Proteomics and neuroscience

Advancing the study of the brain and broader nervous system through the power of proteomics

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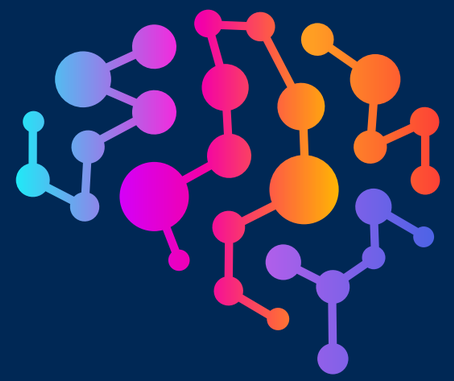


Introduction

- Proteins drive much of the biology of the nervous system and researchers are leveraging proteomics technologies in creative and impactful ways to learn about the mechanistic underpinnings of neurobiology.

Neuroscientists are intrepid researchers who plumb the depths of the brain and broader nervous system. Their work encompasses the study of the intricate series of molecular connections that relay electrochemical signals between cells and tissues in the body to give rise to a plethora of cellular and organismal behaviors. Neurobiological processes enable us to coordinate muscle functions, feel pain, see sights, hear sounds, and so much more. In essence, they enable multicellular organisms to become cohesive, functional wholes. Neuroscience is thus critical to understanding how the body works, and disorders of neurobiology can have broad and devastating impacts on how we think, feel, and perform life's basic functions.

Given their essential role in most all behaviors, there is a deceptively small set of broad cell types in the nervous system. **Neurons** relay electrochemical signals between one another and stimulate cells in other parts of the body. **Glia** provide structure to the nervous system and support neurons by insulating them, eliminating waste, and more. Of course, each of these broad cell types has many, many subtypes, but much of the complexity of the nervous system stems from the ways these cells are connected into networks and the ways they respond to stimuli.

Proteins are at the core of neuroscience

Proteins play a key role in enabling this complexity. The establishment of neuronal connections is largely determined and maintained by protein-based signaling pathways. In addition, the proteins produced and released at the interfaces of neuronal cells can determine the nature of neuronal signaling and do such things as heighten, dampen, or prolong the activation of neuronal networks. Finally, many disorders of the nervous system are a result of protein dysregulation. Often this involves aberrant protein accumulation and misfolding that disrupts cellular function and broader organismal behavior.

For all these reasons, proteomics, the study of all the proteins in a biological sample, has proven essential to basic and applied neuroscience. Proteomics can help researchers understand how cellular connections are made in the nervous system, how these connections relate to various behaviors, and how these connections are disrupted in neurological disorders.

Celebrating inspirational work at the intersection of proteomics and neuroscience

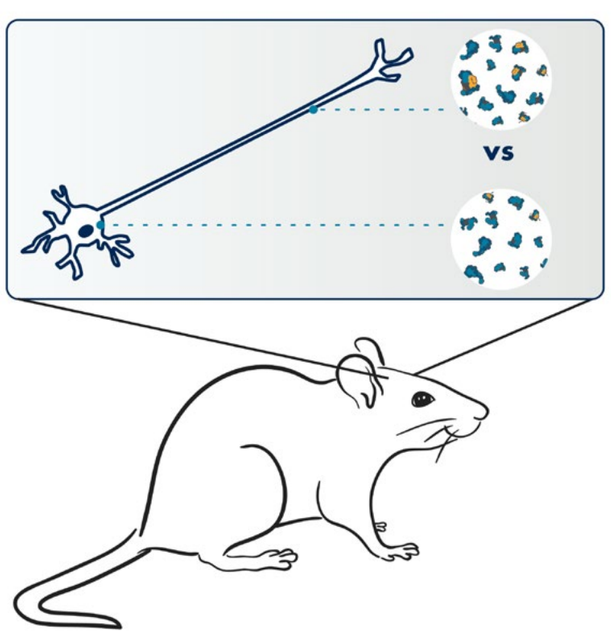
In this eBook, we celebrate some of the many ways proteomics has advanced neuroscience. We also highlight how next-generation proteomics technologies can enable current and future neuroscientists to effectively and efficiently elucidate the roles of proteins, pathways and intercellular communication in developmental processes, organismal behaviors, and neurological disorders.

We hope this eBook will inspire current and future neuroscientists alike to leverage proteomics to advance our understanding of the mind, the body, and their connections. The complexities of neuroscience are daunting, but next-generation proteomics technologies can help researchers navigate that complexity to achieve truly impactful insights that could one day lead to cures for Alzheimer's disease, Parkinson's disease, and much more.

Applications of proteomics in neuroscience: Proximity proteomics for the identification of axonal proteomes

Answer questions like:

- What proteins are abundant during different stages of development?
- What pathways are active during different stages of brain development?
- What known disease-risk genes are associated with which processes and stages of brain development?



Many cells and tissues have complex structures that enable them to carry out their functions. For instance, neurons have long (sometimes incredibly long) extensions called axons that transmit electrochemical signals from one neuron to another. These wire-like connections have different protein compositions than the neuronal cell body or “soma.”

Scientists endeavoring to understand how subcellular structures like axons work at the molecular level often look to the genes expressed within them. They use genomic tools to mutate genes and assess the impacts on cellular activities, but this does not provide direct insights into protein function. They also use transcriptomics and other RNA-based techniques to quantify differences in gene expression. However, special techniques are required to isolate RNA from axons specifically, and RNA expression levels do not always correlate with protein levels. This latter problem is compounded in studies of subcellular gene expression because proteins can be translated in one part of the cell and transported to another leading to a mismatch between local RNA and protein levels. For these reasons, otherwise powerful genomic and transcriptomic technologies cannot reveal what proteins are active in axons and driving function at a molecular, mechanistic level.

Enter proximity proteomics. In this technique, researchers engineer their cells of interest to localize a labeling protein (often APEX) to a subcellular region of interest. Under the appropriate conditions, the APEX labeling protein specifically adds biotin molecules to any nearby proteins. Biotin-labeled proteins can be purified and later identified with a proteomic technique (usually mass spectrometry currently). Scientists can thereby use proximity proteomics to define subcellular proteomes.

This piece highlights recent work by Vasin Dumrongprechachan, et al. They use proximity proteomics to answer an important basic research question, “What proteins are enriched in axons?” Their findings are a great example of the power of proteomics and pave the way for studies investigating the roles of axonal proteins in a variety of behaviors and neurological diseases.

Axonal proximity proteomics in a mouse model

To accomplish the identification of axonal proteins, these researchers created a mouse line expressing the APEX protein after cre-induced activation in a specific subset of neurons. Their APEX protein also had a nuclear export signal that effectively trafficked the protein away from the neuronal nucleus/soma to axons.

The researchers activated axonal APEX in brain tissues derived from this mouse line and did the same in control mice expressing APEX in the soma. Later, they extracted labeled proteins from the brain tissues and identified them via mass spectrometry. Proteins that were more abundant in the mice with axonal APEX were considered axon-enriched proteins.

Using bioinformatics techniques, the researchers clustered the axon-enriched proteins according to developmental expression patterns and thereby identified proteins and pathways important for axon maturation during different developmental time points.

With their mass spectrometry setup, the researchers could also identify phosphorylated proteins and use bioinformatic tools to determine which kinases and kinase pathways were active at the different stages of development. For example, the FYN kinase was particularly active during early postnatal development.

These meticulous efforts generated an extensive dataset mapping protein levels and activity to axonal development. Future researchers can mine this data to identify proteins important for neuronal processes occurring at each time point and even associate subsets of proteins with broader organismal behaviors or disorders.

Associating disease proteins with axonal development

As an example of how this data could be applied in disease research, the authors checked to see if there were any significant associations between known neurological disease-risk genes and the clusters of axonal protein expression. They found significant association between specific protein clusters and disease risk genes for autism spectrum disorders, bipolar disorders, epilepsy, and Alzheimer's disease. Depending on which protein clusters/processes these risk genes were associated with, the researchers hypothesized functional roles for the risk genes.

For example, glutamate is a neurotransmitter involved in neuronal communication throughout the central nervous system. Past work has shown that mutations in glutamate receptor genes such as GRIN2B are associated with epilepsy. In this work, the glutamate receptor encoded in GRIN2B increased in abundance over time along with a cluster of other proteins, many of which were also epilepsy risk genes. In addition, the authors found this cluster was enriched for proteins in glutamate signaling. This reinforces the importance of glutamate signaling in epilepsy and implies that other epilepsy risk genes are linked to this essential process.

New proteomics tools for a better understanding of the brain and its many connections

We glossed over many of the details in the creation of the mouse lines used in this study. Their creation involved the use of CRISPR, Cre-lox, viral vectors, breeding, and more. These studies thus required a ton of genetic, molecular biology, biochemistry, and bioinformatics expertise. Top that all off with the need for extensive expertise in complex mass spectrometry workflows and it's obvious why many labs currently find it difficult to perform this kind of foundational proteomics work.

The data generated from this study alone can be mined to associate proteins with behaviors, neurological processes, diseases, and more for years to come. Nonetheless, to test the hypotheses generated by these associations, in-depth proteomics studies must be more accessible to researchers with diverse kinds of expertise.

We are developing the Nautilus Proteome Analysis Platform with the goal of making comprehensive proteomic analyses like these more accessible to more researchers. Furthermore, we are designing our platform with higher sensitivity, dynamic range, and coverage than current proteomics technologies. With platforms like ours, neuroscientists will hopefully get even richer insights from studies like this one. If this "proteomics revolution" comes to fruition, there's no telling what secrets of the brain scientists might uncover.

Applications of proteomics in neuroscience: Linking genes and proteins to neurological disease

Identifying protein quantitative trait loci (pQTLs) in neurological disorders

- Linking genomics data and proteomics data can help scientists discover the mechanisms of disease.

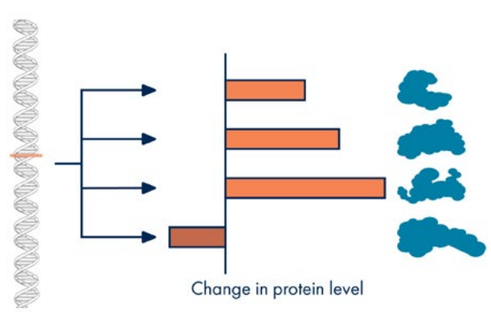
As our understanding of the neurological underpinnings of diseases affecting the brain grows, new therapies for previously intractable diseases like Alzheimer's, Parkinson's, and more may be within reach. As is often the case, these conditions also typically turn out to be far more complex than previously thought.

One example comes from genome-wide association studies, or GWAS. These large-scale studies use data from many different genomes to find correlations between an outcome of interest, like Alzheimer's disease, and specific locations in the genome. One drawback of GWAS is that, while they're good at pointing to genes that may be involved in a trait or disease, they often fail to show the underlying biological mechanisms involving those genes.

Another related issue is that, in some cases, genetic variants are tied to changes in protein expression, but not to changes in RNA, making it difficult to study the effects of these variants using studies of RNA expression.

Researchers can address this problem by turning to proteomics and directly studying the proteins involved in neurological disease. Indeed, next-generation proteomics technologies are making it possible to quickly and thoroughly interrogate the proteomes of diverse samples, opening the door to in-depth studies of the biological mechanisms behind disease.

Genome-wide association studies (GWAS) identify DNA variants associated with disease, but it's often unclear how they are related to biological function.



Proteomics can identify proteins whose abundance is altered in the presence of a DNA variant. These variants are pQTLs and affected proteins may point to a particular function impacted by the pQTL.

In a recent multiomic analysis that combined genomic and proteomic techniques, Yang, et al were able to identify proteins across samples from different types of tissues affected by Alzheimer's disease, stroke, and Parkinson's disease. The research gives insights into the genes as well as the tissue-specific post-translational modifications key to neurological conditions.

The research underscores the power of proteomics to reveal the mechanisms behind biology in exquisite detail. It also highlights the complexities of translating genetic information into physical outcomes in organisms. By helping researchers get a better grip on how genes ultimately control protein levels in different areas of the body, this research could lay the groundwork for identifying new protein biomarkers for diseases, as well as novel drug targets.

Linking genomic and proteomic analysis

To bridge the genome to proteome gap, the researchers looked for protein quantitative trait loci (pQTLs), regions of DNA that affect the abundance of specific proteins. They conducted a broadscale proteomic analysis of 1,744 samples from patients both with and without Alzheimer's disease. The samples came from three different sources: brain tissue, cerebrospinal fluid, and blood plasma. All told, the researchers analyzed 1,305 different proteins, and performed GWAS to compare genetic variants from each patient with levels of the proteins in their tissues.

Identifying proteins associated with Alzheimer's

With their proteomic analysis, the researchers found hundreds of pQTLs correlated with nearly as many proteins in the 3 different sample types. With further analysis, the researchers found 3 proteins in cerebrospinal fluid, 13 in plasma, and 7 in brain tissue that significantly affect the risk of Alzheimer's disease. One such protein was CD33. This protein had been implicated in Alzheimer's in other studies and affects how immune cells in the brain called microglia behave.

Their analysis also identified numerous individual proteins that were affected by multiple genes. They found 10 proteins that each had four genes acting on them, and one protein with five genes acting on it. Such multifactorial interactions highlight the complexity of protein regulation. Some of the identified genes could, for example, be governing when a particular protein is made, while others could be causing modifications to the protein after it's made, changing how it behaves. Some of the genes could even be countering the actions of others, further muddying the waters.

Conversely, a number of genes also acted on more than one protein, an effect known as pleiotropy. One gene in particular, called apolipoprotein E, or APOE, was associated with changes in the levels of up to 13 different proteins. Versions of the APOE gene are known to be some of the strongest genetic risk factors for Alzheimer's.

This data sheds more light on the complex genetic underpinnings of neurological conditions like Alzheimer's disease. Identifying how specific proteins, and the genes that encode them, are involved in disease is a critical step toward developing drugs and other effective treatments for these conditions.

Next-generation proteomics for neurological diseases

In their paper, the researchers made important discoveries with a proteomic analysis covering over 1,300 proteins from patient samples. With millions of proteoforms in the human proteome, researchers will need better and more accessible proteomic analysis technologies to truly unlock the full proteome and expand on this exciting work.

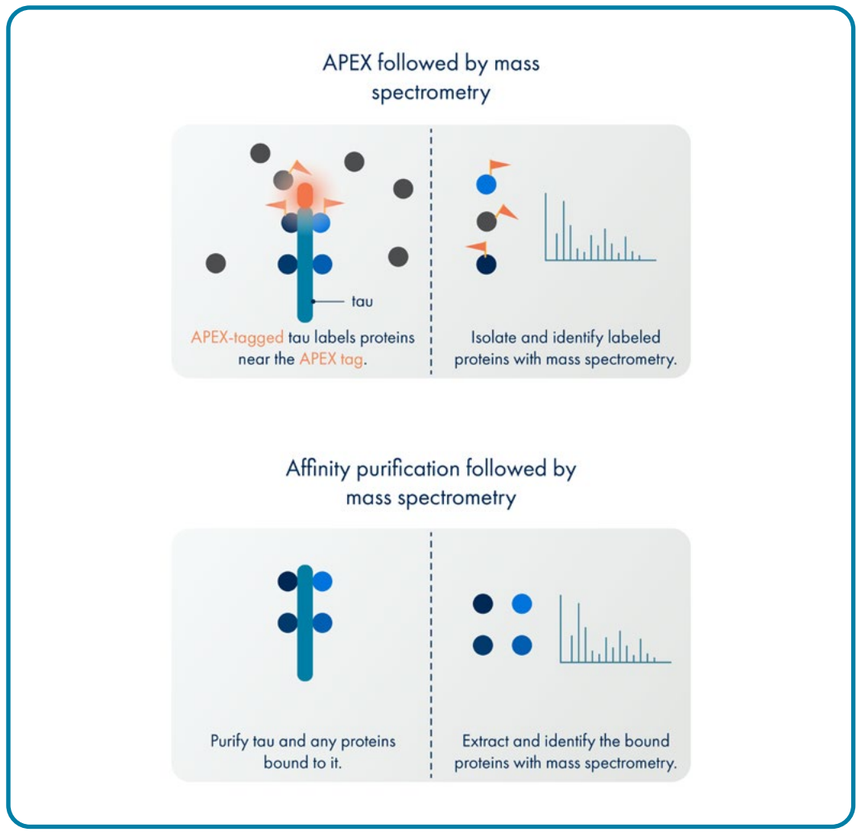
Next-generation proteomics technologies like the Nautilus Proteome Analysis Platform are designed to enable scientists to analyze far more individual proteins at once, and open up proteomics to more labs. With a nanofabricated hyper-dense array, streamlined workflow, and unprecedented sensitivity and dynamic range, protein identification and quantification will hopefully happen more comprehensively and efficiently than ever before.

For multiomics studies digging into the links between genomics, proteomics, and other omics, accessibility and sensitivity may mean being able to see and understand far more connections than was previously possible. There are still genes that affect proteins in unknown ways, and proteins with functions that still aren't understood because researchers haven't been able to study them up close yet. The secrets to diseases like Alzheimer's could lurk within that dark proteome — we'll soon be able to bring them into the light.

Applications of proteomics in neuroscience: Diving into the role of tau in health and disease with interaction proteomics

Learning about protein interactions and functions with the power of proteomics

- Interaction proteomics helps researchers learn more about protein function by identifying the other proteins found in close proximity to a protein of interest.



The molecular mechanisms underlying Alzheimer's disease are poorly understood but are associated with the aggregation of beta amyloid protein outside neurons and tau protein inside neurons. How the aggregation of these proteins relates to the underlying mechanisms of the disease is not well understood. Aggregation could be a symptom or a cause of the disease. To better understand the roles of tau and beta amyloid in both health and disease, researchers have begun to leverage the incredible power of proteomics.

With specific reference to the tau protein, Tracy, et al recently used interaction proteomics to profile the proteins that wildtype and pathogenic varieties of tau interact with. Their work points to roles for tau in synaptic vesicle trafficking and cellular energetics that may be negatively impacted in Alzheimer's disease. These findings may enable the development of biomarkers and treatments focused on these pathways.

Interaction proteomics reveals associations between tau and synaptic proteins as well as with mitochondrial proteins

With interaction proteomics, researchers label and/or isolate proteins that interact with a protein of interest and profile these interaction partners using techniques like mass spectrometry. This reveals associations between a protein of interest and other components of a cell's molecular machinery. Consistent associations between a protein of interest and other proteins with particular functions indicate that the protein of interest is involved in those functions.

In this work, Tracy, et al differentiated human induced pluripotent stem cells (iPSCs) into neurons and performed two types of interaction proteomics with them:

- APEX labeling followed by mass spectrometry
- Affinity purification followed by mass spectrometry

In the APEX labeling experiments, researchers genetically tagged various forms of tau with the APEX protein in iPSC-derived neurons. Under the proper conditions, APEX will add a biotin molecule to any tyrosines on proteins near the APEX-tagged tau. Using biotin binding antibodies, researchers extracted the biotin-labeled proteins from the cells and used mass spectrometry to identify them. This revealed all the proteins that were in close enough proximity to APEX-tau to be labeled.

Results from the APEX experiments showed that associations between tau and other proteins change upon neuronal stimulation. For example, the researchers saw increased association with synaptic vesicle proteins upon neuronal stimulation, suggesting that interactions with these proteins may facilitate tau release at synapses and may enable tau to spread between neurons.

These researchers used affinity purification and mass spectrometry to reveal proteins that have longer-lived interactions with tau. Here, they used antibodies to purify tau and any other proteins that were bound to it. Then these binding partners were released and identified via mass spectrometry.

Results from the affinity purification experiments showed that there were differential associations between wild-type tau and mutated versions of tau known to be associated with genetic forms of dementia. Mutant tau proteins interacted less with mitochondrial proteins and cells producing mutated tau had impaired energetics compared to those producing wildtype tau. In addition, when the researchers looked at the abundance of tau-interacting proteins from mitochondria in people with Alzheimer's, they discovered that decreases in these interacting proteins were associated with increased disease severity.

These results point to impaired energetics as a possible mechanism of tau-induced neuron dysfunction in Alzheimer's. Future treatments could be designed to counteract these energetic effects.

Enabling more studies of protein interaction and function with next-generation proteomics

This study demonstrates the incredible power of proteomics to reveal the functions of proteins in both normal biology and disease. While studies like this are currently restricted to a small number of labs where researchers have the expertise to complete them, next-generation proteomics technologies like the Nautilus Proteome Analysis Platform aim to make such work far more accessible. We are designing our platform to enable many more studies like this and provide unforeseen insights into human health that will make it possible to exquisitely target diseases like Alzheimer's at the molecular level.

Applications of proteomics in neuroscience: Identifying disrupted pathways in dementia

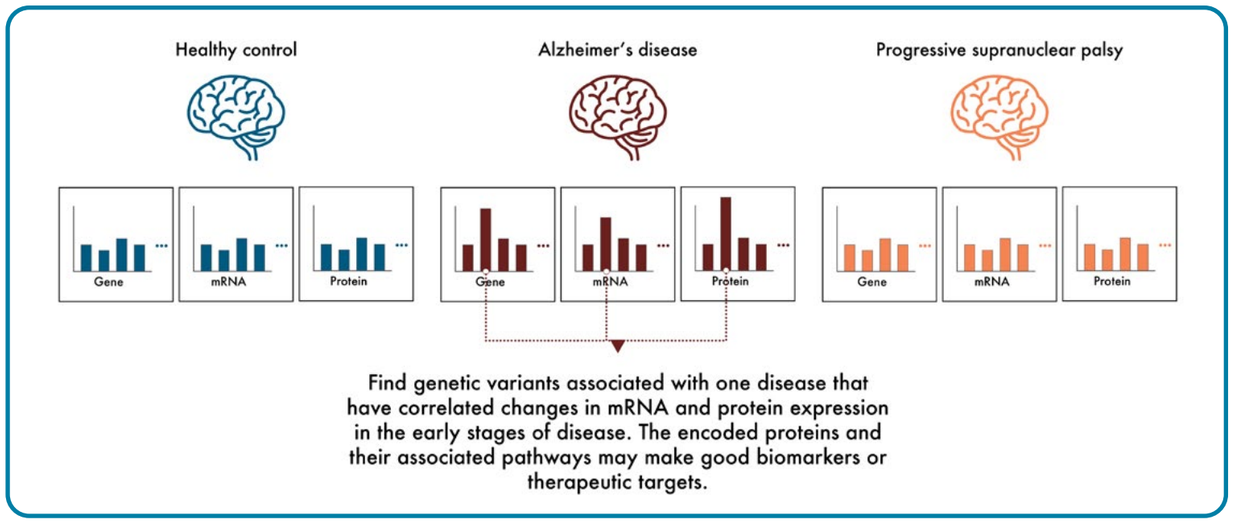
Identifying potential biomarkers and therapeutic targets in neurodegenerative disease

- Looking for salient signals across multiomics data helps researchers discover the genes, proteins, and mechanisms behind disease.

When studying complex neurodegenerative diseases, it can be difficult to sift through mounds of data to determine how underlying biological processes are disrupted. More than that, it can be very hard to distinguish biological perturbations causing a disease from symptoms that occur during disease progression.

One way to get around this issue is to look for salient signals that appear in multiple types of data and see how these signals change across the course of a disease. Signals consistently appearing early on, and only in one specific disease, are more likely to be associated with the cause of the disease.

Swarup and Chang, et al use this strategy in their paper titled, "Identification of Conserved Proteomic Networks in Neurodegenerative Dementia." Here, they leverage a multiomics approach to identify changes in proteomes, transcriptomes, and genomes of post-mortem brain samples from patients with various types of neurodegenerative disease. Concordant signals across various omics methodologies and samples point these researchers toward possible causes of dementia. Their results highlight potential biomarkers and therapeutic targets for neurodegenerative diseases.



Comparing proteomic changes across different neurodegenerative diseases

To understand how different diseases varied at the protein level, these authors analyzed the proteomes of post-mortem brain tissues from people with:

- Alzheimer's disease
- Asymptomatic Alzheimer's disease
- Progressive supranuclear palsy (PSP)
- Frontotemporal dementia
- Parkinson's disease
- No disease (controls)

They found that particular protein modules associated with certain cell types like astrocytes, and functions like synaptic processes were either up-regulated or down-regulated in Alzheimer's disease and these trends progressed along with disease severity. Looking at the other diseases, only those with dementia and not neurodegenerative diseases more broadly, recapitulated the trends found in Alzheimer's disease. These findings implicate the identified protein modules in the specific progression of dementia.

In contrast to the protein modules perturbed early on in Alzheimer's disease, one protein module perturbed in late Alzheimer's was similarly affected across all the neurodegenerative diseases studied and not just those with dementia. This was the mitochondrial protein module. The authors postulate that cellular energetics are unsettled during the progression of many neurodegenerative diseases, and such disruptions may manifest late in disease as a consequence of many possible underlying molecular causes.

Discordance between RNA and protein expression in neurodegenerative disease

Looking at proteomic and transcriptomic differences between Alzheimer's disease and PSP (another type of dementia), the authors noted that, while changes in the transcriptome were often in the same direction as the proteome, they only accounted for ~50% of the variability at the protein level. This is a consistent finding across studies that assess concordance between RNA expression and protein expression (See Buccitelli and Selbach 2020 for a great review on the topic).

While incomplete concordance between RNA and protein expression can be a problem of measurement techniques, it is also an important characteristic of biological systems. There are a variety of cellular regulatory mechanisms that alter protein levels without necessarily impacting mRNA abundance. These can include processes like miRNA-based translational repression and enhanced protein degradation. Indeed, investigating the causes for discordance between mRNA and protein levels can reveal important regulatory mechanisms active in cells.

Nonetheless, in this work, there were distinct overlaps between the important players in the identified proteomic and transcriptomic modules. These may make high priority biomarkers or drug targets given their consistent association with disease across data types.

Even with these findings, the authors highlight that there were many genes whose protein expression correlated poorly with mRNA expression. The authors point to proteins from the electron transport chain and MAPK proteomic modules as prominent examples. Indeed, protein expression was even negatively correlated with mRNA expression for the electron transport chain module. This underscores the incredible importance of posttranscriptional and posttranslational regulation in modulating biological activity. It also calls attention to the need for proteomics to identify markers of biological function and dysfunction.

Combining genomics, transcriptomics, and proteomics to find disease-specific genes

These authors also checked to see if the modules with correlated changes in transcripts and proteins were enriched for genes associated with Alzheimer's or PSP through Genome Wide Association Studies (GWAS). They found significant enrichment for GWAS-identified genes in modules associated with each disease and for early disease modules in Alzheimer's in particular. In addition, they found that the enriched modules were not conserved across the two diseases. These results suggest that the GWAS-identified genes may be disease-specific or causal.

Of course, biology is always complicated. It is possible that the GWAS identified genes do impact late Alzheimer's disease proteomic modules through trans effects. Even if a DNA variant is not directly in or near a particular gene, it may still impact that gene through effects on processes like transcription, translation, or mRNA and protein degradation. Nonetheless, these authors argue that concordance across the genome, transcriptome, and proteome further solidifies the identified genes and their products as potential biomarkers or therapeutic targets.

Enabling stronger comparisons across the omes with next-generation proteomics

The results from this study are particularly powerful because they are derived from multiple analyses across multiple sample types. Unfortunately, such studies are not easy to perform today because proteomic experiments at this scale are often cumbersome and do not cover the proteome in the comprehensive way that transcriptomics and genomics do. We are working tirelessly to ensure that accessible next-generation proteomics platforms like the Nautilus Proteome Analysis Platform will enable many more researchers to analyze comprehensive proteomic data along with genomic and transcriptomic data to identify potential biomarkers and therapeutics with high confidence. The insights obtained as a result will hopefully lead to care strategies and treatments that vastly improve the lives of those with neurodegenerative diseases.

Applications of proteomics in neuroscience: Uncovering protein networks in Alzheimer's

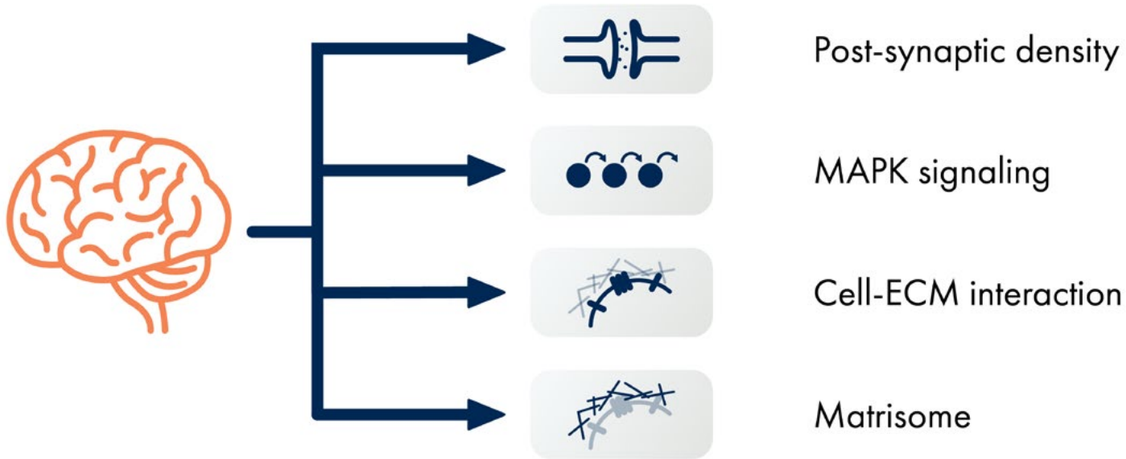
Identifying protein groups or modules associated with Alzheimer's symptoms

- Proteomics can uncover cellular pathways perturbed in Alzheimer’s that are not apparent in transcriptomics.

As researchers hunt for potential Alzheimer’s treatments, multiomics approaches are revealing new avenues of exploration that could lead to breakthroughs. This includes linking the proteome to the genome and other omes to better understand the mechanisms of disease.

Alzheimer’s disease is a degenerative neurological condition that affects nearly 6 million Americans. That number could grow to 14 million by 2050. The disease is associated with buildups of amyloid beta protein and tau protein in the brain, and a corresponding loss of memory and brain function.

Exactly how these two proteins work to degrade neurological function, and what other mechanisms might be involved, aren’t fully clear. Indeed, recent failures of treatments targeting amyloid beta have called into question the role of amyloid beta in Alzheimer’s, and indicate that it may not be as fundamental to disease progression as was thought.



Johnson, et al discovered 4 protein modules strongly correlated with Alzheimer’s disease neuropathology or cognition.

Discovery proteomics uncovers protein modules associated with Alzheimer's

Work linking proteomics to genomics could help get us closer to an understanding of Alzheimer’s disease pathology. In a recent proteomic analysis, researchers associated protein networks with Alzheimer’s and compared them to RNA networks to see whether they match up. In this and similar studies, it is important to look to proteins and not just RNA because

many proteins are modified after they are made. This means RNA expression alone does not reveal the abundance or form of fully functional proteins in many cases.

To get around that limitation and better understand how Alzheimer's works at a fundamental level, Johnson, Carter, and Dammer, et al turned to a technique called discovery proteomics. The team looked at post mortem brain proteomes of people with Alzheimer's, and compared them to post mortem brain proteomes of people without the disease.

Using tandem mass tag mass spectrometry (TMT-MS), the researchers conducted a proteomic analysis of over 1,000 brain tissues from the Accelerating Medicines Partnership for Alzheimer's Disease (AMP-AD) consortium. They identified thousands of proteins, which they grouped into 44 different co-expression modules, or groups of proteins that tend to be produced together. Of the 44 protein co-expression modules, the researchers identified 4 that were most strongly correlated with symptoms of Alzheimer's disease.

The researchers also performed a transcriptomic analysis on post-mortem brain tissue from people with and without Alzheimer's to determine if they could find RNA modules similar to the protein modules. This multiomics approach found that almost half of the protein modules didn't correlate with RNA modules. This is a strong sign that Alzheimer's disease is caused in part by post-translational modifications. The finding also underscores the value of studying the proteome to better understand Alzheimer's.

Two of the protein modules that didn't have any analog in the RNA modules were also ones that had the strongest connection to Alzheimer's symptoms. One of the two modules was associated with the MAPK signaling pathway and metabolism. The other was associated with the matrisome, or the collection of proteins that make up the extracellular matrix. Proteins from both modules could be valuable targets for future research into Alzheimer's treatments.

Furthering the multomic nature of their analysis, the researchers go on to find associations between genomic variants and the identified protein modules. In a sort of validation of this strategy, a single nucleotide polymorphism (SNP) in a gene known to impact Alzheimer's disease risk (APOE) strongly impacted the matrisome protein module. A variety of other modules were impacted by other SNPs and, often, the SNPs were found outside of genes encoding proteins in the modules. This indicates that the SNPs impact the modules through trans effects. These findings both highlight the complexity of multiomic interactions affecting Alzheimer's disease and point to potential targets for therapeutic intervention.

Next-generation tools for in-depth proteomic analysis

One key takeaway from this research is that extending disease studies to the proteome and covering more proteins can help uncover functional networks that were previously hidden. We're designing the Nautilus Proteome Analysis Platform to cover nearly the entire proteome and make similar studies more accessible to more researchers. The platform's single-molecule sensitivity and wide dynamic range are designed to reveal high and low abundance proteins for unprecedented proteome coverage.

Proteomics technologies that identify all the proteins in a sample will hopefully accelerate more fantastic work like the research highlighted here. This acceleration could advance efforts to reveal the biological underpinnings of diseases like Alzheimer's. Such studies may lead to new protein biomarkers that track disease progression and even identify targets for treatments. With next-generation proteomics, new Alzheimer's diagnostics and treatments could be in sight.

Applications of proteomics in neuroscience: Assessing tau modifications during Alzheimer's disease progression

Proteomics reveals increasing tau modifications as Alzheimer's progresses

- Proteomics technologies capable of identifying and measuring the abudance of modified forms of proteins like tau can improve our understanding of cellular functions and disease.

Alzheimer’s disease is a debilitating neurodegenerative disorder that impacts millions of people worldwide and ultimately results in death. While the molecular underpinnings of the disease are poorly understood, it is associated with the aggregation of the tau protein inside neurons. Recent proteomics research is beginning to elucidate the role tau aggregates play in this devastating disease and may lead to new Alzheimer’s diagnostics and therapies.

Here, we cover collaborative work by Wesseling, et al wherein researchers use proteomics to identify the many ways the tau protein is modified during Alzheimer’s progression. Researchers have known for many years that tau is highly phosphorylated in Alzheimer’s, but this groundbreaking work provides a map of the types of modifications present across the tau protein and reveals the cumulative extent of modifications across tau’s component peptides. This and similar work may help researchers identify the mechanisms underlying tau aggregation and its role in disease.

A mass spectrometry-based technique for identifying tau proteoforms - FLEXITau

These researchers leveraged a technique known as FLEXITau to measure tau isoforms and their modifications (collectively known as proteoforms). In this technique, researchers combine internal standards with an optimized bottom-up mass spectrometry protocol to quantify modifications across

tau peptides generated for mass spec. This creates maps identifying the extent of modification to the peptides and further analysis enables researchers to identify the specific modifications present. This methodology does not quantify the amounts of each type of modification but provides a great overview of tau proteoforms.

Tau proteoforms and Alzheimer's progression

The researchers performed FLEXITau on postmortem brain tissue samples derived from people with Alzheimer's as well as matched controls. When analyzing tau from these samples, they were able to correlate specific modification profiles with known levels of AD severity derived from patient data.

As has been reported in the past, these researchers found that tau was more highly modified in patients with more severe cases of Alzheimer's disease. Importantly, in this work, researchers were able to identify the specific kinds of modifications present across tau including acetylation, ubiqutination, methylation, and phosphorylation. In addition, they identified more modified sites than had been reported in the past. The FLEXITau data also revealed which specific modifications and what level of peptide modification are best able to discriminate Alzheimer's disease samples from controls. For instance, the authors discovered that ubiquitination of K311 and K317 and phosphorylation of T217 and S262 are strong differentiators for people with Alzheimer's vs controls. Finally, the FLEXITau data revealed that the 0N and 4R tau isoforms are enriched in insoluble, pathologic tau fractions.

Altogether, this data enabled the development of a model for the accumulation of tau isoforms and modifications during Alzheimer's progression. In this model, the 0N and 4R tau isoforms begin to aggregate at early stages of the disease. These isoforms are modified first by phosphorylation and cleavage of the C-terminus. Later, there is further phosphorylation and subsequent ubiquitination and acetylation to neutralize the negatively charged phosphate groups. Highly modified and neutralized tau forms fibrils that cause disease progression.

These findings show that specific tau isoforms with different modifications may be more amenable targets for diagnostics and therapeutics aimed at the early and late stages of the disease.

Looking forward to future tau studies powered by next-generation proteomics

This powerful study along with others on the topic reveal the many ways tau is modified in Alzheimer's disease. Bottom-up proteomics does not reveal the extent of modification to individual, intact tau molecules, but provides researchers with excellent insights into the modifications present in Alzheimer's. Future work using next-generation proteomics technologies that measure proteins and their modifications at the single-molecule, intact protein level may further resolve tau proteoforms and their modifications.

We're designing the Nautilus Proteome Analysis Platform with such capabilities in mind and have begun collaborating with neuroscience researchers to develop means of measuring tau proteoforms on our platform. We aim to enable researchers to achieve single-molecule resolution of protein abundance and modification and are working tirelessly to make this platform accessible to researchers who may not be familiar with the complex workflows of mass spectrometry. We thereby aim to enable more incredible work like that of the researchers highlighted here and hope this work will elucidate the complex roles of tau and other proteins in health and disease.

3 ways next-generation proteomics can impact neuroscience

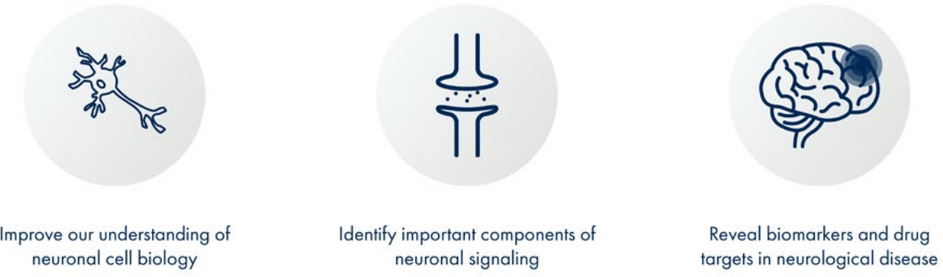
Looking forward to a future rife with neuroscience discovery

- From cell biology, to signalling, to disease, next-generation proteomics platforms can vastly improve our understanding of the role proteins play in neuroscience.

Neuroscience research has advanced dramatically over the last few decades. Researchers now have the ability to trace neuronal connections, activate brain pathways using optogenetics, and even grow mini brains in the lab. Nonetheless, we still have limited understanding of how the brain and broader nervous system work in healthy individuals and malfunction in disease.

Proteomics offers the ability to measure the protein composition of tissues, cells, and specialized cellular structures in the nervous system. This could point researchers to proteins essential for the neurological processes involved in learning, memory, and a wide variety of behaviors. It could also help them identify biomarkers and drug targets for neurological disorders.

While traditional proteomics technologies often fail to measure the full proteome and can be blind to the precise protein modifications that drive changes in cellular activities, next-generation proteomics platforms like the Nautilus Proteome Analysis Platform are designed to measure substantively all of the proteome in just about any cell type. Here, we discuss three ways next-generation proteomics platforms can enable neuroscience research. This is far from an exhaustive list, but it demonstrates how next-generation proteomics can transform neuroscience.



Advancing our understanding of neuronal development with proteomics

The brain is an incredibly complex and heterogeneous organ and understanding how the architecture of the brain and broader nervous system comes to be is a key goal of neuroscience. While scientists have learned a great deal about the cues that establish and maintain neuronal connections, there is much to learn about the molecular mechanisms that guide these connections.

Scientists are using proteomics to learn what proteins are altered as a result of signaling in neuronal development. Techniques such as interaction proteomics can show where these proteins localize and clue researchers into their functions. Techniques like phosphoproteomics, on the other hand, can reveal signaling pathways active during various developmental stages and help reveal the mechanisms behind neural plasticity.

Proteoform profiling is particularly important for studying neuronal development. Things like cytoskeletal proteins are dynamically regulated in neuronal development and regeneration to create and maintain the extensive cellular contacts in the nervous system. The stability of the cytoskeleton is partially determined by posttranslational modifications to cytoskeletal proteins and understanding the composition, distribution, and abundance of these modifications in various neuronal contexts will be essential for a thorough understanding of the nervous system.

Next-generation proteomics platforms are expected to make it much easier to identify all the players involved in neuronal development including their various proteoforms. This may enable scientists to gain a more thorough understanding of things like cytoskeletal modifications that are essential to the dynamics of the nervous system.

Advancing our understanding of neuronal cell signaling with next-generation proteomics

Neurons transmit electrochemical signals to store, process, and relay information as well as to coordinate activities in other cells and tissues. Neuronal networks involved in these functions can be extremely complicated. For example, there are billions of interconnected neurons in the mammalian brain. Researchers have begun mapping these networks and have even mapped full neuronal networks in animals like ***C. elegans***, but one key to understanding their connections and functions is to learn more about the types of signals sent between neurons and how different kinds of neurons receive and respond to those signals.

Synapses are specialized structures at neuronal interfaces where interneuronal communication is largely defined. Here, neurotransmitters are released by pre-synaptic neurons and received by post-synaptic neurons. These neurotransmitters bind to receptors in the post-synaptic neurons and act through various signaling cascades and protein channels to either activate or inhibit the post-synaptic neuron. Understanding what machinery is involved in both the release and receipt of neurotransmitters (of which there are many) is essential to understanding nervous system function.

Researchers have developed a variety of techniques to measure the proteomes of neurons in discrete areas of the brain and more specifically in synapses. In one such technique, researchers genetically encode labeling enzymes such that they are produced in particular regions of the brain, particular types of neurons, and even trafficked to the synapses. These enzymes then add labels to proteins found in these specific regions and the labels can be used to isolate the proteins. Currently, researchers use mass spectrometry to identify such labeled proteins. While useful, mass spectrometry has sensitivity issues and cannot always detect specific proteoforms. This can make it difficult to get a full picture of the proteomes of specific neurons or synapses. For a review on these techniques, see Wang and Savas 2018.

Next-generation proteomics platforms like the Nautilus Proteome Analysis Platform are designed to have single-molecule sensitivity. The Nautilus Platform also analyzes intact proteins and can therefore identify different proteoforms with relative ease. These technologies should make it easier to understand the precise proteomic make-up of particular neurons and synapses and thereby provide researchers with a mechanistic understanding of the neuronal signaling pathways involved in various behaviors and nervous system activities.

Advancing our understanding of neurological disease with next-generation proteomics

Perhaps the best-known neurological disorder is Alzheimer's disease. More than 6 million people in the US alone have this progressive, debilitating, and ultimately lethal disease. It's expected that this number will grow dramatically over the next few decades as the American population ages.

While the precise molecular events causing this disease are not completely understood, Alzheimer's pathology is associated with the aggregation of two proteins:

- Beta amyloid outside brain cells
- Tau inside brain cells

Scientists have already begun developing drugs designed to remove these aggregates and hopefully slow or stop progression of the disease. While there has been moderate success, there is still much to learn about what causes

these proteins to aggregate and how they're involved in neurological dysfunction. In fact, some drugs have effectively removed aggregates without abrogating disease. Clearly more research is needed.

Recent work with traditional proteomics platforms has begun to reveal the role post translational modifications play in the development of aggregates and Alzheimer's disease. For example, Wesseling, et al showed that various phosphorylated, ubiquitinated, and acetylated forms of the tau protein correlate with clinical stages of Alzheimer's disease. This work points to the need to target different forms of tau at different stages of the disease.

Such studies begin to elucidate tau's role in disease biology, but are difficult to perform on standard proteomics platforms. These technologies are not routinely used to identify proteoforms in many labs. Next-generation proteomics platforms aim to be able to routinely measure the collection of proteoforms in cells. Such studies should provide researchers with an in-depth view of protein modification in Alzheimer's and many other diseases involving protein modification such as Parkinson's and prion diseases. These studies may lead to the development of novel treatments targeting pathogenic protein modifications and their effects.

In addition to elucidating the mechanisms underlying diseases like Alzheimer's, next-generation proteomics can also help researchers develop novel biomarkers for these diseases. After cognitive defects are identified, Alzheimer's is currently diagnosed through brain imaging and the measurement of Alzheimer's proteins in cerebral spinal fluid. Blood tests are also in development, but novel tests that are less invasive and help doctors better identify the stage of the disease as well as potential treatments are needed. Efforts are underway in labs like that of Randall J. Bateman to identify new Alzheimer's biomarkers using mass spectrometry. Their efforts may lead to the development of more effective diagnostics and treatments.

Nonetheless, most traditional proteomics platforms lack the sensitivity and dynamic range to assess such biomarkers routinely. Next-generation platforms are designed to measure the majority of the proteome even in a sample as complicated as blood plasma. This may make it possible to treat these diseases early, before neurological impacts become pronounced.

We want to work with you to enable neuroscience research with the power of next-generation proteomics

We're just scratching the surface in terms of the many ways next-generation proteomics platforms can enhance our understanding of neuroscience. Although this field is complex, the single-molecule analysis capabilities and high dynamic range of technologies like the Nautilus Proteome Analysis Platform are designed to be up to the challenge. The Nautilus platform in particular is designed to interrogate intact, full-length proteins at the single-molecule level and is expected to be able to identify the ways individual proteins have been modified in a sample of interest through targeted proteoform studies. If you're working in this field and would like to collaborate to transform neuroscience, please reach out!

Proteomics and neuroscience

Conclusion

- Proteins drive much of the biology of the nervous system and researchers are leveraging proteomics technologies in creative and impactful ways to learn about the mechanistic underpinnings of neuroscience.

In this eBook, we've highlighted a miniscule fraction of the many interesting discoveries forged through the amalgamation of proteomics and neuroscience. Combining knowledge and techniques from these complex and fascinating fields has yielded insights into the development of neuronal networks, protein malfunctions, and neurological disease. Clearly neuroscientists are working incredibly hard to bring knowledge of the proteome to bear on the development and function of the nervous system, and have been highly successful to date.

Nonetheless, there is incredible opportunity for those developing next-generation proteomics platforms to work with neuroscientists to improve the accessibility, throughput, sensitivity, and dynamic range of their technologies in ways that are useful to the field. Given that neuroscientists already need extensive expertise in cell biology, biochemistry, genetics, network analysis, and so much more, we can potentially accelerate their work by replacing the technologically challenging proteomics tools of the past with more accessible platforms. Ideally these will not require neuroscientists to develop extensively customized workflows to achieve the sensitivity and dynamic range required to comprehensively analyze neurological processes. In addition, given the critical importance of proteoforms in neuronal development, maintenance, and disease, such platforms should be developed with single-molecule (single-protein) resolution in mind from the get-go.

We aim for the Nautilus Proteome Analysis Platform to achieve all this and more. When it and similar technologies are available to neuroscientists, there's no telling what advances will come to fruition. One thing is certain however, the proteomics revolution in neuroscience will be rife with discovery. Perhaps neuroscientists will gain a much better understanding of the complex roles proteins play in behaviors like dreaming and depression. Perhaps they'll create and validate better models of the brain. Perhaps still, neuroscientists will gain an understanding of the roles of complex protein networks in consciousness itself.

Neuroscientists are innately driven to ask and answer groundbreaking questions, and we hope to inspire and enable them to let their creativity run wild through the power of next generation proteomics.

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