PROMISE Proteomics solutions for targeted quantification of Tau protein by LC-MS/MS

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Introduction:

Alzheimer's disease (AD) is a common neurodegenerative disease affecting 35 millions patients worldwide. Microtubule-associated protein tau (Tau) concentration in cerebrospinal fluid (CSF) is a specific and sensitive biomarker for AD. As pathological Tau concentrations are very low (> 0.4 ng/mL), sample preparation must be efficient and Tau quantification must be absolute and accurate to obtain reliable results for preclinical and clinical samples. To achieve this goal, published studies[1] have promoted the use of a full-length stable isotope labelled Tau protein as the gold standard for quantification using LC-MS. Promise Proteomics offers a stable isotope labelled Tau-441 isoform protein standard and this poster presents data on both its characterization and its application in an LC-MS workflow for quantifying Tau in serum samples.

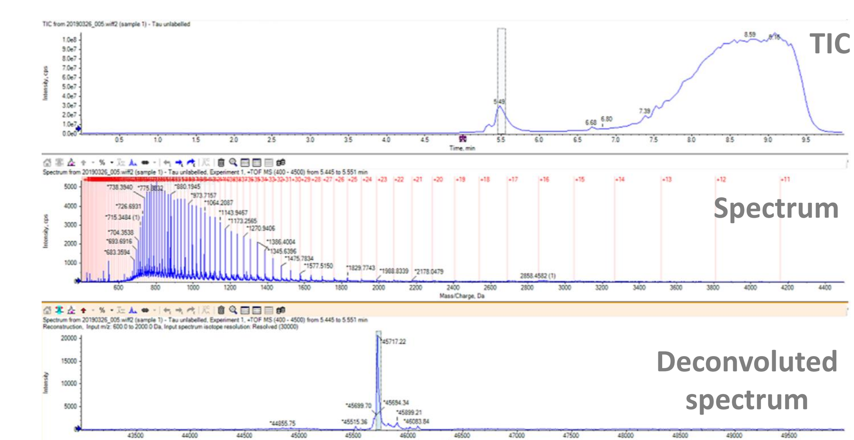
Characterization of the full length Tau-441 protein and its labelled ^{13}C, ^{15}N form

Both labelled and unlabelled full length Tau proteins were produced using an optimized *Escherichia coli* expression system. Produced proteins were analysed using standard quality control procedures: SDS-PAGE; BCA quantitation; intact mass analysis and peptide mapping (SCIEX X500B); isotopic incorporation (SCIEX 6500QTRAP).

Intact mass analysis

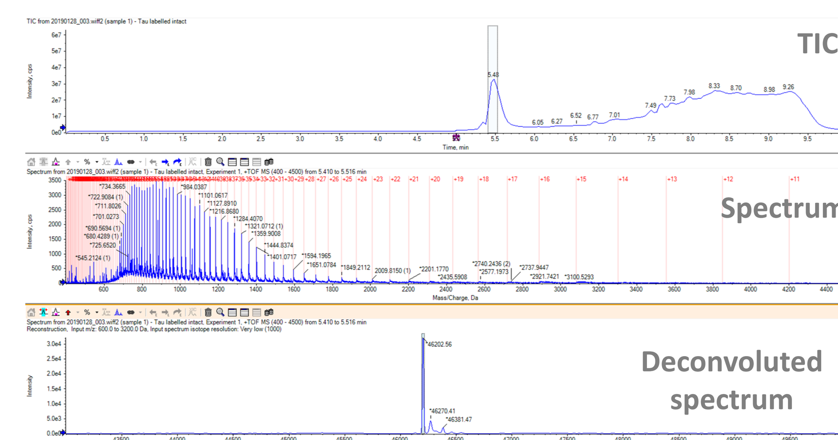
Unlabelled and **13C,15N labelled Tau** proteins were separately diluted to a final concentration of 0.1 mg/mL. Analyses were performed on a X500B QToF equipped with a Exion LC System (SCIEX). Intact proteins were separated using a BioZen 3.6 µm Intact C4 150 x 2.1 mm column (Phenomenex).

- Unlabelled Tau at 0.1 mg/mL



Observed mass (Da)	Expected mass (Da)	Mass deviation (Da)	Interpretation
45717.22	45849.91	132.69	Unlabelled tau 1-441 without Methionine

- Labelled Tau at 0.1 ng/mL



Observed mass (Da)	Expected mass (Da)	Mass deviation (Da)	Interpretation
46202.56	46338.35	135.79	Labelled tau 1-441 without Methionine

Peptide Mapping analysis and isotope incorporation

2 µg of unlabelled and labelled Tau protein were digested separately with trypsin. Digested samples were analyzed on a X500B QToF and 6500QTrap, both equipped with a Exion LC System (SCIEX). Peptides were separated using a BioZen™ 2.6 µm Peptide XB-C18, LC Column 100 x 2.1 mm (Phenomenex).

- Percentage coverage for labelled and unlabelled Tau : 62.4 % (275/441)

MAEPRQEFEVMEDHAGTYGLGDRKDQGGYTMHQDQEGDTDAGLKESPLQTPTEDGSEEPGSETSDAKSTPTAEDVTAP
LVDEGAPGKQAAAQPHTEIPEGTTAEEAGIGDTPSLEDEAAGHVTQARMVSKSKDGTGSDDKKAKGADGKTKIATPRGAA
PPGQKGQANATRIPAKTPPAPKTPPSSGEPPKSGDRSGYSSPGSPGTPGSRSRTPSLPTPPTREPKKVAVVRTPPKSPSSAKS
RLQTAPVPMPDLKNVKSKIGSTENLKHQPGGGKVQIINKKLDLSNVQSKCGSKDNIKHVPGGGSVQIVYKPVDLSKVTSKC
GSLGNIHHKPGGGQVEVKSEKLDFKDRVQSKIGSLDNITHVPGGGNKKIETHKLTFRENAKAKTDHGAEIVYKSPVVSGDTS
PRHLSNVSSTGSIDMVDSPQLATLADEVSASLAKQGL

Detected peptides were underlined.

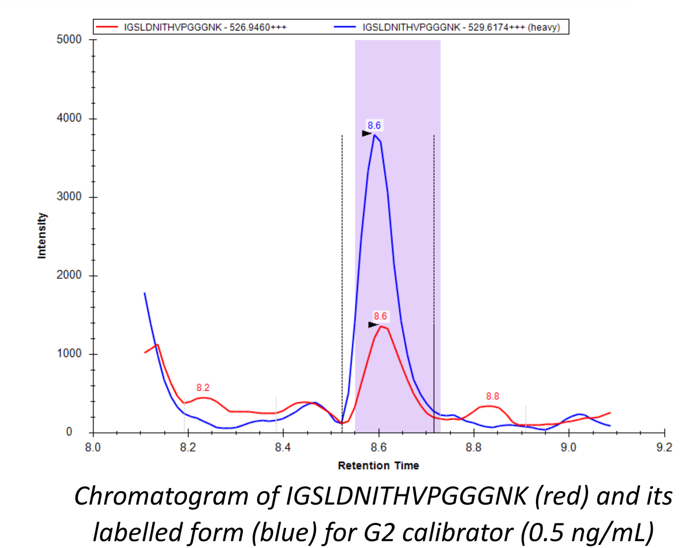
- ^{13}C, ^{15}N isotopic incorporation yield for labelled Tau : > 99%

Conclusions:

- Both Tau standards produced were pure.
- N-terminal Methionine was removed on both proteins
- Percentage coverage was high at 62.4%
- Isotopic incorporation for labelled Tau was > 99%.

Quantification of Tau protein

Seven calibrators were prepared between 0.1 - 10 ng/mL in 0.5% serum and spiked with 0.3 ng/mL of labelled Tau 1-441. As shown in Figure 1, Tau protein was extracted from artificial CSF (0.5% serum) by protein precipitation using perchloric acid 70% followed by solid phase extraction of the supernatant with StrataX Polymeric Reverse Phase. After trypsin digestion, Tau peptides were oxidized with H_2O_2 and analysed on Exion LC- QTRAP (Sciex). Tau peptides (Table 1) were separated on BioZen™ 2.6 µm Peptide XB-C18, LC Column 100 x 2.1 mm (Phenomenex). 3 analytical injections were performed.



Chromatogram of IGSLDNITHVPGGGNK (red) and its labelled form (blue) for G2 calibrator (0.5 ng/mL)

Figure 1

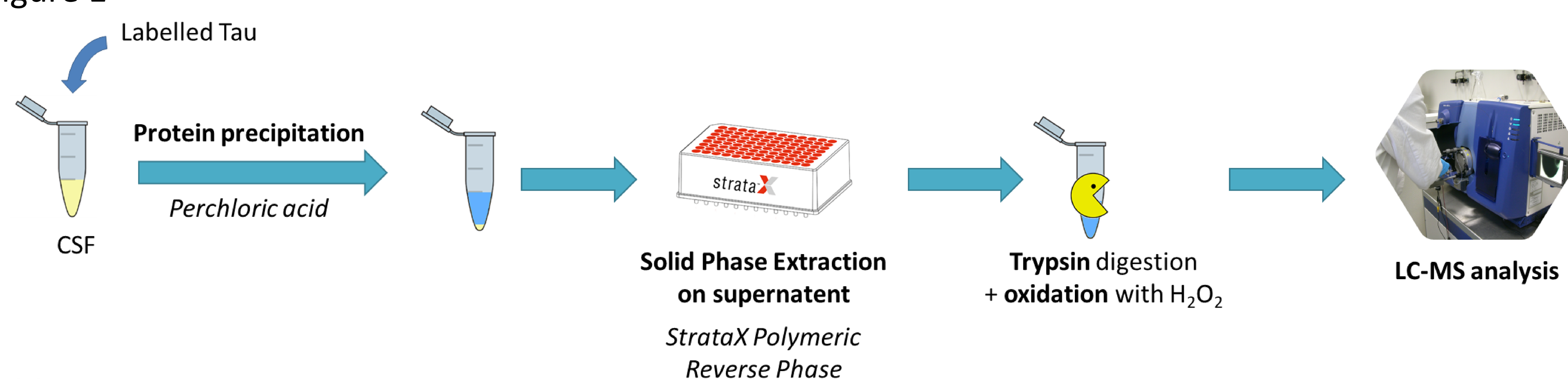


Table 1

Peptide	Precursor ion (m/z)	Charge	Retention time (min)	Collision energy (V)	Fragments
TPSLPTPPTR.light	533.798	2+	8.2	28.1	y_8^+, y_7^+, y_6^+
TPSLPTPPTR.heavy	538.802	2+	8.2	28.1	y_8^+, y_7^+, y_6^+
VQIINK.light	357.729	2+	7.3	21.7	y_4^+, y_3^+
VQIINK.light	361.736	2+	7.3	21.7	y_4^+, y_3^+
IGSLDNITHVPGGGNK.light	526.946	3+	8.6	26.3	y_6^+, y_{15}^{2+}, y_{14}^{2+}
IGSLDNITHVPGGGNK.heavy	529.617	3+	8.6	26.3	y_6^+, y_{15}^{2+}, y_{14}^{2+}
SPVVSGDTSPR.light	551.28	2+	7	28.7	y_8^+, y_7^+
SPVVSGDTSPR.heavy	556.284	2+	7	28.7	y_8^+, y_7^+

Figure 2

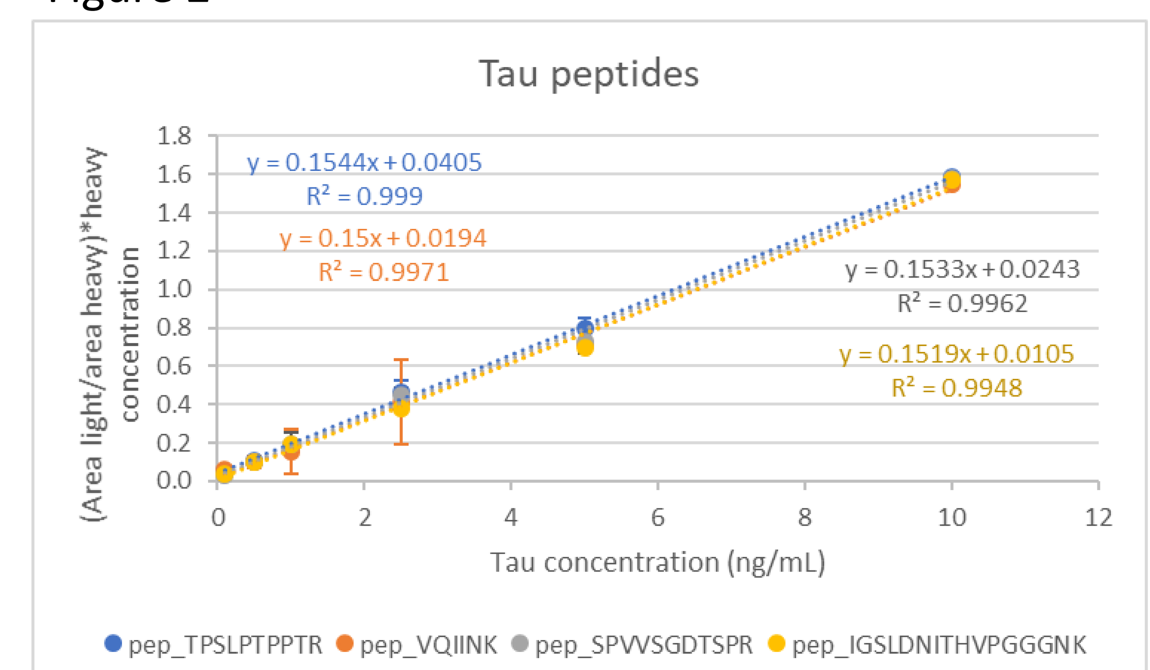


Table 2

Tau concentration (ng/mL)	[(Area light/area heavy)*heavy concentration] mean				CV (%)
	pep_TPSLPTPPTR	pep_VQIINK	pep_SPV…SPR	pep_IGS…NK	
0.1	0.05	0.06	0.03	0.04	24.60
0.5	0.11	0.10	0.10	0.10	4.87
1	0.19	0.15	0.19	0.19	10.03
2.5	0.46	0.41	0.44	0.38	8.67
5	0.80	0.71	0.73	0.69	6.17
10	1.58	1.54	1.58	1.57	1.17

Results:

Tau peptides TPSLPTPPTR, VQIINK, SPVVSGDTSPR and IGSLDNITHVPGGGNK gave consistent results with each other (Table 2). Calibration curves (Figure 2) were linear from 0.1 to 10 ng/mL and results were repeatable (RSD < 25% for LOQ (0.1 ng/mL) and RSD < 20% for calibrator points).

Conclusion:

Combining Promise Proteomics labelled Tau internal standard with this sample preparation procedure allowed quantification of Tau protein at high sensitivity and this can be applied to pre-clinical or clinical samples.

[1] McAvoy T et al. (2014) Quantification of tau in cerebrospinal fluid by immunoaffinity enrichment and tandem mass spectrometry. *Clin Chem*. **60**, 4
Bros P, et al. (2015) Antibody-free quantification of seven tau peptides in human CSF using targeted mass spectrometry. *Front Neurosci* **9**, 302.
Barthélémy NR, et al (2016). Differential Mass Spectrometry Profiles of Tau Protein in the Cerebrospinal Fluid of Patients with Alzheimer's Disease, Progressive Supranuclear Palsy, and Dementia with Lewy Bodies. *J Alzheimer's Dis* **51**, 4.

Authors

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