Proteome Sciences

An update on CSF Tau for the GBSC Workgroup


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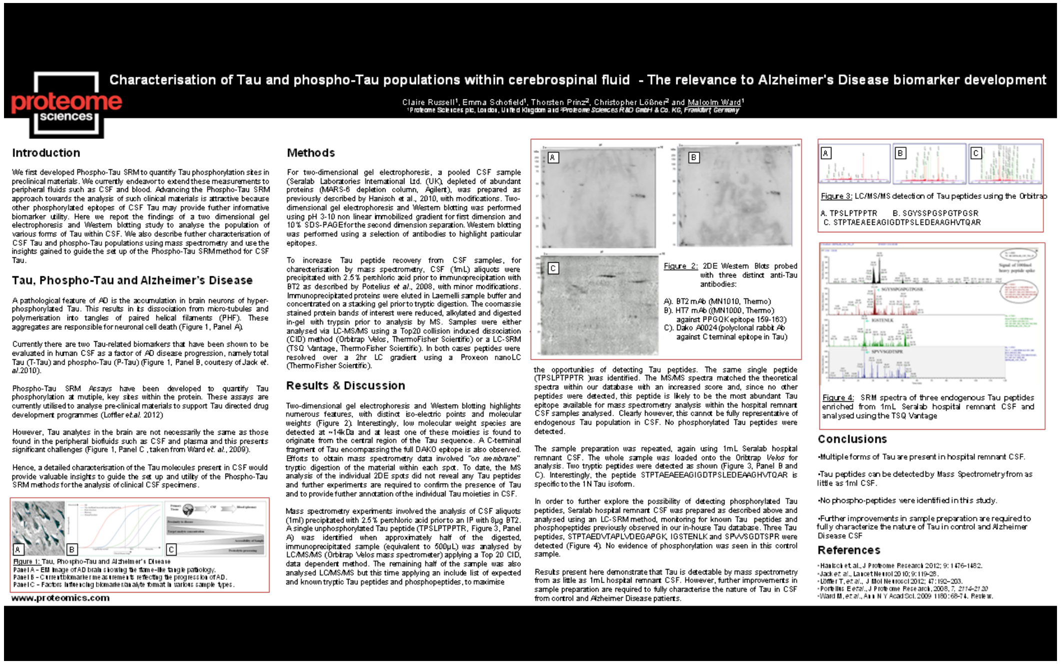
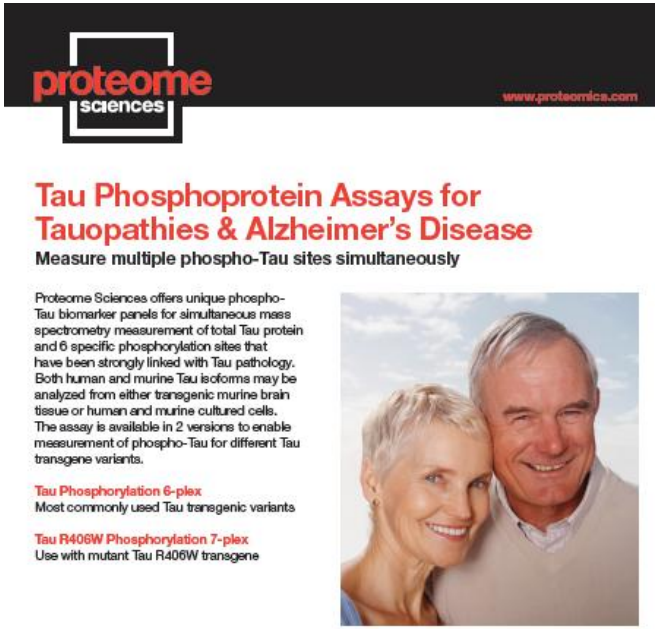
- What forms of Tau do we need to measure in CSF?
- Total Tau?
- Specific phosphosites?
- Truncation products?
- Other PTM's?



- Over 30 pathological phosphorylation sites known in brain
- Glycosylation is increasingly recognised as an important Tau PTM
- The lack of antibodies with required site specificity will make multiplex immunoassay development difficult
- Mass spectrometry may provide an efficient and cost-effective alternative

Using MS to measure Tau

- Proteome Sciences has developed several MS methods for quantitation of Tau and site-specific phosphorylation
- Tau phosphorylation 6plex – AQUA SRM with tryptic digestion for human/murine Tau in brain tissue and neuronal cell culture
- Tau phosphorylation 7plex – AQUA SRM with AspN digestion for R406W mutant human Tau in brain tissue and neuronal cell culture
- TMTcalibrator – isobarically labelled multi-point peptide standards and MS3 for highest sensitivity and precision



Detection of endogenous CSF Tau by MS

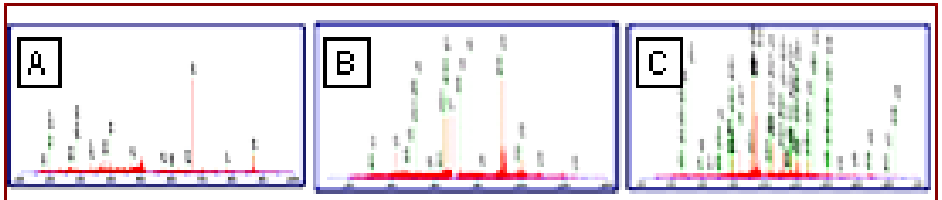


Figure 3: LC/MS/MS detection of Tau peptides using the Orbitrap

A. TPSLPTPPTR B. SGYSSPGSPGTPGSR
C. STPTAEAEEAGIGDTPSLEDEAAGHVTQAR

Orbitrap Velos

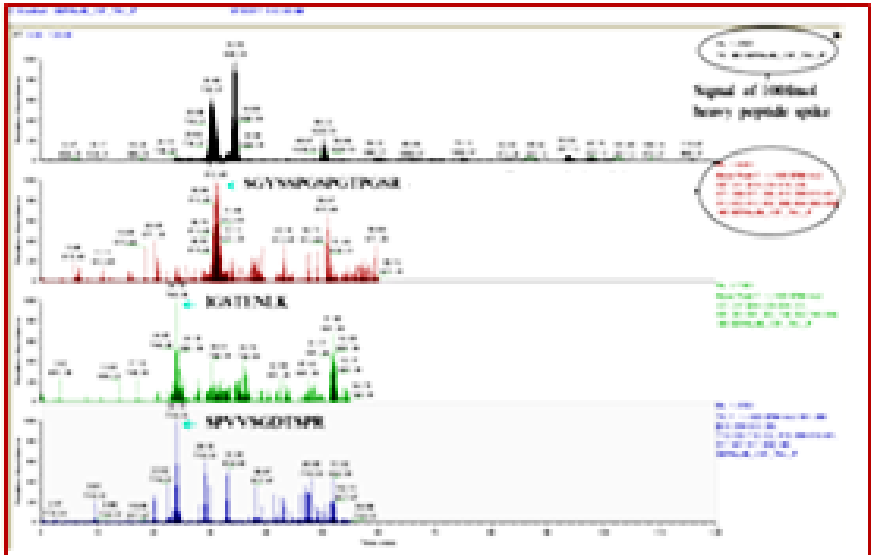


Figure 4: SRM spectra of three endogenous Tau peptides enriched from 1mL Seralab hospital remnant CSF and analysed using the TSQ Vantage

TSQ Vantage

Starting volume of CSF

= 1ml

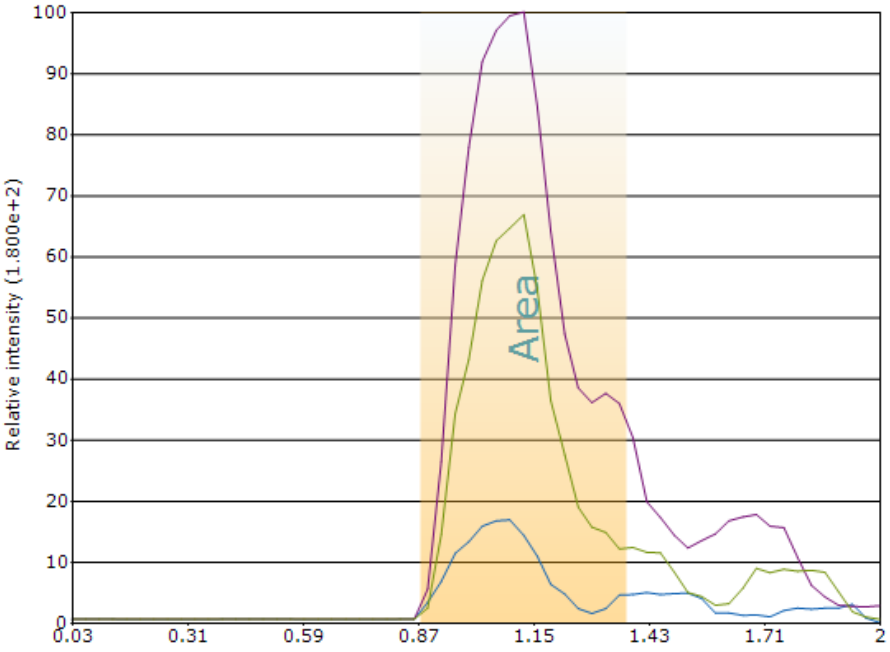
What did we do next ?

- **Development of Quantitative CSF Tau MS assays using synthetic reference peptides**
- **First needed to improve the analytical performance of the Total Tau surrogate peptide**
  - Retention time
  - Sensitivity
  - Precision
- **Combine the use of TMTcalibrator and MS3**
  - Trigger initiated MS/MS and MS3 acquisitions
  - Reduce/eliminate interference from background

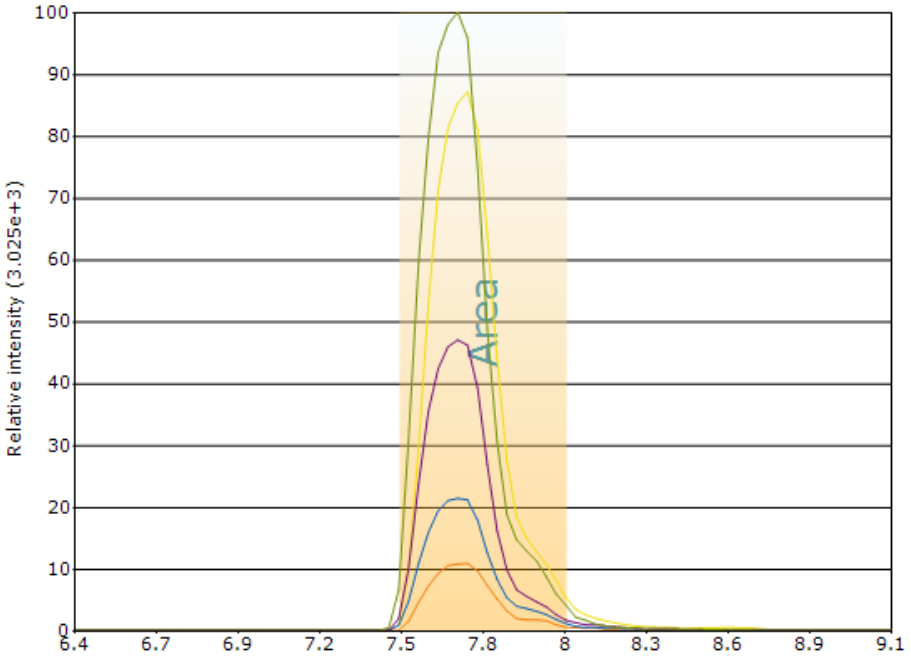
Total Tau Surrogate

Comparison of the elution times of 100fmol total peptide analysed by SRM after micro flow (100µL/min) rate chromatography (TSQ Vantage)

Unlabeled Total Tau peptide elutes at 0.87 mins



TMT labeled Total Tau peptide elutes at 7.5 mins



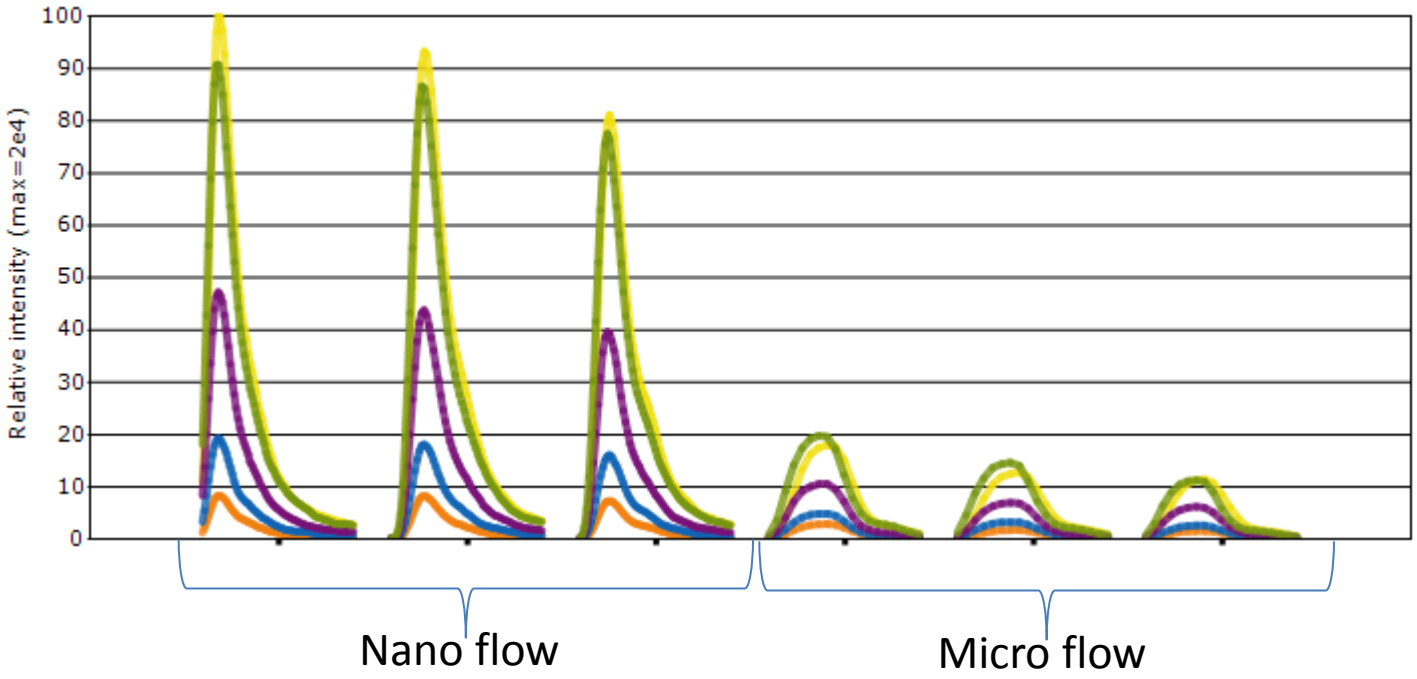
TMT labeling improves retention of Total Tau Surrogate

Total Tau Surrogate

TMT labelled Total Tau peptide

Moving from micro flow (100µL/min) rate to nano flow rate (300nL/min) improves sensitivity and precision

	Nano	Micro
Amount	25 fm	100 fm
Average signal intensity	5.5E4	1.65E4
Flow rate	300 nl/min	100 ul/min
Average total file area	2.01E6	1.394E5
CV	10%	23%
Retention time	17.63 min	7 67 min



TSQ Vantage

Making a viable MS assay for CSF Tau

- We have seen endogenous Tau peptides in 1ml CSF run on Orbitrap (LC/MS/MS) and TSQ Vantage (SRM)
- We can accurately quantify total Tau and several phosphosites in brain tissue by AQUA-SRM
- The AQUA-SRM method may be applicable to CSF but concerns regarding robustness and overall sensitivity for phosphopeptides
- A more sensitive and comprehensive method is desireable
- TMTcalibrator with MS3 quantitation run on the Orbitrap has the potential to reach required levels of sensitivity and can theoretically cover all relevant species and PTM's

TMT Calibrator: Multipoint Calibration

Reference sample

Reduce/alkylate
Digest
Split 1:2:4:8 ratio

TMT^6-128 -129 -130 -131

Label with TMT^6-128 to -131 to generate TMT Calibrator

Individual experimental samples (analyte)

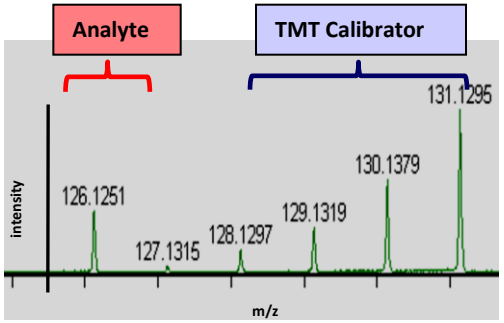
Reduce/alkylate
Digest

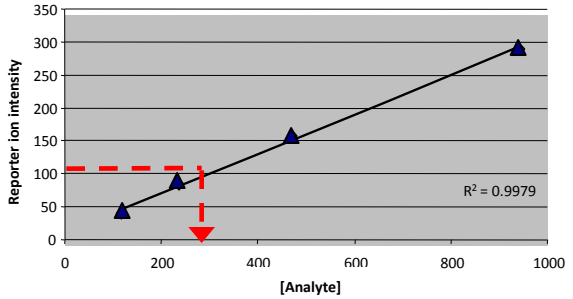
TMT^6-126

Label with TMT^6-126

Mix & analyse

Four point calibration curve generated from TMT Calibrator reporter ion intensities



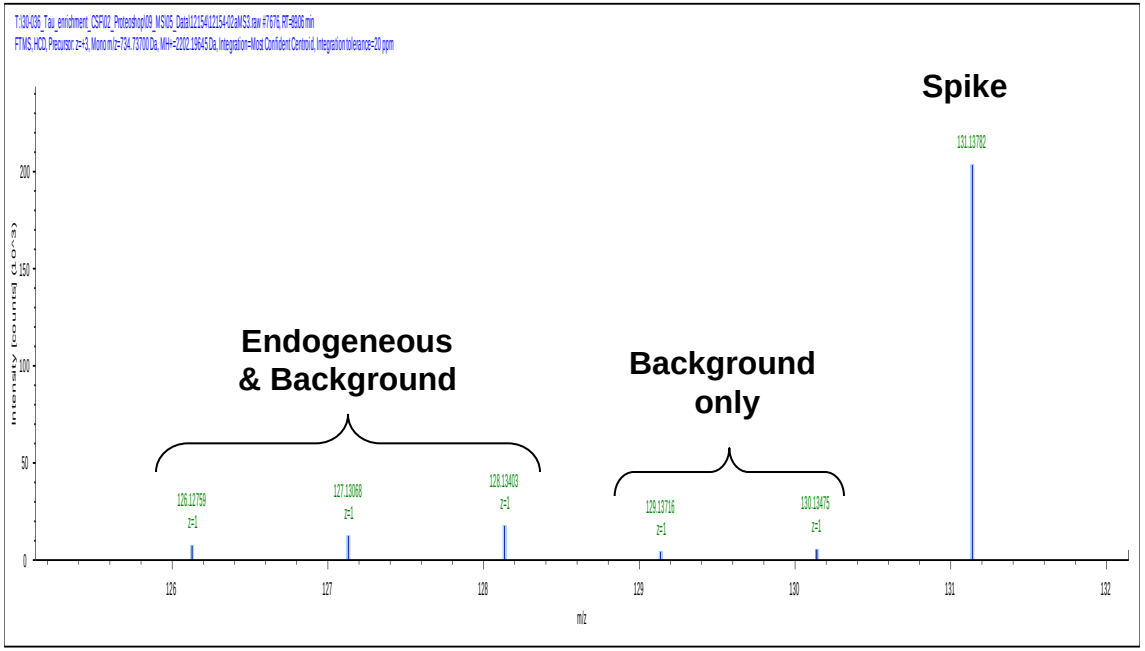


Using reference peptides to trigger MS/MS ?

- Analysis of TMT-labelled synthetic Tau reference peptides and phosphopeptides for method development
  - Confirm detection of phosphorylated tau peptides in buffer
  - Creation of inclusion list
- Analysis of CSF spiked with TMT-labelled synthetic Tau reference peptides and phosphopeptides
  - Use of spiked peptide and include list ensures precursor ions are amongst top 10 selected for MS/MS
  - For PoC study endogenous Tau peptides identified in same MS/MS spectrum as equivalent reference peptide spiked in at high concentration (2ng/ml)
  - Potential influence of contaminating peptides in MS/MS generating TMT reporter ions – switch to MS3 method

TMT^6-131 labelled Tau peptides spiked into CSF MS3 results

- 14 Phosphopeptides
- 10 unphosphorylated peptides



100ul CSF

CSF Tau Enrichment

- Low abundance of Tau in CSF requires enrichment prior to MS
  - Chromatography with depletion column
  - Perchloric acid precipitation*
- For ultra-sensitive detection use trigger concept
  - Isotopic or isobaric peptide spiking at high concentration
  - Using TMTcalibrator can make spike from multi-point standard curve
- Must use MS3 quantitation to ensure precision for ultra-low abundance species due to contaminant peptide co-isolation

*Portelius et al. 2008 J. Proteome Res. 7, 2114–2120

Summary

- We have demonstrated multiple isoforms of Tau in CSF
- Three unmodified peptides seen from 1ml CSF using Orbitrap
- SRM methods lack sufficient sensitivity for robust Tau quantitation in CSF
- TMTcalibrator has potential to detect and quantify multiple Tau phosphorylation sites and total Tau to deliver broadest diagnostic potential