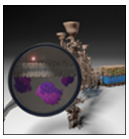

Editorial

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Neuroproteomics: How Many Angels can be Identified in an Extract from the Head of a Pin?*

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Oscar Wilde once defined fox hunting as an activity of "*the unspeakable in pursuit of the uneatable.*" This sentiment may well reflect the reaction of some mass spectrometry laboratories to neuroscience colleagues rushing in with a vial containing a hopelessly inadequate amount of sample originating from a tissue of intractable complexity, or in other words a neuroproteomics project. This issue of *Molecular and Cellular Proteomics* focuses on application of proteomics to current problems in neuroscience. Although some neuroscientists have ventured into proteomics, most of the neuroscience community has yet to embrace proteomics approaches. Despite increasing interest and awareness of the potential of such approaches (1, 2), many neuroscientists are still in the early stages of a learning curve on how proteomics approaches can be leveraged for new insights and knowledge in their field. Conversely, many proteomics laboratories are not aware of the daunting complexity and limited quantities of samples derived from neural tissues. The review and commentary papers presented in this issue highlight current issues in neuroscience that may now be ready for interrogation by modern proteomics approaches. The primary research papers in this issue provide examples of success in overcoming the many hurdles of neuroproteomics projects, and the exciting new insights that such achievements bring.

Despite advances in sensitivity in mass spectrometry (MS), protein yield from neural tissues is often a harsh limiting factor when considering the quantities needed for proteomics. Both the central and peripheral nervous systems (CNS[1] and PNS, respectively) are composed of mixtures of different cell types with intricate architecture and connectivity, presenting major sampling and analysis challenges. These challenges typically require separation of different cell types prior to proteomics, thereby further limiting sample size, or trying to infer cell-type specific changes from tissue lysates. The cover of this issue shows an example of such cellular complexity, illustrating the composition of the mammalian retina with photoreceptors, glia, and neurons assembled into complex layers that are essential for vision. The intricate cytoplasmic extensions of the glial and neuronal cells within the retina, and the axonal extensions from the retina to the brain, add additional layers of complexity that are not depicted in the image. This juxtaposition of different cell types and subtypes, and the intertwined cytoplasmic processes of neurons and glial cells, presents a daunting challenge for proteomics of different neuronal and glial cell types. The work by Grosche *et al.* (3) in this issue tackled the complexity of the retina with a unique fractionation approach tailored to isolate and dissect the proteome of the Mueller glial cells, a specialized glial cell population unique to the retina. Quantitative mass spectrometry on Muller cell enriched *versus* depleted fractions enabled the authors to identify specific functions of this glial population in the retina. Hence, targeted analysis of a cell type of interest by purification directly from its *in vivo* milieu provides insights that may not be achievable from more widely used culture systems. Cultures of primary cells from CNS or PNS or of differentiated stem cells are extensively used in neuroscience, but these may have drawbacks such as incomplete maturation or function of the cells of interest. The review by Shoemaker and Kornblum (4) in this issue further emphasizes issues of cellular heterogeneity in culture in the case of neural stem cells.

The constraints of cellular makeup of the nervous system are further compounded by the need to consider subcellular domains. The contributions of functionally distinct subcellular compartments are perhaps best exemplified by neurons that extend axons and dendrites long distances from their cell bodies, but distinct subcellular domains are also highly relevant for astrocytes, oligodendroglia, and (potentially) microglia in the CNS and Schwann cells in the PNS. Dendrites are post-synaptic processes that extend up to a few millimeters from a neuronal cell body (soma) that is roughly 20–100 microns in diameter. Axons are the presynaptic compartment of a neuron, and can extend from up to centimeters in rodents to over a meter in large mammals. Saito and Cavalli (5) in this issue emphasize the spatial constraints for signaling over the extremely long distances covered by axons. These mechanisms are critical for development, function, and repair of axons after injury. Debaisieux *et al.* (6) took advantage of a

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[1] The abbreviations used are: CNS, central nervous system; PNS, peripheral nervous system.

tetanus toxin that is retrogradely transported in vesicles to selectively assess axon-to-soma signaling in cultured motor neurons. They magnetically isolated endosomes containing tetanus toxin conjugated to iron oxide nanoparticles (MIONs), thereby obtaining a highly enriched neuronal signaling endosome preparation from cultures containing both neurons and non-neuronal cells, and enabling the assembly of a functional map of carriers involved in long-range axonal signaling. The review of van Niekerk *et al.* (7) further emphasizes the multiple influences on neural repair through regeneration of axonal processes. They highlight both intrinsic and extrinsic mechanisms for interrogation by proteomics and other means to identify pathways to improve repair in the injured CNS.

The synaptic region of dendritic processes has been the subject of numerous studies aimed at understanding the proteome of dendritic subdomains and how identified proteins and post-translational modifications relate to synaptic function and plasticity. This has been fueled in part by efficient methods to isolate synaptosomes and postsynaptic density from the CNS, in sharp contrast to the limited purification options for other subcompartments of neural tissues. The review on proteomics of the synapse from Deiterich and Kruetz (8) in this issue emphasizes some limitations in current approaches and highlights the need for integrating temporal analyses. Dynamics of the synaptic proteome are illustrated by Borovok *et al.* (9), who show changes in hippocampal protein levels during spatial learning tasks, and suggest that synaptic protein complexes are dynamically assembled as mice establish memories. Changes in gene expression and protein expression have long been linked to long-term synaptic plasticity. The work from Niere *et al.* (10) in this issue demonstrates that inhibition of neuronal mTOR, the inactivation of which typically decreases overall protein synthesis, can also increase translation of specific proteins such as postsynaptic PARK7 that has been linked to Parkinson's disease. In this case, a proteomics study of normal synaptic functions provides insights on new roles for neurodegeneration-associated proteins. Synapse dysfunction and loss are implicated in neurodegenerative diseases and the review of Moya-Alvarado *et al.* (11) summarizes progress in applying proteomics to the pathophysiology and diagnosis of Alzheimer's disease. Links to neurological diseases are also seen in primary research papers in this issue. Gershoni-Emek *et al.* (12) show that synapses from mouse models of amyotrophic lateral sclerosis (ALS) have distinct protein populations associated with the motor protein dynein. Their data implicate the RNA binding protein Staufen in ALS pathogenesis. Piroli *et al.* (13) show altered succination of proteins in the brain stem of a mouse model for Leigh syndrome, potentially contributing to mitochondrial dysfunction in this disorder.

Glial cells in CNS and PNS present another level of heterogeneity and complexity. These cells play critical functional roles in nervous systems, including synaptic maturation and function, blood-brain-barrier integrity, and myelination. Rasband (14) highlights the diversity of glial types as well as their functions in health and role in neurological diseases in this issue. Intriguingly, interactions between subcellular domains of oligodendrocytes or Schwann cells with axons helps craft nodes of Ranvier in the CNS and PNS, subcellular domains along axons that are essential for the accelerated conduction velocities that myelin conveys to neurons. Specialized roles of astrocytes in providing antioxidant protection to neighboring neurons were studied by Pehar *et al.* (15). They observed regulation of several metabolic and antioxidant pathways at the level of protein expression and lysine acetylation, implicating post-translational regulation of this critical physiology. Similar to neurons, all glia subtypes are highly polarized, a feature that undoubtedly reflects unique functional domains of the glial cytoplasm. The precursors of glial cells are also the source of the most frequent intrinsic brain tumors. Malignant glial tumors come with an exceptionally poor prognosis and over the years treatments have only incrementally advanced survival times. The review from Tian *et al.* (16) outlines the progress made for understanding the proteome of CNS tumors and emphasizes the role that single cell and targeted MS approaches may play to advance future insight in this area. Indeed, the contribution from Demeure *et al.* (17) shows the potential gain from targeted MS approaches, where the investigators xenografted human glioblastoma multiforme into rodent brains and were able to distinguish tumor-derived from host-derived responses.

A shared characteristic of all the contributions to the issue is the challenge of interrogating high complexity samples in limiting or suboptimal quantities. Medieval philosophers allegedly debated the question of how many angels can be found on the head of a pin. Given that the physiological expression levels of many neural proteins are at or below the detection range of current instrumentation, and they originate in tissue or cellular compartments that are literally within the size range of the head of a pin, practitioners of neuroproteomics may occasionally feel that they are revisiting that old philosophical conundrum. Indeed, initial forays into proteomics of the nervous system were focused on abundant components in easily accessible models such as neuropeptides or cytoskeletal proteins in invertebrate giant neurons (*e.g.* see 18–20). More recent efforts range from comprehensive proteomic characterization of mammalian synaptic ensembles (21, 22) to targeted identification of proteins with critical functions in subcellular domains (23, 24). Clearly persistent improvements in resolution and sensitivity of instrumentation, combined with creativity in sample enrichment or purification, are positioning proteomics for significant contributions in neuroscience. Just as one of the cryptic yet satisfying answers to that medieval question was "enough," we are reaching the point where proteomics technologies can rise to cutting edge challenges in neuroscience. We look forward to future developments.

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Editors and staff for their support for this special issue. We regret that only a few of the outstanding advances for neuroproteomics from the current literature can be mentioned herein. We refer the reader to citations across this issue for a broad perspective of current neuroproteomics.

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