CNB REPORT

RESEARCH_DEVELOPMENT_INNOVATION

CSIC
Consejo Superior de Investigaciones Científicas

CNB
Centro Nacional de Biotecnologia

Excelencia Severo Ochoa

CNB REPORT

RESEARCH_DEVELOPMENT_INNOVATION

INDEX

DIRECTOR 2020

Mario Mellado

DIRECTOR 2019

Fernando Rojo

Welcome to the CNB

This report summarises the activities of the CNB-CSIC (Centro Nacional de Biotecnología) through the years 2019 and 2020, a period marked by the SARS-CoV-2 pandemic. The infection caused by this coronavirus has been a great challenge for all scientists around the world, not least for the scientific groups of our Institute.

Taking advantage of their expertise, many CNB scientists have adapted their research activities to provide a rapid response to the society. As a consequence, the Institute has positioned itself at the forefront of the Spanish research against the virus. It is in the nature of a biotechnology centre to rapidly translate new knowledge into useful products for the society; since March 2020, the CNB has made an enormous collaborative effort and has increased its interactions with biotechnology companies to fulfil this commitment.

Our contributions against SARS-CoV-2 have been organised into seven lines of work:

1. Vaccine development. Two out of the three CSIC vaccines are being developed at the CNB, with very promising results.
2. Screening of antiviral compounds. A comprehensive analysis of drug repositioning has been performed and both newly synthesised compounds and natural extracts have been evaluated.
3. Development of neutralising recombinant antibodies against SARS-CoV-2 for therapeutic use.
4. Development of diagnostic kits both to analyse the presence of viruses in biological samples and to detect antibodies in the serum of patients.
5. Structural studies of viral proteins to identify potential therapeutic targets, as well as the effect of new SARS-CoV-2 mutations on viral infection.
6. Computational models to evaluate the effect of non-pharmaceutical measures and the behaviour of the population in the spread of epidemics.
7. Communicate our current knowledge on SARS-CoV2 both to scientists and to the general public.

This complex but coordinated network is already delivering tangible results. One of the vaccine prototypes is close to entering a clinical trial, while we are completing the generation of a second vaccine; we have launched a highly efficient seroprevalence diagnostic kit, and we have created a hub platform for scientists worldwide to access the information generated on the structure of several SARS-CoV-2 proteins. This report includes a specific section where we highlight all the SARS-CoV-2 projects underway at the CNB and the main results obtained to date.

During these two years, and as part of objectives of the Severo Ochoa Project, we have created the CNB Bioimaging platform. The main idea behind this initiative was to strengthen the Institute's bioimaging capabilities and capitalise on the recent acquisition

of advanced electron and light microscopy equipment through new lines of research that exploit the power of integrative and correlative bioimaging techniques. The Instruct Image Processing Centre (I2PC), hosted at the Institute for several years, is thus complemented with the acquisition of state-of-the-art equipment in the fields of cryomicroscopy, correlative microscopy and super-resolution microscopy, and with the development of a new Bioimaging Data Analysis Unit. Our goal now is to integrate all of these efforts to carry out multi-scale, multi-resolution approaches spanning from general anatomy to the single-cell, molecular and atomic scales.

Over the course of 2019 and 2020, CNB groups have contributed to the publication of 467 papers in ISI-listed journals, with an average impact factor of 6. Significantly, 220 of these publications made it to the top 10% of most cited journals. As proof of their dynamism, CNB researchers obtained 75 grants (14 from international agencies), submitted 63 PhD theses, taught more than 3000 hours in Master's degree programs, hosted around 170 seminars, including about 20 webinars in 2020, and organised over 50 international workshops and meetings. The data speak for themselves of the international nature of the CNB; near 58% of the papers published by our scientists are the result of collaborations with international scientific groups. As a result, the CNB is attractive to young scientists from abroad, who currently constitute 10.1% of the pre- and post-doctoral personnel. The INPhINIT initiative by the Fundación La Caixa and the Ministry of Science and Innovation, that we wholeheartedly acknowledge, has been fundamental in this regard, by offering fellowships for international and national PhD students to carry out their thesis projects at the CNB. As a result of the 2019 and 2020 calls, the CNB was selected by 57 excellent students from all over the world to start their doctoral studies at the centre.

We have also made an important effort to strengthen the biotechnological value of the CNB. In this period of time, we have initiated the procedures for 14 patents and 4 licenses to companies so that our research eventually translates into a better quality of life for citizens.

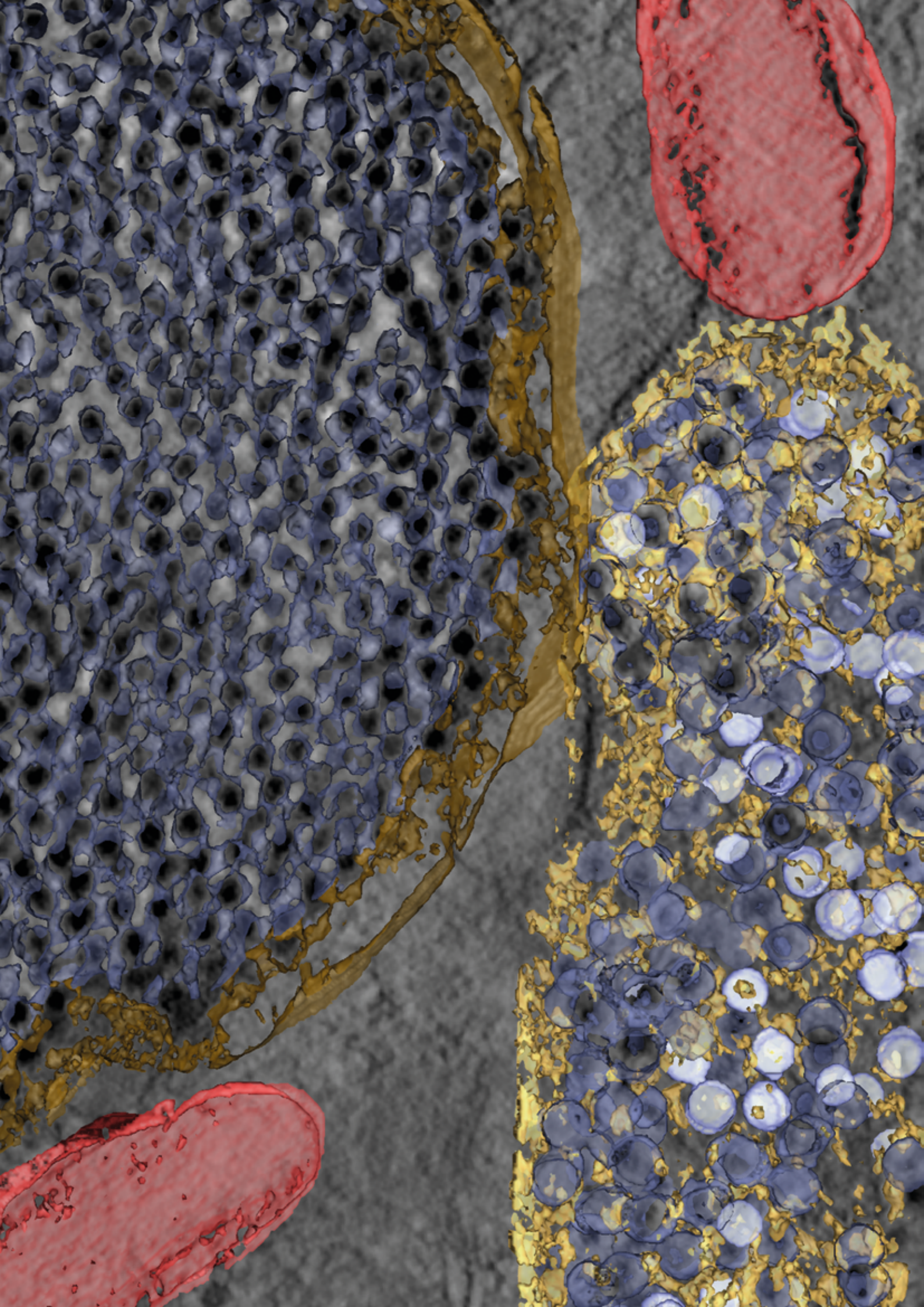
These two years have witnessed an enormous effort in the development of outreach activities and increasing our communications with society. The blog launched in our web site is up and running, our social media accounts have over 4,500 followers in Facebook and 23,500 followers in Twitter and our scientists have been featured more than 2000 times in the media. Although the annual programme of guided visits to the centre have been put on hold due to the pandemic, in the last two years we have participated in outreach events such as the European Researcher's Night, the National Science and Technology Week and the 100xCiencia meetings of SOMMA – the alliance of the Severo Ochoa- and María de Maeztu-accredited Centres.

We would like to express our gratitude to the agencies and institutions that have funded CNB research in the last two years, especially the Spanish Ministry of Science and Innovation and the European Commission which, among others, cofinanced the acquisition of the infrastructure in cryoelectron microscopy and cryocorrelative microscopy (8M€). We are also indebted to the Spanish National Research Council (CSIC) for its continuous support, and we would especially like to acknowledge the generous donations from companies and anonymous people who have contributed to supporting our projects against SARS-CoV-2 during the past year 2020.

Finally, we would also like to express our admiration and gratitude to all CNB personnel who, through their excellent work and commitment, contribute to keep our Institute running and moving forward, even in very difficult times, towards the accomplishment of our objectives.

Mario Mellado
Director 2020

Fernando Rojo
Director 2019



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MACROMOLECULAR STRUCTURES

Scientists in the department work in a large number of biological problems, in particular in the structural and functional characterisation of different molecular machines such as viral structures (Casasnovas, Carrascosa, Castón, Risco, San Martín and van Raaij), DNA repair enzymes (Moreno-Herrero) or molecular chaperones (Valpuesta). These studies are carried out using different structural and biophysical techniques, most of them available at the CNB, which include X-ray diffraction, single-molecule techniques (optical and magnetic tweezers) and various spectroscopic techniques.

Of special note is the development of microscopy techniques such as atomic force, optical and X-ray microscopy, and particularly transmission electron microscopy in its distinct variants (single-particle cryoelectron microscopy, cryoelectron tomography and very recently correlative cryomicroscopy), which is supported by the CNB's cryoelectron microscopy facility, the first one of this kind in Spain. This work is strongly supported by continuous software development in the field of image processing (Carazo, Fernández and Sánchez-Sorzano), which has led to the CNB hosting of the INSTRUCT image processing centre, a pan-European distributed research infrastructure that provides expertise and access to high quality instruments.

Technical developments are also pursued in the field of proteomics (Corrales), which resulted in the CNB being chosen to head the Spanish proteomic facilities network (PROTEORED) and participation in the Human Proteome Project.

Finally, it is important to stress the role played by scientists of this department in different aspects of the COVID19-related investigation carried out by the CNB, which has placed our centre at the forefront of the Spanish research in this field.

HEAD OF DEPARTMENT

José María Valpuesta

The image shows a reovirus-infected cell. Mature virions (blue) are collected by a modified lysosome (brown) from a viral inclusion (yellow), surrounded by mitochondria (red). (From Cristina Risco's lab).

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Biocomputing unit

Electron microscopy of biological samples under cryogenic conditions (cryoEM) has established as a key player in structural biology. Starting from purified samples in the so-called single particle analysis approach, the technique allows elucidating the three dimensional structure of macromolecules up to atomic resolution. For cellular sections, the technique is known as Electron Tomography and it may provide information *in situ*, in the cell. Our group develops image processing algorithms that are able to deal with single particle and electron tomography data. Their goal is to extract the most information from the acquired data in a reliable and as automated as possible way. Particularly in Electron Tomography, our methodological advances are integrated in the context of an ambitious ERC Synergy project, targeting specifically the *in situ* analysis of the Epidermal Growth Factor Receptors family, together with the Medalia, Plueckthun and Olsen laboratories. Our algorithms are available through the Xmipp software package.

In addition to novel image processing algorithms, we also develop Scipion, a workflow engine for the execution of image processing pipelines integrating multiple software suites. Scipion guarantees the traceability and reproducibility of the results, as well as it solves an interoperability problem between the different software packages. Using Scipion, we give support for image processing in cryoEM through the European Infrastructure for Structural Biology Instruct-ERIC and the iNext-Discovery platforms.

Finally, we also have a Structural Bioinformatics role by developing an interface between the structural biology databases (EMDB, PDB) and the biomedical annotations databases containing genomic, proteomic and interactomic information. This connection helps to better understand the biomedical context of the reconstructed structures and has been recognised as one of the few Recommended Interoperability Resources of the European Infrastructure of Life Science Information, ELIXIR.

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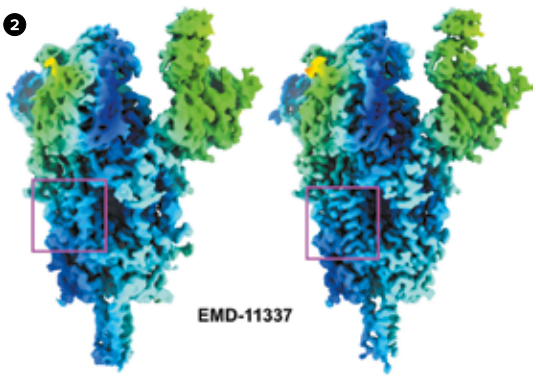

❶ *3DBionotes-Covid19. A Web-based interactive information integration environment (COVID-19 special edition).*

❷ *Effect of local sharpening algorithms (DeepEMhancer, Sanchez-García et al, BioRxiv.2020; LocalDeblurr, Ramirez-Aportela et al., ICUrJ, 2019). The results are shown on our reported data on SARS CoV-2 spike (Melero et al, IUCrJ, 2020).*

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Vilas JL, Tagare HD, Vargas J, Carazo JM, Sorzano COS. Measuring local-directional resolution and local anisotropy in cryoEM maps. Nat Comms 2020; 11: 55..

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Melero R, Sorzano COS, Foster B, Vilas JL, Martínez M, *et al.* Continuous flexibility analysis of SARS-CoV-2 spike prefusion structures. IUCrJ 2020; 7 (6): 1059-1069.

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Structure of macromolecular assemblies

Our group has worked for years in the analysis of the molecular bases of assembly and nanoscopic properties of different macromolecular complexes. We have used a combination of different microscopic approaches in a correlative way, with the idea to cover different resolution levels to provide structural information from the atomic to the cellular level.

We have continued the study on how viral particles incorporate DNA inside virus, how it is stabilised and which are the virus components involved in its ordered delivery upon infection. Using cryo-electron microscopy to solve the structure of different components of phage T7, we have revealed the full atomic structure of the machinery involved in DNA translocation (the connector and several tail components). The structures obtained using a combination of x-ray crystallography and cryo-electron microscopy, and the comparison of these structures in different viruses, have provided the bases for understanding how the DNA is released from the virus particle during viral infection. The cryo-electron microscopy study of the viral core, which is composed of several proteins that dissociate upon viral interaction with the bacterial receptor, has shown that these proteins reassemble to build a conduit for DNA delivery to the cell cytoplasm. The structures obtained at atomic resolution from these core complexes suggest how this process is accomplished.

We have also continued our studies integrating different microscopic approaches at increasing resolution levels. Correlative cryo-electron microscopy, light microscopy, soft –X ray microscopy and spectroscopy have been instrumental in a series of studies where we have defined the intracellular fate of anticancer compounds, the characterisation of different intracellular forms of a parasite, and the structural features of centrosomes in differentiated cells.

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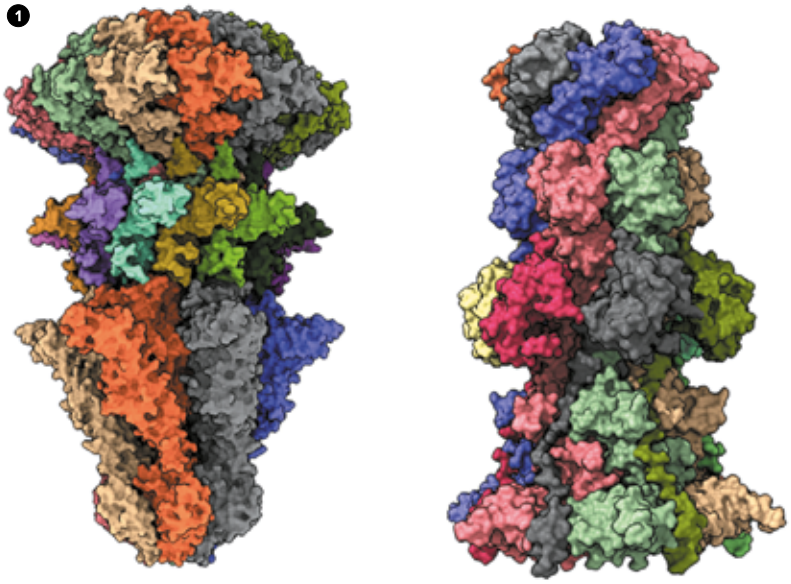

1 *T7 DNA translocation protein complexes involved in viral infection solved by cryo-electron microscopy. Left, three-dimensional reconstruction of T7 tail complex (~1.5 MDa) composed by the connector (gp8), gp11 and gp12 proteins. Right, three-dimensional reconstruction of T7 core complex (~1.4 MDa) composed by gp14 and gp15 proteins. Each protein monomer is colored in a different color.*

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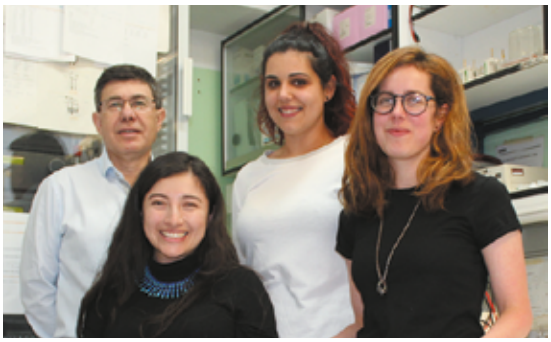

Cell-cell and virus-cell interactions

Our group studies the cell surface molecules that regulate the immune system and virus entry into host cells. We analyse receptor-ligand interactions related to immune processes, as well as virus binding to cells. In addition, we characterise virus neutralisation by humoral immune responses and its correlation with virus cell entry. Our research has provided key observations regarding immune receptor function, and has identified viral epitopes essential for virus infection, some of which are targeted by neutralising antibodies. Our multidisciplinary research applies structural (X-ray crystallography), biochemical and cell biology approaches.

We are characterising antibodies (Abs) that neutralise Human Immunodeficiency Virus (HIV), Ebolavirus (EBOV) or the SARS-CoV-2, responsible of the COVID-19 pandemic. Using electron microscopy, we found that a potent anti-HIV-1 Ab binds to the CD4-receptor binding site in the HIV env protein (Figure 1); this Ab likely neutralises HIV because it inhibits virus cell entry. Using several methodologies we are identifying anti-EBOV antibodies, which are potential therapeutics for the treatment of Ebola disease.

During this year, we have produced the SARS-CoV-2 envelope spike (S) antigen, which is being used in serological tests completed at the CNB-CSIC.

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Tsilingiri K, de la Fuente H, Relaño M, Sánchez-Díaz R, Rodríguez C, *et al.* oxLDL receptor in lymphocytes prevents atherosclerosis and predicts subclinical disease. Circulation 2019; 139: 243-255.

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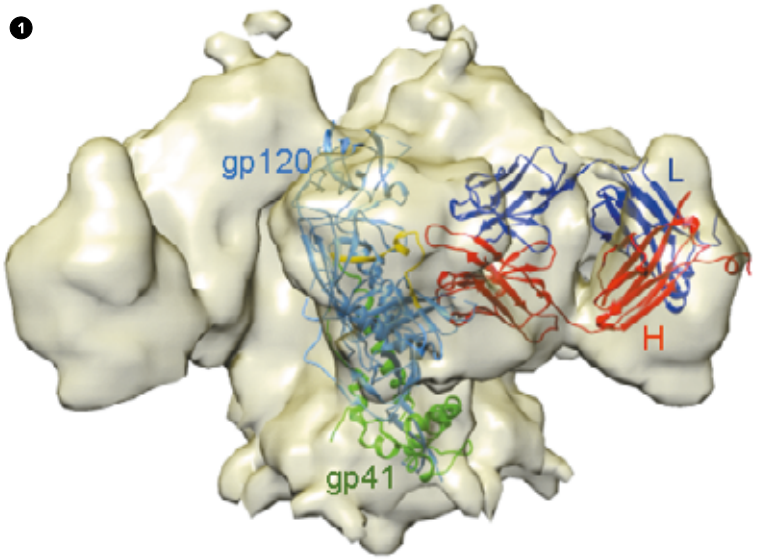

1 *An antibody that neutralises HIV-1 and recognises the CD4 receptor binding site in the virus envelope glycoprotein. Electron density map of a trimeric HIV-1 gp140 protein in complex with a neutralising Ab. A monomer of the HIV-1 protein composed of the gp120 (blue) and the gp41 (green) subunits fitted in the map are shown as ribbons, together with the bound Ab (heavy chain in red y light chain in blue). The CD4 receptor-binding region in gp120 to which the Ab binds is in yellow.*

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Molecular machines laboratory

Our studies address to elucidate structure-function-evolution relationships of viral macromolecular complexes, also known as viral nanomachines, which control many fundamental processes in virus life cycle. For that, we have incorporated state-of-the-art approaches to obtaining high-resolution structures of many viral assemblies in near-native conditions, at resolutions better than 3 Å by combining data from several hundred or often thousands of electron microscope images.

Capsids are dynamic structures whose components have transient conformations associated with specific roles in the viral cycle. In addition, the capsid is a metastable assembly: it is robust enough to protect the genome, and labile enough to allow genome delivery into the host cell. Information on virus structures at the highest possible resolution is essential for identifying their molecular mechanisms and functions. Three-dimensional cryogenic electron microscopy (3D cryo-EM) of viruses and viral capsids is widely used to determine their structures at near-atomic resolution in near-native conditions. Structural analysis of viruses is complemented by study of mechanical properties by atomic force microscopy (AFM), to examine the relationship between physical properties such as rigidity and mechanical resilience, and virus biological function. Our basic structural research shows alternatives for interfering in their function, as well as clues for vaccine and/or antiviral drugs design. These studies are also central to establishing the basis for incorporation of heterologous proteins, nucleic acids, and/or chemicals into viral capsids (considered as nanocontainers), of potential use for future biotechnological applications.

We characterised the structure and conformational polymorphism of several viral systems, including double-stranded RNA (dsRNA) viruses such as birnaviruses (infectious bursal disease virus, IBDV), human picobirnavirus (HPBV) and several fungal viruses, as well as single-stranded RNA viruses such as rabbit haemorrhagic disease virus (RHDV) and human rhinovirus (HRV). We extend our studies to other bacterial and eukaryotic complexes in collaboration with several national and international groups.

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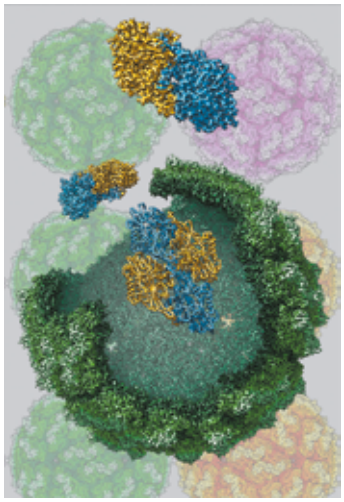

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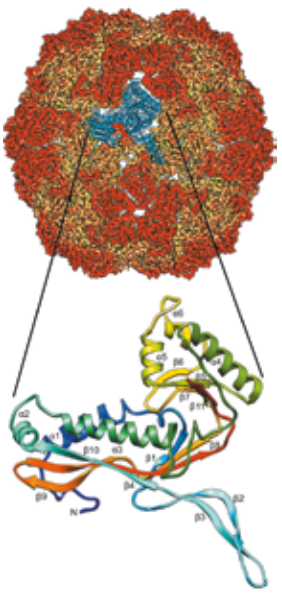

1 *Cryo-EM structure of human picobirnavirus (HPBV) at 2.6 Å resolution. HPBV is a dsRNA virus, broadly extended in the human population. The HPBV capsid is built of 60 capsid protein dimers (blue and yellow). Using an* in vitro *reversible assembly/disassembly system of HPBV, we isolated dimers and tetramers as possible assembly intermediates.*

2 *Cryo-EM Structure of* Brevibacterium linens *encapsulin (BlEnc) at 2.28 Å resolution. Structural comparison between BlEnc and the capsid proteins of the viral lineage HK97 shows high structural similarity, indicating that both compartments descend from a common ancestor.*

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Functional proteomics

The Functional Proteomics laboratory of the CNB has two main research areas of interests: the study of mechanisms underlying the progression of liver diseases and the characterisation of HLA peptide repertoires to define the antigen presentation principles in the context of diseases, including COVID-19.

We have developed a sample processing method that provides a three-fold increased coverage of human bile proteome (Ciordia S *et al*, J Proteomics, 2020). This method allowed identification of a panel of bile proteins that might prove useful for the clinical management of cholangiocarcinoma patients (Urman J *et al*, Cancers, 2020). We have also developed a method for liquid biopsy analysis (Navajas R *et al*, Methods Mol Biol 2019) that is currently being used for stratification of pre-eclampsia patients.

We coordinate a cooperative project with 25 laboratories integrating the Spanish Proteomics Platform (ProteoRed) to investigate the interaction between SARS-CoV-2 and the host cell proteome aiming to discover the bases associated to the COVID-19 progression. We also aim to discover circulating proteins with prognostic value that may lead to an efficient stratification of COVID-19 patients (Calvo E *et al*, J Pers Med 2020). All this work is coordinated with international initiatives, including COVID-19 MS-Coalition (Struwe W *et al*, Lancet, 2020) and the Human Proteome Project (Adhikari S *et al* Nat Comm, 2020), where we are working to define the human proteome (Omenn GS *et al*, J Prot Res, 2020).

Finally, we have leadership in national and international initiatives such as ProteoRed, Spanish Proteomics Society, European Proteomics Association and the Human Proteome Project. The total number of publications in 2019 and 2020 were 23. (average IF 5.52) and 14 (average IF 8.96) respectively.

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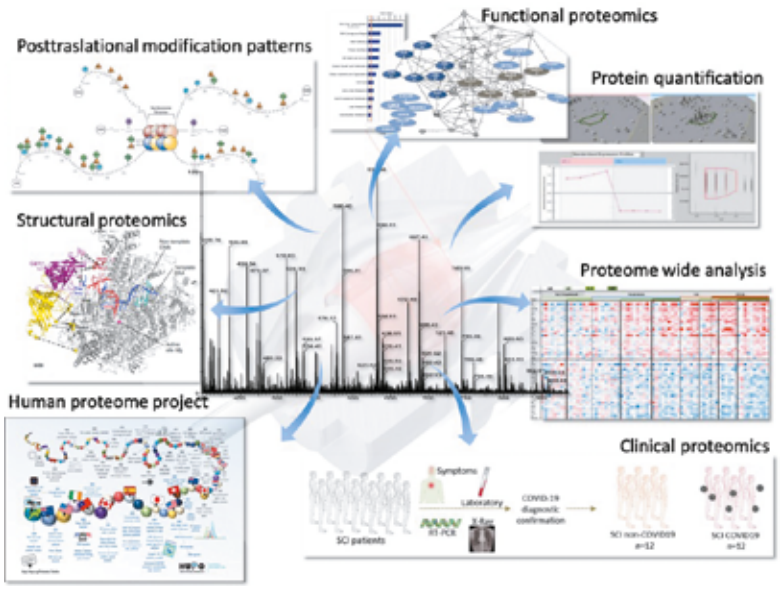

1 *Summary of the main research areas in the CNB Functional Proteomics Laboratory*

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Struwe W, Emmott E, Bailey M, Sharon M, Sinz A, Corrales FJ, *et al.* The COVID-19 MS Coalition-accelerating diagnostics, prognostics, and treatment. COVID-19 MS Coalition. Lancet 2020; 395 (10239): 1761-1762. d

Ciordia S, Alvarez-Sola G, Rullán M, Urman JM, Ávila MA, Corrales FJ. Digging deeper into bile proteome. J Proteomics 2021; 230:103984.

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Li S, Fernandez JJ, Marshall W, Agard DA. Electron cryo-tomography provides insight into procentriole architecture and assembly mechanism. eLife 2019; 8: e43434

Fernandez JJ, Li S, Agard DA. Consideration of sample motion in cryo-tomography based on alignment residual interpolation. J Struct Biol 2019; 205: 1-6

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Electron tomography and image processing of cell structures

Our group is interested in the unique ability of electron tomography (ET) to visualise in three-dimensions the subcellular architecture and macromolecular organisation of cells and tissues *in situ* at a resolution of a few nanometres. Combined with image processing, ET has emerged as a powerful technique to address fundamental questions in molecular and cellular biology.

One of our research interests is focused on the 3D analysis of the neuronal subcellular architecture. Here, ET and image processing are the central techniques along with protocols that ensure preservation of brain tissue samples in close-to-native conditions. With this approach, we are exploring the structural alterations that underlie neurodegenerative diseases, particularly Huntington's disease.

We are also working in close collaboration with Dr. Sam Li (UCSF) in structural elucidation of the microtubule-organising centre (MTOC). This is an important and complex cellular organelle whose dysfunction is linked to many diseases. In addition, we actively collaborate with other teams at the CNB and other international groups in experimental structural studies.

Another important focus of our research is the development of new image processing techniques and tools for the advancement of ET. We are working on new methods for the different computational stages involved in structural studies by ET: image alignment, correction for the transfer function of the microscope, tomographic reconstruction, noise reduction, automated segmentation and subtomogram analysis.

In 2020 the group left the CNB to further focus on the biomedical field and health research.

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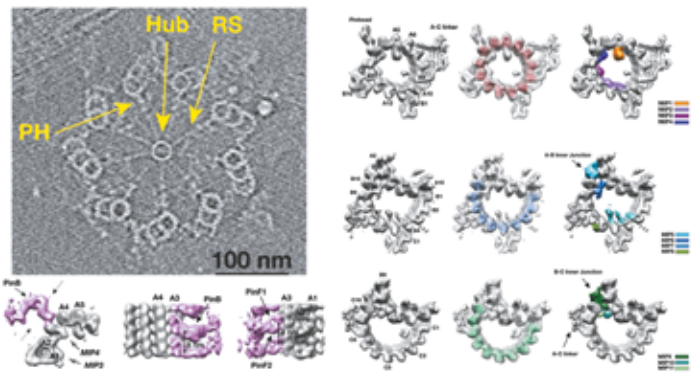

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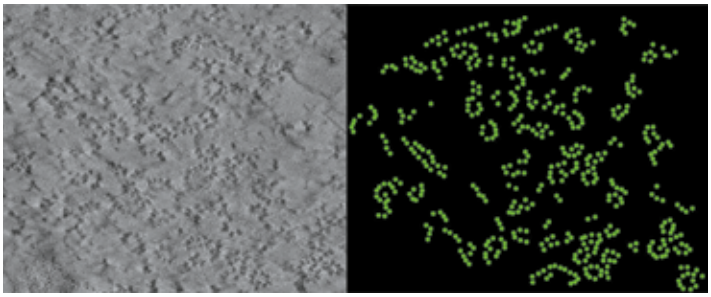

❶ *Molecular architecture of procentrioles revealed by electron cryo-tomography and image processing.*

❷ *Visualisation of the spatial distribution of ribosomes and polysomes in striatal medium-sized spiny neurons by electron tomography of brain tissue samples.*

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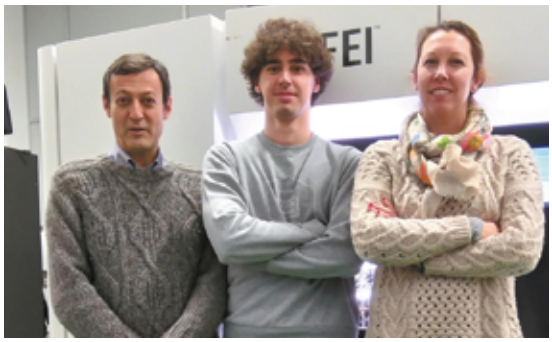

Ultrastructure of viruses and molecular aggregates

The main line of the group is focused on the study of the Influenza A ribonucleoproteins (RNPs) that conforms the virus nucleocapsid. RNPs are macromolecular complexes composed of the genomic RNA bound to multiple monomers of a nucleoprotein and a single copy of the polymerase. In recent years our laboratory has determined the structure of the isolated RNPs at medium resolution and we have verified that this structure is present in native virions using cryoelectron tomography.

Currently our research on this topic is following two major subjects that will extend to the next years. The first one is the resolution improvement of the RNP structure, and for that we will make use of the state-of-the-art cryoelectron microscope equipped with direct electron detector, recently acquired by the CNB. We have already discovered the existence of an enormous conformational variability in the RNPs structure, and this has been possible through the design of a new protocol able to classify and reconstruct helical structures. We have demonstrated that this extreme conformational variability is closely related with the biological roles of the RNPs. With this idea we opened the second major line of our research, the elucidation of the structural basis of transcription and replication mechanisms and for that our plan is to complement structural data with biochemical assays that will allow us to establish the action mechanism. Our research also extends to the study of influenza virus polymerase at high resolution. Our goal is to solve the polymerase dimer structure that represents the functional complex for replication of RNPs.

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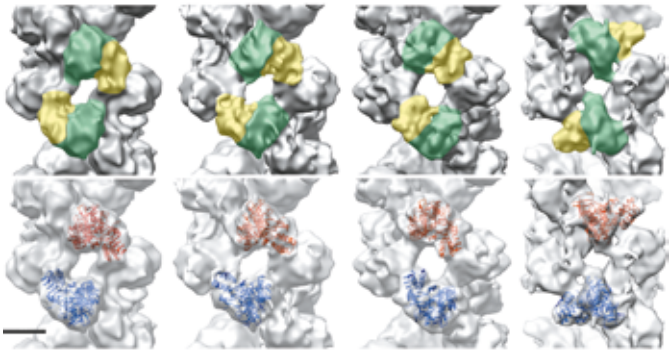

1 *3D reconstructions of different conformations of the helical part of the influenza virus ribonucleoproteins. Top row: the head and body domains of the nucleoproteins are outlined in yellow and green, respectively. Bottom row: the docking of the influenza A nucleoprotein atomic structure (pdb 2IQH) is shown in the opposite strands in red and blue. Scale bar, 50Å.*

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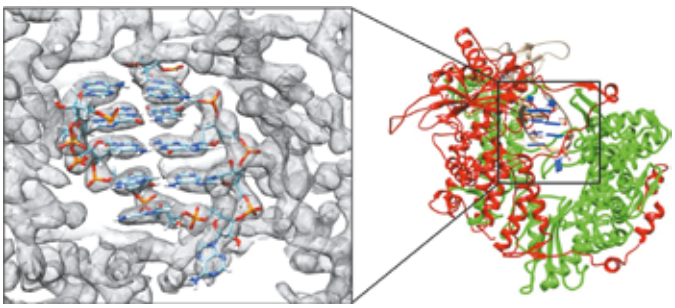

2 *Structure of the polymerase of the influenza A virus at 3.0 Å resolution bound to the cRNA promoter. On the right, the atomic structure of the polymerase obtained by electron cryomicroscopy is shown and on the left, a detail of the atomic density map corresponding to cRNA bound to protein.*

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Molecular biophysics of DNA repair nanomachines

Our group is interested in the development and use of single-molecule techniques to study the mechanical properties of nucleic acids and the mode of action of protein machines involved in the repair, replication and maintenance of chromosome structures. We use novel single-molecule approaches based on atomic force microscopy (AFM) optical and magnetic tweezers, as well as molecular dynamics simulations.

Currently, we are developing and implementing a combined TIRF-AFM system. The combination of high resolution AFM imaging with single-molecule fluorescence will allow us to correlate morphology with the presence of a particular protein. We also manage a hybrid system combining optical tweezers and confocal microscopy (C-Trap™ from Lumicks), which enables manipulating single DNA/RNA molecules while simultaneously imaging the fluorescence between the beads in real time.

Apart from instrument development, in the last two years we have focused on the study of the physical properties of DNA sequences, which has led to the understanding of how they regulate genome folding. In two recent studies, we showed that the crookedness and flexibility of DNA is regulated by nucleotide sequence at short length scales (Marín-González *et al* 2019 and 2020).

We also investigated different molecular motors and DNA-interacting proteins involved in the rolling-circle replication of plasmids (RepC and PcrA) (Carrasco *et al* 2020) and identified a novel DNA2-like helicase-nuclease as a single-stranded DNA looping motor (Wilkinson *et al* 2020).

Lastly, we have used single-molecule assays to investigate the functional and dynamic characteristics of yeast Smc5/6 holocomplex bound to DNA. We showed that the third and less studied eukaryotic SMC complex locks plectonemes and can compact DNAs in an ATP-dependent manner (Gutiérrez-Escribano *et al* 2020). Our results demonstrate that the Smc5/6 complex recognises DNA tertiary structures involving juxtaposed helices, and might modulate DNA topology by plectoneme-stabilisation and compaction.

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Marín-González A, Vilhena JG, Moreno-Herrero F and Pérez R. DNA crookedness regulates DNA mechanical properties at short length scales. Phys Rev Lett 2019; vol 122 (4), 048102.

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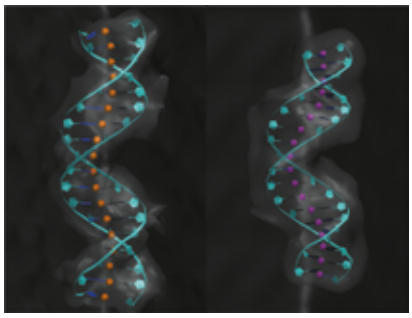

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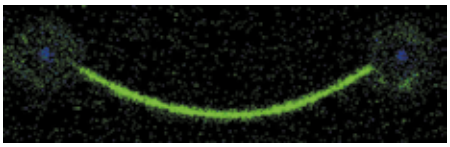

❶ *Molecular dynamics simulation of two DNA molecules showing how their nucleotide sequences affect their crookedness (path given by yellow and pink dots).*

❷ *C-trap fluorescence image of AlexaFluor 488-labelled RPA bound to a single-stranded DNA molecule captured between two beads.*

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Cell structure laboratory

Emerging and re-emerging viruses are a global threat because there are no vaccines nor specific drugs for many of them. Our lab works on the cell biology of viral infections to identify new targets for antiviral therapies. We are currently studying Bunyaviruses, Reoviruses and Coronaviruses. Bunyaviruses are a large group of RNA viruses, many of them transmitted by mosquitoes or ticks, that includes important pathogens for humans, animals and plants. Reoviruses are common pathogens of mammals that have been linked to celiac disease. Coronaviruses cause lethal pathologies such as SARS, MERS and COVID-19.

During the last two years, we have used live cell imaging, correlative light and electron microscopy and electron tomography to study the biogenesis of viral replication factories and virus egress pathways. We have discovered that human reoviruses hijack cell lysosomes. On the periphery of the viral factory, these lysosomes collect mature virions and transport them to the cell surface. This is a new, nonlytic virus egress mechanism and a potential new target for therapeutic intervention.

With state-of-the-art computational tools and databases of clinically approved drugs, we have completed a pre-clinical search for Bunyavirus and Coronavirus inhibitors and we now count with a list of more than one hundred potential antivirals. These include inhibitors of mitochondrial proteins, lipid transfer proteins, proteasome and protein kinases, together with inhibitors of Bunyavirus RNA polymerases, and SARS-CoV-2 MPro, Spike, MTase and RNApol. Ten of these compounds have been selected for subsequent steps of pre-clinical studies. Our research on new antivirals to combat COVID-19 has been supported by almost 3,000 citizens and 9 companies, through the Precipita crowdfunding platform of the Fecyt (Fundación Española para la Ciencia y la Tecnología). The main goal of this project is to validate broad-spectrum antivirals to combat SARS-CoV-2 and many other pathogenic viruses.

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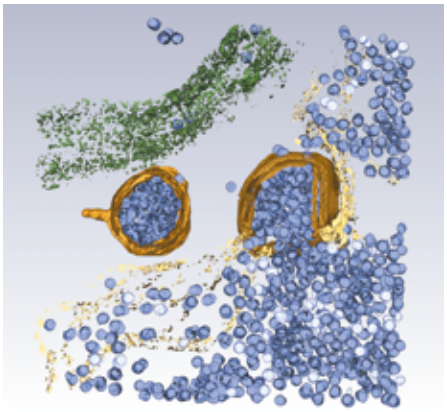

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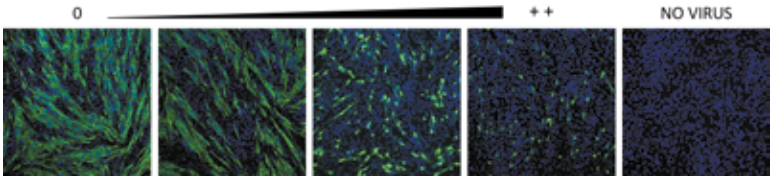

❶ *Egress machinery of human reovirus as visualized by 3D electron tomography. Mature virions (purple) are collected by lysosomes (brown) for their transport to the plasma membrane (green).*

❷ *Testing antivirals for coronaviruses. From left to right, cells infected with the human coronavirus HCoV229E (green) were incubated with increasing amounts of drugs to determine the non-toxic concentration that inhibits viral growth. Nuclei are stained with DAPI (blue).*

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Structural and physical determinants of complex virus assembly

We are interested in the principles governing complex virus assembly. Our main model system is adenovirus, a large non-enveloped icosahedral virus with a 95 nm capsid composed of more than 10 different proteins. Adenoviruses are human pathogens, but can be engineered as therapeutic tools. More than 60 years after their discovery, there are still considerable open questions regarding adenovirus morphogenesis. To address these questions, we use a multidisciplinary approach that combines Biophysics, Structural and Molecular Biology techniques.

Most recently, we have analysed the role of core protein VII, a histone-like protein, in adenovirus assembly. Protein VII was thought to condense the dsDNA genome for packaging within the capsid. Surprisingly, our collaborator P. Hearing (Stony Brook University) showed that protein VII is not required for genome encapsidation, but particles assembled in its absence (Ad5-VII-) are deficient in maturation of minor coat protein VI. Protein VI binds to the inner surface of hexon capsomers, and contains a lytic peptide which must be released during entry to ensure endosome escape. In collaboration with P. Hearing, P. de Pablo (IFIMAC-UAM) and U. Greber (U. Zurich), we have contributed to clarify the role of protein VII and its interplay with protein VI in adenovirus assembly and entry.

We observed that Ad5-VII- particles cannot leave the endosomes during entry due to failure to expose protein VI. A cryo-electron microscopy map (Fig. 1A) showed that, in the absence of core protein VII, the lytic peptide remains trapped inside the hexon cavity (Fig. 1B), unavailable for cleavage by the maturation protease and for endosome membrane disruption. Difference maps between Ad5-VII- and complete particles indicate that proteins VI and VII can interact with the same pocket in hexons. Based on these results, we have proposed a model where the competition between proteins VI and VII for hexon binding during assembly is responsible for releasing the lytic protein from the hexon cavity, facilitating its complete maturation and exposure during uncoating in the endosome (Fig. 1C).

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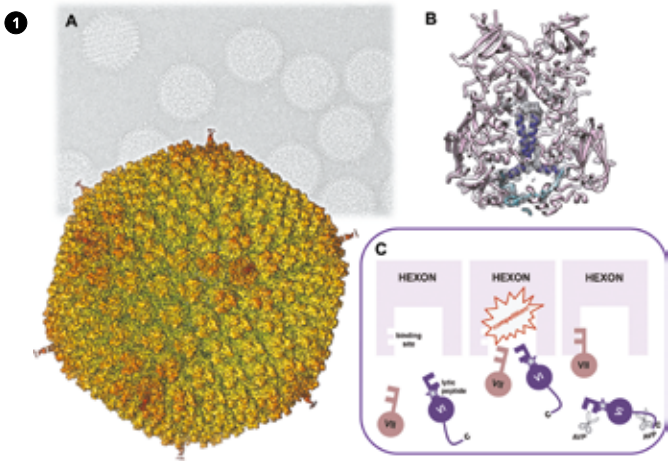

1 *(A) A cryo-electron microscopy map of human adenovirus type 5 lacking core protein VII. (B) Localisation of the coat protein VI lytic peptide (purple) trapped inside a cavity in the major coat protein (hexon, light pink) when protein VII is not present. (C) The information gathered from cryo-EM and other molecular and biophysical techniques indicates that proteins VI and VII compete for binding to the same site in the hexon cavity, and this competition is crucial for the lytic peptide to be exposed during entry to facilitate adenovirus escape from the endosome.*

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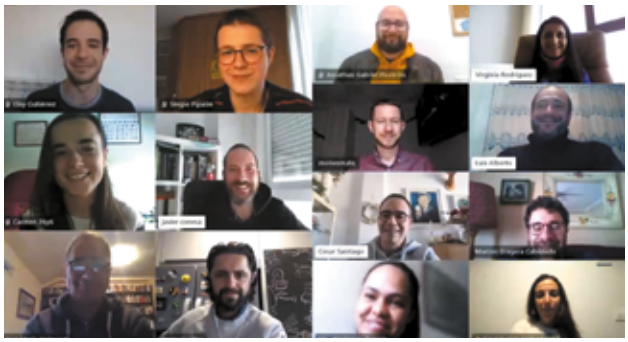
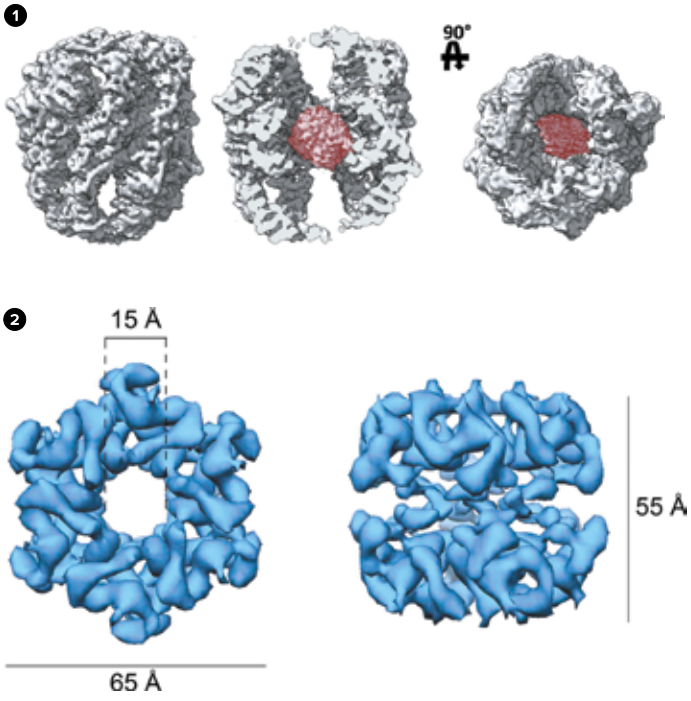


Structure and function of molecular chaperones

We use different various biophysical techniques, chiefly cryoelectron microscopy, to study the structure and function of different macromolecular complexes, in particular those formed by molecular chaperones, a group of proteins involved in cell homeostasis through two opposite functions, protein folding and degradation. These two cellular processes are carried out through the transient formation of complexes between different chaperones and cochaperones, acting like an assembly line and making the process a more efficient one. The two processes are carried out through the transient formation of complexes between different chaperones and cochaperones. Our main goal is the structural characterisation, at the highest possible resolution of some of these complexes, using as a main tool state-of-the-art cryoelectron microscopy and image processing techniques. We also aim to study from a structural point of view the implication of different chaperones in the regulation of complex cellular events as the immune synapse. For that we are implementing correlative approaches to locate and resolve molecular events in a native cellular context.



1 *Three images of the three-dimensional reconstruction (by cryoelectron microscopy) of a complex between the chaperone CCT and its substrate, the protein mLST8 (red mass) (1000 kDa; 3.9 Å resolution). Left, side view of the outer surface. Centre, a view of the interior of the CCT cavity. Right, end-on view showing mLST8 in the CCT cavity.*

2 *Two orthogonal images of the three-dimensional reconstruction (by cryoelectron microscopy) of the engineered protein CI2, which forms a double hexamer in solution (88 kDa; 8.5 Å resolution).*

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Structural biology of viral fibres

Correct recognition of the bacterial cell wall is of crucial importance to the life cycle of a bacteriophage, both in deciding which bacterium to infect as in lysing the host after phage multiplication.

Many bacteriophages bind to their host cell receptors via specialised spike proteins or via specialised fibre proteins. These proteins tend to have the same basic architecture: they are trimeric and contain an N-terminal virus or bacteriophage attachment domain, a long, thin, but stable shaft domain and a more globular C-terminal cell attachment domain. By careful analysis of their domain structure and adapted expression and purification protocols, we obtain suitable quantities of these proteins for crystallisation and structures solution by crystallography.

After infection and generation of multiple infective particles, the bacteriophage needs to lyse the host cell to disperse the daughter phages so they can encounter new bacteria to infect. To this end, the phage produces endolysins that digest the bacterial peptidoglycan layer. In many cases, biotechnologically produced endolysins can also be applied “from the outside” to lyse bacteria.

In the years 2019 and 2020, we have determined the high-resolution structures of receptor-binding proteins of a *Salmonella* and a *Campylobacter* phage and of a *Pseudomonas* phage endolysin protein in complex with a peptidoglycan fragment. In addition, we collaborated with other research groups in crystallisation and structure solution of the proteins and peptides they produce.

Deep knowledge of the structures of bacteriophage receptor-binding and endolysin proteins may lead to different applications. Modification of the bacteriophage fibre receptor binding specificities may lead to improved detection of specific bacteria and to mutant phages with improved host ranges. A better understanding of endolysin structure, stability and specificity may similarly lead to better elimination of pathogenic or otherwise unwanted bacteria, and to mutant endolysins with a different or wider range of target bacteria.

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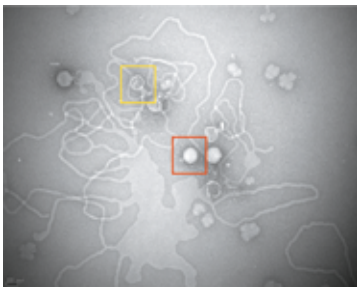

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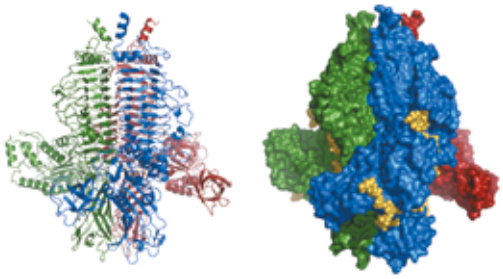
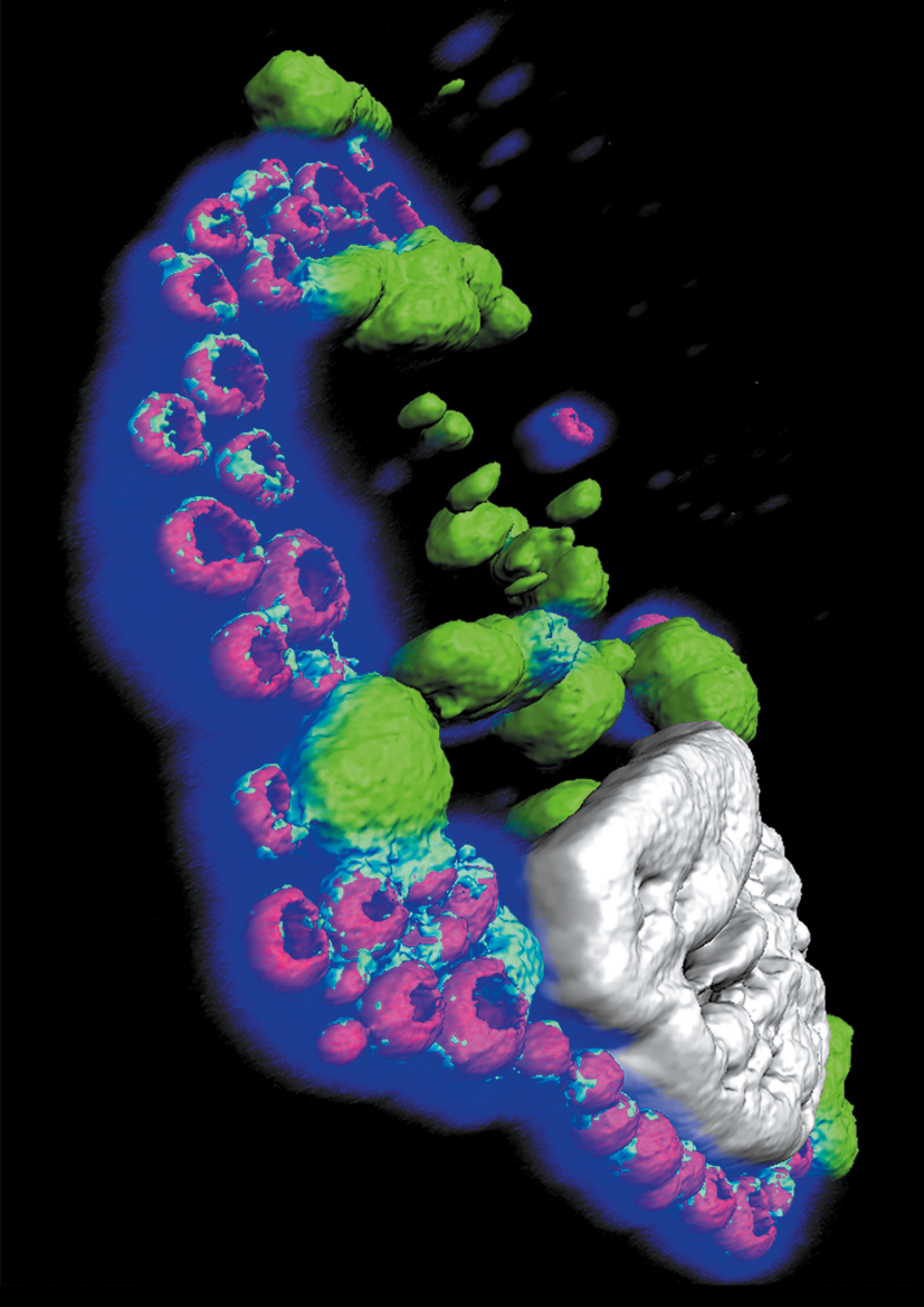


❶ *Electron microscopy image of* Salmonella enterica *subspecies* Enterica, *serovar Anatum A1 bacteriophage epsilon15 in the presence of bacterial lipo-polysaccharide (LPS). The red square highlights a mature phage with a DNA-filled head. The short phage tail and spikes are just visible on the bottom left of the phage head. The yellow square highlights a phage that has transferred the DNA from its head into an LPS vesicle. The size bar in the bottom left corner is 50 nm long.*

❷ *Structure of bacteriophage epsilon15 tailspike gene product 20 (gp20). On the left, a ribbon representation of the trimer is shown, with each monomer coloured differently. On the right, gp20 is shown in space-filled representation, with bound ligands (*Salmonella *O-antigen fragments) shown in yellow.*



2

MOLECULAR AND CELLULAR BIOLOGY

The Department of Molecular and Cellular Biology hosts 14 independent research groups working in two broad, closely interwoven research areas, with the goal of identifying specific therapeutic targets for use in disease prevention and control. The first area focuses on dissecting viral replication mechanisms and on structural studies of key viral proteins, as well as virus-host interactions for important human and veterinary pathogens. The identification of virus and cell elements with key roles in virus replication is essential for the rational design and implementation of new strategies for disease control. Understanding the mechanisms that allow a virus to evade or counteract innate and adaptive host immune responses will allow generation of innovative vaccination strategies and virus-based vaccine vectors. The second area centres on the networks that control mammalian gene expression and on characterising specific genes with critical roles in normal and pathological processes. The aim of this research programme is to identify and exploit molecular targets for diagnostics and therapy. In addition to generating leading edge research, studies in our department help to provide essential scientific background for the development of new biotechnological tools.

Our department also counts with a virus biotechnology platform (VBP) that was created with the aim of providing integral biotechnological solutions to health challenges caused by human and animal viruses. In this regard, in the context of the COVID-19 pandemic several groups have devoted their efforts to fight against SARS-CoV-2 by: i) developing vaccines based on non-replicative SARS-CoV-2 replicons and on poxvirus recombinants; ii) developing a high throughput screening platform to test compound libraries for their antiviral potential against SARS-CoV-2; iii) producing recombinant SARS-CoV-2 proteins as antigens for the development of serological test and potential vaccines; iv) producing monoclonal antibodies for anti-viral therapy; v) controlling viral infection through the modulation of cellular energy metabolism; and vi) using the CRISPR/cas13d technology as a therapeutic tool to target coronavirus RNA genome.

During this period the department has lost one of its research groups due to the retirement of Dr. Amelia Nieto.

HEAD OF DEPARTMENT

Dolores Rodríguez

Image from a 3D reconstruction of IBDV replication complexes. The compact superstructures, exclusively stained with VP2 antibodies, adjacent to viroplasms, correspond to aggregates formed by tightly packaged IBDV virions. Images were captured and processed at the Advanced light microscopy CNB core facility by Daniel Fuentes Martínez, Sylvia Gutiérrez Erlandsson and Ana Oña Blanco. (From José F. Rodríguez's lab).

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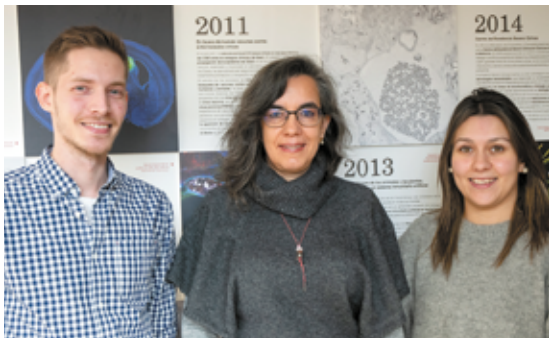

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Menotti M, Ambrogio C, Cheong TC, Pighi C, Mot I, *et al.* Wiskott-Aldrich syndrome protein (WASP) is a tumor suppressor in T cell lymphoma. Nat Med. 2019; 25: 130-140.

Escoll M Lastra D, Robledinos-Antón N, Wandosell F, Antón IM, Cuadrado A. WIP modulates oxidative stress through NRF2/KEAP1 in glioblastoma cells. Antioxidants (Basel). 2020; 9 (9): 773-785.

Antón IM, Gómez-Oro C, Rivas S, Wandosell F. Crosstalk between WIP and Rho family GTPases. Small GTPases. 2020; 11: 160-166.

Molecular bases of actin cytoskeleton reorganisation in cell motility, tumour generation and invasiveness

Actin cytoskeleton is an essential contributor to cell motility and invasiveness and therefore understanding the molecules and mechanisms which regulate its temporal and spatial re-organisation is vital to fight tumour invasion and metastasis, the cause of 90% of cancer-associated deaths.

We study the role of actin-related proteins, mainly (N)WASP (neural Wiskott-Aldrich syndrome protein), WIP (WASP interacting protein), TAZ (Transcriptional coactivator with *PDZ*-binding motif) and YAP (Yes-associated kinase), in tumour generation, progression and dissemination, mostly focusing in central nervous system tumours such as deadly gliomas. Through the analysis of glioblastoma samples using biochemical and molecular approaches, in combination with lentivirus-mediated modification of protein expression and advanced imaging analysis, mouse models and proteomics, we have described that WIP acts as a proto-oncogene; WIP overexpression is sufficient to transform primary human astrocytes following pathways which include Pak, formins (mDia) and the GTPase Rac. WIP levels directly correlate with cell proliferation, anchorage-independent growth, stemness and invasive capability and they also control the cellular amount of the co-transcriptional regulators and mechanosensors TAZ and YAP by protecting them from calpain or proteasome-mediated degradation. In contrast, in haematological malignancies (T cell lymphoma), WASP and WIP turn into tumour suppressors as ALK+ lymphomas developed by transgenic NPM-ALK mice are accelerated in WASP- and WIP-deficient mice.

At present we are following a systematic proteomics approach by mass spectrometry to search for common and unique partners in the WIP interactomes identified from glioblastoma versus haematological samples. We hope to identify novel therapeutic targets to find additional and effective cancer treatments. Our ultimate goal is to understand the molecular basis of the mechanism that regulates actin dynamics, a process that underlies numerous essential cellular functions whose deregulation leads to serious human diseases. We thus hope to provide new diagnostic, prognostic and/or therapeutic tools for neurological disorders, tumour initiation and metastasis.

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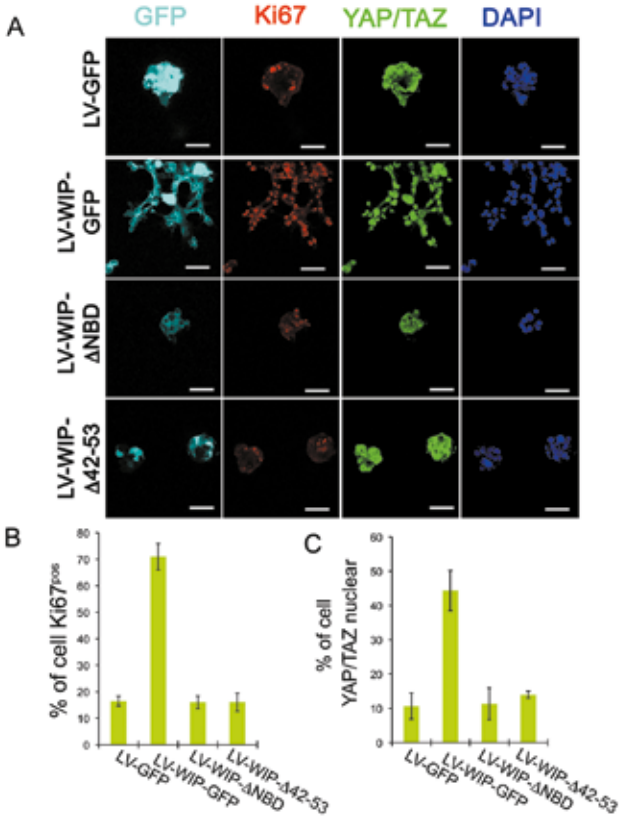

1 *WIP binding to Nck and actin coordinates survival and proliferation to drive cell invasiveness in a YAP/TAZ-dependent manner. MDA-MB-468 cells were transduced with lentivirus encoding GFP, WIP-GFP, WIP-ΔNBD (WIP mutant lacking the Nck binding domain) or WIP-Δ42/53. (WIP mutant lacking the actin binding domain). (A) Confocal images of staining for GFP (cyan), proliferation marker Ki67 (red), YAP/TAZ (green) and nuclei (DAPI, blue) of invasive/non-invasive structures in 3D-Matrigel; bars, 25 µm. (B) Percentage of cells that showed positive staining for Ki67 or (C) nuclear localisation of YAP/TAZ.*

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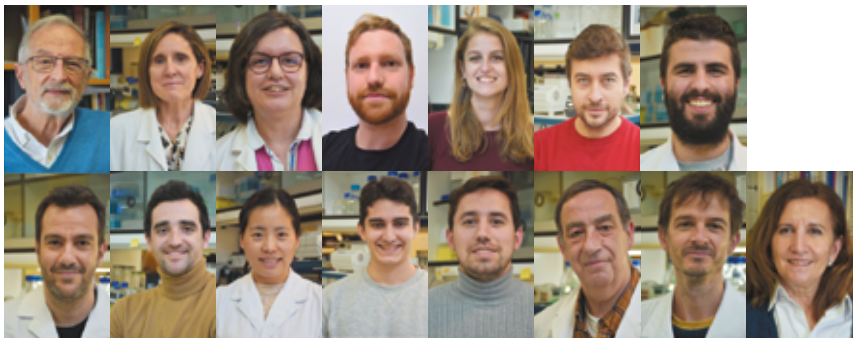


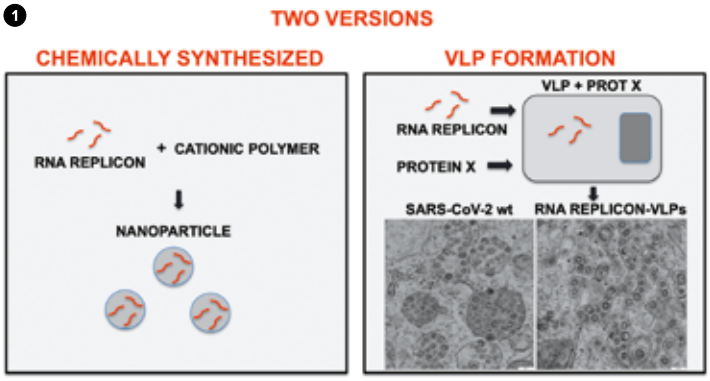
Coronavirus: replication and transcription, virus-host interactions, and protection

Human infections causing pneumonia and acute respiratory distress syndrome (ARDS) are one of the most common cause of death in the EU. The problem is even greater in the elderly population, which responds with lower efficacy to vaccination.

Among the seven known human coronaviruses (CoV), HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1 are the cause of up to 15% of mild respiratory infections. In contrast, SARS-CoV, MERS-CoV, and SARS-CoV-2 cause severe respiratory syndromes. These deadly viruses emerged from animal reservoirs in the 21st century, being SARS-CoV-2 the causative agent of the CoV disease (Covid-19) pandemic. Our laboratory focuses on the study of virus-host interactions, the design of vaccines and the selection of antivirals to protect against severe respiratory CoV infections by modulating the innate immune response in young and elderly populations.

The main aims of our research are:

- **Development of a new generation of SARS-CoV-2 vaccines consisting in replication-competent propagation-deficient RNA replicons, which are safe and promising vaccine candidates, and to determine their efficacy in animal model systems.** Vaccine development includes: (i) Engineering the RNA-replicons by deleting or modifying viral genes responsible for propagation and virulence, using reverse genetics; (ii) Identification of RNA-replicon delivery systems; (iii) Development of packaging cell lines that efficiently complement the generation of virus-like particles (VLPs); (iv) Engineering simplified and safer versions of the replicase complex.
- **To identify cell-signaling pathways involved in CoV replication and pathology** to select antiviral drugs that inhibit these pathways. In particular, we study PBM-PDZ protein-protein interactions involved in the innate immune and inflammatory responses, since overstimulation of these pathways is responsible for increased mortality.
- **To determine the contribution of host miRNAs and virus-derived small RNAs to the inflammatory lung pathology.** These small non-coding RNAs represent antiviral targets. To enhance the efficacy of vaccine candidates in older adults, RNA-replicons delivering immunomodulatory miRNAs will be engineered.



1 *RNA replicon vaccines to protect against highly pathogenic human coronaviruses. Two types of replicon delivery systems have been designed: (i) a chemically synthesised one that includes two components the RNA replicon and a cationic polymer to form nanoparticles. (ii) formation of virus like particles (VLPs) complemented in packaging cells lines with the proteins required for propagation from cell to cell.*

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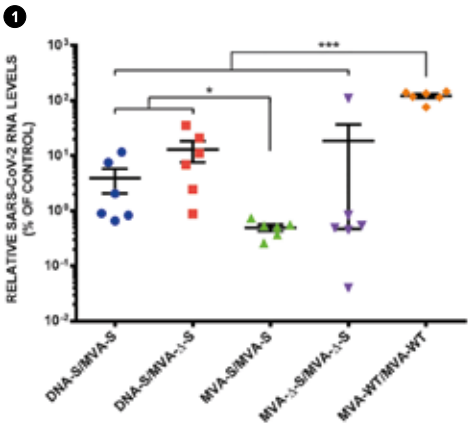
Poxvirus and vaccines

The main objectives of our laboratory are geared towards understanding the molecular basis of the biology of infectious agents and their interaction with the host, as well as to use this knowledge in the development of vaccines that might be effective against emerging viruses, like HIV, chikungunya, ebola, zika, hepatitis C, coronavirus SARS-CoV-2, as well as against cancer. As a model system of an infectious agent and as a delivery vector for expression of genes of interest, we use vaccinia virus (VACV) and the attenuated vaccine strains MVA and NYVAC, members of the poxvirus family. Our goal is to develop the best-in-class immunogens and vaccination protocols to be applied as vaccines against prevalent human diseases.

By studying the behaviour of replication competent and incompetent poxvirus vectors MVA and NYVAC, alone and in combination with other immunogens (DNA, mRNA, alphavirus replicon, VSV vectors, protein), our group has made important contributions in the immune biology of vaccines, the mechanisms of T and B cell immune responses, correlates of protection and the engineering of vaccine candidates against a variety of prevalent human diseases, obtaining in animal models 80-100% efficacy against ebola, chikungunya and zika.

As of January of 2020 the group participates in the fight against the coronavirus SARS-CoV-2 responsible for COVID-19, with the development of a vaccine candidate MVA-CoV2-S that has shown 100% efficacy against SARS-CoV-2 (morbidity, mortality and inhibition of virus replication) in humanised mouse models. Currently with the MVA-CoV2-S vaccine, immunogenicity and efficacy studies are on-going in hamsters and macaque models, as well as there are planned phase I/II clinical trials for 2021. New recombinant vectors and strategies are under development to establish optimal vaccination approaches able to confer wide and long-term protection against different coronavirus variants and strains. This research is supported by national and international grants.



1 *High neutralisation of SARS-CoV-2 by serum from humanised mice immunised with the vaccine candidate MVA-CoV2-S (MVA-S in the graph) in various combinations (from Garcia-Arriaza et al, J Virol 2021).*

2 *The vaccine candidate MVA-CoV2-S administered in one or two doses in humanised mice protects 100% against lethality induced by SARS-CoV-2.*

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Virus-host interactions in hepatitis B virus infection

Our laboratory is interested in understanding virus host interactions that regulate the outcome and pathogenesis of virus infections. Our main objective is to identify vulnerabilities that could be exploited to develop new antiviral therapies. In the last years we have used hepatitis B virus (HBV) and hepatitis C virus (HCV) infection cell culture models. These hepatic viruses are responsible of millions of cases of acute and chronic hepatitis and represent the major etiological agent of liver cancer worldwide.

During the 2019-2020 period, we focused on understanding the role of cellular proteins in the virus life cycle. On one hand, we identified Erlin-1 protein, an endoplasmic reticulum resident protein, as a new host factor required for efficient HCV infection. Gene silencing experiments have demonstrated that Erlin-1 protein regulates early as well as late steps in the HCV life cycle. Interestingly, Erlin-2, a protein with high sequence and functional homology with Erlin-1 protein does not play any important role in HCV infection. Our results provide new insights into functional differences between the two Erlins and identify a new molecular target for therapeutic intervention. On the other hand, we have confirmed and expanded our initial observations that DNA damage response related proteins are key restriction factors for HBV infection. Moreover, we are working on basic aspects of HBV DNA integration, key for cancer development.

Since the SARS-CoV-2 pandemic started our group have teamed up with Dr. Gastaminza's group to establish the CNB Antiviral Screening Platform. The main objective is the identification and characterisation of new antiviral compounds against highly pathogenic human virus infections. We have screened thousands of chemical compounds and identified new families of experimental compounds with antiviral activity against SARS-CoV-2. Moreover, we have identified repurposing drugs that are been considered for clinical testing. Finally, we have signed several research contracts with the pharmaceutical industry.

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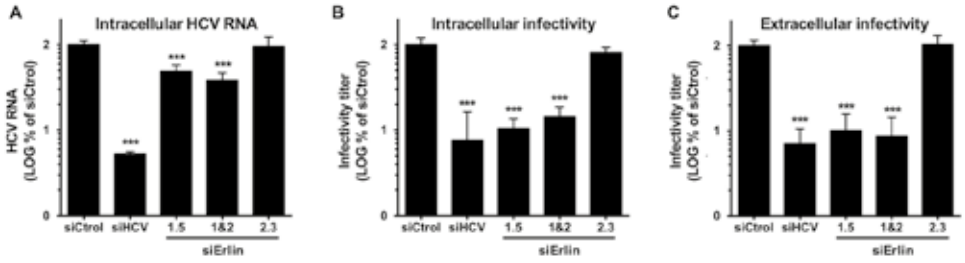

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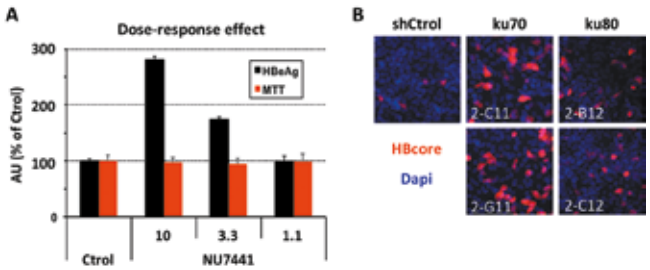

1 *Erlin-1 protein down-regulation interferes with HCV infection, shown by reduced levels of intracellular RNA (A) and infectivity (B) as well as extracellular infectivity (C).*

2 *(A) Treatment with the DNA-PKc inhibitor NU7441 increases HBeAg levels in a de novo HBV infection (B) Silencing of ku70 and ku80 proteins, regulator subunits of DNA-PKc, increases the intracellular accumulation of HBV core protein.*

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Infection by hepatitis C and related viruses

Our laboratory studies pathogenic human viral infections, and focuses on understanding the molecular basis of viral pathogenesis and identifying new molecular targets for antiviral therapy. Our final aim is to propose new therapeutic approaches for antiviral treatment and for reversion of virus-induced pathogenesis. To achieve these general aims, we have implemented cell culture models for infection by hepatitis C virus and other members of the *Flaviviridae* family such as dengue, Zika and West Nile viruses. Given the current health emergency due the COVID-19 pandemic, we have also implemented cell culture models of infection by SARS-CoV-2 coronavirus, including a compound screening platform for antiviral drug discovery.

Although their origin, nature and structure are not identical, a common feature of the aforementioned positive-strand RNA viruses is their ability to subvert host lipids and intracellular membranes to generate replication and assembly complexes. We previously reported that lipin1, a cellular enzyme that converts phosphatidic acid into diacylglycerol, is involved in the formation of the membranous web that hosts hepatitis C virus (HCV) replicase. In the liver, lipin1 cooperates with lipin2 to maintain glycerolipid homeostasis. We extended our previous study of the lipin family on HCV infection, by determining the impact of the lipin2 silencing on viral replication. Our data reveal that lipin2 silencing interferes with HCV virion secretion at late stages of the infection, without significantly affecting viral replication or assembly. Moreover, uninfected lipin2-, but not lipin1-deficient cells display alterations in mitochondrial and Golgi apparatus morphology, suggesting that lipin2 contributes to the maintenance of the overall organelle architecture. Finally, our data suggest a broader function of lipin2 for replication of HCV and other RNA viruses, in contrast with the specific impact of lipin1 silencing on HCV replication. Overall, our studies reveal distinctive functions of lipin1 and lipin2 in cells of hepatic origin, a context in which they are often considered functionally redundant.

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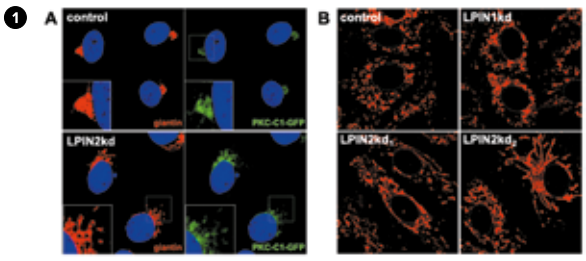
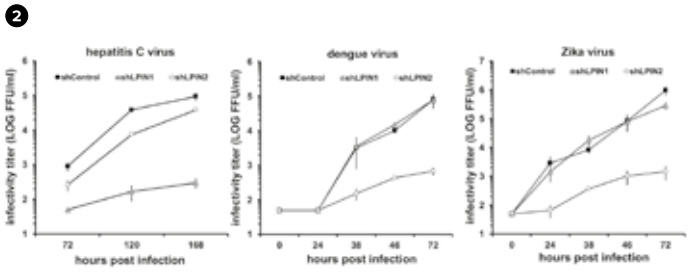
Castro V, Calvo G, Ávila-Pérez G, Dreux M, Gastaminza P. Differential roles of Lipin1 and Lipin2 in the Hepatitis C Virus replication cycle. Cells. 2019; 8 (11): 1456.

Castro V, Ávila-Pérez G, Mingorance L, Gastaminza P. A cell culture model for persistent HCV infection. Methods Mol Biol 2019; 1911: 157-168.

Galindo I, Garaigorta U, Lasala F, Cuesta-Geijo MA, Bueno P, *et al.* Antiviral drugs targeting endosomal membrane proteins inhibit distant animal and human pathogenic viruses. Antiviral Res, 2020; 26: 104990.

Marcos-Villar L, Nistal-Villan E, Zamarreño N, Garaigorta U, Gastaminza P, Nieto A. Interferon-ß stimulation elicited by the Influenza virus is regulated by the histone methylase Dot1L through the RIG-I-TRIM25 signaling axis. Cells 2020; 9 (3): 732.





1 *Lipin2, but not lipin1, silencing causes morphological alterations of the Golgi apparatus and mitochondrial elongation. Huh-7 cells constitutively expressing a DAG sensing probe (PKC-C1-D1-GFP) were transduced with lentiviral vectors expressing non-targeting (control), shRNAs targeting LPIN1 (LPIN1kd) or LPIN2 mRNA (LPIN2kd1 and LPIN2kd2). At day 7 post transduction, control and lipin-deficient cultures expressing the DAG probe were fixed and processed for immunofluorescence microscopy using antibodies against a Golgi Apparatus marker (giantin) or stained with Mitotracker™ red following manufacturer recommendations and imaged in vivo under a confocal microscope at 37oC and 5% CO2 to visualise mitochondria. A-Representative images of the Golgi morphology (red) and subcellular DAG probe (green) PKC-C1-GFP different cell lines. Nuclei were stained with DAPI and are shown in blue. B-Representative images of the mitochondrial morphology in the different cell populations. Cell nucleus is approximately delimited by a dotted white line for reference.*

2 *Lipin2 silencing interferes with hepatitis C, dengue and Zika virus propagation. Huh-7 cells were transduced with lentiviral vectors expressing non-targeting (control), shRNAs targeting LPIN1 (LPIN1kd) or LPIN2 mRNA (LPIN2kd1 and LPIN2kd2). At day 7 post transduction, the different cultures were inoculated with HCV (JFH1-D183v strain), DENV (NGC strain), with ZIKV (BeH819015 strain). Samples of the cell supernatants collected at the indicated time points were used to determine extracellular infectivity titers. Data are shown as average and SD of three biological replicates (n=3).*

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Biological noise and its physiopathological implications

Phenotypic variability of clonal cell populations is mainly due to differential gene expression, in which the mitochondria content is a key factor. This non-genic cellular heterogeneity plays an essential role in many biological processes such as cell differentiation, development, apoptosis, cancer and viral infections. Our laboratory is interested in understanding the origins of this phenotypic variability and its impact on different biological processes to improve our understanding of phenomena like tumour resistance to drugs, virus infection, or cell fate choice.

During years 2019-2020 we have made important contributions in two main areas:

1. Origin of phenotypic variability. We have found that mitochondrial content contributes to heterogeneity in gene products and have a large impact on alternative splicing, which ultimately leads to phenotypic diversity.

2. Physiopathological implications of the variability of mitochondrial content in apoptosis and viral infections. Regarding the apoptosis process, we have described that the cellular mitochondrial content modulates the time to death in response to TRAIL treatment, indicating that this variability could have a great impact on the partial response to chemotherapy observed in the majority of tumours. Regarding viral infections, we have found a correlation between mitochondrial content and virus replication, and that this correlation could be direct or inverse depending of the virus analysed. Due the importance of Zika virus (ZIKV) in human health and the recent COVID-19 pandemic, we have extended these studies to ZIKV and SARS-CoV-2, and we have initiated a new investigation line focused on the study of the molecular bases of the pathogenesis of both viruses. In that sense, we have developed reverse genetic systems for ZIKV and SARS-CoV-2 that has allowed us to identify several virulent factors and several proteins of the cell metabolism important for virus replication. These studies will improve our understanding of ZIKV and SARS-CoV-2 biology and facilitate the development of vaccine and antiviral strategies.

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Ye C, Chiem K, Park JG, Oladunni F, Platt RN, *et al.* Rescue of SARS-CoV-2 from a single bacterial artificial chromosome. mBio 2020; 11 (5): e02168.

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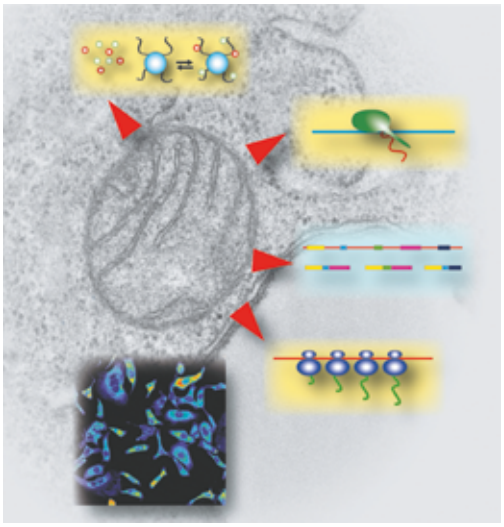

2

1 *Mitochondria and gene expression. Electron micrograph of one mitochondrion with radiating arrows to the steps of gene expression where mitochondria play an important role.*

2 *Pathogenicity of rSARS-CoV-2 rescued from a infectious clone. Gross pathological lung lesions of Golden Syrian hamsters infected with rSARS-CoV-2 or the natural isolate (SARS-CoV-2). Presence of congestion and atelectasis (white arrows) and frothy trachea exudates (black arrows) are indicated. Scale bars, 1 cm.*

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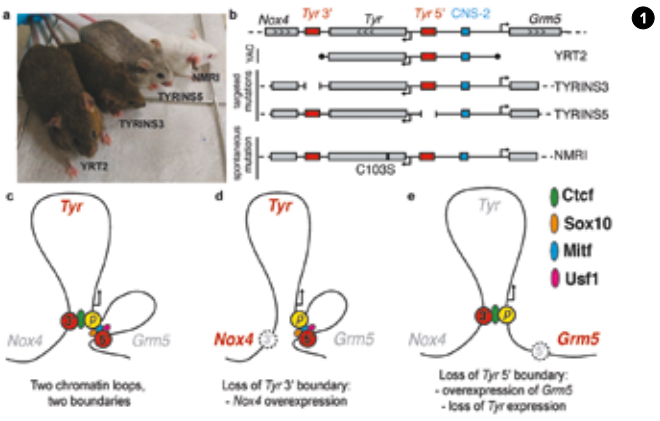
Animal models by genetic manipulation

Our laboratory is interested in understanding the underlying pathological mechanisms of a group of human rare diseases globally known as albinism, a heterogeneous genetic condition associated with mutations in at least 22 genes, characterised by visual impairment and pigmentation alterations. Our work on human rare diseases occurs within our participation in the CIBERER-ISCIII.

Our laboratory has generated and analysed new animal models to study visual abnormalities and different anomalies affecting retina development that are associated with albinism. In collaboration with Angel Carracedo (USC) and Carmen Ayuso (FJD), we have devised, within the CIBERER-ISCIII, a project for the universal genetic diagnostic of all known mutations in albinism. We are already applying this knowledge in cooperation with ALBA, the Spanish association in support of people with albinism and have been able to diagnose more than 120 families.

We are also interested in understanding the function of regulatory elements that are required to define gene expression domains in mammalian genomes. We have used the mouse tyrosinase locus (*Tyr*) as experimental model. This approach has allowed us to identify several key regulatory elements, such as genome boundaries or insulators, which protect the locus from surrounding genes and ensure the faithful gene expression pattern.

As a general strategy, we regularly use transgenic and genome-edited animals, zebrafish and mice to introduce different type of gene constructs in order to investigate the relevance of specific DNA regulatory sequences. The functional analysis of regulatory elements found within the intergenic non-coding genomic sequences can now be addressed more efficiently thanks to the efficient genome editing CRISPR-Cas9 tools. In Spain, where we have pioneered the application of CRISPR technology in mice, we have successfully implemented it in our laboratory and disseminated its use among colleagues by hosting short stays and organising ad-hoc workshops, seminars and courses.



1 *CRISPR-Cas9 genome edited mice used to functionally identify key regulatory elements of the mouse Tyr locus (Seruggia et al. 2020, Sci. Rep.)*

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SELECTED PUBLICATIONS

Momtazi G, Lambrecht BN, Naranjo JR, Schock BC. Regulators of A20 (TNFAIP3): new drug-able targets in inflammation. Am J Physiol Lung Cell Mol Physiol 2019; 316 (3): L456-L469.

Dell'Orco D, Koch KW, Kreutz MR, Naranjo JR, Schwaller B. Neuronal calcium sensors in health and disease. Front Mol Neurosci 2019; 12: 278.

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Gonzalo-Gobernado R, Perucho J, Vallejo-Munoz M, Casarejos MJ, Reimers D, *et al.* Liver growth factor "LGF" as a therapeutic agent for Alzheimer's disease. Int J Mol Sci 2020; 21 (23): 9201.

Functional analysis of transcriptional repressor DREAM

Our major research focus is on the multifunctional protein DREAM and its role in the control of calcium homeostasis in health and disease.

DREAM (downstream regulatory element antagonist modulator), also known as calsenilin or KChIP3, is a Ca2+ binding protein of the neuronal calcium sensors (NCS) superfamily that interacts with specific sites in the DNA to repress transcription of target genes in a Ca2+-dependent manner. In addition, DREAM interacts with specific proteins to exert various specialised functions in different subcellular compartments. Thus, through the control of activity-dependent gene expression and through specific protein-protein interactions, DREAM participates in many physiological processes in and outside the central nervous system. Work reported by us and other groups has shown important regulatory roles for DREAM in learning and memory in the hippocampus, in pain control in the spinal cord as well as in the immune response, in inflammation, in the thyroid gland and in the placenta. Moreover, recent studies have shown the involvement of DREAM in neurodegenerative disorders including Huntington disease (HD), Alzheimer disease (AD) and Amyotrophic Lateral Sclerosis (ALS).

DREAM was originally associated with AD because of its interaction with presenilins, however, altered neuronal calcium and protein homeostasis and early compensatory changes in transcriptional programs are common features of many neurodegenerative disorders which open the opportunity to explore a role for DREAM in these pathologies.

In physiological conditions, binding of calcium or membrane lipids (e.i. arachidonic acid) regulate the interaction with DNA or with other proteins. Newly identified molecules, including glinides, modify DREAM conformation and activity upon binding. In this respect, our interest is to contribute to the definition of more specific DREAM binding molecules, to reveal the molecular mechanisms underlying their effect upon binding to DREAM and to assess their potential therapeutic actions on appropriate cellular and/or mouse models of target pathologies.

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1 *Mouse performing in the Pole test. We use this test to analyse motor coordination in mice expressing the A315T mutation in the TDP-43 gene. In this mouse model of Amyotrophic Lateral Sclerosis (ALS), we assay new DREAM ligands that could ameliorate disease symptoms or delay disease progression. This project is funded by Asahi Kasei Pharma.*

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Mechanisms of interaction between the influenza virus and the infected cell

Influenza A virus (IAV) promotes epigenetic modification in the infected cells. IAV infection increases the methylation of lysine 79 of histone 3 catalyzed by Dot1L enzyme. A decreased antiviral signaling mediated by RIG-I sensor is found in Dot1L-inhibited cells, infected with IAV. Accordingly, Dot1L inhibition decreases the IFN-ß promoter stimulation and RIG-I-MAVS association upon viral infection. Interferon-inducible protein *TRIM25* expression increases in influenza virus infected cells, but Dot1L inhibition reduces both the *TRIM25* expression and *TRIM25* protein levels. *TRIM25* overexpression reverses the defective innate response mediated by Dot1L inhibition elicited upon virus infection or by overexpression of RIG-I signaling intermediates. Thus, TRIM25 is a control point of the RIG-I recognition pathway controlled by Dot1L and may have a general role in RNA viruses recognized by the RIG-I sensor.

Human influenza A virus (hIAV) infection is associated with important cardiovascular complications, although cardiac infection pathophysiology is poorly understood. We evaluated lung and heart viral titers in mice infected with either one of several hIAV strains inoculated intranasally and identified viral replication inside mouse cardiomyocytes, Purkinje cells, and cardiac vessels. In addition, we used human induced pluripotent stem cells-derived cardiomyocytes (hiPSC-CMs) to confirm cardiac infection and studied the underlying molecular alterations associated with the *in vivo* electrophysiological phenotype. Both, pathogenic and attenuated hIAV strains infected and replicated in cardiomyocytes, Purkinje cells, hiPSC-CMs and cardiac endothelial cells. Cardiac conduction alterations and high mortality rates were especially pronounced in mice infected with the highly pathogenic strain, compared with mice infected with the attenuated strain. Thus, human IAV can infect the heart and cardiac specific conduction system, which may contribute to cardiac complications and premature death.

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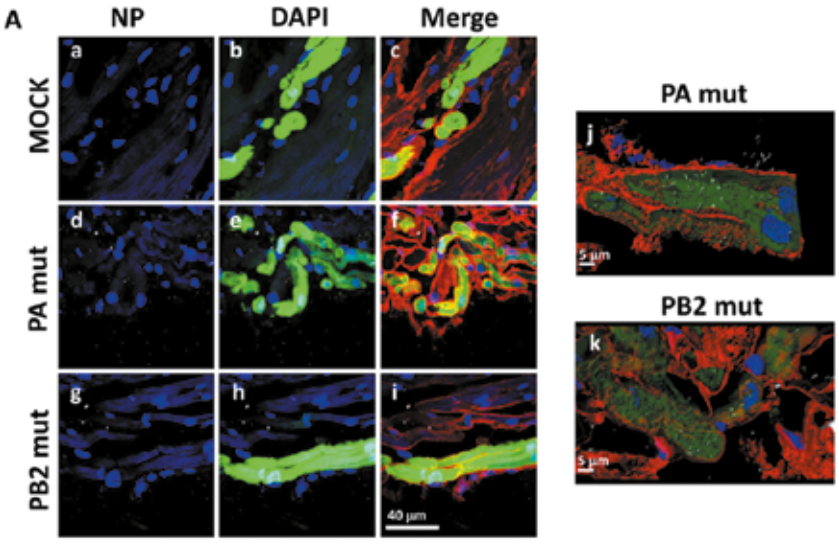

1 *Active replication of influenza A virus in Purkinje cells. (A) Detection of the NP viral protein using immunofluorescence confocal microscopy in heart tissue from Cx40eGFP MOCK-infected mice (upper panels, a–c) and animals infected with 10^6 pfu of either pathogenic strain (PAmut) (middle panels, d–f) or attenuated strain (PB2mut) (lower panels, g–i). Right panels (j–k) show 3D reconstructions of the NP viral protein in Purkinje cells of PAmut- (j) or PB2mut-infected hearts (k) from Cx40eGFP animals. Blue, nuclear staining (DAPI); green, GFP; red, laminin; white, NP viral protein staining*

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Cerebral cortical development

SELECTED PUBLICATIONS

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The cerebral cortex mediates the high functions of the human brain. It contains an extraordinary number and diversity of neurons that after development is completed, form one of the most complex functional networks found in biological systems. Despite their diversity and numbers, cortical neurons wire in following precise and highly stereotyped selective patterns with an apparent invariability. These circuits provide responses to the external world, mediate intellectual processing, and reproduce social behaviors that are optimal and common to all individuals. Still, cortical neurons demonstrate extraordinary plasticity and generate alternative circuits in non-canonical situations, such as the occurrence of genetic mutations or in neurodevelopmental disorders. This includes autism spectrum disorders (ASD), intellectual disabilities, bipolar disorders, schizophrenia, or epilepsy.

Our projects aim to understand the rules of cortical wiring and the emerging of developmental plasticity using the mouse as a model. In recent years, we have focused on the development of corpus callosum connections, which comprise a complex ensemble of interareal circuits responsible for higher-order functions. We aim to understand how neurons encode for the molecular information necessary to build the stereotyped circuits of the CC; how neurons translate this information into selective connectivity while dialoguing with their environment, and how this mechanism of wiring results in non canoncal circuits. We focus on plasticity because of its potential therapeutic implications for treating and managing neurodevelopmental disorders or intervening in others such as the loss of sensory organs or ischemic injury. Our investigation is based on *in vivo* manipulation of circuits by modifying gene expression, sensory input, and circuit activity. We use CRISPR/Cas-mediated knock-in, *in utero* electroporation, electrophysiology, stereotaxic retrotracing injections, pharmacological interventions, and RNA-sequencing among other techniques. In our projects, we collaborate with national and international scientists and clinicians and involve patients and their families.

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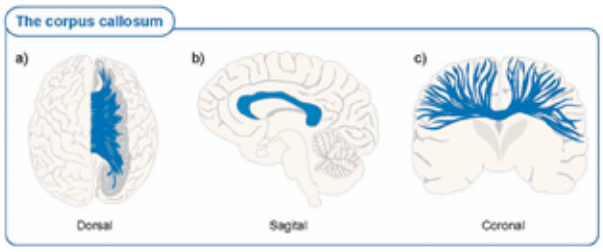

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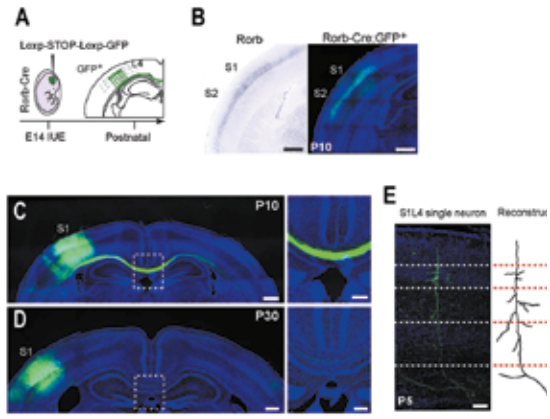

❶ *The general organisation of the corpus callosum (CC) A-C) The CC, shown in blue, is the major commissural tract connecting the cortical hemispheres. A) Dorsal view of the hCC. B) Sagittal view of the hCC. This view is broadly used during clinic diagnosis, as the entire rostro-caudal formation is visible. C) Coronal view of the hCC. Axons of cortical neurons from both hemispheres meet in the midline and cross to the opposite hemisphere to target specific contralateral regions. Most of these projections will connect homotopic regions within the brain, and fewer will connect heterotopic regions. (From De León Reyes et al., Development 2020).*

❷ *Transient callosal projections extend from S1L4 Rorb neurons. A In utero electroporation of Rorb-Cre embryos with a floxed-GFP plasmid at E14. B Left panel, expression of endogenous Rorb expression (Image credit: Allen Institute). Right panel, GFP (green) in a P10 Rorb-Cre animal electroporated at E14 in S1 and S2. DAPI (blue). C –D. Coronal sections of electroporated Rorb-Cre brains. GFP illuminates a subset of L4 neurons and their axons. Right panels show magnification of the CC at the midline. E Single neuron GFP labelling in P5 brains show S1L4 neurons with axons entering the white matter (WM). The reconstruction of that same neuron is shown in the right panel (adapted from De León Reyes et al. Nat Commun 2019).*

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Molecular characterisation and epidemiology of torovirus

Toroviruses are emergent viruses (belonging to *Nidovirales* Order) that cause enteric diseases in different species of domestic animals and could probably represent a zoonotic threat. They are highly distributed worldwide, and yet remain practically ignored. Over the years, our group has developed diagnostic tools allowing us to carry out epidemiological studies that revealed a high prevalence of porcine torovirus in Spanish farms. As it has been evidenced by the COVID-19 pandemic, it is of utmost importance studying new potential zoonotic pathogens like toroviruses. The knowledge acquired from these studies could contribute to adopt therapeutic or preventive measurements in the eventuality of a disease outbreak.

One of the main focuses of our research is the study of the virus-host interaction that would determine the outcome of the disease. During this period we demonstrated that the equine torovirus Berne virus (BEV), the prototype member of the Torovirus genus, induces autophagy at late times post-infection. We have observed that BEV replication also induces ER stress at the time when selective autophagy is taking place, suggesting that the autophagy pathway is activated in response to the hefty accumulation of virus-encoded polypeptides during the late phase of BEV infection.

We maintain a collaboration with Dr. José F. Rodríguez (CNB) to characterise the potential relationship between the innate immune response and pathogenesis caused by infectious bursal disease virus (IBDV). Specifically, we have studied the role of the interferon pathway in acute and persistent IBDV infections. At present, we are involved in a project aimed at elucidating the relevance of defective viral genomes (DVGs) in the establishment of persistent infections by both, IBDV and torovirus.

In the context of the COVID-19 pandemic we have also collaborated with Drs. Lluís Montoliu and Almudena Fernández from CNB, and Dr. Miguel Angel Moreno from the CABD (CSIC/UPO) in a project aimed at using the CRISPR-Cas technology to target coronavirus RNA genome.

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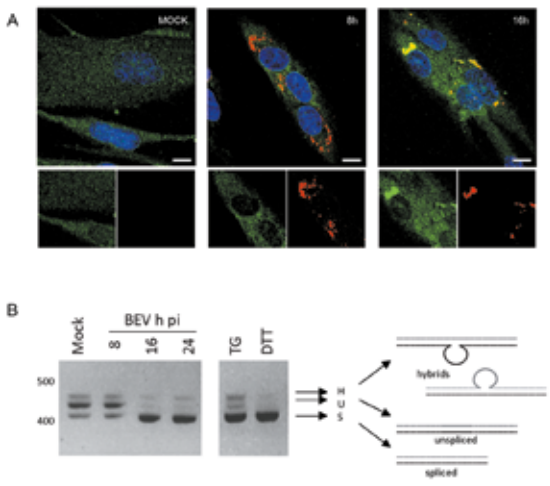

1 *BEV induces selective autophagy and ER stress. (A) Immunofluorescence analysis of mock- and BEV-infected cells showing the colocalization of the viral protease M^{pro} (red) and the cargo protein p62 (green) at late times pi (16h). Cell nuclei were stained with DAPI (blue). Enlarged representative areas showing the fluorescence corresponding to M^{pro} and p62 are shown in the lower panels. Scale bars, 10 µm. (B) Splicing of XBP-1 mRNA, analysed by RT-PCR, was observed in BEV-infected cells at 16 and 24hpi, as well as in cells treated thapsigargin (TG) or dithiothreitol (DTT) used as controls.*

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SELECTED PUBLICATIONS

Broto L, Romero N, Méndez F, Diaz-Beneitez E, Candelas-Rivera O, *et al.* Type I Interferon acts as a major barrier to the establishment of infectious bursal disease virus (IBDV) persistent infections. J Virol 2020; JVI.02017-20.

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Birnavirus molecular biology

During the last few years, our work has been mainly devoted to understanding the interaction between IBDV and host cells and the impact of the innate antiviral response on both virus-induced pathogenesis and the establishment of persistent IBDV infections. Our studies have unveiled the chief role of type I interferons on both phenomena, showing that the activation of the JAK-STAT pathway early after IBDV infection leads to a massive apoptotic response contributing to the deadly cytokine storm that destroys the bursal tissue and eventually finish off infected birds. Conversely, we have shown that the genetic inactivation of the JAK-STAT pathway significantly reduces IBDV cytopathogenicity and largely enhances the susceptibility of infected cells to sustaining long-term, productive, persistent infections.

Currently, in collaboration with Dr. Soubies's group (OIE Reference Laboratory for Gumboro Disease, French Agency for Food, Environmental and Occupational Heath Safety, Ploufragan, France) we are further dissecting mechanisms responsible for the establishment of persistent infections in infected chickens, a major IBDV biological trend likely playing a major role on IBDV dissemination and reemergence. We are particularly interested in deciphering the role of cell/virus genetic elements (e.g. micro-RNAs and defective virus genomes) within this phenomenon. Additionally, we focus on the characterisation of used by IBDV-encoded polypeptides, namely VP3 and VP5, involved in the evasion of innate antiviral cell responses. Finally, we are committed to further knowledge about the IBDV replication process, specifically on the morphogenesis of replicative complexes and progeny assembly and virus egress mechanisms.

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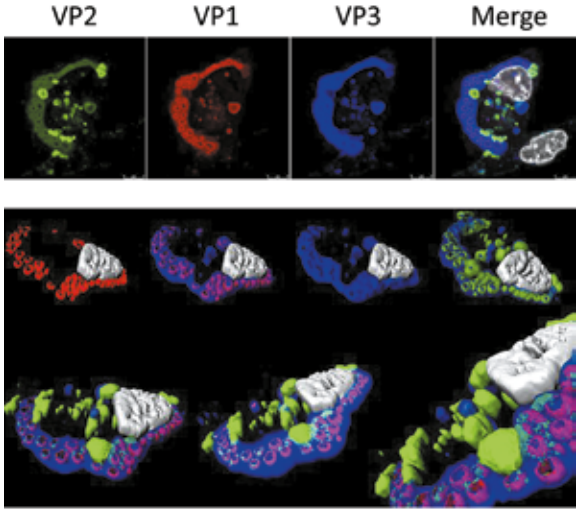

1 *Characterisation of IBDV replication complexes. Upper panels correspond to a single confocal plane captured from an IBDV-infected QM7 cell immuno-stained with antibodies specifically recognising the three structural virus polypeptides, i.e. VP1 (red), VP2 (green) and VP3 (blue). The cell nucleus, stained with DAPI, is shown in white. Lower panel show images from a 3D reconstruction, generated with confocal planes captured along the z-axis, showing the presence of quasi-spherical nucleation domains embedded within large viroplasms. The compact superstructures, exclusively stained with VP2 antibodies, adjacent to viroplasms, correspond to aggregates formed by tightly packaged IBDV virions.*

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Development, differentiation and regeneration in vertebrates

Our group is interested in understanding the molecular and cellular basis of organ formation during embryonic development. Signalling pathways involved in embryogenesis are also required for homeostasis of adult tissues and for repair of damaged organs. Moreover, malfunction of these pathways can lead to disease. Studying developmental genes and signals can therefore offer new avenues for treatment of prevalent diseases such as inflammatory diseases and cancer and also to improve the regenerative ability of tissues in the adult.

We are analysing the relationship between inflammation, regeneration and disease. The role of inflammation in regenerative processes is controversial. In some cases it has been shown to improve tissue healing but in other instances it has been shown to be detrimental for regeneration. In collaboration with the group of Ana Cuenda (Department of Immunology and Oncology, CNB) we are analysing the functions of p38MAPKs in this context, using conditional KO mouse lines. We are using a model of cancer associated to inflammation (colon cancer associated to colitis, CAC) in mice to address this problem. By chemically inducing damage to the colon, which triggers an inflammatory response, we are investigating the role of p38MAPK signalling in the regeneration of the epithelium, the control of inflammation and the activity of different immune cells during tumour initiation and progression.

We are also interested in the functions of p38MAPKs during infections and their role in triggering inflammatory responses. Using a model of candidiasis in mice we have uncovered important regulatory activities for p38γ and p38δ in controlling the extent of inflammation and thus in development of sepsis. The identification of new pharmacological inhibitors of these kinases is very important for research and for novel therapeutic treatments and we are involved in their discovery and characterisation.

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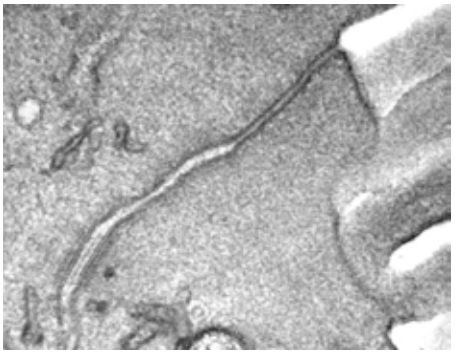

❶ *Electron microscopy image of the intercellular apical junction in colonic epithelial cells.*

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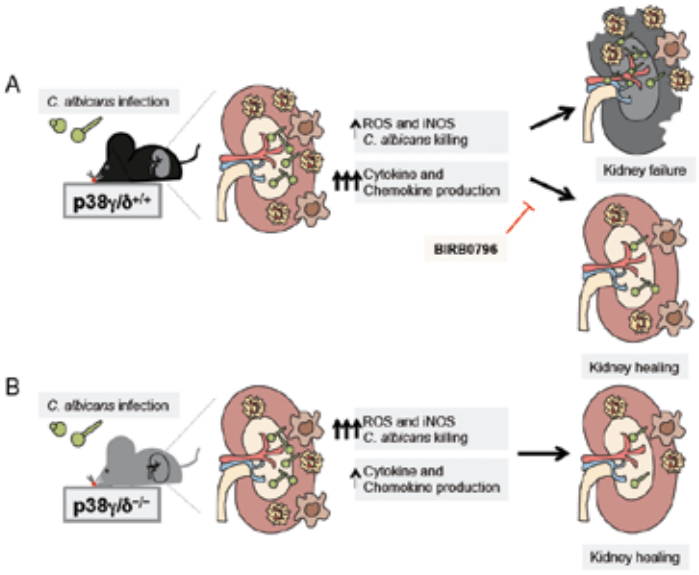

❷ *Schematic diagram showing that p38γ/p38δ regulate both host acute inflammatory response and the killing of C. albicans. The inhibitory action of BIRB0796 ameliorates inflammation in the kidney. (from Alsina-Beauchamp et al. EMBO Molecular Medicine 2018, e8485).*

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SELECTED PUBLICATIONS

Osuna-Pérez J, García-Ferreras R, Veiga E. From cellular microbiology to bacteria-based next generations of cancer immunotherapies. Cell Microbiol 2020; 22: e13187.

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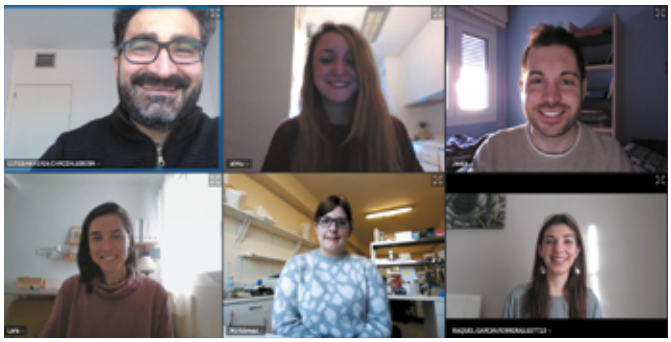

Cellular immunobiology and microbiology

We are focused in generating novel immunotherapies using the ability of bacteria to modify the immune responses. We have discovered that $CD4^+$ T cells contributes to the early immune response capturing bacteria by transfagocytosis. Surprisingly, the transfagocytic $CD4^+$ T cells destroy bacteria and become hyperinflammatory (Cruz-Adalia *et al*. 2014). Moreover, we have discovered that bacteria exposure "trains" conventional $CD4^+$ T cells. Trained $CD4^+$ T cells (bacT), contrary to the role separation dogma in immunology, became potent antigen presenting cells able to (1) cross-present antigens from captured bacteria, activating naïve $CD8^+$ T cells that became effective cytotoxic cells and (2) generating central memory; activities involved in the removal of tumours.

Note that actually there exist huge efforts to generate central memory $CD8^+$ T cells from tumour infiltrating lymphocytes. These effects, together with (3) the localised secretion of inflammatory cytokines by bacT cells, which could block the immunosuppressive environment generated by solid tumors, prompted us to hypothesised that bacT cells could be useful in antitumour therapies. This hypothesis was tested in proof-of-concept model of aggressive mouse tumours. Mice treated with bacT cells that have captured/killed bacteria expressing tumour antigen were protected against tumour development (Cruz-Adalia *et al*. 2017). These discoveries challenged the dogma of adaptive/innate immunity role separation and guided our current research to find novel tumour immunotherapies.

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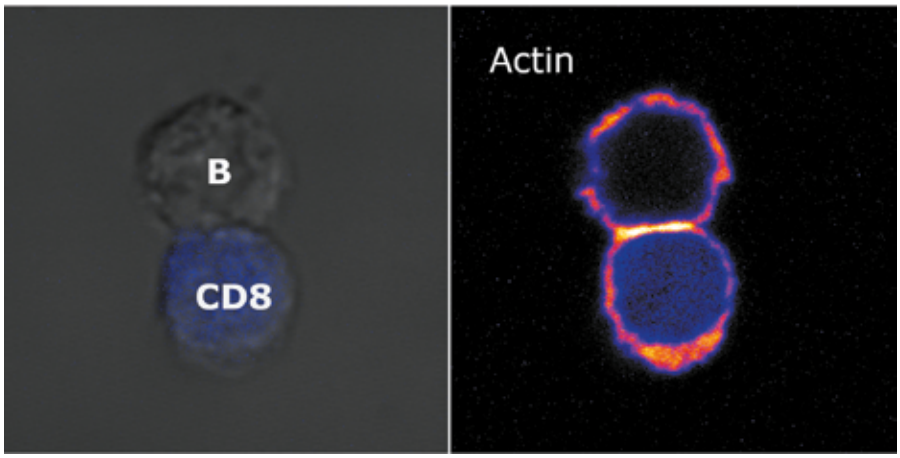
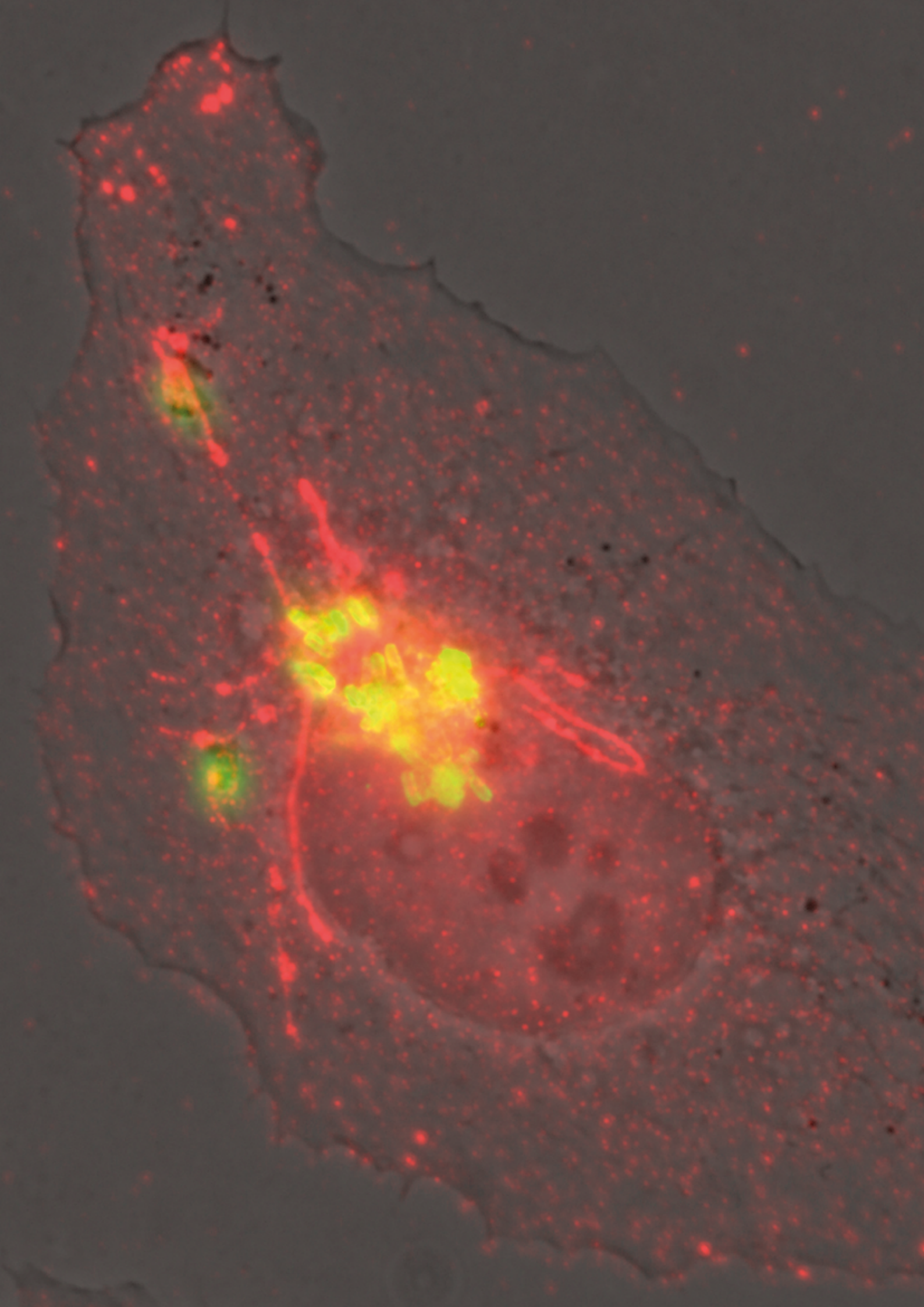


❶ *Immune synapse formed by a bacteria trained lymphocyte (B) and a naïve CD8+ T cell.*



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MICROBIAL BIOTECHNOLOGY

Research on the Department of Microbial Biotechnology is focused on microbes with environmental, industrial or clinical relevance. Work includes several approaches based on molecular genetics, systems and synthetic biology, evolutionary biology, genomics, proteomics and metagenomics. The scientific objectives of the Department are focused on five complementary aspects of microbial biology:

- Environmental microbiology. We aim to characterise the mechanisms underlying the global regulation networks that modulate bacterial metabolism in response to fluctuating environmental conditions. We also study the mechanisms that contribute to horizontal gene transfer in the environment.
- Microbial pathogens. Efforts are directed to understand the host-pathogen interactions occurring in infections caused by different types of microorganisms; the molecular mechanisms underlying the development of bacterial infections are studied as well.
- Microbial resistance to antibiotics. Work aims to understand the evolutionary mechanisms that contribute to bacterial persistence and antibiotic resistance in bacteria, among them, the impact of plasmids and antibiotic-polluted ecosystems. In addition, we study basic processes of microbial physiology, as cell division, which may define antimicrobial targets, and nanobody based therapies to combat bacterial infections.
- Microbial responses to hostile environments. Our focus is to understand bacterial responses to stressful environments, including general stress responses. We study how bacteria replicate and repair damaged DNA.
- Microbial engineering. Our purpose is to generate bacterial strains optimized to obtain products of interest such as antibodies, or to detect and degrade pollutants. In addition, we develop synthetic tools based on amyloids for biotechnological applications.

HEAD OF DEPARTMENT

Silvia Ayora

Secretion of an effector protein by intracellular Salmonella enterica *following infection of an epithelial cell. Shown in red is the signal corresponding to a bacterial effector protein secreted by the type III secretion system encoded in the* Salmonella*-pathogenicity island 2 (SPI-2). In green, signal of lipopolysaccharide (LPS) from intracellular bacteria. In the background, the phase contrast image. Note the translocation of the effector to filamentous structures that are a result of the infection and that are postulated to facilitate nutrient access to the pathogen. (Image from García del Portillo's lab).*

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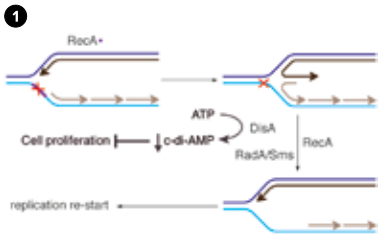
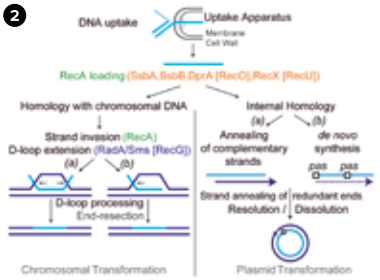
Genetic stability

Our research focuses on the study of the molecular mechanisms that secure genomic stability, promote horizontal gene transfer and control cell proliferation using *Bacillus subtilis* (a representative bacteria of the Firmicutes phylum) as a model. We have shown that the DNA damage response recruits different complex molecular machineries depending on the type of DNA damage and the growth conditions. When the replisome encounters a lesion on the template, the fork stalls and it needs to be stabilised to prevent fork collapse and genome instability. Here, DNA damage tolerance (DDT) subpathways assist fork progression, promoting replication fork reversal, template switching, lesion bypass or translesion DNA synthesis, and finally replication re-initiation. In the presence of a stalled (that mimics a displaced loop [D-loop]) or a reversed (a Holliday junction [HJ]-like structure) replication fork, the recombinase RecA binds to the lesion-containing DNA gap and loads the DNA damage checkpoint protein DisA and the fork remodeller RadA/Sms or RuvAB. DisA recognises and binds D-loop or HJ DNA and suppresses the synthesis of c-di-AMP, that in turn halts cell proliferation until the DNA damage is repaired. Moreover, it contributes to DDT pathways and prevents fork breakage (Fig. 1).

Horizontal gene transfer is a major prokaryotic evolution factor owing to its adaptive value and its power to restore genes inactivated by mutations. Thereby, it prevents the irreversible deterioration of genomes (known as Muller's ratchet). *B. subtilis* cells develop natural competence, with DprA (RecO in a Δ*dprA* strain, SsbA, SsbB, RecX (RecU in Δ*recX* cells), RadA/Sms (RecG in Δ*radA* cells) proteins helping RecA to promote the acquisition of exogenous DNA. Studying the functions that control RecA activities, we are addressing how mediators, modulators and D-loop remodellers contribute to the maintenance of the species and to the acquisition of HGT genes via natural plasmid transformation or viral transfection (Fig 2).





1 *A DDT mechanism. An unrepaired lagging-strand lesion (red cross) causes replication fork stalling. RecA-bound to the lesion-containing gap suppresses DisA dynamic movement and facilitates fork reversal. DisA bound to HJ DNA decreases c-di-AMP synthesis indirectly inhibiting cell proliferation. RecA bound to the nascent leading-strand loads RadA/Sms on the complementary nascent lagging-strand. RadA/Sms unwinds the nascent lagging-strand to provide a substrate for replication re-initiation, with DisA limiting RecA and RadA/Sms activities.*

2 *The DNA uptake apparatus of competent cells takes up linear single-stranded (ss) DNA. RecA, with the help of the mediators (DprA and SsbA) and modulator (RecX) forms a dynamic nucleoprotein filament on the incoming SsbA- and SsbB-coated ssDNA. The RecA filament searches for DNA homology. Once found, RecA initiates strand invasion to form D-loop intermediates (a, b) that are processed by a DNA helicase (RadA/Sms). The integrated strand is ligated, leading to a chromosomal transformant. RecA filamented on heterologous oligomeric plasmid ssDNA undergoes unsuccessful homology search and it must be disassembled. DprA (or RecO) interacts with other independently uptaken complementary strand and catalyses strand annealing, rendering tailed duplex DNA. An intramolecular recombination reaction carried out by DprA (or RecO) circularises the oligomeric plasmid molecule and after DNA replication, leads to plasmid transformation.*

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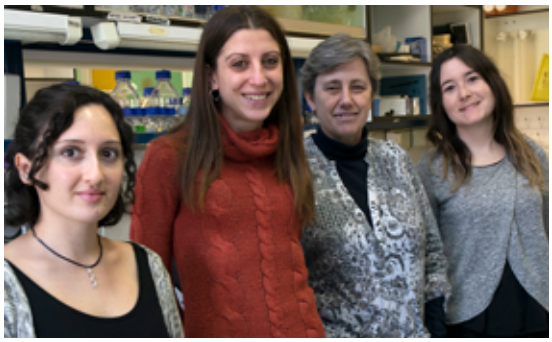

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Serrano E, Ramos C, Ayora S, Alonso JC. Viral SPP1 DNA is infectious in naturally competent Bacillus subtilis cells: inter- and intramolecular recombination pathways Environ Microbiol 2020; 22: 714-725.

Romero H, Serrano E, Hernández-Tamayo R, Carrasco B, Cardenas PP, *et al.* Bacillus subtilis RarA acts as a positive RecA accessory protein. Front Microbiol 2020; 11: A92.

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Recombination-dependent DNA replication

Our research focuses on the study of DNA replication mechanisms, specially on those that cells use to continue DNA replication when this process encounters impediments, which may stall or collapse the replication fork. Replication restart is then mediated by proteins that were initially identified by their roles in homologous recombination. We use *Bacillus subtilis* and its bacteriophage SPP1 as model systems, and biophysics, molecular biology and genetic techniques to study the recombination mechanisms that contribute to genome stability.

In the last years, we have focused on the study of conserved recombination proteins, as an AAA+ ATPase conserved from bacteria to humans. The WRNIP1/RarA AAA+ ATPases play a poorly understood role in the cellular response to blocked replication forks in pro- and eukaryotes. We have observed that RarA is sometimes associated with the replication fork even in the absence of DNA damage, performing a fork protection and regulatory role (Fig 1). We have also started to study the RecD2 helicase, which is the bacterial counterpart of the human helicase B, is also associated with the replisome, and interacts with WRNIP1/RarA.

Recombination also leads to evolution, and we have studied how recombination proteins may contribute to the acquisition of viral DNA or DNA from related species during natural transformation. A proteolysed bacteriophage might release its DNA into the environment. We have observed that RecD2 is required to resurrect an infective lytic phage from inactive environmental viral DNA. This protein, together with DprA, RadA, RecJ and RecX facilitates RecA-dependent gene acquisition from bacteria of related species (Fig 2).

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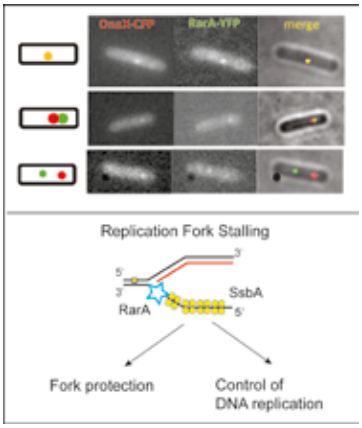

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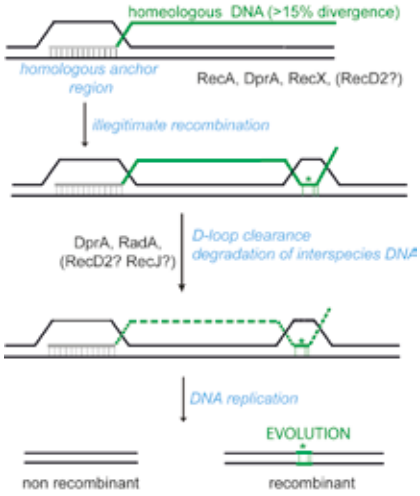

1 *Role of WRNIP1/RarA proteins. Epifluorescence microscopy shows colocalisation of the RarA protein with the clamp loader (DnaX) of the B. subtilis replisome in some cells. The lower image shows the model of RarA action at blocked forks. RarA and the singles-stranded binding protein SsbA bind to the collapsed forks and protect it from undesired recombination and control DNA replication restart.*

2 *Proposed mechanism of acquisition of DNA from related species during natural transformation. When B. subtilis competent cells acquire DNA with high sequence divergence from related species only some regions are fully homologous to the host chromosome. RecA, with the help of some accessory proteins uses this region to catalyse strand exchange and this recombination intermediate is used as an anchor region to facilitate an illegitimate recombination event in another region of the chromosome which leads to the acquisition of some nucleotides from the interspecies DNA. How the RecD2 helicase participates in this process is under study.*

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Stress and bacterial evolution

Our main goal is to understand the genetic mechanisms involved in bacterial genome stability and their roles in evolution and adaptation. We study the genetic basis of both stable and induced hyper-mutation/hyper-recombination as bacterial "strategies" to speed adaptation to stress, particularly to antibiotic stress. Recently we have described a novel non-canonical mismatch repair system in prokaryotes (present in some Archaea and most Actinobacteria), responsible for maintaining genome stability. Disentangle its genetic and biochemical bases in *Mycobacterium* and *Streptomyces* and its relation with the frequency of antibiotic resistance development in *Mycobacterium tuberculosis* is our commitment. This knowledge will be applied to i) understand and prevent the development of antibiotic resistance in this deadly bacterial pathogen and ii) improve prokaryotic species of biotechnological interest.

We collaborate in the development and analysis of new inhibitors of tolerance/resistance to antibiotics in different *Mycobacterium* species. Studies on new molecules to avoid antibiotic-mediated SOS mutagenesis in other bacteria (such as *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) and on inhibitors of β-lactamase activity are also being developed.

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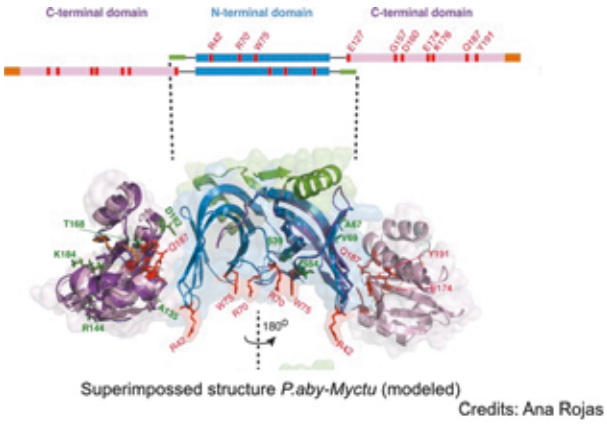
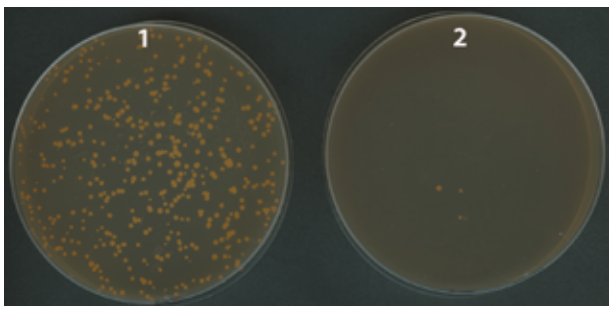


Superimpossed structure *P.aby-Myctu* (modeled)
Credits: Ana Rojas

❷



❶ *Domain characterisation of* M. tuberculosis *NucS, the key protein of the novel non-canonical mismatch repair system. Credits: Ana Rojas.*

❷ *NucS is a guardian of the genome stability in* Mycobacterium. *Increased production of mutant clones resistant to rifampicin in a* Mycobacterium smegmatis *strain lacking nucS gene (1) in comparison with the nucS-proficient strain (2).*

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Bacterial engineering for biomedical applications

Our research is aimed to engineer *E. coli* bacteria for biomedical applications, including the selection of recombinant antibodies and the design of bacteria for diagnostic and therapeutic use. We study protein secretion systems found in pathogenic *E. coli* strains, such as enteropathogenic *E. coli* (EPEC), and engineer them to develop protein nanomachines that can be applied for selection of recombinant antibodies and the delivery of therapeutic proteins by non-pathogenic *E. coli* strains. Among the recombinant antibodies, we employ camelid single-domain antibodies, called nanobodies, which are the smallest antibody fragments with full antigen-binding capacity. We use synthetic biology approaches and genome engineering to combine the expression of these modular parts in the designed bacteria.

In these two years we have been working in the following projects:

1) Expression and selection of nanobodies against pathogens and cancer. We have used *E. coli* surface display and protein secretion systems to screen immune libraries of nanobodies and to select high-affinity clones that, for instance, inhibit the adhesion of enterohemorrhagic *E. coli* (EHEC) to human intestinal cells. We have also selected nanobodies binding relevant antigens in cancer (e.g., EGFR, PD-L1). Further, we started an ongoing work to obtain neutralising nanobodies against SARS-CoV-2.

2) Engineering *E. coli* bacteria as anti-tumour agents. We have continued this synthetic biology project to modify a non-pathogenic *E. coli* chassis with synthetic adhesins and a type III protein secretion system (T3SS) to obtain bacteria with specific anti-tumour activities.

3) Accelerating protein evolution *in vivo*. We have developed a novel *in vivo* mutagenesis system in *E. coli*, called T7-DIVA, based on the recruitment of base deaminases to a target gene with T7 RNA polymerase. This system enables us to accelerate the directed evolution of proteins of interest, such as enzymes and antibodies.

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Álvarez B, Mencía M, de Lorenzo V, Fernández LA. *In vivo* diversification of target genomic sites using processive base deaminase fusions blocked by dCas9. Nature Commun 2020; 11: 6436.

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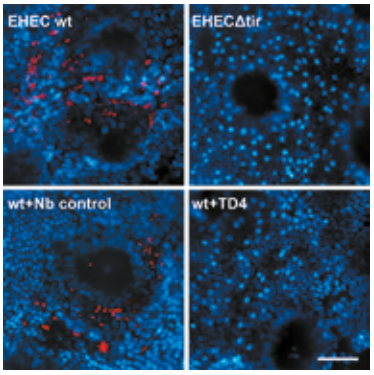

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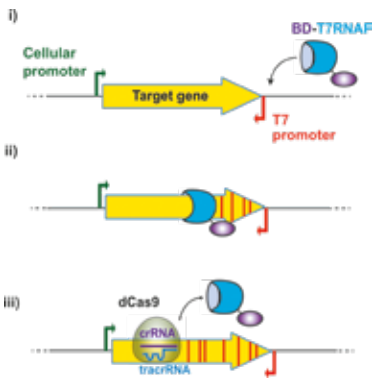

❶ *Attachment of enterohemorrhagic* E. coli *(EHEC) to human colonic biopsies is inhibited by nanobody TD4. Biopsy samples from the transverse colon were infected for 8 h with EHEC wild-type alone (EHEC wt) or in the presence of a nanobody against the extracellular domain of EHEC translocated intimin receptor TirM (wt + TD4) or a control nanobody binding amylase (wt + Vamy). Incubations with mutant EHECΔtir were included as negative control. Tissue samples were stained for EHEC (red) and cell nuclei (blue), bar = 50 µm.*

❷ *Schematic representation of the T7-DIVA in vivo mutagenesis system. The T7 RNA polymerase fusion to a base deaminase (BD-T7RNAP) binds the T7 promoter (i), initiating the transcription and moving along the target gene (yellow filled arrow) introducing mutations (red stripes) in the gene (ii). The fusion stops and detaches from the DNA when encounters a dCas9 molecule bound to a specific sequence determined by the CRISPR RNA (crRNA)(iii).*

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Laboratory of intracellular bacterial pathogens

Our lab is interested in deciphering how an important intestinal pathogen, *Salmonella enterica*, evolved to establish long lasting infections inside eukaryotic cells.

One of our major aims is to understand the changes in the cell wall that take place upon colonisation of the intracellular niche by this pathogen. We are especially interested in *Salmonella* proteins absent in non-pathogenic bacteria bearing activities that alter peptidoglycan structure. Although the peptidoglycan is sensed as a danger signal by the immune system, specific structural modifications may have the opposite effect and facilitate *Salmonella* persistence in the host, a common outcome in infections caused by this and other intracellular pathogens. Therefore, dissecting structural changes of the peptidoglycan triggered in response to eukaryotic signals as well as the responsible enzymes, it is of outmost relevance for designing new anti-infective strategies. In this line, we recently discovered new *Salmonella*-specific peptidoglycan synthases that promote cell elongation and division in the intracellular niche. Remarkably, these enzymes "replace" those that the same bacterium uses to elongate and divide outside the host cell. Such enzymatic switch illustrates the uniqueness of the *Salmonella* intracellular lifestyle in comparison to what it is normally observed in bacteria growing in artificial laboratory media. Some of these pathogen-specific enzymes, not detected in standard growth conditions and only visible *in vivo* in bacteria colonising host tissues, have low affinity for the antibiotics used in clinics.

Our future aims include:

- To unravel how *Salmonella* regulates the switch of peptidoglycan enzymes.
- The search for new drugs targeting these pathogen-specific enzymes.
- The identification of new peptidoglycan enzymes responding to intracellular cues.
- To study the evolution of distinct families of peptidoglycan enzymes.

In collaboration with Drs. Pucciarelli and Ortega, we are also pursuing studies focused on understanding regulation of the adaptative response of *Listeria monocytogenes* to cold.

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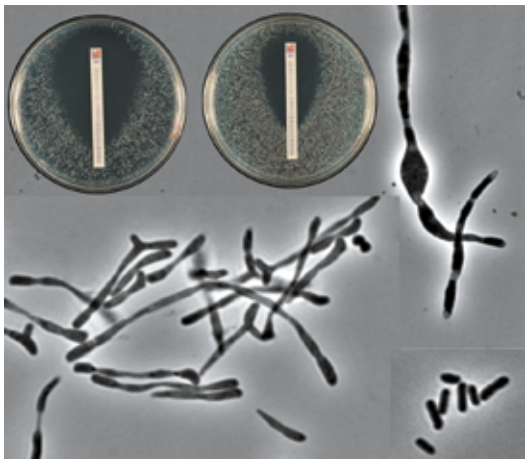

1 *Morphological alterations in* Salmonella enterica *serovar* Typhimurium *mutants lacking elements involved in the switch of PBP2 / PBP3 by the pathogen-specific enzymes PBP2SAL / PBP3SAL. Some of these mutants display increased resistance to antibiotics when grown in minimal media mimicking intracellular conditions*

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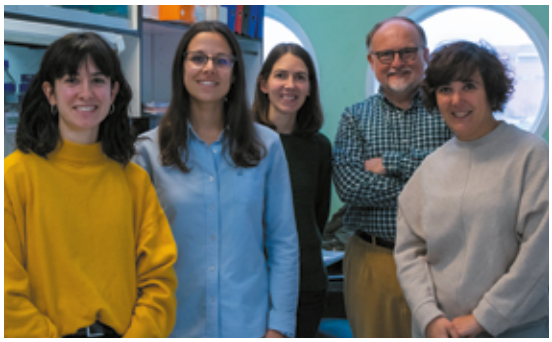

Synthetic bacterial amyloids

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Functional amyloids are protein assemblies that enable the epigenetic inheritance of phenotypes. However, when due to protein misfolding, amyloids can trigger diseases (i.e., human neurodegenerative and systemic amyloidosis). We create, through bottom-up Synthetic Biology, bio-resources based on bacterial amyloids with two major aims: i) understanding the molecular determinants of the shift between function and toxicity in natural amyloids; and ii) generating new constructive resources for Biotechnology and Biomedicine based on amyloids.

RepA is a protein from a bacterial plasmid whose WH1 domain undergoes conformational changes capacitating it as a transcriptional repressor, or as a DNA replication initiator or, through assembling amyloid oligomers, to hinder premature re-replication rounds. RepA-WH1 dimers become metastable monomers upon allosteric binding to plasmid-specific dsDNA sequences or acidic phospholipids, thus triggering amyloidogenesis. We engineered RepA-WH1 to become a biosafe prion-like protein (prionoid) that is transmitted from mother-to-daughter *Escherichia coli* cells, causing a synthetic 'generic' amyloid proteinopathy. RepA-WH1 aggregates propagate as two strains with distinct appearance and cytotoxicity, modulated by the Hsp70 chaperone DnaK. RepA-WH1 amyloidosis recapitulates in bacteria the hallmarks of mitochondrial routes associated with human amyloid diseases, including the formation of oligomeric pores at the internal membrane and the generation of reactive oxygen species.

We have used RepA-WH1 as a benchmark for the design of synthetic tools to probe protein amyloidogenesis, including gold nanoparticles-based sensors, screening devices exploiting amyloid-promoted overriding of translation termination, both in yeast or in bacteria, and *in vitro* expression devices to address amyloidosis within cytomimetic lipid vesicles. Recently, control on RepA-WH1 amyloidogenesis has also been achieved through optogenetics, i.e., the fusion of a blue light-responsive plant domain (LOV2) to the N-terminus of WH1. Expressing LOV2-WH1-mCherry in *E. coli* under blue light illumination leads to the assembly of oligomers that hamper bacterial growth. We are now exploring these devices as novel antimicrobials ('optobiotics').

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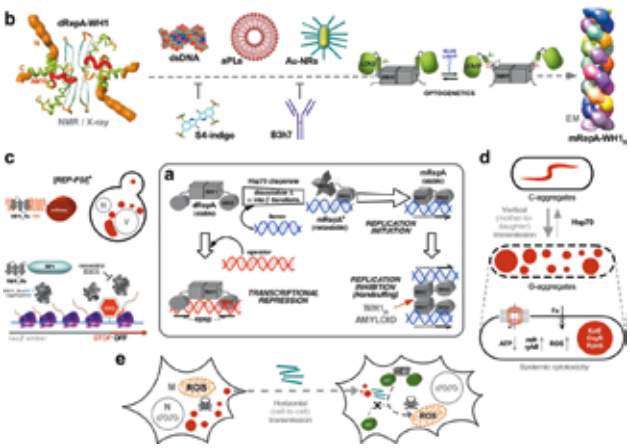

1 *A 'generic' synthetic model of amyloidosis engineered from bacterial RepA.*
(a) RepA is a dimeric transcriptional repressor that dissociates as monomers to initiate plasmid replication. Finally, through its WH1 domain assembles post-replicative, inhibitory amyloid oligomers.
(b) Fraying terminal helices in RepA-WH1 dimers prime RepA-WH1 dissociation and the assembly of the monomers as filaments, involving an amyloidogenic loop (red). Amyloidogenesis can be driven by DNA and acidic phospholipids (aPLs) or by gold nanoparticles (Au-NRs), and inhibited by S4-indigo, or by a conformation-specific antibody (B3h7). Fusion of a plant photosensor domain (LOV2) to the N-terminal helix in RepA-WH1 enables optogenetic modulation of amyloidogenesis: blue light illumination generates cytotoxic oligomers.
(c) The amyloidogenic stretch in RepA-WH1 can functionally replace prionogenic NM sequences in the yeast prion [PSI+]. The same repeats fused to E. coli RF1 enable stop codon read-through by ribosomes, counteracted by anti-amyloid compounds.
(d) RepA-WH1 is vertically inherited in E. coli *as two distinct amyloid strains: cytotoxic globular (G), or harmless comet-shaped (C) particles. Hsp70 chaperone detoxifies aggregates by favoring the C strain. G oligomers make pores at the inner membrane and enhance oxidative stress.*
(e) Horizontal spread of RepA-WH1 can be achieved in mammalian cells, but it is restricted by the need of its heterologous expression.

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Molecular infection biology

A number of bacterial cell processes are confined in platforms termed functional membrane microdomains, some of whose organizational and functional features resemble those of lipid rafts of eukaryotic cells. How bacteria organise these intricate platforms and their biological significance remains an important question. My laboratory is a key laboratory in the field of functional membrane microdomain bacterial compartmentalization and its role during infections, using MRSA (Methicillin-resistance *Staphylococcus aureus*) as model organisms. Our research is supported by competitive funding, such as ERC-StG-2013 or H2020 RIA Biotech-03-2016. We aim to identify the structure and molecular mechanisms that leads to bacterial membrane compartmentalisation and their role during staphylococcal infections that are resistance to antibiotic treatments. To do this, we work in the interface of molecular and cellular biology with other scientific disciplines, such as structural, infection, synthetic and systems biology. This interactive and multidisciplinary environment provides to my laboratory a means to open new areas to study new mechanisms of bacterial infections and to discover new antimicrobial strategies to fight antibiotic resistance and multi-drug resistance pathogens, with special emphasis on those associated with hospital infections.

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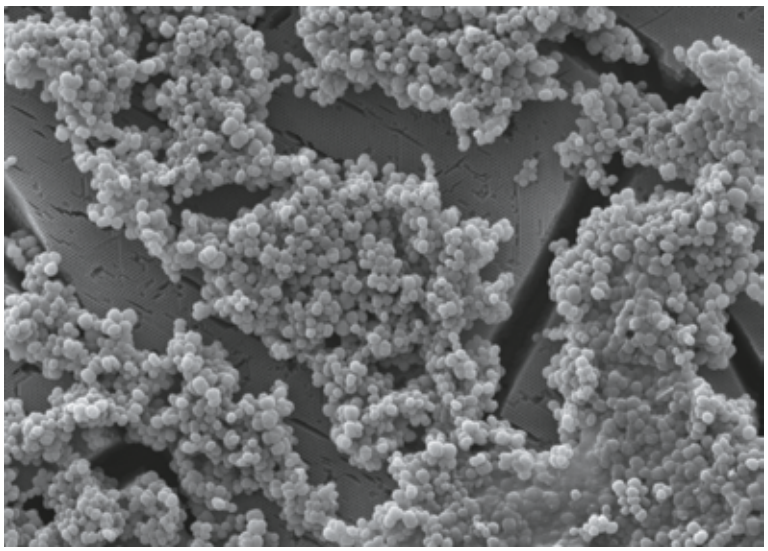
Mietrach N, Damián-Aparicio D, Mielich-Süß B, López D, and Geibel S. Substrate interaction with the EssC coupling protein of the Type VIIb secretion system. J Bacteriol 2020; 202 (7): e00646-19.

❶



❶ *Electron microscopy image of* S. aureus *cells growing attached to a surface.*

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SELECTED PUBLICATIONS

Hernando-Amado S, Sanz-Garcia F, Martinez JL. Antibiotic resistance evolution is contingent on the quorum sensing response in *Pseudomonas aeruginosa*. Mol Biol Evol. 2019; 36 (10): 2238–2251.

Hernando-Amado S, Coque TM, Baquero F, Martinez JL. Defining and combating antibiotic resistance from One Health and Global Health perspectives. Nat Microbiol 2019; 4 (9): 1432-1442.

Ruppe E, Ghozlane A, Tap J, Pons N, Alvarez AS, *et al.* Prediction of the intestinal resistome by a novel 3D-based method. Nat Microbiol 2019; 4: 112-123.

Hernando-Amado S, Sanz-García F, Martínez JL. Rapid and robust evolution of collateral sensitivity in *Pseudomonas aeruginosa* antibiotic-resistant mutants. Sci Adv 2020; 6 (32): eaba5493.

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Ecology and evolution of antibiotic resistance

We study the biology of opportunistic pathogens, focusing on the networks and the evolutionary processes that connect resistance and virulence. In the last years, we have standardised some tools, based on experimental evolution, whole-genome sequencing and functional assays, for predicting the evolution of antibiotic resistance and the consequences of acquiring such resistance for bacterial physiology. Using these approaches, we characterised mechanisms of resistance to last-generation antibiotics and combination of them. Notably, acquisition of resistance is linked to changes in the susceptibility of other antibiotics besides those used for selection. In this regard, we have determined the networks of cross-resistance and collateral susceptibility associated to the acquisition of resistance to different antibiotics.

One important element in our studies is determining the elements that modulate the robustness and predictability of evolutionary trajectories towards antibiotic resistance of bacterial pathogens. Robustness is particularly relevant for exploiting the information concerning collateral susceptibility in order to implement more efficient therapeutic strategies based in antibiotic combinations or cycling. We have been working in this topic and have found some robust collateral susceptibility networks that will be explored in clinical strains in the next future. Among the elements that drive the evolution of antibiotic resistance from stochasticity to determinism, we are particularly interested in the epistatic interactions between elements involved in antibiotic resistance and virulence of bacterial pathogens. Besides mutation-driven resistance, we are studying compounds or conditions that can lead to transient resistance, as well as those that can increase the susceptibility to antibiotics and could hence be used as co-adjuvants in therapy.

A final aspect of our work concerns the One Health and Global Health aspects of antibiotic resistance. We contributed to the development of novel tools and metagenomic analyses for analysing the role of different non-clinical habitats in the evolution and spread of antibiotic resistance.

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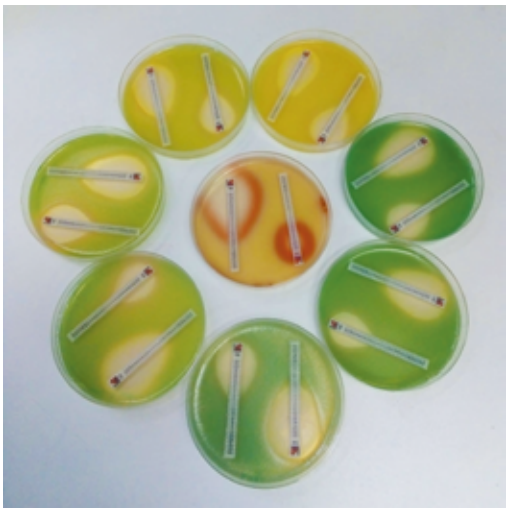

1 *Radiative evolution of antibiotic resistance in presence of antibiotics. Evolution is generally considered a very slow process. However, microorganisms, and bacteria in particular, reach a large number of generations in a very short period of time. This makes it possible to use Adaptive Laboratory Evolution (ALE) experiments to predict bacterial evolution in the presence of different selective pressures, such as antibiotics. This photograph shows the different changes in pigmentation and antibiotic resistance level (measured with E-Test strips), of the opportunistic pathogen* Pseudomonas aeruginosa, *after only 21 days of ALE in the presence of different concentrations of tobramycin.*

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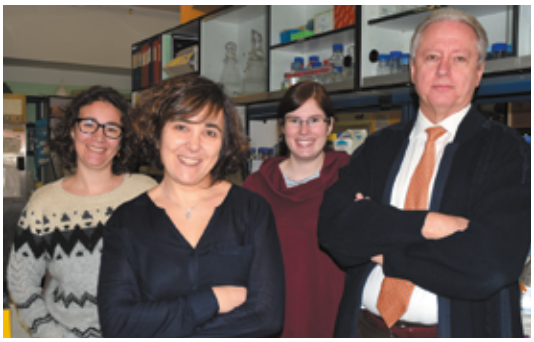

Heterologous gene expression and secretion in Gram-positive bacteria with industrial applications

Our group has a long-standing interest in the physiological and molecular characterisation of the protein secretory routes of the soil Gram-positive bacteria *Streptomyces lividans*, a well-known efficient producer of extracellular hydrolytic enzymes and other compounds of industrial application.

We have described and characterised the bacterial proteins (peptidyl-prolyl *cis-trans* isomerases and thiol-disulphide oxidoreductases) that are involved in the production of extracellular mature active proteins in both secretory pathways, the major Sec secretory pathway, and the minor Tat secretory pathway, which release unfolded and folded proteins respectively.

Four thiol-disulphide oxidoreductases are necessary for the formation of disulphide bonds when protein contains several disulphide bonds and surprisingly two of them are necessary in a protein devoid of disulphide bonds (Tat-dependent agarase) when it is overproduced, supporting the role of Sli-DsbA as a chaperone in the production of active agarase [Gullón S *et al*, 2019]. Additionally, we identified and characterised a Tat-dependent *S. lividans* FKBP-like lipoprotein, Sli-FKBP, that is involved in the folding of secretory proteins when they are overproduced, even in the proteins that are exported by the Tat pathway, so adjusting the level of expression of *sli-fkbp* may facilitate folding of dependent proteins [Vicente RL *et al*, 2019; Figure 1].

Additionally, we have described a dynamic flux balance analysis (DFBA) that adapt to the non-uniform time-dependent patterns that occurs in the protein secretion to study the metabolic changes induced by secretory protein overproduction [Valverde JR *et al*, 2019]. This will allow us to estimate the metabolic cost of that overproduction which, in turn, would enable us to design secretory protein production processes.

The obtained results would be applied first-hand at an industrial level for optimising scaling up secretory protein production, as well as favouring the design and construction of new and efficient secretory strains in *S. lividans*.

SELECTED PUBLICATIONS

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Vicente RL, Marín S, Valverde JR, Palomino C, Mellado RP, Gullón S. Functional identification of a *Streptomyces lividans* FKBP-like protein involved in the folding of overproduced secreted proteins. Open Biol 2019; 9: 190201.

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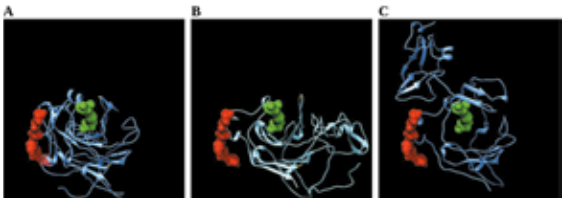

1 *Mature agarase with P127 and P183: both in cis conformation A) P125 in trans, P183 in cis B) both in trans conformation C). Agarose bound to the active site (green) and the allosteric site (red) are indicated. Between trans and cis forms exist differences in dimensions and surface charge. This modelling could explain the increase in agarase activity and the shift in SDS-PAGE mobility when Sli-FKBP is overproduced. Credits: JR Valverde*

IN MEMORIAM

Rafael Pérez Mellado (1950-2019), died suddenly the 27th of March 2019. Rafael was an innovative scientist, passionate about applying science as a way to improve society. He always displayed great intellectual concern that made him a visionary and led him to passionately explore all the activities he undertook.

After a long period at the Centre for Molecular Biology (CBM), he embarked on the beginning of the 80's, together with Victor Rubio and Francisco Malpartida, in the creation of the CNB and especially the Department of Microbial Biotechnology. He was Technical Vice Director for many years and had the difficult mission of organising the development of the CNB as one of the most important and influential center for innovation and translational research in Biology in Southern Europe.

Over the years, his laboratory was involved in a number of important applied projects, including the use of *Streptomyces lividans* as a cell factory. Additionally, he had active participation in numerous national and international commissions where his opinions were heard, and in many cases followed by the competent authorities. Particularly important in the last years was his work in the Ministry of Foreign Affairs and Cooperation as an recognised authority on biological security. In these lines, we want to pay homage to one of the legends of the CNB.

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Regulation of gene expression and metabolism in bacteria

To be competitive in the environments they colonise, bacteria must optimise metabolism to attain maximum gain from available nutrients. Not all potential carbon sources are equally effective in this respect. For this reason, when confronted with a mixture of potentially assimilable compounds at sufficient concentrations, many bacteria preferentially use one of them, leaving others aside until the preferred one is consumed. This implies a complex regulatory process termed catabolite repression. Unravelling the molecular mechanisms involved helps understanding how bacteria coordinate their metabolism and gene expression programs and optimise growth. It also aids in the design and optimisation of biotechnological processes and to understand how bacteria degrade compounds in Nature.

The regulators and molecular mechanisms responsible for catabolite repression differ among microorganisms. Our work is focused on *Pseudomonas putida*, a bacterium with a versatile and robust metabolism much used in biotechnology. Catabolite repression relies on a complex regulatory network that includes the Crc and Hfq proteins, which inhibit translation of mRNAs containing a specific A-rich sequence motif within their translation initiation region. Two small RNAs, CrcZ and CrcY, the levels of which vary greatly depending on growth conditions, antagonise the inhibitory effect of Hfq and Crc. Our aim is to characterise the influence of Crc, Hfq, CrcZ and CrcY in the physiology of *P. putida*, the signals to which they respond, and the molecular mechanisms by which they regulate gene expression.

In addition, we have analysed the role of Hfq in other processes such as iron homeostasis and the regulation of ISPpu9, an insertion sequence of *P. putida* KT2440 in which we have observed that translation of the transposase gene mRNA is inhibited by a highly structured 5' untranslated region, effect that is counteracted by an antisense small RNA and further modulated by a second small RNAs.

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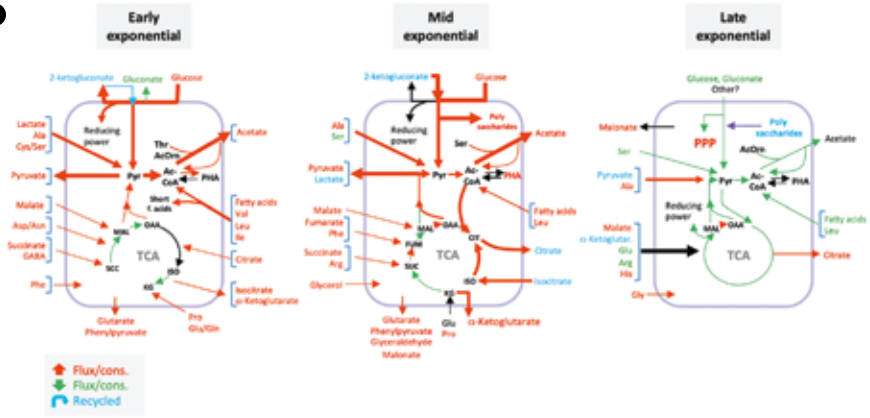

1 *Effect of inactivating the crc gene on the configuration of the metabolite fluxes related to central carbon metabolism during early, mid and late exponential growth. The fluxes that increased (in red), decreased (in green), or remained unchanged (in black) in the Crc-null strain as compared to the wild type, are highlighted. Compounds that were released to the medium and later recycled are indicated in blue.*

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SELECTED PUBLICATIONS

RC MacLean & A San Millan. The evolution of antibiotic resistance. Science 2019; 365(6458): 1082-1083.

Rodríguez-Beltrán J, Sørum V, Toll-Riera M, de la Vega C, Peña-Miller R, San Millan A. Genetic dominance governs the evolution and spread of mobile genetic elements in bacteria. Proc Natl Acad Sci USA. 2020; 117 (27): 15755-15762.

JH DelaFuente, J Rodríguez-Beltrán, A San Millán. Methods to study fitness and compensatory adaptation in plasmid-carrying bacteria. Methods Mol Biol 2020; 2075: 371-382.

Plasmid biology and evolution

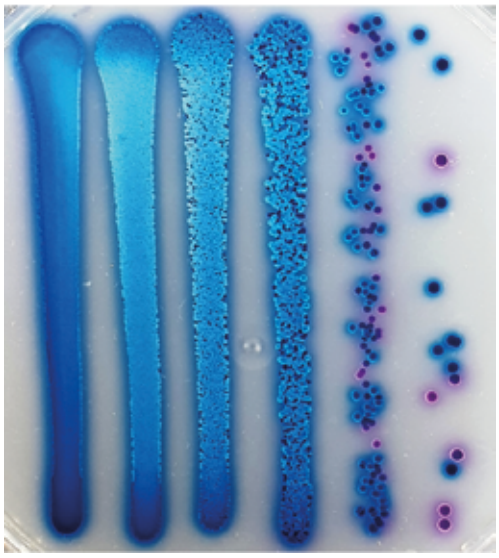
We are interested in the evolutionary forces that drive plasmid dynamics in bacterial populations as well as in the impact of plasmids in bacterial ecology and evolution.

Plasmids play a crucial role in bacterial evolution because they can transfer genes horizontally between different cells. The most striking example of how plasmids drive bacterial evolution is the global spread of plasmid-mediated antibiotic resistance over the last few decades. Plasmids are arguably the main vehicle for the spread of antibiotic resistance genes among clinically relevant bacteria, contributing to the overwhelming antibiotic resistance crisis we are currently facing. In our group we try to understand the population biology of antibiotic resistance plasmids using advanced molecular and evolutionary techniques. Ultimately, we intend to apply the concepts that we learn from the study of the evolution of plasmid-mediated antibiotic resistance to develop more rational intervention strategies to control infectious diseases.

The evolutionary impact of plasmids goes beyond horizontal gene transfer. Plasmids are usually kept at multiple copies per bacterial cell, producing islands of polyploidy in the genome. In the Plasmid Biology and Evolution lab, we are extremely interested in understanding how the multicopy nature of plasmids affects bacterial evolution. Our recent works revealed that multicopy plasmids are able to accelerate gene evolution and maintain allelic diversity, acting as catalysts of bacterial evolution.

This group joined the CNB in July 2020.

❶



❶ *Carbapenem-resistant* Klebsiella pneumoniae *(blue) and* E. coli *(pink) colonies growing on selective agar.*

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SELECTED PUBLICATIONS

Natale P, and Vicente M. (2021) Bacterial Cell Division. In: eLS. John Wiley & Sons, Ltd. Chichester doi.org/10.1002/9780470015902.a0000294.pub3

Genetic control of the cell cycle

The reconstruction of the divisome, the machinery responsible for bacterial division, in the test tube serves to verify that the description of the mechanisms that ensure proliferation is correct and also to use it in the design of new drugs to stop infections. The assembly of the divisome begins with the positioning in the middle of the cell length of a proto-ring in which ZipA contributes, together with FtsA, to place FtsZ, a protein that forms a contractile division ring, in the right place. The reconstruction of ZipA and FtsZ complexes inside artificial vesicles mimics the contractile property of the proto-ring, showing that the vesicles shrink when a GTP analog, an essential compound for the polymerisation of FtsZ, is added.

In addition to providing anchoring of FtsZ to the membrane, ZipA has a role to prevent its degradation.

In *Escherichia coli*, FtsA and FtsZ are encoded by genes located adjacently within the division and cell wall *dcw* cluster. Some peculiar genetic regulatory mechanisms including transcription from a sigma S dependent gearbox promoter operate in the cluster to produce proteins as FtsA and FtsZ that are used to assemble the proto-ring once per cell cycle. On the other hand, the *zipA* gene maps at a different chromosomal region, at min 54.54. We find that the expression of *zipA* is under the control of a housekeeper, and not a gearbox, promoter. The housekeeper regulation suggests that ZipA may play additional roles besides the anchoring of FtsZ to the cytoplasmic membrane. In fact, in addition to anchoring and stabilising FtsZ, ZipA forms part of the cytoplasmic membrane where it needs to be inserted in precise amounts to avoid adverse effects. Furthermore, the disruption of this promoter reduces ZipA protein production by 60% and leads to cell filamentation.

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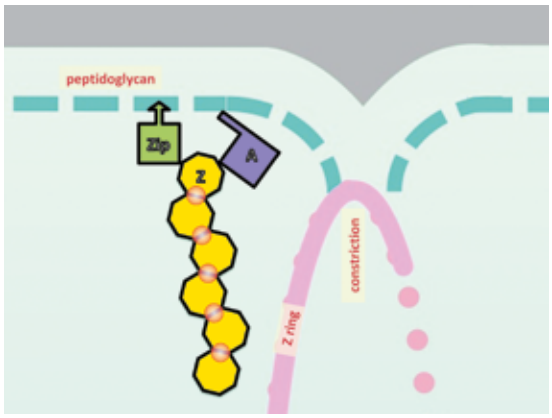

1 *The* E. coli *proto-ring. The interactions of FtsZ (Z), a cytoplasmic protein, with the proto-ring anchors, FtsA (A) and ZipA (Zip) serve to associate it to the cytoplasmic membrane forming the proto-ring, the initial precursor of the division machinery. The FtsZ monomers form polymers in which GTPase active sites, represented as red circles, are formed in the intersection between two monomers. The hydrolytic activity is needed for the function of the FtsZ ring in septation, probably by fuelling constriction of the envelope. The discontinuous blue line represents the cytoplasmic membrane. The division ring is depicted in pink. The location of the peptidoglycan layer is marked but not depicted.*

4

PLANT MOLECULAR GENETICS

The aim of the Plant Molecular Genetics Department is the study of the regulatory mechanism and pathways controlling plant development, adaptation to the environment, and defense responses to biotic and abiotic stresses.

Research lines focused on developmental processes include the study of root architecture, shoot branching, photomorphogenesis and photoperiodism. Plant adaptive responses to nutrient starvation, toxic concentrations of metals or defensive responses to pests and pathogens are also subject to intense research efforts. In addition to the basic interest of the key biological questions that underlie these processes, our work aims at generating new tools and knowledge for improving crop production. For this ultimate goal, we exploit natural diversity resources as well as genetic engineering, including CRISPR/Cas9 technology for precise genome editing, as promising tools and methods. Direct biotechnological applications of plants are also addressed, such as their use as biopharmaceutical factories or as tools for alleviating metal pollution and related environmental conditions. The model species *Arabidopsis thaliana* is the routine system of choice for our research, with much experimental work also carried out in *Nicotiana benthamiana*. Substantial effort has recently been devoted to the development of novel, more amenable model species for plant research, such as the duckweed Lemna spp or the liverwort *Marchantia polymorpha*, in which our Department has already made significant contributions. Crops such as tomato, potato and *Prunus* are also major subjects of our studies, to which knowledge generated in the model species is applied.

HEAD OF DEPARTMENT

Roberto Solano

Aerial tubers formed in the axis of leaves of potato plants lacking the BRANCHED1B function (From Pilar Cubas' lab).

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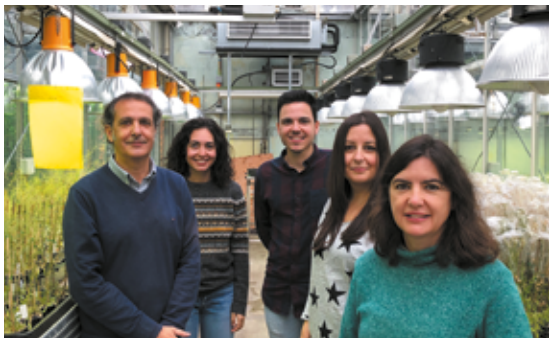

Natural variation of plant development

The main goal of our laboratory is to understand the genetic, molecular and evolutionary mechanisms involved in plant adaptation. In particular, we are interested in understanding how developmental traits, such as flowering time, vegetative growth, or trichome patterning, enable plant adaptation. To address this question we are exploiting the genetic variation that exists in nature within the wild, annual, and model plant *Arabidopsis thaliana*.

Given the relevance of climate change, our research is currently focused in identifying new genes and natural alleles that are involved in the adaptation to different climates. To this end, we are exploiting an *A. thaliana* regional collection of more than 400 wild accessions collected in the Iberian Peninsula (Montes *et al*., 2019; Castilla *et al*., 2020). The analysis of this collection for plant growth has identified an accession from Doñana National Park (Don-0) that is not able to grow at high temperature (Figure 1). Further genetic and molecular analyses identified *ICARUS2* as a new gene involved in adaptation to temperature seasonality. In addition, we are studying *A. thaliana* natural populations for other relevant traits, such as stomata density (Delgado *et al*., 2019), or microbiome composition (Thiergart *et al*., 2020).

Finally, in collaboration with Antonio Leyva's laboratory from the CNB, we are also studying the application of natural varieties of duckweed aquatic plants (*Spirodela polyrhiza* and *Lemna sp*) for water phytoremediation. In particular, our lab is currently involved in the project "Duckweed technology for improving nutrient management and resource efficiency in pig".

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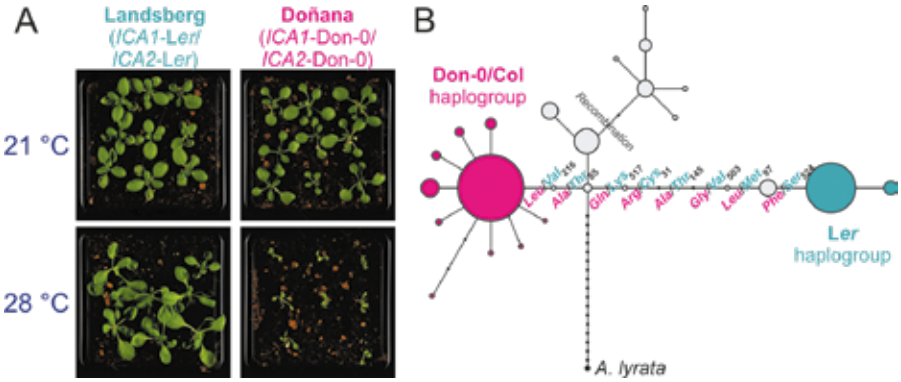

1 *ICARUS2 gene is essential for plant growth at high temperature. A) Growth phenotype of two* Arabidopsis *accessions, Landsberg and Doñana, at 21 and 28 °C. B) Genetic diversity (as haplotype network) of ICA2 proteins from worldwide accessions of* Arabidopsis thaliana *and* A. lyrata. *Each node corresponds to an amino acid substitution. Areas of nodes are proportional to frequency.*

SELECTED PUBLICATIONS

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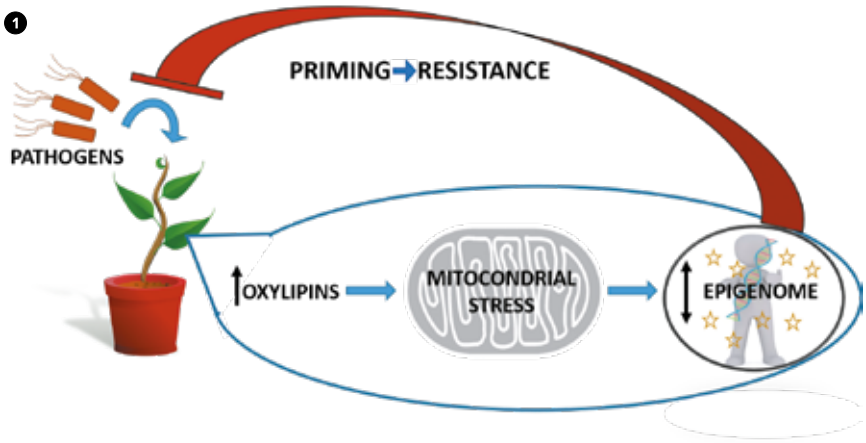
Plant immunity strategies against microbial pathogen infection

Plant pathogens cause diseases in many economically important crop plants, leading to severe losses in food production that are also of fundamental importance for forestry, other plant-derived products and for the sustainability of natural environments. This circumstance, together with an increasing world population, poses a severe threat to agriculture and plant sustainability. An important requirement for the development of successful plant disease control strategies is the understanding of host-pathogen interactions and, in particular, of the molecular mechanisms evolved in plants to avoid pathogen infection. This knowledge will be critical to devise effective approaches to minimise plant losses due to infection by microbes.

To this end, we focus our research on exploring the activities of oxylipins, a family of lipid derivatives activating immune responses in plants. Over the last years, our research has revealed that oxylipins, produced by the biosynthetic pathways initiated by fatty acid alpha-dioxygenases (alpha-DOXs) and 9-lipoxygenases (9-LOXs), contribute to the activation of local and systemic defence. In our studies, we showed that cellular organelles such as lipid droplets and mitochondria are important players during the response to pathogen infection and that global translational reprogramming contributes to activation of plant immunity. Moreover, we found that mitochondrial stress signals trigger the induction of epigenetic changes causing a primed state in which plants activate more effective immune responses leading into long-lasting resistance against different types of pathogens (a schematic representation of our working model is shown in Figure 1). Presently, we focus our research in examining these defence mechanisms and defining the signalling processes activating plant defence responses to control pathogen infection. The characterisation of the mentioned processes will contribute to define new mechanisms, signals, pathways, and genes involved in controlling plant immunity.



1 *Working model. Intracellular signaling process leading to plant defence and priming of immune responses. After a pathogen attack there is an intracellular increment in oxylipin production followed by disruption of mitochondrial function. The induced mitochondrial stress leads into epigenetic changes, which modify the inducibility of defence related genes, mediating a long-lasting resistance phenotype.*

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Genetic control of shoot branching patterns in plants

The control of branch outgrowth is critical for plant fitness, stress resilience and crop yield. We are studying the genetic basis of the control of axillary bud activity and dormancy in the model system Arabidopsis, and in the crop species tomato and potato in which the control of lateral shoot branching is of great agronomical interest. The *Arabidopsis thaliana* transcription factor BRANCHED1 (BRC1) plays a pivotal role in this process as it is a potent growth inhibitor that prevents axillary bud outgrowth in response to environmental conditions. We have combined ChIP-seq, transcriptomic and systems biology approaches to characterise the BRC1-regulated gene network. We have identified a group of BRC1 direct target genes encoding transcription factors (BTFs) that orchestrate, together with BRC1, an intricate transcriptional network enriched in abscisic acid signalling components.

We have also been studying a novel role of a potato *BRC1* gene. The control of carbon allocation, storage and usage is critical for plant growth and development and is exploited for both crop food production and CO_2 capture. Potato tubers are natural carbon reserves in the form of starch that have evolved to allow propagation and survival over winter. They form from stolons, below ground, where they are protected from cold temperatures and animal foraging. We have shown that *BRANCHED1b (BRC1b)* acts as a tuberisation repressor in aerial axillary buds, which prevents buds from competing in sink strength with stolons. *BRC1b* loss of function leads to ectopic production of aerial tubers and reduced underground tuberisation. In buds, *BRC1b* promotes dormancy, ABA signalling and downregulation of plasmodesmata gene expression. This limits sucrose unloading and access of the tuberigen factor SP6A to axillary buds. Moreover, BRC1b directly interacts with SP6A and blocks its tuber-forming activity in aerial nodes. Altogether these actions help promote tuberisation underground.

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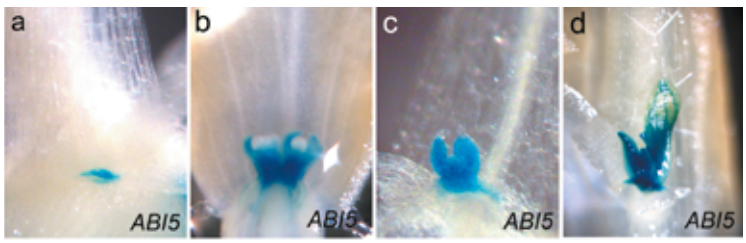

1 *Motifs overrepresented in the BRC1 network. Simplified representation of the BRC1 network, that exemplifies overrepresented motifs (multi-output Feed Forward Loops, regulated Feedback Loops and multi-ouput Feedback Loops) using specific cases. Orange and green circles represent genes encoding BRC1-dependent Transcription factors (BTFs, ATAF1, ABI5, ABF3, GBF2); blue circles, other bona fide BRC1 targets; grey circles, BRC1-dependent genes bound by BTFs but not by BRC1. Green and orange arrows indicate direct binding of the BTFs; red arrows, direct binding of BRC1.*

2 *The BTFs (BRC1-dependent Transcription factors) are expressed in axillary buds. ABI5 is a direct BRC1 target that, consistently, is expressed in axillary buds. GUS activity in axillary buds of transgenic lines carrying the construct ABI5pro:BETA-GLUCURONIDASE*

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Plant-pathogen-host interaction in viral infections

Plants are frequently infected in nature by viruses. Most of these infections are symptomless, or even give rise to mutualist associations, but plant viruses can also cause severe diseases. Breeding for resistance has been useful to fight some viral diseases, however, natural sources of resistance are scarce. The development of genetic engineering has expanded the available arsenal to generate virus-resistant plants. Understanding natural resistance mechanisms and viral amplification processes is essential to find appropriate targets for biotechnological antiviral strategies. Our research aims to contribute to meet this need. We are mainly interested in the family *Potyviridae*, especially in *Plum pox virus*, which causes sharka, a devasting disease of trees of the genus *Prunus*. In these two years we have paid attention to two viral functions that still have not been intensively studied, the proteolytic processing of viral polyproteins and the post-translational modifications (PTMs) of viral proteins. We have shown that the efficiency of the potyviral leader protease may be restricted to avoid that the uncontrolled release of the silencing suppressor HCpro triggers antiviral defences through complex hormonal and transcriptomic changes. We have also obtained data suggesting that alteration of the proteolytic cleavage between NIapro and VPg proteins is involved in the unique known escape of PPV from the HR-like resistance of some *Prunus domestica* cultivars. Regarding PTMs, our results have led us to propose that, whereas joint and opposite action of O-GlcNAcylation and phosphorylation at the N-terminal protrusion of the PPV capsid protein regulates the stability of this factor, phosphorylation at its core region controls assembly and disassembly of viral particles. Other remarkable results have been the finding that the sterol isomerase HYDRA1 restricts PPV infection and the demonstration that the viral genomic sequence is shaped by specific dinucleotide restrictions, so that an increase in UpA frequency causes a strong reduction of virus accumulation. *[Research supported by grants of the Spanish government BIO2016-80572-R and PID2019-109380RB-100 (IPs J.A. García and Carmen Simón) BIO2017-92613-EXP (IP C. Simón), and BIO2015-73900-JIN and PID2019-110979RB-100 (IP A. Valli)]*

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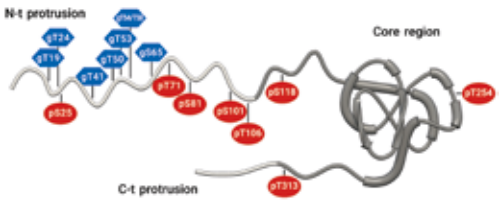

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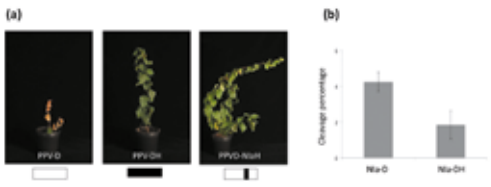

1 *Scheme of Plum pox virus CP and its post-translational modifications. Phosphorylated and O-GlcNAcylated residues are represented as red ellipses and blue hexagons, respectively.*

2 *Unraveling the mechanism of induction of hypersensitive response associated to resistance to Plum pox virus in European plums. (a) Resistant* Prunus domestica *trees inoculated with standard PPV-D isolate, the resistance-escaping isolate PPV-DH or a chimeric virus with the NIa sequence of PPV-DH in a PPV-D backbone. Schematic representation of each virus can be seen on the bottom of each picture. (b) In planta expression of NIa proteins from PPV-D and PPV-DH with a Myc tag to detect self-cleavage activity by western blot. Quantification of the cleavage percentage observed for each protease is shown in the panel.*

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Hervás M, Ciordia S, Navajas R, García JA, Martínez-Turiño S. Common and strain-specific post-translational modifications of the potyvirus *Plum pox virus* coat protein in different hosts. Viruses 2020; 12: 308.

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Regulation of gene expression in plants

Plant plasticity during adaptation to the environment involves specific transcriptional signal-response networks that allow them to reprogram their growth and development. Regulation of these networks relies on sequence-specific transcription factors (TFs), regulatory proteins responsible for the transcriptional activation or repression of target genes.

Research in our group is focused in the study of the components that determine specific recognition of TF target genes and which may influence in the levels of gene expression. During the last few years we have contributed to the characterisation of one of these components, such as the short DNA sequences bound by TFs, known as TF-binding sites (TFBS). Despite TFBS sequence is the major factor determining target recognition, during the last two years we have explored the role of some other components involved in this process. With this regard, we have demonstrated that binding of some TFs extends beyond the TFBS core sequence, as some distant nucleotides, likely determining DNA-shape, are necessary for protein binding. We are also studying the role of the cytosine methylation epigenetic mark in the TFBS region during TF-target recognition, as well as its genetic control, what will allow adding a new layer of regulation of gene expression

In parallel to the experimental approaches, we are developing some easy-to-use bioinformatic tools useful for the interpretation of transcriptional data and for the prediction of TFBS involved in the regulation of biological processes. These tools would contribute to a better and faster interpretation of biological data for the plant biology community, particularly in the case of non-expert researchers in bioinformatics or in the study of non-model species.

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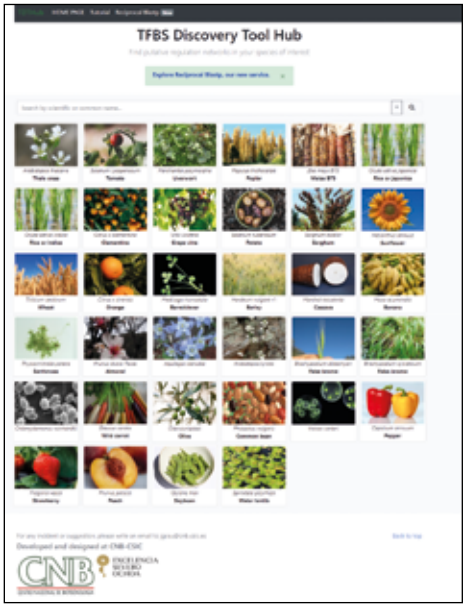

❶ *Selection of bHLH transcription factors target genes in plants. Specific binding of MYC bHLH to targets depends on the recognition of the G-box and of some nucleotides distantly located contributing to confer a particular shape to DNA. This 'double check' mechanism is conserved throughout the plant phylogeny and determines the specification of targets.*

❷ *A web-based tool for the identification of transcription factor binding sites in plants.*

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Mechanisms underlying nutrient uptake and phytoremediation

SELECTED PUBLICATIONS

Mateo C, Navarro M, Navarro C, Leyva A. Arsenic phytoremediation: Finally, a feasible approach in the near future (2019). In Environmental Chemistry and Recent Pollution Control Approaches, ed. Hugo Saldarriaga-Noreña. IntechOpen, London.

Plants have an extraordinary capacity to capture large quantities of nutrients and toxic compounds including heavy metals and arsenic. Arsenic can enter into the food chain through water consumption or crops (particularly rice) and therefore is considered a silent threat to public health.

For the last two years we kept working on the characterisation of the molecular mechanisms involved in arsenic perception and detoxification. Recently we finished the characterisation of a ubiquitination complex involved in the degradation of the transcriptional activator of the arsenate/phosphate transporter (*Navarro et al., under review in Molecular Plant;* Figure 1). We also followed different approaches to identify the key transcriptional activator of the arsenic responses using genetic and in silico strategies. In this context, we identified several transcription factors involved in the regulation of the arsenic response (Figure 2). In parallel we screened an Arabidopsis collection of Iberian natural accessions for arsenic tolerance and performed a Genome-wide association study, identifying several candidate genes.

In the last two years, we also performed a study of the natural variation of arsenic accumulation in duckweed, a hyperaccumulator aquatic plant with tremendous phytoremediation potential. To this end we obtained a new collection of duckweed natural accessions in collaboration with Carlos Alonso-Blanco at the CNB. Furthermore, we just finished a European project funded by the LIFE programme that aimed to use duckweed to extract nitrogen and phosphate form pig slurry in order to be used as a fertilizer (LIFE 15 ENV/ES/000382).

In the near future we aim to study Arabidopsis natural variation of the ionome in relation with arsenic response to understand the interconnected regulatory networks between arsenic and mineral nutrients. The idea will be to identify new mechanisms underlying metal and arsenic extraction in order to improve bio-fortification and phytoremediation capacity in plants.

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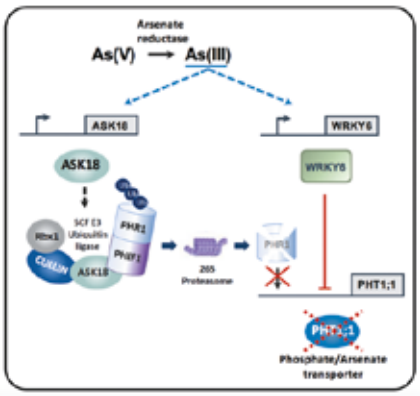

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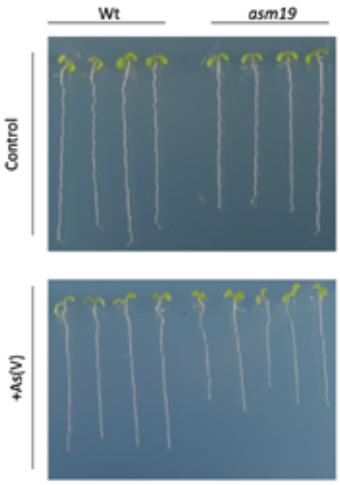

1 *Proposed model for the control of As(V) uptake in* Arabidopsis *roots. As(V) is transported inside the cell by the Pi transporter PHT1;1. As(V) is then rapidly reduced to As(III) by the action of the arsenate reductase ARQ1. As(III) signalling modulates the major regulators of PHT1;1 by inducing the transcription of WRKY6 (PHT1;1 repressor) and ASK18 a component of the SCF complex that interacts with the F-box protein PHIF1. PHIF1 targets the PHR1 (the PHT1;1 activator) for protein degradation. As a result of these coordinated events, PHT1;1 expression is repressed and As(V) uptake is reduced.*

2 *As(V) sensitive phenotype of wild-type and asm19 mutant. Plants were grown in horizontal plates containing 10 µM Pi alone (control) or in combination with 15 µM As(V) for 8 days.*

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Regulation of gene activity in plants. The Phosphate starvation rescue system

We focus our study on the plant phosphate (Pi) starvation rescue system, which consists of an array of developmental, physiological and molecular responses that allow plants to cope with growth under Pi limiting conditions. This rescue system is a suitable model for studies on regulation of gene activity, and in addition, recently it has attracted considerable interest due to its potential to help design plants with increased Pi acquisition and use efficiency, a necessary requirement to implement low-input sustainable agricultural practices. In the past two years, our main activity has been to exploit natural variation to identify QTLs controlling transcription of Pi starvation genes and affecting Pi acquisition and use efficiency. Our transcriptomic analysis of recombinant inbred lines and natural accessions allowed the identification of a large set of transcription factors controlling expression of Pi starvation responsive genes (Figure 1). And the use of GWAS approaches have uncovered candidate genes affecting growth under Pi limiting conditions (Figure 2), whose characterisation is underway.

Additionally, we have examined the dynamics of interchromatin interactions in response to Pi starvation using Hi-C related approaches. We found no large effects of Pi starvation on chromatin interactions, but observed that genes induced by Pi starvation (PSI) tend to display increased chromatin interconnections among themselves, indicating a constitutive predisposition for coordinated PSI gene expression

Finally, we have also initiated an study of extrachromosomal circular DNA formation in response to Pi starvation. It is presently well established that eccDNA formation is a widespread characteristic of eukaryotes, where eccDNAs are originated from thousands of locations of their genomes. We have examined eccDNA formation during Pi starvation and in line with expectations more than 1500 eccDNA have been identified, out of which a 3% appear to be Pi starvation specific. We are presently studying their biogenesis and their potential functional significance

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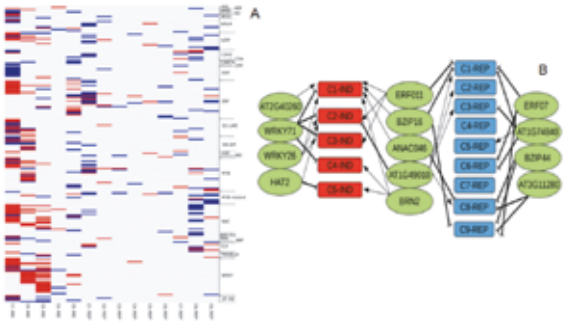

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1 *The Pi starvation co-expression network in* Arabidopsis. *The network was constructed with the WGCNA program using transcriptomic data from a collection of 100 RIL lines. It consists of 5 and 9 co-expression clusters of Pi starvation induced (C-X-IND) and Pi starvation repressed clusters (C-Y-REP). A) Heat map of TFs whose targets are enriched and display positive (red) or negative (blue) correlation with genes in the indicated co-expression clusters. B) Image showing examples of TFs potentially upregulating (displaying positive correlation) and/or downregulating (displaying negative correlation) Pi starvation responsive clusters*

2 *GWAS for root and shoot growth. Images (A)-(D) are Manhattan plots for root (A,B) and shoot (C,D) biomass accumulation after growth for 12 days in low Pi (A, C) and control conditions (B,D). Significance thresholds of -log(P) = 4 or 8 (the first corresponding to that published for potential associations and the latter corresponding to 5% with Bonferroni correction for multiple testing) are shown as dashed horizontal lines.*

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Environmental control of plant growth

Progressive rise in temperature due to global warming negatively impacts on crops productivity and affects wild taxa phenology, interfering with adaptation to their local environment. In Arabidopsis, warm temperatures promote elongation of seedlings hypocotyl and petioles in a thermomorphogenic response addressed to cool the leaves and protect the shoot meristem from the warm soil. Phenotypic analyses of this output unveiled that the red/far red light phytochrome photoreceptors act as main thermosensors, increased temperatures being shown to accelerate bioactive Pfr reversion into the inactive Pr form. Downstream of phyB, the PIF4 factor modulates temperature induced cell elongation by activating auxin and brassinosteroid biosynthesis, and the expression of cell-wall loosening enzymes required for cell expansion. Elevated temperatures cause up-regulated PIF4 expression at night, by impairing function of the circadian clock "evening complex"(EC) loop, consisting of EARLY FLOWERING 3 (ELF3), ELF4 and the LUX ARRHYTMO (LUX) DNA-binding protein. They induce as well nuclear accumulation of the E3 ligase COP1, shown to promote seedlings etiolation by targeting proteasomal degradation of many PIF4-antagonising factors. However, how these signalling events converge to thermal elongation is not well understood. To gain insight on the thermal role of these main signalling hubs, we have measured hypocotyl lengths of different combinations of mutant/over-expression lines grown at 22ºC and 28ºC and variable day length conditions and fitted the hypocotyl growth data into a mathematical model build on the described interactions for these regulators. Notably, the adjusted model fully reproduced thermal elongation of the studied genetic backgrounds and correctly predicted the thermal response of novel genotypes, therefore showing that thermal regulation of phyB, ELF3/EC and COP1 is sufficient to fully explain thermormorphogenic growth of Arabidopsis seedlings. Moreover, the model underscored a main temperature signaling function of the E3 ligase COP1, that acted independently of its inactivation by phyB, and which we validated experimentally. COP1 was shown to act though this thermal signaling activity as a main input for temperature entrainment of the clock, our current research efforts being addressed to the molecular understanding of this entrainment mechanism. Main focus of research in our team is thus directed to:

- Study the role of BR signaling and the master regulator BIN2 kinase in the control of COP1 nuclear shuttling.
- Characterise the cellular mechanisms underlying temperature-induced nuclear COP1 accumulation.
- Test the possible role of ELF3-COP1 interaction in modulating each other's function.
- Gain a better understanding on how this temperature signaling network affects circadian clock function and response of Arabidopsis plants to combined heat and drought stresses.

Overall, results from this research will identify best loci for increased tolerance to heat and drought stress as influenced by day length, and therefore guide smart breeding of seasonal crops for increased resilience to climate change.

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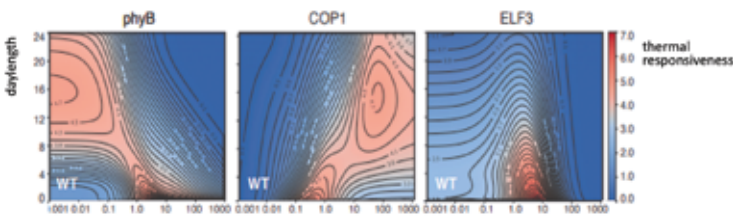

1 *Mathematical modelling estimation of the contribution of phyB, COP1, and ELF3/EC activities to termal elongation as a function of day length (collaboration with Saúl Ares and Pablo Catalán).*

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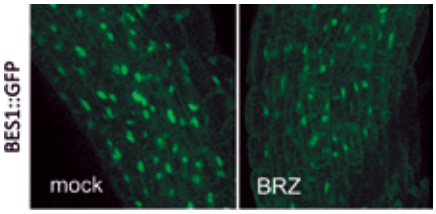

2 *Differential nucleocytoplasmic localisation of the BES1 factor in response to the BR biosynthesis inhibitor brassinazole.*

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Signalling networks in plant development and defense responses

Our group studies how plants adjust their growth and development to challenges from pests and pathogens.

These are some of the questions we are currently addressing:

1) What mechanisms initiate stem cell differentiation in plants and how are they regulated by biotic stresses to modulate organ growth rates? Our working hypothesis is that nuclear migration of the Arabidopsis proteins IYO and RIMA functions as a switch to reprogram the transcriptome and trigger stem cell differentiation in plants. We are studying how IYO/RIMA nuclear localisation and activity is controlled by developmental and biotic cues to control plant growth.

2) What are the roles of vacuoles in plant development and defense? Through a genetic screen, we are characterising genes involved in transport to, and biogenesis of, plant vacuoles, and studying how interfering with their function affects growth and resistance to pests and pathogens.

3) Do non-vascular plants activate systemic defenses against herbivores? When herbivores damage tissues of higher plants, wound signals are transmitted through the vasculature to activate systemic defenses in undamaged tissues. Our studies could provide important clues on the development of systemic signalling systems during the evolution of land plants.

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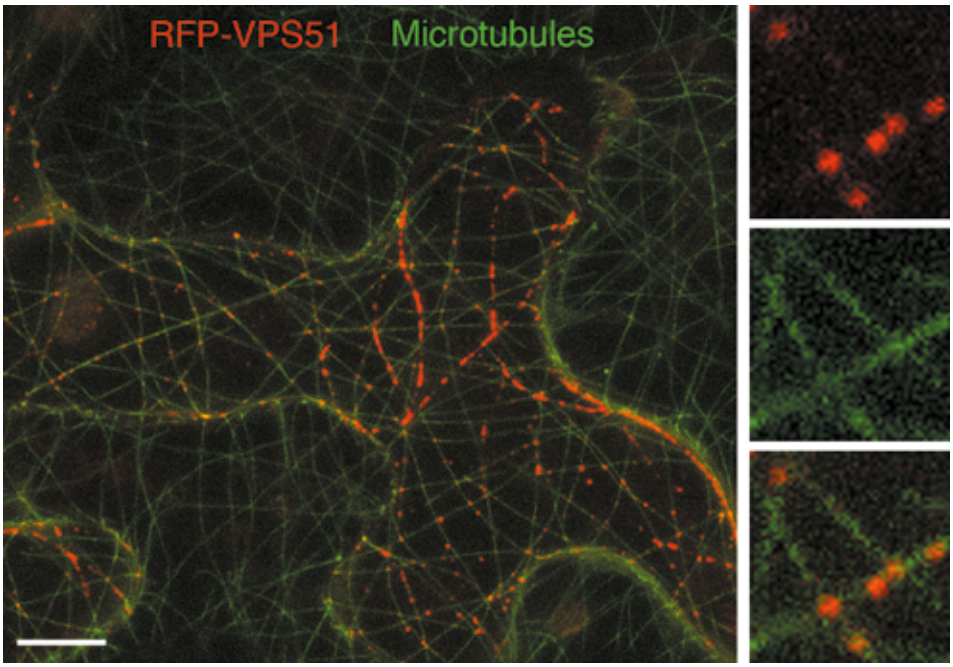

1 *VPS51 localises to Microtubule-Associated Compartments. Max. intensity projection of serial confocal images (depth: 10 µm) of Nicotiana benthamiana epidermal cells co-transformed with pUBI:RFP-VPS51 and the microtubule marker GFP-MAP4. Scale bar: 10 µm.*

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Role of ubiquitin in the control of plant growth and stress tolerance

The relevance of protein ubiquitination as an integral mechanism of many signaling pathways in plants has been demonstrated extensively. Ubiquitin (Ub) conjugation to proteins (i.e. ubiquitination) may trigger degradation of protein targets at the 26S proteasome or changes in their properties (e.g., protein activity, localisation, assembly and interaction ability), depending on the extent or specific Ub chain configurations. Protein ubiquitination is mediated by an enzymatic cascade in which different types of E3 Ub ligases provide the substrate specificity. Among them, Cullin4 RING E3 ubiquitin ligases (CRL4) have been involved in biological processes spanning the plant's whole life, including embryogenesis, seedling photomorphogenesis, circadian clock function, flowering and tolerance to different stresses (i.e. drought, high salinity, cold, osmotic stress) by promoting degradation of specific targets controlling those processes (Fig. 1). As an example, we have recently shown that DDA1, a substrate adaptor of CRL4-CDDD complexes, recognises abscisic acid (ABA) receptors, triggering their ubiquitination and proteasomal degradation (Irigoyen *et al*, The Plant Cell 2014). Therefore, CRL4-CDDD complexes act as repressors of ABA-mediated water stress responses under optimal growth conditions. Interestingly, CRL4-CDDD function is performed in close proximity to chromatin, which should enable rapid translation of environmental and stress signals into changes in gene expression. Indeed, recent results from our laboratory showed that CRL4-CDDD complexes are part of a molecular pathway controlling epigenetic homeostasis (including Histone2B ubiquitination) in response to external stimuli (i.e. light conditions; Nassrallah *et al*, eLife 2018). Our current objectives aim to identify and characterise additional mechanisms by which CRL4-CDDD controls the accumulation of specific epigenetic marks over the plant genome in response to environmental changes, to regulate expression of specific set of genes that lead to plant adaptation to changing climate conditions.

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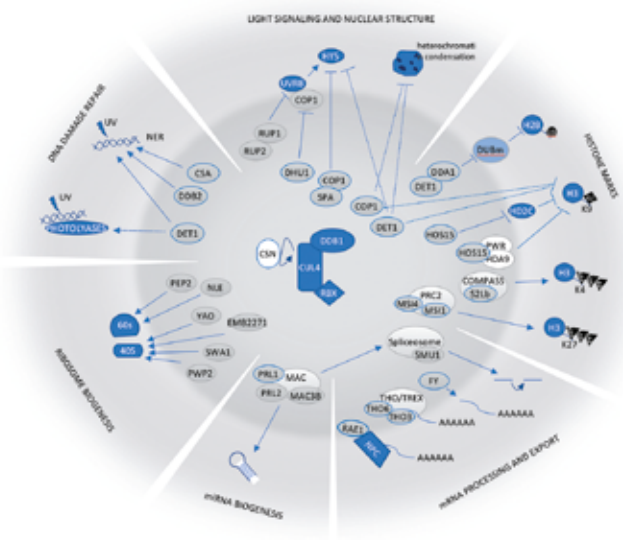

1 *Schematic representation of the chromatin functions in which* Arabidopsis *CRL4-CDDD and DWD-containing proteins play a role (adapted from Fonseca and Rubio, Front Plant Sci 2019)*

SELECTED PUBLICATIONS

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Jasmonate signalling and plant defense

Jasmonates (JAs) are fatty acid-derived signalling molecules that are essential for the survival of plants in nature, since they are important activators of stress responses and developmental programs. The main focus of my lab is to understand the biological mechanims that govern the JA signalling pathway in plants; knowledge that is crucial to design biotech and agronomical applications that improve plant resistance to stresses and plant yield. We have traditionally worked in the model plant *Arabidopsis thaliana*, but have recently focused in the liverwort *Marchantia polymorpha* due to its remarkable genetic advantages, such as very low gene redundancy.

Our major achievements in the last two years are:

- Identification of a new pathway for thermotolerance in plants (Monte *et al.*, *Curr Biol*, 2020)
- Identification of CUL3^{BPM} E3 ubiquitin ligases that regulate MYC transcription factors stability and JA responses (Chico *et al.*, *PNAS*, 2020).
- Characterisation of conserved basal defence mechanisms in land plants (Gimenez-Ibanez *et al.*, *Curr Biol*, 2019).
- Design and obtention of a tomato resistant to bacterial speck by CRISPR/Cas9-based mutation of *SlJAZ2* (Ortigosa *et al.*, *Plant Biotechnology Journal*, 2019).
- Characterisation of MYC2 orthologs in *Marchantia polymorpha* (Peñuelas *et al.*, *The Plant Cell*, 2019).
- Characterisation of the single JAZ repressor in *Marchantia polymorpha* (Monte *et al., Mol. Plant,* 2019).
- Identification of a new function of MYCs in photomorphogenesis (Ortigosa *et al.*, *Plant J*, 2020).
- Collaborated in the characterisation of PIF transcription factors in reproductive development (Costa Galvão *et al., Nat Commun,* 2019).
- Discovery of bioactive hydroxylated derivatives of JA-Ile (Jimenez-Aleman *et al.*, *Biochim Biophys Acta Mol Cell Biol Lipids* 2019).
- Identified the DNA target sequence of many plant transcription factors using previously developed tools and in collaboration with several groups (Ramírez Gonzales *et al.*, *Plant J*, 2020).
- Collaborated in the integrated multi-omics analysis of the plant response to jasmonic acid (Zander *et al.*, *Nat Plants*, 2020)

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Monte I, Franco-Zorrilla JM, García-Casado G, Zamarreño AM, García-Mina JM, *et al.* A Single JAZ Repressor Controls the Jasmonate Pathway in *Marchantia polymorpha*. Mol Plant 2019; 12: 185-198.

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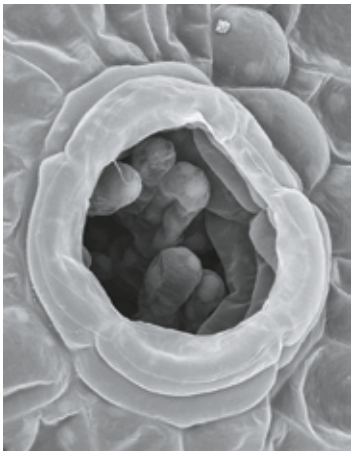

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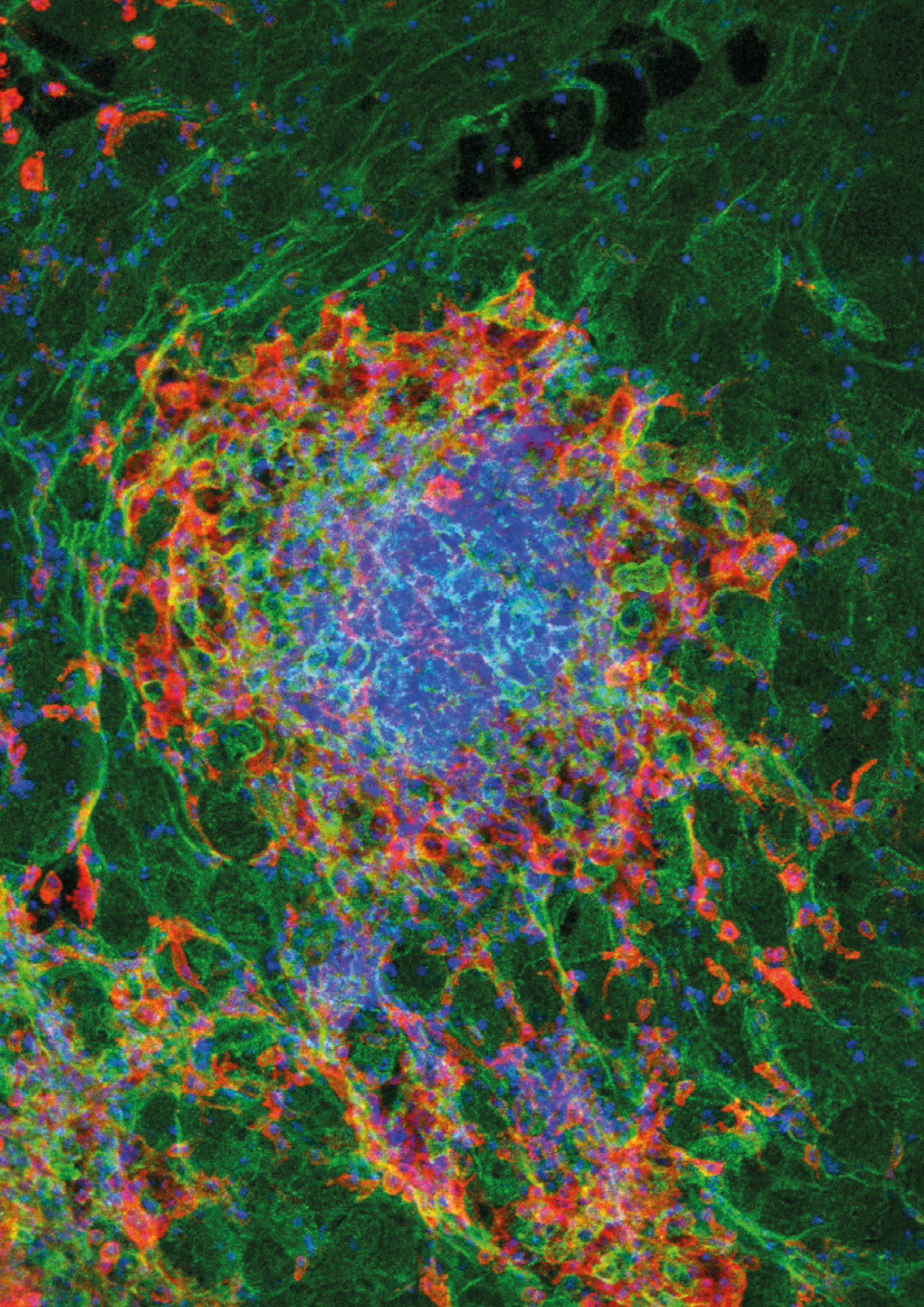
❶ *SEM image of epidermal air pore of* Marchantia polymorpha

❷ *Archegoniophores of* Marchantia polymorpha



5

IMMUNOLOGY AND ONCOLOGY

The Department of Immunology and Oncology (DIO) is devoted to the characterisation of the molecular and cellular bases of immune response in health and disease. We are interested in the study of the immune system function in tumour development, in inflammatory diseases as well as in infection by pathogens. Our aim is to identify new targets for the prevention, diagnosis and treatment of these pathologies, and to develop improved approaches for immune response modulation during cancer.

Various groups in the DIO address several aspects of cancer development and treatment, with special emphasis on the identification of new antitumour targets by characterising the cellular and molecular mechanisms that underlie (i) inflammation-driven carcinogenesis, as well as tumour immunology; (ii) the relationships among stem cells, metastasis, inflammation and cancer; and (iii) immunotherapy and diagnosis. The molecular and cellular mechanisms that underlie the immune response, inflammation and tumour development often overlap, providing many opportunities for collaboration among the groups in the Department as well as with other groups within and outside the CNB in the pursue of common research objectives.

During the COVID-19 pandemic, collaboration between groups in the DIO have led to the development of a serological test that includes several SARS-CoV-2 antigens and determines the presence of SARS-CoV2 antibodies with a 98% reliability. This antibodies test has been commercialised and approved for SARS-CoV2 diagnostics by the Spanish regulatory agency (Agencia Española del Medicamento y Productos Sanitarios, AEMPS).

HEAD OF DEPARTMENT

Ana Cuenda

Whole mount immunofluorescence and confocal microscopy image of the omentum, showing peritoneal resident macrophages infiltrating a milky spot, 4 hours after infection with the Escherichia coli *strain M6L4; anti-F4/80 (macrophages; red), anti-podoplanin (mesothelial cells; green) and DAPI (blue) staining. (Image from Carlos Ardavin's lab).*

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Immunobiology of monocytes, macrophages and dendritic cells

Our research program is currently focused on two major topics, alveolar dysfunction associated to airway allergy, and innate immunity against peritoneal infection and tumour metastasis. Using a mouse model of airway allergy induced by house dust mite extracts, our results demonstrate that airway allergic reactions caused a severe alveolar disorganization, involving the disappearance of alveolar macrophages, later replaced by monocyte-derived alveolar macrophages, and pneumocyte hypertrophy, associated with profound alterations in the composition and biophysical properties of pulmonary surfactant. These data support that the severe respiratory disorders caused by asthmatic reactions not only result from airway pathology due to bronchiolar inflammation, but also from profound alterations in the alveolar system. On the other hand, by using a mouse model of peritoneal bacterial sepsis, based on the intraperitoneal infection with a *E. coli* strain isolated from the mouse intestine, our group has defined the mechanisms by which resident peritoneal macrophages and inflammatory monocyte-derived macrophages control the defence against bacterial infection through the formation of complex, mesothelial bound macrophage aggregates, allowing the containment and elimination of bacteria.

Our results support that the formation of these aggregates require fibrin polymerisation, a process dependent on tissue factor release. The resolution of infection involves the disorganisation of macrophage aggregates, a process that involves fibrinolysis, controlled by monocyte-derived macrophages recruited to the peritoneal cavity. These results demonstrate that the ability of resident macrophages located in body cavities to fulfil their function depends on their attachment to the mesothelium and their clustering in cell aggregates, that in turn require a coagulation process for their formation. Similar cellular structures are formed in response to intraperitoneal injection of tumour-derived organoids, leading to peritoneal colorectal tumour metastasis. Overall these data support an important functional link between coagulation, inflammation and immunity for defence against peritoneal infection and tumour metastasis.

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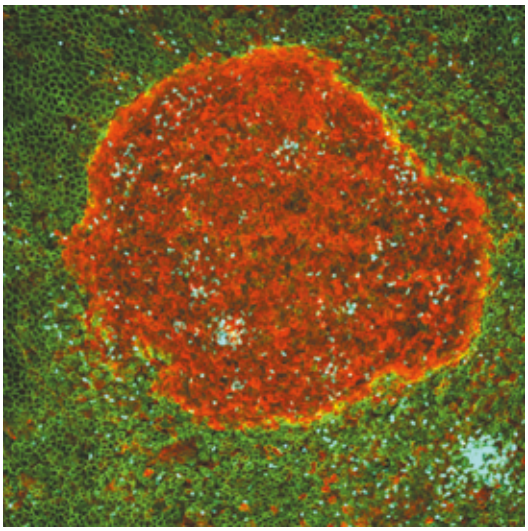

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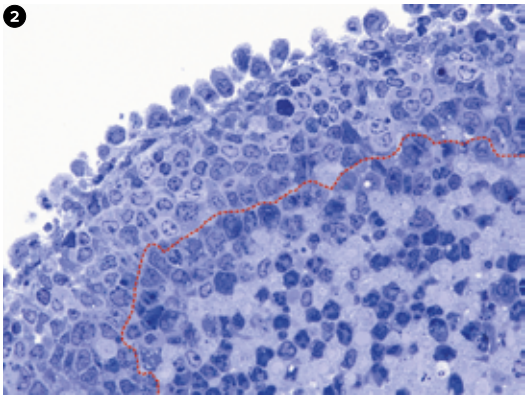

❶ *Whole mount immunofluorescence and confocal microscopy image of a resident macrophage aggregate in the peritoneal wall at 4 hours after infection with the* Escherichia coli *strain M6L4; anti-F4/80 (macrophages; red), anti-Ly6G (neutrophils; cyan) and anti-podoplanin (mesothelial cells; green) staining.*

❷ *Semi-thin section of a resident macrophage aggregate isolated 4 hours after infection with the* Escherichia coli *strain M6L4. The red dashed-line indicates the limits of central area harbouring necrotic macrophages and neutrophils. Toluidine blue staining.*

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Regulation of inflammation by p21 and mitochondrial ROS: from autoimmunity to COVID-19

Increased immune responses and hyperinflammation govern the development and progression of diseases that extend from Autoimmunity to COVID-19. In order to neutralise inflammatory responses, the immune response needs to be supressed. Alternatively, in cancer, immunosuppressed immunity requires reactivation. Therefore is it essential to understand systems that regulate these responses. Notably, our work points to p21 as a regulator of the balance between hyperactivation and immunosuppression by controlling mitochondrial Reactive Oxygen Species (mROS). Our recent work shows that mROS is essential for IFN-gamma production by memory T cells after IL-12 plus IL-18 challenge (Rackov *et al* 2020). IFN-gamma orchestrates inflammatory responses in inflammation-induced diseases. Remakably, Fas controls mROS and IFN-gamma induction independently of its apoptosis inducing potential (Figure 1). Our current work (in preparation) indicates that p21 modulates mROS and IFN-gamma production by memory T cells, corroborating our published data, showing that p21 overexpression tempers autoreactive T cells and IFN-gamma production (Daszkiewicz *et al*, 2015). Therefore, high expression of p21 lowers T cell overactivity, while lack of p21 enhances responses by regulating mROS production (Figure 1).

Similarly to memory T cells, p21 regulates the inflammatory potential in macrophages. We have shown a dual regulatory role for p21; first, in macrophage activation to M1 state (Trakala *et al*, 2009) and, second, in macrophage reprogramming from M1 to the M2 unresponsive state. Lack of p21 prevents macrophage reprogramming to M2 status (Rackov G *et al*, J Clin Invest 2016). Our present results firmly show that mROS, which is regulated by p21, is an early regulator of the inflammatory response of M1 macrophages as it enhances M1 responses as early as five minutes post-activation, and leads to NF-kB activation and ultimately to inflammatory cytokine production. The direct interaction of p21 and mitochondria in M1 macrophages is shown in Figure 2.

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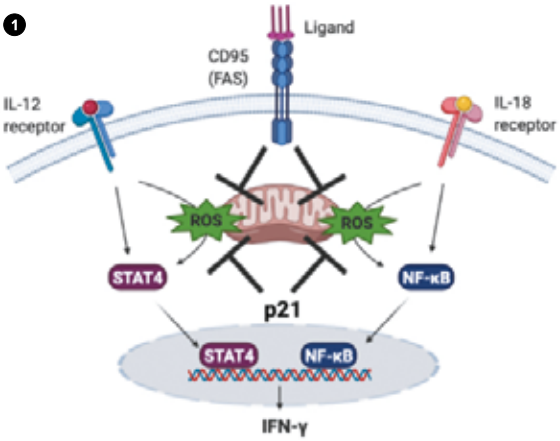

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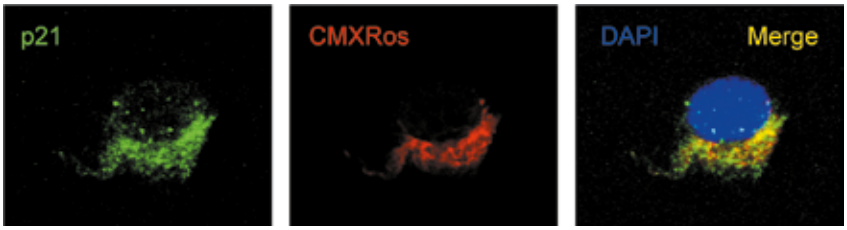

❶ *Schematic representation of how p21 and FAS regulate mitochondrial ROS production and consequently the pathway of memory T cells activation and IFN-gamma production in response to IL-12 plus IL-18.*

❷ *Confocal microscopy of stimulated macrophages shows direct interaction of p21 with mitochondria specifically stained by CMXRos. Polarisation of mitochondria is evident due to their activated status of macrophages.*

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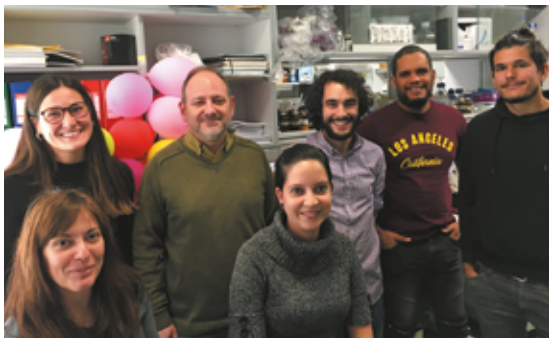

Nanomedicine, cancer immunotherapy and autoimmune diseases

Magnetic Iron oxide nanoparticles (MNPs) have considerable potential to be used as nanomedicines for targeted drug release or magnetic resonance imaging. Recently, we highlighted the promise of using MNPs in other therapeutic approaches to treat cancer, such as the induction of intracellular hyperthermia in tumour cells or the magnetic targeting/retention of lymphocytes in cell transfer therapies. We have also seen that the accumulation of MNPs by different cell types induces oxidative stress and its associated effects as a consequence of MNP degradation. Thus, here we aim to explore whether these responses could be used therapeutically to fight tumours at different levels.

The overall objective of our group is to fully understand the molecular and cellular mechanisms induced by MNPs at their different levels of action. This knowledge can be used to improve the functional design of MNPs for specific biomedical applications, such as therapies to combat tumours and autoimmune diseases, with the aim of bringing them closer to their clinical application. As such, we will pursue five specific objectives: 1) We will expand our studies on the magnetic retention/accumulation of MNP-functionalized anti-tumour lymphoid cells in ACT therapies in order to bring this therapy closer to the clinic; 2) We intend to explore whether the targeting to and/or retention of MNP loaded tolDCs in LNs could ameliorate the symptoms of lupus in the MRL/lpr mouse model of SLE; 3) We will evaluate the capacity of the oxidative stress induced in cells by MNPs to remodel the tumour microenvironment and to improve anti-cancer therapies; 4) We will assess how to improve the efficiency of intracellular heating of MNPs in AMF-induced hyperthermia strategies, studying the biological effects induced by MNPs of different physico-chemical characteristics (size, shape, anisotropy) after the application of an AMF of different intensity and frequency; 5) We will analyse whether oxidative and endoplasmic reticulum (ER) stress caused by MNPs inside tumour cells could affect the processing and presentation of antigens, and whether this might provoke the generation of neoantigens.

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❶ *Lysosomal degradation of superparamagnetic iron oxide nanoparticles inside of macrophages. (Yadileiny Portilla).*

❷ *Retention of tolerogenic dendritic cells (tolDCs) associated to Magnetic nanoparticles (MNPs) using a neodymium magnet of 1.45 T in a flow chamber assay. (Andrés París).*

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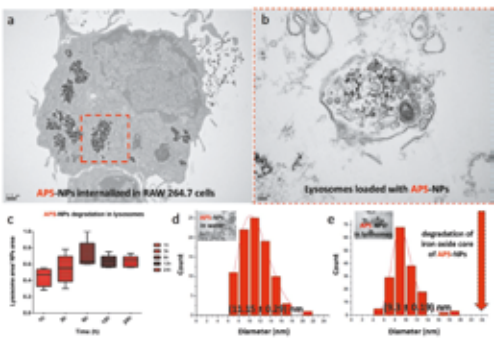

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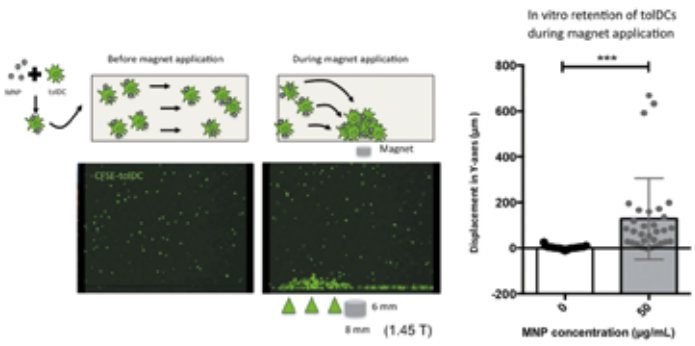

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Cardiac stem cells

Adult mammalian heart refresh damaged or aged cells during their lifetime but with low rate, particularly regarding cardiomyocytes. However, the mechanisms involved in heart turnover remains controversial. We have characterised cardiac progenitor cells that express high levels of the polycomb Bmi1 transcription factor, which contributes to the turnover of the three main cardiac lineages. In response to a variety of cardiac insults these Bmi1^{+} Cardiac Progenitor Cells (B-CPC), get proliferatively activated and their progeny contribution to the mature lineages is enhanced with special proness towards the endothelial lineage. In addition, *in vivo* genetic depletion of the B-CPC population provokes a deleterious condition during acute infarct recovery. Thus, the B-CPC population contains cardiac progenitors contributing both to heart homeostasis and in response to several modes of damage.

In adult tissues, progenitors and stem cells are lodged in specialised structures (niches) that provide a protective microenvironment, essential for their correct regulation. These niches are usually associated to a low oxidative stress environment, where adult progenitors show a restrained proliferative status essential for maintenance of their self-renewal capacity. In good agreement with our working hypothesis, we found that B-CPC show low levels of ROS and, interestingly, in homeostasis conditions, they are located close to the cardiac vasculature, showing a proliferative gradient coincident with Bmi1 expression levels; low-proliferative B-CPC are closer to endothelial structures. These results, together with *in vitro* co-culture experiments, strongly suggested a plausible crosstalk between vessel structures and B-CPC. In addition, we confirmed by transgenic manipulation of ROS levels *in vivo*, that B-CPC cardiac location and their activity are susceptible to oxidative stress modifications. Altogether, we concluded that cardiac vasculature provides a protective and low-stress microenvironment that contributes to the maintenance of B-CPC promoting their self-renewal in adult heart. Currently, we are trying to dissect the specific bidirectional mechanisms involved and defining the B-CPC vascular niche.

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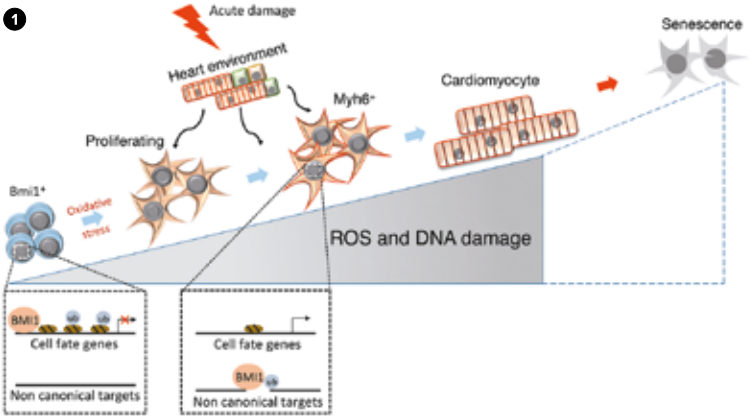
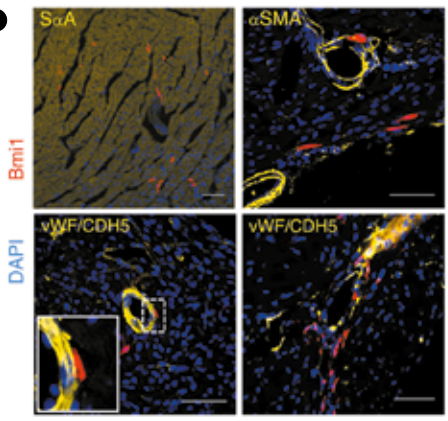
❶ *Scheme of regulation of B-CPC activity in homeostasis and in response to oxidative stress and acute damage.*

❷ *Heart cryosections of reporter mice (B-Tmt) 5-days post Tx induction. B-CPC (Tomato+) are located close to endothelial (yellow markers) cells. Inset (2x).*





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B Lymphocyte dynamics

B lymphocytes patrol our body seeking for pathogen-derived antigens. Recognition of antigen activates the B cell immune response, which leads to the production of highly specific antibodies that will neutralise and eliminate the pathogen, and of memory B cells that confer long-term immunity. The complexity of the B cell response involves changes in lymphocyte behaviour, switching from highly motile states to stable cell-to-cell interactions (immune synapse), adjustments of the cell mechanical properties (flexibility, stiffness) and cell polarity (MTOC and organelle distribution). Gene mutations or functional alterations in proteins related with these events are frequent in B cell pathologies (immunodeficiency, lymphomas), stressing their relevance for B cell function.

Our research focuses on the mechanisms that govern B lymphocyte dynamics, and how their dysfunction leads to B cell pathology. We recently revealed essential new functions of two proteins, Bruton's tyrosine kinase (Btk) and the ζ isoform of Diacylglycerol kinases (DGKζ), both of interest for the clinic as therapeutic targets. Btk has a key role in the signalling of the B cell receptor for antigen and clinical trials with kinase inhibitors are on-going for B cell-lymphoma treatment. We found that Btk promotes the cell-cytoskeleton and adhesion-site remodelling needed for immune synapse formation mainly through its shuttling/scaffold activity. Impairment of that leads to B cell activation defects equivalent to those due to Btk kinase inhibition. Related with DGKζ, known for diminishing antigen receptor signalling through DAG consumption, our findings showed that it also stimulates the B cell immune response. DGKζ facilitates antigen extraction at the immune synapse by promoting actin-cytoskeleton remodelling and mechanical forces. B cell ability of antigen extraction is essential for antigen presentation to CD4 T cells and the germinal centre response. Both events are reduced for DGKζ-deficient B cells compared to wild type (Figure 1).

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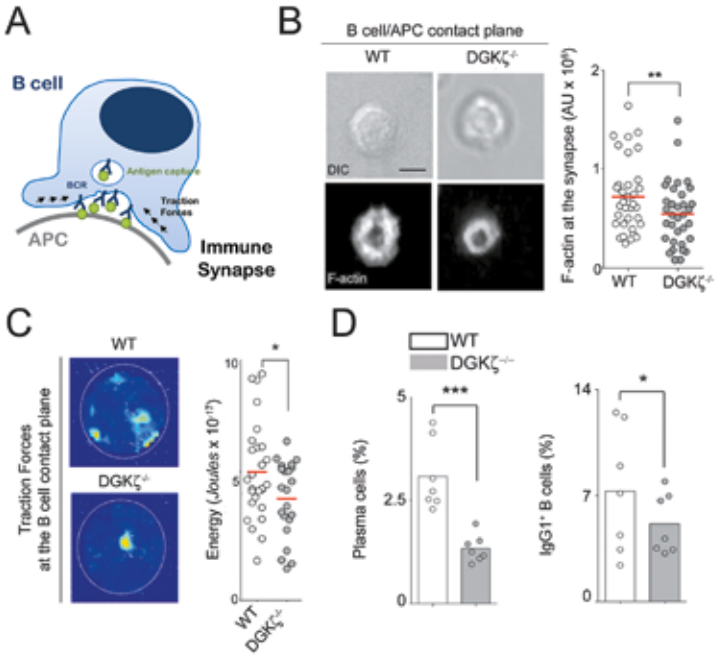

1 *DGKζ stimulates the B cell immune response. A, Events at the B cell immune synapse. B, DIC and fluorescence images of F-actin at the synapse of wild type (WT) and DGKζ-deficient (DGKζ$^{-/-}$) B cells; scale bar, 2.5 µm. Values of the total amount of F-actin at the synapse are shown; each dot is a cell. C, Colour maps of stress forces at the immune synapse of WT and DGKζ-/- B cells, measured by Traction Force Microscopy. Average values of synaptic traction forces per cell over time are shown; each dot is a cell. D, Plasma cells (CD138^{+}) and IgG1+ B cells generated in mice adoptively transferred with WT or DGKζ$^{-/-}$ B cells at day 7 after immunization; each dot is a mouse. *, p<0.05, **, p<0.01, ***, p<0.001.*

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Molecular targets in health and cancer: special focus on PIP3

Our group studies the molecular mechanisms by which signalling proteins control cell behaviour, and how these proteins, when mutated, influence the course of human cancer. In recent years we have focused on the enzymes that control PIP3 (phosphatidylinositol 3-phosphate), a little-abundant molecule in "resting tissues" but which is required when cells need to divide or migrate - including in cancer. We have been involved in the following studies:

1) The action of PI3-kinase beta action on hESC stemness/differentiation decisions
PI3-kinase beta, one of the enzymes generating PIP3, localises to the nucleus and regulates DNA replication, segregation and repair. We are studying its function in human stem cell (hESC) stemness/differentiation decisions.

2) Regulation of PTEN phosphatase activity under near-physiological conditions
PTEN phosphatase, which reduces PIP3 levels, is altered in many human tumours, most commonly during the metastatic phase. The main therapeutic approach for limiting PIP3 action has been to inhibit PI3-kinase enzymes, but boosting tumour PTEN phosphatase activity could be an alternative. We are involved in the study of how PTEN phosphatase activity is modulated after growth factor receptor activation.

3) PIP3 actions in TUMOR microenvironment: hypoxia and oxidative stress
Solid tumours commonly grow under low oxygen conditions (hypoxia). The adaptation of cells to hypoxia is regulated by HIF transcription factors. We are in the process of examining how PI3-kinases modulate HIF-mediated transcription.

Solid tumours also show high levels of reactive oxygen species (ROS), a stress to which their cells have to adapt if they are to survive. Many lung tumours show activation of the **NFE2L2** pathway under high ROS conditions. We are therefore investigating the mechanism of action of NFE2L2 and how to interfere with it in lung cancer.

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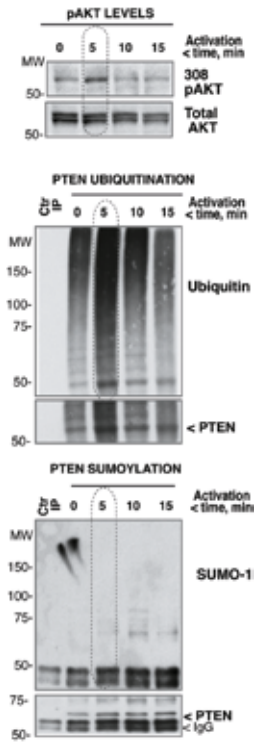

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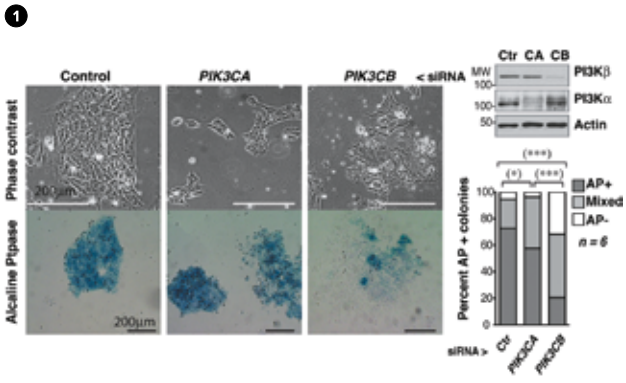

❶ *PI3Kβ is required for human embryonic stem cells stemness. Representative phase-contrast images or alkaline phosphatase (AP)-stained hESC transfected with siRNA control, or for PIK3CB (encoding PI3-kinase beta), or PIK3CA (encoding PI3-kinase alpha) (96 h). Right: Western blotting illustrates silencing efficiency. The graph shows the percentages of AP+, AP- and mixed colonies. (*) P<0.05; (***) P<0.001 (Chi squared test).*

❷ *Maximum pAKT levels correlate with PTEN ubiquitination, while pAKT decrease concurs with PTEN SUMOylation. HEK-293T cells were stimulated with serum (15%) for different times. PTEN was immunoprecipitated from whole cell extracts (for SUMOylation) or from extracts that were enriched in ubiquitinated proteins in appropriate columns (for ubiquitination). WB tested 308-pAKT levels, PTEN ubiquitination, and PTEN SUMOylation.*

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Stress-activated protein kinases in inflammation and cancer

Inflammation is a defensive response against pathogens and a natural process of the immune system to repair tissue damage. However, uncontrolled inflammation is pathological and is the cause of many chronic diseases, which have been steadily increasing in recent decades, especially in western developed countries. This represents a major challenge for modern medicine. Thus, understanding how the inflammatory process is regulated is essential to find new ways to control it, either endogenously or with external therapeutic intervention.

In these two years we have expanded our knowledge on the molecular and cellular mechanisms involved in the inflammatory response in the settings of chronic inflammation leading to tumour development, as occurring in colon cancer associated to colitis; and also, the development of new tools (e.g. kinase inhibitors) for the treatment of inflammation-driven tumours and other inflammatory diseases.

We have also investigated the role of p38MAPK in the development of immune cells such as B lymphocytes in bone marrow and spleen, using mice lacking p38γ and p38δ, or conditional knockout mice that lack both p38γ and p38δ specifically in the B cell compartment. We found that p38γ/δ-deficient mice had reduced numbers of peripheral B cells as well as altered marginal zone B cell differentiation in the spleen. Expression of co-stimulatory proteins and activation markers in p38γ/δ-deficient B cells are diminished in response to BCR and CD40 stimulation; p38γ and p38δ are necessary for B cell proliferation induced by BCR and CD40 but not by TLR4 signalling. Furthermore, p38γ/δ-null mice produced significantly lower antibody responses to T-dependent antigens. Our results identify novel functions for p38γ and p38δ in B cells and in the T-dependent humoral response; and show that the combined activity of these kinases is needed for peripheral B cell differentiation and function.

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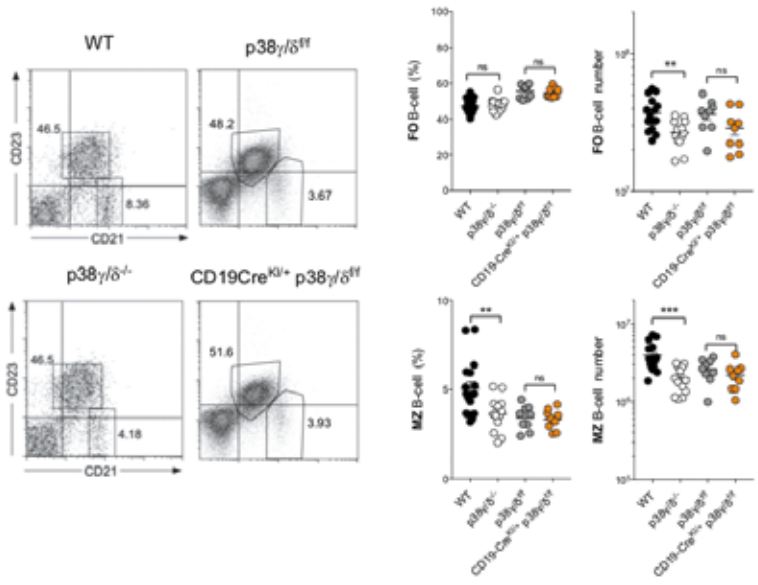

1 *Analysis of B cell populations in the spleen. Representative dot plots for CD21 and CD23 expression in splenocytes from mice of the specified genotypes. Frequencies of gated follicular (FO) B cells (CD21+CD23+) and marginal zone (MZ) B cells (CD21hiCD23-) (gates) are indicated. Frequency and total cell number of FO and MZ B cells in adult mouse spleens. Each dot represents a single mouse.*

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Physiopathology of chemokine receptor interactions

Our group studies the role of chemokines and their receptors in tumour progression and metastasis, since they are involved in tumour cell survival and proliferation, tumour-associated angiogenesis and the antitumour immune response. There is growing interest in the development of new antibody-based immunotherapies for cancer treatment. We have generated a panel of mouse monoclonal antibodies (mAbs) specific for the human CCR9 receptor, which is overexpressed in different haematological malignancies. Two antibodies that were selected for their efficacy in reducing the growth of human CCR9$^+$ tumours in different immunodeficient mouse models have been protected by an international patent and have been licensed to SunRock Biopharma. Chimeric and humanised variants of these antibodies also effectively inhibit tumour growth. Recently, using the CRISPR/Cas9 system, tumour cell lines with modified variants of CCR9, were generated and are being used in ongoing experiments on animal models to evaluate whether the candidate antibodies for clinical use exhibit any off-target side effects.

With the aim to generate antibody cocktails that can simultaneously attack different molecular targets on leukaemia cells, we generated mAbs against surface antigens present on human T-cell acute lymphoblastic leukaemia cells. Several of them strongly reduce tumour size in animal models. Using proteomic techniques to identify the antigens recognized by these mAbs, we are selecting those directed against cell surface molecules that are potential therapeutic targets.

In collaboration with different research groups, we are also generating and evaluating mAbs that can be used to modulate the immune response in other pathologies. We have contributed to analyse the role of the CCL1-CCR8 axis in atherosclerosis, to study CD5L in liver fibrosis and to generate new tools for inhibiting Ebola and HIV-1 viral uptake. We have also generated mAbs against SARS-CoV-2 that are being evaluated as potential therapeutic agents for the treatment of COVID-19.

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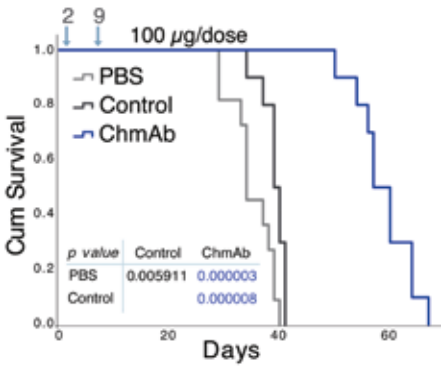

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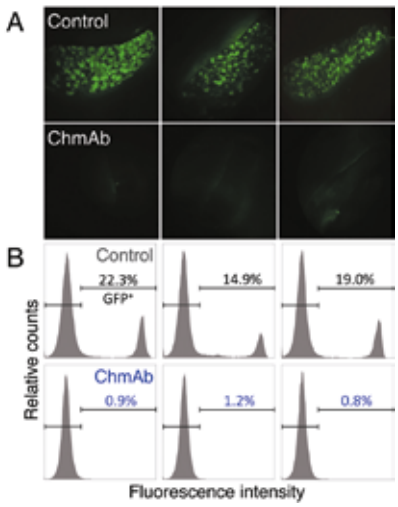

❶ *Kaplan-Meier survival curves of NSG mice carrying xenotransplants. MOLT4-GFP cells were injected into the tail vein of mice on day 1. Mice were subsequently treated on days 2 and 9 with either chimeric anti-CCR9 mAb (ChmAb), isotype control mAb or PBS.*

❷ *A. Stereomicroscopic images of a representative spleen from each treatment group, where the accumulation of tumour cells (MOLT-4-GFP) in the isotype-control treated animals but not in the ChmAb-treated group, could be observed. Data from 3 mice of each 10 mice group are shown. B. Flow cytometry analyses of mouse bone marrow showing the fraction of MOLT-4-GFP cells from isotype control and ChmAb-treated animals. Data from 3 mice of each 10 mice group are shown.*

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Signalling networks in inflammation and cancer

We aim to understand the molecular cues that regulate Inflammation in pathological conditions, such as cancer or Alzheimer's disease. Using multidisciplinary approaches, we study key immune and non-immune cell elements that participate in the inflammatory reaction. In the 2019-2020 period we worked in four areas:

1. Normalisation of tumour-associated vasculature to improve immunotherapy.
Angiogenesis is a common feature of cancer. Tumour vessels are, however, dysfunctional, leading to hypoxia and tumour aggressiveness. We discovered that restoring the levels of Extracellular superoxide dismutase (SOD3) in the tumour microenvironment normalises the tumour vasculature and increases the specific tumour infiltration by effector immune cells (details in Carmona *et al*). Moreover, anti-angiogenic agents synergise with tumour immunotherapy to improve the survival of patients with metastatic breast cancer (details in Quintela *et al*).

2. Identification of signalling pathways downstream of PD-1.
PD-1 blockade is common immunotherapeutic treatment in cancer. Yet little is known about how PD-1 blocks the effector function in T cells. Using RNA-seq and bioinformatics we have identified a PD-1-induced genetic program that elicits immunosuppression by targeting the metabolism and mitochondrial ultrastructure of $CD8^+$ T cells (details in Ogando *et al*).

3. CCR5 effects on T-cell receptor (TCR) organisation and the memory $CD4^+$ T cell response.
The chemokine receptor CCR5 not only causes chemoattraction of immune cells, but also provides costimulatory signals required for optimal $CD4^+$ T cell activation. We have now found that CCR5 regulates the functionality of $CD4^+$ memory T cells. This activity is associated to changes in the nanoscale organisation of the TCR due to alterations on sphingolipid metabolism (details in Martín-Leal *et al*).

4. Innate immune cell differentiation in neurological diseases.
The high co-morbidity of Alzheimer's disease with cardiovascular and metabolic disorders suggest that systemic alterations might be determinant for Alzheimer's evolution. Using isogenic iPSC we have studied the influence of APOEε4 polymorphism in macrophage polarisation, metabolism and cholesterol efflux activity, and the association of these parameters to the development of late-onset Alzheimer's disease (in preparation).

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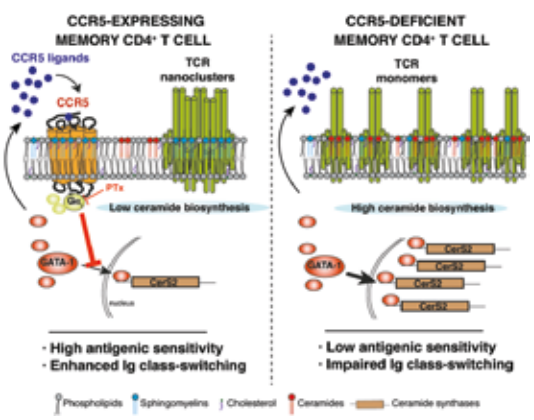

1 *CCR5 primes memory CD4+ T-cell function. In memory cells, CCR5 signals inhibit the nuclear translocation of GATA-1. This reduces ceramide biosynthesis, enables T cell receptor (TCR) nanoclustering and increase the response after antigenic re-encounter. Lack of CCR5 (ccr5Δ32 persons) enhances ceramide levels, which rigidify the cell membrane and impedes TCR nanoclustering.*

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Stem cells and immunity

We have identified *Death Inducer-Obliterator (Dido)*, which produces three protein isoforms termed DIDO1, 2, and 3, as an important gene in stem cell (SC) differentiation. Several lines of investigation link the *Dido* gene to SC biology through its function in transcription and its relation to chromatin biology. Our studies have characterised the effects of the 5' and 3' regions of the Dido gene individually *in vivo* and in *in vitro* cell lines. Our analysis of the *Dido* gene 5' region identified nuclear localisation of all three isoforms and their interaction with chromatin. The recent characterisation of the 3' regions of the gene focused on DIDO3, the only isoform to comprise a complete domain architecture. DIDO3-specific sequences are encoded by a separate exon located in the 3' region, found in all vertebrates but not in organisms without SC such as yeast.

Mammalian cells that lack Dido3 but can produce the other isoforms show widespread defects in the processing of RNA derived from spliced genes. This finding indicates that DIDO3 has a role in splicing (see Figure 1), and possibly in transcription termination (in review process). This hypothesis is supported by preliminary data, since part of the aberrant RNAs found by RNA sequencing involve readthrough beyond the constitutive 3' UTR. Dido3 thus appears to have evolved to compensate for the increased dependency on RNA processing.

In addition, Dido3 mutations cause blockade of stem cell differentiation, defects in chromosome segregation, and genomic instability. Analysis of cells derived from the Dido3 mutant shows centrosome amplification, cytokinesis defects, binucleated cells, and genomic instability. Previous work in our laboratory showed that mice lacking the N-terminal domain of Dido develop myelodysplasia/myeloproliferative disorders (MDS). Based on these results, we propose that Dido3 defects contribute notably to the pathologies associated with the aberrant production of Dido isoforms.

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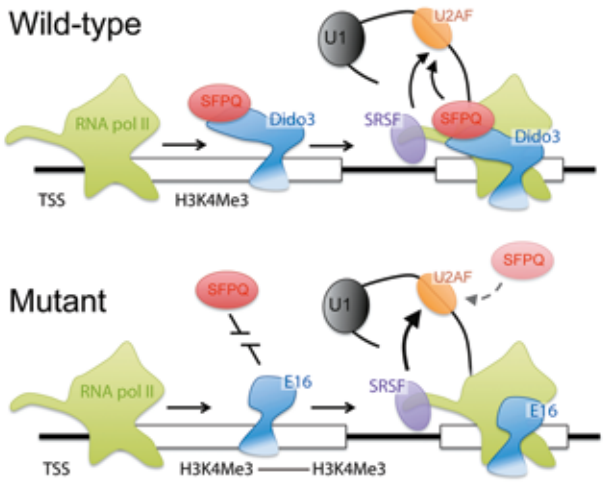

1 *Model for the role of Dido3 in transcription and splicing. Protein interaction studies and deletion mutants attribute a bridging role to Dido3, in which Histones H3 acts as a reservoir from which the protein is recruited by RNA Polymerase II. In turn, Dido3 facilitates the binding of SFPQ to nascent RNA for subsequent spliceosome assembly.*

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Chemokine receptors: New targets for therapeutic intervention

The chemokine receptors are members of the GPCR family that, through interaction with their ligands, induce a wide variety of cellular responses including cell polarisation, movement, immune and inflammatory responses, as well as prevention of HIV-1 infection. Like a Russian matryoshka doll, the chemokine receptor system is more complex than initially envisaged. The chemokines and their receptors exist as monomers, dimers and oligomers, their expression pattern is highly regulated, and the ligands can bind distinct receptors with similar affinities. The use of novel imaging-based technologies, particularly real-time imaging modalities, has shed new light on the very dynamic conformations that chemokine receptors adopt, and that affect chemokine responses. To date, all of the chemokine receptors tested form homo- and heterodimers during their synthesis and maturation, and in such conformations reach the cell membrane.

Knowledge of the dynamic interactions between ligands and receptors, as well as their interplay with other proteins co-expressed by the cell, lipids at the cell membrane, the cellular cytoskeleton, and downstream signalling machinery will be crucial to determine how they modulate cell responses. Using STimulated Emission Depletion (STED) microscopy and single particle tracking and Total Internal Reflection Fluorescence Microscopy (TIRFM) we have evaluated the receptor organisation and signalling in living cells on the spatial and temporal scales and determined the presence of basal nanoclusters of CXCR4 in resting T cells, whose extent, dynamics, and signalling strength are modulated by the orchestrated action of the actin cytoskeleton, other molecules expressed at the cell membrane, and the ligands. This new information will transform our vision of the chemokine-mediated functions, and will hopefully identify exciting opportunities for drug discovery.

In parallel, our group has also a research line to investigate inflammatory and autoimmune disease models to test the targets and hypothesis identified on the chemokine projects.

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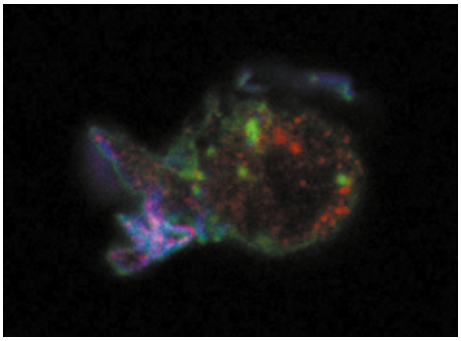

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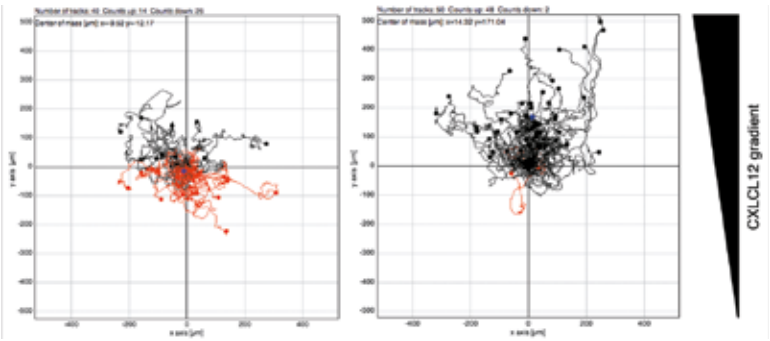

1 *CXCL12 triggers rapid cell polarisation and lamellipodia formation. Jurkat cells expressing CXCR4-AcGFP (green) were added on coverslips coated with fibronectin plus CXCL12, fixed and stained with anti-Rac1-GTP mAb (blue) and phalloidin (red).*

2 *CXCL12 triggers directed cell migration. Spider graphs showing directed JK cells migration towards CXCL12 (right) vs control in the absence of gradient (left). The black triangle indicates the direction of the chemokine gradient.*

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Diacylglycerol kinases in the control of immune response and cancer progression

T cell tolerance is the mechanism that protects healthy tissue from damage during immune attack. Solid tumours employ similar mechanisms, expressing ligands for co-inhibitory receptors to avoid immune destruction (Fig). The Diacylglycerol Kinase (DGK) family of enzymes transform diacylglycerol (DAG) in to phosphatidic acid. Several DGK isoforms have been related to cancer but only the alpha and zeta have been extensively characterised as negative regulators of T cell responses. The abnormal elevation of these two DGK isoforms in tumour infiltrating lymphocytes drives T cells into anergic non-functional states. Manipulation of DGK activity/expression enhances antitumour T cell functions, suggesting potential for pharmacological intervention.

Our group works to better understand the redundant and specific actions of DGK alpha/zeta in cancer. We have identified high DGKα expression, that in healthy cells is mostly restricted to T cells, in mesenchymal cancer types. Targeting DGKα thus not only re-instates immunological tumour recognition and destruction, but may also help to destroy tumours by interfering with oncogenic signals. DGKζ on the other hand, is broadly expressed and operates in others systems different from T cells. In the laboratory we use combinations of genetical and biochemical approaches to explore the consequences of isoform-specific DGK targeting in distinct preclinical models of cancer. We also investigate the immunomodulatory potential of small molecules with potent inhibitory action against purified enzymes.

Finally, we explore the potential adverse effects of DGK manipulation using Down syndrome-associated comorbidities as a model. Our final purpose is dual: on one hand we seek to demonstrate the full potential of DGK targeting so inhibitors of these kinases can be considered in the arsenal of cancer immunotherapies. On the other we want to identify possible adverse consequences derived from targeting DGK-regulated pathways.

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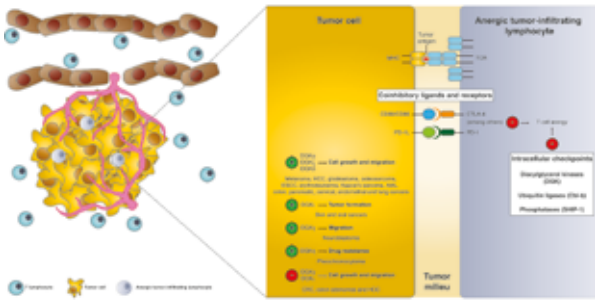

1 *DGK as intracellular checkpoints. Left: cartoon shows how tumour-infiltrating lymphocytes become anergic and unable to destroy tumours. Right: Membrane-bound immune checkpoints in T lymphocytes act as coinhibitory receptors upon recognition of tumour-expressed ligands. In tumours DGKs promote malignant traits whereas in T lymphocytes specific isoforms act as intracellular checkpoints that limit T cell cytotoxic potential. DGK blockade could reinstate T cell attack on tumours, limiting at the same time tumour growth and metastasis. MHC, mayor histocompatibility complex; TCR, T cell receptor; CD80/86, cluster of differentiation 80/86; CTLA-4, cytotoxic T-lymphocyte antigen 4; PD-1/L, Programmed cell death protein 1/ligand; HCC, hepatocellular carcinoma; ESCC, esophageal squamous cell carcinoma; AML, acute myeloid leukemia; CRC, colorectal carcinoma.*

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Mérida I, Arranz-Nicolás J, Rodríguez-Rodríguez C, Ávila-Flores A. Diacylglycerol kinase control of protein kinase C. Biochem J 2019; 476 (8): 1205-1219.

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Arranz-Nicolás J, Martín-Salgado M, Rodríguez-Rodríguez C, Liébana R, Moreno-Ortiz MC, *et al.* Diacylglycerol kinase ζ limits IL-2-dependent control of PD-1 expression in tumor-infiltrating T lymphocytes. J Immunother Cancer 2020; (2): e001521.

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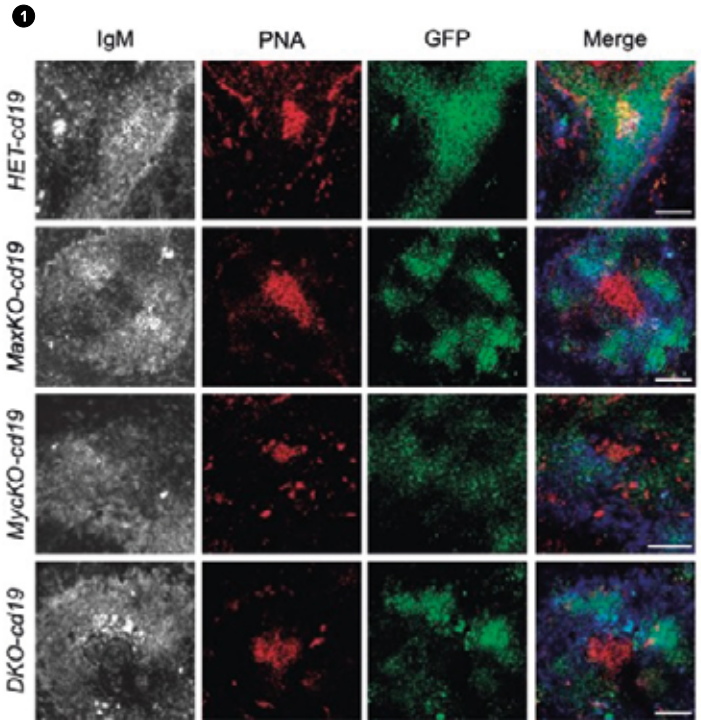
Transcriptional control of lymphocyte differentiation

Our major biological question is to understand how cell differentiation is regulated by transcription factors and how this process is altered in pathological scenarios such as cancer. We approach this question by analysing the transcriptional program in a well-defined setting *in vivo* such as B lymphocyte differentiation. Among the wide spectrum of transcription factors involved in this process we focused our efforts in the function of the proto-oncogene *c-myc* for two reasons. First, the c-Myc protein is a member of the Myc family (N-, L- and c-Myc) of transcription factors involved in numerous biological functions including the regulation of cell proliferation, differentiation and apoptosis in multiple cell types. This pleiotropic function confers this protein an essential and distinct role at different differentiation stages in numerous cell types. Second, in animal models and humans, deregulated c-Myc expression leads to the development of tumours, including B and T lymphomas. This oncogenic potential provides an interesting dimension in terms of possible therapeutic applications of our research.



1 *Analysis of germinal centre (GC) formation in the spleen of Max KO (MaxKO-cd19), Myc KO (Myc-KO-cd19), Double KO (DKO-cd19) and heterozygous control mice immunised with TNP-KLH. Representative images of frozen spleen sections stained with IgM (grey/blue), PNA (GC marker; red), and GFP (Max-, c-Myc- or c-Myc/Max-deficient B cells; green). Scale bar, 80µm.*

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SELECTED PUBLICATIONS

Bravo García-Morato M, Calvo Apalategi A, Bravo-Gallego LY, Blázquez Moreno A, Simón-Fuentes M, *et al,* Impaired control of multiple viral infections in a family with complete IRF9 deficiency. J Allergy Clin Immunol 2019; 144: 309-312. e10.

Pérez-Portilla A, Moraru M, Blázquez-Moreno A, Kolb P, Bravo García-Morato M, *et al,* Identification of the first cases of complete CD16A deficiency: Association with persistent EBV infection. J Allergy Clin Immunol 2020; 145: 1288-1292.

Receptor ligand interactions in immune responses to cancer and viruses

Natural killer (NK) cells kill infected cells and secrete cytokines, to play an important role in defence against viral infection. Although NK cells are often perceived as rather primitive lymphocytes; always ready to kill unless checked by inhibitory receptors binding to MHC Class I molecules. It is now clear that the behaviour of an NK cell when confronted by a potential target cell depends on the integration of multiple signals coming from a range of activating and inhibitory receptors. Inhibitory receptor expression is largely under genetic control, whereas activation receptor expression is heavily environmentally influenced and NK cells adapt their expression of activating receptors in response to pathogens and tumours so giving rise to the multiple discrete NK cell subpopulations that can be found in human peripheral blood. Thus, to understand NK cells in disease requires detailed knowledge of the biochemistry of individual activating and inhibitory receptors and the subpopulations of NK cells expressing different receptor repertoires.

We have contributed extensively to knowledge of the cell biology of various NK cell receptors and their ligands and recently, to address the wider roles of NK cells in immunity, we have initiated collaborations with clinical colleagues to study patients suffering from primary immunodeficiencies that affect NK cell function. Inherited human immunodeficiencies are experiments of nature in which gene defects compromise immune function and our hypothesis is that the study of congenital defects affecting NK cells will help to increase our understanding of NK cell biology and function *in vivo*. We use innovative flow cytometry and molecular genetic technologies to characterise these primary immunodeficiency diseases at high resolution. These studies are complemented and enhanced by *in vitro* experiments involving the study of NK cells and the use of genome-editing technologies to study in detail the molecular bases of the changes observed *in vivo*.

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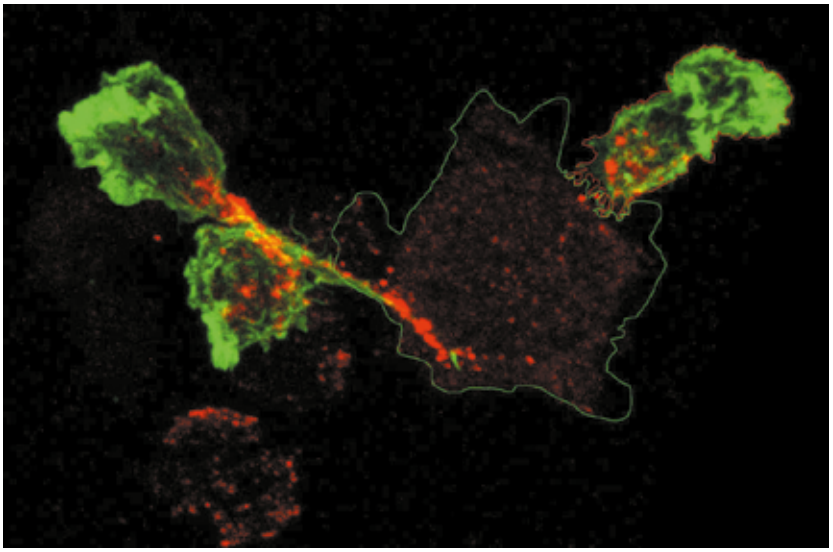

1 *Human natural killer cells attacking a tumour cell induced to express ligands of the activated receptor NKG2D (granzyme stained in red) and polymerised actin in green)*

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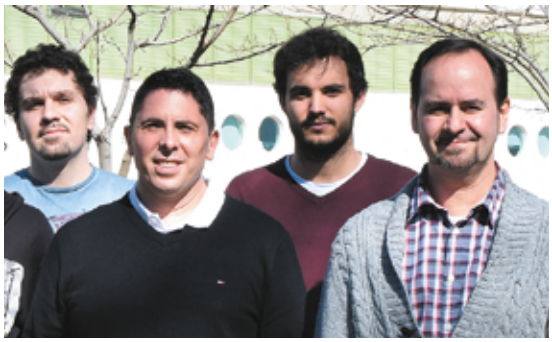

T cell signalling in autoimmune diseases and cancer

The main goal of our group is the characterisation of the molecular mechanisms involved in cell activation, proliferation and apoptosis in the context of inflammation and tumour development. In particular, we are focused on the study of the biological function of the Gadd45 and p38 MAPK family in these processes.

Gadd45 family proteins have an important role in cell cycle control, proliferation, cell survival, and maintenance a genomic stability in response to environmental and physiological stress. In general, there is a large body of evidence that Gadd45 proteins play a key role in tumor suppression. Reduced expression of Gadd45a and Gadd45b has been observed in many tumours and cell lines. Often, this is correlated with promoter methylation in several types of human cancer. In order to identify novel regulators involved in tumorigenesis and/or inflammation, we have developed specific knockout mouse lines of potential autoimmune disease and tumor suppressor genes.

Notably, our recent findings on the role of Gadd45 on carcinogenesis challenge current dogmas on cell regulation and demonstrate a novel role for Gadd45 in tumor promotion. Currently, we are studying the molecular mechanisms that regulate this process. We hypothesise the lack of Gadd45 could affect the molecular mechanisms that control cell death, proliferation, cytokine production or immune cell infiltration in acute and chronic inflammation as well as tumorigenesis. To dissect the molecular pathways involved in carcinogenesis, we are analysing the expression of pro-inflammatory and pro-tumorigenic genes, apoptotic proteins and MAPKs activation by different techniques. The characterisation of novel regulators involved in inflammation-mediated carcinogenesis will help identify new molecular targets for tumour treatment.

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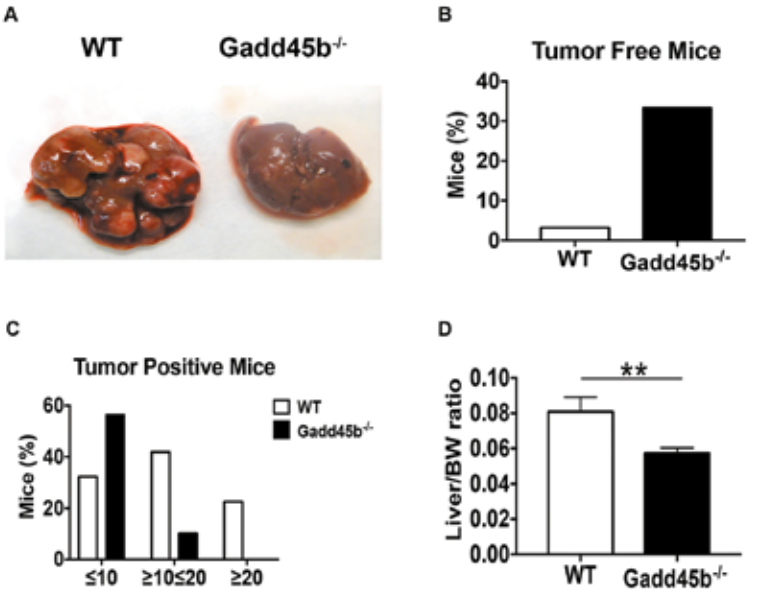

1 *Liver tumour formation in WT and Gadd45b$^{-/-}$ mice. (A) Representative picture of induced liver tumors in wild-type and Gadd45b$^{-/-}$ mice. (B) The percentage of tumour-free mice at 9 months. Statistically significant differences between WT and Gadd45b$^{-/-}$ mice are indicated (P<0,01). (C) The percentage of tumour-positive mice after nine months. (D) The liver/body weight ratio was calculated and expressed as the mean percentage ± S.E.M.*

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Tumour immune activation and evasion

The group is interested in the immune response against cancer, in particular mediated by Natural Killer (NK) cells. These cells respond against tumours after integration of activating and inhibitory signals coming from a large number of receptors. One of the main cytotoxic receptors, NKG2D, recognises ligands that can also be released as soluble molecules either truncated by metalloproteases or in extracellular vesicles (EVs), resulting in immune evasion. In the last years, we have developed several methodologies to examine NKG2D-ligands and other tumour markers carried in EVs. In addition, we have used models of bladder cancer and melanoma to understand successful treatments for cancer that involve activation of the immune system. Since the treatment of bladder cancer patients with intra-vesical instillations of BCG (Bacille Calmette-Guérin) has been used successfully for decades, *in vitro* models that include PBMCs and mycobacteria are used. In parallel, *ex vivo* samples from patients treated with BCG have revealed that urine, collected one week after instillations, provides information on long lasting immune responses that continuously release soluble factors. We have described the presence of CXCL10, a chemokine that could be used to follow the effect of the treatment in patients.

During the COVID-19 pandemic, we have developed a serology test including several SARS-CoV-2 antigens and have described that the main protease of the virus (3CLpro, Mpro) is antigenic in COVID-19 patients. A patent has been filed and the know-how licensed in a non-exclusive manner. The kit commercialised by Immunostep, S.L. was approved for diagnostics by the Spanish regulatory agency (AEMPS).

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Campos-Silva C, Suárez H, Jara-Acevedo R, Linares-Espinós E, Martinez-Piñeiro L, *et al.* High sensitivity detection of extracellular vesicles immune-captured from urine by conventional flow cytometry. Sci Rep. 2019; 9 (1): 2042.

Martínez-Fleta P, Alfranca A, González-Álvaro I, Casasnovas JM, Fernández-Soto D, *et al.* SARS-Cov-2 cysteine-like protease (Mpro) antibodies can be detected in serum and saliva of COVID-19-seropositive individuals. J Immunol 2020; 205 (11) 3130-3140.

Portilla AP, Moraru M, Moreno AB, Kolb P, García-Morato MB, *et al.* Identification of the first cases of complete CD16A deficiency: association with persistent EBV infection. J Allergy Clin Immunol 2020; 145 (4): 1288-1292.

❶ *Detection of immune soluble factors in urine from bladder cancer patients treated with BCG. Treatment of non-muscle invasive bladder cancer consists on weekly instillations with Bacillus Calmette-Guérin (BCG), the tuberculosis vaccine. After instillations, patients activate the immune response with recruitment of cells and soluble factors, such as cytokines and chemokines, to the bladder. The identity of these chemokines can be detected in urine even 7 days after the contact with the mycobacteria. Data in Ashiru et al. Cancers, 2019.*

❷ *Detection of SARS-CoV-2 Mpro-specific antibodies by ELISA. Plates were coated with SARS-CoV-2 Mpro and sera dilutions (1/50 to 1/1600, as indicated) were tested. Black symbols correspond to COVID-19 patients; grey symbols correspond to sera collected pre-COVID.*

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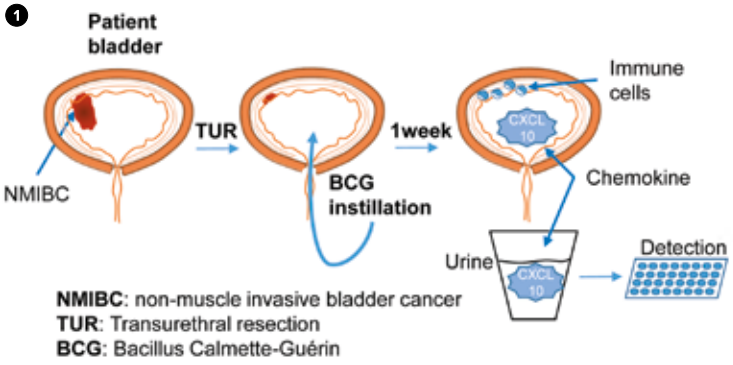

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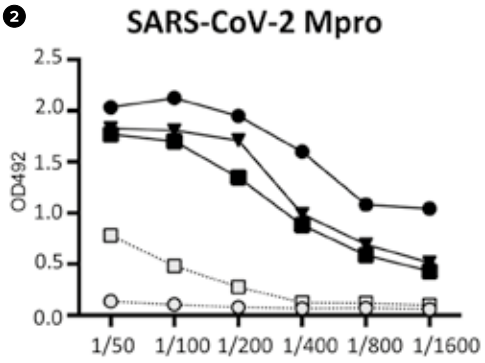


Tetracycline biosynthesis
Amino Acids

6

SYSTEMS BIOLOGY

Systems Biology is a conceptual framework for studying living systems that departs from the reductionism of molecular biology; it pursues the quantitative understanding of complete biological entities rather than the mere comprehension of their parts. One of the key goals of Systems Biology is to reveal the properties embodied in the inner organisation of complete biological objects.

The CNB SysBio Department figures in the contemporary landscape by developing active research lines in environmental genomics, network biology, systemic computation and metabolic engineering. This framework (which many consider a veritable paradigm shift) seeks to address the complexity of living systems as such, not to divide them into smaller parts (at difference from the reductionism of molecular biology). Systems biology offers remarkable scientific and technological potential for the field of biomedicine and for industrial, agricultural and environmental biotechnology.

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SELECTED PUBLICATIONS

Castro M, Ares S, Cuesta JA, Manrubia S. The turning point and end of an expanding epidemic cannot be precisely forecast. Proc Natl Acad Sci USA 2020; 117: 26190-26196.

Clocks and rulers in life

We are interested in spatiotemporal phenomena in living systems: oscillations, pattern formation and dynamics of gene expression, using theoretical and computational methods derived from physics and mathematics. In the past year, following the epidemic emergency, our research has focused on epidemic dynamics, where we have presented a new model considering the effect of confinements, calculated the threshold over which lockdown measures inhibit infection spread, and shown that the predictive power of mathematical models of epidemic dynamics is limited by the exponential growth of uncertainties.

We have also been working on pattern formation in Anabaena, a filamentous cyanobacterium that differentiates specialized cells in the absence of fixed nitrogen. Plant research has also been an important topic: we have been working on the effect of light and temperature in plant growth, focusing on the embryonic stem, the hypocotyl, of *Anabaena thaliana*. We have also developed a theory for the regulation of the effect of nitrogen on the tillering of green revolution varieties of rice. Another relevant topic has been bacterial conjugation in Gram-positive bacteria. Conjugation is one of the mechanisms by which bacteria can exchange genetic material, in particular genes necessary to build antibiotic resistance.

Besides pure research, during 2020 we have made a great effort in science popularisation regarding epidemic dynamics, with a great number of TV, radio and newspaper contributions in Spanish and international media, including The Wall Street Journal, the French public radio, Süddeutsche Zeitung or almost all the major Spanish TV channels. On the Twitter account @omeuxeito we discuss and analyse almost on a daily basis epidemic data from the region of Madrid.

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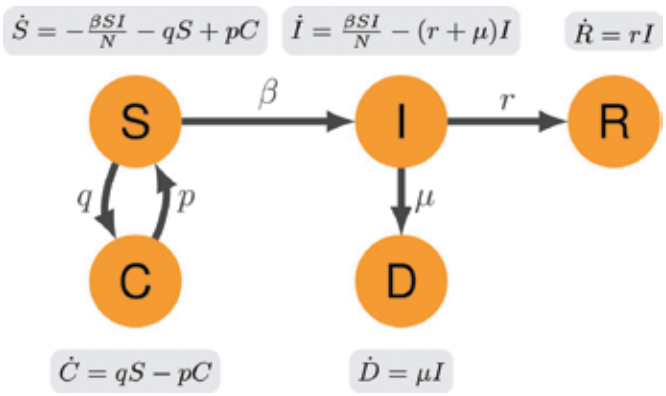

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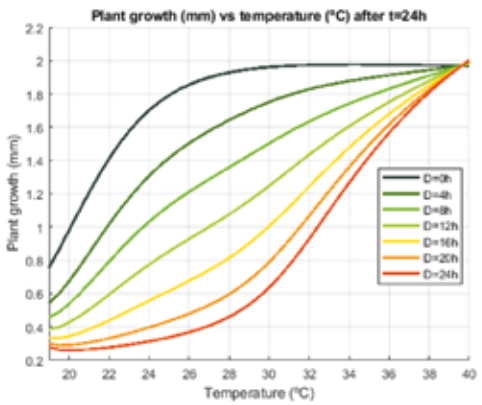

1 *Diagram of the epidemic model along with the equations ruling the dynamics. Susceptible individuals (S) can enter and exit confinement (C) or become infected (I). Infected individuals can recover (R) or die (D). N is the total population. Rates for each process are displayed in the figure; q depends on specific measures restricting mobility and contacts, while p stands for individuals that leave the confinement measures (e.g., people working at essential jobs like food supply, health care, or policing), as well as for defection. We fit I to data on officially diagnosed cases, which are automatically quarantined: The underlying assumption is that the real, mostly undetected, number of infections is proportional to the diagnosed cases. From Castro et al. 2020.*

2 *Model predictions for hypocotyl growth (mm) as a function of temperature (ºC) after 24 hours for different number of light hours in the day, D. From the Master thesis of Gabriel Rodríguez Maroto.*

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Environmental synthetic biology

The longstanding mission our team is the production of biological agents for biosensing, large-scale remediation and valorisation of chemical waste that is otherwise dumped into the Environment by urban and industrial activities. The workhorse to this end is the soil bacterium *Pseudomonas putida*, which combines the ease of genetic programming that is typical of *Escherichia* coli with the safety, robustness and metabolic capabilities required in whole-cell catalysts for applications in harsh biotechnological settings. Specific activities include: [i] Development of *P. putida* as a reliable chassis for implantation of genetic and metabolic circuits. This involves a profound editing of the extant genome of this microorganism for enhancing desirable properties and eliminating drawbacks. Also, the exploitation of surface-display systems for designing complex catalytic properties altogether separated from the cell metabolism and even the design of artificial communities by means of ectopic adhesins. [ii] Genetic tools for deep refactoring of metabolic properties of *P. putida*. The list of new assets that we are developing includes a large collection of standardized plasmid and transposon vectors as well as dedicated reporter systems for parameterization of the gene expression flow and for switching entire metabolic regimes. [iii] The TOL system borne by plasmid pWW0 as a natural example of well-nested metabolic circuit implantation. The two operons for toluene and *m*-xylene biodegradation encoded in pWW0 offer a case of expansion of the metabolic repertoire of environmental bacteria through acquisition of new genes. [iv] Deep metabolic engineering of *P. putida*. Currents efforts attempt to develop strains that can be entirely programmed to deliver catalytic phenotypes of choice upon exposure and computation of both external and internal cues. This endeavour combines direct rational engineering with fine-tuning of gene expression by means of site-specific diversification of genomic sequences of choice through adaptation to *P. putida* of high-efficacy genome engineering technology.

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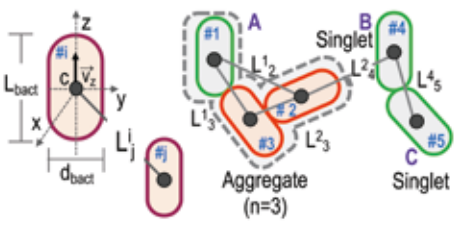

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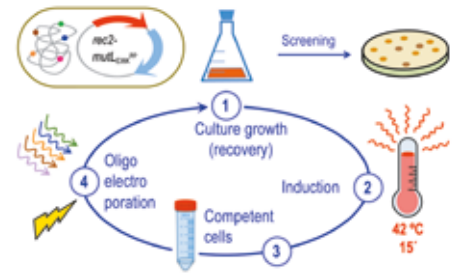

1 *Modelling inter-cell interactions in microbial communities. The figure shows some steps followed by the in house designed computational workflow to identify aggregate clusters within confocal microscopy images. Basically, the starts by obtaining geometrical parameters of individual cells such as the mass center position (c), length (L_{bact}), diameter (d_{bact}) and the axial orientation vector (vz). Then, in step 2 distances between cell pairs are computational arranged into a distance matrix (L^i_j represents the distance between the bacterial centers of #i to #j), where each row contains all distance pair combinations of one bacterium to the rest of cells indicated (L^1_2, L^1_3... L^1_n).*

2 *Scheme of the high-efficacy multiple site genome editing (HEMSE) cycles. The main steps of the procedure are depicted: cultures of P. putida EM42 (pSEVA2314-rec2-mutL_{E36K}^{PP}) are grown and induced by a heat-shock; then competent cells are prepared and transformed with recombineering oligonucleotides. After recovery on fresh media cultures enter in the next round of HEMSE by applying the induction step. Screening of allelic replacements within a given cycle is performed after recovery by plating culture dilutions on the appropriate solid media*

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Evolutionary systems

SELECTED PUBLICATIONS

Catalán P, Elena SF, Cuesta JA, S. Manrubia S. Parsimonious scenario for the emergence of viroid-like replicons de novo. Viruses 2019; 11, 425.

Valdano E, Manrubia S, Gómez S, Arenas A. Endemicity and prevalence of multipartite viruses under heterogeneous between-host transmission. PLoS Comput Biol 2019; 15, e1006876.

Castro M, Ares S, Cuesta JA, Manrubia S. The turning point and end of an expanding epidemic cannot be precisely forecast. Proc Natl Acad Sci USA 2020; 117, 26190-26196.

Catalán P, Manrubia S, Cuesta JA. Populations of genetic circuits are unable to find the fittest phenotype as a result of phenotypic bias in a multilevel genotype-phenotype map, J R Soc Interface 2020; 17, 20190843.

Zanette DH, Manrubia S., Fat tails and black swans: Exact results for multiplicative processes with resets. Chaos 2020, 30, 033104.

The main research topic of the group is the understanding, modelling and analysis of evolutionary mechanisms. For almost two decades, we have investigated the adaptive dynamics of viruses and RNA populations, collaborating closely with experimental groups and addressing broader problems such as the relationship between genotype and phenotype.

In our most recent research, we have been exploring the topological structure that genotype-to-phenotype maps endow in sequence spaces, and its effects in the dynamics of heterogeneous molecular populations. We have uncovered some universal features of sequence spaces topology which are independent of the definition of phenotype and, therefore, of generic consequences for evolution and adaptation. Our results have highlighted, among others, the extent of entropic effects in microscopic evolution, showing that abundant, sufficiently functional phenotypes, might be much more common in nature than highly adapted, but rare ones. A full understanding of microscopic evolution is important to update current evolutionary theories and to derive useful effective models. In this sense, we have questioned the role played by classical metaphors of evolution, such as smooth fitness landscapes, and suggested they must be substituted by network-based representations.

Our research has turned to epidemiology, based on our broad experience with viral evolution and modelling, as a consequence of the crisis caused by COVID-19. Currently, we are exploring the limits of model-based predictions in the face of empirical data, and the effects of the evolution of pathogens and their adaptation to different containment strategies.

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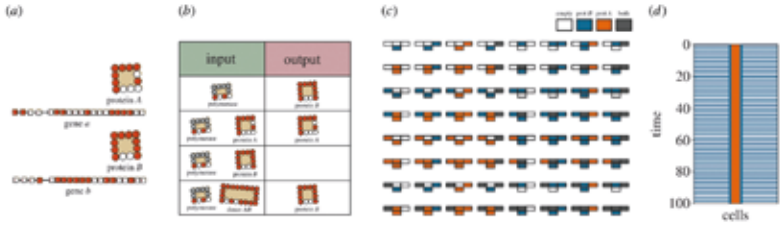
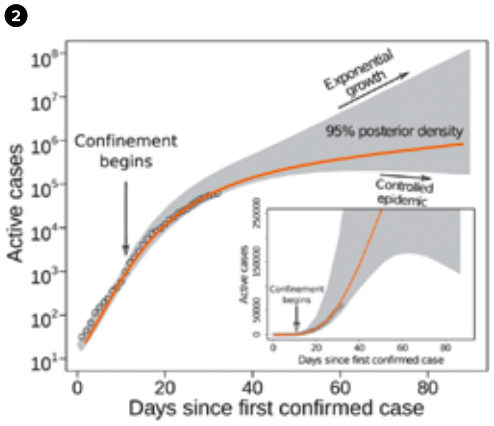


1 *toyLIFE is a multilevel genotype–phenotype map. (a) toyLIFE genotypes are binary strings with promoter and coding regions that, when expressed, yields a lattice folded toy protein. (b) Following toyLIFE's interaction rules, we obtain gene regulatory networks (GRNs) in the form of a truth table. (c) Each GRN determines, under some propagation rules, a unique cellular automaton with cells in state empty (white), expressing protein A (orange), expressing protein B (blue) and expressing both proteins (grey). (d) These cellular automata give rise to spatio-temporal patterns of gene expression (Catalán et al., 2020).*

2



2 *Fit to data obtained in real time for the daily number of COVID-19 active cases in Spain (from March 1st to March 29th). Despite a reasonable agreement between model and empirical observations in the spreading phase of the pandemic, opposite predictions for the future number of active cases can be derived. The solid line represents the number of infected individuals using best-fit parameters. The vertical arrow denotes March 11th, the day when schools and universities closed. The shaded area represents the 95% predictive confident interval: Its increasing width implies that predictability decays exponentially fast.*

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Systems biotechnology

Our foundational aim is the system-level understanding of microbial metabolism as a framework for developing a broad range of novel and non-intuitive biotechnological processes. Taking advantage of metabolic modelling, systems and synthetic biology we are addressing, at different levels, the understanding and full taming of bacterial systems emergence.

Increasing the completeness and scope of metabolic reconstructions

We are involved in the high-quality metabolic modeling of a large set of metabolically diverse bacteria including *P. putida, S. elongatus, A. platensis, Azoarcus* CIB, *S. granuli, P. pseudoalcaligenes* and *B. bacteriovorus*. This effort is enabling the system-level analysis of new metabolic processes while providing new computational test-beds for biotechnological applications. We are particularly interested in the inclusion of new metabolic modules such as the generation of reactive oxygen species and the inclusion of underground metabolisms. We are also developing software for the automatic reconstruction of microbial networks.

System-level analysis of Metabolic Robustness in bacteria

The robustness of a system is the property that allows it to maintain its functions despite perturbations. Through the metabolic modeling analysis of *P. putida*, we have identified metabolic cycles providing robustness. By using synthetic biology, ongoing efforts are focused on the rational engineering of such cycles under diverse biotechnological scenarios.

System-level analysis and designing of microbial communities

The division of labor allows an expanded complexity and functionality in bacteria. We are interested in: i) understanding how these expanded capabilities emerge within a community and ii) how we can engineer this community-level functionality towards biotechnological endeavors. To address these two fundamental questions, we have developed a computational platform called FLYCOP for modeling and engineering synthetic microbial consortia. We are applying this technology in the revalorisation of complex polymers such as lignin and plastic waste as well as in the cost effective production of plant-based secondary metabolites such as flavonoids.

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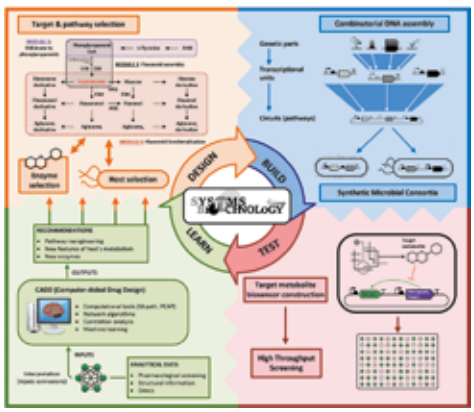

1 *Detail of the Iterative design of DBTL (design–build–test–learn) cycle applied in the lab for addressing complex biotechnological endeavours. Design step includes the selection of target, the in silico design of production pathways, optimal pathway segregation, identification of enzymes and the selection of microbial hosts. Build stage is based on combinatorial DNA assembly methods to construct metabolic pathways that will be finally expressed in different components of a synthetic microbial consortium. Test stage includes production of the target compounds and the development high-throughput screening technologies. Learn stage processes the analytical data from the above steps and finds connections between genotype and phenotype and optimized metabolic fluxes to give recommendations to perform subsequent DBTL cycles.*

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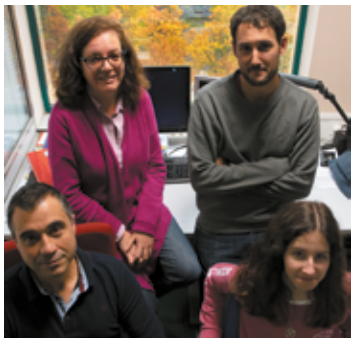

Computational systems biology

Our group is interested in different aspects of Bioinformatics, Computational Biology and Systems Biology. Our goal is to obtain new biological knowledge with an "in-silico" approach which complements the "in-vivo" and "in-vitro" methodologies of Biology. This mainly involves mining the massive amounts of information stored in biological databases. Within this general goal, we work on different research lines that can be framed in three major areas: prediction of protein functional sites, perdition of protein interaction partners, and functional study of biological networks (with an emphasis on networks related to human diseases). Besides our lines of scientific research, we also collaborate with experimental groups providing them with bioinformatics support for their specific needs, and participate in different teaching projects.

In the past two years we were actively working in deciphering the molecular basis of rare diseases combining data on genomic variations with biological networks. We also perform studies on the ecosystem of web servers supporting molecular biology research, with a focus on those dealing with CRISPR/Cas experiments. Additionally, we continued with our previous work on the prediction of protein binding sites and the prediction of the environmental fate of chemical compounds, finishing a couple of projects along these lines.

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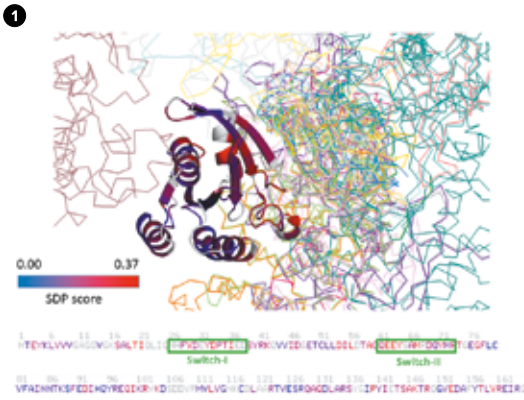

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❶ *Predictions of RasH regions involved in controlling effector interaction specificity (Pitarch et al, 2020), mapped on the interaction structural information available for that protein. RasH is shown in ribbon representation, and its 26 crystallized interactors in thin backbone. The method's score for the RasH residues is shown in a color scale, with red representing the highest scores. The same color schema is used in the primary sequence of RasH (below) where the "switch-I" and "switch-II" regions are highlighted. Figure generated with PyMol (www.pymol.org).*

❷ *Screenshots of WeReview web interface (Torres-Perez et al, 2019), a live repository of computational tools for assisting CRISPR/Cas experiments. The main table, together with some of the dialogs for introducing filters in the search for tools are shown.*

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SELECTED PUBLICATIONS

Sánchez-Gorostiaga A, Bajić D, Osborne ML, Poyatos JF, Sanchez A. High-order Interactions distort the functional landscape of microbial consortia. PLoS Biol 2019; 17 (12): e3000550.

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Logic of genomic systems

Research at the Logic of Genomic Systems Laboratory searches for design principles in biological systems. During the last years, we examined the global transcriptional program controlling genome-wide gene expression, the link between composition and function in microbial consortia, and the factors that determine the impact of mutations in cellular fitness.

The limited availability of the components that influence gene expression, such as the presence of free RNA polymerases, cofactors, ribosomes, etc., and their differential use at the genomic scale determines *the global transcription control program*. We examined this program by 1) developing a methodology to experimentally characterise on a large scale the response to this program in bacteria, which showed that this response contributes to the bacterial genomic organisation, 2) examining how this program integrates with specific –genetic and epigenetic– regulatory strategies in eukaryotes.

To understand how the interaction between members of a microbial community determines its function, we assembled an artificial consortium of soil bacteria in which function represents starch degradation. Combining theory with experiments, we quantified how the contribution of interactions of different order and type shape the action of the community. Functional robustness to pairwise and higher-order interactions critically affects our ability to predict and engineer function.

Understanding the impact of mutations is the focus of Genetics, but many questions remain uncertain. We lately examined two: 1) to what extent part of the fitness cost of a mutation comes from improper rewiring of the transcriptome, and 2) how much any enzyme can act as a modifier of the impact of mutations on other enzymes. In 1), we demonstrated that part of the deleterious effects of mutations is indeed caused by such abnormal rewiring. In 2), we showed that any enzyme can buffer or potentiate the impact of mutations, an effect that has implications in particular cancer therapies.

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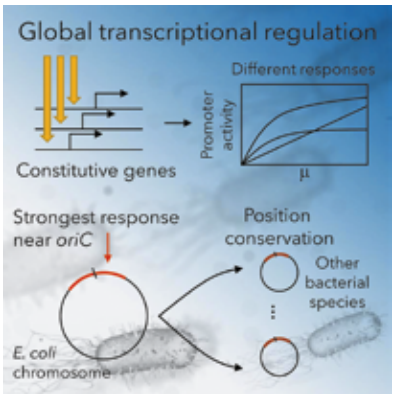

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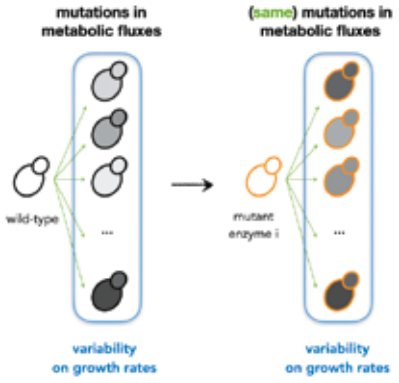

❶ *Bacterial gene expression depends on the allocation of limited transcriptional resources provided a particular growth rate and growth condition. Early studies in a few genes suggested this global regulation to generate a unifying hyperbolic expression pattern. We showed that promoters whose transcriptional response is more dependent on growth rate are preferentially located closer to the origin of replication in the chromosome in* E. coli*, and that the relative location of these genes in other species correlates significantly with their respective growth dynamics, directly related to their habitat.*

❷ *Using a genome-scale metabolic model, we scored the variability of the growth rates in a group of different lines (arrows) in which mutations in the metabolic fluxes are accumulated. We computed this variability in the presence (wild-type) and the absence (mutant metabolism) of a particular enzyme i. Here the difference between growth rates and metabolic backgrounds is represented by the colors of the fill and the border of the yeast cartoons, respectively. We then quantify if these very same lines manifest a different variability depending on the absence of the single enzyme. This led us to identify a set of genes acting as buffers and potentiators whose influence depends on the particular working conditions of the metabolism (i.e., type of available nutrients), and the sources of variability considered.*

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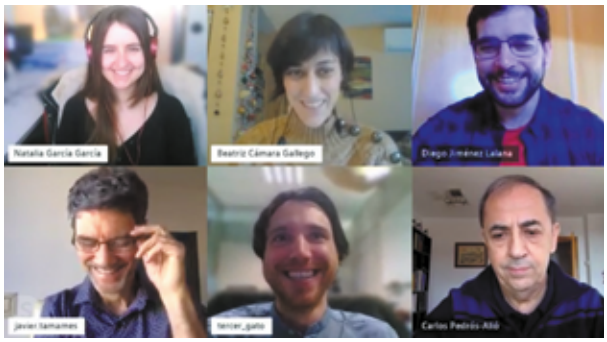

Microbiome analysis

Microbial communities (microbiomes) are key players in many scenarios, from how the biosphere works to industrial and biotechnological processes, as well as human health and wellness. We study microbiomes of diverse environments trying to learn the rules that govern the assemblage of these microbial communities. This knowledge will help to understand how they function, and to predict the effects of disturbances. Eventually, this will lead to rational design and manipulation of microbiomes.

We focus mostly on marine microbial communities, but we are actively working in many other microbiomes from different environments. We study extreme environments because their microbiotas show fascinating adaptations to the harsh conditions. We work with human-associated microbiomes, such as the gut and the vagina, because of their potential to improve our health. We are also interested in other habitats, such as wastewaters and soils.

We use mostly bioinformatics tools to study the composition and functionality of microbiomes. Metagenomics is the basis of our work, since it provides the basic material: DNA sequences from environmental samples. The analysis of these sequences informs about the presence of diverse organisms and the content of their genomes, and the latter can be linked to functionality. We also carry out experimental work addressing interactions between members of microbiomes.

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❶ *Solar salterns in the Salar de Atacama (Chile). Lithium carbonate precipitation ponds where we are looking for the limits of life at low water activity.*

❷ *Time series from winter equinox to summer solstice in Cambridge Bay (Canadian Arctic). Copy number of siderophore synthesis genes (left panel) and siderophore transporter genes (right panel) in metagenomes. Pie charts show taxonomic assignments. Gamma proteobacteria (purple) are the main producers of siderophores, while Bacteroidetes (orange) act as cheaters by increasing the number of outer membrane transporters. Webpage: http://microbiomecnb.com/*

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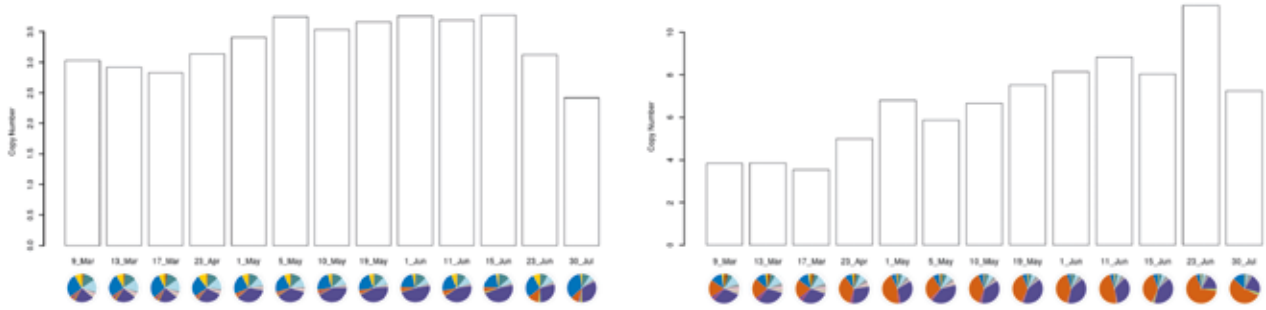
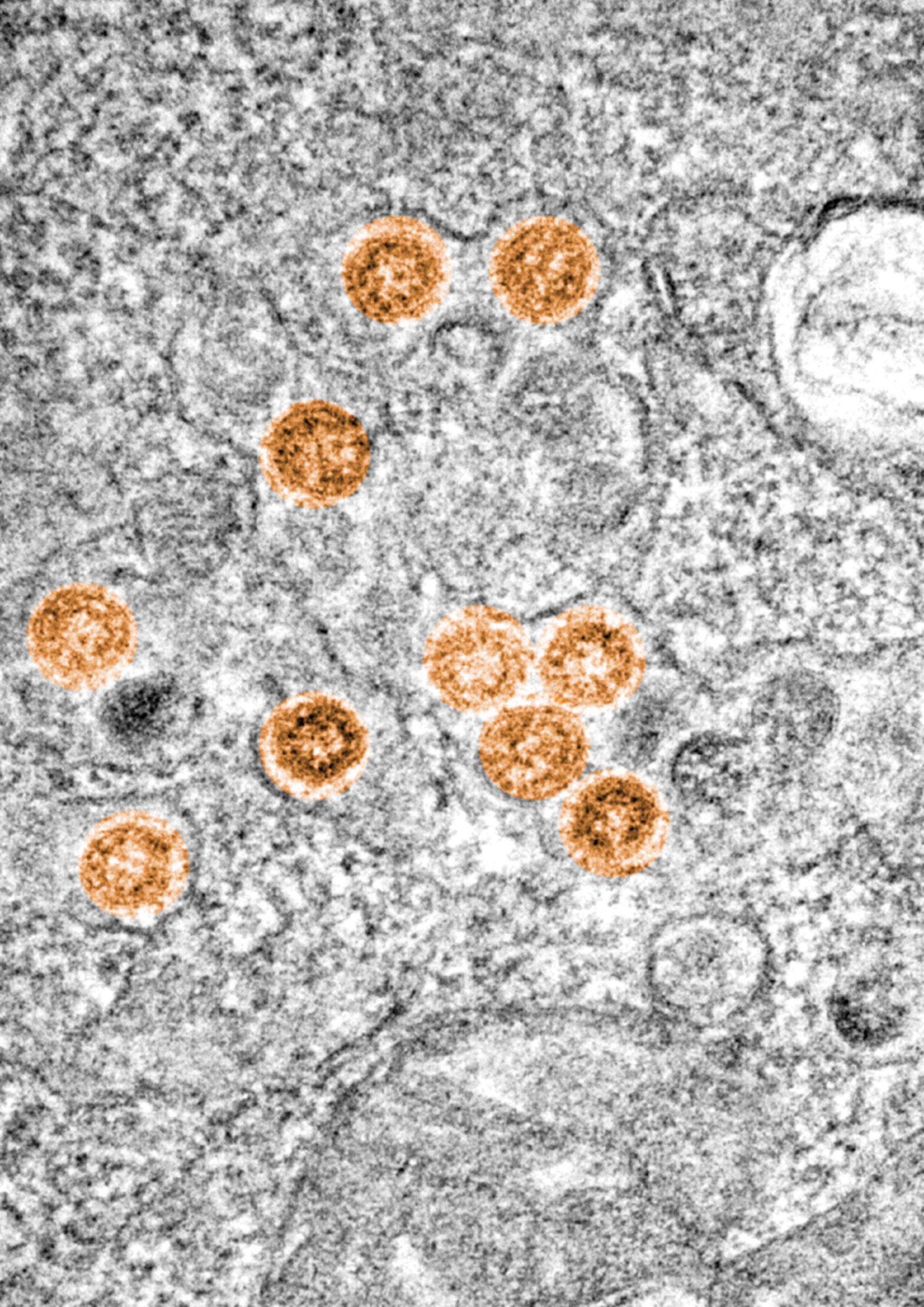




7

THE SARS-CoV-2 PANDEMIC CHALLENGES

The current COVID-19 pandemic represents one of the greatest challenges to humanity. Science and scientists all over the world have joined forces to provide responses to the society. Since March 2020, researchers at the CNB, a multidisciplinary research centre with a long-standing expertise in molecular and structural virology and immunology, have developed collaborative and interdisciplinary studies that exploit synergies between research groups and scientific services.

Our lines of action comprise more than fifteen projects led by researchers from the centre*. Many of these projects are included in the CSIC Global Health Platform**, that counts with more than 200 research groups addressing the scientific challenges posed by COVID-19 pandemic to provide short, medium and long term solutions.

Our contributions against SARS-CoV-2 include the development of vaccines and therapeutical approaches to tackle SARS-CoV-2 infection, structural and computational studies to identify potential therapeutic targets, the development of diagnostic kits to determine the presence of viral antigens or antibodies in biological samples and the development of computational models to evaluate the effect of the populations' behaviour in the spread of epidemics.

The excellent work of the CNB and the CSIC during the pandemic has been recognised by the Consejo General de la Abogacía Española through its Fundación Abogacía. Both the CSIC and the CNB have been awarded the XXII Human Rights Prize in the Institution category. These awards have been dedicated this year to the defense of universal access to health.

** CNB website: http://www.cnb.csic.es/index.php/en/research/sars-cov2-research*

***CSIC Global Health Platform Website: https://pti-saludglobal-covid19.corp.csic.es/*

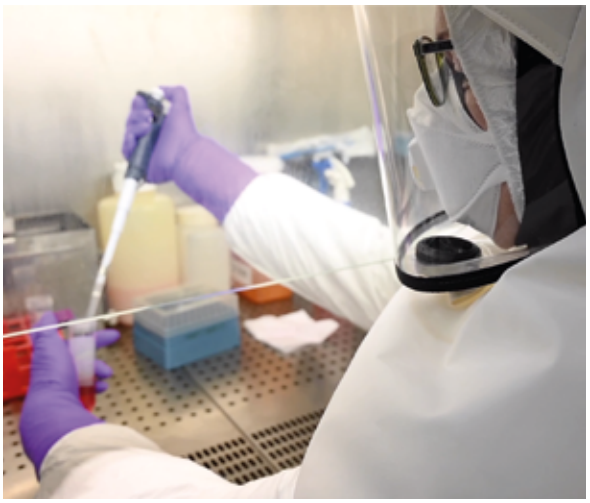
Section of a cell infected with SARS-CoV-2, showing newly formed viral particles (orange) between a double membrane vesicle where the virus genome is replicated and mitochondria (image from Gabriela Condezo, Carmen San Martín and Marta López de Diego).

Development of a SARS-CoV-2 vaccine based in non-infective replicons

PRINCIPAL INVESTIGATORS
Luis Enjuanes, Isabel Sola
Sonia Zúñiga (senior researcher)

The aim of this project has been to generate the SARS-CoV-2 virus by assembling synthetic DNA fragments. Using the full cDNA copy of the genome, and the reverse genetics system based on bacterial artificial chromosomes (BACs), genes responsible for virulence and propagation have been deleted to obtain propagation-deficient, highly immunogenic RNA replicons that can be used as specific SARS-CoV-2 vaccine candidates. In parallel, animal models (transgenic mice) have been developed for the validation of vaccines and other therapeutic agents to protect against COVID-19.

A patent to protect the development of vaccines based in self-replicative propagation-deficient RNAs that induce sterilising immunity has been presented in May 2020



Development of vaccine(s) against SARS-CoV-2/ COVID-19 based on non-replicating viral vector (MVA)

PRINCIPAL INVESTIGATORS
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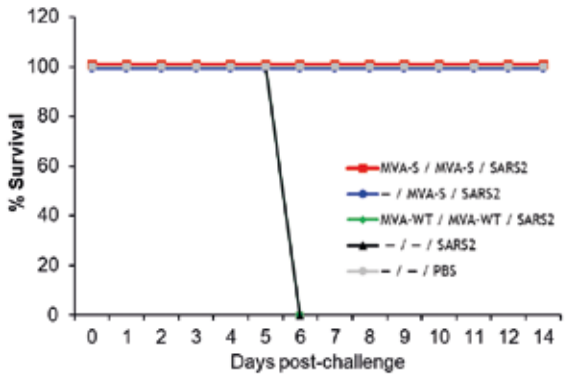
EXTERNAL COLLABORATORS
David Sancho (CNIO), Susana Guerra (UAM)

An aim of the Poxvirus and Vaccines Group is to develop effective vaccines against the prevalent SARS-CoV-2 strain and its variants that might be applicable in humans. This is done using as platform the highly attenuated poxvirus strain MVA expressing different viral antigens of SARS-CoV-2, such as those corresponding to full-length proteins, virus-like particles (VLPs) and conserved multiepitope components.

We have developed a vaccine candidate MVA-CoV2-S expressing the complete S (Spike) protein that in mice triggers the induction of potent S-specific T-cell responses and high titers of neutralising antibodies. Remarkably, susceptible mice immunised with one or two doses of MVA-CoV2-S were 100% protected from SARS-CoV-2 lethality. Moreover, two doses of the vaccine prevented virus replication in lungs. Similar efficacy studies are ongoing with hamsters and macaques. The vaccine MVA-CoV2-S has been produced by a company and phase I/II clinical trials are planned along 2021.



The vaccine candidate MVA-CoV2-S administered in one or two doses in humanised mice protects 100% against lethality induced by SARS-CoV-2.

APPROACHES TO TACKLE SARS-COV-2 INFECTION

The CNB antiviral screening platform

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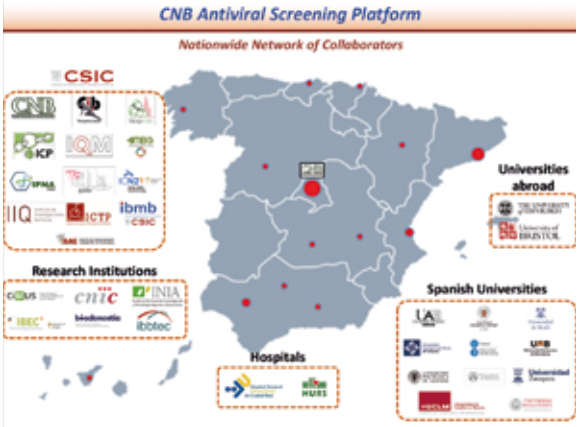
EXTERNAL COLLABORATORS

18 Research Centres (12 from CSIC); 10 Spanish and 2 British Universities; 2 Spanish Hospitals

The objective of the CNB Antiviral Screening Platform is to provide a permanent structure dedicated to the identification and characterisation of antiviral compounds against human pathogenic viruses of biomedical relevance. The working model is based on the use of cell culture systems of viral infections including: SARS-CoV-2, mosquito-borne viruses (dengue, West Nile and Zika flaviviruses), hepatitis B and C viruses as well as influenza A virus. To do so, we have established a nationwide network of collaborators which provide collections of pure compounds and complex extracts to be tested as potential antivirals. So far, we have tested over 2400 repurposing drugs and around 3500 experimental compounds in the SARS-CoV-2 infection cell culture system. A handful of clinically approved drugs have shown antiviral activity in the absence of toxicity in the cell culture systems and they are being considered as potential candidates for clinical testing by the Global Health PTI platform at CSIC. Moreover, we have identified new families of experimental compounds and natural extracts with antiviral activity that have been protected by patent applications. These families of antivirals are being prioritised for further characterisation of their mode of action and preclinical animal studies. Lastly, we are giving support to other SARS-CoV-2-related projects at the CNB by providing our expertise and experimental systems to study the neutralisation capacity of therapeutic recombinant antibodies and sera from mice immunised with vaccine candidates. Finally, five patent applications have been filed in 2020.



Map of Collaborator Institutions.

Development of synthetic biology devises toward the screening of potential inhibitors of the SARS-CoV-2 3CL protease

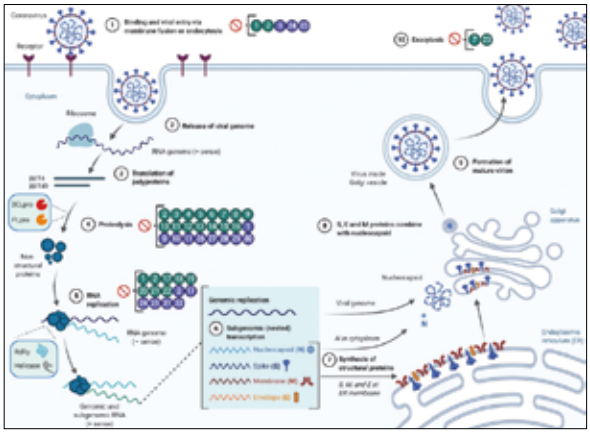
PRINCIPAL INVESTIGATOR

Juan Nogales Enrique

EXTERNAL COLLABORATORS

Felipe Lombó (Universidad de Oviedo); Tobias Goris (German Institute of Human Nutrition Potsdam-Rehbruecke (DIfE); Uwe T. Bornscheuer (Greifswald University)

Different antivirals and natural compounds have been tested against coronaviruses, such as remdesivir, ribavirin or herbacetin. Of the natural anti-coronaviral compounds, flavonoids in particular have shown interesting inhibitory bioactivities. In our group we have evaluated the potential of flavonoids as antivirals by thoroughly reviewing experimental and computational evidences. We identified flavons and flavonols as more active flavonoids and the main viral protease, 3CL, as the more promising flavonoid target. Furthermore, we proposed a way forward for updating a classical industrial biotechnology Design, Built, Test and Lear (DBTL) cycle towards the rational design, construction and screening of combinatorial libraries of flavonoids with antiviral properties. Current efforts are focus on the implementation of synthetic biology devises toward the identification of potential inhibitors of the 3CL based on new-to-nature flavonoids.



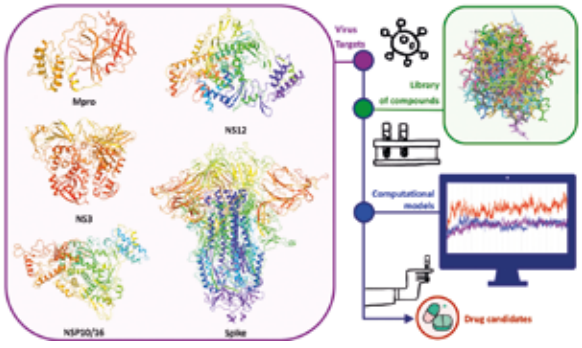
Overview of the coronavirus life cycle, indicating the attachment to the host cell membrane receptor, the translation of the viral (+)ssRNA genome in both polyproteins, the proteolysis carried out by 3CLpro and PLpro proteases, the viral genome replication steps and the virion maturation along endoplasmic reticulum and Golgi apparatus, with final exocytosis across the cell membrane. Numbers encircled in green represent flavonoids tested in vivo against SARS-CoV and/or MERS-CoV. Numbers encircled in purple represent flavonoids identified in silico as promising drugs against SARS-CoV-2 (Goris et al, 2020).

New drugs to combat Covid-19: from computational models to pre-clinical studies

PRINCIPAL INVESTIGATOR
Cristina Risco

EXTERNAL COLLABORATORS
José Pedro Cerón (Universidad Católica de Murcia, UCAM), Nuria Izquierdo-Useros (IrsiCaixa, Barcelona)

The project is a pre-clinical search for anti-SARS-CoV-2 drugs. Our list of potential antivirals includes inhibitors of SARS-CoV-2 MPro, Spike, MTase and RNApol identified by Dr. José Pedro Cerón at the UCAM, using state-of-the-art computational tools and databases of clinically approved drugs (Figure 1). Molecular modelling studies of the interaction of compounds with high-resolution crystal structures of SARS-CoV-2 proteins have produced a list with the best candidates. In addition, our library also includes inhibitors of cell factors used by other RNA viruses such as mitochondrial proteins, lipid transfer proteins, proteasome and protein kinases. These compounds are tested at the Cell Structure Lab (CSL) of the CNB using the human coronavirus 229E, a common cold virus that can be handled in BSL2 labs. The efficacy of compounds is studied by immunofluorescence and confocal microscopy of cell cultures infected in the presence and absence of the drugs. The most promising compounds of the library (10 out of 116 so far) are tested against SARS-CoV-2 in the BSL3 lab by Dr. Nuria Izquierdo-Useros (Irsi-Caixa), before studies with animal models and clinical trials. For those compounds with antiviral activity against SARS-CoV-2, electron microscopy studies are done at the CSL-CNB to obtain details about their mechanism of action.



Workflow for in-silico*, structure-based screening of our chemical library to identify inhibitors of SARS-CoV-2 proteins (José Pedro Cerón, UCAM).*

Identification of antivirals in plant extracts

PRINCIPAL INVESTIGATOR
Roberto Solano

CNB COLLABORATORS
Pablo Gastaminza, Urtzi Garaigorta

EXTERNAL COLLABORATORS
Alejandro Cifuentes (CIAL-CSIC)

SARS-CoV-2 pandemic is having devastating consequences, and has evidenced both a lack of effective treatments and the absence of a global plan to face future pandemics. Search for antivirals has had limited success so far. Therefore, there is an urgent need of new potent and safe antivirals against SARS-CoV-2 and other new viruses, expected to emerge in coming decades. Plants have an extremely rich specialised metabolism that provides them with a broad repertoire of chemicals of pharmaceutical interest. Different plant species use the same metabolic pathways with enzymatic variants to produce a unique blend of metabolites. Therefore, the identification of new plant sources of enzymatic variants and metabolites is key to discover new drugs. In our lab, we are generating suitable model plant systems that allow genetic manipulation and metabolic engineering to produce bioactive metabolites for their pharmacological exploitation.

Identification of antivirals inhibiting essential virus-host interaction during SARS-CoV-infection

PRINCIPAL INVESTIGATORS
Luis Enjuanes, José Manuel Honrubia, José Ramón Valverde

In order to select antivirals that inhibit cell-signalling pathways involved in CoV replication and pathology, our laboratory has previously identified the interaction of a viral motif (PBM) with a cellular protein (PDZ). The inhibition of this interaction prevents virus virulence. Structural studies led to the understanding of the residues involved in this binding, and are facilitating the inhibition of PBM-PDZ interaction, helping the selection of potent antivirals.

APPROACHES TO TACKLE SARS-COV-2 INFECTION

Monoclonal Antibodies against 2019-New Coronavirus (European Project MANCO)

PRINCIPAL INVESTIGATORS
Luis Enjuanes, Isabel Sola
Sonia Zúñiga (Senior researcher)

This project, in collaboration with research groups from Germany and The Netherlands, aims to obtain IgG neutralising antibodies specific for SARS-CoV-2 that elicited full protection, both in experimental models (mice and hamster) and humans. The project includes Phase I and II clinical trials to evaluate protection in persons. The aim is to administer a combination of two neutralising antibodies selected from the pool of more than 70 monoclonal antibodies obtained.

Development of therapeutic antibodies against SARS-CoV-2

PRINCIPAL INVESTIGATORS
Luis Ángel Fernández, José M. Casasnovas

CNB COLLABORATORS
Víctor de Lorenzo, Pablo Gastamiza, Urtzi Garaigorta, Isabel Sola, Luis Enjuanes

EXTERNAL COLLABORATORS
Juan Alberto Corbera Sánchez (Universidad de Las Palmas de Gran Canaria, Spain)

This project aims for the generation of therapeutic antibodies able to block the entry of SARS-CoV-2 into human cells with the final goal of being administered to symptomatic COVID-19 patients, to reduce the risk of progression to severe forms of the disease. To this end, we have focused on the generation of camel-derived nanobodies (Nbs) binding to the receptor binding domain (RBD) of the SARS-CoV-2 envelope Spike (S) protein. Candidates will be expressed and proved in a humanised transgenic mice SARS-CoV-2 infection model to identify neutralising clones with therapeutic potential. In parallel, structural studies will be conducted to define the interaction of Nbs to RBD and S proteins at the molecular level. In addition we are developing antibody engineering technologies to improve Nbs that respond to new and challenging virus variants. Lastly, the Nbs will be also tested in diagnostic applications in collaboration with other Spanish Research Institutions: Institut Català de Nanociència i Nanotecnologia (ICN2), Institute for Advanced Chemistry of Catalonia (IQAC-CSIC), Centro de Astrobiología (CAB, CSIC-INTA), and Centro de Investigación Biomédica (CINBIO, Universidad de Vigo).

Preclinical validation of therapeutical agents for SARS-CoV-2 treatment based in monoclonal antibodies

PRINCIPAL INVESTIGATORS
Luis Enjuanes, Isabel Sola, Leonor Kremer

This research group has obtained neutralising monoclonal antibodies against SARS-CoV-2. Their protection efficiency against SARS-CoV-2 infections is being tested in humanised transgenic mice models.

Control of SARS-CoV-2 infection through the modulation of the energy metabolism of the cell

PRINCIPAL INVESTIGATORS
Fernando Almazán Toral, Francisco José Iborra Rodríguez (IBV-CSIC)

The development of effective therapies against COVID19 disease necessarily involves the knowledge of the fundamental mechanisms of the pathogenesis of SARS-CoV-2. A recurrent mechanism of viral pathogenesis is the metabolic reprogramming. Viruses alter cellular energy metabolism for their own benefit, making it especially attractive to identify these alterations to intervene pharmacologically and prevent or cancel the progression of the viral infection. Each virus uses unique metabolic strategies, so it is sometimes difficult to obtain general treatments, even for viruses of the same family. In this project we are studying the metabolic alterations induced by SARS-CoV-2 infection in cell cultures in order to identify the metabolic pathway affected and explore how the use of drugs targeting the routes identified interfere with SARS-CoV-2 infection. To date, we have identified several proteins of the cellular metabolism that are downregulated during SARS-CoV-2 infection in several cell lines and we are analysing the effect of different pharmacological treatments against the identified proteins on the course of SARS-CoV-2 infection.

Immunosuppressive nanoparticles with lung tropism to stop the cytokine storm and viral replication

PRINCIPAL INVESTIGATOR
Domingo F. Barber

CNB COLLABORATOR
Marta López de Diego

EXTERNAL COLLABORATOR
María del Puerto Morales (ICMM-CSIC)

It has been recently described that iron oxide nanoparticles (IONPs) are capable of inhibiting the replication of the influenza virus. In this project we aim to understand how IONPs, which are already used in clinic for magnetic resonances or for the treatment of anemia, interfere with the replication and infective capacity of different viruses such as influenza and SARS-CoV-2. We also intend to design immunosuppressive nanoparticles that can be used to reduce lung inflammation caused by the cytokine storm generated in the most severe cases of respiratory viral infections, such as those caused by SARS-CoV-2 and the influenza virus.

Immune evasion and immunopathology caused by COVID-19

PRINCIPAL INVESTIGATORS
Isabel Mérida, Margarita del Val (CBMSO)

CNB COLLABORATORS
José María Casasnovas, J. Francisco Rodríguez Aguirre

EXTERNAL COLLABORATORS
Teresa Santos Mendoza (Instituto Nacional de Enfermedades Respiratorias, Mexico)

The SARS-CoV-2 is a betacoronavirus of animal origin closely related to other zoonotic coronaviruses like SARS-CoV and the Middle East Respiratory coronavirus (MERS-CoV). COVID-19 disease comprises two phases: an early period after infection where an adequate and rapid immune response limits virus replication and a second phase where viral-induced inflammatory responses results in immunosuppression and acute respiratory distress syndrome (ARDS). The severe immunopathological features associated to COVID-19 include acute cytokine release syndrome (CRS), characterised by elevated serum levels of inflammatory cytokines. Our team, with a long experience in the study of the mechanism that trigger immune evasion in cancer aims through different approaches to identify the mechanisms by which SARS-CoV-2 triggers immune evasion and inflammatory responses.

Targeting coronavirus RNA genome with CRISPR-Cas13d

PRINCIPAL INVESTIGATORS
Dolores Rodríguez, Lluís Montoliu, Miguel Ángel Moreno Mateos (CABD-UPO/CSIC, Sevilla)

CNB COLLABORATORS
Almudena Fernández (CIBER-ISCIII, Madrid), Fernando Almazán

EXTERNAL COLLABORATORS
Manuel Collado (SERGAS, Santiago de Compostela), Pablo Alfonso del Pino (Universidad de Santiago de Compostela)

In this scientific proposal we will use a new variant of the CRISPR gene-editing tools, Cas13d, with an RNA-guided RNAse specific activity, to target and destroy the RNA genome of the SARS-CoV-2 inside infected cells. This is a direct treatment aiming to inactivate the SARS-CoV-2 genetic material with one of the newest programmable endonucleases. This proposal will proceed stepwise, securing every technological advance, before moving onto the next phase. We want this strategy to be effective but, above all and most importantly, safe. We will first assess the potential toxicity and efficacy of CRISPR Cas13d reagents in zebrafish embryos as an in vivo model. Thereafter, the proof-of-concept of this project will be validated in two related cellular and viral experimental systems. Eventually, upon confirming all previous steps, this strategy will be tested under appropriate BSL3 conditions directly on human epithelial cells infected with SARS-CoV-2 and, next, using adequate mouse models susceptible to this coronavirus. This consortium encompasses proved expertise in CRISPR technology, in Cas13d, in animal models, in cell biology and virology, and in nanobiotechnology.

APPROACHES TO TACKLE SARS-COV-2 INFECTION

SARS-CoV2-host proteomic interactions

PRINCIPAL INVESTIGATORS
Fernando J Corrales, Alberto Paradela

CNB COLLABORATORS
Pablo Gastaminza, Urtzi Garaigorta, Francisco Rodríguez, César Santiago, Hugh Reyburn, Leonor Kremer, Luis Ángel Fernández

EXTERNAL COLLABORATORS
Spanish ProteoRed

Our project aims to consolidate a mass spectrometry-based platform to characterise a) recombinant proteins produced for COVID-19 research and applications, b) serum proteome of COVID-19 patients to define methods for stratification, prognosis and follow-up, c) the immunological response of COVID-19 patients (immunopeptidomics and immunoproteomics) and d) the SARS-CoV-2 host cell interaction at the proteome and phosphoproteome levels.

We have analysed 72 SARS-CoV-2 recombinant protein preparations by MALDI TOF and ESI-MS/MS. Products were identified, the Mr accurately measured and further optimization of purification strategies were implemented to reduce contaminants.

We have identified serum proteins that recapitulate the response of spinal cord injury patients to SARS-CoV-2 infection and suggest treatment strategies to prevent severe symptoms. Additionally, we have identified a 62 serum protein panel that allows stratification of COVID-19 patients by severity and age. Moreover, we have developed a protein array (105 target proteins involved in inflammation, cell adhesion and coagulation) with NAPPA technology to detect complementary blood autoantibody profiles that may help patient stratification

We have designed, synthesised, HPLC purified and characterised by mass spectrometry 70 SARS-CoV-2 peptides containing putative immunogenic epitopes with capacity to activate CD4+ y CD8+ lymphocytes. These peptides have been chosen for their ability to bind to the predominant class I and class II HLA alleles in the Spanish population.

We produced a dodeca-peptide array covering the sequence of the SARS-CoV-2 S protein to characterise the antibody profile raised against this protein by COVID-19 patients.

We set up an immunopeptidomics workflow to identify viral epitopes presented by HLA-I molecules in SARS-CoV-2 infected cells. Up to 13000 sequences were identified. Analysis of cells expressing N, M or E proteins is in process.

Development and experimental validation of sterilisation and decontamination systems for SARS-CoV-2 inactivation

PRINCIPAL INVESTIGATOR
Fernando Usera

EXTERNAL COLLABORATORS
ICV-CSIC, ISCIII, Several public and private entities

The laboratory of Biological safety level 3 (BSL-3) is the key infrastructure of the CNB for carrying out experiments with the SARS-CoV-2, other high-risk coronaviruses and other viruses and bacteria belonging to risk group 3 of human pathogens. The Biosafety Service participates in a series of studies in collaboration with public entities and companies that aim to develop and validate sterilisation and decontamination methods for the inactivation of SARS-CoV-2, other virus, different bacteria, bacterial spores and fungi in different environments and contaminated surfaces.

In addition, we are studying the dynamics of evaporation and persistence of aerosol droplets and the evolution of the viral titer in these droplets and in droplets deposited on different surfaces.

STRUCTURAL STUDIES

Structural analysis of the spike protein of SARS-CoV-2

PRINCIPAL INVESTIGATORS

José María Carazo, Carlos Óscar Sorzano

CNB COLLABORATORS

Mariano Esteban, César Santiago

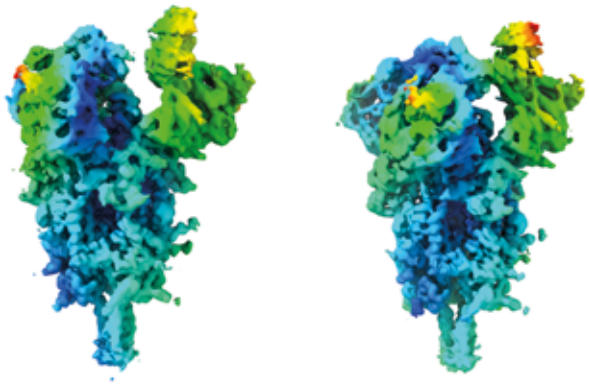
EXTERNAL COLLABORATORS

Pablo Chacón (Instituto Rocasolano, CSIC); Iñaki Comas and José Luis Llacer (Instituto de Biomedicina de Valencia, CSIC); Modesto Orozco (IRB, Barcelona); Heman Tagare (Yale University); Jason S McLellan (University of Texas)

The aim of this project is to describe the structure and dynamics of the spike protein of SARS-CoV2, a macromolecular complex that plays a central role in the infection process of the virus. To achieve this goal, we employ Single Particle Analysis by Cryo-Electron Microscopy (CryoEM) using stablished and new image processing tools, where the emphasis is on analysing the spike continuous flexibility at high resolution. We have studied the wild type virus, in collaboration with McLellan laboratory, continuing now with the analysis of mutants, especially those with a high prevalence in Spain (in collaboration with Comas and Llacer groups`). These fruitful collaborations are being stablished in the context of CSIC internal projects running for the next two years. This knowledge is key to understand how the virus gets into our cells, how the different drugs and vaccines work and how the different mutants of the virus may acquire novel characteristics, potentially impacting therapies.

In addition, we have expanded our information integration portal 3DBionotes, focusing on SARS-CoV2 and making emphasis on quality modeling together with genomic information. It should be noted that 3DBionotes is one of the few Recommended Interoperability Resources of the European Research Infrastructure (RI) on Life Science information (ELIXIR), and that in mid-2020 it was the topic of a joint press release between ELIXIR and the RI for Structural Biology, Instruct.

3D Bionotes-WS: http://3dbionotes.cnb.csic.es/ws/covid19



SARS-CoV-2 Spike protein structural changes

Structural determination of the SARS-CoV-2 nucleocapsid

PRINCIPAL INVESTIGATOR

Jaime Martín-Benito Romero

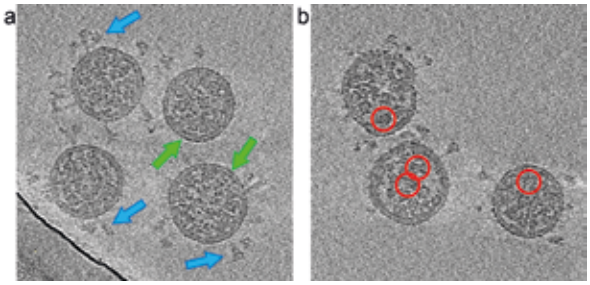
CNB COLLABORATOR

Cryoelectron microscopy service

EXTERNAL COLLABORATORS

Dr. Beata Turoňová (EMBL, Heidelberg, Germany)

The SARS-CoV-2 nucleocapsid is the structure formed by the viral genome bound to multiple copies of a protein called Nucleoprotein (NP). This structure stabilises the genome inside the virion and plays a crucial role in the virus life cycle, participating as a key element in the processes of viral transcription and replication, i.e., in the proliferation of the virus. Nevertheless, and despite its importance, little is known about the nucleocapsid structure and its arrangement inside the virion. Our project aims to determine the nucleocapsid structure using transmission electron cryomicroscopy and image processing techniques. From the acquisition of tilted serial images of SARS-CoV-2 virion samples followed by a reconstruction process, we could determine the 3D structure of individual viruses and how the NP is arranged within the virion. This electron tomography studies are further complemented by other structural techniques and molecular biology studies.



Sections of two three-dimensional reconstructions of SARS-CoV-2 virions obtained by electron tomography. In panel (a) the blue arrows point to the virus spike protein and the green arrows to the viral membrane visualized as a double black line. In panel (b) the red circles mark some details of the nucleocapsid that will be used to determine its structure. The tomographic data have been provided by Dr. Beata Turoňová (EMBL, Heidelberg) and reconstructed at the CNB-CSIC.

STRUCTURAL STUDIES

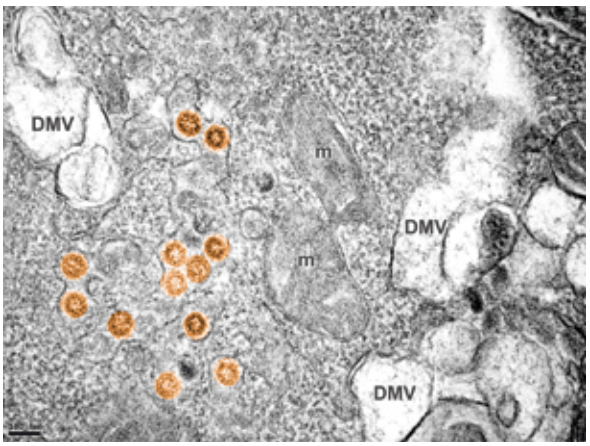
Structural characterisation of SARS-CoV-2 assembly

PRINCIPAL INVESTIGATOR
Carmen San Martín

CNB COLLABORATORS
Marta López de Diego, Mark J. van Raaij

EXTERNAL COLLABORATORS
Marçal Vilar (IBV-CSIC), Daniel Luque (ISCIII)

The global aim of this project is to understand the structural basis of SARS-CoV-2 morphogenesis, to interfere with virus propagation. We are using a combination of fluorescence microscopy, conventional and advanced electron microscopy to analyse key aspects regulating the formation of the SARS-CoV-2 infectious particle.



Section of a cell infected with SARS-CoV-2, showing newly formed viral particles (orange) between a double membrane vesicle (DMV) where the virus genome is replicated and mitochondria (m).

Production and crystallography of COVID-19 related proteins

PRINCIPAL INVESTIGATOR
Mark J van Raaij

CNB COLLABORATORS
Jaime Martín-Benito, Carmen San Martín

EXTERNAL COLLABORATORS
Jorge Pérez Juste (Universidade de Vigo); Maribel Botana Rial (Servicio Galego de Saúde)

In this project, we express COVID-19 related proteins in bacteria for structural biology purposes and for the development of sensors for SARS-CoV-2. The project is financed by a CSIC intramural project and by the Supera COVID fund (SERSforSARS: SERS-based Lateral flow point-of-care immunoassay for ultrasensitive detection of SARS-CoV-2).

Development of chimeric IBDV capsids with SARS-CoV-2 epitopes for therapeutical use

PRINCIPAL INVESTIGATOR
José R. Castón

CNB COLLABORATORS
Luis Enjuanes, Isabel Sola, Sonia Zúñiga, Pablo Gastaminza, Urtzi Garaigorta

Our lab analyses the potential of infectious bursal disease virus (IBDV) capsid to accommodate heterogeneous proteins and peptides fused to the capsid protein. We aim to develop an efficient assembly system of chimeric, IDBV-based virus-like particles where epitopes of different SARS-CoV-2 structural proteins can be inserted to engineer chimeric capsids able to induce protective immunity against SARS-CoV-2.

DIAGNOSTIC SOLUTIONS

Development of an antibody test to asses humoral immunity against Covid-19

PRINCIPAL INVESTIGATORS

José María Casasnovas, Hugh Reyburn, José Miguel Rodríguez Frade, Mar Valés

CNB COLLABORATOR

Salomé Prat

EXTERNAL COLLABORATORS

Francisco Sánchez-Madrid (Hospital de La Princesa), Eduardo López Granados (Hospital La Paz); Immunostep S.L.

Serological tests detect specific antibodies and allow recognition of individuals that have been in contact with the SARS-CoV-2. This technology has been validated in collaboration with La Princesa and La Paz Hospitals in Madrid. The tests are now being manufactured by the Spanish company Immunostep S.L. in ELISA kit format and are already available to the whole country, distributed by Eurofins Megalab. The development of this test in record time demonstrates the benefits of good medical-scientific collaboration.

The test is based on several viral proteins, including some that have not been used in diagnostics previously, that stimulate a strong production of antibodies. Specifically, we have found that the cysteine-like protease, an enzyme produced by the virus during infection, can act as an antigen to generate antibodies that can be detected in patient blood samples.

The tests detect different types of antibodies: IgM, generated usually five or six days after the onset of symptoms; IgG, produced at a slightly later stage of infection, but that persists over time; and IgA, which is produced in early stages, but can also be detected in later phases and which is more localised on mucosal surfaces, such as the respiratory tract, although it is also detected in patient serum.

Commercial ELISA kit developed by CNB-CSIC researchers and manufactured by Immunostep S.L.

Generation of an ELISA test for the detection of SARS-CoV-2 seropositive individuals

PRINCIPAL INVESTIGATORS

José F. Rodríguez, Juan R. Rodríguez

CNB COLLABORATORS

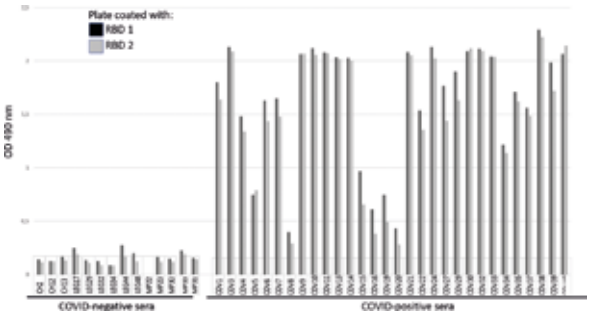
Dolores Rodríguez, Fernando Almazán, César Santiago

EXTERNAL COLLABORATORS

Esther Blanco (CISA-INIA), María Teresa Pérez (CNM-ISCIII)

Our initial aim was to produce recombinant SARS-CoV-2 polypeptides to collaborate in the development and production of serological tests using recombinant versions of the SARS-CoV-2 Spike protein, the Spike's receptor binding domain (RBD) and the cellular receptor ACE2. Recombinant proteins were produced using the baculovirus/insect cell expression system and purified by immobilised metal affinity followed by gel filtration chromatography. Purified polypeptides were initially used to determine optimal conditions to develop highly sensitive ELISA tests (Fig.1). Thereafter, our ELISA test was distributed to different hospitals during the first pandemic wave, before commercial serological tests were available.

Our group has also provided large protein quantities to laboratories of different Spanish institutions working on SARS-CoV-2 related projects. Results obtained with several versions of the RBD polypeptide were the subject of a CNB-CSIC European patent application. Finally, we are interested in applying the protein expression/purification technology developed by our group for the production of novel subunit vaccine candidates against SARS-CoV-2.



Recombinant proteins RBD1 (black bars) or RBD2 (grey bars), were used to determine by ELISA the presence or absence of SARS-CoV-2 in human erum samples.

COMPUTATIONAL MODELS

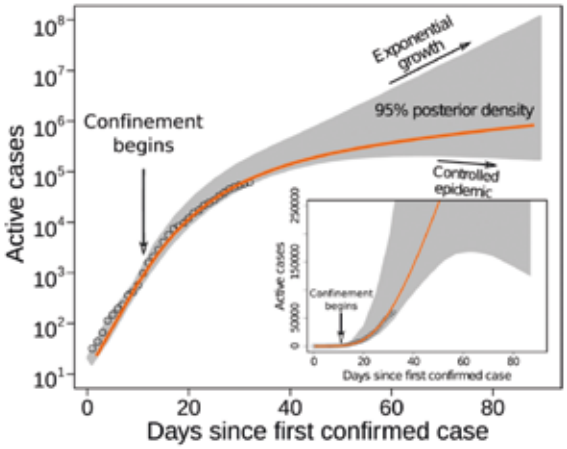
Prediction of the COVID-19 epidemic dynamics

PRINCIPAL INVESTIGATORS
Susanna Manrubia, Saúl Ares

EXTERNAL COLLABORATORS
Mario Castro (Universidad Pontificia Comillas); José A. Cuesta (Universidad Carlos III de Madrid)

The focus of this project is the development of tools to manage, through predictive models, the appropriate social distancing measures to contain or prevent the expansion of COVID-19. The first phase of the project focused on the development of predictive and consensual models. We proposed a new model including reversible confinement of susceptible population. An analytical solution shows that slowing down of epidemic expansion does not guarantee the "flattening of the curve" and could be a transient behavior leading to continuing growth. The theory accurately describes the propagation of COVID-19 in Spain and shows that predictions for its subsequent evolution are disparate, even contradictory. The future of ongoing epidemics is so sensitive to parameter values that predictions are only meaningful within a narrow time window and in probabilistic terms, much as what we are used to in weather forecasts. This work was presented in a PNAS publication.

In a second phase, the refinement of predictive models and potentially useful reports for a future pandemic and the study of explanatory models are being tackled. We have shown that the parameters of a large class of compartmental models are related if properly renormalised, while the effective dynamics do not change. We are also working on stochastic extensions of our results. Finally, we publish analysis of public health data on Twitter, and are in contact with regional and national administrations to discuss data and share our expertise.

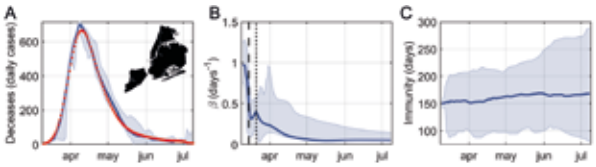


Fit to data obtained in real time for the daily number of active cases in Spain (from March 1st to March 29th) and peak forecast. The shaded area represents the 95% predictive posterior interval: Its increasing width implies that predictability decays exponentially fast. In fact, opposite predictions for the future number of active cases can be derived.

Characterisation of the duration of immunity to SARS-CoV-2 from epidemiological data series

PRINCIPAL INVESTIGATOR
Juan F Poyatos

Among the many open problems associated with the present 2019 coronavirus outbreak, the question about the duration of immunity to SARS-CoV-2 is arguably one of the most significant. This question is traditionally determined through longitudinal serological studies that track antibody prevalence in the same cohort for an extended time. But this method can demand a very long time and requires ample human and technical resources. In this project, we examine an alternative approach to estimate the duration of immunity. This is grounded on the condition that the dynamics of an epidemic where recovered patients become immune for any period should differ significantly from those of one where the recovered promptly become susceptible. We exploit this difference to provide a reliable protocol that can estimate immunity early in an epidemic. We examine this protocol with synthetic data to then apply it to evaluate human immunity to SARS-CoV-2 in mortality data series from New York City. Our results indicate that New York's mortality figures are incompatible with immunity lasting anything below 105 or above 211 days (90% CI.) and set an example on how to assess immune memory in emerging pandemics before serological studies can be deployed. Therefore, we demonstrate that epidemiological models together with state-of-the-art numerical methods are complementary to traditional approaches in providing estimates of the duration of immunity during the COVID-19 pandemic after only four months since the declaration of the pandemic.



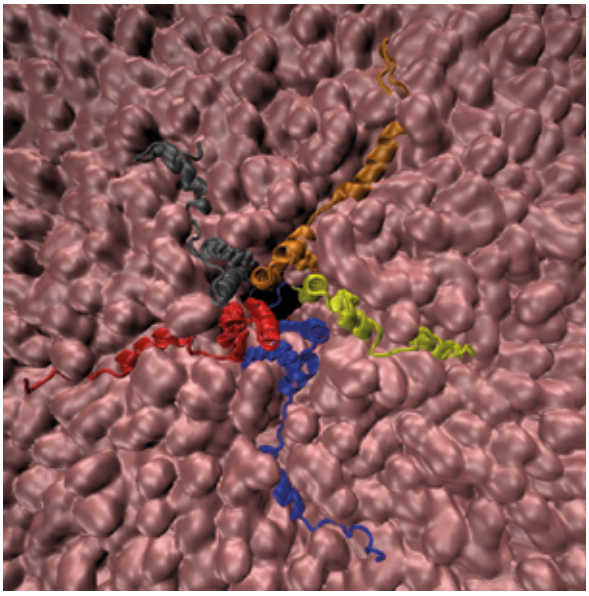
(A) Data (red dots) and algorithm estimate (blue solid, median and 95% CI) of New York City's daily deceases of COVID-19. Data and prediction are in good agreement (ρ => 0.99). (B) Estimate of the infection rate, β, dynamics (median and 95% CI). Drops in β are well aligned with the days on which social distancing measures took place: school closings (black dashed) and the pause order (black dotted). (C) Estimate of the immune memory duration τ (median and 95% CI). The distribution of τ becomes significantly different from that of a control variable δ (two-sample Kolmogorov-Smirnov test p= 0.017) and sets the lower and upper bounds to $\tau \in [80, 288]$ days with (95% CI).

COMPUTATIONAL MODELS

Computer models for the design of therapeutic interventions against SARS-CoV-2

PRINCIPAL INVESTIGATORS
José Ramón Valverde, Luis Enjuanes, J. Manuel Honrubia

This project applies computational methods to several key aspects of SARS-CoV-2 research, including the modelling of mechanisms by which the virus could trigger the different symptoms of COVID-19, the analysis of antibody interaction with SARS-CoV-2 for the design of neutralising antibodies, as well as *in silico* studies of the dynamics of interaction between surface proteins of the virus and its receptor in order to generate a humanised mouse model for pre-clinical studies.



Representation of SARS-CoV-2 E protein pore

Scientific publications

Abian O, Ortega-Alarcon D, Jiménez-Alesanco A, Ceballos-Laita L, Vega S, **Reyburn HT**, Rizzuti B, Velazquez-Campoy A. Structural stability of SARS-CoV-2 3CLpro and identification of quercetin as an inhibitor by experimental screening. Int J Biol Macromol. 2020 Dec 1;164:1693-1703.

Calvo E, Corbacho-Alonso N, Sastre-Oliva T, Núñez E, Baena P, Baena P, Hernández-Fernández G, Rodríguez-Cola M, Jiménez-Velasco I, **Corrales FJ,** Gambarrutta-Malfati C, Gutiérrez-Henares F, López-Dolado E, Gil-Agudo A, Vazquez J, Mourino-Álvarez L, Barderas MG. Why does COVID-19 affect patients with spinal cord injury milder? A case-control study: results from two observational cohorts. J Pers Med 2020; 10 (4): E182.

Castro M, **Ares S**, Cuesta JA, **Manrubia M.** The turning point and end of an expanding epidemic cannot be precisely forecast. Proc Natl Acad Sci USA 2020; 117, 26190-26196.

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Patents

In the context of COVID-19 pandemic, there has been an important increment in the number of patents filed by CNB researchers. More information on the knowledge transfer outcome can be found in the Innovation section of this Report.

CSIC Press Conference held on the 7-7-2020 to announce the development of the serological diagnostic kit. Rosa Menéndez, CSIC President (right) and Mar Valés (left), CNB-CSIC researcher.

Communications to Society

During the pandemic, interest in keeping abreast of scientific developments and understanding the fundamentals of virology and immunology has grown among the general public. CNB research projects have been in the spotlight in national and international media and there have been more than 1,200 appearances in the media related to COVID-19, with interviews and reports featuring the progress of the CNB researchers.

Revista Abogacía Española, diciembre 2020

XL Semanal 18-10-2020

XL Semanal 12-5-2020

El País, 27-1-2020

ABC, 10-5-2020

EuropaPress, 4-5-2020

SINC, 19-5-2020

El País, 29-5-2020

TVE 7-7-2020

El Correo 1-12-2020

EFE 4-6-2020

SINC, 21-12-2020

La Vanguardia, 22-10-2020

COVID-19 funding and sponsorship acknowledgements

CNB COVID-19 research projects have received funding in competitive calls from the European Union, the Spanish Government or private institutions. In addition, the unprecedent social impact of COVID-19 pandemic has motivated private companies, citizens' associations, groups and anonymous individuals to sponsor the research projects carried out in our institute. We would like to express our gratitude to all of them for their support to our research.

European Commission

Gobierno de España · Ministerio de Ciencia e Innovación

CSIC
Consejo Superior de Investigaciones Científicas

Instituto de Salud Carlos III

Fundación "la Caixa"

Catalana Occidente
Grupo asegurador

ferrovial

World Leadership Alliance
Club de Madrid

HELGA DE ALVEAR

multiópticas
número uno en servicios ópticos

GIANT

ayecue

creartelia

Fundación DiabetesCero

RUEDAS Y LETRAS
CONTRA EL COVID

greiner
BIO-ONE

Asociación de Eventuales SAS
aesas

BH
Tempio
Cafés
UCC

Fundación arnhold de la cámara

EPI
INDUSTRIES
family of companies

red // howden

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8

SCIENTIFIC SERVICES

Among the most important assets of the CNB are its core facilities. They provide access to leading-edge technology in the areas of structural and cell biology, genomics, proteomics and bioinformatics. The centre also stands out for its research installations, which include a specific pathogen-free animal facility, a greenhouse, and one of the few high-level biocontainment (BSL-3) laboratories currently operative in Spain. In addition, the CNB hosts two centres of the European Strategic Forum for Research Infrastructures (ESFRI) Project: the Spanish node of INFRAFRONTIER, which includes the European Mouse Mutant Archive (EMMA) and the Instruct Image Processing Centre (I2PC), the Instruct-ERIC facility in the area of Structural Biology. Specialised personnel offer technical support in many other facets of the centre's scientific activities.

STRUCTURAL AND CELL BIOLOGY

Advanced light microscopy
Sylvia Gutiérrez

Quantitative Image analysis
Carlos Óscar S. Sorzano

Cryoelectron microscopy
Rocío Arranz

Electron microscopy image processing
José María Carazo

Electron microscopy
Cristina Patiño

Macromolecular X-ray crystallography
César Santiago

Flow cytometry
Mª Carmen Moreno-Ortiz

Protein tools
Leonor Kremer

Transgenesis
Belén Pintado

Mouse embryo cryopreservation
Lluís Montoliu

Histology
Lluís Montoliu

GENOMICS, PROTEOMICS AND BIOINFORMATICS

Genomics
José Manuel Franco

Proteomics
Fernando Corrales

Bioinformatics for genomics and proteomics
Juan Carlos Oliveros

Sequence analysis and structure prediction
Mónica Chagoyen

Scientific computing
José Ramón Valverde

TECHNICAL SUPPORT

Cell culture, washing and sterilisation
Rosa María Bravo

Photography
Inés Poveda

Instrumentation
Ismael Gómez

Workshop
Daniel Pastora

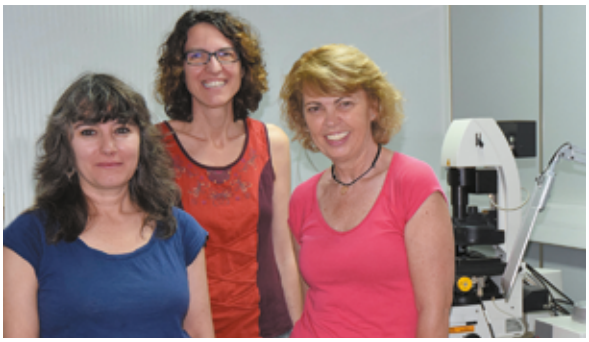
Radiation protection and biological safety
Fernando Usera

RESEARCH INSTALLATIONS

Radioactive facility and biosafety level 3 laboratory
Fernando Usera

Animal facility
Ángel Naranjo

Greenhouse
Tomás Heras



Advanced light microscopy

HEAD
Sylvia Gutiérrez Erlandsson

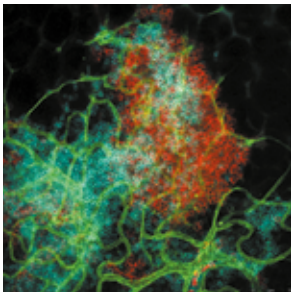
PERSONNEL
Olga Giménez Sáez
Ana María Oña Blanco

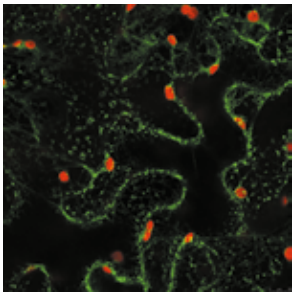
The presence of fluorescent markers in biological samples allows the development of different experimental studies involving single or multiple fluorescent labelling in tissues and living or fixed cells.

The Facility provides state-of-the-art infrastructure for epifluorescence, confocal laser scanning microscopy, TIRFM and STED nanoscopy. The available applications include cell-tracking, multi-position acquisitions, tiling and stitching reconstructions and use of image processing tools, covering main light microscopy experimental imaging approaches.

The equipment and services are available to all CNB personnel and researchers from the public and private sectors.

The technical staff offers assistance and training about equipment use, available methods and image processing and analysis procedures. We also provide cell culture support and aliquots of probes and secondary antibodies with broad use in fluorescence microscopy applications.





Quantitative image analysis unit

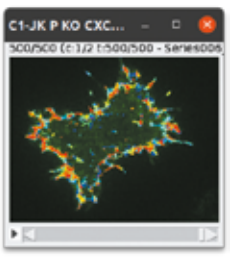
HEAD
Carlos Óscar S. Sorzano

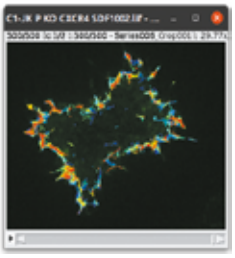
PERSONNEL
Ana Cayuela López (PhD student)

The quantitative image analysis unit complements the advanced light optical microscopes at the cnb by adding the possibility to extract objective information from the acquired images and movies. Quantitative analysis allows objective comparison of the results under different conditions. We develop advanced image processing algorithms that automate the extraction of quantitative features, and facilitates statistical analysis and characterisation.

Examples of the tasks we have tackled are:

- Automatic image segmentation
- Morphological characterisation and classifica objects
- Tracking of objects in videos
- Trajectory classification
- Optical characterisation of the microscope
- Correction by software of some of the microscope aberrations
- Image restoration





Cryoelectron microscopy

HEAD
Rocío Arranz

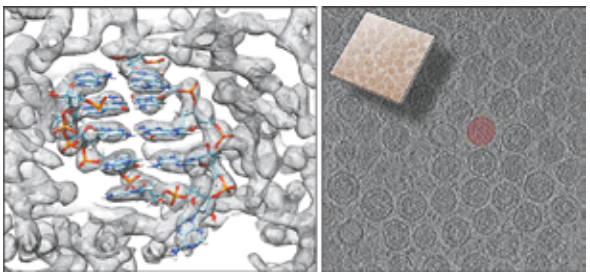
PERSONNEL
Teresa Bueno
Francisco Javier Chichón
Rafael Nuñez (CIB-CSIC)
Noelia Zamarreño

The cryoelectron microscopy core facility is located at the CNB and jointly operated with the Centro de Investigaciones Biomédicas (CIB-CSIC). The services offered by the facility include sample preparation and image collection for cryoelectron microscopy.

The facility provides access to three microscopes for cryoelectron microscopy of biological material: A 300 kV JEOL CryoARM equipped with an autoloader, a Gatan K3 direct electron detector and Omega energy filter and a 200kV FEI Talos Arctica, equipped with autoloader Falcon III direct electron detector. Both can be used for high resolution studies using single-particle methodology. Additionally, the facility hosts a standard 120 KV JEOL JEM 1400 for sample screening.

The service also has four different apparatus for specimen vitrification: a FEI Vitrobot, a Leica EM CPC, a Leica EM GP2 and a high pressure freezer Leica EM ICE.

The facility also offers the cryocorrelative microscopy technique, which allows the analysis by cryo-optical microscopy using a Zeiss LS900 AiryScan microscope and cryoelectron microscopy. The use of a Zeiss CrossBeam 550 cryo-FIB-SEM microscope will increase the cryocorrelative microscopy capabilities of the facility to direct visualisation of cells for tissue-cell resolution or for preparation of thin lamellas in cells for molecular resolution.



Instruct image processing center – I2PC

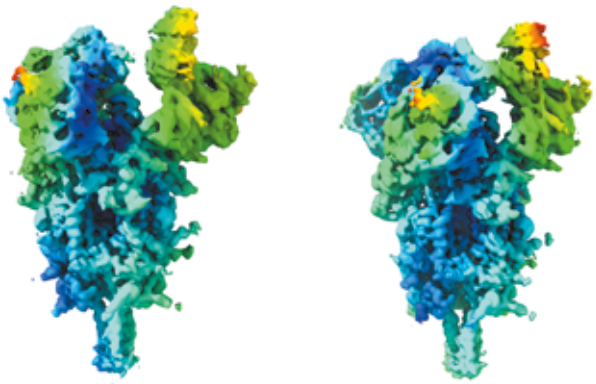
HEAD
José María Carazo

TECHNICAL DIRECTOR
Carlos Óscar Sorzano

PERSONNEL
Blanca E. Benítez
Roberto Melero
Marcos Gragera

Instruct-ERIC is the European Research Infrastructure (RI) in the field of Structural Biology. As part of the Spanish contribution to Instruct-ERIC, the CNB hosts the Instruct Image Processing Center (I2PC), specialised in providing access to researchers from Instruct-ERIC member states with challenging CryoEM image processing projects. Short proposals are directly sent to Instruct Hub, where a review process is coordinated. Selected projects are then conducted at I2PC. Access is at no charge at the point of use for awarded projects, and I2PC personnel is usually acknowledged at publications only, except if particular collaborative developments are needed for the project. We highlight the joint work with GSK on vaccine research and the one in collaboration with a consortium of international laboratories (including Nobel Laureate J. Frank) on the analysis of Ribosome Associated Proteins. COVID-19 has been a special topic this year, and a work on SARS CoV2 spike flexibility was published in IUCrJ.

I2PC also provides support and training in the use of image processing software, at the same time that it develops software and standards oriented towards the standardisation, simplification and reliability of the image processing tasks. During 2019 - 2020 we have hosted 4 courses (2 virtually), which have been attended by more than 86 researchers.



Electron microscopy

HEAD
Cristina Patiño Martín

PERSONNEL
Beatriz Martín Jouve

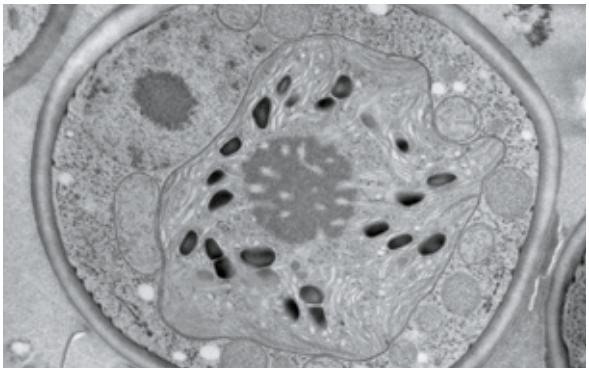
The CNB electron microscopy facility provides scientific and technical support to CNB groups and researchers from public or private institutions.

Technical staff offers assistance and training in the use of equipment and advise on the most appropriate techniques to analyse biological samples (from macromolecular complexes to virus and bacteria, cell cultures and vegetal or animal tissues) using transmission electron microscopy.

We also offer specialised sample preparation, microscopy analysis, data collection and support for data interpretation.

For sample processing the facility disposes of a ultramicrotome, a cryo-ultramicrotome, an automatic freeze-substitution system, two carbon coating equips and a high-pressure vitrification unit. The facility is equipped with a light microscope and a 100 kV transmission electron microscope with digital cameras.

During 2019-2020 the facility has been used by more than 60 research groups.



Macromolecular x-ray crystallography

HEAD
César Santiago

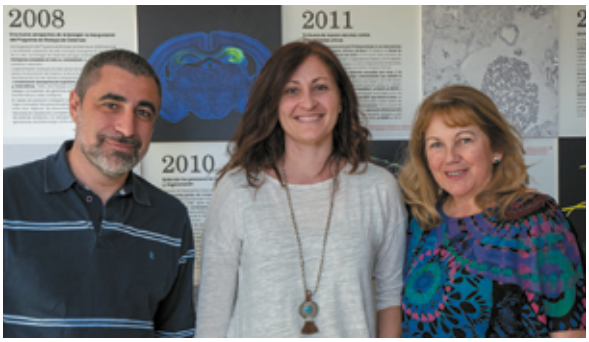
Protein x-ray crystallography is a high-resolution technique that allows us to study protein structure at atomic level. This method provides a detailed view of protein function, ligand and protein interactions, supra molecular organisation and mutants related to human diseases. Great improvements both in crystallisation techniques, and software for structure resolution and refinement have been achieved since the last decade, increasing the chances of solving a macromolecule structure.

The macromolecular X-ray crystallography facility at the CNB provides the following techniques:

- Advice and supervision on protein production from cloning to expression in bacterial, yeast and eukaryotic systems.
- Support and training on protein purification to obtain crystal-grade protein for crystallisation.
- Automated macromolecular crystallisation.
- Crystallization conditions optimisation applying standard and in-house techniques.
- Crystal mounting. Access to synchrotron beam time. X-ray diffraction data collection.
- Data processing and structure resolution and analysis.

Service equipment:

- Mosquito Xtal3 crystallisation robot.
- Genesis RSP 150 workstation (Tecan Trading AG) nanodispenser robot.
- Two temperature controlled crystallisation rooms.



Flow cytometry

HEAD

Mª del Carmen Moreno-Ortiz Navarro

TECHNICIANS

Sara Isabel Escudero García
Miguel A. Sánchez Luengo

Flow cytometry is a powerful tool to analyse multiple parameters on an individual cell. With this technique, we can identify, quantify and isolate different subpopulation of cells based on the levels of expression of fluorescent markers and their relation to each other. It uses a combination of antibodies with fluorophores or fluorescent molecules, both on the surface and intracellularly.

The Unit provides scientific and technological support to the different CNB research groups, as well as to researchers from public and private sectors. We offer the ability to use the different existing applications in flow cytometry and collaborates with the design of experiments, reagents, problem solving, as well as analysis and interpretation of data.

In the Unit there are the following instruments:

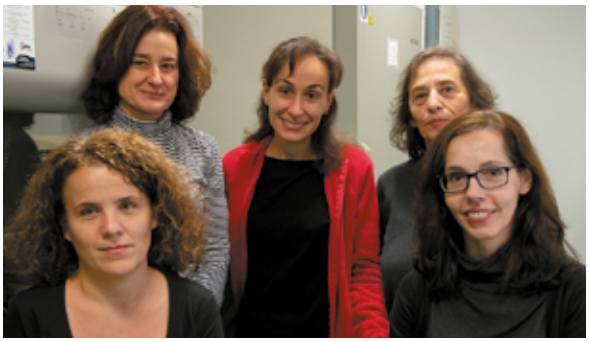
Equipment:

Analysers: BD FACSCalibur: 4 colours. Two Beckman Coulter CYTOMICS FC 500: 5 colours. BD LSRII: 8 colours. Beckman Coulter GALLIOS: 10 colours. Beckman Coulter Cytoflex: 13 colours. Luminex 100 IS Multiparametric Analyser.

Sorter: Cell Sorter Beckman Coulter Moflow XDP: 10 colours.

Analysis Platforms: The Unit also provides 3 PC platforms to analyse data with specific software: WindMDI, CXP, MultiTime, MultiCycle, DIVA, Flowjo, Summit and Kaluza.

We have developed and offer to the users different applications of their interest, such as the analysis of cell viability (in either fresh or fixed cells), apoptosis, cell cycle and ploidy levels in eukaryotic cells, mitotic population studies, proliferation assessment using BrDU, EdU, CFSE or CELLTRACE, gene expression of fluorescent proteins, immunophenotyping up to 13 colours, intracellular signalling, cell's migration, Ca2+ mobilisation, intracellular cytokines, quantitation of soluble molecules by multiplexed assays and cell sorting.



Protein tools unit

HEAD

Leonor Kremer

PERSONNEL

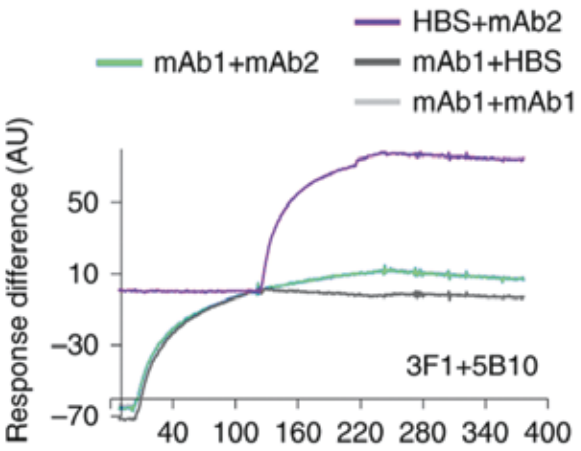
Ana M. García Cabrero
Mercedes Llorente
María Teresa Martín
Elena Ramos

The Protein Tools Unit focuses on immune response studies, generation and characterisation of monoclonal antibodies, design and development of immunoassays, and molecular interaction analyses. The Unit is a founder member of the EuroMAbNet, the European organisation of academic laboratories specialised in mAb production.

Antibodies, assays, research tools and services are provided to scientists from the CNB, other CSIC institutes, universities, public research organisations and private companies. The laboratory offers expertise, technical assistance, advice with data analysis and interpretation, user training and introduction of new methods. The core facility also organises theoretical and practical courses.

In this period, new monoclonal antibodies were raised and characterised against viral proteins (Porcine circovirus type 2, Ebola virus, SARS-CoV-2, etc.) and tumour antigens present on human leukemia cells.

The facility is equipped with an EnVision multilabel reader and a biosensor (Biacore 3000), which allows studies of molecular interactions. The biosensor is used for kinetic constants and affinity analyses of different types of molecules.



Transgenesis

HEAD
Mª Belén Pintado

PERSONNEL
Verónica Domínguez (CBMSO-UAM)
Mª José Palacios

The Transgenesis Service is a join core service shared between the CNB and CBMSO that facilitates access to genetically modified (GM) mouse models to the research groups of both centers, other CSIC institutes, and also external customers, academy or private. Among other services, we incorporate genetically altered mouse lines to the barrier animal facility through embryo transfer or in vitro fertilisation. Our service also provides support in the design and generation of new mouse models, including traditional transgenesis, gene targeting and genome editing. Our aim is to complement the different research groups with technological and scientific support and resources in all the steps involved in the use of GM mice, from genetic modification design to the correct mouse colony management. In addition to fare-based services, we can also establish scientific collaborations when they help us incorporating new state-of-the-art technologies to our services.

For CSIC research groups, we offer the design of guides and edition strategies to generate Knock-out (KO) Knock in (KI) or conditional models using CRSPR/Cas9 technology, either through electroporation or microinjection. For all customers, we generate GM mice through pronuclear microinjection or ES cell-based technologies with ES cell lines from international consortia or generated indoors. We provide embryos in different stages, develop ES lines and perform cell microinjection. We offer training in different assisted reproductive techniques and reproductive phenotyping in mice. Regarding the COVID 19 pandemic, we have generated 8 transgenic lines and 2 KI models susceptible to infection with SARS-Cov-2.

Mouse embryo cryopreservation

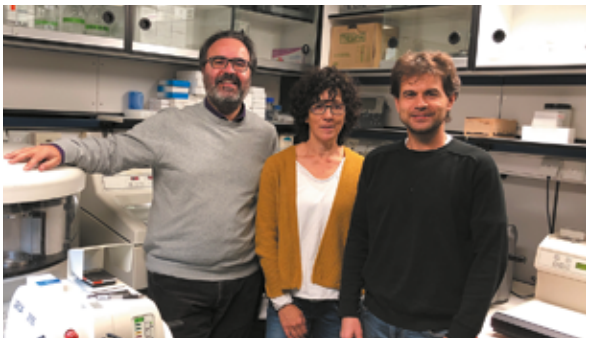
HEAD
Lluís Montoliu

TECHNICAL MANAGER
Julia Fernández

PERSONNEL
Marta Castrillo
María Jesús del Hierro

The CNB mouse embryo cryopreservation facility offers to researchers the possibility to freeze, maintain and rescue transgenic and knockout mouse lines in the form of embryos and/or sperm, hence contributing to current animal welfare recommendations and complying with the associated legislation on animal experimentation. Current methods available include freezing sperm, oocytes and/or embryos, the thawing of sperm, oocytes and/or embryos previously frozen and the subsequent revitalisation of the cryopreserved mouse lines through *in vitro* fertilisation, assessment and/or logistical support for importing/exporting frozen or refrigerated embryos or sperm, from and to the CNB, and quality controls and genotyping procedures. The facility can also produce genome-edited mouse models using the latest CRISPR-Cas9 tools through embryo electroporation.

The CNB hosts the Spanish node of the European scientific research infrastructure (ESFRI) called INFRAFRONTIER, which includes the European Mouse Mutant Archive (EMMA), and whose objective is the generation, phenotyping, cryopreservation, organised archiving and coordinated distribution of mouse lines of interest in biomedicine. EMMA has more than 7,600 mouse mutant lines cryopreserved and is composed of 16 nodes that are present in 13 European countries. About 400 of those mouse lines are cryopreserved and offered from the Spanish node at CNB. The CNB mouse embryo cryopreservation facility has signed scientific cooperation agreements with the Spanish National Cancer Centre (CNIO) and with the Centre for Animal Resources and Development (CARD) at the University of Kumamoto (Japan) for the archiving and distribution of mutant mouse lines of interest in biomedical research.



Histology

HEAD
Lluís Montoliu

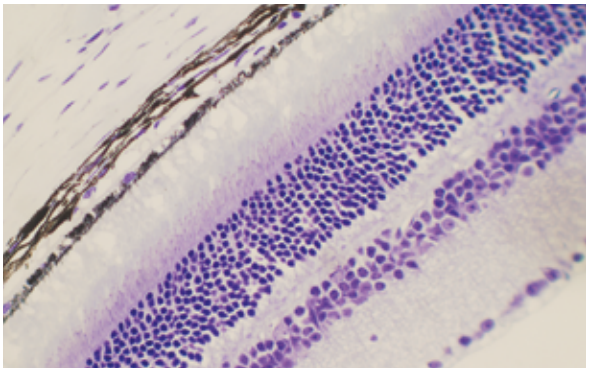
TECHNICAL MANAGER
Soledad Montalbán

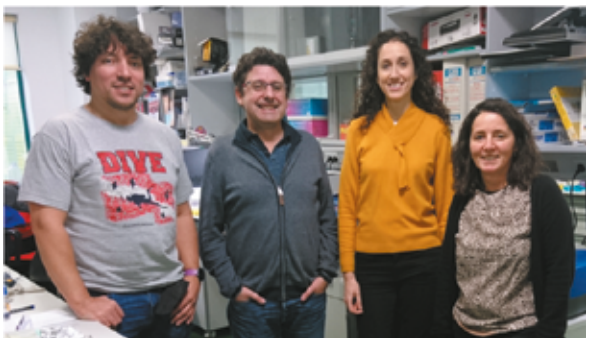
PERSONNEL
Óscar Sánchez

The CNB histology facility offers the preparation of animal and plant biological samples for their histological analyses. All requests are received and processed electronically, through the established facility's registration procedure at the devoted web site, available in Spanish and in English. Offered methods and procedures include the preparation of wax (paraffin) and plastic (resin) blocks with biological specimens embedded, and the corresponding generation of histological sections with one of the two available automated microtomes.

The Histology facility also offers the preparation and sectioning of frozen blocks with the cryostat. The orientation, width and arrangement of the sections can be specified by the user. All sections can be counterstained with any of the available staining procedures (haematoxylin/eosin, cresyl violet, PAS, Mason's trichrome, elastin fibres/Van Gieson/Sirius Red, etc.) or can be processed subsequently for immunohistochemistry.

The facility implements new staining procedures or histological methods upon request. The CNB histology facility has an ample experience in processing a large variety of animal and plant tissues and organs. The CNB histology facility coordinates a joint platform with the IIB-UAM/CSIC histology facility, offering to CNB and IIB researchers a larger processing capacity for histological samples.





Genomics

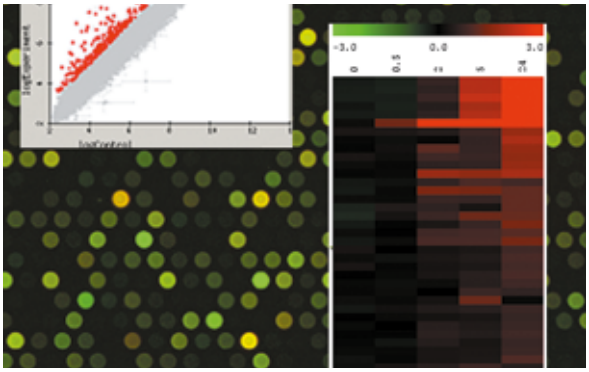
HEAD
José Manuel Franco Zorrilla

PERSONNEL
Luis Almonacid
Marta Godoy
Irene López-Vidriero

The genomics facility is focused on the analysis of gene expression from biological samples using microarrays, interrogating the activity of complete genomes in a single experiment, and contributing to the elucidation of the genetic basis of the biological processes. The facility routinely hybridises and analyses one- and two-channels microarrays, including Agilent, Afymetrix, and custom microarrays.

The services offered by the facility include microarray printing and design, analysis of RNA integrity and microarray hybridisations. Raw data are statistically analysed using "state-of-the-art" algorithms, and filtered results are supplied to customers in a web-based easy-to-use tool developed by the facility.

The facility offers support in the use of several bioinformatics tools for functional analysis, helping customers in the biological interpretation of their results. The facility also offers the possibility of validating gene expression data by real time qPCR.



Proteomics

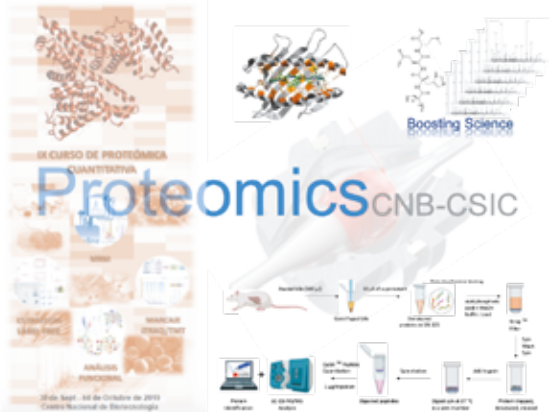
HEAD

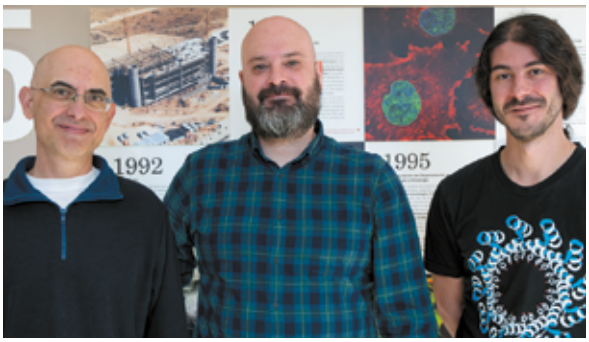
Fernando J. Corrales

PERSONNEL

Alberto Paradela
Sergio Ciordia
Manuel Lombardía
Rosana Navajas
Miguel Marcilla
Lorena Carmona
Laura Guerrero
Fátima Santos
Patricia Gómez

The Functional Proteomics laboratory of the CNB provides resources to identify, characterise and quantify proteins, either purified or as complex mixtures from any biological system. During the 2019-2020 data Independent MS Analysis, processing of challenging samples with high SDS concentrations and S-TRAP columns and functional interpretation of data were implemented. We have performed 4138 proteomic analyses for 404 users including: unsupervised protein quantification by label free or isobaric labeling, targeted quantification, posttranslational modification analysis, analysis of HLA peptide repertoires and structural proteomics. The platform has been upgraded with a new lab and two new state-of-the art instruments Thermo Exploris 240, equipped with ion mobility modules and two nano HPLC Ultimate 3000, one of them with dual capacity to work in nano- and micro-flow modes. We are currently working in: first, structural proteomics: intact protein characterization and protein-protein interaction analysis (combining peptide crosslinking and mass spectrometry). Second, analysis of posttranslational modifications of proteins, including targeted and open-search analysis for epigenetic histone modification patterns.





Bioinformatics for genomics & proteomics (bioinfogp)

HEAD

Juan Carlos Oliveros

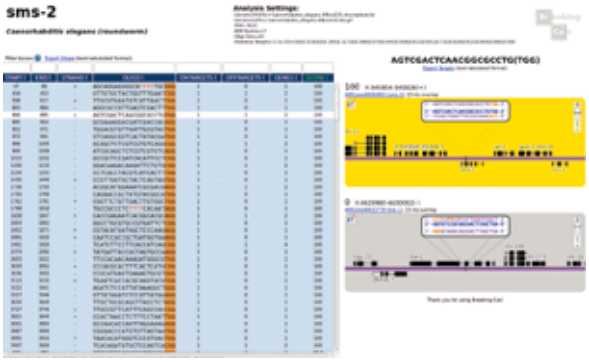
PERSONNEL

Juan Antonio García-Martín
Rafael Torres-Pérez

Our service provides CNB's research groups with bioinformatic support for the analysis, visualisation and interpretation of both genomics and proteomics-related projects. Among other services we provide:

- Assistance on experimental design for deep sequencing and DNA microarrays experiments
- Biostatistical support for extracting quantitative results from genomics or proteomics projects
- Functional annotation of relevant list of genes or proteins
- Periodic courses and tutorials on bioinformatics

In short, at the BioinfoGP service, we try to fill the gap between the complex outcome of the many powerful biostatistical methods available and the final researcher's needs.



Sequence analysis and structure prediction

HEAD
Mónica Chagoyen

Sequence analysis and protein structure prediction methods can explain, simplify and further guide experimental work.

We specialise in ad-hoc analysis of protein sequences to solve specific problems or questions.

In our analysis we commonly:

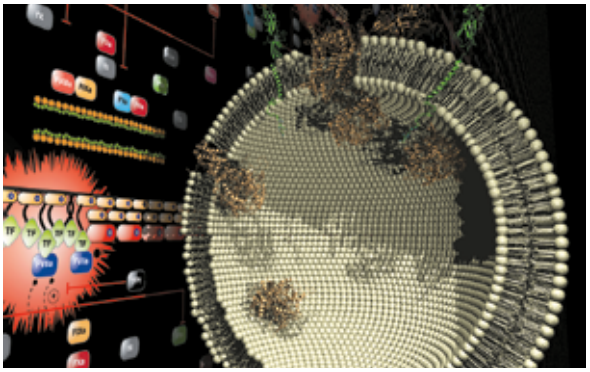
- Predict protein structure
- Search for homologous proteins
- Generate multiple sequence alignments
- Produce structural organization drafts
- Study relevant residues for protein structure/function
- Extract sequence features from full proteomes

Additional services include:

- DNA/RNA motif discovery
- Consultancy in the use of sequence-based methods
- Generation of high-quality protein sequence/structure images for publication

In collaboration with other CNB services, we also organise periodic courses on bioinformatic approaches for sequence analysis and protein structure prediction.

The service is offered to the CNB-CSIC as well as to other academic institutions and private organisations.



Scientific computing

HEAD
José R. Valverde

In the period 2019-2020 our work has concentrated mainly in collaboration analyses with various research groups in several disciplines, concentrating our efforts mainly during 2020 on work related to COVID19.

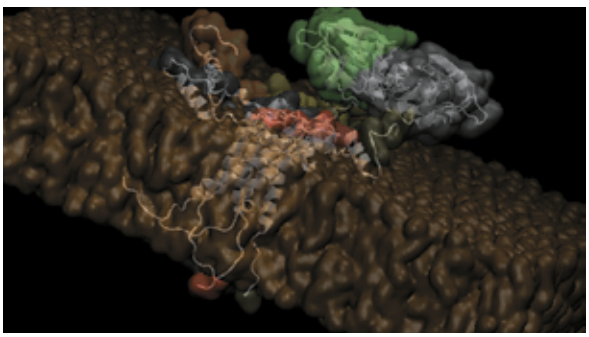
The main lines of work in this period spanned the following topics:

Dynamic metabolic modelling of heterologous protein secretion in *S. lividans,* and of antibiotic resistance in *S. maltophilia* and development of machine-learning based methods to analyse modelling results. We further developed our Adaptive DFBA modelling approach, and applied ML/AI to the analyse of metabolite evolution, prediction of flux evolution based on metabolite concentration, and clustering of internal metabolic fluxes.

Exome analysis of human prostate cancer data from two large cohorts with ~200 individuals each, developing automated protocols based on GATK to clean data, identify variants and annotate them. We have started work on predicting the protein structure of the involved oncogenic proteins with a look towards structure-based prediction of the potential effect of variants.

Structural, bioinformatic and immunogenetic analysis of HIV and CoV vaccines. As a part of this work we have started developing an in-house protocol for *ab initio* protein structure prediction to become independent of external servers.

Study of SARS-CoV2 proteins related to virus entry in the cell and development of COVID-19. We modeled and analysed mutants of viral proteins S and E and their interactions with human targets, and conducted drug screenings for potential treatments against COVID-19.



Photography

HEAD
Inés Poveda

The CNB photography service supports scientists with the photographic material necessary for their research and the dissemination of their results.

Photos are taken on a reprographic table with continuous lighting or with studio flashes against an adjustable background, and illumination with white or ultraviolet light, as needed.

The photography service also manages image processing and, when required, photo retouching; digital images are made accessible to clients on dedicated servers.

The service offers digital color printing of large format posters and, on request, also provides advice for graphic and image design.

Cell culture, washing and sterilisation

HEAD

Rosa Mª Bravo Igual

PERSONNEL

Carmen Berdeal Mera
Margarita Felipe Hombrados
Isabel Martín-Dorado
Ana Montero Moral
Ana Isabel Nieto Jiménez
Josefa Pérez Alfaro
Rosa Ramos Hernández
Aránzazu Rodríguez Martínez
Sonia Rodríguez Murcia
Anunciación Romero
Ángel Valera Lopez

EXTERNAL PERSONNEL (CLECE)
Fernando Oliver Tinuco
Alioune-Aboutalib Sow
Herminia de la Hoz Lorente

Services

- Preparation of cell culture media
- Routine cell culture procedures
- Washing, sterilisation and replacement of laboratory material

Instrumentation

HEAD
Ismael Gómez López

PERSONNEL
Juan Ignacio Golpe de la Fuente
Carlos González Redondo
Rodrigo López Manzano

Services

- Calibration and validation of scientific instrumentation.
- Maintenance and repair of scientific instrumentation.
- Technical advice during the acquisition of scientific-technical equipment.
- Supervision of the installation of scientific-technical equipment.
- User training for scientific-technical equipment.

Workshop

HEAD
Daniel Pastora

Services

- Machining metal and plastic parts
- Custom manufacture of metal structures
- Welding and repair of steel carts

Equipment

- Parallel lathe
- Milling machine
- Power welding set
- Spot welding equipment
- Mitre saw
- Reciprocating saw
- Automatic slitter
- Bending machine
- Grinding machine
- Column drilling machine

Radiation protection and biological safety

HEAD
Fernando Usera Mena

PERSONNEL
María Teresa Bartolomé Jiménez
Aránzazu de la Encina Valencia (coordinator)
Iris Esparza Collado
Jessica Gaspar
Marta Sanz Martínez

OCCUPATIONAL RISK PREVENTION UNIT
Nuria Martín Montes (external)

Service activities

- Evaluation of biological, chemical and radiological risks
- Design of laboratories and other facilities. Management of official authorisations and monitoring of compliance with regulations
- Issuing of guidelines and operating procedures. Risk prevention trainning
- Acquisition of radioisotopes and protection equipment
- Medical and dosimetric surveillance. Management of accidents and emergency
- Management of biological, toxic and radioactive waste

Research activity

Research on SARS-CoV-2 and other high-risk pathogens: new viricides, survival and routes of transmission

Occupational Risk Prevention Unit

Occupational health and safety in areas not related to experimental activities: health, safety and ergonomics. Coordinating business activities regarding safety and health. The Biological Safety Service, in collaboration with the Occupational Risk Prevention Unit, is in charge of the COVID-19 tracing system that operates at the CNB to follow-up COVID-19 positive cases.

This service obtained in 2020 the XI Award for Excellence in Occupational Risk Prevention 'Ramón Tobar' (CSIC) for its management system in risk prevention training.

Biosafety level 3 laboratory and radioactive facility

HEAD
Fernando Usera Mena

PERSONNEL
María Teresa Bartolomé Jiménez
Aránzazu de la Encina Valencia (coordinator)
Iris Esparza Collado
Jessica Gaspar Navarro
Marta Sanz Martínez

Biosafety level 3 laboratory

The laboratory has three sub-laboratories and the necessary equipment for safely handling high risk pathogens: changing room and exit shower, steam sterilizer, air lock, pass through box, effluent treatment plant, data transmission network and remote alarm systems.

Research equipment: biosafety class IIA cabinets, CO_2 cell culture incubators, microbiological incubator, fluorescence microscopes, ultracentrifuge, refrigerated centrifuges and microfuges, ultra-freezers, etc.

Radioactive facility

The CNB radioactive facility is equipped with all the required systems of shielding, containment and detection of ionising radiation.

Research equipment: cabinets for radioisotopes beta and gamma, Biosafety class IIA cabinets, CO2 cell culture incubators, centrifuge and microfuge, inverted optical microscope, etc.

Animal facility

HEAD
Ángel Naranjo

RESEARCH TECHNICIAN
Javier Martín

SHIPMENT COORDINATOR AND ADMINISTRATION
Alberto García García

AREA AND COLONY MANAGERS
Andrés Miguel Acosta Moreno
Sara Flores Solano
Iván Jareño Flores
Raquel Gutierrez Castro
Eladio Martínez Otero
Antonio Morales Martín

ANIMAL TECHNICIANS
Carlos Elías Sánchez
Raul García de la Fuente
Sergio Jímenez Antón
Alfonso Manchado Gonzalez
Guillermo Meza Fernández
Oscar Francisco Montes Carrasco
María Isabel Rodríguez León
Patricia Sanz Arenillas
Miguel Talero Rodríguez

The CNB laboratory animal facility is an area dedicated to the production and maintenance of experimental animals. Most of the experimentation is carried out with genetically modified mice. The laboratory animal service provides a controlled environment for the animals, with periodic control of diet, water, temperature, air, housing, and husbandry conditions. The unit is separated into several areas depending on the microbiological status of the animals, providing special housing conditions for conventional, genetically modified, and immunodeficient animals, depending on the experimental objectives. At the same time, a totally isolated biosafety area is dedicated to in vivo experiments using biological agents.

The animal facility staff delivers services to laboratories for obtaining commercial lines and strains of mice, shipping animals, as well as maintenance, breeding, and generation of transgenic, knock-out and knock-in animals. These services allow control of the microbiological and genetic quality of the animals used in experimentation.

In addition, staff provides services for various techniques used in mouse research models, research assistance in surgical techniques, selection of animal models, animal health surveillance, laboratory animal care, and animal well-being.

The facility also organises courses for continued education specially about management of colonies of genetically modified animals.

The facility's goal is to achieve research excellence following the 3R principles: reduction, refinement, and replacement of animal experiments.

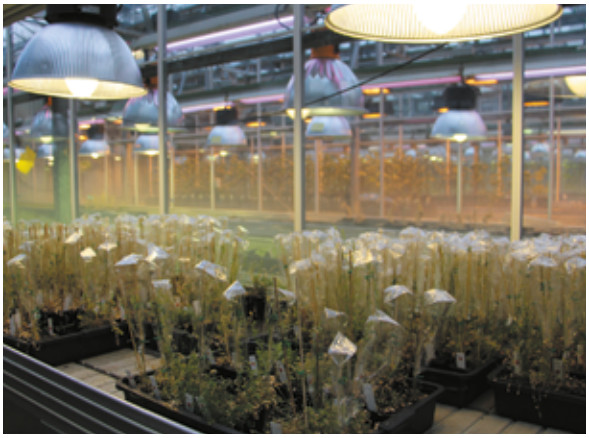
Greenhouse

HEAD
Tomás Heras Gamo

PERSONNEL
Alejandro Barrasa Fuste
Joaquín Rivera Cuesta

The greenhouse service takes care of the following facilities specific for plant cultivation:

- A standard greenhouse with 8 cabinets (total growth surface: 180 m^2)
- A P2 safety level greenhouse with 4 cabinets (total growth surface: 83 m^2)
- 16 climate chambers
- The greenhouse Service carries out the following tasks:
- Growth and propagation of plants under controlled environmental conditions
- Growth and propagation of mutant and transgenic lines under controlled environmental conditions
- Identification, selection and phenotypic analysis of mutant and transgenic plants



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INNOVATION

The unique know-how and cross-disciplinary expertise of CNB's scientists and technologists provides excellent opportunities to transfer leading-edge knowledge and technologies to society and industry. The purpose of the CNB Knowledge Transfer Office (KTO) is to facilitate the process of innovation by:

- raising **awareness** among CNB's researchers about the potential socioeconomic impact of their research and facilitating their implication in technology development and innovation,
- enhancing the **visibility** of the CNB as a source of transferrable knowledge and as a partner for industry in the development of innovative technologies, and
- potentiating the Centre's innovation **capabilities** across all aspects of knowledge protection, commercialisation and entrepreneurship.

KNOWLEDGE TRANSFER MANAGER

Cristina Merino Fernández

Awareness

The CNB knowledge transfer office (KTO) organises and participates in innovation events to foster the entrepreneurial spirit of CNB scientists and familiarise them with the basic principles and benefits of knowledge transfer.

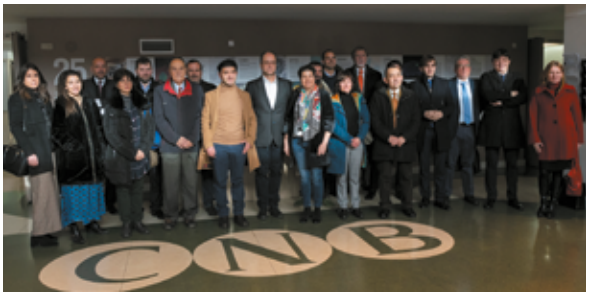
Activities in the 2019-2020 period included the participation in the round table *"Innovation and technology transfer"* held at the *CEU-Innovation Week*; a business visit to our facilities jointly with the FGCSIC and Business Confederation (CEIM-CEOE); and the participation in a workshop, organised by the VATC, about the Nagoya Protocol.

With a focus on the importance of the involvement of women in innovation, the KTO has participated in several forums (EJECON, GIRA Women's Weekend Meeting, Woman's Week Foundation, WStartupC) and organised the event *"Conference: Entrepreneurship and Innovation: Opportunities from a Gender Perspective"*.

KTO also hosts students and participates in mentoring activities (*Fundación Créate, AMCES, Programa steMatEsElla*) aimed at stimulating entrepreneurship in young people.

At the occasion of the Science and Technology Week, with the aim of making visible and dynamising the innovation, the KTO - in collaboration with Inés Poveda and Dolores Aparicio - organised in the CNB hall the exhibition "Transferir", showcasing successful examples of technology transfer spearheaded by CNB scientists.

Science and Technology week 2019: "Transferir", an exhibition to raise awareness of the importance of transferring research results



Attendants to the FGCSIC and Business Confederation (CEIM-CEOE) visit and networking event at the CNB

Visibility

In the context of the COVID-19 pandemic, research outcomes at the CNB have increased their presence in the media, as described in Chapter 12. In addition other initiatives have been undertaken to enhance the visibility of CNB's technology offer, the KTO has established contacts with stakeholders in the public and private sectors, including INNOMADRID, SYVA, DCN, PBL, Ximbio, Nina AD, Ferrer, Proteintech Group, PTGLAB, J&J Asabys, VLX Canaanrd, Health Microbiotics, Erdyn, GENESIS Biomed, COTEC, ASEBIO TTO Circle.

KTO has also participated in meetings, seminars, and congresses related to innovation such as SOUTH SUMMIT, *CSIC-EMPRESAS: tecnología al servicio de la agricultura, European Research and Innovation Days, Hospital Infanta Leonor: II Jornadas de Innovación en el ámbito de la Salud,* RedOTRI, Madrid-TTS Europe, UTM Asia, DRO: Congreso nacional de científicos emprendedores, CEU-San Pablo: XIII Congreso Anual de Biotecnología, Biomedical Innovation Summit, BIOVEGEN: Impacto y futuro de las nuevas tecnologías en producción vegetal, CSIC: I Animal Health Innovation Day: Diagnóstico y Vacunas, TTO Circle: *Connecting with the Entrepreneurial Ecosystem,* InnoUAM, Biospain Transfiere, Farmaindustria and MEDICA.

Furthermore, in close collaboration with CNB's Science Communication and Outreach Office, the KTO promotes the capacities and expertise of its scientists in the media and social networks.


RETEMA

REVISTA ACTUALIDAD ARTÍCULOS Y REPORTAJES PODCASTS AGENDA ENTIDADES NEWSLETTER

AGUA CLIMA EMISIONES ENERGÍA RESIDUOS SOSTENIBILIDAD

Arranca la primera planta semiindustrial para recuperar nutrientes de purines con cultivos de lenteja de agua

El proyecto LIFE LEMNA presenta el primer prototipo semiindustrial de Europa basado en la tecnología de cultivo de lenteja de agua (Lemna) para la recuperación de nutrientes contenidos en los purines excedentarios de porcino

Retema, 18-07-2019

Telediario TVE, 7-7-2020

Capabilities

The CNB KTO works in close collaboration with the CSIC Deputy Vice-presidency for Knowledge Transfer (VATC), covering all aspects of innovation management from the protection of intellectual property to the development and commercialisation of new technologies. In recent years, there has already been a constant upward trend in innovation indicators. Related to the coronavirus pandemic, however, during the year 2020 the number of patents has tripled and the number of contracts with companies has doubled thanks to the unique expertise of CNB researchers in this area.

CONTRACTUAL RESEARCH

The KTO supports and manages contractual relations between the CNB and partners in industry. In the 2019-2020 period, these contracts have generated revenues of 2.5M€, which is more than twice than in the previous period.

Major areas of collaboration and industrial partners include:

- Detection of gluten in food (Damm, R-Biopharm, Ingenasa, Operon, CEA, LETI)
- Production and study of recombinant bacteria, antibodies and proteins for diagnostic and therapeutic purposes (Synlogic, Quantitative Biosciences, eBioscience, Alergovet, Ingenasa, Protein Alternatives, Sanofi-Aventis, Bioncotech, Thrombotargets, Landsteiner, Agroserna, Esteve, Sesderma, Grifols, Pharmamar, Atomwise, Asahi Kasei Pharma, Bayer, Zeulab, Lacer)
- Vaccine development (Sanofi-Aventis, Labopat, Syva, Ceva Santé Animale, Biofabri, UniverCells)
- Development of applications for clinical analytics (Immunostep, Operon, Combat Medical, Lumensia, Samyang, Vircell, PROTEOBOTIC)
- Improvement of crop production, resistance to pathogens and environmental sustainability (Plant Response Biotech, Plant Bioscience Limited, Globachem, TOTAL, Agro Innovation International, CELLBITEC, AINIA, MOA, ERCROS, Semillas Arnedo)
- Electron microscopy and image analysis (FEI Electron Optics, Thermo Fischer Scientific, ATOS, BAI, LEICA, AFMB, BERGEN U., CIM)
- Biotech consulting (Lab Safety Consulting, CEDRION, BLUE WORLD, CTCOM, CTINGENIERING, ATG INGENIERÍA, CLECE, ACTIVHO, ICN2, POLAR, ICS, IRB)

Furthermore, the KTO is in charge of managing material transfer agreements (MTA) with other research institutions and companies all around the world. These agreements, in which the CNB acts as the material provider in more than half of them, reflect on the international reputation of the CNB as a provider of leading-edge materials for research in the life sciences.

PUBLIC-PRIVATE RD ALLIANCES

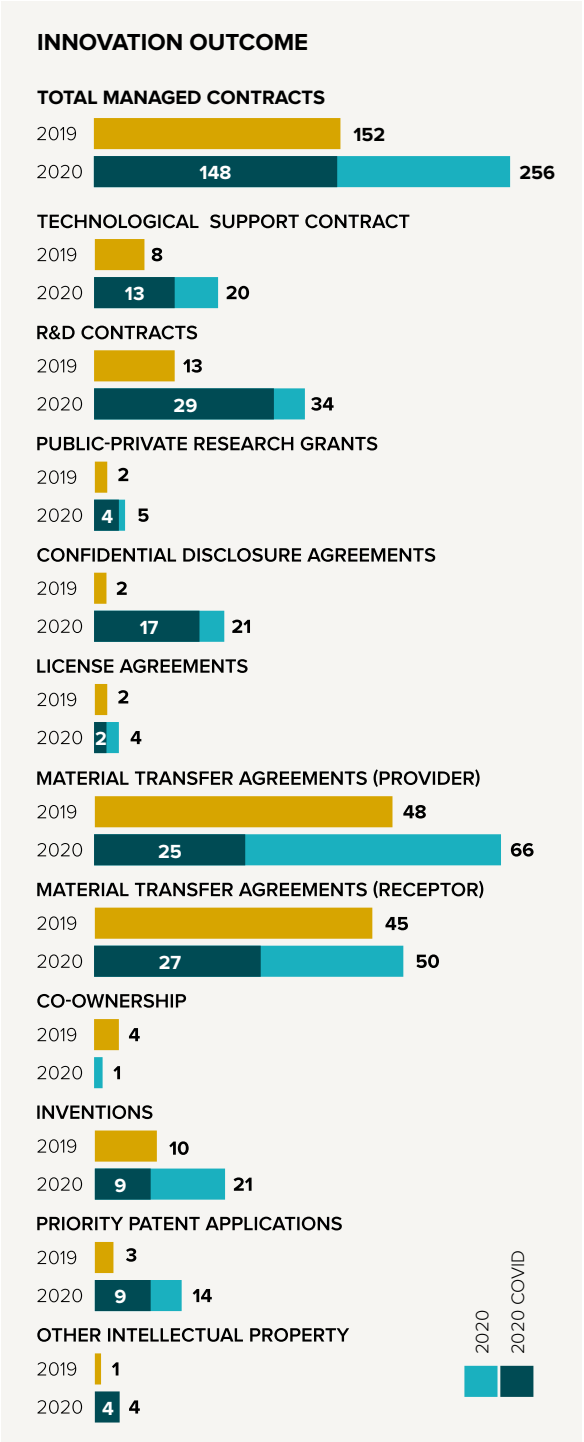
The KTO is in charge of collecting information about skills, results and activities of research at the CNB, with the aim of identifying potential partners for technology transfer and opportunities for joint research projects with companies. Furthermore, the office also provides CNB researchers relevant and timely information on funding opportunities for public-private research projects and assists them in grant preparation, contractual and follow-up issues. During the past two years, CNB scientists presented innovative projects in the framework of private-public partnership funding schemes, such as DINAMIZA-CSIC, CDTI, Retos Colaboración, CAIXA IMPULSE, CEPI, CREU, EITHealth, AECC, ISCIII, PRECIPITA, Sexenios, IRSICaixa or Asahi-Kasei programmes.

ENTREPRENEURSHIP

The KTO helps to identify business opportunities and provides advice on the creation of spin-off companies. In 2019 and 2020, the office supported 5 initiatives for the creation of technology-based companies.

INTELLECTUAL PROPERTY PROTECTION

The KTO provides support in all aspects of intellectual property protection of research results, identifies appropriate business partners for outsourcing the development of new technologies, supervises the activities of license holders and oversees the payment of royalties.



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SCIENTIFIC CAREER DEVELOPMENT

Training of future generations of scientists and technologists is a major priority for the CNB. In the 2019-2020 period, 38 PhD students received competitive fellowships (e.g., INPhINIT, FPU, FPI) to realise their PhD thesis at our institute, and 63 students obtained their PhD degree under a CNB scientist's supervision. Our centre hosted 74 undergraduate and 83 master's students from Spanish and international universities, allowing them to received first-hand experience in biotechnology research. In addition, 42 short-term trainees and visiting scientists chose the CNB for its outstanding training opportunities. Moreover, CNB researchers actively participate in some of the best university and master's degree programmes in Spain.

We are making continuous efforts to attract young people who wish to pursue a scientific career. We have already celebrated the 7th edition of the "CNB course on introduction to research" for undergraduate students. In collaboration with the CSIC and funding from the Severo Ochoa Centres of Excellence Program, we offered fellowships to attract brilliant master's students.

Our PhD training program, launched in 2014 as part of the Severo Ochoa Centres of Excellence Program, is fully established. The PhD Student's and Training Advisory Committees, with the support of the Science Communication and Outreach Officer (Susana de Lucas), organise annual activity programmes to support career development. From courses fostering public presentation skills, how to write a scientific paper, or an interactive workshop on ethics and integrity in research, to welcome events for new PhD students and predoctoral scientific workshops, we aim to improve both their scientific and other soft skills useful in an academic career.

Around 25% of our personnel are postdoctoral researchers, a task force that drives the excellence of our research and participate in the training of younger students. Our centre attracted 12 talented young scientists through international, national and regional calls such as Marie Skłodowska-Curie Actions from the European Commission, Juan de la Cierva, Ramón y Cajal and Talent Attraction Programmes.

A rich program of seminars, conferences, workshops and courses, more than 150 in the 2019-2020 period, provide optimal opportunities for our researchers to keep up with the latest advances in biotechnology. Highlights from the past two years include an international a Congress in collaboration with the CBM-SO on "Chemokines and Cell Migration", involving 120 participants, and a scientific

congress in honor of the scientific career of Prof. J. L. Carrascosa to celebrate 25 years of Electronic Cryomicroscopy in Spain. The Congress attracted two Nobel laureates and had more than 150 participants.

Although the 2020 COVID-19 pandemic has forced changes in the celebration of scientific seminars, now converted in webinars, we have taken this as a new opportunity to reach wider audiences through the use of online platforms.

SCIENTIFIC ACTIVITIES COMMITTEE

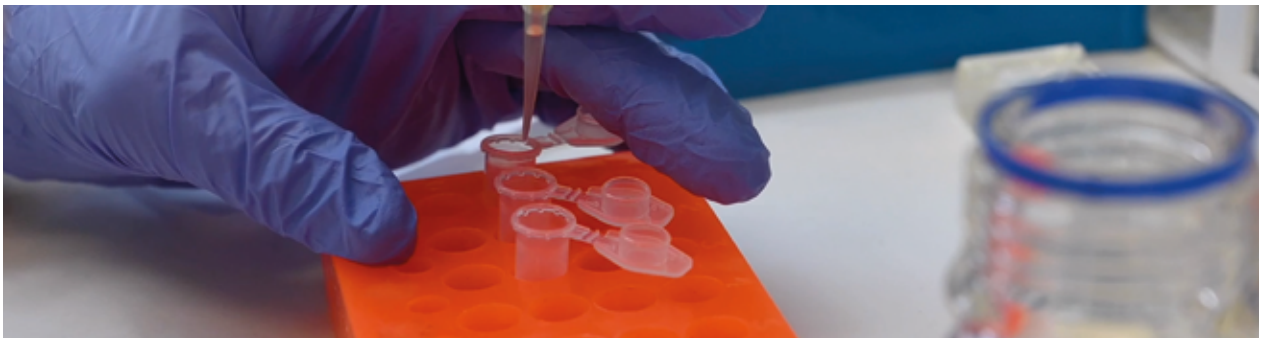
Juan Carlos Alonso
Antonio Leyva
Florencio Pazos
Hugh Reyburn
Juan José Sanz
José María Valpuesta

TRAINING ADVISORY COMMITTEE

Yolanda Carrasco
Mark van Raaij
Vicente Rubio
Juan José Sanz
Javier Tamames
Miguel Vicente

PhD STUDENTS COMMITTEE

Alejandro Asensio
Lorena Bragg
Álvaro Ceballos
Marta Cobo
Alberto Fuster
Sofía Gardeta
Andoni Gómez
Marcos Gragera
Diego Jiménez
Javier López-Ibáñez
Micaela Navarro
Andrés París
Elena Sánchez
Jesús Vallejo



PhD fellowships

2 LA CAIXA INPHINIT FELLOWSHIPS

La Caixa Foundation

María José Felgueres Planells

Arturo Daniel García Vesga

10 FPU FELLOWSHIPS

Ministry Of Education, Culture And Sport

Neus Daviu Bou

Elisabet Díaz Beneitez

Álvaro Fernando García Jiménez

Aitor Jarit Cabanillas

Elia Marcos Grañeda

José Martín Gómez

Almudena Méndez Pérez

María Jesús Rodríguez Espinosa

Ainhoa Ruiz Iglesias

Martín Sastre Gallardo

5 FPI SEVERO OCHOA FELLOWSHIPS

Ministry of Science and Innovation

Ana Cayuela López

Rafael García López

Alejandro López Hurtado

Jonathan Gabriel Piccirillo

Adriana Quijada Freire

1 FIS FELLOWSHIP

Ministry of Science and Innovation

Esmeralda Cebrian Sastre

20 FPI FELLOWSHIPS

Ministry of Science and Innovation

Alba Cabrera Fisac

Christian Camilo Cortés García

David Egea Benavente

Daniel Fernández Soto

Margarita Ferriz Salcedo

Carlos García Crespo

Marta García López

Samuel García Poveda

Sofía Rosa Gardeta Castillo

David Gil Cantero

Teresa Gil Gil

Marina Higuera García

Leticia Lucero López

Luis Miguel Luengo Cerrón

Mikel Marín Baquero

Iris Martínez Hevia

Diego Martínez Rey

Aitor Muñoz López

Elena Pares Guillen

Irene Varela Martínez

Undergraduate and master students fellowships

CSIC Introduction to Research Fellowships

23 JAE INTRO

Gonzalo María Aizpurua de Arteche

Julio César Aragón Lago

Sandra María Camuñas Alberca

Irene Castells Yus

Luis Castillo Cantero

Odette Deen Rozalen

Daniel del Hoyo Gómez

José María Fernández Palacios

Jorge García Condado

Juan García-Agullo Rivera

Darío López García

Iván Martín Martín

Natalia Martínez Puente

Almudena Méndez Pérez

Alberto Manuel Parra Pérez

Sergio Pipaón Alcibar

Julia Purificación Casino

Irati Rincón Santoyo

Marta Sánchez Diez

Paula Sánchez Sánchez

Henry Patricio Secaira Morocho

Carlos Wert Carvajal

Ana Carmen González Brenes

10 JAE INTRO-SOMMA

Yolanda Benítez Quesada

Nicolae Ciobu

Lucia de Dios Blázquez

Jorge Huete Carrasco

Alba Esteli Murillo Sánchez

Sara Otaegi Ugartemendia

Cesar Palacios Cuellar

Álvaro Redondo del Río

Ángel Ruiz Enamorado

Jesús Vílchez García

6 JAE INTRO ICUS CNB (2019)

7th CNB Course Introduction to Research

María González Álvarez

David Gutiérrez Baez

Javier Ortiz Rivero

Sergio Polo Nicoli

Patricia Rus Fernández

Gustavo Adolfo Sánchez Corrales

Doctoral theses

In 2019 and 2020, 63 students obtained the PhD degree under the supervision of CNB researchers.

2019

JAVIER ARRANZ-NICOLÁS

The metabolism of diacylglycerol in T cell tolerance regulation and tumor evasion.

(Isabel Mérida)

NOELIA ARTEAGA RAMOS

Identificación y caracterización de genes implicados en la variación natural para el patrón de tricomas en *Arabidopsis*.

(Carlos Alonso Blanco)

PAULA BLANCO

Inducible and acquired antibiotic resistance in *Stenotrophomonas maltophilia*.

(José Luis Martínez)

JUAN JOSÉ CESTERO

Remodelación del peptidoglicano de *Salmonella* por actividades ausentes en organismos no patogénicos.

(Francisco García del Portillo)

JUAN DÍAZ COLUNGA

Mitochondrial control of gene expression and extrinsic apoptosis.

(Francisco J. Iborra Rodríguez and Raúl Guantes Navacerrada)

ALEJANDRA ESCÓS LÓPEZ

New insights in p38MAPK function and potential value as therapeutic target for high-prevalence diseases.

(Ana Cuenda)

MARTA GARCÍA LEÓN

Unraveling the role of *Arabidopsis* ALIX in the trafficking and turnover of abscisic acid receptors.

(Vicente Rubio)

MOISÉS GARCÍA SERRADILLA

Estudio de la capacidad antiviral de Ribavirina y Nano-partículas de plata en células infectadas con Bunyavirus mediante técnicas de imagen.

(Cristina Risco Ortiz)

MARTA HERVÁS GARCÍA

Estudio de las modificaciones post-traduccionales que afectan a la proteína de la cápsida del Plum pox virus y su papel en el desarrollo del ciclo viral.

(Juan Antonio García and Sandra Martínez Turiño)

Mª DE LOS ÁNGELES HUESO GIL

Refactoring the interplay of *Pseudomonas putida* with solid surfaces for programming lifestyle decisions.

(Víctor de Lorenzo and Belén Calles)

SANTIAGO JOSA DE RAMOS

Functional analysis of the non-coding mouse genome through bioinformatic and CRISPR tools.

(Lluís Montoliu)

JULENE MADARIAGA MARCOS

Magnetic tweezers and fluorescence to study DNA:protein interactions.

(Fernando Moreno-Herrero)

CARMEN MAÑAS TORRES

Engineering *Escherichia coli* to target bladder and colon tumour cells and characterization of the adhesion process.

(Luis Ángel Fernández)

ALEJANDRO MARTÍN GONZÁLEZ

AFM characterization of DNA-binding proteins involved in the repair and organisation of DNA.

(Fernando Moreno-Herrero)

MIGUEL ÁNGEL MARTÍN SERRANO

Validación de las quinasas de éstres p38MAPKs como nuevos biomarcadores tumorales. Análisis de su papel en el cáncer de colon asociado a colitis.

(Ana Cuenda and Juan José Sanz-Ezquerro)

ANA MARTÍN LEAL

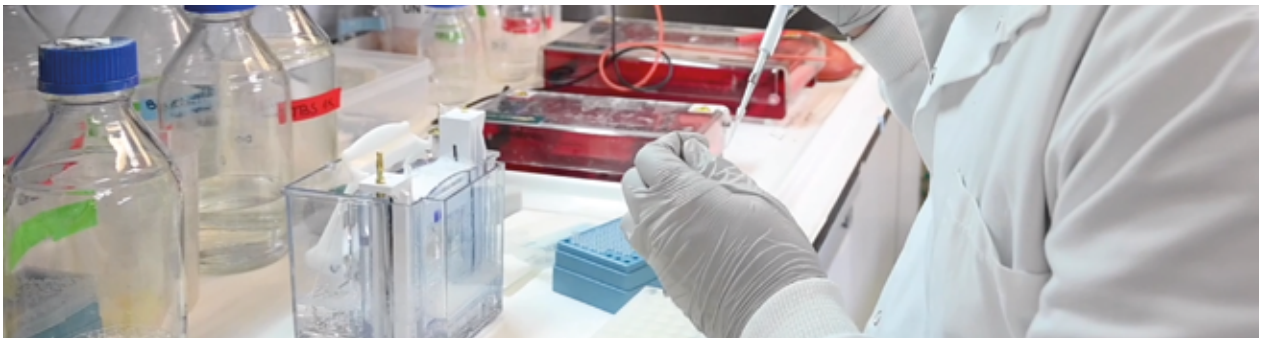
Papel del CCR5 en la oligomerización del TCR y su relevancia en la respuesta de las células T CD4 de memoria.

(Santos Mañes and Raquel Blanco)

GONZALO MARTÍNEZ MARTÍNEZ

Study of membrane proteome of DGKz-deficient cytotoxic T lymphocites.

(Isabel Mérida and Severine Gharbi)



SARA V. MERINO CORTÉS

El ácido fosfatídico producido por la DGKζ regula la respuesta de las células B a través del control del citoesqueleto de actina y la adhesión mediada por integrinas.

(Yolanda R. Carrasco)

MIGUEL MIÑAMBRES

Natural variation for phosphate starvation responses in *Arabidopsis*: new insights from gene expression QTL analyses in a recombinant inbred line population.

(Javier Paz-Ares)

CARMEN MORA GALLARDO

Characterization of the DIDO3-SFPQ axis in alternative splicing.

(Carlos Martínez-A and Karel van Wely)

ANDRÉS ORTIGOSA

Role of MYC transcription factors in photomorphogenesis and stomatal defence.

(Roberto Solano)

MARÍA PEÑUELAS HORTELANO

Functional Characterization of MYCs TFs in *Marchantia polymorpha*.

(Roberto Solano)

MERCEDES PÉREZ-OLIVARES

Max function in B lymphocyte differentiation.

(Ignacio Moreno de Alborán)

ADRIANA PÉREZ PORTILLA

Estudios sobre la inmunogenética de inmunodeficiencias primarias.

(Hugh Reyburn)

PATRICIA PÉREZ RAMÍREZ

Novel vaccines base on poxvirus vector MVA against human viral diseases HIV/AIDS and Zika.

(Mariano Esteban and Juan García-Arriaza)

MARÍA DEL MAR PÉREZ RUIZ

Structure and function of the components of the core of T7 bacteriophage, a DNA translocation complex.

(José L. Carrascosa)

ANTONIO PICHEL BELEIRO

Structure determination of receptor-binding proteins and baseplate of *Staphylococcus* phage K, a therapeutic phage for control of MRSA.

(Mark J. van Raaij)

MARÍA QUIRÓS MARÍN

Aumento de la inmunogenicidad de una vacuna contra la hepatitis C (MVA-HCV) basada en el virus vaccinia modificado de Ankara (MVA).

(Mariano Esteban and Juan García-Arriaza)

AÍDA REVILLA GARCÍA

Transmisibilidad, agregación cruzada y toxicidad de la proteína similar a príones RepA-WH1 en cultivos celulares de mamífero.

(Rafael Giraldo)

ANA ISABEL RODRÍGUEZ

Bases moleculares de la virulencia y la resistencia en *Escherichia coli*: mutación, recombinación y transferencia horizontal.

(Jesús Blázquez and Jerónimo Rodríguez-Beltrán)

SARA ROMÁN GARCÍA

Funciones de la actividad adaptadora y catalítica de la proteína tirosina kinasa de Bruton en la respuesta de las células B.

(Yolanda R. Carrasco)

MARTA SANZ GAITERO

Crystallographic structure determination of bacteriophage-encoded enzymes that specifically target pathogenic bacteria.

(Mark J. van Raaij)

LAURA SANZ ORTEGA

Análisis del uso combinado de nanopartículas magnéticas y campos magnéticos externos para dirigir células linfoides hacia una región de interés y de su potencial en terapias de transferencia adoptiva celular en cáncer.

(Domingo F. Barber)

RUBÉN TORRES SÁNCHEZ

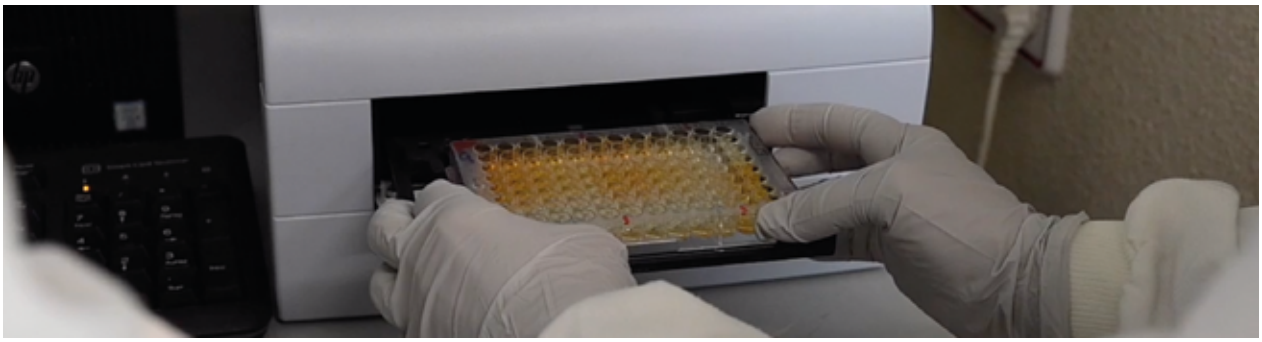
Bacillus subtilis RadA/Sms and RecA contribute in concert to double-strand break repair and natural transformation, and with DisA to DNA damage tolerance.

(Juan Carlos Alonso)

JOSÉ LUIS VILAS PRIETO

Local quality assessment of cryo-EM reconstructions and its applications.

(Carlos Oscar Sorzano-Sánchez and Javier Vargas)



2020

IVÁN CAMILO ACOSTA GARCÍA

A membrane remodelling system for OXPHOS activity in *Staphylococcus aureus*.

(Daniel López)

TERESA BUENO CARRASCO

The quasi-atomic structure of human tyrosine hydroxylase by cryo-electron microscopy: functional implications.

(José María Valpuesta and Jorge Cuellar)

JAVIER CANTÓN BAILÓN

Relevancia de la proteína 4b de MERS-CoV en el antagonismo de la respuesta inmune innata y la virulencia.

(Isabel Sola and Luis Enjuanes)

LIDIA CERDÁN GARCÍA

Construction and validation of a large naïve library of VHHs integrated in the chromosome of *E. coli* for selection of nanobodies using bacterial display.

(Luis Ángel Fernández)

MARTA COBO SIMÓN

Ecology of marine microorganisms: biodiversity, genomics and metagenomics.

(Javier Tamames & Carlos Pedrós-Alió)

DIANA DAMIÁN APARICIO

Mechanism of regulation of flotillin levels by the staphylococcal accessory regulator SarA.

(Daniel López)

CHARLOTTE DESSAUX

Dynamics of *Listeria monocytogenes* stressosome proteins in response to osmotic stress and the intracellular eukaryotic niche.

(Francisco García del Portillo and M. Graciela Pucciarelli)

DANIEL FUENTES MARTÍNEZ

Estudio de los complejos replicativos del virus de la bursitis infecciosa (IBDV) y análisis de la función de la proteína VP5.

(José F. Rodríguez and Dolores Rodríguez)

MARCOS GRAGERA CABEZUDO

Biophysical characterization of a chaperone complex involved in macroautophagy.

(José María Valpuesta and Rosario Fernández)

JAVIER GUTIÉRREZ ÁLVAREZ

Coronavirus causante del síndrome respiratorio de Oriente Medio: Patología y Protección.

(Luis Enjuanes and Isabel Sola)

FERNANDO GUTIÉRREZ DEL BURGO

DIDO3 organiza la red génica que regula la especificación y el destino de las células B.

(Carlos Martínez-A and Ricardo Villares)

LAURA HERNÁNDEZ VILLARRUBIA

Caracterización del sistema inmune Innato de la cavidad peritoneal: papel en la defensa frente a infecciones bacterianas intraperitoneales.

(Carlos Ardavín and María López Bravo)

ADRIÁN LÁZARO FRIAS

Generación de candidatos vacunales basados en el MVA frente a los ebolavirus Zaire y Sudan.

(Mariano Esteban and Juan García-Arriaza)

ALBERTO MARÍN GONZÁLEZ

Combining molecular dynamics simulations and atomic force microscopy experiments to rationalize the mechanical properties of double-stranded DNA and RNA.

(Fernando Moreno-Herrero and Rubén Pérez)

EVA MARTÍN SOLANA

El atasco ribosomal y las alteraciones polisomales como mecanismo de toxicidad en la enfermedad de Huntington.

(María Rosario Fernández Fernández and José Jesús Fernández)

PABLO MARTÍNEZ GÓMEZ

Oligomerización de CXCR4, una nueva diana para modular las funciones mediadas por CXCL12.

(Mario Mellado)

ALEJANDRO PASCUAL IGLESIAS

Virus de la diarrea epidémica porcina: patogénesis y protección.

(Luis Enjuanes and Sonia Zúñiga)

EVA PICO SÁNCHEZ

Engineering of *E. coli* bacteria for targeting human and murine epithelial tumor cells expressing HER2 and PD-L1 markers and their application in the colonization of mouse bladder tumours in vivo.

(Luis Ángel Fernández)

MANUEL OLAZABAL MORÁN

Regulación fisiológica de PTEN tras a estimulación con factores de crecimiento.

(Ana Clara Carrera)

ANA BELÉN PEÑAHERRERA PAZMIÑO

Desarrollo de canales de microfluidica para estudio de crecimiento celular y análisis de flujo en medios porosos.

(José María Casasnovas)

MARTA ROYO LLONCH

Ecogenomics of uncultured marine prokaryotes.

(Silvia Acinas and Carlos Pedrós-Alió)

FERNANDO SANZ-GARCÍA

Predicción de la resistencia a antibióticos, intrínseca y adquirida, en *Pseudomonas aeruginosa*.

(José Luis Martínez and Sara Amado-Hernando)

RUBÉN SÁNCHEZ GARCÍA

Learning from data in structural bioinformatics: a protein-protein interaction study.

(José María Carazo García and Joan Segura Mora)

JAVIER SANTOS ARENAL

Identificación de cisteinil proteasas como mediadores de la disfunción de linfocitos citotóxicos inducida por PD-1. Implicaciones en la inmunoterapia del cáncer.

(Santos Mañes and Rosa Ana Lacalle)

ADRIANA LUCÍA SANZ GARCÍA

Multipartite Viruses. Organization, Emergence & Evolution.

(Susanna Manrubia)

HÜSEYIN TAS

Actualización de *Pseudomonas putida* como chasis de biología sintética mediante la interoperabilidad de dispositivos genéticos.

(Víctor de Lorenzo and Angel Goñi)

MARIA-TSAMPIKA MANOLI

Synthetic and systems biology approaches towards the optimization of polyhydroxyalkanoates metabolism in *Pseudomonas putida* KT2440.

(Juan Nogales)

RABEA WAGNER

The bacterial exo- and endo-cytoskeleton spatially confines functional membrane microdomains.

(Daniel López)

Postdoctoral and Research Fellows

In the last two years, our centre attracted 12 talented young scientists through international, national and regional calls such as Marie Skłodowska-Curie Actions from the European Commission, Juan de la Cierva, Ramón y Cajal and Talent Attraction Programmes.

1 RAMÓN Y CAJAL PROGRAMME
Ministry of Science and Innovation
Adrián Alejandro Valli

1 ATRACCIÓN DE TALENTO PROGRAMME
Comunidad de Madrid
Pablo Pulido

1 YOUNG INVESTIGATOR PROGRAMME
Ministry of Science and Innovation
Selena Giménez Ibáñez

1 MARIE SKŁODOWSKA-CURIE ACTIONS
European Comission
Jorge García Marqués

8 JUAN DE LA CIERVA PROGRAMME
Ministry of Science and Innovation
Alejandra Gutiérrez González
Mercedes Hernando Pérez
Sophie Jayne Kneeshaw
Marcin Krupka
Vladimir Mulens Arias
Fernando Puente Sánchez
Gorjana Rackov
Luis Francisco Seoane Iglesias

Biophysical studies on small protein domains to correlate folding, cooperativity, binding and macromolecular assembly

Luis Alberto Campos Prieto

Ramón y Cajal Fellow
Macromolecular Structures Department
Associated with Dr José María Valpuesta's group

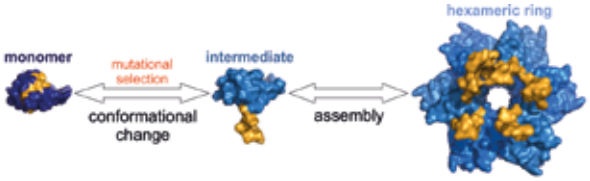
Protein folding cooperativity is the key to expand the protein behaviour in protein folding and binding. Thus, the cooperativity scale goes from intrinsically disordered proteins, with no cooperativity at all, to highly cooperative rigid folders, with few interesting phenomena inbetween, including "downhill" folders, moonlighting binding or metamorphic proteins.

I have focused my scientific interest in the study of small proteins with low cooperativity, applying protein engineering to modulate their folding. With this in mind, I have investigated the oligomerization of small proteins to form big macromolecular assemblies, creating a synthetic system where we have converted by mutations a rigid highly cooperative model into a metamorphic protein that forms stable hexameric rings in solution, and studied its functionalization with metal and/or nucleic acid binding or through protein fusion. Finally, I am applying single molecule techniques to investigate the dynamics of oligomerisation and expanding my studies to vesicles, formed of small proteins, with delivery capabilities.

SELECTED PUBLICATIONS

Campos LA, Sharma R, Alvira S, Ruiz FM, Ibarra-Molero B, *et al.* Engineering protein assemblies with allosteric control via monomer fold-switching. Nat Commun 2019, 10: 5703.

Campos LA, Sadqi M, Muñoz V. Lessons about Protein Folding and Binding from Archetypal Folds. Acc Chem Res 2020; 53: 2180-2188.



Folding diagram for the new synthetic metamorphic protein created in the lab.

Light signalling and chromatin dynamics

Sandra Fonseca

Ramón y Cajal Fellow
Plant Molecular Genetics Department
Associated with Dr Vicente Rubio's group

PERSONNEL

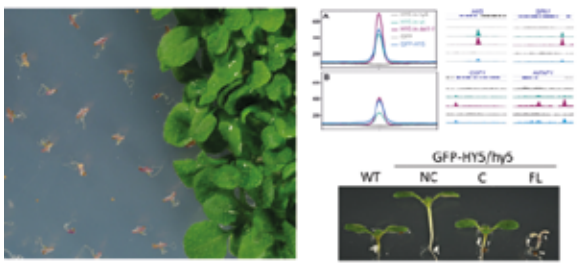
Esther Cañibano (PhD student, co-supervised with Dr V. Rubio)
Laura Gómez (master student)
Leticia Saez (undergraduate student)

Light fuels plant life and is an essential cue that modulates growth and development throughout all the plant life cycle. As sessile photoautotrophic organisms, plants evolved to capture light in an optimal manner and developed sophisticated strategies to perceive light signals and to transduce them into molecular signalling networks. Yet, high light intensities, as well as specific light wavelengths constitute an environmental stress that limits plant growth and development, especially if combined with other abiotic stimuli. My research focus is to understand the molecular mechanisms behind these responses, how they affect transcription and chromatin-associated events by employing, genetic, genomic and proteomic tools.

SELECTED PUBLICATIONS

Fonseca S, Rubio V. Arabidopsis CRL4 complexes: surveying chromatin states and gene expression. Front Plant Sci 2019; 10:1095.

Ortigosa A, Fonseca S, Franco-Zorrilla JM, Fernández-Calvo P, Zander M, et al. The JA-pathway MYC transcription factors regulate photomorphogenic responses by targeting HY5 gene expression. Plant J 2020; 102: 138-152.



The COP/DET/FUS repressors are essential to maintain plant viability by limiting the activity of HY5 transcription factor to primary targets.

Molecular mechanisms regulating plant resistance against bacteria

Selena Giménez Ibáñez

"Retos Jóvenes Investigadores" Fellow
Plant Molecular Genetics Department
Associated with Dr Roberto Solano's group

PERSONNEL

Santiago Michavilla Puente-Villegas (PhD Student, co-supervised with Dr R. Solano)

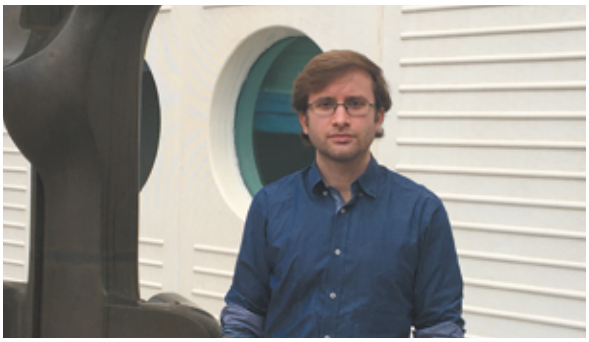
My research line falls into three areas of fundamental research, that are further combined with an additional directed applied line on important crops attacked by phytopathogenic *Pseudomonas* bacteria, such as tomato and kiwifruit. My research uses on one side, model plants such as *Arabidopsis*, *Nicotiana* and the liverwort *Marchantia*, to uncover the basic molecular mechanisms controlling hormonal plant immunity and how *Pseudomonas* bacteria infects hosts though its repertoire of effectors and phytotoxins. On the other side, this generated basic knowledge is directed to study these processes on crops, and to deliver novel strategies for crop protection against two of the most important disease caused by phytopathogenic *Pseudomonas*, the bacterial speck disease of tomato, caused by *P. syringae* pv. *tomato*, and the bacterial canker of kiwifruit, caused by *P. syringae* pv. *actinidiae*, by using biotechnology, genome editing, genetic breeding and searching for anti-infective potential novel chemicals among others. The aim is to gain knowledge into the molecular basis of hormonal plant immunity and infection by phytopathogenic *Pseudomonas*, towards the development of new solutions that could be applied into long-lasting strategies for crop protection against some of the most important diseases caused by *Pseudomonas* in crops, which negatively affect their cultivation worldwide.

SELECTED PUBLICATIONS

Ortigosa A, Gimenez-Ibanez S, Leonhardt N, Solano R. Design of a bacterial speck resistant tomato by CRISPR/Cas9-mediated editing of SlJAZ2. Plant Biotechnol J 2019; 17 (3): 665-673.

Gimenez-Ibanez S, Zamarreño A, Garcia-Mina JM and Solano R. An evolutionarily ancient immune system governs the interactions between *Pseudomonas syringae* and an early-diverging land plant lineage. Current Biology 2019; 29(14): 2270-2281.

Gimenez-Ibanez S. Designing disease-resistant crops: From basic knowledge to biotechnology. Mètode Science Studies Journal, n11, 2020 ISBN/ISSN: 2174-3487.



Homeostatic and pathogenic contribution of Th2 immunity in cardiovascular disease

Rodrigo Jiménez-Saiz

Junior Group Leader
Immunology and Oncology Department

PERSONNEL

Elisa Zubeldia (Visiting PhD student)
Domenico Rosace (Visiting Scientist)

The goal of the Jiménez-Saiz Lab (https://www.jimenezsaizlab.com/) is to understand immunological principles of Th2 immunity in the context of allergic disease, particularly as it pertains to acute allergic reactions (anaphylaxis) and its modulation by the microbiota, the maintenance of IgE immunity (memory responses), and the impact of allergic disease in the development of other pathologies.

Currently, our main line of research merges the fields of allergy (Th2 immunity) and cardiovascular disease (CVD) to answer clinically relevant, fundamental questions, on a serious health, economic and social challenge: understanding the causal relationship amid these two growing and menacing diseases. We use pre-clinical models of allergy and CVD to investigate the impact of allergic pathology on CVD and to define the mechanisms mediating this process. The knowledge generated in our group will provide mechanistic understanding on the putative pathologic effect of allergic responses on CVD, which will pave the way for the identification of therapeutic targets.

SELECTED PUBLICATIONS

Jimenez-Saiz R, Anipindi VC, Galipeau H, Ellenbogen Y, Chaudhary R, *et al.* Microbial Regulation of enteric eosinophils and its impact on tissue remodeling and Th2 immunity. Front Immunol 2020; 11: 155.

Barrio L, Roman-Garcia S, Diaz-Mora E, Risco A, Jimenez-Saiz R, *et al.* B cell development and T-dependent antibody response are regulated by p38gamma and p38delta. Front Cell Dev Biol 2020; 8: 189.

Riggioni C, Comberiati P, Giovannini M, Agache I, Akdis M, *et al.* A compendium answering 150 questions on COVID-19 and SARS-CoV-2. Allergy 2020; 75 (10): 2503-41.

Sokolowska M, Lukasik ZM, Agache I, Akdis CA, Akdis D, *et al.*

Immunology of COVID-19: Mechanisms, clinical outcome, diagnostics, and perspectives-A report of (EAACI). Allergy. 2020; 75(10): 2445-76.

Bruton K, Spill P, Vohra S, Baribeau O, Manzoor S, Gadkar S, *et al.* Interrupting reactivation of immunologic memory diverts the allergic response and prevents anaphylaxis. J Allergy Clin Immunol 2020; S0091-6749 (20) 31763-2.

Innate immunity, respiratory virus replication and pathogenesis

Marta López de Diego

"Atracción de Talento" Fellow
Molecular and Cellular Biology Department
Associated with Dr Luis Enjuanes and Dr Isabel Sola's group

PERSONNEL

Laura Villamayor Coronado (Postdoctoral researcher)
Sandra Gómez López (Technician)
Darío López García (JAE-Intro Graduated student)

Influenza viruses and coronaviruses are respiratory pathogens with drastic health and economic consequences for many animal species, including humans. In our group we are interested in analysing virus host-interactions, particularly the innate immune responses induced after respiratory virus infections, since these host responses affect viral replication and pathogenesis. Our final goal is to use the knowledge generated in our research to develop new antivirals to fight these and other viral infections, and to analyse viral and host genetic factors affecting the severity of respiratory virus diseases. As such we are (i) studying the cellular functions of interferon-stimulated genes and the effect of these genes on virus replication, on the induction of innate immune responses and virus pathogenesis, (ii) studying the functional effects of mutations on influenza virulence genes on virus replication, and pathogenesis, (iii) evaluating the effect of genetic polymorphisms on innate immune response genes in the severity of the diseases induced by influenza and coronaviruses, and (iv) developing antivirals mainly targeting innate immune response proteins and viral proteins.

SELECTED PUBLICATIONS

Nogales A, DeDiego ML. Host single nucleotide polymorphisms modulating Influenza A virus disease in humans. Pathogens 2019; 8 (4): 168.

DeDiego ML, Nogales A, Martinez-Sobrido L, Topham DJ. Interferon-induced protein 44 interacts with cellular FK506-binding protein 5, negatively regulates host antiviral responses, and supports virus replication. mBio 2019; 10 (4): e01839-19.

DeDiego ML, Martinez-Sobrido L, Topham DJ. Novel Functions of IFI44L as a Feedback Regulator of Host Antiviral Responses. J Virol 2019; 93 (21): e01159-19.

Nogales A, Ávila-Pérez G, Rangel-Moreno J, Chiem K, DeDiego ML, Martínez- Sobrido L. A novel fluorescent and bioluminescent bireporter influenza a virus to evaluate viral infections. J Virol 2019; 93(10):e00032-19.

Unravelling chloroplast protein quality control in plants

Pablo Pulido
"Atracción de Talento" Fellow
Plant Molecular Genetics Department
Associated with Dr. Vicente Rubio's group

PERSONNEL

Paloma Cabrerizo (undergraduate student)

Chloroplasts are the organelles that define plants. In plants, they are the unique sites of photosynthesis, the only significant mechanism of energy input into the biosphere. They also mediate numerous essential biosynthetic processes and contribute to many other functions including stress responses. As a result, correct chloroplast performance is absolutely indispensable for plant fitness and agriculture. Plants are sessile organisms that display an astonishing capacity to adapt to adverse conditions including heat, cold, drought, and salinity. However, prolonged exposure to environmental stress inevitably results in productivity losses. These challenging conditions for plant growth are highly relevant in the context of climate change and food security.

One of the main problems that stresses cause at molecular level is protein misfolding and aggregation. Recycling of damaged proteins is achieved by the action of molecular chaperones but, when recycling is not possible, toxic aggregated proteins have to be degraded by the action of proteases to avoid cellular damage. Chaperones and proteases act coordinately and constitute protein quality control (PQC) systems that are required for organismal survival. In our project, we address the characterization of the chloroplast proteostasis network. It is long known for instance that the chaperone HSP70 posttranslationally regulates important chloroplast processes such as photosynthesis. However, the precise molecular mechanisms of the chaperone action remain unresolved. Importantly, the specificity of HSP70 is driven by its DNAJ partners, adaptors that recognise unfolded substrates and transfer them to the chaperone for refolding. Thus, DNAJs are useful tools for plant editing. Besides, disrupted proteostasis results in protein aggregation inside chloroplasts triggering a chloroplast-to-nucleus retrograde signal that regulates the expression of nuclear genes encoding plastid-targeted chaperones. Ultimately, an essential hallmark of the project is to gain knowledge for rational engineering of chloroplast proteostasis and nuclear reprogramming that will assist to manipulate crops stress resistance.

Plant-Virus Coevolution

Adrian A. Valli
Ramón y Cajal Fellow
Plant Molecular Genetics Department
Associated with Dr Juan Antonio García's group

PERSONNEL

Irene Gonzalo Magro (Technician)
Rafael García Lopez (PhD Student)
Alfonso González de Prádena (PhD Student, co-supervised with Dr J.A. García)
Julio César Aragón Lago (Graduate Student JAE-INTRO)

RNA viruses are among the most abundant and economically relevant pathogens infecting plants; indeed, they cause more than 50% of viral crop damage worldwide. Gaining insight about this group of viruses is then critical to reveal and understand new features of them and discover novel plant protein networks acting as defensive barriers. Intriguingly, despite the importance of plant RNA viruses for food security, it is surprising to find that very little is known about their RNA-dependent RNA polymerases (RdRPs), putative RdRP protein partners and the precise role/s of these partners during infection.

As a relevant socio-economical case we currently study the partnership between RdRP and the pyrophosphatase HAM1 deriving from Ugandan cassava brown streak virus, one the agents causing the "Ebola of plants" in cassava, which is a plant that belongs to the huge *Euphorbiacea* family and is the fourth most important crop on earth. To do that we follow a multidisciplinary study that includes (i) synthetic biology to build chimerical infectious clones, (ii) genomics studies to define viral quasispecies variability, (iii) structural studies by cryo-electron microscopy to define protein structures, (iv) metabolomics studies by HPLC-MS/MS to understand viral diseases, and (v) viral ecology to decipher the interaction between viruses and euphorbiaceous in nature. These approaches will greatly help us to fill gaps in our understanding of RdRPs in general, as well as the RdRP-HAM1 partnership.

SELECTED PUBLICATIONS

Ochoa J, Valli A, Martín-Trillo M, Simón-Mateo C, García JA, Rodamilans B. Sterol isomerase HYDRA1 interacts with RNA silencing suppressor P1b and restricts potyviral infection. Plant Cell Environ 2019; 42: 3015-3026.

González de Prádena A, Sánchez-Jiménez A, San León D, Simmonds P, García JA, Valli, AA. Plant virus genome is shaped by specific dinucleotide restrictions that influence viral infection. mBio 2020; 11: e02818-19.

CNB seminars

In 2019 and the beginning on 2020, the CNB hosted around 150 seminars, including talks by international renowned institutions speakers. To overcome the difficulties of inviting speakers during COVID-19 pandemic situation, we started holding online seminars (20), which has become a new opportunity to reach wider audiences.

SEMINARS CYCLE 2019

1 MARCH

Genomics of the origin and evolution of citrus

Manuel Talón

Centro de Genómica IVIA, Spain

22 MARCH

The power of cryo-EM to elucidate biological mechanisms

Stephan Rausner

Max Plank Institute of Molecular Physiology, Germany

5 APRIL

Interplay between mutation supply and relative fitness in the evolution of antibiotic resistance

Diarmaid Hughes

Uppsala University, Sweden

12 APRIL

Uncovering the hidden half of plant development

Malcom Bennet

School of Biosciences, University of Nottingham, UK

26 APRIL

Mechanisms of leukocyte extravasation across post capillary venules of the brain: The role of the endothelial basement membrane and matrix metalloproteinases

Lydia Sorokin

Institute of physiological Chemistry and Pathobiochemistry, University of Muenster, Germany

17 MAY

The Human Protein Atlas and insights from profiling plasma proteomes

Jochen Schwenk

School of Biotechnology, KTH Royal Institute of Technology, Sweden

24 MAY

Immunotherapy and new GTPase-mediated molecular mechanisms for the treatment of ALK tumors

Roberto Chiarle

Boston Children Hospital, Harvard Medical School, USA

20 SEPTEMBER

A lab of one's own: Science and Suffrage in the First World War

Patricia Fara

Clare College Cambridge, UK

15 NOVEMBER

A new perspective into the origin of animals

Iñaki Ruiz-Trillo

Institut de Biologia Evolutiva (CSIC - IBE), Spain

22 NOVEMBER

Role of CRISPR-Cas systems associated to retrotranscriptases in the defence against phages

Antonio Sánchez Amat

Universidad de Murcia, Spain

JUNIOR SEMINARS 2019

11 JANUARY

Forward thinking: pro-active coordination of shoot architecture by long distance hormonal signalling in plants

Tom Bennett

University of Leeds, UK

08 FEBRUARY

Signal and noise – New tools for cryo-EM density interpretation

Arjen Jakobi

Kavli Institute, The Netherlands

22 FEBRUARY

Bacterial cell division: may the force be with you

Mariana Gomes de Pinho

Instituto de Tecnologia Química e Biológica António Xavier, Universidad Nova de Lisboa, Portugal

15 MARCH

Engineering neurogenesis for the postnatal cerebral cortex

Benedikt Berninger

King's College, London

10 MAY

Dealing with change and uncertainty: optimal growth control across environments and individuals

Benjamin Towbin

Friedich Miescher Institute for Biomedical Research, Switzerland

14 JUNE

Nuclear mechanobiology in cancer cell migration and muscular dystrophy

Jan Lammerding

Well Institute for Molecular and Cellular Biology, Cornell University, USA

27 SEPTEMBER

Probing bacterial regulation strategies by quantitative analysis of growth and death in variable environments

Ulrich Gerland

Technical University of Munich, Germany

4 OCTOBER

Chemoreceptor based signaling in bacteria

Tino Krell

Estación Experimental del Zaidín, Spain

8 NOVEMBER

Structural insights into the infection process of bacteriophages

Nicholas Taylor

Novo Nordisk Foundation Center for Protein Research, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

29 NOVEMBER

Plant signal transduction cascades - from phenotypes to atoms and back

Michael Hothorn

Department for Botany and Plant Biology, University of Geneva, Switzerland

SEMINARS CYCLE 2020

10 JANUARY

Engineering Genetic Control Systems

Mustafa Khammash

ETH Zürich, Switzerland

14 FEBRUARY

Tara Oceans: eco-systems biology at planetary scale

Chris Bowler

École Normale Supérieure Paris, France

23 OCTOBER

Conversion of *E. coli* to generate all biomass carbon from CO_2

Ron Milo

Weizmann Institute of Science, Rehovot, Israel

30 OCTOBER

Zooming in on the coronavirus replication organelle

Montserrat Bárcena

Leiden University Medical Center, The Netherlands

6 NOVEMBER

Sensing matrix rigidity: transducing mechanical signals from integrins to the nucleus

Pere Roca-Cusachs

IBEC, Instituto de Bioingeniería de Cataluña, Spain

13 NOVEMBER

Role of titan cells in the virulence of the pathogenic yeast *Cryptococcus neoformans* and new therapeutical approaches

Oscar Zaragoza

National Centre for Microbiology, ISCIII, Spain

27 NOVEMBER

Host microbe interactions in the intestine in health and disease

Fiona Powrie

Kennedy Institute of Rheumatology and Translational Medicine, University of Oxford, UK

JUNIOR SEMINARS 2020

17 JANUARY

The global ocean microbiome through the lens of metaomics

Shinichi Sunagawa

ETH Zürich, Switzerland

31 JANUARY

Influenza virus-host interactions

Adolfo García-Sastre

Icahn School of Medicine at Mount Sinai, USA

21 FEBRUARY

Novel targets and biomarkers of PD-1 inhibitory function

Vassiliki Boussiotis

Beth Israel Deaconess Medical Center. Boston, USA

9 OCTOBER

Plasma membrane-to-chloroplast communication: learning from viruses

Rosa Lozano-Duran

Shangai Center for Plant Stress Biology (Chinese Academy of Sciences), China

16 OCTOBER

Integrins in immune cells: New roles for old players

Susanna Fagerholm

Faculty of Biological and Environmental Sciences, University of Helsinki, Finland

10 NOVEMBER

The immune system of bacteria: Beyond CRISPR

Rotem Sorek

Weizmann Institute of Science Rehovot, Israel

20 NOVEMBER

Breath of life: oxygen sensing across eukaryotic kingdoms

Francesco Licausi

Wadham College, Oxford University, UK

4 DECEMBER

Systems biology and model-based analysis of multi-omic microbiome data

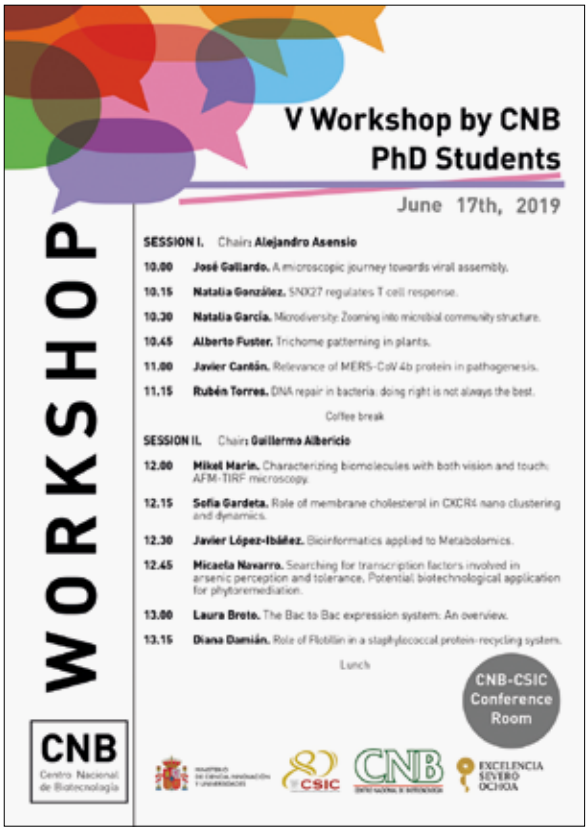
Elhanan Borenstein

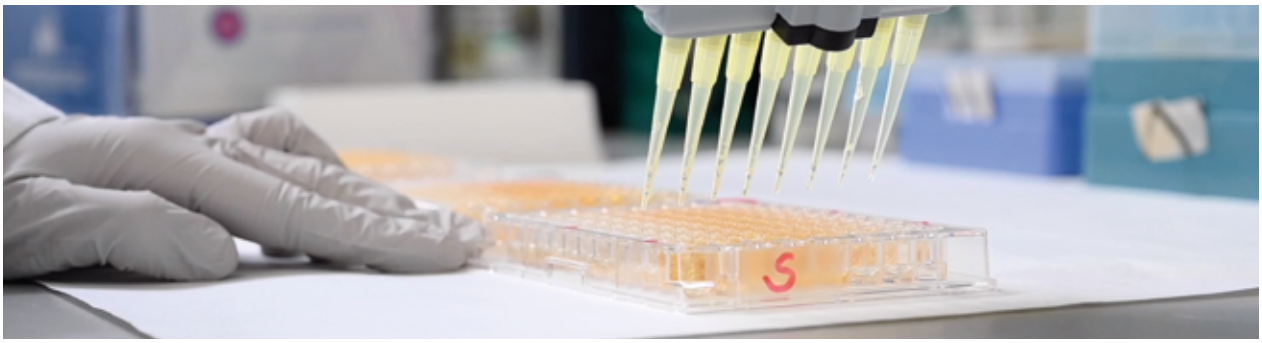
Blavatnik School of Computer Science & Sackler Faculty of Medicine, Tel Aviv University, Israel


Looking at Cell Biology From a Virus Perspective
A tribute to Amelia Nieto on her retirement
Wednesday January 29, 2020 Lecture Hall CNB
11:45 Symposium Opening by the CNB Director
Scientific Program
Session 1. Chair: Pablo Gastaminza
12:00 Ana Falcón
Algenex, Madrid, Spain
Human Influenza A virus causes heart infection, cardiac conduction disorders and premature death
12:15 Santiago F. González
Institute for Research in Biomedicine, Università della Svizzera italiana. Bellinzona, Switzerland
Protection against influenza virus requires early recognition by inflammatory dendritic cells through C-type lectin receptor SIGN-R1
12:30 Emilio Yángüez
Functional Genomics Center (ETH/UZH) Zurich. Zurich, Switzerland
Combination of scRNA-seq strategies to untangle complex cell populations
12:45 Ariel Rodríguez
Sanford Burnham Prebys Medical Discovery Institute. La Jolla, CA, USA.
Identification of new host restriction factors affecting influenza A virus replication in primary human respiratory epithelial cells
13:00 Alicia Pérez
Spanish Agency for Medicines and Medical Devices (AEMPS). Madrid, Spain
From virus-cell interaction to vaccine regulation
13:15 Maite Huarte
Centro de Investigación Médica Aplicada (CIMA-UNAV). Pamplona, Spain
lncRNAs as functional molecules in cancer pathways
13:30 Tomás Aragón
Centro de Investigación Médica Aplicada (CIMA-UNAV). Pamplona, Spain
XBP1 mRNA regulation: A serendipitous path
13:45 Juanjo Sanz-Ezquerro
Centro Nacional de Biotecnología (CSIC). Madrid, Spain
Embryonic development in vertebrates: signalling pathways and genes
Session 2. Chair: Urtzi Garaigorta
15:15 Puri Fortes
Centro de Investigación Médica Aplicada (CIMA-UNAV). Pamplona, Spain
From RNA viruses to oncogenic long non-coding RNAs
15:30 Susana de la Luna
ICREA and Center for Genomic Regulation (CRG). Barcelona, Spain
A tale of RNA polymerases
15:45 Juan Valcárcel
ICREA and Center for Genomic Regulation (CRG). Barcelona, Spain
Mechanisms of alternative splicing regulation in cancer
Amelia Nieto, PhD
Scientific Researcher
CNB-CSIC
16:00 Homenaje a Amelia Nieto
con intervenciones de
Pablo Gastaminza
Urtzi Garaigorta
Juanjo Sanz-Ezquerro
María Ángeles Rodríguez
Margarita Díaz Guerra
Blanca García-Barreno
Tomás Aragón
Nani Marión
Juan Ortín
Laura Marcos
Noelia Zamarreño
The Company of Biologists
https://www.biologists.com/


Biology for the
21st Century
May 20th 2019
Venue:
Centro Nacional de Biotecnología (CNB)
C/ Darwin 3.Carretera de Colmenar Km 16,
Campus UAM, 28049 Madrid
PROGRAM
09:30-09:35
Welcome by the CNB director
09:35-09:45
A few words from the CSIC President
09:45-10:00
A few words from the Deputy Minister of Science
SCIENTIFIC SESSION
10:00-10:45 FREDERICK ALT, Boston Children's Hospital, Harvard Med Sch., Boston, MA, USA
The Fundamental Role of Chromatin Loop Extrusion in Antibody Diversification
10:45-11:30 JUAN CARLOS IZPISUA BELMONTE, Salk Institute for Biological, CA, USA
Aging and Organ Regeneration
11:45-12:30 SERGIO LIRA, Icahn School of Medicine at Mount Sinai, NY, USA
IL-23 + bugs + X = colitis
12:30-13:15 TAK MAK, The Campbell Family Institute for Breast Cancer Research, Toronto, Canada
Does the Immune System Think?
13:15-14:00 Antonio Coutinho, Instituto Gulbenkian de Ciência, Portugal
An Experiment on Graduate Education


Cincuenta Aniversario
UAM
CSIC
25 YEARS OF CRYOELECTRON MICROSCOPY IN SPAIN: A TRIBUTE TO
JOSÉ L. CARRASCOSA
Madrid, 12-13 June 2019
Lecture Hall School of Biology Universidad Autónoma de Madrid
Plenary Speakers
Wolfgang Baumeister
MPI Martinsried
Joachim Frank
Columbia University
Nobel Prize in Chemistry 2017
Richard Henderson
LMB Cambridge
Nobel Prize in Chemistry 2017
Alasdair Steven
NIH Bethesda
Speakers
N. Abrescia (CIC-bioGUNE)
J. Agirre (University of York)
S. Alvira (University of Bristol)
E. Arias-Palomo (CIB-CSIC)
J.M. Carazo (CNB-CSIC)
D. Castaño-Díez (Biozentrum)
P. Chacón (IQFR-CSIC)
M. Coll (IBMB-CSIC)
J. Conesa (ALBA)
S. Connell (CIC-bioGUNE)
J.J. Fernández (CNB-CSIC)
R_F. Fusnadiego (MPI Martinsried)
C. Fernández Tornero (CIB-CSIC)
I. Fita (IBMB-CSIC)
J. Fontana (University of Leeds)
B. Herguedas (LMB)
A. Hierro (CIC bioGUNE)
D. Lietha (CIB-CSIC)
O. Llorca (CNIO)
C. López-Iglesias (Maastricht University)
D. Luque (IISCIII)
J. Martín-Benito (CNB-CSIC)
A. Martínez (MPI Martinsried)
J. Ortega (McGill University)
P. Pérez Navarro (Biozentrum)
V. Rubio (IBV-CSIC)
José R. Castón (CNB-CSIC)
M. Saensó (Virginia C. University)
C. San Martín (CNB-CSIC)
I. Ubarretxena (Biofísika)
I. Usón (IBMB-CSIC)
M. Valle (CIC-bioGUNE)
J.M. Valpuesta (CNB-CSIC)
J. Vargas (McGill University)
ThermoFisher SCIENTIFIC
CNB
EXCELENCIA SEVERO OCHOA


WORKSHOP
V Workshop by CNB
PhD Students
June 17th, 2019
SESSION I. Chairs: Alejandro Asensio
10.00 José Gallardo. A microscopic journey towards viral assembly.
10.15 Natalia González. SNX27 regulates T cell response.
10.30 Natalia García. Microdiversity: Zooming into microbial community structure.
10.45 Alberto Fuster. Trichome patterning in plants.
11.00 Javier Cantón. Relevance of MERS-CoV 4b protein in pathogenesis.
11.15 Rubén Torres. DNA repair in bacteria: doing right is not always the best.
Coffee break
SESSION II. Chairs: Guillermo Albericio
12.00 Mikel Marin. Characterizing biomolecules with both vision and touch: AFM-TIRF microscopy.
12.15 Sofía Gardeta. Role of membrane cholesterol in CXCR4 nano clustering and dynamics.
12.30 Javier López-Ibáñez. Bioinformatics applied to Metabolomics.
12.45 Micaela Navarro. Searching for transcription factors involved in arsenic perception and tolerance. Potential biotechnological application for phytoremediation.
13.00 Laura Brote. The Bac to Bac expression system. An overview.
13.15 Diana Damián. Role of Flotillin in a staphylococcal protein-recycling system.
Lunch
CNB-CSIC Conference Room
CNB Centro Nacional de Biotecnología
CSIC
CNB
EXCELENCIA SEVERO OCHOA



Scientific meetings and courses

CNB researchers have participated in the organisation of almost 50 conferences, workshops and courses in the last two years.

2019

11 FEBRUARY (CNB)
Programmability and predictability of Biological Systems
Víctor de Lorenzo, Juan Nogales, Juan Poyatos

15 FEBRUARY (CNB)
Neurodevelopmental disorders and brain repair symposium
Marta Nieto

5-29 MARCH (CNB)
Training course: Biotechnology facing the challenges of today's society
José Manuel Franco and Leonor Kremer

13-15 MARCH
Madrid, Spain
CECAM workshop: From sequences to functions: challenges in the computation of realistic genotype-phenotype maps
Susanna Manrubia, José A. Cuesta

20 MAY (CNB)
Biology of the 21st Century
Ana Clara Carrera, Isabel Mérida, Mario Mellado

22-24 MAY
Alcalá de Henares, Spain
Instruct Biennial 2019
José María Carazo

12-13 JUNE (CNB)
25 years of cryoelectron microscopy in Spain: a tribute to José L. Carrascosa
Jose María Carazo, José R Castón, Jose María Valpuesta

16-21 JUNE
Miraflores de la Sierra, Spain
VIII National Genetic Course
Almudena Fernández and Lluís Montoliu

17 JUNE (CNB)
Predoctoral Scientific Workshop
CNB PhD Student's Committee

19-22 JUNE
London, UK
Invadosome consortium 7th meeting: Mechano-chemical signals in invasion
Inés M Antón

26-29 JUNE
Salamanca, Spain
3rd European Chemokine and Cell Migration Conference
Mario Mellado

26-28 JUNE
Valencia, Spain
GEIVEX Symposium on Extracellular Vesicles In Biomedicine
Mar Valés-Gómez

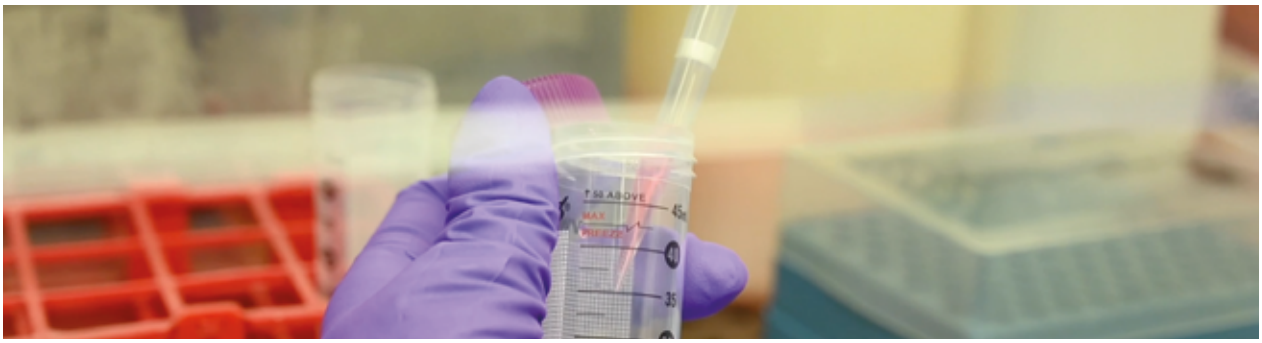
1-2 JULY (CNB)
II Practical course on Genome Editing and Gene Therapy
Almudena Fernández and Lluís Montoliu

08-11 JULY
Madrid, Spain
Instruct course on Image Processing for Electron Microscopy and hybrid modelling
José María Carazo and Carlos Óscar Sorzano

16-19 JULY
Madrid, Spain
42º Congreso de la Sociedad Española de Bioquímica y Biología Molecular
Fernando Moreno-Herrero, Juan José Sanz (Biochemistry in the city)

16-19 JULY (CNB)
Gene Regulation and Cell Signalling Symposium at the 42 SEBBM Congress
Ana Cuenda

20-24 JULY
Madrid, Spain
Evolutionary dynamics Symposium at the 12th EBSA and 10th ICBP-IUPAP Biophysics Congress
Susanna Manrubia



20-24 JULY

Madrid, Spain

12th EBSA and 10th ICBP-IUPAP Congress

José María Valpuesta

1-13 SEPTEMBER

Madrid, Spain

2nd Edition of the Instruct course on Image Processing for Electron Microscopy and hybrid modelling

José María Carazo and Carlos Óscar Sorzano

11-13 SEPTEMBER

Madrid, Spain

Microscopy at the Frontiers of Science 2019 (6th Joint Congress of the Spanish and Portuguese Societies of Microscopy)

Carmen San Martín

15-22 SEPTEMBER

Heidelberg, Germany

EMBO Practical Course: Synthetic Biology in Action: Bridging Natural/ Non-Natural

Víctor de Lorenzo

5 NOVEMBER

Granada, Spain

Biomarkers and EVs: concepts, advances and technical considerations. Hands-on GEIVEX workshop

Mar Valés-Gómez

6-8 NOVEMBER

Madrid, Spain

5th International GEIVEX symposium

Mar Valés-Gómez

8 NOVEMBER

Edinburgh, United Kingdom

From DNA to RNA synthesis, processing and cancer symposium

Susana de Lucas

14 NOVEMBER

Paris, France

2019 ARRIGE (Association for Responsible Research and Innovation in Genome Editing) annual meeting

Lluís Montoliu

19 NOVEMBER (CNB)

Emprendimiento e innovación: oportunidades desde la perspectiva de género

Cristina Merino

22-23 NOVEMBER

Paris, France

Workshop: Grant evaluation assessment for graduate students, Institute Pasteur

Daniel López

25-27 NOVEMBER

Madrid, Spain

IPAD-MD and INFRAFRONTIER Annual Meeting 2019

Lluís Montoliu

28-29 NOVEMBER

Madrid, Spain

2nd ASEICA Educational Symposium

Ana Cuenda

16-17 DECEMBER (CNB)

XXVII Scientific Workshop

Susana de Lucas

19 DECEMBER (CNB)

XXVII CNB Workshop “Advances in Molecular Biology by Young Researchers Abroad

Inés M Antón, Susana de Lucas, Mar Valés, Silvia Ayora, Domingo F Barber, Urtzi Garaigorta, Sandra Fonseca, Juan Poyatos, Carmen San Martín

2020

29 JANUARY (CNB)

Looking at Cell Biology From a Virus Perspective: A tribute to Amelia Nieto on her retirement

Urtzi Garaigorta, Pablo Gastaminza, Laura Marcos, Susana de Lucas, Juan Ortín, Noelia Zamarreño

30-31 JANUARY

Madrid, Spain

Understanding and reprogramming developmental visual disorders: from anophthalmia to cortical impairments

Paola Bovolenta, Marta Nieto

JANUARY-JUNE

e-learning

Curso de especialización en vesículas extracelulares

GEIVEX, Universidad Francisco de Vitoria

Mar Valés-Gómez

7 FEBRUARY (CNB)

Evolution of antibiotic resistance workshop

José Luis Martínez, Álvaro San Millán, Jesús Blázquez

19 FEBRUARY (CNB)

Latest advances in microscopy technologies

Sylvia Gutiérrez-Erlandsson, José María Valpuesta

28 FEBRUARY (CNB)

Colloquium on Systems and Synthetic Biology Mapping, understanding and engineering the microbiome

Víctor de Lorenzo, Juan Nogales, Juan Poyatos, Javier Tamames

11-15 JUNE

Glasgow, UK (Online)

FENS Forum

Marta Nieto

3 AND 17 JUNE (CNB)

2nd Simposium NanoBiocargo: design, development and production of nanocontainers and nanovehicles

José R Castón, José María Valpuesta

29 JUNE

Online

III Practical course on Genome Editing and Gene Therapy

Almudena Fernández, Lluís Montoliu

26-30 OCTOBER

Online

Instruct Course on the development of image processing workflows in streaming and structural data analysis components for Electron Microscopy

José María Carazo, Carlos Óscar Sorzano

4-6 NOVEMBER

Online

17th ASEICA International Congress

Ana Cuenda

4-7 NOVEMBER

Online

5th European Days of Albinism (5EDA)

Lluís Montoliu

14 NOVEMBER

Online

2020 ARRIGE annual meeting

Lluís Montoliu

14-18 DECEMBER

Madrid, Spain

Instruct virtual course on Single Particle Analysis by CryoEM

José María Carazo, Carlos Óscar Sorzano

17 DECEMBER

Online

GEIVEX-UFV / TeNTaCLES 2020 Minisymposium on EVs

Mar Valés-Gómez

16-17 DECEMBER (CNB)

Online

XXVIII CNB Scientific Workshop

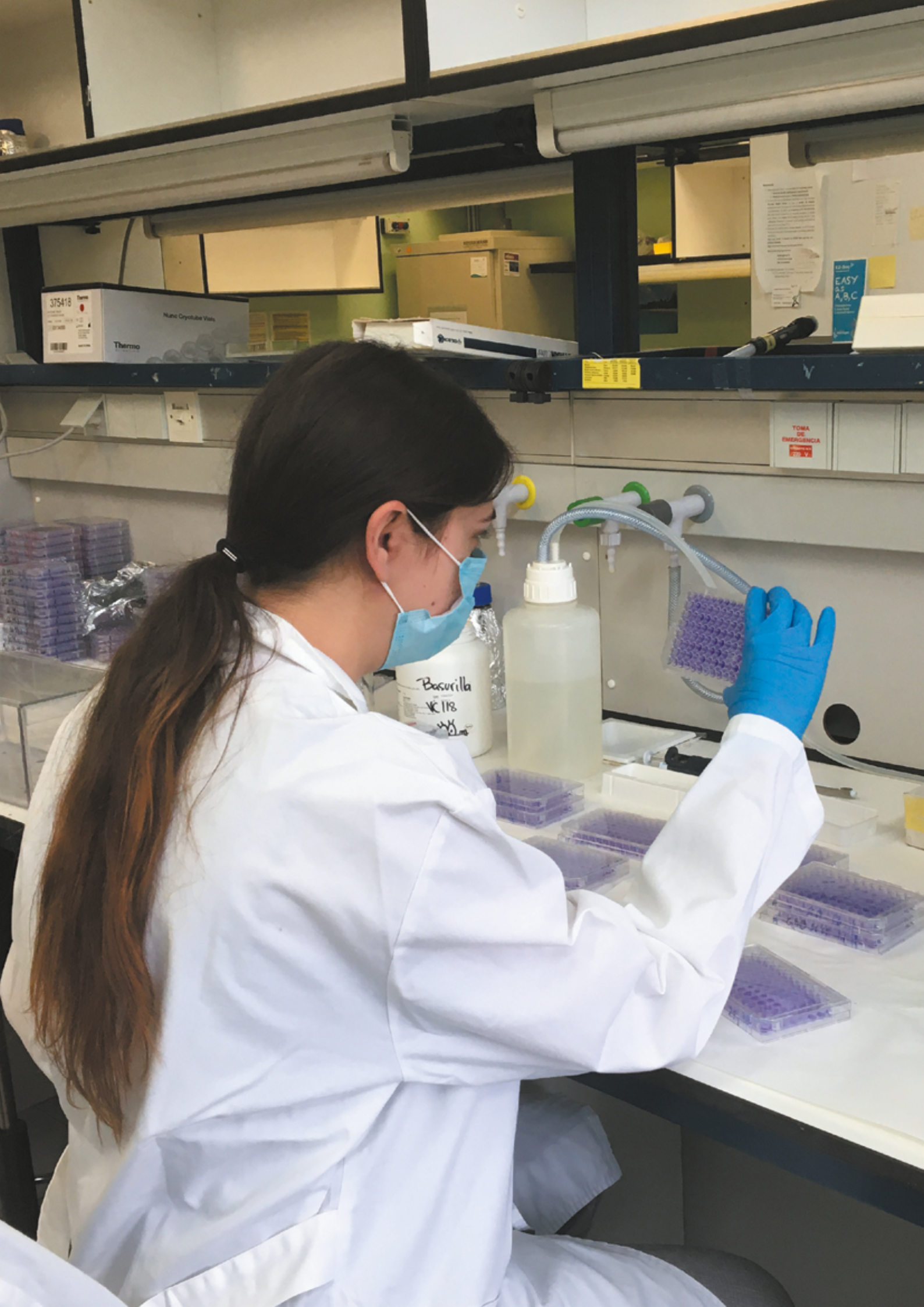
Susana de Lucas, Ricardo Villares

21 DECEMBER (CNB)

Online

XXVIII CNB Workshop Advances in Molecular Biology by Young Researchers Abroad

Inés M Antón, Susana de Lucas, Carmen San Martín, Mar Valés, Urtzi Garaigorta, Alvaro San Millán, Sandra Fonseca, Pablo Pulido, Juan Poyatos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TOMA DE EMERGENCIA

11

EQUALITY

The CNB is strongly committed to promote gender equality in the academic and research environment, and to ensure that the principle of equal opportunities is respected without any discrimination due to gender, ethnicity, religion, political affiliation, sexual orientation or disability.

In the last two years we have actively worked to improve gender equality, with different initiatives such as the facilitation of breastfeeding areas, preparation of a report analysing gender balance in the CNB and promotion of women in innovation with the Conference: *Entrepreneurship and Innovation, opportunities from a Gender Perspective*.

We have continued our collaboration in the 5Th and 6Th edition of the Science by Women programme from The *Women for Africa Foundation,* whose aim is to promote African women's leadership in scientific research and technology transfer and to foster the capacity of the research centres in their home countries.

In addition, we have participated in joint activities organised with other CSIC centres located in the Campus of the Autonomous University of Madrid (UAM). These include training and organisation of courses, outreach campaigns and activities to raise awareness of equity and its importance, and increase the visibility of research made by Women by celebrating *February 11th (#11F)*, the *International Day for Women and Girls in Science*; *March 8th*, the *International Women Day or November 25th*, the *International Day for the Elimination of Violence Against Women*.

Our own Equality Committee has been consolidated in 2020 and is already working to implement a feasible Plan of Equality in our Institute in the next years. This committee has prepared the report *"Women-CNB"* analysing the status of the CNB in terms of gender/sex, in order to detect any possible inequality in this regard and to be able to adopt measures aimed at improving this situation as described in the CNB's Equality Plan.

Since the CNB is bound to public salary schemes, there are no salary differences for the same position between male and female employees at the centre.

From the CNB it is necessary to continue promoting measures that guarantee equal opportunities and contribute to the inclusion and permanence of women in the scientific career. Some of these measures are described in the *CNB Equality Plan* (available in our website www.cnb.csic.es/index.php/es/equality).

CNB EQUALITY COMMITTEE

Saúl Ares
Pilar Cubas
Ana Cuenda
Mónica Chagoyen
Daniel López
Susana de Lucas
Cristina Merino
Carmen San Martín
Juan José Sanz-Ezquerro


Primavera

12

COMMUNICATION AND OUTREACH

The Communications and Outreach Office works to increase the awareness of the research carried out by CNB scientists and to strengthen the bonds of the Centre with other academic institutions, as well as with the media and the general public.

During 2019 and 2020, we have issued more than 50 press releases highlighting the scientific achievements by CNB researchers. Since 2017, we have observed a steady increase in the media coverage of our research and activities, from around 75 appearances in 2017, to 400 appearances in 2019, and more than 1,500 in 2020. This extraordinary increase is mostly due to the involvement of many CNB researchers with long-standing experience in virology and immunology in new COVID-19 studies, from vaccine candidates to diagnostic solutions, treatments, and pandemic evolution. The CNB Communications office, in coordination with the CSIC Communications department, has served as a bridge to respond to inquiries from local, national and international media in these challenging times.

The office also maintains dialogue with the public through social networks, with a community of 4,400 followers on Facebook, 23,500 on Twitter, 5,600 on LinkedIn and 610 subscribers in YouTube, respectively. Our videos in YouTube have been visited by more than 60,000 viewers and the posts in the Blog “CNB Divulga” have received more than 9,000 reads in the last two years.

With the dedicated and indispensable collaboration of the centre’s scientists, the office coordinated activities within the framework of the European Researcher’s Night, the National Science and Technology Week, Plant Fascination Day and the celebration of the International Day of Women and Girls in Science (February 11Th), and participated in the 100xCiencia.4 meeting (San Sebastián, November 2019), an international forum for scientific communication organised by the Severo Ochoa Centre and María de Maeztu Units of Excellence (SOMMA). The office also coordinated monthly guided visits for high school students. Although 2020 has been a challenging year for public engagement, we managed to celebrate online events such as virtual visits, talks and workshops for kids and schools at the European Researcher’s Night and the National Science and Technology Week that were attended by more than 700 people.

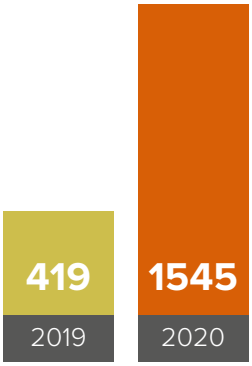
In addition, the office acts as a link with the Scientific Activities Committee, the CNB Training Advisory Committee to organise the CNB seminars, workshops and training activities for PhD students and with the Knowledge Transference Office to promote Innovations events.

We would like to acknowledge the support and involvement of Miguel Vicente, Peter Klatt and Susanna Manrubia in the office’s activities.

COMMUNICATION AND OUTREACH MANAGER

Susana de Lucas

Selected media appearances




EL PAÍS

Materia

El coronavirus, un reto que fomenta la cooperación científica

La lucha contra la pandemia de covid19 ha obligado a abrir los laboratorios y potenciar la colaboración interdisciplinar entre científicos, facilitando la circulación de datos como nunca antes y difundiendo la divulgación entre la sociedad

El País 16-4-2020


ABC ENFERMEDADES

Describen dónde se encuentran las células madre en el corazón y su evolución en función de la edad

ABC, 6-5-2019

Onda Cero, 11-6-2019


elPeriódico

Las plantas se comportan de forma distinta en respuesta al calor según el lugar donde crecen

El Periódico, 17-6-2019


eldiario.es

TECNOLOGÍA

DIRECTO Refugiadas, mujeres perseguidas, en Carne Cruda

Una mutación natural y el cruce de diversas variedades creó la patata europea

El Diario, 24-6-2019


20 minutos, 20-8-2019

Investigador del CNB-CSIC apunta a las bacterias como las "grandes aliadas" contra el cambio climático

El profesor de Investigación del Centro Nacional de Biotecnología del Consejo Superior de Investigaciones Científicas (CNB-CSIC) Víctor de Lorenzo ha apuntado a la utilidad de las bacterias para abordar el cambio climático. "Los grandes aliados para abordar este problema son precisamente las bacterias que viven en nuestro planeta", ha asegurado.


Infosalus, 8-10-2019

infosalus.com

El cerebro en desarrollo tiene una mayor plasticidad de la que se pensaba hasta ahora


SINC, 22-11-2019

Cómo controlar la conexión entre redes complejas


SINC, 30-4-2020

Identifican el mecanismo que emplea el virus de la gripe para provocar daño cardíaco


Madri+d, 8-5-2020

El reovirus humano 'secuestra' parte de la célula infectada tras convertirla en una fábrica de nuevos virus


El Independiente, 21-5-2020

Dromedarios canarios contra el coronavirus, así es el tratamiento anticovid español


SINC, 29-6-2020

Descubierto un nuevo biomarcador que predice la eficacia de la inmunoterapia en cáncer

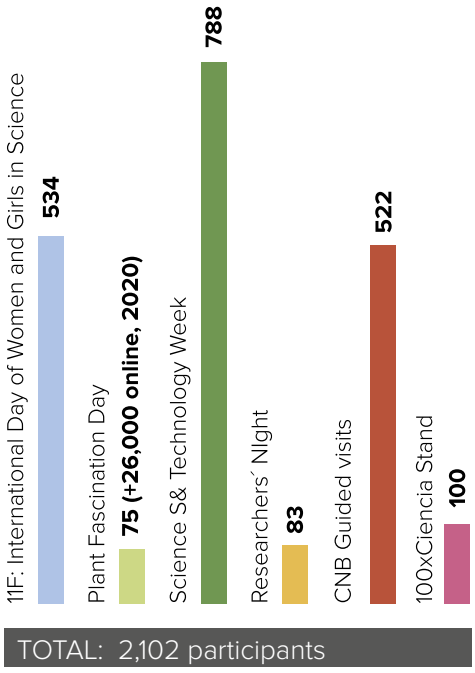

SINC, 5-8-2020

Descubren una estrategia terapéutica eficaz frente a bacterias resistentes

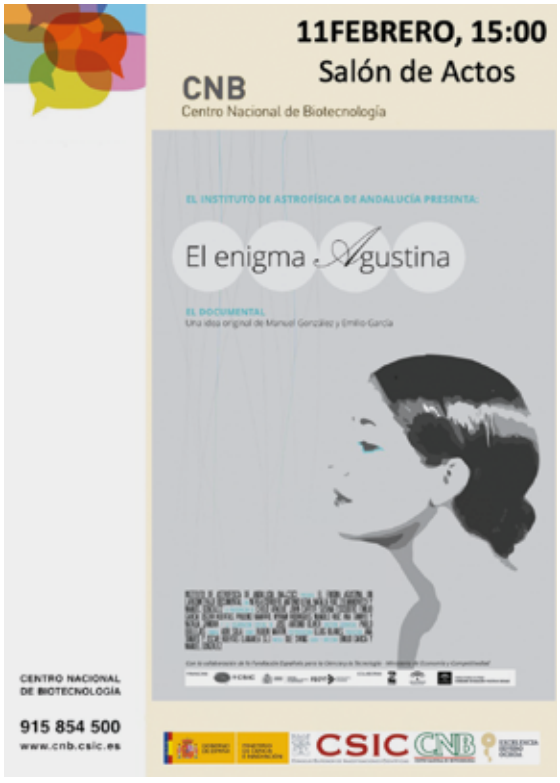
Outreach activities

23,500	4,400	5,650	610

Social Media Followers



Guided visits for secondary school and university students. More than 500 students visited our facilities in 2019 and 2020, including students from schools in the new CSIC program “Ciencia en el Barrio”.

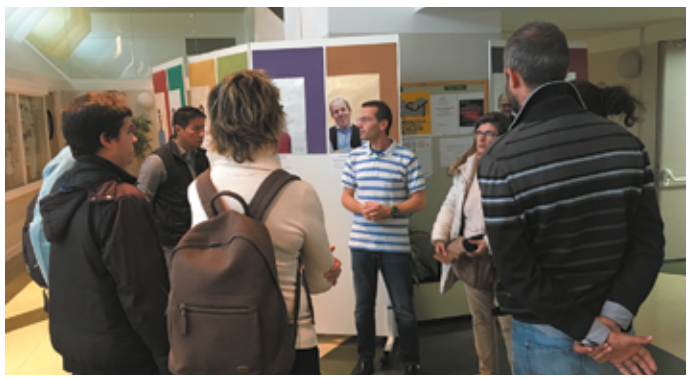


International Day of Women and Girls in Science. In the last 2 years we have developed activities to celebrate the 11F in collaboration with other CSIC centres from the Cantoblanco Campus as well as our own.

100xCiencia 4, San Sebastian. We brought a stand to 100XCiencia Science Fair "How is Science helping us?", celebrated within the frame of the international Science Communication forum by the Severo Ochoa Centre and Maria de Maeztu Units of Excellence (SOMMA).

Plant Fascination Week In 2019, we organised an exhibition in our hall with pictures from Madrid Botanic Garden, developed two workshops based in Plant Biotechnology and invited Dr Manuel Pardo de Santayana from the UAM. In 2020, to overcome lockdown, we organised an online campaign, where researchers from the Genetic Plant Department explained their fascination with plants in short divulgative videos that were distributed in our social media, reaching 26,000 impressions in Twitter and Facebook.

Researchers Night. The CSIC centres from Cantoblanco celebrated the joint event "Research with CSIC at MediaLab Prado", where the institutes offered worskshops for schools, families, and the general public in the city centre.



National Science and Technology Week, every year we offer a broad spectrum of activities, from guided tours to our facilities to divulgative talks, workshops and even an Escape Room. All our activities in 2020 have become virtual, allowing the attendance of a wider audience.

13

DIRECTION AND MANAGEMENT

Scientific Advisory Board

Direction team

Management team

CNB Staff

Scientific advisory board

Our Scientific Advisory Board has been recently renewed having in mind the forthcoming 5-year review of the centre's strategy and performance during the 2016-2020 period. Members of the new SAB are 8 eminent scientists in the Centre's major research areas.

Wolfgang Baumeister

Director of the Department of Structural Biology, Max Planck Institute for Biochemistry, Martinsried, Germany.

Yaakov Benenson

Professor for Synthetic Biology, Department of Biosystems Science and Engineering, ETH Zurich, Switzerland.

Martin Crespi

Director of the Institute of Plant Sciences Paris-Saclay (IPS2), Gif-sur-Yvette, France.

José Luis García-López

CSIC Research Professor for Environmental Biotechnology, Centro de Investigaciones Biológicas (CIB), Madrid, Spain.

George Kollias

President and Director of the Biomedical Sciences Research Center (BSRC) "Alexander Fleming", Vari, Greece.

Christine Orengo

Professor of Bioinformatics, University College London, UK.

Geoffrey L. Smith

Head of the Department of Pathology, Division of Virology, University of Cambridge, UK.

Fiona M. Watt

Director of the Centre for Stem Cells and Regenerative Medicine, King's College London, UK.

Direction and management

DIRECTOR

Mario Mellado *(2020)*
(vicedirector 2019)

VICEDIRECTOR

Fernando Rojo *(2020)*
(director 2019)

VICEDIRECTOR

Susanna Manrubia *(2020)*

TECHNICAL VICEDIRECTOR

Peter Klatt *(until July 2020)*

ADJUNCT MANAGER

Ricardo Villares García
(from September 2020)

ASSISTANT TO THE DIRECTOR

Yolanda García

OUTREACH ADVISOR

Miguel Vicente

SCIENTIFIC COMMUNICATION AND OUTREACH MANAGER

Susana de Lucas

TECHNOLOGY TRANSFER MANAGER

Cristina Merino

General Manager

Isabel Sevillano

Project Management

HEAD

Daniel Martín Hernando
(from July 2020)

Soraya Olmedilla María
(until July 2020)

PERSONNEL

Aránzazu Almendro

Pilar Ara Laúna

Beatriz de los Frailes
(from November 2020)

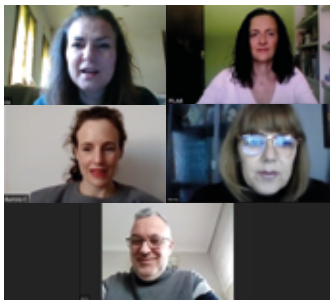
Sergio Gómez
(from March 2020)

Daniel Martín Hernando

Irene López-Vidriero
(until November 2020)

Diana G. Pastor Calero
(until July 2020)

Sergio Sierra
(from October 2020)



Human Resources

HEAD

Marina Hernando Bellido

PERSONNEL

Aurora Cabrerizo Alonso

Pilar Corral Cid

Mario Pérez Arranz
(until June 2020)

Gloria del Sastre Martín
(from March 2020)

Javier Tortosa Nieto

Economic Management

HEAD

Gema Bravo Sanz

PERSONNEL

Francisco Luis Aparicio Reyes
(until January 2020)

Santos Esteban Barranco Sierra

Mª Carmen Berreiros Cano

Francisco Javier Hernández Izquierdo

Mª José Gregorio Usano

Rafael López Laso

Mª Carmen Pascual Martínez
(from June 2019)

Mario Pérez Arranz
(from June 2020)

Mª Carmen Vaz Pereña

Álvaro Vila Hernández
(from December 2020)

Iris Roldán Zuasti

Purchasing And Supplies

HEAD

Julio Díez Álvarez

PERSONNEL

Juan Carlos Bermudo Zamora

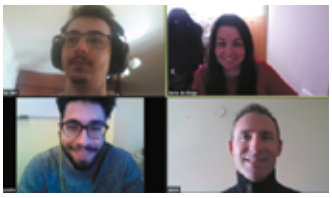
Mª Ángeles Lumbreras Carrasco

Mª Carmen Pascual Martínez
(until June 2019)

Antonio Pastor Encabo
(until December 2019)

Jaime Pastor

Mario Pérez Arranz



Information Technologies

HEAD

Sonia de Diego

PERSONNEL

Alejandro Fernández Ibáñez
(external)

Javier de la Fuente López
(from September 2020)

Carlos Francisco Bell Díaz
(from December 2020)

Librarian

Mª Dolores Aparicio

General Services

HEAD

Gabriel Sánchez de Lamadrid

PERSONNEL

Julián Grande Palomino

Manuel Grande Palomino

(external)

Pilar Cutillas

Sirlene Felix da Silva

Adela García Díaz

Beyca López Milla

Georgia Marcela Valdivia

Socorro Muñoz Ajates

Juan Pardo Prieto

Juana Ruiz González

Lourdes Sánchez Díaz

Vanesa Vera Martínez

(external)

Darianngel H. Bacco Piñango

José Miguel de la Hoz (D.E.P.)

F. Javier Lara Boavent

Santa López Almena

Luis Fernando López Ortega

Carolina Nogales Mauro

Aileen Notario Bonsol

Alberto Peñalva Rubio

Daniel Rodríguez García

Occupational Prevention Risk Unit

HEAD

Núria Martín Montes (external)

Maintenance

HEAD

Antonio Dueñas

PERSONNEL

Juan Carlos Cuenca

Alfonso García

Jesús González

Enrique Mejías

Mario Enrique Rodríguez
(external)

Construction And Infrastructure Planning

Javier Zarco

Security

HEAD

Sócrates Gutiérrez

PERSONNEL

(external)

Fernando Albarrán

Abderrahim Asgais

Lidia Cano
(from August 2019)

Tomás Castro González

Jesús Payán

Juan Tierno
(from August 2019)

CNB committees

Scientific activities

Juan Carlos Alonso
Antonio Leyva
Florencio Pazos
Hugh Reyburn
Juan José Sanz
José María Valpuesta

Equality

Saúl Ares
Pilar Cubas
Ana Cuenda
Mónica Chagoyen
Daniel López
Susana de Lucas
Cristina Merino
Carmen San Martín
Juan José Sanz-Ezquerro

Training advisory

Yolanda Carrasco
Vicente Rubio
Juan José Sanz
Javier Tamames
Mark van Raaij
Miguel Vicente

PhD students

Alejandro Asensio
Lorena Bragg
Álvaro Ceballos
Marta Cobo
Alberto Fuster
Sofía Gardeta
Andoni Gómez
Marcos Gragera
Diego Jiménez
Javier López-Ibáñez
Micaela Navarro
Andrés París
Elena Sánchez
Jesús Vallejo

Biosafety

PRESIDENT

Luis Ángel Fernández Herrero

VOCALS

Carmen San Martín Pastrana
Juan Nogales Enrique
Hugh Reyburn
María Isabel Sola Gurpegui
Juan Antonio García Álvarez
Ángel Fernando Naranjo Pino

SECRETARY

Fernando Usera Mena

Animal research ethics

PRESIDENT

José Miguel Rodríguez Frade

VOCALS

Belén Pintado
Francisco García del Portillo
Carlos Oscar S. Sorzano (statistics)

SECRETARY

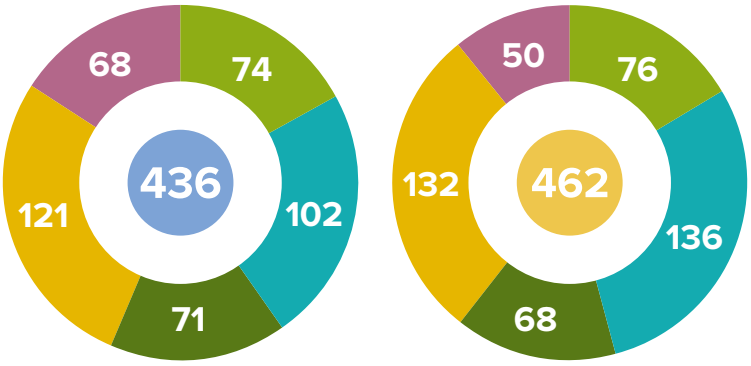
Angel Naranjo (animal welfare)

CNB staff

631 (2019) 656 (2020)

RESEARCH GROUPS

2019		2020	
FEMALE **224** 51.4%	MALE **212** 48.6%	FEMALE **230** 49.8%	MALE **232** 50.2%)
SPANISH **381** 87.4%		SPANISH **427** 92.4%	
INTERNATIONAL **55** 12.6%		INTERNATIONAL **35** 7.6%	



2019 2020

GROUP LEADERS AND CO-LEADERS · POSTDOCTORAL & STAFF SCIENTISTS · RESEARCH TECHNICIANS · PhD STUDENTS · SHORT-TERM TRAINEES AND VISITING SCIENTISTS

SCIENTIFIC SERVICES

101 (2019) 100 (2020)

TECHNICAL SUPPORT

37 (2019) 36 (2020)

ADMINISTRATION

29 (2019) 36 (2020)


25
años
1993
1996
1992
1995
1998
1994
1997
CNB

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FACTS AND FIGURES

Publications

PUBLICATIONS IN JCR-INDEXED JOURNALS

2019	2020
246	238

AVERAGE IMPACT FACTOR

2019	2020
6.68	6.03

PUBLICATIONS IN FIRST QUARTILE (Q1) JOURNALS

2019		2020	
79.7%	196	172	72.3%

PUBLICATIONS IN FIRST DECILE (D1) JOURNALS

2019		2020	
41.1%	101	99	41.6%

PUBLICATIONS WITH CNB SCIENTIST AS SENIOR AUTHOR

2019		2020	
44.3%	109	110	46.2%

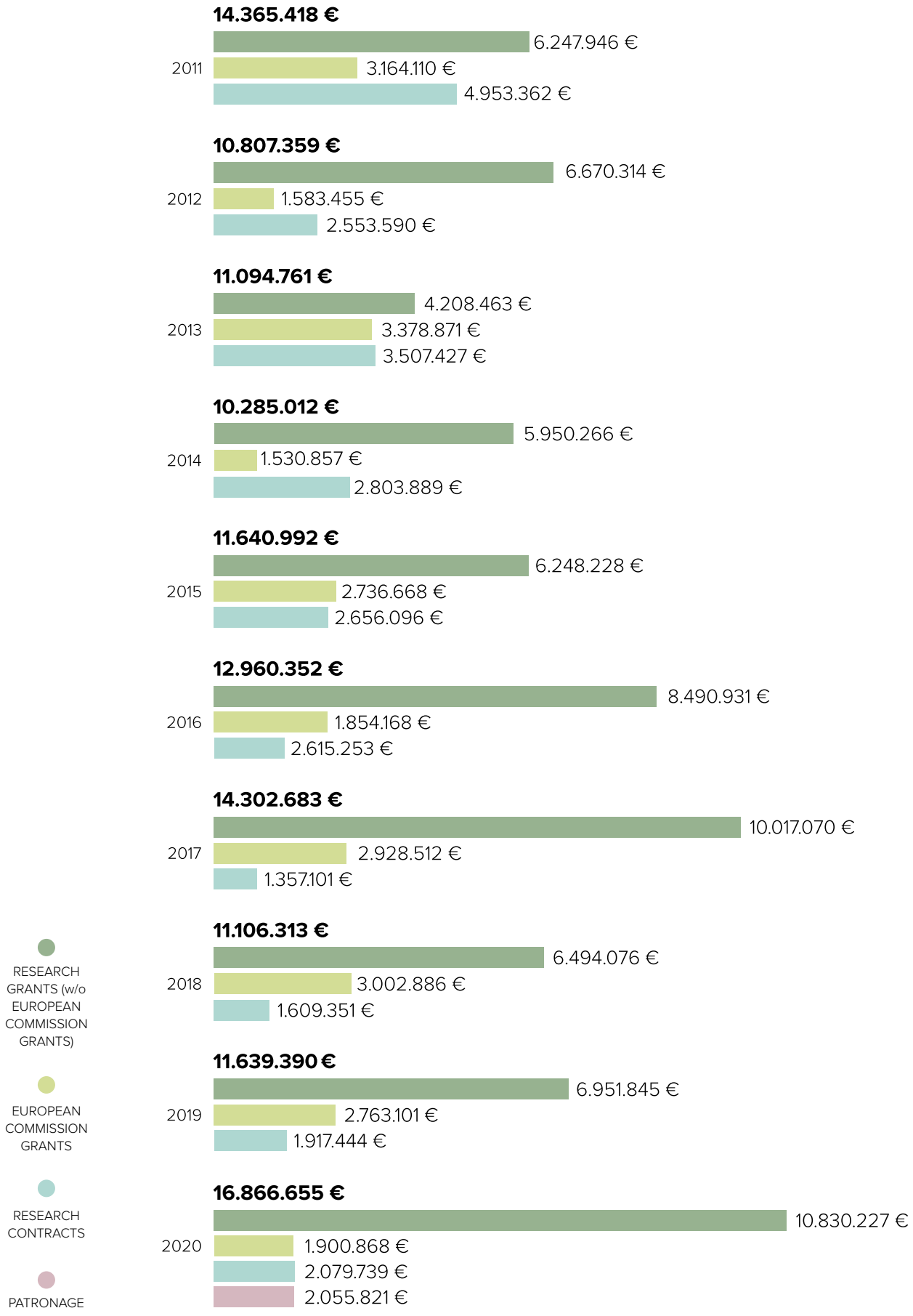
PUBLICATIONS IN INTERNATIONAL COLLABORATION

2019		2020	
63.4%	156	79	33.19%

PUBLICATIONS IN OPEN ACCESS

2019		2020	
63.4%	156	150	63.0%

Research funding evolution



If you want to know more about the CNB, please check the following links:

- CNB website: http://www.cnb.csic.es
- CNB scientific publications: http://www.cnb.csic.es/index.php/en/research/publications
- Blog CNB divulga: http://divulga.cnb.csic.es
- CNB outreach publications: http://www.cnb.csic.es/index.php/en/science-society/publications
- CNB YouTube: http://www.youtube.com/user/CNBcsic
- CNB Facebook: http://www.facebook.com/CNB.csic
- CNB Twitter: http://twitter.com/CNB_CSIC

CNBREPORT

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CNB Directive Team

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Lucía Bajos

Legal deposit
M9079-2021

2019
2020

CNBREPORT

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EXCELENCIA SEVERO OCHOA