

# LCMS methods and traceability of CSF biomarker measurements

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Le progrès, une passion à partager



# Reliability of medical tests is a major public health challenge

- ❖ 60 to 70% of medical decisions are based on an in-vitro diagnostic test
- ❖ Results are not always traceable to internationally recognized references

❖→ **Results may depend on the method used!!**



❖→ **Consequences:**

- 1) **Patient's Health**: risk of inappropriate medical decision
- 2) **Economical**: repetition of measurements - 25 to 30% of costs are due to test repetitions, prevention and error detection instead of diagnostic itself (15-30 billion \$ / year in the US)
- 3) Lack of reliable data for **epidemiological studies and clinical trials**

## Reform of medical biology in France

By 2016-2020, accreditation according to ISO 15189 is mandatory for ALL clinical laboratories (both public and private)

## In vitro diagnostic Directive on medical devices 2017/746

« The metrological traceability of values assigned to calibrators and/or control materials shall be assured through suitable reference measurement procedures and/or suitable reference materials of a higher metrological order »

**LNE's mission : help clinical labs & IVD industrials to meet regulatory requirements regarding metrological traceability of results**

- ✓ Development of reference methods for the main biomarkers used in clinical biology : creatinine, glucose, HbA1c, TCh, LDL-C, HDL-C, TG, ...
- ✓ Production of Certified Reference Materials
- ✓ Assignment of reference values to calibration & quality control materials

## EMPIR Call 2015. Call Scope – Metrology for Health (2015)



*Innovative measurements for improved diagnosis and management of neurodegenerative diseases*

Project Coordinator:  
LGC

### ***WP3: Establishing traceability of AD and PD biomarkers measurements***

➔ *Fit for purpose* reference methods and materials for the measurements of tau protein (AD) and  $\alpha$ -synuclein (PD) in CSF

## Terms of Reference

- ❖ To develop an international reference material for cerebrospinal fluid (CSF)

## Current Projects

- ❖ Collection of CSF material
- ❖ Preparation of the reference material
- ❖ Establishment of reference methods for the key measurands for assignment of values to the reference material

**Calibration of immunoassays for Tau quantification**

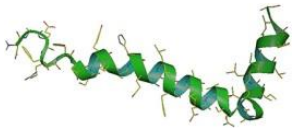


# Principal Biomarkers of Alzheimer's disease

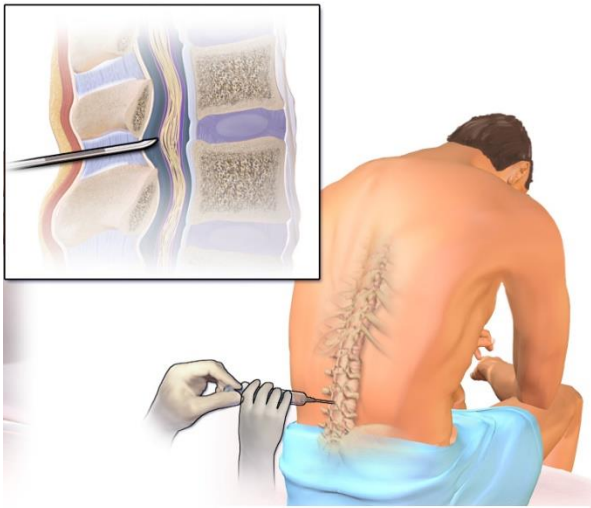
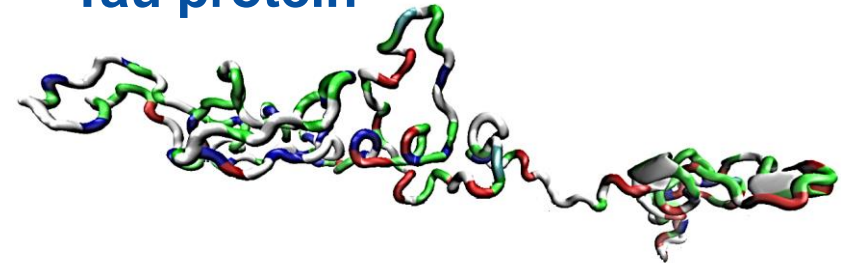
**Alzheimer Disease:** deposit of plaques and tangles in the brain



## Peptide $a\beta$ -42



## Tau protein

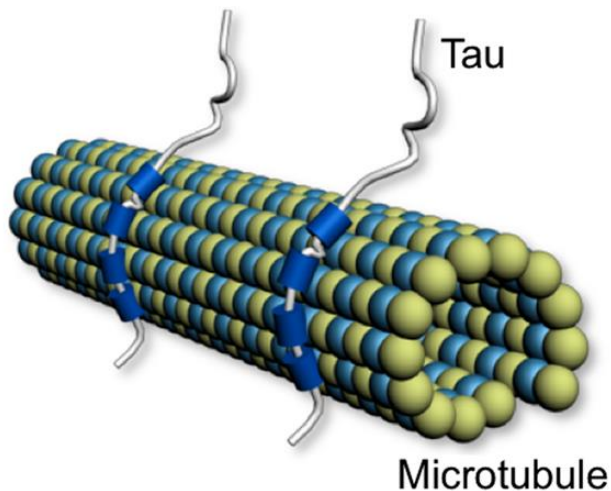


### Why measurements matter?

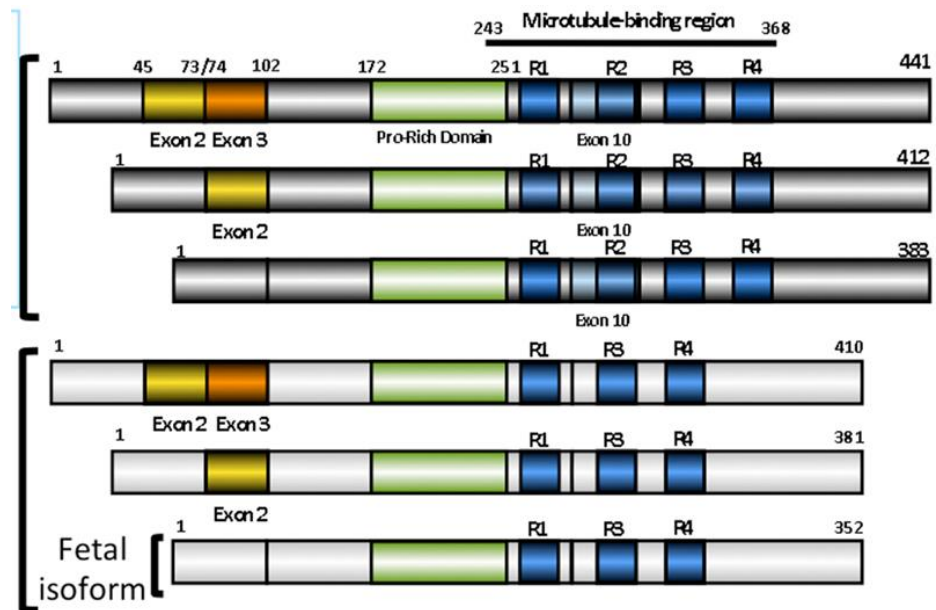
1. Early diagnosis
2. Monitoring of disease progression
3. Monitoring of therapeutic effects (e.g. clinical trials)

## Tau (46 kDa):

- Exists in CSF as 6 isoforms of varying length
- Contains many PTMs, especially phosphorylations.
- Low concentration in CSF (around 5 pM).



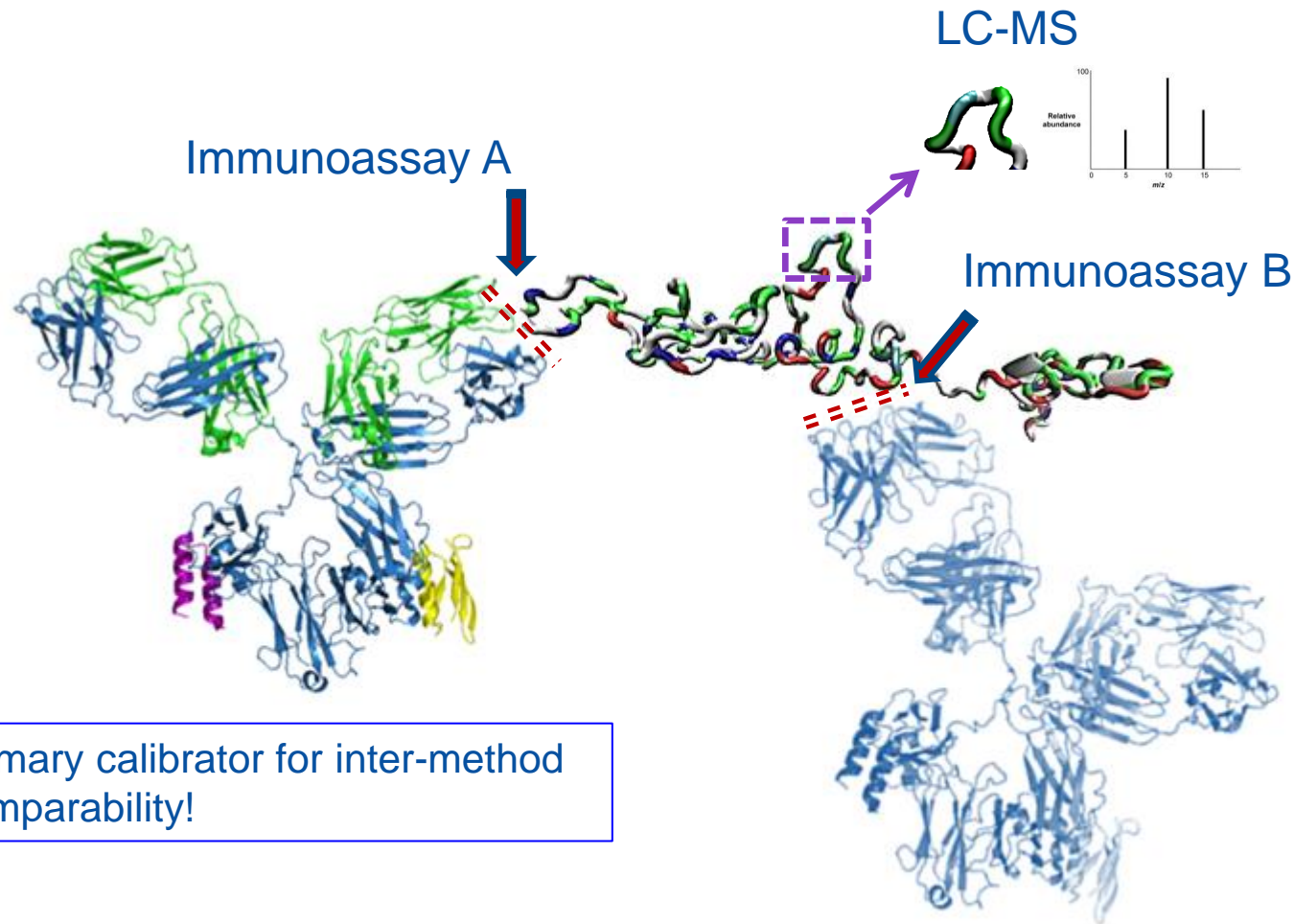
- Lack of inter-method comparability (immunoassays and LC-MS)



# The importance of the definition of the measurand

## Tau quantification: definition of the measurand

### Routine immunoassays vs. LC-MS



- Primary calibrator for inter-method comparability!

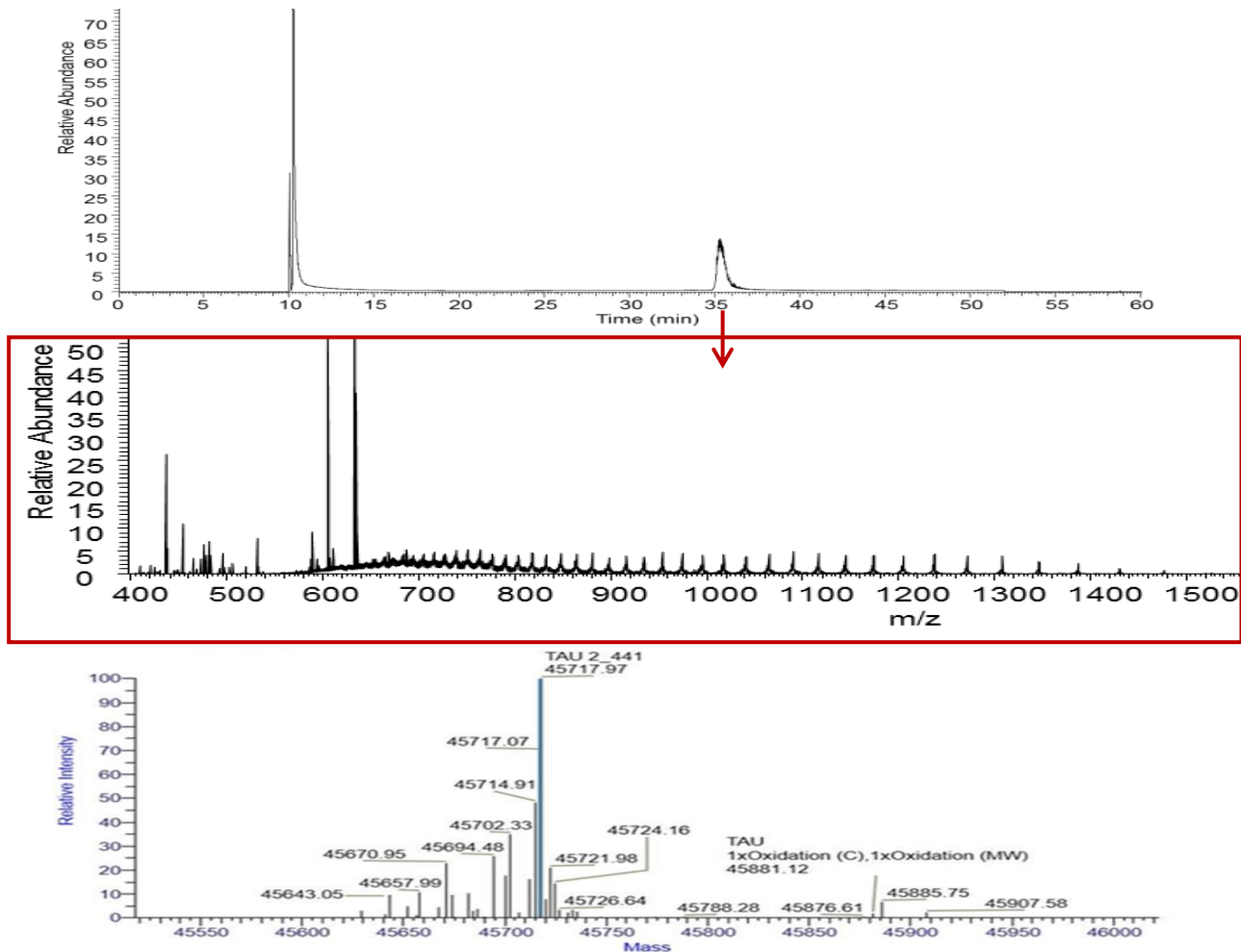


# Tau LC-MS reference method

## SI-traceable primary calibrator: recombinant Tau 441



### 1) Intact mass analysis and impurity profile by high resolution mass spectrometry



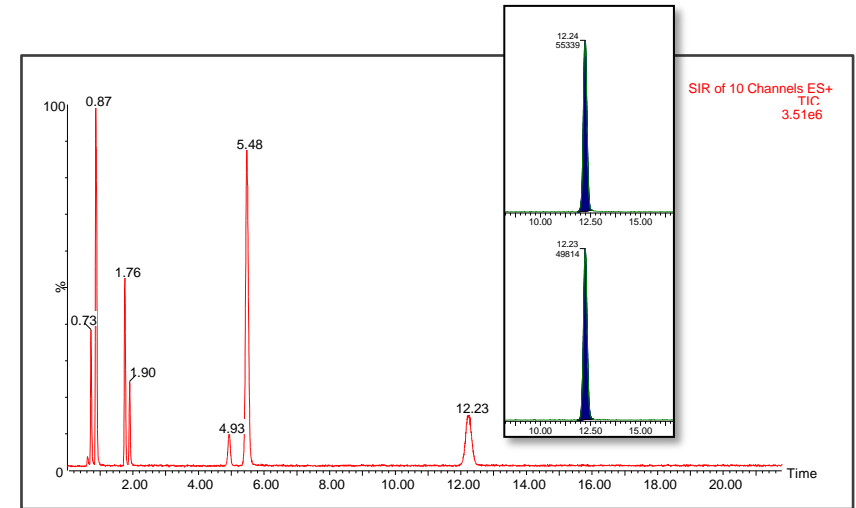
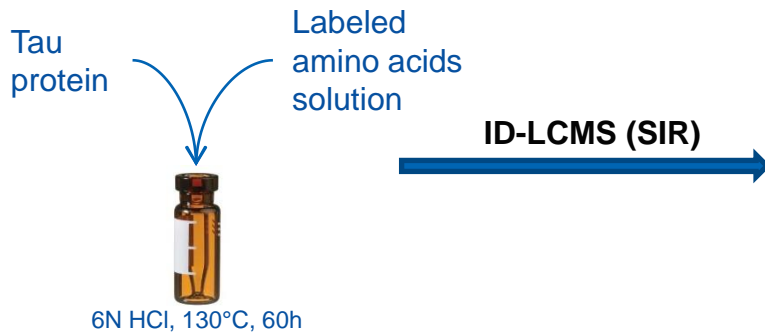
# Tau LC-MS reference method

## SI-traceable primary calibrator: recombinant Tau 441

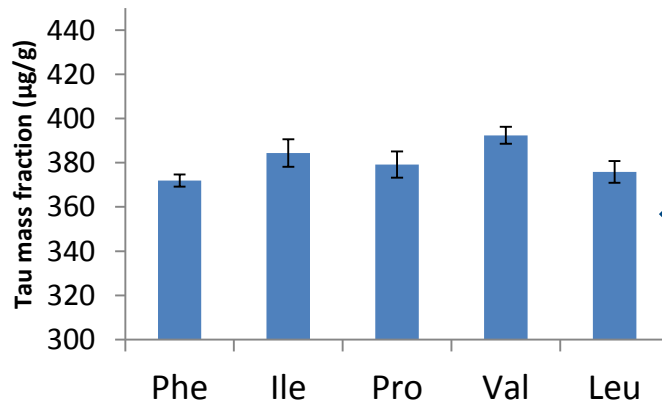
### 2) Tau mass fraction by amino acid analysis ID-LCMS

Peak areas extraction for labelled and unlabelled aminoacids

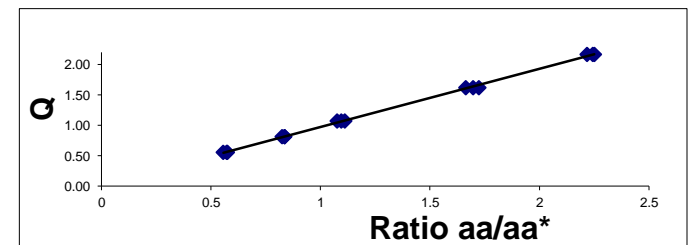
#### Protein hydrolysis



#### Amino acid analysis – Recombinant Tau



#### Standardization curve



# Tau LC-MS reference method

**Measurand:** peptide GAAPPGQK (156-163)

## 1. Protein precipitation

500 µL CSF + Labelled Tau

25 µL perchloric acid (70%)

Centrifugation

50 µL 1% TFA on supernatant

## 2. SPE purification

## 3. Digestion

Reconstitution in Trypsin 1 µg/mL+ **labelled Internal Standard**

Incubation at 37°C for 24h

Stop digestion 10% formic acid

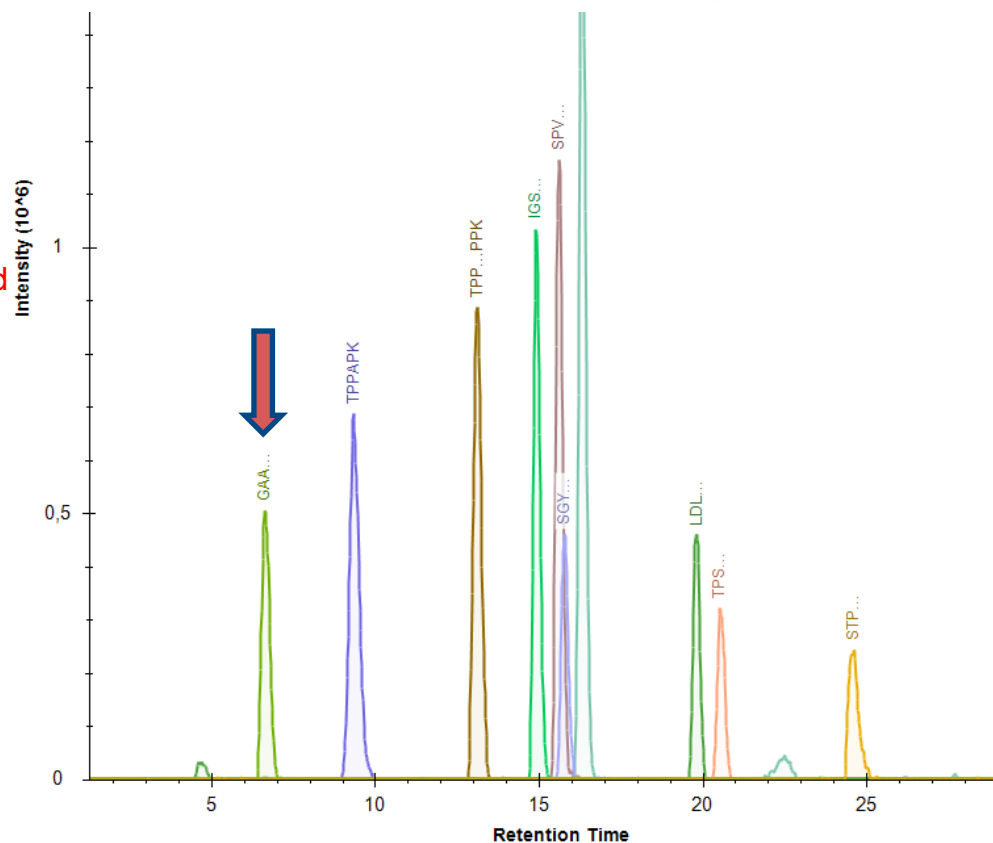
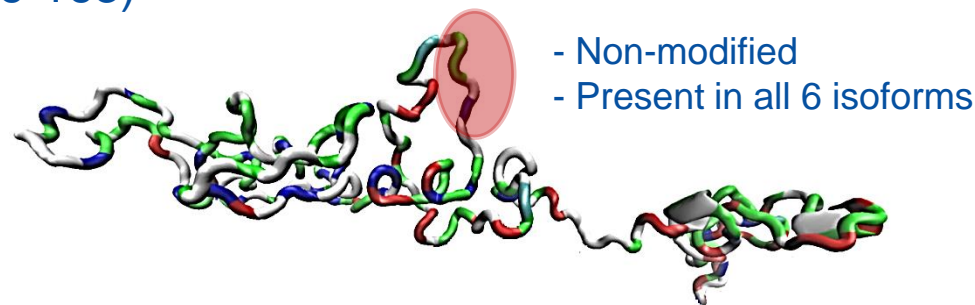
## 4. LC-MS<sup>2</sup>

UPLC separation (C18)

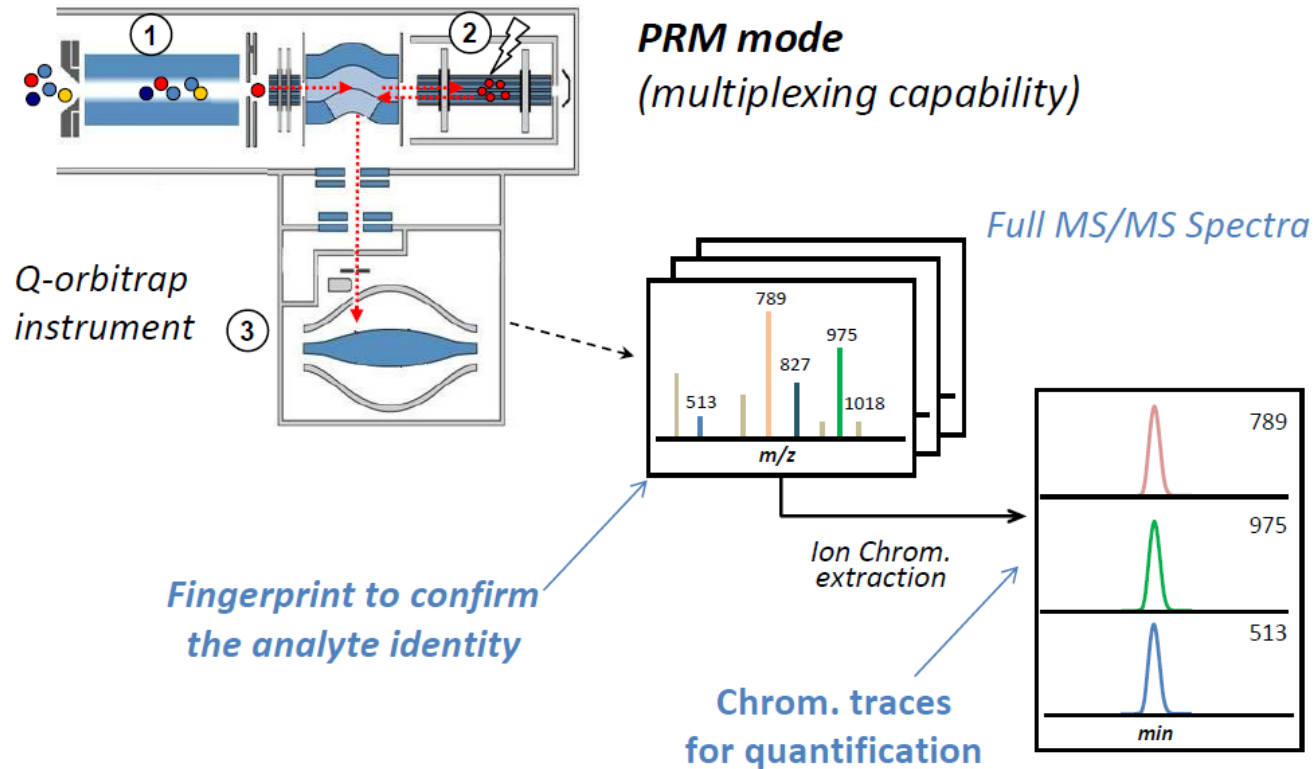
Detection in a Thermo Q-Exactive MS  
(quadrupole orbitrap in PRM mode)

Bros et al. 2015

*Antibody-free quantification of seven tau peptides in human CSF using targeted mass spectrometry*



# Tau LC-MS reference method



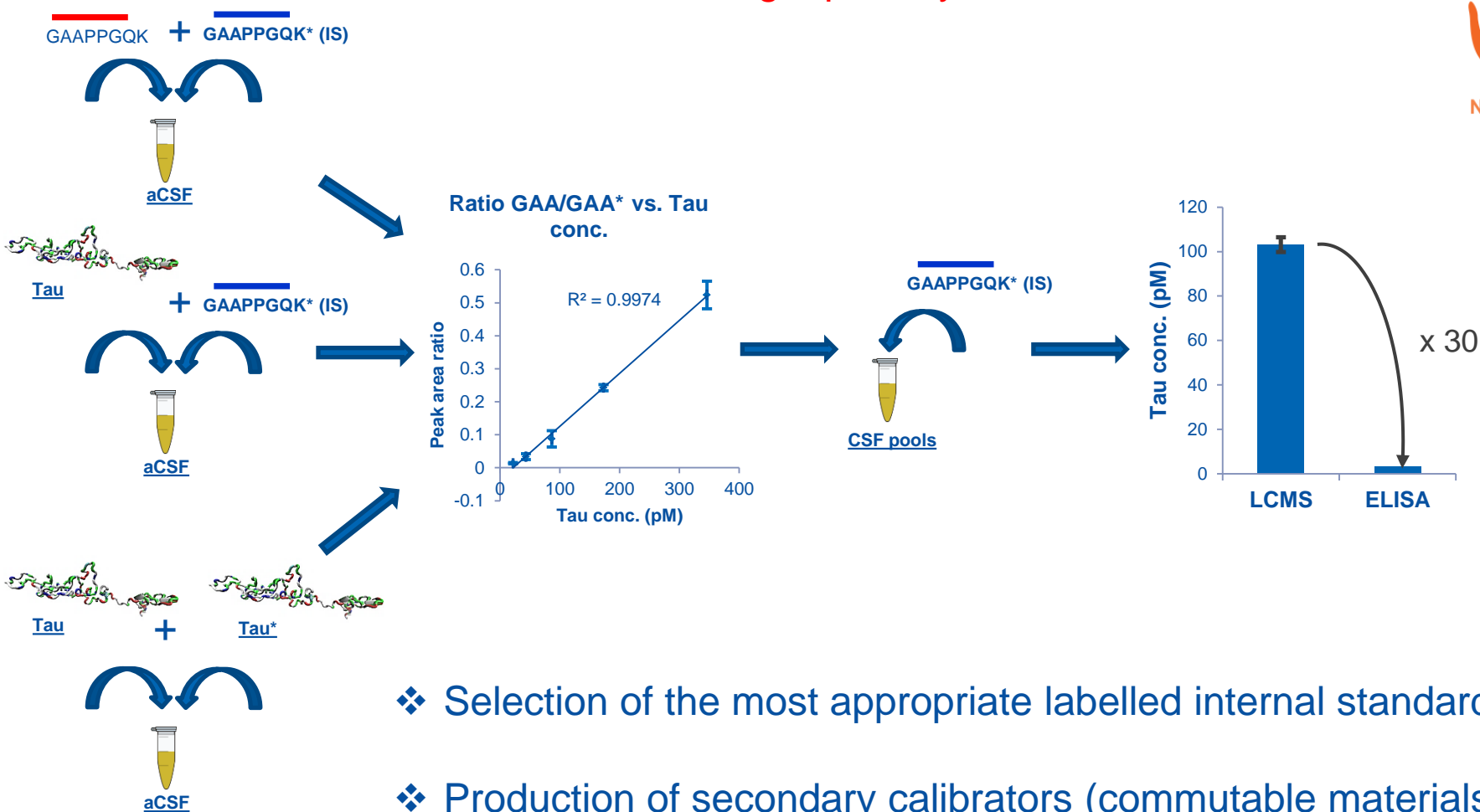
Kim et al., *Proteomics Clin. Appl.* 2013

- ❖ Quantification of the target protein in a complex matrix by exclusively following its peptides and their transitions in predefined m/z and retention time ranges
- ❖ Fragments detection at high resolution



# Tau LC-MS reference method

## Quantification using a primary calibrator



- ❖ Selection of the most appropriate labelled internal standard
- ❖ Production of secondary calibrators (commutable materials)
- ❖ Metrological calibration of immunoassays

# Next steps

- ❖ Primary calibrator
- ❖ Validation of the reference measurement procedure
- ❖ Secondary calibrators
- ❖ Commutability study in 2018



The EMPIR initiative is co-funded by the European Union's Horizon 2020 research and innovation programme and the EMPIR Participating States



- ❖ Submission of a new EMPIR project on NDD diseases (beginning 2018)
- ❖ Any suggestions? Partners?

# Conclusions: take-home messages...

- ❖ The importance of the measurand definition: ELISA vs MS
- ❖ Difficulty of validation of the reference measurement procedures: sensitivity problems, structural heterogeneity, matrix complexity, small sample volumes
- ❖ Importance of industrial cooperation

## NeuroMET

[www.lgcgroup.com/EMPIR-neuromet](http://www.lgcgroup.com/EMPIR-neuromet)



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