PEER REVIEWED

Mapping the Proteome to Understand the Complexity of Neurodegenerative Diseases

Neurological conditions have emerged as the leading global cause of illness and disability, impacting more than 3 billion people.[1] Among these, neurodegenerative diseases stand out for their relentless progression, lack of curative treatments, and their rising prevalence within aging populations. In particular, Alzheimer's disease is known for its slow onset and progressive deterioration, and the lack of a cure underscores the critical need to deepen our understanding of the molecular cues that lead to neurodegeneration, with the goal of identifying effective preventative and therapeutic measures.

Neurodegenerative diseases like Alzheimer's, Parkinson's, amyotrophic lateral sclerosis (ALS) and Huntington's are all characterised by disrupted proteome homeostasis and selective neuronal loss. These conditions have become a significant burden for an ageing society, with one-third of people in industrialised nations expected to develop a neurodegenerative disease during their lifetime.[1] Their extended disease courses, progressive deterioration and necessity for long-term care underscore the urgency of understanding the molecular mechanisms driving neurodegeneration.

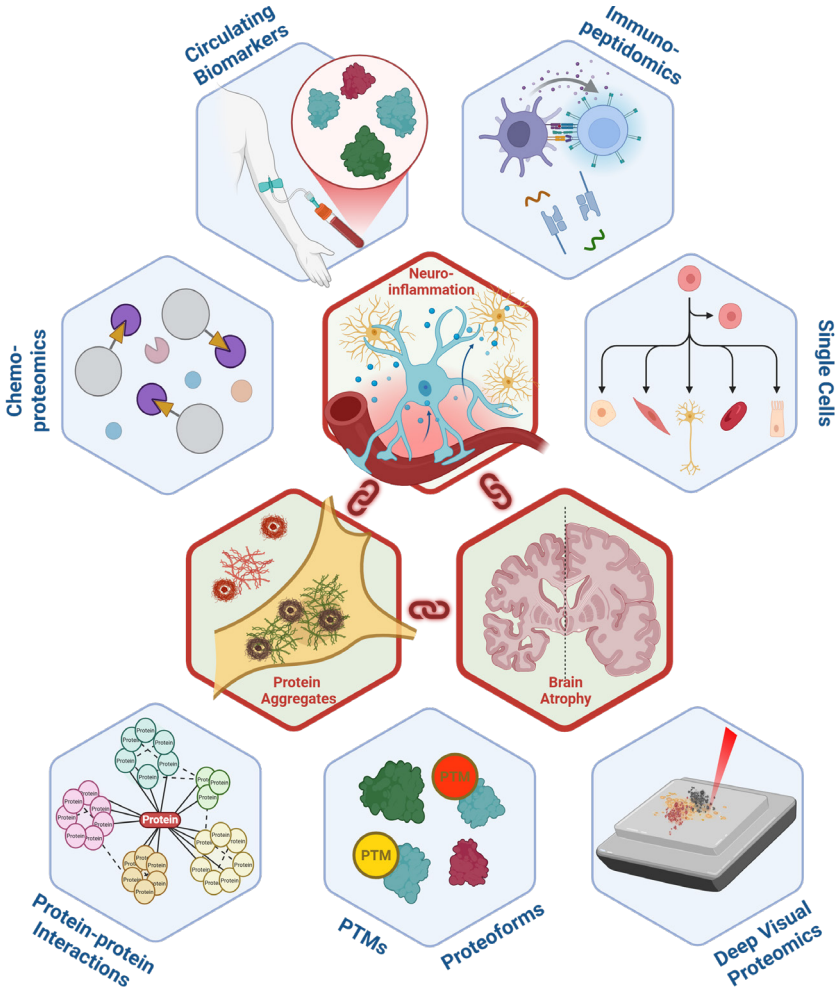

Figure 1: A schematic highlighting key proteomics-driven strategies to dissect the molecular underpinnings of neurodegenerative diseases.

Mass spectrometry (MS)-based proteomics serves as a powerful window into neurodegeneration, enabling researchers to comprehensively analyse changes in protein abundance, modifications, interactions, and degradation pathways. A key advance of this approach is the study of proteoforms, the structurally and functionally diverse protein variants derived from the same gene as a result of genetic variation, alternative splicing and post-translational modifications (PTMs). Investigating proteoforms sheds new light on protein function and dysfunction within neurodegenerative disease contexts and is crucial for resolving complex disease mechanisms.

MS enables highly sensitive, high-throughput identification and quantification of disease-relevant proteome alterations from model systems to human tissues. This deep level of molecular resolution allows researchers to trace neurodegenerative processes from early pathology to advanced disease stages. By capturing a holistic view of the proteome and its diverse proteoforms, MS-based proteomics not only aids in uncovering critical disease biology but also accelerates the discovery of potential therapeutic targets and biomarkers, highlighting biological pathways implicated in disease initiation and progression (see Figure 1).[2] Integrating these advanced techniques into neurodegenerative research will be pivotal for advancing therapeutic development in the field.

Proteostasis, Protein Interactions and Aggregation in Neurodegeneration

It is essential to examine protein homeostasis, to understand the molecular mechanisms underpinning neurodegenerative diseases. Protein homeostasis, or proteostasis, is the regulation and maintenance of the cellular functional protein environment (proteome), ensuring that proteins are correctly folded, modified, and translocated, as well as appropriately degraded when damaged or no longer needed. Characteristic of neurodegenerative disease, disruption of proteostasis leads to the accumulation of misfolded proteins or protein aggregates that impair cellular function and trigger neuronal death. Huntington's disease for example, is characterised by the formulation of extended protein aggregates that cause widespread neuronal toxicity and severe clinical phenotypes. ALS is similarly associated with hundreds of genetic risk factors which converge on pathways that destabilise proteome integrity. A particularly aggressive form of ALS is associated with the genetic

risk factor C9orf72, which has been reported to drive pathology through multiple routes including production of a di-peptide repeat that forms resilient aggregates, sequestering hundreds of different proteins and driving widespread proteostasis collapse.[3]

Protein aggregation is a hallmark of many neurodegenerative diseases. MS-based proteomics enables detailed analysis of the composition and structure of these aggregates, helping to understand their formation and impact on cellular function. Unlike epitope-dependent approaches, MS can detect a vast range of peptides, including PTM-bearing ones, even after harsh denaturation of highly insoluble deposits. Studies have revealed hundreds of proteins sequestered in such aggregates, providing insight into their formation, cellular impact, and neurotoxicity.[4,5] Beyond compositional analysis, chemoproteomics strategies aimed at degrading aggregation-prone proteins may offer novel therapeutic avenues.

MS-based proteomics also enables the mapping of protein-protein interaction networks in neurodegenerative diseases, revealing how both stable physiological complexes and aberrant pathological protein aggregates maintain and disrupt cellular processes, respectively. High throughput proteomics has made large-scale interactions studies feasible, achieving sufficient sensitivity to detect even trace protein interactions in clinical isolates.[6] While changes in interaction networks driven by PTMs or disease-associated mutations cannot be elucidated through genetic or targeted affinity approaches, MS-based methods have proven particularly powerful in these contexts, such as in the study of hyperphosphorylated Tau, offering critical insights into disease-associated interactome remodelling.[7] Similar approaches hold great promise for uncovering pathological interaction networks in other neurodegenerative diseases.

Advanced Proteomic Technologies and Spatial-Temporal Analysis

Recent advancements in MS sensitivity enable proteome analysis at sub single-cell level, enabling comprehensive proteomics insights down to single bacteria and subcellular proteomes.[8,9] This sensitivity is useful for studying heterogeneous cell populations in the brain and understanding cell-specific contributions to neurodegenerative diseases, in particular from organoids or working with limited material. While isolating single neurons can be challenging given their complex and fragile morphology, the ability to detect thousands of proteins and tens of thousands of peptides from only a few picogram (less than a single HeLa cell) opens the opportunity to work with highly limited amounts of material e.g., from axonal cell regions.

Deep visual proteomics (DVP) approaches combine MS with imaging techniques to study the spatial distribution of proteins within tissues and support understanding the regional specificity of protein changes in the brain by resolving distinct brain regions and cell types for their role in disease pathology. Laser capture microdissection (LCM) technologies along with unbiased, high sensitivity MS highlight new opportunities to study diseases at a sub tissue resolution.[10] These technologies hold potential to resolve spatial and temporal disease phenotypes and progression in neurodegeneration from human tissues or organoids. Many neurodegenerative disorders affect specific areas of the brain, with prion-like spreading towards neighbouring regions and intricate neuroinflammatory processes being proposed as key drivers of disease progression.[11] Resolving spatial and temporal progress in model systems or post-mortem human tissue may reveal better insights into the pathological cascade.

Immune-Related Pathogenic Mechanisms

Immunopeptidomics, the study of peptides presented by major histocompatibility complex (MHC) molecules, has significant links to neurodegeneration through various mechanisms like immunogenicity. MS-based proteomics can directly survey cell surface decoration of immunopeptides and monitor MHC shed into circulation. Tailored acquisitions enable highly effective coverage of singly and multiply charged immunopeptides, and de novo sequencing and PTM search strategies significantly enhance the capabilities of MS-based immunopeptidomics to study immune homeostasis.[12] The proteasome which degrades intracellular proteins plays a key role in in both decoration of MHC as well as in dealing with aberrantly folded proteins, hence providing an intriguing yet underexplored connection between immunopeptidomics and neurodegeneration.

Additionally, because neuroinflammation plays a key role in neurodegeneration, peptides derived from aggregated or misfolded proteins could elicit adaptive immune responses and contribute to the problem. Protein-aggregates themselves may also be linked to inflammation mediated by brain-resident cells including microglia and astrocytes, leading to neuronal damage and brain atrophy. By studying the peptides presented by MHC, immunopeptidomics can provide valuable insights into the immunogenicity and inflammatory processes involved in neurodegenerative diseases, helping to identify potential therapeutic targets and improve understanding of disease mechanisms.

Biomarker Identification for Early Detection

Identifying biomarkers is crucial in early diagnosis and monitoring disease progression. MS-based proteomics can discover and validate biomarkers in body fluids like cerebrospinal fluid (CSF) and blood, facilitating early detection and supporting personalised treatment strategies. Many neurodegenerative diseases develop for years at the molecular level before the onset of any symptoms; therefore, it could be assumed that early risk trajectories are key for interventions. With the introduction of trapped ion mobility spectrometry combined with a time-of-flight mass spectrometer (timsTOF), In conjunction with deep workflows using particle technologies, MS is ideally suited to deliver high-quality population scale access and discovery of novel circulating biomarkers.[13,14]

Therapeutic Possibilities

Chemoproteomics and targeted protein degraders offer promising avenues for understanding and treating neurodegenerative diseases.[15] Benefiting from robust, quantitative, and deep insights into a cell's protein repertoire across various model systems, MS has enabled unbiased chemoproteomics at scale. Chemoproteomics involves the use of chemical probes to study small molecule-protein interactions and functions within the cellular environment, providing insights into the molecular mechanisms underlying neurodegeneration. In addition, those modifications could change the physicochemical properties of proteins sufficiently to potentially affect their aggregation/ disaggregation propensity, which may offer a novel therapeutic avenue. Targeted protein degraders, such as proteolysis-targeting chimeras (PROTACs) and molecular glues, leverage the ubiquitin-proteasome system to selectively degrade disease-associated proteins. This approach is particularly valuable in neurodegenerative diseases, where protein aggregates and misfolded proteins play a central role in pathology. By facilitating the removal of these harmful proteins, targeted protein degraders could mitigate neuronal damage and improve cellular function. The

integration of these technologies holds great potential for advancing basic research in neurodegeneration and developing innovative therapeutic strategies.

In Summary

Neurodegenerative diseases pose a key challenge to an ageing society, with disrupted proteome homeostasis at the core of their pathology. MS-based proteomics offers the potential to unravel the intricacies of these disorders, from identifying and quantifying proteins to characterising PTMs, analysing protein aggregates and mapping protein-protein interaction networks. By leveraging these capabilities, researchers can gain critical insights into the molecular mechanisms driving neurodegeneration to provide a clearer picture of disease progression from early events to advanced pathology. As these technologies become further integrated into research, they will hold greater promise for identifying novel biomarkers, illuminating therapeutic targets and paving the way toward innovative diagnostic and treatment strategies.

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Daniel earned his Ph.D. with Matthias Mann at the Max Planck Institute, studying proteome changes in neurodegenerative diseases. He continued research on computational immunoproteomics with Mann and Meissner, then developed multi-omics strategies for metabolic disorders with Mike Snyder's team. Previously VP Proteomics at Seer, Daniel is now VP Biomarkers and Precision Medicine at Bruker. He has served on advisory boards and received the Human Proteome Organization Science and Technology Award.

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Torsten started in proteomics at IBMB Bonn then gained expertise in method development and advanced MS at Boston Children's Hospital and ETH Zürich. From 2016 to 2020, Torsten completed his PhD with Prof. Jeroen Krijgsveld at Heidelberg and DKFZ, improving and automating the SP3 sample preparation method now widely used in proteomics. As a postdoc, he advanced clinical proteomics with the autoSP3 pipeline before joining Bruker Daltonics in 2022.

Stefan Foser

Stefan combines academic excellence with nearly two decades of professional experience in the pharmaceutical and diagnostics sectors. Holding a Ph.D. in Microbiology, in addition to degrees from the Universities of Basel, Switzerland and Mannheim, nearly twenty publications and patents, Stefan was awarded an Executive MBA from the University of St Gallen. Stefan honed his expertise through roles at Roche, Siemens Healthineers and now as VP Global Pharma at Bruker Daltonics