

Toward the Understanding of Brain's Molecular Language

by

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ABSTRACT

What underlies the extraordinary capacity of neurons to process information, form memories, and orchestrate complex behaviors? Over a century of research has established that proteins are the central functional molecules of the cell, yet translating this knowledge into an understanding of emergent neural phenomena and effective treatments for neurological disorders remains elusive. We argue that this paradox stems from studying proteins in isolation, overlooking how their function is fundamentally shaped by spatial context and interactions with DNA, RNA, other proteins, lipids, carbohydrates, and metabolites. This coordinated molecular interplay, we posit, ultimately gives rise to the complex neural circuits and behaviors observed in higher organisms. Intriguingly, Alfred Binet foreshadowed this perspective as early as 1889 when he suggested that even simple, single-celled organisms—lacking anatomically defined nervous systems—might harbor a "diffuse nervous system" of molecular interactions within their cytoplasm enabling complex behaviors. However, the historical progression of neuroscience, largely dictated by available methodologies and oscillating between siloed reductionist molecular approaches and systems-level analyses, has not yet been able to fully capture this intricate molecular choreography underlying neural function. In this review, we examine how studying molecular species in isolation, while yielding important insights, has ultimately proven insufficient for understanding emergent neural functions. We propose that recent technological advances in expansion microscopy, molecular anchoring, machine learning-enabled protein detection, and cryo-fixation now make it possible to map molecular networks in their native context. This integrative approach promises to illuminate the molecular "language" of the brain, shedding light on how collective interactions among biomolecules give rise to neuronal emergent abilities—and guide future therapeutic innovations.

Thesis supervisor: Edward S. Boyden

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Chapter 1

Introduction

How does the brain integrate signals from the environment, interpret them, and generate behavioral outputs? This question has undergone repeated conceptual shifts over the history of neuroscience, each driven and impeded by the methodologies of the time (Yuste 2015). A classic example of the influence of methodology on theory is the "grandmother cell" hypothesis, which posited that individual neurons might respond selectively to a highly specific stimulus, such as the face of one's grandmother (Barlow 1972; Gross 2002). This concept grew out of the prevailing neuron doctrine—that single neurons constitute the fundamental structural and functional units of the nervous system—an idea itself rooted in early single-cell techniques such as Golgi stain and microelectrodes (GOLGI 1873; Hubel 1957; Yuste 2015). Over time, however, the limitations of exclusively single-neuron approaches became evident. As more systems-level methodologies emerged, the field shifted toward analyzing how population-level activity gives rise to emergent properties (Swanson and Lichtman 2016; Yuste 2015). Indeed, recent work has shown that studying higher-order interactions across neural networks can illuminate mechanisms of synaptic plasticity, circuit dynamics, and behavior (Südhof 2017).

Yet focusing solely on circuits without considering molecular details can obscure critical mechanistic insights. For instance, the nematode *Caenorhabditis elegans*, harboring just 302 neurons and a fully mapped synaptic connectome, remains incompletely understood from a purely circuit-level perspective (Südhof 2017). Similarly, studying isolated molecular species—DNA, RNA, proteins, lipids, carbohydrates, and metabolites—has failed to fully explain emergent neural properties.

At the molecular level, many brain functions—and dysfunctions—center on alterations in protein concentration, composition, and interactions. Indeed, as early as 1951, Koshtoyants recognized the importance of specialized proteins in synaptic processes (Gomazkov 2021). However, translating this insight into a comprehensive understanding of neural function or effective therapies has proven difficult. This knowledge gap becomes particularly evident when studying neuropsychiatric disorders, where the molecular basis of neural dysfunction remains poorly understood despite significant advances in identifying genetic risk factors. A key challenge is that genes implicated in these disorders typically exhibit complex expression patterns across diverse cell types and circuits, with their protein products engaging in context-dependent interactions that vary by cellular location and molecular environment. Understanding how these spatially organized molecular networks give rise to emergent neural properties—and how their disruption leads to disease—requires moving beyond traditional

approaches that study molecular species in isolation. Instead, we must develop frameworks that can capture the dynamic interplay between genes, proteins, and their molecular partners across different cellular contexts (Hasin, Seldin, and Lusic 2017; Südhof 2017; Van Den Heuvel, Scholtens, and Kahn 2019; Craft, A. Chen, and Nairn 2013).

Intriguingly, Alfred Binet's 19th-century proposition of a "diffuse nervous system" in the cytoplasm of microorganisms underscored the longstanding realization that behavior can emerge from finely tuned molecular and subcellular interactions. While technological limitations of the time led to his work fading into obscurity (Timsit and Grégoire 2021), we argue that today's emerging technologies offer new avenues to visualize and integrate these multifaceted molecular processes. Expansion microscopy, improved molecular anchoring and cryofixation methods, and machine learning-based image analysis collectively enable unprecedented mapping of protein networks in situ (Jia et al. 2024; Laporte et al. 2022). Crucially, these methods facilitate simultaneous measurement of multiple molecular species at high spatial resolution—an essential step toward linking molecular dynamics with functional outcomes at the cellular, circuit, and behavioral levels.

In this review, we examine how six fundamental molecular classes—DNA, RNA, proteins, lipids, carbohydrates, and metabolites—cooperate to shape protein function in neural cells. We first trace key technological advances that have expanded our understanding of each class and then highlight the limitations of studying these molecules in isolation. Finally, we illustrate how new methodologies enabling simultaneous, high-resolution observation of diverse molecular species may help bridge the longstanding gap between molecular identity and interactions and emergent neural phenomena.

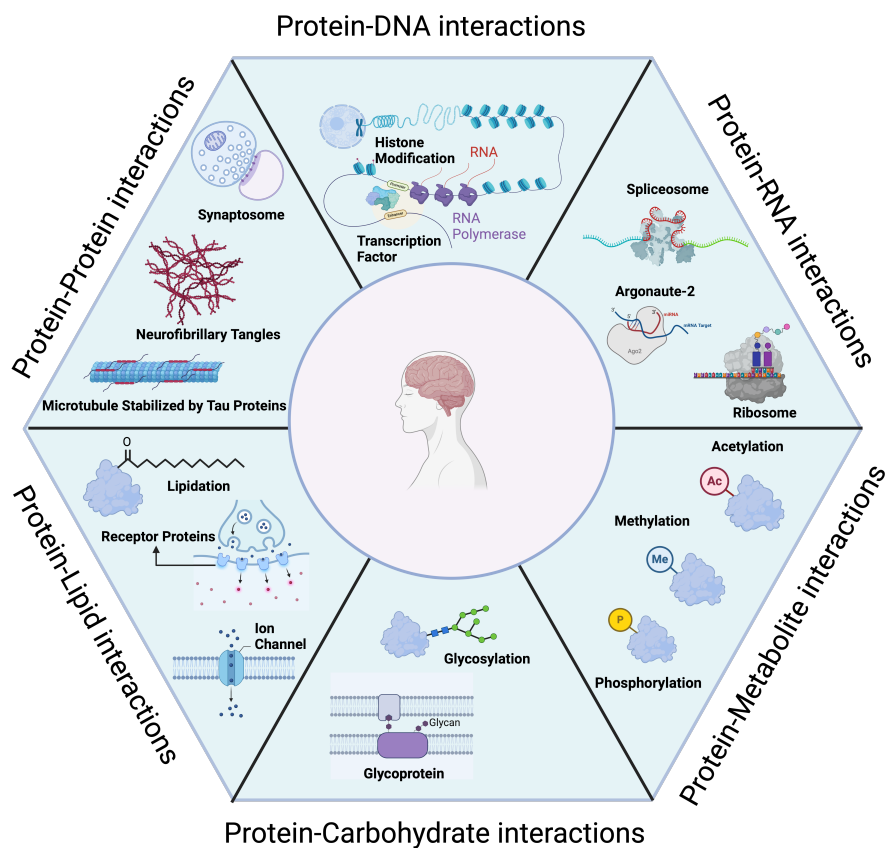


Figure 1.1: Protein interactome in brain

This figure illustrates how proteins interact with major biomolecular classes (DNA, RNA, proteins, lipids, carbohydrates, and metabolites) in neural cells of the brain. This complex molecular cross-talk underscores why studying proteins in isolation provides an incomplete understanding of neural function and highlights the need for integrated approaches to understand the brain's molecular language. This Figure was created with BioRender.com

Chapter 2

DNA

2.1 The Blueprint and Architecture of Brain Function

The remarkable complexity of the nervous system originates at the genetic level, where assemblies of molecular machinery translate the DNA blueprint into each cell's function, identity, connectivity, and capacity for plasticity. Recent technological advances in DNA analysis have dramatically expanded our ability to read and interpret this genetic code, from identifying disease-associated variants to mapping regulatory elements across the genome. Yet these very advances have revealed a crucial insight: DNA does not operate in isolation. Instead, its function is profoundly shaped by interactions with other molecular species, particularly in the nervous system where spatial and temporal regulation of gene expression is essential for neural development and function. This principle is strikingly illustrated by neurological disorders, where genetic risk factors often reside in non-coding regions and exert their effects through complex interactions with other molecular components (Signal et al. 2023). This chapter examines how evolving DNA analysis methodologies have both advanced our understanding and highlighted the limitations of DNA-centric approaches. We review recent technological developments, their applications in neuroscience, and their role in revealing the critical importance of studying DNA within its broader molecular context.

2.2 The Foundational Tools for the Study of DNA

Early efforts to interrogate the genome relied on enzymatic or chemical DNA fragmentation followed by fragment-by-fragment analysis (Heather and Chain 2016; Holley et al. 1965; Satam et al. 2023). The field's first major breakthrough came with the development of Sanger sequencing, which used coupled chain-termination and labeled dideoxynucleotides—termed "first-generation sequencing." This advance enabled the first comprehensive human genome sequencing and led to the first automated sequencer, the Applied Biosystems ABI 370. However, the sequential nature of these reactions limited throughput for whole-genome investigations (Baratta et al. 2022; Hutchison 2007; Satam et al. 2023; Schuster 2008). The emergence of "next-generation sequencing" (NGS) fundamentally transformed genomic analysis by enabling parallel sequencing of nucleotides (Baratta et al. 2022; Rizzo and Buck 2012). This technological leap gave researchers access to multiple sequencing strategies—each

optimized for specific applications: Whole Genome Sequencing (WGS) for comprehensive genomic analysis, Whole Exome Sequencing (WES) for focused investigation of coding regions, targeted sequencing for specific genomic areas, and epigenome sequencing for DNA modifications.

Current NGS platforms fall into two main categories, each with distinct advantages and limitations. Second-generation short-read approaches, including Ion Torrent (which detects released hydrogen ions during DNA synthesis) and Illumina (which uses sequencing-by-synthesis with reversible dye terminators), generate millions of parallel reads of 50–250 base pairs (Baratta et al. 2022; Satam et al. 2023; M. Smith 2022). While these methods offer cost-effective, high-depth coverage, their reliance on reference genome reassembly can introduce errors in repetitive regions.

Third-generation methods, notably Single-Molecule Real-Time (SMRT) sequencing from Pacific Bioscience (PacBio) and Oxford Nanopore Technology (ONT), overcome these limitations by generating reads exceeding 10 kilobases (Baratta et al. 2022; Van Dijk et al. 2018; Satam et al. 2023; M. Smith 2022). These long-read technologies enable the accurate identification of complex variants and repetitive elements. Moreover, their PCR-free library preparation reveals base modifications, providing crucial epigenetic insights.

2.3 The Rise of Genome-Wide Association Studies

These advances in sequencing technology enabled genome-wide association studies (GWAS), which have transformed our understanding of genetic contributions to neural function and disease. GWAS have identified thousands of common variants—particularly single nucleotide polymorphisms (SNPs)—that contribute to complex neurological traits, such as brain volume changes associated with schizophrenia and autism (Adams et al. 2016; García-Marín et al. 2024). However, these studies revealed an unexpected complexity: many risk loci for neurological conditions, including Alzheimer’s disease (AD), map to noncoding regions lacking obvious functional roles (Deplancke, Alpern, and Gardeux 2016; Rao, Yao, and Bauer 2021; Signal et al. 2023). This discovery prompted researchers to investigate how these variants might function through cell-type-specific epigenetic features—such as enhancers, transcription factor binding sites, chromatin accessibility, and three-dimensional genome organization—to modulate gene expression (Gaulton, Preissl, and Ren 2023; Schipper and Posthuma 2022; Xiong et al. 2023).

2.4 Epigenetic Studies Highlight the Need for Investigation of DNA-Protein Interactions

The regulation of epigenomic modifications through interactions with DNA-binding proteins (including histones, methyltransferases, and transcription factors) has shifted research focus from studying DNA in isolation to exploring DNA-protein interactions (DPI). The evolution of DPI analysis techniques spans several generations of technology. Early assays included nitrocellulose filter binding, electrophoretic mobility shift assays (EMSA), and Southwestern blotting, which required purified protein and DNA samples to measure binding strength. Later, more sophisticated *in vitro* technologies emerged—such as proximity ligation assays (PLA),

footprinting approaches, systematic evolution of ligands by exponential enrichment (SELEX), and protein-binding microarrays (PBM)—increasing both sensitivity and throughput of DPI studies. However, these methods still relied on *in vitro* conditions that couldn't fully replicate the native cellular environment(Qu and Du 2024).

Strategies like yeast one-hybrid (Y1H) systems and phage display eliminated the need for extensive protein purification but couldn't always recapitulate the spatiotemporal complexity within cells. This limitation meant they sometimes reported DNA-protein interactions that would be physically impossible in cells due to spatial or temporal constraints. The introduction of chromatin immunoprecipitation (ChIP) transformed the field by enabling analysis of DNA-protein complexes in their native cellular context through chemical or UV crosslinking, followed by isolation of the crosslinked chromatin and analysis of de-crosslinked DNA. The integration of polymerase chain reaction (PCR), quantitative PCR (qPCR), microarrays (ChIP-chip), NGS sequencing technologies (ChIP-seq), and advanced methods (ChIP-exo) provided powerful tools for mapping protein binding sites across the genome. While initial limitations in recovery yield restricted ChIP-based analyses to bulk cell populations, ongoing refinements including microfluidics and DNA barcoding approaches have begun to enable single-cell applications, though data sparsity remains a challenge(Baratta et al. 2022; Qu and Du 2024).

As an alternative to ChIP-seq, cleavage under targets and release using nuclease (CUT&RUN)-derived from chromatin immunocleavage (ChIC)-offers a different approach. This method tethers micrococcal nuclease (MNase) to DNA via transcription factor-specific antibodies, selectively cleaving DNA at protein-binding sites. While CUT&RUN provides high-resolution data on the native chromatin landscape, its supernatant-based recovery of MNase-cleaved fragments and consecutive adapter insertions proved less compatible with single-cell platforms. This limitation led to the development of Cleavage Under Targets and Tagmentation (CUT&Tag), which uses A-Tn5 transposase to simultaneously cleave protein-bound DNA and integrate sequencing adapters. Another significant Tn5-based assay, the assay for transposase-accessible chromatin (ATAC-seq), interrogates chromatin accessibility, a vital determinant of DNA-protein interactions(Qu and Du 2024; M. Smith 2022; F. Sun et al. 2025).

2.5 Beyond Linear DNA: Understanding 3D Organization

While DNA-protein interactions are fundamental to gene regulation, understanding how these interactions are arranged in higher-order nuclear space requires examination of the genome's three-dimensional organization. Conventional chromosome conformation capture methods (3C, Hi-C) and their variants have led our understanding of higher-order genome architecture, revealing features such as topologically associated domains (TADs). However, these approaches require large cell populations, potentially masking rare or dynamic cell-to-cell variations—a particular concern in neurons, which exhibit both physiological homogeneity and subtle subtype-specific state heterogeneity(Rahman et al. 2023). The introduction of single-cell and single-nucleus methodologies has marked a significant advance in the field. These include techniques like single-cell high-throughput chromosome conformation capture (scHi-C) for studying 3D interactions, single-cell ATAC-seq for profiling chromatin accessibility, and single-nucleus methyl-3C (sn-m3C) for examining DNA methylation and

chromatin interactions in individual cells. When integrated with single-cell RNA-seq, these tools have supported the creation of extensive atlases describing chromatin accessibility and gene expression in the brain (D.-S. Lee et al. 2019; Rahman et al. 2023).

A significant limitation of many single-cell methods is their reliance on tissue dissociation, which obscures spatial features crucial for brain architecture. DNA fluorescence in situ hybridization (FISH) and multiplexed Oligopaint approaches partially address this limitation by allowing visualization of chromatin organization in spatially intact nuclei. However, these workflows often require super-resolution microscopy (e.g., STORM), which introduces additional constraints regarding throughput, imaging depth, specialized expertise, and cost (Beliveau et al. 2012; Bintu et al. 2018).

2.6 Expansion Microscopy: A New Window into Nuclear Architecture

The challenge of balancing spatial resolution with comprehensive coverage of the genome’s three-dimensional organization has led to the development of expansion microscopy (ExM) based techniques. ExM involves embedding brain tissue in a swellable polymer matrix, anchoring targeted molecules within, and physically expanding the sample to enable visualization of previously unresolvable structures using conventional microscopy. This process preserves spatial relationships among biomolecules while enabling detailed exploration of nuclear organization. ExM-based studies have provided transformative insights into the three-dimensional organization of DNA in neuronal nuclei, including the spatial partitioning of transcriptionally active and repressed chromatin domains, with clear distinctions in their arrangements between excitatory and inhibitory neurons (Acke et al. 2022).

The technological advances in DNA analysis have revolutionized our understanding of how chromatin organization and epigenetic modifications regulate gene expression in neural cells. However, these insights into DNA’s structural complexity represent only one layer of genetic regulation. A complete understanding of transcriptional control in the nervous system requires further investigation into the interplay between DNA modifications, RNA-protein interactions, and cell-type specific regulatory networks.

Chapter 3

RNA

3.1 Introduction to RNA in Neural Systems

Whereas DNA provides the foundational blueprint for brain function, its influence is manifested through the dynamic processes of RNA transcription and regulation. The complexity of the nervous system is mirrored by the diversity of its RNA species, including messenger RNA (mRNA), long noncoding RNA (lncRNA), and microRNA (miRNA), along with their numerous chemical modifications such as methylation, adenylation, and splicing. These RNA molecules not only mediate the transfer of genetic information from DNA but also play vital regulatory roles in gene expression, neurodevelopment, and synaptic plasticity. Both cell-type-specific programs and activity-dependent changes in RNA dynamics govern processes ranging from neuronal development to memory formation, making them indispensable for decoding how neural systems operate (Lennox, Mao, and Silver 2018).

3.2 Evolution of RNA Methodologies

Technological advances in RNA detection and quantification have drastically enhanced our understanding of how RNA influences brain function. Early approaches utilizing Northern blots and in situ hybridization provided foundational insights into neuronal heterogeneity but were limited by sensitivity and the requirement for prior sequence knowledge. Subsequent development of reverse transcription polymerase chain reaction (RT-PCR) and microarray-based approaches circumvented many of these limitations. RT-PCR offered high-sensitivity detection and amplification of specific transcripts, while microarrays enabled the parallel quantification of thousands of RNA species. These advances led to the discovery of transcriptomic alterations in neuropsychiatric disorders and the creation of first comprehensive gene expression atlas of the human brain; the Allen Human Brain Atlas (Bahn et al. 2001; Shen, Overly, and Jones 2012).

3.3 Methodological Advancements Enable Exploration of Previously Undetected RNA Molecules

The development of RNA sequencing (RNA-seq) technologies marked a significant advance over traditional RT-PCR and microarray-based methods, which were limited to detecting known transcripts. RNA-seq methodologies, particularly single-cell and single-nucleus RNA sequencing (sc/snRNA-seq), revolutionized the field by providing an unbiased, genome-wide survey of the neural transcriptome. These approaches have uncovered previously unknown neuronal subtypes and highlighted the extensive complexity and heterogeneity of neural gene expression, including the identification of novel RNA species and alternative splicing events (Eze et al. 2021; Hrdlickova, Toloue, and B. Tian 2017; Tang et al. 2009; Tasic et al. 2016). Building on these breakthroughs, the implementation of droplet-based microfluidics (e.g., in Drop, Drop-seq) and approaches such as cellular barcoding and unique molecular identifiers (UMIs) further refined sc/snRNA-seq for accurate RNA quantification (Jaitin et al. 2014; Macosko et al. 2015; Piwecka, Rajewsky, and Rybak-Wolf 2023; Pollen et al. 2014; Treutlein et al. 2014). These innovations not only enhanced throughput and sensitivity but also expanded the analytical toolkit with optimized bioinformatic pipelines.

3.4 Reconstructing the Three-Dimensional Map of the Transcriptome

Although scRNA-seq and snRNA-seq have profoundly enhanced our knowledge of neuronal cell-type diversity, they require dissociation of intact cells, which eliminates spatial information. In a neural context, spatial resolution is crucial for reconstructing neuronal networks and elucidating how local transcriptomic changes—particularly at synapses—contribute to synaptic plasticity. The fact that protein synthesis can occur locally in dendrites and axons (far from the soma) underscores the necessity of spatially resolved transcriptomic analyses.

To address this need, spatial transcriptomic (ST) methodologies emerged and can be broadly categorized into image-based and sequencing-based platforms. Image-based ST approaches rely on in situ hybridization (ISH) or in situ sequencing (ISS). ISH-based methods (e.g., split-FISH, EASI-FISH, EEL FISH) use fluorescently labeled probes to detect targeted RNAs at single-cell or subcellular resolution but can be limited by optical crowding and smaller fields of view. ISS-based techniques (e.g., ExSeq, BARseq2) employ rolling circle amplification of padlock probes followed by next-generation sequencing chemistries (F. Chen, Tillberg, and Boyden 2015; Marx 2021; Moses and Pachter 2022; L. Tian, F. Chen, and Macosko 2023; C. G. Williams et al. 2022). While ISS-based approaches can access larger tissue areas, they often have constraints related to transcript coverage and spatial resolution (R. Chen et al. 2024).

3.5 Limitations of Studying RNA Molecules in Isolation

Spatial transcriptomics has revealed that mRNAs encoding synaptic proteins are often localized to dendrites and axons along with polyribosomes, underscoring the importance of local protein synthesis in synaptic function (Steward and Levy 1982; Oswald Steward and Schuman 2001; Cajigas et al. 2012). However, the function of any given RNA is intimately linked and regulated by its molecular context, including interactions with proteins and cellular compartments. This is particularly evident in the brain, where interplay with RNA-binding proteins (RBPs) and other trans-regulators (e.g., miRNAs) orchestrates critical aspects of RNA splicing, transportation, localization, stability, and translation, thereby contributing to cortical development and function (Eliscovich, Shenoy, and Singer 2017; Liu-Yesucevitz et al. 2011; Prashad and Gopal 2021).

These findings highlight the limitations of analyzing RNA molecules in isolation. To capture the activity-dependent dynamics of the local translatoome, multimodal methodologies termed RNA interactome capture (RIC) were developed. Techniques such as RNA immunoprecipitation (RIP) and crosslinking and immunoprecipitation (CLIP), including variants like CLIP-Seq, HITS-CLIP, and iCLIP, offer insights into how RNA-binding proteins regulate RNA metabolism. Additional enrichment strategies based on poly(A)-tail capture (vRIC, RBDmap, serIC, qRIC, eRIC, cRIC), azide-biotin click chemistry (RICK, CARIC), silica-based protocols (2C, TRAPP), and liquid-liquid phase extraction (OOPS, PTex, XRNAX) have further expanded the scope of RNA interactome analyses. Although these methods have advanced our understanding of RNA-protein interactions in the brain, considerable challenges remain in uncovering the intricate networks that regulate local translation and synaptic function (Ramanathan, Porter, and Khavari 2019; J. M. Smith, Sandow, and Webb 2021; Steinmetz et al. 2023).

Chapter 4

Proteins

4.1 The Role of Proteins in the Brain’s Molecular Language

Proteins serve as the primary functional units of brain cells, orchestrating intricate molecular interactions that determine both cellular morphology and function. From controlling DNA expression that drives neurogenesis and neuronal plasticity to catalyzing chemical reactions, transporting key metabolites, and mediating intercellular communication through neurotransmitters, signaling molecules, channels, and receptors, proteins perform an extensive range of critical functions in the brain. Given their wide-reaching roles, the field of “Neuroproteomics” emerged to systematically investigate how protein function, interactions, dynamics, and structure underlie brain development, neuronal connectivity, and disease (Kim et al. 2004; Shevchenko et al. 2015). This discipline aims not only to unveil fundamental neurobiological mechanisms but also to propel biomarker discovery for neurological disorders and to identify novel therapeutic targets (Craft, A. Chen, and Nairn 2013; Gomazkov 2021; Natividad et al. 2018).

4.2 Multiple Layers of Protein Regulation and Neural Plasticity

While genomic and transcriptomic approaches have provided tremendous insight into neuronal identity and function, they do not fully capture the dynamic protein landscape of the brain. The canonical central dogma, depicting a linear flow from DNA to RNA to protein, vastly oversimplifies actual intracellular dynamics, where fine regulation of RNA synthesis, processing, and degradation combined with equally complex control of protein translation, trafficking, and turnover leads to substantial variability between transcript abundance and protein concentration (Timsit and Grégoire 2021; Vogel et al. 2010). Furthermore, although use of new technologies such as TRAP (Translating Ribosome Affinity Purification) transgenic mice have refined our view of mRNA population destined for translation, these techniques are unable to capture post-translational modifications (PTMs) that critically regulate protein function and turnover (Craft, A. Chen, and Nairn 2013; Doyle et al. 2008; Heiman et al. 2008).

Consequently, comprehensive analysis of the brain proteome—encompassing abundance, structure, localization, and modification states—remains indispensable for understanding how proteins enable neural plasticity and higher-order functions.

4.3 Evolution of Protein Analysis Methods

The study of brain protein architecture has undergone dramatic evolution over recent decades. Early investigations relied on traditional biochemical methods such as immunohistochemistry (IHC) and western blotting, which provided important but limited information about protein localization and expression levels. These techniques, while valuable, were limited by antibody availability and were not suited for simultaneously profiling large numbers of proteins. The advent of two-dimensional gel electrophoresis (2D-GE) paired with mass spectrometry (MS) and liquid chromatography (LC) techniques (e.g., 2D-LC-MS/MS) ushered in the first high-throughput mapping of the brain proteome (Mueller et al. 2006). Subsequent development of “shotgun” proteomic approaches facilitated proteome-wide analysis across 18 anatomical brain regions, identifying approximately 7,000 protein species in the healthy human brain (Fernandez-Irigoyen et al. 2015).

Among these approaches, MS technologies proved transformative. Early MALDI-TOF MS gave researchers the means to identify proteins and certain PTMs efficiently, but it showed limited capacity for analyzing highly complex neural tissue. This spurred the development of more sophisticated MS platforms and analytical methods—such as ICAT, iTRAQ, and SILAC—that facilitate isotope labeling and relative quantification of proteins in multiplexed samples (Gygi et al. 1999; Ong et al. 2002; Ross et al. 2004).

4.4 Single-Cell Protein Technologies

Despite these advances in MS technologies, significant challenges remained in analyzing proteins at the single-cell level. While MS systems could detect samples present at hundreds of copies, and technologies such as Laser Capture Microdissection (LCM) and Fluorescence-activated cell sorting (FACS) enabled isolation of specific single cells, substantial sample loss during purification and processing initially made single-cell protein sequencing impossible (Espina et al. 2006; Zhu et al. 2018).

To address this challenge, methods such as NanoPOTS (Nanodroplet Processing in One Pot for Trace Samples) substantially reduce sample loss and enhance sensitivity, enabling the reliable quantification of otherwise undetectable proteins (Zhu et al. 2018). Meanwhile, SCoPE-MS (Single-Cell Proteomics by Mass Spectrometry) incorporates labeled carrier proteomes to mitigate sample loss during chromatography, thus boosting detection sensitivity (Budnik et al. 2018; Mansuri, K. Williams, and Nairn 2023).

4.5 The Need for Spatial Proteomics Technologies

Understanding neuronal function requires not only identifying proteins and their abundance but also mapping their spatial organization and contextual interactions. This spatial context

is particularly crucial given our evolved understanding of protein function. While the traditional one gene-one enzyme paradigm (Beadle and Tatum 1941) oversimplified functional annotation of proteins, contemporary research has revealed that proteins can adopt diverse functions through alternative splicing, PTM-driven functional shifts, and incorporation into multi-subunit complexes (Tawfik 2010). This functional plasticity is especially evident in the brain, where non-stoichiometric and dynamic protein assemblies—ranging from rigid amyloid fibrils to flexible signalosomes—necessitate spatially resolved proteomics approaches (Pancsa et al. 2019). While advancements in techniques such as XL-MS (cross-linking mass spectrometry), APEX (engineered ascorbate peroxidase), BioID, and TurboID enable mapping of protein–protein interaction within defined subcellular compartments, they are limited by antibody availability, multiplexing capacity, and the large amount of starting material required (Leitner et al. 2010; P. Lu et al. 2007; Roux et al. 2012).

4.6 Single-Molecule Proteome Analysis Methodologies

MS-based proteomic methodologies have significantly advanced our understanding of neuroproteome complexity, while simultaneously revealing fundamental technical limitations. A critical challenge emerges from the broad dynamic range of protein abundance in neuronal populations—abundant "housekeeping" proteins (10,000–12,000 proteins) frequently mask the detection of low-abundance proteins that often mediate functionally significant distinctions in brain circuits (Carlyle et al. 2017; Wilhelm et al. 2014). Unlike nucleic acid analysis, which benefits from PCR-based amplification, protein detection lacks analogous amplification strategies, presenting a fundamental obstacle for single-molecule analysis (Mansuri, K. Williams, and Nairn 2023).

Nonetheless, emerging single-molecule protein sequencing platforms are converging on alternative solutions that exploit Edman-based degradation, affinity-based detection, or transit-based sequencing. For instance, the fluorosequencing approach employs site-specific fluorescent labeling of cysteine, lysine, and phosphoserine residues, followed by TIRF-based monitoring of amino acid removal by Edman degradation (Swaminathan, Alexander A Boulgakov, et al. 2018). While this is a key milestone, chemical instability of the labels and loss of spatial information present notable limitations.

Zheng et al. (2024) recently described a “reverse translation” approach using a modified Edman process to convert peptide fragments into DNA-barcoded amino acids, thereby preserving identity and position of the originating peptide in a DNA library. Although promising, it requires efficient conjugation of DNA to peptide’s C-terminus and depends on high-specificity antibodies for each amino acid. Similarly, affinity-based methods (FRET, DNA-PAINT, DNA nanoscopy) leverage DNA hybridization and fluorescent readouts but rely on sophisticated microscopy instruments and robust pre-labeling strategies that impede many in situ applications (Floyd and Marcotte 2022; L. Zheng et al. 2024).

Inspired by the success of nanopore-based DNA sequencing, a “protein sequencing-by-transit” concept uses a motor enzyme, such as Hel308 DNA helicase, to pull peptides tagged with 80-nucleotide DNA strand through a biological pore (MspA). The distinct ionic signals associated with each amino acid have provided another avenue for single-molecule readouts, though challenges related to noise reduction and amino-acid discrimination remain

(Brinkerhoff et al. [2021](#); Floyd and Marcotte [2022](#); Mansuri, K. Williams, and Nairn [2023](#)).

The field of neuroproteomics has progressed from simple antibody-based methods to sophisticated single-molecule approaches. While each new method has expanded our capabilities, none yet provides the perfect combination of sensitivity, throughput, and spatial resolution needed for comprehensive brain protein analysis. Future advances will likely require both technical innovation and creative integration of existing methods.

Chapter 5

Lipids, Carbohydrates, and Metabolites

5.1 Unexpected Players in the Molecular Language of Brain

Despite the traditional emphasis on DNA- and RNA-driven regulation of protein expression and function, an expanding body of evidence highlights how proteins are critically regulated by both complex biomolecules and smaller metabolites. These molecules modulate protein function through direct interactions with complex lipids and carbohydrates (in membranes, cellular compartments, and extracellular spaces) and through post-translational modifications (PTMs), where various substrates including metabolic products can modify proteins, thus reshaping the functional landscape of the brain. Currently, over 620 types of PTMs regulating protein function have been identified, yet many remain underexplored. During PTMs, proteins may undergo proteolytic cleavage or be conjugated with specific biochemical moieties, fundamentally altering their physical properties and functional capacities. Dysregulation of such protein modifications has been implicated in a multitude of neurological disorders (B. Chen et al. 2018; Cifani and Kentsis 2017; Fung and Liu 2018; Torres, Dewhurst, and Sundararaman 2016; R. Wang and Y. Q. Chen 2022; Xu et al. 2018; Zavalova, Zgoda, and Nikolaev 2017).

5.2 The Complex Interplay of Lipids, Carbohydrates, and Metabolites in Neural Function

5.2.1 Lipids

Comprising approximately 35-40% of the neuron-rich gray matter's dry weight, lipids have emerged as crucial regulators of neurotransmitter release, synaptic plasticity, and action potential conduction beyond their known structural roles in cellular membranes, myelin sheaths, and cerebrospinal fluid (Incontro et al. 2025; O'Brien and Sampson 1965; Osetrova et al. 2024). Far from being static, the lipidome exhibits continuous remodeling and reorganization in response to developmental and environmental stimuli, thereby supporting neuronal plasticity for adaptation, and recovery from injury and disease (Corraliza-Gomez, Sanchez, and Ganfornina

2019). The importance of lipids in the nervous system is underscored by the observation that roughly 5% of all protein-coding genes are dedicated to lipid metabolism, and their mutations lead to risk and progression of brain disorders like Alzheimer (Corraliza-Gomez, Sanchez, and Ganfornina 2019; Tumanov and Kamphorst 2017; Van Meer, Voelker, and Feigenson 2008; Xicoy, Wieringa, and Martens 2019). Moreover, protein lipidation—estimated to affect nearly 20% of all proteins—critically modulates membrane association, protein trafficking, and interactions with cofactors, and its dysregulation is strongly linked to neurodegenerative disorders, including Parkinson’s disease (Cho and Park 2016; Yuan et al. 2024).

5.2.2 Carbohydrates

Once regarded predominantly as energy sources, carbohydrates have emerged as pivotal modulators of neurotransmitter synthesis, ion homeostasis, and oxidative stress management in the brain (Dienel 2019). Glycosylation, one of the most prevalent PTMs, involves the addition of glycans to proteins or lipids and impacts protein folding, trafficking, stability and cell–cell communication. Notably, over 70% of brain proteins are glycosylated, and congenital disorders of glycosylation (CDGs) frequently manifest with severe neurological symptoms, including psychomotor retardation and cognitive deficits (Pradeep, Kang, and B. Lee 2023; Sytnyk, Leshchyns’ka, and Schachner 2021; Tena and Lebrilla 2021; Xiao et al. 2018). The pervasive role of glycosylation thus underscores the need to integrate the study of protein–carbohydrate interactions into broader inquiries of brain function and pathology.

5.2.3 Metabolites

The brain metabolome encompasses a vast array of low-molecular-weight biomolecules (≤ 1500 – 2000 Da) produced through endogenous processes and shaped by external factors (Nebert and Vesell 2013). Beyond serving as intermediate or terminal products of metabolic pathways, many metabolites function as signaling molecules that activate adaptive responses, particularly under conditions of stress or nutrient scarcity (Piazza et al. 2018; Yang, Li, and Snyder 2012). These molecules influence protein activity via direct binding (e.g., as substrates, cofactors, or ligands) or through non-enzymatic PTMs (nPTMs). Dysregulation of specific metabolite-driven PTMs has been reported in aging and in neurodegenerative disease states, highlighting the significance of these molecular events in brain function and dysfunction (Baldensperger, Preissler, and Becker 2025; Kabir et al. 2023; J.-Y. Lu et al. 2011).

5.3 Methodological Evolution for Studying Lipids, Carbohydrates, and Metabolites in Neural Systems

5.3.1 Established Approaches

Early explorations into the roles of lipids, carbohydrates, and metabolites in neural cells utilized diverse detection and visualization strategies. Broadly, these methodologies can be grouped into three categories:

1- Spectroscopy-Based Platforms: Nuclear Magnetic Resonance (NMR) is a nondestructive technique that provides high reproducibility and structural insights but suffers from lower sensitivity. Thus, this method is better suited for studying relatively abundant targets or performing multicellular or tissue-level investigations (F. Sun et al. 2025; X. Wang, Peng, and Zhao 2024).

2- Mass Spectrometry (MS)-Based Platforms: MS is prized for its high sensitivity and capacity to detect sub-attomolar concentrations while surveying multiple analytes simultaneously. However, conventional MS is often destructive, limiting the repeatability and spatial mapping of multiple analytes (F. Sun et al. 2025; X. Wang, Peng, and Zhao 2024).

3- Microscopy-Based Platforms: Super-resolution methods such as stimulated emission depletion (STED), photoactivated localization microscopy (PALM), and stochastic optical reconstruction microscopy (STORM) enable nanoscale spatial profiling of biomolecules in cells. While they offer unprecedented resolution, they are typically low-throughput and can provide limited biochemical detail (F. Sun et al. 2025; X. Wang, Peng, and Zhao 2024).

5.3.2 Technological Advancements and Limitations

Continued innovation in ion sources, separation, and sampling methodologies has dramatically expanded our capacity to examine lipids, carbohydrates, and metabolites at unprecedented spatial and temporal resolutions. For instance, coupling liquid chromatography (LC), gas chromatography (GC), or capillary electrophoresis (CE) with MS has improved sensitivity and selectivity, even in low-volumes samples, although such approaches generally require a priori knowledge of the specific features of metabolite under study (Wolthuis et al. 2020). Similarly, innovations in ionization methods (e.g., MALDI, DESI, and SIM) have fueled the evolution of mass spectrometry imaging (MSI), enabling fine-grained spatial mapping of metabolic profiles within tissue sections. Nonetheless, the necessity of vacuum conditions and the risk of substrate interference continue to restrict the applicability of many MSI protocols for certain sample types (X. Wang, Peng, and Zhao 2024). Meanwhile, emerging optical and molecular tools—such as Raman microscopy and expansion microscopy—offer complementary opportunities for metabolite-focused analyses, partially compensating for the inherent constraints of traditional MS- and NMR-based modalities (D.-e. Sun et al. 2021; X. Wang, Peng, and Zhao 2024).

5.4 Studying Non-Enzymatic PTMs and Protein–Metabolite Interactions

Non-enzymatic protein-metabolite post-translational modifications (nPTMs) have emerged as critical regulatory mechanisms that enable rapid protein responses to cellular changes through feedback and feedforward control. The spatial distribution and concentration of specific metabolites within cells are crucial determinants of these modifications (Leutert, Entwisle, and Villén 2021; B. Zhang and Schroeder 2025). The significance of nPTMs is particularly evident in aging and neurodegenerative disorders, where these modifications accumulate and are frequently found in protein aggregates (Baldensperger, Preissler, and Becker 2025).

While conventional analytical techniques such as mass spectrometry (MS), nuclear magnetic resonance (NMR), and immunoblotting are widely employed to study PTM-modified proteins, several technical challenges persist. These include the difficulty in distinguishing structurally distinct but mass-identical PTMs (such as (ω -N^G-monomethylarginine [MMA], ω -N^G,N^G-asymmetric dimethylarginine [ADMA], and ω -N^G,N^G-symmetric dimethylarginine [SDMA])) and the detection of low-abundance PTM-modified proteins against a background of abundant unmodified variants. To address these limitations, researchers have developed complementary approaches, including the generation of site-specific and Pan-PTM antibodies and the application of chemical probes for selective enrichment of PTM-modified proteins (N. Zhang, Wu, and Q. Zheng 2024).

Lipids, carbohydrates, and metabolites are integral to the functional proteome of the brain, regulating protein properties and impacting an array of neural processes. Advances in MS, NMR, super-resolution microscopy, and emerging hybrid approaches are enabling increasingly fine-grained analyses of these molecules and their interactions. Nonetheless, technical challenges persist—particularly regarding the sensitivity, specificity, and throughput of PTM-focused methodologies. Addressing these limitations through continued methodological innovation and interdisciplinary collaboration is vital for exploring the highly interconnected molecular landscape of the brain.

Chapter 6

Future Directions

The continual development of single-molecule and spatial proteomic technologies promises to reshape our understanding of protein dynamics in the brain. Nonetheless, key challenges persist, such as the inability to directly amplify proteins and the need for robust and specific labeling strategies. Below, we discuss emerging tools, potential avenues for integration of these methods with computational approaches, and the conceptual advances they may bring to neuroscience research.

6.1 From Bulk Measurements to Single-Molecule Insights: Advancements and Limitations

Traditional proteomic and transcriptomic approaches, often performed on pooled tissue samples, provide powerful but averaged measurements. While these methods have yielded many insights into neuronal function and dysfunction, they lack the ability to resolve the complexity and heterogeneity of brain cells. Recent single-cell and single-molecule strategies (including single-cell multi-omics) have begun to address this gap by capturing the molecular composition and spatial context of individual cells. These finer-resolution approaches reveal important differences in protein expression and interaction networks across distinct neuronal populations.

Due to the optical diffraction limit (~ 200 nm), conventional microscopes cannot resolve structures or interactions below this scale. Super-resolution fluorescence imaging techniques—such as structured illumination microscopy (SIM), stimulated emission depletion (STED) microscopy, and single-molecule localization microscopy (SMLM)—have partially overcome these constraints. Similarly, mass spectrometry-based imaging (MSI) strategies can achieve subcellular resolution of protein distributions and PTMs. However, many of these technologies still face limitations in throughput, dynamic range, and the ability to monitor complex protein-protein interactions or multiple PTMs simultaneously. As shown in recent comparative evaluations, no single platform currently achieves comprehensive spatial coverage and single-molecule sensitivity for a wide range of protein modifications.

6.2 Overcoming the Challenges of Single-Molecule Protein Sequencing

6.2.1 New ExM Variants: Expanding the Spatial Frontier

Expansion microscopy (ExM) variants—such as High-throughput Expansion Microscopy (HiExM), Iterative ExM, and Single-shot 20-fold expansion protocols—are rapidly evolving. By isotropic physical magnification of biological specimens, these methods circumvent diffraction limits and provide increased resolution of protein distributions and interactions. Other variations of the ExM-based technologies such as One-step nanoscale expansion microscopy (ONE) have recently demonstrated the ability to visualize protein morphology using conventional light microscopy. Ongoing efforts to modify anchoring chemistries and polymer formulations may improve expansion factors even further, potentially allowing the interrogation of previously inaccessible ultrastructural features (Day et al. 2024; Jia et al. 2024; S. Wang et al. 2024).

6.2.2 Combining ExM with Other Emerging Technologies

Combinatorial approaches incorporating robust anchoring chemistries, and Edman degradation—implemented within an ExM framework—could enable the multiplexed identification and quantification of proteins in situ. However, the successful deployment of such methods hinges on designing molecular binders with high affinity and specificity for individual amino acids or post-translational modifications (PTMs). Advances in artificial intelligence (AI)-driven platform development may be particularly valuable here, as computational methods can facilitate the design and optimization of next-generation binders. High-specificity binders that recognize amino acids and their PTMs, coupled with iterative rounds of labeling and chemical cleavage, may allow researchers to reconstruct protein sequences and PTM profiles at single-molecule resolution. Such comprehensive analyses would greatly advance our understanding of protein-level regulation underlying neural plasticity and disease pathology. However, these integrated workflows will likely require significant alterations to traditional protocols—especially with respect to Edman degradation, which typically involves harsh acidic chemicals that could damage other biomolecules (DNA, RNA, metabolites) in the sample. Methodological refinements will be needed to preserve native structures, minimize artifacts, and maintain compatibility with various readout modalities.

6.3 Preserving Native States: Cryo-fixation and Metabolite Imaging

A critical challenge in spatial proteomics is capturing the dynamic states of proteins and metabolites, many of which change within seconds in vivo. By coupling ExM and other spatial proteomic tools with rapid cryofixation of tissues, researchers can stabilize transient molecular configurations (Jia et al. 2024; Vandereyken et al. 2023). This approach reduces the likelihood of perturbations arising from sample preparation and allows the investigation of

fast-evolving cellular processes. Future improvements in cryogenic sample handling, imaging, and polymer expansion chemistry could thus further bridge the gap between static snapshots and real-time views of neuronal function.

6.4 Dynamic Neuronal Identities and Epigenetic Considerations

6.4.1 Revisiting Cell Types and Neural Plasticity

As single-molecule datasets grow, a fundamental question arises: can neurons be classified with immutable identities if they are capable of dynamic changes over time? Hebb's (1949) notion of neuronal plasticity underscores that circuit interactions are subject to rapid and long-term modification, and that the same circuit may respond differently to identical stimuli at different times (Morris 1999). These observations complicate efforts to define "cell types" solely on the basis of single molecular markers.

6.4.2 Epigenetic Clocks and Disease Progression

Recent work has shown that epigenetic modifications, such as methylation patterns, can serve as cell type-specific "clocks," capturing aspects of biological rather than chronological age. In models of Alzheimer's disease, for instance, neuronal and glial cells often display accelerated epigenetic aging (Tong et al. 2024). Thus, refined epigenetic mapping technologies could integrate with proteomic and transcriptomic approaches to categorize cells along a continuum of functional states rather than static cell-type labels. These approaches may facilitate more straightforward multi-modal data integration thus allowing the researchers to map cells to their developmental and disease trajectories in both time and space.

6.5 Computational Approaches and Data Integration

6.5.1 Leveraging AI for Protein Interaction Modeling and Simulation

The volume and complexity of single-molecule proteomic datasets demand robust computational tools. Advances in AI hold the potential to enable more efficient modeling and simulation of protein interaction networks, drastically reducing the experimental burden of exhaustive coverage for every PTM or binding partner. By predicting protein-protein interactions, designing optimal binders, and identifying the most informative subsets of targets for detailed study, AI-based methods are poised to accelerate our ability to generate comprehensive proteomic maps of neural cells (Swaminathan, Alexander A. Boulgakov, and Marcotte 2015; Yao et al. 2015).

6.6 Concluding Remarks

Recent developments in single-molecule and spatial proteomic tools—ranging from ExM variants to AI-assisted binder design—hold great promise for unraveling the intricate molecular architecture of the brain. Continued progress will rely on improving the specificity and throughput of these methods, preserving the native states of molecules, and integrating complementary data sources. As these approaches mature, they are poised to create multi-modal atlases that synthesize multiple data types with high spatial resolution in the same tissue samples. These atlases will integrate single-molecule genomic, epigenomic, transcriptomic, proteomic, and metabolomic data, illuminating how proteins and their connections with other molecules dynamically shape neuronal identities and functions. Ultimately, such insights may drive new strategies for diagnosing, preventing, and treating neurological disorders, advancing our fundamental grasp of the complex molecular language of the brain.

References

- Acke, Aline, Siska Van Belle, Boris Louis, Raffaele Vitale, Susana Rocha, Thierry Voet, Zeger Debyser, and Johan Hofkens (Sept. 2022). “Expansion microscopy allows high resolution single cell analysis of epigenetic readers”. en. In: *Nucleic Acids Research* 50.17, e100–e100. ISSN: 0305-1048, 1362-4962. DOI: [10.1093/nar/gkac521](https://doi.org/10.1093/nar/gkac521). URL: <https://academic.oup.com/nar/article/50/17/e100/6611043> (visited on 01/14/2025).
- Adams, Hieab H H et al. (Dec. 2016). “Novel genetic loci underlying human intracranial volume identified through genome-wide association”. en. In: *Nat Neurosci* 19.12, pp. 1569–1582. ISSN: 1097-6256, 1546-1726. DOI: [10.1038/nm.4398](https://doi.org/10.1038/nm.4398). URL: <https://www.nature.com/articles/nm.4398> (visited on 01/14/2025).
- Bahn, S., S.J Augood, M. Ryan, D.G. Standaert, M. Starkey, and P.C Emson (July 2001). “Gene expression profiling in the post-mortem human brain — no cause for dismay”. en. In: *Journal of Chemical Neuroanatomy* 22.1-2, pp. 79–94. ISSN: 08910618. DOI: [10.1016/S0891-0618\(01\)00099-0](https://doi.org/10.1016/S0891-0618(01)00099-0). URL: <https://linkinghub.elsevier.com/retrieve/pii/S0891061801000990> (visited on 01/14/2025).
- Baldensperger, Tim, Miriam Preissler, and Christian F. W. Becker (2025). “Non-enzymatic posttranslational protein modifications in protein aggregation and neurodegenerative diseases”. en. In: *RSC Chem. Biol.*, 10.1039.D4CB00221K. ISSN: 2633-0679. DOI: [10.1039/D4CB00221K](https://doi.org/10.1039/D4CB00221K). URL: <https://xlink.rsc.org/?DOI=D4CB00221K> (visited on 01/14/2025).
- Baratta, Annalisa M., Adam J. Brandner, Sonja L. Plasil, Rachel C. Rice, and Sean P. Farris (June 2022). “Advancements in Genomic and Behavioral Neuroscience Analysis for the Study of Normal and Pathological Brain Function”. In: *Front. Mol. Neurosci.* 15, p. 905328. ISSN: 1662-5099. DOI: [10.3389/fnmol.2022.905328](https://doi.org/10.3389/fnmol.2022.905328). URL: <https://www.frontiersin.org/articles/10.3389/fnmol.2022.905328/full> (visited on 01/14/2025).
- Barlow, H B (Dec. 1972). “Single Units and Sensation: A Neuron Doctrine for Perceptual Psychology?” en. In: *Perception* 1.4, pp. 371–394. ISSN: 0301-0066, 1468-4233. DOI: [10.1068/p010371](https://doi.org/10.1068/p010371). URL: <https://journals.sagepub.com/doi/10.1068/p010371> (visited on 01/14/2025).
- Beadle, G. W. and E. L. Tatum (Nov. 1941). “Genetic Control of Biochemical Reactions in Neurospora”. en. In: *Proc. Natl. Acad. Sci. U.S.A.* 27.11, pp. 499–506. ISSN: 0027-8424, 1091-6490. DOI: [10.1073/pnas.27.11.499](https://doi.org/10.1073/pnas.27.11.499). URL: <https://pnas.org/doi/full/10.1073/pnas.27.11.499> (visited on 01/14/2025).
- Beliveau, Brian J. et al. (Dec. 2012). “Versatile design and synthesis platform for visualizing genomes with Oligopaint FISH probes”. en. In: *Proc. Natl. Acad. Sci. U.S.A.* 109.52, pp. 21301–21306. ISSN: 0027-8424, 1091-6490. DOI: [10.1073/pnas.1213818110](https://doi.org/10.1073/pnas.1213818110). URL: <https://pnas.org/doi/full/10.1073/pnas.1213818110> (visited on 01/14/2025).

- Bintu, Bogdan, Leslie J. Mateo, Jun-Han Su, Nicholas A. Sinnott-Armstrong, Mirae Parker, Seon Kinrot, Kei Yamaya, Alistair N. Boettiger, and Xiaowei Zhuang (Oct. 2018). “Super-resolution chromatin tracing reveals domains and cooperative interactions in single cells”. en. In: *Science* 362.6413, eaau1783. ISSN: 0036-8075, 1095-9203. DOI: [10.1126/science.aau1783](https://doi.org/10.1126/science.aau1783). URL: <https://www.science.org/doi/10.1126/science.aau1783> (visited on 01/14/2025).
- Brinkerhoff, Henry, Albert S. W. Kang, Jingqian Liu, Aleksei Aksimentiev, and Cees Dekker (Dec. 2021). “Multiple rereads of single proteins at single–amino acid resolution using nanopores”. en. In: *Science* 374.6574, pp. 1509–1513. ISSN: 0036-8075, 1095-9203. DOI: [10.1126/science.abl4381](https://doi.org/10.1126/science.abl4381). URL: <https://www.science.org/doi/10.1126/science.abl4381> (visited on 01/14/2025).
- Budnik, Bogdan, Ezra Levy, Guillaume Harmange, and Nikolai Slavov (Dec. 2018). “SCoPE-MS: mass spectrometry of single mammalian cells quantifies proteome heterogeneity during cell differentiation”. en. In: *Genome Biol* 19.1, p. 161. ISSN: 1474-760X. DOI: [10.1186/s13059-018-1547-5](https://doi.org/10.1186/s13059-018-1547-5). URL: <https://genomebiology.biomedcentral.com/articles/10.1186/s13059-018-1547-5> (visited on 01/14/2025).
- Cajigas, Iván J., Georgi Tushev, Tristan J. Will, Susanne tom Dieck, Nicole Fuerst, and Erin M. Schuman (May 2012). “The Local Transcriptome in the Synaptic Neuropil Revealed by Deep Sequencing and High-Resolution Imaging”. en. In: *Neuron* 74.3, pp. 453–466. ISSN: 08966273. DOI: [10.1016/j.neuron.2012.02.036](https://doi.org/10.1016/j.neuron.2012.02.036). URL: <https://linkinghub.elsevier.com/retrieve/pii/S0896627312002863> (visited on 01/14/2025).
- Carlyle, Becky C., Robert R. Kitchen, Jean E. Kanyo, Edward Z. Voss, Mihovil Pletikos, André M. M. Sousa, TuKiet T. Lam, Mark B. Gerstein, Nenad Sestan, and Angus C. Nairn (Dec. 2017). “A multiregional proteomic survey of the postnatal human brain”. en. In: *Nat Neurosci* 20.12, pp. 1787–1795. ISSN: 1097-6256, 1546-1726. DOI: [10.1038/s41593-017-0011-2](https://doi.org/10.1038/s41593-017-0011-2). URL: <https://www.nature.com/articles/s41593-017-0011-2> (visited on 01/14/2025).
- Chen, Baoen, Yang Sun, Jixiao Niu, Gopala K. Jarugumilli, and Xu Wu (July 2018). “Protein Lipidation in Cell Signaling and Diseases: Function, Regulation, and Therapeutic Opportunities”. en. In: *Cell Chemical Biology* 25.7, pp. 817–831. ISSN: 24519456. DOI: [10.1016/j.chembiol.2018.05.003](https://doi.org/10.1016/j.chembiol.2018.05.003). URL: <https://linkinghub.elsevier.com/retrieve/pii/S2451945618301508> (visited on 01/14/2025).
- Chen, Fei, Paul W. Tillberg, and Edward S. Boyden (Jan. 2015). “Expansion microscopy”. en. In: *Science* 347.6221, pp. 543–548. ISSN: 0036-8075, 1095-9203. DOI: [10.1126/science.1260088](https://doi.org/10.1126/science.1260088). URL: <https://www.science.org/doi/10.1126/science.1260088> (visited on 01/14/2025).
- Chen, Renrui, Pengxing Nie, Jing Wang, and Guang-Zhong Wang (July 2024). “Deciphering brain cellular and behavioral mechanisms: Insights from single-cell and spatial RNA sequencing”. en. In: *WIREs RNA* 15.4, e1865. ISSN: 1757-7004, 1757-7012. DOI: [10.1002/wrna.1865](https://doi.org/10.1002/wrna.1865). URL: <https://wires.onlinelibrary.wiley.com/doi/10.1002/wrna.1865> (visited on 01/14/2025).
- Cho, Eunsil and Mikyoung Park (Sept. 2016). “Palmitoylation in Alzheimer’s disease and other neurodegenerative diseases”. en. In: *Pharmacological Research* 111, pp. 133–151. ISSN: 10436618. DOI: [10.1016/j.phrs.2016.06.008](https://doi.org/10.1016/j.phrs.2016.06.008). URL: <https://linkinghub.elsevier.com/retrieve/pii/S1043661816304534> (visited on 01/14/2025).

- Cifani, Paolo and Alex Kentsis (Jan. 2017). “Towards comprehensive and quantitative proteomics for diagnosis and therapy of human disease”. en. In: *Proteomics* 17.1-2, p. 1600079. ISSN: 1615-9853, 1615-9861. DOI: [10.1002/pmic.201600079](https://doi.org/10.1002/pmic.201600079). URL: <https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/pmic.201600079> (visited on 01/14/2025).
- Corraliza-Gomez, Miriam, Diego Sanchez, and Maria D. Ganfornina (Nov. 2019). “Lipid-Binding Proteins in Brain Health and Disease”. In: *Front. Neurol.* 10, p. 1152. ISSN: 1664-2295. DOI: [10.3389/fneur.2019.01152](https://doi.org/10.3389/fneur.2019.01152). URL: <https://www.frontiersin.org/article/10.3389/fneur.2019.01152/full> (visited on 01/14/2025).
- Craft, George E., Anshu Chen, and Angus C. Nairn (June 2013). “Recent advances in quantitative neuroproteomics”. en. In: *Methods* 61.3, pp. 186–218. ISSN: 10462023. DOI: [10.1016/j.ymeth.2013.04.008](https://doi.org/10.1016/j.ymeth.2013.04.008). URL: <https://linkinghub.elsevier.com/retrieve/pii/S1046202313001126> (visited on 01/14/2025).
- Day, John H, Catherine Marin Della Santina, Pema Maretich, Alexander L Auld, Kirsten K Schnieder, Tay Shin, Edward S Boyden, and Laurie A Boyer (Oct. 2024). *HiExM: high-throughput expansion microscopy enables scalable super-resolution imaging*. DOI: [10.7554/eLife.96025.3](https://doi.org/10.7554/eLife.96025.3). URL: <https://elifesciences.org/reviewed-preprints/96025v3> (visited on 01/14/2025).
- Deplancke, Bart, Daniel Alpern, and Vincent Gardeux (July 2016). “The Genetics of Transcription Factor DNA Binding Variation”. en. In: *Cell* 166.3, pp. 538–554. ISSN: 00928674. DOI: [10.1016/j.cell.2016.07.012](https://doi.org/10.1016/j.cell.2016.07.012). URL: <https://linkinghub.elsevier.com/retrieve/pii/S0092867416309187> (visited on 01/14/2025).
- Dienel, Gerald A. (Jan. 2019). “Brain Glucose Metabolism: Integration of Energetics with Function”. en. In: *Physiological Reviews* 99.1, pp. 949–1045. ISSN: 0031-9333, 1522-1210. DOI: [10.1152/physrev.00062.2017](https://doi.org/10.1152/physrev.00062.2017). URL: <https://www.physiology.org/doi/10.1152/physrev.00062.2017> (visited on 01/14/2025).
- Doyle, Joseph P. et al. (Nov. 2008). “Application of a Translational Profiling Approach for the Comparative Analysis of CNS Cell Types”. en. In: *Cell* 135.4, pp. 749–762. ISSN: 00928674. DOI: [10.1016/j.cell.2008.10.029](https://doi.org/10.1016/j.cell.2008.10.029). URL: <https://linkinghub.elsevier.com/retrieve/pii/S0092867408013664> (visited on 01/14/2025).
- Eliscovich, Carolina, Shailesh M. Shenoy, and Robert H. Singer (Mar. 2017). “Imaging mRNA and protein interactions within neurons”. en. In: *Proc. Natl. Acad. Sci. U.S.A.* 114.10. ISSN: 0027-8424, 1091-6490. DOI: [10.1073/pnas.1621440114](https://doi.org/10.1073/pnas.1621440114). URL: <https://pnas.org/doi/full/10.1073/pnas.1621440114> (visited on 01/14/2025).
- Espina, Virginia, Julia D Wulfkuhle, Valerie S Calvert, Amy VanMeter, Weidong Zhou, George Coukos, David H Geho, Emanuel F Petricoin, and Lance A Liotta (Aug. 2006). “Laser-capture microdissection”. en. In: *Nat Protoc* 1.2, pp. 586–603. ISSN: 1754-2189, 1750-2799. DOI: [10.1038/nprot.2006.85](https://doi.org/10.1038/nprot.2006.85). URL: <https://www.nature.com/articles/nprot.2006.85> (visited on 01/14/2025).
- Eze, Ugomma C., Aparna Bhaduri, Maximilian Haeussler, Tomasz J. Nowakowski, and Arnold R. Kriegstein (Apr. 2021). “Single-cell atlas of early human brain development highlights heterogeneity of human neuroepithelial cells and early radial glia”. en. In: *Nat Neurosci* 24.4, pp. 584–594. ISSN: 1097-6256, 1546-1726. DOI: [10.1038/s41593-020-00794-1](https://doi.org/10.1038/s41593-020-00794-1). URL: <https://www.nature.com/articles/s41593-020-00794-1> (visited on 01/14/2025).

- Fernandez-Irigoyen, Joaquín, Alberto Labarga, Aintzane Zabaleta, Xabier Martínez De Morentin, Estela Perez-Valderrama, María Victoria Zelaya, and Enrique Santamaria (Oct. 2015). “Toward defining the anatomo-proteomic puzzle of the human brain: An integrative analysis”. en. In: *Proteomics Clinical Apps* 9.9-10, pp. 796–807. ISSN: 1862-8346, 1862-8354. DOI: [10.1002/prca.201400127](https://doi.org/10.1002/prca.201400127). URL: <https://onlinelibrary.wiley.com/doi/10.1002/prca.201400127> (visited on 01/14/2025).
- Floyd, Brendan M. and Edward M. Marcotte (May 2022). “Protein Sequencing, One Molecule at a Time”. en. In: *Annu. Rev. Biophys.* 51.1, pp. 181–200. ISSN: 1936-122X, 1936-1238. DOI: [10.1146/annurev-biophys-102121-103615](https://doi.org/10.1146/annurev-biophys-102121-103615). URL: <https://www.annualreviews.org/doi/10.1146/annurev-biophys-102121-103615> (visited on 01/14/2025).
- Fung, To Sing and Ding Xiang Liu (June 2018). “Post-Translational Modifications of Coronavirus Proteins: Roles and Function”. en. In: *Future Virol.* 13.6, pp. 405–430. ISSN: 1746-0794, 1746-0808. DOI: [10.2217/fvl-2018-0008](https://doi.org/10.2217/fvl-2018-0008). URL: <https://www.tandfonline.com/doi/full/10.2217/fvl-2018-0008> (visited on 01/14/2025).
- García-Marín, Luis M. et al. (Nov. 2024). “Genomic analysis of intracranial and subcortical brain volumes yields polygenic scores accounting for variation across ancestries”. en. In: *Nat Genet* 56.11, pp. 2333–2344. ISSN: 1061-4036, 1546-1718. DOI: [10.1038/s41588-024-01951-z](https://doi.org/10.1038/s41588-024-01951-z). URL: <https://www.nature.com/articles/s41588-024-01951-z> (visited on 01/14/2025).
- Gaulton, Kyle J., Sebastian Preissl, and Bing Ren (Aug. 2023). “Interpreting non-coding disease-associated human variants using single-cell epigenomics”. en. In: *Nat Rev Genet* 24.8, pp. 516–534. ISSN: 1471-0056, 1471-0064. DOI: [10.1038/s41576-023-00598-6](https://doi.org/10.1038/s41576-023-00598-6). URL: <https://www.nature.com/articles/s41576-023-00598-6> (visited on 01/14/2025).
- GOLGI, C (1873). “Sulla sostanza grigia del cervello.” In: *Gazzetta Medica Italiana* 33, pp. 244–246. URL: <https://cir.nii.ac.jp/crid/1570572699279815168> (visited on 01/14/2025).
- Gomazkov, O. A. (Mar. 2021). “Neuroproteomics: How a Multitude of Proteins Reflect Brain Functions”. en. In: *Biol Bull Rev* 11.2, pp. 143–153. ISSN: 2079-0864, 2079-0872. DOI: [10.1134/S2079086421020043](https://doi.org/10.1134/S2079086421020043). URL: <https://link.springer.com/10.1134/S2079086421020043> (visited on 01/14/2025).
- Gross, Charles G. (Oct. 2002). “Genealogy of the “Grandmother Cell””. en. In: *Neuroscientist* 8.5, pp. 512–518. ISSN: 1073-8584, 1089-4098. DOI: [10.1177/107385802237175](https://doi.org/10.1177/107385802237175). URL: <https://journals.sagepub.com/doi/10.1177/107385802237175> (visited on 01/14/2025).
- Gygi, Steven P., Beate Rist, Scott A. Gerber, Frantisek Turecek, Michael H. Gelb, and Ruedi Aebersold (Oct. 1999). “Quantitative analysis of complex protein mixtures using isotope-coded affinity tags”. en. In: *Nat Biotechnol* 17.10, pp. 994–999. ISSN: 1087-0156, 1546-1696. DOI: [10.1038/13690](https://doi.org/10.1038/13690). URL: https://www.nature.com/articles/nbt1099_994 (visited on 01/14/2025).
- Hasin, Yehudit, Marcus Seldin, and Aldons Lusic (Dec. 2017). “Multi-omics approaches to disease”. en. In: *Genome Biol* 18.1, p. 83. ISSN: 1474-760X. DOI: [10.1186/s13059-017-1215-1](https://doi.org/10.1186/s13059-017-1215-1). URL: <https://genomebiology.biomedcentral.com/articles/10.1186/s13059-017-1215-1> (visited on 01/14/2025).
- Heather, James M. and Benjamin Chain (Jan. 2016). “The sequence of sequencers: The history of sequencing DNA”. en. In: *Genomics* 107.1, pp. 1–8. ISSN: 08887543. DOI: [10.1016/j.ygeno.2015.11.003](https://doi.org/10.1016/j.ygeno.2015.11.003). URL: <https://linkinghub.elsevier.com/retrieve/pii/S0888754315300410> (visited on 01/14/2025).

- Heiman, Myriam et al. (Nov. 2008). “A Translational Profiling Approach for the Molecular Characterization of CNS Cell Types”. en. In: *Cell* 135.4, pp. 738–748. ISSN: 00928674. DOI: [10.1016/j.cell.2008.10.028](https://doi.org/10.1016/j.cell.2008.10.028). URL: <https://linkinghub.elsevier.com/retrieve/pii/S0092867408013652> (visited on 01/14/2025).
- Holley, Robert W., Jean Apgar, George A. Everett, James T. Madison, Mark Marquisee, Susan H. Merrill, John Robert Penswick, and Ada Zamir (Mar. 1965). “Structure of a Ribonucleic Acid”. en. In: *Science* 147.3664, pp. 1462–1465. ISSN: 0036-8075, 1095-9203. DOI: [10.1126/science.147.3664.1462](https://doi.org/10.1126/science.147.3664.1462). URL: <https://www.science.org/doi/10.1126/science.147.3664.1462> (visited on 01/14/2025).
- Hrdlickova, Radmila, Masoud Toloue, and Bin Tian (Jan. 2017). “RNA -Seq methods for transcriptome analysis”. en. In: *WIREs RNA* 8.1, e1364. ISSN: 1757-7004, 1757-7012. DOI: [10.1002/wrna.1364](https://doi.org/10.1002/wrna.1364). URL: <https://wires.onlinelibrary.wiley.com/doi/10.1002/wrna.1364> (visited on 01/14/2025).
- Hubel, David H. (Mar. 1957). “Tungsten Microelectrode for Recording from Single Units”. en. In: *Science* 125.3247, pp. 549–550. ISSN: 0036-8075, 1095-9203. DOI: [10.1126/science.125.3247.549](https://doi.org/10.1126/science.125.3247.549). URL: <https://www.science.org/doi/10.1126/science.125.3247.549> (visited on 01/14/2025).
- Hutchison, C. A. (Aug. 2007). “DNA sequencing: bench to bedside and beyond”. en. In: *Nucleic Acids Research* 35.18, pp. 6227–6237. ISSN: 0305-1048, 1362-4962. DOI: [10.1093/nar/gkm688](https://doi.org/10.1093/nar/gkm688). URL: <https://academic.oup.com/nar/article-lookup/doi/10.1093/nar/gkm688> (visited on 01/14/2025).
- Incontro, Salvatore, Maria Laura Musella, Malika Sammari, Coralie Di Scala, Jacques Fantini, and Dominique Debanne (Jan. 2025). “Lipids shape brain function through ion channel and receptor modulations: physiological mechanisms and clinical perspectives”. en. In: *Physiological Reviews* 105.1, pp. 137–207. ISSN: 0031-9333, 1522-1210. DOI: [10.1152/physrev.00004.2024](https://doi.org/10.1152/physrev.00004.2024). URL: <https://journals.physiology.org/doi/10.1152/physrev.00004.2024> (visited on 01/14/2025).
- Jaitin, Diego Adhemar et al. (Feb. 2014). “Massively Parallel Single-Cell RNA-Seq for Marker-Free Decomposition of Tissues into Cell Types”. en. In: *Science* 343.6172, pp. 776–779. ISSN: 0036-8075, 1095-9203. DOI: [10.1126/science.1247651](https://doi.org/10.1126/science.1247651). URL: <https://www.science.org/doi/10.1126/science.1247651> (visited on 01/14/2025).
- Jia, Dongling, Minhui Cui, Adeleh Divsalar, Tawfik A. Khattab, Salhah D. Al-Qahtani, Edwin Cheung, and Xianting Ding (Dec. 2024). “Derivative Technologies of Expansion Microscopy and Applications in Biomedicine”. en. In: *ChemBioChem*, e202400795. ISSN: 1439-4227, 1439-7633. DOI: [10.1002/cbic.202400795](https://doi.org/10.1002/cbic.202400795). URL: <https://chemistry-europe.onlinelibrary.wiley.com/doi/10.1002/cbic.202400795> (visited on 01/14/2025).
- Kabir, Fariha, Rachel Atkinson, Anthony L. Cook, Andrew James Phipps, and Anna Elizabeth King (Jan. 2023). “The role of altered protein acetylation in neurodegenerative disease”. In: *Front. Aging Neurosci.* 14, p. 1025473. ISSN: 1663-4365. DOI: [10.3389/fnagi.2022.1025473](https://doi.org/10.3389/fnagi.2022.1025473). URL: <https://www.frontiersin.org/articles/10.3389/fnagi.2022.1025473/full> (visited on 01/14/2025).
- Kim, Sandra I., Hans Voshol, Jan Van Oostrum, Terri G. Hastings, Michael Cascio, and Marc J. Glucksman (June 2004). “Neuroproteomics: Expression Profiling of the Brain’s Proteomes in Health and Disease”. en. In: *Neurochem Res* 29.6, pp. 1317–1331. ISSN: 0364-3190. DOI:

- 10.1023/B:NERE.0000023618.35579.7c. URL: <http://link.springer.com/10.1023/B:NERE.0000023618.35579.7c> (visited on 01/14/2025).
- Laporte, Marine H., Nikolai Klena, Virginie Hamel, and Paul Guichard (Feb. 2022). “Visualizing the native cellular organization by coupling cryofixation with expansion microscopy (Cryo-ExM)”. en. In: *Nat Methods* 19.2, pp. 216–222. ISSN: 1548-7091, 1548-7105. DOI: [10.1038/s41592-021-01356-4](https://www.nature.com/articles/s41592-021-01356-4). URL: <https://www.nature.com/articles/s41592-021-01356-4> (visited on 01/14/2025).
- Lee, Dong-Sung et al. (Oct. 2019). “Simultaneous profiling of 3D genome structure and DNA methylation in single human cells”. en. In: *Nat Methods* 16.10, pp. 999–1006. ISSN: 1548-7091, 1548-7105. DOI: [10.1038/s41592-019-0547-z](https://www.nature.com/articles/s41592-019-0547-z). URL: <https://www.nature.com/articles/s41592-019-0547-z> (visited on 01/14/2025).
- Leitner, Alexander, Thomas Walzthoeni, Abdullah Kahraman, Franz Herzog, Oliver Rinner, Martin Beck, and Ruedi Aebersold (Aug. 2010). “Probing Native Protein Structures by Chemical Cross-linking, Mass Spectrometry, and Bioinformatics”. en. In: *Molecular & Cellular Proteomics* 9.8, pp. 1634–1649. ISSN: 15359476. DOI: [10.1074/mcp.R000001-MCP201](https://linkinghub.elsevier.com/retrieve/pii/S1535947620309208). URL: <https://linkinghub.elsevier.com/retrieve/pii/S1535947620309208> (visited on 01/14/2025).
- Lennox, Ashley L., Hanqian Mao, and Debra L. Silver (Jan. 2018). “RNA on the brain: emerging layers of post-transcriptional regulation in cerebral cortex development”. en. In: *WIREs Developmental Biology* 7.1, e290. ISSN: 1759-7684, 1759-7692. DOI: [10.1002/wdev.290](https://wires.onlinelibrary.wiley.com/doi/10.1002/wdev.290). URL: <https://wires.onlinelibrary.wiley.com/doi/10.1002/wdev.290> (visited on 01/14/2025).
- Leutert, Mario, Samuel W. Entwisle, and Judit Villén (2021). “Decoding Post-Translational Modification Crosstalk With Proteomics”. en. In: *Molecular & Cellular Proteomics* 20, p. 100129. ISSN: 15359476. DOI: [10.1016/j.mcpro.2021.100129](https://linkinghub.elsevier.com/retrieve/pii/S1535947621001018). URL: <https://linkinghub.elsevier.com/retrieve/pii/S1535947621001018> (visited on 01/14/2025).
- Liu-Yesucevitz, Liqun, Gary J. Bassell, Aaron D. Gitler, Anne C. Hart, Eric Klann, Joel D. Richter, Stephen T. Warren, and Benjamin Wolozin (Nov. 2011). “Local RNA Translation at the Synapse and in Disease: Figure 1.” en. In: *J. Neurosci.* 31.45, pp. 16086–16093. ISSN: 0270-6474, 1529-2401. DOI: [10.1523/JNEUROSCI.4105-11.2011](https://www.jneurosci.org/lookup/doi/10.1523/JNEUROSCI.4105-11.2011). URL: <https://www.jneurosci.org/lookup/doi/10.1523/JNEUROSCI.4105-11.2011> (visited on 01/14/2025).
- Lu, Jin-Ying, Yu-Yi Lin, Heng Zhu, Lee-Ming Chuang, and Jef D. Boeke (Oct. 2011). “Protein acetylation and aging”. In: *Aging (Albany NY)* 3.10, pp. 911–912. ISSN: 1945-4589. URL: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3229967/> (visited on 01/14/2025).
- Lu, Peng, Christine Vogel, Rong Wang, Xin Yao, and Edward M Marcotte (Jan. 2007). “Absolute protein expression profiling estimates the relative contributions of transcriptional and translational regulation”. en. In: *Nat Biotechnol* 25.1, pp. 117–124. ISSN: 1087-0156, 1546-1696. DOI: [10.1038/nbt1270](https://www.nature.com/articles/nbt1270). URL: <https://www.nature.com/articles/nbt1270> (visited on 01/14/2025).
- Macosko, Evan Z. et al. (May 2015). “Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets”. en. In: *Cell* 161.5, pp. 1202–1214. ISSN: 00928674. DOI: [10.1016/j.cell.2015.05.002](https://linkinghub.elsevier.com/retrieve/pii/S0092867415005498). URL: <https://linkinghub.elsevier.com/retrieve/pii/S0092867415005498> (visited on 01/14/2025).

- Mansuri, M. Shahid, Kenneth Williams, and Angus C. Nairn (Apr. 2023). “Uncovering biology by single-cell proteomics”. en. In: *Commun Biol* 6.1, p. 381. ISSN: 2399-3642. DOI: [10.1038/s42003-023-04635-2](https://doi.org/10.1038/s42003-023-04635-2). URL: <https://www.nature.com/articles/s42003-023-04635-2> (visited on 01/14/2025).
- Marx, Vivien (Jan. 2021). “Method of the Year: spatially resolved transcriptomics”. en. In: *Nat Methods* 18.1, pp. 9–14. ISSN: 1548-7091, 1548-7105. DOI: [10.1038/s41592-020-01033-y](https://doi.org/10.1038/s41592-020-01033-y). URL: <https://www.nature.com/articles/s41592-020-01033-y> (visited on 01/14/2025).
- Morris, R.G.M (Nov. 1999). “D.O. Hebb: The Organization of Behavior, Wiley: New York; 1949”. en. In: *Brain Research Bulletin* 50.5-6, p. 437. ISSN: 03619230. DOI: [10.1016/S0361-9230\(99\)00182-3](https://doi.org/10.1016/S0361-9230(99)00182-3). URL: <https://linkinghub.elsevier.com/retrieve/pii/S0361923099001823> (visited on 01/14/2025).
- Moses, Lambda and Lior Pachter (May 2022). “Museum of spatial transcriptomics”. en. In: *Nat Methods* 19.5, pp. 534–546. ISSN: 1548-7091, 1548-7105. DOI: [10.1038/s41592-022-01409-2](https://doi.org/10.1038/s41592-022-01409-2). URL: <https://www.nature.com/articles/s41592-022-01409-2> (visited on 01/14/2025).
- Mueller, Michael et al. (Sept. 2006). “Functional annotation of proteins identified in human brain during the HUPO Brain Proteome Project pilot study”. en. In: *Proteomics* 6.18, pp. 5059–5075. ISSN: 1615-9853, 1615-9861. DOI: [10.1002/pmic.200600194](https://doi.org/10.1002/pmic.200600194). URL: <https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/pmic.200600194> (visited on 01/14/2025).
- Natividad, Luis A., Matthew W. Buczynski, Daniel B. McClatchy, and John R. Yates (Dec. 2018). “From Synapse to Function: A Perspective on the Role of Neuroproteomics in Elucidating Mechanisms of Drug Addiction”. en. In: *Proteomes* 6.4, p. 50. ISSN: 2227-7382. DOI: [10.3390/proteomes6040050](https://doi.org/10.3390/proteomes6040050). URL: <https://www.mdpi.com/2227-7382/6/4/50> (visited on 01/14/2025).
- Nebert, Daniel W. and Elliot S. Vesell (2013). “Pharmacogenetics and Pharmacogenomics”. en. In: *Emery and Rimoin’s Principles and Practice of Medical Genetics*. Elsevier, pp. 1–27. ISBN: 978-0-12-383834-6. DOI: [10.1016/B978-0-12-383834-6.00023-9](https://doi.org/10.1016/B978-0-12-383834-6.00023-9). URL: <https://linkinghub.elsevier.com/retrieve/pii/B9780123838346000239> (visited on 01/14/2025).
- O’Brien, John S. and E. Lois Sampson (Oct. 1965). “Fatty acid and fatty aldehyde composition of the major brain lipids in normal human gray matter, white matter, and myelin”. en. In: *Journal of Lipid Research* 6.4, pp. 545–551. ISSN: 00222275. DOI: [10.1016/S0022-2275\(20\)39620-6](https://doi.org/10.1016/S0022-2275(20)39620-6). URL: <https://linkinghub.elsevier.com/retrieve/pii/S0022227520396206> (visited on 01/14/2025).
- Ong, Shao-En, Blagoy Blagoev, Irina Kratchmarova, Dan Bach Kristensen, Hanno Steen, Akhilesh Pandey, and Matthias Mann (May 2002). “Stable Isotope Labeling by Amino Acids in Cell Culture, SILAC, as a Simple and Accurate Approach to Expression Proteomics”. en. In: *Molecular & Cellular Proteomics* 1.5, pp. 376–386. ISSN: 15359476. DOI: [10.1074/mcp.M200025-MCP200](https://doi.org/10.1074/mcp.M200025-MCP200). URL: <https://linkinghub.elsevier.com/retrieve/pii/S1535947620344406> (visited on 01/14/2025).
- Osetrova, Maria et al. (May 2024). “Lipidome atlas of the adult human brain”. en. In: *Nat Commun* 15.1, p. 4455. ISSN: 2041-1723. DOI: [10.1038/s41467-024-48734-y](https://doi.org/10.1038/s41467-024-48734-y). URL: <https://www.nature.com/articles/s41467-024-48734-y> (visited on 01/14/2025).
- Panca, Rita, Eva Schad, Agnes Tantos, and Peter Tompa (Oct. 2019). “Emergent functions of proteins in non-stoichiometric supramolecular assemblies”. en. In: *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* 1867.10, pp. 970–979. ISSN: 15709639. DOI: [10.1016/j.bbaprot.2019.09.001](https://doi.org/10.1016/j.bbaprot.2019.09.001)

- 1016/j.bbapap.2019.02.007. URL: <https://linkinghub.elsevier.com/retrieve/pii/S1570963919300433> (visited on 01/14/2025).
- Piazza, Ilaria, Karl Kochanowski, Valentina Cappelletti, Tobias Fuhrer, Elad Noor, Uwe Sauer, and Paola Picotti (Jan. 2018). “A Map of Protein-Metabolite Interactions Reveals Principles of Chemical Communication”. en. In: *Cell* 172.1-2, 358–372.e23. ISSN: 00928674. DOI: 10.1016/j.cell.2017.12.006. URL: <https://linkinghub.elsevier.com/retrieve/pii/S0092867417314484> (visited on 01/14/2025).
- Piwecka, Monika, Nikolaus Rajewsky, and Agnieszka Rybak-Wolf (June 2023). “Single-cell and spatial transcriptomics: deciphering brain complexity in health and disease”. en. In: *Nat Rev Neurol* 19.6, pp. 346–362. ISSN: 1759-4758, 1759-4766. DOI: 10.1038/s41582-023-00809-y. URL: <https://www.nature.com/articles/s41582-023-00809-y> (visited on 01/14/2025).
- Pollen, Alex A et al. (Oct. 2014). “Low-coverage single-cell mRNA sequencing reveals cellular heterogeneity and activated signaling pathways in developing cerebral cortex”. en. In: *Nat Biotechnol* 32.10, pp. 1053–1058. ISSN: 1087-0156, 1546-1696. DOI: 10.1038/nbt.2967. URL: <https://www.nature.com/articles/nbt.2967> (visited on 01/14/2025).
- Pradeep, Prajitha, Hyeyeon Kang, and Boyoung Lee (May 2023). “Glycosylation and behavioral symptoms in neurological disorders”. en. In: *Transl Psychiatry* 13.1, p. 154. ISSN: 2158-3188. DOI: 10.1038/s41398-023-02446-x. URL: <https://www.nature.com/articles/s41398-023-02446-x> (visited on 01/14/2025).
- Prashad, Shavanie and Pallavi P. Gopal (July 2021). “RNA-binding proteins in neurological development and disease”. en. In: *RNA Biology* 18.7, pp. 972–987. ISSN: 1547-6286, 1555-8584. DOI: 10.1080/15476286.2020.1809186. URL: <https://www.tandfonline.com/doi/full/10.1080/15476286.2020.1809186> (visited on 01/14/2025).
- Qu, Chengyi and Hao Du (Oct. 2024). “Advances and Future Prospects in Technologies for DNA-Protein Interactions Research”. en. In: *Crop Design*, p. 100082. ISSN: 27728994. DOI: 10.1016/j.crope.2024.100082. URL: <https://linkinghub.elsevier.com/retrieve/pii/S2772899424000314> (visited on 01/14/2025).
- Rahman, Samir et al. (Nov. 2023). “Lineage specific 3D genome structure in the adult human brain and neurodevelopmental changes in the chromatin interactome”. en. In: *Nucleic Acids Research* 51.20, pp. 11142–11161. ISSN: 0305-1048, 1362-4962. DOI: 10.1093/nar/gkad798. URL: <https://academic.oup.com/nar/article/51/20/11142/7301278> (visited on 01/14/2025).
- Ramanathan, Muthukumar, Douglas F. Porter, and Paul A. Khavari (Mar. 2019). “Methods to study RNA–protein interactions”. en. In: *Nat Methods* 16.3, pp. 225–234. ISSN: 1548-7091, 1548-7105. DOI: 10.1038/s41592-019-0330-1. URL: <https://www.nature.com/articles/s41592-019-0330-1> (visited on 01/14/2025).
- Rao, Shuquan, Yao Yao, and Daniel E. Bauer (Dec. 2021). “Editing GWAS: experimental approaches to dissect and exploit disease-associated genetic variation”. en. In: *Genome Med* 13.1, p. 41. ISSN: 1756-994X. DOI: 10.1186/s13073-021-00857-3. URL: <https://genomemedicine.biomedcentral.com/articles/10.1186/s13073-021-00857-3> (visited on 01/14/2025).
- Rizzo, Jason M. and Michael J. Buck (July 2012). “Key Principles and Clinical Applications of “Next-Generation” DNA Sequencing”. en. In: *Cancer Prevention Research* 5.7, pp. 887–900. ISSN: 1940-6207, 1940-6215. DOI: 10.1158/1940-6207.CAPR-11-0432. URL: <https://>

- [//aacrjournals.org/cancerpreventionresearch/article/5/7/887/50056/Key-Principles-and-Clinical-Applications-of-Next](https://aacrjournals.org/cancerpreventionresearch/article/5/7/887/50056/Key-Principles-and-Clinical-Applications-of-Next) (visited on 01/14/2025).
- Ross, Philip L. et al. (Dec. 2004). “Multiplexed Protein Quantitation in *Saccharomyces cerevisiae* Using Amine-reactive Isobaric Tagging Reagents”. en. In: *Molecular & Cellular Proteomics* 3.12, pp. 1154–1169. ISSN: 15359476. DOI: [10.1074/mcp.M400129-MCP200](https://doi.org/10.1074/mcp.M400129-MCP200). URL: <https://linkinghub.elsevier.com/retrieve/pii/S153594762032939X> (visited on 01/14/2025).
- Roux, Kyle J., Dae In Kim, Manfred Raida, and Brian Burke (Mar. 2012). “A promiscuous biotin ligase fusion protein identifies proximal and interacting proteins in mammalian cells”. en. In: *Journal of Cell Biology* 196.6, pp. 801–810. ISSN: 1540-8140, 0021-9525. DOI: [10.1083/jcb.201112098](https://doi.org/10.1083/jcb.201112098). URL: <https://rupress.org/jcb/article/196/6/801/36934/A-promiscuous-biotin-ligase-fusion-protein> (visited on 01/14/2025).
- Satam, Heena et al. (July 2023). “Next-Generation Sequencing Technology: Current Trends and Advancements”. en. In: *Biology* 12.7, p. 997. ISSN: 2079-7737. DOI: [10.3390/biology12070997](https://doi.org/10.3390/biology12070997). URL: <https://www.mdpi.com/2079-7737/12/7/997> (visited on 01/14/2025).
- Schipper, Marijn and Danielle Posthuma (Oct. 2022). “Demystifying non-coding GWAS variants: an overview of computational tools and methods”. en. In: *Human Molecular Genetics* 31.R1, R73–R83. ISSN: 0964-6906, 1460-2083. DOI: [10.1093/hmg/ddac198](https://doi.org/10.1093/hmg/ddac198). URL: <https://academic.oup.com/hmg/article/31/R1/R73/6668660> (visited on 01/14/2025).
- Schuster, Stephan C (Jan. 2008). “Next-generation sequencing transforms today’s biology”. en. In: *Nat Methods* 5.1, pp. 16–18. ISSN: 1548-7091, 1548-7105. DOI: [10.1038/nmeth1156](https://doi.org/10.1038/nmeth1156). URL: <https://www.nature.com/articles/nmeth1156> (visited on 01/14/2025).
- Shen, Elaine H., Caroline C. Overly, and Allan R. Jones (Dec. 2012). “The Allen Human Brain Atlas”. en. In: *Trends in Neurosciences* 35.12, pp. 711–714. ISSN: 01662236. DOI: [10.1016/j.tins.2012.09.005](https://doi.org/10.1016/j.tins.2012.09.005). URL: <https://linkinghub.elsevier.com/retrieve/pii/S0166223612001695> (visited on 01/14/2025).
- Shevchenko, Ganna, Anne Konzer, Sravani Musumuri, and Jonas Bergquist (July 2015). “Neuroproteomics tools in clinical practice”. en. In: *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* 1854.7, pp. 705–717. ISSN: 15709639. DOI: [10.1016/j.bbapap.2015.01.016](https://doi.org/10.1016/j.bbapap.2015.01.016). URL: <https://linkinghub.elsevier.com/retrieve/pii/S1570963915000370> (visited on 01/14/2025).
- Signal, Brandon, Thalía Gabriela Pérez Suárez, Phillippa C. Taberlay, and Adele Woodhouse (Oct. 2023). “Cellular specificity is key to deciphering epigenetic changes underlying Alzheimer’s disease”. en. In: *Neurobiology of Disease* 186, p. 106284. ISSN: 09699961. DOI: [10.1016/j.nbd.2023.106284](https://doi.org/10.1016/j.nbd.2023.106284). URL: <https://linkinghub.elsevier.com/retrieve/pii/S0969996123002991> (visited on 01/14/2025).
- Smith, Jeffrey M., Jarrod J. Sandow, and Andrew I. Webb (Feb. 2021). “The search for RNA-binding proteins: a technical and interdisciplinary challenge”. en. In: *Biochemical Society Transactions* 49.1, pp. 393–403. ISSN: 0300-5127, 1470-8752. DOI: [10.1042/BST20200688](https://doi.org/10.1042/BST20200688). URL: <https://portlandpress.com/biochemsoctrans/article/49/1/393/227660/The-search-for-RNA-binding-proteins-a-technical> (visited on 01/14/2025).
- Smith, Moyra (2022). “Advances in methods of genome analyses, nucleotide analyses, and implications of variants”. en. In: *Progress in Genomic Medicine*. Elsevier, pp. 41–61. ISBN: 978-0-323-91547-2. DOI: [10.1016/B978-0-323-91547-2.00008-7](https://doi.org/10.1016/B978-0-323-91547-2.00008-7). URL: <https://linkinghub.elsevier.com/retrieve/pii/B9780323915472000087> (visited on 01/14/2025).

- Steinmetz, Benjamin, Izabela Smok, Maria Bikaki, and Alexander Leitner (Mar. 2023). “Protein–RNA interactions: from mass spectrometry to drug discovery”. en. In: *Essays in Biochemistry* 67.2. Ed. by Hannah Britt, Rebecca Beveridge, and Antonio Calabrese, pp. 175–186. ISSN: 0071-1365, 1744-1358. DOI: [10.1042/EBC20220177](https://doi.org/10.1042/EBC20220177). URL: <https://portlandpress.com/essaysbiochem/article/67/2/175/232665/Protein-RNA-interactions-from-mass-spectrometry-to> (visited on 01/14/2025).
- Steward, O and Wb Levy (Mar. 1982). “Preferential localization of polyribosomes under the base of dendritic spines in granule cells of the dentate gyrus”. en. In: *J. Neurosci.* 2.3, pp. 284–291. ISSN: 0270-6474, 1529-2401. DOI: [10.1523/JNEUROSCI.02-03-00284.1982](https://doi.org/10.1523/JNEUROSCI.02-03-00284.1982). URL: <https://www.jneurosci.org/lookup/doi/10.1523/JNEUROSCI.02-03-00284.1982> (visited on 01/14/2025).
- Steward, Oswald and Erin M. Schuman (Mar. 2001). “Protein Synthesis at Synaptic Sites on Dendrites”. en. In: *Annu. Rev. Neurosci.* 24.1, pp. 299–325. ISSN: 0147-006X, 1545-4126. DOI: [10.1146/annurev.neuro.24.1.299](https://doi.org/10.1146/annurev.neuro.24.1.299). URL: <https://www.annualreviews.org/doi/10.1146/annurev.neuro.24.1.299> (visited on 01/14/2025).
- Südhof, Thomas C. (Nov. 2017). “Molecular Neuroscience in the 21st Century: A Personal Perspective”. en. In: *Neuron* 96.3, pp. 536–541. ISSN: 08966273. DOI: [10.1016/j.neuron.2017.10.005](https://doi.org/10.1016/j.neuron.2017.10.005). URL: <https://linkinghub.elsevier.com/retrieve/pii/S0896627317309340> (visited on 01/14/2025).
- Sun, De-en et al. (Jan. 2021). “Click-ExM enables expansion microscopy for all biomolecules”. en. In: *Nat Methods* 18.1, pp. 107–113. ISSN: 1548-7091, 1548-7105. DOI: [10.1038/s41592-020-01005-2](https://doi.org/10.1038/s41592-020-01005-2). URL: <https://www.nature.com/articles/s41592-020-01005-2> (visited on 01/14/2025).
- Sun, Fengying et al. (Jan. 2025). “Single-cell omics: experimental workflow, data analyses and applications”. en. In: *Sci. China Life Sci.* 68.1, pp. 5–102. ISSN: 1674-7305, 1869-1889. DOI: [10.1007/s11427-023-2561-0](https://doi.org/10.1007/s11427-023-2561-0). URL: <https://link.springer.com/10.1007/s11427-023-2561-0> (visited on 01/14/2025).
- Swaminathan, Jagannath, Alexander A Boulgakov, Erik T Hernandez, Angela M Bardo, James L Bachman, Joseph Marotta, Amber M Johnson, Eric V Anslyn, and Edward M Marcotte (Nov. 2018). “Highly parallel single-molecule identification of proteins in zeptomole-scale mixtures”. en. In: *Nat Biotechnol* 36.11, pp. 1076–1082. ISSN: 1087-0156, 1546-1696. DOI: [10.1038/nbt.4278](https://doi.org/10.1038/nbt.4278). URL: <https://www.nature.com/articles/nbt.4278> (visited on 01/14/2025).
- Swaminathan, Jagannath, Alexander A. Boulgakov, and Edward M. Marcotte (Feb. 2015). “A Theoretical Justification for Single Molecule Peptide Sequencing”. en. In: *PLoS Comput Biol* 11.2. Ed. by David B. Searls, e1004080. ISSN: 1553-7358. DOI: [10.1371/journal.pcbi.1004080](https://doi.org/10.1371/journal.pcbi.1004080). URL: <https://dx.plos.org/10.1371/journal.pcbi.1004080> (visited on 01/14/2025).
- Swanson, Larry W. and Jeff W. Lichtman (July 2016). “From Cajal to Connectome and Beyond”. en. In: *Annu. Rev. Neurosci.* 39.1, pp. 197–216. ISSN: 0147-006X, 1545-4126. DOI: [10.1146/annurev-neuro-071714-033954](https://doi.org/10.1146/annurev-neuro-071714-033954). URL: <https://www.annualreviews.org/doi/10.1146/annurev-neuro-071714-033954> (visited on 01/14/2025).
- Sytnyk, Vladimir, Iryna Leshchyn’ska, and Melitta Schachner (Jan. 2021). “Neural glycomics: the sweet side of nervous system functions”. en. In: *Cell. Mol. Life Sci.* 78.1, pp. 93–116. ISSN: 1420-682X, 1420-9071. DOI: [10.1007/s00018-020-03578-9](https://doi.org/10.1007/s00018-020-03578-9). URL: <https://link.springer.com/10.1007/s00018-020-03578-9> (visited on 01/14/2025).

- Tang, Fuchou et al. (May 2009). “mRNA-Seq whole-transcriptome analysis of a single cell”. en. In: *Nat Methods* 6.5, pp. 377–382. ISSN: 1548-7091, 1548-7105. DOI: [10.1038/nmeth.1315](https://doi.org/10.1038/nmeth.1315). URL: <https://www.nature.com/articles/nmeth.1315> (visited on 01/14/2025).
- Tasic, Bosiljka et al. (Feb. 2016). “Adult mouse cortical cell taxonomy revealed by single cell transcriptomics”. en. In: *Nat Neurosci* 19.2, pp. 335–346. ISSN: 1097-6256, 1546-1726. DOI: [10.1038/nn.4216](https://doi.org/10.1038/nn.4216). URL: <https://www.nature.com/articles/nn.4216> (visited on 01/14/2025).
- Tawfik, Olga Khersonsky And Dan S. (June 2010). “Enzyme Promiscuity: A Mechanistic and Evolutionary Perspective”. en. In: *Annu. Rev. Biochem.* 79.1, pp. 471–505. ISSN: 0066-4154, 1545-4509. DOI: [10.1146/annurev-biochem-030409-143718](https://doi.org/10.1146/annurev-biochem-030409-143718). URL: <https://www.annualreviews.org/doi/10.1146/annurev-biochem-030409-143718> (visited on 01/14/2025).
- Tena, Jennyfer and Carlito B. Lebrilla (Jan. 2021). “Glycomic profiling and the mammalian brain”. en. In: *Proc. Natl. Acad. Sci. U.S.A.* 118.1, e2022238118. ISSN: 0027-8424, 1091-6490. DOI: [10.1073/pnas.2022238118](https://doi.org/10.1073/pnas.2022238118). URL: <https://pnas.org/doi/full/10.1073/pnas.2022238118> (visited on 01/14/2025).
- Tian, Luyi, Fei Chen, and Evan Z. Macosko (June 2023). “The expanding vistas of spatial transcriptomics”. en. In: *Nat Biotechnol* 41.6, pp. 773–782. ISSN: 1087-0156, 1546-1696. DOI: [10.1038/s41587-022-01448-2](https://doi.org/10.1038/s41587-022-01448-2). URL: <https://www.nature.com/articles/s41587-022-01448-2> (visited on 01/14/2025).
- Timsit, Youri and Sergeant-Perthuis Grégoire (Nov. 2021). “Towards the Idea of Molecular Brains”. en. In: *IJMS* 22.21, p. 11868. ISSN: 1422-0067. DOI: [10.3390/ijms222111868](https://doi.org/10.3390/ijms222111868). URL: <https://www.mdpi.com/1422-0067/22/21/11868> (visited on 01/14/2025).
- Tong, Huige, Xiaolong Guo, Macsue Jacques, Qi Luo, Nir Eynon, and Andrew E. Teschendorff (July 2024). *Cell-type specific epigenetic clocks to quantify biological age at cell-type resolution*. en. DOI: [10.1101/2024.07.30.605833](https://doi.org/10.1101/2024.07.30.605833). URL: <http://biorxiv.org/lookup/doi/10.1101/2024.07.30.605833> (visited on 01/14/2025).
- Torres, Matthew P., Henry Dewhurst, and Niveda Sundararaman (Nov. 2016). “Proteome-wide Structural Analysis of PTM Hotspots Reveals Regulatory Elements Predicted to Impact Biological Function and Disease”. en. In: *Molecular & Cellular Proteomics* 15.11, pp. 3513–3528. ISSN: 15359476. DOI: [10.1074/mcp.M116.062331](https://doi.org/10.1074/mcp.M116.062331). URL: <https://linkinghub.elsevier.com/retrieve/pii/S1535947620333405> (visited on 01/14/2025).
- Treutlein, Barbara, Doug G. Brownfield, Angela R. Wu, Norma F. Neff, Gary L. Mantalas, F. Hernan Espinoza, Tushar J. Desai, Mark A. Krasnow, and Stephen R. Quake (May 2014). “Reconstructing lineage hierarchies of the distal lung epithelium using single-cell RNA-seq”. en. In: *Nature* 509.7500, pp. 371–375. ISSN: 0028-0836, 1476-4687. DOI: [10.1038/nature13173](https://doi.org/10.1038/nature13173). URL: <https://www.nature.com/articles/nature13173> (visited on 01/14/2025).
- Tumanov, Sergey and Jurre J Kamphorst (Feb. 2017). “Recent advances in expanding the coverage of the lipidome”. en. In: *Current Opinion in Biotechnology* 43, pp. 127–133. ISSN: 09581669. DOI: [10.1016/j.copbio.2016.11.008](https://doi.org/10.1016/j.copbio.2016.11.008). URL: <https://linkinghub.elsevier.com/retrieve/pii/S095816691630252X> (visited on 01/14/2025).
- Van Den Heuvel, Martijn P., Lianne H. Scholtens, and René S. Kahn (Oct. 2019). “Multiscale Neuroscience of Psychiatric Disorders”. en. In: *Biological Psychiatry* 86.7, pp. 512–522. ISSN: 00063223. DOI: [10.1016/j.biopsych.2019.05.015](https://doi.org/10.1016/j.biopsych.2019.05.015). URL: <https://linkinghub.elsevier.com/retrieve/pii/S0006322319313848> (visited on 01/14/2025).

- Van Dijk, Erwin L., Yan Jaszczyszyn, Delphine Naquin, and Claude Thermes (Sept. 2018). “The Third Revolution in Sequencing Technology”. en. In: *Trends in Genetics* 34.9, pp. 666–681. ISSN: 01689525. DOI: [10.1016/j.tig.2018.05.008](https://doi.org/10.1016/j.tig.2018.05.008). URL: <https://linkinghub.elsevier.com/retrieve/pii/S0168952518300969> (visited on 01/14/2025).
- Van Meer, Gerrit, Dennis R. Voelker, and Gerald W. Feigenson (Feb. 2008). “Membrane lipids: where they are and how they behave”. en. In: *Nat Rev Mol Cell Biol* 9.2, pp. 112–124. ISSN: 1471-0072, 1471-0080. DOI: [10.1038/nrm2330](https://doi.org/10.1038/nrm2330). URL: <https://www.nature.com/articles/nrm2330> (visited on 01/14/2025).
- Vandereyken, Katy, Alejandro Sifrim, Bernard Thienpont, and Thierry Voet (Aug. 2023). “Methods and applications for single-cell and spatial multi-omics”. en. In: *Nat Rev Genet* 24.8, pp. 494–515. ISSN: 1471-0056, 1471-0064. DOI: [10.1038/s41576-023-00580-2](https://doi.org/10.1038/s41576-023-00580-2). URL: <https://www.nature.com/articles/s41576-023-00580-2> (visited on 01/14/2025).
- Vogel, Christine, Raquel De Sousa Abreu, Daijin Ko, Shu-Yun Le, Bruce A Shapiro, Suzanne C Burns, Devraj Sandhu, Daniel R Boutz, Edward M Marcotte, and Luiz O Penalva (Jan. 2010). “Sequence signatures and mRNA concentration can explain two-thirds of protein abundance variation in a human cell line”. en. In: *Molecular Systems Biology* 6.1, p. 400. ISSN: 1744-4292, 1744-4292. DOI: [10.1038/msb.2010.59](https://doi.org/10.1038/msb.2010.59). URL: <https://www.embopress.org/doi/10.1038/msb.2010.59> (visited on 01/14/2025).
- Wang, Rong and Yong Q. Chen (Feb. 2022). “Protein Lipidation Types: Current Strategies for Enrichment and Characterization”. en. In: *IJMS* 23.4, p. 2365. ISSN: 1422-0067. DOI: [10.3390/ijms23042365](https://doi.org/10.3390/ijms23042365). URL: <https://www.mdpi.com/1422-0067/23/4/2365> (visited on 01/14/2025).
- Wang, Shiwei et al. (Nov. 2024). “Single-shot 20-fold expansion microscopy”. en. In: *Nat Methods* 21.11, pp. 2128–2134. ISSN: 1548-7091, 1548-7105. DOI: [10.1038/s41592-024-02454-9](https://doi.org/10.1038/s41592-024-02454-9). URL: <https://www.nature.com/articles/s41592-024-02454-9> (visited on 01/14/2025).
- Wang, Xiaoya, Ruiyun Peng, and Li Zhao (Aug. 2024). “Multiscale metabolomics techniques: Insights into neuroscience research”. en. In: *Neurobiology of Disease* 198, p. 106541. ISSN: 09699961. DOI: [10.1016/j.nbd.2024.106541](https://doi.org/10.1016/j.nbd.2024.106541). URL: <https://linkinghub.elsevier.com/retrieve/pii/S0969996124001402> (visited on 01/14/2025).
- Wilhelm, Mathias et al. (May 2014). “Mass-spectrometry-based draft of the human proteome”. en. In: *Nature* 509.7502, pp. 582–587. ISSN: 0028-0836, 1476-4687. DOI: [10.1038/nature13319](https://doi.org/10.1038/nature13319). URL: <https://www.nature.com/articles/nature13319> (visited on 01/14/2025).
- Williams, Cameron G., Hyun Jae Lee, Takahiro Asatsuma, Roser Vento-Tormo, and Ashraf Haque (June 2022). “An introduction to spatial transcriptomics for biomedical research”. en. In: *Genome Med* 14.1, p. 68. ISSN: 1756-994X. DOI: [10.1186/s13073-022-01075-1](https://doi.org/10.1186/s13073-022-01075-1). URL: <https://genomemedicine.biomedcentral.com/articles/10.1186/s13073-022-01075-1> (visited on 01/14/2025).
- Wolthuis, Joanna C., Stefania Magnusdottir, Mia Pras-Raves, Maryam Moshiri, Judith J. M. Jans, Boudewijn Burgering, Saskia Van Mil, and Jeroen De Ridder (Sept. 2020). “MetaboShiny: interactive analysis and metabolite annotation of mass spectrometry-based metabolomics data”. en. In: *Metabolomics* 16.9, p. 99. ISSN: 1573-3882, 1573-3890. DOI: [10.1007/s11306-020-01717-8](https://doi.org/10.1007/s11306-020-01717-8). URL: <https://link.springer.com/10.1007/s11306-020-01717-8> (visited on 01/14/2025).

- Xiao, Haopeng, Weixuan Chen, Johanna M. Smeekens, and Ronghu Wu (Apr. 2018). “An enrichment method based on synergistic and reversible covalent interactions for large-scale analysis of glycoproteins”. en. In: *Nat Commun* 9.1, p. 1692. ISSN: 2041-1723. DOI: [10.1038/s41467-018-04081-3](https://doi.org/10.1038/s41467-018-04081-3). URL: <https://www.nature.com/articles/s41467-018-04081-3> (visited on 01/14/2025).
- Xicoy, Helena, Bé Wieringa, and Gerard J. M. Martens (Jan. 2019). “The Role of Lipids in Parkinson’s Disease”. en. In: *Cells* 8.1, p. 27. ISSN: 2073-4409. DOI: [10.3390/cells8010027](https://doi.org/10.3390/cells8010027). URL: <https://www.mdpi.com/2073-4409/8/1/27> (visited on 01/14/2025).
- Xiong, Xushen et al. (Sept. 2023). “Epigenomic dissection of Alzheimer’s disease pinpoints causal variants and reveals epigenome erosion”. en. In: *Cell* 186.20, 4422–4437.e21. ISSN: 00928674. DOI: [10.1016/j.cell.2023.08.040](https://doi.org/10.1016/j.cell.2023.08.040). URL: <https://linkinghub.elsevier.com/retrieve/pii/S0092867423009741> (visited on 01/14/2025).
- Xu, Haodong, Yongbo Wang, Shaofeng Lin, Wankun Deng, Di Peng, Qinghua Cui, and Yu Xue (Aug. 2018). “PTMD: A Database of Human Disease-Associated Post-Translational Modifications”. en. In: *Genomics, Proteomics & Bioinformatics* 16.4, pp. 244–251. ISSN: 1672-0229, 2210-3244. DOI: [10.1016/j.gpb.2018.06.004](https://doi.org/10.1016/j.gpb.2018.06.004). URL: <https://academic.oup.com/gpb/article/16/4/244/7225031> (visited on 01/14/2025).
- Yang, Grace Xiaolu, Xiyang Li, and Michael Snyder (Aug. 2012). “Investigating metabolite–protein interactions: An overview of available techniques”. en. In: *Methods* 57.4, pp. 459–466. ISSN: 10462023. DOI: [10.1016/j.ymeth.2012.06.013](https://doi.org/10.1016/j.ymeth.2012.06.013). URL: <https://linkinghub.elsevier.com/retrieve/pii/S104620231200148X> (visited on 01/14/2025).
- Yao, Yao, Margreet Docter, Jetty Van Ginkel, Dick De Ridder, and Chirlmin Joo (Aug. 2015). “Single-molecule protein sequencing through fingerprinting: computational assessment”. In: *Phys. Biol.* 12.5, p. 055003. ISSN: 1478-3975. DOI: [10.1088/1478-3975/12/5/055003](https://doi.org/10.1088/1478-3975/12/5/055003). URL: <https://iopscience.iop.org/article/10.1088/1478-3975/12/5/055003> (visited on 01/14/2025).
- Yuan, Yuan, Peiyuan Li, Jianghui Li, Qiu Zhao, Ying Chang, and Xingxing He (Mar. 2024). “Protein lipidation in health and disease: molecular basis, physiological function and pathological implication”. en. In: *Sig Transduct Target Ther* 9.1, p. 60. ISSN: 2059-3635. DOI: [10.1038/s41392-024-01759-7](https://doi.org/10.1038/s41392-024-01759-7). URL: <https://www.nature.com/articles/s41392-024-01759-7> (visited on 01/14/2025).
- Yuste, Rafael (Aug. 2015). “From the neuron doctrine to neural networks”. en. In: *Nat Rev Neurosci* 16.8, pp. 487–497. ISSN: 1471-003X, 1471-0048. DOI: [10.1038/nrn3962](https://doi.org/10.1038/nrn3962). URL: <https://www.nature.com/articles/nrn3962> (visited on 01/14/2025).
- Zavialova, M.G., V.G. Zgodina, and E.N. Nikolaev (Mar. 2017). “Analysis of contribution of protein phosphorylation in the development of the diseases”. ru. In: *BIOMED KHIM* 63.2, pp. 101–114. ISSN: 2310-6905, 2310-6972. DOI: [10.18097/PBMC20176302101](https://doi.org/10.18097/PBMC20176302101). URL: <http://pbmc.ibmc.msk.ru/en/article-en/PBMC-2017-63-2-101> (visited on 01/14/2025).
- Zhang, Bingsen and Frank C. Schroeder (Jan. 2025). “Mechanisms of metabolism-coupled protein modifications”. en. In: *Nat Chem Biol*. ISSN: 1552-4450, 1552-4469. DOI: [10.1038/s41589-024-01805-z](https://doi.org/10.1038/s41589-024-01805-z). URL: <https://www.nature.com/articles/s41589-024-01805-z> (visited on 01/14/2025).
- Zhang, Nan, Jinghua Wu, and Qingfei Zheng (July 2024). “Chemical proteomics approaches for protein post-translational modification studies”. en. In: *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* 1872.4, p. 141017. ISSN: 15709639. DOI: [10.1016/j.bbapap](https://doi.org/10.1016/j.bbapap).

- 2024.141017. URL: <https://linkinghub.elsevier.com/retrieve/pii/S1570963924000244> (visited on 01/14/2025).
- Zheng, Liwei, Yujia Sun, Michael Eisenstein, and Hyongsok Tom Soh (June 2024). *Peptide sequencing via reverse translation of peptides into DNA*. en. DOI: [10.1101/2024.05.31.596913](https://doi.org/10.1101/2024.05.31.596913). URL: <http://biorxiv.org/lookup/doi/10.1101/2024.05.31.596913> (visited on 01/14/2025).
- Zhu, Ying et al. (Feb. 2018). “Nanodroplet processing platform for deep and quantitative proteome profiling of 10–100 mammalian cells”. en. In: *Nat Commun* 9.1, p. 882. ISSN: 2041-1723. DOI: [10.1038/s41467-018-03367-w](https://doi.org/10.1038/s41467-018-03367-w). URL: <https://www.nature.com/articles/s41467-018-03367-w> (visited on 01/14/2025).