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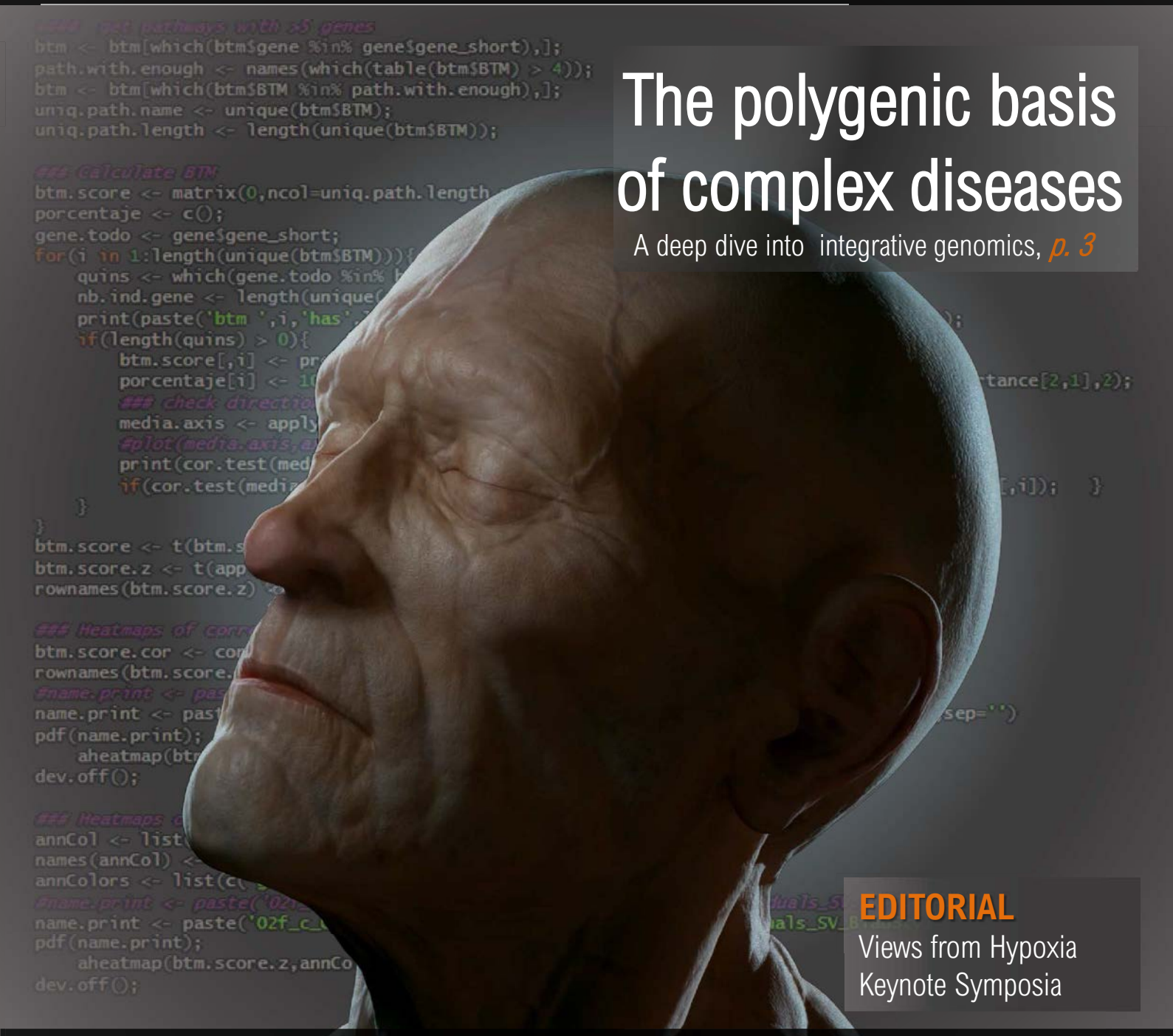

The polygenic basis of complex diseases

A deep dive into integrative genomics, *p. 3*

EDITORIAL
Views from Hypoxia Keynote Symposia

STEM CELLS
Multifaceted Regulation of Tissue Regulation. *p. 4*

STROMA
Metabolism of tumor microenvironment. *p.5*

COMPUTING
Computer Simulation of Biological Processes. *p. 7*

CIC bioGUNE
MEMBER OF BASQUE RESEARCH & TECHNOLOGY ALLIANCE

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 promote development of advanced therapies. Our 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 is member of the Basque Research and Technology Alliance (BRTA).

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.

EDITORIAL

How Cells Sense and Adapt to Oxygen Variations

Edurne Berra, *Cancer Cell Signaling and Metabolism Laboratory*

Tribute to William G. Kaelin, Sir Peter J. Ratcliffe and Gregg L. Semenza (2019 Nobel Laurates in Physiology or Medicine)

Our cells use oxygen (O_2) to produce the energy they need to function. O_2 is, indeed, vital and its deficit or hypoxia, even if transient, can result in irreversible damages that lead living organisms to develop sophisticated strategies throughout evolution to deal with hypoxia. However, understanding how cells/organisms sense O_2 and adapt to O_2 availability remained at issue up to a set of seminal discoveries contributed by William G Kaelin, Peter J Ratcliffe and Gregg L Semenza. These advances have been awarded in 2019 with the Nobel Prize in Physiology and Medicine[1].

Gregg Semenza's identification and later cloning of the Hypoxia Inducible Factor (HIF) as the dimeric transcription factor that promotes EPO expression opened the door to the hypoxia research field. In parallel, an independent work by Peter Ratcliffe shown the ubiquitous hypoxic HIF induction and transactivation of additional target genes (i.e. glycolytic enzymes), which contain the DNA binding sites for HIF, termed HREs (Hypoxia Response Elements). Later on, two back-to-back publications by Willian Kaelin and Peter Ratcliffe proved that hydroxylation in two proline residues of the HIF-α subunit, accounts for the extremely short lived of HIF-α in the presence of O_2. This hydroxylation strongly increases the affinity for pVHL (von Hippel-Lindau) binding and labels HIFα for ubiquitination and degradation. Another remarkable discovery was that of the PHDs/EGLNs (isoforms 1, 2 and 3), the family of prolyl-4-hydroxylases regulating HIF-α. Thereafter, the hydroxylation on an asparagine residue that was also O_2-dependent was found to suppress any residual HIF transcriptional activity. In summary, hypoxia suppresses HIF-α degradation and repression triggering the downstream target genes and therefore, the adaptive hypoxia response.

A myriad of publications has expanded the work of the 2019 Nobel laurates and shown the importance of the Hypoxia/PHD/pVHL/HIF pathway in the pathogenesis of highly prevalent diseases (lung and cardiovascular diseases, neurodegeneration, inflammation and cancer among others). Accordingly, the translation of drugs modulating the "hypoxia response" into clinical practice has emerged as a major milestone in the field. In this regard, while originally HIF was thought to be non-druggable target, a recently developed highly specific HIF-2α antagonist may have immediate use as first-line treatment or in association with other drugs in the treatment of mutant VHL clear cell renal cell carcinoma. On the other hand, PHD inhibitors are also promising pharmacological tools to treat some forms of anaemia.

Last January, I had the honour to participate in the tribute and celebratory toast to the 2019 Nobel Laurates in Physiology or Medicine at the Keystone Hypoxia Meeting. A better understanding of the molecular mechanisms involved in acutely O_2-sensing, the discovery of new O_2-dependent enzymes and the pathophysiological significance of the ones recently identified, the potential hydroxylation of non-HIF targets by PHDs, the biomedical relevance of the study of the "hypoxia response" and the design of new targeting drugs are still challenging topics for future research. Certainly, there is still an opportunity for hypoxia research beyond the Nobel Award.

A Deep Dive into the Polygenic Basis of Complex Diseases

Urko Martinez Marigorta, *Integrative Genomics Laboratory*

The research at the Integrative Genomics lab revolves around the genetic basis of human disease. We analyse -omic profiles (e.g. gene expression) of patient cohorts to illuminate our understanding of disease pathogenesis and, eventually, gear this knowledge towards achieving precision medicine. At present, using inflammatory bowel disease (IBD) as a model, we are focusing on devising new approaches to evaluate genetic risk and tailor health management to the needs of each patient. The biggest challenge in the field of complex disease genetics lies, precisely, in finding ways to gear genetic insights towards new translational solutions that can impact clinical practice. In the last decade we have amassed impressive knowledge about the genetics of disease, and discovery of hundreds of genetic variants robustly associated with each disease is now routine. However, often we simply ignore everything about the nature of these associations, including the underlying causal gene and pathogenic mechanism.

Three particular advances during 2019 merit being mentioned. First, new techniques such as high-throughput reporter surveys and CRISPR-Cas9, as well as refined statistical methodologies, allow for fine mapping with perfect resolution the causal mutations that account for the associations discovered in genetic studies[2,3]. Characterizing susceptibility effects at the single nucleotide level permits to understand the mechanisms that increase risk of developing the disease and hence paves the way towards discovery of new therapeutic approaches. Second, this year we have witnessed the explosion of the polygenic risk scores, also known as PRSs[4]. Useful to summarize risk of disease in each individual, these tools permit to stratify and detect important fractions of individuals (e.g. top 5%) with enhanced risk of developing disease, often equivalent to those seen for classical monogenic mutations that already trigger actionable changes in the clinic. Third, this year has brought a massive burst of single cell transcriptomics for disease. Considering only IBD, this includes ambitious characterizations of the cell subtype landscape in (i) the colon from adult patients[5], (ii) the mucosa of pediatric patients[6], (iii) the role of the mesenchymal component[7], (iv) the underpinning sof response to anti-TNF therapy[8], as well as (v) deep comparisons across many patients[9]. This wealth of studies is key to delineate transcriptional states and identify specific cell populations involved in the pathogenic processes that lead to complex disease, well beyond the insights gained from studies using bulk profiling of biopsies.

The next year will bring a renewed focus on tangible proof-of-concept studies that can be labelled as “achieving precision medicine”. This will include studies attempting to implement genetic profiling (through PRSs) into clinical risk modelling, as well as pioneer inspection of the role of genetic factors in the ample heterogeneity in prognosis and symptoms seen in real-life settings. These efforts will be based on characterization of context-dependent genetic effects and multi-omic profiling of large prospective cohorts. The availability of these datasets will be key for our purpose of developing new tools for longitudinal monitorization of patients and being a lab that allows for bridging genomic medicine with the clinic.

Advances on Metabolic Syndrome

Malu Martínez-Chantar, *Liver Disease Laboratory*

Deregulated reprogramming of liver metabolism is a hallmark of liver disease, a series of multi-factorial conditions involving the progressive destruction and regeneration of the liver parenchyma leading to fibrosis, cirrhosis and liver cancer. Recently, type 2 diabetes and obesity are emerging as the most common causes of liver disease being that Non-Alcoholic Fatty Liver Disease (NAFLD), a spectrum of conditions characterized by the accumulation of fat in the liver, affects about 25% of the adult population worldwide. It ranges from liver steatosis that can progress to inflammation and fibrosis, hallmarks of non-alcoholic steatohepatitis (NASH). Even though, NAFLD is the most common liver disorder in developed countries, current therapies are restricted to lifestyle modifications whereas pharmacological approaches remain scarce and experimental. Overall, an improved knowledge of the regulatory mechanism of liver metabolism during disease progression may provide novel therapeutic targets for the finding novel druggable therapeutic targets which will definitely impact health and economic burden ascribed to liver disease.

An important effort has been made to find a reliable preclinical animal models to develop functional analysis and therapies in the NASH-to-HCC progression, many of them do not faithfully to mimic the human disease and few reliably progress to HCC. In this sense two new different approaches should be highlighted[10] that have generated a human fatty liver using custom-engineered induced pluripotent stem cells with modifiable SIRT1 metabolism and the finding from[11] that have identified MUP-uPA mice that resemble the human pathology and are clearly useful for evaluation for HCC-targeting immunotherapies.

Immune response has emerged strongly in the field of NAFLD. In NASH patients a specific hepatic immune-related gene signature with increased of hepatic CD8 T cells, and alteration of dendritic cells have been identified by[12]. These findings expand the understanding of the mechanism underlying of NASH and identify potential targets for NASH therapy. Finally, microbiota in liver injury has been an area in expansion this year. Relevant findings have positioned Klebsiella pneumoniae as the responsible to produce high levels of alcohol causing fatty liver in a 60% of patients[13].

The liver is a metabolically very active organ that is responsible for vital functions such as digestion, blood detoxification and clearance and metabolism and distribution of nutrients and energy to the rest of the body. All these functions make the liver more exposed to harmful substances than other organs and hence, more vulnerable to injury. Despite the alarming incidence of liver disease, the molecular mechanisms involved in its development and progression have not been fully elucidated, which largely limits the availability of more effective therapeutic treatments. Furthermore, in most cases the disease is detected at an advanced stage and lacks an established treatment. For all these reasons, a better understanding of the mechanisms underlying liver disease is crucial for the development of more effective therapeutic and preventive strategies.

Our main objective will be to identify new mechanisms involved in liver disease in relation to mitochondrial dysfunction and the alteration of post-translational modification (PTM) pathways. As a result of the tremendous metabolic activity of the liver, hepatocytes are one of the cell types with the highest density of mitochondria and one of the most susceptible to suffer alterations in mitochondrial function. Mitochondria are in charge of generating energy for the cell and controlling energy balance and cell death, and their function 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

mechanism and moreover, alterations in their function have been detected in diseases like NAFLD, cholestasis and HCC. On the other hand, Post-translational modifications (PTM) are essential mechanisms for signalling, metabolism and cellular activity, regulating the function and homeostasis of proteins. Since PTMs are necessary for normal liver physiology, alterations in their pathways 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.

Microbial Metabolism and Colorectal Cancer

Juan Anguita and Héctor Rodríguez, *Inflammation and Macrophages plasticity Laboratory*

One of our research lines is focused on the determinants of certain bacterial pathogens to thrive within the gut and contribute to colorectal cancer (CRC) pathogenesis and bad prognosis. The most relevant pathogen associated with this cancer is Fusobacterium nucleatum that has been found overrepresented in CRC tumor tissues all over the world and can increase tumor multiplicity. In particular, we are studying the effect of dietary metabolites produced by the microbiota in F. nucleatum fitness and survival and its possible use as prevention and/or treatment. Some of these metabolites modulate gut microbial composition and are toxic for F. nucleatum but do not affect (or even favor) the growth of beneficial commensals and probiotics. We are also interested in the detoxicant role of enzymatic activities of F. nucleatum over dietary metabolites. As some of these compounds are toxic for the bacteria, we hypothesize that specific enzymatic inhibition of these proteins will result in the loss of bacterial fitness within the gut and furthermore, in the decreased colonization and expansion of the pathogen. We address our basic questions through the use of classical microbiology, molecular microbiology, structural and molecular modeling studies of the proteins in order to 1) Decipher the complex relationship between diet, microbiota and settlement of CRC-related pathogens; 2) Study the effects of microbial metabolites in the growth of F. nucleatum; 3) Study F. nucleatum virulence factors that allow its survival under metabolic stress conditions. The ultimate goal is the identification of metabolites and/or bacteria inhibiting the growth of F. nucleatum as treatment and/or prevention for CRC.

Even though the relationship between certain bacteria and colorectal cancer has been known for quite some time[14], this year several key discoveries have been reported in prominent journals. A report in Nature Medicine has confirmed these results and unveiled for the first time the relationship between gut metabolic dynamics, different stages of colorectal cancer and cancer-associated microbiota unveiling a connection between Fusobacterium prevalence, CRC stage and the abundance of certain metabolites[15]. Further confirmation for the role of the microbiota in cancer progression was also obtained for Li et al. They reported that the transplant of CRC patient microbiota to a murine model of cancer accelerated the expansion of the disease[16]. Another important contribution to the field during 2019 was the confirmation of a hypothesis suggesting an oral origin for the pathogens associated with colorectal cancer[17]. Also of importance, a recent study of the differential microbiota of CRC patients in India (a context with specific regional dietary intake) has unveiled an important role for specific diet components transformation in CRC prevention[18]. New discoveries have also unveiled this year new facets of F. nucleatum mechanisms conducing to the disease. One of the main discoveries has been the epigenetic modifications observed as a result of F. nucleatum infection[19] or the interaction of the bacteria with the Wnt/β catenin pathway[20]. Finally different treatments possibilities targeting F. nucleatum or the microbiota associated with the disease have recently emerged using different approaches[21], the most interesting using phage to kill F. nucleatum populations within the gut[22].

The knowledge of the biology of the microbes recently related to gastrointestinal disease and colorectal cancer is limited and mostly obtained from in vitro cultures in isolation. However, little is known about the factors that trigger the translocation for these pathogens from the mouth to the gut, the interaction of these bacteria with the gut microbiota and the determinants for their establishment in the gastrointestinal tract. Among them, an almost unexplored field is the study of the genetic traits of the CRC-associated bacteria, including Fusobacterium, that facilitate their survival within the gut, their interaction with other microorganisms and diet.

We envisage relevant developments in the field for the 2020 that can shed light into microbial metabolism and how this may affect colon cancer, such as: 1) The identification of compounds and/or probiotics impairing pathobionts establishment within the gut; 2) The understanding of the conditions that initiate the translocation of CRC-associated pathogens from the mouth to the gut and the mechanisms that dictate this 'journey'; 3) The identification of genetic determinants for F. nucleatum survival within the gut in relation to diet and the ecological competition with other microorganisms, and 4) The development of personalized diets protective for CRC-related cancers.

Towards in vivo Stem Cell Reprogramming into Functionally Mature Cells

Antonio del Sol and Sascha Jung, *Computational Biology Laboratory*

The field of regenerative medicine has blossomed in recent decades. However, the ultimate goal of tissue regeneration – replacing damaged or aged cells with healthy functioning cells – still faces a number of challenges. Here, especially the generation of tissue-specific mature and functional cell types is a major goal of regenerative medicine and holds great potential for medical applications. Classical approaches rely on the transplantation of in vitro generated cells into the dysfunctional tissue, but typically fail to produce mature cells mimicking their *in vivo* counterparts due to the niche that transmits cues inducing intracellular signaling. This way, the niche is capable of shaping gene expression and epigenetic patterns of cells and determining their phenotypic states. Hence, the phenotype of a cell is maintained not only by transcription factors (TF) that determine its identity but also by specific TFs activated or inhibited by the niche, an important consideration when designing in vivo reprogramming strategies.

Recently, we have seen a boost of in vivo reprogramming reports aiming to restore tissue functioning without requiring the *in vitro* generation of transplantable cells. A seminal study published in Nature Cell Research identified only three TFs – HNF1A, HNF4A and FOXA3 – whose over-expression reverts hepato-carcinoma cells into normal hepatocyte-like cells and underscores the great potential of direct in vivo reprogramming[23]. In addition, in vivo reprogramming strategies have been developed to treat large cutaneous ulcers by generating expandable epithelial tissue[24] or myocardial infarction by converting fibroblasts into cardiomyocytes[25]. However, these studies, which present approaches at the forefront of regenerative medicine, derive the TFs to be over-expressed by experimental trial-and-error and simple bioinformatics analysis, a strategy that is resource, labor and time consuming. Computational biology approaches can be of great help in this endeavor; however, it is crucial to develop novel methods that consider the niche effect on cellular

phenotypes to generate testable predictions of reprogramming factors in *in vivo* systems. In 2019, we already started addressing this issue and proposed a computational framework for directed *in vivo* stem cell reprogramming[26] that models the effect of niche signals on the transcriptional regulation of stem cells.

In the coming years, we expect increasing efforts on understanding the multifaceted regulation in tissues. Modeling the influence of the niche on shaping and maintaining cellular phenotypes, will enable a computer-guided design of *in vivo* reprogramming protocols and is expected to significantly enhance the development of novel therapeutic strategies for treating diseases or counteracting aging.

Metabolism of Tumor Stroma

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[27]. The complexity of cancer evolution[28,29] has been elegantly illustrated through the annotation and study of the genetic aberrations that govern transformation, proliferation and therapy response[30], in turn leading to the development of targeted treatments. 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 tumor progression. In 2019, cancer research has continued to redefine the concept of a tumor, emphasizing the relevance of environmental factors and co-existing non-cancerous cells modulating the function of transformed counterparts. The adaptive nature of cancer cells is tightly associated to the availability of nutrients and metabolites in the microenviroment. In this regard, nutritional alterations at the systemic level beyond fat composition have been reported to influence cancer biology in experimental models[31]. Western lifestyle is associated with a progressive increase in the intake of sweeteners in food and drinks, which contain high levels of fructose. Interestingly, high-fructose diet was reported in 2019 to increase intestinal tumor growth in the absence of obesity and diabetes[32]. Nutritional and metabolic alterations in the tumor microenvironment also impact on the biology the cancer. Serine is an important aminoacid for the cancer cell that serves as a nutrient for tumor growth. Cancer cells activate the expression of enzymes that support endogenous serine synthesis, and this adaptation enables tumors to grow in organs in which the aminoacid is scarce[33]. Similarly, metastatic breast cancer cells that land in the lung uptake and use environmental pyruvate to remodel of the metastatic niche to their advantage[34]. The strong pressure leading to adaptation upon changes in the tumor microenvironment also offers unprecedented cancer cell vulnerabilities. Cholesterol biosynthesis is impaired in anaplastic large cell lymphoma, thus leading to cholesterol auxotrophy (addiction)[35]. The use of alternative fuels by cancer cells, together with the production of novel lipids to support membrane biosynthesis (e.g. Sapienate[36]), highlight the elevated capacity of tumors to adapt to the ever-changing microenvironment.

With regards to the non-transformed cells co-habiting with cancer cells, 2019 has brought to light some unique phenomena. Fibroblasts represent an important non-transformed population in the tumor stroma. Interestingly, this cell compartment enhances the access of cancer cells to key nutrients in harsh conditions. As an example, fibroblasts can help cancer cells funnelling glucose into glycolysis, by promoting the breakdown of glycogen into glucose[37]. These results support the notion that the stroma is an important contributing factor in the progression of cancer. The extent of their relevance is illustrated by the high content of stromal genes in signatures that harbour prognostic potential in tumors such as colorectal cancer[38]. Moreover, metabolic changes in the cancer-associated fibroblasts account for their capacity to promote tumor aggressiveness[39]. Beyond the support provided by fibroblasts in tumors, the normal cells that compose the metastatic site, such as hepatocytes in cancer that disseminate to the liver, can also contribute to the generation of a niche that is favourable for the tumor[40].Overall, 2019 has brought more complexity into the biology of tumors, and in turn more reasons to explain the emergence of therapy resistance[41].

2019 opens the door to new discoveries genuinely enabled by technological advances. We were made aware of the existence of multiple mutations in a single allele that increase the oncogenic potential and resistance to targeted therapies[42]. In addition, we started to deconstruct cancer genomes and transcriptomes to a deeper extent, revealing that, beyond mutations and gene expression regulation, alternative splicing could play a central role in cancer pathogenesis and progression[43, 44]. The future will unveil how our body functions at single cell resolution[45], and the extent of intratumor heterogeneity in normal or cancerous cell subpopulations, thus revealing new mechanisms underlying cancer progression and therapy resistance[46, 47, 48].

DNA Replication Machines

Francisco J Blanco, *Structural Biology of Cancer Laboratory*

Our research is focused on 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 tumor suppressors, which regulate the compaction state of the chromatin. The protein girdin, 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.

There is a great interest in understanding the effect of post-translational (PTM) modifications in the molecular recognition process of PCNA, especially since PCNA has so many partners, with a strong diversity in the sequence motifs that bind to the ring[49,50]. PCNA interactions are modulated by different PTMs, including ubiquitination, sumoylation, acetylation and phosphorylation. In particular mono or polyubiquitination has a strong impact in translesion synthesis. But not only PCNA but also regulatory proteins are modulated by PTMs. Recently, the effect of ubiquitination on the p15 PCNA associated factor has been studied showing a change in the molecular recognition properties of this intrinsically disordered protein[51]. The structural basis for GPCR-independent activation of heterotrimeric Gi proteins has been unveiled by the examination of the crystal structure of Gαi3 with a fragment of Girdin[52]. Two main advances in the understanding of DNA replication machinery have been recently come out with the cryo-EM structure of the yeast polymerase delta[53] and the time-resolved electron microscopy study revealing the mechanism by which the origin recognition

complex loads pairs of MCM helicases around DNA prior to bidirectional replication[54].

Still missing in the DNA replication field is the determination of the structure of human 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 the structure by 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.

Ubiquitin-like Modifications in Health and Disease

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 and advanced in the understanding of the molecular mechanism of the disease.

UbLs, like SUMO, are 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 an Ubiquitin Code largely unexplored, with consequences in physiology and disease. We develop new technology to identify modified targets in a subcellular manner, as well as factors that only interact with the modified substrates.

Great advances related to SALL1 and kidney formation have been published during 2019, especially involving the role of SALL1 in the generation of in vitro models of kidneys from human pluripotent stem cells (hPSCs). The final aim would be the regeneration of human kidneys in animal models to contribute to transplantation therapies[55, 56, 57, 58, 59, 60, 61]. Also, new evidences of the involvement of SALL1 in cancer were published, like renal cell carcinoma, prostate cancer, oral squamous carcinomas, glioma, colorectal carcinomas[62, 63, 64, 65, 66]. Finally, evidences of the role of SALL1 during development were also published, like lens differentiation, brain macrophages and steroidogenesis[67,68,69,70]. SUMO has also been related to various processes and disorders during 2019: infertility[71,72]; cancer[73, 74, 75]; infection[76, 77, 78, 79], obesity[80]; DNA repair[81,82,83,84] among other processes.

The advances in the mechanisms of cilia dysregulation in TBS individuals opened new possibilities of intervention by treating the cells with specific drugs and/or genome editing. Studies in model systems will be necessary to develop these treatments. The role of members of this family of transcription factors in other diseases like cancer will also benefit. SUMO and other UbLs are involved in a plethora of diseases, many times involving the cross-talk among different modifications[85,86]. New technologies are needed to approach those studies, making proteomics a crucial technology. Biotin-based techniques will be extremely useful in those developments.

Relevance of Glycans in Nature

Jesús Jiménez-Barbero, *Chemical Glycobiology Laboratory*

Carbohydrate molecules are essential actors in key biological events, being involved as recognition points for cell-cell and cell-matrix interactions related to health and disease.

Molecular recognition of glycans is a very complex process. The exquisite selectivity of their biological receptors (lectins, antibodies, enzymes) relies on solving the fragile balance between entropy (dynamics-rigidification, solvation-desolvation, hydrophobicity) and enthalpy elements (hydrogen bonds, CH-π and van der Waals, coulombic, water-receptor and ligand interactions), also considering the role of features as presentation of epitopes and multivalency. 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. Understanding the key recognition features has many implications in chemical biology and for drug discovery[87]. Technical advances in NMR are of paramount importance. The access to new NMR magnets, at or beyond 1 GHz, is providing major enhancements in sensitivity and resolution[88]. This is one of the key developments in the field, with enormous implications for carbohydrate-based drug design and discovery[89]. New molecular biology strategies employing diverse prokaryotic and eukaryotic cell types permit accessing to key glycan receptors, including glycoproteins, also labelled with stable isotopes (2H, 13C, 15N) to perform detailed NMR studies[90]. Fantastic developments are also taking place in glycan synthesis[91], which now provide chemically complex pure glycans in sufficient amounts to provide an exceptional three-dimensional view of large biologically relevant glycan geometries, dynamics, and interactions[92].

Besides our own developments[93], along this year 2019, different approaches have been developed toward monitoring these events using similar conditions to those in vivo[94]. For instance, the synergy of solid-state NMR experiment combined with Dynamic Nuclear Polarization (DNP) has been used to unravel the LecA bacterial lectin residues involved in sugar recognition without sorting to isotope labeling production[95]. One frontier in the application of NMR in glycosciences is the direct study of intact glycoproteins by NMR. Their intrinsic heterogeneity, probably related to its function makes the NMR analysis very puzzling. One of the key milestones has focused on the investigation of the glycan structure of the IgE high-affinity receptor (FcεRIα) expressed in human HEK 293 cells[96]. A three-dimensional structure of the glycoprotein was built, which also allowed to directly detect and unravel the basic interaction features between the N-glycans of the intact glycoprotein and human galectin 3, a lectin involved in cancer. Other paradigmatic illustration of the power of NMR has permitted unravelling the N-glycan structures of several antigenic variants of the major capsid protein from chlorovirus PBCV-1, as initial step to unravel the structure of the associated virus-encoded glycosyl transferases[97]. From the technical side, these methodologies employ uniformly isotope labelled (2H, 13C, 15N) samples generated in insect or mammalian cells[98].

Structural biology is now undertaking the so-called cryo-EM revolution. Outstanding breakthroughs will take place in the near future in the molecular recognition arena, also deciphering fine elements on the role and specificity of the interactions of glycan interactions in biology[99]. Nevertheless, the intrinsic mobility of the glycosidic linkages of glycans[100] makes NMR an essential tool for understanding the conformational and dynamic details of the interactions of these molecules with their receptors[101]. The combination of magnets beyond the GHz with the outburst of well-defined methodologies in chemical biology to access to stable isotope labelling, complex N-glycan synthesis including the ability to produce glycoproteins, including therapeutic glycosylated antibodies, together with the

democratization of novel technologies in NMR will produce an explosion of NMR applications in glycosciences, going into the cell. The future is already here, and the continuous development of new methodologies in liquid and solid state NMR, including DNP and in-cell NMR ensures key steps into understanding the precise glycan roles in nature.

Computer Simulation of Biological Processes

Gonzalo Jiménez-Osés, *Computational Chemistry Laboratory*

Computer simulation allows the virtualization of chemical and biochemical phenomena with ultrahigh spatiotemporal resolution. In this way, complex events occurring at very different size (sub-Angstrom to micrometers) and time scales (femto to microseconds) can be deconvoluted. The combination of conceptually different theoretical frameworks such as quantum and molecular mechanics and powerful computing technologies provides a robust framework for visualizing and understanding chemical reactivity and molecular recognition processes. This technology has been used in our laboratory to accelerate the discovery of efficient reagents for selective protein modification and understand and predict enzyme catalysis, as well as to provide insights into fundamental processes in glycochemistry.

In 2019 we have determined the most accessible and reactive residues for the conjugation of a small-molecule inhibitor of the aggregation of α-synuclein, a process associated with Parkinson's disease (PD), to a specific cysteine residue on human Hsp70[102], and for the chemo- and regioselective functionalization of albumin using lysine instead of the commonly used cysteine residues[103]. Using rigorous quantum mechanics, we have determined the molecular mechanisms operating at the chemically controlled in vitro activation of inactivated prodrugs[104], and the dual-labelling of proteins using novel sulfone-based reagents[105]. The combination of multiscale computational methods allowed us to discover a new reagent for the very efficient modification of proteins, including they global molecular charge, though cysteine residues[106]. We were also able to prepare and use, for the first time, chemical models of very reactive dehydroamino acids[107], and to provide insight into the structural basis for the activity of a protease enzyme involved in the biosynthesis of antimicrobial peptides[108]. We have also contributed to understand the role of ubiquitous protein-carbohydrate interactions in glycosylation reactions at the atomic level[109], and the subtle conformational properties of designed antigens for potential anticancer vaccines[110].

In the incoming future, we will continue developing new methods and reagents for the efficient modification of protein and therapeutic antibodies, with particular emphasis on microenvironmentally-controlled non-standard reactions avoiding the commonly used cysteine and lysine sidechains. In parallel, we will tackle one of the current challenges in the prediction of molecular recognition and in silico drug discovery, namely enthalpy-entropy compensation, with special focus on the binding of carbohydrates to lectins.

Immuno-Oncology Revisited

Asís Palazón, *Cancer Immunology and Immunotherapy Laboratory*

Our understanding of the biology of the immune system and specific features of the tumor microenvironment that dictate escape from immune surveillance has greatly expanded since the development of the first cancer immunotherapies. Regulatory approval of monoclonal antibodies against checkpoint inhibitors (PD-1/PD-L1 and CTLA-4) was a major breakthrough for the treatment of a variety of cancers, which resulted in intense pre-clinical and clinical exploration of novel targets and therapeutic combinations. However, the vast majority of strategies did not translate into significant patient benefit. A strong medical need still remains: to develop more efficacious and safer immunotherapies. The current main challenge is to discover novel targets and drug candidates to be advanced into the clinic, with the ultimate goal of having a positive impact on the survival of the patient.

In 2019, some relevant advances have been made in this direction. An important focus has been patients that do not benefit from established checkpoint receptor blockade, either because they are not eligible for this therapy or because they relapse. In this context, Siglec-15 has been identified as a target expressed by tumor and myeloid cells under conditions that usually don't favor PD-L1 expression[111]. Siglec-15 binds to a yet unidentified receptor expressed by T cells, resulting in immunosuppression. When this interaction is blocked by a novel monoclonal antibody, the adaptive immune system is reinvigorated, resulting in pre-clinical efficacy in cancer[112]. As a result, a Phase 1 clinical trial is ongoing to evaluate the tolerability of anti-Siglec-15 antibodies in different tumor types (lung cancer, melanoma, and others). Another emerging line of work with promising early clinical results during 2019 is focused on potentiating innate immune responses by blocking CD47, a molecule expressed by tumor cells that acts as a "don't eat me signal" against macrophages. When CD47 is blocked with a monoclonal antibody, tumor recognition and elimination by macrophages is stimulated in both solid and hematologic indications[113]. Apart from monoclonal antibodies, cell therapies constitute another tool to combat cancer. CAR-T cells are approved for the treatment of some hematological malignancies, specifically anti-CD19 CAR-T cells for the treatment of ALL, DLBC, and the main current challenge is to develop cell therapies for other cancer indications. In this direction, this year we have seen encouraging clinical results of different cell therapy drug candidates targeting BCMA for the treatment of advanced multiple myeloma[114], and basic research advances that could support the development of CAR-T therapies into solid tumor indications[115, 116].

Finally, this year's Nobel Prize in Physiology or Medicine has been awarded to the discovery of the hypoxia pathway (Semenza, Kaelin, Ratcliffe)[117] (*See Editorial*). This recognition is timely for the field of cancer immunotherapy, because tumors are known to be hypoxic as a result of aberrant vascularization and altered nutrient availability. Oxygen shortage directly influences immune responses, offering an additional pathway for the development of novel immunotherapies.

Vaccines and Elucidation of their Molecular Mechanisms of Action

Alberto Fernández-Tejada, *Chemical Immunobiology Laboratory*

Modern vaccine strategies based on well-defined carbohydrate, glycopeptide and peptide fragments of a pathogen, but especially those overexpressed in cancer cells, have been hampered by the poor immunogenicity of these substructures. To overcome this challenge, the inclusion of an immunological adjuvant that potentiates the immune response is required[118]. The design, synthesis and evaluation of vaccine candidates in which the antigen and adjuvant components are together in a single molecule ("self-adjuvanting") is becoming a promising approach for a safer and more precise targeting of the immune system against important diseases, like cancer. However, elicitation of both humoral and cellular immunity with such synthetic vaccines has been challenging, and no such antitumor carbohydrate-based vaccine has reached clinical approval so far. In this context, our research has a double and

ultimate goal based on applying chemistry to generate novel, improved adjuvants and innovative subunit vaccine approaches for optimal vaccine efficacy, as well as to gain molecular-level insights into the mechanisms of immune-activation of these constructs.

Following last year approval of QS-21 by the US FDA as part of the AS01 adjuvant system in GlaxoSmithKline's shingles vaccine, a recent publication in the New England Journal of Medicine has reported successful results from the latest clinical trial of a M72/AS01 vaccine against tuberculosis[119]. Moreover, two interesting review articles about the mechanisms of action of adjuvants and saponin-derived adjuvants have been published this year[120]. These reports have yielded further insights into the mechanisms of QS-21 adjuvanticity; however, the mechanistic role of QS-21 is still not fully elucidated at the molecular level. Additionally, a review article has been published summarizing the current advances in phytochemical and pharmacological knowledge of saponins from two different Quillaja trees[121]. Notably, in our continued efforts to develop improved, synthetic saponin adjuvants, we have recently published a multidisciplinary study combining chemistry, immunology and conformational analysis using NMR and molecular dynamics simulations. This work has provided expedient synthetic access to streamlined adjuvant-active saponins and molecular-level insights into saponin conformation that correlated with in vivo adjuvant activities[122].

Recent advances on peptide and carbohydrate subunit vaccines as well as particulate adjuvants have also been discussed in three comprehensive review articles appearing this year[123, 124]. Other important studies on the topic that have been published in 2019 include studies on new designs of mucin-based cancer vaccines obtained by attachment of synthetic MUC1-derived glycopeptide antigens to various carriers, such as gold nanoparticles[125] and virus-like particles[126]. Moreover, novel adjuvant platforms and subunit vaccine structures based on Toll-like receptor (TLR) ligands have been recently developed, such as a series of linked TLR triagonist adjuvants[127, 128] and a self-adjuvanting vaccine against HER2 that includes a TLR1/2 agonist (Pam3CSK4) as a built-in adjuvant[129].

Going forward, 2020 is expected to bring outstanding progress in the field, including the development of novel chemical tools to design more effective vaccines. In particular, our current work is focused on the development of new streamlined saponin probes for molecular level mechanistic investigations as well as novel chemical approaches towards synthetic saponin-based self-adjuvanting vaccines.

Bacteria, Archaea and Eukarya

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 Laboratory 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 Point of Care technologies and antiviral therapies constantly challenge our society.

The field of Structural Virology has seen pivotal advances on several fronts in tackling human and animal health threats such as Influenza (infecting humans) and African Swine Fever (infecting pigs) viruses. While for the former virus the quest of a pan-vaccine is undergoing for the latter no licensed vaccine is available. Both viruses cause – apart from the tragic loss of human and animal lives – a huge economical cost to society. Fundamental research has been shown to be essential for progressing in the elucidation of their corresponding life cycles[130,131,132,133,134]. It is worth mentioning that our group is also involved in studying virus members of animal and human threatening Bunyavirus and Flavivirus families. Viruses are also 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. To this end, our Chapter (6) represents a strong contribution in the understanding how the 3D structure can steer evolution. Further, discovering new viruses or engineering new ones for biotech applications as in the case of adeno-associate virus (AAV)[135] can be considered an outstanding scientific advance.

Technical advances in our field of methodological expertise expected in 2020 will mainly concern:

- HR cryo-EM: it will develop even further. Smaller molecules (~50 kDa) and faster data collection with K3 and Falcon IV direct detection cameras (a current reality) will render EM, the technique of choice to determine not only the 3D structure of a wide size range of macromolecules (including the attached carbohydrate) but also the conformational dynamic of such proteins. Pharma companies are heavily involved in drug screening programmes using cryo-EM and cryo-EM will be used in the clinical area.
- FIB/cryo-ET, 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.

Scientific and social opportunities relate also to geographic/governments' interest, outbreaks around the world and possibility of virus spread to Western countries.

Visualizing Antibiotic Targets on the Ribosome

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

Structural information generated by X-ray crystallography and cryo-electron microscopy is used to understand how drugs modulate biological pathways to improve human health leading to the development of new therapeutic strategies. Our group uses these methods to understand molecular events that drive ribosomal subunit biogenesis and understand the mechanism of action of antibiotics and natural inhibitors of protein synthesis. The underpinning theme is to contribute a structural understanding of reaction mechanisms to answer not only essential biological questions but also to combat antibiotic resistance which is one of greatest problems facing mankind.

The World Health Organization has declared antimicrobial resistance a "serious threat that is no longer a prediction for the future, it is happening right now in every region of the world and has the potential to affect anyone, of any age, in any country" (WHO). Overcoming the threat posed by increasing antimicrobial resistance hinges, for example, on our ability to (1) develop more antimicrobial agents that are more effective than their predecessor by derivatizing parental compounds or (2) discover novel targets that are not yet subject to main stream resistance mechanisms. With respect to the first avenue our group has used structural biology approaches to understand molecular recognition principles of several classes of well-known ribosome targeting inhibitors among which telithromycin[136]; dalfopristin and quinupristin[137]; edeine and pactamycin[138]; kasugamycin[139]; linezolid[140]; thiostrepton and micrococcin[141], and, more recently tygacil[142]; Hygromycin A[143] and GE81112[144,145]. In the last year

we have extended this work to look at novel tetracycline derivatives to understand how modifications in their unique C9 extension in the naphthacene core combats the growing problem of antibiotic resistance, namely this extension leads to a distinctive interaction pattern with the 30S subunit allowing it to escape several common resistance mechanism. These studies further expand our understanding of the interaction potential of the primary tetracycline binding pocket allowing for the further derivatization of the tetracycline antibiotic class. Additionally, our group is actively identifying novel antibiotic targets by studying functional ribosomal complexes, such as those involved ribosome biogenesis or initiation of protein synthesis. Within the last year we have further studied the structure of additional ribosome assembly factors that are important for bacterial pathogenesis and therefore represent interesting anti-microbial targets in that they allow one to target specifically disease causing bacteria thus limiting the potential spread of antimicrobial resistance.

In the next year we will provide further insights in the molecular mechanism used by antibiotic to target and inhibit the ribosome as we generate additional ribosome-antibiotic structures and expand our current methodology to include antibiotic stalled ribosomal complexes using versatile structural biology methods like cryo-EM.

3D cryo-Electron Microscopy of Flexible Filamentous Plant Viruses

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

We use cryo-electron microscopy (cryoEM) to explore the structure and to understand the functioning of several biological complexes such as ribosomes and filament plant viruses. In the field of flexible filamentous plant viruses we did contribute with the structure of two viruses from different families. In the last year we have also explored the structure of Viral-like Particles (VLPs) from one representative. In this regard, a recent publication has presented the structure of VLPs from another species, Potato Virus Y (PVY)[146].The VLPs from PVY are different from the previously described ones (including our own structure). It is a filament of stacked rings that allow for high resolution in cryoEM. This sample is very well suited for structural characterization of the interaction of phytosanitary compounds with antiviral properties, a line of research that we are also carrying out. With this in mind, we have already produced these same VLPs from PVY for the next step in our research.

On the other hand, we are lagging in the advance of cryoEM, where our center is acquiring a direct detection camera that will considerably improve the performance (attainable resolution) of our microscope. Also, a new high resolution equipment is being installed in Madrid, at the National Center for Biotechnology (CNB-CSIC). This new equipment is a CRYO ARM 300 TEM (JEOL), and we will be able to access the equipment and collect data with atomic resolution potential.

The Myelin Matters

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 therapeutical strategies for various nerves disorders with a special emphasis on the exploitation of our scientific discoveries. A newly discovered cell type for pain perception. Pain has been thought to be initiated by activation of free nerve endings without end organs in the skin. In contrast to this paradigm, Abdo et al. [147] discovered a previously unknown mesh-like organ covering the skin that senses dangerous environmental stimuli. This organ is built from specialized glial cells, called nociceptive Schwann cells, located in the epidermal-dermal border and is sufficient and required for initiation of mechanical pain transduction. This discovery may offer new insights into future treatments for chronic pain.

Altered human oligodendrocyte heterogeneity in multiple sclerosis. Multiple sclerosis (MS) is clinically the most important disease affecting myelin, but its underlying cause is not well understood. Two recent studies, using 1) 14C-based birth-dating technique[148] and 2) single-cell transcriptomics[149] reveal an unexpected heterogeneity of oligodendrocytes throughout the MS-affected brain and raise questions about the role of oligodendrocyte precursor cells in permanent lesion repair. These two papers have implications for MS as a 'global' brain disease, the requirement of OPCs for lesion repair and the possible contribution of mature oligodendrocytes in this process. While these studies suggest that remyelination of brains is a more challenging task than previously thought, conceptually they are major step forward.

Identification of novel therapeutically targets for myelin disorders would be the stand-out scientific development in 2020, 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). The advent of single cells transcriptomics and epigenomics will also contribute greatly to the delineation of precise molecular mechanisms in specific cell types in a number of different diseases settings in the peripheral and central nervous systems.

Transmissible Spongiform Encephalopathies

Joaquín Castilla, *Prion Research Laboratory*

Transmissible spongiform encephalopathies (TSE) are lethal neurodegenerative disorders caused by prions, aberrantly misfolded isoform (PrPSc) of the prion protein (PrPC). The physiological roles of PrPC are not fully identified and neither the misfolding itself at molecular level nor the neurotoxicity pathway are known and cannot be fully understood until the 3D structure of PrPSc is solved, which is the main challenge of the field. 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. The description in Europe of the first cases of prion disease in cervids (CWD) has become a great public safety issue. Finally, the awareness that other protein misfolding-related neurodegenerative disorders are caused by misfolding-prone proteins such as α-synuclein or Aβ peptide, that share molecular mechanisms with prions, have prompted the application of several techniques originally developed for TSE-causing prions to much more prevalent disorders such as Alzheimer's or Parkinson's diseases.

In 2019, several studies have been published trying to further characterize the pathology and understand the factors governing the dissimilar incubation periods between individuals[150, 151] or the influence of mechanisms governing

protein homeostasis in the pathology of prion disorders[152]. During this year, the use of in vitro prion propagation systems has further extended for mechanistic studies helping to understand how cofactors modulate prion aggregation, define specific infectivity and strain properties or the structural determinants of fibril diversity[153, 154, 155]. Moreover, in vitro prion amplification assays have been widely implemented in prion detection in animals[156, 157]and humans, searching to distinguish different disease subtypes[158], increasing sensitivity and specificity[159] or using new body fluids for diagnosis[160]. These methods and know how have been applied to other neurodegenerative diseases and based on prion disease biomarkers, similar ones have been sought in Parkinson's disease[161]. Finally, prion detection systems in vitro have been dedicated also to risk assessment, using them to screen for prion contamination in human cell lines used to produce biological therapeutics[162]. Other advances in the field during 2019 include the development of new models of prion infection, such as the human cerebral organoids[163] and the identification of new anti-prion compounds that act through the reduction of PrPC levels[164,165]. Finally, the 3D structure of prions continues being unknown but advances have been done with other amyloids with prion behavior and that share also the interspecies transmissibility, fibril structures form systemic AA-amyloidosis have been unraveled by cyro-EM[166], indicating the suitability of this technique for prion structure resolution.

2020 is expected to bring further advances on 3 topics mainly regarding TSEs and prions and other prion-like diseases. First, initial results from the first antibody therapy administered to six patients in UK will be published, indicating the expected efficiency of this strategy. Advances are also expected in the antisense therapies published this year aiming to reduce PrPC levels. Second, diagnosis based on the detection of minute amounts of misfolded proteins in easily accessible tissues and body fluids will be finally developed for its implementation in the clinical practice and this development will likely influence the early diagnosis of diseases like Parkinson's and Alzheimer's based on misfolded species of α-synuclein and tau respectively. Finally, new recombinant prions generated in vitro, more similar to brain-derived prions, propagation systems with much higher yields and the increased control of in vitro generated prion strains together with high resolution biophysical techniques such as ssNMR or cryoEM could finally allow the deciphering of a bona fide prion structure at atomic resolution.

The Circular Economy of Endosomes

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 2019, according to PubMed, is 81. Some outstanding publications include:

1) A work provides evidence that retromer and Snx27 are important for the trafficking of the core planar polarity proteins Fmi and Stbm in Drosophila. This is the first demonstration of a functional coupling between the core planar polarity cargo and endosomal recycling. This connection might have implications which with the ethology of Robinow syndrome, a severe skeletal dysplasia[167]. Rodriguez-Forlan reports the identification of the small synthetic molecule Endosidin17, which interferes with synthetic, endocytic, and autophagic traffic by impairing the fusion of late endosome compartments with the vacuole membrane to deliver cargoes. The mechanism involves the interaction of Endosidin17 with the VPS35 subunit of retromer thereby preventing its normal interaction with RABG3f, the human RAB7 homolog. This work highlights an important checkpoint in the control of cargo traffic toward the vacuole[168].

3) Simonetti uncovers a new mechanism for cargo recognition through the association with the phox homology domains of SNX5 and SNX6. The recognition involves a bipartite sorting motif which folds into a 'promiscuous' β-hairpin structure that binds to the PX domain by β-strand augmentation. The binding mode is similar to that of the C. trachomatis effector protein IncE to promote pathogen survival and replication. The bipartite motif is present in over sixty cargos that are sorted to the plasma membrane or the trans-Golgi network[169].

2) Another work provides evidence of SNX27 as an interactor of the deubiquitinase OTULIN, an essential negative regulator of inflammation and autoimmunity. The work revealed an unexpected role of OTULIN in the regulation of endosome-to-plasma membrane recycling. In this sense, OTULIN is not a trafficking cargo, but counteracts SNX27 recruitment to typical cargos and retromer, thereby preventing assembly of a functional SNX27-retromer complex[170].

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.

New Perspective on Extracellular Vesicles and Exosomes

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.

Each year, more than 2000 articles in the field of extracellular vesicles are reported covering many aspects of the biology of these vesicles from the isolation and phenotyping to

their implications in the development of different diseases. As expected, advances in the field of phenotyping methodologies for single vesicles are gaining strength. During this year, several studies have characterized exosomes via Raman tweezers microspectroscopy[171] and surface enhanced Raman spectroscopy for the early detection of different pathologies such as pancreatic cancer[172], diabetes[173] or for the stratification of Parkinson's patients[174]. In addition, optimizations of imaging flow cytometry for the analysis of single EVs have been perform[175]. Based on the previous study, many researchers will be able to employ high-resolution flow cytometry, which is a sensitive and robust technique, to detect and quantify single sEVs in different experimental contexts. Moreover, CRISPR technology is being used not only for identifying genes involved in EVs biology[176], but also for studying EVs as CRISPR/cas9 machinery carriers to recipient cells, which could be used in the future for targeted gene editing therapies[177]. Apart from techniques, EVs field has increased its knowledge in the field of different diseases. The application of EVs, isolated from stem cells, as cell-free therapy has emerged as a promising therapeutic strategy in regenerative medicine[178, 179, 180]. EVs have been a new tool for research in neurodegenerative medicine, specially in Alzheimer disease, being used for reveal long needed biomarkers and even to constitute a novel tool for drug delivery[181, 182]. Finally, metabolomics is a nascent field in relationship with EVs but has been seen that could be a great potential[183, 184].

The International Society for Extracellular Vesicles (ISEV) had published a comprehensive guide of all resources and knowledge available in the EV field community[185]. But this year has been published in *Cell* journal an article[186] that has generated much controversy, since it rejected some characteristics already described about EVs. This show that the field of EVs is a field that is developing and still needs a lot of research. The perspective for the next year in the field is that drug delivery system via EVs will progress substantially the following years; the improvements in the encapsulation efficiency of desire cargo will allow the use of EVs-based therapeutics.

Precision Medicine & Pharmacological Chaperones

José M Mato and Óscar Millet, *Precision Medicine and Metabolism Laboratory*

Metabolism is the sum of all the chemical reactions that, catalyzed by enzymes, provide the necessary energy to carry out the vital processes of an organism (movement, growth, differentiation, ...) as well as to synthesize and degrade the components of which they are made (mainly lipids, proteins, carbohydrates and nucleic acids). More than 18,000 enzymatic reactions integrated inside thousands of metabolic pathways, through which circa of 100,000 metabolites flow continuously, encompass the human metabolome. The challenge of interpreting and predicting the overall metabolic response of an organism to nutritional changes, a common disease, or a treatment, remains extraordinarily complex and has not been yet achieved. In our laboratory we are generally interested in the relationship between metabolism and disease, including therapeutic agents and focusing in liver disease and selected rare diseases[187].

As an example of it, one of the current projects in our laboratory is to develop a set of techniques, mainly based in NMR spectrometry, which would allow the simultaneous measurement of dozens of phosphorylated metabolites and metabolic drivers and integrate these data into a topological metabolic network; that is, a graphic representation that, in a visual, quantitative and precise manner, could answer the question of what key metabolic alterations occur in response to a physio(patho)logical change or treatment. This approach should allow obtaining a snapshot of the metabolic landscape associated with a disease and its treatment, or identify synergistic combinations of drugs and explore their mechanisms of action to promote clinical studies that lead to replace current standard treatments with more effective and precise therapies with fewer side effects (personalized or precision medicine).

Precision medicine refers to the customization of healthcare with medical decisions, treatments, practices, or products being tailored to the individual patient. Based on data integrated from existing sources, adding genomics, proteomics and metabolomics enables a holistic understanding of the individual. In this context, we currently focus on the problem of obtaining personalized molecular data, mostly at the metabolic level[188]. 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. We have launched Akribea, an ambitious project of precision medicine in the Basque Country, where 10.000 individuals from the working feed samples to be analyzed and to provide an integrative view of such population.

Finally, identifying at a molecular level the mechanism of disease paves the way for therapy. 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 company ATLAS molecular pharma, a spin-off company partially originated from the research in our group is currently undertaking the preparation and development of the clinical phase trials (open label combined phase I/II on patients) for a repurposed drug to be active against congenital erythropoietic porphyria[189, 190].

TECHNOLOGIES

Proteomics landscape: facing analytical challenges by the hand of technological improvements

Felix Elortza, *Proteomics Platform*

After two decades of constant evolution, proteomics can be nowadays truly considered as a high-throughput technique. Latest advances in shot-gun proteomics enable now the identification and quantification of thousands of proteins in fairly routine analyses. Being the proteome a dynamic subject, this approach allows us to get deeper insights into what molecular changes are involved in the biological system we want to study; and in an unbiased manner. Nevertheless, the complexity of the proteome is truly enormous since we already know that in eukaryotic cells proteins can suffer many post-translational modifications such as phosphorylation, acetylation, methylation, glycosylation etc., and they are not exclusive among them[191]. We recognize now that biology uses all these protein decorations to modulate protein activity in diverse ways. Moreover, proteins may work alone or in complexes and location within the cell can also be dynamic. All these events leave us a complex cellular picture to decipher.

It is nowadays accepted that health is a combination of genetics, lifestyle and environmental factors. Precision medicine is the way medicine uses patients' multi –omics information to uncover clues for treating the disease. In this regard, proteomics is getting to the position to face the challenge where thousands of analyses needed have to be accomplished in high throughput manner[192]. From a clinical perspective, early diagnosis is of paramount importance. There is a tremendous interest in liquid biopsy applications within both genomics and proteomics, and blood is a very appealing sample source for clinical testing. It is both easily accessible and potentially highly informative as it contains molecules that could provide insights into all the organs and biological systems it interacts with as it moves throughout the body. However, it is a very challenging sample to work with, especially in the case of molecules like proteins, for which, unlike nucleic acids, there is no method of amplification. This means that any essay must be able to measure proteins in plasma at their endogenous concentrations, a task that is made even more difficult by the plasma proteome's high dynamic range, which can span 10 orders of magnitude or more.

Above mentioned basic research and clinical challenges need best possible analytical tools. Orbitrap type of mass spectrometers have been for over a decade the unbeaten horses in proteomics analytical race since they were launched at the beginning of this century. Lately, the burst in of a quadrupole time of flight based instrument in which an ion mobility device was implemented, has happened to be a technological advance to consider. This cutting-edge mass spectrometer recently installed at Proteomics Platform is named TIMS TOF Pro (Bruker) and is powered by PASEF (Parallel Accumulation Serial Fragmentation). Thanks to PASEF, parent and fragment spectra can be aligned by mobility values achieving >100 Hz sequencing speed. This allows an unprecedented speed in data acquisition together with a very high sensitivity, enabling high performance

proteomic analyses[193]. Besides, this new type of super-fast mass spectrometers together with a new generation of nano-scale chromatographic systems like EVOSEP, are getting proteomics closer than ever to clinical applications[194]. Another promising branch of proteomics is the "tissue MALDI imaging", also known as molecular histology, where proteomics analyses of small proteins or peptides are performed by MALDI TOF on the same tissue. This approach allows pinpointing the localization of observed analytes on studied tissue. The obtained information is being considered as complementary to well stablished techniques like immuno-histochemistry, and may be helpful in clinical practice such as anatomo-pathology.

In next years, we hope that these new applications and instruments shall help paving the way for a deeper understanding of basic molecular biology and precision medicine.

Anticipating the Needs for Genomic Technologies in Biomedical Research

Ana M Aransay, *Genome Analysis Platform*

During the last 20 years, the technology in the field of genomics has advanced vertiginously. Since the development of the polymerase chain reaction (PCR) and automated DNA Sanger sequencing methodologies in the 1980s, the efficient characterization and quantification of DNA and RNA have been major concerns for the biotech companies. As a result, hundreds of genomics strategies have been developed, mainly array and high-throughput sequencing technologies, together with the corresponding panoply of machines and protocols. These techniques allow to search for events such as DNA sequence variants, differential gene expression, epigenetic modifications, chromatin structure or differential microbiome that might be the cause of disease, antibiotic resistance, cancer susceptibility, etc., and therefore, to design among others tools for precision medicine.

The Genome Analysis Platform at CIC bioGUNE is responsible for setting up genomic techniques to aid researchers with all the approaches mentioned above, principally for biomedical investigations, but applicable to many other fields as agronomic improvement. At present, we are able to assist with specific experimental designs and to carry out a total of 77 methods, from low-throughput quantitative PCRs to high-throughput complex sequencing protocols. In addition, we develop basic dry-lab pipelines that help analyzing big data obtained from high-throughput sequencing strategies. In 2019, we have fulfilled a total of 97 projects, being some of them fruitful collaborations using assay for transposase-accessible chromatin sequencing (ATACseq), smallRNAseq, microRNAseq, mRNAseq and metagenomics, that have yielded important public reports in inflammatory disorders[195], glioma[196], central nervous system malignancies and fibromyalgia[197, 198, 199].

Thanks to recent advances in single cell genomic characterizations, several companies are developing exclusive methods and machines, especially for transcriptome and chromatin structure at cell level. Furthermore, we are very interested in the newly designed spatial (multidimensional) transcriptomics strategy that allows describing in-situ mRNA expression of cells in their morphological context. During 2020, the Genome Analysis Platform will select the techniques that best fit for CIC bioGUNE and collaborator's single cell genomic research. Subsequently, we will focus on setting up protocols, first, to obtain parallel catalogues of expressed mRNAs (mRNAseq) and open chromatin regions (ATACseq) for each individual studied cell; next, to additionally classify the studied cells based on their protein contents; and, after, for spatial transcriptomics. Single cell and spatial findings will reveal how complex and rare cell populations interact in healthy and diseased tissues or organs, and thus, will help understanding clue functional regulations and cell communications that cause diseases. Crossing genomics data with proteomics, metabolomics and clinical information by means of system biology algorithms will grant to identify secure sets of biomarkers for precision medicine.

Nuclear Magnetic Resonance

Tammo Diercks, *NMR Platform*

NMR metabolomics is gaining strategic importance worldwide, yet only a few notable NMR methodological advances were recently proposed, e.g. the addition of paramagnetic co-solutes to speed up quantifiable NMR measurements[200] and 1H homodecoupling for spectral simplification and resolution enhancement[201]. Tracer based metabolic NMR studies using 13C[202] or 15N[203] labeled precursors reappear for analysing the metabolism, e.g., in mammalian cell lines.

In-cell NMR, where isotopically labeled tracers or proteins are selectively observed inside cells, is a related emerging application which was recently adopted to detect 13C,15N labeled ubiquitin[204], study the interaction of 15N labeled α-synuclein with chaperones[205], and monitor the metabolism of live mitochondria via the processing of 13C labeled pyruvate[206]. An in-cell diffusion analysis was presented to discriminate unlabeled inter- and intracellular components[207], 13C labeled metabolites were observed and quantified by 2D perfect-HSQC[208], and methylcellulose hydrogel was introduced for NMR studies of extracellular protein binding to receptors on live cells[209].

NMR on large biopolymers, e.g. proteins and their complexes, faces high spectral overlap (poor signal dispersion) and fast signal relaxation. Stable isotope labeling addresses both challenges, where it was shown that stronger media buffering may double protein yields[210] while new selective labeling schemes were presented for sequential assignment[211], protein-ligand complex structure analysis[212], isoleucine 13CH3 labeling in eukaryotic cells[213], and selective HA back-protonation in perdeuterated sidechains[214]. Spectral overlap can then be resolved in multiple heteronuclear dimensions, which exponentially increases conventional NMR sampling times. This resolution limit is now dismantled by Non-Uniform Sampling (NUS) and new processing algorithms. Recently, the distribution of sampled data points was revised to reduce artefacts at very low sampling ratios[215], for aggregating proteins[216], and for homodecoupled pure-shift spectra[217] while new co-processing algorithms were developed for serial 2D NUS relaxation data[218]. Novel protein NMR experiments include a resolution enhanced NOESY[219] and 3D HMBC-HMQC for intraresidual methyl group correlation[220], while MethylFLYA was presented for automatic structure-based methyl signal assignment[221].

NMR on small molecules, e.g. carbohydrates, mainly faces slow polarisation recovery and spectral complexity from homonuclear 1H coupling. The former is greatly accelerated by the new ASAP and ALSOFAST experiment[222] while the latter is addressed by 'Pure Shift' NMR techniques, as recently implemented in DOSY experiments[223, 224].

In 2020 we expect that two hardware advances will drive NMR methods development: the 1.2 GHz magnet and NEO spectrometer with multiple receivers. The former requires new schemes for broadband excitation, decoupling, and Hartmann-Hahn mixing, probably designed with Optimal Control algorithms[225], while the latter enables parallelised NMR experiments to exploit more polarisation especially for small molecules[226].

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