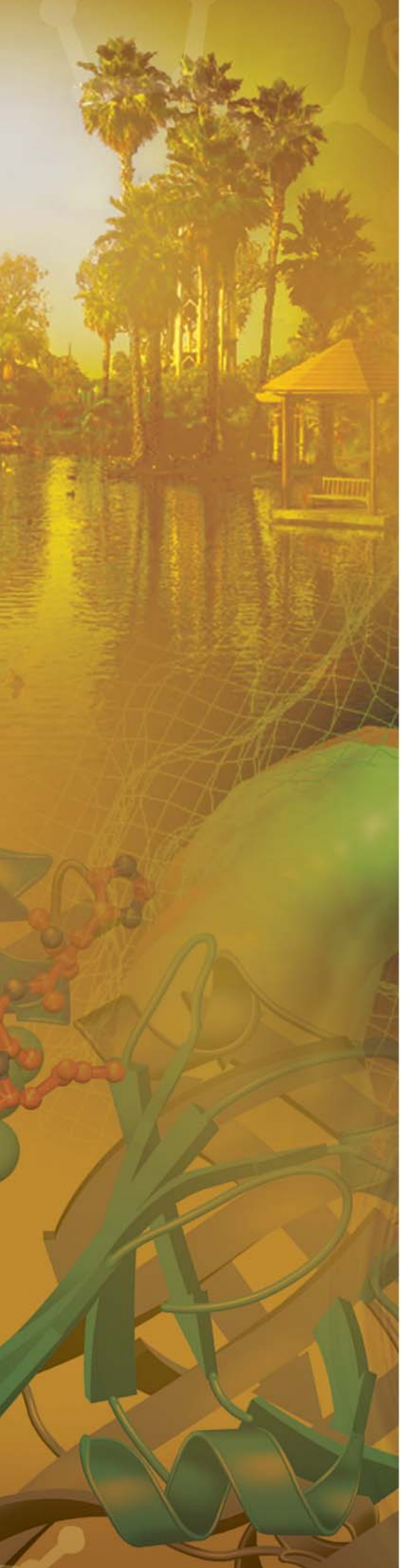

Fragment-based Lead Discovery Conference 2008

San Diego, CA February 18th-20th
Paradise Point Resort & Spa
fbld2008.com

Conference Sessions
Success Stories
Chemical Biology and Drug Discovery
Methods and Emerging Technologies
Lessons Learned

FBLD Technologies
Calorimetry
Nuclear Magnetic Resonance
Surface Plasmon Resonance
X-ray Crystallography

Plus: FBLD Methods & Implementation Workshop
February 17th (separate registration required)

FBLD
Fragment-based Lead Discovery Conference 2008

Premier Sponsors

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ASDI

NANOSYN Chemistry on Demand Accelerating Drug Discovery

Rigaku

MicroCal

GE Healthcare Biacore

Fragment-based Lead Discovery Conference 2008
Welcome!

We hope that you will enjoy the first Fragment-based Lead Discovery Conference, FBLD 2008. We designed the conference to be interdisciplinary and interactive, with an emphasis on high quality science and exhibitors that will provide you with the tools you need to implement FBLD. Thank you for participating!

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Registration: *fbld2008.com/register*

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Exhibit: *fbld2008.com/exhibit*

The Premier Sponsors are Exhibiting and Sponsoring the FBLD 2008 Methods & Implementation Workshop

Conference Overview

Pre-conference Workshop and Registration

Sunday, February 17th

9:00 am-5:00 pm Bay View Room
FBLD Methods & Implementation Workshop
(Separate Registration Required)

2:00-7:00 pm Sunset Foyer
Registration

Conference Agenda

Monday, February 18th

7:00-10:00 am Sunset Foyer
Registration

7:00 am Sunset Ballroom III
Breakfast

8:00 am Sunset Ballroom I-II
Opening Plenary Presentation: *Discovery of Bcl-2 Family Inhibitors for the Treatment of Cancer*—Stephen Fesik, Ph.D.

9:00 am Sunset Ballroom I-II
Morning Session: *Success Stories*—Martin Drysdale, Ph.D., and Daniel Erlanson, Ph.D.

10:30 am Sunset Ballroom III-V
Break, Exhibits

11:00 am Sunset Ballroom I-II
Morning Session, continued: *Success Stories*—Martin Drysdale, Ph.D., and Daniel Erlanson, Ph.D.

12:30-1:30 pm Paradise Lawn (Weather Permitting)
Lunch

1:30-3:00 pm Sunset Ballroom III-V, Bay View Room
Posters, Exhibits, FBLD Scavenger Hunt

3:00-4:00 pm
Free Time

4:00-7:00 pm Sunset Ballroom I-II
Afternoon/Evening Session: *Chemical Biology and Drug Discovery*—Duncan McRee, Ph.D., and Maurizio Pellechia, Ph.D.

7:00-9:00 pm Sunset Ballroom III-V, Bay View Room
Buffet Dinner sponsored by Rigaku, Posters, and Exhibits

Tuesday, February 19th

7:00 am Sunset Ballroom III
Breakfast

8:00 am Sunset Ballroom I-II
Morning Session: *Methods & Emerging Technologies, Part I*—Wolfgang Jahnke, Ph.D., and Vicki Nienaber, Ph.D.

10:10 am Sunset Ballroom III-V
Break, Exhibits

10:40 am Sunset Ballroom I-II
Morning Session, Continued: *Methods & Emerging Technologies, Part I*—Wolfgang Jahnke, Ph.D., and Vicki Nienaber, Ph.D.

12:00-1:00 pm Paradise Lawn (Weather Permitting)
Lunch

1:00-3:00 pm Sunset Ballroom III-V, Bay View Room
Posters, Exhibits, FBLD Scavenger Hunt

3:00 pm Sunset Ballroom I-II
Afternoon Session: *Methods & Emerging Technologies, Part II*—Wolfgang Jahnke, Ph.D., and Vicki Nienaber, Ph.D.

5:00 pm Sunset Ballroom III-V
Break, Exhibits

5:30 pm Sunset Ballroom I-II
Scientific Advisory Board Round Table
—Glyn Williams, Ph.D., Chair

7:00-9:00 pm Paradise Cove (Weather Permitting)
Dinner Reception sponsored by ActiveSight, FBLD Scavenger Hunt and Accelrys Poster Award Winners Announced

Wednesday, February 20th

7:00 am Sunset Ballroom III
Breakfast

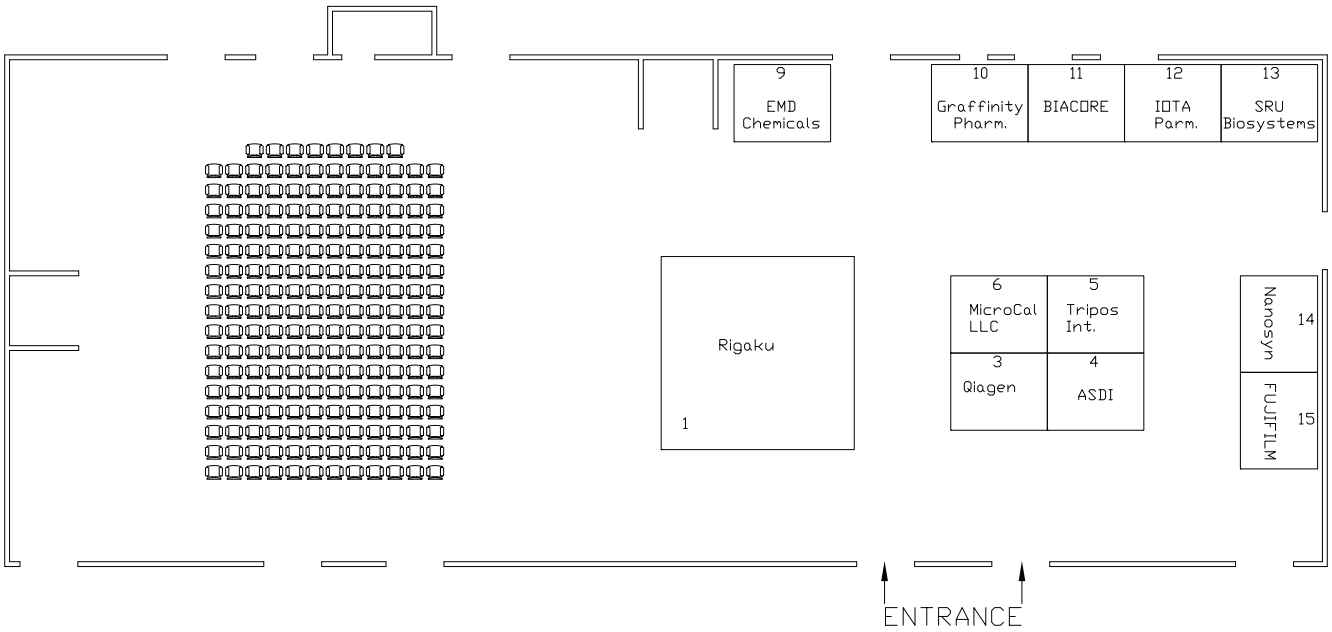
8:00 am Sunset Ballroom I-II
Morning Session: *Lessons Learned*—Roderick Hubbard, Ph.D., and Harren Jhoti, Ph.D.

11:35 pm Sunset Ballroom I-II
Closing Plenary Presentation: *One Discovery Shoe Size Paradigm Does Not Fit All and Could Well Leave Us Barefoot*
—Christopher Lipinski

12:30 pm
Close of Conference

see full conference program on page 12

Conference Floor Plan



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SRU Biosystems ..... Exhibitor (Booth 13)
Tripos International ..... Exhibitor (Booth 5)
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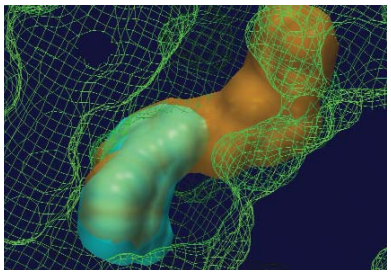
Exhibitor/Sponsor Resources and FBLD 2008 Activities



Implementation of Fragment-based Lead Discovery depends on high quality, specialized products and services. The Exhibitors and Sponsors of FBLD 2008 represent the best companies to find products, services, and collaborations for FBLD Research.

Sponsor Accelrys

Accelrys develops and commercializes Scientific Business Intelligence software for the integration, mining, analysis, modeling and simulation, management and interactive reporting of scientific data. Our solutions are used by biologists, chemists, materials scientists, and information technology professionals for product design as well as drug discovery and development. Our technology and services are designed to meet the needs of today's leading research and development organizations.

Premier Sponsor/Organizer ActiveSight

ActiveSight uses an integrated Fragment-based Lead Discovery (FBLD) platform, LENS™, to develop tightly binding lead compounds quickly. LENS, or Lead Evolution via Novel Synergies, utilizes ActiveSight's expertise in structural biology, advanced binding detection methods, cutting edge diagnostics, automated software, and medicinal chemistry to leverage the power of FBLD efficiently and effectively.

ActiveSight's FBLD Offerings

ActiveSight offers its partners an integrated and modular approach to Fragment-based Lead Discovery (FBLD), working together to generate tightly-binding lead compounds that are more likely to succeed in the clinic. By leveraging ActiveSight's LENS Technology, Partners can take advantage of cutting-edge FBLD technologies without the capital expenditure normally required. In addition, ActiveSight FBLD collaborations can be used as a proof of concept for obtaining the budget to fund future in-house FBLD equipment and programs.

ActiveSight's FBLD 2008 Activities

- **Tuesday, Feb 19th 1:00-2:00 pm**
  A demonstration of fragment screening protocols used at ActiveSight

Premier Sponsor ASDI Inc.

ASDI Inc., established in 1988, is a premier provider of research-enabling technologies, services, and products. ASDI has considerable synthetic chemistry expertise in

providing intermediate scale custom synthesis, hit-to-lead expansion, and lead generation libraries. Product lines include HTS, building block, and fragment screening collections. ASDI also offers complete bioanalytical and ADME/Tox services in support of early drug discovery. Our ability to provide analytical and materials management services enables acceleration of the research process.

ASDI's FBLD Offerings

- **Fragment Collections**
  In-house fragment libraries designed to maximize chemical space
- **Fragment Procurement**
  Sourcing of third party collections
- **Fragment Reformatting**
  Sample preparation for screening
- **Inventory Management**
  Full services supporting storage, preparation, and distribution of fragments

Exhibitor EMD Chemicals, Inc.

EMD, comprising of Calbiochem, Novabiochem, and Novagen brands, is engaged in the development, manufacture, marketing, and distribution of a broad array of research reagents used in life science research. The products these brands provide include tools for peptide synthesis, Proteomics and disease-related research.

Exhibitor FUJIFILM Medical Systems USA, Inc.

FUJIFILM Medical Systems USA, Inc. is a leading provider of medical and life science image, is a wholly owned U.S. subsidiary of FUJIFILM Corporation (FUJIFILM), Tokyo. FUJIFILM will use leading-edge, proprietary technologies to provide top-quality products and services that contribute to the advancement of culture, science, technology and industry, as well as improved health and environmental protection in society. Our overarching aim is to help enhance the quality of life of people worldwide.

Fujifilm's FBLD Offerings

- **AP-3000**
  Fully Automated, Label-Free Drug Screening System

Premier Sponsor GE Healthcare Biacore

Biacore™ (now part of GE Healthcare) systems for label-free interaction analysis generate unique data on molecular interactions, giving insights into biological functions. Applications include drug discovery, life science research, antibody characterization, immunogenicity, biotherapeutic development and manufacturing.

Exhibitor Graffinity Pharmaceuticals

Graffinity Pharmaceuticals is a leader in the field of fragment based drug discovery. The Graffinity fragment screening platform combines chemical microarrays with a proprietary method for the standardized, label-free detection of compound-protein interactions via SPR imaging. The company's rapid and scalable drug discovery technology explores a rich chemical universe to identify drug fragments which address challenging drug targets. With its 110,000-compound library that contains 23,000 true fragments, Graffinity possesses one of the most diverse fragment libraries.

Graffinity's FBLD 2008 Activities

- **Monday, Feb 18th 1:45 pm**
  Graffinity's SPR-based high throughput fragment screening Presentation: An introduction to Graffinity's small molecule fragment screening technology.
- **Monday, Feb 18th 2:30 pm**
  Graffinity drug discovery case studies Presentation: Case studies highlighting Graffinity's capabilities in screening protein-protein interaction targets, identifying allosteric binders as well as highly selective hits.
- **Tuesday, Feb 19th 1:15 pm**
  Graffinity's SPR-based high throughput fragment screening Presentation: An introduction to Graffinity's small molecule fragment screening technology.
- **Tuesday, Feb 19th 2:00 pm**
  Graffinity drug discovery case studies Presentation: Case studies highlighting Graffinity's capabilities in screening protein-protein interaction targets, identifying allosteric binders as well as highly selective hits.

Exhibitor IOTA Pharmaceuticals and Vitas-M Laboratories

IOTA Pharmaceuticals and Vitas-M Laboratories are collaborating to provide a complete FBLD tool kit for our customers, including project-based design of FBLD campaigns, provision of target-focused FBLD libraries, and the execution of FBLD screening and lead optimization programs.

The extensive medicinal chemistry and drug screening capabilities of the two companies, coupled to dedicated, proprietary FBLD resources, enable our customers to progress drug discovery programs to valuable "lead" endpoints in a reliable, cost-effective and efficient manner. Worked examples of such programs will be given at the conference.

IOTA/Vitas FBLD Offerings

- **Fragment Screening Collections**
  Over 5500 proprietary chemical fragments, optimized for screening, available today to IOTA-Vitas customers.
- **Fragment Screening**
  Fragment screening on validated biochemical plat forms available to IOTA-Vitas customers.

Premier Sponsor MicroCal

MicroCal is the leading developer of ultrasensitive calorimeters for the life sciences. For over thirty years, MicroCal Isothermal Titration Calorimeters and Differential Scanning Calorimeters have been used to characterize biomolecular interactions and structure. Microcalorimetry complements structural data and provides a sensitive, direct mode for determining binding affinities. The technique directly measures the heat of interaction, which is common to all binding processes. This removes the need for immobilization, labeling or specific assay design improving the biological relevance of data.

MicroCal's FBLD Offerings

- **VP-DSC**
  Differential Scanning Calorimeter
- **CAPDSC**
  Capillary Differential Scanning Calorimeter
- **ITC 200**
  Isothermal Titration Calorimetry
- **Auto-ITC 200**
  Automated Isothermal Titration Calorimetry

Premier Sponsor Nanosyn

Nanosyn is a state-of-the-art chemistry contract research organization (CRO), providing a wide array of rapid and high quality chemistry products and services for the biotech and pharmaceutical industries worldwide. Products and services include: Medicinal Chemistry, Analytical Chemistry, Small Molecule X-ray, Collaborative Projects and Research, "Plug & Play" Screening Libraries and Proprietary Library Development.

In business since 1998, Nanosyn is conveniently located in the Biotech Hotbed communities of San Francisco and San Diego, California.

Nanosyn's FBLD Offerings

- **Chemistry On Demand™**
  Medicinal chemistry services to support fragment based lead discovery.
- **PureQuality™**
  High throughput LC/MS purification, compound analysis, quantitiation in screening buffer, and formatting. PureQuality™ allows for rapid quality enhancement of your current fragment library or to support the production of new proprietary fragment libraries designed to maximize chemical space.

Nanosyn's FBLD 2008 Activities

- **Monday, Feb 18th 8:00 pm**
  "Chemistry on Demand" Approach to Support Fragment Based Lead Discovery. This presentation will describe Nanosyn's highly efficient technological processes based on heavy use of modern analytical and chemistry instrumentation. Case Studies will be presented.
- **Tuesday, Feb 19th 2:00 pm**
  Nanosyn FBLD Case Studies. This presentation will describe case studies highlighting Nanosyn's medicinal chemistry capabilities allowing for the rapid and cost efficient support of fragment based lead discovery.

Exhibitor QIAGEN

QIAGEN offers the world's largest choice of over 2000 proven crystallization conditions, including JSCG Core Suites - 384 conditions giving the highest hit rates from over 500,000 high-throughput crystallization experiments at the Joint Center for Structural Genomics. Every condition is available as a large-volume Refill Hit, with certificated production reports ensuring reproducible and reliable crystallization for fragment-based or combinatorial library screening - applications further facilitated by the new EasyXtal 15-well Tool, whose SBS footprint makes it ideally suited for automatic visualization systems.

Qiagen's FBLD Offerings

- **EasyXtal 15-well Tool X-seal (20)**
  20 empty 15-well plates with X-Seal crystallization supports.
- **JCSG Core Suite I**
  Pierceable deep-well block containing 96 x 1.5 ml JCSH Core Suite I solutions, piercing tool, adhesive foil.
- **JCSG Core Suite II**
  Pierceable deep-well block containing 96 x 1.5 ml JCSH Core Suite II solutions, piercing tool, adhesive foil.
- **JCSG Core Suite III**
  Pierceable deep-well block containing 96 x 1.5 ml JCSH Core Suite III solutions, piercing tool, adhesive foil.
- **JCSG Core Suite IV**
  Pierceable deep-well block containing 96 x 1.5 ml JCSH Core Suite IV solutions, piercing tool, adhesive foil.

Premier Sponsor Rigaku

Rigaku is the world's leading provider of single crystal X-ray crystallographic hardware, software and contract services. We offer fully integrated macromolecular X-ray diffraction systems and modular protein crystallization systems for the automatic preparation of crystallization trials. The Rigaku product line includes imaging plates and CCD detectors, microfocus X-ray generators, X-ray optics, protein crystallization automation and imaging solutions, cryo-cooling devices, structure solution software and related peripherals.

Rigaku's FBLD Offerings

- **ACTOR™**
  The world's first commercial robotic system for automated crystal sample handling. Eliminates much of the physical handling of protein samples required during crystal screening and data collection.
- **CrystalMation™**
  A fully integrated, configurable platform for protein crystallization, automating every step from custom screen making to crystallization trial imaging and analysis. Can be configured to meet a wide range of requirements and grow with your needs.
- **MIFit+**
  Fully automated processing and structure solution tools that are ideal for fragment screening.
- **MtGUI**
  Bridges the information gap between crystallization and data collection and processing and includes full support for the new Rigaku RFID pins.
- **Ultimate HomeLab™**
  Enables X-ray data collection on the widest range of protein samples, including crystals that diffract too weakly for standard home sources. Features state-of-the-art microfocus X-ray generator technology, Osmic™ optics, Rigaku imaging plate and CCD detectors, and an ACTOR crystal mounting robot to maximize productivity.

Rigaku's FBLD 2008 Activities

- **Monday, Feb 18th 1:30-3:00 pm, 7:00-9:00 pm**
- **Tuesday, Feb 19th 10:10-10:40 am, 1:00-3:00 pm**

  MtGUI
  Learn about MtGUI, new software for tracking crystal samples from the drop to data collection. This demonstration will also include the use of Rigaku RFID pins.

  ACTOR
  A demonstration of automated sample mounting hardware as well as software for scheduling, screening samples, data collection and processing. New developments for the ACTOR system and software will be presented.

Exhibitor SRU Biosystems

BIND© label free technology, from SRU Biosystems, represents a fundamental advance in the ability to monitor biological interactions by providing higher quality assay data with improved throughput and associated cost savings. The BIND© System can rapidly analyze the interactions of cells, proteins, genomic, peptide, antibody or small molecule compound libraries against a wide range of biochemical targets.

SRU Biosystems' FBLD 2008 Offerings

- **BIND Reader**
  High throughput, microplate based assay system that enables label-free detection of drug-target interactions

Exhibitor Tripos International

Tripos International helps researchers in the pharmaceutical industry accelerate the identification and optimization of new compounds that have the potential to become drug products. Most new drugs start as a medicinal chemist's really good idea. AllChem, a new technology from Tripos, helps medicinal chemists find good new chemistry ideas that embody the best chances for the desired biological profile; structural novelty; a complete synthetic route; "drug-like-ness"; and hassle-free access.

Tripos' FBLD Offerings

- **AllChem**
  Most new drugs start as a medicinal chemist's really good idea. AllChem, a new technology from Tripos, helps medicinal chemists find good new chemistry ideas that embody the best chances for the desired biological profile; structural novelty; a complete synthetic route; "drug-like-ness"; and hassle-free access.

Tripos' FBLD 2008 Activities

AllChem demonstration - learn how this new technology can help generate really good new chemistry ideas most likely to become leads. Submit your own structural queries and see the power of this exciting new technology!

- **Monday, Feb 18th 1:30-3:00 pm, 8:00-9:00 pm**
- **Tuesday, Feb 19th 1:00-3:00 pm**

Sponsor ZoBio

ZoBio was founded in 2004 to make the Target Immobilized NMR Screening (TINS) technology available to the pharmaceutical and biotech world. ZoBio makes its TINS technology available primarily through fee-for-service

offerings that with no restrictions on IP or royalties. A limited number of licensing opportunities are also available. ZoBio focuses exclusively on customer projects.

ZoBio has developed an innovative fragment library that is rule of three compliant (Siegal *et al, Drug Discovery Today*, 2007, 12, p.1032). The library is composed of commercially available compounds and is designed so that multiple analogs of each scaffold are readily available for follow-on studies. Screening of the library using TINS has been successfully applied to a number of challenging targets including kinases, small GTPases, molecular chaperones, viral enzymes, and membrane proteins. In all cases we have observed high rates of hit validation in biochemical assays and excellent success in crystallization studies.

ZoBio's FBLD Offerings

- **Fragment Screening**
  TINS screening of our innovative fragment collection using less than 5 mg of protein. TINS is a highly sensitive yet robust technology that typically enables "1st" site and "2nd site" discovery in a single screen. TINS screens are usually offered in conjunction with follow-on competition binding studies to determine ligand binding sites. Projects are typically completed in 1-2 months.
- **Analoging**
  Follow up activity to develop SAR for hit scaffolds. Commercially available analogs are purchased and binding quantitated and characterized using TINS or other applicable methods.
- **Hit Characterization**
  ZoBio has exclusive access to a solid state technology for biochemical characterization of kinases (S/T/Y) and nuclear hormone receptors. The technology can be used for rapid biochemical evaluation of TINS hits for e.g. potency, mode of action studies or profiling.
- **Structure Determination of Target-Fragment Complexes**
  When X-ray crystallography is not applicable ZoBio can uses its expertise in NMR spectroscopy and computation to determine structures of fragments bound to the target. On the one hand rapid, low cost methods, such as chemical shift perturbation mapping; selective NOEs and paramagnetic relaxation enhancement, can be carried out to accurately map the ligand binding site to low resolution. On the other hand, full high resolution structure determination of target-fragment complexes is available.

ZoBio's FBLD 2008 Activities

- **Sunday, Feb 17th 1:00-1:50 pm**
  Workshop Presentation: "The Use of NMR for Hit Detection and Evolution in FBLD"

FBLD Methods & Implementation Workshop

Workshop Schedule

Additional Registration Required
Sign up at: *fbld2008.com/workshop*

9:00 am
Coffee, Pastries, Introductions

9:20 am
Introduction: Case Studies of FBLD Implementation

Roderick Hubbard
Professor, *University of York and Senior Fellow, Vernalis*

This introduction will emphasize how fragment based methods have been implemented within various organizations and integrated with medicinal chemistry for drug discovery. I will begin with a brief overview of the development of the methods over the past ten years, including the central concepts of what fragments are and why they provide a new paradigm for drug discovery against some targets. I will then summarize the main methods for detecting fragment binding and the different approaches used for design of fragment libraries. I will conclude with an overview and examples of the different approaches to fragment evolution. The talk will provide a general introduction to the more detailed treatment of the methods by the rest of the speakers at the workshop.

9:50 am
An Introduction to Biacore Technology for Protein: Small Molecule Interactions

Hans Muller-Kahle
Strategic Market Development, Director, *Biacore–GE Healthcare, Life Sciences Division*

10:00 am
Fragment-Based Discovery Using Optical Biosensors

Tony Giannetti
Ph.D., Research Scientist II, *Roche, Palo Alto*

Advances in surface plasmon resonance (SPR) biosensor technique have allowed applications of the technology, such as that implemented with Biacores, to extend beyond the protein-protein applications into studying small molecule/protein interactions. By combining the latest methods in biosensor operation with the standard methodologies of high throughput screening, we have developed a high throughput procedure for hit identification from fragment libraries for entry into lead generation chemistry. Key features are the ability to screen and verify thousands of compounds in a short time, large

dynamic range of the assay (100 pM to 5 mM) and the low amounts of protein required to complete a fragment screening campaign (<0.5 mg protein from assay development through hit validation). Details from assay to design, to how to convince a chemist to work with fragments, will be discussed.

10:50 am
Break

11:00 am
Lets Stick Together - Thermodynamic Measures of Fragment Binding

Glyn Williams
Ph.D., Director of Biophysics, *Astex Therapeutics*

The interaction of a fragment with a protein has a number of thermodynamic consequences. One is a change in the total free energy of the system, which may also be accompanied by a measurable change in enthalpy (heat). Another is a change in the free energy difference between the native conformation(s) of the protein and its unfolded form(s) which is reflected in a change in the temperature at which the protein unfolds. Both can be used to determine or rank the strength of a protein-fragment interaction. The basis of the methods will be outlined and the relative advantages of both, in the context of Fragment-based Lead Discovery and lead optimization, will be discussed.

11:30 am
The Heat Is On - Accuracy vs. Speed in ITC and DSC

Ronan O'Brien
Ph.D., Applications Research and Development Manager, *MicroCal, LLC*

With the latest developments in ITC, the iTC_{200}, it is now possible to measure the affinities of even weakly interacting fragments with as little as 5 µg of protein. Binding data for a range of ligands and affinities will be presented to demonstrate the wide applicability of this technology. These instruments will be supported by automation that will allow up to 50 measurements a day making high quality affinity data readily available for FBLD platforms.

ITC is a universally applicable method for the determination of accurate affinities. It also provides complementary information to the structural data which is central to most FBLD campaigns. The technique directly measures the heat of interaction, which is common to all binding processes. This removes the need for immobilization, labeling or specific assay design.

Thermal stability by DSC is also becoming increasingly employed in rapid affinity screening. The advantages and limitations of stability shifting will also be discussed.

12:00 pm
Lunch (Provided)

1:00 pm
The Use of NMR for Hit Detection and Evolution in FBLD

Gregg Siegal
Ph.D., Chief Scientific Officer, *ZoBio*

Screening fragment libraries requires tools capable of reliably detecting very weak binding of the compounds to the target. NMR has proven itself a powerful analytical tool for this purpose. In this workshop we will review the use of NMR for finding ligands in fragment libraries, including both target and compound based approaches. We will also discuss the advantages and disadvantages of each and assess the requirements that the various methods place on the library. Finally, we will look at the impact of NMR on the hit evolution stage of FBLD evaluating the potential of low resolution structural methods such as chemical shift perturbation guided docking and paramagnetic NMR constraints.

1:50 pm
Fragment Screening Using High-throughput X-ray Crystallography

Robin Rosenfeld
Principal Scientist, *ActiveSight*

Implementing a Fragment-based Lead Discovery (FBLD) program using X-ray crystallography as an initial screen has many benefits, including the ability to immediately optimize fragment hits based on structural information. Facile fragment screening using X-ray crystallography has been made possible through advancements in instrumentation and software which allow data to be collected, processed and analyzed quickly, often generating up to 20 structures per day. Methods allow for growing, soaking, and mounting 100+ crystals. At ActiveSight, we have successfully screened several human drug targets and have developed techniques that will be of general use to crystallographers at any level who are interested in starting an X-ray crystallography-based FBLD project.

2:40 pm
Practical Design of a Fragment Library for Crystallographic Screening

John Badger
Ph.D., Director, Structural Biology, *ActiveSight*

Both theoretical considerations and practical experience have demonstrated that the chemical space for ligand binding can be covered with ~1000 fragment compounds provided that these molecules are sufficiently small, simple and selected for diversity. Regardless of whether screening library compounds are chosen from an internal collection or

obtained from commercial suppliers, the developer of the library must find a practical system for selecting a relatively small number of compounds from a large collection. Beyond the issue of library composition, a practical screening library must also be formatted for use in crystallographic analysis and be supported by convenient access to chemical information. This talk will describe the approaches used to establish fragment screening libraries at ActiveSight and their implementation for practical protein crystallography. Our successful use of these libraries in crystallographic fragment screening projects has been demonstrated by the identification of chemically diverse hits against multiple classes of protein molecule.

3:10 pm
Break

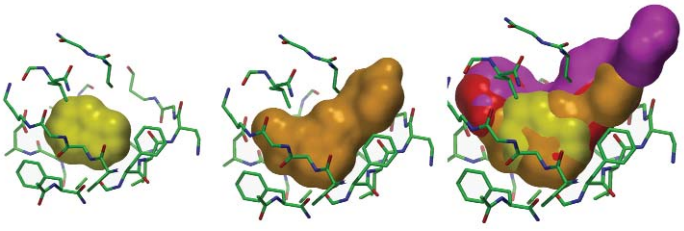
3:20 pm
Fragment Library Creation and Materials Management

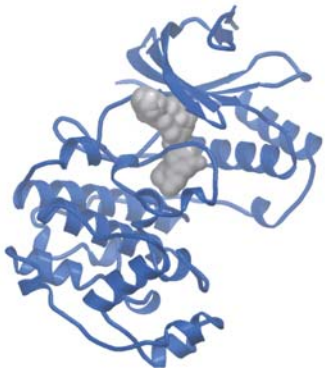
Rick Hammar, Director, *Compound Management Services*, *ASDI Group of Companies*, **Michael J. Kates**, Ph.D. Director of Custom & High Throughput Chemistry, *ASDI Group of Companies*, **Brian Maduskuie**, Director, Customer Service, *ASDI Group of Companies*

A fragment-based discovery program requires a robust infrastructure to enable successful implementation, productive investigation, and viable results. ASDI will discuss options around fragment library identification, creation, analysis, distribution, and screening. Reference will be made to case studies and proven successful practices supporting increased productivity, lead identification, and cost-saving initiatives.

4:10 pm
"Chemistry on Demand" Approach to Support Fragment Based Lead Discovery

Olga Issakova
Ph.D., Executive Vice President, *Nanosyn*





Fragment-based Lead Discovery Conference 2008 Conference Program

Pre-conference Workshop

Sunday, February 17th

9:00 am-5:00 pm
FBLD Methods & Implementation Workshop

Conference Agenda

Monday, February 18th

7:00-10:00 am
Registration

7:00 am
Breakfast

8:00 am
Opening Plenary Presentation: Discovery of Bcl-2 Family Inhibitors for the Treatment of Cancer

Stephen Fesik
Ph.D., Divisional Vice President, Cancer Research,
Abbott Laboratories

9:00 am
Morning Session: Success Stories

Session Chairs: Martin Drysdale, Ph.D., Director of Chemistry and Structural Science, *Vernalis* and Dan Erlanson, Associate Director Medicinal Chemistry, *Sunesis*

Session Intro

Dan Erlanson
Associate Director Medicinal Chemistry, *Sunesis*

9:10 am
Integrating Fragments into Structure Based Medicinal Chemistry–Discovery and Development of Hsp90 Inhibitors for the Treatment of Cancer

Martin Drysdale
Ph.D., Director of Chemistry and Structural Science, *Vernalis*

The use of weak binding "fragments" of molecules is now recognized as an efficient and robust method of hit identification in the drug discovery process. The use and integration of fragment hits into successful lead optimization is the critical determinant of whether this technology will become accepted as a significant tool in drug discovery. We have developed a collection of methods for fragment based drug discovery which we call SeeDs (Structural exploitation of experimental Drug startpoints) which have become integral in our structure based drug discovery efforts. Heat shock protein (Hsp) 90 is a molecular chaperone that is responsible for the correct folding of a large number of proteins allowing them to achieve their functional conformation. Client proteins of Hsp90 include many key overexpressed or mutated oncogenes which are known to be critical for the transformed phenotype observed in tumors. 17-AAG and 17-DMAG are Hsp90 inhibitors derived from the prototypical ansamycin natural product inhibitor geldanamycin, which have shown pre-clinical efficacy in mouse xenograft models, and are now in phase I and II clinical trials. I will discuss our experience in developing and applying the SeeDs technology to target Hsp90, where optimization has been fundamentally informed by the fragments uncovered in our hit identification phase and has led to the identification of clinical and pre-clinical development candidates.

9:50 am
Fragment-to-Clinic: Astex Experience

David Rees
Ph.D., Vice President of Medicinal Chemistry,
Astex Therapeutics

This presentation will outline examples of fragment-based projects that have led to candidates being selected for preclinical development within oncology. For example AT7519 and AT9283 have been progressed into clinical trials in patients.

10:30 am
Break, Exhibits

11:00 am
Successes and Surprises with Fragments

Wolfgang Jahnke
Ph.D., Senior Research Investigator,
Novartis Institutes for BioMedical Research

Fragment-based screening (FBS) is a lead finding technology that is complementary to high-throughput screening (HTS), but leads from FBS often comprise more information than leads from HTS. By probing the protein surface with different fragments, hot spots on the protein surface are identified, and preferential types of interactions are determined separately for each sub-site. In addition, allosteric pockets can be identified which allow the design of novel types of inhibitors. This talk will summarize some of our experiences in fragment-based screening using NMR spectroscopy and X-ray crystallography.

11:40 am
From a Scaffold to the Clinic

Rick Artis
Ph.D., Vice President, Lead Generation, *Plexxikon Inc.*

Plexxikon's Scaffold-based Drug Discovery approach has yielded two clinical programs to date, in diabetes (Phase 2) and oncology (Phase 1). In each case, the time from initiation to first-in-human study was less than two years. In part, success of these efforts are derived from the ability to pick starting points with tractable enabling chemistry in the structural context of a given binding site. With this structural analysis in place, the subsequent design strategy focused on generating compact molecules with high atomic economy. The quality of compounds generated from this process has also allowed for early pharmacokinetic screening and resulted in generally favorable properties when compounds are introduced to *in vivo* profiling. Examples from clinical programs and some early discovery efforts will be discussed.

12:30-1:30 pm
Lunch

1:30-3:00 pm
Posters, Exhibits, FBLD Scavenger Hunt

3:00-4:00 pm
Free Time

Afternoon/Evening Session: Chemical Biology and Drug Discovery

Session Chairs: Duncan McRee, Ph.D., President, *ActiveSight®* and Maurizio Pellechia Ph.D., Professor, *Burnham Institute for Medical Research*

4:00 pm
Session Introduction

Duncan McRee
Ph.D., President, *ActiveSight*

4:10 pm
Fragment-based Approaches to Targeting Enzymes from Parasitic Organisms

Gabriele Varani
Ph.D., Professor, *University of Washington*

Parasitic diseases cause large number of deaths and morbidity in developing countries. Current treatment

options are missing altogether for many parasitic diseases, while long standing chemotherapeutic agents are either too expensive or the parasite population has become resistant to treatment. Fragment-based ligand screening and development provides an ideal strategy for working within these therapeutic conditions. In cases where no inhibitor is known, fragment-based screening provides an entry point for new chemical entities to be discovered. When homologous enzymes have been well-characterized and previously targeted by traditional medicinal chemistry, a structure-based approach can help to isolate chemical moieties that are critical for inhibition, while exploring rational alternatives. Even for enzymes that have previously been targeted, such an approach can yield novel scaffolds with more favorable characteristics, in regards to cost and resistance mechanisms, that can generate new classes of inhibitors. We are applying these methods to critical enzymes from *Vibrio cholerae, Trypanosoma brucei*, and *Plasmodium falciparum*, as well as a series of homologous human targets, and will report on the results of the application of this approach to this area of pharmacology.

4:40 pm
NMR Spectroscopy in Hit Identification and Optimization Process and Reverse Chemical Genetics

Maurizio Pellecchia
Ph.D., Professor, *Burnham Institute for Medical Research*

Recently we reported on an NMR-based approach, named SAR by ILOEs (structure activity relationships by interligand nuclear Overhauser effect), that makes use of protein mediated ligand-ligand NOEs (ILOEs) in complex mixtures to identify initial weak hits that are converted by synthetic chemistry approaches into bi-dentate compounds with higher affinity. In addition, we also reported on combining this approach with pharmacophore based searches of possible linked molecules from large data bases of commercially available compounds (Pharmacophore by ILOEs). Combined with functional studies using the resulting ligands, these methods represent ideal approaches to hit identification and to reverse chemical-genetics studies. Reverse chemical-genetics entails selecting a protein of interest, screening for a ligand for the protein, and finally determine the eventual phenotypic alterations that the ligand induces in a cellular context. Likewise, these methods enable the identification of protein's hot spots by using small molecules, regardless of the knowledge of the function of the protein, and the development of a specific assay. Subsequently, such small organic molecules can be used in cellular assays to investigate the possible role of the target. In particular, the approaches were applied to the identification of the first inhibitor of the pro-apoptotic protein Bid and to find highly selective protein kinase inhibitors . We also will report on the use of paramagnetic probes for the design of potent and selective bi-dentate compounds against kinases and phosphatases.

5:10 pm
Break

5:20 pm
BMSC Fragment Cocktails: Development and Experience in MSGPP

Christophe Verlinde
Ph.D., Associate Professor, *University of Washington*

From a collection of nearly 700 small compounds, called fragments, carefully selected from the MDL ACD database, we created 68 cocktails of 10 compounds that are shape-wise diverse. We have explored the utility of these cocktails for initiating lead discovery in structure-based drug design by soaking numerous protein crystals obtained by the MSGPP (Medical Structural Genomics of Pathogenic Protozoa) consortium. We will report on the fragment selection and cocktail design procedures, and give examples of the successes obtained. The BMSC Fragment Cocktail recipes are available free of charge upon request under the provisions of a university agreement, visit *faculty.washington.edu/verlinde* for more information.

5:50 pm
Dynamics in Drug Design: Application of Freeze-Frame Click Chemistry in the Design of Acetylcholinesterase and Nicotinic Receptor Selective Ligands

Palmer Taylor
Ph.D., Professor, *University of California, San Diego*

The recent elucidation of the crystal structures of the acetylcholine binding protein (AChBP), a surrogate for the extracellular domain of the nicotinic acetylcholine receptor, have provided critical templates for analyzing interaction of ligands with the extracellular domain of the receptor molecule. Since multiple binding proteins with distinct, but homologous, sequences exist and templates resembling the receptor can be constructed through mutagenesis, considerable flexibility is accorded to developing templates. Moreover, the sites of potential interaction extend beyond the classical agonist-antagonist site to non-competitive sites at the non-α-subunit interfaces and sites within the extracellular channel vestibule. AChBP, being a soluble protein, also provides a structure for examining solution dynamics and possible changes in conformation. We have employed H/D exchange and various steady-state and relaxation spectroscopic methods to examine conformation and segmental motion of domains of the molecule. Solution dynamics become important, since ligand binding is associated with conformational changes in the C loop, and some of these changes correlate with agonist-antagonist behavior of the ligands. Using AChBP as a template, we have employed freeze-frame, click chemistry for the *in situ*

synthesis of novel ligands that we hope will confer selectivity with respect to receptor subtype and unique agonist-antagonist behaviors. A large series of lead compounds generated by conventional synthesis and through the click-chemistry approach have now been generated by the Sharpless group in our collaborative studies. The compounds have been screened for affinity at the agonist-antagonist site, and a few have been taken as leads for crystallographic studies. Several of the dissociation constants approach sub-nanomolar values, and the lead complexes reveal the orientation of the acetylenic and azide precursors that form the triazole *in situ*.

6:20 pm
Targeting Drug-resistant Variants of HIV Reverse Transcriptase: High Resolution Crystal Engineering and Fragment Screening

Eddy Arnold
Ph.D., Professor of Chemistry and Chemical Biology, *CABM & Rutgers University*

HIV-1 reverse transcriptase (RT) is a key target for anti-AIDS drug treatment. Emergence of drug-resistant mutations complicates therapy and development of inhibitors effective against a wide range of HIV variants is highly desirable. We participated in the design and discovery of TMC125 and TMC278, non-nucleoside RT inhibitors which have shown promise in clinical trials for treating HIV-1 infections that are resistant to existing treatments. We have proposed that inhibitor flexibility can be useful in evading drug-resistant mutations and recent structural studies of HIV RT complexed with TMC278 have confirmed these concepts (Das, Bauman, *et al*., PNAS, in press). Engineered versions of HIV-1 RT have yielded crystals diffracting to 1.8 Å resolution and we are pursuing fragment cocktail screening by crystallography. Progress in identifying fragments bound at multiple sites on HIV-1 RT will be described.

7:00-9:00 pm
Buffet Dinner, Posters, Exhibits

Tuesday, February 19th

7:00 am
Breakfast

Morning Session: Methods & Emerging Technologies, Part I

Session Chairs: Wolfgang Jahnke, Ph.D., Senior Research Investigator, *Novartis Institutes for BioMedical Research*, and Vicki Nienaber, Ph.D., Chief Scientific Officer, *ActiveSight*

8:00 am
Session Introduction

Vicki Nienaber
Ph.D., Chief Scientific Officer, *ActiveSight*

8:10 am
Fragment-based Screening in Real-time

David Myszka
Director, Center for Biomolecular Interaction Analysis, *University of Utah*

Hear ye! Hear ye! Today's biosensor technology (like Biacore) is capable of detecting the binding of the smallest of molecules (less than 150 Da) interacting with the largest of proteins (>300 kDa). This opens up the possibility of applying biosensors as a primary tool for fragment screening. Believe it or not, biosensors can be used to tell not only which compounds interact with a target, but they root out the badly behaved compounds as well. What's that? You want more? Well, biosensors can also provide detailed information about the affinity of the interaction. Let's see structural methods do that! But unfortunately, biosensor assays can't show you where a compound binds. So, we need to keep structural methods around for a while. However, since biosensors can screen a library of about ~1000 compounds in 3 days, it makes sense to put one upstream of structural analysis, thereby saving you time and money. And it whitens teeth. Step right up and join the biosensor-based fragment-screening drug-discovery bandwagon. No shoving . . . there's plenty of room for everyone.

8:50 am
Fragment Screening of Drug Targets by SPR and Subsequent X-ray Structural Analysis

Michael Hennig
Ph.D., Vice Director, Molecular Structure Research, *F. Hoffmann - La Roche*

The ability of rapid gain in potency of compounds by structure based drug design together with the high sensitivity of biophysical assays like surface plasmon resonance spectroscopy (SPR) enable the use of fragment molecules as starting points for drug discovery efforts. Examples of the application of fragment screening will be presented in order to illustrate the Roche process for this route for hit and lead generation. There will be emphasis on the interplay of biophysical methods for the screening of the fragment library, the subsequent X-ray structural analysis of the hits after affinity ranking and analysis by computational chemistry methods to facilitate the discovery of novel chemical entities. For example, binding of a tyramine molecule to the Alzheimer target β-secretase was detected and the binding mode analyzed by X-ray complex structure

analysis. It binds to the S1 pocket and a follow up chemistry program was initiated to explore this finding. The benefits and challenges of the fragment screening approach will be discussed.

9:30 am
Application of Enthalpy Arrays to Fragment-based Screening

Michael Recht
Ph.D., Member of Research Staff II, *Palo Alto Research Center*

Enthalpy arrays are arrays of nanocalorimeters that enable measurements of the thermodynamics of molecular interactions using small sample volumes and short measurement times. We are investigating the use of enthalpy arrays for screening FBS hits and ranking their ligand efficiencies. The measurements do not require immobilization of the reactants, an attractive feature compared with surface-based methods such as SPR. In principle, isothermal titration calorimetry (ITC) can be used to characterize the thermodynamics of fragment binding to targets, but its use in FBS is severely hampered by the need for large samples (~1.5 mL), long measurement times, and high fragment solubility in the injectant. Our enthalpy array technology addresses this problem by enabling measurements with 250 nL drops that only take a few minutes.

Fragments identified in FBS typically exhibit low binding affinity (0.1 to 1 mM), and it is beneficial to identify the fragments with a high ligand efficiency to take through lead optimization. We have used enthalpy arrays to determine Kd > 0.1 mM, as shown by titration of $BaCl_2$ with 18-crown-6, demonstrating that the approach has adequate sensitivity for characterizing FBS interactions. In this talk we will discuss the application of enthalpy arrays to confirm and rank hits identified in an X-ray crystallographic FBS.

10:10 am
Break, Exhibits

10:40 am
HT Fragment Screening using Fluorescence Correlation Spectroscopy

Thomas Hesterkamp
Ph.D., Vice President, Fragment Based Drug Discovery, *Evotec AG*

The most widely described fragment screening methods are NMR and X-ray crystallography but the use of high concentration biochemical assays has emerged as a viable alternative. In particular the use of high concentration screening of fragments has been successfully demonstrated using single-molecule Fluorescence Correlation Spectroscopy detection techniques to ensure both high data precision and reproducibility and pharmacological sensitivity. In Evotec's fragment screening process bioassay hits are confirmed by NMR (or vice versa), followed by X-ray and structure driven optimization of the weak binders.

11:20 am
In vivo Incorporation of NMR-active Unnatural Amino Acids

Bernhard Geierstanger
Ph.D., Group Leader, Protein Sciences, *Genomics Institute of the Novartis Research Foundation*

Recently developed methods for genetically encoding the incorporation of unnatural amino acid in *E. coli*, yeast, and mammalian cells can be used to introduce NMR-active labels site-specifically into proteins (Deiters, A., Geierstanger, B. H., Schultz, P. G. (2005) ChemBioChem 6, 55-8). The process requires encoding the incorporation site by an amber non-sense codon, TAG, in the gene of interest, and an orthogonal tRNA/tRNA synthetase pair evolved specifically for each unnatural amino acid. We will report on our progress to incorporate fluorinated, ^{15}N and ^{13}C labeled unnatural amino acids into proteins for the characterization of small molecule-protein interactions.

12:00-1:00
Lunch

1:00-3:00
Posters, Exhibits, FBLD Scavenger Hunt

Afternoon Session: Methods & Emerging Technologies, Part II

3:00 pm
From Fragments to Leads: Seed, Assemble and Grow

Gerhard Klebe
Prof. Dr., *Philipps-Universität*

Virtual screening has been developed as an alternative for lead discovery. It departs from the 3D structure of a target protein and tries to predict putative ligands by docking and molecular similarity analyses. Large collections of candidate molecules are screened which exhibit the size of usual pharma molecules. Successive hierarchical filtering strategies are applied to reduce the initial sample of several million entries to some hundred prospective hits. The properties of the target protein binding pocket are considered in terms of hot spots appropriate to accommodate functional groups of putative ligands. Once such interaction sites are detected, a protein-based pharmacophore is defined to anchor a ligand in the binding pocket. This concept has been transferred to a screening sample of fragments with MW >250 Da. Special care is needed to define a relevant pharmacophore model and to

tailor the applied docking and scoring tools to fragment-based screening. Once discovered as a seed, a fragment is further assembled and grown to molecules of usual drug size. To obtain a more detailed insight into the different contributions gained by adding molecular portions to a given lead fragment, systematic studies by stepwise optimizing ligands in the thrombin binding pocket were performed. This process of ligand growing has been studied by recording isothermal titration calorimetry, crystal structure analysis and computational simulations.

3:40 pm
Integrating Biophysical Methods into Fragment-Based Screening

Glyn Williams
Ph.D., Director of Biophysics, *Astex Therapeutics*

There are a number of established and emerging biophysical methods which are capable of detecting fragment which bind to protein targets with low affinities. The relative strengths and weaknesses of these methods are now better understood and there are a number of ways in which they can be integrated into an effective and efficient screening process. This talk will focus on factors which influence the selection of techniques and the ways in which they can be integrated, with particular reference to X-ray crystallography, NMR and thermal methods.

4:20 pm
Pulling it all together: Faster Better Lead Discovery through Integration of Technologies

Vicki Nienaber
Ph.D., Chief Scientific Officer, *ActiveSight*

"We have the capabilities to make higher quality clinical candidates . . . Better than they were before. Better. Stronger. Faster." Since Fragment-based Lead Discovery (FBLD) was first introduced in the mid-1990s, significant progress has been made in the field. Technologies have progressed both in the detection of fragment binding and in their optimization to lead compounds. Biophysical techniques such as SPR and calorimetry are becoming central assets to an FBLD program. Furthermore, the line between a traditional FBLD process and optimization of these early leads into clinical candidates is becoming blurred. Technologies such as parallel synthesis, SPR and calorimetry may be used hand-in-hand with high-resolution crystal structures to rapidly advance leads through optimization into clinical candidates. Fragment co-crystal structures may be used to scaffold hop towards the goal of expanding or generating IP or improving the drug-like properties of a late-stage lead series. ActiveSight's LENS™ technology pulls together multiple biophysical techniques with proprietary software tools to focus synthetic efforts in the transformation of fragment hits into drugs.

5:00 pm
Break, Exhibits

5:30 pm
Scientific Advisory Board Round Table

Chair: Glyn Williams, Ph.D., Director of Biophysics, *Astex Therapeutics*

7:00-9:00 pm
Dinner Reception, Poster & FBLD Scavenger Hunt Winners Announced

Wednesday, February 20th

7:00 am
Breakfast

Morning Session: Lessons Learned

Session Chairs: Roderick Hubbard, Professor, *University of York and Senior Fellow, Vernalis* and Harren Jhoti, CEO and Founder, *Astex Therapeutics*

8:00 am
A Decade of Thinking Small: Highlights and Lessons Learned from Building a Fragment Discovery Company

Harren Jhoti
CEO and Founder, *Astex Therapeutics*

8:20 am
Fragmentology

Roderick Hubbard
Professor, *University of York and Senior Fellow, Vernalis*

Over the past six years, we have developed, refined and applied fragment-based methods in a number of structure-based discovery projects. In this presentation, I will review some of the phenomena observed. These include:

- hit rates for different target classes and relationship to library design methods
- conformational change induced by fragments
- virtual screening and docking of fragments
- similarities and differences in fragment binding to members of the same protein family
- comparison of NMR and SPR for identifying fragments

From these experiences, I will review what we believe are the next steps for developing and applying the methods. These include improved characterization of library content, design of novel fragments, decision support for fragment evolution and targeting protein-protein interactions.

9:05 am
How then Shall we Screen?

Phil Hajduk
Ph.D., Project Leader, *Abbott Laboratories*

Fragment-based screening is only one of many tools that can be brought to bear on the discovery of new drug leads. Especially at large pharmaceutical companies, the proper balance of resources between conventional HTS, fragment-screening, and fast-follower strategies is not always clear. This presentation will discuss the integration of fragment-based screening into the array of screening technologies available at Abbott, the optimal application of FBDD, and opportunities for continued contributions to development candidates.

9:50 am
Break, Exhibits

10:05 am
Integrating Fragment-Based Methods into Drug Discovery: Some Examples

Siegfried Reich
Ph.D., Vice President of Drug Discovery, *SGX Pharmaceuticals*

Fragment-Based Methods continue to evolve as a powerful tool in the identification and optimization of useful leads for Drug Discovery. Integrated into an overall drug discovery process which includes iterative structure-based design and a focus on retaining intrinsic favorable drug-like properties of the original hit, viable development candidates can be discovered. We have optimized our FAST approach to allow rapid crystallographic screening of our fragment library against oncology targets of high therapeutic value. Several examples of the application of FAST toward the identification of advanced leads targeting BCR-Abl, HCV Pol, and MET will be described.

10:50 am
Fragment-based Discovery for Challenging Targets

James Wells
Ph.D., Professor in Pharmaceutical Chemistry and Molecular & Cellular Pharmacology, *UCSF*

Drug discovery has been challenged when targets do not present small molecule precedents for binding such as protein-protein interfaces and novel allosteric regulatory sites in enzymes. Fragment discovery approaches have been showing promise in addressing of these targets. I'll review some of this work and the general outlook for attacking what has been considered "undruggable" territory.

11:35 am
Closing Plenary Presentation
One Discovery Shoe Size Paradigm Does Not Fit All and Could Well Leave Us Barefoot

Christopher Lipinski
Ph.D., Scientific Advisor, *Melior Discovery*

For orally active drugs we live in a target poor environment. There is nothing wrong with using high-throughput screening (HTS) to find a superbly selective drug for a new target. It is just that we do not know which the really good targets are. So we search for complements to the one shoe size fits all mentality of the single ligand for a novel target discovered by HTS paradigm. All the alternatives have a common feature; they expand opportunity space on the biology side, the chemistry side or the computational side. On the biology side we have phenotypic screening; letting the experimental observation rather than biology/genomics opinion point us to the correct target. We have non reductionist approaches such as systems biology. We have the most relevant experiment; keen clinical observation and we even have the revolutionary concept that maybe it is not the target per se that is critical but the physiological set point/state of a cell. On the chemistry side we have fragment screening with advantages of chemistry space coverage and better interrogation of dubious targets. We have the search for multiple activities in a single compound or a mixture of compounds and we have the rediscovery that medicinal chemistry pattern recognition and expertise often works better than automated technology. On the computational side we have new software to assist in fragment generation and reassembly and we have software and databases in the commercial and increasingly in the public domain to predict biological activity from chemistry structure alone. Finally, we are trying approaches to fix a major disconnect. The innovative biology resides in academia but the drug development capability resides in industry.

12:30 pm
Close of Conference

Poster Abstracts

Session 1: Monday, February 18th

10:30 to 11:00 am or 12:30 to 1:30 pm
Set up

7:00 to 9:00 pm
Presentation

Session 2: Tuesday, February 19th

10:10 to 10:40 am or 12:00 to 1:00 pm
Set up

1:00 to 3:00 pm
Presentation

Session 1-01
NMR-based hit validation and optimization

Ziming Zhang, John Stebbins, Hongbin Yuan and Maurizio Pellecchia, *Burnham Institute for Medical Research*

Despite the tremendous progress of high-throughput (HT) screening and automation in testing larger collections of compounds, the number of compounds experimentally screened (10,000-100,000) still represents only a very small fraction of all the possible "lead-like" compounds that could be imagined (up to 10^{16}, considering "lead-like empirical constraints). Therefore, it is highly unlikely that selective and potent compounds are found directly from a HT screen. Rather, it is expected that initial hit compounds can be identified which will exhibit only moderate affinity and selectivity for a given target. As part of our San Diego Center for Chemical Genomics (SDCCG), one of the nine NIH funded centers belonging to the Molecular Library Screening Center Network (MLSCN), we will present strategies aimed at the design of optimized inhibitors based on NMR-based strategies that rely on a chemical fragment linking approach. In some cases, particularly compelling for targets for which a defined biochemical assay cannot be developed, or for targeting proteins with unknown function or substrates, or protein involved in more complex macromolecular interactions such as protein-protein and protein –nucleic acid interactions, we also propose to use NMR-based screening techniques for de novo hit discovery. Finally, simple NMR binding assays are presented to provide rapid validation of compounds arising from HTS projects.

Session 1-02
Synthesis of Pyrone Derivatives as Inhibitors of Matrix Metalloproteinases: Targeting Primed and Nonprimed Sites

Yi-Long Yan and Seth M. Cohen, *Department of Chemistry and Biochemistry, University of California, San Diego*

Matrix metalloproteinases (MMPs) are a class of hydrolytic zinc(II)-dependent enzymes that catalyze peptide bond hydrolysis. They are involved in tissue remodeling, wound healing, and growth. Misregulated activity of these enzymes is associated with a variety of diseases such as cancer, arthritis, atherosclerosis, and heart diseases. Many MMP inhibitors have been developed as potential chemotherapeutics. In this report, we developed a new synthetic scheme for the preparation of hydroxypyrones with unprecedented substitution patterns. The scheme is applied to the development of new hydroxypyrone-based MMP inhibitors with backbones designed to interact with both the MMP primed and non-primed pockets. The inhibitory activities of these inhibitors against a variety of MMPs were determined using fluorescence-based enzyme assays.

References:
1. Page-McCaw, A.; Ewald, A. J.; Werb, Z. Nat. Rev. Mol. Cell Biol. 2007, 8, 221.
2. Whittaker, M.; Floyd, C. D.; Brown, P.; Gearing, A. J. H. Chem. Rev. 1999, 99,
3. Skiles, J. W.; Gonnella, N. C.; Jeng, A. Y. Curr. Med. Chem. 2004, 11, 2911.
4. Yan, Y.-L.; Cohen, S. M. Org. Lett. 2007, 9, 2517.

Session 1-03
Docking Stydies of a Bridge Head Fused Heterocyclic Library Into Hepatitis C Virus Binding Sites

Mohamed A. Eldawy and Khaled M. Elokely, *Tanta University*

Hepatitis C represents a major health problem for the world. It is a chronic disease that affects mainly the liver and currently there is no cure. Computer Aided Drug Design/Discovery (CADD) algorithms were employed for thorough study of the molecular mode of action of in house library of bridge head fused heterocyclic compounds at the molecular level. The study of the possible docking of the tested compounds was done on specific HCV targets including: human CD81 large extracellular loop (LEL), thus blocking viral entry into human cells, HCV NS3-4A Serine protease and HCV NS5B Polymerase, thus interfering with viral replication. The docking experiments utilized the linear scoring of Molegro® Virtual Docker (MVD).

Session 1-04
Fragment-based design of isoform specific PI3K inhibitors

Si Wang, Surya De, Li-Hsing Chen and Maurizio Pellecchia, *Burnham Institute for Medical Research*

The PI3-Kinase pathway is important for regulation of cell survival and proliferation. Central to this pathway is the phosphoinisitol-3,5 phosphate kinase (PI3K) which exists in four different isoforms. In order to obtain isoform specific PI3K inhibitors, we designed molecules that can bind simultaneously to the ATP and substrate sites of the target. These molecules include three basic ATP mimic building blocks, a moiety targeting the substrate site, and a linker coupling the ATP mimic. In the present study, we have

identified that novel indazole-alkyne derivatives have excellent binding affinity to the ATP pocket of the α-isoform of PI3K, with IC50 values in the low nM range in vitro and cellular activity in the 5-10 μM range. The molecules have been derivatized to reach the substrate binding pocket and current efforts focus on the identification of suitable substrate mimics.

Session 1-05

Anthrax Lethal Factor Protease Inhibitors

Sherida Johnson[1], Rebecca Harbach[1], Li-Hsing Chen[1], Mojgan Sabet[2], Martino Forino[1], Dawoon Jung[1], Alex Strongin[1], Robert Liddington[1], Donald Guiney[2], Maurizio Pellecchia[1], *Burnham Institute for Medical Research*

Inhalation anthrax is a deadly disease for which there is currently no effective treatment. Bacillus anthracis[1] lethal factor (LF) metalloproteinase is an integral component of the tripartite anthrax lethal toxin secreted from the pXO1 plasmid that is essential for the onset and progression of anthrax[2]. We recently identified a series of compounds that efficiently inhibit LF utilizing a fragment-based approach[3,4]. This method formed the basis for the rational design of additional compounds with improved activity and selectivity. Here we present the fragment based approach adopted and further structure-activity relationship (SAR) and 3D QSAR studies on newly derived inhibitors[2]. Moreover, we will discuss selectivity of the inhibitors in comparison with other metalloproteases[5,6], ADME-T properties[7] and preliminary in vivo studies[8]. Our data indicates that the LF inhibitors we have identified are the foundation for the development of novel, safe and effective emergency therapy against post-exposure inhalation anthrax.

[1]Smith, H.; Keppie, *J. Nature*, 1954, 173, 869-870.
[2]Hanna, P. et al., *Proc. Natl. Acad. Sci.* USA 1993, 90, 10198-10201
[3]Forino, M. et al., *Proc. Natl. Acad. Sci.* U S A 2005, 102, 9499-504.
[4]Johnson, S. et al. *J. Med. Chem.* 2006, 49, 27-30.
[5]Johnson, S. et al. *J. Med. Chem*. submitted.
[6]Johnson, S; Pellecchia, M.* *Curr. Top. Med. Chem.* 2006, 6, 317-29.
[7]Asinex; *www.asinex.com*
[8]Johnson,S. et al. *Chem. Biol. and Drug Design*, in press.

Session 1-06

Synthesis and SAR of bi-dentate compounds as potent JNK inhibitors

Surya K. De, Megan Riel-Mehan, John Stebbins, Li-Hsing Chen, and Maurizio Pellecchia, *Burnham Institute for Medical Research*

c-Jun-N-terminal kinases (JNKs) are serine threonine protein kinases and are members of the mitogen activated protein kinase (MAPK) family. The JNK pathways are implicated in many diseases, including diabetes, cancer, atherosclerosis, stroke, Alzheimer's, and Parkinson's disease, making JNKs attractive targets for drug therapy. The phosphorylation of substrates by JNK is regulated by binding to JNK interacting protein (JIP-1), via a D-domain. Binding to JIP-1 is necessary for JNK activity, and can be inhibited by a peptide containing the sequence of the D-domain (pep-JIP-1). Thus by tethering an ATP mimic to a pep-JIP-1 mimic the resulting bi-dentate compound exhibits both the affinity of ATP and the selectivity of JIP-1.

By using an innovative fragment based approach to JNK inhibition, we designed mono- and bi-dentate compounds that target either the substrate docking site alone or simultaneously block this site and the ATP binding pocket. The resulting compounds are substrate competitive and inhibit JNK in the micromolar to low nanomolar range. The results of this study will be presented in further detail.

Session 1-07

A Program for Automated Fitting of Fragments on Noisy X-ray Density Maps from Fragment Screening

Qing Zhang, Charles D. Stout, Arthur J. Olson Department of Molecular Biology, *The Scripps Research Institute*

Fragment-based lead discovery employs structural determination tools to screen a fragment library of hundreds to thousands compounds. The method is currently utilizing a highly automated procedure from X-ray diffraction, to data collection, to protein structure refinement. However, fitting fragments on resulting tens to hundreds of X-ray density maps is still by hand in most practices. This is a tedious step and sometimes quite challenging if maps are noisy. Here we present a novel docking-based program to automate the step. The program searches the entire density difference map and interprets fragment binding sites and conformations as well as binding fragments in cocktail screenings. It first finds fragment-like density peaks and then dock fragments on these peaks with AutoDock. Docking is performed twice: docking with a density-dominating scoring function and then with the AutoDock4 energy scoring function. Combination of both density and energy information not only evaluates a binding conformation comprehensively but also alleviate the inaccuracy in the protein structure and scoring function. In this poster, we present one of the program's applications—HIV protease cocktail soakings, in which ActiveSight provides the fragment library and automated X-ray diffraction, data collection and protein refinement.

Session 1-08

Identification of a Novel Class of Nitropyrazole Leads for Plasmodium berghei Orotidine 5'-Monophosphate Decarboxylase using Fragment-Based Screening

Robert Lam[1], Kevin Battaile[2,3] , Roni Gordon[1], Gera Kisselman[1], Jennifer Artz[4], Raymond Hui[4], Lisa Keefe[2,3], Cheryl Arrowsmith[1,4], Aled Edwards[4], Emil Pai[1,5], and Nickolay Chirgadze[1,6]

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Fragment-based screening by X-ray crystallography was used to screen four therapeutically relevant targets against a library of 610 small molecules. The compound library was assembled from internal as well as commercially available sources. Automated processes were utilized throughout for data collection including "FedEx crystallography" with IMCA-CAT synchrotron beamline, crystal handling robotic system, and automated data processing and electron density map generation scripts. Complete screening of a malaria target, Plasmodium berghei orotidine 5'-monophosphate decarboxylase, led to the discovery of a novel class of nitropyrazole leads.

Session 1-09

Design, synthesis and evaluation of novel Apogossypol derivatives targeting anti-apoptotic Bcl-2 family proteins

Jun Wei, Michele F. Rega, Shinichi Kitada, Hongbin Yuan, John C. Reed and Maurizio Pellecchia, *Burnham Institute for Medical Research*

Apogossypol, a semi-synthetic compound derived from the natural product Gossypol, possess anti-cancer activity by targeting anti-apoptotic Bcl-2 family proteins such as Bcl-2 and Bcl-xL. Apogossypol retains most of cytotoxic activity against cancer cells but is much less toxic than Gossypol for normal cells because it lacks two reactive aldehyde functionalities. These observations make Apogossyol a better lead compound than Gossypol for further optimizations. In order to obtain compounds which have improved potency, stability and drug-like properties than Apogossypol, novel synthetic routes were designed to obtain novel derivatives. The resulting compounds are evaluated for their ability to bind in the BH3 binding groove of Bcl-xL, by using a combination of nuclear magnetic resonance (NMR) binding assays and in vitro displacement assays. Computer docking studies supported by the NMR data provide a detailed picture of the possible interaction mode of Apogossypol and its derivatives with Bcl-xL. Preliminary cell-based evaluations and in vivo studies with most promising compounds will be presented.

Session 1-10

Development of new inhibitors for the PTPase YopH through second site screening employing radical probes

Jesus Vazquez, Lutz Tautz, Tomas Mustelin and Maurizio Pellecchia, *Burnham Institute for Medical Research*

Bubonic plague is caused by the bacterium Yersinia Pestis and its mode of action implies the use of a type III secretion system (TTSS) to deliver a set of effector proteins into the cytoplasm of eukaryotic cells and avoid detection and targeting by the immune system. One of those effector proteins, YopH, is a very active tyrosine phosphatase (PTPase) which dephosphorylates critical tyrosine kinases and signal transduction molecules, leading to paralysis of lymphocytes and macrophages. Our previous work identified potential inhibitors of YopH that target its catalytic site [1]. Albeit relatively potent, these molecules lack of selectivity, inhibiting also several human PTPases. While the phosphotyrosine binding pocket is well conserved among human and bacterial proteins, the observation of additional binding crevices surrounding their active sites suggests that selective bi-dentate inhibitors can be attained by bridging non-selective phosphotyrosine mimics with small molecule fragments that occupy such sites. We report on the discovery of such small molecule fragments employing a synthetic radical probe [2] and the development of new bidentate inhibitors based on those fragments and aided by in silico docking [3].

[1] Tautz, L.; Bruckner, S.; Sareth, S.; Alonso, A.; Bogetz, J.; Bottini, N.; Pellecchia, M. and Mustelin, T. Inhibition of Yersinia tyrosine phosphatase by furanyl salicylate compounds. J. Biol. Chem. 2005, 280, 9400-9408.
[2] Vazquez, J.; Tautz, L.; Ryan, J. J.; Vuori, K.; Mustelin, T. and Pellecchia, M. Development of molecular probes for second site screening and design of protein tyrosine phosphatase inhibitors. J. Med. Chem. 2007, 50, 2137-2143.
[3] Vazquez, J.; Tautz, L.; Mustelin, T. and Pellecchia, M. A fragment-based approach to the design of potent and selective bi-dentate inhibitors of PTPases, 2008 (in preparation).

Session 1-11

A fragment-base approach to the design of novel SMAC mimetics

Jui-Wen Huang, Ziming Zhang, Xiyun Zhang, Bainan Wu, John Stebbins and Maurizio Pellecchia, *Burnham Institute for Medical Research*

The XIAP (X-Linked Inhibitor of Apoptosis Protein) Bir3 (baculovirus IAP repeat 3) domain binds directly to the N-terminal of Caspase-9 and thus inhibiting programmed cell death. It has been shown that in the cell this interaction can be displaced by the protein SMAC (second mitochondrial activator of caspases) and that its N-terminal tetrapeptide region (AVPI) is responsible for the binding. However, synthetic SMAC tetrapeptides do not exert pro-apoptotic activity in cultured cells likely due to their limited cell-permeability, hindering their use as potential drug candidates against cancer cells. Because of the

modular nature of peptides, we developed a novel fragment-based strategy in which individual amino-acids are replaced in an iterative manner with more drug-like scaffolds. We will report on the design, synthesis and characterizations of novel SMAC mimetic compounds obtained by using such strategy.

Session 1-12
Fragments of Life™ for Lead Discovery

Doug R. Davies, *deCODE biostructures, Inc.*

Small molecules of life carry natural born information content, and through co-evolution with proteins they are likely to have high ligand efficiency for protein binding. Inspired by this concept, we developed a novel Fragments of Life™ library for lead identification and optimization in conjunction with protein X-ray crystallography. The Fragments of Life™ library includes molecules of life, secondary metabolites, mimetics of protein architecture, and hetero-functional derivatives thereof, each selected for pharmaceutic properties, water solubility, and potential for chemical elaboration to facilitate medicinal chemistry efforts. The application of Fragments of Life™ to both metabolic and infectious disease targets has yielded hit rates of 2-8%, allowing the identification of multiple lead series templates. The fragment binding information has expanded our understanding of the structure activity relationships for known inhibitors of a metabolic target, and has facilitated chemotype switching in two synthetic cycles to produce a potent back-up compound to a clinical candidate.

Session 1-13
Integrating computational and X-ray crystallographic methods to obtain vantage co-structures

Vivek Sharma, *Locus Pharmaceuticals*

Fragment-based X-ray crystallography methods are now widely employed to explore new or alternate hits in druggable sites. The active site structure of most drug targets, however, exhibit a plasticity that is neither sufficiently explored nor represented by small molecular fragments used in such methods. Moreover, crystallography-based fragment screening that rely on broad diverse libraries suffer from low hit rate. At Locus Pharmaceuticals, we have used our core in silico fragment-based design technology to select novel compounds that are reasonably potent to bind in the target site and to yield a co-structure, as well as that are sufficiently large to induce changes in active site that are pertinent to further design. Using Hsp90, a key target in cancer, we show that a successful fragment-based approach to discover new hits desires effective integration of both computational and crystallographic methods.

Session 2-01
Automated Fragment-based Screening using High Throughput Surface Plasmon Resonance

Toshihide Ezoe, *FUJIFILM MEDICAL SYSTEMS USA Inc.*

Here we introduce new SPR system, AP-3000, allows the identification of weak interactions (~10mM) with high throughput (3840cmds/day) and automated hands free operation and is therefore particularly suited for initial fragment-based screening (FBS). The fragment is significantly smaller (Mr 120-300) exhibit lower binding affinity (~mM) compared to HTS hits and is therefore difficult to detect using traditional bioassay-based screening methods. Instead, biophysical techniques such as NMR and X-ray crystallography are routinely used in FBS, because they possess the high sensitivity needed to detect low affinity fragments. However, both methods have their limitations. For many pharmacological targets, it can be difficult to obtain the significant amounts of 15N-labeled protein required for FBS using NMR. The use of X-ray crystallography in FBS is often limited by the need for a soakable, well-diffracting and robust crystal system. The surface plasmon resonance (SPR) can be additional biophysical techniques, in which biomolecular interactions are analyzed in a sensitive and label-free format. However the conventional SPR is not suitable for drug screening because of their low throughput (<1000cmds/day) and complicated operation. AP-3000 allows high throughput FBS with complete hands off automation and minimize running cost. Selected case studies demonstrate the successful detection of fragment bindings for pharmacologically relevant targets.

Session 2-02
Assembling Fragment Libraries

Stephen P. East, *Evotec (UK) Ltd*

One of the key considerations when designing and assembling fragment libraries is the type of the screening platform that will be used to detect the interaction between the biological target and the fragment. We have constructed two libraries of fragments. One library has been built for use in high-throughput biochemical screening assays and the other was purposefully designed for screening using NMR techniques. The composition of each library is approximately 20000 compounds; however, there is an overlap of less than 1000 compounds between them. This poster will describe the approaches we used to compile the two complementary libraries and our efforts to ensure the dynamic evolution of our fragment-library screening capability.

Session 2-03
Dual Polarization Interferometry: A Biophysical Technique Suitable for Fragment Based Drug Discovery

Neville Freeman, *Farfield Scientific Inc*

FBLD offers the basis for a dramatic reduction in the throughput volume required in the drug discovery process. However as typical fragment sizes are less than 250 Da and affinities are in the uM to mM range FBLD challenges even the most sensitive biophysical detection techniques. This poster introduces a biophysical technique known as Dual Polarization Interferometry (DPI) which has been used to study low affinity, low molecular weight binders to proteins. In addition to detecting the binding of such entities, the technique is also capable of measuring structural changes which occur during the binding process.

Session 2-04

Rigid and Flexible Pharmacophore Search for Pyrantel Analogs

Mohamed A. Eldawy, Manal A. Nayel, *Tanta University*

In an attempt to explore the binding at molecular level of the anthelmintic drug, pyrantel, to its reported biological target, nicotinc AcetylCholine receptor (nAChR), two pharmacophore search methods were utilized for a series of pyrantel analogs previously synthesized and tested for their nematocidal activity. These compounds include tetrahydropyrimidines derivatives, 1-(2-arylvinyl) pyrimidinium salts, non-cyclic amidines, thiazolines and dihydrothiazolines. Ligandscout® Software version 1.03 was used for rigid alignment and Galahad® software was used for flexible search (torsional space based) the obtaining pharmacophore features were analyzed and the two methods were compared regarding model validation and its specificity.

Session 2-05

Docking of Praziquantel Analogs Into the Schistosomal Enzyme Glutathione-S-Transferase (GST)

Mohamed A. Eldawy, Manal A. Nayel, *Tanta University*

Schistosomaisis is a major health problem that affects about 200 millions world wide. The treatment of this health condition depends heavily on a single drug, praziquantel, the mode of action of which is not fully understood, however a complex of the drug with schistosomal glutathione-S-transferase enzyme (GST) was isolated and this enzyme is considered as potential target for antischistosomal drugs. In this study docking of certain praziquantel analogs into this enzyme was attempted using two docking algorithms which utilizes different scoring functions .These analogs include 3-benzyl-5-benzylidene-1-methyl-2-thioxoimidazolidine derivatives, pyrazino[2,l-a][2]benzazepine derivatives and trioxolanes derivatives. The utilized algorithms are melgro® virtual docker (MVD) which use linear scoring function and Surflex which use nonlinear scoring function .The results obtained by the two algorithms were analyzed and compared.

Session 2-06

QSAR Study of Pyrantel and Levmisole Analogs

Mohamed A. Eldawy, Manal A. Nayel, *Tanta University*

The search for new nematocidal compounds is mandatory as nematodal infections affect more than two billions throughout the world. In this study different quantitative structure activity relationship (QSAR) techniques were used to correlate the chemical structure of a series of compounds including tetrahydropyrimidines derivatives, 1-(2 arylvinyl) pyrimidinium salts, non-cyclic amidines, thiazolines and dihydrothiazolines with nematocidal activity exhibited by clinically used drugs. These studies employed traditional QSAR method using Accelyrs' Materials Studio® (MS) Modeling to correlate the nematocidal activity with certain molecular descriptors, molecular fingerprinting technique employing Hologram QSAR (HQSAR®) and 3D QSAR method using CoMFA® to explore the target binding properties. The results of these studies were analyzed.

Another two models were constructed for levamisole analogs using MS® 4.0 QSAR module based on [7H]-S-triazolo[3,4-b][1,3,4]thiadiazine derivatives for the first model and compounds containing thiazoline or dihydrothiazine rings substituted by alkylamino or arylamino groups for the second model and the results obtained were analyzed.

Session 2-07

Chk2 is a checkpoint kinase involved in the ATM-Chk2 checkpoint pathway

George T. Lountos, *National Cancer Institute-Frederick*

This pathway is activated by genomic instability and DNA damage and results in either cell death or arrest of the cell cycle to allow DNA repair to occur. Chk2 has been identified as a promising target for anti-cancer drug design. Our primary rationale for development of Chk2 inhibitors is focused on two areas. Selective inhibition in p53-defective tumor cells may provide chemo/radiosensitization as Chk2 is activated in tumor cells by a wide range of chemotherapeutic drugs and ionizing radiation. Thus, selective inhibition of Chk2 could increase the therapeutic indices of DNA-targeted agents in p53-defective tumors. Furthermore, inhibition of Chk2 in normal cells may also protect normal tissues from p53-induced apoptosis. We have initiated a drug design effort targeting Chk2 by screening over 100,000 compounds in the Open Repository Library and identified a bis-guanylhydrazone, NSC109555, as a potent and selective inhibitor with an IC50= 240 nM. A co-crystal structure of Chk2 in complex with NSC109555 has been solved and provides an important first step in a structure-based drug design effort to provide a rational approach to improving the structure of the inhibitor to achieve greater potency and selectivity for Chk2.

Session 2-08

In silico fragment-based screening for SARS 3CLpro inhibitors

M.E. Johnson, D.C. Mulhearn and W.-T. Fu, *Center for Pharmaceutical Biotechnology, University of Illinois at Chicago*

Following its introduction in China, severe acue respiratory syndrome (SARS) spread rapidly to other Asian countries, North America and Europe, with more than 8,000 reported cases and nearly 800 deaths. The chymotrypsin-like protease (3CLpro) is a primary target for SARS drug development. We have reported the design, synthesis and biological activity for several peptidomimetic 3CLpro inhibitors (1-2). To expand the potential lead repertoire, we have initiated in silico screening of low molecular weight fragments against the active site of 3CLpro. Using the ZINC database fragment library, we are using a variety of software strategies to evaluate docking, including the DOCK, Autodock, Glide and Gold programs. We are additionally evaluating the receptor flexibility to refine potential docking. Details of the docking strategies and initial results will be discussed.

Supported by NIH grant P01 AI060915 (MEJ).

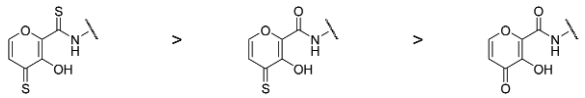
1. A.K. Ghosh, K. Xi, K. Ratia, B.D. Santarsiero, W. Fu, B.H. Harcourt, P.A. Rota, S.C. Baker, M.E. Johnson and A.D. Mesecar (2005) J. Med. Cehm. 48:6767-71.
2. A.K. Ghosh, K. Xi, V. Grum-Tokars, X. Xu, K. Ratia, W. Fu, K.H. Houser, S.C. Baker, M.E. Johnson and A.D. Mesecar (2007) Bioorg. Med. Chem. Lett. 17:5876-80.

Session 2-09

Structure-activity-relationship of novel Anthrax Lethal Factor inhibitors

Arpita Agrawal and Seth M. Cohen, *Department of Chemistry and Biochemistry, University of California San Diego*

Anthrax Lethal Factor (LF) is one of three key proteins involved in the pathogenesis and virulence of Anthrax by the bacterium Bacillus anthracis. LF is a zinc-dependent proteolytic enzyme which cleaves the N-terminal of MAPKKs, thereby disrupting downstream signaling pathways. Inhibition of anthrax has been of growing concern due to its use as a bioweapon before and after 9-11. The current study focuses on a bioinorganic approach toward the design of novel LF inhibitors. The design is based on a two-part strategy incorporating a zinc-binding group (ZBG) that chelates the catalytic Zn2+ ion and a peptidomimetic backbone which interacts with the active site of the protein. A set of hydroxypyrone and hydroxythiopyrone compounds were synthesized with varying backbones. As hypothesized, the (O, S) hydroxythiopyrone subset of compounds showed higher potency than the (O, O) hydroxypyrone compounds against LF. The potency was evaluated in vitro using a fluorogenic MAPKKide substrate and showed ZBG potency in the following order:



Session 2-10

Identification of MMP-12 inhibitors by a biosensor-based screen of a fragment library

Helena Nordström [1], Thomas Gossas [1], Markku Hämäläinen [2], Per Källblad [3,4], Susanne Nyström [3], Hans Wallberg [3], U Helena Danielson [1], *Beactica AB*

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A biosensor-based strategy has been used to screen a library of 245 fragments for novel inhibitors of matrix metalloproteinase 12 (MMP-12). The interaction between single concentrations of fragments injected alone or in competition with ilomastat, and three variants of the target protein and a reference protein (carbonic anhydrase II) was measured continuously by surface plasmon resonance. The experimental design overcame the inherent instability of MMP-12 and allowed the identification of fragments that interact with the active site of MMP-12 but do not interact with carbonic anhydrase II. Selected compounds which exhibited a concentration-dependent and saturable interaction were further evaluated by an activity-based assay, verifying MMP 12 inhibition. The most potent compound was confirmed to be a competitive inhibitor and had a ligand efficiency of 0.69 kcal/mol. The procedure integrates selectivity and binding site identification into the screening procedure and does not require structure determination.

Session 2-11

Therapeutic approaches to treat HIV infection: development of HIV-1 integrase inhibitors using a combined NMR and X-ray crystallography approach

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HIV/AIDS continues to affect the livelihoods of millions of people all over the world. The viral enzyme integrase is the most significantly underdeveloped HIV target. Using Saturation Transfer Difference (STD) NMR we identified a set of small molecules that interact with integrase. The hit fragments were soaked into crystals of integrase and a number of complexes have been solved. Superimposition of the fragment complexes revealed overlapping but distinct fragment binding regions. By merging the two fragments and exploiting both binding sites we have shown improved

binding affinity and in vitro activity. In this work we highlight how NMR and X-ray crystallography can be used together to improve the efficiency of lead development.

Session 2-12

Picky Pezzi: screening for host-parasite selectivity at the fragment level using NMR spectroscopy

Darren Begley, *University of Washington, Chemistry Department*

Thymidylate synthase (TS) is an essential enzyme in all living organisms. It has long been a focus of human cancer studies, and more recently investigated as a target for treating bacterial and parasitic infections. Traditional medicinal chemistry has yielded many antifolate analogues, but high sequence similarity and structural homology across species make it difficult to develop species-selective inhibitors. By screening enzymes from multiple species using a fragment-based NMR strategy, we are seeking to discover new species-selective inhibitors that are easily synthesized, for applications to diseases such as malaria and other parasitic infections. We have studied human TS and the essential RNA-editing uridylyl transferase from *Trypanosoma brucei* (TbRET2) by screening a chemically diverse, low-molecular weight compound library using NMR methods. We are now using NMR, crystallography, and computational modeling tools to characterize our screening hits and propose modifications which will increase their specificity and potency, in some cases by linking fragments together. By incorporating TS enzymes from other organisms into future NMR studies, we plan to develop novel lead fragments as candidates for species-specific TS drug therapies.

Session 2-13

Exploring fragment selectivity between different classes of protein

Kerrin Bright, *Structural Biology Laboratory, University of York*

The aim of my project is to explore fragment selectivity between different classes of protein. A commercially available and structurally diverse fragment library has been developed and acquired by my industrial sponsor, Organon. In addition, a small library focused towards one protein has also been developed. Both libraries have been screened against two protein targets (a kinase and glucosidase) using saturated transfer difference nuclear magnetic resonance, a rapid screening technique suitable for detecting ligands binding with μM affinity [1]. Competitive hits for both proteins have been identified, and future work will focus on exploring the protein-ligand interactions and investigating possible routes for improving potency.

[1] Angew. Chem. Int. Ed. 1999, 38, 12, 1784-1788

Session 2-14

Design of a druglike and leadlike Fragment Library

Herman Verheij, *Pyxis Discovery*

In recent years, fragment-based screening has become an established technology to identify small active molecules that have a high potential for optimization, and thus frequently yield high-affinity binders that still possess desirable (physico-) chemical properties.

To accommodate this increased demand for fragments, many fragment libraries have been introduced recently. However, to yield hits that possess a high chance of successfully progressing through the lead optimization process, the fragments in such a library should, just like "traditional" screening compounds, possess certain " leadlike" and "druglike" features. From most fragment libraries that are commercially available, this aspect is partially or fully missing.

Pyxis Discovery has therefore designed a fragment library of which all compounds are "leadlike" (i.e., do not contain undesirable (physico-)chemical properties), and furthermore contain scaffolds that are identical or very similar to those found in existing drugs.

This poster will describe the design process used to construct the library, both with respect to filtering for "leadlike" properties, and the selection of "drug scaffolds".

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