 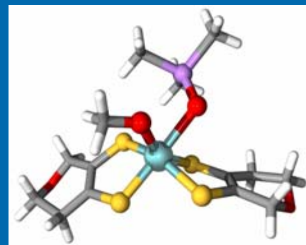 

 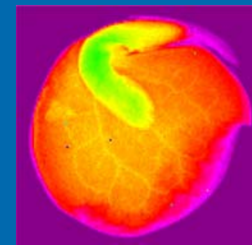 

Life Sciences at the NSLS-II XAS Damping Wiggler Beamline

Ingrid J. Pickering

Canada Research Chair,

University of Saskatchewan

XAS NSLS-II Workshop – January 16, 2008 – Brookhaven National Laboratory

Acknowledgments

Graham George

- Pickering and George group (University of Saskatchewan)
  - Graham George
  - Christian J. Doonan
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  - Manuel Gnida (Stanford U)
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- Staff at SSRL and CLS
- As-Se Molecule
  - Jürgen Gailer (U. Calgary)
  - Roger Prince (ExxonMobil)
- Bangladesh Clinical Trial
  - Julian Spallholtz (Texas Tech)
  - Paul LaPorte (U. Chicago)
  - www.bangladesh-selenium.org
- Lentil Project
  - Dil Thavarajah and Bert Vandenberg (Pulse Crop Research Group, U. Saskatchewan)

Overview

- Why life science XAS is important
- Examples of life science XAS
- Significance of Damping Wiggler XAS beamline

Background

- From University of Saskatchewan
- Chaired CLS Life Science Workshop Nov. 2007
- Co-lead on BioXAS
  - 2 new CLS beamlines for life science XAS

University of Saskatchewan is home to Canadian Light Source

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The Potential

- Life Science X-ray absorption spectroscopy is poised for huge expansion

- NSLS-II XAS beamline will be positioned to take advantage of this
- XAS of intact cells and tissues is the single area with potentially greatest gain

Life Science
X-ray Absorption Spectroscopy

- Metals in purified biomolecules
  - Traditional mode of study
  - Still important

- Metals in intact cells and tissues
  - Relatively new mode of study
  - Potential for great expansion
  - Huge potential for human benefit

Metals in Intact Cells and Tissues: Environmental Parallel

- XAS is ideal for investigating chemical forms of metals and metalloids in complex systems
- XAS is element-specific, and can give local information including oxidation state and speciation, electronic and structural information
- X-rays pass through any matter and can detect the element of interest in any phase or form
- Gives quantitative information on multiple forms
- Little or no pretreatment required, such as extractions
- Can be applied to dilute systems

Metals in Intact Cells and Tissues: Environmental Parallel

	Environmental Science	Life Science
Target Element	various, including actinides	mostly Z=42 and below for essential elements, some toxic or pharmaceutical elements higher
Matrix	various, often heavier elements abundant (e.g. soil, sediment with mineral content)	almost always light (C, H, N, O)

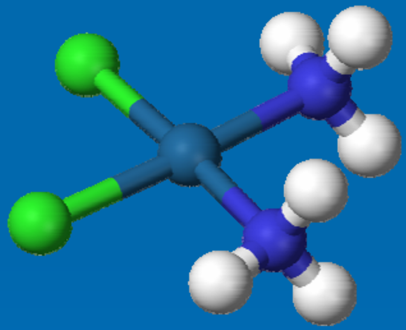
Metals in Intact Cells and Tissues: Environmental Parallel

- Field of XAS of whole cells about a decade behind environmental XAS
- Improved sensitivity allows measurement of physiologically relevant levels
- Huge potential benefits, particularly in human health

Metals in Intact Cells and Tissues: Types of Study

- Essential elements

- Metal-based drugs



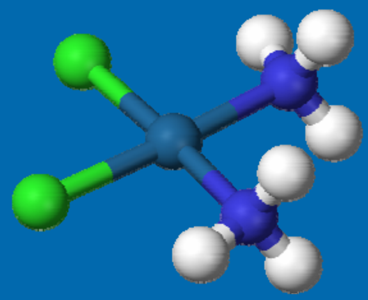
- Toxic elements

Essential Elements

- Metals and metalloids needed for wellbeing

- Fundamental studies of mechanisms/mode of action
- What happens when mechanisms malfunction? How can we fix it?

Metal-Based Drugs



- Particularly anti-cancer drugs
- Cis-platin is classic example
- Many novel metal-based drugs appearing
  - Are they optimized?
  - A detailed understanding of the mechanism of action aids in developing better drugs which are more targeted and less generally toxic
  - XAS of metal in cell culture provides vital information

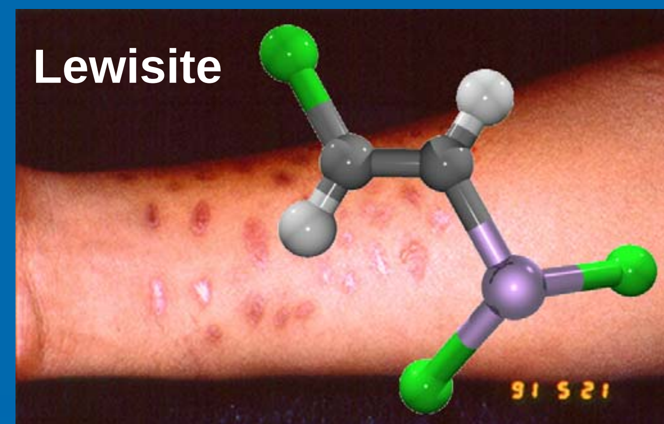
Toxic Elements

- Examples include mercury, arsenic, cadmium, lead
  - However, all essential metals are toxic at high concentrations
- How do they poison us?
- Can XAS help to find the antidote?

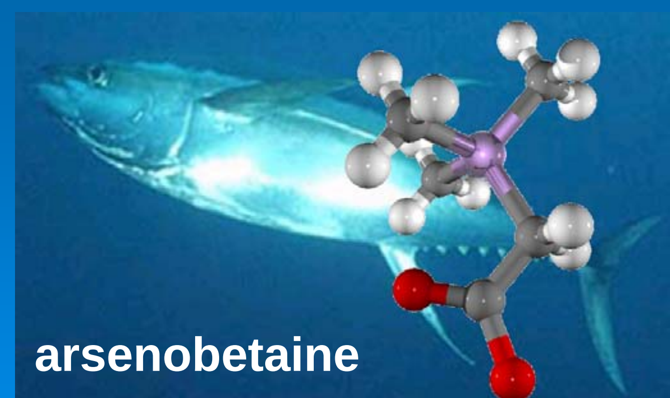
Chemical Form Matters!

Arsenic – sometimes poisonous, sometimes not

Lewisite – war gas – known as the “Dew of Death”.
It is a deadly poison.



Arsenobetaine – 0.02% in fish.
It is not poisonous at all.



Need for Spatial Information

- XAS can look at:
  - Subcellular structures
  - Individual cells, cell cultures
  - Tissues, organs
  - Organisms

- Biological materials are inherently structured, so there is a need for simultaneous spatial and chemical information

Fluorescence Imaging of Metals

- *Experiments in three different length scale ranges*

- **Macro** (>50µm)

- **Micro** (1-5 µm)

- **Sub-Micro** (<1µm)

Scientific Disciplines

- Biochemistry
- Biology
- Biomedical sciences
- Agriculture
- Environmental sciences

Examples

- Methyl mercury in zebrafish larvae
- Wide-format imaging of brain
- Arsenic, selenium and Bangladesh

Data from BL 9-3 at SSRL
(unless otherwise indicated)

Wide Format Imaging of Metals in Brain and other Tissues

Helen Nichol
and many other collaborators at the
University of Saskatchewan

Metals and Neurodegenerative Disease

- Metal accumulations in brain have been implicated in a number of neurodegenerative diseases

- Example: **Alzheimer's:**
  - "Plaques" of β-amyloid protein form in brain cortex.
  - These plaques accumulate zinc, iron, and copper, and form in tandem with the progress of the disease (cognitive problems, initially as memory loss, followed by impairment of language and planning).

Alzheimer's disease – How can XAS help?

- XAS can help develop a deeper understanding of the disease

- Open Questions:
  - Are metals a cause or an effect of the disease?
  - New U of S finding – not all plaques are alike; some contain more zinc, some more iron. Are there correlations between the metal in plaques and disease development?
  - What are the molecular forms of the metals?

Wide Format Imaging: A Different Approach

- Microbeam work often uses the smallest beam possible
- However, sometimes lower resolution and greater area is much more valuable
- In this approach, we scan rapidly to cover large areas such as a complete section of human brain

Wide Format Imaging

- Rapidly shows localizations of metals on a scale similar to medical X-ray, MRI, etc.
- Chemical information upon metals available
- Potential for understanding and treating disease:
  - Showing how metals may be implicated in disease
  - Testing effect of drug treatments in animal models

3. Arsenic, selenium and Bangladesh

www.bangladesh-selenium.org

As

Se

Arsenic and Selenium

H																	He
Li	Be											B	C	N	O	F	Ne
Na	Mg											Al	Si	P	S	Cl	Ar
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	**As**	**Se**	Br	Kr
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	La	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	Ac															

Lanthanides

Actinides

Metals

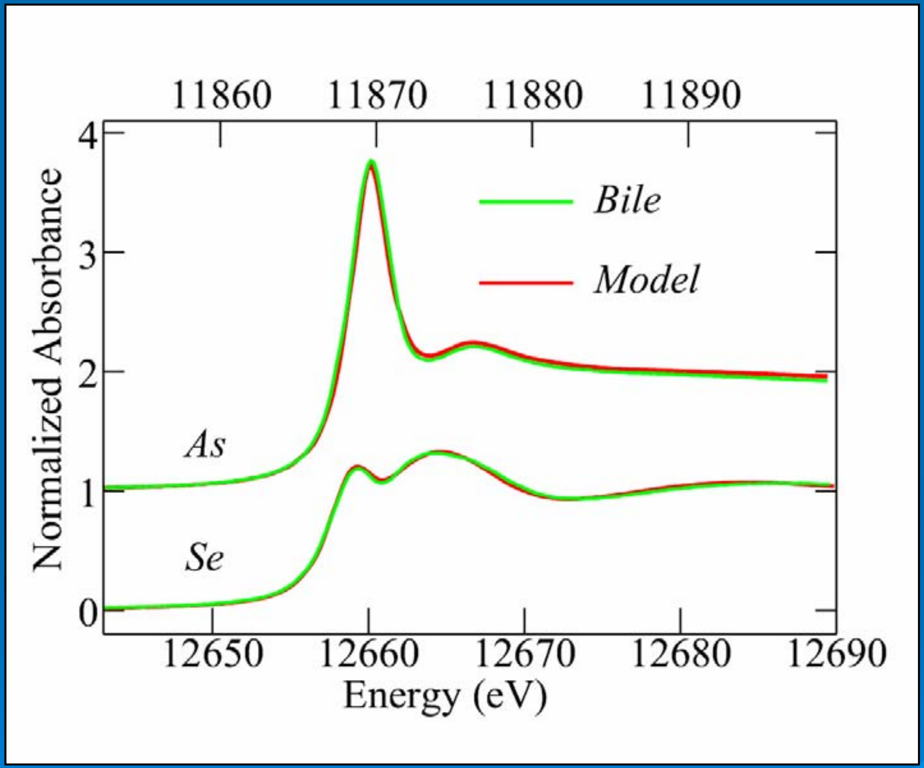
Non-metals

Arsenic and Selenium

- Arsenic has no confirmed biological role
- Selenium is an essential ultra-trace element
  - Arsenite and selenite have approximately equal toxicities at higher amounts
- But taken together…:
  - A lethal dose of arsenite can be completely counteracted by an equal and otherwise lethal dose of selenite (or vice versa).

Arsenic-Selenium Antagonism

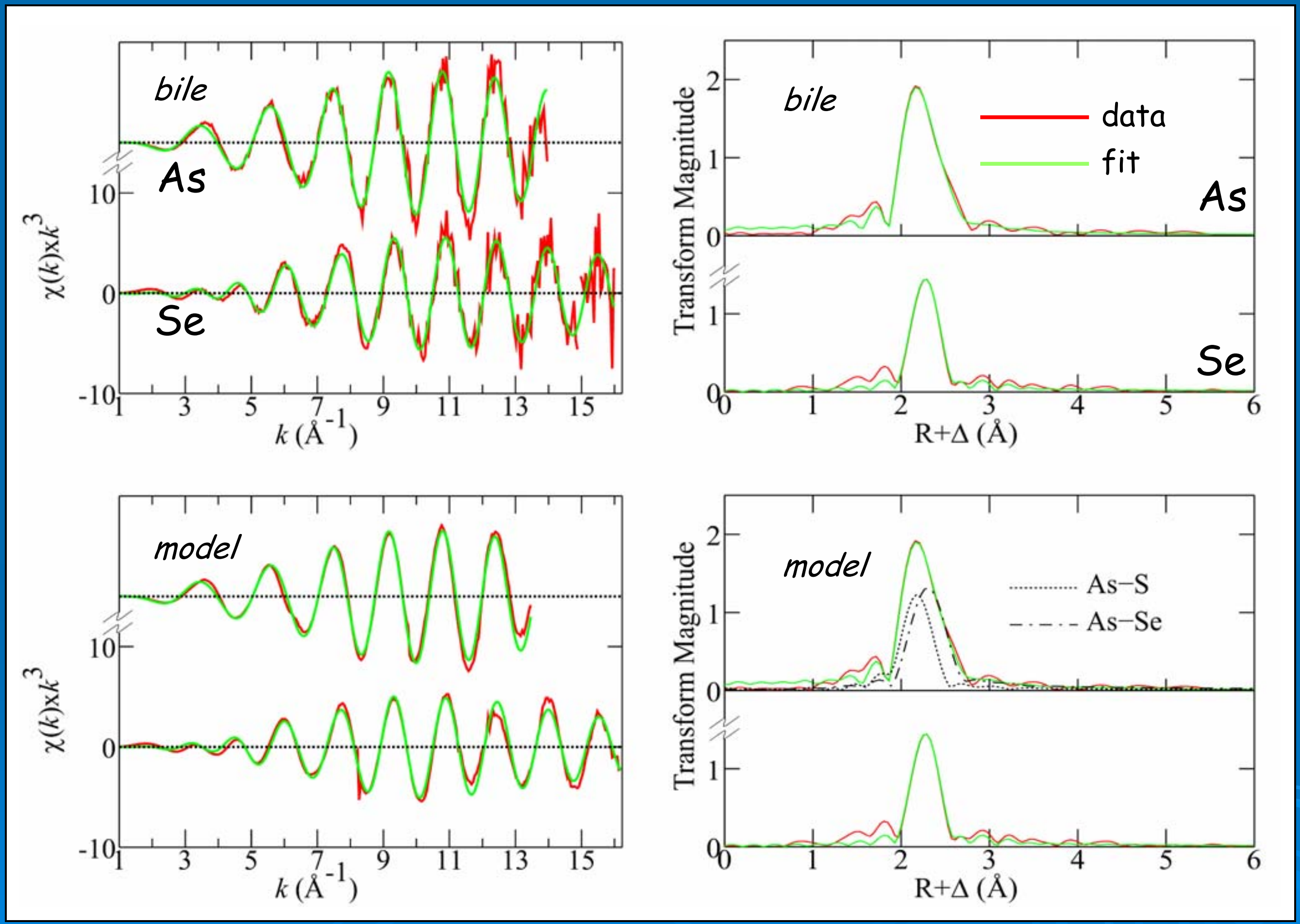
• Bile As and Se K near-edge spectra identical with model synthesized from arsenite, selenite and glutathione.



Gailer et al., *J. Am. Chem. Soc.* **122**, 4637-4639 (2000).

Arsenic-Selenium Antagonism

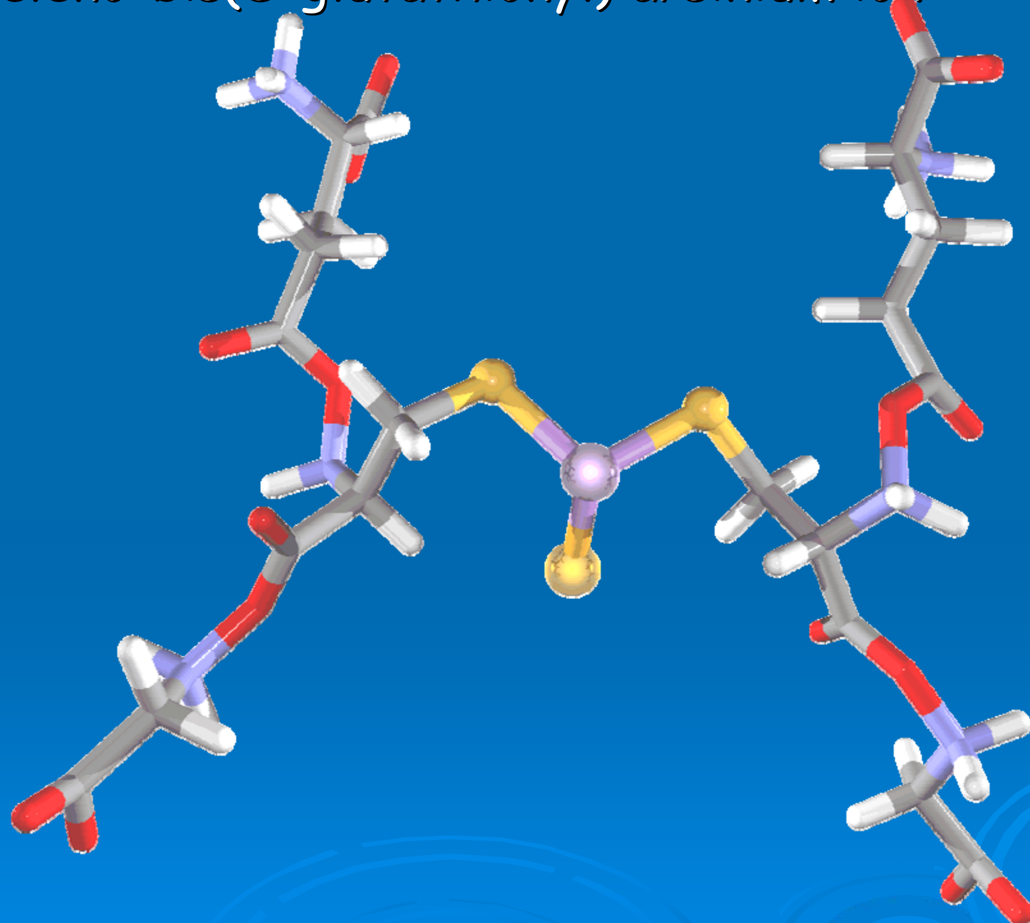
- Bile As and Se EXAFS used to structurally characterize species in bile



Gailer et al., *J. Am. Chem. Soc.* **122**, 4637-4639 (2000).

As-Se Species Identified in Bile

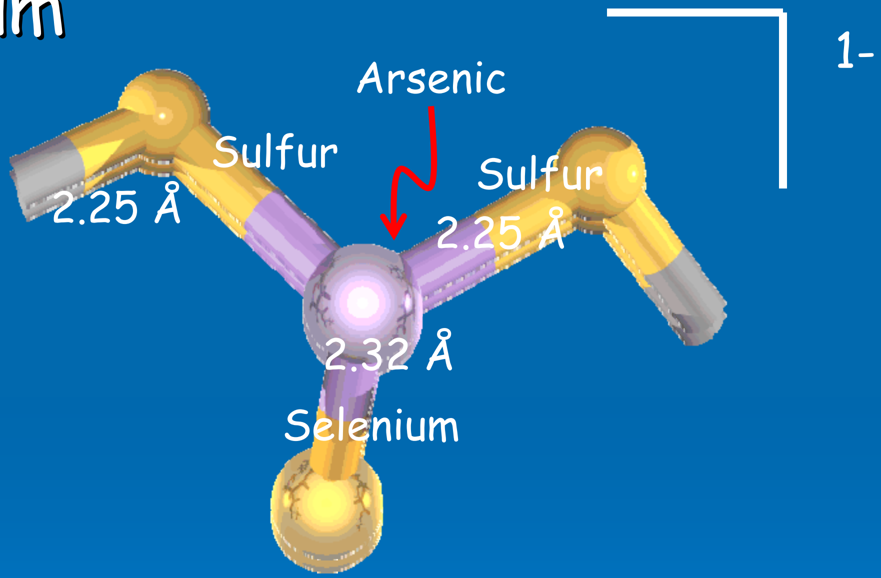
- An unusual molecule with 1:1 Se:As
  - The seleno-bis(S-glutathionyl) arsinium ion



Gailer et al., *J. Am. Chem. Soc.* **122**, 4637-4639 (2000)

As-Se Species Identified in Bile

- Formation of this species in the body is a mutual detoxification mechanism for arsenic and selenium



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Arsenic Poisoning in Bangladesh

LOCATION: Bangladesh & parts of India

PROBLEM: In 1960's contaminated surface water is causing disease

SOLUTION: Sink tube wells to provide clean drinking water from deep underground

NEW PROBLEM: Tube well water contains low-levels of arsenic which gradually makes people sick

The World's Worst Mass Poisoning

- Disease called:
  - Arsenicosis
- Symptoms:
  - Dermatitis and skin disorders
  - Malignant tumors
  - Death
- Estimated number of people affected:
  - 35 to 77 million*

Typical lesions

* Estimated by World Health Organization, 2000

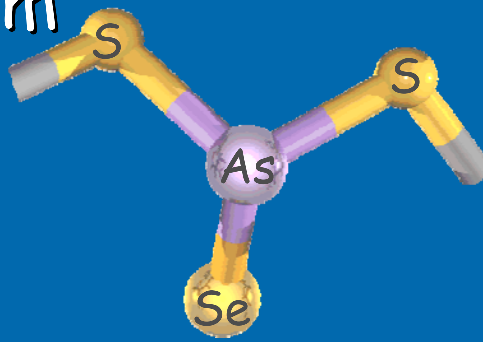
Arsenic Poisoning in Bangladesh

- Puzzles:
  - Not all people drinking from same well get sick
  - Other areas in the world have high arsenic but not the same symptoms
  - Biochemistry of arsenicosis is (or was) unknown

- Clues:
  - Bangladesh is low in dietary selenium
  - Very low selenium levels in livers of victims
  - Other arsenicosis areas also have low selenium

Selenium and Arsenic

- Our bodies use the arsenic-selenium molecule to get rid of arsenic
  - For every arsenic that is eliminated, one selenium is lost too



- But humans need selenium!
  - deficiency results in skin disorders, cancers, and death

What We Think is Happening

- Our hypothesis:
  - The people are not being poisoned by arsenic but are becoming selenium deficient!
- Proposed solution:
  - Add selenium to diet
- Testing the hypothesis:
  - Phase III clinical trial ongoing in Bangladesh

Clinical Trial of Selenium Supplementation

www.bangladesh-selenium.org

TEXAS TECH UNIVERSITY THE UNIVERSITY OF CHICAGO শিশু-মাতৃ স্বাস্থ্য ইনস্টিটিউট INSTITUTE OF CHILD AND MOTHER HEALTH Apollo Hospitals DHAKA

Selenium Treatment of Arsenic Toxicity & Cancers in Bangladesh [SETAC]

Phase III, Double-Blind, Randomized, Placebo-Controlled Trial on the Use of Long-term, Dietary Selenium in Countering Arsenic Toxicity | **Sponsors: NIH/NCI, American Cancer Society**

- Project Leaders:
  - Julian Spallholz (Texas Tech)
  - Paul LaPorte (U. Chicago)
  - Selim Ahmed
    (Institute of Child & Mother Health, Bangladesh)

Clinical Trial of Selenium Supplementation

www.bangladesh-selenium.org

- Patients exposed to arsenic are given selenite
- We measure As and Se K-edge XAS of blood from treated patients

Clinical Trial in Bangladesh: Breaking News

- Blood samples taken from treated patients on Christmas Day, 2007

- XAS measured January 2008 at SSRL

Arsenic, Selenium and Bangladesh

- As-Se detoxification molecule has been identified
- Selenium supplementation as a solution to arsenicosis in Bangladesh is currently under test by clinical trial
- XAS of ultra-dilute species is key to this project

NSLS-II Damping Wiggler XAS

- Proposed beamline will provide:
  - Exceptionally wide, continuous energy range
  - Exceptionally high flux

- How will these characteristics be important in life science studies?

Wide, Continuous Energy Range

- Continuous source
  - No need to tune undulator gap
  - No residual undulator structure (unlike some high-flux, multi-pole wigglers)
- Should lead to excellent stability with time and energy
  - improved signal to noise for more challenging low-concentration experiments
- Very attractive for life science applications

High Energies (<90 keV)

- Life science studies
  - Mostly use light matrices
  - Mostly investigate elements with edges below 30 keV

- The hard X-ray spectroscopy can be achieved using “conventional” energy ranges

- Specialized applications of higher energies might include:
  - Cross-over with biomedical imaging (i.e. probing heavier elements in thick objects)
  - Spectroscopy of trace elements in heavier matrices such as bone or teeth

High Flux

- Higher flux (if no other limits) should enable:
  - Lower concentrations

    OR
  - Higher throughput

    OR
  - "Improved" data e.g. longer *k*-ranges

- All important in Life Sciences
  - Ultra-low concentrations most important

Concentration: How Low Can We Go?

- Se in blood
  - SSRL BL 9-3:
    - 2 μM Se
    - 150 ppb Se
- How low at NSLS-II DW-XAS?
- Lower concentrations facilitate study of metals at physiologically relevant levels

Low Concentration Needs

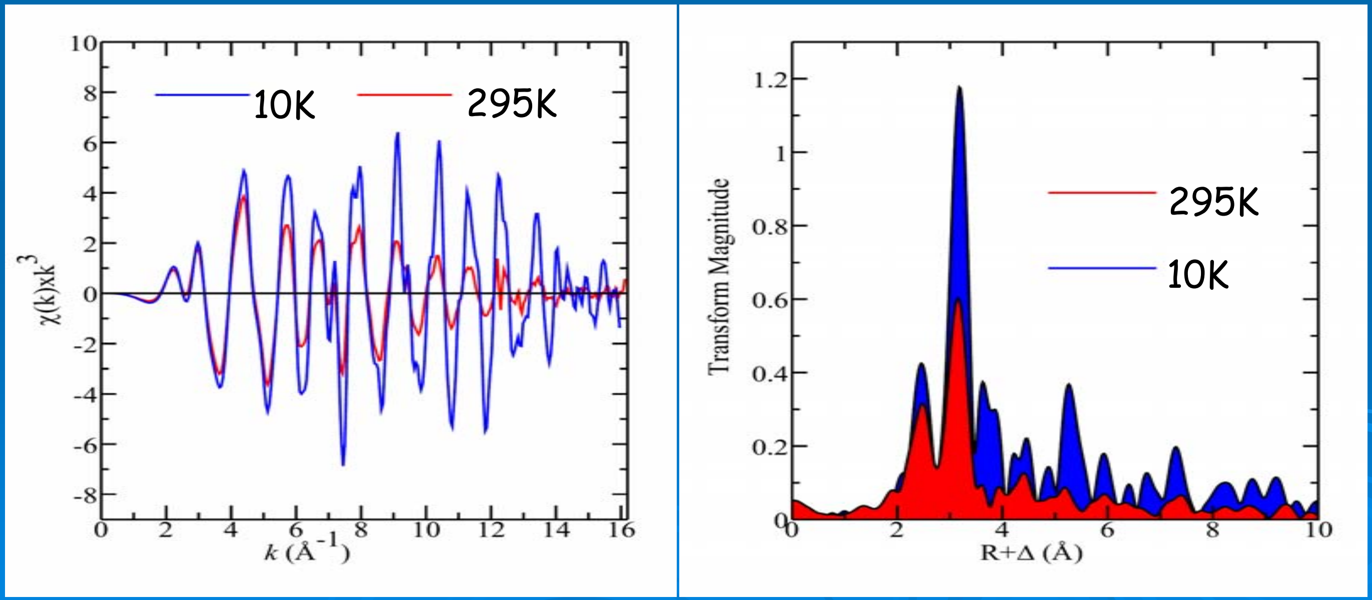
- High flux
- High stability
- State of the art detectors
  - (to capitalize on the flux)
- Low temperatures
  - (to stop sample being blown away)
- Low background signals
  - (to avoid “contamination”)

Detectors

- Bulk XAS usually is a detector-limited experiment
  - Need detectors to capitalize on the flux
- Current options:
  - Solid state (Ge, Si-drift) arrays (pixels or elements)
  - Bent-Laue diffraction-based
- Advice:
  - Buy late – to capitalize on latest developments
  - BUT retain enough money to buy the best

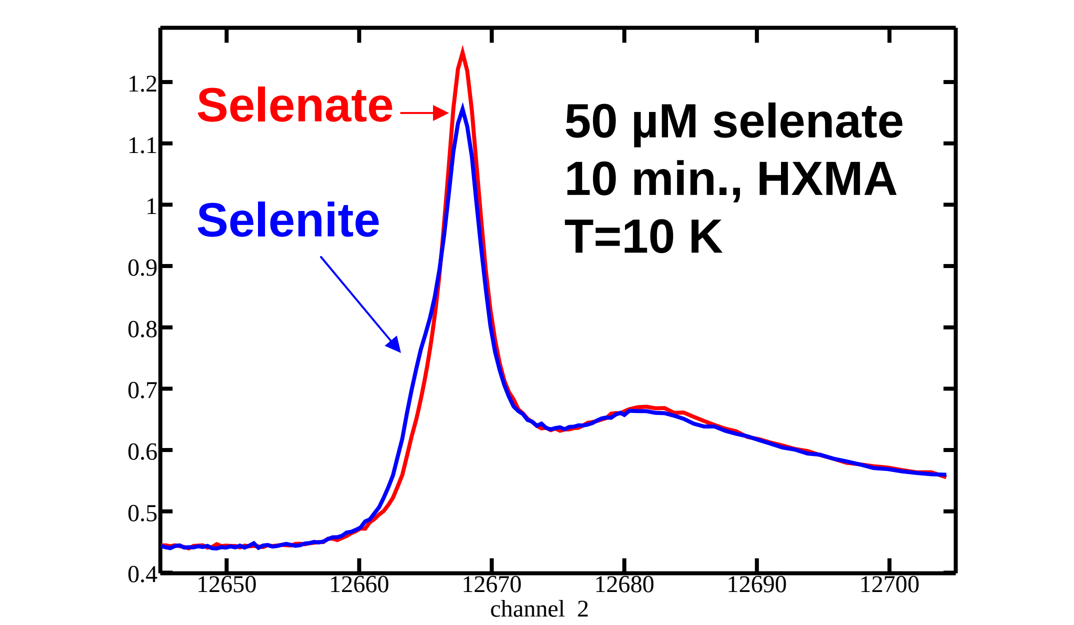
Low Temperatures

- Preserve biological samples
- Improve EXAFS amplitudes



Low Temperatures

- Preserve biological samples
- Improve EXAFS amplitudes
- Minimize "photo-induced chemistry"



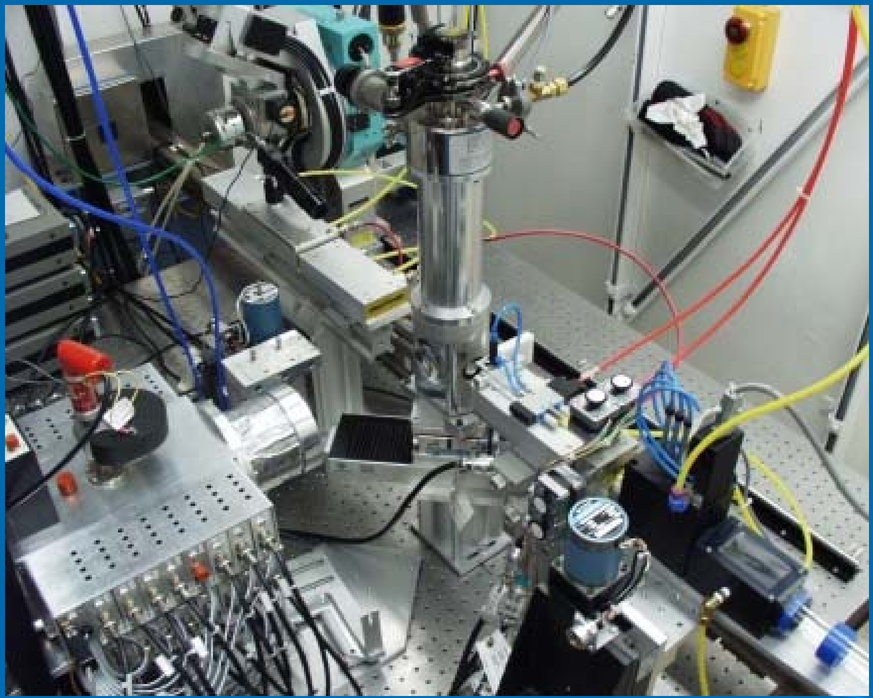
Beam damage is bigger problem with:

•Higher flux density

•Slower data collection

Low Temperatures

- Temperature?
  - LN2 NOT low enough
  - Still see effects at 10 K Would lower help?
- lHe displex
  - Economical in lHe
- Flowing lHe cryostat
  - Rapid sample change
  - Low mechanical vibrations
- Other cryotechnologies?



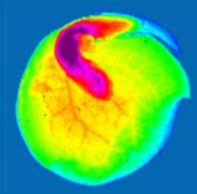
BL 9-3, SSRL

Background Contamination

- For lower concentrations, background signals become increasingly significant
- Background contamination can be:
  - Inherent, from construction materials (e.g. cryostat, detectors)
  - Introduced, e.g. dust, previous samples
- A big problem for e.g. Fe, Cu, Zn (which are important bio elements)

Conclusions

- Life science is a fertile area of potential growth for XAS
- Measurements on intact cells and tissues show great potential in terms of human benefit
- NSLS-II DW XAS is well-suited for low-concentration life science measurements



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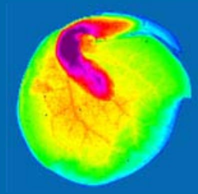

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