STRUCTURAL BIOLOGY PROGRAMME

ÓSCAR LLORCA Programme Director

The Programme's research areas and strategic goals

Scientists at the Structural Biology Programme (SBP) use the enormous opportunities provided by advances in structural and computational biology to improve our understanding of fundamental processes in the origin and progression of cancer, and to generate new knowledge and tools that can ultimately benefit patients. The Programme currently encompasses 5 Groups and 5 Units organised according to 2 major strategic lines: (a) structural biology and (b) computational and cancer genomics. The strategic line in structural biology comprises 3 Groups (Macromolecular Complexes in DNA Damage Response; Kinases, Protein Phosphorylation and Cancer; Genome Integrity and Structural Biology) and 4 Units (Electron Microscopy (EM); Spectroscopy and Nuclear Magnetic Resonance (NMR); Protein Crystallography; Protein Production). Their main aim is to determine the structures of proteins and macromolecular complexes relevant in cancer in order to resolve how they work and to support drug discovery efforts. The strategic line in computational and cancer genomics consists of 2 groups (Computational Cancer Genomics and Computational Oncology) and 1 Unit (Bioinformatics). They use bioinformatics, computational biology, and cancer genomics to better understand the complexity of cancer, predict therapy responses, design new therapeutic strategies, and develop new tools. In addition, the Units at SBP provide support in cryoEM, NMR, protein purification, protein crystallography, bioinformatics and biophysics to all CNIO researchers, particularly to groups outside SBP that do not have the sufficient expertise in these methodologies.

Summary of milestones & major achievements during 2022

During 2022, scientists at SBP made interesting discoveries in several areas of cancer research and developed new tools. The Computational Oncology Group evaluated chromosomal instability across thousands of tumours, defining "signatures" with predictive value for drug response and possible new drug targets. They also developed a computational tool to help select cell lines for cancer research. The Bioinformatics Unit studied the clinical relevance of tandem exon duplication-derived substitutions in cancer, developed bioinformatics tools to help clinicians to prioritise available drugs for treatment, and assisted several groups across the CNIO. The

> **"During 2022, research at SBP generated knowledge on fundamental processes in cancer and about the proteins involved, and developed new tools aiming to help cancer research and eventually patients."**

Kinases, Protein Phosphorylation and Cancer Group integrated structural, computational and biochemical data to identify a druggable vulnerability in the RET kinase that could serve to search for new inhibitors useful for patients with RET-driven cancers. The Macromolecular Complexes in DNA Damage Response Group and the Genome Integrity and Structural Biology Group used cryoEM to understand the assembly of the spliceosome, DNA replication, and cytosolic DNA sensing. The Ku70-Ku80 complex is part of an innate immunity system that serves against viral infection and contributes to autoimmune diseases and cancer. The Macromolecular Complexes in DNA Damage Response Group discovered how some viruses inactivate the capacity of Ku70-Ku80 to detect cytosolic DNA. The Electron Microscopy, Protein Production and Protein Crystallography Units made substantial contributions to the research of many groups across the CNIO. The Protein Production Unit also developed nanobodies to be used as probes for triple negative breast cancer imaging. As a final remark, I want to emphasise the involvement of many of the scientists at SBP, especially our junior faculty and the younger members of the groups, in numerous activities to bring science closer to society and make the CNIO a better place to train young scientists. ∎

MACROMOLECULAR COMPLEXES IN DNA DAMAGE RESPONSE GROUP

Óscar Llorca
Group Leader

Research Scientists
Javier Coloma, Ana Isabel Hernández, María Ibarra, Andrés López, Ángel Rivera, Marina Serna

Graduate Students
Natalia Cuervo, Carmen García (since September), Nayim González

Post-Doctoral Fellows
Sofía Cabezudo, María Martínez

Technicians
Paloma Escudero (until March) (TS)*, Ana González

Titulado Superior (Advanced Degree)

Students in Practice
Carmen Arroyo (Feb-June) (Bacherlor's Degree Final Project, *Univ. Autónoma de Madrid*, Spain), Carmen García Martín (March-July) (Master's Thesis, *Univ. Complutense de Madrid*, Spain)

Visiting Scientists
Raquel Arribas (Feb-March) (Univ. of Sussex, UK), Evangelina Nogales (Jan-July) (University of California Berkeley, USA)

OVERVIEW

Our Group uses cryo-electron microscopy (cryoEM) to determine the 3D structure of large macromolecular complexes of relevance in cancer at high resolution. Structural information, in combination with molecular and cell biology and biochemistry, is then used to propose how these molecules work and increase our understanding of the molecular basis of cancer. Most of our efforts are currently focused on 2 major areas of research: the study of i) chaperones essential for the activation of several macromolecular complexes relevant in cancer and ii) complexes implicated in the repair of DNA damage and in genomic instability. In collaboration with other groups, we are also studying the structure and mechanisms of several amino acid transporters.

"We have improved our understanding of the molecular mechanisms involved in spliceosome maturation, and cytosolic DNA sensing by the DNA repair protein Ku70/Ku80 and its subversion by some poxviruses."

RESEARCH HIGHLIGHTS

Understanding the maturation of the spliceosome, a cellular process involved in some types of cancer

Splicing is a cellular mechanism that facilitates the reading of DNA and multiplies the number of potential protein sequences in a cell by allowing the synthesis of several different proteins from a single gene. Alternative splicing is an extraordinarily complex process that requires the coordinated action of multiple proteins, each specialised in very specific functions. These proteins are assembled and matured, forming large macromolecular complexes, a process that is tightly controlled, and any failure can result in genetic diseases (FIGURE 1A). Several types of cancer present failures in the splicing processes, which is an advantage for tumour cells since these failures improve their rate of survival.

We have investigated some of the factors that enable the assembly and maturation of the spliceosome, particularly PRPF8, one of U5 snRNP's main components. We used biochemistry, interaction mapping, mass spectrometry and cryoEM to study the role of RUVBL1 and RUVBL2 ATPases and the ZNHIT2 protein in the biogenesis of PRPF8. We found that ZNHIT2 forms a network of contacts between several assembly factors required for PRPF8 biogenesis including ECD and AAR2, and that ZNHIT2 connects PRPF8 with the R2TP-HSP90 chaperone machinery, which is required for PRPF8 maturation. In addition, cryoEM showed how ZNHIT2 binds RUVBL1-RUVBL2 and affects the conformation of RUVBL2 (FIGURE 1B), which regulates RUVBL1-RUVBL2 ATPase activity.

Taken together, our results reveal part of the complex mechanisms that regulate the maturation of the splicing machinery, an essential process for the cell that can cause diseases such as cancer when perturbed.

Mechanism that helps some poxviruses to evade our cellular defence system

The Ku70-Ku80 complex is an essential component of the non-homologous end-joining (NHEJ) machinery that repairs DNA double strand breaks. Its structure shows that the protein comprises a preformed ring that can encircle duplex DNA. Ku70-Ku80 is the first protein to detect the presence of a break in the DNA thanks to this capacity to bind DNA like a ring encircles a finger.

Interestingly, Ku70-Ku80 is also present in the cytoplasm of cells, but its role there is not to detect and repair broken DNA but to alert the cell of the presence of viruses and activate cellular defences. The capacity of Ku70-Ku80 to encircle a linear dsDNA is used in the cytoplasm to detect viral DNA and initiate an inflammatory and innate immunity response. But some of these viruses have evolved countermeasures against these DNA sensors to attempt to block or delay the host immune response and allow the proliferation and spread of the disease. Vaccinia virus (used in the development of the smallpox vaccine and belonging to the poxvirus family) produces 2 proteins, C4 and C16, that bind to Ku70-Ku80 and inactivate its downstream signalling to the cellular immune response; however, the mechanism has not been well understood.

Using cryoEM, we have determined the 3-dimensional structure of C16 and its complex with Ku70-Ku80 (FIGURE 2A). In collaboration with L. H. Pearl's group (University of Sussex) and the Institute of Cancer Research in UK, we discovered that C16 and C4 proteins produced by the virus act as plugs that insert into the central hole of Ku70-Ku80, which it uses to thread itself into DNA, inhibiting Ku70-Ku80's ability to recognise viral DNA (FIGURE 2B). The structure of the C16 – Ku70-Ku80 complex was determined at high resolution, which allowed us to identify atomic details of how C16 binds and inactivates Ku70-Ku80, identifying key residues. Interestingly, by comparing the protein sequences of the C4 and C16 homologues in other viruses of the same family, we found that the regions involved in Ku inactivation are conserved in several orthopoxviruses, including smallpox and monkeypox. ∎
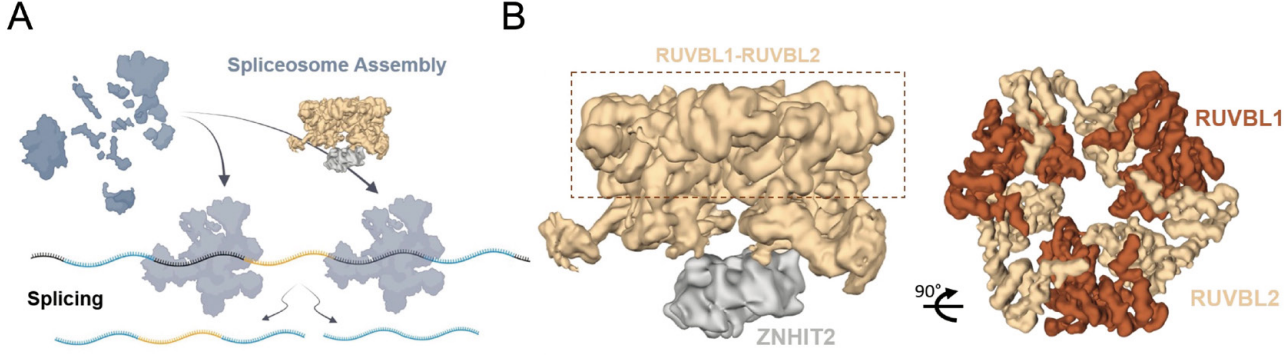

FIGURE 1 RUVBL1, RUVBL2 and ZNHIT2 form a complex required for the maturation of PRPF8 and the spliceosome. (**A**) Drawing representing the need of several assembly factors during the assembly of the spliceosome. (**B**) CryoEM map of the RUVBL1-RUVBL2-ZNHIT2 complex. Right panel highlights RUVBL1-RUVBL2 in the complex.
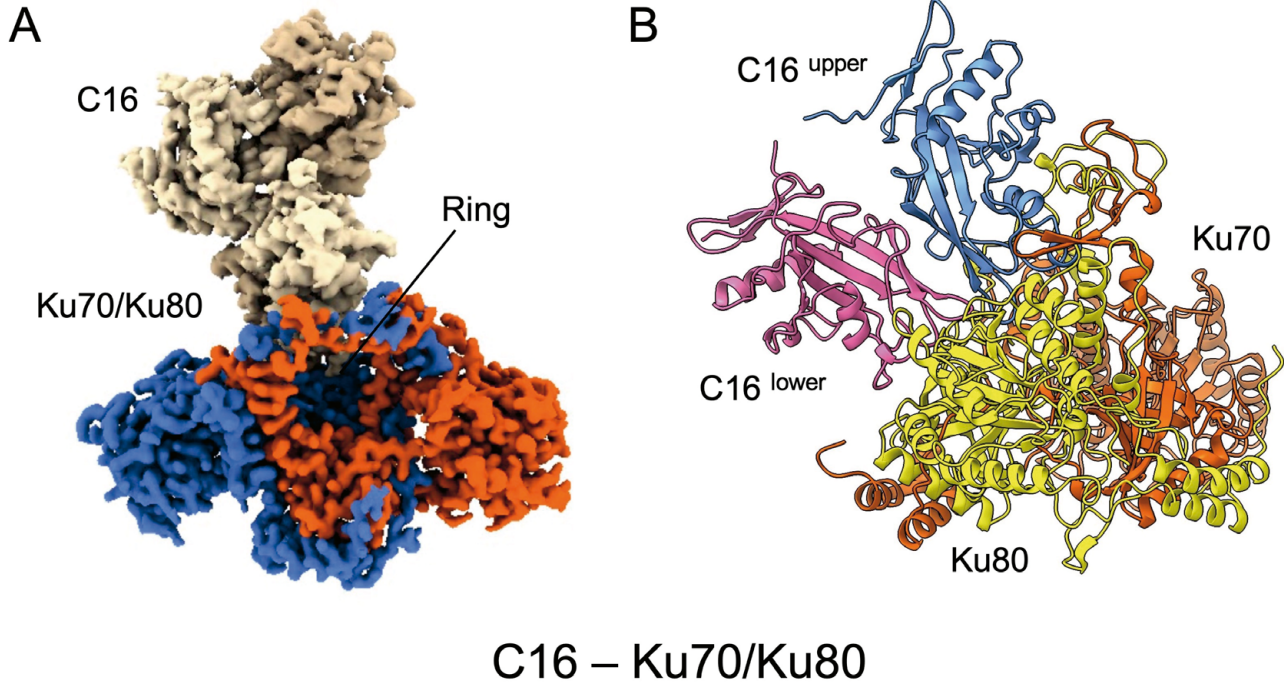

C16 – Ku70/Ku80

FIGURE 2 Structure of C16 — Ku70-Ku80 complex. (**A**) CryoEM map of the C16 protein from vaccinia bound to Ku70-Ku80. (**B**) One view of the atomic model of C16 — Ku70-Ku80, showing 2 copies of C16 C-terminal domain binding to Ku70-Ku80.

▸ **PUBLICATIONS**

▸ Rivera-Calzada A, Arribas-Bosacoma R, Ruiz-Ramos A, Escudero-Bravo P, Boskovic J, Fernandez-Leiro R, Oliver AW, Pearl LH, Llorca O (2022). Structural basis for the inactivation of cytosolic DNA sensing by the vaccinia virus. *Nat Commun* 13, 7062.

▸ Rullo-Tubau J, Bartoccioni P, Llorca O, Errasti-Murugarren E, Palacín M (2022). HATs meet structural biology. *Curr Opin Struct Biol* 74, 102389.

▸ Serna M, González-Corpas A, Cabezudo S, López-Perrote A, Degliesposti G, Zarzuela E, Skehel JM, Muñoz J, Llorca O (2022). CryoEM of RUVBL1-RUVBL2-ZNHIT2, a complex that interacts with pre-mRNA-processing-splicing factor 8. *Nucleic Acids Res* 50, 1128-1146.

▸ **AWARDS AND RECOGNITION**

▸ Marina Serna: *Premio Josep Tormo* Award for Structural Biology 2022, the Spanish Biochemical and Molecular Biology Society (*SEBBM*).

▸ María Ibarra Dauden: "*Premios Fundación Merck Salud-ASEICA por el Impulso de las Vocaciones Científicas – Investigadoras*", *Merck Salud* Foundation and the Spanish Association for Cancer Research (*ASEICA*).

KINASES, PROTEIN PHOSPHORYLATION AND CANCER JUNIOR GROUP

Iván Plaza Menacho
Junior Group Leader

Post-Doctoral Fellow
Julia M. Contreras

Gradutate Students
Nicolás Cuesta (until November), Ana Martín-Hurtado, Moustafa Ahmed Shehata (until April)

Visiting Gradutate Student
Yanara Astudillo (*Universidad Tecnológica Equinoccial - Fundación Carolina,* Quito, Ecuador)

Students in Practice
Marina Rodríguez (Bachelor's Degree Final Project, *Univ. Autónoma de Madrid;* Master's Thesis, *Univ. Complutense de Madrid,* Spain), Alejandro Sánchez (March–

September) (Master's Thesis, *Univ. de Alcalá de Henares,* Spain)

OVERVIEW

Rational and precise targeting of oncogene-driven signalling is a crucial and yet today outstanding challenge in cancer research. Understanding the structural and molecular bases of oncogene activation and signalling is key for the design and development of better therapeutics. Our research focuses on the structural and molecular understanding of protein kinase function: how protein kinases are activated and regulated by post-translational modifications and allosteric inputs, and how they assemble into macromolecular protein complexes to transmit signals inside the cell. We put a special emphasis on how these mechanisms are corrupted in cancer due to oncogenic mutations and other oncogenic insults. Crucially, such atomic and molecular information can be translated into the design and development of next generation protein kinase inhibitors for targeted and personalised therapies.

We apply an integrated and multidisciplinary approach by combining: molecular biology for the generation of suitable constructs; protein biochemistry and biophysics for protein purification, quality assessment and functional evaluation; mass spectrometry (MS) for the identification and quantification of post-translational modifications; X-ray crystallography for the 3D-visualisation of proteins; and *Drosophila* as an *in vivo* model for data validation. Furthermore, we use structure-guided drug discovery and MD simulation approaches to exploit structural and functional vulnerabilities for drug design and development.

RESEARCH HIGHLIGHTS

Our main strategic lines are:

1. Structural and molecular determinants that control protein phosphorylation. Auto-phosphorylation controls the transition between discrete functional and conformational states in protein kinases, yet the structural and molecular determinants underlying this fundamental process remain unclear. In our recent work, we show that c-terminal Tyr 530 is a *de facto* c-Src auto-phosphorylation site with slow time-resolution kinetics and strong intermolecular component. By contrast, activation-loop Tyr 419 undergoes fast kinetics and a cis-to-trans phosphorylation-switch that controls c-terminal Tyr 530 auto-phosphorylation, enzyme specificity, and strikingly, c-Src non-catalytic function as a substrate. In line with this, we visualised by X-ray crystallography a snapshot of Tyr 530 intermolecular phosphorylation in which a c-terminal palindromic phospho-motif flanking Tyr 530 on the substrate molecule engages the G-loop of the active kinase for ready entry prior catalysis. Perturbation of the phospho-motif accounts for c-Src dysfunction as indicated by viral and a colorectal cancer (CRC) associated c-terminal deleted variants. We showed that c-terminal residues 531 to 536 are required for c-Src Tyr 530 and global auto-phosphorylation, and this detrimental effect is caused by the substrate molecule inhibiting allosterically the active kinase. Our work reveals a bi-directional crosstalk between the activation and c-terminal segments that controls the allosteric interplay between substrate and enzyme acting kinases during auto-phosphorylation (Cuesta and Contreras *et al.*, under revision. *BioRXiv*. doi: https://doi.org/10.1101/2022.10.16.512342).

2. Structure, function, and pharmacology of protein kinase-gene fusion products. Gene fusion products are known drivers in human cancers and are current drug targets for personalised therapy. A second strategic line in the lab was established and directed to dissect the functional and structural determinants for two RET oncogenic fusion products, namely CCDC6-RET and KIF5B-RET, which are drivers and therapeutic targets in lung (NSCLC) and thyroid cancers. We have successfully purified these challenging proteins using a baculovirus expression system in different isoforms and length-variants. By applying an integrated approach, we demonstrated that full-length constructs behave like active dimers in solution. Auto-phosphorylation and enzymatic assays demonstrated

fast kinetics compared to wild-type RET, and further phospho-proteomic characterisation by mass spectrometry highlighted important roles for catalytic activity and substrate specificity through unexpected allosteric inputs by distant elements to the catalytic site (Hurtado *et al.*, submitted).

3. Structure-guided drug discovery for next generation protein kinase inhibitors. A third main research line is focused on the exploitation of structural and functional vulnerabilities in RET for the rational design and development of highly specific inhibitors. Our current paradigm is based on the recently developed second generation RET inhibitors LOXO-292 and BLU-667 that showed excellent results in both preclinical models and early clinical trials, resulting in timely FDA approval for the treatment of RET-rearranged or -mutated cancers. We are applying an integrated approach combining structural data, molecular docking, structure-guided molecular dynamics simulations, and screening with both virtual and chemical libraries together with biophysical and biochemical tools for functional validation. Following this approach, we identified an allosteric interface in RET with good druggability score that can be potentially targeted with allosteric inhibitors. Furthermore, we found a cryptic and druggable pocket within the ATP-binding site that is exploited by LOXO-292 and BLU-667 *(*Shehata and Contreras *et al., J Adv Res 2022)*. This information will be crucial to designing and developing highly specific third generation RET inhibitors able to overcome refractory RET mutations. Based on these results we are optimising chemical scaffolds of second generation RET inhibitors to maximise contacts and interactions with the cryptic pocket, in collaboration with CNIO's Experimental Therapeutics Programme. ■

▸ **PUBLICATIONS**

▸ Shehata MA, Contreras J, Martín-Hurtado A, Froux A, Mohamed HT, El-Sherif AA, Plaza-Menacho I (2022). Structural and dynamic determinants for highly selective RET kinase inhibition reveal cryptic drug-gability. *J Adv Res.* PMID: 35595215.

▸ Cuesta N, Contreras J, Sánchez-Walder-mer J, Soriano-Maldonado P, Martín-Hur-tado A, Muñoz IG, Llimargas M, Muñoz J, Plaza-Menacho I (2022). An allosteric switch between the activation loop and

a c-terminal palindromic phospho-motif drives c-Src function. *BioRXiv.* doi: https://doi.org/10.1101/2022.10.16.512342.

▸ **PATENT**

▸ Plaza Menacho I. Identification of a cryp-tic and druggable pocket in the active site of RET with therapeutic potential: the post-lysine pocket. PCT application (2022). *PCT/EP2022/077036. WO2023052462A1.*

GENOME INTEGRITY AND STRUCTURAL BIOLOGY JUNIOR GROUP

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Junior Group Leader

Post-Doctoral Fellow
Maria Dolores Moreno

Gradutate Students
Ester Casajús, Samuel Míguez

Technician
Araceli Grande (TS)*

*Titulado Superior (Advanced Degree)

Students in Practice
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Univ. Autónoma de Madrid, Spain), Raquel Cuesta (Bachelor's Degree Final Project; Master's Thesis, *Univ. Complutense de Madrid*, Spain), Marta Seijo (*Univ. Complutense de Madrid*, Spain)

RESEARCH HIGHLIGHTS

DNA replication & repair - focus on mitochondria

Mitochondrial DNA (mtDNA) replication is critical for human health. Deficiencies in the operation of mtDNA replication machinery underlie various devastating multi-systemic mitochondrial disorders. Importantly, mtDNA defects have been linked to other prominent diseases, including Parkinson's and Alzheimer's disease, autism spectrum disorders, diabetes, and several cancer types. However, how the mitochondrial genome's integrity is maintained through the equilibrium between DNA replication, repair and degradation, and organelle dynamics, remains unclear. We are interested in understanding these pathways because of their implications for ageing and disease, particularly their relationship to cancer.

Genome integrity - focus on telomeres

Telomeres are essential nucleoprotein structures that protect the end of our chromosomes. These structures are shaped by the protective shelterin complex that specifically binds to telomeric TTAGGG DNA repeats. Shelterin is composed of 6 proteins – TRF1, TRF2, RAP1, TIN2, TPP1, and POT1 – and their proper arrangement and function protect telomeres from degradation and activation of a persistent DNA damage response. Shelterin function is therefore crucial for telomere and genome integrity. Despite the key role of the shelterin complex in cell viability and tissue homeostasis, as well as its potential use as a target for anti-cancer therapeutic strategies, its mechanistic details and architecture are poorly understood.

Biochemistry & cryo-EM

By combining *in vitro* reconstitution and native purification of protein-DNA complexes and by taking advantage of the recent developments in cryo-EM imaging, we can capture these protein machineries in different functional states to study their structures. With this information we will be able to unveil their molecular mechanisms, rationalise pathological mutations and their physiological consequences, and aid in the development of future cancer therapeutic strategies. ∎
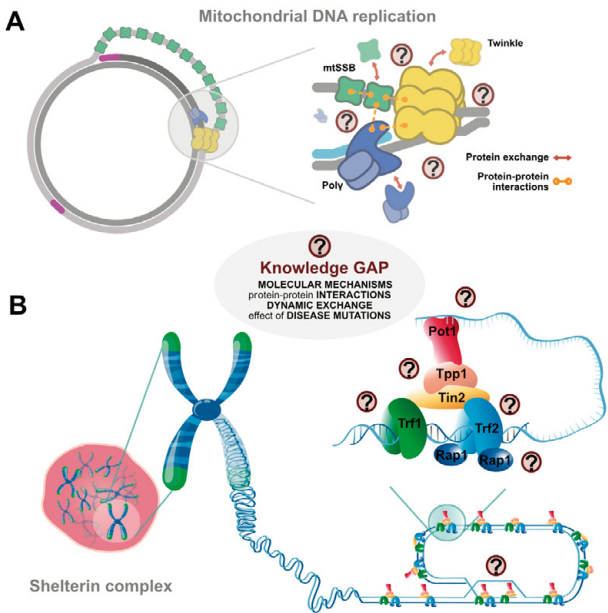

FIGURE 1 (**A**) Mitochondrial DNA replication machinery. (**B**) The shelterin complex shapes, protects, and regulates enzymatic activities at telomeres. Several key aspects of their regulation, and the molecular mechanisms through which both protein complexes exert their activities, remain unknown.

OVERVIEW

Safeguarding genetic information is essential to avoid malignant transformation. Two key cellular processes keep it free from errors: DNA replication and DNA repair. Importantly, when these do not work correctly, genetic information may be damaged or lost, ultimately leading to disease. Deregulation and malfunction of the protein machinery that safeguards our genome are all hallmarks of cancer, but it remains unclear how this happens at the molecular level. The devil is in the detail, and we aim to understand what goes wrong with these molecular machines, and when, so we can act on it to correct it and prevent it from happening.

These macromolecules are like real-life machines, with intricate mechanisms that allow them to perform their

activities. To understand how they work, we use cryo-electron microscopy (cryo-EM) and biochemistry in an integrative approach. Beyond fundamental research, this structural information provides the necessary detail for drug development.

> **"Macromolecules are like real-life machines. To understand how they work, we study their structures using cryo-EM. Beyond basic research, this provides the necessary information for drug development."**

▸ **PUBLICATION**

▸ Rivera-Calzada A, Arribas-Bosacoma R, Ruiz-Ramos A, Escudero-Bravo P, Boskovic J, Fernandez-Leiro R, Oliver A.W, Pearl LH, Llorca O (2022). Structural basis for the inactivation of cytosolic DNA sensing by the vaccinia virus. *Nat Commun* 13, 7062.

▸ **PATENT**

▸ Lamers MH, Fernández-Leiro R (2022). Device and method for cryogenic electron microscopy sample preparation. *Dutch Patent Application No. 2033291*.

COMPUTATIONAL CANCER GENOMICS JUNIOR GROUP

Solip Park
Junior Group Leader

Post-Doctoral Fellow
Seulki Song (since October)

Gradutate Students
Pelayo González de Lena, Moustafa
Ahmed Mohamed Shehata (since
May)

Technicians
Luis García (until June), Manuel
Moradiellos (since August), Laia Ollé
(since July)

Visiting Master's Students
Adrián Maqueda (since September)
(Master's Thesis, *Univ. Autónoma de
Madrid*, Spain), Laia Ollé (February-

May) (Master's Thesis, *Univ.
Politécnica de Madrid*, Spain)

OVERVIEW

Cancer is a complex disease whereby cells grow and reproduce uncontrollably. One important feature necessary to understand cancer is its heterogeneity, which indicates that the effect of alterations could be different depending on the cellular context. In the Computational Cancer Genomics (CCG) Lab, we aim to understand the context-dependent cancer fitness landscape both by applying a computational approach and by setting up experimental collaborations. For example, we are specifically interested in changing the cancer fitness landscape depending on time, by analysing the associations between germline variants and somatic alterations, or by comparing the differences between the primary tumour and metastasis. In addition, we aim to further pursue how protein-protein interaction networks of cancer driver genes can be perturbed by their somatic or germline variants. We expect that our

"Through large-scale cancer genomics analysis, we aim to understand the complete cancer fitness landscape, analysing both germline variant- and somatic mutation-based perturbation of protein interaction."

context-dependent cancer fitness landscape will provide a crucial direction for personalised medicine, since we are aiming to address the heterogeneity across patients, conditions, and cellular contexts.

RESEARCH HIGHLIGHTS

Context-specific genetic interaction perturbations

Metastasis is the main cause of death in cancer patients. However, most current cancer consortia have focused on primary cancer states. To gain a better understanding of the context-specific cancer fitness landscape across cancer statements, we systematically measured the association between somatic mutations and copy-number changes within the same genes across cancer types and compared their strengths of interaction between cancer statements. We found that several cancer types and cancer genes present significantly different preferences of interaction between mutations and copy-number changes and also proved that these differences are not due to medical treatments or genomic differences (*manuscript in preparation*). We expect that our findings will provide new insights to understand statement-specific perturbations and clues to develop better treatments for cancer patients.

Defining new cancer predisposition genes

Although large-scale cancer genomics data are rapidly accumulating, our understanding of cancer genes is highly biased towards somatic alterations and not germline variants. Germline frequencies are usually low, and there are several technical difficulties associated with their analysis. Since only 130 cancer predisposition genes (CPGs) are currently available, their contributions to cancer risk are underestimated. We hypothesised that germline variants in Mendelian-associated genes (OMIM genes) could contribute to increasing cancer risk. First, we proved that OMIM genes tend to have more pathogenic germline variants in cancer compared to controls (*manuscript under revision*). We then focused on a *PAH* that is associated with phenylketonuria, which presents the strongest enrichment in cancer compared to controls, and this enrichment is reproduced in other cancer data sets. Furthermore, through collaborations in South Korea, we addressed how metabolic dysfunction increases cancer risk experimentally, and we identified the possible contribution of OMIM genes as new CPGs. Currently, we are expanding this concept to predict novel CPGs, not only OMIM genes, by integrating multiple features using a machine learning approach. ∎
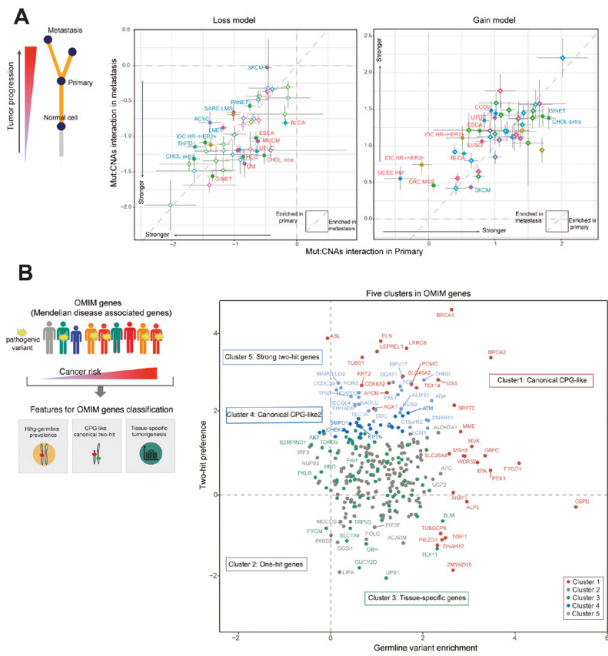

FIGURE 1 Understanding the cancer fitness landscape through both germline and somatic alterations. (**A**) Genetic interaction differences between primary tumours and metastases using 25,000 tumour samples. (**B**) Elucidating the role of Mendelian disease-associated genes as possible new cancer predisposition genes.

COMPUTATIONAL ONCOLOGY JUNIOR GROUP

Geoff Macintyre
Junior Group Leader

Research Scientist
Maria José García (until October)

Post-Doctoral Fellow
Bárbara Hernando

Gradutate Students
Blas Chaves, María Escobar, Ángel Fernández (since May), **David Gómez**

Technician
Alice Nicole Cádiz (PEJ, CAM) ¹

¹ *Plan de Empleo Joven de la Comunidad de Madrid* (Youth Employment Plan, Community of Madrid)

Students in Practice
Diego García (since February) (Master's Thesis, *Universidad Politécnica de Madrid*, Spain), **Sara Vettorazzo** (August-December)

(Erasmus + Fellowship, *Università di Trento*, Italy)

OVERVIEW

In the Computational Oncology Group, we are tackling some of the deadliest cancers by targeting the causes of chromosomal instability (CIN). By therapeutically targeting CIN, we aim to improve patient outcomes.

Our main research areas include:

→ Using model systems to develop therapeutic strategies to target CIN.
→ Predicting therapy response using CIN signatures in patient biopsies.
→ Developing single cell sequencing approaches for ultrasensitive CIN detection.

> **"We have developed a new computational framework to evaluate the extent, diversity and origin of chromosomal instability pan-cancer."**

We aim to apply these technologies at the earliest stages of tumour development in patients with premalignant lesions, with the goal of preventing aggressive, difficult to treat cancers.

RESEARCH HIGHLIGHTS

2022 was a great year for the Computational Oncology Group. We welcomed a new lab member and saw our senior staff scientist, Maria José Garcia, move to CSIC as a PI! Another key highlight was seeing our CIN signature study published in *Nature*. We also secured the front cover artwork with an abstract interpretation of the research painted by Geoff's cousin Julian Aubrey Smith (FIGURE 1). This study was the culmination of years of computational work during the pandemic period in collaboration with the University of Cambridge. We developed a framework to evaluate the diversity and origin of chromosomal instability pan-cancer, identifying 17 genomic signatures of different types of CIN. Barbara was crucial to the success of the project demonstrating that the signatures can predict drug response and identify new drug targets. The work attracted significant press coverage, including appearing on the front page of *El País*. Barbara is now extending this technology to work at single cell resolution to enable a number of other projects in the lab.

Ángel joined the lab as a computational PhD student to understand how CIN contributes to tumour evolution. Working closely with Barbara, they have recently developed an approach to forecast oncogenic amplification in tumours using the CIN signatures. David is combining this approach with his new method to extract robust copy number profiles from targeted gene sequencing data to forecast MET amplification-driven resistance to EGFR inhibitors in lung cancer.

Maria and Blas were busy performing single cell DNA sequencing of organoids and KO cell lines – expect exciting

FIGURE 1 Our CIN signature article on the cover of *Nature*.

results in 2023! Two masters' students also completed their projects: Diego developed a new method for mis-match repair deficiency prediction, and Sara uncovered new patterns of CIN in premalignant oesophageal lesions.

Much of this work will be submitted in 2023 for publication, so hopefully there will be another great year ahead! ∎

▸ **PUBLICATIONS**

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▸ Cheng Z, Mirza H, Ennis DP, Smith P, Morrill Gavarró L, Sokota C, Giannone G, Goranova T, Bradley T, Piskorz A, Lockley M; BriTROC-1 Investigators; Kaur B, Singh N, Tookman LA, Krell J, McDermott J,

Macintyre G, Markowetz F, Brenton JD, McNeish IA (2022). The genomic landscape of early-stage ovarian high-grade serous carcinoma. *Clin Cancer Res* 28, 2911-2922.

▸ Chaves-Urbano B, Hernando B, Garcia MJ, Macintyre G (2022). CNpare: matching DNA copy number profiles. *Bioinformatics* 38, 3638-3641.

▸ Gallego A, Mendiola M, Hernando B, Berjon A, Cadiz A, Chaves-Urbano B, Heredia-Soto V, Spagnolo E, Hernández Gutiérrez A, Hardisson D, Macintyre G, Redondo A, Garcia MJ (2022). Prognostic markers of inflammation in endometrioid

and clear cell ovarian cancer. *Int J Gynecol Cancer* 32, 1009-1016.

▸ Vias M, Gavarró LM, Sauer CM, Sanders D, Piskorz AM, Couturier DL, Ballereau S, Hernando B, Hall J, Correia-Martins F, Markowetz F, Macintyre G, Brenton JD (2022). High-grade serous ovarian carcinoma organoids as models of chromosomal instability. *bioRxiv*. doi: https://doi.org/10.1101/2022.09.01.506155.

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Macintyre G, Markowetz F, Brenton JD, McNeish IA, The BriTROC Investigators (2022). The genomic landscape of recurrent ovarian high grade serous carcinoma: the BriTROC-1 study. *medRxiv*. doi: https://doi.org/10.1101/2022.10.21.22280992.

▸ **PATENT**

▸ Macintyre G, Drews R, Markowetz F, Hernando B. Method of characterising a DNA sample. PCT application (2022). *PCT/EP2022/077473. WO2023057392A1.*

SPECTROSCOPY AND NUCLEAR MAGNETIC RESONANCE UNIT

Ramón Campos-Olivas
Unit Head

Technician
Clara M. Santiveri (TS)*

*Titulado Superior (Advanced Degree)

RESEARCH HIGHLIGHTS

The Unit provides a broad range of instrumentation for the biophysical characterisation of biomolecules and their interactions, including spectrophotometers, a fluorimeter, a nanoDSF (Differential Scanning Fluorimetry) device, isothermal titration and differential scanning calorimeters, a circular dichrograph, dynamic and multi-angle static light scattering (MALS) equipments, 2 biosensor instruments — surface plasmon resonance (SPR) and biolayer interferometry (BLI) — and a multiple-well microplate reader with numerous technologies. Research groups mostly from but not limited to (i.e., DNA Replication Group, Metabolism and Cell Signalling Group, Experimental Oncology Group) the Structural Biology Programme used these technologies throughout the year. For example, in collaboration with the Protein Production and Molecular Imaging Core Units, using nanoDSF and MALS, we validated that 2 anti-MT1-MMP nanobodies are well-folded, stable and monomeric proteins (FIGURE 1, panels A and B). In addition, we used SPR to characterise the affinity and kinetics of the interaction of each antibody with human MT1-MMP protein (FIGURE 1, panels C and D). This research is useful to further develop labelled nanobodies as PET probes for triple negative breast cancer imaging.

The Unit hosts a 700 MHz NMR spectrometer that is equipped with probes and a sample changer to run up to 120 samples automatically. This provides medium throughput for the screening of small molecule protein binders (together with the Experimental Therapeutics Programme), as well as for metabolite quantification that in 2022 was done in collaboration with the Growth Factors, Nutrients and Cancer, and Transformation and Metastasis Groups (Molecular Oncology Programme) and the Hereditary Endocrine Cancer Group (Human Cancer Genetics Programme). Collectively with our client groups, we will continue implementing sample preparation protocols and developing spectroscopic and analytical tools to characterise metabolites present in different biological samples. ∎
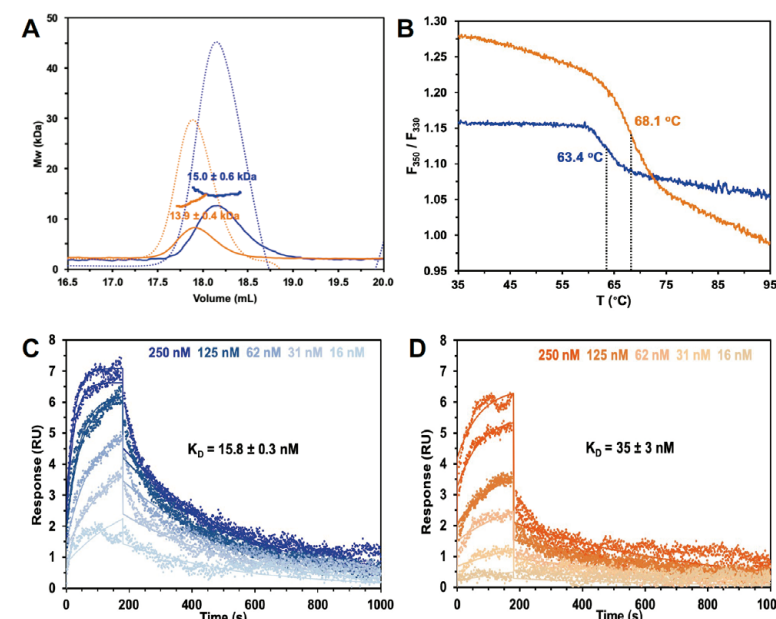

FIGURE 1 Characterisation of 2 nanobodies targeting a matrix metalloproteinase. (A) Superimposed MALS chromatographic traces of light scattering (solid lines), refractive index (dashed lines) and the calculated molecular weight from each nanobody. (B) NanoDSF thermal unfolding profiles of the 2 nanobodies. Inflection temperatures (Ti) are shown. (C, D) Overlay of the SPR sensorgrams and corresponding kinetic fits to a 1:1 binding model (solid lines) for the interaction of each nanobody with MT1-MMP. Nanobody concentrations and the calculated dissociation constants (K_D) of the complexes are indicated.

OVERVIEW

This Unit focuses on the technical and scientific management of Nuclear Magnetic Resonance (NMR) spectroscopy and molecular biophysics instrumentation available through the Structural Biology Programme. It provides CNIO researchers with equipment and experimental support for biophysical techniques used in studies of molecules involved in cancer. This includes the *in vitro* characterisation of i) the structure and dynamics of proteins using NMR and ii) the affinity and kinetics of protein interactions with other biopolymers and small molecules that could represent initial hits in drug discovery or research compounds for biophysical and functional studies. Furthermore, we use NMR to screen libraries of fluorinated fragments against macromolecular targets and to characterise the metabolic profiles of biofluids, cell growth media, and cell and tissue extracts from both animal models of cancer and human samples. The Unit is also endowed with a state-of-the-

"In 2022, we characterised biophysically 2 nanobodies targeting a matrix metalloproteinase and quantified the affinities and association and dissociation kinetics of both complexes. These results will help to validate the nanobodies as potential tools for breast cancer diagnosis."

art, multiple-well microplate reader equipped with diverse detectors (absorbance; intensity, polarisation and time-resolved fluorescence; luminescence; and AlphaScreen) for in-solution and adherent cells measurements.

▸ **PUBLICATIONS**

▸ Garrido A, Kim E, Teijeiro A, Sánchez Sánchez P, Gallo R, Nair A, Matamala Montoya M, Perna C, Vicent GP, Muñoz J, Campos-Olivas R, Melms JC, Izar B, Schwabe RF, Djouder N (2022). Histone acetylation of bile acid transporter genes plays a critical role in cirrhosis. *J Hepatol* 76, 850-861.

▸ García-Alonso S, Mesa P, Ovejero LP, Aizpurua G, Lechuga CG, Zarzuela E,

Santiveri CM, Sanclemente M, Muñoz J, Musteanu M, Campos-Olivas R, Martínez-Torrecuadrada J, Barbacid M, Montoya G (2022). Structure of the RAF1-HSP90-CDC37 complex reveals the basis of RAF1 regulation. *Mol Cell* 82, 3438-3452.

▸ Sanz-Castillo B, Hurtado B, Vara-Ciruelos D, El Bakkali A, Hermida D, Salvador-Barbero B, Martínez-Alonso D, González-Martínez J, Santiveri C, CamposOlivas R, Ximénez-Embún P, Muñoz J, Álvarez-Fernández

M, Malumbres M (2022). The MASTL/PP2A cell cycle kinase-phosphatase module restrains PI3K-Akt activity in an mTORC1-dependent manner. *EMBO J.* PMID: 36354735.

▸ Chaves-Arquero B, Martínez-Lumbreras S, Camero S, Santiveri CM, Mirassou Y, Campos-Olivas R, Jiménez MA, Calvo O, Pérez-Cañadillas JM (2022). Structural basis of Nrd1-Nab3 heterodimerization. *Life Sci Alliance* 5, e202101252.

▸ Mulero F, Oteo M, Garaulet G, Magro N,

Rebollo L, Medrano G, Santiveri C, Romero E, Sellek RE, Margolles Y, Campos-Olivas R, Arroyo AG, Fernández LA, Morcillo MA, Martínez-Torrecuadrada JL (2022). Development of anti-membrane type1-matrix metalloproteinase nanobodies as immunoPET probes for triple negative breast cancer imaging. *Front Med* 9, 1058455.

BIOINFORMATICS UNIT

Fátima Al-Shahrour
Unit Head

Research Scientist
Michael Tress

Gradutate Students
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*Plan de Empleo Joven de la Comunidad de Madrid (Youth Employment Plan, Madrid)

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Elena Piñeiro (TS)**, Francisco J Soriano (since Oct.) (TS)**

**Titulado Superior (Advanced Degree)

Students in Practice
Lucas Friedman (June-Dec.) (Master's

in Biocomputing, ENS-ISCIII, Madrid, Spain), Paula Gómez (June-Aug.) (Summer Trainee, Univ. Carlos III de Madrid, Spain), Adel Samir Saleh (June-Aug.) (Summer Trainee, Nile University, Abuja, Nigeria), Víctor

Sánchez (June-Dec.) (ENS-ISCIII, Madrid, Spain)

Visiting Scientist
Carlos Carretero (Hosp. 12 de Octubre, Madrid, Spain)

OVERVIEW

Bioinformatics is a key discipline for understanding the cancer genome and for the future of cancer therapeutics. Bioinformatics-based approaches have the ability to transform the vast amount of biological data into comprehensible models that provide a deep understanding of cancer disease and the complex genotype-phenotype relationships needed to identify molecular cancer-driving alterations and novel therapeutic targets.

The CNIO Bioinformatics Unit (BU) has several objectives: (i) to develop new computational methodologies and bioinformatics tools to enable the integration of biological and clinical data, (ii) to achieve genome analysis in cancer patients' data to identify new biomarkers and drug response mechanisms, (iii) to provide bioinformatics support with data analysis and interpretation using computational and statistical

"We develop bioinformatics methods to therapeutically characterise differentially drug-responsive tumour cell subpopulations, proposing cell-specific anticancer treatments at single-cell resolution."

methods, (iv) to maintain the scientific computing facilities at the CNIO, and (v) to provide training in bioinformatics tools and methods.

RESEARCH HIGHLIGHTS

In 2022, the Bioinformatics Unit published more than 10 peer-reviewed articles as a result of our ongoing research projects and scientific collaborations (see the full list of our activities in our website: https://bioinformatics.cnio.es/). We studied cancer cell biology, inter- and intra-tumour heterogeneity, and drug response, using single-cell and spatial transcriptomics. In particular, we reviewed current approaches for the selection of anticancer therapies based on the type of tumour heterogeneity being targeted and the type of sequencing data available (FIGURE 1, Jiménez-Santos et al. 2022).

During 2022, our group participated in whole-genome screenings, identifying N-glycosylations as a genetic and therapeutic vulnerability in calreticulin-mutant myeloproliferative neoplasms, thus advancing the development of clonally selective treatments for this disease (Jutzi et al. 2022). We also collaborated in identifying the deficiency in the FBXW7 tumour suppressor gene that leads to multi-drug resistance (Sanchez-Burgos et al. 2022). In addition, our laboratory released an updated version of the APPRIS database (Rodríguez et al. 2022), which selects principal protein isoforms according to protein structure, function features and conservation.

Beyond the above-mentioned activities, the BU is an active node of the European network ELIXIR (https://www.elixir-europe.org/), leading the ELIXIR Cancer Data Focus Group to provide the framework and expertise for the systematic analysis and interpretation of cancer genomes. BU also co-coordinates the ISCIII IMPaCT-Data project (https://impact-data.bsc.es/), in which our activity focuses on leading training activities and genomics data management. Our
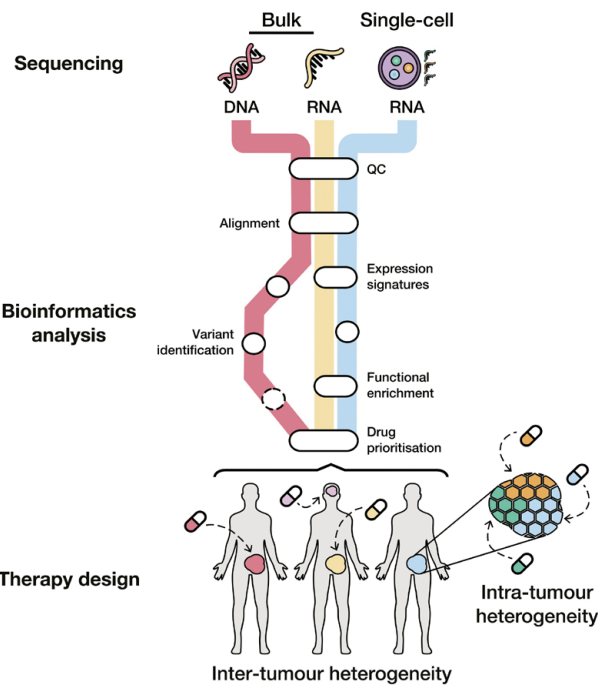

FIGURE 1 Summarised roadmap for drug prioritisation and therapy design to address inter- and intra-

tumour heterogeneity using multi-omics data.

training and knowledge-transfer activities include the co-organisation of the MSc in "*Bioinformática aplicada a la medicina personalizada y la salud*" at the *ISCIII*. ∎

▸ **SELECTED PUBLICATIONS***

▸ Rodriguez JM, Pozo F, Cerdán-Vélez D, Di Domenico T, Vázquez J, Tress ML (2022). APPRIS: selecting functionally important isoforms. *Nucleic Acids Res* 50, D54-D59.
▸ Sanchez-Burgos L, Navarro-González B, García-Martín S, Sirozh O, Mota-Pino J, Fueyo-Marcos E, Tejero H, Antón ME, Murga M, Al-Shahrour F, Fernandez-Capetillo O (2022). Activation of the inte-

grated stress response is a vulnerability for multidrug-resistant FBXW7-deficient cells. *EMBO Mol Med* 14, e15855.
▸ Jiménez-Santos MJ, García-Martín S, Fustero-Torre C, Di Domenico T, Gómez-López G, Al-Shahrour F (2022). Bioinformatics roadmap for therapy selection in cancer genomics. *Mol Oncol* 16, 3881-3908.
▸ Pozo F, Rodriguez JM, Martínez Gómez L, Vázquez J, Tress ML (2022). APPRIS principal isoforms and MANE Select tran-

scripts define reference splice variants. *Bioinformatics* 38(Supplement_2), ii89-ii94.
▸ Sanchez-Burgos L, Gómez-López G, Al-Shahrour F, Fernandez-Capetillo O (2022). An in silico analysis identifies drugs potentially modulating the cytokine storm triggered by SARS-CoV-2 infection. *Sci Rep* 12, 1626.
▸ Rogado J, Pozo F, Troulé K, Sánchez-Torres JM, Romero-Laorden N, Mondejar R,

Donnay O, Ballesteros A, Pacheco-Barcia V, Aspa J, Al-Shahrour F, Alfranca A, Colomer R (2022). Excess weight and anti-PD-1 immune checkpoint inhibitor's outcomes in non-small cell lung cancer. *Clin Transl Oncol* 24, 2241-2249.
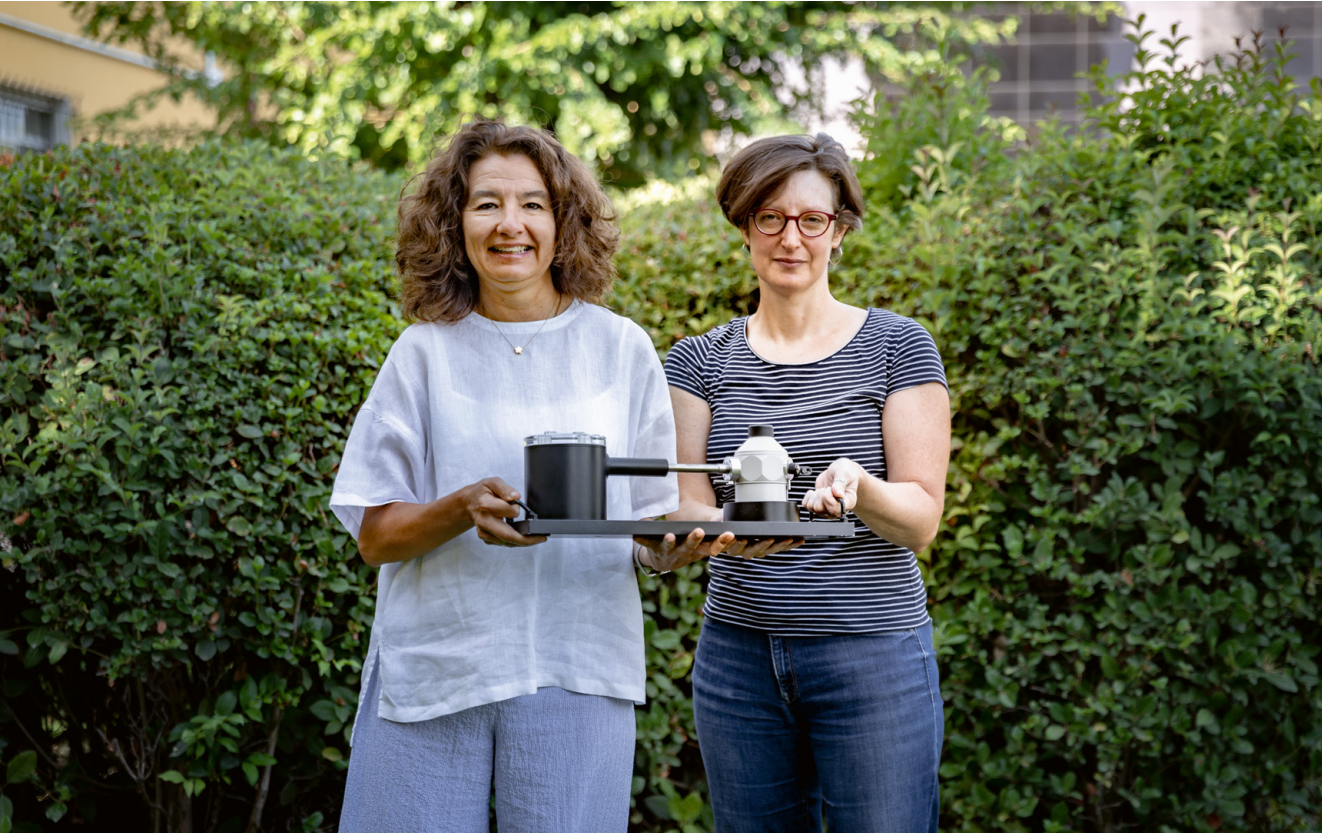
*please see BU's web site for a list of all publications.

ELECTRON MICROSCOPY UNIT

Jasminka Boskovic
Unit Head

Post-Doctoral Fellow
Johanne Le Coq

Technician
Carmen García (until February) (TS) [*]
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OVERVIEW

The principal goal of the Electron Microscopy (EM) Unit is to offer scientific-technical support to researchers to resolve their scientific questions using different transmission EM techniques. We routinely use cryo-EM and negative staining to prepare samples. We also perform data collection and help in image processing, through 2D analysis and 3D reconstruction. Support is offered in choosing adequate EM techniques and performing sample preparation. Moreover, we manufacture our own sample supports (EM grids) for better quality control and lower cost. In addition, we provide the training necessary for the use of our microscopes and auxiliary equipment. More advanced structural studies are generally carried out through research collaboration.

"In the Electron Microscopy Unit we dedicate our central effort to securing efficient access to all our infrastructure. We also offer the training necessary for the use of our microscopes and auxiliary equipment."

RESEARCH HIGHLIGHTS

In 2022, we gave support to several CNIO Groups in their research activities. In collaboration with the Transformation and Metastasis Group, we analysed mitochondrial morphology in human breast cancer patient-derived xenografts (PDX). Together with the Microenvironment & Metastasis Group, we studied different types of vesicles, and with the Growth Factors, Nutrients and Cancer Group, we optimised cryoEM grids and collected data for structural studies of the URI complex. We also started a collaboration with the H12O-CNIO Haematological Malignancies Clinical Research Unit to structurally characterise hnRNPK.

We continued collaborating closely with all the groups from the Structural Biology Programme, performing single-particle EM grid preparation, cryo-EM grid screening, data collection, and 2D and 3D analysis of different samples. We collaborated in several projects carried out by the Macromolecular Complexes in DNA Damage Response Group, performing EM grid preparation, data collection, and analysis of different samples: ARN helicase DDX11; RuvBL complex of *Arabidopsis thaliana* (a collaboration with D. Alabadí, *Universitat Politècnica de València*); lncRNA (a collaboration with M. Huarte, *CIMA*, *Universidad de Navarra*); and different heteromeric amino acid transporters (a collaboration with M. Palacin and J. Fort, *IRB Barcelona*). With the Genome Integrity and Structural Biology Group, we provided cryoEM grid screening and data collection of different samples, and with the Kinases, Protein Phosphorylation and Cancer Group, we performed EM grid optimisation, data collection and processing of PTC1 Kinase, as well as EM grid preparation and imaging of KIF5B-RET kinesin samples. Outside our Centre, together with Rafael Fernández Leiro, we are collaborating with J.A. Costoya Puente (*Universidad de Santiago de Compostela*) on characterising the structure of human hPARP1. Furthermore, together with E. Lara (*CNIC*), we are studying mitochondrial structure in brown adipose tissue (BAT) of KO CnAbeta1 mice. ∎
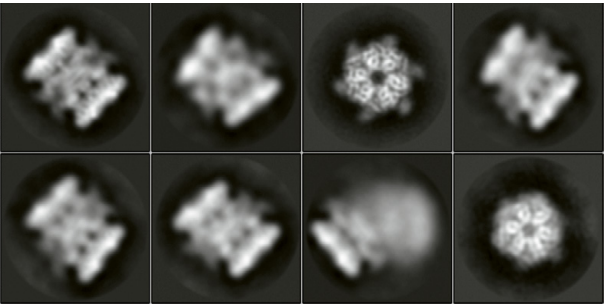

FIGURE 1 Reference-free 2D class averages demonstrating that cryo-electron microscopy is a powerful tool to view the structural flexibility of protein and protein complexes.

▸ **PUBLICATIONS**

▸ Le Coq J, Acebrón I, Rodrigo Martin B, López Navajas P, Lietha D (2022). New insights into FAK structure and function in focal adhesions. *J Cell Sci* 135, jcs259089.

▸ Rivera-Calzada A, Arribas-Bosacoma R, Ruiz-Ramos A, Escudero-Bravo P, Boskovic J, Fernandez-Leiro R, Oliver AW, Pearl LH, Llorca O (2022). Structural basis for the inactivation of cytosolic DNA sensing by the vaccinia virus. *Nat Commun* 13, 7062.

PROTEIN CRYSTALLOGRAPHY UNIT

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(PEJ)**, Lluvia Rebollo (until
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University, Japan), Eleonora Bado

(Feb-July) (Master's Thesis, Univ.
Autónoma de Madrid, Spain), Laura
Fernández (July-Aug) (AECC
Traineeship, Univ. Rey Juan Carlos,
Madrid, Spain)

OVERVIEW

The Protein Crystallography Unit is a core facility that provides on-demand services at different levels, from the cloning, expression, and purification of proteins to the determination of their 3D structures, with the purpose to fulfil the demands of our users and to understand the function of their protein targets. Thus, we produce high-quality proteins for different types of assays and structural determination at low resolution by small-angle X-ray scattering (SAXS) or at atomic resolution by X-ray crystallography. The latter includes protein co-crystallisation in the presence of inhibitors or small fragments, a method that we routinely combine with the quantification of protein thermal stability (thermofluor assay) to aid the drug discovery process.

"Fragment screening on crystals helps to map new binding sites in the target proteins."

RESEARCH HIGHLIGHTS

Our Unit works closely with the Experimental Therapeutics Programme on several projects: human TRF1 dimerisation domain; TRF1 DNA binding domain; and kinase domains of human MASTL and HASPIN for biochemical and structural analyses. Furthermore, to support drug discovery projects, we perform several thermal shift assays (thermofluor) in the presence of compounds developed in the Medicinal Chemistry Section.

The Unit is also engaged in several internal collaborations with other CNIO groups (Growth Factors, Nutrients and Cancer; Transformation and Metastasis; Metabolism and Cell Signalling; Experimental Oncology; Microenvironment and Metastasis; Topology and DNA Breaks; DNA Replication; Macromolecular Complexes in DNA Damage Response; Kinases, Protein Phosphorylation and Cancer Groups; and the H12O-CNIO Lung Cancer Clinical Research Unit), providing some of them with recombinant proteins that can be used for protein crystallography, SAXS or thermofluor assays analysis and, in some cases, for other biophysical, biochemical, cell-based functional assays and cryoEM studies.

Throughout 2022, the Unit also continued working on its own scientific project, supported by a grant from the BBVA Foundation. Carried out in collaboration with the Immuno-oncology and Immunotherapy Unit at the *Hospital 12 de Octubre*, this work generated a new synthetic bispecific antibody capable of targeting the spike protein of the SARS-CoV-2 virus, inducing neutralisation while promoting T cell cross-priming. We also revealed the cryo-EM structure, which shows how the trimerbody (TN[T]) binds the trimeric RBD spike ectodomain in a 1:1 equimolar ratio. ∎
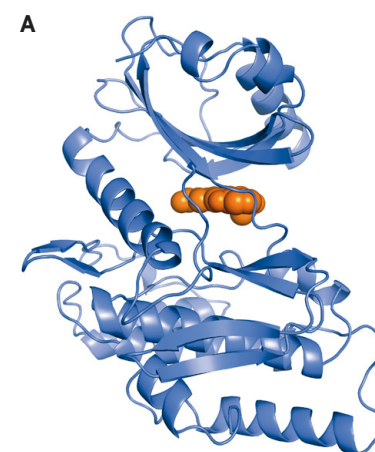
A

B

FIGURE 1 (**A**) Three-dimensional crystal structure of HASPIN kinase (in steel blue) in complex with the drug ETP-53005 (in orange). (**B**) Side view of the spike protein/TN[T] complex model showing TN[T] embracing the spike protein in the 3-up RBD prefusion conformation. The spike protein subunits are coloured in yellow, steel blue, and olive green, while V[HH]E and V[HH]V chains from the antibody are in purple and magenta, respectively. The cryo-EM map is coloured in light grey.
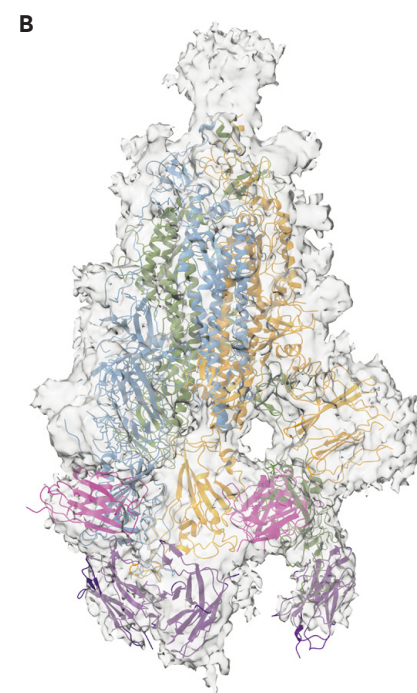
▸ **PUBLICATION**

▸ Lama R, Xu C, Galster SL, Querol-García J, Portwood S, Mavis CK, Ruiz FM, Martin D, Wu J, Giorgi MC, Bargonetti J, Wang ES, Hernandez-Ilizaliturri FJ, Koudelka GB, Chemler SR, Muñoz IG, Wang X (2022). Small molecule MMRi62 targets MDM4 for degradation and induces leukemic cell apoptosis regardless of p53 status. *Front Oncol* 12, 933446.

PROTEIN PRODUCTION UNIT

Jorge L. Martínez-Torrecuadrada
Unit Head
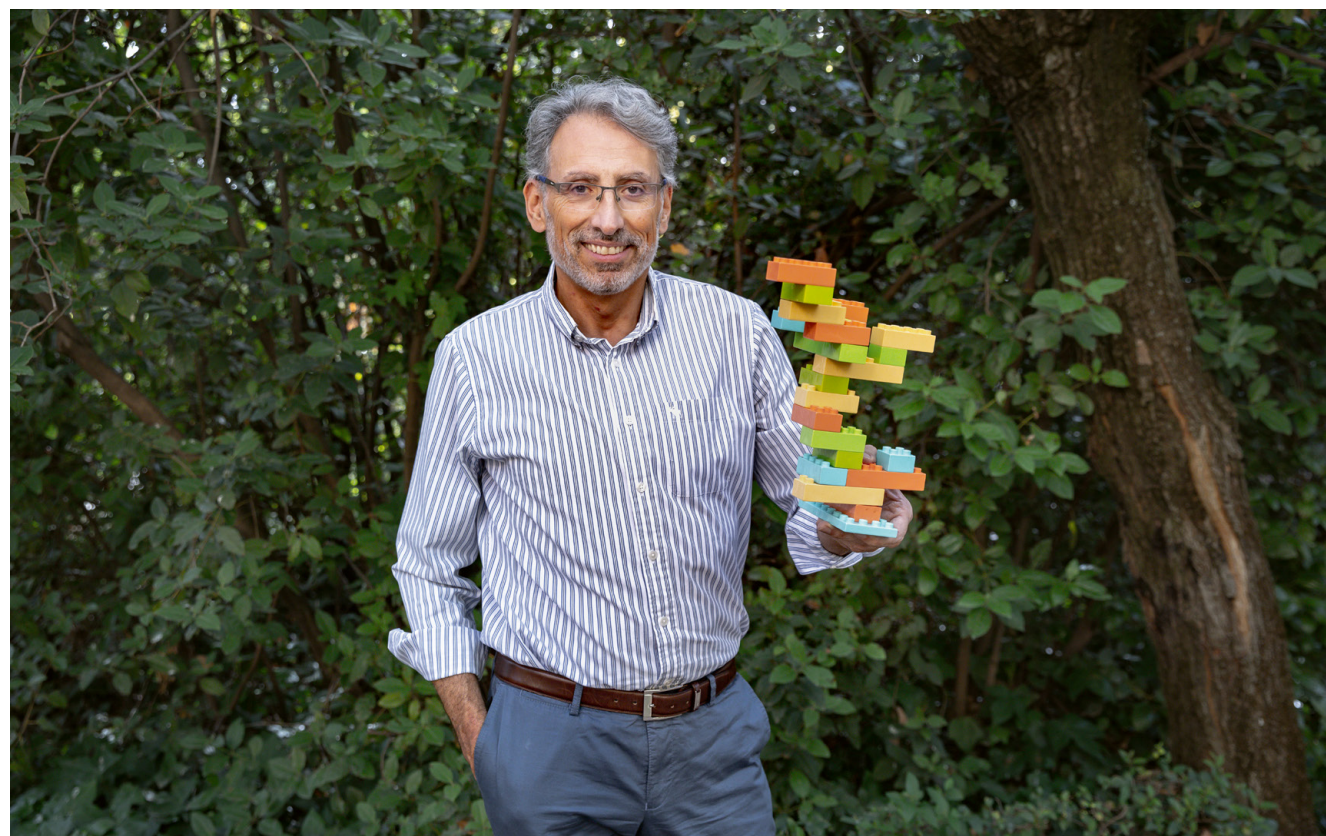
OVERVIEW

The biological functions of thousands of proteins, especially those involved in cancer development, remain unexplored, and understanding their structures, tissue and cellular distributions and functions is critical for biomedical progress. However, researchers often face the challenge of insufficient supply, since proteins of interest identified in a particular process can be very difficult to produce in high quality and in adequate amounts for analysis, causing a bottleneck in how quickly they can be characterised. The Protein Production Unit is a core service lab that aims to address this bottleneck by offering expertise and state-of-the-art technologies to develop effective processes for producing recombinant proteins. These can be used in diverse downstream applications such as the generation of highly-specific antibodies, biophysical, biochemical or functional analyses, or structural studies, with

"The Protein Production Unit provided many high quality recombinant proteins that were essential for drug discovery projects and for cancer diagnosis through the development of specific antibodies."

the final goal of accelerating cutting-edge cancer research for CNIO and external research groups.

RESEARCH HIGHLIGHTS

The Protein Production Unit was created at the beginning of 2022, with the aim of providing high-quality recombinant proteins to meet the needs of CNIO Research Groups and external collaborators. During the year, the laboratory was refurbished with state-of-the-art technologies for heterologous recombinant protein expression and purification, to implement efficient production protocols for each particular protein. With its portfolio of services ranging from cDNA cloning in expression vectors to purification in milligrams of purified protein, the Unit contributed to the research projects of several CNIO Groups. It is worth mentioning the elucidation of the structure of the RAF1-HSP90-CDC37 complex, in collaboration with the Experimental Oncology Group; the production of active MIDKINE protein for functional assays and the generation of specific monoclonal antibodies for the Melanoma Group; and the production of exosome-secreted micropeptides to develop antibody-based detection tools in collaboration with the Microenvironment and Metastasis Group; among others. In addition, we worked closely with the Monoclonal Antibodies Unit, providing purified proteins to generate highly-specific monoclonal antibodies, such as CSF3R, IL4I1, TACI and PILRA; and with the Biology Section of the

Experimental Therapeutics Programme, producing active full-length human MASTL for functional assays. Other tool proteins for in-house use (i.e. sortases and AG-MNase) were also produced in the Unit.

Apart from providing such services, the Unit carries out research activities focused on the development of specific recombinant antibodies and antibody fragments for diagnostic and therapeutic purposes. In 2022, llama-derived nanobodies against the metalloprotease MT1-MMP were developed and have shown their great potential as radiotracers in PET imaging for the detection of triple-negative breast cancer in mouse models. This project was a joint CNIO-*CIEMAT* collaboration and was supported by a grant from the BBVA Foundation. ∎
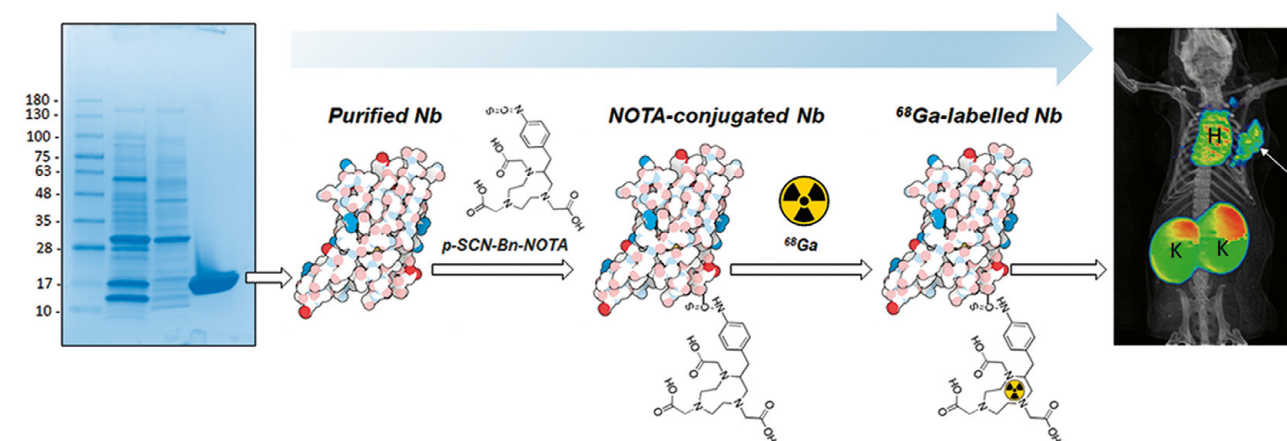

FIGURE 1 Strategy to obtain nanobody-based radiotracers for tumour detection by PET imaging. After nanobody expression and purification, conjugation with NOTA, and labelling with ^{68}Ga, the biodistribution of the ^{68}Ga-labelled nanobodies was monitored by PET. Arrow indicates tumour position, K: kidney, H: heart.

› **PUBLICATIONS**

› García-Alonso S, Mesa P, Ovejero LP, Aizpurua G, Lechuga CG, Zarzuela E, Santiveri CM, Sanclemente M, Muñoz J, Musteanu M, Campos-Olivas R, Martínez-Torrecuadrada J, Barbacid M, Montoya G (2022). Structure of the RAF1-HSP90-CDC37 complex reveals the basis of RAF1 regulation. *Mol Cell* 82, 3438-3452.e8.

› Mulero F, Oteo M, Garaulet G, Magro N, Rebollo L, Medrano G, Santiveri C, Romero E, Sellek RE, Margolles Y, Campos-Olivas R, Arroyo AG, Fernández LA, Morcillo MA, Martínez-Torrecuadrada JL (2022). Development of anti-membrane type 1-matrix metalloproteinase nanobodies as immunoPET probes for triple negative breast cancer Imaging. *Front Med (Lausanne)* 9, 1058455.