2017

Report

Institute of Molecular Biology of Barcelona

2018

ibmb

Institut de Biologia Molecular de Barcelona

Molecular Biology Institute of Barcelona CSIC

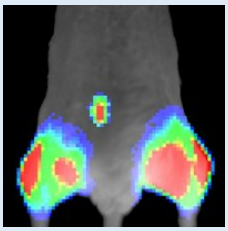
Index

Welcome to IBMB

The Institute of Molecular Biology of Barcelona (IBMB), an interdisciplinary research centre of the Spanish Research Council (CSIC), hosts research groups devoted to the understanding of major molecular mechanisms sustaining life, with significant biomedical and biotechnological implications.

A distinctive feature of the IBMB is its strong nucleus of researchers with leading-edge expertise in Structural Biology, the field of Biology that describes the architecture of biomolecules at the atomic level. Such expertise benefits from a scientific community within the IBMB that interrogates the physiological functions of those biomolecules in living experimental model systems and from multiple angles. In order to potentiate the generation of knowledge and applications based on these models and technologies, the IBMB has implemented in recent years a major effort to promote interdisciplinary projects between researchers with expertise in X-ray crystallography, cryo-electron microscopy and computational biology, on the one hand, and researchers tackling shared biological problems through cell and developmental biology or molecular genomics approaches, on the other hand. This strategy should lay the ground for the development of what we call Structural Physiology, aimed at solving the dynamic structure of macromolecular complexes supporting essential biological processes at atomic resolution and in physiological contexts.

Research



Cell biology

The primary goal of the Department of Cell Biology (DCB) is to understand major cellular processes and the underlying molecular mechanisms, including the regulation of cell growth, organelle biogenesis and function, signalling pathways, synaptic plasticity or protein degradation. The knowledge thus garnered affords improved understanding of mechanisms underlying human disease or physiological decline, such as aging, neoplastic diseases or neurodegenerative disorders, and how to tackle them on the basis of robust mechanistic insights. These fundamental questions are addressed through the use of in vivo (including yeast and mice) and in vitro (cultured cells and purified proteins) model systems. All research groups at the DCB have a common interest in the mechanistic bases of key cellular functions, and share approaches that include the use of advanced microscopy to analyse molecular dynamics at the single-cell and single-molecule levels, genomic and proteomic methodologies to study gene expression and protein interactions, and state-of-the-art genetic manipulation techniques to test molecular models.

HEAD OF DEPARTMENT

Carme Gallego

RESEARCH GROUPS

1. Spatial control of cell cycle entry. Aldea Malo, Martí
2. Signaling through G proteins. Aragay Combas, Anna
3. Regulation of the proteasome. Crosas Navarro, Bernat
4. Control of local mRNA expression. Gallego González, Carme
5. The endocytic pathway and the actin cytoskeleton. Geli Fernández-Peñaflor, Mª Isabel
6. Neural Proliferation Control. Pons, Sebastian
7. Cell Cycle and Signaling. Roig, Joan
8. Cell signalling, ubiquitin and cancer. Thomson Okatsu, Timothy

Spatial control of cell cycle entry

Cell Biology

Protein misfolding and accumulation of proteotoxic aggregates has a direct impact in cell aging, but the underlying molecular mechanisms are poorly understood. Although it has been mainly associated to neurodegenerative diseases, recent evidence has linked proteostasis defects to cell precursor damage in proliferative tissues. As in many cell types of different origin, prion-like aggregates are asymmetrically distributed during cytokinesis in budding yeast, and we have found that their progressive accumulation through consecutive generations eventually inhibits cell cycle entry and leads the cell to an irreversible senescent state. Since prion-like and proteotoxic aggregates act as chaperone sinks, our data point to the idea that chaperome alterations would mediate the observed decline in cell proliferation during cell aging. With these hypotheses we aim at the identification of key molecules and mechanisms that restrain cell proliferation during cell aging and, hence, at a better understanding of aging at a systems level.

Research Lines

1. Prions and aggregons as START inhibitors: a path to cell aging
2. Molecular competition and cell size control
3. Inntag: applications to the diagnostic of pathological protein-protein interactions

GROUP LEADER
Martí Aldea

POSTDOCTORAL RESEARCHERS
David Moreno

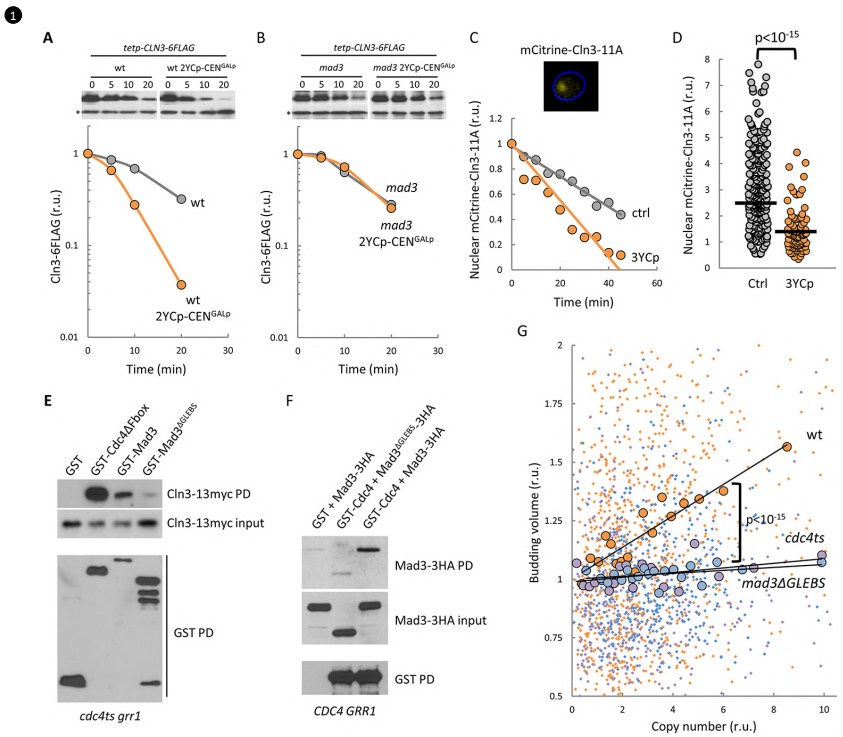
PhD STUDENTS
Pedro Jesús Vidal
Alexis Pérez

PUBLICATIONS

Martínez-Láinez JM, Moreno DF, Parisi E, Clotet J, **Aldea M.** Centromeric signaling proteins boost G1 cyclin degradation and modulate cell size in budding yeast. PLoS Biology 16:e2005388 (2018)

Parisi E, Yahya G, Flores A, **Aldea M.** Cdc48/p97 segregase is modulated by Cdk to determine cyclin fate during G1 progression. The EMBO Journal 37:e98724 (2018)

Saarikangas J, Caudron F, Prasad R, Moreno DF, Bolognesi A, **Aldea M**, Barral Y. Compartmentalization of ER-bound chaperone confines protein deposit formation to the aging yeast cell. Current Biology 27, 773–83 (2017)



1 Degradation of cyclin Cln3 by exceeding CENs: Mad3 physical and functional interactions with SCF.
(A) Analysis of Cln3 stability by promoter shut-off experiments in the presence (orange circles) or absence (gray circles) of two YCp–CENGALp vectors in wild-type cells grown under permissive conditions. After tetracycline addition, cells were collected at the indicated times, and obtained Cln3–6FLAG levels are plotted relative to an unspecific cross-reacting band (asterisk) used as loading control. (B) Analysis of Cln3 stability in Mad3-deficient cells as in (A). (C) Analysis of mCitrine–Cln3–11A stability by time-lapse microscopy in the presence (orange circles) or absence (gray circles) of three YCp vectors. Nuclear levels of mCitrine–Cln3–11A in cells were determined at the indicated times after cycloheximide addition, and mean values (N = 100) are plotted. (D) Analysis of mCitrine–Cln3–11A accumulation in the nucleus in the presence (orange circles) or absence (gray circles) of three YCp vectors. Nuclear levels of mCitrine–Cln3–11A were determined in G1 daughter cells with 50–60 μm3 of volume. Individual data (N = 90) and median values are plotted. (E) Cell extracts (input) and GST PDs of cdc4ts grr1 cells expressing Cln3–13myc and GST fusions to Cdc4ΔFbox, Mad3, or Mad3ΔGLEBS were analyzed by immunoblotting with either αmyc (top panels) or αGST (bottom panel) antibodies. (F) Cell extracts (input) and GST PDs of cells expressing Mad3–3HA or Mad3 ΔGLEBS–3HA and either GST or GST–Cdc4 were analyzed by immunoblotting with either αHA (top panels) or αGST (bottom panel) antibodies. (G) Cells with the indicated genotypes carrying three YCp vectors were analyzed as in Fig 1B to determine cell size at budding as a function of copy number. Individual budding volumes (small dots) were binned, and mean values (large circles, N = 50) and a regression line are plotted. Correlation analysis and pairwise comparisons were performed with nonparametric tests as described in Materials and methods. Underlying data can be found in S1 Data. GST, glutathione S-transferase; PD, pulldown; YCp, yeast centromeric plasmid.

Signaling through G proteins

Cell Biology

GROUP LEADER
Anna Aragay

PhD STUDENTS
Ismael Izquierdo
Helena Delgado
Matías Soto

The aim of this subline is to understand the process of signal transduction that let to changes in cell structure and function in physiology and diseases. We focus our attention on G proteins that are molecular switches that signal through their receptors, GPCRs, at the plasma membrane. However, a novel localization of G proteins at the mitochondria and other endomembranes where they regulate the physiology of these organelles has been recently demonstrated by our group. We focus our attention in understanding how the G proteins control the proper balance between mitochondria fusion and fission, their dynamic movement and the mitochondrial physiology. Mitochondria are dynamic organelles that produce most of the energy of the cell. Broken mitochondria cristae, and loss of internal structure impairs many of the functions of mitochondria and its present in neurons of several neurodegenerative diseases.

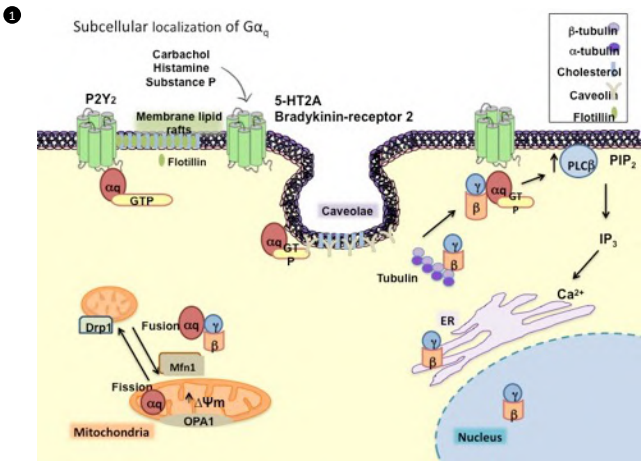
In order to unveil the putative effectors of G proteins that mediate those effects at the mitochondria, our group has undertaken a mass-spectrometry analysis based on G protein-immunoprecipitates from cellular endomembranes using different cell lines: “mito-intereactome”. In addition, our projects relay heavily on the use of “state of the art” imaging technologies that helps us to answer crucial questions on mitochondria dynamics and neuronal physiology.

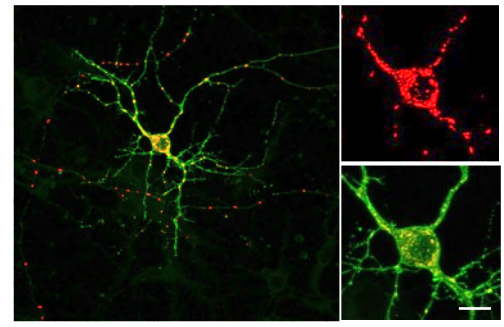
Research lines:

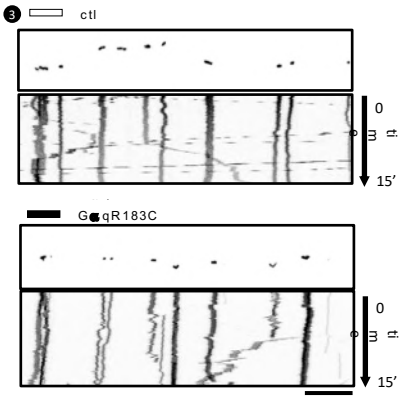
1. To study the dynamics of the mitochondria in hypocampal neurons.
2. To investigate if G proteins regulate mitophagy.

PUBLICATIONS

Pons M, Izquierdo I, Andreu-Carbó M, Garrido G, Planagumà J, Muriel O, Del Pozo MA, Geli MI, **Aragay AM**. Phosphorylation of filamin A regulates chemokine receptor CCR2 recycling. J Cell Sci. 2017 Jan 15;130(2):490-501.







1. Subcellular localization of Gαq
2. Mitocondria in a hippocampal neurons expressing GFP-GαqR183C.
3. Kymographs showing movement of mitocondria through the axons over time.

Regulation of the proteasome

Cell Biology

GROUP LEADER
Bernat Crosas

POSTDOCTORAL RESEARCHERS
Alice Zuin

PhD STUDENTS
Bernat Coll

The goal of the lab is to examine novel levels of regulation of the proteasome pathway focusing on the mechanisms that control proteasome function and its interaction with protein substrates. Our ongoing studies pursue basic and translational research areas. Our objective is to characterize the regulatory interactions that control the process of protein degradation in the proteasome. We also aim to identify novel ubiquitin pathway substrates by means of an optimized biochemical fractionation of ubiquitin conjugates. The development of these projects will uncover mechanisms of action of the proteasome, which will be integrated in the comprehension of the role of the proteasome in cell physiology and molecular pathology. One of our priorities will be to reveal novel targets in the proteasome pathway for the design of drug therapies. The use of S. cerevisiae, the reference model in the ubiquitin-proteasome system, will allow us to address simultaneously biochemical, genetic, molecular and cell biology approaches in feasible time frames.

Research Lines

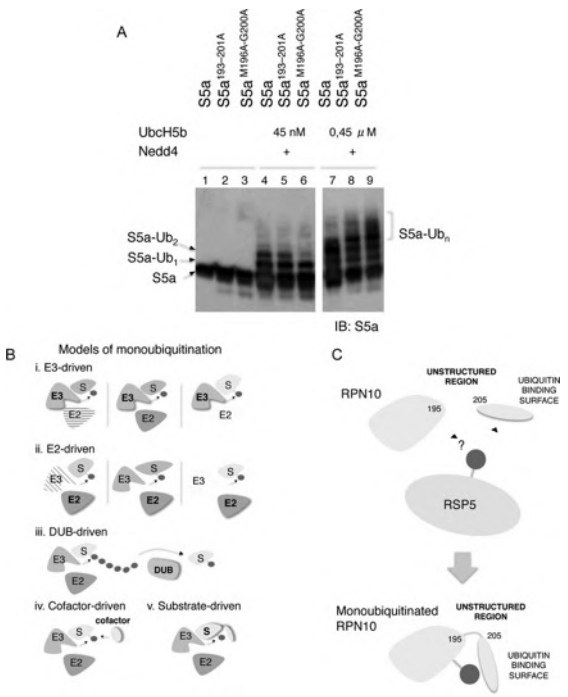
1.To study the proteasome mechanism, focusing on the role of the regulatory particle.

We are interested in understanding how the proteasome processes substrates delivered from multiple cellular pathways, in terms of selectivity, priority and homeostatic feedback. The regulatory particle of the proteasome is a functional network of interacting subunits and associated factors which define several levels of control of the proteolytic pathway. Our aim is to uncover this network.

2. To define the physiological scope of the ubiquitin-proteasome system.

We are interested in characterizing novel substrates of the system. Inversely, we are also interested in developing a method of conditional degradation by ubiquitin independent approaches, in order to create artificial substrates of the proteasome.

1



1 Mutations of the unstructured region before the UIM1 of S5a cause a change in the process of ubiquitination
(A) Ubiquitination reaction of S5a, S5a193–201A and S5aM196A,G200A carried out for 3 h at 37°C. The indicated amounts of UbcH5b were used. IB, immunoblot. (B) (A) E3-driven model: The ubiquitin ligase determines the specificity. Different E2 enzymes may be involved, with no influence on the product. (B) E2-driven model: The ubiquitin-conjugating enzyme determines the specificity. The E2 binds distinct cognate E3s to promote monoubiquitination to different substrates. (C) DUB-driven model: a deubiquitinating activity trims the polyubiquitin chain of a polyubiquitinated protein producing a monoubiquitinated one. (D) Cofactor-driven model: a cofactor promotes monoubiquitination of the substrate by preventing the polyubiquitination. (E) Substrate-driven model: structural properties of the substrate prevent it to be polyubiquitinated. (C) Model representing the involvement of the characterized linker between the VWA domain and the UIM in Rsp5-dependent Rpn10 monoubiquitination and showing a putative role for the flexibility of the linker in a fold-back disposition.

Control of local mRNA expression

Cell Biology

The connections in our brains are constantly changing. As we interact with our environment and with each other, the connections between our neurons are remodeled so that we can retain these interactions as learning and memory. At a molecular level, the brain accomplishes this remodeling in part by making new proteins at the specific sites where our neurons interact with each other (known as synapses). The development of new synaptic connections and the pruning away of old ones through the process of protein synthesis is the molecular basis for learning, memory, and behavior. The process of synaptic remodeling, of strengthening and weakening of connections between neurons, is known as synaptic plasticity. Our long-standing goal is the identification of molecular components of neuronal signaling cascades and the determination of their role in synaptic plasticity.

Our specifics aims are:

1. To understand the role of KIS kinase in neuronal plasticity. KIS is a protein kinase that associates with stathmin, a modulator of the tubulin cytoskeleton. KIS is found in RNA granules and stimulates translation of AMPA receptor subunits and PSD-95. Furthermore, KIS enhances β-actin polymerization in dendritic spines accordingly the absence of KIS produces morphological defects in synaptic spines. All these data suggest that KIS is a particularly attractive protein for the study of dendritic plasticity. Search for the synaptic inputs that activate KIS and identify endogenous targets will allow us to better understand the molecular mechanisms of synaptic plasticity.
2. To study the functional relevance of eEF1A2 regulation in synaptic plasticity. Synthesis of many synaptic proteins is under local control, and recent evidence suggests that modulation of the elongation steps of translation may be the key regulatory process underlying synaptic plasticity. Notably, the essential elongation factor eEF1A that binds the aminoacyl-tRNA to drive the first steps of translation elongation displays two very similar forms in vertebrates, eEF1A1 and eEF1A2. While the first is expressed throughout life in almost all tissues, the second form is specific to brain and skeletal muscle, pointing to specific roles of eEF1A2 in tissues where cellular plasticity is most relevant. The recent discovery that missense de novo mutations in eEF1A2 have causal effects in autism and epilepsy underline the relevance of this elongation factor in cognitive functions.
3. To analyze the presence of intron-containing mRNAs in dendrites. Several recent studies have consolidated the notion that many introns are actively retained in mature mRNAs. Intron retention (IR) has recently been revealed as an independent mechanism of controlling and enhancing the complexity of gene expression. IR facilitates rapid responses to biological stimuli, is involved in disease pathogenesis, and can generate novel protein isoforms. On the other hand, our findings suggest that intron retention could have key roles in the assembly and stabilization of an mRNA subpopulation to fine-tune CaMKIIα activity at synapses.

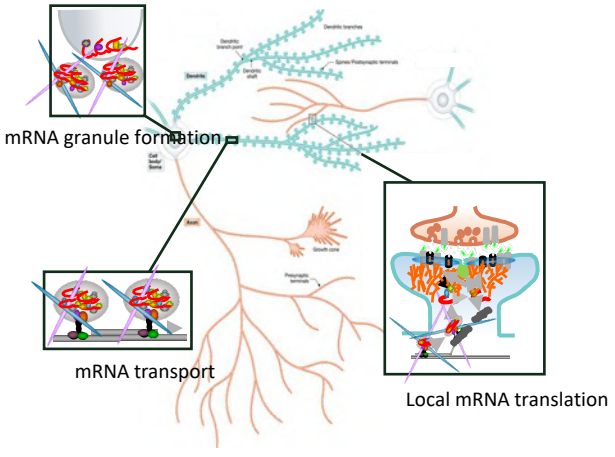
GROUP LEADER
Carme Gallego

PhD STUDENTS
Mònica Mendoza
Marcos Moreno

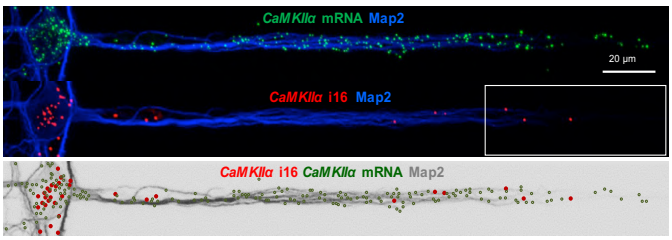
PUBLICATIONS

Ortiz R, Georgieva MV, Gutierrez S, Pedraza N, Fernandez-Moya SM, **Gallego C**. Recruitment of Staufen2 enhances dendritic localization of an intron-containing CaMKIIα mRNA. Cell Reports 20:13-20 (2017).

Asensio-Juan E, Fueyo R, Pappa S, Iacobucci S, Badosa C, Lois S, Balada M, Bosch-Prsegue L, Vaquero A, Gutierrez S, Caelles C, **Gallego C**, De La Cruz X, Martinez-Balbas MA. (2017) The histone demethylase PHF8 is a molecular safeguard of the IFNγ response. Nucleic Acids Research 45:3800-3811.



1 Molecular mechanisms involved in synaptic plasticity: mRNA granule formation, mRNA transport and local mRNA translation.



2 Hippocampal cells were seeded in microfluidic chambers in order to isolate dendrites from cell bodies. At 14 DIV, chambers were removed from coverslips and FISH was performed using custom ViewRNA probes against intron i16 (red) or exonic (green) CaMKIIa sequences. Dendrites were decorated with an αMap2 antibody (blue). The bottom panel shows identified foci in the red and green channels with the aid of FociJ software. Image shows the first 220 mm of the microchannel as well as part of the main channel where somas and nuclei can be observed.

The endocytic pathway and the actin cytoskeleton

Cell Biology

Our group is interested in understanding the molecular mechanisms supporting membrane traffic within the endocytic pathway, which can selectively extract material from the cell surface and either bring it to the degradative compartments (the lysosome in mammals and the vacuole in yeast and plants) or relocate it to other subcellular organelles or plasma membrane subdomains. Thereby, endocytosis not only supports nutrient uptake, but also plays essential functions in the spatiotemporal control of signaling, in metabolic reprogramming or cellular morphogenesis, and its miss-function is at the etiology of human pathologies such as cancer or neurodegeneration. To understand the molecular mechanisms of endocytic traffic and its physiological functions, we use the yeast S. cerevisiae and different mammalian cultured cell lines. In S. cerevisiae, we apply advanced fluorescence and electron microscopy techniques to decipher the conserved mechanisms involved in membrane deformation, with a particular focus in those involving assembly of actin supra-structures. In this context, we have uncovered an essential role in endocytosis of the yeast VAPs (VAMP-associated Proteins) and ORPs (OSBP-Related Proteins), whose miss-function in humans leads to ALS (Amyotrophic Lateral Sclerosis) (Encinar del Dedo (2017) Dev Cell). These proteins form transient endoplasmic reticulum-endocytic contact sites (ERECS), which are needed to initiate actin polymerization and membrane invagination. In mammalian cells, we focus on human genes not present in lower eukaryotes, which adapt the evolutionary conserved hard-core machinery to the complex physiological functions that endocytosis plays in multicellular organisms (Schmelzl (2002) EMBO Rep). Within this research line, we study the role of Kazrin C, a protein that might link clathrin to the actin and tubulin cytoskeletons, in fibroblast migration and invasion.

GROUP LEADER
Mª Isabel Geli

LABORATORY MANAGER
Isabel M. Fernández

POSTDOCTORAL RESEARCHER
Javier Encinar

PhD STUDENTS
Inés Hernández

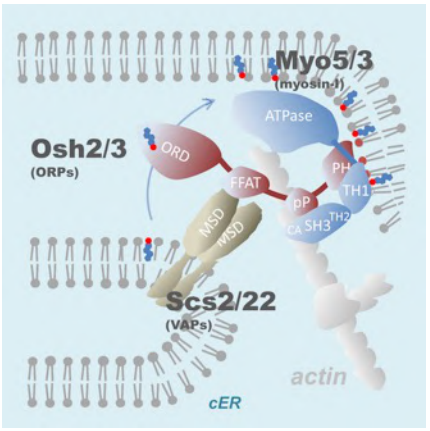
MASTER STUDENT
Laura Pastor

PUBLICATIONS

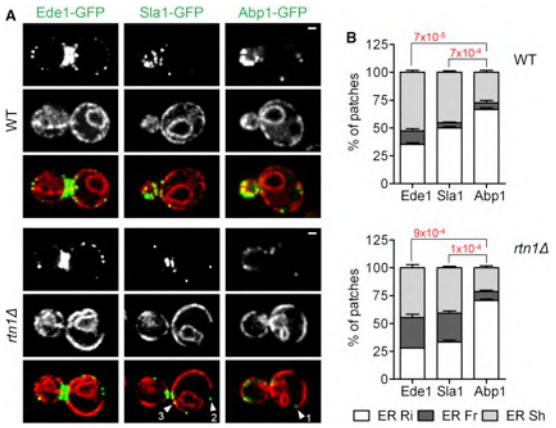
Encinar Del Dedo J, Idrissi FZ, Fernandez-Golbano IM, Garcia P, Rebollo E, Krzyzanowski MK, Grötsch H, **Geli MI**. ORP-Mediated ER Contact with Endocytic Sites Facilitates Actin Polymerization. Dev Cell. 2017 Dec 4;43(5):588-602.e6.

Pons M, Izquierdo I, Andreu-Carbó M, Garrido G, Planagumà J, Muriel O, Del Pozo MA, **Geli MI**, Aragay AM. Phosphorylation of filamin A regulates chemokine receptor CCR2 recycling. J Cell Sci. 2017 Jan 15;130(2):490-501.

❶



❷



❶ ORP-Mediated ER Contact with Endocytic Sites Facilitates Actin Polymerization

❷ cER Contact with Endocytic Sites Occurs at the Onset of Actin Polymerization (A) Representative confocal fluorescence micrographs of WT or rtn1Δ cells expressing Sec61-mCherry and either Ede1-GFP, Sla1-GFP, or Abp1-GFP. The individual channels and the merge are shown. In the merge, Sec61-mCherry is red and GFP fusion is green. Arrowheads indicate endocytic patches: 1, associated with a cER rim; 2, assembled at a cER-free region; 3, assembled at a cER-sheltered region. Scale bars, 1 µm. (B) Average ± SEM frequency of Ede1-GFP, Sla1-GFP, or Abp1-GFP patches associated with either cER-free (Fr) or -sheltered (Sh) regions, or overlapping with cER rims (Ri). Student's t test p values are indicated for the cER rim association. Significant differences are in red. At least 150 patches in at least 15 cells were analyzed per 3 experiments.

Neural proliferation control

Cell Biology

Our research focusses on the mechanisms that control the proliferation and differentiation processes that take place during the nervous system development and the consequences of their malfunction. In particular, we are interested in molecular alterations that induce aberrant growth and predispose to malignancy. Our results are important not only to gather knowledge on the steps that led to neoplastic transformation but also to find new strategies to fight it.

The group has strong expertise in molecular biology, cell biology and biochemistry techniques that we use to study the effect of oncogenic mutations at molecular cellular and whole organism level.

At the present, we are working on three main subjects where our contributions have been:

1. Stablish the relevance of N-Cdherin/b-Catenin complex, as a master builder of the apical polarity in neural stem cells and its relevance on primary neuro ectodermal tumours (PNETs).
2. Expose the importance of PI3K as the main link between protein and lipid signaling during nervous system development and the catastrophic consequences of its malfunction.
3. Demonstrate the importance of Wnt independent functions of b-Catenin during the neurogenic process.

GROUP LEADER
Sebastian Pons

RESEARCH ASSISTANT
Anghara Menéndez

POSTDOCTORAL RESEARCHERS
Antonio Herrera

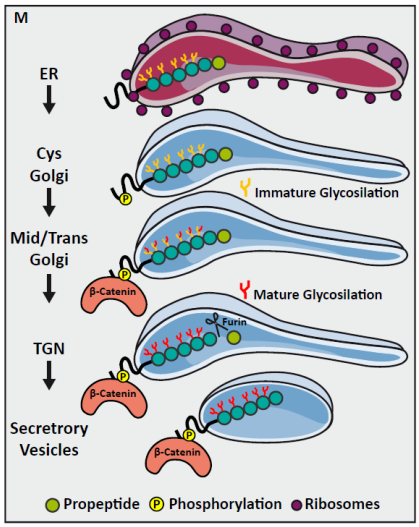
PhD STUDENTS
Andrea Ochoa

PUBLICATIONS

Le Dreau G, Escalona R, Fueyo R, Herrera A, Martinez JD, Usieto S, Menendez A, **Pons S**, Martinez-Balbas MA, Marti E (2018) E proteins sharpen neurogenesis by modulating proneural bHLH transcription factors' activity in an E-box-dependent manner. eLife 2018; 7

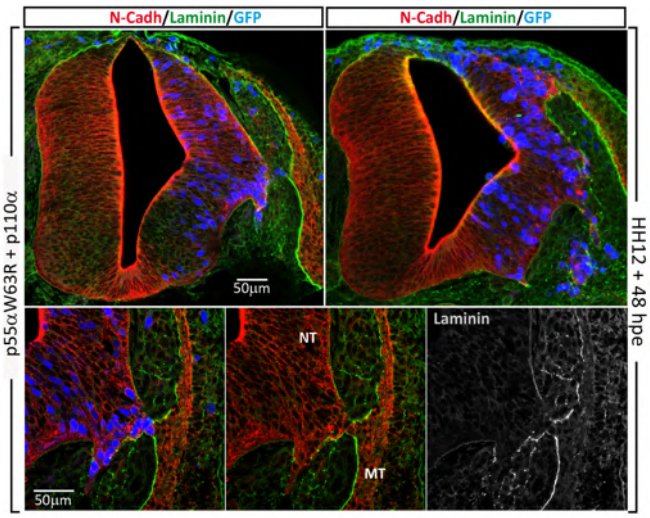
Torroba B, Herrera A, Menendez A and **Pons S**. PI3K regulates intraepithelial cell positioning through Rho GTP-ases in the developing neural tube. Developmental Biology 2018; 436:42-54.

❶



❶ Pro-N-Cadherin needs to interact with β-Catenin to excise the propeptide and complete its maturation process. Model displaying the sequence of events occurring in the course of N-Cadherin maturation, ER: endoplasmic reticulum, TGN: trans Golgi network.

❷



❷ Oncogenic mutations of PI3K causes unrestraint basal migration and mesenchyme invasion. HH12 neural tubes were transfected for 48h with WT p110α plus p55αW63R (active mutant), N-Cadherin labels the neural tube and the myotome, Laminin de basal membrane and GFP denotes the transfected cells. The lower panels show higher magnification images of transfected cells over- migrating towards the basal side at the moment of breaching the basal membrane invading the mesenchyme and fussing with the myotome. (NT) Neural Tube, (MT) Myotome.

Cell cycle and signaling

Cell Biology

We are interested in studying how the different processes that allow cells to physically divide are controlled in time and space. We focus on protein phosphorylation and the important role that different protein kinases have reorganizing cellular components during mitosis, in order to correctly build the mitotic spindle and segregate chromosomes into the daughter cells.

We work with members of the NIMA, CDK, Polo and Aurora family of protein kinases and investigate their regulation and interconnections, as well as how their substrates respond to phosphorylation in the context of the centrosome cycle and the organization of the mitotic spindle. We pay special attention to how the deregulation of any of these processes can lead to different pathologies, including the apparition of cancer or different developmental defects.

Our major past scientific contributions are:

- The description of the activation mechanism of the signaling module composed by the NIMA-family kinases Nek9, Nek6 and Nek7.
- The identification of these kinases as major regulators of centrosome maturation and separation, downstream of Plk1 and CDK1.
- The molecular dissection of the mechanism that regulates the mitotic motor Eg5/KIF11 during centrosome separation in early mitosis.

Active lines of research are:

1. The identification of additional functions of Nek9, Nek6 and Nek7 during the centrosome cycle.
2. The study of the importance of Nek9, Nek6 and Nek7 for the normal development and hemostasis of different tissues in model animals.
3. The importance of phosphorylation for the regulation of different molecular motors during G2 and mitosis.

GROUP LEADER
Joan Roig

PhD STUDENTS
Núria Gallisà

MASTER'S STUDENT
Celia Lerma

UNDERGRADUATE STUDENT
Meritxell Serra

PUBLICATIONS

Eisa NH, Jilani Y, Kainth K, Redd P, Lu S, Bougrine O, Abdul Sater H, Patwardhan CA, Shull A, Shi H, Liu K, ElSherbiny NM, Eissa LA, El-Shishtawy MM, Horuzsko A, Bollag R, Maihle N, **Roig J**, Korkaya H, Cowell JK, et al (2019) The co-chaperone UNC45A is essential for the expression of mitotic kinase NEK7 and tumorigenesis. J. Biol. Chem. In press (pii: jbc.RA118.006597. doi: 10.1074/jbc.RA118.006597)

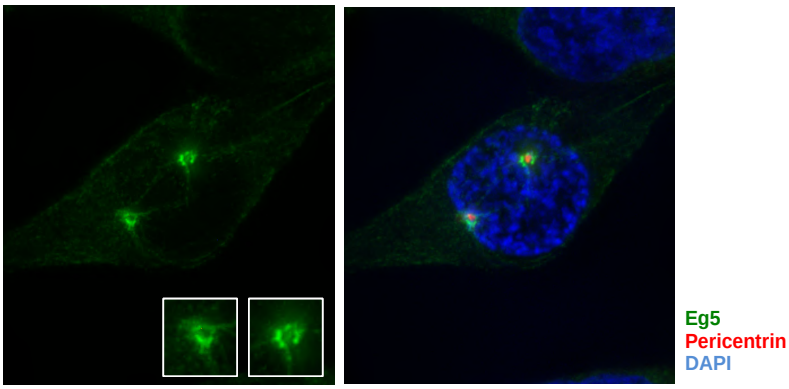
Freixo F, Martinez Delgado P, Manso Y, Sánchez-Huertas C, Lacasa C, Soriano E, **Roig J** & Lüders J (2018) NEK7 regulates dendrite morphogenesis in neurons via Eg5-dependent microtubule stabilization. Nat Commun 9: 2330

Eibes S, Gallisà-Suñé N, Rosas-Salvans M, Martínez-Delgado P, Vernos I & **Roig J** (2018) Nek9 Phosphorylation Defines a New Role for TPX2 in Eg5-Dependent Centrosome Separation before Nuclear Envelope Breakdown. Curr. Biol. 28: 121-129.e4

Cota RR, Teixidó-Travesa N, Ezquerra A, Eibes S, Lacasa C, **Roig J** & Lüders J (2017) MZT1 regulates microtubule nucleation by linking γTuRC assembly to adapter-mediated targeting and activation. J. Cell. Sci. 130: 406–419

Fry AM, Bayliss R & **Roig J** (2017) Mitotic Regulation by NEK Kinase Networks. Front Cell Dev Biol 5: 102

1



1 The accumulation of the molecular motor Eg5 (green) around centrosomes (red) in prophase, a process that is key for centrosome separation during early mitosis and normal chromosome (blue) segregation, and we have shown is controlled through phosphorylation by the NIMA kinases Nek9 and Nek6/7 (Image, Susana Eibes; see Eibes et al. Curr. Biol. 28: 121-129.e4).

Cell signalling, ubiquitin and cancer

Cell Biology

We work on three principal research lines:

1. Study of mechanisms of neoplasia and malignant progression
Discovery of new genes and pathways relevant in malignancy and in the development of metastasis, useful as markers useful in diagnosis and prognosis and the identification of novel pathways for the development of effective new therapeutic schemes to tackle the problem of metastatic spread of cancer.
2. Study of the role of the ubiquitin-proteasome system in relevant cellular processesRole of non-canonical ubiquitylation (through K63 of ubiquitin), mediated by the ubiquitin-conjugating enzyme Ubc13-Uev, and role of the ubiquitin ligase RNF8 in checkpoint control and in the establishment of the DNA damage response.
3. Drug design and development
Discovery of novel antagonists of protein-protein interaction with relevance in the regulation of the ubiquitin-proteasome system. After in vitro and in vivo validation in cancer models, our aim is to take compounds with effective anti-tumor activity to preclinical and, eventuall, clinical phases.

GROUP LEADER
Timothy Thomson

TECHNICIAN
Mònica Pons

PUBLICATIONS

Thomson TM, Fernández PL. Epithelial plasticity in cancer: beyond metastasis. Aging (Albany NY). 2018 Jan 22;10(1):3-4.

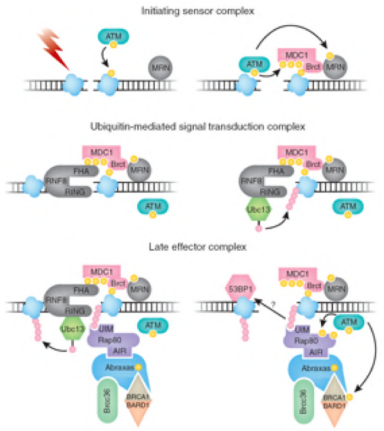
Marín de Mas I, Aguilar E, Zodda E, Balcells C, Marin S, Dallmann G, **Thomson TM**, Papp B, Cascante M. Model-driven discovery of long-chain fatty acid metabolic reprogramming in heterogeneous prostate cancer cells. PLoS Comput Biol. 2018 Jan 2;14(1):e1005914.

Sánchez-Cid L, Pons M, Lozano JJ, Rubio N, Guerra-Rebollo M, Soriano A, Paris-Coderch L, Segura MF, Fueyo R, Arguimbau J, Zodda E, Bermudo R, Alonso I, Caparrós X, Cascante M, Rafii A, Kang Y, Martínez-Balbás M, Weiss SJ, Blanco J, Muñoz M, Fernández PL, **Thomson TM**. MicroRNA-200, associated with metastatic breast cancer, promotes traits of mammary luminal progenitor cells. Oncotarget. 2017 Sep 7;8(48):83384-83406.

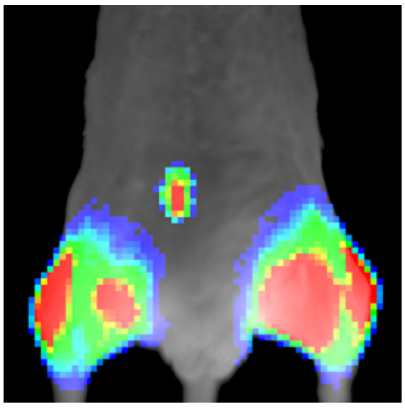
Tarrado-Castellarnau M, de Atauri P, Tarragó-Celada J, Perarnau J, Yuneva M, **Thomson TM**, Cascante M. De novo MYC addiction as an adaptive response of cancer cells to CDK4/6 inhibition. Mol Syst Biol. 2017 Oct 4;13(10):940.

Campbell CT, Haladyna JN, Drubin DA, **Thomson TM**, Maria MJ, Yamauchi T, Waters NJ, Olhava EJ, Pollock RM, Smith JJ, Copeland RA, Blakemore SJ, Bernt KM, Daigle SR. Mechanisms of Pinometostat (EPZ-5676) Treatment-Emergent Resistance in MLL-Rearranged Leukemia. Mol Cancer Ther. 2017 Aug;16(8):1669-1679.

❶

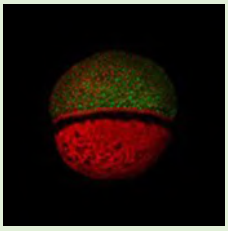


❷



❶ Orchestration by RNF8 of DNA damage recognition-repair and checkpoint control

❷ ?????



Developmental biology

The Department of Developmental Biology (DDB) investigates the organization of cells in time and space to form multicellular organisms. In order to gain comprehensive insights into these processes, all research groups in the Department, although each team focused on specific topics, employ an extensive range of experimental approaches, from gene expression analysis to the generation of shape and tackle a multiplicity of biological processes relevant to distinct phases of organismal development, including mechanisms of regulation of gene expression, cell proliferation and differentiation, cell communication, cell rearrangements, regulation of the cytoskeleton or cell migration. For this, researchers in the DDB use a variety of well-established model systems, from the fruitfly to vertebrates such as zebra fish, chick and mouse. From a methodological perspective, we combine molecular biology, classical genetics, cell biology, advanced microscopy, and genomic and proteomic approaches.

HEAD OF DEPARTMENT

Marta Llimargas

RESEARCH GROUPS

1. Proliferation and differentiation of the nervous System. Arbones, Mariona
2. Cell signalling and morphogenesis. Casanova, Jordi
3. Gene expression and signaling. Jiménez, Gerardo
4. Mechanisms of morphogenesis and organogènesis. Llimargas, Marta
5. Development of spinal cord in health and disease. Martí, Elisa
6. Signalling events controlling cell migration during morphogenesis. Martín-Blanco, Enrique

Proliferation and differentiation of the nervous system

Developmental Biology

Our laboratory studies mechanisms that control cell numbers and generate cell diversity in the central nervous system. We are particularly interested in the neocortex, which is the brain region responsible for cognitive function, sensory perception and consciousness. During development the many type of neurons that form this layered structure are generated from neural stem cells or from progenitors with a more restricted fate, in a space and time regulated manner. Disturbances in the proliferation rates and type of division of these progenitors (proliferative or differenciative) may lead to brain size defects and are on the basis of common disorders such as autism and schizophrenia. Our experimental approach includes morphological and functional studies in mouse models of human diseases, as well as proteomics and high-throughput sequencing techniques.

Research lines

1. DYRK1A-regulated activities in neocortical development.

2. Temporal regulation of gene expression in progenitors during neurogenesis.

GROUP LEADER
Mariona Arbonés

POSTDOCTORAL RESEARCHERS
María José Barallobre

PhD STUDENTS
Isabel Pijuan
Alejandro Trujillano

MASTER'S STUDENT
Laia Caudet

PUBLICATIONS

Luna J, Boni J, Cuatrecasas M, Bofill-De Ros X, Núñez-Manchón E, Gironella M, Vaquero EC, Arbones ML, de la Luna S, Fillat C. DYRK1A modulates c-MET in pancreatic ductal adenocarcinoma to drive tumour growth. Gut. 2018 Oct 20.

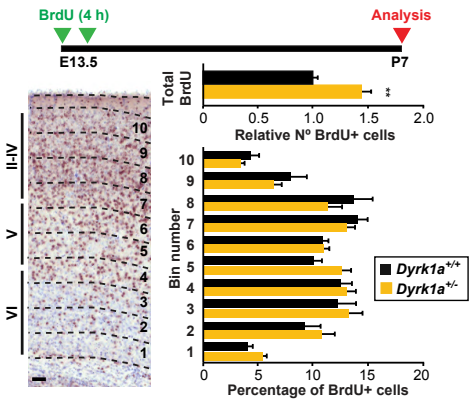
Arbones ML, Thomazeau A, Nakano-Kobayashi A, Hagiwara M, Delabar JM. DYRK1A and cognition: A lifelong relationship. Pharmacol Ther. 2018 Sep 28.

Latour A, Gu Y, Kassis N, Daubigney F, Colin C, Gausserès B, Middendorp S, Paul JL, Hindié V, Rain JC, Delabar JM, Yu E, Arbones M, Mallat M, Janel N. LPS-induced inflammation abolishes the effect of DYRK1A on IkB stability in the brain of mice. Mol Neurobiol. 2018 May 30.

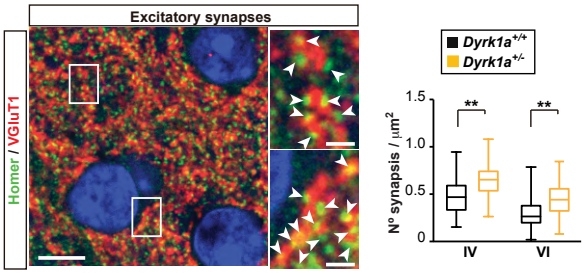
Rozen EJ, Roewenstrunk J, Barallobre MJ, Di Vona C, Jung C, Figueiredo AF, Luna J, Fillat C, Arbonés ML, Graupera M, Valverde MA, de la Luna S. DYRK1A kinase positively regulates angiogenic responses in endothelial cells. Cell Rep. 2018 May 8;23(6):1867-1878.

Janel N, Alexopoulos P, Badel A, Lamari F, Camproux AC, Lagarde J, Simon S, Feraudet-Tarisse C, Lamourette P, Arbones M, Paul JL, Dubois B, Potier MC, Sarazin M, Delabar JM. Combined assessment of DYRK1A, BDNF and homocysteine levels as diagnostic marker for Alzheimer's disease. Transl Psychiatry. 2017 Jun 20;7(6):e1154.

❶



❷



❶ DYRK1A haploinsufficiency increases cortical neurogenesis and alters synaptic connectivity. BrdU birthdating experiments showing an augmented production of cortical excitatory neurons in Dyrk1a+/- embryos.

❷ DYRK1A haploinsufficiency increases cortical neurogenesis and alters synaptic connectivity. Immunofluoresce against presynaptic and postsynaptic markers (white arrows points to interactions) and excitatory synapse numbers in the external and internal neocortical layers of Dyrk1a+/+ and Dyrk1a+/- mice.

Cell signalling and morphogenesis

Developmental Biology

The work of many laboratories has allowed beginning to understand the genetic logic behind development. However, while genes operate in cells and cells are the constituent units that contribute to morphogenesis of organs and of the whole organism, we are still trying to understand how the genetic information that impinges on cell behaviour allows for changes in individual cells that sum up to generate organs. To address this issue, studies need to be done in vivo and in the context of the whole organism. Thus, we approach this issue by combining the study of three Drosophila morphogenesis models, namely, the embryonic tracheal and gut morphogenesis and the embryonic terminal patterning system. In particular, we focus our study in the role of progenitor cells and epithelial plasticity in these morphogenetic events, with a specital attention on the interaction between different germ layers.

Research Lines

1. Spatial and temporal triggers taking tracheal adult precursors from quiescence into proliferation
2. Cytoskeleton changes lying behind the transition of tracheal cells from a proliferative to a migratory program.
3. Triggers for migration initiation and arrest in the gut morphogenesis, with a particular attention to the interaction between the gut (endoderm) and its surrounding tissue (mesoderm).
4. Interaction between the egg chamber and the early embryo, with special attention on the mechanisms that dictate Torso receptor tyrosine kinase activation.

GROUP LEADER
Jordi Casanova

POSTDOCTORAL RESEARCHERS
Panos Giannios
Juan José Fraire

PhD STUDENTS
Ana Elena Gaspar
Beatriz Pino
Sandra Casani

TECHNICIAN
Nicolás Martín
Esther Fuentes
Natalia Plana

PUBLICATIONS

Mineo, A., Furriols, M. and **Casanova, J.** (2017). Transfer of dorsoventral and terminal information from the ovary to the embryo by a common group of eggshell proteins in Drosophila. Genetics 205, 1529–1536.

Hernández de Madrid, B. and **Casanova, J**. (2018) GATA factor genes in the Drosophila midgut embryo. PLoS ONE 13(3):e0193612

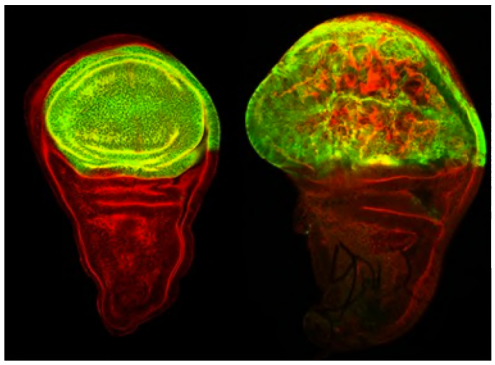
Campbell, K., Lebreton, G., Franch-Marro, X. and **Casanova, J**. (2018). Differential roles of the Drosophila EMT-inducing transcription factors Snail and Serpent in driving primary tumour growth. PLOS Genetics 14(2): e1007167.

Casanova, J. (2018). Biology, a science of grays. Embo Reports DOI: 10.15252/embr.201846332

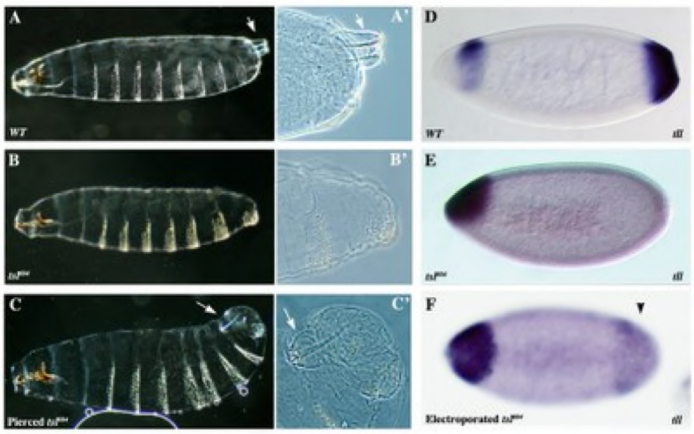
Mineo, A., Fuentes, E., Furriols, M. and **Casanova, J.** (2018). Holes in the Plasma Membrane Mimic Torso-Like Perforin in Torso Tyrosine Kinase Receptor Activation in the Drosophila Embryo. Genetics 210, 257–262.

Öztürk-Çolak,A., Stephan-Otto Attolini, C., **Casanova, J***. and Araújo, S.* (2018). Blimp-1 Mediates Tracheal Lumen Maturation in Drosophila melanogaster. Genetics 210, 653-663. (* correspondig autors)

❶



❷



❶ Both Srp and Sna drive an EMT in wing disc cells

❷ Holes in the Plasma Membrane Mimic Torso-Like Perforin in Torso Tyrosine Kinase Receptor Activation in the Drosophila Embryo.

Gene expression and signaling

Developmental Biology

During animal development, the differentiation of cells, tissues and organs is tightly regulated through specific gene expression programs. Our group is particularly interested in studying the transcriptional and cell signaling mechanisms responsible for this control. Using Drosophila as a model system, we have been dissecting the activities of repressor and co-repressor factors, as well as the responses induced by receptor tyrosine kinase (RTK) pathways during pattern formation and differentiation. One emerging theme has been the identification of the Capicua (Cic) transcriptional repressor as a general sensor targeted by multiple RTK-initiated signals. Because the molecules and pathways that we study are conserved in evolution, our results have direct implications for human biology and disease.

Research lines

1. Cic is a nuclear HMG-box protein that functions downstream of RTK signaling in multiple developmental processes. In mammals, Cic has been directly implicated in neurodegeneration and tumorigenesis, where it behaves as a tumor and metastasis suppressor. We are studying Cic function from different perspectives, including the analysis of its mechanism of action, its interaction with RTK signaling and other signal transduction pathways, and the functional significance of its two conserved isoforms, Short and Long.
2. We are studying the mechanisms and pathways that regulate embryonic pattern formation. In Drosophila, this process is under elaborate maternal control, and we are dissecting different maternal systems that organize both the anteroposteior and dorsoventral embryonic axes.
3. We are characterizing the activities of different repressor and corepressor proteins such as Tailless, Atrophin and Groucho, which play important roles in cell fate decisions and are also involved in human pathologies.

GROUP LEADER
Gerardo Jiménez

SENIOR SCIENTIST
Sergio González

RESEARCH ASSISTANT
Núria Samper

PhD STUDENTS
Laura Rodríguez

MASTER'S STUDENT
Claudia Lagares

PUBLICATIONS

Papagianni A, Forés M, Shao W, He S, Koenecke N, Andreu MJ, Samper N, Paroush Z, González-Crespo S, Zeitlinger J, **Jiménez G** (2018) Capicua controls Toll/IL-1 signaling targets independently of RTK regulation. Proc Natl Acad Sci USA 115, 1807-1812.

Simón-Carrasco L, **Jiménez G**, Barbacid M, Drosten M. (2018) The Capicua tumor suppressor: a gatekeeper of Ras signaling in development and cancer. Cell Cycle 17, 702-711.

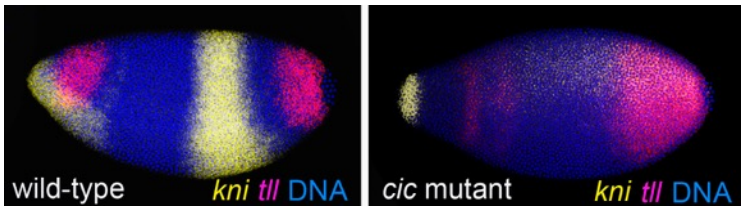
Simón-Carrasco, L., Graña, O., Salmón, M., Jacob, H. K. C., Gutierrez, A., **Jiménez, G.**, Drosten, M. and Barbacid, M. (2017) Inactivation of Capicua in adult mice causes T-cell lymphoblastic lymphoma. Genes & Dev 31, 1456-1468.

Forés, M., Simón-Carrasco, L., Ajuria, L., Samper, N., González-Crespo, S., Drosten, M., Barbacid, M. and **Jiménez, G.** (2017) A new mode of DNA binding distinguishes Capicua from other HMG-box factors and explains its mutation patterns in cancer. PLOS Genetics 11, e1006622.

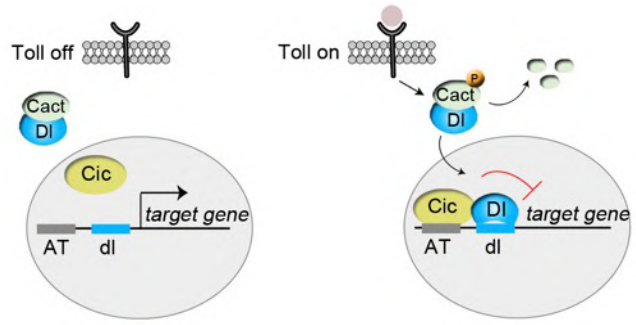
Jiménez, G. (ed) (2017) ERK Signaling: Methods and Protocols. Methods in Molecular Biology Series, Springer, New York (ISBN 978-1-4939-6424-6).

Forés, M., Papagianni, A., Rodríguez-Muñoz, L. and **Jiménez, G.** (2017) Using CRISPR-Cas9 to study ERK signaling in Drosophila. Methods Mol Biol 1487, 353-365.

❶



❷



❶ Expression of patterning genes in wild-type and cic mutant embryos. The cic mutation studied on the right panel impairs Cic DNA binding and is equivalent to human cic mutations that cause oligodendroglioma and other tumors.

❷ Capicua represses Toll/IL-1 signaling targets via suboptimal, low-affinity sites (designated 'AT') that it cannot recognize on its own (left). Instead, Capicua DNA binding requires the Dorsal/NF-kB protein, which enters the nucleus upon Toll activation and binds next to the Capicua sites (right).

Mechanisms of morphogenesis and organogenesis

Developmental Biology

Our lab is interested in understanding how organs and tissues form during development. To approach this issue we investigate the morphogenesis of epithelial tissues in Drosophila melanogaster, an excellent model system for animal development. We mainly focus on the formation of the tracheal (respiratory) system, which consists of a network of interconnected epithelial tubes that has become a paradigm for the analysis of tubulogenesis (morphogenesis of branched tubular structures). The aim in our lab is to analyse the cellular mechanisms that underlie epithelial/tracheal morphogenesis and to understand how these cellular mechanisms are genetically controlled at the molecular level. In addition we ask how these genetically controlled changes in morphology and behaviour at single cell resolution contribute to the formation of the tissue.

Our research focuses around 3 main questions: How do tissue remodelling takes place and how is it controlled? How is the epithelial structure maintained during remodelling and how epithelial features contribute to morphogenesis? How are the different morphogenetic events coordinated to give rise to a functional final structure? To advance in these objectives we use direct approaches (analysing candidate genes to affect these processes) or indirect approaches (through the characterisation of lines emerged from our previous genetic screens) and we combine approaches that lie at the interface between genetics, developmental biology and cell biology, with special attention to the use of advanced microscopy techniques.

GROUP LEADER
Marta Llimargas

RESEARCH ASSOCIATE
Annalisa Letizia

POSTDOCTORAL RESEARCHERS
Bastian J. Klussmann-Fricke

PhD STUDENTS
Ettore de Giorgio

MASTER'S STUDENT
Carlos Rojano

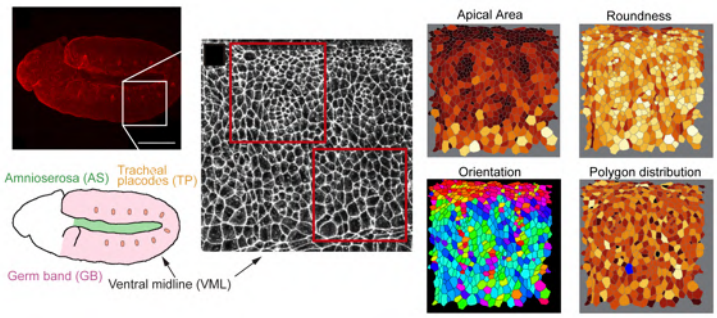
PUBLICATIONS

Olivares-Castiñeira I, **Llimargas M**. (2018) Anisotropic Crb accumulation, modulated by Src42A, is coupled to polarised epithelial tube growth in Drosophila. PLoS Genet. 2018 Nov 26;14(11):e1007824.

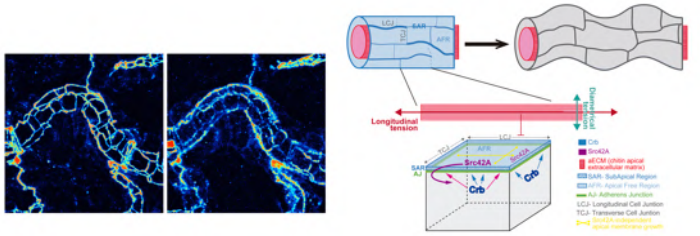
Letizia A, Tosi S, **Llimargas M**.(2018) Morphogenetic movements affect local tissue organisation during embryonic Drosophila morphogenesis. Eur J Cell Biol. 2018 Mar 15. pii: S0171-9335(17)30311-4. doi: 10.1016/j.ejcb.2018.03.004

Olivares-Castiñeira I, **Llimargas M**. (2017). EGFR controls Drosophila tracheal tube elongation by intracellular trafficking regulation. PLoS Genet. 2017 Jul 5;13(7):e1006882.

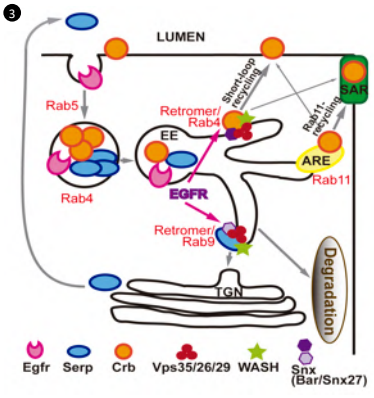
❶



❷



❸



❶ We investigated how tissue organisation is influenced by extrinsic forces derived from concomitant morphogenetic events. We used the epidermis of Drosophila embryos and by computational tissue segmentation we provided a quantitative description of tissue packing in control and experimental conditions

❷ Longitudinal growth of tracheal tubes depends on Crb, Src42A and the apical extracellular matrix. We reported that Crb becomes anisotropically accumulated in longitudinal junctions, and we hypothesised that this orients cell elongation, and as a consequence tube elongation. Src42A is required to promote Crb anisotropic distribution.

❸ We identified a new role for the conserved EGFR in regulating the length of the main tracheal branch in Drosophila by controlling the intracellular trafficking of the apical determinant Crb and the extracellular matrix regulator Serp. We also provided new insights into Crb trafficking and its apical subcellular accumulation.

Development of spinal cord in health and disease

Developmental Biology

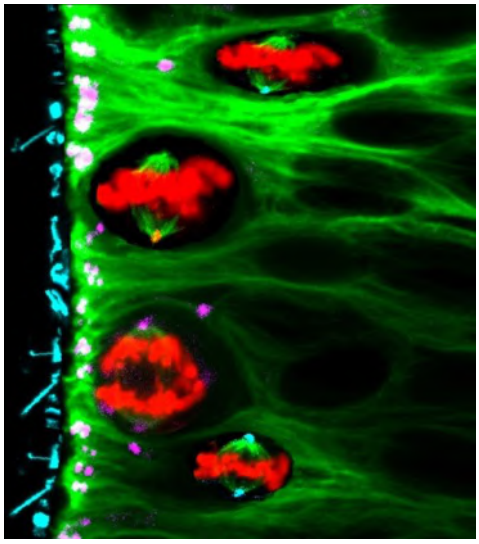
Research in our laboratory aims to understand the genetic networks and cellular events that generate the proper cell numbers and neuronal subtypes in the developing spinal cord. Our work holds the promise to uncover pathways relevant for various neurodevelopmental malformations, active research projects in the group include

1. Morphogenesis of the spinal cord and related neural tube defects, in which our aim is to study the generation of the secondary neural tube. Development of the posterior spinal cord involves the elongation and cavitation of the tail bud in a poorly understood process called secondary neurulation (SN). Importantly, faulty SN is the cause of closed spina bifida. We are combining live imaging and data from transcriptomics and functional genetics, to unveil the gene regulatory networks and tissue dynamics of secondary neural tube formation and to generate computational models with predictive capabilities.

2. Growth of the embryonic nervous system and associated microcephaly, to that aim our group developed molecular markers to trace neural progenitors on the basis of their mode of division, and validated them as powerfull tools to asses for normal growth of the embryonic central nervous system and the pathological condition associated to congenital primary microcephaly.

3. The dynamics of neural crest (NC) cells and associated Neuroblastomas (NB). NC cells, a multipotent stem cell-like population that originate from the dorsal spinal cord, differentiate into a wide range of cell types including the entire peripheral nervous system, and genetic lesions in these cells generate the paediatric tumour NB. We have identified a collection of NC specific genes which are common in high risk NB and we are currently investigating the function of these genes in normal and pathological NC development. This project is currently being financed by AECC.

❶



❶ Transient expression of H2B-RFP in the chick NT after electroporation (HH14, 16 hours post electroporation) reliably labels the chromosome plates in dividing neural progenitors. FOP (purple) formed pairs of dots at the spindle poles during mitosis that co-localize with anti-β-Tubulin (green). Acetylated tubulin (magenta) stain the cilium shaft. Saade, and cols Nature Cell Biology 19, 493–503 (2017)

GROUP LEADER
Elisa Martí

POSTDOCTORAL RESEARCHERS
Gwenveal Le Dreau
Murielle Saade

PhD STUDENTS
Lucia Fanlo
Elena Gonzalez-Gobartt
Jose Blanco

TECHNICIAN
Susana Usieto

PUBLICATIONS

Murielle Saade, Jose Blanco-Ameijeiras, Elena Gonzalez-Gobartt, and **Elisa Martí** (2018) A centrosomal view of CNS growth Development 145: dev170613.

Gwenvael Le Dréau, René Escalona, Raquel Fueyo, Antonio Herrera, Juan D Martínez, Susana Usieto, Anghara Menendez, Sebastián Pons, Marian A Martinez-Balbas, and **Elisa Marti** (2018). E proteins sharpen neurogenesis by modulating proneural bHLH transcription factors activity in an E-box-dependent manner. Elife. 2018 Aug 10;7. pii: e37267. doi: 10.7554/eLife.37267

Kim Dale and Elisa Martí (2017) Introduction to the special section of YDBIO on: Spinal cord a model to understand CNS development and regeneration. Dev Biol. 2017 Dec 1;432(1):1-2.

Demian Burguera , Yamile Marquez , Claudia Racioppi , Jon Permanyer , Antonio Torres, Rosaria Esposito, Beatriz Albuixech , Lucía Fanlo , Ylenia d'Agostino , Enrique Navas-Perez , Ana RiesgY, Claudia Cuomo , Giovanna Benvenuto, Lionel A. Christiaen, Elisa Martí, Salvatore D'Aniello, Antonietta Spagnuolo, Filomena Ristoratore, Maria Ina Arnone, Jordi Garcia-Fernàndez, Manuel Irimia (2017) Evolutionary recruitment of flexible Esrp-dependent splicing programs into diverse embryonic morphogenetic processes. Nat Commun. 2017 Nov 27;8(1):1799.

Murielle Saade, Elena Gonzalez-Gobartt, Rene Escalona, Susana Usieto and Elisa Martí (2017) Shh-mediated centrosomal recruitment of PKA promotes symmetric proliferative neuroepithelial cell division. Nature Cell Biology 19, 493–503.

Signalling events controlling cell migration during morphogenesis

Developmental Biology

Cell migration is a universal process involving distinct mechanisms and morphologies in different cell types and tissue environments. During development, cell migration is detected from the earliest stages becoming responsible for most morphogenetic processes. In healing and regeneration, cells migrate to close the wound following the same behavioural patterns than during morphogenesis.

Important progress has been achieved at the molecular and cellular level in determining the guidance and locomotion mechanisms of individual migratory cells. However, an equivalent analysis for collective cell movements is missing. The difficulties to combine genetic analysis and direct observation of moving clusters or sheets has prevented to obtain detailed information on the regulatory networks coordinating the distinct actions of cells within the group in movement. Further, the isolation of moving cells in clusters to undergo genomic studies has proven to be extremely difficult. Finally, the unavailability of methods to follow in vivo and at real time the activation or inhibition of signalling events; or the lack of quantitative measurement algorithms allowing to statistically discriminate in time and space specific parameters of the behaviour of cells in movement, has strongly precluded further progress in understanding these fundamental events.

Starting from the model systems of dorsal closure, imaginal disc fusion and histoblasts spreading in Drosophila, we aim to deepen in the regulatory control of these events and to explore the conservation of the signalling elements and cellular behaviours involved in vertebrate counterparts in amenable systems, Zebrafish development and wound healing in vertebrates.

GROUP LEADER
Enrique Martín-Blanco

POSTDOCTORAL RESEARCHERS
Katerina Karkali

PUBLICATIONS

Mangione F, **Martín-Blanco E**. The Dachsous/Fat/Four-Jointed Pathway Directs the Uniform Axial Orientation of Epithelial Cells in the Drosophila Abdomen. Cell Rep. 2018 Dec 4;25(10):2836-2850.e4.

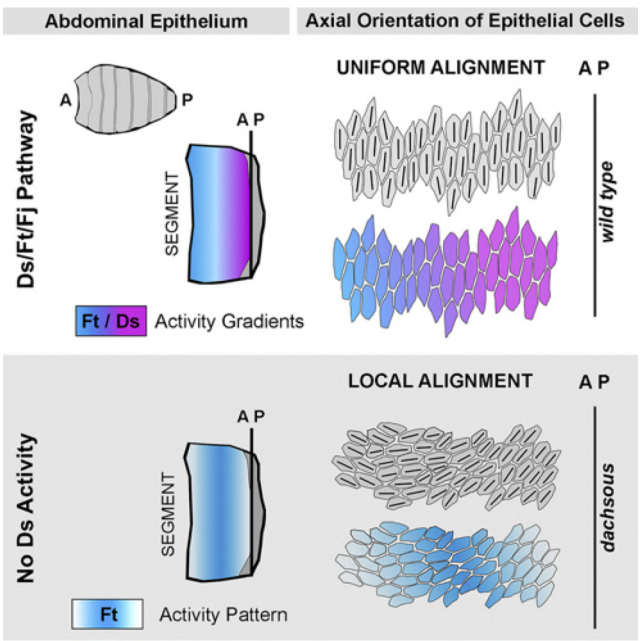
Marsal M, Jorba I, Rebollo E, Luque T, Navajas D, **Martín-Blanco E**. AFM and Microrheology in the Zebrafish Embryo Yolk Cell. J Vis Exp. 2017 Nov 29;(129).

Karkali K, **Martin-Blanco E**. Mechanosensing in the Drosophila nervous system. Semin Cell Dev Biol. 2017 Nov;71:22-29.

Marsal M, Hernández-Vega A, **Martín-Blanco E**. Contractility, differential tension and membrane removal lead zebrafish epiboly biomechanics.vCell Cycle. 2017 Jul 18;16(14):1328-1335. Similar articles

Hernández-Vega A, Marsal M, Pouille PA, Tosi S, Colombelli J, Luque T, Navajas D, Pagonabarraga I, **Martín-Blanco E**. Polarized cortical tension drives zebrafish epiboly movements.vEMBO J. 2017 Jan 4;36(1):25-41.

1



1 The Dachsous/Fat/Four-Jointed Pathway Directs the Uniform Axial Orientation of Epithelial Cells in the Drosophila Abdomen

Molecular genomics

The Department of Molecular Genomics (DMG) encompasses five research groups working on the molecular mechanisms that allow the eukaryotic genomes to perform their most critical functions across evolution. The main research areas are DNA topology, chromatin structure, epigenetics, transcription control and pre-mRNA splicing. Work is done taking advantage of a variety of model systems, from budding yeast to human.

HEAD OF DEPARTMENT

Mª Lluisa Espinàs Janer

RESEARCH GROUPS

1. Chromatin structure and function. Azorín Marín, Ferran
2. Chromatin domains in transcriptional regulation. Espinàs Janer, Mª Lluïsa
3. Chromatin regulation of human and viral gene expression. Jordan Vallès, Albert
4. Molecular signaling and chromatin. Martinez, Marian
5. Molecular mechanisms of pre-mRNA splicing. Vilardell Trench, Josep

Chromatin structure and function

Molecular Genomics

Genomic functions take place in chromatin, not in naked DNA. In recent years, our knowledge about the regulation of chromatin functions has improved thanks to the identification of components and mechanisms that modify its structural and functional properties, such as remodelling complexes, histone modifications (acetylation, methylation...) and the corresponding enzymes, histone variants that localise to specific chromosome locations, structural non-histone proteins that contribute to the functional properties of specific chromatin domains, among others. Our research focuses on the study of the molecular basis of chromatin function, their regulation during cell cycle and development, and their alteration in human pathologies.

Research Lines:

1. Centromere structure and function:chromosome segregation.
2. Epigenetic regulation of transcription.
3. Chromatin-mediated long-distance interactions.
4. Higher-order chromatin organisation.

GROUP LEADER
Ferran Azorín

CO-IP
Jordi Bernués

SENIOR RESEARCH ASSOCIATE
Mònica Torras

SENIOR POSTDOCTORAL RESEARCHER
Olga Moreno

POSTDOCTORAL RESEARCHERS
Albert Carbonell
Srividya Tamirisa

PhD STUDENTS
Paula Escudero
Paula Climent
Paula Buyosa
Marta Puerto

TECHNICIAN
Alicia Vera

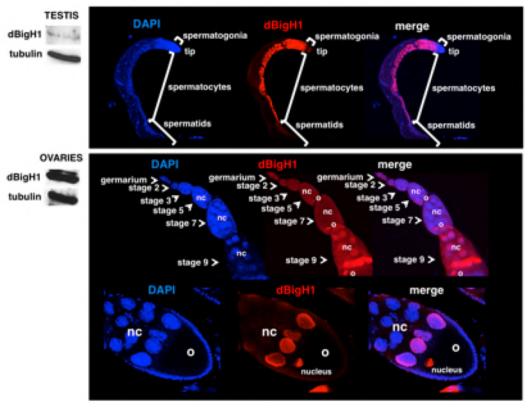
PUBLICATIONS

E. Šatović, J. Font-Mateu, A. Carbonell, M. Beato and **F. Azorín** (2018) "Chromatin remodeling in Drosophila preblastodermic embryo extract" Sci Rep, 8, 10927.

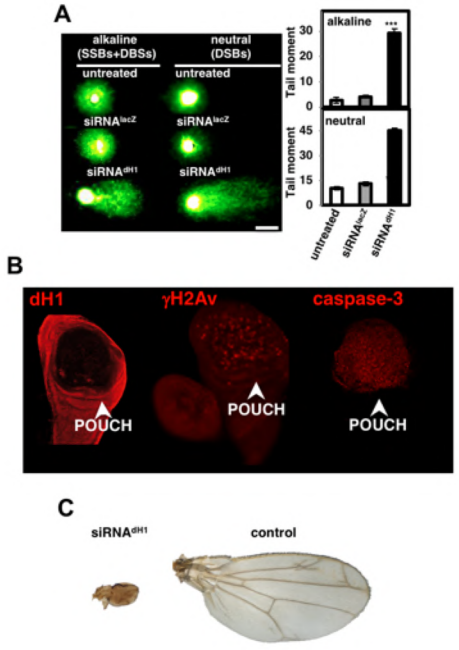
A. Carbonell, S. Pérez-Montero, P. Climent, O. Reina and **F. Azorín** (2017) "The germilne linker histone dBigH1 and the translational regulator Bam form a repressor loop essential for male germ stem cell differentiation" Cell Rep, 21, 3178-3189.

Bayona-Feliu, A. Casas-Lamesa, O. Reina, J. Bernués and **F. Azorín** (2017) "Linker histone H1 prevents R-loop accumulation and genome instability in heterochromatin". Nature Commun, 18, 283.

O. Moreno-Moreno, M. Torras-Llort and **F. Azorín** (2017) "Variations on a nucleosome theme: the structural bases of centromere function" BioEssays, 39.



❶ The pattern of expression of the germline specific linker histone dBigH1 in Drosophila testes (top) and ovaries (bottom).



❷ Somatic linker histone dH1 is essential for maintenance of genome integrity.
A) Comet assay for detects DNA breaks in control cells (top and center) and cells depleted for dH1 (bottom)
B) dH1 depletion in the wing imaginal disc induces DNA and JNK-dependent apoptosis, which results in strong wing defects

Chromatin domains in transcriptional regulation

Molecular Genomics

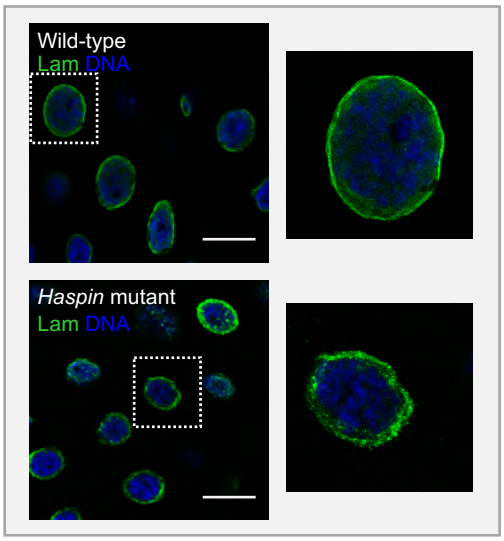
GROUP LEADER
Mª Lluïsa Espinàs

PhD STUDENTS
María Rodríguez

We are interested in factors and mechanisms involved in the formation and maintenance of chromatin domains and their implication in cell differentiation and development. We ask how interactions between distant sites in the genome are set up, maintained and controlled, and what are the consequences of genome organization inside the nucleus in gene expression. In particular, we study the role of chromatin architectural proteins in alternative promoter selection, response to stimuli and cell-type specific regulation of gene expression. We are also working to identify new proteins involved in insulator activity and chromatin organization.

Research lines:

1. New architectural proteins and their role in chromatin organization during cell differentiation and development
2. Role of insulator proteins in genomic organization into domains of gene expression
3. Identification of insulator-binding complexes in a cell and locus-specific manner



1 Insulator/architectural proteins modulate nuclear organization of interphase cells

Chromatin regulation of human and viral gene expression

Molecular Genomics

We focus our research on the control of gene expression in human cells by chromatin organization, components and modifications, with a focus on the linker histone. The linker histone in mammals, participating in nucleosome spacing and higher-order chromatin structure, is a family of different histone H1 subtypes, including 7 somatic variants. It is poorly understood why so many H1 variants exist and whether they have particular features relevant to its functionality. Knock-out experiments have suggested that the different variants are redundant and one isoform can substitute for another, but slowly new reports including ours are suggesting specific roles for the H1 variants, including involvement in specific gene expression regulation, and differential distribution in the human genome. In a second line of research, we use an HIV promoter model to investigate the influence of chromatin organization at the integration site on HIV expression, with a focus on the role of heterochromatin on the establishment of viral latency, and we search for therapies for reactivation.

Research Lines

1. Whether human histone H1 variants have distinct properties and roles in chromatin organization, gene expression control and, ultimately, cell growth.
2. Whether H1 variants are distributed distinctively in the genome, or with specific participation in particular gene promoters, or interact with different nuclear components.
3. What is the participation of H1 variants in tumor progression and cell differentiation.
4. How the organization and compaction of chromatin at the integration site influences the transcriptional activity of the HIV promoter and leads to viral latency.
5. What are the molecular mechanisms involved in HIV reactivation from latency by newly identified drugs.

GROUP LEADER
Albert Jordan

RESEARCH ASSOCIATE
Carles Bonet

PhD STUDENTS
Daniel García
Mónica Salinas

MASTER'S STUDENT
Stefany Montúfar
Francesc Torrent
Núria Serna

PUBLICATIONS

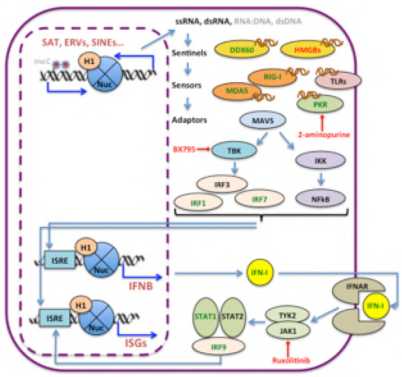
Carbonell A, Fueyo R, Izquierdo-Bouldstridge A, Moreta C, **Jordan A** (2018) Epigenetic mechanisms in health and disease: BCEC 2017. Epigenetics 13(3): 331-341.

Abner E, Stoszko M, Zeng L, Chen H-C, Izquierdo-Bouldstridge A, Konuma T, Zorita E, Fanunza E, Zhang Q, Mahmoudi T, Zhou M-M, Filion G, **Jordan A** (2018) A new quinoline BRD4 inhibitor targets a distinct latent HIV-1 reservoir for re-activation from other 'shock' drugs. Journal of Virology 2018 Apr 27;92(10). pii: e02056-17.

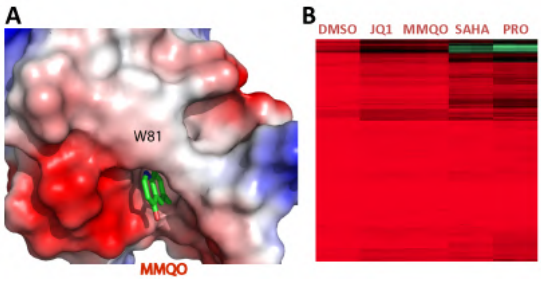
Izquierdo-Bouldstridge A*, Bustillos A*, Bonet-Costa C, Aribau P, Garcia D, Dabad M, Esteve-Codina A, Pascual L, Peiro S, Esteller M, Murtha M, Millán-Ariño Ll, **Jordan A** (2017) Histone H1 depletion triggers an interferon response in cancer cells via activation of heterochromatic repeats. Nucleic Acids Research 45(20): 11622-42.

Perearnau A, Orlando S, Islam A, Gallastegui E, Martínez J, **Jordan A**, Bigas A, Aligué R, Pujol MJ, Bachs O (2017) p27Kip1, PCAF and PAX5 cooperate in the transcriptional regulation of specific target genes. Nucleic Acids Research 45(9):5086-99.

❶



❷



❶ Histone H1 depletion triggers an interferon response in cancer cells.
Simultaneous depletion of particular histone H1 variants in human breast cancer cells triggers the cellular interferon response, originally designed to fight against invading pathogen-associated nucleic acids such as DNA or RNA viruses. We have shown that this is due to the derepression of heterochromatic non-coding RNAs such as satellites and endogenous retroviruses, which may be naturally repressed with the participation of particular histone H1 subtypes such as H1.2 and H1.4.

❷ A new quinoline BRD4 inhibitor targets a distinct latent HIV-1 reservoir for re-activation from other 'shock' drugs.
We have shown that a previously identified Human Immunodeficiency Virus (HIV) latency-reversing agent (MMQO) acts as a bromodomain inhibitor. A) Despite of very different chemical structures, MMQO mimics the action of JQ1, a well-known bromodomain inhibitor, and binds to the bromodomain and extraterminal domain (BET) family protein BRD4. B) Utilizing barcoded HIV-1 minigenomes, we demonstrate that PKC pathway activators, HDAC and bromodomain inhibitors all target different subsets of proviral integrations.

Molecular signaling and chromatin

Molecular Genomics

Multipotent cells are capable either self-renew or differentiate. This process is regulated by interactions between transcription factors, signalling proteins and epigenetic factors. Our group is focused on understanding the role of epigenetic regulators in gene transcription during development. We use as main experimental model gene induction and silencing that take place during neurogenesis. Our work will help to understand the molecular basis of chromatin function during the development as well as in pathological processes, helping to identify new therapeutic targets.

Research Lines

1. The role of histone methylation during neurogenesis and cell fate using chick embryo neural tube.
2. Epigenetic defects in mental retardation. Role of histone demethylase PHF8.
3. Genome architecture contribution to cell fate determination

GROUP LEADER
Marian Martinez

PhD STUDENTS
Stella Pappa
Simona Lacobucci
Marta Vicioso

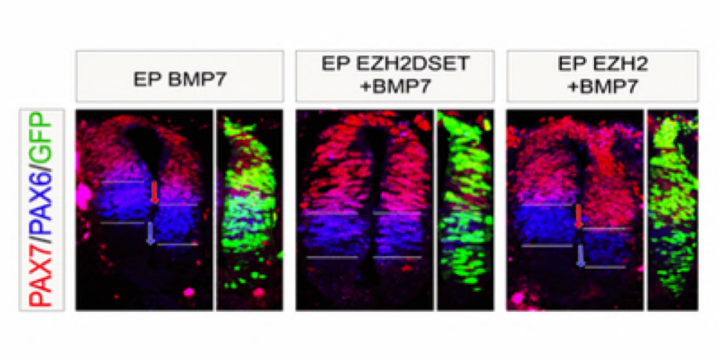
PUBLICATIONS

Fueyo R, Lacobucci S, Pappa S, Estarás C, Lois S, Vicioso-Mantis M, Navarro C, Cruz-Molina S, Reyes JC, Rada-Iglesias Á, de la Cruz X, **Martínez-Balbás M.A.** Lineage specific transcriptio factors and epigenetic regulators mediate TGFb-dependent enhancer activation. 2018. Nucleic Acids Research. 46, 3351-3365.

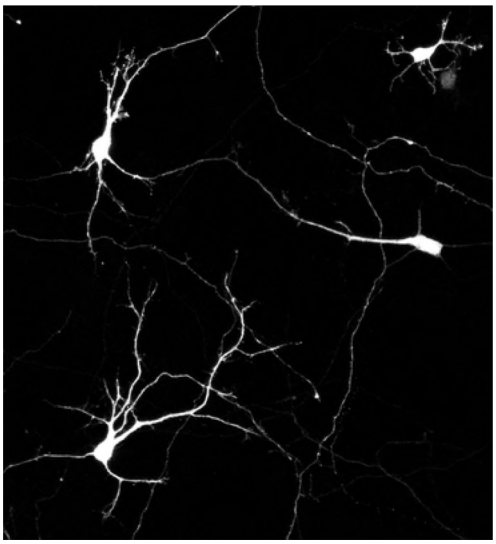
Asensio-Juan, E., Fueyo, R., Pappa, E., Iacobucci, S., Badosa, C., Lois, S., Balada, M., Bosch-Presegué, L., Vaquero, A., Gutiérrez, S., Caelles, C., Gallego, C., de la Cruz, X., and **Martínez-Balbás, M.A**. The histone demethylase PHF8 is a molecular safeguard of the IFNγ response. 2017. Nucleic Acids Research,45, 3800-3811.

Sánchez-Cid L, Pons M, Lozano JJ, Rubio N, Guerra-Rebollo M, Soriano A, Paris-Coderch L, Segura MF, Fueyo R, Arguimbau J, Zodda E, Bermudo R, Alonso I, Caparrós X, Cascante M, Rafii A, Kang Y, **Martínez-Balbás M**, Weiss SJ, Blanco J, Muñoz M, Fernández PL, Thomson TM. MicroRNA-200, associated with metastatic breast cancer, promotes traits of mammary luminal progenitor cells.2017. Oncotarget.8, 83384-83406.

❶



❷



❶ Neural differentiation requires general changes in gene expression in which epigenetic changes are involved. Our work has allowed to identify several signalling pathways that cooperate with the enzymes responsible of H3K27me3 to control neurogenesis. Our goal is to describe the molecular mechanisms responsible of this cooperation (Akizu et al., 2010).

❷ A very high number of genes mutated in mental retardation encode regulators of chromatin structure. One of these genes is PHF8, a histone demethylase enzyme. The PFH8 function and the molecular mechanisms responsible for it role in mental retardation are not clearly established. In the laboratory we are interested in establishing the role of PHF8 in vivo.

Molecular mechanisms of pre-mRNA splicing

Molecular Genomics

GROUP LEADER
Josep Vilardell

POSTDOCTORAL RESEARCHERS
Joan Marc Martínez

Our overall motivation is to contribute to understanding pre-mRNA splicing and its impact on gene expression. While we follow a reductionist approach with a well-defined working model (yeast), we aim to decipher mechanisms relevant to human and, given the link between splicing and disease, to health. Our research covers the following subjects: (a) sequence elements that act on the core spliceosome, (b) how early spliceosome assembly can be regulated, (c) changes in splicing at cellular level in response to diverse stimuli.

Research Lines

1. **Exonic and intronic sequences that act on the spliceosome**

We are developing a working model for the identification of intronic ends by the spliceosome. This model has helped us in the identification of several novelties: an RNA thermosensor that links temperature an alternative splicing, a novel intron that requires an unlikely fold to be identified by the spliceosome, and a set of sequences that are not being identified as introns for reasons that still escape us.

2. **Exon recognition by the core spliceosome**

Substrate recognition by the spliceosome is not fully understood. Our data on yeast cryptic introns suggest that even in yeast cells, supposedly with a simplified splicing machinery, there are unknown elements that act on this recognition. Because the evolutionary conservation of pre-RNA splicing, these elements are likely to be at the core of strategies for splicing regulation followed in other systems. In accordance with this, we have shown that the yeast spliceosome recognizes a human cassette exon and that this recognition is modulated by changes in the exonic sequence. How this happens at molecular level is what we are trying to solve.

3. **Links between regulated splicing, chromatin, and signal transduction**

We are developing the reagents needed to determine possible genetic interactions between histones and splicing. In addition, we have a set of UV-induced mutants on the L30/*RPL30* system of splicing regulation, where excess L30 fails to block *RPL30* splicing. We have found that this phenotype can be suppressed by an extra copy of the PRP45 gene. Prp45 human homologue, SKIP, is a transcription factor that is phosphorylated. Thus, our working hypothesis is that Prp45 is part of a link between regulated splicing, transcription, and MAPK kinases. As before, there is evidence for such links in metazoans, but with little information on mechanisms. Here we may have a valuable opportunity to investigate this process.

4. **Splicing at a higher level: gene families and genomes**

Using Bioinformatics and RNASeq data we aim to perform meta-analyses to monitor how the yeast spliceosome responds to a number of stresses (including aging and genomic stability). In addition, we explore the transcriptome of the mRNAs encoding ribosomal proteins (RP) in healthy vs tumoral human cells

PUBLICATIONS

Chakraborty A, Lyonnais S, Battistini F, Hospital A, Medici G, Prohens R, Orozco M, Vilardell J, Solà M. DNA structure directs positioning of the mitochondrial genome packaging protein Abf2p. Nucleic Acids Res. 2017 Jan 25;45(2):951-967.

Structural biology unit

The Structural Biology Unit (SBU) is one of the leading research poles in the field of macromolecular X-ray crystallography in Spain. It consists of eight research groups, featuring over 60 researchers, all involved in cutting-edge projects of medical, biological or biotechnological relevance. The SBU aims to understand the cell machinery and physiological processes from structural and functional perspectives. The main interest of SBU researchers is the study of proteins, nucleic acids and their interactions, to help elucidate the molecular bases of physiological processes and pathology. The major research lines are focused on the regulation of nucleic acid replication and transcription, structure-function analysis of proteases and host-microbiome interactions, the study of different pathogens -including viruses-, and organelles like peroxisomes and mitochondria. A separate but highly complementary line of research is centred on the development of computational methods for X-ray crystallography.

In 2015, the SBU was designated a María de Maeztu Unit of Excellence by the Spanish Ministry of Economy and Competitiveness (MINECO), which recognizes exceptional quality in research, training, human resources, outreach and technology transfer. In October 2017, the Severo Ochoa and María de Maeztu alliance (SOMMa) was created. The SBU participates in all the meetings and actions fomented by this initiative to strengthen the R+D+i system and to play a significant role in Science policies in Spain and in Europe.

HEAD OF DEPARTMENT

F. Xavier Gomis-Rüth

SCIENTIFIC DIRECTOR OF SBU EXCELLENCE UNIT

Núria Verdaguer Massana

RESEARCH GROUPS

1. Structural Biology of Protein-Nucleic Acid Complexes and Molecular Machines. Coll Capella, Miquel Coll
2. Protein Interactions and Docking. Fernández-Recio, Juan
3. Structural Biology of Oxidative Stress Systems. From Organelles to Membrane Proteins. Fita Rodríguez, Ignacio
4. Proteolysis Lab. Gomis-Rüth, F. Xavier
5. DNA Topology. Roca, Joaquim
6. Structural Biology of Mitochondrial Macromolecules - Structural MitoLab. Solà Vilarrubias, Maria
7. Crystallographic methods. Usón, Isabel
8. Structural virology and large biological complexes. Verdaguer Massana, Núria

Protein-nucleic acid complexes and molecular machines

Structural Biology Unit

Our research focuses on the 3D structure of proteins, nucleic acids and their complexes with the aim to further our understanding of several essential mechanisms in the cell. We use a number of molecular biology and structural biology techniques for our purposes, including X-ray diffraction—using synchrotron radiation, and electron microscopy. The final outcome is a detailed 3D view of the molecular structures of interest. This information, together with complementary molecular biology and biochemistry experiments, unveils how these proteins and molecular machines function.

We address the regulatory mechanisms of gene expression and DNA replication. In addition, we examine systems related to horizontal gene transfer that involve DNA translocation across cell membranes. We also study unique DNA structures, like DNA junctions, and novel drugs that target DNA and proteins.

GROUP LEADER
Miquel Coll

RESEARCH ASSOCIATE
Albert Canals

POSTDOCTORAL RESEARCHERS
Cristina Machón
Olga Borisovna
Montserrat Fàbrega

PhD STUDENTS
Mireia Muriel
Luis Daniel Toribio
Jorge García
Sara Muriel

TECHNICIAN
Rosa Pérez
Esther Ferrando

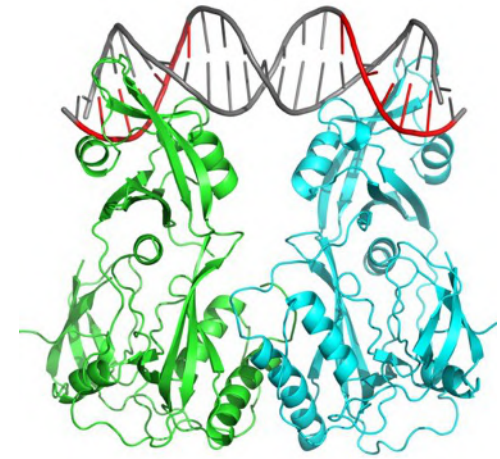
PUBLICATIONS

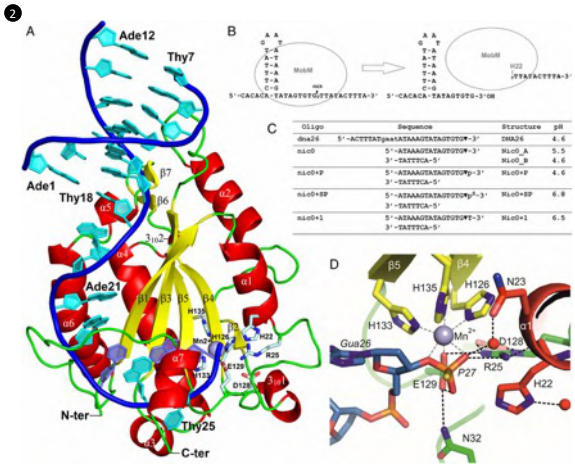
Roura Frigolé H, Camacho N, Castellví Coma M, Fernández-Lozano C, García-Lema J, Rafels-Ybern À, Canals A, **Coll M**, Ribas de Pouplana L. tRNA deamination by ADAT requires substrate-specific recognition mechanisms and can be inhibited by tRFs. RNA. 2019 Feb 8. pii: rna.068189.118.

Peña-Soler E, Aranda J, López-Estepa M, Gómez S, Garces F, **Coll M**, Fernández FJ, Tuñon I, Vega MC. Insights into the inhibited form of the redox-sensitive SufE-like sulfur acceptor CsdE. PLoS One. 2017 Oct 18;12(10):e0186286.

Pluta R, Boer DR, Lorenzo-Díaz F, Russi S, Gómez H, Fernández-López C, Pérez-Luque R, Orozco M, Espinosa M, **Coll M.** Structural basis of a histidine-DNA nicking/joining mechanism for gene transfer and promiscuous spread of antibiotic resistance. Proc Natl Acad Sci U S A. 2017 Aug 8;114(32):E6526-E6535.

Canals A, Arribas-Bosacoma R, Albericio F, Álvarez M, Aymamí J, **Coll M**. Intercalative DNA binding of the marine anticancer drug variolin B. Sci Rep. 2017 Jan 4;7:39680.





1 Crystal structure of an ARF1-DBD/ER7 complex shown in two orientations (rotation axis indicated). ARF1-DBD monomers are differently colored.

2 Structure of the MobM-DNA complex. (A) Crystal structure of the N-terminal MobM relaxase domain bound to the nic0+SP oligonucleotide containing the scissile phosphate (Nic0+SP structure). (B) Scheme of oriT processing by MobM. (C) Oligonucleotides used for crystallization, structure names, and pH. (D) Active site details.

Protein interactions and docking

Structural Biology Unit

Our group works on theoretical and computational approaches to study protein-protein association at structural and energetic level. We are developing new computational methods for modeling protein-protein complex structure by docking, prediction of binding sites and hot-spot residues, and understanding the specificity of protein-nucleic acid interactions. On the most practical side, we are modeling protein interactions of biological and therapeutic interest, related to signal transduction, amino acid transport or gene regulation. The ultimate goal of our research is to apply our models to the interpretation of pathological mutations involved in protein interactions, and to drug discovery.

The main goal of the group is to contribute to solve some of the important challenges in the structural biology of protein-protein and protein-nucleic acid interactions. These are our major research lines:

1. Development of new computational tools for protein-protein docking and binding site prediction.
2. Structural bioinformatics for the characterization and modeling of protein-nucleic acid interactions.
3. Understanding binding affinity, kinetics and mechanism of macromolecular association.
4. Structural modeling of cases of biomedical interest and drug discovery.
5. Molecular interpretation of pathological mutations involved in protein interactions.

GROUP LEADER
Juan Fernández-Recio

PhD STUDENTS
Miguel Romero
Mireia Rosell
L. Ángel Rodríguez

RESEARCH VISITORS
Lucía Díaz
Brian Jiménez

PUBLICATIONS

Ordan M, Pallara C, Maik-Rachline G, Hanoch T, Gervasio FL, Glaser F, **Fernandez-Recio J**, Seger R. Intrinsically active MEK variants are differentially regulated by proteinases and phosphatases. Sci Rep. 2018 Aug 7;8(1):11830.

Jankauskaite J, Jiménez-García B, Dapkunas J, **Fernández-Recio J**, Moal IH. SKEMPI 2.0: An updated benchmark of changes in protein-protein binding energy, kinetics and thermodynamics upon mutation. Bioinformatics. 2018 Jul 18.

Rosell M., **Fernández-Recio J**. Hot-spot analysis for drug discovery targeting protein-protein interactions. Exp. Opin. Drug Discov. 2018 Apr 13; 13:327-338.

Jiménez-García, B., Roel-Touris, J., Romero-Durana, M., Vidal, M., Jiménez-González, D., **Fernández-Recio, J**. LightDock: A new multi-scale approach to protein-protein docking. Bioinformatics. 2018 Jan 1;34(1):49-55.

Barradas-Bautista, D., Rosell, M., Pallara, C., **Fernández-Recio, J**. Structural prediction of protein-protein interactions by docking: application to biomedical problems. Adv Protein Chem Struct Biol. 2018;110:203-249

Barradas-Bautista, D., **Fernández-Recio, J**. Docking-based modeling of protein-protein interfaces for extensive structural and functional characterization of missense mutations. PLoS One 12, e0183643. (2017)

Barradas-Bautista, D., Moal, I.H., **Fernández-Recio, J**. A systematic analysis of scoring functions in rigid-body protein docking: the delicate balance between the predictive rate improvement and the risk of overtraining. Proteins 85, 1287-1297. (2017)

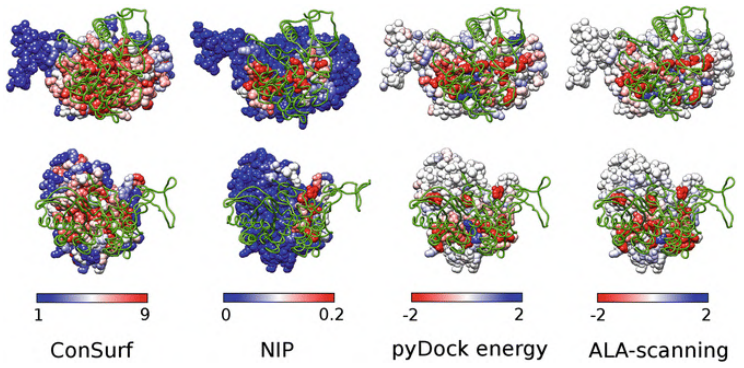
Moal, I.H., Barradas-Bautista, D., Jiménez-García, B, Torchala, M., van der Veide, A, Vreven, T., Weng, Z., Bates, P.A., **Fernández-Recio, J**. (2017) RaPPA: Information retrieval based integration of biophysical models for protein assembly selection. Bioinformatics 33, 1806-1813 (2017)

Romero-Durana, M., Pallara, C., Glaser, F., **Fernández-Recio, J**. Modeling binding affinity of pathological mutations for computational protein design. Methods Mol.Biol. 1529, 139-159. (2017)

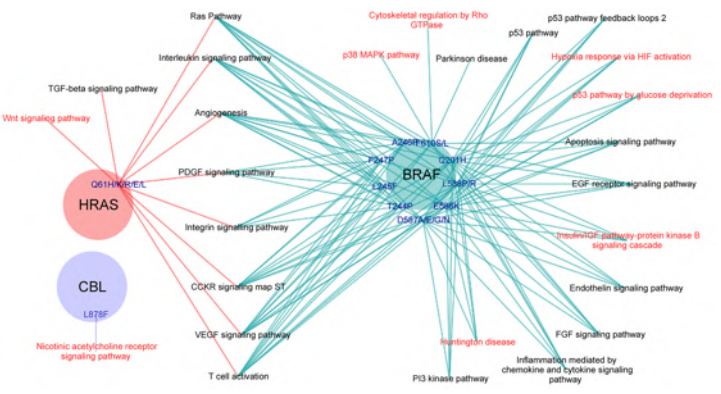
Pallara, C., Jiménez-García, B., Romero-Durana, M., Moal, I.H., **Fernández-Recio, J**. pyDock scoring for the new modeling challenges in docking: Protein-peptide, homo-multimers, and domain-domain interactions. Proteins 85, 487-496. (2017)

Pérez-Cano, L., Romero-Durana, M., **Fernández-Recio, J**. Structural and energy determinants in protein-RNA docking. Methods 118-119, 163-170. (2017).

❶



❷



❶ MEK1–BRAF interface characterization. MEK1 and BRAF interface characterization using different computational techniques (first and second line, respectively): ConSurf evolutionary conservation, pyDock NIP calculation, pyDock binding energy decomposition, binding free energy change (ΔΔG) estimated by in silico alanine scanning

❷ Proteins of the RAS/MAPK pathway are shown as colored circles, showing pathological mutations that were not previously characterized due to the lack of structural data, but that have been predicted here to be binding hot-spots for docking partner proteins involved in other pathways (linked to the corresponding mutation). Pathways shown in red are those that could not have been found using only available structural data.

Oxidative stress systems

Structural Biology Unit

The major expertise of the lab is in Macromolecular X-Ray Crystallography. Currently we are especially interested in studies requiring the integration of different structural methodologies with research centered on two biological systems, one prokaryotic and one eukaryotic:

1. The Functional and Structural Study of Mycoplasmas Terminal Organelle
2. The Structure and Interactions of Peroxisomal Proteins

Several targets of our research lines include macromolecular aggregates and membrane-associated or integral membrane proteins. In addition, we also have a number of different collaborative projects mainly related with oxidative-stress processes and pathologies.

GROUP LEADER
Ignasi Fita

STAFF SCIENTIST
Alicia Guasch

POSTDOCTORAL RESEARCHERS
David Aparicio

PhD STUDENTS
David Vizarraga
Jesús Martín

TECHNICIAN
Rosa Pérez

PUBLICATIONS

Aparicio D, Torres-Puig S, Ratera M, Querol E, Piñol J, Pich OQ, **Fita I**. *Mycoplasma genitalium* adhesin P110 binds sialic-acid human receptors. Nat Commun. 2018 Oct 26;9(1):4471.

Teixeira JMC, Guasch A, Biçer A, Aranguren-Ibáñez Á, Chashmniam S, Paniagua JC, Pérez-Riba M, **Fita I,** Pons M. Cis-trans proline isomers in the catalytic domain of calcineurin. FEBS J. 2018 Dec 7.

Gordo V, Aparicio D, Pérez-Luque R, Benito A, Vilanova M, Usón I, **Fita I,** Ribó M. Structural Insights into Subunits Assembly and the Oxyester Splicing Mechanism of Neq pol Split Intein. Cell Chem Biol. 2018 Jul 19;25(7):871-879.e2.

Loewen PC, De Silva PM, Donald LJ, Switala J, Villanueva J, **Fita I**, Kumar A. KatG-Mediated Oxidation Leading to Reduced Susceptibility of Bacteria to Kanamycin. ACS Omega. 2018 Apr 30;3(4):4213-4219.

Adell M, Calisto BM, **Fita I**, Martinelli L. The nucleotide-bound/substrate-bound conformation of the *Mycoplasma genitalium* DnaK chaperone. Protein Sci. 2018 May;27(5):1000-1007.

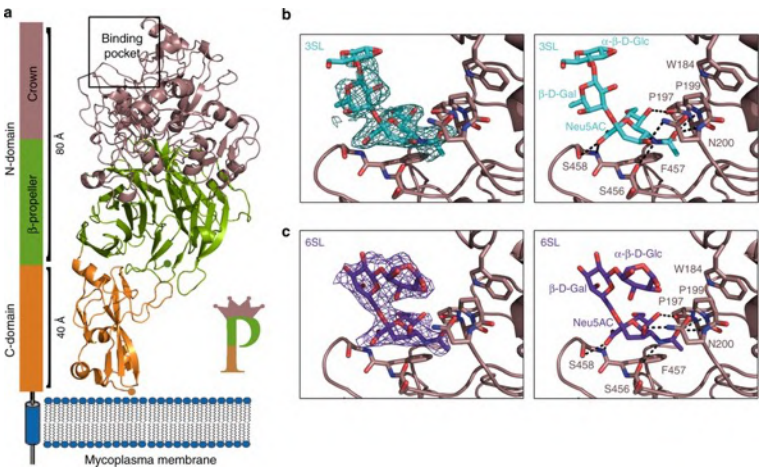
Wang B, **Fita I**, Rovira C. Theory Uncovers the Role of the Methionine-Tyrosine-Tryptophan Radical Adduct in the Catalase Reaction of KatGs: O2 Release Mediated by Proton-Coupled Electron Transfer. Chemistry. 2018 Apr 6;24(20):5388-5395.

Goethe M, Gleixner J, **Fita I**, Rubi JM. Prediction of Protein Configurational Entropy (Popcoen). J Chem Theory Comput. 2018 Mar 13;14(3):1811-1819.

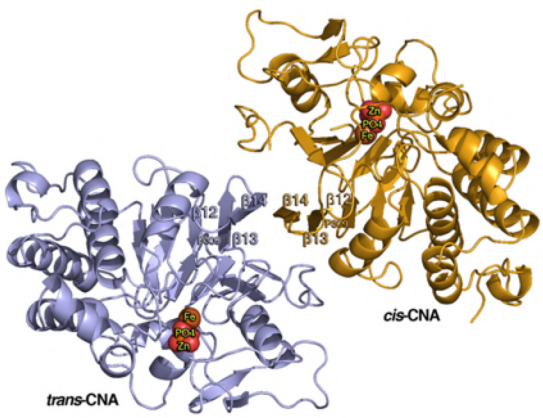
Goethe M, **Fita I**, Rubi JM. Testing the mutual information expansion of entropy with multivariate Gaussian distributions. J Chem Phys. 2017 Dec 14;147(22):224102.

Scheffer MP, Gonzalez-Gonzalez L, Seybert A, Ratera M, Kunz M, Valpuesta JM, **Fita** I, Querol E, Piñol J, Martín-Benito J, Frangakis AS. Structural characterization of the NAP; the major adhesion complex of the human pathogen *Mycoplasma genitalium*. Mol Microbiol. 2017 Sep;105(6):869-879.

Machuqueiro M, Victor B, Switala J, Villanueva J, Rovira C, **Fita I**, Loewen PC. The Catalase Activity of Catalase-Peroxidases Is Modulated by Changes in the pKa of the Distal Histidine. Biochemistry. 2017 May 2;56(17):2271-2281.



1 *Mycoplasma genitalium* adhesin P110 binds sialic-acid human receptors. Binding of sialic acid oligosaccharides to P110. a Schematic representation of the disposition of P110 with respect to the mycoplasma membrane (same color code as in Fig.). The predicted transmembrane helix follows in sequence to the C-domain, which accordingly is expected to be close to the cell membrane. The overall structure of P110 can be sketched as a capital letter P with the sialic binding site (indicated in the figure with a rectangle) located in the crown, away from the cell membrane. Detail of the binding to P110 of sialic acid oligosaccharides b 3SL and c 6SL. Left panels show the electron density corresponding to oligosaccharides in a sigma weighted (Fo–Fc) omit map at two sigma. The binding site and the binding interactions with P110 for both oligosaccharides are very similar, but not identical



2 Structures of the interacting CNA catalytic domains with trans- and cis- conformations.
(a) Ribbon representation of two neighboring CNA subunits in the crystal presenting the trans- (blue) and cis- (brown) conformations, respectively. The metal atoms and the phosphate group in the active site are shown as spheres.

Proteolysis lab

Structural Biology Unit

The work carried out in the Proteolysis Lab is centered on the analysis of the molecular determinants of function and regulation of proteolysis at the protein level. We study peptidases and their zymogens, mostly from host-microbiome interactions, as well as their complexes with small-molecule and protein inhibitors. Another line of research deals with the molecular analysis of other interaction mediators between animal hosts and their microbiomes. Employed techniques include molecular biology, biochemistry, biophysics, and X-ray crystallography, among others. Our lab is fully furnished with state-of-the-art equipment, which enables us to carry out cutting-edge science projects.

Recently, among other projects, we unveiled the mechanism of action of a multidomain, 180 kDa, α_2-macroglobulin (α_2M)-like protease inhibitor encoded in the *Escherichia coli* genome, by applying biochemical and structural techniques. We found that the protein is a target for proteases of diverse catalytic mechanism and specificity, which cut in an unstructured bait region. This triggers a large conformational rearrangement in the molecule from a native to an induced form, which has been extensively studied. However, the inhibitor remains monomeric, contrarily to the tetrameric state of some mammalian α_2Ms, and the entrapment of the protease is necessarily accomplished by covalent binding of a surface lysine of the protease through a conserved and highly reactive thioester bond. As a consequence, the protease becomes sterically hindered to reach globular substrates of high molecular weight, so its proteolytic activity is inhibited. Taking into account the periplasmatic localization of the inhibitor, we hypothesize that it is acting as an *E. coli* defense mechanism against invading proteases that may damage cell wall components.

Proteolysis lab website

GROUP LEADER
F. Xavier Gomis-Rüth
Google Scholar

LABORATORY MANAGER
Tibisay Guevara

RESEARCH ASSOCIATE
Ulrich Eckhard

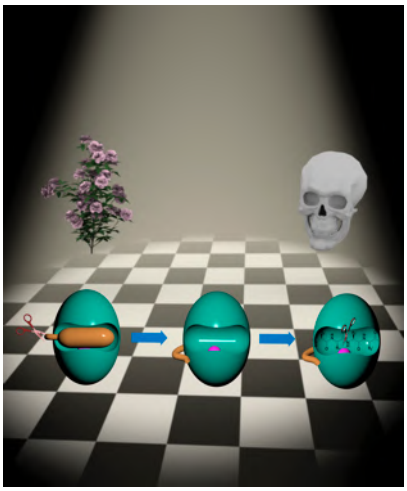
POSTDOCTORAL RESEARCHERS
Artur Rodríguez-Banqueri

PhD STUDENTS
Laura Mariño-Puertas
Laura del Amo-Maestro
Soraia dos Reis-Mendes

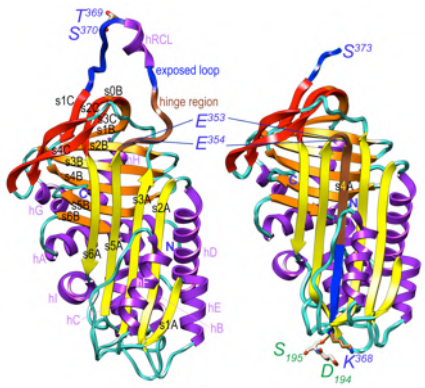
MASTER'S STUDENT
Marina Moliner

TECHNICIAN
Miguel Ángel Blázquez

❶



❷



❶ In the playground of life and death, metallopeptidases exert a key role by activating or inactivating proteins through peptide bond cleavage. To keep their potentially deleterious activity in check, metallopeptidases are biosynthesized as zymogens, in which a prosegment (in orange) blocks access to the active-site cleft, at whose bottom the catalytic zinc ion (magenta sphere) residues. Autolytic or heterolytic limited proteolysis (scissors) remove the prosegment in a timely and locally adequate context, thus yielding the mature, competent enzyme. The latter now binds peptidic substrates in its cleft and cleaves them (scissors).

❷ (Left) Native miropin in the reference "front view" (according to (32)) as a Richardson-type plot, with strands as arrows and helices as purple spirals (labeled hA-hH plus hRCL). The strands are arranged in three β-sheets (sheet sA, yellow strands s6A, s5A, s3A-s1A; sheet sB, orange strands s0B-s6B; and sC, red strands s1C-s4C). The RCL (E353-P376) connects strand s5A with s1C and is subdivided into the "hinge region" (E353-V361; brown ribbon) and the "exposed loop" (T362-P376; blue ribbon). The residues flanking the theoretic RSB (P1-P1'; T369-S370) are shown for their side chains and labeled. The position of the P16 (E353) and P17 (E354) residues is indicated by lines. (Right) Same as (A) but showing trypsin-induced wild-type miropin as representative of the induced miropin structures. β-Strand s4A from sheet sA, absent in (A), is shown in the colors of the corresponding segment of (A) and labeled. Dipeptide D194-S195 of trypsin is covalently attached through atom S195 Oγ to the carbonyl of K368 after cleavage of bond P2-P1 (K368-T369). On the primed side, the chain is only defined from S373 (P4) onwards.

PUBLICATIONS

I. Garcia-Ferrer, A. Marrero, **F.X. Gomis-Rüth** & T. Goulas (2017). α2-Macroglobulins: structure and function. In Macromolecular Protein Complexes, series Subcellular Biochemistry, Vol. 83 (J.R. Harris & J. Marles-Wright, eds.), Springer, Cham (Switzerland), pp. 149-183 (Book chapter).

F.X. Gomis-Rüth (2017). Third time lucky? Getting a grip on matrix metalloproteinases. J. Biol. Chem., 292, 17975-17976 (JBC Editors' Pick Highlight).

A. Pomowski, I. Usón, Z. Nowakowska, F. Veillard, M.N. Sztukowska, T. Guevara, T. Goulas, D. Mizgalska, M. Nowak, B. Potempa, J.A. Huntington, J. Potempa & **F.X. Gomis-Rüth** (2017). Structural insights unravel the zymogenic mechanism of the virulence factor gingipain K from Porphyromonas gingivalis, a causative agent of gum disease from the human oral microbiome. J. Biol. Chem., 292, 5724-5735 (highlighted in the March 2017 issue of the JBC Editorial Member Digest, "Bringing the gingipain").

T. Goulas, M. Ksiazek, I. Garcia-Ferrer, A.M. Sochaj-Gregorczyk, I. Waligorska, M. Wasylewski, J. Potempa & **F.X. Gomis-Rüth** (2017). A structure-derived snap-trap mechanism of a multispecific serpin from the dysbiotic human oral microbiome. J. Biol. Chem., 292, 10883-10898.

T. Goulas, I. Garcia-Ferrer, A. Marrero, L. Marino-Puertas, S. Duquerroy & **F.X. Gomis-Rüth** (2017). Structural and functional insight into pan-endopeptidase inhibition by α2-macroglobulins. Biol. Chem., 398, 975-994 (highlighted in SCIENCE Discoveries "Large multi-modular proteins inhibit proteolytic enzymes like Venus flytraps", 22.02.2018; http://sciencediscoveries.degruyter.com/multi-modular-proteins-inhibit-proteolytic-enzymes-traps).

L. Marino-Puertas, T. Goulas & **F.X. Gomis-Rüth** (2017). Matrix metalloproteinases outside vertebrates. Biochim. Biophys. Acta – Mol. Cell Res., 1864, 2026-2035.

R. García-Castellanos, N.S. Nielsen, K. Runager, I.B. Thøgersen, M.V. Lukassen, E.T. Poulsen, T. Goulas, J.J. Enghild & **F.X. Gomis-Rüth** (2017). Structural and functional implications of human transforming growth factor β-induced protein (TGFBIp) in corneal dystrophies. Structure, 25, 1740-1750.

J.L. Arolas, T. Goulas, A. Cuppari & **F.X. Gomis-Rüth** (2018). Multiple architectures and mechanisms of latency in metallopeptidase zymogens. Chem. Rev., 118, 5581-5597.

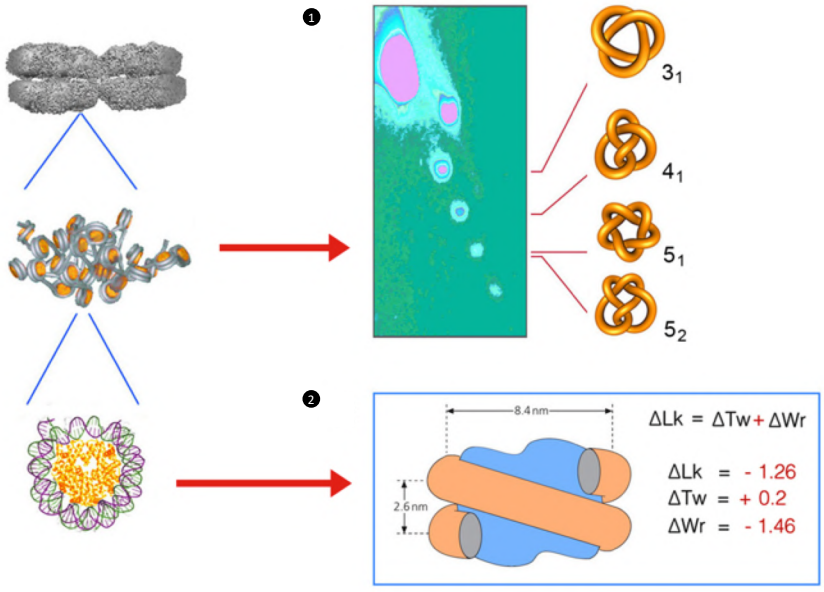
DNA topology

Structural Biology Unit

Full understanding of the mechanisms that govern genome biology and cell fate will be achieved only after uncovering the complex interplay of intracellular DNA topology with chromatin architecture and functions. To this aim, our research focuses on the mechanism and activity of DNA topoisomerases, the processes that generate and dissipate DNA twisting forces, and the mechanisms that produce and dissolve chromosomal DNA entanglements. We develop also new experimental procedures to assess the topology of intracellular DNA, both at local and genome-wide scale. Our research integrates cellular and molecular biology approaches with DNA biophysics and molecular simulations.

Some of our major scientific contributions comprise:

- The three-gate mechanism of DNA transport by type-2 topoisomerases
- Regulation of cellular topoisomerase activity by chromatin structure.
- Response of chromatin structure to DNA torsional stress.
- Genome-wide analysis of DNA helical tension by psoralen-DNA photo-binding.
- DNA topology constrained by chromatin elements (centromeres, telomeres …)
- Capture of the 3D path of DNA by means of knot analysis.



1 2D-gel electrophoresis of the DNA in a yeast circular minichromosome. The gel blot reveals that topoisomerase II produces DNA knots within the clusters of nucleosomes, that constitute eukaryotic chromatin. The probability and complexity of these knots indicate the flexibility of nucleosome arrays and the path of DNA within chromatin fibers in vivo (Valdes et al 2018).

2 Since the 80s, there had been an enduring discrepancy between theoretical and observed measurement of the DNA linking number (ΔLk) constrained by nucleosomes. We used a novel approach to determine the ΔLk constrained by individual nucleosomes in vivo. Our results, along with calculated values of ΔTw and ΔWr, reconcile the so called "Lk paradox of nucleosomal DNA" (Segura et al 2018).

GROUP LEADER
Joaquim Roca

RESEARCH ASSISTANT
Belén Martínez-García

POSTDOCTORAL RESEARCHERS
Ricky Joshi
Ofelia Díaz-Ingelmo

PhD STUDENTS
Antonio Valdés
Joana Segura
Sílva Dyson

PUBLICATIONS

Joana Segura, Ricky S. Joshi, Ofelia Díaz-Ingelmo, Antonio Valdés, Silvia Dyson, Belén Martínez-García and **Joaquim Roca**. Intracellular nucleosomes constrain a DNA linking number difference of -1.26 that reconciles the Lk paradox. Nature Com Sep 28;9(1):3989 (2018)

Valdes, A., Segura, J., Dyson, S., Martinez-Garcia, B., and **Roca, J.** DNA knots occur in intracellular chromatin. Nucleic Acids Res 46, 650-660 (2018)

Martinez-Garcia, B., Valdes, A., Segura, J., Dyson, S., Diaz-Ingelmo, O., and **Roca, J.** Electrophoretic Analysis of the DNA Supercoiling Activity of DNA Gyrase. Methods Mol Biol 1805, 291-300 (2018)

Joshi, R.S., Nikolaou, C., and **Roca, J.** Structure and Chromosomal Organization of Yeast Genes Regulated by Topoisomerase II. Int J Mol Sci 19 (2018).

Tsochatzidou, M., Malliarou, M., Papanikolaou, N., **Roca, J.**, and Nikolaou, C. Genome urbanization: clusters of topologically co-regulated genes delineate functional compartments in the genome of Saccharomyces cerevisiae. Nucleic Acids Res 45, 5818-5828 (2017)

Structural biology of mitochondrial macromolecules

Structural Biology Unit

A unique feature of mitochondria is that they possess their own genome, the mitochondrial DNA (mtDNA), which codes for subunits of the electron transport chain that eventually leads to the synthesis of the ATP molecule. Dysregulation of this genome causes complex diseases and syndromes that are difficult to diagnose and treat. These are mostly classified as rare diseases. In the Structural MitoLab,we are interested in the structural basis underlying mtDNA regulation and packaging. Our research is multidisciplinary and combines macromolecular X-ray crystallography with biochemical and biophysical analyses (chromatography, electrophoresis, SAXS, MALLS, etc.), which can be completed with additional techniques in collaboration with other groups.

Our group is interested in understanding the structural basis of mitochondrial genome regulation and focuses on the following research lines.

1. Transcription of the mtDNA strands
2. The replicative helicase Twinkle
3. Termination factor mTERF, which functions as a roadblock, preventing collision between transcription and replication machineries by binding to specific sites ofmtDNA.

The structural MitoLab is headed by Dr. Maria Solà. After a tenure-tracked period of four years funded by a Ramón y Cajal contract, Maria Solà was awarded a permanent position as tenured Assistant Professor at the Spanish Research Council (Consejo Superior de Investigaciones Científicas, CSIC) in 2008, joining the Institute of Molecular Biology Barcelona (Institut de Biologia Molecular Barcelona, IBMB), where she established a research laboratory on the structural biology of mitochondrial DNA regulation.

GROUP LEADER
Maria Solà

POSTDOCTORAL RESEARCHERS
Hida Yuliana

PhD STUDENTS
Aleix Tarrés
Claus Schmitz
Arkadiusz Szura
Ramiro Illanes

TECHNICIAN
Elena Ruiz

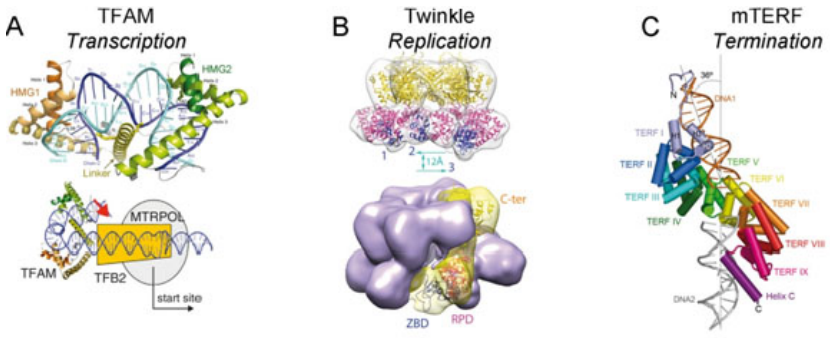
PUBLICATIONS

Rubio-Cosials A, Battistini F, Gansen A, Cuppari A, Bernadó P, Orozco M, Langowski J, Tóth K, **Solà M.** Protein Flexibility and Synergy of HMG Domains Underlie U-Turn Bending of DNA by TFAM in Solution. Biophys J. 2018 May 22;114(10):2386-2396.

Lyonnais S, Tarrés-Solé A, Rubio-Cosials A, Cuppari A, Brito R, Jaumot J, Gargallo R, Vilaseca M, Silva C, Granzhan A, Teulade-Fichou MP, Eritja R, **Solà M**. The human mitochondrial transcription factor A is a versatile G-quadruplex binding protein. Sci Rep. 2017 Mar 9;7:43992. Erratum in: Sci Rep. 2017 Apr 06;7:45948.

Chakraborty A, Lyonnais S, Battistini F, Hospital A, Medici G, Prohens R, Orozco M, Vilardell J, **Solà M**. DNA structure directs positioning of the mitochondrial genome packaging protein Abf2p. Nucleic Acids Res. 2017 Jan 25;45(2):951-967.

1



1 Gallery of representative projects of the Structural Mitolab.(A) Mitochondrial Transcription Factor A, TFAM. Top panel, the domains of TFAM, HMGbox1 and HMGbox2 are depicted in orange and green respectively; the connecting Linker is in yellow. TFAM's cognate DNA sequence at LSP is represented in blue. Bottom, representation of the DNA U-turn imposed by TFAM at the transcription initiation site ('start site'), to which mtRNA polymerase (MTRPOL) and transcription factor B2 (TFB2) bind. The red arrow signals the position of TFAM's C-terminal tail and its interaction with the transcription machinery. (B) Replicative helicase Twinkle. The top panel shows the different domains of a homology model based on the T7 phage gp4 helicase and E.coli primase fitted into the EM map (1Q57 and 2AU3 PDB codes, respectively). The Zinc-binding domain (ZBD) is shown in blue, the RNA-polymerase domain (RPD) in magenta, and the C-terminal domain in yellow; ZBDs 2 and 3 show different relative positions. Bottom: The EM map is represented in purple and the contacts between subunits are shown. (C) Mitochondrial Termination Factor mTERF. The different TERF motifs I – IX from the termination factor mTERF are represented. Two DNA molecules (one from the asymmetric unit and the second from a symmetry mate) lay along the central axis of the Zurdo left-handed domain.

Crystallographic methods

Structural Biology Unit

Our group pursues two distinct, but nevertheless, complementary kinds of scientific goals. On one hand the development of crystallographic methods for the solution of macromolecular structures and on the other their application on projects of medical, biological or biotechnological relevance.

Crystallography allows us to see with our own eyes the main players in biological processes, even in functional complexes: we can see how molecules work. Nevertheless, this structural model cannot be directly calculated from the experimental data as we cannot measure the phases of the diffracted X-ray beams. Pushing the limits to solve this “phase problem” is the main aim of my research. Our program ARCIMBOLDO, downloaded by over 300 laboratories worldwide has allowed to phase ab Initio equal atom structures over twice as large and at resolutions of average, rather than exceptional experimental quality than previous methods. Our program SUBIX introduces a new approach to solve the structures of nucleic acids and their complexes, relying rather than on sequence-based molecular replacement, on a prediction of the packing accounting for the geometrical constraints of the unit cell and symmetry and taking into account the main kinds of interactions driving nucleic acid structure.

Our goal is to provide computational methods for macromolecular crystallography to better exploit the available experimental diffraction data and previous chemical knowledge resulting in the most effective, accurate, model-bias free and user-friendly structural determination, both in typical average cases and problem structures.

GROUP LEADER
Isabel Usón

RESEARCH ASSOCIATE
Nicolas Soler

POSTDOCTORAL RESEARCHERS
Giovanna Petrillo
Claudia Millán

PhD STUDENTS
Iracema Caballero
Ana del Rocio

PUBLICATIONS

Dunce JM, Dunne OM, Ratcliff M, Millán C, Madgwick S, **Usón I**, Davies OR. Structural basis of meiotic chromosome synapsis through SYCP1 self-assembly. Nat Struct Mol Biol. 2018 Jul;25(7):557-569.

Gordo V, Aparicio D, Pérez-Luque R, Benito A, Vilanova M, Usón I, **Fita I**, Ribó M. Structural Insights into Subunits Assembly and the Oxyester Splicing Mechanism of Neq pol Split Intein. Cell Chem Biol. 2018 Jul 19;25(7):871-879.e2.

Millán C, Sammito MD, McCoy AJ, Nascimento AFZ, Petrillo G, Oeffner RD, Domínguez-Gil T, Hermoso JA, Read RJ, **Usón I.** Exploiting distant homologues for phasing through the generation of compact fragments, local fold refinement and partial solution combination. Acta Crystallogr D Struct Biol. 2018 Apr 1;74(Pt 4):290-304.

McCoy AJ, Oeffner RD, Millán C, Sammito M, **Usón I**, Read RJ. Gyre and gimble: a maximum-likelihood replacement for Patterson correlation refinement. Acta Crystallogr D Struct Biol. 2018 Apr 1;74(Pt 4):279-289.

Oeffner RD, Afonine PV, Millán C, Sammito M, **Usón I**, Read RJ, McCoy AJ. On the application of the expected log-likelihood gain to decision making in molecular replacement. Acta Crystallogr D Struct Biol. 2018 Apr 1;74(Pt 4):245-255.

Lee M, Batuecas MT, Tomoshige S, Domínguez-Gil T, Mahasenan KV, Dik DA, Hesek D, Millán C, **Usón I**, Lastochkin E, Hermoso JA, Mobashery S. Exolytic and endolytic turnover of peptidoglycan by lytic transglycosylase Slt of *Pseudomonas aeruginosa*. Proc Natl Acad Sci U S A. 2018 Apr 24;115(17):4393-4398.

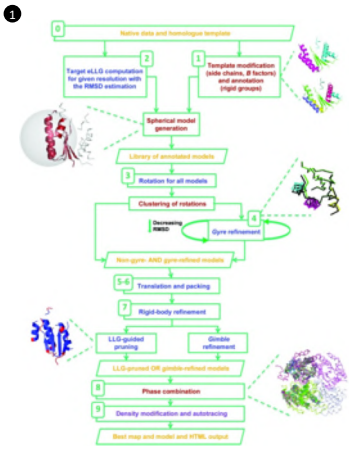
Usón I, Sheldrick GM. An introduction to experimental phasing of macromolecules illustrated by SHELX; new autotracing features. Acta Crystallogr D Struct Biol. 2018 Feb 1;74(Pt 2):106-116.

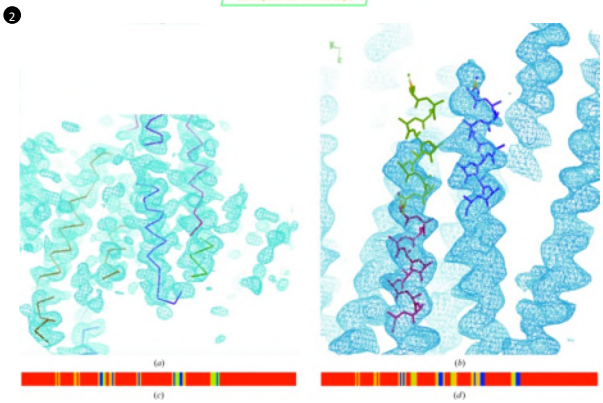
Caballero I, Sammito M, Millán C, Lebedev A, Soler N, **Usón I**. ARCIMBOLDO on coiled coils. Acta Crystallogr D Struct Biol. 2018 Mar 1;74(Pt 3):194-204.

Bustamante N, Iglesias-Bexiga M, Bernardo-García N, Silva-Martín N, García G, Campanero-Rhodes MA, García E, **Usón I**, Buey RM, García P, Hermoso JA, Bruix M, Menéndez M. Deciphering how Cpl-7 cell wall-binding repeats recognize the bacterial peptidoglycan. Sci Rep. 2017 Nov 28;7(1):16494.

Hernández K, Parella T, Petrillo G, **Usón I**, Wandtke CM, Joglar J, Bujons J, Clapés P. Intramolecular Benzoin Reaction Catalyzed by Benzaldehyde Lyase from Pseudomonas Fluorescens Biovar I. Angew Chem Int Ed Engl. 2017 May 2;56(19):5304-5307.

Pomowski A, **Usón I**, Nowakowska Z, Veillard F, Sztukowska MN, Guevara T, Goulas T, Mizgalska D, Nowak M, Potempa B, Huntington JA, Potempa J, Gomis-Rüth FX. Structural insights unravel the zymogenic mechanism of the virulence factor gingipain K from *Porphyromonas gingivalis*, a causative agent of gum disease from the human oral microbiome. J Biol Chem. 2017 Apr 7;292(14):5724-5735.





1 ARCIMBOLDO_SHREDDER workflow. The numbers reference the steps described in §3.1. Orange colour refers to input/output, blue to Phaser steps, red to ARCIMBOLDO steps and purple to SHELXE steps.

2 Issues in map tracing and additional constraints. (a) The trace corresponding to an electron-density map of the coiled coil autophagy-related protein 38 at 2.4 Å resolution illustrates how the helical geometry is degraded in the regions where the map is poor. (b) shows how an originally placed seed (lime) continuing a correct seed (red) is refined by the unrestrained simplex into the density of a neighbouring helix (purple). The neighbouring helix happens to be in the reversed direction. (c) Seed coverage in the first autotracing cycle of fibronectin for the unrestrained versus (d) restrained simplex refinement of β-sheets. In (c) and (d) the sequence numbers increase from left to right and the r.m.s.d. of the seeds versus the correct structure is represented in blue (<0.36 Å), green (0.5 Å) and yellow (<1 Å). Restraining the simplex refinement of the seeds (d) renders a better and more accurate coverage than in (c).

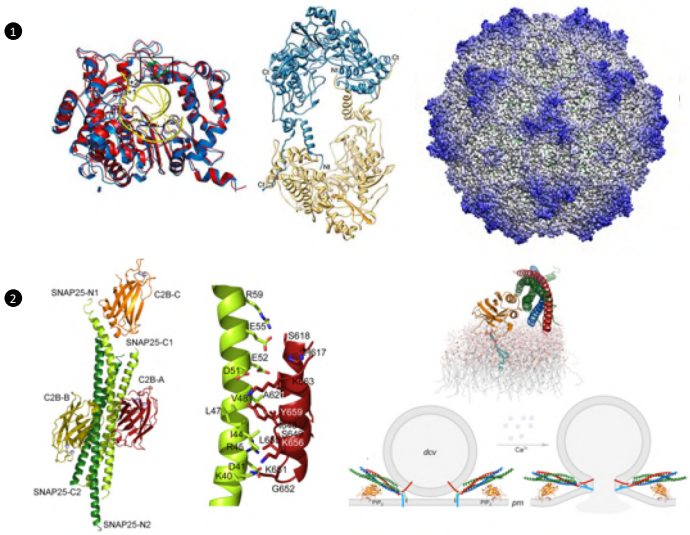
Virus and large Biologial Complexes

Structural Biology Unit

Our research focuses on the **structure-function relationships of proteins and macromolecular complexes directly implicated in pathological processes.** In particular, **virus particles, viral proteins and protein-complexes** involved in RNA replication as well as other large macromolecular assemblies. The information obtained would be instrumental not only for improving the knowledge about the functioning of these biological systems but also in the development of new therapeutic strategies. We use a number of **molecular and structural biology techniques.** In particular, X-ray crystallography and combinations of crystallography and cryo-electron microscopy. Our approaches usually start with the cloning of relevant genes and the expression and purification of the encoded proteins. **Proteins, nucleic acids and their complexes are then crystallized and analyzed by X-ray diffraction**. The final outcome is a detailed three dimensional view of the molecular structures at atomic resolution.

Our research interests cover three main topics:

a. **The structural and functional studies of proteins involved in replication of RNA viruses,** including: capsid dynamics, RNA uncoating and protein priming and nucleotide incorporation by RNA-dependent RNA polimerases.
b. **Structural analyses of the vault ribonucleoprotein complex,** including structural dynamics of the vault shell and the minor vault components: TEP1, VPARP and vRNA.
c. **Peripheral membrane proteins involved in membrane fusion.** In particular, we aim to elucidate how Rabphilin 3A (Rph3A), a protein involved in synaptic vesicle fusion, coordinates its interactions with Ca2+, the phosphoinositide PIP2 and the SNARE complex to control key events at the presynaptic neuron.



1 Structures of RNA-dependent RNA polimerases from two single stranded RNA viruses: the 3Dpol-RNA complex of foot-and-mouth disease virus (Left) and the RdRP from the permutatetravirus TaV (middle). The right panel shows the capsid structure of the minute virus of mice.

2 X-ray structures of the C2B domain of Rph3A in complex with the SNARE component SNAP25 (left). The right panel shows comparative binding models of the Rph3A C2B–SNARE and Sytl C2B–SNARE complexes on the plasma membrane, showing that Rph3A adopts a specific PIP2-dependent conformation at the membrane that facilitates the interaction, not only with SNAP25 molecules but also with the SNARE complex, shedding new light to understand the fine tuning control in the vesicle fusion process.

GROUP LEADER
Núria Verdaguer

RESEARCH ASSOCIATE
Cristina Ferrer

POSTDOCTORAL RESEARCHERS
Diego S. Ferrero
Pablo Guerra

PhD STUDENTS
Victor M. Arroyo
María González

MASTER'S STUDENT
Nastasia Ablanque

TECHNICIAN
Mònica Buxaderas

PUBLICATIONS

de la Higuera I, Ferrer-Orta C, Moreno E, de Ávila AI, Soria ME, Singh K, Caridi F, Sobrino F, Sarafianos SG, Perales C, **Verdaguer N**, Domingo E. Contribution of a Multifunctional Polymerase Region of Foot-and-Mouth Disease Virus to Lethal Mutagenesis. *J Virol.* 2018 Sep 26;92(20). pii: e01119-18.

Ferrero D, Ferrer-Orta C, **Verdaguer N**. Viral RNA-Dependent RNA Polymerases: A Structural Overview *Subcell Biochem*. 2018;88:39-71.

Ferrer-Orta C, Pérez-Sánchez MD, Coronado-Parra T, Silva C, López-Martínez D, Baltanás-Copado J, Gómez-Fernández JC, Corbalán-García S, **Verdaguer N**. Structural characterization of the Rabphilin-3A-SNAP25 interaction. *Proc Natl Acad Sci U S A.* 2017 Jul 3;114(27):E5343-E5351.

Miras M, Truniger V, Silva C, **Verdaguer N**, Aranda MA, Querol-Audí J. Structure of eIF4E in Complex with an eIF4G Peptide Supports a Universal Bipartite Binding Mode for Protein Translation. *Plant Physiol.* 2017 Jul;174(3):1476-1491

Guerra P, Valbuena A, Querol-Audí J, Silva C, Castellanos M, Rodríguez-Huete A, Garriga D, Mateu MG, **Verdaguer N**. Structural basis for biologically relevant mechanical stiffening of a virus capsid by cavity-creating or spacefilling mutations. *Sci Rep*. 2017 Jun 22;7(1):4101.

de la Higuera I, Ferrer-Orta C, de Ávila AI, Perales C, Sierra M, Singh K, Sarafianos SG, Dehouck Y, Bastolla U, **Verdaguer N**, Domingo E. Molecular and Functional Bases of Selection against a Mutation Bias in an RNA Virus. *Genome Biol Evol*. 2017 May 1;9(5):1212-1228.

Facilities

Molecular imaging platform

Facilities

The Molecular Imaging Platform (MIP IBMB-PCB) has been set up as a co-operation between the Molecular Biology Institute of Barcelona (IBMB) and the Barcelona Scientific Park (PCB). It offers a collection of state-of-the-art light microscopy equipment, including high-resolution and high-speed confocal microscopy, multiphoton microscopy, automated wide-field imaging, fluorescence correlation spectroscopy and image processing tools.

Available applications

- Automated Wide-Field Microscopy
- Laser Scanning Confocal Microscopy
- Spinning Disk Confocal Microscopy
- Multiphoton Microscopy
- Fluorescence Correlation Spectroscopy
- Image processing and analysis

PLATFORM HEAD
Elena Rebollo

RESEARCH TECHNICIAN
Jaume Boix

CONFOCAL & MULTIPHOTON MICROSCOPES

ZEISS LSM 780 MULTIPHOTON

- Motorized inverted Zeiss Axio Observer Z1
- Objectives: 10x Air, 25x Multi Imm., 32x W MP, 40x Oil DIC, 40x water DIC FCS MP, 63x Oil DIC, 63x Water DIC
- Laser lines (nm): 405, 458, 488, 514, 561, 594, 633, 690-1040 IR
- Detectors: 3 fluorescence detectors (2PMTs and a GaAsP array) and 1 transmission detector for DIC imaging
- Galvanometric Scanner up to 6144x6144 pixels, free rotation.
- Scanning x-y stage for multiposition acquisition
- Environmental control (temperature and CO2) for live specimens
- Acquisition Software ZEN
- Applications: Optical sectioning, multi channel 3D imaging and time lapse, FRAP, FRET, FLIP, FCS, Multiphoton

LEICA TCS SP5 CONFOCAL

- Motorized inverted Leica DMI6000
- Objectives: 10x Air, 20x Air, 40x Oil DIC, 63x Oil DIC
- Laser lines: 405, 458, 476, 488, 496, 514, 561 and 633 nm
- Detectors: 3 fluorescence PMTs and 1 transmission PMT for trans imaging
- Galvanometric Scanner up to 2800 Hz.
- Galvanometric Z-stage for fast z-stepping
- Scanning x-y stage for multiposition imaging
- Environmental control (temperature and CO2) for live specimens
- Acquisition Software Las AF
- Applications: Optical sectioning, multi channel 3D imaging, time lapse, FRAP, FRET, FLIP

ULTRAVIEW ERS6 SPINNING DISK

- Manual inverted Zeiss Axiovert 200
- Objectives: 10x Air Ph1, 20x Air, 40x Oil, 63x Oil, 100x Oil
- Laser lines: 405, 440, 488, 514, 568 and 640 nm
- Cool-CCD high sensitivity camera
- Fast Spinning disk (5000rpm), MODEL CSU22.
- Filter sets suited for Dapi, CFP, GFP, YFP, dsRED, mCherry, mPlum or DRAQ5
- Fast z-stepping driven by a piezo objective
- Scanning x-y stage for multiposition imaging
- Environmental control (temperature and CO2) for live specimens
- Photokinesis Unit for laser bleaching
- Acquisition Software Volocity
- Applications: Optical sectioning, multichannel 3D imaging and time lapse, FRAP/Photoconversion

MOTORIZED WIDE-FIELD MICROSCOPE

LEICA AF7000

- Motorized inverted Leica DMI6000
- Objectives: 5x Air, 10x Air, 20x Air DIC, 40x Oil DIC, 63x Oil DIC, 100x Oil DIC, 100 Oil Ph3
- Filtersets suited for Dapi, CFP, GFP, YFP, Kusabira Orange, mCherry, Alexa 555, RFP and Cy5
- External fast filter wheel for excitation filter CFP, GFP, YFP and m-Cherry.
- Illumination source: SOLA SE II White Led 375-675 nm
- Monochrome camera: ORCA R2 Hamamatsu
- Color camera: DFC-550 Real Leica
- Environmental control (temperature and CO2) for live specimens
- Acquisition software Leica LasX
- Applications: motorized fluorescence multichannel wide-field acquisition, motorized color acquisition, time-lapse assays, screening.

IMAGE ANALYSIS WORKSTATIONS

WORKSTATION 1

- Huygens professional (confocal module)
- LAS AF Simulator (SP5, AF7000)
- Metamorph (Leica)
- Imaris (basic)
- FijiImageJ

WORKSTATION 2

- VOLOCITY off-line
- LAS AF Simulator (SP5, AF7000)
- FijiImageJ
- Zen Blue lite
- Cell profiles

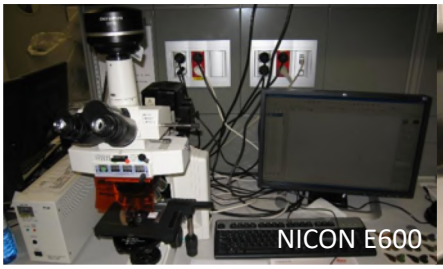
MANUAL WIDE FIELD MICROSCOPES

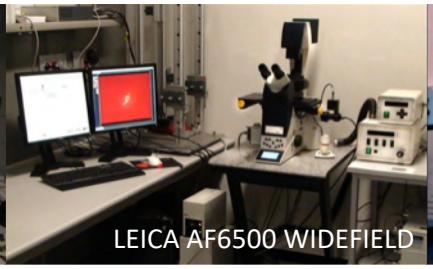
NIKON E-600

- Manual upright Nikon E-600
- Objectives: 4x Air, 10x Air Ph1, 20x Air Ph1, 40x Air, Ph2 63x Oil Ph2, 100x Oil Ph3
- Filtersets for DAPI, FITC, GFP and TRITC
- Color CCD digital camera
- Acquisition software: Cell-F

LEICA DM-IRBE

- Manual Inverted Leica DM-IRBE
- Objectives: 10x Air Ph1, 20x Air Ph1, 40x Air Ph2, 63x Air Ph2, long working distance
- Filtersets for DAPI, FITC and TRITC
- Monochromatic CCD digital camera
- Acquisition Software Metamorph

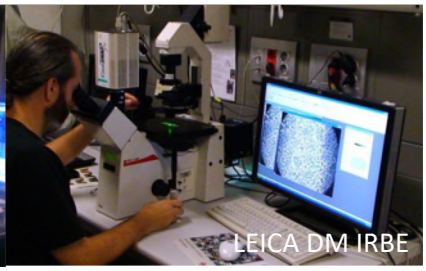

NICON E600


LEICA AF6500 WIDEFIELD


SPINNING DISK CONFOCAL


LEICA TCS-SP5 CONFOCAL


ZEISS LSM780 CONFOCAL & MULTIPHOTON


LEICA DM IRBE

PUBLICATIONS

Marsal M, Jorba I, **Rebollo E**, Luque T, Navajas D, Martín-Blanco E. AFM and Microrheology in the Zebrafish Embryo Yolk Cell. J Vis Exp. 2017 Nov 29;(129). doi: 10.3791/56224.

Encinar Del Dedo J, Idrissi FZ, Fernandez-Golbano IM, Garcia P, **Rebollo E**, Krzyzanowski MK, Grötsch H, Geli MI. ORP-Mediated ER Contact with Endocytic Sites Facilitates Actin Polymerization. Dev Cell. 2017 Dec 4;43(5):588-602.e6.

Publications that mentioned the Molecular Imaging Platform.

Asensio-Juan E, Fueyo R, Pappa S, Iacobucci S, Badosa C, Lois S, Balada M, Bosch-Presegue L, Vaquero A, Gutierrez S, Caelles C, Gallego C, de la Cruz X, Martinez-Balbas MA. The histone demethylase PHF8 is a molecular safeguard of the IFNgamma response. Nucleic Acids Res. 2017 Jan 18.

Pons M, Izquierdo I, Andreu-Carbo M, Garrido G, Planaguma J, Muriel O, Del Pozo MA, Geli MI, Aragay AM. Phosphorylation of filamin A regulates chemokine receptor CCR2 recycling. J Cell Sci. 2017 Jan 15;130(2):490-501.

Hernandez-Vega A, Marsal M, Pouille PA, Tosi S, Colombelli J, Luque T, Navajas D, Pagonabarraga I, Martin-Blanco E. Polarized cortical tension drives zebrafish epiboly movements. EMBO J. 2017 Jan 04;36(1):25-41.

Automated crystallography platform

Facilities

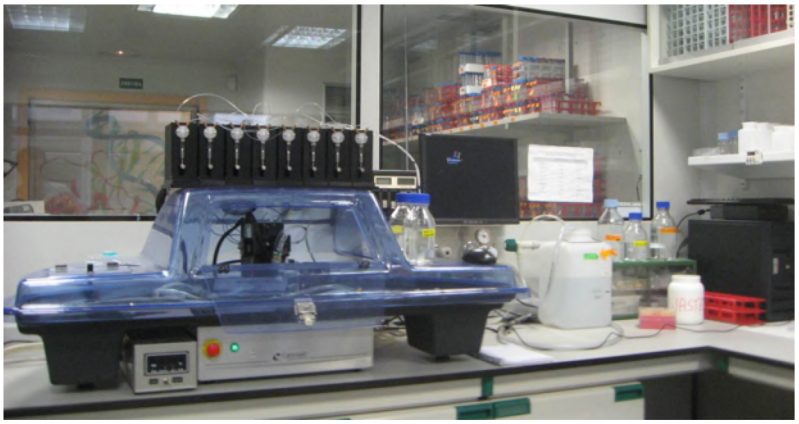
The Automated Crystallography Platform (PAC) is a technology platform that offers researchers the necessary tools to find adequate crystallization conditions from a purified protein sample, followed by X-Ray diffraction data collection and processing of the resulting macromolecular crystals. The PAC Facility is shared with the Institute for Research in Biomedicine (IRB).

PLATFORM HEAD
Joan Pous

RESEARCH TECHNICIAN
Xandra Kreplin

Services offered

- Protein crystallization.
- X-ray diffraction.
- SEC-MALS analysis of biological macromolecules
- Use of equipment on a self-service basis for users in academia and for private companies.
- Equipment use for private companies.
- Design and development of research projects.
- Scientific and technical consulting.



Protein Purification service

Facilities

PLATFORM HEAD
Roman Bonet

The Protein Purification Service (PPS) at Barcelona Scientific Park provides customers reliable analytical and preparative purification tools to be run at room temperature and at 4ºC. The service is supervised by personnel from the SBU.

Equipments are used on a self-served basis. Personal advising and troubleshooting are offered by our personnel.

Equipment available:

- 3 AKTA Purifier 10
- AKTA Purifier 10 with sample pump P-950
- AKTA Purifier 10 with sample pump P-960 + air-sensor
- AKTA Purifier 10 with sample pump P-960 + air-sensor @4ºC
- 1 AKTA FPLC
- 1 AKTA Explorer 100
- 1 BioRad NGC QUEST+ with sample pump + air sensor

Facts and figures

Publications

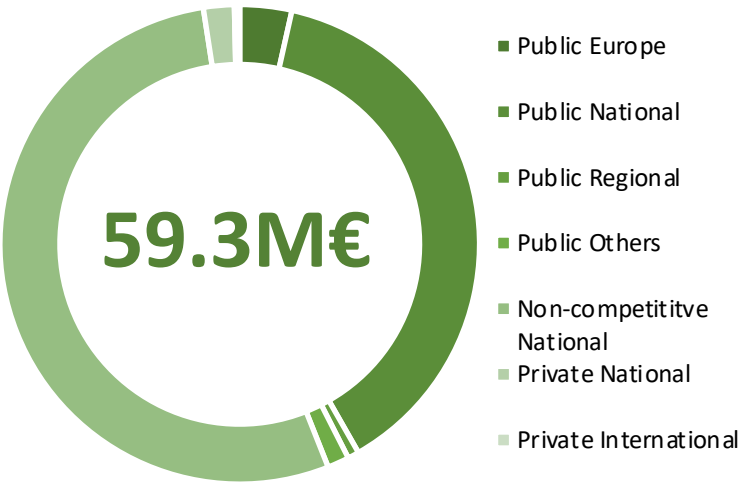
137 Total publications

47% Open Access

91% Publications Q1

77% Publications D1

Funding



Staff

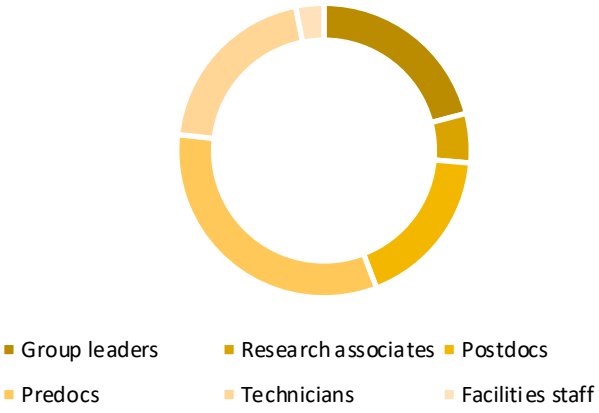
141 Total

129 Research staff

12 Administrative staff

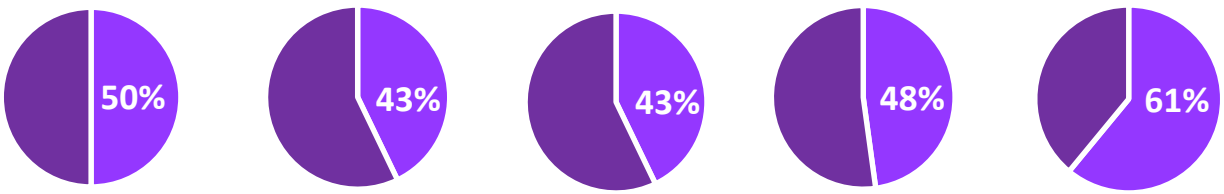
12 Research groups

Research categories



Gender

FEMALE BY PROFESSIONAL CATEGORIES



HEADS OF DEPARTMENT | GROUP LEADERS | RESEARCH ASOCIATES | POSTDOCS | PREDOCS

Direction and management

Direction and management

DIRECTOR

Prof. Núria Verdaguer

VICEDIRECTORS

Prof. M. Isabel Geli
Prof. Josep Vilardell
Prof. Mariona Arbonés

ADMINISTRATION MANAGING

Olga Gallardo

HUMAN RESOURCES

Leonor Calopa

PROJECT MANAGEMENT

Adélaïde saint-Léger
Mónica Aceves
Carme Llorach

ACCOUNTING OFFICE

Gemma Molla
Alex Dopico

PURCHASING OFFICE

Joan Lambea
Jaume Vaqué

INFORMATION TECHNOLOGY SERVICES

Luis Fernando Montoya
Marc Hsiao
Dani Butjosa

OUTREACH AND COMMUNICATION OFFICE

Laia Vives

Communication and Outreach

Communication is part of the IBMB vision. The institute has a number of Program Seminars to foster scientific discussion. These Programs are organized by departments or by the Institute, can be co-organized with other research institutions, and include a monthly series run by the PhD Students.

In addition to this, the IBMB strives to spread its findings by participating in scientific meetings, including those devoted to training present and future scientists. Importantly, the institute aims to contribute to society's knowledge on current, critical, questions that we are facing, and outreach activities have greatly benefited by the "María de Maeztu" award. Some of these activities target early audiences: "Microbes" is addressed to children from 4 to 7 years old. Currently being offered to primary schools, it recruits more than 200 participants per year. The IBMB is part as well of the BAXT2LAB Project, aimed at high school students, from the 'Research in Society' Program of the Barcelona Science Park. The Institute participates regularly at two Science Fairs every year, the "Saló de l'Ensenyament", organised by Barcelona's Council, and the "Fira Recerca en Directe", organised by the Barcelona Science Park.

All these efforts go together with the use of the new technologies and social network platforms.

Weekly Programme Seminars

SBU Annual Scientific Meetings

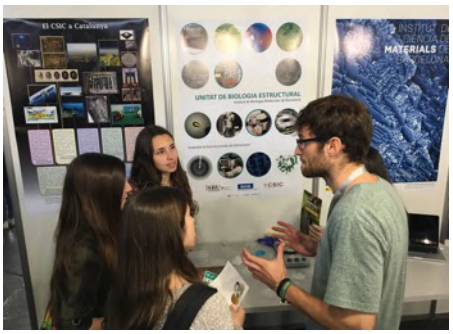
IBMB Christmas Meetings

International **Crystallography** School Madrid

Conference on methods and Applications in the frontier of MX and CryoEM

Symposium "30 years of Macromolecular Crystallography in Barcelona Ignasi Fita's 65th birthday

Fair “Research in Live” (2017 and 2018) Cosmocaixa-Barcelona



Fair “Professional Education” (2017 and 2018) Barcelona

Primary School activity: MICROBES

BATX2LAB Project

Dissemination talks to general public

Institut de Biologia Molecular de Barcelona
Parc Científic de Barcelona
C/ Baldiri Reixac, 4 Torre R 3a planta
08028 Barcelona