

X-ray spectroscopy methods in Bioinorganic Chemistry

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X-ray Absorption Spectroscopy

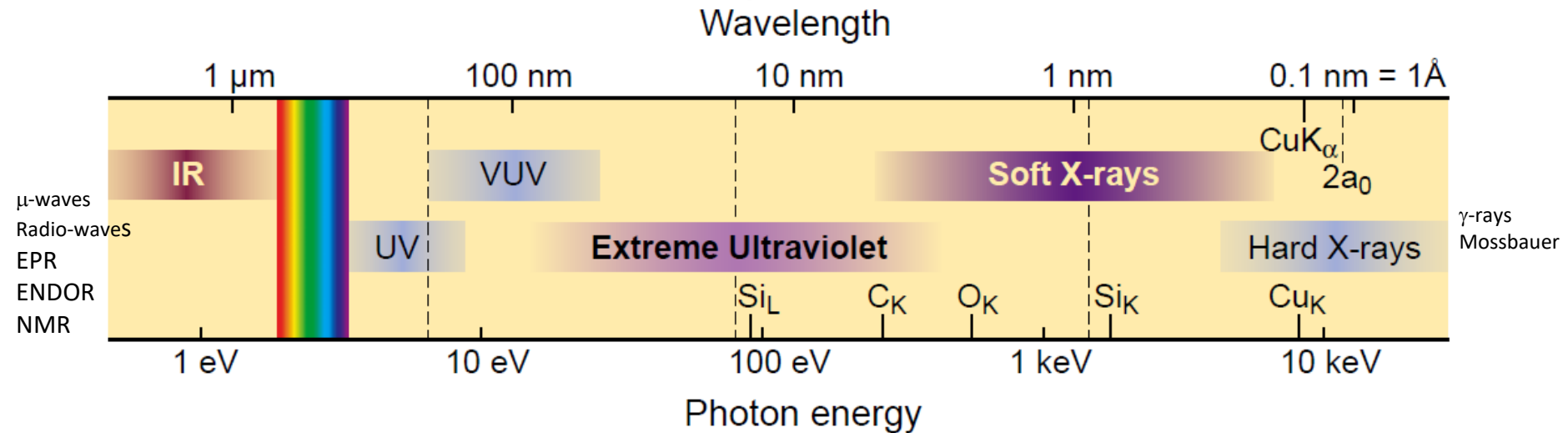
Lecture outline:

- Overview of XAS Spectroscopy and its uses
 - Synchrotron sources
- Theory (a little)
- Experiment
- Information content
- Data Analysis examples
 - BioXAS examples from literature

lecture slides and learning material available

X-ray Absorption Spectroscopy

The electromagnetic spectrum



X-rays are high energy photons: Energy from about 200 eV (soft) to 120 keV (hard)

wavelength λ : from 100 Å to 0.1 Å

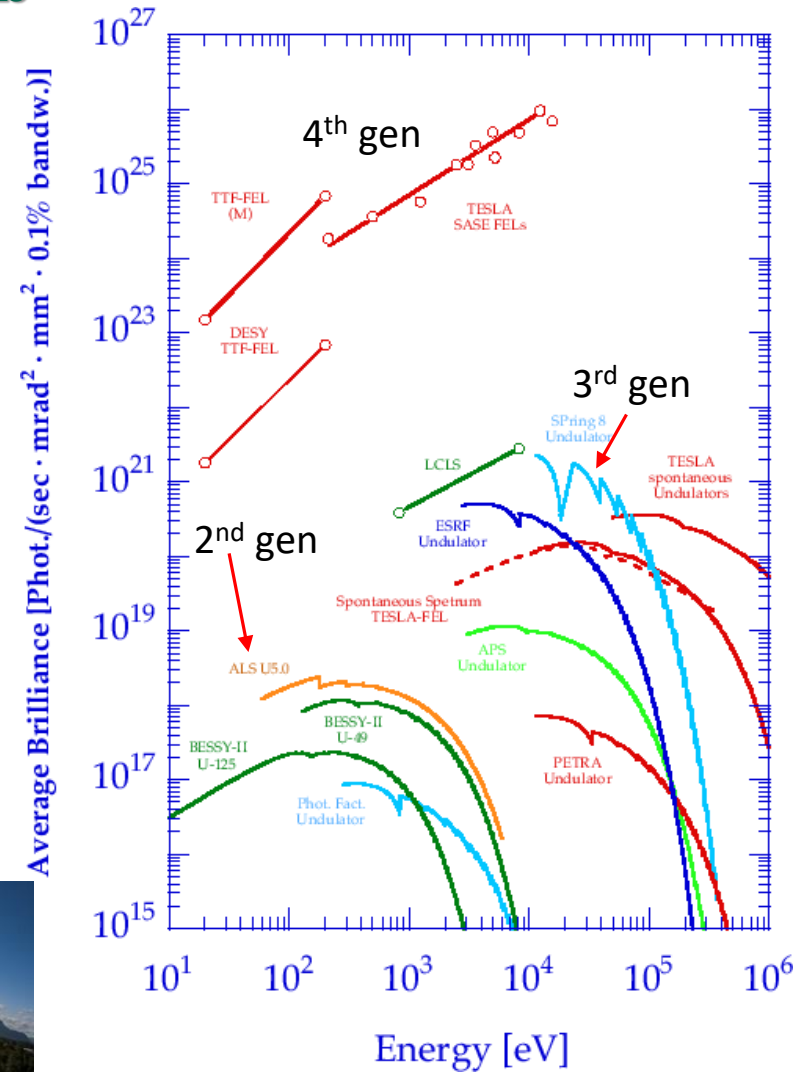
E is usually expressed in electron volts (eV) or kilo eV (keV) where 1 eV = $1.6 \cdot 10^{-19}$ J.

E.g.: blue light has E = 3 eV

$$E = \frac{hc}{\lambda} \quad E(\text{eV}) = \frac{1.24 \cdot 10^4}{\lambda(\text{in } \text{\AA})}$$

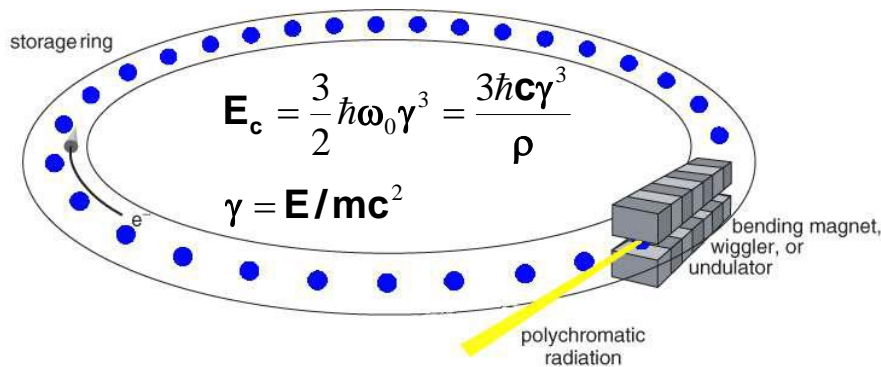
For X-ray spectroscopy we need tunable, intense, X-ray sources

Modern synchrotrons & XFELS



Synchrotron radiation

When charged particles, moving at **RELATIVISTIC** speeds ($v \approx c$), are forced to **change** the direction of their motion (acceleration), under the effect of **magnetic fields**, in circular particle accelerators, like synchrotrons, the radiation produced is called **Synchrotron Radiation**.



ω_0 = angular frequency in classical approximation
 ρ = radius of instantaneous curvature of electron trajectory

E_c : critical photon energy

E (or E_e) = electron beam energy

Brightness = Flux of photons of freq. ω emitted from unit source area into unit solid angle.

Flux/ $S \cdot \Omega$

Brilliance = brightness/ mm^2

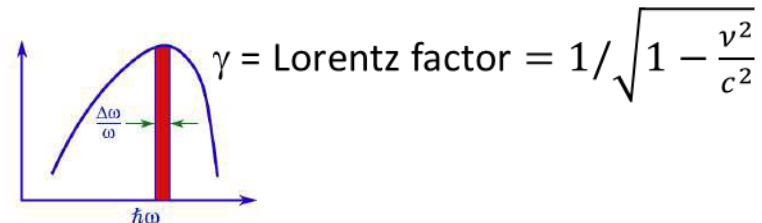
Spectral brightness $(\Delta\omega/\omega) = \frac{\text{photons}}{s \cdot \text{mrad}^2 \cdot \text{mm}^2 \cdot 0.1\% \text{BW}}$

Synchrotron radiation is characterized by:

- **Continuum source** from IR to γ -rays
- **High brightness** and **high intensity**, many orders of magnitude more than with X-rays produced in conventional X-ray tubes
- **High brilliance**, exceeding other natural and artificial light sources by many orders of magnitude:
 3rd generation sources typically have a brilliance larger than 10^{18} photons/s/mm²/mrad²/0.1%BW, where 0.1%BW denotes a bandwidth $10^{-3}\omega$ centered around the frequency ω .
- **High collimation**, i.e. small angular divergence of the beam.
- **Low emittance**, i.e. the product of source cross section and solid angle of emission is small
- **Widely tunable** in energy/wavelength by monochromatization
- High level of **polarization** (linear or elliptical)
- **Pulsed light emission** (pulse durations less than one nanosecond)

The relativistic electron has an energy:

$$E = \gamma m_0 c^2$$



The universal curve of BM synchrotron radiation

The integrated spectral density up to the critical frequency contains half of the total energy radiated, the peak occurs approximately at $0.3 \omega_c$

For *electrons*, the **critical energy**:

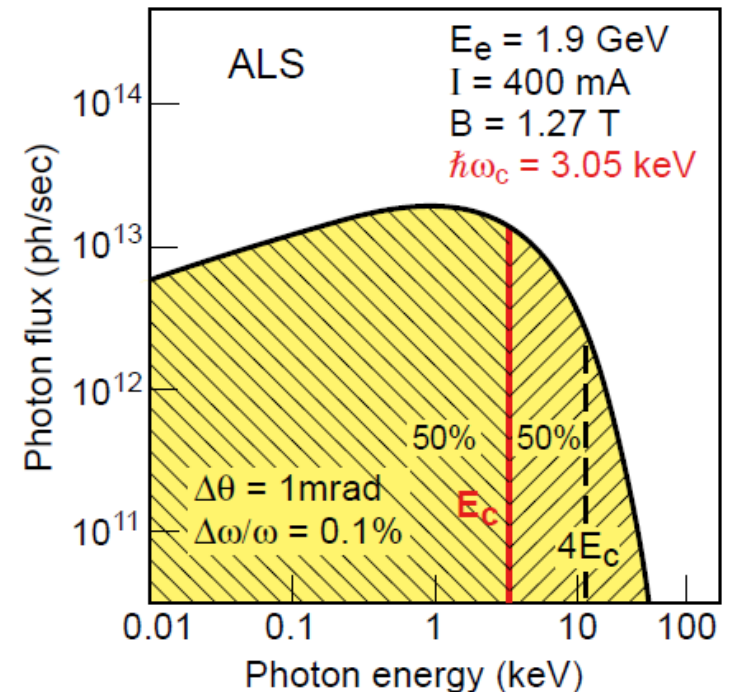
$$E_c = 3hc\gamma^3 / (4\pi R)$$

Where R is the ring radius.

In practical units is: $E_c(keV) = 2.218 E_{ring}^3(GeV) / R(m)$

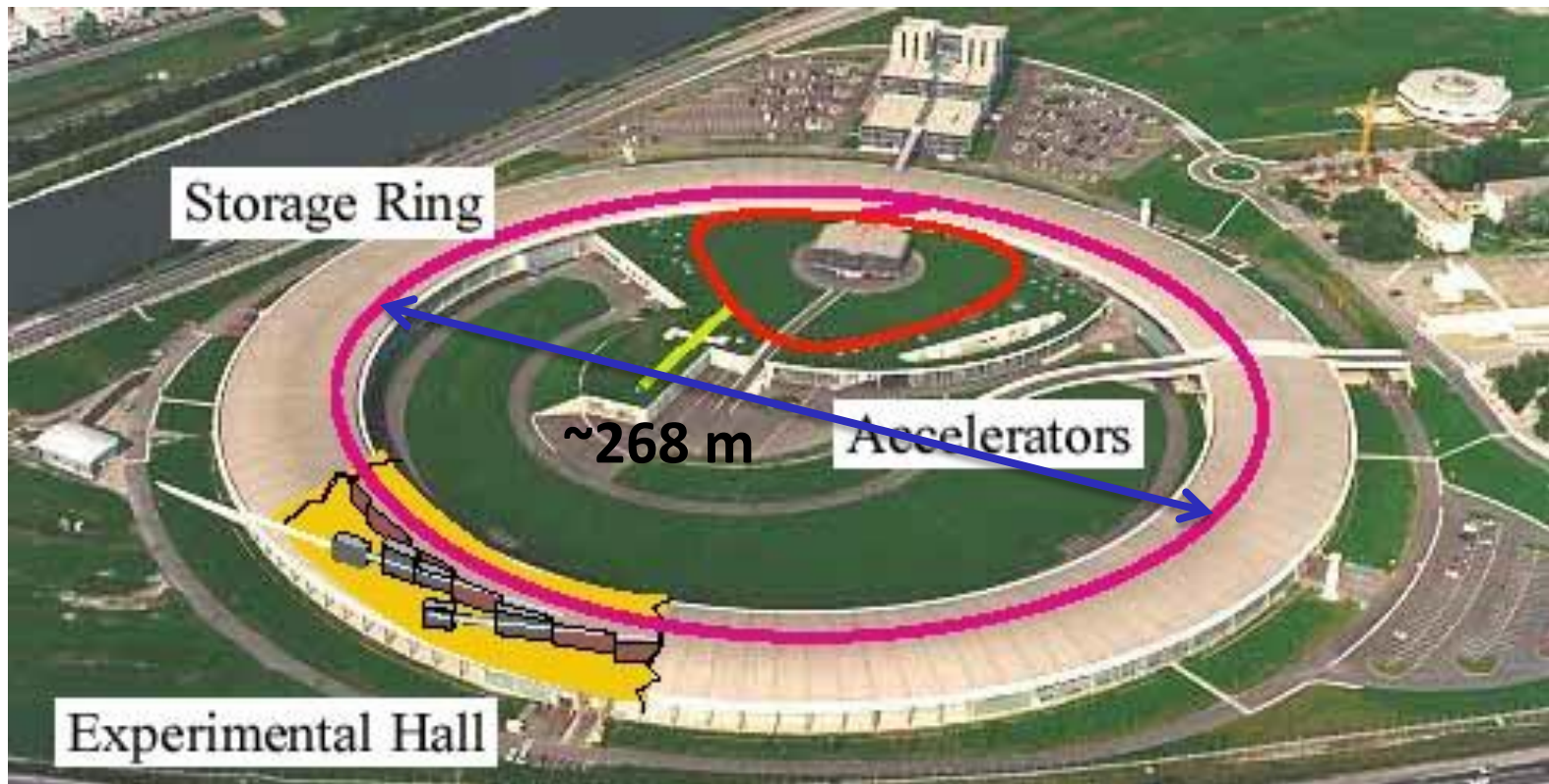
For *electrons and positrons*, in practical units γ is:

$$\gamma = 1957 E_{ring} (GeV)$$



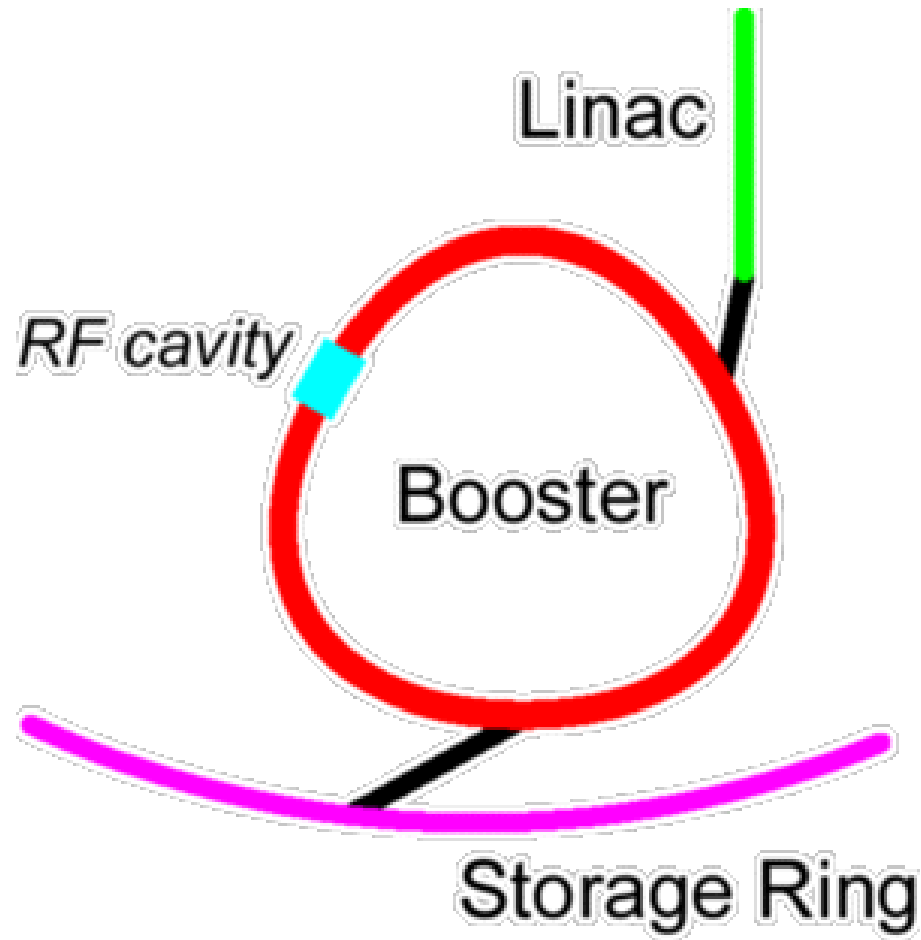
Synchrotron radiation

ESRF - Grenoble



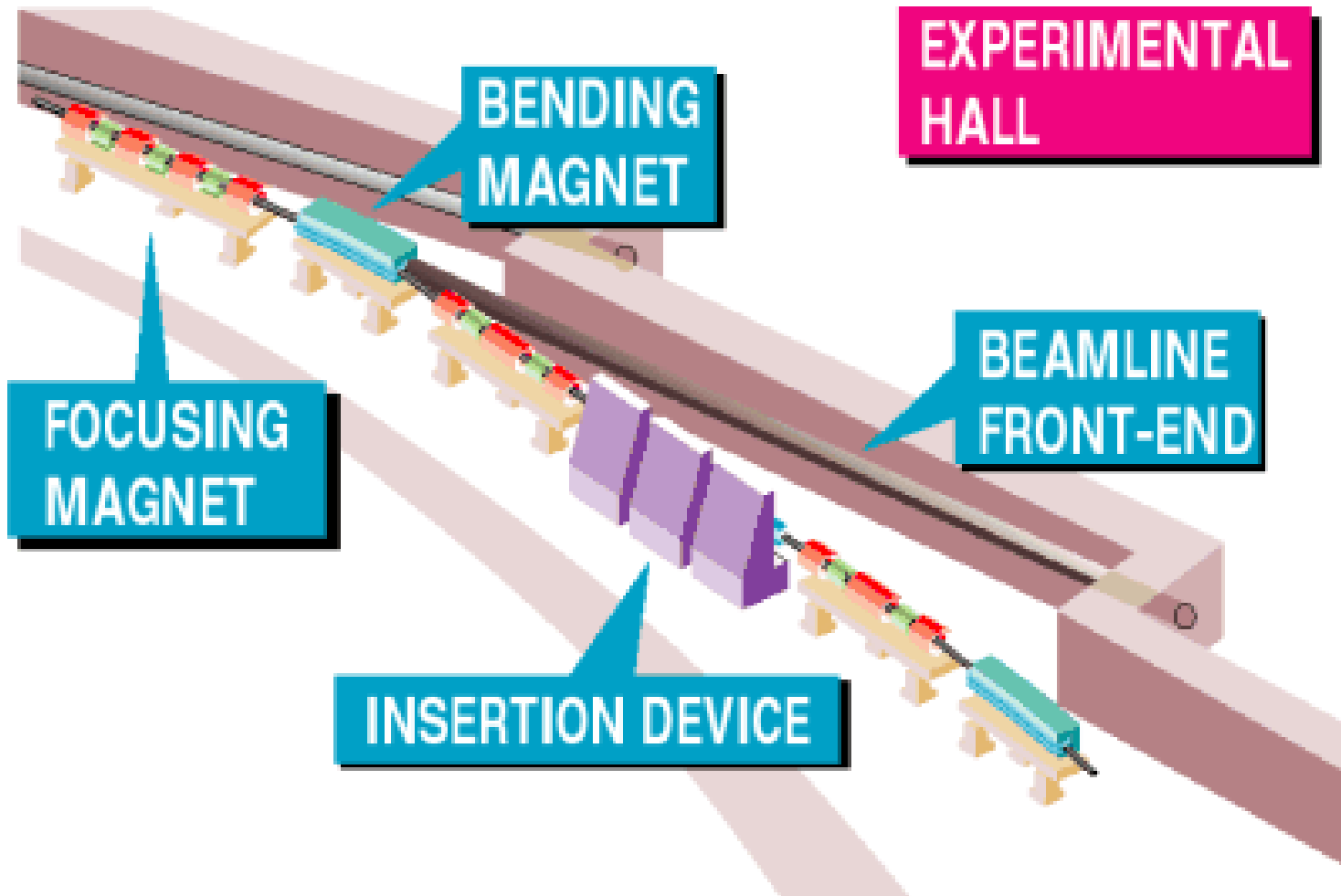
Synchrotron radiation

Injection process



Synchrotron radiation

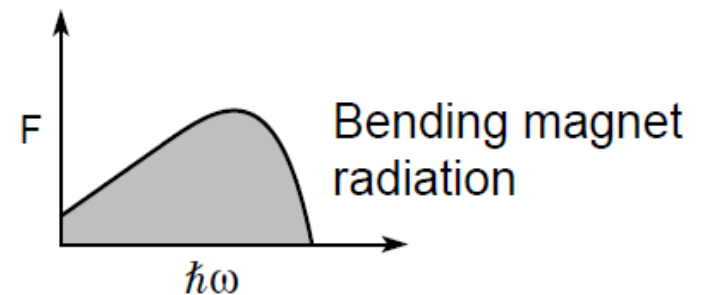
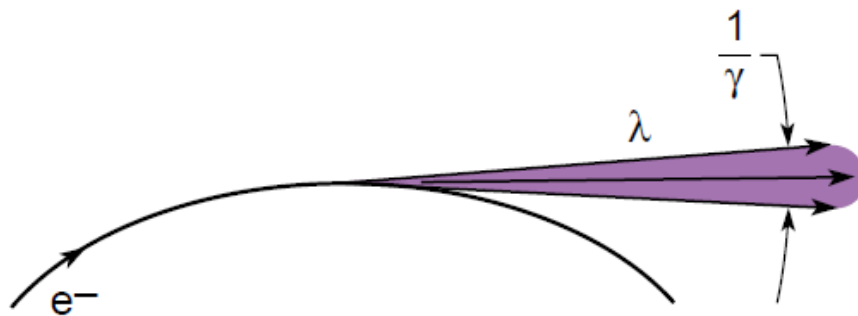
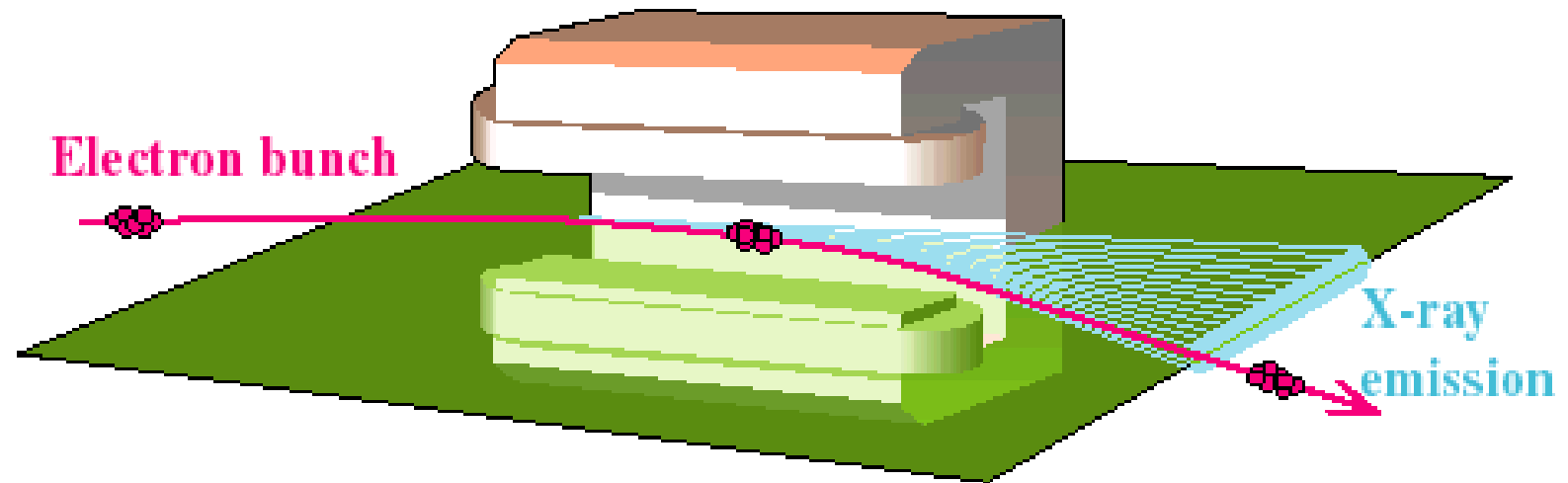
SR tunnel



Synchrotron radiation

Bending magnet

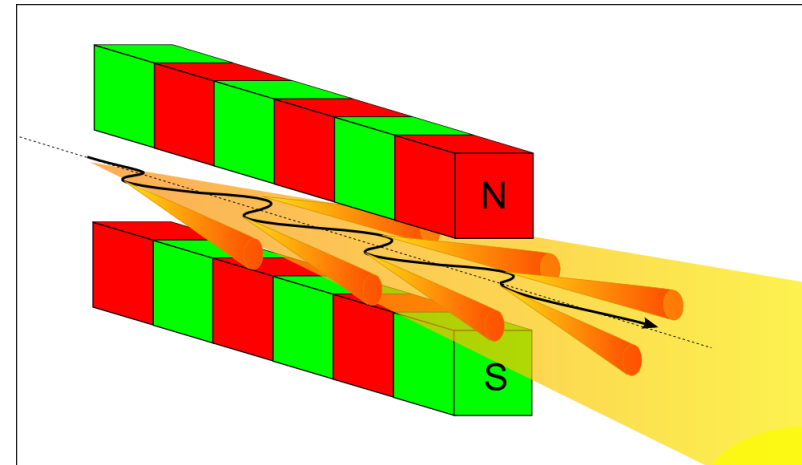
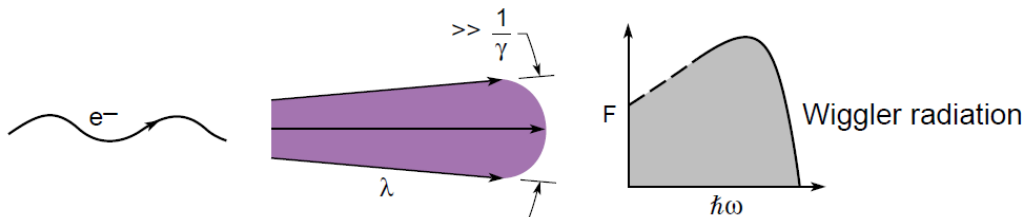
Bending Magnet



Synchrotron radiation

Wigglers

Wigglers are “insertion devices” used to increase the intensity of synchrotron radiation by lining up a series of bending magnets which enhance the intensity simply by the number of magnet poles. In order to add up the intensity of the individual emission cones, the dipoles are arranged with alternating polarity such that the electrons are essentially moving straight except for small “wiggles” where the radiation is emitted. The emission cones overlap and the intensity adds up by positive interference.

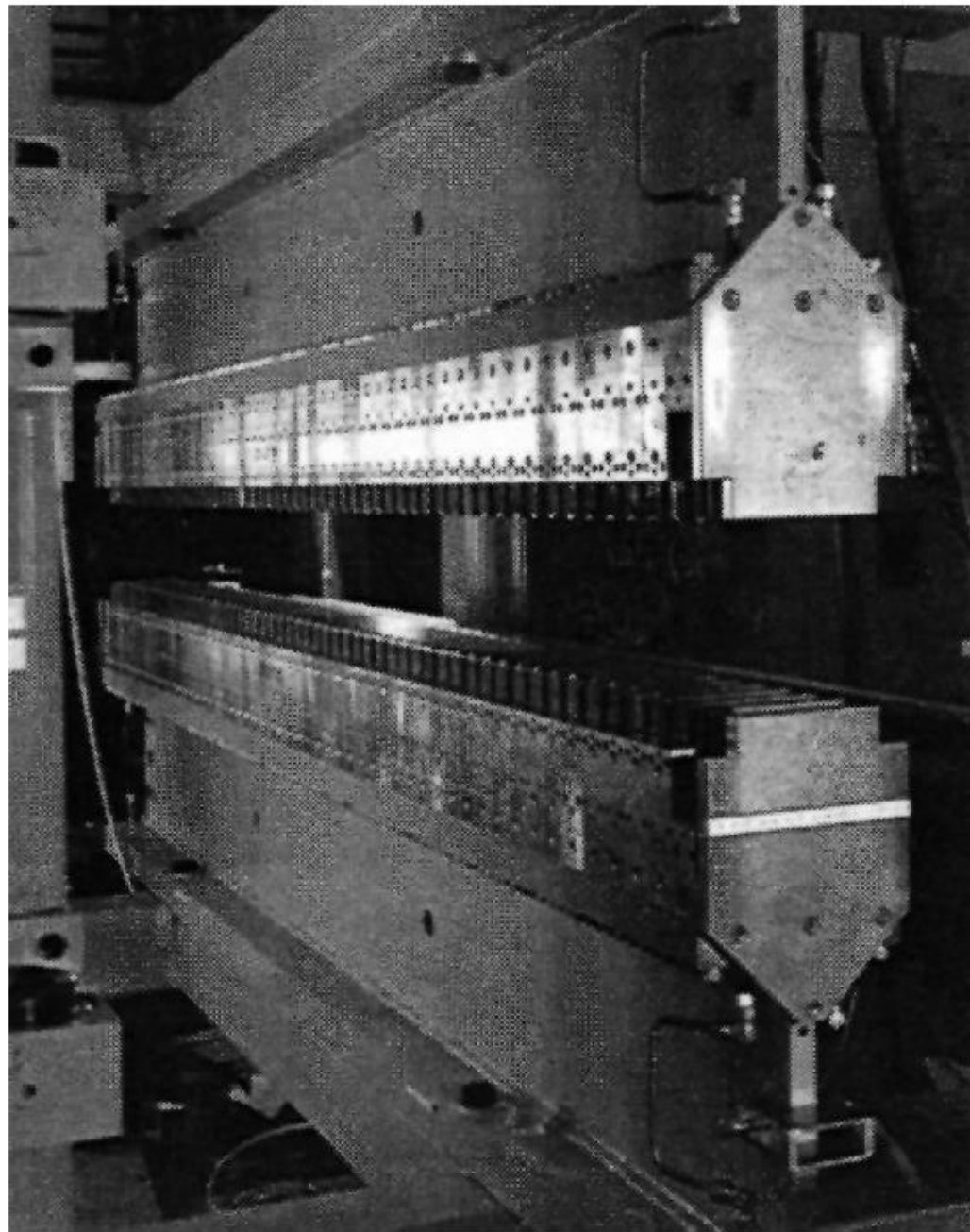


A real wiggler

The advantage of a wiggler is that it emits intense radiation over a wide spectral range, very much like a bending magnet.

Short pulses of SR = wide spread of wavelengths.

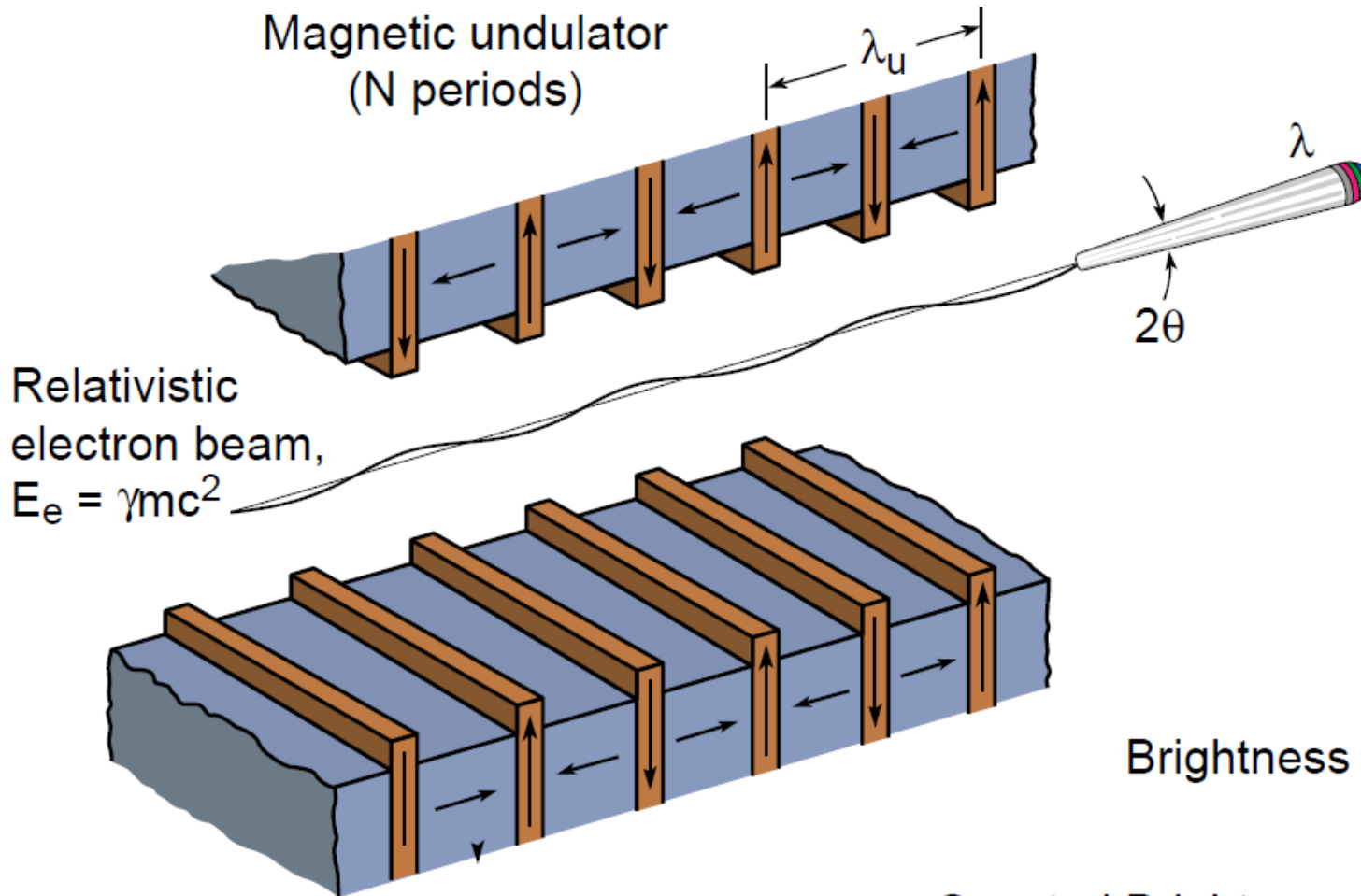
$$\theta_{cen} > \frac{1}{\gamma}$$



LBNL/EXXON/SSRL (1982), SSRL Beamline VI
55 pole ($N = 27.5$), $\lambda_w = 7$ cm

Synchrotron radiation

Undulator



$$\lambda \approx \frac{\lambda_u}{2\gamma^2}$$

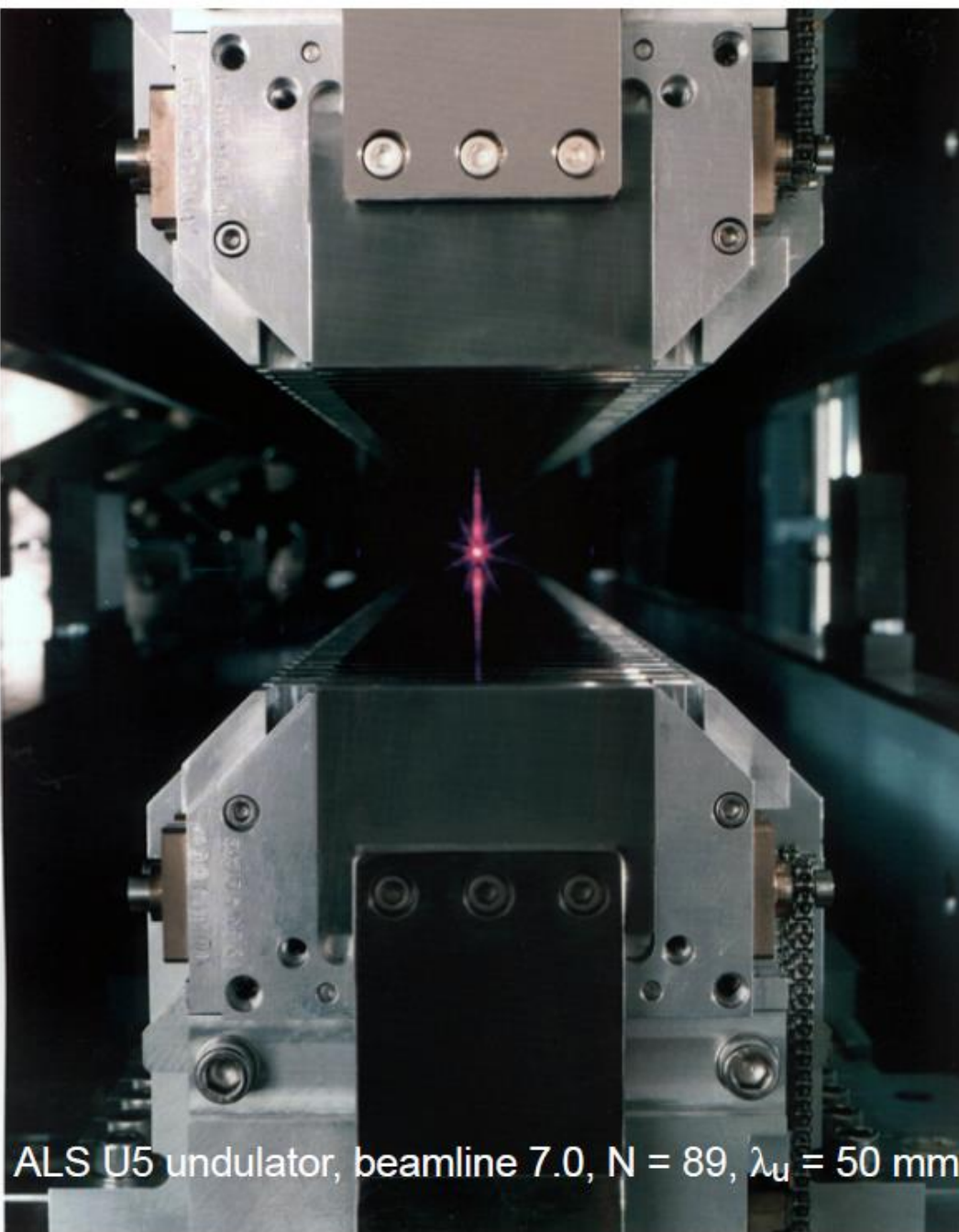
$$\theta_{\text{cen}} \approx \frac{1}{\gamma\sqrt{N}}$$

$$\left[\frac{\Delta\lambda}{\lambda} \right]_{\text{cen}} = \frac{1}{N}$$

$$\text{Brightness} = \frac{\text{photon flux}}{(\Delta A) (\Delta\Omega)}$$

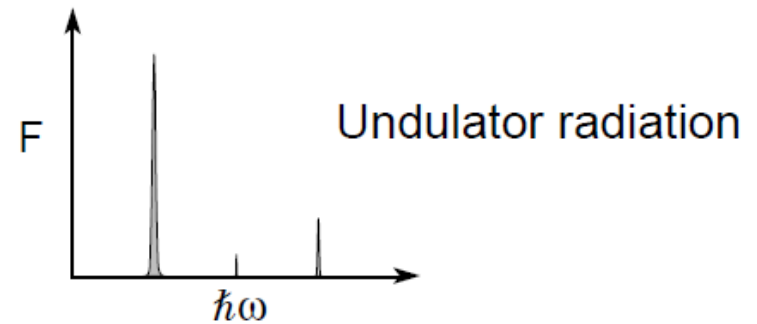
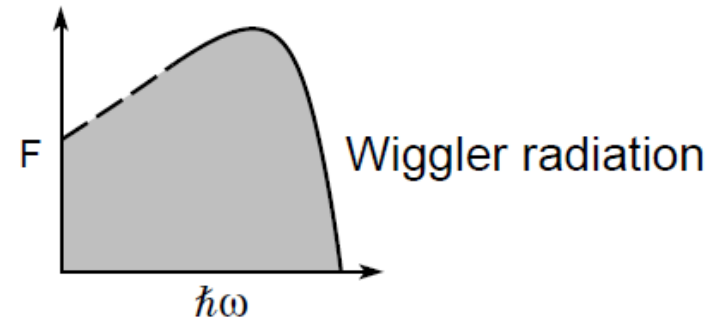
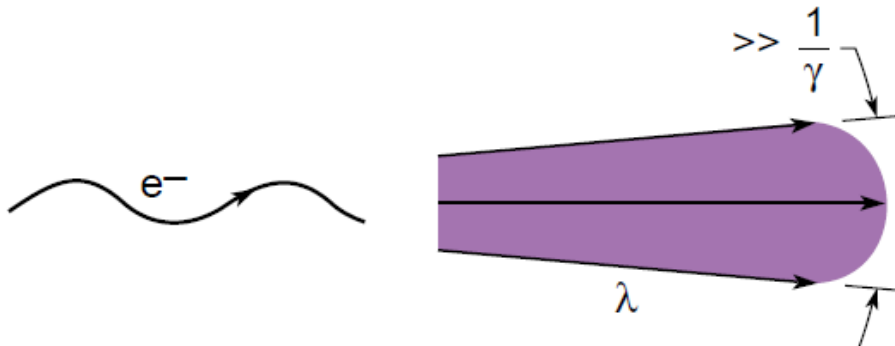
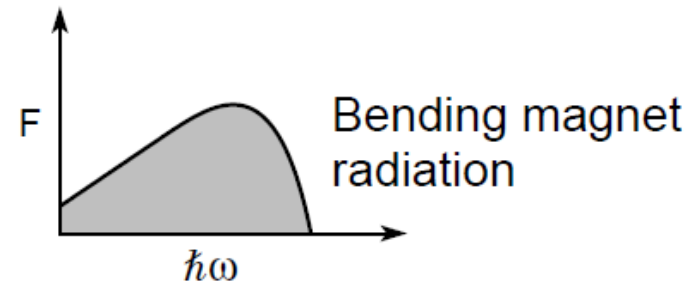
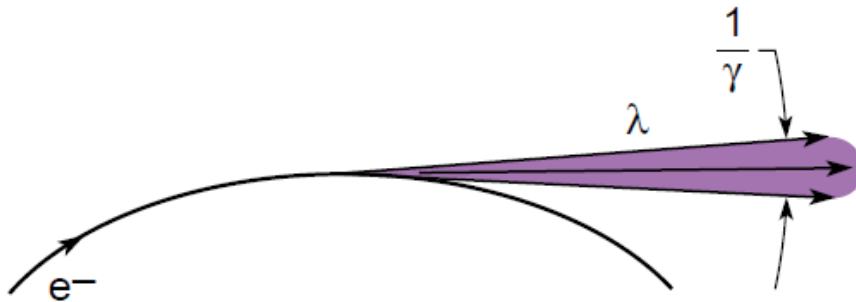
$$\text{Spectral Brightness} = \frac{\text{photon flux}}{(\Delta A) (\Delta\Omega) (\Delta\lambda/\lambda)}$$

Undulator



ALS U5 undulator, beamline 7.0, $N = 89$, $\lambda_u = 50$ mm

Synchrotron radiation



Courtesy of University of
California - Berkeley



Typical Parameters for Synchrotron Radiation



| Facility | ALS | BESSY II | ESRF | SP-8 |
|--|----------------------|----------------------|----------------------|----------------------|
| Electron energy | 1.90 GeV | 1.70 GeV | 6.04 GeV | 8.00 GeV |
| γ | 3720 | 3330 | 11,800 | 15,700 |
| Current (mA) | 400 | 200 | 200 | 100 |
| Circumference (m) | 197 | 240 | 884 | 1440 |
| RF frequency (MHz) | 500 | 500 | 352 | 509 |
| Pulse duration (FWHM) (ps) | 35-70 | 20-50 | 70 | 120 |
| <i>Bending Magnet Radiation:</i> | | | | |
| Bending magnet field (T) | 1.27 | 1.30 | 0.806 | 0.679 |
| Critical photon energy (keV) | 3.05 | 2.50 | 19.6 | 28.9 |
| Critical photon wavelength | 0.407 nm | 0.50 nm | 0.634 Å | 0.429 Å |
| Bending magnet sources | 24 | 32 | 32 | 23 |
| <i>Undulator Radiation:</i> | | | | |
| Number of straight sections | 12 | 16 | 32 | 48 |
| Undulator period (typical) (cm) | 5.00 | 4.90 | 4.20 | 3.20 |
| Number of periods | 89 | 84 | 38 | 140 |
| Photon energy ($K = 1, n = 1$) | 457 eV | 373 eV | 5.50 keV | 12.7 keV |
| Photon wavelength ($K = 1, n = 1$) | 2.71 nm | 3.32 nm | 0.225 nm | 0.979 Å |
| Tuning range ($n = 1$) | 230-620 eV | 140-500 eV | 2.6-7.3 keV | 4.7-19 keV |
| Tuning range ($n = 3$) | 690-1800 eV | 410-1100 eV | 7.7-22 keV | 16-51 keV |
| Central cone half-angle ($K = 1$) | 35 μ rad | 33 μ rad | 17 μ rad | 6.6 μ rad |
| Power in central cone ($K = 1, n = 1$) (W) | 2.3 | 0.95 | 14 | 16 |
| Flux in central cone (photons/s) | 3.1×10^{16} | 1.6×10^{16} | 1.6×10^{16} | 7.9×10^{15} |
| σ_x, σ_y (μ m) | 260, 16 | 314, 24 | 395, 9.9 | 380, 6.8 |
| σ'_x, σ'_y (μ rad) | 23, 3.9 | 18, 12 | 11, 3.9 | 16, 1.8 |
| Brightness ($K = 1, n = 1$) ^a [(photons/s)/mm ² · mrad ² · (0.1%BW)] | 2.3×10^{19} | 4.6×10^{18} | 5.1×10^{18} | 1.8×10^{20} |
| Total power ($K = 1$, all n , all θ) (W) | 83 | 32 | 480 | 2,000 |
| Other undulator periods (cm) | 3.65, 8.00, 10.0 | 4.1, 5.6, 12.5 | 2.3, 3.2, 5.2, 8.5 | 2.4, 10.0, 3.7, 12.0 |
| <i>Wiggler Radiation:</i> | | | | |
| Wiggler period (typical) (cm) | 16.0 | 12.5 | 8.0 | 12.0 |
| Number of periods | 19 | 32 | 20 | 37 |
| Magnetic field (maximum) (T) | 2.1 | 1.15 | 0.81 | 1.0 |
| K (maximum) | 32 | 12.8 | 6.0 | 11 |
| Critical photon energy (keV) | 5.1 | 2.11 | 20 | 43 |
| Critical photon wavelength | 0.24 nm | 0.59 nm | 0.62 Å | 0.29 Å |
| Total power (max. K) (kW) | 13 | 1.8 | 4.8 | 18 |

^aUsing Eq. (5.65). See comments following Eq. (5.64) for the case where $\sigma'_{x,y} \approx \theta_{\text{cen}}$.

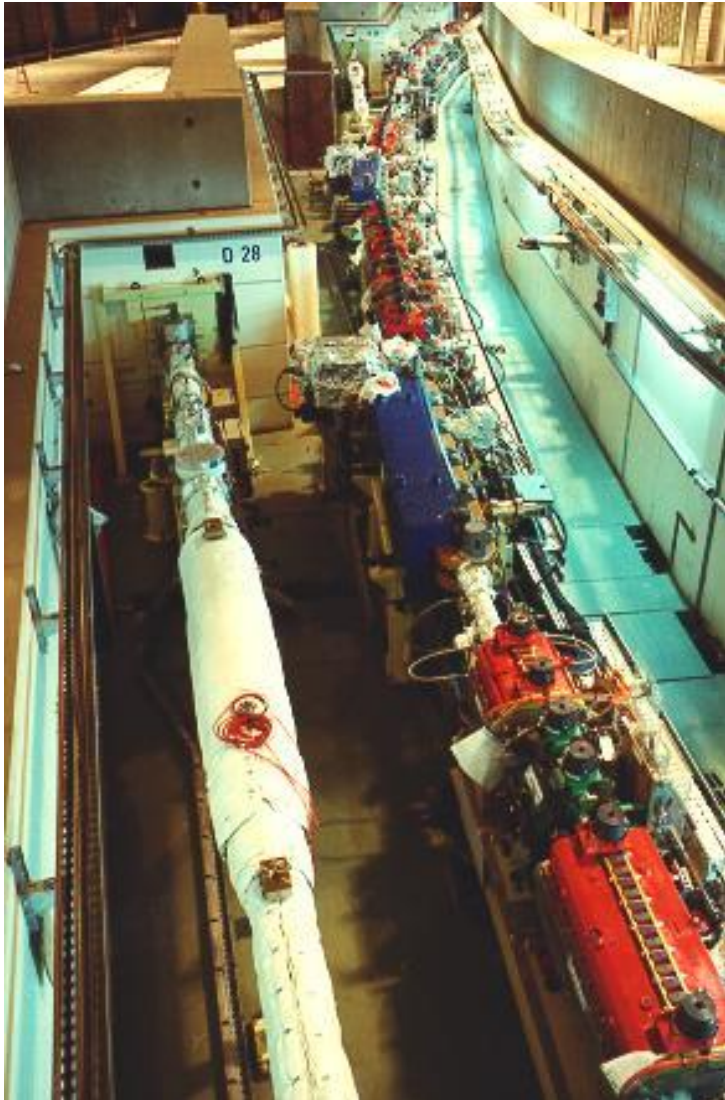
Courtesy of University of
California - Berkeley

Synchrotron radiation

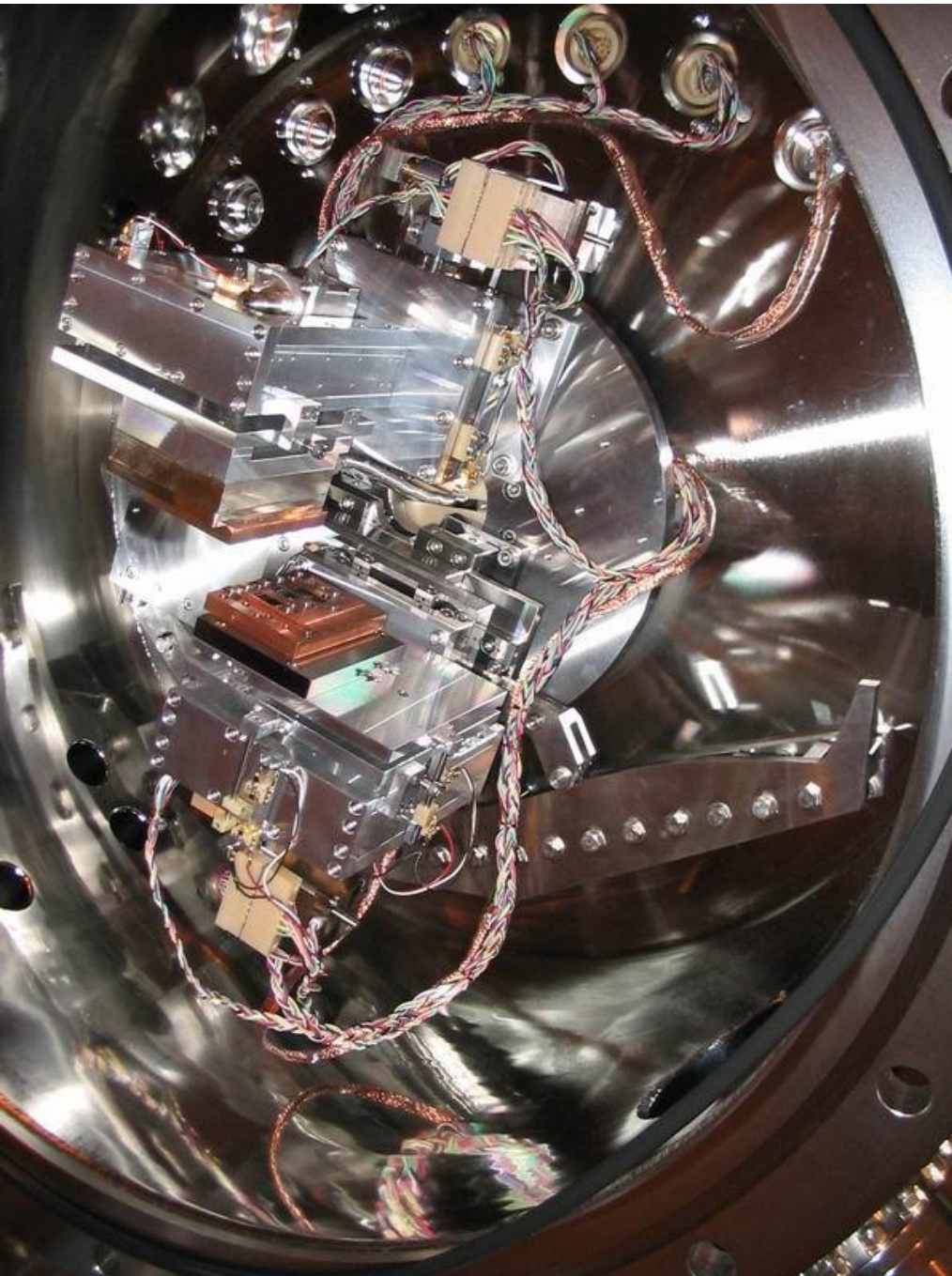
Focusing magnet



Synchrotron radiation



Front - end

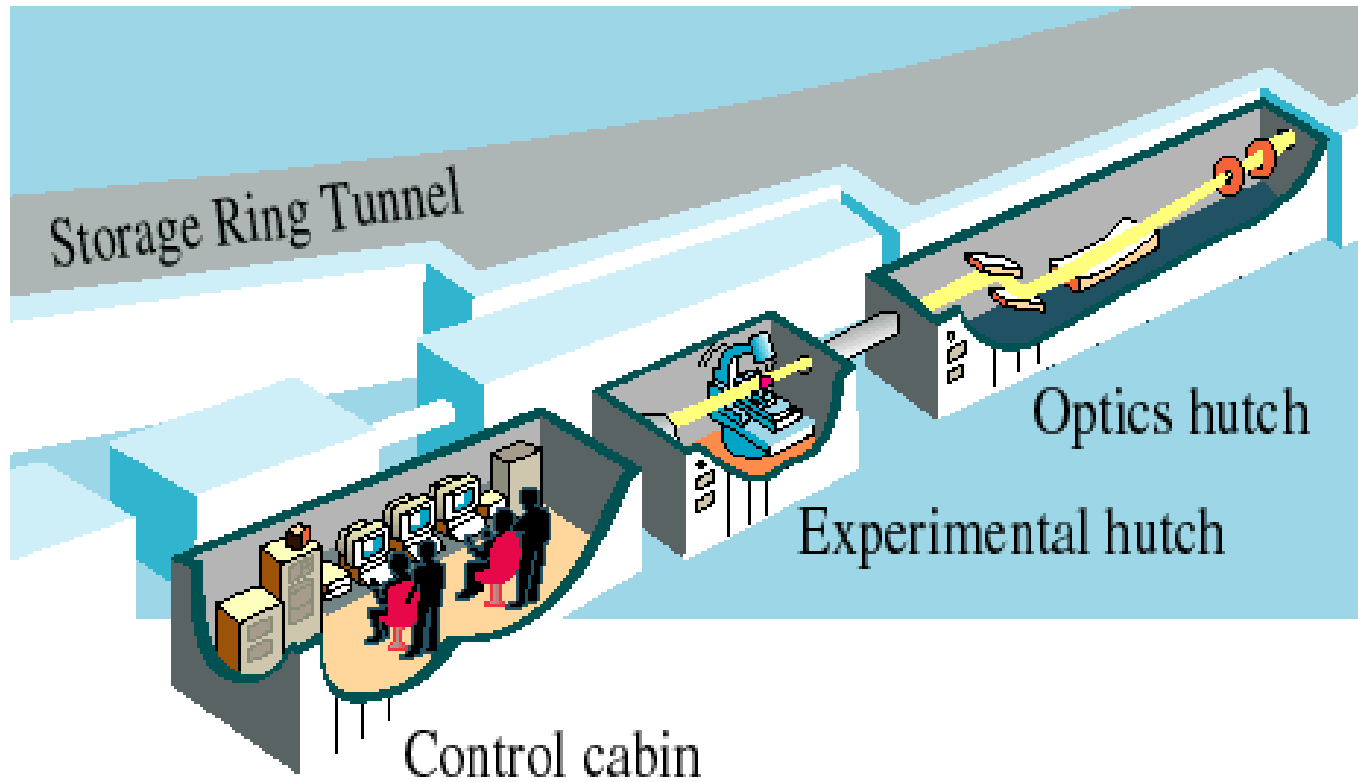


Double crystal monochromator

Si(111), Si(220), Si(311),
Ge(111)

Synchrotron radiation

Beamline



X-ray Absorption Spectroscopy

Acronyms

XAS X-ray **A**bsorption **S**pectroscopy: Generic term for various techniques involving absorption of X-rays

XAFS X-ray **A**bsorption **F**ine **S**tructure: Name resuming the following two techniques

XANES(NEXAFS) X-ray **A**bsorption **N**ear **E**dge **S**tructure: Studies the region just before and after the edge (about -20 eV +50 eV from edge). It is a *spectroscopic* technique

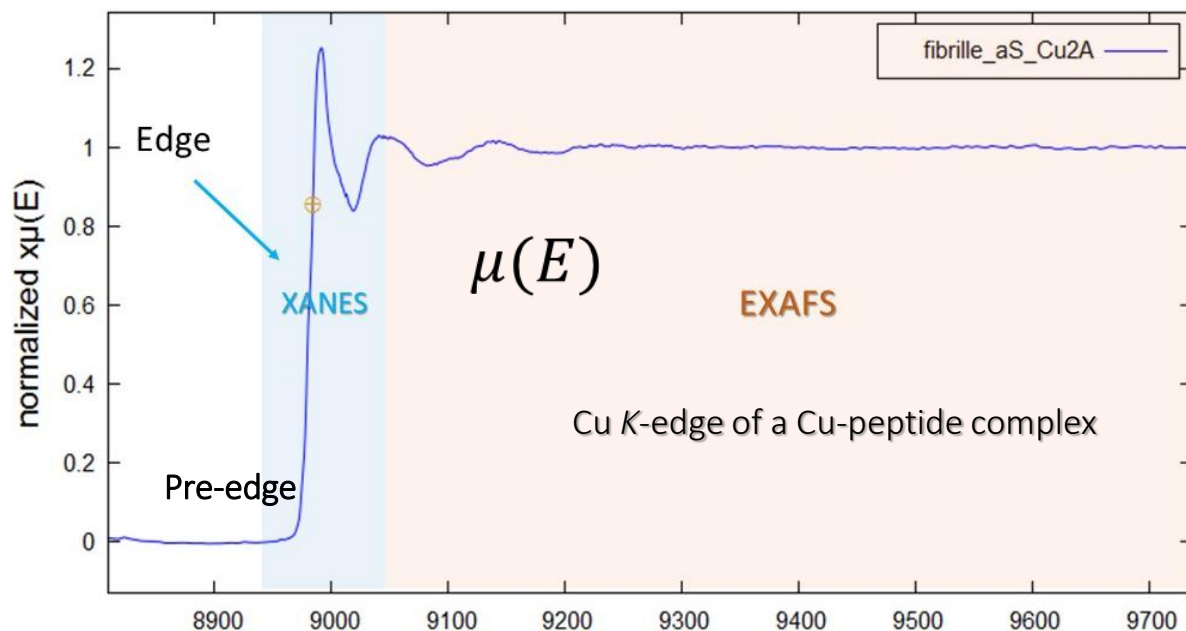
EXAFS Extended X-ray **A**bsorption **F**ine **S**tructure: Studies the region from about 50 eV up to 1000 (or more) eV from the edge. It is a *structural* technique

X-ray Absorption Spectroscopy

What is XAS?

- X-ray Absorption Spectroscopy exploits the x-ray photoelectric effect and the wave nature of the electron to study the modulation of the X-ray absorption coefficient at energies in the vicinity of the absorption edge of selected atomic species in the sample. The edge region and the extended region of the spectrum contain information about the chemical state and coordination environment of the absorber.
- Unlike X-ray diffraction, it does not require long range translational order – it works equally well in amorphous materials, liquids, (poly)crystalline solids and molecular gases.

XAS spectrum (Cu-edge)

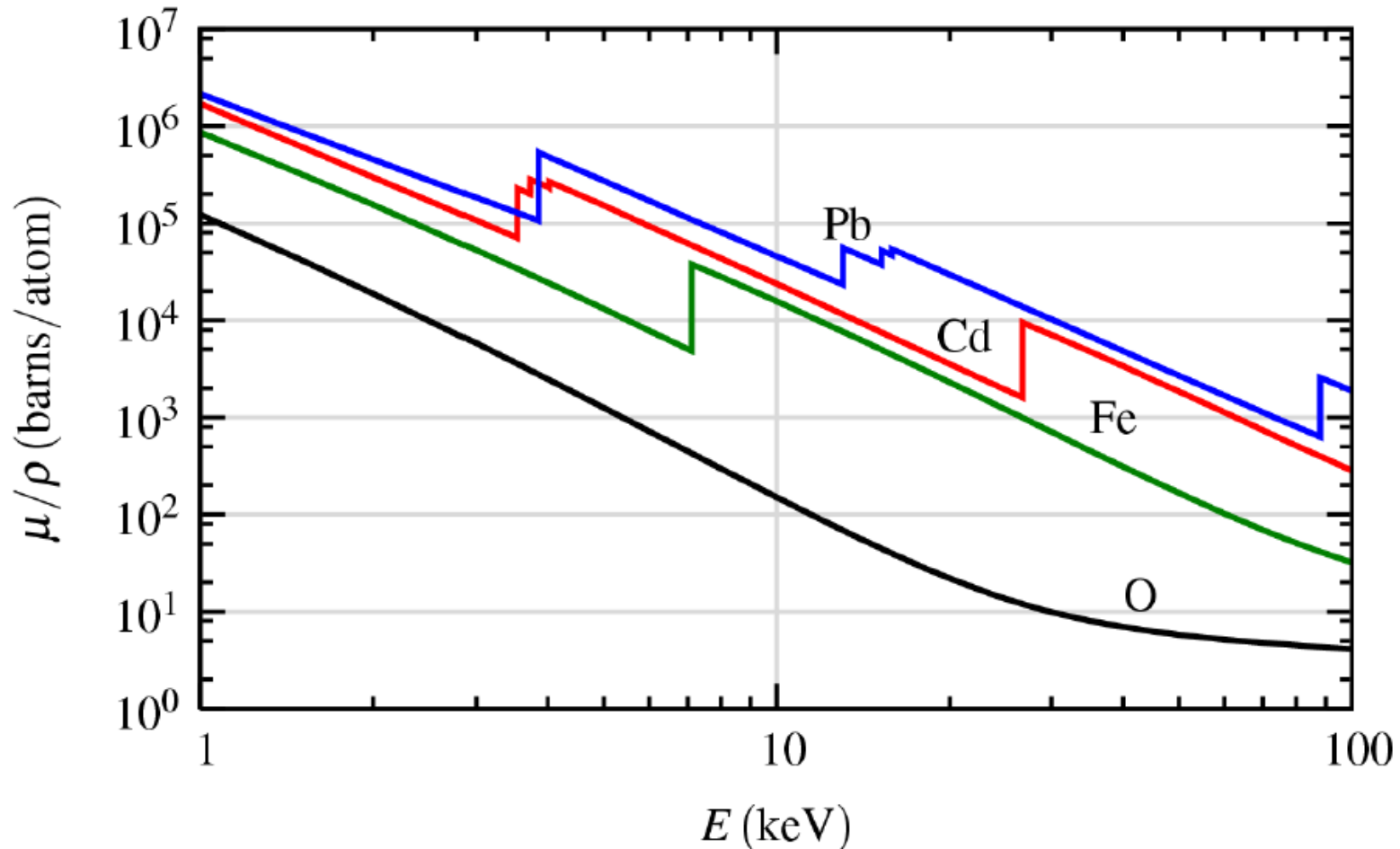


XAS characteristics

- interatomic distances, atom types
- coordination number (geometry) of absorber
- oxidation state of absorber
- covalency of coordination bonds

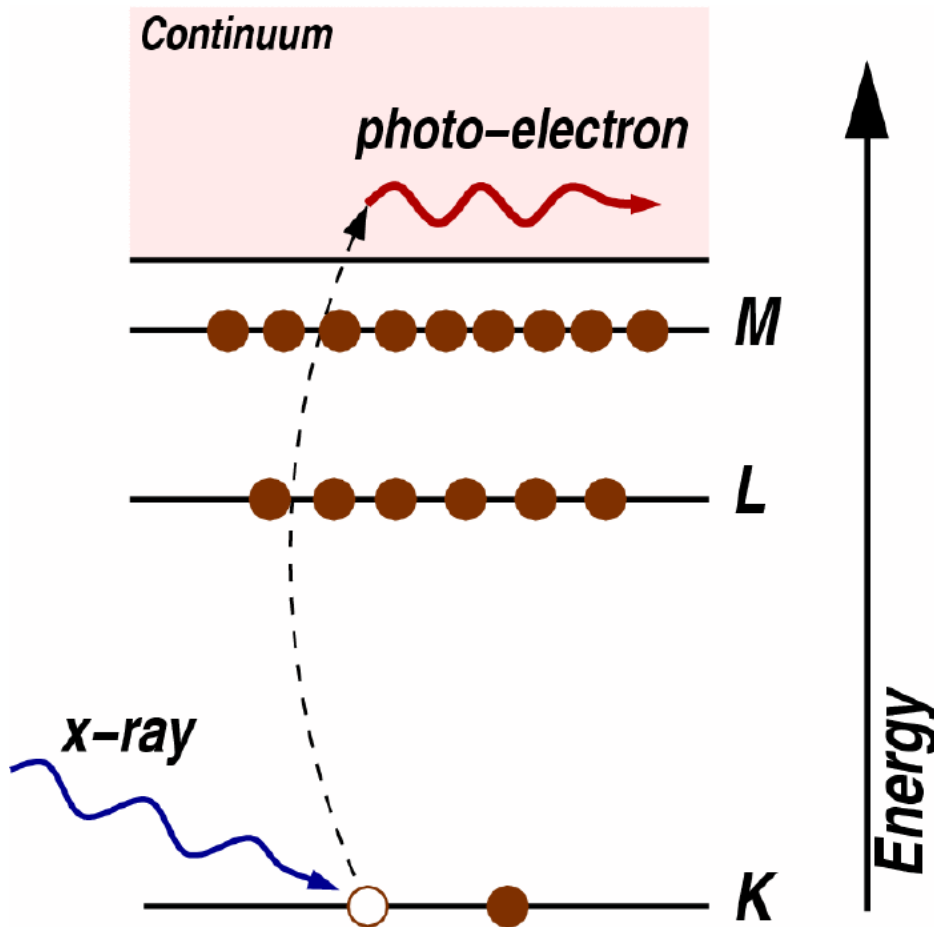
X-ray Absorption Spectroscopy

Mass absorption coefficient vs. E of different elements



X-ray Absorption Spectroscopy

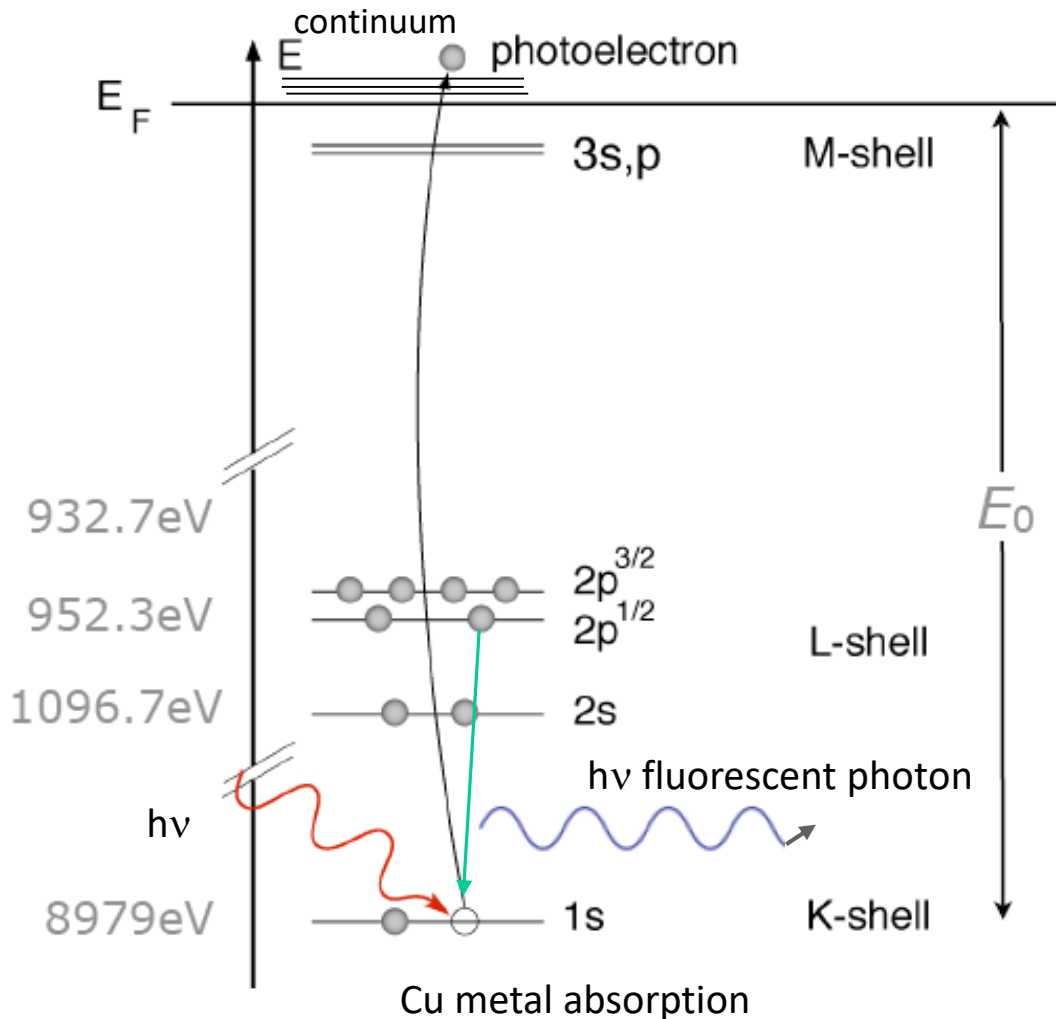
The photoelectric effect



A core-level electron is extracted out of the absorbing atom that is now in an *excited state*.

The kinetic energy of the free photoelectron is the *difference* between that of the absorbed photon and that of the initial electron state.

X-ray Absorption Spectroscopy



Electrons are arranged in shells. Photoionization: atom can be ionized by absorption of an (X-ray) photon if:

$$E_{\text{photon}} > E_{\text{ionization}}$$

Absorption/Emission Selection rules:

$$\Delta L = \pm 1; \Delta S = 0; \Delta J = 0, \pm 1$$

Core-hole decay may occur by **x-ray fluorescence emission** or by **Augier** electron emission

X-ray Absorption Spectroscopy

How XAS happens – the physical process:
photoabsorption/photoionization

Transition rate: The Fermi golden rule

$$\mu(E) \propto |\langle \psi_f | \boldsymbol{\varepsilon} \cdot \mathbf{p} | \psi_i \rangle|^2$$

Here $\boldsymbol{\varepsilon}$ is the electric dipole vector associated with the photon and \mathbf{p} is the electron momentum, ψ_i and ψ_f are the electron initial and final states wave-functions.


This relation is valid if considering only «*elastic transitions*», (i.e. all the photon energy is transferred to the photoelectron so that only one core electron changes its state and the remaining $N - 1$ electrons, passive electrons, simply relax their orbitals around the core hole) and under the so-called «*electric dipole approximation*», i.e. when the photon electric field is constant over (almost all) the extension of the electron wave function. Under such approximation, the electromagnetic field interacts only with a core orbital, whose extension is smaller than the X-ray wavelength. For 1s electrons, this is the case.

X-ray Absorption Spectroscopy

How XAS happens – the physical process:
photoabsorption/photoionization

Example: Cu 1s orbital
Cu 1s electron radius:

Bohr radius of H in ground state

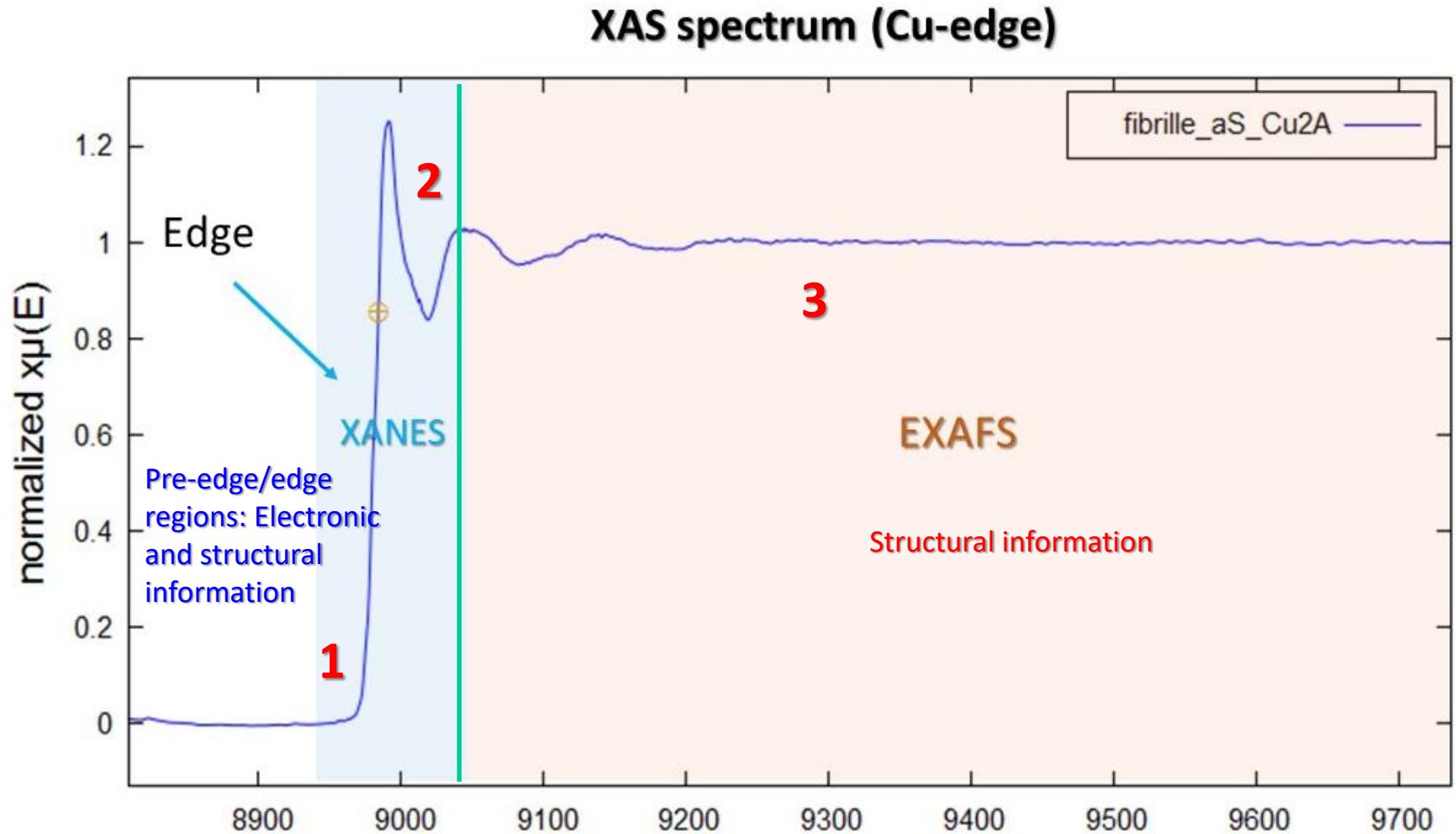

$$\frac{a_0}{Z} = \frac{0.53 \text{ Å}}{29} = 0.02 \text{ Å}$$

Wavelength of photon at the Cu absorption edge:

$$\lambda = \frac{hc}{E} = \frac{12.4 \text{ keVÅ}}{8.9282 \text{ keV}} = 1.38 \text{ Å}$$

$\lambda \gg \frac{a_0}{Z}$: dipole approximation is well fulfilled

X-ray Absorption Spectroscopy



X-ray Absorption Spectroscopy

How XAS happens – the physical process

Electric-dipole electronic transitions between atomic quantum states.

$$\mu(E) \propto |\langle \psi_f | \boldsymbol{\varepsilon} \cdot \mathbf{p} | \psi_i \rangle|^2$$

Transition probability

$$\mu(E) \propto |\langle \psi_e | \boldsymbol{\varepsilon} \cdot \mathbf{p} | \psi_i \rangle|^2$$

1. *Core to bound* states (pre-edge, edge regions): the excited electron remains within the atom nuclear potential. The electronic structure of the emitter bound to the molecule is probed.
2. *Core to quasi-bound* states (XANES): multiple scattering resonances between the excited electrons with low kinetic energy dominate. Low kinetic energy electrons - Multiple scattering (typically up to ~ 50 eV above the threshold, the region where bound to quasi-bound transitions take place); e is scattered primarily by valence and shallow inner shell electrons of the neighboring atoms - XANES region.

X-ray Absorption Spectroscopy

How XAS happens – the physical process

3. *core to continuum* (electron with sufficient kinetic energy to escape into the continuum) - photoelectric effect. (EXAFS). High kinetic energy electrons (50 -1000 eV) are scattered primarily by the core electrons of the neighboring atoms, single scattering pathway dominates - EXAFS region.

It is suggested that the boundary between XANES and EXAFS regions is that where the wavelength of excited electron is equal to the distance between the absorbing atom and its nearest neighbors. $(\lambda(\text{\AA}) = 12/[E(\text{eV})]^{1/2})$.

X-ray Absorption Spectroscopy

How XAS happens – electron scattering

Free electron (plane wave) scattered by an atom (spherical potential) travels away as a spherical wave.

Electron with kinetic energy: $KE > 0$ in a molecular environment is scattered by the surrounding atoms.

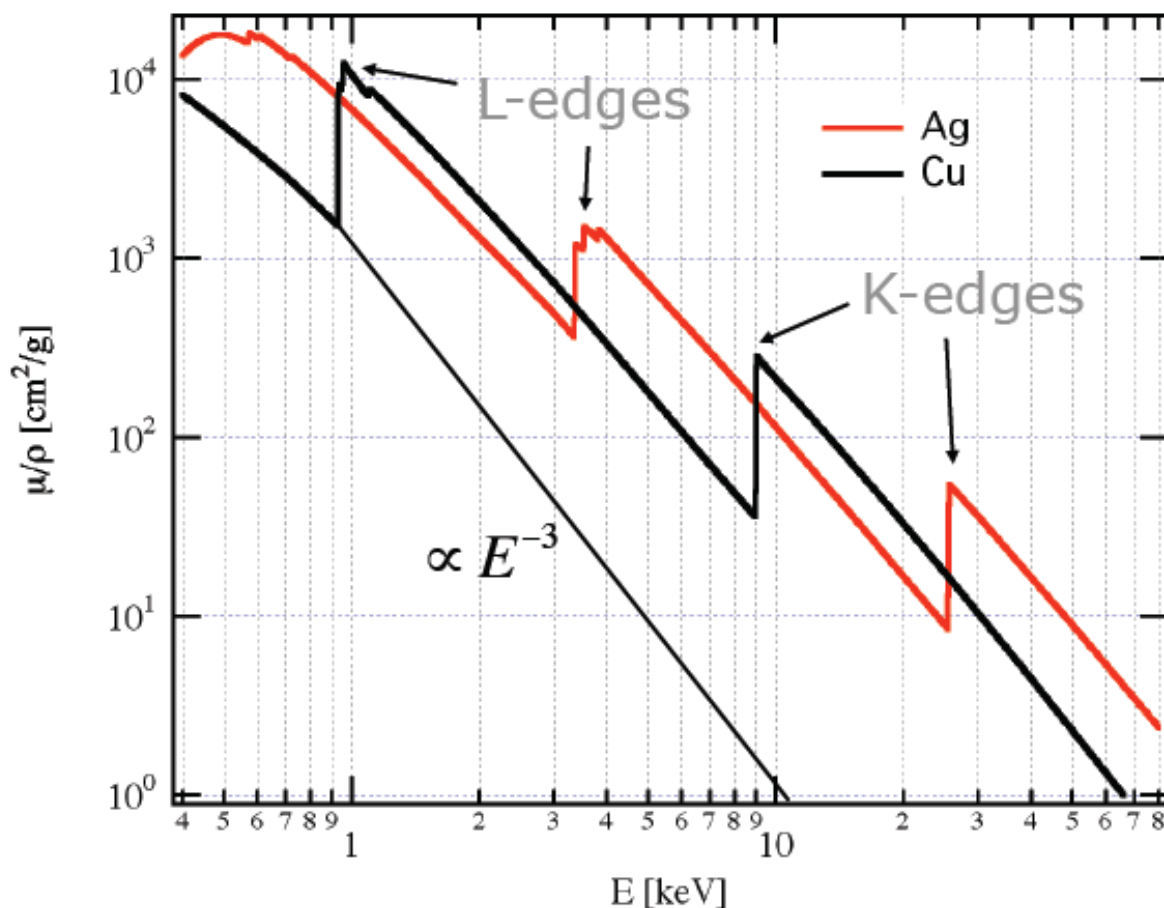
Low KE e^- is scattered by valence electrons, undergoes multiple scattering in a molecular environment (XANES).

High KE e^- is scattered by core electrons, favors single scattering (EXAFS).

The number of absorbed photons is proportional to the absorption probability (the absorption cross-section for the absorber A is given by: $\sigma = \frac{\mu}{\rho} \cdot \frac{m_a}{N_A}$; where $\frac{\mu}{\rho}$ is the mass absorption coefficient, m_a is the atomic molar mass and N_A is the Avogadro's number).

X-ray Absorption Spectroscopy

Example: Linear Absorption Coefficient of Cu & Ag

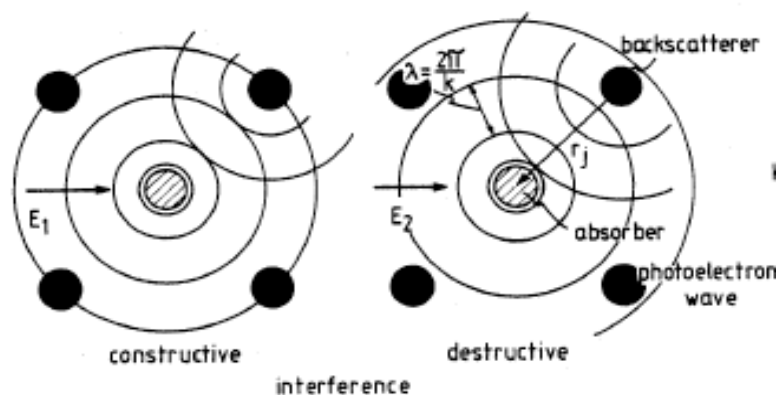


- 💡 mainly atomic effect!
- 💡 strong dependence on energy
 $\propto E^{-2.78}$
- 💡 strong dependence on atomic number:
 $\propto Z^{2.7}$
- 💡 inner shell electr. contribute most strongly!

X-ray Absorption Spectroscopy

The EXAFS equation

Absorber has neighboring atoms in condensed matter. They scatter back the emitted photoelectron.



Interference occurs between outgoing and incoming waves

EXAFS monitors the variable behavior of the absorption coefficient above the edge energy

$$\chi(k) = \frac{\mu - \mu_0}{\mu_0}$$

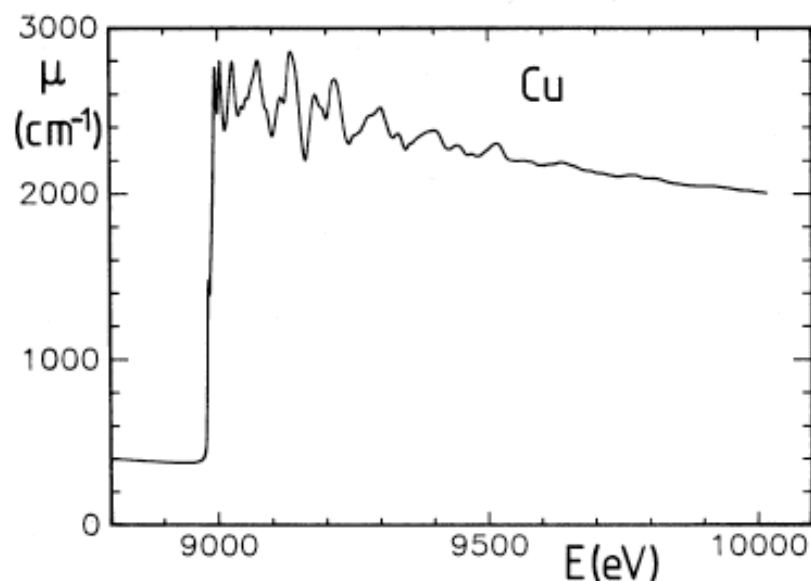
$$\lambda = \frac{2\pi}{k}, \quad k = \frac{1}{\hbar} \sqrt{2m(E - E_0)}$$

where E_0 is the absorption edge energy and m is the electron mass. $k = 0.513 \Delta E^{1/2} (\text{\AA}^{-1})$

X-ray Absorption Spectroscopy

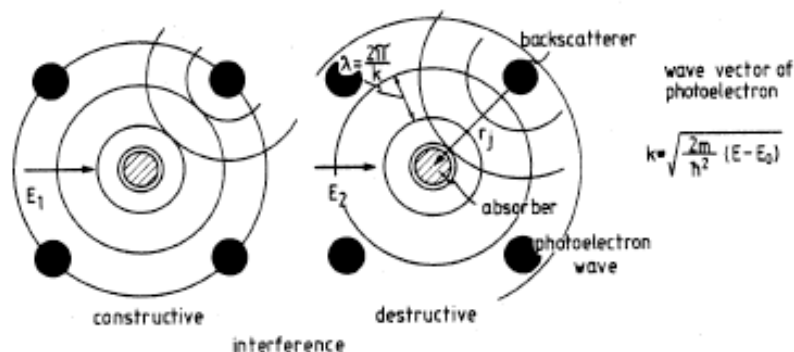
The EXAFS equation

EXAFS: Oscillatory Part of μ beyond the edge



$$\chi(k) = \frac{\mu - \mu_0}{\mu_0}$$

EXAFS



Constructive (destructive) interference:

probability to excite electron is increased (reduced)

μ has maximum (minimum)

X-ray Absorption Spectroscopy

EXAFS

How can the emitted electron influence the absorption of the photon?

Naive picture:

1. photon is absorbed
2. electron is emitted
3. electron is scattered back by neighbors

How can scattering of electron (step 3) influence absorption (step 1)?

X-ray Absorption Spectroscopy

EXAFS

How can the emitted electron influence the absorption of the photon?

Quantum mechanical process:

photon (with given energy) is absorbed by atom and electron is excited into (free) state!

This is a one step process!

Final state of electron is given by the local environment of the atom!

Final state determines transition probability!

$$\mu(E) \propto |\langle \psi_e | \boldsymbol{\varepsilon} \cdot \mathbf{p} | \psi_i \rangle|^2$$

X-ray Absorption Spectroscopy

EXAFS

How can the emitted electron influence the absorption of the photon?

Other way of looking at the same process:

absorption of photon takes a certain time τ_0
[given by the length of the wave train of the photon
(monochromaticity)]

During that time the excited electron has time to probe the local environment of the atom.

X-ray Absorption Spectroscopy

EXAFS: Monochromaticity and Coherence Time

Example:

energy: 9000eV

band width: $\sim 1\text{eV}$

$$1\text{eV} = 1.60218 \times 10^{-19} \text{ J}$$

$$\begin{aligned} h &= 4.13667 \times 10^{-15} \text{ eV} \cdot \text{s} = \\ &= 6.62607 \times 10^{-34} \text{ J} \cdot \text{s} \end{aligned}$$

$$\nu = \frac{E}{h} = 2.3 \cdot 10^{18} \text{ s}^{-1}, \quad T = 4.4 \cdot 10^{-19} \text{ s}$$

wave train contains ~ 9000 oscillations:

$$\tau_0 = 4 \cdot 10^{-15} \text{ s}$$

In copper:

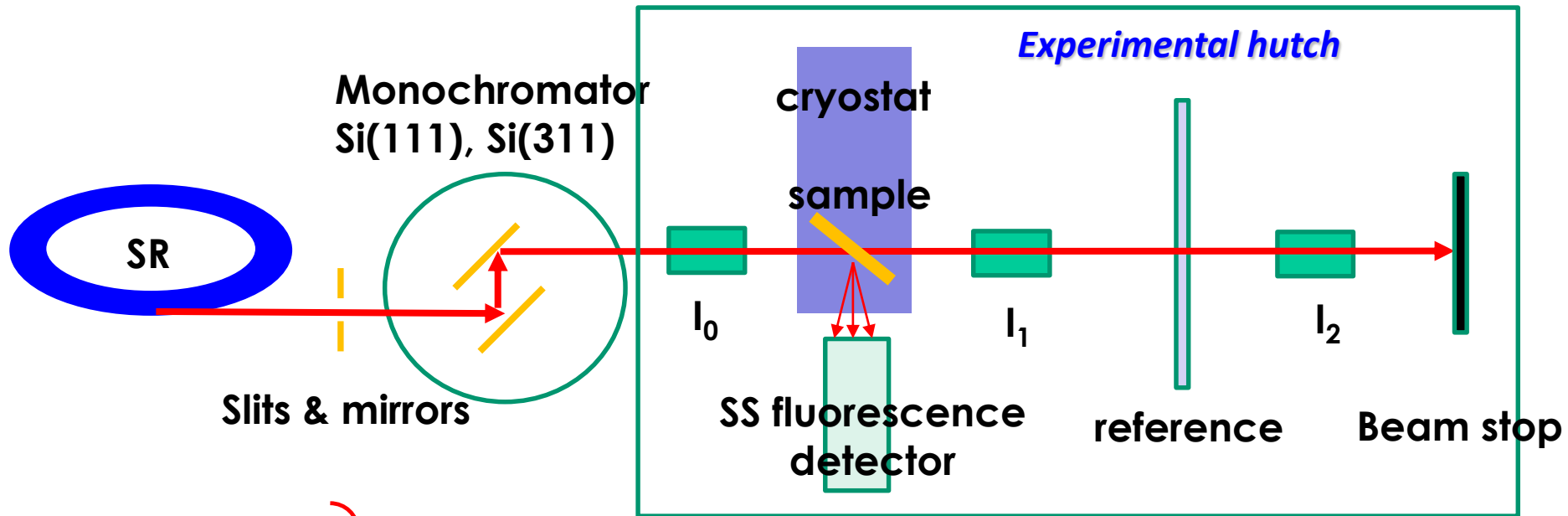
energy of electron: $\sim 20\text{--}100 \text{ eV}$

In time τ_0 electron could travel $\sim 50 - 100 \text{ \AA}$ and back!

In practice, travel range limited by inelastic scattering processes to about 10\AA .

X-ray Absorption Spectroscopy

X-ray Absorption Spectroscopy: the experiment



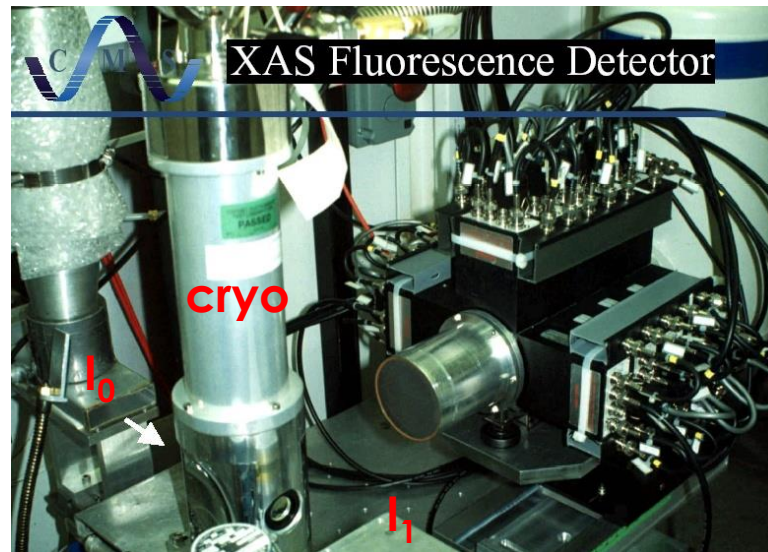
$$\frac{I}{I_0} = e^{-\mu(E)x}$$

$$\mu(E)x = \ln \frac{I_0}{I}$$

Absorption mode

$$\mu(E) = \frac{I_F}{I_0}$$

Fluorescence mode



X-ray Fluorescence Emission

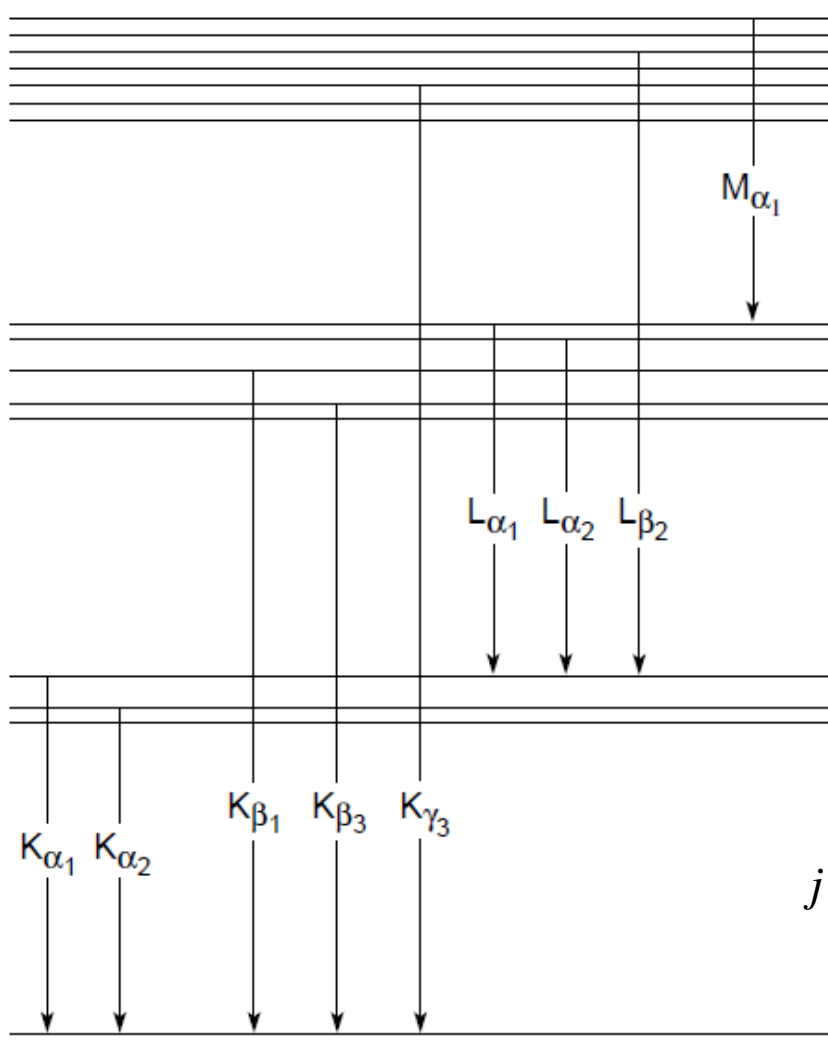
| n | ℓ | j |
|---|---|-----|
| 4 | 3 | 7/2 |
| 4 | 3 | 5/2 |
| ⋮ | ⋮ | ⋮ |
| 4 | 0 | 1/2 |
| 3 | 2 | 5/2 |
| 3 | 2 | 3/2 |
| 3 | 1 | 3/2 |
| 3 | 1 | 1/2 |
| 3 | 0 | 1/2 |
| 2 | 1 | 3/2 |
| 2 | 1 | 1/2 |
| 2 | 0 | 1/2 |
| 1 | 0 | 1/2 |

N

M

L

K



N_{VII} 4f_{7/2}
 N_{IV} 4d_{3/2}
 N_I 4s

M_V 3d_{5/2}
 M_{IV} 3d_{3/2}
 M_{III} 3p_{3/2}
 M_{II} 3p_{1/2}
 M_I 3s

L_{III} 2p_{3/2}
 L_{II} 2p_{1/2}
 L_I 2s

K 1s

Absorption edges
for copper (Z = 29):

$E_{N_1, \text{abs}} = 7.7 \text{ eV}$

$E_{M_3, \text{abs}} = 75 \text{ eV}$

$E_{M_1, \text{abs}} = 123 \text{ eV}$

$E_{L_3, \text{abs}} = 933 \text{ eV}$

$E_{L_2, \text{abs}} = 952 \text{ eV}$

$E_{L_1, \text{abs}} = 1,097 \text{ eV}$

$E_{K, \text{abs}} = 8,979 \text{ eV}$
 (1.381Å)

$j = |l+s|, |l+s-1|, \dots, |l-s|$ subshells

Cu $K_{\alpha_1} = 8,048 \text{ eV}$ (1.541Å)

Cu $K_{\alpha_2} = 8,028 \text{ eV}$ (1.544Å)

Cu $K_{\beta_1} = 8,905 \text{ eV}$

Cu $L_{\alpha_1} = 930 \text{ eV}$

Cu $L_{\alpha_2} = 930 \text{ eV}$

Cu $L_{\beta_1} = 950 \text{ eV}$

Sample requirements and limitations

- ✓ Samples can be measured in any state:
 - Solid (pure or solution)
 - Liquid (pure or solution)
 - Gas (also mixtures)
 - Frozen solutions

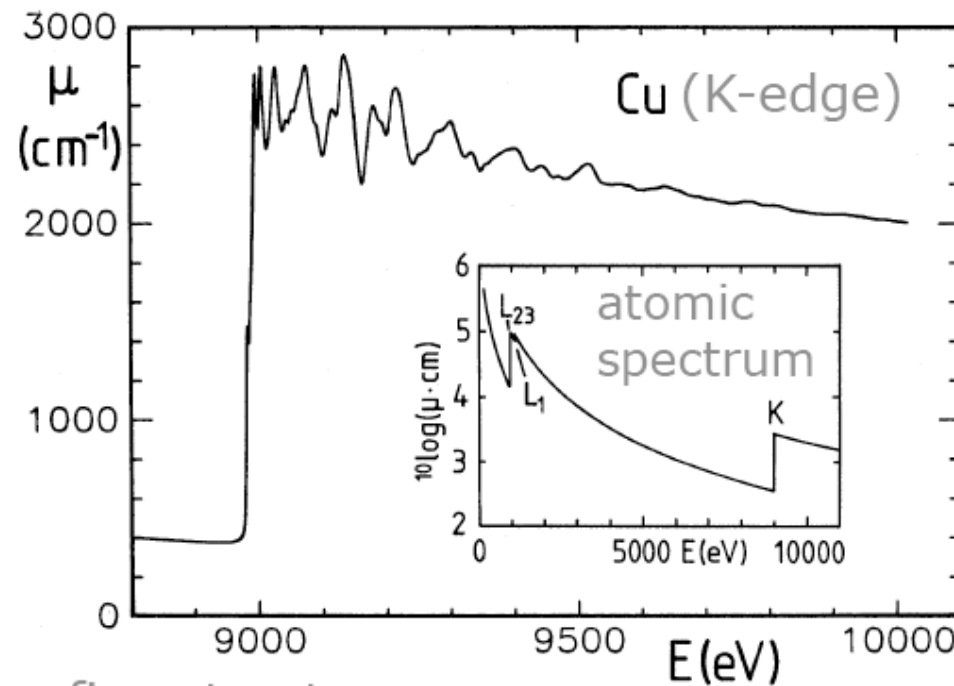
- ✓ Concentrations and volume requirements depend on energy, flux, beamline characteristics
 - 1 mM (typical) - 50 μ M (possible)
 - Small volume (10 μ L with focused beam)

The experimental XAS spectrum

Example: Absorption of Cu

$\mu(E)$: linear absorption coefficient

metallic
copper:



In condensed
matter: absorption fine structure

X-ray Absorption Fine Structure

X-RAY & ABSORPTION EDGE ENERGIES (keV)

| Element | K_{abs} | $K\alpha_1$ | $K\beta_1$ | $L\text{-III}_{\text{abs}}$ | $L\alpha_1$ | $L\beta_1$ | $M\text{-V}_{\text{abs}}$ | $M\alpha_1$ | $M\beta$ |
|--------------|------------------|-------------|------------|-----------------------------|-------------|------------|---------------------------|-------------|----------|
| 9 F | 0.687 | 0.677 | | | | | | | |
| 11 Na | 1.072 | 1.041 | 1.067 | | | | | | |
| 12 Mg | 1.305 | 1.253 | 1.295 | | | | | | |
| 13 Al | 1.559 | 1.486 | 1.553 | | | | | | |
| 14 Si | 1.838 | 1.740 | 1.829 | | | | | | |
| 15 P | 2.142 | 2.013 | 2.136 | | | | | | |
| 16 S | 2.472 | 2.307 | 2.464 | | | | | | |
| 17 Cl | 2.822 | 2.622 | | | | | | | |
| 18 Ar | 3.202 | 2.957 | 3.190 | | | | | | |
| 19 K | 3.607 | 3.313 | 3.589 | | | | | | |
| 20 Ca | 4.038 | 3.691 | 4.012 | 0.346 | 0.341 | 0.345 | | | |

X-RAY & ABSORPTION EDGE ENERGIES (keV)

| | | | | | | | | | |
|--------------|---------|--------|---------|--------|--------|--------|-------|-------|-------|
| 21 Sc | 4.496 | 4.090 | 4.460 | 0.403 | 0.395 | 0.400 | | | |
| 22 Ti | 4.965 | 4.510 | 4.931 | 0.454 | 0.452 | 0.458 | | | |
| 23 V | 5.465 | 4.951 | 5.426 | 0.513 | 0.511 | 0.519 | | | |
| 24 Cr | 5.989 | 5.414 | 5.946 | 0.574 | 0.573 | 0.583 | | | |
| 25 Mn | 6.540 | 5.898 | 6.489 | 0.641 | 0.637 | 0.649 | | | |
| 26 Fe | 7.112 | 6.403 | 7.057 | 0.709 | 0.705 | 0.718 | | | |
| 27 Co | 7.709 | 6.929 | 7.648 | 0.779 | 0.776 | 0.791 | | | |
| 28 Ni | 8.333 | 7.477 | 8.263 | 0.855 | 0.851 | 0.869 | | | |
| 29 Cu | 8.979 | 8.046 | 8.904 | 0.932 | 0.930 | 0.950 | | | |
| 30 Zn | 9.659 | 8.637 | 9.570 | 1.021 | 1.012 | 1.034 | | | |
| 38 Sr | 16.105 | 14.163 | 15.833 | 1.940 | 1.806 | 1.871 | | | |
| 40 Zr | 17.998 | 15.772 | 17.665 | 2.223 | 2.042 | 2.124 | | | |
| 56 Ba | 37.441 | 32.188 | 36.372 | 5.247 | 4.465 | 4.827 | | | |
| 57 La | 38.925 | 33.436 | 37.795 | 5.483 | 4.650 | 5.041 | | | 0.854 |
| 58 Ce | 40.449 | 34.714 | 39.251 | 5.724 | 4.839 | 5.261 | | | 0.902 |
| 60 Nd | 43.571 | 37.355 | 42.264 | 6.208 | 5.229 | 5.721 | | | 0.996 |
| 72 Hf | 65.351 | 55.781 | 63.222 | 9.561 | 7.898 | 9.021 | | | 1.697 |
| 82 Pb | 88.006 | 74.965 | 84.922 | 13.035 | 10.550 | 12.612 | 2.484 | 2.345 | 2.442 |
| 90 Th | 109.646 | 93.334 | 105.591 | 16.300 | 12.967 | 16.199 | 3.332 | 2.996 | 3.145 |
| 92 U | 115.036 | 98.422 | 111.281 | 17.167 | 13.612 | 17.217 | 3.552 | 3.170 | 3.336 |

The experimental XAS spectrum



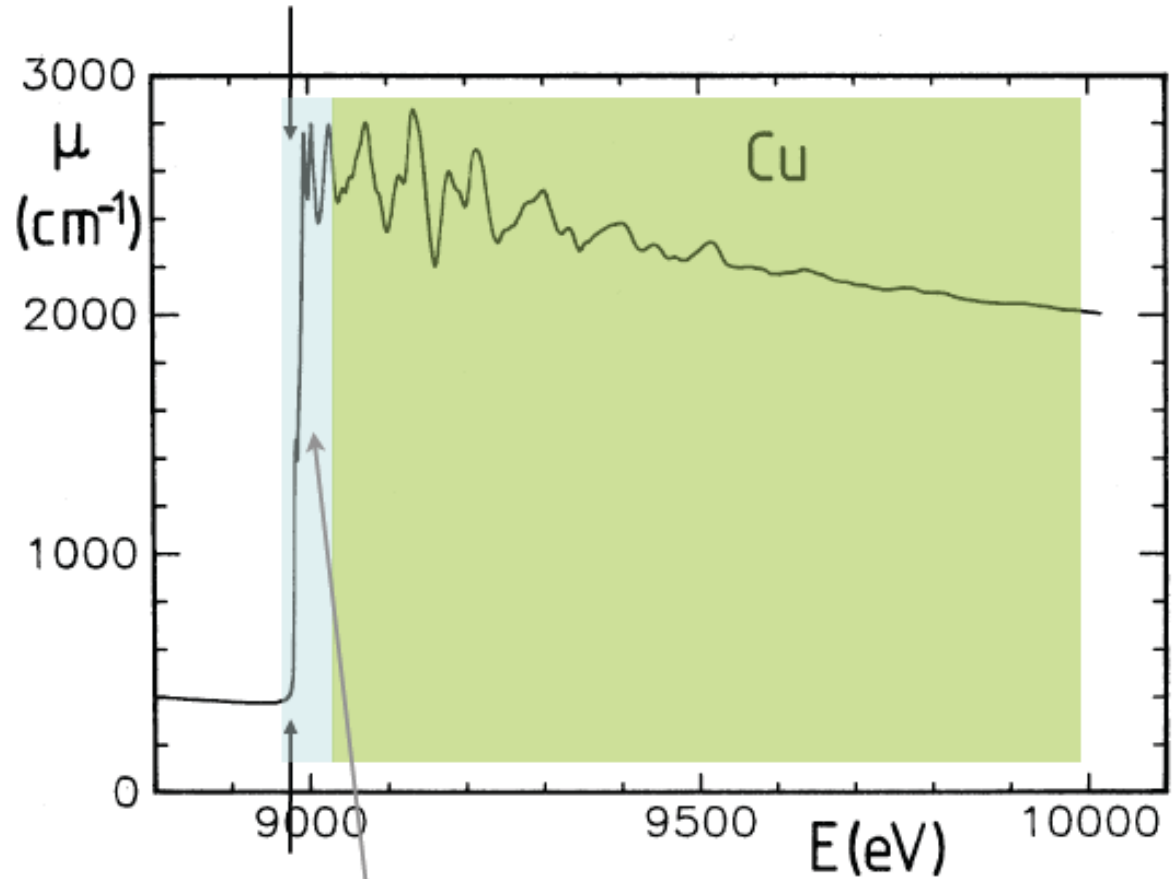
The experimental XAS spectrum



The experimental XAS spectrum

X-Ray Absorption Spectrum

- edge position:
oxidation state
- near edge spectrum:
local free projected density of states
- extended fine structure:
local atomic neighborhood



XANES

X-ray Absorption Near Edge Structure

$$k = 0.26245 (E - E_0)$$

The EXAFS formula

Elementary EXAFS formula (Lytle, Sayers, Stern)

$$\chi(k) = \sum_j \frac{N_j S_0^2}{k r_j^2} F_j(k) D_j(k) \exp(-2\sigma_j^2 k^2) \sin(2kr_j + \Phi_j(k))$$

interference:

$$\sin(2kr_j) = \sin\left(\frac{2\pi}{\lambda} 2r_j\right)$$

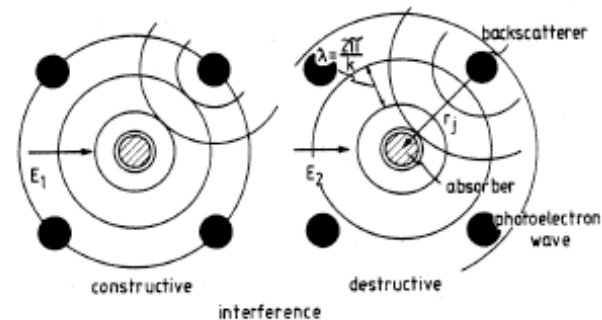
r_j : distance to neighboring shell j

$F_j(k)$: backscattering amplitude

$\Phi_j(k)$: scattering phases in absorber and backscatterer

N_j : number of neighbors in shell j .

S_0^2 : amplitude reduction factor due to intrinsic inelastic effects within the absorbing atom (0.7 – 0.9)



The EXAFS formula

$$\chi(k) = \sum_j \frac{N_j S_0^2}{k r_j^2} F_j(k) D_j(k) \exp(-2\sigma_j^2 k^2) \sin(2k r_j + \Phi_j(k))$$

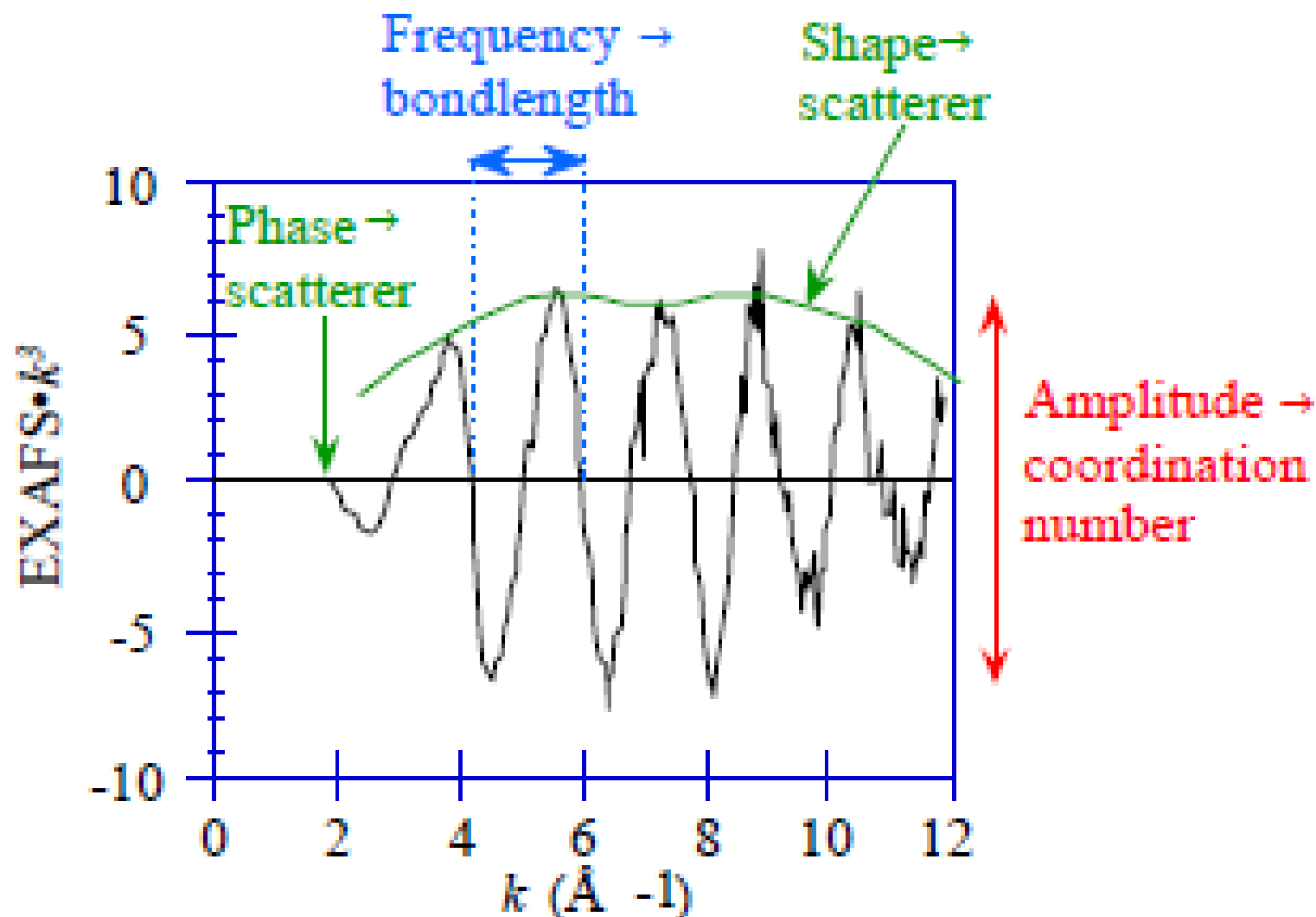
$$D_j = \exp\left(-\frac{2r_j}{\Lambda(k)}\right)$$

$$\Lambda^{-1} \cong \sqrt{3} \frac{a_0 R}{E_{kin}} r_s^{3/2} \ln \left[\left(\frac{4}{9\pi} \right)^{3/2} \frac{E_{kin}}{R} r_s^2 \right]$$

Essential structural information from EXAFS

| Observable | Information | Accuracy |
|---------------------------|--|---|
| Frequency of oscillations | distance | $0.005 - 0.02 \text{ \AA}$ |
| Phase shift | Type of atoms in shell | $Z \pm 2$ ($Z = 6 - 17$) $Z \pm 3$ ($Z = 20 - 35$) |
| Amplitude of oscillations | Type of atoms in shell Number of atoms in shell (coordination number) | $N \pm 5 - 25 \%$ Depending on the system and data quality |

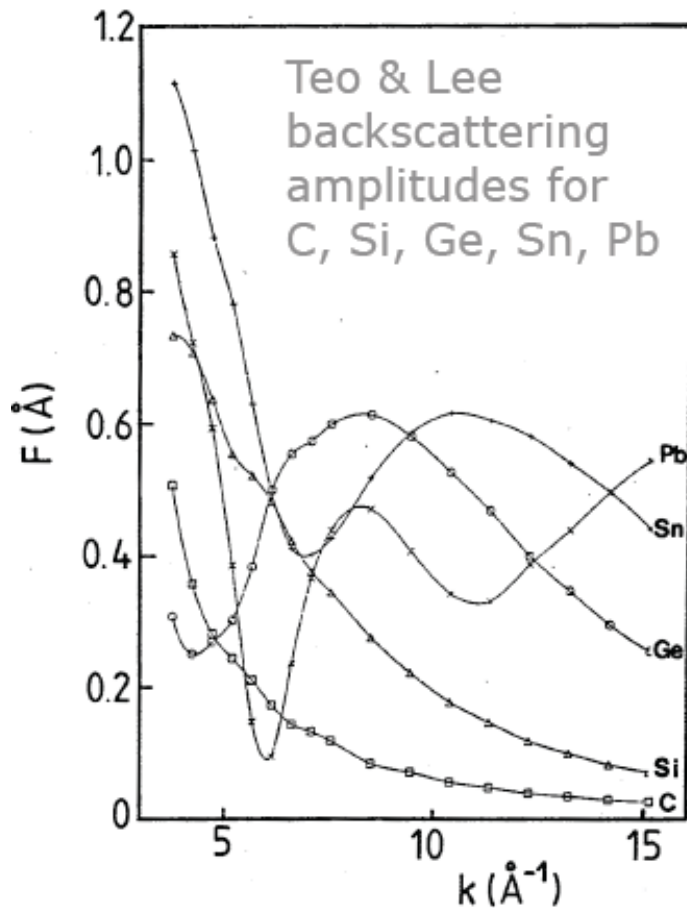
The EXAFS signal



The EXAFS formula

$$\chi(k) = \sum_j \frac{N_j S_0^2}{k r_j^2} F_j(k) D_j(k) \exp(-2\sigma_j^2 k^2) \sin(2k r_j + \Phi_j(k))$$

Backscattering Amplitudes (and Phases)



photoelectron is scattered in absorber and backscatterer:

k -dependent

● backscattering amplitude $F(k)$

● phases $\Phi(k) = \Phi_a(k) + \Phi_b(k)$

● $F(k)$ decreases with increasing k .

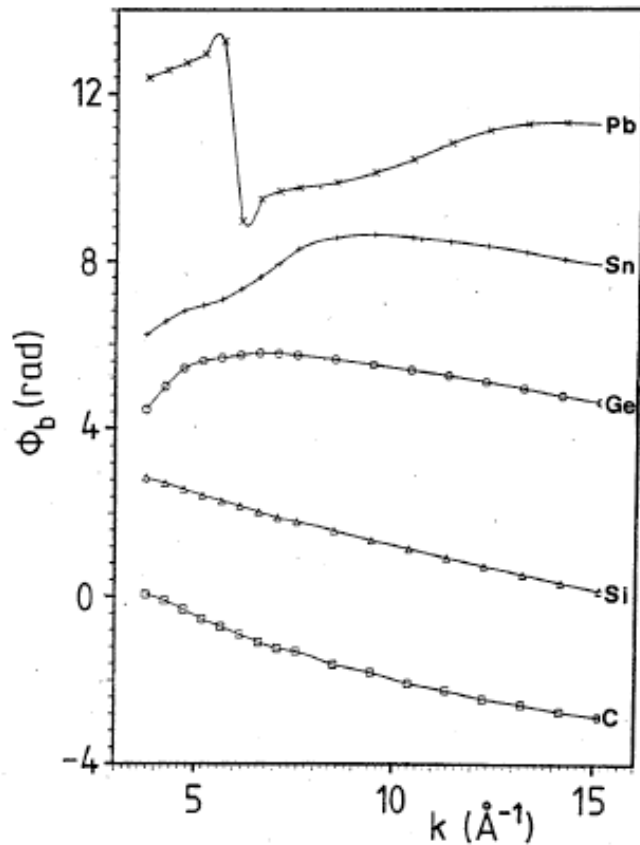
● superposed may be 1, 2 or 3 resonances:

Ramsauer-Townsend-resonances

(Backscatterer becomes transparent for certain energies of the photoelectron!)

Scattering Phases

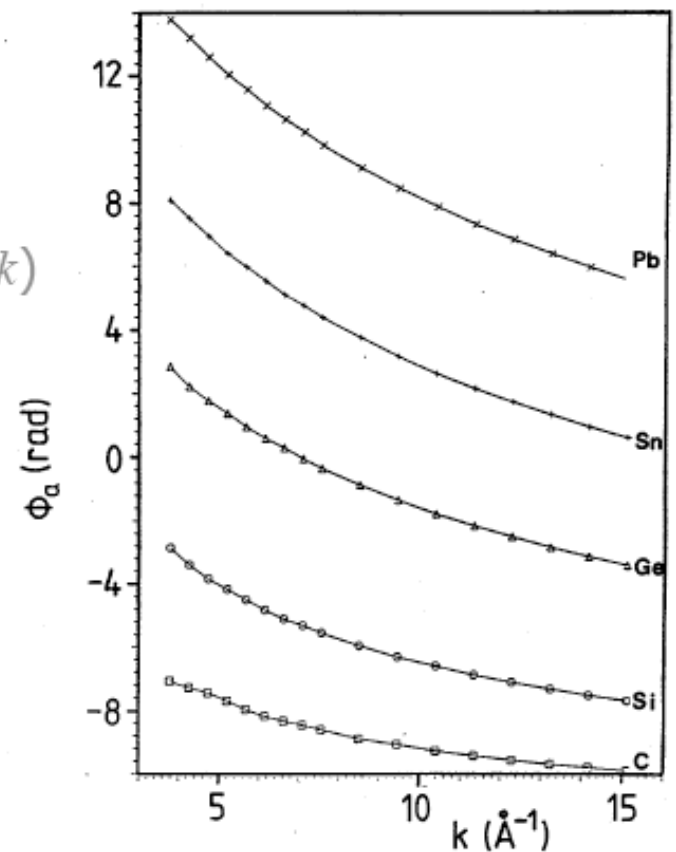
backscatterer



with Ramsauer-Townsend-Osc.

$$\Phi(k) = \Phi_b(k) + \Phi_a(k)$$

absorber



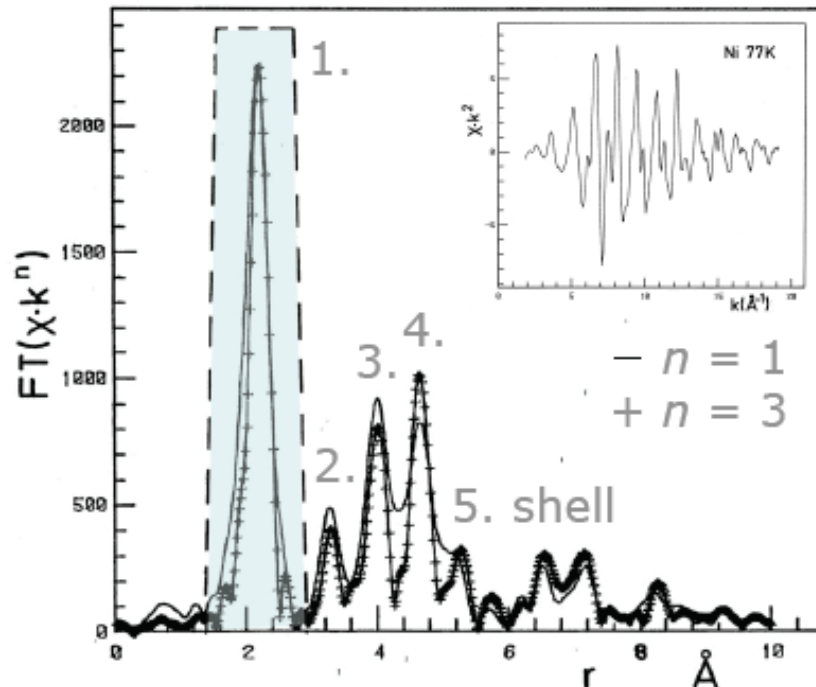
monotone in k

Fourier analysis

Identify Different Shells: Fourier Transform of $\chi(k) \cdot k^n$

$$\chi(k) = \sum_j \frac{N_j S_0^2}{k r_j^2} F_j(k) D_j(k) \exp(-2\sigma_j^2 k^2) \sin(2k r_j + \Phi_j(k))$$

Multiplication with k^n



$n = 0, 1$: short data set
 $n = 2, 3$: long data set
 (with low noise)

$n = 3$: enhances high k data,
 makes data set effectively
 longer and separates shells
 better in Fourier transform

Fourier analysis

Isolate Shell

For given shell j fit

$$\chi_j(k) = \frac{N_j S_0^2}{k r_j^2} F_j(k) D_j(k) \exp(-2\sigma_j^2 k^2) \sin(2k r_j + \Phi_j(k))$$

Example:

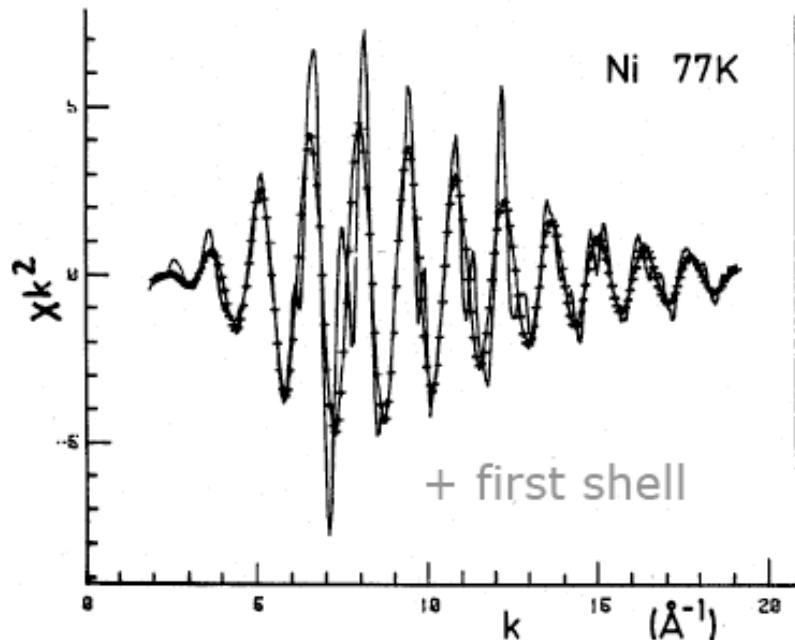
determine $\Phi_1(k)$ from model
substance

oscillations:

$$2k r_1 + \Phi_1(k)$$

with known $r_1 = 2.486 \text{\AA}$

$$\longrightarrow \Phi_1(k)$$



Fourier analysis

Isolate Shell

For given shell j fit

$$\chi_j(k) = \frac{N_j S_0^2}{k r_j^2} F_j(k) D_j(k) \exp(-2\sigma_j^2 k^2) \sin(2kr_j + \Phi_j(k))$$

Example:

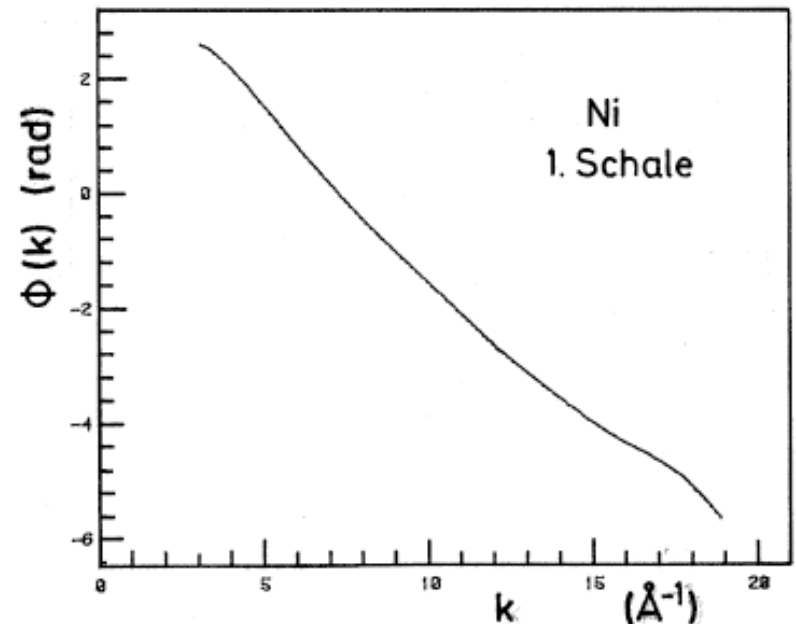
determine $\Phi_1(k)$ from model
substance

oscillations:

$$2kr_1 + \Phi_1(k)$$

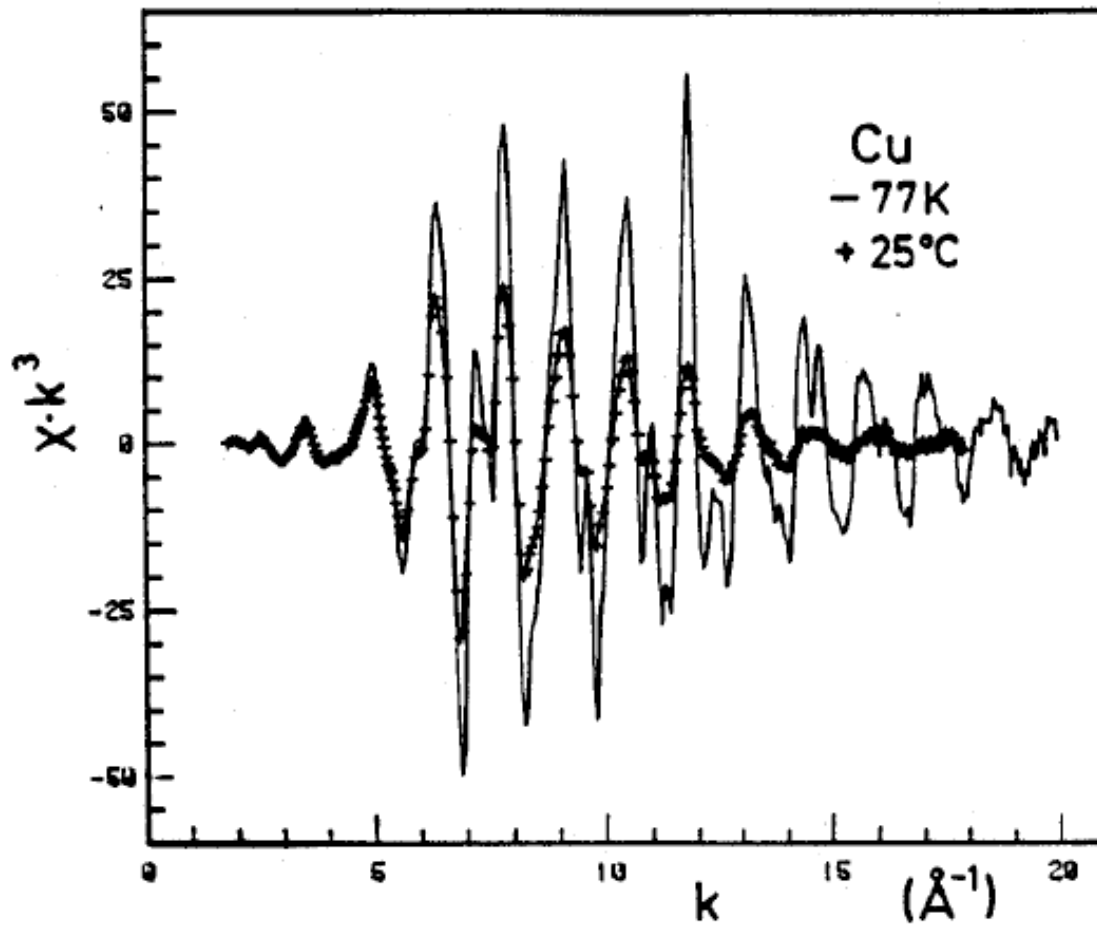
with known $r_1 = 2.486 \text{ \AA}$

$$\longrightarrow \Phi_1(k)$$



Fourier analysis

Example: Cu at Room Temp. Analysed with 77K Data



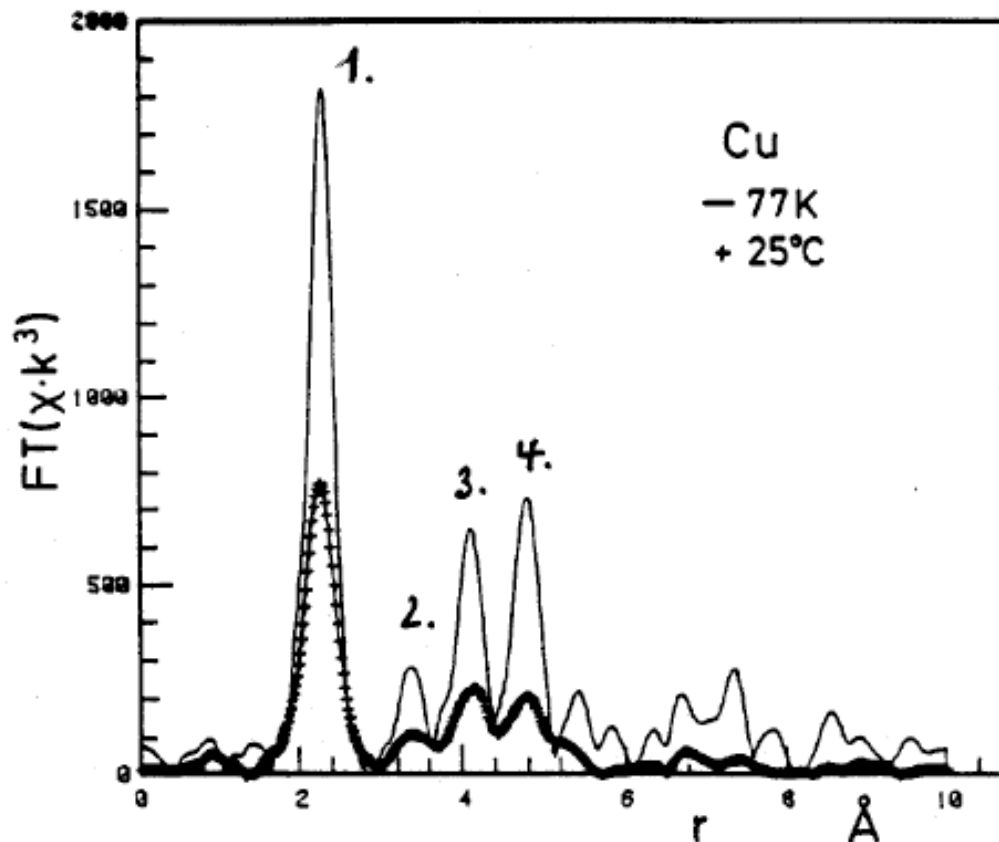
reference data for
 $\Phi(k)$ and $F(k)$:

copper at 77K

Fourier analysis

Example: Cu at Room Temp. Analysed with 77K Data

Fourier transform:



Main difference:

damped oscillations due
to thermal motion of
atoms



Fit of first shell at
RT with 77K data

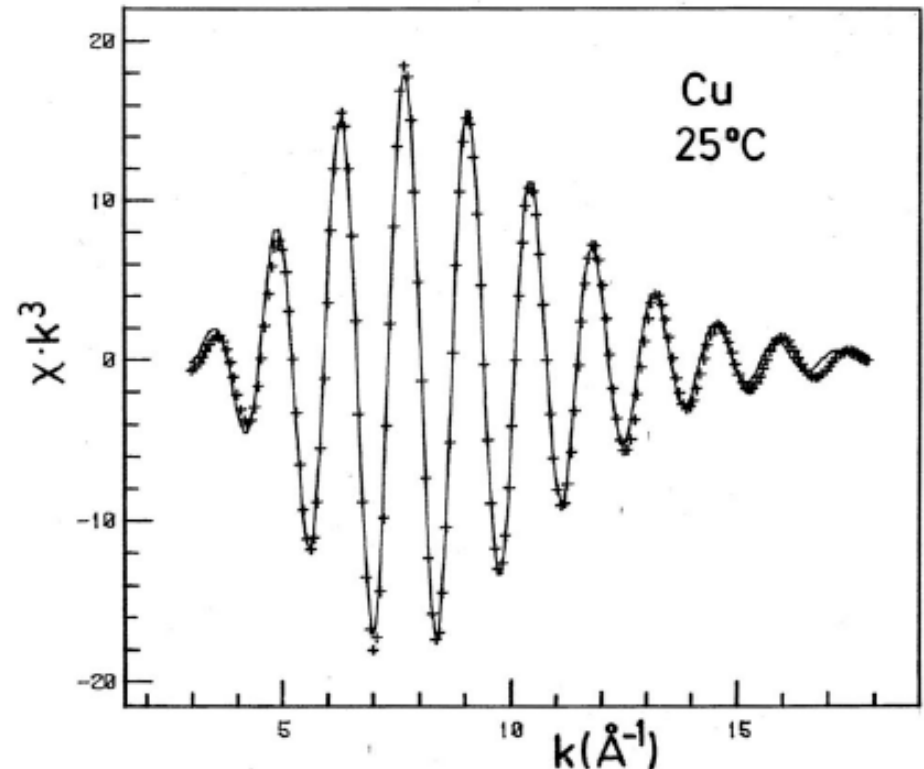
Fourier analysis

Example: Cu at Room Temp. Analysed with 77K Data

Input: F_f , Φ_j , D_j , r_j , N_j , σ_j , E_{0j}

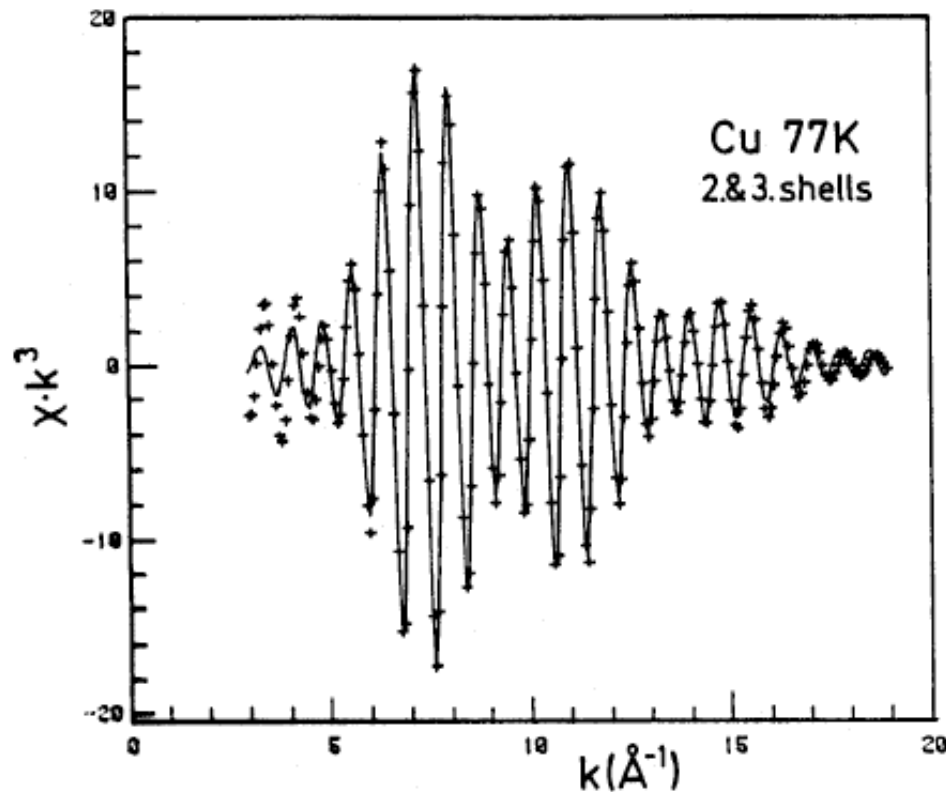
for Cu at 77K

| | fit | expected |
|--------------------|----------------------|----------|
| r_1 | 2.546Å | 2.556Å |
| N_1 | 12.06 | 12 |
| $\delta\sigma_1^2$ | 0.0044Å ² | |
| δE_{01} | -1.48eV | |



Fourier analysis

Example: Cu at Room Temp. Analysed with 77K Data



| | fit | expected |
|--------------------|----------------------|----------|
| r_2 | 3.609Å | 3.603Å |
| N_2 | 6.4 | 6 |
| $\delta\sigma_2^2$ | 0.0030Å ² | |
| δE_{02} | 2.57eV | |
| | fit | expected |
| r_3 | 4.409Å | 4.413Å |
| N_3 | 19.4 | 24 |
| $\delta\sigma_3^2$ | 0.0020Å ² | |
| δE_{03} | -4.61eV | |

inelastic scattering length: $\Lambda = 21\text{\AA}$

Accuracy

Accuracy of r , E_0 , N & σ^2

$$\chi_j(k) = \frac{N_j S_0^2}{k r_j^2} F_j(k) D_j(k) \exp(-2\sigma_j^2 k^2) \sin(2kr_j + \Phi_j(k))$$

r_j has little correlation with other parameters:

$$\chi(k) \propto \sin(2r_j k + \Phi(k))$$

$$k = \frac{1}{\hbar} \sqrt{2m(E - E_0)}$$

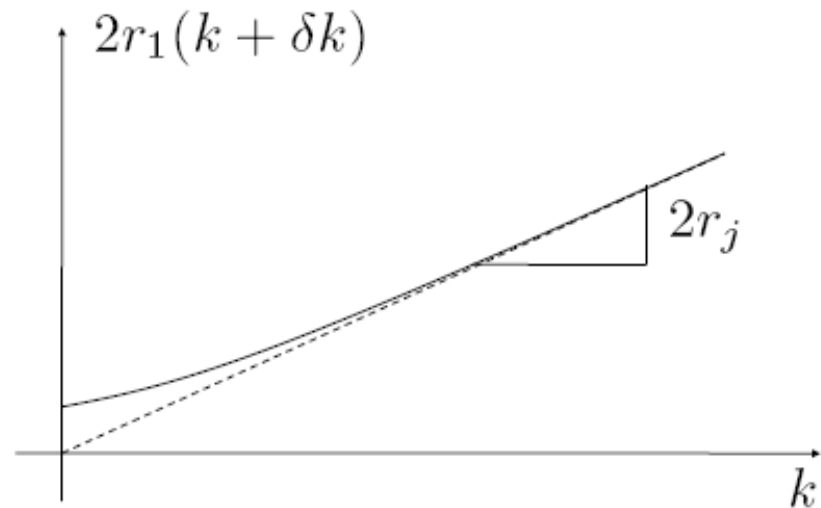
High accuracy r_j : 0.01Å and less

However:

N and σ are 100% correlated!

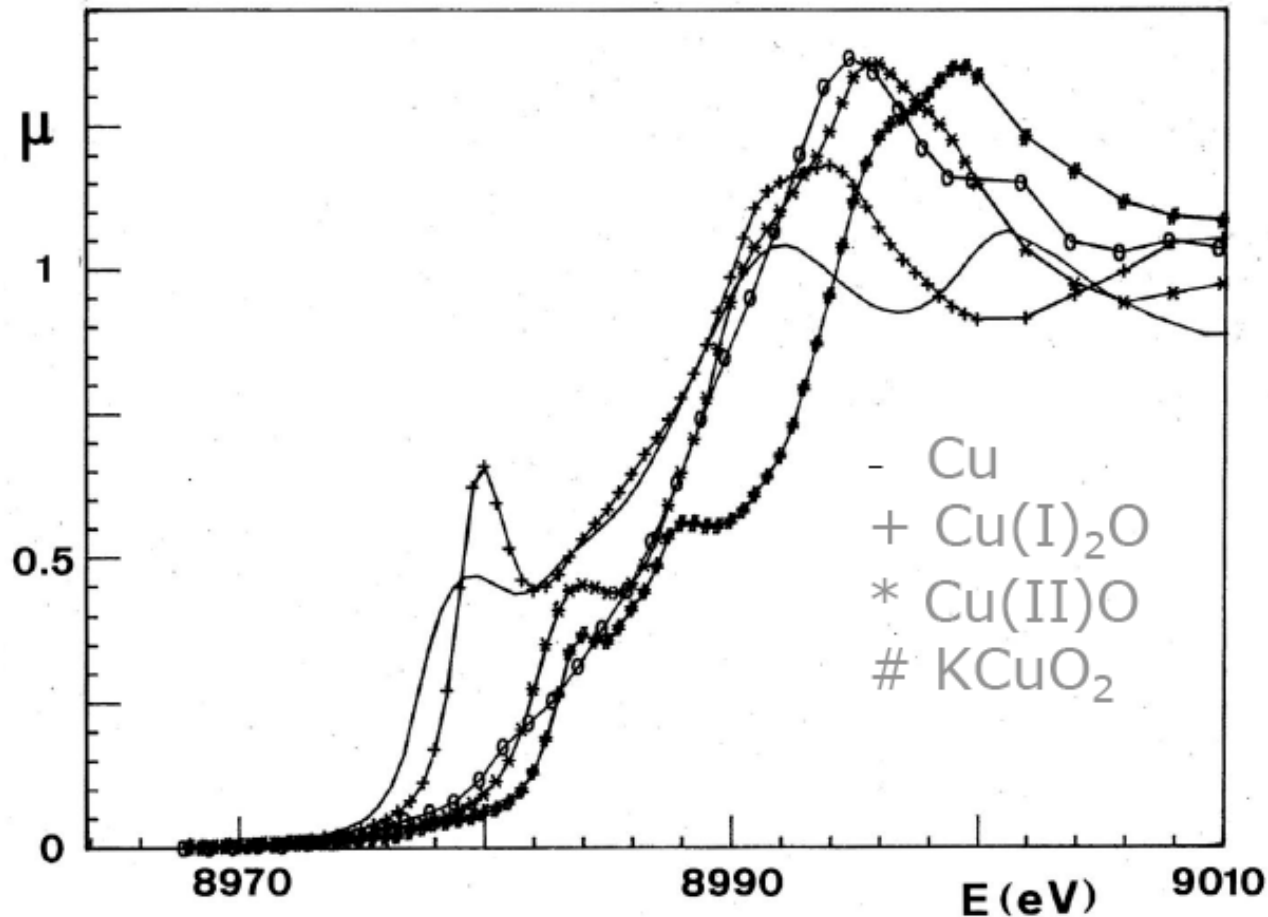
→ errors are compensated

→ accuracy: 5 – 25 %



The X-ray Absorption Edge

Position of Absorption Edge



Edge is shifted toward higher energies with increasing formal valence

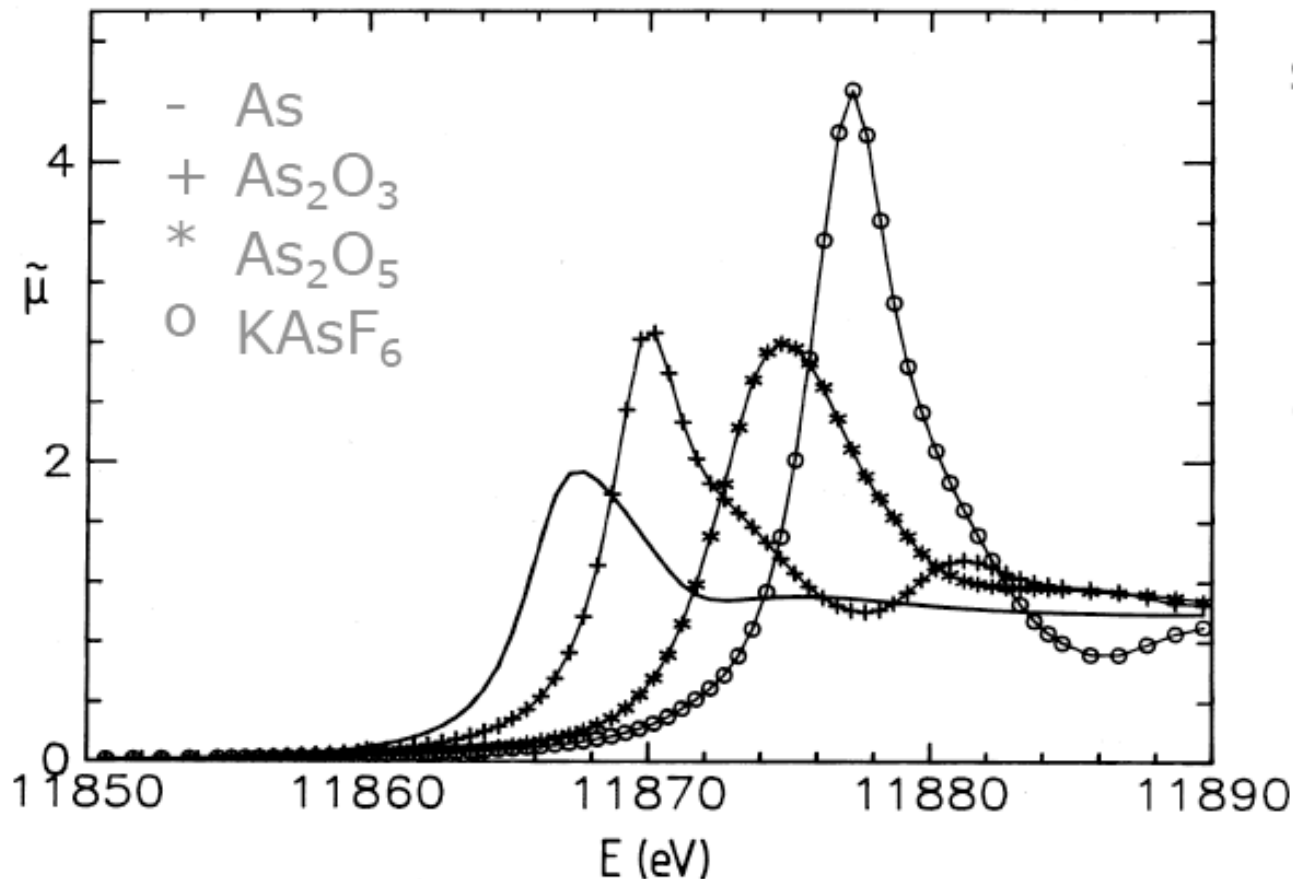
Screening of nucleus by valence electrons decreases:

Core electrons are more tightly bound!

The X-ray Absorption Edge

Position of Absorption Edge

Shift increases with electronegativity of ligands



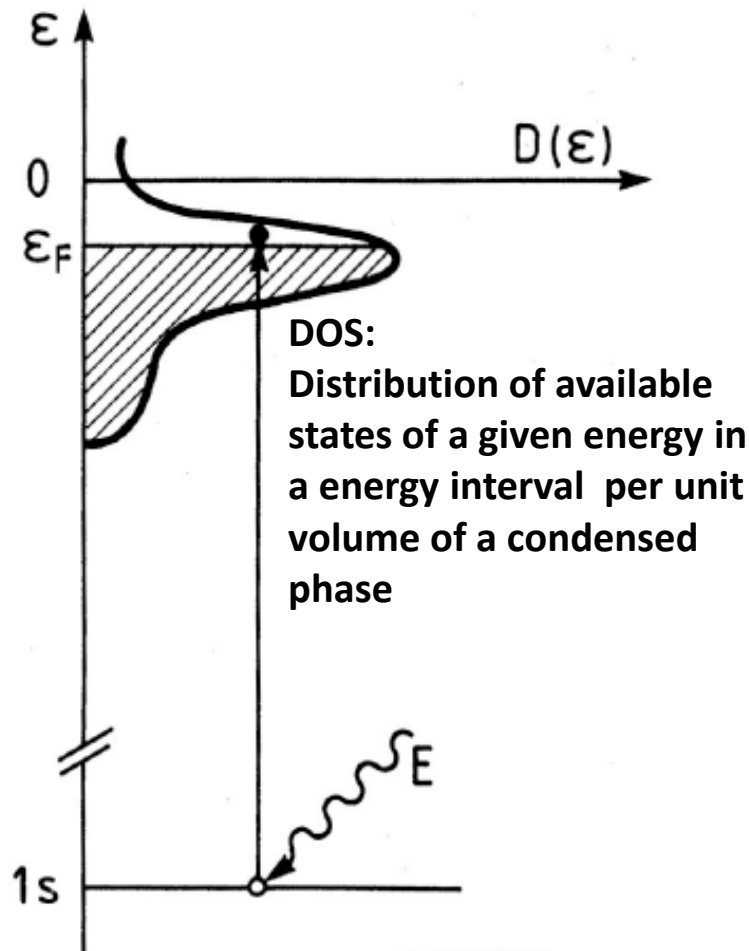
strongest for:

oxides and
fluorides

(up to 13eV)

The X-ray Absorption Edge

Shape of Absorption Edge



local projected density of empty states:

μ proportional to transition rate:

$$R_{fi} = \frac{2\pi}{\hbar} \sum_f |\langle f|H_1|i\rangle|^2 \delta(E_f - E_i - \hbar\omega)$$

matrix element $M(E) = \langle f|H_1|i\rangle$ only weakly energy dependent:

$$\mu \propto M(E) \cdot \rho(E)$$

density $\rho(E)$ of free final states determines edge structure.

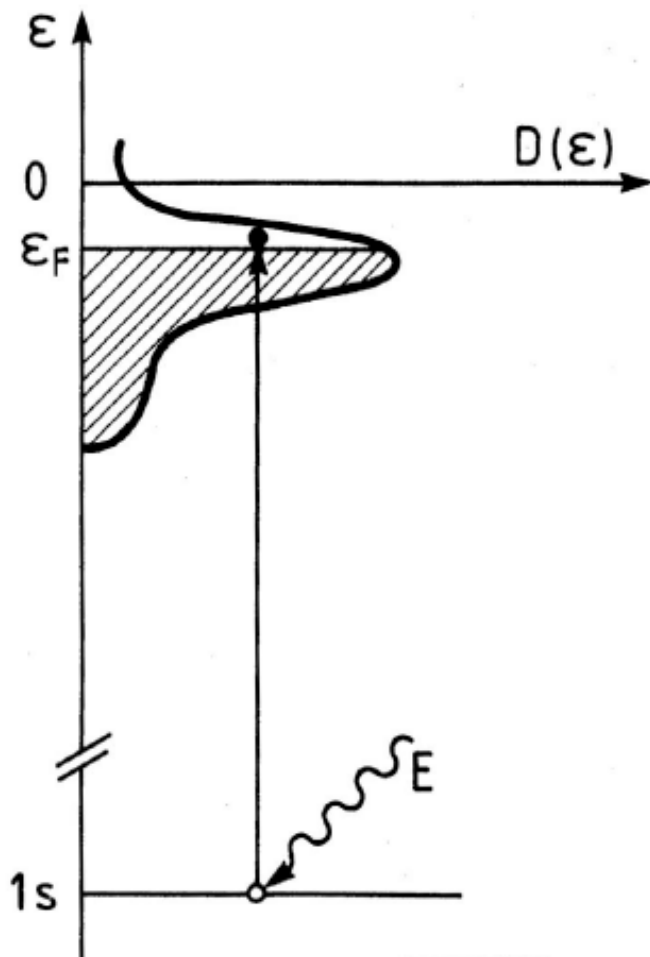
local: at atom of interest

free: Pauli-principle

projected: selection rule $\Delta L = \pm 1$

The X-ray Absorption Edge

Shape of Absorption Edge



local projected density of empty states:

density $\rho(E)$ of free final states determines edge structure.

local: at atom of interest

free: Pauli-principle

projected: selection rule $\Delta L = \pm 1$

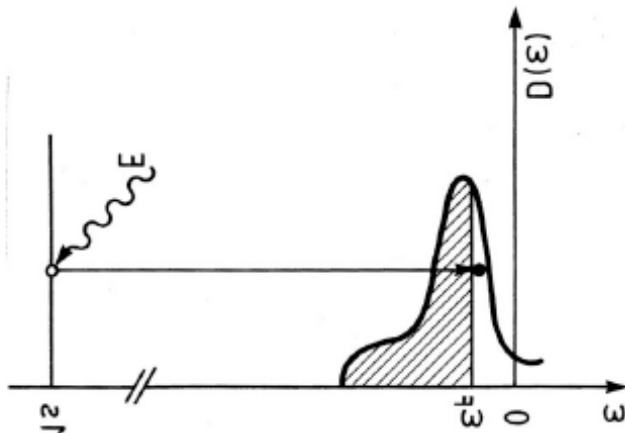
Angular momentum conservation:

K-, L_1 -shell: $s \rightarrow p$

L_2 -, L_3 -shell: $p \rightarrow d, (s)$

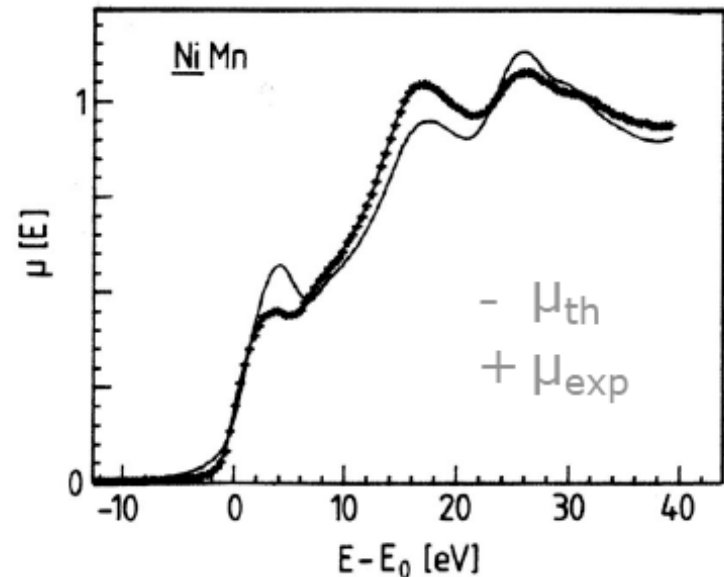
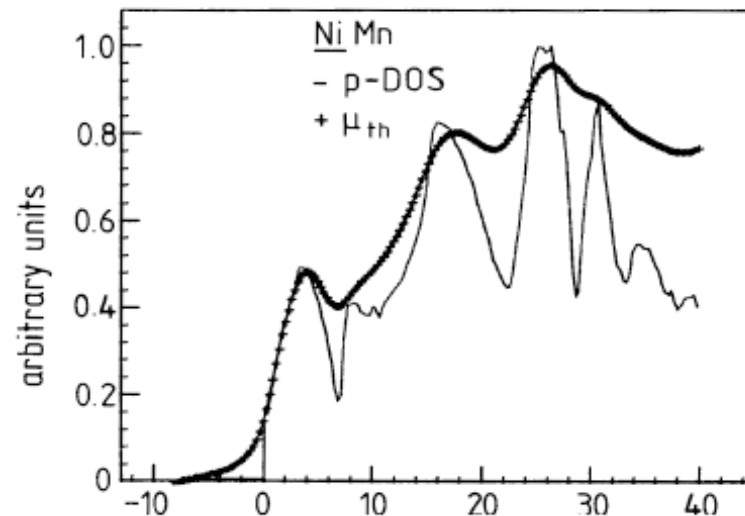
The X-ray Absorption Edge

Shape of Absorption Edge



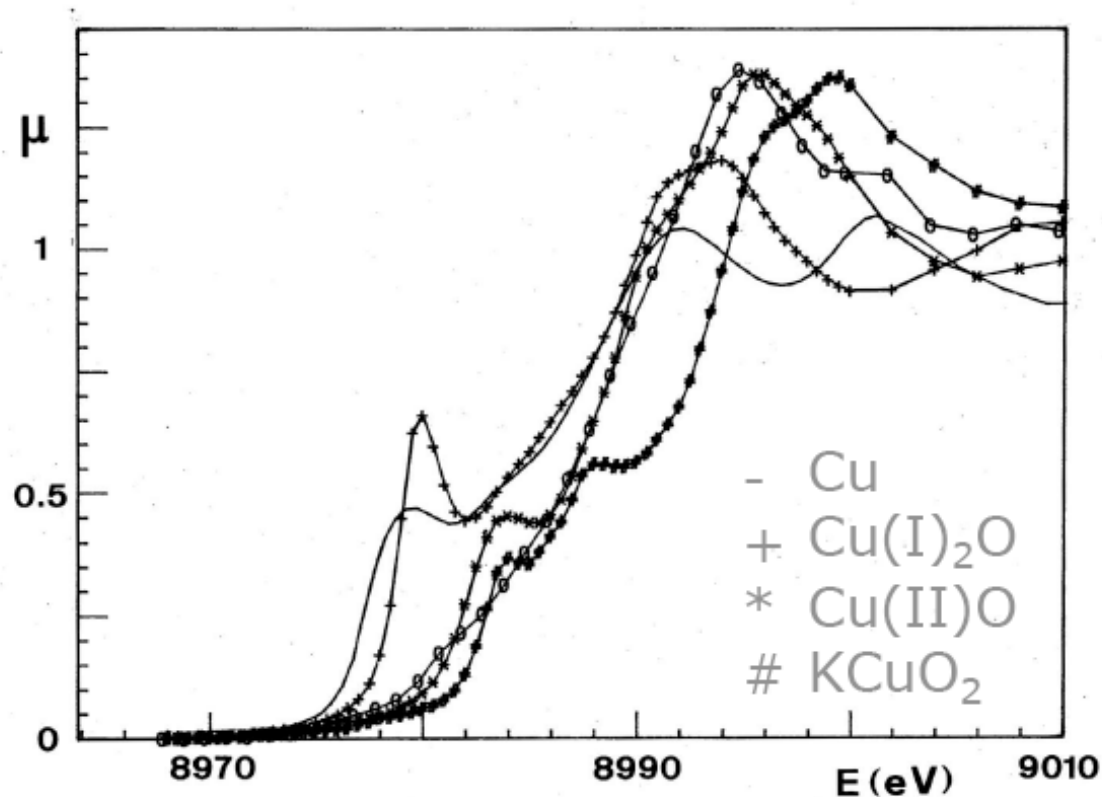
life time of excitation and
experimental energy
resolution:

→ edge smoothed



The X-ray Absorption Edge

Near Edge Spectrum



Finger print for
chemical state of
element of interest

Determine
concentration of
chemical compounds
in mixtures

Example:
inhomogeneous
specimens

X-ray Absorption Spectrum

Summary: X-Ray Absorption Spectrum

● edge position:

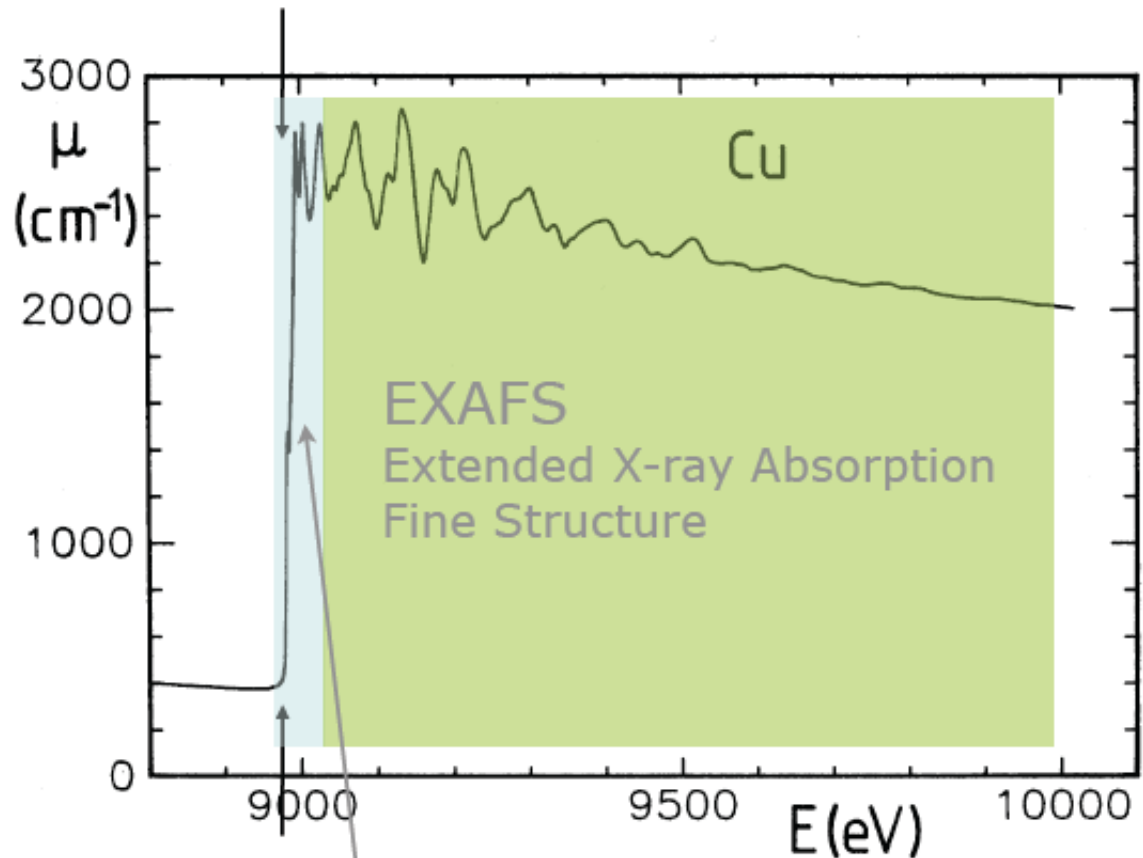
oxidation
state

● near edge
spectrum:

local free projected
density of states

● extended fine
structure:

local atomic
neighborhood



XANES

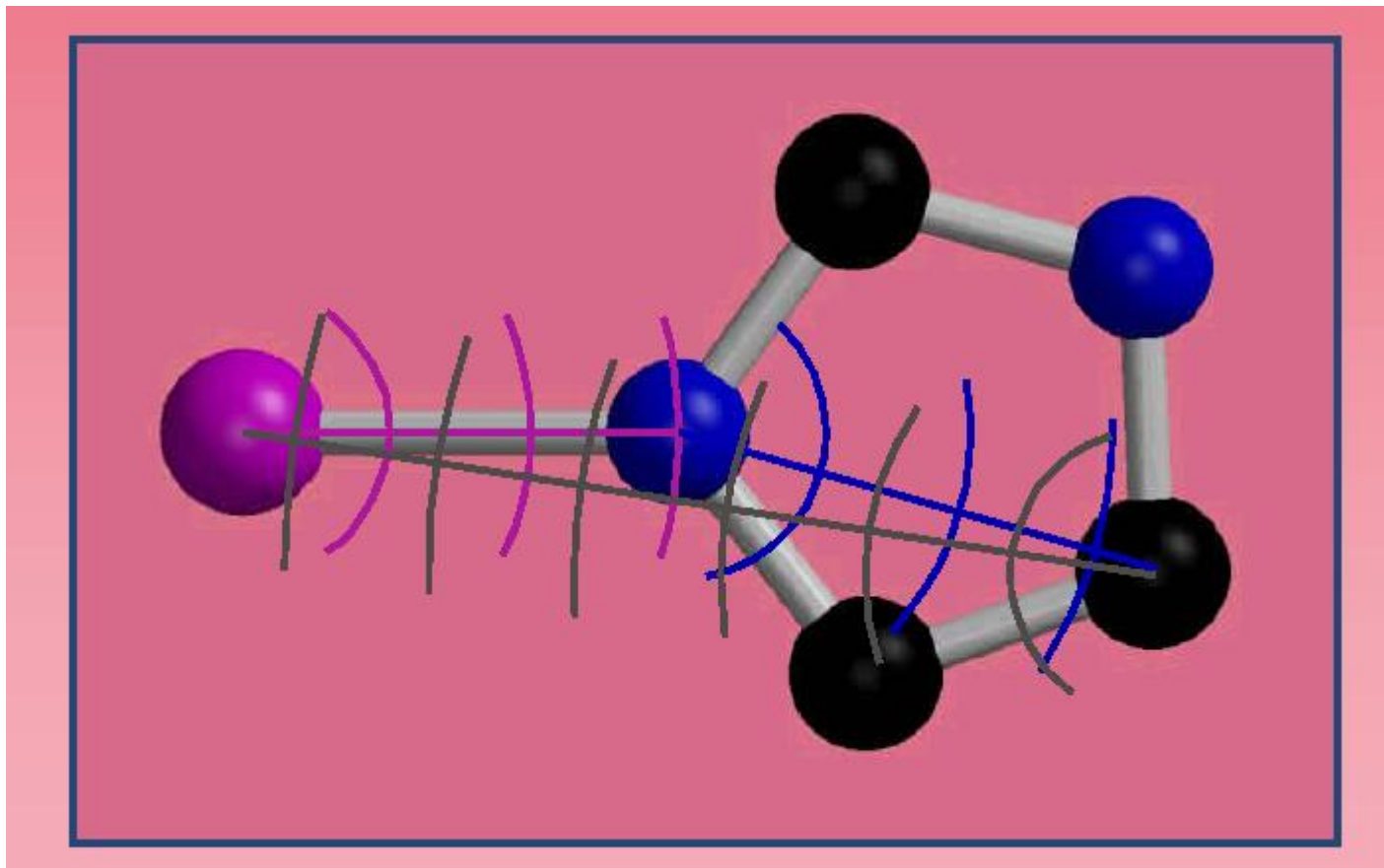
X-ray Absorption Near Edge Structure

EXAFS spectrum analysis

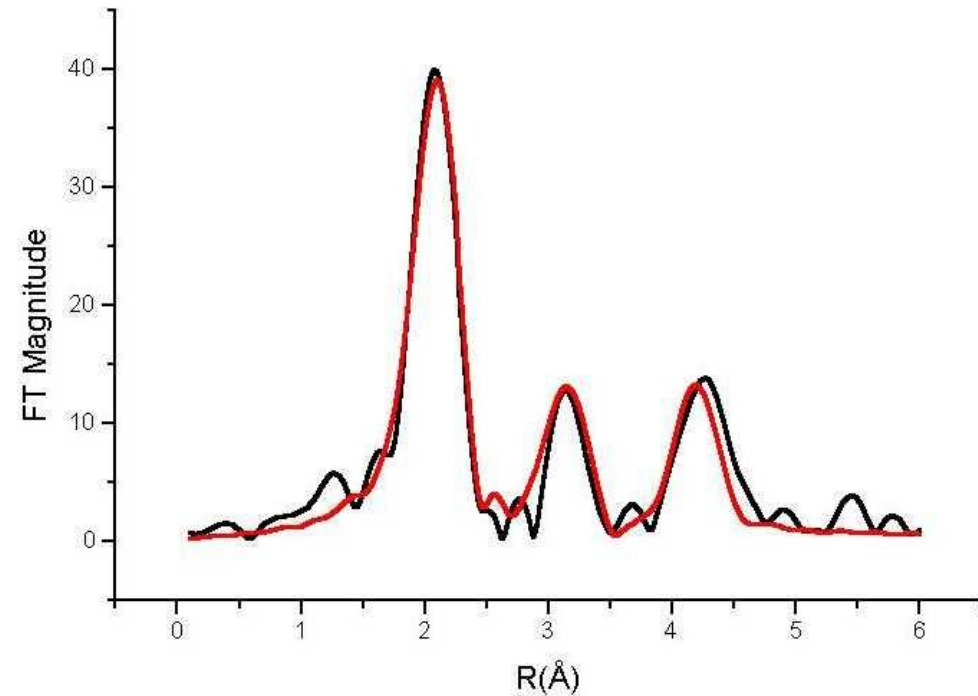
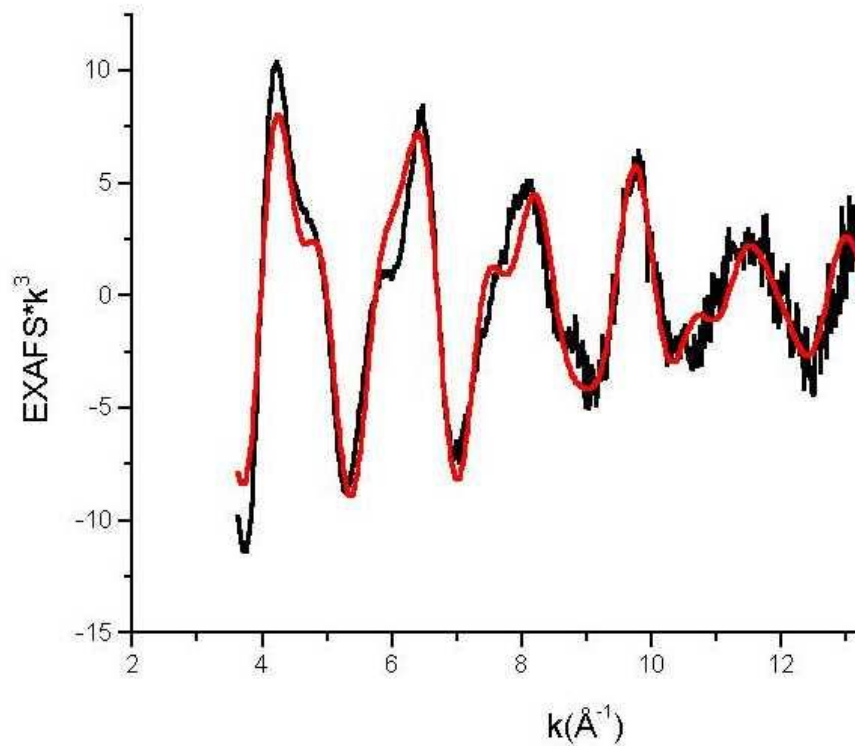
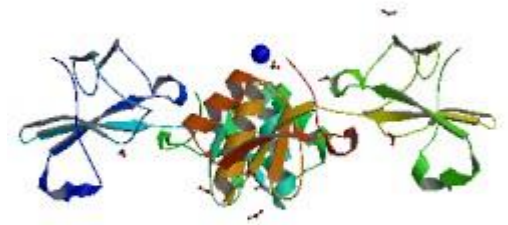
EXAFS Curve fitting

- Evaluate data quality (how many shells can be analysed?)
- Make hypothesis about the structure of atom shells around absorber
- Use theoretical amplitude and phase shift functions for each atom species
- Choose initial values for r_j , N_j , σ_j^2 (for each shell)
- Optimize fit of $\chi(k)$ by varying r_j , N_j , σ_j^2 , ΔE_0

BioXAS case studies



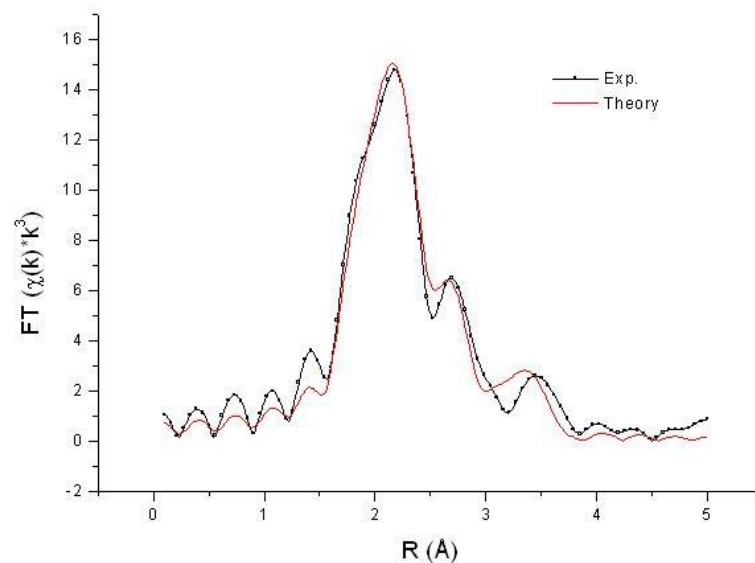
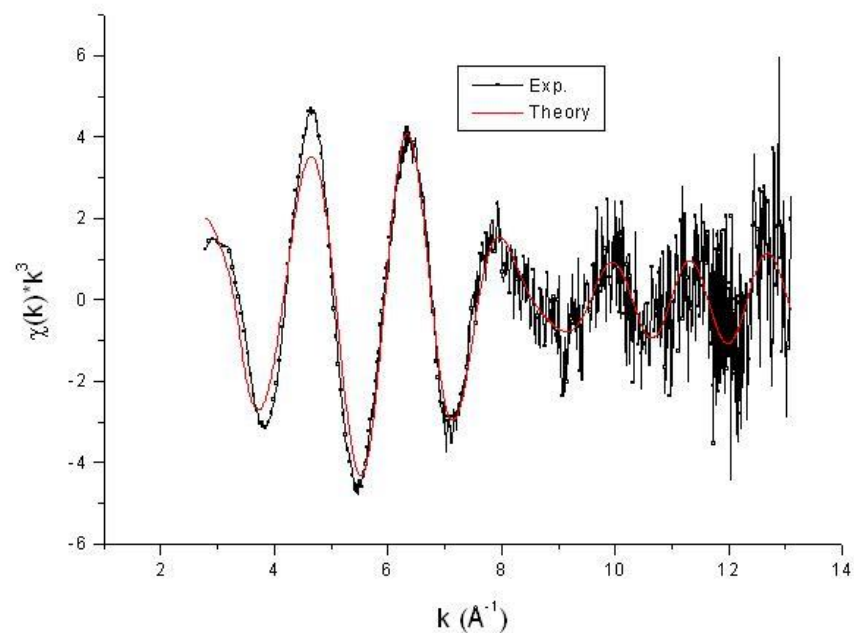
The case of Ni-UreE



Ni – UreE

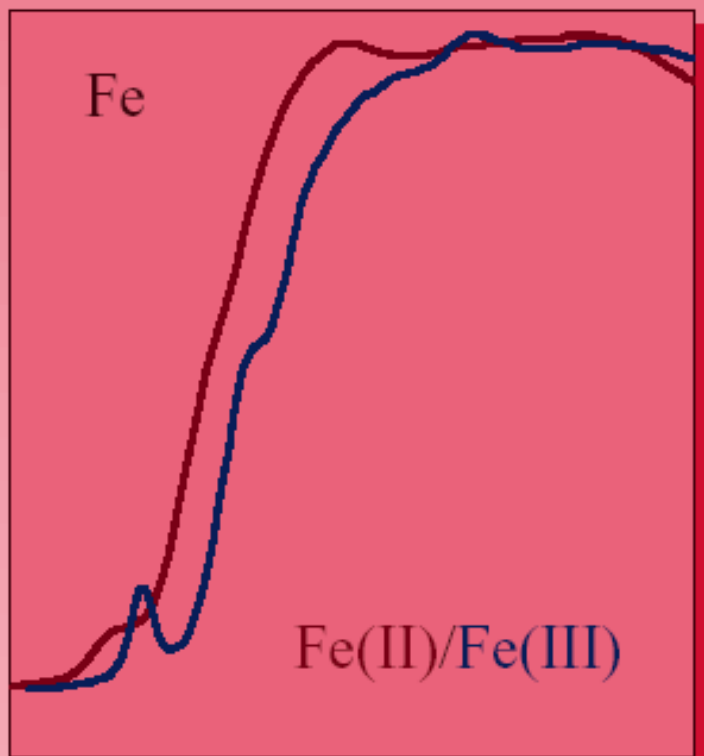
Biochemistry 2006, 45, 6495-6509

Close shells in Cu(I)-CopZ

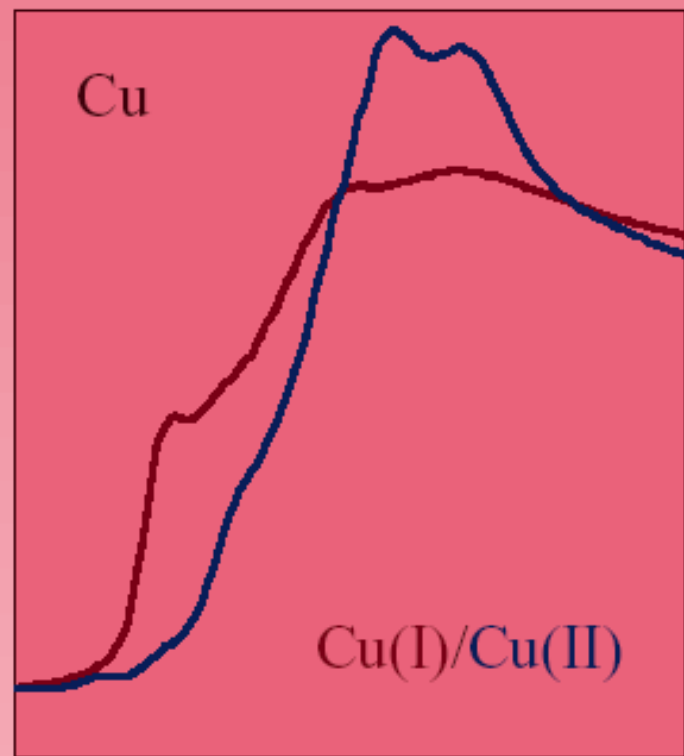


X-ray Absorption Edges in proteins

edge energy reflects redox state



Rubredoxin

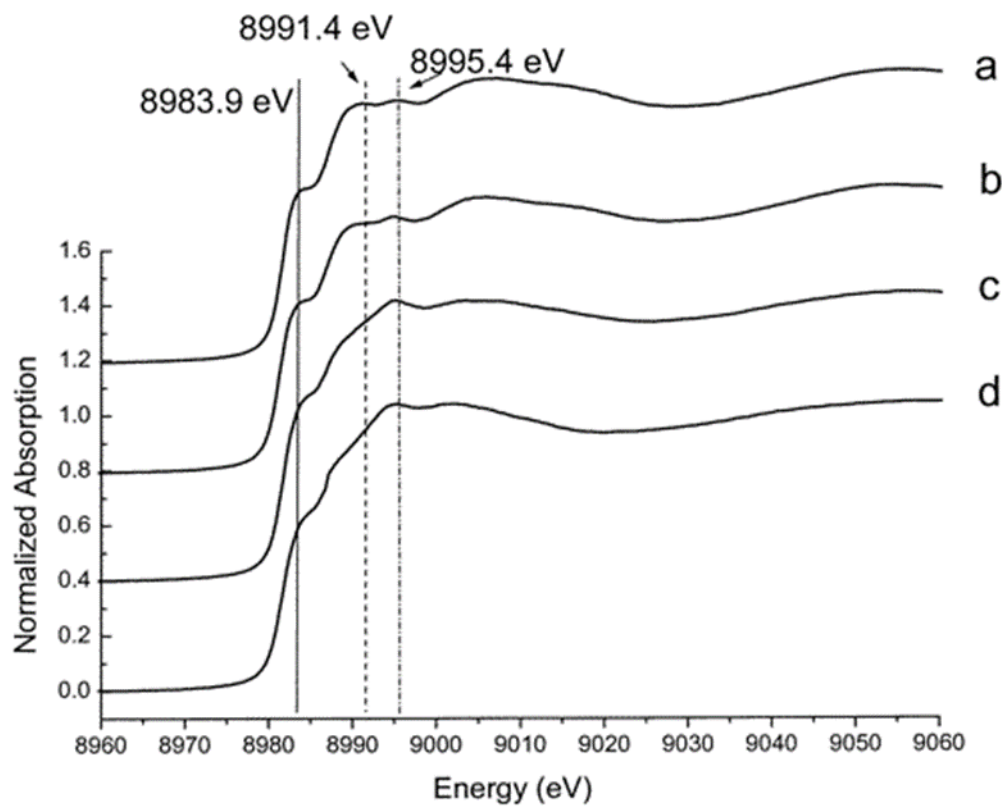


Amine Oxidase

X-ray Absorption Edges in proteins

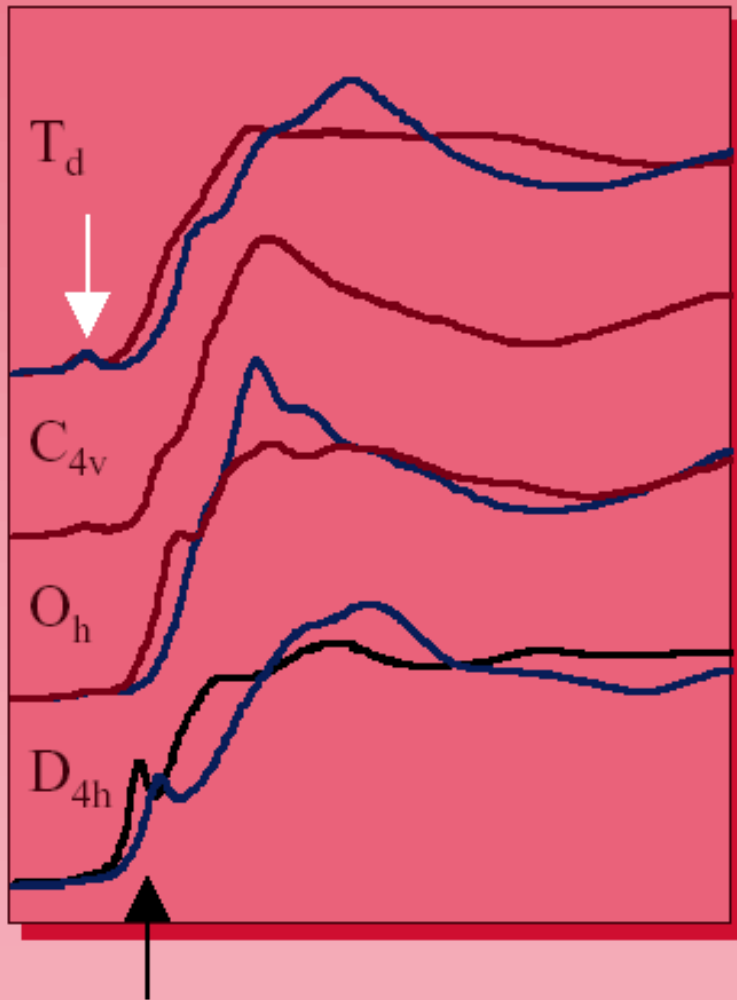
**Cu(I)-edges
In different
coordination
environments**

- a) DTT reduced 3mM Cu(I)**
- b) DTT reduced 1 mM Cu(I)**
- c) Dithionite reduced 3mM Cu(I)**
- d) Ascorbate reduced 3mM Cu(I)**



X-ray Absorption Edges in proteins

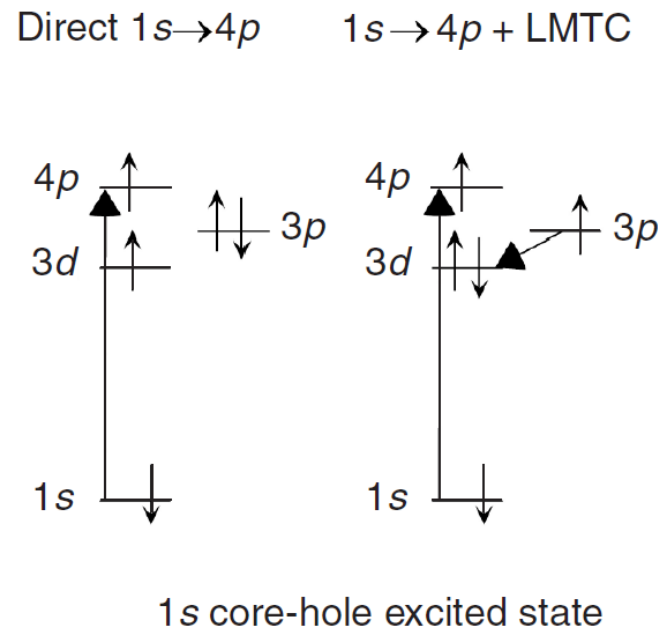
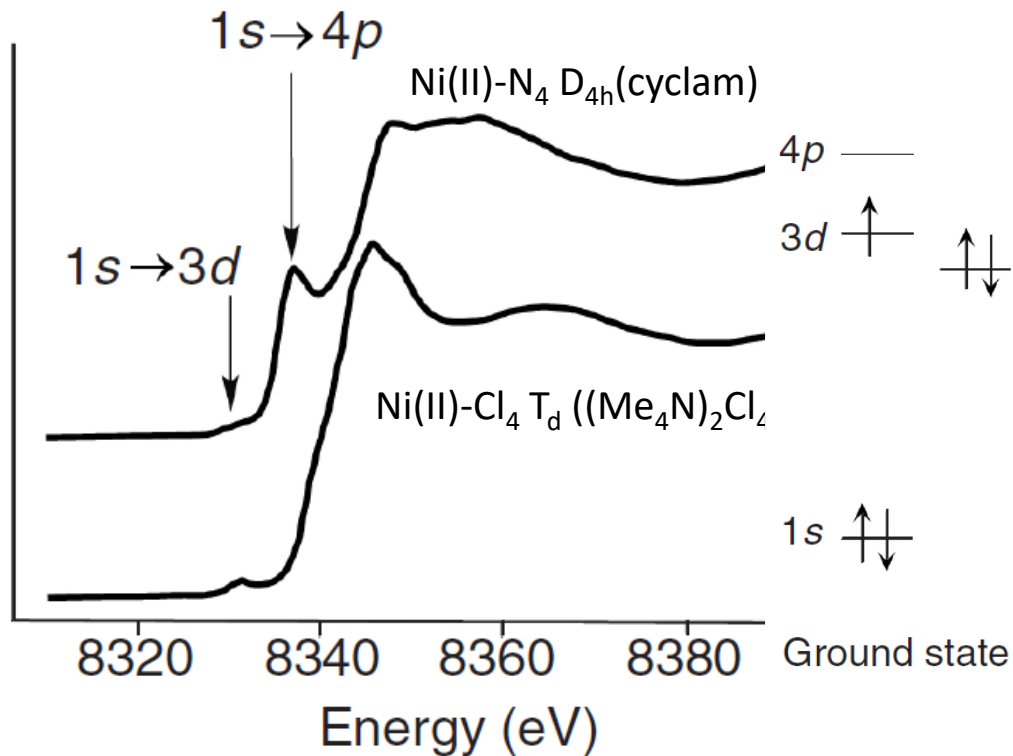
***pre-edge transitions reflect coordination symmetry
(model compounds)***



| | |
|----------|--------------------------------|
| T_d | NiN_4 NiS_4 |
| C_{4v} | NiN_4Br |
| O_h | NiN_6 NiS_6 |
| D_{4h} | NiN_4 NiS_4 $NiSe_4$ |

Support
coordination
number
determination
from EXAFS
analysis

X-ray Absorption Edges in proteins (and model compounds)

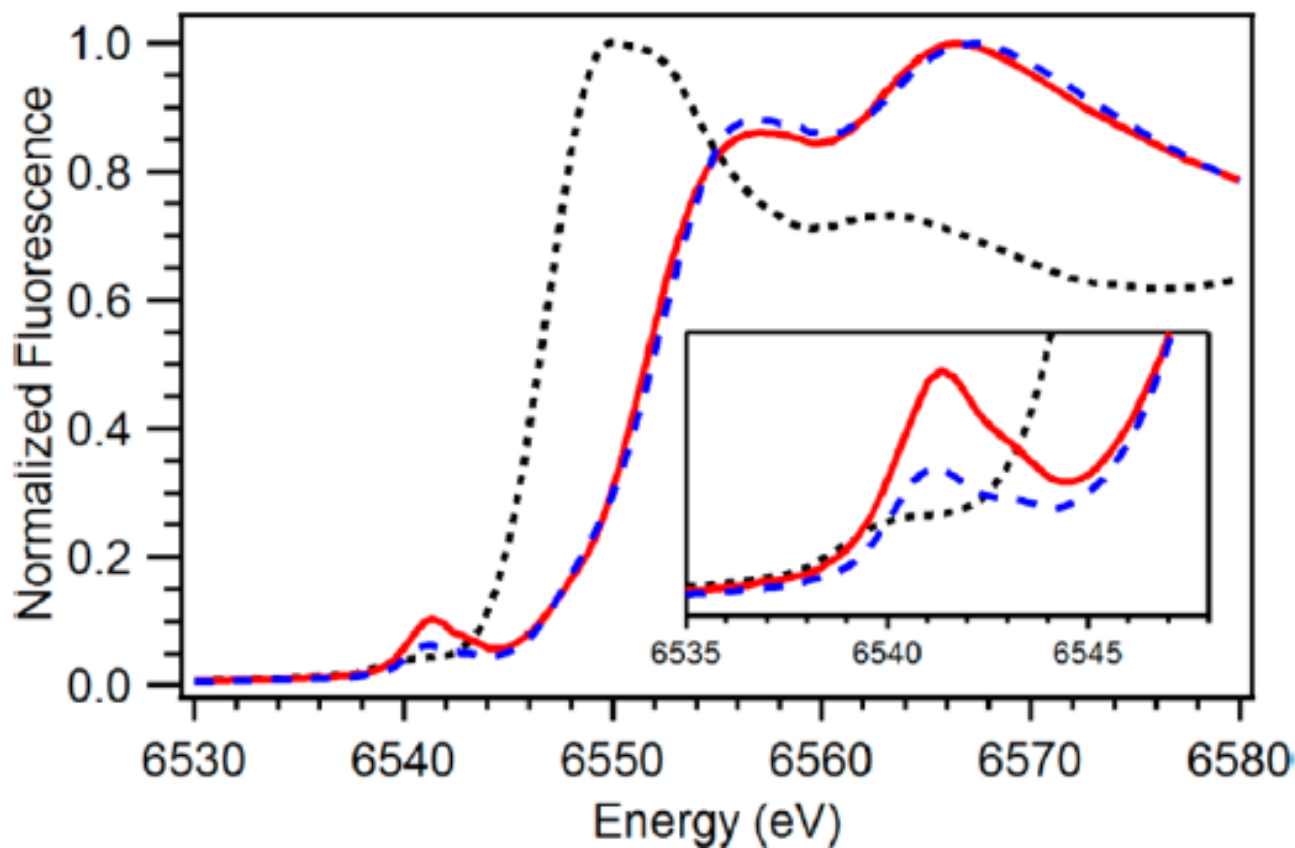


Example for d¹

Colpas, G. J et al. *Inorg. Chem.* 1991, 30, 920–928

The greater intensity for square-planar complexes may be due to decreased mixing between the empty 4p orbital (4p_z) and the ligand orbitals. This intensity of the 1s → 4p transition is even more dramatic for 2-coordinate Cu(I)

X-ray Absorption Edges in proteins (an model compounds)



..... $\text{Mn(II)N}_4\text{Cl}_2$
- - - $\text{Mn(IV)N}_4(\text{OH})_2$
— $\text{Mn(IV)N}_4(\text{O})(\text{OH})$

6-coordinated
complexes with
different ligands

D.F. Leto & T.A. Jackson *Inorg. Chem.* 2014, 53, 6179-61-94

Multi-technique approach for determining the structure of a metallo-protein

Establishing the nature, the oxidation state and the coordination parameters of a metal ion bound to a protein is usually beyond the capability of either X-ray crystallography or NMR spectroscopy

Many experimental difficulties:

- Limitations of the technique
- Redox reactions occurring during data acquisition
- Radiation damage (X-ray)
- Chemistry occurring with sample components (buffer, additives)
- Contamination of the sample by other metal ions

Multi-technique approach for determining the structure of a metallo-protein

NMR \Rightarrow structure of the protein in solution (little info on the metal center)

PX \Rightarrow structure of the protein in the crystal

XAS-EXAFS \Rightarrow Accurate metal-ligand distances (constraints for NMR). Oxidation state, electronic structure, and coordination geometry of the metal ion

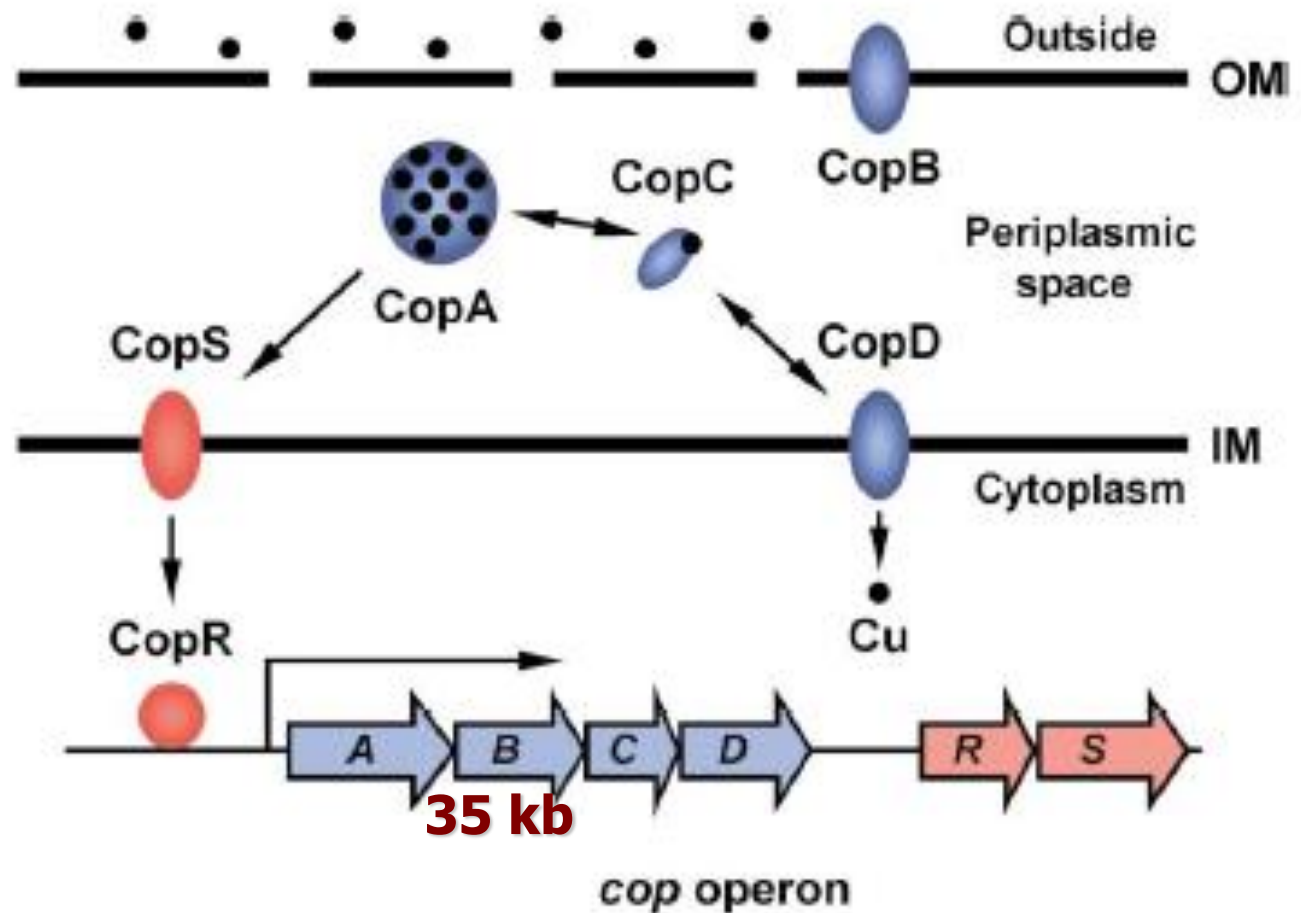
XAS-NMR; XAS-PX \Rightarrow complete structure determination of the protein in solution or solid state

Multi-technique approach for determining the structure of a metallo-protein

One example from previous work:

The structure of the copper resistance protein CopC from *P. syringae*

The copper resistance system in Gram negative bacteria (*P. syringae* - *E. coli*)



Plasmid for Cu resistance

Cha and Cooksey, *PNAS* 88, 8915 (1991)

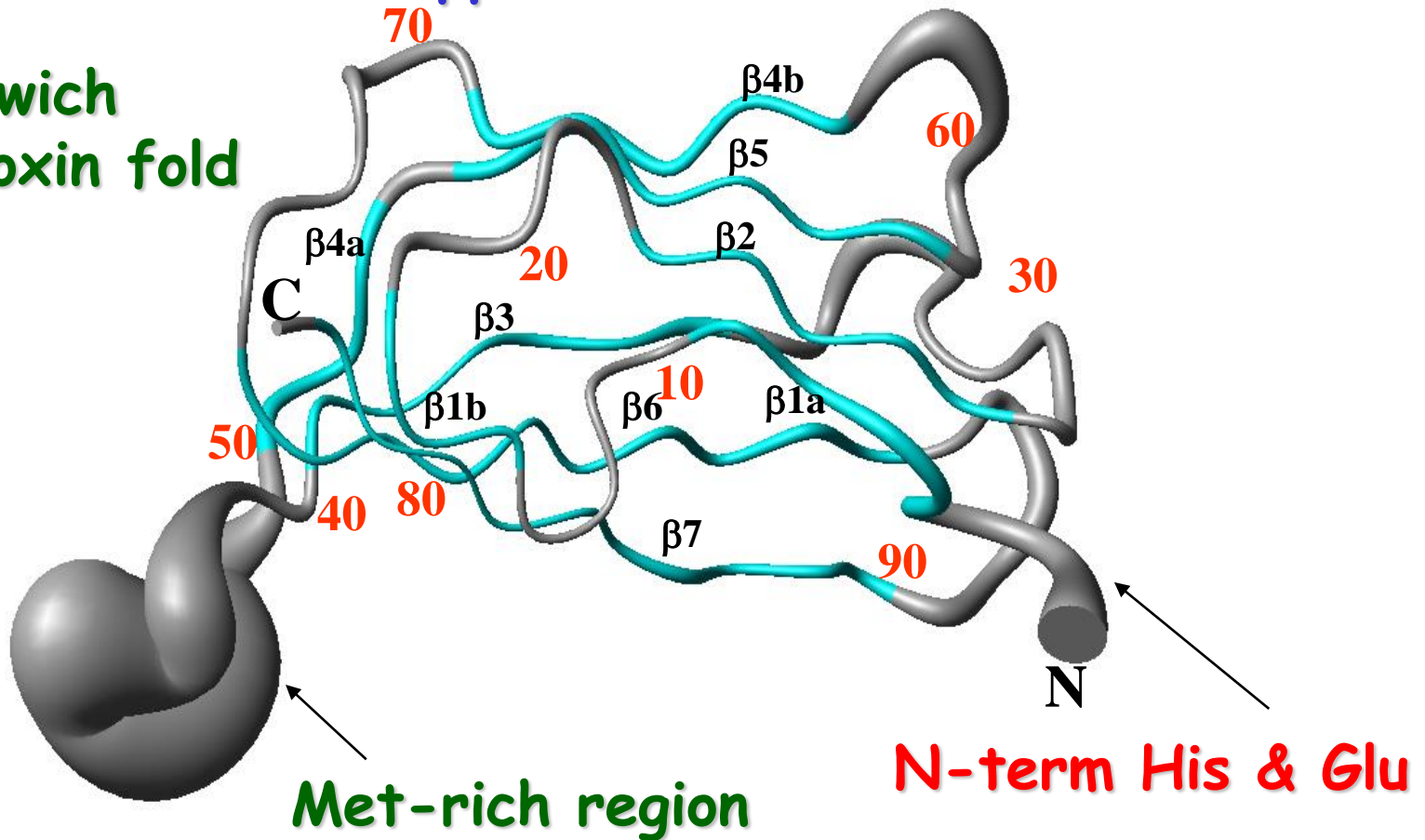
Puig, Rees & Thiele

Structure, 10, 1292 (2002)

Solution structure of apoCopC

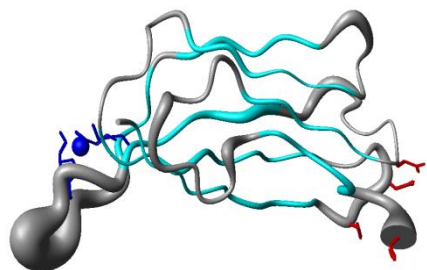
A cupredoxin-like protein involved in copper homeostasis

β -sandwich
cupredoxin fold



Redox-driven metal transfer

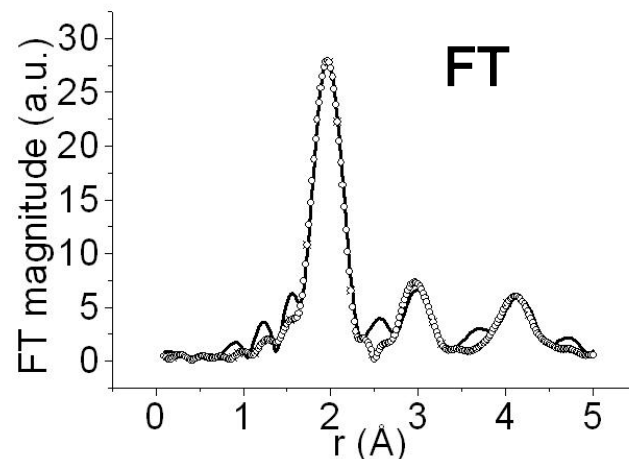
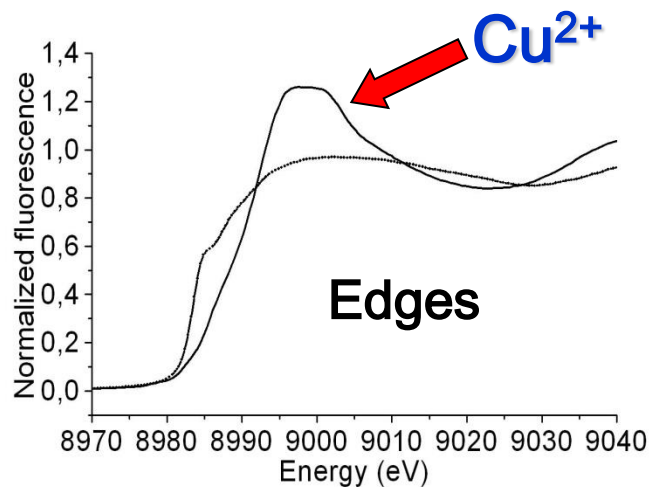
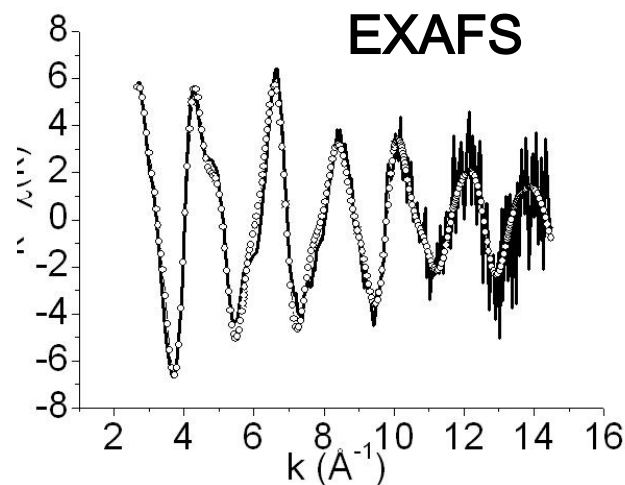
The Cu(II) - CopC sample



EXAFS results:

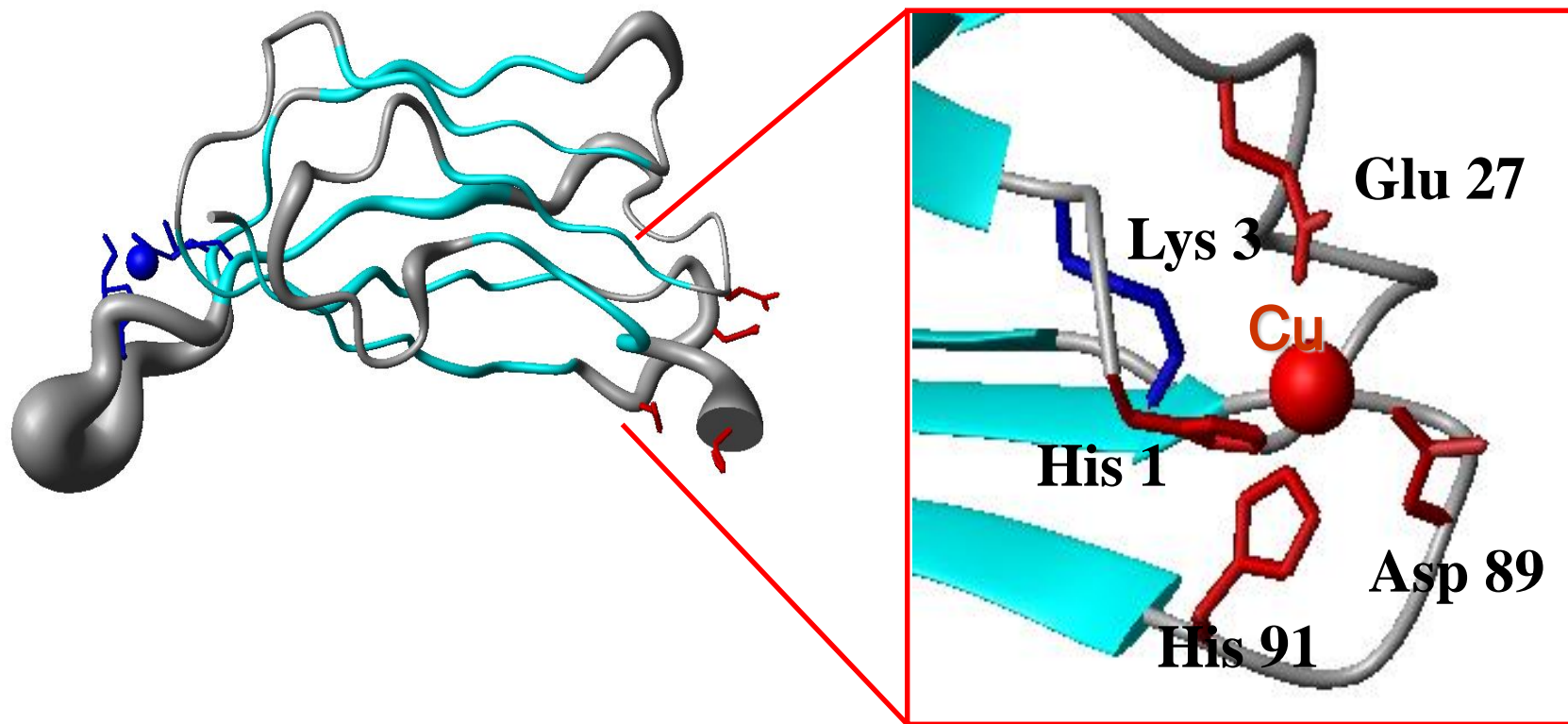
2 Cu - His 1.99 Å

2 Cu - O/N 1.97 Å



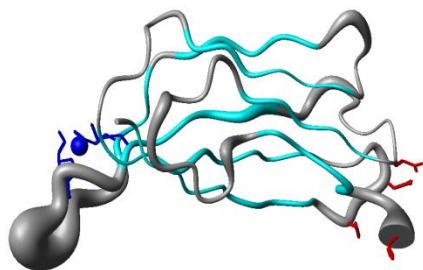
Redox-driven metal transfer

The Cu(II) - CopC sample



Redox-driven metal transfer

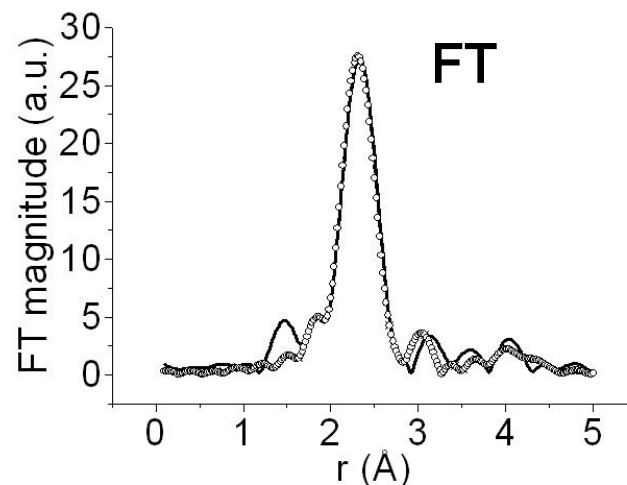
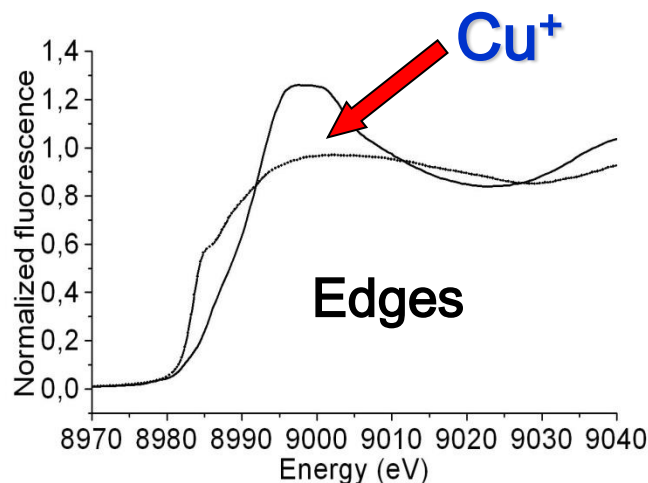
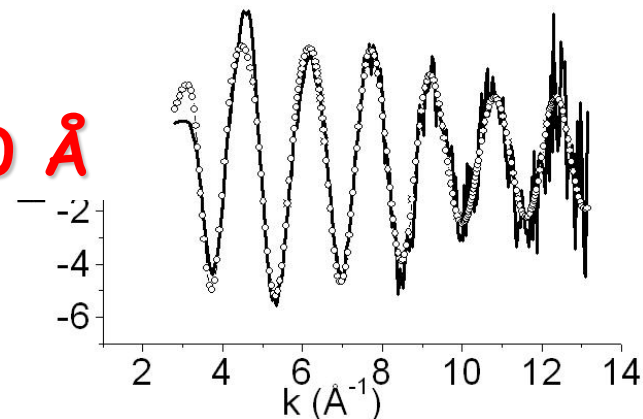
The Cu(I) - CopC sample reduced from an aliquot of the Cu(II) sample



EXAFS results:

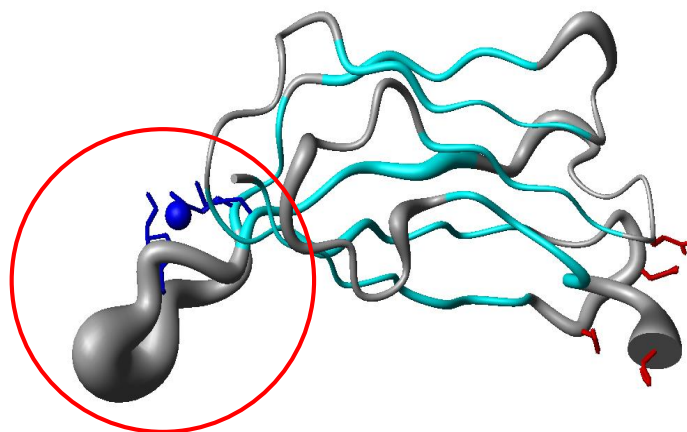
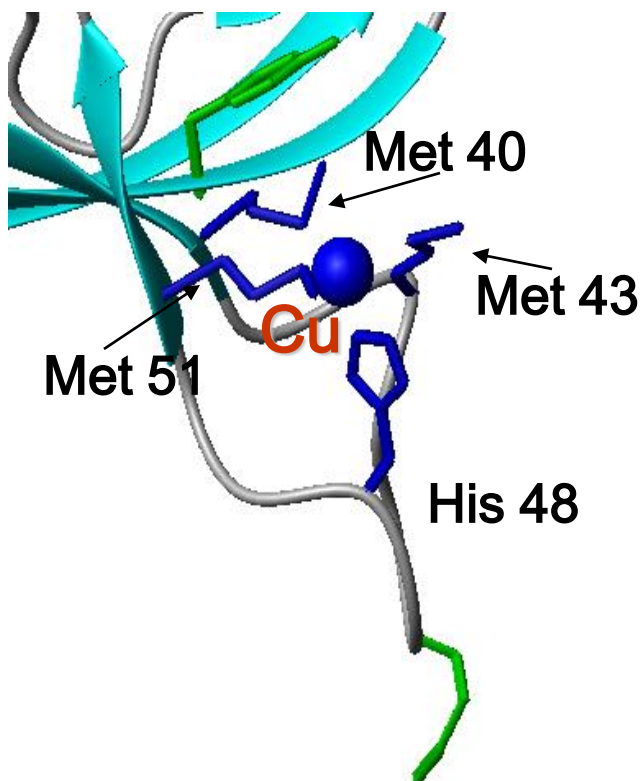
1 Cu - His 1.95 Å
3 Cu - S (Met) 2.30 Å

EXAFS



Redox-driven metal transfer

The Cu(I) - CopC sample reduced from an aliquot of the Cu(II) sample



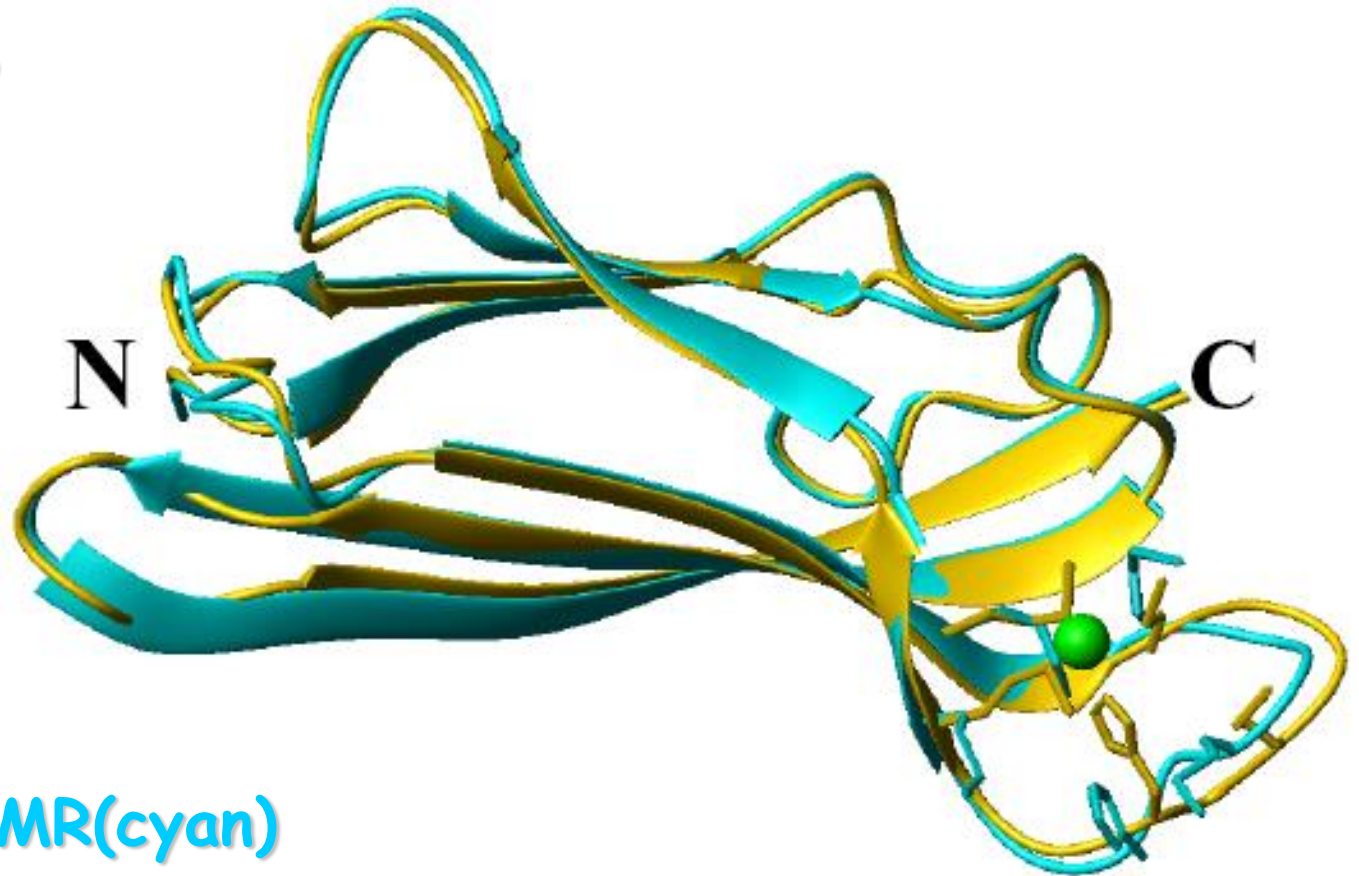
EXAFS results:

1 Cu - His 1.95 Å

3 Cu - Met 2.30 Å

Redox-driven metal transfer

NMR + EXAFS
constraints



Fit

Cu(I)-CopC NMR(cyan)

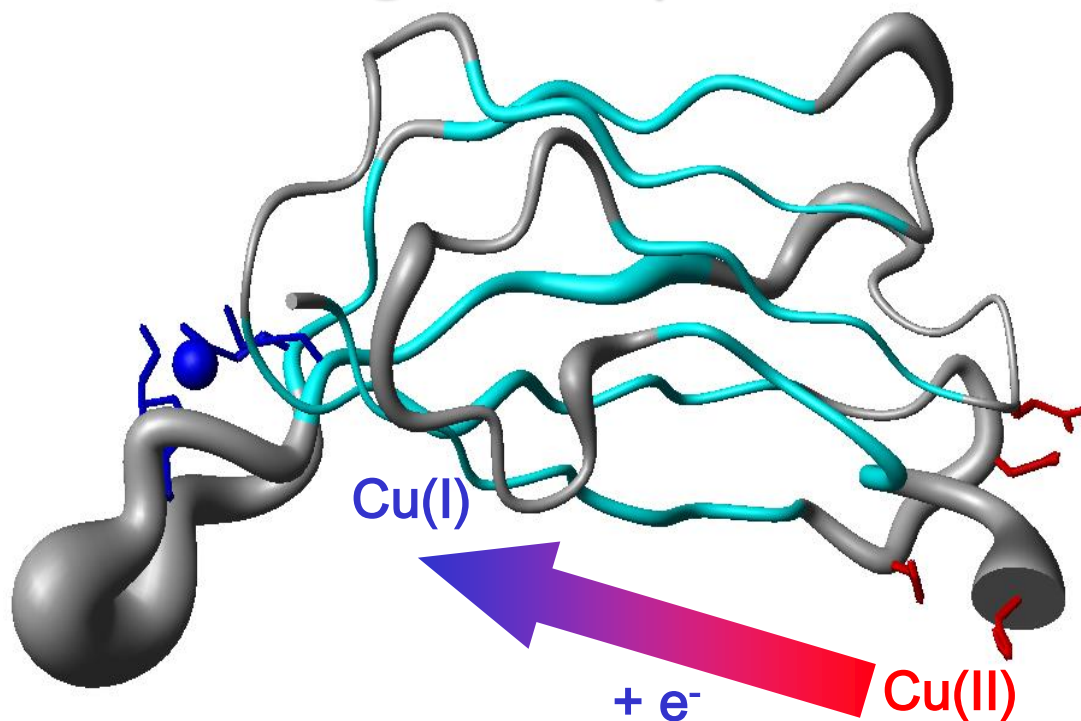
Cu(I)-CopC

NMR+EXAFS(yellow)

Arnesano, Banci, Bertini, Mangani, Thompsett, *PNAS* 100, 3814 (2003)

Redox-driven metal transfer

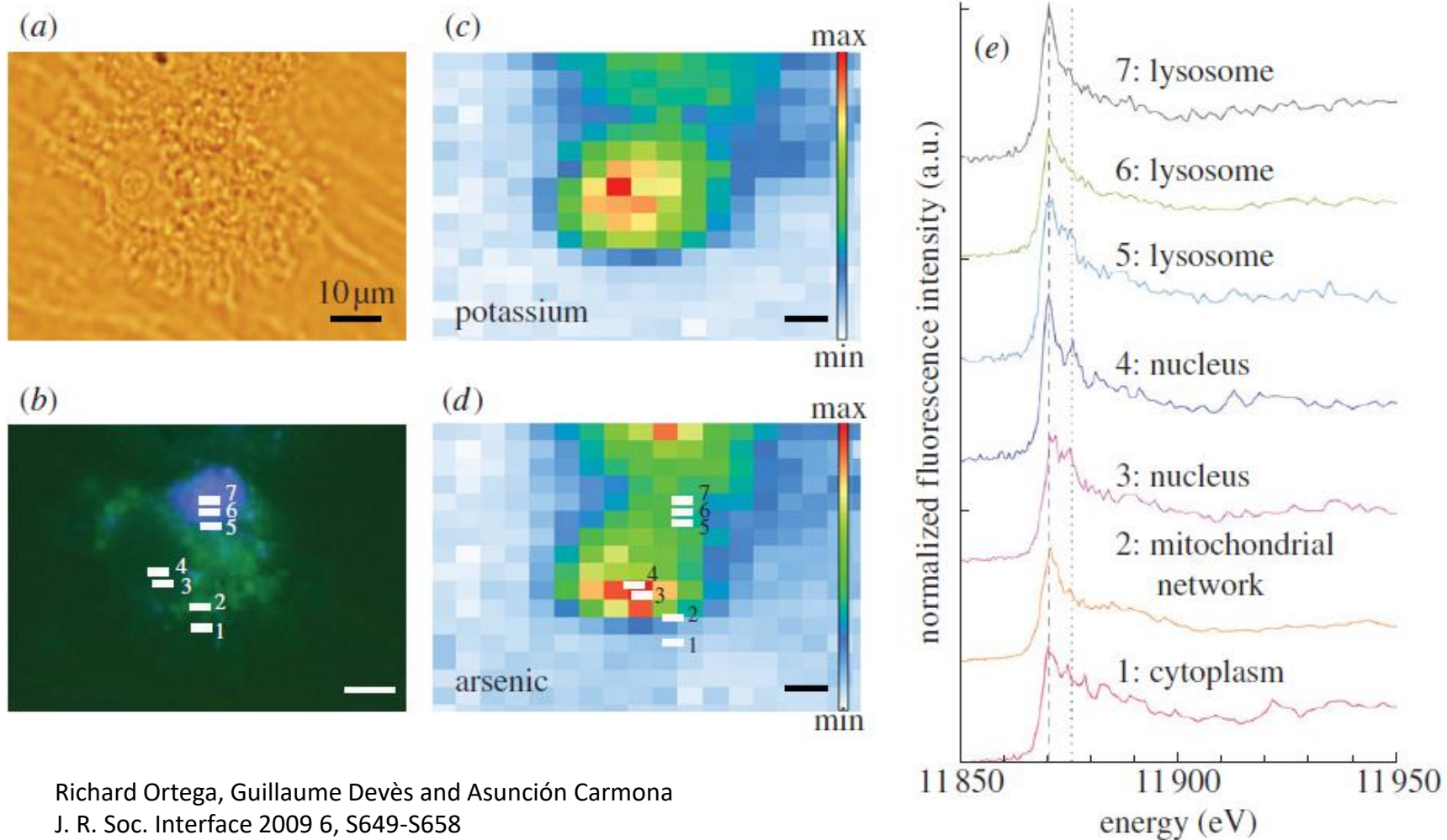
Cu migrates from Cu(II) binding site to Cu(I) binding site upon reduction!

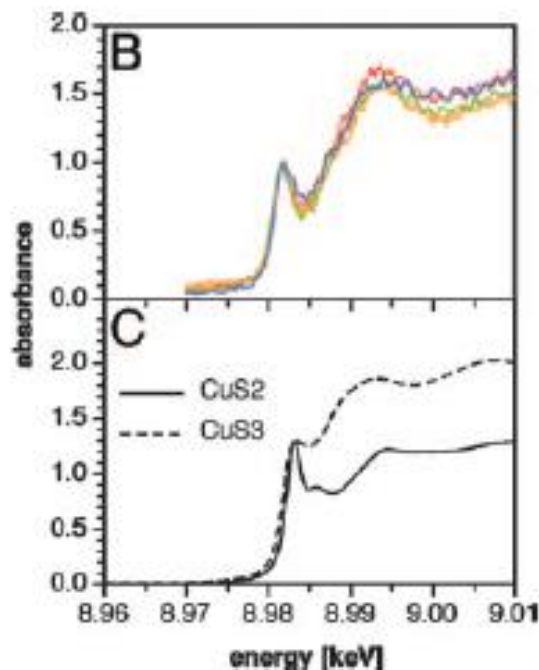
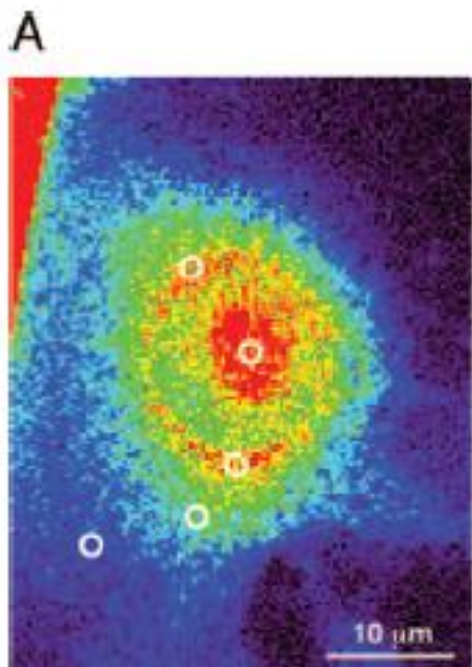


The process is reversible and it can be proposed as a trigger mechanism for copper trafficking

XRF+ μ XANES coupled to optical
microscopy of
fluorescent-tagged proteins

Normalized XANES spectra at arsenic K-absorption edge in the cytosol, mitochondrial network, nucleus and lysosomes of HepG2 cells exposed to $\text{As}(\text{OH})_3$. Light microscopy of a single cell (a); epifluorescence microscopy of the same cell showing in green the mitochondrial network (labelled with rhodamine 123) and in blue the lysosomes (labelled with lysotracker) (b); micro-XRF distribution of potassium (c) and arsenic (d). XANES spectra in the subcellular compartments show that the main arsenic oxidation state is As(III) in all organelles, with a mixture of trivalent and pentavalent arsenic in the nucleus (e).





NIH 3T3 fibroblast cell grown in medium supplemented with 150 μM CuCl_2 .

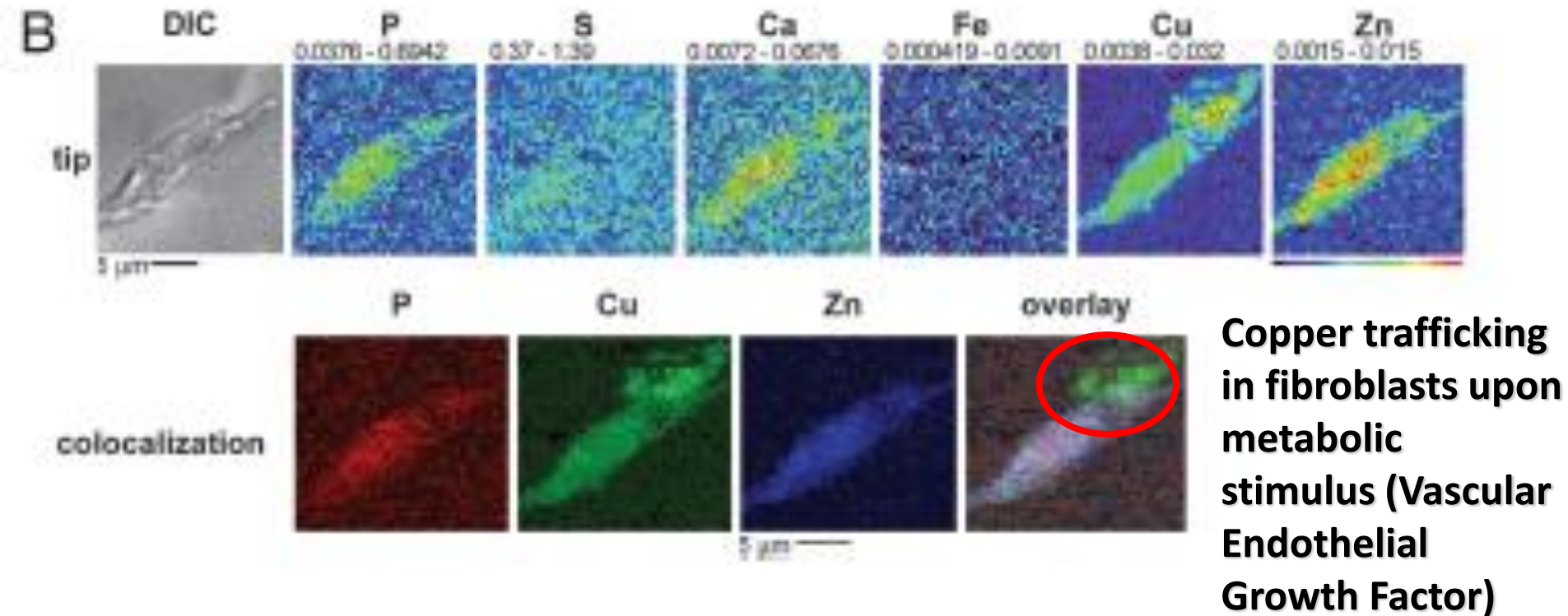
(A) False-color XRF micrograph showing the copper distribution

(B) XANES spectra acquired at various locations (marked with white rings in A).

(C) XANES reference spectra: CuS_2 : $[\text{Cu}(\text{SC}_{10}\text{H}_{13})_2][\text{N}(\text{C}_3\text{H}_7)_4]$ and CuS_3 : $[\text{Cu}(\text{SC}_6\text{H}_5)_3][\text{P}(\text{C}_6\text{H}_5)_4]$

μXRF experiments at the **Advanced Photon Source** of the Argonne National Laboratory.

The instrument at the 2-ID-D beamline: detection limit of $10^{-19} \text{ mol} \cdot \mu\text{m}^{-2}$ for Mn, Fe, Cu, or Zn and a **spatial resolution of 200 nm**.



When cells are stimulated with VEGF and bFGF and plated onto the basement membrane surface, **copper translocates** outwards toward the periphery of the cell within 30- 60 min and a substantial amount is excreted.

XAS reference material

International XAS Society:

<https://www.ixasportal.net/ixas/>

Books and Review Articles:

EXAFS: Basic Principles and Data Analysis, B.K. Teo, Springer, 1986

X-ray Absorption: Principles, Applications, Techniques of EXAFS, SEXAFS, and XANES, in *Chemical Analysis* 92, D. C. Koningsberger and

R. Prins, ed., John Wiley & Sons, 1988.

Basic Principles and Applications of EXAFS, Chapter 10 in *Handbook of Synchrotron Radiation*, pp 995–1014. E. A. Stern and S. M. Heald, E. E. Koch, ed., North-Holland, 1983.

G. Bunker *Introduction to XAFS*, Cambridge University Press 2011

Tutorials and other Training Material:

<http://gbxafs.iit.edu/training/tutorials.html> Grant Bunker's tutorials

<http://srs.dl.ac.uk/XRS/courses/> Tutorial from Daresbury Lab, UK

<http://bruceravel.github.io/XAS-Education/> Bruce Ravel's XAS Education materials.

Software Resources:

<https://bruceravel.github.io/demeter/>

<http://www.esrf.fr/computing/scientific/exafs/>

http://gnxas.unicam.it/pag_gnxas.html

<http://leonardo.phys.washington.edu/feff>

BioXAS beam lines

- <https://www.elettra.trieste.it/elettra-beamlines/xaafs.html> Elettra general purpose XAS
- <http://www.esrf.eu/UsersAndScience/Experiments/CRG/BM08> ESRF Italian XAS beamline
- <http://www.esrf.eu/home/UsersAndScience/Experiments/MEx/BM23.html> ESRF multi purpose beamline
- <http://www.esrf.eu/home/UsersAndScience/Experiments/MEx/ID24.html> ESRF Energy dispersive XAS
- http://photon-science.desy.de/facilities/petra_iii/beamlines/p64_advanced_xafs/index_eng.html Petra III
- <https://www.diamond.ac.uk/Instruments/Spectroscopy/B18.html> Diamond general purpose XAS
- <https://www.psi.ch/sls/superxas/superxas> Swiss Light Source XAS & XES
- <https://bioxas.lightsource.ca/> CLS spectroscopy & imaging