases responsible for the synthesis of their carbohydrate ligands.
To date, Core E has screened 279 RNA samples – 106 Core E-generated samples and the rest provided by investigators. The Core E-generated samples, in addition to those involved in the development of the microarray, constitute a survey of gene expression in wild type mouse tissues. (To view this data on the Consortium website, visit [http://web.mit.edu/glycomics/consortium/organization/sciCores/dataCoreE.shtml][1]) The investigator-provided samples represent an inquiry into the expression of glyco-genes in a wide range of experimental models and biological systems.

An invited talk and several posters presented at the Annual Conference of The Society for Glycobiology highlighted data from the Consortium’s microarray. Jim Paulson presented a talk on the highly annotated and focused Glyco-gene Chip. Information obtained about the expression of glycosyltransferases in ten mouse tissues and in resting and activated murine lymphocytes illustrates the utility of this resource. It is hoped that this information, in conjunction with glycan profiling, will illuminate how changes in gene expression are reflected in the glycan structures produced by cells.¹ Linda Baum’s laboratory is studying the changes in glycosylation-specific gene expression during memory T cell differentiation. She examined changes in glycosyltransferase and glycan-binding protein gene expression during this process by submitting RNA samples from in vivo generated antigen-specific T cell populations for Glyco-gene Chip analysis.² In a study of the role(s) of genes relating to glycan synthesis and function in the endoplasmic reticulum (ER) stress response, Mark Lehrman’s laboratory submitted RNA samples from human fibroblasts subjected to a series of characterized ER stresses for Glyco-gene Chip analysis.³ In a study of the altered sialylation in cystic fibrosis (CF) airway epithelial cells, Thomas Scanlin’s laboratory submitted RNA samples from airway epithelial cell lines with differing expression of the gene mutated in CF, cystic fibrosis transmembrane conductance regulator (CFTR), for Glyco-gene Chip analysis.⁴

Core E is currently upgrading version 1 of the Glyco-gene Chip to version 2; this process has involved extensive feedback from Participating Investigators in contributing new genes and annotating the gene list. The result has been the addition of 149 new probe sets for mouse genes and 104 new probe sets for human genes. Improvements in the chip based on an evaluation of the performance of redundant probe sets on the current version have also been made. The second version of the chip is expected to be available for screening investigator’s samples by this spring.

Presentations Available For Viewing

Slides from the introductory remarks and the Scientific Core presentations from the Participating Investigators’ meeting can be viewed by clicking the links below:

- Introduction Jim Paulson [PPT]
- Scientific Cores: Results to Date
  - Protein-Carbohydrate Interaction Core (H) Rick Alvarez [PPT]
  - Carbohydrate Synthesis Core (D) Ola Blixt [PPT]
  - Carbohydrate Analysis Core (C) Anne Dell [PPT]
  - Gene Microarray Core (E) Tim Gilmartin [PPT]
  - Mouse Transgenics Core (F) Peter Sobieszczuk [PPT]
  - Mouse Phenotype Core (G) Sally Orr [PPT]
  - Bioinformatics Core (B) Rahul Raman [PPT]

Available Resources and Services

The Consortium for Functional Glycomics is producing novel resources and services for Participating Investigators and interested scientists. These reagents and tools are detailed on the Consortium website, where 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.

- **Carbohydrate compounds** (available at [http://web.mit.edu/glycomics/consortium/resources/resourcecored.shtml](http://web.mit.edu/glycomics/consortium/resources/resourcecored.shtml))
  - 45 saccharides, from monosaccharides up to decasaccharides, in 1 and 5 mg quantities
  - 44 biotinylated structures in 250 µg quantities
  - 8 polyacrylamide derivatives in 50 - 250 µg quantities

  - Human Siglec-2 and human Siglec-10 in 100 µg vials
  - Mouse Siglec-4, 50 µg in 10 ml, as a non-purified protein in media
  - Mouse CD1d in 500 µg vials

  - 24 glycosyltransferase and GBP knockout strains, including several double knockout strains

- **Glycan array screening** (available at [http://web.mit.edu/glycomics/consortium/resources/resourcecoreh.shtml](http://web/mit.edu/glycomics/consortium/resources/resourcecoreh.shtml))
  - Glycan array version 2.2, a 384-well plate using a streptavidin/biotin format, with 170 unique glycans plus controls
  - Instructions for human and murine lectin preparation

  - A custom-designed glyco-gene chip array (GLYCOv1) contains probe sets for 1,814 human and mouse transcripts relevant to the Consortium (developed using Affimetrics technology)
    - Gene list and probe set annotation
    - Instructions for RNA sample purification and shipping
  - Glyco-gene chips for analysis in your own DNA microarray facility

### People and News

- The Consortium is pleased to announce Dr. Rahul Raman’s appointment as Director of the Information and Bioinformatics Core (B). He has been a major contributor and effectively co-directed Core B operations with Dr. Ganesh Venkataraman, who will continue to participate as a consultant to Core B. Dr. Raman will also maintain the responsibilities of Bioinformatics Liaison.

- The Mouse Genomic Nomenclature Committee (MGNC) has accepted a proposal by Dr. Peter Sobieszczuk, Director of the Mouse Transgenics Core (F), to adopt a nomenclature for the sialyltransferase gene family that conforms to that used in the biological literature.
The Consortium welcomes the following new members:

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Institution</th>
<th>Subgroup</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bruce Bochner, MD Professor</td>
<td>Johns Hopkins</td>
<td>Siglec</td>
</tr>
<tr>
<td>Moonjae Cho, PhD Assistant Professor</td>
<td>Cheju National University Medical School</td>
<td>Galectin</td>
</tr>
<tr>
<td>Mary Cloninger, PhD Assistant Professor</td>
<td>Montana State University</td>
<td>Other</td>
</tr>
<tr>
<td>Steven Domino, MD, PhD Assistant Professor</td>
<td>University of Michigan</td>
<td>Other</td>
</tr>
<tr>
<td>Siamon Gordon, MD, PhD Professor</td>
<td>University of Oxford</td>
<td>C-type Lectin</td>
</tr>
<tr>
<td>Jenny Gumperz, PhD Assistant Professor</td>
<td>University of Wisconsin</td>
<td>TCR/CD1/MHC</td>
</tr>
<tr>
<td>Amy Howell, PhD Associate Professor</td>
<td>University of Connecticut</td>
<td>TCR/CD1/MHC</td>
</tr>
<tr>
<td>Gordon Lauc, PhD Associate Professor</td>
<td>University of Zagreb</td>
<td>Galectin</td>
</tr>
<tr>
<td>Timothy Logan, PhD Associate Professor</td>
<td>Florida State University</td>
<td>Other</td>
</tr>
<tr>
<td>Petr Maly, PhD Head of Laboratory</td>
<td>Academy of Sciences of the Czech Republic</td>
<td>Galectin</td>
</tr>
<tr>
<td>Paul Martin, PhD Assistant Professor</td>
<td>University of California San Diego</td>
<td>Other</td>
</tr>
<tr>
<td>Subash Sad, PhD Staff Scientist</td>
<td>National Research Council of Canada</td>
<td>Other</td>
</tr>
<tr>
<td>P. Sriramarao, PhD Professor</td>
<td>La Jolla Institute for Molecular Medicine</td>
<td>Galectin</td>
</tr>
<tr>
<td>Mark Tykocinski, MD Professor</td>
<td>University of Pennsylvania</td>
<td>Other</td>
</tr>
<tr>
<td>Will York, PhD Assistant Professor</td>
<td>University of Georgia CCRC</td>
<td>Other</td>
</tr>
<tr>
<td>Hermann Zillener, PhD Professor</td>
<td>The University of British Columbia</td>
<td>C-type Lectin</td>
</tr>
</tbody>
</table>

Acknowledgements:

- Pictures taken at the Participating Investigators’ meeting used in this newsletter were contributed by Dr. Nahid Razi, Dr. Peter Sobieszczuk and Anna Tran-Crie. Their photographic efforts are greatly appreciated.
- The Consortium gratefully acknowledges contributions of enzymes, glycan-binding proteins and mouse lines from a number of investigators. Their names are on the website acknowledgement page at [http://web.mit.edu/glycomics/consortium/acknowledgement.shtml](http://web/mit.edu/glycomics/consortium/acknowledgement.shtml).