CNB REPORT

2021_2022

RESEARCH
DEVELOPMENT
INNOVATION

CSIC
Consejo Superior de Investigaciones Científicas

CNB
Centro Nacional de Biotecnología

Excelencia Severo Ochoa

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2021_2022

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INDEX

Welcome to the CNB

This report summarises the activities of the CNB-CSIC (Centro Nacional de Biotecnología) through the years 2021 and 2022, a period still marked by the end of the pandemic caused by SARS-CoV-2.

The activity developed by CNB researchers during the pandemic has been outstanding, culminating with the application for a Phase I Clinical Trial for a CNB vaccine candidate. This candidate, based on the Modified Vaccinia Ankara (MVA) virus has shown, in preclinical trials, to generate a very robust cellular and humoral immune response in three different animal models (mice, hamsters and macaques). In addition, the CNB has developed a serological diagnostic kit which use has been made available to the World Health Organization (WHO) to be manufactured in African and other developing countries through the C-TAP WHO initiative to provide rapid, equitable and affordable access to Covid-19 health products. These and other activities are summarised by some very relevant scientific indicators, with more than 60 scientific publications on topics related to SARS-CoV-2, 12 patents developed and 17 contracts for technological support to companies.

We are now in a transition period connecting the hyperactivity during the pandemic with the regular scientific activity of a multidisciplinary research center such as ours. The CNB is nevertheless very interested in the continuance of the infrastructures developed to respond to SARS-CoV-2, as a way to be prepared to respond to the emerging viruses that are likely to play a key role in the next future. Another important aspect that can be nurtured by this experience is the development of platforms for the study of bacterial resistance, probably one of the major challenges for the global health system in the coming years. The CNB also wants to be a leader in facing this challenge.

During this period, we have also improved our technological offer and finished the development of the Bioimaging platform as a way to integrate all the CNB efforts to carry out multi-scale and multi-resolution microscopy approaches, as well as the two image analysis units to process the vast amount of information generated. In this context, the CNB hosts two facilities that belong to the INSTRUCT-ERIC network of European structural biology facilities: the cryoEM CNB-CSIC facility for data acquisition in cryoelectron microscopy and the Instruct Image Processing Center (I2PC) for processing electron microscopy data. This makes the CNB a unique Center where the whole process, from sample preparation to sample characterisation, data acquisition and advanced data processing can be carried out by the user.

Over this period of time, CNB researchers have contributed to the publication of 545 papers in ISI-listed journals with an average impact factor of 8.8. Significantly, 50% of these publications were among the top 10% of the most cited journals according to the Scimago database. As proof of their dynamism, CNB researchers obtained 220 grants (20 from international agencies), submitted 44 PhD theses,

taught more than 65 hours in Master's degree programs, hosted around 150 seminars, including webinars, and organised over 30 international workshops and meetings. The data speak for themselves of the international nature of the CNB; near 55% of the papers published by our scientists are the result of collaborations with international scientific groups from 120 countries. As a result, the CNB offers an attractive career destination for young scientist, with 10 new tenured scientists joining in 2022, which facilitate the renewal of the scientific staff.

As a continuance of their earlier support during the pandemic, in 2022, we have signed an agreement with the Jesus Serra Foundation to promote the career development of early career researchers at the CNB. Their support has offered the possibility to create 4 grants for emerging groups and a scholarship to pursue a doctoral thesis project in the CNB. This initiative is a great example of the fruitful collaborative efforts made to promote CNB scientific research in future years.

We have also continued the effort to strengthen the biotechnological value of the CNB. In 2021-2022, we have initiated the procedures for 24 patents and 3 licenses to companies with the idea that our research facilitates the quality of life for citizens.

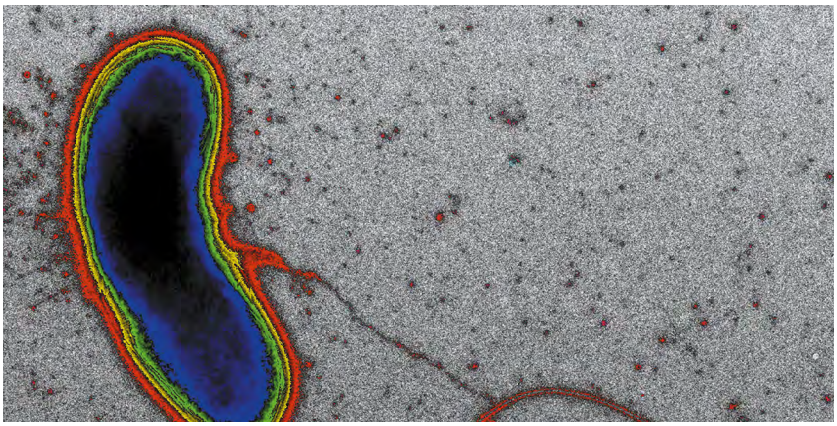
Similarly, we have also reinforced our commitment to communicate with society. Our Scientific Culture Unit manages the CNB presence in social media, with accounts in Facebook (4,7K followers), Twitter (28K followers), LinkedIn (12K followers) and a new account in Instagram with over 700 followers. Our science and scientists have been featured more than 2000 times in the media (press, TV, radio and digital media). Although some outreach activities such as high school guided tours to the center were put on hold during the pandemic, we are back to receive schools' visits. The annual outreach events, such as the European Researcher's Night, the National Science and Technology Week or the celebration of February 11th (#11F), the International Day for Women and Girls in Science, have been celebrated the last two years with both virtual or in-person events, when possible, with the invaluable collaboration of CNB volunteers.

We have completed a second period as a Severo Ochoa Center of Excellence, fulfilling expectations and obtaining an excellent return on the investments made. The CNB has proven to be a mature research center capable of responding with interdisciplinary excellence to the challenges that society demands.

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, supported the acquisition and maintenance of the new infrastructures. We are also indebted to the Spanish National Research Council (CSIC) for its continuous support to our projects.

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 towards the accomplishment of our objectives.

Mario Mellado
Director



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, Castón, Martín-Benito, Risco, San Martín and van Raaij), proteins involved in DNA repair (Moreno-Herrero), in the control of the genome stability (Ortega) or in protein homeostasis (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 cryoelectron microscopy in its distinct variants (single-particle cryoelectron microscopy, cryoelectron tomography and very recently cryocorrelative microscopy), which is supported by the CNB cryoelectron microscopy facility, the first of this kind in Spain. This work is strongly supported by continuous software development in the field of image processing (Carazo and Sánchez Sorzano).

All this has led to the CNB being the Spanish node of the Instruct ERIC network of European structural biology facilities, hosting two of them, the CryoEM CNB-CSIC facility and the Instruct Image Processing Center (I2PC). 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 its participation in the Human Proteome Project.

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José María Valpuesta

Figure legend: Vibrio anguillarum*, a fish pathogen, imaged by negative staining electron microscopy (Sara Otaegi).*

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Biocomputing unit

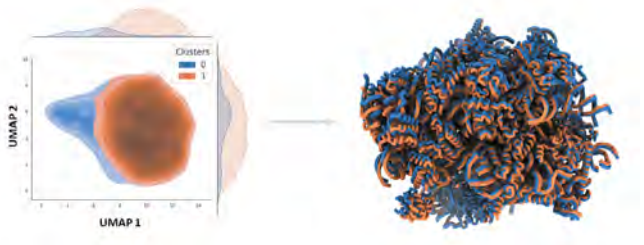
Our group develops and applies image processing algorithms for the analysis of the images of macromolecular structures acquired by an Electron Microscope under cryogenic conditions. The goal is to understand how the structure of a macromolecule and its dynamics captured by the microscope explains its physiological and pathological behavior.

Our image processing algorithms cover the whole pipeline from the microscope to the final structure. We develop algorithms to elucidate this structure when the particles are isolated (Single Particles technique) and when they are in their cellular context (Electron Tomography). Our algorithms are open-source and publicly available under the software package Xmipp. We also develop a workflow engine especially well-suited to image processing in Structural Biology called Scipion that integrates Xmipp and many other software suites also solving the same problem. Scipion provides a traceability, reproducibility and interoperability layer on top of the scientific calculation layer offered by Xmipp and the other software.

We are particularly committed to Open Science and to provide the software tools that allow the FAIR use of the electron microscopy data (FAIR=Findable, Accessible, Interoperable, and Reusable). Once the structure of a macromolecule is solved, our tools allow going further by helping to construct an atomic model and to explore possible ligands that could interact with it.

Finally, we also have a role in Structural Bioinformatics by integrating genomic, proteomic, and interactomic information onto the solved structure. Our tool in this area is one of the few recommended Interoperability Resources of the European Infrastructure of Life Science Information, ELIXIR. Finally, part of our group uses the image processing tools developed by us to solve specific structures. In this way, we know first-hand the problems encountered in the leading edge of Structural Biology.

Our group also provides access to the whole European scientific community through the Instruct-ERIC Image Processing Center (I2PC) as well as to collaborate in Joint Research Activities in the iNext-Discovery platform. I2PC belongs to the European infrastructure project for Structural Biology and supports the community by having an access program by which users can solve the structures of the macromolecules of their interest, hosting short-term internships, and by a training program through which we give at least 4 courses per year on the technology related to image processing in Electron Microscopy.



Example of Zernike3D continuous heterogeneity analysis. (Left) Contour level representation of the Zernike3D conformational landscape associated with the molecular motions of the Plasmodium falciparum 80S ribosome from EMPIAR-10028. (Right) Synthesis of the two main ribosome states captured by the landscape through the application of the Zernike3D deformation fields.

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*

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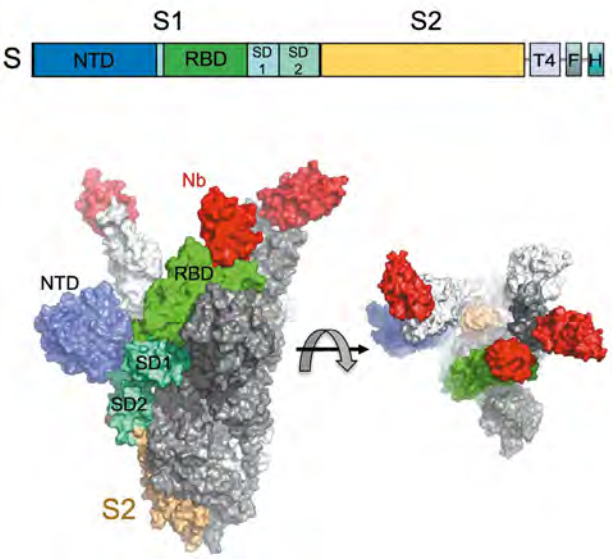
Cell-cell and virus-cell interactions

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 (Abs). Our multidisciplinary research applies structural, biochemical and cell biology approaches.

During the last two years, we have been deeply involved in several collaborative projects related to SARS-CoV-2 detection and COVID-19 prevention with Abs and vaccines. We produced the SARS-CoV-2 envelope spike (S) antigen for serological test development at the CNB-CSIC and at the company Immunostep S.L. Recently, SARS-CoV-2 materials prepared in our laboratory were transferred to WHO for serological tests implementation in Africa.

Most of our recent research work concentrated on the generation of Abs to neutralise SARS-CoV-2. We prepared Abs derived from nanobodies coming out of immunised camels, most of which neutralised the SARS-CoV-2 virus and four of them hindered COVID-19 progression in animals challenged with the virus. The Nbs recognised the S receptor binding domain and bound to different epitopes overlapping with receptor-binding motifs. We generated highly potent neutralising Abs that bound to all SARS-CoV-2 variants except omicron. They are promising candidates for the COVID-19 treatment in infected people and they have been used in a highly sensitive detection test.

Our group also cooperated in the generation of other technologies related to SARS-CoV-2 prevention, such as mouse Abs that neutralise the virus, as well as in the design and characterisation of vaccines that protected animal models from COVID-19 progression.



Structure of the SARS-CoV-2 spike S in complex with a neutralising nanobody (Nb). Top. Scheme of the S protein used to generate the structure. The extracellular S1 with the N-terminal domain (NTD), receptor-binding domain (RBD), subdomains SD1 and SD2, and the S2 region are shown colored. The soluble S contained a T4 trimerization domain (T4), a FLAG peptide (F), and a 6xHis-tag (H) at its C-terminal end. Bottom. Cryo-EM structure of the trimeric S with a Nb bound to its RBD. The S monomers are represented as surfaces, those with the open RBD are shown in white or grey, whereas the monomer with the closed RBD is with the domains colored as in A. The three Nbs bound to the RBD are shown in red. Lateral (left) and frontal (right, from the top) views of the complex..

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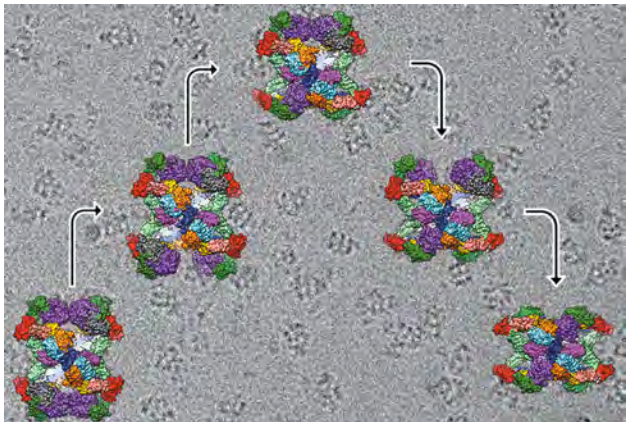
Viral molecular machines

When viruses are viewed as dynamic containers of an infectious genome, their structural, physical, and biochemical analyses become necessary to understand the molecular mechanisms that control their successful life cycle. Information on virus structures at the highest possible resolution is essential for identifying the principles of their structure-function relationship, and could lead to development of antivirals, vaccines, and the advancement of new platforms for virus-based nanotechnology.

Three-dimensional cryogenic electron microscopy (cryo-EM), which has revolutionised structural biology, is central to determining high-resolution structures of many viral assemblies in near-native conditions. We use Cryo-EM to solve near-atomic structures of infectious virions with helical or icosahedral symmetry. State-of-the-art approaches now extend beyond purified symmetric capsids and focus on the asymmetric components as the genome and viral polymerases. Asymmetric structures have important functions in many steps of the virus replication cycle, and many of these will be key targets for the development of new antiviral drugs.

Our group studies several viruses with varying levels of complexity, with focus on a number of double-stranded RNA viruses such as infectious bursal disease virus, the human picobirnavirus, and several fungal viruses (*Saccharomyces cerevisiae* virus L-A, *Penicillium chrysogenum* virus, and Yadonushivirus), as well as single-stranded RNA viruses such as human rhinovirus and rabbit haemorrhagic disease virus. We extended our studies to other macromolecular assemblies such as α_2-macroglobulin, a blood plasma proteinase inhibitor of broad specificity, and encapsulins, bacterial nanocages that naturally confine a functional protein cargo such as an enzyme. Structural analysis is complemented by study of mechanical properties by atomic force microscopy, to examine the relationship between physical properties such as rigidity and mechanical resilience, and virus biological function.

Finally, our research establishes the basis for incorporation of heterologous proteins and/or chemicals into viral capsids, considered as nanocontainers or nanocarriers, of potential use for future biotechnological applications.



Cryo-EM structures of human α_2-macroglobulin functional states. Human plasma α_2-macroglobulin is a 1451-residue protein built of 11 domains. Four protomers associate to a ~720-kDa polyglycosylated complex [$(h\alpha_2M)_4$] with pan-peptidase inhibitory functions that transits between an open native conformation (bottom, left) and a closed induced state (bottom, right), in which endopeptidases are trapped upon cleavage of an accessible bait region. The background shows a cryo-EM image of $(h\alpha_2M)_4$.

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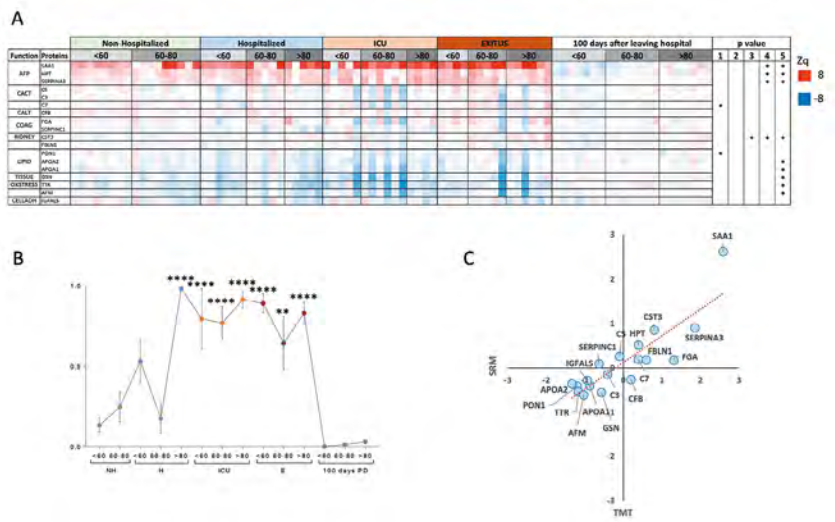
Functional proteomics

The activity of the Functional Proteomics laboratory of the CNB is focused in two main research areas of interests: the study of mechanisms underlying the progression of liver diseases and COVID-19 using proteomics. We have developed sample processing methods that increase three-fold the coverage of human bile proteome (Ciordia S *et al*, J Proteomics, 2022 and Meth Mol Biol 2022), allow for the study of HLA-presented phosphopeptides (Marcilla M, Meth Mol Biol 2022) and to quantitate one carbon metabolism (OCM) enzymes (Guerrero L *et al*, Meth Mol Biol 2022). The analysis of serum samples from COVID-19 patients revealed a protein panel feasible to assess disease severity (Núñez E *et al*, Biomedicines 2022) and liver proteome and phosphoproteome dynamics shed light on the molecular basis of liver cancer progression (Colyn L *et al*, J Ex Clin Cancer Res 2022) and point to OCM cycle as a functional biomarker for liver disease patients prognosis (Guerrero L. *et al*, J Physiol Biochem 2022; Guerrero L *et al*, Metabolites 2022). We have also identified potential biomarkers of pre-eclampsia in exosomes from afflicted patients (Navajas R *et al*, Clin Proteomics. 2022). Our research is granted by CSIC (2021-2023), MICIN (2022-2026) and CAM (2023-2027). Finally, we have leadership in national and international initiatives such as the Spanish Proteomics Society, European Proteomics Association and the Human Proteome Project.



COVID-19 severity markers monitored by SRM. *(A) Protein abundance changes (Zq) normalised by the average values of the 100 days after leaving hospital patient group. Up-regulated proteins are in red and down-regulated proteins in blue. (B) Prediction of disease severity using a logistic regression model. (C) Protein quantification from TMT and SRM experiments.*

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Ultrastructure of viruses and macromolecular aggregates

The main line of the group is focused on the study of viral ribonucleoproteins (RNPs) that conforms the virus nucleocapsid of some enveloped single stranded RNA viruses. RNPs are macromolecular complexes composed of the genomic RNA bound to multiple monomers of a nucleoprotein and, in some cases, a single copy of the viral polymerase.

In recent years our laboratory has determined the structure of the influenza A RNPs at medium resolution and we have verified that this structure is present in native virions using cryogenic electron tomography. We have also elucidated the structural basis of the transcription process, that is, how RNP produces messenger RNA in influenza A. We are currently extending these studies to the genome replication process of this virus.

Within the framework of research on Covid-19, our laboratory has developed a model for structural and biophysical studies on coronaviruses, using the transmissible gastroenteritis virus (TGEV) as a surrogate, on which we have carried out studies on its biomechanical properties and its resistance to the use of virucidal agents.

On the other hand, our laboratory is also developing a system for the determination of the structure of membrane proteins based on the use of nanodiscs and liposomes by means of cryogenic electron microscopy. In this field, we have solved the structure of two actinoporins, pore-forming proteins, at a resolution of 2 Å. These studies allow us to determine the structure of membrane proteins in the bilayer and to show the interactions of the proteins with lipids.

1 *The left panel shows high-resolution averages of a pore formed by a protein on lipid nanodiscs, front (top) and side (bottom) views. The right panel shows two details of the high-resolution structure solved using cryogenic electron microscopy, including some amino acid and lipid constituents of the pore structure.*

2 *The left panel presents a cryoelectron tomogram section of a TGEV virion showing the spike proteins (blue arrowheads), the double layer membrane (green arrowheads) and the RNP inside the envelope (red arrowheads). The right panel shows intact virions visualised by Atomic Force microscopy in buffered solution. Blue arrowheads point to putative spike. The colour palette shows the surface height. Scale bar represents 50 nm.*

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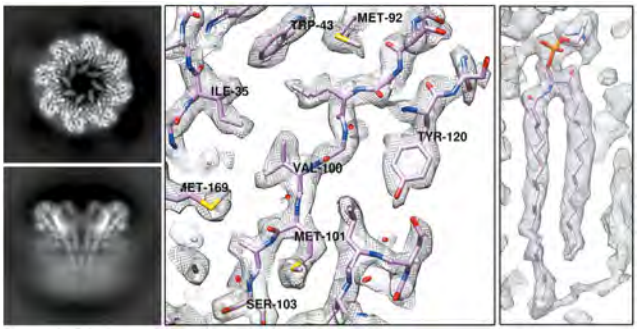

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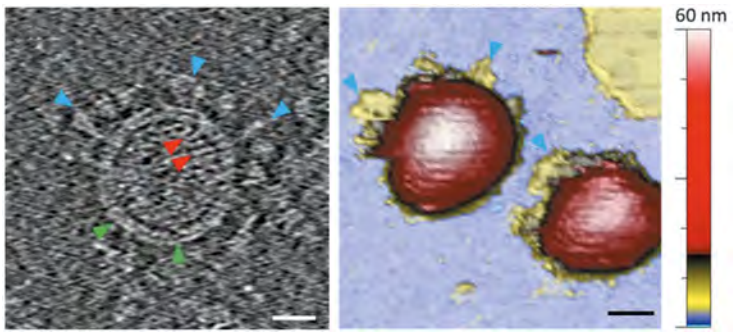

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Molecular biophysics of DNA repair nanomachines

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 DNA structures. We use novel single-molecule approaches based on atomic force microscopy (AFM), optical and magnetic tweezers, and their combination with confocal and TIRF microscopy.

During the last two years, we have continued developing 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 had also mastered the use of C-Trap™ from Lumicks, a hybrid system combining optical tweezers and confocal microscopy, which enables manipulating single DNA/RNA molecules while simultaneously imaging the fluorescence between the beads in real time.

Using our expertise in AFM, we showed that NIHCOLE, a lncRNA induced in hepatocellular carcinoma, folds into dsRNA secondary structures and binds the Ku heterodimer, a component of the NHEJ pathway of DSB repair machinery, cooperatively (Unfried et at. 2021). This AFM work involved the development of new methodology to analyse AFM images and to assign sequence regions to folded domains. We have also captured the diffusion of ParB along dsDNA for the first time using the C-trap™ instrument (Balaguer et. al. 2021); and characterised the biochemical activities of the human DNA helicase B (HELB). We showed that HELB is a monomeric protein that binds ssDNA and uses ATP hydrolysis to translocate along ssDNA in the 5'-3' direction. We also showed that HELB translocation is accompanied by the formation of DNA loops (Hormeño *et al*. 2022). Our work required the development of new methods to fabricate long duplex and hybrid single-stranded/double-stranded DNA molecules for single-molecule experiments (Aicart-Ramos *et al*. 2022).

1 *(A) High-resolution AFM image where the double-helix structure of the DNA molecule can be observed. (B) Confocal image (C-trap) where three DNA molecules held by two optically trapped microspheres are visualised. (C) Confocal image (C-trap) where the binding between a protein and a DNA molecule trapped using 2 optical traps is observed.*

2 *AFM image of lncRNA molecules.*

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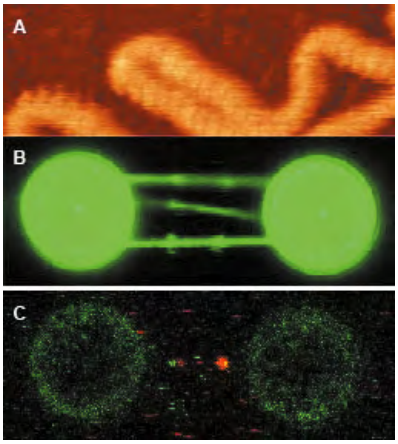

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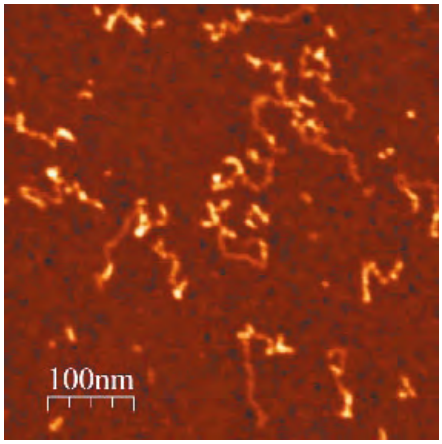

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Structural biology of bacteriophage and virus proteins

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. We perform high-resolution structural studies on the proteins involved in these processes, using X-ray crystallography, preferably in complex with their receptors or substrates.

Tailed bacteriophages bind to their host cell receptors via specialised spike or fibre proteins. In the last two years, we have solved structures of the fibre protein gp20 of *Salmonella* phage epsilon15 in complex with fragments of its lipopolysaccharide receptor and of the Staphylococcus phage S24-1 fibre protein orf16 in complex with a teichoic acid analogue. In an international collaboration, we also analysed structures of antibody Fab fragments in complex with teichoic acid (Figure 1; Di Carluccio *et al*, 2022), identifying important teichoic acid recognising features.

To lyse their host cells, bacteriophages produce endolysins that digest the bacterial peptidoglycan layer. In the last two years, among others, we have solved structures of the Pseudomonas phage JG004 endolysin Pae87 in complex with a peptidoglycan fragment. We identified a previously unknown intra-domain substrate binding domain likely to be common to a whole subfamily of monodomain endolysins (Figure 2; Vázquez *et al*, 2022). Other endolysins have separate cell wall binding domains, while Pae87 has incorporated a cell-binding subdomain in its enzymatic domain.

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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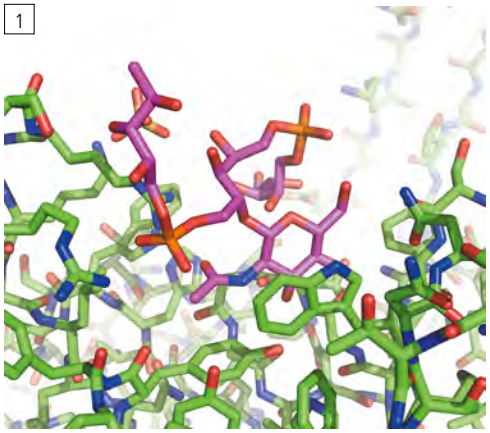

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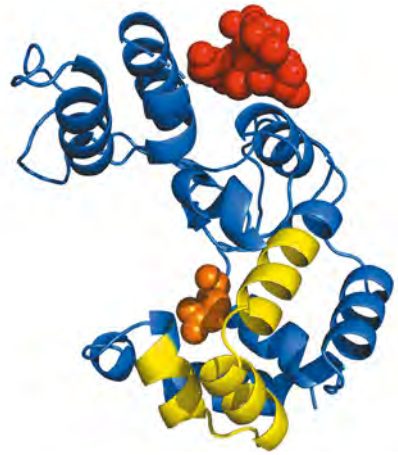

1 *Detail of a crystal structure of a teichoic acid fragment (pink backbone) bound to a Staphylococcus antibody Fab domain (green backbone).*

2 *Crystal structure of the Pseudomonas phage JG004 endolysin Pae87 (in blue and yellow). In orange, a buffer molecule in the active site is shown; in red a peptidoglycan fragment is bound to the opposite side of the protein.*

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Cell structure laboratory

The Cell Structure Laboratory makes use of state-of-the-art imaging techniques to study virus-host interactions. Together with cell biology, virology, biochemistry and proteomics, we use light microscopy, transmission electron microscopy (TEM) and correlative light and electron microscopy (CLEM) to unveil new targets for antiviral treatments.

The pandemic caused by SARS-CoV-2 made evident that we count with very few virus-fighting drugs. The current list of priority diseases of the World Health Organization includes pathologies caused by three coronaviruses and by three bunyaviruses.

During the last two years we have studied the mechanism of action of several compounds that inhibit bunyavirus growth. We have also discovered how Plitidepsin blocks SARS-CoV-2 infection in cultured cells. Plitidepsin is a potent anti-SARS-CoV-2 antiviral that targets a cell protein used by the virus and is currently being studied in clinical trials (phase III) to treat Covid-19. We have also validated four more repurposed drugs from our library in cells infected with two different coronaviruses. One of the compounds is a promising candidate to be used in the therapeutic treatments for SARS-CoV-2 and possibly other respiratory viruses. This compound is currently being tested *in vivo* with one of the animal models for Covid-19.

With our studies on the cell biology of viral infections, we have discovered the role of the lipid transfer protein (LTP) NPC1 in early, post-entry steps of reovirus infection. In addition, CLEM studies helped to discover a new pathway for bunyavirus propagation that bypasses the secretory pathway of the cell. The role of several LTPs, lipid flows and mitochondrial proteins in bunyavirus infection has been also studied in detail. This knowledge will be crucial to characterise promising compounds that interfere with host factors whose implication in key biological processes can be applied to design pan-antiviral strategies.

1 *Live-cell imaging and CLEM of bunyavirus-infected cells uncovers a new mechanism for virus propagation. Scale bars, 20 µm; 200 nm (image on the right).*

2 *TEM of cells infected with SARS-CoV-2 in the absence (image on top) and presence (bottom image) of Plitidepsin. The viral replication organelle (asterisks) is not assembled in cells treated with the drug. N, nucleus. P, plasma membrane. Scale bars, 1 µm.*

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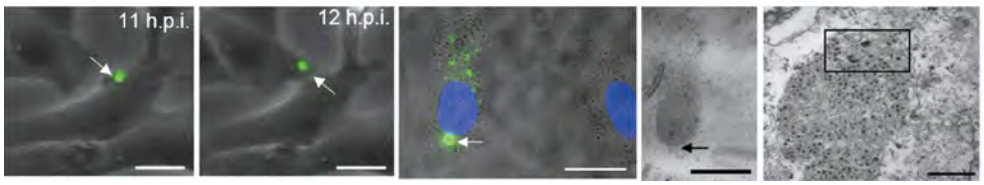

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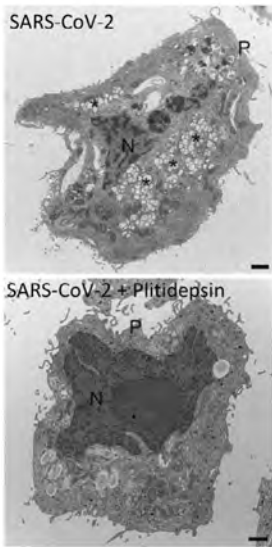

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Structural and physical determinants of complex virus assembly

We investigate the principles governing complex virus assembly. Our main model system is adenovirus, an icosahedral virus with a capsid composed of more than 10 different proteins. Adenoviruses are pathogens, but they can be engineered as therapeutic tools. Understanding adenovirus assembly poses many challenges due to the virion complexity. Although several hundred adenovirus types are described, most of our knowledge focuses mainly on HAdV-C5. From the 2021-2022 period, we highlight our work elucidating two new adenovirus structures.

Enteric human adenoviruses are one of the main global causes of viral diarrhoea, but knowledge on their biology lags behind that of adenoviruses with respiratory or ocular tropisms. We solved the structure of an enteric adenovirus, HAdV-F41. We focused on relating its structural features with survival to the deleterious conditions found in the gastrointestinal tract. We described differences in the capsid surface that may account for resistance to neutralisation by intestinal defensins, as well as partially ordered structural elements that could have a role in enhancing the stability of the HAdV-F41 capsid.

We also solved the first high-resolution structure of an adenovirus infecting a non-mammalian host: lizard adenovirus LAdV-2. LAdV-2 virions carry on their surface a protein called LH3 that does not exist in human adenoviruses. Instead, human adenoviruses have a protein called IX, with a very different fold (even mostly disordered in HAdV-F41). They also produce another protein with certain sequence similarity to LH3, E1B-55K, which is not part of the viral particle, but alters the cell division cycle. Our analysis showed that the LH3 gene was probably captured from a bacterial gene, incorporated as capsid decoration in reptilian adenoviruses, and later on duplicated to become protein IX and the non-structural, oncoprotein E1B 55K in mammalian adenoviruses. This work provided information on the evolution of complex viruses, and protein function exaptation across evolution.

Structures of HAdV-C5 (left), the most studied adenovirus and used as reference in the field; the enteric adenovirus HAdV-F41 (centre); and the reptilian adenovirus LAdV-2 (right). Proteins IX and LH3 are depicted in gold.

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Structure and function of molecular chaperones

We use different various biophysical techniques, mostly cryoelectron microscopy (cryoEM), 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. 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 cryoEM 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.

We are also interested in the quantitative study of molecular events at the membrane interface, focusing on the role of the membrane physical properties in the functional outcome of molecular interactions. Our toolbox majorly includes advanced optical microscopy and single molecule optical spectroscopy techniques (such as single-molecule tracking and Fluorescence Correlation Spectroscopy).

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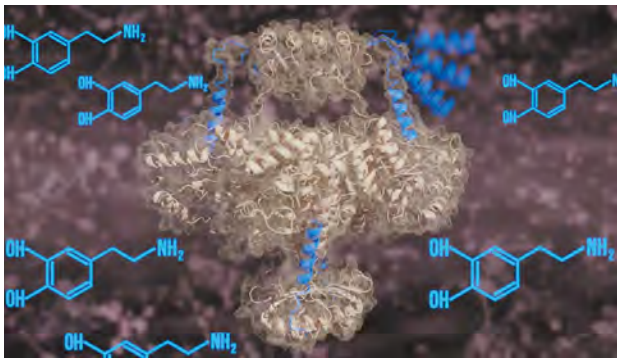

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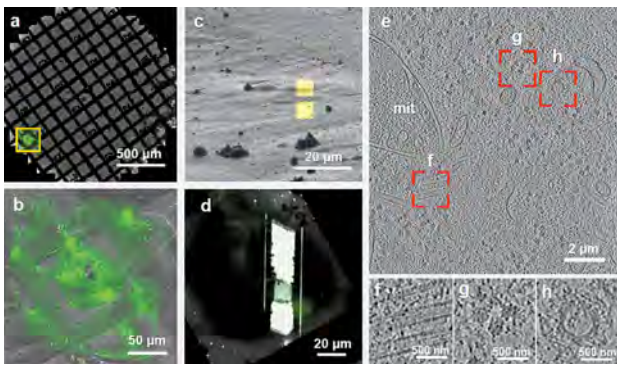

1 Artistic image of the tetrameric enzyme tyrosine hydroxylase with the 3D reconstruction obtained by cryoelectron microscopy (envelope) in which the atomic model has been fitted. The blue *α*-helices are a flexible part which can block the active site depending on the level of dopamine (its chemical formula drawn in the background) present in the solution

2 Cryocorrelative light and electron microscopy (CLEM) workflow for lamellae preparation of Jurkat cells expressing GFP-EB3 protein. (a) overlay of bright field (gray) and maximum intensity projection of cryoconfocal microscopy stack of images of Jurkat cells attached to the grid. (b) higher magnification of the area yellow squared in (a). (c) Focused Ion Beam image of the area in (b) where the milling pattern positioned using the confocal fluorescence information is shown in yellow. (d) cryo-TEM image of the area shown in (c) overlayed with a single slice of a confocal stack showing EB3 protein signal. (e) cryoelectron tomogram section selected based on the confocal imaging information; mit, mitochondrion. (f-h), higher detail of the areas squared in (e);(f) microtubules; (g) ribosomes and (h) a coated vesicle.

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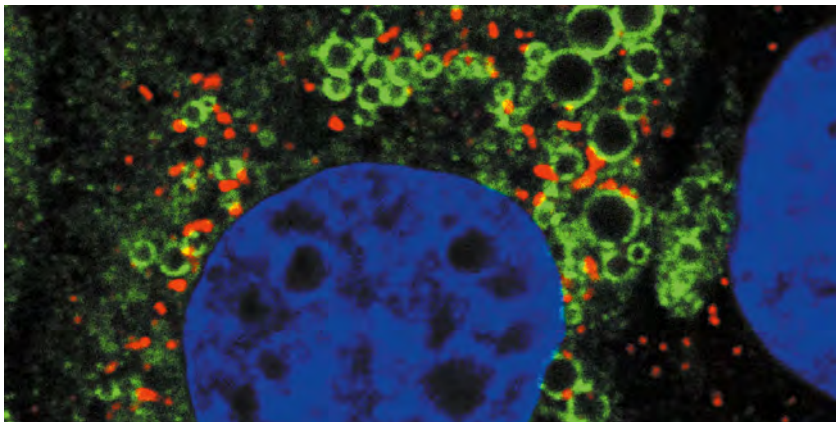

Molecular and Cellular Biology

The Department of Molecular and Cellular Biology hosts 19 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 Virology, 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 regards different aspects of Biomedicine, from gene expression, to cellular biology of tumors and the development of novel antitumor therapies to understand the molecular networks allowing the generation of neurons and circuits of the mammalian cerebral cortex. The main aim of this research program, is to improve current diagnostics and therapies for different human diseases.

Our department developed the virus biotechnology platform (VBP), created with the aim of providing integral biotechnological solutions to health challenges caused by viruses. 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) developing ultra-deep sequencing techniques to study intra-host variability of SARS-CoV-2; iv) producing recombinant SARS-CoV-2 proteins as antigens for the development of serological test and potential vaccines; v) producing monoclonal antibodies for anti-viral therapy; vi) controlling viral infection through the modulation of cellular energy metabolism; and vii) using the CRISPR/cas13d technology as a therapeutic tool to target coronavirus RNA genome.

HEAD

Esteban Veiga

Figure legend: Confocal microscopy image of hepatitis C virus-infected Huh7 cells. HCV core protein (green) localises at the surface of lipid droplets, which are prominent in this hepatoma cell line. A host factor required for efficient initiation for viral RNA replication, LPIN1, was labeled in red. (63X magnificaton with a 7X digital zoom) (Pablo Gastaminza´s lab).

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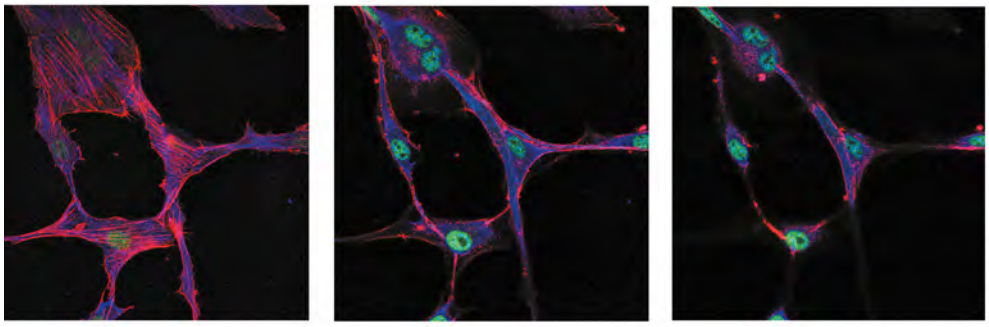
Molecular bases of actin cytoskeleton reorganisation in cell motility, tumour generation and invasiveness

Cancer can generate from oncogene-mediated transformation of stem cells. Aged stem cells are more vulnerable to malignant transformation, making aging a risk factor for developing cancer. Altered integrity of the actin cytoskeleton perturbs cell metabolism and tissue homeostasis contributing to functional decline in older individuals. Thus, modifiers of the actin cytoskeleton dynamics can be additional inducers of age-associated diseases like cancer. WIP (Wiskott-Aldrich Syndrome Protein (WASP) Interacting Protein) serves as an appropriate model to study cancer development and aging, as it regulates the organization of the actin cytoskeleton and participates in cell proliferation, migration, invasion, differentiation and tumour progression. Our group aims to define the molecular and physiological bases of WIP function in relation to stem cell activity during cell transformation and organism aging.

A combination of biochemical, proteomic and transcriptomic approaches, advanced imaging and 2/3D cell cultures, have led us to describe the pro-oncogenic activity of WIP in solid tumours like GBM (glioblastoma), colorectal and breast cancer mediated by transcription regulators YAP/TAZ. Database analysis confirmed that low WIP levels correlate with a higher overall survival of cancer patients (GBM, head and neck, gastric and breast). Interestingly, WIP acts as a tumour suppressor in ALK+ (anaplastic lymphoma kinase) haematological cancers. Our proteomic analyses of the WIP interactome have identified potential candidates that could explain WIP specific activity in solid tumours, focusing on GBM. Complementary, we have observed that WIP-deficient mice present shorter lifespan and phenotypic characteristics compatible with premature aging, such as immunological disorders or homeostatic alterations of tissues relying on proper stem cell activity. Our results reinforce the importance of WIP as a promising therapeutic target both for cancer and aging. They also open new venues to study the contribution of actin cytoskeleton regulatory proteins in (cancer) stem cell development and functionality, and their contribution to associated human diseases.



Confocal microscopy image of WIP and YAP/TAZ distribution in glioblastoma cells. Low, medium and upper stacks (from left to right) of U-87 human glioblastoma cells fixed and stained for actin (red), YAP/TAZ (green) and WIP (blue).

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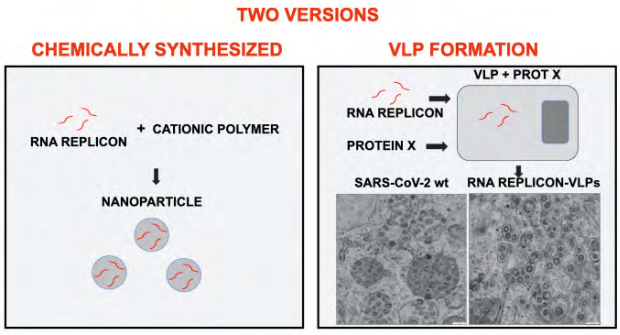
Coronavirus: replication and transcription, virus-host interactions, and protection

Coronaviruses are emerging viruses with pandemic potential. To date, seven coronaviruses (CoV) that infect humans are known. HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1 cause up to 15% of mild respiratory infections. In contrast, SARS-CoV, MERS-CoV, and SARS-CoV-2 cause severe pneumonia and acute respiratory distress syndrome (ARDS), which are potentially deadly conditions. The problem is even greater in the elderly population, which responds with lower efficacy to vaccination. SARS-CoV, MERS-CoV, and SARS-CoV-2 emerged from animal reservoirs in the 21st century, being SARS-CoV-2 the causative agent of the 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 next generation of SARS-CoV-2 vaccines consisting in replication-competent propagation-deficient RNA replicons and to determine their efficacy in animal model systems.** Vaccine development includes: (i) Engineering the SARS-CoV-2 RNA-replicons by deleting or modifying viral genes responsible for propagation and virulence, using reverse genetics; (ii) Development of packaging cell lines that efficiently complement the generation of virus-like particles (VLPs); (iii) Identification of RNA-replicon delivery systems; (iv) Engineering simplified and safer versions by reducing the replicase size.
- **To identify cell-signaling pathways involved in CoV replication and pathology** in order to select antiviral drugs that inhibit these pathways. We study PBM-PDZ protein-protein interactions and viral accessory genes involved in the innate immune and inflammatory responses, since activation of these pathways is responsible for virulence.
- **To determine the relevance of post-transcriptional regulation of gene expression to the inflammatory pathology.** We study the contribution to dysregulated inflammation of small non-coding RNAs (host miRNAs and virus-derived RNAs) and RNA-protein complexes. RNAs and proteins involved in these regulatory networks represent potential antiviral targets.



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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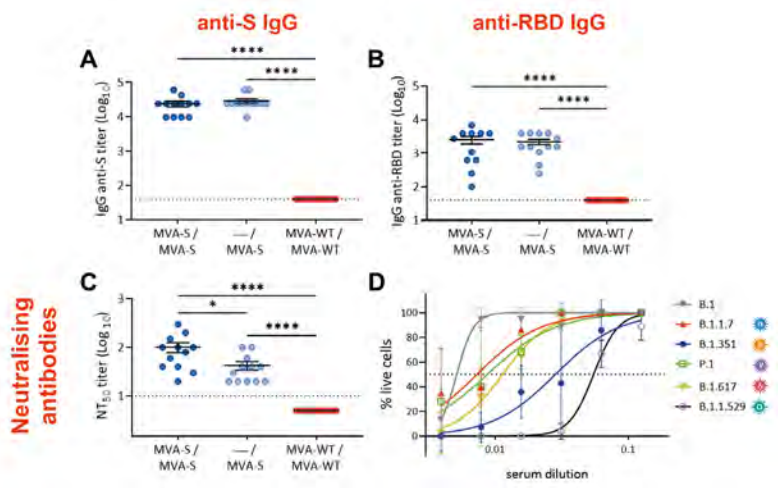
Poxvirus and vaccines

The main objectives of our laboratory are directed to understand 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 and reemerging 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 used 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.**

Due to the SARS-CoV-2/COVID-19 pandemic, during the period of 2021-2022 we focused our research in the development of a vaccine against this coronavirus. We generated modified vaccinia virus MVA vectors expressing the S (spike) protein of SARS-CoV-2, as well as conserved domains of the main structural proteins of the virus. In three animal models (mouse, hamsters and macaques), we demonstrated that the vaccine MVA-CoV2-S triggered potent immune responses and complete protection against SARS-CoV-2 infection. Moreover, the vaccine induced long-term high titers of binding antibodies to S and of virus neutralising antibodies, together with activation of specific CD4+ and CD8+ T cells, markers that correlated with protection against infection. In addition, the vaccine produced sterilising immunity in the brain and broad spectrum of neutralisation against SARS-CoV-2 variants of concern (VoCs). GMP lots of the vaccine were produced by the company Biofabri in Spain and a phase I clinical trial is under consideration. The vaccine was patented and received wide coverage in the media (radio, press and TV). Financial support was obtained from various private and public institutions.



MVA-CoV2-S activates markers of efficacy in hamsters. One or two doses of MVA-CoV2-S induced high levels of binding IgGs against S and RBD and neutralising antibodies against parental SARS-CoV-2 and several variants of concern. Boudewijns et al, Front. Immun. 13:845969 (2022)

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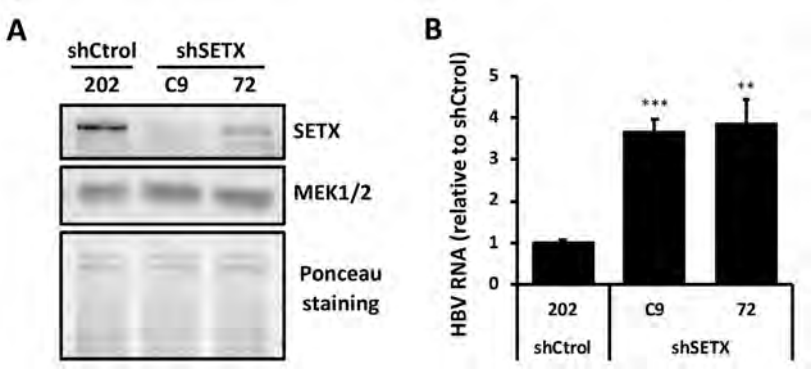
Virus-host interactions in hepatitis B virus infection

Our laboratory is interested in understanding the virus host interactions that regulate the outcome and pathogenesis of hepatitis B virus (HBV) infection in order to identify vulnerabilities that could be exploited to develop new antiviral therapies against this important human pathogen. Indeed, HBV is responsible of millions of cases of acute and chronic hepatitis and represent the major etiological agent of liver cancer worldwide.

During the 2021-2022 period, we focused on understanding the role of cellular proteins in the replicative cycle of HBV. We have confirmed our initial observations that DNA damage response related proteins, such as Senataxin and Ku70/Ku80, are key restriction factors for HBV infection and described their role as negative regulators of HBV gene expression. Moreover, we have expanded these observations to other viral and non-viral systems which gene expression relies on episomal DNA as template for transcription. These results suggest that the identified factors are master regulators of episomal gene expression.

Complementary to those studies, we are also working on the identification and characterisation of cellular genes and pathways that are required for HBV DNA integration in the host cell, an aspect that is important for HBV-related hepatocellular carcinoma development.



Effect of Senataxin silencing on HBV gene expression. *(A) Senataxin downregulation efficiency measured by WB analysis by two different shRNA sequences (C9 and 72). (B) Effect of Senataxin silencing on the accumulation of intracellular HBV RNA during HBV infection.*

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Hepatitis C and related virus infection

The main objective of the laboratory is to understand the molecular mechanisms underlying efficient infection by pathogenic human viruses, with the ultimate goal of identifying novel targets for antiviral therapy. The lab uses multidisciplinary approaches studying functional and structural aspects infection. The approaches and methodologies implemented in our laboratory for hepatitis C virus (HCV) infection are already being applied to study other pathogens, mainly Flaviviruses and recently SARS-CoV-2.

We used models of persistent HCV infection to determine the ability of direct-acting antiviral (DAAs) drugs of restoring normal cell homeostasis and ultrastructure in formerly infected cells. We have focused on the differential transcriptomic profiles of human hepatoma cells persistently infected with HCV, before and after infection elimination by treatment with DAAs. Our studies suggest that persistent HCV infection causes irreversible transcriptional alterations in the host. Studies in differentiated, growth arrested cell cultures enabled identifying a de-differentiation profile of the formerly infected cells, providing insight on the contribution of HCV replication on hepatocarcinogenesis, a biomedical problem that remains elusive for patients that have cleared the infection. Cell culture HCV models enabled monitoring reversion of the ultrastructural alterations caused by persistent HCV replication using cryo soft X-ray tomography, a technique producing 3D maps of whole cells in a quasi-native state that facilitate investigating the host mechanisms of viral replication organelle elimination after treatment with DAAs.

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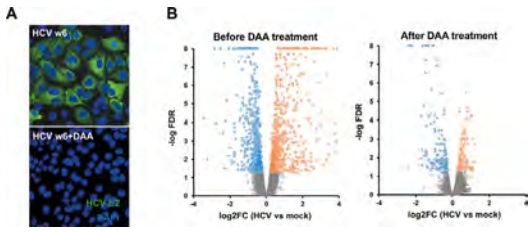

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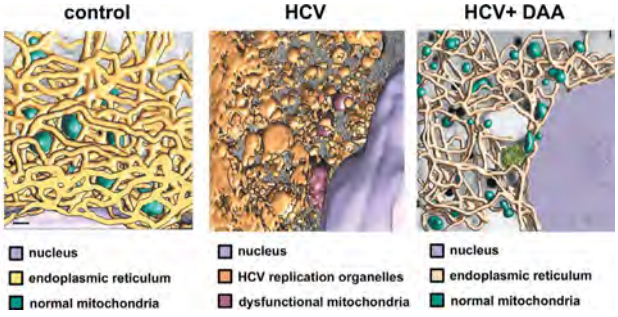

1 ***Irreversible transcriptional alterations in persistently HCV infected cells.*** *A-Immunofluorescence microscopy showing the loss of viral antigen staining (green) in cells counterstained using the nuclear stain DAPI (blue). B-Vulcano plot (log2 fold change in expression versus -log of false discovery rate (FDR) for individual transcripts illustrating the impact of HCV infection on the host cell transcriptome before and after virus elimination using direct acting antivirals. Data from 4 biological replicates show how virus elimination does not entail complete normalization of the cell transcriptome despite elimination of all viral proteins and RNA. Significantly (FDR<0.05) upregulated transcripts are shown in orange and downregulated transcripts are shown in blue.*

2 ***Tridimensional model of cells replicating HCV before and after antiviral treatment.*** *Cryo-SXT of control, HCV-replicating cells untreated or treated with DAAs. Manual segmentation of the surface boundaries identifying the organelles: normal mitochondria in dark green, abnormal mitochondria in dark purple, ER in yellow-brown, nuclear envelope in light purple and HCV-modified ER in orange. The scale bar represents 1 µm.*

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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 known as albinism, a heterogeneous genetic condition associated with mutations in at least 22 genes, characterised by visual impairment, present in all types, and pigmentation alterations, not obvious for some cases. This work on human rare diseases is associated with 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 apply this knowledge in cooperation with ALBA, the Spanish association in support of people with albinism and have been able to genetically diagnose numerous Spanish 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 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 pioneered the application of CRISPR technology in mice, we have generated a series of genome-edited mice carrying patient-specific mutations that we are currently phenotyping and using to explore new therapies for albinism.

CRISPR genome edited mice as animal models of (from left to right): OCA2, wild type, OCA1A and OCA1B oculocutaneous albinism types. (Fernandez et al 2021 Pigment Cell Melanoma)

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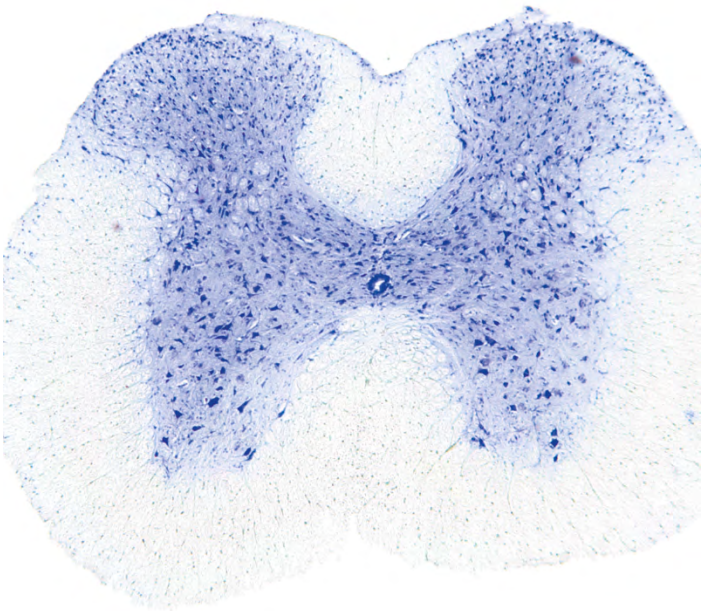
Functional analysis of transcriptional repressor DREAM

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



Nissl staining of lumbar spinal cord (coronal) in transgenic mice overexpressing human TDP-43 with the A315T mutation, a mouse model of familial ALS (Wegorzewska et al. PNAS 106:18809, 2009). Project funded by Asahi Kasei Pharma (Japan). Tissue preparation and staining was performed in the Histology service at the CNB.

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Cerebral cortical development

The cerebral cortex mediates the high functions of the human brain. It provides optimal responses to the external world, intellectual processing, and social behaviours. It is one of the most complex functional networks in biological systems and comprises an extraordinary number and diversity of neurons. Despite their complexity, cortical circuits, which ensemble during a protracted period of embryonic and postnatal development, wire stereotypically and reproducibly in all individuals of the same species. We think, see, feel, or interpret social behaviours similarly because our circuits are similar, allowing us to interact and evolve as a population.

We study the developmental mechanisms that build cortical circuits. We aim to improve the understanding, diagnosis and treatment of neurodevelopmental disorders that appear when these mechanisms fail, such as epilepsy, dyslexia, autism, schizophrenia, mental retardation, and many syndromic and nonsyndromic disorders. During these two years, our principal focus has been investigating the development of corpus callosum (CC) connections. The CC connects the cerebral hemispheres and creates a more complex information processing. It is an evolutive addition to the mammalian brain, and focusing on the CC facilitates us to delve into the specific properties of the mammalian neurons. We have found that one of the hallmarks of mammalian neurons is extraordinary molecular and axonal plasticity under the control of activity. Bragg *et al*., discuss these ideas in a beautiful review. We also investigated the axonal cues that guide specific connectivity. Neuropilin-1 (Nrp1) is a receptor that binds to various ligands and signals differentially upon its association with distinct coreceptors. We demonstrated that dynamic expression of Neuropilin-1 during postnatal development is key to establishing a topographic organization of axonal projections in the contralateral hemisphere. Together our data support a model of cortical assembly due to the temporal evolution of molecular and wiring trajectories.

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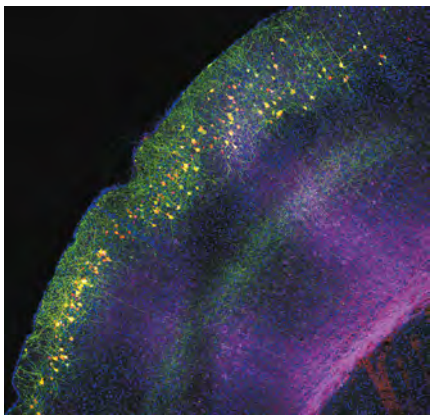
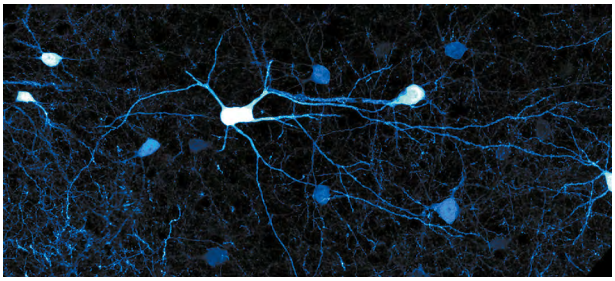


1 *Neurons of the cerebral cortex were labeled genetically with fluorescence proteins Dsred Express (red) and GFP (green). Fluorescence-CTB (magenta) illuminates callosal neurons and their axons. Cell nuclei (blue).*

2



2 *Genetic labeling of Parvalbumin GABAergic inhibitory neuron from the mouse cerebral cortex.*

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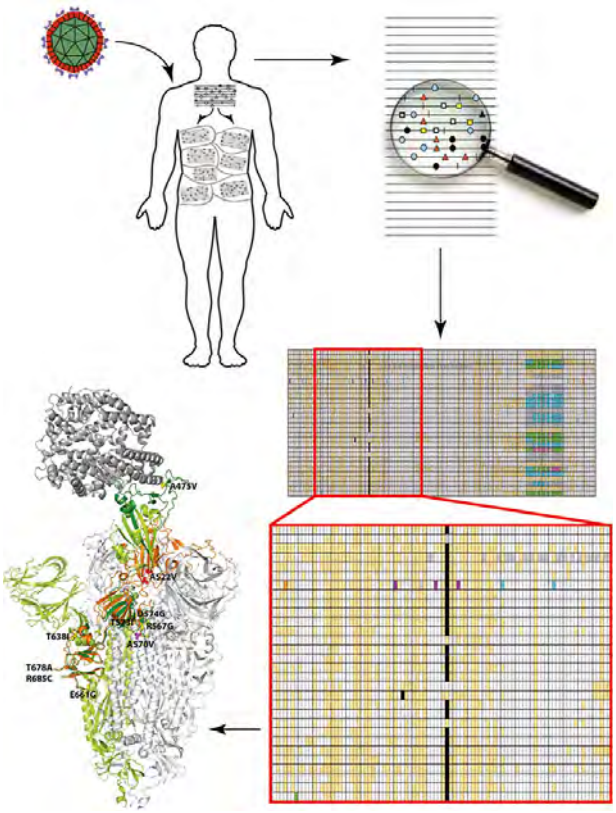
Dynamics of RNA viruses in infected patients. New antiviral designs

Virus variability is one of the main obstacles for the effective prevention and treatment of viral diseases. The main objective of our laboratory is to understand dynamics of RNA viruses, based on deep sequencing data of *in vivo* and ex vivo viral populations. Genomes present at low frequency in a mutant spectrum fuel virus adaptability, and may also influence the behaviour of the population ensemble. Selective pressures (such as antiviral agents or vaccination) may favour replication of some components of a mutant spectrum over others. Quasispecies dynamics demands that new approaches be investigated for the prevention and treatment of diseases associated with RNA viruses, to counteract the adaptive capacity conferred by the mutant clouds.

In our laboratory, we are extending previous studies on population dynamics with other RNA viruses to SARS-CoV-2 with the aim of increasing our understanding of quasispecies implications in a comparative manner in cell culture and *in vivo*. To this aim, we have available more than 7,000 SARS-CoV-2 positive nasopharyngeal swabs from the Fundación Jiménez Díaz in Madrid that cover all pandemic waves. We are currently analysing the intra-host mutant composition of SARS-CoV-2 populations from infected patients by ultra-deep sequencing, and exploring synergistic combinations between inhibitors to achieve viral extinction. Most of these compounds are nucleotide analogues and some of them act as lethal mutagens, driving virus extinction by an excess of mutations. These projects are performed in collaboration with other teams, as reflected in recent publications.



When a viral particle enters into a host, rapidly replicates and becomes a distribution of different mutants called a viral quasispecies. Ultra-deep sequencing analyses are revealing a huge complexity of viral populations represented here through heat maps. Possible structural and functional effects of amino acid substitutions are routinely analysed in the three-dimensional structure of the corresponding proteins.

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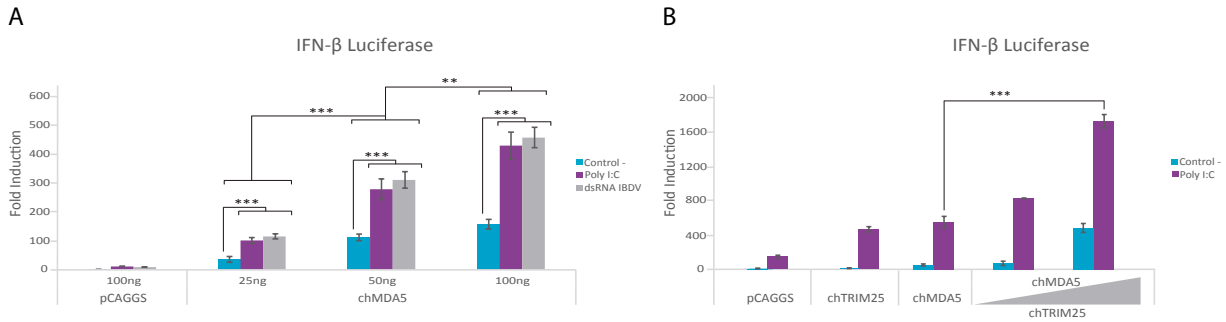
Molecular characterisation and epidemiology of torovirus

One of the main focuses of our research is the study of the virus-host interaction that would determine the outcome of the disease. For many years our efforts have been concentrated on toroviruses, emergent viruses (*Nidovirales* Order) that cause enteric diseases in different species of domestic animals and could represent a zoonotic threat. Related with them, and in the context of the COVID-19 pandemic, during this period we initiated new research projects on SARS-CoV-2 for the development of new strategies of control. We found that SARS-CoV-2 downregulates several enzymes of the Krebs cycle affecting the mitochondrial function and that the use of therapies directed to maintain mitochondrial function could be effective in combating SARS-CoV-2 infection (manuscript submitted). In addition, we have also collaborated with Drs. Montoliu and Fernández (CNB), and Dr. Moreno (CABD-CSIC)/UPO) in a project aimed at using the CRISPR-Cas technology to target coronavirus RNA genome.

In addition, we maintain a longstanding collaboration with the group of Dr. J.F. Rodríguez (CNB) to study the molecular bases of infectious bursal disease virus (IBDV) pathogenesis. IBDV infection is responsible for the immunosuppression and/or death of infected birds, causing heavy losses to the poultry industry worldwide. IBDV infection causes an exacerbated expression of proinflammatory cytokines, including IFN. Our initial results revealed that IFN contributes to exacerbate apoptosis of infected cells and therefore may collaborate to aggravate the disease caused by this virus in chickens. Additionally, we have determined that IBDV can establish long-term persistent infections in cultured cells. Significantly, the characterization of persistently infected cell clones revealed that they lack the capacity to respond to type I IFN. All the above highlights the importance of IFN in the outcome of IBDV infection. Our knowledge of the chicken IFN system is still very limited. Therefore, during this period we have approached the regulation of the signalling pathway initiated in chicken cells upon recognition of the dsRNA IBDV genome. We uncovered the regulatory role of TRIM25 on the MDA5 signalling pathway in chicken cells, and its contribution to control IBDV infection.



Activation of IFN-β promoter by the chicken pathogen recognition receptor MDA5, and its regulation by chicken TRIM25. *(A) DF-1 cells were co-transfected with different amounts of chMDA5 expression vector together with plasmids, pLucter, carrying the firefly* luciferase *gene under the IFN-β promoter, and pR-null, harboring the* Renilla luciferase *gene. At 8 h pt, cultures were either mock transfected (control) or transfected with IBDV dsRNA or synthetic dsRNA (Poly I:C) and harvested 24 h after plasmid transfection. (B) DF-1 cells were transfected with pLucter and pR-null in combination with the plasmids expressing either chTRIM25 or chMDA5, or chMDA5 with two amounts of the chTRIM25 expression plasmid. At 8 h pt the cells were transfected with Poly I:C. 24 h after plasmid transfection the firefly luciferase expression level of each sample was determined and normalized using* Renilla *values. ** and *** indicate P values of <0.01 and <0.001, respectively, as determined by unpaired Student´s test.*

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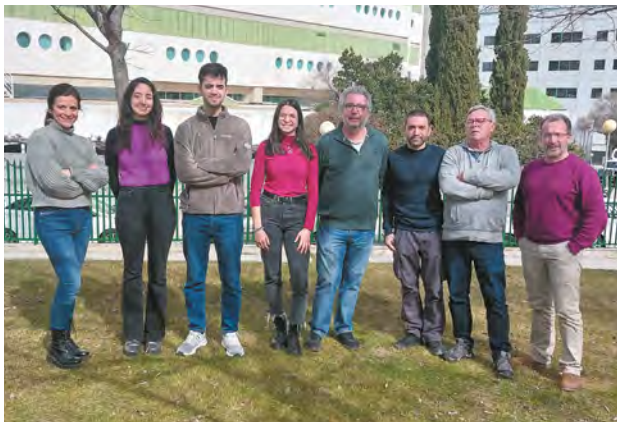

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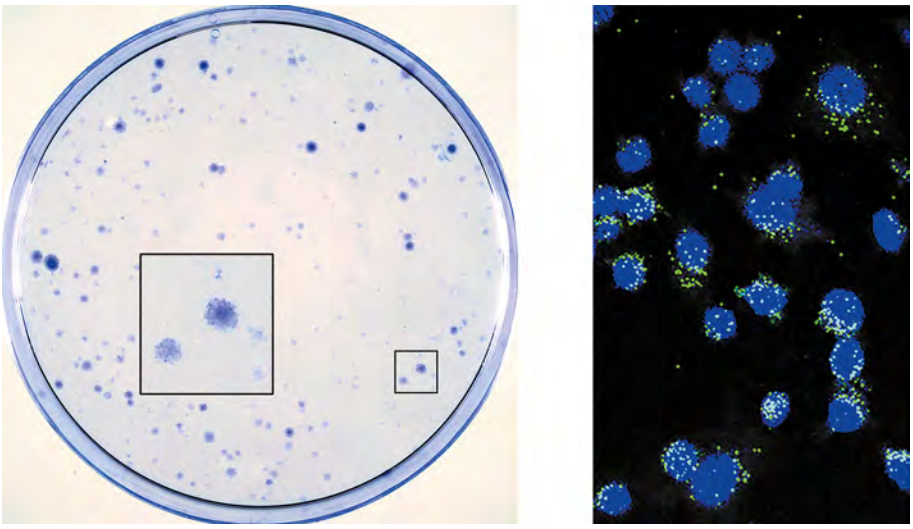
Molecular biology of birnaviruses

The *Birnaviridae* family groups a small number of species of non-enveloped, single shelled, icosahedral viruses harboring bipartite dsRNA genomes. Members of this unconventional dsRNA virus family include pathogens affecting a wide variety of animal species. The infectious bursal disease virus (IBDV), our main study model, is the etiological agent of an extremely contagious, immunosuppressive disease affecting domestic chickens with a major socio-economic impact to the Poultry Industry world-wide. During the past few years, our work has been mainly focused to deciphering the molecular basis underlying IBDV pathogenesis and the establishment of persistent infections. In addition to this, our team has devoted a great deal of effort to SARS-CoV-2 research, developing new diagnostic tools as well as a subunit vaccine candidate using dimers of the receptor binding domain (RBD) of the SARS-CoV-2 spike polypeptide.



Generation of DF-1 cell cultures persistently infected with IBDV. DF-1 cell monolayers were infected with IBDV using a multiplicity of infection of 3 plaque forming units per cell. Cultures were maintained for three weeks and then either stained with crystal violet, to visualise surviving cell clones (left panel), or processed for immunofluorescence microscopy (right panel) to detect the virus-encoded VP3 polypeptide (green). Cell nuclei (blue) were stained with DAPI. The inset at the left panel show a x2.5 magnification of the boxed area.

SELECTED PUBLICATIONS

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Diaz-Beneitez E, Cubas-Gaona LL, Candelas-Rivera O, Benito-Zafra A, Sánchez-Aparicio MT, *et al*. Interaction between chicken TRIM25 and MDA5 and their role in mediated antiviral activity against IBDV infection. Front Microbiol 2022, 13:1068328.

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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 adult tissue homeostasis and repair. Moreover, malfunction of these pathways can lead to disease. Therefore, studying developmental genes and signals can offer new avenues for treatment of prevalent diseases such as inflammatory diseases and cancer and also to improve the regenerative ability of tissues.

The focus of our research in embryology is the development of limb and heart, studying the mechanisms of their morphogenesis. In a long-standing collaboration with James Sharpe (EMBL Barcelona) on this topic, we have participated in the generation of a computer model of organ formation in the mouse embryo. The result allows for a data-driven quantitative 4D description of limb and heart morphogenesis (Figure 1).

We are also interested in analysing the relationship between inflammation, regeneration and disease. We want to understand how uncontrolled or chronic inflammation can lead to disease, particularly in the context of inflammatory bowel disease (IBD) and colon cancer. In collaboration with the group of Ana Cuenda (Department of Immunology and Oncology, CNB) we are studying the functions of p38MAPKs in those pathologies. Using mice models and also samples from patients, we have shown that an increase in p38*γ* and a decrease in p38*δ* protein expression correlates with more inflammatory bowel disease and tumour development, making p38*γ*/*δ* useful biomarkers for colitis and early colon cancer. We are also investigating the role of gut microbiota in inflammation and cancer.

Finally, we have studied the evolution of p38MAPK along the tree of life in collaboration with Iñaki Ruiz-Trillo (Institute of Evolutionary Biology, Barcelona), showing that a p38MAPK homolog was already present in the closest unicellular relatives of animals, where it can respond to osmotic stress (Figure 2).

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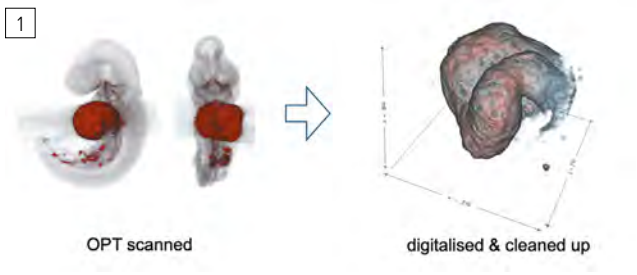

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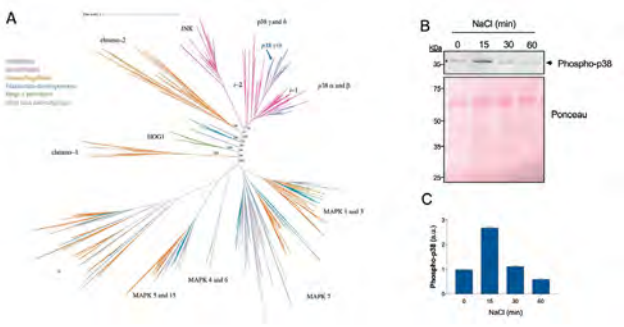

1 *Mouse embryo showing heart stained with myosin heavy chain, imaged with Optical Projection Tomography and digitised for computer reconstruction.*

2 *(A) Maximum-likelihood phylogenetic tree of the p38MAPK subfamily, including the closest unicellular relatives of animals. (B, C) Osmotic stress induces the phosphorylation of Capsaspora owczarzaki p38MAPK. B, Western blot and C, quantification of band intensity*

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Fajardo P, Cuenda A, Sanz-Ezquerro JJ. A Mouse Model of Candidiasis. Methods Mol Biol 2021, 2321, 63-74.

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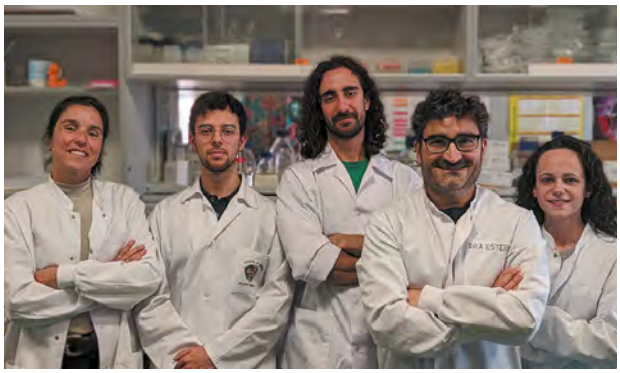

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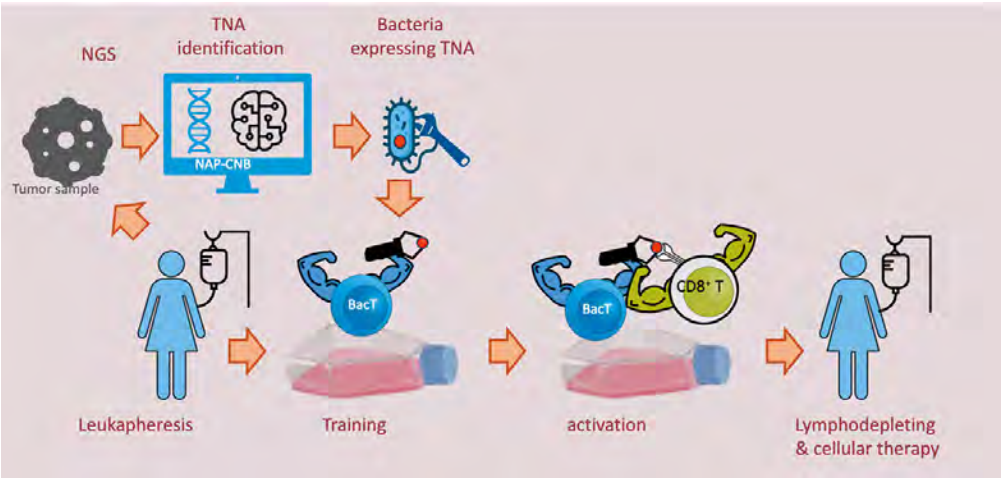
Andrea González Gómez

Bacteria-based immunotherapies against cancer

Our group is focused on generating novel cellular immunotherapies using the ability of bacteria to modify immune responses. We discovered that CD4+ T cells could be *"trained"* with some bacteria expressing tumor antigens to generate potent antitumor responses. This discovery that challenged the dogma of adaptive/innate immunity role separation are at the leading edge of the immunology and oncology fields as recognised by different prizes, for example, the XIX FERO foundation award for the best project against breast cancer.

CD4+ T cells can capture and destroy bacteria by transphagocytosis. Moreover, bacteria exposure "trains" conventional CD4+ T cells. Trained T cells (bacT), cross-present antigens from captured bacteria, activating naïve CD8+ T cells that became effective cytotoxic cells and differentiated into central memory cells expressing very low amounts of PD1 or CTLA-4; desired features for antitumor cells. The antitumor effects of bacT cell therapies are being tested in proof-of-concept experiments against different mouse model of cancer.

In addition, in order to fight one of the major bottlenecks in cancer immunotherapies, we are generating an easy-to-use platform supported in machine-learning based algorithms that would allow to rapidly identify tumor neoantigens (TNAs). Our predictions were more accurate than any competing algorithms. The (NeoAntigen Prediction; NAP-CNB) platform is open and only requires RNAseq data from malignant and healthy tissues. NAP-CNB is being generated under the direction of Drs. Carlos Oscar Sánchez Sorzano (CNB) and Arrate Muñoz Barrutio (Universidad Carlos III de Madrid).



Summary of bacT therapy. Tumour neoantigens (TNA) will be identify by using NAP-CNB platform developed by us from NGS sequencing. TNA will be cloned in engineered bacteria generated to optimise cellular training. Lymphocytes from the patients will be trained with bacteria expressing TNA and bacT cell-activated CD8+ T cells will be re-infused as therapy.

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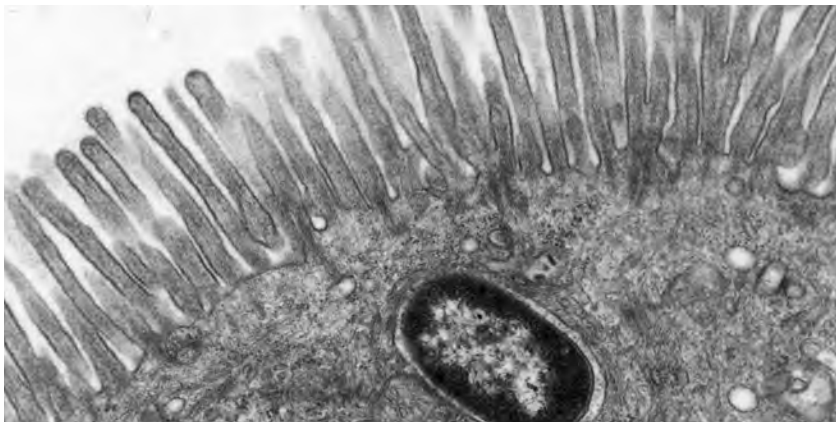

Microbial Biotechnology

Research in the Department of Microbial Biotechnology focuses on the molecular aspects of the biology of bacteria, their interaction with the environment or the host during infection, the spread of antibiotic resistance in the clinic, and on exploiting their biotechnological potential for biomedical and environmental applications. Our work includes multiple approaches using molecular genetics, systems and synthetic biology, evolutionary biology, genomics, proteomics and metagenomics. The scientific objectives of the Department are grouped on five complementary aspects of microbial biology:

- Environmental microbiology. We aim to characterise the mechanisms underlying the global regulatory networks that modulate bacterial metabolism in response to changes in environmental conditions, which can compromise biotechnological applications. We also study the the assembly of microbial communities and the mechanisms that contribute to horizontal gene transfer among environmental and pathogenic microbes.
- Microbial pathogenesis. Efforts are directed to understand the molecular mechanisms underlying extracellular and intracellular infections, from tissue colonisation to cell invasion and persistent infections in different host cell types.
- Microbial resistance to antibiotics. Work aims to understand the evolutionary mechanisms that contribute to 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 infections.
- Microbial responses to hostile environments. Our work focuses on the understanding of 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 engineered bacterial strains optimised to obtain products of interest such as nanobodies, and able to combat tumors, pathogens or environmental pollutants. In addition, we develop synthetic tools based on bacterial proteins, including amyloids, for biotechnological applications.

HEAD

Luis Ángel Fernández

Figure Legend: Salmonella enterica *in the interior of an enterocyte following invasion of the intestinal epithelial barrier. The micrograph was taken in a sample obtained from a mouse challenged with the pathogen per the oral route (Francisco García-Portillo´s lab).*

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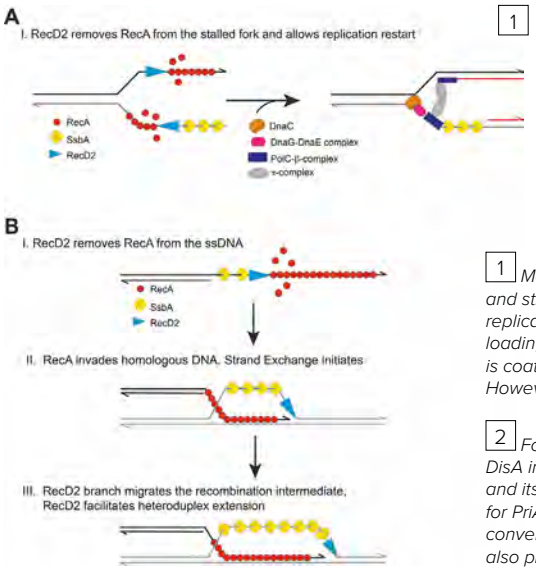
Genetic stability

We investigate the factors that govern genetic stability in bacteria and the mechanisms of horizontal gene transfer, using *Bacillus subtilis* as the model.

In the presence of endogenous threads, the replisome disassembles roughly five times during each cell cycle, leaving single-stranded DNA (ssDNA) regions coated by a single-stranded binding protein (SsbA). RecA recombinase may assemble at this ssDNA region and inhibit DNA replication restart. We found that the RecD2 helicase avoids this, and facilitates thereby replication restart. Another helicase, PcrA, also modulates RecA activities. In addition, we analysed how the damage checkpoint (DisA) is implicated in the preservation of stalled replication forks and genome integrity in *B. subtilis*. DisA catalyses the formation of c-di-AMP, and we found that levels of this essential second messenger are altered by replication stalling. RecA interacts with and loads DisA at the stalled fork. DisA-mediated synthesis of c-di-AMP is suppressed upon DisA binding to DNA structures that mimic stalled or reversed forks (gapped forks or Holliday junctions [HJ]) and c-di-AMP synthesis is blocked in the presence of helicases RecG, RuvAB and RadA/Sms bound to these structures. Low c-di-AMP levels indirectly inhibit DNA replication by inhibiting DNA primase activity. *In vitro* assays also revealed that DisA assembled at stalled or reversed forks limits RecG- and RuvAB-mediated branch migration and fork remodeling. Finally, how these helicases that act on recombination intermediates (RecG, RuvAB, RecD2 and RadA/Sms) contribute to the acquisition of divergent sequence during natural competence was analysed.



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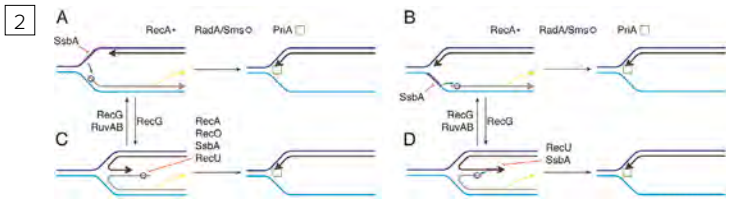

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1 *Model depicting how RecD2 helicase may modulate RecA during replication restart and strand exchange. (A) On a stalled fork, RecA may bind to the ssDNA regions inhibiting replication restart. RecD2 removes RecA from the stalled fork and thereby, facilitates replisome loading and replication restart. (B) During homologous recombination the invading strand is coated with RecA. RecD2 may translocate 5´➔3´ along the ssDNA and remove RecA. However, once RecA initiates strand exchange it may accelerate heteroduplex extension.*

2 *Fork remodelling after DisA detection of stalled leading- (A) or lagging-strand (B). The DisA interacting protein RadA/Sms is loaded on the stalled fork in coordination with RecA and its mediators and unwinds the nascent lagging-strand to yield the optimal substrate for PriA binding and replication restart (A and B). Alternatively, the RecG remodeller converts the stalled forks into Holliday junction-like structures (C and D), which can be also processed by the RadA/sms helicase facilitating PriA replication restart.*

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Stress and bacterial evolution

The research of the "Stress and Bacterial Evolution" group is focused on the genetic mechanisms involved in genome stability and their roles in bacterial evolution and adaptation. The group´s interest is to understand the genetic basis of both stable and induced hyper-mutation/hyper-recombination as bacterial "strategies" to speed adaptation to stress, particularly to antibiotic challenge. Recently, the group described the genetic characteristics of a novel non-canonical mismatch repair (nc-MMR) system in some Prokaryotes, including Mycobacteria and *Streptomyces*, a key pathway for maintaining their genome stability. Currently, we are trying to disentangle the possible relationship between genome stability/instability and antibiotic resistance development in pathogenic Mycobacteria, such as *M. tuberculosis, M. abscessus* and *M. avium*.

From a practical perspective, the lab wants to apply this knowledge to i) prevent and fight antibiotic resistance in bacterial pathogens by searching for new antibiotics (such as anti-mutagenic molecules and inhibitors of *β*-lactamases) and new target genes, and ii) engineer prokaryotic species (eg *Streptomyces*) to improve their biotechnological features.

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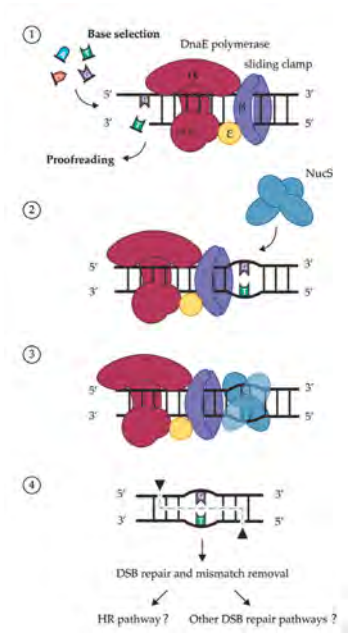

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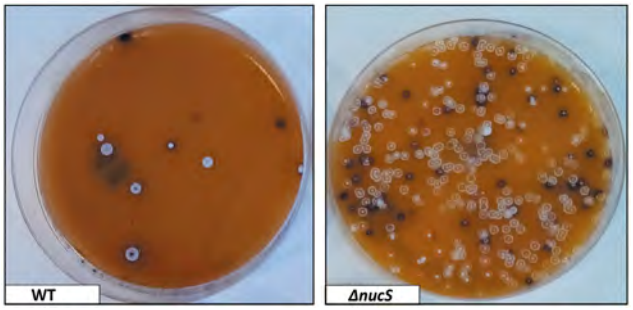

1 *Model of action of the non-canonical MMR pathway in Actinobacteria. DnaE core polymerase (α subunit, red; ε subunit, yellow), sliding clamp (β subunits, purple) and NucS dimer (blue). (1) During replication DnaE polymerase performs base selection and, through its PHP domain, proofreading activity (3'–5' exonuclease). In mycobacteria, ε subunit has no proofreading activity. (2) The mismatches that escape these correction processes are the substrate of NucS. (3) NucS binds to the dsDNA containing a mismatch and its activity is stimulated by interaction with the sliding clamp. (4) NucS nicks both strands around the mismatch leaving a DSB. Finally, the DSB and the mismatch may be repaired through either HR pathway or other DSB repair mechanisms. Source: Cebrián-Sastre. Cells. 2021. 10(6):1314.*

2 *A* Streptomyces coelicolor *nucS mutant (right) produces a high number of mutants resistant to the antibiotic (rifampicin) in comparison with the wild type strain (left).*

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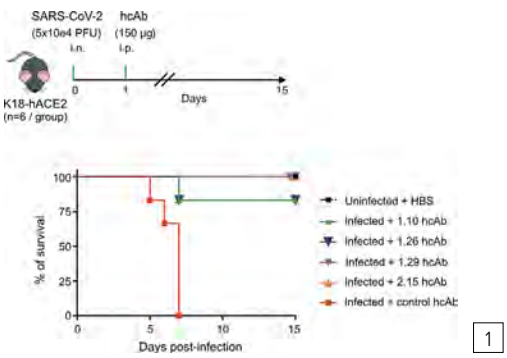
Bacterial engineering for biomedical applications

Our research is aimed to engineer *E. coli* bacteria for biomedical applications, including the selection of single-domain antibodies (nanobodies) and the design of synthetic bacteria for diagnostic and therapeutic use. Nanobodies are the smallest recombinant antibody fragments with full antigen-binding capacity, and are derived from heavy-chain-only antibodies found in camelids (e.g. dromedaries, llamas). We study protein secretion systems found in pathogenic *E. coli* strains, such as enteropathogenic *E. coli* (EPEC), and engineer them to produce protein nanomachines that can be applied in the selection and expression of nanobodies, or the delivery of therapeutic proteins into tumour cells. We use synthetic biology and genome engineering to combine the expression of these modular parts in our engineered bacteria.

These years our work has focused on: 1) The expression and selection of nanobodies that neutralise SARS-CoV-2 infection in vivo; 2) The engineering *E. coli* bacteria as anti-tumour agents able to bind to tumour cells and deliver a protein cargo; 3) The directed evolution of proteins with a novel *in vivo* mutagenesis system in *E. coli*, called T7-DIVA.



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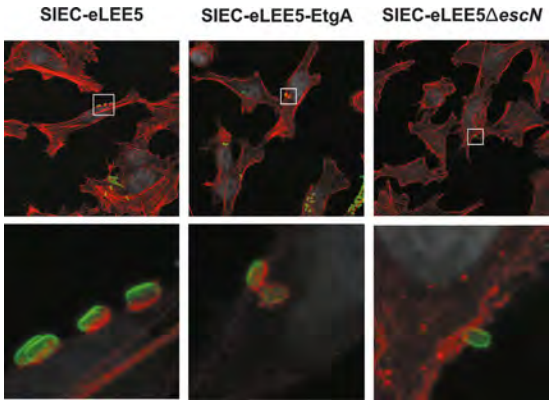

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1 *Protection of hACE2-trangenic mice after a lethal SARS-CoV-2 infection by selected nanobodies. Groups of K18-hACE2 mice (n=6/group) were either infected intranasally (i.n) with a lethal dose of SARS-CoV-2 (infected groups) or mock infected with PBS (uninfected group). On day 1 postinfection, 150 µg of nanobodies 1.10, 1.26, 1.29, 2.15 or control, fused to human IgG1 Fc domain (hcAbs) were administered intraperitoneally (i.p.) to animals in the infected groups. The uninfected group was treated i.p. with buffer (HBS). Graph represents the percentage of daily mice survival in each experimental group up to 15 d.p.i.*

2 *Translocation of Tir protein into mammalian cells by synthetic injector* E. coli *(SIEC) strains. Confocal fluorescence microscopy images of HeLa cells infected with the indicated bacterial strains SIEC-eLEE5, SIEC-eLEE5-eEtgA and SIEC-eLEE5ΔescN (T3SS mutant). F-actin in cells is stained red, and DNA and nuclei are stained grey. Bacteria are stained in green. The F-actin accumulations can be visualized as strong red fluorescence signals associated with attached bacteria, except in the ΔescN mutant, indicating the translocation of Tir protein by the T3SS. Bottom images are magnifications of the regions marked with white squares.*

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Intracellular bacterial pathogens

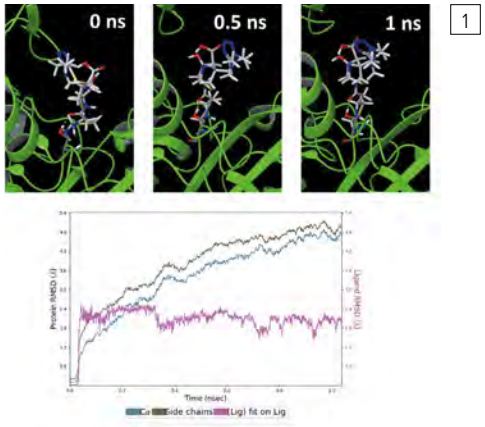
Intracellularity as lifestyle has been selected by an array of successful bacterial pathogens in which *Salmonella* is included. Our lab aims to understand how *Salmonella* evolved to colonise acidic vacuolar compartments of eukaryotic cells and the changes occurring in its cell envelope during the residence in such unique niche. A common phenomenon occurring in this vacuolar compartment is the entry of the pathogen into a state of reduced metabolic activity (latency) that favours persistence. This metabolic switch and in such particular environment represents one of the major obstacles to eradicate the intracellular infections caused by *Salmonella*.

Morphological studies

We have identified several proteins upregulated by intravacuolar *Salmonella* in response to the acidic environment that encounters within the infected host cell. Some of these proteins are peptidoglycan synthases involved in morphogenesis, which are pathogen-specific and "replace" enzymes extensively characterised in bacteria grown in laboratory media. This feature is currently exploited to identify drugs acting selectively on targets expressed by intracellular bacteria. Besides this applied investigation, we are much interested in determining at the molecular and cellular level how *Salmonella* morphogenesis is regulated within the phagosomal compartment, considering the spatial constrains that are imposed by the surrounding phagosomal membrane. We have found unique morphogenetic complexes involved in cell elongation and division of intracellular *Salmonella* and our aim is to characterise the involved components. We also seek to understand how these new morphogenetic systems interact with the virulence factors that promote survival and proliferation of intracellular bacteria.

Evolutionary studies

We are starting studies focused on the analysis of enzymatic activities of uncultivated bacteria predicted to be linked to peptidoglycan metabolism. These studies are offering new insights into what it could be the origin of the cell wall in unicellular microbes, a major event that marked the split of the Archaea and Bacteria domains.



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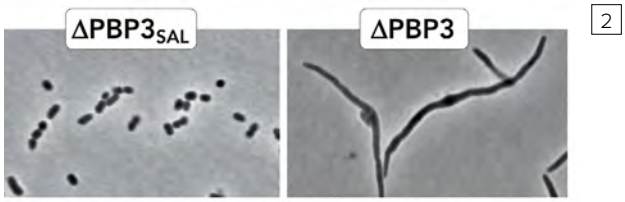

2

1 *Molecular dynamics showing binding of a drug to the morphogenetic protein PBP3SAL upregulated by intracellular Salmonella. ns = nanosecond.*

2 *Morphological alteration caused by a drug that shows higher affinity to PBP3SAL than to PBP3 as inferred by the response of the respective mutants.*

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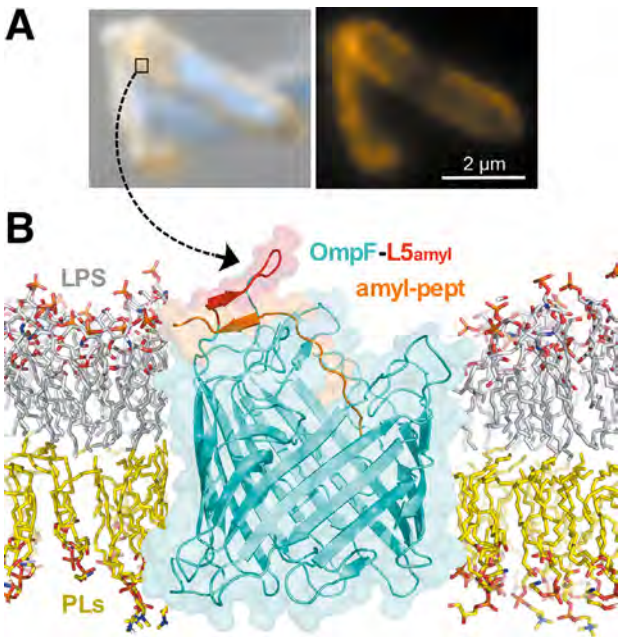
Synthetic bacterial amyloids (SynBAmyl)

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

RepA is a protein from a bacterial plasmid whose WH1 domain undergoes conformational changes enabling it as a transcriptional repressor, or as a DNA replication initiator or inhibitor, the latter by assembling a functional amyloid, that hinders 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 strains with distinct appearance and cytotoxicity, modulated by an Hsp70 chaperone. 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.

Recently, control on RepA-WH1 amyloidogenesis has 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 ('optobiotics').

Along the last two years, we have engineered the bacterial outer membrane porin OmpF by grafting its extracellular loops with an amyloidogenic peptide sequence, thus enabling homotypic self-recognition of the same sequence when either presented in solution or displayed on functionalized surfaces. We are exploring the potential of such synthetic bacterial devices as biosensors for environmental amyloids, to then further expand their abilities to achieve amyloid clearance.



Engineering the E. coli *outer membrane (OM) porin OmpF as a scavenger of amyloid peptides and proteins. (A) Rhodamine-labelled amyloid RepA-WH1 peptide probe (orange) is selectively titrated on the envelope of bacteria expressing OmpF having the same amyloidogenic sequence grafted into its extracellular loop 5 (L5). (B) 3D model of the OmpF β-barrel (cyan) embedded in the OM with the target amyloidogenic peptide (orange) docked, forming a β-sheet with the amyloid-grafted L5 in the porin (red).*

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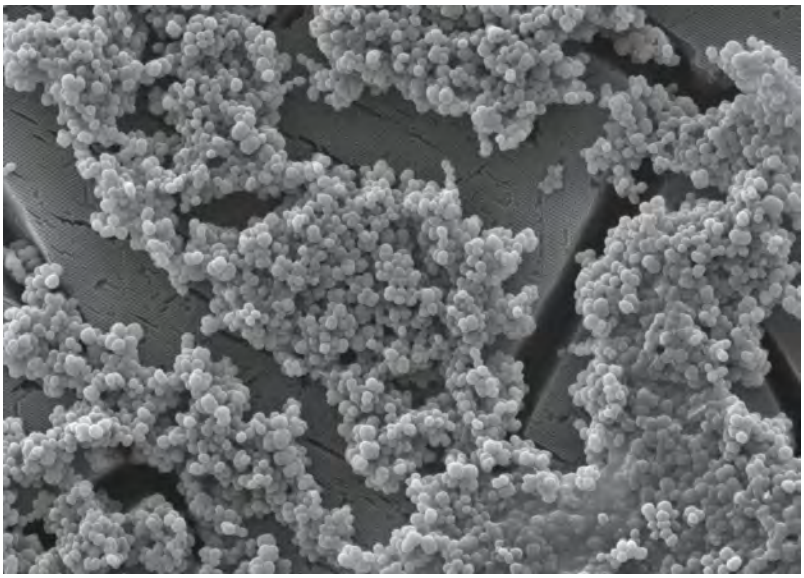
Molecular infection biology

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. This laboratory is a key group in the field of functional membrane microdomain bacterial compartmentalisation 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 resistant to antibiotic treatments. To do this, we work in the interface of molecular and cellular biology with other scientific disciplines, such as structural, infectious diseases, synthetic and systems biology. This interactive and multidisciplinary environment provides to the laboratory 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.



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

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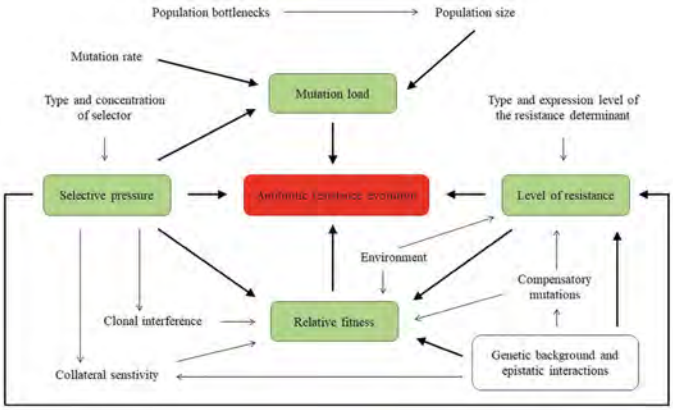
Ecology and evolution of antibiotic resistance

One of the most cumbersome human health problems is the increased prevalence of antibiotic resistant pathogens. As stated by the WHO, this is not just a problem dealing with regular infections. Several therapeutic procedures as transplantation, anti-cancer therapy or surgery require a good prevention and treatment of potential associated infections. Antibiotic resistance can comprise such treatments and, hence several aspects of current clinical practice. With few new antibiotics introduced into the market, a better use of those currently available is mandatory.

Our laboratory has been involved in the development of tools for predicting the pathways of emergence of antibiotic resistance. In addition of their predictive potential, the use of these tools has allowed to determine the emergence of some trade-offs associated to the selection of antibiotic resistance. These trade-offs constitute bacterial weaknesses that could be exploited for improving current anti-bacterial therapeutic strategies. Among them, we have explored collateral sensitivity and fitness costs. Collateral sensitivity consists on the increased susceptibility to one antibiotic when bacteria acquire resistance to a different one. This situation can be exploited to design sequential or combinatory treatments. However, its implementation requires that the phenotype is conserved in different bacteria and in different growing conditions. We have been able to identify some robust collateral sensitivity patterns that could be exploited for treating *Pseudomonas aeruginosa* infections. Regarding fitness costs, it is generally accepted that resistant bacteria can present a growth impairment in such a way that they will be outcompeted by their wild-type susceptible counterparts. However, it was soon evident that resistant bacteria can acquire compensatory mutations that restore their fitness. We have identified antibiotics that select mechanisms of resistance that impose high fitness costs. These fitness costs are compensated when bacteria grow in absence of the antibiotic, but resistance also declines. Further compensatory evolution preserves a conserved collateral sensitivity phenotype in a variety of strains indicating that this phenotype is robust.



Conceptual scheme bringing together the factors that constrain the evolution of antibiotic resistance and showing the interplay among them. *Four factors have a central effect on the evolution of antibiotic resistance: the relative fitness of the resistant mutant, the level of resistance conferred by the resistance mechanism, the strength of selection pressure, and the frequency of mutation. Genetic background and epistatic interactions have a major effect on antibiotic resistance evolution: resistance level, as well as the potential genetic modifications to compensate it, fitness cost and collateral sensitivity associated with a specific mutation, are strongly dependent on genetic background. The central factors are also influenced by other aspects, such as the emergence of compensatory mutations or the population size. Reproduced with permission from Pablo Laborda's PhD Thesis.*

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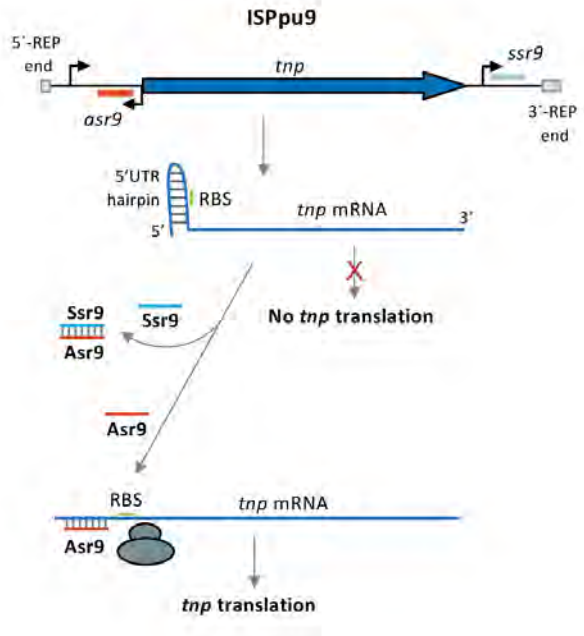
Regulation of gene expression and metabolism in bacteria

Pseudomonas putida KT2440 is a model bacterium that has become a valuable tool in biotechnology. It is not virulent, it is resistant to various types of stress, and has a great metabolic versatility. To optimise its performance, it is necessary to understand in detail the complex regulatory networks that coordinate the expression of its genome and the configuration of its metabolic fluxes. These regulatory processes optimise growth, but also limit the performance of many biotechnological processes. An additional problem derives from the elements that affect the stability of its genome. Strain KT2440 has a large genome with many insertion sequences, genomic islands and repeated sequence elements that can lead to mutations, rearrangements, or the deletion of large DNA segments. Obtaining stable strains useful in biotechnological processes requires understanding how the activity of mobile genetic elements is regulated. Our efforts are directed to address these problems.

The optimisation of metabolic fluxes relies on a regulatory network that includes the Crc and Hfq proteins, and two small RNAs named CrcZ and CrcY. Crc and Hfq inhibit translation of mRNAs containing a specific sequence motif within their translation initiation region, a process that is antagonised by CrcZ and CrcY. We have characterised the influence of these regulatory elements on *P. putida* physiology and the molecular mechanisms involved. Our interest on the Hfq protein led us to analyse its role in other processes such as iron homeostasis and the regulation of ISPpu9, an insertion sequence present in *P. putida* KT2440. We have observed that translation of the mRNA encoding the ISPpu9 transposase 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 RNA. We are currently studying the influence of Hfq on this regulatory process.



Expression of the transposase gene (tnp) from the insertion sequence ISPpu9 present in Pseudomonas putida *KT2440 is inhibited by a strong secondary structure that blocks the translation of the tnp mRNA. The Asr9 small RNA binds to this region, weakening the secondary structure and facilitating translation. The Ssr9 small RNA likely counteracts Asr9, by binding to it.*

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Rojo F. A new global regulator that facilitates the co-metabolization of polyaromatic hydrocarbons and other nutrients in Novosphingobium. Enviro Microbiol 2021, 23, 2875-2877.

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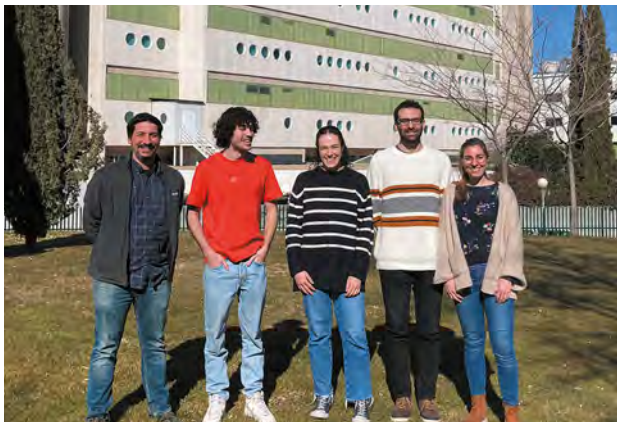

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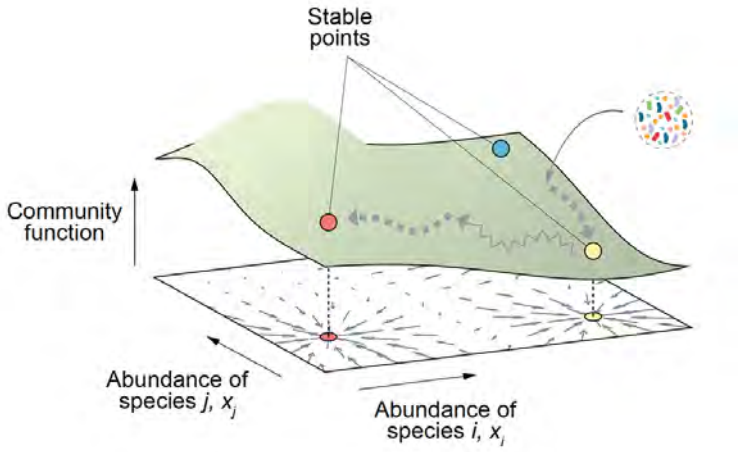
Microbial evolution and ecology

Our research centres on exploring how evolutionary biology may help us make sense, predict, and engineer microbial communities. To this end, we employ a combination of laboratory experiments, computer simulations, and mathematical modeling.

Our laboratory is highly interdisciplinary and quantitative in nature, and both predoctoral and postdoctoral researchers typically combine theory and experiment in their projects, acquiring a broad range of technical skills across the wet-lab / dry-lab divide. They also get exposed to a broad scientific intellectual background in ecology, evolution and systems biology.

We have two main research lines. The first consists of engineering microbial consortia from the top down, using evolutionary engineering. The second one consists of developing quantitative and predictive models linking microbial community composition and function.

This group joined the CNB in Summer 2022.



Conceptualisation of our basic protocol of directed evolution of microbial consortia.

SELECTED PUBLICATIONS

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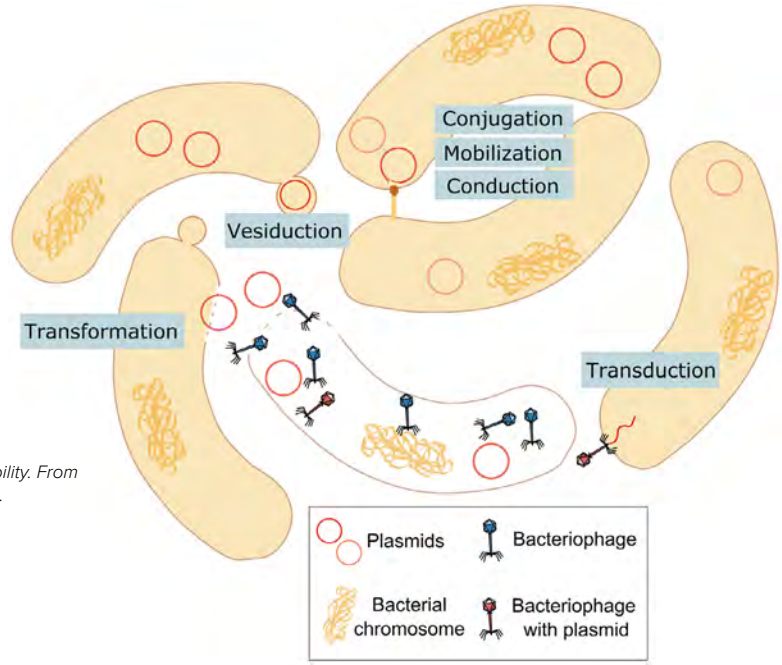
Plasmid biology and evolution

In the PBE lab we are interested in the evolutionary forces that drive plasmid dynamics in bacterial populations, with special focus in the evolution of antibiotic resistance in bacteria. Plasmids play a crucial role in bacterial ecology and evolution because they can transfer genes horizontally between different bacteria. 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.



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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 and shoot branching. 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. We use model species, such as the angiosperms *Arabidopsis thaliana, Nicotiana benthamiana* and the duckweed Lemna spp., and the liverwort *Marchantia polymorpha*. 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

Roberto Solano

Figure legend: Arabidopsis thaliana *mutants in SNOWY COTYLEDON 2 (Pablo Pulido).*

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Alba Murillo Sánchez

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 enable plant adaptation. To address this question we are exploiting the genetic variation that exists in nature in the wild, annual, and model plant *Arabidopsis thaliana*. In the past few years we have analysed the natural variation for the amount and distribution (pattern) of trichomes, showing that Arabidopsis has evolved trichomes in fruits exclusively in the Iberian Peninsula (Figure 1 and 2). Genetic analyses have demonstrated that three loci named as *MALAMBRUNO* (*MAU*) 2, 3 and 5, showing strong epistatic interactions, are necessary and sufficient to display this trait. Molecular analyses show that synergistic mutations in three genes encoding MYB transcription factors, TCL1, TRY and GL1, have driven evolutionary innovations in fruit trichome patterning in Arabidopsis (Arteaga *et al*., 2021).

Genome-wide association analyses for trichome pattern in other organs, such as leaves, stems and pedicels, have shown that partly independent genomic architectures underlie vegetative and reproductive phases. Furthermore, climatic associations suggest a precise fit between trichome patterning and climate throughout the Arabidopsis life cycle. In addition, in collaboration with other laboratories, we have also analysed Arabidopsis natural variation for flowering time and virus tolerance (Shukla *et al*., 2022), as well as for oxygen dependent traits (Abbas *et al*., 2022), thus identifying new adaptive mechanisms. Finally, we have also developed a new collection of natural strains of the Arabidopsis relative *Cardamine hirsuta*, which will enable future comparative genomic analyses of developmental traits (Fuster, 2022).

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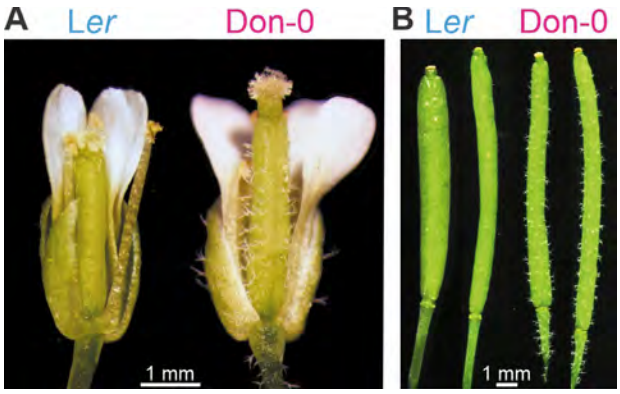

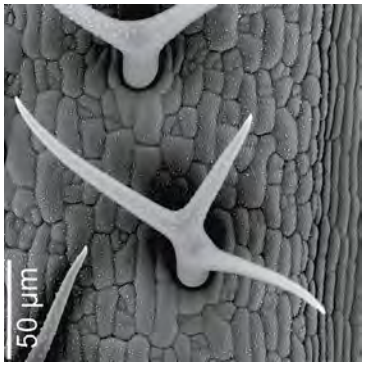

2

1 *Natural variation for trichome development in carpels (A) and fruits (B) of Arabidopsis. Ler and Don-0 are two natural accessions of Arabidopsis from Poland and Spain, respectively.*

2 *Branched trichome developed in a fruit of Don-0 accession from Spain.*

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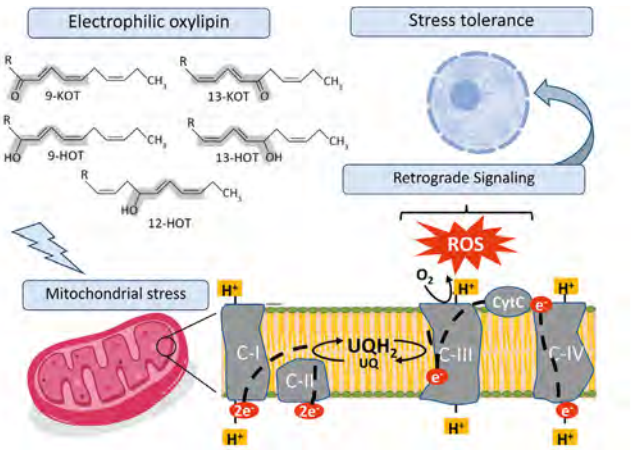
Plant immunity strategies against microbial pathogen infection

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, pose 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; a knowledge critical for the devise of effective approaches to minimise plant losses due to infection. We focus our research in exploring the activities of oxylipins, a family of lipid derivatives activating immune responses in plants. Our research has revealed that oxylipins produced by the biosynthetic pathways initiated by fatty acid alpha-dioxygenases (alpha-DOXs) and 9-lipoxigenases (9-LOXs) contribute to the activation of local and systemic defense. Analyses with 9-HOT and 9-KOT (two members of the 9-LOX pathway) and signaling mutants (*noxy* for *non-responding to oxylipins)* showed the role of mitochondria in 9-LOX signaling and the action of 9-HOT and 9-KOT by enhancing ROS production at complex III of the respiratory mitochondrial chain; a response that protects plants against subsequent mitochondrial damage and activates plant defense. Protection against mitochondrial damage was found in *noxy* mutants affecting mitochondria, cytoplasmic or chloroplast proteins. This indicated that mitochondrial protection is a common response to distinct stresses and likely a critical process to maintain energy supply and facilitate plant survival. Furthermore, our results support the action of oxylipins as inducers of retrograde signaling pathways mediating communication and functional coordination of organelles during the activation of plant immunity. Evidence suggests that these responses are mediated by covalently binding of oxylipins to their targets, presumably mitochondrial proteins. The characterisation of the processes mentioned will contribute to define new defense mechanisms, as well as the signals, pathways, and genes involved in controlling plant immunity.



In environmental stress conditions, electrophilic oxylipins from both enzymatic and non-enzymatic sources cause a mild, ROS-associated mitochondrial stress. This process triggers a retrograde signalling pathway, leading to induction of nucleus-encoded genes which protect mitochondria against subsequent stress.

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Genetic control of shoot branching patterns in plants

We are studying the genetic basis of the control of axillary bud development in the model system Arabidopsis, and in the crop species tomato and potato in which control of lateral shoot branching is of great agronomical interest. We have characterised the Arabidopsis *BRANCHED1* (*BRC1*) gene, which acts as a central switch of axillary bud development and outgrowth. We have recently combined ChIP-seq, transcriptomic and systems biology approaches to characterise the Arabidopsis 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. The *BRC1* network is enriched in feed-forward and feed-back loops, robust against noise and mutation and reversible in response to stimuli. This knowledge is fundamental to adapt plant architecture and crop production to ever-changing environmental conditions.

In Solanaceae we are exploring new roles of BRC1-like genes in relation to the control of carbon allocation storage and usage, a process critical for plant growth and development. A good model system to study this is potato tuberisation, a developmental program important for both crop food production and CO2 capture. Potato tubers are natural carbon reserves in the form of starch that have evolved to allow propagation and survival over winter. 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 tuberization. In aerial axillary buds, *BRC1b* promotes dormancy, abscisic acid responses and a reduced number of plasmodesmata. This limits sucrose accumulation and access of the tuberigen protein SP6A. *BRC1b* also directly interacts with SP6A and blocks its tuber-inducing activity in aerial nodes. Altogether, these actions help promote tuberisation underground.

In potato plants a single gene, BRANCHED1b, blocks the accumulation of sugars in above-ground plant organs. Its loss results in tubers growing on aerial shoots.

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Regulation of gene expression in plants

Plant adaptation to environmental stimuli involves the activation of specific transcriptional cascades and networks that allow plants to reprogram their growth and development in a changing environment. 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, the short DNA sequences bound by TFs, known as TF-binding sites (TFBS) and 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 addition we are developing some bioinformatic and computational approaches for a biological interpretation of genomic 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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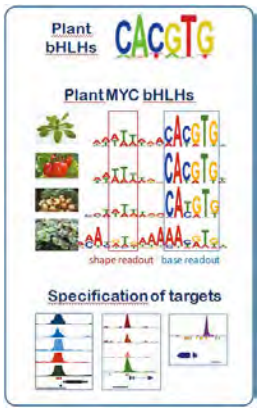
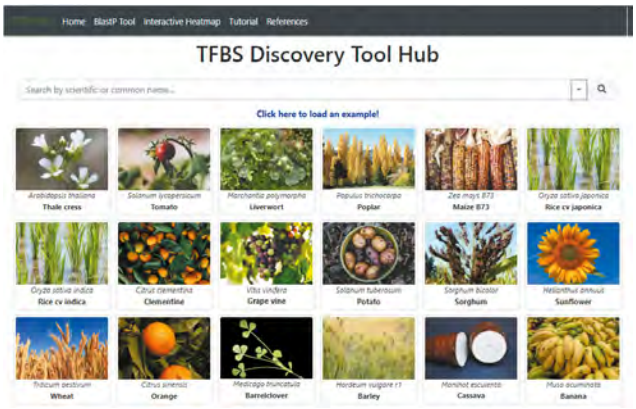


1 *Different components contribute to specification of bHLH targets.*



2

2 *TDTHub helps identifying relevant TFBS in model plants and crops*

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Plant-pathogen-interaction in viral infections

Although metagenomic studies show that most viral infections do not produce noticeable damage in wild plant hosts, viruses also cause severe diseases in all type of cultured plants. Understanding the infection process is essential to uncover factors involved in viral pathogenicity and find weak points on which to act in order to prevent plant diseases of agronomical relevance. This is the general objective of our research line.

We are mainly interested in the family *Potyviridae*, the largest group of plant RNA viruses, especially in plum pox virus (PPV), which causes sharka, an important disease of trees of the genus *Prunus*. We have shown the peculiar nature of the P1 protein of the potyvirus sweet potato feathery mottle virus (SPFMV), which is able to functionally replace both P1 and HCPro of PPV. The wide variety of viral species selected in plants infected with the PPV-SPFMV chimera highlights the strong adaptation capacity of P1 and provides hints about the evolution of the family *Potyviridae*. We have also obtained evidence that the fusion of pyrophosphatase of non-canonical nucleotides to the viral polymerase facilitates adaptation of viruses to hosts of the family *Euphorbiaceae*. Also related with host adaptation, our results have shown that mutations emerged in a PPV isolate of the cherry strain during adaptation to *Arabidopsis thaliana* facilitates its infection in *Chenopodium foetidum*, a non-host species for this PPV strain. These results reveal how virus host jumping can be promoted by pre-adaptation into an intermediate host. Regarding engineering resistance, we have demonstrated that targeting the viral minus strand RNA contributes to the antiviral resistance against PPV conferred by artificial miRNAs (amiRNAs), and that in partially resistant plants, the selection pressure posed by the dual activity of both amiRNA strands on the genomic and minus viral RNA strands causes an evolutionary explosion resulting in the emergence of a wide range of virus variants. *(Research supported by grants of the Spanish government BIO2016-80572-R PID2019-109380RB-100 to J.A. García and C. Simón, and PID2019-110979RB-100 to A. Valli).*

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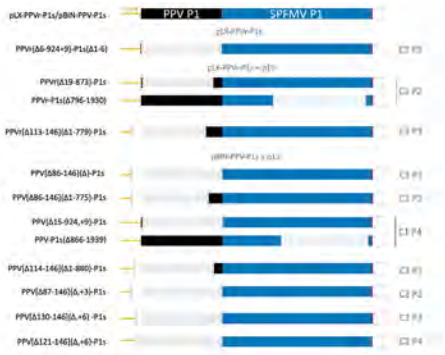

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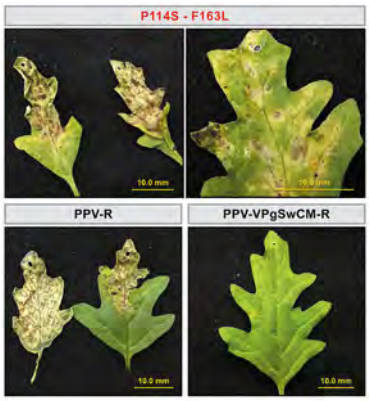

1 *Schematic representation of the different viral species found in plants infected with a chimeric plum pox virus carrying P1 of sweet potato feathery mottle virus.*

2 *Effect of VPg mutations emerged in* Arabidopsis thaliana *on* Chenopodium foetidum *infection by a plum pox virus chimera.*

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Mechanisms underlying nutrient uptake and phytoremediation

Understanding the mechanisms underlying stress perception and growth adaptation to stress severity is a major goal of biology. This is particularly relevant at present, since climatic models predict the sudden availability of toxic compounds in the biosphere. Among all toxic compounds, the presence of arsenic in soils and waters is particularly serious in rice, being the most important entry of arsenic in the human food chain.

In our laboratory we are involved in the characterisation of the molecular mechanisms underlying arsenic perception in plants.

In the last two years we uncover a complex regulatory network that sense arsenite tightly coordinating the amount of arsenic perceived by plants with its detoxification capacity and accumulation (Navarro *et al*., 2021, Molecular Plant). Indeed, in collaboration with our colleague, Javier Paz-Ares, our results contributed to provide a perspective review of the cross talk between arsenate and phosphate uptake regulation (Paz-Ares *et al*., 2021, Molecular Plant). Finally, in collaboration with our colleague Carlos Alonso-Blanco, we performed a study of the natural variation of nitrogen, phosphorous and arsenic accumulation in a collection of duckweed natural isolates from the Iberian Peninsula. Currently, we are identifying regulators of the arsenic response critical for arsenic perception (Figure 1 and 2).

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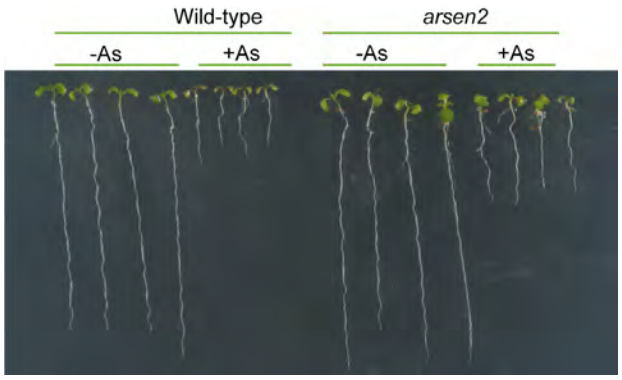
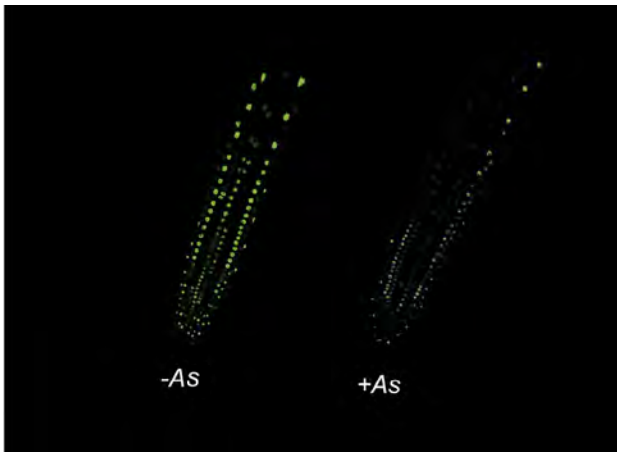


1 *ArSen2 mutant (arsen2) exhibits an arsenic tolerance phenotype. Wild-type and Arsen2 KO mutant plants (arsen2) were grown on MS vertical plates with (+As) or without (-As) arsenite.*

2



2 *ArSen2 is down regulated in response to arsenic. Confocal microscopic analysis of Arabidopsis plants expressing the ArSen2 tagged with GFP with or without arsenite (As).*

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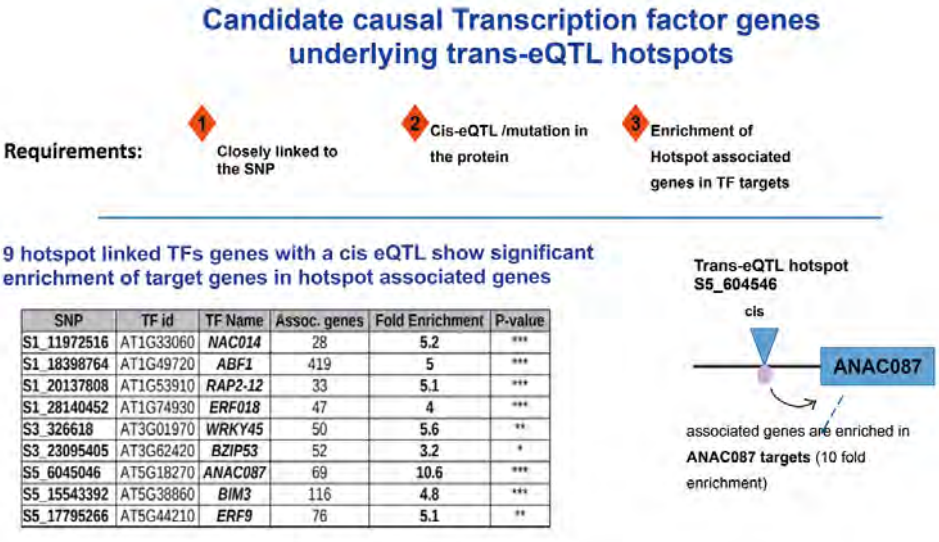
Regulation of gene activity in plants. The phosphate starvation rescue system

The phosphate starvation rescue system has been a model for studies on the regulation of gene activity since the beginning of molecular genetics. In plants this system has recently received great attention due to its potential to provide tools and strategies towards improving phosphate use efficiency, a key objective towards the effective implementation of sustainable agriculture practices. In the past, we identified several key components of the phosphate starvation signalling pathway following forwards genetics approaches (see Paz-Ares *et al* 2022)

Recently we embarked in the analysis of natural variation of the Pi starvation rescue system. Specifically, we performed a Genome wide Association Study (GWAs) of the Pi starvation transcriptome and metabolome in the Iberian collection of Arabidopsis accessions (kindly provided by Carlos Alonso-Blanco). We identified 15891 QTls associated to changes in 5999 metabolites (out of 8869 detected) and 18892 genes (out of 20808 detected), indication that a large proportion of the metabolome and transcriptome displays variation among different accessions. Of note is that we identified 1296 hotspots QTls each affecting the expression/accumulation more than 20 genes(eQTL)/metabolites (mQTL). Following the strategy depicted in Figure 1, we have identified 9 transcription factor (TF) genes as candidate causal genes underlying eQTL hotspots.



SNP	TF id	TF Name	Assoc. genes	Fold Enrichment	P-value
S1_11972516	AT1G33060	*NAC014*	28	5.2	***
S1_18398764	AT1G49720	*ABF1*	419	5	***
S1_20137808	AT1G53910	*RAP2-12*	33	5.1	***
S1_28140452	AT1G74930	*ERF018*	47	4	***
S3_326618	AT3G01970	*WRKY45*	50	5.6	**
S3_23095405	AT3G62420	*BZIP53*	52	3.2	*
S5_6045046	AT5G18270	*ANAC087*	69	10.6	***
S5_15543392	AT5G38860	*BIM3*	116	4.8	***
S5_17795266	AT5G44210	*ERF9*	76	5.1	**

Strategy towards the identification of candidate causal TF genes underlying trans eQTL hotspots. *The causal TF gene should be 1) closely linked to the SNP; 2) the SNP should be associated to an alteration of the expression of the TF gene (cis-eQTL) or to a plausible strong mutation in the protein and 3) hotspot- associated genes should be enriched in targets of the candidate TF*

SELECTED PUBLICATIONS

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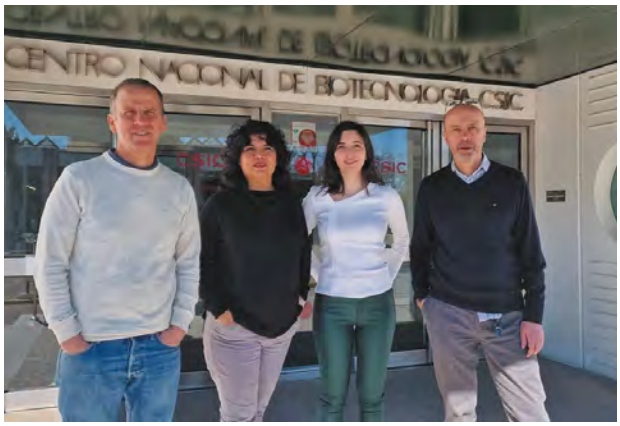

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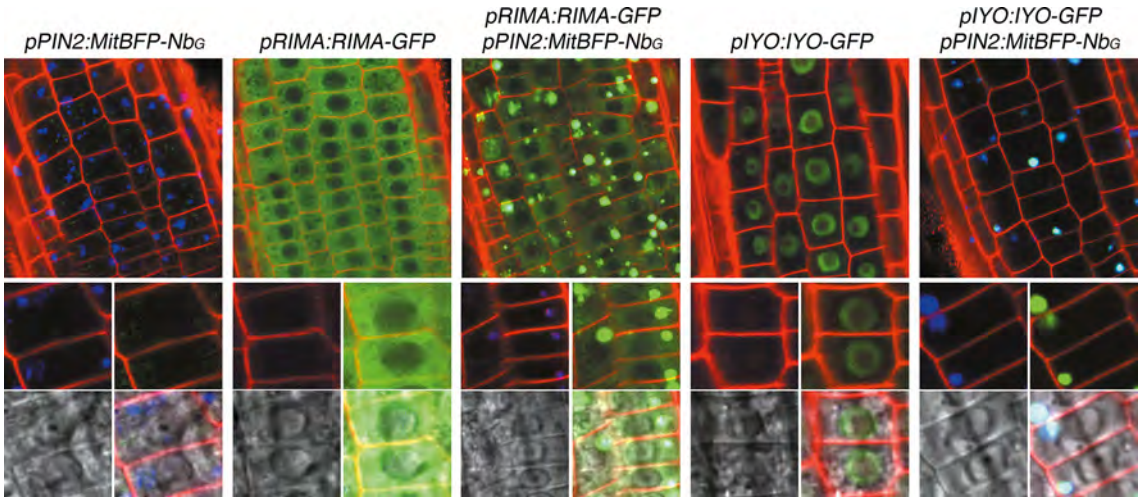
Signalling networks in plant development and defense responses

Plants are powerful model systems for genetically dissecting the mechanisms controlling stem cell fate. In our group we have identified two Arabidopsis genes, IYO and *RIMA*, which are required for initiating all events of stem cell differentiation in the plant. Our working model is that RIMA-dependent nuclear IYO accumulation functions as a switch that activates stem cell differentiation by directly regulating RNA Polymerase II activity and mediating transcriptional reprogramming in stem cell progeny. In the last two years, we have focused in determining the molecular mechanisms that regulate IYO nuclear migration and in identifying direct transcriptional targets of the IYO/RIMA module that drive cell differentiation. We are investigating whether the phosphorylation status of IYO determines its subcellular distribution and how it is controlled by endogenous and external stimuli. In addition, we have identified gene promoter regions bound by RIMA and are now developing genetic tools (Figure 1) for conditional activation and disruption of IYO and RIMA with the aim of determining early transcriptional responses to alterations in their activity. Ultimately, we want to use the knowledge gained on the function of this cell differentiation switch to alter organogenesis and/or regeneration capacity at will, and are currently pursuing some proof of concept experiments to demonstrate the usefulness of this technology for crop breeding.



Anti-GFP nanobody-mediated lockdown of IYO-GFP and RIMA-GFP in mitochondria of null mutants complemented with the GFP fusions (in collaboration with Dr. D. Van Damme, VIB).

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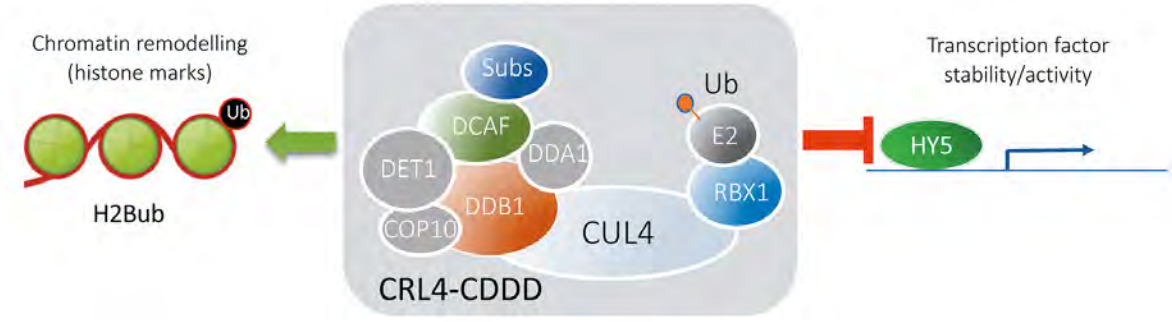
Role of ubiquitin in the control of plant growth and stress tolerance

Protein ubiquitination is an integral regulatory mechanism of many signaling pathways in plants. Ubiquitin (Ub) conjugation to proteins may trigger degradation of protein targets at the 26S proteasome or changes in their properties (e.g., protein activity, localization, 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 by promoting degradation of specific targets controlling those processes. Research at my group has been focused in the characterisation of CRL4 E3s that regulate developmental and stress responses in plants, including light and abscisic acid (ABA)-mediated stress signaling. Thus, we have reported novel mechanisms to modulate ABA responses in plants based on targeted destabilization of the ABA receptors (Irigoyen *et al*., 2014 Plant Cell; García-León *et al*., 2019 Plant Cell). Interestingly, CRL4 function is performed in close proximity to chromatin, which should enable rapid translation of environmental and stress signals into changes in gene expression. Indeed, we found that a CRL4-DET1 complex mediates a molecular pathway controlling epigenetic homeostasis (including Histone2B ubiquitination) in response to external stimuli (i.e. light conditions; Nassrallah *et al*., 2018 eLife). Our current objectives aim to identify and characterise new mechanisms by which CRL4 controls accumulation of specific epigenetic marks over the plant genome in response to environmental changes, to regulate expression of specific gene sets that lead to plant adaptation to changing climate conditions, as it is the case of COP1-DET1-complex-mediated destabilization of HY5 (Figure 1. Cañibano *et al*., 2021 Molecular Plant). As a highlight of our contributions to this field, we organised an International Conference on Plant Proteostasis in 2022 at the CNB-CSIC.



DET1 complexes play multiple roles at the plant chromatin by controlling accumulation of specific epigenetic marks and the stability of transcription factors.

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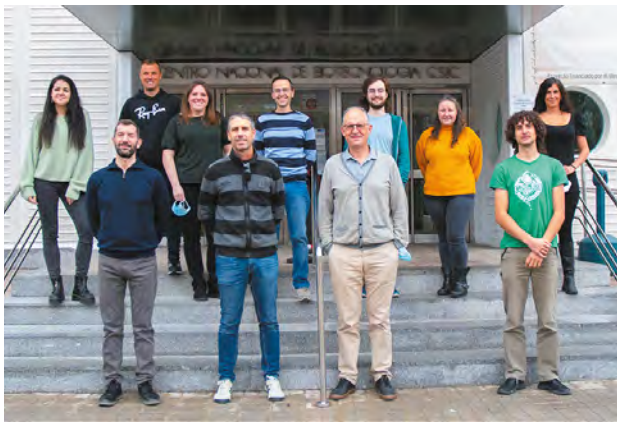

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Jasmonate signalling in plants

Jasmonates (JAs) are fatty acid-derived signalling molecules essential for the survival of plants in nature since they are important activators of stress responses and developmental programs. The main focus of our lab is to understand mechanistically the JA signalling pathway in plants; knowledge that is basic 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 the binding determinants of the dn-OPDA co-receptor MpJAZ (Monte *et al*., PNAS, 2022)
- Discovery of a new ligand of the MpCOI1/MpJAZ co-receptor that co-operate with dn-OPDA in the activation of the JA pathway in *M. polymorpha* (Kneeshaw *et al*., PNAS, 2022)
- Elucidation of the biosynthesis pathway for dn-OPDA in M. polymorpha (Soriano *et al*., New Phytologist. 2022)
- Identification of a general antagonist of the JA receptor that functions in vascular and non-vascular plants (Chini *et al*., Plant Phys. 2021)
- Identification of a potent anti-SARS-Cov2 antiviral using Marchantia extracts (Jimenez-Aleman *et al*., *Pharmaceuticals*. 2021).
- Collaborated in the characterisation of DNA-binding determinants of MYC TFs (Lopez-Vidriero *et al*., Plant Communications, 2021)
- Collaborated in the discovery of the role of ANAC089 in seed germination and stress response (Albertos *et al*., Cell Reports, 2021).
- Collaborated in the characterisation of responses to Fusarium pathogens in *M. polymorpha* (Redkar *et al*., New Phytologist, 2021; Redkar *et al*., The Plant Cell, 2022)
- Collaborated in the characterisation of CDF1 and StFlore in tomato tuber development and drought (Ramirez *et al*., TPJ, 2021)

Like other bryophytes, Marchantia is dioecious, so the fertilisation occurs only by mating of male and female plants. The receptacle bearing female sex organs, called archegoniophore, is shown here.

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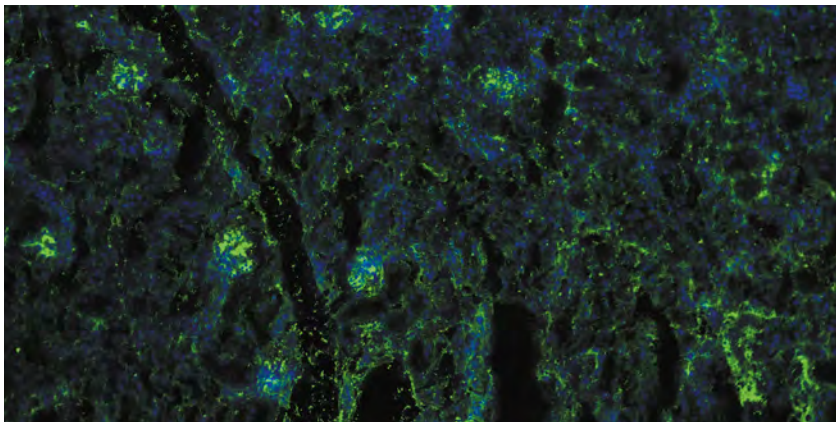

Immunology and Oncology

Research in the Department of Immunology and Oncology focusses on the molecular and cellular bases of immune system function and on tumour development. The Department comprises 16 Research Groups, including 1 Junior Group. Some of our groups are working in inflammatory and in autoimmune diseases; in the study of the innate and adaptive immunity in inflammation and in the tumour microenvironment; in the immune response against pathogens. Others are addressing several aspects of cancer development and treatment, with special emphasis on the identification of new anti-tumour targets by characterising the cellular and molecular mechanisms that underlie inflammation-driven carcinogenesis; in the relationships among stem cells, metastasis, inflammation and cancer; in tumour immunology; in tumour diagnosis and immunotherapy. Some research groups are focused on translational technologies such as nanoparticle-based nanomedicines, monoclonal antibodies, exosomes, or targeting peptides.

The department expertise combines multidisciplinary cutting-edge technologies such as advanced microscopy (total internal reflection fluorescence microscopy or real-time confocal microscopy), multi-parameter flow cytometry, nanoparticle production and characterization, exosome isolation and characterization, *in vivo* peptide phage display for the identification of targeting peptides, and next-generation "omics". In addition, we have extensive experience in the generation and use of genetically modified mouse models using the latest techniques.

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.

Since its origins, the DIO has maintained stable, productive collaborations with public and private partners that include prominent national and international research institutes, hospitals and pharmaceutical companies. As a recent example, during the COVID-19 pandemic, the 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 test has been approved for SARS-CoV2 diagnostics by AEMPS, and commercialised by the Spanish company Immunostep. An agreement with the public health organization Medicines Patent Pool (MPP), supported by the United Nations, and supervised by the World Health Organization (WHO) will allow this technology to reach developing countries.

Figure Legend: Immunofluorescence image from frozen kidney sections (Andrés París-Muñoz).

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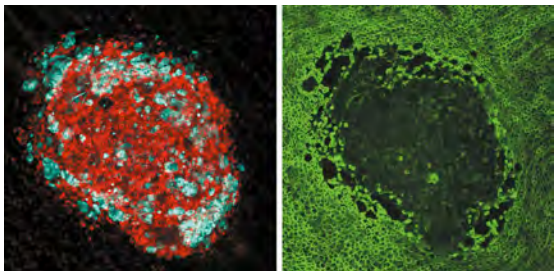
Immunobiology of macrophages and dendritic cells

Carlos Ardavín´s lab is focused on the immunobiology of monocytes, macrophages and dendritic cells during airway allergy and bacterial and fungal infections, and over the last years on the crosstalk between inflammation and coagulation in the control of innate immunity in the peritoneal cavity, using murine experimental models of bacterial sepsis and peritoneal metastasis.

Studies carried out in Ardavín's lab demonstrate that large peritoneal macrophages are crucial for defense against *E. coli* infection by coordinating the formation of mesothelium-bound, multicellular structures, composed by sequentially-recruited large peritoneal macrophages, B1-cells, neutrophils and monocyte-derived cells, that we termed resMØ-aggregates. ResMØ-aggregates are dynamic, transient structures, that provide a physical scaffold allowing the interaction and function of peritoneal immune cells, free in the peritoneal fluid in homeostasis, and enable efficient control of infection. The formation of resMØ-aggregates requires the development of a fibrin scaffold resulting from mesothelial tissue factor-dependent fibrin polymerisation.

Ongoing research in Ardavin's lab is focused on defining the molecular events driving fibrin polymerisation during peritoneal antimicrobial immunity, and in exploring whether resMØ-aggregate-like structures may also be formed during infection in other body cavities, such as the pleural cavity or the brain ventricular system, harboring MØs in a fluidic environment, that may need to attach to the epithelium lining these cavities to fulfill their immune defense functions.

An in-depth understanding of the response of the peritoneal immune system to peritoneal tumour metastasis is needed to develop immunotherapeutical treatments as an alternative to the current therapeutical strategies associated to severe adverse effects. By using an experimental model of colorectal cancer peritoneal metastasis based on intraperitoneal transfer of mouse tumor organoids derived from primary tumours, ongoing research in Ardavin's lab aims at exploring the interplay between peritoneal innate immunity and coagulation in the control of peritoneal metastatic growth.



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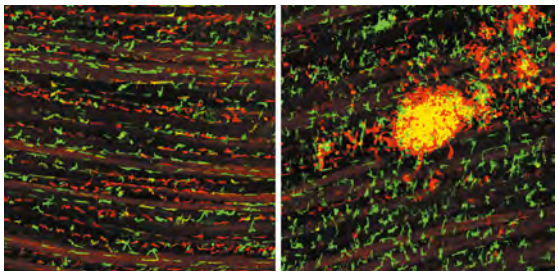

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1 *Whole mount immunofluorescence and confocal microscopy images of an early colorectal cancer (CRC) metastasis in the peritoneal wall, at 4 hours after intraperitoneal injection of mCherry-labeled mouse CRC-derived organoids. Turquoise blue: mCherry-labeled tumor cells. Red: resident peritoneal macrophages; anti-F4/80 staining. Green: mesothelial cells; anti-podoplanin staining.*

2 *Whole mount immunofluorescence and confocal microscopy images of the peritoneal wall in the steady state (left), and at 4 hours after infection with the Escherichia coli strain M6L4 (right), showing the response of MHC-II+ Tim4- and MHC-II- Tim4+ submesothelial macrophages to bacterial infection. Red: anti-Tim4 staining. Green: anti-MHC-II staining.*

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Regulation of inflammation by p21 and mitochondrial ROS in autoimmunity and cancer

Increased immune responses and hyper-inflammation govern the development and progression of diseases that extend from Autoimmunity, Cancer or COVID-19. In order to neutralise autoimmune inflammation, immune responses needs to be supressed. Alternatively, in cancer, immunosuppressed immunity requires reactivation. Therefore is it essential to understand how we could regulate the inflammatory responses. Notably, we have identified p21 as a regulator of the balance between hyper-activation 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*, 2022) and that inhibition of mROS results in reduction memory T cells (Figure 1). IFN-gamma orchestrates inflammatory responses in inflammation-induced diseases. Remarkably, Fas controls mROS and IFN-gamma induction independently of its apoptosis inducing potential. 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 p21expression lowers T cell overactivity, while lack of p21 enhances mROS production and cell responses.

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 *et al*, 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 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.

1 *Confocal microscopy of naïve and memory-like T cells shows increased p65 nuclear translocation in the later and supports the increased responses of memory T cells, which is linked to mROS. DPI is an inhibitor of mROS generation and lowers the p65 translocation.*

2 *Confocal microscopy of stimulated macrophages showed direct interaction of p21 with mitochondria stained by CMXRos. Lack of p21 reduced staining by CMXRos, which reflects lower mitochondrial membrane potential in p21 macrophages at 15 and 30 minutes post LPS-stimulation.*

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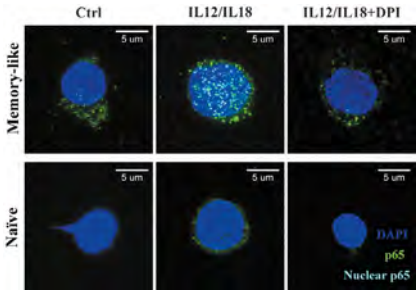

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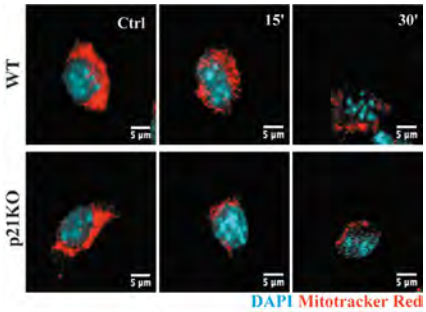

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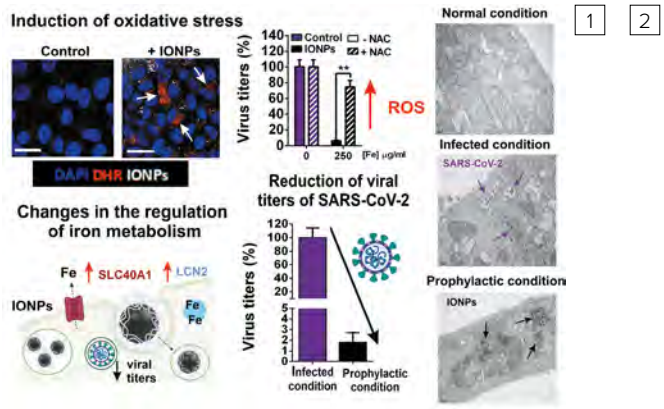
Nanomedicine, cancer immunotherapy and autoimmune diseases

During these years, our group has contributed to demonstrate the potential of iron oxide nanoparticles (IONPs) to be used as nanomedicines in different biomedical applications for cancer treatment: targeted drug release, reprogramming of macrophage responses and the tumour microenvironment, induction of intracellular hyperthermia in tumour cells, and magnetic targeting/retention of lymphocytes functionalised with IONPs in cell transfer therapies (ACT). We have also seen that the accumulation of MNPs in tumour cells induces oxidative stress as a consequence of IONP degradation, which affects mitochondrial metabolism. We are therefore investigating whether this effect could be used therapeutically to fight tumours at different levels.

Our group has also found a link between alterations in the tolerogenic dendritic cell (tolDC) compartment and the absence of CD8+ Tregs in two lupus-prone mouse models (MRL/MPJ and MRL/lpr), as well as the differential expression of Helios in several T-cell populations associated with lupus pathology. Based on these findings, we have extended the study of IONPs applications to other pathologies, and have investigated whether the functionalisation of TolDCs with IONPs and their subsequent magnetic retention could be used as an autoimmune therapy in the aforementioned mouse models of lupus.

We have also tested the antiviral capacity of some types of IONPs against some respiratory viruses such as influenza and SARS-CoV-2.

The overall goal of our group for the coming years is to fully understand the molecular and cellular mechanisms induced by IONPs at their different levels of action. This knowledge will be used to improve the functional design of IONPs for specific biomedical applications in the treatment of cancer and autoimmune diseases.



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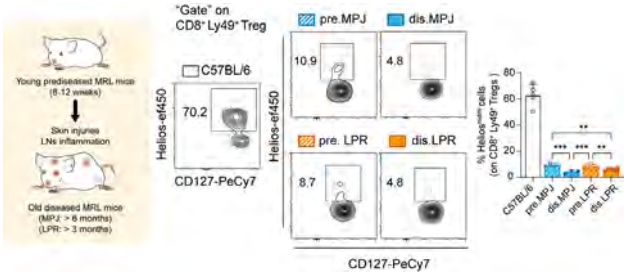

2

1 *Iron oxide nanoparticles impair SARS-CoV-2 infection of cultured cells through two processes induced by the internalization of IONPs: the induction of oxidative stress and changes in the regulation of iron metabolism. (Yadileiny Portilla).*

2 *Helios expression on splenic CD8+ Ly49+ Tregs from C57BL/6, MRL/LPR and MRL/MPJ mice. (Andrés París).*

SELECTED PUBLICATIONS

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Cardiac stem cells

Maintenance of organ functionality in mammals is determined by an efficient cell turnover. There are, however, large differences among adult organs both in steady-state, as well as in response to damages. It has been estimated that in adult mammalian heart only a 40% of total cardiomyocytes are replaced along lifespan but the mechanisms involved remains controversial.

We have identified and characterised a cardiac progenitor population, determined by the high levels of expression of the Bmi1 transcription factor (Bmi1+ Cardiac Progenitor Cells; B-CPC) which contributes to the turnover of the three main cardiac lineages. In response to a variety of cardiac damages B-CPC get proliferatively activated and their contribution to the mature lineages is enhanced. Thus, the B-CPC population contains cardiac progenitors contributing both to heart homeostasis and repair response to several modes of damage. In addition, *in vivo* genetic depletion of the B-CPC population provokes a pathological condition, when coupled to acute infarct, similar to human dilated cardiomyopathy (Figure 1). This is the first time that an association between a putative cardiac progenitor population and a human pathology (the most common cause of heart failure) has been established.

We have demonstrated self-renewal capacity in B-CPC that seems to be linked to their loading in specialised structures, known as niches, in a perivascular 3D distribution (vascular niches). This 3D distribution seems to be functionally relevant because it is distorted by conditions that increase ROS. Both cell-cell interactions and locally secreted factors, within the niches, restrain proliferation, promoting concomitantly self-renewal programs (Figure 2). Altogether, results strongly suggest a plausible crosstalk between vessel structures and B-CPC implying bidirectional mechanisms to be studied. Furthermore, to gain a better knowledge of the B-CPC intrinsic activity, we are going deeper in the study of some identified putative players (Tbx3 and Mbd3) which seem involved in the regulation of the critical quiescence/differentiation equilibrium.

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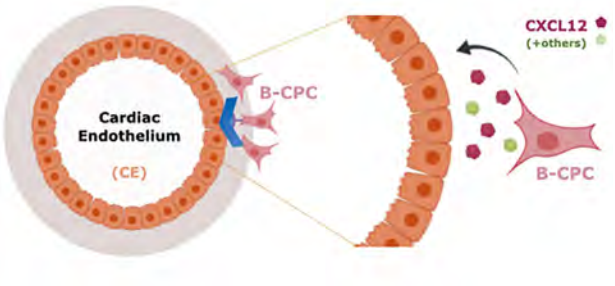

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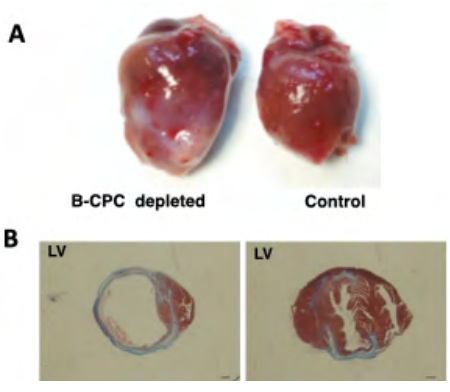

1 ***The vascular niche.*** *B-CPC secrete high levels of CXCL12 that promotes migration of cardiac endothelium to the close vicinity of B-CPC.*

2 ***Figure 1. B-CPC are essential for heart post-infarct repair.*** *Animals depleted of B-CPC (>95%), when a simultaneous infarct (AMI) was provoked, developed a lethal condition (2-3 months post-AMI), accompanied by heart hypertrophy (A) that derives from a large dilatation (B) of the left ventricle (LV).*

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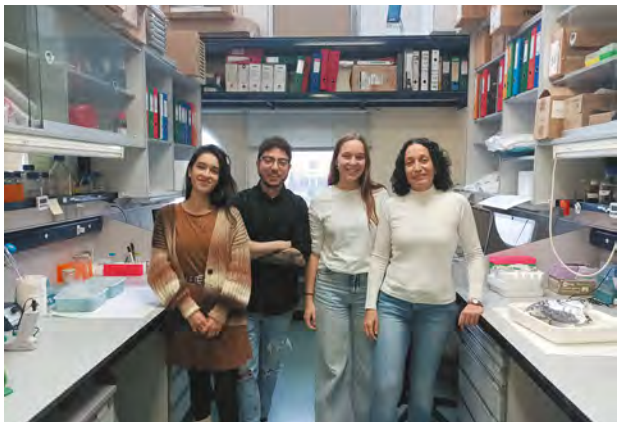

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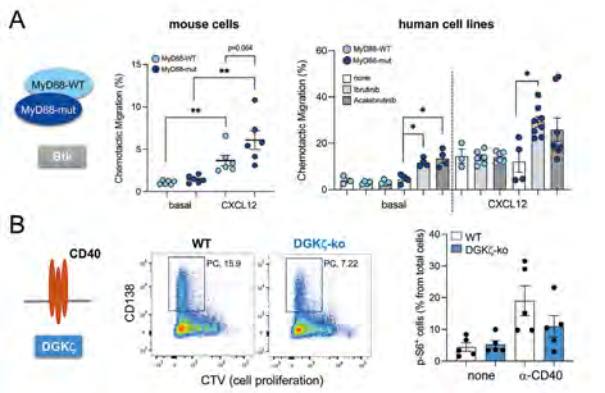
B lymphocyte dynamics

We investigate the molecular mechanisms that govern B lymphocyte dynamics and response, and how their dysfunction leads to or associates with B lymphocyte pathologies, in particular with B cell lymphomas. Our previous studies (Frontiers Immunol, 2018; Science Signaling, 2020) showed essential new functions for two proteins of clinical interest as therapeutic targets, the Bruton's tyrosine kinase (Btk) and the ζ isoform of Diacylglycerol-kinases (DGKζ). In the 2021-2022 period, we have studied the functional relation between Btk and the mutant active-form of MyD88 (MyD88-L265P), that features certain lymphomas, and the relevance of DGKζ for Plasma Cell differentiation downstream CD40 signalling, a main-actor at the germinal centre response.

While Btk mutations have not been linked to lymphoma development, increased protein levels and activation mediated by MyD88-L265P associates with Waldenström Macroglobulinemia and Activated B cell-Diffuse Large B cell Lymphoma (ABC-DLBCL). Using human ABC-DLBCL cell lines derived from patients and preclinical mouse models generated by our collaborator JA Martinez-Climent, CIMA-Navarra, we observed that the presence of mutant-MyD88 enhances cell invasion ability in mouse B cells. Its combination with the use of Btk pharmacological inhibitors (ibrutinib, acalabrutinib) augmented cell migration in human ABC-DLBCL cell lines (Figure 1A). Somehow MyD88 and Btk cooperate to regulate B lymphocyte motility/tissue residence, and this might be of clinical relevance. We are now investigating the molecular mechanisms behind. Regarding the CD40-DGKζ axis, we found that DGKζ-deficient B lymphocytes generate less Plasma Cells compared to wild type when stimulated *in vitro* via CD40. No differences in proliferation rate or CD98/CD71 markers upregulation were detected. Nonetheless, mTORC1 complex activity was reduced in deficient cells, estimated by measuring the levels of phosphorylated S6 ribosomal protein (Figure 1B). The data suggest that DGKζ has an important role downstream CD40 in regulating Plasma Cell fate via the mTORC1 pathway.



A, Migration frequency in basal and stimulated (CXCL12 chemokine) conditions of B lymphocytes carrying or not the mutant form of MyD88, in mouse models and human ABC-DLBCL cell lines. In the latter, they were also pre-treated with the specified Btk inhibitors. B, Representative dot-plots of Plasma Cell (PC) generation and cell proliferation for wild type and DGKζ-knockout B lymphocytes after 96 h stimulation with anti-CD40 antibodies plus IL-4/IL-5. Right, Quantification of S235/236-phosphorylated S6 protein levels by intracellular flow cytometry at 96h in the indicated culture conditions.

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Molecular targets in health and disease: focus on PI3-kinase

1) Boosting PTEN phosphatase as an anti-tumoral strategy

PIP3 (3-poly-phosphoinositides) control cell survival, division, and migration. Whereas PI-3-kinase (PI3K) upregulates PIP3, PTEN phosphatase reduces PIP3 levels. The mechanisms connecting PI3K and PTEN activities were unknown.

We have examined the activation kinetics of PTEN and those of the PIP3-effector AKT after growth factor addition for different times. AKT and PTEN activities were complementary (Figure 1). Maximal pAKT levels coincided with low PTEN activity and vice versa. The inactivation of PTEN was induced by UBIquitination and required cCBL and CBLb expression. At later time points, when pAKT levels decreased and PTEN phosphatase activity is recovered, PTEN exhibits high SUMOylation and translocates to the plasma membrane. These results reveal a mechanism for PI3K/PTEN crosstalk, and suggest that cCBL and SUMO machinery could be actionable strategies to rescue PTEN activity in PTEN +/-tumours.

2) The action of PI3-kinase beta on hESC stemness/ differentiation decision

The mechanism governing the transition of human embryonic stem cells (hESCs) toward differentiated cells is only partially understood. To gain insight of the role of PI3K pathway in this process, the activity and expression of the ubiquitous PI3Kα and PI3Kβ have been modulated in primed hESCs using different approaches.

The study reveals a pathway that dismantles the restraint imposed by the EZH2 (Enhancer Of Zeste 2 Polycomb Repressive Complex 2) repressor on an essential stemness gene, NODAL. PI3Kβ requirement for stemness is illustrated by OCT4 faded signal in cells with low PI3Kβ content (Figure 2). Control cells were greener (high OCT4 content), similar to the single cell in the image centre (not PI3Kβ depleted). At later time points, when ESC are prone to differentiate through the formation of the primitive streak (the site where gastrulation begins), PI3Kβ will contribute to tissue differentiation by modulating EZH2 release from promoters of transcription factors essential for primitive streak formation.

The pathway involves a noncatalytic action of PI3Kβ that controls nuclear- RAC1 levels, activation of Jun N-terminal kinase and nuclear β -catenin accumulation. These findings highlight how EZH2 is erased from promoters and points at new targets for directing tissue regeneration.

Additionally, our results support that PI3Kβ expression is also required for cancer stem cells phenotype maintenance in Lung Squamous Cell Cancer.

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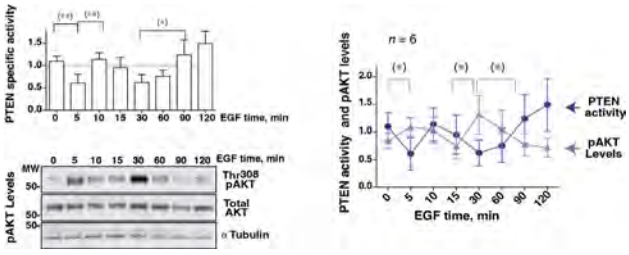

1 *Kinetics of PTEN activity and pAKT levels at different time points after EGF addition suggest PTEN inactivation contribution for maximal AKT activation.*

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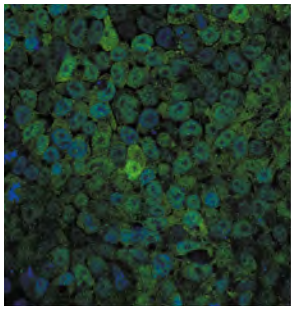

2 *Human stem cells with low PI3Kbeta levels (most of them) show low levels of the stemness marker OCT4 (green).*

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Stress-activated protein kinases in inflammation and cancer

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 colorectal cancer (CRC) associated to colitis. CRC is the second leading cause of cancer death. Patients with inflammatory bowel disease (IBD), ulcerative colitis or Crohn's disease are at increased risk of developing CAC; however, our understanding of the interplay inflammation-cancer at the molecular level is limited. We have reported that p38γ/p38δ have a pro-oncogenic function in CRC by regulating the production of inflammatory molecules and miRNAs, in humans. We found that p38γ/p38δ protein can be detected in human plasma, and that p38γ is significantly upregulated in IBD and CRC patients indicating that can mediate inflammation signaling to promote tumorigenesis. Our work suggests that p38γ/p38δ can be useful biomarkers for CRC/IBD diagnostic, as well as potential treatment targets, for colitis and early-stage CRC. Additionally, our data support the role of p38γ/p38δ in promoting tumour growth and give further evidence that one of the mechanisms by which p38γ promotes tumorigenesis is linked to its elevated protein expression and activation, thus increasing inflammation and providing an inflamed tumour environment that would promote tumour growth.

We have further investigated the molecular mechanism by which p38γ/p38δ regulate cytokine production and found that these kinases regulate inflammation, in part by controlling the expression of the ERK1/2 upstream kinase, tumour progression locus 2 (TPL2), in myeloid cells. We have demonstrated that TPL2 protein level is regulated by p38γ/p38δ at two different levels: 1) interacting with the TPL2/A20 Binding Inhibitor of NF-κB2 (ABIN2)/Nuclear Factor κB1p105 (NF-κB1p105) complex, increasing TPL2 protein stability; and 2) controlling TPL2 mRNA translation by modulating the repressor function of TPL2 3' Untranslated region (UTR) mediated by its association with aconitase-1 (ACO1).

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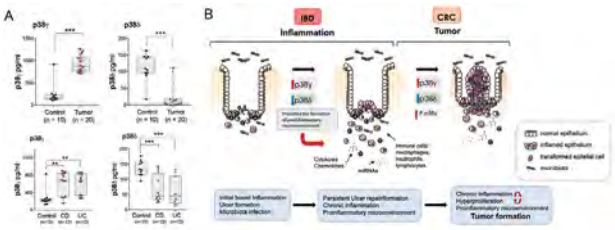

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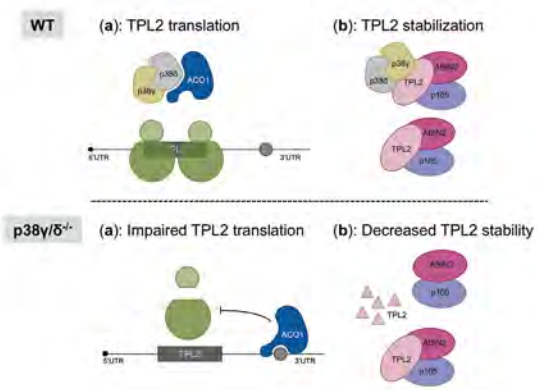

1 ***A***. *p38γ and p38δ expression in the plasma of healthy donors (control), colon cancer patients (tumor) or of IBD (CD and UC) patients. Each dot is a single patient or donor. (Upper panel) p38γ expression in CRC patients with (grey square) or without (red square) IBD; p38δ expression in CRC patients with (grey triangle) or without (blue triangle) IBD. Student's t-test. **P ≤ 0.01; ***P ≤ 0.001.* ***B.*** *Schematic illustration of the function of alternative p38MAPKs, p38γ and p38δ, in IBD and CRC formation.*

2 *Model for the regulation of TPL2 protein levels by p38γ and p38δ. In WT cells, p38γ and p38δ associate with ACO1 and also with the TPL2/ABIN2/p105 complex. (a) In WT cells, the p38γ/p38δ/ACO1 complex prevents ACO1 from binding to TPL2 3´UTR, and TPL2 mRNA is translated. In p38γ/δ-/- cells, free ACO1 binds to TPL2 3´UTR, and impairs TPL2 mRNA translation. (b) In WT cells, the p38γ/p38δ/TPL2/ABIN2/p105 complex stabilises TPL2 protein, whereas p38γ/p38δ absence decreases TPL2 stability and increases its degradation.*

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Physiopathology of chemokine receptor interactions

Chemokines and their receptors play an important role in homeostasis and inflammation, but they also regulate the tumour microenvironment, the generation of metastases and the anti-tumour immune response. Our group has generated monoclonal antibodies (mAbs) against human CCR9, which is overexpressed in different types of haematological malignancies. Two of these mAbs strongly inhibit the growth of human CCR9+ tumours in immunodeficient mouse models. These mAbs have been protected by an international CSIC patent, licensed to SunRock Biopharma. We have also generated human leukaemia cell lines expressing a modified CCR9 epitope which is not bound by these antibodies. Using these gene-edited cells in animal models of tumour progression, we have confirmed that the presence of the native epitope is a necessary and sufficient condition for the antitumor activity of our anti-CCR9 mAbs.

With the aim of developing naked antibody and antibody-drug conjugate (ADC) cocktails that allow simultaneous targeting of multiple tumour antigens, we generated a wide panel of new hybridomas producing antibodies from immunizations with whole human leukemic cells, from both primary cultures and stable cell lines. Twelve hybridomas were selected and cloned, and the corresponding mAbs were purified for their characterisation. Using flow cytometry, immunoprecipitation, protein arrays, and proteomics analyses, we have identified three different proteins, highly expressed on the surface of malignant cells, which are recognised with high affinity and specificity by their respective antibodies. These novel mAbs strongly reduce leukaemia tumour growth in xenograft models.

In collaboration with other research groups, we have generated and characterised mAbs against the macrophage protein CD5L, which modulate the immune response and are being evaluated in mouse models of liver fibrosis and liver cancer. We have also generated a broad panel of mAbs against the spike protein of the SARS-CoV-2 virus. Four of these mAbs protect mice from a lethal SARS-CoV-2 infection.

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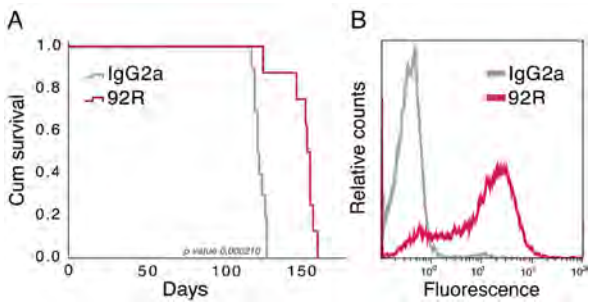

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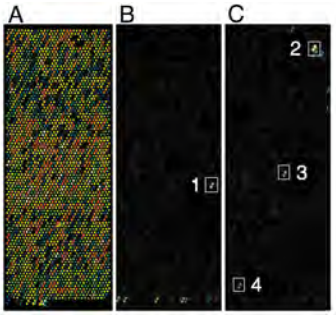

1 *92R increases survival of mice inoculated with CCR9+ early T-cell precursor lymphoblastic leukaemia cells. (A) Kaplan-Meier cumulative survival curves of mice carrying human xenotransplants injected in the tail vein on day 1. Mice were treated on days 2, 9 and 70 with either an anti-CCR9 mAb (92R) or an isotype control mAb (IgG2a). (B) Flow cytometry analysis of the leukaemia cells labelled with 92R or IgG2a.*

2 *Discovery of antibody specificity using HuProtTM v4.0 array analysis, representing more than 16,000 different human genes. Control subarray block with glutathione S-transferase staining (A). Detection of individual hits for mAbs 21 (B) and 35 (C). Staining intensity is shown in a false colour scale: blue-green-yellow-orange-red, increasing.*

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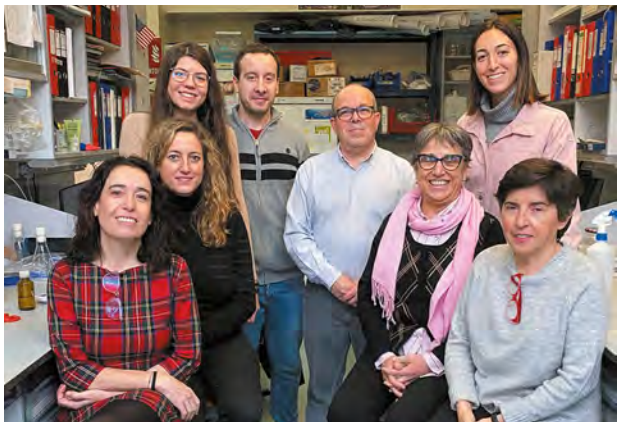

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Signaling networks in inflammation and cancer

Immune evasion is a fundamental hallmark of cancer. The immune system recognises tumour cell-derived neoantigens and generates tumour-specific T cell responses, distinguishing neoplastic from healthy cells. But cancers escape this control. We aim to identify, understand and manipulate tumour-induced resistance mechanisms and to develop new immunotherapies against cancer.

Our main research topics are:

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 are working on basic aspects of a new normaliser of the vasculature (SOD3), and collaborate in clinical trials to demonstrate the synergistic activity of anti-angiogenic and immunotherapy in breast cancer treatment.

2. Signals involved in T cell dysfunction

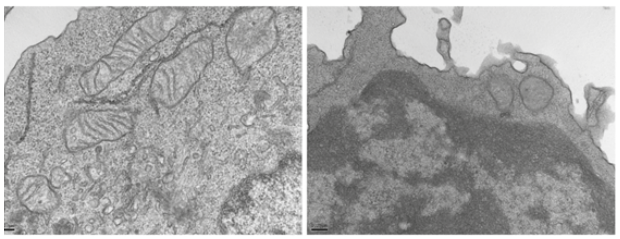
Tumour-specific T cells are usually dysfunctional in the tumour microenvironment (TME). Using RNA-seq and bioinformatics, we identified a genetic program associated to T cell exhaustion. In collaboration with Industry, we are deciphering the mechanisms of new intermediates causing T cell dysfunction and their potential clinical application.

3. Rewiring tumour cell metabolism

Metabolic reprogramming is another new hallmark of cancer. Neoplastic cells develop a number of metabolic adaptations to survive and proliferate without restrictions, but also induce metabolic stress affecting the activity of endothelial and T cells in the TME. We aim to manipulate the metabolism of tumour, cells to turn the immunosuppressive TME into an immunodominant one.

4. CD4 T cell differentiation in the TME

The number and nature of the immune populations that infiltrate tumours determine the immune-mediated control of cancer and the response to immunotherapy. The cells may come from the bloodstream or lymph, or can be differentiated in the TME. We are investigating the function of the extracellular matrix in the generation of immunosuppressive immune cell phenotypes and its relevance in tumour progression.



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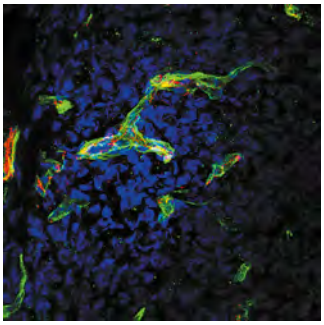

2

1 *PD-1 signalling reduces mitochondrial cristae length. Note the different structure of mitochondria in activated (left) and PD-1-exhausted CD8+ T cells (right).*

2 *Restoration of SOD3 expression in the tumour microenvironment normalises tumour vasculature. Tumour section stained with SOD3 (red), CD31 (green) and DAPI (blue).*

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Stem cells and immunity

We previously identified the *DIDO* locus, which regulates chromatin remodeling and RNA metabolism such as RNA splicing and alternative termination. We now used mouse embryo fibroblast (MEF) mutants in the Death Inducer Obliterator (*DIDO*) gene to study the effect on 3′ end processing and on regulation of alternative polyadenylation. In *in vivo* experiments in mutant mice lacking *Dido* exon 16, we also observed the development of mild hepatitis, testicular degeneration, and progressive ataxia, in association with systemic alterations in mRNA splicing and transcriptional readthrough.

As we determined previously, the *DIDO* locus also participates in early embryonic development and ESC differentiation through processes involving chromatin remodeling and RNA metabolism; these include RNA splicing and alternative termination. Moreover, as embryonic stem cell (ESC) differentiation and somatic cell reprogramming are highly regulated biological processes that display a largely common set of genes, we characterised the role of *Dido* in somatic cell reprogramming.

Using a conditional deletion mutant for *Dido* in combination with transcriptomic, protein interaction, and cellular studies, we have identified the underlying molecular mechanism through which *Dido* orchestrates the effects on both differentiation and reprogramming. We show that truncation of the exons 16 of the *DIDO* gene alters RNA splicing and transcription termination in ESC and MEF. In addition, we found that DIDO3, the largest *Dido* isoform, interacts with the helicase DHX9, which is involved in R-loop processing and transcription termination, and that DIDO3-exon16 deletion increases nuclear R-loop content and causes DNA replication stress. These effects result in failure of ESC to differentiate and of MEF to be reprogrammed. MEF immortalization restored impaired reprogramming capacity. DIDO3 therefore has essential functions in ESC differentiation and somatic cell reprogramming by supporting accurate RNA metabolism, with its exon16-encoded domain playing the main role. These data allow us to postulate an underlying molecular mechanism of an additional *DIDO* role in somatic cell reprogramming.

In an exhaustive computational study that integrated multi-omics data and *Dido* domain composition, we analyzed the significance of the *Dido* gene in EMT and MET processes, in differentiation, cell reprogramming, and tumour formation (see figure). Experiments in progress will allow us to understand Dido gene function in tumour formation and metastasis, and will permit exploration and clarification of its role in oncogenic transformation and cancer progression, in which EMT and MET are fundamental.

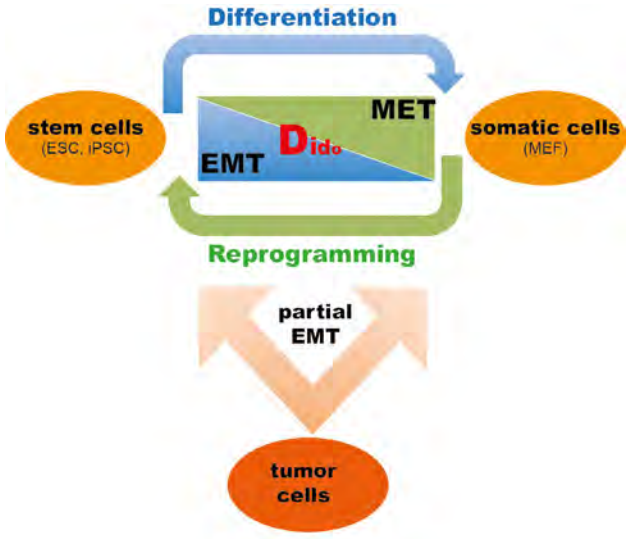

Figure from Archives of Cancer Biology and Therapy

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Chemokine receptors: biology and clinical relevance in inflammation, cancer and AIDS

Cell migration involves myriad signaling proteins and receptors that act coordinately to activate intracellular pathways and promote polarised cell states and directional migration. To migrate directionally in response to external stimuli, the internal machinery of cells needs to be spatially organised, which involves the integration of biochemical and mechanical factors to generate force in a specific direction to move the cell forward.

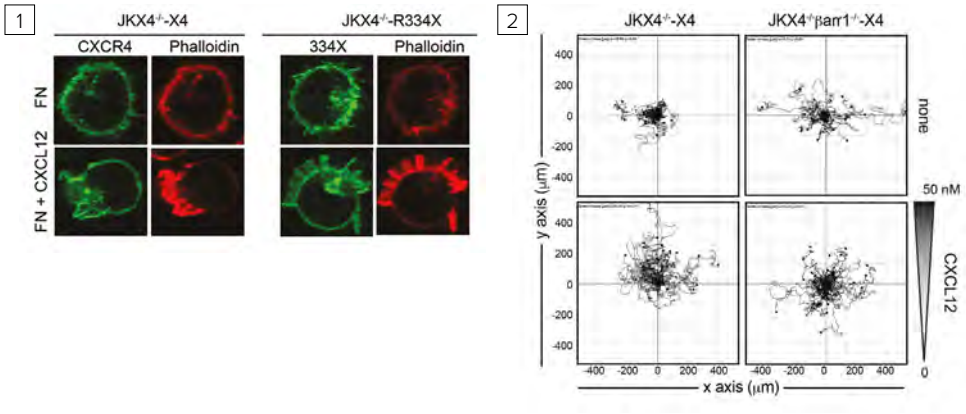
Chemokine receptors are membrane-expressed seven-transmembrane receptors linked to G proteins. Through interaction with the corresponding ligands, they induce a wide variety of cellular responses including cell polarisation, movement, immune and inflammatory responses, as well as prevention of HIV-1 infection. For achieving their function, chemokine receptors undergo ligand-dependent conformational changes that are influenced by the local cellular microenvironment.

Using quantitative single-molecule spatio-dynamic imaging, our group studies how the formation of large receptor nano-clusters in response to the ligand is critical for the maintenance of correct actin dynamics in migrating cells that allows directed cell migration. We have determined that altered cluster formation, using natural mutant chemokine receptors or altering the lipid composition of the cell membrane, results in the inability of these cells to correctly sense chemokine gradients. This inability to form clusters is responsible of the complex phenotype manifested by individuals carrying this mutated receptor and suggest that receptor clustering can be exploited as therapeutic target in inflammatory diseases and cancer.

In addition, our group is also involved in studying the effect of Growth hormone (GH) pretreatment of immune cells and its functional relevance. For instance, we observe that GH treatment prevents inflammatory joint destruction in a CIA model or that GH improved remission of inflammation and mucosal repair during recovery in the acute dextran sodium sulfate-induced colitis. The effects occur in several immune cells, i.e. GH reprograms inflammatory macrophages to an anti-inflammatory phenotype and improves resolution during pathologic inflammatory responses.

1 ***CXCL12 induces multiple lamellipodia through interaction with mutant CXCR4R334X.*** *F-actin (phalloidin-TRITC, red) and CXCR4 (AcGFP, green) visualised by confocal microscopy in JKX4-/--X4 and JKX4-/--R334X cells adehered to fibronectin and treated or not with CXCL12.*

2 ***β-arrestin regulates directional cell migration in response to CXCL12.*** *Spider plots showing the trajectories of tracked JKX4-/--X4 and JKX4-/-βarr-/--X4 cells migrating on fibronectin-coated μ-slide chemotaxis chambers along a CXCL12 gradient.*



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Diacylglycerol kinases in the control of immune response and cancer progression

Over the past years the emergence of immunotherapies, namely adoptive T cell transfer (ATC) and immune checkpoint blockade (ICB) has shifted the paradigm of cancer therapies, directing them to rescue the ability of T lymphocytes to eliminate tumours. The efficacy of these treatments is still quite limited due to the development of either innate or acquired resistance. Diacylglycerol Kinase (DGK) alpha and zeta are two cytosolic checkpoint inhibitors that limit T cell function by transforming diacylglycerol (DAG) into phosphatidic acid. The abnormal elevation of these two DGK isoforms in tumour-infiltrating natural and engineered T lymphocytes contributes to driving these cells into non-functional states.

Our group works to better understand the consequences of limiting the activity and/or expression of these two DGK isoforms in the regulation of antitumor T cell responses. To this we use cell-based and mouse preclinical models to investigate the consequences of 1) genetic deletion 2) pharmacological inhibition and 3) altered subcellular distribution of DGK alpha and zeta in the immune response against tumours. Some of our recent results have demonstrated that DGK alpha targeting potentiates the effect of targeting PD-1.

Unleashing the potential of targeting these kinases offers additional possibilities to enhance the effectiveness of actual therapies and improve its success rates in the treatment of advanced tumours. 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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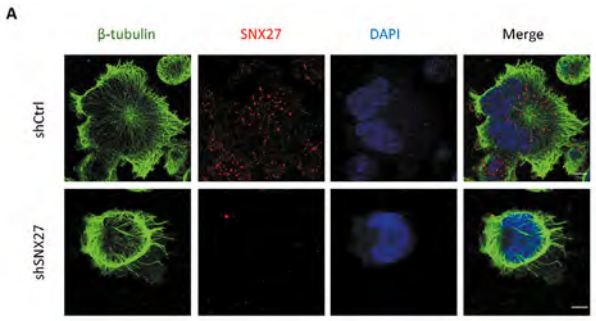

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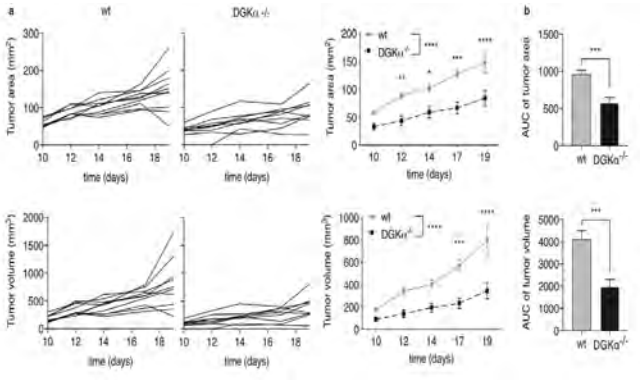

1 *SNX27 silenced Jurkat T cells display abnormal microtubule reorganization and cell shape*

2 *DGKa deficiency limits subcutaneous implanted MC38 tumor growth*

SELECTED PUBLICATIONS

Arranz-Nicolás J, Martin-Salgado M, Adán-Barrientos I, Liébana R, Del Carmen Moreno-Ortíz M, *et al*. Diacylglycerol kinase α inhibition cooperates with PD-1-targeted therapies to restore the T cell activation program. Cancer Immunol Immunother 2021, 70(11):3277-3289

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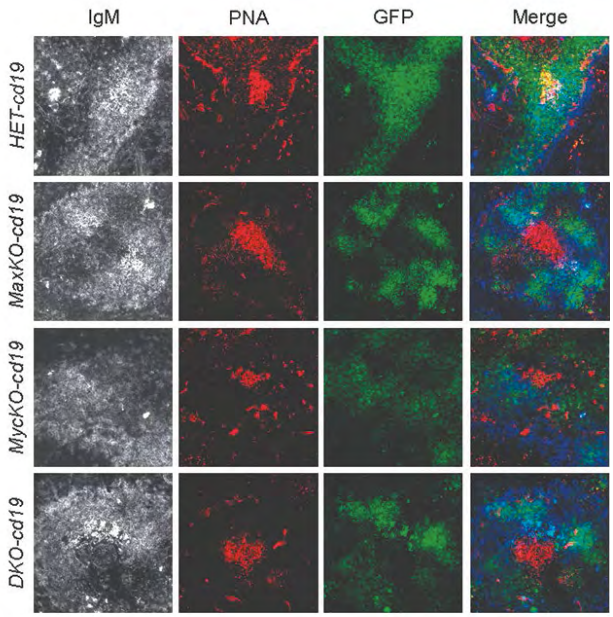
Transcriptional regulation of B lymphocyte differentiation

With more than 3 million new cases and 1.7 million deaths each year, cancer is currently the second most important cause of death in Europe. In up to 50% of all human cancers, constitutively enhanced expression of MYC family proto-oncogenes is one of the hallmarks (Burkitt lymphoma, breast and lung cancers). Myc proto-oncogenes are deregulated by various mechanisms such as rearrangements or other mutations in one of the three myc genes or by alteration of the signaling pathways that control their expression. The Myc proteins are members of a basic region/helix-loop-helix/leucine zipper (bHLHZip) transcription factor family (N-, L- and c-Myc) and are implicated in many biological functions such as regulation of cell cycling, differentiation, and apoptosis. Experiments in mouse tumour models strongly suggest that interference with the function of deregulated myc will have clear therapeutic impact on a wide range of aggressive and hitherto incurable tumors.

To activate or repress target genes, Myc proteins bind to conserved DNA sequences (E-boxes) on gene regulatory regions, for which Myc must form heterodimers with its partner, Max. The vast majority of related scientific reports to date assume that Myc/Max partnership is needed for all Myc functions. Max is highly conserved in evolution and is constitutively expressed in many cell types. Max can also heterodimerize with other proteins and antagonise Myc functions. Max thus has a central role in modulating the complex Myc protein network.

Our group is interested in characterising whether Myc action on developing and mature B cells relies exclusively on its association with Max. Thus, we will challenge the current idea and explore the possibility of Myc function without Max *in vivo* using unique genetically modified mouse models.

The prevailing strategies for combating cancer in patients rely mainly on the use of non-specific cytotoxic drugs, whose severe side effects are a major drawback. New therapies that target specific key molecules involved in cell transformation are therefore pursued. c-Myc is a therapeutic target for human cancer, due to the broad range of malignancies in which this gene is activated. As the contribution of Max to Myc-induced B lymphoma *in vivo* is also unknown, we will also study whether Max is needed for generation and/or maintenance of these lymphomas. Hopefully, our work will open new strategies to tackle Myc-induced tumorigenesis and thus, improve human health.



*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 immunized 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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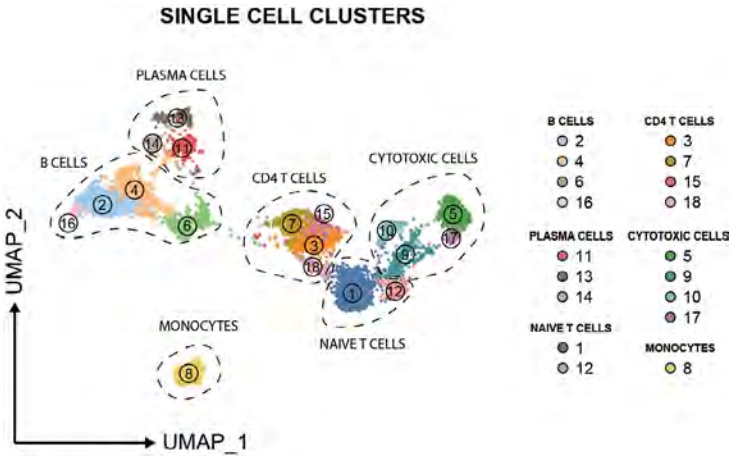
Receptor ligand interactions in immune responses to cancer and viruses

Immunity to virus infection, particularly the innate immune response to viruses, has been the central focus of my research since my PhD working on immune responses occurring in lymph nodes. Current research in the lab is still focussed on innate immune recognition of cells infected by viruses. This research is driven by the study of patients with primary immunodeficiencies as a source of *in vivo* insights into the effects of defective innate immunity on disease susceptibility in these individuals. Once the genetic defect has been identified, we aim to characterise the phenotypic and functional defects in detail with the goal of achieving a mechanistic explanation for the genotype-phenotype observations. These studies typically involve the use of genome-editing technologies to establish relevant in vitro models to study in detail the molecular bases of the changes observed in vivo. For example, we have studied a number of individuals with novel genetic deficiencies including patients unable to make an interferon αβ response after virus infection and patients that lack expression of the cellular Fcγ receptor CD16.

Since infection with Epstein-Barr virus is a major cause of mortality for these patients we have also embarked on a systematic characterisation of the cellular immune response to this virus in healthy, yet seropositive individuals where the host manages to achieve sufficient control of viral replication that an equilibrium consistent with health is established. These experiments are enhanced by an extensive network of collaborations, both national and international, that we have established with basic scientists and clinical colleagues.

Single cell RNASeq analysis of peripheral blood mononuclear cells responding to Epstein-Barr Virus identifies clusters of B and plasma cells, CD4 T cells, cytotoxic lymphocytes and monocytes.



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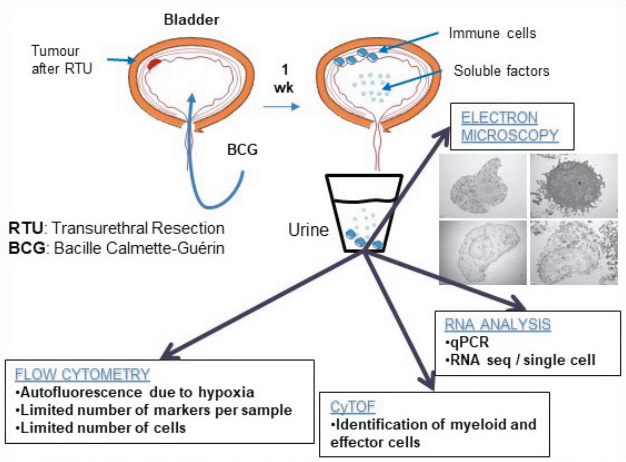
Tumour immune activation and evasion

To understand the immune response against cancer, we study a successful therapy for bladder cancer that involves activation of the immune system. Intra-vesical instillations of BCG (Bacille Calmette-Guérin) have been used for decades achieving great response rates, with 70% of patients free of tumour five years after therapy. In the last years, we have developed *in vitro* models that allow us to explore in detail the mechanisms underlying these therapeutic effects, and have described the activation phenotype of anti-tumour Natural Killer (NK) cells, both in the presence of live and dead mycobacteria. In parallel, analysis of ex vivo urine samples from patients treated with BCG, collected one week after instillations, has provided information on long lasting immune responses, such as the presence of cells with a phenotype consistent with tumour associated neutrophils (TAN).

Moreover, we study the biology of NK receptors, that can also be expressed by other effector lymphocytes, including αβ and γδ T cells. One key activating receptor, NKG2D, recognises tumour-associated ligands that mediate immune evasion when released as soluble molecules, either by metalloprotease cleavage or in extracellular vesicles (EVs). We have identified an unexpected role of this system in targeted therapies, using BRAFV600E and other MAPK inhibitors, for melanoma. After studying the physico-chemical properties of EVs, we optimised immunocapture assays using flocculation methods, that allow us to examine NKG2D-ligands, and other EV-associated tumour markers directly in biological fluids.

Finally, as part of the CNB response to the COVID-19 pandemic, we developed a multi-antigenic serology test (4 viral proteins + 3 antibody isotypes in one test tube) using flow cytometry, that has been commercialised by Immunostep, S.L., as well as conventional ELISAs that have since been transferred to the WHO.



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. We use several approaches to analyse cells one week after instillations. Data in Castellano et al. Front Immunol, 2022.*

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Cáceres-Martell Y, Fernández-Soto D, Campos-Silva C, ..., Valés-Gómez M. Single-reaction multi-antigen serological test for comprehensive evaluation of SARS-CoV-2 patients by flow cytometry. Eur J Immunol 2021, 51(11):2633-2640.

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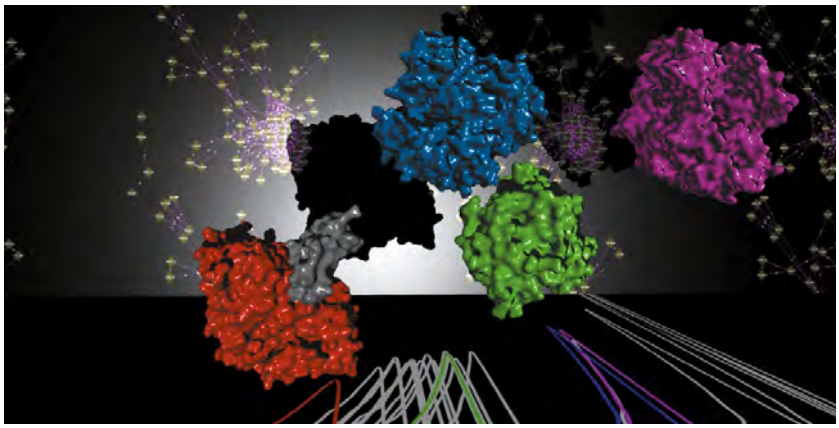


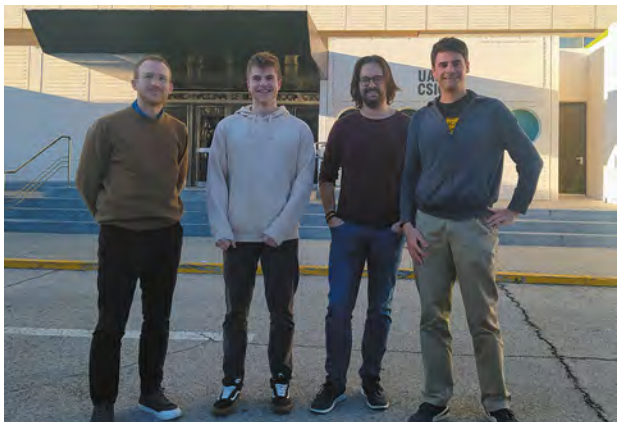
Systems Biology

The successful application of Molecular Biology over the last decades is providing many of the molecular agents underpinning fundamental cellular processes. While this reductionist approach is surely going to be valid in the near future, Biology is now facing a new age, in which questions about how molecular elements act together are of great demand. This is particularly significant for the development of a new Biotechnology based on the rational control of biological processes.

The Systems Biology Department of the Spanish National Biotechnology Center (CNB) tries to promote this new discipline and its Biotechnological applications. Our vision is to bring together researchers with quantitative, computational and experimental backgrounds to understand and engineer complete biological systems. The program is also planned to act as a core unit of a broader Systems Biology initiative at the Spanish National Research Council (CSIC).

HEAD

Florencio Pazos



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Clocks and rulers in life

During the period 2021-2021, different projects have been developed simultaneously. Following our collaboration with the group of Wilfried Meijer at CBMSO (see Meijer *et al*, 2021), we have been working on mathematical models of bacterial conjugation in Gram-positive bacteria. We have also been working on the pattern formation of nitrogen-fixing cells in filamentous cyanobacteria (Figure 1, Casanova-Ferrer 2022), and on models for the regulation by nitrogen of tillering in Green Revolution rice varieties.

Modelling plant growth has been a big topic (Figure 2): in collaboration with the group of Salomé Prat we described the effects of light and temperature on the growth of *Arabidopsis thaliana* (Nieto *et al*, 2022). Furthermore, we have been working towards generalising this model to incorporate circadian rhythms.

We are also interested in understanding the physics of how cells and plant organs grow. Currently, we are working in collaboration with Pilar Cubas' group on an experimental and modelling project to understand the control of dormancy in *Arabidopsis*' axillary buds.

In this period, James Pelletier, a postdoctoral researcher in the group, has also obtained funding to start a project on genomically minimal cells, bacteria where all non-essential genes have been removed.

We have also been working on epidemiological models (with Susanna Manrubia) and control of organ size during embryonic development (with Fernando Casares, CABD).

Finally, we have been actively analysing COVID-19 data on Twitter through Saúl Ares account @omeuxito.

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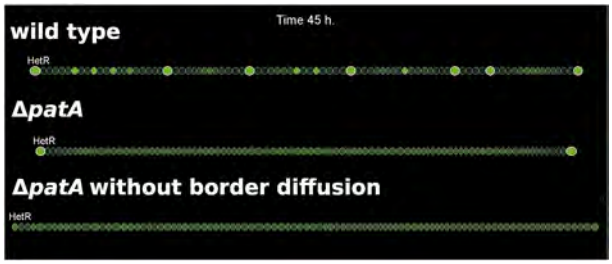

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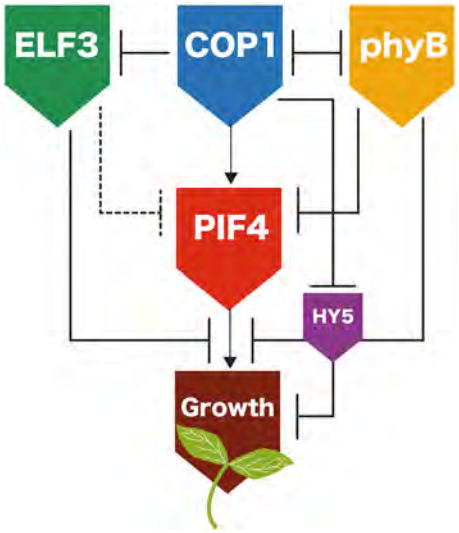

1 *Simulation of an Anabaena filament under three different conditions. The importance of boundary conditions is apparent in the terminal cells of patA mutant filaments.*

2 *Regulatory network controlling the effect of light and temperature on hypocotyl elongation in* Arabidopsis thaliana.

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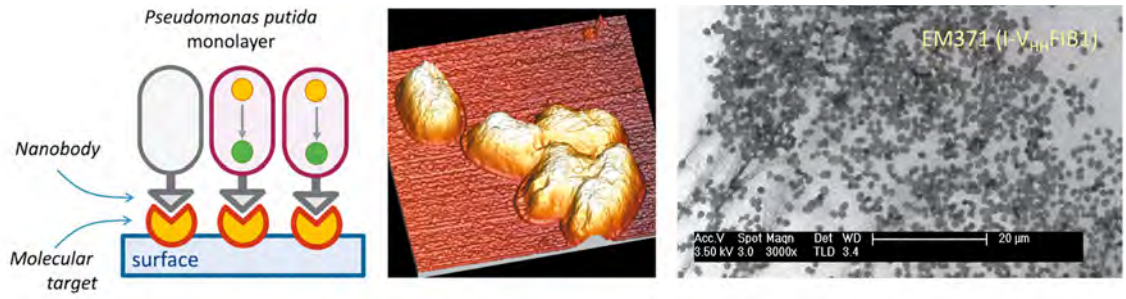
Environmental synthetic biology

The longstanding mission our team is the production of biological agents for biosensing, remediation and (wherever possible) 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. [iii] The TOL system borne by plasmid pWW0 as a reference for metabolic circuit implantation. The two operons for toluene and m-xylene biodegradation encoded in pWW0 offer a natural case of expansion of the metabolic repertoire of environmental bacteria through acquisition of new genes. [iv] Deep metabolic engineering of *P. putida*. Our long-term ambition is engineering propagation of the thereby designed bioremediation agents at a very large scale much beyond Laboratory, bioreactor or microcosm setups, for which we are placing a considerable effort in domestication of horizontal gene transfer.



Strategy for generating monolayers of Pseudomonas putida *cells specifically stuck to a solid surface by means of ectopic display of single-chain camel antibodies (nanobodies).*

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Velázquez V, Al-Ramahi Y, Tellechea-Luzardo J, Krasnogor N, de Lorenzo V. Targetron assisted delivery of exogenous DNA sequences into *Pseudomonas putida* through CRISPR-aided counterselection. ACS Synth Biol 2021 10 (10): 2552-2565 .

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Evolutionary systems

The main research topic of the group is the understanding, modelling and analysis of evolutionary mechanisms in biological and social systems. For almost two decades, we have investigated the adaptive dynamics of viruses and RNA populations and addressed broader problems, such as the relationship between genotype and phenotype (figure 1). Recently, we have explored 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, have generic consequences for evolution and adaptation. Our results highlight the role of entropic effects in microscopic evolution: abundant, sufficiently functional phenotypes, are 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 question the role played by classical metaphors of evolution, and suggest that smooth fitness landscapes must be substituted by network-based representations. General evolutionary and adaptive processes affect multiple disciplines beyond biology. Game theory, understood as the search for strategies that optimise fitness, can be applied to economic and other processes involving agents able to take decisions. In rigged economies, where market rules allow agents to artificially modify stock market prices, we have shown that economies increase in complexity: while growing economic complexity spontaneously defuses cartels, it also leads to large-fluctuations regimes that threaten the system's stability. In the context of epidemic propagation, we have shown that, even in the absence of non-pharmaceutical measures, epidemic waves and a convergence towards the critical propagation rate (figure 2) can originate from a self-adapting population behaviour, where individuals vary their degree of exposure according to their subjective perception of the external threat.

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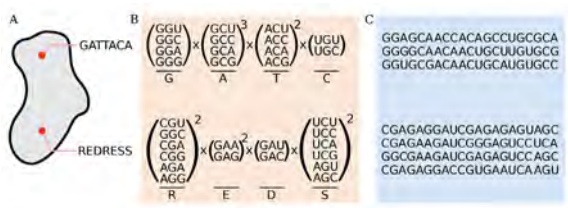

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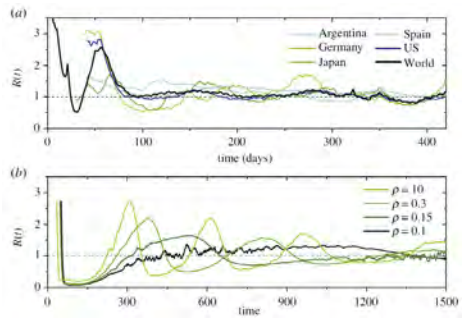

1 *Illustration of phenotypic redundancy in a neutral nucleotide-to-amino-acid-sequence model. (A) The space of protein sequences of the same length is vast and contains a variety of functional sequences. (B) The number of codons representing each amino acid varies: codons coding for glycine (G), alanine (A), threonine (T), cysteine (C), arginine (R), glutamic acid (E), aspartic acid (D), and serine (S) are shown here explicitly as examples. (C) Possible nucleotide sequences coding for GATTACA (above) and REDRESS (below). From Villanueva et al., Biophysica 2022.*

2 *Effective reproduction number R_0 as a function of time. (a) Empirical estimation of COVID-19 R0 for various countries and the World since 23 January 2020. (b) Evolution of R_0 value for a model that incorporates the risk-aversion response of individuals to the pandemic state. The model generates epidemic waves and a value of R_0 around 1, in agreement with natural progression (from Manrubia and Zanette, RSOS 2022).*

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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

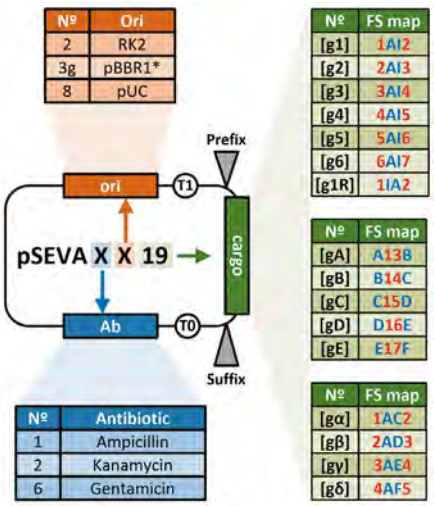
We are involved in the high-quality metabolic modeling of a large set of metabolically diverse bacteria including *P. putida, S. elongatus, A. platensis, Azoarcus* CIB, *S. granuli, P. pseudoalcaligenes, B. bacteriovorus, H. influenzae and Bifidubacterium sps*. This effort is enabling the system-level 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, underground metabolisms and metabolic heterogeneity.

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. The synthetic biology assisted validation of such cycles is allowing the rational engineering of superior microbial biocatalyst 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 bacterial populations and communities and ii) how we can engineer this supracellular-level functionality towards biotechnological endeavors. To address these two fundamental questions, we have developed systems and synthetic biology tools for modeling and engineering synthetic microbial populations and 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.



Structure and nomenclature of Golden Standard pSEVA vector collection for modular cloning developed at Systems Biology Group. Golden Standard cargo is denoted with the number 19.

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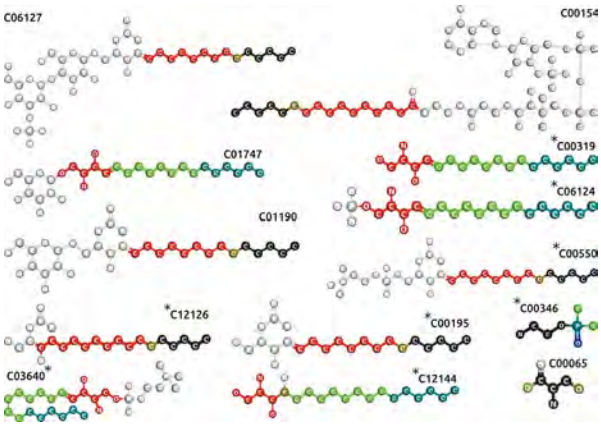
Computational systems biology

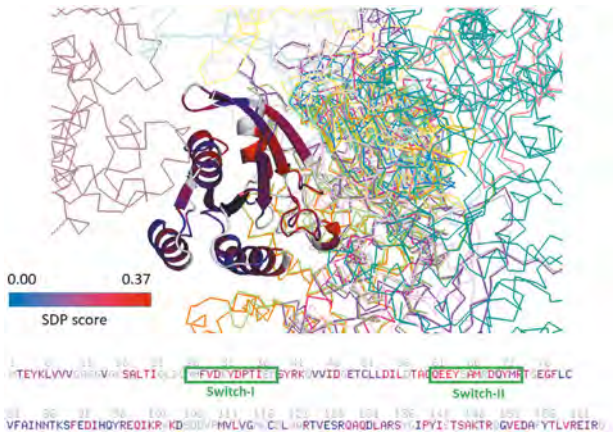
In the last two years, we continued working in the application of network-based approaches to the study of human pathologies. Within this line, in collaboration with the group of Prof. Juan. A.G. Ranea (U. Málaga), we worked on the systematic identification of genetic systems associated with phenotypes in patients with rare diseases. Along the same line, we also developed a novel methodology for the comprehensive detection of relationships between biomedical concepts in the scientific literature, using a co-mention based approach. Along our research lines dealing with protein structure and function, we developed a methodology for finding protein sites related to interaction specificity, as well as a new approach for the concomitant detection of residues and physicochemical properties related to functional specificity. Within our Systems Chemoinformatics research line, we finished the development of a profile-based approach for assigning chemical compounds to functional classes.

1



1 *Chemical fragments determining the pertenence of chemical compounds to the "sphingolipid metabolism" KEGG pathway KEGG (map00600), highlighted in the structures of the compounds of that pathway.*

2



2 *Prediction of regions determining interaction specificity for RasH, mapped on the interaction structural information available for that protein. RasH is shown in ribbon representation, and its 26 crystallised interactors in thin backbone. The method's score for the RasH residues is shown in a color scale, with red representing the highest scores.*

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Pazos F, Chagoyen M, Seoane P, Ranea J. CoMent: relationships between biomedical concepts inferred from the scientific literature. J Mol Biol 2022, 434(11), 167568.

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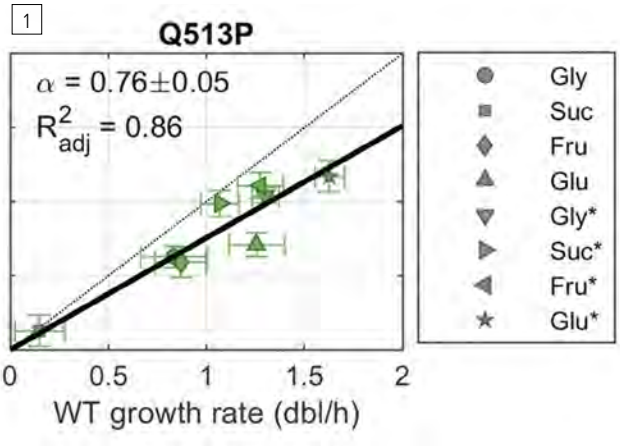
Logic of genomic systems

How does a biological system deteriorate? Genetic mutations could be a dominant factor. Are there ways to buffer the function of the system against these types of disturbances? By examining genome-wide expression patterns in response to gene deletions, we found that responses are stereotyped, and in some cases do not buffer but rather potentiate functional disruption resulting from the deletion itself.

This result made us wonder to what extent we could anticipate the functional impact of a mutation. We studied this problem using *E. coli* RNA polymerase (RNAP) as a model. RNAP mutations can affect multiple phenotypes that are apparently unrelated. We examined the direct effects associated with RNAP function but also the system-level response that causes additional "indirect" effects. Our work proposes that an important driver of the functional costs produced by mutations is the indirect effect of altering the so-called global transcriptional regulatory program; a program that connects the physiological state of the cell with its gene expression.

1

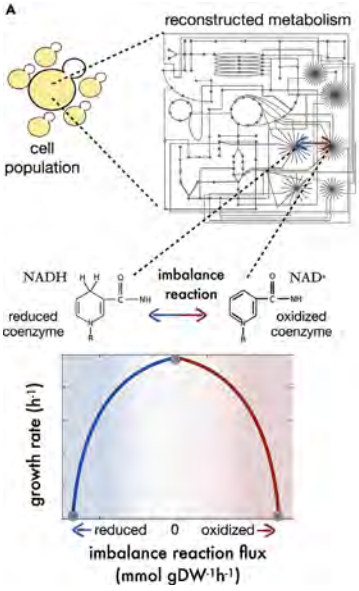


1 *The growth rate of a rpoB Q513P mutant strain and its relative WT in eight different growth media shows a global fitness cost.*

A second way of analysing how a biological system deteriorates is by focusing on the appearance of metabolic imbalances. To study this, we focused on the NADH/NAD+ redox couple in yeast. Using large-scale metabolic models, we showed that reductive imbalances generate metabolic syndromes comparable to those seen in cancer cells and identify the underlying mechanisms of pathology, lifespan-protecting molecules, or caloric restriction mimetics. Tolerance to redox imbalances thus becomes a robust framework for recognising deteriorating system properties while providing a consistent biological rationale for evaluating protective interventions.

Work during this period has led us to be interested in understanding the limitations in prediction and causal inference in biological systems. We did a sabbatical in the CSIC Institute of Mathematics (ICMAT) that will have a sure impact on our research at the Logic of Genomic Systems Laboratory in the coming years.

2



2 *We can control the interconversion of the two conjugated forms of a NADH/NAD+ pair in a yeast in silico metabolic model and then apply flow balance analysis to calculate growth rate. The predicted values of growth are plotted against the values of the rate of this imbalance as an indicator of the tolerance of metabolism when faced with the chosen perturbation*

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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.

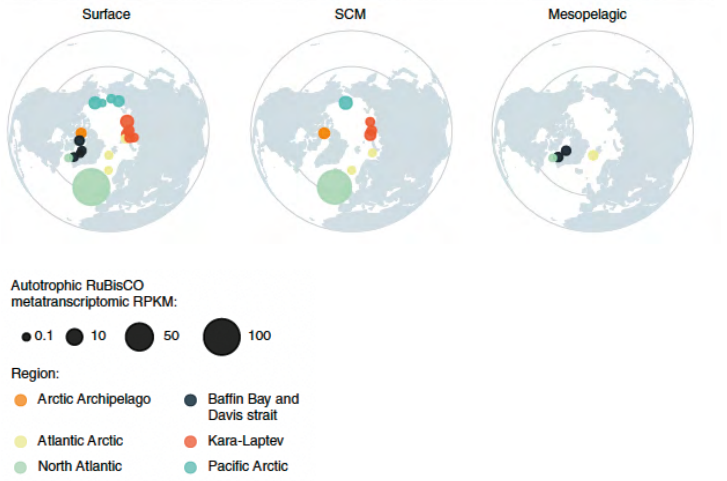
We focus mostly on marine microbial communities, but we are actively working in many other microbiomes from different environments. We study extreme environments because their microbiotas show fascinating adaptations to the harsh conditions. We work with human-associated microbiomes, such as the gut 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.

1

1 *Testing the* in situ *protocol for sequencing and annotation of metagenomes in La Palma lavas.*

2



2 *Gene expression in the TARA Arctic cruise. Transcript abundance of RuBisCO forms I and II, involved in the Calvin cycle pathway (K01601), color-coded by Arctic region. The size of the dots is proportional to the accumulated metatranscriptomic RPKMs.*

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Scientific Facilities and Research Platforms

Scientific Facilities at the CNB are one of the most important assets of the centre. They provide access to leading-edge technology in the areas of imaging, structural and cell biology, genetically modified mouse models, -omics and bioinformatics. In the last years we have received funding for the acquisition of new state-of-the-art technologies and equipment such as Microscopes and Flow Cytometers to improve the quality or our services.

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. Specialised personnel offer technical support in many other facets of the centre's scientific activities.

Our core technologies are well established locally and nationally and act as host or partners in several European Research initiatives. In this regards, the CNB hosts two centres of the European Strategic Forum for Research Infrastructures (ESFRI) Projects, the Spanish node of INFRAFRONTIER, that includes the European Mouse Mutant Archive (EMMA), and the Spanish Instruct-ERIC Center, that includes the Instruct Image Processing Centre (I2PC) and the CNB-CSIC cryoelectron microscopy Facility.

New National and International Scientific Research Platforms are running in the institute, such as the CNB Antiviral Platform, created during COVID-19 pandemic or the Standard European Vector Architecture 4.0 (SEVA).

Advanced light microscopy

HEAD

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Jaime Fernández de Córdoba

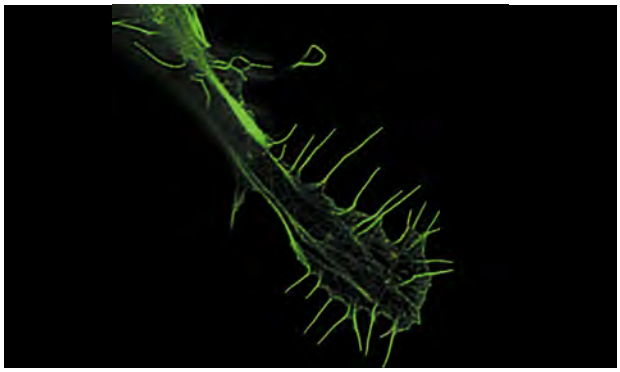
Optical microscopy, and in particular fluorescence microscopy, is one of the most powerful tools for the study of key problems in the life sciences, allowing the study of unlabelled and/or fluorescently labelled species in the space and their evolution over time with macromolecular spatial resolution.

The Facility provides state-of-the-art infrastructure for epifluorescence, transmission imaging, confocal laser scanning microscopy, TIRFM and STED nanoscopy. Applications available include: cell dynamics, multi-position acquisitions, multi-channel imaging (2D, 3D, 4D), sub-cellular localisation, FRET, FRAP, mosaic and 3D reconstructions, as well as the use of image processing and analysis tools, covering the main experimental imaging approaches in optical microscopy.

The Facility will be equipped in 2023 with a new confocal nano- and mesoscopy system that will allow the use of new imaging techniques: STED, FLIM, FCS and a Lightsheet.

Our staff offers scientific-technical assistance in the use of the equipment, experimental protocols, image processing and analysis procedures. We also provide aliquots of probes, antibodies and supports for widespread use in light microscopy.

Super-resolution (STED) image of a HeLa cell filopodium with actin filaments labelled with Alexa Fluor™ 488 Phalloidin. The image was acquired with the Leica TCS SP8 multispectral confocal system with a 3X STED module.



Quantitative image analysis unit

HEAD

Carlos Óscar Sorzano Sánchez

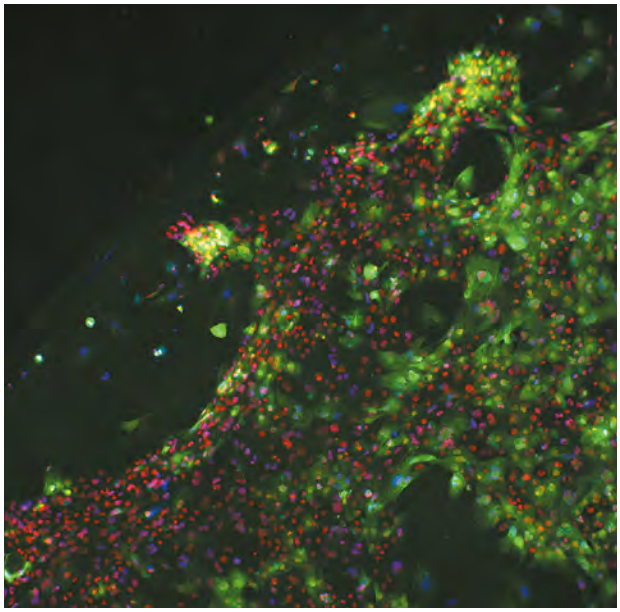
PERSONNEL

Ana Cayuela

This unit develops image processing algorithms to perform a quantitative analysis of the images produced by the Advanced Light Microscopy facility of the CNB.

In particular, we help to automatically track single particles or cells, characterising their trajectories, diffusion features and fission or fusion events. We are also able to classify cells into different phenotypes, quantitatively assessing the fluorescence intensity under various conditions or identify spatial relationships beyond colocalisation and image segmentation.

Our expertise includes also the development of deep learning algorithms to be applied in light microscopy.



Cryoelectron microscopy

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The cryoelectron microscopy facility offers services including sample preparation, screening and image collection for cryoelectron microscopy and cryoelectron tomography. It hosts tree microscopes: A 300 kV JEOL CryoARM microscope equipped with autoloader, a Gatan direct electron detector and Omega energy filter and a 200kV FEI Talos Arctica microscope equipped with autoloader and a Falcon III direct electron detector can be used for high resolution studies using single-particle methodology and cryoelectron tomography and additionally, a 120 KV JEOL JEM 1400 microscope for sample screening. The service has different apparatus for specimen vitrification: a FEI Vitrobot, a Leica EM CPC, a Leica EM GP2 and a high pressure freezer Leica EM ICE. We are also offering two new services: cryocorrelative microscopy and microelectron diffraction. The cryocorrelative microscopy technique 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 allows direct visualisation of cells for tissue-cell resolution or for preparation of thin lamellas.In MicroED diffraction of nano crystals, the TEM is used to diffract crystals of the complete range of molecules that could be crystallised: from small compounds to macromolecules.

Cryoelectron microscopes: 200kV FEI Talos Arctica and JEOL CryoARM300

Instruct image processing center – I2PC

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(Technical Director)
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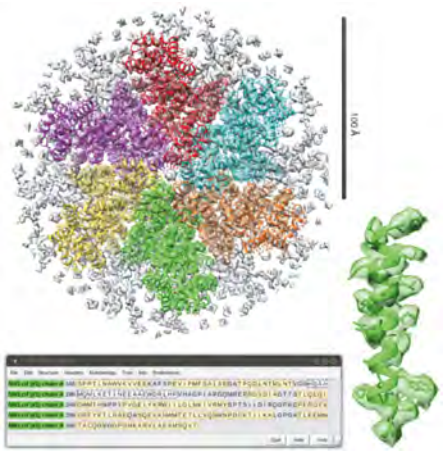
Roberto Melero

The Instruct Image Processing Centre (I2PC) is the reference laboratory for the image processing of Cryo-Electron Microscopy (CryoEM) acquired data within Instruct-ERIC (the European infrastructure for Structural Biology). Our contribution is two-fold. On one-hand we develop infrastructure software for the support of image processing in CryoEM throughout Europe and the world. On the other hand, we serve the community by helping experimentalists to obtain three-dimensional structures of the biological macromolecules of their interest. These projects must be approved by Instruct and are channelled to us for their solution. Experimentalists can submit their data, come to learn how to process it, or even have a short internship (3-6 months) through Instruct. The I2PC is very active in giving training courses and seminars on all the image analysis techniques related to CryoEM.



Electron microscopy

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Pablo Sola
Miriam Guerra
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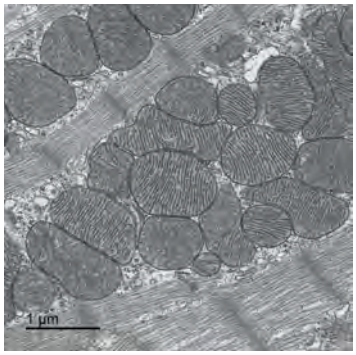
The CNB electron microscopy service provides scientific-technical support to research groups for the study of biological samples by transmission electron microscopy.

Technical staff offers training in the use of equipment and advise on the appropriate techniques for ultrastructural and immunocytochemical analysis of macromolecular complexes, viruses, bacteria, eukaryotic cells and tissues.

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

In addition, techniques that combine optical and electronic microscopy are performed. These CLEM (correlative light and electron microscopy) techniques enables to obtain a complete overview of a cell at the same time analyse biomolecules in that same cell on the scale of a few manometers. It also allows the observation of rare objects or events in light microscopy and their subsequent ultrastructural localization in an electron microscope.

The facility is equipped with a 120-kV transmission electron microscope (Jeol 1400 Flash) with a high-resolution camera (Gatan OneView) and for sample processing the facility disposes of an ultramicrotome Reichert-Jung Ultracut, a cryo-ultramicrotome Leica UC6-FC6, a specimen trimming device Leica EM Trim, an automatic freeze-substitution system Leica AFS-2, a carbon coating equipment Leica AC600, a high-pressure vitrification unit Leica EM PACT2 and a light microscope Leica DM2500 with digital camera.



Ultrathin section of cardiac muscle embedded in epoxy resin. Jeol 1400, magnification 10.000.

Macromolecular X-ray crystallography

HEAD
César Santiago

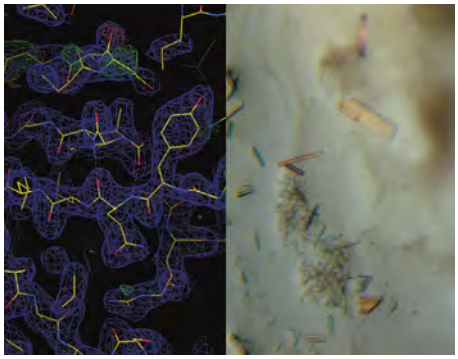
Protein x-ray crystallography is a high-resolution technique that allows us to study protein structure at atomic level. This method provides a detailed view of protein function, ligand and protein interactions, supra molecular organization and mutants related to human diseases. Great improvements both 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 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.
- Crystallisation conditions optimsation 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

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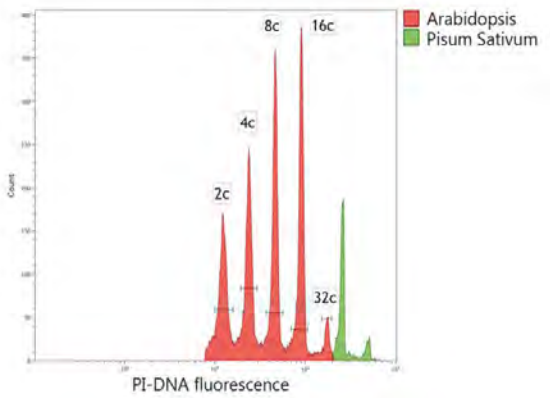
Miguel A. Sánchez Luengo

Flow cytometry is a powerful tool used in different fields (plants, microbiology, oncology,...). Is a high throughput and multiparametric technique that allows the characterisation and separation of cells and particles in suspension using different lasers and optical detectors.

In the Unit there are two types of instruments: analysers that can be used for cell analysis only or cell sorters which can perform cell analysis and cell sorting simultaneously. Currently, the Unit has 5 analysers based in conventional cytometry with the capacity to detect up to thirteen colors simultaneously, and one high speed cell sorter with the capacity to separate up to four population of cells at the same time. Recently the Unit has been equipped with a new analyser based in spectral cytometry that allow detection of more than 40 colors simultaneously which will allow to solve complex problems in research.

The Unit offers training for the self-use of analysers, assistance on the design of experiments, sourcing and supply of reagents, support and courses of data analysis, presentation and interpretation as well as troubleshooting of machines and experiments.

Ploydia´s analysis of homogenates prepared from Arabidopsis thaliana Col-1 leaf tissue and internal standard Pisum sativum.



Protein tools unit

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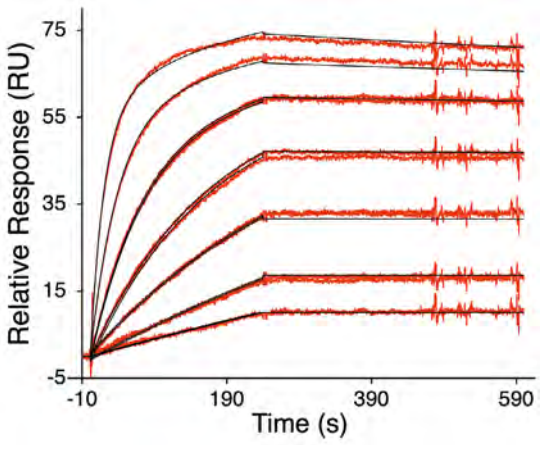
María Teresa Martín
Elena Ramos Serrano

The Protein Tools Unit is a scientific facility focused on immune response studies, generation and characterisation of monoclonal antibodies, design of immunoassays, and real time analysis of molecular interactions. The Unit is a founding member of EuroMAbNet, the non-profit European organisation of academic laboratories specialised in the production of mAbs.

Antibodies, immunoassays and related services are provided to CSIC scientists, universities, public organisations and private companies. The laboratory also offers technical assistance and advice on the analysis and interpretation of data, the training of external users, and the development of new techniques. We organise theoretical and practical courses and help with the preparation of manuscripts. In this period, new mAbs against SARS-CoV-2 and human tumour antigens were generated and characterised.

The facility is equipped with a BIAcore 3000 instrument that integrates surface plasmon resonance (SPR) technology with a microfluidic system to monitor molecular interactions in real time. Its high sensitivity allows the detection and studies of kinetic constants and affinity of small drug-protein, protein-protein, RNA-protein, DNA-protein, carbohydrate-protein, and lipid-protein interactions.



Transgenesis

HEAD

Mª Belén Pintado

PERSONNEL

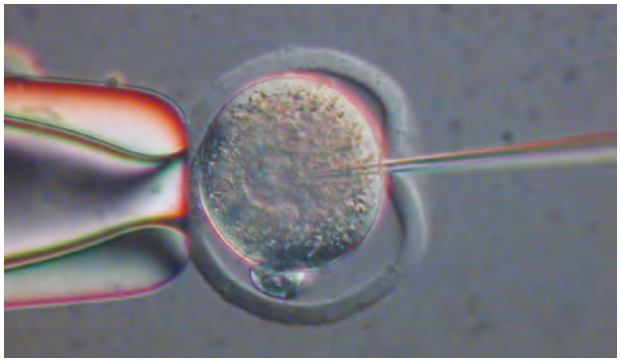
Verónica Domínguez Plaza
(CBMSO)
Mélani Margullón Cardoso
Mª José Palacios Barea
(2021)

The Transgenesis Service is a joint scientific service of CNB and CBMSO that provides support to internal and external research groups in the creation, interchange and management of genetically modified mouse models. The service covers all the required steps for this purpose: from founder generation to breeding and management of lines. Models are generated by additive transgenesis, ES cell derived gene targeting and CRISPR/Cas9 based genome edition either with embryo microinjection for large insertions or electroporation for KO or small insertion models.

The Transgenesis Service offers technical and scientific support, complementing the expertise of our customers, advising on the best strategy to obtain the desired model and also providing genotyping support if needed.

The service counts with two fully equipped microinjection settings, one electroporator specifically designed for embryo edition, a standard molecular biology laboratory and a laboratory for ES cells. The service is integrated in the scientific-technological platform INNOTEK (UAM+CSIC). We also provide rederivation of mouse lines and on-demand design and testing of guides for the genome edition of embryos and mammalian cell lines. The general activity of the service is complemented with the organisation and the participation in specialisation courses and master programs.

Pronuclear CRISPR/Cas9 microinjection in a 1-cell mouse embryo x200



Mouse embryo cryopreservation

HEAD

Lluís Montoliu

PERSONNEL

Julia Fernández
(technical manager)

María Jesús del Hierro
Marta Castrillo
Cristina Robledo Bernal
Inés Arroba
(CIBER)

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 8,400 mouse mutant lines cryopreserved and is composed of 16 nodes that are present in 13 European countries. More than 550 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

PERSONNEL

Soledad Montalbán
(technical manager)
Óscar Sánchez
Inés Arroba
(CIBER)

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

Marta Godoy

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 analyses several commercial and custom microarrays, and the services 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
Alberto Paradela

PERSONNEL

Lorena Carmona
Miguel Marcilla
Sergio Ciordia
Manuel Lombardía
Fátima Santos
Patricia Gómez
Rosana Navajas
Jorge Vindel
José Ramón Lamas
Laura Guerrero
Irene Blázquez
(TFM)
Carla Díaz
(TFG)

The Functional Proteomics Service at the CNB provides resources to identify, characterise and quantify proteins, either purified or as complex mixtures from any biological system. Unsupervised protein quantification by label free or isobaric labeling, targeted quantification, posttranslational modification analysis, analysis of HLA peptide repertoires and structural proteomics are some of the main routine workflows already set up in the lab. Moreover, there is a continuous update of our technological portfolio to maintain competitivity in a highly dynamic field such as proteomics.

During the 2021-2022 term new state-of-the-art instruments have been installed and are 100% operative. Moreover, a top LC-platform has been granted and will be installed in early 2023. In the 2021-2022 period, we have performed 4138 proteomic analyses for 404 users, that included sample prep, nLC-MS/MS analysis and data processing with a preliminary functional interpretation.

In regard of COVID-19 pandemic, we have synthetised and purified more than 100 peptides.

We are currently working in two main aims, first, to enhance our capacities in structural proteomics: intact protein characterisation and protein-protein interaction analysis (combining peptide crosslinking and mass spectrometry). Second, to define new strategies for the analysis of posttranslational modifications of proteins (including targeted and open-search analysis for epigenetic histone modification patterns).

Bioinformatics for genomics and proteomics

HEAD

Juan Carlos Oliveros Collazos

PERSONNEL

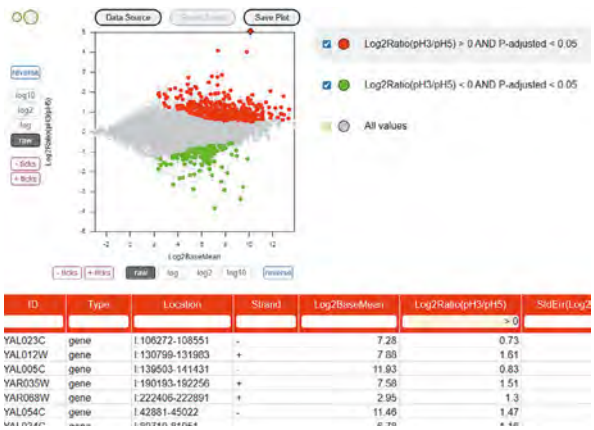
Juan Antonio García-Martín
Rafael Torres-Pérez

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 experiments involving deep sequencing and other high-throughput technologies
- 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
- Development of computational tools to make popular algorithms and pipelines more accessible to researchers.

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.

SeqNjoy: Complete RNA-Seq workflows in your Desktop



Scientific computing

HEAD

José R. Valverde

PERSONNEL

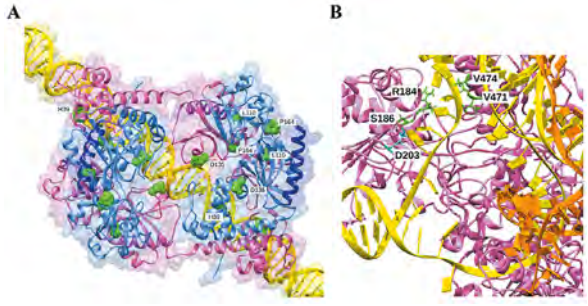
Alejandro Melones
Antonios Kapogiannatos
Irene Sainz de la Maza

The Scientific Computing Service cooperates with researchers inside and outside the CNB to perform complex data analyses. It currently provides support on Bioinformatics, Metabolic Modeling, Structural Biology, Immunoinformatics, Statistics, Artificial Intelligence and Data Science.

The service participated in two funded national projects, to study IBDV infections in *C. japonica* and *G. gallus*, and to study autophagy in *D. discoideum*. We also cooperated in the analysis of vaccines for HIV and SARS; drug screening against COVID-19; the pangenomic analysis of 27000 complete E. coli genomes; the study of antibiotic resistance in *P. aeruginosa* and *S. maltophilia*; and the development of advanced metabolic analysis tools, an ab initio protocol for protein structure prediction; and various AI/ML tools.

The Service regularly organises courses in Biostatistics and R programming; run an online course on Metagenomics (with U. of La Rioja and U. of Cuyo, Argentina). We received national and international (ERASMUS+) students to carry out undergraduate and master internships and participated in outreach activities such as Nanociencia and the 4ºESO+EMPRESA program.

Modeling of the effect of antibiotic resistance and compensatory mutations in the structures of AmpR and RpoB (Hernando-Amado, S., Laborda, P., Valverde, J. R., & Martínez, J. L. (2022). Rapid decline of ceftazidime resistance in antibiotic-free and sublethal environments is contingent on genetic background. Molecular Biology and Evolution, 39(3), msac049).



Biological safety and radiation protection

HEAD

Fernando Usera Mena

PERSONNEL

Aránzazu de la Encina Valencia
(coordinator)
María Teresa Bartolomé Jiménez
Marta Sanz Martínez
Luis Carlos Guardado Guerra

OCCUPATIONAL RISK PREVENTION UNIT

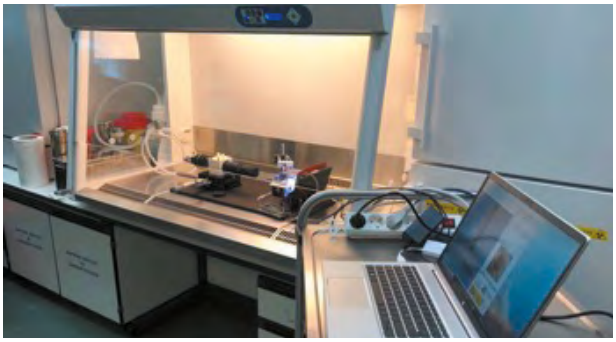
Nuria Martín Montes

Service activities:

- Evaluation of biological Chemical and radiological risks
- Design of laboratories
- Management of official authorizations and monitoring of compliance with regulations
- Issuing of guidelines and operating procedures
- Risk prevention training
- Acquisition protection equipment
- Medical and dosimetric surveillance
- Management of accidents and emergencies
- 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.



Animal facility

HEAD

Ángel Naranjo

RESEARCH TECHNICIAN

Javier Martín

SHIPMENT COORDINATOR AND ADMINISTRATION

Alberto García

AREA AND COLONY MANAGERS

Eladio Martínez
Antonio Morales
Raquel Gutiérrez
Iván Jareño
Isabel Rodríguez
Patricia Sanz

ANIMAL TECHNICIANS

Raul García
Alfonso Machado
Sergio Jiménez
Carina Solange
Marcelle Gómez
Elena Olivas
Guillermo Meza
Ignacio Ureña
Selvin Rápalo

The CNB laboratory animal facility is an area dedicated to the production and maintenance of experimental animals, aiding in research, essential techniques, and legal support for this duty. 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: quarantine, conventional, and specific pathogen-free (SPF), depending on the microbiological status of the animals. The facility provides 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 animals, shipping animals for collaboration with other institutes, 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. The animal facility 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 and to obtain accreditation for working with animals and manage 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 m2)
- A P2 safety level greenhouse with 4 cabinets (total growth surface: 83 m2)
- 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

Biosafety level 3 laboratory and radioactive facility

HEAD

Fernando Usera Mena

PERSONNEL

Aránzazu de la Encina Valencia
(coordinator)
María Teresa Bartolomé Jiménez
Marta Sanz Martínez
Luis Carlos Guardado Guerra

Biosafety level 3 laboratory

The three BSL 3 sub-laboratories are equipped with the installation necessary for safely handling of high risk pathogens, including a changing room and shower for personnel and a steam steriliser, air lock, pass through box for material, an effluent treatment plant, data transmission network and remote alarm systems.

Research equipment includes biosafety class IIA cabinets, CO2 cell culture incubators, microbiological incubators, 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 includes a gamma irradiator, cabinets for radioisotopes beta and gamma, Biosafety class IIA cabinets, CO2 cell culture incubators, centrifuge and microfuge, inverted optical microscope, and others.

Photography

HEAD

Inés Poveda
(until February 2021)

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
Anunciación Romero
Ángel Valera López

EXTERNAL PERSONNEL (CLECE)

Alioune-Aboutalib Sow
María Teresa Amado Jiménez de los Galanes
Vanesa Vara Martínez

Services

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

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

Instrumentation

HEAD

Ismael Gómez López

PERSONNEL

Juan Ignacio Golpe de la Fuente
Carlos González Redondo

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

Antiviral screening platform

SCIENTIFIC COORDINATORS

Pablo Gastaminza
Urtzi Garaigorta

TECHNICIANS

Jennifer Moya Vaquero
Laura Barbado Fernández

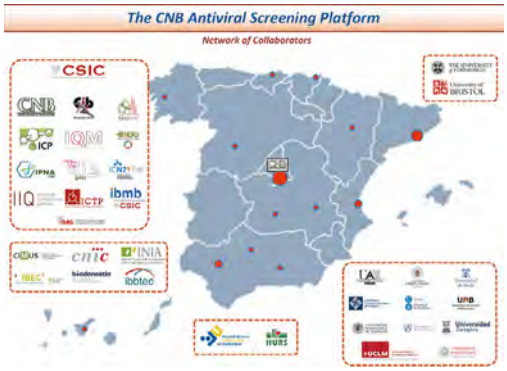
PhD RESEARCHER

Paula Bueno Fernández

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 unbiased phenotypic cell culture screening systems of viral infections including: SARS- CoV-2, dengue, West Nile, 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. We have already tested over 2400 repurposing drugs and around 7000 experimental compounds in the SARS-CoV-2 infection cell culture system. Several 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 at CSIC. Moreover, we have identified new families of experimental compounds and natural extracts with antiviral activity that have been protected by patent applications. We are currently characterising their mode of action and performing preclinical animal studies. Finally, we have performed several R&D contracts with public and private institutions in the antiviral discovery field.

Geographic distribution of the Network of Collaborators that provide chemical compounds and natural extracts for antiviral screening purposes.



Bioimaging platform

One of the priorities of the centre is to strengthen our bioimage capabilities. A bioimaging platform has been recently established to capitalise on the recent acquisition of advanced electron and light microscopy equipment, by launching novel research endeavours that exploit the power of integrative and correlative bioimaging techniques.

Efforts have been carried out to integrate multi-scale and multi-resolution approaches to cover the whole range of resolution from gross anatomy down to single cell, molecular and atomic scales. A strong coordination will be promoted among the **Advanced Light Microscopy** facility (which has recently benefited from the acquisition of state-of-the-art instrumentation that largely expands mesoscopy and functional imaging capabilities), the **Electron Microscopy** facility and the **Cryoelectron Microscopy** facility, the latter offering a wide range of techniques for which it is a pioneer in Spain.

Among others, single particle analysis, cryoelectron tomography, cryocorrelative microscopy (the combined use of cryolight microscopy, cryoscanning electron microscopy and cryotransmission electron microscopy) and very recently, cryoelectron diffraction, a very useful technique for the structural determination of crystalline structures of organic and biological molecules, has been offered to the national and international communities.

A major challenge associated to the bioimaging platform is the need for robust algorithms and user-friendly data analysis pipelines, capable to extract meaningful information from a massive amount of data generated by single molecule, light and electron microscopy imaging experiments in virus, bacteria, plants, animal and human cells. In the case of optical microscopy, and to overcome this bottleneck, a Quantitative **Image Analysis Unit** has been created. In the case of cryoelectron microscopy, the **Instruct Cryoimage Processing** Center (I2PC) and the above mentioned Cryoelectron microscopy are the only Spanish facilities belonging to INSTRUCT, the European network of structural biology facilities. I2PC provides continuous support to data processing and has been involved in the organisation of several practical courses at the international level.

Associated to this bioimaging effort is the development and application of single molecule biophysics techniques. The CNB hosts a good infrastructure and it has been organised as part of the Single Molecule Optical Spectroscopy Unit.

Standard european vector achitecture (SEVA) platform

HEAD

Víctor de Lorenzo

PERSONNEL

Sofía Fraile
Esteban Martínez

The SEVA platform is a web-based resource and a material clone repository to assist the choice of optimal plasmid vectors for de-constructing and re-constructing complex prokaryotic phenotypes based in the SEVA database (SEVA-DV), originally launched in 2013.

The updated SEVA database 4.0 (SEVA-DB 4.0) is a resource for implementation of a standard for physical assembly of vector plasmids and for their non-ambiguous nomenclature as well as the index for a repository of functional sequences and actual constructs available to the community. The database was designed to simplify the choice of a given vector for the sake of specific applications, in such a way the user can easily decide the best configuration of replication origins, antibiotic resistance and business segments.

The SEVA-DB adopts simple design concepts imported from Systems Engineering into vector architecture and development to facilitate the swapping of functional modules and the extension genome engineering options to microorganisms beyond typical Laboratory strains. This platform has been implemented in the Molecular Environmental Microbiology Laboratory (CNB-CSIC).

Since their launching in 2013, the SEVA platform has sent over 3700 plasmids to more than 43 countries.

E Martínez-García, S Fraile, E Algar, T Aparicio, E Velázquez, *et al*. SEVA 4.0: an update of the Standard European Vector Architecture database for advanced analysis and programming of bacterial phenotypes. Nucleic Acids Res 2022, 51, D1558–D1567.

SEVA | Standard European Vector Architecture

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.

KNOWLEDGE TRANSFER MANAGER

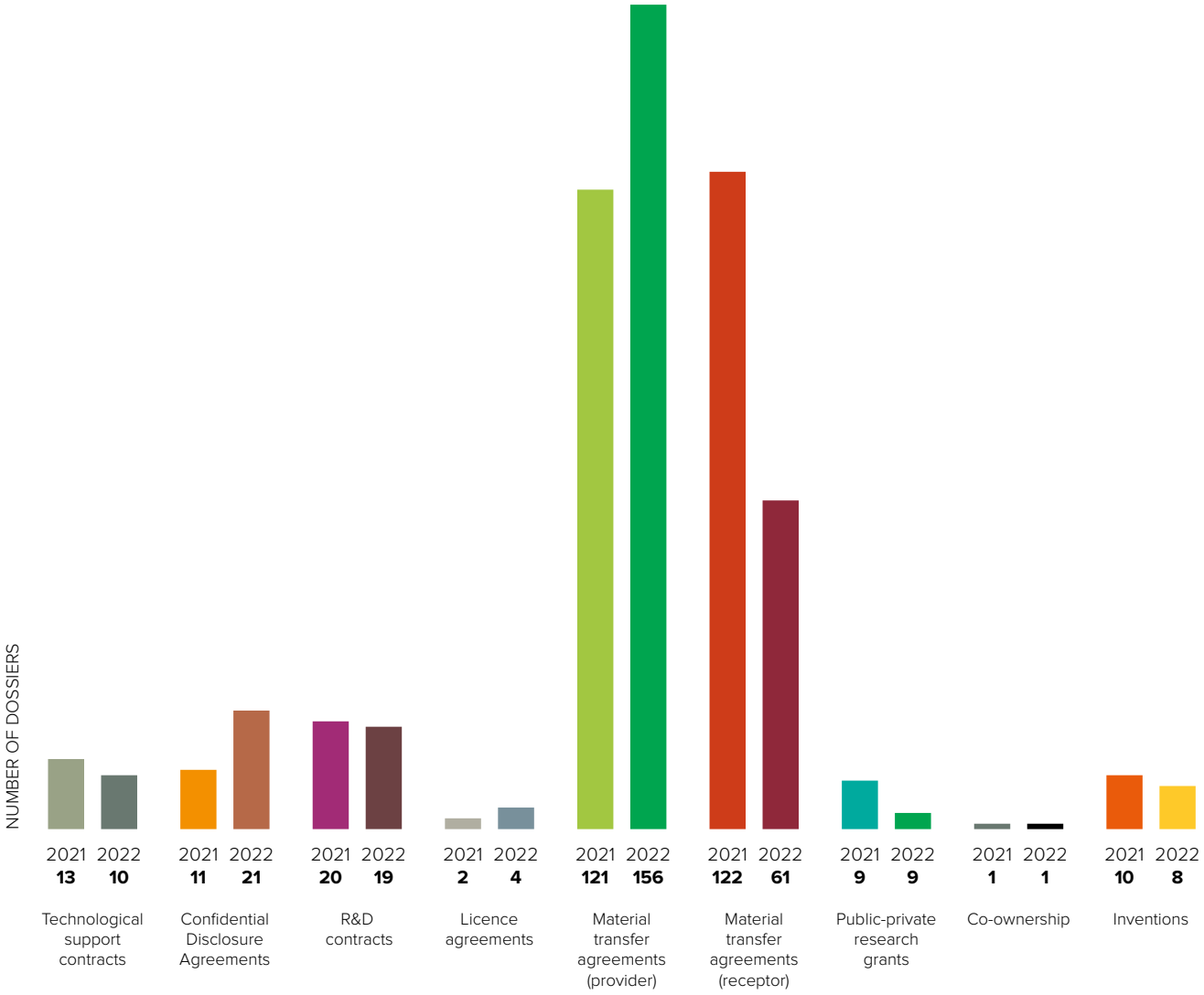
Cristina Merino Fernández

Innovation

The pourpose 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 transferable knowledge and partner for industry in the development of innovative technologies,
- potentiating the centre's innovation capabilities across all aspects of knowledge protection, commercialisation and entrepreneurship.

OUTCOME OF THE SUPPORT PROVIDED BY THE KTO



Scientific Activities, Facts and Figures

A rich program of seminars, conferences, workshops and courses (over 150 in the 2021-2022 period) provides optimal opportunities for our researchers to keep up with the latest advances in biotechnology. Each academic year, the scientific activities committee or the PhD researchers committee, both with the support of the Science Communication and Outreach Officer (Susana de Lucas), organise the main seminar series and junior seminar series (respectively). These series attract around 25 excellent speakers from around the world; this is complemented by CNB department seminars, *ad-hoc* seminars by visiting researchers and a new scientific techniques seminar series celebrated on 2022.

Highlights from the past two years include the celebration of the CNB XXX Scientific Anniversary workshop in 2022 and the EMBO International Conference on Plant Proteostasis organised by Vicente Rubio. Through online platforms, many of the scientific seminars have also been available online, thus reaching wider audiences.

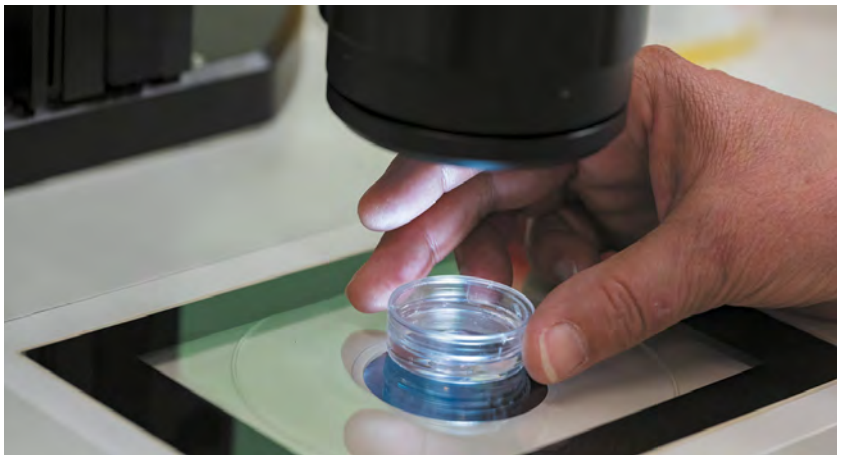
To acknowledge the financial support received from many companies, institutions or individuals during the pandemic, in September 2022 we invited their representatives to visit our institute and our research. The event called “CNB Actions against COVID-19” was inaugurated by Eloísa del Pino, president of CSIC, accompanied by the deputy presidents José María Martell (VICYT), Ana Castro (VATC) and the director of the CSIC cabinet, Isabel Martínez.

Over this period of time, CNB researchers have contributed to the publication of more than 500 papers in ISI-listed journals with an average impact factor of 8.8. Significantly, 50% of these publications were among the top 10% of the most cited journals according to the Scimago database. The international nature of the CNB is shown in the high number of papers published in collaboration with scientific groups from 120 countries.

The expertise of our researchers has been recognised with more than 80 Scientific Awards in the past 5 years, and they are present in the scientific boards of more than 20 international and national Scientific Societies that promote conferences, international collaborations and the discussion of new research results.

SCIENTIFIC ACTIVITIES COMMITTEE

Juan Carlos Alonso
Saúl Ares
Ana González
Antonio Leyva
Fernando Moreno- Herrero
Hugh Reyburn
José María Valpuesta
Esteban Veiga
Karel van Wely



CNB Seminars

During 2021 and 2022, the CNB hosted around 150 seminars. From the traditional departments´ seminars, we have also started hosting a new monthly cycle to raise awareness of the capabilities of the center's scientific facilities. In addition we have continued inviting (both in person or in virtual events, according to the pandemic restrictions) 48 internationally recognised speakers for our CNB Cycle and CNB Junior Seminar series.

SEMINARS 2021

CYCLE

19 FEBRUARY
Orphan CpG islands dictate enhancer-gene compatibility.
Álvaro Rada-Iglesias. IBBTEC-UNICAN, Santander, Spain.

26 FEBRUARY
New perspectives on eukaryogenesis.
Purificación López-García. CNRS – Université Paris-Sud – AgroParisTech, France.

5 MARCH
Photoperiod and temperature mediated control of phenology in trees.
Rishi Bhalerao. Umea Plant Science Centre, Sweden.

23 APRIL
Origin and evolution of auxin response.
Dolf Weijers. Wageningen University & Research, Wageningen, the Netherlands.

14 MAY
Structure and assembly of respiratory syncytial virus - a highly ordered filamentous virion.
David Bhella. MRC-University of Glasgow Centre for Virus Research, UK.

16 APRIL
A genome on the move: gene regulation and adaptation in malaria parasites.
Elena Gómez Díaz. Instituto de Parasitología y Biomedicina López Neira, IPBLN-CSIC, Granada, Spain

28 MAY
Directed evolution of microbial communities.
Álvaro Sánchez. Microbial Science Institute, Advances Biosciences Center, Yale University, USA

4 JUNE
Modulating expression of type IV glandular trichomes: a sustainable insect-resistance approach to control whitefly-transmitted viruses in tomato.
Enrique Moriones. Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora", CSIC-Universidad de Málaga, Málaga, Spain.

17 SEPTEMBER
Revolution in High Performance Computing to accelerate scientific progress.
Mateo Valero Cortés. Barcelona Supercomputing Centre, Barcelona, Spain.

29 OCTOBER
Computational approaches to quantify tissue organization.
Luis María Escudero Cuadrado. Instituto de Biomedicina de Sevilla (IBiS) & Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla.

12 NOVEMBER
Function of the microbiota in the control of tissue immunity and pathogen infection.
Yasmine Belkaid. NIH Center for Human Immunology, Bethesda, USA.

24 NOVEMBER
Organizing and governing principles of the bacterial cell.
Christine Jacobs-Wagner. Howard Hughes Medical Institute, Stanford University, USA.

3 DECEMBER
Imaging lipid-enveloped virus assembly and entry.
Peter Rosenthal. Crick Institute, London, UK.

JUNIOR

15 JANUARY
Ubiquitin signalling in immunity and vesicle trafficking.
Marco Trujillo. Institut for Biology II, Freiburg University, Germany.

29 JANUARY
Quantitative microbiome profiling in health and disease**.**
Jeroen Raes. Rega Institutte VIB Center for the Biology of the Disease, Leuven, Belgium.

26 MARCH
The 'Hunger Games' - Structural Studies of the MC4 Receptor Reveal Mechanism for Satiety.
Moran Shalev-Benami. Weizmann Institute of Science, Rehovot, Israel.

9 APRIL
Understanding cognition and its perturbation in intellectual disability: a holistic approach.
Mara Dierssen. Centre for Genomic Regulation, Barcelona, Spain.

7 MAY
Metagenomics of the human microbiome: uncovering the hidden diversity and exploring biomedical applications
Nicola Segata. CIBIO – University of Trento, Italy.

12 MAY
Dendritic cells therapy combined with immunomodulatory treatment in multiple sclerosis (Phase II).
Daniel Benítez. Institut de Ínvestigacions Biomédicques August Pi i Sunyer. Hospital Clínic de Barcelona. Fundació GAEM, Barcelona, Spain.

11 JUNE
The Role of subepithelial macrophages in colon-microbiota interactions
Ana-Maria Lennon-Duménil. Institut Curie, Institut Curie, Paris Sciences et Lettres University París, France.

24 SEPTEMBER
Breaking the restriction barrier in Staphylococci and application to essential signalling.
Ian Monk. Department of Microbiology and Immunology, University of Melbourne (Melbourne, Australia).

1 OCTOBER
Chemical and Molecular Ecology of Belowground Ecosystems.
Ricardo A. R. Machado. Institute of Biology. University of Neuchâtel, Switzerland.

8 OCTOBER
The protective role of type 1 conventional dendritic cells against obesity.
Salvador Iborra Martín. Medical School, University Complutense Madrid, Spain.

5 NOVEMBER
AAV-HDV: An Attractive Platform for the In Vivo Study of HDV Biology and the Mechanism of Disease Pathogenesis.
Gloria Gonzalez. CIMA Universidad de Navarra, Pamplona Spain.

VISIBLES CNB

11 FEBRUARY
Robótica: Aplicaciones y retos
Concepción Monje. Universidad Carlos III, Madrid

2022 SEMINARS

CYCLE

21 JANUARY
The bacterial epigenome.
Josep Casadesus. Facultad de Biología, Universidad de Sevilla, Sevilla.

13 JUNE
Simulating large conformational transition from multiscale modelling
Modesto Orozco. Institut de Reserca Biomédica. Barcelona, Spain.

25 FEBRUARY
Noncoding roles for RNA in the regulation of the cancer genome
Maite Huarte. CIMA Universidad de Navarra, Pamplona Spain.

4 MARCH
Emerging roles of D-amino acids in bacteria.
Felipe Cava. University of Umea, Sweden.

11 MARCH
Seeing the invisible – perception and signaling of UV-B radiation in plants
Roman Ulm. University of Geneva, Switzerland.

9 JUNE
Reorganization of chromatin architecture and global amplification of nuclear transcription by light: towards a role for plastid organelles
Clara Richet-Bourbousse. Institut de Biologie de l'Ecole Normale Supérieure (IBENS) – Paris, France.

13 MAY
Towards the utilization of cannabinoids as anticancer agents...and other stories inspired by cannabis.
Guillermo Velasco. School of Biology, University Complutense Madrid, Spain.

24 JUNE
Inferring tumor and immune cell interactions from spatial transcriptomics with SpaceMarkers.
Elana Fertig. Johns Hopkins University, Baltimore USA.

30 SEPTEMBER
Nanobiosensor devices for label-free and ultrasensitive diagnostics at the point-of-care.
Laura Lechuga. ICN2-CSIC, Barcelona, Spain.

28 OCTOBER
Expanding horizons in cryo-EM, with cryo-STEM
Michael Elbaum. Weizmann Institute, Rehovot, Israel.

11 NOVEMBER
Functional insights into bacterial chromosome organization
Virginia Lioy. Université Paris-Saclay, Gif-sur-Yvette, France.

25 NOVEMBER
Role of innate lymphoid cells in tissue homeostasis.
Andreas Diefenbach. Institute of Microbiology, Infectious Diseases and Immunology, Charité - Universitätsmedizin Berlin, Germany.

16 DECEMBER
The concept of virus in the giant virus era.
Chantal Abergel. CNRS, Marseille, France.

JUNIOR

14 JANUARY
CRH release from the infra-limbic area to the lateral septum regulates social preference.
Félix Leroy. Instituto de Neurociencias de Alicante, Spain.

28 JANUARY
Improving plant immunity by synthetic exploitation of the ubiquitin system.
Beatriz Orosa Puente. Institute of Molecular Plant Science. University of Edinburgh, UK.

18 MARCH
Adding New Pieces to the Lupus Puzzle.
Virginia Pascua. Gale and Ira Drukier Institute for Children's Health, Weill Cornell Medicine, New York, USA.

25 MARCH
Key automated tools to streamline the Design-Build-Test-Learn biomanufacturing pipeline. **Pablo Carbonell.** Universidad Politécnica de Valencia, Spain.

1 APRIL
Multiplexed imaging to investigate DNA organization and transcription in embryos.
Marcello Nollmann. Center for Structural Biochemistry of the CNRS/ INSERM, Montpellier, France.

22 APRIL
Antiviral defense during embryonic development
Sara Macias. Institute of Immunology and Infectious Diseases, University of Edinburgh, UK.

29 APRIL
The ecology and evolution of bacterial genomes.
Michael Brockhurst. Division of Evolution and Genomic Sciences. University of Manchester, UK.

20 MAY
Modeling the metabolism and adaptation of extremophilic microbes.
Ying Zhang. Rhode Island University, Kingston, USA.

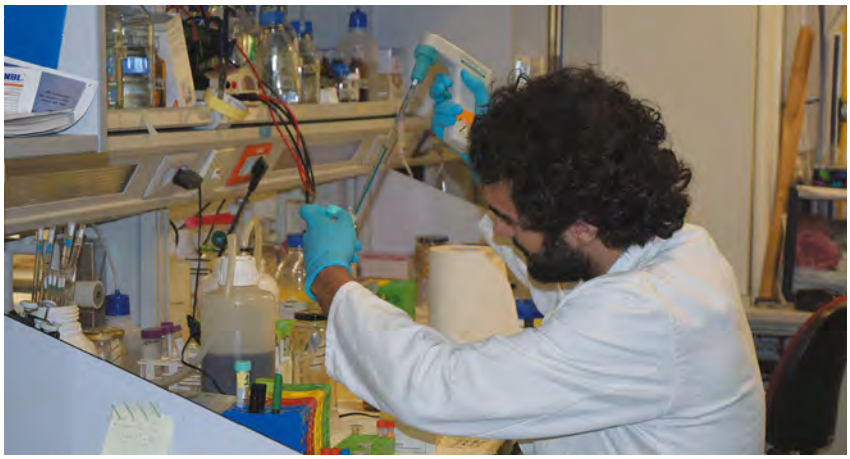
17 JUNE
Replenishing the ends: Visualisation of human telomerase holoenzyme by cryo-EM.
Kelly Nguyen. MRC-Laboratory of Molecular Biology, Cambridge, UK.

14 OCTOBER
Discovering the metabolic underpinnings of angiogenesis: A systems biology perspective.
Abhishek Subramanian. KU Leuven Center for Cancer Biology, Belgium.

21 OCTOBER
Culturing and managing complex soil microbiomes.
Jan Roelof van der Meer. University of Lausanne, Switzerland.

VISIBLES CNB

23 DECEMBER
Structural insights into the regulation of the gene silencer PRC2
Eva Nogales. University of Berkeley, USA.



Scientific-technical seminars 2022

To promote the technical capabilities available at the CNB´s scientific core units, a new cycle of seminars has been running on a monthly basis during 2022, organised by Jose Requejo-Isidro and José María Valpuesta.

25 JANUARY
Electron cryo-microscopy, **Rocío Arranz.**

22 FEBRUARY
Advance Optical Microscopy, **Ana Oña.**
Electron Microscopy, **Cristina Patiño.**

29 MARCH
Single-Molecule Infrastructure, **Fernando Moreno.**
Bioimaging system for animal research, **Ángel Naranjo.**
Quantitative optical microscopy and single molecule imaging, **José Requejo-Isidro.**

26 APRIL
Image analysis, **Carlos Oscar Sorzano.**
Instruct Image Processing Centre (I2PC), **Jose María Carazo.**

31 MAY
Proteomics, **Fernando Corrales.**
Molecular Crystallography, **César Santiago.**

28 JUNE
Flow Cytometry, **Mª Carmen Moreno.**
Protein Tools, **Leonor Kremer.**

27 SEPTEMBER
BioinfoGP, **Juan Carlos Oliveros.**
Sequence Analysis and Structure Prediction, **Florencio Pazos.**

11 OCTOBER
Histology, **Lluís Montoliu.**
Embryo cryopreservation, **Lluís Montoliu.**

25 OCTOBER
Spectroscopy techniques to analyse biological macrostructures, **Luis Alberto Campos.**

29 NOVEMBER
Transgenesis, **Belén Pintado.**
Animal Experimentation, **Ángel Naranjo.**

Scientific courses and meetings organised by CNB researchers

In the past two years, our scientists have contributed to the organisation of 85 international or national workshops, meetings and courses.

2021

JANUARY-JUNE 2021 (ONLINE)
Curso de especialización en vesículas extracelulares.
Universidad Francisco de Vitoria. Escuela de Postgrado y Formación Permanente.
Mar Valés-Gómez.

17-21 MAY (CNB)
Gestión de la producción y utilización de organismos modificados genéticamente. Procedimientos de autorización de actividades e instalaciones.
Fernando Usera & CSIC Gabinete de Formación.

18-21 MAY (ONLINE)
14th International Adenovirus Meeting.
Carmen San Martín & Mark van Raaij.

20/05/ (ONLINE)
Conversation on metabolic engineering.
Juan Nogales.

25-28 MAY (MADRID)
Cultivos de células animales. Metodología e Instrumentación.
Celia Perales & CSIC Gabinete de Formación .

07-11 JUNE (ONLINE)
Criomicroscopía en Biología.
Rocío Arranz & CSIC Gabinete de Formación.

22-25 JUNE (MADRID)
Aplicación de técnicas de cultivo celular en virología.
Celia Perales & CSIC Gabinete de Formación.

7-9 JUNE (CNB)
Redacción y seguimiento de proyectos para autorización del uso de animales de experimentación en el marco del RD 53/2013.
Belén Pintado & CSIC Gabinete de Formación.

JUNE (ONLINE)
XXVIII Congress of the Spanish Society of Microbiology (SEM).
Rafael Giraldo.

JUNE 28 JUNE– JULY 02 JULY (ONLINE)
2nd Edition: Instruct virtual course on Single Particle Analysis by CryoEM.

12 JULY (ONLINE)
IV Practical course on Genome Editing and Gene Therapy.
Lluís Montoliu, Almudena Fernández.

AUGUST (SANTANDER, SPAIN)
VII UIMP-CSIC Summer School of Integrative Synthetic Biology: "The Challenge of Bacterial Pathogens".
Rafael Giraldo.

SEPTEMBER-DECEMBER (MADRID)
Exhibition at the National Museum of Natural History, "Microbiology: Exploring beyond what is visible".
Rafael Giraldo.

23 SEPTEMBER (ONLINE)
1st Albrecht Müller ARRIGE (AMA) seminar on "Gene drives".
Lluís Montoliu.

29 SEPTEMBER- 1 OCTOBER (ONLINE)
Joint Congress of the Portuguese and Spanish Microscopy Societies.
José R. Castón, Carmen San Martín, José María Valpuesta.

18-19 OCTOBER (MADRID)
FlagEra SoundSight Meeting. The sight of sound: how vision shapes the development of auditory inputs to the occipital cortex.
Marta Nieto.

19 OCTOBER (CNB)
4th Tec4Bio Biannual Meeting on New Technologies Applied to the Study of Biological Nanomachines.
Fernando Moreno-Herrero.

OCTOBER 2021 (MADRID)
Theoretical-Practical Course of Immunochemical Techniques: Characterization and Quantification of Proteins Using Antibodies.
Leonor Kremer, María Teresa Martín and M García-Gallo. Protein Tools Unit-CNB & Gabinete de Formación del CSIC.

25-29 OCTOBER (CNB)
Introduction to programming in R.
José Ramón Valverde.

1-5 NOVEMBER (LLEIDA)
Biannual meeting SENC (Spanish Neuroscience Society).
Marta Nieto.

22-26 NOVEMBER (ONLINE)
Tomografía electrónica en Biología.
Rocío Arranz & CSIC Gabinete de Formación.

NOVEMBER (MADRID)
Master of Biotechnology, Biotechnology of Microorganisms.
Universidad Autónoma de Madrid. Sonia Gullón Blanco.

23-25 NOVEMBER (MADRID)
3rd ASEICA Educational Symposium.
Ana Cuenda.

22-25 NOVEMBER (CNB)
Descontaminación, desinfección y esterilización.
Fernando Usera & CSIC Gabinete de Formación.

3 DECEMBER (ONLINE)
ESPCR annual meeting.
Lluís Montoliu.

13-16 DECEMBER (ONLINE)
Instruct virtual course on Electron Tomography by CryoEM.
Francisco Javier Chichón.

13-17 DECEMBER (CNB)
Biostatistics.
José Ramón Valverde.

20-21 DECEMBER (CNB, ONLINE)
XXIX CNB Scientific Workshop.
Scientific Activities Committee, Susana de Lucas, Ricardo Villares.

22 DECEMBER (CNB, ONLINE)
XXIX Wokshop Advances in Molecular Biology by Young Researchers Abroad.
Mar Valés Gómez, Inés Antón, Urtzi Garaigorta, Pablo Pulido, Esther Ortega, Jesús Salvador, Juan Poyatos, Álvaro San Millán, Susana de Lucas, Domingo F. Barber.

2022

SCHOOL YEAR 2021-2022 (MADRID, SANTANDER)
UIMP-CSIC Official Master in "Integrative Synthetic Biology (MISB).
Rafael Giraldo.

JANUARY-JUNE 2022 (ONLINE)
Curso de especialización en vesículas extracelulares. **Universidad Francisco de Vitoria.** Escuela de Postgrado y Formación Permanente.
Mar Valés-Gómez.

14-18 FEBRUARY (CNB)
Biostatistics.
José Ramón Valverde.

28FEBRUARY 4 MARCH (CNB)
Introduction to programming in R.
José Ramón Valverde.

21-23 MARCH (MADRID)
The Mathematics of Living Matter, ICMAT-LifeHUB Worksho.
Juan F Poyatos.

28 MARCH (MALLORCA)
Curso de especialización: "Biología Sintética. Definiciones, metodología y aplicaciones en microbiología",
Universidad de les Illes Baleares.
Programa de doctorado en Microbiología ambiental i Biomédica.
Víctor de Lorenzo.

18-20 APRIL (ONLINE)
I2PC - Instruct virtual course on Single Particle Analysis by CryoEM.

3 MAY (CNB)
Variability in Biological Systems meeting.
Juan F Poyatos.

5 MAY (CNB)
5th Tec4Bio Biannual Meeting on New Technologies Applied to the Study of Biological Nanomachines.
Fernando Moreno-Herrero.

10 MAY
Looking for synergies between IQM and CNB scientists.
Mario Mellado.

13 MAY (MADRID)
Conversations on Metabolic Engineering: towards a new partnership with nature.
Juan Nogales.

22 MAY (ONLINE)
CNB XXX Anniversary Workshop.
CNB Scientific Activities Committee, Susana de Lucas.

23 MAY (CNB)
PhD students Scientific Workshop.
PhD Students committee.

23 - 27 MAY (MADRID)
Gestión de la producción y utilización de organismos modificados genéticamente. Procedimientos de autorización de actividades e instalaciones.
Fernando Usera & CSIC Gabinete de Formación.

23-27 MAY (ONLINE)
Criomicroscopía en Biología.
Rocío Arranz & CSIC Gabinete de Formación.

24-27 MAY (MADRID)
Cultivos de células animales. Metodología e Instrumentación.
Celia Perales & CSIC Gabinete de Formación.

30 MAY-1 JUNE (CNB)
Redacción y seguimiento de proyectos para autorización del uso de animales de experimentación en el marco del RD 53/2013.
Belén Pintado & CSIC Gabinete de Formación .

3 JUNE (CNB)
Workshop: Functional Genomics in Development, Evolution and Disease.
Juan Jose Sanz.

10 JUNE (CNB)
Ecology and evolution of antimicrobial resistance.
Sara Hernando-Amado, Alvaro San-Millan.

22–24 JUNE (ONLINE)
I2PC – Instruct course: introduction to model building.

1 JULY (CNB)
Workshop: Structural Proteomics Conference.
Fernando Corrales.

8 JULY (ONLINE)
V Practical course on Genome Editing and Gene Therapy.
Almudena Fernández, Lluís Montoliu.

11 JULY (PARIS, FRANCIA)
Biannual meeting FENS (Federation of European Neuroscience Societies).
Workshop: Development and Evolution of Bilateral Sensory Circuits.
Marta Nieto.

18-19 JULY (MADRID)
3º symposium Nanobiocargo: design, development and production of nanocarriers.
José María Valpuesta, José R. Castón.

31 JULY- 4 AUGUST (JUPITER FL USA)
FASEB Meeting. The phospholipids Conference: Dynamic lipid signaling in health and disease.
Isabel Mérida.

AUGUST (SANTANDER)
VIII UIMP-CSIC Summer School of Integrative Synthetic Biology: "Frontiers in Synthetic Biology".
Rafael Giraldo.

15-19 AUGUST (LAUSANNE, SWITZERLAND)
ISME 18 Session Convenor (2022). ISME Conference.
Álvaro Sánchez.

21-25 AUGUST (CORK, IRELAND)
16th International Meeting of the European Calcium Society Workshop: "Neuronal calcium sensors: from physiological processes to disease".
José Ramón Naranjo.

4-9 SEPTEMBER (BRNO, CZECH REPUBLIC)
16th Multinational Congress on Microscopy.
Jose María Valpuesta.

16 SEPTEMBER (CNB)
Acciones del CNB contra la pandemia por SARS-CoV-2.
Mario Mellado, Susana de Lucas.

21-23 SEPTEMBER (CNB)
International Conference on Plant Proteostasis.
Vicente Rubio.

OCTOBER 2022 (MADRID)
Theoretical-Practical Course of Immunochemical Techniques: Characterization and Quantification of Proteins Using Antibodies.
Leonor Kremer, María Teresa Martín and M García-Gallo. Protein Tools Unit-CNB and Gabinete de Formación del CSIC.

24 OCTOBER (SANTIAGO DE COMPOSTELA)
Hands-on workshop: GEIVEX / Tentacles [red traslacional para la aplicación clínica de vesículas extracelulares].
Mar Valés Gómez.

24-28 OCTOBER (CNB)
X Curso de Proteómica Cuantitativa
Alberto Paradela, Sergio Ciordia.

25-28 OCTOBER (SANTIAGO DE COMPOSTELA)
6th International GEIVEX [Grupo Español de Investigación en vesículas extracelulares] symposium.
Mar Valés Gómez.

NOVEMBER (MADRID)
Master of Biotechnology, Biotechnology of Microorganisms,
Universidad Autónoma de Madrid Sonia Gullón Blanco.

2 NOVEMBER (ONLINE)
2nd Albrecht Müller ARRIGE (AMA) seminar on "Genome editing in livestock and pets".
Lluís Montoliu.

9 NOVEMBER (ONLINE)
ESPCR annual meeting.
Lluís Montoliu.

15 NOVEMBER (CNB)
6th Tec4Bio Biannual Meeting on New Technologies Applied to the Study of Biological Nanomachines.
Fernando Moreno-Herrero.

14-17 NOVEMBER (MADRID)
Aplicación de técnicas de cultivo celular en virología.
Celia Perales & CSIC Gabinete de Formación.

15-18 NOVEMBER (CNB)
Descontaminación, desinfección y esterilización.
Fernando Usera & CSIC Gabinete de Formación.

16-18 NOVEMBER (SANTIAGO DE COMPOSTELA)
18th ASEICA International Congress.
Ana Cuenda.

16-19 NOVEMBER (ONLINE)
International Scientific Conference on Albinism (ISCA).
Global Albinism Alliance (GAA) and Lluís Montoliu.

22-26 NOVEMBER (ONLINE)
Tomografía electrónica en Biología.
Rocío Arranz, Francisco Javier Chichón & CSIC Gabinete de Formación .

23-25 NOVEMBER (SALAMANCA)
6th Spanish & Portuguese Advanced Optical Microscopy Meeting – SPAOM 2022.
Ana Mª Oña Blanco, Gianluca D`Agostino, Jaime Fernández de Córdoba, José Requejo-Isidro, Carlos Óscar Sorzano Sánchez José Javier Conesa.

28-29 NOVEMBER (MADRID)
I Encuentro CIBERESP sobre visualización de la resistencia antimicrobiana como problema de salud púbica.
Álvaro San Millán .

29 NOVEMBER (BRUSSELS, BELGIUM)
EAM EB meeting Delegación CSIC – Bruselas.
Víctor de Lorenzo.

4-8 DECEMBER (MEXICO)
HUPO World Congress.
Fernando Corrales.

6-8 DECEMBER (CHILE)
Determinación Estructural de Proteínas y Complejos Macromoleculares Mediante Técnicas de Microscopía Electrónica y Procesamiento de Imágenes.
Jaime Martín-Benito, Rocío Arranz.

13-16 DECEMBER (CNB)
Instruct course on Cryo-Electron Tomography Image Processing.
Francisco Javier Chichón.

19-20 DECEMBER (CNB)
XXX CNB Scientific Workshop,
Scientific Activities Committee Susana de Lucas.

22 DECEMBER (CNB)
XXX Workshop Advances in Molecular Biology by Young Researchers Abroad
Mar Valés Gómez, Inés Antón, Urtzi Garaigorta, Antonio Bernad, Esther Ortega, Esteban Martínez, Jesús Salvador, Álvaro San Millán, Pablo Pulido.

Scientific and social events in the last 2 years

CryoEM facility official Inauguration by Pedro Duque, Science and Innovation Ministry, 26 May 2021. The Minister of Science and Innovation, Pedro Duque, inaugurated the new cryomicroscopy facilities at the CNB-CSIC. This new infrastructure allows the observation of cells and their components with an unprecedented level of detail.

CNB actions against COVID-19. Group picture, September 2022. Event celebrated to acknowlege the financial support received from many companies, institutions or individuals during the pandemic.

30th CNB Scientific workshop, December 2022. After 2 years celebrating online workshops, we were looking forward to finally celebrate in person events (Full Lecture Hall view).

CNB actions against COVID-19. September 2022 During the event, Eloísa del Pino, president of CSIC, visited the CNB.

Publications

TOTAL

2021	2022
274	**262**

AVERAGE IMPACT FACTOR	PUBLICATIONS SCOPUS Q1	PUBLICATIONS IN SCOPUS D1
2021 **7.8**	2021 **214** 78.1 %	2021 **141** 51.5 %
2022 **9.7**	2022 **213** 81.3 %	2022 **137** 52.3 %

CNB CORRESPONDING AUTHORS	INTERNATIONAL COLLABORATION	OPEN ACCESS
2021 **147** 53.6 %	2021 **155** 56.6 %	2021 **246** 89.7 %
2022 **136** 51.9 %	2022 **131** 48.2 %	2022 **226** 86.3 %

Funding

TOTAL

2021	2022
€15.085.987	**€16.076.878**

RESEARCH GRANTS (without European Commission grants)	EUROPEAN COMMISSION GRANTS	PATRONAGE	CONTRACTS & AGREEMENTS
2021 **€11.914.990**	2021 **€2.255.718**	2021	2021 **€915.279**
2022 **€12.640.615**	2022 **€1.191.726**	2022 **€286.219**	2022 **€1.958.318**

Staff

TOTAL

2021	2022
674	**698**

RESEARCH

TOTAL

2021	2022
475	**489**

	PI	PI Postdoc + Senior	Technicians	PhD candidates	Trainees & visitors
2021	**69**	**129**	**79**	**156**	**42**
2022	**71**	**128**	**86**	**158**	**46**

GENDER

	2021	2022
Women	**53,3%**	**51,9%**
Men	**46,7%**	**48,1%**

NATIONALITY

	2021	2022
Spanish	**88,6%**	**86,5%**
Non-Spanish	**11,4%**	**13,5%**

ADMINISTRATION

2021	2022
34	**31**

SCIENTIFIC FACILITIES

2021	2022
68	**76**

TECHNICAL SUPPORT

2021	2022
43	**46**

Scientific Career Development

Training of future generations of scientists and technologists is a priority and the CNB continues attracting young people who wish to pursue a scientific career. In the 2021-2022 period, 41 PhD students obtained competitive fellowships (e.g., INPhINIT, FPU, FPI) to realise their PhD thesis at our institute, and 45 students obtained their PhD degree under a CNB scientist's supervision. Our centre hosted 73 undergraduate and 99 master's students from Spanish and international universities, allowing them to experience first-class biotechnology research in a lab setting. In addition, 35 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 degree programmes in Spain. We have already celebrated the 8th 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 students.

Our PhD training programme is fully established. The PhD Student and Training Committees, with the support of the Scientific Culture Unit, organise an annual programme of events to promote career development. In our effort to improve the excellence of the PhD Training Program, in 2023 we are launching the new Thesis Advisory Committee (TAC), based on the Salzburg Principles. This tool is established in internationally recognised research institutes, and has two main goals: (i) provide a complete individual quality assessment of PhD researchers for their professional growth and thesis achievements and (ii) help to detect earlier conflicts and problems that otherwise might truncate the thesis success and satisfaction of PhD researchers and supervisors.

Around 20% 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 14 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, la Caixa Junior Leader, and Talent Attraction Programmes. At the end of 2022 we launched a new Postdoctoral Researchers Committee to improve their structure, visibility and involvement in CNB´s scientific activities. In collaboration with the CNB Training Committee, they will work on the implementation of a specific Postdoctoral Training Program to enhance the career development of the scientists at this stage including soft-skills, profesonal career and complementary training.

TRAINING COMMITTEE

Inés Antón
Yolanda Carrasco
Sandra Fonseca
Vicente Rubio
Álvaro San Millán
Juan José Sanz
Javier Tamames
Mark van Raaij
Miguel Vicente

PhD RESEARCHERS COMITTEE

Alfonso Aguilera
Lorena Bragg
Alberto Fuster
Margarita Ferriz
Arturo García Vesga
Sofía Gardeta
Álvaro Gómez
Andoni Gómez
Diego Jiménez
Javier López-Ibáñez
Leticia Lucero
Moisés Maestro
Iris Martínez
Kateryna Matveyeva
Santiago Michavila
Micaela Navarro
Sara Otaegi
Andrés París
Sergio Pipaon
Martín Sastre

Undergraduate and master students fellowships

CSIC introduction to Research Fellowships (JAE INTRO)

7 JAE INTRO

Irene Bragado García
Alba Carballo Castro
Gema Castillo García
Carlos Quero Dotor
Margarita Roda Herreros
Ainhoa Romo Valera
Paula Vázquez Utrilla

6 JAE INTRO ICU

Beatriz Deltoro Bernardes
Álvaro López-Maroto Quiñones
Inés Muniesa Martínez
Ismael Nizialek Puerto
Uxia Pérez de José
Adrián Sansiñena Rodríguez

6 JAE INTRO Severo Ochoa Excellence Center

Inés Carmen Almena Domínguez
Enrique Álvarez Coruña
Diego Crespo Roche
Oier Lauzirika Zarrabeitia
Ariadna Villanueva Marijuán
Marta Villarejo Torres

New PhD candidates

1 Fundacion Jesús Serra Fellowship

Ana Fernández Barrecheguren

2 Fundación La Caixa Fellowship

Alfonso Aguilera Vera
Darío López García

1 FIS Programme
Ministry of Science and Innovation

Brenda Martínez Gonzalez

25 FPI Programme
Ministry of Science and Innovation

Martín Albacete Rodríguez
Julio César Aragón Lago
David Astorgano López
Paula Carballeira Peñacoba
Gema Castillo García
Emma Diaz Piñero
Marta García López
Álvaro Gargantilla Becerra
Jesús Hurtado Tamayo
Daniel Alejandro Marchán Torres
Isabel Martín Blecua
Eva María Martín Cuevas
Altea Martín Martínez
Miguel Martín Salgado
Samuel Martínez Alcalá
Juan Manuel Martínez Romero
Kateryna Matveyeva
Santiago Michavila Puente-Villegas
Daniel Mora Diego
Alba Esteli Murillo Sánchez
Elena Nonnast Fornieles
Sergio Pipaón Alcibar
Juan Rivas Santisteban
Noelia Santander Acerete
Víctor Venturini Juarez

6 FPU Programme
Ministry of Education, Culture and Sport

Tamara Alonso Blanco
José María Fernández Palacios
Silvia López Borrego
Sara Otaegi Ugartemendia
Cesar Palacios Cuéllar
Ángel Ruiz Enamorado

6 FPI Severo Ochoa Programme
Ministry of Science and Innovation

José Manuel Aguilella Porcar
María Luz Blasco Santamaría
Francisco Javier Canalejo Molero
Adrián Fernández Rego
Alicia Lou Gracia
Eva Álvarez Medrano

Doctoral Theses

45 researchers obtained their PhD under the supervision of CNB researchers in the years 2021 and 2022.

2021

Lucía Agudo Alguibe

Aplicación de la biología de sistemas para mejorar las propiedades biotecnológicas de la cianobacteria *Arthrospira platensis*.
(Juan Nogales Enrique, José Luis García López)

Guillermo Albericio

Análisis de los mecanismos centrales de regulación de los progenitores cardiacos Bmi1+ en ratón adulto.
(Antonio Bernad, Carmen Mora)

Carolina Allende Ballestero

Análisis estructural y aplicaciones biotecnológicas del nanocontenedor de encapsulina de *Brevibacterium linen*.
(José R. Castón, Javier María Rodríguez)

Alejandro Asensio Calavia

Engineering of *E. coli* bacteria for protein translocation into target mammalian cells and its *in vivo* application in tumor therapy.
(Luis Ángel Fernández, Beatriz Álvarez González)

Diego Carlero Carnero

Bases estructurales y moleculares de la proliferación viral. Mecanismo de replicación y transcripción en gripe A.
(Jaime Martín-Benito Romero)

Victoria Castro Illana

Cellular models of persistent hepatitis C virus infection reveal non-reversible transcriptomic alterations after infection clearance by direct-acting antiviral treatment. (Pablo Gastaminza, Celia Perales)

Sergio Díaz Díaz

Insights on the mode of inheritance of eQTLs and on the contribution of stabilizing and directional selection in shaping the evolution of the phosphate starvation transcriptome in Arabidopsis.
(Javier Paz-Ares)

Karolina Gmurczyk

The evolutionary analysis of Cdc13 protein between yeast exemplified by *Saccharomyces cerevisiae* and *Candida glabrata*.
(Fernando Moreno Herrero)

Guillermo Gómez García

Regulación de la secuencia de inserción ISPpu9 de Pseudomonas putida KT2440.
(Fernando Rojo, Renata Moreno)

Natalia González Mancha

Characterisation of SNX27 function in immune synapse formation and activation in T lymphocytes.
(Isabel Mérida)

Eva María Martín Cuevas

Discrete step analysis of DNA condensation by SMC proteins using Magnetic Tweezers.
(Fernando Moreno Herrero)

Fernando Martín Fernández

Estudio de la conectividad interhemisférica del cuerpo calloso.
(Marta Nieto López)

Natalia Martín González

Mechanics of Adenovirus: role of core proteins and environmental conditions in virion uncoating.
(Carmen San Martín, Pedro J. de Pablo)

Cristian Mateo Elizalde

Molecular mechanisms underlying arsenic tolerance and ER stress response in plants.
(Antonio Leyva)

Andrea Montero Atalaya

Análisis funcional de las variantes S192Y y R402Q del gen de la tirosinasa y su implicación en Albinismo Oculocutáneo de tipo I.
(Lluís Montoliu)

Rosa Ana Navajas Morillas

Análisis proteómico cualitativo y cuantitativo por espectrometría de masas en tándem para la identificación de biomarcadores de naturaleza proteica asociados a preeclampsia.
(Alberto Paradela)

Jesús Osuna Pérez

Mitochondria drive a bacteria-induced metabolic reprogramming in CD4+ T cells that orchestrates CD8+ T cell responses.
(Esteban Veiga Chacón)

Marta Pérez Illana

Structure of two stable, complex capsids: enteric and avian adenoviruses.
(Carmen San Martín)

Yadileiny Portilla Tundidor

Estudio de la influencia en los procesos de internalización, tráfico intracelular y biodegradación de nanopartículas superparamagnéticas de óxido de hierro de los recubrimientos empleados para su uso en biomedicina, y de la corona proteica que se forma sobre estos recubrimientos en entornos biológicos.
(Domingo F. Barber)

Sergio Rivas Muñoz

Análisis de la vía protumoral WIP-YAP/TAZ mediante proteómica y transcriptómica diferencial.
(Inés M Antón, Francisco Wandosell)

Elena Sanchez Martin-Fontecha

Mechanisms involved in the degradación of the strigolactone receptor DWARF14 in *Arabidopsis thaliana*.
(Pilar Cubas)

Mateo Seoane Blanco

Structure and function of Salmonella virus epsilon15 and Campylobacter virus F358 tailspikes.
(Mark J. van Raaij)

Adrián Vega Pérez

Response of peritoneal macrophages to infection and tumor metastasis.
(Carlos Ardavín)

2022

Laura Broto Campo

Estudio del establecimiento de infecciones persistentes por el virus de la bursitis infecciosa. (José F. Rodríguez)

Carmen Campos Silva

Analysis of extracellular vesicles in cancer immunomodulation and liquid biopsy. (Mar Valés Gómez)

Chang-Yu Chang

Evolutionary engineering of microbial communities. (Álvaro Sánchez)

Antonie Cossa

Bacterial morphology analysis by cryo-soft X-ray tomography and cryo-(scanning) transmission electron microscopy. (José María Carazo, Carlos Oscar Sorzano, Veronique Arluison)

Cesar Omar Domínguez Márquez

Spatial confinement of an ethanologenic route into *Bacillus subtilis* functional membrane microdomains to improve ethanol bioproduction. (Daniel López)

Alberto Fernández Oliva

De la biología celular a los tratamientos antivirales: Estudio del flujo de lípidos y la dinámica mitocondrial como dianas terapéuticas frente a bunyavirus.
(Cristina Risco Ortiz)

Alberto Fuster Pons

Disección genómica y ambiental de la variación natural para el patrón de tricomas en Cardamine. (Carlos Alonso Blanco)

Raquel García Ferreras

Mecanismos moleculares y aplicaciones terapéuticas de la transfagocitosis mediada por linfocitos. (Esteban Veiga Chacón)

Arturo García Vesga

Single molecule optical microscopy for the quantitative study of protein-lipid interactions. (Jose Requejo-Isidro)

José Manuel Honrubia Belenguer

Relevancia del motivo PBM de la proteína E en la replicación y virulencia de los coronavirus. (Luis Enjuanes, F. Javier Gutierrez Alvarez)

Pablo Laborda Martínez

Understanding evolution to tackle antibiotic resistance in *Pseudomonas aeruginosa*. (José Luis Martínez, Sara Hernando-Amado)

Aleksandra Lazarova

Modulación de la actividad de iyo y rima para entender la autorrenovación y diferenciación de células madre en plantas. (Enrique Rojo)

Bran López Luengo

Acción de las oxilipinas en la inmunidad vegetal: nuevos integrantes en las rutas de señalización. (Carmen Castresana)

Moisés Maestro López

Biochemical and structural characterization of a complex involved in chaperone mediated proteasomal degradation. (Jorge Cuéllar, José M. Valpuesta)

Javier Mendia García

Hybrid single-stranded – double stranded DNA substrates for magnetic tweezers experiments. (Fernando Moreno Herrero, Francisco de Asis Balaguer Pérez)

Elena Pedrero Vega

Caracterización del fenotipo de semi-dependencia a antibióticos ribosomales en un aislado clínico de *Staphylococcus aureus*. (Daniel López)

David Střelák

Acceleration of image processing algorithms for single particle analysis by electron microscopy. (José María Carazo, Carlos Oscar Sorzano, Ludek Matyska).

Amaia Talavera Gutiérrez

A study of the Death-Inducer Obliterator (Dido) function in somatic cell reprogramming to pluripotency. (Carlos Martínez-A)

Elena Velázquez Muñoz

Recombination-independent genomic editing and Chromosomal site-focused diversification of Gram-negative bacteria. (Víctor de Lorenzo)

Jean CC Vila

On the evolutionary ecology of microbial metabolic niche construction. (Álvaro Sánchez)

Li Wang

Human coronavirus-host interactions: pathogenesis and antiviral response. (Sonia Zúñiga, Luis Enjuanes)

Pablo Yubero Bernabé

Trade-offs in the architecture and predictability of complex phenotypes. (Juan F Poyatos)

Postdoctoral and Research Fellows

In the last two years, our center has attracted 14 early career researchers through international, national and regional calls such as Marie Sklodowska Curie Actions, from the European Commission, Juan de la Cierva, Ramón y Cajal and Talent Attraction Programmes.

3 RAMON Y CAJAL PROGRAMME

Ministry of Science and Innovation

Selena Giménez Ibañez
Esther Ortega Portero
Pablo David Scodeller

4 MARIE SKLODOWSKA CURIE ACTIONS

European Commission

Eduardo González Grandío
James Michael Krieger
Sonia Marcela Villegas Plazas
Marta Ukleja

5 JUAN DE LA CIERVA PROGRAMME

Ministry of Science and Innovation

Francisco de Asís Balaguer Pérez
Lara Del Campo Milán
Rafael Laso Pérez
Elena Pajares Martínez
Alonso Sánchez Cruz

2 LA CAIXA JUNIOR LEADER PhD FELLOWSHIPS

La Caixa Foundation

Cristina Díez Vives
Alfonso Santos López

4 FUNDACIÓN JESÚS SERRA FELLOWSHIPS

María Antonia Ávila
James Pelletier
Luis Seoane
Adrián Valli

1 AECC FOUNDATION FELLOWSHIP

Alejandra Gutiérrez

Biophysical studies on synthetic oligomeric proteins to correlate cooperativity, binding and macromolecular assembly

Luis Alberto Campos Prieto

Ramón y Cajal Fellow

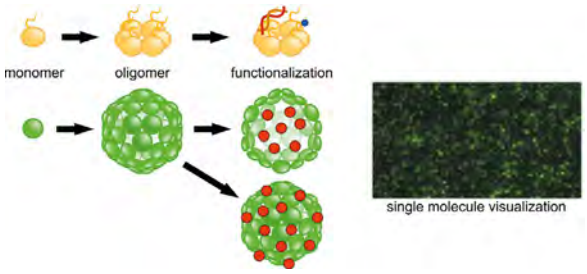
MASTER STUDENT

Ana Paula Lóbez

Proteins are involved in the majority of biological events, forming macromolecular assemblies with sometimes complex geometrical structures. In recent years, big efforts have been made in the design of non-natural protein assemblies with new geometries and functionalities for multiple applications.

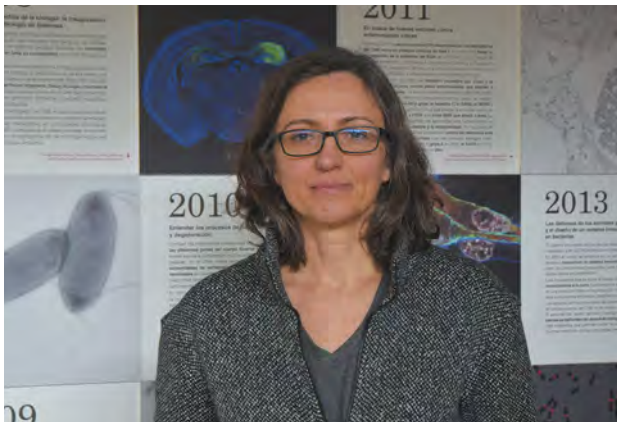
I have focused my scientific interest in the rationalized modification of proteins to obtain designed assemblies. Thus, I have created a synthetic hexameric ring by mutations of a monomeric protein, which can be regulated using different activators, and studied their functionalization with different molecules. Finally, I have designed modified viral capsids, useful for delivery. These models have been fluorescently labelled for dynamic and structural experiments using single molecule techniques.

Scheme representing the designed oligomerisation and functionalisation of de novo hexamers (top) and modified viral capsids (bottom). All processes can be followed by single molecule techniques (right).



SELECTED PUBLICATION

Campos LA. Mutational analysis of protein folding transition states: phi values. Methods Mol Biol 2022, 2376: 3-30.



Light signalling and plant adaptation to the environment

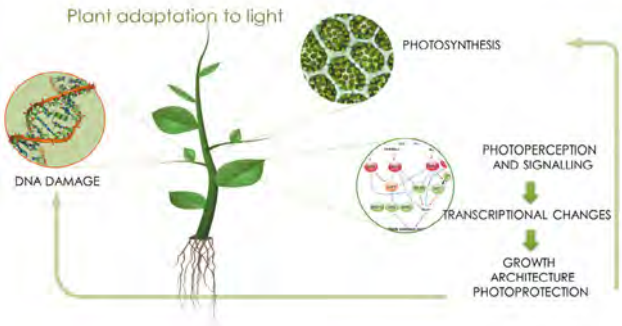
Sandra Fonseca
2021, Ramón y Cajal Fellow
2022, Investigador Distinguido

PhD RESEARCHERS
(Co-supervised with Vicente Rubio)
Esther Cañibano
Martín Albacete

MASTER STUDENT
Alexandra Oliveira

UNDERGRADUATE STUDENT
Alicia Arenas

As sessile photoautotrophic organisms, plants evolved sophisticated strategies to perceive light environmental signals and to transduce them into molecular signalling networks. Though light is essential for plant growth and development, often plants have to cope with damaging or excessive light conditions, which generate stress and limits growth. We aim to understand the molecular mechanisms that allow plants to integrate beneficial and damaging effects of light and respond to them with striking plasticity. We are especially interested in the events that lead to coordinated transcriptional changes during light adaptation as changes in chromatin states, transcription factor stability and protein homeostasis. To understand the molecular mechanisms that coordinate these processes we are using genetic, genomic, biochemical and proteomic tools.



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Cañibano E, Bourbousse C, Garcia-Leon M, Wolf L, Garcia-Baudino C, *et al*. DET1-mediated COP1 regulation avoids HY5 activity over second-site targets to tune plant photomorphogenesis. Mol Plant 2021, 14(6): 963-982.

Lee B-D, Yim Y, Cañibano, E, Kim S-H, García-León M, *et al*. CONSTITUTIVELY PHOTOMORPHOGENIC1 promotes seed germination by destabilizing RGA-LIKE2 in Arabidopsis. Plant Physiol 2022, 189(3): 1662-1676.

Molecular mechanisms regulating plant resistance against phytopathogenic bacteria

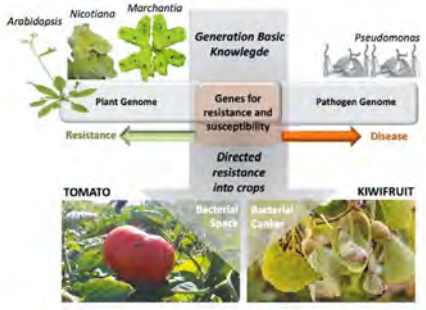
Selena Giménez-Ibáñez
Ramón y Cajal Fellow

POSTDOCTORAL RESEARCHER
Loreto Espinosa Cores

PhD RESEARCHER
(Co-directed with R. Solano)
Santiago Michavila

Our research uses on one side, model plants such as *Arabidopsis* 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.

Research Workflow of Molecular Mechanisms Regulating Plant Resistance Against Phytopathogenic Bacteria



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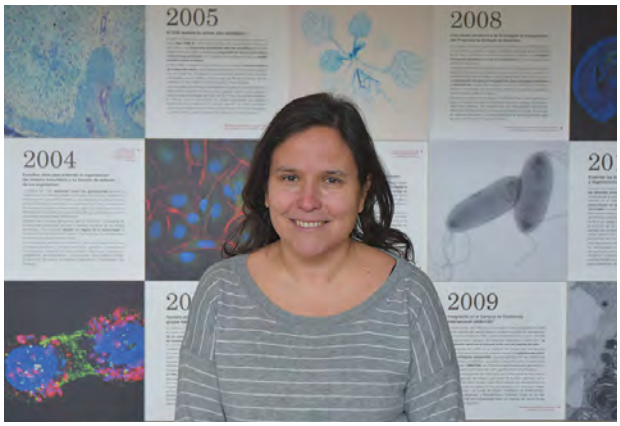
Pardal AJ, Piquerez SJM, Dominguez-Ferreras A, Frungillo L, Mastorakis E, *et al*. Immunity onset alters plant chromatin and utilizes EDA16 to regulate oxidative homeostasis. PLOS Pathog 2021, 17(5):e1009572.

Gimenez-Ibanez S, Espinosa-Cores L, Solano R. Reversible acetylation fine-tunes plant hormone signaling and immunity. Mol Plant 2022, 15(9):1415-1417.

Redkar A, Gimenez Ibanez S, Sabale M, Zechmann B, Solano R, Di Pietro A. *Marchantia polymorpha* model reveals conserved infection mechanisms in the vascular wilt fungal pathogen *Fusariumoxysporum*. New Phytologist, 2022, 234(1):227-241.



Effect of viral and host factors on innate immunity, respiratory virus replication and pathogenesis

Marta López de Diego
Atracción de Talento Fellow

POSTDOCTORAL RESEARCHERS
Vanessa Rivero Perdomo
Raúl López Fernandez
Laura Villamayor Coronado

TECHNICIAN
Sandra Gómez López

PhD RESEARCHERS
Darío López García
Paula Vázquez Utrilla

UNDERGRADUATE STUDENT
Laura Palomo Sánchez-Grande

Influenza viruses and coronaviruses are respiratory pathogens causing drastic health and economic consequences for many animal species, including humans.

In our group we analyse 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 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) analysing the cellular functions of interferon-stimulated genes and the effect of these genes on virus infections, (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 viral diseases, and (iv) developing antivirals targeting innate immune response proteins and viral proteins.

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Saiz ML, DeDiego ML, López-García D, Corte-Iglesias V, Baragaño Raneros A, *et al*. Epigenetic targeting of the ACE2 and NRP1 viral receptors limits SARS-CoV-2 infectivity. Clin Epigenetics 2021, 13(1):187.

Nogales A, Villamayor L, Utrilla-Trigo S, Ortego J, Martinez-Sobrido L, DeDiego ML. Natural selection of H5N1 avian influenza a viruses with increased PA-X and NS1 shutoff activity. Viruses 2021, 13(9):1760.

Chiem K, Martinez-Sobrido L, Nogales A, DeDiego ML. Amino acid residues involved in inhibition of host gene expression by influenza A/Brevig mission/1/1918 PA-X. Microorganisms 2021, 9(5):1109.

DeDiego ML, Portilla Y, Daviu N, López-García D, Villamayor L, *et al*. Iron oxide and iron oxyhydroxide nanoparticles impair SARS-CoV-2 infection of cultured cells. J Nanobiotechnology 2022, 20(1):352.

Chiem K, López-García D, Ortego J, Martinez-Sobrido L, DeDiego ML, Nogales A. Identification of amino acid residues required for inhibition of host gene expression by influenza virus A/Viet Nam/1203/2004 H5N1 PA-X. J Virol 2022, 96(5):e0040821.

Molecular mechanisms of transcription-replication conflicts (TRCs) in eukaryotes

Esther Ortega
Ramón y Cajal Fellow

TECHNICIAN
Eduardo Muñoz

PhD RESEARCHER
Jose Manuel Aguilella

MASTER STUDENTS
Chiara Saccanis
Zisis Millioris

Our group has been stablished in 2021 and aims to understand the molecular established mechanisms that maintain eukaryotic genome stability during DNA replication, using a combination of biochemical, biophysical, cellular and structural techniques.

DNA replication and RNA transcription are two essential processes required for accurate cell function and the propagation of genetic information. As both machineries need to access to the same DNA substrate, efficient coordination between these two processes is essential to maintain the integrity of the genome. However, these machineries can meet in space and time, causing transcription-replication conflicts (TRCs), which are a main cause of genomic instability. In eukaryotic organisms, TRCs interfere with the progression and stability of the replication forks and also trigger the accumulation of dangerous recombinant DNA structures (as R-loops) which slows down or stalls replication forks due to the physical impediments that prevents its advance. Stalled forks are a threat for DNA duplication and genome stability, causing neurodegeneration and cancer. Our group is focused on understanding the role of several factors important in the resolution of TRCs. We aim to elucidate how human Senataxin and PCNA_unloading complexes protect the integrity of the replication machinery and resolve R-loops structures formed during these TRCs.

SELECTED PUBLICATION

Cvetkovic M.A, Ortega E, Bellelli R, Costa A. Multiple roles of Pol epsilon in eukaryotic chromosome replication. Biochem SocTrans 2022, 50(1): 309–320.

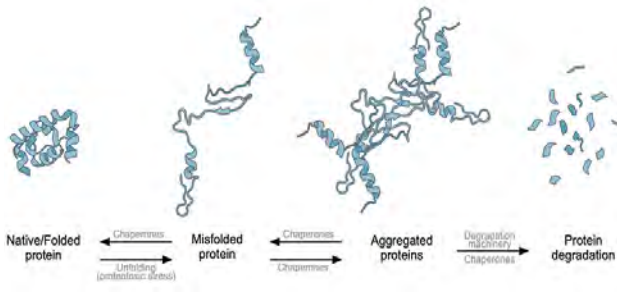
Chloroplast protein quality control (chloroquality)

Pablo Pulido
Atracción de Talento Fellow

POSTDOCTORAL RESEARCHERS
Bran Lopez Luengo
Jorge Vicente Conde

Protein quality control (PQC) systems are formed by chaperones and proteases that together regulate the correct folding and activity of proteins in every compartment of the cell, including chloroplasts. Misfolded or aggregated proteins are either recycled by chaperones (such as DNAJ and HSP70) or degraded by proteases (such as CLP) in chloroplasts. In our group we aim to elucidate and engineer the molecular machinery that protect proteins in chloroplasts allowing plants to cope with adverse environmental conditions such as high temperature or drought. These abiotic stresses are the leading cause of yield loss in crops in the current context of climate change.

Currently we are focused on the study of chloroplast DNAJE type of assembly factors of photosynthetic complexes and their role in plant stress tolerance. Besides, we have identified novel components of the chloroplast-to-nucleus retrograde signalling pathway that regulate plant survival under protein aggregation within the organelle



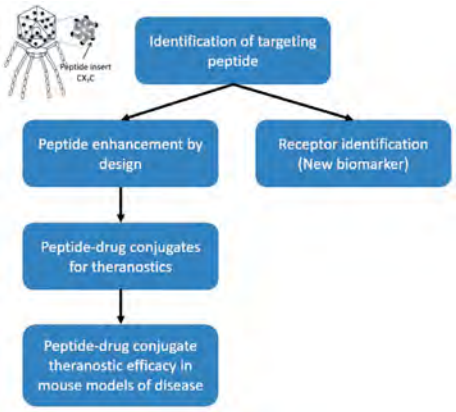
SELECTED PUBLICATION

Llamas E, Pulido P. A proteostasis network safeguards the chloroplast proteome. Essays Biochem 2022, EBC20210058.

Peptide-guided theranostics

Pablo Scodeller
Ramón y Cajal Fellow

Targeting peptides can be used to carry relevant cargo to specific tissue or cells in the disease, to improve treatment and diagnosis. Targeting peptides provide selectivity, versatility, deep tissue penetration, low immunogenicity and ease and low cost of synthesis. The aim of the Peptide-Guided Theranostics Lab is to identify new targeting peptides and perform precision delivery for cancer, immunomodulation, and Alzheimer's disease, by designing peptide-guided theranostics that can be translated to the clinic.



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Figueiredo P, Lepland A, Scodeller P, Fontana F, Torrieri G, *et al*. Peptide-guided resiquimod-loaded lignin nanoparticles convert tumor-associated macrophages from M2 to M1 phenotype for enhanced chemotherapy. Acta Biomater 2021 133: 231-243.

Ewert KK, Scodeller P, Simón-Gracia L, Steffes VM, Wonder EA, *et al*. Cationic liposomes as vectors for nucleic acid and hydrophobic drug therapeutics. Pharmaceutics 2021, 13(9): 1365.

Lepland A, Malfanti A, Haljasorg U, Asciutto EK, Pickholz M, *et al*. Depletion of mannose receptor-positive tumor-associated macrophages via a peptide-targeted star-shaped polyglutamate inhibits breast cancer progression in mice. Cancer Res Comms 2022, crcres.0043.2022.

Simón-Gracia L, Scodeller P, Fisher WS, Sidorenko V, Steffes VM, *et al*. Paclitaxel-Loaded cationic fluid lipid nanodiscs and liposomes with brush-conformation peg chains penetrate breast tumors and trigger Caspase-3 activation. ACS Appl Mater Interfaces 2022, 14(51): 56613-56622.

Simón-Gracia L, Loisel S, Sidorenko V, Scodeller P, Parizot C, *et al*. Preclinical validation of tumor-penetrating and interfering peptides against Chronic Lymphocytic Leukemia. Mol Pharm 2022, 19(3): 895-903 .

Plant-virus coevolution

Adrian A. Valli
Ramón y Cajal Fellow

MASTER STUDENT
Alberto Angulo Roiz

TECHNICIAN
Irene Gonzalo Magro

UNDERGRADUATE STUDENT
Adrian Zhou

PhD RESEARCHERS
Rafael García López
Alfonso González de Prádena
(co-supervised with JA García)

Our group studies plant-virus coevolution with the aim of (i) understanding the arms race taking place when viruses infect plants, (iii) discovering new layers of antiviral responses exerted by hosts, and (ii) developing innovative strategies to fight viruses affecting the production of relevant crops. To do that, we pay special attention to RNA viruses of the *Potyviridae* family, the most relevant group of plant RNA viruses. For studies carried out over the last two years, we follow a multidisciplinary approach that includes (i) synthetic biology to build and manipulate viral infectious clones; (ii) genomics/transcriptomics studies aiming to decipher the effects of plants over viruses, and vice versa; (iii) metabolomics studies by HPLC-MS/MS to untangle plant physiological changes due to viral diseases, (iv) viral ecology to understand the interaction occurring between plants and viruses in the field.

Some of our recent findings are:

- The host-specific role of viral-derived ITPases expressed by potyvirids infecting plants from the *Euphorbiaceae* family in nature.
- The potential antiviral activity of non-canonical nucleotides produced by plants.
- The presence of an inheritable antiviral RNA-based immune system against pararetroviruses in certain plants.

Our research is supported by grants RYC2018-025523-I, PID2019-110979RB-100 from MICINN, and COOPA20452 from CSIC.

SELECTED PUBLICATIONS

Valli A, García Lopez R, Ribaya M, Martínez FJ, García Gómez D, *et al*. Maf/ham1-like pyrophosphatases of non-canonical nucleotides are host-specific partners of viral RNA-dependent RNA polymerases. PLOS Pathog 2022, 18(2): e1010332.

Valli A, Gonzalo I, Sanchez D. Rearranged endogenized plant pararetroviruses as evidence of heritable RNA-based immunity. Mol Biol Evol 2022, 40(1):msac240.

Communication and Outreach

The CNB Communication and Outreach Office works to increase the awareness of the research carried out by CNB scientists and to strengthen the bonds of the Center with other academic institutions as well as with journalists and media. It also acts as the intermediary between the CNB and citizens, through activities of different types such as talks, dissemination events, or training, with the main objective of promoting scientific, technological culture and innovation.

Since April 2022, the CNB is an accredited member of the Spanish Foundation for Science and Technology (FECYT) Network of Scientific Culture and Innovation Units (UCC+I). This UCC+I Network aims to promote the exchange of experiences and the search for synergies between entities, improving the quality of UCC+I products and services and the optimisation of resources.

In addition, the office acts as a link in the organisation of annual scientific activities, such as the CNB Seminar Series, the CNB Scientific Workshop, the training activities for PhD researchers organised by the CNB Training Committee and the Programme for High School Students “4 ESO and Empresa” held in March-April.

COMMUNICATION AND OUTREACH MANAGER

Susana de Lucas

VISITING JOURNALISTS

(CSIC-BBVA Foundation Scientific Communication Awards)

Leyre Flamarique
2021

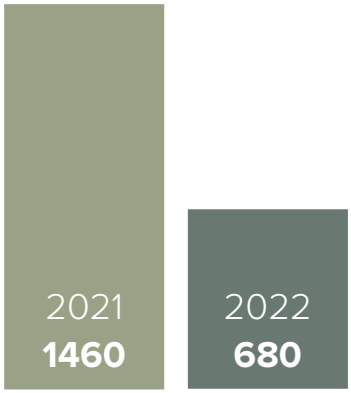
Lucía Casas
2022

POSTGRADUATE STUDENT

Mónica Blanco
(UAM)

Communication

The CNB communication office, in coordination with the CSIC Communication department, serves as a bridge to respond to inquiries from local, national and international media. In the last two years, more than 50 press releases highlighted the scientific achievements made by CNB researchers. We continued to increase the coverage of our research and initiatives in the media, with around 2000 appearances in the 21-22 period. In addition, we have received two young scientific journalists beneficiaries of the first "CSIC-BBVA Foundation Scientific Communication Awards", Leyre Flamarique and Lucía Casas to learn first-hand about the entire scientific research process, interact with researchers and learn about the development of our scientific projects.



MEDIA APPEARANCES

SINC. 21-3-22

Infosalus. 25-4-21

Grupo Noticias (DEIA, Noticias de Navarra, Diario de Álava y Noticias de Gipuzkoa). 28-2-21

El Diario, 15-6-21

EFE Verde. 26-4-21

COPE. 5-9-21

SINC. 23-11-21

National Geographic. 3-1-22

El País. 19-3-22

The Conversation. 29-4-22

La Vanguardia. 1-8-22

Social media

The CNB maintains dialogue with the public through social media, a community that keeps increasing, with more than 4,5K followers on Facebook, 28,5K on Twitter, 11,7K on LinkedIn and 825 subscribers in YouTube, respectively. We have also joined Instagram in 2022, reaching 800 followers by the end of the year. In this time, our YouTube channel has become the adequate scenario to present our activities during the pandemic, when face-to-face events were restricted, reaching more than 27K viewers. In addition, the new 14 posts published in the "CNB Divulga" Blog have received more than 17,000 reads in this time.

TWITTER
Followers
28,500
Impressions
1689,4K

LINKEDIN
Followers
11,750
Impressions
15K

FACEBOOK
Followers
4,700
Impressions
39,1K

INSTAGRAM
Followers
810
Impressions
4,3K

YOUTUBE
Followers
825
Viewings
27,1K

CNB DIVULGA
Followers
-
Readers
17K

iLeon. 13-10-22

Public engagement

With the invaluable collaboration of the center's scientists, the office coordinates outreach 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 11). Despite the challenges posed by the pandemic, we celebrated online talks and workshops for kids and schools in 2021, or adapted our capacity to the restrictive measures imposed during the pandemic.

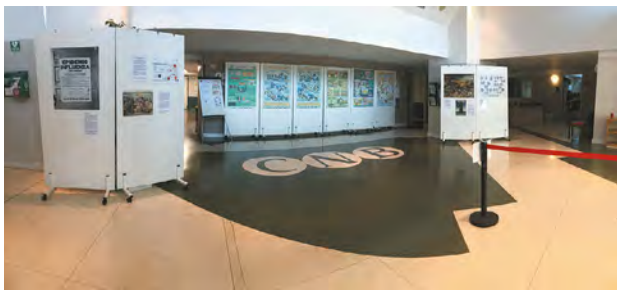
We would like to draw attention to the project "Science and Responsibility against the COVID-19" developed in 2021 with the support from Fundación General CSIC to Susanna´s Manrubia. This proposal contained an exhibition and a video showcasing the SARS-CoV-2 research carried out in our institute. The exhibition featured six CNB´s research projects in comics and six infographics from other historical epidemics such as Variola or HIV that were displayed at the CNB, the as part of 2021 European Researchers´ Night Event and in several schools in collaboration with the CSIC project "Ciencia en el Barrio" (Science in the Neighbourhood). In 2022, we presented the project at ComCiRed, the annual meeting of FECYT Scientific Culture Units.

Escape Room, 2021

Plant Fascination Day, 2021

European Researchers´ Night, 2021

Science week, 2022



Science and Responsibility against COVID-19, CNB hall, 2021

We also have a fruitful ongoing collaboration with other CSIC neighbouring research institutes located in the same campus to celebrate the European Researcher's Night. To make it easier for citizens to attend, we moved our event to different locations in Madrid city center, such as Madrid-Atocha Train Station Tropical Garden in 2021 or the CSIC headquarters in 2022. This collaboration serves also to vertebrate the "Escape Road: Looking for Female Nobel Prizes", an exhibition-gymkana to visualise and valorise the research work made by women. This exhibit has been "on tour" in commemorative dates as the International Day for Women and Girls in Science (11F) or the 8th of March, the International Women Day, but not only on those, as during the year it is borrowed by schools to use in their teaching programmes as a transversal activity.

Promotional Flyer for "A Research Night at CSIC" Event

Escape Road at CSIC Headquarters, Researchers Night, 2022

Escape Road at Atocha Train Station Tropical Garden, 2021

Science Week, 2022

Science Week, 2022

Science and Responsibility against COVID-19, IES Arcipreste de Hita, 2022

Audience reached

11F: INTERNATIONAL DAY WOMEN AND GIRLS IN SCIENCE DAY

2021	2022
306	**1952**

TOTAL **2258**

PLANT FASCINATION DAY

2021	2022
198	**40**

TOTAL **238**

SCIENCE & TECHNOLOGY WEEK

2021	2022
282	**205**

TOTAL **487**

EUROPEAN RESEARCHERS´ NIGHT

2021	2022
420	**970**

TOTAL **1390**

EUROPA´S DAY (9TH MAY)

2021	2022
-	**400**

TOTAL **400**

COVID-19 EXHIBITION (ONLINE/ FACE-TO-FACE)

2021	2022
12239	**200**

TOTAL **12439**

COVID-19 VIDEO

2021	2022
-	**620**

TOTAL **620**

NOBEL PRIZES ESCAPE ROAD

2021	2022
1470	**5772**

TOTAL **25074**

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 June 2020 the Equality Committee of the CNB was formally established to implement measures promoting gender equality and diversity, to inform on equality issues and to mediate in possible conflict situations. In their composition (gender-balanced) both men and women from all professional levels are represented.

The committee works to implement a feasible Equality Plan in the institute, analysing the centre's staff data broken down by gender, producing documents related to gender equality plans and protocols for the prevention of sexual harassment that are now in force at the CNB-CSIC.

EQUALITY COMMITTEE

Saúl Ares
Alicia Calvo Villamañán
Pilar Cubas
Ana Cuenda
Mónica Chagoyen
Daniel López
José Martín Gómez
Carmen San Martín
Juan José Sanz-Ezquerro

Igualdad CNB

Since the creation of the Committee, there has been an increase in the participation in equality training courses by CNB personnel, going from 4 women in 2019-2020, to 32 people in 2021-2022, including men. Moreover, in 2021 the Committee co-organised, in collaboration with the neighbouring centers Instituto de Catálisis y Petroleoquímica (ICP-CSIC) and the Instituto de Ciencias Materiales de Madrid (ICMM-CSIC), a Workshop on the "Inclusion of the gender perspective in research projects". Also, we have ensured gender parity in the speakers of all the seminars cycles and workshops organised by the center, and for example, 45% of speakers in the CNB main seminar series in 2022 were women.

During these two years, the committee has continued organising and participating in activities to make visible the work of CNB women scientists. In special dates such as #11F, International Day of Women and Girls in Science, our female scientists have participated in numerous outreach activities both in-house and at external venues (schools, civic centres, media). Moreover, in 2022 we produced 12 short videos (CNB's YouTube channel) of researchers from the CNB (women and men) where they describe either their work or the inspiring female scientists and mentors in their professional career. It is worth noting the relevance and impact of the communication work by CNB female researchers during the Covid19 pandemic, due to their scientific expertise in coronaviruses and vaccines.


Día de la mujer y la ciencia

"Ser científica no es el mundo de la alfombra roja o los Oscar, pero también resulta fascinante"

La ciencia es la base de nuestra sociedad, por eso hay que visibilizar a quienes están detrás de un microscopio y darles el papel que merecen, uno en el que las mujeres tienen un espacio enorme.

Isabel Sola Gurpegui, bióloga española experta en coronavirus y coinventora de tres patentes.

We have also implemented the Conference series "Visibles-CNB" to show the professional success of women despite inequalities and gender biases. By making the work of these great women visible, we want to provide female references to young people who are starting their careers. We inaugurated this series on #11F in 2021, with Concha Monje, electronics engineer, professor and researcher in 'Humanoid Robotics and Systems Control' from Carlos III University, Madrid (UC3M). A second conference presented by Eva Nogales, structural biologist at University of California Berkeley was held on December 2022,

These two years, the CNB has continued participating in the program "Science by Women" from Women for Africa Foundation to promote the leadership of African women in

scientific research. The CNB has hosted 2 researchers, from Tunisia and Nigeria, who have worked on projects related to agriculture and systems biology.

Additionally, in 2022 the CNB equality committee has coordinated the Inter-Centre Equality Commission of the Campus of International Excellence CSIC+UAM. In this context, we have organised the joint celebration of #11F, with a talk about Katalin Karikó´s achievements, that is available at the CNB Youtube channel. Also, for the 25th November, the International Day for the Elimination of Violence against Women (#25N), we have organised a colloquium about sexual harassment in Spanish academia, inviting Ángela Bernardo, journalist and author of the book #Metoo en la ciencia española (#Metoo in Spanish academy), Zulema Altamirano, director of the Women and Science Committee, Spanish Science and Innovation Ministry, and Laura Chaparro, scientific journalist from the Spanish Science Media Center (SMC). This debate was recorded and can be viewed at the CNB Youtube channel.

Finally, the CNB won one of the accessits from the CSIC Equality Award in 2022, in recognition of the work carried out for the implementation of equality measures and the outreach and mentoring activities to promote women in research.

DISTINTIVO DE IGUALDAD CSIC

Sustainability

Mother Earth is clearly urging a call to action. Nature is suffering, and we must immediately change our energy consumption, resource management and collective behaviour to prevent the catastrophic destruction of our environment. The Sustainability Committee of the CNB was created in the Mother Earth Day (April 22nd, 2022) with the commitment to promote in the CNB awareness, improve the care of our environment and reduce the environmental impact derived from our daily activity in it. The committee was initially formed by twelve volunteers from all professional levels.

SUSTAINABILITY COMMITTEE

Silvia Ayora
María López Sanz
Cristina Martínez
Santiago Michavila
Álvaro Gargantilla
Nuria Fernández
Lara del Campo
Juan Nogales
Esther Ortega
Enrique Rojo
Raquel Tenorio
Ignacio Moreno de Alborán

In these eight months, several actions have been taken to improve sustainability at the CNB. First, we produced a report analysing whether we were doing enough to alleviate the environmental crisis. This report identified weaknesses in the current waste recycling management, that led us to set up new initiatives. We have installed new recycling bins for plastic and paper in areas that lacked them. We also installed bins to separate organic waste in the cafeteria and other lunch areas. In collaboration with the company Terracycle we are now recycling writing supplies such as pens, correction markers or highlighters.

We have produced and distributed informative guides to encourage CNB workers to use properly the new recycling bins.

A further step was to launch a car-sharing initiative, to reduce pollution in the daily commute to work. In addition, the CNB has replaced its institutional vehicle for an electric one, and the parking-lot has now a charging point for electric cars that can be used by CNB workers.

It is necessary to continue promoting measures in the CNB that reduce energy comsumption, and conserve resources. Energy use varies widely across the institute, from energy-dense laboratories to offices, each posing different challenges in reducing emissions. We are currently working with researchers, staff, and building managers to envision and implement sustainable practices and technologies that will contribute to CNB's goals and commitments to confront the challenges of climate change.

CNB management

The CNB is headed by an executive director and two vice-directors elected every four years by the General Board, formed by the Centre's academic staff (Faculty).

The general manager is responsible for the economic and administrative management of the institute and the efficient use of the material, economic means and personal resources assigned to it.

The CNB governing board is headed by the Director and Vice-Directors and formed by the six department heads, two personnel representatives and the general manager. They meet regularly to plan, discuss and implement the decisions taken by the Centre's management.

The CNB has several committees that actively collaborate in activities such as the organisation of scientific events or the promotion of career development, in order to improve the quality of our science and professional environment.

In addition, an external Scientific Advisory Board monitors the scientific activities of the Centre through regular evaluations and acts as an advisory body to the Director.

Scientific advisory board

Our Scientific Advisory Board (SAB) has reviewed the last 5-years centre's strategy and performance (the 2018-2022 period). Members of the 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.

Board of directors

DIRECTOR
Mario Mellado

VICEDIRECTOR
Fernando Rojo

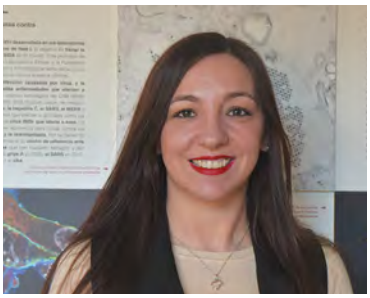
VICEDIRECTOR
Susanna Manrubia
(2021)

VICEDIRECTOR
Lluís Montoliu
(2022)

Director´s office

ADJUNCT MANAGER
Ricardo Villares

ASSISTANT TO THE DIRECTOR
Yolanda García
(until July 2022)



ASSISTANT TO THE DIRECTOR
Alexandra Rodríguez
(from Nov 2022)

SCIENTIFIC CULTURE UNIT MANAGER
Susana de Lucas

TECHNOLOGY TRANSFER MANAGER
Cristina Merino

General manager

Isabel Sevillano

Project management

HEAD

Daniel Martín Hernando

PERSONNEL

Aránzazu Almendro
Pilar Ara Laúna
Beatriz de los Frailes
Sergio Gómez
Sergio Sierra

Human resources

HEAD

Marina Hernando Bellido

PERSONNEL

Aurora Cabrerizo Alonso
Pilar Corral Cid
Alexandra Rodríguez
Gloria del Sastre Martín
Javier Tortosa Nieto

Economic management

HEAD

Gema Bravo Sanz

PERSONNEL

Santos Esteban Barranco Sierra
Mª Carmen Berreiros Cano
Eva Castillo
(from August 2022)
Francisco Javier Hernández Izquierdo
(until August 2022)
Mª José Gregorio Usano
Rafael López Laso
Mª Carmen Pascual Martínez
Mª Carmen Vaz Pereña
Álvaro Vila Hernández
Iris Roldán Zuasti

Purchasing and supplies

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Julio Díez Álvarez

PERSONNEL

Juan Carlos Bermudo Zamora
Mª Ángeles Lumbreras Carrasco
(until August 2021)
Jaime Pastor
Mario Pérez Arranz
Rocío Rodríguez Vázquez

Information technologies

HEAD

Sonia de Diego

PERSONNEL

Carlos Francisco Bell Díaz
(until July 2021)
Alejandro Fernández Ibáñez
Javier de la Fuente López
(until August 2021)
Inigo Oficialdegui García de Blas
Mario Yuste García
(from September 2021)

Occupational risk prevention unit

HEAD

Núria Martín Montes (external)

General services

HEAD

Gabriel Sánchez de Lamadrid Herranz

PERSONNEL

Julián Grande Palomino
Manuel Grande Palomino

EXTERNAL

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Beyza Zulema López Milla
Cristina Mendiola Martínez
Juan Pardo Prieto
Anna Pawelczyk
Pablo Pradp Capilla
Perla Portillo De Alegre
Lourdes Sánchez Díaz
Manuela Sánchez Herrera

EXTERNAL

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Carolina Nogales Mauro
Aileen Notario Bonsol
Darianngel Hivvizay Bacco Piñango
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Fco. Javier Lara Boavent

Maintenance

HEAD

Antonio Dueñas

PERSONNEL

Juan Carlos Cuenca
Alfonso García
Jesús González
Enrique Mejías
Mario Enrique Rodríguez (external)

Construction and insfrastructure planning

Javier Zarco

Security

HEAD

Sócrates Gutiérrez

PERSONNEL (EXTERNAL)

José Fernando Albarrán Aparicio
Abderrahim Asgou Achtouk
Lidia Cano Rojilla
Tomás Castro López
Dragos Dumitru Calistecu
Emilio Encada Pereira
José Luis Hernando
Marcos Manzano Diez
Ignacio Rodríguez
Ignacio Sánchez Chávez

Governing board and CNB committees

GOVERNING BOARD

Isabel Sevillano
Mario Mellado
Fernando Rojo
Lluís Montoliu
Roberto Solano
Esteban Veiga
Domingo F Barber
Luis Ángel Fernández
José María Valpuesta
Florencio Pazos
Julia Fernández
Luis Yuste

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Isabel Sola
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Fernando Usera

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Ana González
Antonio Leyva
Fernando Moreno Herrero
Hugh Reyburn
José María Valpuesta
Esteban Veiga
Karel van Wely

TRAINING ADVISORY COMITTEE

Inés Antón
Yolanda Carrasco
Sandra Fonseca
Vicente Rubio
Álvaro San Millán
Juan José Sanz
Javier Tamames
Mark van Raaij
Miguel Vicente

PhD RESEARCHERS COMITTEE

Alfonso Aguilera
Lorena Bragg
Alberto Fuster
Margarita Ferriz
Arturo García Vesga
Sofía Gardeta
Álvaro Gómez
Andoni Gómez
Diego Jiménez
Javier López-Ibáñez
Leticia Lucero
Moisés Maestro
Iris Martínez
Kateryna Matveyeva
Santiago Michavila
Micaela Navarro
Sara Otaegi
Andrés París
Sergio Pipaon
Martín Sastre

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Enrique Rojo
Raquel Tenorio

CNB REPORT

2021_2022

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