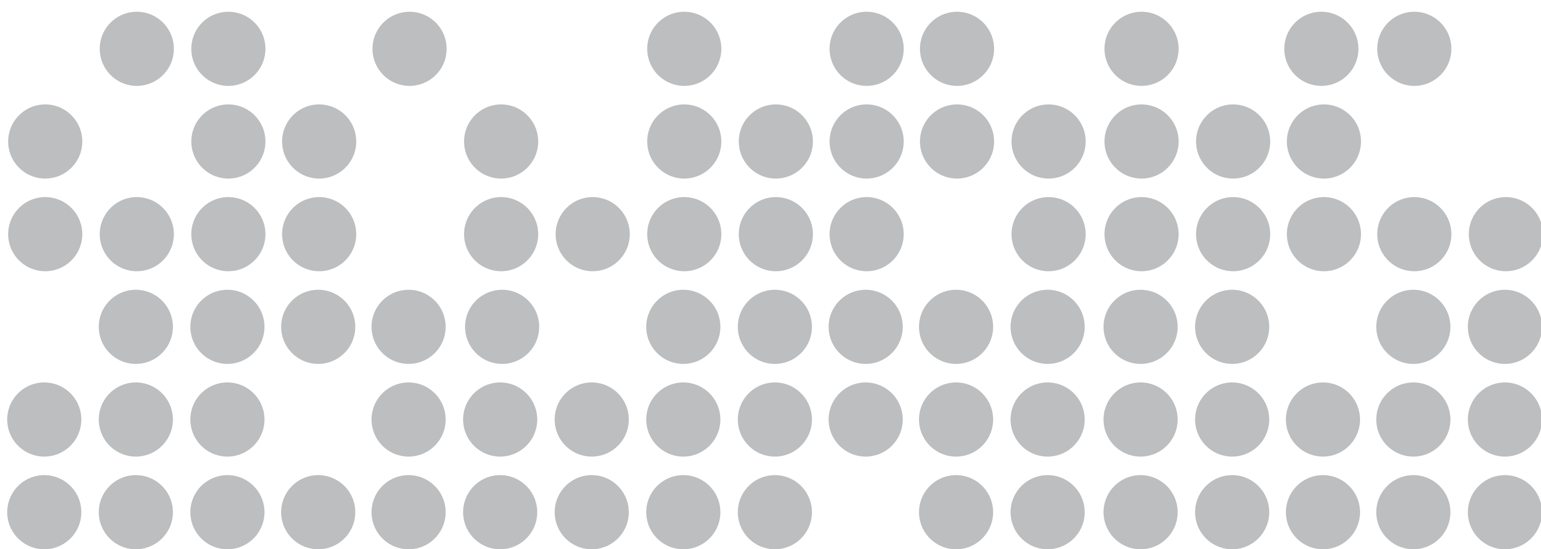
Institut Pasteur
de Montevideo

SCIENCE THAT CHANGES THE FUTURE

Annual Report 2013

Institut Pasteur de Montevideo
Annual Report 2013



PREFACE

On behalf of the Institut Pasteur de Montevideo (IP Montevideo), I invite you to go over the following pages and learn more about the activities we developed during 2013.

Inaugurated in 2006, the Institute is nowdays mainly financed by the Uruguayan State. It also receives program funding from the European Union (Uruguay Innova) and FOCEM (MERCOSUR Regional Convergence Fund). It also generates its own resources through grants and technological services. The total budget in 2013 was close to 7 million US dollars.

The IP Montevideo's mission is to establish a state-of-the-art research center with international projection dedicated to investigate the biological mechanisms of human and animal diseases, and eventually pave the way for new treatments and cures. As part of this mission, IP Montevideo seeks to train new human capacities through education and laboratory training, thus contributing to the development of science and biotechnology in the country.

The Institut Pasteur de Montevideo belongs to the International Pasteur Institute Network (IPIN). It includes 32 research institutes around the world with expertise to fight against infectious diseases. The IP Montevideo is one of the youngest institutes within this network, and from its inception, shares the same Pasteurian principles in life sciences research and its applications.

IP Montevideo is organized around a central core facilities area, in which an important investment was done to acquire state-of-the-art equipment serving to genomic, proteomic, structural biology, cell biology and transgenic animal research. In the last years, we set up two Next Generation Sequencing equipments, which allow researchers from different specialties to study in detail the genome, transcriptome, and epigenome of any organism.

In 2013, the number of research groups has increased significantly. One young research leader was recruited through the Uruguay INNOVA II program (UE) and four other groups have been incorporated through a visionary collaboration agreement with the Universidad de la República. The number and impact of the publications further increased in 2013, with 60 publications, reaching a total of 270 publications since 2007.

Our research laboratories provide an environment for the training of advanced undergraduate and graduate students who are interested in pursuing careers in sciences. In 2013, more than 80 PhD, MSc students and postdoctoral researchers were trained at IP Montevideo's laboratories. In addition, we received more than 600 school and high school students in different activities of science pop.

We have experienced great success in the organization of international or regional Courses on different topics of molecular medicine and bioinformatics. In 2013, more than 40 of distinguished professors and dozens of advanced students from abroad attended 7 main international courses.

With FOCEM funding, the IP Montevideo has inaugurated the "innovation space", a new space for laboratories and officesat the ground floor area, covering 1300 m2. This new extension will serve to reinforce collaborations with other institutions from the MERCOSUR region and to accommodate more research groups as well as pre-incubation and incubation star-ups. The innovation space hosts the "Bioespinn" incubator funded by the Uruguayan Agency for innovation and research (ANII).

I wish to thank all of our researchers and Institute members for their dedication, continued support and commitment with our Institute.

Luis Barbeito
Executive Director
Institut Pasteur Montevideo

INDEX

PREFACE ... 3

INDEX ... 5

TECHNOLOGICAL UNITS ... 7

Analytical Biochemistry and Proteomics Unit ... 9
Recombinant Proteins Unit Laboratory on Chronic Lymphocytic Leukemia ... 13
Protein Crystallography Unit ... 17
Bioinformatics Unit ... 21
Molecular Biology Unit ... 25
Cell Biology Unit ... 29
Transgenic and Experimental Animal Unit ... 35
Protein Biophysics Unit ... 39

RESIDENT LABORATORIES ... 43

Funcional Genomics Laboratory - Research Program in Molecular Oncology ... 45
Tumor Immunology and Glicobiology Laboratory - Research Program in Molecular Oncology ... 49
Neurodegeneration Laboratory ... 51
Biomolecular Simulations Laboratory ... 55
Molecular and Human Genetics Laboratory ... 59
Cellular Membranes Laboratory ... 63
Redox Biology of Trypanosomes Laboratory ... 67

YOUNG GROUP LEADERS LABORATORIES ... 71

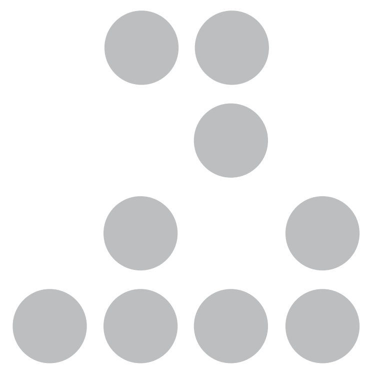
G5 - Epigenetics of Cancer and Aging Laboratory ... 73

ASSOCIATED AND MIXED UNITS ... 75

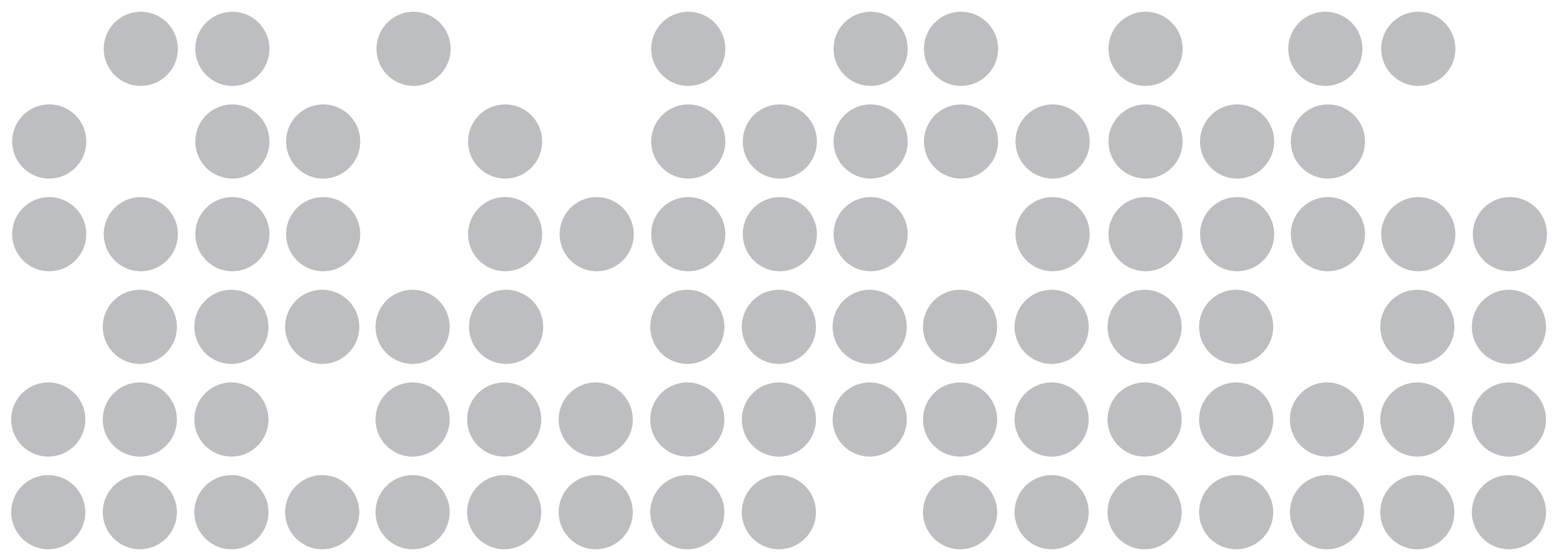
Laboratory of Immunology and Inflammation ... 77

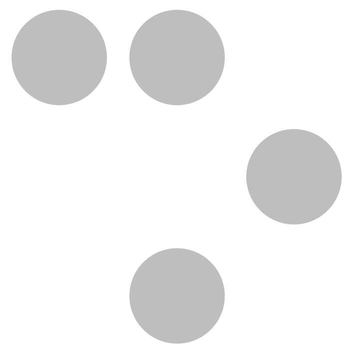
IP Montevideo Courses and Publications ... 81

PUBLICATIONS ... 83
COURSES ... 101



TECHNOLOGICAL UNITS





Analytical Biochemistry and Proteomics Unit

Head: Rosario Durán, PhD (IIBCE – IP Montevideo, Interim Head)

Members:

- Carlos Cerveñansky, PhD (IIBCE – IP Montevideo)
- Carlos Batthyány, MD, PhD (Associate Investigator-IP Montevideo; Adjunct Professor of Biochemistry, School of Medicine, UdelaR)
- Magdalena Portela (Technical Assistant – School of Sciences/IP Montevideo)
- Analía Lima, MSc (Technical Assistant, PhD student)
- Magdalena Gil, Biochemist (Technical Assistant -IIBCE; PhD student)
- Jorge Rodriguez, Biochemist (PhD student)
- Jessica Rosello, Biochemist (Graduate student, Technician)
- Bernardina Rivera Biochemist (Graduate student, Technician)
- Rosina Toledo (Graduate student)

Associate members:

- Horacio Botti, MD, PhD (Adjunt Investigator, IP Montevideo)
- María Noel Alvarez, PhD (Associate Investigator, Adjunct Professor of Biochemistry, School of Medicine, UdelaR, Uruguay)
- Leonel Malacrida, Biochemist (Associate Investigator, Assistant Proffesor, Pathophysiology Department, School of Medicine, UdelarR, Uruguay)

The mission of the Analytical Biochemistry and Proteomics Unit (UByPA) at the Institut Pasteur de Montevideo is to:

1. perform and support mass spectrometry (MS)/ analytical biochemistry based research
2. provide researchers with training, scientific assistance and access to MS and proteomic related technologies
3. improve available MS techniques for biomedical research
4. contribute to local and regional training and education programs

Our specific goals are:

- to provide open access to analytical biochemistry, mass spectrometry and proteomics technologies to local and regional researchers,
- to pursue biomedical research projects based on mass spectrometry and proteomics,
- to facilitate collaborative scientific projects with other national and international research groups, supporting a join-effort to seek funding,
- the education of graduate students and researchers in analytical biochemistry, mass spectrometry and proteomics related technologies.

Research

In the past years, members of our research group have been involved in different areas of biological/biochemical research. A major contribution made by the UByPA´s scientist was the incorporation of modern mass spectrometry (MS) and 2D-electrophoresis to our local academy, bringing totally new analytical capabilities to perform comprehensive protein studies, including posttranslational modifications of proteins and the ability to decode cell signaling networks. Now a day, we are involved in two main areas of research concerning protein-mediated cell signalling events.

1. PROTEOMIC PROFILING OF HOST-PATHOGEN INTERACTIONS

A. Effects of *Mycobacterium tuberculosis* Ser/Thr kinase PknG on the macrophage. PR_FCE_2009_1_2479, PIs: Rosario Durán, Carlos Batthyany, 2011-2012.
B. Characterization of the phosphoproteome and acetylome of phagosomes during the maturation process: effects of PKnG. PhD Thesis Project MSc Analía Lima Raimondo, Pro.In.Bio., UdelaR, 2011-2013.
C. Identification of proteins associated to *Pseudomonas aeruginosa* transition from a planktonic to surface-associated and multicellular states by proteomic approaches. PIs: R. Durán, A. Kierbel. (J. R Msc thesis project)

2. DESGN AND DEVELOPMENT OF SECOND GENERATION NITROALKENES FOR USE IN THE TREATMENT AND PREVENTION OF INFLAMMATION RELATED CONDITIONS:

A. Nitroalkene Tocopherol and Analogs for Use in the Treatment and Prevention of Atherosclerosis. CABBIO 2013-2015. PI: C. Batthyany.
B. Electrophilic/Nitrated-fatty acids mediated protein modifications:
C. Inhibition of PknG of *Mycobacterium tuberculosis* by Nitrated Fatty Acids. PIs: Rosario Durán & Carlos Batthyany.

Services

Our Unit received in January 2007 a MALDI TOF-TOF MS instrument (AB-SCIEX, Framingham, USA) and, in December 2009, completed the MS platform with the arrival of a nano-electrospray/ion trap LTQ Velos instrument (Thermo, USA). Both instruments together complement each other and expand the quality and type of mass analytical procedures we can offer to local and regional research groups.

Two main modes to get access to the Unit are available:

1. Routine Service
For routine analysis, users are welcome to access the UByPA as a "fee for service facility" supported by the Institut Pasteur de Montevideo. The facility offers this kind of service to researchers in the region, with priority given to users from the Institute and local academy. The analysis will be performed by members of our technical staff and will be done following standard protocols. The routine analysis includes analysis and interpretation of raw data based on routine practices only.

Routine analysis includes:

- 2-D gel electrophoresis
- Protein sample preparation for MS analysis: in-gel digestion, in-solution digestion, desalting
- Molecular mass determination for peptides and small proteins by MS
- Protein identification by MALDI-TOF/TOF MS (peptide mass fingerprinting, MS/MS ion search) and database search.

In 2013 we analyzed **1178 different samples** from **83 different groups: 55%** of the samples were from our **local academy**, **42%** were from the **regional academy** (Argentine, Brazil, Chile, Colombia, and Venezuela) and **3%** from **private industry**.

2. Non-Routine Service
Collaborative research projects, beyond routine services, are welcome. Members of the Unit are expected to significantly contribute to the conception, design of experiments and custom-design protocols, original ideas as well as data analysis and interpretation beyond routine practice.

Non routine analysis includes:

- Custom sample preparation
- Post-translational modification analysis
- 2-D gel electrophoresis based proteomics
- "shotgun" based proteomis
- Quantitation
- De novo peptide sequencing
- Gycomycs and glycoproteomics

During the last year (2013) we got involved in several collaborative research projects, mainly in the area of post-translational modification of proteins and shotgun proteomics (9 projects granted in 2013). In this case most of the samples analyzed were from the local academy with some contribution of Brasil and Argentina research groups.

Publications

1. Alvarez G, Aguirre-López B, Cabrera N, Marins EB, Tinoco L, **Batthyány CI**, de Gómez-Puyou MT, Puyou AG, Pérez-Montfort R, Cerecetto H, González M. 1,2,4-thiadiazol-5(4H)-ones: a new class of selective inhibitors of *Trypanosoma cruzi* triosephosphate isomerase. Study of the mechanism of inhibition. J Enzyme Inhib Med Chem. 28: 981-989 (2013) .
2. **Gil M**, Graña M, Schopfer FJ, Wagner T, Denicola A, Freeman BA, Alzari PM, **Batthyány C, Durán R**. Inhibition of *Mycobacterium tuberculosis* PknG by non-catalytic rubredoxin domain specific modification: reaction of an electrophilic nitro-fatty acid with the Fe-S center. Free Radic Biol Med. 65:150–161. (2013)

Grants

1. Development of a novel class of anti-atherogenic agents: electrophilic nitroalkenes-Vitamin E (α-tocopherol) analogs. 2013 - 2015 CABBIO Carlos Batthyany - Amount Granted USD 30.000
2. N Glycan fingerprint of exosomes in Chagas and Cancer diseases. Interdisciplinary projects IP Montevideo, 2013. Coordinator C. Baththyany Amount granted USD 20.000
3. "Exploring the role of mosquito's saliva in the transmission of Rift Valley fever" Actions Concertees Interpasteuriennes (ACIP) 2012-2014. Scientific coordinator: V. CHOUMET (Paris). Uruguayan Researchers: C. Batthyany; R. Durán. EUR 18.000
4. Doctoral Fellowship – Magdalena Gil – 2012 (2 years) – ANII
5. Doctoral fellowship-Jorge Rodriguez -2013(2 years) – ANII
6. Master Fellowship – Jessica Rosello – 2012 (2 years) – ANII

Other Main Grants (where scientist of the UByPA are participating as investigators):

"Functional evaluation of soy draught response genes involved in hydric stress tolerance." PI: S.Vidal, Facultad de Ciencias, UdelaR; CSIC, UdelR, 2012-2014

"Nitro-oxidative modifications of -Synuclein"PI: Dr. J. Souza; Depto. de Bioquímica, Facultad de Medicina, UdelaR. FCE 2011 ANII (2012-2014)

"Identification of Proteins involved in visual cortex plasticity". PI: F. Rossi, Facultad de Ciencias, UdelaR; FCE 2011 ANII. (2012-2014).

"***Trypanosome cruzi*** Leucine-Rich Proteins as virulence factors". PI: A. Parodi, Insitut Pasteur deMontevideo & Facultad de Ciencias, UdelaR; FCE 2011 ANII. (2012-2014)

Other Acitivities

TRAINING OF STUDENTS

1. Training of three PhD degree students:
   - Analía Lima. Pro.In.Bio. "Caracterización molecular del proceso de inhibición del fagosoma por una quinasa de *Mycobacterium tuberculosis*" Academic Directores: C. Batthyány, R. Durán, MN. Álvarez.
   - Magdalena Gil. PhD student PEDECIBA Química, "Regulación de la actividad quinasa de PknG de *M. tuberculosis* y su rol en las primeras etapas de la infección". Director: A. Denicola; Co-Director: R. Durán.
   - Jorge Rodriguez-Post-graduate student PEDECIBA Química. I+D de análogos de la vitamina E liberadores de óx ido nítrico como potenciales fármacos para prevención primaria de aterosclerosis. Director: V. López. Co-director. C. Batthyany

2. Training of two Master degree students:
   - Jessica Rossello PEDECIBA Biología. "Estudio de la adhesión y agregación de *Pseudomonas aeruginosa* en células epiteliales mediante aproximaciones proteómicas" Director: R. Durán Co-Director: A. Kierbel
   - Dr. Gonzalo Spera. Master student Pro.In.Bio. "Proteomica diferencial de líneas celulares de cáncer de mama metástasico HER2 negativo sensibles y resistentes a Docetaxel". Academic Directors: Dr. C. Batthyány, Dra. C. Touriño, Dra.L. Delgado.

3. Training of undergraduate students:
   - Bernardina Rivera, Facultad de Ciencias, Uruguay

4. Training stages at the Unit:
   - Lic. Pablo Yunes (PhD student, Departamento de Química Biológica-CIQUIBIC, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina) Análisis de proteínas purificadas a partir de veneno de Yarará Chica utilizando electroforesis bidimensional y técnicas de espectrometría de masa. July 2013.
   - Mariana Lingua (PhD student, Departamento de Química, Universidad Nacional de Córdoba); UNU- Biolac; Nov – Dic 2013
   - Lorena Pardo (MSc student, Microbiology Department, School of Medicine, UdelaR); Oct 2013 to day

5. Training of the Unit´s staff
   - Analyzing Shotgun Proteomic Data. Instituto Carlos Chagas, Fiocruz Paraná (Curitiba, Brasil). Coordinador: Dr. Paulo Costa Carvalho, 2 al 6 de Setiembre de 2013.
   - Bioinformática estrutural e análises do proteoma. Curso CABBIO, Instituto de Ciências Biológicas (Universidade Federal de Minas Gerais, Brasil). Coordinador: Dr. Vasco Ariston Carvalho de Azevedo, 15 al 26 de Abril de 2013.

6. Scientific Communications
   - 3er Encuentro Nacional de Ciencias Químicas, Uruguay, 4 al 6 de Noviembre de 2013 (4 posters)
   - V Congreso da BrMASS, Campinas, Brasil, 8 al 11 de Diciembre de 2013 (1 invited lecture)
   - VIII Meeting of the SFRBM - South American Group, Buenos Aires, Argentina, 14 al 17 de Octubre de 2013 (1poster)
   - 8as Jornadas de Bioquímica y Biología Molecular, Sociedad de Bioquímica y Biología Molecular, Uruguay, 12 y 13 de Setiembre de 2013 (6 posters)
   - *3rd International Congress on Analytical Proteomics, 2013, Brasil (1 invited lecture)*

Recombinant Proteins Unit Laboratory on Chronic Lymphocytic Leukemia

Head: Pablo Oppezzo, PhD

Members:

Cecilia Abreu, M.Sc. (Doctoral student-staff Research Assistant)

Agustín Correa, M.Sc. (Doctoral student-staff Research Assistant)

Florencia Palacios, PhD (Staff Research Assistant)

Claudia Ortega, PhD (Staff Research Assistant)

Pablo Morande, PhD (post-doctoral position)

Natalia Sotelo, PhD (post-doctoral position)

Daniel Prieto, M.Sc. (Staff Research Assistant)

Virginia Patiño, MD. (Doctoral student)

Research

Summary of Activity Report and Short Scientific Project

Our scientific proposal is aimed at consolidating the current lines of research. As a first general goal, we focus to understand how the immunologic microenvironment and subsequent cell interactions with the tumoral B-lymphocyte are involved in tumor progression of haematopoietic B-cell malignancies. In a second line, we wish to use this information to develop therapeutic and/or prognostic agents for the lymphoproliferative disorders. The development of these therapeutic tools focus to disrupt the crosstalk between malignant B cells and their microenvironment, whereas prognosis approach involves the characterization of new molecules isolated from tumoral proliferative subpopulations from B cell disorders.

Our work concentrates in the study of Chronic Lymphocytic Leukemia (CLL) as a biological model. This haematopoietic B-cell disease follows an extremely variable course and despite the fact that treatments often induce remissions, most patients relapse and CLL remains incurable. The dissection of the molecular basis of the interactions between cancer cells and their microenvironment is leading to the development of new treatment modalities which are aimed at manipulating the communication of tumor cells with their milieu. In this regard, CLL is an instructive example of how these relationships influence the natural history of a disease. Our work is framed by the haematology and tumoral immunology. It lies on the interface between biochemistry and the molecular and cell biology fields, which in combination with protein expression approaches constitute the core of our experimental designs.

Background

From its beginning in 2007, our group has had a double mission at the IP Montevideo: to develop original lines of research, and to set up a technological core facility for the expression of recombinant proteins. The group leader (P. Oppezzo) has background in the area of tumoral immunology and recombinant proteins production. Immuno-haematological B cell malignancies, adaptive immunity, as well as recombinant antibody production has been Oppezzo's main investigation area for the last 10 years.
The first impact work in CLL area was published in Leukemia Journal where we provided evidences that clarified the origins of the B-leukemic clone in CLL *(Oppezzo et al, Leukemia, 2002)*. Next, we went deep insight in the physiological mechanism of Somatic Hypermutation (SHM) and Class Switch Recombination (CSR) events studying these process in CLL B cells. At this time, Honjo group's described one of the most exciting molecules discovered in the last decades in B lymphocyte Immunology *(Maramatsu et al, JBC,1999)*. Activation-Induced Cytidine Deaminase, "AID" appeared as the enzyme responsible for the origins of SHM and CSR process and is triggered after immune microenvironment signalling mainly, in secondary lymphoid organs. Our work in the field linked CSR, SHM and AID expression in CLL disease. We demonstrated that in contrast to normal circulating B-lymphocytes, in some CLL cases, the leukemic cells express high levels of an active AID enzyme *(Oppezzo et al, Blood, 2003)*. Interestingly, we also observed that all these cases corresponded to progressive forms of this disease *(Oppezzo et al, Blood, 2005)*. These results and those from other groups, suggest that, over-expression of AID could play an important role in CLL disease progression.
In the context of therapeutics tools related with cancer, we also produced two chimeric anti-tumoral monoclonal antibodies (mAbs) constructed by fusion of the V_H and V_L fragments of the murine mAb 83D4. Both recombinant antibodies were found to bind breast carcinoma cells expressing the Tn antigen *(Oppezzo et al., 2000, Hybridoma)* and further demonstrated that the chimeric form IgG1 -83D4 efficiently inhibits the growth of human carcinomas in mouse xenograft models *(Hubert et al, Cancer Research, 2011)*. As the Tn antigen is widely expressed on tumor cell, this monoclonal antibody could provide a way to treat cancer based on the chi-83D4 *(Huber et al, European Patent, 2010)*.
Finally, in relation to the development of prognostic markers in CLL, we described that the expression ratio of Lipoprotein Lipase (LPL) and metalloprotease ADAM29 is an important additional marker for the prognosis of CLL *(Oppezzo et al, Blood, 2005)*. This data was confirmed by several groups working in CLL in the consecutive years and at present, the prognostic marker LPL is used as one of the strongest prognostic factor in a comparative analysis of RNA-based markers in CLL disease *(Kaderi et al., Haematologica, 2011)*.

Research Lines

The dissection of the molecular basis of CLL progression focusing in the interactions between leukemic B cells and their microenvironment is becoming one of our main scientific interests. In this regard two research lines have been pursued since the establishment of our group in 2007.

A. Role of microenvironment interactions in CLL progression.

At present, many questions remain unsolved concerning the role of the microenvironment interactions in the progression of tumoral diseases. We think that CLL represents an excellent model to study these relationships between the leukemic B lymphocyte and their milieu. A detailed characterization of proliferative tumoral subsets that exist in this leukemia may shed light on the association between lymphoid tumours progression and malignant transformation.

Representative publications:

1. **Palacios and Moreno et al.** High expression of AID and active class switch recombination might account for a more aggressive disease in unmutated CLL patients: Link with an activated microenvironment in CLL disease. **Blood, 2010**. Jun 3; 115 (22):4488-96. **IF=9.88.**
2. **Palacios F. et al**. Microenvironment interactions in Chronic Lymphocytic Leukemia: A delicate equilibrium linking the quiescent and the proliferative pool. Chronic Lymphocytic Leukemia. Book Open Access. **InTech 2011**. ID 647. p 1-8.
3. **Nannini P. et al**. CCR4 expression in a case of cutaneous Richter's transformation of chronic lymphocytic leukaemia (CLL) to diffuse large B cell lymphoma (DLBCL) and in CLL patients with no skin manifestations. **Eur J Haematol. 2011** Jul; 87 (1):806. **IF = 5.1**
4. **Moreno and Abreu et al.** Lipoprotein lipase expression in unmutated CLL patients is the consequence of a demethylation process induced by the microenvironment. **Leukemia, 2013,** Jul;87(1):80. **IF = 9.56**
5. **Palacios et al.** miRNA-22 is a key regulator molecule of CLL B-cells survival through PTEN/AKT pathway in the proliferative subset expressing AID. ***Manuscript in revision.***

B. Development of new prognostic and therapeutic tools in CLL

Despite the fact that the experience in this area is supported by some publications as has been mentioned before, this is a recent research line that we expect to reinforce. This research project is outlined by our double profile as a research/facility group. In this context, the production of recombinant antibodies as well as recombinant proteins constitute a very useful tools to evaluate different prognostic and/or therapeutic molecules in cancer. One of the goals of this line aim to develop new prognostic methods based on the differential expression of LPL protein in CLL B-cells. In this regard, we recently demonstrated the expression of LPL protein in CLL patients and we obtained funds to carry out the project entitled *"Red-iberoamericana de Leucemia Linfoide Crónica: hacia el desarrollo de nuevos marcadores pronósticos"* at the International call CYTED (Centro Iberoamericano de Tecnología y Desarrollo). In addition, new methodologies such as Ribosome display and Affitins production by high-throughput screening are being incorporated to our unit in order to attain the best technology to carry out this research line.

Representative publications:

1. **Abreu et al.** Methylation status regulates LPL expression in Chronic Lymphocytic Leukemia. **Leukemia & Lymphoma, April** 25, 2013. **IF=2.5**
2. **Correa A. and Oppezzo P.** Tuning different expression parameters to achieve soluble recombinant proteins in E. coli: Advantages of high-throughput screening. **Biotechnol J. 2011** Jun;6(6):715-3**0. IF=3.2**
3. **Correa et al.** Affitins as enzymatic glycosidases inhibitors. ***Manuscript in revision***

To accomplish the research lines mentioned above, it is mandatory to constitute a CLL network, that engages a continuous and coordinate work between our group (focused in the CLL biology) and a medical group (specialized in the management of this disease). To initiate this, in the last years our group has become a reference laboratory that performs the molecular analysis of the immunoglobulin V_H genes (IgV_H) in the leukemic clone. This method allows to separate CLL cases in two prognostic groups: Patients whose B cells express mutated V_H genes have a more indolent disease and longer overall survival those which patients express UM genes. Publications in this area has been achieved in collaboration with Pritsch group in the Biophysical Proteins Unit at IPmont *(Bianchi et al, Leuk Lymphoma, 2010).*

The establishment of this standard procedure as a routine laboratory practice allowed us to start a strong collaboration with the clinical hematologic group of Hospital Maciel in Montevideo, with the clinical hematologic group of Academy of Medicine in Buenos Aires and recently, with the hematologic group of the Haematology chair at Hospital de Clínicas in Montevideo. These collaborations resulted in the first regional CLL group as well as in the first regional cellular bank of CLL placed in IPMont.

The consolidation of this network was recently achieved after obtaining the funds supported by CYTED. Oppezzo's lab is the principal coordinator of this programme (2011-2014) devoted to join efforts from the principal experts in lymphoproliferative disorders in the Iberoamericana region and to consolidate the regional CLL groups. Presently, a number of successful events have been achieved including workshops and student training. In this context, the first international CLL meeting (http://www.ibamcll2013.com/) has been carried out in November 15th to 17th, 2013 in Punta del Este, receiving 285 participants.

Current situation and major achievements

Most significant results over the last three years (2010-2013):

- Median Impact factor of Recombinant Protein Unit (UPR) publications = 6.4
- Eleven peer-reviewed articles: P. Oppezzo is corresponding author in four of these: Blood, Leukemia, Leukemia & Lymphoma and Biotechnology Journal.
- P. Oppezzo is the editor of the book entitled "Chronic Lymphocytic Leukemia" published in 2011.
- Five grants obtained from national and international research agencies (160 Ku$s for our laboratory).

- Attendance to national and international scientific meetings, among which P. Oppezzo has lectured in four international meetings as invited speaker (Brazilian Hematology Congress, 2010; International Workshop in CLL, 2011, USA; VIth Young Investigators´ Meeting on CLL, 2012, Germany and IXth International Workshop of European CLL group, 2012, Germany)
- P. Oppezzo served as reviewer for a number of Journals (Blood, Leukemia, Haematologica, Int. Journal of Hemat., Biotech. Journal, etc) and research agencies (ANII, Uruguay, CONICET, Argentina; Italian Cancer Research Association (AIRC) and International Union Against Cancer (UICC).

Grants

1. Fondo Clemente Estable – Dra. Cecilia Abreu – "Estudios genómicos del perfil de metilación del ADN en una población tumoral leucémica sobre-expresando la enzima AID" – 2013-2014 – ANII
2. Fondo Clemente Estable – Dr. Pablo Oppezzo – "Implicancias de la expresión anómala de la enzima mutagénica AID en los procesos leucémicos: Desarrollo de un modelo tumoral" – 2013-2015 – ANII
3. Fondo María Viñas – Dr. Pablo Oppezzo – "Expresión de la Lipoproteína Lipasa en las células B de la Leucemia Linfoide Crónica (LLC): Hacia el desarrollo de un nuevo marcador pronóstico" – 2013-2015 – ANII
4. Redes Temáticas – Dr. Pablo Oppezzo – "Red-iberoamericana de Leucemia Linfoide Crónica: hacia el desarrollo de nuevos marcadores pronósticos" – 2011-2014 – CYTED.

Publications

1. Libisch G, Casás M, Chiribao ML, Moreno P, Cayota A, Osinaga E, **Oppezzo P**, Robello C. GALNT11 as a new molecular marker in Chronic Lymphocytic Leukemia. Gene. 2013 Sep 26. doi:pii: S0378-1119(13)01257-2. 10.1016/j.gene.2013.09.052.
2. **Oppezzo P,** Dighiero G. "Role of the B-cell receptor and the microenvironment in chronic lymphocytic leukemia". Blood Cancer J. 2013 Sep 20;3:e149. doi: 10.1038/bcj.2013.45.
3. **Abreu C, Moreno P, Palacios F,** Borge M, Morande P, Landoni A, Gabus R, Dighiero G, Giordano M, Gamberale R, Oppezzo P. Methylation status regulates lipoprotein lipase expression in chronic lymphocytic leukemia. Leuk&Lymphoma. 2013 Aug;54(8):1844-8. doi: 10.3109/10428194.2013.796057. Epub 2013 Jun 21.
4. **Correa A**, Trajtenberg F, Obal G, Pritsch O, Dighiero G, **Oppezzo P**, Buschiazzo A. Structure of a human IgA1 Fab fragment at 1.55 Å resolution: potential effect of the constant domains on antigen-affinity modulation. Acta Crystallogr D Biol Crystallogr. 2013 Mar;69:388-97. doi: 10.1107/S0907444912048664. Epub 2013 Feb 16.
5. Montamat-Sicotte D., **Palacios F,** Di Noia JM, **Oppezzo P.** Origins and consequences of AID expression in lymphoid neoplasms. Current Immunology Reviews, 2013, 9, 72-85

Other activities

TRAINING COURSES

1. International Course "Expression, Purification and Crystallization of Recombinant Proteins by High-throughput Methodologies", 18th to 27th February, 2013.

CONGRESS

1. First Ibero-American Meeting on Chronic Lymphocytic Leukaemia (IbAM-CLL), 15th to 17th November, 2013

Head: Alejandro Buschiazzo, PhD

Members:

Horacio Botti, MD/PhD (staff Research Scientist, until Nov 2013)

Sofía Horjales, MSc (PhD student)

Juan Imelio (Undergraduate Intern)

Nicole Larrieux (staff Technician)

Ariel Mechaly, PhD (Postodoctoral fellow)

Natalia Morero, PhD (Postdoctoral fellow)

Fabiana San Martin (Undergraduate Intern)

Felipe Trajtenberg, PhD (staff Research Scientist)

Research

As our main scientific interest, we are committed to understanding how signaling and subsequent cell regulation work at the molecular level, with particular emphasis to their link with microbial pathogenesis. To these ends we study different species, both pathogenic as well as non-pathogenic models, with a molecular structural perspective.

Among the bacterial models, we have been actively working with *Leptospira spp.* (prokaryotic Spirochetes), studying proteins from *L. interrogans* (one of the principal etiologic agents of leptospirosis) and *L. biflexa* (a saprophytic model, highly related to the pathogenic relatives). In 2012 we have started to work *in vivo*, establishing the experimental setup to culture *L. biflexa* focused on the Spirochetal motility machinery and its regulation. We are still engaged in also studying *Bacillus subtilis* (Firmicutes), a well known prokaryotic model of Gram+ bacteria, to answer questions of temperature sensing and downstream regulation.

On the other hand, we wish to better understand signaling mechanisms in unicellular eukaryotic protozoa of the Trypanosomatid family. Concentrating efforts in the study of Ser/Thr protein kinases of *Leishmania major*, one of the species causing leishmaniasis. Key proteins implicated in sialic acid metabolism, expressed by the other important American Trypanosomatid parasite, *Trypanosoma cruzi*, have also been matter of structural studies due to their involvement in pathogenesis mechanisms.

Apart from our own main lines of research, we carry on several projects as collaborators, contributing with our expertise in protein science and structural biology.

In terms of the scientific approach, we intend to understand protein function at the molecular level. This is why it is essential for us to explore a diverse set of organisms, with interest in eventually extending our studies to other clinically relevant bacterial species. Our methods lie on the interface between biology, chemistry and physics: protein crystallography in combination with biochemistry, biophysics and molecular biology constitute the core of our experimental approaches.

1. SIGNALING AND REGULATION IN PATHOGENIC MICROORGANISMS

Bacterial two-component systems (TCSs) and Ser/Thr protein kinases (MAPKs and CK1) in eukaryotes, constitute the main protein systems that we work on. The common theme is how cells use proteins to sense extra- and intra-cellular signals in order to regulate specific functions.

Signaling through TCSs in bacteria

To understand the molecular means by which bacteria transduce signals, adapting to a changing environment, during the last few years we have been using a non-pathogenic model (*Bacillus subtilis*) focusing our efforts in elucidating the molecular mechanisms of signaling and regulation of lipid synthesis in Gram+ bacteria. Our main contribution concerns the structural studies of the TCS DesK/DesR. DesK is a trans-membrane histidine kinase that, together with its cognate response regulator DesR, regulates the membrane's fluidity in response to cold shock in *B. subtilis*. Previous structural and biochemical work with the entire cytoplasmic region of DesK from *B. subtilis* (Albanesi et al., Proc Natl Acad Sci U S A. 2009, 106:16185-90; Trajtenberg et al., J Biol Chem 2010, 285:24892-903), allowed us to propose a mechanistic model that appears to be general for histidine kinase-mediated signal transduction. More recently, we have turned our attention to the response regulator DesR. The crystal structure of full-length DesR has been obtained, in its activated state. Several crystal forms of the receiver domain were also determined in the active and inactive configurations, revealing molecular details of the activation switch (Trajtenberg et al., submitted).
Since 2012, and with the aim of studying how TCS-mediated signaling regulates pathogenesis, we have launched a different line of research focused on *Leptospira spp*., spirochetal bacteria that cause leptospirosis. This disease is the most widespread zoonosis in the world, reemerging as a major health problem. In Uruguay its prevalence as a veterinary issue is very significant, identified as the second most serious problem after brucellosis (Plan Nacional de Investigacion en Salud Animal http://www.fvet.edu.uy/planisa). A collaborative partnership has been established with Albert Ko's lab (Yale Univ) and Mathieu Picardeau's (Institut Pasteur). We have progressed in the understanding of heme sensing and metabolic regulation, which at difference with other Spirochetes, is critical for *Leptospira* survival. Controlled via a TCS, we have been able to solve the structure of the receiver domain of the response regulator HemR (Morero et al., submitted), understanding its role as an transcriptional activator and repressor of key genes in a heme regulon. Finally, we are studying proteins that constitute the leptospiral motility apparatus, which in spirochetes displays important differences compared to similar Gram-negative bacteria.

Signaling through MAPKs in Trypanosomatid parasites
With regards to eukaryotic MAPKs, we are studying their mode of action and regulation in *Leishmania major*, a trypanosomatid protozoa that causes human leishmaniasis. Trypanosomatids cause severe human diseases such as sleeping sickness, Chagas disease, and leishmaniasis. Together these diseases provoke millions of deaths in endemic areas around the world and represent a major burden for the socio-economic development of affected countries. No vaccine exists for any of these diseases. Current treatment options have serious side-effects and are increasingly threatened by the spread of drug-resis-

tant parasite strains. Apart from the previous progress im learning about specific structure-function features, from the first reported crystal structure of a Trypanosomatid MAPK, LmaMPK10 (Horjales et al., Structure 2012), we have also performed *in silico* work to propose a list of candidate inhibitor molecules (Horjales et al., unpublished data). The proposed role of LmaMPK10 in parasite differentiation shall thus be tested, if some of these small molecules show kinase inhibition potency *in vivo*.
A procedure to produce and purify recombinant LmaCK1 (isoform 2 of the casein kinase1) as a soluble homogeneous sample, will allow us to progress into crystallogenesis screening assays. LmaCK1 has been shown by our collaborators to be important in *Leishmania* viability and virulence, and initial inhibitory compounds have already been identified (Rachidi N. et al., Antimicrob Agents Chemother 2014, 58:1501-15). Our structural work shall thus be valuable to advance in the lead optimization process within a rational perspective.
Finally, we started a new collaboration with the Helenic Pasteur Institute (Athens, Greece), with the team led by Dr Despina Smirlis, together with Gerald Spaeth (Inst Pasteur, Paris, France) and Milena Soares (Fiocruz, Salvador, Brazil). The focus has been set into structure/function studies of a new group of *Leishmania* protein kinases, dual specifity tyrosine-regulated kinases (DYRKs), given that a *Leishmania major* DIRK1 knock-out is not viable. Ultimately a structure-based drug design strategy is envisaged.

2. COLLABORATIVE WORK

i- We continue an active collaboration with Dr Otto Pritsch (Unit of Protein Biophysics, IP Montevideo) and his team, in the area of structural virology. Our group has concentrated in crystallographic studies of the capsid protein p24 from the bovine leukemia virus (BLV), a deltaretrovirus. We report the first structures of a full-length retroviral capsid protein in its native (non-engineered) configuration (Obal, Trajtenberg, et al, manuscript in preparation).
This collaborative project has recently received extended support from CNRS (Centre Nationale de la Recherche Scientifique, France), through an Associated International Laboratory program (LIA). This program, led by Drs Pritsch (Uruguay) and Felix Rey (France), links our group, as well as Jean Lepault's (CNRS, Gif-sur-Yvette, France), into a structural virology network focused on BLV.
ii- We continue an active collaboration with Dr Hugo Gramajo (Instituto de Biologia Molecular y Celular IBR, Rosario, Argentina) and his team, aimed at elucidating the crystal structure of two transcription factors from *Mycobacterium tuberculosis*, MabR and FasR, which are important regulators of the lipid metabolism in this pathogenic bacterium. We have obtained crystals from both proteins, needing further optimization to be able to determine their 3D structures.
iii- A collaboration with Drs Gustavo Schujman and Diego de Mendoza (IBR- Rosario, Argentina) resulted in a recent publication (Trajtenberg et al., 2014, FEBS J, in press), disclosing the structural bases of resistance to the antibiotic cerulenin in Gram+ bacteria, based on crystal structures of one of its target enzymes, the fatty acid condensing FabF from *Bacillus subtilis*.
iv- A collaboration with Drs Lucia Piacenza and Rafael Radi (Dept Bioquimica, UdelaR- Montevideo, Uruguay) resulted in a recent publication (Martinez et al., 2014, J Biol Chem, in press), contributing with structural data on the differential inactivation of the mitochondrial Fe-superoxide dismutase from *Trypanosoma cruzi*, by the action of peroxynitrite.

Technological Facility

The Protein Crystallography Facility (PXF) is up and running, with a sustained number of users and trainees, from Argentina and Uruguay.
The web page is kept up to date, and for the last 5 years now, our facility has become fully operational to receive and process all the users' requests (mainly from IPMontevideo, from the Uruguayan community and several users from Argentina).
The web page informs on the detailed specifics of the available equipment and ways of using the platform.

Experimental approaches currently available for users

1. Protein crystallization screenings (manual and robotic [Honeybee963® 96-well robot)
2. Follow-up and optimization of initial crystallization hits (manually and robot-assisted with an Alchemist® instrument)
3. X ray Diffraction – Testing & Crystal Characterization
4. X ray Diffraction – single crystal data collection
5. Crystal Structure Determination & Refinement

Progress 2013

1. Ten (10) structures determined, two released in 2013 in the Protein Data Bank: 2YOC, 4DVH; and 8 on hold for publication: 4LS5, 4LS6, 4LS7, 4LS8, 4LDZ, 4LE0, 4LE1 and 4LE2.
2. The Varimax-HF optics module of our X ray system was upgraded, allowing from now on to work under vacuum instead of purging it with costly helium gas.
3. Two trainees from abroad were hosted to use the facility: PhD student Nicolás González Bardeci (Universidad de Buenos Aires – Argentina) (one month), Dr Clarisa Alvarez (Centro de Estudios Fotosintéticos y Bioquímicos, Rosario – Argentina) (three months).

Publications

1. Correa A, **Trajtenberg F,** Obal G, Pritsch O, Dighiero G, Oppezzo P, **Buschiazzo A.** Structure of a human IgA1 Fab fragment at 1.55 Å resolution: potential effect of the constant domains on antigen-affinity modulation. Acta Crystallogr D Biol Crystallogr. 2013 Mar;69(Pt 3):388-97.
2. Albanesi D, Reh G, Guerin ME, Schaeffer F, Debarbouille M, **Buschiazzo A**, Schujman GE, de Mendoza D, Alzari PM. Structural basis for feed-forward transcriptional regulation of membrane lipid homeostasis in Staphylococcus aureus. PLoS Pathog. 2013 Jan;9(1):e1003108.

Grants

3. "Cell signaling in bacterial pathogenesis: iron metabolism regulation in Leptospira as a working model" – A Buschiazzo, Principal Investigator – Institut Pasteur, Paris, Projet Transversal de Recherche PTR – 2011 (2 years) – Extended until 2014 after favorable evaluation.
4. "Consolidacion del Centro de Biologia Estructural del Mercosur CeBEM" – A Buschiazzo, R Radi (heads of the 2 Uruguayan CeBEM nodes) – Direccion de Innovación, Ciencia y Tecnología para el Desarrollo (DICYT), Miniterio de Educacion y Cultura (MEC), Gobierno Nacional, Uruguay – 2012 (1 year)
5. "Structure/function studies of MAP kinases with key relevance in the biology of *Leishmania spp.*" – A Buschiazzo (IP Montevideo) & G Spaeth (IP, Paris) – ECOS-Sud, Researchers/students exchange program between France and Uruguay – 2012 (3 years)
6. "Evaluation of Leishmania DYRK family of kinases as molecular targets for the development of anti-leishmanial drugs" – A Buschiazzo, collaborative partner (PI: Despina Smirlis) – Actions Concertees Interpasteuriennes (ACIP) – 2013 (2 years)

Other activities

TRAINING COURSES

1. As a joint initiative between CeBEM and CCP4 (Collaborative Computational Project N°4, Science & Technology Facilities Council, UK), the international Workshop Joint CeBEM-CCP4 initiative "Macromolecular Crystallography School - From data processing to structure refinement and beyond", held at the IP Montevideo from April 9th to 17th, 2013. Apart from IP Montevideo, CCP4 and CeBEM, complementary supporting funds have been obtained from the Reseau International des Instituts Pasteur (RIIP) and the International Union of Crystallography (IUCr).

FURTHER ACTIVITIES

1. Continued contribution of our group to the Center for Structural Biology of the Mercosur (Centro de Biologia Estructural del Mercosur, CeBEM). www.cebem.org.ar A grant from the Ministerio de Educacion y Cultura (Uruguay) was obtained, allowing us to contribute for the first time in 4 years to the regional funds sustaining the training and exchange activities.
2. The head of the Unit has organized and/or attended to several national and international meetings and scientific activities, including in 2013: First Latin American meeting of Crystallography (AB member of the international scientific committee, and organizer of the Biological Macromolecules symposium; Nov 2013 – Cordoba, Argentina); 3rd National Meeting of Chemistry ENAQUI 3.0 (AB invited speaker; Nov 2013 – Montevideo, Uruguay); 4th Latin American Protein Society LAPS meeting & XII Congress of the Panamerican Association for Biochemistry and Molecular Bioology PABMB (AB invited speaker; Nov 2013 – Puerto Varas, Chile); 2nd meeting of the uruguayan Biophysics society "+Biofisica" (AB co-chair of a macromlecular structure and function symposium, Montevideo, Nov 2013); among others.
3. A Buschiazzo - Reviewer of >5 peer-reviewed journals, including J Biol Chem, J Synchr Rad, Biochemistry, J Med Chem, PLoS ONE, PNAS, Protein Sci, among others. Acted as reviewer of scientific projects for FAPESP (CEPID program, Brazil), FONCYT (Argentina agency).
4. A Buschiazzo – Associate Editor of the journal PLoS Neglected Tropical Diseases.
5. Integration of three new members to our group: Ariel Mechaly (Postdoctoral scholarship, ANII); Fabiana San Martín (undergraduate intern scholarship, ANII); Juan Imelio (undergraduate intern scholarship, ANII).
6. Our Unit has hosted a total of four (4) traineeships for students and postdoctoral fellows in 2013: Oct '12 - Mar '13, Fabiana San Martín (School of Sciences, Universidad de la Republica – Uruguay); Oct '12 - Mar '13, Juan Andres Imelio (School of Sciences, Universidad de la Republica – Uruguay); Mar'13 – Apr'13, Nicolás González Bardeci (Universidad de Buenos Aires – Argentina); Jun'13 – Sep'13, Clarisa Alvarez (Centro de Estudios Fotosintéticos y Bioquímicos, Rosario – Argentina).

Bioinformatics Unit

Head: Hugo Naya, PhD

Members:

- Martín Graña (PhD, AI)
- Natalia Rego (TA, MSc student in Zoology)
- Lucía Spangenberg (PhD student in biology)
- María Inés Fariello (PhD student in biology)
- Tamara Fernandez (MSc in biology, Electrical Engineering student)
- Sebastián Valenzuela (MSc student in bioinformatics)
- Gregorio Iraola (MSc student in bioinformatics)
- Daniela Megrian (undergrad student in biochemistry)
- Gabriel Martinez (undergrad student in biology)

Research

In the past 20 years, the development of new technologies has led to amazing discoveries in biology. In particular, nano-technologies, automatization and computer science allowed a series of High-Throughput analysis in molecular and cell biology that completely changed the existent paradigm. However, these new instruments also changed unexpectedly the landscape of research conception. The promise of hypothesis-free data has conducted, in several cases, to careless experimental design that precluded full exploitation of results, increasing the experimental turnover and the storage of waste in data-repositories. Technology evolves extremely fast, but analytical methods aren't automatized enough yet, leading to the well-known effect of "Next-Generation gap". The gap is in expansion now (with the 2nd generation sequencing) and will be enormous with 3rd generation technologies. In fact, analysis teams simply can't analyze exhaustively each dataset before a new dataset arrives, just scratching the surface and sending to the warehouse (or even garbage) tons of data.

In this context, any methodological effort towards better usage of data should be viewed as benefiting the scientific community. Our research, although diverse, is united by this underlying goal and combines the methodological strengths of bioinformatics, statistics, evolutionary genomics and quantitative genetics.

We recently proposed a method that identifies associations between amino acid changes in potentially significant sites in an alignment (taking into account several amino acid properties) with phenotypic data (Spangenberg et al., 2011), through the phylogenetic mixed model. The latter accounts for the dependency of the observations (organisms). It is known from previous studies that the pathogenic aspect of many organisms may be associated with a single or just few changes in amino acids which have a strong structural and/or functional impact on the protein. Discovering these sites is a big step towards understanding pathogenicity. Our method was able to discover such sites in proteins (RpoS) associated to the pathogenic character of a group of bacteria, highlighting several sites with significant differences in biological relevant regions. In addition, we developed a freely available R package named "bcool" (http://cran.r-project.org/web/packages/bcool/index.html). In the near future, we think to apply this strategy to search for differences in biofilm related genes.
We also assessed the question of how bacteria cause pathogenicity in humans from other perspective. Our motivation was try to give integrative information about general genome-coded signatures that explains pathogenicity for all bacterial pathogens, and not restricted to particular taxa. In this case, we explained pathogenicity based on the hypothesis that it is caused by the presence of a reduced set of virulence-related genes. To do this, we explored the presence/absence patterns of virulence genes in all available genomes of pathogenic and non-pathogenic strains. Then, this information was used to build a Support Vector Machine model that, once trained, is capable of predicting if a new sequenced genome is a human pathogen or not. This model has an average accuracy of 95%, and to the best of our knowledge, is the statistical model with this purpose that achieves the highest accuracy reported so far. Moreover, our method can classify bacterial genomes independently of their taxonomic context, in contrast with other similar approaches that only take into account a certain part of bacterial diversity, being useful only to classify specific taxa. Our statistical learning approach is grounded on the biological meaning of the selected genes and supporting the fact that bacterial pathogenicity can be explained by the presence or absence of a set of specific genes that code for virulence determinants. Based on this, we developed "BacFier", a freely available software that may be useful for practical purposes. Beyond the implementation of our model in a program, capable to accurately classify bacteria in human pathogens or non-pathogens, we determined and discussed the biological significance of the core set of genes that mostly explains the pathogenic phenotype in bacteria. Finally, we have shown which functional categories of virulence genes (i.e: toxins, motility proteins, etc.) were likely pathogenicity signatures within each taxonomic division (i.e: Actinobacteria, Gammaproteobacteria, Firmicutes, etc.), which seems to be a completely new kind of information and could lead to important evolutionary conclusions. Nowadays, we are working in enhancing model sensitivity and exploring the possibility of developing a multiclass classifier, that could predict pathogenicity in other hosts besides human, like cattle, plant or fish.

As part of our general interest in bacterial pathogenicity, we are involved in a more specific problem; the study of biofilms formation determinants in *Leptospira*. This genus includes animal and re-emerging human pathogens, as well as non-pathogenic strains. Despite its importance for human health and animal production, genetic features that determine pathogenic phenotypes in *Leptospira* proved to be elusive. Recently, biofilms formation capability has been suggested as a key factor in pathogenesis of leptospirosis but, as mentioned above, there is a lack of knowledge regarding its genetic basis. In this ground, we are implementing comparative genomics analysis to find orthologous genes with functions associated to biofilms formation. Moreover, in the near future we plan to perform transcriptome analysis that could give data regarding expression patterns of genes involved in biofilms formation, providing with a new kind of information that could be useful to understand the pathogenesis mechanisms of these bacteria.

Services

1. NGS and microarrays data analysis.
2. Sequence alignment and phylogenetic inference software.
3. Sequence analysis software.
4. 3D molecular modeling software.
5. Database hosting and querying.
6. Tools for complex systems analysis.
7. Basic biostatistics and use of specific software advice.
8. Software development.

Publications

1. Garcia-Silva MR, das Neves RF, Cabrera-Cabrera F, Sanguinetti J, Medeiros LC, Robello C., **Naya H, Fernandez-Calero T**, Souto-Padron T, de Souza W, Cayota A. Extracellular vesicles shed by Trypanosoma cruzi are linked to small RNA pathways, life cycle regulation, and susceptibility to infection of mammalian cells. Parasitol Res. 2014;113:285-304.
2. Gil M, **Graña M**, Schopfer FJ, Wagner T, Denicola A, Freeman BA, Alzari PM, Batthyány C, Durán R. Inhibition of Mycobacterium tuberculosis PknG by non-catalytic rubredoxin domain specific modification: reaction of an electrophilic nitro-fatty acid with the Fe-S center. Free Radic Biol Med. 2013 Jun 19. pii: S0891-5849(13)00302-X. doi: 10.1016/j.freeradbiomed.2013.06.021.
3. **Iraola G**, Pérez R, **Naya H**, Paolicchi F, Harris D, Lawley TD, **Rego N**, Hernández M, Calleros L, Carretto L, Velilla A, Morsella C, Méndez A, Gioffre A. Complete Genome Sequence of Campylobacter fetus subsp. venerealis Biovar Intermedius, Isolated from the Prepuce of a Bull. Genome Announc. 2013 Aug 1;1(4). doi:pii: e00526-13. 10.1128/genomeA.00526-13.
4. Naya DE, **Spangenberg L, Naya H**, Bozinovic F. 2013 Thermal conductance and basal metabolic rate are part of a coordinated system for heat transfer regulation. Proc Biol Sci. 2013 Jul 31;280(1767):20131629.. http://dx.doi.org/10.1098/rspb.2013.1629
5. **Spangenberg L**, Correa A, Dallagiovanna B, **Naya H**. Role of Alternative Polyadenylation during Adipogenic Differentiation: An In Silico Approach. PLoS One. 2013 Oct 15;8(10):e75578. doi: 10.1371/journal.pone.0075578.
6. **Spangenberg L,** Shigunov P, Abud AP, Cofré AR, Stimamiglio MA, Kuligovski C, Zych J, Schittini AV, Costa AD, Rebelatto CK, Brofman PR, Goldenberg S, Correa A, **Naya H**, Dallagiovanna B. Polysome profiling shows extensive posttranscriptional regulation during human adipocyte stem cell differentiation into adipocytes. Stem Cell Res. 2013 Jun 10;11(2):902-912. doi: 10.1016/j.scr.2013.06.002. [Epub ahead of print]

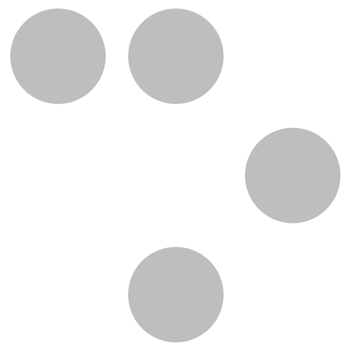
Grants

1. "Investigação dos Mecanismos Genéticos e Moleculares em Biofilmes de Leptospira". Funded by CAPES – Brazil 2012/2015. A Schnadelbach/P Ristow. Special Visiting Professor H Naya. Granted R$ 100000.
2. "Análisis transcripcional en Leptospiras formadoras de biofilms". Funded by ANII 2013/2015. H Naya. Granted U$S 20000.

Other activities

We are currently involved in several teaching activities, mainly on bioinformatics-related topics. The recently created MSc in Bioinformatics is currently highly demanding, courses design and impartment being in charge of the Faculty of Sciences, School of Engineering, and our group at Pasteur. We also have punctual participations in several PEDECIBA courses, including topics in bioinformatics and quantitative genetics.

Human resources are clearly needed in this somewhat new research domain; this calls for our effort in such teaching activities, as well as for maximizing the number of graduate and undergraduate students in our lab (eight persons at the moment).



Head: Carlos Robello, PhD

Members:

Adriana Parodi-Talice (PhD - Senior, Facultad de Ciencias)

Dolores Piñeyro (PhD - Postdoc, Facultad de Medicina)

Luisa Berná (PhD - Postdoc, INNOVA)

Ma. Laura Chiribao (PhD Student, Facultad de Medicina)

Paula Faral (MSc Student)

Gabriela Libisch (Technological activities)

Gonzalo Greif (PhD Student)

Cecilia Portela (Tecnician, Facultad de Ciencias)

Florencia Díaz (Intern)

Research

The Unit of Molecular Biology research is focused on human and animal pathogens, in particular the protozoan parasites *T. cruzi*, *T. vivax* and Leishmania, and the prokariote Mycobacterium, with emphasis in genomics and functional genomics of those pathogens, and host-pathogen interactions.

A. **Functional Genomics of Host-Parasite Interaction**

Trypanosoma cruzi, the causative agent of Chagas disease, has the peculiarity, when compared with other intracellular parasites, that it is able to invade almost any type of cell. This property makes Chagas a complex parasitic disease in terms of profilaxis and therapeutics. The identification of key host cellular factors that play a role in the *T. cruzi* invasion, are important for understanding of disease pathogenesis. In Chagas disease most of the focus was on the response of macrophages and cardiomyocytes, since they are responsible for host defenses and cardiac lesions respectively. We studied the early response to infection of *T. cruzi* in human epithelial cells, which constitute the first barrier for establishment of infection. These studies identified up to 1700 significantly altered genes regulated by the immediate infection. The global analysis indicates that cells are literally reprogrammed by *T. cruzi*, which affects cellular stress responses (neutrophil chemotaxis, DNA damage response), a great number of transcription factors (including the majority of NF B family members) and host metabolism (cholesterol, fatty acids and phospholipids). These results raise the possibility that early host cell reprogramming is exploited by the parasite to establishment of the initial infection and posterior systemic dissemination.

B. **Benznidazole Biotransformation and Multiple Targets in *Trypanosoma cruzi* Revealed by Metabolomics**

The first line treatment for Chagas disease involves administration of benznidazole (Bzn). Bzn is a 2-nitroimidazole pro-drug which requires nitroreduction to become active, although its mode of action is not fully understood. By using a non-targeted MS-based metabolomics approach we studied the metabolic response of *T. cruzi* to Bzn. Parasites treated with Bzn were minimally altered compared to untreated trypanosomes, although the redox active thiols trypanothione, homotrypanothione and cysteine were significantly diminished in abundance post-treatment. In addition, multiple Bzn-derived metabolites were detected after treatment. These metabolites included reduction products, fragments and covalent adducts of reduced Bzn linked to each of the major low molecular weight thiols: trypanothione, glutathione, -glutamylcysteine, glutathionylspermidine, cysteine and ovothiol A. Bzn products known to be generated in vitro by the unusual trypanosomal nitroreductase, TcNTRI, were found within the parasites, but low molecular weight adducts of glyoxal, a proposed toxic end-product of NTRI Bzn metabolism, were not detected. Our data is indicative of a major role of the thiol binding capacity of Bzn reduction products in the mechanism of Bzn toxicity against *T. cruzi*.

C. **Tuberculosis: Genomics and molecular typing**

The incidence of tuberculosis (TB) is increasing in high-risk populations in Uruguay, possibly owing to emerging resistance. Mycobacterial interspersed repetitive units (MIRU) genotyping and katG sequence analysis of isoniazid (INH) resistance-associated mutations were performed in 45 INH-resistant *Mycobacterium tuberculosis* isolates in Uruguayan patients. The genotype distribution among INH-resistant isolates shares features of that of neighbouring countries, with a predominance of Latin American and Mediterranean, T and Haarlem genotypes, although the S genotype was particularly frequent among our isolates. Forty-four per cent of INH-resistant strains harboured the S315T mutation in katG; we found novel katG mutations (W321X, G269T, P232R and G221Wfs1) that could explain INH resistance. More recently, we reported an unusual tuberculosis (TB) outbreak centered on a professional basketball team in Montevideo. The strain, named MtURU-001, was fully sequenced: MtURU-001 has a circular chromosome of 4,378,296 bp, with an average G+C content of 65%, including 4,314 protein-encoding genes, 1 rRNA operon, and 45 tRNA genes. In comparison with M. tuberculosis H37Rv, 4,096 orthologous groups were defined with OrthoMCL and 1,016 polymorphisms were identified using the Burrows-Wheeler Aligner (BWA) and GATK. A subset of 849 polymorphisms (802 single-nucleotide polymorphisms [SNPs] and 47 indels) were inside coding sequences, and 480 affect protein sequences, especially 24 that introduced stop codons disrupting several hypothetical proteins, one transcriptional regulator, 2 genes for the haloacid dehalogenase (HAD) superfamily, and 3 involved in lipid metabolism. Further comparative genomics across this genome may provide genotype-phenotype associations that might explain the rapid progression of this unusual outbreak

D. **Trypanosoma vivax transcriptome**

Trypanosoma vivax is the earliest branching African trypanosome. This crucial phylogenetic position makes *T. vivax* a fascinating model to tackle fundamental questions concerning the origin and evolution of several features that characterize African trypanosomes, such as the Variant Surface Glycoproteins (VSGs) upon which antibody clearing and antigenic variation are based. Other features like gene content and trans-splicing patterns are worth analyzing in this species for comparative purposes. We present a RNA-seq analysis of the bloodstream stage of *T. vivax* from data obtained using two complementary sequencing technologies (454 Titanium and Illumina). Assembly of 454 reads yielded 13385 contigs corresponding to proteins coding genes (7800 of which

were identified). These sequences, their annotation and other features are available through an online database presented herein. Among these sequences, about 1000 were found to be species specific and 50 exclusive of the *T. vivax* strain analyzed here. Expression patterns and levels were determined for VSGs and the remaining genes. Interestingly, VSG expression level, although being high, is considerably lower than in Trypanosoma brucei. Indeed, the comparison of surface protein composition between both African trypanosomes (as inferred from RNA-seq data), shows that they are substantially different, being VSG absolutely predominant in *T. brucei*, while in *T. vivax* it represents only about 55%. This raises the question concerning the protective role of VSGs in T. vivax, hence their ancestral role in immune evasion.It was also found that around 600 genes have their unique (or main) trans-splice site very close (sometimes immediately before) the start codon. Gene Ontology analysis shows that this group is enriched in proteins related to the translation machinery (e.g. ribosomal proteins, elongation factors). This is the first RNA-seq data study in trypanosomes outside the model species *T. brucei*, hence it provides the possibility to conduct comparisons that allow drawing evolutionary and functional inferences. This analysis also provides several insights on the expression patterns and levels of protein coding sequences (such as VSG gene expression), trans-splicing, codon patterns and regulatory mechanisms. An online *T. vivax* RNA-seq database described herein could be a useful tool for parasitologists working with trypanosomes.

Core Facilities/Services

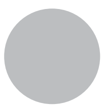
1. DNA sequencing (Sanger methodology)
2. Real Time PCR
3. Microarrays
4. Bioanalyzer
5. Illumina Genome Analyzer
6. Illumina MiSeq

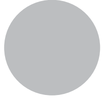
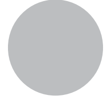
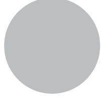
Publications

1. **Faral-Tello P**, Liang M, Mahler G, Wipf P, **Robello C**. Imidazolium compounds are active against all stages of Trypanosoma cruzi. Int J Antimicrob Agents. 2013 Nov 20. pii: S0924-8579(13)00376-2.
2. Garcia-Silva MR, das Neves RF, Cabrera-Cabrera F, Sanguinetti J, Medeiros LC, **Robello C**, Naya H, Fernandez-Calero T, Souto-Padron T, de Souza W, Cayota A. Extracellular vesicles shed by Trypanosoma cruzi are linked to small RNA pathways, life cycle regulation, and susceptibility to infection of mammalian cells. Parasitol Res. 2014 Jan;113(1):285-304.
3. Coitinho C, **Greif G, Robello C**, Laserra P, Willery E, Supply P. Rapidly progressing tuberculosis outbreak in a very low risk group. Eur Respir J. 2013 Oct 10. [Epub ahead of print]
4. **Libisch G**, Casás M, **Chiribao ML**, Moreno P, Cayota A, Osinaga E, Oppezzo P, **Robello C**. GALNT11 as a new molecular marker in Chronic Lymphocytic Leukemia. Gene. 2013 Sep 26. doi:pii: S0378-1119(13)01257-2. 10.1016/j.gene.2013.09.052.
5. Arias DG, Marquez VE, **Chiribao ML**, Gadelha FR, **Robello C**, Iglesias AA, Guerrero SA. Redox metabolism in Trypanosoma cruzi: functional characterization of tryparedoxins revisited. Free Radic Biol Med. 2013 Oct;63:65-77.
6. **Greif G**, Ponce de Leon M, Lamolle G, Rodriguez M, **Piñeyro D**, Tavares-Marques LM, Reyna-Bello A, **Robello C**, Alvarez-Valin F. Transcriptome analysis of the bloodstream stage from the parasite Trypanosoma vivax. BMC Genomics. 2013 Mar 5;14:149.
7. Michelini FM, **Zorrilla P, Robello C**, Alché LE. Immunomodulatory activity of an anti-HSV-1 synthetic stigmastane analog. Bioorg Med Chem. 2013 Jan 15;21(2):560-8.
8. Narancio R., **Zorrilla P., Robello C.**, Gonzalez M., Vilaró F., Pritsch C., Dalla Rizza M. Insights on gene expression response of a characterized resistant genotype of Solanum commersonii Dun.against Ralstonia solanacearum. Eur J Plant Pathol DOI 10.1007/s10658-013-0210-y

Grants

1. Fondo María Viñas – Identificadores de factores del hospedero necesarios para la invasión de *Trypanosoma cruzi* – Paula Faral – (2 years - 2013-2015) – ANII
2. Proyecto Transversal – Chagas disease: secreted microvesicles as predictors of persistence and pathogenicity – Carlos Robello – (1 year - 2013-2014) – IP Montevideo
3. Fondo Clemente Estable – Proteínas con motivos repetidos ricos en leucina de *Trypanosoma cruzi* y su rol en la virulencia: un abordaje bioinformático, funcional y proteómico" – Adriana Parodi – ANII (2 years – 2013-2015)



Cell Biology Unit

Head: Mariela Bollati, PhD

Members:

Soledad Astrada, MSc (PhD student, Staff TA)

María Belén Harreguy (Undergraduate Intern)

Giuliana Mastropietro (Undergraduate Intern)

Romina Pagotto, PhD (Postdoctoral fellow)

Karen Perelmuter, MSc (Staff TA)

Valentina Porro, MD (PhD student, Staff TA)

Inés Tiscornia, MSc (Staff TA)

Sabina Victoria, MSc (Staff TA)

Research

CELL CULTURE TECHNOLOGY:
During the last years, our group has generated a variety of reporter cell lines (NF- kB, type I IFN, redox biosensors, among others). These stable cell lines are being widely used to search for substances that interfere with the type I IFN signaling pathways (Burgi et al, 2012), for the improvement of metabolism / productivity of cells with biotechnological interest (redox biosensors: Perelmuter et al, manuscript under preparation), or for *in vitro* models of inflammation (NF- kB, Tiscornia et al, 2012).

ENVIRONMENTAL TOXICOLOGY:
A wide variety of anthropogenic substances in the environment, known as endocrine disruptors (EDs), are able to alter the homeostasis of the endocrine system of organisms. Its growing use and its ability to bioaccumulate have encouraged public entities to promote the control of their utilization, generating the need of flexible and efficient tools for evaluating their effects. In the field of endocrine disruption, the working hypothesis is that the increase of certain reproductive disorders (testicular and breast cancers, decreased fertility, hypospadias and reduced sperm count) is caused, at least in part, by an increased exposure to substances classified as EDs that are present in the environment. These pathologies associated with the exposure to EDs, could be the result of developmental defects of the reproductive tract and/or germ cells, produced during critical organizational periods with high sensitivity to the actions of the EDs.

In this context, we aim to design and develop *in vitro* and *in vivo* models for toxicological studies of EDs. For the *in vitro* approach, we propose to obtain a dual reporter cell line, in order to assess in a single assay the estrogenic or androgenic activity of a putative ED. For the *in vivo* studies, we selected Oct4 -GFP transgenic mouse. We have studied the GFP expression pattern in testis cells collected from animals at 3, 5, 7, 10, 14 days old and at adult stage and we defined the appropriate parameters for studying the EDs in germ cell development. By using this approach, we have demonstrated that the Oct4 -GFP transgenic mouse is sensible to the action of a known EDs, ethinyl estradiol (manuscript under preparation). At the moment we are studying the effects of perinatal exposure to different xenoestrogens on the development of gametes population and their potential causal relationship to impaired fertility. To achieve the above mentioned studies, we are actively collaborating with Horacio Rodríguez, PhD (Laboratorio Endocrinología y Tumores Hormonodependientes, UNL, Santa Fe, Argentina).

For this project we have the financial support from ANII (PR_ FMV 2_ 2011 1 6046), one post-doctoral fellow (R. Pagotto), one PhD student (V. Porro) and one undergraduate fellow (MB. Harreguy).

All together, the knowledge derived from this project will help to clarify the role of endocrine disruption on the development of reproductive alterations, to implement toxicological screening test and to design possible technology-health strategies in the area of environmental toxicology.

COLLABORATIVE PROJECT:
Since 2011 we are collaborating with Maribel G. Vallespi, PhD, from the Pharmaceuticals Division, Center for Genetic Engineering and Biotechnology (CIGB), Habana, Cuba in the project entitled “CIGB -552: novel peptide with antitumor properties useful for cancer treatment“. From this collaboration, one article was published (Fernández Massó et al, 2013), one manuscript is under preparation and one student (S Astrada) is performing her PhD thesis under the supervision of Bollati M and Vallespi M.

We are collaborating with Estela Castillo, PhD, Facultad de Ciencias, UdelaR, Montevideo in the frame of the project “Abriendo camino a la transgénesis en cestodos: aislamiento, caracterización y cultivo de células madre en Mesocestoides corti“. The obtained results were recently published (Domínguez et al, 2014).

With Mónica Marín, PhD, Facultad de Ciencias, UdelaR, Montevideo we are collaborating on the project entitled “Efecto de mutaciones sinónimas en la actividad del receptor de estrógenos alfa“. Recently, one article was accepted for its publication (Fernández-Calero et al, 2014).

Finally, the work in association with Susana Etcheverry, PhD (Cequinor, UNLP, Argentina) have yielded three manuscripts (Leon et al, 2013a and 2013b and one is under its second revision).

References:

- Bürgi M., Prieto C., Etcheverrigaray M., Kratje R., Oggero M., Bollati-Fogolín M. WISH cell line: From the antiviral system to a novel reporter gene assay to test the potency of human IFN- and IFN- . J Immunol Methods. 2012, 381(1-2):70-4.
- Domínguez MF, Koziol U, Porro V, Costábile A, Estrade S, Tort J, Bollati-Fogolin M, Castillo E. A new approach for the characterization of proliferative cells in cestodes. Experimental Parasitology 138C (2014), pp. 25-29.
- Fernández-Calero T, Astrada S, Alberti A, Horjales S, Rovira C, Bollati-Fogolín M, Flouriot G, Marin M. The transcriptional activities and cellular localization of the human estrogen receptor alpha are affected by the synonymous Ala87 mutation. Accepted in The Journal of Steroid Biochemistry and Molecular Biology
- Fernández Massó JR, Oliva Argüelles B, Tejeda Y, Astrada S, Garay H, Reyes O, Delgado-Roche L, Bollati-Fogolín M, Vallespí MG. The Antitumor Peptide CIGB-552 Increases COMMD1 and Inhib-

its Growth of Human Lung Cancer Cells. J Amino Acids. 2013; 251398.

- Leon IE, Porro V, Di Virgilio AL, Naso LG, Williams PAM, Bollati-Fogolín M and Etcheverry SB. Antiproliferative and apoptosis-inducing activity of an oxidovanadium(IV) complex with the flavonoid silibinin against osteosarcoma cells. J Biol Inorg Chem. 2013a Nov 14.
- Leon IE, Di Virgilio AL, Porro V, Muglia CI, Naso LG, Williams PAM, Bollati-Fogolín M and Etcheverry SB. Antitumor properties of a vanadyl(IV) complex with the flavonoid chrysin [VO(chrysin)2EtOH]2 in a human osteosarcoma model: role of the oxidative stress and apoptosis. Dalton Trans. 2013b; 42(33):11868-80. doi: 10.1039/c3dt50524c.
- Tiscornia I., Sánchez-Martins V., Hernández A., Bollati-Fogolín M. Human monocyte-derived dendritic cells from leukoreduction system chambers after plateletpheresis are functional in an in vitro co-culture assay with intestinal epithelial cells. J Immunol Methods. 2012, 384(1-2):164-70.

Services

1. Culture of different cell lines, cell banking.
2. Detection of mycoplasma contamination in cell culture by PCR.
3. Citotoxicity and proliferation assays.
4. Adaptation of different cell lines to the suspension growth mode and to serum free medium.
5. Generation of reporter cell lines, with broader applications.
6. Flow cytometry analysis: DNA content and cell cycle analysis, fluorescent proteins detection, apoptosis, multicolor analysis, etc.
7. Sorting of heterogeneous cell populations into homogeneous populations: sterile sorting, single cell deposition, 4 way sorting.

Publications

1. Leon IE, **Porro V**, Di Virgilio AL, Naso LG, Williams PAM, **Bollati-Fogolín M** and Etcheverry SB. Antiproliferative and apoptosis-inducing activity of an oxidovanadium(IV) complex with the flavonoid silibinin against osteosarcoma cells. J Biol Inorg Chem. 2013 Nov 14. [Epub ahead of print]. PMID: 24233155
2. Mazal D, Lo-Man R, Bay S, Pritsch O, Dériaud E, Ganneau C, Medeiros A, Ubillos L, Obal G, Berois N, **Bollati-Fogolin M**, Leclerc C, Osinaga E. Monoclonal antibodies toward different Tn-amino acid backbones display distinct recognition patterns on human cancer cells. Implications for effective immuno-targeting of cancer. Cancer Immunol Immunother. 2013; 62(6):1107-22. doi: 10.1007/s00262-013-1425-7.
3. Noya V, Bay S, Festari MF, García E, Rodriguez E, Chiale C, Ganneau C, Baleux F, **Astrada S**, **Bollati–Fogolín M**, Osinaga E and Freire T. Mucin-like peptides from Echinococcus granulosus induce antitumor activity. Int J Oncol. 2013; 43(3):775-84. doi: 10.3892/ijo.2013.2000.
4. Chenoll E, Codoñer FM, Silva A, Ibáñez A, Martinez-Blanch JF, **Bollati-Fogolín M**, Crispo M, **Ramírez S**, Sanz Y, Ramón D and Genovés S. Genomic Sequence and Pre-Clinical Safety Assessment of Bifidobacterium longum CECT 7347, a Probiotic able to Reduce the Toxicity and Inflammatory Potential of Gliadin-Derived Peptides. J Prob Health 2013, 1: 106. doi: 10.4172/ jph.1000106
5. Leon IE, Di Virgilio AL, **Porro V**, Muglia CI, Naso LG, Williams PAM, **Bollati-Fogolín M** and Etcheverry SB. Antitumor properties of a vanadyl(IV) complex with the flavonoid chrysin [VO(chrysin)2EtOH]2 in a human osteosarcoma model: role of the oxidative stress and apoptosis. Dalton Trans. 2013; 42(33):11868-80. doi: 10.1039/c3dt50524c.
6. Fernández Massó JR, Oliva Argüelles B, Tejeda Y, **Astrada S**, Garay H, Reyes O, Delgado-Roche L, **Bollati-Fogolín M**, Vallespí MG. The Antitumor Peptide CIGB-552 Increases COMMD1 and Inhibits Growth of Human Lung Cancer Cells. J Amino Acids. 2013; 251398. doi: 10.1155/2013/251398.
7. **Victoria S**, Temerozo JR, Gobbo L, Pimenta-Inada HK, Bou-Habib DC. Activation of Toll-like receptor 2 increases macrophage resistance to HIV-1 infection. Immunobiology, 2013; 218(12):1529-36.
8. Massari NA, Medina VA, Cricco GP, Martinel Lamas DJ, Sambuco L, **Pagotto R**, Ventura C, Ciraolo PJ, Pignataro O, Bergoc RM, Rivera ES. Antitumor activity of histamine and clozapine in a mouse experimental model of human melanoma. J Dermatol Sci. 2013; 72(3):252-62. doi: 10.1016/j.jdermsci.2013.07.012.
9. Perona M, Dagrosa MA, **Pagotto R**, Casal M, Pignataro OP, Pisarev MA, Juvenal GJ. Protection against radiation-induced damage of 6-propyl-2-thiouracil (PTU) in thyroid cells. Radiat Res. 2013; 179(3):352-60. doi: 10.1667/RR2658.1.

Grants

- Monitoreo de compuestos naturales y sintéticos que modulen la actividad biológica de los Interferones de Tipo I utilizando un nuevo ensayo de gen-reportero. PI: M. Bollati (Uruguay), M. Oggero (Argentina), Cooperación bilateral Argentina–Uruguay (AR-UR/11/01), 2012 – 2013 (2 years).
- Toxicología ambiental aplicada: evaluación del riesgo por exposición a estrógenos ambientales antropogénicos en un modelo de ratones transgénicos Oct4-GFP. PI: Mariela Bollati, ANII: Fondo María Viñas PR_FMV_2_2011_1_6046 (Uruguay), 2013 – 2015 (2 years).
- Compuestos naturales y sintéticos que modulen la actividad biológica de los Interferones humanos de tipo I: análisis mediante una nueva herramienta biológica. PI: M. Oggero (Argentina), M. Bollati (collaborative partner, Raíces), PICT-Raíces 01789, 2010 –2013 (3 years).
- Toxicología ambiental aplicada: evaluación del riesgo por exposición a estrógenos ambientales antropogénicos en un modelo murino transgénico Oct4-GFP. PI: M. Crispo (Uruguay), H. Rodríguez (Argentina), M. Bollati (collaborative partner). Cooperación bilateral Argentina–Uruguay (AR-UR/12/02), 2013 – 2015 (2 years).
- Modulación de la respuesta innata epitelial por levaduras probióticas: determinación de los mecanismos genéticos de levaduras involucrados en esta propiedad. PI: P. Aguilar (Uruguay), M. Rumbo (Argentina), M. Bollati (collaborative partner). Cooperación bilateral Argentina–Uruguay (AR-UR/12/03), 2013 – 2015 (2 years).
- Postdoctoral fellowship CONICET-IPMontevideo, R. Pagotto, 2012-2014 (2 years)
- Doctoral fellowship ANII- POS_NAC_2012_1_8523, S. Astrada, 2013-2015 (2 years)
- Student fellowship ANII- INI_X_2011_1_3830, G. Mastropietro, 2012- 2013 (1 year)
- Student fellowship ANII- INI_X_2012_1_4184, M.B. Harreguy, 2013- 2014 (1 year)
- Travel Fellowship to attend the 23rd ESACT Meeting,Lille, France, K. Perelmuter, June 2013
- Starting in 2014
- Postdoctoral fellowship ANII- PD_NAC_2013_1_10903, R. Pagotto, April 2014 (2 years).

Other activities

TRAINING COURSES

As organizers:
"Fundamentos y aplicaciones de la citometría de flujo", PEDECIBA – BIOLOGIA. Montevideo, Uruguay. Organizers: V. Porro, M. Bollati, G. Folle. October 2013.

As participants:
"Curso Sistemas de Expresión para la Producción de Proteínas: desde el diseño del vector al primer escalado", PEDECIBA - BIOLOGIA. Facultad de Ciencias-UdelaR, Montevideo, Uruguay. M. Bollati participated as conferencist. July 2013.
"Fifth International School on Production of Biopharmaceuticals in Animal Cell Cultures", UFRJ, Rio de Janeiro, Brazil. M. Bollati participated as conferencist. August 2013.
"Curso Básico de Cultivo de Células", PEDECIBA – BIOLOGIA. Montevideo, Uruguay. V.Porro, I.Tiscornia and M. Bollati participated in the theoretical and practical activities. August 2013.
"Curso Básico de Citometría de Flujo". Buenos Aires, Argentina. V. Porro participated in the theoretical and practical activities. September 2013.
"Curso Cultivos de células eucarióticas y su utilidad para modelar la interacción entre los microorganismos y el hospedador".CIDCA-Facultad de Ciencias Exactas, UNLP, La Plata, Argentina. M. Bollati participated as conferencist. September 2013.
"Curso Producción, purificación y caracterización estructural de proteínas", PEDECIBA-QUÍMICA. Instituto de Higiene, Facultad de Química, UdelaR, Uruguay. M. Bollati participated as conferencist. October 2013.

TRAINING OF STUDENTS

1. PhD students:
- V. Porro. Pro.In.Bio, Uruguay. Academic Directors: M. Bollati and H. Rodriguez
- S. Astrada. Pro.In.Bio, Uruguay. Academic Directors: M. Bollati and M. Guerra Vallespi
- M. Burgi. FBCB, UNL, Santa Fe, Argentina. Academic Directors: R. Kratje and M. Bollati

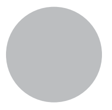
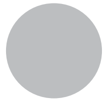
2. Undergraduate students:
- G. Mastropietro, Facultad de Ingeniería, Universidad ORT, Uruguay
- M.B. Harreguy, Facultad de Ingeniería, Universidad ORT, Uruguay

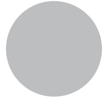
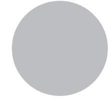
3. Stages at the Unit:

- MSc Luis Eduardo Hinojosa. PhD student, Centro de Inmunología Molecular, La Habana, Cuba (4 months).
- Ignacio León. PhD student, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Argentina (1 month).
- MSc. Andrea Blanc, Laboratorio Microsules, Montevideo, Uruguay (9 months).

RECEIVED TRAINING BY THE CELL BIOLOGY UNIT STAFF

- S. Victoria atended the “Fifth International School on Production of Biologicals using Animal Cell Cultures”. Federal University of Rio de Janeiro, Rio de Janeiro. August 2013.
- S. Astrada attended the RIIP Regional Course “Digital image processing/analysis tools in Light Microscopy:From the basics and beyond”,Hellenic Pasteur Institut, June 2013.
- Tiscornia attended the courses “Redox biology and chemistry of thiols” (June 2013, Montevideo) and “Laboratory biosafety” (May 2013,Buenos Aires, Argentina).
- K. Perelmuter atended the course “Bioprocess Engineering”, Facultad de Ingeniería, Montevideo, Uruguay.
- Ma Belén Harreguy atended the intramural course “Manipulación y administración de sustancias en ratones de laboratorio” Instituto Pasteur de Montevideo. March 2013.

Transgenic and Experimental Animal Unit

Head: Martina Crispo, DVM, PhD

Members:

Geraldine Schlapp, MSc (Full time technician)

María Noel Meikle, MSc (Technician)

Ana Paula Arévalo, TMN (Technician)

Gabriel Fernández, Biol (Technician, animal caretaker)

Sergio Anchetta, (Animal caretaker)

Martín Mereles, (Animal caretaker)

Ana Paula Mulet, MSc (Research assistant)

Tali Korytnicki (Degree student)

Pia Jacques (Degree student)

Research

During 2013 five full articles in peer review journals were published, with a PhD thesis finished in October 2013 and others posgraduate thesis running. Worldwide diffusion of the generation of GFP transgenic sheep through lentiviral technology was launched, with good scientific and public perception of the research team and the institutions involved. This project led to a full article that was recently accepted.

We reinforced the transgenic technologies, focusing in increase the efficiencies for pronuclear DNA microinjection after a particular year with construction noise and vibrations that affected the animal's wellbeeing and overall production of the Unit. We incorporated a new transgenic technology mediated through transposons, known as Sleeping Beauty Transposons (SBT100x). Also, collaboration was stablished to start working with CRISPRs technology, one of the latest advances in animal transgenesis to generate KO models in several species.

New PI's that uses different transgenic mice models were recluted by the Institution, demanding thus many services that included mice importation, embryo and sperm cryopreservation, rederivation, breeding and mainteinance of several transgenic lines. This demand has increased the amount of work at the Unit, with an occupation of physical area close to 80%. This triggered plans to expand the animal house in a conventional area.
We continue working with national and international biotechnological companies, with grants close to 65.000 USD and the service offered resulting in a publication in Journal of Probiotics and Health.

The second edition of the internal course for researchers of Institut Pasteur was organized: *Manejo, técnicas de administración de sustancias y obtención de muestras en ratones*, beeing mandatory for working with mice at our facility. Several lectures for undergraduated and posgraduated students were given by members of the UATE.
In summary, the amount of services increased substantially, research area was reinforced, human resources formation continues developing and original information was published during the period.

Projects

2011 - to date	Rol of micovesicles in murine embryonic stem cells. PI.
2012-2015	Estudio de los mecanismos responsables de potenciales efectos probióticos de la cepa Lactobacillus rhamnosus CNCM I-3690. Co-Tutor.
2013-2014	Caracterización de un modelo murino inducible para el estudio de las vías de señalización de la insulina. Tutor.
2013-2014	Derivación y caracterización de una línea de células madre embrionarias (ESC) murinas Oct4-GFP en ausencia de suero y de fibroblastos embrionarios murinos (MEF). Co-tutor.
2011 - 2013	Transgenic sheep developed by somatic cell nuclear transfer (SCNT). Co - PI
2011 - 2013	New alternatives for gamete cryopreservation in female ruminants. Co-PI
2010 - 2013	Creation of an International Consortium for preclinical evaluation of probiotic strains for diary food prototypes. Collaboration.
2012 - 2014	Applied environmental toxicology: risk evaluation to environmental antropogenic estrogen exposition in a transgenic murine model Oct4-GFP. Collaboration.
2011 - 2013	Differential genic expression during meiosis: identification and characterization of specific products in male meiotic prophase in rodents. Collaboration.
2011 - 2013	Rol of circulating endothelial progenitors from bone marrow in the tumoral neovascularization of NHL. Murine model. Collaboration.
2009 - to date	Trypanothione biosynthesis as a basis for drugs against trypanosomes. Collaboration.
2008 - to date	Rol of IL-17 in immunoprotection of respiratory airways mucosae. Collaboration.
2008 - to date	Colon anticancer immunotherapy using Trypanosoma cruzi antigens. Collaboration.

Services

- Generation of transgenic mice by pronuclear microinjection of DNA fragments (5 projects for researchers from Argentina, Chile and Uruguay).
- Generation of transgenic mice by homologous recombination in embryonic stem cells (2 projects - mgc-1203 and Spats1).
- Set up of Sleeping Beauty Transposons (SBT 100x) technology (one project in progress – Redox GFP)
- Embryo and sperm cryopreservation (7 projects running).
- Rederivation of mouse lines (11 projects running).
- *In vitro* fertilization: successfully set up of a new protocol
- Breeding and housing of SPF and conventional mice (C57BL/6J, BALB/cJ, DBA/2J, SWISS, SJL/J, Nude, several hybrids and aprox. 30 different transgenic lines). Actual production: aprox. 1200/month.
- Trials of biological activity for recombinant eritropoyetin (Lab. Clausen & LCB) (aprox. 12 per month). The animal facility is certified by the Ministry of Health.
- Trials of toxicity for biotechnological products (EPO, Filgen, Interferon) for Lab. Clausen & LCB (8 per month).

Publications

Full article in peer review journals:

- **M. CRISPO**; L VAN MAELE; J TABAREAU; D CAYET; A ERREA; AM FERREIRA; M RUMBO; JC SIRARD. Transgenic mouse model harboring the transcriptional fusion Ccl20-luciferase as a novel reporter of pro-inflammatory response. PLoS ONE 2013, 12; 8(11):e78447.
- **M. CRISPO; SCHLAPP G**; CÁRDENAS M; D. GONZÁLEZ-MACIEL; M RUMBO. Optimization of transgenesis conditions for the generation of CXCL2-luciferase reporter mice line. EJB Electronic Journal of Biotechnology 2013, 16 (6): DOI: 10.2225
- E CHENOLL; FM CODOÑER ; A SILVA; A IBÁÑEZ; JF MARTINEZ-BLANCH ; M. BOLLATI FOGOLIN; **M. CRISPO**; S RAMIREZ; Y SANZ ; D RAMÓN ; S GENOVÉS. Genomic Sequence and Pre-Clinical Safety Assessment of Bifidobacterium longum CECT 7347, a Probiotic able to Reduce the Toxicity and Inflammatory Potential of Gliadin-Derived Peptides. Journal of Probiotics and Health 2013, 12:1-6.
- BERRIEL E, RUSSO S, MONIN L, FESTARI MF, BEROIS N, **FERNÁNDEZ G**, FREIRE T, OSINAGA E. Antitumor activity of human hydatid cyst fluid in a murine model of colon cancer. Scientific World Journal 2013:230176. doi: 0.1155/2013/230176.

Abstracts in meetings

- M RUMBO; **M. CRISPO**; L VAN MAELE; J TABAREAU; D CAYET; A ERREA; AM FERREIRA; JC SIRARD. Transgenic mouse model harboring the transcriptional fusion Ccl20-luciferase as a novel reporter of pro-inflammatory response, 2013. Evento: Internacional, 11th World Congress on Inflammation, Natal.
- **M. CRISPO**; AM FERREIRA; AP AREVALO; AP MULET; JC SIRARD; M RUMBO. Caracterización in vivo de un modelo murino CCL20-luciferasa reportero de inflamación, 2013. Evento: Regional, I Congreso AUCyTAL, Punta del Este.
- AP MULET; **M. CRISPO**; SCHLAPP G; L. GOYENECHE; G FERNÁNDEZ; G. GROMPONE. Obtención de un modelo murino para el estudio de la función de FOXO1 en el intestino, 2013. Evento: Regional, I Congreso AUCyTAL, Punta del Este.
- P. DOS SANTOS; M VILARIÑO; **M. CRISPO**; A MENCHACA. Vitrificación de mórulas y blastocistos ovinos producidos por fertilización in vitro utilizando el método espátula, 2013. Evento: Internacional, X Simposio Internacional de Reproducción Animal, Córdoba.

Grants

- ”Desarrollo de modelos transgenicos mediante transferencia nuclear de celulas somaticas en ovinos” – Alejo Menchaca (PI) and **Martina Crispo** (Co-responsible) ANII INNOVAGRO (2011 – 2013) – USD 60.000.
- “Nuevas alternativas para la criopreservación de gametos en hembras rumiantes”. Co-responsible. ANII Maria Viñas (2011-2013). USD 20.000.
- “Una innovación tecnológica para la reproducción ovina en Uruguay: producción in vitro de embriones y su aplicación a gran escala”. Union Agriculture Group (UAG). Co-responsable **Martina Crispo** (2012 – 2013) USD 100.000.
- “Ensayo de ingesta aguda de dos cepas probióticas en ratones BALB/cJ”. Biópolis. Responsables Mariela Bollati, **Martina Crispo** (2013) USD 25.000.
- “Estudio de la actividad hipolipemiante, capacidad antioxidante y actividad anti-inflamatoria de los componentes del extracto de pericarpio derivado de girasol “violeta” (EPGv)”. Igra Semillas. Responsables Mariela Bollati, Carlos Battyhany, **Martina Crispo** (2013-2014) USD 38.500.

Other activities

Human resources formation

Ana Paula Mulet, MSc - Estudio de los mecanismos responsables de potenciales efectos probióticos de la cepa Lactobacillus rhamnosus CNCM I-3690. **PhD Thesis** (2012-2015). UdelaR PRO.IN.BIO (Co-Tutor).

Pedro Claudino dos Santos Neto, DMV – Criopreservacion de embriones ovinos. **MSc Thesis** (2011-2014), Facultad de Veterinaria - UDeLaR (Co-Tutor).

Pia Jacques, student - Caracterización de un modelo murino inducible para el estudio de las vías de señalización de la insulina. **Research initiation fellowship** (2013-2014), ANII (Tutor).

Pia Jacques, student – Obtención de herramientas moleculares para el estudio del estado redox de tripanosomátidos. **Degree thesis** (2012-2013)

Tali Korytnicki, Biol – Derivación y caracterización de una línea de células madre embrionarias (ESC) murinas Oct4-GFP en ausencia de suero y de fibroblastos embrionarios murinos (MEF).

Research initiation fellowship (2013-2014), ANII (Co-tutor).

Tali Korytnicki, Biol – Derivación de Células Madre Embrionarias (ESC) murinas como alternativa a su adquisición en otros laboratorios para uso en la producción de ratones genéticamente modificados. **Degree thesis** (2012-2013) (Co-tutor)

Meetings

- 64th American Asociation for Laboratory Animal Science (AALAS) meeting. Baltimore, USA, October 2013.
- I Congreso Asociación Uruguaya de Ciencia y Tecnología de Animales de Laboratorio (AUCyTAL). Punta del Este, Uruguay, April 2013.

Oral presentations in national and international meetings

- Banco de embriones y esperma murino. I Congreso Asociación Uruguaya de Ciencia y Tecnología de Animales de Laboratorio (AUCyTAL), Punta del Este, Uruguay, April 2013.

Internships & Courses

- 6th HKU PASTEUR IMMUNOLOGY COURSE, Hong-Kong, November 2013.
- Laboratory Animals Science Course, Monterotondo, Italy, april 2013.
- Curso CHEA: Cuidado y manejo de animales para experimentación. Categoría A y B, Montevideo, Uruguay, July 2013.

Teaching

- Organization of the IP Montevideo internal course 2013: *Manejo, técnicas de administración de sustancias y obtención de muestras en ratones*, for 20 researchers that uses mice at the animal facility.
- Several lectures in postgraduate national and regional courses.

Other

- Member of Comite de Ética en el Uso de Animales (CEUA). IP Montevideo (2009 - to date)
- Member of Comite de Ética en el Uso de Animales (CEUA). Facultad de Ciencias, UdelaR (2011 to date)
- Member of Scientific Comitee of Centro Multidisciplinario para Investigação Biológica (CEMIB) Universidad de Campinas (2010 - to date).
- Member of Comisión Nacional de Experimentación Animal (CNEA) (2010 - 2014).
- Member of Comisión de Evaluación del Riesgo en Bioseguridad, MGAP (2009 - to date).

Protein Biophysics Unit

Head: Otto Pritsch, PhD

Members:

Gonzalo Obal (PhD student, Technical Assistant)

Sergio Bianchi (MD, MSc, PhD student)

Gonzalo Moratorio (MSc, PhD student)

Lorena Tomé (MSc, PhD student)

Federico Carrión (Technical Assistant)

Gonzalo Rama (MSc student)

Natalia Olivero (Intern)

Andrés Addiego (Intern)

Research

Our scientific activity is focused on the study of viral pathogenesis of bovine leukemia. Indeed, viral infections, and in particular those affecting cattle, are major health problems in Uruguay.
Enzootic Bovine leukemia (EBL) is an infectious disease caused by a retrovirus, the bovine leukemia virus (BLV), which affects more than 60% of dairy cattle in Uruguay. Given the high percentage of infected cattle, and the importance of livestock for the Uruguayan economy, EBL has become an issue of great concern. At the moment, no vaccine against BLV is available. The main objective of this project was to integrate a national team to study the epidemiology and pathophysiology of EBL, its causative agent and the oncogenic process triggered by BLV.
In the context of this project we have organized a multidisciplinary group to work on BLV, funded by the National Institute of Agronomic Research of Uruguay and the University of the Republic in Montevideo. Moreover, we have participated in the First Latin American Workshop on EBL (2012) and created the Regional Network of EBL that integrates a diverse group of research laboratories in Latin America.

The specific aims are:

A. **To analyze the genetic variability of BLV in Uruguay:** EBL can be divided into three clinical forms: a) Asymptomatic (AS), b) Persistent Lymphocytosis (PL), characterized by a malignant polyclonal expansion of CD5+ B cells and c) Lymphosarcoma (LS), characterized by the formation of tumors. The general objective of this work is to contribute to knowledge on EBL, by molecular characterization and analysis of viral strains present in infected cattle with different clinical manifestations of the disease. We obtained for first time the complete genomic sequence of a BLV strain from a lymphosarcoma. The amplified BLV genome was cloned, sequenced, and bioinformatically studied with other BLV full-length sequences from other manifestations of the disease including PL and asymptomatic ones. These results were included in Moratorio et al (2013)

B. **To develop new methods for diagnosis of BLV infection:** we have developed a rapid and sensitive real time PCR assay using SYBR green chemistry to detect and quantify BLV proviral DNA by amplifying gp51 gene from bovine peripheral blood. A comparative analysis with validated diagnostic tests (AGID, ELISA and direct nested PCR) was performed in 45 dairy cattle samples. In summary, our results reveal that real-time PCR is comparable to nested PCR, and confirm an increased sensitivity of the PCR (real-time and nested) over the ELISA and AGID tests respectively. Overall, our results show that this SYBR Green -based PCR assay may be a useful, simple, and rapid tool to detect BLV infection in dairy cattle samples that could be adapted to high-throughput diagnostic procedures.

C. **To characterize the biophysical and structural bases of BVL capsid self-assembly:** Like other retroviruses, assembly of BLV virions is driven by Gag, a polyprotein precursor composed of three major domains: MA (matrix), CA (capsid), and NC (nucleocapsid). After particle budding, the virus-encoded protease PR cleaves Gag and releases the individual domains: the N-terminally myristoylated MA remains anchored at the viral envelope, NC condenses with the viral RNA, and CA spontaneously self-assembles to form a closed structure: the mature "core"or capsid. This dramatic structural rearrangement, known as maturation, is essential for infectivity, and thus constitutes an attractive target for novel antiretroviral strategies. The mechanism of viral capsid formation via self-assembly of thousands of copies of the capsid protein (CABLV) represents a key event in the retrovirus cycle. The objective of this part of the project is to characterize the biophysical and structural bases of BVL capsid self-assembly. We have characterized in vitro the self-assembly process of the purified recombinant BLV-CA protein. Specifically, we have analysed the effect ofr protein concentration, pH, ionic strength, temperature, phosphate and polyphosphates on self-assembly kinetics. In parallel, we performed electron microscopic analysis of assembly products in order to evaluate the physical characteristic of the material formed under these conditions. This work provides the first description of the BLV capsid protein assembly properties, which may also be of relevance for understanding other Deltaretrovirus assembly characteristics (Obal et al, manuscript in preparation). On the other hand, in collaboration with IP-Mont Protein Cristallography Unit, we have performed crystallographic studies of the BLV capsid protein p24. Full-length and separate N-terminal and C-terminal domain constructs were generated, expressed and purified to homogeneity. Crystallization conditions were obtained for the three variants, and they diffract X rays to near atomic resolution.

D. **To characterize the biochemical and structural bases of BLV envelope protein:** As in all members of Retroviridae family, the BLV env complex plays a crucial role in determining viral infectivity, since it is responsible for the recognition of a specfic cell-surface receptor required for virus entry. It is also responsible for inducing fusion of viral and cellular membranes for entry. This project proposes to characterize the 3D structure of the BLV env glycoprotein complex in its pre-fusion conformation. The difficulties rely in the heavy glycosylation and instability of the protein complex, which is maintained in a metastable confor-

mation in the pre-fusion form. We have an advantage with respect to HIV because BLV gp51 is smaller and is less glycosylated than its gp120 HIV counterpart. In addition, since BLV virions are not pathogenic to humans they can be produced in large quantities to allow its complete characterization by electron microscopy. Also, recent advances in electron tomography with vitrified samples at liquid nitrogen temperature ("electron cryo-tomography", or cET) together with sub-tomogram averaging have made it possible to study the structure of peomorphic particles at a resolution of about 20Å. We will use this approach to characterize the structure of whole BLV virions. We have also optimized the production of the soluble ectodomain (i.e., eliminating the transmembrane segment and downstream C-terminal cytosolic tail) in Drosophila S2 cells. The env protein is truncated at two different positions upstream the TM segment. We have designed 4 different constructs in total, with a natural and an altered furin cleavage site taking into account codon-optimization for efficient production in Drosophila. This system should therefore allow the production of sufficient material for crystallization trials, electron cryo-microscopy of isolated trimers, and biophysical studies of the multimeric complex formed by the recombinant proteins. This work will be done in collaboration with Dr. Félix Rey (Unité de Virologie Structurale - CNRS URA 3015, Institut Pasteur, Paris, France) and Dr. Jean Lepault (Laboratoire de Virologie Moléculaire et Structurale, CNRS UPR3296, Gif-sur-Yvette, France) in the context of the CNRS International Associated Laboratory on Structural Virology.

Services

1. Thermodynamic analysis of protein-protein and protein-ligand interaction through determination by isothermal titration microcalorimetry (ITC) of binding constants (KB), reaction stoichiometry (n), enthalpy (ΔH) and entropy (ΔS).
2. Thermodynamic analysis of conformational changes of proteins including assessment of stability and folding of recombinant proteins through differential scanning microcalorimetry (DSC) to study a wide range of thermal transitions in biological systems, to determine melting temperatures as well as thermodynamic parameters associated to these changes.
3. Kinetic analysis of protein - ligand interaction through Surface Plasmon Resonance (SPR) measurements, determination of kinetic association (kass) and dissociation (kdiss) constants.
4. Determination of the hydrodynamic radius of macromolecules or particles through dynamic light scattering measurements coupled to size exclusion chromatography SEC-HPLC.

Publications

1. **Moratorio G**, Fisher S, **Bianchi S, Tome L, Rama G, Obal G, Carrion F, Pritsch O,** Cristina J. A detailed molecular analysis of complete Bovine Leukemia Virus genomes isolated from B-cell lymphosarcomas. (PMID: 23506507) *Veterinary Research*, 44(1): 19, 2013.
2. Meikle A, Cavestany D, Carriquiry M, Adrien ML, Artegoitia V, Pereira I, Ruprechter G, Pessina P, **Rama G**, Fernandez A, Breijo M, Laborde D, **Pritsch O**, Ramos JM, de Torres E, Nicolini P, Mendoza A, Dutour J, Fajardo M, Astessiano AL, Olazábal L, Mattiauda D, Chilibroste P. Avances en el conocimiento de la vaca lechera durante el período de transición en Uruguay: un enfoque multidisciplinario. *Agrociencia Uruguay* 17(1): 141-152, 2013.
3. Mazal D, Lo-Man R, Bay S, **Pritsch O**, Dériaud E, Ganneau C, Medeiros A, Ubillos L, **Obal G**, Berois N, Bolatti-Fogolin M, Leclerc C, Osinaga E. Monoclonal antibodies toward different Tn-amino acid backbones display distinct recognition patterns on human cancer cells. Implications for effective immuno-targeting of cancer. (PMID: 23604173) *Cancer Immunology and Immunotherapy* 62: 1107-1122, 2013
4. Correa A, Trajtenberg F, **Obal G, Pritsch O**, Dighiero G, Oppezzo P, Buschiazzo A. Crystal structure of a human IgA1 Fab fragment at 1.55Å resolution: potential effect of the constant domains in antigen-affinity modulation. (PMID: 23519414) *Acta Crystallografica* D 69(3): 388-397, 2013.
5. **Moratorio G**, Iriarte A, **Moreno P**, Musto H, Cristina J. A detailed comparative analysis on the overall codon usage patterns in West Nile virus. Infect Genet Evol. 2013 Mar;14:396-400. doi: 10.1016/j.meegid.2013.01.001. Epub 2013 Jan 16.

Grants

1. **"International Associated Laboratory on Structural Virology".** Centre National de la Recherche Scientifique - IPMont-LIA. Period: January 2013 – December 2016. Felix Rey, CNRS URA 3015 Virology, Institut Pasteur, Paris - Otto Pritsch. Institut Pasteur de Montevideo. Amount granted: 15.000 euros / year.
2. **"Desarrollo de nuevos métodos para el diagnóstico del Virus de la Leucosis Bovina".** Ministerio de Industria y Energía – Dirección Nacional de Industria, uruguay. Period: December 2012 – December 2013. PI: Otto Pritsch. Amount granted 17.000 USD.
3. **Doctoral Fellowship – Lorena Tomé** – 2012 - 2014 – ANII
4. **Master Fellowship – Gonzalo Rama** – 2012 - 2013 – ANII

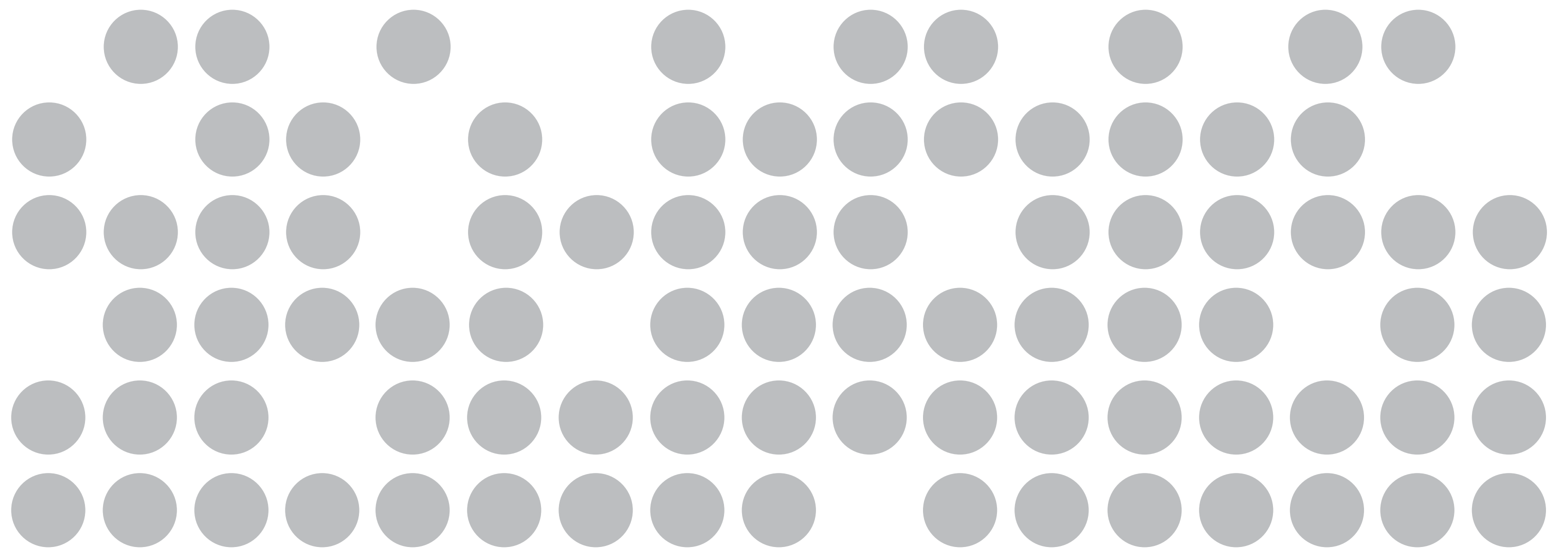
Other Acitivities

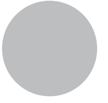
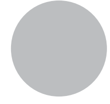
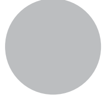
TRAINING OF STUDENTS

1. **Training of PhD degree students:**
   - Gonzalo Obal, PhD Student, PEDECIBA. “Estudios Biofísicos y Estructurales del Ensamblado de la Cápside del Virus de la Leucemia Bovina” Director: O. Pritsch.
   - Lorena Tomé, PhD Student, PEDECIBA. “Estudio de la interacción entre el Virus de la Leucosis Bovina y la célula hospedera”. Directors: J. Arbiza, O. Pritsch.
   - Rodrigo Puentes, PhD Student, Faculty of Veterinary. “Epidemiología molecular y efecto de la presencia del virus de la Leucosis bovina enzoótica en animales asintomáticos sobre parámetros productivos, reproductivos y sanitarios”. Directors: Silvia Llambí, Gonzalo Moratorio and Otto Pritsch.

2. **Training of Master degree students:**
   - Gonzalo Rama, Master in Biotechnology, Faculty of Science. “Desarrollo y análisis comparativo de una nueva herramienta para el diagnóstico de la Leucosis Enzoótica Bovina; Impacto del descenso de anticuerpos anti-VLB circulantes en el periparto para el diagnóstico serológico” Directors: A. Meikle, O. Pritsch. Defense date: March 2013.
   - Jorge de los Santos, Master in Biotechnology, Faculty of Science. “Desarrollo de kits para genotípado de SNPs en los genes humanos, FII, FV y MTHFR , utilizando la tecnología PCR en Tiempo Real”. Directors: Andrés Abin and Otto Pritsch. Defense date: August 2013.

RESIDENT LABORATORIES



Funcional Genomics Laboratory

Research Program in Molecular Oncology

Head: Alfonso Cayota, MD, PhD

Members:

Mª Rosa Silva (Postdoctoral Fellow)

Juan Pablo Tosar (Doctoral Student)

Braulio Bonilla (MSc Student)

Florencia Cabrera (Msc Student)

Julia Sanguinetti (Msc Student)

Fabiana Gambaro (Undergraduate Student)

Research

In the last 2 years, our main focus of research has been centered on the biology of small RNAs in the regulation of gene expression with especial emphasis in extracellular small RNAs and their role in cell-to-cell communication in human cancer. Ongoing work deal with three main related research areas:

1. "THE SECRETED RNAome": AN UNEXPECTED PATHWAY OF INTERCELLULAR COMMUNICATION AND NEW SOURCE OF BIOMARKERS IN CANCER

Cell-free DNA/RNA are normally secreted from a variety of normal and diseased cells to the extracellular media either through membrane-bound vesicles or included in ribonucleoprotein complexes. Studies over the past few years showed that these structures contain bioactive molecules, lipids, nucleic acids and proteins, which like hormones can influence normal homeostasis and many aspects of cancer progression including tumor development, invasion and metastasis.

Circulating mRNAs and microRNAs are detectable in the serum and plasma of healthy individuals and cancer patients. It is known that RNA released into the circulation is surprisingly stable in spite of the high levels of RNases in the blood. High stability and resistance to degradation is achieved through its packaging into either membrane-bound structures (i.e. exosomes, ectosomes and apoptotic blebs) or circulating ribonucleoprotein complexes.

Cancer is currently the second leading cause of death worldwide. Despite the advances in cancer therapeutic approaches during the last decades, the morbidity and mortality rates still remain high. The earliest possible diagnosis and treatment is still the best approach to improve survival. The National Cancer Institute of USA estimates that premature deaths, which may have been avoided through screening, range from 3% to 35% (www.cancer.gov). Screening for cancer is usually attempted whenever worrying symptoms arise, having as a result the diagnosis of cancer as a latest age disease. The current methods for diagnosis of the disease are usually invasive and expensive whereas the existing biological markers are not definitive and lack high sensitivity and specificity. At present, growing scientific efforts in human cancer are aimed to find and develop new, sensitive, non-invasive and inexpensive biomarkers to identify high risk individuals, detect cancer at an early stage, to predict outcome, to monitor treatment and to screen for disease recurrence. Detection of extracellular or cell-free nucleic acids (DNA or RNA) in blood or body fluids has been recently suggested as surrogates for non-invasive and cost effective biomarkers in human cancer.

Our present work is aimed to analyze in depth the total repertoire of RNA transcripts and small RNAs secreted by tumor cells to the extracellular media and their contribution to different fractions of circulating species in normal plasma as well as in cancer patients. Methodological and conceptual results issues from this study should be useful to establish new working hypothesis in the near future and to better understand their diagnostic and predictive value in human cancer and possibly other human diseases.

Thus, vesicular and non-vesicular horizontal transfer of small RNAs could emerge as a relatively wide-spread process that may complement intercellular communication by other mechanisms. One of the most intriguing questions in this regard is how, and to what extent, is this process involved in various forms of cellular pathology. Of particular interest is the role of MVs in cancer whose exploration may both afford new avenues in cancer biology and inspire new therapeutic and diagnostic approaches in biomedicine.

2. NON-HUMAN SMALL RNAS IN HUMAN PHYSIOLGY AND PATHOLOGY

This project is aimed to determine the potential of foreign small RNAs (from plant, insect, fungi or bacteria) as new actors in human physiology and cancer. In a recent meta-analysis of small RNA datasets we found a widespread distribution of exogenous small RNAs in normal human tissues. Recent experimental evidence suggested that a significant fraction of these "non human" sequences could result from a transfer of exogenous small RNAs between unrelated organisms. Some experimental evidences suggested that plant microRNAs are present in the sera and tissues of some animals which were assumed to be primarily acquired orally through food intake. Surprisingly, functional studies showed that the some plant microRNAs are functional in mammal tissues by inducing down-regulation of specific target mRNAs. The analysis of small RNA datasets from various vertebrate and invertebrates animals revealed that the presence of plant microRNAs is a widespread phenomenon across the animal kingdom. Thus, it can be assumed that several classes of small non-coding RNAs are actually transferred from one species to another, inducing epigenetic changes in distant species, even in a cross-kingdom manner.

Therefore this is a very attractive area of research able to afford new mechanisms in understanding the role of diet and environmental factors influencing human health and the risk of cancer.

3. BIOLOGICAL SIGNIFICANCE OF SMALL RNAs SECRETED BY ***TRYPANOSOMA CRUZI***. A ROLE ON LIFE CYCLE REGULATION AND SUSCEPTIBILITY TO INFECTION OF MAMMALIAN CELLS

The protozoan parasite *Trypanosoma cruzi*, has a complex life cycle characterized by intracellular and extracellular forms alternating between invertebrate and mammals. To cope with these changing environments, T. cruzi undergoes rapid changes in gene expression which are achieved essentially at the post-transcriptional level by mechanisms do not completely elucidated. At present, expanding families of small RNAs are recognized as key players in novel forms of post-transcriptional gene regulation in most eukaryotes. However, T. cruzi lacks canonical

small RNA pathways. In a recent work aimed to identify the presence of alternate small RNA pathways in T. cruzi, we reported the presence of a homogeneous population of small RNAs derived from mature tRNAs (tsRNAs). In T. cruzi epimastigotes submitted to nutrient starvation, tsRNAs co-localized with an argonaute protein distinctive of trypanosomatids (TcPIWI-tryp) and were recruited to particular cytoplasmic granules. By using epifluorescence and electronic microscopy, we observed that tsRNAs and the TcPIWI-tryp protein were recruited mainly to reservosomes and other intracellular vesicles including endosome-like vesicles and the trans-Golgi. These data suggested that in T. cruzi, tsRNAs biogenesis is probably part of endocytic/exocytic routes. We also demonstrated that epimastigotes submitted to differentiation media by nutrient starvation shed high levels of vesicles to the extracellular medium which carry small tRNAs and TcPIWI-tryp proteins as cargo. Extracellular vesicle cargo was efficiently transferred between parasites and to mammalian susceptible cells. Our data afford experimental evidence indicating that extracellular vesicles shed by T. cruzi promote not only life cycle transition of epimastigotes toward the infective trypomastigote forms but also infection susceptibility of mammalian cells.
The transfer of tsRNAs and other small RNAs from these parasites toward mammalian cells through shed vesicles represent a cross-kingdom transfer of small RNAs and associated proteins which could conduct us to rethink some concepts in host-pathogen biology.

Publications

1. Libisch G, Casás M, Chiribao ML, Moreno P, **Cayota A**, Osinaga E, Oppezzo P, Robello C. GALNT11 as a new molecular marker in Chronic Lymphocytic Leukemia. Gene. 2013 Sep 26. doi:pii: S0378-1119(13)01257-2. 10.1016/j.gene.2013.09.052.
2. **Garcia-Silva MR**, das Neves RF, **Cabrera-Cabrera F, Sanguinetti J**, Medeiros LC, Robello C., Naya H, Fernandez-Calero T, Souto-Padron T, de Souza W, **Cayota A**. Extracellular vesicles shed by Trypanosoma cruzi are linked to small RNA pathways, life cycle regulation, and susceptibility to infection of mammalian cells. Parasitol Res. 2014;113:285-304.

Grants

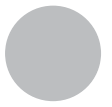
Starting in 2013

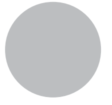
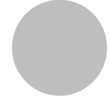
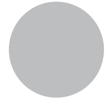
1. “ARNs extracelulares y cáncer: caracterización e implicancias en la modulación recíproca entre células malignas y no malignas” Juan Pablo Tosar ANII Amount Granted USD 13.000.

Other activities

Participation in Multicentric Cancer Programs

- Latin American Breast Cancer Pilot Project: **“Molecular Profiling of Breast Cancer Study”** NCI (United States) – Brazil – Argentina – Mexico – Chili – Uruguay - Colombia – Puerto Rico



Tumor Immunology and Glicobiology Laboratory

Research Program in Molecular Oncology

Head: Eduardo Osinaga, MD, PhD

Members:

Nora Berois (MD, PhD, Associate Investigator)

Edgardo Berriel (MD, MSc, PhD student)

María Florencia Festari (PhD student)

Patricia Moerzinger (MSc, student)

Diego Touyá (MSc, student)

Claudia Schvartzman (MSc, student)

Patricia Solari (MSc, student)

Guillermo Tramontín (undergraduate student)

Research

The most abundant form of O-linked glycosylation in higher eukaryotes, termed “mucin-type”, is characterized by the covalent linkage of an -N-acetylgalactosamine residue (GalNAc) to the hydroxyl group of Ser/Thr residues. Mucin core O-glycosylation is catalyzed by a group of UDP-GalNAc:polypeptide N-acetylgalactosaminyl-transferases (ppGalNAc-Ts) (EC. 2.4.1.41). Subsequent elongation of *O*-linked sugar chains is achieved by the transfer of additional saccharide units, catalyzed by specific glycosyltransferases. Malignant transformation of epithelial cells is commonly associated with changes in the expression level and/or glycosylation pattern of mucins, including exposure of simple mucin-type carbohydrates, such as Tn, sialyl-Tn and TF antigens. These determinants contribute to the phenotype and biology of cancer cells and are involved in their metastatic activity. Moreover, they are considered among the most specific cancer-associated structures, and are thus being evaluated as promising targets for tumor immunotherapy. We have recently identified some apomucins and glycosyltransferases, which are abnormally expressed in certain cancer cells. One of these enzymes, ppGalNAc-T13, is probably associated to the aggressiveness of some tumors. We investigate the molecular mechanisms underlying the regulation of the initial steps of mucin-type *O*-glycosylation in human cancer, and evaluate how this abnormal process influences malignant cell behavior.

Publications

1. Medeiros A, **Berois N**, Incerti M, Bay S, Franco Fraguas L and **Osinaga E**. A Tn antigen binding lectin from Myrsine coriacea displays toxicity on human cancer cell lines. J Natural Med 67:247-54 (2013) doi: 10.1007/s11418-012-0671-x.
2. Dalotto-Moreno T, Croci DO, Cerliani JP, Martinez-Allo VC, Dergan-Dylon S, Mendez Huergo SP, Stupirski JC, Mazal D, **Osinaga E**, Toscano MA, Sundblad V, Rabinovich GA, Salatino M. Targeting galectin-1 overcomes breast cancer associated immunosuppression and prevents metastatic disease. Cancer Res. 73:1107-17 (2013) doi: 10.1158/0008-5472.CAN-12-2418
3. **Berois N**, De Cremoux P, Mazal D, Sica A, Cedeira M, Caserta B, Barrios E, **Osinaga E** and Sastre-Garau X. Prevalence and distribution of high-risk human papillomavirus genotypes in invasive carcinoma of the uterine cervix in Uruguay. Int. J. Gynecol. Cancer 23:527-532 (2013) doi: 10.1097/IGC.0b013e318285e753.
4. Mazal D, Lo-Man R, Bay S, Pritsch O, Dériaud E, Ganneau C, Medeiros A, Ubillos L, Obal G, **Berois N**, Bollati-Fogolin M, Leclerc C and **Osinaga E**. Monoclonal antibodies toward different Tn-amino acid backbones display distinct recognition patterns on human cancer cells. Implications for effective immuno-targeting of cancer. Cancer Immunol. Immunother. 62:1107-22 (2013) doi: 10.1007/s00262-013-1425-7
5. Noya V, Bay S, **Festari MF**, García E, Rodriguez E, Chiale C, Ganneau C, Baleux F, Astrada S, Bollati – Fogolín M, **Osinaga E** and Freire T. Mucin-like peptides from Echinococcus granulosus induce antitumor activity. Int. J. Oncology 43:775-84 (2013) doi: 10.3892/ijo.2013.2000
6. **Berriel E**, **Russo S, Monin L, Festari MF, Berois N**, Fernández G, Freire T and **Osinaga E**. Anti-tumor activity of human hydatid cyst fluid in a murine model of colon cancer. The Scientific World Journal 2013:230176 (2013) doi: 10.1155/2013/230176.
7. Libisch G, Casás M, Chiribao ML, Moreno P, Cayota A, Osinaga E, Oppezzo P, Robello C. GALNT11 as a new molecular marker in Chronic Lymphocytic Leukemia. Gene. 2013 Sep 26. doi:pii: S0378-1119(13)01257-2. 10.1016/j.gene.2013.09.052.

Grants

Producción por ingeniería genética de diabodies e inmunotoxinas anti-antígeno tumoral Tn. Aplicación en imagenología molecular y tratamiento del cáncer.
ANII - Fondo María Viñas. U$S 47.000, 2013-2015

Other activities

TRAINING COURSES

“Glycobiology and Cancer: from Chemistry to Clinical applications”, 29th November – 7th December, 2013. Organized by Eduardo Osinaga and Nora Berois

Patents

Berois N, Touyà D, Varangot M and **Osinaga E.**
A novel method to detect resistance to chemotherapy in patients with lung cancer.
PCT International Application PCT/US2013/051904 (2013)

Berois N, Touyà D, Varangot M and **Osinaga E.**
Use of GalNAc-T13 as a marker in breast or colon cancer diagnostics.
U.S. Provisional Application Serial No. 61/858,038 (2013)

Neurodegeneration Laboratory

Head: Luis Barbeito, MD, PhD

Members:

- Hugo Peluffo (PhD, patternship with Faculty of Medicine (UR)
- Natalia Lago (PhD)
- Monique Richter (PhD)
- Natalia Puig (Student)
- Luciana Negro (Student)
- Patricia Solari (Student)
- Valentina Varela (Student)
- Daniela Alí (Student)
- Emilia Villamil (Student)
- Alejandra Silva Santisteban (Student)

Past members:

- Andrea Cragnolini (until 2010)
- Javier Ganz (until 2009)
- Andres de León (until July 2013)
- Mandi Gandelman (until 2008)

Research

1. **CHARACTERIZATION OF ABA CELLS AND THEIR PATHOGENIC MECHANISMS**

In Amyotrophic Lateral Sclerosis, glial cells are toxic to motor neurons in rodent models as well as in ALS patients. AbA cells (from aberrant astrocytes) are a new type of glial cells recently isolated by our group from degenerating spinal cord from SOD1G93A rats and mice. Nowdays, AbA cells are the most toxic cell yet identified to motor neurons. The biology and pathogenic potential of AbA cells are being studied in the context of a collaborative study involving local and international collaborators. The aim of our research at the IP Montevideo is to characterize the transcriptome and secretome of AbA cells, using microarrays and mass spectrometry technologies respectively. Also, we attempt to identify markers to label these cells in different models of neurodegenerative diseases and to test whether or not these cells exert a pathogenic role.
Recently, we have reported that AbA cells are derived from activated microglia that proliferate around damaged motor neurons by an apparent phenotypic transition. Since the appearance of AbA cells is closely associated with the progression of paralysis in SOD1G93A rats, a better understanding of the mediators inducing such phenotypic transition may allow intervention to slow the progressive spread of disease in ALS patients.

Related lab publications :

- Díaz-Amarilla et al, Proc Natl Acad Sci U S. 108:18126-31, 2011.
- Miquel et al. PLoS One. 2012;7:e34776
- Trias et al Front. Cell. Neurosci., 2013 7:274 1-8
- Gandelman et al. J Neurochem. 2013 126:382-8

2. **NEW NEUROTROPHIN SPECIES: THEIR ROLE IN NEURODEGENERATIVE DISEASES AND PAIN**

The neurotrophins are growth factors required by discrete cell types for survival and maintenance, with a broad range of activities in the nervous system and beyond. In 2006 we have described new species of NGF resulting from posttranslational modification of the mature neurotrophin resulting from nitration of tyrosine residues. Importantly, nitrated NGF form high molecular weight oligomers and display a gain-of-function through interaction with p75NTR. We propose nitrated NGF is formed during inflammation, playing a specific activity including neuronal apoptosis and nociception. The aims of the present research are to identify the specific activity of nitrated neurotrophins in different models including neurodegenerative conditions, inflammatory pain and to develop neutralizing antibodies with potential therapeutic applications.

Related lab publications :

Pehar et al. J Neurochem. 2004 89:464-73.
Pehar et al. Free Radic Biol Med. 2006 41:1632-44.
Pehar et al. J Neurosci. 2007 27:7777-85.

3. **INNOVATIVE GENE THERAPY STRATEGIES FOR TRAUMATIC BRAIN INJURY TARGETING NEUROINFLAMMATION AND IMMUNORECEPTORS. (PI: Hugo Peluffo)**

Inflammatory and immune reactions are present in all acute and chronic neurological pathologies. Interestingly, these processes are not only a consequence of neurodegeneration but also a critical mediator of the neurotoxic or neuroprotective mechanisms. Thus their modulation has emerged as an important therapeutic opportunity. The existence of different types of immune receptors with the capability to regulate microglia/macrophage and astrocytic phenotype opens a new range of molecular targets for the treatment of acute CNS damage. This project aims to analyse the therapeutic potential of some of those proteins (CD200R and the CD300 family of receptors) and their ligands in different experimental in vitro and in vivo models. We use a Controlled Cortical Impact traumatic brain injury model both with rats and Thy1-YFP-H mice and CD300f knock out mice. Our hypothesis is that modulation of glial, macrophage and mast cell responses by targeting novel immune receptors regulating cell activation and phenotype will provide neuroprotection after acute CNS damage. Development of tools such as fusion proteins or gene therapy vectors may provide innovative therapeutic strategies. In fact, we are focused on the design of novel non-viral modular recombinant nano-vectors, obtained by the rational engineering of functional protein domains. When combined with DNA these vectors adopts compact nanoparticle conformation capable of in vitro and in vivo transfection. In addition, non-integrating lentiviral vectors are also being evaluated as a promising therapeutic tool. The neuroprotective strategies outcomes are analysed by different neurological tests, Positron Emission Tomography, histology and immunohistochemistry.
External collaborators: Patricia Cassina (Faculty of Medicine, UR), Joan Sayós (Hospital Vall d´Hebron, Autonomous University of Barcelona), Antoni Villaverde (Autonomous University of Barcelona), Rafael Yáñez-Muñoz (Royal Holloway University of London), Henry Engler (Uruguayan Center for Molecular Imaging).

4. **INVOLVEMENT OF INFLAMMATION IN ACUTE INJURY TO THE NERVOUS SYSTEM AND IN CHRONIC PAIN (PI: Natalia Lago)**

Central and peripheral nerve injuries can result in substantial functional loss and have major social consequences. Spinal cord injury leads to a marked neuropathology and a reduced functional recovery. One of the reasons of this poor recovery is the secondary lesion characterized by a chronic inflammation, cell death and glial scar. In comparison with the Central Nervous System (CNS), the Peripheral Nervous System (PNS) has the ability to regenerate its axons after nerve injury although target reinnervation remains inaccurate with axons

growing into wrong distal targets leading to poor sensation and motor control as well as the establishment of chronic pain. The difference in the regenerative capabilities between the CNS and the PNS is partially due to the efficacy to remove myelin and axonal debris distally to the lesion during Wallerian Degeneration, and to the exhaustive control of the inflammatory response in the PNS in comparison with the CNS. Moreover, the communication between Schwann cells and macrophages seems to be important during injury induced Wallerian degeneration and for the establishment of chronic pain.
In our lab we are focused in the study of the inflammation after acute injuries to the CNS and PNS and how the modulation of the inflammation might be relevant in neuroprotection and regeneration. In fact, we are interested in the therapeutic potential of different types of immune receptors (CD200R, TREM-2 and the CD300 family of receptors) with the capability to regulate microglia/macrophage, astrocytic and Schwann cell phenotype. Moreover, we are interested in the modulation of these immune receptors in order to develop new strategies in the treatment of several painful inflammatory states.
To achieve this goal, we use models of acute peripheral nerve injury and spinal cord injury as well as animal models of inflammatory pain, combined with transgenic mice Thy1-YFP-H which express the YFP in a percentage of the neurons, CD300f knock out mice, fusion proteins and gene therapy vectors.
External collaborators:
Patricia Cassina (Faculty of Medicine), Joan Sayós (Hospital Vall d´Hebron, Autonomous University of Barcelona), Xavier Navarro (Faculty of Medicine, Autonomous University of Barcelona), Rafael Yáñez-Muñoz (Royal Holloway University of London).

Publications

1. **Trias E, Díaz Amarilla P**, Olivera-Bravo S, Isasi E, Drechsel D, Lopez N, Ireton E, Beckman JS, **Barbeito L**. Phenotypic transition of microglia into astrocyte-like cells associated with disease onset in a model of inherited ALS. Front. Cell. Neurosci., 7:274 1-8 (2013)
2. Olivera-Bravo S, Isasi E, Fernández A, Rosillo JC, Jiménez M, Casanova G, Sarlabós MN, **Barbeito L**. White Matter Injury Induced by Perinatal Exposure to Glutaric Acid. Neurotox Res. 2013 Dec 3. [Epub ahead of print]
3. Gandelman M, Levy M, Cassina P, **Barbeito L**, Beckman JS. P2X7 receptor-induced death of motor neurons by a peroxynitrite/FAS-dependent pathway. J Neurochem. 2013 126:382-8.
4. Seminotti B, Amaral AU, Da Rosa MS, Fernandes CG, Leipnitz G, Olivera-Bravos S, **Barbeito L**, Ribeiro CA, De Souza DO, Woontner M, Goodman SI, Koeller DM, Wajner M. Disruption of brain redox homeostasis in glutaryl-CoA dehydrogenase deficient mice treated with high dietary lysine supplementation. Mol Genet Metab. 2013 108:30-39.

Grants

1. Project Fundació Marató TV3, Catalunya, España. “Modulation of immune receptors function as a novel therapeutic strategy for acute CNS damage”. (2012-2014) Amount Granted € 80.000.
2. Project Collaboration Agreement - IP Paris/IP Montevideo/RECALCINE (Chile) – Development of Mabs against nitrated NGF (2010-2013)– USD 300.000.
3. Collaborative project IP Montevideo-Oregon State University. Pathogenic role of astrocytes in ALS. USD 25.000.
4. Project CSIC-UDELAR: “Gene therapy applied to brain truma: comparative preclinical studies using modular recombinant bectors and lentiviral vectors”. (2013-2014) Amount granted USD 40.000.
5. Fondo Clemente Estable ANII. FCE_1_2011_1_7342. Astrocitos fenotípicamente Aberrantes (células AbA): identificación de mecanismos y genes neurotóxicos. (2013-2014) Amount granted aprox USD 40.000
6. Movilidad Bilateral Uruguay-Brasil – DICYT (MEC) (2013-2015 - 18 month) Amount Granted USD 6.000.

Other activities

Monique Richter (ANII postdoc contract)
Luciana Negro (PhD Scholarship)
Emilia Villamil (Research Initiation Scholarship)
Alejandra Silva Santisteban (Research Initiation Scholarship)

Biomolecular Simulations Laboratory

Head: Sergio Pantano, PhD

Members:

Matías Machado, PhD. (Staff Member)

Humberto Gonzalez (Msc. Student)

Astrid Brandner (Msc. Student)

Sebastian Ferreira (Undergraduate Student)

Research

Development of a coarse-grained force field for molecular simulation.
The use of simplified molecular representations allows for a significant reduction in the computational cost of molecular simulations, helping to bridge the gap existing between the time and size scales of relevant biological systems and those accessible to state-of-the-art computer simulations.
During 2013 we continued with the development of a general purposes CG force field for biomolecular systems, for which we coined the name SIRAH. Two research papers were published about this topic (listed as number 1 and 5 in the publication list).
As a result from an exhaustive testing on a number of protein systems, we decided to continue refining some of the parameters representing intermolecular interactions. A significantly improved version is currently undergoing the final stages of testing and a research article describing this work will be submitted during 2014.
A substantial effort has been devoted to make the implementation of SIRAH user-friendly and straightforward in popular simulation packages. This resulted in a series of scripts, tutorials and input files freely available from our web site (www.sirahff.com).
Currently, CG simulation techniques have been applied by the group to study the problems described in the next two paragraphs.

Development of novel FRET indicators for cAMP.
In 2013 we continued applied some of the above mentioned models to the development of genetically encoded FRET sensors for local concentrations of cAMP. The relevance of this research resides in the fact that monitoring cascades of events in living cells requires non-destructive methods to follow biological processes. In collaboration with the groups directed by Drs. Buschiazzo and Oppezzo we are running a project to unravel the mechanistic details of functioning of one of the most widely used cAMP sensors, which is based on the allosteric mechanism of the protein EPAC1. Using CG simulations we determined the minimal extension of the protein fused to a GFP variant, which is able to bind cAMP, producing a measurable conformational change. After expression and purification, preliminary results indicate that the modeled chimerical protein behaves according to the theoretical predictions. Crystallization trials are on their way to further refine the 3D guesses furnished by simulations. Provided that an X-ray structure will be obtained, we will proceed to a second round of modeling aimed to design a new generation of sensors with improved dynamic range and localization properties. This project is being carried out in the framework of an intramural transversal project.

SNARE Mediated Membrane Fusion.
Neuroexocytosis is the fundamental physiological process that leads a cytosolic synaptic vesicle to bind and fuse with the presynaptic membrane, thereby releasing neurotransmitters. The SNARE proteins VAMP/Synaptobrevin, SNAP-25 and Syntaxin are core components of the apparatus that mediates neurotransmitter release. They first form a heterotrimeric complex and then an undetermined number of SNARE complexes assemble to form a supercomplex. During 2013, in collaboration with the group of Prof. Cesare Montecucco at the University of Padua, Italy, we published a paper providing direct evidence of protein-protein interactions between SNARE complexes at the neuromuscular junction (ref. 3 in publication list). On the base of this result and the mode of action of botulinic toxins on the SNARE proteins, we also proposed a new model for the assembly of a radial super-complex of SNARE proteins, which explains the role of the two regulatory proteins Complexin and Synaptotagmin (see ref. 2 in publication list).

Modeling *A.nidulans'* proteins related with calcium homeostasis and transport of urea.
As a side-line of research we undertook a collaborative work with different groups to apply standard modeling techniques. This helped to gain structural insights on different proteins of interest for which experimental 3D information is not available. In particular we modeled Calineurin variants to provide molecular explanations for the genetic bypass of the transcription factor crzA in calcium homeostasis. The identification of point mutants within EF-hands Calcium binding motifs provided support to the higher tolerance to Ca^{2+} levels in some particular *A. nidulans* strains (see ref. 4 in publication list).
We also carried out structure/function modeling of the urea/H^{+} combined with site-directed mutagenesis performed by the group of Dr. Ana Ramon, Fac. de Ciencias, UdelaR. In this case we were able to identify specific amino acids affecting the binding, recognition and/or translocation of urea. A research paper describing these findings was submitted for publication.

Modeling posttranscriptional modifications on VEGFR2.
The tyrosine kinase receptor VEGFR2 is a key regulator of angiogenesis interacting primarily with members of the VEGF family, and involving phosphorylation of various tyrosine residues in the intracytoplasmic portion of the receptor. Results obtained at the lab. of Prof. Mauro Giacca (ICGEB, Trieste) provided the first evidence that membrane-associated VEGFR2 in endothelial cells undergoes Lysine acetylation at a single lysine located in the kinase activation loop. While VEGFR2 acetylation counteracts the process of receptor desensitization following VEGF stimulation, it still allows for receptor phosphorylation and intracellular signaling. VEGFR2 mutant cells unable to be acetylated show reduced levels of receptor phosphorylation and impaired migratory capacity. Consistently, MD simulations suggest that acetylation of the lysine in the activation loop contributes to the transition to an open active state, in which tyrosine phosphorylation is favored by better exposure of the kinase target residues. A research paper describing these findings was submitted in Dic. 2013.

Publications

1. **Gonzalez HC, Darré, L. Pantano, S.** Transferable Mixing of Atomistic and Coarse-Grain Water Models. J. Phys. Chem. B, 2013, 117 :14438.
2. **Pantano S**, Montecucco C. The Blockade of the Neurotransmitter Release Apparatus by Botulinum Neurotoxins. Cell. Mol. Life Sci. 2013, DOI:10.1007/s00018-013-1380-7.
3. Megighian A, Zordan M, **Pantano S**, Scorzeto M, Rigoni M, Zanini D, Rossetto O, Montecucco C. Evidence for a radial SNARE super-complex mediating neurotransmitter release at the Drosophila neuromuscular junction. J. Cell. Sci., 2013, 136: 3134.
4. Almeida RS, Loss O, Colabardini AC, Brown NA, Bignell E, Savoldi M, **Pantano S**, Goldman MH, Arst HN Jr, Goldman GH. Genetic Bypass of Aspergillus nidulans crzA Function in Calcium Homeostasis. G3 (Bethesda), 2013, 3:1129.
5. Dans, P. D.; **Darré, L.; Machado, M. R.; Zeida, A.; Brandner, A. F.; Pantano, S.** Assessing The Accuracy Of The Sirah Force Field To Model Dna At Coarse Grain Level. In *Advances In Bioinformatics And Computational Biology*, Setubal, J. C., Almeida, N. F., Eds.; Springer International Publishing: 2013; Pp 71-81.

Grants

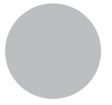
Proyecto Transversal – Sergio Pantano – “Rational design of FRETsensors to monitor cyclic nucleotides concetration in vivo” – 2013-2014 – IP Montevideo

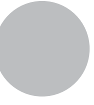
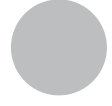
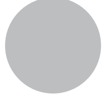
Other activities

1. We organized the “FOCEM Course: Introduction to Structural Biology and Bioinformatics” (Practical Tips for non Practitioners), 11th to 15th November, 2013, held at the Institut Pasteur de Montevideo.

2. SP and MM have delivered invited seminars and tutorials at:
   - First and second International Seminar at the University of Talca, Chile.
   - Argentinean Biophysical Society, Carlos Paz, Cordoba, Argentina
   - Universidad de Cs. Exactas, UBA, BsAs, Argentina
   - ICGEB, Trieste, Italy.

3. SP worked as member of the Program Committee of the Brazilian Symposium on Bioinformatics in 2013.



Molecular and Human Genetics Laboratory

Head: José Badano, PhD

Members:

Florencia Irigoín, PhD (Research associate)

Victoria Prieto, PhD (Postdoctoral Fellow)

Magdalena Cárdenas, MSc (PhD student – Graduated in 2013)

Cecilia Gascue, MSc (PhD student – Graduated in 2013)

Paola Lepanto (PhD student)

Rossina Novas, Bach (MSc student – Graduated in 2013)

Belén Torrado (MSc student)

Matías Fabregat (MSc student)

Research

Since 2007, the Molecular and Human Genetics lab has been focused in the study of ciliary biology, trying to understand how cilia are formed/maintained and how do they function in order to gain insight into the role of this organelle in diverse aspects of the cell´s life. In recent years, different lines of evidence have shown that ciliary dysfunction underlies the pathogenesis of a broad group of human disorders collectively known as ciliopathies. This group of human pathologies is characterized by a number of phenotypes including retinal degeneration, cystic kidney disease, obesity, and diabetes (1-3). One example of a ciliopathy, and the model we study in the lab, is Bardet-Biedl syndrome (BBS), a genetically heterogeneous disorder for which 18 genes have been cloned to date (Refs. 4-8 and references within). The vast majority of BBS proteins tested to date localize to centrosomes, basal bodies and in some cases the ciliary compartment, and they participate in the transduction of important developmental signaling pathways such as Shh and Wnt (both canonical and the planar cell polarity "PCP" pathway) (9-22). However, the exact biological role of these proteins is still not completely understood. Therefore, on one hand we are studying individual ciliary proteins, such as several of the BBSs, and on the other hand we are tackling more general questions related to cilia biology.

One protein in which we have been working is CCDC28B (coiled-coil domain containing protein 28b), a protein originally identified as a second site modifier of the BBS phenotype given that the mutation found in *CCDC28B* was not sufficient to cause BBS but did collaborate with mutations at *bona fide* BBS loci to modulate the penetrance and expressivity of the disorder (23). Thus we started working in this protein of unknown function to both gain information regarding its role in cilia biology and to understand, at the cellular and molecular level, why it behaves as a modifier of BBS. Through a combination of bioinformatics, cellular and *in vivo* (zebrafish) studies we were able to determine that CCDC28B is a conserved protein restricted to metazoa that participates in the regulation of ciliary length. We showed that depletion of this protein both in cultured cells and zebrafish results in shortened cilia and thus *ccdc28b* morphant zebrafish embryos present with a number of cilia-associated phenotypes such as shortening of the body axis, smaller eyes, defects in the establishment of the left-right axis of symetry and hydrocephalus (24).

To understand the mechanism by which CCDC28B modulates cilia length we sought to identify proteins that physically interact with it. In a yeast two-hybrid screen we identify an interaction with the mTORC2 component SIN1. We were able to show that the CCDC28B/SIN1 interaction is relevant both in the context of cilia length regulation as well as modulating mTORC2. In the context of the mTOR complex our data showed that CCDC28B participates in its assembly and/or mediates its stability and thus, a depletion of CCDC28B results in decreased activity of the complex whereas its overexpression has the converse effect. Regarding the role of CCDC28B in cilia length regulation, we were able to show that this activity of the BBS modifier depends, at least in part, on its interaciont with SIN1 but independently of mTORC2 since i) *sin1* morphant embryos, but not other mTORC2 component (rictor), present with shortened cilia, ii) *ccdc28b* and *sin1* interact genetically and iii) overexpression of *sin1* can partially ameliorate the cilia defect in *ccdc28b* morphant embryos (25). Interestingly, mTORC2 dysfunction resulted in cilia-related phenotypes albeit not affecting cilia directly. One possibility that we are currently exploring is that mTORC2 dysfunction could contribute to the pathogenesis of cilia-associated defects through a "PCP-like" phenotype, thus potentially providing a cellular explanation to the observed modifier effect of *CCDC28B*. Therefore, while we keep studying CCDC28B/SIN1 to gain mechanistic insight into their role in cilia regulation (CSIC Grant), the study of this particular protein has open new avenues of research in the lab.

Our initial studies on BBS7, which led to the demonstration that at least some BBS proteins play extraciliary roles in the nucleus modulating gene transcription (26), resulted in a similar process in the lab leading to a new line of research. In this project, which is being guided by Dr. Irigoín, we are interested in understanding the process of protein targeting to the cilium focusing on proteins that can localize to both the cilium and nucleus. A growing number of reports in the literature are highlighting striking similarities in the process of nuclear and cilia protein import (for example see Ref. 27). We are focusing on a number of proteins that shuttle between these two cellular compartments, including some of the BBSs, to understand whether they used similar mechanisms and if so, identify the signals that allow them to choose between destinations. To this end, we are working on an interdisciplinary collaboration with another unit at the IP-Mon (UByPA) where we plan to use a combination of cell/molecular biology and mass spectrometry to explore this cilia-nucleus connection (Intramural IPMon Grant).

More recently we have started working on another BBS protein, BBS4, since we have identified interesting protein interactors potentially linked to at least some of the BBS typical phenotypes. Our preliminary results started to highlight a role of BBS4 and other BBS proteins on intracellular trafficking, an area of research in which we became especially interested through a collaboration with Dr. Norann Zaghloul at University of Maryland, Baltimore, USA (manuscript accepted at Journal of Cell Science). Lastly, through a collaboration with Dr. Flavio Zolessi, we are studying the role of cilia during the formation and differentiation of neuronal cell types, in particular retinal ganglion cells (FCE Grant).

References

1. Badano JL, Mitsuma N, Beales PL, & Katsanis N (2006) The Ciliopathies: An Emerging Class of Human Genetic Disorders. *Annu Rev Genomics Hum Genet* 22:125-148.
2. Cardenas-Rodriguez M & Badano JL (2009) Ciliary Biology: Understanding the Cellular and Genetic Basis of Human Ciliopathies. *Am J Med Genet Part C Semin Med Genet* 151C:263-280.
3. Irigoin F & Badano JL (2011) Keeping the balance between proliferation and differentiation: the primary cilium. *Current genomics* 12(4):285-297.
4. Kim SK, *et al.* (2010) Planar Cell Polarity Acts Through Septins to Control Collective Cell Movement and Ciliogenesis. *Science* 329:1337-1340.
5. Leitch CC, *et al.* (2008) Hypomorphic mutations in syndromic encephalocele genes are associated with Bardet-Biedl syndrome. *Nat Genet* 40:443-448.
6. Marion V, *et al.* (2012) Exome sequencing identifies mutations in LZTFL1, a BBSome and smoothened trafficking regulator, in a family with Bardet--Biedl syndrome with situs inversus and insertional polydactyly. *J Med Genet* 49:317-321.
7. Otto EA, *et al.* (2010) Candidate exome capture identifies mutation of SDCCAG8 as the cause of a retinal-renal ciliopathy. *Nat Genet* 42:840-850.
8. Scheidecker S, *et al.* (2014) Exome sequencing of Bardet-Biedl syndrome patient identifies a null mutation in the BBSome subunit BBIP1 (BBS18). *J Med Genet* 51(2):132-136.
9. Ansley SJ, *et al.* (2003) Basal body dysfunction is a likely cause of pleiotropic Bardet-Biedl syndrome. *Nature* 425:628-633.
10. Blacque OE, *et al.* (2004) Loss of *C. elegans* BBS-7 and BBS-8 protein function results in cilia defects and compromised intraflagellar transport. *Genes Dev* 18:1630-1642.
11. Fan Y, *et al.* (2004) Mutations in a member of the Ras superfamily of small GTP-binding proteins causes Bardet-Biedl syndrome. *Nat Genet* 36:989-993.
12. Gerdes JM, *et al.* (2007) Disruption of the basal body compromises proteasomal function and perturbs intracellular Wnt response. *Nat Genet* 39:1350-1360.
13. Jin H, *et al.* (2010) The conserved Bardet-Biedl syndrome proteins assemble a coat that traffics membrane proteins to cilia. *Cell* 141:1208-1219.
14. Kim JC, *et al.* (2004) The Bardet-Biedl protein BBS4 targets cargo to the pericentriolar region and is required for microtubule anchoring and cell cycle progression. *Nat Genet* 36:462-470.
15. Kim JC, *et al.* (2005) MKKS/BBS6, a divergent chaperonin-like protein linked to the obesity disorder Bardet-Biedl syndrome, is a novel centrosomal component required for cytokinesis. *J Cell Sci* 118:1007-1020.
16. Li JB, *et al.* (2004) Comparative genomic identification of conserved flagellar and basal body proteins that includes a novel gene for Bardet-Biedl syndrome. *Cell* 117:541-552.
17. Loktev AV, *et al.* (2008) A BBSome subunit links ciliogenesis, microtubule stability, and acetylation. *Dev Cell* 15:854-865.
18. Marion V, *et al.* (2009) Transient ciliogenesis involving Bardet-Biedl syndrome proteins is a fundamental characteristic of adipogenic differentiation. *Proc Natl Acad Sci U S A* 10:1820-1825.
19. Nachury MV, *et al.* (2007) A core complex of BBS proteins cooperates with the GTPase Rab8 to promote ciliary membrane biogenesis. *Cell* 129(6):1201-1213.
20. Ross AJ, *et al.* (2005) Disruption of Bardet-Biedl syndrome ciliary proteins perturbs planar cell polarity in vertebrates. *Nat Genet* 37:1135-1140.
21. Wiens CJ, *et al.* (2010) Bardet-Biedl syndrome-associated small GTPase ARL6 (BBS3) functions at or near the ciliary gate and modulates Wnt signaling. *J Biol Chem* 285:16218-16230.
22. Zhang Q, Seo S, Bugge K, Stone EM, & Sheffield VC (2012) BBS proteins interact genetically with the IFT pathway to influence SHH-related phenotypes. *Hum Mol Genet* 21:1945-1953.
23. Badano JL, *et al.* (2006) Dissection of epistasis in oligogenic Bardet-Biedl syndrome. *Nature* 439:326-330.
24. Cardenas-Rodriguez M, *et al.* (2013) Characterization of CCDC28B reveals its role in ciliogenesis and provides insight to understand its modifier effect on Bardet-Biedl syndrome. *Hum Genet* 132(1):91-105.
25. Cardenas-Rodriguez M, *et al.* (2013) The Bardet-Biedl syndrome-related protein CCDC28B modulates mTORC2 function and interacts with SIN1 to control cilia length independently of the mTOR complex. *Hum Mol Genet* 22(20):4031-4042.
26. Gascue C, *et al.* (2012) Direct role of Bardet-Biedl syndrome proteins in transcriptional regulation. *J Cell Sci* 125:362-375.
27. Dishinger JF, *et al.* (2010) Ciliary entry of the kinesin-2 motor KIF17 is regulated by importin-beta2 and RanGTP. *Nat Cell Biol* 12(7):703-710.

Publications

1. **Cardenas-Rodriguez M**, **Irigoín F**, Osborn DP, **Gascue C**, Katsanis N, Beales PL, **Badano JL** (2013) The Bardet-Biedl syndrome-related protein CCDC28B modulates mTORC2 function and interacts with SIN1 to control cilia length independently of the mTOR complex. Hum Mol Genet 22:4031-4042

Human Resources

In the year 2013 three students from the lab defended their thesis and graduated:

- Cecilia Gascue completed her PhD studies with the thesis "Análisis funcional de proteínas del síndrome de Bardet-Biedl: vinculando proteínas ciliares con la regulación de la expresión génica"
- Magdalena Cárdenas-Rodríguez completed her PhD studies with the thesis "Caracterización del rol biológico de CCDC28B, un modificador secundario del síndrome de Bardet-Biedl"
- Rossina Novas completed her MSc. Studies with the thesis "Análisis de los mecanismos que regulan la expresión, localización y función de CCDC28B, un modificador del Síndrome de Bardet-Biedl"

Grants and Fellowships

1. Doctoral Fellowship – Magdalena Cárdenas – 2012-2013 – CSIC
2. Master Fellowship - Rossina Novas – 2011-2013 – ANII
3. Master Fellowship – Belén Torrado – 2013-2015 – ANII
4. Doctoral Fellowship – Paola Lepanto – 2013-2016 – ANII
5. Master Fellowship – Matías Fabregat – 2014-2016 – ANII
6. Fondo Clemente Estable – Dr. Flavio Zolessi – "Rol de las cilias y proceso de ciliogénesis durante la generación y diferenciación de neuronas en el sistema nervioso central de vertebrados."- 2013-2015 – ANII
7. CSIC Project – Florencia Irigoín – "Estudios funcionales y estructurales de CCDC28B, un modificador del Síndrome de Bardet-Biedl." – 2013-2015 – I+D Program, CSIC, UDELAR
8. Proyecto Transversal – Florencia Irigoín – "Protein sorting and transport to the ciliar and nuclear compartments: common and distinctive mechanisms" – 2013-2014 – IP Montevideo

Head: Pablo S. Aguilar, PhD

Members:

Agustina Olivera-Couto (PhD Student, full time, since October 2013). *Plasma membrane organization*.

Milagros Mailhos (M. Sc. Student, part time, since March 2011). *Insecticidal Yeasts*.

Marcos Nieves (M. Sc. Student, part time, since Aug 2012). *Plasma membrane signaling*.

Valentina Salzman (Postdoc, full time, since Sept 2012). *Cell-cell fusion*.

Natalia Carbó (Postdoc, full time, since April 2013). *Cell-cell fusion*.

Daniela Megrian (undergraduate student, part time, since Dec 2012). *Cell-cell fusion*.

Carolina do Pazo (undergraduate student, part time, since August 2012). *Plasma membrane organization*.

Research

Focusing on the plasma membrane, we seek to understand how the cell organizes at its boundaries.

1. **EISOSOMES AND PLASMA MEMBRANE ORGANIZATION**

Eisosomes define sites of endocytosis in *Saccharmoyces cerevisiae* (*Nature*, 439: 998-1003, 2006). These structures, lay underneath the plasma membrane and are mainly composed by thousands of copies of two paralog proteins Pil1 and Lsp1 (*Mol Bio Cell*, 20, 809-818, 2009). Eisosomes are plasma membrane domain organizers that concentrate lipids and proteins around them. In doing so, they are thought to help coordinate spatial and temporal execution of diverse plasma membrane functions such as small molecules transport, signaling and bulk endocytosis (*EMBO J.*, 26, 4946-4955, 2007; *J Cell Biol.* 185:1227-1242, 2009; *Nat Struct Mol Biol*, 17: 901-908, 2010).
We have found that Pil1 and Lsp1 belong to a superfamily of proteins that work as membrane shape modifiers. Overall, our results support a model where eisosome core proteins-mediated bending of the plasma membrane is followed by local recruitment of proteins and lipids resulting in stable plasma membrane domains (*Mol Bio Cell, 22: 2360-2372*, 2011, *Mol Genet Genomics* 287: 607-620, 2012).
We are currently challenging this model applying different live fluorescence microscopy approaches. By studying the dynamics of eisosome biogenesis we expect to build a quantitative model of plasma membrane domain organization.

2. CELL-CELL FUSION

Eukaryotic cells use intracellular membrane fusion events to move cargo through the various compartments while maintaining compartmental identity. Membrane fusion also when gametes fuse their cell membranes to form zygotes and when enveloped viruses deliver their genome into host cells during infection. Membrane fusion reactions are catalyzed by proteins collectively referred to as "fusases". Remarkably, to this day, only intracellular and viral fusases have been identified and characterized. Cell-cell fusion stands as the field where the fusases still remain elusive.
We use yeast mating as a model of cell-cell fusion. We identified new proteins involved in this process and studied the role that extracellular calcium plays in cell fusion and cellular lysis (*Mol Biol Cell.* 18, 547-556, 2007). We also determined the structural role that sterols play during cell polarization and cell fusion (*Proc Natl Acad Sci USA.*, 107: 4170-4175, 2010). Overall, our results support a model in which the still unidentified cell fusion machinery promotes both: fusion and lysis. In collaboration with the Bioinformatics Unit of the IP Montevideo we are currently executing a battery of genetic and bioinformatic screens to identify cellular fusases. Envisioning a large set of data to be experimentally analyzed we are currently devolping a high-throughput cell-cell fusion assay in collaboration with the Cell Biology Unit of our institute. We are also analyzing the role of different genes involved in pheromone-induced calcium uptake as lysis enhancers.

3. DEVELOPMENT OF YEASTS WITH INSECTICIDAL CAPACITY

Development of new biocontrol agents is a valuable alternative to the vast use of chemical pesticides. In the recent years, the use of different yeasts as biocontrol agents has been widely reported. In collaboration with Celia Carlini (Universidade Federal do Rio Grande do Sul, Brasil) and Enrique Castiglioni (Estación Experimental Dr. Mario A. Cassinoni, Universidad de la República, Uruguay) we are developing yeast strains that produce the plant-derived entomotoxic peptide Jaburetox. With this project we expect to develop new biocontrol agents that can be used against different insects that affects soybean, cotton and corn production.

4. CELL BIOLOGY OF BACTERIAL SIGNAL TRANSDUCTION

In collaboration with Alejandro Buschiazzo's lab (IP Mont) and Mathieu Picardeau (IP Paris) we are addressing the subcelular localization of the two-component fluidity sensor DesK-DesR. In Gram-positive bacteria this system is crucial to adjust plasma membrane fluidity according to need (*EMBO J.* 20, 1681-1691, 2001; *Proc Natl Acad Sci USA*, 106: 16185-16190, 2009). Subcelular compartmentalization of signal transduction systems is a common eukayotic feature that is currently poorly studied in prokaryotic organisms. We hope to expand the knowledge we gain throughout this model to study similar systems in the pathogenic bacteria of the genus *Leptospira*.

Publications 2013

1. **Aguilar, P.S.**; Baylies, M.K.; Fleissner, A.; Helming, L.; Naozaku, I.; Podbilewicz, B.; Wang, H-M. and Wong, M. (2013) Genetic Basis of cell-cell fusion mechanisms. *Trends Genet*., 29: 427-437.
2. Zhang, S.; Zheng, H.; Long, N.; **Carbó, N.**; Chen, P.; **Aguilar, P.S.** and Lua, L. (2014) FigA, a Putative Homolog of Low-Affinity Calcium System Member Fig1 in *Saccharomyces cerevisiae*, Is Involved in Growth and Asexual and Sexual Development in *Aspergillus nidulans. Eukaryot Cell*.doi: 10.1128/EC.00257-13.

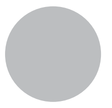
Grants

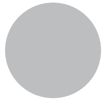
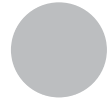
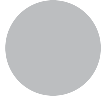
1. “Yeast Mating as a model of Cell-Cell Fusion”. International Centre for Genetic Engineering and Biotechnology Collaborative Research Programme (ICGEB-CRP). Funding period 2012-2015. € 57.000.
2. “Cell signaling in bacterial pathogenesis: iron metabolism regulation in Leptospira as a working model”. Réseau International des Institut Pasteur (RIIP). Funding period: 2012-2013. € 21.900.
3. “Apareamiento de levaduras como modelo de fusión célula-célula”. ANII-FCE-6682. Funding period: 2013-2014. USD 28.000.
4. “Análisis cuantitativo de ensamble de dominios de membrana mediante microscopía de fluorescencia in vivo.” ANII-FCE-5942. Funding period: 2013-2014. USD 13.000.

Other funding

FELLOWSHIPS and AWARDS

- Milagros Mailhos: ANII Masters fellowhip, 2012-2013.
- Valentina Salzman: CONICET postdoctoral fellowship, 2012-2014. EMBO Traveling Award, 2013.
- Agustina Olivera-Couto, ANII Doctoral fellowship, 2013-2015. Journal of Cell Science and American Society and international Union for Biochemistry and Molecula Biology, (ASBMB/IIUBMB) Traveling Awards, 2013.
- Natalia Carbó, ANII- Caldeyro Barcia postdoctoral fellowship, 2013-2015.



Redox Biology of Trypanosomes Laboratory

Head: Marcelo Comini, PhD

Members:

Andrea Medeiros (Postdoc)

Mariana Bonilla (Postdoc, from October 2012 on)

Katerina Doleckova (Calmette-Postdoc, from January 2013 on)

Bruno Manta (PhD Student)

Cecilia Ortíz (PhD Student)

Diego Benitez (Technical Assistant)

Lucía Fiestas (MSc Student)

Florencia Sardi (MSc Student)

Diego Charquero (Undergraduate student, until September 2013)

Research

Several trypanosomatid species cause highly disabling and often fatal diseases of human and live-stock (e.g. African sleeping sickness, Chagas' disease, black-fever and Nagana cattle-disease, and Sura disease), for which safe and efficacious treatments are lacking. Trypanosomatids have evolved a specific thiol-redox metabolism that relies on the use of bis-glutathionylspermidine (trypanothione) as low molecular mass thiol cofactor and ligand. Trypanothione is involved in a variety of essential cellular processes and, important from a therapeutic point of view, is absent in mammals. By means of a multidisciplinary approach, research in our laboratory aims at:

1. gain further understanding into trypanothione-depedent metabolism by studying its synthesis, recycling and role in metal and redox homeostasis.
2. exploit the use of novel redox biosensors to unravel fundamental questions on parasite biology and host/parasite interaction.
3. identify and characterize anti-parasitic compounds and novel drug target candidates.

1. **Fundamental aspects of trypanothione metabolism: synthesis, reduction and utilization.**

Several proteins involved in the synthesis, recycling of the reduced form and utilization of trypanothione are indispensable for parasite survival and/or virulence, and have evolved specific structural, biochemical and biological features that make them suitable drug target candidates (Manta et al. 2013, Comini et al. 2013a and b, Sardi et al. 2013). The functional and structural analysis of several key components from the trypanothione system remains poorly investigated and deems extremely important not only for understanding the role they play in parasite biology and pathogenesis but also to guide novel drug development strategies.

2. **Monitoring intracellular redox changes with novel redox biosensors**

Short lived reactive oxygen or nitrogen species act as second messengers producing changes in the redox poise of relevant redox couples within biological systems. A steadily increasing number of evidences support a key role for redox signaling in the regulation of a wide diversity of cellular and (patho)physiological processes. The measurement of physiological oxidants and intracellular redox changes on real-time and by non-invasive methods has recently been possible due to the development of fluorescent redox biosensors [Meyer and Dick 2010]. Transgenic cell lines of *T. brucei* and *T. cruzi* expressing a redox biosensor have been generated in our lab. and are currently used to address the role of redox signaling and oxidative stress in events such as parasite-host interaction, cell differentiation, cell cycle, apoptosis and metabolic dysfunction.

3. **Early phase drug discovery pojects**

Several hundreds of compounds have been screened against TryS from three major trypanosomatids (*L. infantum*, *T. brucei* and *T. cruzi*) using a colorimetric assay on a HTS format. The main conclusions of this study were the detection of a remarkable species-specific inhibition of TrySs and the identification of the potential residues responsible for this behavior in the *T. brucei* enzyme (Benítez et al. in preparation). Several of these compounds are being further optimized by our collaborators on organic chemistry (www.costcm0801.org). With our collaboration several metal-based and organic compounds from local and international groups have been identified as potent and selective anti-trypanosomal agents [Férnandez et al. 2013, Peña et al. 2012, Demoro et al. 2012]. The relevance of several drug target candidates (e.g. lipoamide dehydrogenase, tryparedoxin peroxidase, 1-C-Grx1, TryS, O-Phosphoseryl-tRNA:selenocysteinyl-tRNA synthase) was assessed in a murine infection model. Ongoing validation studies include several other potential drug target candidates. Future compounds to be evaluated in animal infection models include novel inhibitors of tryparedoxin and glucose-6-phosphate dehydrogenase and compounds that arose as potent trypanocidal agents from cell-based *in vitro* screening conducted in our lab.

The ultimate goal of our research is to provide valuable information for the development and implementation of safe and efficacious drugs and/or therapies to treat trypanosomiasis.

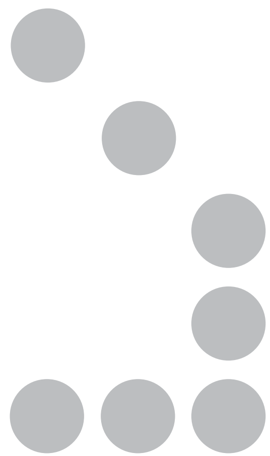
Publications

1. Fernández M., Becco L., Correia I., Benítez J., Piro O.E., Echeverria G.A., **Medeiros A.**, **Comini M.**, Lavaggi M.L., González M., Cerecetto H., Moreno V., Pessoa J.C., Garat B. and Gambino D. (2013) Oxidovanadium(IV) and dioxidovanadium(V) complexes of tridentate salicylaldehyde semicarbazones: Searching for prospective antitrypanosomal agents. *J. Inorg Biochem.*
2. **Manta B., Comini M., Medeiros A.**, Hugo M., Trujillo M. and Radi R. (2013) Trypanothione: A unique bis-glutahionyl derivative in trypanosomatids. *Biochim. Biophys. Acta.* 1830, 3199-3216
3. **Sardi F.**, **Manta B.**, Portillo-Ledesma S., Knoops B., **Comini M.** and Ferrer-Sueta G. (2013) Determination of acidity and nucleophilicity in thiols by reaction with monobromobimane and fluorescence detection. *Anal. Biochem.* 435, 74-82
4. **Comini M.A.**, Flohé L. (2013) Chapter: The Trypanothione System, In: Drug Discovery for Trypanosomatid Diseases. Ed. Leopold Flohé, Timo Jäger and Oliver Koch. *Wiley-Blackwell*, Oxford, UK. ISBN: 978-3-527-33255-7.
5. **Comini M.A.**, Cazzulo J. J. and **Ortíz C.** (2013) Chapter: The Pentose Phosphate Pathway, In: Drug Discovery for Trypanosomatid Diseases. Ed. Leopold Flohé, Timo Jäger and Oliver Koch. *Wiley-Blackwell*, Oxford, UK. ISBN: 978-3-527-33255-7.

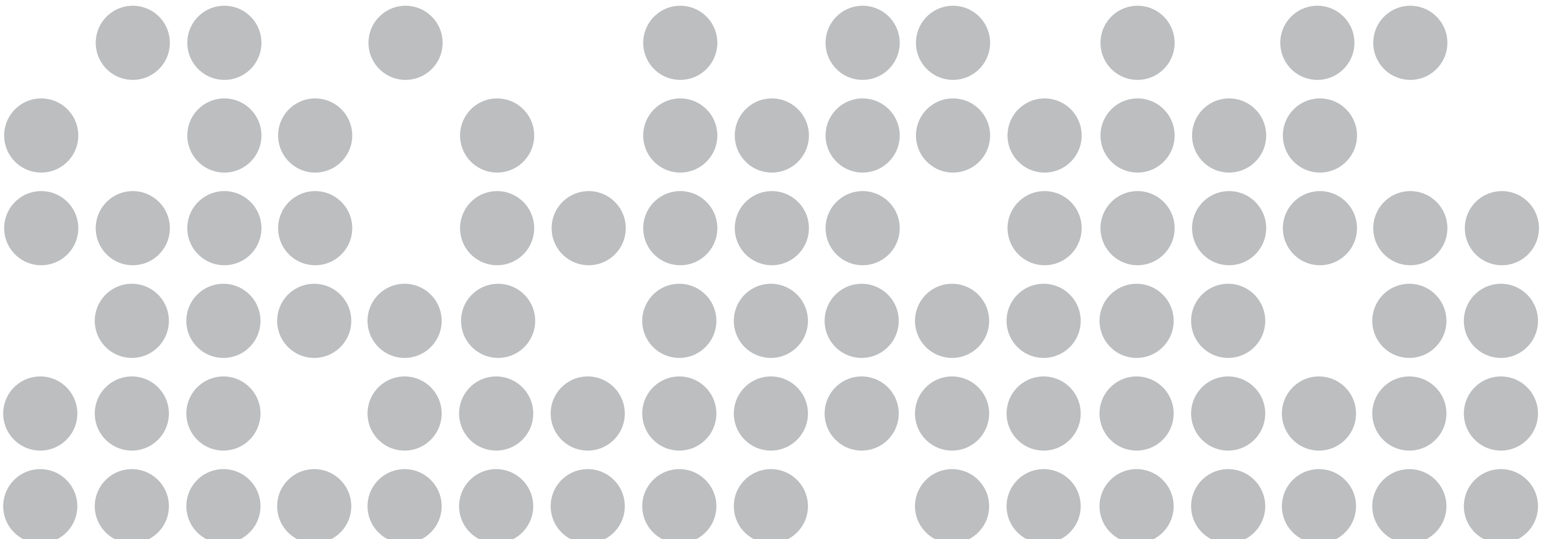
Other activities

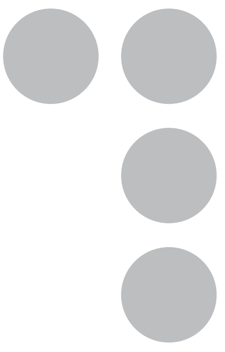
Education and Training

1. Lab. members (Bonilla M., Manta B., Sardi F. and Comini M.) collaborated with the organization of the Course "Química y Biología Redox de Tioles - 2013" PEDECIBA Biología y Química. 10/06/13 - 24/06/13, Montevideo, Uruguay.
2. Diego Benítez dictated a practical workshop ("Expresión, purificación y cribado de compuestos contra la tripanotión sintetasa de tripanosomátidos") at the Centro Nacional de Investigaciones Científicas de El Salvador (CICES), El Salvador, July 15th-17th 2013.
3. The development of expertise in NMR techniques within the group has been promoted by supporting a 2 months traineeship of Dr. Bruno Manta at the Department of Chemical Sciences, University of Padova, Italy (Oct.-Dec. 2013).



YOUNG GROUP LEADERS LABORATORIES





G5 - Epigenetics of Cancer and Aging Laboratory

Head: Ruben Agrelo, MD

Members:

Miguel Arocena Sutz (Postdoctoral Fellow)

Santiago Gonzalez Volpe (MSc Student)

Fabian Aldunate (BSc Student)

Valeria Da Costa (BSc Student)

Research

Our group is interested in the influence of epigenetics on cell proliferation, cell senescence and cell differentiation. We want to understand how epigenetics impacts cell and tissue aging, cancer progression and response to cancer therapies.

1. **EPIGENETICS AT THE AGING-CANCER INTERFACE**

Abnormal epigenetic signaling plays a critical role in tumorigenesis. Epigenetic changes can also be important determinants of cellular senescence and organism aging. The best-defined epigenetic modifications are DNA methylation and histone posttranscriptional modifications. The best known examples are global loss of DNA methylation in aging and cancer and the promoter hypermethylation of genes with a dual role in tumor suppression and progeria, such as WRN and LMNA.
In particular we are interested in exploring how epigenetic alterations are accumulated during aging and how these events contribute to the cell transformation process.

2. **EPIGENETICS AND NUCLEAR MECHANICS**

A-type lamins are essential components of the nuclear lamina. Together with B-type lamins, they are the most prominent intermediate filaments forming this network of 10-nM diameter filaments located on the inner side of the nuclear membrane. LMNA has been found to be hypermethylated in hematological tumors. Moreover LMNA is mutated in aging related diseases such as Hutchinson Guilford progeria (HGPS). By the activation of a cryptic donor splice site progerin is expressed in HGPS cells affecting the epigenetic control of facultative and constitutive heterochromatin.
We want to elucidate how these epigenetic modifications impact in nuclear mechanics.

3. **EPIGENETIC CONTEXT OF CANCER PROGENITORS**

Using tools derived from the mammalian dosage compensation system are useful for defining the epigenetic context for Xist mediated silencing. This has led to the identification of an epigenetic context that is linked to the potential of Xist to induce chromosome-wide gene silencing in cancer progenitors.
The aim of this project is to better characterize this cellular context and its components. As a consequence we expect to gain insight into normal cell biology and identify novel therapeutic targets in cancer.

Publications

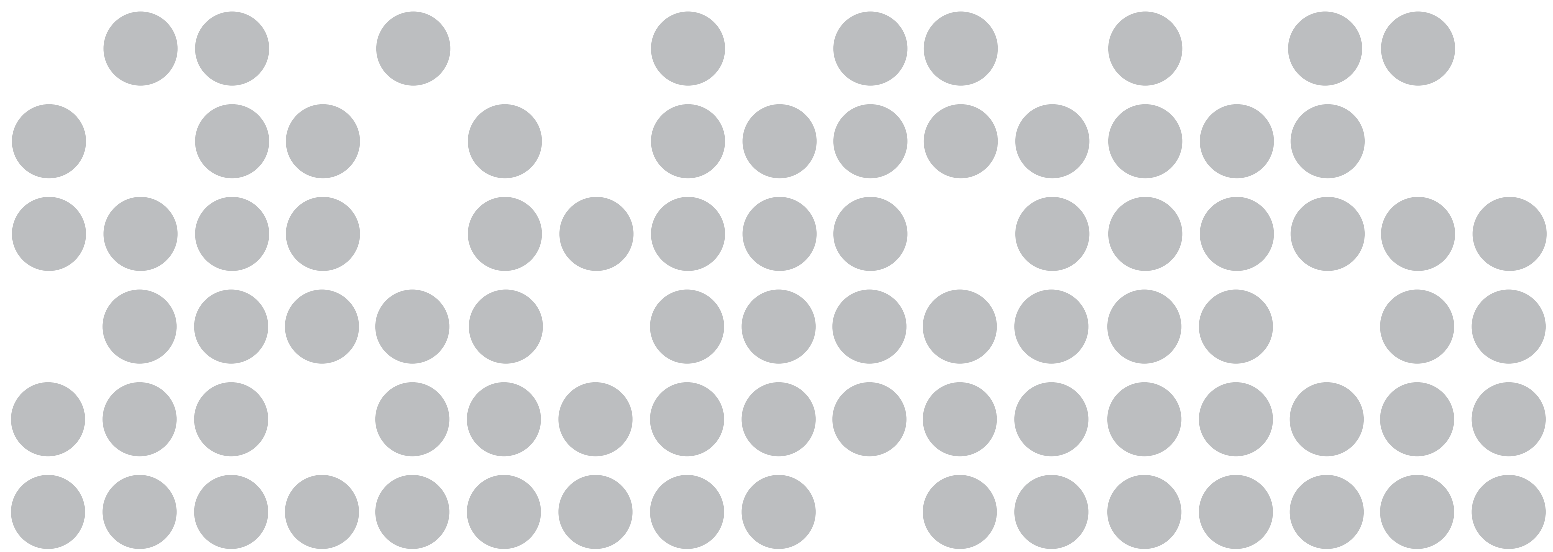
1. **Agrelo R**, Kishimoto H, Novatchkova M, **Peraza V**, Paolino M, Souabni A, Wutz A. SATB1 collaborates with loss of p16 in cellular transformation. Oncogene. 2013 Nov 28;32(48):5492-500. doi: 10.1038/onc.2013.158. Epub 2013 May 20.

Other activities

TRAINING COURSES

1. Course "Aproximaciones modernas al estudio epigenético del envejecimiento y cancer", 29th April to 31st May, 2013.

ASSOCIATED AND MIXED UNITS



Laboratory of Immunology and Inflammation

Head: Marcelo Hill, MD, PhD

Members:

Mercedes Segovia (Postdoc)

Sofia Russo (MSc student)

Maria Eugenia Schroeder (MSc student)

Mariana Seija (Trainee)

Florencia Rammauro (Trainee)

Research

Dysregulation of immune responses leads to chronic inflammatory disorders collectively called immune-mediated inflammatory diseases (IMIDs). More than 80 clinically distinct diseases have been identified within this category, including classical autoimmune diseases, graft rejection and graft-versus host disease, asthma and atopy, psoriasis, immunodeficiencies, and chronic inflammatory diseases such as inflammatory nephropathies and atherosclerosis (Baeten 2009). IMIDs affect approximately 10 percent of the population and, for reasons that are not yet clear, the prevalence of these diseases appears to be rising. Cancer is another pathophysiological scenario where inflammation is part of the natural history of the disease. Furthermore, the biological basis of the effect obtained with pharmacological agents such as hydroxychloroquine (HCQ), currently used to impair immune-mediated damage, are not fully understood. Basic research is therefore needed to better characterize immunoregulatory mechanisms and new targets to control inflammation.

We have recently described new physiologic mechanisms which can control immune-mediated damage (Guillonneau et al. 2007; Hill et al. 2007a; Hill et al. 2007b; Hill et al. 2011). This knowledge can help to understand the natural history of IMIDS at the cellular and molecular level. Moreover, characterization of novel immunoregulatory mechanisms is an important issue to rationalize immunointerventional strategies (Hill et al. 2011) as well as to understand the pharmacodynamics of currently used anti-inflammatory drugs.
The laboratory of immunoregulation and inflammation has been established at the IP Montevideo in October 2013. Our laboratory studies cellular and molecular mechanisms which can control inflammation and adaptive immune responses. We are focused on the biology of dendritic cells (DCs). This is a particular subset of leukocytes which can trigger effector but also regulatory immune responses.

We are interested in the study of inflammasomes activation. Inflammasomes are cytoplasmic multi-protein complexes composed of a sensor protein (NOD-like) and an adaptor (ASC) which, once assembled, are able to activate pro-caspase-1 into caspase-1. Some of the targets of activated caspase-1 are pro-IL-1beta and pro-IL-18. Proteolytical process of these precursors by caspase-1 transforms them into active cytokines with a powerful pro-inflammatory action. In the last years, the cytoplasmic ionic composition has emerged as a one of the most studied mechanisms by which inflammasome activation is regulated (Latz et al. 2013). Indeed, characterizing new ion channel will certainly shed light on original mechanisms of inflamasomme activation. This context gives the main rational bases of our lines of investigation.

1. Study of the impact of new ion channels on the activation of inflammasomes.
2. Analysis of novel ion channels in cancer patients and in tumoral experimental models.
3. Characterization of novel anti-inflammatory mechanisms triggered by hydroxychloroquine through the inhibition of ion channels.
4. Study of the expression of new ion channels in autoimmune patients.

Baeten, D. (2009). “Memorandum of understanding for the implementation of a European Concerted Research Action designates as COST Action BM0907: European Network for Translational Immunology Research and Education (ENTIRE): From Immunomonitoring to personalized immunotherapy.”

Guillonneau, C., M. Hill, F. X. Hubert, E. Chiffoleau, C. Hervé, X.-L. Li, M. Heslan, C. Usal, L. Tesson, S. Ménoret, A. Saoudi, B. Le Mauff, R. Josien, M. C. Cuturi and I. Anegon (2007). «CD40Ig treatment results in allograft acceptance mediated by CD8+CD45RClow T cells, IFN-gamma and indoleamine 2,3-dioxygenase.» J Clin Invest **117**(4): 1096-106.

Hill, M., S. Tanguy-Royer, P. J. Royer, C. Chauveau, K. Asghar, L. Tesson, F. Lavainne, S. Rémy, R. Brion, F. X. Hubert, M. Heslan, M. Rimbert, L. Berthelot, J. Moffett, R. Josien, M. Gregoire and I. Anegon (2007a). “IDO expands human CD4+CD25high regulatory T cells by promoting maturation of LPS-treated dendritic cells.” Eur J Immunol. **37**(11): 3054-62.

Hill, M., R. Zagani, C. Voisine, C. Usal and I. Anegon (2007b). “Nitric oxide and indoleamine 2,3-dioxygenase mediate CTLA4Ig-induced survival of heart allografts in rats.” Transplantation **84**(8): 1060-3.

Hill, M., P. Thebault, M. Segovia, C. Louvet, G. Beriou, G. Tilly, E. Merieau, I. Anegon, E. Chiffoleau and M. C. Cuturi (2011). “Cell therapy with autologous tolerogenic dendritic cells induces allograft tolerance through interferon-gamma and epstein-barr virus-induced gene 3.” Am J Transplant **11**(10): 2036-45.

Latz, E., TS. Xiao and A. Stutz. (2013). “Activation and regulation of the inflammasomes”. Nat Rev Immunol **13**: 397-411

Publications

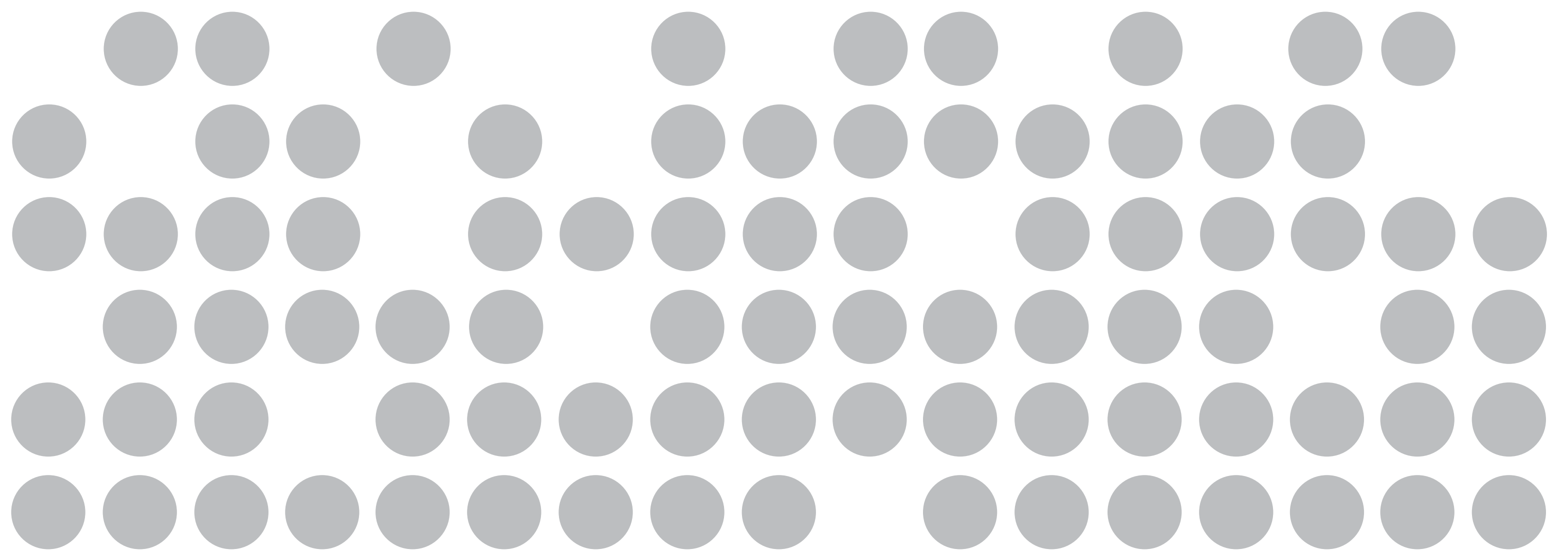
1. Tardif V, Riquelme SA, Remy S, Carreño LJ, Cortés CM, Simon T, **Hill M,** Louvet C, Riedel CA, Blancou P, Bach JM, Chauveau C, Bueno SM, Anegon I, Kalergis AM. Carbon monoxide decreases endosome-lysosome fusion and inhibits soluble antigen presentation by dendritic cells to T cells. Eur J Immunol. 2013 Nov;43(11):2832-44

Other activities

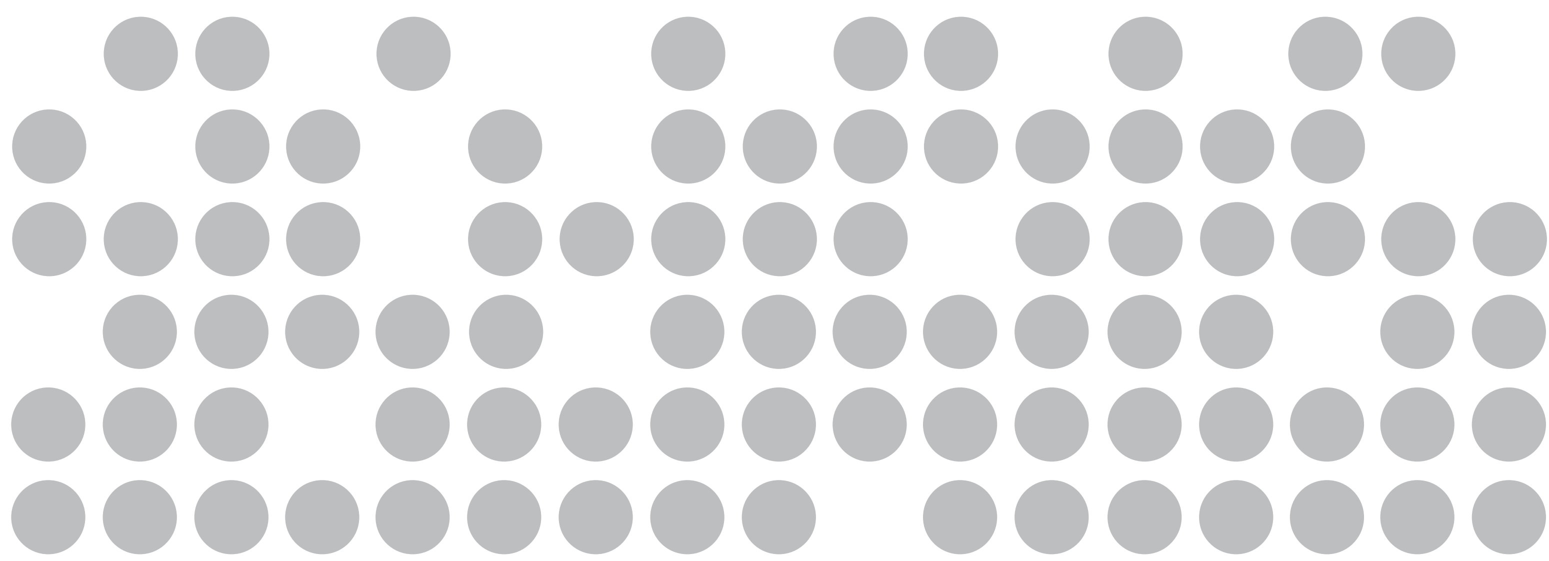
Conferences and courses
2013

- Pharmacodynamics of immunological biological drugs used in medicine. Post-graduate course. Faculty of Medicine. Coordinators: Drs Marcelo Hill, Caroline Agorio and Eduardo Osinaga.
- VIII Jornadas de la Sociedad de Bioquímica y Biología Molecular. Regulation of inflammation through ion metabolism in dendritic cells. Dr Marcelo Hill. Montevideo, Uruguay.

IP Montevideo
Courses and Publications



PUBLICATIONS



2007

1. **Parodi-Talice A,** Monteiro-Goes V, Arrambide N, Avila AR**, Duran R, Correa A,** Dallagiovanna B, **Cayota A,** Krieger M, Goldenberg S, **Robello C.** Proteomic analysis of metacyclic trypomastigotes undergoing *Trypanosoma cruzi* metacyclogenesis. (2007) J Mass Spectrom. 42(11):1422-32.
2. Pérez-Díaz L, Duhagon MA, Smircich P, Sotelo-Silveira J, **Robello C**, Krieger MA, Goldenberg S, Williams N, Dallagiovanna B, Garat B**.** *Trypanosoma cruzi*: Molecular characterization of an RNA binding protein differentially expressed in the parasite life cycle. (2007) Exp Parasitol. 117: 99-105.
3. Guerin ME, Kordulakova J, Schaeffer F, Svetlikova Z, **Buschiazzo A**, Giganti D, Gicquel B, Mikusova K, Jackson M, Alzari PM. Molecular recognition and interfacial catalysis by the essential phosphatidylinositol mannosyltransferase PimA from mycobacteria. (2007) *J Biol Chem.* 282:20705-14.
4. Neres J, Bonnet P, Edwards PN, Kotian PL, **Buschiazzo A**, Alzari PM, Bryce RA, Douglas KT. Benzoic acid and pyridine derivatives as inhibitors of *Trypanosoma cruzi* trans-sialidase. (2007) *Bioorg Med Chem.* 15:2106-19.
5. Shepard W, Haouz A, **Graña M**, **Buschiazzo A**, Betton JM, Cole ST, Alzari PM. The crystal structure of Rv0813c from *Mycobacterium tuberculosis* reveals a new family of fatty acid-binding protein-like proteins in bacteria. (2007). *J Bacteriol.* 189:1899-904.
6. **Graña M**, Haouz A, **Buschiazzo A**, Miras I, Wehenkel A, Bondet V, Shepard W, Schaeffer F, Cole ST, Alzari PM. The crystal structure of *M. leprae* ML2640c defines a large family of putative S-adenosylmethionine-dependent methyltransferases in mycobacteria. (2007) *Protein Sci.* 16:1896-904.
7. Menchaca A, Vilariño M, **Crispo M**, Pinczak A and Rubianes E. Day 0 Protocol: Superstimulatory treatment initiated in the absence of a large follicle improves ovarian response and embryo yield in goats. (2007) Theriogenology 68: 1111-1117.
8. Urioste JI, Chang YM, **Naya H,** Gianola D. Genetic variability in calving success in Aberdeen Angus cows under extensive recording. (2007). Animal 1:1081-8.
9. Sabbia V, Piovani R, **Naya H**, Rodriguez-Maseda H, Romero H, Musto H. Trends of amino acid usage in the proteins from the human genome. (2007). J. Biomol Struct Dyn. 25:55-9.
10. Diehl, , Furstenau de Oliveira, L, Sánchez, G, Camboim, C, de Oliveira Alvares,L, Bispo Lanziotti, V, **Cerveñansky, C**, Kornisiuk, E, Jerusalinsky, D y Quillfeldt, J A. Facilitatory effect of the intra-hippocampal pre-test administration of MT3 in the inhibitory avoidance task. (2007) Behavioural Brain Research 177:221-231, 2007.
11. Pehar M, Vargas MR, Robinson KM, **Cassina P**, **Diaz-Amarilla PJ**, Hagen TM, Radi R, **Barbeito L,** Beckman JS. Mitochondrial superoxide production and nuclear factor erythroid 2-related factor 2 activation in p75 neurotrophin receptor-induced motor neuron apoptosis. (2007) J Neurosci. 27:7777-85.
12. **Peluffo H.**, González P., Arís A., Acarin L., Villaverde A., Castellano B., González B. RGD domains neuroprotect the immature brain by a glial dependent mechanism. (2007) Annals of Neurology. 62:251-261
13. Walther, T.C., **Aguilar P.S.,** Frölich, F., Chu, F. Moreira, K., Burlingame, A.L. and Walter, P. Pkh-kinases control eisosome assembly and organization. (2007) *EMBO J.*, 26, 4946-4955.
14. Gerdes JM, Liu Y, Zaghloul NA, Leitch CC, Lawson SS, Kato M, Beachy PA, Beales PL, Demartino GN, Fisher S, **Badano JL**, Katsanis N. Disruption of the basal body compromises proteasomal function and perturbs intracellular Wnt response. (2007) Nat Genet 39:1350-1360.
15. Hallek M, Cheson BD, Catovsky D, Caligaris-Cappio F, **Dighiero G**, Döhner H, Hillmen P, Keating MJ, Montserrat E, Rai KR, Kipps TJ. International Workshop on Chronic Lymphocytic Leukemia. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines (2007) BLOOD 111 :5446-5456
16. Véronèse L, Tournilhac O, Verrelle P, Davi F, **Dighiero G**, Chautard E, Veyrat-Masson R, Kwiatkowski F, Goumy C, Vago P, Travade P, Tchirkov A. Low MCL-1 mRNA expression correlates with prolonged survival in B-cell chronic lymphocytic leukemia (2007) LEUKEMIA Volume: 22 Issue: 6 Pages: 1291-1293
17. Rebecca L., A; **Guillaume, D.**; Lynn, R., G.; Syndercombe-Court, D.; Jones, C.; McElwaine, S.; Newland, D.C.; Fegan, C.D.; Caporaso, N.; Cotter, F.E.. Trinucleotide repeat dynamic mutation identifying susceptibility in familial and sporadic chronic lymphocytic leukaemia (2007) BRITISH JOURNAL OF HAEMATOLOGY Volume: 136 73-79
18. Stamatopoulos K, Belessi C, Moreno C, Boudjograh M, Guida G, Smilevska T, Belhoul L, Stella S, Stavroyianni N, Crespo M, Hadzidimitriou A, Sutton L, Bosch F, Laoutaris N, Anagnostopoulos A, Montserrat E, Fassas A, **Dighiero G,** Caligaris-Cappio F, Merle-Béral H, Ghia P, Davi F. Over 20% of patients with chronic lymphocytic leukemia carry stereotyped receptors: pathogenetic implications and clinical correlations (2007) BLOOD 109 259-270

2008

19. Sudarman E; **Bollati-Fogolin M;** Hafner M; Mueller W; Scheller J; Rose-John S; Eichler J. Synthetic mimetics of the gp130-binding site for viral Interleukin-6 as inhibitors of the vIL-6 – gp130 interaction. (2008) Journal of Peptide Research, 2008, v. 71 5, p. 494-500.
20. Medeiros A, Chiribao ML, Ubillos L**, Festari MF,** Saldaña J**, Robello C,** Domínguez L, Calvete JJ **and Osinaga E.** Mucin-type *O*-glycosylation in *Mesocestoides vogae* (syn. corti)-Int. (2008) J. Parasitol 38 (3-4): 265-276.
21. Piñeyro MD, Parodi-Talice A, Arcari T, Robello C. Peroxiredoxins from *Trypanosoma cruzi*: Virulence factors and drug targets for treatment of Chagas disease? (2008) Gene. 2008 Jan 31;408 (1-2):45-50.
22. Galletti J; Cañones C; **Oppezzo P;** Morande P; Geffner J; Bezares FR; Giordano M. Chronic lymphocytic leukemia cells bind and present the erythrocyte protein band 3: possible role as initiators of autoimmune hemolytic anemia. (2008) Journal of Immunology, (5): Sep 1, p.3674-3683,
23. Buschiazzo, A. and Alzari, P.M. Structural insights into sialic acid enzymology. (2008) *Curr. Opin. Chem. Biol.* 12: 565-72.
24. Buchini, S., **Buschiazzo, A.** and Withers, S.G. A new generation of specific *Trypanosoma cruzi* trans-sialidase inhibitors. (2008) *Angew Chem Int Ed Engl.* 47:2700-03.
25. Damager, I., Buchini, S., Amaya, M.F., **Buschiazzo, A**., Alzari, P., Frasch, A.C., Watts, A. and Withers S.G. Kinetic and mechanistic analysis of Trypanosoma cruzi trans-sialidase reveals a classical ping-pong mechanism with acid/base catalysis. (2008) *Biochemistry* 47:3507-12.
26. Turell L, **Botti H**, Carballal S, Ferrer-Sueta G, Souza JM, **Durán R**, Freeman BA, Radi R, Alvarez B. Reactivity of sulfenic acid in human serum albumin. (2008) *Biochemistry* 47:358-67.
27. González-Techera A, Umpiérrez-Failache M, Cardozo S, **Obal G, Pritsch O**, Last JA, Gee SJ, Hammock BD, González-Sapienza G. High-throughput method for ranking the affinity of peptide ligands selected from phage display libraries. (2008) Bioconjug Chem. 19(5):993-1000.
28. O'Hare HM, **Durán R, Cerveñansky C**, Bellinzoni M, Wehenkel AM, **Pritsch O, Obal G,** Baumgartner J, Vialaret J, Johnsson K, Alzari PM. Regulation of glutamate metabolism by protein kinases in mycobacteria. Mol Microbiol. (2008) 70(6):1408-23.
29. Naya H, Urioste JI, Chang YM, Rodrigues-Motta M, Kremer R, Gianola D. A comparison between Poisson and Zero-inflated Poisson regression models with an application to number of black spots in Corriedale sheep. (2008) Gen Sel Evol 40:379-394.
30. Wehenkel, A, Bellinzoni M, **Graña M, Durán R, Villarino A,** Fernández P, André-Leroux G, England P, Takiff H, **Cerveñansky C**, Cole ST, Alzari PM. Mycobacterial Ser/Thr protein kinases and phosphatases: physiological roles and therapeutic potencial (2008). Biochimica et Biophysica Acta, v. 1784, p. 193-202.
31. Marton S, Garcia M**, Robello C,** Persson H, **Trajtenberg F, Pritsch O,** Rovira C, **Naya H, Dighiero H, Cayota A.** Small RNAs analysis in CLL reveals a deregulation of miRNA expression and novel miRNA candidates of putative relevance in CLL pathogenesis. (2008) Leukemia, v. 22, p. 330-338.
32. Naya H, Urioste J, Chang Y, Rodrigues-Motta M, Kremer R, Gianola D. A comparison between Poisson and Zero-inflated Poisson regression models with an application to number of black spots in Corriedale sheep. (2008) Genetics Selection Evolution, v. 40, p. 379-394.}
33. Jubany S, Tomasco I, Ponce De León I, Medina K, Carrau F, Arrambide N, **Naya H**, Gaggero C. Toward a global database for the molecular typing of Saccharomyces cerevisiae Straits. (2008). Fems Yeast Research, v. 8, p. 472-484.
34. Carballal, S, Madzelan P, Zinola CF, **Graña M,** Radi R, Banerjee R, Alvarez B. Redox Potential of Truncated Human Cystathionine B-Synthase. (2008). Biochemistry, v. 47 10, p. 3194-3201.
35. S. G. Irañeta; Acosta Dm; **Durán, R**; Apicella C; Orlando Ud; Seoane Ma; Alonso A; Duschak Vg. MALDI-TOF MS analysis of labile Lolium perenne major allergens in mixes. (2008) Clinical and Experimental Allergy 38:1391-1399.
36. Cancela M; Acosta D; Rinaldi G; Silva E; **Durán, R**; Roche L; Zaha A; Carmona C; Tort JF. A distinctive repertoire of cathepsins is expressed by juvenile invasive Fasciola hepatica. (2008) Biochimie, 90(10):1461-75.
37. Binolfi A; Lamberto Gr; **Durán, R**; Quintanar L; Bertoncini Cw; Souza Jm; **Cerveñansky C**; Zweckstetter M; Griesinger,C; Fernandez CO. Site-specific interactions of Cu(II) with alpha- and beta-synuclein: bridging the molecular gap between metal binding and aggregation. (2008) Journal of the American Chemical Society 130(35):11801-11812.
38. Ferreira, A.M.; Ferrari M.; Trostchansky A.; **Batthyany, C**.; Souza, Jm.; Alvarez M.N. ; López G. V. ; Baker P.R.S.; Schopfer F.J.; O`Donnell V.; Freeman B.A. ; Rubbo H. Macrophage Activation Induces Nitration of Cholesteryl Linoleate- . (2008) Biochemical Journal,
39. Souza Jm; Castro L; Cassina Am; **Batthyany, C**.; Radi R. Nitrocytochrome c: synthesis, purification, and functional studies. (2008) Methods in Enzymology 441: 197-215.

40. **Batthyany, C**.; Kelley, E.E.; Hundley, N.J.; Woodcock, S.R.; Bonacci, G.; Del Rio, M.J.; Schopfer, F.J.; Lancaster Jr., J.; Freeman, B.A.; Tarpey, M.M. Nitro-oleic Acid: A Specific and Irreversible Inhibitor of Xanthine Oxidoreductase. (2008) Journal of Biological Chemistry, 283(52):36176-84.
41. Cairoli, E.; Scott-Algara, D.; **Pritsch, O.; Dighiero, G; Cayota, A**. HIV-1 induced decrease of nitric oxide production and inducible nitric oxide synthase expression during in vivo and in vitro infection. (2008) Clinical Immunology, v. 127 1, p. 26-33, 2008.
42. Dupuis L, Pehar M, Cassina P, Rene F, Castellanos R, Rouaux C, Gandelman M. Dimou L, Schwab ME, Loeffler JP**, Barbeito L**, Gonzalez de Aguilar JL. Nogo receptor antagonizes p75NTR-dependent motor neuron death. (2008) Proc Natl Acad Sci USA. 105:740-5
43. Cassina, P; Cassina, A; Pehar, M; Castellanos,R; Gandelman, M; de Leon, A; Robinson, KM; Mason, RP; Beckman, JS; **Barbeito, L**; Radi R. Mitochondrial dysfunction in SOD1(G93A)-bearing astrocytes promotes motor neuron degeneration: Prevention by mitochondrial-targeted antioxidants. (2008) J. Neurosci. 28: 4115-4122.
44. Vargas MR, Pehar M, **Díaz-Amarilla PJ**, Beckman JS**, Barbeito L.** Transcriptional profile of primary astrocytes expressing ALS-linked mutant SOD1. (2008) J Neurosci Res. 86:3515-25.
45. Olivera S.; Fernandez A, Latini A., Rosillo J., Casanova G., Wajner M., **Cassina P., Barbeito L.** Astrocytic proliferation and mitochondrial dysfunction induced by accumulated glutaric acidemia I (GAI) metabolites: possible implications for GAI pathogenesis. (2008) Neurobiol Dis. 32:528-34.
46. Favre, C; **Aguilar, P.S**. and Carrillo, M.C. Oxidative stress and chronological aging in glycogen-phosphorylase-deleted yeast. (2008) *Free Radic Biol Med*., 45, 1446-1456.
47. **Pantano S;** Zonta F; Mammano F. A fully atomistic model of the Cx32 connexon. PLoS ONE, (2008) v. 3, p. 2614-2625.
48. **Pantano S.** In silico description of fluorescent probes in vivo. (2008) J. Mol. Graph. Model, v. 27, p. 563-567,
49. Leitch CC, Zaghloul NA, Davis EE, Stoetzel C, Diaz-Font A, Rix S, Al-Fadhel M, Lewis RA, Eyaid W, Banin E, Dollfus H, Beales PL, **Badano JL**, Katsanis N. Hypomorphic mutations in syndromic encephalocele genes are associated with Bardet-Biedl syndrome. (2008) Nat Genet 40:443-448
50. Filser M.; **Comini M.A**.; Dirdjaja N.; Molina Navarro Mm; Herrero E.; Krauth-Siegel RL. Cloning, functional analysis and localization of monothiol glutaredoxin-1 from Trypanosoma brucei. (2008) Biol. Chem. Hoppe-Seyler, v. 389 1, p. 21-32,
51. Buchholz K; **Comini M.A.**; Wissenbach D; Schirmer H; R. Luise Krauth-Siegel; Gromer S. Cytotoxic interactions of methylene blue with trypanosomatids-specific disulfide reductases and their dithiol products. (2008) Molecular and Biochemical Parasitology, v. 160 1 , p. 65-69,
52. **Irigoín F.;** Cibils L.; **Comini M.A**.; Wilkinson S.; Flohé L.; Radi R. Insights into the redox biology of Trypanosoma cruzi: Trypanothione metabolism and oxidant. (2008) Free Radical Biology and Medicine, v. 45, p. 733-742,
53. Krauth-Siegel RL; **Comini M.A.** Redox control in trypanosomatids, parasitic protozoa with trypanothione-based thiol metabolism. (2008) Biochimica et Biophysica Acta-General Subjects, v. 1780, p. 1236-1248,
54. **Comini M.A.,** Rettig J., Dirdjaja N., Hanschmann E.M., Berndt C., Krauth-Siegel R.L. Monothiol Glutaredoxin-1 Is an Essential Iron-Sulfur Protein in the Mitochondrion of African Trypanosomes. (2008) J Biol. Chem. v v. 283, p. 27785-27798,
55. **Dighiero G.** Monoclonal B-cell lymphocytosis - A frequent premalignant condition (2008) NEW ENGLAND JOURNAL OF MEDICINE 359 : 638-
56. **Dighiero G.;** Hamblin T. J. Chronic lymphocytic leukaemia (2008) LANCET Volume: 371 Issue: 9617 Pages: 1017-1029.

2009

57. Rodríguez-Casuriaga R, Geisinger A, López-Carro B, **Porro V**, Wettstein R, Folle GA. Ultra-Fast and Optimized Method for the Preparation of Rodent Testicular Cells for Flow Cytometric Analysis. (2009). Biol Proced Online.
58. Fasnacht N, Greweling MC, **Bollati-Fogolín M**, Schippers A, Müller W. T-cell-specific deletion of gp130 renders the highly susceptible IL-10-deficient mouse resistant to intestinal nematode infection. (2009). Eur J Immunol., 39(8):2173-83.
59. Madan R, Demircik F, Surianarayanan S, Allen JL, Divanovic S, Trompette A, Yogev N, Gu Y, Khodoun M, Hildeman D, Boespflug N, **Bollati-Fogolin M,** Gröbe L, Greweling M, Finkelman FD, Cardin R, Mohrs M, Müller W, Waisman A, Roers A, Karp CL. Nonredundant roles for B cell-derived IL-10 in immune counter-regulation. (2009). J Immunol. 183(4):2312-20.
60. Nogueira FB, Ruiz JC, **Robello C**, Romanha AJ, Murta SM. Molecular characterization of cytosolic and mitochondrial tryparedoxin peroxidase in *Trypanosoma cruzi* populations susceptible and resistant to benznidazole. (2009) Parasitol Res. 104(4):835-844.
61. Marquez VE, Arias DG, Piattoni CV, **Robello C**, Iglesias AA, Guerrero SA. Cloning, expression and characterization of a dithiol glutaredoxin from *Trypanosoma cruzi*. (2009) Antioxidants & Redox Signaling, 12(6):787-792.
62. Albanesi D, Martín M, **Trajtenberg F**, Mansilla MC, Haouz A, Alzari PM, de Mendoza D, **Buschiazzo A.** Structural plasticity and catalysis regulation of a thermosensor histidine kinase. (2009) *Proc. Natl. Acad. Sci. U. S. A.* 106:16185-90.
63. Patenaude AM, Orthwein A, Hu Y, Campo VA, Kavli B, **Buschiazzo A,** Di Noia JM. Active nuclear import and cytoplasmic retention of activation-induced deaminase. (2009) *Nat. Struct. Mol. Biol.* 16:517-27.
64. **Graña M**, Bellinzoni M, Bellalou J, Haouz A, Miras I, **Buschiazzo A**, Winter N, Alzari PM. The crystal structure of *Mycobacterium tuberculosis* LppA, a lipoprotein confined to pathogenic mycobacteria. (2009) *Proteins* 78:769-72.
65. **Graña M**, Bellinzoni M, Miras I, Fiez-Vandal C, Haouz A, Shepard W, **Buschiazzo A**, Alzari PM. The crystal structure of *Mycobacterium tuberculosis* Rv2714, representative of a duplicated gene family in Actinobacteria. (2009) *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.* 65: 972-7.
66. Neres J, Brewer ML, Ratier L, **Botti H, Buschiazzo A**, Edwards PN, Mortenson PN, Charlton MH, Alzari PM, Frasch AC, Bryce RA, Douglas KT. Discovery of novel inhibitors of *Trypanosoma cruzi* trans-sialidase from *in silico* screening. (2009) *Bioorg. Med. Chem. Lett.* 19: 589-96.
67. Tiscornia A, Cairoli E, Marquez M, Denicola A, **Pritsch O, Cayota A.** Use of diaminofluoresceins to detect and measure nitric oxide in low level generating human immune cells. (2009) J Immunol Methods. Mar 15;342(1-2):49-57.
68. Rich RL, Papalia GA, Flynn PJ, Furneisen J, Quinn J, Klein JS, Katsamba PS, Waddell MB, Scott M, Thompson J, Berlier J, Corry S, Baltzinger M, Zeder-Lutz G, Schoenemann A, Clabbers A, Wieckowski S, Murphy MM, Page P, Ryan TE, Duffner J, Ganguly T, Corbin J, Gautam S, Anderluh G, Bavdek A, Reichmann D, Yadav SP, Hommema E, Pol E, Drake A, Klakamp S, Chapman T, Kernaghan D, Miller K, Schuman J, Lindquist K, Herlihy K, Murphy MB, Bohnsack R, Andrien B, Brandani P, Terwey D, Millican R, Darling RJ, Wang L, Carter Q, Dotzlaf J, Lopez-Sagaseta J, Campbell I, Torreri P, Hoos S, England P, Liu Y, Abdiche Y, Malashock D, Pinkerton A, Wong M, Lafer E, Hinck C, Thompson K, Primo CD, Joyce A, Brooks J, Torta F, Bagge Hagel AB, Krarup J, Pass J, Ferreira M, Shikov S, Mikolajczyk M, Abe Y, Barbato G, Giannetti AM, Krishnamoorthy G, Beusink B, Satpaev D, Tsang T, Fang E, Partridge J, Brohawn S, Horn J, **Pritsch O, Obal G,** Nilapwar S, Busby B, Gutierrez-Sanchez G, Gupta RD, Canepa S, Witte K, Nikolovska-Coleska Z, Cho YH, D'Agata R, Schlick K, Calvert R, Munoz EM, Hernaiz MJ, Bravman T, Dines M, Yang MH, Puskas A, Boni E, Li J, Wear M, Grinberg A, Baardsnes J, Dolezal O, Gainey M, Anderson H, Peng J, Lewis M, Spies P, Trinh Q, Bibikov S, Raymond J, Yousef M, Chandrasekaran V, Feng Y, Emerick A, Mundodo S, Guimaraes R, McGirr K, Li YJ, Hughes H, Mantz H, Skrabana R, Witmer M, Ballard J, Martin L, Skladal P, Korza G, Laird-Offringa I, Lee CS, Khadir A, Podlaski F, Neuner P, Rothacker J, Rafique A, Dankbar N, Kainz P, Gedig E, Vuyisich M, Boozer C, Ly N, Toews M, Uren A, Kalyuzhniy O, Lewis K, Chomey E, Pak BJ, Myszka DG. A global benchmark study using affinity-based biosensors. Anal Biochem. (2009) Mar 15;386(2):194-216.
69. Sabbia V, Romero H, Musto H, **Naya H.** Composition profile of the human genome at the chromosome level. (2009). J Biomol Struct Dyn. 27(3):361-70.
70. Weigel KA, de los Campos G, González-Recio O, **Naya H,** Wu XL, Long N, Rosa GJM, and Gianola D. Predictive ability of direct genomic values for Lifetime Net Merit of Holstein sires using selected subsets of single nucleotide polymorphism markers. (2009). J Dairy Sci 92(10):5248-57.
71. Romero H, Pereira E, **Naya H,** Musto H. Oxygen and GC profiles in marine environments. (2009). J Mol Evol 69(2):203-6.
72. de Los Campos G, **Naya H,** Gianola D, Crossa J, Legarra A, Manfredi E, Weigel K, Cotes JM. Predicting Quantitative Traits with Regression Models for Dense Molecular Markers and Pedigrees. (2009). Genetics 182(1):375-85.

73. Garcia JM, Gao A, He PL, Choi J, Tang W, Bruzzone R, Schwartz O, **Naya H**, Nan FJ, Li J, Altmeyer R, Zuo JP. High-throughput screening using pseudotyped lentiviral particles: a strategy for the identification of HIV-1 inhibitors in a cell-based assay. (2009).Antiviral Res. 81(3):239-47.
74. Boucontet L, **Graña M**, Alzari P, Pereira P. Mechanisms determining cell membrane expression of different TCR chain pairings. (2009). European Journal of Immunology 39(7): 1937-1946.
75. González S, Fló M, Margenat M, **Durán R,** González-Sapienza G, **Graña M**, Parkinson J, Maizels RM, Salinas G, Alvarez B, Fernández C. A family of diverse Kunitz inhibitors from Echinococcus granulosus potentially involved in host-parasite cross-talk. (2009) PLoS One: 17; 4(9):e7009.
76. Díaz A, Fontana EC, Todeschini AR, Soulé S, González H, Casaravilla C, **Portela M**, Mohana-Borges R, Mendonça-Previato L, Previato JO, Ferreira F. The major surface carbohydrates of the Echinococcus granulosus cyst: mucin-type O-glycans decorated by novel galactose-based structures. (2009) Biochemistry 48(49):11678-1169.
77. Sánchez G, Colettis N, Vázquez P, **Cerveñansky C**, Aguirre A, Quillfeldt JA, Jerusalinsky D, Kornisiuk E. Muscarinic inhibition of hippocampal and striatal adenylyl cyclase is mainly due to the M(4) receptor. (2009) Neurochem Res. 34(8):1363-1371.
78. Sánchez G, Alvares Lde O, Oberholzer MV, Genro B, Quillfeldt J, da Costa JC, **Cerveñansky C**, Jerusalinsky D, Kornisiuk E. M4 Muscarinic Receptors Are involved in modulation of Neurotransmission at Synapses of Shaffer Collaterals on CA1 Hippocampal Neurons in Rats. (2009) J Neurosci Res. 87(3):691-70.
79. Celano L, Gil M, Carballal S, **Durán R,** Denicola A, Banerjee R, Alvarez B. Inactivation of cystathionine beta-synthase with peroxynitrite. (2009) Arch Biochem Biophys 491: 96-105.
80. Khoo NK, Rudolph V, Cole MP, Golin-Bisello F, Schopfer FJ, Woodcock SR, **Batthyany C,** Freeman BA. Activation of vascular endothelial nitric oxide synthase and heme oxygenase-1 expression by electrophilic nitro-fatty acids. (2009) Free Radic Biol Med. 2010 Jan 15;48(2):230-9.
81. Schopfer FJ, **Batthyany C**, Baker PR, Bonacci G, Cole MP, Rudolph V, Groeger AL, Rudolph TK, Nadtochiy S, Brookes PS, Freeman BA. Detection and quantification of protein adduction by electrophilic fatty acids: mitochondrial generation of fatty acid nitroalkene derivatives. (2009) Free Radic Biol Med. 46(9): 250-259.
82. Cairoli E, **Cayota A**, Iriarte MJ, Irureta S, Rocha A. Circulating CD4+ T cells levels in active and non active systemic lupus erythematosus patients. (2009) Acta reumatologica portuguesa 34:559-60.
83. Sotelo-Silveira JR, **Lepanto P**, Elizondo MV, **Horjales S, Palacios F,** Martinez-Palma L, Marin M, Beckman JS, **Barbeito L.** Axonal mitochondrial clusters containing mutant SOD1 in transgenic models of ALS. (2009) Antioxid Redox Signal 11:1535-45
84. Gonzalez P., Burgaya F., Acarin L., **Peluffo H.**, Castellano B. Gonzalez. B. IL-10 and IL-10RI are upregulated in glial cells after an excitotoxic injury to the postnatal rat brain. (2009) Journal of Neuropathology and Experimental Neurology. 68:391-403
85. Fröhlich, F., Moreira, K., **Aguilar, P.S.**, Hubner, N.C., Mann, M., Walter, P. and Walther, T.C. A genome-wide screen for genes affecting eisosomes reveals Nce102 function in sphingolipid signaling. (2009) *J Cell Biol.* 185:1227-1242.
86. Moreira, K.E.; Walther, T.C., **Aguilar, P.S**. and Walter, P. Pil1 controls eisosome biogenesis. (2009) *Mol Bio Cell*., 20, 809-818.
87. Machado MR, Dans PD, Pantano S. Isoform Specific Determinants in the Binding of HP1 to Histone 3: Insights from Molecular Simulations, Amino Acids, (2009) May;38(5):1571-81.
88. De Marco, **Dans, P. D.** Knezevich, A., Maiuri, P., **Pantano, S.** and Marcello, A. Subcellular localization of the interaction between the human immunodeficiency virus transactivator Tat and the nucleosome assembly protein 1. (2009) Amino Acids, 2010 May;38(5):1583-93.
89. Herrera F, **Pantano S.** Salt induced asymmetry in membrane simulations by partial restriction of ionic motion. (2009) J. Chem. Phys., v. 130 p. 195105,
90. Carnevale V, Raugei S, Neri M, **Pantano S**, Micheletti C, Carloni P. Multi-scale modeling of the HIV-1 proteins, (2009) Journal of Molecular Structure, v. 898 p. 97-105.
91. de Pontual L, Zaghloul NA, Thomas S, Davis EE, McGaughey DM, Dollfus H, Baumann C, Bessling SL, Babarit C, Pelet A, Gascue C, Beales P, Munnich A, Lyonnet S, Etchevers H, Attie-Bitach T, **Badano JL,** McCallion AS, Katsanis N, Amiel J. Epistasis between RET and BBS mutations modulates enteric innervation and causes syndromic Hirschsprung disease. (2009) Proc Natl Acad Sci USA 106:13921-13926
92. Cardenas-Rodriguez M, Badano JL. Ciliary biology: Understanding the cellular and genetic basis of human ciliopathies. (2009) Am J Med Genet Part C Semin Med Genet 151C: 263-280
93. Comini M.A., Dirdjaja N., Kaschel M., Krauth-Siegel R.L. Preparative enzymatic synthesis of trypanothione and trypanothione analogues. (2009) Int. J. Parasitol. v. 39, p. 1059-1062,
94. Véronèse L, Tournilhac O, Verrelle P, Davi F, **Dighiero G**, Chautard E, Veyrat-Masson R, Kwiatkowski F, Goumy C, Gouas L, Bay JO, Vago P, Tchirkov A. Strong correlation between VEGF and MCL-1 mRNA expression levels in B-cellchronic Lymphocytic leukemia. (2009) LEUKEMIA RESEARCH 33 : 1623-1626

2010

95. Garavaglia PA, Cannata JJ, Ruiz AM, Maugeri D, **Duran R**, Galleano M, García GA. Identification, cloning and characterization of an aldo-keto reductase from Trypanosoma cruzi with quinone oxido-reductase activity. (2010) Mol Biochem Parasitol. 173(2):132-41.
96. Rama G, Meikle A, Puentes R, **Moratorio G,** Nicolini P, Pessina P, Furtado A, **Pritsch O.** Estudio comparativo de tres técnicas diagnósticas para la Leucosis Enzoótica Bovina y análisis del efecto de enfermedades concurrentes sobre la fórmula leucocitaria. (2010) Veterinaria (Montevideo) 46: 15-22
97. Guimarães FS, Ferrari LA, Martins SH, Sene RV, Wanderer C, **Tiscornia I, Bollati-Fogolín M,** Trindade ES, de Freitas Buchi D and Abud APR. In vitro and in vivo anticancer properties of a Calcarea carbonica derivative complex (M8) treatment in a murine melanoma model. (2010) BMC Cancer; 10:113.
98. Palacios F, Moreno P, Morande PE, **Abreu C, Correa A, Porro V,** Landoni AI, Gabus R, Giordano M, **Dighiero G, Pritsch O and Oppezzo P.** High expression of AID and active class switch recombination might accounts for a more aggressive disease in unmutated CLL patients: link with an activated microenvironment in CLL disease. (2010) BLOOD: 115:4488-96
99. Moratorio G, Obal G, Dubra A, Correa A, Bianchi S, Buschiazzo A, Cristina J, **Pritsch O.** Phylogenetic analysis of bovine leukemia viruses isolated in South America reveals diversification in seven distinct genotypes. (2010) *Arch Virol.* 155(4):481-9.
100. Menchaca A.; Vilariño M.; **Crispo M.**; de Castro T; Rubianes E. New approaches to superovulation and embryo transfer in small ruminants. (2010) Reproduction Fertility and Development. 22 (1): 113-118.
101. Garcia-Silva MR, Frugier M, **Tosar JP,** Correa-Dominguez A, Ronalte-Alves L, **Parodi-Talice A,** Rovira C, **Robello C,** Goldenberg S, **Cayota A**. A population of tRNA-derived small RNAs is actively produced in Trypanosoma cruzi and recruited to specific cytoplasmic granules. (2010) Molecular and biochemical parasitology. (DOI: 10.1016)
102. Barbeito L, Cassina P**,** Beckman JS. What makes astrocytes neurotoxic in ALS? **(2010)** J. Neurochem.
103. Barbeito AG, Martinez-Palma L, Vargas MR, Pehar M, Mañay N, Beckman JS, **Barbeito L,** Cassina P. Lead exposure stimulates VEGF expression in the spinal cord and extends survival in a mouse model of ALS. (2010) Neurobiol Dis. 37:574-580.
104. Aguilar, P.S.; Fröhlich, F.; Rehman, M.; Shales, M.; Ulitsky, I.; **Olivera-Couto, A.**; Braberg, H.; Shamir, R.; Walter, P.; Mann, M.; Ejsing, C.S.; Krogan, N.J. and Walther, T.C. A Plasma Membrane E-MAP Reveals Links Between the Eisosome, Sphingolipid Metabolism and Endosomal Trafficking. (2010). Nat Struct Mol Biol. 17(7):901-8.
105. Aguilar, P.S.; Heiman, M.G.; Walther, T.C.; Engel, A.; Schwudke, D.; Guswha, N.; Kurzchalia, T. and Walter, P. Structure of sterol aliphatic chains affects yeast cell shape and cell fusion during mating. (2010) *Proc Natl Acad Sci USA.*, 107: 4170-4175.
106. Dans PD, Zeida A, Machado MR, Pantano S. A Coarse-Grained Model for DNA Simulations at Nearly Atomistic Resolution. **(2010)** Journal of Chemical Theory and Computation.
107. Zaghloul, N.A., Liu, Y., Gerdes, J.M., **Gascue, C.**, Oh, E.C., Leitch, C.C., Bromberg, Y., Binkley, J., Leibel, R.L., Sidow, A., **Badano, J.L.**, Katsanis, N. Functional analyses of variants reveal a significant role for dominant negative and common alleles in oligogenic Bardet-Biedl syndrome. (2010) Proc Natl Acad Sci USA Sci USA 107: 10602-10607
108. Botti H, Möller MN, Steinmann D, Nauser T, Koppenol WH, Denicola A, Radi R. Distance-dependent diffusion-controlled reaction of •NO and O2•- at chemical equilibrium with ONOO-. (2010) J Phys Chem B. 114(49):16584-93.
109. Pils, Marina; Bleich, André; Fasnacht, Nicolas; **Bollati-Fogolin, Mariela**; Schippers, Angela; Rozell, Björn; Müller, Werner. Commensal gut flora reduces susceptibility to experimentally induced colitis via T-cell derived Interleukin-10 ***Inflammatory Bowel Diseases.*** (2010) (10):2038-46. doi: 10.1002/ibd.21587.
110. Piñeyro MD, Arcari T, Robello C, Radi R, Trujillo M. Tryparedoxin peroxidases from Trypanosoma cruzi: high efficiency in the catalytic elimination of hydrogen peroxide and peroxynitrite. (2010) Arch Biochem Biophys. 507(2):287-95.
111. Dujardin JC, Herrera S, do Rosario V, Arevalo J, Boelaert M, Carrasco HJ, Correa-Oliveira R, Garcia L, Gotuzzo E, Gyorkos TW, Kalergis AM, Kouri G, Larraga V, Lutumba P, Macias Garcia MA, Manrique-Saide PC, Modabber F, Nieto A, Pluschke G, **Robello C**, Rojas de Arias A, Rumbo M, Santos Preciado JI, Sundar S, Torres J, Torrico F, Van der Stuyft P, Victoir K, Olesen OF. Research priorities for neglected infectious diseases in Latin America and the Caribbean region. (2010) PLoS Negl Trop Dis. Oct 26;4(10):e780
112. Binolfi A, Rodriguez EE, Valensin D, D'Amelio N, Ippoliti E, **Obal G, Duran R**, Magistrato A, **Pritsch O**, Zweckstetter M, Valensin G, Carloni P, Quintanar L, Griesinger C, Fernández CO. Bioinorganic chemistry of Parkinson's disease: structural determinants for the copper-mediated amyloid formation of alpha-synuclein. (2010) Inorg Chem. Nov 15; 49(22):10668-79.

113. Bianchi S, Moreno P, Landoni AI, **Naya H, Oppezzo P, Dighiero G**, Gabús R, **Pritsch O**. Immunoglobulin heavy chain V-D-J gene rearrangement and mutational status in Uruguayan patients with chronic lymphocytic leukemia. (2010) Leuk Lymphoma. 51(11):2070-8.
114. Rovira C, **Güida MC, Cayota A**. MicroRNAs and other small silencing RNAs in cancer. (2010) IUBMB Life. 62(12):859-68
115. Garré JM, Retamal MA, Cassina P, **Barbeito L**, Bukauskas FF, Sáez JC, Bennett MV, Abudara V. FGF-1 induces ATP release from spinal astrocytes in culture and opens pannexin and connexin hemichannels. (2010) Proc Natl Acad Sci U S A. 107(52):22659-64.
116. Agrelo R, Wutz A. ConteXt of change--X inactivation and disease. (2010) EMBO Mol Med. 2(1):6-15.
117.Gascue C, Katsanis N, **Badano JL.** Cystic diseases of the kidney: ciliary dysfunction and cystogenic mechanisms. (2010), Pediatr Nephrol, DOI 10.1007/s00467-010-1697-5
118. Aebischer J, Cassina P, Otsmane B, Moumen A, Seilhean D, Meininger V, **Barbeito L**, Pettmann B, Raoul C. IFN triggers a LIGHT-dependent selective death of motoneurons contributing to the non-cell-autonomous effects of mutant SOD1. (2010) Cell Death Differ. 18(5):754-68. PMID: 21072055
119. Gandelman M, **Peluffo H**, Beckman JS, Cassina P, **Barbeito L**. Extracellular ATP and the P2Xreceptor in astrocyte-mediated motor neuron death: implications for amyotrophic lateral sclerosis. (2010) Journal of Neuroinflammation, 7:33 PMID: 20534165
120. Darré L, Machado MR, Dans PD, Herrera FE, and **Pantano S**, Another Coarse Grain Model for Aqueous Solvation: WAT FOUR? (2010) *J. Chem. Theory Comput.* DOI: 10.1021/ct100379f
121. Garcia Silva MR, Tosar JP, Frugier M, **Pantano S, Bonilla B**, Esteban L, Serra E, Rovira C, **Robello C, Cayota A.** Cloning, characterization and subcellular localization of a Trypanosoma cruzi argonaute protein defining a new subfamily distinctive of trypanosomatids. (2010) Gene. 466(1-2):26-35.
122. Robles A, **Medeiros A**, **Berois N**, Balter HS, Pauwels EK, **Osinaga E.** In-site interaction evaluation of Tn density by inhibition/competition assays.(2010) ***Nucl Med Biol.*** 37(4):453-8.
123. Trajtenberg F, Graña M, Ruétalo N, Botti H, Buschiazzo A. Structural and enzymatic insights into the ATP binding and autophosphorylation mechanism of a sensor histidine kinase. (2010) J Biol Chem. 285(32):24892-903.
124. González-Recio O, Weigel KA, Gianola D, **Naya H**, Rosa GJ. L2-Boosting algorithm applied to high-dimensional problems in genomic selection. (2010) Genet Res (Camb). 92(3):227-37.
125. Hamon T, **Graña M**, Raggio V, Grabar N, **Naya H**. Identification of relations between risk factors and their pathologies or health conditions by mining scientific literature. (2010) Stud Health Technol Inform. 160(Pt 2):964-8
126. González JM, **Buschiazzo A**, Vila AJ. Evidence of adaptability in metal coordination geometry and active-site loop conformation among B1 metallo-beta-lactamases. (2010) Biochemistry. 49(36):7930-8.
127.Megighian A, Scorzeto M, Zanini D, **Pantano S**, Rigoni M, Benna C, Rossetto O, Montecucco C, Zordan M. Arg206 of SNAP-25 is essential for neuroexocytosis at the Drosophila melanogaster neuromuscular junction. (2010) J Cell Sci. 123:3276.
128. Cabrera M, Lavaggi ML, Croce F, Celano L, Thomson L, Fernández M, Pintos C, Raymondo S, **Bollati M**, Monge A, López de Ceráin A, Piro OE, Cerecetto H, González M. Identification of chalcones as in vivo liver monofunctional phase II enzymes inducers. (2010) Bioorganic & Medicinal Chemistry 18 14, p. 5391-5399.
129. Hill M, Mazal D, Biron V, Pereira L, **Ubillos L, Berriel E**, Ahmed H, Freire T, Rondán M, Vasta GR, Liu FT, Iglesias MM and **Osinaga E**. A novel clinically-relevant animal model to study galectin-3 and its ligands during colon carcinogenesis. J. Histochem. (2010) Cytochem. 58:553-65
130. Binolfi A, Valiente-Gabioud AA, **Duran R**, Zweckstetter M, Griesinger C, Fernandez CO. Exploring the Structural Details of Cu(I) Binding to -Synuclein by NMR Spectroscopy. (2010). J Am Chem Soc. 133 (2), pp 194–196

2011

131. Zazzetti F, **Buschiazzo A**, Villamil FG, Barreira JC. Liver transplantation in systemic lupus erythematosus: case report and review of the literature. (2011) Lupus. (1):90-4.

132. Musumeci MA, **Botti H, Buschiazzo A**, Ceccarelli EA. Swapping FAD binding motifs between plastidic and bacterial ferredoxin-NADP(H) reductases. (2011) Biochemistry. 50(12):2111-22.

133. Ferrer-Sueta G, **Manta B, Botti H**, Radi R, Trujillo M, Denicola A. Factors affecting protein thiol reactivity and specificity in peroxide reduction. (2011) Chem Res Toxicol. 24(4):434-50.

134. Oppezzo P, Obal G, Baraibar MA, **Pritsch O**, Alzari PM, **Buschiazzo A**. Crystal structure of an enzymatically inactive trans-sialidase-like lectin from Trypanosoma cruzi: The carbohydrate binding mechanism involves residual sialidase activity. (2011) Biochim Biophys Acta. 1814(9):1154-61.

135. Roldán A, **Comini MA**, **Crispo M**, Krauth-Siegel RL. Lipoamide dehydrogenase is essential for both bloodstream and procyclic Trypanosoma brucei. (2011) Mol Microbiol. doi: 10.1111/j.1365-2958.

136. Persson H, Kvist A, **Rego N**, Staaf J, Vallon-Christersson J, Luts L, Loman N, Jonsson G, **Naya H**, Hoglund M, Borg A, Rovira C. Identification of new microRNAs in paired normal and tumor breast tissue suggests a dual role for the ERBB2/Her2 gene. (2011). Cancer Res. 71(1):78-86.

137.Peñagaricano F, Urioste JI, **Naya H**, de los Campos G, Gianola D. Assessment of Poisson, probit and linear models for genetic analysis of presence and number of black spots in Corriedale sheep. (2011). J Anim Breed Genet 128(2):105-13.

138. Schijman AG, Bisio M, Orellana L, Sued M, Duffy T, Mejia Jaramillo AM, Cura C, Auter F, Veron V, Qvarnstrom Y, Deborggraeve S, Hijar G, Zulantay I, Lucero RH, Velazquez E, Tellez T, Sanchez Leon Z, Galvão L, Nolder D, Monje Rumi M, Levi JE, Ramirez JD, **Zorrilla P**, Flores M, Jercic MI, Crisante G, Añez N, De Castro AM, Gonzalez CI, Acosta Viana K, Yachelini P, Torrico F, **Robello C**, Diosque P, Triana Chavez O, Aznar C, Russomando G, Büscher P, Assal A, Guhl F, Sosa Estani S, DaSilva A, Britto C, Luquetti A, Ladzins J. International study to evaluate PCR methods for detection of Trypanosoma cruzi DNA in blood samples from Chagas disease patients. (2011). PLoS Negl Trop Dis. 5(1):e931

139. Piñeyro MD, Parodi-Talice A, Portela M, Arias DG, Guerrero SA, **Robello C**. Molecular characterization and interactome analysis of Trypanosoma cruzi Tryparedoxin 1. (2011) J Proteomics. 74(9):1683-92.

140. de Figueiredo Peloso E, Vitor SC, Ribeiro LH, **Piñeyro MD, Robello C**, Gadelha FR. Role of Trypanosoma cruzi peroxiredoxins in mitochondrial bioenergetics. (2011) J Bioenerg Biomembr. 43(4):419-24.

141. Manta B, Obal G, Ricciardi A, Pritsch O, Denicola A. Tools to evaluate the conformation of protein products. (2011) Biotechnol J. (6):731-41.

142. Laschuk A, Monteiro KM, Vidal NM, Pinto PM, **Duran R, Cerveñansky C**, Zaha A, Ferreira HB. Proteomic survey of the cestode Mesocestoides corti during the first 24 hours of strobilar development. (2011). Parasitol Res. 108, 645-56

143. Lima A, Durán R, Schujman GE, Marchissio MJ, **Portela MM, Obal G, Pritsch O**, de Mendoza D, **Cerveñansky C**. Serine/threonine protein kinase PrkA of the human pathogen Listeria monocytogenes: Biochemical characterization and identification of interacting partners through proteomic approaches. (2011) J Proteomics. 74(9):1720-34. 1720-1734.

144. Bonacci G, Schopfer FJ, **Batthyany CI**, Rudolph TK, Rudolph V, Khoo NK, Kelley EE, Freeman BA. J Electrophilic fatty acids regulate matrix metalloproteinase activity and expression. (2011) J. Biol Chem 286(18):16074-81.

145. Correa A, Oppezzo P. Tuning different expression parameters to achieve soluble recombinant proteins in E. coli: advantages of high-throughput screening (2011) Biotechnol J. 6(6):715-30.

146. Nannini PR, Borge M, Mikolaitis VC, Abreu C, Morande PE, Zanetti SR, **Oppezzo P**, **Palacios F**, Ledesma I, Bezares RF, Giordano M, Gamberale R. CCR4 expression in a case of cutaneous Richter's transformation of chronic lymphocytic leukemia (CLL) to diffuse large B-cell lymphoma (DLBCL) and in CLL patients with no skin manifestations. (2011) Eur J Haematol. 87(1):80-6

147.Hubert P, Heitzmann A, Viel S, Nicolas A, Sastre-Garau X, **Oppezzo P, Pritsch O, Osinaga E**, Amigorena S. Antibody-Dependent Cell Cytotoxicity Synapses Form in Mice during Tumor-Specific Antibody Immunotherapy. (2011) Cancer Res. 71(15):5134-43.

148. Olivera-Bravo S, Fernández A, Sarlabós MN, Rosillo JC, Casanova G, Jiménez M, **Barbeito L.** Neonatal astrocyte damage is sufficient to trigger progressive striatal degeneration in a rat model of glutaric acidemia-I. (2011) PLoS One.;6(6):e20831.

149. Olivera-Couto A, Graña M, Harispe L, Aguilar PS. The eisosome core is composed of BAR domain proteins. (2011) Mol Biol Cell. 22(13):2360-72.

150. Barthélémy I, Barrey E, **Aguilar P**, Uriarte A, Le Chevoir M, Thibaud JL, Voit T, Blot S, Hogrel JY. Longitudinal ambulatory measurements of gait abnormality in dystrophin-deficient dogs. (2011) BMC Musculoskelet Disord. 12:75.

151. Lepanto P, Bryant DM, **Rossello J**, Datta A, Mostov KE, **Kierbel A**. Pseudomonas aeruginosa interacts with epithelial cells rapidly forming aggregates that are internalized by a Lyn-dependent mechanism. (2011) Cell Microbiol. 13(8):1212-22.

152. Elwell CA, **Kierbel A**, Engel JN. Species-specific interactions of SRC family tyrosine kinases regulate Chlamydia intracellular growth and trafficking. (2011) Bio. 2(3)

153. Romero S, Grompone G, Carayol N, Mounier J, Guadagnini S, Prevost MC, Sansonetti PJ, Tran Van Nhieu G. 1ATP-Mediated Erk1/2 Activation Stimulates Bacterial Capture by Filopodia, which Precedes Shigella Invasion of Epithelial Cells. (2011) Cell Host Microbe 9(6):508-19.

154. Briva A, Santos C, Malacrida L, Rocchiccioli F, Soto J, Angulo M, **Batthyany C**, Cairoli E, Piriz H. Adenosine triphosphate-dependent calcium signaling during ventilator-induced lung injury is amplified by hypercapnia. (2011) Exp Lung Res. 37(8):471-81.

155. Spangenberg L, Battke F, **Graña M**, Nieselt K, **Naya H**. Identifying associations between amino acid changes and meta information in alignments. (2011) Bioinformatics.

156. Gonzalez P, Peluffo H, Acarin L, Villaverde A, Gonzalez B, Castellano B. Interleukin-10 overexpression does not synergize with the neuroprotective action of RGD-containing vectors after postnatal brain excitotoxicity but modulates the main inflammatory cell responses. (2011) J Neurosci Res. doi: 10.1002/jnr.22741.

157.Peñagaricano F., Zorrilla P., Naya H., Robello C., Urioste J.I. Gene expression analysis identifies new candidate genes associated with the development of black skin spots in Corriedale sheep. (2011) Journal of Applied Genetics. Ref.: Ms. No. JOAG-D-11-00054R3.

158. Machado MR, Dans PD, Pantano S. A hybrid all-atom/coarse grain model for multiscale simulations of DNA. (2011) Phys Chem Chem Phys.

159. Domingo-Espín J, Vazquez E, Ganz J, Conchillo O, García-Fruitós E, Cedano J, Unzueta U, Petegnief V, Gonzalez-Montalbán N, Planas AM, Daura X, **Peluffo H**, Ferrer-Miralles N, Villaverde A. Nanoparticulate architecture of protein-based artificial viruses is supported by protein-DNA interactions. (2011) Nanomedicine (Lond).

160. Peluffo H. Modular Multifunctional Protein Vectors for Gene Therapy. (2011) Non-viral Gene Therapy, Prof. Xubo Yuan Ed., Editorial INTECH, ISBN: 9789533075389

161.Cabrera M, López GV, Gómez LE, Breijo M, Pintos C, **Botti H**, Raymondo S, Vettorazzi A, Ceráin AL, Monge A, Rubbo H, González M, Cerecetto H. Genetic toxicology and preliminary in vivo studies of nitric oxide donor tocopherol analogs as potential new class of antiatherogenic agents. (2011) Drug Chem Toxicol. 34(3):285-93.

162. Duhagon MA, Smircich P, Forteza D, **Naya H**, Williams N, Garat B. Comparative genomic analysis of dinucleotide repeats in Tritryps. (2011) Gene. 487(1):29-37.

163. Tabares-da Rosa S, Rossotti M, Carleiza C, **Carrión F, Pritsch O**, Ahn KC, Last JA, Hammock BD, González-Sapienza G. Competitive Selection from Single Domain Antibody Libraries Allows Isolation of High-Affinity Antihapten Antibodies That Are Not Favored in the llama Immune Response. (2011) Anal Chem. 83(18):7213-7220.

164. Perez DI, Palomo V, Perez C, Gil C, **Dans PD**, Luque FJ, Conde S, Martinez A. Switching Reversibility to Irreversibility in Glycogen Synthase Kinase 3 Inhibitors: Clues for Specific Design of New Compounds. (2011) J Med Chem. 54(12):4042-4056.

165. Irigoín F, **Badano JL.** Keeping the balance between cell proliferation and differentiation: the primary cilium. (2011) Curr Genomics 12:285-297

166. Díaz-Amarilla P, Olivera-Bravo S, Trias E, Cragnolini A, Martínez-Palma L, Cassina P, Beckman J, **Barbeito L**. Phenotypically aberrant astrocytes that promote motoneuron damage in a model of inherited amyotrophic lateral sclerosis. (2011) Proc Natl Acad Sci U S A. [Epub ahead of print]

167.Peluffo H, Alí-Ruiz D, Ejarque-Ortíz A, Heras-Alvarez V, Comas-Casellas E, Martínez-Barriocanal A, Kamaid A, Alvarez-Errico D, Negro ML, **Lago N**, Schwartz S Jr, Villaverde A, Sayós J. Overexpression of the immunoreceptor CD300f has a neuroprotective role in a model of acute brain injury. (2011) Brain Pathol. doi: 10.1111/j.1750-3639.2011.00537.x. [Epub ahead of print]

168. Ortiz C, Larrieux N, Medeiros A, Botti H, Comini M*, Buschiazzo A*. Expression, crystallization and preliminary X-ray crystallographic analysis of glucose-6-phosphate dehydrogenase from the human pathogen Trypanosoma cruzi in complex with substrate. Acta Crystallogr Sect F Struct Biol Cryst Commun. 2011 Nov 1;67(Pt 11):1457-61

169. Bürgi M, Prieto C, Oggero M, **Bollati-Fogolín M**, Etcheverrigaray M, Kratje R. New reporter cell clones to determine the biological activity of human type I interferons. BMC Proceedings. 2011 Nov 22;5 Suppl 8:P4.

170. Pils MC, Bleich A, Prinz I, Fasnacht N, **Bollati-Fogolin M**, Schippers A, Rozell B, Müller W. Commensal gut flora reduces susceptibility to experimentally induced colitis via T-cell-derived interleukin-10. Inflamm Bowel Dis. 2011 Oct;17(10):2038-46

171.D'Alessandro B, Lery LM, von Krüger WM, **Lima A**, Piccini C, Zunino P. Proteomic analysis of Proteus mirabilis outer membrane proteins reveals differential expression in vivo vs. in vitro conditions. FEMS Immunol Med Microbiol. 2011 Nov;63(2):174-82

2012

172. Herrera FE, **Pantano S**. Structure and dynamics of nano-sized raft-like domains on the plasma membrane. (2012) J Chem Phys. 136(1):015103.

173. Buschiazzo A, Muiá R, **Larrieux N**, Pitcovsky T, Mucci J, Campetella O. Trypanosoma cruzi trans-Sialidase in Complex with a Neutralizing Antibody: Structure/Function Studies towards the Rational Design of Inhibitors. (2012) PLoS Pathog. (1):e1002474.

174. Gascue C, Tan PL, **Cardenas-Rodriguez M, Libisch G**, Fernandez-Calero T, Liu YP, Astrada S, **Robello C, Naya H**, Katsanis N, **Badano JL**. Direct role of Bardet-Biedl syndrome proteins in transcriptional regulation. (2012) J Cell Sci. 125(Pt 2):362-75.

175. Basika T, Muñoz N, Casaravilla C, **Irigoín F, Batthyány C**, Bonilla M, Salinas G, Pacheco JP, Roth J, **Durán R**, Díaz A. Phagocyte-specific S100 proteins in the local response to the Echinococcus granulosus larva. (2012) Parasitology. 139(2):271-83.

176. Demoro B, Sarniguet C, Sánchez-Delgado R, Rossi M, Liebowitz D, Caruso F, Olea-Azar C, Moreno V, Medeiros A, **Comini MA**, Otero L, Gambino D. New organoruthenium complexes with bioactive thiosemicarbazones as co-ligands: potential anti-trypanosomal agents. (2012) Dalton Trans. Gene. 498(2):147-54.

177. Chiribao ML, Libisch MG, Osinaga E, Parodi-Talice A, Robello C. Cloning, localization and differential expression of the Trypanosoma cruzi TcOGNT-2 glycosyl transferase. (2012) Gene. 498(2):147-54.

178. Peloso EF, Gonçalves CC, Silva TM, Ribeiro LH, **Piñeyro MD, Robello C**, Gadelha FR. Tryparedoxin peroxidases and superoxide dismutases expression as well as ROS release are related to Trypanosoma cruzi epimastigotes growth phases. (2012) Arch Biochem Biophys. 520(2):117-22.

179. Hernández P, Cabrera M, Lavaggi ML, Celano L, **Tiscornia I**, Rodrigues da Costa T, Thomson L, **Bollati-Fogolín M**, Miranda AL, Lima LM, Barreiro EJ, González M, Cerecetto H. Discovery of new orally effective analgesic and anti-inflammatory hybrid furoxanyl N-acylhydrazone derivatives. (2012) Bioorgan Med Chem. 20(6):2158-71.

180. Rego N, Bianchi S, Moreno P, Persson H, Kvist A, Pena A, **Oppezzo P, Naya H,** Rovira C, **Dighiero G, Pritsch O**. Search for an aetiological virus candidate in chronic lymphocytic leukaemia by extensive transcriptome analysis. (2012) Br J Haematol. doi: 10.1111/j.1365-2141.2012.09116.x

181. García G, **Libisch G**, Trujillo-Cenóz O, **Robello C**, Russo RE. Modulation of gene expression during early stages of reconnection of the turtle spinal cord. (2012) J Neurochem. doi: 10.1111/j.1471-4159.2012.07750.x

182. Drechsel DA, Estévez AG, **Barbeito L**, Beckman JS. Nitric Oxide-Mediated Oxidative Damage and the Progressive Demise of Motor Neurons in ALS. (2012)Neurotox Res. [Epub ahead of print]

183. Peluffo H, Alí-Ruiz D, Ejarque-Ortíz A, Heras-Alvarez V, Comas-Casellas E, Martínez-Barriocanal A, Kamaid A, Alvarez-Errico D, Negro ML, **Lago N**, Schwartz Jr S, Villaverde A, Sayós J. Overexpression of the Immunoreceptor CD300f Has a Neuroprotective Role in a Model of Acute Brain Injury. (2012) Brain Pathol. 22(3):318-328.

184. Herrera FE, Bouchet A, Lairion F, Disalvo EA, **Pantano S**. Molecular Dynamics Study of the Interaction of Arginine with Phosphatidylcholine and Phosphatidylethanolamine Bilayers. (2012) J Phys Chem B. [Epub ahead of print].

185. Puig N, Agrelo R. From aging to cancer: a DNA methylation journey, Ageing Res Rev, Vol 3, No 1 (2012) Abstract views: 215

186. Miquel E, Cassina A, Martínez-Palma L, Bolatto C, Trías E, Gandelman M, Radi R, **Barbeito L**, Cassina P. Modulation of astrocytic mitochondrial function by dichloroacetate improves survival and motor performance in inherited amyotrophic lateral sclerosis. (2012) PLoS One. 2012;7(4):e34776

187. Bürgi MD, Prieto C, Etcheverrigaray M, Kratje R, Oggero M, **Bollati-Fogolín M**. WISH cell line: From the antiviral system to a novel reporter gene assay to test the potency of human IFN- and IFN- . (2012) J Immunol Methods.

188. Torres MJ, Turell L, **Botti H**, Antmann L, Carballal S, Ferrer-Sueta G, Radi R, Alvarez B. Modulation of the reactivity of the thiol of human serum albumin and its sulfenic derivative by fatty acids. (2012) Arch Biochem Biophys. 521(1-2):102-10.

189. Obal G, Ramos AL, Silva V, L**ima A, Batthyany C**, Bessio MI, Ferreira F, Salinas G, Ferreira AM. Characterisation of the Native Lipid Moiety of Echinococcus granulosus Antigen B. (2012) PLoS Negl Trop Dis. 6(5):e1642.

190. Naya DE, **Spangenberg L, Naya H**, Bozinovic F. Latitudinal patterns in rodent metabolic flexibility. (2012) Am Nat. 179(6):E172-9.

191. Medeiros A, Berois N, Incerti M, Bay S, Franco Fraguas L, **Osinaga E**. A Tn antigen binding lectin from Myrsine coriacea displays toxicity in human cancer cell lines. (2012) J. Nat Med.

192. Giorgetti A, Ruggerone P, **Pantano S**, Carloni P. Advanced computational methods in molecular medicine. (2012) J Biomed Biotechnol. 2012:709085.

193. Moreno P, Abreu C, Borge M, **Palacios F**, Morande P, **Pegazzano M, Bianchi S**, Landoni AI, **Agrelo R**, Giordano M, **Dighiero G**, Gamberale R, **Oppezzo P.** Lipoprotein lipase expression in unmutated CLL patients is the consequence of a demethylation process induced by the microenvironment. (Jul 2012) Leukemia

194. Iriarte A, Sanguinetti M, Fernández-Calero T, **Naya H**, Ramón A, Musto H. Translational selection on codon usage in the genus Aspergillus. (Jun 2012) Gene

195. Greif G, Coitinho C, Rivas C, van Ingen J, **Robello C**. Molecular analysis of isoniazid-resistant Mycobacterium tuberculosis isolates in Uruguay. (Jul 2012) Int J Tuberc Lung Dis. 16(7):947-9

196. Cecchetto G, Richero M, Oestreicher N, Muro-Pastor MI, **Pantano S**, Scazzocchio C. Mutations in the basic loop of the Zn binuclear cluster of the UaY transcriptional activator suppress mutations in the dimerisation domain. (Jul 2012) Fungal Genet Biol.

197.Olivera-Couto A, Aguilar PS. Eisosomes and plasma membrane organization. (Jul 2012) Mol Genet Genomics

198. Tiscornia I, Sánchez-Martins V, Hernández A, **Bollati-Fogolín M**. Human monocyte-derived dendritic cells from leukoreduction system chambers after plateletpheresis are functional in an in vitro co-culture assay with intestinal epithelial cells, J. Immunol. Methods (2012), doi:10.1016/j.jim.2012.07.005

199. Darré L, Tek A, Baaden M and **Pantano S**. Mixing atomistic and coarse grain solvation models for MD simulations: let WT4 handle the bulk, JCTC 2012 DOI: 10.1021/ct3001816

200. Darré L, Machado MR, Pantano S. Coarse-grained models of water, DOI: 10.1002/wcms.1097

201. Iraola G, Vazquez G**, Spangenberg L, Naya H**. Reduced set of virulence genes allows high accuracy prediction of bacterial pathogenicity in humans. (Agosto 2012) Plos ONE 7(8):e42144

202. Horjales S, Schmidt-Arras D, Limardo RR, Leclercq O, **Obal G**, Prina E, Turjanski AG, Späth GF, **Buschiazzo A**. The Crystal Structure of the MAP Kinase LmaMPK10 from Leishmania Major Reveals Parasite-Specific Features and Regulatory Mechanisms. Structure 2012 Oct 10; 20(10):1649-60

203. Freire T, **Osinaga E**. The sweet side of tumor immunotherapy. (Jul 2012) Immunotherapy. 4(7):719-34.

204. Domingo-Espín J, Petegnief V, de Vera N, Conchillo-Solé O, Saccardo P, Unzueta U, Vazquez E, Cedano J, Negro L, Daura X, **Peluffo H**, Planas AM, Villaverde A, Ferrer-Miralles N. RGD-based cell ligands for cell-targeted drug delivery act as potent trophic factors. (Jul 2012) Nanomedicine NBM.

205. Vo Cam Q, **Pantano S**, Rossetti G, Giacca M and Carloni P. HIV-1 Tat Binding to PCAF Bromodomain: Structural Determinants from Computational Methods. (Ago 2012) Biology.

206. Cardenas-Rodriguez M, Osborn DP, **Irigoín F, Graña M**, Romero H, Beales PL, **Badano JL**. Characterization of CCDC28B reveals its role in ciliogenesis and provides insight to understand its modifier effect on Bardet-Biedl syndrome. Hum Genet. 2012 Sep 27.

207.Reifur L, **Garcia-Silva MR**, Poubel SB, Alves LR, Arauco P, Buiar DK, Goldenberg S, **Cayota A**, Dallagiovanna B. Distinct subcellular localization of tRNA-derived fragments in the infective metacyclic forms of Trypanosoma cruzi. Mem Inst Oswaldo Cruz. 2012 Sep;107(6):816-9.

208. Dans PD, Pérez A, Faustino I, Lavery R, Orozco M. Exploring polymorphisms in B-DNA helical conformations. Nucleic Acids Res. 2012 Sep 24

209. Comini MA, Krauth-Siegel L, Bellanda M. Mono- and Dithiol Glutaredoxins in the Trypanothione-based. Redox Metabolism of Pathogenic Trypanosomes. Antioxid Redox Signal. 2012 Sep 16. [Epub ahead of print] Pubmed PMID : 22978520

210. Peluffo H, Foster E, Ahmed SG, **Lago N**, Hutson TH, Moon L, Yip P, Wanisch K, Caraballo-Miralles V, Olmos G, Lladó J, McMahon SB and Yáñez-Muñoz RJ. Efficient gene expression from integration-deficient lentiviral vectors in the spinal cord. Gene Therapy (2012), 1–13.

211. Zeida A, Machado MR, Dans PD and Pantano S. Breathing, bubbling, and bending: DNA flexibility from multimicrosecond simulations. (Ago 2012) PHYSICAL REVIEW E.

212. Berois N, Gattolliat CH, Barrios E, **Capandeguy L**, Douc-Rasy S, Valteau-Couanet D, Bénard J and **Osinaga E**. GALNT9 gene expression is a prognostic marker in neuroblastoma patients. Clinical Chemistry (doi:10.1373/clinchem.2012.192328)

213.Albanesi D, Reh G, Guerin ME, Schaeffer F, Debarbouille M, **Buschiazzo A**, Schujman GE, de Mendoza D and Alzari PM. Structural basis for feed-forward transcriptional regulation of membrane lipid homeostasis in Staphylococcus aureus. PLoS Pathog., in press (2012)

214. Correa A, Trajtenberg F, Obal G, Pritsch O, Dighiero G, Oppezzo P and Buschiazzo A. Crystal structure of a human IgA1 Fab fragment at 1.55A resolution: potential effect of the constant domains in antigen-affinity modulation Acta Crystallogr. D Biol. Crystallogr., (2012)

215. Almejun MB, Cols M, Zelazko M, Oleastro M, Cerutti A, **Oppezzo P**, Cunningham-Rundles C, Danielian S. Naturally occurring mutation affecting the MyD88-binding site of TNFRSF13B impairs triggering of class switch recombination. Eur J Immunol. 2012 Dec 7. doi: 10.1002/eji.201242945.

216. Bonacci G, Baker PR, Salvatore SR, Shores D, Khoo NK, Koenitzer JR, Vitturi DA, Woodcock SR, Golin-Bisello F, Cole MP, Watkins S, St Croix C, **Batthyany CI**, Freeman BA, Schopfer FJ. Conjugated linoleic Acid is a preferential substrate for Fatty Acid nitration. J Biol Chem. 2012 Dec 28;287(53):44071-82. doi: 10.1074/jbc.M112.401356.

217. Gadelha FR, Gonçalves CC, Mattos EC, Alves MJ, **Piñeyro MD, Robello C,** Peloso EF. Release of the cytosolic tryparedoxin peroxidase into the incubation medium and a different profile of cytosolic and mitochondrial peroxiredoxin expression in H(2)O(2)-treated Trypanosoma cruzi tissue culture-derived trypomastigotes. Exp Parasitol. 2012 Dec 22;133(3):287-293. doi: 10.1016/j.exppara.2012.12.007.

218. Grompone G, Martorell P, Llopis S, González N, Genovés S, **Mulet AP**, **Fernández-Calero T**, **Tiscornia I, Bollati-Fogolín M,** Chambaud I, Foligné B, Montserrat A, Ramón D. Anti-Inflammatory Lactobacillus rhamnosus CNCM I-3690 Strain Protects against Oxidative Stress and Increases Lifespan in Caenorhabditis elegans. PLoS One. 2012;7(12):e52493. doi: 10.1371/journal.pone.0052493.

219. Manta B, Pavan C, Sturlese M, **Medeiros A, Crispo M,** Berndt C, Krauth-Siegel L, Bellanda M, **Comini MA.** Iron-sulfur cluster (ISC) binding by mitochondrial monothiol glutaredoxin-1 of Trypanosoma brucei: molecular basis of ISC coordination and relevance for parasite infectivity. Antioxid Redox Signal. 2012 Dec 24. 19(7):665-682

220. Peluffo H, Gonzalez P, Acarin L, Arís A, Beyaert R, Villaverde A, Gonzalez B. Overexpression of the nuclear factor kappaB inhibitor A20 is neurotoxic after an excitotoxic injury to the immature rat brain. Neurol Res. 2012 Dec 26.

221. Peña S., Scarone L., **Medeiros A.**, Manta E., **Comini M.A.**, Serra G. Synthesis of precursors and macrocycle analogs of aerucyclamides as anti-trypanosomal agents. (2012) Med. Chem. Comm. 3, 1443-1448.

222. Schlapp G; Goyeneche L.; Cárdenas Rodriguez M; Crispo M. Eficiencia de la transgénesis murina en el Institut Pasteur de Montevideo. Revista da Sociedade Brasileira de Ciência em Animais de Laboratório – RESBCAL v.: 1 4, p.: 310 - 316, 2012.

223. Seminotti B, Amaral AU, da Rosa MS, Fernandes CG, Leipnitz G, Olivera-Bravo S, **Barbeito L**, Ribeiro CA, de Souza DO, Woontner M, Goodman SI, Koeller DM, Wajner M. Disruption of brain redox homeostasis in glutaryl-CoA dehydrogenase deficient mice treated with high dietary lysine supplementation. Mol Genet Metab. 2013 Jan;108(1):30-9. doi: 10.1016/j.ymgme.2012.11.001. Epub 2012 Nov 9.

224. Trujillo AI, Peñagaricano F, Grignola MP, Nicolini P, Casal A, Espasandín AC, **Naya H**, Carriquiry M, Chilibroste P. Using high resolution melting analysis to identify variation of NPY, LEP and IGF-1 genes in Angus cattle. (2012) Livestock Science 146:193-198.

2013

225. Abreu C, Moreno P, Palacios F, Borge M, **Morande P**, Landoni AI, Gabus R**, Dighiero G**, Giordano M, Gamberale R, **Oppezzo P**. Methylation status regulates lipoprotein lipase expression in chronic lymphocytic leukemia. Leuk Lymphoma. 2013 Aug; 54(8):1844-8. doi: 10.3109/10428194.2013.796057. Epub 2013 Jun 21.

226. Agrelo R, Kishimoto H, Novatchkova M, Peraza V, Paolino M, Souabni A, Wutz A. SATB1 collaborates with loss of p16 in cellular transformation. Oncogene. 2013 May 20. doi: 10.1038/onc.2013.158. [Epub ahead of print]

227. Aguilar PS, Baylies MK, Fleissner A, Helming L, Inoue N, Podbilewicz B, Wang H, Wong M. Genetic basis of cell-cell fusion mechanisms. Trends Genet. 2013 Feb 27. pii: S0168-9525(13)00023-1. doi: 10.1016/j.tig.2013.01.011. [Epub ahead of print]

228. Albanesi D, Reh G, Guerin ME, Schaeffer F, Debarbouille M, **Buschiazzo A**, Schujman GE, de Mendoza D, Alzari PM. Structural basis for feed-forward transcriptional regulation of membrane lipid homeostasis in Staphylococcus aureus. PLoS Pathog. 2013 Jan;9(1):e1003108.

229. Almeida RS, Loss O, Colabardini AC, Brown NA, Bignell E, Savoldi M, **Pantano S**, Goldman MH, Arst HN Jr, Goldman GH. Genetic Bypass of Aspergillus nidulans crzA Function in Calcium Homeostasis. G3 (Bethesda). 2013 May 11. pii: g3.113.005983v1. doi: 10.1534/g3.113.005983. [Epub ahead of print]

230. Alvarez G, Aguirre-López B, Cabrera N, Marins EB, Tinoco L, **Batthyány CI**, de Gómez-Puyou MT, Puyou AG, Pérez-Montfort R, Cerecetto H, González M. 1,2,4-thiadiazol-5(4H)-ones: a new class of selective inhibitors of Trypanosoma cruzi triosephosphate isomerase. Study of the mechanism of inhibition. J Enzyme Inhib Med Chem. 2013 Oct; 28(5):981-9. doi: 10.3109/14756366.2012.700928

231. Arias DG, Marquez VE, **Chiribao ML**, Gadelha FR, **Robello C**, Iglesias AA, Guerrero SA. Redox metabolism in Trypanosoma cruzi: Functional characterization of tryparedoxins revisited. Free Radic Biol Med. 2013 May 8;63C:65-77. doi: 10.1016/j.freeradbiomed.2013.04.036. [Epub ahead of print]

232. Berois N, De Cremoux P, Mazal D, Sica A, Cedeira M, Caserta B, Barrios E, Osinaga E, Sastre-Garau X. Prevalence and Distribution of High-Risk Human Papillomavirus Genotypes in Invasive Carcinoma of the Uterine Cervix in Uruguay. Int J Gynecol Cancer. 2013 Mar;23(3):527-532.

233. Berriel E, **Russo S**, Monin L, **Festari MF, Berois N**, Fernández G, Freire T, **Osinaga E**. Anti-tumor activity of human hydatid cyst fluid in a murine model of colon cancer. The Scientific World Journal. 2013 Aug 18;2013:230176

234. Cardenas-Rodriguez M, Irigoín F, Osborn DP, **Gascue C**, Katsanis N, Beales PL, **Badano JL**. The Bardet-Biedl syndrome-related protein CCDC28B modulates mTORC2 function and interacts with SIN1 to control cilia length independently of the mTOR complex. Hum Mol Genet. 2013 Jun 13. [Epub ahead of print]

235. Chenoll E; Codoñer FM; Silva A; Ibáñez A; Martinez-Blanch JF; **Bollati Fogolin M.; Crispo M.; Ramirez S**; Sanz Y; Ramón D; Genovés S. Genomic Sequence and Pre-Clinical Safety Assessment of Bifidobacterium longum CECT 7347, a Probiotic able to Reduce the Toxicity and Inflammatory Potential of Gliadin-Derived Peptides. Journal of Probiotics and Health, v.: 1 2, p.: 1 - 6, 2013

236. Coitinho C, **Greif G, Robello C**, Laserra P, Willery E, Supply P. Rapidly progressing tuberculosis outbreak in a very low risk group. Eur Respir J. 2013 Oct 10. [Epub ahead of print]

237. Comini M.A., Cazzulo J. J. and **Ortíz C.** (2013) Chapter: The Pentose Phosphate Pathway, In: Drug Discovery for Trypanosomatid Diseases. Ed. Leopold Flohé, Timo Jäger and Oliver Koch. *Wiley-Blackwell*, Oxford, UK. ISBN: 978-3-527-33255-7.

238. Comini M.A., Flohé L. (2013) Chapter: The Trypanothione System, In: Drug Discovery for Trypanosomatid Diseases. Ed. Leopold Flohé, Timo Jäger and Oliver Koch. *Wiley-Blackwell*, Oxford, UK. ISBN: 978-3-527-33255-7.

239. Correa A, Trajtenberg F, Obal G, Pritsch O, Dighiero G, Oppezzo P, Buschiazzo A. Structure of a human IgA1 Fab fragment at 1.55 Å resolution: potential effect of the constant domains on antigen-affinity modulation. Acta Crystallogr D Biol Crystallogr. 2013 Mar;69(Pt 3):388-97. doi: 10.1107/S0907444912048664. Epub 2013 Feb 16.

240. Crispo M.; Schlapp G; Cárdenas M; González-Maciel D.; Rumbo M. Optimization of transgenesis conditions for the generation of CXCL2-luciferase reporter mice line. EJB Electronic Journal of Biotechnology, v.: 16 6, 2013

241. Crispo M.; Van Maele L; Tabareau J; Cayet D; Errea A; Ferreira Am; Rumbo M; Sirard Jc. Transgenic mouse model harboring the transcriptional fusion Ccl20-luciferase as a novel reporter of pro-inflammatory response. PLoS ONE, 2013

242. Dalotto-Moreno T, Croci DO, Cerliani JP, Martinez-Allo VC, Dergan-Dylon S, Méndez-Huergo SP, Stupirski JC, Mazal D, **Osinaga E**, Toscano MA, Sundblad V, Rabinovich GA, and Salatino M. Targeting galectin-1 overcomes breast cancer-associated immunosuppression and prevents metastatic disease. Cancer Res; 2013; 73:1107-17

243. Dans PD, **Darré L, Machado MR, Zeida A, Brandner AF, Pantano S**. Assessing the Accuracy of the SIRAH Force Field to Model DNA at Coarse Grain Level. Lect. Notes in Comp. Science, 2013. DOI: 10.1007/978-3-319-02624-4_7

244. Espasandín AC, Urioste JI, **Naya H**, Alencar MM. Genotype x Production Environment Interaction for Weaning Weight in Angus Populations of Brazil and Uruguay. Livestock science (2013). 151(2): 264-270. (1.249)

245. Fariello MI, Boitard S, **Naya H**, San Cristobal M, Servin B. Detecting Signatures of Selection Through Haplotype Differentiation Among Hierarchically Structured Populations. Genetics. 2013 Mar;193(3):929-41. (4.389)

246. Fernández M, Becco L, Correia I, Benítez J, Piro OE, Echeverria GA, Medeiros A, Comini M, Lavaggi ML, González M, Cerecetto H, Moreno V, Pessoa JC, Garat B, Gambino D. Oxidovanadium(IV) and dioxidovanadium(V) complexes of tridentate salicylaldehyde semicarbazones: Searching for prospective antitrypanosomal agents. J Inorg Biochem. 127:150-160. 2013 Mar 4. pii: S0162-0134(13)00050-0. doi: 10.1016/j.jinorgbio.2013.02.010. [Epub ahead of print]

247. Fernández Massó JR, Oliva Argüelles B, Tejeda Y, Astrada S, Garay H, Reyes O, Delgado-Roche L, Bollati-Fogolín M, Vallespí MG. The Antitumor Peptide CIGB-552 Increases COMMD1 and Inhibits Growth of Human Lung Cancer Cells. J Amino Acids. 2013; 2013:251398.

248. Gandelman M, Levy M, Cassina P, **Barbeito L**, Beckman JS. P2X7 receptor-induced death of motor neurons by a peroxynitrite/FAS-dependent pathway. J Neurochem. 2013 Aug;126(3):382-8. doi: 10.1111/jnc.12286. Epub 2013 Jun 14.

249. Garcia-Silva MR, Ferreira Cura das Neves R, **Cabrera-Cabrera F, Sanguinetti J**, Medeiros LC, **Robello C., Naya H, Fernandez-Calero T**, Souto-Padron T, de Souza W, **Cayota A**. Extracellular vesicles shed by Trypanosoma cruzi are linked to small RNA pathways, life cycle regulation, and susceptibility to infection of mammalian cells. Parasitol Res (in press).

250. Gil M, Graña M, Schopfer FJ, Wagner T, Denicola A, Freeman BA, Alzari PM, **Batthyány C, Durán R**. Inhibition of Mycobacterium tuberculosis PknG by non-catalytic rubredoxin domain specific modification: reaction of an electrophilic nitro-fatty acid with the Fe-S center. Free Radic Biol Med. 2013 Jun 19. pii: S0891-5849(13)00302-X. doi: 10.1016/j.freeradbiomed.2013.06.021. [Epub ahead of print]

251. Gonzalez HC, Darré L, Pantano S. Transferable Mixing of Atomistic and Coarse-Grain Water Models. J. Phys. Chem. B, Just Accepted Manuscript. DOI: 10.1021/jp4079579

252. Greif G, de Leon MP, Lamolle G, Rodriguez M, Piñeyro D, Tavares-Marques LM, Reyna-Bello A, Robello C, Alvarez-Valin F. Transcriptome analysis of the bloodstream stage from the parasite Trypanosoma vivax. BMC Genomics. 2013 Mar 5;14(1):149. [Epub ahead of print]

253. Iraola G, Pérez R, **Naya H**, Paolicchi F, Harris D, Lawley TD, **Rego N**, Hernández M, Calleros L, Carretto L, Velilla A, Morsella C, Méndez A, Gioffre A. Complete Genome Sequence of Campylobacter fetus subsp. venerealis Biovar Intermedius, Isolated from the Prepuce of a Bull. Genome Announc. 2013 Aug 1;1(4). doi:pii: e00526-13. 10.1128/genomeA.00526-13.

254. Leon IE, Di Virgilio AL, **Porro V**, Muglia CI, Naso LG, Williams PA, **Bollati-Fogolin M**, Etcheverry SB. Antitumor properties of a vanadyl(iv) complex with the flavonoid chrysin [VO(chrysin)2EtOH]2 in a human osteosarcoma model: the role of oxidative stress and apoptosis. Dalton Trans., 2013, 42 (33), 11868 - 11880. [Epub ahead of print]

255. Libisch G, Casás M, **Chiribao ML, Moreno P**, **Cayota A, Osinaga E, Oppezzo P, Robello C.** GALNT11 as a new molecular marker in Chronic Lymphocytic Leukemia. Gene. 2013 Sep 26. doi:pii: S0378-1119(13)01257-2. 10.1016/j.gene.2013.09.052.

256. Manta B, Comini M, Medeiros A, Hugo M, Trujillo M, Radi R. Trypanothione: A unique bis-glutathionyl derivative in trypanosomatids. Biochim Biophys Acta. 1830(5):3199-3216. 2013 Feb 8. pii: S0304-4165(13)00017-2. doi: 10.1016/j.bbagen.2013.01.013. [Epub ahead of print]

257. Massari NA, Medina VA, Cricco GP, Martinel Lamas DJ, Sambuco L, **Pagotto R**, Ventura C, Ciraolo PJ, Pignataro O, Bergoc RM, Rivera ES. Antitumor activity of histamine and clozapine in a mouse experimental model of human melanoma. J Dermatol Sci. 2013; 72(3):252-62. doi: 10.1016/j.jdermsci.2013.07.012.

258. Mazal D, Lo-Man R, Bay S, Pritsch O, Dériaud E, Ganneau C, Medeiros A, Ubillos L, Obal G, Berois N, Bollati-Fogolín M, Leclerc C, Osinaga E. Monoclonal antibodies toward different Tn-amino acid backbones display distinct recognition patterns on human cancer cells. Implications for effective immuno-targeting of cancer. Cancer Immunology, Immunotherapy. 2013 Jun; 62(6):1107-22.

259. Medeiros A, **Berois N**, Incerti M, Bay S, Franco Fraguas L and **Osinaga E**. A Tn antigen binding lectin from Myrsine coriacea displays toxicity on human cancer cell lines. J Natural Med 67:247-54 (2013) doi: 10.1007/s11418-012-0671-x.

260. Megighian A, Zordan M, **Pantano S**, Scorzeto M, Rigoni M, Zanini D, Rossetto O, Montecucco C. Evidence for a radial SNARE super-complex mediating neurotransmitter release at the Drosophila neuromuscular junction. J Cell Sci. 2013 May 17. [Epub ahead of print]

261. Meikle A, Cavestany D, Carriquiry M, Adrien ML, Artegoitia V, Pereira I, Ruprechter G, Pessina P, **Rama G**, Fernandez A, Breijo M, Laborde D, **Pritsch O**, Ramos JM, de Torres E, Nicolini P, Mendoza A, Dutour J, Fajardo M, Astessiano AL, Olazábal L, Mattiauda D, Chilibroste P. Avances en el conocimiento de la vaca lechera durante el período de transición en Uruguay: un enfoque multidisciplinario. *Agrociencia Uruguay* 17(1): 141-152, 2013.

262. Michelini FM, **Zorrilla P, Robello C**, Alché LE. Immunomodulatory activity of an anti-HSV-1 synthetic stigmastane analog. Bioorg Med Chem. 2013 Jan 15;21(2):560-8. doi: 10.1016/j.bmc.2012.10.054

263. Montamat-Sicotte D., **Palacios F**, Di Noia JM, **Oppezzo P**. Origins and consequences of AID expression in lymphoid neoplasms. Current Immunology Reviews, 2013, 9, 72-85

264. Moratorio G, Fischer S, Bianchi S, Tomé L, Rama G, Obal G, Carrión F, Pritsch O, Cristina J. A detailed molecular analysis of complete Bovine Leukemia Virus genomes isolated from B-cell lymphosarcomas. Vet Res. 2013 Mar 18;44(1):19. [Epub ahead of print]

265. Moratorio G, Iriarte A, **Moreno P**, Musto H, Cristina J. A detailed comparative analysis on the overall codon usage patterns in West Nile virus. Infect Genet Evol. 2013 Mar;14:396-400. doi: 10.1016/j.meegid.2013.01.001. Epub 2013 Jan 16.

266. Narancio R., **Zorrilla P., Robello C.**, Gonzalez M., Vilaró F., Pritsch C., Dalla Rizza M. Insights on gene expression response of a characterized resistant genotype of Solanum commersonii Dun.against Ralstonia solanacearum. Eur J Plant Pathol DOI 10.1007/s10658-013-0210-y

267. Naya DE, **Spangenberg L, Naya H**, Bozinovic F. How does evolutionary variation in Basal metabolic rates arise? A statistical assessment and a mechanistic model. Evolution. 2013 May;67(5):1463-76. doi: 10.1111/evo.12042. Epub 2013 Jan 23.

268. Naya DE, **Spangenberg L, Naya H**, Bozinovic F. Thermal conductance and basal metabolic rate are part of a coordinated system for heat transfer regulation. Proc Biol Sci. 2013 Jul 31;280(1767):20131629. http://dx.doi.org/10.1098/rspb.2013.1629

269. Noya V., Bay S., **Festari MF.**, García E., Rodriguez E., Chiale C., Ganneau C., Baleux F., **Astrada S, Bollati-Fogolín M., Osinaga E.** and Freire T. Mucin-like peptides from Echinococcus granulosus induce antitumor activity. Int. J. Oncology 2013: 43:775-84

270. Olivera-Bravo S, Isasi E, Fernández A, Rosillo JC, Jiménez M, Casanova G, Sarlabós MN, Barbeito L. White Matter Injury Induced by Perinatal Exposure

to Glutaric Acid. Neurotox Res. 2013 Dec 3. [Epub ahead of print]

271. Oppezzo P, Dighiero G. Role of the B-cell receptor and the microenvironment in chronic lymphocytic leukemia. Blood Cancer J. 2013 Sep 20;3:e149. doi: 10.1038/bcj.2013.45.

272. Pantano S, Montecucco C. The blockade of the neurotransmitter release apparatus by botulinum neurotoxins. Cell Mol Life Sci. 2013 Jun 11. [Epub ahead of print]

273. Perona M, Dagrosa MA, **Pagotto R**, Casal M, Pignataro OP, Pisarev MA, Juvenal GJ. Protection against radiation-induced damage of 6-propyl-2-thiouracil (PTU) in thyroid cells. Radiat Res. 2013; 179(3):352-60. doi: 10.1667/RR2658.1.

274. Sardi F, Manta B, Portillo-Ledesma S, Knoops B, **Comini MA,** Ferrer-Sueta G. Determination of acidity and nucleophilicity in thiols by reaction with monobromobimane and fluorescence detection. Anal Biochem. 2013 Jan 4. doi:pii: S0003-2697(12)00645-8. 10.1016/j.ab.2012.12.017.

275. Seminotti B, Amaral AU, Da Rosa MS, Fernandes CG, Leipnitz G, Olivera-Bravos S, Barbeito L, Ribeiro CA, De Souza DO, Woontner M, Goodman SI, Koeller DM, Wajner M. Disruption of brain redox homeostasis in glutaryl-CoA dehydrogenase deficient mice treated with high dietary lysine supplementation. Mol Genet Metab. 2013 108:30-39.

276. Spangenberg L, Correa A, Dallagiovanna B, **Naya H**. Role of Alternative Polyadenylation during Adipogenic Differentiation: An In Silico Approach. PLoS One. 2013 Oct 15;8(10):e75578. doi: 10.1371/journal.pone.0075578.

277. Spangenberg L, Shigunov P, Abud AP, Cofré AR, Stimamiglio MA, Kuligovski C, Zych J, Schittini AV, Costa AD, Rebelatto CK, Brofman PR, Goldenberg S, **Correa A, Naya H**, Dallagiovanna B. Polysome profiling shows extensive posttranscriptional regulation during human adipocyte stem cell differentiation into adipocytes. Stem Cell Res. 2013 Jun 10;11(2):902-912. doi: 10.1016/j.scr.2013.06.002. [Epub ahead of print]

278. Tardif V, Riquelme SA, Remy S, Carreño LJ, Cortés CM, Simon T, **Hill M**, Louvet C, Riedel CA, Blancou P, Bach JM, Chauveau C, Bueno SM, Anegon I, Kalergis AM. Carbon monoxide decreases endosome-lysosome fusion and inhibits soluble antigen presentation by dendritic cells to T cells. Eur J Immunol. 2013 Jul 15. doi: 10.1002/eji.201343600. [Epub ahead of print]

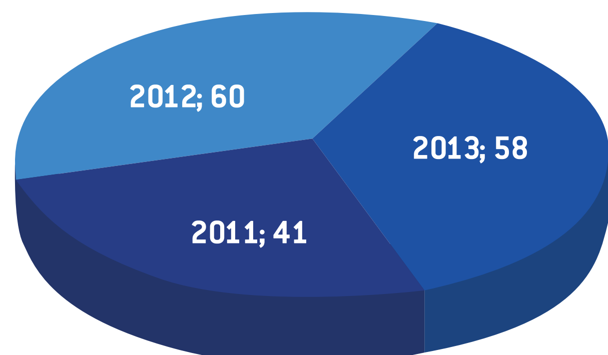
279. Trias E, Díaz Amarilla P, Olivera-Bravo S, Isasi E, Drechsel D, Lopez N, Ireton E, Beckman JS and Barbeito L. Phenotypic transition of microglia into astrocyte-like cells associated with disease onset in a model of inherited ALS. Front. Cell. Neurosci., 7:274 1-8 (2013)

280. Victoria S, Temerozo JR, Gobbo L, Pimenta-Inada HK, Bou-Habib DC. Activation of Toll-like receptor 2 increases macrophage resistance to HIV-1 infection. Immunobiology, 2013; 218(12):1529-36.

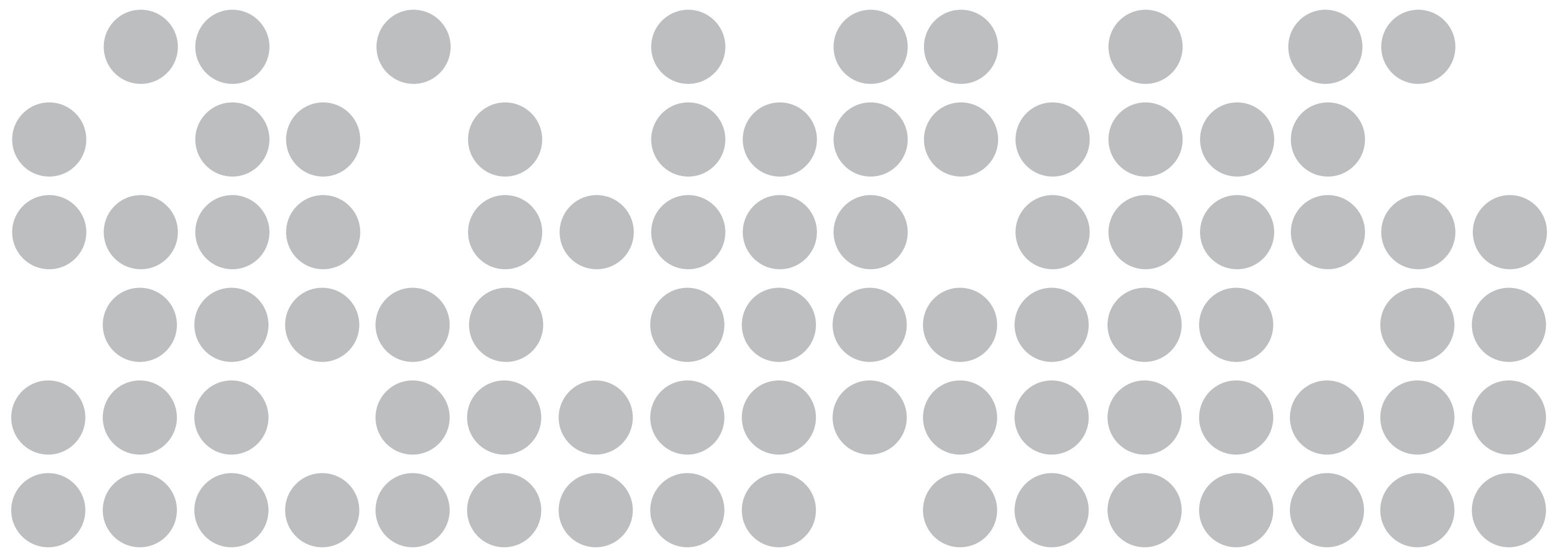
281. Zarantonelli ML, Skoczynska A, Antignac A, El Ghachi M, Deghmane AE, Szatanik M, Mulet C, Werts C, Peduto L, d'Andon MF, Thouron F, Nato F, Lebourhis L, Philpott DJ, Girardin SE, Vives FL, Sansonetti P, Eberl G, Pedron T, Taha MK, Boneca IG. Penicillin Resistance Compromises Nod1-Dependent Proinflammatory Activity and Virulence Fitness of Neisseria meningitidis. Cell Host Microbe. 2013 Jun 12;13(6):735-45. doi: 10.1016/j.chom.2013.04.016.

282. Zhang, S.; Zheng, H.; Long, N.; Carbó, N.; Chen, P.; Aguilar, P.S. and Lua, L. (2014) FigA, a Putative Homolog of Low-Affinity Calcium System Member Fig1 in *Saccharomyces cerevisiae*, Is Involved in Growth and Asexual and Sexual Development in *Aspergillus nidulans. Eukaryot Cell*.doi: 10.1128/EC.00257-13.

Publicaciones 2011-2013



COURSES



Courses 2011

1. Redox Chemistry and Biology of Thiols
2. Hands on Course: Coarse grain methods for biomolecular simulations
3. International Course in Yeast Systems Biology
4. Functional genomics and its applications in biomedicne
5. Genetics of Laboratory Rodents

Courses 2012

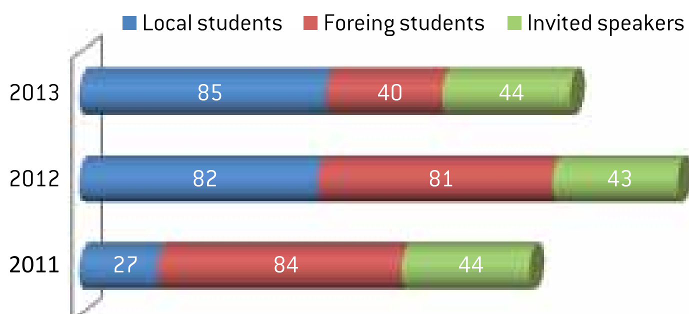
1. Data Analysis Using STATA
2. Generation of Libraries for Next Generation Sequencing
3. Molecular-based Multiscale Modeling and Simulation
4. Neuron-Glia interactions in health and disease: from basic biology to translational neuroscience
5. Biología Molecular de Tripanosomátidos
6. Mass Spectrometry (MS) in Proteomics

Courses 2013

1. Expression, Purification and Crystallization of Recombinant Proteins by High-throughput Methodologies
2. Aproximaciones modernas al estudio epigenético del envejecimiento y cáncer
3. Macromolecular Crystallography School 2013
4. Química y Biología Redox de Tioles
5. Fundamentos y aplicaciones de la Citometría de Flujo
6. FOCEM Course: Introduction to Structural Biology and Bioinformatics
7. Glycobiology and Cancer: From Chemistry to Clinical applications

Obtained funds

Participants' distribution



SCIENCE THAT CHANGES THE FUTURE

Institut Pasteur de Montevideo
Mataojo 2020 - Montevideo, Uruguay
Tel.: + 598 2522 0910 - Fax: + 598 2522 4185
www.pasteur.edu.uy