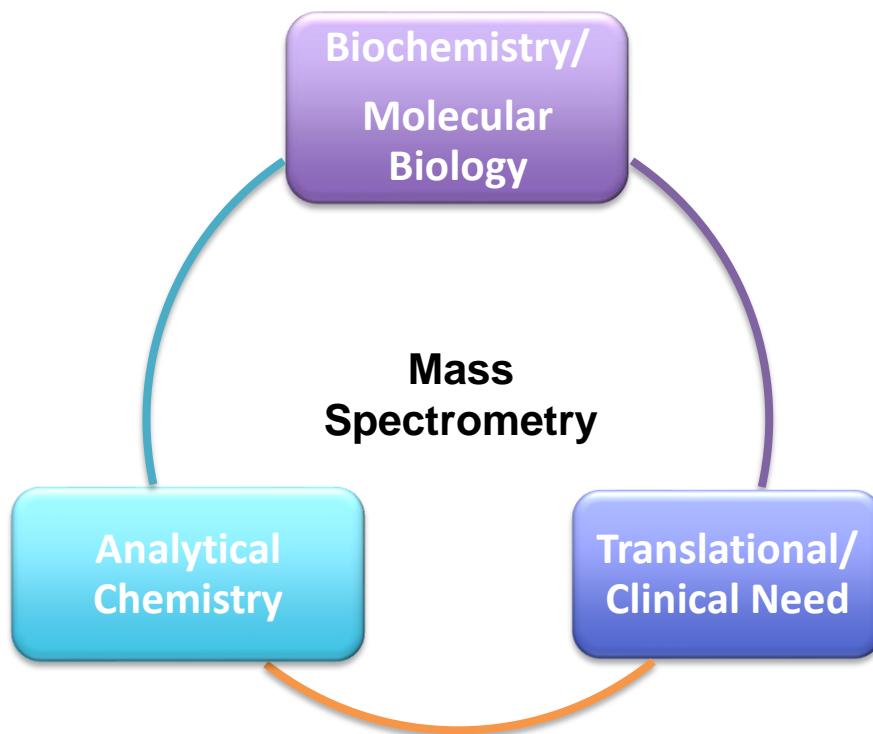


Jet Stream Thermal Focusing Electrospray in Quantitative Proteomics

Stephanie M. Cologna, Ph.D.

Department of Chemistry, Laboratory for
Integrative Neuroscience

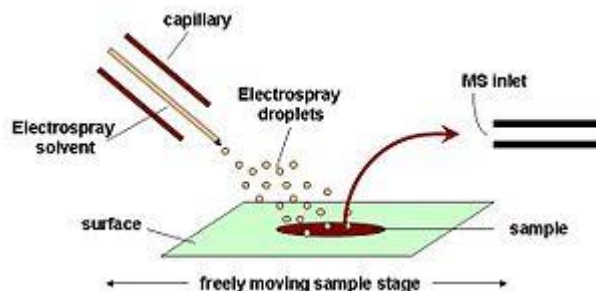
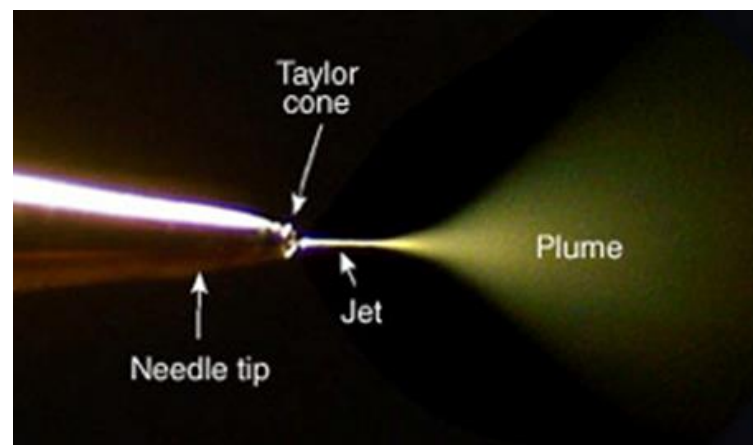
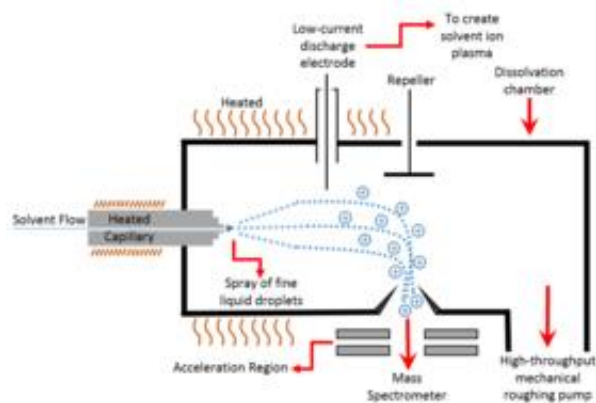
Molecular Processes of Neurodegeneration: Proteins and Lipids



Advances for Proteomics

- Soft ionization
- High resolution/mass accuracy mass spectrometers
- Database and informatic tools
- Separation science

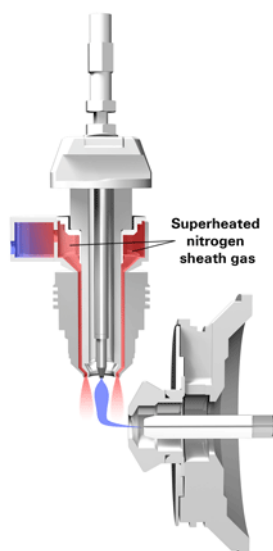
Electrospray Approaches



www.wikipedia.com
www.newobjective.com

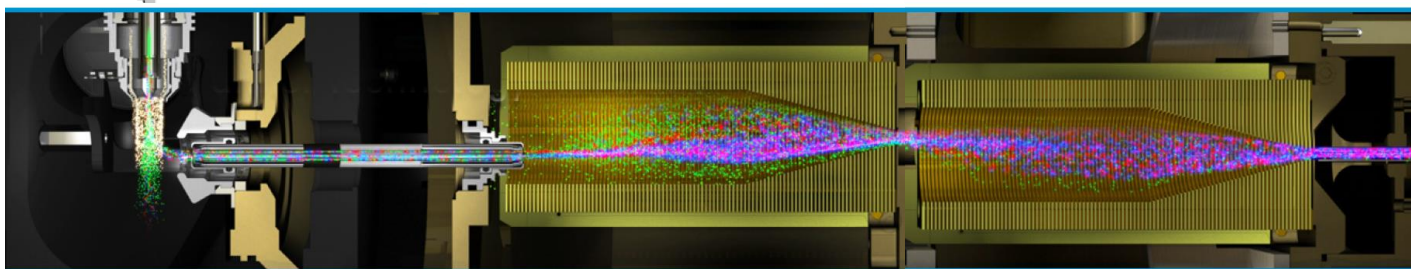
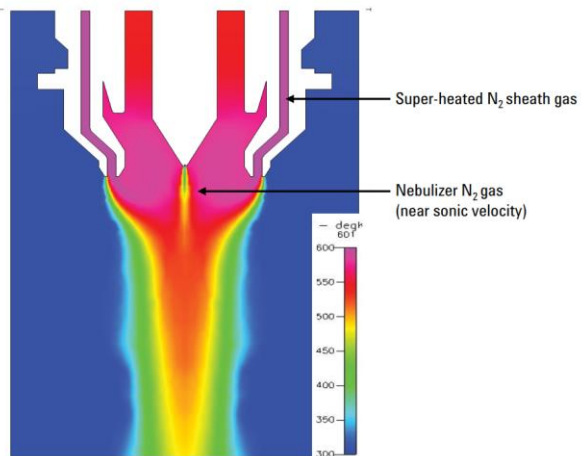
High Flow ESI?

Agilent Jet Stream (AJS) Electrospray



Thermal energy is focused to the nebulizer spray

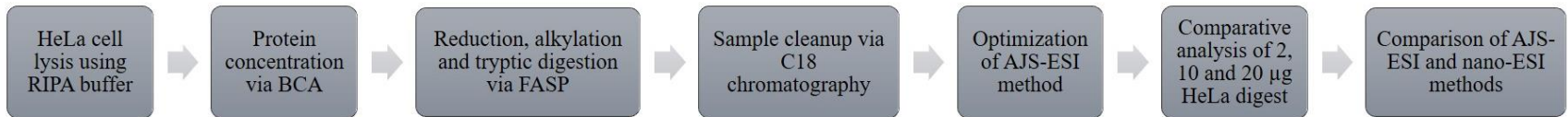
Thermal focusing produces the most efficient desolvation and ion generation possible



Towards Label-Free Proteomics

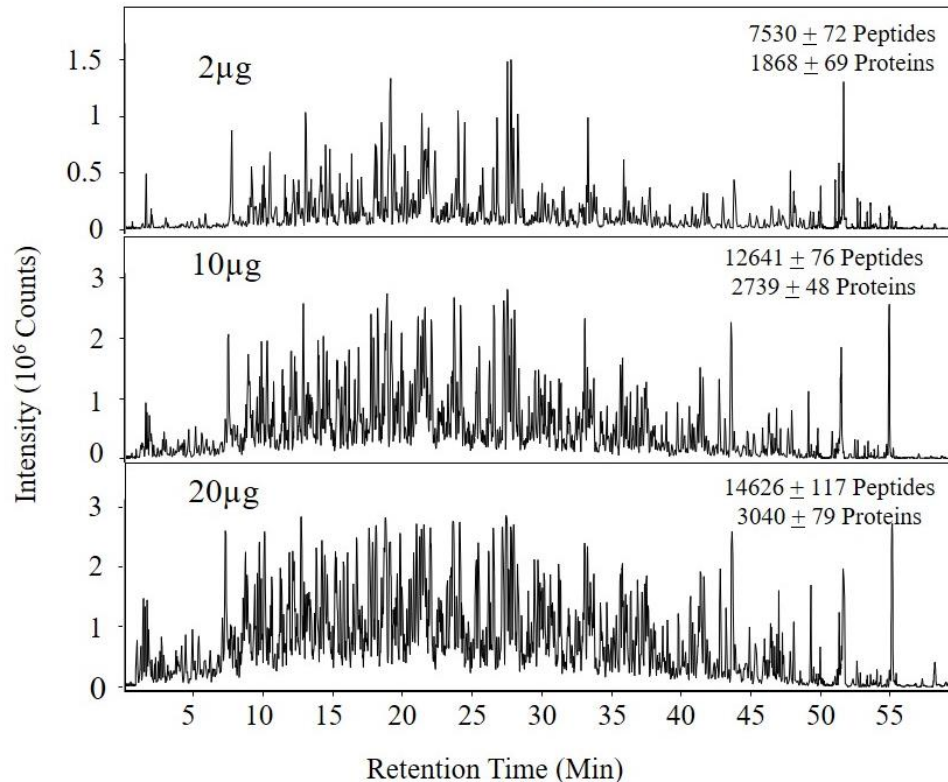
A

Workflow



B

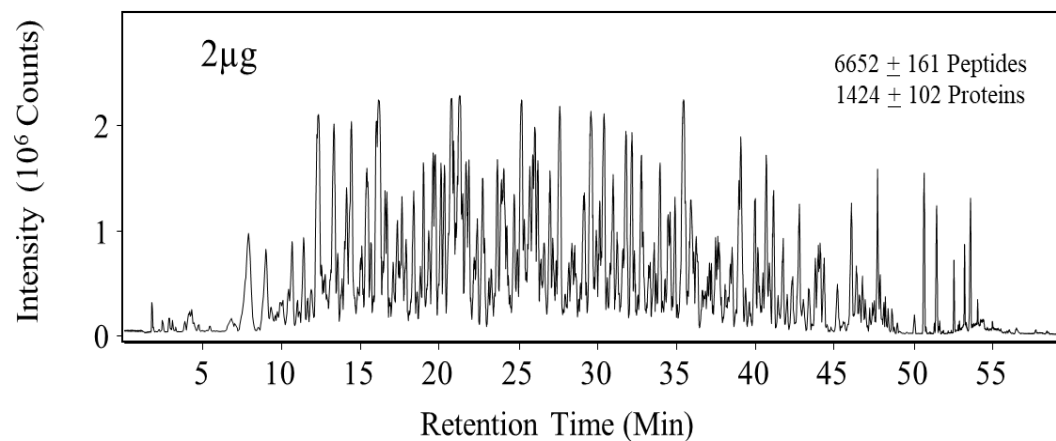
Analysis of HeLa Digest via AJS-ESI



Pergande, et al., Proteomics. 2019.

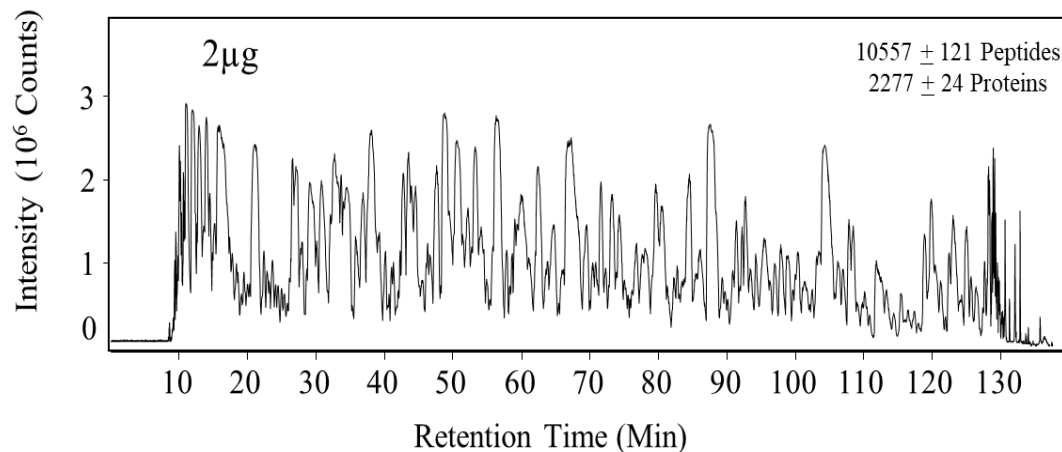
A

Analysis of Cerebella Digest via AJS-ESI



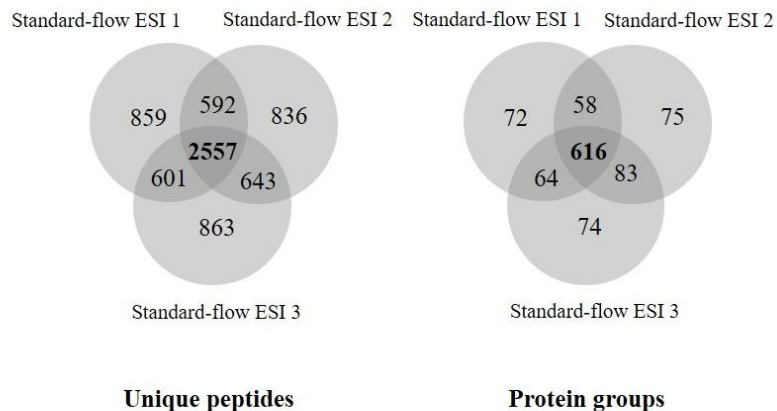
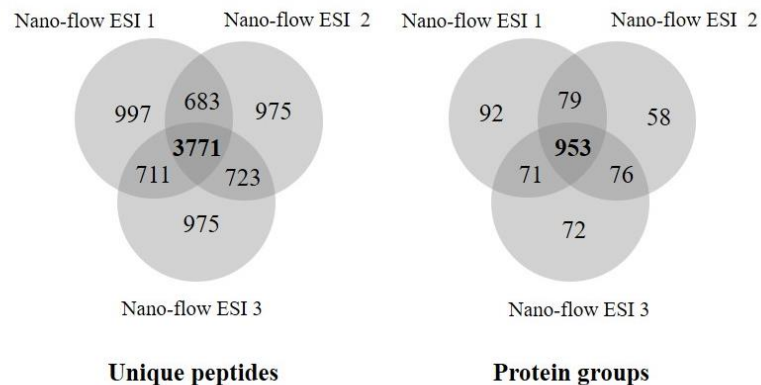
B

Analysis of Cerebella Digest via nano-ESI



Pergande, et al., Proteomics. 2019.

AJS vs ChipCube NanoESI



Pergande, et al., Bioanalysis. 2019.

Research Program Question

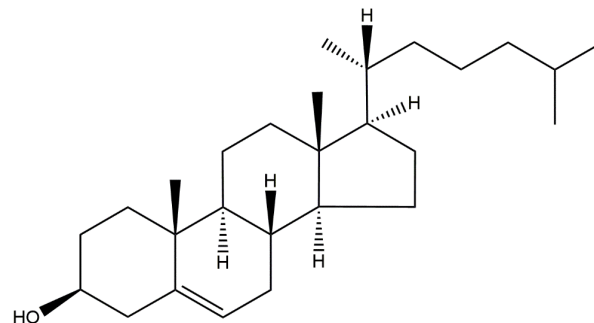
What chemical signal(s) drive a neuron (or other cell in the CNS) to survive or die?

Neurodegenerative Disorders

- Class of diseases with progressive neuron loss
- Degeneration of the central nervous system
- Neurodegenerative disorders include:
 - Alzheimer
 - Parkinson
 - Huntington
 - Lysosomal storage disorders
- Oftentimes, mis-folded proteins, fibrillation or deposits are observed

Cholesterol

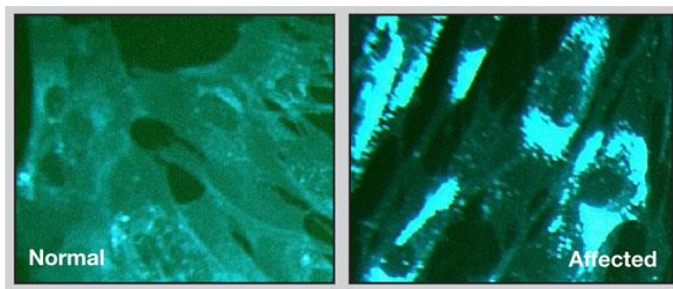
- In mammals, synthesized enzymatically from acetyl-CoA and obtained from the diet peripherally
- Brain cholesterol is synthesized endogenously and tightly regulated
- Required for cell membrane structure and function
- Cholesterol has been implicated in neurodegenerative disorders



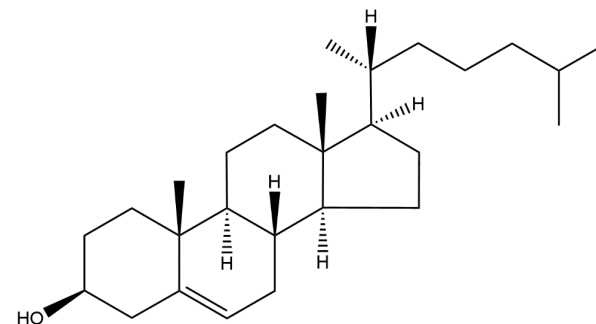
J.E. Vance, Disease Models and Mechanisms, 2013, Vol 5 (6).

Niemann Pick Disease, Type C (NPC)

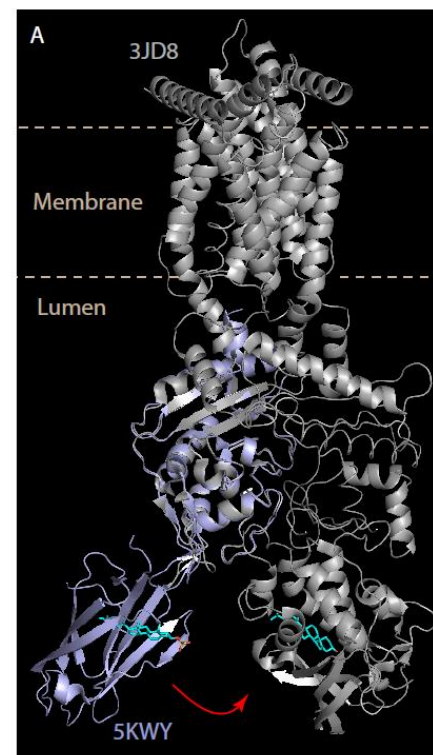
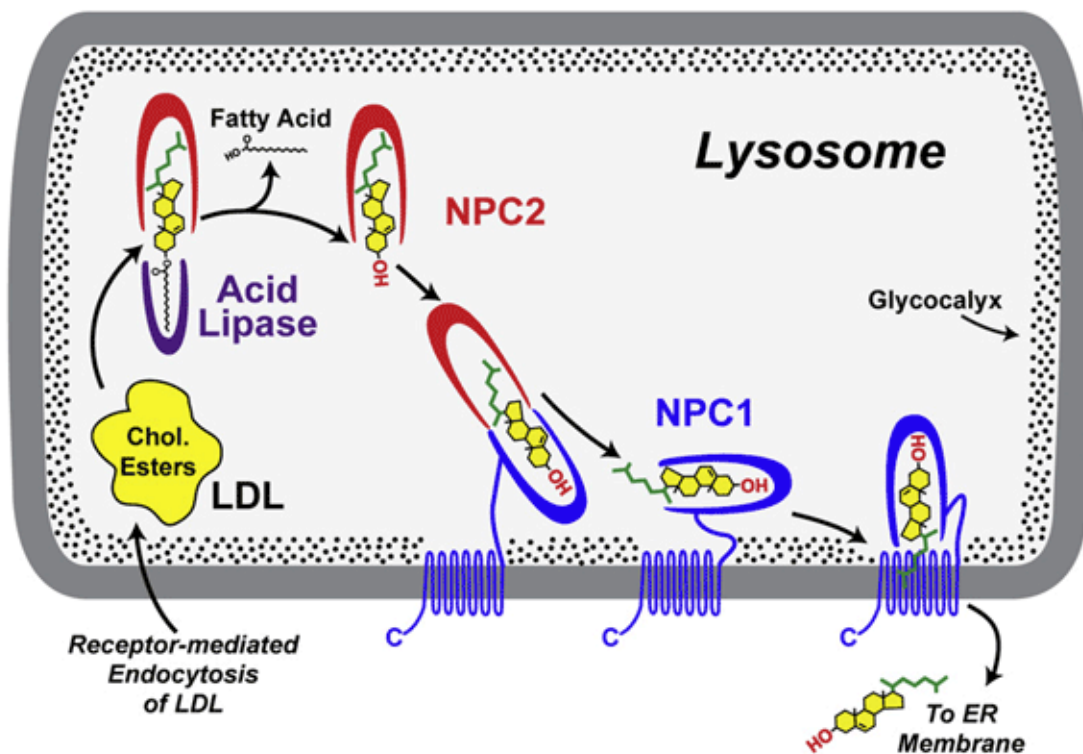
- A fatal, autosomal recessive neurodegenerative disease
- Accumulation of unesterified cholesterol and glycosphingolipids in the endosomal/lysosomal system
- Due to mutations in the *NPC1* or *NPC2* gene
Mutations in the *NPC1* gene account for approximately 95% of diagnoses



www.mayoclinic.org



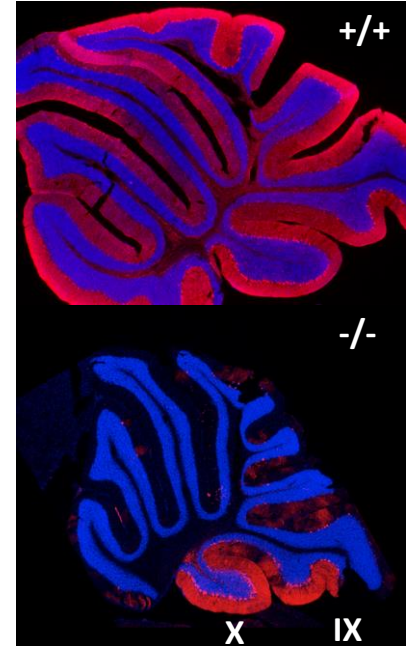
NPC1 and cholesterol transport



Kwon, et al., Cell 137, (2009)1213–1224
Cologna, S.M., Rosenhouse-Dantkster, in revision.

NPC Neuropathology

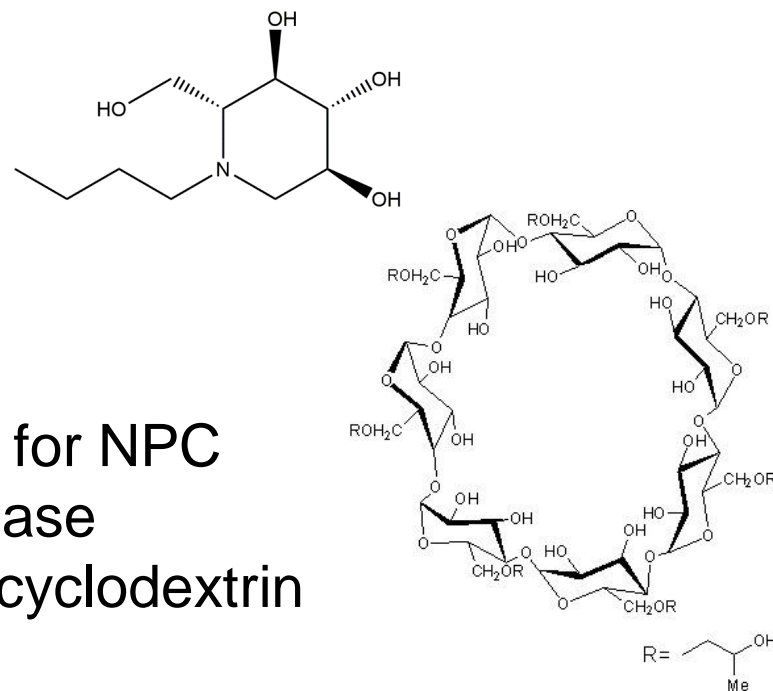
- Neuronal apoptosis in the cerebral cortex and cerebellum
- Progressive neuronal loss, especially of **cerebellar Purkinje neurons**
- Neuron degeneration mechanism is unclear
- Some pathological overlap with Alzheimer Disease, such neurofibrillary tangles and tau hyperphosphorylation



Courtesy of Ian Williams, Ph.D., NIH

Clinical Presentations of NPC

- **Large variation in age of onset, and severity**
- NPC has a wide spectrum of clinical presentations including **neurological** symptoms such as:
 - Ataxia
 - Progressive dementia
 - Seizures
 - Vertical gaze palsy
- Currently no FDA-approved therapy for NPC
 - Off-label miglustat, Gaucher Disease
 - Clinical Trial: 2-hydroxypropyl- β -cyclodextrin



Differential Proteomics in the Symptomatic Mouse

1 – 4 weeks
(Pre-symptomatic)

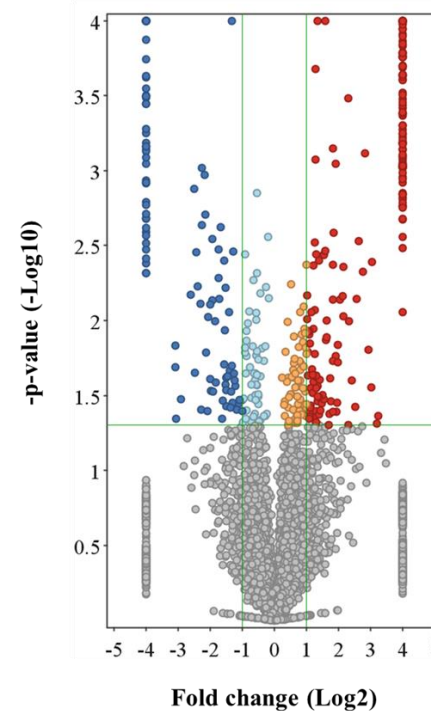
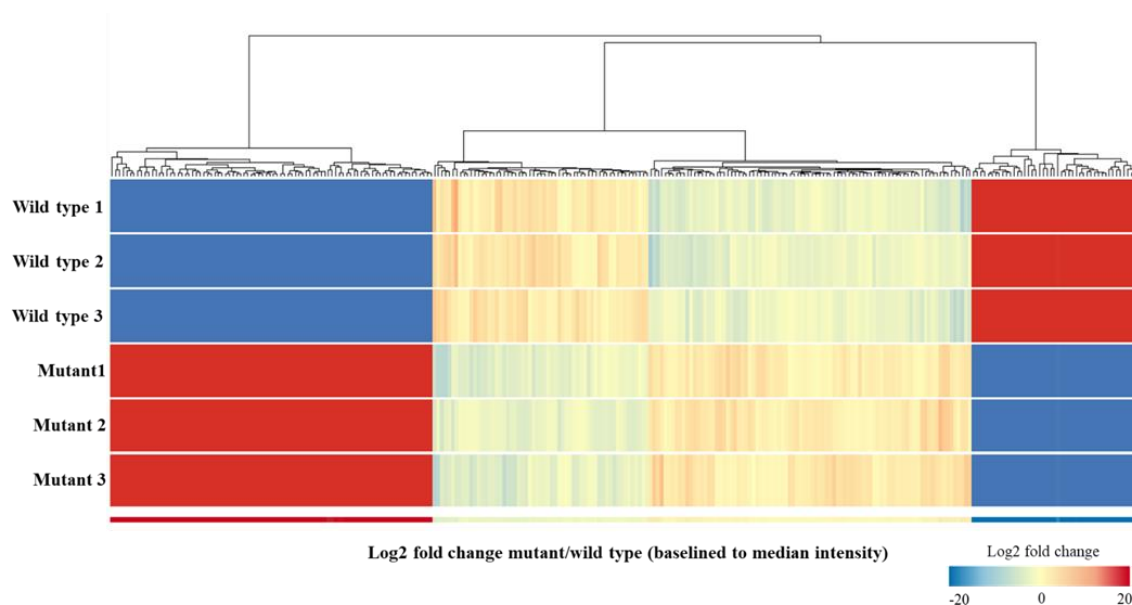
5 – 6 weeks
(Tremors)

7 – 8 weeks
(Ataxia)

10 – 12 weeks
(Terminal)



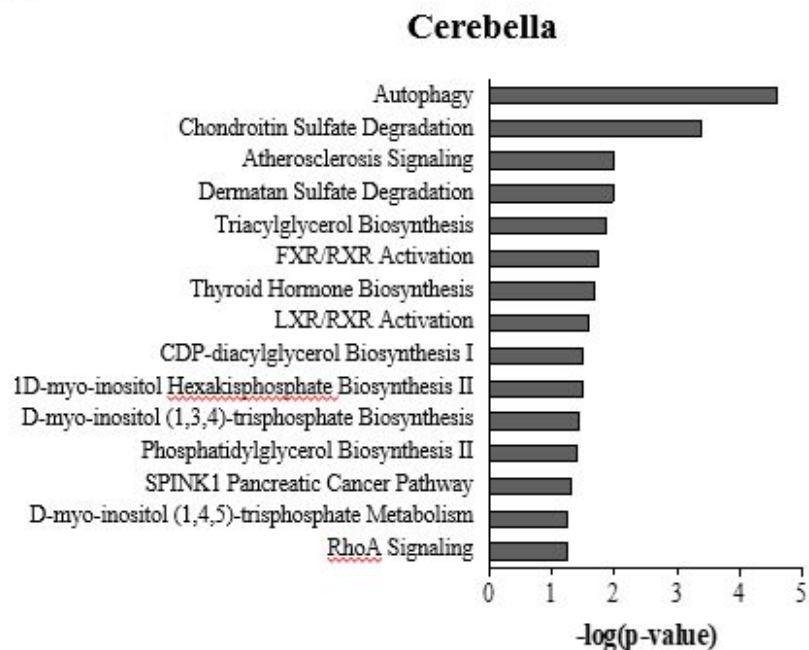
Label-Free Quantitation in NPC1 Mouse Cerebellum



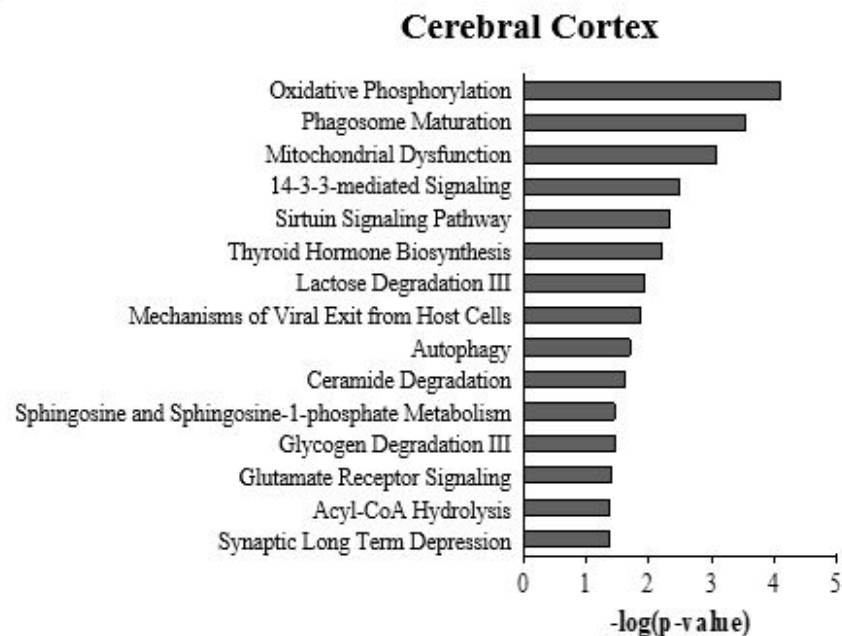
Pergande, et al., Proteomics. 2019.

Pathway Analysis – Cerebellum

A



B



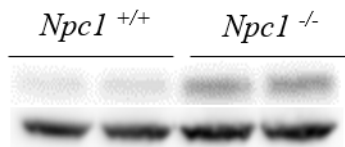
Pergande, et al., Proteomics. 2019.

Secondary Validation of Differential Proteins

Fatty Acid Transport

A

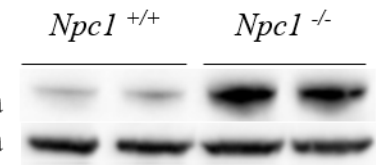
FABP5 15kDa
GAPDH 37kDa



Lysosomal Proteins

B

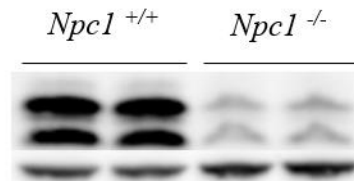
LIMP2 85kDa
GAPDH 37kDa



Purkinje Neuron Proteins

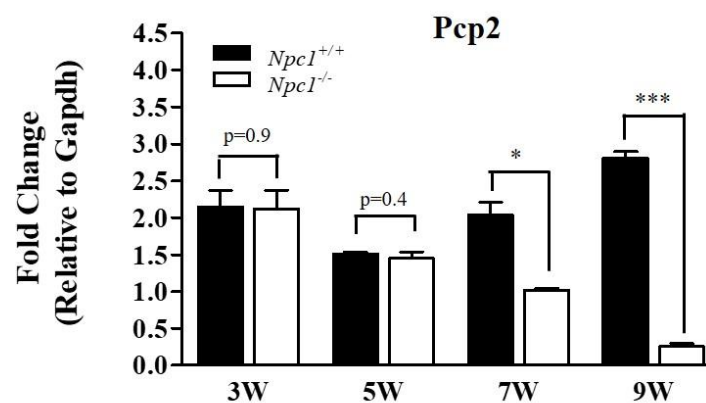
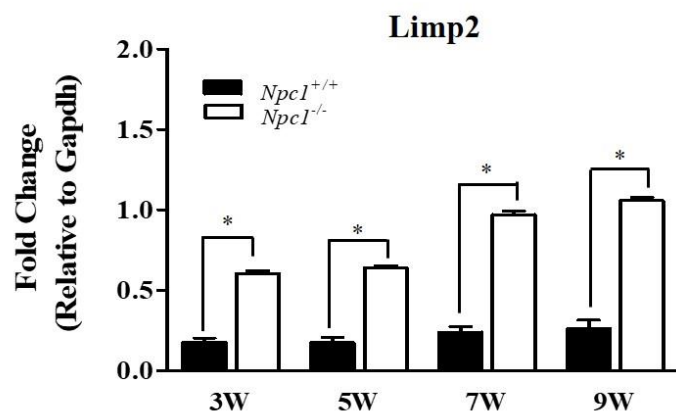
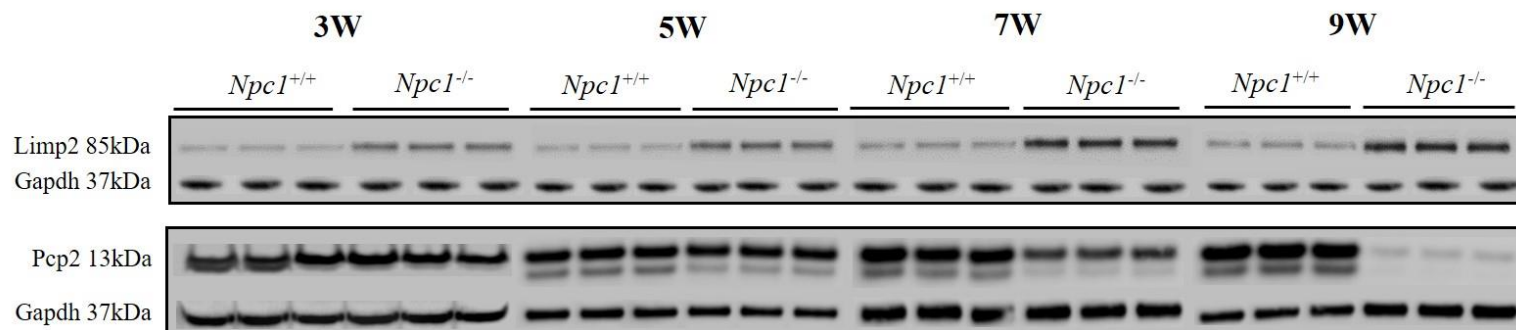
C

PCP2 13kDa
GAPDH 37kDa



Pergande, et al., Proteomics, 2019

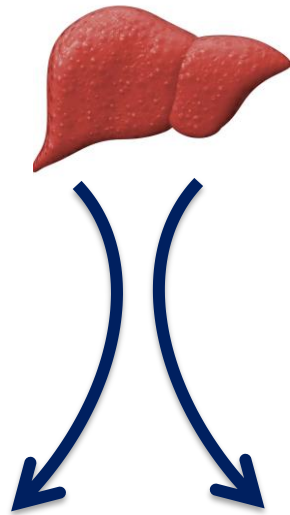
Cerebellar Disease Progression



What have we learned?

- Label-free differential proteomics with AJS
- New markers CNS pathophysiology?
- LIMP2 changes with progressive neurodegeneration

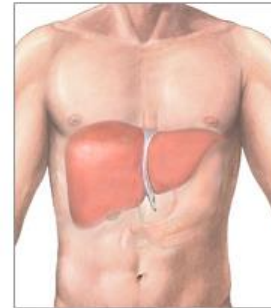
Altered Fatty Acid Metabolism in NPC



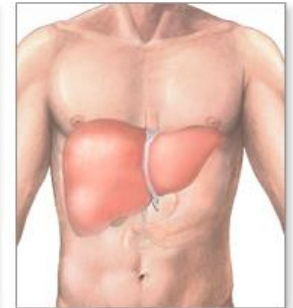
Untargeted
Proteomics

Untargeted
Lipidomics

Normal liver

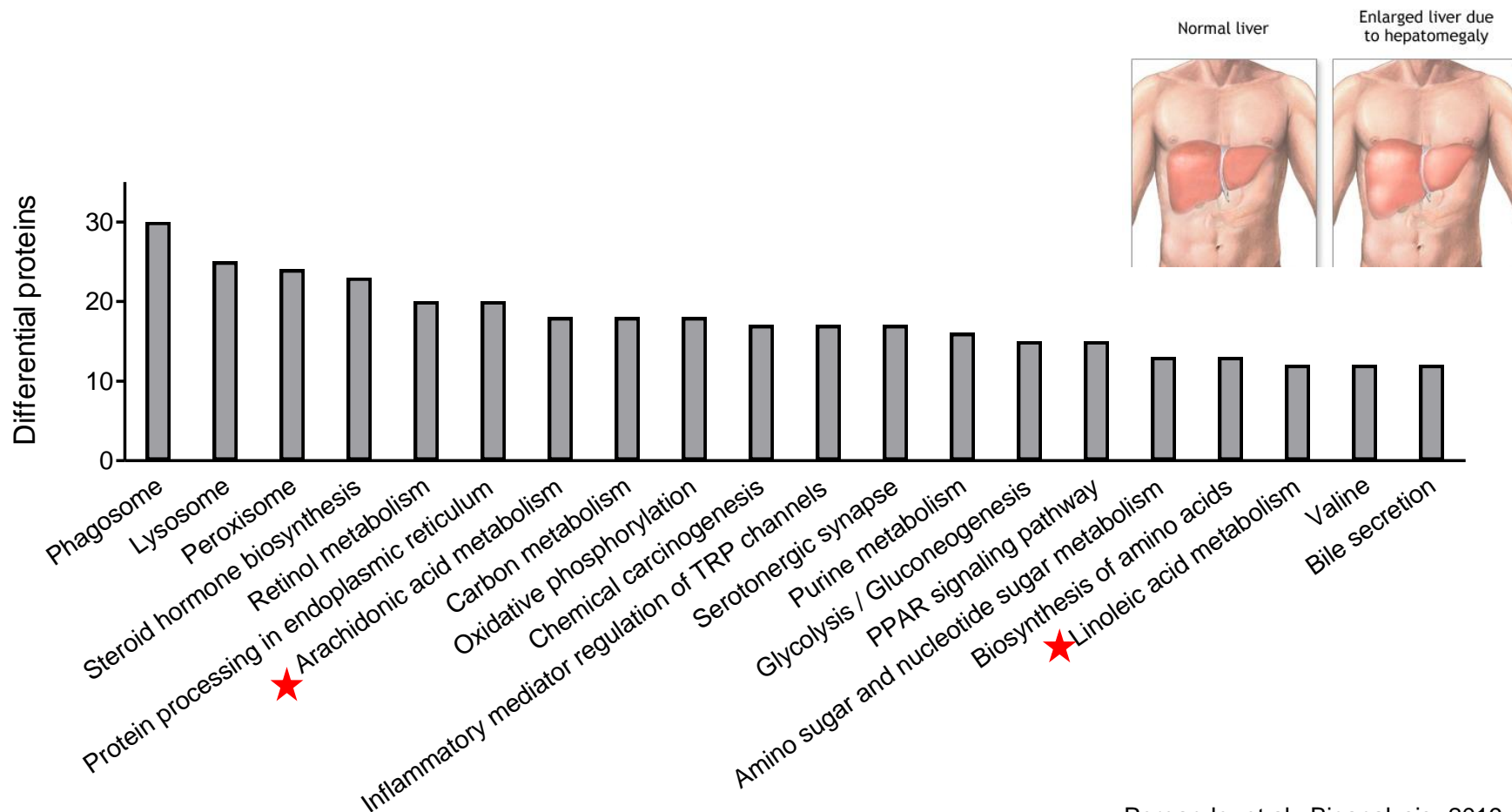


Enlarged liver due
to hepatomegaly

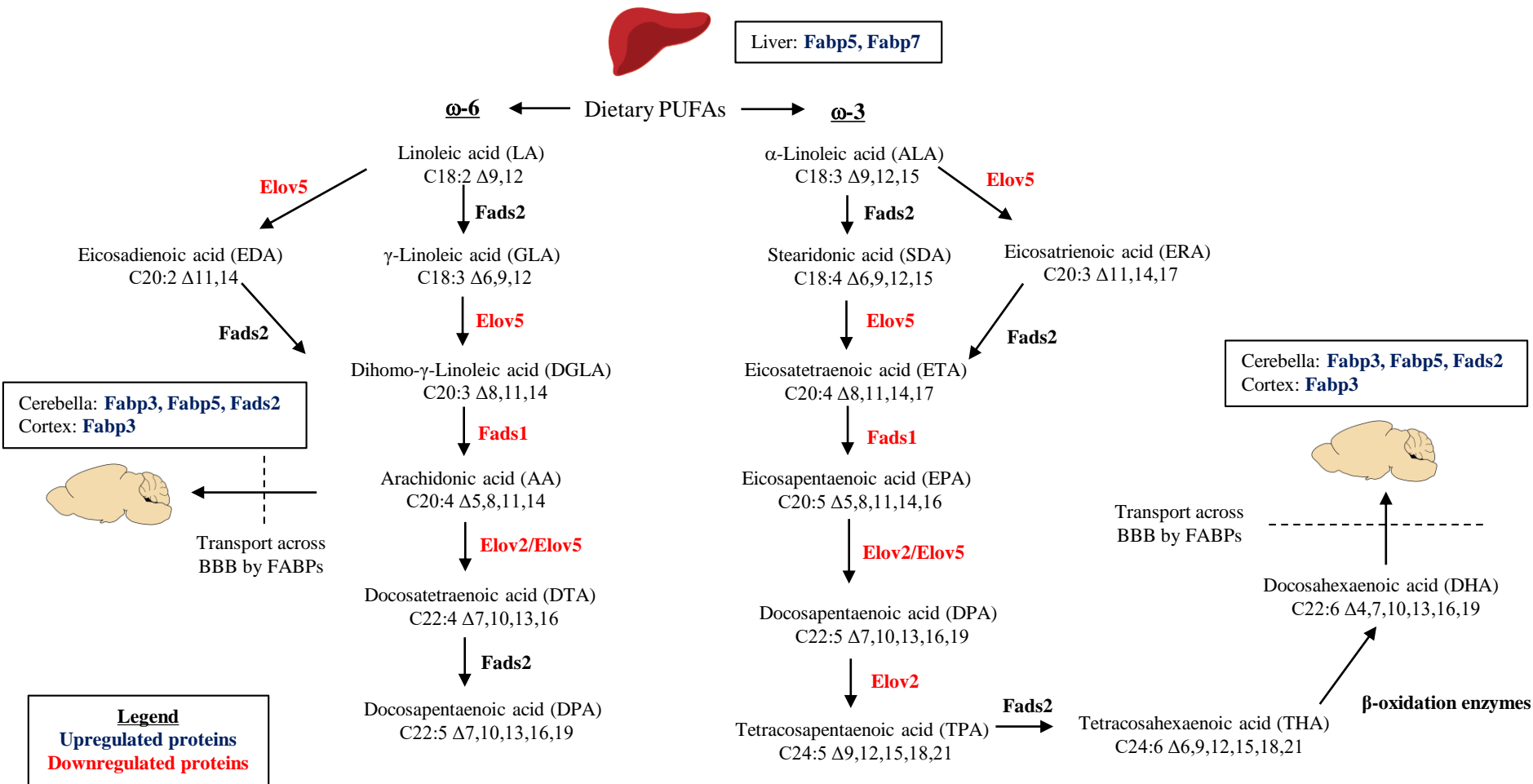


ADAM.

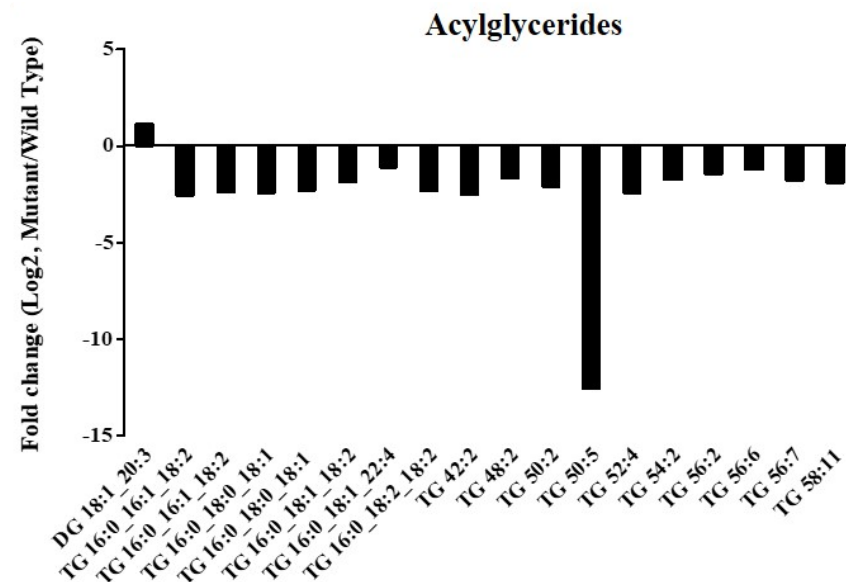
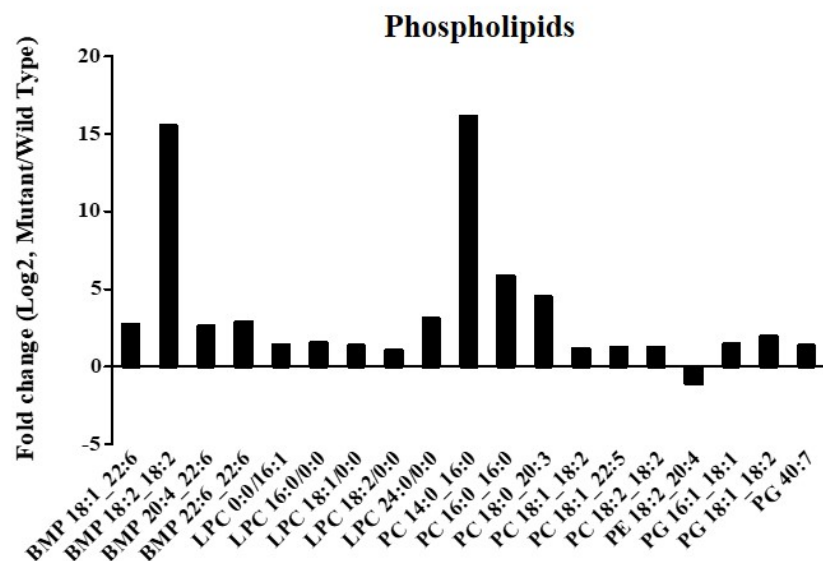
Differential Proteome and Lipidome of the Liver



Pergande, et al., Bioanalysis. 2019

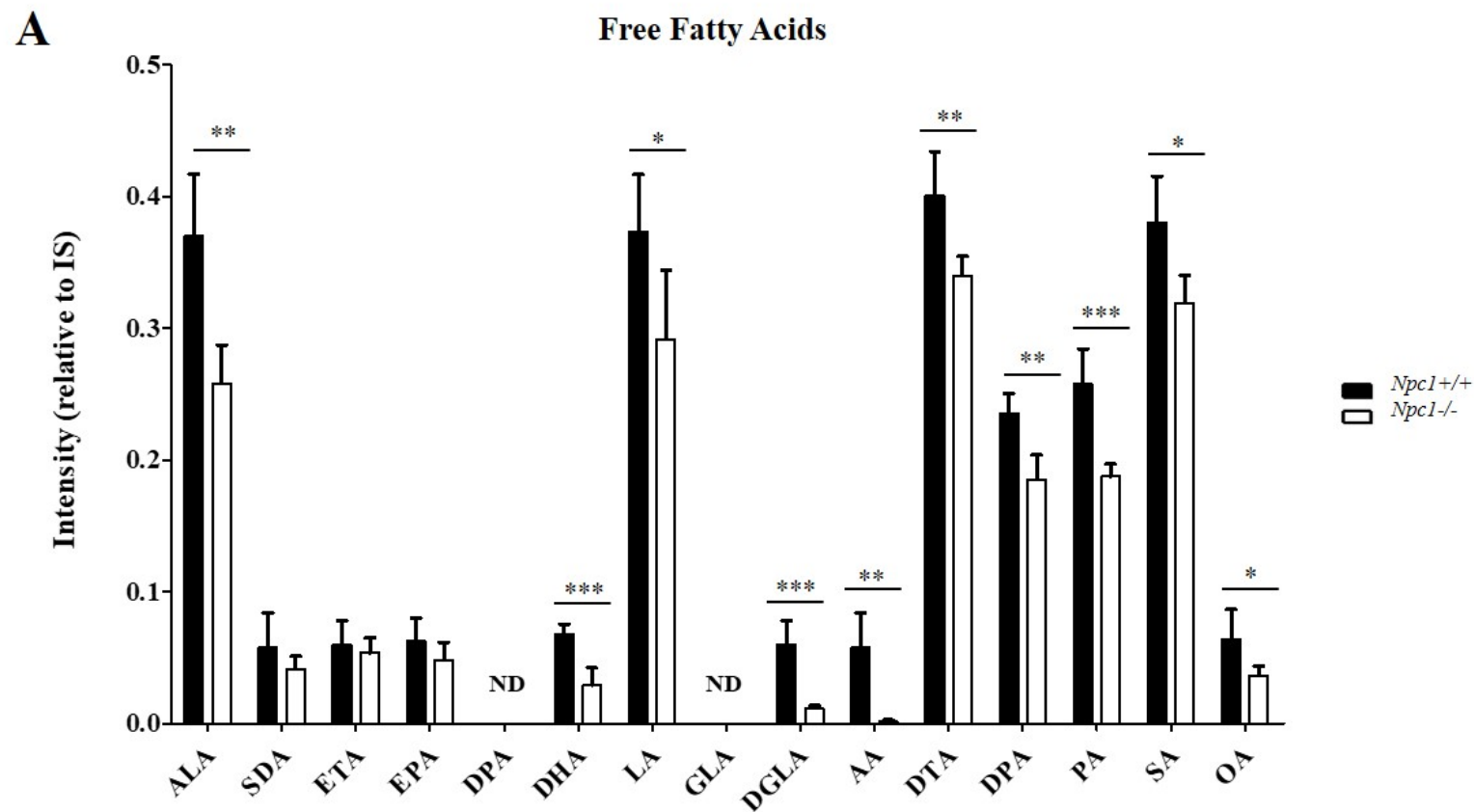


Lipidomic Results



Pergande, et al., Proteomics. 2019

Free Fatty Acids in NPC



Pergande, et al., Proteomics. 2019

Summary and Outlook

- NPC1 is a fatal, lysosomal storage disease without an approved therapy
- Jet Stream (AJS) has been used for differential proteomic and lipidomics studies in NPC1
- Proteomic studies reveal new NPC1 biomarkers in the cerebellum and liver
- Integrated studies reveal altered ω -3 and ω -6 fatty acid metabolism in the liver

Acknowledgements



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Mark Hoppe
Sheher Mosin



Graduate Studies:
<http://chem.uic.edu/graduate-studies/>

