

Sulfur X-ray Absorption Spectroscopy as an *in-situ* Probe of Plant Biochemistry



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Overview

- Introduction, why bother with sulfur XAS?
- How do we do it?
- Examples

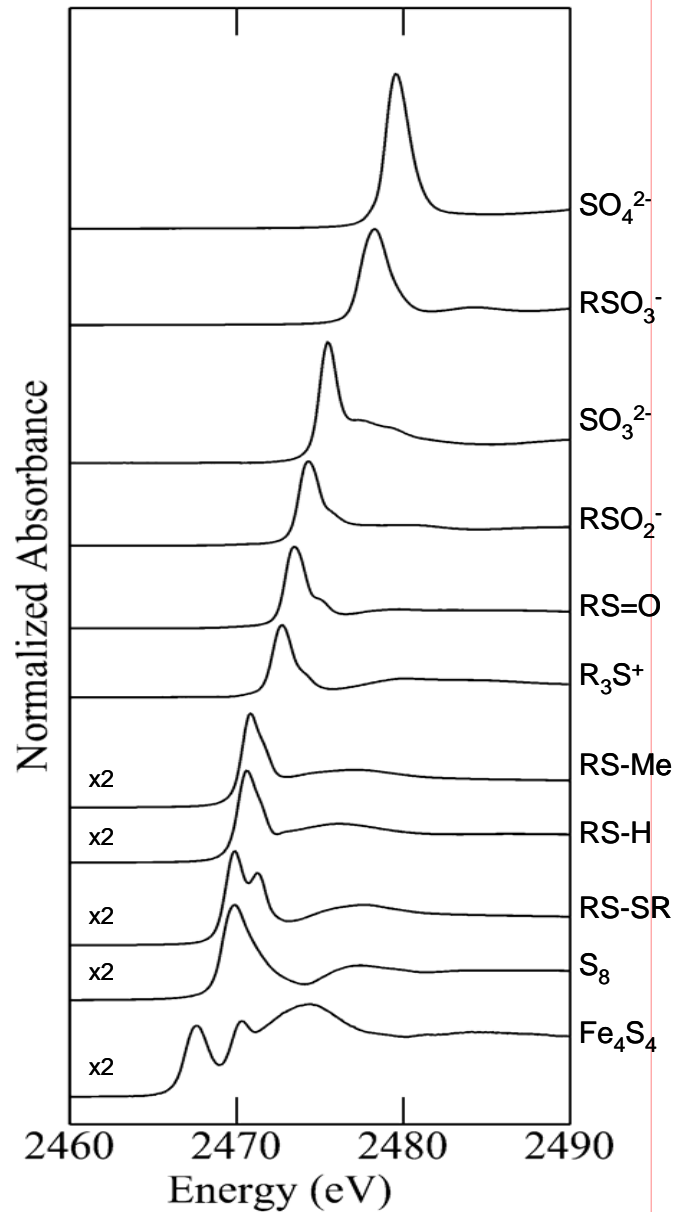
Why Study Sulfur?

- Important
 - Sulfur is an essential biological element
- Spectroscopically silent
 - Sulfur biochemistry only partly understood because there are so few tools for studying it in biological systems

Why Use Sulfur X-ray Absorption Spectroscopy ?

- Sensitive to chemical form (more on this)
- Can be an *in situ* probe
 - No pretreatment necessary
 - Looks at all occurrences of sulfur (solid, solution, gas etc.)
 - Quantitative estimations of chemical species
 - Ideal for complex samples such as whole tissues

Sulfur K X-ray Absorption Near-Edge Spectrum is Rich in Structure

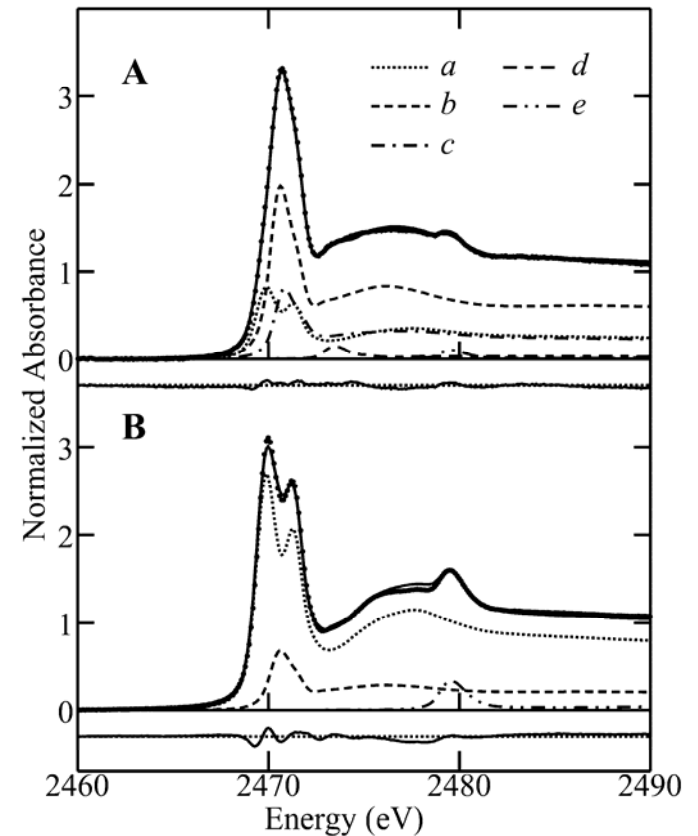


- Observed variations:
 - Oxidation state: -2 to +6
 - Energy of peak: 14 eV
- Spectrum sensitive to local structure
 - can be used as a "fingerprint" of the chemical "class" of species present

Chemical Analysis using Sulfur XAS

We can use least-squares fitting and other statistical tools (e.g. principal component analysis) to obtain a quantitative chemical analysis of all the sulfur present in a sample.

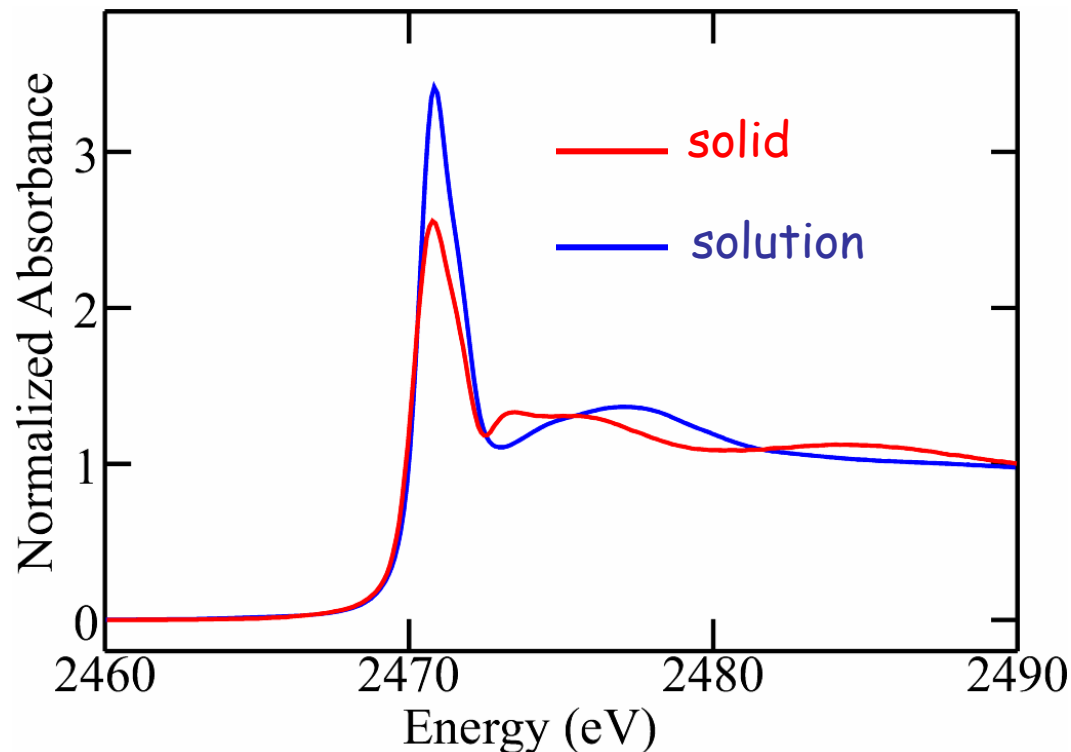
Pickering, I. J., Prince, R. C., Divers, T. C. and George, G. N.
“Sulfur K-edge X-ray Absorption Spectroscopy for Determining
the Chemical Speciation of Sulfur in Biological Systems”
FEBS Letters 1998, **441**, 11-14.



Requirement - Must have good models for reference spectra, and these models must be in the correct form.

Solids vs. Solutions...

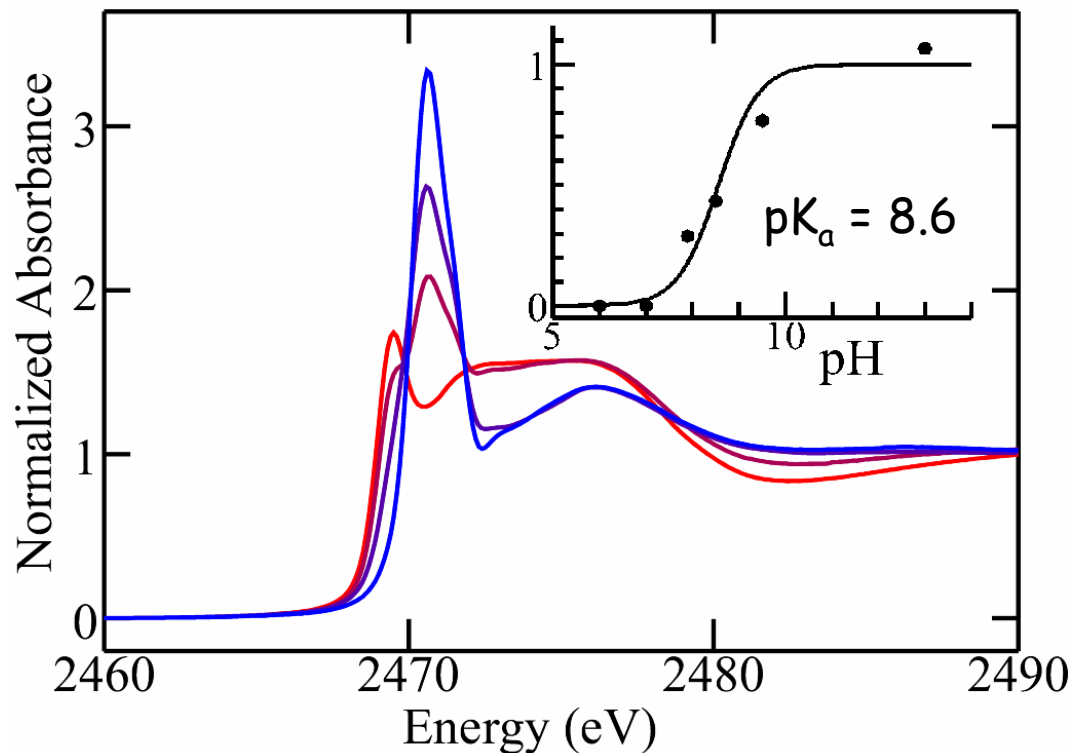
solid vs. aqueous solution spectra of methionine



In general peaks of solution spectra are more pronounced than with solids. The reason for this is that crystal packing forces in the solid distort the molecule lifting orbital degeneracy and cause bound state transitions to be more spread out in energy.

pH can be important

Spectra of an aqueous solution of cysteine taken at different pH values.

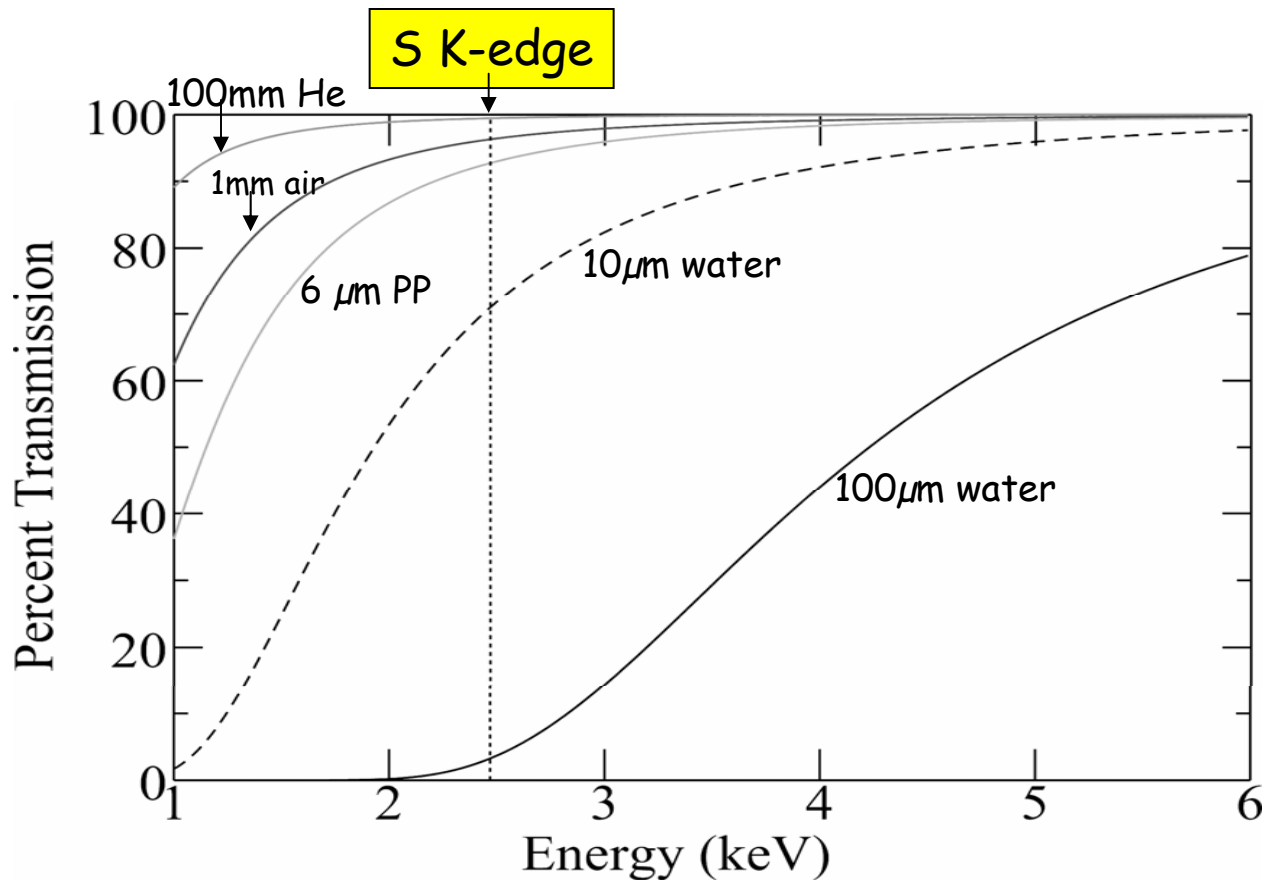


Experimental Aspects

- Attenuation issues
- Experimental setup

Why is Sulfur K-edge XAS Different?

Attenuation is more significant than for higher energies



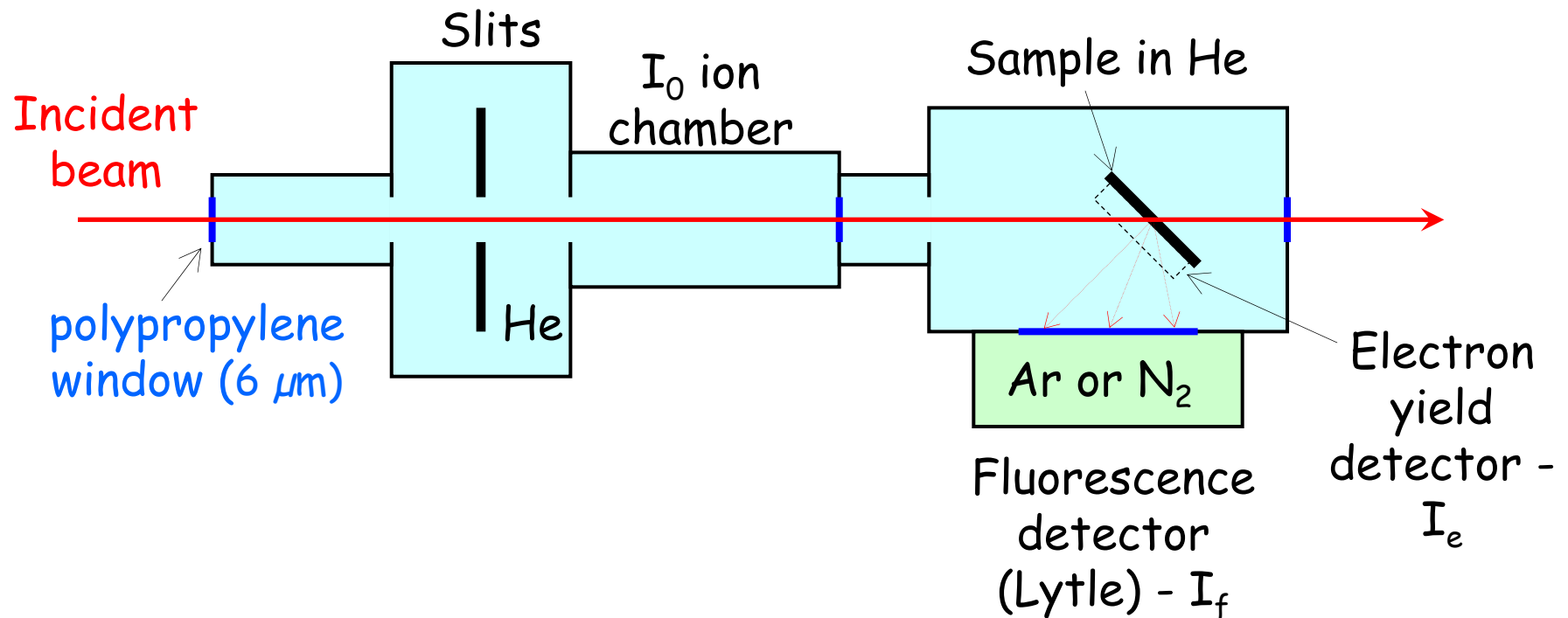
Can use:

- Thin polymer windows
- 1mm of air
- Long helium flight paths

P, Cl K-edges similar issues

Experimental Setup

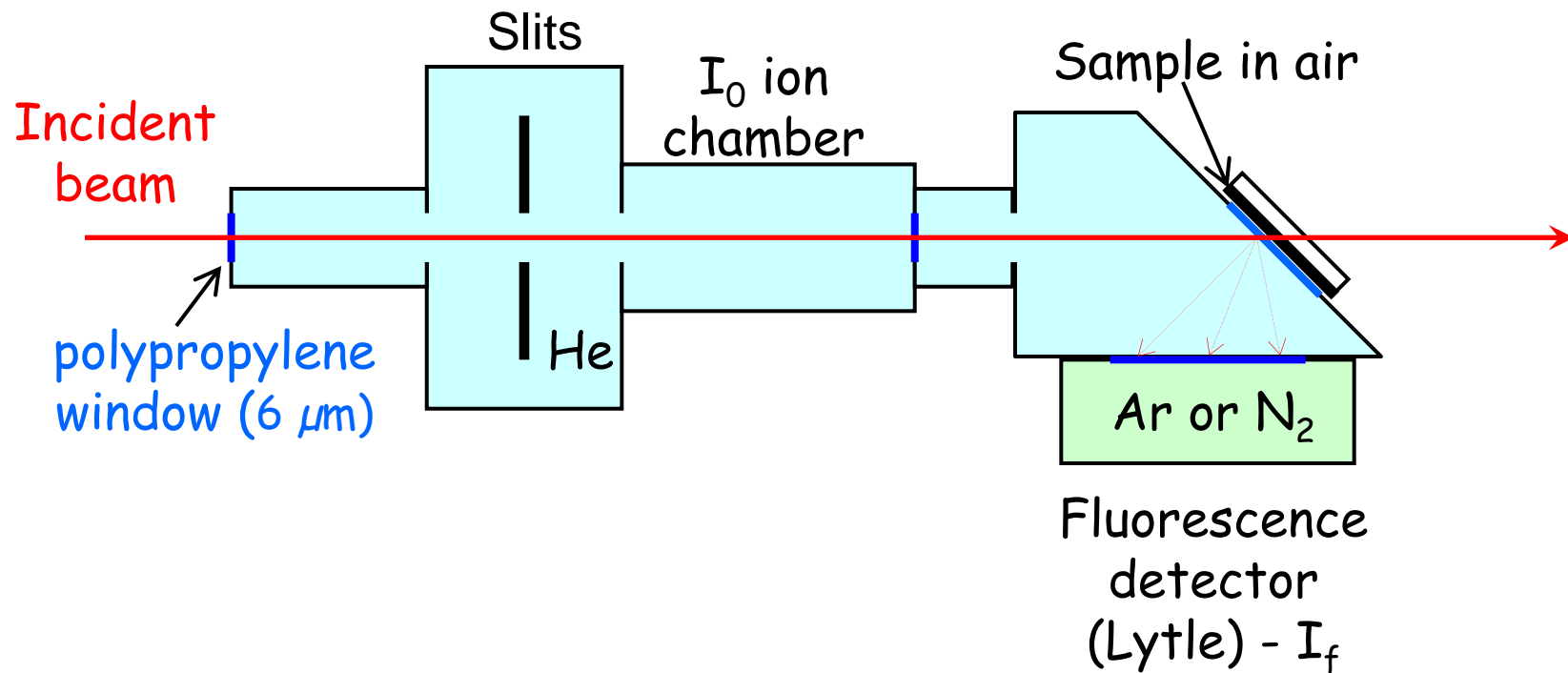
- In-helium setup for air-sensitive samples or electron yield



Beam line 6-2, SSRL

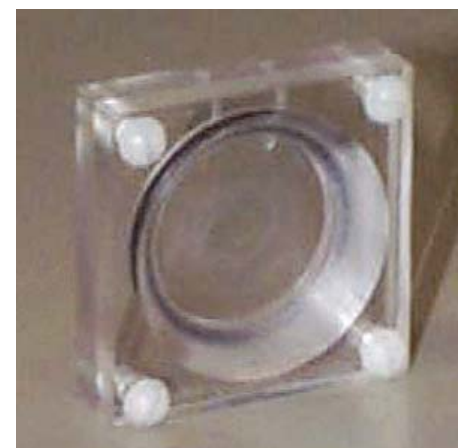
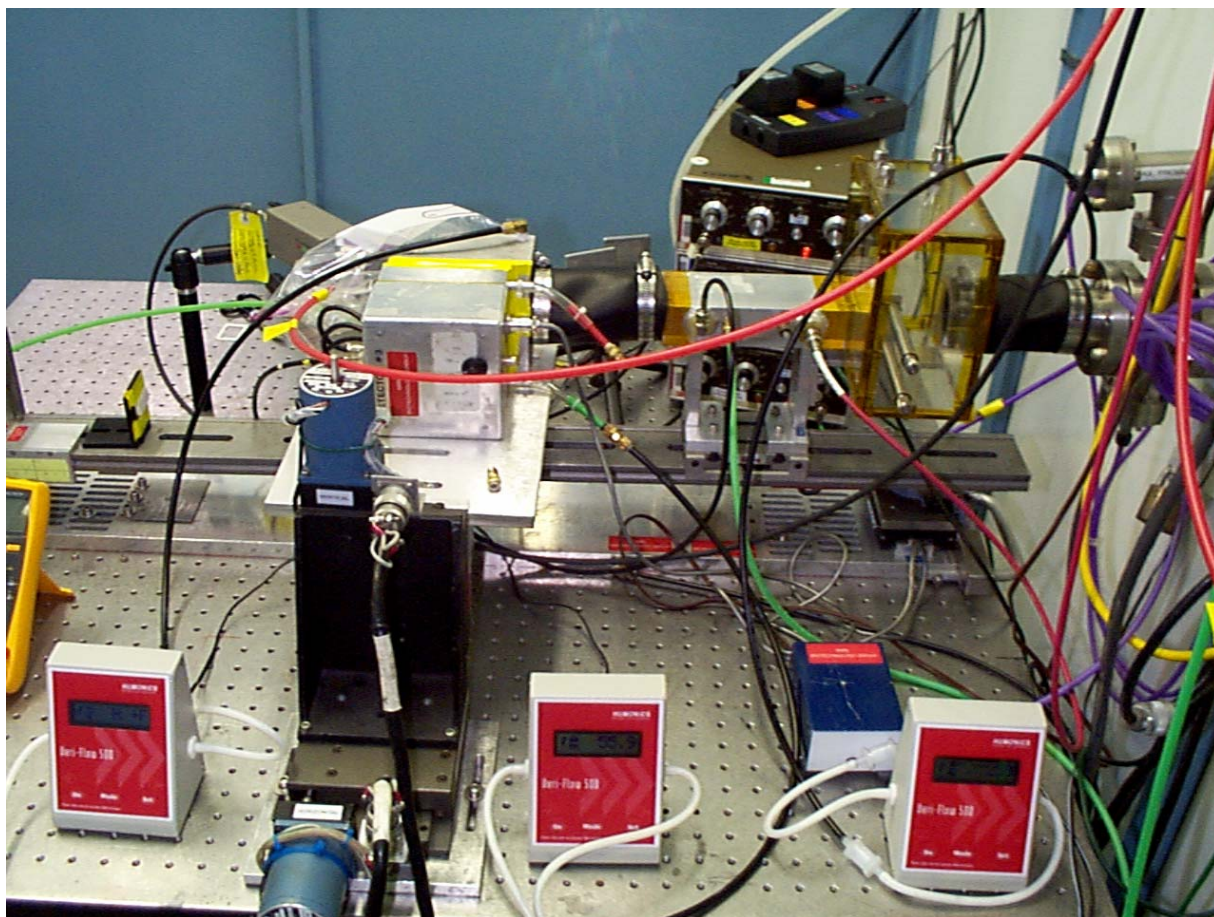
Experimental Setup

- In-air setup for living tissues and easy sample change



Beam line 6-2, SSRL

Sulfur K-edge XAS - Experimental Setup



Beam Damage

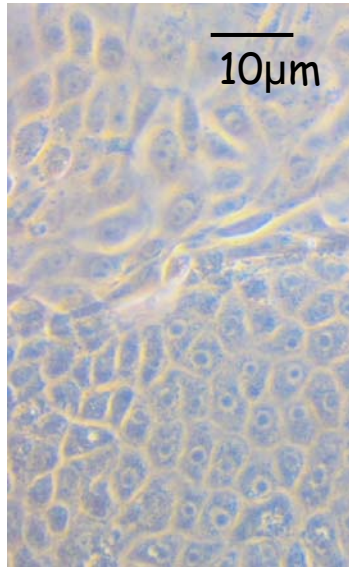
- Is a concern as beam is absorbed in small sample depth
- Duplicate scans - check for any changes
- Minimize beam exposure time
 - Quick, repetitive scans
 - Expose new portion of sample each scan
- Use external tests for sample integrity
- Low temperatures (can be problematic)

Beam Damage

Trypan Blue test for cell health - all cells take up trypan blue (an intense blue stain), but healthy cells actively pump it out, so that sick cells stain blue.

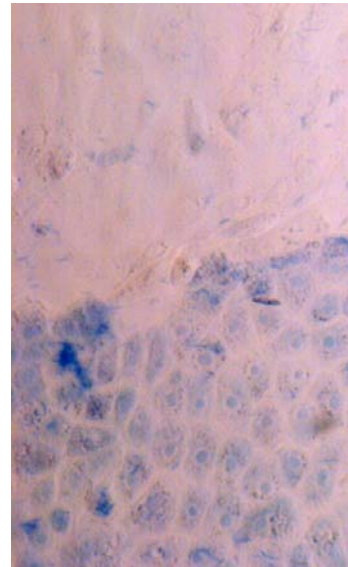
MDCK cells, 24 hours after exposure to X-ray beam for 6 minutes. Cells remain apparently healthy for several hours following beam exposure.

Phase contrast

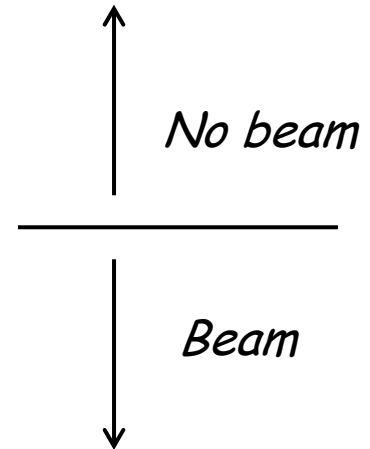


All cells visible

Bright field

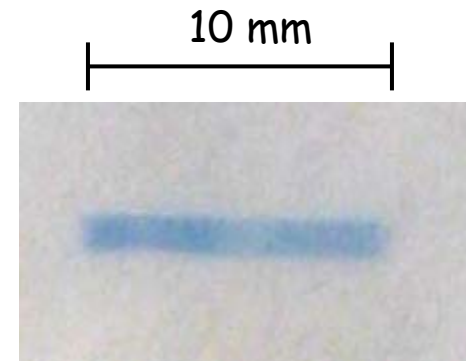


Invisible healthy cells, blue sick cells



Trypan Blue Test for Beam Damage.

MDCK cells, 24 hours after exposure to X-ray beam for 6 minutes (one scan). Cells remain healthy for several hours following beam exposure but after 24 hours show progressive signs of being unhealthy.



- Little difference when different fluxes used (tested over a factor of 10).
- Time is the most important factor - minimize beam exposure.
- Experiments completed as quickly as possible.



Wasabi and Horseradish have similar chemistries



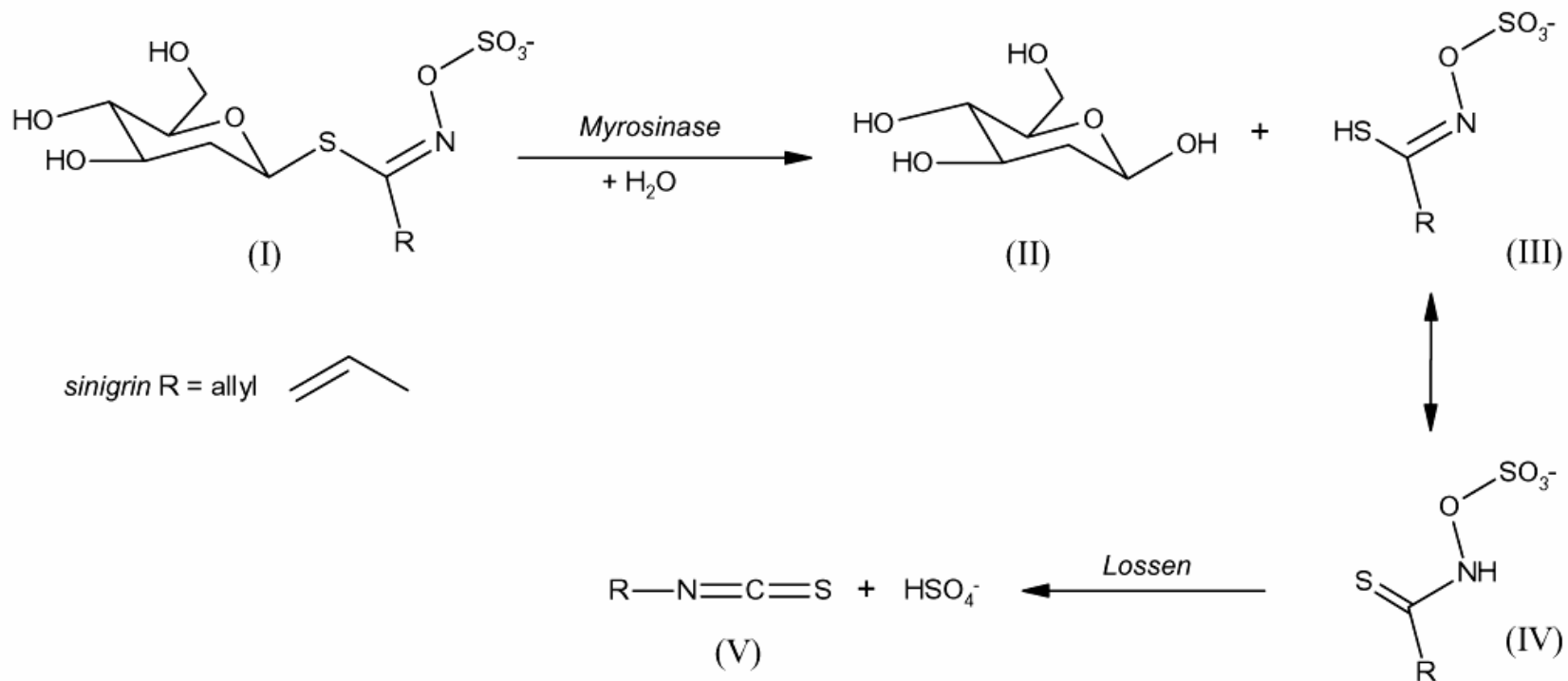
Most commercial "wasabi" is actually horseradish and food coloring.

INGREDIENTS : HORSERADISH, MUSTARD, MUSTARD EXTRACT, CITRIC ACID, VITAMIN C (ASCORBIC ACID), ARTIFICIAL COLORING (YELLOW NO.5, BLUE NO.1)



Chemistry of horseradish: *In situ* observation

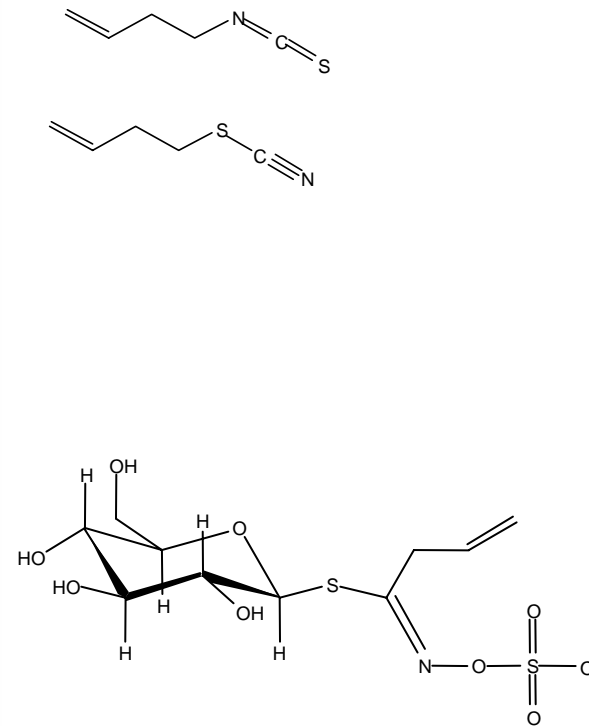
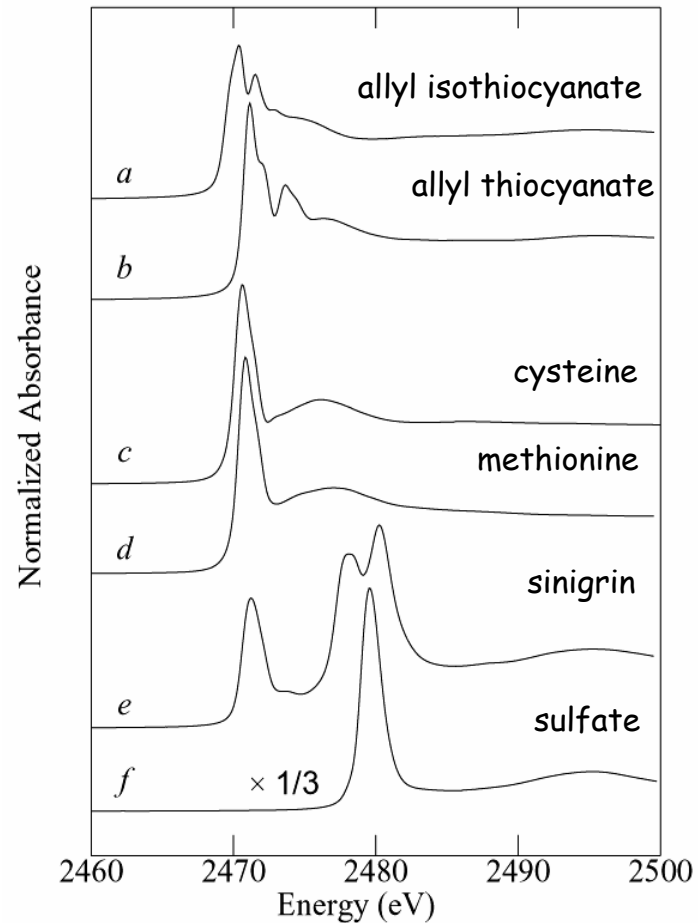
- Horseradish and Wasabi are quite benign until attacked, and then it releases an intensely lachrymatory compound, allyl isothiocyanate



Yu et al, (2001) *BBA* **1527**, 156-160

Chemistry of horseradish: *In situ* observation

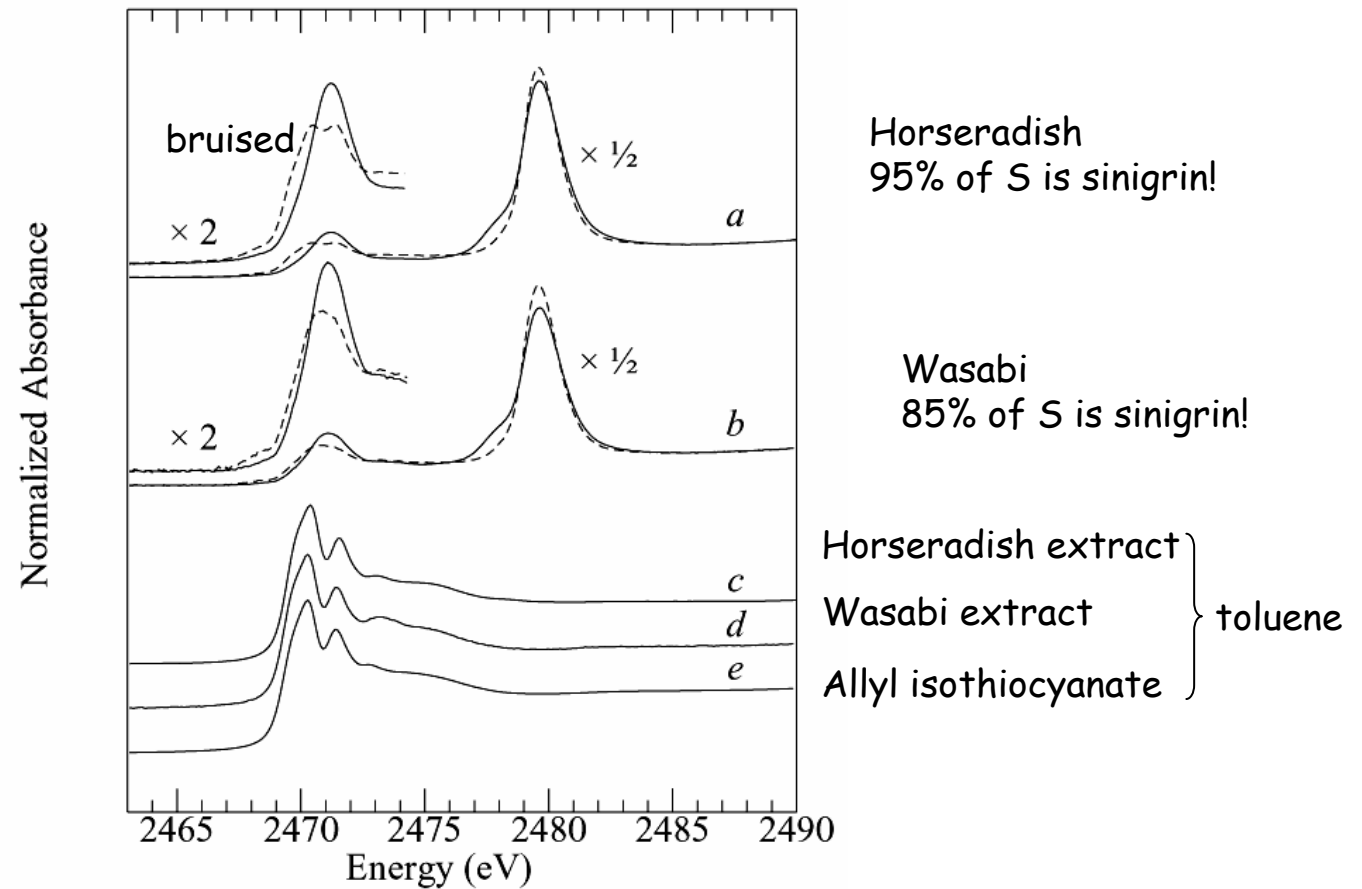
- The potential intermediates, and the other cellular components have distinctly different spectra.



Yu et al, (2001) *BBA* 1527, 156-160

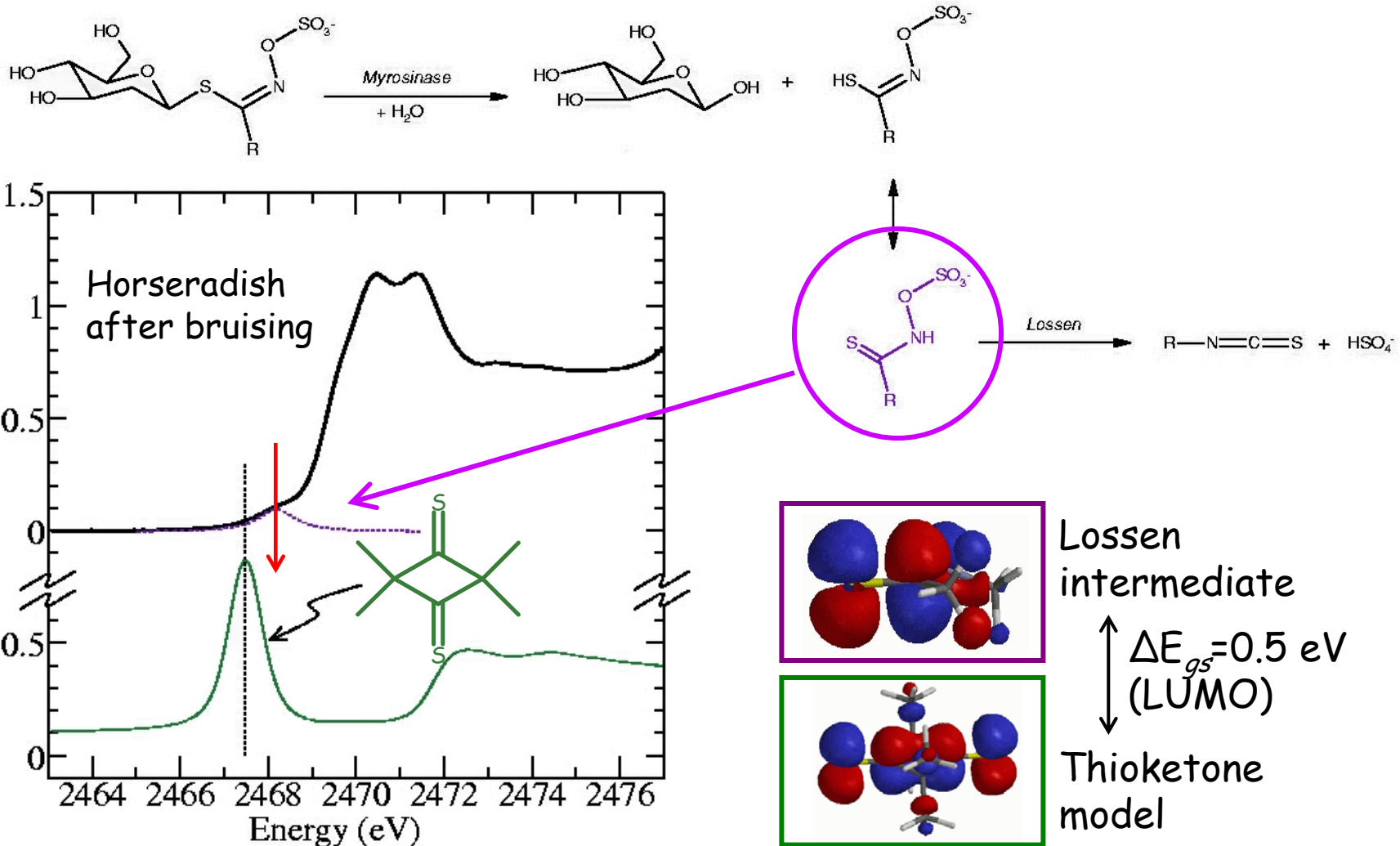
The release of allyl isothiocyanate

- Sinigrin disappears on bruising, and the sulfur reappears as allyl isothiocyanate and sulfate



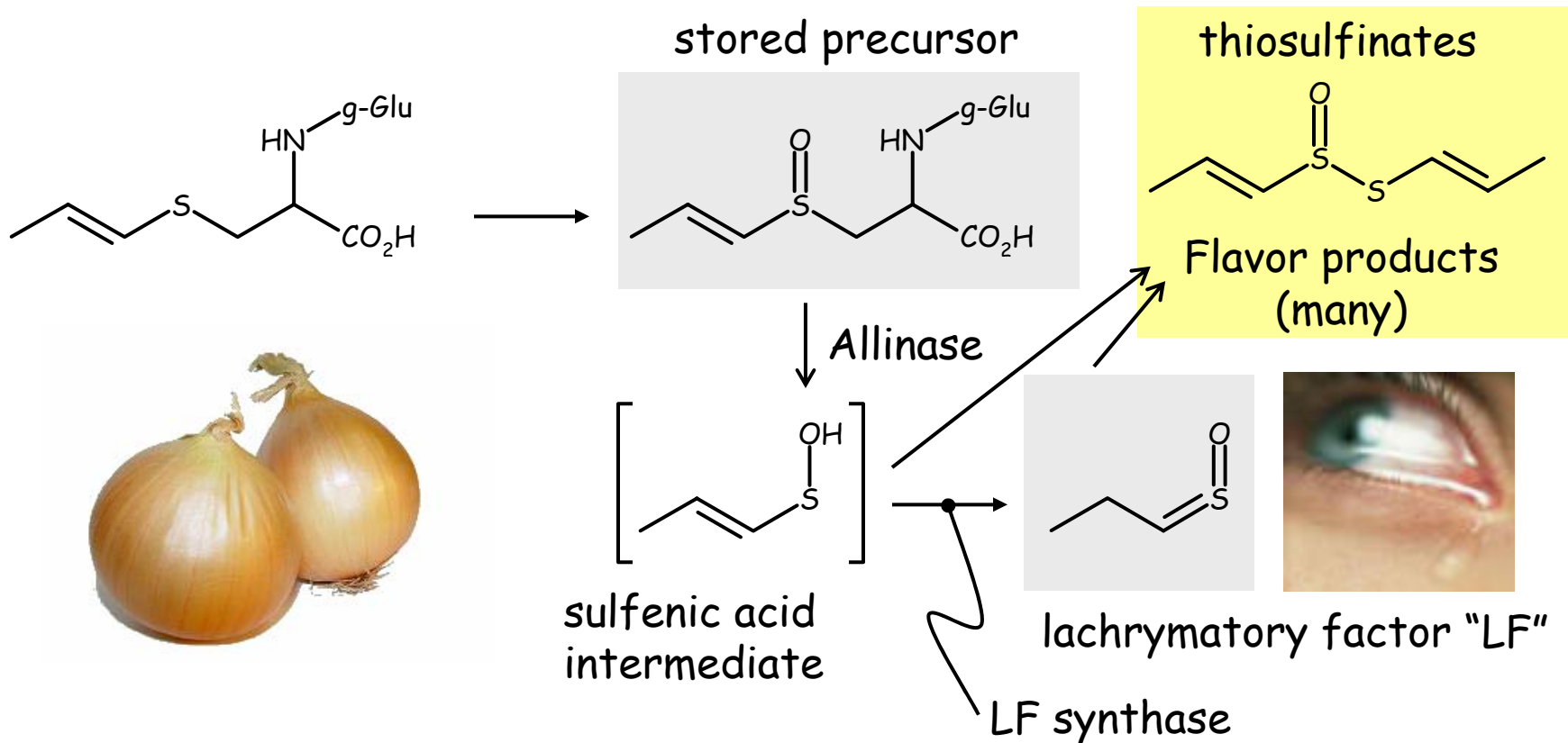
Yu et al, (2001) *BBA* 1527, 156-160

Feature indicates novel intermediate



Yu et al, (2001) *BBA* 1527, 156-160

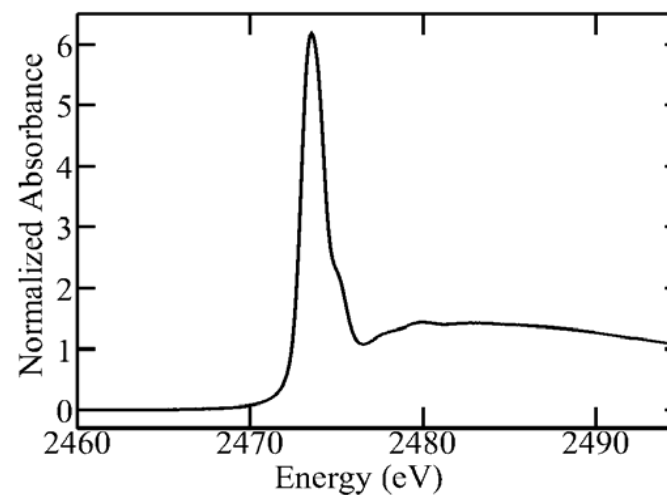
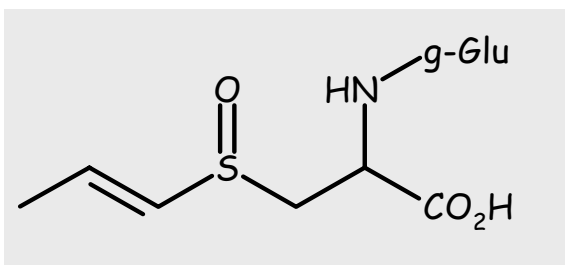
Onion Chemistry



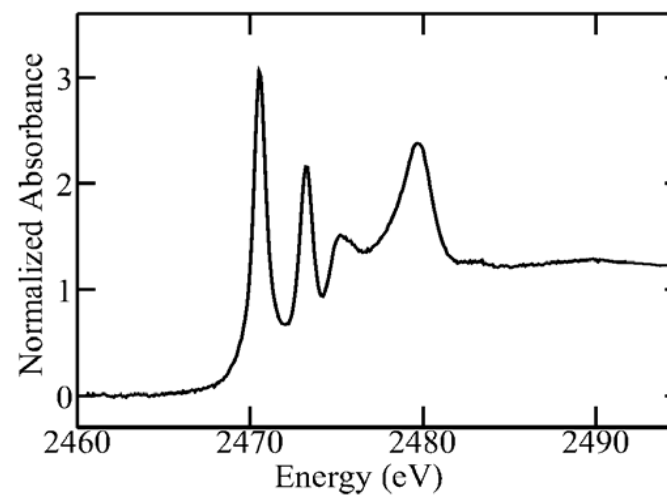
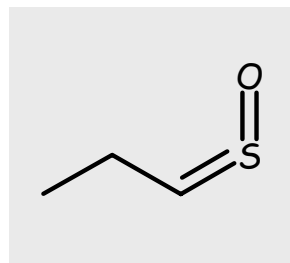
The stored precursor and the enzyme Allinase are mixed upon cell breakage. This results in release of the lachrymatory factor.

Onion Chemistry

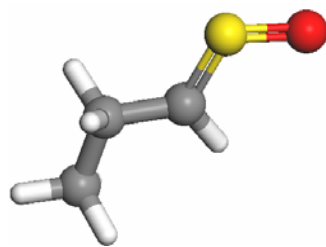
stored precursor



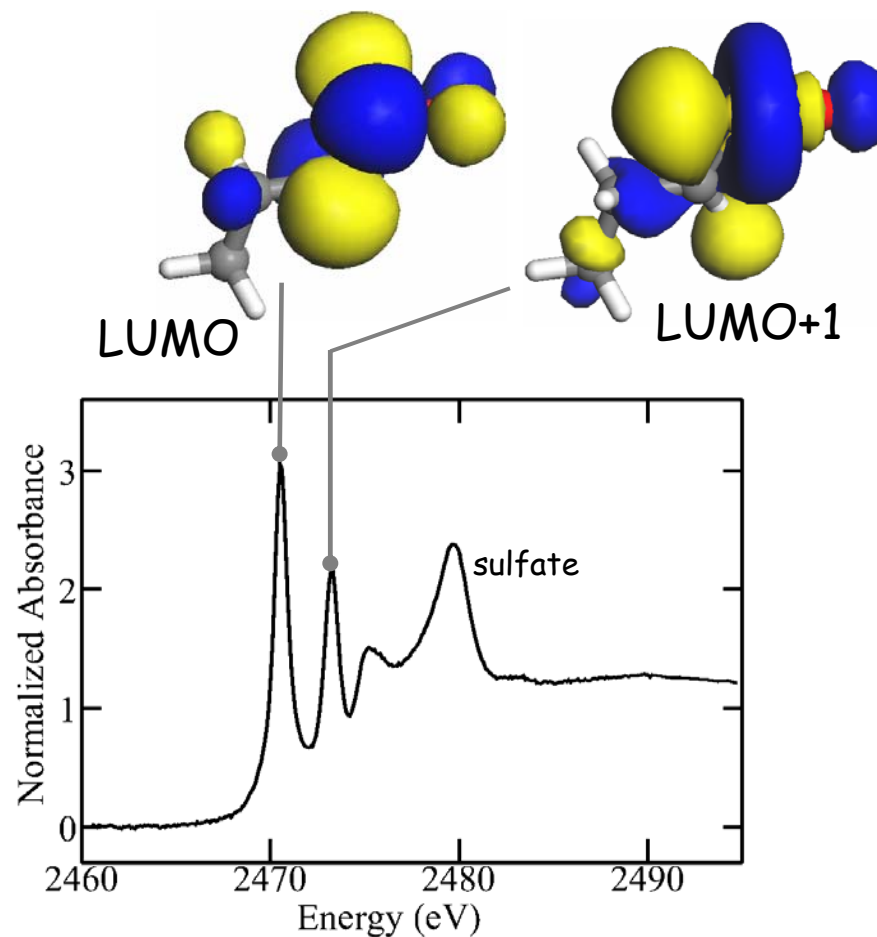
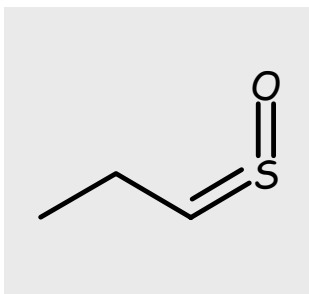
lachrymatory factor "LF"



Onion Chemistry



lachrymatory factor "LF"



Highly characteristic spectrum due to low lying excited state

Where in onion is the precursor located?

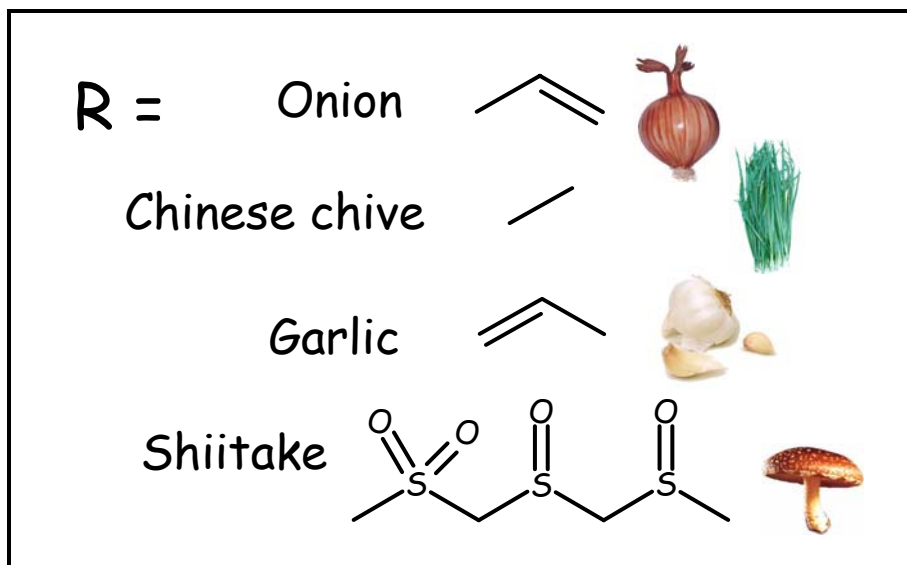
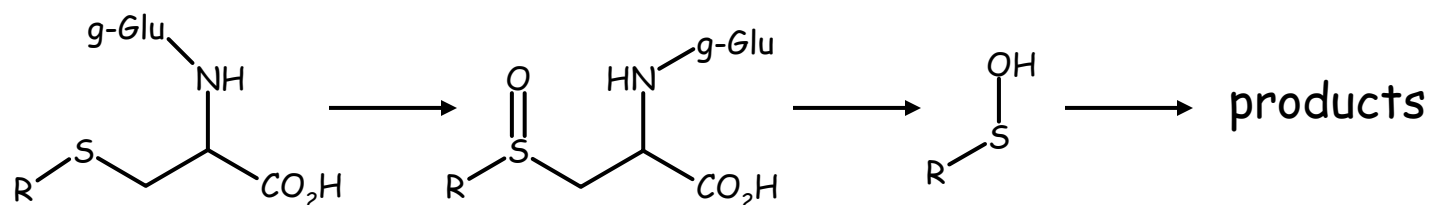
This cannot be discovered with conventional analysis.

XAS Imaging* can help.

Method: Use a microfocused beam, with carefully chosen X-ray energies to give sensitivity to different chemical types, and raster the sample to build up a map of different species.

* Also called spectromicroscopy by some groups

Allium plants and Shiitake mushrooms are thought to share the same chemistry



The biological function of this chemistry is thought to be to generate noxious compounds to deter herbivores.

Allium plants and Shiitake mushrooms are thought to share the same chemistry

These conclusions were based on conventional chemistry - solvent extraction and chromatography.

The problem with this is that how quantitative this is is not known - i.e. it could be probing 100% of the sulfur forms present - or it could be probing just 1%.

XAS provides a probe of all the sulfur present.

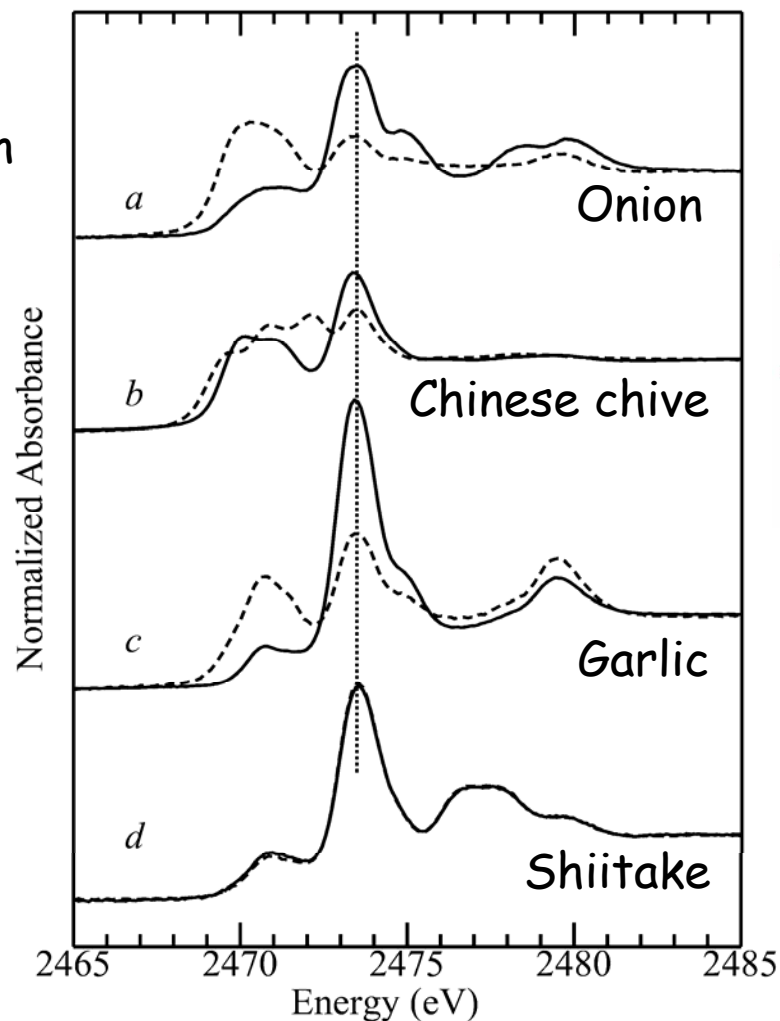
XAS of Allium plants and of Shiitake mushroom

—— intact
----- cells broken

All Allium plants change significantly on cell breakage, with sulfoxides being lowered.

Shiitake mushrooms show no change at all.

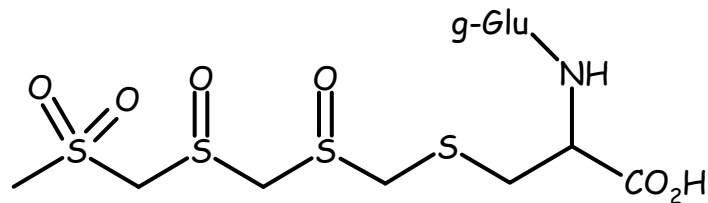
The chemistry proposed for shiitake is not important for most of the sulfur present.



Sneeden et. al. *J. Am. Chem. Soc.* **2004**, *125*, 458-459.

Shiitake mushroom

So, why does the shiitake mushroom make quantities of exotic sulfur compounds?



The previous suggestion - that it generates noxious products to deter herbivores using *Allium*-like chemistry - is incorrect.

At present this is not known, perhaps the sulfoxide tastes unpleasant to insect herbivores, but not to mammals (obviously).

Summary

- Sulfur K-edge XAS can provide unique insights into the sulfur biochemistry of living systems.
- This technique is well suited to the study of plants because they are robust (compared to mammalian cells), and the cells are large.

Acknowledgements - Group Members

Ingrid J. Pickering

Satya Pal Singh

Christian J. Doonan

Ruth Hoffmeyer

Gosia Korbas

Dil Thavarajah

Manuel Gnida

Limei Zhang

Ruwandi Andrahennadi

Jamie Ruszkowski

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Post-doctoral fellow

Post-doctoral fellow

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Graduate student

Graduate student

Alumni

Eileen Yu Sneed

Hugh H. Harris

Post-doctoral fellow

Post-doctoral fellow

Acknowledgements

Canada Research Chair Award



Canada Research
Chairs

Canada Foundation for Innovation



Natural Science and Engineering Research Council



Canadian Institute for Health Research



CIHR IRSC

National Institutes of Health



Province of Saskatchewan



University of Saskatchewan



Saskatchewan Health Research Foundation



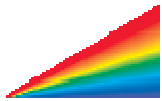
Saskatchewan
Health Research
FOUNDATION



Acknowledgements



Stanford Synchrotron Radiation Laboratory



Advanced Photon Source



U.S. DOE and NIH

Canadian Light Source
and its many sponsors
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Source synchrotron*