Yale/NIDA Neuroproteomics Center

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Current and Future Challenges in Quantitative Neuroproteomics
General challenges with particular nuances in studies of the CNS
- Application of robust and sensitive mass spectrometric methods
- Accurate reproducible and quantitative procedures
- Appropriate experimental design and assessment of factors that influence technical and biological variance

Specific challenges for proteomic studies of the CNS
- Huge amount of cell type variability with specific and distinct patterns of gene/protein expression and regulation
- Complex intermingling of neuronal sub-types
- Complex cell shapes and sub-compartments
- Low amounts of proteins to analyze
Diversity of neuronal subtypes

Deisseroth et al Nature 2013 - Three-dimensional view of hippocampus in c showing eYFP-expressing neurons (green), parvalbumin-positive neurons (red) and GFAP (blue). Alv, alveus. Scale bar, 200 μm
Current and Future Challenges in Quantitative Neuroproteomics – Sub-cellular analysis

- However, particularly in neurons there is also the added feature of local protein synthesis and possibly local proteosome-mediated degradation.

- Finally, there is the desire to identify many different types of post-translational modifications, alternative splicing etc which are key in understanding the control of protein function.
Can proteomics keep up with and complement other genomic approaches?

- The major advances in generation of BAC transgenic mice under the control of gene promoters with specific cell type expression combined with conditional viral expression is driving many aspect of neuroscience, including functional anatomy, definition of novel neuronal circuits and the rapidly progressing field of optogenetics

- And is also enabling –omic level analysis of DNA methylation, epigenetic modifications, mRNA expression and ribosomal profiling
BAC transgenic mice expressing tagged proteins can allow expression analysis of ribosomal mRNA in defined populations of neurons.

GFP expression in either direct (Drd1a) or indirect (Drd2) pathways of striatum – BAC transgenic mice generated by GENSAT (Rockefeller Univ)

- BacTRAP uses a GFP-tagged ribosomal protein expressed in unique populations of neurons under the control of a specific promoter - method has raised the bar in terms of analyzing mRNA translation

Heiman et al. 2009
Cell type based analysis of microRNA profiles in mouse brain

- Used Cre-loxP methods to selectively express tagged-Argonaute (AGO2) and identify differential miRNA expression

Huang et al Neuron 2012
Wide array of transgenic mice and different approaches that now allow for GFP labeling of defined cell populations – can we apply to proteomics

1. FACS sorting?

2. Isolation of sub-proteomes from defined neuronal sub-types using rapid immunoprecipitation methods

3. Development of large-scale targeted mass spectrometry methods for quantitative analysis of sub-proteomes
FACS sorting D1- and D2-Medium spiny neuron nuclei from mouse striatum

Jordi et al PNAS In Press (2013)
FACS sorting D1- and D2-Medium spiny neuron nuclei from mouse striatum

Histone Phosphorylation and Acetylation

Histone methylation

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Jordi et al PNAS In Press (2013)
2. Proteomics of Neuronal Subtype-Specific Organelles

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2. Isolation of Neuronal Subtype-Specific Organelles

- Identify candidate proteins that are part of the cytosolic side of organelles.
- Label organelles by expression EGFP tagged candidate proteins.
- Pull down organelles through immunoprecipitation

Genetically tag organelles with EGFP and pull down through affinity isolation

Nuclei were tagged by expression of EGFP-fused Nup214

Synaptic vesicles were labeled by expression of EGFP-tagged synaptophysin.

Mitochondria were labeled by expression of EGFP-tagged Fis1

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2. Isolation of Neuronal Subtype-Specific Organelles

Affinity isolation methods were optimized to allow for specific, efficient and reproducible isolation of pure EGFP-tagged mitochondria, nuclei, and synaptic vesicles respectively.

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High efficiency of affinity isolation. Immunoprecipitation elution fractions from transfected or naïve control cells samples were analyzed using SDS-PAGE and immunoblotting with antibodies to mitochondrial markers (porin and cytochrome C oxidase), nuclear makers (lamin B and histone H3), and synaptic vesicle marker (synaptobrevin and synaptophysin) respectively.

<table>
<thead>
<tr>
<th></th>
<th>Mitochondria</th>
<th>Nuclei</th>
<th>Synaptic Vesicles</th>
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<tr>
<td>Porin</td>
<td>![Porin]</td>
<td>![Lamin B]</td>
<td>![Synaptobrevin]</td>
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<tr>
<td>Cytochrome C Oxidase</td>
<td>![Cyto]</td>
<td>![Histone H3]</td>
<td>![Synaptophysin]</td>
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<tr>
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<td>EGFP-tagged</td>
<td>![EGFP-tagged]</td>
<td>![EGFP-tagged]</td>
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Anshu Shen
EM analysis reveals that organelles have been enriched and labeled with superparamagnetic microbeads. Image was acquired with an FEI Tenai Biotwin 80-120kV TEM.

Anshu Shen
Top five high-abundance proteins identified by LC-MS/MS analysis

<table>
<thead>
<tr>
<th>Protein Name</th>
<th>Protein ID</th>
<th>Expectation</th>
<th>Score</th>
<th>MW</th>
<th>% Coverage</th>
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<td>1 chaperonin (HSP60)</td>
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<td>306890</td>
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<td>60986</td>
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<td>2 malate dehydrogenase, mitochondrial precursor</td>
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2. Cell-Specific Expression Of tagged Ubiquitin Using Tg-BAC

MQIFVKTLTGKITLEVEPSDTIENVKA
KIQDEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVR

6xHis-HA

MG132 (µM) | 0 | 1 | 2 | 5
---|---|---|---|---
6xHis-Ubiquitin | + | + | + | +

Hicke et al, 2005

George Craft
Distribution of DA neuron cell groups in the developing (a) and adult (b) rodent brain

Bjorklund and Dunnett, TINS 2007

Midbrain DA neurons – 20-30,000 in mice, to 400-600,000 in humans
The axons of the DA neurons in the SNc mainly project to the dorsal striatum, with a small percentage innervating specific areas of the cortex and the ventral striatum.
2. Cell-Specific expression of Ubiquitin using Tg-BAC under the control of the dopamine transporter
The green-red-blue merged image indicates the co-localization of EGFP and TH in caudate putamen.
The green-red-blue merged image indicates the co-localization of EGFP and TH. Compare with previous founder, the signal to noise ratio of EGFP channel is relatively lower.
The green-red-blue merged image indicates the co-localization of EGFP and TH.

The localization of EGFP-Fis1 fluorescence is consistent with mitochondrial localization.

Verification EGFP-Fis1 Expression Profile (Genotyping Positive Founder 1, SNigra)
Strategy for ongoing experiments with BAC transgenic mice and Dopamine signaling in mice models

- Examine consequences on organellar proteomes and ubiquitylated proteome in DA neuron sub-compartments – eg axon terminals and projections

Midbrain DA neurons – 20-30,000 in mice, to 400-600,000 in humans

Iversen and Iversen, 2007
Leuner and Muller, 2006
Matsuda 2009
3. Development of large-scale targeted mass spectrometry methods for quantitative analysis of sub-proteomes

- In parallel develop large-scale MRM and SWATH methods to quantitatively interrogate sub-proteomes of interest; eg mitochondria, nuclei, transcription factors, histones, synaptic vesicles
- Optimize MS-based methods to converge at same level of protein isolated from tissue

- Multiple approaches are possible
  - eg Target excitatory or inhibitory synapses – proof of principle from studies from Brian Chait and Nat Heintz
  - expansion to combined use of viral expression and selective expression of tagged-proteins in BAC-Cre Tg mice
  - Potential for combination with FACS sort to isolate specific pre-synaptic/post-synaptic syapses
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• Lisa Chung
• Can Bruce

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