NEWS FROM THE ADVANCED TECHNOLOGY PROGRAM

ATP Update

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Director's Point of View

Tim Harris, Ph.D., Director, ATP

On behalf of the NCI, we are developing an "Advanced Technology Partnerships Initiative" to encourage commercial companies to work with us in public–private partnerships (PPPs). We have hosted meetings with several companies to show them our capabilities, and at least one of them is keen to work with us. These kinds of partnerships are of value to both parties: NCI enjoys the benefit of having their research translated into the clinical setting more rapidly than would otherwise be possible; private companies benefit from working with a complex set of technologies in the same place, at the same time. Even though many large companies have access to most of the technology that we have, such technology is not always located in the same place or even in the same time zone. Another significant part of our outreach program is the Work for Others (WFO) program. In this issue, Dr. Charmaine Richman outlines the WFO, which enables us to work with other companies on some interesting projects, generally at a smaller scale than that envisaged in the PPP setting. It is important that we continue to present good science and technology at outside meetings, so that companies that are potential partners, both large- and small-scale, gain a better understanding of our capabilities. Internal presentations are equally important: I still hear from NCI PIs that they do not really know what we do, so it is necessary for us to speak and present posters at internal meetings, too. This newsletter is designed to increase the awareness about the ATP and what we do. I hope it serves that purpose.

Inside This Issue

Work for Others Program Presents Opportunities

By Dr. Charmaine Richman

The Work for Others (WFO) program at SAIC-Frederick, Inc., presents an opportunity for ATP laboratories to provide services to non–federal government customers, such as university researchers, nonprofit entities, and businesses. The WFO services must meet specific criteria: (1) services must be unique, i.e., available only at SAIC-Frederick, Inc., and (2) in no way can providing the services to the non–federal government customer be construed as competition with the private sector.

Cost estimates developed following a formal WFO request must reflect full recovery of all the costs associated with providing the service and include the appropriate overhead rates. Jeff Lake, Lead Financial Analyst, is available to advise and review the cost estimates. He may be reached at 301-846-5758, or lakejeff@mail.nih.gov.

The program is coordinated through Dr. Charmaine Richman, Scientific Administrator. She may be reached at 301-846-6308, or richmanc@mail.nih.gov.

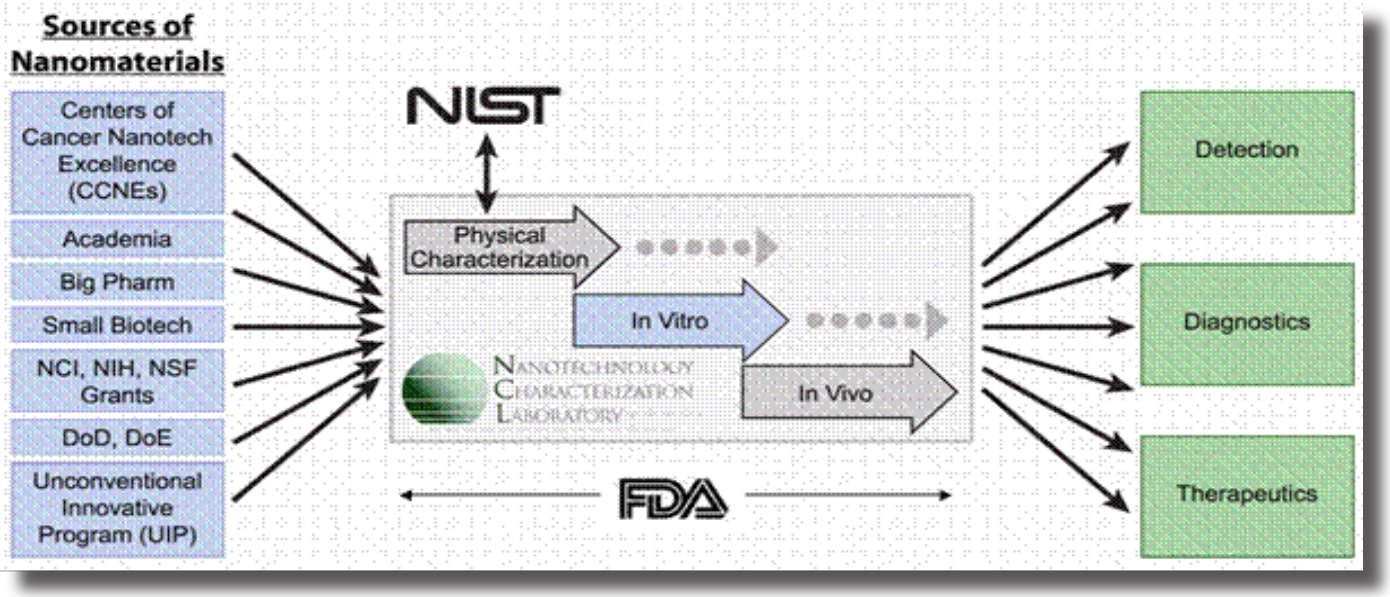
Moving Nanotechnology Concepts to the Clinic

by Dr. Jennifer Hall

The National Cancer Institute's Alliance for Nanotechnology in Cancer created the Nanotechnology Characterization Laboratory (NCL) in collaboration with the U.S.

Food and Drug Administration (FDA) and the National Institute of Standards and Technology (NIST). Part of the Advanced Technology Program (ATP) at NCI-Frederick, the NCL has a simple mission: to accelerate the transition of nanotechnology-based research into clinical applications for cancer. For many nanotechnology developers, the prospect of preparing, characterizing, and submitting these products for regulatory approval can be daunting.

The NCL is a national resource that provides a smoother path to clinical trials by offering characterization of cancer diagnostics, imaging agents, and therapeutics based on standard protocols of preclinical toxicology, pharmacology, and efficacy developed especially for nanoscale concepts and strategies. The NCL's services are offered free of charge to nanotechnology developers from academia, government, and industry.



NCL's mission is to accelerate the transition of nanotechnology-based research into clinical applications for cancer.

Clone Optimization Group Reaches Milestone

By Dr. James Hartley

A small celebration recently in the Clone Optimization Group of the Protein Expression Laboratory (PEL) marked the 5,000th oligonucleotide ordered since 2001 to tailor genes for protein expression. "It's actually an underestimate, since we didn't keep track of individual oligos for the first year or so," explained Dr. Dominic Esposito, the group's head.

The oligos are used for PCR amplification of genes of interest; introduction of translation start and stop signals; signal peptides, protease sites, and epitope tags; mutagenesis; and sequence confirmation. "We're getting close to 4,000 clones constructed with the oligos," noted Dr. Jim Hartley, the PEL's director. "Dom and his people do a great job tracking not only the oligos, but also the clones, strains, and data that come from them. They've developed a smooth work flow that makes it seem easy, but, of course, it's not. It really comes from constant improvement and effort."

Dr. Esposito can be contacted for clone and vector construction at domespo@ncifcrf.gov or 301-846-7376. Dr. Hartley is available to discuss other aspects of protein expression and purification at hartley@ncifcrf.gov or 301-846-7375.

LPAT Develops New Method for Labeling Phosphoproteins

By Dr. Timothy Veenstra

Scientists in the Laboratory of Proteomics and Analytical Technologies (LPAT) have developed a method that uses stable-isotope labeling and mass spectrometry to unambiguously identify phosphorylated residues generated during in vitro kinase reactions. Instead of relying on radioactive ^{32}P-labeling, the new method utilizes an adenosine triphosphate (ATP) molecule, in which the four oxygen atoms surrounding the terminal phosphate molecule are each substituted with a stable

oxygen isotope (i.e., ^{18}O). When this novel reagent ($\gamma(^{18}O_4)$-ATP) is mixed with an equimolar of normal ATP, phosphopeptides generated via an in vitro kinase reaction are observed as a doublet of peaks within the mass spectrum. The peaks are separated by a defined mass based on the difference between $\gamma(^{18}O_4)$-ATP and ATP (i.e., 6.01 Da). The exact number of phosphorylation sites within a peptide can also be determined by the number of observed peaks separated by 6.01 Da. For example, a doubly phosphorylated peptide will give rise to a grouping of three peaks, all separated by integral values of 6.01 Da.

This method, developed by Drs. Ming Zhou and Zhaojing Meng of the LPAT, has a number of advantages over using radioactive ^{32}P-labeling. Because of the health risks associated with exposure to radioactivity, special precautions must be taken with ^{32}P-labeling techniques. In addition, careful planning and timing are necessary when using ^{32}P-labeling, so that the precise amount needed for a specific experiment is ordered and consumed within 3 to 5 days of delivery. Once the experiment is completed, any unused reagent and consumable materials that it came into contact with must be disposed of properly. With the new method, however, no special precautions or disposal procedures are required because the isotope is stable and the shelf-life of $\gamma(^{18}O_4)$-ATP is comparable to normal ATP.

The ability to use $\gamma(^{18}O_4)$-ATP to monitor phosphorylation modification within various proteins was determined by performing in vitro kinase reactions in the presence of a 1:1 mixture of $\gamma(^{18}O_4)$-ATP and normal isotopic abundance ATP (ATP). After tryptic digestion, the peptides were analyzed using mass spectrometry (MS). Phosphorylated peptides are easily recognized within the MS spectrum owing to the presence of doublets separated by 6.01 Da, representing versions of the peptide modified by ATP and $\gamma(^{18}O_4)$-ATP. Standard peptides phosphorylated using $\gamma(^{18}O_4)$-ATP via in vitro kinase reactions showed no exchange loss of ^{18}O with ^{16}O. The identity of these doublets as phosphorylated peptides could be readily confirmed using tandem MS. This method provides the first direct stable-isotope labeling method to definitely detect phosphorylation sites within proteins.

For more information on this method, contact Dr. Veenstra at 301-846-7286 or veenstra@ncifcrf.gov.

Ninety Years and Still Learning

By Butch Hopkins

Can an old dog learn new tricks? Well, it seems so. With 90 years of combined experience and more than 2,000 completed projects under their belts, the Eukaryotic Expression Group (EEG) staff is still finding ways to improve and streamline current technologies while developing new ones.

For more than 20 years, the EEG has supported NCI's Drug Screening Program by characterizing the cell lines and maintaining the frozen cell stocks of the NCI-60, a panel of (now) 59 human tumor cell lines from all over the world. Over the years, more than 46,000 vials of cells have been prepared as a valuable resource to investigators in 30 countries. Most recently, the EEG has improved by fivefold recombinant protein expression in mammalian cells by adapting cells to grow at very high cell densities.

Left to right: Ronnie Roberts, Butch Hopkins, and Cammi Bittner of the EEG.

Since 1985, the EEG has been operating as a core service group supporting the research efforts of various government agencies. Once a component of the former Fermentation Production Facility (FPF), the EEG is now run by a group of three "veterans" as part of the Protein Expression Laboratory (PEL).

Group leader Butch Hopkins began his lab experience as a technician in 1970 at Flow Laboratories in Rockville, MD, while completing his undergraduate studies. Following graduation, he joined Litton Bionetics in Kensington, MD, as a contractor for NCI. He transferred to NCI-Frederick in 1976 and has been with the contract ever since.

Ronnie Roberts began working at NCI-Frederick in 1976, in the (then) Chemotherapy Fermentation Program, later the FPF. After a year, she left to finish her undergraduate degree and then returned to the FPF in 1978. A number of restructurings led to her current position as a senior research associate in the EEG.

Cammi Bittner began her career at NCI-Frederick in 1985 in the Cell Acquisition and Characterization Laboratory in support of the In Vitro Drug Screening Program of the Developmental Therapeutic Program (DTP). For the next 20 years, Ms. Bittner worked in various DTP support laboratories until coming to the EEG in 2006 as a senior research technician.

According to Mr. Hopkins, the EEG has evolved a great deal over the years; however, because the challenges of science do not let a sleeping dog lie, the "old dogs" will continue to learn new tricks. Fetch!

On Effective Communication

By Ken Michaels

Pointer of the month: Give special attention to the introduction.

I'll skip the cliché about first impressions and just mention that the very first words a speaker utters when taking the podium can be the most important words of the whole talk. That's because they set the tone and give the audience an idea of what's coming. And lots of people will decide right then and there how valuable the seminar is going to be.

Oral presentation advice is sometimes expressed as:

- Tell them what you're going to tell them.
- Tell them.
- Tell them what you told them.

This advice is not urging you to treat your audience like morons. It's reminding you that an oral presentation is enhanced in terms of ease of understanding by an effective introduction and an effective conclusion. It's surprising how often scientific presentations tend to skip both.

Bear in mind as you plan your next talk that somebody in the audience, or perhaps several somebodies, have no idea what you've been doing in your lab, or why you've been doing it. If you launch right into the talk, some peopvle may already be trying to catch up with you. And when a speaker and the audience are out of sync, understanding suffers. So take a few moments before getting into your work to set the stage with a few words of introduction; explain to your audience what you'll be taking about for the next 30 minutes or so, and why it's important. The first 60 seconds of your talk may well be the most important.

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