BIOLOGICAL SCIENCES

# MULTI-USER EQUIPMENT

## ADVANCED TECHNOLOGY TO REACH OF RESEARCHERS

## Biological Sciences

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SÃO PAULO
RESEARCH FOUNDATION

Multi-user Equipment

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# ACQUISITION OF A MASS SPECTROMETER COUPLED TO A LIQUID CHROMATOGRAPHY SYSTEM FOR INCREASING THE CAPACITY TO MEET THE NEEDS OF USERS AND FOR MAKING NEW TECHNOLOGIES AVAILABLE IN THE LABORATORY OF MASS SPECTROMETRY

Adriana Franco Paes Leme

National Synchrotron Light Laboratory

FAPESP Grant 2009/54067-3

The approved multi-user equipment was installed in August 2011 at Mass Spectrometry Laboratory, located at Brazilian Biosciences National Laboratory (LNBio), ABTLuS-CNPEM.

The multi-user equipment is ETD enabled Orbitrap Velos instrument (Thermo Fisher Scientific) connected to nanoflow liquid chromatography EASY-nLC II system (Proxeon Biosystem) through a Proxeon nanoelectrospray ion source. These instruments combine mass accuracy and high resolution of the Orbitrap mass analyzer, with the increased sensitivity of LTQ Velos. It can provide multiple fragmentation techniques: CID, HCD and ETD, which have extended the possibilities within tandem mass spectrometry, increasing the speed and confidence of identification of large-scale complex proteomes, binding partners and post-translational modifications, analysis of cross-linked complexes to map protein interaction, and likely quantitative proteomic approaches.

The equipment is available to the Brazilian scientific community in the same manner as is the other multi-user equipment of the Mass Spectrometry Laboratory. The Laboratory started the operation in continuous mode, in terms of the submission of proposals and analyses in June 2009. One of the characteristics of the Laboratory is that users are provided with technical support in the operation of the mass spectrometers and the data analysis, as well as with training in the area of mass spectrometry/proteomics that is fundamental for the development of individual research.

The implementation of the new cutting-edge technology ETD enabled Orbitrap Velos instrument at Mass Spectrometry Laboratory has included the Brazilian scientific community in the international panorama of competitiveness in the area of mass spectrometry/proteomics.

## EQUIPMENT GRANTED

- ETD enabled Orbitrap Velos instrument (Thermo Fisher Scientific) connected to nanoflow liquid chromatography EASY-nLC II system (Proxeon Biosystem) through a Proxeon nanoelectrospray ion source.

# ASSOCIATED PROJECTS

**Brazilian Biosciences National Laboratory, LNBio, ABTLuS-CNPEM**

*Proteomics applied to the study of ADAMs in oral cancer*
Adriana Franco Paes Leme

*Pathogenesis of cardiac hypertrophy and heart failure: mechanisms activated by mechanical stimulation*
Kleber Gomes Franchini
FAPESP Grant 2006/54878-3

**Heart Institute/University of São Paulo School of Medicine**

*Development of biomarkers as predictors of treatment response and injury to target organs in systemic arterial hypertension*
Eduardo Moacyr Krieger
FAPESP Grant 2007/58942-0

*Identification of protein targets with diagnostic and prognostic potential in coronary disease*
José Eduardo Krieger
FAPESP Grant 2008/51892-0

## Contacts for instructions for the use of the equipment

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# FLUORESCENCE SYSTEM FOR INVESTIGATIONS OF PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL ASPECTS IN CELLULAR MODELS

Adriana Karaoglanovic Carmona

Paulista School of Medicine

Federal University of São Paulo (Unifesp)

FAPESP Grant 2009/53840-0

In this project, we intend to enable pathophysiology studies in experimental models that are being developed by the researchers involved. The topics that will be explored by the researchers are of great medical and biological interest and could answer important questions related to pathologies of high incidence in humans. Among other topics, the projects presented are related to the following: arterial hypertension and its involvement in heart disease (participation of the renin-angiotensin and kallikrein-kinin systems); liver metabolism in the setting of inflammation; the effects of sleep deprivation on physiology and cellular responses; deficiency and activation of various membrane receptors; apoptosis (cell death); study of intracellular proteases; and the development of new fluorescent probes. These projects could allow us to develop and apply new methodologies for study of the intracellular metabolism in various experimental models, identifying associations with the mechanisms of signaling, ionic mobility, and morphology of organelles, as well as with the expression and activity of proteins, or rather, making it possible to understand the complexity of the cell physiology involved in certain pathologies. Therefore, the fluorescence system requested is essential for the analysis (distribution/morphometry and quantification) of fluorescence in fixed preparations or in cell cultures. Accessories such as an environmentally controlled chamber (with regulation of temperature, $CO_2$, and humidity) and slide holders could be employed in the study of live cells, making it possible to create a controlled environment for metabolic, pharmacological and studies of cell signaling in multiple culture plates.

## EQUIPMENT GRANTED

- Axio Observer Z1 inverted microscope (motorized Z axis), with incubator (for Petri dishes and 24-well plates) and AxioCam HSm high-speed digital camera (Carl Zeiss AG)
- F-7000 fluorescence spectrophotometer, with xenon lamps, intracellular cation measurement program, microplate reader, micro-sampling assembly, and thermo.cell holder w/stirring (Hitachi High Technologies)
- DG-4 high-speed fluorescence system (Sutter Instruments); and the Axiovision 4.8 software driver for image analysis, with modules for physiology, time laps, commander, mark find, and multi-channel fluorescence (Carl Zeiss AG)

# ASSOCIATED PROJECTS

**Department of Biophysics/Unifesp**

*Proteolytic enzymes involved in physiological and pathophysiological processes*
Adriana Karaoglanovic Carmona
FAPESP Grant 2008/56340-6

*Synthesis and biology of peptides: update and adaptation of high-performance chromatography systems for the analysis and sequencing of amino acids*
Luiz Juliano Neto
FAPESP Grant 2009/51038-2

**Department of Biosciences/Unifesp**

*Evaluation of biochemical factors and of epigenetic events in macrophages with different levels of chitotriosidase activity*
Vânia D'Almeida
FAPESP Grant 2009/02499-4

Contacts for instructions for the use of the equipment

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# ACQUISITION OF A JACKETED AUTOCLAVE FOR THE PRODUCTION AND EXPERIMENTATION ANIMAL FACILITY OF THE UNIVERSITY OF SÃO PAULO SCHOOL OF PHARMACEUTICAL SCIENCES AND INSTITUTE OF CHEMISTRY

Alícia Juliana Kowaltowski

Institute of Chemistry

University of São Paulo (USP)

FAPESP Grant 2009/53847-5

The Production and Experimentation Animal Facility of the USP School of Pharmaceutical Sciences and Institute of Chemistry is a large multi-user facility that breeds and houses experimental animals for these two major branches of the University. Because of the growing demand for experimental animals of different lineages and for the use of animal housing systems with better environmental controls, together with constant updates of the international standards for sanitation and ethics, this facility is in need of a new jacketed autoclave for the sterilization of materials employed in the field of animal breeding. This project is aimed at equipping the facility with a jacketed autoclave and the appropriate accessories, including the installation of the equipment.

## EQUIPMENT GRANTED

- Horizontal jacketed autoclave (model 39206; Luferco)

Contacts for instructions for the use of the equipment

Alícia Juliana Kowaltowski

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# ACQUISITION OF A MICROARRAY PLATFORM FOR THE DIADEMA CAMPUS OF THE FEDERAL UNIVERSITY OF SÃO PAULO

André Luiz Vettore de Oliveira

Federal University of São Paulo - Diadema Campus

Processo FAPESP 2009/53955-2

The project envisages acquisition of a microarray platform for the Diadema Campus of Universidade Federal de São Paulo (the Federal University of São Paulo). The microarray technology consists of utilization of a microarrangement in which DNA samples are fixed on spots for hybridization with a pool of mRNAs extracted from biological samples (targets) that were previously marked with fluorescent dyes and allows for simultaneous evaluation of the expression of thousands of genes in different tissues of a given organism. This approach can also be applied in studies to analyze DNA methylation, pharmacogenetis, cytogenetics, genotyping, mRNA expression, etc. Scientists chose the Agilent platform based on its better price and lower operational costs at the existing authorized dealer in Brazil (GE Healthcare Bio-Sciences Corp.) and above all, because of the flexibility of the system, which allows operators to use blades made by different manufacturers. Additional equipment necessary for the operating the platform will be acquired. A management plan will be created with the main objective of ensuring management, maintenance and multi-user access to the equipment, allowing for and facilitating usage of this equipment by researchers affiliated to this and other institutions. The management commission will be initially formed by researchers associated with this proposal and by the technicians assigned by the host-institution. And finally, there are several complementary projects, many of which at other institutions, which will also benefit from this request, allowing for future collaboration and greater scientific impact. In this manner, acquisition of the microarray platform will not only contribute to new scientific discoveries within associated and complementary projects, but will give a major boost to research from different areas that are part of this campus.

## EQUIPMENT GRANTED

- Scanner for high-density microarrays (Agilent Technologies)

# ASSOCIATED PROJECTS

**Unifesp, Diadema Campus**

*Evaluation of the methylation profile as molecular marker for the early identification of recurrence in patients with head and neck tumors*
André Luiz Vettore de Oliveira
FAPESP Grant 2008/58460-9

*Thermal stress-induced cellular and molecular alterations in oocytes of Bos indicus and* Bos taurus *cattle*
Fabíola Freitas de Paula Lopes
FAPESP Grant 2007/53323-0

*Functional genome of the citrus canker: study of plant-pathogen interactions*
Julio Cezar Franco de Oliveira
FAPESP Grant 2004/02007-6

*Regulation of BMP-9 expression in animals receiving chronic supplementation with different doses of L-arginine*
Lucianda Chagas Caperuto
FAPESP Grant 2009/50041-0

Cryptococcus neoformans*: Study of the signal transduction pathway that controls growth at 37°C, employing molecular biology techniques and epidemiological characterization*
Marcelo Afonso Vallim
FAPESP Grant 2007/50536-3

**Institute of Environmental, Chemical, and Pharmaceutical Sciences/Unifesp**

*Evaluation of lung function and pulmonary histopathology in an experimental model of reduced cholinergic function in genetically modified mice*
Carla Máximo Prado
FAPESP Grant 2008/55359-5

**Paulista School of Medicine/Unifesp**

*Studies of the gene expression in multiple myeloma: identification of tumor markers and possible therapeutic targets*
Gisele Wally Braga Colleoni
FAPESP Grant 2004/13213-3

Contacts for instructions
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# INSTALLATION OF A SURFACE PLASMON RESONANCE SYSTEM AT THE FEDERAL UNIVERSITY OF SÃO PAULO

Aparecida Sadae Tanaka

National Institute of Pharmacology

Federal University of São Paulo (Unifesp)

FAPESP Grant 2009/53844-6

The diversity of biological activities occurring in hematophagous arthropods, together with the importance of such arthropods as disease vectors, has motivated our group to investigate the presence of new anti-hemostatic molecules and protease inhibitors in three species of arthropods that are major vectors of diseases affecting humans and animals in Brazil: the reduviid bug *Triatoma infestans*, the cattle tick *Rhipicephalus* (*Boophilus*) *microplus*, and the mosquito *Aedes aegypti*. Over the last 10 years, our group has described more than ten active proteins present in these vectors, two of which have been patented, and we are currently working on the characterization of at least 15 other proteins. Using the techniques available, we have characterized the biochemical properties of the following: the inhibitors of thrombin and subtilisin A, the *Aedes aegypti* thrombin inhibitor (AaTI) and infestina 1R, respectively; lipocalins—tilipo33 (*T. infestans* platelet aggregation inhibitor), as well as tilipo37, tilipo39, and tilipo77 (putative anticoagulant proteins); and the pro-apoptotic protein *Boophilus microplus* chymotrypsin inhibitor (BmCI). However, we are currently in need of state-of-the-art equipment to perform techniques that allow the interactions between two molecules to be quantified. For example, the use of the surface plasmon resonance (SPR) technique would allow us to determine whether the AaTI inhibitor binds to thrombin, heparin, and fibrinogen. Using the SPR system in the laboratory of Dr. Lauro Kubota, at the State University of Campinas, we recently made two attempts at such determination. However, in order to standardize the experiments, it is necessary to have continuous access to the equipment for a number of weeks. Therefore, we decided to postpone our work. We believe that the acquisition of an SPR system is essential, so that we can address this and other questions, such as which platelet receptors interact with the *T. infestans* salivary protein tilipo33 and what is the mechanism of action by which BmCI induces apoptosis. This equipment will be the first of its kind at Unifesp and will undoubtedly also further the investigations of the associated and complementary research groups that support this request.

## EQUIPMENT GRANTED

- Biacore T100 surface plasmon resonance interaction analysis device (GE Healthcare) and XPRO computer (Compaq), with laser printer and 17" monitor (Hewlett-Packard)

# ASSOCIATED PROJECTS

**Paulista School of Medicine/Unifesp**

*Membrane microdomains rich in glycolipids, sphingolipids, and sterols: organization , function, and signaling*
Helio Kiyoshi Takahashi
FAPESP Grant 2006/07005-4

*Peptide synthesis and peptide libraries for the study of proteases*
Maria Aparecida Juliano
FAPESP Grant 2008/54894-4

Contacts for instructions for the use of the equipment

Aparecida Sadae Tanaka

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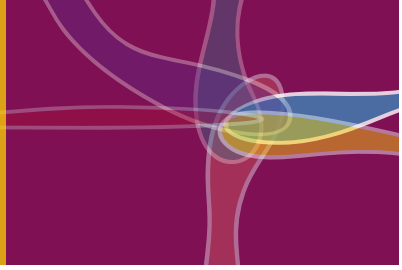

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# MULTI-USER CENTRALIZED LABORATORY AT THE SÃO PAULO STATE UNIVERSITY CENTER FOR BIODIVERSITY STUDIES

Célio Fernando Baptista Haddad
Rio Claro Biosciences Institute
São Paulo State University (Unesp)
FAPESP Grant 2009/54208-6

This request is for the equipment needed for sample collection and polyphasic studies in order to characterize biodiversity. The equipment will be installed in a multi-user centralized laboratory at the Unesp Center for Biodiversity Studies. This laboratory will focus on the collection and processing of biological samples of various groups of organisms, including microorganisms, plants, insects, mammals and amphibians, serving the groups led by researchers at the various Unesp campuses. We expect this laboratory to foster research in biodiversity through the use of state-of-the-art equipment for morphological, physiological, biochemical and genetic analyses, allowing researchers to work in a collaborative and unified manner, with the objective of producing polyphasic descriptions of new biological species.

## EQUIPMENT GRANTED

- LI-6400XTR portable photosynthesis and fluorescence system and accessories (LI-COR Biosciences)
- LI-3100C leaf area meter and accessories (LI-COR Biosciences)
- LI-610 portable dew point generator (LI-COR Biosciences)
- LI-7200 CO2/H2O analyzer and accessories (LI-COR Biosciences)
- TurboFox portable metabolic measurement system and accessories (Sable Systems International)
- FoxBox Portable Oxygen Analysis System and accessories (Sable Systems International)
- Chameleon V multiplate reader and accessories (Hidex)
- L200 Outdoor 4×4 sports utility vehicle (Mitsubishi)
- Light-duty van
- Fitotron acclimatized greenhouse room for the acclimatization of samples (Eletrolab Equipamentos)

# ASSOCIATED PROJECTS

**Rio Claro Biosciences Institute/Unesp**

*Speciation of anuran amphibians in high-altitude environments*
Célio Fernando Baptista Haddad
FAPESP Grant 2008/50928-1

*Phenology of Atlantic Forest species in the state of São Paulo: comparison between extracts, influence of natural boundaries, and importance of the family Myrtaceae*
Leonor Patrícia Cerdeira Morellato
FAPESP Grant 2008/54386-9

**Center for the Study of Social Insects**

*Molecular phylogeny, genomics, and metagenomics in select Attine ants*
Maurício Bacci Júnior
FAPESP Grant 2008/54386-9

Contacts for instructions for the use of the equipment

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# ACQUISITION OF A REFRIGERATED ULTRACENTRIFUGE FOR THE DEPARTMENT OF BIOCHEMISTRY OF THE INSTITUTE OF BIOLOGY STATE UNIVERSITY OF CAMPINAS INSTITUTE OF BIOLOGY

Eneida de Paula

Institute of Biology

State University of Campinas (Unicamp)

FAPESP Grant 2009/54209-2

Analytical ultracentrifuges and refrigerated centrifuges are both of great utility in biochemical preparations and analyses. For example, they can be employed in the separation of cell organelles, in the preparation of plasma membranes or its detergent-resistant fractions, liposomes, in the determination of partition coefficients, for enzyme purification,... Therefore, devices that use centrifugal force for separation have become indispensable in biochemistry laboratories.

In 1984, the researchers of the Department of Biochemistry, Institute of Biology /Unicamp acquired an ultracentrifuge (model L8-80M; Beckman) and a refrigerated centrifuge (model J2-21; Beckman) for shared use among its various research groups, benefiting a large number of researchers, graduate and undergraduate students. After more than 25 years of use such equipments are obsolete and some replacement parts are no longer available. Therefore, the acquisition of these multi-user equipments (analytical ultracentrifuge and refrigerated centrifuge) provides continuity to the research projects currently underway in the Department of Biochemistry laboratories, as well as those conducted by other research groups at the Institute of Biology / Unicamp. The direct benefits of the acquisition of the ultracentrifuge and centrifuge include the maintenance/ expansion of the scientific productivity of the researchers in the Department of Biochemistry, Institute of Biology/Unicamp, support for scientific collaborations with research groups abroad, as well as making the Institute a reference for multi-user facilities in the field of biochemistry.

## EQUIPMENT GRANTED

- Optima L-90 K preparative ultracentrifuge, with microprocessor-based control and a range of rotors, plus accessories (Beckman Coulter)
- Avanti J-E high-performance refrigerated centrifuge (Beckman Coulter)

# ASSOCIATED PROJECTS

**Institute of Biology/Unicamp**

*Detergent-resistant membranes [DRMs] in erythrocytes: induction by different agents, effects of the reduction of cholesterol, temperature variation, ionic strength, and local anesthetics*
Eneida de Paula
FAPESP Grant 2009/00904-1

*Biochemical, structural, and biological characterization of a class III secretory PLA2 isolated from marine cnidarians of the species* Bunodosoma caissarum
Sérgio Marangoni
FAPESP Grant 2008/53250-6

*Vaccine potential of viruses inactivated by high hydrostatic pressure and mapping of epitopes of biotechnological interest*
Carlos Francisco Sampaio Bonafé
FAPESP Grant 2008/09835-0

Contacts for instructions for the use of the equipment

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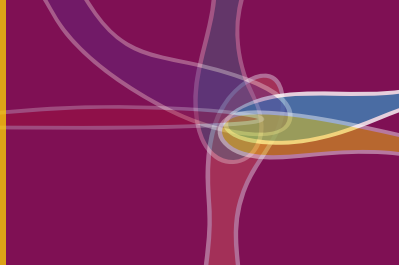



# ACQUISITION OF A BIOPHOTONIC IMAGING SYSTEM AND A MULTIPHOTON MICROSCOPY SYSTEM FOR *IN VIVO* IMAGING

Enilza Maria Espreafico

Ribeirão Preto School of Medicine

University of São Paulo (USP)

FAPESP Grant 2009/54014-7

## EQUIPMENT GRANTED

- LSM 780 NLO microscope, with two-photon excitation, and ZEN 2011 software (Carl Zeiss)
- IVIS Spectrum biophotonic imaging system (Caliper Life Sciences) and Biolum CCD Camera (Xenogen)

The objective of the present request is to fill a major gap in the availability of *in vivo* imaging methodologies in the state of São Paulo, through the acquisition of two modern pieces of equipment in this area: the IVIS Spectrum biophotonic *in vivo* imaging system (Caliper Life Sciences) and the LSM 780 NLO multiphoton microscopy system for intravital imaging (Carl Zeiss). This equipment will be installed in the currently functioning Center for Cell and Tissue Imaging of the Department of Cell and Molecular Biology and Pathogenic Bioagents of the USP Ribeirão Preto School of Medicine, a multi-user center that functions up to the standards of modern and traditional facilities in developed countries. The equipment to be acquired will function in a complementary manner. The biophotonic *in vivo* imaging system has low resolution but high sensitivity for the acquisition and quantification of fluorescence and luminescence in live animals, allowing the luminosity within the organism to be mapped in organs and defined anatomical regions. The multiphoton microscopy system is applied to the acquisition of high-resolution multifluorescence images, obtained over the surface of any organ, capable of revealing details of the extracellular matrix and of subcellular compartments, allowing dynamic events of cell proliferation and cell death to be examined, as well as cell-to-cell interaction, vascularization, innervation, and interactions with pathogens. It also makes it possible to detect intrinsic fluorescence emitted by molecules that function as markers of the metabolic state and oxidative stress, as well as facilitating the study of the dynamics of molecules, organelles, and structural components, through the use of techniques of photoactivation, uncaging, and photoconversion. Together, the two systems are powerful tools for the capture of *in vivo* images and provide data at complementary levels of resolution, for the evaluation of physiological and pathological events, being applied especially to the study of cancer, stem cells, tissue regeneration, immunology, inflammation, infection, neurobiology, development, aging, and metabolism, as well as to accelerating the process of gene characterization and drug development.

# ASSOCIATED PROJECTS

**Ribeirão Preto School of Medicine/USP**

*Myosin Va and DLCs as targets for antitumor therapy mediated by shRNA and apoptogenic peptides*
Enilza Maria Espreafico
FAPESP Grant 2009/50167-3

*Properties and functions of myosin V in the nucleus and centrosome*
Roy Edward Larson
FAPESP Grant 2007/59939-3

*Biological effects and pharmaceutical applications of lectins*
Maria Cristina Roque Antunes Barreira
FAPESP Grant 2006/60642-2

*Development of a bioengineered drug for tuberculosis immunotherapy*
Celio Lopes Silva
National Council for Scientific and Technological Development (CNPq)

*Different gene therapy approaches for the treatment of and elucidation of the mechanisms involved in Duchenne muscular dystrophy*
Arlete Aparecida Martins Coelho-Castelo
FAPESP Grant 2008/00052-2

*Structure and function of new* Drosophila melanogaster *genes whose orthologs in mammals are associated with cell and tissue dysfunction*
Maria Luisa Paçó Larson
FAPESP Grant 2007/50173-8

*Characterization of genes identified at the H locus of* Leishmania spp.
Luiz Ricardo Orsini Tosi
FAPESP Grant 2007/56187-0

*Elucidation of the cellular and molecular mechanisms of HIV-1 Nef downregulation of cell surface proteins*
Luis Lamberti Pinto da Silva
FAPESP Grant 2009/50650-6

*Effects of hypertension and pulsatile flow on the* in vitro *endothelial function of human saphenous veins*
Paulo Roberto Barbosa Evora
FAPESP Grant 2009/51511-6

*Influence of acid-base disorders on endothelium-dependent vascular reactivity*
Paulo Roberto Barbosa Evora
FAPESP Grant 2006/51329-9

*Study of the involvement of the opioid, serotoninergic and noradrenergic systems of the endogenous system of inhibition of pain in antinociceptive processes of the medial hypothalamus*
Norberto Cysne Coimbra
FAPESP Grant 2007/01174-1

## Contacts for instructions for the use of the equipment

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# CENTRAL LABORATORY OF HIGH-PERFORMANCE TECHNOLOGIES IN THE LIFE SCIENCES

Fernando Ferreira Costa
School of Medical Sciences
State University of Campinas (Unicamp)
FAPESP Grant 2009/54129-9

The LCTAD, Central Laboratory of High-Performance Technologies, has the mission of making advanced technologies in the areas of genomics, bioinformatics, proteomics, and cell biology available to the Unicamp community and to those of other universities and research institutes, in order to meet the growing demand for world-class research involving sophisticated high-performance techniques. The LCTAD will feature modern facilities in a space of approximately 2000 m$^2$ located in the Unicamp Technology Park. The building will house laboratories with high-performance genome sequencing equipment, equipment for analyzing the mass, sequence, and interaction of proteins, image analysis equipment, and computers with high processing speeds, as well as other equipment. The LCTAD will also have an auditorium for training sessions and multidisciplinary encounters. The LCTAD is a service provider and will not develop its own lines of research. In addition to providing the venues for the activities offered, the LCTAD will coordinate the formation of a network of accredited laboratories certified to provide services to the standards of those provided by the LCTAD. These laboratories will constitute a Virtual Network of Laboratories (VNL). Any professors or researchers that have underutilized high-performance equipment in their laboratories will be able to apply for membership in the VNL. The technologies that will be made available to the academic community will focus on genomics, bioinformatics, protein analysis, and cell biology. The LCTAD will be active in specific areas of the life sciences: 1) genomics: Sanger sequencing (ABI 3730) and high-throughput sequencing (454 Illumina/Solexa), transcriptome sequencing (array and sequencing of tags); 2) high-performance bioinformatics; 3) analysis of proteins: separation of samples by high-resolution chromatography or bidimensional chromatography (gel), mass spectrometry, and evaluation of protein-binding interactions; 4) flow cytometry, confocal microscopy and microdosage.

## EQUIPMENT GRANTED

- FLX high-performance genome sequencer and accessories (454 Life Sciences/Roche)
- Genome analyzer IIx (GAIIx), paired-end module, cluster station, and accessories (Illumina Inc.)
- 3730XL large-scale DNA analyzer and accessories (Applied Biosystems)
- MicroCal iTC200 isothermal titration calorimetry instrument (GE Healthcare)
- FV10i compact integrated confocal laser microscopy system with water-immersion objetives and accessories (Olympus Corporation)
- FACSCalibur flow cytometer with FACStation software and accessories (Becton Dickinson)
- Bio-Plex Suspension Array System sample reader and accessories (Bio-RAD Laboratories Inc.)
- 7500 Fast Real-Time high-performance PCR system with Tower computer (Applied Biosystems)
- Z1 dual threshold particle counter (Beckman Coulter)
- Agilent 2100 Bioanalyzer (GE Healthcare)
- Tissuelyser II sample disruptor for molecular analysis (100-240 V, 50/60 Hz), with microtubes, dispensers, and adapters (QIAGEN)
- 5305 Plus vacuum concentrator centrifuge (Eppendorf)
- 5430 R refrigerated microcentrifuge with FA-45-30-11 fixed-angle rotor (Eppendorf)
- MiniBIS Pro gel documentation system (110 V with Transilluminator) with 25-mm lens and accessories (BioAmerica Inc.)

# ASSOCIATED PROJECTS

**Blood Bank/Center for Hematology and Transfusion Medicine/Unicamp**

*National Institute of Science and Technology for Hematology Research*
Fernando Ferreira Costa
FAPESP Grant 2008/57895-1

*Chemical and molecular alterations in hemoglobinopathies and in other hereditary forms of hemolytic anemia*
Fernando Ferreira Costa
FAPESP Grant 2008/57441-0

**Institute of Biology/Unicamp**

*Green pathways for propene - Research Partnership for Technological Innovation, phase 3*
Gonçalo Amarante Guimarães Pereira
FAPESP Grant 2007/58336-3

*Integrated comparative study of three fungal diseases of cacao trees - witches' broom, moniliasis, and wilt disease - toward understanding the mechanisms of pathogenicity in order to develop control strategies*
Gonçalo Amarante Guimarães Pereira
FAPESP Grant 2009/50119-9

*Protein stability, protein structure, and protein folding pathway*
Carlos Henrique Inacio Ramos
FAPESP Grant 2005/00462-8

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Multi-user Equipment

FAPESP

BIOLOGICAL SCIENCES

# INFRASTRUCTURE FOR METABOLIC, MOLECULAR BIOLOGY, AND CELL BIOLOGY ANALYSES IN PROJECTS RELATED TO FUNCTIONAL AND COMPARATIVE GENOMICS

Gabriel Marroig Zambonato

Biosciences Institute

University of São Paulo (USP)

FAPESP Grant 2009/54203-4

The USP Biosciences Institute is a center of excellence in research, participating in cutting-edge projects, such as the Center for Human Genome Studies (funded by the FAPESP Centers for Research, Innovation, and Dissemination Program), FAPESP Thematic Projects, genome projects, projects funded by the FAPESP Program for Research on Bioenergy, and National Institute of Science and Technology projects. Currently, the Biosciences Institute is in a phase of expansion, having recently opened 16 new teaching positions and constructed a new building, with the objective of bringing together professors working in various specialties. The academic excellence acquired by the various groups at the Biosciences Institute can be strengthened by the establishment of a multi-user facility, tentatively designated the Laboratório Multiusuário de Análises Celulares e Moleculares (Lamcemol, Multi-User Laboratory for Cellular and Molecular Analyses). Therefore, the present project is aimed at equipping the Lamcemol, on the basis of the "facility" concept, with a high-resolution microtomograph (micro-CT) and a mass spectrometer (LTQ Orbitrap). The micro-CT (SkyScan 1076) combines the resolution of microscopes with the study of living systems through computed microtomography, allowing three-dimensional reconstructions, with a precision (pixel size) as fine as 7 microns, of organisms or entire structures, without the need for dissection. This makes it possible to obtain morphological data for cells, organs, and systems in a non-destructive manner. The other multi-user apparatus included in this proposal is a high-performance mass spectrometer, which allows the mass of molecules to be determined with great precision. The spectrometer has a number of applications, including those related to post-translational modifications, interactions among biomolecules, proteomics, and metabolic studies. The spectrometer requested features a combination of high resolution, precision in the determination of mass, and sensitivity (down to the subfemtomole level).

## EQUIPMENT GRANTED

- SkyScan 1076 high-resolution in vivo microtomography system (SkyScan Systems)
- LTQ Orbitrap high-performance mass spectrometer (Thermo Scientific)

The Lamcemol will be beneficial for various groups of the Biosciences Institute, because it will enable the execution of procedures including, but not limited to, the following: sequencing of DNA and proteins; identification of proteins that bind to biomolecules; analyses of protein-protein interactions; proteomic analyses; and metabolomic analyses.

# ASSOCIATED PROJECTS

**Biosciences Institute/USP**

*Biological aspects of thiols: protein structure, antioxidant effects, redox signaling, and redox states*
Luis Eduardo Soares Netto
FAPESP Grant 2007/58147-6

*Center for Human Genome Studies*
Mayana Zatz
FAPESP Grant 1998/14254-2

*National Institute of Science and Technology in Biofuels Research and Development*
Marcos Silveira Buckeridge
FAPESP Grant 2008/57908-6

*Sugarcane genome sequence: plant transposable elements are active contributors to gene structure variation, regulation and function*
Marie-Anne Van Sluys
FAPESP Grant 2008/52074-0

*Embryogenesis studies as the bases for reproduction and conservation strategies in tree species*
Eny Iochevet Segal Floh
FAPESP Grant 2004/03333-1

*Functional genomics in Plasmodium*
Celia Regina da Silva Garcia
FAPESP Grant 2007/52924-0

*Modularity and morphological diversification in mammals*
Gabriel Henrique Marroig Zambonato
FAPESP Grant 2009/05687-9

*Chemotaxonomy, molecular phylogeny, and pharmacological potential of croton [Euphorbiaceae], with an emphasis on native species*
Antonio Salatino
FAPESP Grant 2007/02518-6

*Alternative splicing and functional diversity of the FMR1 gene*
Luciana Amaral Haddad
FAPESP Grant 2008/53857-8

*Identification and characterization of genes linked to metabolic changes of interest in tomatoes*
Maria Magdalena Rossi
FAPESP Grant 2008/50946-0

*Molecular aspects of heterochromatin in species of the family Sciaridae (Diptera: Nematocera)*
Eduardo Gorab
FAPESP Grant 2008/50653-2

**Institute of Biomedical Sciences/USP**

*Effect of growth hormone replacement on bone formation in female rats with hypothyroidism treated with the selective thyromimetic of the thyroid hormone receptor beta*
Cecília Helena de Azevedo Gouveia Ferreira
FAPESP Grant 2005/52910-4

**São Paulo State University (Unesp), Campus Litoral (Shoreline Campus)**

*Functional and structural analysis of thiol-dependent antioxidant proteins: an investigation of the molecular mechanisms of catalysis and of the formation of protein complexes containing mixed disulfides*
Marcos Antonio de Oliveira
FAPESP Grant 2007/50930-3

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BIOLOGICAL SCIENCES

# LABORATORY OF MULTIMODAL MICROSCOPY – COMPLEMENTARY PROJECT FOR THE CREATION OF THE MAIN INCT-INFABIC LABORATORY

Hernandes Faustino de Carvalho

Institute of Biology

State University of Campinas (Unicamp)

FAPESP Grant 2009/54164-9

The objective of this project is to acquire equipment and accessories to be incorporated into the INFABiC, National Institute of Science and Technology in Photonics Applied to Cell Biology, which will be the first multimodal microscopy laboratory in Latin America, integrating modern techniques of microscopy based on linear and nonlinear optics. The structure suggested is particularly appropriate for the study of cellular phenomena, the biochemical, physiological, and biomechanical aspects of which can be monitored in a nondestructive manner and in real time. Therefore, it is important to combine the various techniques in simultaneous analyses, collecting all of the data relevant to the phenomenon under investigation. The INFABiC will make available tools that are fundamental for the understanding of the cellular processes based on a broad set of photonic methodologies. That will allow the development of cutting-edge basic research projects, in various areas, with the potential to have an immediate socioeconomic impact. The entire community involved in research at the tissue, cellular, or subcellular level will benefit from the creation of this laboratory. It will be a facility, with a considerable number of large-scale equipment, which require, for their efficient use, intensive knowledge in the areas of optics, photonics, and cell biology. Therefore, the only efficacious way in which to use the laboratory will be to share the knowledge in these areas, as well as the associated infrastructure, clearly making it a multi-user laboratory. Appropriate management of the laboratory will ensure its use by the greatest possible number of researchers. One of the results of the creation of the institute will be the natural formation of a network of multidisciplinary research, with the dissemination of the methodologies and their application in various biological models.

## EQUIPMENT GRANTED

- Axio Imager Z2 spectral confocal NLO microscope – for LSM 780 (Carl Zeiss)
- MMI CellManipulator Optical Tweezers for microdissection under confocal microscopy (Molecular Machines & Industries)
- NanoWizard 3 Bioscience AFM confocal microscope and accessories – tip scanner and NanoWizard 3 Vortis SPMControl station (JPK Instruments)
- Simple-Tau 154 stand-alone time-correlated single photon counting system for FLIM and FCS (Becker & Hickl GmbH)
- SPEC-10:100BR/LN monochromator and CCD camera for Raman spectroscopy (Princeton Instruments)

# ASSOCIATED PROJECTS

**Institute of Biology/Unicamp**

*Androgen regulation, sinalization and cellular interactions in prostate development, physiology and regression*
Henandes Faustino de Carvalho
FAPESP Grant 2009/16150-6

*INFABiC, National Institute of Science and Technology in Photonics Applied to Cell Biology*
Henandes Faustino de Carvalho
FAPESP Grant 2008/57906-3

**Institute of Physics/Unicamp**

*CEPOF, Center for Research in Optics and Photonics*
Carlos Lenz Cesar
FAPESP Grant 2005/51689-2

**Institute of Biomedical Sciences/USP**

*Dynamics of formation in tumor cell lines treated with bioactive laminin-derived peptides: Study in 4D microscopy*
Ruy Gastaldoni Jaeger
FAPESP Grant 2008/57103-8

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BIOLOGICAL SCIENCES

# FACILITY FOR ADVANCED STUDIES OF BIOSYSTEMS AND NANOSTRUCTURED MATERIALS

Igor Polikarpov
São Carlos Institute of Physics
University of São Paulo (USP)
FAPESP Grant 2009/54035-4

This proposal is aimed at modernizing and expanding a multi-user centralized laboratory (facility) that has been in operation for more than 20 years at the USP São Carlos Institute of Physics (IFSC), doing research and providing services in the area of inorganic and organic materials with techniques of scanning electron microscopy, atomic force microscopy and X-ray diffraction, meeting the needs of researchers in the state of São Paulo and in other Brazilian states. The modernization and the expansion will bring together modern techniques of confocal microscopy and dual-wavelength X-ray diffraction, as well as high-resolution electron microscopy, field-emission gun scanning electron microscopy, and state-of-the-art atomic force microscopy. With this expansion of the laboratory, it will be possible to broaden the gamut of materials investigated and to serve a greater number of researchers, with personnel highly qualified. In addition, the growing multidisciplinary emphasis at the IFSC has brought new demands for methodologies that make it possible to investigate organic and inorganic nanostructures, soft matter, biomass, and biosystems in general (confocal microscopy). The project is supported by two FAPESP Centers for Research, Innovation and Dissemination grants, three National Institute of Science and Technology grants, three FAPESP Thematic Project grants, one FAPESP Regular Project grant, and one Financing Agency for Studies and Projects grant, together with other associated project grants, totaling over 75 million Brazilian reals in financial resources, as well as various complementary grants. Through these projects, we seek to understand physical, physicochemical, and biological processes on the atomic and nanometric scales, in cutting-edge areas to which the groups participating in this proposal have made significant contributions at the international level. Because of the broad (statewide and nationwide) scope of some of the associated projects, a considerable number of researchers and specialists in technological innovation in the state of São Paulo will benefit, principally in terms of the infrastructure and the training of technicians at the IFSC to facilitate the sharing of the multi-user equipment.

## EQUIPMENT GRANTED

- SP5 AOBS broadband confocal microscope (Leica Microsystems)
- Dimension ICON scanning probe microscope and accessories (Bruker AXS/Veeco)
- X-RAY Difractometer Kappa Apex II Duo

# ASSOCIATED PROJECTS

**São Carlos Institute of Physics/USP**

*Structure and function of enzymes and auxiliary proteins from* Trichoderma, *active in cell-wall hydrolysis*
Igor Polikarpov
FAPESP Grant 2008/56255-9

*Structural biophysics of nuclear receptors and related proteins*
Igor Polikarpov
FAPESP Grant 2006/00182-8

*Photodynamic system for microbiological treatment and control*
Vanderlei Salvador Bagnato
FINEP/GNATUS

*National Institute of Optics and Photonics*
Vanderlei Salvador Bagnato
FAPESP Grant 2008/57858-9 and CNPq, National Council for Scientific and Technological Development

*Center for Optical Sciences and Photonics*
Vanderlei Salvador Bagnato
FAPESP Grant 1998/14270-8

*CBME, Center for Molecular and Structural Biotechnology*
Glaucius Oliva
FAPESP Grant 1998/14138-2

*INBEQMeDI, National Institute of Structural Biotechnology and Medicinal Chemistry in Infectious Diseases*
Glaucius Oliva
FAPESP Grant 2008/57910-0

*National Institute of Organic Electronics*
Roberto Mendonça Faria
FAPESP Grant 2008/57706-4 and CNPq

*Use of spectroscopic techniques for the study of molecular interactions on Langmuir, Langmuir-Blodgett, and self-assembled films*
Osvaldo Novais de Oliveira Junior
FAPESP Grant 2008/55587-8

*Microcellulose, nanocellulose, and their composites with cellulose, silica and plaster*
Milton Ferreira de Souza
FAPESP Grant 2006/57117-3

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BIOLOGICAL SCIENCES

# ACQUISITION OF AUTOMATED PLATFORMS FOR THE ANALYSIS AND PHOTODOCUMENTATION OF CRYSTALLIZATION ASSAYS OF BIOLOGICAL MACROMOLECULES AND HIGH-PERFORMANCE SCANNING OF BIOACTIVE COMPOUNDS

Íris Concepcion Linares de Torriani

National Synchrotron Light Laboratory

MCT, Science and Technology Ministry

FAPESP Grant 2009/54077-9

The principal objective of this project is to obtain the complementary instrumentation needed in order to achieve full automation and efficiency in the field of crystallization and in the performance of bioassays of biological macromolecules. The Center for Molecular and Structural Biology is equipped with all of the infrastructure necessary for cloning, expressing, and purifying proteins, as well as for developing crystallization assays, this last being partially automated. The full automation and miniaturization of the crystallization process will allow thousands of conditions for the growth of crystals to be tested in a rapid and efficient manner. A system for the identification, photodocumentation, and remote analysis of crystals is indispensable for consolidating the substantial gains made (time saved) with automated crystallization. The National Synchrotron Light Laboratory has two beamlines dedicated to protein crystallography. This places the Laboratory in a privileged position in Brazil and in South America, allowing competitive, world-class experiments to be conducted. With the synchrotron light source instrumentation that is currently on the market, a wiggler beamline in particular, we would be able to achieve even greater resolution of the structures of biological macromolecules. The crystallization process is recognized as a true stumbling block to advances in molecular and structural biology, and it is therefore essential to focus our efforts on overcoming this obstacle. The possibility of performing *in situ* assays is also of great importance for research into the interactions between proteins and inhibitors, which could lead to applications for the identification of bioactive compounds. Consequently, this proposal is aimed at the acquisition of a system of analysis and documentation of protein crystals that allows the visualization and photodocumentation of the assays. A plate reader that allows the large-scale automated scanning of bioactive compounds would complement this equipment, allowing the production of samples to reach a level in keeping with the methods of structural analysis available in the laboratory.

## EQUIPMENT GRANTED

- Minstrel HT UV system of crystal visualization and photodocumentation, with Gallery 700 Incubator (Rigaku Americas)
- Envision Multilabel plate reader (PerkinElmer Inc.)
- Freedom EVO 150 robotic platform for pipette handling (Tecan Trading AG)

# ASSOCIATED PROJECTS

**Center for Molecular and Structural Biology/Brazilian Association for Synchrotron Light Technology**

*Functional and structural studies of human regulatory proteins involved in cancer*
Jörg Kobarg
FAPESP Grant 2008/04849-2

**Boldrini Center for Children**

*Microenvironment of the bone marrow and PI3K in the drug resistance of pediatric acute lymphocytic leukemia*
José Andrés Yunes
FAPESP Grant 2008/10034-1

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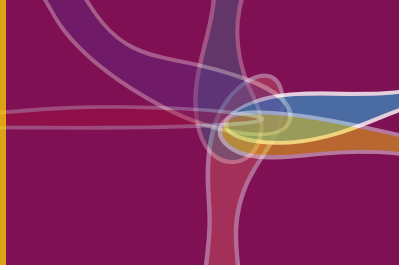



# ACQUISITION OF A CONFOCAL MICROSCOPE WITH CELL MICROINJECTOR AND FLOW CYTOMETER FOR THE ADVANCED INVESTIGATION OF MECHANISMS OF CELL DEATH RELATED TO OXIDATIVE STRESS

Iseli Lourenço Nantes

Center for the Natural and Human Sciences

Federal University of the ABC (UFABC)

FAPESP Grant 2009/54130-7

In the present project, we propose the acquisition of a confocal microscope with a microinjection system and a flow cytometer for investigating the molecular mechanisms of cell death related to oxidative stress. The associated projects are related to the following: the investigation of the mechanisms of cell death promoted by hemoproteins, hemopeptides, and porphyrins in the aortic cells of rabbits, focusing on the structure and the redox center of each agent of cell death; the investigation of the mechanisms of cell death promoted by phenothiazins in tumor cells, including its effects on the mitochondrial bioenergy and calcium homeostasis; and the investigation of the effects of copper and zinc imbalance on the generation of reactive species in cell culture, with repercussions for the viability of those cells. In addition, the project will benefit a great number of complementary projects in a wide variety of areas, including a project conducted abroad by Professor Rafael Radi, who is also our collaborator on the hemoprotein/hemopeptide project. Therefore, the equipment requested will make an enormous contribution to research in the state of São Paulo in terms of the publication of articles, the filing of patents, and the development of human resources.

## EQUIPMENT GRANTED

- Cell Lab Quanta SC flow cytometry system and accessories (Beckman Coulter)

# ASSOCIATED PROJECTS

**UFABC Center for the Natural and Human Sciences**

*Structure and reactivity of hemoproteins, hemopeptides, and porphyrins in homogeneous and heterogeneous media: basic aspects and applications in nanotechnology*
Iseli Lourenço Nantes
FAPESP Grant 2008/04948-0

*Characterization of the antitumor properties of phenothiazins in their ground and excited states, based on their effects on mitochondria, lysosomes, and biological membranes*
Tiago Rodrigues
FAPESP Grant 2006/00995-9

*Studies of oxidative and free-radical processes involving copper in a biological medium: an investigation of the molecular mechanisms of the oxidative activity of complexes and proteins*
Giselle Cerchiaro
FAPESP Grant 2007/50765-2

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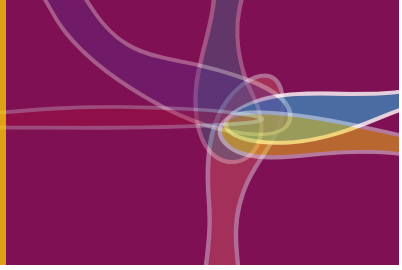



# ACQUISITION OF A DNA SEQUENCER FOR THE STUDY OF GENE POLYMORPHISMS IN MICROORGANISMS, THE REPERTOIRE OF T AND B CELLS, AND OTHER GENES OF INTEREST IN HEART DISEASE

Luiza Guilherme Guglielmi

Heart Institute

University of São Paulo (USP) School of Medicine Hospital das Clínicas

FAPESP Grant 2009/53859-3

The use of state-of-the-art automated DNA sequencers, which employ capillary electrophoresis, presents a series of advantages in relation to that of microplate readers. The sequencing process is almost entirely automated, without the need to prepare plates or gels, or to adjust the sequenced samples after electrophoretic separation. In addition, the runtime in a capillary-based system is shorter, allowing a greater number of samples to be sequenced within a given time frame. This technique has various applications in the study of DNA (sequencing, the study of polymorphisms, and the analysis of fragments, as well as other applications, depending on the software employed). The multi-user device requested will directly aid in the genotyping of microorganisms and of polymorphisms in the repertoire of T and B cells, as well as of other genes of interest in heart disease. By the end of the project, we expect to have gained a better understanding of the distribution of the most prevalent strains of *Streptococcus pyogenes* in the state of São Paulo, allowing us to evaluate the efficiency of the vaccine in development, which is aimed at preventing the rheumatic fever and other pathologies resulting from infection with *S. pyogenes*. The sequencer will also facilitate the identification of single-nucleotide polymorphisms in Chagas disease and of alterations in the repertoire of T and B cells in the peripheral blood of patients who have undergone transplantation (solid organ transplant or cell therapy). In addition, the equipment will be employed for the structural and functional analysis of the LEE region and of fimbrial and afimbrial adhesins of atypical enterogenic *Escherichia coli*. It will also be a valuable tool in the evaluation of various genes cloned in vectors, the objective being to determine the integrity of the DNA and the position of the insert, as well as to identify the mutations induced in genes of interest, for a variety of functional studies and for the production of recombinant proteins.

## EQUIPMENT GRANTED

- Model 3500 8-capillary Genetic Analyzer and accessories (Applied Biosystems)

# ASSOCIATED PROJECTS

**Heart Institute/USP School of Medicine Hospital**

*Identification of the strains of* Streptococcus pyogenes *isolated in the city of São Paulo through genotyping*
Luiza Guilherme Guglielmi
FAPESP Grant 2007/59262-3

*Coupling of the stress of the endoplasmic reticulum with the oxidative stress in vascular cells by the pathway of interaction between protein disulfide-isomerase and NAD(P)H-oxidase: role of thiol-oxidoreductase*
Francisco Rafael Martins Laurindo
FAPESP Grant 2004/13683-0

**School of Medicine/USP**

*Operational tolerance in human kidney transplantation: repertoire of B lymphocytes*
Verônica Porto Carreiro de Vasconcellos Coelho
CNPq, National Council for Scientific and Technological Development

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Multi-user Equipment

FAPESP

BIOLOGICAL SCIENCES

# FUNCTIONAL CORRELATION BETWEEN MAST CELLS AND TUMOR ANGIOGENESIS

Maria Célia Jamur

Ribeirão Preto School of Medicine

University of São Paulo (USP)

FAPESP Grant 2009/54013-0

In adult organisms, mast cells originate from the precursor cells present in the bone marrow, which migrate to peripheral tissues where they proliferate and mature. Recent studies have shown that these cells participate in pathological disorders of the nervous system, such as Alzheimer's disease, and multiple sclerosis, as well as in cardiovascular diseases, such as congestive heart failure and neo-angiogenesis. Some researchers have observed an accumulation of mast cells at sites where tumor angiogenesis is occurring, which suggests that mast cells directly contribute to the process of formation of new blood vessels. Mast cells are involved with tumorigenesis and angiogenesis due to ther production of chemical mediators as well as of certain angiogenic factors (vascular endothelial growth factor, platelet-derived growth factor, and basic fibroblast growth factor) that have a chemotactic effect on the mast cells. The objective of our current project is to characterize the relationship between mast cells and the initial phases of tumor angiogenesis. Initially, we will investigate the mediators produced in this phase of angiogenesis, which are responsible for the recruitment of mast cells. Subsequently, we will evaluate whether the increase in the number of mast cells during angiogenesis correlates with tumor progression and whether mediators released by mast cells participate in this process. Preliminary results show that mast cells accumulate at the site where tumor formation initiates and play a role in this process by liberating histamine, chymase, and tryptase, producing cytokines (FGF-α, FGF-β, TGF-β, G-CSF) and metalloproteases. To confirm these results, we will perform *in vitro* assays using mast cell produced chemical mediators that can act selectively in the formation of blood vessels. The three-dimensional configuration of the blood vessels will be analyzed by scanning electron microscopy. This project will contribute to the development of new treatments to inhibit tumor angiogenesis and, consequently, tumor progression.

## EQUIPMENT GRANTED

- JSM-6610LV low vacuum scanning electron microscope (JEOL Ltd.), with detectors for backscattered electrons, energy dispersive spectometry, wavelength dispersive X-ray spectometry, and accessories

# ASSOCIATED PROJECTS

**Ribeirão Preto School of Medicine/USP**

*Functional correlation between mast cells and tumor angiogenesis*
Maria Célia Jamur
FAPESP Grant 2007/54231-2

*Biological effects and pharmaceutical applications of lectins. Subproject: Lectin-carbohydrate interactions in the activation and regulation of cell functions*
Constance Oliver
FAPESP Grant 2006/60642-2

*The effects of the use of insulin and a temporary implant (natural latex biomembrane) in an experimental model of traumatic perforation of the tympanic membrane*
Miguel Angelo Hyppolito
FAPESP Grant 2009/05467-9

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Multi-user Equipment

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BIOLOGICAL SCIENCES

# SYSTEM FOR THE DETECTION OF SINGLET OXYGEN AND TRIPLETS IN CELLS AND IN OTHER COMPLEX ENVIRONMENTS

Maurício da Silva Baptista

Institute of Chemistry

University of São Paulo (USP)

FAPESP Grant 2009/53845-2

Reactive oxygen species, including free-radical and electronically excited species, are important in a variety of phenomena in nature, from the processes of photosynthesis inhibition and skin damage caused by sun exposure to pathological manifestations, such as neurodegenerative diseases and cancer, and even in clinical treatment protocols for photodynamic therapy. The understanding of these phenomena in complex systems, principally of biological origin, is gained through the development of tools designed to detect triplets and singlet oxygen. In this project, our proposal is to utilize the infrastructure available in our laboratory for the detection of singlet oxygen in solution, as well as other equipment also available in the laboratory, and to develop a multi-user centralized laboratory for the detection of triplets and singlet oxygen in cells and on surfaces. At this facility we will be able to characterize the specific site of singlet oxygen generation, as well as to quantify triplets with precision. In subproject 1, the research group led by Professor Baptista will employ molecular and nanometric photosensitizers, which the group has synthesized, in order to establish relationships among the structure, activity, and mechanism of cell death of photosensitizers, as well as to provide a detailed characterization of the process of injury in giant vesicle membranes. We believe that these studies will allow the development of clinical protocols adapted for photodynamic therapy, as well as furthering the understanding of other physiological and pathological conditions in which an oxidative imbalance occurs, as in neurodegenerative diseases and in skin damage caused by exposure to the sun. In subproject 2, the research group led by Professor Medeiros will use the facility in order to characterize damage to biomolecules and specific markers in proteins and nucleic acids in a model of amyotrophic lateral sclerosis. In subproject 3, the research group led by Professor Miyamoto will use the new facility in order to elucidate the molecular details of the biological damage that can be caused by polyunsaturated fatty acids. Various associated projects will benefit from this facility. These projects involve researchers in the fields of physics, chemistry, and biochemistry, engaged in studies of artificial photosynthesis, nanomaterials, antioxidants, photodynamic therapy etc.

## EQUIPMENT GRANTED

- NIR-CCD image detector for microscope coupling (Hamamatsu Photonics)
- Surelite optical parametric oscillator for laser flash photolysis and accessories (Photonic Solutions)
- Accessories for a Zeiss microscope: Cube 660 nm and 405 nm excitation lasers (Coherent Inc.); Cube Heat Sink (Coherent Inc.); and Axiovert objectives (Carl Zeiss MicroImaging)

# ASSOCIATED PROJECTS

**Institute of Chemistry/USP**

*Photodynamic therapy: physical, biochemical, and clinical aspects*
Maurício da Silva Baptista
FAPESP Grant 2005/51598-7

*Modified lipids and biological implications: study of the products of docosahexaenoic acid oxidation*
Sayuri Miyamoto
FAPESP Grant 2007/00025-2

*Damage to biomolecules promoted by redox systems. Study of the mechanisms and development of sensitive biomarkers*
Marisa Helena Gennari de Medeiros
FAPESP Grant 2006/56663-4

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Multi-user Equipment

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BIOLOGICAL SCIENCES

# CREATION OF A MULTI-USER LABORAT ORY FOR MOLECULAR GENETICS: ACQUISITION OF A FLUORESCENCE MICROSCOPE WITH STRUCTURED ILLUMINATION FOR ANALYZING 3D IMAGES FROM OPTICAL SECTIONS OF TISSUES AND ORGANS

Nilce Maria Martinez Rossi

School of Medicine at Ribeirão Preto

University of São Paulo (USP)

FAPESP Grant 2009/54034-8

We are requesting a fluorescence microscope with structured illumination for three-dimensional analysis of the images captured (images of sections from organs or tissues). The equipment

is state-of-the-art and will provide us with the image analysis capability and technical sophistication generated through hybridization and immunofixation protocols, thereby promoting the expansion of knowledge and sparking the process of innovation in molecular biology and biotechnology.

## EQUIPMENT GRANTED

- Axio Imager.Z2 fluorescence microscope with ApoTome attachment and accessories (Carl Zeiss AG)

# ASSOCIATED PROJECTS

**Ribeirão Preto School of Medicine/USP**

*Functional and comparative genomics in fungi*
Nilce Maria Martinez Rossi
FAPESP Grant 2008/58634-7

*Transcriptome analysis in* Diabetes mellitus
Geraldo Aleixo da Silva Passos Junior
FAPESP Grant 2008/56594-8

*Differential development of XX and XY embryos in the preimplantation period and pattern of expression of the genes TSPX and SRY*
Ester Silveira Ramos
FAPESP Grant 2005/00616-5

*Differential expression and methylation pattern in the MHM region during gonadal development in chickens*
Ester Silveira Ramos
FAPESP Grant 2009/08313-2

**Ribeirão Preto School of Philosophy, Science, and Literature/USP**

*Functional genomics of* Apis mellifera *– the search for new genes and functional networks in the contexts of development, cast differentiation, and reproduction*
Zilá Luz Paulino Simões
FAPESP Grant 2005/03926-5

*Validation of genes related to the anhydrobiosis by RNA interference (RNAi)*
Tiago Campos Pereira
FAPESP Grant 2008/54236-7

**Ribeirão Preto School of Philosophy, Science, and Literature/USP and Ribeirão Preto School of Medicine/USP**

*Responses to the silencing of DNA repair genes and of transcription factors in the chemoresistance and radioresistance of glioblastoma cells*
Elza Tieme Sakamoto Hojo
FAPESP Grant 2009/10925-6

*Cell and molecular signaling in response to genotoxic stress or oxidative damage in glioma lineages, evaluated in the lymphocytes of patients with diabetes or Alzheimer's disease*
Elza Tieme Sakamoto Hojo
FAPESP Grant 2006/01947-8

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FAPESP

BIOLOGICAL SCIENCES

# IMPLEMENTATION OF MASS SPECTROMETRY TECHNIQUE MALDI-TOF-TOF AT THE CENTER FOR ANALYTICAL SCIENCES OF THE UNIVERSITY OF SÃO PAULO INSTITUTE OF CHEMISTRY

Paolo di Mascio

Institute of Chemistry

University of São Paulo (USP)

FAPESP Grant 2009/53851-2

The objective of this project is the installation, at the USP Institute of Chemistry (IQ) Center for Analytical Sciences, of the maldi-tof-tof technique of mass spectrometry. The USP-IQ Center for Analytical Sciences was created in 1986 to meet the needs of USP-IQ researchers in the performance of techniques such as resonance spectrometry, mass spectrometry, infrared spectroscopy, and elemental analysis. Over time, the Center became a referral center within the academic community, not only within the state of São Paulo but in all of Brazil, providing services to the USP-IQ and to various research institutes and institutions of higher learning. In addition, the Center offers its services to private enterprises in the state of São Paulo, as a means of partnering with society in terms of the techniques of analysis that are not available on the market. The project envisions the installation of a high-resolution mass spectrometer that combines, in a single instrument, the more advanced technology of maldi-tof-tof with high analytical power and versatility (high-resolution and determination of exact mass available for routine analyses in a user-friendly device). Mass spectrometry has become an extremely popular technique because of its versatility and broad variety of applications. Tandem mass spectrometry has promoted a revolution in this field. The great majority of mass spectrometry users have reacted favorably to the potential of this technique to meet the needs of their projects. However, the equipment required to perform the maldi-tof-tof technique is costly, and the expense is rarely justifiable for a single project. The USP-IQ Center for Analytical Sciences, being an institutional center for the support of research, is fully capable of managing the equitable sharing of its resources among the researchers at the USP-IQ, as well as with those working at other public institutions or in the private sector.

## EQUIPMENT GRANTED

- UltrafleXtreme maldi-tof-tof mass spectrometer and accessories (Bruker Daltonics)
- High-performance liquid chromatography (HPLC) system

# ASSOCIATED PROJECTS

**Institute of Chemistry/USP**

*Singlet oxygen and hydroperoxides in chemical and biological systems*
Paolo Di Mascio
FAPESP Grant 2006/59458-2

*Synthetic polymers applied to biomaterials*
Luiz Henrique Catalani
FAPESP Grant 2005/02855-7

*Development of beta-glycosidases designed to improve the efficiency of noncomplexed cellulase systems*
Sandro Roberto Marana
FAPESP Grant 2008/55914-9

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# ACQUISITION OF A NUCLEAR MAGNETIC RESONANCE SPECTROMETERS FOR STUDIES OF BIOMOLECULES

Raghuvir Krishnaswamy Arni
São José do Rio Preto Institute of Biosciences, Literature, and Exact Sciences
São Paulo State University (Unesp)
FAPESP Grant 2009/53989-4

The NMR equipment will be installed at The Multiuser Center for Biomolecular Innovation, a multiuser facility established at UNESP- São Jose do Rio Preto. The Center, housed in a dedicated building of 640 m$^2$ (financed by UNESP) is located near the Department of Physics. This center will provide the experimental and technical support to conduct a wide range of experiments in structural biology ranging from protein expression, purification, crystallization and structure determination (NMR Spectroscopy, SAXS and Protein Crystallography).

Since this equipment will utilized for the study of a wide range of molecules, the configuration of the two NMR spectrometers acquired in the FAPESP Multiuser Equipment Program was chosen after consulting the potential users. The 600 MHz spectrometer and Cryo platform is suited for the study of the conformations and interactions of macromolecules in solution whereas, the 400 MHz spectrometer is suited for solid state studies such as membrane-peptides interactions and also for the study of small organic molecules.

The equipment acquired through earlier FAPESP programs for robotic crystallization, protein crystallography and small-angle X-ray scattering will be integrated into this center.

Additionally, as a means of supporting this center, UNESP is hiring a NMR research scientist and a technician for routine maintenance of the equipment and to provide assistance for potential users.

## EQUIPMENT GRANTED

- Bruker Superconducting Fourier NMR Spectrometer Advance III 600 MHz with 3 channels, 54mm bore UltraShieldPlus magnet, TXI-Z and TCI-Cryo probe heads and a fully automatic Cryo platform
- Bruker Superconducting Fourier NMR Spectrometer Advance III 400 MHz with 2 channels, 54mm bore ASCEND magnet, BBFO-Z and VTN/CPMAS-15N-31P+1H probe heads and a fully automated pneumatic unit

# ASSOCIATED PROJECTS

**Butantan Institute/São Paulo State Department of Health**

*Center For Applied Toxicology (CAT), a FAPESP Center for Research, Innovation, and Dissemination*
Hugo Aguirre Armelin
FAPESP Grant 1998/14307-9

**São José do Rio Preto Institute of Biosciences, Literature, and Exact Sciences/Unesp**

*Applied Venom Proteomics*
Raghuvir Krishnaswamy Arni
FAPESP Grant 2007/54865-1

*The search for the most promising compounds in the rational development of new drugs and pesticides through bioprospecting of the arthropod fauna in Brazil*
Mario Sergio Palma
FAPESP Grant 2006/57122-7

*Synthesis of enantioselective solids catalysts: the intellectual, technological and synthetic challenge of chiral zeolites*
José Geraldo Nery
FAPESP Grant 2005/54703-6

*Electrostatic contribution to the interaction between lytic peptides and model membranes*
João Ruggiero Neto
FAPESP Grant 2007/03657-0

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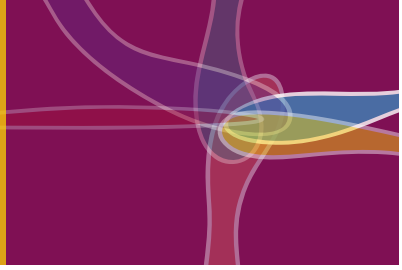



# ACQUISITION OF A SPINNING-DISK CONFOCAL MICROSCOPE WITH HIGH SPATIAL AND TEMPORAL RESOLUTION

Renato Arruda Mortara

Paulista School of Medicine

Federal University of São Paulo (Unifesp)

FAPESP Grant 2009/53833-4

This project is aimed at the acquisition of a fast imaging system. The equipment chosen (Leica SP5 TS) provides excellent spacial and time resolution both in live and fixed biological specimens.

## EQUIPMENT GRANTED

- TCS SP5 II Tandem Scanner Spinning Disk Confocal Imaging System, with optional AOBS (Leica Microsystems)

# ASSOCIATED PROJECTS

**Federal University of São Paulo (Unifesp)**

*Invasion and intracellular transit of infective forms of* Trypanosoma cruzi *(subproject of the thematic project:* In vitro *and* in vivo *molecular studies of* Trypanosoma cruzi *and its interaction with host cells and factors)*
Renato Arruda Mortara
FAPESP Grant 2006/61450-0

*Immunological characterization of the fine-point pattern tending toward homogeneity, observed through indirect immunofluorescence in Hep-2 cells*
Luiz Eduardo Coelho Andrade
FAPESP Grant 2009/51887-0

*Cellular organization in the differentiation and cell cycle of* Trypanosoma
Sérgio Schenkman
FAPESP Grant 2007/54621-5

## Contacts for instructions for the use of the equipment

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BIOLOGICAL SCIENCES

# CORE FACILITY FOR SCIENTIFIC RESEARCH UNIVERSITY OF SÃO PAULO CEFAP-USP

Rui Curi

Institute of Biomedical Sciences

University of São Paulo (USP)

FAPESP Grant 2009/53994-8

The present proposal aims to implement a core facility that will provide services to the research community of this university, as well as to researchers working at other institutions. Facilities are currently being established in four separate themes: 1) Biological Imaging with two photon microscopy to study living cells and Bioluminescence system to perform *in vivo* animal images; 2) Cell sorting by flow cytometry; 3) Genomics with next generation sequencing; and 4) Proteomics and mass spectrometry. An area of approximately 200 $m^2$ is being prepared for CEFAP-USP, located at Institute of Biomedical Sciences. Six research specialists are being hired to manage and operate the equipment. We expect these efforts will provide feasible means to support researches of the community of this university, and of the state of Sao Paulo, as a whole, which will improve the quality of the scientific production of our scientists.

## EQUIPMENT GRANTED

- Imaging of Live Cells, Tissues and Animals – Confocal two photon microscopy and IVIS.
  - IVIS Spectrum in vivo imaging system (Caliper Life Sciences) with Biolum CCD Camera (Xenogen)
  - Two photon (LSM710) confocal microscopy system (Carl Zeiss).
- Flow Cytometry for cell sorting.
  - Flow cytometer and sorter ARIA III (Becton Dickinson)
- Genomics and New generation DNA sequencing
  - SOLID 4 and SOLID 5500XL plataforms (Life Technologies)
- Proteomics and Mass Spectrometry
  - Nano LC-MS/MS Platform - LTQ Orbitrap Velos Mass Spectrometer equipped with electron transfer dissociation (ETD) source (Thermo Scientific)
  - LC-MS/MS Platform - TSQ Quantum Access Triple Stage Quadrupole Mass Spectrometer (Thermo Scientific)
  - MALDI TOF/TOF Platform - Autoflex Speed Mass Spectrometer (Bruker Daltonics)
  - Surface Plasmon Resonance Detection system - Biacore 3000 (GE Healthcare)

# ASSOCIATED PROJECTS

**Institute of Biomedical Sciences/USP**

*Study of the mechanisms of action of fatty acids in leukocytes*
Rui Curi
FAPESP Grant 2004/12137-1

*Cellular responses to genome damage*
Carlos Frederico Martins Menck
FAPESP Grant 2009/52417-7

*Investigating the role of the hemi-oxygenase-1 in various renal inflammatory processes in animal models*
Niels Olsen Saraiva Câmara
FAPESP Grant 2007/07139-3

*Behavioral and biochemical effects of calorie restriction in the setting of a inflammatory stimulus in the rat central nervous system*
Cristóforo Scavone
FAPESP Grant 2008/58865-9

*Virulence factors of* A. actinomycetemcomitans*: participation in disease, regulation of expression, diversity, and immune response*
Marcia Pinto Alves Mayer
FAPESP Grant 2003/08598-0

*Signaling mechanisms involved in chronic hyperglycemia: effects on cell migration*
Marinilce Fagundes dos Santos
FAPESP Grant 2006/57508-2

*Genetic diversity, population structure, and transmission dynamics of* Plasmodium vivax *in the rural Brazilian Amazon*
Marcelo Urbano Ferreira
FAPESP Grant 2007/51199-0

*Neural bases of motivated behaviors*
Newton Sabino Canteras
FAPESP Grant 2005/59286-4

*Biosynthesis of isoprenoids in* Plasmodium falciparum*. Potential target for the development of new antimalarial drugs*
Alejandro Miguel Katzin
FAPESP Grant 2007/59567-9

*Molecular and functional characterization of cultures of adrenal tumor cells obtained from fragments of human adrenal tumors*
Claudimara Ferini Pacicco Lotfi
FAPESP Grant 2009/01338-0

*Viral genetic diversity network (VGDN)*
Paolo Marinho de Andrade Zanotto
FAPESP Grant 2000/04205-6

In vivo *targeting of recombinant antigens to dendritic cells: a new strategy for vaccine development*
Silvia Beatriz Boscardin
FAPESP Grant 2007/08648-9

*Glucose transporters and diabetes mellitus: contribution to knowledge of glycemia regulation and of the development of complications*
Ubiratan Fabres Machado
FAPESP Grant 2007/50554-1

*The role of melatonin in energy metabolism control: hormonal interactions, central and peripheral effects (pineal, obesity, diabetes, and aging)*
José Cipolla Neto
FAPESP Grant 2004/06767-2

*Cellular and molecular aspects of muscle plasticity*
Anselmo Sigari Moriscot
FAPESP Grant 2006/61523-7

*Activation of the immune system in pulmonary paracoccidioidomycosis: host and fungus factors that influence disease severity*
Vera Lúcia Garcia Calich
FAPESP Grant 2004/14518-2

*Molecular mechanisms involved in the regulation of pancreatic B cell function*
Angelo Rafael Carpinelli
FAPESP Grant 2005/60232-6

*Molecular aspects of the microbicidal and inflammatory effects of leukocytes in the lung*
Sonia Jancar Negro
FAPESP Grant 2006/03982-5

*Study of vascular function in an experimental model of the metabolic syndrome*
Maria Helena Catelli de Carvalho
FAPESP Grant 2007/59702-3

*Cellular characterization and analysis of the signaling pathways of the G protein-coupled receptor GPR30: participation in the estrogen-induced neuroprotective effects on the central nervous system*
Carolina Demarchi Munhoz de Souza
FAPESP Grant 2008/55178-0

## Contacts for instructions for the use of the equipment

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FAPESP

BIOLOGICAL SCIENCES

# ACQUISITION OF A HIGH-PERFORMANCE PLATFORM FOR COMPUTATIONAL ANALYSES APPLIED TO THE FIELD OF MEDICINE

Wilson Araújo Silva Júnior
Ribeirão Preto School of Medicine
University of São Paulo (USP)
FAPESP Grant 2009/53853-5

The proposal is aimed at the acquisition and setup of a high-performance platform for computational analyses applied to the field of medicine, with an emphasis on genomics. The availability of various state-of-the-art models of sequencers has resulted in a great volume of raw data. However, data processing capacities have not kept pace with that increase and therefore represent a stumbling block to the computational analysis of the data generated. The groups involved in this proposal have extensive experience with sequencing data, being (and having been) directly involved in various projects in the area of genomics. The data processing power of genomics platforms has also been used in various analyses, including drug design and the modeling of biological systems. The platform requested here will also benefit projects in these areas of knowledge. We expect this platform to have a significant impact on the projects carried out by the groups involved. The proposal also encompasses the organization of two courses on the processing and analysis of genomic data, which will be open to all interested parties within the scientific community.

## EQUIPMENT GRANTED

- ProLiant DL385 G6 servers (64 GB and 32 GB) for data processing and storage and accessories: MSL2024 tape library (backup) and rack (Hewlett-Packard Development Company, L.P.)
- 3CBLSG24 baseline switch (3Com Corporation)

# ASSOCIATED PROJECTS

**Ludwig Institute for Cancer Research**

*Alternative splicing: integration of computational strategies for the functional characterization of variants identified by mutations in splicing regulatory sites in tumors of color, breast and glioblastoma*
Sandro José de Souza
FAPESP Grant 2007/55790-5

*Identification of molecular markers of breast cancer through the study of the methylation pattern in tumor cells*
Anamaria Aranha Camargo
FAPESP Grant 2004/09088-9

**Antônio Cândido Camargo Hospital**

*Microarray analysis of gene expression at the various stages of kidney and liver development: implications in embryonal tumors*
Dirce Maria Carraro
FAPESP Grant 2006/00054-0

**Heart Institute/USP School of Medicine Hospital das Clínicas**

*Rational design of protein kinase C-specific peptide inhibitors: a computational and experimental validation approach*
Paulo Sérgio Lopes de Oliveira
FAPESP Grant 2008/52695-4

Contacts for instructions for the use of the equipment

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BIOLOGICAL SCIENCES

# ACQUISITION OF A CONFOCAL LASER SCANNING MICROSCOPE FOR A MULTI-USER MICROSCOPY CENTER AT SÃO PAULO STATE UNIVERSITY

Sebastião Roberto Taboga

São José do Rio Preto Institute of Biosciences, Literature, and Exact Sciences

São Paulo State University (Unesp)

FAPESP Grant 2009/53990-2

Confocal laser scanning microscopy has become an extremely important tool in various areas of biology, medicine, and material sciences. The researchers at our Institute have acquired experience with this technique through collaborations, within Brazil and abroad. This has increased our interest in acquiring the necessary equipment for performing the technique in our multi-user laboratory, established with FAPESP resources a decade ago. The acquisition of a confocal laser scanning microscope for the Professor Celso Abbade Mourão Multi-User Microscopy Center, located on the São José do Rio Preto campus of Unesp, will benefit a number of FAPESP-funded research projects that are currently underway, or were recently concluded, in various areas of knowledge: reproductive cell biology (headed by Professors Sebastião Taboga and Clássius de Oliveira); inflammation biology (headed by Professor Sonia Oliani); cell biology and insect cytogenetics (headed by Professors Maria Tercilia V. A. Oliveira and Mary Itoyama); molecular biology and molecular cytogenetics of cancer (headed by Professor Ana Elizabete Silva); microscopy in food sciences (headed by Professor Célia Landi-Franco); histophysiology of diabetes and obesity (headed by Professors José R. Bosqueiro and Rejane Maira Góes); virology (headed by Professor Paula Rahal); and biodiversity (headed by Professors Orlando Nechi Jr., Luis H. Zanini Branco, and Nelson Bittencourt Jr.). All of these projects are dependent upon the Microscopy Center and will benefit considerably from the acquisition of the equipment requested. Because the assessment by confocal microscopy combines an important component to all research cited above, making it possible to employ immunocytochemical and topochemical techniques in the identification of specific markers, including their colocalization, which is extremely difficult to detect with conventional fluorescence microscopy. We emphasize that some of the researchers, including the General Coordinator, have published articles involving the use of confocal microscopy.

## EQUIPMENT GRANTED

- TCS SP5 broadband confocal microscope and accessories (Leica Microsystems)

# ASSOCIATED PROJECTS

**São José do Rio Preto Institute of Biosciences, Literature, and Exact Sciences/Unesp**

*Morphofunctional aspects of the female prostate during the estrous cycle in gerbils*
Sebastião Roberto Taboga
FAPESP Grant 2006/06876-1

*Comparison of the effects of 5-alpha reductase and aromatase enzyme blockers in the prostate of gerbils at different ages*
Sebastião Roberto Taboga
FAPESP Grant 2008/00608-0

*Effects of androgenic and estrogenic suppression in the prostate microenvironment of the aged gerbil*
Sebastião Roberto Taboga
FAPESP Grant 2005-04670-4

*Morphophysiology of the prostate of bats: comparative study in the families Phyllostomidae, Molossidae, and Vespertilionidae*
Sebastião Roberto Taboga
FAPESP Grant 2009/03470-2

*Systematic analysis of the genus Batrachospermum [Batrachospermales, Rhodophyta] in Brazil based on morphological and molecular data*
Orlando Necchi Junior
FAPESP Grant 2008/00708-5

*Study of the role that the protein annexin 1 and formyl peptide receptors [FPRs] play in epidermoid carcinoma of the head and neck*
Sonia Maria Oliani
FAPESP Grant 2008/08187-4

*Studies of TP53, FHIT, and MTS-1 gene mutations and protein expression in megaesophagus secondary to Chagas' disease: association with the genetic variability of* Trypanosoma cruzi
Ana Elizabete Silva
FAPESP Grant 2005/54489-4

*Evaluation of the pattern of gene expression in precancerous gastric lesions: association with carcinogenesis in the stomach*
Ana Elizabete Silva
FAPESP Grant 2007/58661-1

## Contacts for instructions for the use of the equipment

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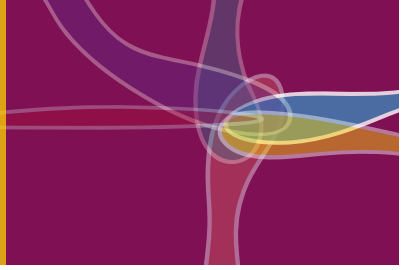

Multi-user Equipment

FAPESP

BIOLOGICAL SCIENCES

# ESTABLISHMENT OF AN ANALYTICAL SCIENCES FACILITY FOR DNA SEQUENCING AND MOLECULAR STUDIES OF NEW CHEMOTHERAPEUTIC TARGETS IN MICROORGANISMS AND THE USE OF METAGENOMICS FOR THE ISOLATION AND IDENTIFICATION OF NEW MICROBIAL-DERIVED ANTIBIOTICS

Sérgio Akira Uyemura

Ribeirão Preto School of Pharmaceutical Sciences

University of São Paulo (USP)

FAPESP Grant 2009/54099-2

In the last two decades, opportunistic infections caused by microorganisms have often been diagnosed inimmunocompromised patients and are a leading cause of morbidity and mortality in hospitalized patients the world over. With the rapid increase in the number of AIDS cases, the incidence of some diseases, such as those caused by microorganisms, has increased drastically. In addition to the high incidence of opportunistic infections, the number of multidrug-resistant microorganisms is one of the principal challenges in the treatment of these diseases. The molecular aspects involved in such multidrug- resistance include modifications of the target enzymes, overexpression of genes integrons, transposons, and stress response proteins. In this context, the discovery of new chemotherapeutic agents, particularly those that act via multiple metabolic pathways, as well as improving the understanding of their mechanisms of action is of great therapeutic importance. Therefore, it is essential the introduction of new classes of more selective and less toxic drugs, active on multidrug-resistant microorganisms, into clinical practice. However, the majority of the new therapeutic agents introduced are based on the already known chemotherapeutic targets and, in recent years, practically no new targets have been described, making it difficult to control the mechanisms of resistance. Nevertheless, the introduction of new techniques creates new possibilities for studies in areas such as pharmacogenomics, bioinformatics, molecular modeling, profile of expression/transcriptome profiling, proteomics, and metagenomics. The associated projects involve the study of new targets for the treatment of microorganisms, using molecular approaches, use of metagenomics for the isolation and identification of new microbial-derived antibiotics, and the study of the molecular mechanisms of resistance involved in multidrug-resistant bacteria. Therefore, having the possibility to conduct large-scale sequencing at the institution is essential for the development of these projects, as well as of the complementary projects, or even new projects, in this area.

## EQUIPMENT GRANTED

- Model 454 GS Junior System and accessories – Next Generation Sequencing (Roche Life Sciences)

# ASSOCIATED PROJECTS

**Ribeirão Preto School of Pharmaceutical Sciences/USP**

*Alternative mitochondrial pathways: biochemical and molecular studies of a UCP-like sequence of* Aspergillus sp.
Sérgio Akira Uyemura
FAPESP Grant 2009/08470-0

*Molecular analysis of mechanisms that determine antibiotic resistance in* Pseudomonas aeruginosa *and* Acinetobacter spp.
Ana Lucia da Costa Darini
FAPESP Grant 2008/56370-0

*New strategies in the quest for natural antibiotics: symbiotic microorganisms as sources of substances and in vivo assay based on a model of* Caenorhabditis elegans *infection*
Mônica Tallarico Pupo
FAPESP Grant 2008/09540-0

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Multi-user Equipment

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BIOLOGICAL SCIENCES

# ACQUISITION OF A 700 MHZ NUCLEAR MAGNETIC RESONANCE INSTRUMENT WITH CRYOGENICALLY COOLED PROBE FOR THE ANALYTICAL CHEMISTRY CENTER OF THE UNIVERSITY OF SÃO PAULO

Shaker Chuck Farah

Institute of Chemistry

University of São Paulo (USP)

FAPESP Grant 2009/54009-3

The Analytical Chemistry Center of the USP Institute of Chemistry (IQ) has maintained one of the largest nuclear magnetic resonance (NMR) equipment facilities in the country and has made that equipment available to a great number of research groups (including those of private sector companies) that employ NMR as a high-resolution analytical tool for studying the composition, structure, and conformational behavior/dynamics of chemical materials. The services offered (sample analysis, training, and technical support for advanced users) are possible due to the team of highly qualified technicians and the modern infrastructure of the USP-IQ, as well as the maintenance and modernization of the equipment, funded, in part, by resources obtained from the fees charged for providing services. In this project, we intend to acquire an NMR instrument, equipped with a cryogenically cooled probe, to be incorporated into to the Analytical Chemistry Center of the USP-IQ. The equipment will benefit a great number of users of the NMR services administrated by the Center, researchers at our Institute in particular, together with other researchers in the city and state of São Paulo, as well as in the rest of the country. This equipment, in combination with the cryogenically cooled probe requested, will increase the capacity of the users to analyze systems and samples of greater complexity (polymers, biological macromolecules, and mixtures of natural products) with greater sensitivity and resolution (for example, with the cryogenically cooled probe, macromolecules can be studied by employing concentrations in the micromolar range). The multi-user nature of this project, combined with the open administration of the Center, ensures that the new instrument will be available to a panoply of users and collaborators at the IQ and at other research centers in the state of São Paulo. This will also effectively expand the small group of centers in the country specializing in macromolecular NMR.

## EQUIPMENT GRANTED

- 700-MHz nuclear magnetic resonance instrument with cryogenically cooled probe and accessories
- Uninterrupted power supply (15 kV)

# ASSOCIATED PROJECTS

**Institute of Chemistry/USP**

*Structural and functional analysis of multiprotein systems that play important roles in the pathogenicity of* Xanthomonas axonopodis pv citri
Shaker Chuck Farah
FAPESP Grant 2005/59243-3

*Structural and dynamic characterization of polymers and micellar aggregates by nuclear magnetic resonance (NMR)*
Omar Abou El Seoud
FAPESP Grant 2004/15400-5

*Ordered arrays of macromolecules and nanostructures for the development of sensors and microbatteries*
Roberto Manuel Torresi
FAPESP Grant 2003/10015-3

*Preparation and application of chiral compounds containing chalcogenides*
João Valdir Comasseto
FAPESP Grant 2008/55401-1

**São Paulo State University (Unesp) at Araraquara and University of São Paulo (USP)**

*Conservation and sustainable use of the diversity from Cerrado and Atlantic Forest: chemical diversity and prospecting for potential drugs - phase II*
Vanderlan da Silva Bolzani
FAPESP Grant 2003/02176-7

Contacts for instructions
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Shaker Chuck Farah

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# ACQUISITION OF A CONFOCAL LASER SCANNING MICROSCOPY SYSTEM AND A TRANSMISSION ELECTRON MICROSCOPE FOR THE AUXILIARY ELECTRON MICROSCOPY CENTER

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FAPESP Grant 2009/54141-9

Since its creation, in the 1980s, the CME, Electron Microscopy Center, has distinguished itself as one of the most organized microscopy centers in the country, being recognized by renowned researchers in the areas of biological sciences and of material sciences. Using conventional and advanced techniques of scientific investigation, the CME has met the needs related to instruction, research, and extension education, not only of the Biosciences Institute but also of other sectors on the Botucatu Campus of Unesp, for approximately 30 years. The Center also provides services to public and private entities in the off-campus community. The recent scientific production of researchers who are CME users has distinguished those researchers within the scientific community. The Center is located in the central region of the state of São Paulo, which makes it accessible to a great number of researchers from across the state. In addition, for the third consecutive year, the Botucatu Institute of Biosciences has received the Unesp award for outstanding scientific productivity. A large part of this production was the result of studies developed at the CME. Therefore, we are interested in increasing the level of technological innovation at the Center by acquiring an LSM 780 Quasar ZEN 2010 confocal laser scanning microscopy system, which will be the only of its kind in the state of São Paulo, as well as a new transmission electron microscope with a digital image capture system. To that end, we report that the Center is currently involved in associated and complementary projects conducted by researchers working at various institutions and departments, thus meeting the definition of a multi-user facility. As previously mentioned, the central location of the CME also promotes its use by multiple users. The necessary infrastructure is already in place, as are the human resources, although additional advanced support technicians will be hired. The Unesp administration has pledged its full, duly documented, support, and we believe that the acquisition of the abovementioned equipment will contribute considerably to the scientific and technological betterment of a great number of users, as well as to the training of highly qualified personnel, and will promote international cooperation among researchers.

## EQUIPMENT GRANTED

- Leica TCS SP5 laser confocal microscope
- Tecnai Spirit transmission electron microscope

# ASSOCIATED PROJECTS

**Botucatu Institute of Biosciences (Unesp)**

*Secretory structures in vegetable species of the Brazilian cerrado (scrubland): morphological, chemical, and ecological approach*
Silvia Rodrigues Machado
FAPESP Grant 2008/55434-7

*Biology of renal development in a rat model of protein restriction during gestation*
Patricia Aline Boer
FAPESP Grant 2005/54362-4

*Expression of the factors of myogenic regulation in the striated skeletal muscle of rats with heart failure submitted to to the exercise: metabolic, hormonal, and inflammatory relationships*
Maeli Dal Pai Silva
FAPESP Grant 2007/57048-4

*Gene and protein expression of myogenic regulatory factors and of myostatin in the skeletal muscle of the pirarucu* (Arapaima gigas *Cuvier 1817) during growth*
Maeli Dal Pai Silva
FAPESP Grant 2007/58896-9

*Muscle growth and differential expression of myogenic regulatory factors and of myostatin in the striated skeletal muscle of the pirarucu* (Arapaima gigas)*, during the growth*
Maeli Dal Pai Silva
FAPESP Grant 2007/59584-0

*Morphofunctional adaptations and molecular responses of the skeletal muscle of rats submitted to to resistance training (doctoral scholarship work)*
Maeli Dal Pai Silva
FAPESP Grant 2007/58905-8

**School of Medical Sciences/Unicamp**

*Experimental models of human diseases: improving the multi-user infrastructure and defraying the costs of research at the Medical and Experimental Surgery Center*
José Antônio Rocha Contijo
FAPESP Grant 2008/54658-9

## Contacts for instructions for the use of the equipment

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Multi-user Equipment

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# ACQUISITION OF A LASER CAPTURE MICRODISSECTION PLATFORM FOR APPLICATION IN CELL AND MOLECULAR BIOLOGY STUDIES

Siu Mui Tsai

Center for Nuclear Energy in Agriculture (Cena)

University of São Paulo (USP)

FAPESP Grant 2009/53998-3

This project request is for the acquisition of a laser capture microdissection (LCM) system based on gravity and the movement of a laser beam over the samples, which will make it possible to perform dissection studies and to capture cells from tissue or rhizosphere samples and, at the same time, will ensure high performance in terms of the speed and precision of the microdissection of samples of different thicknesses. This technology has been applied to obtaining cells of plants and animals, as well as, more recently, to the study of plant-microorganism interactions. Particularly, the techniques used in studies in the plant-microorganism interactions are of particular relevance for research into mutualistic plant-pathogen and plant-microorganisms relationships. The USP-CENA research team will use the LCM system in their analyses of gene (proteomic) expression at specific sites (transcription and spatial expression), because it allows RNA to be isolated, provides data related to transcript profiles, facilitates the investigation of gene functionality, and can identify the molecular basis of the active processes in samples. The system is accompanied by devices for the motorization of the microscope, in order to capture and overlap fluorescence images, as well as to monitor the functions of the LCM system. The estimated cost is € 182,720.
The system will serve to support projects conducted by USP-CENA researchers, as well as those conducted by researchers affiliated with the following institutions: the State University of Campinas (Unicamp); Institute of Biology; the University of São Paulo (USP) Ribeirão Preto School of Pharmaceutical Sciences; the USP Higher School of Agriculture Luiz de Queiroz; the Unicamp Faculty of Dentistry of Piracicaba; the Federal University of Rio de Janeiro Institute of Biology; and the Brazilian Agricultural Research Corporation. The projects involve techniques such as *in situ* hybridization, immunolocalization, and visualization of reporter genes and have required the cell-specific analysis of the expression of individual genes and of the accumulation of individual proteins. The LCM technique is a cutting-edge method that can furnish such cellular data for large-scale studies in genomics and proteomics.

## EQUIPMENT GRANTED

- LMD7000 laser microdissection system (Leica Microsystems)
- Platform with Axio Observer Z1 inverted microscope (motorized Z axis), AxioCam HSm high-speed digital camera and accessories (Carl Zeiss AG)

# ASSOCIATED PROJECTS

**Center for Nuclear Energy in Agriculture/USP**

*Monitoring the microbial diversity and functional activities in response to land-use changes and deforestation under soybean and sugarcane cultivations*
Siu Mui Tsai
FAPESP Grant 2008/58114-3

**Ribeirão Preto School of Philosophy, Science, and Literature/USP**

*Characterization of genes preferentially expressed in the stigma/stylus of* Nicotiana tabacum*: identification by macroarray and SSH*
Maria Helena de Souza Goldman
FAPESP Grant 2006/54431-9

**Institute of Biology/Unicamp**

*Flowering and fructification of Valencia orange trees: anatomical, physiological, biochemical, and molecular aspects*
Marcelo Carnier Dornelas
FAPESP Grant 2005/57862-8

Contacts for instructions for the use of the equipment

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