

SCIENTIFIC
REPORT
2023-2024

Decoding life

Director Report



Paola Bovolenta
CBM Director

In March 2023, I assumed the role of Director of the Centro de Biología Molecular Severo Ochoa (CBM), succeeding Lourdes Ruiz-Desviat, who led the centre with dedication for more than four years. I would like to express my sincere gratitude to her for her commitment and contributions during this period, including the first two months of 2023.

It is now my turn and my pleasure to introduce this 2023–2024 report, covering a period of significant changes and new activities at the CBM. These two years have been marked by an important reorganization and a variety of scientific and institutional milestones. All of these initiatives have been made possible thanks to the constant support of the CBM Deputy Director, Beatriz López-Corcuera, the guidance of the Internal Scientific Advisory Committee, the commitment of the CBM Manager, German Lerma, and the generous, dedicated contributions of many CBM faculty members. Equally essential has been the continuous and efficient support of the CBM's administrative and technical services, whose professionalism and dedication have been fundamental to the successful implementation of many of the activities outlined in this report, in addition to their daily responsibilities that are critical for sustaining research at CBM.

A key development has been the implementation of a new collaboration agreement between our parent institutions, the CSIC and the UAM. This agreement introduces internal regulations specific to the CBM, which have led to important updates in our governance structure. These include the establishment of a Supervisory Board, a revised Steering Committee, and a new Internal Scientific Advisory Committee. We also began the implementation of the scientific program proposed in our Centre of Excellence project, launching several associated initiatives. Another highlight of this period was the visit, in May 2023, of our External Scientific Advisory Board (SAB). The visit included interviews with the majority of CBM principal investigators, and culminated in a detailed report. This report offered valuable general recommendations regarding the organization and scientific activity of the CBM, as well as individual feedback for PIs.

As a result of these developments, and with the support of the Internal Scientific Advisory Committee, the Direction has undertaken a major reorganization of laboratory space. This redistribution follows newly established internal criteria, aiming to support the growth of research groups expanding their activities, while promoting interaction and mentorship between senior researchers and recently appointed PIs. We have also made a concerted effort to optimize the use of shared spaces and equipment, enhancing access and efficiency for all researchers across the centre.

A second major area of focus has been the optimization of our scientific and technical services, through the acquisition of new equipment and—perhaps more importantly—the incorporation of new staff. While I cannot mention every change in this introduction, I will highlight the most significant ones. Both the Electron Microscopy and Protein Biotechnology services have welcomed new facility heads, and several other services have added staff, where possible, to enhance their operations. A particularly noteworthy achievement has been the establishment of a joint Proteomics Service in collaboration with the neighbouring CNB, physically located within the CNB facilities. This joint service now benefits from the support of CBM staff, whose expertise has been essential in managing the high volume of requests. Both the CBM and CNB leadership are especially proud of this collaborative effort, which we see as a model for future service optimizations. We have also restructured the former Genomics/NGS Service into two independently managed units: Functional Genomics and Biocomputational Analysis. This division has significantly improved efficiency and specialization. The Functional Genomics service has implemented several new technologies, including RNA-seq and ChIP-seq library preparation, as well as various approaches to single-cell transcriptomics—supported by the acquisition of dedicated equipment. Meanwhile, the Biocomputational Analysis service has enhanced its computational pipelines and developed custom software solutions tailored to specific user needs. In addition, the Advanced Light Microscopy service has expanded its microscopy capabilities with the incorporation of a light-sheet system, broadening the range of imaging techniques available at the centre. Other important improvements include the expansion and reorganization of services offered by the Protein Biotechnology and Preclinical Biomedicine platforms, further strengthening our technical infrastructure.

The CBM has a long-standing tradition in scientific outreach, and in recent years, we have taken a significant step forward by formally establishing a dedicated Communications and Science Outreach office. This initiative has greatly enhanced both our internal and external communication efforts and significantly expanded the number and scope of outreach activities in which the CBM participates or leads. A full list of activities is included in this report, but I wish to highlight the tremendous success of the “Ciencia Contigo” events—particularly the 2024 edition focused on “The Microlife”. Held at the Caixaforum in Madrid, the event attracted more attendees than the auditorium could seat, demonstrating the growing public interest in our initiatives.

The creation of this office has also had a positive impact on our predoctoral and postdoctoral communities, encouraging early-career researchers to take an active role in organizing events and engaging with institutional life. One outstanding outcome of this empowerment is the formation of a strong and dynamic Predoc-Postdoc Committee, which has significantly enriched the scientific and social life of the centre. It is a pride to have such an active collective of early career scientists. Congratulations to all involved!

The work of early-career researchers at the CBM has been further supported by the creation of the Train@CBM program, which includes a variety of activities designed to complement the PhD training provided by the University. Each doctoral student is also assigned a PhD Thesis Advisory Committee to help ensure the successful development and completion of their research project. An associated innovation has been the establishment of the Help@Hand office, which serves as a first point of contact for addressing academic or workplace-related concerns of PhD students and postdoctoral researchers. Training the next generation of scientists remains a core mission of the CBM. Many of our faculty members actively participate in undergraduate and graduate teaching, and the centre regularly hosts a large number of summer as well as bachelor and master's students, who engage in hands-on research and complete their final degree projects (TFG and TFM) within our laboratories.

I wish to take this opportunity to express the CBM's—and my own—gratitude to the members of the Equality and Sustainability Committees. The first, active since 2021, continues to position the CBM at the forefront of innovation in this area through meaningful initiatives and educational activities. The Sustainability Committee, although less than a year old, has already made a significant impact by raising awareness of the critical importance of sustainability in both everyday life and scientific practice.

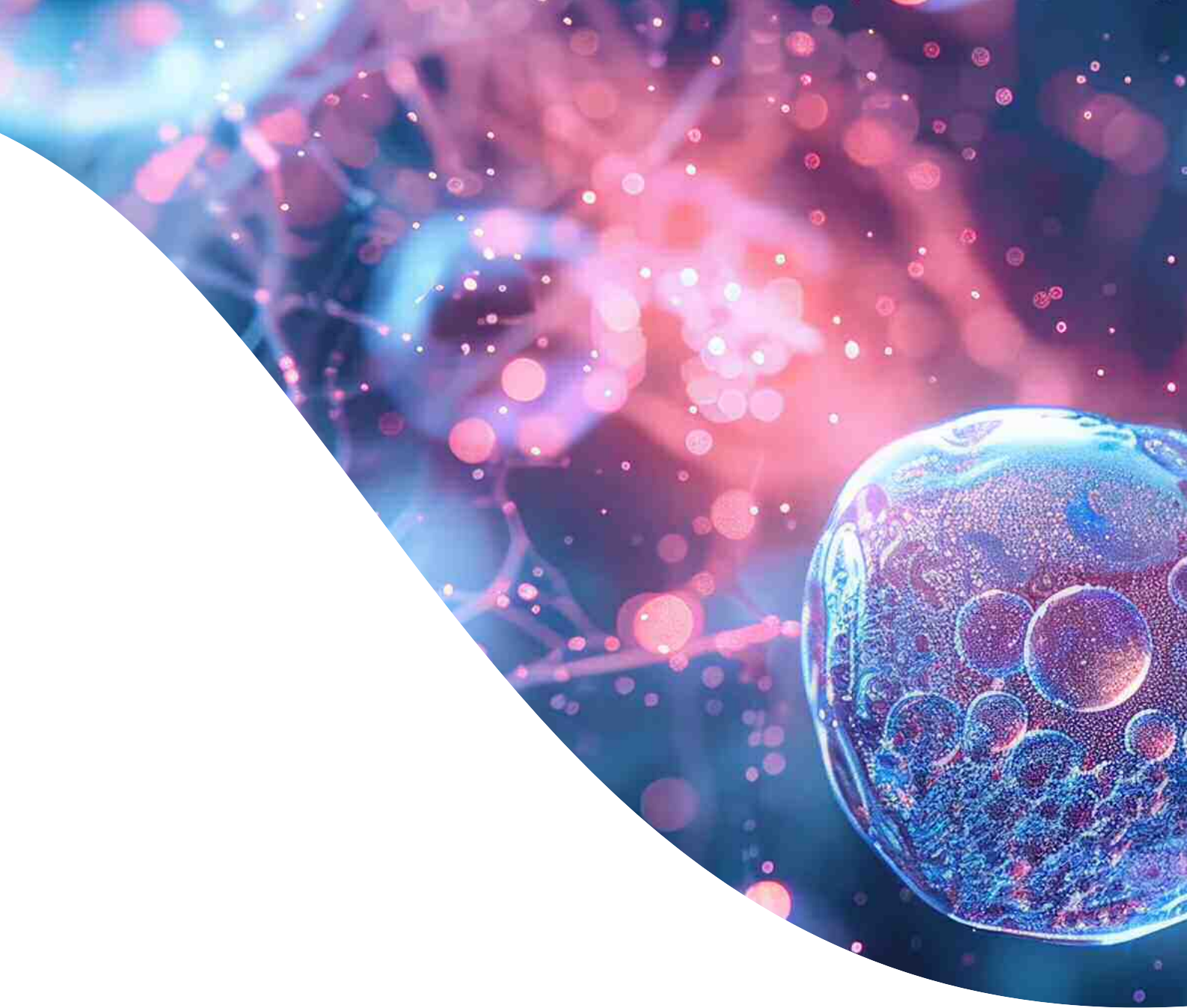
The past reorganization of the CBM into four scientific programs and eight research units, along with the incorporation of highly promising tenure-track scientists—many of whom have already secured permanent positions—had already significantly revitalized the scientific life of the centre. To maintain this momentum and foster collaboration, we launched an “Intramural Exploratory Grants Program Severo Ochoa” and a dedicated “Postdoctoral Call” aimed at attracting international talent. Both initiatives were supported through funding from the Severo Ochoa Centre of Excellence grant and awarded based on the evaluation and recommendations of our SAB, to whom we are deeply grateful for their continued support. During this period, five new tenure-track scientists joined the CBM.



As previously noted, eight non-tenured researchers secured permanent positions, and five additional tenured researchers at various career stages were incorporated into the CBM. Scientific accomplishments include securing highly competitive national and international grants, such as ERC Grants. In total, the CBM produced over 500 scientific publications during this time, many of which appeared in leading international journals, as detailed in the individual team reports. A substantial number of CBM researchers have received national and international awards, distinctions, and have taken on key leadership and management roles in the scientific community. Their efforts have also translated into successful knowledge transfer activities, establishing valuable connections with both the clinical and industrial sectors, as highlighted in the following pages.

I would like to close this introduction by acknowledging those who have left the CBM over the past period. Many have been students or postdoctoral researchers who have moved on to continue their careers at other research institutions or have chosen to pursue new directions in related fields. To all of them, we extend our best wishes for the journey ahead. Several more senior colleagues have entered retirement. Both the CBM and I are deeply grateful for their dedication, time, and passion throughout the years. We will miss them all, but we also wish them the best in this new—and hopefully more relaxed—chapter of their lives. Sadly, a few members of our community have passed away. Their absence is deeply felt, and we have highlighted their invaluable contributions to the CBM and to Spanish science in the following pages.

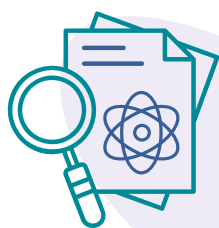




The CBM in a nutshell



2023-24 IN NUMBERS



600
scientific
publications

31

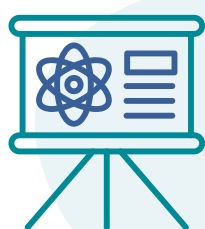
awards to
our staff



28,6
million euros obtained
in competitive funding



45
nationalities



211
scientific
seminars



42
patent
applications



+4500 attendees to our science
outreach activities & events



+19.000

followers gained across
social media platforms



55.547

website visits



946

CBM staff



67

PhD theses
completed



223

degree and master's
theses completed



110

outreach
volunteers



13

projects with
industry

PEOPLE AT THE CBM



SCIENTIFIC STAFF

Principal investigators (PIs)

▶ Emeritus/Ad honorem 8

▶ Senior Group Leaders¹ 95

▶ Tenure Track Group Leaders² 9

112

Staff scientist

49

Postdoctoral researchers

102

PhD researchers

235

Undergraduate students

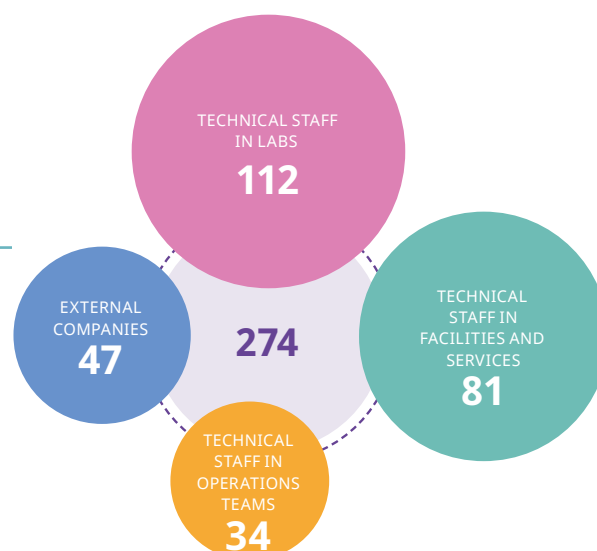
219

TOTAL 717

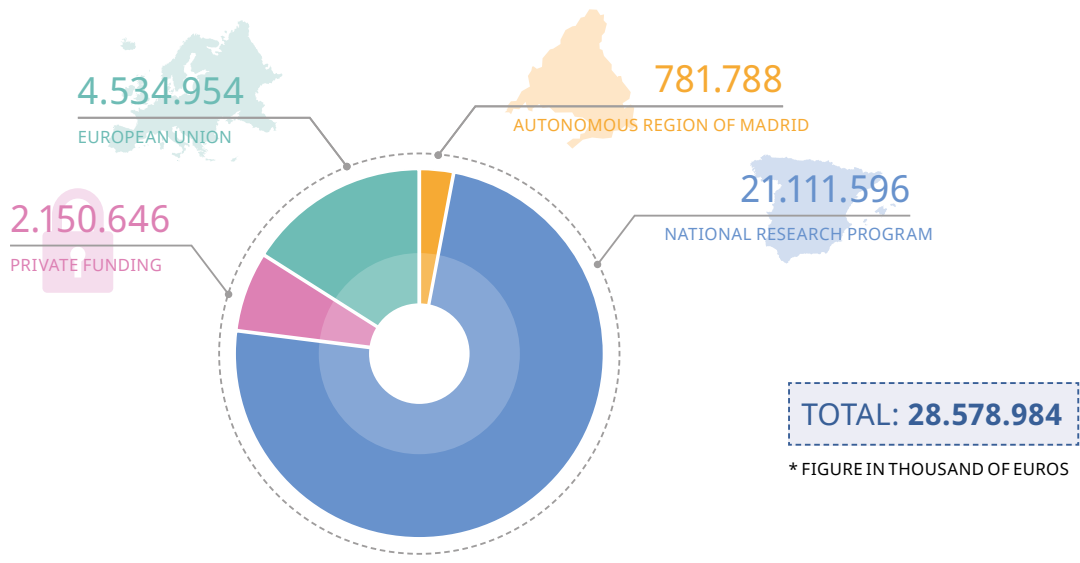
¹ Includes the following categories: Profesor de Investigación, Científico Titular, Investigador Científico, Doctor FC1, Catedrático, Profesor Titular, Profesor Contratado Doctor, Profesor Permanente Laboral, Profesor Ayudante Doctor

² Includes the following categories: Ramón y Cajal, Talento, La Caixa Junior, Profesor Contratado Doctor, Profesor Permanente Laboral, Profesor Ayudante Doctor

RESEARCH SUPPORT STAFF



PROJECTS AND FUNDING



SCIENTIFIC FACILITIES



- | | | |
|---------------------------|---------------------|-------------------------|
| Advanced light microscopy | Electron microscopy | Preclinical biomedicine |
| Animal facility | Flow cytometry | Protein biotechnology |
| Biocomputational analysis | Genomics | Proteomics |
| Bioinformatics | Library | |
| Drosophila transgenesis | Mouse gene editing | |

TECHNICAL SERVICES

- Biosafety and radioprotection
- Cell culture, glass wash and media preparation
- Facilities Maintenance
- Information Technology and Services
- Occupational Health and Safety
- Technical Maintenance
- Photography



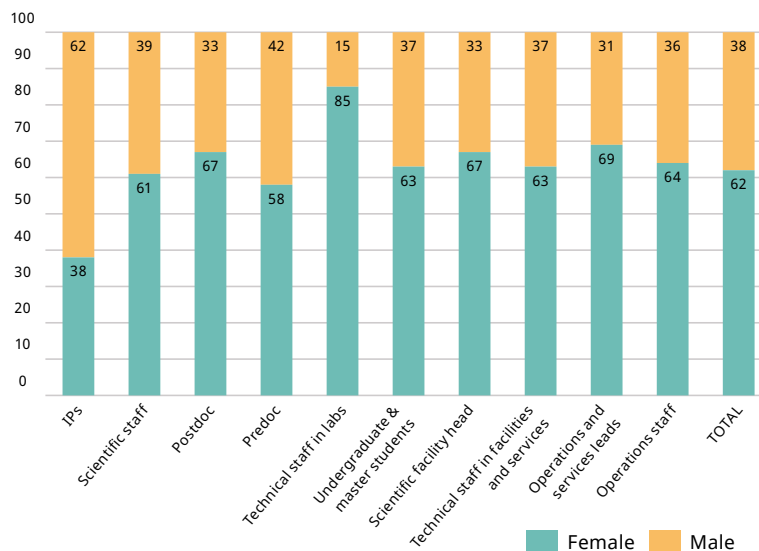
OPERATIONS TEAMS

- Communications & Scientific Outreach
- Finance and grants
- Grant support
- Human resources
- National and international programs
- Institutional relations
- Procurement
- Quality management

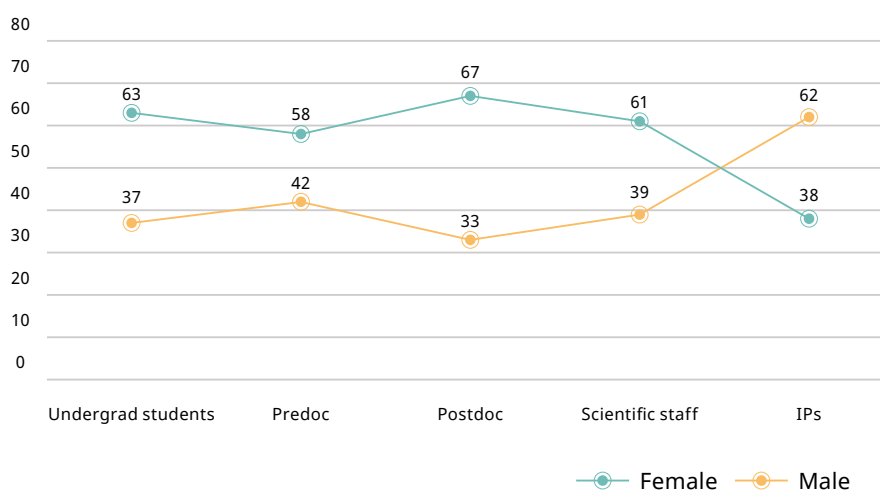


GENDER

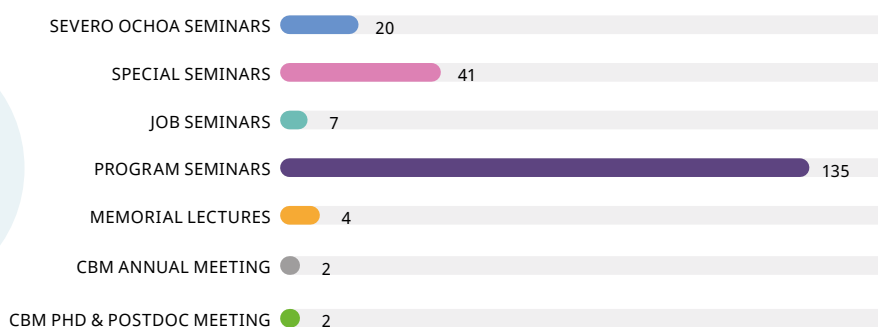
GENDER BY STAFF GROUP (%)



GENDER IN ACADEMIC CAREER PATH (%)



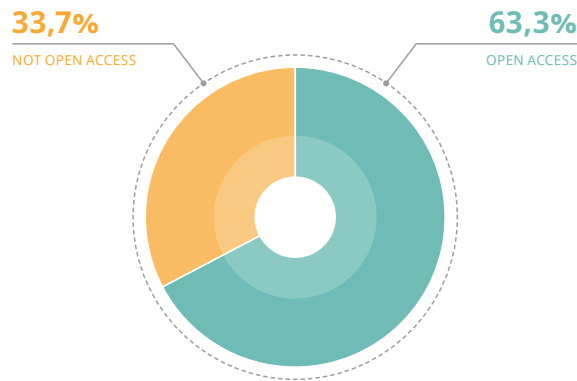
SEMINARS AND LECTURES



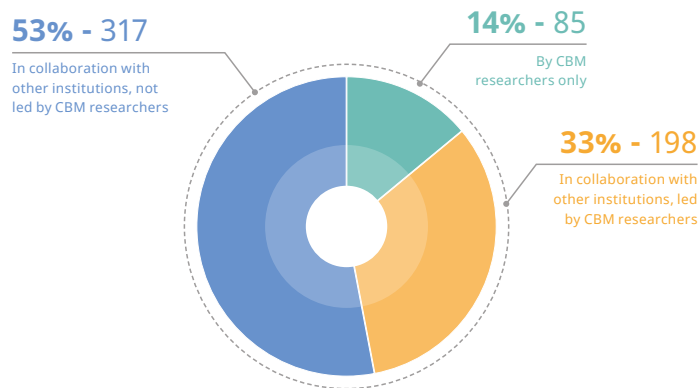
PUBLICATIONS



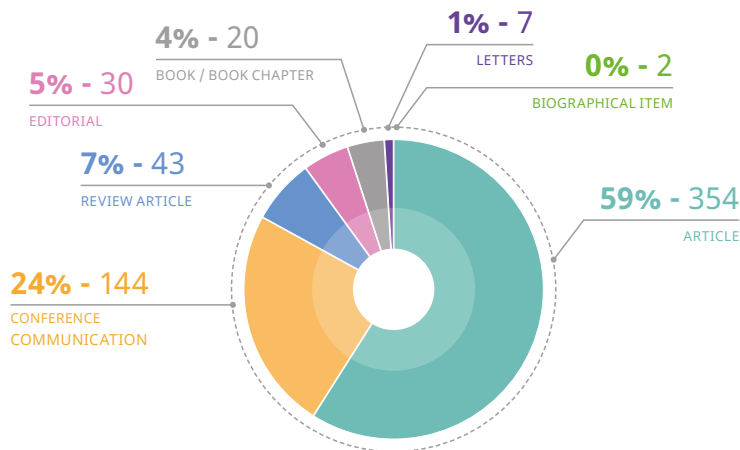
OPEN ACCESS



COLLABORATIONS IN SCIENTIFIC PUBLICATIONS



PUBLICATION TYPES



TRAINING ACTIVITIES

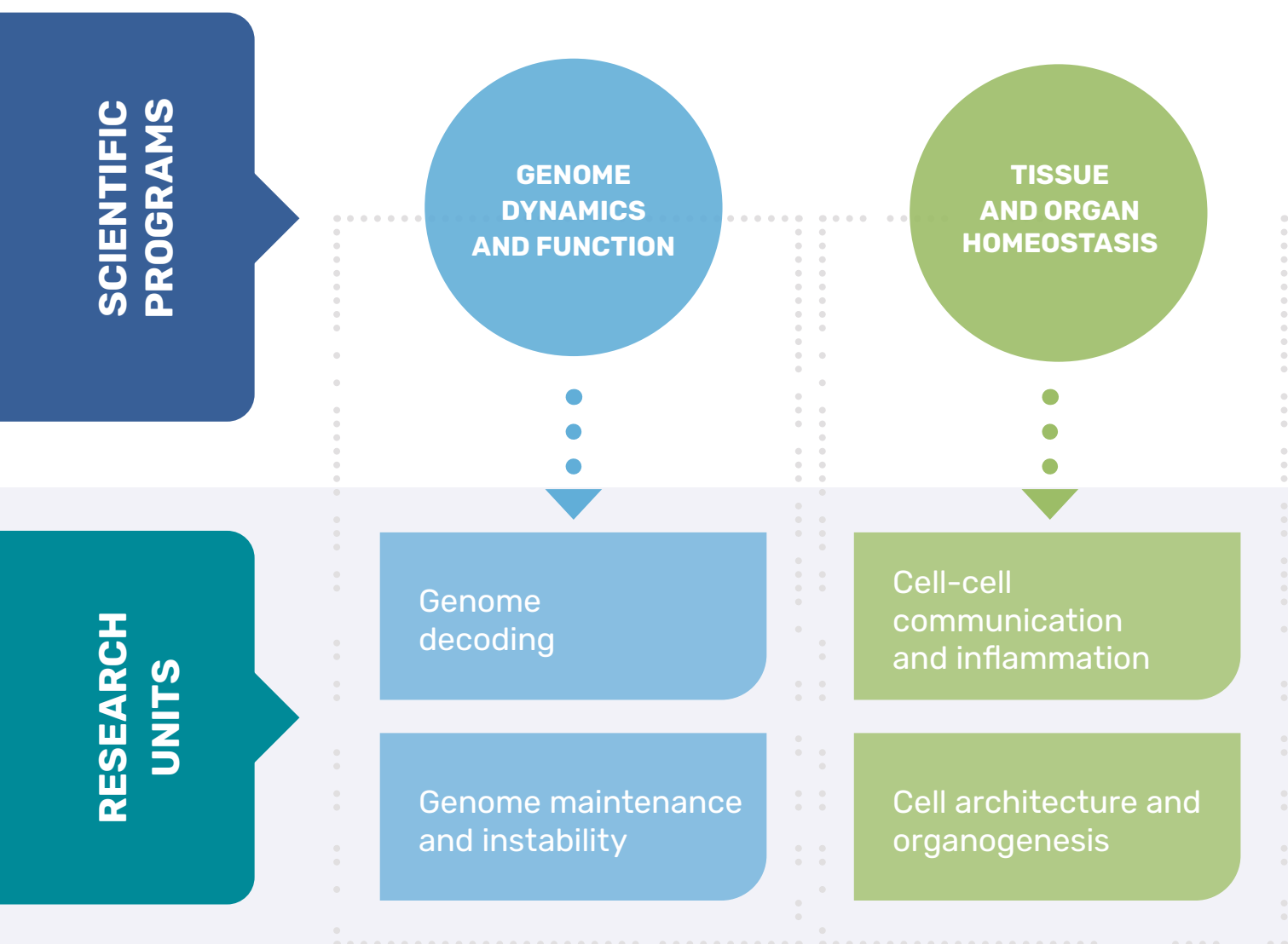
DOCTORAL THESES ▶

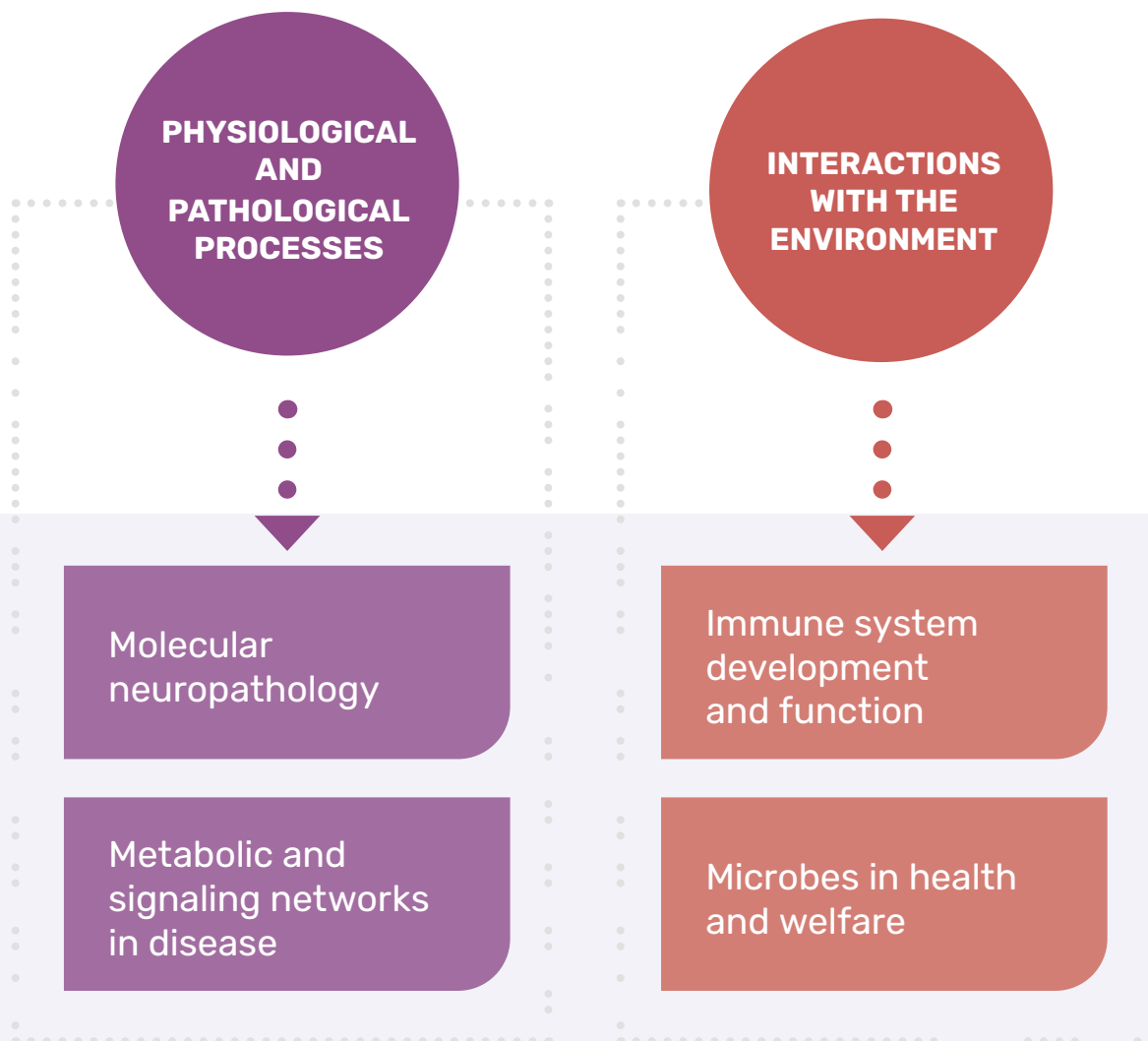
67

223

◀ MASTER AND DEGREE THESES

SCIENTIFIC ORGANIZATION OF THE CBM







EXTRACELLULAR MATRIX REMODELING IN THE CARDIOVASCULAR SYSTEM

Group members

Principal investigator:

Fernando Rodríguez Pascual

Predoctoral fellows:

Marta Navarro Gutiérrez

Verónica Romero Albillo

Technicians:

Raquel Jiménez Sánchez

Undergraduate and Master students:

Lucía Rosario Paredes Carbajo



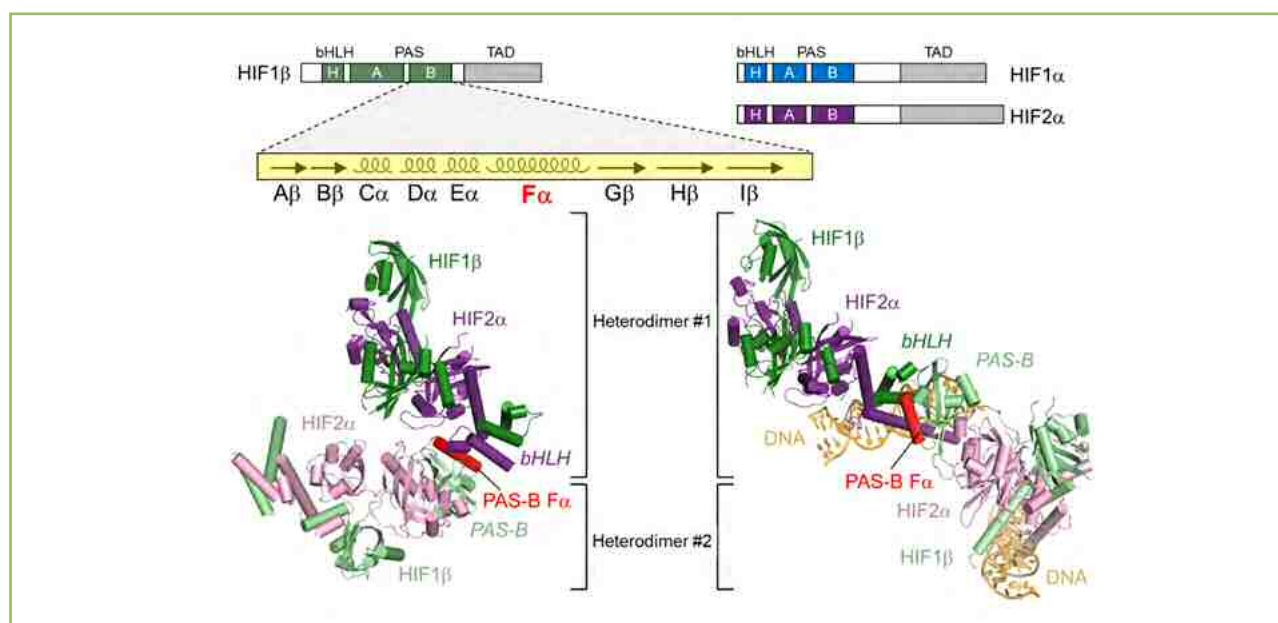
<https://www.cbm.uam.es/frodriguez>



Summary

The extracellular matrix (ECM) constitutes an intricate molecular network that surrounds and integrates cells and tissues in multicellular organisms. Traditionally considered a static mass, today it is widely recognized as a highly dynamic biomaterial that confers mechanical strength to tissues, provides attachment and movement points to cells, and is also essential for the intercellular communication. Therefore, proper synthesis and assembly of the components of the ECM is essential for cell and tissue homeostasis, and defects or alterations in these processes are associated with the development of several human disorders, particularly in the cardiovascular system. The biosynthesis of collagen, the main component of the ECM, is a complex and highly regulated process involving numerous steps, including chain association and folding, extracellular secretion, proteolytic processing and cross-linking. A set of prolyl- and lysyl-hydroxylases, glycosidases, isomerases and lysyl oxidases catalyze an extensive series of post-translational modifications, most of which are unique to collagen. In the last few years our group has investigated different aspects of this biosynthetic pathway. To this respect, we have focused our work in the study of the biology of lysyl oxidases (LOX), the enzymes responsible for the collagen cross-linking, an essential post-translational modification that largely determines the biomechanical properties of the collagen.

Another interest of the laboratory has been the regulation by hypoxia of several collagen remodeling enzymes, including lysyl- and prolyl-hydroxylases. Here, we have described the molecular mechanism by which hypoxia through the hypoxia inducible factor (HIF) increases the expression of these matrix biosynthetic enzymes and how this contributes to the deposition of a stiff collagen matrix. Currently, the group explores the functional and molecular links between the stiffness of the matrix, the metabolism and the hypoxia in the development of matrix-related diseases such as the pulmonary fibrosis and other fibrotic disorders.



Model for the interaction between HIF heterodimers. HIF domain organization and schematic of the HIF1β PAS-B showing the position of the the Fa helix in red. Crystallographic structures show two HIF2α/HIF1β heterodimers alone (left) and bound to the HIF element (right) through the Fa helix.



List of publications

- Miguel V, Alcalde-Estévez E, Sirera B, Rodríguez-Pascual F, Lamas S. (2024) Metabolism and bioenergetics in the pathophysiology of organ fibrosis. *Free Radical Biology and Medicine*, 222: 85–105, DOI: 10.1016/j.freeradbiomed.2024.06.001.
- Rosell-García T, Rivas-Muñoz S, Kin K, Romero-Albillo V, Alcaraz S, Fernandez-Tornero C, Rodríguez-Pascual F. (2023) Multimerization of HIF enhances transcription of target genes containing the hypoxia ancillary sequence. *Biochimica et Biophysica Acta - Gene Regulatory Mechanisms*, 1866 (4), art. no. 194963, DOI: 10.1016/j.bbagr.2023.194963.



Other activities

- Member of the Editorial Board (Molecular Biology Section) of the journal "Scientific Reports", Asia-Pacific Journal of Ophthalmology (Visual Sciences Section), Molecular Biology Reports (Extracellular Matrix Biology Section) and the International Journal of Molecular Sciences.
- Coordinator of the SEBBM group on Extracellular Matrix (2024).



Research projects

- PID2022-136703OB-I00. Ministry of Science, Innovation, and Universities. "Impact of the extracellular matrix on the cellular biology and metabolic rewiring of organ fibrosis." PI: Fernando Rodríguez Pascual, Santiago Lamas. 09/01/2023-08/31/2027
- CPP2022-009582. Public-Private Partnership Projects of the Ministry of Science, Innovation, and Universities. "Evaluation of M2R therapy in an experimental model of SDRA". Coordinator: Pablo García de la Riva. PI CSIC: Fernando Rodríguez Pascual, Santiago Lamas. 2023-2026
- S2022/BMD-7221 (INNOREN-CM). Biomedical Projects of the Community of Madrid. New diagnostic and therapeutic strategies in chronic kidney disease. PI and coordinator: Fernando Rodríguez Pascual. 01/01/2023-31/12/2026.



Scientific
facilities





ADVANCED LIGHT MICROSCOPY FACILITY

Group Members

Technical director:

Álvaro Sahún Español

Scientific supervisor:

Francisco Javier Díez Guerra

Staff:

Carmen Sánchez Jiménez

Elena Calvo Casalilla

Carlos Gallego García

Maite Villalba Villacorta



Description

The **Advanced Light Microscopy Facility (SMOA)** is a core research support platform at the Centro de Biología Molecular Severo Ochoa, offering access to advanced optical microscopy technologies. Its instrumentation includes four widefield microscopes, seven laser scanning confocal microscopes—one featuring STED super-resolution and FLIM capabilities—and a spinning disk fluorescence microscope equipped with dual cameras and a high-resolution SoRa disk. The facility is also equipped for sample preparation, with two stereomicroscopes and a vibratome, and provides a wide range of microscopy-related reagents, such as primary and secondary antibodies, fluorescent dyes, mounting media, and imaging chambers. High-performance computing systems are available for image processing and analysis.

SMOA's technical staff is responsible for the maintenance and operation of all microscopy and computational resources. They offer comprehensive support to researchers, including expert guidance, troubleshooting, and training through theoretical and practical courses, workshops, and seminars on microscopy techniques.

Recognized as a reference center by Evident/Olympus, SMOA is integrated into national networks such as the Spanish Network for Advanced Optical

Microscopy (REMOA) and the Red de Laboratorios e Infraestructuras (RedLab). The facility also holds ISO 9001:2015 (AENOR) certification, ensuring compliance with international standards in quality management.



Courses

CBM courses:

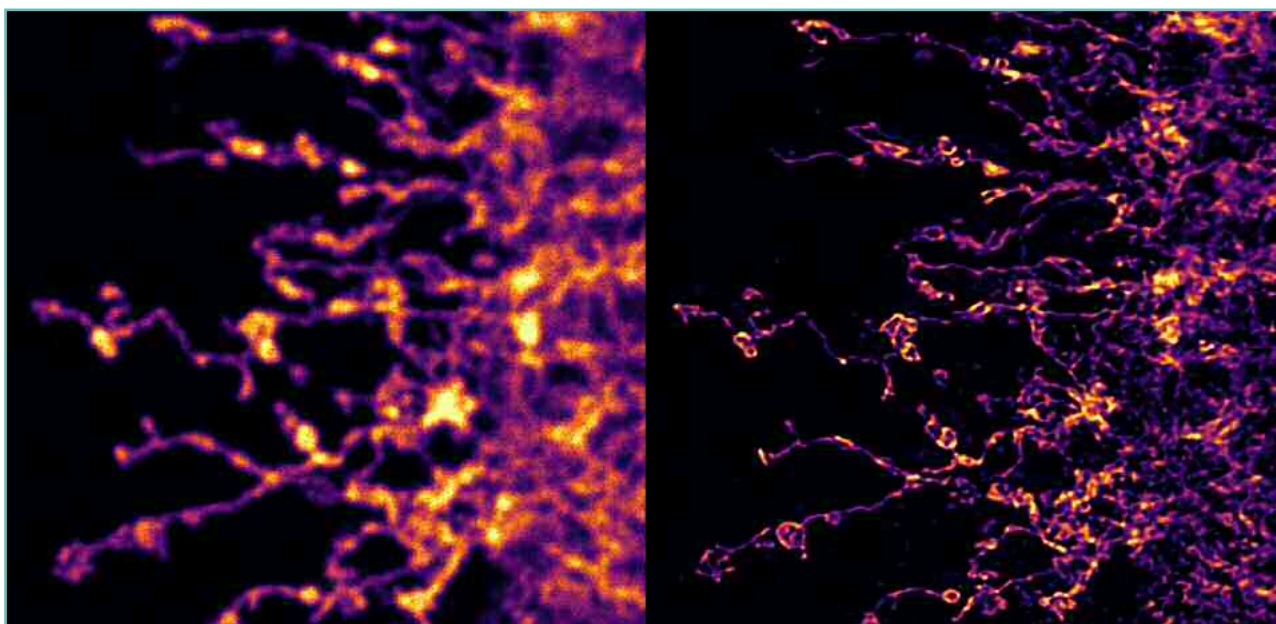
- Good practices in optical microscopy

Visits:

- Spanish Biology Olympiad
- Latin-American Biology Olympiad
- 4°ESO+Empresa programme
- Cientific@s en prácticas programme
- Monthly scholar visits

Organised conferences:

- Spanish & Portuguese Advanced Optical Microscopy Meeting 2024 (SPAOM24) - Toledo (Spain), 20-22 November 2024 (Organiser: Alvaro Sahún).
- 1st Junior Group Workshop for the European Society for Virology (ESV): Imaging Viruses - Madrid (Spain), 13-14. February 2025 (Organiser: Carlos Gallego).



Cytonemes in the imaginal wing disc of *Drosophila melanogaster* are emitted from Hedgehog-producing cells to receptor cells. Cytonemes are labelled through Ihog protein fused with RFP fluorescent protein. On the left, confocal microscopy; on the right, STED super-resolution microscopy.

Sample preparation by Clara Fernandez (Isabel Guerrero's Lab). Image acquisition by Carmen Sánchez (SMOA).



Services

- Preventive and corrective **maintenance** of equipment to ensure optimal performance.
- Scientific and **technical assistance** (experimental design, sample preparation, image acquisition, image visualization and analysis and results publication).
- **Training.**
- Organization of **seminars and tutorials**, as well as the **development of theoretical and practical guides.**
- Storage and distribution of **common reagents and consumables.**
- **Control of access** to the service and the use of its resources.
- Management of **billing and reservations.**
- Regular maintenance and updates of the **website.**
- Assess, evaluate, and discuss researchers' needs and advancements in optical microscopy to **ensure cutting-edge scientific capabilities.**
- **Collaborate and negotiate with commercial companies** to explore their offerings and maximize the benefits of their systems, capabilities and expertise.
- **Secure funding** to facilitate necessary and timely upgrades.



ANIMAL FACILITY

Group Members

Technical director:

Blanca Estévez Santiago

Scientific supervisor:

María Mittelbrum Herrero

Staff:

Miguel A. Bordallo Martín-Fontecha

Alfonso Gutiérrez García

Marta González Mella

José María Sedano Torres

Laura López Martínez

Beatriz García Martínez

Virginia González de la Torre

José Ignacio Herrero Lahuerta

Isidro García Gómez

María Soledad López Martín

Haisul CY Chang Hsiao



Description

This is a modern facility, inaugurated in January 2011, covering approximately 1,500 m². It was designed and equipped with the latest advances in laboratory animal housing and care. The facility breeds and maintains mice (mostly transgenic), frogs, and fish.

The animal facility is divided into two main areas:

- An SPF (Specific Pathogen Free) zone dedicated to the breeding and maintenance of mouse lines, where new lines are introduced exclusively via embryo rederivation, thanks to the Reproductive Biotechnology Service.
- An experimental zone that includes rooms classified as BSL1, BSL2, and BSL3 (Biosafety Levels 1, 2, and 3).

There are dedicated rooms for behavioral studies both in the SPF area and in BSL1 and BSL2.

The facility works in close collaboration with the Preclinical Biomedical Services—housed within the animal facility—as well as with the Biosafety Service, which enables the irradiation of mice.

All mice are housed in individually ventilated cages (IVCs) placed on ventilated racks, and are handled inside dedicated Biological Safety Cabinets using forceps to prevent contamination. Fish (zebrafish—many of them genetically modified—and medaka fish) and frogs (*Xenopus laevis*) are kept in dedicated aquatic systems specifically designed for their maintenance.

To support the maintenance and handling of mice, the facility is equipped with more than 50 ventilated racks, 18 Biological Safety Cabinets, 5 changing stations, 2 gas exposure cabinets, 6 anesthesia units, microsurgical magnifiers, and other standard laboratory equipment.

The designated veterinarian has a dedicated pharmacy for administering drugs necessary to support research activities, promote successful experimental outcomes, and ensure animal welfare at all times.

For facility maintenance, there is a dedicated technical area housing HVAC systems and other equipment required to maintain stable environmental conditions.



Culture of Care: commitment to improving animal welfare, scientific quality, care of the staff and transparency for the stakeholders.

In the washing area—where all materials are cleaned and processed—the facility is equipped with a rack washer, a tunnel washer, a bedding aspiration system, a bottle washer and filler, and a water treatment unit. Two double-door autoclaves and a hydrogen peroxide pass-through chamber (SAS) are available for material entry into the SPF zone. The BSL3 area of the facility has an additional 2,000-liter double-door autoclave and two Security Airlocks (SAS). One of these connects directly to the BSL3 Cell Culture facility, a rare and highly valuable feature for conducting certain types of studies.

Access to the facility and its various rooms is controlled via magnetic key cards, ensuring that users can only enter authorized zones. Access to biocontainment areas also requires approval from the Center's Biosafety Service. The center is fully authorized to work with genetically modified organisms at Biosafety Levels I and II.



Services

Internal Services Provided by the Animal Facility:

- Breeding and maintenance of mice under SPF conditions, conventional housing, and Biosafety Levels 2 and 3.
- Management of transgenic mouse colonies.
- Breeding and maintenance of zebrafish and medaka fish.
- Technical support for researchers in the execution of various experimental procedures.
- Technical and ethical advisory services.
- Coordination of national and international animal shipments and arrivals.
- Veterinary supervision and oversight.
- Health monitoring of housed animals, conducted through accredited external services.

- Reproductive biotechnology services.

- Transgenesis service in collaboration with the CNB (National Center for Biotechnology).

All activities are carried out in strict compliance with current regulations, with particular attention to animal health and welfare, as well as the training and qualification of personnel involved in animal care and handling.

Courses

- 4°ESO+Empresa programme
- Científic@s en practicas programme
- Spanish Biology Olympiad



BIOCOMPUTATIONAL ANALYSIS FACILITY

Group Members

Technical director:
Sandra González de la Fuente

Scientific supervisor:
Begoña Aguado (until november 2023 in the Genomics and NGS Core Facility and later in the Biocomputational Analysis Core Facility).

Staff:
Sandra González de la Fuente (until november 2023, as technical supervisor of the NGS section of the Genomics and NGS Core Facility, and since then, of the Biocomputational Analysis Core Facility)
Paula Martínez García (until november 2023 in the Genomics and NGS Core Facility and since then in the Biocomputational Analysis Core Facility)

Iván Lorca Alonso (until november 2023 in the Genomics and NGS Core Facility and since then in the Biocomputational Analysis Facility)
Maria Santos Galindo (until november 2023 in the Genomics and NGS Core Facility and since then in the Biocomputational Analysis Facility)
Maria Santos Romero (since 20/11/24)
Laura Tabera Moreno (until november 2023, as technical supervisor of the Genomics section of the Genomics and NGS Core Facility)
Maria Gabriela Atencia Cibreiro (until november 2023 as part of the Genomics and NGS Core Facility)

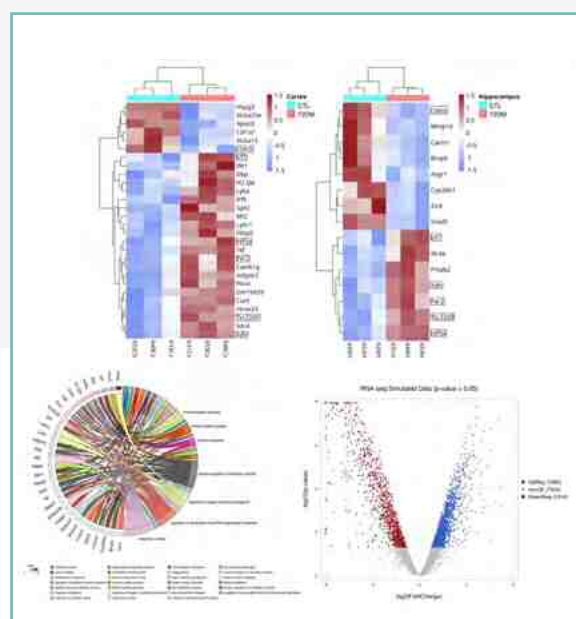


Description

In November 2023, the Genomics and NGS Core Facility was divided into two independent facilities: The Genomics Core Facility and the Biocomputational Analysis Core Facility.

The Biocomputational Analysis Service (SABio) is specialized in providing state-of-the-art solutions for computational analysis of massive data, covering a wide range of technologies and applications. From meticulous selection of preprocessing and analysis methods to custom software development, our expert team is qualified to handle and integrate all types of multi-omics data. We offer:

- Technical scientific advice, experimental design, training to research groups with data analysis needs.
- Multiomics data analysis: massive sequencing (NGS), proteomics, metabolomics, etc.; omics integration, specific software development and biostatistical data analysis.



Example of personalized graphics generated in the Biocomputational Analysis Core Facility

- Secure and confidential management of raw data acting as collaborators ("brokers") of the European ENA repository (EBI-EMBL).
- Support to users in accessing CSIC scientific computing resources (NodoBio CCC, DRAGO).

Depending on the objectives, the technology used for data generation, types of samples and sample size and study organizations among others, we design the best method of data processing.



List of publications

- Aguado, B., Begeman, L., Günther, A., Iervolino, M., Soto, F., Vanstreels, R. E. T., Reade, A., Coerper, A., Wallis, B., Alcami, A., & Dewar, M. (2024). Searching for high pathogenicity avian influenza virus in Antarctica. *Nature microbiology*, 9(12), 3081–3083. <https://doi.org/10.1038/s41564-024-01868-7>
- Adán-Jiménez, J., Sánchez-Salvador, A., Morato, E., Solana, J. C., Aguado, B., & Requena, J. M. (2024). A Proteogenomic Approach to Unravel New Proteins Encoded in the *Leishmania donovani* (HU3) Genome. *Genes*, 15(6), 775. <https://doi.org/10.3390/genes15060775>
- Torres-Sánchez, E., Lorca-Alonso, I., González-de la Fuente, S., Hernández-Ledesma, B., & Gutiérrez, L. F. (2024). Antioxidant Peptides from Sacha Inchi Meal: An In Vitro, Ex Vivo, and In Silico Approach. *Foods* (Basel, Switzerland), 13(23), 3924. <https://doi.org/10.3390/foods13233924>
- Oggerin, M., Del Moral, C., Rodriguez, N., Fernandez-Gonzalez, N., Martínez, J. M., Lorca, I., & Amils, R. (2024). Metal tolerance of Río Tinto fungi. *Frontiers in fungal biology*, 5, 1446674. <https://doi.org/10.3389/ffunb.2024.1446674>
- Carús-Cadavieco, M., González de la Fuente, S., Berenguer López, I., Serrano-Lope, M. A., Aguado, B., Guix, F., Palomer, E., & Dotti, C. G. (2024). Loss of *Cldn5* -and increase in *Irf7* -in the hippocampus and cerebral cortex of diabetic mice at the early symptomatic stage. *Nutrition & diabetes*, 14(1), 64. <https://doi.org/10.1038/s41387-024-00325-y>
- Cacheiro-Llaguno, C., Mösges, R., Calzada, D., González-de la Fuente, S., Quintero, E., & Carnés, J. (2024). Polysensitisation is associated with more severe symptoms: The reality of patients with allergy. *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology*, 54(8), 607–620. <https://doi.org/10.1111/cea.14486>
- González-de la Fuente, S., Requena, J. M., & Aguado, B. (2023). Datasets of Iso-Seq transcripts for decoding transcriptome complexity in four *Leishmania* species. *Data in brief*, 52, 109838. <https://doi.org/10.1016/j.dib.2023.109838>
- Sánchez-Salvador, A., González-de la Fuente, S., Aguado, B., Yates, P. A., & Requena, J. M. (2023). Refinement of *Leishmania donovani* Genome Annotations in the Light of Ribosome-Protected mRNAs Fragments (Ribo-Seq Data). *Genes*, 14(8), 1637. <https://doi.org/10.3390/genes14081637>
- Carús-Cadavieco, M., Berenguer López, I., Montoro Canelo, A., Serrano-Lope, M. A., González-de la Fuente, S., Aguado, B., Fernández-Rodrigo, A., Saido, T. C., Frank García, A., Venero, C., Esteban, J. A., Guix, F., & Dotti, C. G. (2023). Cognitive decline in diabetic mice predisposed to Alzheimer's disease is greater than in wild type. *Life science alliance*, 6(6), e202201789. <https://doi.org/10.26508/lsa.202201789>
- Camacho, E., González-de la Fuente, S., Solana, J. C., Tabera, L., Carrasco-Ramiro, F., Aguado, B., & Requena, J. M. (2023). *Leishmania infantum* (JPCM5) Transcriptome, Gene Models and Resources for an Active Curation of Gene Annotations. *Genes*, 14(4), 866. <https://doi.org/10.3390/genes14040866>



Services

- Selection of Preprocessing and Analysis Methods: We identify the tools and methods of preprocessing and data analysis suitable for the type of sequencing.
- Quality Control Planning: We define strategies for quality control of sequencing data, such as removal of low quality sequences, artifact detection and coverage assessment.
- Computational analysis of massive sequencing (NGS) data: re-sequencing (DNA-seq), de novo assembly, RNA-seq, ChIP-seq, ATAC-seq, CLIP-seq, Exome-seq, Ribo-seq, IsoSeq, Single-cell, metabolomics, 16S, 18S, ITS and other amplicon and shotgun metagenomics. Pathway enrichment analysis, search for germline or somatic variants, SNP/InDel, CNV and structural variants, microarrays, etc. Graphical representation of results in a customized way according to user requirements.
- Computational analysis of proteomics and metabolomics data: Multivariate statistical analysis of proteomics and metabolomics data, principal component analysis (PCA) or linear discrimination analysis (LDA) for clustering of samples depending on the expression of certain proteins/metabolites. Mapping of proteins/metabolites to known metabolic pathways to analyze differential profiles. Graphical representation of results in a customized way according to user requirements.
- Multiomics data integration analysis: Combination of NGS omics data such as genomics, transcriptomics, etc. with metabolomics and proteomics data for complete understanding of biological processes. Machine learning analysis to build predictive models. Graphical representation of results in a customized way according to user requirements.
- Development, implementation and creation of customized software for data analysis: Programming in different languages according to the needs of the Service (Phyton, R, Bash, Tcsh, etc.) to develop ad hoc scripts.
- Statistical analysis of experimental data: exploratory analysis, hypothesis testing with parametric and non-parametric methods, robust statistical methods, ANOVA, principal component analysis (PCA), data mining (machine learning), ROC curves, etc. Graphical representation of results in a customized way according to user requirements.
- Sending sequences to European repositories, specifically to the European Nucleotide Archive (ENA) and European Genome-phenome Archive (EGA) belonging to the European Bioinformatics Institute (EBI). It is a secure and confidential way of maintaining the data, in addition to being an essential requirement when publishing the results in scientific journals. As collaborating users of ENA (Brokers), we take care of the maintenance of the data, as well as renewing the privacy of the data.



Courses

- Specialized courses tailored to the needs of the users.
- Gabinete de formación del CSIC: Análisis de datos de NGS 2023
- Gabinete de formación del CSIC: Análisis de datos de NGS 2024 (First edition)
- Gabinete de formación del CSIC: Análisis de datos de NGS 2024 (Second edition)



BIOINFORMATICS FACILITY

Group Members

Technical director:

David Abia Holgado

Scientific supervisor:

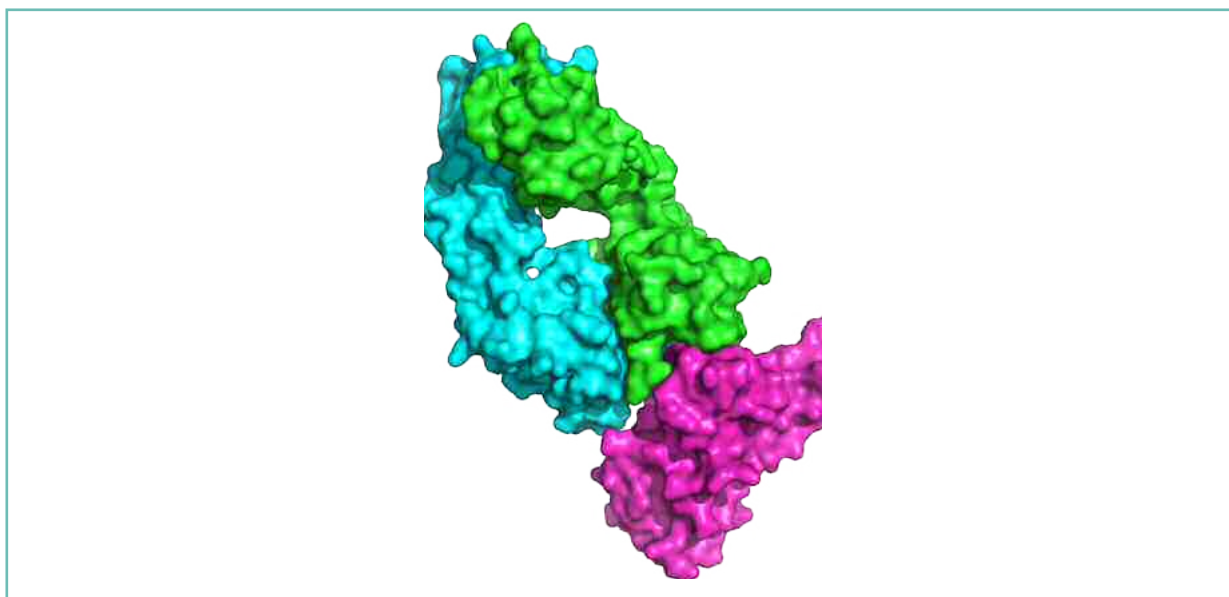
Ugo Bastolla Bufalini



Description

The mission of the Bioinformatics Facility is to support research groups of the CBMSO (and occasionally other centres) in the bioinformatics analysis of their data, to orient their research through computational predictions and to advice on the computational aspects of their projects. The personnel in the Facility teach at master's courses and participate in bioinformatics training of master students. Nowadays, bioinformatics analyses and predictions must complement experiments in order to rationalize their results and to maximize the knowledge that they provide. This is particularly true for high throughput experiments, whose bioinformatics analysis is necessary for extracting the information of interest. In addition, in silico predictions may help optimizing the design of the experiments. We perform these analyses and

predictions through computer programs that are publicly available or we develop specific programs to tackle specific problems, and we run the computations in the computing infrastructure of the Bioinformatics Facility, which consists of a high performance computing cluster, with infiniband connection, a shared parallel file system and computational nodes with up to 512GB of memory and 60 CPU cores.



List of publications

The bioinformatics service staff has participated in 12 scientific publications between 2021-2022. Below are some of the most relevant ones.

- Bastolla, Ugo; Chambers, Patrick; Abia, David; Garcia-Bermejo, Maria-Laura; Fresno, Manuel; (2022) Is Covid-19 severity associated with ACE2 degradation? *Frontiers in Drug Discovery* Vol 1, page 789710
- Horndler, Lydia; Delgado, Pilar; Abia, David; Balabanov, Ivaylo; Martínez-Fleta, Pedro; Cornish, Georgina; Llamas, Miguel A; Serrano-Villar, Sergio; Sánchez-Madrid, Francisco; Fresno, Manuel; (2021) Flow cytometry multiplexed method for the detection of neutralizing human antibodies to the native SARS-CoV-2 spike protein. *EMBO molecular medicine* Vol 13, Num 3, page e13549
- Erkelens, Amanda M; Qin, Liang; van Erp, Bert; Miguel-Arribas, Andrés; Abia, David; Keek, Helena GJ; Markus, Dorijn; Cajili, Marc KM; Schwab, Samuel; Meijer, Wilfried JJ; (2022) The B. subtilis Rok protein is an atypical H-NS-like protein irresponsive to physico-chemical cues. *Nucleic Acids Research*. Vol 50, Num 21, pages 12166-12185
- Hortal, Alejandro M; Oeste, Clara L; Cifuentes, Claudia; Alcoceba, Miguel; Fernández-Pisonero, Isabel; Claváin, Laura; Tercero, Rut; Mendoza, Pilar; Domínguez, Verónica; García-Flores, Marta; (2022) Overexpression of wild type RRAS2, without oncogenic mutations, drives chronic lymphocytic leukemia. *Molecular Cancer*. Vol 21, Num 1, page ene-24
- Bovolenta, Elena R; García-Cuesta, Eva M; Horndler, Lydia; Ponomarenko, Julia; Schamel, Wolfgang W; Mellado, Mario; Castro, Mario; Abia, David; van Santen, Hisse M; (2022) A set point in the selection of the $\alpha\beta$ TCR T cell repertoire imposed by pre-TCR signaling strength. *Proceedings of the National Academy of Sciences* Vol 119, Num 22, page e2201907119



Services

- The main functions of the Facility are the analysis of proteins at the level of sequence (homologous search, family characterization, prediction of properties) and structure (modeling of non-crystallized or mutant proteins and characterization of protein interactions using molecular dynamics).

Summary list of services offered

- Among the services provided during these years, we would highlight the research projects that concerned the Covid-19 pandemics. The first one, in collaboration with Balbino Alarcón's group, consisted in the computational design and experimental validation of antibodies against the SARS-CoV2 Spike protein, and it generated a highly efficient method for detecting specific antibodies against Spike protein in blood serum, which was patented and licensed. The second one, in collaboration with Ugo Bastolla, Manuel Fresno and Laura García-Bermejo from the Ramon y Cajal hospital, investigated the relationship between ACE2 levels in the blood of patients and the severity of symptoms of SARS-CoV2 infection. The third project, in collaboration with the CNB-CBMSO transgene facility, was an in silico study for humanizing the mouse ACE2 receptor using the CRISPR technique in order to generate a murine model of Covid-19.



DROSOPHILA TRANSGENESIS FACILITY

- **Group Members**

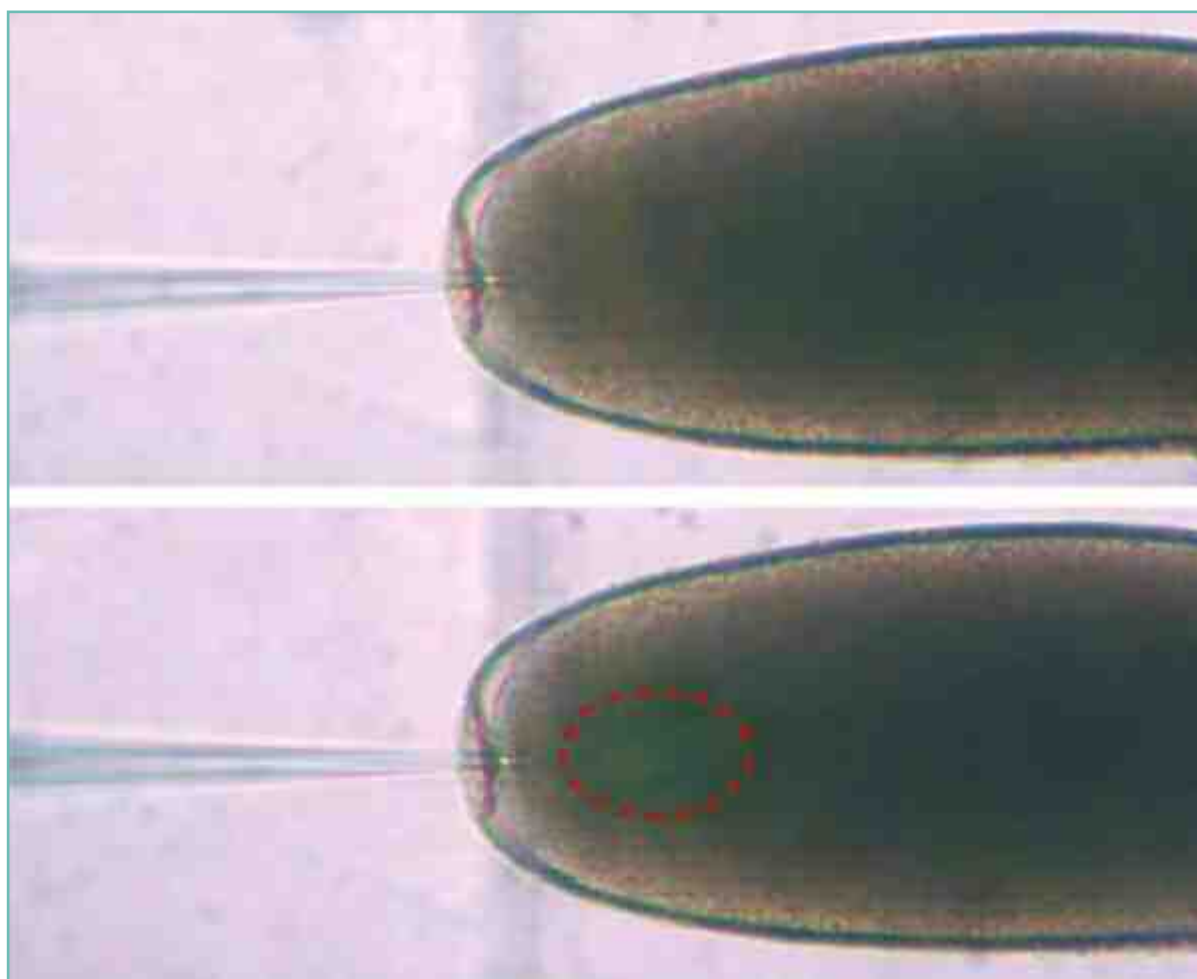
- **Technical director:**
 - Mar Casado García
 - Nuria Esteban Delgado

- **Scientific supervisor:**
 - Carlos Estella



Description

The *Drosophila* Transgenesis Facility was established in 2014 to provide technical support to research teams at CBMSO, CSIC and other national and international groups, by generating genetically modified *Drosophila* strains. It is equipped with two microinjection setups including microinjectors, stereomicroscopes with cold light source units and one micropipette puller as well as several incubators for desiccating embryos and for maintenance of *Drosophila* stocks. The main activity of the transgenesis facility is to generate *Drosophila* transgenic lines and to inject cocktails for CRISPR/Cas genome editing. The facility also maintains a collection of over 500 *Drosophila* stocks and a *Drosophila* cDNA plasmid collection, available to researchers upon request.



Drosophila embryos injection



Services

- Generation of transgenic strains mediated by the transposon P (random insertion into yw or w strains) Helper DNA is provided by the facility, that also prepares the injection mixture.
- Targeted insertion of transgenes mediated by the PhiC31 integrase into the following acceptor strains: ZH-attP22A, ZH-attP-51D, ZH-attP-68E, ZH-attP-86Fb.
- Transgenesis with BAC vectors [P(acman) collection] » Injection of DNA cocktails for gene editing using CRISPR/Cas technology in the strains: 25C, 68A, Nos-cas9, Vasacas9 and Vasa-cas9 RFP-.
- Insertion of DNA into specific strains provided by the customer.
- Shipment of injected embryos or generation and shipment of transgenic lines according to the requested service.
- Shipment of Drosophila stocks from the collection.
- Plating and shipment of cDNA plasmids.



ELECTRON MICROSCOPY FACILITY

Group Members

Technical director:

María Teresa Rejas

Staff:

Milagros Guerra Rodríguez

Tania Matamoros Grande

María Josefa Rodríguez Gómez

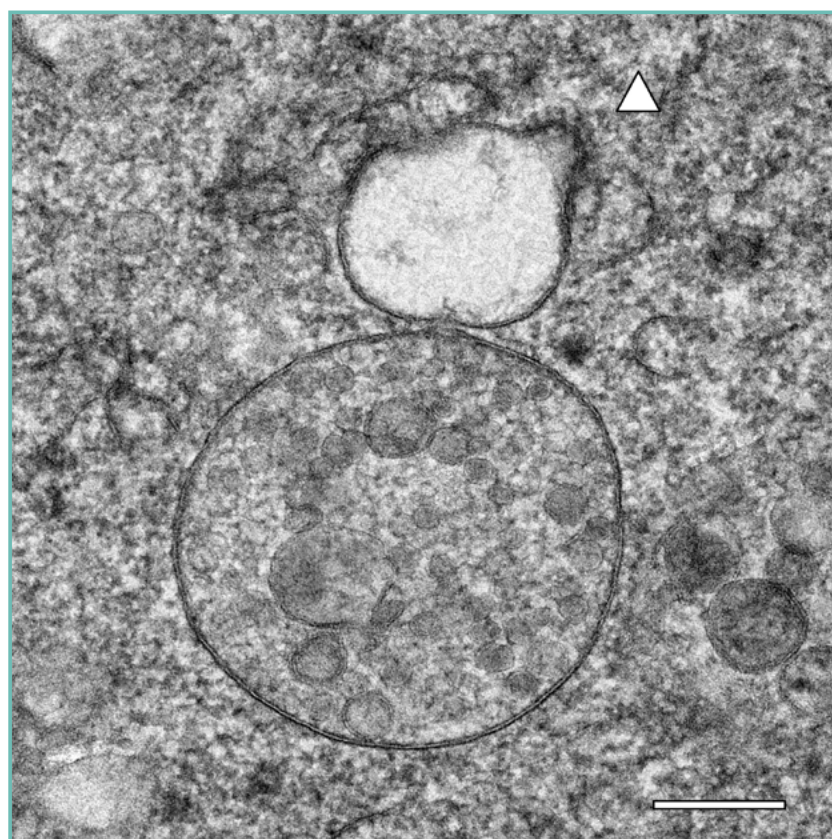
Martin Christoph Sachse



Description

The Electron microscopy service (SME) of the CBM provides help to scientists of the CBM and from outside the institute to address their morphological questions within their research. For this, the SME is specialized in conventional and cryo-sample preparation for transmission electron microscopy of cell biological samples, such as bacteria, fungi, cultured cells, and tissues. In addition, bacteria, purified viruses or proteins can be analysed by negative staining, as well as nanoparticles for material science. The service has a 100 kV and a 120 kV TEM, both equipped with a 4x4 K CMOS camera.

We offer a full service for sample preparation for morphological and immunohistochemical studies. The analysis at the microscope can be done by the scientists themselves after a training, as well as by the service. The members of the SME can provide help with the analysis and interpretation of the results.



In 2023 a high pressure freezing machine was installed at the SME. The image shows a late endosome of a high pressure frozen Vero cell. Next to it is an electron lucent vacuole with a clathrin coated bud (white arrowhead) at the limiting membrane. Scale bar 200 nm.



List of publications

- Gurriaran-Rodriguez U, Kodippili K, Datzkiw D, Javandoost E, Xiao F, Rejas MT, Rudnicki MA. (2024) Wnt7a is required for regeneration of dystrophic skeletal muscle. *Skelet Muscle*. 14(1):34. doi: 10.1186/s13395-024-00367-x.
- Iborra-Pernichi M, Ruiz García J, Velasco de la Esperanza M, Estrada BS, Bovolenta ER, Cifuentes C, Prieto Carro C, González Martínez T, García-Consuegra J, Rey-Stolle MF, Rupérez FJ, Guerra Rodríguez M, Argüello RJ, Cogliati S, Martín-Belmonte F, Martínez-Martín N. (2024). Defective mitochondria remodelling in B cells leads to an aged immune response. *Nat Commun*. 15:2569. doi: 10.1038/s41467-024-46763-1.
- Martin V, Guerra B, Hernaez B, Kappler-Gratias S, Gallardo F, Guerra M, Andres G, Alejo A. (2024). A novel live DNA tagging system for African swine fever virus shows that bisbenzimidazole Hoechst 33342 can effectively block its replication. *Antiviral Res*. 230:105973. doi: 10.1016/j.antiviral.2024.105973.
- Alejo A, García-Castey M, Guerra M, Hernáez B, Martín V, Matamoros T, Andrés G. (2023). African swine fever virus transmembrane protein pEP84R guides core assembly. *PLoS Pathog*. 19:e1011136. doi: 10.1371/journal.ppat.1011136.



Services

- Technical and scientific supervision on experimental design and data analysis.
- User assistance and training.
- Negative staining of macromolecular complexes, extracellular vesicles, viruses and nanoparticles.
- Chemical fixation and resin-embedding of biological specimens.
- Cryofixation, freeze-substitution and low temperature embedding.
- Ultramicrotomy of resin embedded samples.
- Tokuyasu cryosectioning.
- Immunoelectron microscopy with gold conjugates.
- Correlative light-electron microscopy.
- Freeze-fracture, freeze-etching and Pt-C replication.
- Electron microscopy of nucleic acids.



FLOW CYTOMETRY FACILITY

Group Members

Technical director:

Berta Raposo

Scientific supervisor:

Maria Luisa Toribio

Staff:

Silvia Andrade

Raquel Nieto



Description

The flow cytometry facility provides access to state-of-the-art multiparametric flow cytometry, spectral flow cytometry and cell sorting. Available flow cytometry techniques range from classic cell immunophenotype, cell cycle and apoptosis determinations, quantifications of cytokine production, cell signaling and metabolic pathways, to analyses of prokaryote organisms, dissociated discs of *Drosophila* larvae, or protoplasts and various plant cell types. Spectral flow cytometry maximizes sensitivity, decreases background, and allows investigation of an increasing number of molecules of interest. Cell sorting provides high purity cell separations, single-cell sorting, and particle enrichment. Determinations of protein-protein interactions based on FRET are also provided. Highly experienced and dedicated personnel offers training and advice on flow cytometry principles and applications, cell-sorting operation and equipment calibration and maintenance.

Equipment includes one analogical cytometer with two lasers and four fluorescence detectors (FACScalibur), three digital cytometers, one with two lasers and six fluorescence detectors (FACSCanto A), and two with three lasers and eight fluorescence detectors (FACSCanto II), one equipped with High Throughput Sampler. Spectral flow cytometry

equipment includes two Cytex cytometer (AURORA) equipped with 4 lasers (V/B/YG/R) and 5 lasers (UV/V/B/YG/R), 4 analysis stations equipped with a Spectroflo and Flowjo licenses, and a next-generation software (OMIQ) containing the most commonly used algorithms for multidimensional data analysis. The facility also includes a cell sorter, FACS Aria Fusion integrated inside a Class II biosafety cabinet, equipped with four lasers and sixteen fluorescence detectors. For cell metabolism analysis, the facility is equipped with a Seahorse metabolic analyzer, that enables the detection of discrete changes in cellular bioenergetics in real-time using living cells in a 96-well platform, and with a multiwell motorized microscope (Cytation) that allows normalization of seahorse assays and other assays.

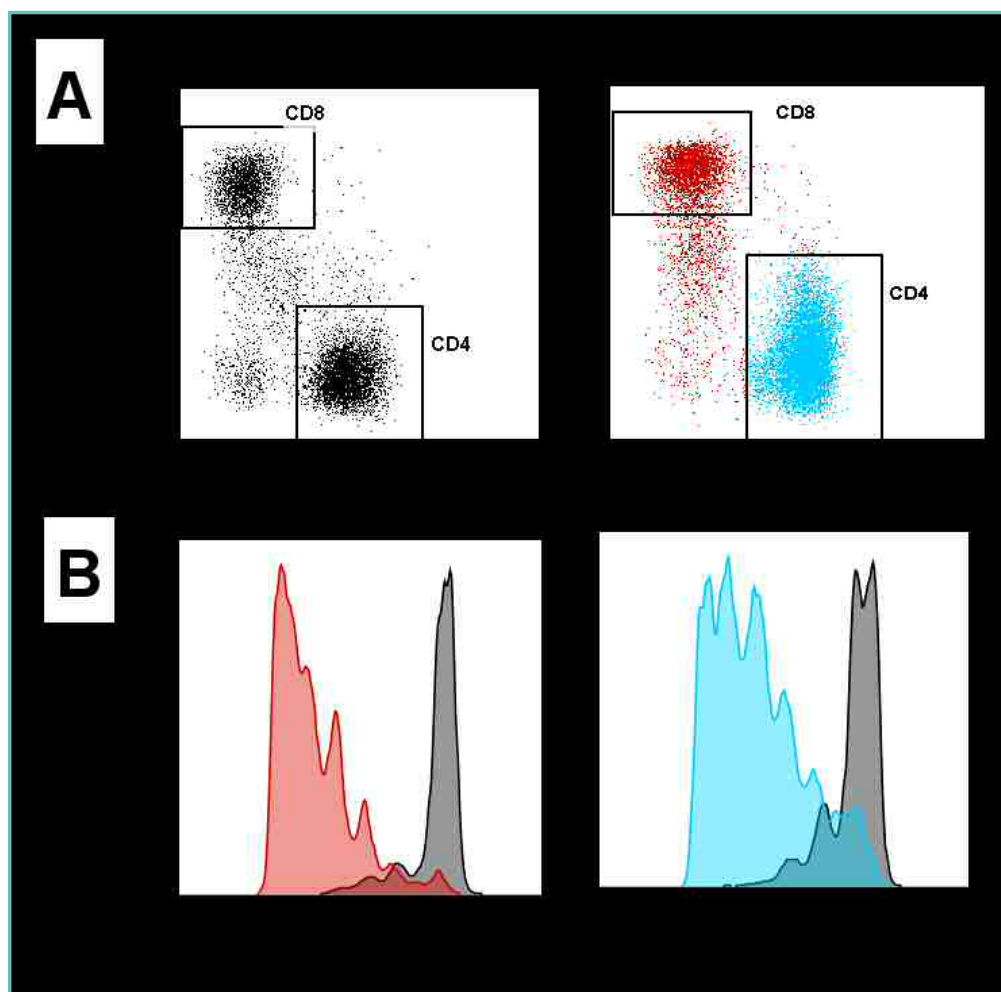


FIGURE 1. Tracking human T cell division. Human peripheral blood lymphocytes, either non-stimulated or stimulated with anti-human CD3 antibody were labeled with anti-CD3-PE, anti-CD4-FITC and anti-CD8-PECy7 antibodies and Cell Trace Violet.



Services

- Training and advice on instrument operation.
- Advice on new experimental designs and adaptive setups.
- Training on post-acquisition data analysis using FlowJo, FACSDiva, Spectroflo and OMIQ softwares.
- Equipment operation and maintenance.
- Availability to commonly used reagents.



Courses

- III CURSO ANÁLISIS AVANZADO de datos en citometría de flujo (co-organized by CBM, CIEMAT and PALEX)
- Gabinete de Formación del CSIC (2024): Introducción a la citometría de flujo (co-organized by CBM, CNB and CIB)
- Gabinete de Formación del CSIC (2024): Análisis de datos de citometría de flujo: DIVA y FlowJo
- Gabinete de Formación del CSIC (2024): Aplicaciones y uso de la citometría de flujo en investigación (co-organized by CNB, CBM and CIB)



GENOMICS FACILITY

Group Members

Technical director:

Laura Tabera Moreno

Scientific supervisor:

Begoña Aguado Orea (until november 2023 in the Genomics and NGS Core Facility)

Leonardo Beccari (since november 2023 in the Genomics Core Facility)

Staff:

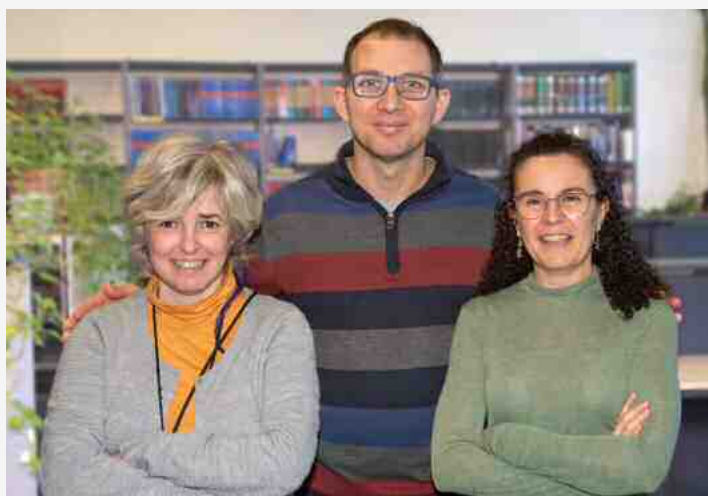
Maria Gabriela Atencia Cibreiro (until november 2023 as part of the Genomics and NGS Core Facility and since then in the Genomics Core facility)

Sandra González de la Fuente (until november 2023 as technical supervisor of the NGS section of the Genomics and NGS Core Facility)

Paula Martínez García (until november 2023 in the Genomics and NGS Core Facility)

Iván Lorca Alonso (until november 2023 in the Genomics and NGS Core Facility)

María Santos Galindo (until november 2023 in the Genomics and NGS Core Facility)



Description

In november 2023, the Genomics and NGS Core Facility was divided into two independent facilities: the Genomics Core Facility and the Biocomputacional Core Facility.

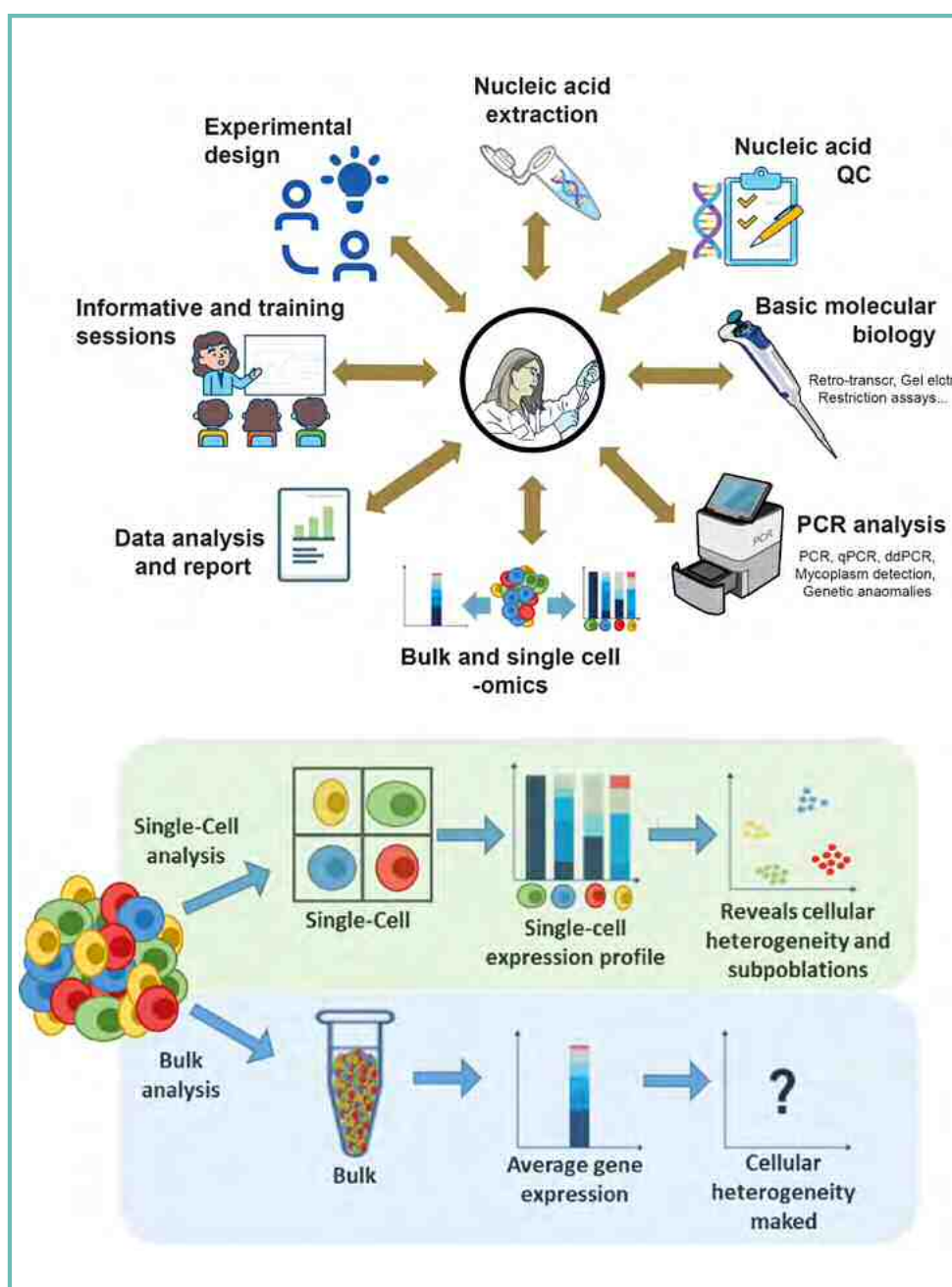
The Genomics Core Facility provides a comprehensive range of molecular biology and -omics services, technical advice, and experimental design support to research teams at CBMSO and external users. Additionally, it offers specialized training courses.

With extensive experience in PCR, qPCR, RT-qPCR, and digital droplet PCR (ddPCR), the facility also supports nucleic acid extraction and their quantification, and quality control. In the past two years, its capabilities have significantly expanded to include genomic abnormality analysis, mycoplasma detection in cell cultures, and NGS library preparation for bulk and single-cell experiments using diverse technologies.

Notably, we have integrated various scRNA-seq methodologies, including microfluidic-based (10x Genomics), combinatorial cell barcoding, and gel-separation techniques, enabling adaptation to different research needs.

Keeping pace with rapid advancements in -omics technologies, the facility is committed to incorporating new technologies (long-read sequencing, scATAC-seq, CITE-seq, spatial transcriptomics), when required to respond user's needs. Furthermore, it actively informs CBMSO users about the latest technological developments through seminars and periodic reports.

The Genomics Core Facility also offers users the option to independently access its equipment via an electronic reservation system, allowing flexibility in their level of involvement based on specific research requirements.



Overview of the services offered by the Genomics core facility and differences between bulk and single cell RNA seq experiments



Services

- Experimental design, performance and data analysis.
- Training courses.
- Primer design
- Nucleic acids extraction
- Nucleic acids quantification (using spectrophotometry or fluorimetry)
- RNA, DNA and NGS libraries quality control
- Reverse transcription (RT)
- PCR, RT-PCR, qPCR, RT-qPCR
- Droplet Digital PCR
- Single cell experiments



MOUSE GENE EDITING FACILITY

Group Members

Technical director:

M^a Belén Pintado Sanjuanbenito

Staff:

Verónica Domínguez Plaza

Coral Pedrero García



Description

The Service is shared between two CSIC centres: CBM and CNB. Our mission is to generate new genetically altered mouse models supporting the activity of research groups. We implement traditional technologies like additive transgenesis or ES cell mediated gene targeting, with genome edition with CRISPR/Cas9. We perform the technical generation of models through microinjection, electroporation or ES cell handling. We also support researchers on the design of the most appropriate mouse model for the specific purpose of their project: transgenic, KO, KI or conditional. The Service can design and test guides for genome edition and for additive transgenesis and ES-based gene targeting. We support research groups on the design of constructs, vectors, and genotyping if requested.

We also advise in breeding strategies of genetically altered mouse colonies, and facilitate the location and the incorporation of existing mouse lines from different sources. We can generate new mouse ES cell lines or collect embryos at different developmental stages. We perform cell line gene edition on request, designing guides and electroporating cells provided by the different research groups. All our services are open to any researcher who demand our help, except those under patent restriction, that are only provided to internal users from CSIC or UAM.

As part of our supporting activities for the research community, we organize two courses included in the annual CSIC catalogue, one focused on advanced handling of complex genotypes of genetically altered mouse models: conditional and inducible and a second one to help researchers to write the official request for authorization of experiments with animals according to Directive 2010/63/EU.

The service occupies physically three rooms at CNB, plus specifically assigned space in animal facilities of CNB and CBM. We count with the following equipment: P2 laboratory of ES cells with 2 CO₂ incubators for cell culture, Class II Biological Safety cabinet, Laminar flow hood, stereomicroscope, inverted microscope for tissue culture with camera, Electroporator, Cell electrofuser, Refrigerated centrifuge, Microinjection laboratory with two microinjection settings, consisting of inverted microscope with micromanipulator system, piezo drill, stereomicroscopes, pipette puller, Nepa 21 electroporator for embryos, CO₂ incubator for embryo culture and a molecular biology laboratory with: PCR Thermocycler, basic laboratory equipment.



Chimeric mice generated by microinjecting embryonic stem (ES) cells into blastocysts



List of publications

- Martínez-Campanario, M.C., Cortés, M., Moreno-Lanceta, Han, L., Ninfali, C., Domínguez, V., Andrés-Manzano M.J., Farràs, M., Esteve-Codina, A., Enrich, C., Díaz-Crespo, F.J., Pintado, B., Escolà-Gil, J.C. García de Frutos, P. Andrés, V., Melgar-Lesmes, P. and Postigo, A. (2023) Atherosclerotic plaque development in mice is enhanced by myeloid ZEB1 downregulation. *Nat Commun.* 2023; 14: 8316. doi: 10.1038/s41467-023-43896-7 PMID: PMC10721632
- Cortés, M., Agnese Brischetto, A., Martínez-Campanario, M.C., Ninfali, C., Domínguez, V., Fernández, S., Celis, R., Esteve-Codina, A., Lozano, J.J., Sidorova, J., Garrabou, G., Siegert, A.M., Enrich, C., Pintado, B., Morales-Ruiz, M., Castro, P., Cañete, J.D. and Postigo, A. (2023). Inflammatory macrophages reprogram to immunosuppression by reducing mitochondrial translation. *Nat Commun.* 2023; 14: 7471. doi: 10.1038/s41467-023-42277-4 PMID: PMC10656499
- Ninfali, C., Cortés, M., Martínez-Campanario, M.C., Domínguez, V., Han, L., Tobías, E., Esteve-Codina, A., Enrich, C., Pintado, B., Garrabou, G. and Postigo, A. (2023). The adaptive antioxidant response during fasting-induced muscle atrophy is oppositely regulated by ZEB1 and ZEB2. *Proc Natl Acad Sci U S A.* 120(46): e2301120120. doi: 10.1073/pnas.2301120120



Courses

- Maintenance and management of conditional and inducible mouse lines
- Drafting and monitoring of projects for the authorization of the use of experimental animals within the framework of Royal Decree 53/2013



Services

- Survey in international repositories and web resources to locate existing mice lines with the desired genotype
- Advise and technical support in the design of targeting vectors or constructs for microinjection and guides for genome editing with CRISPR/Cas9 technology
- Collection and culture of mouse embryos from 1 cell to blastocyst
- Pronuclear microinjection of DNA
- Vector electroporation in ES cell lines
- CRISPR/ Cas9 based genome edition of mouse embryo including design of guides
- CRISPR/Cas9 edition of cell lines, including design of guides
- International consortia ES cells handling and injection to generate chimeras
- Derivation of murine ES cell lines
- Genotyping by PCR, LR-PCR and sequencing
- Reproductive biotechnology to solve breeding problems of genetically modified mice
- DNA construct design and cloning for genome modification on demand.
- Support in establishment and management of genetically altered mouse lines
- Specific training in collection, handling and culture of mouse embryos in preimplantational stages
- Advise in creation and establishment of genetically altered mouse models according to Directive 63/2010/EU, including preliminary welfare assessment



PRECLINICAL BIOMEDICINE FACILITY

Group Members

Technical director:

Haisul CY Chang Hsiao

Scientific supervisor:

Konstantinos Stamatakis Andriani



Description

Preclinical Biomedicine is a scientific facility inaugurated in 2024, located in the CBM's animal facility. It provides scientific-technical guidance and support for experimental design and the use of specialized equipment with mice.

Our goal is to offer reliable, high-quality services that meet the needs and expectations of researchers, while complying with ethical and regulatory standards in animal research. The service is available to all institute researchers as well as external collaborators interested in using the equipment.

Training and guidance are offered on equipment usage, along with support for data analysis and interpretation. The personnel have expertise and qualifications in preclinical biomedicine and can assist researchers in designing and conducting their experiments.

Haisul CY Chang Hsiao, our experimental microsurgeon with experience more than 20 years in the field of animal experimental surgery, provides creative and practical advises for the experimental surgical settings and, if some are not skilled enough to maximize the animal wellness and ethical issues to obtain the samples for our services, Haisul also helps our investigators in small animal manipulations. In the near future, the first IVIM Intravital Microscope in Europe is incorporated in our unit in CBMSO. Until then,

Haisul takes charge of the training of the IVIM Intravital Microscope operations to access observation windows for the IVIM Intravital Microscope in brain, lung, skin, liver, small intestines, spleen, kidney, mammary glands, adipose tissue, lymph nodes, y peritoneum. By using IVESTA 3 Greenough Microscope, sophisticated microsurgeries can be done like heterotopic heart transplantation or orthotopic kidney transplantation in mice by Haisul.

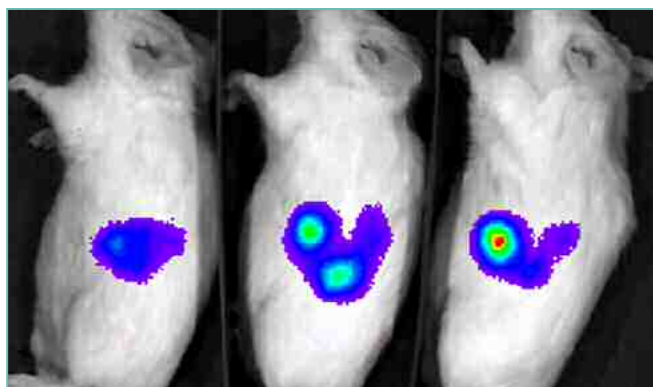
Konstantinos Stamatakis Andriani, our experienced expert without doubts in IVIS imaging experiment settings, gives full usefulness of our two IVIS imaging systems in two animal rooms with different sanitary status: the Room P2 (where virus vectors and mouse tumor can be employed) and the Room Preclinical Biomedicine (conventional space free of potential pathogenic agents).

BELOW IS A DESCRIPTION OF THE EQUIPMENT AVAILABLE:

Metabolic Cages for Mice

PhenoMaster (TSE Systems) and CLAMS (Comprehensive Lab Animal Monitoring System):

These systems allow for the measurement of various metabolic parameters in mice, including food and water intake, energy expenditure, activity, temperature, bodyweight, and oxygen consumption. Capacity for housing 12 mice individually.



Provide scientific-technical advice and support for experimental design and the use of equipment for research with mice ("mus musculus") in the field of biomedicine



Services

- Training and guidance on how to use the available equipment.
- Advice on data analysis and interpretation.
- The service staff have experience and qualifications in the field of preclinical biomedicine and can assist researchers with their experimental design and execution.

Stereoscopic Magnifier

IVESTA 3 Greenough Microscope: Unlike conventional stereoscopic microscopes, the Ivesta 3 incorporates **FusionOptics** technology, combining high resolution with a large depth of field simultaneously. Includes downloadable proprietary software for streamlined image use on laptops and smartphones.

Mouse Endoscope

COLOVIEW Mainz by Karl Storz: Enables visualization of the colon, fluid injection, and color video recording in anesthetized mice.

In Vivo Imaging Systems

IVIS Lumina II and IVIS Spectrum: Provide non-invasive imaging of live animals using optical, fluorescence, and bioluminescence modalities.

Element HT5 Hemocytometer

A multi-channel analyzer using laser flow cytometry, impedance technology, and colorimetric detection for rapid and accurate hematological profiling across 20 parameters in under a minute using only 15 µL of whole blood. Also supports safe analysis of non-blood cavity fluids, delivering immediate cellular content information. Results are displayed numerically and as WBC scatter plots and WBC/PLT histograms.

Biochemical Analyzer

VetScan VS2: A compact system for chemical, gasometric, and electrolyte blood analysis completed in 12 minutes with just 100 µL of blood. Includes an intelligent quality control system (**iQC®**) for reliability. Each disposable rotor contains all necessary reagents and diluent for multivariate analysis. Available rotor profiles:

Rotor	Description	Parameters
Comprehensive Diagnostic Profile	Full chemical and electrolyte analysis	ALB, ALP, ALT, AMY, BUN, Ca, CRE, GLOB, GLU, K+, Na+, PHOS, TBIL, TP
Preventive Care Profile Plus	Comprehensive health screening	ALB, ALP, ALT, AST, BUN, Ca, Cl-, CRE, GLOB, GLU, K+, Na+, TBIL, tCO2, TP
Electrolytes Plus	Acid-base and osmotic balance	Cl-, K+, Na+, tCO2
Prep Profile II	Basic health assessment	ALP, ALT, BUN, CRE, GLU, TP
Critical Care Plus	Serial testing and reassessments	ALT, BUN, Cl-, CRE, GLU, K+, Na+, tCO2
Kidney Profile Plus	Renal monitoring and evaluation	ALB, BUN, Ca, Cl-, CRE, GLU, K+, Na+, PHOS, tCO2
Mammalian Liver Profile	Liver function baseline	ALB, ALP, ALT, BA, BUN, CHOL, GGT, TBIL
Phenobarbital Profile	Drug-level monitoring + liver markers	ALB, ALP, ALT, AST, BUN, GGT, PHB, TBIL
T4/Cholesterol Profile	Thyroid screening	T4, CHOL



PROTEIN BIOTECHNOLOGY FACILITY

Group Members

Technical director:

Dionisio Ureña Rodríguez (until May 2024)
María Gimeno Pérez (since Jan 2024)

Scientific supervisor:

José Berenguer Carlos (until Jun 2023)
Aurelio Hidalgo Huertas (since Jan 2024)

Staff:

Isabel Carrascal Blanco



Description

The Biotechnology Protein Facility, formerly known as the Fermentation Facility, provides scientific and technical support to research groups at CBM and other public or private institutions, offering guidance on the best strategies for biomass and protein production. We advise users on selecting appropriate bacterial strains, plasmid expression systems, and conditions for the overproduction of recombinant proteins. The facility also enables scaling up production processes and cultivating a wide range of non-recombinant microorganisms in large volumes, utilizing the fermenters available and monitoring key parameters—such as temperature, stirring, pH, oxygen concentration, foam, and biomass—in compliance with cGMP (current Good Manufacturing Practice) regulations. To evaluate protein production, we offer cell culture disruption services and SDS-PAGE analysis.

Additionally, the Biotechnology Protein Facility provides ready-to-use competent preparations of various *E. coli* strains suitable for gene cloning or recombinant protein expression. It also produces DNA size markers for internal use by most research groups at CBMSO.



Prueba de expresión de proteínas fluorescentes en matraz



Services

- Bioprocesses for microbial culture production at laboratory and pilot scale.
- Optimization of batch, feed-batch and continuous cultures.
- Heterologous protein expression in prokaryotes and yeasts.
- Ready-to-use *Escherichia coli* competent cells.
- DNA molecular weight markers.
- Disruption of cell cultures.
- SDS-PAGE electrophoresis and gel staining.



PROTEOMICS FACILITY

Group Members

Technical director:

Carlos García García
Esperanza Morato López

Scientific supervisor:

José Manuel Cuezva Marcos
María Yáñez Mo

Staff:

Tamara Rosell García
Alberto Rodríguez Izquierdo



Description

The Proteomics Facility offers advance proteomics analytical services to the CBM scientific community, and also to any researcher from other public research centers, universities, hospitals and companies. As a support service for research activity, we provide personalized scientific/technical support to each project, which may include the stages before and after analysis: experimental design, projects review, data analysis and advice/support on the biological interpretation of generated data.

Since 2005, our laboratory has been part of ProteoRed, a proteomics platform whose main objective is to integrate and coordinate the activities of the Spanish Proteomics Services as well as to evaluate the quality of the services offered (participating in the human proteome sequencing project (SpHP)). We are also members of the Spanish Proteomics Society (SEProt) and the European Proteomics Association (EuPA). Besides, we are the Proteomics Service of the IIS- Princesa.



Services

Protein QC (Quality Control):

- Single-protein analysis
- Confirmation of protein overexpression in complex samples
- Protein identification in a gel spot/band

Protein-protein interactions:

- Characterization of protein-protein interactions: IPs, Pull-down, TAP system, BioID,
- Avidin/Biotin....
- Interactomics ID

Proteins identification/characterization by LC-MS/MS and bioinformatic analysis:

- High-throughput protein identification
- PTMs (Post-translational modifications) identification and characterization
- Discovery/validation of proteins of interest/biomarkers
- Targeted Proteomics (Peptide selection by Single Mass Ion Monitoring (SMIM))
- Characterization of molecular mechanisms

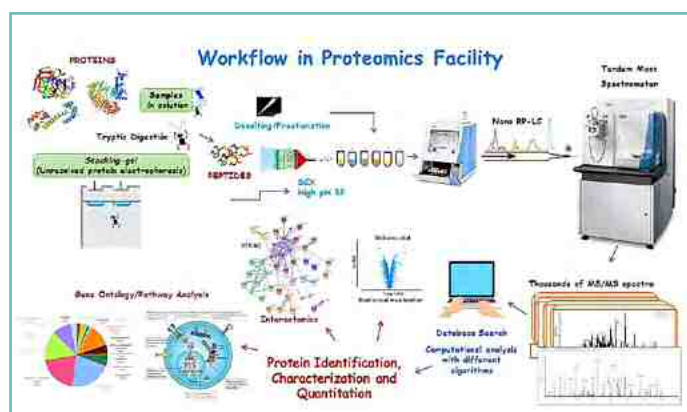
Quantitative Proteomics:

- Label-free protein quantitation in complex samples
- Protein quantitation in complex samples by iTRAQ/TMT
- Differential expression analysis in two or more sample groups
- Quantitation of post-translational modifications (PTMs)
- Proposals/Budget review for projects calls
- Collaboration in research projects
- Training in proteomics and bioinformatics tools



Courses

- Training in proteomics and bioinformatics tools



Workflow in Proteomics Facility



List of publications

- Rosell-García, T., Rivas-Muñoz, S., Kin, K., Romero-Albillo, V., Alcaraz, S., Fernandez-Tornero, C., & Rodríguez-Pascual, F. (2023). Multimerization of HIF enhances transcription of target genes containing the hypoxia ancillary sequence. *Biochimica et biophysica acta. Gene regulatory mechanisms*, 1866(4), 194963. <https://doi.org/10.1016/j.bbagr.2023.194963>
- Franca-Oliveira, G., Martínez-Rodríguez, A. J., Morato, E., & Hernández-Ledesma, B. (2023). Contribution of Proteins and Peptides to the Impact of a Soy Protein Isolate on Oxidative Stress and Inflammation-Associated Biomarkers in an Innate Immune Cell Model. *Plants*, 12(10), 2011. <https://doi.org/10.3390/plants12102011>
- Pablo Martí-Andrés, Isabela, Salvador Pérez, Sergio Rius-Pérez, Isabel Torres-Cuevas, Hildegard Colino-Lage, David Guerrero-Gómez, Esperanza Morato, Anabel Marina, Patrycja Michalska, Rafael León, Qing Cheng, Antonio Miranda-Vizuete, Edward E. Schmidt, Antonio Martínez-Ruiz, Elias S.J. Arnér, Juan Sastre. (2023). TRP14 is the cellular cystine reductase and also reduces cysteinylated proteins. *Free Radical Biology and Medicine*, Volume 201, Supplement 1, Page 15 <https://doi.org/10.1016/j.freeradbiomed.2023.03.078>.
- Romina Medeiros Figueredo, Elizabeth López-Achigar, Jorge Cantero Piñanez, Margot Paulino Zunini, Esperanza Morato López, Carlos García García, Antonio Martínez Ruiz (2023). Identification of tyrosine nitration as a nitroxidative posttranslational modification in fibrinogen. *Free Radical Biology and Medicine*. Volume 201, Supplement 1, Pages 63-64. <https://doi.org/10.1016/j.freeradbiomed.2023.03.258>.
- Pablo Martí-Andrés, Isabela Finamor, Isabel Torres, Salvador Pérez, Sergio Rius-Pérez, Hildegard Colino-Lage, David Guerrero-Gómez, Esperanza Morato, Anabel Marina, Patrycja Michalska, Rafael León, Qing Cheng, Eszter Petra Jurányi, Klaudia Borbényi-Galambos, Iván Millán, Péter Nagy, Antonio Miranda-Vizuete, Edward E. Schmidt, Antonio Martínez-Ruiz, Elias S.J. Arnér, Juan Sastre. (2024). Beyond reduction: unveiling TRP14's dual role in cysteine homeostasis and redox dynamics. *Free Radical Biology and Medicine*, Volume 218, Supplement 1, Page 8. <https://doi.org/10.1016/j.freeradbiomed.2024.04.034>.
- Romina Medeiros Figueredo, Elizabeth López-Achigar, Esperanza Morato López, Carlos García, García, Antonio Martínez Ruiz. (2024). Identification of nitrated proteins in human thrombi from ischemic cerebrovascular events. *Free Radical Biology and Medicine*, Volume 218, Supplement 1, Page 48. <https://doi.org/10.1016/j.freeradbiomed.2024.04.171>.
- Benito-Vázquez, I., Muñoz-Labrador, A., Garrido-Romero, M., Hontoria-Caballo, G., García-García, C., Díez-Municio, M., & Moreno, F. J. (2024). New Pipeline for Analysing Fruit Proteolytic Products Used as Digestive Health Nutraceuticals. *International journal of molecular sciences*, 25(19), 10315. <https://doi.org/10.3390/ijms251910315>
- Benito-Vázquez, I., Garrido-Romero, M., Hontoria-Caballo, G., García-García, C., Díez-Municio, M., & Moreno, F. J. (2024). Carob (*Ceratonia siliqua*) Flour as Source of Bioactive Compounds: Production, Characterization and Nutraceutical Value. *Foods (Basel, Switzerland)*, 13(19), 3024. <https://doi.org/10.3390/foods13193024>
- Martí-Andrés P, Finamor I, Torres-Cuevas I, Pérez S, Rius-Pérez S, Colino-Lage H, Guerrero-Gómez D, Morato E, Marina A, Michalska P, León R, Cheng Q, Jurányi EP, Borbényi-Galambos K, Millán I, Nagy P, Miranda-Vizuete A, Schmidt EE, Martínez-Ruiz A, Arnér ES, Sastre J. (2024). TRP14 is the rate-limiting enzyme for intracellular cystine reduction and regulates proteome cysteinylation. *EMBO J*. 43(13):2789-2812. doi: 10.1038/s44318-024-00117-1.
- Adán-Jiménez, J., Sánchez-Salvador, A., Morato, E., Solana, J. C., Aguado, B., & Requena, J. M. (2024). A Proteogenomic Approach to Unravel New Proteins Encoded in the *Leishmania donovani* (HU3) Genome. *Genes*, 15(6), 775. <https://doi.org/10.3390/genes15060775>
- Torres, A., Bernardo, L., Sánchez, C. et al. (2024). Comparing the Proteomic Profiles of Extracellular Vesicles Isolated using Different Methods from Long-term Stored Plasma Samples. *Biol Proced Online* 26, 18. <https://doi.org/10.1186/s12575-024-00243-4>
- Herreros-Cabello, A., del Moral-Salmoral, J., Morato, E., Marina, A., Barrocal, B., Fresno, M., & Gironès, N. (2024). Quantitative Proteomic Analysis of Macrophages Infected with *Trypanosoma cruzi* Reveals Different Responses Dependent on the SLAMF1 Receptor and the Parasite Strain. *International Journal of Molecular Sciences*, 25(13), 7493. <https://doi.org/10.3390/ijms25137493>
- Torres-Sánchez, E., Morato, E., Hernández-Ledesma, B., & Gutiérrez, L.-F. (2024). Proteomic Analysis of the Major Alkali-Soluble Inca Peanut (*Plukenetia volubilis*) Proteins. *Foods*, 13(20), 3275. <https://doi.org/10.3390/foods13203275>
- Paterson, S., Alonso-Pintor, L., Morato-López, E., González de la Fuente, S., Gómez-Cortés, P., & Hernández-Ledesma, B. (2025). Microalga *Nannochloropsis gaditana* as a Sustainable Source of Bioactive Peptides: A Proteomic and In Silico Approach. *Foods*, 14(2), 252. <https://doi.org/10.3390/foods14020252>



Participation in projects

- Teaching Innovation Project INNOVA "Coeducating in Equality to Teaching and Research Staff of UAM".
Funded by: Universidad Autónoma de Madrid
IP: Equality Unit and IUEM (2023-2024)
Alejandra Gamez is part of the research team
- Teaching Innovation Project IMPLANTA: "Gender and Intersectionality in Teaching Practice".
Funded by: Universidad Autónoma de Madrid
IP: Equality Unit and IUEM (2024-2025)
Alejandra Gamez is part of the research team



Other activities

- ACCESSIT ACCREDITATION IN GENDER EQUALITY 2024 Centro de Biología Molecular Severo Ochoa (CBM).

Driving Inclusion: The CBM Equality Committee's activities Towards Diversity and Equality



Activities

SEMINARS

- Dr. Silvia Gallego, ICM-SCIC, "Equality Networks. My Experience at CEI UAM+CSIC" March 21, 2024, commemorating International Women's Day.
- Dr. Paola Boloventa, Director of CBM and CSIC Research Professor, "Breaking the Glass Ceiling without Quotas" June 10, 2024.
- Professor José Ignacio Pichardo, tenured at UCM, "LGBTIQ+ Diversity at in university and work environments". Pride Month. June 2024.
- Dr. Aitor Villafranca, CSIC Research Professor and Director of Education at the PRISMA association, "LGBTIQ+ Diversity in Science: Current Situation and Actions for Equality. July 2024

WORKSHOPS

- Lola Ledesma "Do you want to be a (Neuro)Scientist?" IES Palomeras-Vallecas 2024
- Esther Serrano-Saiz "When I grow up, I want to be... a scientist". Webinar for ESO students as part of the seminar series "Women and Science at CBM" (2024).
- Educational workshop by María José Martín Pereira in collaboration with the Spanish Association Against Cancer at Arcángel Rafael International School. 2024
- Meeting with students, featuring Margarita del Val (organized by IRNASA - CSIC) 2024
- Talk in the "Women Scientists Cycle" by Dr. Natalia Azpiazu at IES Francisco Nieva de Valdepeñas for 8M - International Women's Day. 2024
- Educational workshop by Alejandra Gámez Abascal at Greenwich School, February 11th 2024.
- School workshop at CBM to award a 1st-grade student who won the "Draw a Scientist" prize from SEBMM. 2024
- "Microbiology is Written with an M for Woman" event organized by the Microbiology Area - E.S. of Experimental Sciences and Technology at Rey Juan Carlos University by Dr. Bruna Fernanda de Sousa. 2024.
- Reading of names of murdered women and a family picture with signs against gender violence. November 25, 2024.

DIGITAL CONTENT

- Creation of a commemorative video for March 8, 2024: International Women's Day.
- Creation of a video and poster for the IV Equality Committees Encounter at CSIC.

PROJECTS: VISIBILITY AND CONCILIATION

- Setup and inauguration of a lactation room at CBM (January 2024)
- Violet space in CBM hall (March 8, 2024)

DISSEMINATION

- Monthly newsletter from the CBM Equality Commission to disseminate activities related to Equality, Diversity, and Inclusion. Distributed at IUEM, Inter-center CSIC Commission, CBM, IU UAM.

CONTRIBUTION TO THE ESCAPE ROAD OF THE INTERCENTERS CSIC-UAM NETWORK

- Creation of Gerty Cori and Christiane Nüsslein-Volhard posters that are part of the escape road "In Search of Nobel and Non-Nobel Women"

MULTICULTURAL DAY

- A interactive world map celebration the cultural diversity day (21st May) in the CBM hall where individuals can pin their country of origin to celebrate the worldwide diversity of the CBM community

GAY PRIDE MONTH

- Seminars by Aitor Villafranca and José Ignacio Pichardo
- Stand with diverse information about the LGTBIQA community with posters of 8 key LGTBIQA+ scientists and lists of movies and books related to the community.
- Game to visualize affective-sexual diversity at our center with 6 flasks: Heterosexual, Bisexual, Gay, Lesbian, Asexual, and Others to put a small colored ball inside the flask that most represents you



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2023-2024