Mikroskopi i andre dimensioner – Fra massespektometribaseret antistofuafhængig histologi til højopløsningsmikroskopi af hjernevæv fra Alzheimers-patienter

By Allan Stensballe; PhD. (ATV)
Translational Biomarker Unit
AAU

Thermo Sci Qexactive HF-X
Pharma-option

Bruker timsTOF PRO
Multi-omics setup

Bruker UltrafleXtreme
Imaging MS setup

4D Setup for biomolecule characterization down to low cell number

Extensive bioinformatics platform including multiple commercial solutions for quantitative proteomics and metabolomics

MS, Multiplex & Protein Array for cell signalling & autoantibody profiling

Imaging from cells to tissue

FLUO
ThioflavinS staining

Plaques in AD brain

AALBORG UNIVERSITY DENMARK
Omics and the Brain

• Despite the advances in sensitivity in mass spectrometry and in the development of proteomic workflows applied in neuroscience, protein yield from neural tissues is often a harsh limiting factor when considering the quantities needed for proteomics.

• The nervous system is composed by tangled mixtures of different cell types, presenting major sampling and analysis challenges
Proteomics and Alzheimer’s disease

AD is a multifactorial and complex neurodegenerative disorder that is characterized by progressive and severe dementia with neuropsychiatric symptoms.

- Proteomics studies have contributed to two aspects of AD research:
  - The development of biomarkers for clinical diagnostics.
  - The recognition of proteins that can help elucidate the pathways leading to AD brain pathology.
Amyloid plaque proteome and metabonomic fingerprints in AD senile plaques

- Tau protein and amyloid beta (Aβ) peptides are the two principal aggregation hallmarks

- Several very expensive phase III clinical trials targeting molecules like Aβ have failed to show an effect on cognition or significantly halted the disease

We need a much better understanding of the underlying biology in AD

Aim: Can we use imaging techniques to study the amyloid plaque proteome and metabonomic fingerprints in AD senile plaques and in tissue surrounding tissue?
Enhanced amyloid beta proteoform detection in Alzheimer’s disease.

- Laser Microdissection combined with proteomics allows investigation of minute amounts of protein
- An AD mouse model system was used to investigate the plaque proteome
Microproteomics based investigation of the PD plaque proteome in a mouse model

5 x TgAPP-PS1-21
6 x WT littermates (L/R hemisphere)

25µm sections, add to PEN membrane glass slides, EtOH fixation and -80°C storage

Thioflavin-T staining, Acturus LCM system (Veritas)
Groups: Plaques, adjacent control, WT

Data processing with MaxQuant, Perseus, Cytoscape (STRING)

UPLC-MS, Q-Exactive mass HF spectrometer

Optimized In-solution digest (Trypsin)
Microproteomics in AD, PD and depression

- Laser Microdissection combined with proteomics allows investigation of minute amounts of protein
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**Advanced Imaging by combined MALDI IMS and Expansion /CLARITY microscopy**

Optimized novel sample preparation enhances S/N >100x

- **Advanced microscopy**
- **MALDI MS Imaging**

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Optimized CLARITY technique detects reduced parvalbumin density in a genetic model of schizophrenia.
MALDI IMAGING - Overview

Sample preparation → MS → Data analysis
MALDI IMAGING – Overview State-of-the-art

Rat brain lipid image acquired on the timsTOF fleX. Measured at 20 µm spatial resolution, this contains 312,095 spectra with acquisition time of five hours.

Lower panel shows segmentation analysis generated from this dataset in SCiLS Lab. The spectra are clustered into regions corresponding to different brain regions.
Novel MALDI MSI sample preparation enhances consistent detection of a-beta proteoforms

Tg APP/PS1-21 (n=3) → 12µm sections, add to ITO glass slides, EtOH fixation and -80°C storage → Carnoy’s wash

Data analysis in SCiLS lab → LP_Imaging 2-20 kDa on Ultraflextreme (MALDI TOF/TOF 500 shots per spot, raster 300µm) → ImagePrep robot, DHB/PA (30mg/ml)
Novel MALDI MSI sample preparation enhances consistent detection of a-beta proteoforms

Novel Matrix additives to the S-DHB matrix to investigate the ionization difference of the Aβ1-42 peptide when utilizing LCM and MALDI-MSI approach
Improving detection by selective protein extraction from AD plaques

FA based extract LMD
Plaque Enhances detection

= Matrix 1
= Matrix 2
Avanced Imaging by tissue Expansion microscopy

Expansion Microscopy (ExM) is a recently developed super-resolution microscopy (SRM) method in which fluorescent labels on fixed specimens are linked to a **swellable polymer hydrogel** that is physically expanded with remarkably low distortion.
ExM

- Idea behind ExM: introduce water absorbing polymer → link to biomolecules → digestion → add water → expansion → **enable nanoscale imaging with conventional microscopes**
3D Tissue ExM

Mice do not naturally develop Alzheimer’s disease. Credit: The Jackson Laboratory

A

B

Pre-expansion

Post-expansion

E

AALBORG UNIVERSITY
DENMARK
Enolase (ENO1)
β-Amyloid (D54D2)
Translational Biomarker Research Unit, Aalborg University, Denmark

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Proteome Complexity

- Genome: ~20,000 genes
- Transcriptome: ~100,000 transcripts
- Proteome: >1,000,000 proteoforms

Improved sample preparation from Biobank samples

- Most biological samples are preferably snap-frozen and stored at -80°C
- Typical samples are serum, plasma, tissue and immune cells

Improved sample preparation from Biobank samples

**FFPE biopsy**

- Optimized FFPE removal and NaDOC based protein extraction
- In solution digest (trypsin; IAM)
- UPLC tandem MS (Qexactive 2hr gradient)
- Protein ID
Improved sample preparation from Biobank samples

Pathway analysis of quantitative MS data

Comparable proteomic information

Biological based separation possible