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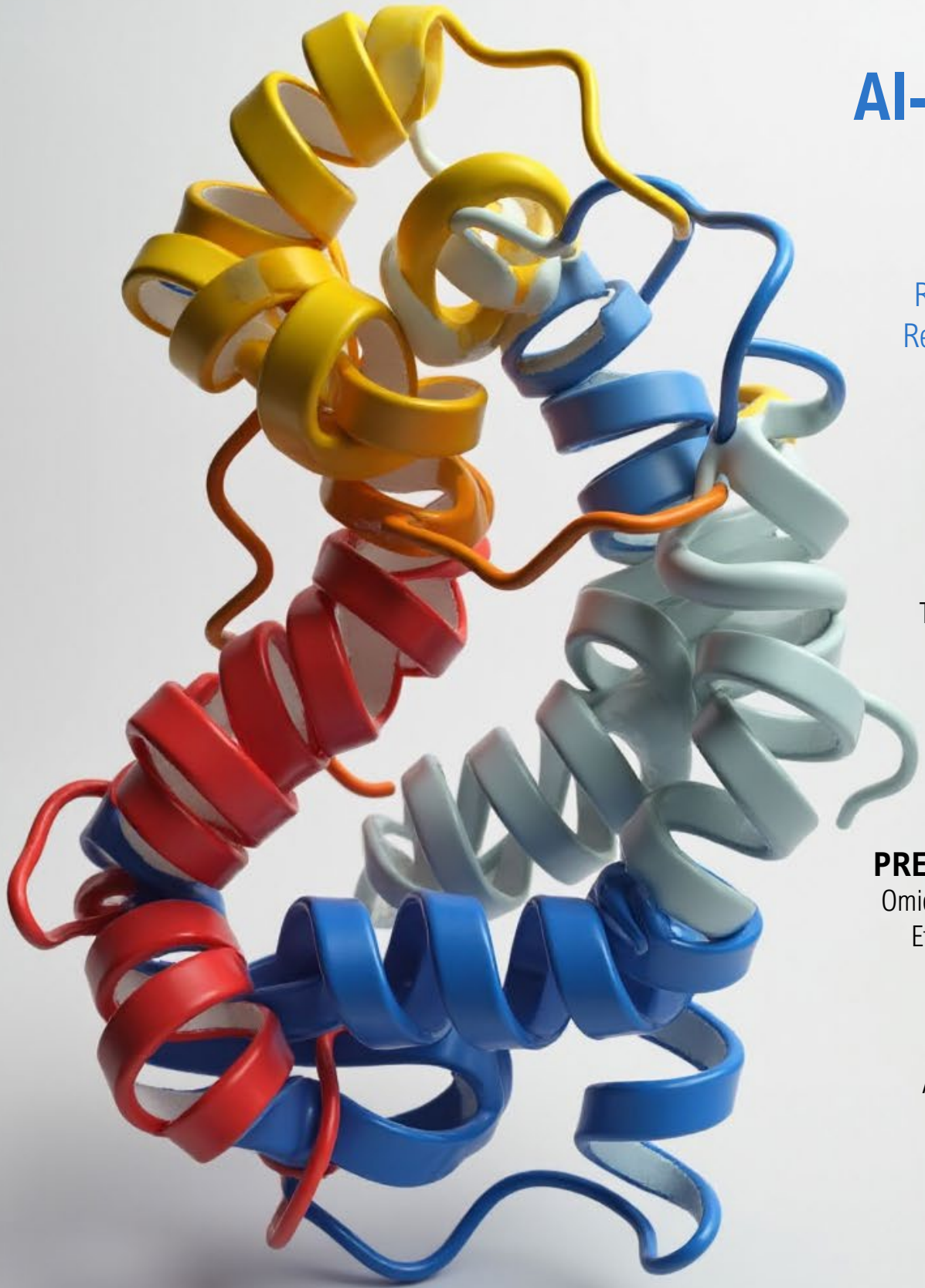
A collection of scientific advances in the research lines of CIC bioGUNE



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CIC bioGUNE
MEMBER OF BASQUE RESEARCH & TECHNOLOGY ALLIANCE

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The Center

CIC bioGUNE is a multidisciplinary research center dedicated to advancing Life Sciences, bridging the fields of Chemistry and Biomedicine, and spanning the spectrum from fundamental to translational science. Our mission centers on uncovering the molecular mechanisms underlying diseases, aiming to drive the development of innovative therapies. Research at CIC bioGUNE is focused on four key areas of biomedicine: Cancer, Metabolic Diseases, Rare Diseases, and Infectious Diseases. These efforts are structured into two core programs: "Metabolism and Cell Signaling in Disease" and "Molecular Recognition and Host-Pathogen Interactions."

Recognized for its scientific excellence, CIC bioGUNE has been awarded the prestigious Severo Ochoa Seal of Excellence by the Spanish Ministry of Science and has received special acknowledgment from the Spanish Association Against Cancer (AECC) for its contributions to cancer research. Guided by a spirit of collaboration, CIC bioGUNE fosters partnerships with researchers, clinicians, and technology specialists at local, national, and international levels. The center is an active member of the Basque Research and Technology Alliance (BRTA) and operates within a diverse network of academic institutions, clinical organizations, and research and technology centers.

Our work is supported by state-of-the-art infrastructures and cutting-edge technological platforms. These include advanced facilities for nuclear magnetic resonance (NMR), designated as ICTS, electron microscopy, monoclonal antibody production integrated with our animal facility, and comprehensive core platforms for genome, proteome, and metabolome analysis.

General View

Direction

CIC bioGUNE's mission is to establish a leading European hub in biosciences, leveraging cutting-edge technologies to drive innovation in life sciences and health. By enhancing collaboration within the Basque Research and Technology Alliance (BRTA) and partnering with academic, social, and healthcare entities in the Basque Country, CIC bioGUNE optimizes resources to deliver a high-value scientific and technological offering that strengthens the region's biotech and pharmaceutical sectors.

Our research spans from gene studies to animal models, focusing on biomolecular structure, key mechanisms of health and disease, and their clinical applications. With a strong emphasis on precision medicine, CIC bioGUNE aims to unravel the molecular basis of immune defence, cell proliferation, and development to advance healthcare solutions.

Transformative Advances in Computational Protein Design: The 2024 Nobel Prize in Chemistry

Gonzalo Jiménez-Osés, *Computational Chemistry Laboratory*

Computational Protein Design (CPD) is changing how we address some of chemical biology's most pressing issues, from developing new medicines to creating sustainable industrial catalysts. In essence, CPD merges biology, chemistry, and advanced computational methods - particularly artificial intelligence (AI) - to predict and engineer protein structures with unprecedented accuracy. The potential impact is immense: improved vaccines, potent therapeutic nanobodies, and enzymes that accelerate green chemical reactions. In 2024, these possibilities were recognized at the highest level when the Nobel Prize in Chemistry was awarded to pioneers in the field (David Baker, John Jumper and Demis Hassabis), acknowledging how tools like AlphaFold, RoseTTAFold, RFDiffusion and ProteinMPNN have revolutionized our understanding of protein structures and their functions. David Baker's laboratory, long at the forefront of this revolution, has continued to push the boundaries with AI-based protein design methods, setting the stage for even more transformative breakthroughs worldwide in the years ahead.

The year 2024 witnessed the consolidation of AI-driven CPD as a mature, indispensable tool. AlphaFold 3, developed by DeepMind, represented a key milestone. Beyond the already remarkable predictive accuracy of AlphaFold 2, this new iteration introduced a diffusion-based architecture that enhances its ability to tackle posttranslational modifications such as glycosylation, intrinsically disordered regions, nucleic acids, ligands, cofactors and multiprotein complexes, providing detailed insights into challenging targets.[1] Moreover, acceleration in protein structure prediction and design was further amplified by the code release of AlphaFold 3, which democratized access to these cutting-edge capabilities.

Central to this year's advances is the pioneering work from David Baker's lab, which continues to produce landmark achievements in de novo protein design. Their integrative approaches, blending generative AI models with structural biology insights, have led to entirely new protein topologies and functional motifs.[2,3,4] Recent studies highlight how these methods not only predict protein folds but also create novel enzymes that outperform natural counterparts in catalyzing specific reactions, and nanobodies that bind to therapeutically relevant targets with exceptional affinity.[5]

A groundbreaking development in 2024 was the use of AI systems as "virtual scientists" for designing nanobodies - small antibody fragments with immense therapeutic potential. The Virtual Lab project integrated tools like AlphaFold, ESMFold, and Rosetta to design 92 nanobodies targeting SARS-CoV-2 variants.[6] Experimental validation revealed nanobodies with enhanced binding specificity and affinity, showcasing AI's ability to rapidly generate viable therapeutic candidates.

These advances highlight how AI is revolutionizing molecular design by automating complex tasks traditionally reliant on human expertise.

As we look toward 2025, multiple trends emerge. The closed-loop optimization cycle - AI-based design, rapid prototyping, and automated high-throughput validation - will continue to refine protein functions at an unprecedented pace, leveraging generative frameworks to produce ever more complex designs and moving beyond single proteins to modular assemblies and synthetic biosynthetic pathways. The stage is set for another year of extraordinary innovation, with CPD firmly at the heart of transformative biomedical and environmental solutions.

Revolutionizing Aging Research with Artificial Intelligence

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

The worldwide population is aging rapidly with more than two billion people projected to be over the age of 60 by 2050. However, the increase in people's lifespan is not necessarily accompanied by an increase in healthspan, resulting in more people living longer in poor health. Thus, it is imperative to investigate effective ways to increase the healthspan of an aging population. Slowing down or reverting the aging process by devising effective rejuvenation interventions constitutes a promising strategy to achieve this goal. In this regard, the rise of artificial intelligence (AI) in all areas of our life is prone to revolutionize aging research as well. Increasing efforts have been devoted to catalogue vast amounts of molecular and demographic data, which for the first time allowed the development of AI-based computational methodologies for the characterization of existing strategies as well as the discovery of novel rejuvenation interventions.

In the previous year, we have witnessed the emergence of groundbreaking AI-based strategies to identify novel target genes and drugs for rejuvenation. In this regard, a computational approach combining machine learning techniques and multiscale modeling to pinpoint target genes for mimicking complex interventions, including calorie restriction and exercising.[7] As a result, the AP-1 transcription factor complex has been found to mediate the rejuvenating effect of multiple interventions across a variety of cell types and tissues. Moreover, although the genes Jun and Fos emerge as common master regulators, the analysis suggests that cell type specific effects are exerted by distinct AP-1 dimers. In addition to target identification, two seminal studies have been published that employ AI-based computational methods to discover and design rejuvenating drugs. In the first case, the computational platform AgeXtend has been introduced that identifies geroprotectors based on bioactivity descriptors such as chemical structure, known target genes and clinical evaluation.[8] Using this platform, 1.1 billion chemical compounds were screened several of which show high geroprotective effects. In the second case, an AI-based target discovery method was combined with a generative chemistry approach to design molecules with desired properties.[9] Although a proof-of-principle study has been conducted in the context of idiopathic pulmonary fibrosis, its implications for the discovery of novel rejuvenation interventions are staggering. Considering that it took only 18 months from the initial design of a small molecule to the preclinical candidate nomination demonstrates that AI-based methodologies can significantly accelerate the design of novel drugs. Despite these promising approaches, an important aspect in the discovery of novel interventions is the evaluation of their rejuvenation effects. In this regard, a computer vision approach has been designed that can accurately capture different aspects of biological aging based on imaging data.[10] As such, this approach offers a cost-effective biomarker aging that can be employed in the future to assess the efficacy of rejuvenation interventions.

The remarkable developments in AI-based computational methods make leading a longer life in good health a tantalizing prospect. Nevertheless, many studies still disregard the possibility of inadvertently learning associations that can lead to disparities in the outcome of different groups of people.[11] We expect to see an upsurge in explainable AI in the coming years that allows interpreting the model's decision-making in the process of discovering and designing novel drugs for rejuvenation.

Resistance to Targeted Cancer Therapies: The Other Side of the Coin

Uxue Armendariz-Martínez, Samuel Pasco, Roos Vincken, and Ana Ruiz-Sáenz, *Cancer Therapy Resistance Laboratory*

Breast cancer (BC) is the most common type of cancer among women. Up to 30% of BC tumors classify as HER2-positive (HER2+), due to overexpression of the tyrosine kinase receptor HER2 driving tumor growth. Since its identification as a biomarker, HER2-targeted therapies revolutionized BC treatment for a cancer subtype previously associated with poor prognosis. Trastuzumab, the first humanized monoclonal antibody targeting HER2, marked a significant advancement in the treatment of this type of cancer. This therapeutic antibody binds the extracellular domain of the receptor inhibiting intracellular signaling pathways and eliciting antibody-dependent cell-mediated cytotoxicity. Subsequent developments, like pertuzumab, which prevents HER2 heterodimerization, and small molecule tyrosine inhibitors, both reversible (lapatinib) or irreversible (neratinib), further improved outcomes for HER2+ BC patients.[12] More recently, antibody-drug conjugates (ADCs) have emerged as a transformative strategy. These agents link trastuzumab to cytotoxic payloads like emtansine (T-DM1) or deruxtecan (T-DXd) to shuttle the chemotherapy agent directly to BC cells, achieving improved survival in metastatic patients.[13] However, despite these advancements, therapeutic resistance and subsequent patient relapse remains a challenge.

Research on HER2-targeted therapy resistance has moved beyond HER2 loss, mutations in HER2 signaling pathways, and activation of alternative signaling.[14] Some ADC payloads induce DNA alterations, prompting resistant cells to activate DNA repair pathways for survival. Consequently, the inhibition of these pathways can improve T-DXd efficacy.[15] Other factors such as HER2 heterogeneity[16] and the interaction with immune and stromal cells of the tumor microenvironment, can also contribute to resistance. For instance, cancer intrinsic factors such as metabolism affect immune synapse formation leading to resistance to trastuzumab-dependent cytotoxicity.[17] Furthermore, lower immune infiltration and a more immunosuppressive phenotype at baseline correlates with non-response to T-DM1.[18] Moreover, when HER2+ BC metastasizes to the brain, resident stromal cells induce the expression of several mucins and other glycoproteins, diminishing the response to neratinib.[19]

Future anti-HER2 strategies aim to overcome or prevent resistance by addressing different factors contributing to therapeutic response, particularly the extracellular component of breast cancer. The bulky layer of glycoproteins forming the glycocalyx hinders treatment efficacy, alters cell signaling, and facilitates immune evasion.[20] And in this context, strategies to modulate the glycocalyx present a promising avenue for enhancing the effectiveness of HER2-targeted therapies and preventing resistance.[21]

Other improvements to HER2-targeted therapies include bispecific multifunctional antibodies capable of stimulating T-cell mediated cytotoxicity, representing a cutting-edge approach against HER2+ cancers.[22] Similarly, expanding the application of the ADC, T-DXd, to HER2-low patients[23] opens a new avenue of research, enabling the exploration of novel mechanisms of action of HER2-targeting therapies through tumor heterogeneity and optimizing the use of these promising therapeutic agents. Finally, extending HER2-targeted therapies beyond BC[24] signifies a step forward to enhance therapeutic outcomes across other cancer types.

The Right Time with the Right Friends: Increasing Complexity in Cancer Management

Mikel Pujana, Jaione Auzmendi, Marco Piva, and Arkaitz Carracedo, *Cancer Cell Signaling and Metabolism Laboratory*

In 2024, cancer research brought renewed focus to two vital and interconnected fields of interest in our lab: cancer metabolism and the tumor microenvironment. Cancer is now seen as a highly adaptive ecosystem that reprograms both its metabolic landscape and immune interactions to ensure survival and progression. Metabolism, long viewed as a byproduct of rapid cell division, is now understood as a central driver of tumor growth, signaling, and adaptability. Similarly, breakthroughs in immunotherapy, from immune checkpoint inhibitors to CAR T-cell therapies, highlight the crucial role of the immune system in counteracting the expansion of cancer cells. However, challenges such as metabolic reprogramming, immune cell exhaustion, and the influence of external factors limit therapeutic success. These areas of research are essential to design strategies that disrupt the adaptability of cancer cells while enhancing immune efficacy.

Recent studies shed light on key discoveries in cancer metabolism and the tumor microenvironment. Research on the "resistance continuum" revealed that cancer cells adapt to therapy through non-genetic mechanisms like transcriptional reprogramming and metabolic shifts, creating intermediate states that eventually lead to drug resistance.[25] Targeting these early transitional phases could revolutionize therapeutic approaches, emphasizing the need to outpace cancer's adaptability.

Mitochondrial dynamics emerged as a critical area of focus. A study showed how mitochondria specialize under stress, dividing tasks between energy production and biosynthesis to sustain tumor survival.[26] While this flexibility supports cancer's resilience, it presents an opportunity for therapies to disrupt tumor metabolism. Another breakthrough revealed that metastatic cells retain the metabolic traits of their tissue of origin, limiting their adaptability in new environments.[27] Exploiting this metabolic rigidity could provide new avenues to inhibit metastasis.

On the intersection of nutrition and cancer, research highlighted the delicate balance of fasting and refeeding cycles. While refeeding boosts tissue repair and regenerative capacity, it also increases cancer risk through pathways like mTORC1 signaling.[28] This underscores the complexity of metabolic interventions and the need to balance benefits with potential risks.

In the realm of immunotherapy, circadian biology demonstrated its potential to optimize treatment. Timing therapies to align with T-cell rhythms improved immune responses in preclinical models, suggesting that treatment schedules could enhance efficacy.[29] Similarly, studies on mitochondrial transfer via nanotubes revealed how boosting T-cell metabolism could enhance immune cell performance and CAR T-cell therapy outcomes.[30]

Age-related changes in the hematopoietic system were also linked to cancer progression. Enhanced myelopoiesis in aging led to the accumulation of pro-inflammatory myeloid cells, promoting tumor growth. Blocking IL-1α signaling showed promise in reversing this effect, offering potential therapeutic strategies for older cancer patients.[31]

The impact of chronic stress on cancer metastasis was also unveiled. Stress-induced changes in neutrophils and the formation of extracellular traps (NETs) created a pro-metastatic microenvironment.[32] Targeting NET formation emerged as a

promising approach to mitigate stress-driven cancer progression.

Overall, these studies highlight the central role of metabolism and the immune tumor microenvironment in cancer evolution and therapy. Metabolism enables tumor cells to adapt, resist treatment, and survive under stress, but it also exposes vulnerabilities connected to energy pathways and tissue-specific constraints. Simultaneously, understanding the interactions between immune cell metabolism, circadian biology, and ageing is essential for developing personalized and effective therapies. Together, these works pave the way for developing novel therapeutic strategies that could balance targeting tumor vulnerabilities while avoiding adaptive resistance.

Glycans in Action

Ana Ardá, Ana Gimeno, Luca Unione, and Jesús Jiménez-Barbero, *Chemical Glycobiology Laboratory*

Glycans are everywhere. Glycans (carbohydrates, sugars, saccharides) are extensively presented in nature, in all living life forms, and involved in key processes related to health and disease. From the basic research perspective, we have contributed to the development of groundbreaking experimental advances for unraveling the mechanism of glycosylation[33] and for designing and generating novel carbohydrate scaffolds with precise spatial topologies,[34] and for monitoring glycan-lectin interactions within the cell and on the surface of the cell by using NMR methods.[35] Besides these fundamental breakthroughs, more and more evidence has been accumulated on the regulatory roles of lectin-glycan molecular recognition events in key biological functions. Focused on galectins, the chemical glycobiology group[36,37,38] and other labs worldwide[39,40,41,42,43] have provided novel discoveries in the field, which provide the basis for future translational applications. In a parallel manner, the scientific community continues to strive to decipher the mechanisms that regulate the complex process of cell and protein glycosylation. In this context, a recent study, utilizing neural networks, has uncovered correlations between specific protein sequences and glycosylation, leading to the development of an algorithm capable of predicting most human glycosylation events documented in databases.[44] Other studies have focused on specific glycan signatures, such as the presence of high-mannose N-glycans, where new regulatory genes have been disclosed,[45] or N-glycan branching, where a self-regulatory mechanism has been proposed for the responsible glycoenzymes.[46] At the same time, the tools to advance in our understanding of the roles of glycans in Nature continue to evolve. As expected, rapid advancements in genetic engineering tools are also making a significant impact on glycosciences. An interesting example is the proposal to leverage glycoengineered HEK293 and CHO cell cultures - commonly used for protein overexpression - for the sustainable and cost-effective production of glycans in formats and quantities that allow structural studies of molecular interactions and use of glycans in biomedical applications, by designing Glycocarriers that incorporate the desired glycan when expressed in the aforementioned cell lines.[47] In the field of molecular recognition, an impressive library of 150 site-specifically fluorinated LewisX analogues ('glycofluoroforms') was developed using efficient enzymatic modular assembly from ^{19}F-monosaccharides as building blocks. This library enabled the creation of distinct binding fingerprints for lectins with overlapping LewisX binding specificities.[48] This promising strategy could be harnessed to address glycan-binding promiscuity in lectins, paving the way for diverse biotechnological and biomedical applications.

Glycans are also involved in infection processes. Since January 2022, the highly pathogenic avian Influenza H5N1 virus clade 2.3.4.4b has caused millions of deaths in domestic birds. Given the remarkable ability of the virus to cross species barriers, robust measures to prevent and control infection and further spread in mammals are needed. This would reduce the risk of virus adaptation in mammalian host species, thereby decreasing the pandemic risk to humans. A recent study[49] has described the spillover of a new reassorting of this virus (genotype B3.13) into dairy cattle and provided evidence of efficient transmission among cattle and other mammalian species. The tropism of the H5N1 virus for the cattle mammary gland tissues is consistent with the high expression of sialic acid receptors with an α2,3 (avian-like receptor) galactose linkage in milk-secreting epithelial cells. Yet, those cells also abundantly express sialic acid receptors with an α2,6 (human-like receptor) galactose linkage. These data highlight the zoonotic potential for bovine-to-human or even human-to-human transmission. Consistently, in early 2024, the first human-infecting bovine H5N1 case was reported in Texas. In October 2024, the US Centers for Disease Control and Prevention (CDC) reported 15 human infections worldwide. Several studies from the past decades have shown how punctual mutations in avian viral hemagglutinin (HA) can promote binding to human receptors. In that sense, in 2024, in collaboration with international leaders, we have contributed to this field by providing atomic-level structural studies about the adaptation of HA proteins from the H3N2 avian and human viruses to extended glycan receptors that abundantly decorate the human respiratory tract tissues.[50,51] Also, this year, a seminal work by Wilson and Paulson[52] has demonstrated that a single Texas H5 HA Gln226Leu mutation can switch receptor specificity from avian-like to human-like glycan receptors. Thus, it is important to continue to monitor for signs of such change in the currently circulating viruses, as well as to advance in the field of HA inhibitors. In that sense, the development of small-molecule inhibitors targeting influenza hemagglutinin by using Sulfur-Fluoride Exchange (SuFEx) click chemistry–based high-throughput medicinal chemistry (HTMC) strategy has been just presented.[53] The design led to the identification and validation of ultrapotent influenza fusion inhibitors with subnanomolar EC50 cellular antiviral activity against several influenza A group 1 strains.

Improving Cancer Immunotherapy by Targeting Glycoimmune Checkpoint Receptors

June Ereño-Orbea and Asís Palazón, *Cancer Glycoimmunology Laboratory*

The year 2024 has been marked by significant advances in the exploration of alternative immune checkpoints for cancer immunotherapy, with a focus on the potential of glycans as an emerging target class. More than 50% of cancer patients fail to respond or are not eligible for therapies with classic immune checkpoint inhibitors (CTLA-4 and PD-1/PD-L1), resulting in an unmet clinical need. Glycans and their glycan-binding proteins can act as immune checkpoints, promoting immune suppression and reshaping the tumor microenvironment (TME). In this context, the upregulation of sialic acid glycans has been associated with immune evasion in cancer patients, and hypersialylation of tumor and immune cells inhibit anti-tumor immune responses. The Siglec family, which binds to sialic acids, plays a pivotal role in mediating this suppression.

Recent research from Prof. Wu's group (Scripps Research Institute) has provided breakthrough insights into the impact of altered sialylation and Siglec expression on cancer progression. Notably, Siglecs-7 and -9, constitutively expressed on myeloid cells, act as inhibitory modulators of cytokine production during inflammation. In 2024, Prof. Wu's team demonstrated that T cells acquire Siglec-7/9 from neighboring myeloid cells through trogocytosis - a novel mechanism for Siglec receptor acquisition.[54] This process results in impaired T cell

effector functions and reduced anti-tumor responses, with Siglec-7/9+CD8+ tumor-infiltrating lymphocytes present diminished cytotoxic activity compared to their Siglec-7/9−negative counterparts. These findings underscore the importance of exogenous checkpoints acquired within the TME and their implications for designing effective checkpoint blockade therapies. A novel Siglec-7/9 degrader developed by this laboratory targets these receptors for lysosomal degradation.[55] This degrader selectively enhances T cell anti-tumor immunity and provides a new therapeutic approach.

In parallel, innovative platforms targeting the Siglec-sialoglycan axis have emerged, exemplified by the $30 million seed funding secured by Valora Therapeutics to advance its AbLec Antibody-Lectin chimera) platform. This technology, licensed from Stanford University and developed by Nobel Laureate Dr. Carolyn Bertozzi and Dr. Jessica Stark, consists of bispecific molecules that combine glycan-binding domains of Siglecs with antibodies targeting tumor-associated antigens.[56] The AbLec platform, alongside Siglec degraders, represents a pioneering approach to reprogramming the immune system to overcome cancer immune evasion. Potential therapeutic applications extend beyond oncology to autoimmune and other diseases.

Looking forward, these advances could unlock glyco-immune checkpoint modulation as a novel strategy for cancer treatment. Combining these emerging strategies with existing ICIs could yield synergistic effects, expanding the arsenal against cancer.

Breast Cancer Research Insights from AI Precision Medicine and Cancer Stem Cells

Maria dM Vivanco and Robert Kypta, *Cancer Heterogeneity Laboratory*

Several exciting advances in breast cancer research are improving our understanding of the disease and offering new hope for treatment in the future. These breakthroughs span a variety of areas, including some key developments in the use of artificial intelligence and precision medicine. In particular, the use of single cell RNA sequencing and spatial transcriptomics continues to contribute to the human breast cell atlas (HBCA) project,[57] which aims to generate a comprehensive reference of cell types and cell states in adult human breast tissue.

Identification of women at high risk of developing the disease can contribute to reducing incidence and minimizing impact. Somatic mutations are known to accumulate in normal tissues over time and increasingly so due to an aging population and, although the vast majority are inconsequential, they contribute to cancer. Gene dosage mutations due to somatic copy number alterations (CNAs) occur in many tumor types and they also represent an important source of transcriptional variation in breast cancer.[58] Aparicio and colleagues[59] have used single-cell whole-genome sequencing of breast epithelium to compare BRCA1 and BRCA2 carriers with wild-type individuals, and found that CNAs were enriched in luminal cells, while absent in basal myoepithelial cells, suggesting that these cells would be more tolerant of stress, allowing defective mutant cells to expand and eventually progress to cancer.

Hormone receptor-positive breast cancer remains one of the most common forms of the disease. Advances in hormone therapies are showing promising results,[60] however, resistance to therapies still develops, representing an unmet clinical challenge. In this context, cancer stem cells (CSCs), a small subset of cancer cells believed to drive tumor initiation and resistance, remain an area of intense research.[61] We previously showed that SOX factors are potential therapeutic targets in breast cancer[62] and SOX2 has potential as biomarker of resistance to hormone treatment.[63] Although targeting transcription factors is challenging, a variety of strategies have been used to target SOX2 in other tumor types. We identified a polyoxometalate that could block SOX2 binding to DNA, leading to reduced cancer cell migration, invasion and stem cell depletion in vitro and in vivo. Importantly, this compound restored sensitivity hormone therapy-resistant cancer cells to tamoxifen (a frequently used in hormone therapy), suggesting the potential use of this inhibitor as part of a combination therapy for tamoxifen-resistant breast cancer.[64] This is an example of how understanding how CSCs contribute to breast cancer recurrence can help develop therapies that specifically target and eradicate CSCs. An important aspect of this research is that the polyoxometalate selectively targeted CSCs without damaging normal stem cells. A remaining challenge is that CSCs can evolve and adapt, making it difficult to develop long-term cures.

Research into CSCs in breast cancer continues, as targeting these cells could potentially lead to more effective treatments and reduce the likelihood of relapse and metastasis. Thus, while there is still much to learn, these breakthroughs are a testament to the continued progress being made in the fight against breast cancer, with the final aim of improving patient outcomes.

Omics Expanding Goals and Effects Towards Precision Medicine

Urko Martínez Marigorta, *Integrative Genomics Laboratory*

At the Integrative Genomics lab, we continue to focus on methods to analyze and integrate multi-omic data, ideally collected over time, to better understand the complex pathways driving multifactorial disease. This year marks a significant milestone for us, with the launch of the recruiting component of our EARLY study for preclinical preclinical inflammatory bowel disease. Focusing on preclinical disease and prevention is a strategic choice for us. By characterizing molecular signatures before symptoms appear, we aimed at a better understanding of the cascade that leads to disease onset. Here, we discuss three relevant developments in genomics for precision medicine.

The first one involves the incorporation of large-scale omics, beyond genomics, into established disease cohorts. Multi-omic integration is transforming cohort studies, with resources like the UK Biobank or the AllOfUs initiative expanding to include metabolomic and proteomic profiles for hundreds of thousands of participants. These multi-omic datasets allow researchers to connect genomic data with protein and metabolite profiles, revealing intricate molecular patterns. For instance, the Pharma Proteomics Project has profiled the plasma proteome of 54,219 UK Biobank participants, yielding significant insights.[65] This initiative mapped over 14,000 links between genetic variation and plasma protein levels, hence offering mechanistic insights into known drug targets and biomarkers. As sample sizes and assay coverages grow, established large-scale biobanks will become even more useful to develop multi-omic research programs at full strength.

The second development revolves around the increasing focus on predicting preclinical disease through longitudinal approaches. The availability of follow-up data in many of these large biobanks enables a proactive approach to discover molecules that indicate an increased risk of disease, oftentimes before disease onset. For example, the UK Biobank has generated proteomic profiles from the participants using samples obtained around 2010. By checking the health records of these individuals up to 2023, many groups are obtaining predictive proteomic signatures with promising prognostic value.[66] For our lab, these developments illustrate the clinical power of

longitudinal datasets for identifying individuals at risk for immune-mediated diseases. In this regard, the recent FDA approval of Teplizumab for high-risk individuals before type 1 diabetes symptoms appear marks a pivotal step in using preclinical multi-omic studies to find biomarkers and molecular profiles that may help delay or prevent disease. As a lab, we want to develop an arm focused on this incoming anticipatory medicine.

The final development is associated with the potential of genomics to fuel drug discovery and safety prediction. The utility of genetic data in this area is increasingly well-evidenced. For example, a recent paper discussed how genomics facilitates drug discovery by revealing genetic links to disease pathways.[67] A similar global overview of the landscape of drugs approved recently highlighted this same theme.[68] These resources illustrate how the integration of big data into drug discovery workflows is enhancing our ability to target disease mechanisms and anticipate challenges before drugs reach the market. In our lab, we are using decades' worth of accumulated genetic data to predict adverse events for new drugs, demonstrating how large-scale data can trickle down to inform unanticipated areas of research.

Looking forward, I anticipate further integration of multi-omic data with clinical and exposomic data, particularly in preclinical settings. By merging genetic data with environmental factors, we can refine predictive models and move closer to comprehensive risk profiling. Our goal is to help in this concerted effort to push the boundaries towards anticipatory approaches to health.

Genomics for precision medicine in functional gastrointestinal disorders

Andreea Zamfir-Taranu, Leire Torices and Mauro D'Amato, *Gastrointestinal Genetics Laboratory*

The Gastrointestinal Genetics Lab[69] is at the cutting edge of research in the genetics of gastrointestinal (GI) diseases. Integrating genomic, computational and experimental methodologies, the lab aims to unravel pathogenetic mechanisms underlying complex GI conditions, refining precision medicine strategies.

The leading focus of the Gastrointestinal Genetics Lab lies on irritable bowel syndrome (IBS), a common functional GI disorder characterized by altered bowel habits and abdominal pain, which manifest without observable structural or biochemical abnormalities. The unclear pathophysiology of IBS underscores the importance of research endeavors uncovering its molecular mechanisms, which may represent a breakthrough in the clinical setting. Often IBS patients report postprandial symptoms, with carbohydrate-restricted diets proving effective in some but not all sufferers. Our large-scale genetic (GWAS) studies identified the first IBS risk loci,[70] while we recently showed that polymorphisms in the sucrase-isomaltase (SI) gene not only increase IBS risk but also influence the response to diets targeting SI substrates,[71,72] as well as food preference.[73] Based on our discoveries, we postulate that besides SI, the whole enzymatic cascade governing human carbohydrate digestion (human carbohydrate-active enzymes, hCAZymes) plays a pivotal role in IBS predisposition and its therapeutic management.

Analyzing the genetic makeup of hundreds of thousands of European individuals from population-based cohorts (using data obtained from primary care physicians, hospital admissions, and health-related questionnaires) we revealed that, together with SI, dysfunctional genetic variations in the salivary amylase AMY1B and the pancreatic amylase AMY2A, both involved in starch digestion, increase the risk for IBS.[74] We have further explored the influence of carbohydrate restrictive diets by stratifying DOMINO trial participants according to their hCAZymes genetic make-up.[75] We have shown that IBS patients with impaired hCAZymes activity respond better to a carbohydrate-reduced diet, a first-line approach for IBS. The therapeutic advantage was most pronounced in patients with multiple dysfunctional hCAZymes, reinforcing the potential of a gene-dosage effect in improved clinical response. Hence, we are providing extensive evidence that hCAZyme genotype overall may impact IBS pathophysiology via impaired digestive capacity, possibly leading to re-classification of some IBS patients as "carbohydrate malabsorbers". This constitutes rationale to tailor (dietary) therapeutic approaches based on patients' genetic profiling, with important repercussions on their management and the optimization of therapeutic approaches.

An important research effort is also made to explore the pathophysiology of GI conditions through the study of endophenotypes, quantitative intermediate traits that permit effective inference of the biological context of a disease. Through the analysis of physiological proxies of gut motility (such as stool frequency and consistency) in multiple population-based cohorts and biobanks via GWAS, we pursue actionable mechanisms that may be targeted to prevent and treat bowel (dys)function.

The Gastrointestinal Genetics Lab is leading efforts to decode genetic factors contributing to common GI disorders. By leveraging a multi-disciplinary approach, the lab aims to advance precision medicine, providing novel insights into GI pathogenesis via the identification of therapeutic targets, which may allow patients' stratification based on their genetic profiles.[76]

Balancing the Balance: Diet, Microbiota and Innate Immune Education

Juan Anguita, *Inflammation and Macrophage Plasticity Laboratory*

The now 'classic' model of innate immune memory focuses heavily on secondary responses mediated by innate and other immune cells to subsequent challenges, with dichotomous and opposing effects: either an increase (training) or a decrease (tolerance, historically referred to as 'immunoparalysis') in proinflammatory cytokine induction compared to the primary response. Training is generally associated with improved infection control and is, therefore, a more actively pursued area of research. However, there is also growing interest in understanding how tolerance can be reversed.[77] This dichotomy, however, becomes less clear when evaluating memory responses under persistent infectious or homeostatic conditions, especially when broadening the scope of innate immune cell function beyond proinflammatory cytokine production.

The role of microbiota components in these processes is particularly compelling, as their metabolic outputs are increasingly recognized as powerful drivers of both peripheral and central innate immune "education" events. These have critical implications for maintaining homeostasis and regulating pathological processes. A primary purpose of homeostatic innate immune memory induction is to sustain a permissive environment for these microorganisms. Thus, it is not surprising that the direct interaction of microbiota components with myeloid cells often leads to a lower induction of proinflammatory responses.

This phenomenon is exemplified by two prominent members of the intestinal microbiota, *Lactiplantibacillus plantarum*[78] and *Akkermansia muciniphila*,[79] both described by our group. Moreover, transplantation of fecal microbiota from parasite-exposed patients into germ-free mice resulted in reduced intestinal proinflammatory cytokine production,[80] further supporting the idea that this phenomenon extends beyond exposure to bacteria. Although impossible to accomplish in its entirety, the effect of individual, relevant microorganisms at the

local level will further enhance our understanding of this important ecological niche.

A key aspect of intestinal microbiota is its modulation by dietary components, as well as the effect of microorganisms and, importantly, their metabolites, in the generation of systemic and central innate immune memory. A recent seminal study in human volunteers[81] clearly indicated the key role played by the diet (ketogenic and vegan diets during 2 weeks) in the subsequent immune response, regardless of the order in which the diets were taken and primary to factors such as age, sex, ethnicity or body mass index. How changes in dietary habits affect the immune system through microbiota changes is still a growing field, but some studies have addressed the effects of microbial metabolites on innate immune cells, both short- and long-term. Some of these studies have seen the light this year, including studies by Liu and cols.,[82] Bulut and cols.[83] and our own group,[84] as well as reviews by Ferreira and cols.[85] or Takeuchi and cols.[86]

Advancing Gene Editing Technologies

Raúl Pérez-Jiménez, *Synthetic Biology Laboratory*

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a transformative technology that allows scientists to precisely edit DNA sequences and modify gene function. It uses an enzyme called Cas (CRISPR-associated) to cut DNA at specific sites, guided by RNA matching the target sequence, enabling targeted genetic modifications. Despite its potential, CRISPR faces several challenges. Off-target effects can cause unintended DNA changes, leading to harmful mutations. Efficient delivery of gene-editing tools to target cells is also problematic, often triggering immune responses. Ethical and regulatory concerns, especially regarding human embryos, raise questions about long-term impacts. Technical limitations, such as designing effective guide RNAs and ensuring precise DNA cuts, add to the complexity. Public acceptance is crucial, requiring transparent communication and engagement to build trust. Our lab is developing synthetic CRISPR systems to overcome some of these limitations, aiming for safer and more effective gene-editing technologies. Continued research and robust regulatory frameworks are essential for responsible gene editing.

In 2024, several significant advancements in CRISPR technology were made, highlighting new discoveries and improved methods for gene editing. Peter H. Yoon and colleagues[87] investigated the origins of CRISPR-Cas systems, with a particular focus on RNA-guided enzymes. While Cas9 and Cas12 are derived from transposon-encoded nucleases, the origins of Cas13 had remained unclear. Their study uncovered a group of small Cas13 enzymes in E. coli with antiphage defense properties, sharing characteristics with DNA-targeting CRISPR systems. This discovery suggests that, despite their distinct evolutionary roots, RNA- and DNA-targeting CRISPR-Cas systems exhibit convergent evolutionary traits, which could pave the way for discovering new CRISPR systems.

Another breakthrough came from Zhao, Qin, Yu, and colleagues,[88] who developed polymer-locking fusogenic liposomes, known as "Plosomes," for targeted delivery of gene editors, such as CRISPR-Cas systems, to the central nervous system. These Plosomes can cross the blood-brain barrier (BBB) in both in vitro and in vivo models. The liposomes are designed with a locking mechanism that controls the fusion process, preventing premature cargo release. This innovation allows for targeted gene editing in brain tumor cells by delivering gene editors across the BBB encapsulated in Plosomes. Lastly, Zhang, Neugebauer, Krasnow, and their team[89] enhanced cytosine base editors (CBEs) through phage-assisted evolution, improving their activity, selectivity, and sequence compatibility. These refined CBEs outperform previous models, such as TadA-derived editors and BE4max, by offering higher precision and reduced off-target effects in both *E. coli* and mammalian cells. The study demonstrated that the new CBE6 variants are state-of-the-art for precision gene editing, making them safer and more versatile for clinical and research applications.

In 2025, CRISPR technologies are expected to make significant strides. Researchers will focus on improving the precision of CRISPR systems to minimize off-target effects and enhance the accuracy of gene edits. This precision will be crucial as more CRISPR-based therapies enter clinical trials, targeting a broader range of diseases such as genetic disorders, cancers, and infectious diseases. Additionally, CRISPR technology will expand its applications beyond medicine, playing a vital role in agriculture by developing crops with improved traits, like drought resistance and higher nutritional value. Ethical and regulatory developments will keep pace with these advancements, ensuring the safe and responsible use of gene editing. Increased public awareness and education about gene editing will help build trust and acceptance, fostering broader support for its applications. These advancements will push the boundaries of what's possible with gene editing, offering new hope for treating diseases and improving various aspects of life.

Advancing Insights into MASLD: Pathophysiology, Diagnostics and Therapeutics

David Fernandez-Ramos, Fernando Lopitz-Otsoa, Oscar Millet, and Jose M. Mato, *Precision Medicine and Metabolism Laboratory*

Our research emphasizes the use of metabolomics and lipidomics to identify biomarkers and develop non-invasive diagnostic tests for Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD). The laboratory's work aims to understand the metabolic pathways and molecular mechanisms underlying liver diseases, which is critical for developing new therapeutic strategies and improving patient outcomes.

Metabolic dysfunction-associated steatotic liver disease (MASLD), previously termed non-alcoholic fatty liver disease (NAFLD), highlights the role of systemic metabolic dysfunction in liver pathology. MASLD, closely linked to obesity, type 2 diabetes (T2D), and metabolic syndrome, affects a significant portion of the global population and ranges from simple steatosis to metabolic dysfunction-associated steatohepatitis (MASH). Severe cases carry elevated risks of fibrosis, cirrhosis, and hepatocellular carcinoma.[90,91,92]

MASLD pathogenesis involves lipid dysregulation, mitochondrial dysfunction, oxidative stress, and chronic inflammation. The forkhead box M1 (FOXM1)-methionine adenosyltransferase 2A/2B axis plays a central role in liver inflammation and fibrosis by driving hepatocellular injury and extracellular matrix remodeling, making it a therapeutic target.[93] Impaired very-low-density lipoprotein (VLDL) metabolism exacerbates hepatic lipid accumulation and dyslipidemia and has been linked to tumorigenesis in advanced MASLD stages.[94,95] Additionally, S-adenosylmethionine (SAMe), a critical mitochondrial regulator, modulates oxidative stress and improves energy homeostasis by influencing the respiratory chain repressor MCJ.[96]

Innovative diagnostics address MASLD's complexity. The metabolomics-advanced steatohepatitis fibrosis (MASEF) score identifies at-risk MASH patients using metabolic profiles, offering a scalable alternative to biopsies.[97,98] Machine learning models and genetic algorithms also improve risk stratification and guide personalized care by leveraging translational insights from murine dietary studies.[99]

Lifestyle changes remain the keystone of MASLD management, yet pharmacological advancements are transforming treatment. Agents targeting metabolic, inflammatory, and fibrotic pathways, such as FXR and PPAR agonists, improve insulin sensitivity and reduce hepatic fat accumulation.[93] Translational research based on murine models has ranked dietary interventions by their relevance to human MASLD, aiding preclinical testing.[99]

MASLD has significant systemic implications, including heightened cardiovascular risk. Metabolomic analyses reveal distinct MASLD subtypes with unique cardiovascular profiles, underscoring the importance of tailored strategies.[98] Furthermore, MASLD increases the risk of extrahepatic malignancies, such as colorectal, pancreatic, and breast cancers, driven by impaired lipid metabolism and systemic inflammation.[94,95]

Pediatric MASLD is a growing concern as childhood obesity rises. Unique pediatric metabolic profiles necessitate specialized diagnostic and therapeutic strategies to prevent long-term complications.[92] Efforts to standardize MASLD nomenclature have further aligned global research and clinical practices, enabling unified approaches to patient care.[90]

Towards a Cure for Prion Diseases: Emerging Therapies, Diagnostic Tools and Molecular Insights

Joaquín Castilla and Hasier Eraña, *Prion Research Laboratory*

Transmissible spongiform encephalopathies (TSE) are rare, rapidly progressing, and fatal neurodegenerative disorders caused by prions. These prions arise from PrPC, a protein expressed on neuronal surfaces in all mammals. Through unknown mechanisms, PrPC misfolds into PrPSc, a pathogenic form capable of inducing misfolding in other PrPC molecules, triggering a cascade of protein aggregation in the brain and neuronal death.

This year brought hopeful news for patients with prion diseases, including Creutzfeldt-Jakob disease, Fatal Familial Insomnia, and Gerstmann-Sträussler-Scheinker syndrome, with the launch of a clinical trial for antisense oligonucleotides (ASOs) designed to reduce PrPC levels, delaying onset and progression.[100] Given potential limitations, other therapeutic strategies are also in development, including epigenetic approaches to silence PrPC expression.[101,102,103] Combination therapies targeting multiple mechanisms are likely needed, prompting the exploration of additional treatment platforms[104,105] and advanced compound screening methods.[106,107] For the design of new therapeutic approaches, gaining insight on the largely unknown mechanisms of these diseases is necessary and thus, significant resources have been made available this year such as studies on PrPC misfolding across mammalian species[108] and CRISPR-based screening libraries,[109] advancing understanding of disease mechanisms. Research has also focused on the properties of prion strains[110,111,112] and elucidating their structures, including the strain causing Chronic Wasting Disease (CWD) in deer,[113] as well as genetic and cellular factors influencing disease onset and progression.[114,115,116] These studies are deepening insights into neurotoxicity and prion propagation mechanisms.[117,118,119] Many of these findings are being translated to more prevalent neurodegenerative diseases that exhibit prion-like mechanisms.[120,121] Notable discoveries this year include evidence of Alzheimer's disease iatrogenic transmission,[122,123] prion-based vaccines for Parkinson's disease,[124] and seeding assays for synucleinopathy diagnostics.[125,126] Conversely, further developments in seeding assays, such as using skin punctures for prion detection[127,128] and identification of new biomarkers for early genetic case detection,[129,130] highlight the potential for early diagnosis considering emerging treatments. Finally, research on CWD in cervids, a growing epidemic in North America and now detected in Europe, is worth mentioning, since it has provided insights into its zoonotic potential,[131,132] strain variability influenced by infection routes,[133,134] and the transmissibility and origins of the disease.[135,136,137]

In summary, the past year has been transformative for prion research, marked by a promising clinical trial and advancements in diagnostic methods, potentially enabling early detection through minimally invasive means soon, which will likely continue being adapted to other neurodegenerative disorders caused by misfolded proteins. In the coming years, attention will focus on the trial's results while new therapeutic strategies continue to emerge, likely requiring combination therapies targeting multiple aspects of these diseases. On the molecular front, we anticipate new high-resolution structures of diverse prion strains, shedding light on the structural features that drive their unique biological behaviors.

New Insights, Therapies and Global Advancements in Liver Disease Research

Malu Martínez-Chantar, *Liver Diseases Laboratory*

Over the past year, the Liver Disease Lab has concentrated its efforts on uncovering the mechanisms underlying a diverse range of liver pathologies, from acute liver injury induced by paracetamol toxicity to rare liver cancers such as cholangiocarcinoma and pediatric hepatoblastoma. The mechanisms we have identified and published throughout this year have not only deepened our understanding of these complex diseases but have also laid the groundwork for novel therapeutic approaches. By dissecting the molecular and cellular pathways involved, our research has contributed to advancing diagnostic tools and treatment strategies, offering new hope for patients battling these challenging conditions.

In addition to these critical investigations, we have expanded our focus to explore the effects of aging on liver function, particularly its connection to chronic liver disease. Aging is now widely recognized as a key driver that accelerates liver damage, yet the mechanisms behind this progression remain incompletely understood. Our lab is particularly concerned with the rising prevalence of metabolic dysfunction among populations subjected to shift work and nocturnal rotations. These disrupted circadian rhythms appear to impact liver metabolism profoundly and contribute to systemic disease. To address this, we are investigating hepatic signalling molecules that may serve as mediators influencing multiple organs, including the brain, heart, and adipose tissue. Our goal is to identify systemic signals that can act as therapeutic targets to mitigate the long-term effects of metabolic disruption on overall health.

Furthermore, our lab has opened a new research line exploring hepatic zonation and its implications in liver disease. This line of research focuses on identifying mechanisms that regulate liver cell heterogeneity and how they relate to metabolic imprinting. Of particular interest is the impact of parental nutrition, both maternal and paternal, on the establishment of liver zonation patterns. These findings provide critical insights into developmental programming and highlight the significance of early nutritional interventions in preventing liver disease later in life.

At an international level, 2024 has marked a transformative year in hepatology, with several key breakthroughs advancing the field. These achievements, built upon decades of research, have reshaped clinical guidelines and

introduced new therapeutic strategies. 1) The World Health Organization (WHO) released updated guidelines for the management of hepatitis B, emphasizing tailored strategies for resource-limited settings. These revised recommendations aim to improve screening, vaccination, and treatment protocols, particularly in regions where hepatitis B prevalence remains high, and resources are scarce;[138] 2) Significant progress has been made in the management of primary liver tumors through the integration of advanced technologies. Innovations such as artificial intelligence (AI)-driven diagnostics, precision imaging, and targeted therapies have drastically improved the accuracy of diagnosis and treatment outcomes. The multidisciplinary approach, combining expertise from radiology, oncology, and hepatology, is now central to optimizing patient care. These advancements are transforming the landscape of liver tumor management and improving patient survival rates.[139] 3) Immunotherapy has emerged as a transformative treatment strategy for patients with advanced hepatocellular carcinoma (HCC). Several phase III clinical trials conducted this year have demonstrated significant improvements in patient outcomes, with immunotherapeutic agents showing enhanced efficacy and safety profiles. This marks a paradigm shift in the treatment of HCC, offering new therapeutic options for patients who previously had limited alternatives.[140]

The progress achieved both within our laboratory and by the broader scientific community underscores a pivotal year for hepatology. By advancing our understanding of liver diseases - ranging from acute injury to aging-related dysfunction - and addressing critical challenges such as systemic metabolism and novel diagnostics, we are paving the way for more effective treatments and preventative measures. These collective efforts will undoubtedly improve patient outcomes and set the stage for future breakthroughs in liver disease management.

Modifications by Ubiquitin-Like Proteins in Health and Disease, Tools and Strategies

Rosa Barrio and James D. Sutherland, *Ubiquitin-likes and Development Laboratory*

Our research focuses on understanding how developmental processes and diseases are regulated through post-translational modifications mediated by the Ubiquitin-like (UbL) family. UbL proteins conjugate to target proteins, modulating their functions and governing key cellular processes. Studying UbL modifications presents challenges due to the scarcity of modified proteins and the transient nature of these modifications. To address these challenges, we have developed biotin-based strategies,[141,142,143,144] the most recent being BioE3[145] for the dentification of specific targets of E3 ligases. These methods are particularly valuable in the context of targeted protein degradation (TPD), a cutting-edge approach that exploits the ubiquitin-proteasome system to eliminate disease-related proteins. We focus on rare diseases, particularly Townes-Brocks Syndrome (TBS), caused by mutations in Spalt-like 1 (SALL1), as well as other rare diseases.

After the publication of the BioE3, two related strategies were published in 2024: E-STUB, by the group of WR Sellers at the Broad Institute[146] and Ub-POD, by the group of S Bhogaraju at EMBL Grenoble.[147] This evidence the need for this type of technology in the fields of UbL and TDP.

Recently, a captivating new avenue of research has emerged, focusing on non-canonical substrates of ubiquitin. Non-protein substrates (e.g. lipopolysaccharides and adenosine diphosphate ribose (ADPr) have been described. DELTEX family of E3 ligases catalyses ubiquitylation of ADPr linked to nucleic acids.[148,149] The N-glycan-recognizing E3 ubiquitin ligase SCFFBS2, together with ARIH1 ubiquitinate Nrf1 through N-acetyl glucosamine residues.[150] The unconventional ubiquitination is sustained on the specificity of certain E3 ligases.

The rapid advancement of novel TPD strategies and degraders have led to a significant rise in the number of PROTACs entering Phase I/II clinical trials, two of them entering in Phase III: ARV-471 and ARV-766 against estrogen and androgen receptors.[151] New drugs are being developed around the concept of "proximity biology" which holds promise to be able to tackle the undruggable proteome.

During this year, more evidence was collected on the role of SALL factors in development and disease, e.g. SALL1 in congenital anomalies of the lower genitourinary tract.[152] An interesting analysis of a large cohort of patients with hearing loss showed underdiagnosed TBS cases and a higher penetrance of phenotypes in offspring respect to the affected parents.[153] In addition, SALL2, 3 and 4 have been related to numerous cancer types.

TPD field will continue growing in the next period. The identification and characterization of new E3 ligases with diverse specificities is of utmost importance in the field. The characterization of new forms of ubiquitination will also expand in the new year's. There is a need for new, specific and sensitive strategies to support these new fields of research.

Chemical Immunology Approaches to Advance Molecular Adjuvants and Vaccines

Alberto Fernández-Tejada, *Chemical Immunology Laboratory*

Current subunit vaccine approaches based on molecularly defined antigens are less immunogenic than traditional whole-pathogen vaccines. Therefore, they require the use of adjuvants to increase antigen immunogenicity and potentiate the immune response, improving overall vaccine efficacy. However, not many adjuvants show sufficient potency and acceptable toxicity for human use; in addition, their mechanisms of action are poorly understood. Among the few immunostimulatory molecules currently approved in human vaccines, the Toll-like receptor 4 (TLR4) agonist monophosphoryl lipid A (MPLA) and the saponin natural product QS-21, stand out. They have been coadministered in combination with several vaccine antigens, leading to the clinically licensed adjuvant system AS01, which has been approved in various human vaccines. QS-21 has also been licensed as part of a saponin-based nanoparticle formulation (Matrix-M™) recently used in the Novavax Covid-19 vaccine and in the newest, more effective R21 malaria vaccine.[154] Despite these exceptions, natural QS-21 suffers from several intrinsic liabilities (scarcity, low-yielding extraction/purification, heterogeneity, structural instability, and dose-limiting toxicity) that have hampered its approval as a stand-alone adjuvant in absence of any lipid-based formulation. To address these challenges, the development of novel strategies towards more practical, improved alternatives to the suboptimal, bark-extracted QS-21 has been a long-standing goal in the field. Thus, the primary research program in the Chemical Immunology Lab has a dual far-reaching mission based on developing and exploiting innovative chemical immunology approaches to tackle the above gaps in the adjuvant/vaccine arena.

In 2024, critical advances have been disclosed in significant articles reporting new tools and methods to access QS-21 independently of the laborious bark extraction. The first study described the complete biosynthetic pathway to QS-21 and its reconstitution in tobacco, demonstrating the production of QS-21 in a heterologous expression system.[155] In a transformative work published in Nature, the complete biosynthesis of QS-21 and its precursors was accomplished in

engineered yeast strains,[156] providing additional access to related structural derivatives. Another article described an in vitro platform for plant cell culture production of QS-21 and demonstrated the bioequivalence of this material to the naturally derived saponin isolated from the bark.[157] This year has also seen the publication of an article reporting a modular saponin-based nanoparticle platform incorporating several TLR agonists, which induced specific T helper responses and humoral immunity in SARS-CoV-2 and HIV experimental vaccines in mice.[158] Notably, our laboratory has also contributed actively to this theme in 2024 with a study published in the Journal of Medicinal Chemistry,[159] where we have reported the development of a novel adjuvant system based on the combination of our synthetic QS-21 variant lead compound and a designed TLR4 agonist (FP20). The coformulation of both molecules resulted in stable structures with properties that contributed to a robust synergistic immune response in mice, with induction of significantly higher levels of antigen-specific antibodies compared to the individual adjuvants. Moreover, in a collaboration led by the group of Prof. Juan Anguita, we have participated in a multidisciplinary research work showing that phloroglucinol, a microbiota metabolite, moderates macrophage responses to pro-inflammatory insults, inducing central trained immunity conducive to long-term protection against inflammation *in vivo*.[160]

On a separate topic of interest to our laboratory, a comprehensive review article on O-GlcNAc glycosylation, a key post-translational modification implicated in the pathogenesis of several diseases and immunological disorders, has been published this year.[161] This manuscript describes the biochemistry and development of inhibitors of the O-GlcNAc enzymes, cellular regulation of O-GlcNAc and its implications in pathophysiology, opportunities for therapeutic modulation of O-GlcNAcylation and clinical advancement of O-GlcNAc modulators. Notably, this is an important area of research in the Chemical Immunology Laboratory, whereby we are investigating potential links between QS-21 immunostimulation and O-GlcNAc regulation and have also established interesting international collaborations with several research groups worldwide. Going forward, 2025 is expected to bring important advances in the field, with the development of new immune-activating adjuvant technologies and further (pre)clinical advancement of the platforms developed so far. Moreover, we will continue to apply our chemical tools to gain key molecular insights into saponin immunopotentiation and O-GlcNAc glycosylation in immunity, always with an innovative perspective and attentive to new scientific opportunities and alliances.

New Classifications, Technologies and Clinical Impacts in Extracellular Vesicle Research

Juan Manuel Falcón, *Exosomes Laboratory*

These days, the Extracellular Vesicles family[162] include exosomes, microvesicles, arrestin-domain-containing protein 1(ARRDC1)-mediated microvesicles (ARMMS), migrasomes, apoptotic EVs, Autophagic EVs, Stressed EVs (Stressome), Mitochondial extracellular vesciles, Matrix vesicles, Exosphers, oncosomes and non-vesicular extracellular nanoparticles including exosomers and supermeres. All of them have been introduced in the updated guideline for the EV field that was published this year in *Journal Extracellular Vesicles*.[163] In these updated guidelines also the last advanced technologies particularly focused on the single vesicles analysis are detailed including flow cytometry and super-resolution microscopy. An increasing interest in the EV field during last year has been the implementation of methods to study different body fluids including salivary EVs[164] and cerebrospinal fluid[165] with diagnostics potential for aging-related diseases. In addition, technologies for detecting EV-mRNA remains challenging due to low mRNA levels and the small proportion of tumor-derived EVs in the bloodstream, which often necessitates large sample volumes and advanced techniques. To overcome this, researchers repurposed Cas13a to amplify both the target mRNA and detection signals. This novel assay amplifies fluorescence signals upon mRNA recognition, allowing the detection of single-nucleotide polymorphisms (SNPs) in EVs with high specificity. By enabling dual amplification, the method detects mRNA at sub-attomolar concentrations. The assay successfully identifies circulating EVs with tumor-specific genetic mutations, reflecting those found in corresponding tumor tissues.[166]

In physiology one of the areas more developed during 2024 related to the EVs has been the impact of these vesicles in the neuronal functioning with more than 330 publications. Special interest in their role in controlling neuronal plasticity.[167,168] Their impact in mental disorders including Alzheimer´s disease, Parkinson´s disease, Huntington, schizophrenia and additions has been highlighted during this year.[169] The use of circulating nucleic acid associated with the EVs was supported as a low-invasive diagnostic method for glioblastoma.[170] The increasing interest of the study or the role of EVs in the brain has also been reflected in the creation of the Scientific Interest Group by ISEV[171] with more than 400 members.

Globally, there has been more than 8840 publications on exosomes or EVs (source: PubMed), and there are 218 clinical trials on exosome studies, of which 140 trials are evaluating exosome-based therapeutics and 190 are testing exosome-based diagnostic tests.[172] These numbers reflect the impact that these vesicles are having in biomedicine. In therapeutics, a systematic review of 40 studies[173] shows that they are all small pilot trials with a large heterogeneity in terms of administration route and target disease. Moreover, the absence of a placebo control in most of the studies, the predominant local application of EV formulations and the inconsistent administration dose metric still impede comparison across studies and firm conclusions about EV safety and efficacy.

In conclusion, the study of the Extracellular Vesicles (EV) family represents a groundbreaking area with implications for understanding cell biology, signaling, disease mechanisms, and innovative therapeutic strategies.

'Google-Maps' of Cells and Tissues by Cellular Cryo-Electron Tomography

Ane Martinez-Castillo, Juan Diego Riveros, and Nicola GA Abrescia, *Structure and Cell Biology of Viruses Laboratory*

The past years have underscored the critical importance of virology and immunology research. Rapid responses to pandemic events depend on decades of accumulated fundamental knowledge and continuous technological advancements. The study of viruses - whether they infect Bacteria, Archaea, or Eukaryotes, and whether approached from basic, clinical, or translational perspectives - benefits society.

At the Abrescia Lab, we focus on uncovering the molecular mechanisms that drive viral pathogenesis using advanced structural methods. Our research focuses on three key areas: virus assembly, virus-cell entry, and virus-antibody recognition. By deciphering the principles of virus assembly, we aim to develop molecular strategies to interfere with viral morphogenesis. Expanding our structural understanding of the viral world, or virosphere, enables us to identify evolutionary relationships that remain undetectable at the sequence level.

Studying virus-cell entry mechanisms offers opportunities to disrupt these critical key-lock processes.

Antibodies often serve as tools for such interventions, and understanding their recognition and neutralization mechanisms can significantly enhance their effectiveness. To achieve these goals, we employ an integrative approach that combines X-ray crystallography, electron microscopy (EM), and functional studies. A particular long-standing focus of our lab has been the direct visualization of viral infection processes within cells. Notably, our early work in 2012, published in *Virology*[174] marked a significant milestone for the Abrescia Lab, demonstrating our capability to establish electron tomography in the Basque Country - pioneering, to our knowledge, the first study of its kind from a research center in the region. Today, we leverage state-of-the-art imaging technologies to further unravel the cellular landscape under viral siege and to decipher the mechanisms of viral assembly within the cell.

The knowledge we generate not only advances our understanding of viral molecular mechanisms but also provides a foundation for developing practical molecular tools, including drugs, diagnostics, and vaccines, to improve both human and animal health. Our work aligns with the One Health concept, addressing societal challenges through innovative strategies to combat infectious diseases.

Structural biology has advanced with cryo-electron microscopy (cryo-EM), enabling the study of biological structures in their native cellular context. This technique provides near-native, high-resolution insights into cellular environments, overcoming the limitations of traditional light and electron microscopy methods. Cryogenic techniques allow for the preservation of cells in their natural state, revealing detailed cellular architecture. Researchers are now able to map viral infection stages through cryo-electron tomography and computational techniques, gaining valuable insights into viral processes, such as those observed in SARS-CoV-2 and Reovirus infections.

Even entire organisms, such as C. elegans larvae, can now be sampled at the molecular level using advanced cryo-electron tomography (cryo-ET).[175] In this study, the authors demonstrated the power of serial lift-out cryo-ET in mapping the molecular anatomy of whole organisms. Additionally, a comprehensive review in *Current Opinion in Cell Biology*[176] delves into the molecular architecture of the actin cytoskeleton across different organisms using cryo-ET. Cryo-ET is also being employed in human brain tissue research. A latest study used cryo-ET to examine Alzheimer's disease brain tissue, revealing intact subcellular structures, including autophagy components and potential tau fibrils. These findings demonstrate how plasma cryo-FIB milling coupled with cryo-ET can provide nanoscale insights into the human brain, offering potential breakthroughs in neurodegenerative disease research.[177]

In 2024, the Basque region made significant strides in cryo-ET with the installation of two cryo-focused ion beam (cryo-FIB) machines. One is at CIC nanoGUNE, enabling cell and tissue investigation, while the other, located at the Biofisika Institute at BREM, supports cellular correlative microscopy. These machines, along with a Titan Krios G4 and JEOL JEM2200FS microscopes, create a robust infrastructure for cryo-EM and cryo-ET research. Additionally, a virtual network connecting microscopy facilities at CIC bioGUNE, CIC biomaGUNE, CIC nanoGUNE, and CIC energiGUNE led to the creation of the Basque Research Technological Alliance in Electron Microscopy (BRTA-EM), aimed at fostering collaboration and establishing the region as a global microscopy hub.

As Abrescia Lab, we are consolidating biological cryo-FIB milling procedures and developing a pipeline for cellular structural cryo-electron tomography. Furthermore, the combination of soft X-ray tomography (a cellular structural technique enabled by synchrotron radiation) and high-resolution correlative microscopy, such as cryo-structured illumination microscopy (cryo-SIM), will allow for direct correlation of cellular morphological changes with viral entry pathways and subsequent antiviral treatments. The structural techniques mentioned above are helping to create a "Google Map" of distinct (pathological) human cells, providing predictive capabilities for navigating the landscape of human diseases. Targeting physiological processes and elucidating biological mechanisms in whole cells will increasingly become a common approach.

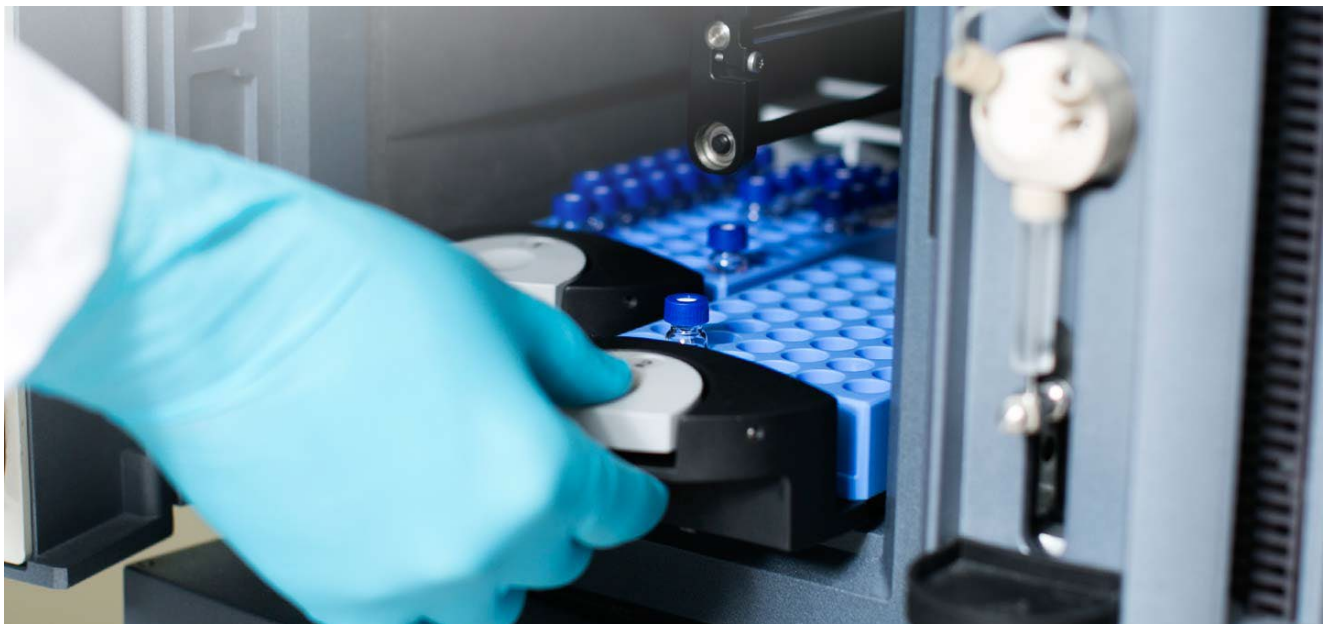
Looking for Elusive Metabolons

Mikel Valle, *CryoEM of Biological Macromolecules Laboratory*

To fully understand cell functioning we need to explore the interplay between macromolecules inside the cellular environment. Some of these interactions between molecular players are transient, weak, and can be regulated by unknown mechanisms. Metabolons, associations of enzymes participating in the regulation of a particular metabolic pathway, are among these transient interactions, and their characterization is rather challenging. Our team has recent experience in the structural studies of oligomeric enzymes, but now we want to move a step forward and explore the presence of metabolons in different cell systems. Our main aim is to isolate these enzymatic complexes directly form cultured cells and characterize their structure by cryo-electron microscopy (cryoEM), with special interest in tumor cell lines.

This task requires the analysis of endogenous proteins and the ability to model their structures and possible interactions. During these years we have seen several progresses that might pave the way for this type of studies. In the first place, labelling of endogenous protein using CRISPR/Cas systems allows for affinity purification of isolated and/or complexed specimens suitable for cryoEM studies.[178] Also, if the cryoEM samples are complex mixtures of different nature, there are new cryoEM methods that can deal with a high level of structural heterogeneity[179] and deliver several high-resolution structures even starting with fractionated cell lysates.[180] Importantly, AI based methods now can predict not only the structure of proteins, but also their oligomeric forms and their interaction with other molecules, including other proteins and nucleic acids.[181] This new tool has been rapidly incorporated into the studies of potential metabolons.[182]

In the near future, we can expect an explosion of newly described metabolons. Their composition, structure, and posttranslational modifications could shed light on their function and regulation. The identity of the partners in large complexes could also unveil crossroads between different metabolic and/or signalling pathways. This has the potential to expand our understanding of the transformation mechanisms behind tumor development where metabolic rewiring plays a central role.



TECHNOLOGIES

Decoding the Complexity of Ever-Changing Genomes

Ana M Aransay, *Genome Analysis Platform*

Genomes are intricate and dynamic blueprints, continuously adapting and interacting with environmental factors to govern life processes. Their complexity lies not only in its vast amount of information but also in the way they adjust and respond to external conditions. To keep up with these challenges, scientists must rapidly develop innovative methods to analyze and understand the genome with greater precision.

In this vein, at CIC bioGUNE's Genome Analysis Platform, efforts this year have been dedicated to adopting and applying cutting-edge methods to better understand the genome. Particular focus has been placed on single-cell analysis, a revolutionary approach that allows scientists to deeply understand transcriptomes (which genes are active) and epigenomes (how genes are turned on or off) in biological samples. To meet the needs of specific projects, technical strategies for bulk tissues, single-cell preparations and extracellular-vesicle isolates have been followed. Relevantly, most outputs of these projects have been reported in several collaborative, scientific articles.[183-195]

The genomes' complexity and ever-changing nature demand the rapid development of tools and strategies which ensures that the scientific community remains at the forefront of discovery. Accordingly, the Genome Analysis Platform works hard in the aims:

- To establish consistent protocols to get reproducible microRNAs expression signatures (MicroRNomes)

- Another key advancement is the implementation of long-read sequencing technologies. Unlike traditional short-read methods, which break DNA into small fragments, long-read sequencing captures much larger sections of DNA in a single read. This approach provides a completer and more accurate picture of the genome, enabling the identification of structural variations, complex rearrangements, and repetitive sequences,

which play vital roles in gene regulation and evolution but have historically been difficult to analyze.

- By combining single-cell RNAseq, ATACseq (Assay for Transposase-Accessible Chromatin using sequencing) and Methyl-seq techniques with long-read sequencing, we will be able to achieve unprecedented resolution in studying the genome.

Looking ahead, the future of genomic research lies in System Biology, which brings together data from multiple biological levels to create a comprehensive view of life's processes. By integrating genomic, transcriptomic, epigenomic, proteomic, and metabolic data, CIC bioGUNE envisions constructing dynamic models that reveal how genes interact with each other and with their environment, unlocking the interconnected systems that sustain life itself. This holistic approach not only deepens our understanding of life's complexities but also promises to transform how we diagnose diseases, cultivate crops, and study evolution, paving the way for a future of groundbreaking discoveries.

New Key Areas in Proteomics Include Immunopeptidomics, Drug Discovery and Spatial Proteomics

Félix Elortza, *Proteomics Platform*

Mass spectrometry (MS) based proteomics is evolving rapidly. Advances in instrument sensitivity and overall performance, combined with optimized methods and dedicated software tools, have significantly accelerated research across various proteomics fields bringing them closer to clinical applications.

Immunopeptidomic research to study neoantigens has gained momentum lately. A neoantigen is a type of protein fragment that arises from mutations within cancer cells, making it unique to those cancer cells and not found in normal, healthy cells. Because neoantigens result from the specific genetic mutations in a given tumor, they are highly specific to that particular tumor's cells. This uniqueness makes neoantigens a valuable target for immunotherapy because they can be recognized by the immune system as "non-self" or foreign, sparking an immune response to attack and destroy the cancer cells. In this regard we implemented a pipeline in our proteomics platform for immunopeptide analysis based on dual acquisition method for singly charged and multiple charged peptides. For that we took advantage of the unique ion-mobility feature of the TIMS Tof Pro. This methodology was applied to samples sent from FPC center in Findland (Dr. Tuija Kekarainen) resulting in thousands of peptides identified. The obtained results are included in a manuscript in preparation. Following this line, one of the latest advances in mass spectrometry for this type of analysis is the "Thunder-DDA-PASEF". This method semi-selectively fragments singly and multiply charged Human leukocyte antigen (HLA) class I peptide ligands based on their "ion mobility spectrometry" (IMS) and m/z. Besides, the method employs the high sensitivity mode and extended IMS resolution with fewer MS/MS frames, increasing the coverage of regular immunopeptidomics analyses.[196]

Proteomics is also making valuable contributions to the field of drug discovery. The lab of Bernhard Küster has introduced a new methodology called "decryptE." This proteome-wide approach measures the full dose-response characteristics of drug-induced protein expression changes, providing insights into cellular drug mechanisms of action.[197] In this context, due to the challenges posed by polypharmacology, a significant portion of the human kinome still lacks selective chemical probes. To address this, Küster´s lab has profiled over 1,000 compounds from drug discovery projects in lysates of cancer cell lines using Kinobeads. The resulting dataset of 500,000 compound-target interactions is now accessible in ProteomicsDB, demonstrating how this molecular resource can advance medicinal chemistry.[198]

Another area of focus that has evolved significantly is spatial proteomics. Deep visual proteomics (DVP) is an approach that combines image-based artificial intelligence with automated microdissection and ultra-high sensitive MS. Among others, this approach enables the characterization of cellular heterogeneity within distinct tissue regions and across patients. The potential of this multi-focus approach is to refine early-stage detection, contribute to personalized patient management strategies and provide novel insights into metabolic reprogramming.[199] Noteworthy, spatial proteomics has been chosen as method of the year 2024 by *Nature Methods*.[200]

Above mentioned immunopeptidomics, MS driven drug discovery analyses and spatial proteomics are just some of the areas benefited by the advances of proteomics techniques and methodologies. The proteomics community envisions continuously expanding its applications to other challenging yet highly relevant fields for basic and clinical research.

Advances in Mass Spectrometry

Oihane Albóniga, Diana Cabrera, Sebastiaan van Liempd and Juan M. Falcón-Pérez, *Metabolomics Platform*

The metabolomics platform uses mass spectrometry coupled to liquid chromatography to dig deep into the metabolite pool. Mass spectrometry is especially useful for this since it combines unprecedented sensitivity with a high level of structural selectivity. Although it is an old technique, big advancements are still being made regarding both these properties. Also scan speed and dynamic range has increased dramatically. Moreover, MS data analysis has seen big improvements with the introduction of machine learning methods.[201] These advancements allow new use-cases to be explored. Here we want to highlight some new technological features and recent use cases in the field of mass spectrometry.

Technologically there have been great improvements in Time-of-Flight (ToF)spectrometry. Multiple reflection ToF allows resolutions up to 100.000 for low molecular weight analytes such as metabolites.[202] This surpasses the performance of orbitrap mass separation at these low masses. Together with scan rates up to 100 Hz and a dynamic range close to 5 orders of magnitude, these new generation ToF instruments will be instrumental in many analytical applications.

Another interesting development is the separation of enantiomeric compounds.[203] By using ion mobility MS (IMMS) it was possible to induce symmetry-breaking circular motions in the ions. This made it possible to separate D and L isomers of amino acids, sugars and drug molecules. The authors state that implementation of this technology on a commercially available device should be fairly straightforward. Another advancement involving ion mobility is cyclic IM separation[204] with which it is possible to separate structural isomers with exceptionally small differences in collision cross sections.

Technological advances, especially in sensitivity and resolution, created some new analytical opportunities such as single-cell metabolomics, mass spectrometry imaging and oligonucleotide analysis. Single-cell mass spectrometry is a significant advancement, allowing scientists to analyse metabolites within individual cells rather than averaging across cell populations.[205] This level of detail is essential when studying heterogeneous cell populations, like tumours or immune cells, where individual cells may exhibit unique metabolic behaviours that could impact disease progression or treatment efficacy.

Another development is MS imaging, which captures spatial information of metabolites and lipids within tissues.[206] This technology allows researchers to monitor the distribution of drugs and their metabolites in tissues with high precision. Advances in

this area are crucial for early disease detection and drug development. Researchers have also been integrating machine learning with MS to process massive data sets more efficiently, enabling deeper metabolic network analysis and aiding in identifying metabolic biomarkers for diseases

Finally, mass spectrometry has become essential for oligonucleotide mapping.[207] Oligonucleotides are now used in diagnostics and therapeutics, making their correct analysis important. New chromatographic and mass spectrometric methods have been developed to facilitate their analysis. Specifically hydrophilic interaction liquid chromatography coupled to high resolution MS is becoming the workhorse in this field.

We at the metabolomics platform will keep track of new advancements and implement them in our workflow. We will help you with your metabolic analyses be it untargeted metabolic finger printing, stable-labelled metabolic flux analysis or custom targeted pathway analysis.

Recent Advancements in NMR Spectroscopy

Tammo Diercks, *NMR Platform*

NMR spectroscopy is a most versatile analytical technique offering atomic resolution under near-native liquid conditions to derive molecular quantities, identities, structures, dynamics, and even the lowest interaction affinities. The drawbacks of NMR include low detection sensitivity, long measurement times, molecular size limits, and spectral complexity, and have been addressed by advances in the following key enabling areas:

Isotope labeling of proteins benefits from novel protocols for alternative bacterial expression in *V. natriegens*,[208] expression in mammalian cells,[209] segmental labeling,[210] and antibody labeling.[211,212] New site-selectively labeled precursors generate $^{13}CγH_2$-Ile,[213] $^{13}CβH$-Val and $^{13}CγH$-Leu,[214] 2Hα labeled amino acids,[215] and phosphorylated pTyr with alternate aromatic $^{1}H^{13}C$, $^{2}H^{12}C$ labeling.[216] An efficient protocol for $^{13}CH_3$ (I,L,V) labeling with background deuteration was presented for powerful methyl TROSY applications.[217]

Fluorine labeling gives access to extremely well resolved, simplified, intense, and background free ^{19}F NMR spectra that benefit a wide range of biomolecular and especially in-cell applications.[218] Methods to introduce fluorine into proteins[219,220,221,222] include the feeding of fluorinated amino acids like F-Gln,[223] F-Leu,[224,225] F-Val,[226] F-Pro,[227] F-Trp,[228,229] or unnatural p-CF_3-Phe during expression.[230] F-Trp, the best established ^{19}F NMR sensor for local protein dynamics,[231,232] electrostatics,[233] and polymorphism,[234] is now available with ^{13}C labeling,[235] as in ^{13}C, ^{19}F, ^{2}H labeled Phe,[236] to exploit the aromatic ^{13}C,^{19}F TROSY effect.[237] Post-expression modifications of Cys,[238] Thr,[239] His,[240] and Tyr[241] use diverse fluorinated F-tags.[242,243] F-tagging also facilitated easier metabolite distinction[244] or biomarker tracking.[245] ^{19}F NMR assays use fluorinated probes for generalized competitive ligand screening[246] also in in-cell studies.[247] A fluorinated probe glycan allowed to rank its recognition by different influenza viral hemagglutinins.[248]

New processing software increasingly uses Artificial Intelligence to enhance spectra beyond conventional NMR, as by virtual ^{13}C homodecoupling and ^{1}H,^{13}C line sharpening,[249] by reconstructing a pure-phase from a phase-modulated spectrum,[250] cleansing ^{1}H homodecoupled spectra,[251,252] recovering exact chemical shifts and coupling constants from *J*-resolved spectra of strongly coupled spins,[253] or by compensating for B0 and B1 drift.[254] New software for NMR metabolomics aims to improve metabolite assignment and quantification[255-262] and could generate metabolite and protein subspectra from one standard ^{1}H spectrum.[263]

Certainly, new AI-driven software and further refined (isotopic) labeling schemes will be the key drivers of further NMR development.

Transformative Advances in High-Resolution Molecular Imaging in 2024

Isaac Santos and Adriana L. Rojas, *Electron Microscopy (EM) and Crystallography Platforms*

The work at the Electron Microscopy (EM) and Crystallography platforms focuses on studying biological structures at the atomic level. In EM, we specialize in sample vitrification, grid screening, automated image collection using SerialEM, and data processing. Our expertise extends to the quality control of nanocarriers, exosomes, and AAVs, which are critical for gene therapy. We evaluate particle integrity and filled/empty ratios through rigorous statistical analysis. Since 2023, we have been ISO 9001:2015 certified. As part of the BRTA-EM network, we support our users with cutting-edge methods such as cryogenic electron tomography (cryo-ET). Additionally, we provide crystallization services for X-ray crystallography and support data collection at synchrotron facilities like ALBA and the Diamond Light Source (DLS), along with data processing and structure determination.

In 2024, a significant development in cryo-electron microscopy (cryo-EM) was the achievement of ultra-low temperatures using liquid helium, reaching sub-25 K with exceptional thermal stability. Khusainov and co-workers introduced a side-entry specimen holder capable of liquid helium cooling while achieving atomic resolution.[264] This breakthrough enables high-resolution imaging of temperature-sensitive biological samples.

Improvements in phase contrast techniques have also been made, particularly with the development of advanced phase plates and the use of ponderomotive interactions between light and electrons. These innovations enhance image contrast without compromising resolution, facilitating the study of low-contrast specimens.[265]

Another notable recent advancement is One-step Nanoscale Expansion (ONE) microscopy. This technique enables the visualization of the shapes of individual membrane and soluble proteins with approximately 1-nm resolution. The method achieves this by physically expanding the specimen to separate labeling sites, followed by labeling with fluorophores. ONE is a simple and easily applicable technology for studying proteins with high resolution and has the potential to bridge the gap between X-ray crystallography and EM-based techniques.[266]

On the data processing front, Ducrocq and co-workers introduced cryoSPHERE,[267] a deep learning method that takes the protein structure from AlphaFold as input. The method divides the structure into segments and move these segments as approximately rigid bodies to fit the various conformations present in the cryo-EM dataset.

Other new sofware, spIsoNet addresses the challenge of particle orientation bias using artificial intelligence. This end-to-end, self-supervised software improves particle alignment and angular isotropy, significantly enhancing 3D reconstructions of complex biological systems.[268]

In the field of crystallography, Khusainov and co-workers present a provocative perspective on the rise of time-resolved crystallography as the new frontier of macromolecular structure determination. They argue that crystallography now stands on the threshold of directly observing biological function, a capability that could be as revolutionary as structural biology itself. This advancement may ultimately make the prediction of dynamic protein motions as routine as the prediction of protein folding is today.[269]

Looking ahead, electron microscopy (EM) and X-ray crystallography techniques are expected to continue revolutionizing our understanding of biology. The integration of quantum computing and AI could enable researchers to model biomolecular dynamics with unprecedented accuracy within living cells, unveiling intricate details of cellular processes and guiding personalized medicine.

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