Proteomics and neuroscience

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

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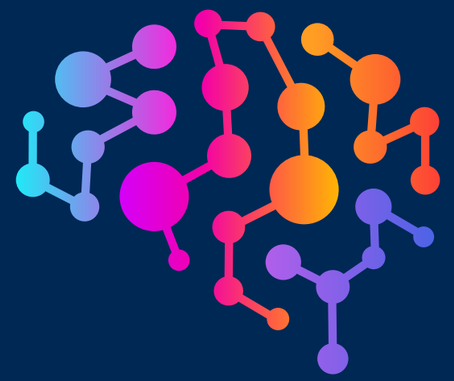


Introduction

- 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 in 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 located 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 modification, 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, proteoforms, 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 and knowledge of proteoforms 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 impactful insights that could one day lead to cures for Alzheimer's disease, Parkinson's disease, and much more.

Uncovering protein networks in Alzheimer's

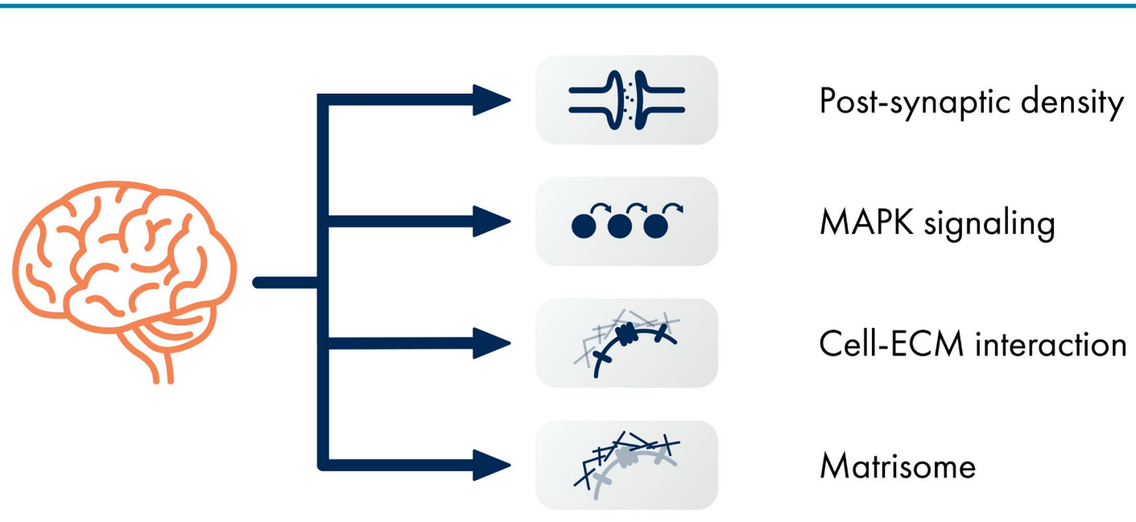
Identifying protein groups or modules associated with Alzheimer's symptoms

- 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., 2022 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 et al., 2022 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 went 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 substantively 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.

Recent further research

Aiming to deepen our understanding of Alzheimer's pathology, Levites et al., 2024 compared proteome changes in Alzheimer's disease mouse models with those in brains from humans with Alzheimer's. They discovered that changes in the matrisome protein module were the most conserved across mouse and human samples, that many proteins from this module localized to amyloid plaques, and that two proteins from this module, Mdk and PTN, enhanced amyloid beta aggregation. Furthermore, Mdk and PTN localized with the heart disease amyloid, TTN. These results led Levites et al. to hypothesize that amyloids associated with various diseases may, in addition to their direct impacts, serve as scaffolds for proteins that cause further pathological effects.

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.

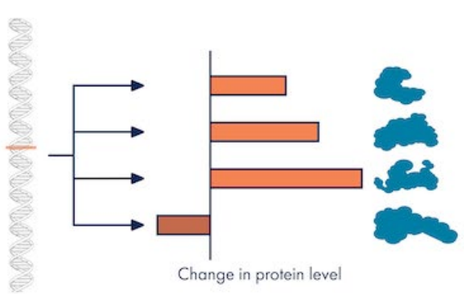
As our understanding of the neurological underpinnings of diseases affecting the brain grows, new therapies for previously intractable diseases like Alzheimer's, Parkinson's, and more may be within reach. As is often the case, these conditions also typically turn out to be far more complex than previously 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., 2021 identified proteins across samples from different types of tissues affected by Alzheimer's disease, stroke, and Parkinson's disease. The research provides insights into the genes as well as the tissue-specific post-translational modifications underlying neurological conditions.

The research also underscores the power of proteomics to reveal the mechanisms behind biology in exquisite detail while highlighting 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, Yang et al. 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 modifying 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 using the Nautilus Platform.

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 may soon be able to bring them into the light.

Recent further research

Ali et al., 2025 used multiple proteomics platforms, samples, and kinds of analysis to identify potential Alzheimer's biomarkers in cerebrospinal fluid. Their efforts enabled them to identify changes in proteins that had not been identified before and develop models for identifying cases of Alzheimer's disease. Their final model had performance comparable to standard measures of CSF amyloid beta and phosphorylated tau. This model also had higher specificity for Alzheimer's when compared to previous proteomics models. Finally, their extensive datasets enabled them to track changes in the proteome across clinical stages of Alzheimer's disease and identify changes in biological processes that may be important to disease progression.

Identifying disrupted pathways in dementia

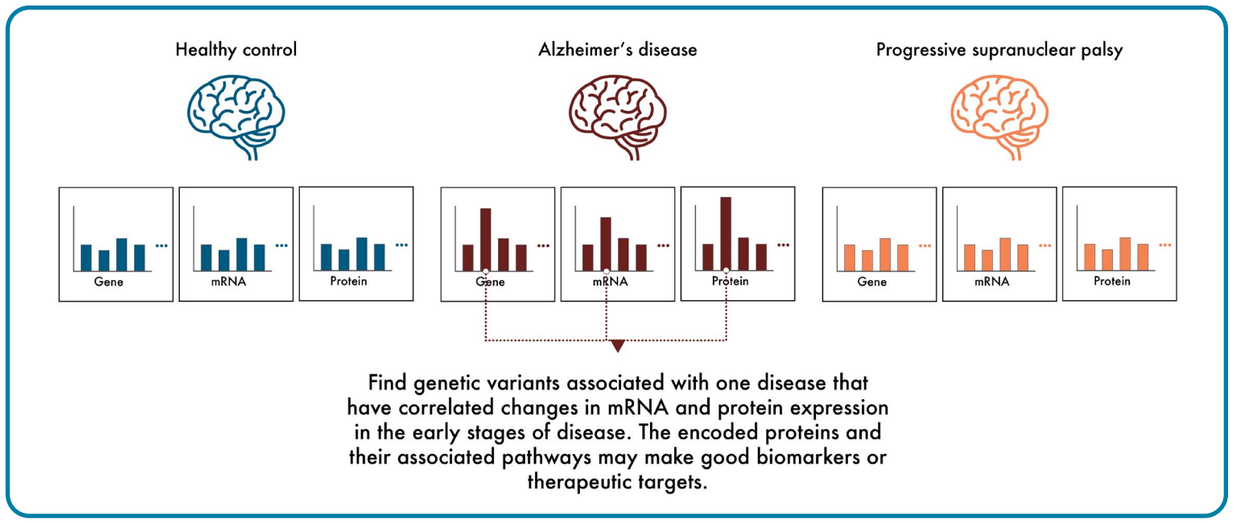
Identifying potential biomarkers and therapeutic targets in neurodegenerative disease

- 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 et al., 2020 used this strategy in their paper titled, "Identification of Conserved Proteomic Networks in Neurodegenerative Dementia." Here, they leveraged 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 pointed 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, mirrored the trends found in Alzheimer's disease. These findings specifically implicate the identified protein modules the 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 highlighted that there were many genes whose protein expression correlated poorly with mRNA expression. The authors pointed to proteins from the electron transport chain and MAPK proteomic modules as prominent examples. 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.

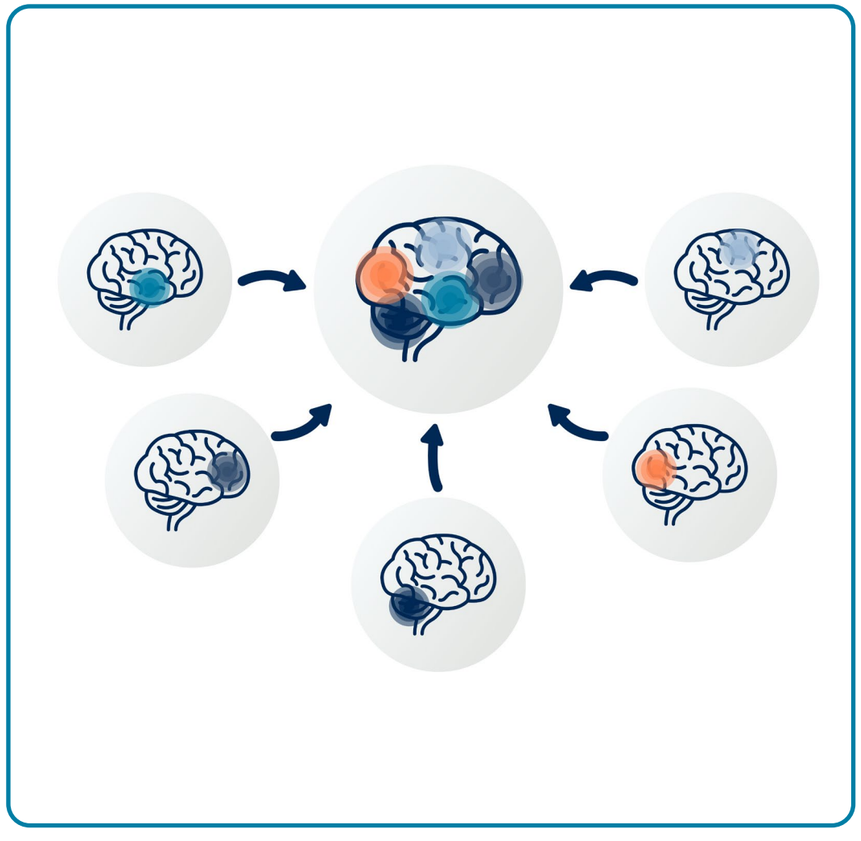
Enabling stronger comparisons across the omes with next-generation proteomics

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.

Creating an atlas of protein changes during Alzheimer's disease progression

A systematic review of studies covering:

- 3 clinical stages of Alzheimer's disease
- 13 brain regions
- Neurofibrillary tangles, amyloid plaques, and cerebral amyloid angiopathy



Alzheimer's disease (AD) is a complex neurodegenerative disorder involving many brain regions, many stages of disease, and many pathological features (e.g. amyloid plaques, neurofibrillary tangles [NFTs], and cerebral amyloid angiopathy [CAA]). This complexity has been captured in the hundreds of proteomic studies of Alzheimer's and the even more numerous publications on the roles of individual proteins in the disease. However, each individual study provides a narrow perspective on Alzheimer's disease as most are limited by small sample sizes, the inclusion of few brain regions, or the inclusion of few AD stages.

A comprehensive study encompassing all stages of AD and all brain regions sounds like a monumental task, especially if you take into account the number of samples you'd need for such an experiment. Rather than start from scratch, a recent study took another approach: a systematic literature review of multiple proteomics studies.

Askenazi et al., 2023 selected 38 publications that used proteomics to study Alzheimer's disease in humans. With data from these publications in hand, they set out to determine how the proteome changes across 3 clinical stages of disease, 13 brain regions, and 3 pathological features (or "lesions"). The amalgamation of data from their systematic review (which can be viewed in the NeuroPro database) allowed them to answer questions that would have been difficult with just one study on its own, for example:

- What proteins are enriched in amyloid plaques, NFTs, and cerebral amyloid angiopathy?
- What are the earliest protein changes in Alzheimer's disease?
- How do protein abundance changes associated with AD vary between brain regions during AD progression?

Below, we take a dive into the data to see how the authors' systematic review answers these questions and discuss how next-generation proteomics platforms like the Nautilus™ Proteome Analysis Platform can propel future studies of AD and other diseases.

Identifying common proteome changes in AD

In their review, Askenazi et al., 2023 found 54 proteins were differentially expressed in at least 15 of the studies included. Because they were altered in so many studies encompassing different techniques, brain regions, and clinical stages of disease, it's likely these are the most prevalent proteome changes in AD. These differentially expressed proteins provide a great opportunity for future scientific inquiry: 46% of these proteins are currently understudied in AD (≤ 10 previous publications) and 4 of these proteins have never been examined in the context of AD.

Pathological features of AD show different patterns of protein enrichment

While the 54 differentially expressed proteins mentioned above highlight widespread changes in AD, focusing on specific, localized features of the AD brain can help identify proteins involved in their formation. Here, the authors identified many protein expression changes associated with amyloid plaques, NFTs, and CAA. They found that only a few proteins were enriched in all three while the majority of the proteins enriched/depleted in each pathological feature likely reflected the processes involved in that feature's formation.

These findings demonstrate the importance of doing proteomic studies on localized brain features in addition to bulk tissue samples. In bulk tissue experiments, the enrichment of these proteins could have been missed.

A temporal wave of protein dysfunction

With their systematic review, Askenazi et al., 2023 could simultaneously examine proteome changes during different stages of disease. They could also examine different regions of the brain known to be "vulnerable" or "resistant" to pathological changes that occur during AD. For example, amyloid beta and tau (proteins that aggregate to form amyloid plaques and NFTs, respectively) are known to progressively spread throughout the brain affecting vulnerable regions first.

One of the key findings from their analysis was there is a widespread decrease in synapse-related protein expression in early AD suggesting general synaptic dysfunction. These changes occur before amyloid beta and tau accumulation and are likely the initiating drivers of disease. They may also be potential drug targets for therapeutics treating early AD.

Overall, Askenazi et al., 2023 found 258 altered proteins in early and late AD with very few proteins that trended in opposite directions in early vs. advanced stages (i.e. proteins that were highly expressed early generally remained highly expressed and vice versa). Furthermore, they saw the same patterns of protein changes in resistant brain regions in advanced AD as vulnerable brain regions in early AD. This patterning hints at a "temporal wave of progressive protein dysfunction" throughout the brain where resistant brain regions undergo the same changes as vulnerable regions, just at a later time.

Next-generation proteomic tools empower Alzheimer's disease research

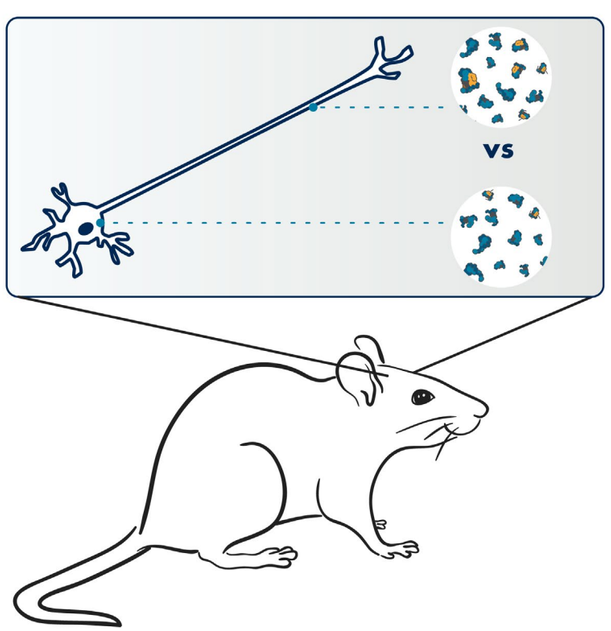
Askenazi et al., 2023 uncovered several key proteomic changes in the progression of AD in different brain regions, in different neuropathological features, and at different stages in disease progression. As the authors demonstrated, examining the collection of proteomics publications for a given disease or biological process can paint a comprehensive picture of the entire proteomic landscape that would not have been possible with just one proteomic study alone.

However, the authors did note that some of the past proteomic studies included in the analysis were "underpowered." With low-throughput, underpowered studies, low abundance proteins could be missed and there could be higher variability in the data. Rapid throughput, next generation platforms, such as the Nautilus™ Proteome Analysis Platform, can drive the detection of rare proteome changes, capture more data, and bring even more power to systematic literature reviews of protein changes in AD and beyond.

Proximity proteomics for the identification of axonal proteomes

Answer questions like:

- What proteins are abundant during different stages of axonal 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 neuronal cell bodies 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 causes biotin molecules to attach 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 Dumrongprechachan et al., 2022. 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, Dumrongprechachan et al. 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 at various developmental time points and identified the proteins 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.

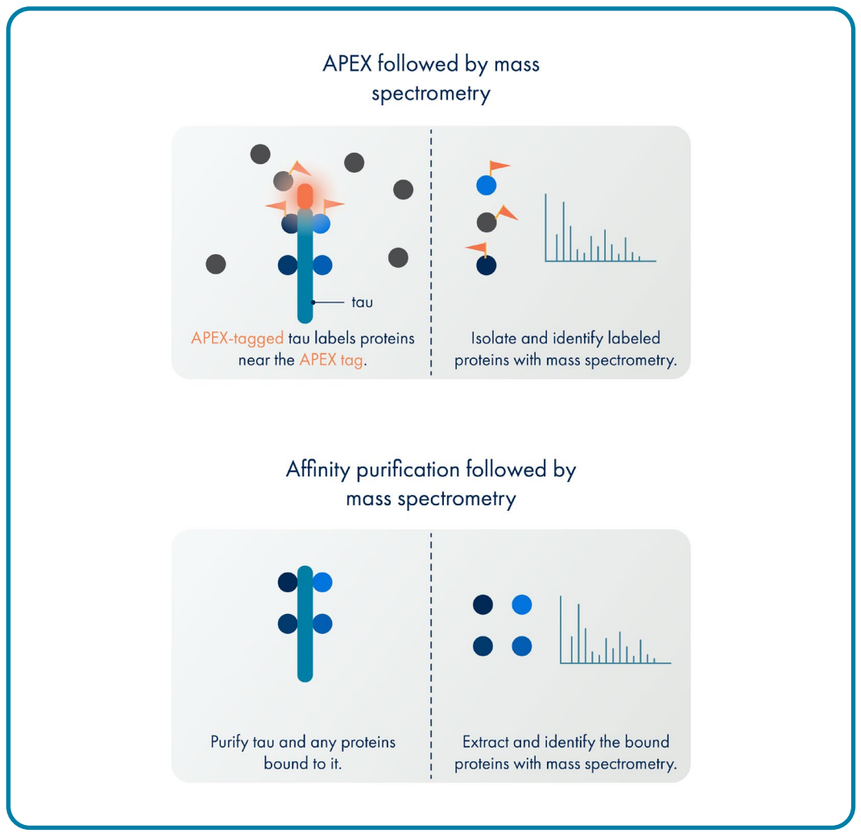
The data generated from this study alone can be mined to associate proteins with behaviors, neurological processes, diseases, and more for years to come. Nonetheless, to test the hypotheses generated by these associations, in-depth proteomics studies must be more accessible to researchers with diverse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

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 amyloid beta outside neurons and tau 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. To better understand the roles of tau and amyloid beta 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., 2022 recently used interaction proteomics to profile the proteins that interact with wildtype and pathogenic varieties of tau. 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., 2022 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 cause biotin molecules to be added to any electron-rich amino acids in proteins near the APEX-tagged tau. Using biotin-binding antibodies, researchers purified the biotin-labeled proteins from the cells and used mass spectrometry to identify them. This revealed 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.

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 work by Wesseling et al., 2020 wherein researchers used 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 amount of each proteoform but provides a great overview of tau modifications.

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 patterns of modification.

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.

Recent further research

Wenger et al., 2023 used the FLEXITau technique to quantify tau modifications in mouse models of Alzheimer's and compared the modifications observed to those in post-mortem samples from people with Alzheimer's. For the specific models studied, Wenger et al. found that modifications present in late-stage human Alzheimer's samples were not present in the mouse models. Thus, these models may be suitable for early human disease but not late-stage disease. Similar analyses of tau proteoforms across patient samples and model systems may reveal the best models to use when studying different facets of Alzheimer's disease.

Access recent tau proteoform data from Nautilus.

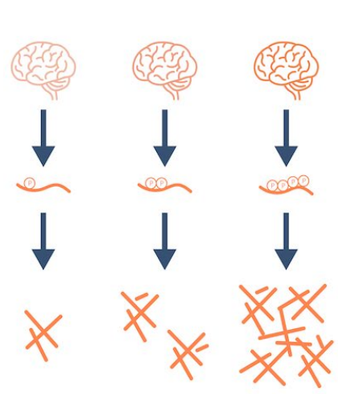
Tau proteoforms as potential drivers of cognitive decline

Linking tau proteoforms to aggregation and clinical outcomes

- Dujardin et al., 2020 measured post-translational modifications found on tau proteins extracted from post mortem brain samples from 32 Alzheimer's patients and identified associations between tau modifications, aggregation, and cognitive decline.

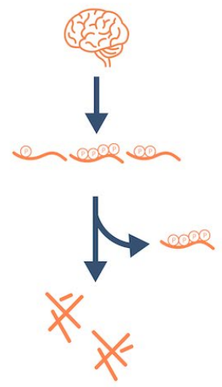
The classic pathological features of Alzheimer's disease are amyloid beta aggregates outside neurons (amyloid plaques) and tau aggregates inside neurons (neurofibrillary tangles or NFTs). Plaques have been primary therapeutic targets, yet immunotherapies successfully eliminating them slow but do not stop cognitive decline (Golde and Levey 2023, the section of this eBook focused on amyloid beta proteoforms). These immunotherapies are powerful advances but not cures. For many, the next step in the fight against Alzheimer's is a renewed focus on tau.

Tau has many molecular variants or proteoforms. It comes in 6 isoforms in the central nervous system, and these can be modified in various ways including phosphorylation, acetylation, and ubiquitination to produce a wide variety of proteoforms (Reviewed in Alquezar et al., 2021). In the work highlighted below, Dujardin et al., 2020 provided evidence that tau proteins extracted from Alzheimer's patients with varying rates of cognitive decline have heterogenous proteoform profiles. These are associated with varying propensities to seed aggregate formation, and higher seeding potential is correlated with faster rates of cognitive decline. Finally, these authors showed that depleting brain extracts of groups of tau proteoforms with shared epitopes lowers their seeding potential. This work suggests depleting tau proteoforms associated with seeding may be a promising therapeutic avenue.



Extracts from brains of Alzheimer's patients with different rates of cognitive decline contain different mixtures of tau proteoforms with different propensities for seeding aggregates.

Greater seeding potential is correlated with faster cognitive decline.



Depleting extracts of groups of tau proteoforms associated with Alzheimer's progression decreases their seeding potential. This suggests that depleting these proteoforms in patients may be a good therapeutic strategy.

Tau seeding potential is correlated with cognitive decline

Dujardin et al. extracted proteins from the brains of 32 Alzheimer's patients with varying levels of cognitive decline. Then, they tested each extract's propensity to seed tau aggregation in a variety of models:

- An in-vitro FRET biosensor – HEK293 cells expressing tau proteins fused to fluorescent proteins were treated with extracts from patients. Increased FRET signals resulting from treatment indicated seeding. Extracts from different patients had highly variable seeding by this assay.
- Mouse primary neurons – Mouse primary neurons expressing human tau were cultured and treated with the Alzheimer's brain extracts. Staining with antibodies that detect hyperphosphorylated tau – an indicator of aggregation – showed extracts that induced more hyperphosphorylation also had greater seeding potential in the FRET assay.
- Live mice – Patient extracts were injected into the brains of live mice expressing human tau isoform 1N4R. Two months after injection, the mouse brains were assessed for tau aggregate formation using an antibody that detects hyperphosphorylated tau. Hyperphosphorylated tau was more prevalent in brains injected with extracts that had high seeding potential according to the FRET assay.

These results showed extracts from different patients had varied seeding potential. Dujardin et al. went on to show there was a positive correlation between seeding potential and rate of cognitive decline. These intriguing results suggest seeding may be an important factor in disease progression.

Groups of tau proteoforms are associated with seeding potential

Knowing tau is heavily modified during Alzheimer's disease progression (Wesseling et al., 2020), Dujardin et al. assessed whether seeding and cognitive decline were associated with groups of tau proteoforms defined by their higher order structures, post-translational modifications, or patterns of modification. They discovered that extracts with higher seeding potential also had:

- More oligomeric tau
- More hyperphosphorylated tau
- More high molecular weight tau

Bottom-up mass spectrometric analysis later revealed that some single phosphorylation events (e.g. pSer262) and some double phosphorylation events (E.g. pThr321 and pSer235) were positively correlated with seeding potential and cognitive decline while others were negatively correlated with seeding and cognitive decline.

Highlighting the need for better resolution of tau proteoforms, some single phosphorylation events within clusters of potential phosphosites were negatively correlated with seeding while the simultaneous phosphorylation of multiple sites within these clusters was positively associated with seeding. It can be difficult to determine which sites in a cluster are phosphorylated using bottom-up mass spec, but these results show that patterns of phosphorylation found on specific tau proteoforms or groups of tau proteoforms may help control seeding potential. Technologies that are capable of more fully identifying tau proteoforms and disambiguating their patterns of modification may reveal the links between these specific proteoforms and their pathological effects.

Targeting tau proteoforms as a potential path for precision medicines against Alzheimer's

Given the association between groups of tau proteoforms, seeding, and cognitive decline, Dujardin et al. suggested that depleting these proteoforms may make a viable strategy for the development of precision medicines against Alzheimer's disease. To test this concept, the authors depleted various groups of tau proteoforms from patient extracts using antibodies targeting tau epitopes and phospho-epitopes. Depletion of different epitopes had different effects on seeding depending upon which extracts and which epitopes were used. This may be anticipated given the heterogenous proteoform make-up in each patient. Nonetheless, depletion of proteoform groups known to be associated with Alzheimer's progression was a consistently effective strategy for attenuating seeding, suggesting this approach has therapeutic promise.

A future for tau-targeted precision medicines

Taken together, these results indicate that particular tau proteoforms or groups of proteoforms are associated with Alzheimer's progression. In addition, individual Alzheimer's patients likely have distinct proteoform profiles contributing to their progression. By developing technologies that can resolve these profiles, it may be possible to effectively target and deplete a given patient's specific set of pathological tau proteoforms. Such treatments may make effective precision medicines for Alzheimer's disease.

Tau proteoforms as potential drivers of precision medicine

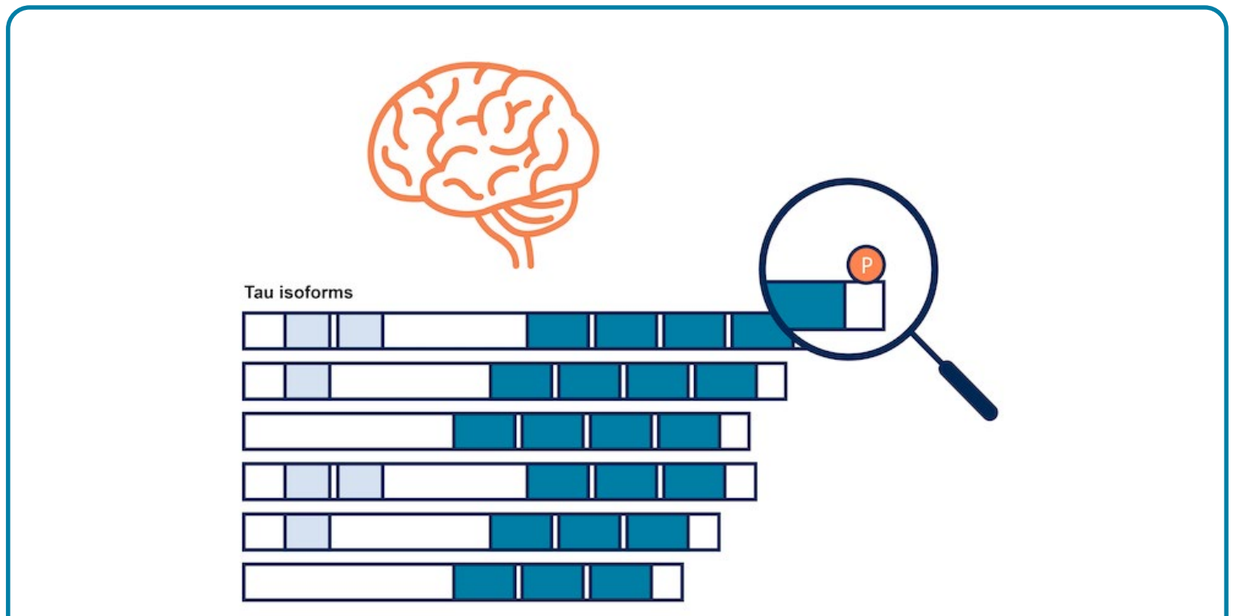
Tau plays roles in many diseases that may superficially look similar

- Kyalu Ngoie Zola et al., 2023 measured tau post translational modifications and associated them with specific diseases involving tau including Alzheimer's disease, corticobasal degeneration, Pick's disease, and frontotemporal lobe degeneration.

Alzheimer's disease is generally quite difficult to diagnose. Until recently, definitive Alzheimer's diagnoses were only established postmortem through the identification of aggregates in the brain – insoluble amyloid plaques seeded by amyloid beta and insoluble neurofibrillary tangles (NFTs) seeded by the tau protein. Now, researchers are establishing means of diagnosing Alzheimer's through the visualization of plaques and tangles with positron emission tomography (PET) and the detection of certain forms of amyloid beta and tau in the blood (see Palmqvist et al., 2024 for example). However, PET is not widely available, and it is not clear if blood biomarkers are specific to Alzheimer's disease as opposed to other forms of dementia. For instance, tau blood biomarkers may be shared amongst diseases involving tau ("tauopathies"). These diseases may have similar effects on cognition yet different molecular causes.

To effectively diagnose and treat Alzheimer's disease as well as other tauopathies, it is critical to gain a better understanding of their molecular nature. This may make it easier to use precision medicines to treat tauopathies and attack them at their molecular roots.

One potential means of distinguishing between tauopathies is to map the tau proteoforms associated with each disease and use any disease-specific proteoforms as biomarkers. Proteoforms are any variants of gene-encoded proteins arising from mutation, alternative splicing, post-translational modifications (PTMs), or any other source. The mixture of proteoforms present in a cell will shape its function, but few technologies achieve proteoform resolution.



Nonetheless, researchers are already seeing clues that patterns of modification on specific tau isoforms may be indicative of AD progression (Wesseling et al., 2020). In the work summarized below, Kyalu Ngoie Zola et al., 2023 show how particular tau PTMs and patterns of PTMs on tau peptides may make effective biomarkers that help diagnose Alzheimer's and other tauopathies. Furthermore, these authors suggest how their data may point to the molecular mechanisms underlying tauopathies.

The potential importance of analyzing soluble tau as opposed to insoluble tau

Prior to this work, Wessling et al., 2020 did extensive work cataloging insoluble tau PTMs and isoforms present in post-mortem brain tissue extracts from Alzheimer's patients with varying degrees of Alzheimer's severity (see previous section in this eBook). Insoluble tau forms aggregates classically associated with Alzheimer's disease. Wessling et al., 2020 showed that 4R tau isoforms as well as increased tau PTMs are found in insoluble tau fractions and are associated with disease progression.

In contrast, Kyalu Ngoie Zola et al., 2023 focused on soluble tau isolated from postmortem brain tissues of patients with various tauopathies and not just Alzheimer's. They did this for two main reasons:

1. They thought soluble tau from brain tissue might be more like tau found in cerebral spinal fluid (CSF) which can be relatively readily analyzed when drawn from live patients. Although insoluble tau forms aggregates, soluble tau may still have modifications indicative of Alzheimer's disease. Thus, investigating soluble tau may point to more clinically significant tau biomarkers than insoluble tau.
2. They wanted to determine if there were differences in soluble tau form different tauopathies. If so, they could use these differences to develop biomarkers that distinguish between the tauopathies.

Soluble tau extracts from various tauopathies have minimal differences in tau isoform levels but significant differences in tau PTMs

Kyalu Ngoie Zola et al., 2023 investigated differences between soluble and insoluble tau extracted from post-mortem brain tissues from patients with the following tauopathies:

- Alzheimer's disease (AD) (n = 15)
- Corticobasal degeneration (CBD) (n = 5)
- Pick's disease (PiD) (n = 5)
- Frontotemporal lob degeneration (FTLD) (n = 10)

While there were clear differences between the relative levels of insoluble 3R and 4R tau isoforms extracted from these samples, there were far less prevalent differences in the soluble isoforms.

When the researchers then used LC-MS to quantify modifications on tau peptides, they observed significant differences between soluble tau in the different diseases. For example, K317 was ubiquitylated in AD but not the other diseases. Similarly, peptides containing both Ub-K267 and P-S262 were also specific to Alzheimer's disease.

Patterns of modification to soluble and insoluble tau suggest impacts on tau aggregation

Kyalu Ngoie Zola et al., 2023 used LC-MS to identify tau PTMs, and in their workflow, tau was enzymatically digested before analysis. The resulting examination of tau peptides made it impossible to determine all the PTMs and other alterations found together on intact tau protein molecules (i.e. it was impossible to identify tau proteoforms). Nonetheless, these researchers did identify tau peptides with multiple modifications and their observations suggest certain modifications may have aggregation-preventing effects.

For example, any soluble tau peptides that had P-T217 also had P-T212 whereas peptides with only P-T217 were observed in insoluble tau aggregates. The authors suggest that such associations may indicate that modifications like P-T212 (found only in soluble tau) prevent aggregation. They observed similar associations for a few other PTM patterns as well.

A need for studies with proteoform resolution

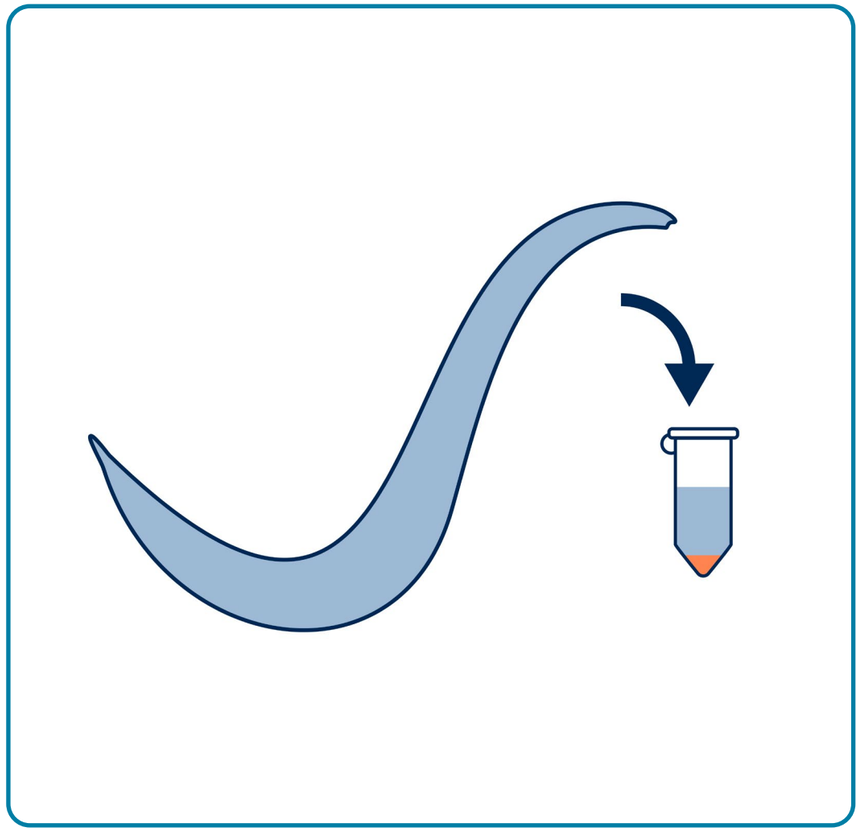
These findings are important because they point to the need to study patterns of modification as opposed to individual PTMs. Even with low resolution views of peptides, these authors have hints that combinations of modifications may be associated with specific processes and outcomes. With full proteoform resolution, researchers may resolve many additional, similar associations. Much more work must be done to determine how the patterns identified here are associated with tau aggregation, but such associations should be decipherable with new, proteoform-aware technologies.

Furthermore, studies like this one suggest that many of the molecular details of disease are obscured because researchers don't have enough resolution in their views of proteins. Here PTM and isoform resolution provide clues to associations with specific diseases and disease processes, but these are difficult to interpret because they are established through the analysis of peptides. With bona fide proteoform resolution, researchers may be able to determine exactly how proteins are modified in diseases, determine how those modifications change protein structure/function in combination, and how interactions between proteoforms impact disease. Specific proteoforms may then be used as biomarkers for disease processes or targeted with novel treatments. Even if these treatments target the same protein, they may be made disease-specific through proteoform targeting and thus advance us to a new era of proteoform-enabled precision-medicine.

Alzheimer’s insights from amyloid beta expression in nematodes

Discover important characterisitics of the amyloid beta-induced insoluble proteome:

- The amyloid beta-induced insoluble proteome has extensive overlap with the age-related insoluble proteome.
- The overlapping **core insoluble proteome** may play an important role in Alzheimer’s and other age-related diseases.



Isoforms of the amyloid beta protein aggregate and form insoluble clumps of protein. These “plaques” play an important role in the progressions of Alzheimer’s disease, and therapeutics designed to clear them have been approved. These promising drugs slow but do not prevent Alzheimer’s progression.

Alzheimer’s therapies may be improved through research into the processes underlying disease progression. Indeed, exactly how amyloid beta aggregation contributes to Alzheimer’s pathology is not understood. In their 2024 work published in GeroScience, Anderton et al. from the Buck Institute set out to elucidate more of the biology underlying this process. They knew other proteins aggregate along with amyloid beta in Alzheimer’s and wanted to identify which of these proteins amyloid beta causes to aggregate. They also wanted to determine if altering these proteins could mitigate amyloid beta’s effects.

Read on to learn how Anderton et al., 2024 leveraged a *C. elegans* model of amyloid beta overproduction and proteomics to discover proteins that may play a role not just in Alzheimer’s, but many age-related diseases.

Watch this animation to learn how next-generation proteomics platforms can fuel neuroscience.

Discovery proteomics reveals age-related and amyloid beta induced protein insolubility

Many human genes have orthologs in *C. elegans*. These nematode worms are often used in Alzheimer's research because they are multicellular, have well-mapped nervous systems, produce many Alzheimer's proteins, and are easier to work with than common mammalian model organisms (see Alexander et al., 2014 for a review). Previous studies in *C. elegans* showed:

- Amyloid beta over-production results in *C. elegans* paralysis.
- Many *C. elegans* proteins become insoluble as the worms age.
- These age-related insoluble proteins can exacerbate the effects of amyloid beta overproduction.

Anderton et al., 2024 first hypothesized amyloid beta production could trigger age-related protein insolubility. Such triggering could potentially drive a feedback loop of insolubility and pathology.

To test this hypothesis, they overproduced amyloid beta in *C. elegans*, lysed the worms, pelleted the insoluble proteins, and used discovery proteomics techniques to determine what proteins were in the pellet. They found that the number of insoluble proteins increased ~3x with amyloid beta overproduction. Furthermore, more than half of these proteins overlapped with those that become insoluble in aging worms. They termed these overlapping proteins the "core insoluble proteome." Many proteins in the core insoluble proteome are associated with proteostasis, mitochondrial processes, and lifespan.

Interestingly, when Anderton et al., 2024 computationally predicted solubility, they found the average protein in the core insoluble proteome had a higher solubility score than the average protein in the proteome. Core insoluble protein expression also failed to consistently increase with age. This indicates proteins in the core insoluble proteome are not intrinsically insoluble. Instead, their insolubility may be induced by amyloid beta and other stimuli.

Mitigating the effects of amyloid beta overproduction in worms

As a proxy measure for potential impacts on disease, Anderton et al., 2024 tested if they could mitigate amyloid beta-induced paralysis by modulating the core insoluble proteome. They knocked down 23 members of the core insoluble proteome using RNAi and found that knock down sometimes delayed and sometimes advanced paralysis. This makes it clear that modulating the core insoluble proteome can alter the effects of amyloid beta, albeit in complex and sometimes opposing ways.

Given that many proteins in the core insoluble proteome are associated with mitochondrial function, Anderton et al., 2024 went on to determine if safeguarding mitochondrial function by enhancing mitophagy could delay amyloid beta-induced paralysis. They treated amyloid beta overproducing worms with Urolithin A, a drug that promotes mitophagy, and showed treatment reduced paralysis.

Altogether, these results support the notion that manipulating the biology underlying the core insoluble proteome can protect against amyloid beta. In addition, manipulating the core insoluble proteome may be a productive route to treating Alzheimer's disease.

An "encompassing strategy" for treating age-related diseases like Alzheimer's

Amyloid beta plaque formation is a key feature of Alzheimer's, but Anderton et al., 2024 showed proteins in the core insoluble proteome may be involved in a wide range of diseases. Indeed, these proteins are enriched for processes associated with many broad categories of age-related diseases like cancer and metabolic disorders. Importantly, the core insoluble proteome is more enriched for functions associated with age-related diseases than non-age-related diseases.

Given these associations, Anderton et al., 2024 posited that targeting the core insoluble proteome may help treat many age-related diseases. Determining the specific ways to target these proteins requires much further work, but these results point to a potential broad "encompassing strategy" for drug development.

This work could have far-reaching impacts on the ways researchers think about treating a wide variety of ailments. Anderton et al., 2024 thus provide a great demonstration of the power of proteomics supported by animal models in which physiologically meaningful hypotheses can be tested quickly. We hope the Nautilus™ Proteome Analysis Platform will accelerate similar research endeavors and aid the development of novel treatments for age-related diseases.

Amyloid beta proteoforms and Alzheimer's disease

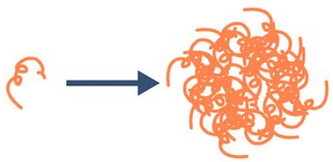
Top-down proteomics reveals a diversity of amyloid beta proteoforms

- Work by a variety of researchers shows that amyloid beta comes in a varitey of proteoforms that may have differential impacts on the molecular mechanisms underlying Alzheimer’s and its progression.

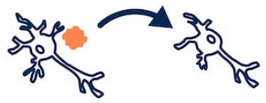
Alzheimer’s disease is incredibly complex, difficult to diagnose, and difficult to treat. It was traditionally diagnosed postmortem through the identification of amyloid beta plaques and tau neurofibrillary tangles in the brain. More recently, researchers have discovered they can diagnose live patients by imaging plaques and tangles in the brain and by measuring tau and amyloid beta biomarkers in cerebral spinal fluid and the blood. Aggregates of amyloid beta and tau have been firmly associated with cognitive decline, but there is still much to learn about Alzheimer’s development and progression. This includes but is not limited to:

- The causal links between aggregate formation or clearance and cognitive decline
- The ties between amyloid beta and tau
- The mix of pathologies that lead to aggregate formation

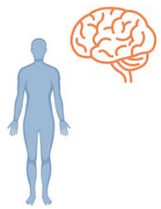
Work from a variety of researchers shows amyloid beta proteoforms may...



Differentially impact plaque seeding



Differentially impact plaque spread



Associate with different physiological effects

What has become clearer over the last few years is that aggregates of amyloid beta and tau are heterogenous mixtures. Not only are there many proteins in these aggregates (Xiong et al., 2018, Wang et al., 2005, Cardoso Ferreira et al., 2023), but the amyloid beta and tau molecules within them are composed of many discrete proteoforms (molecular variants of each protein). As we've discussed in this eBook, tau proteoforms may arise in a specified order and may differentially contribute to Alzheimer's pathology. Understanding these contributions may be crucial to understanding Alzheimer's. In this section, we provide a brief look at work showing that enhanced understanding of amyloid beta proteoforms may be critical to understanding Alzheimer's disease as well. This is not an exhaustive review of the literature but nonetheless highlights the important role amyloid beta proteoform research may play in the battle against Alzheimer's.

Diverse amyloid beta proteoforms in the Alzheimer's brain

All the studies discussed here make it clear that amyloid beta comes in diverse forms, and Wildburger et al., 2017 directly assessed this diversity in the brains of Alzheimer's patients. They used mass spectrometry to investigate proteoforms found in amyloid beta plaques and discovered 26 different amyloid beta proteoforms with variable combinations of N-terminal truncations, C-terminal truncations, and post-translational modifications (PTMs) in the brains of Alzheimer's patients. They additionally identified trends in the differential abundance of amyloid beta proteoforms between soluble and insoluble amyloid beta fractions. While these investigators studied a small number of Alzheimer's patients (N=6) and only looked at post-mortem samples, their work highlights the heterogeneity of this single protein in plaques and points to the need to learn more.

This work raises intriguing questions about whether the different amyloid beta proteoforms observed play distinct roles in the seeding and development of plaques. These proteoforms may also be associated with different downstream physiological effects. The papers referenced in the examples below begin to answer such questions.

Cell and animal models provide insights into Alzheimer's development

Given how difficult it is to obtain brain samples from living Alzheimer's patients, *in vitro* and *in vivo* models play a large part in elucidating the impacts and etiology of amyloid beta plaques. Various studies leveraging such models point to differential impacts of amyloid beta proteoforms on aggregate formation and Alzheimer's pathology.

In 2013, Bouter et al. tested the ability of a variety of amyloid beta proteoforms to form aggregates *in vitro* and *in vivo*. They found that different amyloid beta proteoforms aggregate at different rates and have different structures. Of the proteoforms they tested, amyloid beta pE3-42 (which begins with pyroglutamate at what would be position 3 in full-length amyloid beta and ends at position 42) had the highest propensity to aggregate, amyloid beta 4-42 (starting at position 4 and ending at position 42) had the second highest propensity to aggregate, and proteoforms with C-terminal truncations (some of which also had N-terminal truncations) had lower propensity to aggregate.

Furthermore, amyloid beta proteoforms were differentially toxic to primary neurons. Of the proteoforms tested, amyloid beta 1-42, pE3 4-42, and 4-42 lowered cell viability the most. Injecting all the amyloid beta proteoforms tested into the brains of live mice also impaired working memory as assessed by a maze test. Finally, transgenic mice expressing amyloid beta 4-42 and thereby having chronic exposure to this proteoform had decreased hippocampal neuron abundance and impaired spatial memory as assessed by a separate maze test. The effects on memory were also stronger in older mice as compared to younger mice.

Overall, the results in Bouter et al., 2013 show that amyloid beta proteoforms can cause different effects at the molecular and cellular levels and that a specific amyloid beta proteoform can cause detrimental effects *in vivo*.

Beretta et al., 2024 similarly used biological models to determine how various amyloid beta proteoforms are generated. In their work, they treated iPSC-derived astrocytes with *in vitro* generated amyloid beta fibrils consisting of amyloid beta truncated at position 42. These fibrils were taken up by the astrocytes (along with magnetic beads), stored in lysosomes, and truncated further to generate additional proteoforms. Some of these new, truncated amyloid beta proteoforms were excreted in extracellular vesicles and others were released directly into the growth media. Interestingly, the proteoforms inside astrocytes were often N-terminally truncated and formed high molecular weight aggregates that were resistant to denaturation. The authors suggested that these stable proteoforms may be transferred to other cells to seed further aggregate formation. If true, this would be yet another way specific amyloid beta proteoforms can impact disease pathology. It may also be possible to develop novel drugs that target the proteoforms best at spreading between cells and thereby abrogate disease.

In a final example using an *in vivo* model, Kandi et al., 2023 studied the 5xFAD mouse model which expresses five mutations found in cases of familial Alzheimer's disease. They monitored the amyloid beta proteoforms produced in this model over time, used hierarchical clustering to put the proteoforms into 3 groups based on expression levels across the study, and determined which proteoforms were found in soluble and insoluble fractions. Those in the insoluble fraction were likely associated with aggregation. One particular group (demarcated as Group 1 in this study) was consistently found in the insoluble fraction and increased in abundance in this fraction with aging. This group contained many proteoforms with N-terminal truncations.

Finally, they also observed differences in the abundance of proteoforms with particular PTMs. For example, amyloid beta pE3-42 increased in relative abundance with age in both the soluble and insoluble fractions.

While causal relationships between proteoforms and dementia were not determined in this work, clear associations between certain groups of proteoforms, amyloid beta fractions, and age once again suggest that amyloid beta proteoforms may have differential impacts on the pathology and trajectory of Alzheimer's disease.

Amyloid beta proteoforms and clinical trials

Given the association between amyloid beta aggregates and Alzheimer's, researchers have been developing drugs that clear these aggregates for many years. Indeed, three recently approved drugs, Aducanemab (since discontinued by its developer), Lecanemab, and Donanemab all consist of monoclonal antibodies targeting amyloid beta (Golde and Levey 2023, Zhang et al., 2024). These drugs all clear amyloid plaques and slow, but do not prevent cognitive decline. All also target epitopes found in insoluble forms of amyloid beta:

- Aducanemab: Binds amino acids 3-7 in amyloid beta
- Lecanemab: Binds structures formed by amyloid beta aggregates
- Donanemab: Targets pyroglutamate at position 3 in amyloid beta

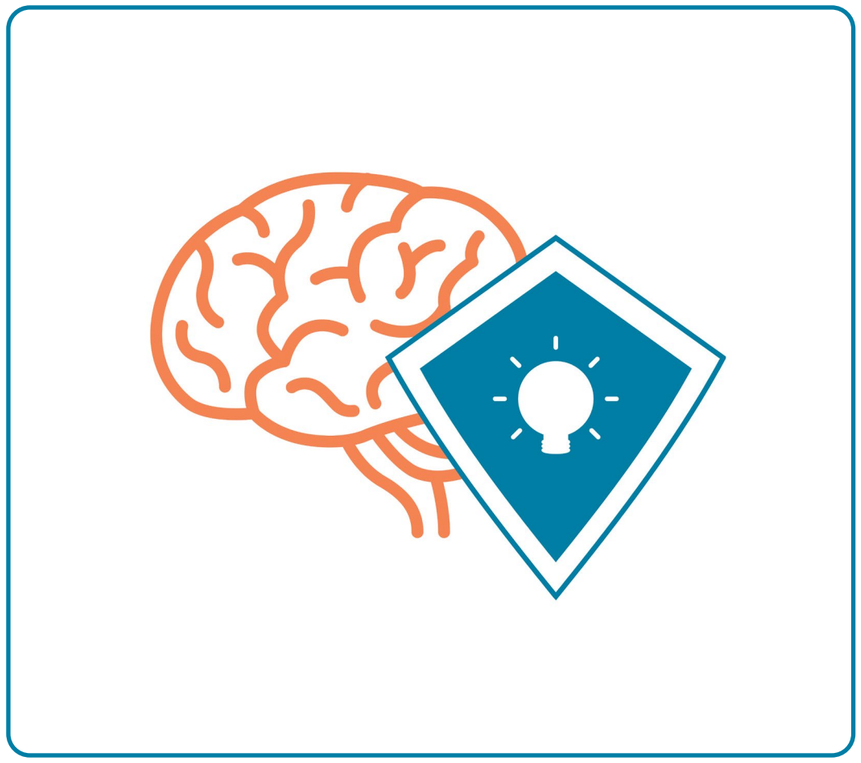
These antibodies may slow cognitive decline due to their propensity to bind amyloid beta epitopes associated with plaques as opposed to soluble amyloid beta (Golde and Levey 2023, Zhang et al., 2024). In the future, It may be possible to associate these epitopes with groups of proteoforms that arise during different stages of Alzheimer's, and it will be interesting to see if one of these drugs (or a new one) ultimately proves best at slowing cognitive decline. Such a result may point to a group of amyloid beta proteoforms as particularly important to Alzheimer's progression and may spur the development of additional drugs that either clear these proteoforms or prevent their generation. In this way, greater knowledge of proteoforms may help researchers understand the results of these and future clinical trials targeting amyloid beta. They may also point to additional avenues for productive drug development.

As companies like Nautilus develop technologies that can quantify proteoforms, we may gain a much better understanding of Alzheimer's and many other diseases. Hopefully this understanding will lead to new ways to prevent, treat, and cure ailments of all kinds.

Finding drivers of cognitive resilience in Alzheimer's

Key points:

- People with asymptomatic Alzheimer's disease have amyloid beta and tau aggregates but not dementia and are considered cognitively resilient.
- Hurst et al., 2023 used proteomics to identify NRN1 and a set of proteome modules as promoters of cognitive resilience.
- Promoting cognitive resilience may be productive for Alzheiimer's treatment.



Cognitive defects in Alzheimer's disease follow the accumulation of extracellular amyloid beta plaques and intracellular tau tangles. Tau, however, may be a marker for many kinds of neurological pathology and there are many unsolved mysteries surrounding its role in Alzheimer's development and progression. One huge mystery is why some people accumulate plaques and tangles but don't develop cognitive symptoms. Understanding how these asymptomatic individuals achieve such "cognitive resilience" could enable the development of therapies promoting resilience or biomarkers determining what treatments should be pursued.

Hurst et al., 2023 from the Seyfried Lab at Emory University set out to solve the mystery of cognitive resilience by applying proteomic analysis to brain samples from healthy individuals, those with asymptomatic Alzheimer's, and those with cognitive defects due to Alzheimer's. These samples come from the Religious Orders Study/Memory and Aging Project (ROSMAP) wherein Catholic nuns, priests, and brothers 65 and older as well as older persons from retirement communities and senior subsidized housing facilities in the Chicago area volunteer to get medical and physiological (including cognitive) evaluations each year and donate their brains after death. All participants enter the project without known dementia but may develop it over the course of the project.

Using this powerful resource, Hurst et al., 2023 discovered protein modules associated with resilience. They also integrated their findings with previous Alzheimer's proteomics studies to zero in on the NRN1 protein as a driver of cognitive resilience in Alzheimer's. Later, they demonstrated NRN1 can protect rat neurons from some of the detrimental effects of amyloid beta and began to elucidate the mechanism through which NRN1 achieves its effects. Their results strongly suggest targeting NRN1 could help prevent or slow the cognitive impacts of Alzheimer's disease.

Identifying proteome modules associated with cognitive resilience and zeroing in on the NRN1 protein

Leveraging the ROSMAP samples, Hurst et al., 2023 measured over 7,700 proteins from brain regions BA6 and BA37 across healthy individuals, individuals with asymptomatic Alzheimer's, and individuals with Alzheimer's and cognitive impairment. In both brain regions, they discovered hundreds of proteins with altered abundance in asymptomatic individuals and thousands of proteins with altered abundance in cognitively impaired individuals. As expected, amyloid beta and tau increase in cognitively impaired compared to healthy individuals. To better conceptualize the role of other altered proteins, Hurst et al., 2023 grouped them into modules of co-expressed proteins and functionally annotated them using the cWGCNA algorithm and GO annotation, respectively.

In both brain regions, five modules were increased in asymptomatic Alzheimer's compared to Alzheimer's with cognitive impairment while also having a positive correlation with cognition. These included:

- M22 – Synapse
- M5 – Synapse
- M25 – Ribosomal
- M30 – Mitochondria/ER
- M36 – Exocytosis

Modules elevated in Alzheimer's with cognitive impairment compared to asymptomatic Alzheimer's and negatively correlated with cognition included:

- M15 – MAPK signaling
- M16 – Gluconeogenesis

To narrow in on some particularly important proteins for cognitive resilience, Hurst et al., 2023 compared their results to those from a previous proteome-wide association study testing protein associations with cognitive resilience in ROSMAP samples (only 30 of which overlapped with Hurst et al., 2023).

Four of the modules from Hurst et al., 2023 were enriched for proteins identified as positively associating with cognitive resilience in the previous study. These included:

- M22 – Synapse
- M5 – Synapse
- M36 – Exocytosis
- M30 – Mitochondria/ER

Being that M22 and M5 were largely associated with synapses and neuronal markers, they narrowed in on these modules and discovered that NRN1 was the most significantly elevated protein in asymptomatic Alzheimer's vs. Alzheimer's with cognitive impairment and was also highly associated with measures of cognition in both studies.

NRN1 promotes the formation of neuronal protrusions that can enhance signaling between neurons and can improve learning in animal models. These roles line up well with its potential for improving cognitive resilience.

NRN1 protects neurons from amyloid beta in *in vitro* models

Amyloid beta decreases the abundance of critical neuronal structures called dendritic spines while also altering their morphology. Dendritic spines are protrusions from neurons that receive signals from other neurons. Impaired dendritic spine biology disrupts neuronal networks and decreasing dendritic spine abundance decreases the total surface area of the cell, making the neurons more compact. This can cause neurons to fire action potentials more readily – i.e. makes them more excitable.

By culturing rat hippocampal neurons *in vitro* and exposing them to recombinant amyloid beta and NRN1, Hurst et al., 2023 showed that, while amyloid beta alone does decrease dendritic spine abundance and alter spine morphology, NRN1 prevents these effects. Furthermore, either amyloid beta or NRN1 alone increase neuronal excitability, but combining them restores excitability to normal levels. It is unclear why NRN1 alone increases excitability, but its impacts on the effects of amyloid beta are promising signs of its powerful role in cognitive resilience.

How does NRN1 cause cognitive resilience?

Diving into the potential mechanisms underlying cognitive resilience, Hurst et al., 2023 first showed that NRN1 does not prevent amyloid beta aggregation. Then, they treated rat primary neurons with recombinant NRN1 protein and measured changes in the proteome. They discovered 216 proteins with significantly altered abundance at a false discovery rate of 10% and found that 7 human brain modules are enriched with these proteins. Four human modules were enriched with proteins that increased in response to NRN1:

- M22 – Synapse
- M5 – Synapse
- M4 – Synaptic vesicle
- M19 – ATPase activity

Three modules were enriched with proteins that decreased in response to NRN1 treatment:

- M8 – RNA splicing
- M31 – Translation initiation
- M12 – Hydrolase activity

The consistent association of M22 and M5 with cognitive resilience highlights these modules as particularly important for resilience and warrants further study.

A role for cognitive resilience in Alzheimer's treatments

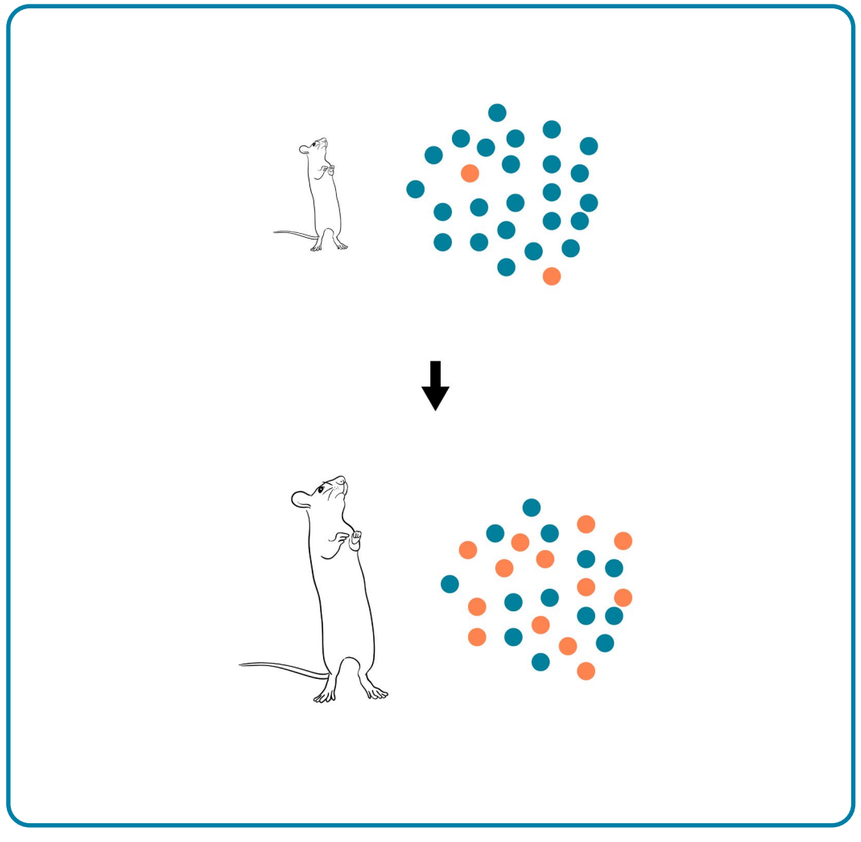
Approved Alzheimer's treatments and clinical trials largely focus on amyloid beta and tau. This is reasonable considering the strong correlation between these proteins, their aggregation, and the progression of Alzheimer's disease. Nevertheless, Hurst et al., 2023 make it clear that cognitive resilience plays an important role in the Alzheimer's story. Their cross-disciplinary work leverages human samples, proteomics, *in vitro* models, and a keen understanding of neuronal biology to identify many potential points of intervention that could enhance cognitive resilience. They show that NRN1 is particularly important, but many proteins in the altered modules may play important roles as well.

It will be interesting to follow how the networks of interactions between these proteins are studied and hopefully manipulated to improve Alzheimer's treatment. This kind of work is only possible with powerful proteomics tools, and we're excited to see how next-generation tools like the Nautilus™ Proteome Analysis Platform accelerate these studies in the near future.

Spatiotemporal changes to the proteome in Angelman syndrome

Discover insights like:

- UBE3A knockout mice display symptoms of Angelman syndrome and changes in the proteome that get progressively more prominent with time.
- Alterations to tRNA synthetase and proteasome proteins appear early.
- Alterations to synaptic proteins become more prominent later.



Angelman syndrome is a neurological disorder that leads to developmental delays, intellectual disability, and a range of other symptoms. It is a rare genetic disorder caused by mutations in the UBE3A gene with a prevalence of 1/20,000 – 1/12,000 live births. Because the paternal allele of UBE3A is normally silenced (imprinted), mutations in the maternal allele are sufficient to cause the disorder (See Madaan and Mendez 2024 for more information).

Many people with Angelman syndrome have a normal paternal allele for UBE3A, and drugs designed to activate this allele are currently in clinical trials. Indeed, studies in mice have shown that reinstating UBE3A protein levels soon after birth can largely rescue the physiological effects of the disease. The impact is much less pronounced if UBE3A is reinstated in adulthood (Silva-santos et al., 2015).

To better understand the biology underlying Angelman syndrome and the intricacies of rescue, Pandya et al., 2022 leveraged proteomic and targeted protein analysis across mouse, rat, and human cell line-based models of the disease. Their work not only identified biological processes prominently impacted by the disease and its rescue but also demonstrated varying degrees of rescue from UBE3A reinstatement at different developmental time points. Finally, their work showed a canonically metabolic enzyme called transketolase may play a surprising role in the disorder.

Proteomic analyses of mouse, rat, and human Angelman syndrome models reveal aberrations in proteostasis and synaptic function

Pandya et al., 2022 used mass spectrometry to quantify proteins with altered abundance in UBE3A knockout mice and rats. In both cases, the rodents displayed developmental delays and other symptoms of Angelman syndrome. At the level of the proteome, the rodents had significant changes in proteins involved in:

- **tRNA synthetase pathways** – The levels of tRNA synthetase pathway proteins had both increases and decreases compared to controls. tRNA synthetases are essential components in protein production and alterations in these pathways could have wide-ranging effects.
- **Proteasomal pathways** – The levels of these proteins were generally increased compared to controls. Proteasomal proteins coordinate protein degradation and these too could have broad-ranging effects on biological function.
- **Synaptic factors** – Synaptic proteins were both increased and decreased in the rodent models compared to controls. Synapses form the hub of signal transmission between neurons, and alterations in these proteins could have broad impacts on neurological function.

Similar patterns of protein expression were seen in iPSCs derived from Angelman syndrome patient neurons compared to controls.

Spatiotemporal proteomics in rodent models of Angelman syndrome

In the rats, the researchers also looked at differences in patterns of protein abundance across brain regions. While the patterns above were generally recapitulated across regions, they did observe broad proteomic differences between regions indicating differences in their development. Further work could elucidate how these differences impact development.

Pandya et al., 2022 additionally looked at changes in mouse protein expression at birth, during adolescence, and in adulthood. Changes in tRNA synthetase and proteasome pathways are prominent at birth, but changes in synaptic proteins became gradually more prominent over time. This may reflect a gradual impairment of brain development stemming from more immediate impacts on tRNA synthetase and proteasomal pathways.

Discrepancies between Angelman syndrome proteomic and physiological rescue at different developmental time points

Pandya et al., 2022 also set out to determine how changes in the proteome correlate with the rescue of physiological function brought about by reinstating UBE3A expression at different developmental time points. To do so, they used a tamoxifen-inducible UBE3A mouse line and reinstated UBE3A expression at adolescence and adulthood. Early reinstatement rescues physiological function while late reinstatement does not. Interestingly, however, reinstating UBE3A during adolescence rescues many impacts on the proteome, while reinstating during adulthood partially rescues proteomic changes. This may indicate that further intervention is necessary with later UBE3A reinstatement.

Proteomics identifies a surprising target of UBE3A

Across all their models, Pandya et al., 2022 observed that the abundance of an enzyme called transketolase increases in Angelman syndrome. Further analysis showed that nuclear levels of this protein particularly increase in neurons. Although transketolase nuclear localization had been reported in non-neuronal cells prior to this work, given that transketolase canonically catalyzes a reaction in the pentose phosphate pathway, and this pathway occurs in the cytoplasm, this prominent nuclear localization points to a non-canoncial role for transketolase. Pandya et al., 2022 further showed that UBE3A likely directly ubiquitinates transketolase in the nucleus.

The findings that transketolase is consistently up-regulated across Angelman syndrome models, and that it is likely a direct target of UBE3A indicate that transketolase plays an important role in Angelman syndrome. More work is necessary to determine this role, but these results are a great demonstration of the insights that can come from proteomics.

Implications for future work on Angelman syndrome

The results presented by Pandya et al., 2022 have a variety of implications for future academic and clinical research into Angelman syndrome. On the academic side, it will be interesting to see how modifying levels of proteins downstream of UBE3A impact neuronal networks across the brain. In addition, studying the function of transketolase in neuronal nuclei may reveal interesting new functions for this enzyme. In the clinic, these results reinforce previous studies suggesting early reinstatement of UBE3A expression has greater impacts than later reinstatement. They also point to some downstream proteins that may need further intervention to rescue the pathology of older Angelman syndrome patients.

Overall, this work represents a thorough integration of proteomics, *in vivo* models, and *in vitro* models. This kind of work requires researchers with various types of expertise and experimental skills to collaborate extensively. We hope next-generation proteomics technologies like the Nautilus™ Proteome Analysis Platform will make integrative and collaborative efforts like this one far more accessible.

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 neurobiology.

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 patterns of modification found on proteoforms 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 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.

Improve our understanding of neuronal cell biology

Identify important components of neuronal signaling

Reveal biomarkers and drug targets in neurological disease

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 develops 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 cytoskeletal and other proteoforms 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 with 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 usually cannot identify 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 at the single-molecule level making it well suited to proteoform identification, quantification, and analysis. Technologies like ours 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 has been associated with the aggregation of:

- Amyloid beta 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 while others slow, but do not stop disease progression. 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. Some of this work is covered in previous sections of this eBook. 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 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 proteoforms 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 resolve the patterns of modification on individual proteoforms in a sample of interest through proteoform analyses. 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 neurobiology.

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 functions, 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 they 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.

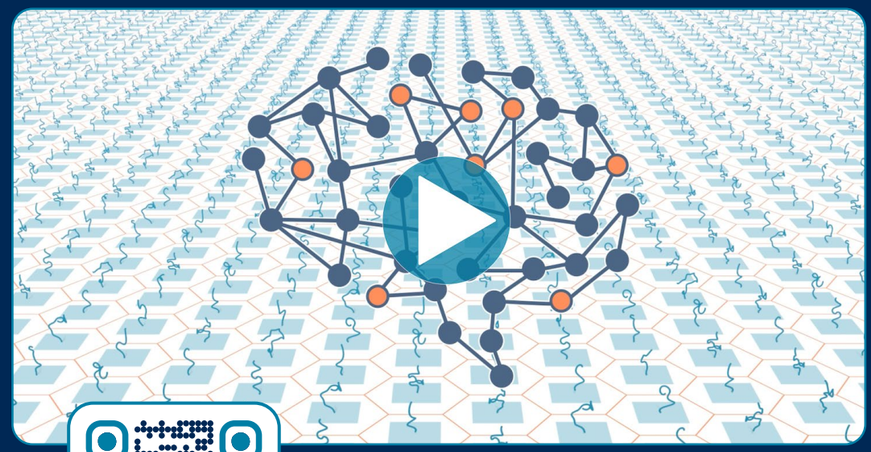
We aim for the Nautilus™ Proteome Analysis Platform to achieve all this and more. When it and similar technologies are available to neuroscientists, there's no telling what advances will come to fruition. One thing is certain however, the proteomics revolution in neuroscience will be rife with discovery. 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.

Additional proteomics and neuroscience resources from Nautilus

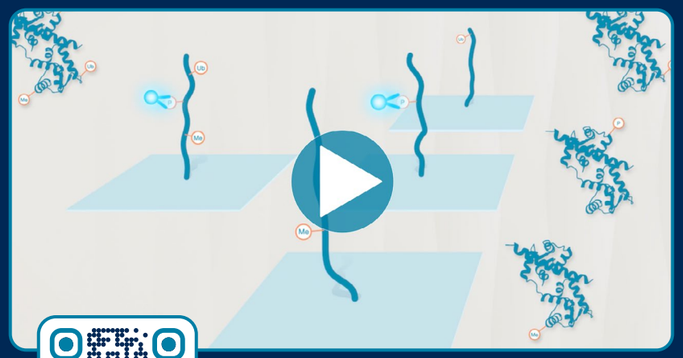
Animations, our podcast, and more!

- In the resources linked here, you'll discover even more ways proteomics and the Nautilus™ Proteome Analysis Platform will advance neuroscience research.



ANIMATION

Unlocking the potential of proteomics to fuel neuroscience



ANIMATION

Proteoform analysis on the Nautilus Proteome Analysis Platform

PODCAST

A New Era in Alzheimer's Research with special guest Sarah DeVos Ph.D.

PRESENTATION

Curated Nautilus Data from US HUPO 2025

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