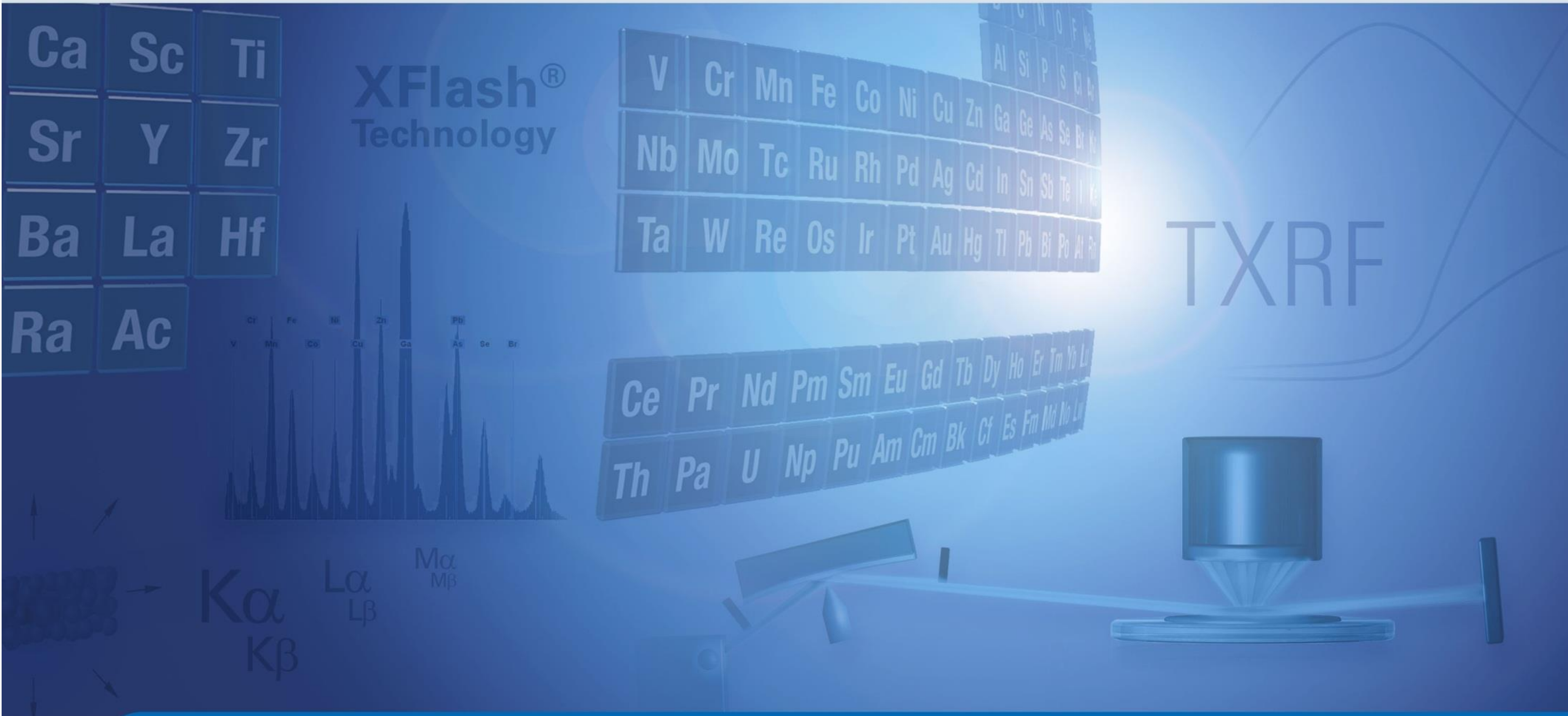
Do it yourself - Metal analysis in biological and medical samples

Bruker Nano Analytics, Berlin
Webinar, February 27th, 2019



Welcome

Speakers

Dr. Hagen Stosnach
Applications Scientist TXRF
Berlin, Germany

Dr. Po-Wah So
Senior lecturer in biomedical imaging and spectroscopy
King's College London

Dr. Armin Gross
Global Product Manager TXRF
Berlin, Germany

Itinerary

Part I: Introduction and background

- Motivation
- TXRF spectroscopy

Part II: Po-Wah So

- TXRF-based element profiling in Alzheimer‘s Disease

Part III: Protein analysis

Part IV: Summary/Q & A

Part I: Introduction and background

Introduction
Motivation

Metals play a crucial role for cellular and subcellular functions

- The divalent cations Zn^{2+} , Ca^{2+} and Mg^{2+} prevent cytotoxicity and in vivo antagonize Cd-induced carcinogenesis.
- Lack of body iron is common in cancer patients and it is associated with complications in surgery and in animal experiments. The transport of iron and other metal ions by the blood plasma is achieved through the formation of protein complexes.
- Copper is recognized as an essential element and is primarily associated with copper-dependent cellular enzymes triggering redox reactions or for the stabilization of protein particulates.
- In addition it is well known that a number of toxic metals can inhibit enzyme functions, which leads to serious health issues.

Introduction
Motivation

Common methods for metal analysis

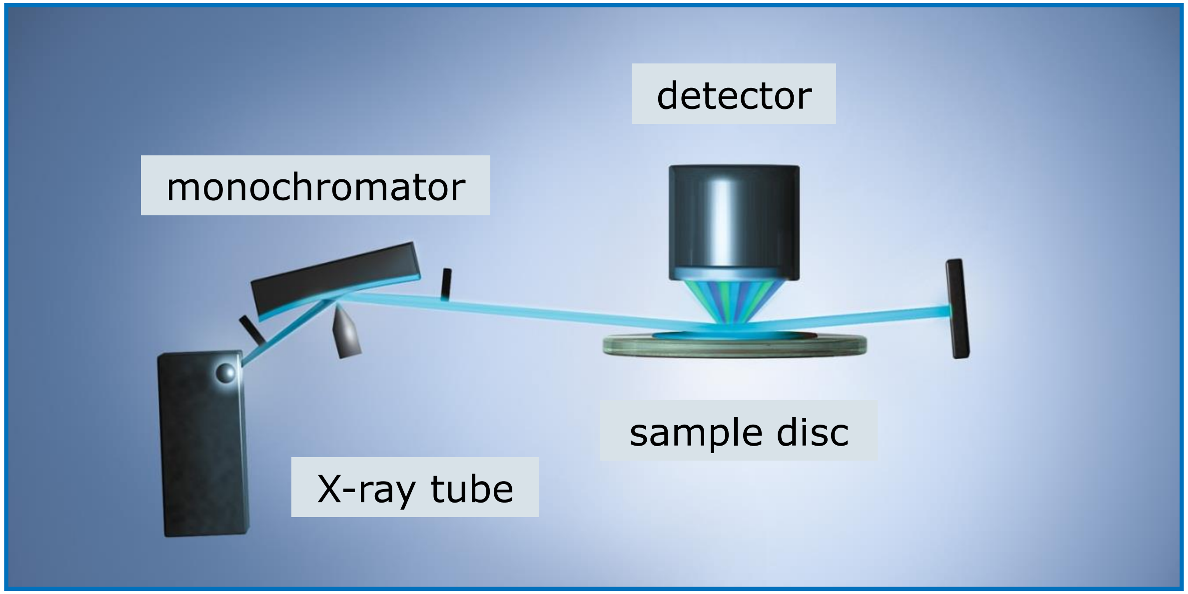
- Atomic absorption spectroscopy (AAS)
- Inductively-coupled plasma optical emission spectroscopy (ICP-OES)
- Inductively-coupled plasma mass spectrometry (ICP-MS)
  - High demands on laboratory environment and staff
  - Necessary sample amounts are often too high

⇨ Samples are sent to central or service labs for analysis

⇨ restricted numbers of analyzed samples
⇨ high costs
⇨ long waiting time for samples

Introduction
TXRF spectroscopy

Total reflection X-ray fluorescence spectroscopy



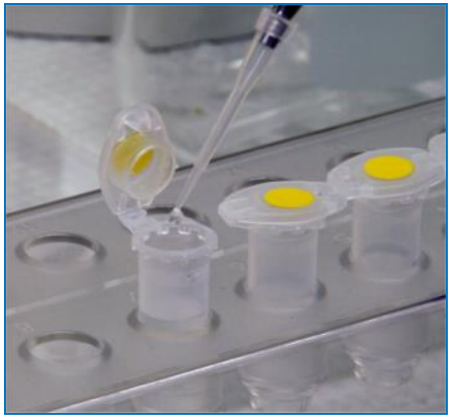
Beam angle: 0° / 90°

- Samples must be prepared on a reflective media
- Polished quartz glass or polyacrylic glass disc
- Dried to a thin layer, or as a thin film or microparticle

Introduction
TXRF spectroscopy

Sample preparation of minute liquid samples

- Aliquotation of sample (10 µl – 10 ml)
- Addition of internal standard (mono-element solution)
- Homogenization (mixing)
- Transfer of 10 µl sample to sample carrier
- Drying



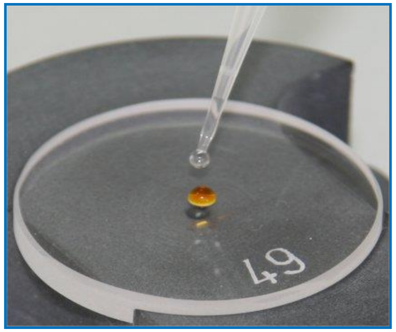
Introduction
TXRF spectroscopy

Sample preparation of minute liquid samples (< 10 µl)

- Preparation of sample droplet on sample carrier
- Direct addition of internal standard onto sample droplet
- Drying

⇨ Results are not as reproducible as for external mixing but in most cases still sufficient

⇨ Also possible: Weighing of microsamples directly on carrier and subsequent internal standardization and ashing or digestion



Introduction
TXRF spectroscopy

Sample preparation of solid or very viscous samples

- Weighing of sample (amount depending on balance working range)
- Suspension in water or alcohol (ultrasonic bath optional)
- Addition of internal standard
- Homogenization
- Transfer of 10 µl sample to sample carrier
- Drying

Introduction
TXRF spectroscopy

S2 PICOFOX

- Mo-tube 50 kV/1000 µA
- 60 mm² XFlash SDD
- 25 position sample changer

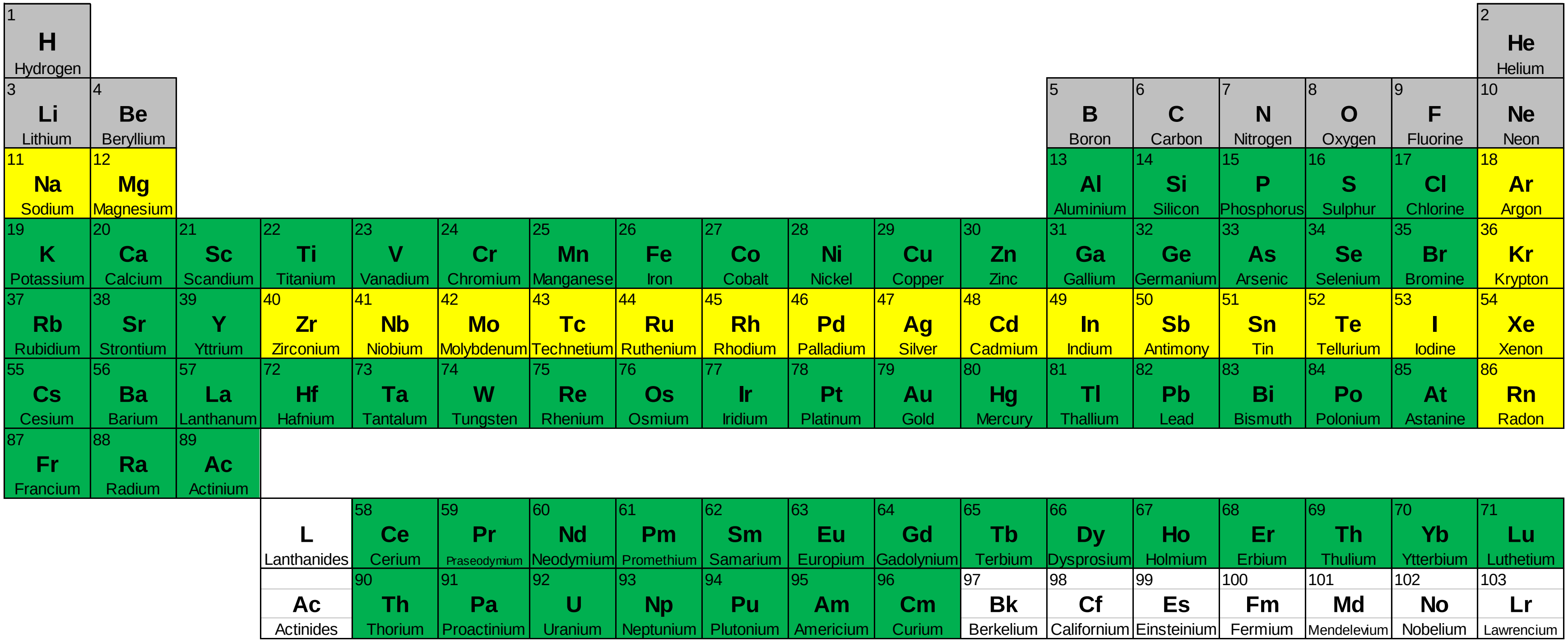
S4 T·STAR

- Mo tube, 50 kV/1000 µA
- W-tube, 50 kV/1000 µA
- Monochromator system for Mo-K, W-L and W-Brems monochromatisation
- 60 mm² XFlash SDD
- 90 position sample changer

Introduction

TXRF spectroscopy

Element range S2 PICOFOX



⬜ 1 **H** Hydrogen																	⬜ 2 **He** Helium
⬜ 3 **Li** Lithium	⬜ 4 **Be** Beryllium											⬜ 5 **B** Boron	⬜ 6 **C** Carbon	⬜ 7 **N** Nitrogen	⬜ 8 **O** Oxygen	⬜ 9 **F** Fluorine	⬜ 10 **Ne** Neon
🟨 11 **Na** Sodium	🟨 12 **Mg** Magnesium											🟩 13 **Al** Aluminium	🟩 14 **Si** Silicon	🟩 15 **P** Phosphorus	🟩 16 **S** Sulphur	🟩 17 **Cl** Chlorine	🟨 18 **Ar** Argon
🟩 19 **K** Potassium	🟩 20 **Ca** Calcium	🟩 21 **Sc** Scandium	🟩 22 **Ti** Titanium	🟩 23 **V** Vanadium	🟩 24 **Cr** Chromium	🟩 25 **Mn** Manganese	🟩 26 **Fe** Iron	🟩 27 **Co** Cobalt	🟩 28 **Ni** Nickel	🟩 29 **Cu** Copper	🟩 30 **Zn** Zinc	🟩 31 **Ga** Gallium	🟩 32 **Ge** Germanium	🟩 33 **As** Arsenic	🟩 34 **Se** Selenium	🟩 35 **Br** Bromine	🟨 36 **Kr** Krypton
🟩 37 **Rb** Rubidium	🟩 38 **Sr** Strontium	🟩 39 **Y** Yttrium	🟨 40 **Zr** Zirconium	🟨 41 **Nb** Niobium	🟨 42 **Mo** Molybdenum	🟨 43 **Tc** Technetium	🟨 44 **Ru** Ruthenium	🟨 45 **Rh** Rhodium	🟨 46 **Pd** Palladium	🟨 47 **Ag** Silver	🟨 48 **Cd** Cadmium	🟨 49 **In** Indium	🟨 50 **Sb** Antimony	🟨 51 **Sn** Tin	🟨 52 **Te** Tellurium	🟨 53 **I** Iodine	🟨 54 **Xe** Xenon
🟩 55 **Cs** Cesium	🟩 56 **Ba** Barium	🟩 57 **La** Lanthanum	🟩 72 **Hf** Hafnium	🟩 73 **Ta** Tantalum	🟩 74 **W** Tungsten	🟩 75 **Re** Rhenium	🟩 76 **Os** Osmium	🟩 77 **Ir** Iridium	🟩 78 **Pt** Platinum	🟩 79 **Au** Gold	🟩 80 **Hg** Mercury	🟩 81 **Tl** Thallium	🟩 82 **Pb** Lead	🟩 83 **Bi** Bismuth	🟩 84 **Po** Polonium	🟩 85 **At** Astanine	🟨 86 **Rn** Radon
🟩 87 **Fr** Francium	🟩 88 **Ra** Radium	🟩 89 **Ac** Actinium															
			L Lanthanides	🟩 58 **Ce** Cerium	🟩 59 **Pr** Praseodymium	🟩 60 **Nd** Neodymium	🟩 61 **Pm** Promethium	🟩 62 **Sm** Samarium	🟩 63 **Eu** Europium	🟩 64 **Gd** Gadolynium	🟩 65 **Tb** Terbium	🟩 66 **Dy** Dysprosium	🟩 67 **Ho** Holmium	🟩 68 **Er** Erbium	🟩 69 **Th** Thulium	🟩 70 **Yb** Ytterbium	🟩 71 **Lu** Luthetium
			Ac Actinides	🟩 90 **Th** Thorium	🟩 91 **Pa** Proactinium	🟩 92 **U** Uranium	🟩 93 **Np** Neptunium	🟩 94 **Pu** Plutonium	🟩 95 **Am** Americium	🟩 96 **Cm** Curium	97 **Bk** Berkelium	98 **Cf** Californium	99 **Es** Einsteinium	100 **Fm** Fermium	101 **Md** Mendelevium	102 **No** Nobelium	103 **Lr** Lawrencium

⬜ Impossible to analyse

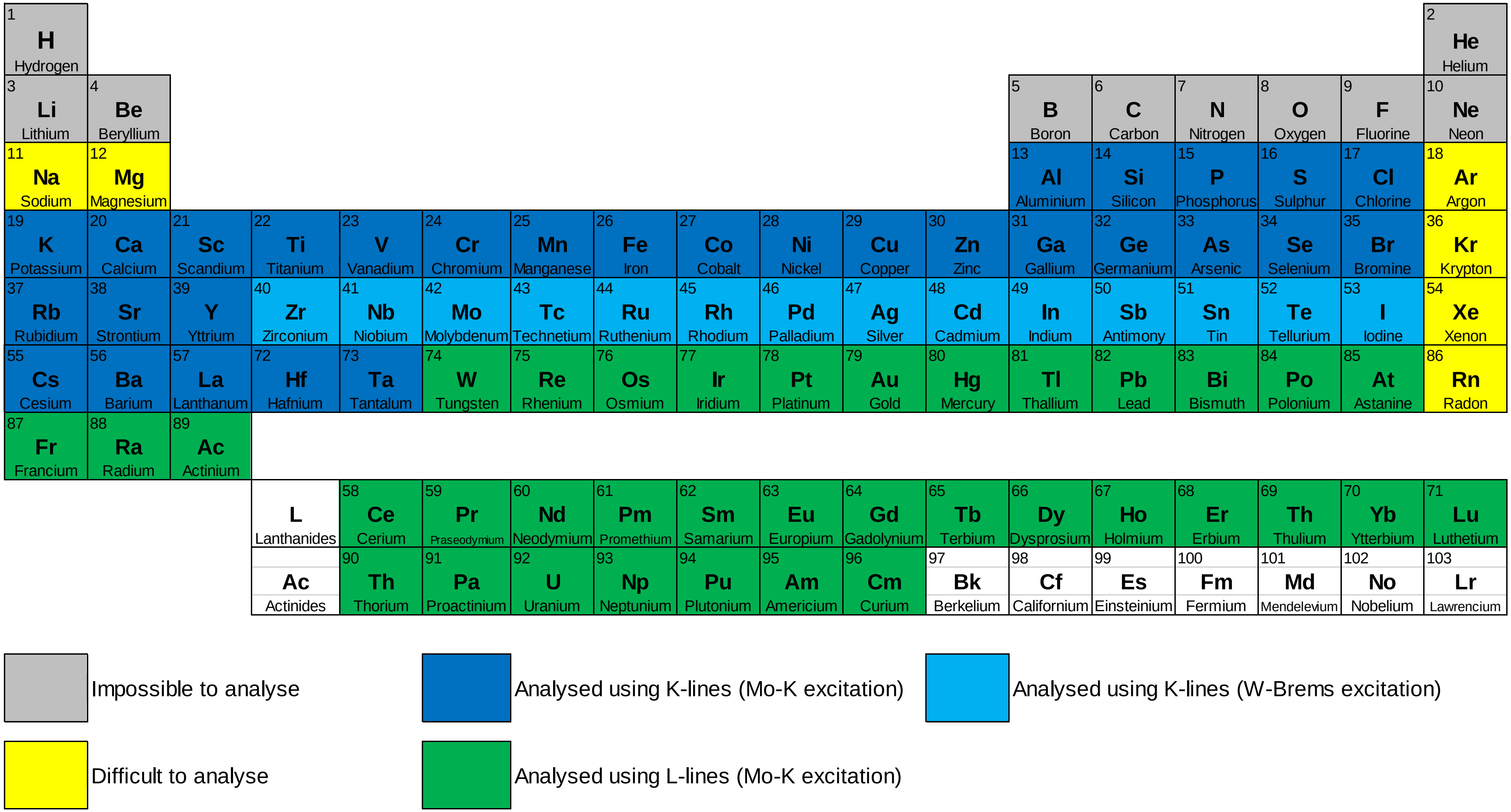
🟦 Analysed using K-lines

🟨 Difficult to analyse

🟩 Analysed using L-lines

Introduction
TXRF spectroscopy

Element range S4 T-STAR



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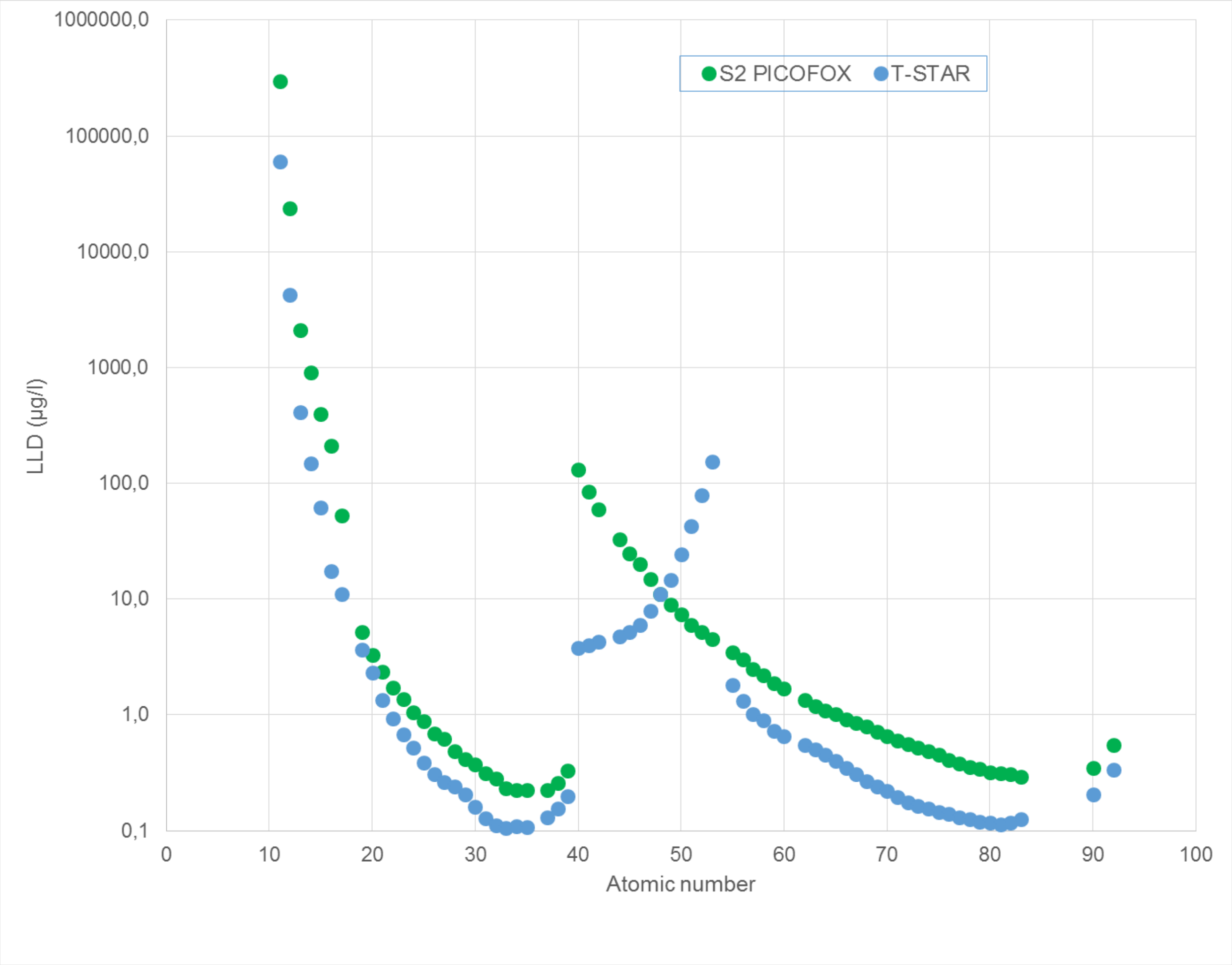
- ▨ Impossible to analyse
- 🟦 Analysed using K-lines (Mo-K excitation)
- 🔷 Analysed using K-lines (W-Brems excitation)
- 🟨 Difficult to analyse
- 🟩 Analysed using L-lines (Mo-K excitation)

Introduction
TXRF spectroscopy

Sensitivity S2 PICOFOX vs. S4 T-STAR



Part II: Dr. Po-Wah So

TXRF elemental profiling in Alzheimer‘s disease

TXRF-based Elemental Profiling in Alzheimer's Disease

Dr Po-Wah So

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Head, Phenomics Lab

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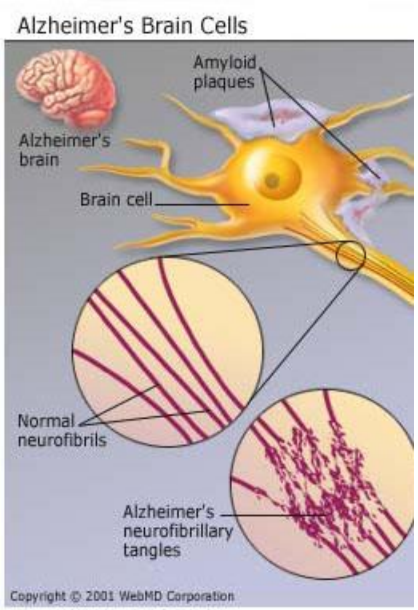
Department of Neuroimaging,

Maurice Wohl Clinical Neuroscience Institute,

Institute of Psychiatry, Psychology and Neuroscience

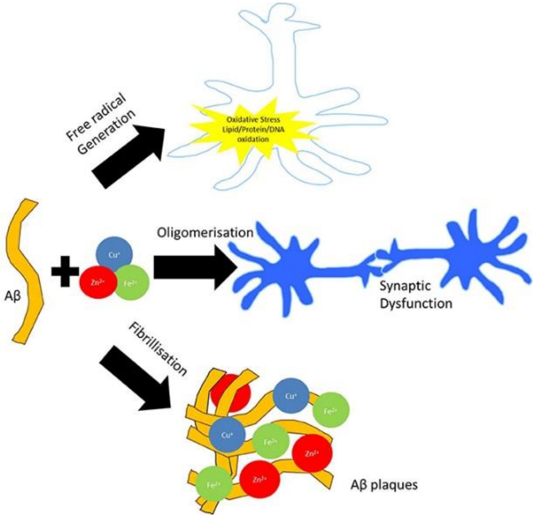
KING'S College LONDON

Causes of Alzheimer's Disease



Amyloid and Tau

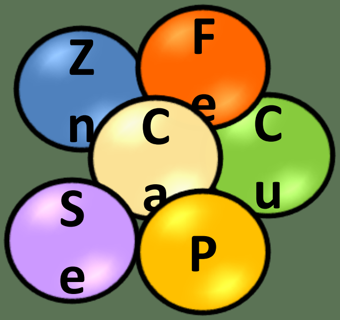
Metal Dyshomeostasis



Braidy et al., 2014. Front. Aging Neurosci., https://doi.org/10.3389/fnagi.2014.00138

Aim

Discriminate between control and Alzheimer’s Disease by plasma TXRF elemental profiling



Blood Metals/Elements in Alzheimer’s Disease

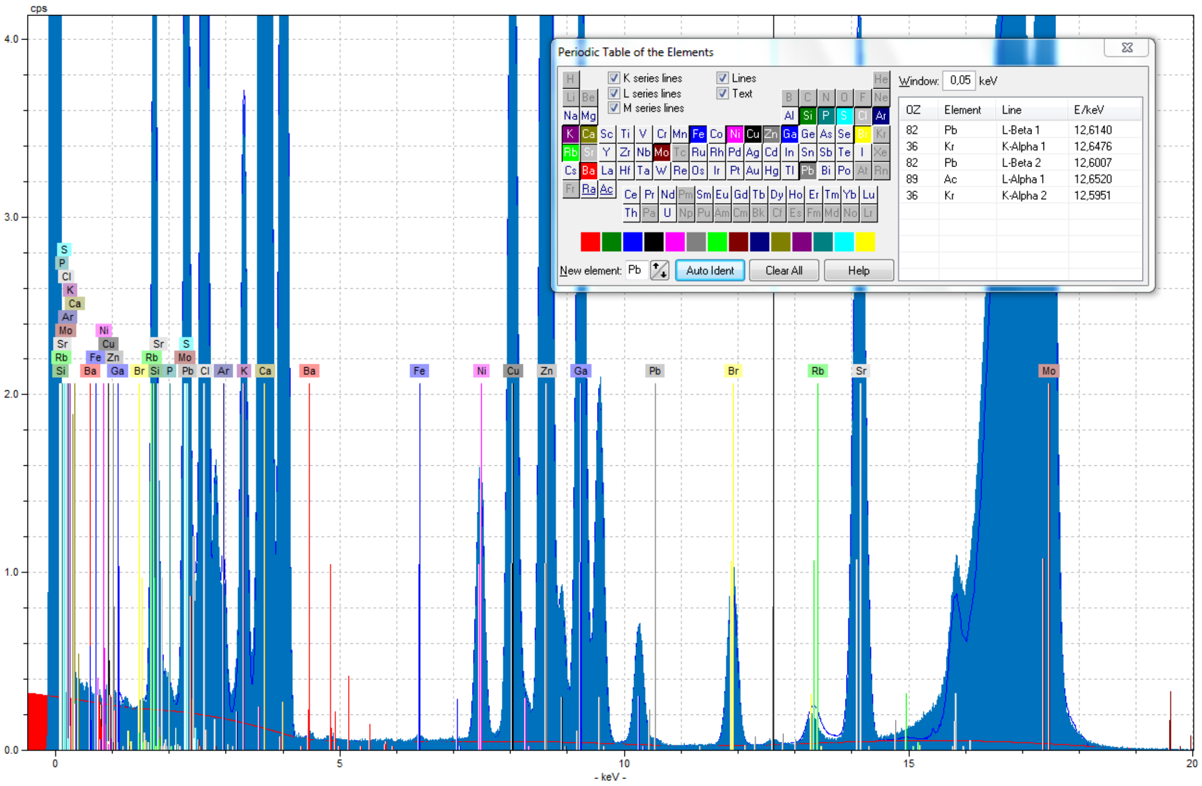
No consensus

Traditional elemental/metal analysis methods

matrix effects

limited elements measured concomitantly

Typical TXRF Spectrum of Human Plasma

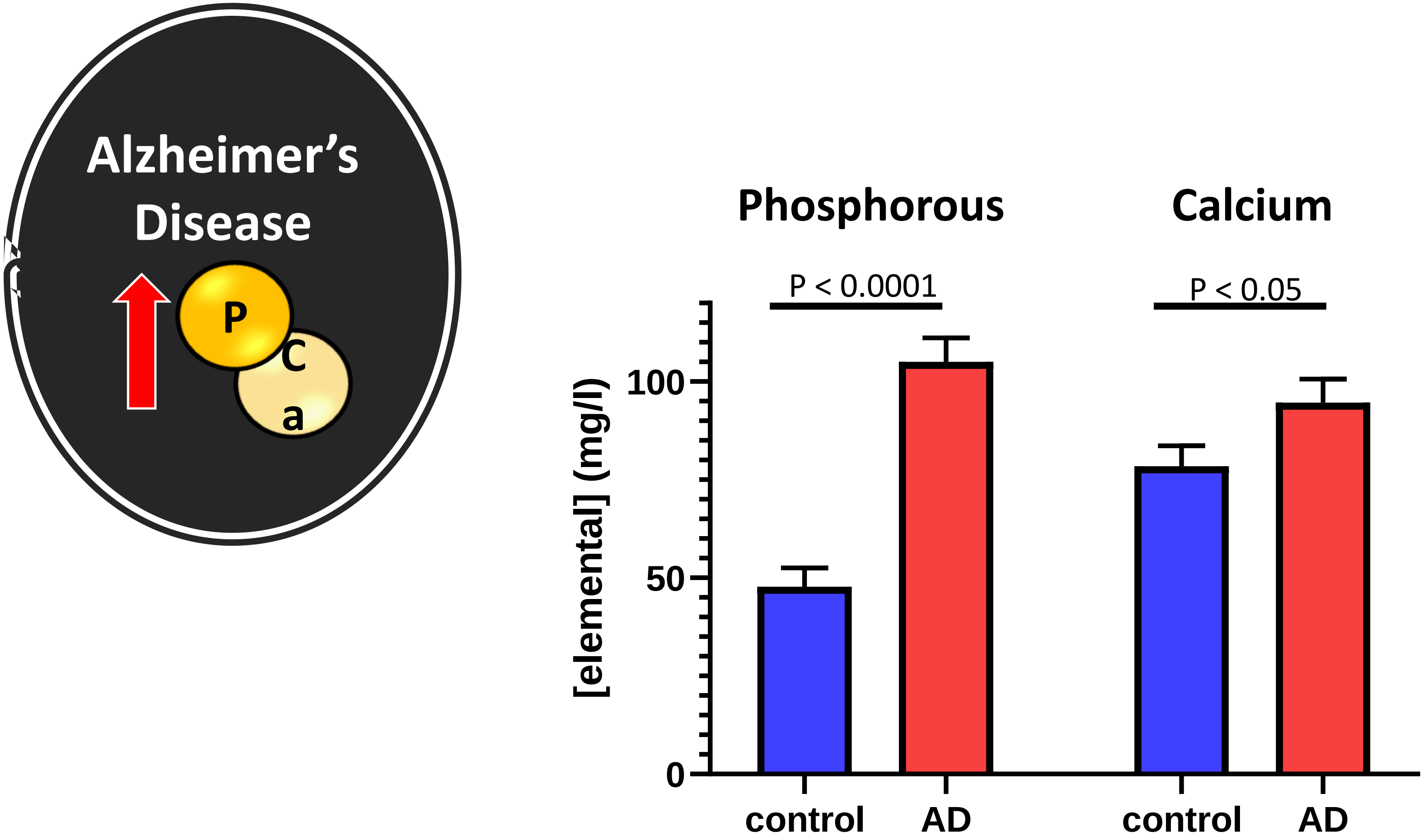


☑ K series lines
☑ L series lines
☑ M series lines
☑ Lines
☑ Text

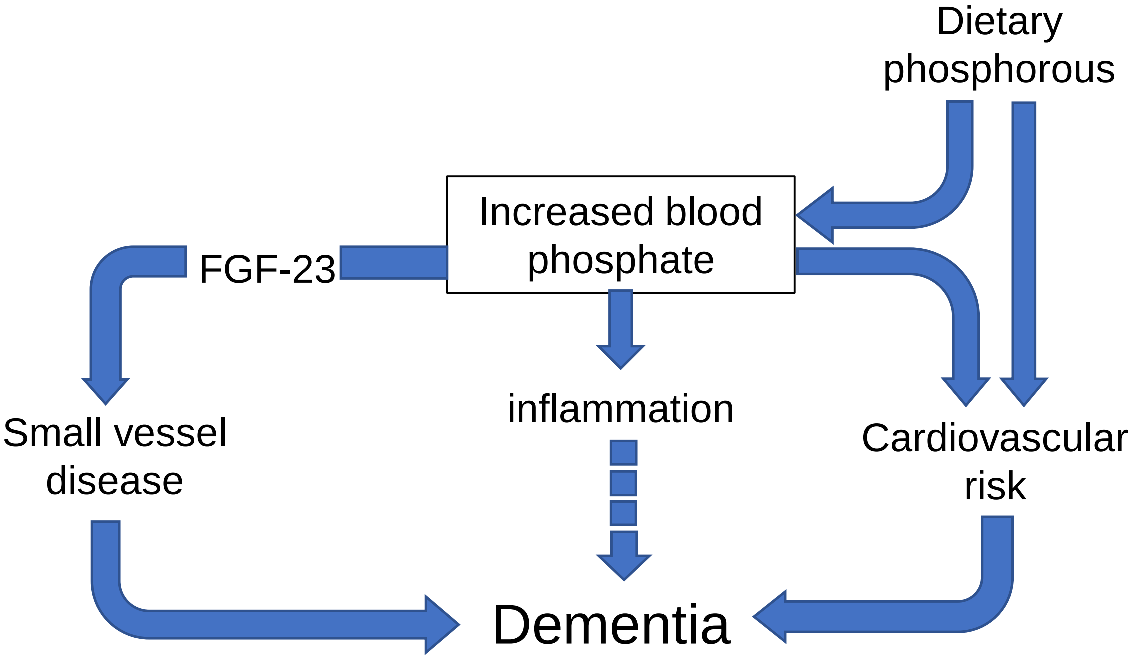
Window: 0,05 keV

New element: Pb

OZ	Element	Line	E/keV
82	Pb	L-Beta 1	12,6140
36	Kr	K-Alpha 1	12,6476
82	Pb	L-Beta 2	12,6007
89	Ac	L-Alpha 1	12,6520
36	Kr	K-Alpha 2	12,5951


Alzheimer's Disease
P
C
a
Phosphorous
Calcium
P < 0.0001
P < 0.05
[elemental] (mg/l)
100
50
0
control
AD
control
AD

Best discriminators of control and Alzheimer's Disease



Dietary phosphorous

Increased blood phosphate

FGF-23

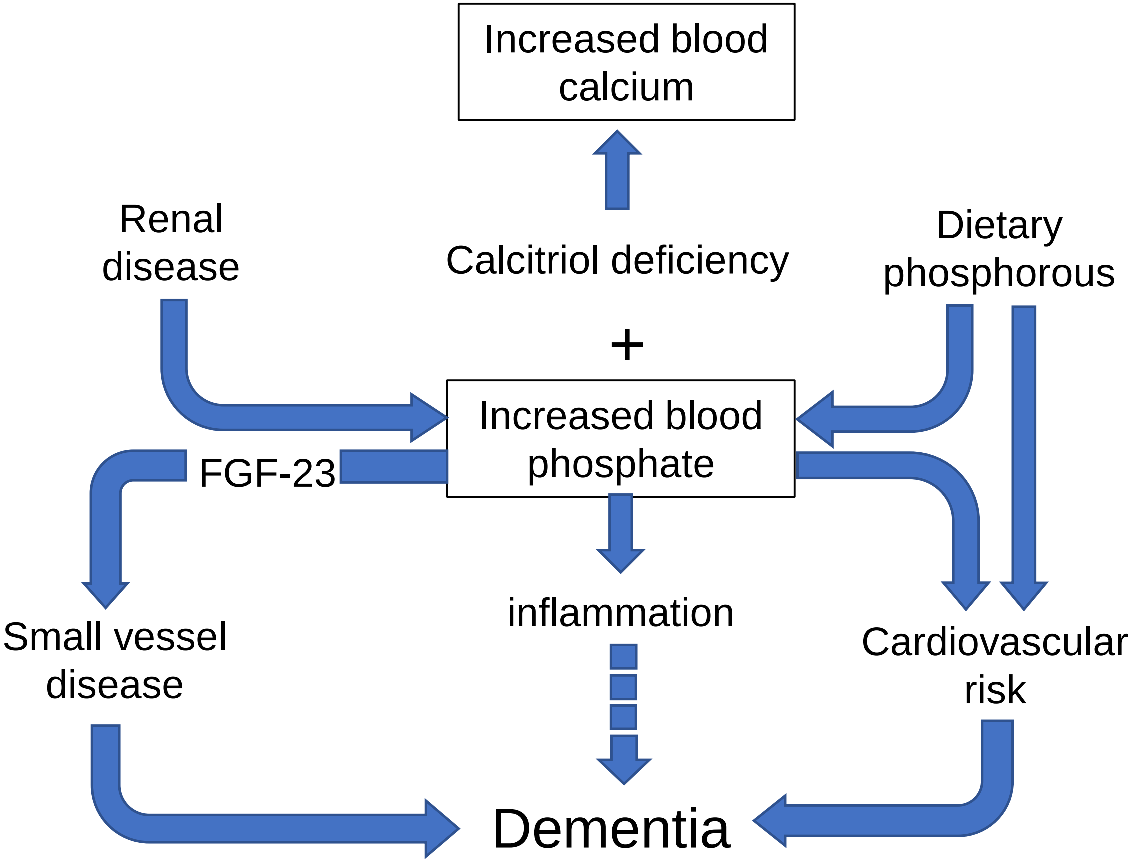
Small vessel disease

inflammation

Cardiovascular risk

Dementia

Best discriminators of control and Alzheimer's Disease



Increased blood calcium

Calcitriol deficiency

Renal disease

Dietary phosphorous

\+

Increased blood phosphate

FGF-23

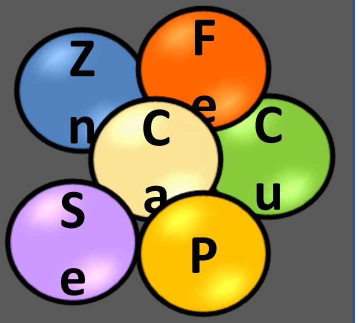
Small vessel disease

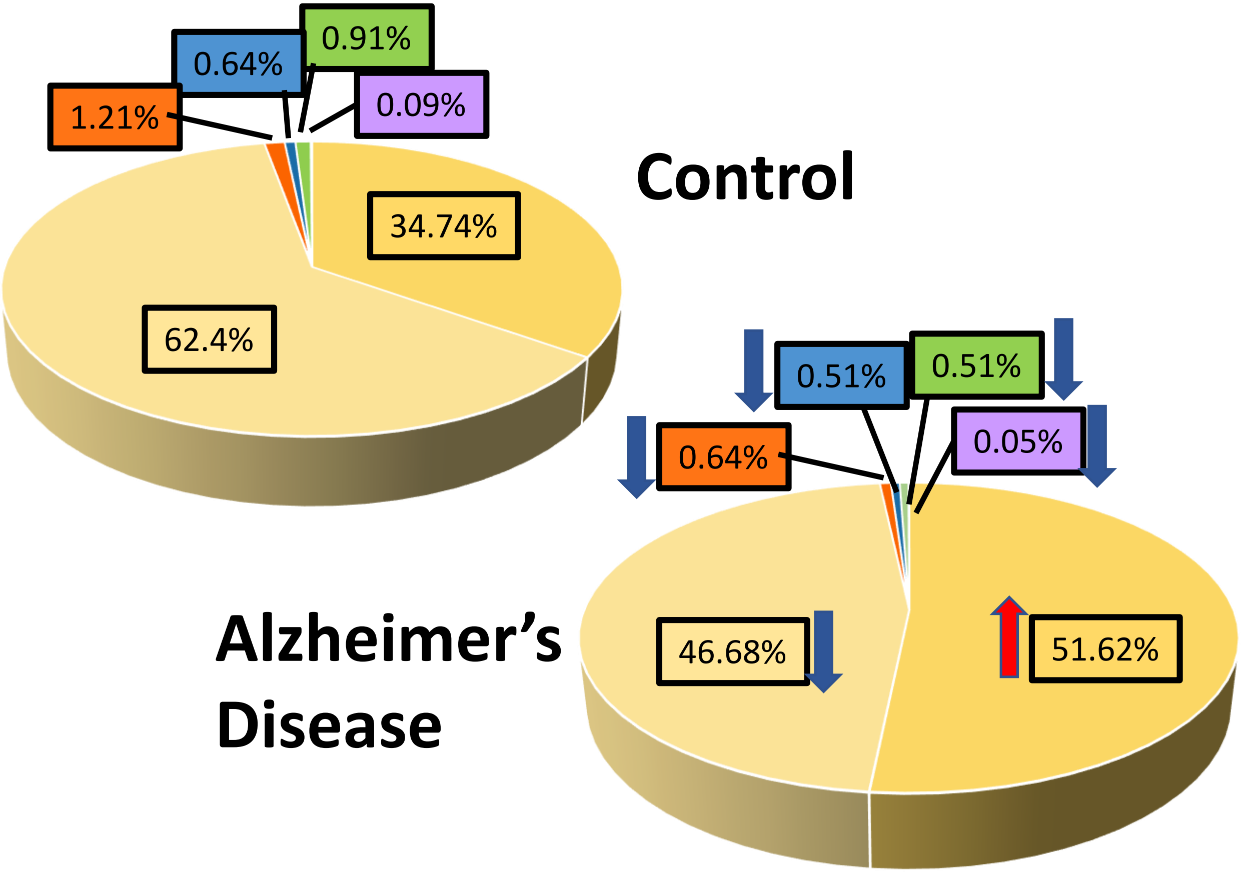
inflammation

Cardiovascular risk

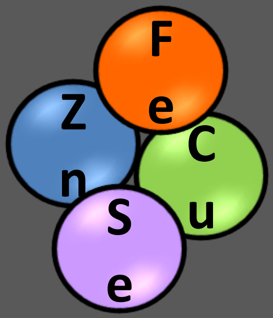
Dementia

Percentages of Elements in Plasma

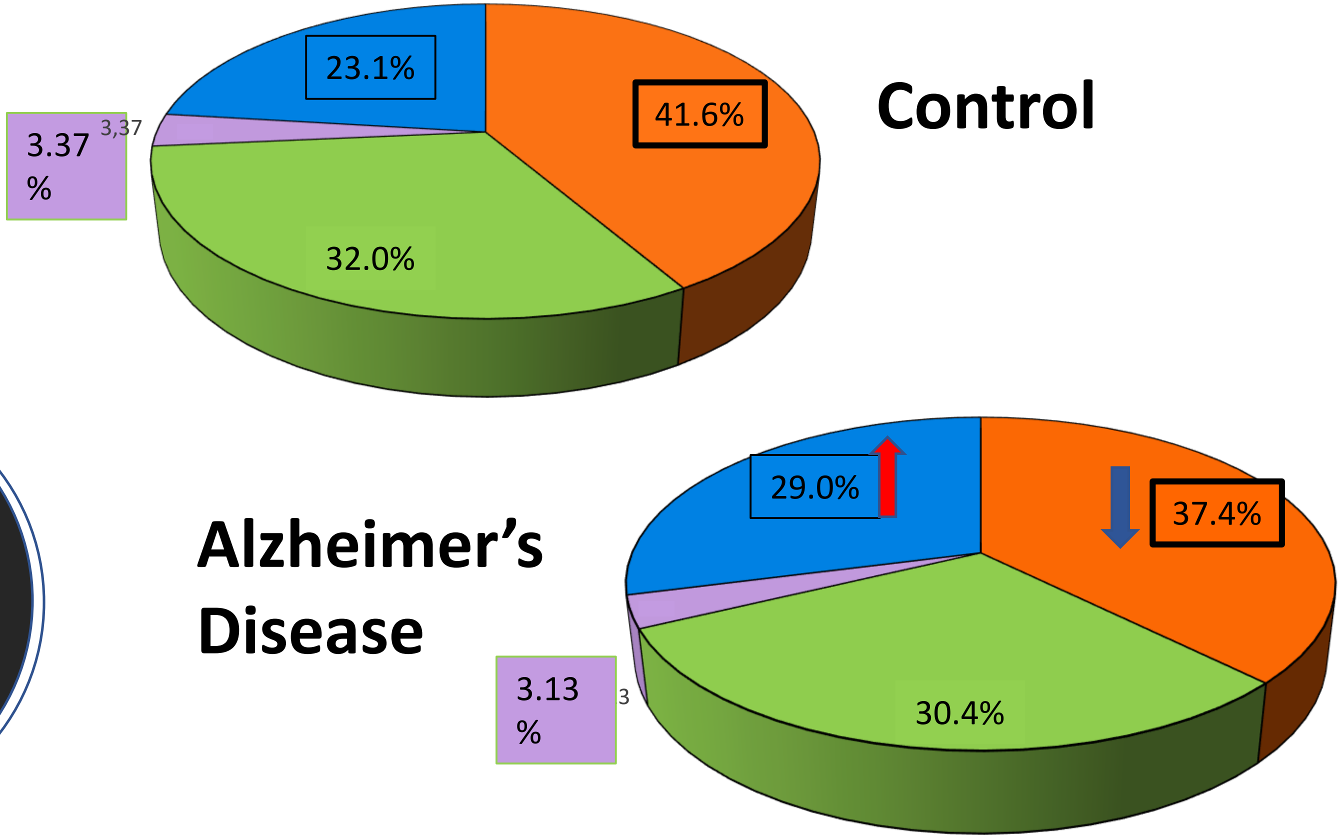




Significant difference between control and Alzheimer's Disease



Percentages of Elements in Plasma



Significant difference between control and Alzheimer's Disease

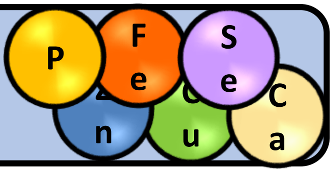
Control

Alzheimer's Disease brain

Absolute levels

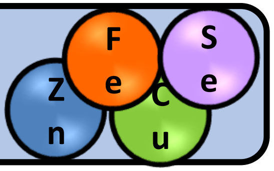
No difference

Normalised percentage



↓ Alzheimer's Disease

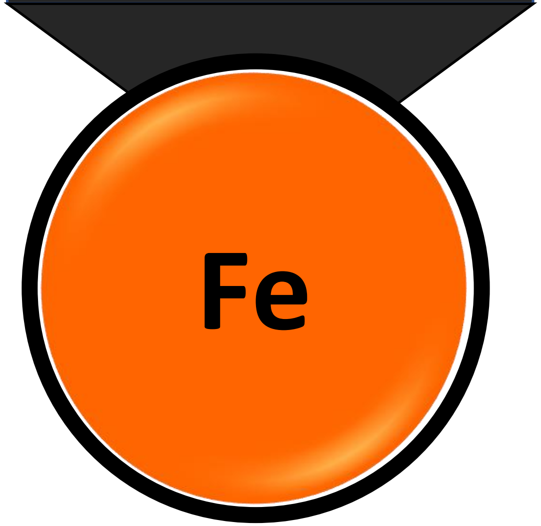
Normalised percentage

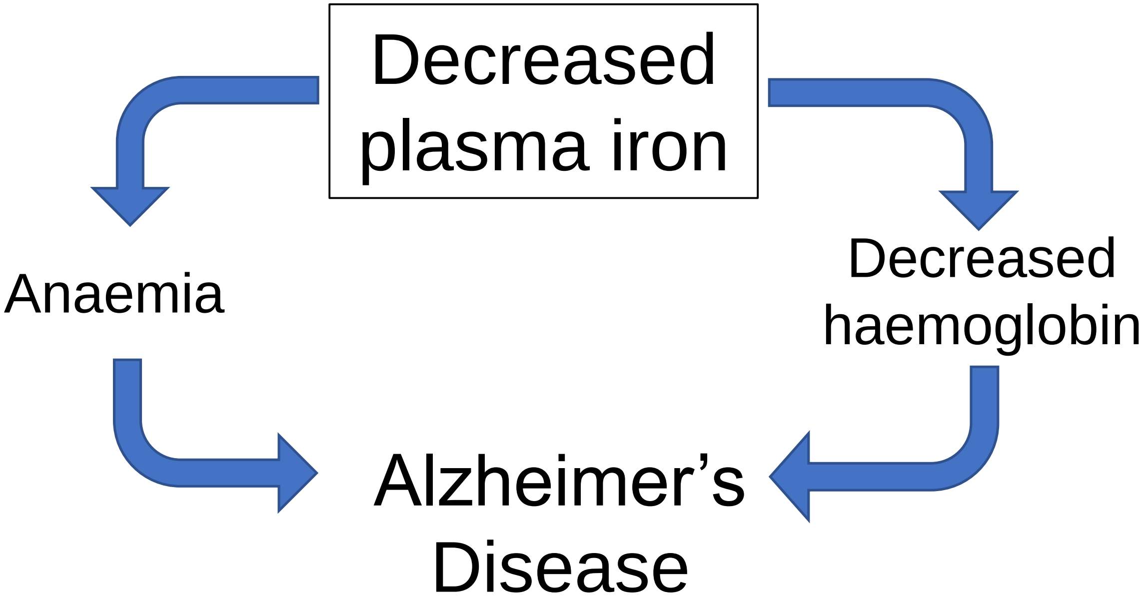


↑ Alzheimer's Disease

Zn

Iron Deficiency In Alzheimer's Disease





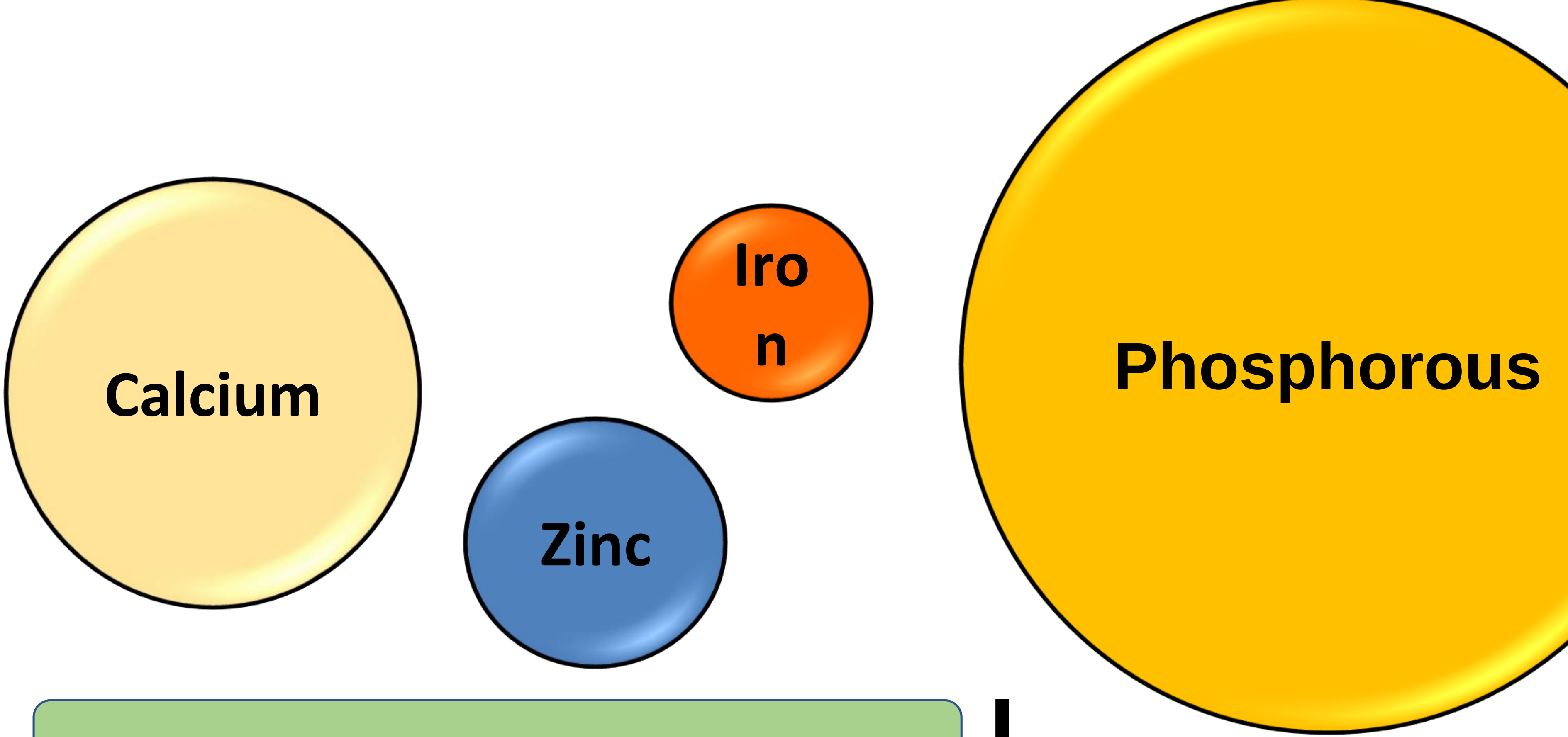
Decreased plasma iron

Anaemia

Decreased haemoglobin

Alzheimer's Disease

Conclusions



Calcium

Zinc

Iron

Phosphorous

Elemental profiling discriminates between control and AD

Crucial to measure panel of elements

Ashraf, Stosnach, Parkes, Hye, Lovestone and So, to be published in Scientific Reports

Acknowledgements

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Azhaar Ashraf

Harry G. Parkes,

Abdul Hye,

John Powell

Simon Lovestone

Samples from AddNeuromed Consortium

Alzheimer's Research UK
The Power to Defeat Dementia

BBSRC
bioscience for the future

LIDo London Interdisciplinary Doctoral Programme
@ UCL, KCL, QMUL, Birkbeck, LSHTM, RVC

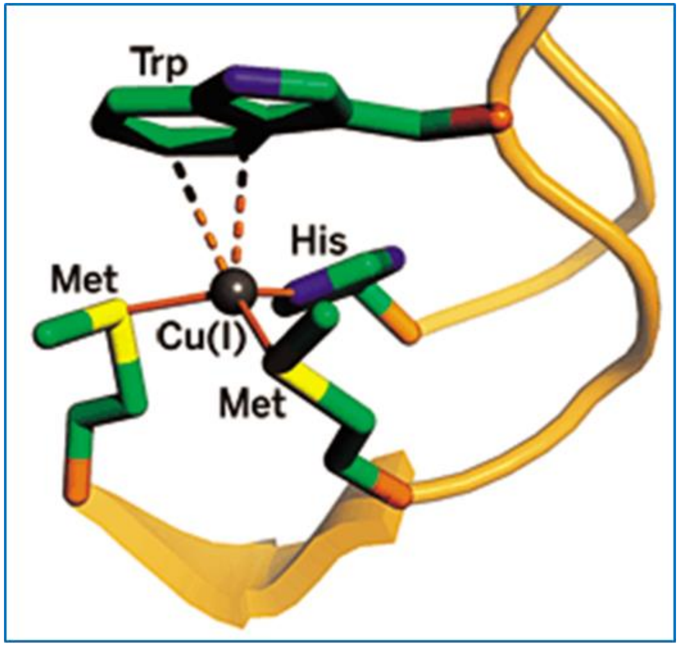
Part III: Protein analysis

Protein analysis
Introduction

Metal ions and enzymes

- Metal ions are important for the biological function of enzymes
- Various modes of metal-protein interaction: metal-, ligand-, enzyme-bridge complexes
- Metals serve as electron donors or acceptors, Lewis acids or structural regulators

(Riordan JF.: "The role of metals in enzyme activity.", Ann Clin Lab Sci. 1977 Mar-Apr;7(2):119-29



C. Arnaud: CEN, January 7, 2008
Volume 86, Number 1, p. 8

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Protein analysis
Introduction

Examples of metal ions in enzymes

Role	Metals	Protein
Oxygen transport and storage	Fe, Cu	Haemoglobin
Electron transport	Fe, Cu	Cytochromes
Nitrogen fixation	Fe, Mo, V	Nitrogenase
Oxygen atom transfer	Mo, W	Oxidases, Reductases
Alkyl group transfer	Co	Vitamin B_{12}
Hydrolysis	Zn, Cu, Mn	Hydroxylases, Peptidases
Storage and transport	Fe, Cu, Zn	Ferritin, Metallothioneins

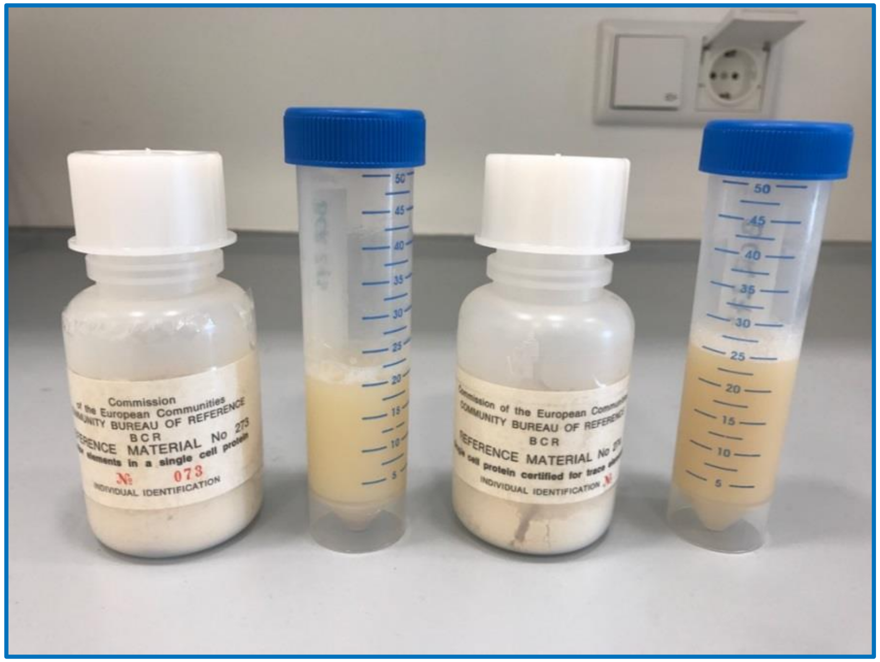
Protein analysis
Feasibility tests

Samples

Certified reference materials

- “BCR 273 – Single cell protein”
- “BCR 274 – Single cell protein”

500 mg powderous sample were suspended in 25 ml pure water



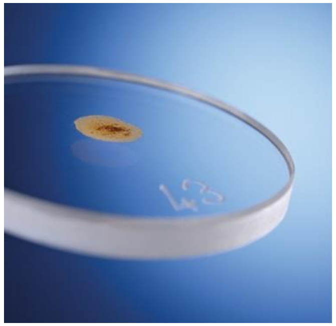
Protein analysis
Feasibility tests

Sample preparation

First approach:
mixing of microsamples in **vials**

- 100 µl sample + internal standard
  + 10 µl Sc (10 mg/l)
  + 10 µl Ga (10 mg/l)

- Preparation of
  - 10 µl
  - 5 µl
  - 3 µl
  - 1 µl
  - 0,5 µl

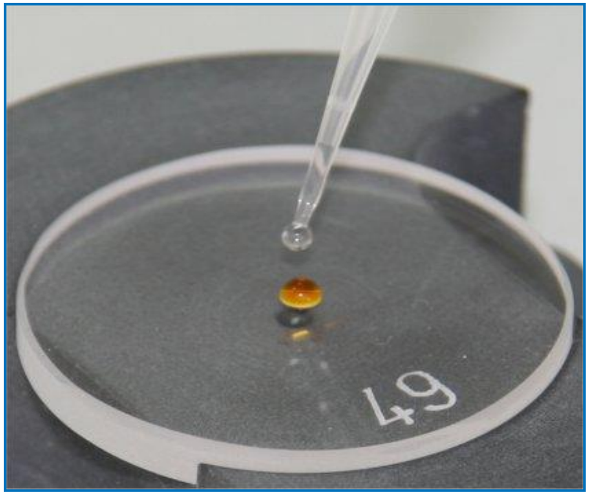


Protein analysis
Feasibility tests

Sample preparation

Second approach:
mixing of microsamples on **discs**

- 5 µl sample + 5 µl Sc/Ga solution (20 mg/l)
- 3 µl sample + 3 µl Sc/Ga solution (20 mg/l)
- 1 µl sample + 1 µl Sc/Ga solution (20 mg/l)



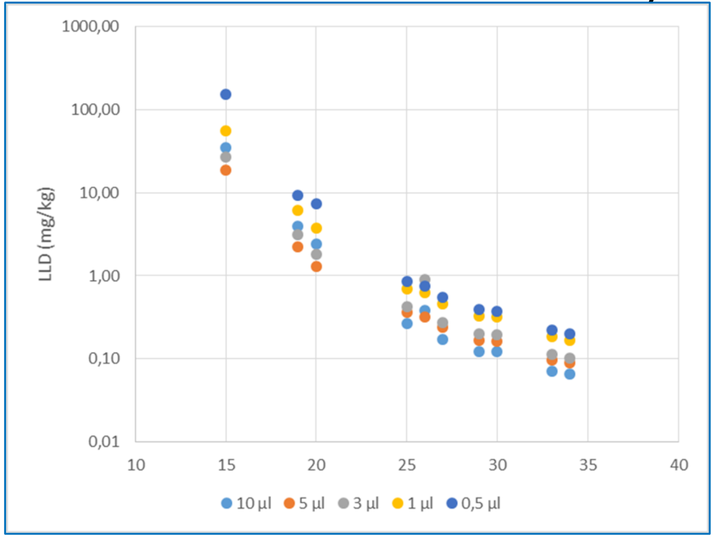
Protein analysis
Feasibility tests

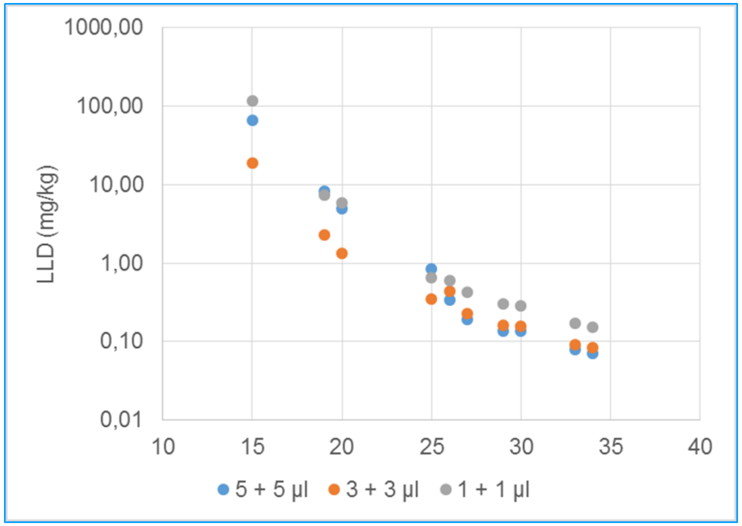
S4 T·STAR

- Mo tube, 50 kV/1000 µA
- W-tube, 50 kV/1000 µA
- 60 mm^2 XFlash SDD
- 90 position sample changer
- Mo-K excitation, 1000 s
- W-L excitation, 1000 s
- W-Brems, 1000 s

Protein analysis
Feasibility tests

- Accuracy and reproducibility for the light elements P (S and Cl) fluctuates with deposited sample amount ⇨ effect of sample height and size
- Other major and trace elements (K, Ca, Mn, Fe, Cu, Zn and Se) are not affected
- Reproducibility of samples, mixed in vessels is better that for those, directly mixed on the carrier
- The detection limits are directly related to the deposited amount

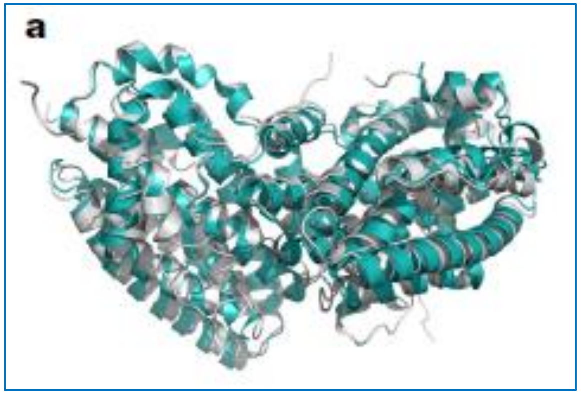




Protein analysis
Application example

Background

- Ribonucleotide reductase (RNS) catalyses the only known de novo pathway for the production of all four deoxyribonucleotides that are required for DNA synthesis
- Fe is required for function in the aerobic, class I RNR in all eukaryotes and many bacteria
- A dinuclear metal site has been viewed as necessary to generate and stabilze the catalytic radical, essential for RNR activity
- This study*) describes a group of RNR proteins that possess a metal independent stable radical



*): Srinivas, V. et al. (2018): „Metal-free ribonucleotide reduction powered by a DOPA radical in Mycoplasma pathogens‟, Nature, Vol. 563, 416 - 420

Protein analysis
Application example

Samples

- Proteins (*Mf*R2 active, *Mf*Nrdl, *Mf*R1, *Ec*R2a) and buffer solutions
- Control sample: metal-free E. coli class 1a R2 protein, Fe-reconstituted by incubation and subsequent removal of all unbound Fe

Preparation

- Addition of a gallium standard solution (2 mg/l) with a volume ratio of 1:1 with a few µl sample
- Preparation on quartz glass carriers

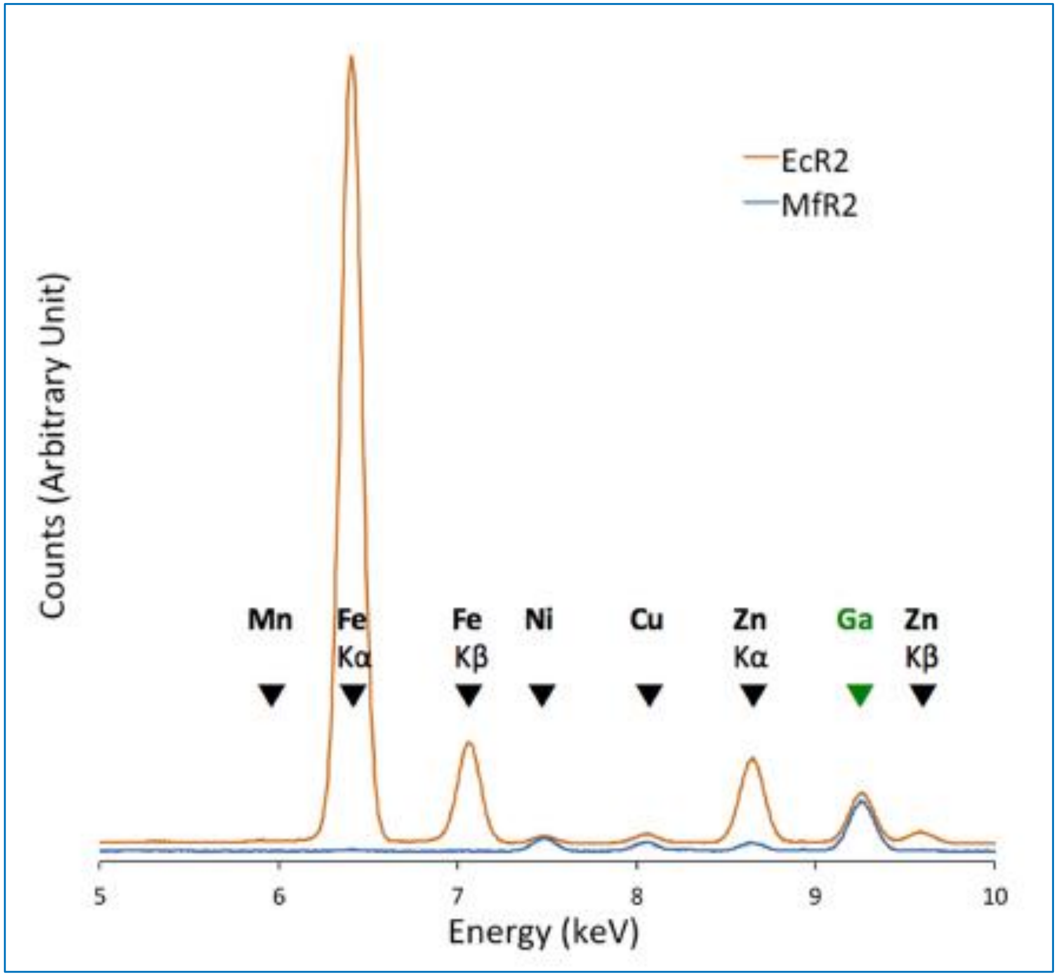
Measurements

- Threefold preparation and measurement with a S2 PICOFOX system for 1000 s

Protein analysis
Application example

Results

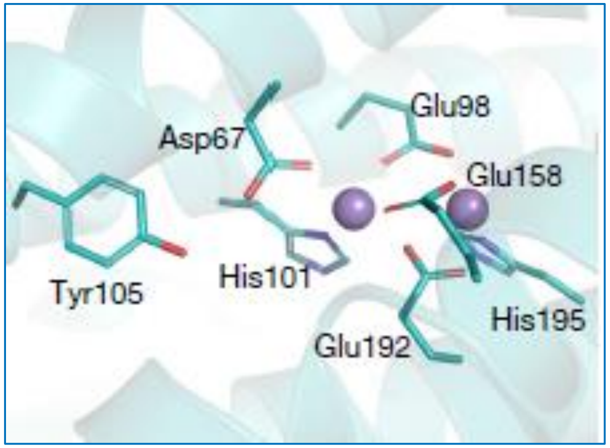
- None of the *Mf*RNR proteins contains a substantial amount of metal
- The *Ec*R2a contains in the order of two metal ions per monomer, also after the desalting step



Protein analysis
Application example

Conclusions

- The cumulative amount of transition metals is less than 4 % per protein. It is therefore not possible that a metal ion is required to stabilze the observed radical species
- Comprehensive structural, EPR, UV-vis absorption, TXRF and mass spectrometric data support the hypothesis that the novel radical species is metal-independent



Summary

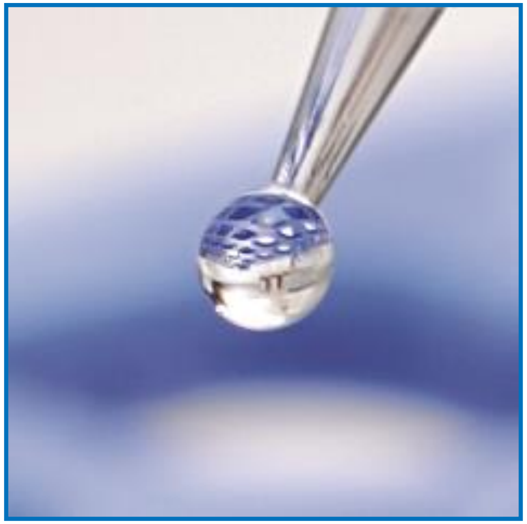
TXRF offers an ideal analytical solution for elemental analysis in medicical and biological research

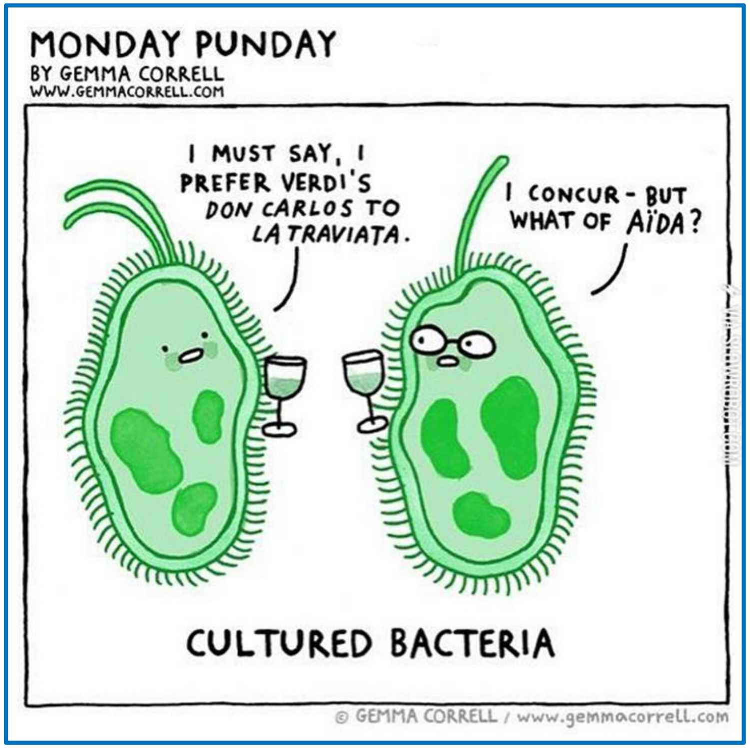
- Analysis of small sample amounts in the low µl-range
- Simultaneous analysis of main- and trace elements
- Simultaneous analysis of other important samples types like buffer solutions
- Instruments can be operated in normal laboratory environments (small footprint, no external gases or cooling water necessary)
- Moderate analytical demands on laboratory staff
- Low analytical and lifetime costs

Q & A

Any Questions?

Please **type in** the questions you may have for our speakers in the **Questions Box** and click **Submit**





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