TI-3D

Texas Institute for Drug and Diagnostic Development

"Tranforming basic research into tools for better health."

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Director's letter

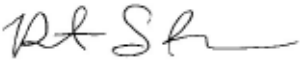
Brent Iverson, Ph.D. - Interim Director

It is the responsibility of all Texas research universities to provide an effective pathway for the translation of basic science breakthroughs into products and technologies that benefit Texans as well as all Americans. Indeed, the promise of benefits to society largely justifies the substantial government investment in the research side of public universities. Societal benefits from research are manifest not only in the products or technologies that improve lives, but also the tremendous economic development catalyzed by the commercialization of university discoveries. Major economic centers such as those in California's Silicon Valley or the Greater Boston area in Massachusetts have naturally emerged around research universities and their discoveries.

New treatments, drugs, or diagnostic technologies are the ultimate practical benefits that result from biomedical research. The challenge of academic biomedical research is to coordinate all of the complementary expertise and resources necessary to take an idea from the research laboratory bench to a patient's bedside. Fortunately, all of the required components exist within the University of Texas System, although they are scattered around the state. The University of Texas at Austin is the state's recognized leader in basic science and engineering research. The Texas Institute for Drug and Diagnostics Development (TI-3D) was founded by a group of UT Austin scientists and engineers from several departments, generously supported by then provost Sheldon Ekland-Olsen and the Robert A. Welch Foundation, to provide the organizational infrastructure necessary for the successful translation of scientific and technological breakthroughs into benefits to society. Our approach is to provide a focal point for communication between UT Austin scientists and medical center researchers around Texas. This has already been achieved. In addition, it is our intent to provide UT Austin scientists the resources and facilities they need to translate their breakthroughs into more than just a publication in a noteworthy scientific journal. To this end, we have established, or have helped to establish, important new facilities such as the TI-3D high throughput screening facility to enable more rapid drug discovery, a facility in Pharmacy to look at drug candidate metabolism, an X-ray structural core facility to better understand how drugs interact with their target, and a genomic sequencing center to push biological research on campus well into the genomic frontier. Most important, we have offered several million dollars worth of seed funding to facilitate nascent collaborations between UT Austin faculty and medical researchers around the state.

Our timing could not be better. The major pharmaceutical companies are drastically downsizing their drug discovery efforts, and as a result, most have a widely publicized lack of new drugs in their pipelines. Many companies are turning to academic institutions for their new drug, treatment, and diagnostic technology candidates. The Texas Cancer Fund, through the Cancer Prevention and Research Institute of Texas (CPRIT), will provide an unprecedented level of support within the state for drug and diagnostic research aimed at fighting cancer. By coordinating the efforts of UT Austin scientists with researchers at Texas medical centers, we are in a much better position to benefit from CPRIT funding.

As TI-3D extends into the future, with continued support from Provost Steve Leslie, we will extend our vision of facilitating translation of the most promising basic science and engineering breakthroughs at UT Austin into tangible benefits for the citizens of Texas and the country.



Mission Statement

TI-3D Transforming Basic Research Into Tools for Better Health

The Texas Institute for Drug and Diagnostic Development was founded in 2005 at The University of Texas at Austin with the following mission:

> To increase scientific collaboration between chemists, biochemists, chemical engineers and medical researchers grappling with complex challenges such as cancer detection and treatment.
>
> To facilitate and foster interaction between these scientists and commercial entities so that life-changing results of chemistry and medical research can be made available more quickly to individuals who will directly benefit from new diagnostic tools and improved treatments.
>
> To train a cadre of research and clinical professionals who will understand the drug development process and support the pharmaceutical and diagnostic industries, with the intent of facilitating the staffing of ventures that arise from technologies conceived and implemented in the state of Texas.

This report describes how TI-3D has utilized funding to make considerable progress in its first three years of existence.

People of TI-3D

Founding Members

Brent Iverson, PhD - Interim Director
Department of Chemistry and Biochemistry

Jonathan Sessler, PhD
Department of Chemistry and Biochemistry

Andrew Ellington, PhD
Department of Chemistry and Biochemistry

Edward Marcotte, PhD
Department of Chemistry and Biochemistry

George Georgiou, PhD
Department of Chemical Engineering
Cockrell School of Engineering

Eric Anslyn, PhD
Department of Chemistry and Biochemistry

Stephen F. Martin, PhD
Department of Chemistry and Biochemistry

Steering Committee

Eric Anslyn, PhD
Department of Chemistry and Biochemistry

Kevin N. Dalby, PhD
Department of Medicinal Chemistry
College of Pharmacy

Andrew Ellington, PhD
Department of Chemistry and Biochemistry

George Georgiou, PhD
Department of Chemical Engineering
Cockrell School of Engineering

Edward Marcotte, PhD
Department of Chemistry and Biochemistry

Stephen F. Martin, PhD
Department of Chemistry and Biochemistry

Robert O. Williams III, PhD
Department of Pharmacology
College of Pharmacy

People of TI-3D

Administrative Staff

Yvonne Murray - Sr. Grants and Contracts Specialist

Ms. Murray was hired in August 2008 to manage TI-3D's financial accounts and expenditures. Prior to joining TI-3D, she was employed in UT's Office of Sponsored Projects for 10 years, where she reviewed grant proposals and award documents, managed the establishment of grant accounts, and processed post-award requests such as no-cost extensions and budget revisions. She brings an understanding of both UT financial requirements and funding agency terms and conditions to her current position within TI-3D. Ms. Murray holds a BA in Communications from Fordham University and an MA in Higher Education Administration from Teachers College, Columbia University.

Angel Syrett - Grants and Contracts Specialist

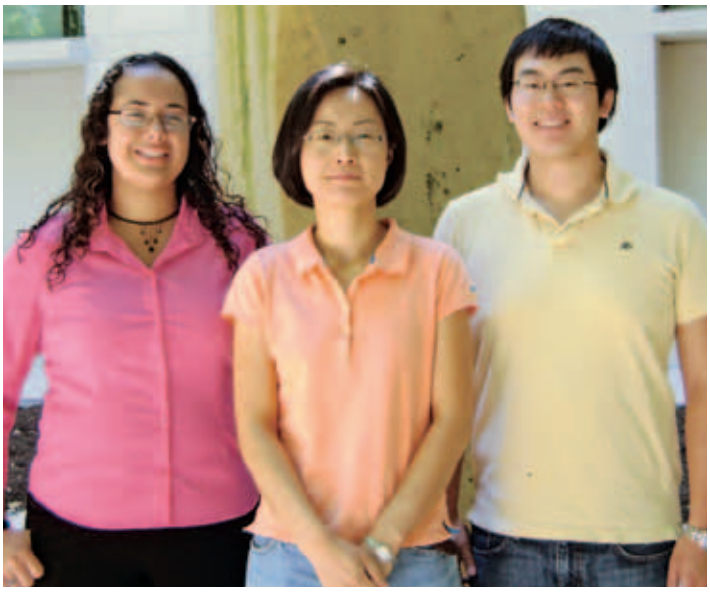
Ms. Syrett was hired in 2009 as a project coordinator and liason for collaborative and inter-institutional grant writing and development. She earned her undergraduate degree in Biochemistry from Southern Oregon University and attended graduate school here at the University of Texas at Austin as a member of the Molecular Biology program. Her scientific experience includes a variety of relevant research including her dissertation project in HIV-1 detection using a novel diagnostic approach. Ms. Syrett has several years of grant writing and editing experience and a strong background in the visual arts. As a member of the TI-3D team, she has assisted in writing, illustrating and editing a variety of individual, collaborative and multi-PI proposal submissions to the NIH, DOD and NSF.

People of TI-3D

Automation and High Throughput Screening Facility

Eun Jeong Cho, Ph. D. - Director

Dr. Cho has been directly involved in the establishment of the TI-3D since 2006. Dr. Cho is the initial point of contact for access to the facility prior to screening activities. She consults with users for assay development, optimization, chemical library screen, hit validation, and lead optimization. She oversees the High throughput screening facility; manages instruments, database, chemical libraries, and facility users. She trains and approves new users for instrumental access and coordinates scientific meetings to stimulate high throughput screening research between scientists on and off UT's campus. Dr. Cho also assists researchers in writing and preparing grant applications. She earned her Ph.D. in analytical chemistry from The State University of New York at Buffalo and worked under Professor Andrew D. Ellington at University of Texas-Austin as a post-doctoral fellow until she joined TI-3D. Her research focused on design and development of chemical and biosensors utilizing various analytical technologies. Before she moved to the state, she worked for Korea's Institute for Science and Technology as a research scientist for six years developing new organosilicon chemicals and their industrial applications. Dr. Cho has published numerous scientific papers and has been an inventor on a total of eighteen patents, several of which she receives royalties from. Her strong expertise in a broad range of chemistry, biochemistry, and molecular biology techniques has been successfully adapted to the multi-disciplinary area of high throughput screening and has resulted in the completion of six screening campaigns over the last two years.



Left to right

Erica Merchant
Eun Jeong Cho - Director
Jonathan Lu

People of TI-3D

Automation and High Throughput Screening Facility

Erica Merchant - Technical Assistant

Erica is a student technician, serves the IT needs of TI-3D. This includes managing user accounts, maintaining the computers, and performing any manner of functions relating to technology in the lab. "It's very rewarding being part of such a friendly, focused team and I'm glad to be able to contribute to the effort at TI-3D." She is an undergraduate linguistics major with a minor in Computer Science and will be graduated in the Fall 2009 semester.

Jonathan Lu - Research Assistant

Jonathan is a student technician, manages the research equipment in the lab, performing periodic maintenance and running assays when necessary. "Scientific research always requires teamwork and I'm happy to be a part of that team." He graduated with a degree in biochemistry in the Fall 2009 semester and continues to work as a research assistant.

Mission Statement

As a major infrastructural component of the Texas Institute for Drug and Diagnostics Development we have brought on-line an Automation and High Throughput Screening Facility. This facility has been established to foster drug discovery and development by enabling access to fully automated compound synthesis and screening of commercial chemical libraries as well as in-house compounds. As a part of the Automation facility, the High Throughput Screening Facility (HTSF) is designed to be highly flexible in order to meet the needs of multiple investigators performing a variety of assays. In addition we aim to provide cost-effective and time-efficient services including:

- Access to high throughput screening technology for researchers across the UT-Campus and for off-campus users.
- Assistance and consultation in assay development.
- Assistance and consultation in assay miniaturization.
- User training on a variety of instrumentation.
- Data analysis consultation.

Operating the Phoenix liquid handling robot.

Equipment and Instrumentation

Teledyne Isco Combiflash Companion 4X: This robot is set-up to purify up to 4 reactions in sequence, using normal-phase flash chromatography (typically with silica gel columns). It features a touch screen for rapid programming of conditions, dual pumps for forming solvent gradients, a dual wavelength UV detector, and an automated fraction collector. It can purify crude reactions from milligram to multigram scale.

Mettler Toledo Minimapper Liquid Handler: This instrument was purchased with a combination of Welch grant and institutional funds. This liquid-handling robot can be easily programmed to add numerous solvents and reagent solutions to reaction vessels or microtiter plates. It can handle small quantities of corrosive reagents under an inert atmosphere.

Thermo Electron Quad Ion Trap Mass Spectrometer: This instrument was purchased with a combination of Welch grant and institutional funds. This spectrometer generates structural information using multiple dissociation techniques including Pulsed Q Collision Induced Dissociation and Electron Transfer Dissociation.

Tech Quip Nitroflow Lab: This instrument is an accessory for the quad ion trap mass spectrometer; it is used to produce commercially sterile nitrogen from a compressed air supply.

Thermo Scientific Microplate Heat Sealer: This instrument securely seals various plate types, which contain either chemical libraries dissolved in 100% DMSO solution or assay reagents for high throughput screening.

Equipment and Instrumentation

Shimadzu Scientific Analytical and Preparative HPLC Systems: Two custom designed Shimadzu HPLC systems for high throughput analytical and preparative work were purchased. Both systems have cooled autosamplers with adjustable configurations for either two 96-well plates, or up to 70 standard HPLC vials, automated eight-solvent and six-column switching and PDA detectors. The preparative system pumps can deliver up to 20 ml/minute and the preparative collection system can also be modified for large volume fraction collection.

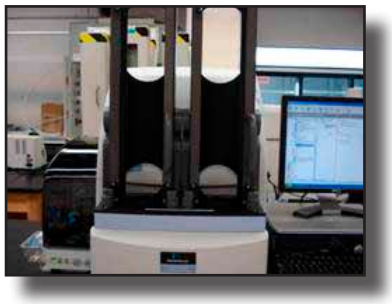
Jasco CD Spectrometer: This instrument was purchased with a combination of Welch grant and institutional funds. It enables high-throughput circular dichroism (CD) measurements from 96-microwell plates. CD spectra are very useful for studying the secondary structure of proteins and their changes under different conditions.

Art Robbins Instruments Phoenix Liquid-Handling Platform: This multiposition, high-throughput liquid-handling platform uses a combination of fixed 384 syringe heads and non-contact dispenser to perform low volume (lower than 1 uL) liquid handling tasks. Its main tasks are library transferring and assay plate formation.

Perkin Elmer Janus Automated Workstation: This laboratory automation workstation performs versatile liquid handling tasks. Its main tasks are plate reformatting, large scale assay plating, and cherry picking.

Equipment and Instrumentation

Perkin Elmer Envision Microplate Reader: This instrument is used for general assay development utilizing various detection technologies such as absorbance (UV-Vis), fluorescence intensity (FI), fluorescence polarization (FP), time- resolved fluorescence (TRF), alphascreen, and luminescence (Lum).



CEM Corporation 48-Position Automated Microwave Synthesizer: This instrument was purchased with a combination of Welch grant and institutional funds. Many reactions which require forcing conditions (high temperatures and reaction times) can benefit greatly from microwave irradiation. The Explorer 48 is easily programmed to automatically run up to 48 reactions in sequence, using 4- 10- or 35- mL reaction vials.

Biotek Microflo Select: This instrument is a self-contained, user programmable dispenser that distributes a wide variety of liquids, including buffered saline and reagent solutions, to micro-well plates.

Equipment and Instrumentation

Miniblock Peptide Synthesizer: This is a flexible, easy-to-use reaction block designed for parallel synthesis and reaction screening. It enables parallel synthesis for solution phase, solid phase, and purification, all on the same platform.

Genevac Centrifugal Evaporator: This evaporator conveniently removes both low- and high-boiling solvents (including DMSO) from test-tubes (e.g. generated by Mini-Block or prep. HPLC purifications) and microtiter plates.

Eppendorf 5810R Centrifuge: This instrument is used for sample re-hydration, including separation in PCR tube strips and test tubes, as well as high-volume centrifugation of tubes, flasks and filter plate systems.

Zebra Z4M Bar Code Printer: This printer is used to prepare bar code labels for all screening processes and compound library.

Perkin Elmer MicroBeta TriLux Microplate scintillation and luminescence counter: Specific for radioactive assay utilizing liquid scintillation or luminescence detection. Applications for use with samples in microplates, tubes or on filters; sample capacitites of up to 16 or 32 plates; updated model is fitted with an external loading platform for fully automatic loading by robot systems.

Equipment and Instrumentation

CEM Liberty Automated Microwave Peptide Synthesizer: The Liberty is an automated solid-phase peptide synthesizer which uses microwave irradiation to minimize reaction times and often give improved yields and purity over standard conditions. The system is capable of making up to 12 peptides a day at scales up to 5 mmol.

VWR Incubating Microplate Shaker: This instrument is used for defrosting and mixing and can shake up to four microplates or two microtube racks with adjustable temperature at a speed range: 100 - 200 rpm.

Collaborating Facilities

Genomic Sequencing & Analysis Facility

Scott Hunicke-Smith, Ph. D.

Dr. Hunicke-Smith joined UT Austin in August of 2008 to start next-generation sequencing activities at UT. Prior to joining UT, he was VP and General Manager of the molecular biology services division of Asuragen, a spin-out of Ambion. He joined Ambion in July of 2003 as Director of Business Development, Instrumentation. He was promoted to Vice President, Business Development in March of 2004 and to Vice President, Ambion Services in September of 2005. Prior to joining Ambion, Dr. Hunicke-Smith served as founder and CEO of GeneMachines, a molecular biology equipment firm, from 1997 through March of 2003. Dr. Hunicke-Smith also served on the Board of Directors of Silicon Genetics, a bioinformatics software firm, from 1998 until Agilent acquired it in 2004. He received his Ph.D. from Stanford University in the laboratory of Ron Davis in 1997 and is an inventor on seven issued patents.

Michelle Byrom - Research Assistant

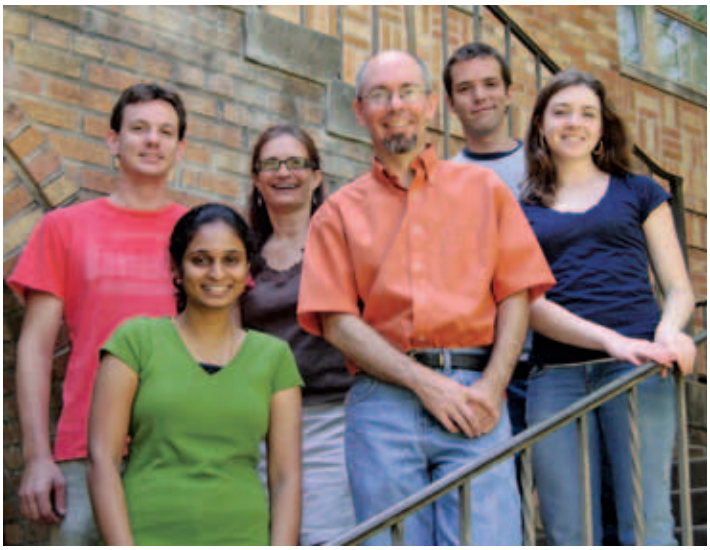
Michelle graduated with a BS in Microbiology with Honors from the University of Texas in Austin in 1997. She immediately began working for UT MD Anderson Cancer Research Center, Science Park Division in Smithville, Texas, where she gained skills in molecular and cell biology techniques. She began working at the Austin campus of the University of Texas and the GSAF in October of 2008.

Dhivya Arasappan - Bioinformatician

Dhivya earned a Masters in Bioinformatics from Virginia Commonwealth University and a Bachelors in computer science from Anna University in India. She joined the GSAF team in April 2008 and primarily deals with analysis of Next-gen sequencing data. Her experience includes work with the National Center of Toxicological Research, FDA where she was involved in analysis of gene expression data and testing and maintenance of the FDA array data anlaysis tool, ArrayTrack.

Andrew Adey - Research Technician

Andrew is a recent graduate from the University of Texas in biochemistry. While pursuing his undergraduate degree he worked at the University of Texas Microarray Core Facility (UTMCF) for nearly three years giving him extensive experience with the technology.



Left to right

Aaron Chevalier
Dhivya Arasappan
Michelle Byrom
Scott Hunicke-Smith - Director
Andrew Adey
Meredith Corley

Meredith Corley - Research Technician

Meredith is currently a double-major biochemistry/music undergraduate at UT. Meredith works primarily on next-gen DNA sequencing applications

Aaron Chevalier - Research Assistant

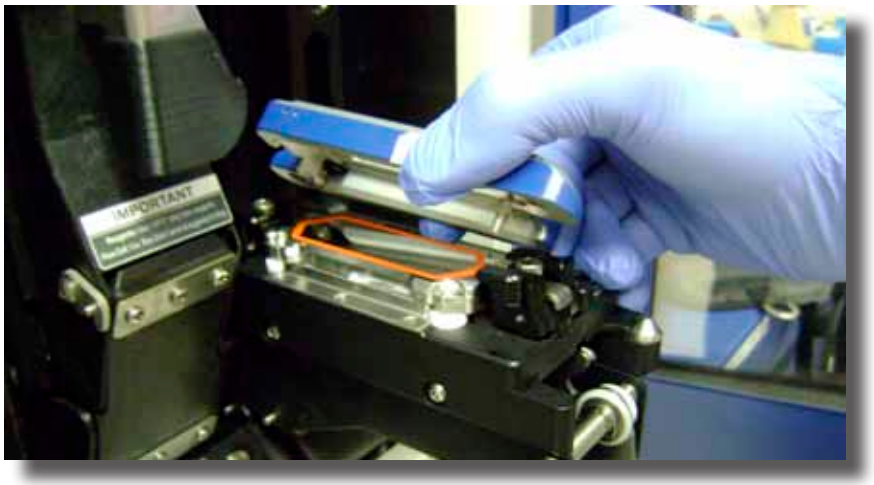
Aaron is currently a graduate student in biomedical engineering with an undergraduate degree in physics from UT-Austin. In addition to working at the facility he researches biosensor assay development and real-time microarrays with Dr. Arjang Hassibi and Dr. Andrew Ellington. He is specifically focused on determining and improving the quantitation limits of microarrays and developing integrated biosensors.

Genomic Sequencing & Analysis Facility

Mission Statement

The Genomic Sequencing and Analysis Facility (GSAF) is poised to be a world-class genomic analysis center in terms of data quality, breadth of available methods, and productivity. The mission of the Genome Sequencing and Analysis Facility is to provide the best quality analytical results with the best value to life sciences researchers. This mission is currently fulfilled through the following GSAF activities:

- Conduct fee-for-service genomic analyses customers with qualified staff and tested procedures.
- Provide access to GSAF analytical and computational equipment for trained and qualified users.
- Train faculty and students in genomic methods for their own independent research.
- Evaluate, acquire, and demonstrate proficiency in leading-edge genomic analysis tools including laboratory methods, reagent systems, analytical instrumentation, and data analysis software.
- Standardize procedures and train staff on an ongoing basis to ensure all results are reproducible and reliable.
- Consult with customers and users to assist them in using GSAF resources effectively.



Loading a slide onto the ABI SOLiD sequencer.

Collaborating Facilities

TherapeuTex Core Laboratory : Drug Dynamics Institute

Carla Van Den Berg, Pharm D.

My lab has had a long-standing interest in various functions of JNK in breast cancer models. JNK is a kinase traditionally characterized as pro-apoptotic and necessary for many anti-cancer agents to induce cell death. Recent studies have demonstrated that JNK is also activated by growth factors to increase cell migration and survival, properties essential for cancer cells to move to distant sites and metastasize. Thus far, we have shown that loss of JNK expression inhibits mammary tumorigenesis and metastatic burden using mouse models. Ultimately we hope to evaluate if JNK can be therapeutically targeted to treat breast cancer patients.

Jamye O'Neal -TherapeUTex core lab manager

In 1998 Jamye earned a B.S. in Biology from Lipscomb University in Nashville, TN. In 2000, she began working at the bench for Dr. R. Stokes Peebles, Jr., M.D. in the Allergy, Pulmonary, and Critical Care Division of the Vanderbilt Medical Center concentrating on infectious diseases and lung inflammation, predominantly Respiratory Syncytial Virus (RSV). In 2006, she moved to Austin to manage the animal colony of Dr. Carla Van Den Berg, Pharm.D, and study the role of JNK in breast cancer. In 2007, she also took over as Dr. Van Den Berg's Lab Manager and in January of 2009 began helping to establish the TherapeUTex Preclinical Core Lab.

Facility

The Drug Dynamics Institute's TherapeUTex Core Facility provides novel and innovative solutions in the modeling, evaluation and development of therapeutically active agents utilizing state of the art translational research. A diverse cross-section of faculty from The University of Texas College of Pharmacy created TherapeUTex making their novel preclinical models and evaluation methodologies for drug development available to scientists on a broad basis.

TherapeUTex is part of the Drug Dynamics Institute, which promotes collaboration with University of Texas faculty, Texas Institute for Drug Diagnostics and Development (TI-3D) investigators, Austin Technology Institute (ATI), along with numerous other academic and industry partners.

TherapeUTex Core focus is on animal models in the areas of cancer, lung inflammation, including asthma, and infectious disease, and will facilitate data generation by processing and imaging tissue or cell samples. Additional animal models will be available and custom models can be developed upon request. The work performed by TherapeUTex is based on the expertise of University faculty and its ability to build collaborations between academia, federally funded research, corporate grants and sponsored research.

Collaborating Facilities

Therapeutex Core Laboratory : Drug Dynamics Institute

Animal Models

- Disease specific animal models for testing therapeutic efficacy, including oncology, lung inflammation infectious diseases
- Customized animal models for special needs including administration by oral, buccal, inhalation, intravenous, Intramuscular dosing
- Evaluation and Analysis
- *In vivo* and *in vitro* target validation studies
- Tissue, dose response, metabolism and toxicology evaluation
- Imaging capabilities
- Pharmacokinetic and pharmacodynamic studies
- State of the art analytical technologies to characterize biological samples and dosage forms, including mass spectroscopy and immunohistochemistry
- Strategic study design to develop intellectual property and commercialization opportunities

Dose form development

- Tailored delivery system development, characterization, and optimization
- Comprehensive and custom development of novel and standard delivery modalitie

Instrumentation

- Hanson SR8-Plus Dissolution Bath, DS-8 AutoPlus DissoScan, MF-8 AutoPlus MultiFill Collector and 8453 UV/Vis-System
- Waters HPLC system including 1525 Micro Binary HPLC pump, 2707 Autosampler, 2489 UV/Visible detector and Empower 2 software
- Malvern STP2000 Spraytec with STP2300 Inhalation Cell and STP2520 Wet Sample Dispersion unit
- Malvern Zen1600 Zetasizer
- MSP Corporation Next Generation Pharmaceutical Impactor
- TA Instruments AR-G2 Rheometer
- Texture Tech TA.XTplus Texture Analyzer
- Thermo Multiskan FC Plate Reader
- Spex SamplePrep 6870 CryoMill
- Thermo Forma Chromatograpy Refrigerator, Forma Enzyme/Lab Freezer, and Forma Ultra Low Freezer
- Eppendorf Refrigerated Clinical Benchtop Centrifuge and 5415R Refrigerated MicroCentrifuge
- Bio-Rad C1000 Thermal Cycler 48/48
- Leica-Meyer Instruments M205 FA Stereomicroscope System
- Leica TP1020 Automatic Tissue Processor
- Leica EG1150H Paraffin-embedding station and EG1150C Cold Plate
- Leica RM2255 Fully Motorized Rotary Microtome
- Leica CM1950 Cryostat
- Leica-Meyer Instruments DMI1600B Inverted Fluorescent Microscope

Collaborating Facilities

Macromolecular Crystallography Core Facility

Arthur Monzingo

Arthur Monzingo is the manager of the Macromolecular Crystallography Facility and has over 25 years experience as a protein crystallographer with more than 40 published research papers. He received a B.A. in biochemistry from Rice University and a Ph.D. in chemistry from the University of Texas at Austin. He was a postdoctoral fellow in the laboratory of Dr. Brian Matthews at the University of Oregon and has, for several years, been a Research Associate at UT-Austin. In addition to managing the Crystallography Facility, he continues to do biochemical research using the methods of protein crystallography in association with the laboratory of Dr. Jon Robertus.

Facility

The Macromolecular Crystallography Facility allows users to solve three-dimensional structures of macromolecules, using X-ray crystallography. The capacities of this center have recently been expanded into a modern core facility. The Macromolecular Crystallography Facility is staffed to carry out structural analysis on a service basis, or to train and assist interested users in both crystallization and the collection, processing, and interpretation of X-ray diffraction data. The Facility is equipped to address a variety of crystallography studies and applications. Our facility houses a host of state-of-the-art equipment including a crystallization robot capable of extremely small volumes to enable high-throughput crystallization experiments. We have the capacity to perform differential scanning fluorimtry to assist in protein-ligand studies in finding ligands that stabilize the structure of a protein molecule, perhaps enhancing its ability to crystallize. We also perform dynamic light scattering studies of a macromolecular solutions to help determine whether the macromolecule is likely to crystallize.

X-ray Data Collection

The Macromolecular Crystallography Facility features three complete X-ray crystallography systems. A VariMax HighRes optical system is mounted on one port of the high flux Rigaku MicroMax 007HF X-ray generator, facilitating the resolution of diffraction data from crystals with large unit cell dimensions. A VariMax HighFlux optical system mounted on the other port of the MicroMax 007HF enables data collection from small and weakly diffracting crystals. A third X-ray port is on a Rigaku RUH3R generator. An Rigaku R-AXIS IV++ image plate detector and Oxford Cryosystems cryo-cooling system (purchased with TI-3D funds) are mounted on each X-ray port.

The facility supports cryocrystallography with tools from Hampton Research and has a dewar for the storage of frozen crystals for future data collection. Macromolecular crystal samples may be submitted to the facility for data collection, and training and assistance is available for users of the X-ray data collection equipment.

The facility also assists users with data collection from synchrotron sources and has a dewar for the shipping of crystals and six Universal V1 pucks and associated tools for remote, automounted data collection.

Macromolecular Crystallography Core Facility

X-ray Data Processing & Structure Determination

To support data processing and structure determination, the Macromolecular Crystallography Facility has two dual core HP workstations installed with current popular crystallographic software, including HKL2000, CCP4, Phenix, CNS, COOT, O, Solve/Resolve, and more. Storage of diffraction data is supported with a 2 terabyte RAID5 file system mounted on a Dell PowerEdge T300 server. The workstations are available for use by trained users. Diffraction data may be submitted to the facility for processing and structural analysis.

Documentation & Training

Training is an important component of the facility mission. Individual training will be provided for the first time user of an instrument, method, or software used in the facility. Subsequent individual training will be provided, by request. Workshops are offered in Crystallization, X-ray Data Collection & Processing, Structure Determination.

Virtual Drug Screening

Virtual drug screening is supported with the TI-3D Drug Discovery Cluster housed within the facility. The idea behind "structure-based" or "rational" drug design is to use a three-dimensional model of a protein active site as the physical template against which to evaluates potential inhibitors. Powerful computer programs search can be used to match thousands of ligands from a database with the target protein. This method is referred to as "virtual screening", a potentially potent tool in drug design that is used by most pharmaceutical houses. The TI3D Drug Discovery Cluster contains 16 HP Proliant BL35P blade servers, each with 2 dual core AMD Opteron 2.4 GHz processors for a total of 64 processors. Each blade contains 8 GB of memory and a 6 GB ATA hard disk drive. The front-end of the cluster is an HP xw9300 Workstation, equipped with a dual core AMD Opteron 2.4 GHz processor, an NVIDIA Quadro FX4500 graphics card, 4 GB memory, and two 500 GB SATA hard drives. Also attached to the cluster is an HP Proliant DL380 G4 storage server with an Intel Xeon Processor (3.4 GHz) which supports a RAID5 network attached storage system with five 500 GB SATA disk drives.

Dr. Monzingo operating the Cryostream 700 ex.

Macromolecular Crystallography Core Facility

Instrumentation

HP Proliant Blade Cluster: This is a computer with 32 dual core processors that be independently addressed, which renders the cluster equivalent to 64 computers acting in parallel. It is used to perform high throughput virtual drug screening, the process of docking three-dimensional models of drug-like compounds into three-dimensional models of potential drug receptors.

Cryostream 700 EX: This instrument is used in X-ray data collection for protein crystallography. Protein crystals are flash frozen in a small fiber loop and are maintained in this frozen state by a stream of super- cooled nitrogen blown over it by this device.

The R-AXIS IV++: This instrument is the most popular X-ray area detector for macromolecular crystallography. It combines two large active area imaging plates with fast readout speeds and a wide dynamic range, making it ideal for collecting accurate diffraction data from the widest ranges of samples in the home laboratory.

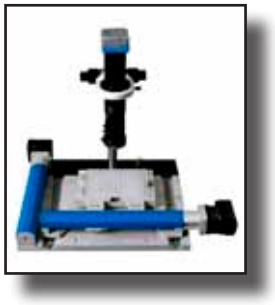
Art Robbins Instruments Phoenix crystallization robot: The Macromolecular Crystallography Facility is equipped with an Art Robbins Instruments Phoenix crystallization robot. It uses extremely small volumes, as small as 50 nanoliters, and is ideal for high throughput crystallization experiments. Leading crystallization screening kits from Hampton Research and Qiagen are available for use. Storage of crystallization plates both at 4 ºC and room temperature is supported. For the automated review of crystallization experiments, the facility is equipped with the Art Robbins CrysCam. High power stereo microscopes are also available for manual review.

Macromolecular Crystallography Core Facility

Instrumentation

Rigaku MicroMax 007 X-ray generator: This microfocus rotating anode X-ray source for structural biology generates 8 × 1010 X-rays/mm²/sec within a focal spot. It is ideal for rapid screening of samples and for collection of high quality data for in-house structure determination. It is designed to be ideal for small and poorly diffracting crystals.

Art Robbins Instruments CrysCam: This digital microscope allows for the acquisition of high resolution images and crystal observation. The user may capture images for an entire plate or manipulate the microscope stage via software user interface.



Walter Fast

Inhibitors of the amidinotransferase enzyme superfamily

The aim of this project is to use a chemical-rescue strategy along with a focused library of amines to develop isoform-selective inhibitors for enzymes in the amidinotransferase superfamily. We have developed a novel method to determine structure-activity relationships by the combinatorial chemical rescue of an impaired enzyme (SAR by chemical rescue) and are using this information for inhibitor design. Expression, purification and characterization of a human isoform, development of the 'SAR by chemical rescue" methodology, an X-ray structure of a trapped covalent reaction intermediate (used as a reagent in the SAR studies), and the synthesis and characterization of one inhibitor series based on these results (including a bound X-ray structure) have all been published, or are in preparation. One of these inhibitors has been forwarded to MD Anderson Cancer Center for biological testing. We are extending our studies on the mechanism of chemical rescue and improving the scoring function for determining "hits" in combinatorial rescue experiments, the planned topics for two future papers.

With this effort, we have started and substantially pursued a new strategy for developing enzyme inhibitors. This work is detailed in two publications, one manuscript in preparation, and experiments for approximately two additional papers are currently underway. This preliminary data and a supporting letter from TI-3D aided a successful NIH R01 grant application, and reviewers described these experiments as creative, interesting and highly innovative.

New Grants Generated

Enzymology of Dimethylargininase
5 R01 GM069754; Walter Fast (PI)
02/01/08 – 01/31/13
National Institutes of Health

Tissue, cell and small-molecule libraries for diagnosing and treating melanoma
In collaboration with Suhendan Ekmekcioglu

The specific aims of this proposal are 1) to develop small-molecule inhibitors of DDAH, along with a paired diagnostic reagent; and 2) to develop a melanoma cell line microarray and a plate-based panel of melanoma cell lines to evaluate the potency, efficacy and biochemical pathways involved in killing melanoma by these DDAH/NOS inhibitors.

In the first few months of this grant, we have made substantial and exciting progress. At UT, Austin, we developed high-throughput screens for two different DDAH isoforms (Z' factors > 0.8). 6500 compounds have been screened as inhibitors of one DDAH isoform, with a second isoform underway. 50 compounds inhibit ≥ 50%. Secondary assays and hit analyses are now prioritizing future work. This work has already provided adequate preliminary data to justify an NIH screening grant, although patent protection for some compounds is under consideration.

At UT, MDACC, we established a melanoma cell line microarray (MCL-MA) containing 60 lines, including radial and vertical growth phases of primary melanoma lines, metastatic melanoma cells and normal control cells, such as human melanocytes, fibroblasts and keratinocytes. Initial stainings to show tissue (in this case, individual cell line pellets) recovery and to test staining techniques, such as antigen retrieval process, were successful. We have established a database to dissect characteristics of these lines, such as mutational status, some of which play a role in NO signaling. We are currently standardizing conditions for a 96-well format to determine DDAH inhibitor efficacy for growth inhibition and cell killing, and to correlate these to the molecular aspects of the biologically diverse cell lines.

New therapeutics of multiple sclerosis

The goals of this grant are 1) to ascertain the specificity determinants of peptidylarginine deiminase; 2) to develop small molecule inhibitors of peptidylarginine deiminase activity and 3) to test these inhibitors in a mouse model of multiple sclerosis (MS).

To determine substrate specificity, an array of 38,000 proteins was incubated with peptidylarginine deiminase (PAD) and probed for posttranslational citrulline modifications. 44 unreported putative protein substrates were identified. We are verifying and characterizing several hits before publishing. Several hits are of particular interest, including: FEZ1, a schizophrenia-associated protein that plays a role in neuronal development; RPS2, a ribosomal protein and therapeutic target in prostate cancer; ING4, a tumor suppressor; among others. We have characterized the RPS2 hit *in vitro* and found that an alternative posttranslational modification, arginine methylation by PRMT3, occurs at the same residues! These two modifications are antagonistic to each other and they suggest an interesting and unexpected avenue for future studies.

We have synthesized inhibitors, but their potency is less than desired, and turned our efforts to high-throughput screening (HTS). The extant 96-well plate assay proved to be unsuitable for HTS, so we are currently establishing a fluorescence polarization competition assay compatible with the TI-3D automation core. A recent paper reports that PAD over expression in mice leads to myelin loss, underscoring the need to develop PAD inhibitors as MS therapeutics. We look forward to continuing these studies and moving potent inhibitors into cell and animal models.

Edward Marcotte

Directed identification of ciliogenic genes impacting neural tube defects
In collaboration with John Wallingford

The major goal of this project has been to identify ciliogenesis genes—implicated in diverse neural tube birth defects including spina bifida—by bioinformatics, testing candidate genes by reverse genetics in Xenopus (frog), a model of vertebrate embryogenesis.

Neural tube defects, including spina bifida, are among the most common and debilitating human birth defects, affecting ~1 in 1000 pregnancies. This project's goal was to apply new computational methods for discovering candidate genes involved in neural tube birth defects, then to test those genes in a vertebrate model of neural tube closure (the frog Xenopus laevis). At the end of our year of TI-3D funding, we have already successfully found 2 new genes whose inactivation can induce neural tube birth defects in a vertebrate, as shown by us for the first time. With these data, we successfully applied for a federal grant from the National Institutes of Health, leveraging our TI-3D grant moneys by >14-fold. We filed a patent on the new computational methods for identifying candidate disease genes and are moving towards licensing the technology. Our computational methods also

revealed candidate genes in diverse other diseases and disease-relevant processes, including Waardenburg syndrome (characterized by craniofacial, hearing, and pigmentation abnormalities), breast cancer, and angiogenesis. We confirmed one gene as a new regulator of angiogenesis in animals and human *in vitro* cell-based angiogenesis assays, serving as a possible target for angiogenesis inhibitors suitable for anti-tumor chemotherapy.

Resulting publications

Kriston L. McGary, Tae Joo Park, John O. Woods, Hye Ji Cha, Srimoyee Ghosh, John B. Wallingford, and Edward M. Marcotte. Systematic discovery of non-obvious human disease models through orthologous phenotypes. Submitted to Science.

Ryan S. Gray, Phil B. Abitua, Bogdan J. Wlodarczyk, Otis Blanchard, Insuk Lee, Greg Weiss, Edward M. Marcotte, John B. Wallingford and Richard H. Finnell. The planar cell polarity effector protein Fuzzy is essential for targeted membrane trafficking, ciliogenesis, and mouse embryonic development. Submitted to Nature Cell Biology.

Patents Filed

Edward M. Marcotte, John B. Wallingford, Kriston L. McGary, Tae Joo Park. Systematic Discovery of Non-Obvious Human Disease Models and Candidate Disease Genes Through Phenologs.

New Grants Generated

Network-directed discovery of disease genes
2R01 GM067779-01A1; Edward Marcotte (PI)
04/01/09 – 03/31/13
National Institutes of Health

Sponsored Research Projects

Eric Anslyn

Development of novel small molecule anti-cancer compounds
Our role in this project was to make small molecule inhibitors of a proprietary cancer target discovered at M.D. Anderson Cancer Center. Overall, the project has allowed for synthetic advances toward the key heterocyclic ring system found in the lead compound. While the parent heterocycle is commercially available, it is extremely expensive. We have discovered a single step transformation which creates it. However, we have now found that further dervitization is not successful. We are therefore returning to making the ring system with appropriate substituents already attached to allow for further functionalization.

Identifying inhibitors of NS1α
Our role in this project is to create small molecule inhibitors for NS1α. We specifically are seeking to understand what structural elements are good leads. Several thousand compounds have been screened for inhibition of NS1α. A series of lead compounds have been identified. The leads are structurally homologous and derive from tannins, flavones, isoflavones and structurally similar ring systems. We have devised two simple routes for ring system construction that should yield facile library creation using parallel synthesis.

Katherine Brown

Development of new protein-based therapies for pre and post exposure to *Burkholderia mallei*, the causative agent of glanders
Burkholderia mallei, the causative agent of glanders, has the 'potential for large scale dissemination with resultant illness' and has been designated a category B biological agent by the Centers for Disease Control. Due to its high level of drug resistance there is no licensed vaccine for glanders. This work is focused upon the development of vaccines for *B. mallei* and *B. pseudomallei*, and the development of reagents for use in both vaccine and diagnostic applications. Surface and secreted proteins from both Burkholderia strains were purified and assessed for protective efficacy in a mouse model for glanders infection with our TI-3D collaborator, Professor Mark Estes at UTMB – Galveston. *B. pseudomallei* recombinant proteins and purified lipopolysaccharide (LPS) from a related organism, *B. thailandensis*, have shown protective efficacy in a mouse model for melioidosis. Several Burkholderia proteins are now being used for the generation of high affinity aptamers and antibodies for use in diagnostic and therapeutic testing.

New grants generated

Novel Molecular Recognition Reagents for Detection of Pathogenic Burkholderia
U01 AI078008; Kathering Brown (PI)
06/15/08 – 05/31/11
National Institutes of Health

Kevin Dalby

Targeting PERK and the tumor's stress response for cancer therapy
The overall goal of this project was to develop a highly potent and selective inhibitor of the important transcription factor protein kinase JNK for the treatment of breast cancer. Because of the important role of this protein kinase in transcription, it is a highly attractive cancer therapeutic target. Thus we have screened for effective inhibitors to this and other transcription related kinases such as mitogen-activated protein (MAP) kinase. A combinatorial 96-well peptide library approach was developed, to facilitate the generation of high affinity MAP kinase inhibitors, and several viable candidates for kinase inhibition studies were obtained. The mechanism of these inhibitors - ranging in affinity from 10-200 nM - is currently being assessed using *in vitro* kinetic approaches and tissue culture models.

Development of novel analgesics
This project, still in early stages, aims to develop selective inhibitors of the protein kinase ERK5. ERK5 expression and activation is currently being optimized in insect cells. A fluorescent peptide substrate for ERK5 has been developed in anticipation of high throughput screening.

Identification of inhibitors of EEF2 kinase
A screening assay will be developed. eEF2 kinase, suitable for structural studies, will be prepared from insect cells. We successfully achieved the first purification of recombinant eEF2K suitable for biophysical studies and then identified of the most efficiently phosphorylated eEF2K substrate peptide. This enabled the first analysis of eEF2K peptide substrate kinetics to be performed and facilitated the development of a 96-well assay format for the screening for eEF2K activity against compound libraries of small molecules. The synthesis and characterization of a fluorescent peptide substrate for eEF2K, suitable for automated high throughput 384-well screening was achieved. Preliminary crystals of full-length recombinant eEF2K were obtained after screening 1400 different buffer conditions.

Sponsored Research Projects

Andrew Ellington

Targeting micro RNAs with small molecules: a novel approach

We are developing a high-throughput assay for compounds that can modulate the function of microRNAs. In collaboration with the TI-3D HTSF and with Dr. George Calin at MD Anderson, we will identify and screen compounds with this assay. In collaboration with **Chris Sullivan** (Assistant Professor in MGM) a cell-based assay for microRNA function has been developed wherein luciferase constructs containing microRNA binding sites in their UTRs were stably transfected into tissue culture cells. The luciferase reporter is down regulated by a specific viral microRNA (from the JC virus, the etiologic agent of a neurological disease called progressive multifocal leukoencephalopathy (PML) that occurs in immunodeficient patients. In control experiments, treatment with anti-sense inhibitors specifically down-regulates microRNA activity and up-regulates luciferase. The dynamic range of the assay is at least six-fold and the reporter cells have proven to be stable for at least two months. We are currently optimizing this assay for large-scale screens in the TI-3D Automation and High Throughput Screening Facility. We will work with our collaborators at MD Anderson to carry out virtual screens for compounds that may interact with the JC virus microRNA, and introduce these into the assay system.

Christine Schmidt

Identifying nucleic acids and small molecules that promote nerve growth

In collaboration with Andrew Ellington

Our objective is to adapt aptamers selected against the Nogo-66 receptor (NgR) to function in nerve regeneration models. Breakdown products of myelin inhibit nerve regeneration, in part through interactions with the Nogo-66 receptor (NgR). The Ellington lab has selected RNA aptamers against NgR. We have assayed these aptamers for their ability to promote neurite outgrowth in dorsal root ganglia isolated from rats. An increase in both the number and length of neurite outgrowths were observed. The aptamer proved superior to a peptide inhibitor and could block the effects of several different myelin breakdown products. These results are being submitted to PLoS Biology. A modified, 2-fluoropyrimidine aptamer that should be more stable *in vivo* is being selected. We are also attempting to determine if the anti-NgR aptamers can be endocytosed, and thus might be used to carry cargoes such as siRNAs to neurons. As a model system we chose PC12 cells, which have previously been reported to express NgR, but we could not detect expression using either anti-NgR aptamers or antibodies. We are currently seeking additional, non-primary cell lines that may express NgR and are also attempting to express NgR in tissue culture cells. Ultimately, we hope to assay the aptamers for their ability to repair spinal cord injury in an animal model.

George Georgiou

Humanizing arginine deiminase for cancer therapy
In collaboration with Walter Fast

The goal of this research is to develop human enzyme variants that destroy the amino acid L-Arginine and are thus cytotxic for arginine auxotrophic cancers, namely hepatocellular carcinomas and melanomas. Certain human cancers that are highly refractile to chemotherapy. Hepatocellular carcinomas and melanomas are auxotrophic for the non-essential amino acid L-Arginine and experience cell cycle arrest and apoptosis in its absence. L-Arginine depletion by PEGylated bacterial arginine deiminase has shown significant promise in animal models and clinical trials but its use is confounded by immunogenicity. We use bioinorganic and saturation mutagenesis strategies to resolve the therapeutic shortcomings of hArg I. Replacing the metal cofactor with Co2+ resulted in a 10-fold decrease in binding for substrate L-Arg and a concomitant increase in kcat/Km as well as increased enzyme. Fine tuning the metal coordination by saturation mutagenesis in the dinuclear metal binding site led to the isolation of variants displaying an even further increase in catalytic efficiency binding affinity at physiological than for the parental enzyme. *In vitro*, the modified enzymes displayed approximately 15-fold lower IC50 values in killing human hepatocellular carcinoma and melanoma cells, indicating the promise of this enzyme as a therapeutic candidate.

Resulting Publications

Everett M. Stone, Lynne Chantranupong, Evan S. Glazer, Paul Cherukuri, Steven A. Curley, and George Georgiou. Engineering of Human Arginase for Improved Chemotherapeutic Dynamics in Treating Melanomas & Hepatocellular Carcinomas. Submitted to PNAS.

Targeted therapy of malignant melanoma using engineered human arginases

We are using existing arginase structures to pick putatively important residues for randomization followed by screening for activity and stability. We hope to find variants with greater k_{cat}/KM than human arginase and the bacterial homolog. We achieved the activity goal and matched the bacterial enzyme in cancer cytotoxicity, but only a few of those variants were also stable.

Co-hArgI displayed markedly improved cytotoxicity against a human melanoma and a hepatocellular carcinoma cell line relative to the Mn-hArgI. In fact, the Co-hArgI displayed an IC50 on par with that of the bacterial arginine deiminase (ADI) currently undergoing advanced clinical evaluation. As a human enzyme, Co-hArgI is expected to have a much more favorable immunogenicity profile compared to the heterologous *M. arginii* ADI. To use Co-hArgI as a cancer therapeutic, it will be necessary to engineer the protein for long serum persistence. Similar protein engineering and directed evolution approaches can be deployed to change the catalytic properties of human enzymes to develop catalysts of clinical interest. Efforts to develop human enzymes displaying asparaginase activity that might be used to replace the FDA-approved *E.coli* enzymes currently in clinical use for the treatment of acute lymphoblastic malignancies and also human L-methioninase to replace the Pseudomonas enzyme are currently under way.

Sponsored Research Projects

Michael Krische

Ansamycin analogues: Hsp90 inhibitors for cancer treatment
The specific aims of this proposal are: (a) to prepare libraries of ansamycin analogous using catalytic C-C bond formations developed by Krische, and (b) to submit these analogs to the i*n vitro* assays of Obermann in order to identify potent analogues, and especially (c) analogs that preferentially act upon the Hsp90α isoform.

After establishment of the subaward agreement between UT Austin and UT Galveston, a post-doctoral research associate was recruited to the project. Dr. Michael Rössle began work in the laboratory and is now actively engaged in the synthesis of the ansamycin antibiotic micotrienol. The total synthesis of micotrienol will set the stage for the synthesis of the more complex ansamycin antibiotic, geldanamycin, and related analogous for *in vitro* assay.

Hung-wen Liu

Development of cell surface glycan-based cancer vaccines
We are working toward the development of cell-based methods to construct pancreatic and melanoma cancer-specific cell surface glycans containing sialic acid moieties modified with biocompatible functional groups (e.g., azido and keto groups). Added functional groups might serve as anchors for the chemoselective attachment of various adjuvants to the glycan antigen for stimulation of the immune response upon vaccination. Both peptide-based and non-peptide-based immunity will be exploited. This is expected to facilitate antigen processing and subsequent presentation. Cancer vaccines with the above properties will be generated. It is our hope that when these conjugated glycans are used in vaccination, cancer-specific peptide-based and non-peptide-based effector cell maturation will be achieved.

We have focused on preparation of azido-N-acetyl-mannosamine, a synthetic monosaccharide that can be incorporated into sialic acids at the cancer cell surface by the cellular sialic acid biosynthetic machinery. The alkynyl conjugated TLR7 agonist resiquimod is also being prepared. Progress has been made in the former synthesis, but the latter was hampered by the unexpected difficulty to secure resiquimod which we have undertaken to prepare ourselves. Once the synthesis of the azido sugar is complete, growth of the pancreatic cancer cell line PANC02 and the melanoma cancer line B16F10 in medium containing the azido sugar will be commenced. When the alkynyl conjugated resiquimod is synthesized, it will be used to react with the azido-modified cell surface sialic acid by a Cu(I) catalyzed cyclization reaction. The resulting resiquimod-cancer cell conjugate will be used for vaccination. The direct conjugation of the adjuvant, resiquimod, to the surface antigen can facilitate antigen processing and subsequent presentation by both class I and class II MHC molecules, resulting in a dramatic reduction of the required antigen dose. Recently, we have also set up our own cell culture laboratory, which is now fully operational.

Exploration of the therapeutic potential of poly(ADP-ribose)-polymerase-1: Developing PARP-1 inhibitors for neurological diseases

PARP-1 is a eukaryotic nuclear protein which catalyzes poly(ADP-ribosylation) of numerous nuclear acceptor proteins using NAD+ as the ADP-ribose donor. Inhibition of PARP-1 activity has been shown to significantly reduce injury in acute CNS disorders such as ischemic stroke, traumatic brain injury, and hypoglycemia. PARP-1 inhibition also facilitates long-lasting neuronal survival following ischemia reperfusion. Clearly, PARP inhibitors show great promise for the treatment of both acute and chronic neurological injuries. However, most of the known PARP inhibitors are NAD+ mimics that can react with other NAD(P)+-dependent enzymes, producing undesirable side effects. The development of specific PARP-1 inhibitors is the main goal of this research. To facilitate this drug discovery effort, we are also studying the basic biochemical properties of PARP-1, which remain largely unexplored.

Our studies on domain C of PARP-1, which lies between the N-terminal DNA-binding domains and the C-terminal catalytic domain, suggest that upon DNA binding domain C activates PARP-1 by inducing a rearrangement in the protein, giving rise to a new conformation which is more favorable for catalysis. Thus, any chemicals that bind to domain C or at the interface between domain C and other domains of PARP-1 could be potential inhibitors. To facilitate the identification of lead compounds, we have devoted our initial effort to the development of high-throughput assays that can be used to screen the chemical libraries available from the TI-3D collection for PARP-1 inhibition. Using biotinylated NAD+ as the substrate, we have detected poly(ADP-ribosyl)ation of immobilized histones on 96-well plate surfaces by PARP-1 (and by the domain C and ABDEF complex). The modified proteins have biotin moieties within their associated PAR polymers and can be readily detected by flourophore-labeled streptavidin. However, the biotinylated NAD+ is no longer available from commercial sources. Currently, we are synthesizing a few NAD+ derivatives that will be coupled with various fluorescence and chemical tags using click chemistry. These NAD+ analogues will be tested for their suitability in high throughput assays.

Resulting publications

Z. Tao, P. Gao, H.W. Liu. Identification of the ADP-ribosylation sites in the PARP-1 automodification domain: analysis and implications. J Am Chem Soc. 2009 Oct 14;131(40):14258-60.

Stephen F. Martin

Design and development of agents that block the viral protein docking site in alix

The objectives of the proposed research are to: (i) design and prepare short peptides and pseudopeptides derived from viral YPXnL late domain sequences of the retroviral proteins HIV-1 p6Gag and the EIAV p9Gag; (ii) determine the binding free energies, enthalpies and entropies of these synthetic molecules for the recombinant V domain of Alix; (iii) determine the structures of complexes of the Alix V domain with selected peptides; and (iv) determine whether selected peptides and pseudopeptides compete with retroviral proteins HIV-1 p6Gag and the EIAV p9Gag for binding to Alix in cellular settings.

Analysis of the x-ray structure of the Alix V domain with the late domain sequence of the EIAV p9Gag protein suggested that peptides derived from the sequence LYPDL would have high affinity for the Alix V domain. In order to verify this hypothesis, we have prepared or have nearly prepared N-terminal acylated derivatives of peptides LYPDL, YPDL, LYPD and LYP. We have not been able to get an expression vector for a fusion construct of the Alix V domain, although efforts toward this goal are in progress. However, we now have an expression vector for the GST fusion protein of the Alix Bro1–V domain and have begun studies to purify and express the protein so we can determine binding affinities of our synthetic peptides for this domain. Although we prefer to conduct *in vitro* binding and x-ray crystallographic studies with the Alix V domain alone, it is known that the Alix Bro1–V domain and the Alix V domain have comparable binding affinities for the late domain sequence of EIAV p9Gag. We anticipate completing preliminary binding studies prior to the termination of this grant. The results should then serve as the basis for a collaborative NIH proposal.

Identification of novel potassium ion channel modulators

The goals of the proposed research are to identify and identify novel analogs of 6-bromo-2-mercaptotryptamine dimer (BrMT)2 as modulators of potassium ion channels and to establish preliminary SAR for these compounds. Adequate quantities of active compounds will be prepared for structural studies.

A reliable synthetic procedure for the synthesis of the natural product 6-bromo-2-mercaptotryptamine dimer (BrMT)2 and a series of six other analogs having different substitution patterns on the indole ring was developed. Procedures were also developed for the synthesis of analogs of (BrMT)2 in which the unstable disulfide linker was replaced with either a –(CH2)4– or a –CH2OCH2– linker, thereby leading to five additional compounds. Nine of these novel derivatives of (BrMT)2 have been tested in preliminary patch clamp assays to ascertain their ability to modulate the opening of potassium channels. Three compounds having interesting activities have been identified. For example, 6-chloro-2-mercaptotryptamine dimer appears to be approximately an order of magnitude more potent than the natural product (BrMT)2; the 6-methyl analog was also more potent than (BrMT)2. Of the compounds having different linkers between the indole rings tested thus far, those having the ether linker appear to be the most active and interesting for further examination. Based upon these results, we are presently in the process of preparing additional analogs of (BrMT)2 to evaluate their ability to modulate potassium ion channels.

Identification of novel fortilin inhibitors

The goals of the proposed research were to: (1) establish *in vitro* and *in vivo* assays suitable for screening the effects of small molecules on secondary structure and anti-apoptotic effects of fortilin; and (2) identify small molecules, some of which will be artemisinin analogs, that disrupt the helical secondary structural elements of fortilin and/or that are antagonists of fortilin anti-apoptotic activity.

We demonstrated that dihydroartemisinin (DHA), a derivative of the natural product artemisinin (ART), reduces cellular fortilin levels in U2OS cells—a osteosarcoma cell line. ART also reduces cellular fortilin levels, albeit to a lesser extent. We then determined that DHA reduces cellular fortilin levels in various cell types. Based upon these results, we initiated SAR studies and prepared deoxy-DHA (dDHA) and deoxy-ART (dART) to ascertain whether they have the same effects as DHA. dDHA reduced fortilin expression in U2OS cells significantly less than did DHA, whereas dART did not reduce fortilin expression in this system. These results suggested that the peroxy bridge in DHA and ART is important for fortilin reducing activity. Additional studies are needed, but difficulties with reproducing the cellular assays rendered continuing the SAR studies problematic. Hence, we tried to develop *in vitro* assays of binding of small molecules to fortilin based upon preliminary work of Dr. Fugise, but we were unable to develop a reliable assay. Dr. Fugise moved to UTMB during the course of this collaboration and was unable to continue his participation in the project. We then turned to establishing parallel synthesis capability within TI-3D and to developing new methods for generating diverse libraries.

Resulting publications

J. H. Clements, J. E. DeLorbe, M. G. Teresk, A. P. Benfield, H. R. Plake, L E. Millspaugh, and S. F. Martin, "Thermodynamic and Structural Effects of Conformational Constraints in Protein-Ligand Interactions. Entropic Paradoxy Associated with Ligand-Preorganization."

J. D. Sunderhaus, C. Dockendorff, and S. F. Martin, "Novel Applications of Multicomponent Reactions and Functional Group Pairing to the Synthesis of Diverse Heterocyclic Scaffolds."

New grants generated

Generating Diverse Pilot-Scale Libraries for Screening
P41GM086192 ; Stephen Martin (PI)
9/1/08 to 8/31/11
National Institutes of Health

John Mihic

***In vitro* screening of novel therapeutic agents for the treatment of anxiety**

It is the purpose of this project to identify novel molecules that affect the functioning of $GABA_A$ receptors specifically mediating the anxiolytic effects of benzodiazepines *in vivo*. The ultimate goal is the development of compounds that have anxiolytic effects without producing sedation, a major drawback of benzodiazepines currently used to treat anxiety.

Using the technique of phage display we conducted cell-based selections in an attempt to identify novel peptides that bind specifically to a particular $GABA_A$ receptor subtype believed responsible for mediating the anxiolytic effects of benzodiazepines. Using the New England Biolabs Ph.D.-C7C phage display library we identified 37 phage each bearing a unique heptapeptide amino acid sequence that allowed it to bind to $\alpha2\beta2\gamma2$ $GABA_A$ receptors. This $GABA_A$ receptor subtype has been implicated in mediating the anxiolytic effects of benzodiazepines. Negative selection was done using $\alpha1\beta2\gamma2$ $GABA_A$ receptors that are responsible for the sedating effects of benzodiazepines. Some of the phage sequences identified showed clear amino acid homology with others that were also found during this screen, suggesting that specific motifs of amino acids work can distinguish between $\alpha2\beta2\gamma2$ and $\alpha1\beta2\gamma2$ $GABA_A$ receptors. Thus Aim 1 of the project has been completed successfully and we are currently synthesizing five peptides to test electrophysiologically to determine if they can enhance $\alpha2\beta2\gamma2$ but not $\alpha1\beta2\gamma2$ $GABA_A$ receptor function.

New grants generated

In vitro screening of novel therapeutic agents for the treatment of alcohol abuse
R03 AA018197-01; John Mihic (PI)
8/1/09 to 7/31/11
National Institutes of Health

Jon Robertus

Computer aided virtural screening for drug discovery

The goal of this project was to use computer assisted docking to facilitate enzyme inhibitor identification. The process, called virtual screening, may expedite drug development. The objective was to use virtual screening on several projects at the University of Texas, and to initiate work on novel screening algorithms.

As part of this project we acquired and implemented two commercial programs commonly used for virtual screening: ICM and GOLD. These programs are now accessible from the TI-3D server. We also established several large virtual screening libraries. These include Sigma (50K compounds), ChemBridge Diversity (50K), Maybridge Diversity (50K), ChemBridge Kinase (13K), ChemBridge Fragment (5k), Spectra (12K, and NIH Clinical (0.5K). We used these tools to assist in several inhibitor design projects. These include as targets: ricin A chain (RTA), influenza NS1, fungal methionine synthase, and several protein kinases. An effort was made to carry out screening of the dengue fever virus in collaboration with the team at UTHSCG. The goal was to use domain 3 of the E protein as a target. Unfortunately no robust assay for binding could be developed to evaluate the effectiveness of the virtual screen.

In the RTA project virtual screening successfully identified two novel inhibitor platforms which are being explored; these are the 2-amino dihydroxy pyrimidines and the amino thiol-diazoles. The solubility of the 2-amino dihydroxy pyrimidine makes it an attractive target for inhibitor diversification and this is being pursued with TI-3D collaborators in the Anslyn group.

Resulting publications

Bai,Y., Monzingo, A.F, and Robertus, J.D. The X-ray structure of ricin A chain with a novel inhibitor. Arch. Biochem. Biophys 483, 23-28 (2009).

New grants generated

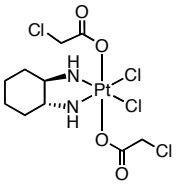
Small molecule inhibitors of ricin and shiga toxins
AI075509; Jon Robertus (PI)
National Institutes of Health

Jonathan Sessler

Platinum-texaphyrin cisplatin conjugates and drug resistance interactions

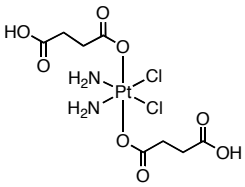
The goal of this project has been to develop new potential leads by combining texaphyrins, invented by the Sessler group, with various cytotoxic platinum agents that are being extensively studied by the Siddik group. Texaphyrins (e.g., motexafin gadolinium; MGd) have an ability to localize well to many cancers, including metastatic non-small cell lung cancer (NSCLC). They have been studied in recent years as potential anticancer agents and appear to operate via a novel mechanism of action that involves perturbations of redox balance and metal homeostasis. In contrast, cytotoxic platinum complexes, including the FDA-approved Pt(II)-based drugs cisplatin, carboplatin, and oxaliplatin, are thought to mediate their action through promoting irreparable DNA lesions. This mode of action has made them mainstays in cancer chemotherapy, however, it may also account in part for the resistance to Pt drugs seen after initial treatments. This resistance often becomes dose-limiting and, in the case of NSCLC, is manifest in a ca. 5% five-year survival rate.

The fact that texaphyrins localize well to tumors means that they could be used to deliver Pt-type drugs to cancerous sites, thereby increasing potentially the local concentration and overcoming the effects of resistance. This project is designed to test this possibility via the synthesis and study of texaphyrin-Pt conjugates. These efforts will be complemented by detailed analyses of mechanism that are designed to probe in particular whether the different modes of action of texaphyrin and Pt complexes give rise to synergistic activity, as some initial (and unpublished) studies with cisplatin (and carboplatin) and MGd would suggest.



1

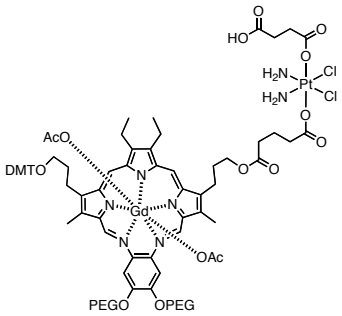
To date, platinum(IV) complexes 1 and 2 have been prepared by the Sessler Group. Complex 1 is reminiscent of DAP, the Pt (IV) derivative currently under heavy investigation by the Siddik group. The chloride leaving group in the α-positions of the axial chloromethyl acetate ligands is thought to provide a synthetic handle for conjugation to a texaphyrin. As yet this approach has not been explored.



2

A second approach centers around the use of complex 2. This species has been previously conjugated to estrogen derivatives, as reported by Keppler, B. K. et al. (J. Med. Chem. 2007, 50, 6692–6699); this is precedence for its conjugation to texaphyrin. With an exploration of this chemistry in mind, Mitsonobu reaction conditions were used in an effort to produce conjugate 3. Unfortunately, however, minimal conjugate could be detected by HPLC and ESI-MS analyses. Rather, multiple potential products were detected.

Selective protection of one peripheral hydroxyl may alleviate the formation of some undesired side products. While this route is currently being explored, it is just one of many other routes that will be studied in due course.



3

REG SGC function by non-heme tetrapyrrole macrocycles

Soluble guanylyl cyclase, the only recognized receptor for signaling molecule nitric oxide (NO), is involved in a number of important physiological processes. These processes include, but are not limited to, smooth muscle relaxation and blood pressure regulation, platelet aggregation, neurotransmission and memory. Mice with deficient sGC enzyme develop chronic high blood pressure, making this enzyme a candidate for the pharmacological therapy or prevention of hypertension,

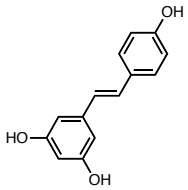
This project combines the expertise of the Sessler group in oligopyrrole synthesis with that of the Martin laboratory in soluble guanylyl cyclase (sGC) regulation and is designed to test the hypothesis that replacing the normal heme found at the regulatory site of sGC with vitamin B12 and other corrin derivatives will modulate activity of this all-important enzyme. It thus involves an iterative process of synthesis and testing.To date, 36 corrin derivatives with structures similar to factor B12 have been synthesized and tested for sGC activation.

On the basis of these studies, it is concluded that only a subset of peripheral modifications allow for sGC activation. In fact, initial analyses support the conclusion that the use of substituted phenols in more than one position produces a compound that is either ineffective or even inhibitory, with the same being true for alkyl substituents (data not shown). On the other hand, one derivative, termed fBm3a, with polar groups shows activity that makes it attractive for further study. Several other compounds also demonstrated activity. Comparison of effective derivatives with the same modifications, but different axial ligands (e.g., fBm6/fBm7 or fBm2/fBm3 in Table 1), gives rise to a tentative conclusion that aqua-cyano ligation of the cobalt ion is superior to the dicyano ligation, also present in factor B12, or diaqua ligands. There is thus a pathway for further optimization that comes to mind based on the work carried out to date, namely maintain polarity of the substitutents while controlling the axial ligation.

Dionicio Siegel

Redesign resveratrol to overcome chemoresistance in human multiple myeloma cells
The objective of the proposed research has been to synthesize resveratrol analogs that are predicted to possess improved metabolic profiles and improved biological activity against multiple myeloma (MM). Biological testing at the MD Anderson Cancer Center will be conducted to determine if the analogs cause:

- Suppression of the proliferation of drug-resistant MM cell lines and potentiate the apoptotic effect of Velcade and thalidomide.
- Accumulation of MM cells in sub G1 phase, increased release of Bax protein accu mulation and activation of caspase-3.
- Suppression of AKT activation and inhibition of the expression of antiapoptotic proteins in MM cells.
- Inhibition of constitutively active NF-KB in MM cells.
- Inhibition of constitutively active and IL-6 inducible STAT3 activation in MM cells.
- Potentiation of the effect of Velcade and Thalidomide on NF-KB and STAT3 activation in MM cells.

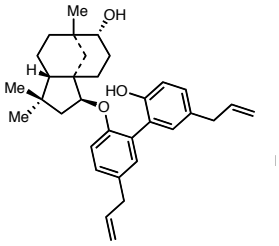


Resveratrol

We have completed the syntheses of nine resveratrol analogs to date with different substitution patterns on the three phenolic hydroxyl groups. Resveratrol analogs with acetoxy and methoxy groups at the phenolic positions have been prepared on the 200 mg scale for testing. These analogs are predicted to slow metabolism of the compounds, occurring by glucuronidation of the free phenolic groups, relative to the natural product. These analogs are currently being transferred to the MD Anderson Cancer Center for testing in the Aggarwal lab. After testing of the first set of compounds has been performed a second round of structural optimization will be conducted. By repeating this process between biologists at MD Anderson and chemists at UT Austin we hope to develop a new compound based on the resveratrol scaffold that can be transitioned to pre-clinical studies for the treatment of multiple myeloma.

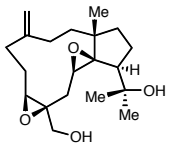
Synthesis and neurobiological evaluation of clovanemagnolol and stolonidiol

The first objective of the proposed research has been to develop practical, efficient, and enantioselective laboratory syntheses of two natural products clovanemagnolol and stolonidiol that have both been shown to possess neurotrophic activity. The long-term objective is to use these compounds and their derivatives to aid in the discovery of new drugs and drug targets for neurodegenerative diseases. Currently we are working to characterize the actions of these small molecules in primary neuronal cultures in an effort to examine their neurotrophic effects and to identify their molecular targets. We are also seeking to characterize the neurotrophic properties of the compounds as they relate to neurite outgrowth and synaptogenesis. In addition, we are attempting to validate our hypothesis that these natural products interact with proteins that control gene expression.



Clovanemagnolol

We have completed the synthesis of the neurotrophic small molecule clovanemagnolol in a six-step sequence. The synthesis has provided the natural product as a single enantiomer and generated over 250 mg of clovanemagnolol to date. This material will support further biological investigation. The syntheses of clovanemagnolol analogs have also been initiated and the current synthetic route will be used for the synthesis of the isomeric natural product caryolanemagnolol.



Stolonidol

Significant progress towards completing the synthesis of stolonidiol has been achieved and we have charted a synthetic route that will provide the small molecule inducer of choline acetyltransferase activity in twelve or fewer steps. With an advanced intermediate in hand, prepared in five steps from geraniol, we are attempting one of the key transformations an allylic Hiyama coupling reaction. As planned the successful synthesis should be easily scalable providing stolonidiol on the gram scale.

Preliminary biological testing has been accomplished with a structurally related natural product, magnolol, also prepared by synthesis in the Siegel lab. The addition of magnolol to developing neurons increased the length and number of dendrites in primary neuronal cultures relative to control. Similar biological testing of clovanemagnolol is underway as is the identification of a putative biomolecular target.

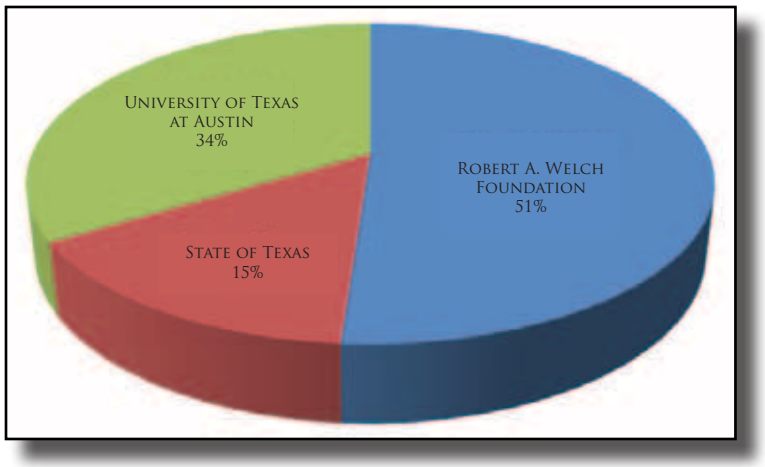
Financial Report : Funding Sources

Funding Sources

TI-3D has received financial support from three important funding sources:

$3,500,000 from the Robert A. Welch Foundation
$1,040,199 from the State of Texas
$2,304,000 from The University of Texas at Austin.

Total financial support to date - $6,844,199



Robert A. Welch Foundation

The Robert A. Welch Foundation is one of America's oldest and largest private funding sources for basic chemical research. In January 2006, TI-3D received a $3,500,000 grant from the Welch Foundation. This grant was primarily utilized to finance equipment purchases for TI-3D's Automation and High Throughput Screening Facility and fund drug and diagnostic applications "Seed Projects" at UT.

Funding Sources

The State of Texas Science and Technology Acquisition and Retention "STARs" Awards

Funding from the State of Texas was received in the form of STARs funding. Retention and recruitment of the most gifted university faculty members is a priority of the State of Texas, as evidenced by the existence of Faculty Science and Technology Acquisition and Retention awards, known as STARs. The State allocates this funding to universities that have demonstrated a need to retain or recruit top faculty, particularly in areas of emerging technology that have the potential to positively impact the economy of the State. In the Fall of 2006, five of TI-3D's founding members received STARs funding from the State of Texas:

Eric Anslyn Andrew Ellington George Georgiou Brent Iverson Edward Marcotte Jonathan Sessler

These founding members made the decision to utilize a portion of their STARs financial support to purchase state of the art laboratory equipment necessary for automation, high throughput screening and protein expression. The combination of their STARs contributions used for these equipment purchases totaled $1,040,199.

The University of Texas

As a direct result of STARs and Welch Foundation funding, The University of Texas has committed $2,304,000 to date in seed funding to TI-3D. A total of $868,249 of these institutional funds were utilized to purchase equipment for two UT facilities with missions that parallel TI-3D's goal of drug discovery and development: the Genomic Sequencing Facility, and the TherapeUTex Core of the College of Pharmacy's Drug Dynamics Institute. Institutional funds were also used for TI-3D staff salaries ($218,613) and scientific supplies ($145,338).

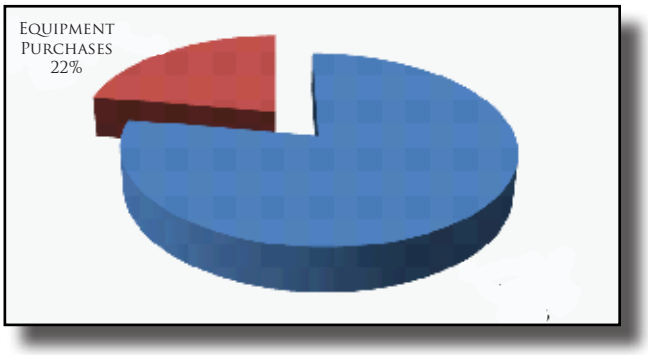
Welch grant funds have been utilized for approximately 50% of TI-3D's major expenditures to date, including equipment purchases, seed grants, scientific supplies, and salaries. State of Texas STARs funding has contributed an additional 16% to TI-3D's equipment outlays. UT's institutional funding has been utilized for equipment, salaries, and supplies, accounting for 15% of TI-3D expenses to date.

The TI-3D has outfitted its Automation Facility as well as underwritten equipment purchases for three closely allied UT facilities, the Genomic Sequencing Facility, the TherapeUTex Core of the College of Pharmacy's Drug Dynamics Institute, and the Macromolecular Crystallography Facility with $2,686,081 worth of essential laboratory equipment.

Financial Report : Equipment Purchases

Laboratory Equipment purchased from the Welch Foundation Grant

A total of $777,633 of the Welch Foundation's $3.5 million grant was utilized by TI-3D researchers to purchase laboratory equipment necessary to perform high throughput screening, and protein crystallography . All of the equipment listed below is used in TI-3D's Automation and High Throughput Screening Facility, which fosters drug discovery and development through fully automated compound synthesis and screening of commercial chemical libraries as well as in-house compounds.



Instrumentation

- Teledyne Isco Combiflash Companion 4X
- Mettler Toledo Minimapper Liquid Handler
- Thermo Electron Quad Ion Trap Mass Spectrometer
- Tech Quip Nitroflow Lab
- Thermo Scientific Microplate Heat Sealer
- Shimadzu Scientific Analytical and Preparative HPLC Systems
- Jasco CD Spectrometer
- Art Robbins Inst. Phoenix Liquid-Handling Platform
- Perkin Elmer Janus Automated Workstation
- Perkin Elmer Envision Microplate Reader
- CEM Corporation Automated Microwave Synthesizer
- Biotek Microflo Select
- Miniblock Peptide Synthesizer

General Laboratory Equipment

Welch grant funds were used to purchase freezers and a professional balance which are essential items in any research laboratory.

- Revco Ultima II Freezer
- Two VWR Upright Freezers
- VWR Professional Balance

Computational and Protein Crystallography Equipment

The Welch Foundation grant funds were also used to purchase two major pieces of equipment for UT's Protein Crystallography Core Facility, a newly instituted unit aimed at supporting macromolecular structure research.

- HP Proliant Blade Cluster
- Cryostream 700 EX

Financial Report : Equipment Purchases

Laboratory Equipment purchased from State of Texas STARs Funding

As further evidence of the strong collaborative nature of TI-3D, five of its founding members made the decision to dedicate a portion of their STARs financial support to the purchase equipment for high throughput screening and protein expression. A total of $1,040,199 of STARs funding was utilized for these purchases. The equipment described below was purchased for the automation facility.

One hundred percent of all STARs funding that was dedicated to the TI-3D by its founding members has been utilized for the purchase of the instrumentation listed below.

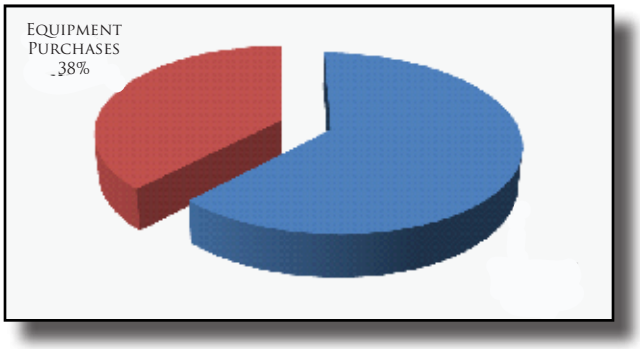
Instrumentation

- Genevac Centrifugal Evaporator
- Teledyne Isco Companion 4x Automated Flash Chromatography
- Universal Precision Microplate Pipetting System
- Perkin Elmer Envision Microplate Reader
- Eppendorf 5810R Centrifuge
- Jasco CD Spectrometer
- Mettler Toledo Minimapper Liquid Handler
- Thermo Electron Quad Ion Trap Mass Spectrometer
- CEM Corporation 48-Position Automated Microwave Synthesizer
- CEM Corporation Liberty Automated Microwave Peptide Synthesizer
- Art Robbins Instruments Phoenix Base Module Workstation
- Zebra Z4M Bar Code Printer
- NanoDrop ND-1000 UV-Vis Spectrophotometer
- New Brunswick Stackable Orbital Incubator Shakers

Financial Report : Equipment Purchases

Laboratory Equipment purchased from Institutional Funds

A total of $868,249 of these institutional funds was utilized to purchase equipment for two UT facilities with missions that parallel TI-3D's goal of drug discovery and development, the Genomic Sequencing and Analysis Facility and the TherapeUTex Core of the College of Pharmacy's Drug Dynamics Institute.



The equipment described below was purchased for the TherapeUTex Core of the Drug Dynamics Institute in the College of Pharmacy. The TherapeUTex Core's mission is to enhance the discovery, development, and commercialization of human disease therapies; this mission parallels TI-3D's goals of drug discovery and commercialization. In keeping with the inter-disciplinary nature of TI-3D, the core's researchers work closely with their colleagues in TI-3D.

Instrumentation

- Malvern Spraytec System
- Leica M205 FA Stereomicroscope
- Leica DMI6000 B Inverted Microscope
- Dell Precision T3400 Computers (2)
- Rheometer System
- Texture Analysis System
- Thermal Cycler System
- Pharmaceutical Impactor
- HPLC System
- Freezer Mill
- AutoPlus Dissoscan System
- AutoPlus MultiFill Collector
- Collection Racks & Small Dissolution Vessel Set-up (2)
- Tissue Processing System
- Centrifuges
- Multiskan
- Flow Meter
- Laboratory Refrigerator and Freezer
- Dell Optiplex 760 Computers (3)
- HP Printer
- InsituPro VSi

Financial Report : Equipment Purchases

Laboratory Equipment purchased from Institutional Funds

Genomic Sequencing and Analysis Facility: Applied Biosystems SOLiD Sequencing System

ICMB focuses its efforts on the revolutionary developments occurring in molecular biology, genetics, cell biology, and genomics, all of which are intertwined with the research efforts of TI-3D. The Genomic Sequencing and Analysis Facility provides advanced analytical resources for analysis of DNA and RNA at whole-genome scales and extends these resources through ongoing research and development.

The SOLiD System Genetic Analysis Platform was purchased for the Genomic Sequencing and Analysis Facility with a combination of institutional funding from TI-3D and UT's Institute for Cellular and Molecular Biology. A new Director for the facility was hired by CSSB to oversee the purchasing, setup, and operation of this powerful new tool.

The SOLiD System is one of a new generation of instruments that enable remarkably higher output and lower cost access to the DNA sequence. New methodologies based on these instruments allow similarly significant improvements in the study of DNA structure and variation, and RNA transcription and regulation.

The SOLiD System is utilized for *de novo* DNA sequence generation, ChIP-sequencing, RNA-sequencing, and small RNA discovery.

The SOLiD System is supported by a Dell R900 high-end computer server required to process the vast amounts of data generated by the SOLiD instrument.

Genomic Sequencing and Analysis Facility: Roche Nimblegen Microarray Platform

In addition to the SOLiD system, the GSAF evaluated new microarray platforms. The Roche Nimblegen system was chosen by the staff of the Genomic Sequencing Facility after a small benchmarking study was performed by Asuragen Services ($2,646). This study established that the Nimblegen system was capable of providing the quality of data required by UT researchers. This choice required no additional capital outlay because the GSAF was able to inherit the microarray instrumentation of UT's Microarray Core Facility which is capable of processing Nimblegen arrays. The competing platform would have required an initial capital outlay of over $300,000.

Financial Report : Sponsored Research

"Seed Projects" Funded Under the Welch Foundation Grant

TI-3D has made significant progress toward its goal of increasing collaboration between chemists, biochemists, chemical engineers, and medical researchers. Scientific collaboration requires a solid foundation of research funding, and TI-3D's steering committee made the decision to utilize a large portion of the Welch Foundation's grant to sponsor research in the form of "seed projects" at UT. As a result, a total of $2,165,000 in seed funding has been awarded to TI-3D researchers investigating issues in three categories: protein expression and purification, high throughput screening, and chemistry and molecularly-targeted therapeutic development.

Three calls for research were subsequently announced, and starting in Spring 2006, the following "seed projects" were awarded to UT faculty in Chemistry, Biomedical Engineering, and Pharmacy:

Calls for Proposals

I. Protein Expression

This call for proposals sought to establish a fully functioning expression and purification facility accommodating protein expression in *E. coli, Pichia*, insect cells, and mammalian cell lines. The facility's fermentation capabilities allow for preps in the 2-10 L scale as well as small to medium scale (less than 1 g protein) purifications, and provide access to downstream processing equipment.

Identifying Inhibitors of NS1 Alpha
Eric Anslyn, Principal Investigator
Total Award: $100,000
Research Personnel Support: $81,293

Identification Inhibitors of EEF2 Kinase
Kevin Dalby, Principal Investigator
Total Award: $100,000
Research Personnel Support: $80,788

Inhibitors of the Amidinotransferase Enzyme Superfamily
Walter Fast, Principal Investigator
Total Award: $100,000
Research Personnel Support: $71,289

Therapeutics for Autoimmune Diseases via the Targeting of Compliment Components: Engineered Antibodies
George Georgiou, Principal Investigator
Total Award: $100,000
Research Personnel Support: $73,778

Identification of Novel Fortilin Inhibitors
Stephen Martin, Principal Investigator
Total Award: $100,000
Research Personnel Support: $87,987

Computer Aided Virtual Screening for Drug Discovery
Jon Robertus, Principal Investigator
Total Award: $100,000
Research Personnel Support: $87,449

"Seed Projects" Funded Under the Welch Foundation Grant

II. High Throughput Screening

In order to establish and bring into full operation the Automation and High Throughput Screening Facility, TI-3D chose to support projects with up to $100,000 funding. This call for proposals allowed support for postdoctoral level researchers who would use the facility for research. Since a major focus of TI-3D is to facilitate interactions between the university and researchers at medical schools, a key criterion sought for this mode of support was a high degree of interaction with one or more medical institutions. A major focus of these proposed projects was to seed and facilitate drug or diagnostics development that would transition lines of research into NIH support.

Development of New Protein-Based Therapies for Pre- & Post-Exposure to *B. mallei*, the Causative Agent of Gladers
Katherine Brown, Principal Investigator
Total Award: $100,000
Research Personnel Support: $56,878

Development of Highly Potent and Selective c-jun-n-terminal Kinase Inhibitors for the Treatment of Metastatic Breast Cancer
Kevin Dalby, Principal Investigator
Total Award: $90,000
Research Personnel Support: $52,688

Development of Novel Analgesics
Kevin Dalby, Principal Investigator
Total Award: $75,000
Research Personnel Support: $52,099

Identifying Nucleic Acids and Small Molecules that Promote Nerve Growth
(in collaboration with Christine Schmidt)
Andrew Ellington, Principal Investigator
Total Award: $37,500
Research Personnel Support: $5,848

Humanizing Arginine Deiminase for Cancer Therapy
Walter Fast, Principal Investigator
Total Award: $50,000
Research Personnel Support: $49,195

New Therapeutics of Multiple Sclerosis
Walter Fast, Principal Investigator
Total Award: $75,000
Research Personnel Support: $47,216

Ansamycin Analogues: Hsp90 Inhibitors for Cancer Treatment
Michael Krische, Principal Investigator
Total Award: $75,000
Research Personnel Support: $20,729

Exploration of the Therapeutic Potential of Poly (ADP-Ribose) Polymerase-1 Inhibitors in Neurological Disorders
Hung-wen Liu, Principal Investigator
Total Award: $75,000

"Seed Projects" Funded Under the Welch Foundation Grant

III. Chemistry and Molecularly-Targeted Therapeutics Development

In Fall 2008, 10 "seed projects" were funded at UT under the Chemistry and Molecularly-Targeted Therapeutic Development call for proposals. This call for proposals was issued in collaboration with UT MD Anderson Cancer Center's Center for Targeted Therapy (CTT), with the goal of promoting collaborative research efforts between basic and translational investigators from CTT and synthetic chemists from both CTT and TI-3D. Each project includes a CTT biologist and a TI-3D synthetic chemist as co-principal investigators.

Development of Novel Small Molecule Anti-Cancer Compounds Eric Anslyn, Co-Principal Investigator
Zhen Fan, CTT Co-Principal Investigator
Total Award: $50,000
Research Personnel Support: $8,325

Targeting PERK and the Tumor's Stress Response for Cancer Therapy
Kevin Dalby, Co-Principal Investigator
Taly Spivak-Kroizman, CTT Co-Principal Investigator
Total Award: $50,000
Research Personnel Support: $32,645

Targeting MicroRNAs with Small Molecules: A Novel Approach
Andrew Ellington, Co-Principal Investigator
George Calin & Shuxing Zhang, CTT Co-Principal Investigators
Total Award: $50,000
Research Personnel Support: $17,117

Tissue, Cell, and Small-Molecule Libraries for Diagnosing and Treating Melanoma
Walter Fast, Co-Principal Investigator
Suhenden Ekmekcioglu, CTT Co-Principal Investigator
Total Award: $50,000
Research Personnel Support: $31,296

Targeted Therapy of Malignant Melanoma Using Engineered Human Arginases
George Georgiou, Co-Principal Investigator
Macus Tien Kuo, CTT Co-Principal Investigator
Total Award: $50,000
Research Personnel Support: $39,903

Caffeic Acid Phenethyl Ester (CAPE) - Based Specific Inhibitor of Inflammatory Pathways
Sean Kerwin, Co-Principal Investigator
Bharat Aggarwal, CTT Co-Principal Investigator
Total Award: $50,000
Research Personnel Support: $26,106

Development of Cell Surface Glycan-Based Cancer Vaccines
Hung-wen Liu, Co-Principal Investigator
Elizabeth Grimm, CTT Co-Principal Investigator
Total Award: $50,000
Research Personnel Support: $27,092

Design and Development of Agents that Block the Viral Protein Docking Site in Alix
Stephen Martin, Co-Principal Investigator
Jian Kuang, CTT Co-Principal Investigator
Total Award: $50,000
Research Personnel Support: $32,800

Platinum-Texaphyrin Cisplatin Conjugates and Drug Resistance Interactions
Sessler, Jonathan, Co-Principal Investigator
Zahid Siddik, CTT Co-Principal Investigator
Total Award: $50,000
Research Personnel Support: $24,415

Redesign Resveratrol to Overcome Chemoresistance in Human Multiple Myeloma
Dionicio Siegel, Co-Principal Investigator
Bharat Aggarwal, CTT Co-Principal Investigator
Total Award: $50,000
Research Personnel Support: $49,960

Financial Report : Newly Generated Funding

New Grants Generated as a Result of TI-3D Funding

To date, TI-3D researchers have received a total of $16,483,039 in federal grants. These highly-competitive awards are tangible evidence of the exciting drug and diagnostic development-related research currently underway on the UT-Austin campus.

Eric Anslyn
Robert Krug, Lead Principal Investigator
Grant # 5 UO1 AI74497
National Institutes of Health
Targeting the Ns1 Protein for the Development of Influenza Virus Antivirals
Period of Performance: 08/17/07 – 07/31/12
Total Funding: $4,965,428

Katherine Brown
Andrew Ellington, Co-Investigator
George Georgiou, Co-Investigator
Brent Iverson, Co-Investigator
Grant # 5 UO1 AI078008
National Institutes of Health
Novel Molecular Recognition Reagents for Detection of Pathogenic Burkholderia
Period of Performance: 06/15/08 – 05/31/11
Total Funding: $1,833,640

Grant # 09-052
University of Texas Medical Branch-Galveston
Vaccine Development for Burkholderia Mallei and Pseudomallei
Period of Performance: 04/21/09 – 02/28/14
Total Funding: $330,425

Walter Fast
Grant # 5 R01 GM069754
National Institutes of Health
Enzymology of Dimethylargininase
Period of Performance: 02/01/08 – 01/31/13
Total Funding: $987,819

George Georgiou
Grant # 1 R01 CA139059
National Institutes of Health
Human Engineered Enzymes for L-Arg Depletion Chemotherapy
Period of Performance: 03/01/09 – 02/28/12
Total Funding: $1,016,638

Edward Marcotte
Grant # 2 R01 GM067779
National Institutes of Health
Network-Directed Discovery of Disease Genes
Period of Performance: 04/01/09 – 03/31/13
Total Funding: $2,073,195

Stephen F. Martin
Grant # 5 P41 GM086192
National Institutes of Health
Generating Diverse Pilot-Scale Libraries for Screening
Period of Performance: 9/1/08 to 8/31/11
Total Funding: $1,071,214

John Mihic
Grant # R03 AA018197-01
In vitro screening of novel therapeutic agents for the treatment of alcohol abuse
National Institutes of Health
Period of Performance: 08/01/09 - 07/31/11
Total Funding: $144,200

Jon Robertus
Grant # 5 UO1 AI075509
National Institutes of Health
Small Molecule Inhibitors of Ricin and Shiga Toxins
Period of Performance: 08/01/07 – 07/31/12
Total Funding: $4,060,480

Financial Report : Total Expenditures

Total Expenditures from all Funding Sources

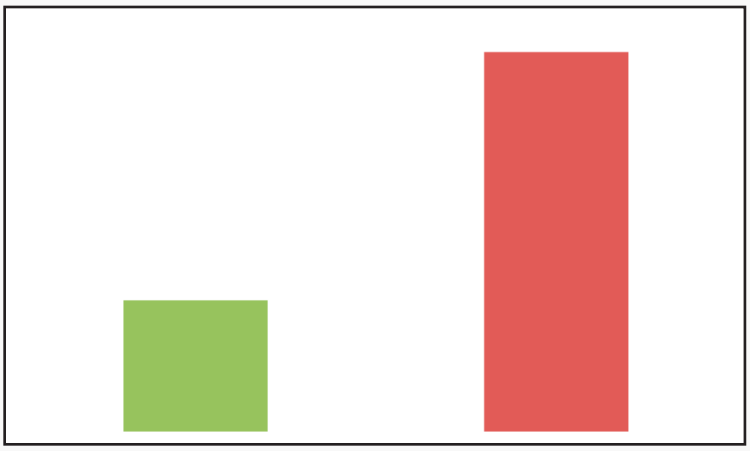
To date, TI-3D has spent $5,715,711 from its three funding sources. This funding has enabled TI-3D to accomplish the following goals and improvements on drug and diagnostics related research and infrastructure during its first three years of existence:

- Purchase crucial instrumentation for its Automation Facility and three closely related campus facilities
- Issue "seed project" funding to University of Texas researchers
- Hire scientific and administrative staff
- Purchase small equipment and consumable lab supplies

Financial Report : Return on Investment

Total Expenditures Compared to New Federal Funding "Return on Investment"

We are pleased to report that the Welch Foundation, State of Texas STARs and University of Texas Institutional support has already been leveraged to garner substantial Federal funding.



Total Expenditures (Welch, UT, STARs)	Total Federal Funding
$5,715,711	$16,483,039

TI-3D (512) 471-3680 1 University Station, A5300, Austin, TX 78712