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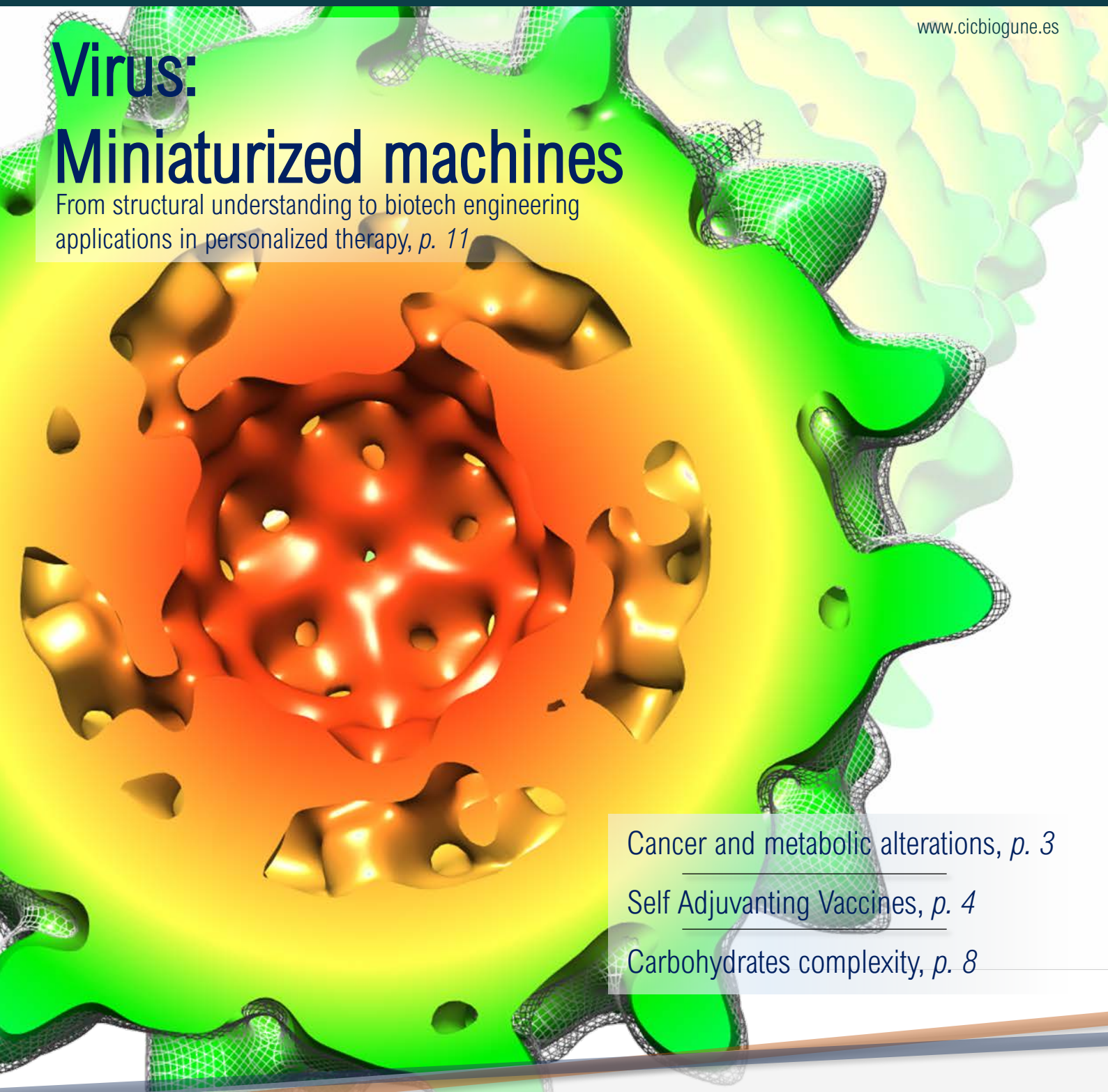

Virus: Miniaturized machines

From structural understanding to biotech engineering applications in personalized therapy, *p. 11*

CIC bioGUNE
CENTER FOR COOPERATIVE RESEARCH IN BIOSCIENCES
EXCELENCIA SEVERO OCHOA

2018

Contents

The Center

CIC bioGUNE is a biomedical research center focused on Biochemical, Cellular and Molecular Biology. Our cutting-edge scientific activity concentrates on discovering the molecular bases and mechanisms of disease to create new diagnostic methods and to promote development of advanced therapies. The activity explores four main biomedical research themes like Cancer, Metabolic Diseases, Rare Diseases, and Infectious Diseases organized in two research programmes "*Metabolism and Cell Signaling in Disease*" and "*Molecular Recognition and Host-Pathogen Interactions*".

With our collaborative philosophy we are deeply engaged in multidisciplinary research collaborations with local, national and international colleagues and technology experts. The center is impinged in a heterogeneous network of Academic and Clinical Entities, Research and Technology Centers, and the vibrant Biotech and Pharma ecosystem.

The scientific activity is supported by cutting-edge infrastructures and technology platforms, including advanced equipment for nuclear magnetic resonance (NMR), now recognized as ICTS, electron microscopy, a facility for monoclonal antibody production, as well as different core technology platforms where genomes, proteomes and metabolomes can be analyzed.

General View

Management Direction

CIC bioGUNE activities are strongly related to our specific mission: to build up a EU-referent knowledge pole in biosciences, which should be able to favour the development of the emerging sectors in the bioscience and health fields, and the incorporation of the proper technologies to be able to enhance the competitiveness of the corresponding industrial (biotech, pharma, etc) sectors. Specifically, CIC bioGUNE acts with a strong commitment of collaboration and coordination with the rest of social and scientific agents in the Basque Country to optimize the existing capacities, and jointly conform an integrative scientific and technological offer of excellence. This offer should be able to boost the evolution of the economy by strongly increasing its intrinsically high added-value. Our research activities cover from the gene to animal models of cellular processes through the determination of biomolecular structure and assembly, and elucidating the key mechanisms and interaction patterns at the highest resolution. Our scientific objectives are transversal, and target the complete characterization of the molecular basis of protein-based processes in human pathophysiology and immunological defence, cell proliferation, and development. The final aim is to translate our findings to the clinic, with special interest in precision medicine.

Cancer and metabolic alterations

Arkaitz Carracedo, *Cancer Cell Signaling and Metabolism Laboratory*

Our view of cancer has quickly changed in the recent decades. Intensive cancer multidisciplinary research has revisited the classical notion of a static disease based on aberrant proliferation, to a group of diseases that differs between individuals and within the same individual over time. The complexity of cancer evolution has been elegantly illustrated through the annotation and study of the genetic aberrations that govern transformation, proliferation and therapy response, in turn leading to the development of targeted treatments. However, despite the initial excitement of targeted approaches, resistance frequently emerges, preceded by a poorly characterized drug-tolerant stage. In this context, the mechanisms underlying the adaptation of cancer cells to new hostile microenvironments remain obscure. Biological aspects related tumour cell adaptability beyond genomic aberrations have neither been pursued nor therapeutically exploited. In this regard, the study of cellular metabolic alterations in tumours offered a complementary an exciting opportunity to increase therapeutic efficacy, in addition to serve for the development of novel biomarkers. Yet, our understanding is limited around the preferred metabolic alterations in the different stages of tumour progression.

In 2018, cancer researchers have made unique discoveries altering our perception on the toolbox that we have at our disposal to treat this disease. Genetic engineering our immune cells to recognize and attack tumour cells has consolidated as an exciting therapy for some types of leukaemia[1]. The strategies to wake up our immune system against tumour cells has taken very interesting and important turns as well. Immunotherapy (the pharmacological activation of our immune cells against cancer) has provided clinical results that were thought unreachable[2]. In turn, the Nobel award has recognized scientists that discovered key molecules involved in the process of immune regulation by tumours cells, which are now the target of such immunotherapy (James P. Allison, and Tasuku Honjo). Mutational burden revealed as a vulnerability of tumours due to the generation of neoantigens that are recognized by immune cells, a process that could be dictated by metabolic alterations affecting the equilibrium between nucleotide pools[3]. Despite the clarifying relevance of mutations in the development and progression of cancer, the field also accumulated evidence on a surprising fact: normal cells in our organism also accumulate mutations that lead to cancer, without resulting in the formation of a tumour[4]. This surprising finding forces us to rethink how cancers are born, as well as to differentiate between mutations being essential *versus* being sufficient. The progress in innovative cancer therapies was accompanied in 2018 with an increase capacity to detect and monitor the disease in biofluids. 2018 has opened and closed with two exciting studies that bring technology closer to the patient, opening new avenues towards the establishment of screening programs for early cancer detection[5,6].

2019 opens with cumulative evidence showing that we can exploit our immune system for therapy. Yet, the stratification criteria that defines patients that will benefit from such approaches are lacking, and next year will likely shed light on this issue. In addition, early detection is revolutionized by technological progress, and this will drive us to re-evaluate therapeutic regimes that originally failed in advanced cancers. Last, but not least, the design of precision medicine-based therapies will be dependent on the fundamental understanding of cancer cell function. Only uncovering the strategies developed by cancer cells in the face of new milieu (therapies, immune activation, dissemination) will empower us to design smart strategies to eradicate tumours, thinking globally but acting locally.

Macrophage plasticity and metabolism

Juan Anguita, *Inflammation and Macrophages plasticity Laboratory*

Our work is focused on the response of macrophages to inflammatory insults in the context of infectious and non-infectious conditions, including colitis and cancer. We are interested in the evolution over time of the signaling pathways initiated by stimuli that activate complex arrays of pathogen recognition receptors that are dependent or independent of phagocytic events, how these signals affect the long-term responses of macrophages as well as their metabolic status and the differential response elicited by pathogenic and homeostatic factors. We address our basic questions through the use of multi-omic approaches (metabolomics, proteomics, metagenomics, and transcriptomics, ATACseq) in order to identify 1) Regulatory pathways that determine the specific response of macrophages to homeostatic and pathogenic signals, and 2) Specific metabolic adaptations that distinguish both responses. The ultimate goal is the identification of specific points of therapy intervention in order to reduce the inflammatory response under pathological conditions without affecting the homeostatic responses of macrophages that are essential for a proper balance in a healthy mammalian host.

Even though the long-term consequences of the activation of macrophages to specific stimuli has been known for quite some time[7], this year several key discoveries have been reported in high ranking journals. Innate memory responses are divided into trained and tolerant, depending on the secondary response to unrelated stimuli, such as LPS. A key report on Nature described the miRNA-ome associate with the tolerant response to lipolisaccharide, identifying miR221 and miR222 as key players in the maintenance of the tolerant state[8]. Furthermore, another manuscript reported in Nature demonstrated that the effect of the prototypical tolerance-inducing stimulus, LPS, is dramatically distinct systemically and in the nervous system. This results from differential effects of peripheral innate cells and microglia[9]. The induction of innate immune memory is accompanied by metabolic changes that are rooted in, and can influence, epigenetic alterations. Innate immune memory development generally invokes an increased glycolytic output and a reduced oxidative phosphorylation capacity compared to acute responses, and may result in the production of immunomodulatory metabolites, including lactate, fumarate, succinate or itaconate[10,11].

The development of innate immune memory has been studied almost exclusively in response to pathogen associated molecular patterns or dead microorganisms, but little is known on the response of macrophages under infectious and homeostatic conditions. Expected developments in the field are:

- Identification of long-term responses of macrophages to pathogens and commensal bacteria during inflammation
- Understanding of the regulatory mechanisms of these responses both at the gene expression and metabolic levels and the development of therapies to control pathogenic processes while keeping intact those responses important for the hemostatic balance of the host.
- Development of novel adjuvants to induce better and more protective vaccine formulations
- Study of innate memory development and characterization under various inflammatory conditions, including cancer
- Study of the role of innate immune memory on the microbiota composition.

Saponin adjuvants and self-adjuvanting vaccines

Alberto Fernandez Tejada, *Chemical Immunobiology Laboratory*

The clinical success of anticancer and antiviral vaccines often requires the use of an adjuvant, a substance that helps stimulate the body's immune response to the vaccine, making it work better. However, few adjuvants are sufficiently potent and non-toxic for clinical use, although, it is not really known how they work. Current vaccine approaches based on weak carbohydrate and glycopeptide antigens are not being particularly effective to induce the human immune system to mount an effective fight against cancer. Despite intensive research and several clinical trials, no such carbohydrate-based antitumour vaccine has yet been approved for public use. In this context, the research topic in my group has a double, ultimate goal based on applying chemistry to address the above clear gaps in the adjuvant-vaccine field.

The past year has brought interesting advances on the topic as it refers to vaccine adjuvants based on the saponin natural product QS-21. Notably, QS-21 was approved by the US FDA in GlaxoSmithKline's shingles (herpes zoster) vaccine[12] as part of the AS01 combination of immunostimulants, marking the first time ever that QS-21 is approved in a human commercial vaccine. This key milestone adds to the approval of the QS-21 containing RTS,S/A01 malaria vaccine by European regulators in 2015[13]. Moreover, this year has witnessed the publication of two reference review articles about the mechanisms of action of saponin-derived adjuvants[14,15]. It is since recently that some more precise mechanisms of QS-21 adjuvanticity have been proposed. However, the molecular role of QS-21 in the high efficacy of these vaccines still remains to be understood mechanistically. Concerning the development of synthetic self-adjuvanting vaccines incorporating the adjuvant and antigen components in a single molecule, new advances have become available along this year. These include new insights into and designs of mucin-based cancer vaccines obtained by linking synthetic MUC1-derived antigens to various carriers and immunostimulating glycans, such as virus-like particles[16], β-glucan[17] and β-galactosylceramide[18]. Moreover, other built-in adjuvants included in these vaccine structures are based on Toll-like receptor (TLR) ligands, such as a TLR2 agonist (Pam_3CSK_4)[19] a TLR4 agonist (monophosphoryl lipid A)[20], and a TLR7 agonist[21].

Looking forward, significant achievements can be anticipated for 2019 from my perspective, including the development of novel, improved saponin chemical probes for molecular level mechanistic investigations. It is expected that novel insights into the molecular mechanisms of QS-21 and saponin-derived adjuvants can be obtained, although it is unlikely that a universal mechanism of action would be relevant to the range of saponin adjuvants and new synthetic variants. Finally, the development of self-adjuvanting vaccines that include saponin-derived in-built adjuvants in their structure will also be a major goal in my research plan, providing new synthetic constructs and potentially improved vaccine approaches.

Breast cancer stem cells

María del Mar Vivanco, *Breast Cancer Stem Cells Laboratory*

Breast cancer is a heterogenous disease that remains the cancer with the highest incidence in women and, despite all advances, has the highest mortality for cancer in women in the world. The majority of breast tumours express the estrogen receptor (ER) and are therefore usually treated with hormone therapy, for example, tamoxifen. Unfortunately, approximately 30% of tumours develop resistance to treatment and cancer returns. This treatment failure is associated with poor prognosis and remains an area of unmet clinical need that affects more than 8 million patients worldwide and is the focus of our studies.

Various studies in the field have shown the existence of different subtypes of breast cancer with clinical implications. Furthermore, complexity has also been demonstrated at the intratumoral level, by the presence of cells with properties of stem cells. These cells (cancer stem cells, CSCs) display increased tumour initiation potential and are also more resistant that non-CSCs to current forms of therapy, including radiotherapy, chemotherapy and hormone therapy. Previous work in the our laboratory had shown that development of resistance to tamoxifen leads to the enrichment in cells with properties of stem/progenitor cells and that the transcription factor Sox2 plays a key role in this process. Another interesting approach to gain further understanding of the molecular alterations that occur during endocrine resistance is to explore posttranslational modifications that may affect tumour susceptibility to external stimuli. TRAIL is an immune-related apoptotic protein that has been shown to specifically target cancer cells while sparing normal cells. However, limited success in the past could likely be attributed to poor patient stratification. Indeed, studies published by the group of Richard Clarkson have shown that the selective use of TRAIL agonists to target the CSC compartment in breast tumours that have acquired endocrine resistance may improve clinical outcome with potential long-term benefits to patients[22]. Elucidation of the molecular regulation of stem/progenitor cells that are likely the origin of various types of breast tumours is key in cancer research. Mammary ER-negative luminal progenitors are characterised by the expression of the membrane tyrosine kinase receptor c-KIT, as well as the SRC-family kinase LYN. Work by Smalley´s group, another collaborator, has demonstrated that LYN kinase is a transducer of c-KIT growth signals in the normal mammary epithelium and that dual mechanisms, uncoupling from upstream signals and changing splice isoform ratios, drive the activity of LYN in aggressive breast cancers[23]. These mechanisms have the potential to be targeted therapeutically and could benefit a subset of breast cancer patients.

Identification of patients who will respond to targeted, novel, or repurposed therapies remains a major goal of clinical research and, in particular, of breast cancer research. Two major lines of active research may yield outstanding results in the near future: 1) identification and characterisation of biomarkers that allow patient stratification to improve treatment specificity and personalised medicine and2) further understanding of tumour heterogeneity to enable design of new compounds that target the complexity of the tumour and reduce or eliminate the likelihood of recurrence.

Post-translational modification in cancer cell migration

Francisco J Blanco, *Structural Biology of cancer Laboratory*

We are interested in the mechanism of copying the genetic material (DNA replication) and how the cell copes with DNA damage. In this context we study the molecular recognition of the human DNA sliding clamp (PCNA), an essential factor in DNA replication and repair involved in cell proliferation. PCNA interacts with numerous enzymes and regulatory proteins and is a target for anticancer therapies. It also interacts with ING tumour suppressors, which regulate the state of chromatin. The protein girdin is one of the "master regulators" of metastasis, is up-regulated in highly invasive cancers and its expression correlates with cancer metastasis and predicts patient death in breast, colorectal, and esophagus cancer. Girdin binds the Galphai3

subunit of G proteins. We study the binding of a class of small molecule inhibitors of this protein-protein interaction. The insufficient mechanistic information on metastasis is hampering the development of efficient therapeutics for it.

PCNA interactions are modulated by posttranslational modifications, including ubiquitination, sumoylation, acetylation and phosphorylation. In particular mono or polyubiquitination has a strong impact in translation synthesis. There is now great interest in understanding the effect of these modifications in the molecular recognition process of PCNA, especially since PCNA has so many partners. It has recently been described that NEDDylation antagonizes ubiquitination of PCNA and regulates the recruitment of polymerase eta in response to oxidative DNA damage[24]. SUMOylation of PCNA by PIAS1 and PIAS4 promotes template switch in the chicken and human B cell lines[25].It has been also found that ING5 inhibits cell proliferation and invasion in esophageal squamous cell carcinoma through regulation of the Akt/NF-κB/MMP-9 signaling pathway[26]. It has been found that girdin regulates collective cancer cell migration by controlling cell adhesion and cytoskeletal organization[27], and that promotes cell survival during endoplasmic reticulum stress[28].

One of the major issues on DNA replication is the determination of the structure of polymerase delta, free and bound to DNA and PCNA. It consists of 4 subunits (a catalytic one and three regulatory subunits). A possible development is the determination of this structure, especially with the new techniques of cryo-electron microscopy. The interplay between the different covalent modifications of PCNA will also be a fertile ground for new discoveries. Since there are currently no small molecule inhibitors that target girding interaction with G proteins the discovery of such molecules would represent a major advance for the study of a fundamental process of intercellular communication. In addition to serving as novel investigational tools, they will become new leads to develop anti-metastatic drugs, an area of cancer therapeutics greatly underserved.

Wnt signaling in cancer

Robert Kypta, *Breast Cancer Stem Cells Laboratory*

Aberrant Wnt signaling underlies a wide range of pathologies, from bone and metabolic disorders to neurodegenerative diseases and cancer. The Wnt field is at a stage where most of the key players have been characterized, many at the structural level, and the knowledge generated from basic research in cell and developmental biology is beginning to be employed with the aim of improving human health. Several drugs targeting the Wnt pathway have been developed, some of them now in clinical trials. The global challenges for Wnt-targeted therapies in cancer are the risk of side-effects that may result from altering signals that also play roles in tissue homeostasis and repair and the inherent heterogeneity of tumours that may lead to development of resistance to therapy. These challenges can be met by the generation of more specific inhibitors tailored to the patient's tumour (personalized medicine). In the context of Wnt signaling, this translates to targeting a specific ligand or receptor, rather than all of them, or targeting an associated protein that confers tissue and/or tumour stage selectivity.

Two studies showed how Wnt signals can be decoded and identified structural determinants for Wnt functional diversification by the associated protein RECK, which confers selectivity for Wnt7 signalling in brain endothelial cells via GPR124/FZD/LRP5/6[29,30]. Our group identified a novel interaction between FZD_8 and TGF-β receptor I that provides a new nexus to target in prostate cancer[31]. Two studies unexpectedly found that R-spondins directly inhibit the E3 ubiquitin ligases ZNRF3 and RNF43, which destabilize FZD and are mutated in cancer[32,33]. findings with implications for cancer and regenerative medicine. In the area of infectious diseases, the crystal structure of FZD_2 with the *C. difficile* virulence factor TcdB[34] provided a new target for the most common cause of diarrhoea in developed countries. Genentech identified a peptide that binds a novel site on FZD_7, causing a conformational change in its lipid-binding groove, thereby inhibiting cancer stem cell function[35] and the Stanford Wnt group further developed FZD-LRP5/6 heterodimerizers as surrogate Wnt agonists that enhance osteogenesis and regulate metabolic liver zonation[36]. Unlike Wnts, these proteins are easily produced and will facilitate translational applications in regenerative medicine.

Recent investments in start-ups such as Surrozen and Samumed highlight the interest in exploiting Wnt signaling as a therapy. Developments are now commercially sensitive, so it is difficult to speculate on what will be revealed in 2019. We expect that interest in Wnt signaling in prostate cancer will continue to grow, building on recent reports that Wnt inhibition overcomes tumour resistance to enzalutamide. There are also indications that inhibiting Wnt signaling overcomes resistance to immunotherapy. Our discovery linking Wnt and TGF-β signaling and the Wnt blocking antibodies we have developed put us in an excellent position to benefit from the interest in these areas. Imminent advances are described in the Prostate Cancer Foundation State of Science Annual Report, published in early 2019.

The global epidemics of liver diseases

Malu Martinez-Chantar, *Liver disease Laboratory*

Chronic Liver diseases is a term that includes a broad group of hepatic pathologies from different etiology that last longer than 6 months and are commonly ended in cirrhosis and hepatocellular carcinoma (HCC), one of the deadliest types of cancer. Chronic liver disease is one of the leading cause of mortality in the U.S and Europe and it can be caused by different pathologies, including viral infection of hepatitis B and C, alcohol and drug abuse, and Non-alcoholic Fatty Liver Disease (NAFLD). Independently of aetiology, chronic liver diseases are characterized by a slow, and frequently indolent progression. The main objective of our research group has been to identify new mechanisms involved in liver disease in relation to mitochondrial dysfunction and the alteration of post-translational modification (PTM) pathways.

One of the most common chronic liver disease is Non-alcoholic fatty liver disease (NAFLD), which comprehends a group of conditions closely related to obesity, insulin resistance, and metabolic syndrome[37]. There are estimations of about 1.8 billion people (30-40%) suffering from NAFLD in the world, which clearly emerges as global health problem. Patients with fatty liver, a condition usually considered rather benign on the NAFLD spectrum, have excellent prognosis from a liver standpoint. On the other hand, non-alcoholic steatohepatitis (NASH), a condition characterized by aberrant oxidative stress, inflammation and fibrosis localized on the most harmful part of the spectrum of NAFLD progression, is an important risk factor for cirrhosis and liver cancer[38]. Recent evidences shows that gut microbiota and microbiota-derived compounds have a role in the progression of steatosis to NASH, modulating pro- and anti- inflammatory effectors disturbing lipid and carbohydrate metabolism[39]. Pharmacological approaches targeting NASH can provide the means for rapidly achieving a reversal of liver injury and blocking the progression of chronic liver disease whilst protecting from cardiovascular hazardous, disregarding possible lacks of compliance of the harsh dietary and lifestyle interventions recommended.

In spite of the huge increase of investment forecast by the pharmacological industry (34% in the next decade) there are

still no approved therapies targeting NASH[40]. Nevertheless, the development of multitarget mechanism therapies and the identification of non-invasive biomarkers remains an important goal for the diagnosis and treatment of NAFLD in the future. Hepatocytes are one of the cell types with the highest density of mitochondria, tremendous metabolic activity and one of the most susceptible to suffer alterations in mitochondrial function. Mitochondrial functions may be altered by mutations in mitochondrial genes as well as by exogenous substances such as viruses, alcohol or drugs. In the last few years the role of mitochondria in liver injury has begun to be considered as a major suspect, and alterations in their function have been detected in diseases like NAFLD, DILI, cholestasis and HCC. PTMs are necessary for normal liver physiology and their alterations may be involved in liver disease. Indeed, aberrant acetylation, ubiquitination and NEDDylation have been already described in different disorders such as liver cancer and cholestasis, and new data will corroborate that both a correct mitochondrial function and protein homeostasis are crucial for the proper function of the liver and that alterations in both processes are associated with liver injury.

Molecular mediators of Hypoxia

Edurne Berra, *Cancer Cell Signaling and Metabolism Laboratory*

Today most of the cells are addicted to oxygen as they rely on oxygen to produce energy. Hence, any reduction in oxygen availability, or hypoxia, could be dramatic, even when transient. In spite of the seminal contributions made during the last two decades, to understand how cells and tissues respond to changes in oxygen availability is still a fundamental problem in biomedicine. Indeed, hypoxia occurs in physiology but hypoxia is also a characteristic of a wide range of disorders including inflammatory, neurodegenerative or cardiorespiratory diseases and cancer.

The publications in the field keeps on the plateau reached 4 years ago (around 7000 indexed manuscripts) and uncover two main research areas:

- To understand the molecular mechanisms that control the hypoxia signaling pathway: In this regard, López-Barneo's group has elegantly demonstrated the relevance of a high CoQH2/coQ ratio, NADH and ROS production at the mitochondrial complex I to trigger the acute oxygen sensing response by the carotid body[41]. A manuscript by Ratcliffe and Mole reports the inherent DNA binding specificities of HIF-α subunits helping along the differential but complementary HIF1- versus HIF2-dependent targets[42]. p53 and the transcription factor ZHX2 add to the so far not extensive and controversial list of HIF-independent PHD/EGLN targets. Moreover, several papers have shown the relevance of new molecular mediators in the transcriptional output to hypoxia including but not limited to new miRNAs and lncRNAs, SIN3A, BICD1 or the Tet proteins.
- To decipher the role played by the signaling pathway in physio-pathology: Two genomic studies show new natural adaptations, PDE10A and PTPN1, in the context of the Bajau people with a lifestyle based on breath-hold diving and insects at high-altitude, respectively[43],[44]. Following the notion reported last year, a new manuscript highlights the need to buffer lactic acid production to maintain circadian oscillations[45]. Two recent papers clearly show the role played by HIF1 in endometrial repair during menstruation as well as the preferential role played by HIF2 in preeclampsia[46],[47]. Interestingly, chronic hypoxia induced-hypomyelination appears to be the cause of synaptic loss and neurodegeneration[48]. It is also worth noting the role of hypoxia and HIF-signaling in allergy, tumour T-cells function and acquired resistance in cancer[49],[50]. Furthermore, a very recent paper shows the excessive oxygen consumption in adipocytes contributing to metabolically abnormal obesity[51].

Visualizing hypoxia, deciphering the molecular mediators including the role of epigenetic in the hypoxia transcriptional outcome and elucidating specificities of such mediators in hypoxia-related disorders and biodiversity are among the opportunities to face in the next year. 2019 will certainly be a crucial year in this field as we will know about the final data from the ongoing clinical trials. Indeed, PHD/EGLN inhibitors are in phase 3 trials to treat anaemia triggered by chronic renal failure, and are promising in preclinical models of ischemia. Conversely, a HIF2 inhibitor has induced responses in a subset of patients with ccRCC, though transcription factors have been largely considered as undruggable proteins.

Neuropathies, PNS cancer and nerve regeneration

Ashwin Woodhoo, *Nerve Disorders Laboratory*

Peripheral nerves are important for movement, our senses and unconscious control of most organs. Nerve disorders, induced by insults such as nerve injury, metabolic disturbances, microbial infections or genetic defects, can be severely debilitating and even fatal. At present, there are no cure for many of these disorders, some of which have already high incidences, and rising alarmingly. For example, diabetic neuropathy (DN), the most common and debilitating complication in diabetes, a high-burden disorder that is estimated to affect over 6% of the global population (400 million people), is the leading cause of non-traumatic lower-extremity amputations and has an annual cost estimated to be more than $10 billion in the US. Similarly, traumatic injury of peripheral nerves, which can result in significant disability, is a worldwide problem with an average of 10 million people affected. Our research is focused on identifying therapeutic strategies for various nerves disorders with a special emphasis on the exploitation of our scientific discoveries.

In 2018, the major scientific advances in the field has been related to uncovering novel clinically relevant targets in nerve disorders, including neuropathies, nerve regeneration and PNS cancer. In the first outstanding study, the authors identified histone deacetylase 3 as a potent inhibitor of myelin formation, and showed that it could be targeted for improving peripheral nerve repair[52]. In the second study, the authors showed that YAP/TAZ signaling drives Malignant Peripheral Nerve Sheath Tumourigenesis, identifying potential therapeutic targets in these untreatable tumours[53]. Both studies clearly show the importance of identifying new targets for these untreatable disorders, and the potential applicability of these findings in clinic. Similarly, recent studies have shown that mitochondria represent an important drug target for highly prevalent diseases, and several strategies aimed at therapeutically restoring mitochondrial function have emerged. Notably, 2 studies have shown that enhancing mitochondrial function using genetic and pharmacological means has good therapeutic potential for Alzheimer´s disease, and kidney and liver diseases[54],[55]. These work could be of special relevance to several nerve disorders, which can be characterized by severe mitochondrial dysfunction that lead to development of pathology.

Identification of novel therapeutic targets for nerve disorders would be the stand-out scientific development in 2019, which could be perfectly achievable by proposed studies combining computational-based analyses, including big data analysis, and experimental paradigms that would include latest OMICs technologies (metabolomics by NMR, transcriptomics or proteomics). Notably, strategies aimed at targeting particularly debilitating and high-burden disorders, such as diabetic neuropathy, could have important societal impacts, given its high prevalence (5–10% of European people, and increasing 5% yearly) and the fact that there is currently no specific medication

that prevents or reverses diabetic neuropathy in humans. In addition, strategies focused on improving functional recovery after traumatic injury of nerves, could also have important benefits for patients, and here the focus would be on harnessing experimental data to devise new types of artificial conduits for nerve regeneration in collaboration with bioengineering companies.

Transmissible spongiform encephalopathies

Joaquín Castilla, *Prion Research Laboratory*

The description in Europe of the first cases of prion disease in cervids (CWD) has become a great public safety issue due to its rapid spread in the US and Canada. Transmissible spongiform encephalopathies (TSE) are lethal neurodegenerative disorders caused by prions, aberrantly misfolded isoform (PrP^{Sc}) of the prion protein (PrP^{C}), which upon misfolding becomes amyloidogenic, able to induce its conformation on PrP^{C}, neurotoxic and transmissible to other individuals. The physiological roles of PrP^{C} are not fully identified and nor the neurotoxicity pathway and cannot be fully understood until the 3D structure of PrP^{Sc} will be solved. Also the development of new early diagnostic methods and the search of a therapy need to be highlighted as the main challenges in clinical practice, as well as understanding the interspecies transmission barrier and the determinants of prion strains.

During 2018 new approaches have been developed to increase our knowledge on the physiology of PrP^{C} as the identification of regulatory miRNAs in humans[56]. Regarding the 3D structure of prions, the end of 2017 brought the first structure at high resolution using ssNMR and a synthetic prion as model[57]. However its biological significance is unclear and the few advances done on prion structure in 2018 were scarce and made with low resolution techniques[58]. Prion transmissibility through environmental materials has been further characterized[5] and an important molecular key to the high transmission barrier to prions presented by canines has been deciphered[59].Similarly, prion strain phenomenon has been reproduced *in vitro* using recombinant prions in different propagation systems, providing important clues on the possible mechanisms of strain variability *in vivo*[60,61]. Few new diagnostic methods have been also explored, some that used novel and more accessible tissues for detection of minute amounts of prions[62], a completely new amyloid fibril detection system through plasmon resonance[63], and an several high throughput screening (HTS) methods based on *in vitro* prion propagation assays[64]. Regarding the arrival of CWD to Europe, few new cases have been described in Norway and classified as different from the US and Canadian isolates[65]. Finally, the debate in still opened in the International Prion Community about other misfolded proteins being prions, such as α-synuclein or Aβ peptide, rising issues of new terminology and classifications of prions.

2019 is expected to be an important year in the field mainly regarding the 3D structure of prions. New recombinant prions generated *in vitro*, more similar to brain-derived prions, new propagation systems with much higher yields and the increased control of *in vitro* generated prion strain heterogeneity together with high resolution biophysical techniques such as ssNMR or cryo-EM could finally allow the deciphering of a *bona fide* prion structure at atomic resolution. News on therapeutic strategies are also expected as the first experimental treatment will be administered to a single patient in UK. Nonetheless, novel therapeutic approaches will be also sought using new HTS methods. Further efforts will be also done to study the interspecies transmission properties and the zoonotic risk of the new European CWD strains. Therefore, 2019 will bring new opportunities mainly regarding 3D structure, distinct therapeutic approaches and assessment of public safety risk due to CWD spreading in Europe.

Molecular and Functional characterization of Extracellular Vesicles.

Juan M. Falcón-Perez, *Exosomes Laboratory*

As part of the intercellular communication mechanism, all cells in the organisms secrete, different types of extracellular vesicles (EVs) that are present in the different fluids of the body. They are actively been investigated as a source for low-invasive biomarkers, also as a therapeutic agent for targeted delivery, and as a pathological agent in different pathologies including neurological and metabolic diseases. However, the isolation procedures and phenotyping methodologies are still not well established, and are areas of intense development in the field. During this year, more than 2000 articles in the field of exosomes have been reported covering many aspects of the biology of these vesicles from the isolation and phenotyping to their implications in the development of different diseases.

A big effort has been performed by the International Society for Extracellular Vesicles (ISEV) in compiling most of the problems and possible solutions to them, and it has published a comprehensive guide of all resources and knowledge available in the EV field community[66]. Novel methodologies to isolate EVs based on microfluidics has started to be applied in the field[67]. The combination of different methodologies to get higher purity preparations has also received a lot of attention specially by applying size-exclusion chromatography coupled to density gradient[68] and asymmetrical-flow field-flow fractionation[69]. Preliminary studies supporting the application of imaging flow cytometry has also been published during this year. Surface proteomics has been applied to the study of EVs in order to identify novel biomarkers of cancer[70]. Novel technologies to analyze metabolites associated with EVs has been reported[71]. Advances, in the isolation of EVs from saliva, at the same time that their possible role in development of systemic diseases has been suggested also suggested during this year[72]. In addition, significant advance in the isolation and analysis of sweat EVs has been reported[73], as well as, suggested their role in skin immunity. In addition, the possible pathological role of EVs has been extended to other clinical areas such as joint[74] and musculoskeletal[75] diseases.

The perspective for the next year in the field is that there will be a big development in technologies able to isolate single vesicles mostly based in microfluidic devices. In addition, another related area that is expected to suffer an advance in the field will be in phenotyping methodologies for single vesicles, and in particular Raman spectroscopy and high-resolution flow cytometry. In addition, the role of EVs in the development of pathologies will also be a growing area especially in neurological, infectious and metabolic diseases.

Sorting of integral membrane proteins

Aitor Hierro, *Membrane Trafficking Laboratory*

Recycling prevents waste, reduces consumption and saves energy. Living cells constantly recycle proteins and lipids, with a direct impact on nutrient uptake, re-sensitisation to environmental signals, immune surveillance and waste management. Endosomes are key intracellular recycling compartments where the biosynthetic and endocytic pathways intersect. Here, the fate of sorting receptors is directly linked to

their selective recruitment into tubulo-vesicular carriers. Retromer is a multiprotein complex that assembles on endosomes and forms tubular vesicles that return specific integral membrane proteins to a variety of cellular compartments. Retromer's cargo includes signalling receptors, ion channels, nutrient transporters and enzymes that are essential for a wide range of physiological processes. Nonetheless, our understanding of the mechanisms that regulate the recruitment of retromer to endosomes, the concentration of cargo in prebudding domains and the coordinated assembly of the tubular coat remains very limited. Spatiotemporal control of these events not only is essential for general proteostasis and neuroprotection, but also is subverted by numerous pathogens. Our goal is to elucidate the molecular mechanisms for recognition, packaging and sorting of integral membrane proteins into retromer-coated tubulo-vesicles.

The number of scientific papers published in the field of retromer during 2018 (updated Dec 20th), according to PubMed, is 81. Some outstanding work described: 1) the structure of the retromer complex from the thermophilic fungus *C. thermophilum* at subnanometer resolution assembled around tubular membranes. Critical to this achievement has been the use of cryo-EM tomography and reference-free subtomogram averaging. The structure revealed a two-layer organization with an oligomeric helical array of VPS5 proteins in the inner layer and an outer layer of retromer molecules forming arch-like dimers[76]; 2) the identification of retromer-dependent Wnt signaling to propagate cell-non-autonomous mitochondrial unfolded protein response (UPR^{mt}). The UPR^{mt} can communicate local stresses across multiple tissues to be prepared beforehand. This protective response may be of fundamental importance in neurodegenerative proteinopathies such as Alzheimer's and Parkinson's diseases[77]; and 3) the identification of a cell penetrating peptide at the C-terminal region of the human papillomavirus L2 protein. The peptide contains a conserved cationic sequence that drives the passage throughout the endosomal membrane and a retromer binding motif responsible for retrograde transport of viral particles from endosomes to the trans-Golgi network[78].

Protein recycling has a direct impact on metabolic balance and cellular homeostasis. Retromer is a multiprotein complex responsible for recycling protein channels and receptors involved in a wide range of physiological processes such as nutrient intake, cell signalling, polarised transport, cell differentiation, immune response, and nerve transmission. These pathways are complex and have a large number of components and interactions regulating the sequential formation of retromer-coated vesicles. Selective dissection of these steps at the molecular level will allow the use of target-focused libraries in order to find small molecules that can enter cells and act acutely and specifically on given steps of a selected pathway. For example, delaying or enhancing the delivery of receptors to the plasma membrane is a way to modulate cellular de-sensitization or re-sensitization to extracellular stimuli.

Understanding carbohydrates complexity: Concepts and applications

Jesús Jimenez Barbero, *Chemical Glycobiology Laboratory*

Carbohydrates (glycans, sugars) play key roles in virtually all biological events. Given their chemical complexity, understanding their roles in nature requires a multidisciplinary approach. Research in the field is growing, since advances in the area could be part of the solution to many health issues. We address glycan recognition by using a multidisciplinary approach, combining chemical synthesis, molecular biology and biophysics, with a prominent role for NMR and molecular modelling. We are developing new 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. However, understanding sugar recognition remains a major challenge to ultimately precisely manipulate them and create new probes and eventually drugs.

Besides our own developments, along this year 2018, novel key methodological and conceptual advances in the study of sugar recognition events have been described with applications in biology and biomedicine. In particular, single-molecule force spectroscopy (SMFS) by atomic force microscopy (AFM) has enabled the molecular interactions of sugars to be studied[79]. Moreover, the modulation of Immune Tolerance via Siglec-Sialic Acid Interactions have been reviewed, providing a hallmark for the ongoing and future studies in our group[80]. As fantastic application of glycosyl transferases, it has been shown that α1,3-galactosyltransferase-knockout pig hearts that express human CD46 and thrombomodulin permits the maximum survival of a baboon after heart replacement with a porcine xenograft[81]. In the heparan sulfate arena, an HS-mutant mouse lung endothelial cell library by systematic deletion of HS genes expressed in the cell has been described. In this context, it has been demonstrated that it is the strictly defined fine structure of HS, not its overall degree of sulfation, the key factor for FGF2-FGFR1 signaling. Moreover, the fine inter-regulation networks by which HS genes modify HS and chain length in mammalian cells at a cell-type-specific level has been delineated[82]. In the N-glycan field, the results of a systematic study on the effect of sequence on the structure and dynamics of increasingly larger, complex biantennary N-glycoforms, in the Fc region of human IgGs, have shown that while core fucosylation and sialylation do not affect the intrinsic dynamics of the isolated (unbound) N-glycans, galactosylation of the α(1-6) arm shifts dramatically its conformational equilibrium from an outstretched to a folded conformation[83]. In the biomedical context, the levels of α2–6 sialylation and β1–6 branching have been correlated to C-reactive protein concentration, an inflammation marker and prognostic indicator for bladder cancer, further strengthening the link between inflammation and abnormal plasma protein glycosylation.

One of the major expectations for next year is the development of protocols to monitor glycan recognition by NMR not *in vitro*, but directly *in-cell*, a crowded ambient where viscosity is doubled respect to water. Moreover, novel advances in understanding the role of glycans (shape, structure, dynamics) in infection processes, such as influenza (avian and human) and other viral infections (Ebola, HIV, Zika) are expected.

Glycobiology of enzymes

Marcelo Guerin, *Structural Glycobiology Laboratory*

Enzymes play a central role in nature essentially due to their capacity to catalyse a great number of stereospecific chemical reactions in all living organisms[84]. Enzymes accelerate the speed of such reactions more than a million times. Thus, chemical reactions that would take years in the absence of enzymes, can occur in fractions of seconds if they are catalysed by the appropriate enzyme. Most of the enzymes responsible for the biosynthesis, degradation and modification of glycan structures are Carbohydrate-Active Enzymes[85]. They are highly selective in nature, allowing the recognition of subtle structural differences in the sequences and stereochemistry of their glycan substrates. The understanding of the structural determinants and the modulation of substrate specificity remains a major challenge in the field.

This year a tremendous advanced has been made in the understanding of the N-linked glycosylation process at the molecular level, a post-translational modification of asparagine residues found in all domains of life[86]. Specifically, oligosaccharyl-transferase (OST) is an essential membrane protein complex in the endoplasmic reticulum, where it transfers an oligosaccharide from a dolichol-pyrophosphate-activated donor to glycosylation sites of secretory proteins. The atomic structures of eukaryotic OSTs were determined by cryo-electron microscopy, revealing a conserved subunit arrangement[87,88]. The active site of the catalytic STT3 subunit points away from the center of the complex, suggesting how eukaryotic OSTs efficiently glycosylate a large number of polypeptides before their folding. In that sense, the development of systematic approaches to understand site specific modifications of proteins, as glycosylation. We created a single molecule assay where mechanical force both activates the enzymatic reaction and controls precisely the energy landscape of the disordered substrate peptide modulating its kinetics of recognition with distinct orders of magnitude. Furthermore, we built an energetic model with atomistic details that successfully predicts the impact of substrate entropy on the enzymatic recognition and allows, for new interpretations of biopolymer chemistry and post-translational modification of proteins[89].

Considerable challenges remain to be overcome to fully understand the mode of action of these enzymes. Specifically, the emerging importance of conformational dynamics in substrate recognition and enzyme catalysis anticipates exciting times. We will apply a combination of Nucleic Magnetic Resonance (NMR) and in particular cryo-electron microscopy (Cryo-EM) on enzyme models to contribute to the elucidation of this major challenge (7). This knowledge will undoubtedly enable to understand the molecular basis of these mechanisms from a fundamental perspective and to generate a variety of important applications in biomedicine and biotechnology.

Pharmacological chaperones NMR-based diangostic

Oscar Millet, *Protein Stability and Inherited Disease Laboratory*

Pharmacological chaperones are organic entities that correct the misfolding issues of a protein introduced by a mutation and/or assist in the protein trafficking towards the functional final destination. The main challenge here resides in to validate the proof-of-concept of the entire strategy with limited overall toxicity.

The field of pharmacological chaperones is experiencing significant advances with the first product in the market (Migalastat), which is a pharmacological chaperone for the treatment of Fabry disease. In parallel, many different projects are applying this strategy to a plethora of rare diseases, mostly lysosomal disorders. In our laboratory, we have stablished the proof-of-concept in rodents for a pharmacological chaperone that allosterically binds to uroporphyrinogen III synthase, being active against Congenital Erythropoietic Porphyria[90]. Moreover, the compound is a repurposed drug and it has been granted the orphan drug designation by both, the FDA and the EMA[91]. The company ATLAS molecular pharma is currently undertaking the preparation and development of the clinical phase trials for the compound (open label combined phase I/II on patients).

It is expected that in the following years an avenue of new compounds acting as pharmacological chaperones will reach the market of rare disorders. Theoretical studies about proteostasis and about the required kinetic properties of the compound-protein association are also of the utmost relevance.

Precision medicine is a very broad concept that refers to the customization of healthcare with medical decisions, treatments, practices, or products being tailored to the individual patient. In this context, we focused on the problem of obtaining personalized molecular data, mostly at the metabolic level. NMR-based metabolomics is a powerful technique that allows the absolute quantification of a large set of metabolites in biofluids (i. e. urine, serum, faeces) in a non-costly and non-destructive way. Again, the main challenge for this approach is the validation of the obtained biomarkers. There is a significant number of on-going initiatives that aim for the use of NMR-based metabolomics in precision medicine, with the "all of us" initiative from the NIH and an upscaled version from the Chinese government as good examples of it. In our laboratory, we are implementing an equivalent strategy to initially target the population to the Basque Country, by addressing significant health problems such as metabolic syndrome, liver disease and the early diagnose of congenital inborn errors of metabolism. The obtained metabolic information will combine well with other molecular analyses (i. e. genetics, proteomics, transcriptomics) and with the medical records of the individual.

It is expected for this field to produce new commercial tests that will define the metabolic and genetic basis of each individual in the context of health and disease. If is important to emphasize that this data can always be analyzed retrospectively, as long as new relevant information is ascribed to already measured variables.

Ubiquitin-likes and Development processes

Rosa Barrio, *Ubiquitin-likes and Development Laboratory*

We are interested on the regulation of developmental processes and diseases by post-translational modifications by the Ubiquitin-like (UbL) SUMO of specific transcription factors. Among those, the Spalt-like (SALL) family are necessary for numerous biological processes. Mutations in *SALL1* are associated to Townes-Brocks Syndrome (TBS), a rare disease causing kidney defects, deafness and polydactyly. TBS patients might develop kidney failure, requiring dialysis or transplant. We discovered that TBS interferes with the function of cilia, cellular antennas that play crucial roles in cell signalling, which opened new opportunities of intervention. SUMO is attached to target proteins altering their function, thus regulating nuclear integrity, proliferation and transcriptional regulation, contributing to diseases like cancer or neurodegeneration. UbLs can be conjugated to each other generating hybrid chains, creating a Ubiquitin Code largely unexplored, with consequences in physiology and disease.

SALL1 has been related to a number of processes and disorders during 2018, like heart diseases (hypoplastic right heart syndrome[92]); kidney formation and disease (renal agenesis or hypoplasia[93]); retina formation[94]; and to cancer (uterine leiomyomas[95], esophageal squamous cell carcinoma[96], acute myeloid leukemia[97], non-small cell lung cancer[98], head and neck squamous-cell carcinoma[99], and breast cancer[100]). Furthermore, we described for the first time the mechanism of action of truncated SALL1 in the rare disease TBS though cilia dysregulation[101]. SUMO has also been related to various processes and disorders during 2018, like DNA stability, cellular stress and cancer. For instance, its role in cellular differentiation has been elucidated by stabilizing changes in chromatin states[102]. Other roles of SUMO in inflammation[103], cancer though destabilization of c-Myc[104] senescence through PML-nuclear body associated proteins[105], etc, have been reported. Importantly, advances in the identification and isolation of SUMOylated proteins have been also described, like peptide-level immunoprecipitation enrichment[106], E2-thioester-driven

identification[107], chemical probes for profiling the activity of SUMO-specific proteases[108], among others.

Our discovery of cilia dysregulation in TBS individuals opened new possibilities of intervention by treating the cells with specific drugs and/or genome editing. To study the role of SALL proteins in cilia formation and function will shed light in the role of members of this family of transcription factors in other diseases like cancer. The development of new technologies for the identification and isolation of proteins modified by Ubiquitin and SUMO chains will be crucial to understand the function of hybrid chains, how they are created and the interaction with other factors involved in their possible functions. Isolation of modified proteins in an organelle-specificity manner and determination of specificity of ligases and deSUMOylases are some of the big challenges of the field. Technological development is thus crucial in those studies.

Cryo-Electron Microscopy advances

Sean Connell and Paola Fucini, *Ribosome Structural Biology Laboratory*

Cryo-electron microscopy (cryo-EM) is a biophysical method that can be used to elucidate the structure of molecules important for human health and biotechnology. For example, structural information generated by this approach can be used to understand how drugs modulate biological pathways to improve human health and also lead to the development of new completely new therapeutic strategies based on understanding how the structure of biological molecules is altered in a disease state and how drugs can correct this alteration. Currently we use cryo-EM to understand how antibiotics improve human health by targeting a bacterial molecular machine called the ribosome, and aim to use this information to combat antibiotic resistance which is one of greatest problems facing mankind.

This year has seen some amazing advances in cryo-EM, punctuated by the fact that three pioneers in the field, Jacques Dubochet, Joachim Frank and Richard Henderson, won the 2017 Nobel Prize in chemistry for their contributions to the methodological development of cryo-electron microscopy. Notable advances include:

- Cryo-EM in Personalized Medicine: The use of cryo-EM in personalized medicine and diagnostic applications is highlighted by its use to image tau filaments extracted from patient samples[109].
- Cryo-EM studies of Biomedical Targets: Cryo-EM has revealed structure-function relationships for many molecules with biomedical importance including the insulin receptor–insulin complex[110] the CRISPR complex[111], GPCR complexes[112] and membrane ion transporters[113]. These structures will potentially impact human health.
- Sample Preparation Technology: Two new industrial players in cryo-EM vitrification technologies emerged, namely cryosol and spotiton, indicating that significant advances can still be made through engineering solutions.
- Increased target suitability: New cryo-EM technologies like more stable supports, more sensitive cameras, and phase plates are increasing the application of cryo-EM to smaller protein targets[114]. This increases the use of cryo-EM by pharmaceutical and biotechnology industries.
- Software Developments: Advances in software packages like Relion[115], cryosparc and Warp[116] are making cryo-EM image processing more reproducible, user friendly, automated and fast.

Cryo-EM will continue to mature and develop as a field and become increasingly scientifically and commercially relevant in the fields of biomedicine (drug development, personalized medicine) and chemical biology owing to its ability to provide atomic structural data on samples refractory to other biophysical characterization methods. We expect to see major developments in European countries with strong centralized facilities aimed at increasing the throughput of cryo-EM by multiplexing samples and employing automated intelligent data collection and processing schemes made possible by the use of big data and machine learning. Other breakthrough areas will include sample preparation where we expect microfluidic based screening methodologies to make significant impacts on solving orientation bias problems.

Flexible filamentous plant viruses and ribosomes

Mikel Valle, *Cryo-EM of biologicals macromolecules Laboratory*

We use cryo-EM to explore the structure and to understand the functioning of several biological complexes such as ribosomes and filament plant viruses. Cryo-EM can provide structures of biological macromolecules at atomic resolution. The advances in direct detection cameras have brought the new potential for atomic resolution. During 2018 a new generation of direct detection cameras has been presented to the community. Essentially they provide higher speed, larger field, and better efficiency, allowing to collect a large number of images of high quality during the cryo-EM experiment. The group of Sjors Scheres at the LMB-MRC (Cambridge) has published and new version of their software package Relion for cryo-EM. This is currently the most used software in the field. Also, software packages that processes the images from cryo-EM have greatly improved. Rather than future challenges, cryo-EM faces a brilliant future and has a lot more potential to deliver. Regarding specific topics we cover. The structure of flexible filamentous plant viruses has been solved essentially by our own group (two samples from different families), and now the challenge is to decipher their mechanism of assembly, and the role of the protein-RNA interactions in the process. Also, how can we make use of the structural information to manipulate (biotechnology) or block (phytosanitary applications) these viruses. Concerning ribosomal structures, the field has exploded and there are numerous groups working on structure of prokaryotic, eukaryotic, and organelle ribosomes. However there is a lack of *in vitro* translation systems (apart from prokaryotic models) to face structure-function studies of different steps of protein synthesis.

For the next year (2019) we are expecting few changes in cryo-EM at international level, but some others at local and national level might greatly impact the field and the cryo-EM community here. For instance, the acquisition of a new transmission electron microscope in Madrid, at the National Center for Biotechnology (CNB-CSIC). This new equipment is a Titan Krios 300 kV microscope (FEI, Thermo Fisher Scientific) and includes a direct detection camera. This is the most advanced microscope available nowadays, and the first of this type to be installed in Spain. It will boost the performance of Spanish cryo-EM groups. For flexible filamentous plant viruses we expect to find compounds that bind to their nucleoproteins and block the propagation of the viruses. This would have a large economic impact in agriculture worldwide. Also, studies of viral like particles (VLPs) of the virions might bring some light to their unknown assembly mechanism. We are not aware of any other lab involved in this type of studies. We hope to see the development of new *in vitro* translation systems in the near future. We are aware of attempts by collaborating groups focused on mitochondrial ribosomes, a system that could allow to study mitochondrial diseases related to translation defects.

Viruses: miniaturized machines

Nicola G. A. Abrescia, *Structure and Cell Biology of Viruses Laboratory*

Viruses are pathogens to humans and animals others are allied of humans in controlling bacteria proliferation. Others are manipulated and used as delivery systems into humans of drugs or repairing genes (gene therapy). Our group studies viruses infecting organisms across the three domain of life: Bacteria, Archaea and Eukarya. Our recent projects involve mainly the study of eukaryotic animal and human viruses (enveloped and not) at the stage of assembly and virus entry. The ultimate goal is to provide a solid conceptual/knowledge-based framework for exploring new avenues for therapeutic interventions or biotechnological applications. Research on viral pathogenesis at basic or translational level remains a global health challenge as the emerging of new viruses or the need of new POC technologies and antiviral therapies constantly challenge our society.

The field of Structural Virology has seen pivotal advances on several fronts in tackling human health threats such as Ebola and Zika viruses. For none of the two viruses a licensed vaccine or an antiviral drug is available and fundamental research has been shown to be essential for progressing in their understanding[117,118,119]. It is worth mentioning that our group is also involved in studying virus members of animal and human threatening *Bunyavirus* and *Flavivirus* families. Also, viruses are miniaturized machines that perform complex tasks and comprehending how members of the Virosphere are related beyond the similarity in their primary sequence (which is easily lost when analyzing viruses) remains a powerful tool to exploit/discover new virus applications. Our on-going work is shedding light into the origin of double-beta barrel viruses of which human adenovirus is member of. Further, discovering new viruses or engineering new ones for biotech applications as in the case of Adeno-Associate Virus (AAV) can be considered an outstanding scientific advance[120].

Technical advances in our field of methodological expertise expected in 2019 will mainly concern:

- HR cryo-EM: it will develop even further and using very little amount of sample (e.g. through Spotiton devices). It is currently used by Pharma companies in drug screening programmes and it will be used in the clinical area.
- Soft X-ray tomography and high-resolution correlative microscopy: their combination allows a direct correlation of cellular morphological changes with virus entry pathways and consequent antiviral treatment.

Although a powerful technique current state for nanocrystallography and time-resolved experiments (X-FELS), relies on systems previously well explored with classical X-ray crystallography. Specifically to the above viruses, delivery of vaccines for Ebola or Zika is unlikely to occur in 2019 although there are candidate vaccines in phase-clinic III for Ebola. Scientific and social opportunities relate also to geographic/governments' interest, outbreaks around the world and possibility of virus spread to Western countries.

References

1 CAR T-cell Therapy: A New Era in Cancer Immunotherapy. *Curr Pharm Biotechnol*, 2018, 19, 5-18.

2 Nivolumab plus Ipilimumab in Lung Cancer with a High Tumor Mutational Burden. *N Engl J Med*, 2018, 378, 2093-2104.

3 Urea Cycle Dysregulation Generates Clinically Relevant Genomic and Biochemical Signatures. *Cell* 174, 1559-1570 e1522 (2018).

4 Somatic mutant clones colonize the human esophagus with age. *Science* 362, 911-917 (2018).

5 Detection and localization of surgically resectable cancers with a multi-analyte blood test. *Science*, 2018, 359, 926-930.

6 Epigenetically reprogrammed methylation landscape drives the DNA self-assembly and serves as a universal cancer biomarker. *Nat Commun* 2018, 9, 4915.

7 Long-term reprogramming of the innate immune system. *J Leukoc Biol*. 2018 Jul 12. doi: 10.1002/JLB.MR0318-104R.

8 Induction of innate immune memory via microRNA targeting of chromatin remodelling factors. *Nature*, 2018, 559, 114-119.

9 Innate immune memory in the brain shapes neurological disease hallmarks. *Nature,* 2018, 556, 332-338.

10 Electrophilic properties of itaconate and derivatives regulate the IkappaBzeta-ATF3 inflammatory axis. Nature, *2018*, 556, 501-504.

11 Itaconate is an anti-inflammatory metabolite that activates Nrf2 via alkylation of KEAP1. Nature, *2018*, 556, 113-117.

12 [Food and Drug Administration, Shingrix. https://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm581491.htm

13 https://www.ema.europa.eu/en/news/first-malaria-vaccine-receives-positive-scientific-opinion-ema

14 Elucidating the Mechanisms of Action of Saponin-Derived Adjuvants. *Trends Pharmacol Sci*. 2018, Jun;39(6):573-585. doi: 10.1016/j.tips.2018.03.005.

15 Correlates of adjuvanticity: A review on adjuvants in licensed vaccines. *Semin Immunol*, 2018 Oct;39:14-21. doi: 10.1016/j.smim.2018.05.001.

16 Protective Epitope Discovery and Design of MUC1-based Vaccine for Effective Tumor Protections in Immunotolerant Mice. *J Am Chem Soc.* 2018, Nov 19. doi: 10.1021/jacs.8b08473.

17 β-Glucan as an immune activator and a carrier in the construction of a synthetic MUC1 vaccine. *Chem Commun (Camb).* 2018 Dec 20;55(2):253-256. doi: 10.1039/c8cc07691j.

18 Synthesis, Liposomal Formulation, and Immunological Evaluation of a Minimalistic Carbohydrate-α-GalCer Vaccine Candidate. *J Med Chem*. 2018 Jun 14;61(11):4918-4927. doi: 10.1021/acs.jmedchem.8b00312.

19 Syntheses and Immunological Evaluation of Self-Adjuvanting Clustered N-Acetyl and N-Propionyl Sialyl-Tn Combined with a T-helper Cell Epitope as Antitumor Vaccine Candidates. *Angew Chem Int Ed Engl.* 2018, Jul 2;57(27):8219-8224. doi: 10.1002/anie.201804437.

20 Recent Advances in Toll Like Receptor-Targeting Glycoconjugate Vaccines. *Molecules* 2018, 23, 1583.

21 Design, Synthesis, and Immunological Evaluation of a Multicomponent Construct Based on a Glycotripeptoid Core Comprising B and T Cell Epitopes and a Toll-like Receptor 7 Agonist That Elicits Potent Immune Responses. *J Med Chem.* 2018,61(21):9568-9582. doi: 10.1021/acs.jmedchem.8b00960.

22 Dual Mechanisms of LYN Kinase Dysregulation Drive Aggressive Behavior in Breast Cancer Cells. Cell Rep. 2018 Dec 26;25(13):3674-3692.e10. doi: 10.1016/j.celrep.2018.11.103

23 Dual Mechanisms of LYN Kinase Dysregulation Drive Aggressive Behavior in Breast Cancer Cells. Cell Rep. 2018 Dec 26;25(13):3674-3692.e10. doi: 10.1016/j.celrep.2018.11.103.

24 NEDDylation antagonizes ubiquitination of proliferating cell nuclear antigen and regulates the recruitment of polymerase η in response to oxidative DNA damage. *Protein Cell,* 2018,9(4):365-379. doi: 10.1007/s13238-017-0455-x.

25 SUMOylation of PCNA by PIAS1 and PIAS4 promotes template switch in the chicken and human B cell lines. *Proc Natl Acad Sci U S A.* 2018,115(50):12793-12798.doi: 10.1073/pnas.1716349115.

26 ING5 inhibits cell proliferation and invasion in esophageal squamous cell carcinoma through regulation of the Akt/NF-κB/MMP-9 signaling pathway.*Biochem Biophys Res Commun*. 2018, 496, 387-393.

27 Girdin/GIV regulates collective cancer cell migration by controlling cell adhesion and cytoskeletal organization. *Cancer Sci.* 2018 109, 3643-3656.

28 GIV/Girdin promotes cell survival during endoplasmic reticulum stress. *Mol Cell Biochem*. 2018, doi: 10.1007/s11010-018-3433-6

29 A molecular mechanism for Wnt ligand-specific signaling. *Science,* 2018,361(6403). pii: eaat1178. doi: 10.1126/science.aat1178.

30 A RECK-WNT7 Receptor-Ligand Interaction Enables Isoform-Specific Regulation of Wnt Bioavailability. *Cell Rep,* 2018,25(2):339-349.e9. doi: 10.1016/j.celrep.2018.09.045.

31 Frizzled-8 integrates Wnt-11 and transforming growth factor-β signaling in prostate cancer. *Nat Commun*. 2018,9(1):1747. doi: 10.1038/s41467-018-04042-w.

32 inhibition of RNF43 and ZNRF3 governs limb development independently of LGR4/5/6. *Nature*, 2018,557(7706):564-569. doi: 10.1038/s41586-018-0118-y. RSPO2

33 R-spondins can potentiate WNT signaling without LGRs. *Elife.* 2018,7. pii: e33126. doi: 10.7554/eLife.33126.

34 Structural basis for recognition of frizzled proteins by Clostridium difficile toxin B. *Science,* 2018,360(6389):664-669. doi: 10.1126/science.aar1999.

35 A selective peptide inhibitor of Frizzled 7 receptors disrupts intestinal stem cells. *Nat Chem Biol*, 2018,14(6):582-590. doi: 10.1038/s41589-018-0035-2.

36. Surrogate Wnt agonists that phenocopy canonical Wnt and β-catenin signalling. *Nature*, 2017,545(7653):234-237. doi: 10.1038/nature22306

37 Clinical workup of fatty liver for the primary care provider. *Postgrad Med.* 2018,29:1-12. doi: 10.1080/00325481.2019.1546532.

38 The incidence and risk factors of hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. *Hepatology.* 2010,51(6):1972-8. doi: 10.1002/hep.23527.

39 The role of the microbiome in NAFLD and NASH. *EMBO Mol Med*. 2018,27. pii: e9302. doi: 10.15252/emmm.201809302.

40 Recent Insights into the Pathogenesis of Nonalcoholic Fatty Liver Disease. *Annu Rev Pathol*. 2018,13:321-350.

41 Acute O2 Sensing: Role of Coenzyme QH2/Q Ratio and Mitochondrial ROS Compartmentalization. *Cell Metab*. 2018,28(1):145-158.

42 Inherent DNA-binding specificities of the HIF-1α and HIF-2α transcription factors in chromatin. *EMBO Rep*. 2019, 20(1). pii: e46401.

43 Physiological and Genetic Adaptations to Diving in Sea Nomads. *Cell*. 2018 Apr 19;173(3):569-580.

44 Genetic variation in PTPN1 contributes to metabolic adaptation to high-altitude hypoxia in Tibetan migratory locusts. Nat Commun. 2018 Nov 26;9(1):4991.

45 Acid Suspends the Circadian Clock in Hypoxia through Inhibition of mTOR. *Cell*, 2018,174(1):72-87.

46 Hypoxia and hypoxia inducible factor-1α are required for normal endometrial repair during menstruation. *Nat Commun*, 2018,9(1):295.

47 HIF-2α, but not HIF-1α, mediates hypoxia-induced up-regulation of Flt-1 gene expression in placental trophoblasts. *Sci Rep*, 2018,8(1):17375.

48 Enhancing Oligodendrocyte Myelination Rescues Synaptic Loss and Improves Functional Recovery after Chronic Hypoxia. *Neuron*, 2018,99(4):689-701.

49 E3 Ligase VHL Promotes Group 2 Innate Lymphoid Cell Maturation and Function via Glycolysis Inhibition and Induction of Interleukin-33 Receptor. *Immunity*, 2018, 48(2):258-270.

50 Cancer Lipid Metabolism Confers Antiangiogenic Drug Resistance. *Cell Metab*. 2018,28(1):104-117.

51 Knockdown of ANT2 reduces adipocyte hypoxia and improves insulin resistance in obesity. *Nature Metabolism,* 2019,1, 86–97.

52 A histone deacetylase 3-dependent pathway delimits peripheral myelin growth and functional regeneration. *Nat Med*, 2018,24,338-351

53 Programming of Schwann Cells by Lats1/2-TAZ/YAP Signaling Drives Malignant Peripheral Nerve Sheath Tumorigenesis. *Cancer Cell*, 2018,33,292-308 e297.

54 Enhancing mitochondrial proteostasis reduces amyloid-beta proteotoxicity. *Nature*, 2017, 552, 187-193.

55 De novo NAD(+) synthesis enhances mitochondrial function and improves health. Nature, 2018,563, 354-359.

56 Genome-Wide Identification of microRNAs regulating the Human Prion Protein. *Brain Pathol*, 2018 Nov 19. doi: 10.1111/bpa.12679. PMID: 30451334

57 Species-dependent structural polymorphism of Y145Stop prion protein amyloid revealed by solid-state NMR spectroscopy. *Nat Commun*, 2017, 8(1):753. doi: 10.1038/s41467-017-00794-z. PMID:28963458

58 Recombinant PrPSc shares structural features with brain-derived PrPSc: Insights from limited proteolysis. *PLoS Pathog.* 2018,14(1):e1006797. doi: 10.1371/journal.ppat.1006797.

59 An Amino Acid Substitution Found in Animals with Low Susceptibility to Prion Diseases Confers a Protective Dominant-Negative Effect in Prion-Infected Transgenic Mice. *Mol Neurobiol*, 2018,55(7):6182-6192. doi: 10.1007/s12035-017-0832-8.

60 Artificial strain of human prions created in vitro. *Nat Commun*, 2018,9(1):2166. doi: 10.1038/s41467-018-04584-z. PMID: 2986716

61 Cofactors influence the biological properties of infectious recombinant prions. *Acta Neuropathol*. 2018,135(2):179-199. doi: 10.1007/s00401-017-1782-y. Epub 2017 Nov 1.

62 Prion Seeds Distribute throughout the Eyes of Sporadic Creutzfeldt-Jakob Disease Patients. *MBio*, 2018,9(6). pii: e02095-18. doi: 10.1128/mBio.02095-18.

63 Detection of amyloid fibrils in Parkinson's disease using plasmonic chirality. *Proc Natl Acad Sci U S A*. 2018,115(13):3225-3230. doi: 10.1073/pnas.1721690115.

64 Cell-free prion protein conversion assays in screening for anti-prion drug candidates. *Curr Opin Pharmacol*, 2018,6;44:1-7.

65 Novel Type of Chronic Wasting Disease Detected in Moose (Alces alces), Norway. *Emerg Infect Dis*. 2018,24(12):2210-2218.

66 Minimal Information for Studies of Extracellular Vesicles (MISEV2018). https://www.tandfonline.com/action/doSearch?AllField=MISEV2018&SeriesKey=zjev20

67 Microfluidics-based on-a-chip systems for isolating and analysing extracellular vesicles. *J Extracell Vesicles*. 2018,7(1):1508271. doi: 10.1080/20013078.2018.1508271.

68 Comparison of an Optimized Ultracentrifugation Method versus Size-Exclusion Chromatography for Isolation of Exosomes from Human Serum. *J Proteome Res.* 2018,17(10):3599-3605. doi:10.1021/acs.jproteome.8b00479.

69 Ultrafiltration and size exclusion chromatography combined with asymmetrical-flow field-flow fractionation for the isolation and characterisation of extracellular vesicles from urine. *J Extracell Vesicles*. 2018,7(1):1490143.

70 Surfaceome profiling enables isolation of cancer-specific exosomal cargo in liquid biopsies from pancreatic cancer patients. *Ann Oncol*, 2018,29(1):223-229. doi: 10.1093/annonc/mdx542.

71 Exosome isolation from distinct biofluids using precipitation and column-based approaches. *PLoS One*. 2018 Jun 11;13(6):e0198820. doi: 10.1371/journal.pone.0198820.

72 Salivary Exosomes: Emerging Roles in Systemic Disease. Int J Biol Sci. 2018 Apr 30;14(6):633-643. doi: 10.7150/ijbs.25018. eCollection 2018.

73 Proteomic Profiling of Sweat Exosome Suggests its Involvement in Skin Immunity. J Invest Dermatol. 2018 Jan;138(1):89-97.

74 Emerging Role of Exosomes in the Joint Diseases. Cell Physiol Biochem. 2018;47(5):2008-2017. doi: 10.1159/000491469.

75 Emerging role of extracellular vesicles in musculoskeletal diseases. Mol Aspects Med. 2018 Apr;60:123-128. doi: 10.1016/j.mam.2017.09.006.

76 Structure of the membrane-assembled retromer coat determined by cryo-electron tomography. *Nature*, 2018,561(7724):561-564. doi: 10.1038/s41586-018-0526-z.

77. The Mitochondrial Unfolded Protein Response Is Mediated Cell-Non-autonomously by Retromer-Dependent Wnt Signaling. *Cell,* 2018,174(4):870-883.e17. doi: 10.1016/j.cell.2018.06.029

78 Cell-Penetrating Peptide Mediates Intracellular Membrane Passage of Human Papillomavirus L2 Protein to Trigger Retrograde Trafficking. *Cell*, 2018,174(6):1465-1476.e13. doi: 10.1016/j.cell.2018.07.031.

79 Studying glycobiology at the single-molecule level. *Nature Reviews Chemistry*, 2018, 2,148–159.

80 Modulation of Immune Tolerance via Siglec-Sialic Acid Interactions, *Front Immunol*, 2018 doi.org/10.3389/fimmu.2018.02807

81 Consistent success in life-supporting porcine cardiac xenotransplantation. *Nature*. 2018 Dec;564(7736):430-433. doi: 10.1038/s41586-018-0765-z.

82 A mutant-cell library for systematic analysis of heparan sulfate structure–function relationships. *Nature Methods*, 2018, 15, 889-99.

83 Sequence-to-structure dependence of isolated IgG Fc complex biantennary N-glycans: a molecular dynamics study. *Glycobiology*, 2018, doi: 10.1093/glycob/cwy097

84 Flexibility, diversity, and cooperativity: pillars of enzyme catalysis. *Biochemistry*, 2011,50:10422-30.

85 The Carbohydrate-active enzymes database (CAZy) in 2013. *Nucleic Acids Res*, 2013,42:D490–D495.

86 Mechanisms and principles of N-linked protein glycosylation. *Curr. Opin. Struct. Biol.* 2011,21:576-82.

87 Structure of the yeast oligosaccharyltransferase complex gives insight into eukaryotic N-glycosylation. *Science*, 2018,359:545-550.

88 The atomic structure of a eukaryotic oligosaccharyltransferase complex. *Nature*, 2018,555:328-333 (2018).

89 Conformational entropy of a single peptide controlled under force governs protease recognition and catalysis. *Proc. Natl. Acad. Sci. USA.* 2018,115:11525-11530.

90 Repourposing ciclopirox as a pharmacological chaperone in a model of congenital erythropoietic porphyria. *Sci Transl Med,* 2018,10(459). pii: eaat7467.

91 Orphan drug designation by the FDA. DRU-2018-6297. https://www.cicbiogune.es/news/european-medicines-agency-ema-favorably-comments-orphan-drug-designation-molecule-activity

92 Copy number variants in hypoplastic right heart syndrome. *Am J Med Genet A*. 2018. doi: 10.1002/ajmg.a.40527.

93 Constitutive metanephric mesenchyme-specific expression of interferon-gamma causes renal dysplasia by regulating Sall1 expression. *PLoS One*, 2018,13(5):e0197356. doi: 10.1371/journal.pone.0197356.

94 Sall1 Regulates Microglial Morphology Cell Autonomously in the Developing Retina. Adv Exp Med Biol, 2018,1074:209-215. doi: 10.1007/978-3-319-75402-4_26.

95 Genetic predisposition to uterine leiomyoma is determined by loci for genitourinary development and genome stability. *Elife*. 2018 Sep 18;7. pii: e37110. doi: 10.7554/eLife.37110.

96 Identification of Hyper-Methylated Tumor Suppressor Genes-Based Diagnostic Panel for Esophageal Squamous Cell Carcinoma (ESCC) in a Chinese Han Population. *Front Genet*,2018,9:356. doi: 10.3389/fgene.2018.00356..

97 SALL1 expression in acute myeloid leukemia. *Oncotarget*, 2017,9(7):7442-7452. doi: 10.18632/oncotarget.23448.

98 Genome-wide identification of transcription factors that are critical to non-small cell lung cancer. *Cancer Lett*. 2018 Oct 10;434:132-143. doi: 10.1016/j.canlet.2018.07.020.

99 Genome-wide identification of transcription factors that are critical to non-small cell lung cancer. *Cancer Lett*, 2018,434:132-143. doi: 10.1016/j.canlet.2018.07.020.

100 SALL1 functions as a tumor suppressor in breast cancer by regulating cancer cell senescence and metastasis through the NuRD complex. *Mol Cancer*, 2018 Apr 6;17(1):78. doi: 10.1186/s12943-018-0824-y.

101 Truncated SALL1 Impedes Primary Cilia Function in Townes-Brocks Syndrome. *Am J Hum Genet*, 2018,102(2):249-265.

102 SUMO Safeguards Somatic and Pluripotent Cell Identities by Enforcing Distinct Chromatin States. *Cell Stem Cell*, 2018,23(5):742-757.e8. doi: 10.1016/j.stem.2018.10.001.

103 SUMOylation of ROR-γt inhibits IL-17 expression and inflammation via HDAC2. *Nat Commun*, 2018,9(1):4515. doi: 10.1038/s41467-018-06924-5.

104 SUMO protease SENP1 deSUMOylates and stabilizes c-Myc. *Proc Natl Acad Sci U S A*, 2018115(43):10983-10988. doi: 10.1073/pnas.1802932115.

105 Quantitative SUMO proteomics reveals the modulation of several PML nuclear body associated proteins and an anti-senescence function of UBC9. *Sci Rep*, 2018,8(1):7754. doi: 10.1038/s41598-018-25150-z.

106 Site-specific characterization of endogenous SUMOylation across species and organs. *Nat Commun*. 2018 Jun 25;9(1):2456. doi: 10.1038/s41467-018-04957-4.

107 An E2-ubiquitin thioester-driven approach to identify substrates modified with ubiquitin and ubiquitin-like molecules. *Nat Commun*, 2018,9(1):4776. doi: 10.1038/s41467-018-07251-5.

108 Total Chemical Synthesis of SUMO and SUMO-Based Probes for Profiling the Activity of SUMO-Specific Proteases. *Angew Chem Int Ed Engl*. 2018,57(29):8958-8962. doi: 10.1002/anie.201803483.

109 Structures of filaments from Pick's disease reveal a novel tau protein fold. *Nature*. 2018,561(7721):137-140. BioRvix doi: 10.1101/302216

110 Structure of the insulin receptor–insulin complex by single-particle cryo-EM analysis. *Nature*, 2018,556, 122–125.

111 Cryo-EM structure of Type III-A CRISPR effector complex. Cell, 2018,28,1195–1197.

112 Cryo-EM structure of the serotonin 5-HT1B receptor coupled to heterotrimeric Go. *Nature*, 2018,558, 620–623.

113 Cryo-EM structure of a fungal mitochondrial calcium uniporter. Nature, 2018,559,570–574.

114 Near-atomic cryo-EM imaging of a small protein displayed on a designed scaffolding system. *PNAS*, 2018,115(13),3362-3367.

115 RELION-3: new tools for automated high-resolution cryo-EM structure determination. BioRvix doi: 10.1101/421123

116 Real-time cryo-EM data pre-processing with Warp. View ORCID ProfileDimitry Tegunov, Patrick Cramer. BioRvix doi: 10.1101/338558

117 Cryo-EM structure of the Ebola virus nucleoprotein-RNA complex at 3.6 Å resolution. *Nature*. 2018,563(7729):137-140. doi: 10.1038/s41586-018-0630-0.

118 Electron Cryo-microscopy Structure of Ebola Virus Nucleoprotein Reveals a Mechanism for Nucleocapsid-like Assembly. *Cell*. 2018 Feb 22;172(5):966-978.e12. doi: 10.1016/j.cell.2018.02.009.

119 Altered vector competence in an experimental mosquito-mouse transmission model of Zika infection. doi: 10.1371/journal.pntd.0006350. *PLoS Negl Trop Dis*. 2018,12(3):e0006350.

120 Designer viruses could be the secret to cheaper and better gene therapy. *Nature*, 2018,564(7735):S18-S19. doi: 10.1038/d41586-018-07647-9.

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