

ReMiND

Role of metals and metal containing biomolecules in neurodegenerative diseases such as Alzheimer's disease

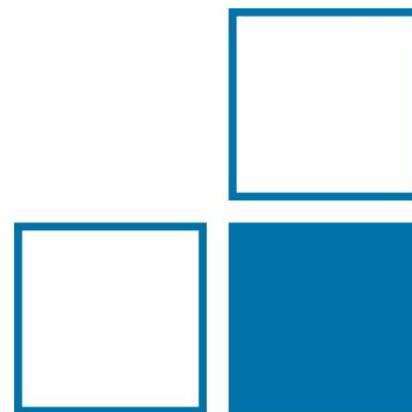
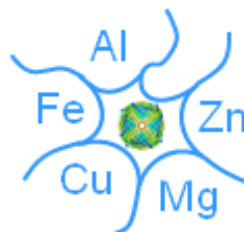
C. Swart¹, C. Frank¹, J. Gleitzmann¹, E. Griffin², A. Raab², N. Solovyev³,
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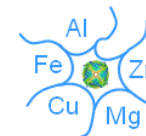
- ~**10.5 million patients** with dementia in EU
- Number predicted to double in next 20 years
- Estimated costs for health care systems **€286.1 billion** in 2015
- **Lack in comparability** between results of different kits and laboratories \Rightarrow hampers large-scale studies
- Traceability in accordance with EC-directive 98/79/EC required (2017 evolved into the EU regulation 2017/746)

		N	Median ng/L	Ref ng/L	normal	borderline	pathological
Aβ1-42	Kit 1	39	517	med. 500	18	11	10
Aβ1-42	Kit 2	8	330	651	0	0	8
tot Tau	Kit 1	42	442	med. 450	21	6	12
tot Tau	Kit 2	8	501	466	4	2	2
P Tau	Kit 1	40	33		40	0	0

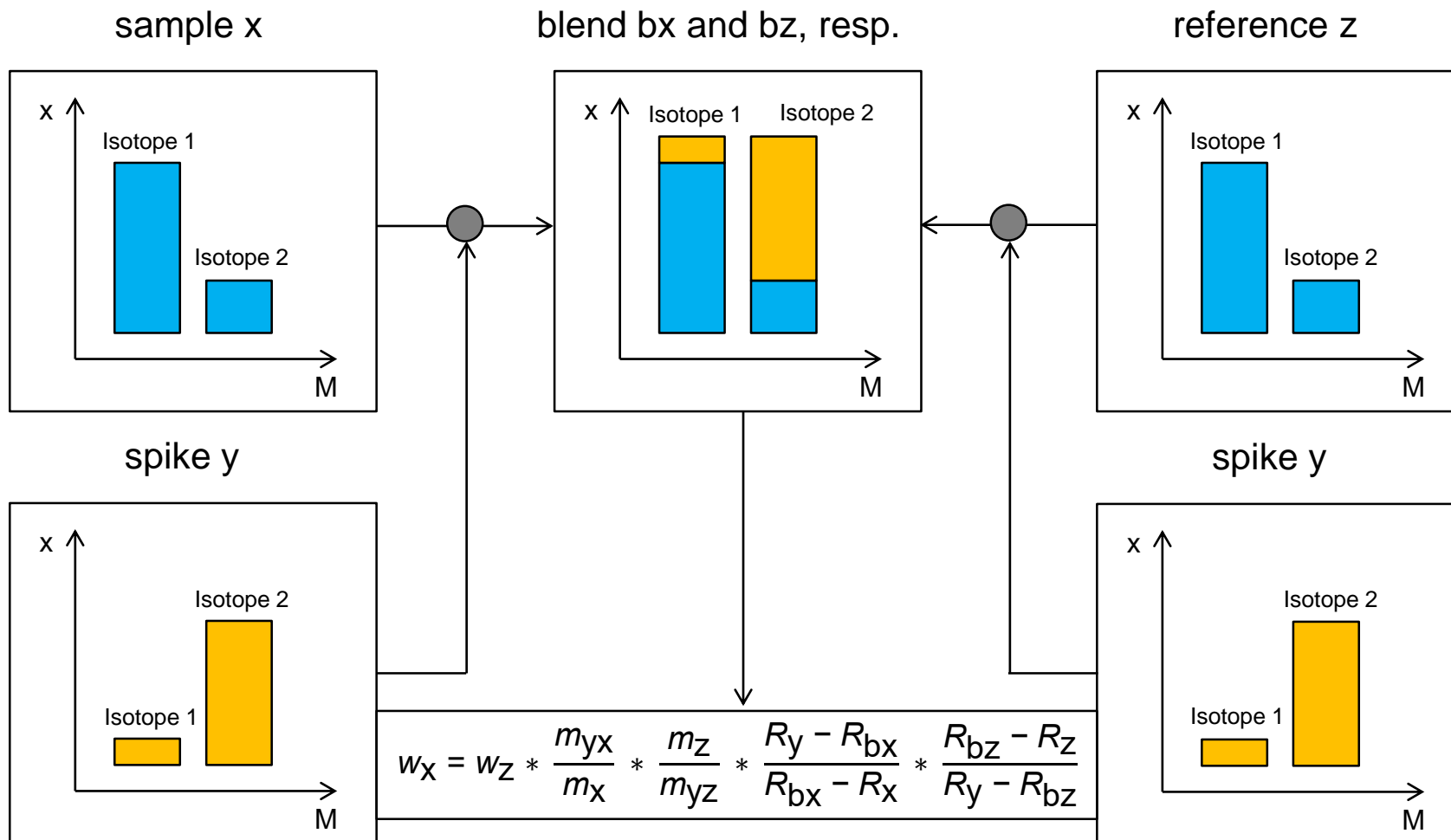
Reiber *et al.*, J Alzheimers Dis Parkinsonism 2014, 4(3))

- Development of **new and accurate methods** for measuring peptide and protein biomarkers
- Development of methods for the **traceable quantification** of metals and metal containing biomolecules
- Production and characterisation of isotopically labelled spike materials
- Characterisation of uptake, metabolism and transport of metals and metal containing biomolecules to the brain
- Facilitation of uptake of the technology and measurement infrastructure

Underlying measurement principle

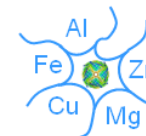


Double Isotope Dilution



Ideally, spike and analyte have the same chemical form.

Technical Work Packages of ReMiND



WP1

Reference measurement procedures for β -amyloid, T-tau and P-tau

Correlation metal content \leftrightarrow biomarkers

WP2

Reference measurement procedures for total metal content and isotope ratios

Correlation metalloprotein \leftrightarrow established biomarkers

WP3

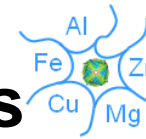
Reference measurement procedures for metalloproteins

Correlation metal content \leftrightarrow isotope ratio

WP1

Quantification of established peptide and protein biomarkers

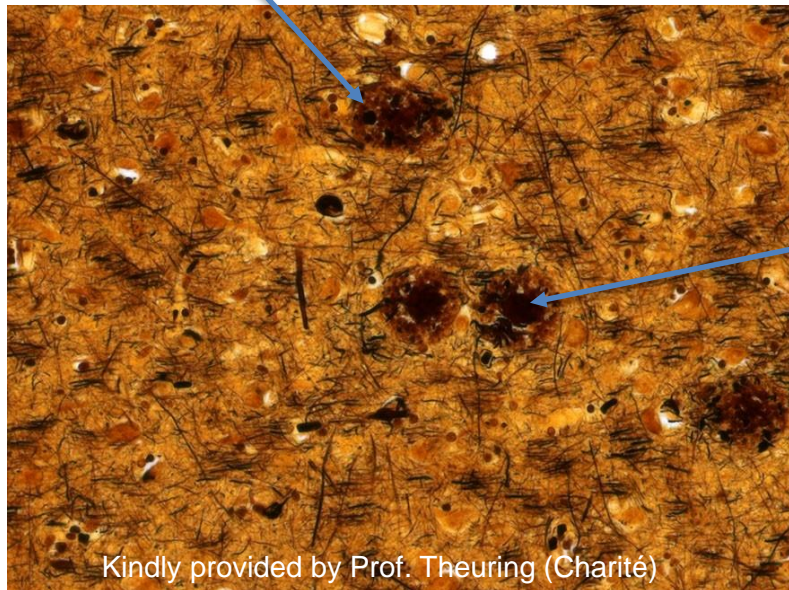
Quantification of established biomarkers



Established biomarkers

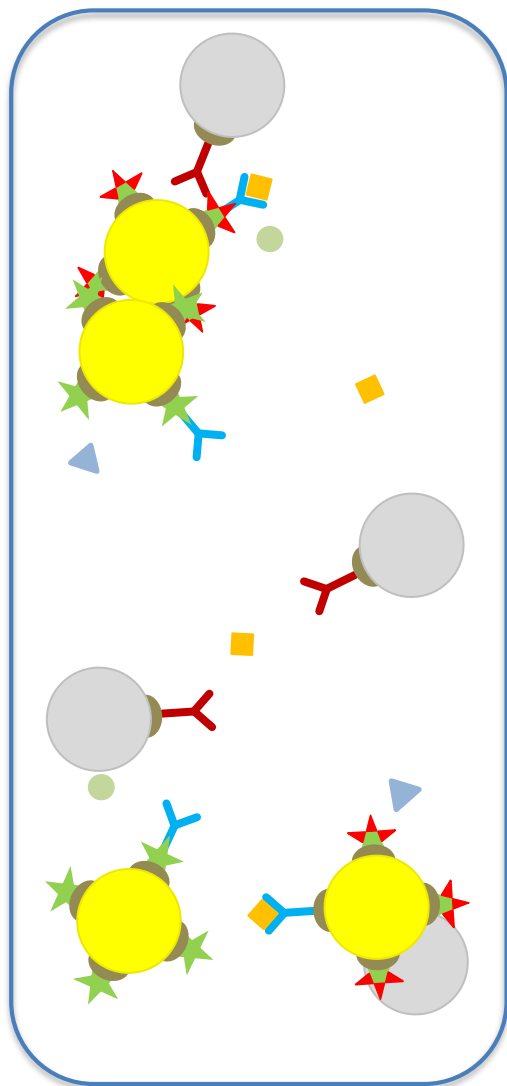
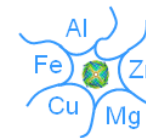


- Physiological function unknown
- Formation of plaques in the brain during aging
- Decreased in Alzheimer patients



- Stabilisation of microtubules in neurons
- Formation of tangles in the brain during aging
- Increased in Alzheimer patients

τ -Protein quantification with ID-Raman



proteins

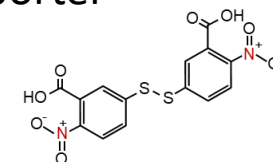
gold NP
Raman reporter
and m-antibody

magnetic NP
and p-antibody

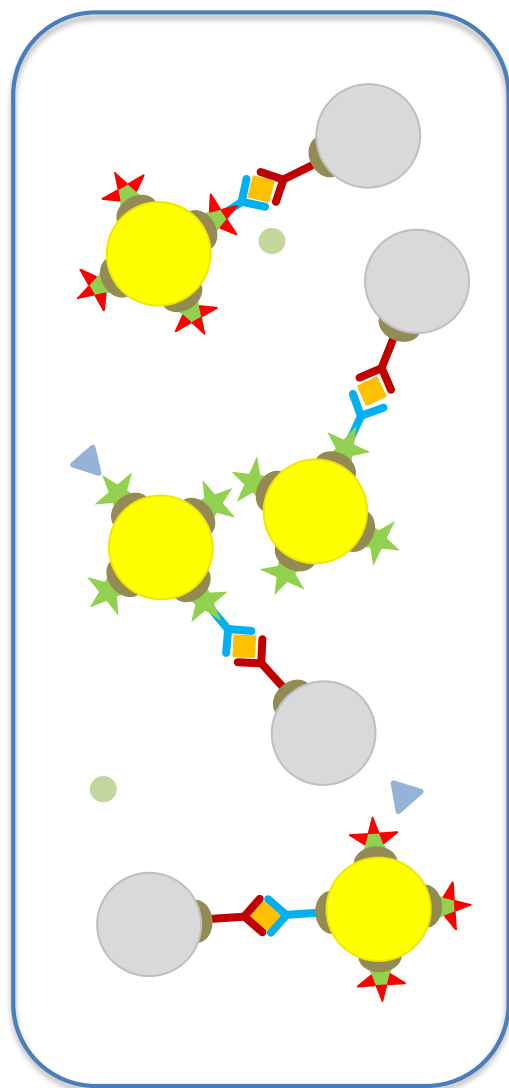
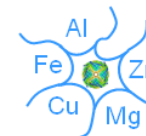
spike with isotopic enriched Raman reporter

specific interactions

separation detection,
quantification



τ -Protein quantification with ID-Raman



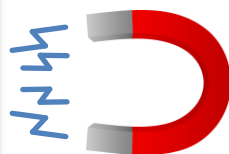
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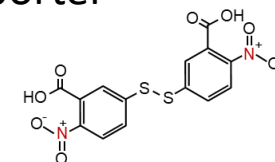
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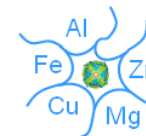
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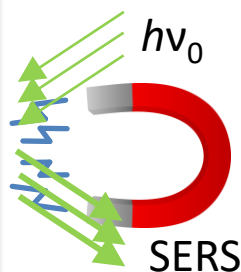
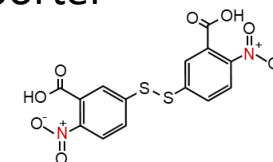
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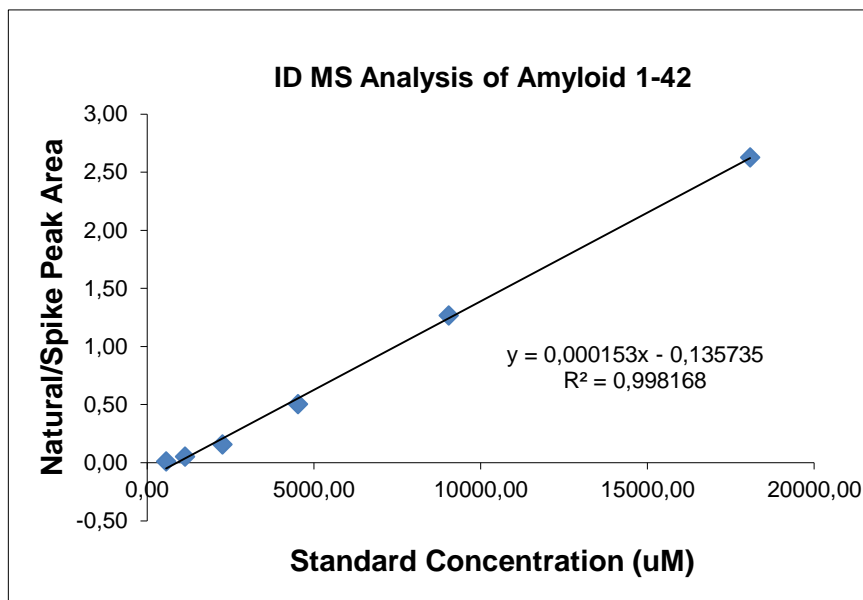
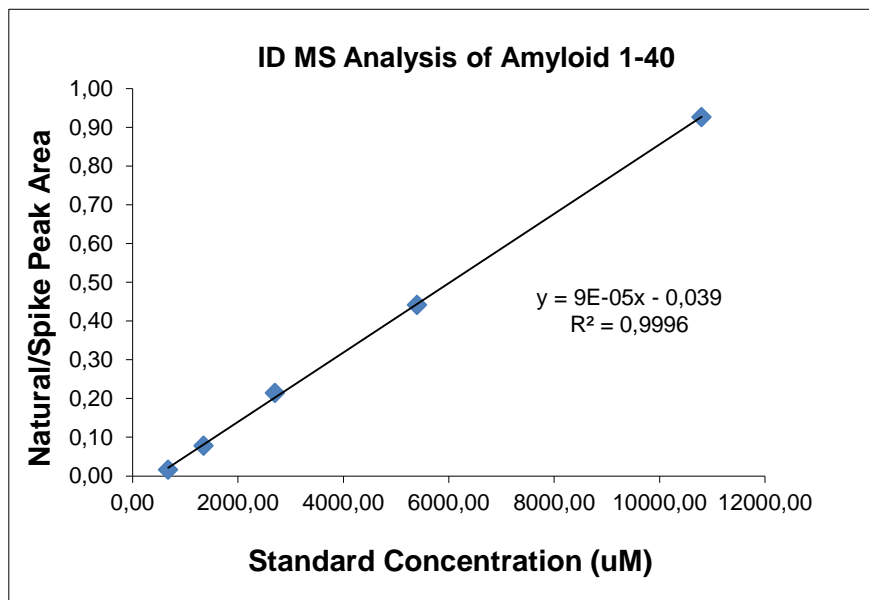
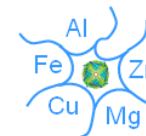
specific interactions

separation detection,
quantification



V. Maurer et al., J Bio (2019), <https://doi.org/10.1002/jbio.201960090>

β-amyloid quantification using MS/MS

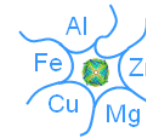


Peptide		Expected Concentration (ng/mL)	Measured Concentration (n=4)(ng/mL)	Combined Uncertainty U (ng/ml) k=2	Precision (%CV)	Recovery (%)
aβ 1-40	QC-1	2.41	2.64	0.32	6.65	109.58
	QC-2	6.93	6.97	0.61	2.66	100.61
	Pooled CSF	2.00-4.00	4.95	0.47	3.35	N/A
aβ 1-42	QC-1	2.03	2.08	0.35	7.98	102.40
	OC-2	5.84	5.26	0.57	6.74	90.03
	Pooled CSF	0.35-0.70	0.70	0.12	7.63	N/A

WP2

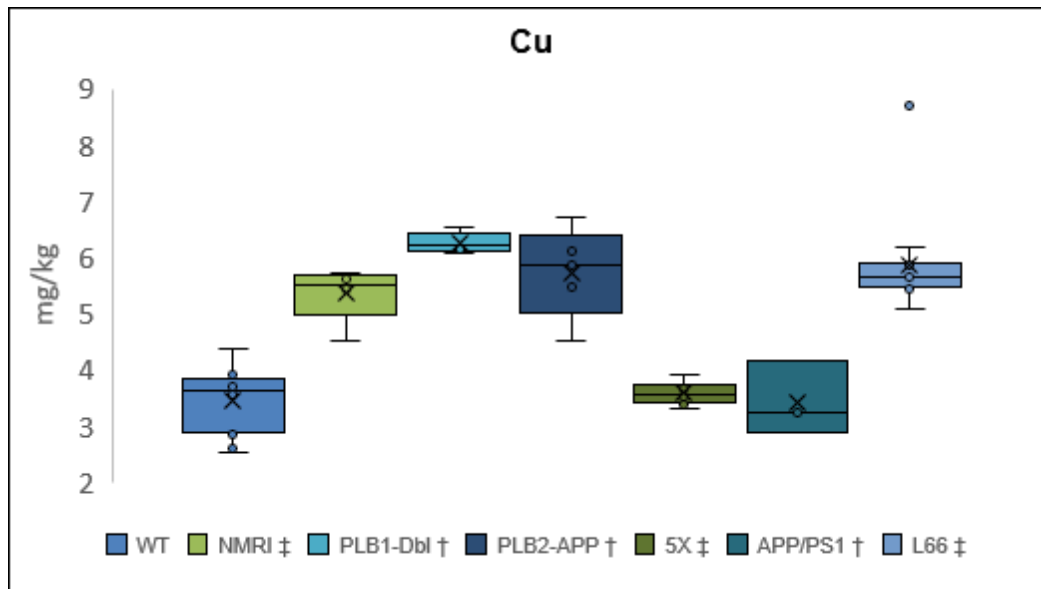
Multielemental and isotopic analysis

Total metal amounts



Results

- Generally, higher Fe, Cu and Zn concentrations in disease model brains compared to healthy controls
- Highest metal concentrations in the disease model that has both disease pathologies present



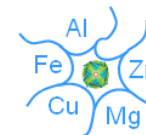
WT, n = 13. NMRI, n = 5. PLB1 - Dbl, n=5.
 PLB2 – APP, n = 5. 5X, n=7. APP/PS1, n = 3. L66, n = 13.

Are the differences significant?

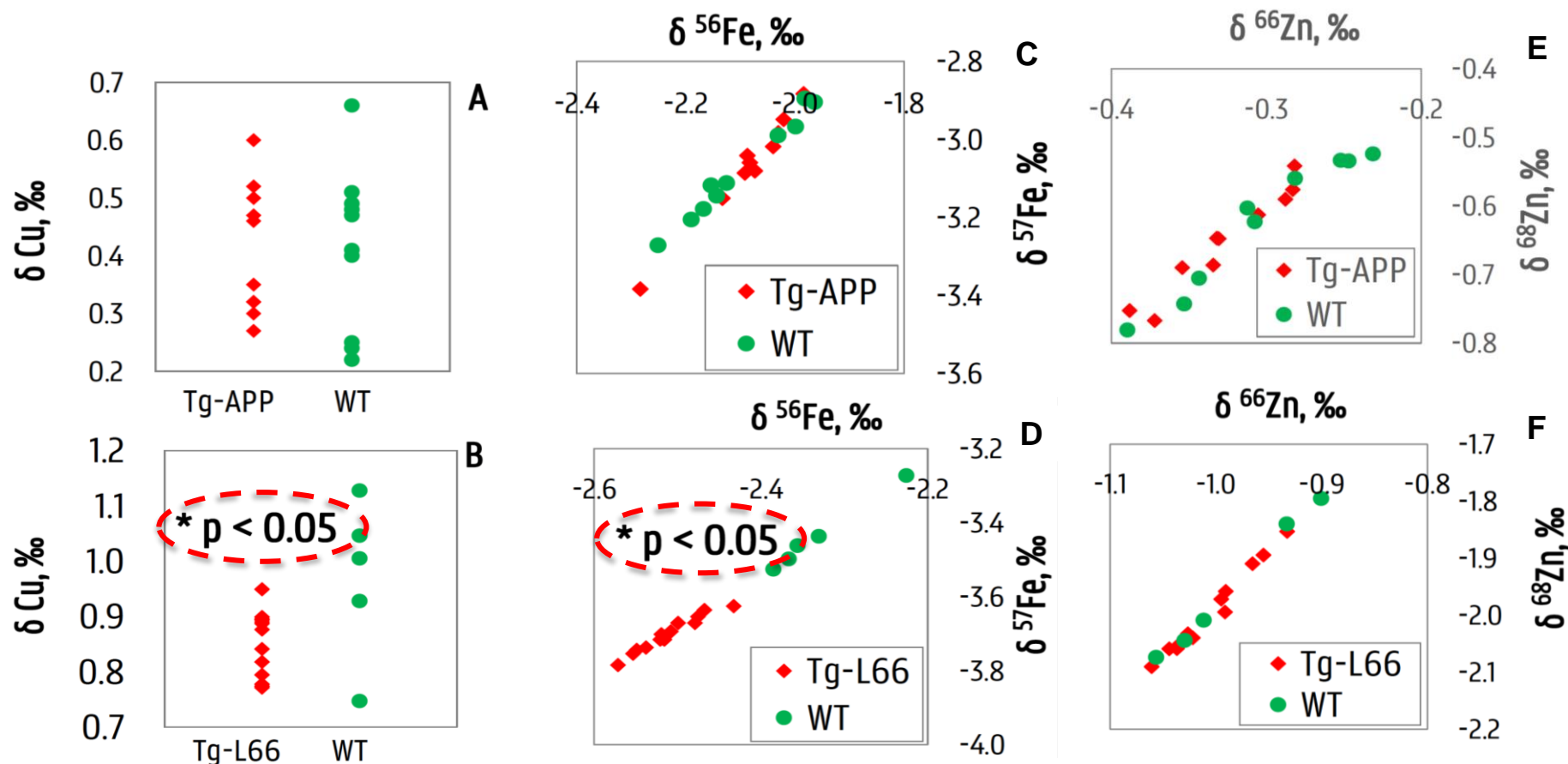
WT						
NMRI±	✓					
PLB1 - Dbl†	✓	✓				
PLB2 - APP†	✓					
5X±		✓	✓	✓		
APP/PS1†		✓	✓	✓		
L66±	✓				✓	✓
WT	NMRI±	PLB1 - Dbl†	PLB2 - APP†	5X±	APP/PS1†	L66±

† - None of the individual samples are perfused
 ± - All individual samples are perfused

Isotopic signatures in AD models



Brain tissue of APP and L66 tau mice vs. matched WT controls

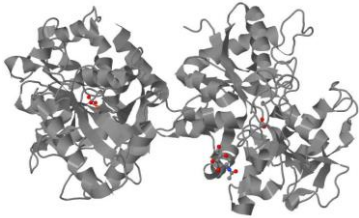
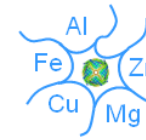


Brain Cu, Fe and Zn isotopic composition respectively for **A, C and E**: APP mice (n=10) vs. matched wild-type (n=10); and **B, D and F**: L66 tau mice (n=13) vs. matched wild-type (n=5). Significant difference ($p < 0.05$, Student's t-test) between the Tg-L66 and the WT tau-transgenic mice for Cu and Fe (**B and D**).

WP3

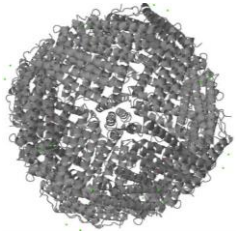
Metal containing biomolecules as potential biomarkers

Metalloproteins



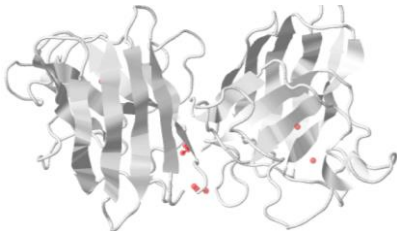
Transferrin

Fe transport protein, Fe is suspected to be involved in plaque formation, free Fe causes oxidative stress



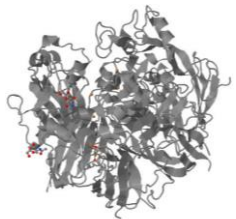
Ferritin

Fe storage protein, incorporation also of other metals (including toxic ones) with similar properties



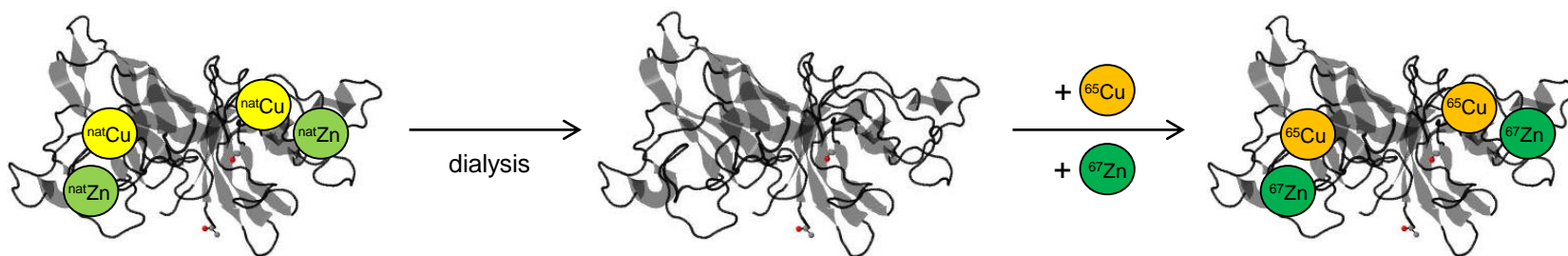
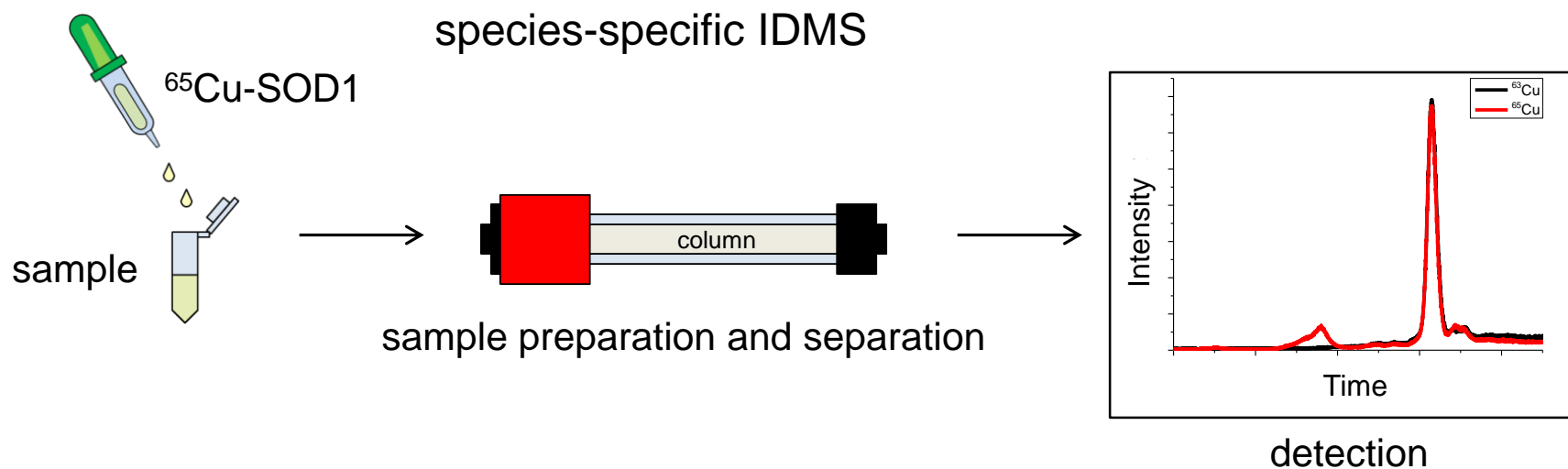
Cu, Zn-superoxide dismutase

Oxidative stress prevention, acute phase protein, indication for inflammation processes

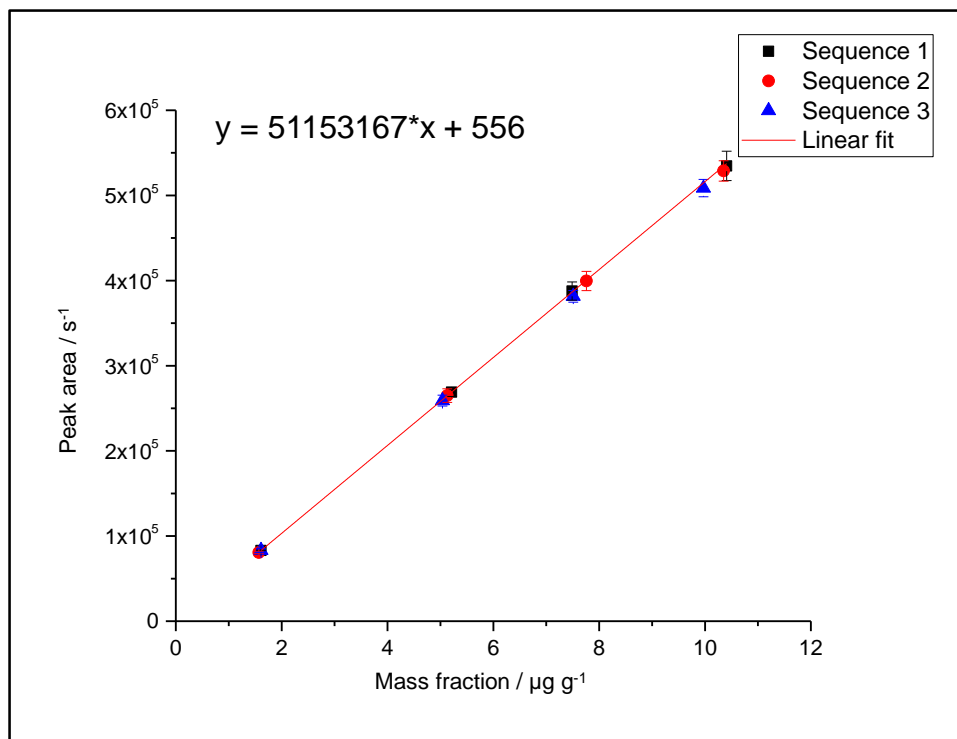


Ceruloplasmin

Cu storage protein, ferroxidase, Cu is suspected to be involved in plaque formation



- Mass fraction of SOD1 in erythrocytes $(63.95 \pm 0.93) \mu\text{g g}^{-1}$
- Mass fraction of SOD1 in CSF much smaller
- Dilution series for the determination of LOD and LOQ



analyte	blank / μg g ⁻¹	LOD / μg g ⁻¹	LOQ / μg g ⁻¹
SOD1 in CSF	0.013	0.039	0.117

Clinical Impact

- Support in establishing **global cut-off values** for biomarkers by providing comparable and reliable measurement procedures for interlaboratory studies
- Investigation of **potential biomarkers** such as metalloproteins and isotope ratio analysis may lead to new approaches of dementia treatment

Social Impact

- Potential biomarkers investigated in proposed project can enable **earlier diagnosis**
- Earlier diagnosis allows for earlier intervention and, thus, delay of severe symptoms and hospitalisation
⇒ **improvement of quality of life** for patients and their caretakers

Thank you to all the partners...



Coordination, lead WP1

Determination of proteins using ICP-MS
and Raman



Lead WP2

Multielemental and
isotopic analysis



universität
wien

Lead WP 3

Quantification of metal containing
biomolecules



Lead WP4 (Impact)

Quantification of potential biomarkers,
provision of animal models



Multielemental and
isotopic analysis



Quantification of metal and
provision of animal models



Quantification of metals and
metalloproteins



Isotopic analysis in
metalloproteins





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