CIC bioGUNE
CENTER FOR COOPERATIVE RESEARCH IN BIOSCIENCES

EXCELENCIA SEVERO OCHOA

INPhINIT
"la Caixa" Fellowship Programme

CIC bioGUNE - INPhINIT INCOMING Doctoral Fellowship Program

"la Caixa" Foundation

CIC bioGUNE (http://www.cicbiogune.es/) is a key research center within the national and international scientific landscape and has emerged as a knowledge source in the area of health science. The cutting-edge scientific activity of CIC bioGUNE researchers explores the interface between Chemistry and Biomedicine, with emphasis on Structural, Molecular and Cell Biology. Two research programmes - "Molecular Recognition and Host-Pathogen Interactions" and "Metabolism and Cell Signaling in Disease" - are made up of more than a hundred scientists and technicians who concentrate on the molecular bases and mechanisms of disease to create new diagnostic methods and promote development of advanced therapies.
Located in the Science and Technology Park of Bizkaia (Derio), the activity of CIC bioGUNE includes generation of both fundamental and oriented knowledge, training of research talent, and collaboration-building with local, national and international institutions. CIC bioGUNE has modern scientific infrastructures, led by prestigious scientists, including advanced equipment for nuclear magnetic resonance (NMR), electron microscopy and X-ray diffraction, as well as different core technology platforms where genomes, proteomes and metabolomes can be analyzed.

For detailed information please visit the Caixa website.

PhD Positions available in the INPhINIT Incoming Program **(deadline February 6th 2019)**:

- Understanding the mode of action of ribosome targeting antibiotics using cutting-edge cryo-EM methodologies: A path to **discovering** new clinically relevant antibiotics (**Sean Connell** and **Paola Fucini**)
- Townes-Brocks Syndrome: mechanisms of disease and cilia manipulation. (**Rosa Barrio**)
- Targeting the interaction Gαi3-GIV to stop tumor invasion and metastasis. (**Francisco José Blanco Gutiérrez**)
- Study of the molecular mechanisms involved in the spontaneous misfolding of prion protein/Predoctoral position at Prion biology laboratory at CIC bioGUNE (**Joaquín Castilla Castrillón**)
- Role of retrotransposition in Schwann cell development and pathology (**Ashwin Woodhoo**)
- Mechanisms of adaptation (**Arkaitz Carracedo**)
- Improving human and animal welfare through structure-function studies of emerging viral threats. (**Nicola G.A. Abrescia**)
- GLYCONCOLOGYNMR: Breaking the limits in understanding tumour-related Glycan recognition by NMR (**Jesús Jiménez-Barbero**)
- Conformational analysis of synthetic saponin vaccine adjuvants by NMR spectroscopy for molecular-level mechanistic elucidation at the chemistry-immunology frontier. (**Alberto Fernández-Tejada**)
- Chemoenzymatic glycoengineering of therapeutic monoclonal antibodies. (**Marcelo E. Guerin**)
- CARBOHYDRINCELLNMR: Breaking the limits in Carbohydrate recognition by NMR: In-cell NMR. (**Jesús Jiménez-Barbero**)
- Angiogenesis and NEDDylation (PhD Position In Liver Disease Laboratory/CIC bioGUNE)(**Maria L. Martínez-Chantar**)

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RESUME of Available Projects

- **Understanding the mode of action of ribosome targeting antibiotics using cutting-edge cryo-EM methodologies: A path to discovering new clinically relevant antibiotics.**

Sean Connell and Paola Fucini (pfucini@cicbiogune.es) (https://www.cicbiogune.es/people/pfucini)

Until few years ago, cryo-EM was limited to providing low resolution structures (10-20Å) but today cryo-EM has the power to resolve the atomic structure of nature's biological machines (>3Å). At this resolution we can describe the molecular structure of vital macromolecular complexes and understand how their structure changes to elicit a functional activity. Moreover, we can observe substrates bound in catalytic sites and begin to define how interactions and reactive centers drive reactions or promote conformational changes essential to the machine's cellular role. Understanding these details allows one to harness the activity of the machine for applications in biotechnology (chemical biology) or designing molecules that regulate the activity of the machine for biomedical applications (i.e. antibiotics). The research conducted in the Connell and Fucini groups aims at understanding and further developing the mechanism of action of clinically relevant or newly discovered antibiotics that target the ribosome, one of the most vital macromolecular machines of the cell, devoted to protein synthesis. The perspective student will join a highly collaborative and multi-disciplinary research team to study at the structural level the assembly and initiation phases of protein synthesis to identify targets and describe the mode of action of novel antibiotics with clinical potential. Uncovering the structural basis of antibiotic action can yield solutions to the ever-threatening problem of growing antibiotic resistance.

- **Townes-Brocks Syndrome: mechanisms of disease and cilia manipulation**

Rosa Barrio (rbarrio@cicbiogune.es) (http://personal.cicbiogune.es/rbarrio/)

Townes-Brocks Syndrome (TBS) is a rare genetic disease that is characterized by polydactyly, hearing loss and kidney malformations. These symptoms overlap with those characteristic of ciliopathies, which are diseases caused by defects in the operation or assembly of the primary cilium. Mutations in the *SALL1* gene are the cause of TBS. These mutations produce the expression of a truncated protein that establish aberrant interactions with cytoplasmic proteins.

We have proved that the truncated form of SALL1 interacts with ciliary proteins preventing their correct functioning and therefore altering the function of the cilia (Bozal-Basterra et al 2018 Am J Hum Genet 102:249-265). We propose here to generate and use cellular and animal models to prove the reversion of the cilia phenotype by CRISPR–mediated gene editing and drugs treatment. If successful, this work will open new possibilities for future therapies in human patients. In addition, we will explore the role of full length SALL1 in cilia function and formation by using cells and tissue derived from a murine TBS model and human cells modified by CRISPR-Cas9. Our project builds upon a strong foundation, and will generate new knowledge, powerful reagents for the research community, and novel ideas for therapeutic approaches to TBS and related genetic syndromes.

➢ **Targeting the interaction Gαi3-GIV to stop tumor invasion and metastasis**

Francisco José Blanco Gutiérrez (fblanco@cicbiogune.es) (https://www.cicbiogune.es/people/fblanco)

GIV is a metastasis biomarker whose expression correlates with the metastatic potential of cancer cell lines and with advanced clinical stage of tumors. GIV controls tumor cell behavior by an unconventional signaling mechanism. GIV is a non-receptor Guanine nucleotide Exchange Factor (GDP-GTP) for the α-subunits of Gi subfamily of G proteins. The attractiveness of the GIV-Gαi3 interface as a promising target lies in its broad impact on cancer pathways and its high sensitivity and specificity. Recently we have mapped the binding site of GIV to Gαi3 by NMR using a peptide that recapitulates the binding properties of GIV. A screen of small molecules from the LOPAC library already identified two compounds that displace GIV from Gαi3. These results prove that the Gαi3-GIV interaction is druggable. But the two molecules identified in this limited screening are not specific for Gαi3 and not cell permeable, preventing their use as therapeutic inhibitors of an intracellular target. Our goal is to characterize the binding to Gαi3 of compounds that specifically inhibit the interaction. Our collaborator at Boston University has already screened a large library of compounds for inhibitors with the desired properties. Our specific aims are: 1) Identification of the binding site of the inhibitors and measurement of their affinity and effect on the nucleotide exchange rate; 2) Measurement of the dynamics of Gai3 and its changes when bound to GIV and selected inhibitors; 3) Measurement of the effect on the GDP-GTP exchange rate of Gαi3 in lysates of 10 different cancer cell lines and in the presence of selected inhibitors. To meet these aims will use predominantly NMR taking advantage of our previous results.

➢ **Study of the molecular mechanisms involved in the spontaneous misfolding of prion protein**

Joaquín Castilla Castrillón (jcastilla@cicbiogune.es) (https://www.cicbiogune.es/people/jcastilla)

Prion diseases belong to a group of fatal neurodegenerative disorders that affect humans and animals and for which no therapy is available. They are characterized by an extreme variability in their clinical presentation, neuropathological patterns and the existence of molecular subtypes. The diseases of sporadic origin seem to imply the spontaneous misfolding of prion protein that results in a great diversity of prion strains which show differential biological and physicochemical properties. To decipher what factors determine this misfolding and therefore the pathogenesis, in-depth knowledge of these spontaneous processes as well as the decrypting of the three-dimensional structure of the infectious protein are required, for what *in vitro* prion propagation methods are necessary. The main objective of this project is to understand the role of the biological and physicochemical factors that are involved in the spontaneous protein misfolding and the way in which they favor the generation of structural diversity.

The candidate will have the opportunity to do short stays out of his laboratory thanks to more than 20 international collaborations that Castilla's group has opened. His group is the leading expert in a unique technique able to replicate prions *in vitro,* what allows him to establish fruitful collaborations with laboratories all over the world.

➢ Role of retrotransposition in Schwann cell development and pathology

Ashwin Woodhoo (awoodhoo@cicbiogune.es) (https://www.cicbiogune.es/people/awoodhoo)

Schwann cells, the main glial cells of the peripheral nervous system, are highly plastic cells and have a remarkable ability to radically change their cellular identity in pathological situations, a feature that is highly unusual in mammals. The fundamental aim of the work in the group is to deconstruct the essential mechanisms that govern this plasticity, which forms the cornerstone of several debilitating and even fatal PNS neurological disorders that include demyelinating neuropathies e.g. Charcot-Marie-Tooth disease, and PNS cancers, including Neurofibromatosis.

The research project we are offering is to examine the role of transposable elements in regulating gene expression patterns during Schwann cell development and in pathological situations. New research is showing that transposition of mobile elements can have significant impact on human development and physiology (e.g. see Elbarbary *et al*., Science 2016). This project is focused on 3 key interlocking aspects of retrotransposition on Schwann cell development, myelination and pathology: (1) generation and analysis of different mice mutant models to examine the impact of retrotransposition, (2) identification of key retransoposition events, and (3) harnessing the potential of these mobile elements for controlling these processes using CRISPR-Cas9 technology.

➢ Mechanisms of adaptation

Arkaitz Carracedo (acarracedo@cicbiogune.es) (https://www.cicbiogune.es/people/acarracedo)

The career of Arkaitz Carracedo is based on answering and contributing to a fundamental question: what are the characteristics of the tumour cells that differentiate them from normal cells, and that can allow us to exploit this knowledge for the establishment of stratification strategies for patients and new therapies? His research group is aimed at deconstructing the essential requirements of cancer cells with special emphasis on the translation of the acquired knowledge from bench to bedside. In order to define the genuine features of cancer cells, we focus on the signalling and metabolic alterations in tumours. Through a multidisciplinary approach with increasing complexity, his lab works on bioinformatics, cell lines and primary cultures (using cell and molecular biology technologies), mouse models of prostate cancer that are faithful to the human disease and the analysis of human specimens through the development of prospective and retrospective studies. The research project under this call is focused on the study of molecular drivers of aggressive tumors, with special emphasis on cell signalling, metabolism and prostate cancer. The proposal will require of the integration of cell biology, mouse modelling and human specimen analysis, together with strong bioinformatics support.

➢ **Improving human and animal welfare through structure-function studies of emerging viral threats**

Nicola G.A. Abrescia (nabrescia@cicbiogune.es) (http://www.ikerbasque.net/es/nicola-g-abrescia)

Infectious diseases represent the major causes of human death in underdeveloped countries (http://www.who.int/). Some of these diseases are caused by viruses, entities that permeate the entire biosphere and infect organisms of the three domains of life: Bacteria, Archaea and Eukarya. Strictly related to the human food-chain, to the agricultural activities of small, medium and large size farms, animal welfare represents a continuous challenge in both poor and rich countries. Indeed a great amount of emerging diseases have an animal origin; in a study of 1415 pathogens known to affect humans, 61% were zoonotic [Taylor LH et al., 2001] and in several cases animal and human diseases may become interconnected. Our target is Schmallenberg virus (SBV), an enveloped and pleomorphic virus infecting livestock, a member of the Bunyaviridae family, and transmitted by midges (Culicoides spp.). SBV is an emerging EU animal threat and it serves us as a model system for studying Bunyaviruses, which is the largest virus family on the planet.
The goal of the current proposal is to determine the molecular mechanisms governing SBV viral pathogenesis using integrative cellular and structural methods. The results deriving from this study serve (i) to enhance the effectiveness of virus research through a cross-sectional project; (ii) to impact on our society providing not only fundamental knowledge but also enabling the development of new therapeutic strategies in this and other areas.

➢ **GLYCONCOLOGYNMR: Breaking the limits in understanding tumour-related Glycan recognition by NMR**

Jesús Jiménez Barbero (jjbarbero@cicbiogune.es) (https://www.cicbiogune.es/people/jjbarbero)

GLYCONCOLOGYNMR aims at developing novel molecules to address one major health problem: Cancer. We are focused on developing novel glycan-based molecules to preclude the inherent tumour immune suppression and therefore, prevent tumour growth and metastasis. Altered glycosylation is a hallmark of cancer. Tumour-associated glycome signature is critical in tumour biology and immunology. Understanding how the tumour glycocode affects immune cell activity within tumour microenvironment should provide a breakthrough in the field. Tumour-associated glycans with terminal sialylated and fucosylated glycan epitopes are expressed among cancers and in cancer stem cells and are recognized by the immune system through lectin-mediated interactions. Specifically, Siglecs-9/15 and DC-SIGN, expressed in immune cells, recognize tumour-related structures, suppressing anti-tumour immune responses. Thus, blocking the specific interaction between tumour glycocode and those lectins may interfere cancer immunological events. The specific project will focus on deciphering tumour associated glycan recognition by lectins and anti-cancer antibodies, using integrated methodologies: NMR, microarrays, modelling. In particular, the molecular specificity and structural features that govern interactions between immune-related lectins and normal vs aberrant glycome signature will be deciphered.

- **Conformational analysis of synthetic saponin vaccine adjuvants by NMR spectroscopy for molecular-level mechanistic elucidation at the chemistry-immunology frontier**

Alberto Fernández Tejada (afernandeztejada@cicbiogune.es)
(*https://www.cicbiogune.es/people/afernandeztejada*)

The clinical success of anticancer and antiviral vaccines often requires coadministration of an adjuvant, a substance that enhances the immunogenicity of the antigen and potentiates the immune response. However, few adjuvants exhibit sufficient potency and negligible toxicity to be suitable for clinical use; moreover their mechanisms of action are generally not fully understood. Saponin-based adjuvants are a promising class of immunopotentiators used in a number of vaccine clinical trials but present several liabilities and an unknown mechanism of action. My research group possesses the technology to develop novel improved synthetic variants and chemical probes of the saponin adjuvants and to evaluate their adjuvant activity in mice. With this expertise at our disposal, and using a multidisciplinary approach involving synthetic chemistry, immunology, and NMR spectroscopy, this project aims at establishing the links between the adjuvant activity of the synthetic molecules and their conformational preferences in solution by using NMR methodologies. There is some evidence suggesting that overall saponin conformation is correlated to adjuvant activity, including my previous computational studies on synthetic variants, but in this project we will experimentally probe this relationship, for the first time, by performing detailed conformational analysis in solution by in-depth NMR experiments. These studies at the chemistry-immunology frontier will provide key insights into the conformational behavior and structural features of the saponin adjuvants, yielding experimental correlations between saponin structure, conformation and adjuvant activity and important molecular-level information at atomic resolution to shed light on the unknown mechanisms of saponin immunopotentiation.

- **Chemoenzymatic glycoengineering of therapeutic monoclonal antibodies**

Marcelo E. Guerin (mrcguerin@cicbiogune.es) (http://www.ikerbasque.net/marcelo.guerin)

Therapeutic immunoglobulin G (IgG) antibodies are a prominent and expanding class of biologicals used for the treatment of several human disorders including cancer, autoimmunity, and infectious diseases. The presence of this N-linked glycan is critical for IgG function contributing both to Fc γ receptor binding and activation of the complement pathway. *S. pyogenes*, one of the most common human pathogens, secretes the endoglycosidase EndoS to remove complex N-glycans linked to the Fc of IgG antibodies. Because antibodies are central players in many human immune responses, the understanding of the substrate and catalytic mechanisms of EndoS impacts diverse fields in biomedicine. EndoS already showed great promise in animal models as a treatment for diverse autoimmune diseases that rely on autoantibodies; fine-tuning the specificity of this endoglycosidase is needed for its development as protein therapeutic for safe and effective use in humans. Moreover, EndoS glycosynthase variants attach glycans specifically to IgG antibodies. Expanding the N-glycan substrate repertoire of newly designed EndoS variants will more fully realize the potential of antibody engineering, a crucial step in increasing the therapeutic impact of antibody drugs. We recently determined the X-ray crystal structures of EndoS in complex with the glycan portion of the substrate. In this project we propose (i) to elucidate the structural determinants of antibody recognition by EndoS and (ii) to determine the molecular basis of N-glycan specificity of the GH18 family of endoglycosidases. We will utilize this information to engineer enzymes with novel substrate specificity and expand the toolkit for customized chemoenzymatic synthesis of homogeneously glycosylated IgG antibodies, hastening the next generation of monoclonal antibody immunotherapies.

- **CARBOHYDRINCELLNMR: Breaking the limits in Carbohydrate recognition by NMR: In-cell NMR**

Jesús Jiménez Barbero (jjbarbero@cicbiogune.es) (https://www.cicbiogune.es/people/jjbarbero)

The project fits within one of our key programmes. CARBOHYDRINCELLNMR aims to understand the molecular mechanisms that govern glycan recognition by different receptors (proteins, nucleic acids) using a multidisciplinary approach, combining synthesis, molecular biology, biophysics, and computational techniques with a prominent role for NMR. Every cell is enveloped by a sugar coat called *glycocalix*. Glycans are attached to proteins or lipids, but our knowledge about it is still rudimentary. Even though glycans are involved in a wide range of vital cellular processes like pathogen recognition, immune modulation, or tumor growth, we lack detail in the mechanism of these sugar mediated processes. This is due to the fact that glycans have a colossal chemical complexity. Anti-influenza drugs (Relenza, Tamiflu) were developed when the molecular basis of the interaction of the viral proteins and the glycans in human cells was fully understood. On this basis, we will apply state-of-the-art NMR protocols to decipher key glycan recognition aspects beyond current knowledge: the role of presentation and dynamics and understanding the mechanisms behind the exquisite receptor and ligand selectivity. Importantly, till now, sugar recognition NMR studies have been exclusively limited to in vitro. We will break the limits of NMR, studying the interactions in-cell, a crowded ambient where viscosity is doubled respect to water. We are in a unique position to approach this project due to our expertise in NMR, synthetic chemistry, and the network of collaborators we have established for years, enabling us to access a large variety of synthetic sugars. Different receptors have been chosen due to their key implications in the development of diseases. Discovering the molecular bases of these interactions will provide groundbreaking information on chemical glycobiology and will open unexplored avenues for approaching sugar-associated diseases, as inflammation and viral infections.

- **Angiogenesis and NEDDylation (PhD Position In Liver Disease Laboratory. CIC bioGUNE)**

Mª Luz Martínez Chantar (mlmartinez@cicbiogune.es) (https://www.cicbiogune.es/people/mlmartinez)

Angiogenesis consists on the formation of new blood vessels from pre-existing ones. Although this process is essential for embryonic development, postnatal growth and wound healing, a deregulation can also contribute to tumor development or ischemia. There have been developed several research advances about this process such uncovering the role of VEGF or angiopoietins and their receptors. Indeed, the treatments with anti-VEGF molecules has been shown in a certain percentage of patients to have a reverse effect in promoting the invasive phenotype through the induction of alternative pathways such as fibroblast growth factors, the selection of hypoxia-resistant cells or the induction of tumor growth. Nevertheless, the mechanisms underlying those adverse effects are poorly understood so new approaches need to be investigated in order to develop suitable therapies. The existing crosstalk among transcriptional, post-transcriptional and post-translational regulation in the wide spectrum of signaling pathways involved in different pathologies is a novel cutting-edge research topic. Alterations in the transcriptome provide means to buffer rapid shifts in several extracellular and intracellular signals, but post-translational modifications (PTMs) have emerged as a faster and more effective mechanism to securely regulate signaling pathways and metabolic reactions. Our group has been focused on the research in NEDDylation for treating liver pathologies such as fibrosis and hepatocellular carcinoma. NEDDylation is increased in bad prognosis HCC patients and interestingly, neoangiogenesis is stimulated in those patients (Villa et al., 2016). Therefore, the crosstalk between angiogenesis, bad prognosis and NEDDylation emerges as a new approach to understand tumor progression and the development of metastasis.