Biomarker Assays for Alzheimer's Disease

- An update for the GBSC Workgroup

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Biomarkers for AD

Ideally, we require the ability to measure biochemical markers within peripheral biofluids to:

- Assist early diagnosis and stratify patients for PET imaging
- Discriminate between MCI convertors and non convertors
- Predict fast/slow rate of decline for individual patients
- Correlate with response to experimental medicines during clinical trials

Novel Assays for AD biomarkers

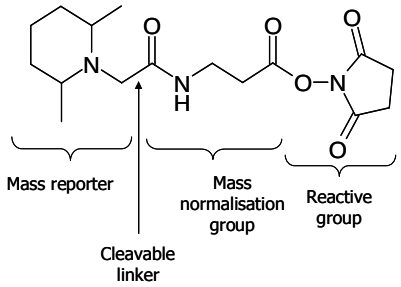
We have developed several new mass spectrometry based multiplexed assays to measure key analytes associated with Alzheimer‘s disease.

Abeta isoforms

CSF16plex

Total CSF Tau

TMTcalibrator ^{IV} (Hybrid)



Thompson *et. al*

***Anal. Chem.* 2003, *75*, 1895-1904**

CSF Sample Cohort

- CSF samples have kindly been provided by
  Dr. Henrik Zetterburg, Mölndal (Sweden)

- 31 non AD but memory impaired and 31 AD CSF samples

- Following metadata has been provided
  - Diagnosis, gender, age, and concentration of tau, beta-amyloid and phospho-tau

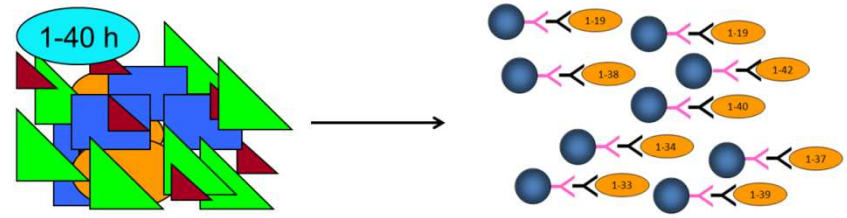
- Additional Metadata such as vial type and protein concentration after Bradford assay has also been considered

ABeta Isoforms Assay

CSF samples were processed according to the Abeta-IP-MALDI process (adapted from Portelius *et al* with slight modifications)

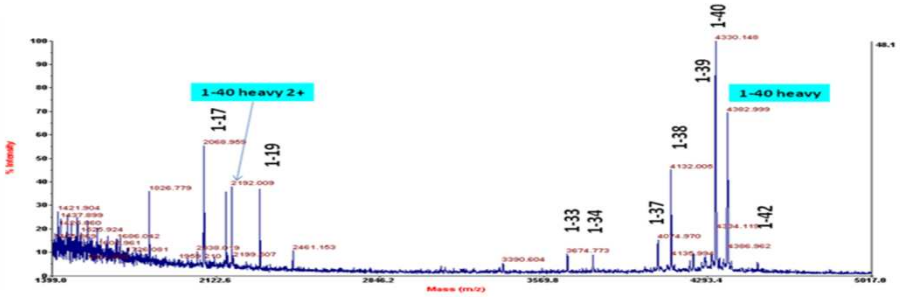
CSF sample spiked with heavy standard

Enrichment of Aß- peptides by immunoprecipitation



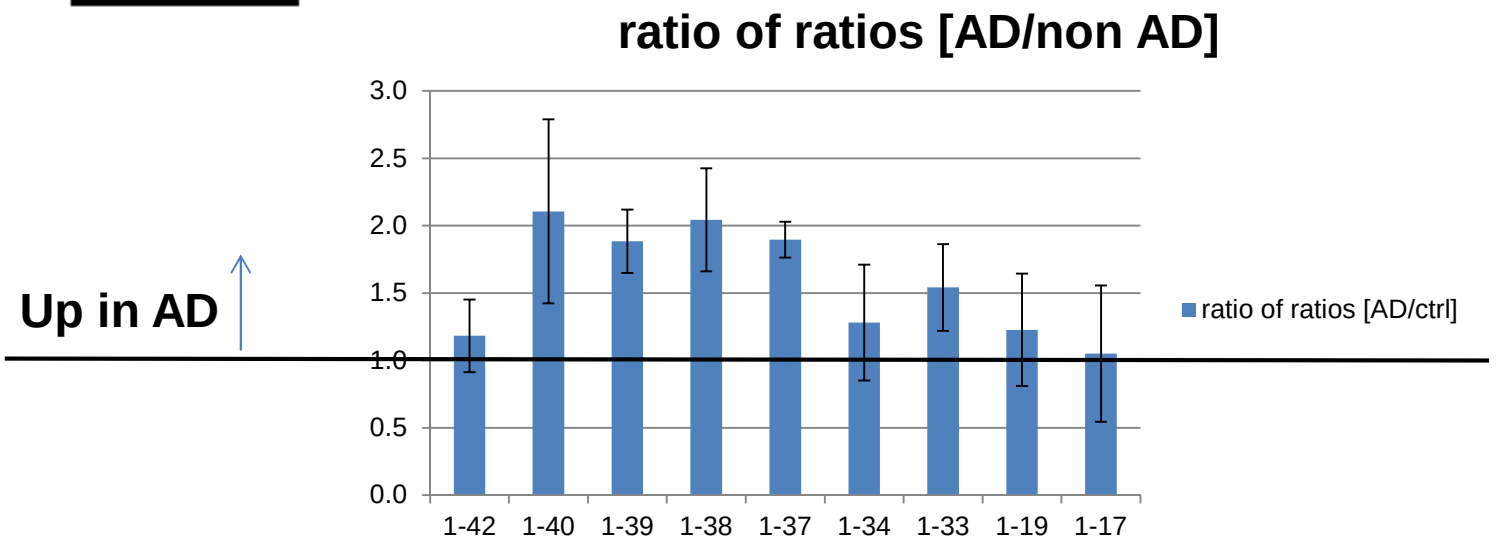
200 µl of CSF spiked with 10 ng/ml heavy 1-40 and used for IP MALDI preparation in triplicate

Referencing of endogenous peptides to spike enables comparison of spectra in each batch of Immunoppts.



Aβ-IP-MALDI workflow. Enrichment from CSF and simultaneous MS-detection of different Aβ-peptides and heavy internal standard Aβ 1-40.

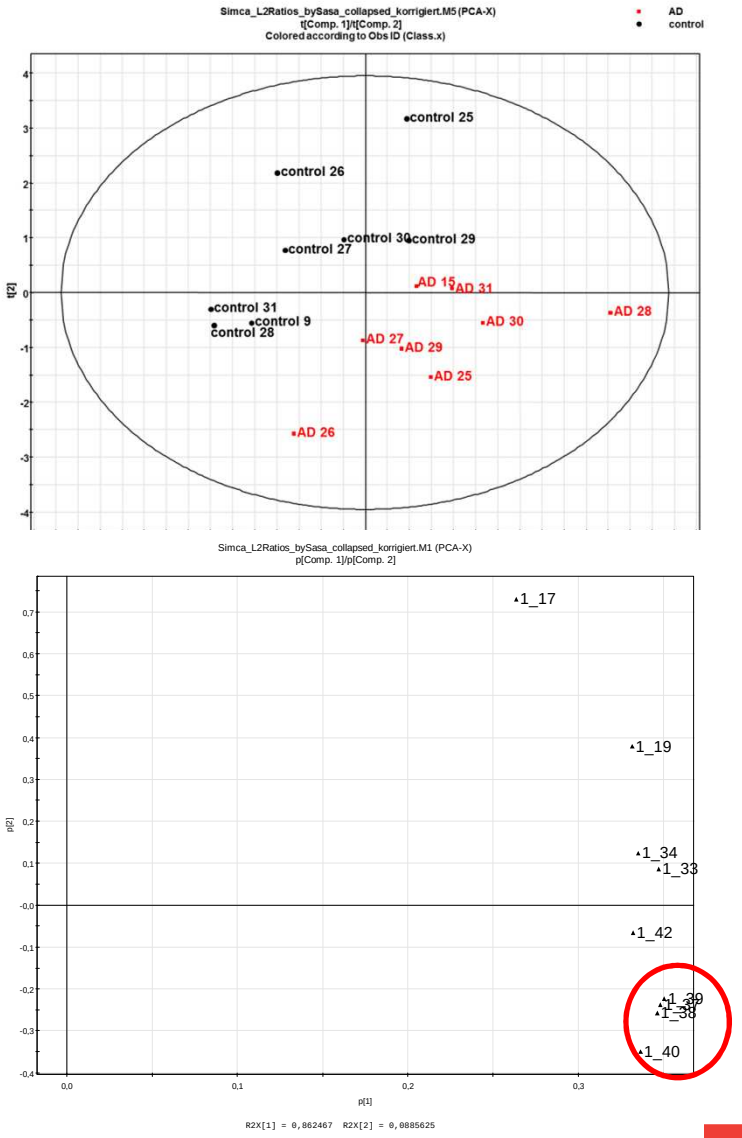
Mean AD/non AD ratios of 4 IP-batches



A mean ratio of [AD/non AD] and each endogenous peptide was calculated for each IP batch and the mean of these 4 ratios is displayed above for each of the peptides (error bars display the variation of the relative ratio between the 4 IP-batches.

- **A clear upregulation of a factor up to ~2 is seen for peptides 1-40, 1-39, 1-38 and 1-37 in ADs.**
- **An upregulation trend is less pronounced but visible in 1-34, 1-33 and 1-19 in ADs.**
- **These IP-MALDI data do not demonstrate differences in 1-17 and 1-42 between AD cases and non AD subjects (1-17 has high variations, 1-42 is a very weak signal, close to background)**

PCA Scores/Loadings Plots



Measurement of Aβ-peptides in the IP-MALDI assay enables a clear separation between AD cases and non AD samples.

The PCA Scores Plot shown relates to single IP batch (8 vs 8) to illustrate typical outcome.

Overall the separation is mainly driven by higher levels of Aβ1-37, Aβ1-38, Aβ1-39 and Aβ1-40 isoforms in AD cases as indicated in the Loadings Plot.

Statistics: Linear Mixed Effect Modelling

	Diagnosis AD/non AD						
	IP Batch 1	IP Batch 2	3	IP Batch 4	Gender	Age	Total protein conc.
1-17	0.789	0.000	0.000	0.000	0.049	0.036	0.000
1-19	0.117	0.000	0.000	0.000	0.098	0.009	0.000
1-33	0.001	0.000	0.000	0.000	0.282	0.001	0.038
1-34	0.018	0.000	0.000	0.000	0.174	0.378	0.188
1-37	0.000	0.000	0.000	0.000	0.462	0.008	0.052
1-38	0.000	0.000	0.000	0.000	0.502	0.005	0.081
1-39	0.000	0.000	0.000	0.000	0.423	0.015	0.196
1-40	0.000	0.000	0.000	0.000	0.220	0.023	0.426
1-42	0.051	0.000	0.000	0.000	0.253	0.689	0.040

A subset of Aβ-peptides deliver excellent p-values for the diagnosis of 31 vs 31 AD cases and non AD subjects.

TMT-SRM CSF 16plex Assay

Content: 16 proteins represented by 31 peptides with 236 transitions

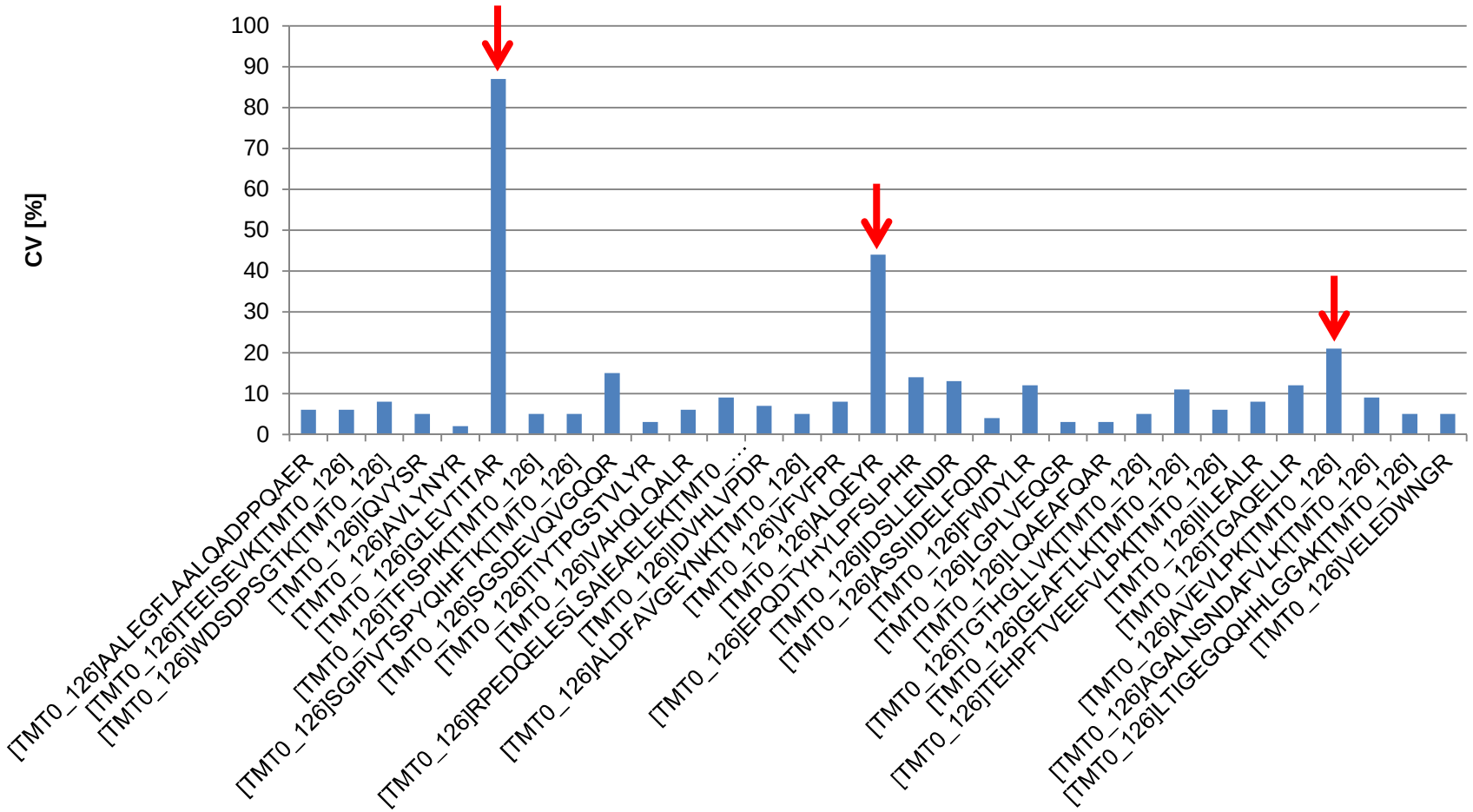
- Amyloid-like protein 1
- Amyloid beta A4 protein
- Beta-2-microglobulin
- Complement C3 alpha and beta
- Chromogranin A
- Complement factor H
- Cystatin C
- Serum amyloid P-component
- Clusterin alpha chain
- Clusterin beta chain
- Apolipoprotein E
- Alpha-2-macroglobulin
- Secretogranin-2
- Gelsolin
- Fibrinogen gamma chain

Isotopic TMTduplex workflow

- TMTzero: Individual samples (50 µL)
- TMT6-127: Universal reference
- (Seralab #CSF-123-S-26975) →
- Volumetric 1:1 mixture
- QC samples: Universal reference
  - TMTzero and TMT6-127 1:1
- 1h nLC gradient
- TSQ Vantage
- Triplicate analysis
- 2 µL CSF o/c
- PinPoint 1.1 including manual editing

TMT-SRM CSF 16plex Assay

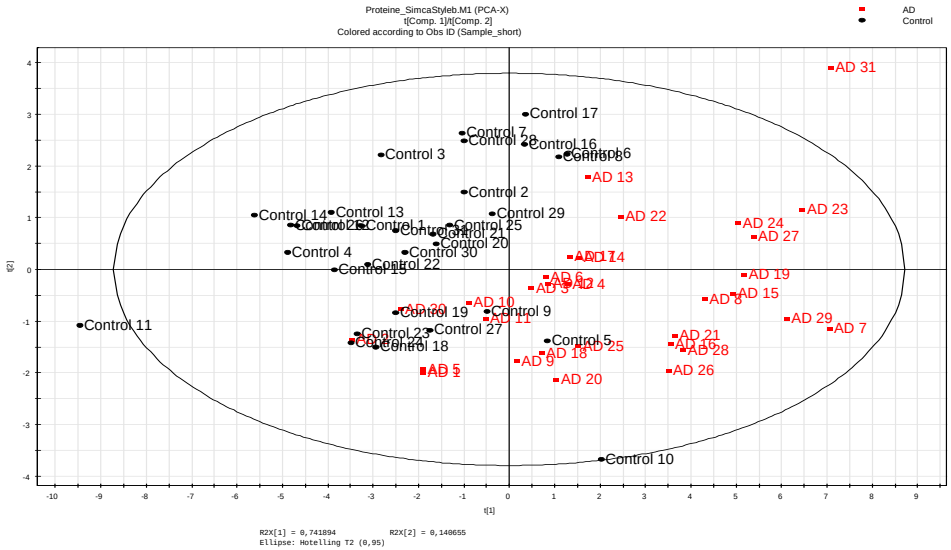
Assay Precision - All CV's below 15% except three peptides



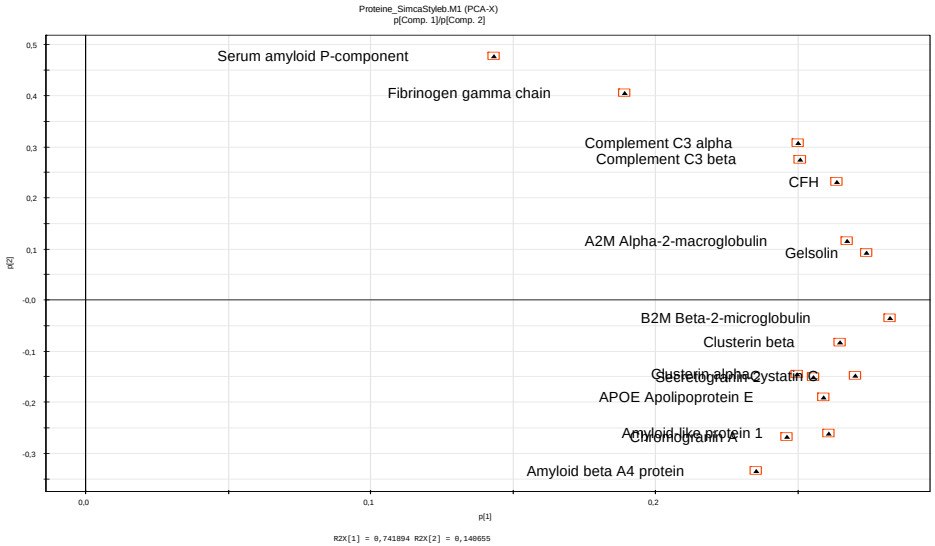
Decreased performance of peptides GLEVTITAR, ALQEYR and AVEVLPK due to reduced quality of SRM transitions

Statistical Analysis

PCA Scores Plot

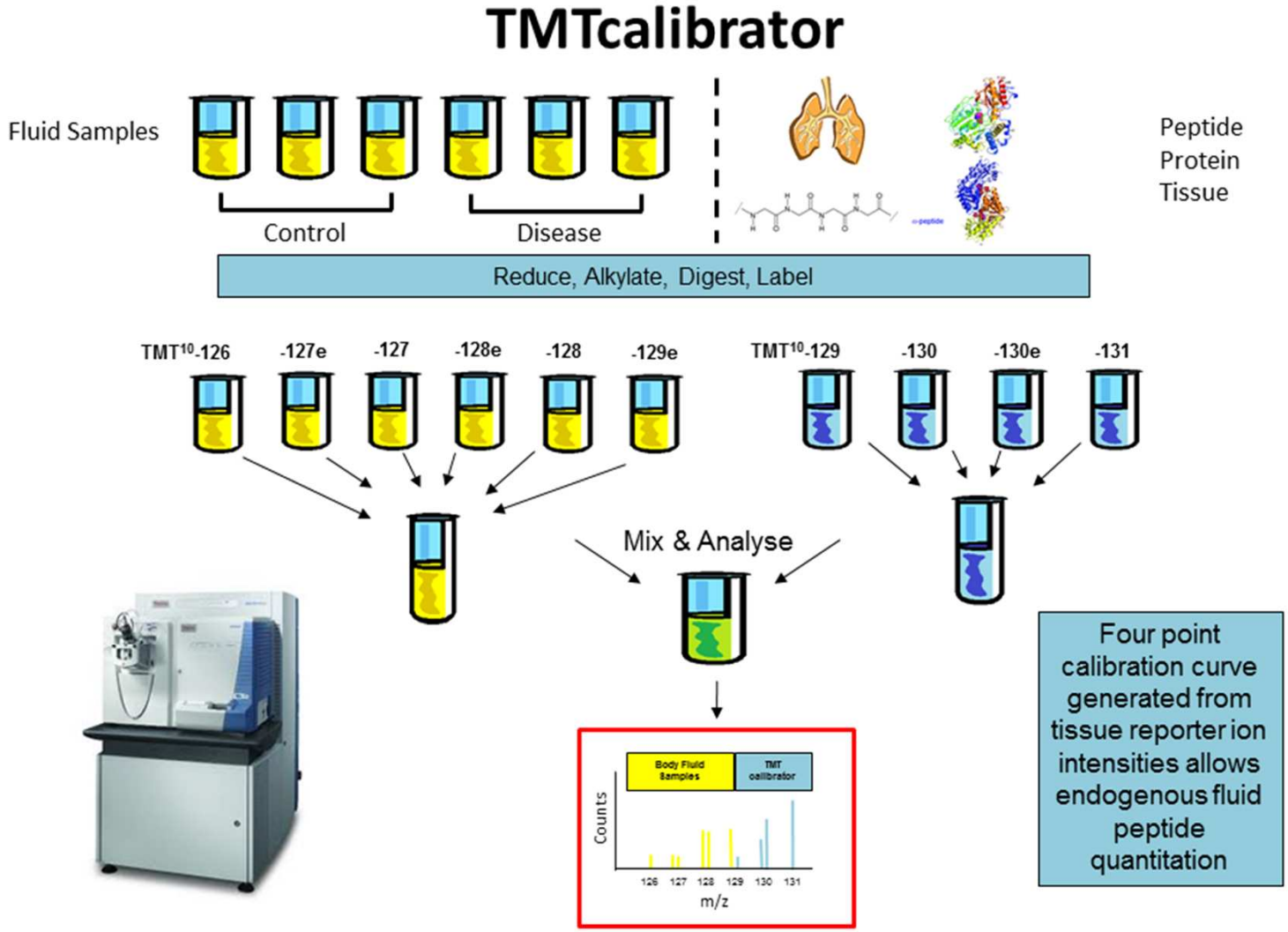


PCA Loadings Plot

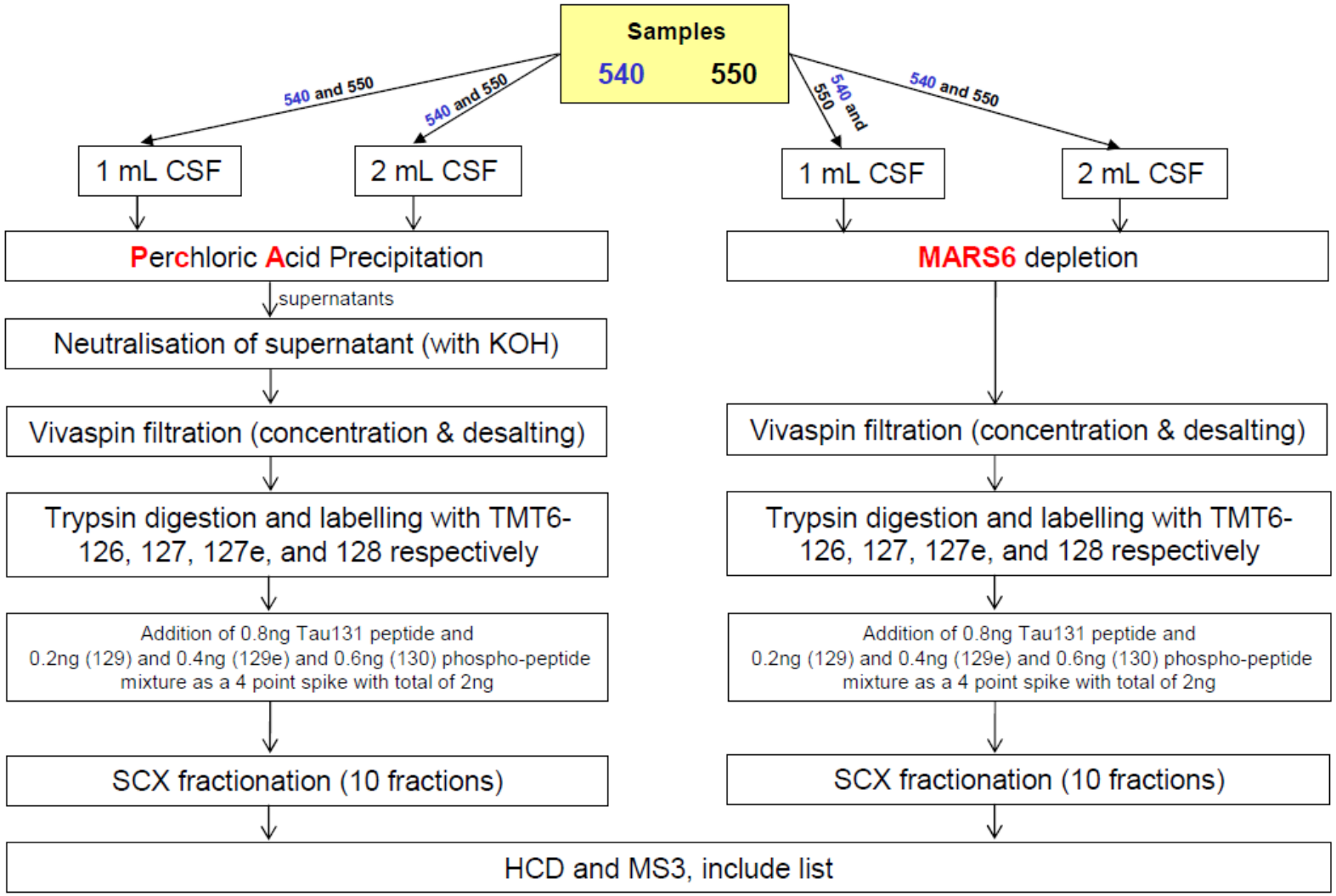


→ **For this cohort, the 16plex CSF assay gives remarkable separation of AD cases and non AD subjects with most analytes having a strong influence on the differentiation of the clinical groups**

TMTcalibrator CSF-Tau

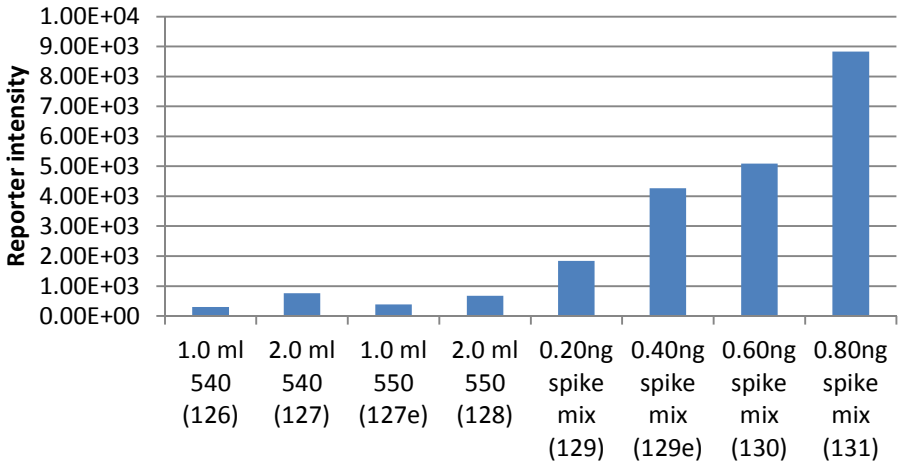


TMTcalibrator for CSF Tau

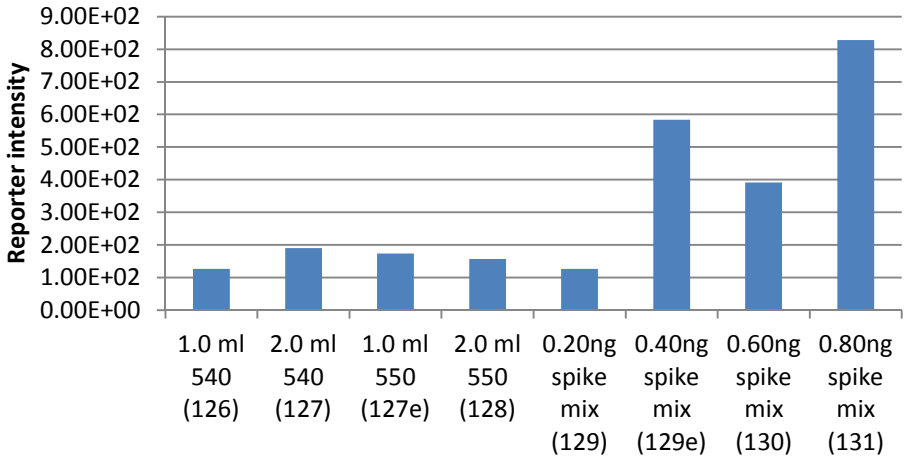


TMTcalibrator affords more scope

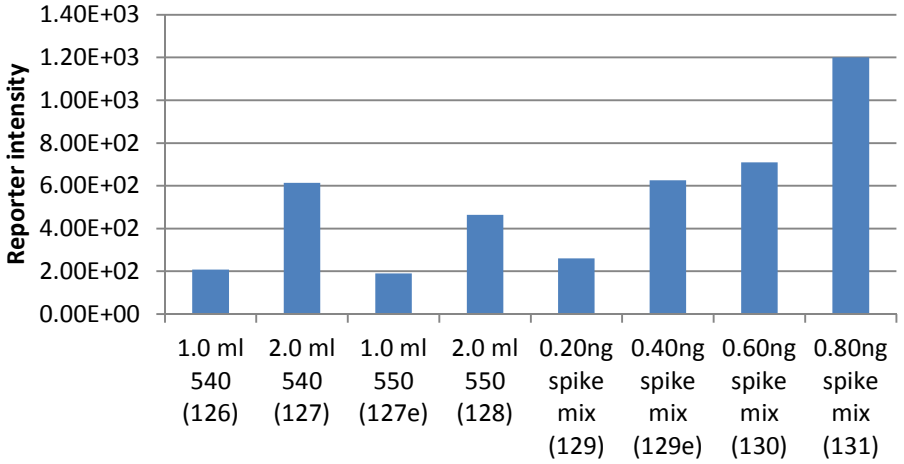
SPVVSGDTSPR



SGYSSPGSPGTPGSR



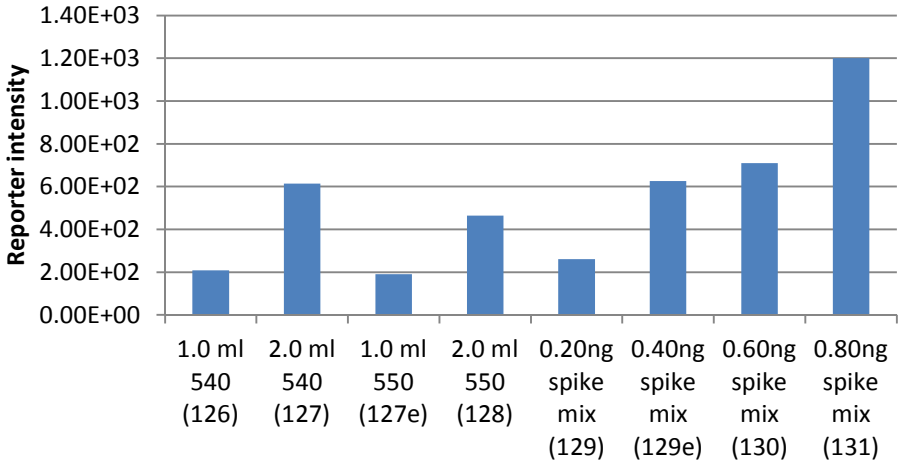
TPPSSGEPPK

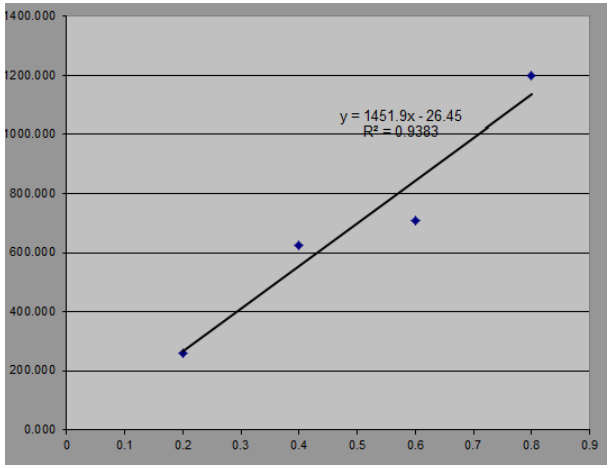


Several Tau peptides as surrogates for Total Tau measurement each with a four-point internal calibration curve

Illustration of TMTcalibrator results

TPPSSGEPPK



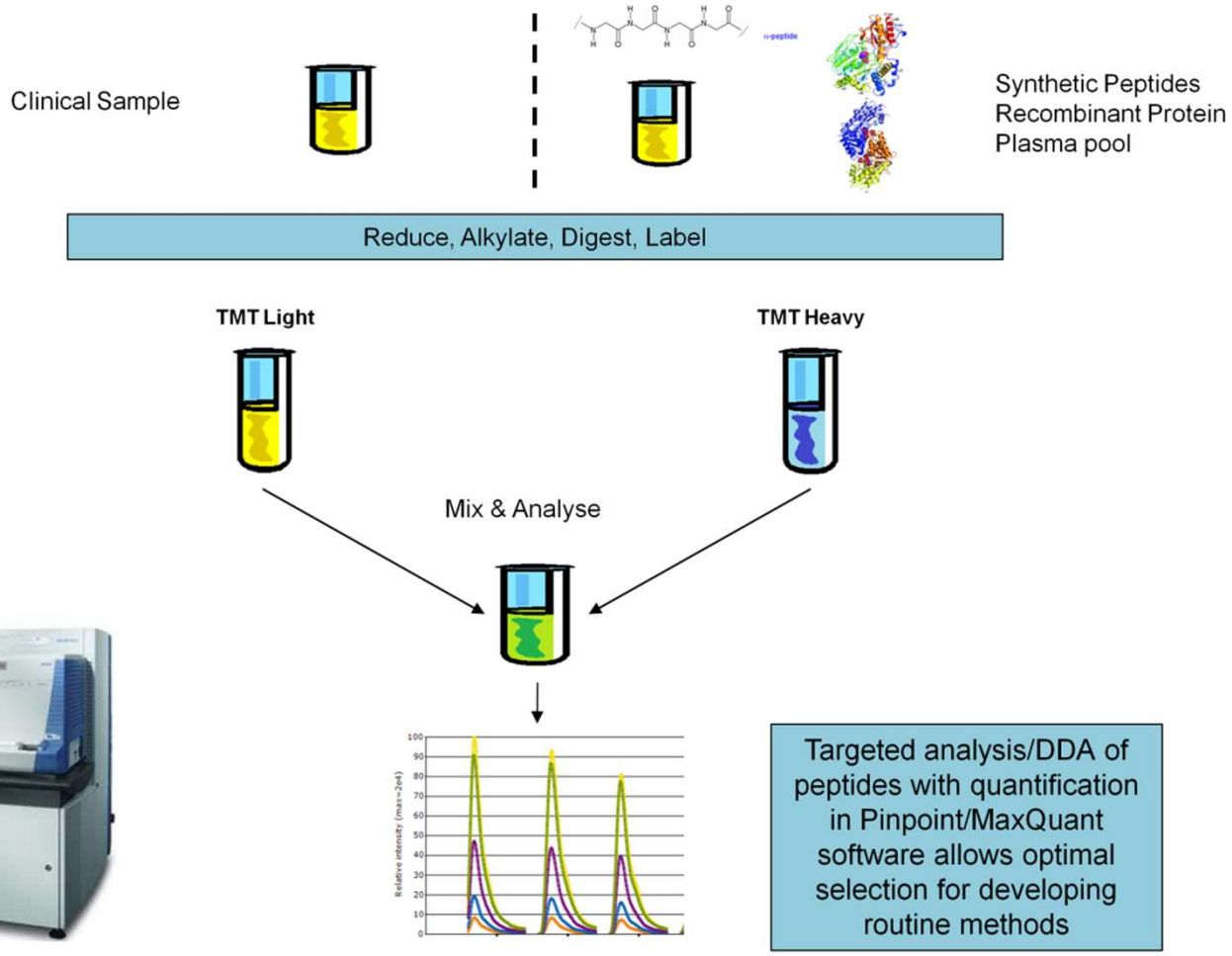


sample	slope	y axis offset	R^2	Intensity average	amount peptide in sample [ng]	used volume in preparation	amount peptide per mL [ng/mL]
1.0 ml 540	1451.9000	26.4500	0.9383	208.70	0.13	1.00	0.13
2.0 ml 540	1451.9000	26.4500	0.9383	613.20	0.40	2.00	0.20
1.0 ml 550	1451.9000	26.4500	0.9383	190.60	0.11	1.00	0.11
2.0 ml 550	1451.9000	26.4500	0.9383	463.70	0.30	2.00	0.15

Our hunt for Tau phospho-peptides in CSF continues…

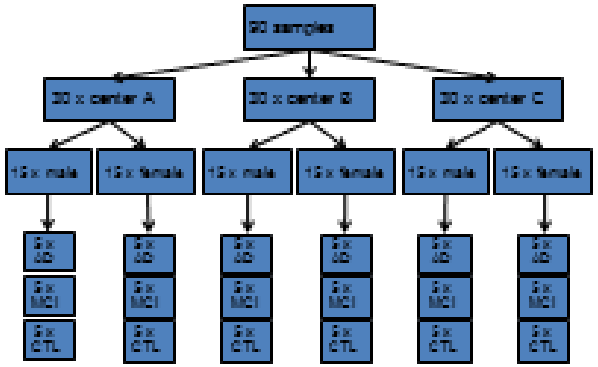
- Further exploration of Tau/pTau enrichment prior to MS
  - Immuno-ppt and TiO_2
- Create optimal TMTcalibrator standard
  - Post mortem AD brain or RecTau+Kinase(s) + ATP
- Use of additional TMT related fragment ions for quantitation purposes (TMT^C ions Wühr & Gygi 2012)
- We are still looking for additional partners to help
  - Access to CSF cohorts
  - Advice on tau fragments for inclusion
  - Funding

TMTcalibrator IV



A hybrid targeted and non targeted screening approach

Input A: Samples (n=90)



Input B: Include List Content

Protein	Number of peptides
Alpha-2-macroglobulin	15
Apolipoprotein E	13
Complement C3	14
Complement factor H	10
Gelsolin	12
Clusterin	11
Fibrinogen gamma chain	12
Serum amyloid P-component	8

Table 1: AD SRM proteins and number of peptides included in the LTQ Orbitrap Velos method

Output: New Biomarker Panels

Group modeling of data handling (GMDH)

– Four peptide panel for AD vs MCI/Control

	Condition AD	Condition Control/MCI	
model >0.5	TP = 17	FP = 1	Positive predictive value = 0.944
model <0.5	FN = 12	TN = 60	Negative predictive value= 0.833
	Sensitivity = 0.58	Specificity = 0.98	

– Six peptide panel for MCI vs AD/Control

	Condition MCI	Condition Control/AD	
model >0.5	TP = 22	FP = 3	Positive predictive value = 0.88
model <0.5	FN = 9	TN = 56	Negative predictive value = 0.86
	Sensitivity=0.71	Specificity =0.95	

2089 distinct peptides corresponding to 199 identified protein groups

Reference for GMDH: Alexey G. Ivakhnenko 1968

To Conclude

- **Use of MS combined with TMT facilitates rapid and inexpensive set up and assay validation**
- **Multiple analytes are measured with high quantitative precision and accuracy**
- **Several AD Biomarker Assays are “Ready to Use”**
- **Further details on our posters at AAIC tomorrow**

 # P1-141: **Quantitative mass spectrometry assays for Amyloid beta, tau and phospho-tau in CSF**

 # P1-233: **Further exploration of Plasma Biomarkers for Alzheimer’s disease using isotopic Tandem Mass Tags and a combined targeted /non-targeted LC/MS/MS method**