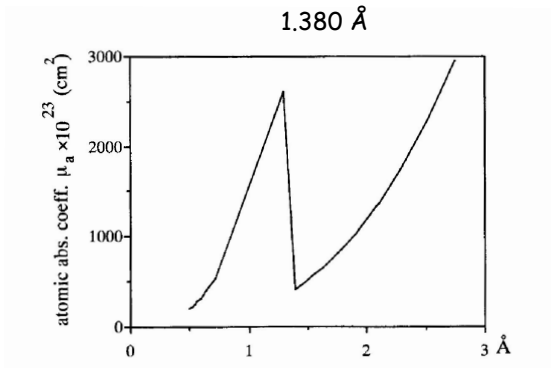
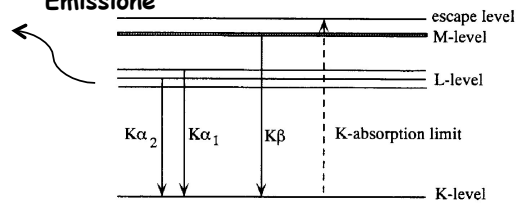


Diffusione anomala = assorbimento

Assorbimento



Emissione



$$\lambda_{\min} = \frac{h \times c}{e \times V} = \frac{12.4}{V}$$

where V is in kilovolts. At $V = 40 \text{ kV}$ the cut-off edge is at 0.31 \AA .

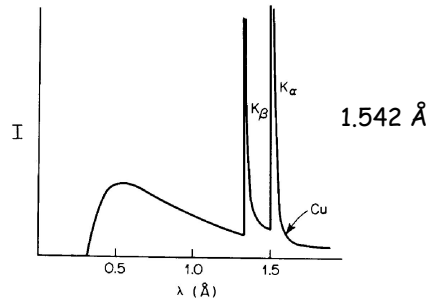


Figure 2.2. The spectrum from an X-ray tube with a copper anode. It shows a continuous spectrum and in addition two sharp peaks due to quantized electrons

http://skuld.bmsc.washington.edu/scatter/AS_periodic.html

1

Sommario di un esperimento MAD: multiple wavelength anomalous dispersion

1. introduzione del diffusore anomalo (atomo pesante) nel campione (*se non già presente*)
2. analisi dello spettro di fluorescenza del campione: scelta delle 3(4) λ sperimentali
3. raccolta dati su 1 cristallo (3(4) λ sperimentali)
4. individuazione delle posizioni dei diffusori anomali (atomi pesanti):
Patterson delle *differenze anomale*
5. ottenimento delle fasi usando 2-4 datasets raccolti a diverse lunghezze d'onda

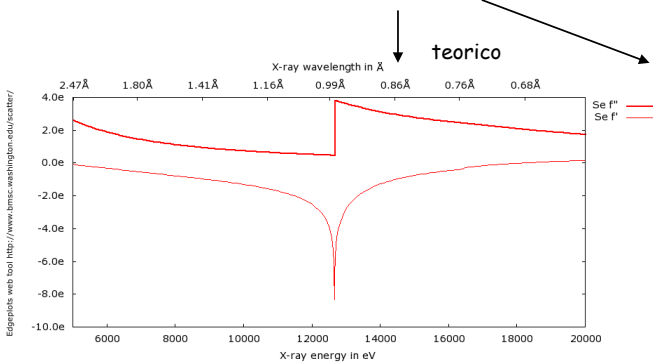
2

1. introduzione del diffusore anomalo nel campione:

- il diffusore anomalo potrebbe essere già presente: ad es. metalloproteine
- soaking del cristallo con un atomo pesante
- produzione della proteina con seleniometionine

2. analisi dello spettro di fluorescenza del campione:

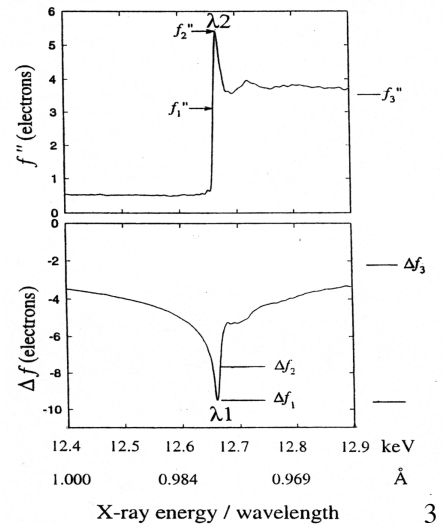
lo spettro di emissione (fluorescenza) genera lo spettro di assorbimento



http://www.bmsc.washington.edu/scatter/AS_periodic.html

$$f_0 \Rightarrow f(\lambda) = f_0 + \Delta f(\lambda) + if''(\lambda)$$

Sperimentale: misura di λ_1 e λ_2



Scattering anomalo

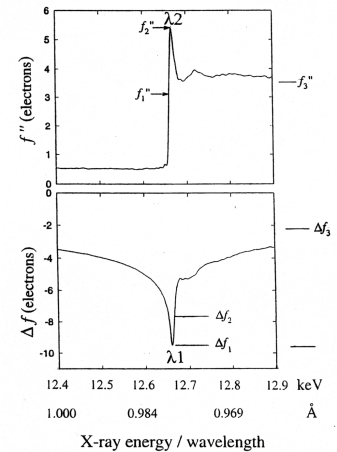
fattore di scattering atomico:

$$f_0 \Rightarrow f(\lambda) = f_0 + \Delta f(\lambda) + if''(\lambda) = f' + if''$$

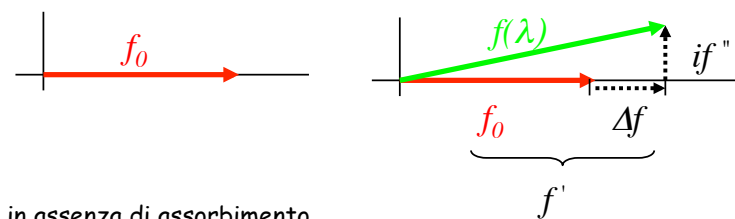
\uparrow \uparrow \uparrow
 ricavato da f'' misurati (fluorescenza)

$$\Delta f(\omega) = \frac{2}{\pi} \int_0^{\infty} \frac{\omega' f''(\omega')}{(\omega'^2 - \omega^2)} d\omega'$$

Solitamente la fluorescenza viene usata per misurare λ_1 e λ_2



L'assorbimento produce uno **sfasamento** della radiazione diffusa (diverso da π)



in assenza di assorbimento

in presenza di assorbimento

Coppie di Friedel

$$F(h,k,l) = \int_{cell} \rho(x,y,z) \exp[-2\pi i(hx + ky + lz)] dV$$

la densità elettronica è una funzione reale

$$\rho(x,y,z) = \rho^*(x,y,z)$$

$$F^*(h,k,l) = \int_{cell} \rho(x,y,z) \exp[2\pi i(hx + ky + lz)] dV = F(\bar{h}, \bar{k}, \bar{l})$$

$$F^*(h,k,l) = |F(h,k,l)| e^{-i\alpha(h,k,l)} = F(\bar{h}, \bar{k}, \bar{l}) = |F(\bar{h}, \bar{k}, \bar{l})| e^{i\alpha(\bar{h}, \bar{k}, \bar{l})} \longrightarrow \begin{cases} |F(h,k,l)| = |F(\bar{h}, \bar{k}, \bar{l})| \\ \arg[F(h,k,l)] = -\arg[F(\bar{h}, \bar{k}, \bar{l})] \end{cases}$$

in presenza di scattering anomalo **non vale più la legge di Friedel**

$$\begin{cases} |F(h,k,l)| \neq |F(\bar{h}, \bar{k}, \bar{l})| \\ \arg[F(h,k,l)] \neq -\arg[F(\bar{h}, \bar{k}, \bar{l})] \end{cases}$$

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$$f(\lambda) = f_0 + \Delta f(\lambda) + i f''(\lambda) = f' + i f'' \longrightarrow \arg(F_H(+)) \neq -\arg(F_H(-))$$

$$F_H = F'_H + i F''_H$$

$$F_{PH}(+) = F_P(+) + F'_H(+) + i F''_H(+) = |F_P(+)| e^{i\alpha_P(+)} + |F'_H(+)| e^{i\alpha_H(+)} + i |F''_H(+)| e^{i\alpha_H(+)}$$

$$i = e^{i\frac{\pi}{2}}$$

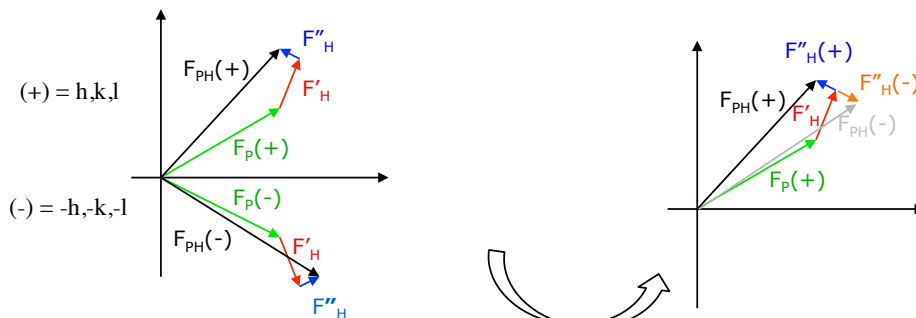
sfasamento

$$\begin{cases} |F(h,k,l)| = |F(\bar{h}, \bar{k}, \bar{l})| \\ \alpha(h,k,l) = -\alpha(\bar{h}, \bar{k}, \bar{l}) \end{cases}$$

$$F_{PH}(-) = |F_P(+)| e^{-i\alpha_P(+)} + |F'_H(+)| e^{-i\alpha_H(+)} + |F''_H(+)| e^{-i(\alpha_H(+)-\frac{\pi}{2})}$$

(+) = h,k,l

$$|F_{PH}(+)| \neq |F_{PH}(-)|$$



riflessione rispetto all'asse R

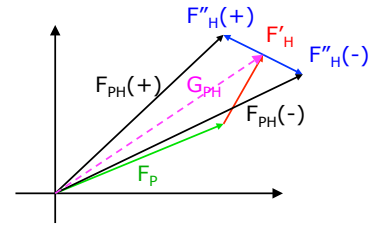
6

3. individuazione delle posizioni dei diffusori anomali: Patterson delle differenze anomale

$$\Delta|F|_{ano} \equiv \{|F_{PH}(+)| - |F_{PH}(-)|\} \frac{f'}{2f''}$$

da dimostrare: ↓

$$(\Delta|F|_{ano})^2 \equiv \frac{1}{2}|F'_H|^2 + rumore$$



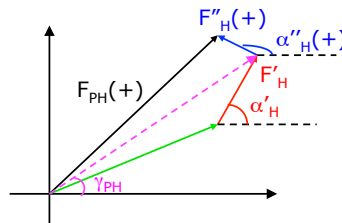
$$F_H = F'_H + iF''_H$$

$$\begin{cases} F_{PH}(+) = |F_P|e^{i\alpha_P} + |F'_H|e^{i\alpha'_H} + |F''_H|e^{i(\alpha'_H + \frac{\pi}{2})} = |G_{PH}|e^{i\gamma_{PH}} + |F''_H|e^{i(\alpha'_H + \frac{\pi}{2})} \\ F_{PH}(-) = |F_P|e^{-i\alpha_P} + |F'_H|e^{-i\alpha'_H} + |F''_H|e^{-i(\alpha'_H - \frac{\pi}{2})} = |G_{PH}|e^{-i\gamma_{PH}} + |F''_H|e^{-i(\alpha'_H - \frac{\pi}{2})} \end{cases}$$

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$$\begin{aligned} |F_{PH}(+)|^2 &= |G_{PH} + F''_H(+)|^2 = |G_{PH}|^2 + |F''_H|^2 + 2|G_{PH}||F''_H|\cos(\gamma_{PH} - \alpha''_H(+)) = \\ &= |G_{PH}|^2 + |F''_H|^2 + 2|G_{PH}||F''_H|\cos\left(-\frac{\pi}{2} + (\gamma_{PH} - \alpha'_H)\right) = |G_{PH}|^2 + |F''_H|^2 + 2|G_{PH}||F''_H|\sin(\gamma_{PH} - \alpha'_H) \end{aligned}$$

$$\begin{aligned} \alpha''_H(+) &= \alpha'_H + \frac{\pi}{2} \\ \alpha''_H(-) &= \alpha'_H - \frac{\pi}{2} \end{aligned}$$



$$\begin{aligned} |F_{PH}(-)|^2 &= |G_{PH} + F''_H(-)|^2 = |G_{PH}|^2 + |F''_H|^2 + 2|G_{PH}||F''_H|\cos(\gamma_{PH} - \alpha''_H(-)) = \\ &= |G_{PH}|^2 + |F''_H|^2 + 2|G_{PH}||F''_H|\cos\left(\frac{\pi}{2} + (\gamma_{PH} - \alpha'_H)\right) = |G_{PH}|^2 + |F''_H|^2 - 2|G_{PH}||F''_H|\sin(\gamma_{PH} - \alpha'_H) \end{aligned}$$

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$$|F_{PH}(+)|^2 - |F_{PH}(-)|^2 = 4|G_{PH}| |F_H''| \sin(\gamma_{PH} - \alpha'_H)$$

vale inoltre la relazione seguente:

$$|F_{PH}(+)|^2 - |F_{PH}(-)|^2 = (|F_{PH}(+)| - |F_{PH}(-)|)(|F_{PH}(+)| + |F_{PH}(-)|) \cong 2|G_{PH}| (|F_{PH}(+)| - |F_{PH}(-)|)$$

approssimazione $|F_{PH}(+)| \approx |F_{PH}(-)| \approx |G_{PH}| \Rightarrow |F_{PH}(+)| + |F_{PH}(-)| = 2|G_{PH}|$

$$(|F_{PH}(+)| - |F_{PH}(-)|) \cong 2|F_H''| \sin(\gamma_{PH} - \alpha'_H)$$

$$|F_H''| = \left| \sum_j f_j'' \exp(2\pi i \mathbf{h} \cdot \mathbf{r}_j) \right| = \left| \sum_j \frac{f_j''}{f_j'} f_j' \exp(2\pi i \mathbf{h} \cdot \mathbf{r}_j) \right| = \frac{f''}{f'} |F_H'| \quad \downarrow$$

$$(|F_{PH}(+)| - |F_{PH}(-)|) \cong 2 \frac{f''}{f'} |F_H'| \sin(\gamma_{PH} - \alpha'_H)$$

$$\Delta|F|_{ano} \cong \{|F_{PH}(+)| - |F_{PH}(-)|\} \frac{f'}{2f''} \cong |F_H'| \sin(\gamma_{PH} - \alpha'_H)$$

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$$\Delta|F|_{ano} \cong \{|F_{PH}(+)| - |F_{PH}(-)|\} \frac{f'}{2f''} \cong |F_H'| \sin(\gamma_{PH} - \alpha'_H)$$

$$(\Delta|F|_{ano})^2 \cong |F_H'|^2 \sin^2(\gamma_{PH} - \alpha'_H)$$

$$\cos 2x = \cos^2 x - \sin^2 x = 1 - 2\sin^2 x$$

$$\sin^2 x = \frac{1}{2} - \frac{1}{2} \cos 2x$$

$$(\Delta|F|_{ano})^2 \cong \frac{1}{2}|F_H'|^2 - \frac{1}{2}|F_H'|^2 \cos 2(\gamma_{PH} - \alpha'_H)$$

γ_{PH}, α'_H indipendenti

$$(\Delta|F|_{ano})^2 \cong \frac{1}{2}|F_H'|^2 + \text{rumore}$$

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Mappa di Patterson delle differenze anomale: posizione degli atomi pesanti

$$P(\mathbf{u}) = \frac{1}{V} \sum_{\mathbf{h}} (\Delta |F_{\mathbf{h}}|_{\text{ano}})^2 \cos(2\pi i \mathbf{h} \cdot \mathbf{u}) = \frac{1}{V} \sum_{\mathbf{h}} (|F_{PH}(+)| - |F_{PH}(-)|)^2 \left(\frac{f'}{2f''} \right)^2 \cos(2\pi i \mathbf{h} \cdot \mathbf{u}) \approx$$

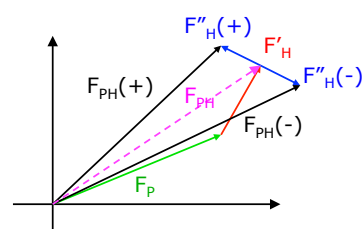
$$\approx \frac{1}{V} \sum_{\mathbf{h}} \frac{1}{2} |F'_H|^2 \cos(2\pi i \mathbf{h} \cdot \mathbf{u}) + \text{rumore} \quad \longrightarrow \quad \text{deconvoluzione:} \quad \text{posizione degli atomi pesanti} \quad \longrightarrow \quad F_H$$

$$F_H(\lambda) = \sum_{j=1}^n f_j(\lambda) \exp[2\pi i(\mathbf{h} \cdot \mathbf{r}_j)] = [f_0 + \Delta f(\lambda) + i f''(\lambda)] \sum_{j=1}^n \exp[2\pi i(\mathbf{h} \cdot \mathbf{r}_j)] = F'_H + i F''_H$$

$$F'_H = [f_0 + \Delta f(\lambda)] \sum_{j=1}^n \exp[2\pi i(\mathbf{h} \cdot \mathbf{r}_j)] = F_{H0} \left(1 + \frac{\Delta f}{f_0} \right)$$

$$F_{H0} = \sum_{j=1}^n f_0 \exp[2\pi i(\mathbf{h} \cdot \mathbf{r}_j)] \quad \downarrow$$

deconvoluzione: posizione degli atomi pesanti



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SIRAS: single isomorphous replacement anomalous scattering

2 esperimenti: $|F_P|$, $1/2(|F_{PH}(+)| + |F_{PH}(-)|)$, $|F_{PH}(+)|$ e $|F_{PH}(-)|$

differenze isomorfe

differenze anomale

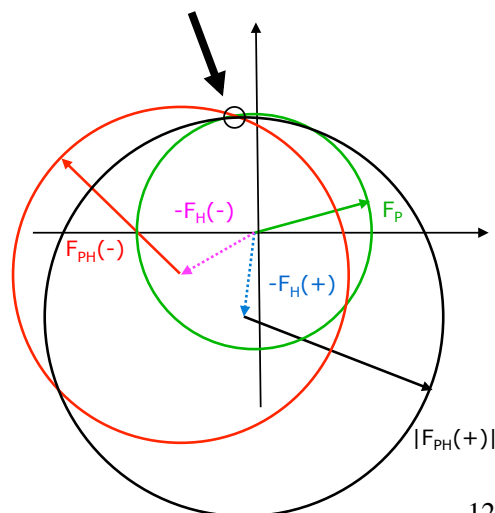
posizione degli atomi pesanti

F_H

fasi

$$\begin{cases} F_{PH}(+) = F_P + F_H(+) \\ F_{PH}(-) = F_P + F_H(-) \end{cases}$$

$$\begin{cases} F_P = F_{PH}(+) - F_H(+) \\ F_P = F_{PH}(-) - F_H(-) \end{cases}$$



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4. ottenimento delle fasi usando 2-4 dataset raccolti a diverse lunghezze d'onda: MAD

$$F_H(\mathbf{h}, \lambda) = \sum_{j=1}^n f_j(\lambda) \exp[2\pi i(\mathbf{h} \cdot \mathbf{r}_j)] = [f_0 + \Delta f(\lambda) + i f''(\lambda)] \sum_{j=1}^n \exp[2\pi i(\mathbf{h} \cdot \mathbf{r}_j)] = F_A + a$$

$$f(\lambda) = f_0 + \Delta f(\lambda) + i f''(\lambda) = f' + i f''$$

parte anomala

$$\begin{cases} a = [\Delta f(\lambda) + i f''(\lambda)] \sum_{j=1}^n \exp[2\pi i(\mathbf{h} \cdot \mathbf{r}_j)] \\ F_A = f_0 \sum_{j=1}^n \exp[2\pi i(\mathbf{h} \cdot \mathbf{r}_j)] \Rightarrow \sum_{j=1}^n (\dots) = \frac{F_A}{f_0} \end{cases} \longrightarrow a = \left[\frac{\Delta f(\lambda)}{f_0} + i \frac{f''(\lambda)}{f_0} \right] F_A$$

$$F_{PH} = F_P + F_H = F_P + F_A + a = F_{PA} + a \longrightarrow |F_{PH}|^2 = |F_{PA}|^2 + |a|^2 + 2|F_{PA}||a|\cos\Delta\varphi$$

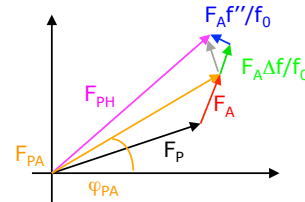
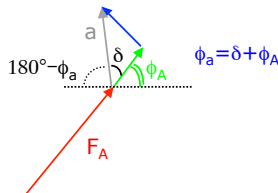
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$$|a|^2 = \left[\frac{\Delta f}{f_0} - i \frac{f''}{f_0} \right] F_A^* \left[\frac{\Delta f}{f_0} + i \frac{f''}{f_0} \right] F_A = \frac{\Delta f^2 + (f'')^2}{f_0^2} |F_A|^2$$

$$a = \left[\frac{\Delta f(\lambda)}{f_0} + i \frac{f''(\lambda)}{f_0} \right] F_A$$

angolo tra F_{PA} e a

$$\Delta\varphi = \varphi_{PA} - \varphi_a = \varphi_{PA} - \varphi_A - \delta$$



$$\begin{cases} |a|\cos\delta = \frac{\Delta f}{f_0}|F_A| \\ |a|\sin\delta = \frac{f''}{f_0}|F_A| \end{cases} \longrightarrow \begin{cases} |a|\cos^2\delta = \frac{\Delta f}{f_0}|F_A|\cos\delta \\ |a|\sin^2\delta = \frac{f''}{f_0}|F_A|\sin\delta \end{cases} \quad |a|(\cos^2\delta + \sin^2\delta) = |a| = \left(\frac{\Delta f}{f_0}\cos\delta + \frac{f''}{f_0}\sin\delta \right) |F_A|$$

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$$|F_{PH}|^2 = |F_{PA}|^2 + |a|^2 + 2|F_{PA}||a|\cos\Delta\varphi$$

$$\cos\Delta\varphi = \cos(\varphi_{PA} - \varphi_A - \delta) = \cos(\varphi_{PA} - \varphi_A)\cos(\delta) + \sin(\varphi_{PA} - \varphi_A)\sin(\delta)$$

$$\begin{aligned} |a|\cos\Delta\varphi &= |a|(\cos(\varphi_{PA} - \varphi_A)\cos(\delta) + \sin(\varphi_{PA} - \varphi_A)\sin(\delta)) = \\ &= \cos(\varphi_{PA} - \varphi_A)\frac{\Delta f}{f_0}|F_A| + \sin(\varphi_{PA} - \varphi_A)\frac{f''}{f_0}|F_A| \end{aligned}$$

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$$\begin{aligned} |F_{PH}|^2 &= |F_{PA}|^2 + |a|^2 + 2|F_{PA}||F_A|\left(\frac{\Delta f}{f_0}\cos(\varphi_{PA} - \varphi_A) + \frac{f''}{f_0}\sin(\varphi_{PA} - \varphi_A)\right) = \\ &= |F_{PA}|^2 + \frac{\Delta f^2 + (f'')^2}{f_0^2}|F_A|^2 + 2|F_{PA}||F_A|\left(\frac{\Delta f}{f_0}\cos(\varphi_{PA} - \varphi_A) + \frac{f''}{f_0}\sin(\varphi_{PA} - \varphi_A)\right) \end{aligned}$$

$$\boxed{|F_{PH}|^2 = |F_{PA}|^2 + p(\lambda)|F_A|^2 + 2|F_{PA}||F_A|(q(\lambda)\cos(\varphi_{PA} - \varphi_A) + r(\lambda)\sin(\varphi_{PA} - \varphi_A))}$$

misura sperimentale

$$\forall \lambda \Rightarrow |F_{PH}(+)|, |F_{PH}(-)| \quad \text{2 equazioni in 3 incognite: } |F_{PA}|, |F_A|, \varphi_{PA} - \varphi_A$$

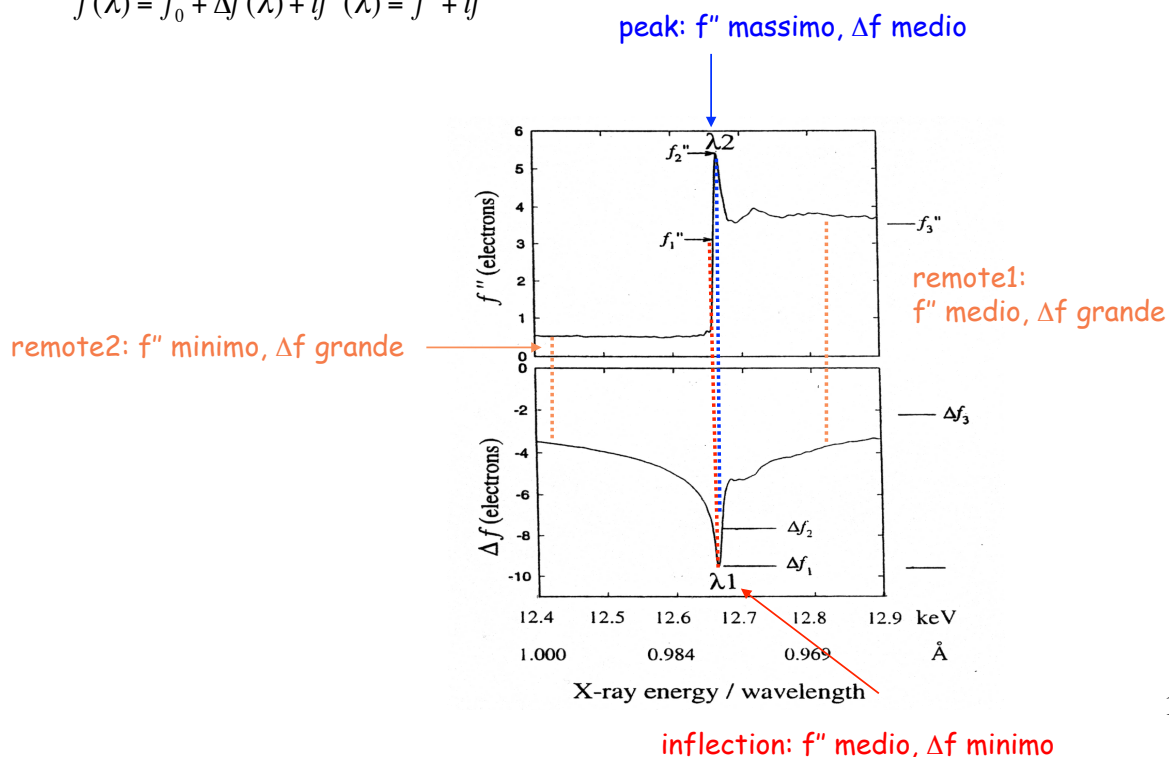
sono sufficienti 2 esperimenti (2 terne di valori p, q, r) con 2 lunghezze d'onda differenti:
4 equazioni in 3 incognite

φ_A è noto dalle posizioni degli atomi pesanti: mappa di Patterson diff. anomale

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Scelta delle lunghezze d'onda sperimentali

$$f(\lambda) = f_0 + \Delta f(\lambda) + if''(\lambda) = f' + if''$$



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Configurazione assoluta degli atomi pesanti nel MIR: uso del segnale anomalo

$$\Delta|F|_{ano} \equiv \{|F_{PH}(+)|-|F_{PH}(-)|\} \frac{f'}{2f''} \equiv |F'_H| \sin(\alpha_{PH} - \alpha'_H)$$



$$|F'_H| = \frac{k}{\sin(\alpha_{PH} - \alpha'_H)} \{|F_{PH}(+)|-|F_{PH}(-)|\} \geq 0$$



sperimentale: dai dati isomorfi



sperimentale

2 configurazioni degli atomi pesanti



$$(\alpha_{PH} - \alpha'_H) = \begin{cases} +|\alpha_{PH} - \alpha'_H| \\ -|\alpha_{PH} - \alpha'_H| \end{cases} \longrightarrow \begin{cases} |F'_H| \geq 0 \\ |F'_H| \leq 0 \end{cases} \begin{array}{l} \text{configurazione "giusta"} \\ \text{configurazione "sbagliata"} \end{array}$$

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