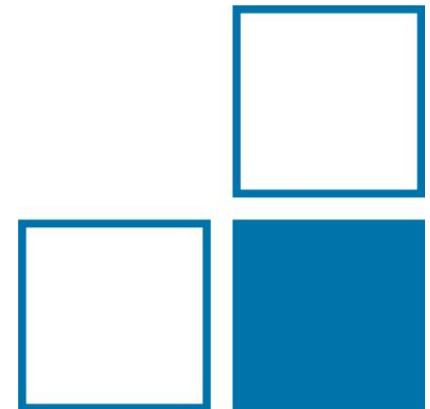


New approach for the development of candidate reference measurement procedures

C. Swart, J. Gleitzmann, N. Tokman, A. Raab,
E. Del Castillo, C. Brauckmann, S. Zakel,
C.-G. Arsene, C. Frank



EMRP

European Metrology Research Programme
 Programme of EURAMET



The EMRP is jointly funded by the EMRP participating countries within EURAMET and the European Union

Metrology for metalloproteins

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EMRP Project "Metrology for Metalloproteins" - HLT05



Metalloproteins are especially important in medical diagnosis as they represent around 30 % of the whole proteome. Many of them such as haemoglobin (Hb), transferrin (Tf), superoxide dismutase (SOD) or ceruloplasmin (Cp) are important markers for diseases such as Down's syndrome in the prenatal diagnostic (SOD), inflammation (acute-phase proteins like Tf or C-reactive protein (CRP)) or deficiency diseases (Hb, Tf, Cp). Moreover, they are used for the control of treatment efficiency e.g. total Hb as the most important marker for anaemia treatment. Other metalloproteins are important in cancer treatment like selenoproteins or platinum (Pt) containing drugs that form adducts to biomolecules.

[→ Contact](#)

The research leading to these results has received funding from the European Union on the basis of Decision No 912/2009/EC.
 Last updated: 2013-10-17

JRP h-17: Metrology for Metalloproteins

Human Resources: 359.4 FM
 Total eligible costs: 4.3 ME

Need

- Metroproteins represent about 30% of the whole proteome
- No primary reference measurement procedures available for metalloproteins
- Therefore no reference values for laboratory comparison available
- Insufficient comparability

Objectives

- General approach for the quantitative determination of different types metalloproteins using isotopic dilution (ID) and/or laser plasma micro spectrometry (LPMS)
- Reference measurement procedures
- Complementary methods for the separation, identification and quantification of proteins

Consistency based

- Interdisciplinary, multi-centre and multi-institutional approach
- Sample characterization and treatment
- WPM 77 FM, HREC1, HREC2
- Oxyhaemoglobin (HbO₂)

Non-covalently bound metalloproteins

- Ceruloplasmin (Cp)
- Dr. Th. Knebel, Dresden (PTB)
- WPM 78 FM, HREC1, HREC2
- Ceruloplasmin (Cp)

Non-covalently bound metalloproteins

- Haemoglobin (Hb)
- WPM 79 FM
- Haemoglobin (Hb)

WPM Creating Impact (HLS, HREC1, HREC2)

- JRP website
- Exchange with stakeholders
- Publications in journals
- Participation in international conferences
- Reference values for clinical trials and inter-laboratory comparisons
- Workshop
- Dissemination of the results at ICCOM and ACTM

WPM Development of new and complementary methods (HLS, HREC1)

- Light absorption
- "Fe" - "Fe" in Hb
- Organic EMS
- ID Raman spectroscopy

Partners

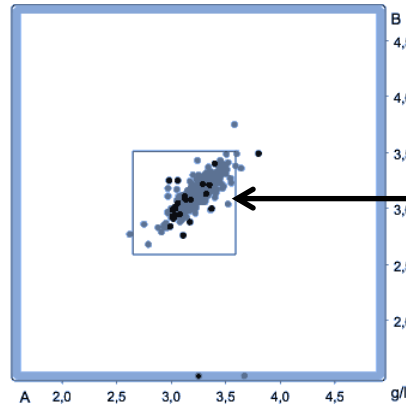
Co-ordinators

Partners

Co-ordinators

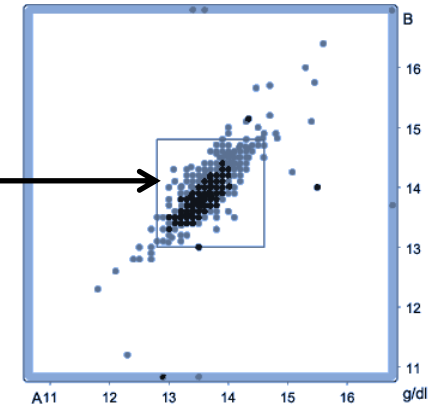


IG3/14
Transferrin
Kit 28



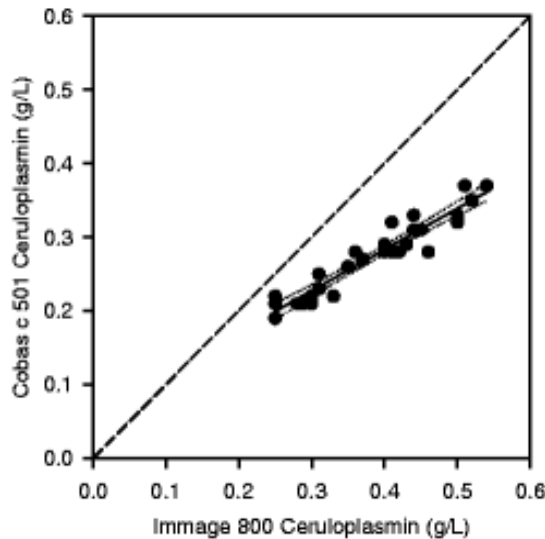
15 %

HA3/14
Haemoglobin
Kit 70



6 %

Reference institute for bioanalytics
<http://www.dgkl-rfb.de/>



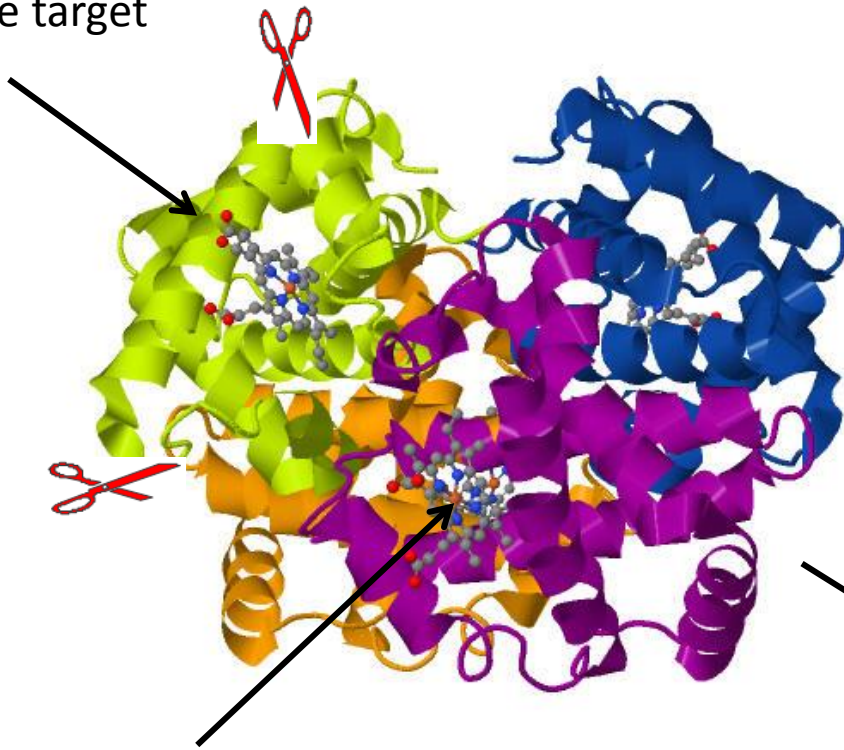
Example: Ceruloplasmin (CER)

- Cu storage protein
- marker for Wilson's disease - a genetic defect in 1:30 000 to 1:300 000 persons
- if untreated ⇒ liver failure and neurological defects
- methods have to distinguish between functional and non-functional protein forms

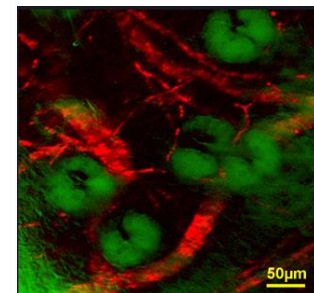
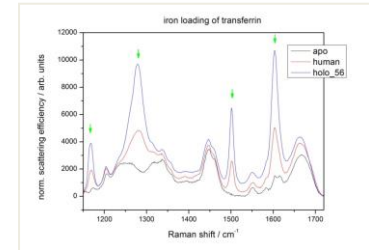
¹Infusino I. et al., Anal Bioanal Chem (2010) 397:521-525

Multimodal approach for protein quantification

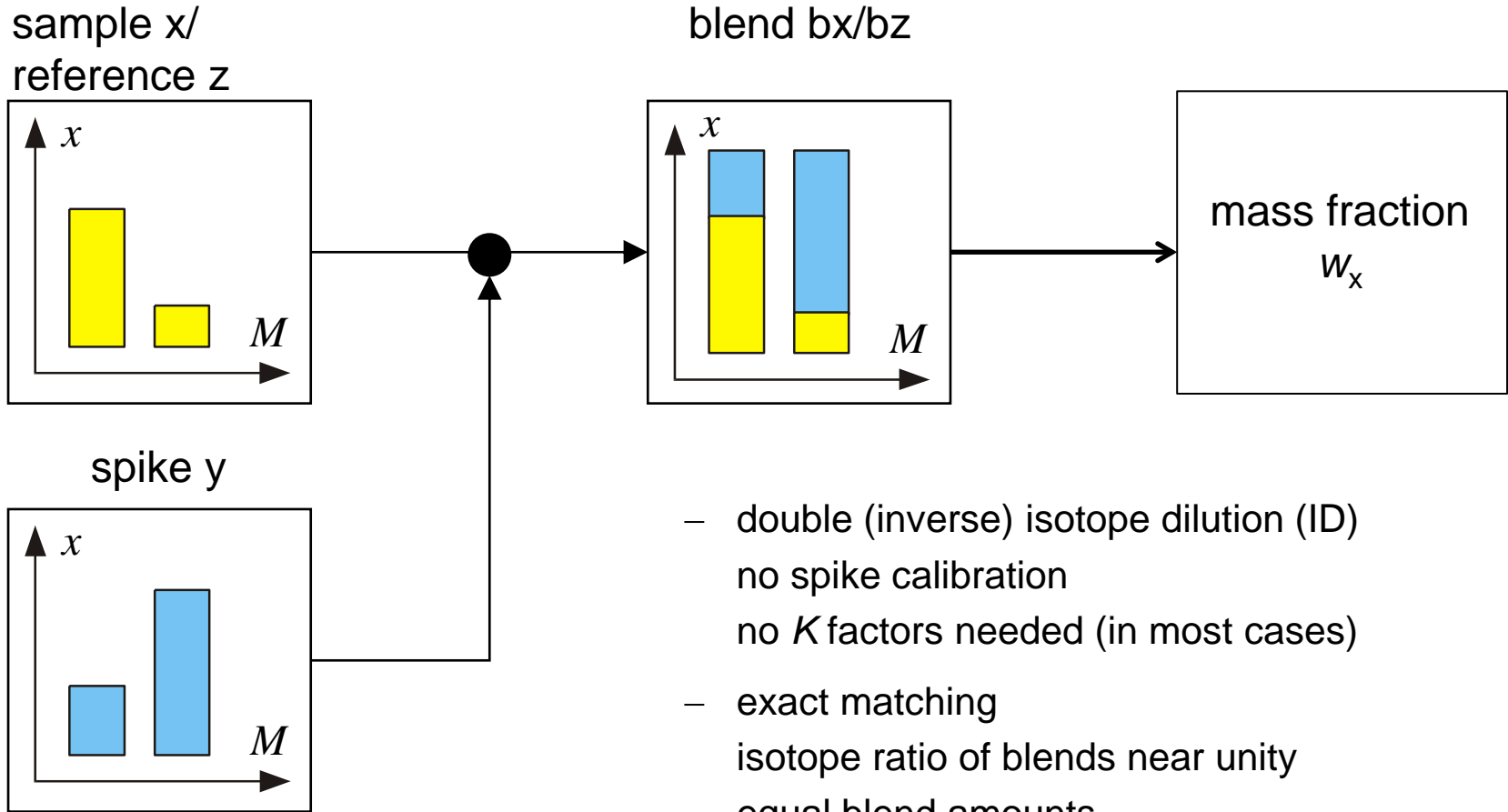
organic MS
peptide target



ICP-MS
determination of elements

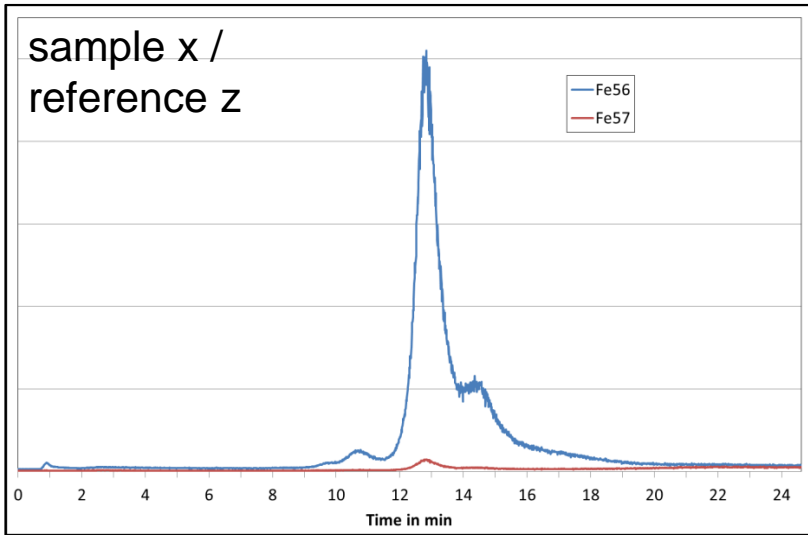


Isotope dilution approach

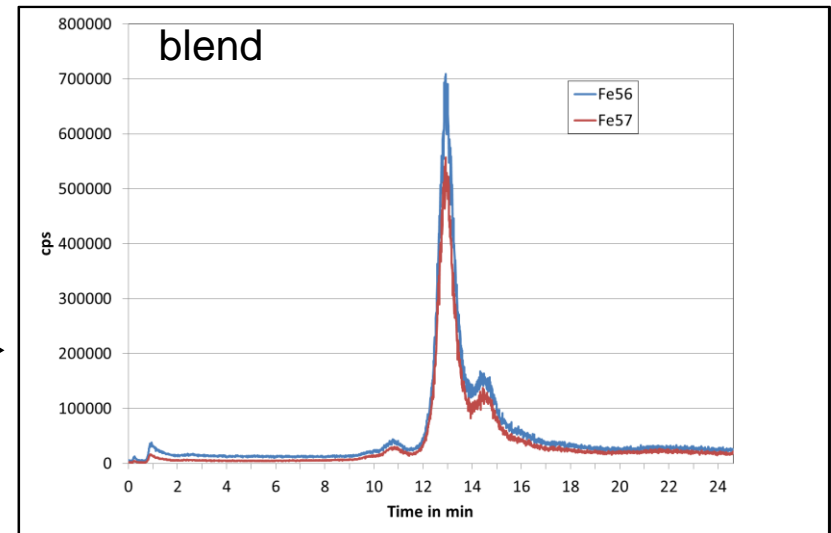
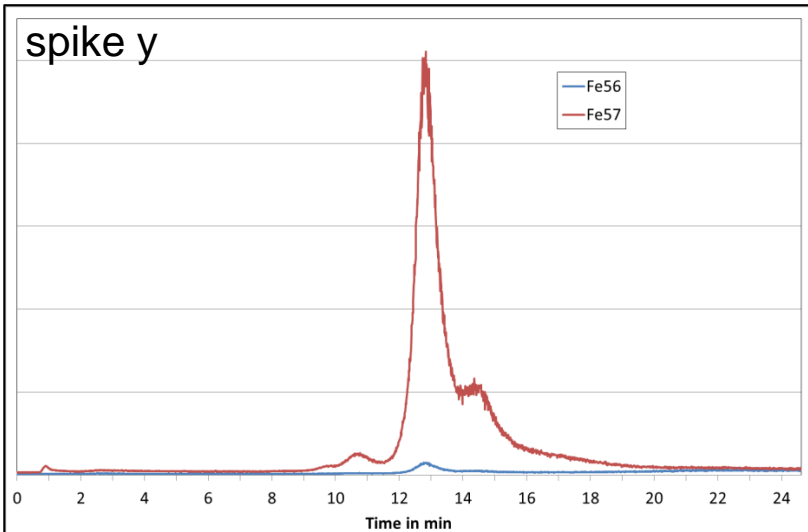


- double (inverse) isotope dilution (ID)
no spike calibration
no K factors needed (in most cases)
- exact matching
isotope ratio of blends near unity
equal blend amounts
no dead time, back ground, ... correction
both blends share the same "fate"

Isotope dilution approach

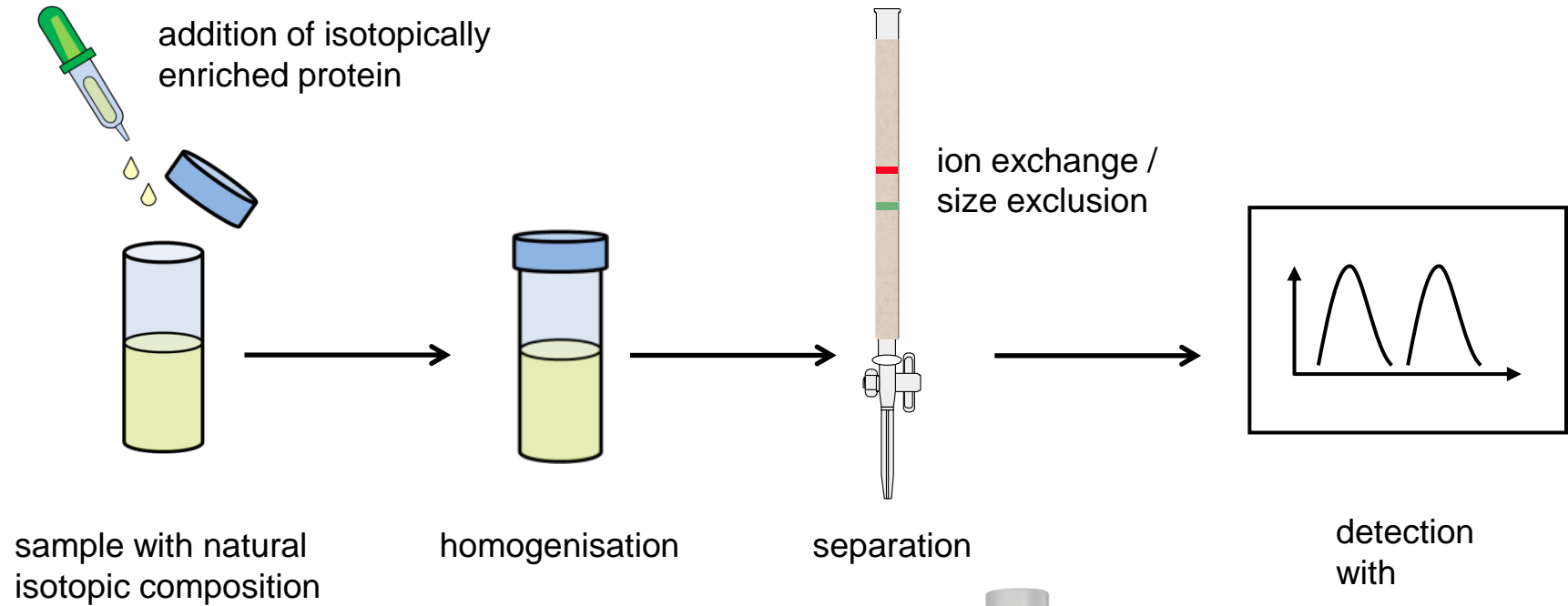


+



$$w_x = w_z \cdot \frac{m_{xy}}{m_x} \cdot \frac{m_z}{m_{zy}} \cdot \frac{R_y - R_{bx}}{R_{bx} - R_x} \cdot \frac{R_{bz} - R_z}{R_y - R_{bz}}$$

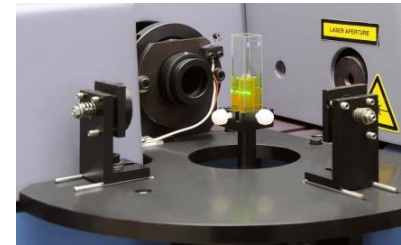
Quantification of proteins using ID



ICP-MS



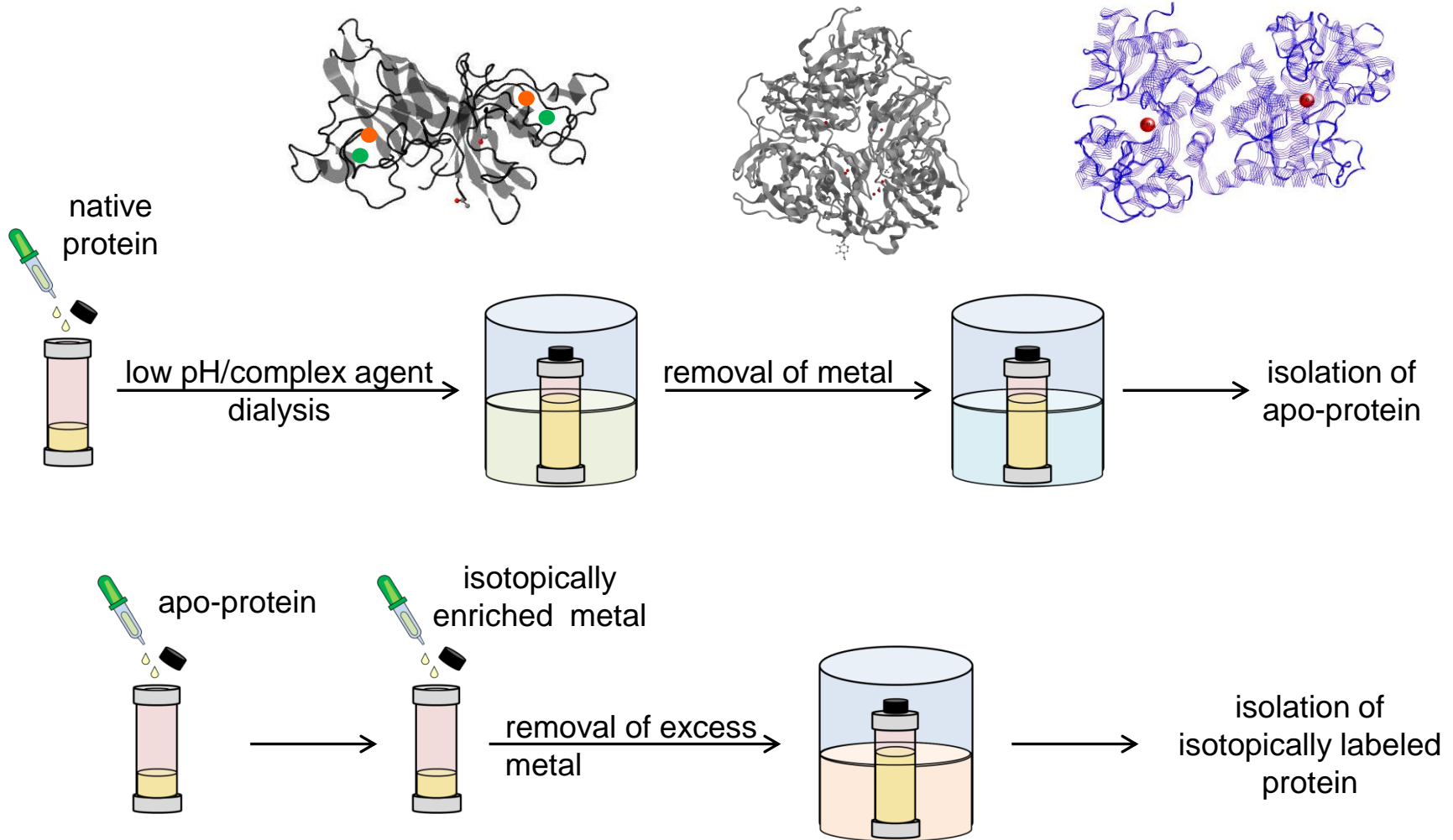
MS/MS



Raman

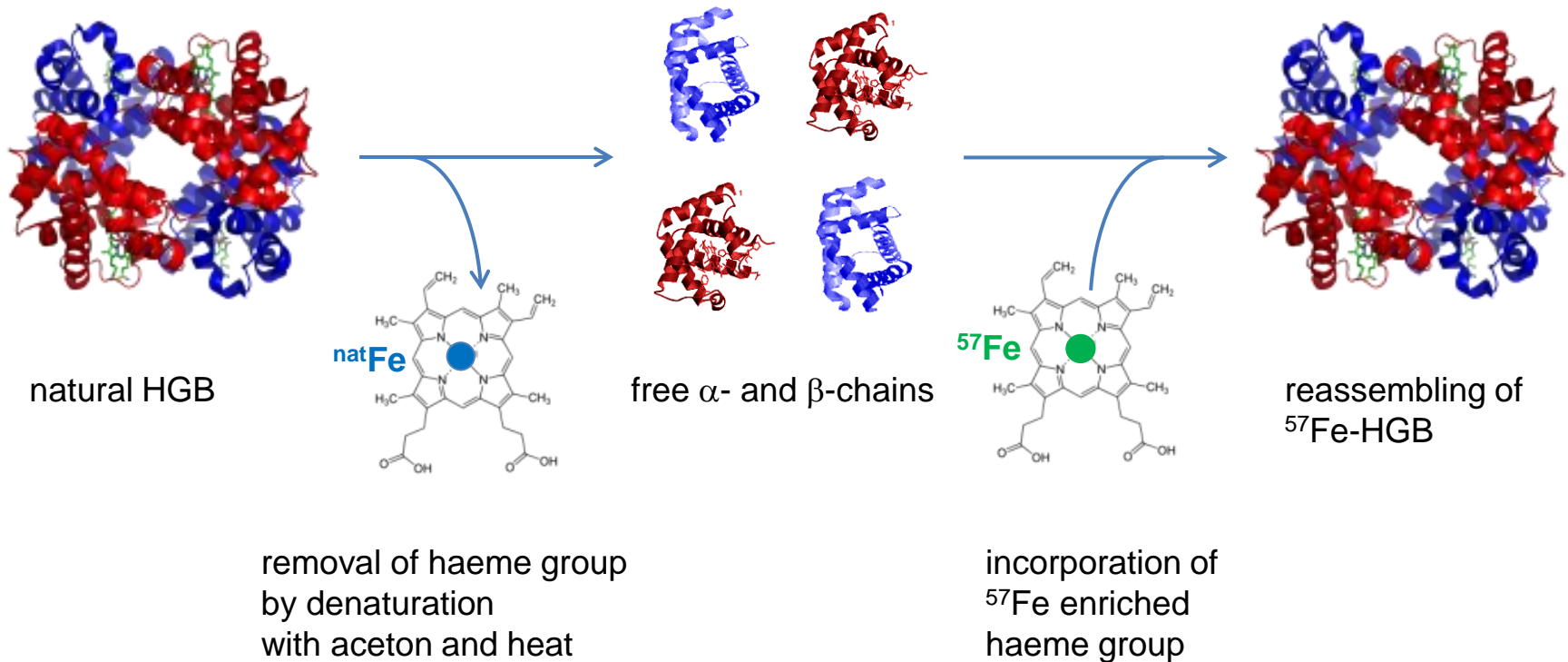
Species specific spike production

metals easily exchangeable such as Cu in SOD or CER or Fe in TRF



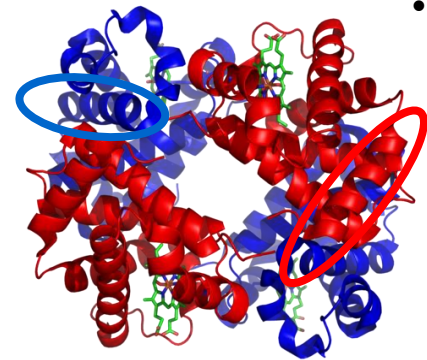
Species specific spike production

proteins with metals more strongly bound such as Fe in HGB



HGB quantification via characteristic peptides

- measurand of clinical importance: percentage of HBA₂ in blood
- IDMS for determination of HBA₂ and total HGB in reference materials



HBA₀/A₂ ($\alpha_2\beta_2$ / $\alpha_2\delta_2$)

proteolysis
(trypsin)

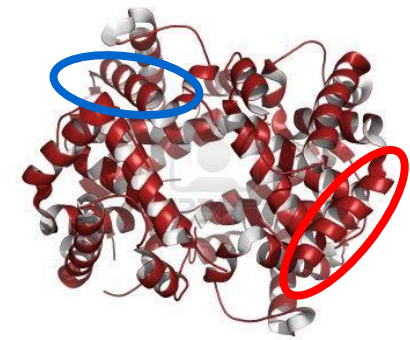
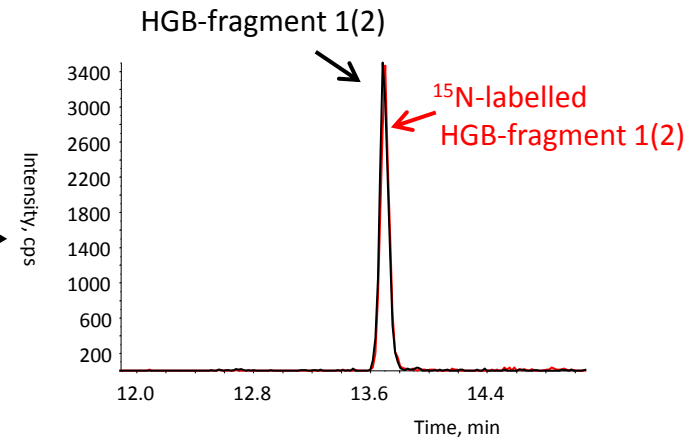


proteolytic fragments of
HGB

LC/MS-MS



¹⁵N -labelled
proteolytic fragments of HGB



U-¹⁵N-HBA₀/A₂ (internal standards)

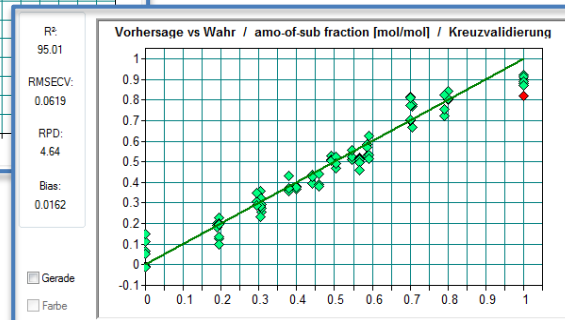
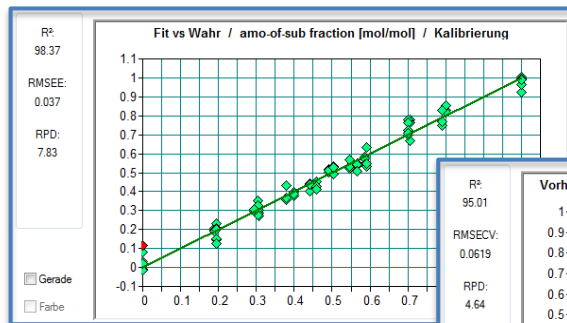
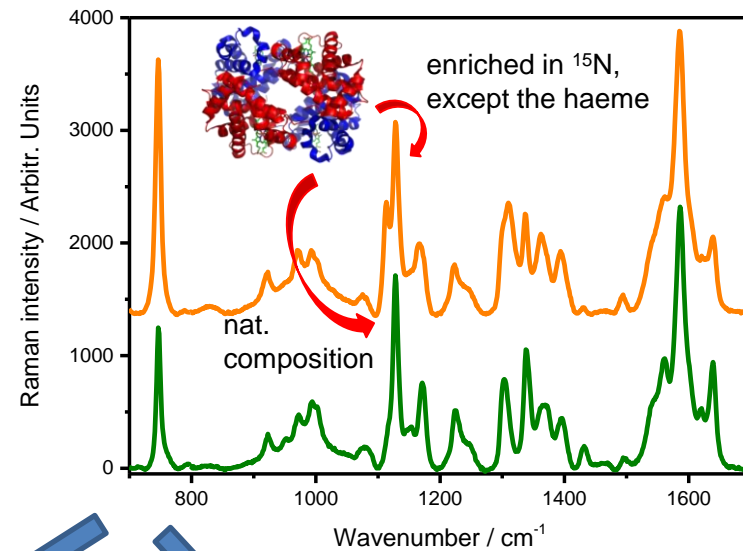
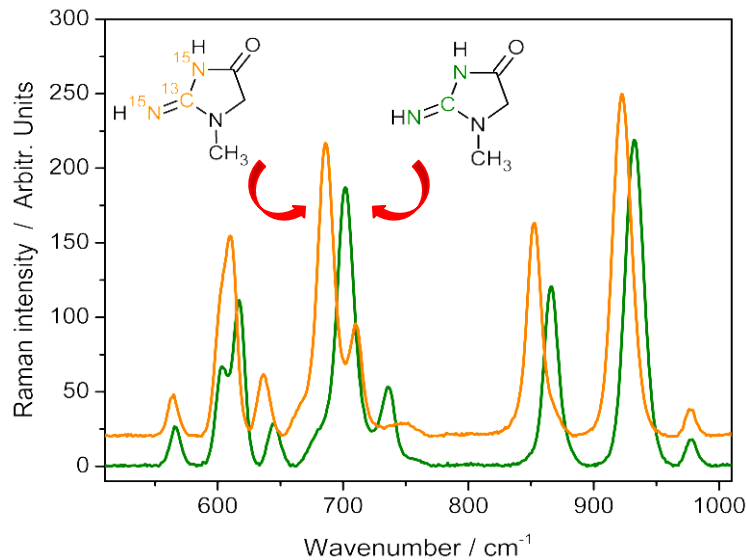
- added to sample in defined amounts

percentage of HBA₂ = $\frac{[HBA_2]}{[total\ HGB]} * 100$

result 1 for HBA₀ or HBA₂
by quantifying **Hb-fragment 1** of **β-** or **δ-globin**

result 2 for „total HGB“
by quantifying **HGB-fragment 2** of **α-globin**

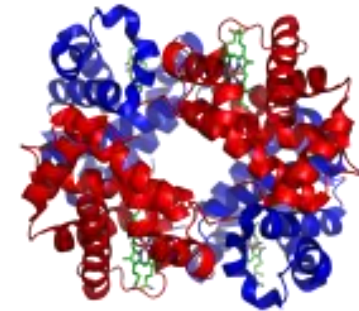
Protein quantification with ID-Raman



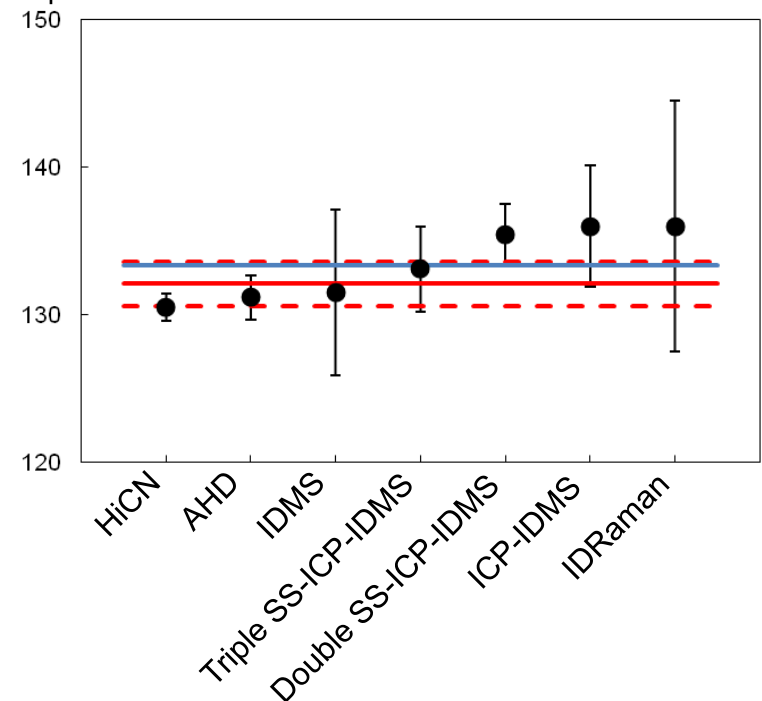
method	value g/dL Hb
ID Raman	16.20 ± 1.78
clinical lab	14.4 ± 0.9

S. Zakel, S. Wundrack, G. O'Connor, B. Guettler, R. Stosch, Journal of Raman Spectroscopy, 2013, 44 (9), 1246-1252

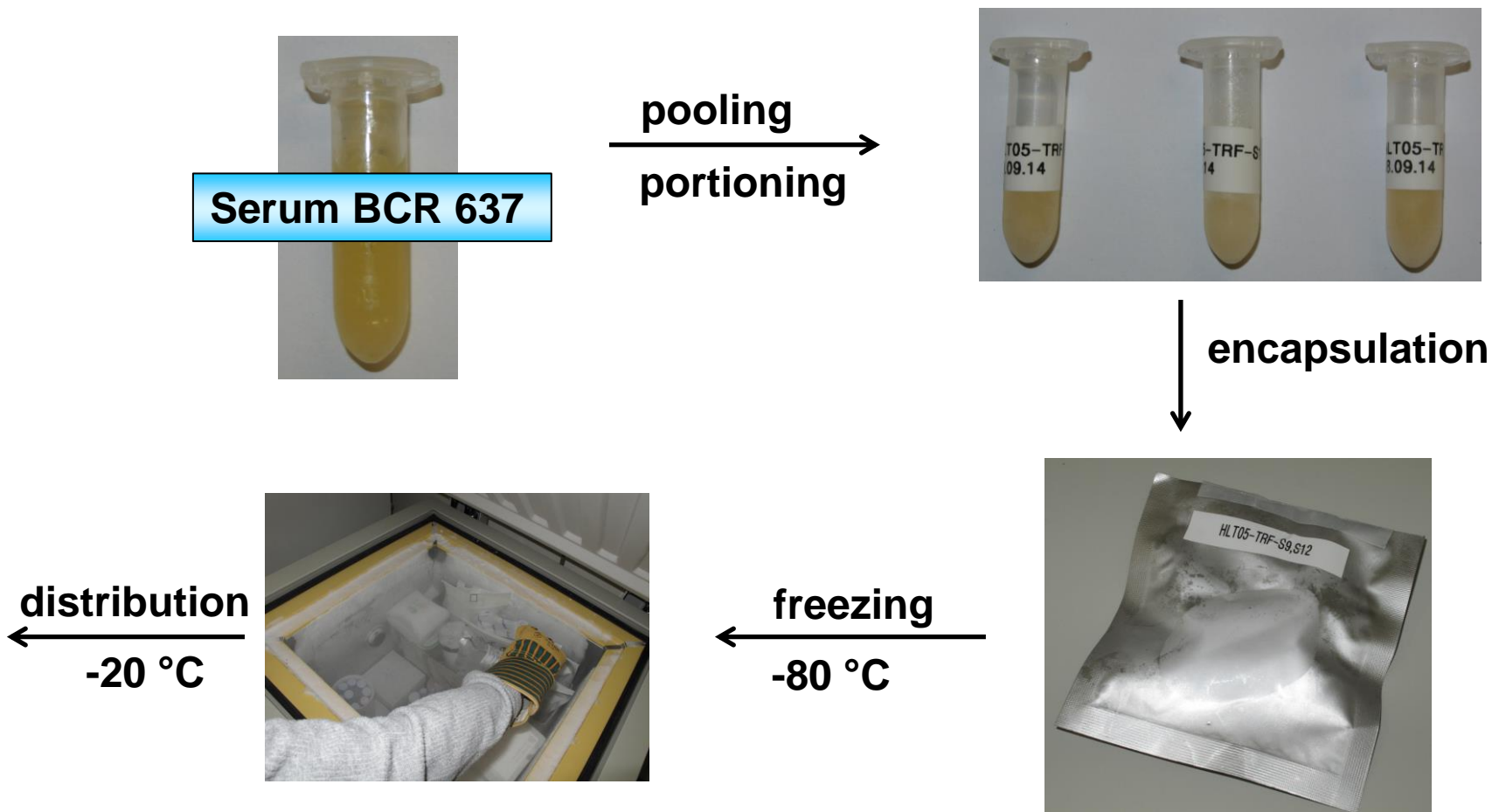
Comparison of HGB results



Method	Mass fraction <i>w</i> / g/kg	Uncertainty (<i>k</i> =2) <i>u</i> _{rel} / %
certified value JCCRM 912-2M	132.1 ± 1.5	1.1
HiCN-method	130.5 ± 0.9	0.7
AHD-method	131.2 ± 1.5	1.1
IDMS	131.5 ± 5.6	3.9
triple SS-ICP-IDMS	133.1 ± 2.9	2.2
double SS-ICP-IDMS	135.4 ± 2.1	1.6
ICP-IDMS	136.0 ± 4.1	3.0
ID-Raman	136.0 ± 8.5	6.2



Transferrin in human serum (Ref. 1351)



Transferrin in human serum (Ref. 1351)

Sample: BCR[®]-637 human Serum (untreated Serum)

Instrumentation: HPLC-ICP-MS

Monitored elements: Fe (participants 1-3), S (participant 4)

4 Participants

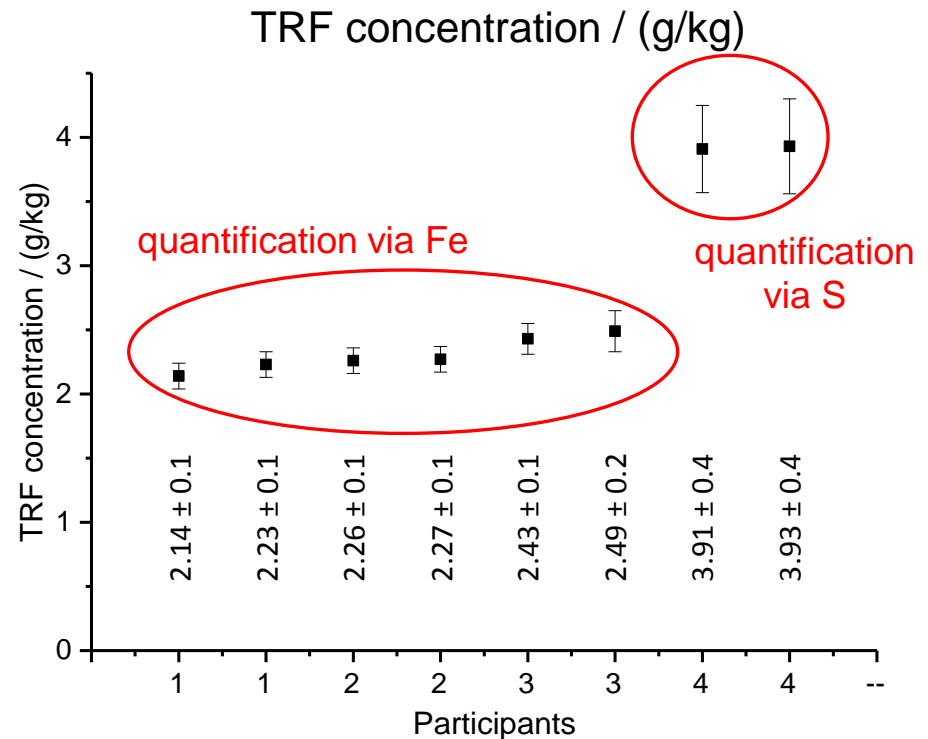
Shipped and stored at -80 °C

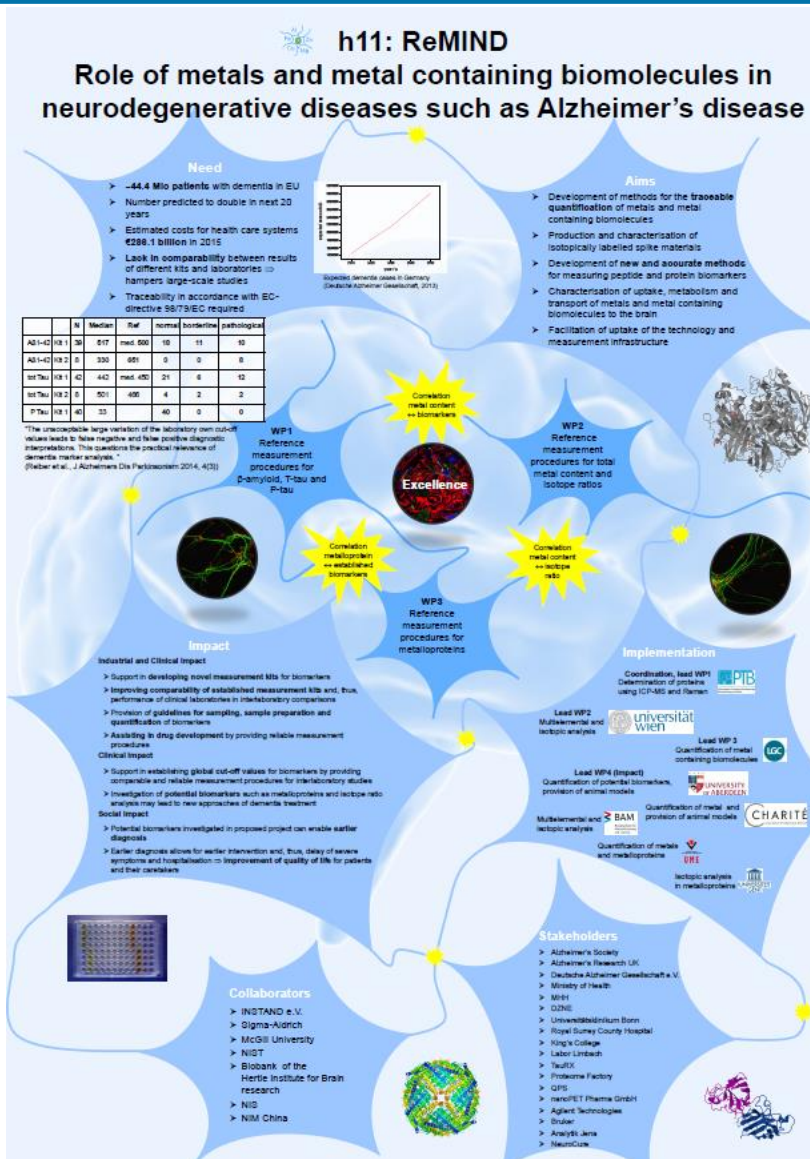


Results:

Deviation between analysis of Fe or S

Problems with interferences in untreated Serum





Aims

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



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