

Metrology for biomarker standardisation in neurodegenerative diseases

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Introduction

Accurate quantification of protein biomarkers in neurodegenerative diseases (NDDs) is essential for reliable disease diagnosis and monitoring. Standardised methods are crucial to ensure comparability across laboratories. However, the complexity and heterogeneity of NDDs make biomarker standardisation challenging, particularly due to the structural variability of key proteins like amyloid, tau, α -synuclein (α -Syn), and neurofilament light chain (NfL).

This poster highlights the NML's efforts in developing reference measurement procedures (RMPs) and materials based on isotope dilution mass spectrometry (ID-MS), as well as its structural protein characterisation capabilities. These efforts are supported by participation in key consortia such as NeuroMET and NEuroBioStand, strong networks with academia, industry, and hospitals, and engagement with the IFCC-WG-BND, supporting both clinical and research applications.



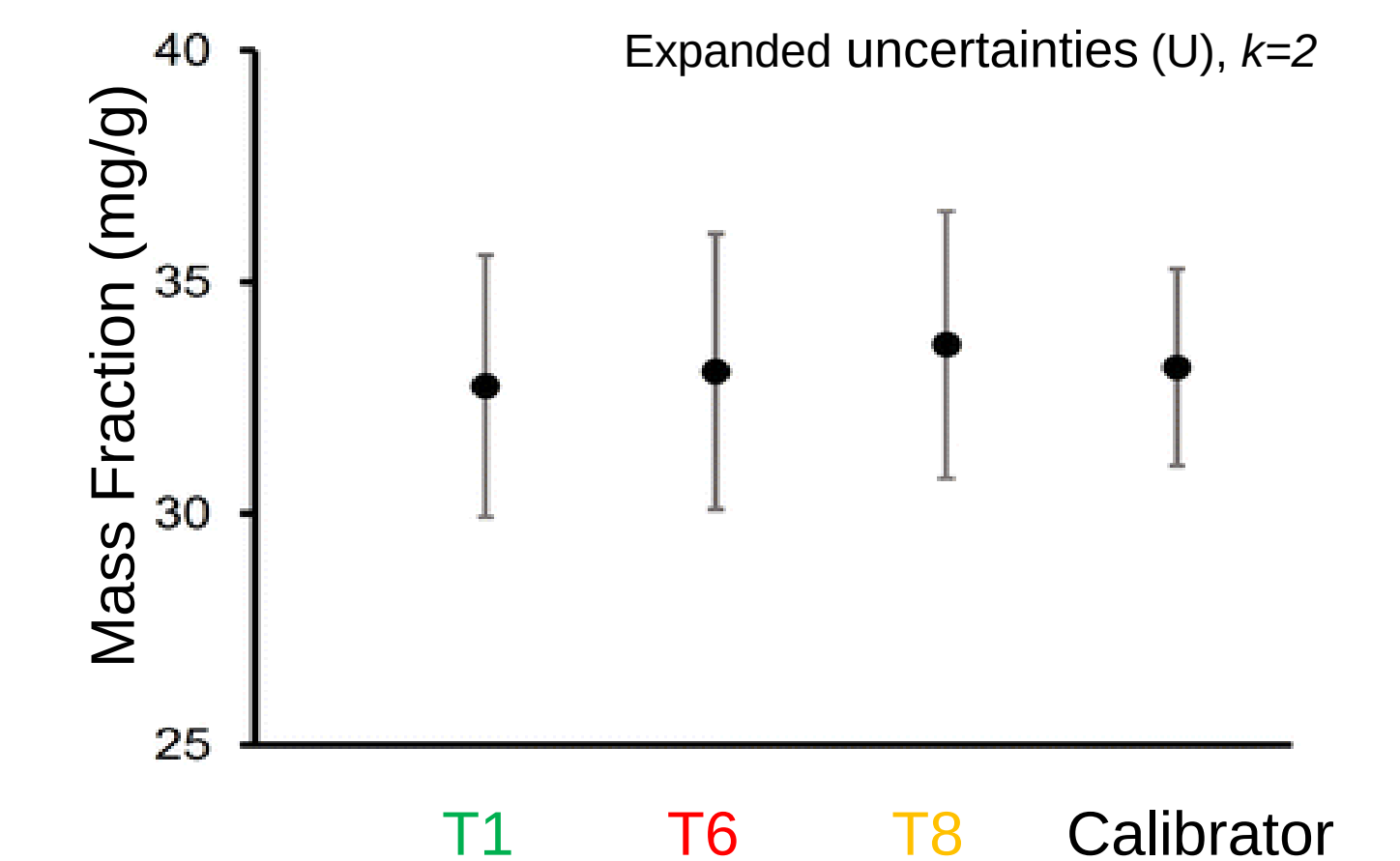
Standardisation of α -Syn measurements

Sequence of α -Syn and monitored peptides

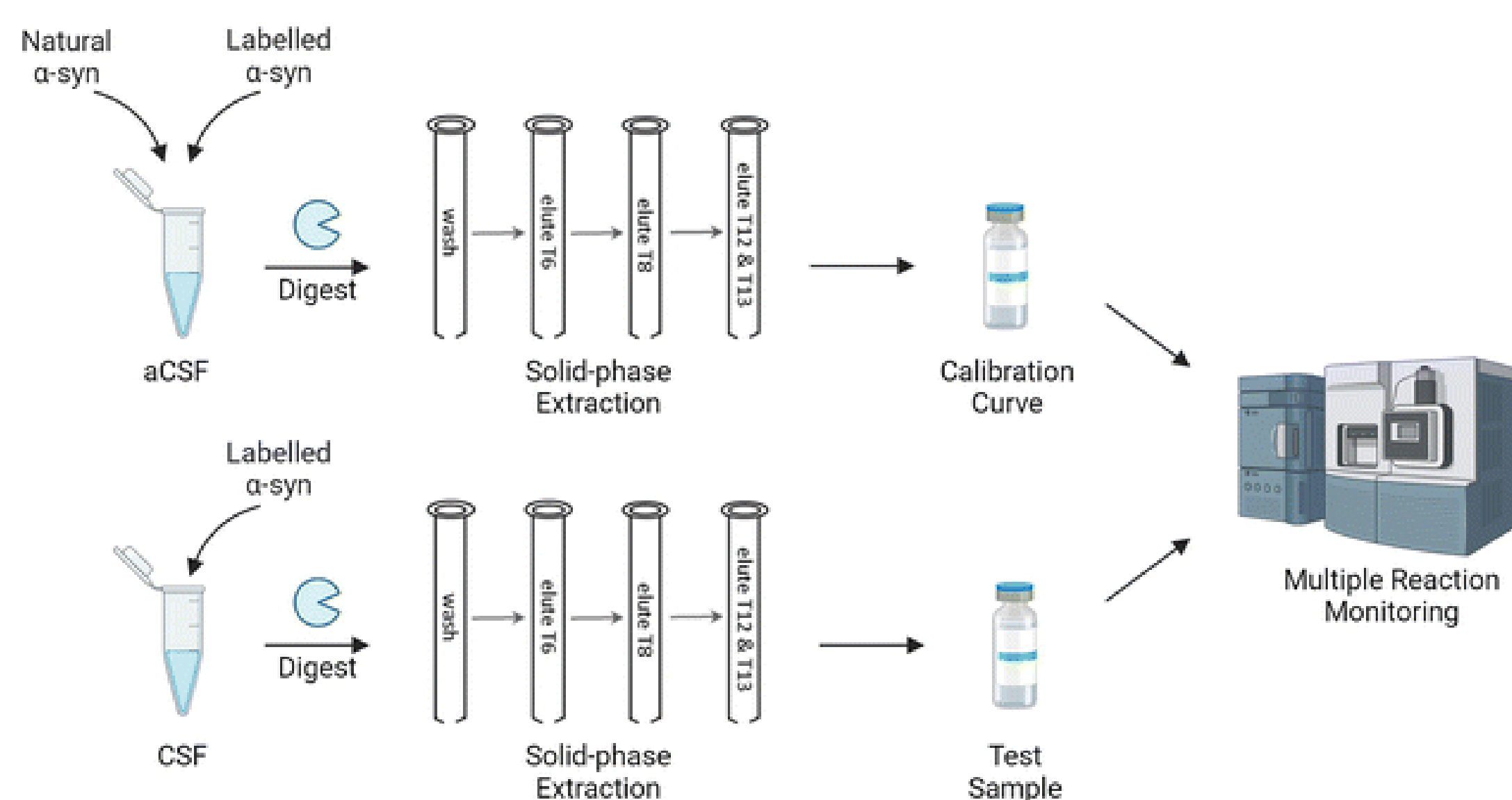
T1, T6, T8, T12, T13:

MDVFMKGLSKAKEGVVAAAEKTKQGVAAAGKTKEGVLYVGSKTKEGV
VHGVATVAEKTKEQVTNVGGAVVTGVTAVAQKTVEGAGSIAAATGFVKK
DQLGKNEEGAPQEGILEDMPVDPDNEAYEMPSEEGYQDYEPEA

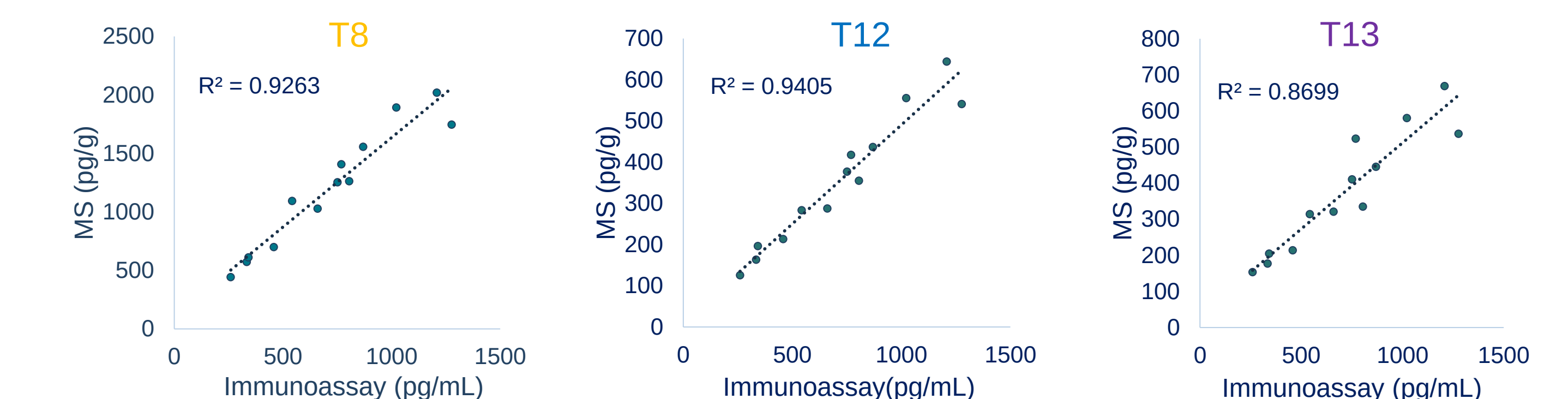
α -Syn primary calibrator
quantification using T1, T6, T8
peptide calibrators by ID-MS



Sample preparation and analysis workflow for the candidate RMP of α -Syn quantification in cerebrospinal fluid (CSF):



Analysis of 15 CSF samples from Charite University hospital by immunoassay and developed candidate RMP:

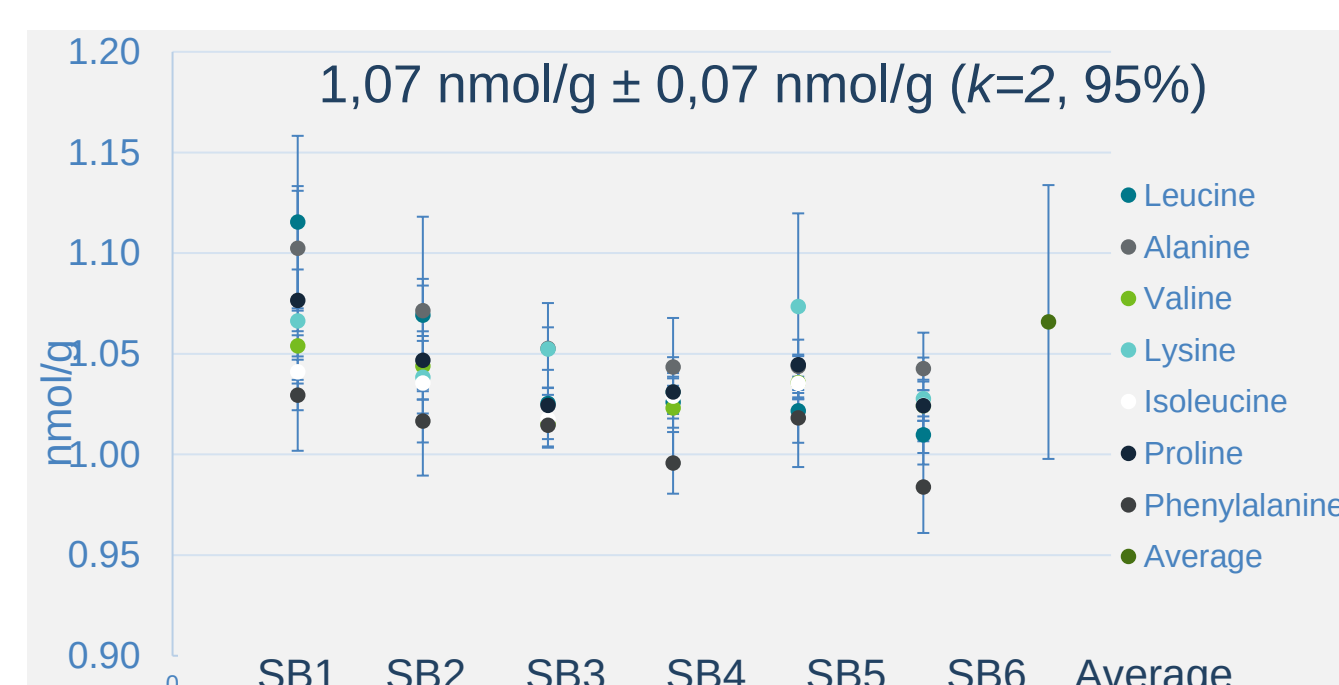


- α -Syn samples were quantified by IDMS using the lower range of calibration curves (total range: 0.1 ng/g–10 ng/g, and a linearity (r^2) of 0.996–0.999)
- Quantified T12 and T13 amounts are lower while T8 amount is higher compared to immunoassay results
- T12+T13 is specific for α -Syn, whereas T8 is present in α -Syn and β -Syn

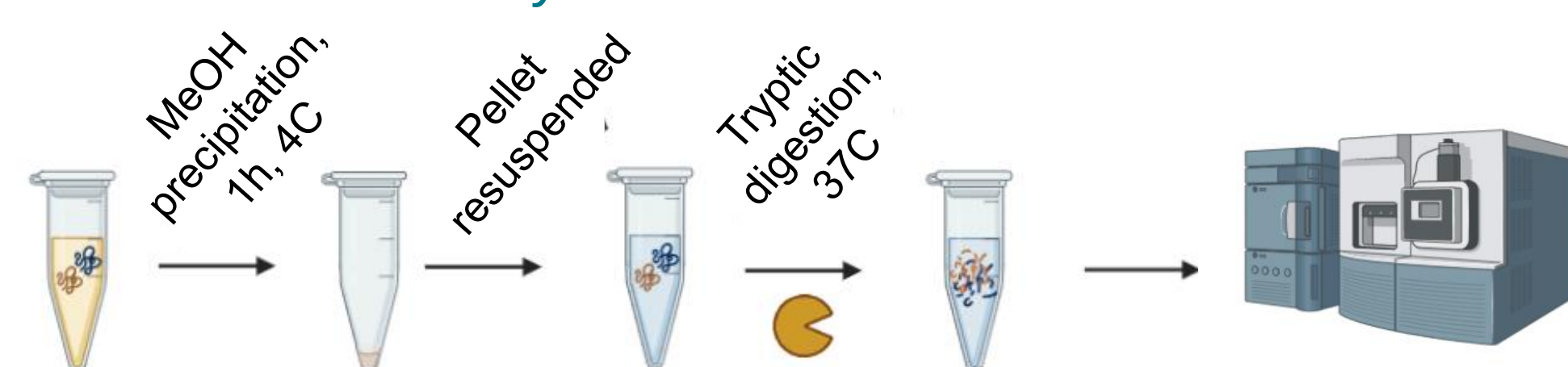


Standardisation of NfL measurements and structural protein characterisation

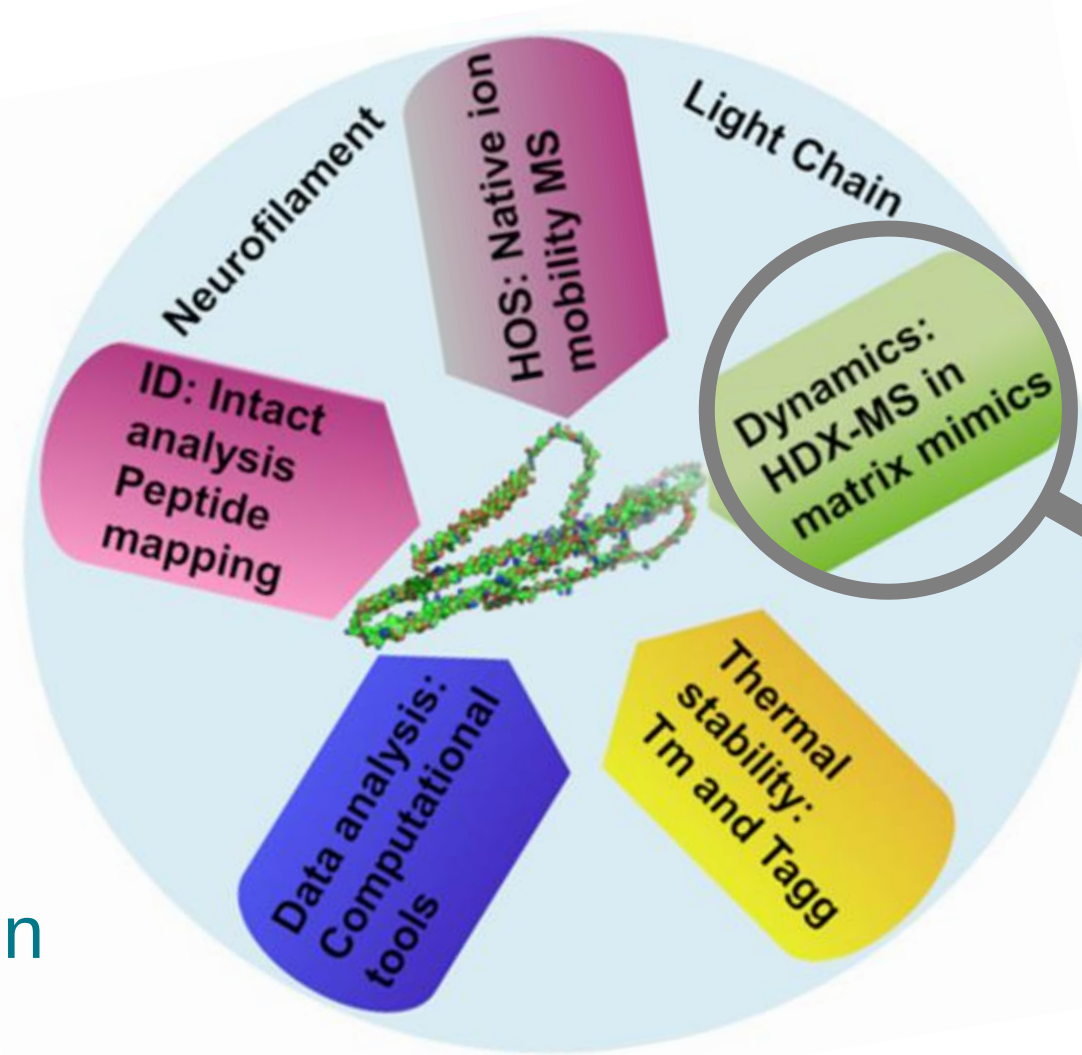
Primary calibrator quantification by amino acid analysis and ID-MS:



Method development and optimisation for NfL quantification in CSF and blood by ID-MS:



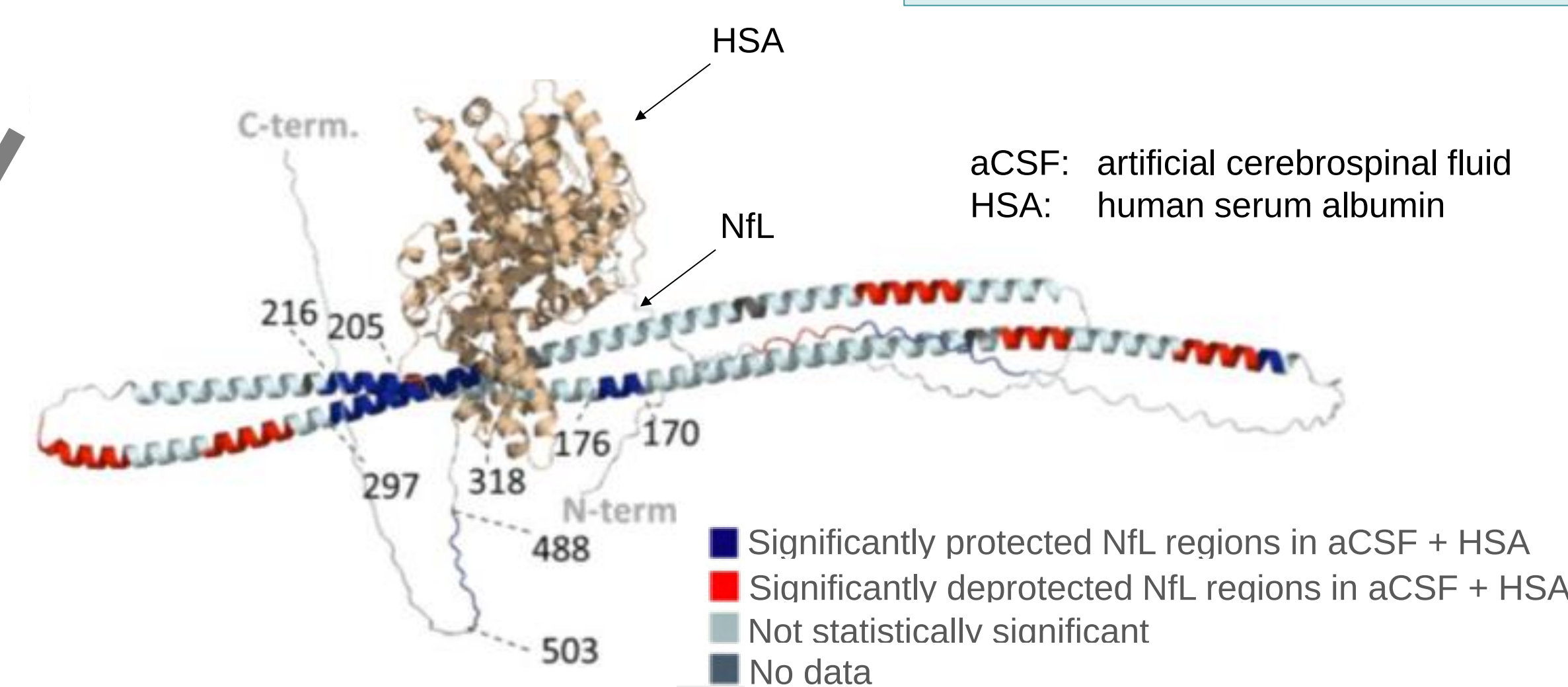
Tools of structural protein characterisation at the NML:



Hydrogen-deuterium-exchange mass spectrometry (HDX-MS) to determine the impact of sample matrix on NfL structure and dynamics:

Difference in deuterium uptake between:

NfL in aCSF
vs.
NfL in aCSF + HSA



Summary

- A candidate RMP for α -Syn quantification in CSF has been developed and successfully applied to real patient samples (NeuroMet I/II, 2016-2023)¹
- Development of a RMP for NfL quantification in CSF and blood is ongoing (NEuroBioStand)²
- Structural characterisation of α -Syn¹ (not shown) and NfL³ primary calibrator has been undertaken to underpin protein quantification efforts

Publications

- Zhang *et al.*, 2024: A candidate reference measurement procedure for the quantification of α -synuclein in cerebrospinal fluid using an SI traceable primary calibrator and multiple reaction monitoring. *Analyst*, 149, 4842-4850
- Coppens *et al.*, 2023: Neurofilament-Light, a Promising Biomarker: Analytical, Metrological and Clinical Challenges. *Int. J. Mol. Sci.*, 24(14), 11624
- Coppens *et al.*, 2025: Neurofilament Light Chain under the Lens of Structural Mass Spectrometry. *ACS Chem Neurosci*, 16(2):141-151.