VANDERBILT
Institute of Chemical Biology
Quarterly Newsletter
January 2009

CORE NEWS: New Leadership for Chemical Synthesis

To solve important biological problems through chemistry requires the ability to synthesize novel molecules at will. The VICB Chemical Synthesis Core (CSC) has provided that capability to VICB researchers and the University at large since 2005. Now the CSC expects to achieve even greater proficiency and productivity under the leadership of its new Director, Alex Waterson. Alex joins the VICB after a successful career in Medicinal Chemistry at GlaxoSmithKline, where he participated in and led a number of program teams focused primarily on developing novel kinase- and nonkinase-directed anticancer agents. His experience includes target validation, hit identification, and lead optimization efforts to solve problems of drug potency, selectivity, distribution, and metabolism. He brings to the CSC his extensive expertise in focused library synthesis, singleton iterative synthesis, combinatorial library synthesis, large scale synthesis, HTS data mining, and molecular modeling-based drug design.

Alex Waterson

The CSC, originally established by VICB member Gary Sulikowski, was charged with the mission of providing organic synthesis, purification, and characterization services. When the VICB High-Throughput Screening Core joined the NIH Molecular Library Screening Centers Network (MLSCN) in 2005, the CSC became a key collaborator, providing target validation, hit identification, and lead optimization services for MLSCN projects. Craig Lindsley's move to Vanderbilt in 2006 brought his Technology Enabled Synthesis approach to the CSC, greatly increasing its productivity. During 2007 and 2008, the CSC has executed 72 projects for 30 different investigators. Projects have ranged from synthesizing a single known compound (often at great cost savings compared to commercial vendors) to full-scale medicinal chemistry support for drug/probe discovery programs.

As the mission of the CSC expanded, the need for dedicated, full-time leadership emerged. Padma Portonovo, who joined the CSC as Laboratory Manager in 2007 takes care of day-to-day operations. Now with the addition of Alex's expertise, the CSC is fully prepared to meet all of your chemical synthesis needs.

Padma Portonovo

For more information, go to http://www.vanderbilt.edu/syncore/ or contact Padma at padma.s.portonovo@vanderbilt.edu.

RESEARCH HIGHLIGHTS: What Does Phospholipase D Really Do?

Phospholipase D (PLD) is a deceptively simple, ubiquitous enzyme that removes the alcohol from the head group of phospholipids, primarily phosphatidylcholine, to yield phosphatidic acid (PA). Although it is easy to dismiss PLD as just another phospholipid metabolizing enzyme, numerous reports have suggested that it is important for a variety of cell signaling events, and aberrant PLD function has been implicated in a range of pathologic states, including neurodegenerative, inflammatory, cardiovascular, and malignant diseases. Often these conclusions have been drawn from experiments carried out using cells treated with short-chain alcohols that displace water at the PLD active site, leading to the formation of a phosphatidyl alchohol rather than PA. In such experiments, the production of PA is prevented, but PLD-dependent phospholipid hydrolysis itself is not blocked, and the potential nonspecific effects of the alcohol may lead to misinterpretations of the findings.

Alex Brown

Definitive research on the physiologic function of PLD has been stymied by the lack of highly potent and specific small molecule inhibitors, a problem that has now been solved through the combined efforts of VICB members Alex Brown and Craig Lindsley. In this highly productive collaboration, the leading PLD research team in the Brown lab set up the necessary activity screens to characterize isoform-selective inhibitors of both PLD1 and PLD2. The Lindsley lab applied their Technology Enabled Synthesis approach to generating the necessary compounds. Beginning with the literature report that halopemide has PLD inhibitory activity, the Lindsley lab started by dividing the structure of this lead compound into a scaffold, linker, and amide cap. They then created three alternate scaffolds based on the halopemide structure and generated a 263 compound library through combining the 3 scaffolds with 30 amide caps using the 3 different linkers.

Craig Lindsley

Extensive screening of the library and subsequent follow-up studies yielded both isoform-selective and dual inhibitors with potency in the nanomolar range and specificities (for isoform-selective compounds) of 20- to 40-fold. With these tools in hand, the Brown lab lost no time in addressing some of the key questions about the possible role of PLD in the pathophysiology of cancer. Collaborative experiments with Carlos Arteaga's lab (Vanderbilt Ingram Cancer Center) have revealed that the PLD inhibitors block migration of breast cancer cells. These results are just the beginning of what may be learned through these exciting new molecular probes of PLD activity.

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RESEARCH HIGHLIGHTS: A Revolutionary View of Molecular Interactions

Imagine a molecular detection technique that uses remarkably simple equipment to monitor the interaction of as few as 12,000 molecules, free in solution, with no need for labels or tags. Such a feat can be accomplished with backscatter interferometry (BSI), a technique Darryl Bornhop brought to Vanderbilt when he moved from Texas Tech in 2004. Having shown that BSI is amenable to multiple applications, including high-throughput screening and capillary-scale universal detection, the Bornhop lab is now poised to use BSI's remarkable combination of sensitivity, simplicity, economy, and diversity to revolutionize the study of biomolecular interactions.

Darryl Bornhop

The key to BSI is the poly(dimethyl-siloxane) microfluidic chip containing a Y-shaped micrometer-dimensional channel through which two reactant solutions can be rapidly introduced and mixed before entering a rectangular reaction chamber. When light from a fiber-coupled He-Ne laser beam (similar to the sources used for barcode scanning) passes through the solution in the chamber, the interaction of the laser beam with the chamber and its contents produces a reflected fan of scattered light. The pattern of this reflected light consists of a set of high contrast interference fringes that is collected by a high-resolution linear charge-coupled device (as found in a digital camera) and subjected to fast Fourier transform analysis. Simply stated, the pattern of light reflected back from the chip is dependent upon the solution in the channel. Changes in the state of the species in solution that cause even minute fluctuations in refractive index are captured as differences in the interference fringe pattern. The Fourier transform analysis software developed by the Bornhop lab enables the researchers to use the information contained in the light patterns to monitor the kinetics and extent of interactions between biological macromolecules (proteins, DNA, RNA, carbohydrates) and their binding partners.

Since publishing the use of BSI to monitor molecular interactions in *Science* [(2008) **317**:1732], the Bornhop lab has made exciting new discoveries. They have used BSI to study the interaction of the heat shock protein α-crystallin with its in vivo target βB1-crystallin and with mutants of T4 lysozyme having varying stability. The extensive kinetic and thermodynamic data (in press in *Analytical Chemistry*) allowed them to determine forward and reverse rate constants for two mode binding processes. The results demonstrated the expected correlation between the affinity of α-crystallin for the T4 lysozyme mutants and their degree of instability (free energy of unfolding). The studies also demonstrated abnormal binding kinetics for a mutated α-crystallin that is associated with autosomal dominant cataract.

Additional studies have shown the value of BSI to evaluate the interaction between proteins and small molecule ligands. Thus, the binding of carbonic anhydrase with known inhibitors was evaluated free in solution using single endpoint determinations, yielding binding affinities in good agreement with those previously reported. Another series of ongoing studies are evaluating the interactions between sugars and surface-bound lectins providing a means to study a range of simple and aggregate interactions. These initial studies provide just a glimpse into the power of BSI to explore molecular interactions. We predict a bright future for this promising new technology.

UPCOMING EVENTS

Seminars

The VICB Seminar Series continues into spring.
All of the following seminars are at 12:15 PM in room 1220 MRBIII.

Jan 21 .... Andrew Byrd, National Cancer Institute
Feb 11 .... Josh Rabinowitz, Princeton University
Feb 18 .... Jay Keasling, Univ. of California, Berkeley
Mar 04 .... Amos Smith, University of Pennsylvania
Mar 11 .... Jennifer Laurence, University of Kansas

For more information, please visit:
http://www.vanderbilt.edu/vicb/seminars2008-09.htm

Funding Opportunity

The National Center for Research Resources Shared Instrumentation Grants program offers an excellent opportunity to secure funding for equipment that costs more than $100,000.
The earliest date for grant submission is Feb. 23, and the deadline is March 23, 2009.

For more information, go to:
http//www.ncrr.nih.gov/biomedical_technology/shared_instruments