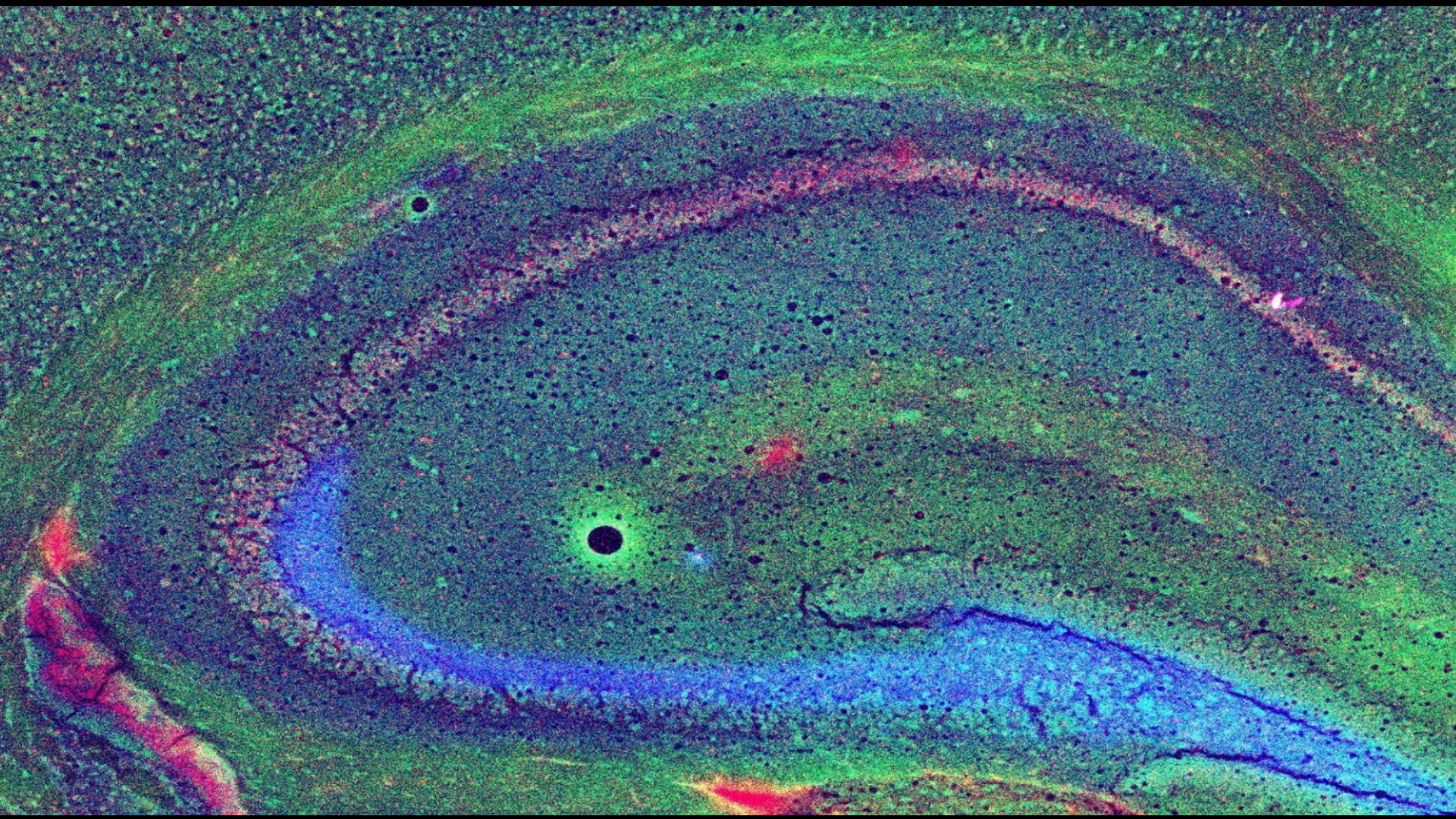
Possibilities for Studying Sulfur Speciation in Biological Systems Using MEX



XAS & MEX Workshop, August 26th 2021

Dr Mark Hackett – Curtin University

Curtin University

BACKGROUND

- BSc (Hons) Analytical Chemistry (Curtin University, 2006)
- PhD Chemistry (The University of Sydney, 2007 - 2010)
  - *Spectroscopic investigation of cerebral malaria*
    - *ischemia and blood-brain-barrier*
- Research Fellowships in Canada (University of Saskatchewan 2011 - 2015)
  - *Spectroscopic studies of neurodegeneration after stroke*
    - *ischemic stroke, intra-cerebral haemorrhage*
- Senior Lecturer in Analytical Chemistry at Curtin University (2016)
  - *Developing and applying spectroscopic approaches to study chemical mechanisms of memory loss during ageing and dementia*

Analytical Chemistry
Applied Spectroscopy

Neuroscience
Neurochemistry

CURTIN HEALTH INNOVATION RESEARCH INSTITUTE

Curtin University

dementia australia

Australian Government
Australian Research Council

CHEMISTRY @ CURTIN

CURTIN HEALTH INNOVATION RESEARCH INSTITUTE

Curtin University

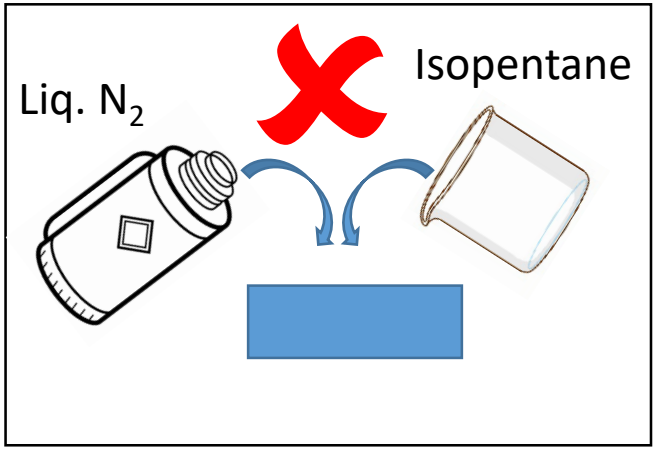
TALK OUTLINE

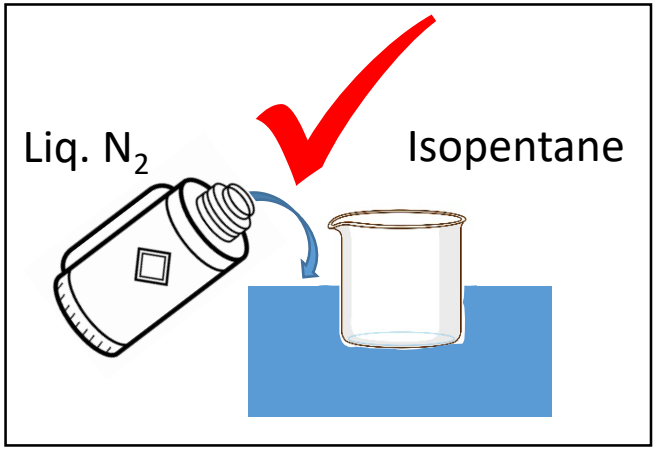
- Application of XAS to image Zn speciation in brain tissue
- Development of chemically specific XANES sulfur imaging
- Pitfalls with chemically specific sulfur imaging
- Applications of chemically specific sulfur imaging to study brain tissue

CURTIN HEALTH INNOVATION RESEARCH INSTITUTE
Curtin University
dementia australia
Australian Government
Australian Research Council

SAMPLE PREPARATION – RAPID FLASH FREEZING

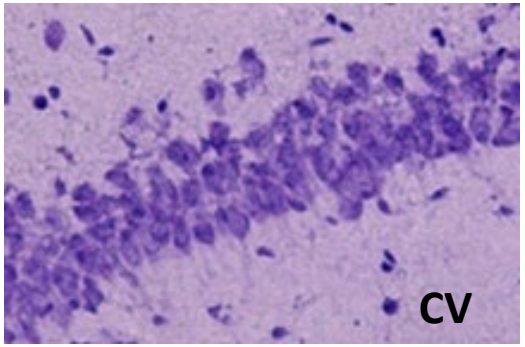
- Tissues samples are rapidly dissected and immediately flash frozen in liquid-nitrogen cooled iso-pentane
  - *(no perfusion, no fixation, no sucrose crytoprotection, no plastic or paraffin embedding)*
- Samples stored in -80 ^{0}C until ~ 1 week before analysis
- Samples sectioned on a *cryo-microtome at -16 ^{0}C* for brain (liver -18 ^{0}C), *10-µm-thick*. This is warmer than most SOPs, which are written for sucrose-cryoprotected, formalin-fixed tissue
- Tissue sections "melted" onto substrate, and either *transferred back to -80 ^{0}C freezer, or air-dried*
- Analysis typically within 1 week of sectioning
- If your samples are cracking, or tearing when you section, they are too cold. It takes about 30 – 40 min for tissue to warm from -80 ^{0}C to – 16 ^{0}C in cryostat
- Substrates
  - 500 nm, 1 µm, or 2 µm thick Si_3N_4
    - *XFM, or multi-modal XFM/IRM*
  - 500 µm or 1 mm thick CaF_2, BaF_2
    - *For IRM*



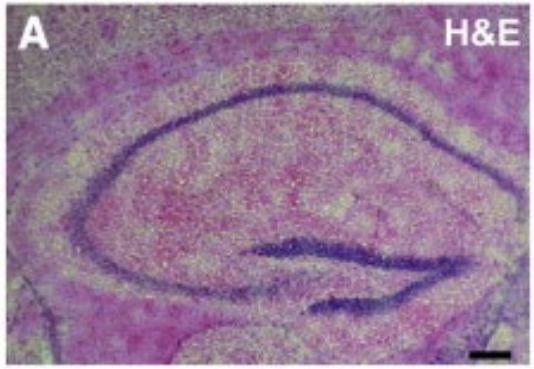


RAPID FLASH FREEZING, HISTOLOGY, & ICE CRYSTALS

- Histology and immuno-histochemistry can easily be performed on flash frozen tissues
- Either on the same tissue analysed at synchrotron, or serial sections
- Post-sectioning immersion fixation (typically 10 % PFA)

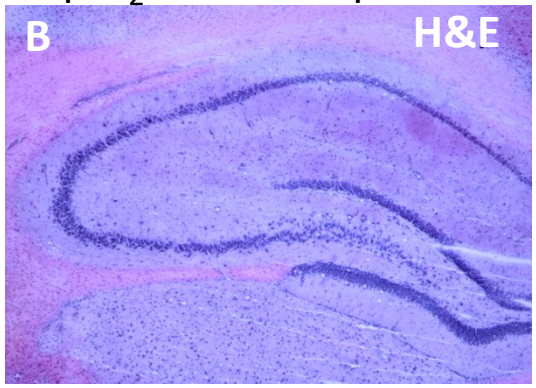


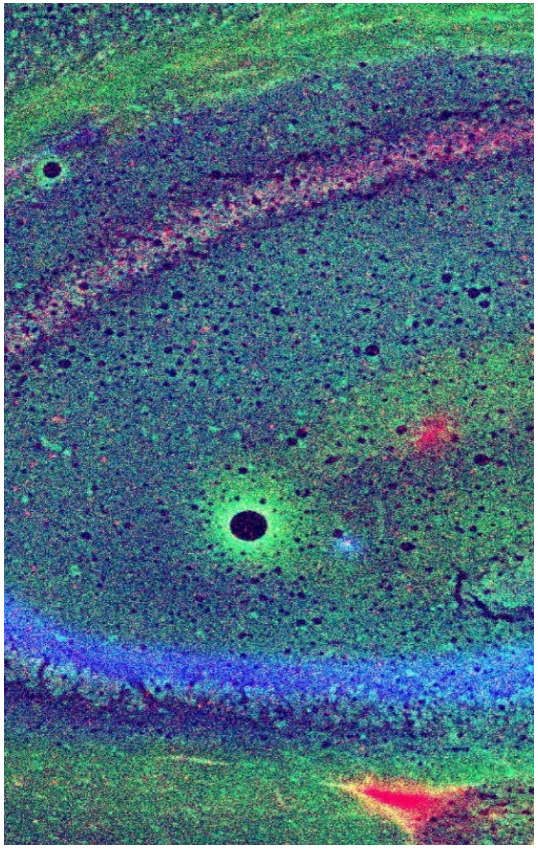
Freezing on dry ice



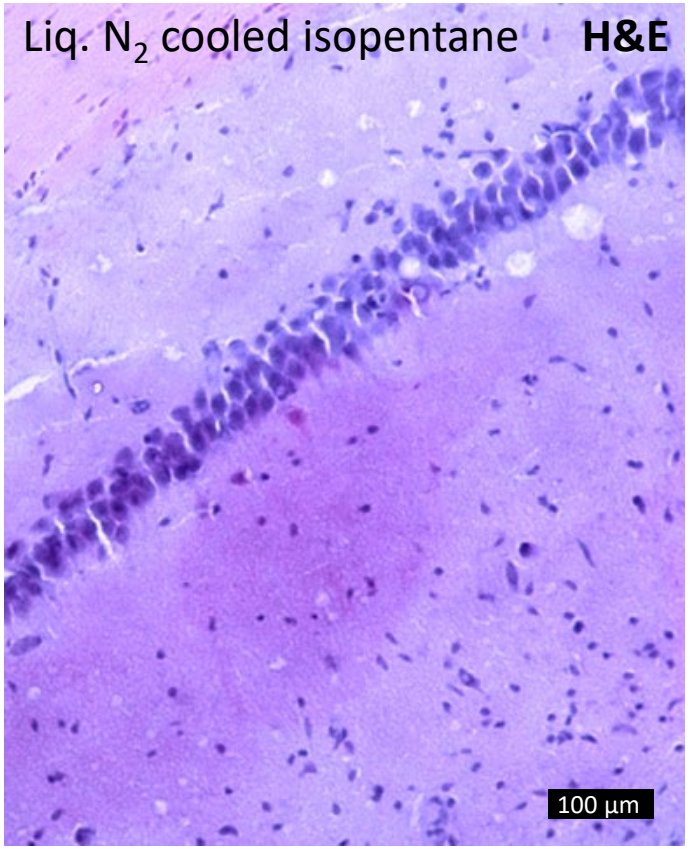
Neuroimage. 2011, 55(1): 32–38.

Liq. N_2 cooled isopentane



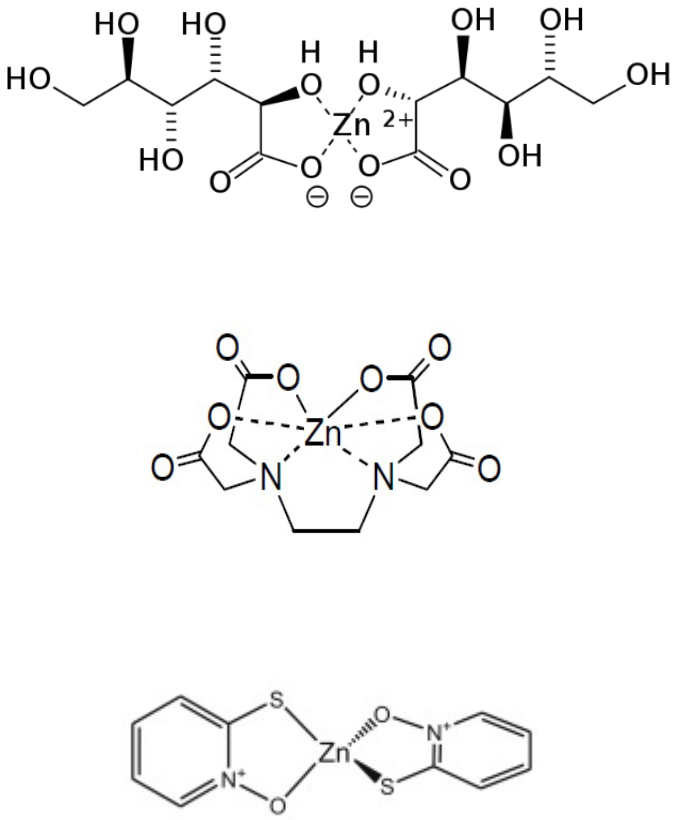


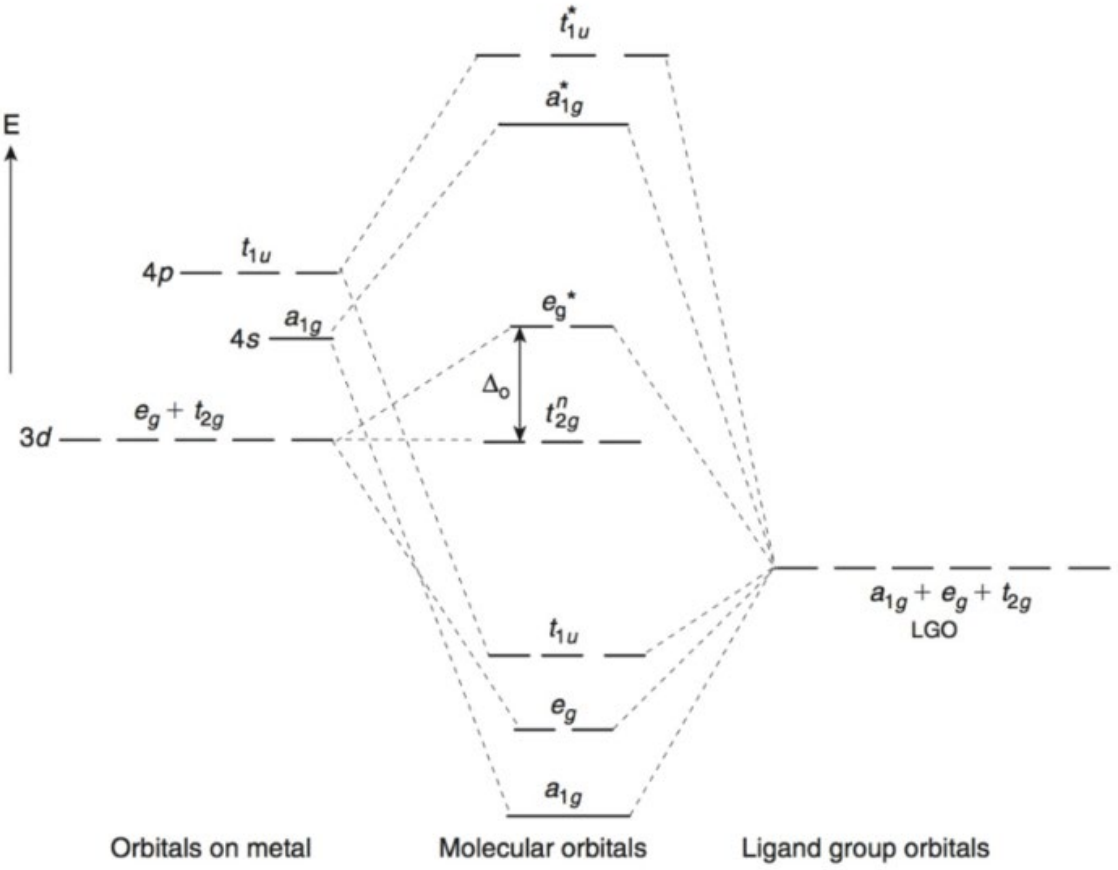
Liq. N_2 cooled isopentane **H&E**



DEVELOPING XANES Imaging – Zn in Brain Tissue

XANES spectroscopy is highly sensitive to the coordination geometry, number of ligands, and ligand type

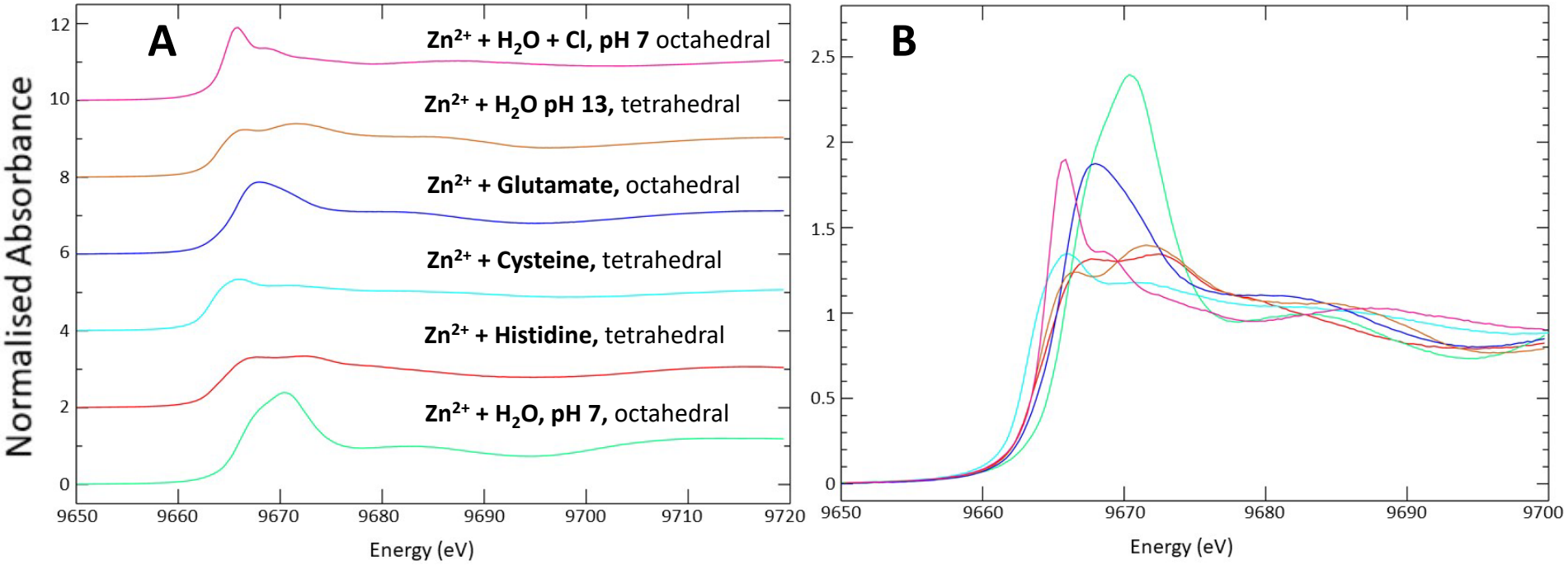




Archives of Biochemistry and Biophysics 611 (**2016**) 3-19.

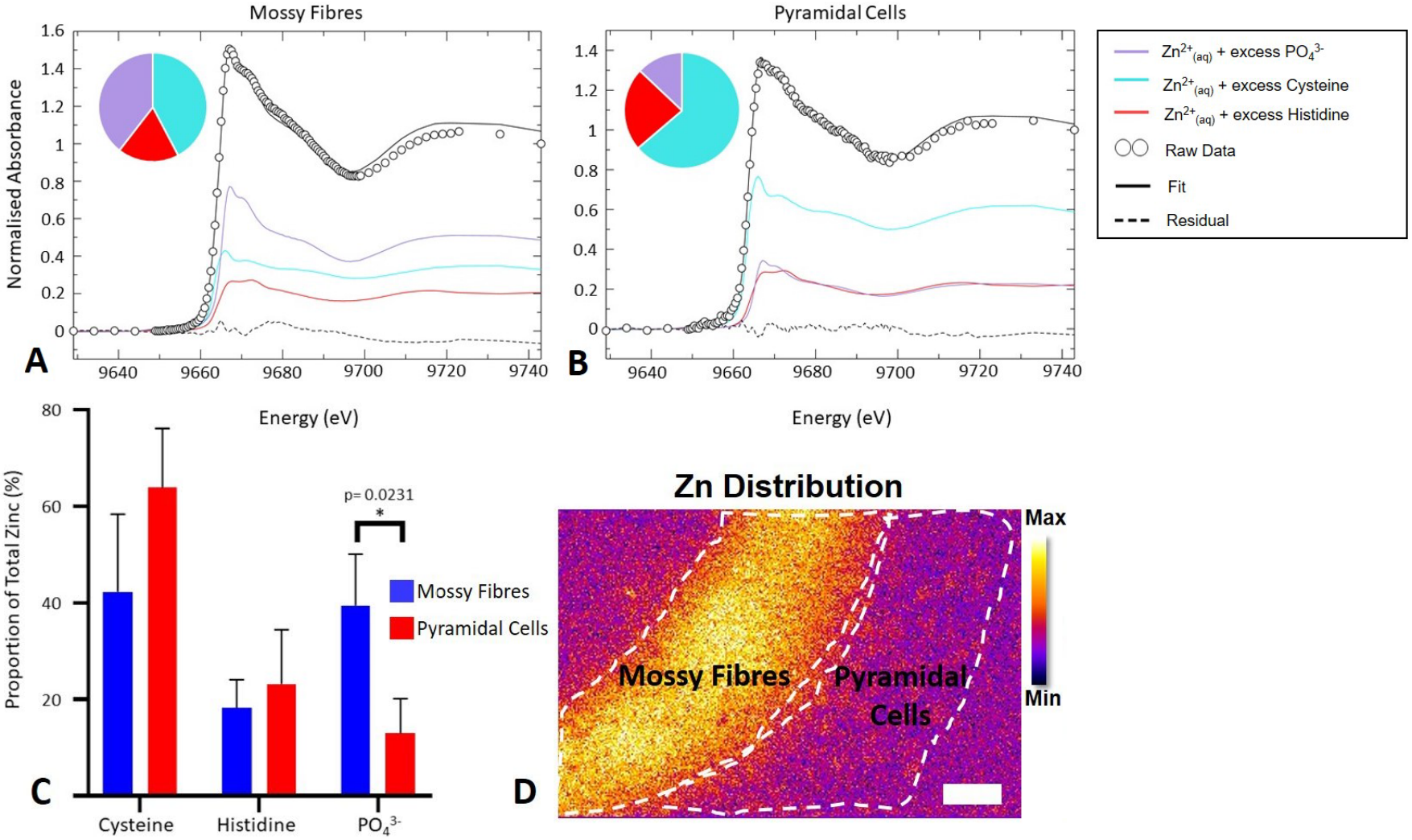
IS THE ZINC K-EDGE SENSITIVE TO COORDINATION CHEMISTRY? (YES)

Modelling Zn Bonding in Biological Environments:
Zn^{2+} in presence of excess biological ligands



Hollings et. al., *Metallomics*, 12, 2143-2144, **2020**.

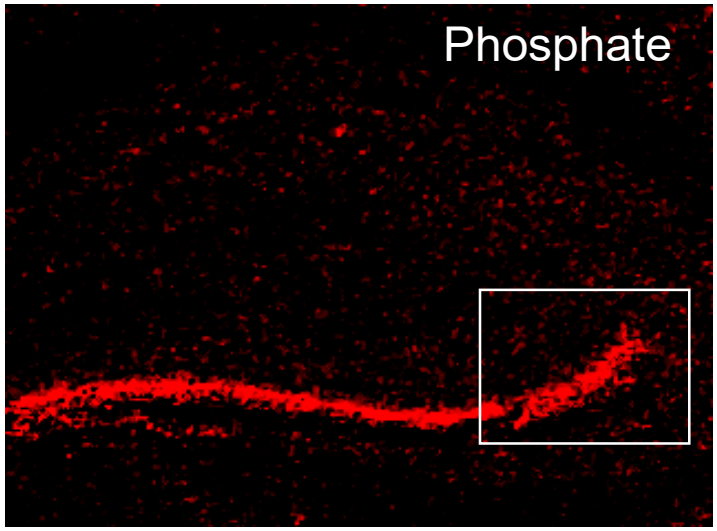
ZN SPECIATION IN BRAIN TISSUE

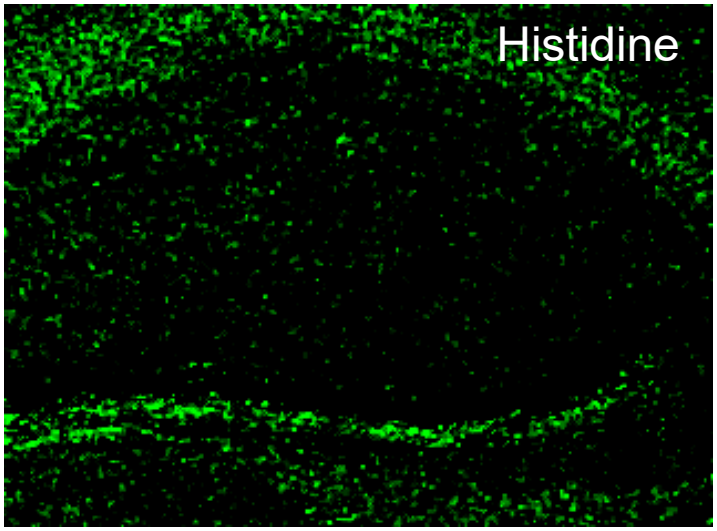


Ashley Hollings PhD

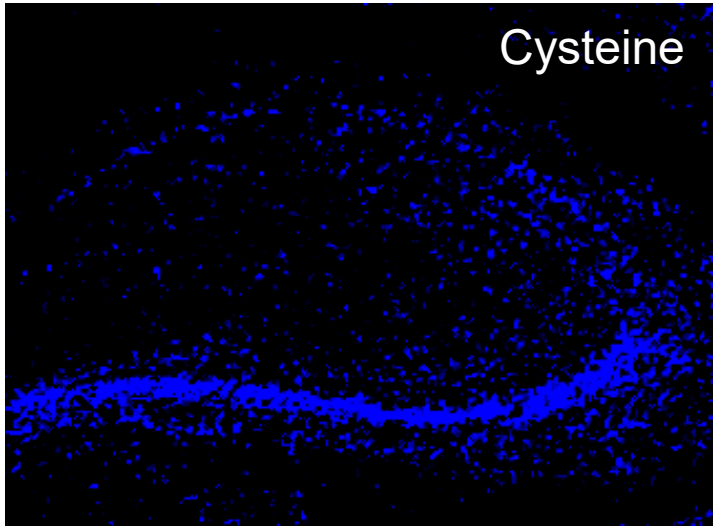
Hollings et. al., *Metallomics*, 12, 2143-2144, **2020**.

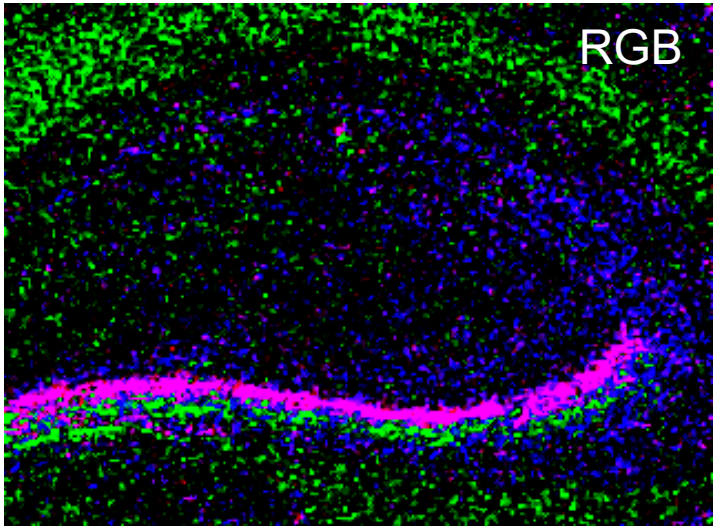
CHEMICALLY SPECIFIC XANES ZN IMAGING (XFM)



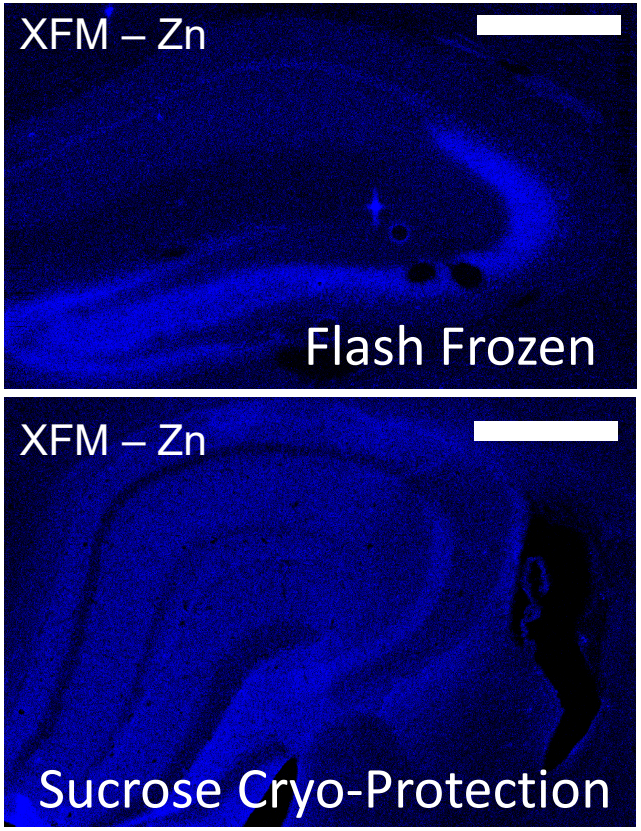


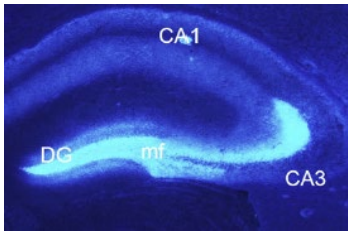
~1 ms dwell
~ 2 x 2 mm area
~2 – 5 µm pixel
~ 8 hours
~1 hours (ROI)





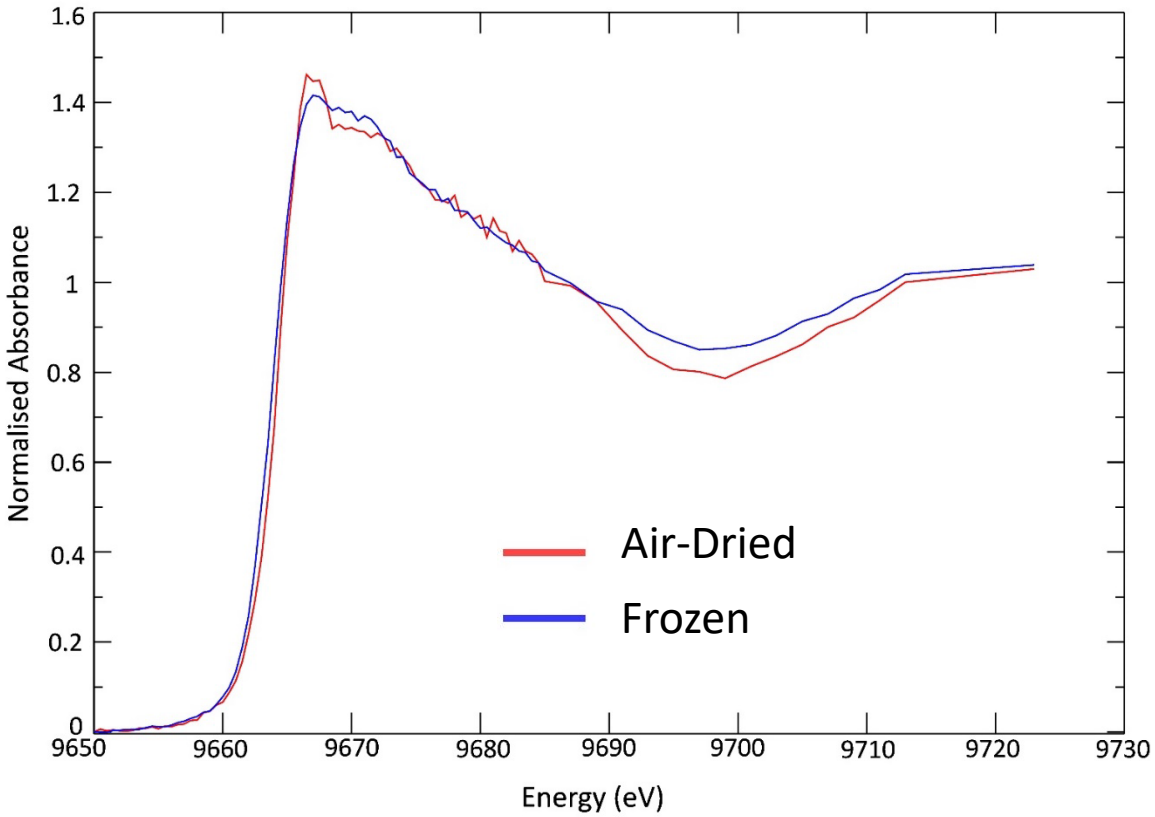
A CAUTIONARY TALE: SAMPLE PREPARATION & ANALYSIS CONDITIONS ARE CRITICAL!!



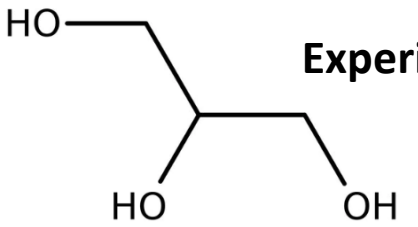


Lee, Joo-Yong, et. al., *Brain research* 1418, 12-22, **2011**

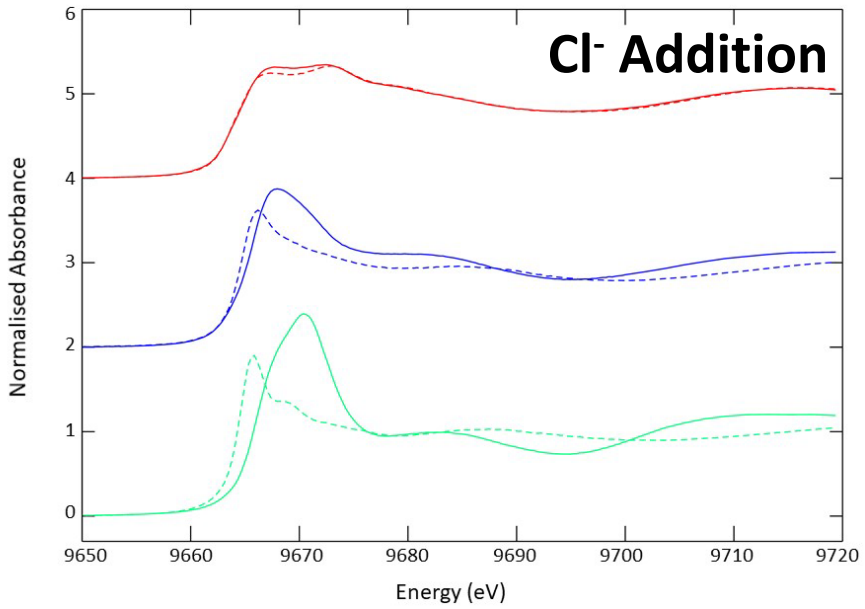
Hollings et. al., *JAAS*, 35, 2498-2508. **2020**

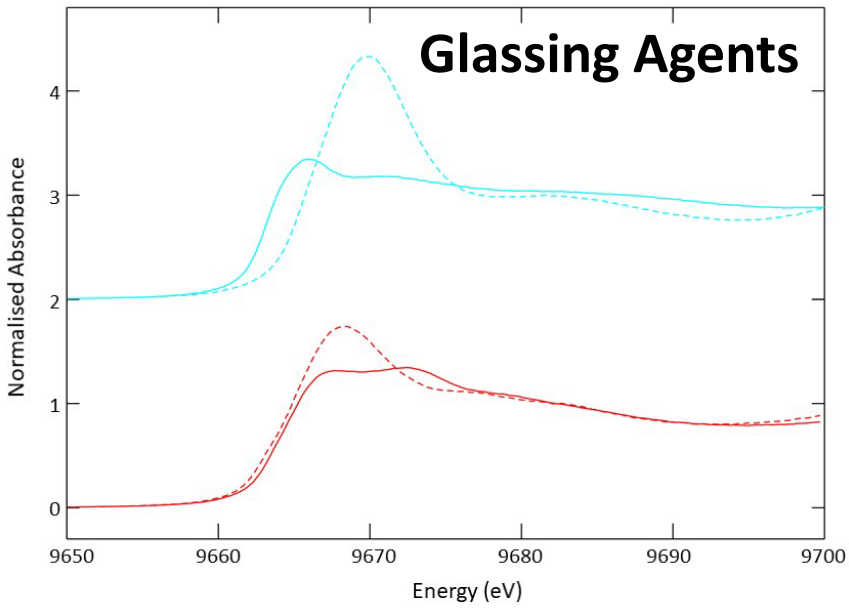


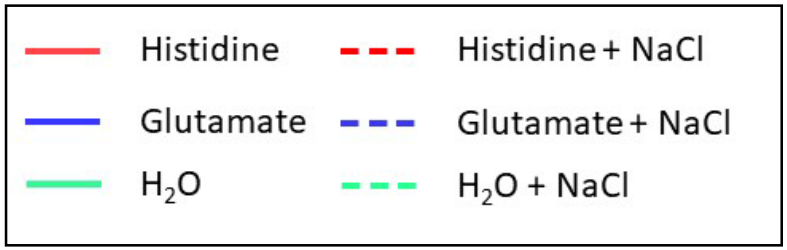
A CAUTIONARY TALE: SAMPLE PREPARATION & ANALYSIS CONDITIONS ARE CRITICAL!!

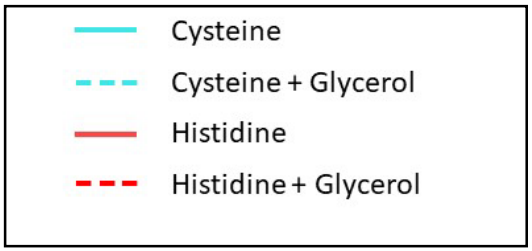


Experimental considerations for the XANES library: buffer ions, and "glassing agents"

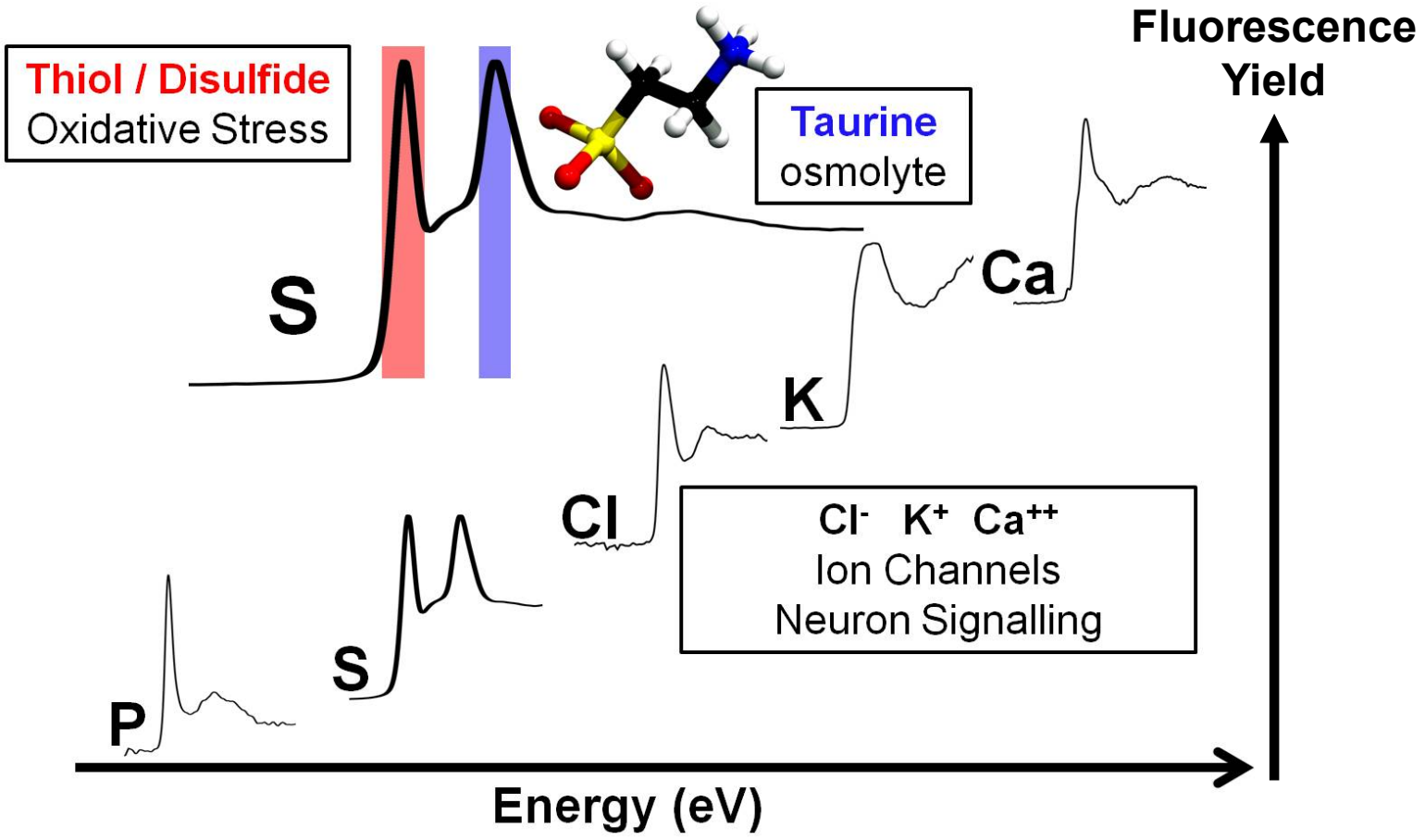








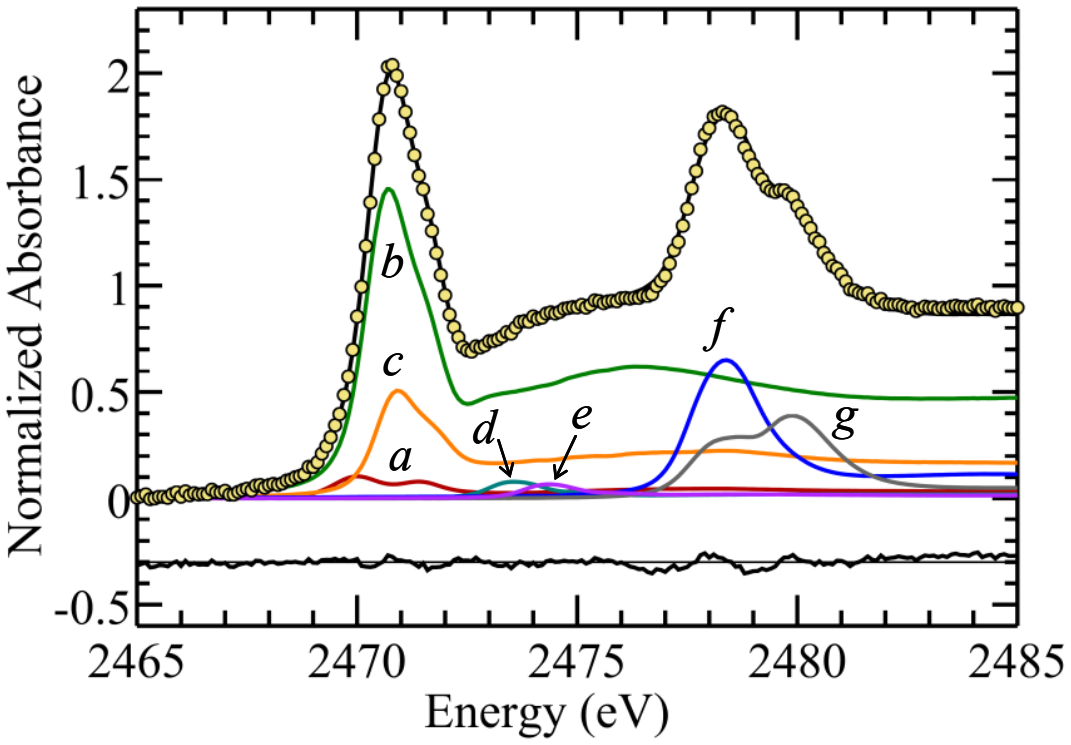
MEDIUM ENERGY X-RAY ABSORPTION SPECTROSCOPY (MEX) @ THE SYNCHROTRON

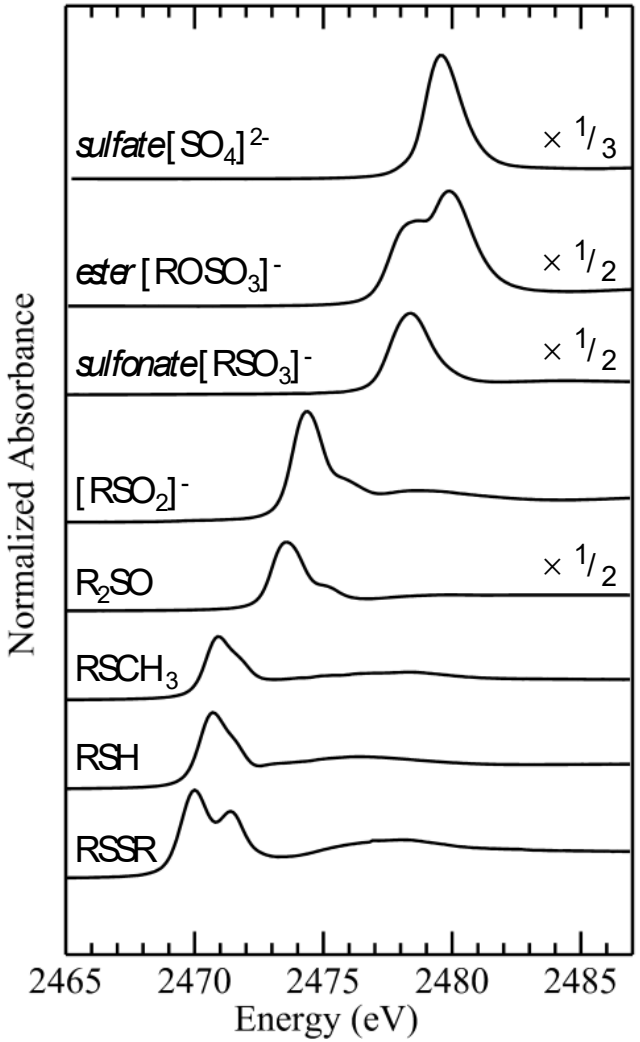


MEDIUM ENERGY X-RAY ABSORPTION SPECTROSCOPY

The Sulfur K-edge Is Very Sensitive To Sulfur Speciation

Fitting Sulfur K-edge XANES of Brain Tissue





Hackett et. al., *ACS Chemical Neuroscience*, 3 178-185, **2012**

BE WARY OF SAMPLE THICKNESS, AND CONCENTRATION

- Self-Absorption distorts XANES spectra
- Fitting errors
- Self-absorption is often not negligible in medium-energy range
- We aim for thin samples (< 20 um)
- Aqueous standard solutions

- CXRO Website
- https://henke.lbl.gov/optical_constants/

Research article

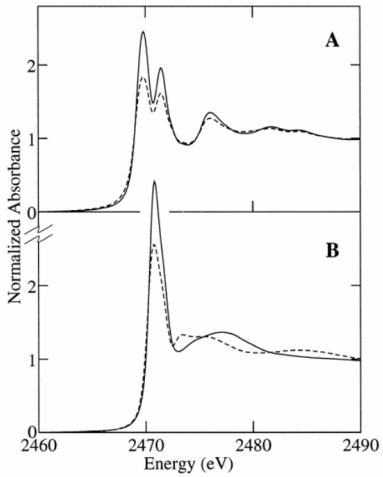
X-RAY Spectrometry

Received: 11 April 2012 Accepted: 31 May 2012 Published online in Wiley Online Library: 29 June 2012

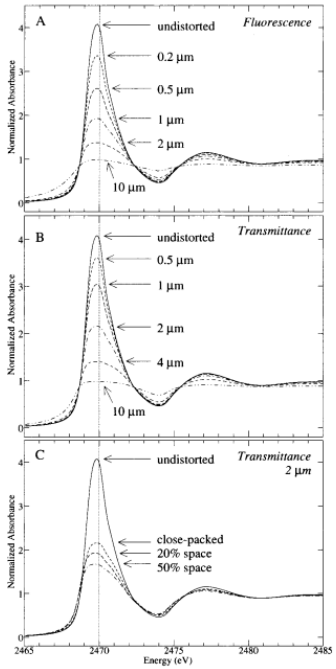
(wileyonlinelibrary.com) DOI 10.1002/xrs.2407

Sample thickness considerations for quantitative X-ray fluorescence analysis of the soft and skeletal tissues of the human body – theoretical evaluation and experimental validation

Magdalena Szczerbowska-Boruchowska*



Pickering et. al., FEBS Letters 2008

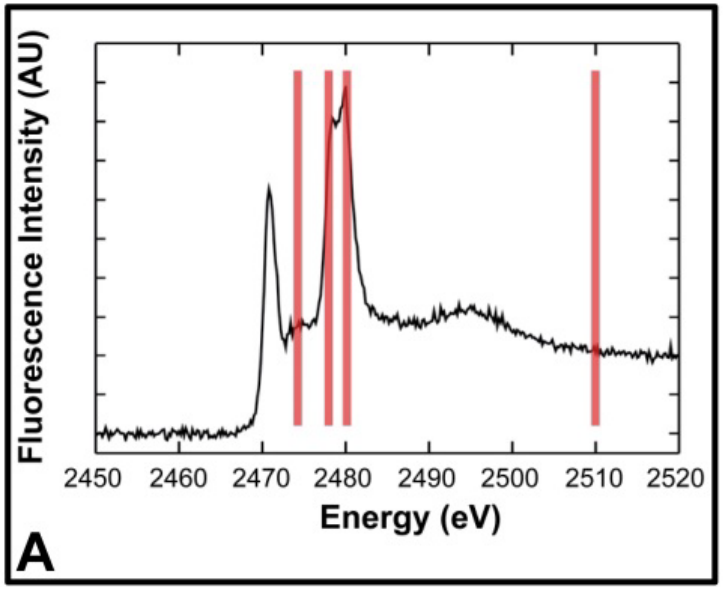


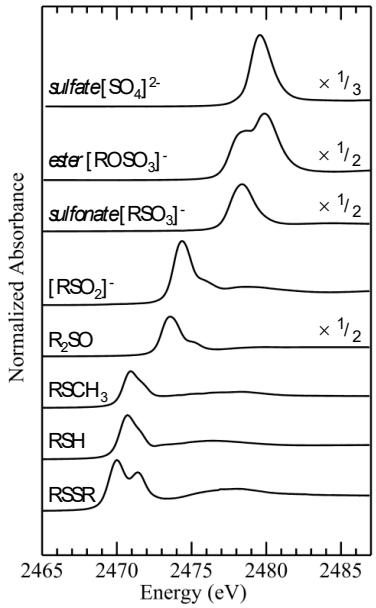
Pickering et. al., Biochemistry 2001

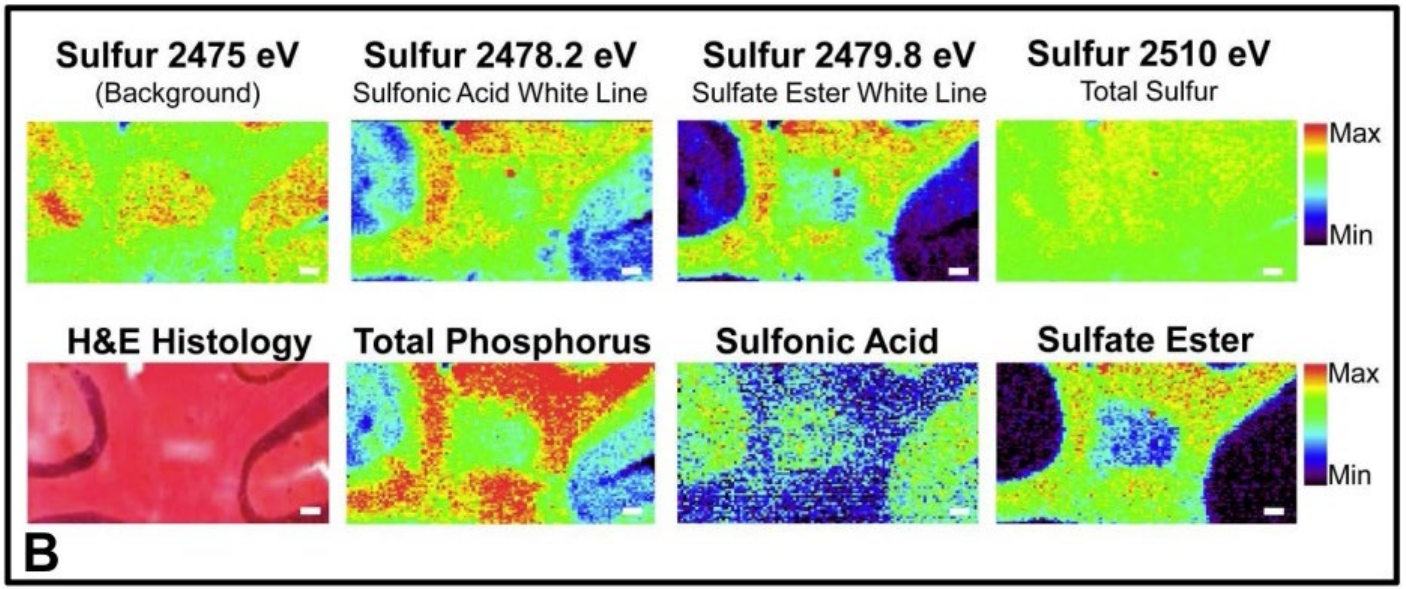
MEDIUM ENERGY X-RAY ABSORPTION SPECTROSCOPY TIME CONSTRAINTS

- Typically less flux at MEX beamlines
  - (relative to hard X-ray microprobes)
- Dwell time (50 – 500 ms)
- Full XANES per pixel, generally not feasible (time)

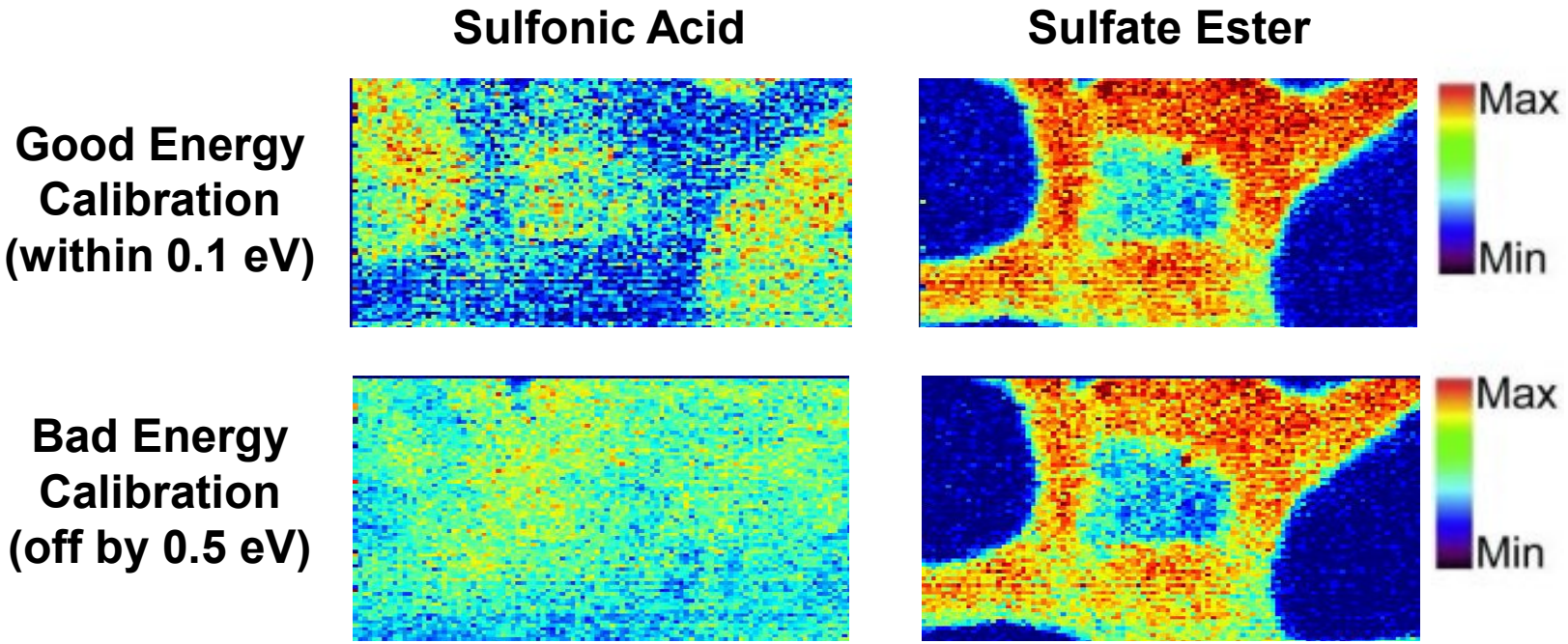
$$I_e = A_{\text{sulfonic acid}} \times I_{N(\text{sulfonic acid})} + A_{\text{sulfate ester}} \times I_{N(\text{sulfate ester})}$$







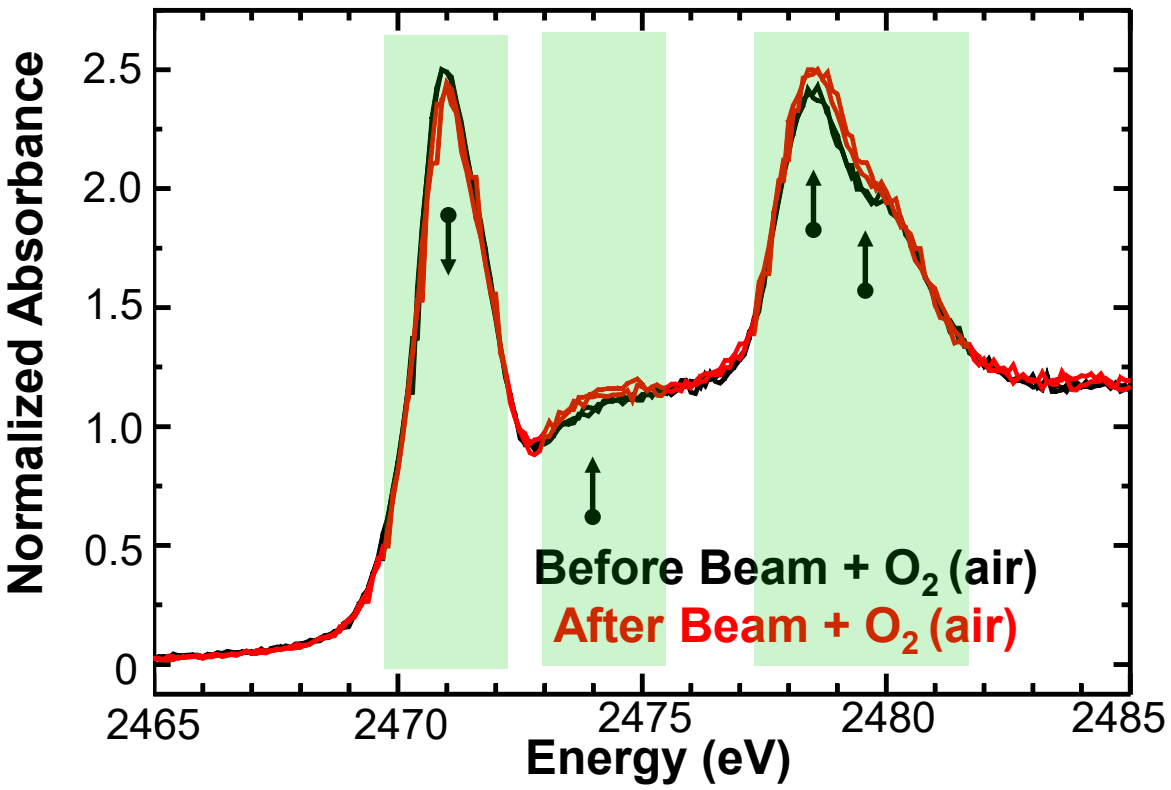
PIT FALLS WITH CHEMICALLY SPECIFIC XFM

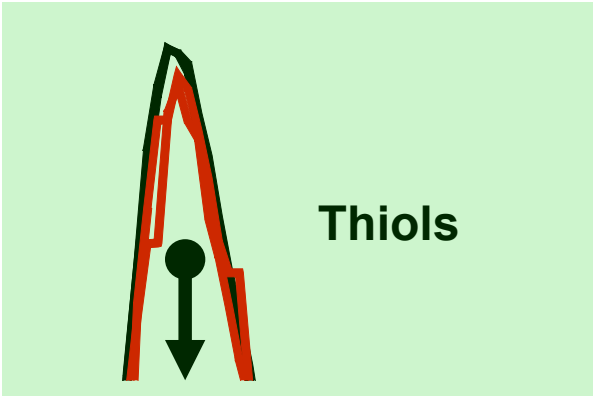


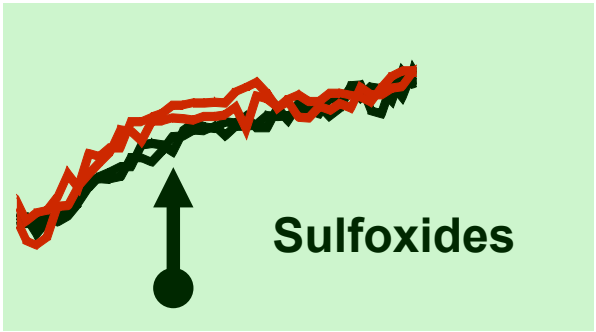
Energy Calibration & Stability is Critical!

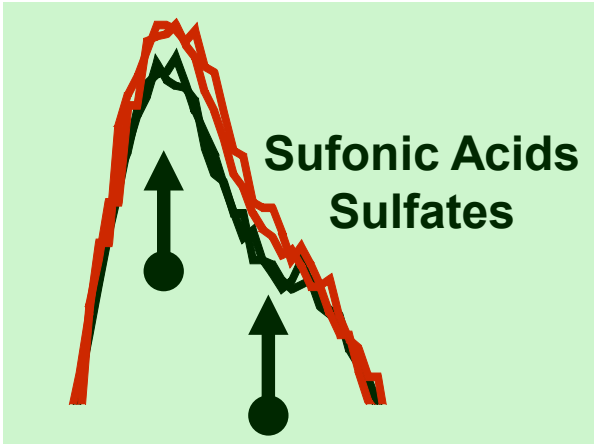
Hackett et. al. (2016) Analytical Chemistry
Hackett et. al. (2016) Biochemistry

X-RAY PHOTO-DAMAGE



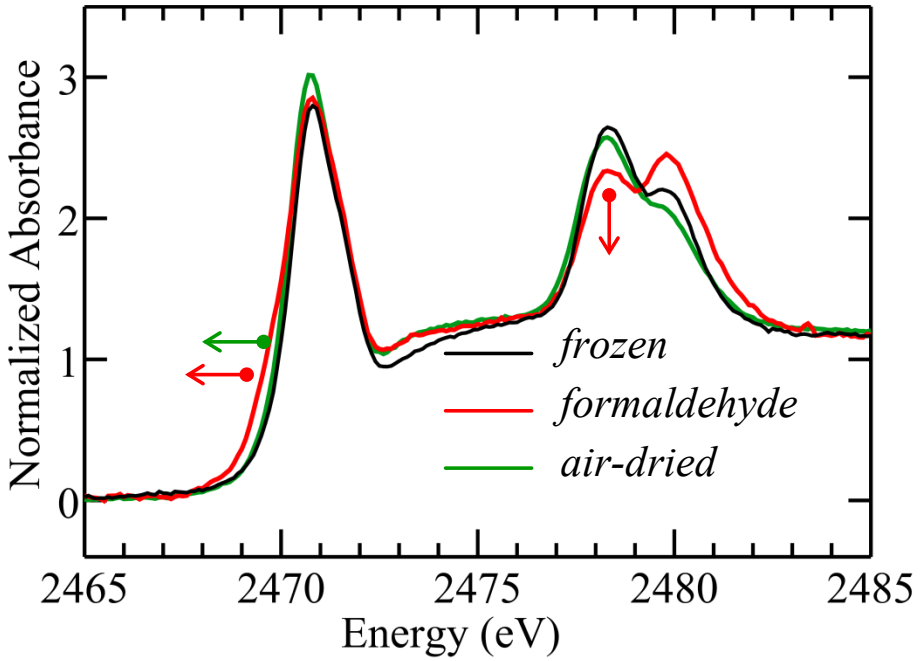


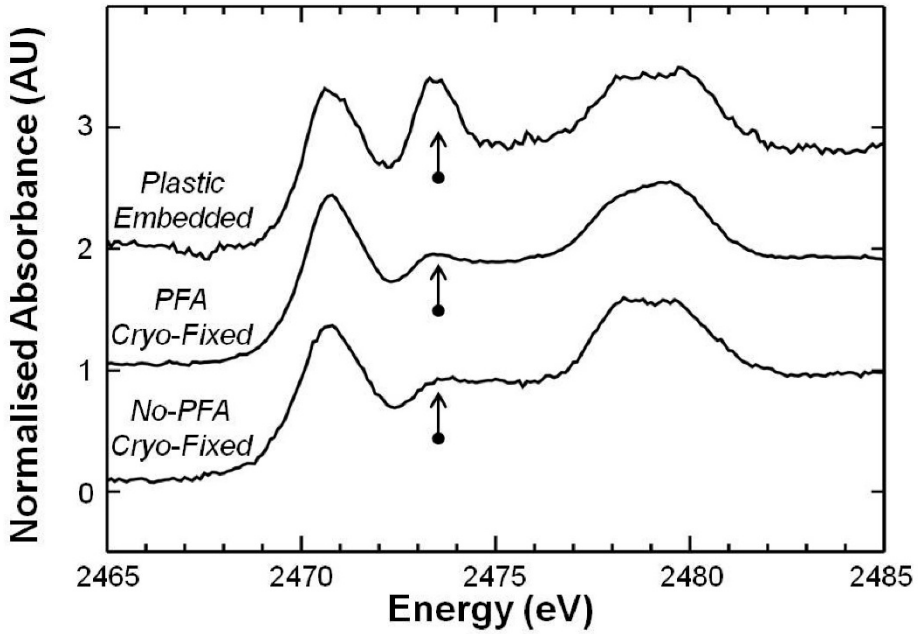




SAMPLE PREPARATION – DON'T USE CHEMICAL FIXATION

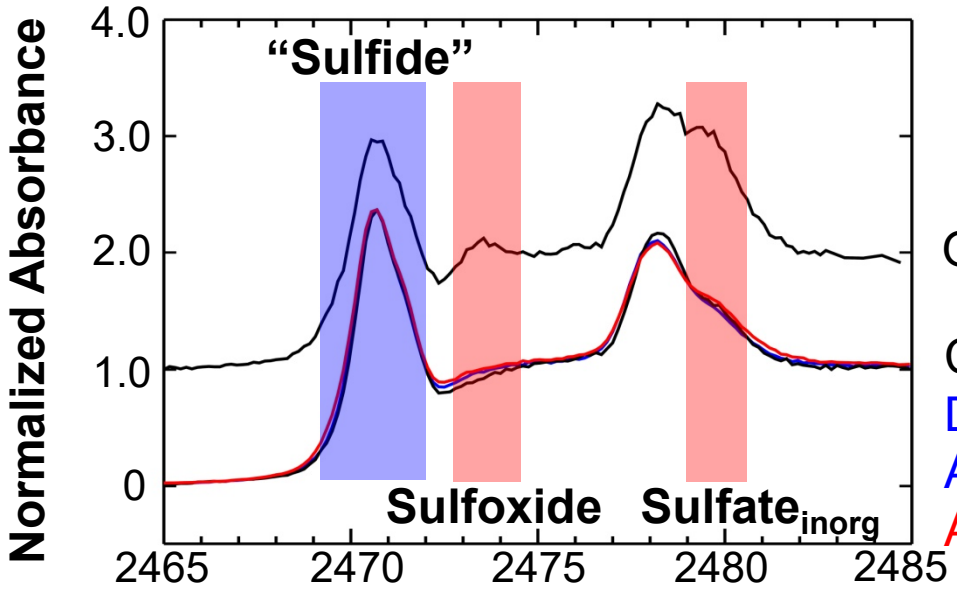
Chemical fixation = sulfonic acid leaching & thiol oxidation
Paraffin or plastic embedding = massive sulfur oxidation





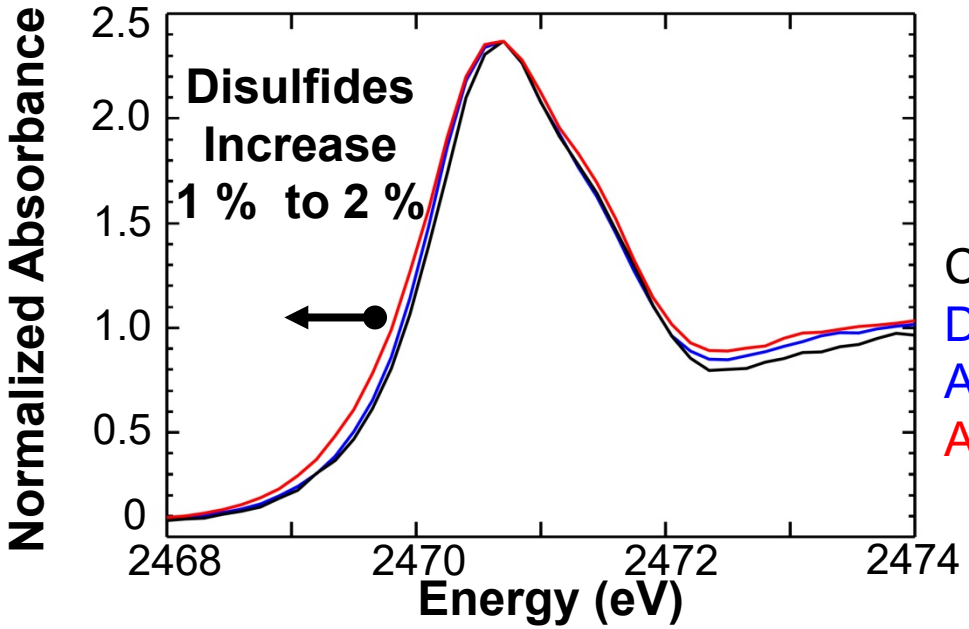
Hackett et al. (2012) ACS Chemical Neuroscience
Hackett et al. (2016) ACS Biochemistry

EFFECTS OF SAMPLE PREPARATION



Old sample (2 months)

Cold-Finger
Dried in Inert Atmosphere
Air Dried

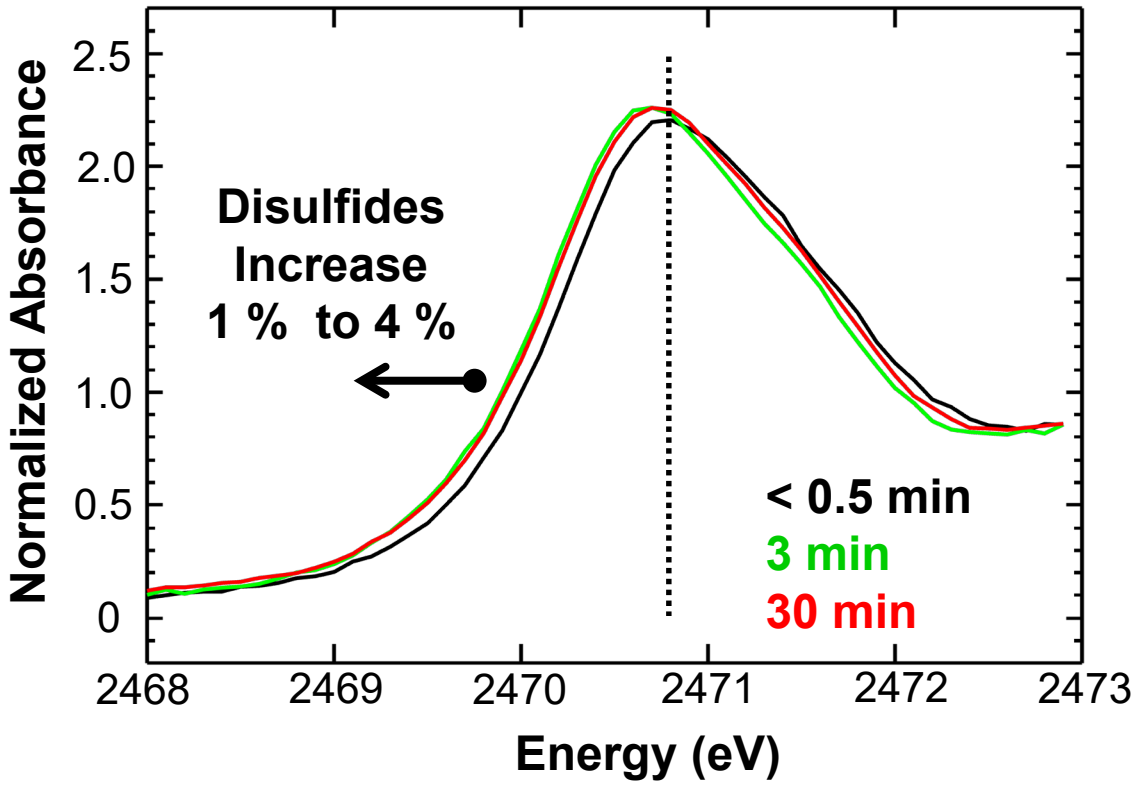


Cold-Finger
Dried in Inert Atmosphere
Air Dried

Hackett et al. (2017) J. Syn. Radiat.

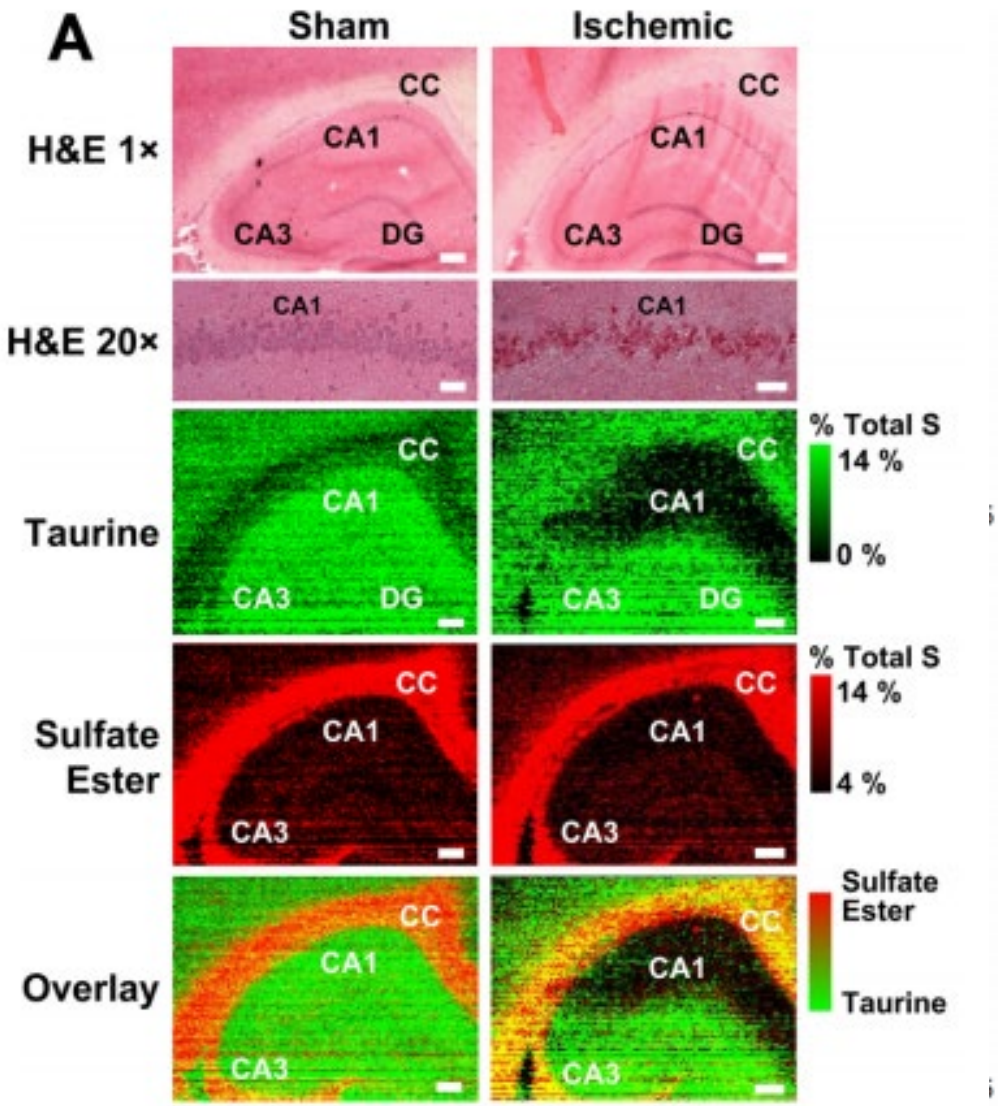
EFFECTS OF POST-MORTEM INTERVAL

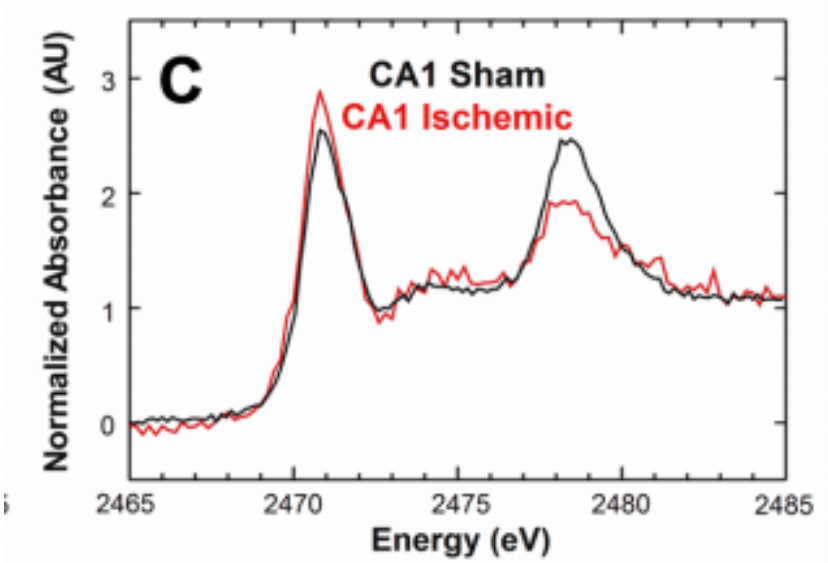
The time it takes to sacrifice an animal and remove its brain (30 seconds – 3 minutes) induces ischemia and oxidises thiols to disulfides

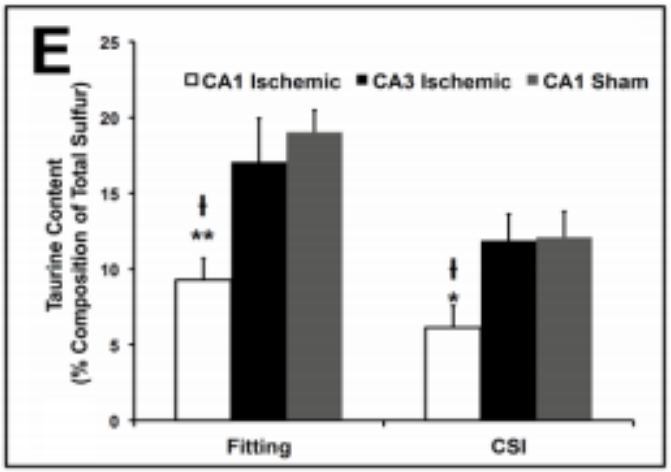


Hackett et al. (2015) ACS Chemical Neuroscience

MEDIUM ENERGY X-RAY ABSORPTION SPECTROSCOPY BRAIN TAURINE LEVELS AFTER STROKE

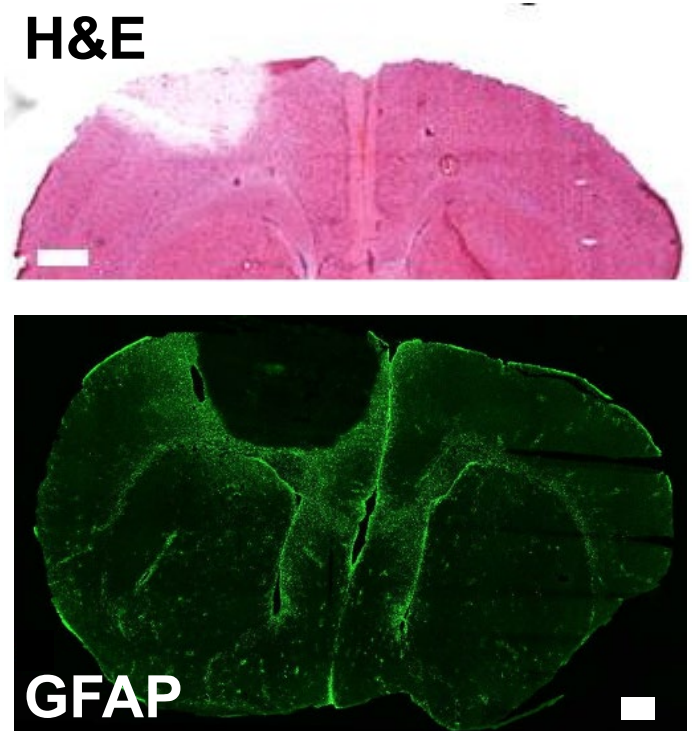




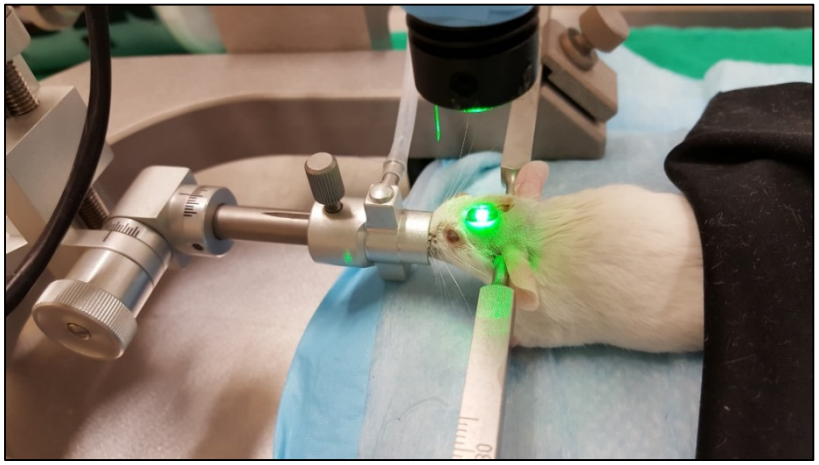


Hackett et. al. (**2015**) *Free Rad. Biol. Med.*, Hackett et. al. (**2016**) *Analytical Chemistry*. Hackett et al. (**2016**) *Biochemistry*

MEX: IS THERE AN ISCHEMIC PENUMBRA IN THE MURINE PHOTOTHROMBOTIC STROKE MODEL?

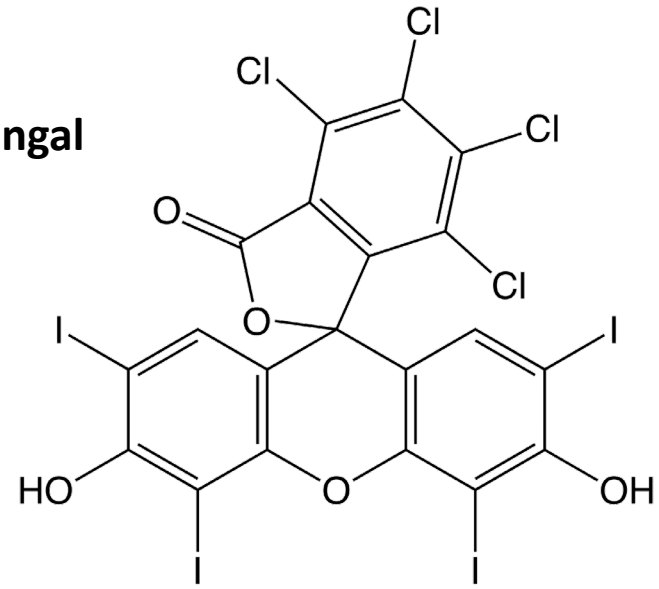


Scale = 500 µm



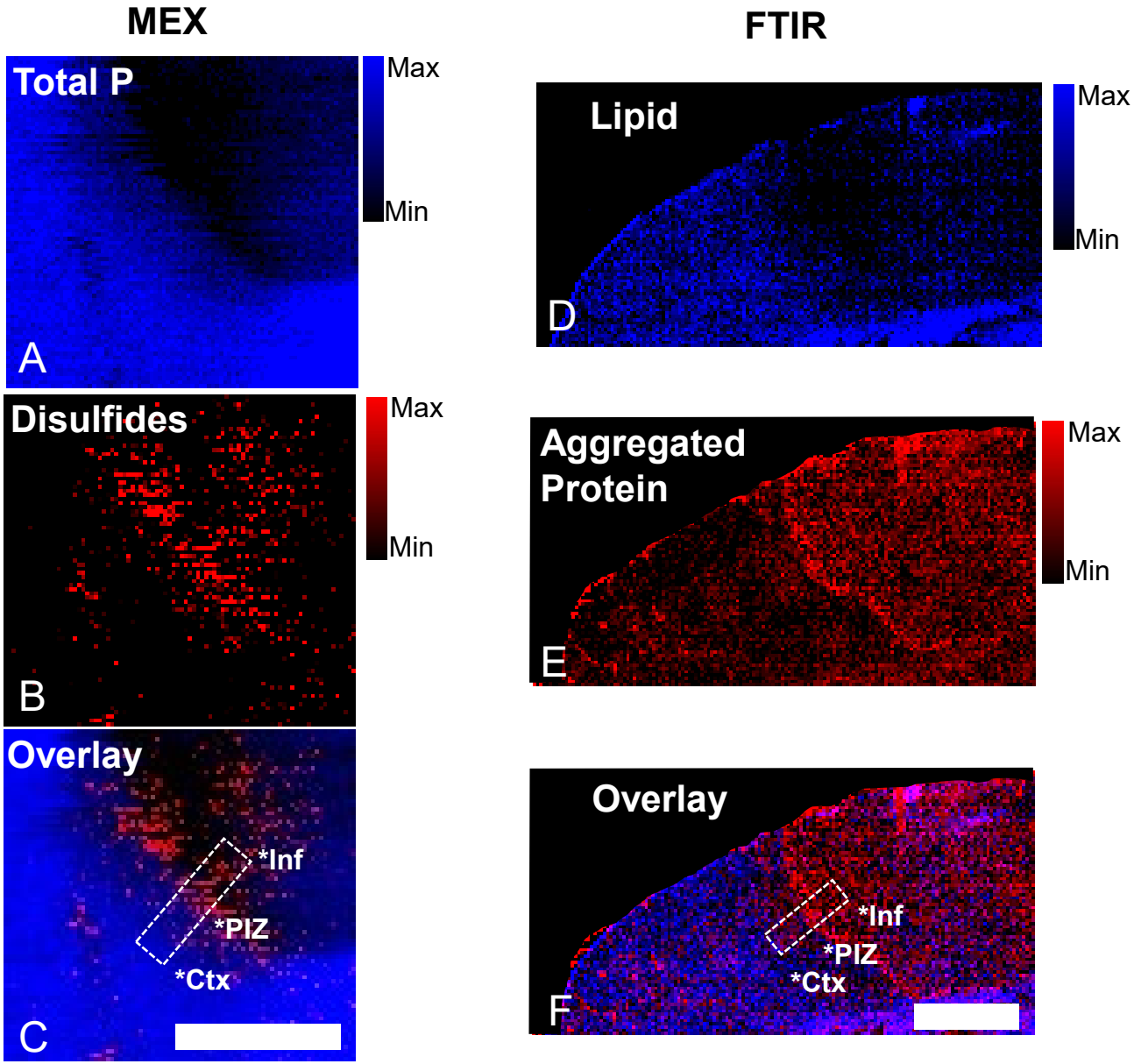
Saskatchewan Cerebrovascular Unit

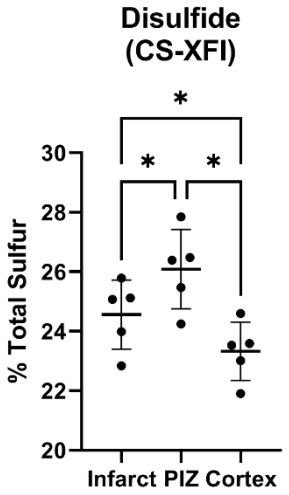
Rose Bengal

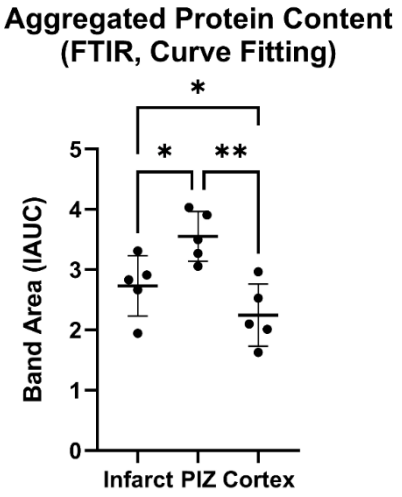


Is there an ischemic penumbra in the photothrombotic stroke model?

MEX: IS THERE AN ISCHEMIC PENUMBRA IN THE MURINE PHOTOTHROMBOTIC STROKE MODEL?







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ANSTO

dementia australia™

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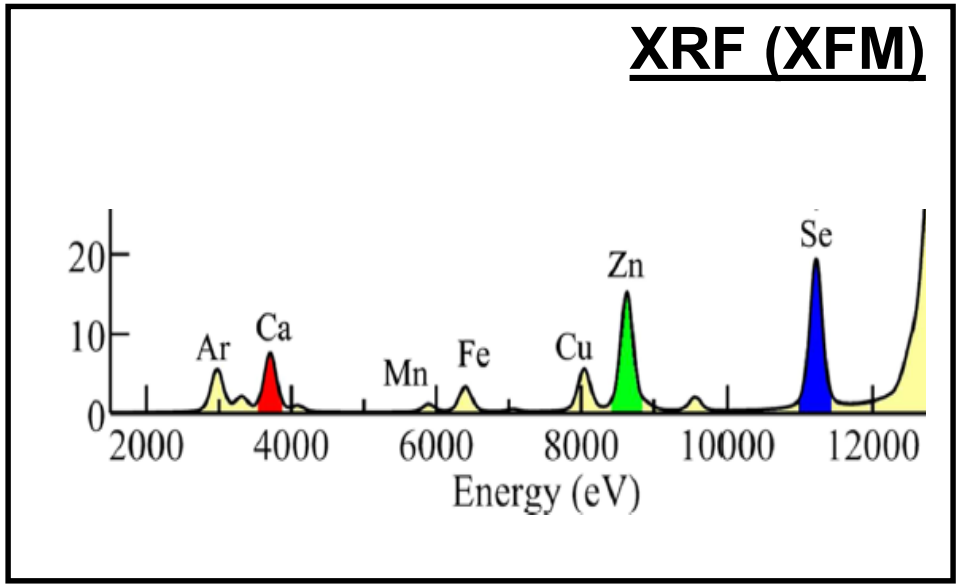
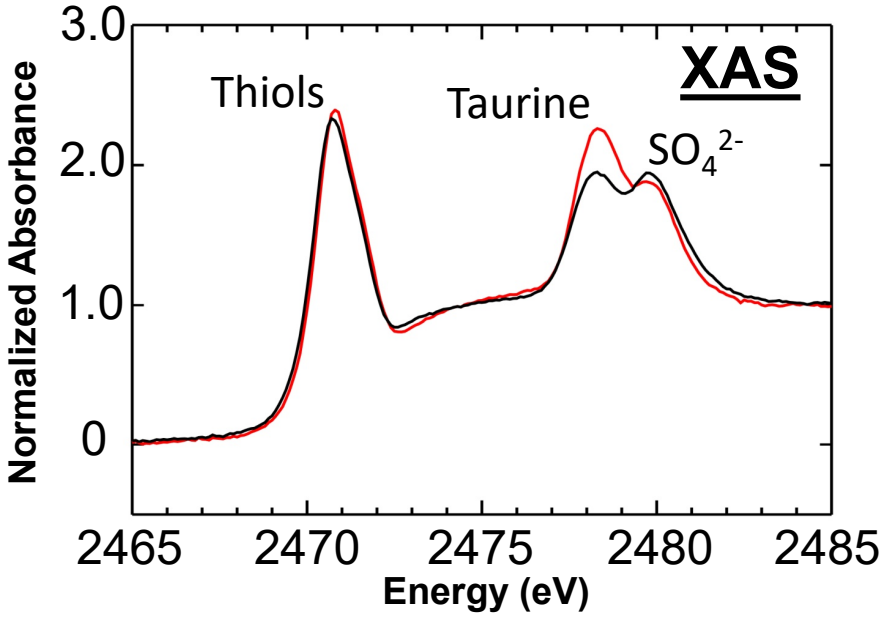
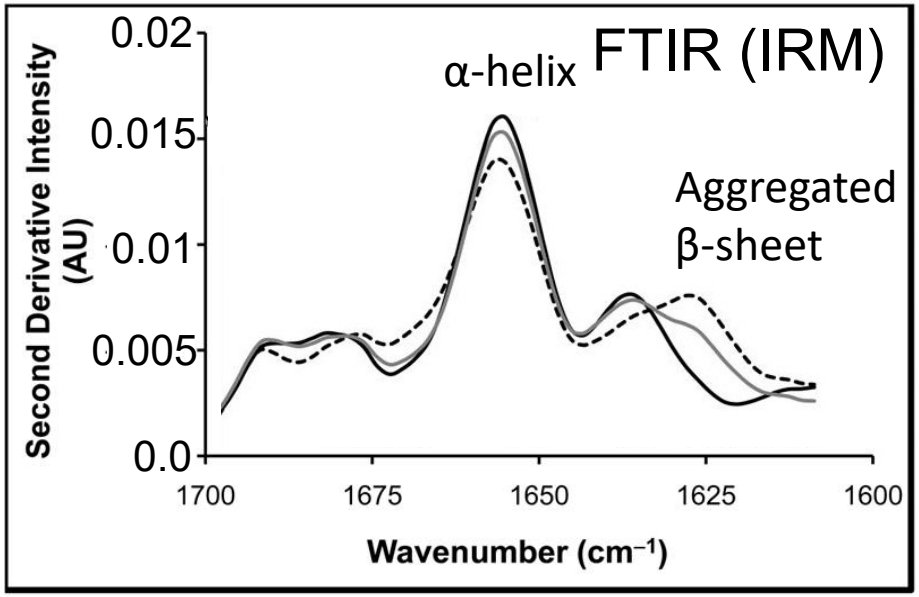
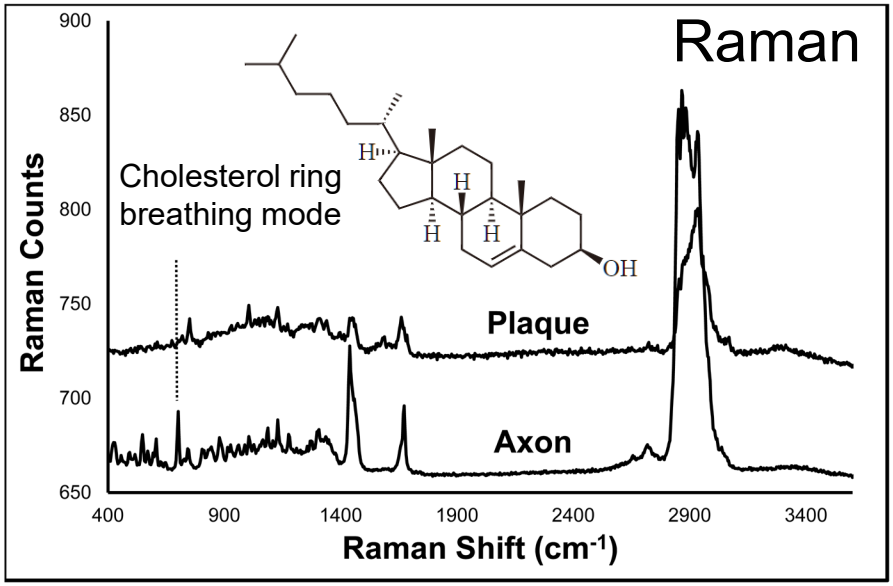
Australian Research Council

INTRODUCTION: THE TECHNIQUES I USE…….







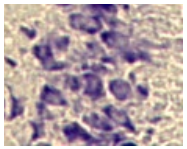


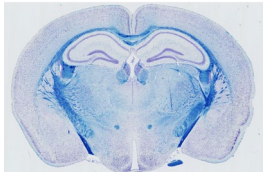
WHAT IS THE ROLE OF SPECTROSCOPIC IMAGING?

Morphology
(**WHERE**)

Chemical / Composition
(**WHAT/HOW MUCH**)

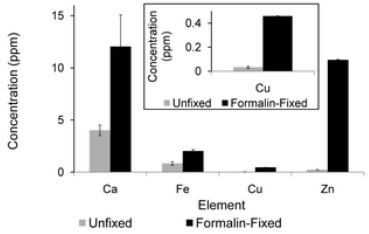
Light Microscopy
Electron Microscopy





IRM, XFM,
XAS, Raman
LA-ICP-MS, TOF-SIMS, PIXE……

Assays
Bulk Techniques
Mass Spectrometry



High Spatial Resolution
Low Amount of Chemical Information

Poor Spatial Resolution
Information Rich