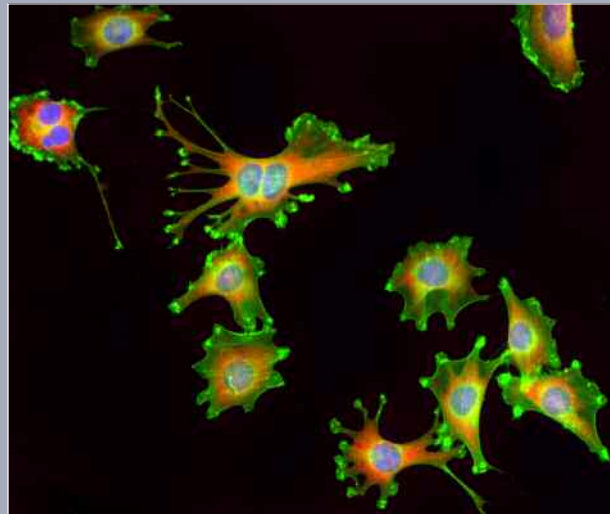


Translational Research Institute



Src overexpression in NIH-3T3 fibroblasts. Overexpression results in actin relocalization toward structures known as "podosomes," which are thought to be involved in cell motility. In order to examine actin localization and apoptosis activity after treatment with src inhibitors, the cells were stained with Alexa Fluor 488 phalloidin, Bcl-2, and Alexa Fluor 647 F(ab')₂ goat antirabbit fragment of IgG. The nuclei were stained with Hoechst dye 33342. The cells were plated in a 96-well plate and visualized with the IN Cell 1000. The image was analyzed for granularity by using Developer Toolbox. Further processing was done with Adobe Photoshop. Work done by Evelyn Griffin, research assistant, in the laboratory of Thomas Schroeter, Ph.D., senior scientist.



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Patrick R. Griffin, Ph.D.

Director's Overview

The Translational Research Institute merges drug discovery efforts at Scripps Florida with advanced technology platforms to rapidly identify and validate biological pathways that can be targeted for therapeutic intervention. The technology platforms are grouped into genomics, cell-based screening, and proteomics cores.

The goal of the drug discovery operation is to discover and develop small-molecule therapeutic agents for unmet medical needs in neurodegeneration, Parkinson's disease, acute respiratory distress syndrome, spinal cord injury, cardiovascular disease, cancer, and metabolic disorders such as insulin resistance and type 2 diabetes. Therapeutic areas and targets, which include G protein-coupled receptors, proteases, channels, and kinases, are selected on the basis of unmet needs and the ability to attract external funding. The drug discovery operation is fully integrated with the following groups: Lead Identification and High-Throughput Screening, headed by Peter Hodder, Department of Molecular Therapeutics; Medicinal Chemistry, headed by William Roush, Department of Chemistry; Discovery Biology, headed by Phil LoGrasso, Department of Molecular Therapeutics; and Drug Metabo-

lism and Pharmacokinetics, headed by Mike Cameron, Department of Molecular Therapeutics.

The Lead Identification department enables drug-target lead identification via ultra-high-throughput screening technology. Using state-of-the-art automation and instrumentation, members in this department are responsible for developing and executing biochemical or cell-based high-throughput screening assays in a miniaturized 1536-well microtiter plate format. In addition to its support of internal Scripps Research objectives, the group participates in the National Institutes of Health Molecular Libraries Screening Centers Network, in which qualified assays are screened against the network's high-throughput screening compound library. Several internal and external investigators have accessed the department's expertise via collaborative or core-charge mechanisms.

The genomics core is headed by Mathew Pletcher. Scientists in this core oversee genotyping and gene expression profiling. The services provided by the core allow Scripps Research investigators to examine the genome at both the genetic and the transcriptional levels for the genes that underlie common diseases. In collaboration with Scripps Florida colleagues, members of the core have been involved in projects to identify the genes responsible for pathologic conditions, such as addiction and alcoholism, systemic lupus erythematosus, autism, obsessive-compulsive disorder, diabetes, obesity, and prion diseases.

The cell-based screening platform is headed by Julie Conkright, Department of Molecular Therapeutics. The faculty advisor to the core is Michael Conkright, Department of Cancer Biology. In this group, high-throughput technologies are used to provide a systematic description of the function of genes encoded by the human genome and a more comprehensive understanding of the genetic basis for human disease. Members of the group provide investigators access to genome-wide collections of cDNAs and short interfering RNAs that can be used to examine cellular models of signal transduction pathways and phenotypes.

The proteomics platform is headed by Jennifer Caldwell-Busby, Department of Molecular Therapeutics. The focus of this core is using liquid chromatography and state-of-the-art mass spectrometry technology to identify, quantify, and characterize proteins and protein modifications. Researchers in the core are involved in scientific collaborations in which novel technologies are used to identify biologically important proteins and protein modifications. Large-scale differential analysis is

being used to map the pathways related to insulin sensitization and adipogenesis. In other projects, chromatographic enrichment techniques are used to identify sites of phosphorylation and other posttranslational modifi-

cations. Researchers in the proteomics core collaborate with other scientists to create experiments that will provide meaningful mass spectrometric results.

INVESTIGATORS' REPORTS

Drug Metabolism and Pharmacokinetics Laboratory

M.D. Cameron, L. Lin, C. Ruiz, S. Khan

The Drug Metabolism and Pharmacokinetics Laboratory at Scripps Florida provides *in vitro* and *in vivo* evaluation of the pharmacokinetic and pharmacodynamic properties of new chemical entities. We work on project teams within the Drug Discovery group of the Department of Molecular Therapeutics and support chemistry efforts within the Scripps Research Institute Molecular Screening Center. We help bridge medicinal chemistry and pharmacology by evaluating the metabolic fate and identifying the liabilities of compounds. Pharmacokinetic studies provide basic parameters, including peak plasma concentration, bioavailability, exposure, half-life, clearance, volume of distribution, and tissue distribution. Research interests include P450 structure-function relationships and the formation of reactive intermediates during metabolism. The laboratory is equipped with liquid chromatography–tandem mass spectrometry system and a Q-trap hybrid triple quadrupole/linear ion-trap mass spectrometer.

PUBLICATIONS

Cameron, M.D., Wright, J., Black, C.B., Ye, N. *In vitro* prediction and *in vivo* verification of enantioselective human tofisopam metabolite profiles. *Drug Metab. Dispos.*, *in press*.

Feng, Y., Cameron, M.D., Frackowiak, B., Griffin, E., Lin, L., Ruiz, C., Schröter, T., LoGrasso, P. Structure-activity relationships, and drug metabolism and pharmacokinetic properties for indazole piperazine and indazole piperidine inhibitors of ROCK-II. *Bioorg. Med. Chem. Lett.* 17:2355, 2007.

Proteomics Laboratory

J.A. Caldwell-Busby, V. Cavett

The Proteomics Laboratory at Scripps Florida provides proteomics services and expertise to scientific collaborators at Scripps Research facilities

in both Florida and California, universities within the state of Florida, and other educational institutions. We use cutting-edge mass spectrometry technology to identify proteins, map modifications that occur after translation, and do relative quantitation experiments with a variety of samples.

In its lifetime, a protein it can have several locations and functions within a cell. Location, function, and 3-dimensional structures of proteins are all influenced by static and dynamic chemical modifications that occur after translation. These modifications vary from small methyl and acetyl groups, which are a part of the histone codes, to large lipid and glycosylation modifications, which act as cellular markers and signaling molecules. With mass spectrometry, we can detect both the small and the large changes in mass that occur in proteins because of these modifications, and we can identify the specific amino acids modified.

Relative changes in protein levels between multiple samples provide biologically relevant information about cellular pathways and proteins of interest. Large-scale studies of this type require rigorous sample preparation and highly tuned algorithms for comparing different mass spectrometric analyses. We are currently validating methods for both sample fractionation and data analysis for these types of large-scale differential protein experiments.

Mass spectrometers at the facility include an ion-trap spectrometer, which is used mostly to identify proteins and peptides, and a triple quadrupole mass spectrometer, which is used for relative quantitation experiments. A new addition is a mass spectrometer that can be used to perform accurate mass and high-resolution experiments. Each mass spectrometer is interfaced to nanoflow electrospray ionization sources and capillary high-performance liquid chromatography columns.

Data analysis is performed primarily via automated workflow on a cluster maintained by the bioinformatics group. Automation of the front-end processing allows for a more thorough review of the resultant data and more time for development of innovative software in collaboration with information technology groups at Scripps Research and beyond.

PUBLICATIONS

Godeny, M.D., Sayyah, J., VonDerLinden, D., Johns, M., Ostrov, D.A., Caldwell-Busby, J., Sayeski, P.P. The N-terminal SH2 domain of the tyrosine phosphatase, SHP-2, is essential for Jak2-dependent signaling via angiotensin II type AT₁ receptor. *Cell Signal*. 19:600, 2007.

Soley, S., Smith, S., Algeciras, M., Cavett, V., Caldwell Busby, J.A., London, S., Clayton, D.F., Bhattacharya, S.K. Proteomic analyses of songbird (zebra finch; *Taeniopygia guttata*) retina. *J. Proteome Res.* 6:1093, 2007.

Soley, S., Smith, S., Gandhi, S., Caldwell Busby, J.A., London, S., Luksch, H., Clayton, D.F., Bhattacharya, S.K. Proteomic analyses of zebra finch optic tectum and comparative histochemistry. *J. Proteome Res.* 6:2341, 2007.

Probe and Drug Discovery: The Lead Identification Department

P. Hodder, P. Baillargeon, P. Chase, L. DeLuca, F. Madoux, B. Mercer, D. Minond, S. Saldanha, L. Scampavia, T. Spicer, P. Subramaniam, L. Sullivan

The Lead Identification Department is responsible for developing and executing high-throughput screening (HTS) assays and for supporting downstream medicinal chemistry and probe development efforts. The anchors of the department are 2 fully automated robotic platforms (Fig. 1). One supports screen-



Fig. 1. The Scripps Research uHTS platform. A, An industrial anthropomorphic robotic arm moves assay and compound microtiter plates. B, A pin tool is used to transfer test compounds rapidly from compound plates to assay plates. C, Liquid handlers capable of dispensing up to 32 different reagents and of washing plates are integrated into the platform. D, Incubators, each capable of holding approximately 700,000 samples in 1536-well format, are used to store microtiter plates at a variety of temperatures and gas concentrations. E, A multimode plate reader measures absorbance, luminescence, fluorescence, or fluorescence resonance energy transfer (with time resolution) from microtiter plates. F, A kinetic imaging plate reader allows measurement of second-messenger or ion channel activity in live cells. Not shown is a compound management robot capable of storing and retrieving desirable compounds from the Scripps Research screening file.

ing of 384- and 1536-well microtiter plates in a variety of biochemical and cell-based assay formats. The other is used to manage and characterize the screening library of more than 600,000 compounds used for drug disc-

covery at Scripps Research. The facility also contains an assay development laboratory with equipment for tissue culture and semiautomated liquid handling and detection. Supporting this operation is an integrated laboratory information management system, which is used to track HTS data and compound usage and quality. Additionally, we are involved in developing metallo- β -lactamase chemical probes.

THE SCRIPPS RESEARCH INSTITUTE MOLECULAR SCREENING CENTER

Established in 2005, the Scripps Research Institute Molecular Screening Center is a national resource for small-molecule screening and the development of chemical probes. It is 1 of 10 members in the Molecular Libraries Screening Centers Network, a translational research initiative sponsored by the National Institutes of Health (NIH) and part of the NIH Roadmap Initiative. The mission of the Scripps center is to screen the NIH library of more than 150,000 individual compounds against peer-reviewed targets; the goal is to discover proof-of-concept probes. The results are available to the scientific community through the PubChem Web site of the National Center for Biotechnology Information: <http://pubchem.ncbi.nlm.nih.gov>. Currently, the Lead Identification Department serves as the HTS core within the Scripps screening center; our responsibilities are to develop biological and biochemical assays, perform HTS campaigns, manage the resulting data, steward the NIH screening library, and provide assay support for the development of probes.

OTHER SCREENING ACTIVITIES

Since the inauguration of the ultra-HTS (uHTS) operation in November of 2005, we have also been actively screening the Scripps collection of compounds against drug discovery targets not only from the MLSCN but also from scientists at Scripps Research and from outside partners. So far, members of the department have initiated and successfully completed more than 30 uHTS-related collaborations (Table 1).

DISCOVERY AND DEVELOPMENT OF CLASS B METALLO- β -LACTAMASE INHIBITORS

The diversity of bacterial β -lactamases continues to outpace the development of useful β -lactam-based antibiotics. Although the development of class B β -lactamase inhibitors has been an active area of research, an array of potent, class-specific small-molecule inhibitors has yet to be fully characterized in the clinically relevant VIM-2 metallo- β -lactamase system. Additionally, VIM-2 inhibitors that are effective inhibitors of other class B β -lactamases will be of great interest. Such compounds

Table 1. Summary of HTS and HTS assay development collaborations

Target class	Target name (abbreviation)	Collaboration	Collaborator
G protein-coupled receptor	5HT _{1a}	β-Lactamase reporter gene uHTS campaign	M. Teitler ^a
	5HT _{1e}		S. Brown ^b
	GalR2		
	S1P ₁		
	S1P ₂		H. Rosen ^b
	S1P ₃		
	AGTRL-1 (APJ)	FLIPR/TETRA HTS assay development	L. Smith ^c
	GLP-1		P. LoGrasso ^d
	GPR119		P. McDonald ^d
μ-Opioid heterodimers	L. Devi ^e		
	NPY-Y1	FLIPR/TETRA uHTS campaign	C. Wahlestedt ^d
	NPY-Y2		
Kinase	JNK3	TR-FRET uHTS campaign	P. LoGrasso ^d
	ROCK2	Luminescence uHTS campaign	T. Schroeter ^d
	PKA		
	FAK	TR-FRET uHTS campaign	P. Hodder ^d
Transcription factor	PPARγ/Src1	TR-FRET uHTS campaign	P. Griffin ^d
	PPARγ/Src2	TR-FRET HTS assay development	
	PPARγ/Src3	TR-FRET uHTS campaign	
	NF-κB	Luciferase reporter gene uHTS campaign	J. Reed ^f
	STAT3	Luciferase reporter gene uHTS campaign	D. Frank ^g
	STAT5		
Protein/protein	EphB4-ephrinB2	FP uHTS campaign	P. Kuhn ^b
	HCV core homodimer	TR-FRET HTS assay development	D. Strosberg ^d
	NS5B/CYPB		
Protein/RNA	HIV Rev-RRE RNA	FRET uHTS campaign	J. Williamson ^b
Nuclear receptor	Estrogen receptor	Luciferase reporter gene HTS assay development	K. Nettles ^d
Orphan nuclear receptor	SF1 (NR5A1)	Luciferase (transient-transfection) reporter gene uHTS campaign	X. Li ^h
	RORα (NR1F1)		
Metalloproteinase	ADAMTS4	QFRET HTS assay development	G. Fields ⁱ
	MMP13	QFRET uHTS campaign	
β-Lactamase	VIM-2	Absorbance HTS assay development	P. Hodder ^d
	IMP-1		
Protein misfolding	Hsp70	Luciferase reporter gene HTS assay development	R. Morimoto ^j
	AL-09	QFRET HTS assay development	M. Ramirez-Alvarado ^k
Ubiquitin proteolysis	WEE1	Luciferase reporter gene HTS assay development	N. Ayad ^d
Proliferation/viability	Jurkat E6.1Cells	Luminescence uHTS campaign	P. Hodder ^d
Reductase	msr A	Absorbance HTS assay development	H. Weissbach ⁱ
Ion channel	Aquaporins (AQP)	Yeast-spheroplast HTS assay development	M. Yeager ^b
Stem cell proliferation	Notch	Luminescence (cell-based) HTS assay development	H. Petrie ^d

Abbreviations: FLIPR, fluorescence imaging plate reader; FP, fluorescence polarization; FRET, fluorescence resonance energy transfer; QFRET, quenched FRET; TETRA, brand name for a FLIPR system; TR-FRET, time-resolved FRET.

^a Albany Medical College, Albany, New York

^b Scripps Research

^c Burnham Institute, Orlando, Florida

^d Scripps Florida

^e Mt. Sinai School of Medicine, New York, New York

^f Burnham Institute, La Jolla, California

^g Dana-Farber Cancer Institute, Boston, Massachusetts

^h Orphagen Pharmaceuticals, San Diego, California

ⁱ Florida Atlantic University, Boca Raton, Florida

^j Northwestern University, Chicago, Illinois

^k Mayo Clinic, Rochester, Minnesota

will be useful as tools for characterizing gram-negative pathogens or as adjuvants in antibiotic therapy.

One of our goals is to develop HTS-ready assays suitable for rapid identification of compounds that modu-

late the activity of Ambler molecular class B (Bush-Jakoby-Medeiros group 3) metallo-β-lactamases, specifically the VIM-2 and IMP-1 enzymes. In preliminary research efforts, we have developed HTS-ready

fluorescence- and absorbance-based VIM-2 and IMP-1 inhibition assays. In collaboration with K.B. Sharpless, Department of Chemistry, we have screened a diverse click-chemistry library of compounds designed specifically to inhibit metallo- β -lactamases. Currently, we are developing several novel scaffolds that appear to be specific inhibitors.

PUBLICATIONS

Lauer-Fields, J.L., Spicer, T.P., Cudic, M., Burstein, G.D., Nagase, H., Hodder, P., Fields, G.B. Screening of potential ADAMTS-4 inhibitors utilizing a collagen-model FRET substrate. *Anal. Biochem.*, *in press*.

Schröter, T., Minond, D., Weiser, A., Dao, C., Habel, J., Spicer, T., Chase, P., Baillargeon, P., Scampavia, L., Schürer, S., Chung, C., Mader, C., Southern, M., Tsinoremas, N., LoGrasso, P., Hodder, P. Comparison of miniaturized time-resolved fluorescence energy transfer and enzyme-coupled luciferase high-throughput screening assays to discover inhibitors of Rho-kinase II (ROCK-II). *J. Biomol. Screen.*, *in press*.

Development of Protein Kinase Inhibitors

C. Liang, M. Koenig, P. Holmberg, Y. He

Our goal is to discover protein kinase inhibitors that can be used as therapeutic agents for the treatment of human diseases such as cancer and arthritis. Protein kinases are a class of enzymes that catalyze the transfer of the γ -phosphate from ATP to protein substrates. These enzymes play critical roles in signal transduction for a number of cellular functions. In particular, they regulate most of the hallmarks of cancer: cell proliferation, cell survival, cell motility/metastasis, cell cycle/division, and angiogenesis. Protein kinases are also implicated in inflammatory diseases such as arthritis and asthma. For these reasons, protein kinases are being investigated as valuable therapeutic targets by almost every pharmaceutical company, and according to estimates, about 25% of all current pharmaceutical research is devoted to these targets.

In the past year, we successfully completed the first drug discovery project in collaboration with Poniard Pharmaceuticals (formerly NeoRx Corporation), Seattle, Washington. The collaboration was then expanded to include D.D. Schlaepfer, Department of Immunology. Our goal was to identify lead compounds that would inhibit focal adhesion kinase. By April, we had exceeded the original goal; we have identified novel compounds with excellent potency and oral bioavailability. Preclinical studies are under way to identify potential clinical candidates. Provisional patent applications have been filed.

Pharmacology Services

L.H. Smith, A. Pachori, S. Khan, M. Lopez, D. Zadory

We provide comprehensive pharmacology services and expertise in support of drug discovery efforts for Scripps Research at both the Florida and California sites. We use cutting-edge technology and established models of disease to validate and confirm the efficacy of new chemical compounds and to assess the druglike characteristics of the compounds. Our disease models include tumor xenografts, hypertension, angiogenesis, hyperlipidemia, obesity induced via diet or genetic manipulation, diabetes, and Parkinson's disease. Studies of this kind require expertise in many scientific disciplines. Thus, we work with researchers in Medicinal Chemistry, Drug Metabolism and Pharmacokinetics, and Cell Biology and the leaders of each project team to move drug discovery projects from the test tube to in vivo models and, ultimately, to clinical trials.