WORKSHOP TITLE: Future-Proofing Isotope Metallomics Measurement Science/Services with Medical Needs and Scalability Requirements

WORKSHOP DATE AND TIME: Sunday, July 9, 2023 @ 08:30 – 17:15 CEST

WELCOME:

Dear Workshop Participants, Presenters, and Isotope Metallomics enthusiasts*:*

We would like to thank you for your contribution, participation and/or attendance in Goldschmidt 2023, and more specifically in our workshop: "Future-Proofing Isotope Metallomics Measurement Science/Services with Medical Needs and Scalability Requirements". The workshop is quickly coming up and we wanted to provide you with some general information for everyone's reference to help you get prepared. Below you will find abstracts and biographies for all the presenters as well as the Schedule for the workshop with presentation times and breaks for each of the workshop sections. We will have a 45-minute discussion session at the end of each topic section which will provide additional time for questions along with discussion. The goal for the discussion section portions of the workshop will be to compile your feedback and ideas on each of the topics with the intention of developing a publishable blueprint for National Metrology Institutes (NMIs) and fellow academic and industrial expert partners to begin addressing the gaps identified. Participants are strongly encouraged to make their voices heard and to actively contribute their ideas, thoughts, and insights to develop a communal strategy and path forward that can be used to build a comprehensive measurement science and services portfolio for the Isotope Metallomics community.

For those attending the workshop remotely the workshop will be accessed through a ZOOM link which will be linked online next to the event on the Workshop page. The link will become a live link 20 mins (8:10 am CEST) before the start of the workshop. A "Join now" button should appear in your personal programs to link to the workshop. To find the "join Now" button sign into the conference website (using the email address you used to register) and navigate to the "Program by Day" page and find our workshop. The "Join Now" button should be there. We recommend you update the Zoom app to the latest version of Zoom and also check your registration is fully paid (you can check your registration has been fully paid by logging into the conference website and Go to My Goldschmidt > My Registration > View My Registration). If you have any technical issues please refer to the end of this document.

For those who are attending in-person please be aware you will need to check-in in advance of the workshop which can be done one half hour prior to the start of the workshop starting at 8:00 am (CEST) at the Helpdesk at Entrance A (this is located on the River side) of Building A. Our workshop is currently assigned to room R2 (Level 1) (see map below); however, this is subject to change, and you will want to be sure to check upon arrival to confirm the location of the workshop. Please be sure to bring any adapters required for your laptop/tablets.

We are eager to meet you all and look forward to a successful workshop!

Your Organizers,

Jacqueline Mann, Rebecca Kraft, Clay Davis (National Institute of Standards and Technology, USA)
Corday Selden (Rutgers University, USA)
Kaj Sullivan (Ghent University, Belgium)
Brandon Mahan (James Cook University, Australia)
Vincent Balter (CNRS, Ecole Normale Superieure de Lyon, France)
Emmanuelle Albalat (CNRS, Ecole Normale Superieure de Lyon, France)
Anton Eisenhauer (GEOMAR, Germany)

SCHEDULE DETAILS: I:In-person, R: Remote

PRESENTATION TIME:	PRESENTATION TITLE:	NAME:	AFFILIATION:
8:30-8:40 am	Opening Remarks	Jacqueline Mann (I) & Brandon Mahan (I)	NIST, USA and James Cook University, Australia
8:40-9:10 am	Introductions	All Participants & Speakers	
Topic Area 1: Rapid and Novel Techniques & Reference Materials (RMs) Moderator: Brandon Mahan, James Cook University, Alternate Moderator: Jacqueline Mann, NIST			
9:10-9:30 am	Neoma MC-ICP-MS: simplified, high-precision isotope ratio analysis	Nicholas Lloyd (I)	Thermo Fisher Scientific
9:30-9:50 am	Metal stable isotope analysis by Orbitrap IRMS	John Eiler (R)	California Institute of Technology, USA
9:50-10:10 am	**COFFEE/TEA BREAK**		
10:10-10:30 am	Considerations for the Development of Reference Materials for Clinical Applications	Johana Camara (R)	NIST, USA
10:30-10:50 am	Fresh-Frozen Biological Reference Materials at the National Institute of Standards and Technology: Considerations for Development and Production	Debra Ellisor (R)	NIST, USA
10:50-11:10 am	Reference Materials for Metalloprotein Measurements: Updates on Production	Clay Davis (R)	NIST, USA
11:10-11:55 am	**DISCUSSION: Measurement Science & RM Needs of the Isotope Metallomics Community**		
11:55-13:00 pm	**LUNCH BREAK**		
Topic Area 2: Isotope Metallomics – Current & Future Moderator: Corday Selden, Rutgers University, Alternate Moderator: Kaj Sullivan, Ghent University			
13:00-13:20 pm	Linking isotope metallomic to other omics	Vincent Balter (I)	Ecole Normale Superieure de Lyon, France
13:20-13:40 pm	Isotopic analysis of essential mineral elements in samples from patients, cell line studies and animal experiments for obtaining an enhanced insight into isotope ratio variations in health and disease	Frank Vanhaecke (I)	Ghent University, Belgium
13:40-14:00 pm	Using Stable Isotopes in the Detection of Alzheimer's Disease	Fred Moynier (I)	Institut de Physique du Globe de Paris, France
14:00-14:20 pm	Principles of Density Functional Theory for Isotope Geochemistry	Mark Nestmeyer (R)	James Cook University, Australia
14:20-14:40 pm	Experimental and theoretical investigation of gallium isotopic fractionation with solvent extraction method	Chizu Kato (I)	Osaka University, Japan
14:40-15:00 pm	**COFFEE/TEA BREAK**		
15:00-15:20 pm	Metal isotopes as potential *new techniques and tools* in Environmental Health Sciences	Kathrin Schilling (I)	Columbia University, USA
15:20-15:40 pm	The influence of physiological and lifestyle factors on essential mineral element isotopic compositions in the human body: implications for the design of isotope metallomics research	Kaj Sullivan (I)	Ghent University, Belgium
15:40-16:00 pm	Building Interdisciplinary Teams in Isotope Metallomics: Bridging the Earth Science-Medical Divide	Franco Marcantonio (I)	Texas A&M University, USA
16:00-16:45 pm	**DISCUSSION: Isotope Metallomics – Current & Future**		
16:45-17:15 pm	**Workshop Wrap-up and Announcements**		

ABSTRACTS:

Vincent Balter (email: vincent.balter@ens-lyon.fr)**:**

Linking isotope metallomic to other omics

Laboratoire de Géologie de Lyon. Ecole normale Supérieure de Lyon. 46, Allée d'Italie. 69342 Lyon cedex 07, France.

The metallome, including metal concentrations and isotopic compositions, is suspected to be variable in health and disease, having thus the potential for capturing some fundamental aspects of metabolic and pathologic processes. To study the evolution of the isotope metallome during aging, we have measured a suite of trace element concentrations as well as the Cu and Zn isotope compositions in organs (liver, muscle, kidney, brain and heart) at different time points (6, 16 and 24 months) of aging mice. The mice were also characterized by phenomic, metabolomic and proteomic analysis. The results show a wealth of associations between the (isotopic) metallome and other omic layers. We show for instance that changes in hepatic Cu isotope compositions are correlated to age and recapitulate several aspects of the glucose/fat metabolism. For the first time for animals, we introduce metallomic as a new omic layer and adapt classic bioinformatic tools to integrate the metallome as a new component of biological systems.

Johanna Camara (email: Johanna.camara@nist.gov)**:**

Considerations for the Development of Reference Materials for Clinical Applications

Chemical Sciences Division, National Institute of Standards and Technology (NIST), Gaithersburg, MD 20899

Reference materials (RMs), including certified reference materials (CRMs), are provided by the National Institute of Standards and Technology (NIST) and other RM producers to support global clinical measurement standardization. These materials are available in various forms, including neat powders, solutions, and clinically relevant biological matrices. The intended RM uses include calibration and validation, depending on the material. Calibration with RMs may provide traceability to higher-order references when incorporated into specific measurement schemes. The choice of which RM to use and how to incorporate it into a measurement system depends on laboratory goals. There are many specifications which must be considered when designing a new RM. The material can be a high-purity standard or based on a biological matrix. Most biological matrix RMs consist of pools of material from many individuals in order to obtain sufficient volume to provide enough RM to support measurements over an extended number of years. In addition to determining which components require value assignment, a plan for determining both short-term and long-term stability must be in place to support the integrity of a material throughout its lifespan. Other details to consider include storage temperature, shipping conditions, amount per container, type of container, and the necessity for additives. To determine these design elements, it is important to gather input from prospective user communities to ensure the resulting RM will have an impact for a significant number of eventual users. Useful input often comes from established working groups with specific interest in a measurement area. More recently, NIST has incorporated surveys into our planning process for some RMs to ensure that new and renewal materials are designed for maximum impact while using resources wisely within NIST. While the processes of designing, preparing, and distributing RMs are often intensive, the benefits to clinical laboratory quality and patient care through standardization or harmonization of measurements is well worthwhile.

Clay Davis (email: clay.davis@nist.gov):

Reference Materials for Metalloprotein Measurements: Updates on Production

Chemical Sciences Division, National Institute of Standards and Technology (NIST), Gaithersburg, MD 20899

As has been frequently observed, analytical data from instrumental platforms can be more variable than the actual changes attributable to biological treatment and/or condition. These isotopic, proteomic and metabolomic data and spectral profiles are used to discriminate pheno-typical differences (disease processes, histopathological changes, drug effects, etc.) associated with biological specimens, and are used to classify healthy and/or non-treated samples from diseased and/or treated biological samples. NIST is focused on developing a large supply of stable QC materials, including a suite of materials used to establish definitive levels of analytes in differential based studies, that could be used as harmonization materials within the fields of genomics, metabolomics, proteomics, and the elemental isotopic community. Therefore, there is a significant need for tools that will increase data harmonization, comparability, and reproducibility between laboratories, studies, and fields. Currently, the National Institute of Standards and Technology (NIST) is developing quality control materials with multi-omic measurements that include isotopic analysis, which can be used to assess complete analytical workflows and analytical data platforms. An update on current material production and the status of new materials will be presented.

John Eiler (email: eiler@caltech.edu):

Metal stable isotope analysis by Orbitrap IRMS

Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, CA

The application of natural-abundance isotopic measurements to the biochemistry of metals generally requires isolation of a pure metal of interest from a biomaterial followed by MC-ICP mass spectrometry. While this approach has led to a variety of discoveries and useful tools, it has several disadvantages: The required techniques and instruments are relatively specialized, labor intensive and not commonly available in facilities dedicated to medical research or diagnostics; low concentrations and complex matrices make some measurements technically challenging; and such measurements are difficult to connect directly to 'context clues' that might be provided by compound-specific isotopic measurements and/or correlations between metal isotopes and other stable-isotope properties (especially of H, C, N, O, and S) of metal-complexing molecules.

I will review the recent development of Orbitrap mass spectrometry as a platform for natural-abundance, high precision isotope ratio measurements and discuss its potential for biomedical metallomics. Orbitrap IRMS calls for specialized sample introduction protocols and mass spectrometric methods, but its core technology is highly automated and widely available in life-science analytical laboratories; thus, it provides a relatively accessible and scalable platform for applied metallomics studies. This method focuses on direct mass spectrometric study of compounds of interest (e.g., metal-ligand complexes) with little or no chemical modification, and in some cases relatively little off-line purification. And, perhaps most promising, a metal isotope measurement made by Orbitrap IRMS can simultaneously provide a sophisticated set of constraints on other isotopic properties of metal-bearing compounds.

The principal challenge to Orbitrap IRMS is that we have only recently established protocols for achieving usefully precise and well-standardized isotope ratio measurements. Current state of the art with this approach reaches uncertainties of approximately ±0.1 ‰, which is sufficient for some biomedical studies of some elements, but not for others. Further advances to errors of ~ ±0.01 ‰ are conceivable but will require a concerted push to engineer systems that deliver sample and standard to the mass spectrometer with careful control over periods of 10's of minutes to hours.

Debra Ellisor (email: debra.ellisor@nist.gov):

Fresh-Frozen Biological Reference Materials at the National Institute of Standards and Technology: Considerations for Development and Production

Chemical Sciences Division, National Institute of Standards and Technology (NIST), Gaithersburg, MD 20899

A vast array of biological reference materials has been developed at NIST, including food-based materials for nutrient and contaminant studies, sediments and soils for environmental contaminant and elemental analyses, and human-derived materials for biomedical applications. To produce a homogenous and stable material that is fit for purpose, many important factors must be considered including the identification of appropriate matrices, specification of preparation methods, and suitable storage conditions to ensure stability. Recently, an archived collection of fresh frozen human livers was accessed to develop a metabolomics reference material suite. In partnership with the Environmental Protection Agency (EPA), these livers had been collected from decedents between 1980 and 1994 using strict sampling protocols to monitor environmental contaminants in human populations across the United States and have since been stored at the NIST Biorepository under cryogenic conditions. NIST researchers surveyed the historical data to identify subjects from different life history categories and accessed liver tissues from these subjects to generate three cohorts of fresh frozen liver homogenate: Normal, Fatty and Congested. The livers were cryogenically homogenized, and the final products will be used to assist the community in harmonizing measurements across laboratories and benchmark instrument performance for differential metabolomic analysis.

Chizu Kato (email: chizu@see.eng.osaka-u.ac.jp):

Experimental and theoretical investigation of gallium isotopic fractionation with solvent extraction method

Chizu Kato[1], Frédéric Moynier[2], Toshiyuki Fujii[1], Kurumi Asai[3], Minori Abe[4]

[1]Osaka University, [2]Institut de Physique du Globe de Paris, [3]Tokyo Metropolitan University, [4]Hiroshima University

Gallium (Ga) has two stable isotopes, 69 and 71, and their abundances are 60.10% and 39.89% respectively [1]. Although Ga is classified as a siderophile element, because of its unusually high abundance in the mantle (~ 4ppm [2]), it is thought to have some characteristics of a lithophile element. Recently, isotopic fractionation values of Ga in terrestrial [3], meteoritic [4] and lunar samples [5] along with adsorption experiments [6] show a wide variety. However, despite these results, the mechanism between the chemical ligands and Ga during isotope fractionation remains poorly understood. In this study, we applied *ab initio* calculations of aqueous Ga in a chlorine environment and compared that with solvent extraction experiments using a dicyclohexano-18-crown-6 (DC18C6) with a gallium chloride solution in order to understand the isotopic fractionation of Ga.

Orbital geometries and vibrational frequencies of aqueous Ga(III) species were computed with the density function theory (DFT) as implemented by the Gaussian09 code [7, 8]. The nuclear field shift effects were estimated by the infinite-order Douglas-Kroll-Hess method with the mean-field spin-orbit interaction (IODKH+MFSO) for 2-component relativistic implemented in the DIRAC12 software package [9]. Molecules were modeled without any forced symmetry. Gallium (III) chloride was dissolved in 0.9 M to 5.7 M HCl to prepare a 0.1 M Ga solution for the aqueous phase. DC18C6 was diluted by 1,2-dichloroethane to achieve a concentration 0.1 M. The two phases were stirred and centrifuged for fore extraction and then Ga was stripped back to pure water. The collected solution was dried down and passed through a column to purify the Ga following the method by [3]. The Ga concentration and isotopic values were measured with a Thermo-Fisher Neptune Plus Multi Collector-Inductively Coupled Plasma-Mass Spectrometer (MC-ICP-MS). Results are shown in the delta notation (‰) and defined as:$\delta^{71}Ga = \{(^{71}Ga/^{69}Ga)_{sam}/(^{71}Ga/^{69}Ga)_{Ga\ IPGP\ std} - 1\} \times 1000$, $\Delta^{71}Ga = \delta^{71}Ga_{org} - \delta^{71}Ga_{aq}$.

The calculated isotopic values were in agreement within the error of the experimental values for most of the samples. The estimated NFS effects were minimal (-0.006 ‰ and -0.007 ‰ for $GaCl(H_2O)5$ and $GaCl_2(H_2O)_4$, respectively) compared to the error from the isotopic measurements (δ 71Ga 2SD = ~0.05 ‰) and is therefore negligible. As the HCl concentration increased, the isotopic fractionation decreased from Δ 71Ga = -0.99 ‰ to -0.41 ‰, demonstrating that Ga ligands fractionate at a larger degree in lower Cl^- concentrations. The most abundant ligands in this concentration were Ga^{3+}, $GaCl^{2+}$ and $GaCl^{2+}$ in descending order.

References:

[1] Meija et al., (2016) *Pure Appl. Chem.* **88**, 293-306 [2] McDonough (1990) *Geochim. Cosmochim. Acta* **54**, 471-473 [3] Kato et al., (2017) *Chem. Geol.* **448**, 164-172 [4] Kato & Moynier (2017) *Earth Planet. Sci. Lett.* **479**, 330-339 [5] Kato & Moynier (2017) Sci. Adv. **3**, e1700571 [6] Yuan et al., (2018) *Geochim. Cosmochim. Acta* **223**, 250-363 [7] Frisch et al. (2016) Revision B.01 Gaussian, Inc., Wallingford CT [8] Becke (1993) *J. Chem. Phys.* **98**, 5648-5652 [9] Jensen et al., (2012) DIRAC, a relativistic ab initio electronic structure program, Release DIRAC12 https://www.diracprogram.org/

Nicholas S. Lloyd (email: nicholas.lloyd@thermofisher.com):

Neoma MC-ICP-MS: simplified, high-precision isotope ratio analysis

G. Craig[1*], J. Roberts[1], N. S. Lloyd[1], C. Bouman[1], J. Schwieters[1] and N. S. Lloyd[1]

[1]Thermo Fisher Scientific, Hanna-Kunath Str. 11, 28199 Bremen, Germany (send correspondence to: grant.craig@thermofisher.com)

It has been observed that future development of the field of isotope metallomics by MC-ICP-MS is reliant on a significant increase in sample throughput and the development of fully integrated workflows[1]. Although progress towards these two aims has already been achieved, limitations within existing hardware and software platforms has been a barrier to further automation. Technological developments to the market-leading Thermo Scientific Neptune™ Series MC-ICP-MS, including advanced technologies such as the Jet Interface, XHR and 10^{13} Ω Amplifier Technology, improved the quality of isotope metallomics measurements, by increasing sensitivity, reducing sample size, and improving isotope ratio precision, but did not necessarily address the sample throughput challenge.

To take MC-ICP-MS technology further, Thermo Fisher Scientific™ have introduced the Thermo Scientific™ Neoma™ MC-ICP-MS, blending cutting-edge and field proven technology, and at its heart a new limit-breaking 11 Faraday mobile Faraday cup linked to up to 24 customizable amplifier slots. Technologies retained from the Neptune Series have been refined, for example the extended dynamic range of the 10^{13} Ω Amplifier Technology has been doubled and enhanced with a focus on increasing integration and productivity. Common MC-ICP-MS peripherals, including desolvating nebulizer systems and autosamplers, are fully integrated within the Qtegra™

ISDS software for significant improvements in productivity. ChromControl™, also offers the potential to merge the software of the Neoma MC-ICP-MS with numerous GC and LC systems for even greater automation.

The latest Neoma MS/MS MC-ICP-MS technology, including a groundbreaking, patented combination of a collision/reaction with a pre-cell mass filter design [2], offers the possibility to significantly reduce the requirement for highly purified sample solutions for MC-ICP-MS [3], a major barrier to greater uptake of isotope metallomics in the wider community.

References:
1 B. Mahan, et al., *Cell. Mol. Life Sci.*, 2020, **77**, 3293–3309.
2 G. Craig, et al., *Anal. Chem.*, 2021, **93**, 10519–10527.
3 P. Telouk, et al. *AGU Fall Meeting 2022.* 2022AGUFM.V35A..02T

Franco Marcantonio (marcantonio@geos.tamu.edu):

Building Interdisciplinary Teams in Isotope Metallomics: Bridging the Earth Science-Medical Divide

Department of Geology and Geophysics, Texas A&M University

Using internal pilot funds granted by Texas A&M University, I completed a project in which lead (Pb) isotope ratios were used to track the source of contaminant lead in children's blood. For this project, I assembled and lead a team from Texas A&M University (isotope geochemists in the Department of Geology and Geophysics and epidemiologists in the School of Public Health), the University of Texas, San Antonio (environmental scientists), and Children's Mercy Hospital in Kansas City, MO (doctor, nurse, and environmental hygienists). After multi-institute, human-subject, IRBs were evaluated and approved, the work began. We identified children with high blood lead levels in inner-city homes in Kansas City, with help from the Kansas City health department. Eligible parents were introduced to the research project, filled out questionnaires and, if interested, gave written consent for their participation. After agreeing to participate, a nurse collected blood from the child in the home and environmental hygienists sampled all potential sources in the home from which the elevated blood lead levels likely resulted. All blood and environmental samples were transported to Texas A&M, and the blood was decontaminated in a BSL2 lab. Upon separation and purification of the lead, Pb isotope ratios were measured by multi-collector ICPMS.

Using my experience from this pilot project, I am attempting to begin work within the colonias of South Texas, economically impoverished communities that lack infrastructure such as clean and safe potable water, sewer systems, electricity, paved roads, and safe housing. In this vulnerable region, I will focus on an analysis of the Pb concentrations and isotope ratios of private well water which, unfortunately, is not regulated by federal or state governments for contaminants. In an effort to delineate the extent, location, and cause of metal contamination, I am assembling a team of isotope geochemists, environmental geoscientists, epidemiologists, spatial analysts, community resilience and outreach specialists, and state of Texas Agricultural extension professionals.

Fred Moynier (moynier@ipgp.fr):

Using Stable Isotopes in the Detection of Alzheimer's Disease

F. Moynier[1], E. Lahoud, B. Mahan, M. Le Borgne

[1]Institut de Physique du Globe de Paris, Sorbonne Paris Cite´, Universite´ Paris Diderot, CNRS, 1 rue Jussieu, 75005 Paris, France

Alzheimer's disease (AD) is a leading cause of death in high-income countries and affects approximately one in ten individuals aged 65 or older worldwide. One of the prominent physiological characteristics of AD is the development of senile plaques, which are associated with the extracellular buildup of metal-rich amyloid β (Aβ) fibrils containing elements such as Cu and Zn, as well as the accumulation of tau proteins. The natural stable isotopic composition of metals like Zn, Cu, and Fe differs across bodily organs and exhibits minimal natural variations. Consequently, these isotopes serve as valuable indicators of metal imbalances in the body. Notably, the isotopic composition of Zn, Cu, and Fe in blood differs from that in the brain. The speciation, or bonding, of metals within Aβ fibrils differs from that in a healthy brain, which can consequently alter the isotopic distribution of metals between the brain and body fluids.

In this study, we examined the Cu isotopic compositions of both serum and brain samples from (i) APPswe/PSEN1dE9 transgenic mice, a widely used AD model, (ii) wild-type (WT) control mice at different ages (3, 6, 9, and 12 months), (iii) human brain tissues obtained from both AD patients and healthy individuals (Moynier et al., 2020), and (iv) cerebrospinal fluids and serum from both AD patients and healthy individuals (work presented at the meeting). Our findings reveal that the Cu isotopic composition in the brains of AD-afflicted mice and humans is lighter compared to that of healthy mice and patients. This isotopic shift suggests an increase in Cu(I) associated with the formation of Aβ fibrils and the accumulation of tau proteins. Furthermore, in mice, we observed a correlation between the Cu isotopic composition of the brain and serum, indicating the transport of copper between these two reservoirs, specifically the transfer of Cu(I) from the brain to the serum. These results suggest that the stable Cu isotopic composition of body fluids has the potential to be utilized as a detection tool for monitoring the formation of Aβ fibrils in the brain.

In summary, our study demonstrates that the Cu isotopic composition in AD-afflicted brains differs from that of healthy brains in mice and humans. This difference likely arises from the increased presence of Cu(I) associated with the formation of Aβ fibrils and tau protein accumulation. Additionally, our findings indicate the possibility of using the stable Cu isotopic composition of body fluids as a potential detection method for monitoring Aβ fibril formation in the brain.

Mark Nestmeyer (mark.nestmeyer@my.jcu.edu.au):

Principles of Density Functional Theory for Isotope Geochemistry

IsoTropics Geochemistry Laboratory, James Cook University, Australia

Density functional theory (DFT) is a computational method for calculating properties in molecules and solids “from first principles” which means that the calculations are based on fundamental quantum mechanical laws. The main properties of a molecule or solid which are of interest in Isotope Geochemistry, are the vibrational frequencies of the atomic bonds. Mass-independent isotope fractionation in equilibrium is solely controlled by vibrational frequencies and temperature, whereas temperature only effects the magnitude of fractionation, while the vibrational frequencies predict in which bonding environment the heavy or light isotopes preferentially incorporate.

To quantify isotope fractionation, the vibrational frequencies are used to calculate reduced partition function ratios or β-factors for each relevant phase such as molecules, metal complexes, or minerals. The β-factors can be used to calculate the isotope enrichment factor α or the difference in isotopic composition between two phases relative to a widely used standard expressed as ‘big delta’ Δ.

To set up a DFT calculation we need to know our system in terms of its molecular structure, charge, and electron configuration. Also, we need to choose a functional that relates the electron density of a system to its total energy. There a several functionals available, and a reasonable choice must be made for our system to obtain valuable results. The choice of our functional can be evaluated by comparing the results with experimental data. For example, the distance between atoms or specific vibrational frequencies which have been determined experimentally are commonly used. As we have a first idea of our system, we optimize its geometry to find the internal coordinates of the atoms that lead to the lowest possible energy. This is a prerequisite for a vibrational frequency calculation. As we obtain the vibrational frequencies of our system for both isotopes, we can calculate the β-factor for the temperature we are interested in using the equations from Bigelesien & Mayer 1947 and Urey 1947.

Although isotope fractionation is only controlled by vibrational frequencies and temperature, there are several correlations that occur which are used to predict isotope fractionation without using DFT. One useful example is the correlation of β with the electronegativity of the bonding partner. This implies, for example, that heavier isotopes will favor a bonding to oxygen and nitrogen over sulfur in an amino acid.

Overall, DFT is a valuable tool in Metallomics as we can combine it well with isotope fractionation experiments and measurements in natural samples to obtain a holistic idea of isotope fractionation of metals in biological systems.

Kathrin Schilling (email: kathrins@ldeo.columbia.edu):

Metal isotopes as potential *new techniques and tools* in Environmental Health Sciences

Lamont-Doherty Earth Observatory, Columbia University, Palisades, NY, USA

About 90% of causes for disease development are environmental factors. So, we must study environmental factors to understand disease development and prevention. Natural metal isotopic biomarker can help characterize environmental exposure as well as pharmacokinetics of metals in the human body, mechanisms of toxicity, and changes of metabolic processes caused by disease development and progression. Our team at Columbia University has established a systems framework to interpret metal isotope and speciation metallomics for "personalized" disease and treatment fingerprints. At this workshop, I will review examples of how metal stable isotopes can be used in Environmental Health Sciences and will discuss its future potential.

Kaj Sullivan (email: kaj.sullivan@ugent.be):

The influence of physiological and lifestyle factors on essential mineral element isotopic compositions in the human body: implications for the design of isotope metallomics research

Department of Analytical Chemistry, Ghent University, Ghent, Belgium

In the last 20 years, the application of high-precision isotopic analysis of essential mineral elements (Mg, S, K, Ca, Fe, Cu and Zn) to biomedicine (sometimes referred to as *isotope metallomics*), has revealed that their isotopic compositions are altered by the metal dysregulation that is fundamental to the pathogenesis of many cancers and other diseases [1,2]. Despite many published works showing the diagnostic and prognostic potential of this approach, a number of factors that may influence the stable isotopic composition of these essential mineral elements in healthy individuals remain unstudied. In this presentation, the available evidence will be summarized from trophic level studies, animal models, and ancient and modern humans, relating to physiological and lifestyle factors that appear likely (there is evidence indicating their influence) or unlikely (there is evidence indicating their lack of influence) to require controlling for when investigating variations in essential mineral element isotopic compositions in human subjects. Factors that require additional data to properly assess will also be discussed. There is evidence that sex, menopausal status, age, diet, vitamin and metal supplementation, genetic variation, and obesity influence the isotopic composition of at least one essential mineral element in the human body [3]. The task of investigating potential influences on essential mineral element isotopic compositions in the human body is sizeable but presents an exciting research opportunity. To that end, new Cu isotope ratio data determined from significant sources of Cu in the human diet will also be presented and implications for study design discussed.

[1] Vanhaecke & Costas-Rodríguez (2021), *View* 2, 20200094.
[2] Mahan, Chung & Pountney *et al.* (2020), *Cellular and Molecular Life Sciences* 77, 3293–309.
[3] Sullivan, Moore & Vanhaecke (2023), *Metallomics* 15.3, mfad012.

Frank Vanhaecke (email: Frank.Vanhaecke@UGent.be):

Isotopic analysis of essential mineral elements in samples from patients, cell line studies and animal experiments for obtaining an enhanced insight into isotope ratio variations in health and disease

Atomic & Mass Spectrometry – A&MS research unit, Department of Chemistry, Ghent University, Campus Sterre, Krijgslaan 281–S12, 9000 Ghent, Belgium

By now, it is common knowledge that all elements with ≥2 isotopes show natural variation in their isotopic composition as a result of isotope fractionation. Multi-collector ICP-mass spectrometry (MC-ICP-MS) shows a sufficient isotope ratio precision to reveal and quantify such differences. Although mainly used in the domain of geo- and cosmochemistry, the past 15-20 years of research have demonstrated that also in a biomedical context, high-precision isotopic analysis is a powerful tool. Isotope ratios of essential mineral elements have been proven to pick up some metabolic changes more sensitively and more reproducibly than element concentrations do and/or they may contain information not embedded in element concentrations.[1]

Ghent University's Atomic and Mass Spectrometry research group (UGent-A&MS) has focused on the essential transition metals Cu, Fe and Zn.

It has, e.g., been demonstrated that the isotopic composition of serum Cu is affected by liver disorder.[2,3] Cell line experiments (HepG2) and experiments with murine models (mice) have provided more insight into the factors driving the change in Cu isotopic composition observed.[4,5] A further level of detail could be obtained by specifically studying the isotopic composition of the exchangeable + ultrafiltrable (EXCH+UF) and the non-exchangeable + non-ultrafiltrable (NEXCH+NUF) serum fractions of Cu separately.[6]

The Fe isotopic composition of whole blood/serum was demonstrated to be a robust measure for an individual's iron status.[7] Cell line experiments (CaCo-2) allowed verification of the occurrence of isotope fractionation during

intestinal uptake.[8] Isotopic analysis of serum Fe was shown capable of distinguishing between anemia caused by Fe deficiency or by problems with the production of erythropoietin (the hormone regulating the rate of production of red blood cells in bone marrow) in the kidneys, respectively.[9]

Also, the prognostic value of isotope ratios has been demonstrated as it has been shown that amidst a population of patients with hematological malignancy, those showing a deviating plasma Cu and/or Zn isotopic composition have a significantly lower chance of surviving a 5-year time window.[10]

Currently, UGent-A&MS is investigating potential changes in the isotopic composition of essential mineral elements induced by neurological disorders. In a first phase, murine models (mice) are relied upon to evaluate isotopic heterogeneity among brain compartments and changes induced in these brain parts and in blood plasma upon encephalopathy or development of Alzheimer's disease symptoms.[11-13]

In this lecture, the main findings will be presented, while the added value of working with cell lines and murine models will be illustrated.

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PRESENTER BIOGRAPHIES:

Vincent Balter: I am a CNRS Directeur de Recherche biogeochemist at the Ecole Normale Supérieure de Lyon. My work primarily concerns the use of isotopes to study biological and paleoenvironmental processes. These studies include metals isotopic fractionation in living systems to investigate normal metabolism and highlight pathological disorders. Collaborations with industrial partners help to develop these interdisciplinary studies. Stable isotopes of metals are also measured in fossils to study past biological processes and reconstruct past trophic chains and their environments.

Johanna Camara: Johanna Camara received a Ph.D. in Biochemistry and Molecular Biology from Georgetown University in 2004 and immediately began her employment at NIST as an NRC Postdoctoral Associate. Most recently, her laboratory activities at NIST have encompassed the quantification of small organic molecules in food and biological SRMs. Johanna is the Clinical SRM Program Coordinator, Quality Manager, and Deputy Group Leader for the Organic Chemical Measurement Science Group in the Chemical Sciences Division at NIST. Outside of NIST, Johanna has served on several expert panels and document development committees for the Clinical and Laboratory Standards Institute (CLSI). She is the NIST delegate to CLSI, and a voting member on the US Technical Advisory Group to ISO/TC 212-Clinical laboratory testing and *in vitro* diagnostic test systems. Johanna also participates as a member on multiple International Federation of Clinical Chemistry (IFCC) Working Groups and a Joint Committee for Traceability in Laboratory Medicine (JCTLM) Task Force.

Clay Davis: Clay Davis received his B.S. in chemistry from Georgia Southern University and Ph.D. from Clemson University under the direction of Ken Marcus. Clay is currently a Research Chemist in the Biochemical and Exposure Science Group at the National Institute of Standards and Technology (NIST) in Charleston, SC. He first started at NIST as a National Research Council (NRC) Postdoctoral Fellow to develop novel techniques to measure small molecule organometallic species and metalloproteins using atomic and molecular mass spectrometry. Clay's current work sits broadly within analytical chemistry applied to investigating interesting biological questions utilizing elemental, isotopic, and multi-omic applications in proteomics, metabolomics and lipidomics. Current research projects focus on improving metrological quality and reproducibility including the optimization of sample preparation and data acquisition, quantification, and generating standardized multi-omic data sets to assess all steps of the measurement process.

Clay is also heavily involved in developing resources for stakeholders including advancing and harmonizing measurement science via the production of reference materials with the principal goal of promoting best practices to produce high quality multi-omic data. Specific reference material applications include clinical, non-model organisms, soil, and plant biota, as well as materials for understanding microbial systems.

John Eiler: John Eiler is the Robert P. Sharp Professor of Geology and Geochemistry at the California Institute of Technology. He directs a laboratory for stable isotope geochemistry that specializes in the mass spectrometric study of intramolecular distributions of naturally occurring isotopes, including site-specific and multiply-substituted isotopologues of diverse molecules, with applications to the earth, planetary and environmental sciences.

Debra Ellisor: Debra Ellisor is a Research Biologist in the Biospecimen Science Group of the Materials and Measurement Laboratory at the National Institute of Standards and Technology. She aims to advance cryopreservation sciences by assessing the effects of pre-analytical variables on biospecimen quality using advanced measurement techniques and developing standardized protocols and methods for sample collection and storage to preserve biospecimen integrity. Deb also generates cryogenic reference materials (RMs) and has collaborated with other National Metrology Institutes to evaluate the effects of reference material production methods on downstream analyses, particularly with regard to food safety and authentication.

Chizu Kato: Chizu Kato is an assistant professor at the Nuclear Engineering Chemistry Lab at Osaka University, Japan. She completed her Bachelor's and Master's degrees at Hokkaido University, where she specialized in oxygen isotopes and Al-Mg dating techniques on meteorites using the Secondary Ion Mass Spectrometer (SIMS). To further understand the formation of the Solar System, she expanded her studies to the isotopic composition of volatile elements in terrestrial and extraterrestrial (lunar/meteorites) materials. She developed the gallium chromatography and high-precision isotope measurement method with the Multi Collector-Inductively Coupled Plasma-Mass Spectrometer (MC-ICP-MS) and received her MA at Washington University in St Louis (USA) and later her Ph.D. at the Institut de Physique du Globe de Paris (France). Currently, Chizu's scientific interests primarily revolve around volatile elements and their isotopes. Her research encompasses exploring the cycle of these elements within the Earth's lithosphere, advancing isotope analysis methods, and investigating their applications beyond the field of geochemistry.

Nicholas S. Lloyd: Dr. Nicholas S. Lloyd earned his doctorate in environmental sciences from the University of Leicester in collaboration with the British Geological Survey. In 2010 he joined Thermo Fisher Scientific as a product specialist for the Neptune MC-ICP-MS. His current role as Senior Manager for Product Line Management is responsible for the Thermo Scientific™ portfolio of market leading isotope ratio mass spectrometers.

Franco Marcantonio: Marcantonio is a Professor of Geology and the Jane and Ken R. Williams '45 Chair in Ocean Drilling Science, Technology and Education at Texas A&M University. He received his PhD in Earth and Environmental Sciences from Columbia University's Lamont-Doherty Earth Observatory. He pursues several areas of research, all of which involve the measurement of radiogenic isotopes in the environment. One focus of his research is on the use of isotope and trace element tracers to better understand the relationship between climate change and its forcing and response mechanisms. Another recent focus is on using radiogenic isotope ratios as "geo-health" tracers. His recent work on lead isotopes as a tracer of contamination in children's blood involved forming and leading a team of medical professionals, epidemiologists, and isotope and environmental geochemists.

Frédéric Moynier: Frédéric Moynier is a professor of geochemistry at the Institut de Physique du Globe de Paris, where he leads the cosmochemistry laboratory and the Origins theme. While his primary research revolves around comprehending planet formation, his contributions extend beyond planetary science. Over the past 15 years, he has developed an interest in applying stable isotopes of metals to the field of medical sciences. This has led to his recent achievement in securing funding for an ERC POC grant, specifically aimed at exploring the applications of stable isotopes in understanding Alzheimer's disease.

Mark Nestmeyer: I am a PhD candidate at the IsoTropics Geochemistry laboratory at James Cook University, Australia. After completing my Master thesis in Geoscience on marine hydrothermal systems, I am now working on isotope fractionation in banded iron formations to reconstruct long term trends in the ancient environment. I have learned DFT during my PhD candidature and I am using it now to understand isotope fractionation between the ocean and marine sediments. My supervisor Brandon Mahan invited me to join the Metallomics community to share my knowledge in computational chemistry.

Kathrin Schilling: Dr. Schilling is an analytical and isotope (geo)chemist. Her interdisciplinary research brings high-precision metal isotope analysis into a synergistic space with multiple new approaches in Environmental Health Sciences. Dr Schilling explores various metal isotope systems (e.g., selenium, zinc, copper) as biomarker for environmental carcinogens, nutrient status and as source tracers of metal exposure. Dr Schilling received the Young Investigator Award from the Royal Society of Chemistry in 2019 for her work on zinc stable isotope as potential diagnostic biomarker for pancreatic cancer and NIEHS P30 Junior Faculty Development Award in 2022 for her work in isotope metallomics.

Kaj Sullivan: Kaj Sullivan received his BSc (Honours) in Geological Sciences in 2015 and his PhD in 2020, both from Queen's University (Canada). During his PhD, Kaj received the inaugural Hugh C. Morris Experiential Learning Fellowship and Mitacs Globalink Research Award which led to collaborations with researchers at National Researcher Council Canada and Imperial College London on projects related to i) expanding the range of biological reference materials characterized for their Cu isotopic composition, ii) investigating postprandial variations in Zn isotopic compositions in blood serum, and iii) exploring the heterogeneous distribution of Zn in the breast tumour environment using Zn isotopic analysis. After his PhD, Kaj received the Mitacs Elevate Postdoctoral Fellowship and used this to collaborate with Queen's University, IGO Ltd, and ALS Geochemistry on a project focused on developing geochemical methods to improve the ability to locate magmatic Ni-Cu deposits in areas with post-mineral cover. In February 2022, Kaj joined the A&MS research unit of Prof. Dr. Frank Vanhaecke at Ghent University (Belgium) as a postdoctoral researcher, where his focus returns to applications of biomedical isotopic analysis. In his spare time, Kaj is an avid hiker and cyclist and can occasionally be seen on the streets of Ghent playing the highland bagpipes.

Frank Vanhaecke: Frank Vanhaecke received a PhD in Chemistry from Ghent University (UGent, Belgium) in 1992. He carried out postdoctoral research at UGent and the Johannes Gutenberg University of Mainz (Germany). He is now Senior Full Professor in Analytical Chemistry at UGent, where he also leads the 'Atomic & Mass Spectrometry – A&MS' research group, specialized in the determination, speciation and isotopic analysis of (ultra)trace elements via ICP-MS. His group studies fundamentally-oriented aspects of the technique and develops methods for solving challenging scientific problems in interdisciplinary contexts. Specific topics of research include 2-D and 3-D elemental mapping by means of laser ablation (LA) – ICP-MS, single-event (single-particle and single-cell) ICP-MS and high-precision

isotopic analysis using multi-collector ICP-MS. Frank is (co)author of ca. 470 papers in peer-reviewed journals. In 2011, he received a ‘European Plasma Spectrochemistry Award’, in 2013, he was designated ‘Fellow of the Society for Applied Spectroscopy – SAS’, in 2017, he received the ‘Lester Strock Award’ from SAS and in 2023 the ‘Theophilus Redwood Prize’ from the Royal Society of Chemistry – RSC. At UGent, Frank is currently vice-president of the Department of Chemistry, member of the Executive Board and chairman of the Business Development Center ChemTech.