
NEWS OF THE ALLIANCE FOR CELLULAR SIGNALING

The Alliance is six months old, sitting up, and ready to crawl. A great deal has been accomplished during this time, and the pace is accelerating. We realize that there is only modest evidence of this progress visible on our Web site (http://cellularsignaling.org), but this should change soon, and we hope dramatically.

Staffing has reached acceptable levels, although some technical positions remain to be filled. Most renovations are complete. Most of the major items of equipment have been installed and are being used routinely.

Newly Renovated Dallas AFCS Labs

For those not familiar with the organizational structure of the Alliance, please see the document entitled Administrative Management Plan at http://cellularsignaling.org

PERSPECTIVE

In addition to the progress made in the seven Alliance Laboratories (highlighted below), a great deal of additional effort has gone into development of the AFCS Molecule List, construction of the Molecule Page Database, and drawing of initial pathway maps.

The AFCS Molecule List. This list of more than 2400 proteins is an attempt by the AFCS to identify a reasonably comprehensive set of cellular signaling elements. The list includes a primary name (and AFCS number), synonyms (in many cases), a functional category, and GI (gene index) numbers for nucleotide and protein sequences (mouse preferred). The list also names those who have volunteered to contribute individual Molecule Pages (see below) and indicates if a Mini Molecule Page has been completed and posted.

This list will soon be available to you via the Internet. It will be searchable, and you may also download it as a Microsoft Excel file, which will enable you to sort the entries in ways that make the list quite useful (e.g., group all listed Rho GEF's, RGS proteins, etc.).

We have constructed this list for several reasons, including standardization of nomenclature and assignment of an unambiguous identifier (AFCS number) to a given sequence. Importantly, the list serves as a starting point for prioritization of our efforts. For example,
the System Committees are using the list to assign priorities to molecules for recruitment of Molecule Page authors, acquisition of antibodies, performance of yeast two hybrid screens, construction of GFP fusion proteins, and several other tasks. The list will be useful to you, even in its current simple form. Examples include searches for molecules using synonyms, grouping of molecules by functional categories, and location of sequence and other information using GI numbers.

The list also serves as an important starting point for assembly of Molecule Pages. A great deal of information about each molecule on the list (particularly sequences and structure) can be extracted automatically from a number of other databases and used as the foundation for each Molecule Page. This should be a great aid for Molecule Page authors. These partially completed Molecule Pages will be extremely useful in their own right and should be available for everyone in the foreseeable future.

The AFCS Molecule List will be posted along with three simple forms for your input. One form will encourage your suggestions of additional molecules for the list. It will be particularly helpful if you include as much information as possible: name, synonyms, functional category, and GI number. A second form will permit your input on errors or suggestions on nomenclature or other issues. The third, truly important form will of course allow you to volunteer to be the author of a Molecule Page for some poor orphan on the list in need of a champion.

The Molecule Page Database.
The AFCS is establishing a comprehensive literature-derived, object-relational database of signaling molecules. Its core element is the “Molecule Page”. Thanks to the efforts of many of you, we are currently producing Mini Molecule Pages as the first stages of this effort. More than 100 are currently posted on our Web site. These are simple documents, designed to provide useful basic information about proteins of interest to the Alliance. But the mini pages are only a prelude to our real goal – the full length Molecule Page.

With your help this database should become the model for future annotation of genomes. It will enable you to access conveniently virtually all information about the molecule of interest. As just a few examples, you will be able to use the Molecule Page database to:

• Query the database about complex relationships among molecules
• View mutations or domains in the context of protein structure
• View or create de novo signaling pathways assembled from knowledge of interactions between molecules and the flow of information among them
• Evaluate or establish quantitative relationships among the components of complex pathways.

In addition, of course, you will be able simply to browse individual pages to find information about a given molecule.

We Need You. Rather than hire curators to extract information from the literature about signaling molecules, the AFCS seeks “the experts” — that is, you. We know that you, the research sci-
entists, are best qualified to extract the best from the literature and incorporate the information in its most useable form. As authors of Molecule Pages you will make use of automated searches designed by the AFCS; judgments about quality of information, however, will be yours.

The Molecule Page Database will be independent of (but linked to) the experimental databases of the Alliance.

We answer the obvious question — what’s in it for me, the author? — in several ways. Most important, you (and many others) will create a community resource of enormous value. Moreover, a Molecule Page will be a scholarly work, attributable to the author and edited by a distinguished Editorial Board. We are establishing mechanisms for indexing and archiving these electronic publications. You will be credited and recognized. But to return to the high ground, we hope that you will examine the first set of molecule pages (Summer, 2001) and recognize that your favorite molecule(s) must be represented . . . and that only you can do it justice. Members of the Alliance will create this resource.

Pathway Maps. Members of the System Committees have constructed maps of signaling modules to help us keep clearer pictures in our minds of complex pathways. At the moment they are nothing more than cartoons, and they reflect the bias of the authors. In the future they will have links to Molecule Pages and they will be derived automatically from our own data and/or from data supplied in Molecule Pages. We will share our first efforts with you when the finishing touches are complete. We

are grateful to Roger Sunahara, Ph.D., whose artistic talents are a near match for his scientific skills. One of these maps will be found at the end of this letter.

Please address comments to Al Gilman at the University of Texas Southwestern Medical Center, Dallas, TX: alfred.gilman@UTSouthwestern.edu

ALLIANCE LABORATORIES

There are seven Alliance Laboratories, and most of our budget supports the research in these dedicated facilities. Participating Investigators in the Alliance are Directors, Co-Directors, or Associate Directors of each laboratory. Laboratory staff includes Ph.D. scientists, technical staff, and administrative personnel.


AFCS activities in San Diego are currently focused in two major areas: design and construction of the Molecule Page Database discussed above and design and construction of our experimental databases (for B cells and cardiac myocytes). Once cell preparations are standardized, assays are validated, and results can be reproduced among sites, we will launch our first major set of experiments – the ligand screen in B lymphocytes (see Program Summary, Experimental Strategies on our Web site). The Bioinformatics Laboratory must be able to acquire, store, and analyze all of the different types of data to be acquired during this screen. Most challenging are the large data sets generated by analysis
of DNA microarrays and two dimensional protein gels.

**Cell Preparation and Analysis.** University of Texas Southwestern Medical Center. Paul Sternweiss, Director; Donald Hilgemann and Richard Scheuermann, Associate Directors.

This laboratory has the responsibility for preparation of requisite numbers of B cells and cardiac myocytes, incubation of cells with ligands, performance of certain of the assays on these cells, and preparation of fractions of these cells for analysis by others.

The lab is currently preparing $1.6 \times 10^9$ B cells from 32 mice each week; the goal is to increase production to $2.4 \times 10^9$ cells each week (in three preparations from 16 spleens each). Viability is reasonably well maintained for 24 hours, although further improvement is sought. More rigorous criteria will now be used to define the stability of the preparation at shorter times.

The preparation of cardiac myocytes from the mouse is more challenging, but substantial improvements have been made. We particularly appreciate the help received during a visit by Dr. Randa Dandan, who has extensive experience in preparing mouse myocytes in Larry Brunton’s lab at UCSD. We routinely obtain $> 2 \times 10^6$ rod-shaped cells from each heart, and the cells respond vigorously to catecholamines soon after isolation. Current goals are to assess the stability of their responses during culture and to explore conditions that will promote maintenance of a stable plateau.

Assays for cyclic AMP, Ca$^{2+}$, and cell surface markers (FACS) are now routine in the lab, and samples are prepared for analysis of mRNA and proteins (2D gels and immunoblots) by others.

![Freshly prepared adult mouse cardiac myocytes. The left panel is a low magnification view (bar equals 100 μ). The right panel is a high magnification view (bar equals 20 μ).](image)

**Assay Development.** University of California, San Francisco; San Francisco Veteran’s Administration Medical Cen-
The San Francisco lab has focused initially on cardiac myocytes, given the difficulties in their preparation. They have optimized yield by modifications of tissue digestion procedures and have worked with different attachment matrices and culture media to improve survival in culture.

In addition, B cells are now being prepared with yields essentially identical to those obtained in Dallas. The San Francisco and Dallas labs will continue to work collaboratively to improve isolation and culture conditions and to standardize all procedures between the two sites. Experiments (particularly on B cells) will soon be conducted in parallel at the two sites, and RNA and protein samples will be sent to the Molecular Biology and Protein Chemistry labs for direct and detailed comparison of the similarity of the two cell preparations and their responses to a set of five test ligands. Reproducibility must be assured before the ligand screen can begin. The Bioinformatics Lab will assist in the statistical evaluation of these data and advise on the need for replication of experiments and assays.

The group in San Francisco has also begun to evaluate the chemotactic responses of several B cell lines. We will need to use a B cell line for several experimental approaches to be pursued later, and the choice of this line will be made according to criteria noted in our research plan.


cDNA arrays have been the major focus. Large numbers of mouse cDNA clones have been obtained and amplified, and high-quality batches of arrays are being prepared with over 15,000 clones. The first samples from both Dallas and San Francisco are being analyzed now.

Although we are starting with cDNA arrays, we hope to switch to arrays made with long synthetic oligonucleotides in the near future. We are collaborating with Rosetta InPharmatics (Seattle) in the design of a mouse oligonucleotide array, and we are considering the logistic and financial issues involved in the ultimate choice of the comprehensive mouse gene array that we will want to use.

Preliminary experiments with antisense oligonucleotides (from Isis) will begin using a B cell line (probably BCL-1 initially). Arrangements with Myriad have been finalized and construction of libraries for yeast two hybrid screens will begin as soon as mRNA is supplied from B cells (resting and stimulated) and cardiac myocytes.

Protein Chemistry. University of Texas Southwestern Medical Center. Marc Mumby, Director; Yingming Zhao, Associate Director.

The focus here is on the newly acquired DIGE system for 2D gel electrophoresis (described in the first Newsletter). Procedures for extracting and labeling proteins with Cy3 and Cy5 have been optimized. Equipment for running large format 2D gels has been received, and we are learning to optimize its use. The gel-casting chamber permits 25 homogeneous or gradient gels to be pre-
pared at once, and, with two iso-DALT gel boxes, we should be able to run 20 gels at a time. To support the ligand screening exercise, the goal of the Protein Chemistry Lab is to scale up initially to about 100 gels per week.

Two mass spectrometers are functional: the Voyager DE MALTI-TOF instrument and the LCQdeca LC-MS system. The Applied Biosystems Qstar Pulsar mass spectrometer is currently being installed in the newly occupied Protein Chemistry Laboratory. The high-throughput capabilities of the latter instrument will be particularly valuable in systematic identification of protein spots that are altered in response to ligands.

Antibodies. University of Texas Southwestern Medical Center. Susanne Mumby, Director.

An important current goal of the Antibody Lab is to define a cocktail of phospho-specific antibodies that can be used to track the phosphorylation of several different proteins simultaneously after resolution on a one-dimensional gel. This will be one of the assays utilized in the ligand screen. Antibodies must provide visualization of single bands for such analysis and, of course, there must be an adequate amount of the phosphoprotein in preparations made from ligand-treated B cells.

We will perform a so-called Power Blot experiment in collaboration with BD Transduction Labs to screen over 700 of their antibodies for reactivity with proteins from our B cells and cardiac myocytes. This will be an enormous help in identification of commercial antibodies that will be useful for many of our purposes, particularly including protein quantification and subcellular localization.

When Will You See Data? We are of course mindful of our commitment to share our data and methods with you in a timely manner. We must develop databases, as well as analysis and visualization tools in order to do this. This is an obvious priority, and these capabilities must be in hand before we initiate the ligand screen. We will test our ability to display data in meaningful and helpful ways as we do the preliminary experi-

---

**Microscopy.** Stanford University. Tobias Meyer, Director; Stephen Smith, Associate Director.

The Microscopy Lab at Stanford is about to start imaging experiments with a B cell line, initially with BCL-1 because of the superior surface adhesion properties of these cells compared with others. B cell lines will be evaluated with regard to transfection and live cell imaging properties. Different fluorescent translocation biosensors are being made in collaboration with the Pasadena group and are being tested. The lab is also perfecting a standardized protocol for the preparation of cell lines and multiwell coverslips for imaging experiments.
ments necessary to validate our cell preparations and assays. The ligand screen will not commence until we are capable of sharing the data with you. Our goal is to have all of these capabilities in hand within the next three months.

**ANNUAL MEETING**

The first annual meeting of the Alliance will take place at the Natcher Building Conference Center at NIH in Bethesda, Md. on May 24 and 25, 2001. All who receive this newsletter are most welcome to attend, although we must again note that funds available to us will support travel and accommodations only for our Participating Investigators and the Alliance Laboratory Scientists.

We hope to broadcast the meeting on the Internet for those who are unable to come.

All members should have received (by email) a tentative schedule for the meeting, as well as information about hotels, receptions, a dinner, *etc.* If you have not received this information, please request it from Wendy Deaner (wendy.deaner@utsouthwestern.edu).

**LOGO: VOTE!**

We have received a modest response to our request for ideas about a logo, but three creative individuals have come forth, and we thank them. If you would like to express an opinion about these entries (*i.e.*, vote: #1, #2, #3, or none of these), please do so by email to Al Gilman.

#1. Artist’s Description: The different sized circles represent different molecules that are involved in signaling. They are different sizes to represent movement or intracellular communication.

#2. Artist’s Description: The design represents the “network machine” that we hope to understand. The spheres or nodes connect with each other at a focal point, such that the network can turn like a wheel or very basic machine.
#3. Artist’s Description: The stick figures in the logo represent the many talented individuals involved in this large-scale effort. The center circle represents the cell, the target of our study. The arrows signify the energy and focus required of Alliance personnel and also the cellular signaling pathways.

**SPONSORS**

We acknowledge our sponsors with gratitude:

**The National Institutes of Health:**
The National Institute of General Medical Sciences.
The Alliance Project was conceived under the NIGMS Glue Grant Initiative. See: www.nigms.nih.gov/funding/gluegrants.html
The National Institute of Allergy and Infectious Diseases
The National Cancer Institute

**The Pharmaceutical Industry:**
Eli Lilly and Co.
The Merck Genome Research Institute
Aventis Pharmaceuticals, Inc.
Johnson & Johnson
Novartis Pharma AG
Chiron Corporation

It is notable that these corporate sponsors support the investigative aims of the Alliance in full compliance with our policy on Intellectual Property (see [http://cellularsignaling.org](http://cellularsignaling.org)). No sponsor views Alliance data before it is posted on the public Internet.

**Philanthropic Foundations:**
The Agouron Institute
Anonymous Foundation, Dallas TX

**Others:**
The University of Texas Southwestern Medical Center

**We have established collaborative relationships with the following entities:**
Isis Pharmaceuticals, Inc.
Myriad Genetics, Inc.
Amersham-Pharmacia Biotech UK Ltd.

These relationships involve either (1) collaborative research agreements in which services are performed for the Alliance at cost or below (with full and prompt disclosure of data) or (2) substantial reductions in pricing for equipment and/or reagents with the hope that successful use of these technologies by the Alliance (and promulgation of such data) will encourage widespread adoption of relevant technologies.

We hope to add additional collaborators to this list.