NEWS FROM THE ADVANCED TECHNOLOGY PROGRAM

ATP Update

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Director's Point of View

Tim Harris, Ph.D., Director, ATP

Welcome to the second issue of the ATP Update. I hope you will find it interesting. Our feedback on the first issue indicated that no one seemed to dislike it, and at least one man of influence said it was "about time, too." So we are continuing. You'll find an article below, entitled "Business Opportunities at the Advanced Technology Program," which describes the technical capabilities of each of the ATP groups. In these difficult budget times, it is our job to go out there and speak to our customers (largely the NCI), so that they will spend money for technically challenging (larger-scale) experiments with the ATP, rather than going to other vendors. To consider NCI PIs and staff scientists as customers as well as a collaborators is not inappropriate. Our metric for success is not just publications, but also return on investment. We have to answer the question "Do we (the ATP) provide value for money?" as quantitatively as possible. Our site visits have gone very well, and the general view is that our applied science is as good as it gets. There is still the nagging question that some people do not know who we are or what the ATP is. I hope this newsletter helps make it clear and that you'll bear with us when we come after your business.

Business Opportunities at the Advanced Technology Program

by Dr. Bruce Crise

The Advanced Technology Program (ATP) at SAIC-Frederick, Inc., consists of 10 laboratories dedicated to the development and application of state-of–the-art technology in the applied life sciences. The ATP offers investigators highly specialized support to help them succeed in today's fast-paced biomedical research environment. Through an integrated approach, the laboratories of the ATP bring cutting-edge technologies in genomics, proteomics, and imaging to answer complex biological research questions.

The **Core Genotyping Facility (CGF)** in Gaithersburg, MD, carries out genome-wide association studies on behalf of NCI and others to identify regions of the genome that are associated with susceptibility to disease. The CGF is one of the groups who recently discovered that the chromosomal region 8q24 is associated with prostate cancer and that FGFR2 is strongly associated with breast cancer risk. The CGF also is heavily involved in developing bioinformatics tools, which are made available as resources to the entire scientific community. The **Laboratory of Molecular Technology (LMT)** emphasizes development and deployment of technologies that provide customers with a unique means to detect mutations and their frequency in different genes and regions of chromosomal deletion or amplification. This is accomplished primarily by using very-high-throughput Affymetrix and Agilent microarrays. Both the LMT and the CGF also have access to state-of-the-art DNA sequencing technologies, and the ATP is presently evaluating the next-generation DNA sequencing platforms. The LMT has also developed other microarrays, most notably for the identification of all viruses (the virus chip) and for all siRNAs, and has made antibody-based chips for protein identification. The virus chip is being used to identify viruses in various samples followed up by qPCR by the ATP's **Virus Technology Laboratory (VTL)**. Additionally, the VTL has developed and customized both protein- and nucleic acid–based detection assays, and, along with the

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LMT, provides assessment of gene expression levels using multiple platforms.

One of the most recent chip developments is the production of a chip in which the proteins are made in situ using cell-free protein synthesis. This new chip was developed in collaboration with the ATP's **Protein Expression Laboratory (PEL)** and is based on the discovery of a novel high-affinity tagging system that allows a newly synthesized protein to bind to the DNA that encoded it. The PEL uses this and other tags to enable the purification of recombinant proteins made in surrogate systems. The PEL is expert in synthesizing and purifying recombinant proteins from *E.coli,* yeast, insect, and mammalian cells (both in transiently transfected and permanent cell lines) and in scaling the systems to provide multiple milligrams of proteins for structural and other studies. Recently, the group further adapted the TAP affinity tag system for use in mammalian cells to study protein–protein interactions.

The ATP also has a collection of adenoviral and lentiviral vectors that can be used to transduce mammalian cells and to vaccinate animals. One current initiative is to produce proteins as immunogens for making and characterizing monoclonal antibodies. This initiative is part of a collaboration that includes our world-class **Protein Chemistry Laboratory (PCL)**, which focuses on measuring the thermodynamics of protein–protein and protein–small molecule interactions using surface plasmon resonance (SPR) and fluorescence spectroscopy. The laboratory is using new array-based SPR machines to compare the affinities of several single-chain antibody fragments and has been examining other affinity reagents such as affibodies.

The **Laboratory of Proteomics and Analytical Technologies (LPAT)** is the third of our protein-focused laboratories and is a center of excellence for mass spectrometry (MS). They have developed state-of-the-art methods for top-down proteomics, examining complex protein mixtures, including membrane proteins, and have a particular interest in post-translational modifications, such as phosphorylation. The LPAT also has considerable interest in metabolomics and has developed rapid, supercritical fluid chromatographic MS methods for the rapid separation and analysis of metabolites. Additionally, the LPAT has developed methodologies for identifying and quantifying steroid hormones in complex body fluids.

The **Nanotechnology Characterization Laboratory (NCL)** is also part of the ATP. This laboratory was set up with NCI, NIST, and the FDA to provide the capability to characterize nanoparticles physically and to determine their in vitro and in vivo characteristics. This type of analysis will produce comparative data sets to allow customers to assess structure–function relationships for their nanoparticles. Targeted nanoparticles and nanoparticles carrying drug payloads are currently being analyzed. The NCL, in collaboration with the **Image Analysis laboratory (IAL)**, another ATP laboratory, is developing the capability to examine nanoparticles with high-resolution 3D electron microscopic tomography, building on the expertise developed by Sriram Subramanian, Ph.D., at NCI in Bethesda. The IAL also has a suite of confocal microscopes and is expert in developing new mathematical modeling methods for image analysis. The **Advanced Biomedical Computing Center (ABCC)** is an ATP group that provides computational and bioinformatics support to the ATP and other laboratories at NCI-Frederick and elsewhere. ABCC has developed LIMS systems to hold and interrogate complex data, and it also manages the local, large computer cluster. The **Scientific Publications Graphics & Media (SPGM)** group provides expert service to the ATP and to NCI-Frederick in making our capabilities known to the outside world through assistance with papers and development of posters, newsletters, and brochures. They also manage the conference facilities at NCI-Frederick and the video links to NCI in Bethesda.

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PCL's New Instrument Cuts Screening Time to a Fraction

by Dr. Robert Fisher

The Protein Chemistry Laboratory uses surface plasmon resonance (SPR) spectroscopy to study molecular interactions. The lab is currently evaluating an innovative SPR platform that allows the investigator to monitor 36 molecular interactions simultaneously. This process is made possible by the Bio-Rad ProteOn XPR36, which contains 6 parallel flow cells that are used to immobilize 6 targets on the sensor surface. The fluidic system can be rotated through 90° to allow the flow of 6

different ligands over the 6 surfaces, thereby creating a 36-target array.

Investigators have already used this instrument to measure the binding kinetics of an affibody to Her2 (see related report) in a fraction of the time the experiment would take using traditional SPR instrumentation. The obvious advantage of this instrument is greater sample throughput. One potential application for this technology is screening antibodies. In the next experiment, the lab will screen a series of hybridoma supernatants.

PCL Characterizes Affibodies

by Dr. Robert Fisher

To obtain images of HER2-positive breast cancer tumors, radiolabeled trastuzumab (Herceptin®) and F(ab') fragments of trastuzumab labeled with In-111 and Ga-68 have been used for single photon emission computed tomography (SPECT) and positron emission tomography (PET) imaging, respectively. However, antibodies are rather bulky proteins as compared with the molecular probes typically used for PET imaging, making tumor penetration and clearance from the circulation rather slow, and, in turn, considerably affecting imaging studies.

As no known natural ligands for the HER2 receptor have been identified, Dr. Jacek Capala (National Cancer Institute, National Institute of Biomedical Imaging and Bioengineering) chose to use Affibody® molecules (http://www.affibody.com). Affibodies are highly stable proteins that are four times smaller than single-chain antibody fragments (scFv) and 20 times smaller than monoclonal antibodies. The three-alpha-helix affibody molecules are based on 58 amino acids derived from the B-domain of the IgG-binding region of Staphylococcal protein A. The three alpha-helices in their structure make them highly stable. The cysteine-free affibody molecules provide a strong framework independent of disulfide bonds for its folding, and their small size enables penetration into solid tumors and rapid clearance from the bloodstream.

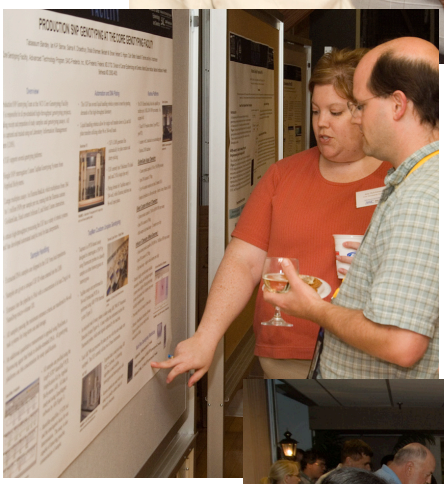
The Protein Chemistry Laboratory (PCL) of the Advanced Technology Program has characterized affibodies that display low picomolar binding affinities. As such, these molecules are highly suitable as carriers of radioisotopes or toxins to HER2-positive tumor cells because of their specific target-binding capability and lack of specific non-receptor interactions.

Dr. Capala has asked the PCL to provide biophysical characterization of the affibodies and the labeled variants, using their expertise in peptide purification and surface plasmon resonance (SPR) spectroscopy.

ATP Hosts Scientific Exchanges

by Ken Michaels

ATP has initiated regular get-togethers for scientific exchange. These gatherings give scientists from NCI-Frederick and other entities a chance to interact with investigators from the ATP. The events, held at the Community Activity Center, include an introduction from Dr. Harris and short talks by scientists from ATP labs, followed by informal discussions. Scientific posters are also on display. All are encouraged to attend.



Robert Welch and Meredith Yeager (above) from the ATP Core Genotyping Facility (CGF) in Gaithersburg were featured speakers at the June 20 event. Posters on display (left) also profiled the CGF and its work.

"Next-Generation" Sequencing Technologies

By Claudia Stewart

Recent advances in DNA sequencing technology have led to the development of "next-generation" DNA sequencing platforms. These platforms are capable of sequencing up to 150 Mbp of DNA in a single sequencing run over a period of 2 to 3 days. To date, next-generation sequencing technology has been utilized by investigators at many leading research institutions in applications as diverse as whole genome re-sequencing, high-throughput mutation detection/discovery, SAGE, ChIP-Seq, whole genome chromosomal translocation breakpoint mapping, and whole genome bi-sulfite sequencing (methylation detection). This technology is revolutionary, not evolutionary, as it will not only replace some older and lower-throughput sequencing technologies, but it will also enable new research avenues and approaches. There is considerable demand among NCI investigators for access to this instrumentation. Procurement of such next-generation sequencing technology is essential to NCI's maintaining its state-of-the-art genomics capabilities.

Roche/454 GS 20 Sequencer: a next-generation DNA sequencer

On Effective Communication

by Ken Michaels

Pointer of the month: Don't abuse the pointer.

The recommendation I most frequently offer to speakers on using the laser pointer in an oral presentation is this: If you must use the pointer, hold it in both hands and point directly at your target; then put it down and continue your presentation.

Why both hands? Two reasons: it keeps you aware that you're holding the pointer, and it helps you hold it steady. Many speakers who pick up the pointer the moment they begin talking not only point to things of interest on the screen, but often start pointing at *everything*. Presently, that little red dot is dancing around so actively that the audience wishes they'd pre-medicated with Dramamine®.

Why put it down? So you won't use the pointer as a crutch. When we're holding the pointer, we tend to point it at something, usually the screen; and when we focus on the screen, we're not focused on our audience.

And finally: "If you must." With a little planning, we can avoid using a pointer altogether. Showing bullets one at a time, or bringing in graphics like arrows, boxes, and circles add detail incrementally as the discussion progresses. These techniques are child's play for PowerPoint.

So why not try putting the pointer down? Probably, you'll look at the screen less and your audience more. After all, they came to your talk mainly to hear *you* talk, not to see a laser light show.

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