Institut Pasteur de Montevideo

Scientific Report 2018

INDEX

PREFACE

The Institut Pasteur de Montevideo aims to be a "state-of the-art" research center with international projection in the field of biomedicine with focus on molecular mechanisms of human and animal diseases. We are also committed to find new diagnostic tools, treatments and cures, contributing to the development of drugs, vaccines and biomarkers of disease.

By December 2018, around 270 people worked or studied at the IP Montevideo, including almost 100 established researchers, technicians or assistants coming from several partner institutions and 40 collaborators working in administration and research support units.

Research groups are organized into institutional programs focused on the One-Health concept, with emphasis in multidisciplinary molecular approaches in human and veterinary medicine including environmental interactions. These programs are mainly funded by institutional grants or grants from several agencies including National Agency for Innovation and Research (ANII) National Institute for Agricultural Research (INIA), National Republic University FOCEM (Mercosur), Interamerican Development Bank (IDB), and the Institut Pasteur International Network (IPIN).

The IP Montevideo has established central core facilities with "state-of-the-art" equipment to study genomic, proteomic, structural & cell biology and animal research. In 2018, a significant investment was made to purchase one equipment to study “circular dichroism”, one device for “in vivo imaging” and a new confocal microscopy.

The number and impact of the publications have reached the average of 90 publications/year. According to international databanks, publications from the IP Montevideo have a cumulated average of >20 citations per publication, which can be considered of competitive international standard. Remarkably, new patents on a new drug-class technology have been filed in the last year; some of them have been licensed to a start-up company. More interestingly, 2 scientific groups won a position in our international call for startup creation, in a join effort with an incubator from Argentina, CITES (http://cites-gss.com/en/).

Our research laboratories provide an environment for the training of advanced graduate students. The IP Montevideo also contributes to the training of human

capacities in collaboration with the national and international postgraduate programs. In 2018, we harbour more than 120 MSc, doctoral and postdoctoral fellows. We have also organized several international courses on different topics of molecular medicine. In 2017, 79 distinguished professors and around 20 advanced students from abroad attended the courses. In addition, we have received hundreds of elementary and high school students for different science pop activities. All over the year, this activity allows us to spread science among hundreds of young students.

Transfer technology to public or private companies is also a major activity of the IP Montevideo, contributing to the development of biotechnology and supporting the creation of startup companies.

Finally, the 2018 annual budget of the IP Montevideo was close to 8,5 million dollars, mainly coming from the Uruguayan national budget (≅ 60%) and from own incomes by service sales, grants and research contracts (≅ 40%)

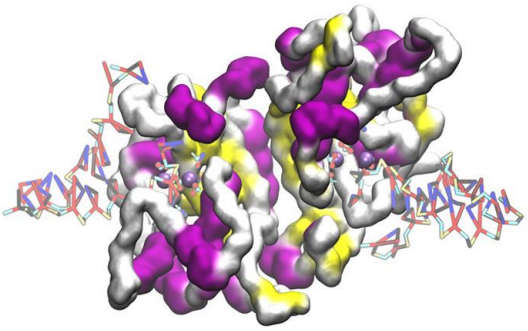
I wish to thank all our researchers and members for their dedication, continued support and commitment. In addition, I wish to acknowledge the great contribution and trust from our partner institutions in Uruguay and France.

Carlos Batthyany, MD. PhD
Executive Director
Institut Pasteur Montevideo

RESEARCH

LABORATORIES

1. BIOMOLECULAR SIMULATIONS LAB



Biomolecular simulations make use of computer programs to recreate and visualize the behavior and phenomena that rule biological processes at the molecular level. These tools make it possible to simulate experiments in more controlled conditions than in cells or living organisms, and to perform "theoretical experiments" that would be technically impossible.

These possibilities have resulted in important advances in biomedicine, facilitating the understanding of mechanisms of diseases and the development of drugs, for example.

In the Biomolecular Simulations Laboratory, we apply different molecular modelling techniques and simulations to several problems of biomedical interest, such as the stability of viral particles of Zika and Dengue or interactions between proteins that participate in the contraction of the cardiac muscle. These activities are carried out in collaboration with experimental groups in Uruguay and abroad.

Finally, we dedicate an important part of our work to the development of coarse-grained methods to perform advanced simulations at low computational cost. These methods offer the possibility of improving the comparability of theoretical studies with biochemical / biophysical or molecular biology experiments.

MEMBERS

Sergio Pantano, PhD, Head
Matías Machado, PhD, Associate researcher
Leonardo Darré, PhD, External collaborator
Exequiel Barrera, PhD, IP Montevideo Postdoc
Pablo Garay, PhD, CONICET Postdoc
Florencia Klein, BSc, Postgraduate student
Martín Sóñora, MSc, PhD Student

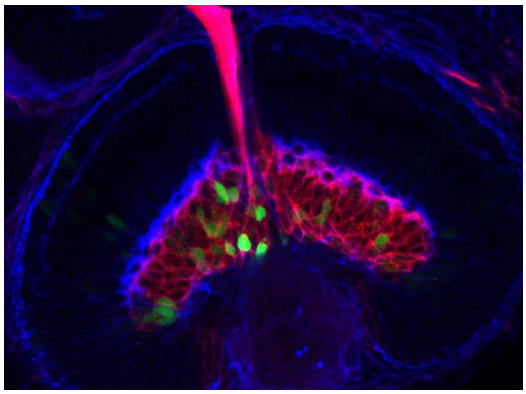
RESEARCH LINES

- **Development of the SIRAH coarse-grained force field (Southamerican Initiative for a Rapid and Accurate Hamiltonian).** Our group develops and maintains one of the broadest coarse-grained force fields for existing biomolecular simulations. SIRAH (www.sirahff.com) uses a Top Down approach and a classic Hamiltonian, common to atomic force fields. SIRAH is freely distributed with easy-to-use analysis tools, parameters and topologies to simulate DNA, proteins, explicit solvent and phospholipids. Currently, representations are being developed for metal ions, glycans and RNA. This line is entirely developed by our group.

- **Development of FRET sensors for cyclic nucleotide and redox signalling pathways.** Using bioinformatics and structural modelling, together with coarse-grained simulations, we have developed a new generation of FRET sensors for signaling cAMP, cGMP and redox conditions. These biosensors allow us to reach an unprecedented spatial resolution since they can be genetically fused to the C-terminal of virtually any protein, directing them to any cell compartment. This research line continues with the design of new generations of biosensors in the framework of the ProTeMCA program and in collaboration with foreign experimental groups.

- **Flavivirus stability studies.** Using multiscale simulations, we study the different factors that affect the stability of viral particles (Virus-like Particles). The reduced computational cost of our simulation scheme allows us to perform comparative simulations of different flaviviruses varying the temperature and pH conditions. The availability of experimental structures of viral particles of Zika, Dengue, Japanese encephalitis (JEV) and tick-borne Encephalitis virus (TBEV) allows us to study the accessibility of different epitopes, helping to understand the mechanisms of viral neutralization by antibodies. Additionally, the computational approach enables the identification of amino acids involved in the acid firing mechanism of flaviviruses, which could contribute significantly to the development of vaccines through the creation of attenuated viruses. These studies are part of collaborations with national and foreign experimental groups.

2. CELL BIOLOGY OF NEURAL DEVELOPMENT LAB



The laboratory is a joint group integrated by researchers from the IP Montevideo and the Faculty of Sciences (Udelar). Its work is focused on the relationship between cell polarity —that is, the asymmetries that allow cells to have specific functions— and neural differentiation, the phase of development of neurons in which they acquire the morphological and physiological characteristics of its mature stage.

To study these phenomena, the laboratory uses experimental systems in vertebrates such as zebrafish and chicken, to analyse of the function of diverse proteins that have an impact on neuronal development.

Based on this, the group has characterized on both species the functions of a family of proteins ("MARCKS") and the genes that encode them, in the initial formation of the embryonic nervous system (phase known as "neurulation").

The lab also has characterized the function of primary cilia have in neuronal formation in the retina of zebrafish, and has presented evidence indicating that the extracellular matrix protein Laminin 1 is essential for the formation of axon of those same neurons, among other investigations.

The group also participates in undergraduate and postgraduate teaching activities, as well as in various scientific dissemination initiatives.

MEMBERS

Flavio R. Zolessi, PhD, Head, Associate Professor of Cell Biology, Faculty of Science, Universidad de la República.

Gonzalo Aparicio, MSc, PhD student, assistant at Cell Biology Department, Faculty of Science

Camila Davison, BSc, PhD student, assistant at Cell Biology Department, Faculty of Science.

Magela Rodao, BSc, MSc student, assistant at the Electronic Microscopy Unit, Faculty of Science

Lucía Veloz, BSc, MSc student

RESEARCH LINES

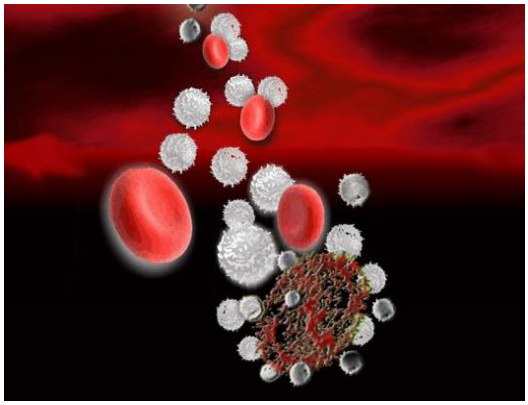
- **Role of Laminin 1 and Slit-Robo signalling in retinal ganglion cells differentiation.** We have previously demonstrated that Laminin 1 from the retinal basal lamina acts as a positive signal for axon outgrowth at the basal side. Our current working hypothesis is that negative signals inside the retina, like Slit factors, would reinforce the correct orientation of retinal ganglion cells, preventing axon outgrowth in other directions.

- **Characterization of primary cilia in early stages of photoreceptor cell differentiation.** After showing that retinal ganglion cells and their progenitors have a small primary cilium, which is important for their genesis and correct localization in the retina, we have started to analyse the possibility that this organelle also has a function at early stages of photoreceptor cell differentiation.

- **Polarized signals in the localization and orientation of photoreceptors.** We propose that, like retinal ganglion cells, photoreceptors respond to extracelular signals present in the retina, which are necessary for giving them their apropriate orientation. We investigate the possible role in this process of subapical adhesion molecules, such as N-Cadherin, and others necessary for cell polarity, like Pals1/Nok.

- **Role of MARCKS proteins in the modulation of polarity transitions in vertebrate neurulation.** We have found that MARCKS family proteins are important for neurolation in both chick and zebrafish embryos, aparently by modulating cell polarity in the neural plate. However, there are different cell mechanisms for neurulation in each of these species, which could be related with the duplication of Marcks genes in the fish.

3. CHRONIC LYMPHOID LEUKEMIA LAB



Our laboratory focuses on the study of the mechanisms involved in the origins and progression of Chronic Lymphoid Leukemia (CLL), the most common type of leukemia in people over 50 years, whose incidence in Uruguay is 5 cases per 100,000 people. CLL originates in cells of the immune system known as B lymphocytes, which lose their ability to die and begin to accumulate in the blood.

Although many patients with CLL respond to current treatments, some don't. The B lymphocyte is one of the most specialized cells of the immune system and is able to re-edit its DNA, thanks to the action of the cytidine deaminase (AID), an enzyme necessary to respond in the presence of different infections

However, the mutagenic action of the AID enzyme also has its negative aspects since B lymphocytes are continually exposed to damage of their DNA. Although the control mechanisms on AID are many and redundant, they sometimes fail, and in their absence this enzyme can be overexpressed in tumour cells, causing cancer progression and / or refractoriness in its treatment.

Our advances are related to the characterization of the expression of the AID enzyme in patients with CLL, and the development of animal models to study the causes of the progression of the disease and the refractoriness in the treatment.

MEMBERS

Pablo Oppezzo, PhD, Head
Agustín Correa, PhD, Associate Technician
Claudia Ortega, PhD, Technical assistant
María Elena Márquez, PhD, External associated researcher
Catalina Berca, MSc, PhD Student
Angimar Uriepero, MSc Student
Florencia Palacios, Associate researcher (currently on postdoc abroad)

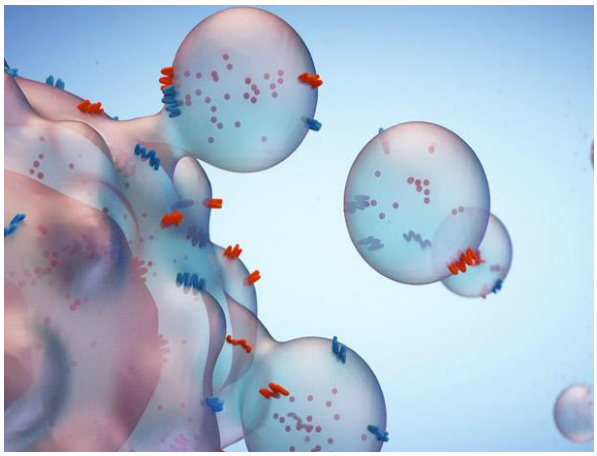
RESEARCH LINES

- **Characterization of the origins of the immunological microenvironment in tumour progression and its correlation with the constitutive expression of AID in progressive patients with CLL.** The objective is to corroborate if the constitutive expression of the enzyme cytidine deaminase (AID) during the evolution of the disease is a key event in the progression of CLL.

- **Deciphering of the effect of new inhibitors of kinases and proapoptotic molecules on the proliferative subpopulations of CLL.** It aims to characterize the molecular and phenotypic effects of new kinase inhibitors and the action of proapoptotic molecules in proliferating subpopulations of the tumour clone during treatment.
- **The S100A9 protein as a new therapeutic target in CLL. Linkage of inflammation, microenvironment and clinical evolution.** We aim to deepen the knowledge of the role of inflammation in the clinical evolution of patients with CLL.

- **Development of an artificial binding protein (ABP) platform to generate new prognostic and treatment tools in the LLC.** The objective is to create a therapeutic tool (ABP) capable of simultaneously recognizing the tumor cell and recruiting natural killer cells (NK) to destroy the tumor clone.

4. FUNCTIONAL GENOMICS LAB



Our scientific proposal aims to clarify the role of small regulatory RNAs in the biology of human cancer. In addition, we work in close collaboration with the University Hospital and the National Cancer Program, providing technological and experimental support for research in Clinical Oncology and the development of new diagnostic biomarkers in cancer.

In recent years, our main focus of research has focused on the study of a new class of molecules generically called small non-coding RNAs.

Our work has shown that the fragmentation of these RNAs generates molecules capable of regulating the cell survival pathways to stress and proliferation.

We focus primarily on fragments derived from transfer RNAs and Y-RNAs, and their secretion by normal and tumor cells.

We study how other cells are capable of capturing and sensing these RNAs released into the extracellular environment, thus representing a new communication mechanism between cells. This and other lines of work of the unit are aimed at identifying new molecular pathways in the initiation and progression of cancer, with special emphasis on new therapeutic targets and diagnostic biomarkers.

MEMBERS

Alfonso Cayota, MD, PhD, Head
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Fabiana Gambaro, Msc
Tania Possi, PhD student
Marco Li Calzi, MSc student
Braulio Bonilla, PhD student

RESEARCH LINES

- **Small RNAs derived from tRNA and Y-RNAs as new molecular pathways in cancer and their potential as a source of new diagnostic biomarkers in cancer.** Our main line of research is oriented to the role of new classes of small regulatory RNAs derived from tRNA and Y-RNA as central actors in the proliferation and survival responses to stress, and its potential as new molecular mechanisms in the initiation and progression of cancer. The focus of our current research activities is on the extracellular biology of these regulatory RNAs, and their ability to work as signaling molecules between cells. Part of our work has shown that these small RNAs are actively secreted by cells through extracellular vesicles or extra-vesicular fractions, being transferred to other cells, constituting a new mechanism of intercellular communication and transfer of genetic information. Additionally, its unique stability in the extracellular environment allows its detection in biological fluids, positioning itself as potential molecular biomarkers in human cancer.

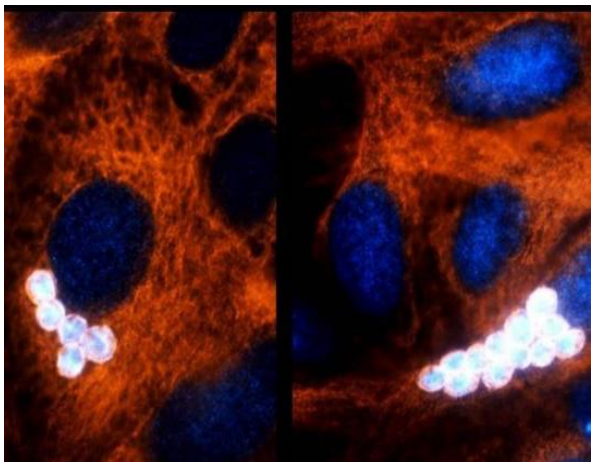
  Currently, in collaboration with the Clinical Oncology Service of the Hospital de Clínicas and the National Cancer Institute, we are studying the diagnostic value of small circulating RNAs derived from tRNAGlu, tRNAGly and Y4-RNA in patients with lung cancer.

- **Research and Development activities with health centers.** Our laboratory is currently participating in a series of initiatives to incorporate new diagnostic biomarkers in oncology, as well as genomic and molecular tools.

  Genetic susceptibility to breast cancer. Together with the Hospital de Clínicas of the Faculty of Medicine (Udelar), we have incorporated in the oncology routine the study of mutations of a panel of 11 hereditary predisposition genes to breast and ovarian cancer. This procedure uses next-generation deep sequencing for analysis including the BRCA1 and BRCA2 genes among others.

  Biomarkers of therapeutic prediction in lung cancer. Our laboratory performs fluorescent in situ hybridization (FISH) assays to detect translocations of the ALK gene, as a way to predict sensitivity to treatment with inhibitors (Crizotinib) in lung cancer.

5. HOST-PATHOGEN INTERACTIONS LAB



The Host-Pathogen Interaction Laboratory is focused on the study of human and animal pathogens, in particular the protozoan parasites Trypanosoma cruzi —which causes Chagas disease—, T. vivax and T. evansi; the causative agent of Leishmania, and the Mycobacterium prokaryote —associated with tuberculosis—, with emphasis on its functional genomics and its interactions with the host.

On Chagas Disease, our main focus of study, we have described a set of proteins related to the redox metabolism of trypanosomatids.

We seek to deepen its inhibition, as well as its use in the development of possible therapeutic strategies and preventive. Likewise, we work on the characterization of gene expression changes produced by T. cruzi in human cells. We have shown that there is a cellular reprogramming by this parasite, which allows the establishment and persistence of the infection.

We are focus on the identification of virulence factors, the development of new prophylactic strategies, and the improvement of diagnostic techniques, as well as in understanding aspects of the basic biology of T. cruzi and other related pathogens that are important for human and animal health.

MEMBERS

Carlos Robello, PHD, Head
Adriana Parodi-Talice, PhD, External associated research (Faculty of Science)
Dolores Piñeyro, PhD, External associated research (Faculty of Medicine)
María Laura Chiribao, PhD, Faculty of Medicine
María Eugenia Francia, PhD, Associate researcher
Gonzalo Greif, PhD, Associate Researcher
Luisa Berná, PhD, Associate Researcher
Sebastián Pita, PhD, Research assistant
Paula Faral-Tello, PhD student, Technical Assistant
Gabriela Libisch, PhD student
Florencia Díaz Viraqué, Research assistant, PhD student
Fernanda Matto, MSc

Lucía López, MSc
Ramiro Tomasina, MSc student
Fabiana González, Undergraduate student
Andrés Cabrera, PhD student

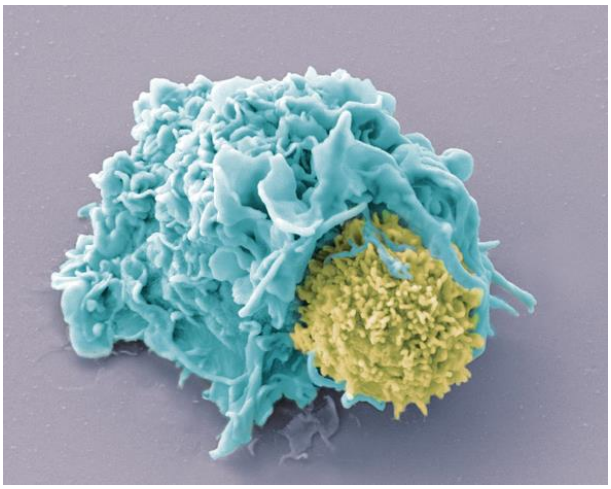
RESEARCH LINES

- **Study of the intracellular host-pathogen interaction with a systemic approach.** At the lab we have studied virulence factors of intracellular pathogens, as well as the host genes and routes necessary for the establishment of the infection, and the interface between them. We set out as main objectives the design of new strategies for the treatment or prevention of various infectious pathologies, such as Chagas disease, African trypanosomiasis, leishmaniasis, and tuberculosis

- **Determination of virulence factors necessary for infection with *T.cruzi*.** Regarding the establishment and persistence of T. cruzi infection, we have described a set of proteins related to the redox metabolism of trypanosomatids (triparedoxin cytosolic and mitochondrial peroxidase, triparredoxins, glutarredoxins, pteridin reductase), and we have characterized their structure and function. We are developing strategies for its inhibition, as well as its application in the development of therapeutic and preventive strategies.

- **Characterization of the changes in gene expression produced by intracellular parasites in human cells.** We have shown that there is a cellular reprogramming led by T. cruzi that allows the establishment and persistence of the infection in human cells. One of the main objectives we are currently pursuing is to identify host proteins, which, when inhibited, prevent *T. cruzi* infection. We are also extending this strategy for genomic and molecular biology studies in leishmaniasis, African trypanosomiasis, neosporosis and tuberculosis.

6. IMMUNOREGULATION AND INFLAMATION LAB



The deregulation of the immune system can lead to chronic conditions that are known as immune-mediated inflammatory diseases (IMIDs).

IMIDs include more than 80 clinical entities such as autoimmune and autoinflammatory diseases that affect up to 10% of the population in the western world. Basic research has allowed to characterize physiological mechanisms responsible for controlling the development of inflammatory and adaptive responses mediating pathological effects, and this knowledge is critical to innovate at the level of strategies directed at the immune system and to understand the mechanism of action of drugs currently being used in the clinic.

Our group is interested in the study of cellular and molecular mechanisms that control the inflammatory process and the adaptive immune response.

We focus on the biology of dendritic cells (DCs), since they constitute a sub-population of leukocytes able to orchestrate effector adaptive immune responses, while having powerful strategies capable of regulating the development of the inflammatory process and the adaptive response. Our work tries to cover relevant and original aspects at the level of molecular mechanisms while looking for pertinence in human health.

In this framework, the laboratory has characterized the emerging ion transporters TORID-1 (Tmem176b) and TORID-2 (Tmem176a), which are critical regulators of NLRP3 inflammasome activation. Inflammasome regulation by TORID-1 has been shown to be relevant in the anti-tumor immune response. We have characterized pharmacological inhibitors of TORID-1 and TORID-2 that have been characterized at a pre-clinical level as promising anti-tumor drugs.

MEMBERS

Marcelo Hill, Head, Associate Professor, Immunobiology Department, Faculty of Medicine, Udelar

Mercedes Segovia, Assistant Profesor, Immunobiology Department, Faculty of Medicine, Udelar

Sofía Russo, PhD student. Assistant at Immunobiology Department, Faculty of Medicine, Udelar

Sabina Victoria, PhD student

Valentina Pérez, MSc student. Assistant at Immunobiology Department, Faculty of Medicine, Udelar

Daniela Olivera, Intern. Assistant at Immunobiology Department, Faculty of Medicine, Udelar

Javier Noboa, Intern. Assistant at Immunobiology Department, Faculty of Medicine, Udelar

RESEARCH LINES

- Role of TORID-1 as an innate checkpoint in tumor immunity.

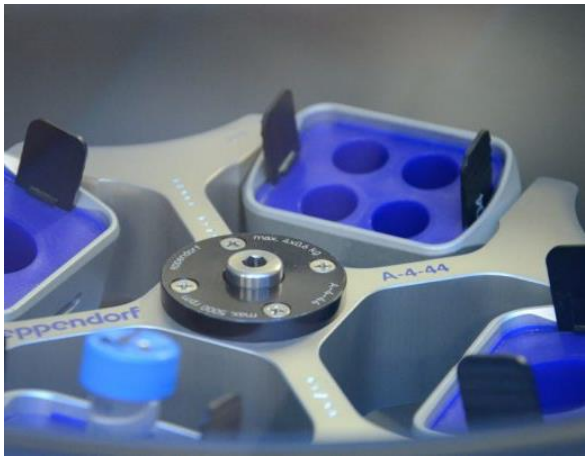
- Role of TORID-1 and TORID-2 in the biology of chronic lymphocytic leukemia. (Collaboration with Pablo Oppezzo)

- Characterization of small molecules capable of inhibiting or activating the conductance mediated by TORID-1 and 2.

- Role of the intracellular protein TORID-1 in obesity and inflammation induced by obesity. (Collaboration with Carlos Escande)

- Study of non-conventional anti-inflammatory drugs as immunomodulators in organ transplantation. (Collaboration with Carlos Batthyany)

7. IMMUNOVIROLOGY LAB



At the Immunovirology Laboratory, we study the cellular and molecular processes involved in leukemic transformation by oncogenic viruses. In particular, we work with the Enzootic Bovine Leukemia (LBE) caused by the Bovine Leukemia Virus (BLV), which has a high prevalence in dairy cattle, generating important economic losses for our country.

With the aim of elucidating the mechanisms involved in viral infection, we have analyzed the genetic variability of BLV in Uruguay comparing their circulating genotypes with those described in other countries.

We have characterized at the molecular and structural level the main BLV proteins: the glycoprotein of envelope (ENV), capsid (CA) and protease (PR). Also, we studied the interaction between these proteins with different components of the infected cell.

Besides, using transcriptomic analysis, we studied the differences of gene expression between infected and non-infected animals with BLV, to know possible mechanisms involved in the control of the infection.

The knowledge we have obtained of this pathology and its causal agent has also allowed us to develop new technologies for the diagnosis both serologically and molecularly, as well as the generation of immunogenic preparations from the viral proteins that are being tested in animal models.

The results of our work will allow us to better understand the mechanisms that cause the leukemic transformation, generating new tools to optimize its diagnosis, and new procedures to improve the control and prevention of viral transmission.

MEMBERS

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Natalia Olivero, MSc, Technical assistant, PhD student

Sergio Bianchi, PhD, MD, PostDoc, Assistant professor at the Department of Basic Medicine, Faculty of Medicine, Udelar.

Martín Fló, PhD, PostDoc, Assistant at the Immunobiology Department, Faculty of Medicine, Udelar

Mariana Margenat, PhD, PostDoc

Florencia Rammauro, PhD, Assistant at the Immunobiology Department, Faculty of Medicine, Udelar

Andrés Addiego, MD, MSc

Natalia Ibañez, MD **,** MSc student, Assistant at the Immunobiology Department, Faculty of Medicine, Udelar

RESEARCH LINES

- **Biophysical and structural characterization of the BLV capsid protein (BLV-CA).** The mechanism of BLV capsid formation through the self-assembly of thousands of copies of BLV-CA represents a key event in the retrovirus cycle. To understand this mechanism we have characterized the biochemical and biophysical properties that affect this process, and in collaboration with the Laboratory of Molecular and Structural Microbiology of the IPMontevideo we elucidate the three-dimensional structure of BLV-CA, showing that it is organized in a pseudohexagonal with an important conformational plasticity. On the other hand, we generated nano-antibodies directed against the viral capsid and we studied the effect of the interaction with capsid on the modulation of self-assembly.

- **Characterization of the interactions between BLV-CA and intracellular components of the host cell.** The transit of the viral capsid between the plasma membrane and the cell nucleus depends on the interaction with various cellular proteins. Other retroviral models have described restriction factors that disturb the conformation of the capsid generating anti-viral conditions. For the case of delta-retroviruses such as BLV we have no confirmed evidence on these mechanisms. In the HIV model it has been demonstrated that the interaction between capsid and nucleoporins would participate in this transit and in the entrance to the nucleus through the nuclear pore. Based on the ability of BLV-CA to self-assemble in vitro in tubular or supramacromolecular structures, and using affinity and mass spectrometry techniques, our objective is to identify and characterize the interactions between BLV-CA and the factors of the cellular guest involved in this traffic. We will also look for partners of BLV-CA that can act as innate immune sensors when analyzing cell lysates of permissive and non-permissive cells to BLV infection. The engineering cells generated by Francesca Di Nunzio in IP Paris will be used to identify new restriction factors or functional viral partners by mass spectrometry. We will also design and purify nanobodies against BLV-CA that will be labeled as microscopy approaches. The results obtained in BLV will then be transferred to the HTLV-1 research to define

common and diverse mechanisms adopted by these delta retroviruses when establishing the viral infection.

- **Caracterización bioquímica, estructural e inmunológica de la proteína de la envoltura BLV.** The env of BLV has a crucial role in the determination of viral infectivity, and is responsible for inducing the fusion of viral and cellular membranes after recognition of specific cell surface receptors.

We optimized the expression of soluble env ectodomain in Drosophila S2 cells, with a natural and altered furin cleavage site. We induced protein expression and secretion in the supernatant by divalent metals, and we performed protein purification by affinity chromatography using a StrepTactin column followed by size exclusion chromatography. The quality control of the proteins was evaluated by mass spectrometry. This system should allow the production of sufficient material for crystallization assays, cryo-electron microscopy of isolated trimer and biophysical studies of the multimeric complex formed by the recombinant proteins.

Env is one of the main targets of antiviral immune responses, which generate both humoral neutralizing antibodies and T-cell specific adaptive immunity. Other retroviruses have been reported to be the presence of an immunosuppressive peptide (isu) in the structure of the glycoprotein Env. It could be important in its ability to immunomodulate immune responses. We are interested in studying the effect of amino acid modifications in the isu domain on humoral and cellular adaptation responses to the challenge with modified Env glycoproteins. This will allow us to understand one of the mechanisms involved in the generation of resistance used by BLV to escape the antiviral immune response. On the other hand, we also hope to identify the modifications that reduce the immunosuppressive activity of this domain and, therefore, increase its immunogenicity. This result could be useful for the rational design of effective vaccines against this retrovirus.

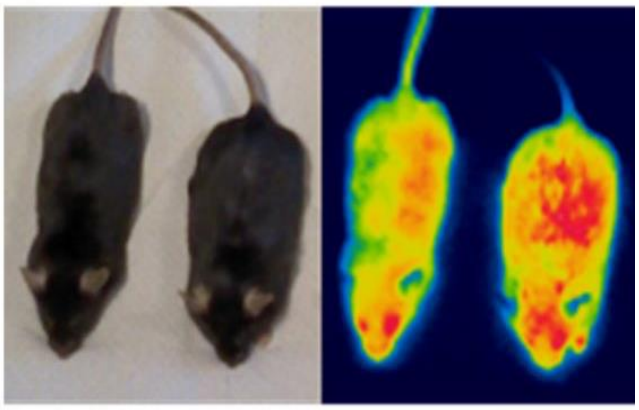
Using BLV Env purified protein we have also developed a new ELISA test to use it for the diagnosis of enzootic bovine leukosis. In collaboration with ATGen we are now generating a new EBL diagnostic kit that will be used in a field experiment with more than 50.000 dairy cows.

- **Genetic characteristics identification associated with the Enzootic Bovine Leukemia (LBE) natural control.** Given the high prevalence of Enzootic Bovine Leukemia (LBE) in Uruguay, the strategy to eradicate the disease implemented in Europe and Oceania is impracticable in our country. An alternative control strategy using vaccines is promising, but there are still no effective products on the market. Considering that recent results show that the LBE has a hereditary component that reaches 8%, a third strategy to control the disease would involve the herding of flocks by increasing the frequency of genotypes associated with resistance to infection.

In an experimental herd with high prevalence of BLV infection, we have analyzed a the groups of animals: one control group characterized by a low proviral load and low titers of anti-BLV antibodies; one “non-control” group with high proviral load and high specific antibody titers, and a “negative” group, without detectable presence of BLV.

Using peripheral blood mononuclear cells (PBMC) from these animals, we are characterizing, in collaboration with Natalia Rego and Hugo Naya of the Bioinformatics Unit (IP Montevideo), the transcriptomic representatives of these groups by mass sequencing of mRNA (ARNseq). We hope to identify genes and isoforms differentially expressed in control animals and interpret these differences in the context of biological processes, ontologies of sub or overrepresented metabolic pathways.

8. METABOLIC DISEASES AND AGING LAB



Obesity is a serious medical problem that involved a high percentage of the world population. Traditionally conceived as a disease of developed countries, it is now recognized as a pandemic by WHO. In Uruguay, nearly 60% of the adult population are considered overweight or obese. Moreover, about 10% of the child population is overweight or obese, and they suffer hypertension and diabetes, pathologies associated with obesity.

Understanding the molecular mechanisms involved in pathophysiology of obesity, diabetes and other associated diseases is our main goal. Research aims on Metabolic Diseases and Aging Lab are:

a) to do basic science focused on the molecular mechanisms of metabolic diseases, with a strong emphasis on Sirtuins;

b) To develop novel pharmacological strategies to treat obesity and metabolic diseases.

MEMBERS

Carlos Escande, PhD, Head

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Aldo Calliari, PhD, External associated researcher, Faculty of Veterinary (Udelar) – Biochemestry Departament (Biophysics Area)

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Leonardo Santos, Research assitant, PhD student

Laura Colman, MSc, Research assistant, PhD student

Pía Garat, Biotech engineer, CEO at EOLO Pharma (Argentina)

Alejandro Rodriguez, BSc, MSc student

Karina Cal, MSc, Lab technician

María Caggiani, MD, MSc student

Adriana Carlomagno, MD, MSc, PhD student

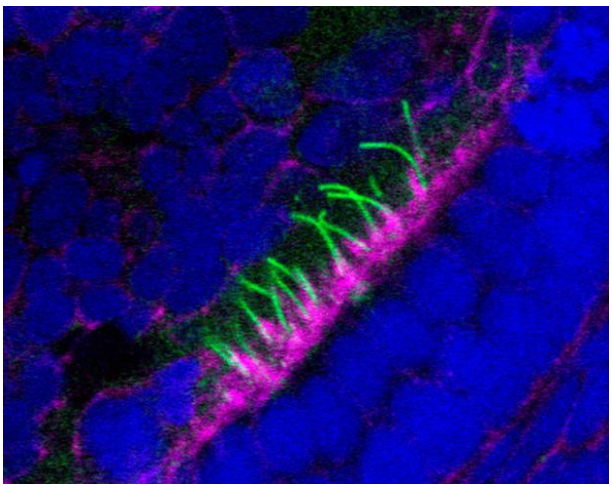
RESEARCH LINES

- **DBC1 as modulator of metabolic function.** The main focus of our research has been to continue to understand the role of the protein Deleted in Breast Cancer-1 (DBC1), a SIRT1 regulator, in the control of metabolism and metabolic diseases. In order to achieve that, we took four different scientific approaches. A) We continued using the genetic deletion of DBC1 mouse model as an experimental paradigm in metabolism regulation; B) we engaged in studying how DBC1 function is regulated in vivo. C) We decided to generate a loxp/loxp DBC1 mouse model as a tool for tissue-specific knockout of DBC1. D) Based on our previous data that DBC1 regulates the "healthy obesity" phenotype, we began to search for secreted targets of DBC1 that may account for its effects.

- **Novel regulators of metabolism and metabolic diseases**: focus on Inflammation. Chronic inflammation has emerged in the past few years as a major player in the development of metabolic diseases, with accumulating evidences showing that both innate and adaptive immune cells are involved in the onset and progression of obesity, type II diabetes and atherosclerosis. During this period, we begun to work with two different proteins in context of chronic inflammation and metabolic diseases: SIRT6 and "TMEM176B.

- **Research and development in anti-obesity drugs**. The development of novel compounds for pharmacological treatment of metabolic diseases was a seminal part of our G5 proposal. We associated in close collaboration with Dr. Carlos Batthyany, who together with Dr. Virginia Lopez had already designed several compounds aimed to treat atherosclerosis. Together, we showed that these compounds are effective in vivo for atherosclerosis. In the course of this scientific collaboration, we designed together a novel family of compounds, one of which is showing striking results on prevention of obesity, insulin resistance and non-alcoholic liver steatohepatitis (NASH).

9. MOLECULAR AND HUMAN GENETICS LAB



In the lab we are interested in understanding different aspects of the biology of primary cilia, organelles that are present in the vast majority of cell types in the human body and that play a role as signaling hubs, acting as cellular antennae that are critical for the interaction between cells and the environment.

It has been shown that ciliary dysfunction underlies a number of human conditions collectively known as ciliopathies.

Among them, we focus on Bardet-Biedl Syndrome (BBS), a ciliopathy characterized primarily by obesity, polydactyly, mental retardation, retinal degeneration, renal and gonadal malformations and that can include additional features such as asthma, diabetes, anosmia and congenital heart disease.

We are focused on BBS and other cilia-associated proteins and perform both in vitro and in vivo assays to determine their function. The goal is to gain insight into basic aspects of ciliary biology as well as to understand the cellular and molecular basis of different phenotypes that characterize the ciliopathies. To this end, a significant effort of the lab is centered on the institutional program InDICyO ("Investigación en Diabetes, Inflamación, Enfermedades Cardiovasculares y Obesidad"), where we aim to understand the role of cilia and proteins of interest in the development of obesity and atherosclerosis.

MEMBERS

José Badano, PhD, Head
Florencia Irigoín, PhD, Honorary Associated researcher
Victoria Prieto-Echagüe, PhD, Associate Researcher
Paola Lepanto, PhD, PostDoc
Rossina Novas, PhD student
Belén Torrado, PhD Student
Matías Fabregat, PhD student
Lucía Guggeri, PhD student
Carolina Silberstein, MSc student
Florencia Levin, Intern, biotech thesis in progress, Universidad ORT Uruguay
Leonardo Santos, PhD student (co-tutored by Carlos Escande)

Adriana Carlomagno, MD, MSc student (co-tutored by Carlos Escande and Dr. Danza)

María Eugenia Cruces, MSC student

RESEARCH LINES

- **CCDC28B and BBS proteins in the regulation of ciliogenesis and cilia length.** In the lab we have been dissecting the biological role of a number of BBS proteins and the BBS-associated protein CCDC28B (coiled-coil domain containing protein 28B). In patients it was first reported that a reduction in CCDC28B protein levels, in the presence of mutations in BBS genes, results in a more severe presentation of the syndrome. CCDC28B interacts with a number of BBS proteins and we have demonstrated that it is a novel regulator of ciliary length, both in cells and in vivo in zebrafish. We know that the function of CCDC28B in the cilium relies, at least in part, on its interaction with SIN1 and the molecular motor kinesin 1. Currently, we continue characterizing CCDC28B with the goal of understanding the mechanim by which it regulates cilia.

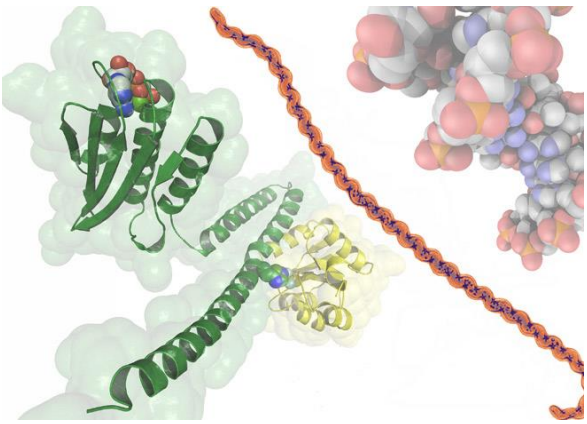
- **Cilia targeting: similarities with nuclear transport.** Cilia are highly conserved organelles that protrude from the cellular plasma membrane while their interior is connected to the cytosol. However, the composition of cilia appears to be highly regulated and different from that of the membrane and cellular interior. Importantly, this particular composition is important for the function of the organelle. For example, receptors and mediators of different signaling cascades are concentrated inside the cilium and ciliary entry of molecules has been shown to be regulated. However, the mechanisms involved in directing molecules into the cilium as well as in mediating their entry are not completely understood. Different lines of evidence show a similarity with nuclear import. In this context, we have been studying the role of the nuclear import machinery in transporting proteins into the cilium, looking at proteins that are able to localize to both compartments. In particular, we have focused on Gli2, a transcription factor for Hedgehog signaling, that changes its localization in response to pathway activation. We were able to show that Gli2 used importins to enter both the nucleus and the cilium but importantly, it uses different importins in each case. We continue studying this process with the aim of gaining a better understanding of the mechanism.

- **Bardet-Biedl associated proteins in intracellular trafficking**. The functional characterization of BBS proteins has led us to uncover different extra-ciliary roles. For example, we showed that BBS7 is able to enter the nucleus where it modulates the activity of RNF2, a chromatin remodeling factor. Thus, defects in BBS7 result in changes in gene expression. More recently, we also documented a role for CCDC28B in

the nucleus that is linked to cilia length regulation in a mechanism that we still do not completely understand. In collaboration with Dr. Norann Zaghloul at the University of Maryland, USA, we have shown that BBS proteins not only transport molecules to the base of cilia but have a broader role in intracellular transport and secretion of at least a subset of proteins. We have shown that BBS4 is required for the correct secretion of FSTL1, a function that we believe relevant to understand the etiology of BBS.

- **The role of BBS proteins and cilia in the development of ciliopathy associated phenotypes.** Dissecting the biological role of the BBS proteins and CCDC28B is critical to understand the mechanism underlying the development of BBS associated phenotypes. Therefore, in addition of studying the function of these proteins at a cellular and molecular level, we also evaluate their function in models that are relevant to the pathology. For example, we have studied the role of cilia in the development of the retina in zebrafish. Currently, we are studying the role of BBS proteins and cilia in adipogenesis and the development of obesity. Our results show that defects in BBS4 lead to a reduction in the secretion of FSTL1 (as previously mentioned). Importantly, we uncovered a novel role for FSTL1 in ciliogenesis and adipogenesis. This line of research is being followed in a collaboration with the laboratory of Dr. Escande in the context of the INDICyO Program.

10. MOLECULAR AND STRUCTURAL MICROBIOLOGY LAB



The Laboratory of Molecular and Structural Microbiology (LMSM) seeks to understand how bacteria sense signals from their environment and internal milieu, to thereafter respond and adapt. Pathogenic bacteria are a particular interest, focused on the Spirochete genus Leptospira.

Signaling is mediated by proteins, which change their 3D structures in a signal-dependent way, structures that are often stabilized by phosphorylation.

Phosphoryl-transfer along such signal transduction pathways is thus a key enzymatic reaction, the mechanistic features of which our lab wants to uncover at the molecular level.

Our lab uses X-ray crystallography, to image the 3D structures of target proteins, such as sensory histidine kinases and response regulators, alone and in complex. By combining these high-resolution images with other sources of information —especially issued from biochemistry, genetics and microbiology experiments— we wish to understand the function and then contribute to the development of applications, such as vaccines against microbial diseases.

The Leptospira genus comprises many species, at least 10 of them cause a serious disease: leptospirosis. This zoonosis (that is, a disease that transmits from animals to humans), affects the reproductive capacity of cattle in Uruguay. It also causes an acute disease in humans, sometimes deadly, for which there are still no effective vaccines. Signaling systems in Leptospira shall uncover virulence and pathogenesis mechanisms, still poorly understood in these spirochetes. Key virulence proteins will be attractive targets to develop intervention strategies, and effectively control the zoonosis, with anticipated veterinarian and public health applications.

MEMBERS

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Juan Andrés Imelio, MSc, PhD Student
Nicole Larrieux, Biochemist, Technician

Sofía Lima, BSc, MSc student
Fabiana San Martin, BSc, PhD student
Leticia Zarantonelli, PhD, Associated research scientist – INIA
Felipe Trajtenberg, PhD, Research scientist
Marcos Nieves, BSc, PhD student

RESEARCH LINES

- **Signaling and regulation in microorganisms**. We are particularly interested in two component systems (TCS) in bacteria. TCSs are central in mediating signaling and regulation, almost ubiquitous in prokaryotes and archaea, they are also present in fungi and plants. Other regulatory proteins, such as one component systems, are also being studied. The general question behind this line of research is: how do cells use these sensory and regulatory proteins to detect extra- and intra-cellular signals, and then regulate specific functions? To answer this question, saprophytic bacteria (such as Bacillus subtilis and Leptospira biflexa) are studied, as well as pathogenic ones (Leptospira interrogans, L. borpetersenii, L. noguchii, Enterococcus faecium, Mycobacterium tuberculosis). In these models, several key biological processes are studied, such as the regulation of lipid synthesis (Albanesi et al., Proc Natl Acad Sci USA 2009, 106: 16185, Trajtenberg et al., J Biol Chem 2010, 285: 24892, Trajtenberg et al. al., mBio 2014, 5: e02105; Trajtenberg et al., eLife 2016, 5: e21422; Imelio et al., Bio-protocol 2017, 7: e2510; Lara et al., 2018 submitted), heme metabolism (Morero et al., Mol Microbiol 2014 , 94: 340), or virulence in pathogenicity (Adhikarla et al., Front Cell Infect Microbiol 2018, 8:45).

- **Molecular and structural biology of Leptospira** Different species of Leptospira cause leptospirosis. This zoonosis is the most widespread one around the world, reemerging as an important problem in human and animal health. In Uruguay, it is a significant issue, provoking cattle abortions and reproductive failure, and transmitting to humans causing acute disease. Our lab wants to elucidate the molecular mechanisms that determine and/or regulate virulence and pathogenicity in Leptospira. There are currently two main projects to do this: the study of the motility apparatus and systematic efforts to isolate and type local Leptospira strains in Uruguay.

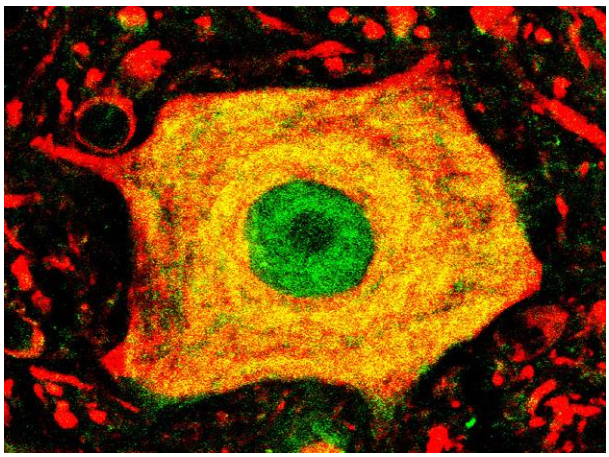
i- The motility apparatus of Leptospira. Active translational motility of leptospira (swimming) is central for the virulence of the pathogenic species that cause leptospirosis. The essential organelle used for swimming is the flagellum. Our lab is currently studying the detailed molecular architecture of the flagellar appendage. The filament of these spirochetes' flagella is confined within the periplasm, a unique feature shared by all Spirochetes, including bacteria like Treponema pallidum (the agent of syphilis) or Borrelia burgdorferi (Lyme disease). In collaboration with the Ko and Sindelar labs in Yale University, as well as the Picardeau lab at the Institut Pasteur

(Paris), we have shown that the flagellar filament from Leptospira is much more complex compared to better-known bacterial models used so far as a paradigm of swimming motility in bacteria. As an example, filaments from Salmonella and other Enterobacteria are built as homopolymers of a single protein species (flagellin), whereas Leptospira comprise at least eight different proteins polymerized into the periplasmic filament assembly (Wunder et al., Mol Microbiol 2016, 101: 457; San Martin et al., Acta Crystallogr F 2017, 73:123; Wunder et al., Front Cell Infect Microbiol 2018, 8:130). We have recently crystallized two new proteins from both pathogenic and saprophytic Leptospira spp. (San Martín et al., Acta Crystallogr F 2017, 73:123),and solved their 3D structures (unpublished results), revealing novel protein folds. We are now moving forward towards their physiological interactions and interaction partners, enabling for the flagellar filament role in spirochete motility.

ii- Isolation and typing of autochthonous Leptospira spp. strains. This line is being developed in the context of a multicentric collaborative project, aims to isolating native strains from Leptospira spp. from biological samples obtained from infected cattle and other animal reservoirs. These isolates are typed by complementary techniques: classic serologic methods and novel molecular approaches, ultimately achieving greater sensitivity and specificity (Zarantonelli et al., PLoS Negl Trop Dis 2018, 12:e0006694). This effort has led to the creation of a biobank of strains of Leptospira, which up to now was not available in Uruguay, reporting the identity of the serovars that circulate in natural infections. This biobank will be useful in the formulation of more efficacious vaccines, in the improvement of diagnostic methods, as well as in further investigations of leptospirosis in Uruguay.

- **Collaborations**. Our lab works in collaboration with Dr. Hugo Gramajo (Institute of Molecular and Cellular Biology, IBR, Rosario, Argentina) and his team, to elucidate crystallographic structures and mechanisms of action of Mycobacterium tuberculosis transcription factors (one component regulator systems), playing essential roles in regulating fatty acid metabolism in this pathogen.
- We also collaborate with Dr. Mathieu Picardeau (Institut Pasteur, Paris, France) and Dr. Albert Ko (Yale University, New Haven, USA) on motility of Leptospira and pathogenesis mechanisms of these bacteria at the molecular level.
- In addition, our lab integrates a multicentric consortium in Uruguay, working together with teams from the Veterinary Laboratories Division ("Miguel C Rubino" DILAVE) of the Ministry of Livestock, Agriculture and Fishery (Alejandra Suanes, Rodolfo Rivero), National Institute of Agricultural Research (Franklin Riet), Hygiene Institute at the Medical School (Felipe Schelotto), addressing problems of isolation, typing, diagnostics and genomics of Leptospira.

11. NEURODEGENERATION LAB



In most neurodegenerative diseases such as Alzheimer, Parkinson and Amyotrophic Lateral Sclerosis (ALS), neuronal pathology begins as a focal process that extends to other regions of the nervous system.

Our research aims to understand the biological mechanisms underlying the neurodegenerative process in ALS. Likewise, our research points to the development of new drugs that could stop or slow disease progression, which would allow a significant improvement in patient's quality of life.

The experimental approach is based on characterizing the neurodegenerative "cellular microenvironment", unravelling the role of neurons and glial cells as well as immune and vascular cells. We have recently identified new cell types with aberrant phenotype as well as new protein mediators and receptors that promote the degenerative process and that can be targeted by specific drugs.

The results of these studies will impact on a better understanding, diagnosis and treatment of neurodegenerative diseases.

MEMBERS

Luis Barbeito, MD, Head
Emiliano Trías, PhD, Research Assistant
Valentina Varela, Technical Assistant
Sofía Ibarburu, PhD student
Mariangeles Kovacs, MSc student

RESEARCH LINES

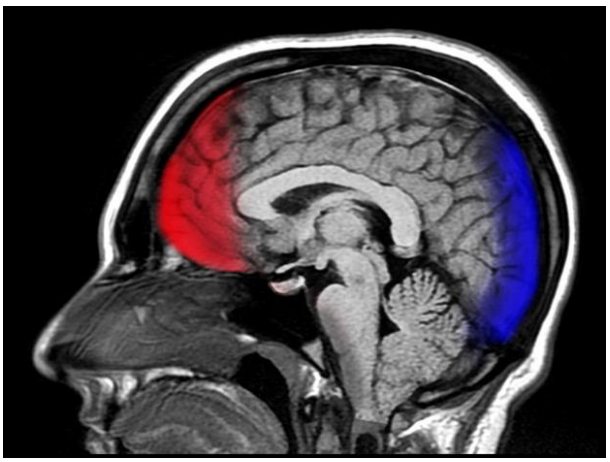
- Characterization of aberrant phenotypes of glial cells during ALS.

- Influence of mast cells and neutrophils on the degeneration of motor neurons and loss of neuromuscular synapses.

- Development of new neuroinflammatory modulators for the treatment of ALS.

- Nitro-NGF as a new glial factor that mediates the apoptosis of the motor neuron.

12. NEUROINFLAMMATION AND GENE THERAPY LAB



Traumatic injuries affecting the brain or spinal cord due to traffic or work accidents, sports activities or violence, constitute in Uruguay the main cause of death and disability in people under 40 years. These injuries are in many cases progressive, and could trigger psychiatric pathologies, in particular depression and dementias like Alzheimer disease.

The objective of our group is to understand the consequences that these traumas could have for the nervous system, and in this way decipher how to reduce them.

The main responsible for the progression of the traumatic damage is the inflammatory process that is triggered after the trauma.

Our hypothesis is that by stimulating the positive part of this process (tissue cleansing and healing) and restraining its negative components (oedema-swelling, toxic mediators) by means of modulating immune receptors, we may promote functional recovery.

We focus on the study of molecules capable of acting as checkpoints of inflammation, called immune inhibitory receptors such as CD300f or CD200R1. Also, working with experts from the State Insurance Bank of Uruguay (Banco de Seguros del Estado-BSE), we search for possible blood molecules that may represent biomarkers of the progression of these injuries in traumatic brain injury patients.

Through the administration of recombinant proteins (proteins produced outside our body) or the use of gene therapy (introduction or manipulation of genes in cells of the brain or spinal cord), we aim to stimulate cells to produces a specific therapeutic protein that modulates the response of the tissue towards the alleviation of inflammation.

MEMBERS

Hugo Peluffo, PhD, Head
Natalia Lago, PhD, Associate researcher
Bruno Pannunzio, MSc
Daniela Alí, BSc, PhD student
Andrés Cawen, BSc, Invited researcher
Nathalia Vitureira, PhD, Honorary associated researcher
Luciana Negro, PhD, Currently Posdoc at NIH

RESEARCH LINES

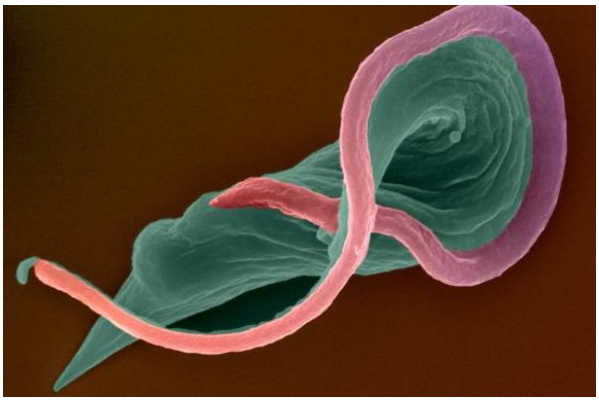
- Modulation of the microglial and macrophage phenotype by activation of the CD200R1 immune receptor after traumatic injuries in the brain, spinal cord and peripheral nerve.

- Modulation of the microglial and macrophage phenotype by the CD300f immune receptor and its role after traumatic injuries of the brain, spinal cord and peripheral nerve.

- Role of the CD300f immune receptor in psychiatric conditions such as major depressive disorder or autistic spectrum disorder.

- Preclinical and clinical research in traumatic brain injuries in association with the State Insurance Bank (BSE). Determination of blood biomarkers that could contribute to improve patient stratification and precision medicine approaches.

13.REDOX BIOLOGY OF TRYPANOSOMES LAB



By means of a multidisciplinary approach, we study the biochemical, structural and biological features that distinguish several key components of the redox system from pathogenic trypanosomatids, parasites that are causative agents of severe diseases in animals and humans (Chagas disease, Leishmaniasis and African sleeping sickness).

These studies allow us to identify and understand the role these components play in parasite biology (e.g. growth, infection, and pathogenesis).

Our research aims to gain understanding into the redox biology of trypanosomatids to guide novel strategies and the development of the safer and more efficacious drugs against this disease.

MEMBERS

Marcelo Comini, PhD, Head
PhD Andrea Medeiros, PhD, Associated research
Mariana Bonilla, PhD, Assistant research
Diego Benítez, PhD, PostDoc
Estefanía Dibello, PhD, PostDoc
Cecilia Ortiz, PhD, Investigadora Asistente
Jaime Franco, MSc, Investigador Asistente
Florencia Sardi, MSc, Estudiante de Doctorado
Matías Deambrosi, BSc, MSc student

RESEARCH LINES

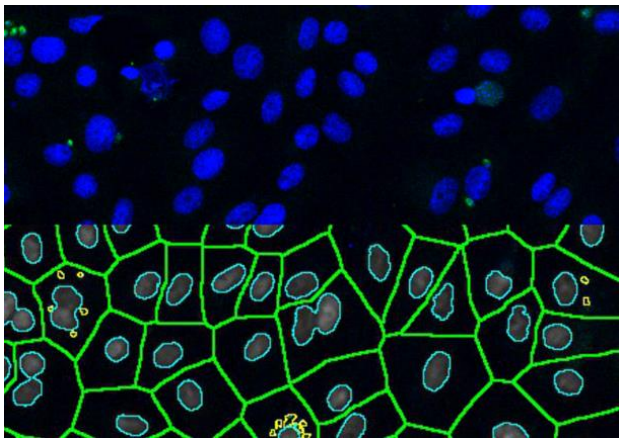
- **Fundamental aspects of trypanothione metabolism: synthesis, reduction and utilization.** We study the biochemical, structural and biological features that distinguish several key components of the trypanothione system from pathogenic trypanosomatids. Using animal infection models, we investigate the role these molecules play in parasite biology and pathogenesis. The data from these studies allows to validate new drug target candidates, their inhibitors as well as to guide novel drug development strategies.

- **Development of biosensors for non-invasive and high-content studies.** Our laboratory is interested in the development of different types of biosensors (fluorescence- and luminescence-based) that allow the real time and non-invasive monitoring of parasite proliferation, redox state and major signaling pathways. The transgenic cell lines expressing the biosensors allow us to study the role played by the oxidative stress and redox signaling in a variety of cellular processes (e.g. host-pathogen interaction, cell differentiation, cell cycle, apoptosis and metabolic dysfunction). The reporter cell lines are also used in phenotypic drug-screening campaigns and to investigate drug mode of action.

- **Early phase drug discovery projects.** We apply target- and phenotypic-based approaches to screen synthetic and natural compounds that affect, in a selective manner, the growth of the infective form of different trypanosomatid species. Drug repurposing is also an active area of research in our lab.

- **Drug mode of action at cellular and enzymatic level** is addressed to foster and guide drug optimization. Our laboratory relies on an important network of local and international groups working on (medicinal) chemistry to fulfill this goal.

14.SIGNAL PROCESSING LAB



The Signal Processing Laboratory is an interdisciplinary group focused on research in signal processing and biomedical imaging with special interest in microscopy. It is also dedicated to the training of life science researchers in image processing in order to establish a common language and a critical vision in these techniques.

The signal processing offers an objective approach that allows to automatize and systematize the analysis of data generated by the wide spectrum of techniques and equipment used in the IP Montevideo, from the quantification of microscopy images to genomic sequence data.

An interdisciplinary approach permits developing methodologies and algorithms that incorporate knowledge of different disciplines in all stages of research.

This is a joint group with members of the Signal Processing Department of the Faculty of Engineering (Udelar) and the Institut Pasteur of Montevideo.

MEMBERS

Federico Lecumberry, PhD, Eng, Head, Associated professor at the Signal Processing Department of the Faculty of Engineering (Udelar)

Martín Etchart, Eng, Electrical Engineer, MSc student

Mauricio Ramos, Undergraduate student in Electrical Engineer

Alfredo Solari, Undergraduate student in Electrical Engineer

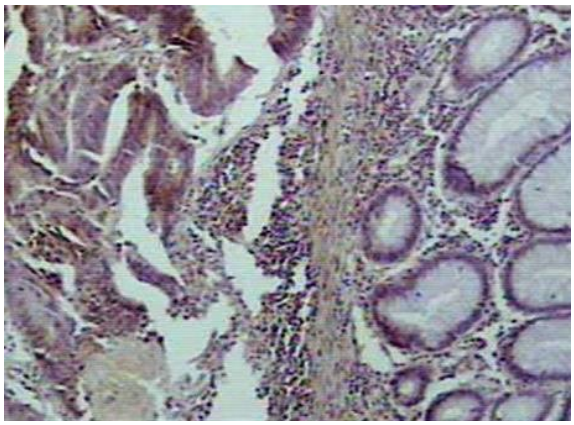
Daniel Soria, Undergraduate student in Electrical Engineer

RESEARCH LINES

- **Processing of biomedical images.** We work on the development and integration of algorithms and tools for the quantification and analysis of images from various sources of imaging in biomedicine: optical microscopy, fluorescence, electronics, or in-vivo imaging system. Some of our lines of research and applications are: detection and long quantification and fluorescence of primary cilia, detection of parasites and calculation of infection index, monitoring of neutrophil trajectories, quantification of fluorescence dynamics in different cell and tissue treatments, reconstruction of structures three-dimensional, among others. At the Microscopy Unit of IP Montevideo, we collaborate with researchers from various groups within the institute and with researchers from the Faculty of Medicine of the Universidad de la República.

- **Signal processing applied to bioinformatics**. Analysis of genomic data and application of automatic learning methods to the analysis and visualization of the evolution of viruses or the identification of proteins with fusogenic capacity. We work in collaboration with the Bioinformatics Unit (IP Montevideo) and the Institute of Mathematics of the Faculty of Engineering (Udelar).

15.TUMOR IMMUNOLOGY AND GLYCOBIOLOGY LAB



Proteins and carbohydrates (sugars) interaction plays an essential role in several cellular processes, such as cell proliferation and differentiation, as well as in different diseases, including viruses, bacteria and parasites infections. To perform these specialized functions, some types of carbohydrates bind to proteins creating complex structures called glycoproteins, which are found on the surface of cells and which influence cellular communication.

Glycobiology is a discipline that studies the structure and function of carbohydrates as entities linked to cell function.

Our laboratory is focused on the identification and study of some alterations of glycosylation in cancer. Altered glycosylation produces some structures that are tumour-specific. Against them, we generate different types of biotechnological developments in view of their biomedical application. Our lines of research are oriented to understand characteristics of tumour biology and, especially, to develop new molecular procedures in view of their application to the diagnosis and treatment of the disease.

MEMBERS

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Sabrina Fischer, MSc, PhD student
Mariel Flores, MSc, PhD student
Stephanie González Barceló, MD, PhD student
Santiago Garat, MSc student
Ruben Azar Scarone, Intern
Alina Brosque, Intern

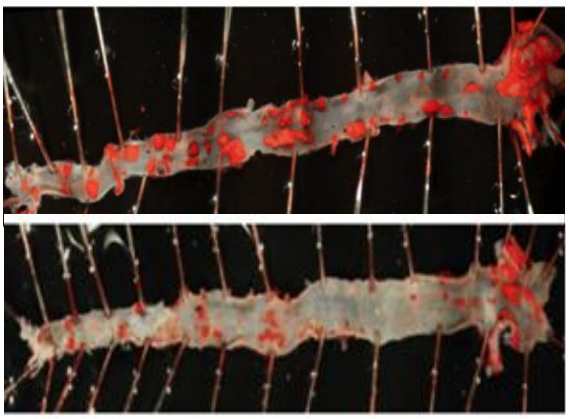
RESEARCH LINES

- **Anti-TN antibody engineering in molecular imaging and cancer immunotherapy.** Using different recombinant antibodies and fragments of them, we found that the Tn antigen is a molecular target of interest for the diagnosis and treatment of human tumours. We develop fragments of antibodies (scfv, minibodies and nanobodies) for their application in molecular imaging. We also investigated the development of immuno-nanoparticles to direct drugs towards tumours.

- **O-glycosylation enzymes as predictive and predictive biomarkers in oncology.** We evaluated whether the expression of GalNAc-Ts enzymes, which catalyse the first stage of O-glycosylation, can influence the biological properties of cancer cells (invasiveness, chemo-resistance, etc.), to subsequently develop useful diagnostic procedures in clinical oncology.

- **Anti-tumour immunotherapy using parasite molecules.** We found that some cancer-specific glyco-antigens are also expressed in certain parasites. We observed that immunization with extracts of Trypanosoma cruzi and Echinococcus granulosus induces anti-tumour immune responses in animal models. We seek to elucidate the molecular and cellular bases of this type of immune responses in order to generate a new type of anti-tumour therapeutic strategy.

16. VASCULAR BIOLOGY AND DRUG DEVELOPMENT LAB



The Laboratory of Vascular Biology and Drug Development has focused on the understanding of the molecular and cellular bases of atherosclerosis and other diseases in which chronic inflammation and low noise play a central pathogenic role (obesity, type II diabetes, neurodegenerative diseases).

In collaboration with other groups of our institute, we develop new strategies for the prevention and treatment of these diseases.

The initial objective of the laboratory was to develop a new pharmacological strategy for the prevention and treatment of atherosclerosis, based on the design of a hybrid compound analogous to Vitamin E that possesses non-conventional anti-inflammatory properties.

Subsequently, we focus our research activities on the design and development of other non-conventional anti-inflammatory drugs that can be used for the treatment of other diseases in which chronic inflammation plays a central role (insulin resistance induced by obesity, hypertension). In this way, we have currently designed and developed four new families of non-conventional anti-inflammatory compounds that are patented in the US and internationally. We have recently licensed our portfolio of intellectual property and are committed to conducting clinical trials in humans (phase I /II) with our leading compound.

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Williams Porcal, PhD, External Associated researcher, Faculty of Chemistry, Udelar
Jorge Rodríguez, PhD, Research assistant
Germán Galliussi, MSc, Research assistant, PhD student
Alejandro Leyva, Research assistant, PhD student
Lucía Collela, MSc, Research assistant, PhD student
Mariana Ingold, MSc, assistant researcher, PhD student
Federico Ortiz, MSc student
María Varela, MSc student, Intern

RESEARCH LINES

- **Development of a new pharmacological strategy for the treatment and prevention of atherosclerosis.** We designed a hybrid compound analogous to α-tocopherol to which we added a nitroalkenyl group. The rationale of our idea was that the nitroalkene analogous to tocopherol should be selectively incorporated into lipoprotein particles (particularly LDL) during its normal metabolism, due to the presence of chromanol in its structure and as does -tocopherol. Once incorporated, LDL would act as carriers of our compound to the whole organism but including atherosclerotic damage, where the compound could exert the anti-inammatory and anti-atherogenic properties of nitroalkenes. In order to test our hypothesis and test our compound in vivo, we developed dierent models of cardiovascular diseases in mice and demonstrated the ecacy of our compound for the prevention of atherosclerosis (J Rodríguez-Duarte et al., 2018; British Journal of Pharmacology)

- **Development of new pharmacological strategies for the prevention and treatment of diseases related to chronic inflammation.** After the development of our first generation of synthetic nitroalkenes, we focus our research activities on the design and development of non-conventional anti-inflammatory drugs for the prevention and treatment of different diseases in which chronic inflammation plays a central pathogenic role. We design and develop three new families of unconventional anti-inflammatory compounds that are protected in the USA. We licensed our portfolio of intellectual property and created a “Startup” (EOLO Pharma S.A.) to test our leading compound in clinical trials (phase I / II).

17.WORM BIOLOGY LAB

Our laboratory studies how worms harvest energy. Parasitic worms —also known as helminths— infect a quarter of the world's population, livestock and crops. Eight of the twenty diseases catalogue by WHO as unattended are caused by helminths, while infections by these organisms constitute a major economic problem for developing countries.

For these parasites there are no commercial vaccines available and there is an urgent need for new antihelmintics.

We seek to identify "Achilles heels" of parasite metabolism. We also established whole organism motility tests in order to discover new antihelmintics that paralyze worms. Our laboratory also investigates in selenium biology, an essential micronutrient for most organisms, including mammals. For most of our research we use the C. elegans worm as a model.

MEMBERS

Gustavo Salinas, PhD, Head
Lucía Otero, PhD
Jorge Pórfido, PhD
Laura Romanelli, PhD
Cecilia Martínez, PhD
Gastón Risi, MSc

RESEARCH LINES

- **Malate dismutation in helminths.** We aim to elucidate aspects of this metabolic pathway that allows parasitic worms to harvest energy under hypoxic conditions, such as those found in the gastrointestinal tract of their hosts.

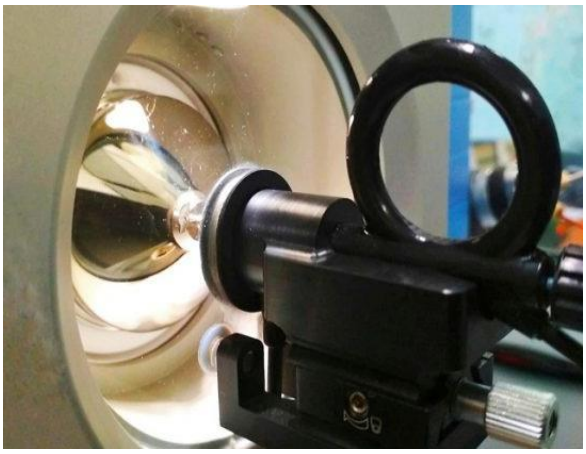
- **Thioredoxin and glutathione systems of parasitic flatworms.** These organisms have linked pathways of thioredoxin and glutathione, with thioredoxin glutathione reductase as the sole enzyme serving both pathways. We are currently investigating structural aspects of this enzyme and determinants of redox function and iron-sulfur binding of thioredoxins and glutaredoxins.

- **Search for new anthelmintics.** Based on a motility test of *C. elegans* we aim to identify, from libraries of natural and synthetic products, new anthelmintics.

- **Selenium metabolism.** We seek to understand pathways of metabolization and response to the trace element selenium in animals, by direct and reverse genetic approaches using C.elegans as a model organism.

TECHNOLOGICAL UNITS

18. ANALYTICAL BIOCHEMISTRY AND PROTEOMICS UNIT



The Analytical Biochemistry and Proteomics Unit (Ubypa) —an IP Montevideo and Instituto de Investigaciones Biológicas Clemente Estable (IIBCE) Mixed Unit— has the objective of carrying out and supporting biomedical research projects based on mass spectrometry (MS) and proteomics. It also offers training, scientific assistance and access to EM-based proteomic technologies to the local scientific community; and contributes to local and regional education programs in this area.

During the last years, the Unit incorporated mass spectrometers and developed the know-how to expand the quality and type of analytical procedures available. Currently, our analytical portfolio includes “shotgun” proteomic strategies, as well as gel-based strategies; in vivo and in vitro interactome studies; and analysis of post-translational protein modifications.

The research projects of our group focus on the study of signaling mechanisms in mycobacteria using proteomic approaches, with emphasis on the analysis of protein phosphorylation. In particular, we are interested in understanding some key processes for pathogenic mycobacteria, such as Mycobacterium tuberculosis, which are related to their ability to survive inside the host.

MEMBERS

Rosario Durán, Head
Magdalena Portela, Technical Assistant
Analía Lima, Technical Assistant, PhD student
Jessica Rossello, PhD student
Bernardina Rivera, Technical Assistant
Alejandro Leyva, Technical Assistant, PhD student

RESEARCH LINES

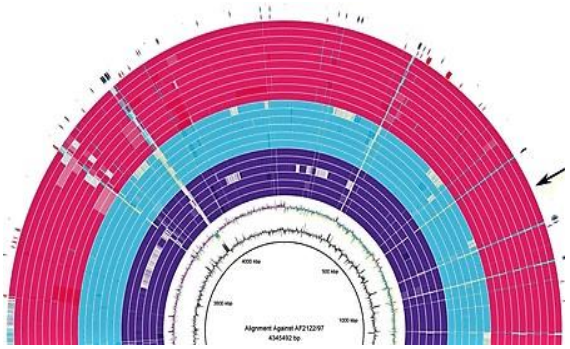
- **The role of protein phosphorylation in the regulation of biological processes in Mycobacterium tuberculosis.**

We aim to understand the mechanisms of Ser/Thr kinases and identified some of their substrates and targets “downstream” in the signaling pathways. At present, we are using in vivo cross-linking in combination with mass spectrometry to obtain a “snapshot” of the protein-protein interactions in the living bacteria, with emphasis in the study of the interactome of the previously identified kinase-substrates. This has allowed us to begin to elucidate phosphorylation-dependent interactions that participate in the regulation of nitrogen uptake and cell division.

MAIN EQUIPMENT

- HPLC, Agilent 1200
- Capilar HPLC, Agilent 1200;
- Nano HPLC, Easy-nLC 1000, Thermo
- Nano HPLC Ultimate 300, Thermo
- 2D Electrophoresis, EttanIPGphor + EttanDaltSix
- Typhoon FLA 9500, GE Healthcare
- 4800 MALDI TOF/TOF Mass Spectrometer, Abi Sciex
- LTQ Velos + ETD Mass Spectrometer, Thermo
- Q-exactive (Q-Orbitrap), Thermo

19. BIOINFORMATICS UNIT



Bioinformatics is the application of computational and statistical techniques for the analysis of data of biological origin, which also includes its organization and storage as well as the development of tools to manage them.

The volume of data currently generated by sequencing techniques promotes a constant development of methods to deal efficiently with numerous genomes and proteomes, without losing sight of the biologically relevant data.

At the Bioinformatic Unit we apply various tools to the comparative study of genomes on a global scale, as well as specific families of genes and proteins. Historically we have focused on bacteria, human pathogens and the human genome. We have also developed specific tools to deal with the new methodological challenges posed by the information explosion. These applications and developments have a place in genetics and biomedicine, and most recently in metagenomics, the genomic characterization of environmental samples.

MEMBERS

Hugo Naya, Head
Martín Graña, Associate research
Natalia Rego, Associate technician – PhD student
Lucía Spangenberg, Research assistant
Tamara Fernández, Research Assistant – PhD student
Gregorio Iraola, Associate research
Pablo Fresia, Research assistant – Postdoc
Ignacio Ferrés, MSc student in Bioinformatic
Daniela Costa, PhD student in Biology
Cecilia Salazar, PhD student in Biology
Verónica Antelo, Research assistant – PhD student in Biology
Matías Giménez, MSc student in Biology
Guillermo Cabrera, BSc student in Biology
Gastón Rijo, MSc student in Bioinformatic
Camila Simoes, MSc student in Biomedical Engineering

RESEARCH LINES

Human genomics:

The URUGENOMES project seeks to create local capacities in the area of human genomics and especially medical genomics. In this context, 50 genomes were sequenced, which are being analyzed from the point of view of population genomics (ancestry), and 30 other genomes of patients with rare diseases, which are analyzed in the context of medical genomics.

Human transcriptomics:

One of the areas in which the unit specializes is human transcriptomics. Several projects are currently being developed within this line. One of them is dedicated to the study of differentiation processes of stem cells to different cell types (adipogenesis, osteogenesis and cardiomyogenesis). Of special interest is the post transcriptional regulation that takes place during these processes. Another project within this research line aims to analyze the response of human cells to the infection of pathogens of different origins.

Structural bioinformatics:

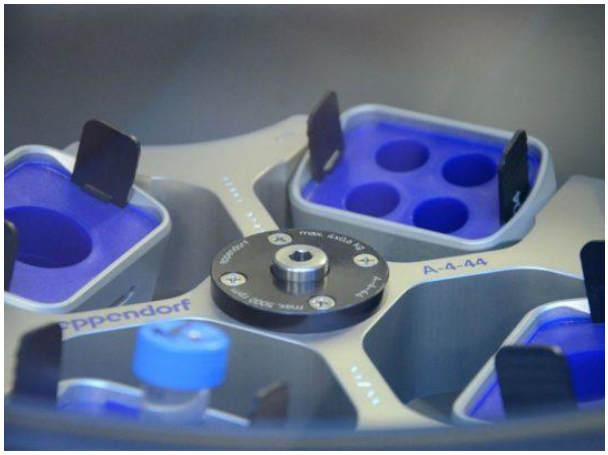
We seek to exploit data on protein structures, generated by X-ray crystallography and other techniques (NMR, cryo-electromicroscopy), in the functional understanding of protein families. Using three-dimensional structures and genomic sequences often provides clues about the function of a protein and, sometimes, generates functional hypotheses about whole families of 'hypothetical' genes (genes without known function).

Genomics and microbial evolution:

It seeks to understand the evolution of pathogens that affect livestock and domestic animals using phylodynamics; and fundamentally the discovery of the evolutionary forces that shape the genomes of zoonotic bacteria such as Campylobacter, Leptospira and Mycobacterium. Among the ongoing projects within this line, there is currently a Latin American joint effort to study the population dynamics of Clostridium difficile using genomic epidemiology approaches. Recently, one of the projects of this line seeks to analyze the dynamics of resistance to antibiotics in enterobacteria of urban environments through the application of metagenomics.

Statistical genetics and its application to evolution.

20. BIOPHARMACEUTICAL QUALITY CONTROL & DEVELOPMENT UNIT



The Biopharmaceutical Quality Control & Development Unit offers vast experience in bioassays and protein chemistry, as well as a wide range of analytic techniques and lab equipment.

It provides solutions in the field of analytical control of biopharmaceuticals, either using pre-established methodologies based on international guides and pharmacopeia, or developing new analytic tools in order to meet and follow bioanalytical strategies.

Our assays follow ICH guidelines and the FDA and EMA regulations.

From its creation in June 2009, it was designated by the Ministry of Public Health as a national reference laboratory for the control of biopharmaceuticals sold in the Uruguayan market.

MEMBERS

Alejandro Ricciardi, Pharmaceutical Chemist, Head
Larissa Armas, Technical Assistant
Julia Sanguinetti, Biochemist, M.SC.
Bernardina Rivero, Clinical Biochemist, M.Sc.
Verónica Marco, Chemist, M.Sc.

RESEARCH LINES

The unit has developed biopharmaceuticals kits and analytical methodologies for the productive sector. An example of this have been the following projects:

- **Development of Methodologies to Quantify Proteins and DNA Contaminant Derivatives of the Host Cell in Recombinant Bio-Pharmaceuticals.** Funded by the National Research and Innovation Agency. (Project ALIANZA – Laboratorio Celsius S.A. and IP Montevideo) (2011 – 2012)

- **Methodological development for quantification of immunogenicity generated by administration of Interferon beta1a in patients, by means of a Bioassay based on cell culture and Real Time PCR.** Funded by Laboratorios Clausen S.A. (2010).

- **Participation in a Multicenter Study for the Determination of the Biological Potency of the First Filgrastim Standard of the United States Pharmacopeia** (USP). (2012)

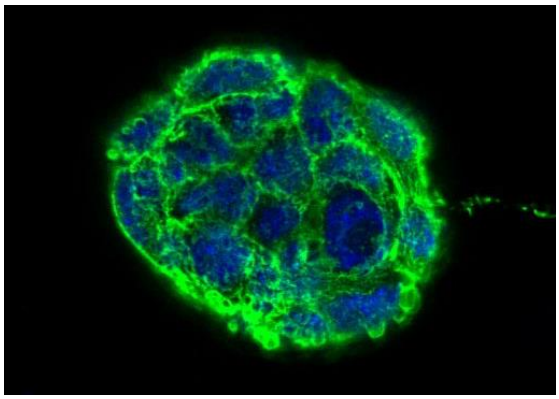
EQUIPMENT

- PA 800 Plus Capillary Electrophoresis (Beckman Coulter)
- HPLC Prominence with DAD, RID and Fluorescence detectors (Shimadzu)
- Multiskan Spectrum Spectrophotometer and Plate Reader (Thermo Scientific)
- Class II, Type A2 Biological Safety Cabinet (Thermo Scientific)
- CO2 Incubator (Thermo Scientific)
- Inverted Microscope (Nikon)
- Freezer -20 °C and Fridge (Angelantonni)
- PLA® 2.0 , Stegmann Systems.
- Combistats®, EDQM.

21.CELL BIOLOGY UNIT

The Cell Biology Unit is a technology-based platform whose mission is to offer advice and support to research groups or private entities in different projects that use cell cultures and flow cytometry as a central methodology.

With these technologies, we develop research projects specific to the biotechnology area. The UBC also integrates the ProTeMCA group, one of the broad programs of the institute.



MEMBERS

Mariela Bollati-Fogolín, PhD, Head
Romina Pagotto,PhD, Associate researcher
Cecilia Abreu, PhD, Associate researcher
Vanesa Piattoni, PhD, Associate researcher, PosDoc
Karen Perelmuter, MSc, Associate Technician
María Paula Céspedes, Associate Technician, MSc student
Tatiana Basika, PhD, Assistant Technician
Hellen Daghero, MSc, Research assistant
Karin Grunberg, Assistant Technician, MSC student
Constanza Silvera, Assistant Technician, undergraduate student

RESEARCH LINES

- **CELL TECHNOLOGY:** Our group has been dedicated to the generation and characterization of recombinant cell lines of biomedicine and biotechnological interest. Among them, we use a great variety of reporter cell lines (IFN type I, NF-kB, redox biosensors, among others) to search for and characterize substances that interfere with the signaling pathways of type I IFNs (Burgi et al, 2012 and Burgi et al, 2016). Also we use them in in-vitro models of inflammation (NF-kB, Tiscornia et al, 2012 Mastropietro et al, 2015; Rolny et al, 2016) or to improve the metabolism/productivity of cells of biotechnological interest (redox biosensors within the framework of ProTeMCA).

- **ENVIRONMENTAL TOXICOLOGY:** Endocrine disruptors (ED) are anthropogenic substances present in the environment, capable of altering the homeostasis of the endocrine system of organisms, contributing to the development of pathologies. Our working hypothesis is that the increase of certain reproductive pathologies is caused, in part, by the increasing exposure to EDs present in the environment. In this context, we have validated an in vivo model —the transgenic mouse Oct4-GFP— for toxicological monitoring of environmental estrogens (Porro et al, 2015). We have also developed an in vitro assay that uses a dual reporter cell line and allows us to evaluate in a single test the estrogenic or androgenic activity of a putative ED. In this line of research, we are collaborating with Dr. Rodríguez (ISAL, CONICET-UNL, Argentina).

- **ANTITUMORAL PEPTIDES:** Since 2011, we have collaborated with Dr. Vallespi, from the Center for Genetic Engineering and Biotechnology (CIGB), in Cuba, on the project "CIGB-552: novel peptide with antitumor and anti-inflammatory properties useful for cancer treatment". We demonstrate that CIGB-552 is effective in reducing the size of tumours present in mice and we identify the COMMD1 protein as a key mediator for its antitumor activity (Fernández Massó et al, 2013, Vallespí et al., 2014, Núñez de Villavicencio et al., 2015).
Recently, we described the minimum functional unit of CIGB-552 necessary to exercise its biological activity (ability to penetrate tumor cells, interact with COMMD1 and induce apoptosis, Astrada et al, 2016 and 2018).

- **TISSUE ENGINEERING: INTESTINAL ORGANOIDS AND 3D CROPS:** Traditional cell cultures, in two dimensions, offer a simple, rapid, economic and reproducible evaluation system. However, they lack the ability to recreate the cellular interactions that take place in a tissue in vivo, limiting its predictive power. Our goal is to generate and characterize cellular models in three dimensions (3D) that resemble living systems, improving the correlation of results. In particular, we propose to establish a murine intestinal organoid culture, either from primary tissue or by differentiation of induced pluripotent stem cells (from the English iPSC) and apply them in the study of inflammation, cancer and probiotic screening.

EQUIPMENT

LABORATORY OF CELLULAR CROPS

We have several laboratories equipped to work in an aseptic environment, such as biological safety cabinets, CO2incubators, cytological centrifuges and inverted microscopes.
We also have an Automatic Analyzer for Glucose and Lactate Bioprofile Basic 2 Analyzer (Nova Biomedical).

FLOW CYTOMETRY LABORATORY

Cellular analyzers:

- CyAn™ ADP (Beckman Coulter)
- BD Accuri™ C6 (BD Biosciences)
- Attunne Nxt (Thermo Fisher Scientific)
- BD FACSAria™ Fusion (BD Biosciences)
- High speed cellular classifier

Software for data analysis:

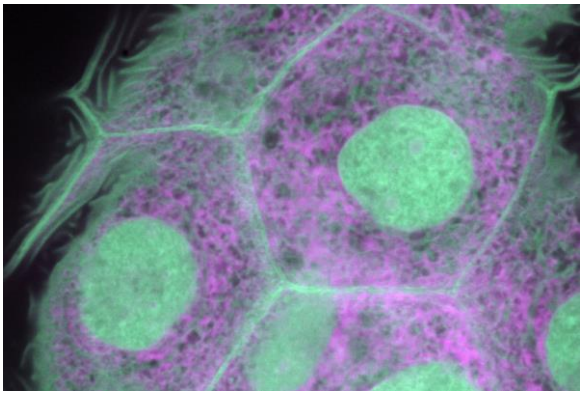
– Summit V4.3

– BD Accuri C6 Software V1.0.264.21

– FACSDiva

– FlowJo

22.MICROSCOPY UNIT



The Microscopy Unit has equipment for performing fluorescence and confocal microscopy. These microscopes are available to all researchers in the public or private sector who wish to view and take pictures of fluorescent or confocal microscopy.

Our service is dedicated to analyse and process images as well as to provide technical advice. We have high quality equipment that allow us to obtain high-resolution images of biological and non-biological materials.

MEMBERS

Flavio Zolessi, PhD, Head
Federico Lecumberry, PhD, Head
Marcela Díaz, MSc, Technician
Tabaré de los Campos, Technician

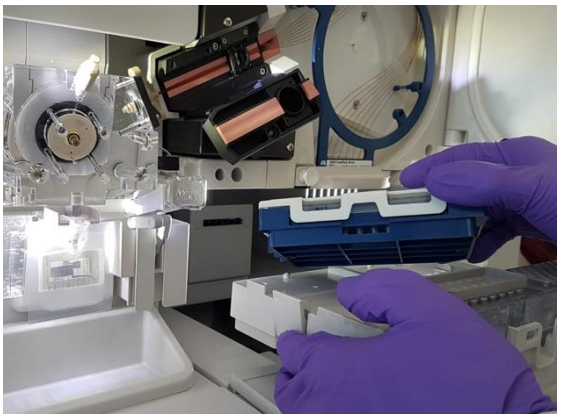
EQUIPMENT

- Olympus IX81 conventional epifluorescence microscope

- Zeiss LSM 800CyAn ™ ADP semi-spectral confocal laser scanning microscope (Beckman Coulter)

- Zeiss LSM 880 spectral confocal laser scanning microscope (as of September 2018)

23.MOLECULAR BIOLOGY UNIT



The Molecular Biology Unit (UBM) offers standardized nucleic acid sequencing services and fragment analysis by capillary electrophoresis. These services are aimed to provide technical support and scientific advice to researchers of the institute and other public or private research organizations, companies and centers across the region.

It also offers advice for the experimental design of genomic and transcriptomic studies through DNA microarrays or massive sequencing.

It has a microarray scanner (Agilent) and a high capacity sequencer (Illumina) that allows the realization of a wide spectrum of experiments.

MEMBERS

Carlos Robello, Head
Gonzalo Greif, Associate researcher
Luisa Berná, Associate researcher
Gabriela Libisch, Technical assistant
Paula Faral-Tello, Technical assistant, PhD student in Biology
Florencia Díaz Viraqué, Research assistant, PhD student
Cecilia Portela, Technician

RESEARCH LINES

- **Study of the intracellular host-pathogen interaction with a systemic approach.** At the UBM we studied virulence factors of intracellular pathogens, as well as the host genes and routes necessary for the establishment of the infection, and the interface between them. Based on this, one of our objectives is the design of new strategies for the treatment or prevention of various infectious pathologies, like Chagas disease, African trypanosomiasis, leishmaniasis, and tuberculosis.

- **Determination of virulence factors necessary for *T.cruzi* infection.** For the establishment and persistence of *T. cruzi* infection, we have described a set of proteins related to the redox metabolism of trypanosomatids (triparedoxin cytosolic and

mitochondrial peroxidase, triparredoxins, glutarredoxins, pteridin reductase), characterizing them from the structural and functional point of view. We are developing strategies to inhibit them, and applying them in the development of therapeutic and preventive strategies.

- **Characterization of changes in gene expression produced by intracellular parasites in human cells.** We have shown that *T.cruzi* triggers a cellular reprogramming that allows the establishment and persistence of the infection in human cells. One of our objectives is to identify host proteins, which, when inhibited, prevent *T. cruzi* infection. We are also extending this strategy for genomic and molecular biology studies in leishmaniasis, African trypanosomiasis, neosporosis and tuberculosis.

EQUIPMENT

- DNA sequencer / analyzer
- Real-time PCR
- Microarray reader
- Microarray hybridizer
- BioAnalyzer
- General molecular biology instrumentation
- MiSeq Illumina

24.PASTEUR+INIA MIXED UNIT

The Institut Pasteur of Montevideo (IP Montevideo) and the National Institute of Agricultural Research (INIA) Joint Unit was created in October 2014 with the aim of promoting and adding value to knowledge and combining research areas of both institutions. It also seeks to provide solutions to the agricultural sector based on the use of technologies developed from multidisciplinary approaches in animal health, microbiology, bioinformatics, molecular genetics and generation of bio-inputs.

This Unit intends to carry out joint research projects, train human resources and create associative networks in areas of mutual interest, meeting the needs of the agricultural sector. As an open platform, the unit is available to researchers from IP Montevideo, INIA and other research institutions, promoting the linkage of the productive sector, academy and industry.

MEMBERS

Leticia Zarantonelli, PhD, Head
Camila Hamond, PhD, Postdoc fellow – INIA

RESEARCH LINES

- **Bioinputs**: We study new bio-inputs formulated with microorganisms or microbial bioactive compounds to improve the productivity, quality and health of the plants or the characteristics of the soils. We seek the development of products and technologies that allow improving the sustainability of food production systems, complying with safety standards and reducing the use of agrochemicals.

- **Infectious diseases affecting livestock:** We study Enzootic Bovine Leukosis, Tuberculosis, Campylobacteriosis, Leptospirosis and Neosporosis. These diseases affect animal health and have a negative impact on livestock production. Some of these infectious diseases can also affect humans and put at risk the health of those who work in contact with infected animals. The objective is to develop technologies for the

diagnosis, treatment and control of these diseases, seeking to improve the global health status of Uruguayan cattle.

EQUIPMENT

The UMPI has a laboratory with Biosafety level II to work with pathogenic microorganisms. This makes it possible to strengthen the work capacities of the researchers that work under the Animal Health program at the institute.

The laboratory has areas equipped and dedicated for:

- Cultivation of aerobic microorganisms
- Cultivation of eukaryotic cells
- Extraction of nucleic acids and proteins
- Preparation of reaction mixtures for the amplification of nucleic acids (Pre-PCR area)
- Amplification by conventional or real time PCR (PCR zone)
- Quantification and analysis of nucleic acid amplification products (Post-PCR area)
- Protein analysis (ELISA / Western Blot)

25. PROTEIN CRYSTALLOGRAPHY UNIT

The purpose of the Unit is to set up and maintain a Protein Crystallography Facility PXF, to provide equipment, training, assistance, and technological innovations for determining three-dimensional structures of proteins and other macromolecules and macromolecular assemblies.

X-ray crystallography is one of the most powerful techniques to study the 3D structures of macromolecules and it has transformed our understanding of biological processes.

Our setup allows users to crystallize macromolecules and solve their three-dimensional structures using X-ray diffraction.

MEMBERS

Alejandro Buschiazzo, PhD, Head
Joaquín Dalla Rizza, MSc, Technician
Nicole Larrieux, Biochemist, Technician
Felipe Trajtenberg, PhD, Research scientist

EQUIPMENT

- **Crystallization robot – Honeybee963®.** The Honeybee963® (Digilab) robot is a bench-top system for the automation and miniaturization of vapor diffusion in sitting-drop protein crystallization experiments. Proprietary Cartesian synQUAD® dispensers couple high-speed micro-solenoid valves with high resolution syringe pumps, dispensing volumes down to 100 nL. We currently use 200-300 nL nanodrops to maximize precision and crystallizability. The 96-needle arm allows for very fast dispensing of reservoir solutions on a 96-well setup. Three independent protein synQUAD® needles then proceed to dispense up to three different proteins, variable volumes are defined using the robot's software. Automation enables the assay of typically hundreds of different potential crystallogenesis conditions in a matter of minutes, allowing to greatly increase the search space, and thus the probability of finding hits.

- **Automatic setup and optimization of screen assays – Alchemist DT®.** The Alchemist DT ® (Rigaku) is a bench-top liquid handling robot for the screen production and optimization of crystallization conditions. It provides consistent, precise and accurate liquid dispensing in a volume range of 1 µl to 10 ml into SBS, Linbro ® and Nextal® footprint plates. Due to its technology, elimination of tubing means no waste and removes the possibility of cross-contamination. CrystalTrak™, the integrated software package, is designed specifically for protein crystallography. Once the screen is designed, CrystalTrak™ automatically calculates the recipe and defines the necessary stock solutions for use with the Alchemist. 26 different stock solutions can be stored on the deck at one time. Stock management tools and barcode tracking ensure that the correct stock solutions and necessary volumes of solutions are available on the deck before any plate generation begins.

- **X ray generator – Rigaku MicroMax-007HF®.** Micromax007-HF® (Rigaku) is an X-ray generator with a 0.07 mm diameter effective focal size at the source. Equipped with a Cu rotating anode, it provides an output of 1200 W and a brightness of 31 kW/mm2. In combination with the installed optics (Varimax-HF®, Rigaku) which consists of confocal multilayer mirrors, the resulting X rays focused on the crystalline sample are ultra-bright, and can be used effectively for various measurement purposes. We can solve structures with atoms that scatter anomalously at 1.5418Å wavelength (S, I, Cs, lanthanides, among the most used). Molecular replacement problems can be tackled, as well as data collection for high-resolution refinement (including ligands, inhibitors, drugs, point-mutation protein variants, etc).

- **Image plate area detector – MAR345®.** The MAR345® (Mar Research) detector installed on a MAR345dtb®table is an image plate detector that enables us to collect data up to 1.2Å resolution on our geometric setup (taking full advantage of the 2θ angle). It is a single Φ-angle oscillation setup, equipped with a convenient χ-motor that facilitates crystal mounting under cryogenic conditions. Read-out cycles range from 108 to 34 seconds, depending on pixel size and effectively scanned plate diameter. The read-out system of the Mar345 is unique in its use of a single high performance 85mW laser which delivers more than 0.8 µJ/pixel at the plate. This ensures that an extremely high percentage (>95%) of trapped F-centers are transformed into photostimulated luminiscence.

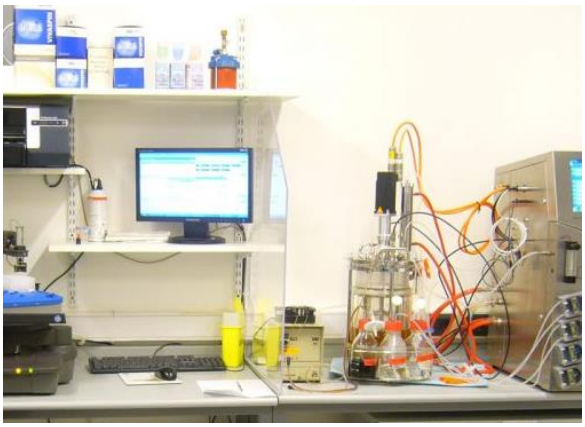
- **X ray cryosystem – 700 series Cryostream®.** The Cryostream® (Oxford Cryosystems) allows a continuous laminar flow of gas nitrogen at cryogenic temperatures during single crystal data collection. Fast cool-down to 100 Kelvin is achievable in 20 minutes. It has a fairly low liquid nitrogen consumption with a variable flow from 5 to 10 litre/minute. We have an accessory auto-fill system that uses a level

probe in the cryosystem's Dewar, that automates the topping-up of the Dewar basically during data collections that last for several hours/days.

- **Liquid nitrogen generator – LN40®.** The LN40® (Rigaku) is a helium compressor-based machine able to produce up to 40lts/day of highly pure (>98%). liquid nitrogen. The gas input comes from the air pumped by the included PSA (Pressure Swing Adsorption) system.

- The operation of the cryogenic refrigeration system is based on a closed-loop Gifford-McMahon (GM) helium expansion cycle. The PSA system consists of two basic components: vessels containing "carbon molecular sieve" (CMS) and an air compressor as a source of clean dry air.

26.RECOMBINANT PROTEIN UNIT



Recombinant proteins —those produced in the laboratory by genetic engineering using cells different from nature— have shown a high impact in basic research and in the biomedical field for drug manufacture. However, in many cases it is not possible to obtain a stable, soluble and homogeneous product, and this limits its applications. Several strategies were developed in the last decades to overcome these limitations.

In this sense, our group has generated a set of vectors that facilitates cloning and allows the evaluation of several parameters that can improve the soluble expression of a target protein.

In the context of cancer therapeutic tools, our group recently focused on the generation of artificial binding proteins known as Affitins. This class of proteins has a wide range of advantages compared to classical therapeutic antibodies that could be taken into account in the development of therapeutic approaches.

MEMBERS

Pablo Oppezzo, PhD, Head
Agustín Correa, PhD, Associate Technician
Claudia Ortega, PhD, Technical assistant

RESEARCH LINES

Vector Suite and Artificial Binding Proteins (ABP). Our laboratory has developed a set of vectors that allow the evaluation of different promoters and proteins that enhance solubility, through an efficient cloning strategy. This vector suite was extended in order to generate a set of vectors to include the evaluation of expression of recombinant proteins in different cellular compartments and cellular hosts. This helps to overcome the limitations encountered when working with a single subcellular location and a single type of host. In addition, these vectors also allow the evaluation

of alternative purification strategies for the improvement of the yields of the target protein.

In the context of cancer therapeutic tools, our group recently focused on the generation of artificial binding proteins known as Affitins. This class of proteins has a wide range of advantages compared to classical therapeutic antibodies that could be taken into account in the development of therapeutic approaches.

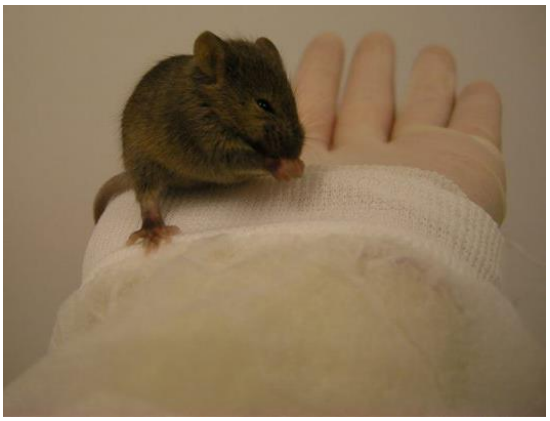
Compared to classical therapeutic antibodies, Affitins are able to maintain constant high affinity even when their molecular weight remains small. This could be very useful in lymphoid neoplasms, in order to gain access to solid tissues as secondary lymphoid organs, where leukemic cells receive survival signals that acquire favourable conditions of proliferation.

In this line, a new generation of combinatorial protein engineering technologies has recently been created in our laboratory. The results have made it possible to propose the use of these artificial binding proteins as versatile selective glycosidase inhibitors and, potentially, as enzymatic inhibitors in general, which could be foreseen for future tumour therapy strategies (Correa et al., PlosOne, 2014).

EQUIPMENT

- ÄKTAxpress / ÄKTA Pure and ÄKTA Purifier
- Benchtop Bioreactor BIOSTAT® B plus (Prokaryotic culture)
- CelliGen 310 Bioreactor (Eukaryotic culture)
- BelloCell 3000 Bioreactor (Eukaryotic culture)
- EmulsiFlex-C5 Homogenizer
- Multitron 2 Incubated Shaker

27.TRANSGENIC AND EXPERIMENTAL ANIMAL UNIT



The unit provides high-level support in the field of animal genome modification (mice, rats and ruminants). We offer several techniques, such as pronuclear microinjection, homologous recombination in embryonic stem cells, lentiviral injection, transposons and the revolutionary CRISPR / Cas9 system. We also provide cryopreservation, in vitro fertilization and embryo rederivation in mice. Besides, the unit supplies mice and rats with high genetic and health status for researchers within the institute and the region.

Since 2007 we have worked with national and international biotechnology companies offering in vivo biological assays in mice and rats, under GLP standards.

We receive undergraduate and graduate students who do thesis using different technologies to obtain animal models. We also organize courses for technicians and scientists, and offer internships for technicians or veterinarians who administer their own services.

Our staff is member of organizations, such as the International Society for Transgenic Technologies (ISTT), the Institutional Articulation Committee (CAI), the National Commission of Animal Experimentation (CNEA), the Uruguayan Association of Animal Science and Technology Laboratories (AUCyTAL), and the Federation of South American Associations for Laboratory Animal Science (FESSACAL).

MEMBERS

Martina Crispo, PhD, DVM, Head
Ana Paula Mulet, PhD, Molecular Biology Technician
Geraldine Schlapp, MSc, Reproductive technology expert
María Noel Meikle, MSc, Reproductive technology expert
Ana Paula Arévalo, TMN, Radioisotope technician, MSc student
Gabriel Fernández, BSc, Animal Facility technician
Sergio Ancheta, Amimal keeper
Andrés Pereyra, Amimal keeper
Nicolás Fiore, Amimal keeper
Joaquín González, Maintenance

RESEARCH LINES

- Gene editing in rodents
- Gene editing in ruminants
- Cryopreservation of embryos and mammals sperm
- In vitro fertilization of rodents and ruminants

EQUIPMENT

Facilities

- Animal Facility: SPF (Specific Pathogen Free) and Conventional area
- Microinjection Laboratory
- Assisted Reproduction Techniques Laboratory (ART)
- Molecular Biology Laboratory

Services

- Generation of transgenic animals using CRISPR / Cas9 technology, pronuclear microinjection of DNA fragments, gene-targeting in embryonic stem cells, Sleeping Beauty Transposons technology.
- Cryopreservation of embryos and sperm.
- Rederivation of murine lines
- In vitro fertilization of murine lines
- Crosses and maintenance under SPF and conventional conditions of various strains mice (C57BL / 6J, BALB / cJ, DBA / 2J, Nude, several hybrids and about 30 different transgenic lines). Approximate production (currently): 2.000 mice per month.
- Immunomodulatory capacity assays of probiotic bacteria
- Biological activity assays of recombinant erythropoietin. Our Bioterio is certified by the Ministry of Public Health
- Toxicity tests for biotechnological products (EPO, Filgen, Interferon)
- Production of polyclonal antibodies in mice

COURSES

Title	Organizers	Date	Foreign Speakers	Foreign Students	Financial Agencies
Urugenomes: Introduction to Medical Genomics	H. Naya	March 5 - 9	1	20	IADBank
Congreso y Simposio: "Trauma Encefálico: desde la investigación preclínica a la clínica"	H. Peluffo I. Kasek V. Patiño	April 9 – 14	5	1	BSE IBRO IP Montevideo
Métodos Alternativos para el uso de Animales de Experimentación	M. Crispo M. Bollati	May 21 - 25	6	7	IP Montevideo
CARD-IP Montevideo Mouse Embryo and Sperm Cryopreservation Hands-on Course	M. Crispo G. Schlapp MN. Meilkle	Sept 10 - 13	3	12	UNU BIOLAC IP Montevideo
Curso Internacional de Citometría de Flujo	M. Bollati D. Lens	Oct 1 – 5	7	30	ANII PEDECIBA BD/BECKMAN COULTER VISUR IP Montevideo
Proteome Analysis in Mass Spectrometry	R. Durán P. Carvalho C. Batthyány	Oct 15 - 23	7	20	UNU BIOLAC ICGEB TECNOFORM IP Montevideo
Conference on Latin American Initiative for Molecular Simulations	S. Pantano	Nov 5 – 7	21	---	ICTP IP Montevideo
VII curso internacional Biologia molecular de Tripanosomátidos VII Simposio biología molecular de la enfermedad de chagas	A. Parodi F. Álvarez C.Robello	Nov26-Dec1	15	15	UNU BIOLAC CSIC PEDECBA IP Montevideo
Hands-on microbiome data analysis: tools for understanding microbial communities in health and disease	G.Iraola A.Bordería	Dec 3 - 7	5	14	COOPERACIÓN FRANCESA

PUBLICATIONS (2018)

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IP Montevideo at a glance

Staff

Human Resources	Dec 2010	Dec 2015	Dec 2016	Dec 2017	Dec 2018
Scientific &Technical staff	70	189	203	217	225
Administration support staff	30	37	40	41	46
Total	**150**	**226**	**243**	**258**	**271**

Publications and Citations (Scopus)

Year	2007-2011	2012	2013	2014	2015	2016	2017	2018	Total
Publications	167	48	49	82	90	77	79	91	**683**

Aggregate Record	2007 -2017	2018
Number of Publications	592	91
Accumulated citations	11.253	13.761
Citations per publication	20	24

Human Resources Training

	2014	2015	2016	2017	2018
Post-docs	24	22	26	14	**14**
PhD students	32	33	44	40	**47**
Master students	44	47	51	37	**40**
Undergraduate students	25	35	19	5	**17**
TOTAL	**125**	**137**	**140**	**96**	**118**

Courses

	2013	2014	2015	2016	2017	2018
Courses	7	8	6	9	9	**11**
Students	85	174	49	260	217	**163**
Regional students	40	51	68	91	139	**121**
Invited professors	44	36	65	57	71	**79**
Funding (k US$)	140	211	190	201	250	**148**

Budget Overview

Evolution of countable incomes 2007-2018