Programme’s research areas and strategic goals

The goal of the Structural Biology Programme (SBP) is to provide mechanistic insights at the molecular level of proteins and macromolecular complexes that contribute to cancer progression. A better understanding of how these macromolecules work, together with knowledge of their three-dimensional structures, provide information to guide the design of new strategies against cancer. The groups at SBP are currently focused on the study of protein kinases as well as complexes involved in the DNA damage response. A special emphasis has been placed on setting up high-resolution cryo-electron microscopy (cryo-EM) methods, a powerful technique for high-resolution structural characterisation of individual molecules that is reshaping biological research.

SBP is composed of 1 Senior Group, 2 Junior Groups and 4 Units. During 2019, 2 new Junior Groups joined the Programme to reinforce the computational studies applied to cancer research.

Summary of milestones & major achievements during 2019

It has been roughly two years since the Structural Biology Programme was restructured to incorporate 3 new Groups and high-resolution cryo-EM technologies. 2019 was the year when these changes were firmly consolidated. The new cryo-EM microscope was installed and started to generate high-resolution data; we also set up all the computational resources and methodologies essential for high-resolution image processing. The new Groups are fully operational having published remarkable results in *Nature*, *ACS Chemical Biology*, *Journal of the American Chemical Society* and *Proceedings of the National Academy of Sciences of the USA*. In addition, the on-going activity of the Units contributed to the research conducted at CNIO, resulting in publications in *Nucleic Acids Research*, *Journal of Experimental Medicine*, *Bioinformatics* and *Cancer Cell*, among other journals. During 2019, the Groups and Units at SBP also secured access for data collection, based on competitive applications, to the eBIC Biological Cryo-Imaging - Diamond Light Source (UK) and the ALBA synchrotron Light Source (Spain).

In 2019, SBP was deeply engaged in organising scientific meetings. Groups and Units at SBP participated in the organisation of four meetings: two CNIO “la Caixa” Frontiers Meetings (on ‘Structural and Molecular Biology of the DNA Damage Response’ and ‘Heterogeneity and Evolution in Cancer’), the ‘CCP-EM High Resolution EM Model Building and Validation Workshop’ and the ‘Workshop in Advances in the R2TP / URI-Prefoldin Complex in Cancer’. These meetings were an excellent opportunity to discuss the latest advances in these areas, but also to advertise the good science performed at SBP and the CNIO as a whole. In addition, excellent speakers were invited to our seminar series on topics connecting Structural Biology and cancer research; of particular interest was the visit of Sjors Scheres (MRC-Laboratory of Molecular Biology, Cambridge, UK), one of the main references in cryo-EM applied to biomedical research.

Finally, 2 new Groups, led by Solip Park and by Geoff Macintyre respectively, recently joined the CNIO to strengthen computational science in SBP. They will combine high-throughput technologies, big data and computational modelling to characterise the complexity of tumours and to develop better diagnostic and therapeutic tools for personalised medicine.
Our key mission is to provide in-depth structural and molecular understanding of how macromolecular complexes implicated in cancer work. This information is essential to comprehend why and how some proteins are involved in the development of cancer. This fundamental knowledge is the basis to start the search for potential strategies to interfere with the function of these macromolecules. To accomplish this, we make use of several biochemical and molecular biology tools in combination with cryo-electron microscopy (cryo-EM). Cryo-EM is used to visualise large macromolecular complexes, and to observe their motions. Two main objectives drive our current research: (i) the study of macromolecular complexes that function in the cellular response to DNA damage; and (ii) an HSP90 co-chaperone network implicated in the assembly, activation and regulation of several complexes that are essential for cancer progression. In addition, we also address other relevant questions about human disease, in collaboration with other groups.

“R2TP/URI Prefoldin-like complex is a molecular chaperone that contributes to cancer by poorly understood mechanisms. In 2019, we showed how 2 essential ATPases in this complex are regulated.”
RESEARCH HIGHLIGHTS

Regulation of RUVBL1–RUVBL2 ATPases in the R2TP co-chaperone

Activation and assembly of several protein complexes implicated in cancer require the assistance of Heat Shock Protein 90 (HSP90), a molecular chaperone; thus, HSP90 inhibitors are being evaluated as potential anticancer agents. HSP90, working in concert with the R2TP/URI Prefoldin-like complex, is needed for the activation and stability of the PI3-kinase-like family of kinases (PIKKs), regulating the DNA damage response and cell growth. R2TP/URI Prefoldin-like complex is the most complex HSP90 co-chaperone yet described; it is involved in cancer progression and is the focus of several studies looking for potential inhibitors.

We are using cryo-EM to fully understand the structure of this co-chaperone complex and how it works. R2TP forms a core sub-complex containing 2 ATPases. RuvB-like protein 1 (RUVBL1) and RuvB-like protein 2 (RUVBL2). These ATPases form a hexameric ring that interacts with RNA polymerase-associated protein 3 (RPAP3) and PIH1 domain-containing protein 1 (PIH1D1) to assemble the R2TP complex. Several studies have demonstrated that the ATPase activity of RUVBL1 and RUVBL2 is required for their biological functions in vivo, but how this is controlled and regulated is still mysterious.

In our new study, cryo-EM is used to reveal one of the mechanisms regulating these ATPases. RUVBL1 and RUVBL2 cannot efficiently hydrolyse ATP because the access to their nucleotide-binding site is obstructed by hexamerization, making ATP/ADP exchange more difficult. Cryo-EM of RUVBL1 and RUVBL2 in complex with PIH1D1, one of the components of the R2TP complex implicated in client recruitment, reveals that the interaction of PIH1D1 with RUVBL2 induces large conformational rearrangements that lead to the destabilization of an N-terminal segment of RUVBL2 acting as a gatekeeper to nucleotide exchange (FIGURE 1). These results identify PIH1D1 as a factor that regulates the accessibility to the nucleotide-binding site in RUVBL2, thereby facilitating nucleotide exchange and activation.

Architecture of the Type VII secretion system in mycobacterium

Tuberculosis is an infectious disease and one of the 10 leading causes of death worldwide. The mycobacterium causing this disease uses a membrane-embedded secretion system (Type VII secretion system, T7SS) to inject virulence factors required for infection into the host immune cells, some of which block the defensive response. Targeting the function of this secretion mechanism has been proposed as a way to stop infection, but the lack of information on how T7SS works at the atomic level has prevented progress in designing these new therapeutic strategies. In a joint effort between our group and the group led by Sebastian Geibel at the University of Würzburg, we managed to describe in detail the structure of T7SS at the atomic level.

We used M. smegmatis as a model to study M. tuberculosis, a bacteria that shares the same secretion system. Our work showed that T7SS is a sophisticated nanomachine in which several proteins cooperate to inject the virulence factors produced by the bacterium into the cells of the immune system (FIGURE 2). Most interestingly, EccC3, one of the proteins in the cytosolic side of the membrane, comprises 4 linked but flexible ATPase domains involved specifically in recruiting the virulence factors and energising secretion. Our structure shows that this ATPase protein makes direct contact with an alpha helix from the periplasmic pore protein, which inserts all the way into the cytoplasm. The architecture of T7SS and the experiments performed suggest that conformational changes induced by ATP hydrolysis most likely regulate the opening and closing of the secretion pore.

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: (i) how protein kinases are activated and regulated by posttranslational modifications and allosteric inputs, and (ii) 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 and disease due to oncogenic mutations and other oncogenic insults. Crucially, such atomic and molecular information can be translated into the design and development of more potent and specific protein kinase inhibitors, eventually leading to more effective drugs for the treatment of cancer patients.

KINASES, PROTEIN PHOSPHORYLATION AND CANCER JUNIOR GROUP

Iván Plaza Menacho
Junior Group Leader

Post-Doctoral Fellows
Pablo Sortano, Rubén Julio Martínez
(since December)

Graduate Students
Nicolás Cano, Moustafa Ahmed Shehata

Students in Practice
Carolina Hernández (June-August, Universidad Católica de Valencia), Fabiola Piscopo (March-September, Università degli Studi Napoli Federico II), David Sánchez (until June, Universidad Autónoma de Madrid).

RESEARCH HIGHLIGHTS

We established 4 main research lines in 2019, and at the same time the lab expanded with the arrival of 2 ‘CNIO Friends’ fellowship recipients: Rubén Julio Martínez Torres (postdoctoral contract) and Moustafa Ahmed Shehata (Carmen Gloria Bonnet predoctoral contract).

→ Structural and molecular determinants of RET catalytic activity and signalling, both in cis by intrinsic elements and in trans by effector kinases and adaptor proteins. We paid special attention to the crosstalk between RET and non-receptor tyrosine kinases (NRTKs).

→ Structure-function studies of RET oncogenic variants, i.e. point mutations targeting the kinase domain and oncogenic fusions generated by DNA-rearrangements, with a special emphasis on the latter in the context of aggressive types of cancers.

→ Structure-based drug-discovery for new (allosteric) RET inhibitors.

→ Histidine phosphorylation and structure-function studies of histidine kinases.

We also focused on less known phospo-specific modifications such as histidine phosphorylation and the regulation of histidine kinases. The current understanding of this type of phosphorylation is poor. Together with T. Schirmer’s group at the Biozentrum (University of Basel), we directly contributed to the first crystal structure of a full-length hybrid histidine kinase and the molecular dissection of its full catalytic cycle (FIGURE). This work was recently published in PNAS (Dubey et al., 2020).

“A understanding protein kinase function and inhibition for better cancer therapeutics.”

“Awards and Recognition”

Rubén Julio Martínez Torres was the recipient of a ‘CNIO Friends’ Postdoctoral Contract.

Moustafa Ahmed Shehata was awarded the ‘CNIO Friends’ Carmen Gloria Bonnet Predoctoral Contract.

“Publication”


“Figure”

Crystal structure of full-length hybrid histidine kinase ShkA. (A) Domain organisation (colour coded) of hybrid histidine kinase ShkA. Crystal structure representations, front-back and top-bottom views of AMP-PNP-bound protein, depict a compact dimeric and catalytic incompetent arrangement of the multidomain protein, from Dubey et al. PNAS, 2020. (B) Functional evaluation of dual histidine kinase and phosphatase activities. Western blots (WBs) of ShkA wild-type (WT) and phospho-transfer deficient mutant (D430A) in the presence or absence of second messenger c-di-GMP using histidine phospho-specific antibodies, upper panel. Phosphate sensor analyses capturing phosphatase activity (i.e. phosphate release) of ShkA WT and D430A, lower panel, from Plaza-Menacho et al. unpublished.
OVERVIEW

Safeguarding genetic information is essential to all forms of life. Two key cellular processes keep it free from errors: DNA replication and repair. Importantly, when they 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 a hallmark of cancer, but it remains unclear how this happens. We are interested in understanding these pathways because of their implications for ageing and disease, and, in particular, their relation to cancer.

Cryo-electron microscopy (cryo-EM)

Combined with many other approaches already established at the CNIO, we use cryo-EM to study diverse macromolecular complexes involved in cancer. Recent technological developments in microscopes, detectors and image processing tools have significantly improved the resolution of the technique, enabling the structural analysis of many elusive macromolecules to an unprecedented level of detail. Last year, we worked together with Oscar Llorca’s Group and the EM Unit to bring the cryo-EM facility at the CNIO to a state-of-the-art level. Moreover, we were awarded access to high-end microscopes at the Biological Electron Bio-Imaging Centre (eBIC) in Oxford (UK).
Cancer is one of the most complex human diseases, involving genetic, environmental and even unknown factors. Over the past several decades, large-scale genomics analyses of cancer patients have been made in order to understand this complex disease. One of the most striking findings of large-scale cancer genomics is the remarkable heterogeneity in cancer driver (oncogene or tumour suppressor gene) alterations across different patients and cancer types. However, even though various important biological characteristics are commonly measured in cancer patients, little is currently known about the cancer type- or context-specific tumour progression for each gene in each cancer type. Furthermore, by analysing large-scale cancer genomics data, many exceptions have been observed, including haploinsufficiency in tumour suppressor genes, or amplification-linked mutations in oncogenes and even in dual-functional genes. Clearly, activity levels of genomic alterations in cancer genes are disparate across cancer types, and their optimal models for tumour progression may also vary depending on contexts or cancer types.

“Large-scale public cancer genomics has provided new insights to understand the contribution of inherited mutations to cancer risk.”

**OVERVIEW**

**RESEARCH HIGHLIGHTS**

**Finding novel cancer predisposition genes**

We established 3 collaborations (2 in Spain and 1 in South Korea) to find novel cancer predisposition genes using our previous method (Park et al., 2018, Nature Communications). We applied it to 3 different cancer studies and this will increase the scientific impact of our method.

**Cancer-type specific fitness landscape**

To understand the comprehensive cancer fitness landscape, we established the dosage (by copy-number changes)-fitness function when it is mutated (either somatic or germline), for each gene in each cancer type. We applied it to all possible human genes (~20,000 genes) across more than 30 cancer types. Based on this analysis, we could classify many different classes of cancer genes, including unexpected, novel cancer types. We expect to validate our observations by computational (independent dataset) and experimental analysis in 2020.

**PUBLICATIONS AT OTHER INSTITUTIONS**

Different types of chromosomal instability leave distinct “scars” in the DNA of a tumour which we can detect using whole-genome sequencing. These genetic scars, or “signatures of CIN”, represent a way to detect and quantify different causes of CIN in a tumour.

Using tumour organoids to target CIN
Tumour organoids can be treated in vitro providing a way to link genomic features with drug response. By determining the activity of different types of CIN and linking them to drug response we are building a rational framework for therapy selection in the clinic using CIN as a biomarker.

Predicting therapy response in patients
Our lab is performing a series of retrospective clinical studies looking at predicting response to therapies using signatures of CIN (FIGURE A). The long-term goal is to predict response in prospective clinical trials in order to improve patient stratification and trial success.

Single cell DNA sequencing to detect ongoing CIN
Standard “bulk” whole-genome sequencing does not allow us to separate CIN that is ongoing in a tumour, from CIN that is historical (occurred during the evolutionary history of the tumour). We are using single cell DNA sequencing to interrogate the changes unique to each cell, which enables us to separate ongoing CIN from historical CIN (FIGURE B). Our lab is applying this technology to premalignant lesions allowing us to observe CIN at its earliest stages in tumour development, before it causes aggressive, resistant tumours, with the goal of developing chemo-preventive treatment strategies.

OVERVIEW

In the Computational Oncology Group we are tackling some of the deadliest cancers by targeting the causes of chromosomal instability. Pancreatic, oesophageal, lung and ovarian cancers have the lowest survival rates, but they also share a common trait which we can exploit – extreme chromosomal instability (CIN). By therapeutically targeting CIN, we aim to improve outcomes in these tumours.

Our main research areas include:

- Developing single cell/nucleus sequencing approaches to detect ongoing CIN.
- Predicting therapy response using genomic signatures of CIN in patient biopsies.

“Tackling some of the deadliest cancers by targeting the causes of chromosomal instability.”

Figure (A) An overview of the workflow for predicting drug response from clinical samples. (B) A schematic outlining how a normal cell can acquire ongoing CIN through TP53 mutation and NF1 deletion. The plots on the right show genome-wide copy number profiles obtained from bulk or single cell sequencing approaches. As the bulk is an average across cells, the newly acquired CIN cannot be detected.
OVERVIEW

This Unit focuses on the technical and scientific management of Nuclear Magnetic Resonance (NMR) Spectroscopy and molecular biophysics instrumentation available at the Structural Biology Programme. It provides CNIO researchers with equipment and experimental support for a variety of techniques used for biophysical studies of molecules involved in cancer. This includes the application of NMR to the in vitro characterisation of the structure and dynamics of biomolecules (proteins in particular). Together with NMR, diverse biophysical techniques are used to study the affinity and kinetics of the interactions of proteins with other biopolymers, as well as with small molecules that could represent initial hits in the drug discovery process or research compounds for biophysical and functional studies. Furthermore, we use NMR to characterise the metabolic profiles of biofluids, cell growth media, and cell and tissue extracts from both animal models of cancer and human samples.

“In 2019, we conducted NMR experiments in cell media and extracts to quantify metabolite changes induced by gene silencing or by chemical inhibitors, thereby contributing to the elucidation of the cellular function of the silenced genes and the specificity and mode of action of the inhibitors.”

RESEARCH HIGHLIGHTS

The Unit provides a broad range of instrumentation for the biophysical characterisation of biomolecules and their interactions, including spectrophotometers, a fluorimeter, isothermal titration and differential scanning calorimeters, a circular dichrograph, dynamic and multi-angle static light scattering devices, and a surface plasmon resonance (SPR) instrument. Research groups mostly from, but not limited to (i.e. the Haematological Malignancies Clinical Research Unit, the Epithelial Carcinogenesis Group and the Experimental Therapeutics Programme – ETP), the Structural Biology Programme extensively used these technologies throughout 2019.

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 CNIO’s Structural Biology and ETP Programmes), as well as for metabolite quantification that in 2019 was done in collaboration with the CNIO-Lilly Cell Signalling and Immunometabolism Section, from the ETP; and the Cell Division and Cancer and the Metabolism and Cell Signalling – MCS – Groups, from the Molecular Oncology Programme. As an example, in collaboration with the MCS Group, we conducted C tracing intracellular metabolite measurements by NMR to characterise the metabolic changes associated with exposure to a small molecule chemical inhibitor (FIGURE). Collectively, with this and other groups, we continue to implement sample preparation protocols and to develop spectroscopic and analytical tools to characterise the metabolites present in different biological samples.

Figure NMR quantification of inhibitor-affected 13C-labelled polar intracellular metabolites derived from L-13C-glucose in MDA-MB468 breast cancer cells. Each colour-coded square corresponds to the relative variation of each metabolite in a particular sample upon inhibitor treatment (INH) relative to the average value of the 3 independent control (DMSO) experiments. The putative metabolic enzyme inhibitor was discovered in a cell phenotypic screening performed by MCS and ETP. With the exception of the glutamate in glutathione, the compound produces a decrease of the metabolic flux from glucose to most metabolites, including: the Krebs cycle intermediates citrate, alpha-ketoglutarate (αKG) and malate; and the glutamic and aspartic acids that are proxies for αKG and oxalacetate, respectively.
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 huge amount of biological data into comprehensive models that provide an in-depth understanding of cancer disease and the complex relationships among genotype and phenotype that are needed to identify cancer driver molecular alterations and new therapeutic targets.

The CNIO Bioinformatics Unit (BU) has several goals: (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 in order to identify new biomarkers and mechanisms of drug response; (iii) to provide bioinformatics support with data analysis and interpretation using computational and statistical methods; and (iv) to maintain the scientific computing facilities at the CNIO and provide training in bioinformatics tools and interpretation.

In 2019, the CNIO Bioinformatics Unit published 19 peer-reviewed articles (see full list on our web site https://bioinformatics.cnio.es/) generated from our ongoing research projects and scientific collaborations. We studied the impact of inter- and intra-tumour heterogeneity using our tool PanDrugs and its predictive power of clinical outcome (Piñeiro-Yáñez et al., 2019). Our results showed that patients labelled as responders according to PanDrugs predictions had significantly increased overall survival compared to non-responders.

Additionally, PanDrugs’ usefulness was assessed considering spatial and temporal intratumor heterogeneity and showed that it can propose drugs or combinations to target clonal diversity. Additionally, we published vulcanSpot (Perales-Patón et al., 2019) for detecting and targeting cancer genetic dependencies. All our tools are freely available and have been used in our scientific collaborations, such as the use of PanDrugs in a case of T-ALL (Fernández-Navarro et al., 2019) or mutational analysis during tumour clonal evolution (González-Rincón et al., 2019).

Additionally, the Unit extended its analysis of misannotated coding genes (Frankish et al., 2019) from the human genome to the mouse genome, and manual annotators are currently revising the annotations of coding genes in Ensembl/GENCODE for both genomes. Finally, the Unit analysed the importance of SINE Alu transposable elements in the human genome and found that these elements are preferentially incorporated into human coding genes.

The Bioinformatics Unit, as a node of INB-ELIXIR, is also involved in the organisation of events and training activities. During 2019, we organised the ONCONET-SUDE Workshop on Innovative IT for Healthcare as well as a Software Carpentry workshop. With regard to training, we co-organised the Master en Bioinformática Aplicada a Medicina Personalizada y Salud (ISCIII-ENS) (visit our web page for a full list of activities).

**OVERVIEW**

“PanDrugs predictions can be correlated with clinical outcome and can be useful to manage intratumor heterogeneity in patients while increasing therapeutic options and demonstrating their clinical utility.”

**SELECTED PUBLICATIONS**


Please see BU’s web site for a list of all publications.
Nowadays, cryo-electron microscopy (cryo-EM) has become a main structural biology method to study macromolecules in more ‘native,’ i.e. biochemically functional, buffer conditions. At the CNIO, we use our 120 kV Tecnai G2 Spirit microscope, equipped with the TVIPS CMOS detector, to screen cryo-samples and to carry out low resolution analysis of different biological specimens. For high-resolution data collection, CNIO’s Structural Biology Programme has been granted access to high-end cryo-EM microscopes at the Electron Bio-Imaging Centre (eBIC) (Oxford, UK) through a peer-reviewed Block Allocation Group (BAG). In addition, we are performing the final set-up of our new cryo-electron microscope (JEM-2200FS) equipped with a K3 direct electron detector for medium-high resolution data collection.

During 2019, our scientific activity was developed through collaborations with all the research groups of the Structural Biology Programme, as well as with several groups from other Programmes and with scientists outside the CNIO. For instance, in collaboration with the Kinases, Protein Phosphorylation and Cancer Group (Structural Biology Programme), we worked on the structural characterisation of an oncogenic fusion of RET tyrosine kinase; in collaboration with the Macromolecular Complexes in DNA Damage Response Group (Structural Biology Programme), we were involved in the structural characterisation of several protein complexes (e.g. DNA repair and RUVBL1/2 complexes), together with M. Palacín’s Group (IRB Barcelona), we collaborated on the high-resolution structural characterisation of heteromeric amino acid transporter complexes; with the Genome Integrity and Structural Biology Group (Structural Biology Programme), we collaborated to set up a pipeline to use cryo-EM as a tool for drug discovery; with the Experimental Oncology Group (Molecular Oncology Programme), we worked on optimising, for cryo-EM, the purification of c-Raf’s protein complex; and, lastly, together with the Cell Division and Cancer Group, we analysed how the mutations in a centrosomal protein affect the architecture of centrioles.

**OVERVIEW**

The aim of the Electron Microscopy (EM) Unit is to help researchers to solve their biological questions using various transmission electron microscopy techniques. We have extensive knowledge in sample preparation, negative staining and cryo-electron microscopy, as well as experience in image processing, 2D analysis and 3D reconstruction. We offer guidance for selecting suitable techniques, specimen preparation and training on the use of our microscopes and support equipment. More advanced studies are usually provided through research collaboration.

“Over the past year, we devoted our efforts to provide technical and scientific support to the users of the Unit in addition to fine-tuning the recently installed high-resolution cryo-electron microscope.”

**PUBLICATION**

CRystallography and protein engineering unit

Overview

The Crystallography and Protein Engineering Unit (XTCEU) is a core facility that provides on-demand services at different levels, from protein cloning to the determination of its 3D structure, with the purpose to fulfill the demands of our users and to comprehend how their protein targets work. Thus, we produce proteins for different types of biochemical/ biophysical/in vitro/in vivo assays, for monoclonal antibody generation, and we also offer macromolecular 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 in parallel with the quantification of protein thermal stability (thermofluor assay) – during the guided drug discovery process.

Research Highlights

Fragment-based screening service was recently included in our portfolio. It is a well-established and powerful approach to early drug discovery. The purpose of this method is to expose protein to libraries of fragments and to solve the crystal structures of the complexes. Our first target was the dimerization domain of TRF1, a project in which we joined efforts with the Telomeres and Telomerase Group. The work was financially supported after approval of an XChem proposal by the synchrotron Diamond Light Source (UK) in the context of an H2020 INEXT European project. As in previous years, we worked closely with the Experimental Therapeutics Programme on several projects that have since led to the production of recombinant proteins (full-length human MASTL, tyrosine kinase domain of human DDR1, full-length mouse TRF1 and human TRF1 dimerization domain) for biochemical and structural analyses. Also, in support of drug discovery projects, we performed several thermal shift assays (thermofluor) in the presence of compounds developed in the Medicinal Chemistry Section.

We have also continued our work on the production of proteins for the generation of antibodies by the Monoclonal Antibody Unit (Biotechnology Programme), including several cancer-related proteins such as POT1A, HIPA, Syncytin1, CDK6, TRIB2, TRIB3, and FNIP2, among others. Furthermore, we have combined efforts with the Haematological Malignancies Clinical Research Unit in the structural and biophysical characterisation of boronated complexes. Additionally, we undertake a number of internal collaborations with other CNIO Groups and Units, providing them with recombinant proteins for biochemical and/or cell-based functional assays, for example, with the Telomeres and Telomerase Group, the Experimental Oncology Group, the Genomic Instability Group, the Cell Division and Cancer Group, the Melanoma Group, the Lung Cancer Clinical Research Unit, and the Macromolecular Complexes in DNA Damage Response Group.

Publications


Awards and recognition

- Members of the Board of Directors, Asociación de Cheiros de Sahianz de España
- National Cancer Research Centre, CNIO