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SI traceable RMPs and CRMs are essential to underpin a worldwide standardisation of results, thus facilitating regulatory approvals of new assays and their implementation in clinical practice

NEuroBioStand is a European consortium funded within the EPM Health call 2022, aimed at developing a metrological research framework for standardizing biomarkers of neurodegenerative diseases (NDDs), ensuring equivalent results among different measurement procedures, and facilitating the establishment of common reference intervals and cut-off values. In clinical chemistry, the highest level of standardization for end-user measurement procedures is achieved through the development of reference materials (RMs) and/or reference measurement procedures (RMPs) to establish a calibration hierarchy to the International System of Units (SI). This work is accomplished through tight collaboration among NMIs/Dis, academics, clinicians, and industry, under the umbrella of the IFCC WG-BND.



P-tau measurements in plasma

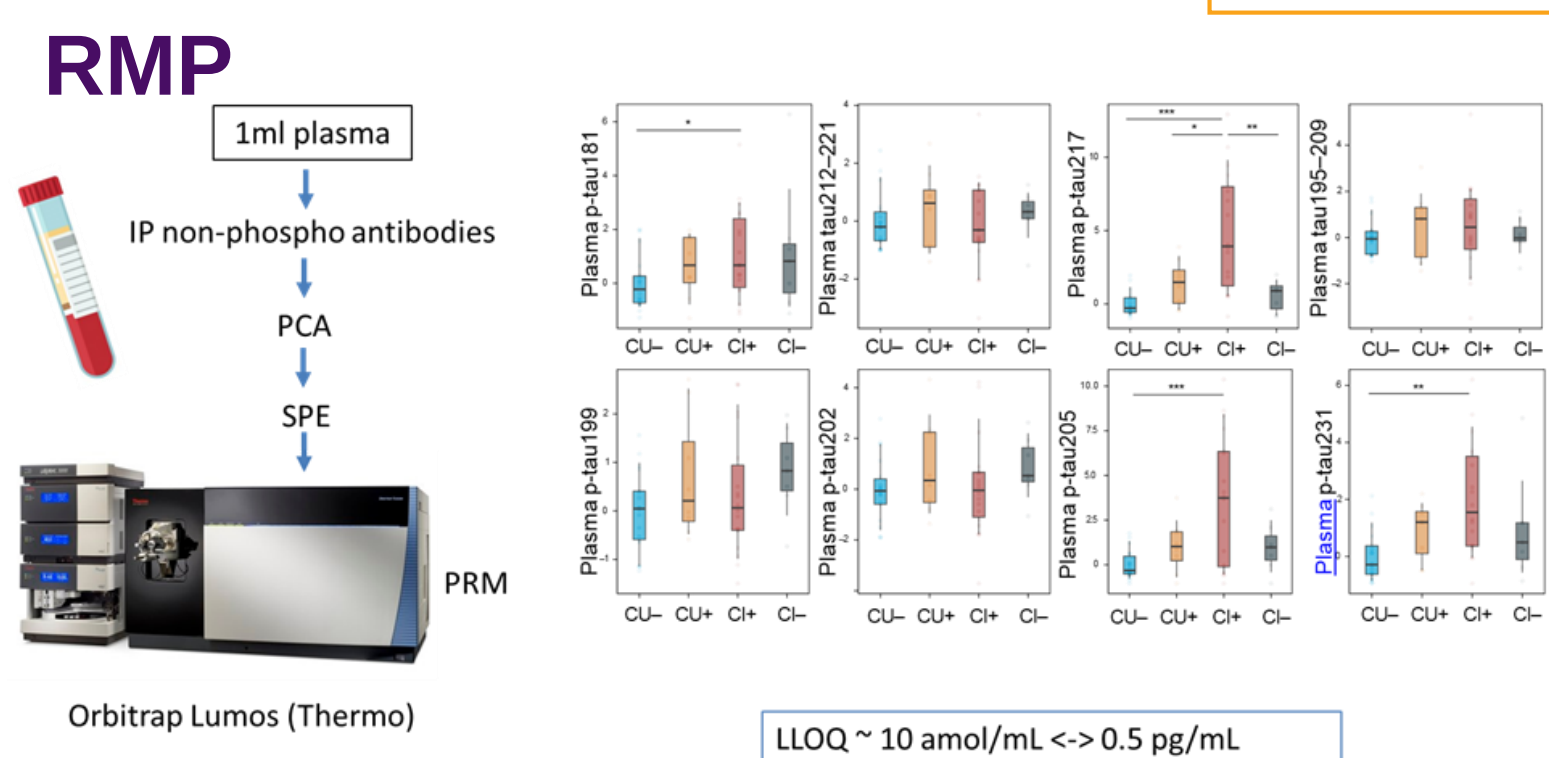
P-tau is a promising biomarker for Alzheimer's disease. Its measurement in plasma constitute a breakthrough for early diagnosis and patients stratification in clinical trials. The first step in standardising this biomarker is defining the measurand, focusing on specific phosphorylated isoforms, including P-tau181, P-tau217, and P-tau231.

Development of phosphorylated calibration materials

Phosphopeptide primary calibrators have been developed. A protein primary calibrator for tau was already available within the consortium (1) and a recombinant GSK-3β-phosphorylated Tau protein is also under investigation to assess its suitability as primary calibrator for the different P-tau isoforms. In particular, its purity (a), mass fraction (b) and the site occupancy (c) (% of phosphorylation sites actually modified) are under investigation.

P-tau181 peptide	TPPAKIPSPSGEPK
P-tau217 peptide	TPSLPTPTTR
P-tau231 peptide	VAVVRIPK
P-tau231 peptide	VAVVRIPKSPSSAK
P-tau231 peptide	KVAVVRIPKSPSSAK
Recombinant protein	Tau-441
Recombinant protein	GSK-3β-phosphorylated Tau-441

Site occupancy is around 0.3% for the phosphosites 181 and 217



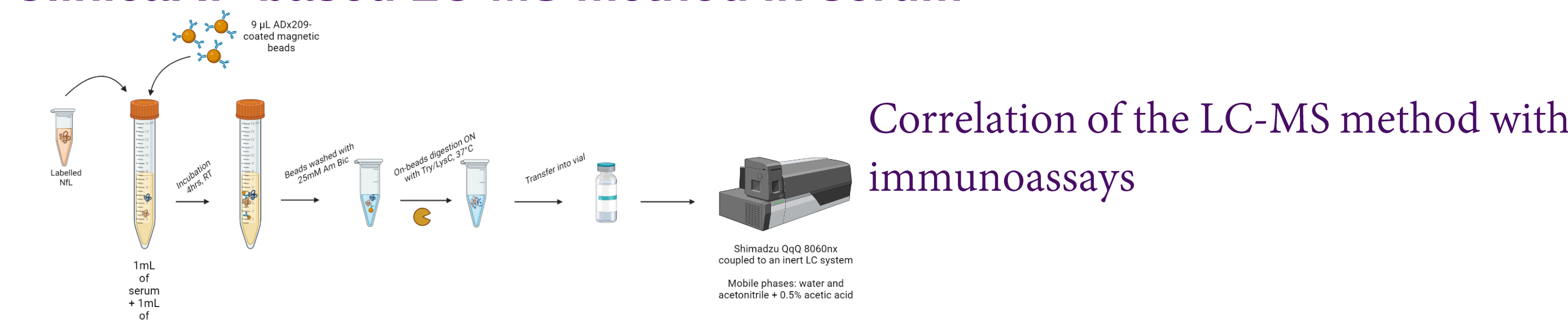
[Concentration of P-tau] = 1711,9 ± 90,4 µg/g (k=2) [5,3%]

The sample preparation workflow is based on an IP protocol (2): gravimetric sample preparation and the spiking of the labelled internal standards are being included. The candidate RMP will certify P-tau concentration in pilot matrix-based reference materials.

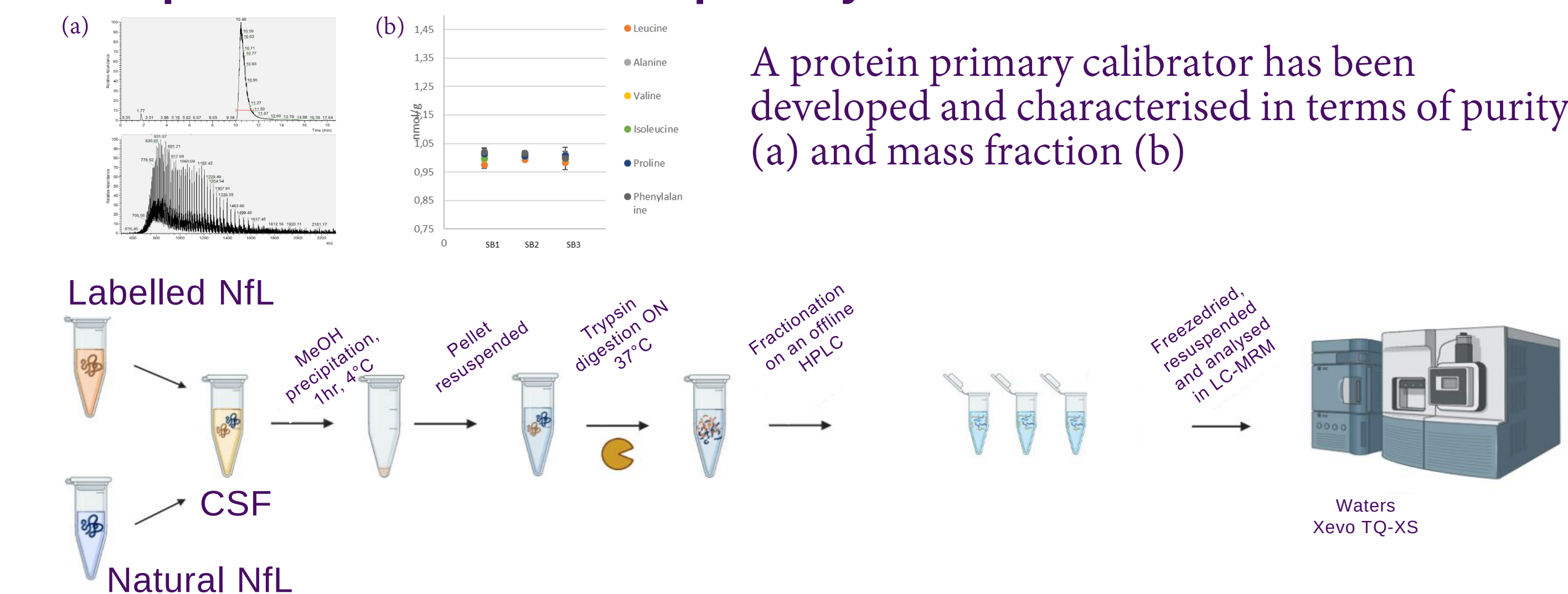
Standardisation of NfL in blood derivatives

Neurofilament light chain (NfL) has been established as a robust biomarker for neuroaxonal damage in various neurological conditions.

Clinical IP-based LC-MS method in serum

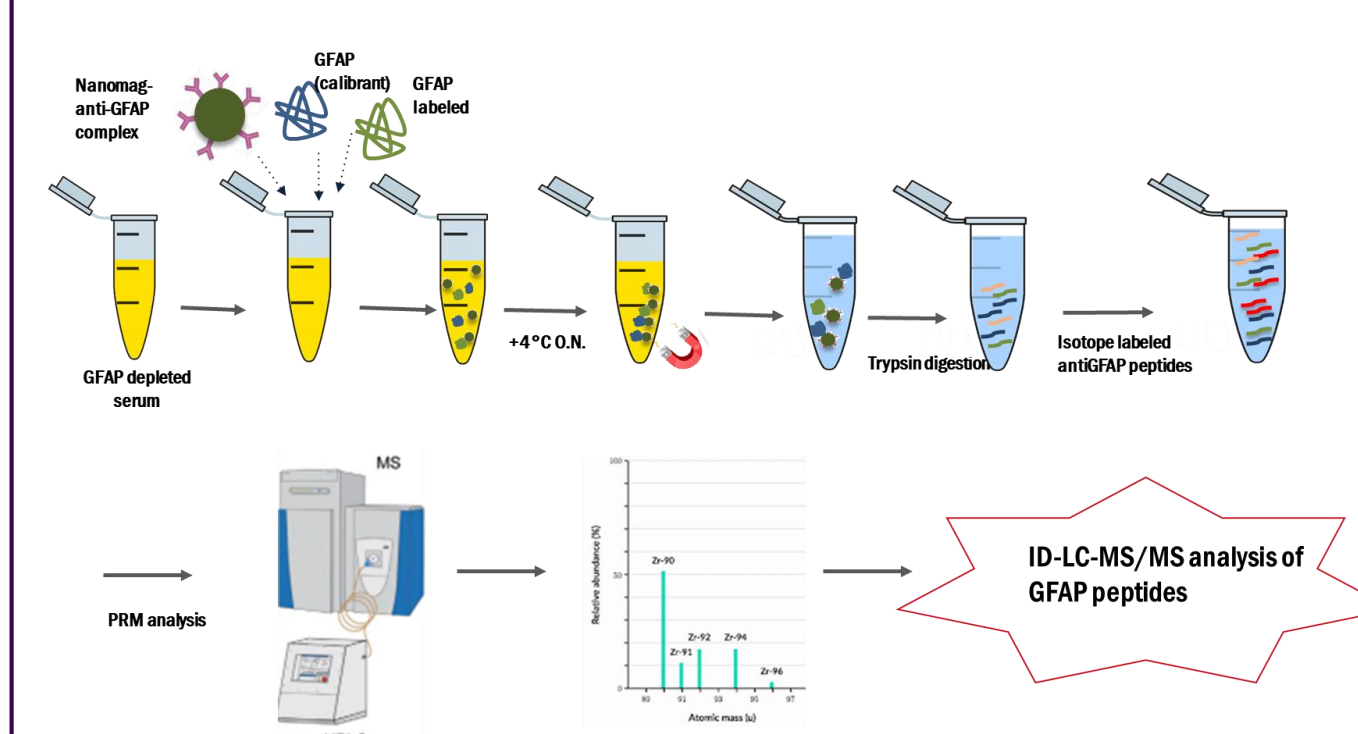


Development of a SI-traceable primary calibrator and RMP

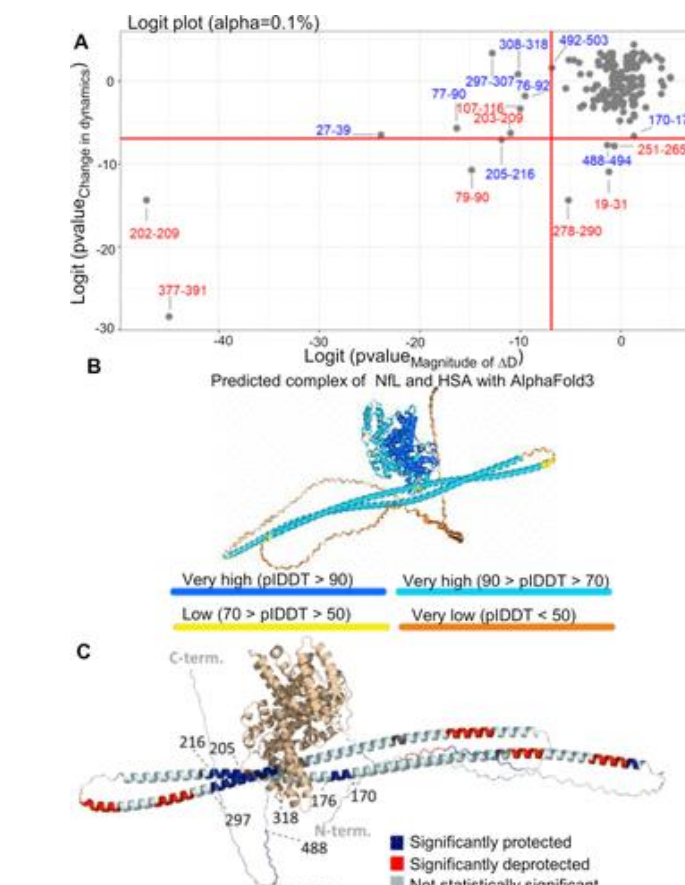


An antibody-free method is under validation in CSF. Samples spiked with CSF have already shown high commutability (3) and the RMP in CSF will be used to certify CRMs.

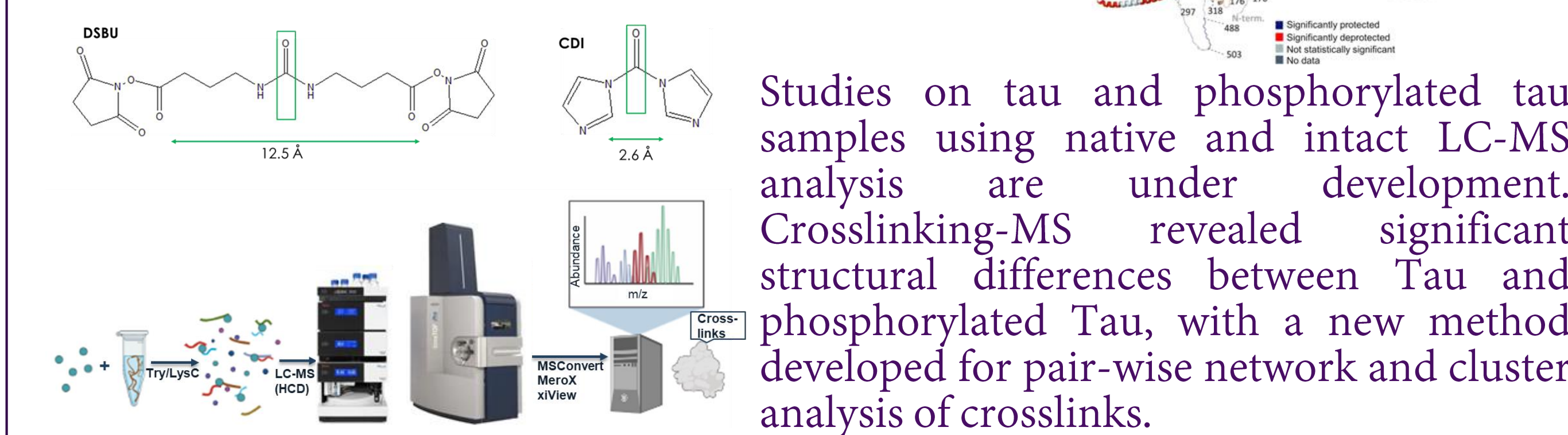
Emerging biomarkers and novel approaches for protein structural conformation determination



The consortium has selected GFAP as an emerging biomarker candidate to promote its implementation in clinical practice by developing an LC-MS method to evaluate the feasibility for SI-traceable quantification in biological fluids.



HDX-MS approach revealed that NfL has a conformational heterogeneity and adopts different conformations in various environments. Interactions with human serum albumin (HSA) were identified, and higher-order structures such as dimers and tetramers were observed (4).



Outcomes for industrial and other users communities

- Metrologically validated methods and certified reference materials
- Regulatory approval of IVD devices
- Implementation of emerging biomarkers in clinical practice
- Metrological approaches for protein structural analysis to better select therapeutic targets in clinical trials

Outcomes for the metrological and scientific communities

- New metrological approaches for PTMs
- Better understanding of the protein conformational analysis and of the impact of three-dimensional structure on pathological mechanisms
- Wide impact on proteomics/mass spectrometry community through the development of ultra-sensitive methods for biomarkers analysis

Outcomes for relevant standards

- IFCC, GBSC, Society for CSF analysis, JCTLM, CCQM, ISO committees

Wider Impact

- Global Access to not invasive, automated, cost-effective, time-effective and certified tests
- Accurate biomarker measurements to conclude on the efficacy of clinical trials
- Facilitation of the European agencies' decisions on drug marketing applications
- Accurate measurements to be used in urgent care units
- Early stage diagnosis of dementia in a culturally appropriate and gender inclusive manners and for all patients without regard to their economic condition and wherever they live

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