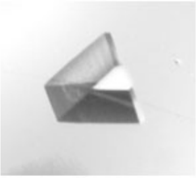
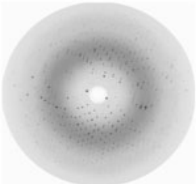
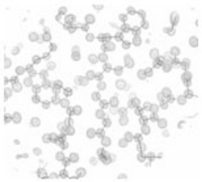
Phase Determination Using Synchrotron Radiation





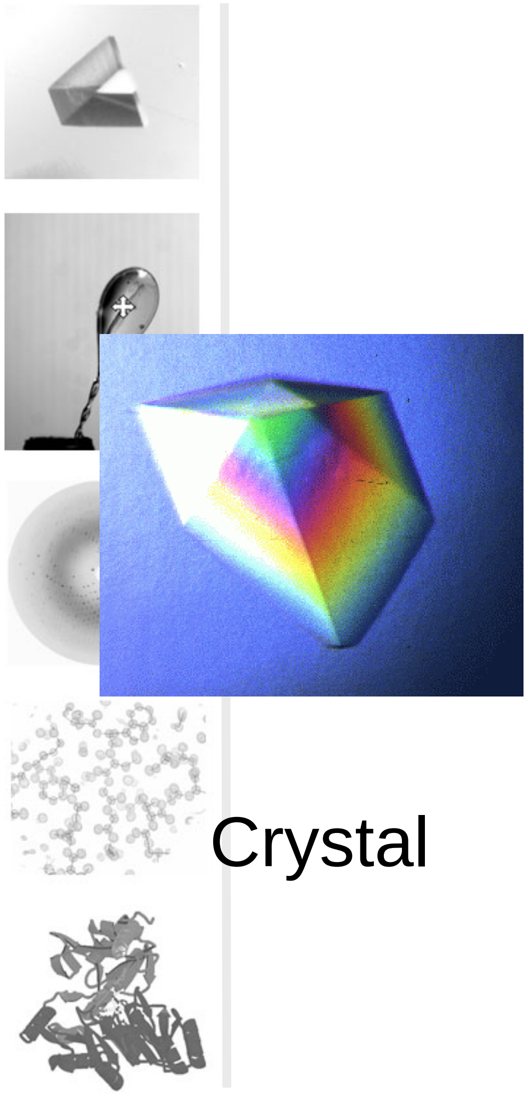


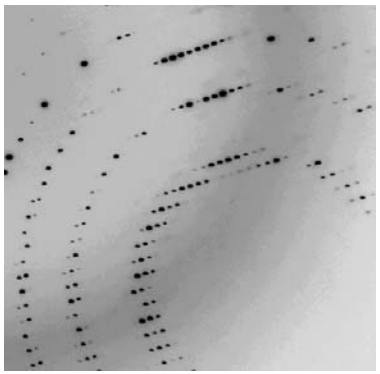
Manfred S. Weiss

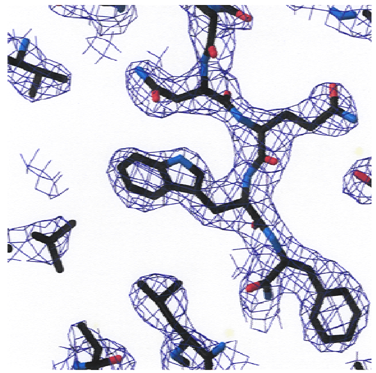
Helmholtz-Zentrum Berlin für Materialien und Energie
Macromolecular Crystallography (HZB-MX)
Albert-Einstein-Str. 15
D-12489 Berlin, Germany
msweiss@helmholtz-berlin.de

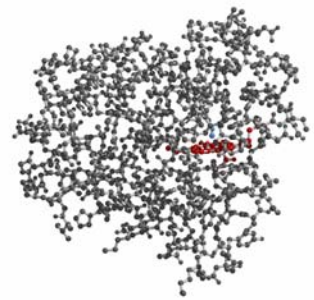
Structure Determination

Phases $\alpha(hkl)$









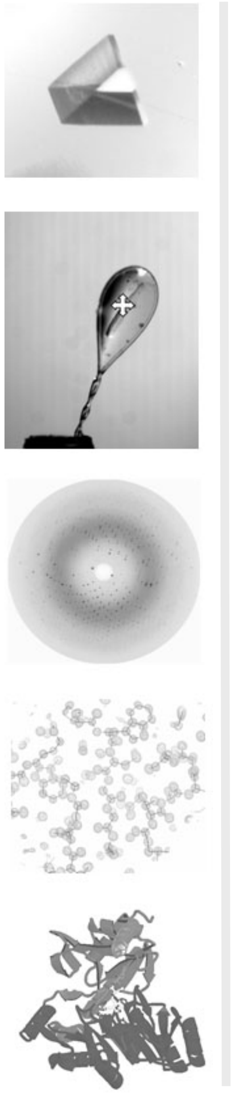
Crystal

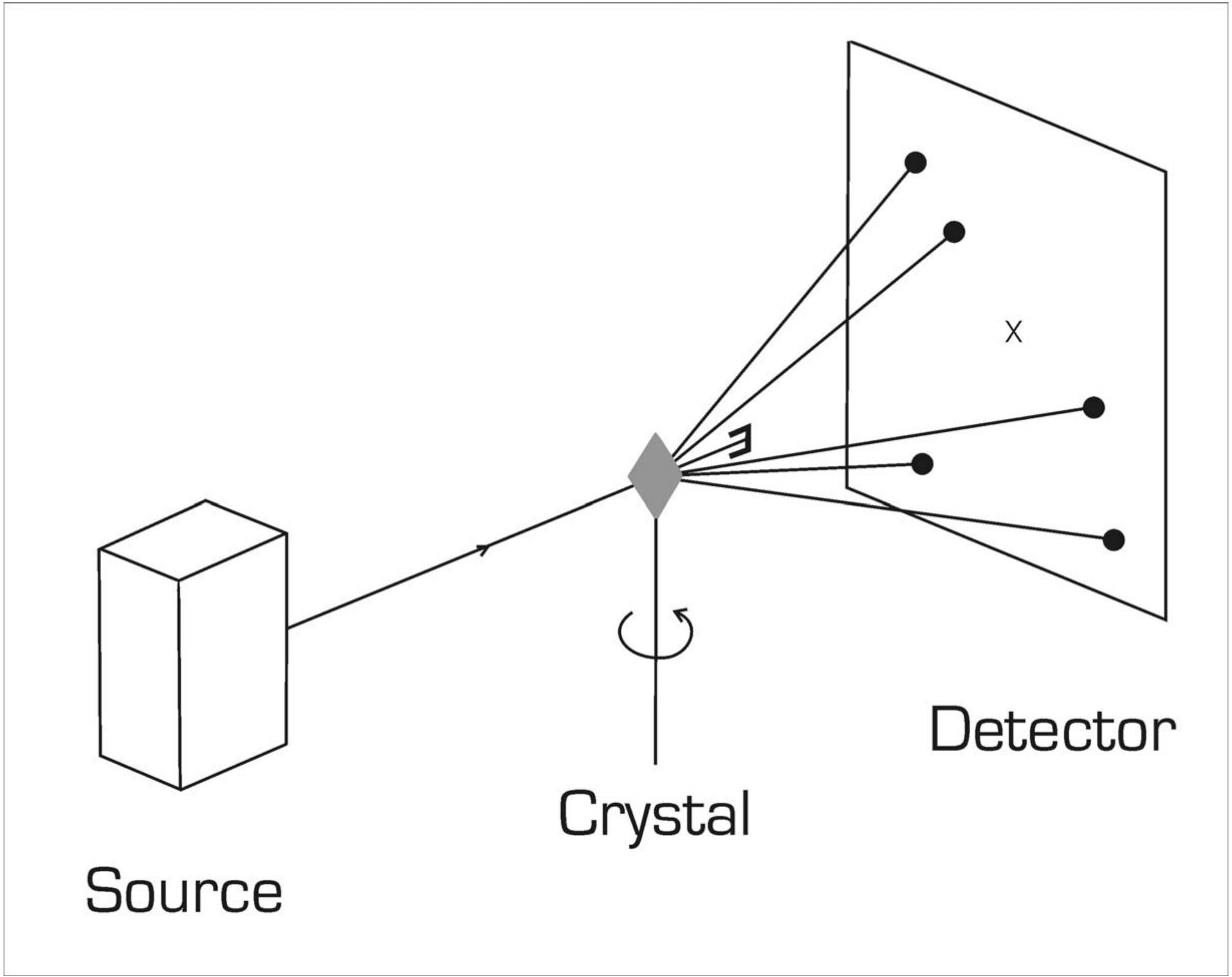
$h, k, l, I, \sigma(I)$

$\rho(x,y,z)$

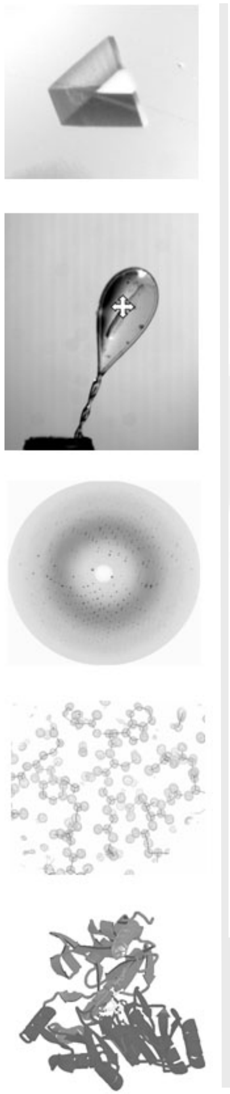
Structure

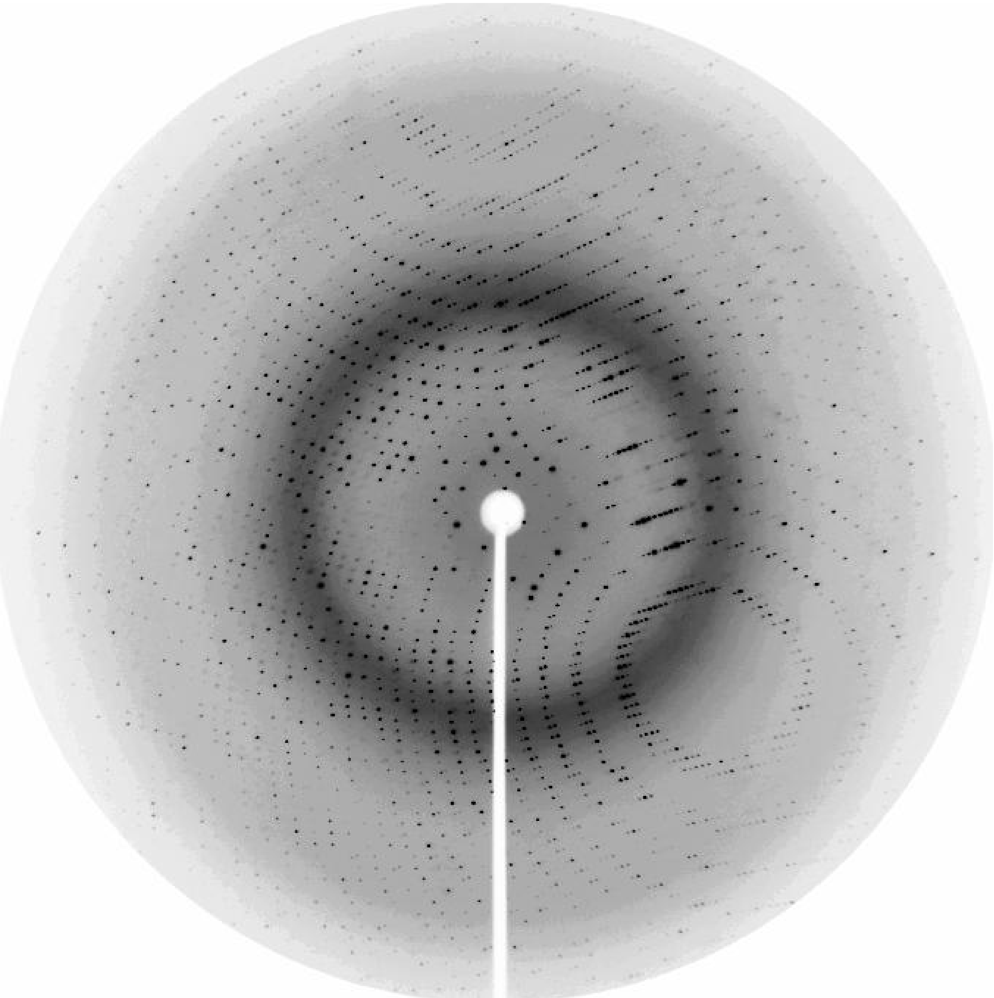
Diffraction Data Collection

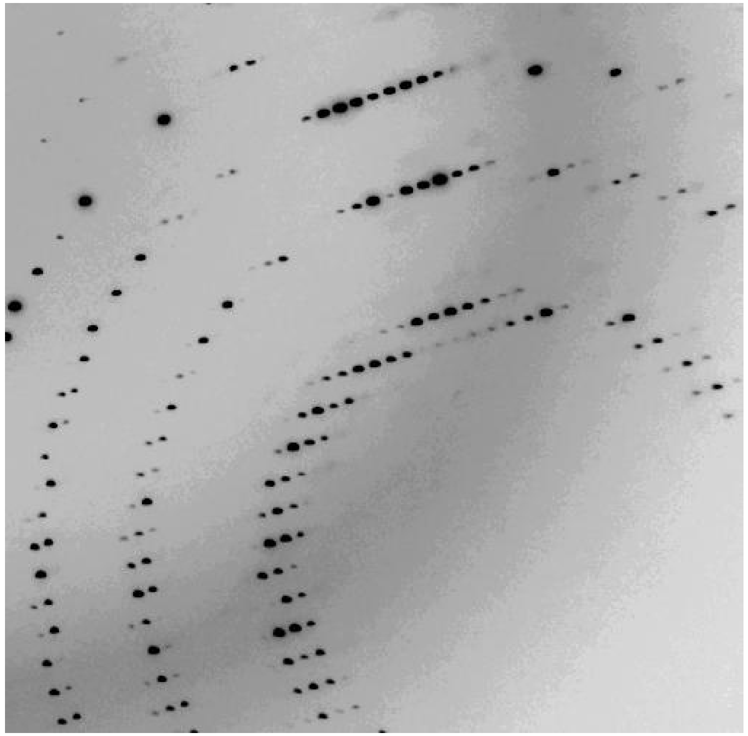




Diffraction Data Collection



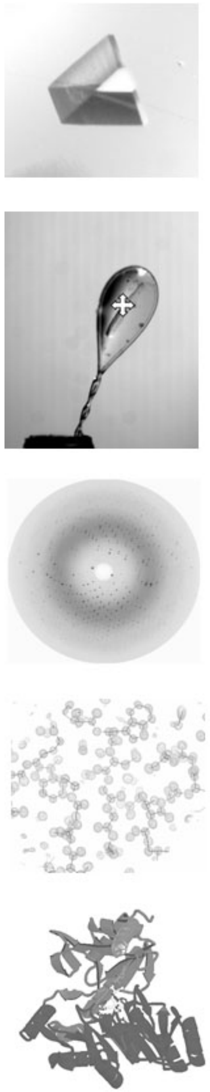


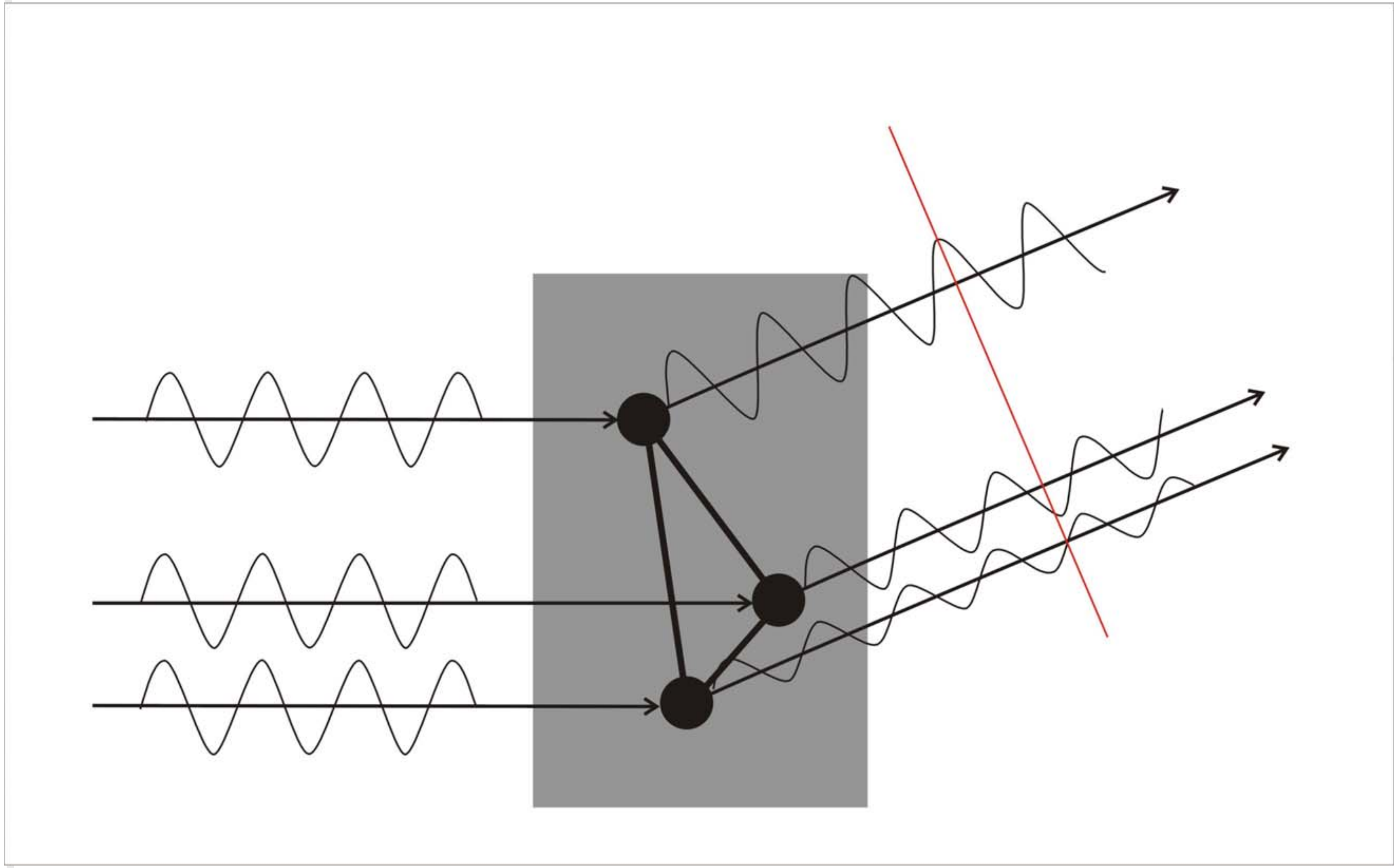


h, k, l **Miller indices**
I(h,k,l) **intensity**
σI(hkl)

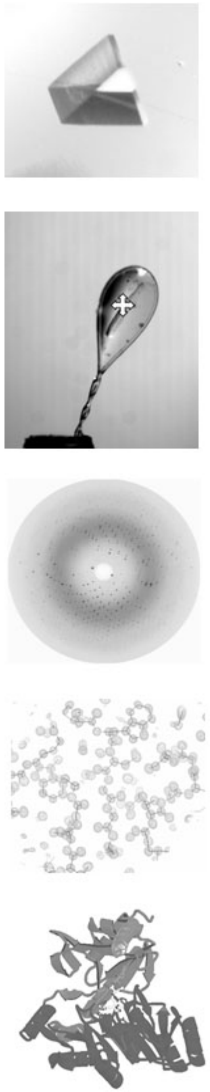
423	427	430	438	446	429	466	463	471	465	436	452	457	420	429
428	437	452	444	479	463	493	523	481	498	508	492	468	454	443
444	443	462	467	511	539	552	553	569	577	542	517	510	487	468
448	457	491	502	594	615	655	685	662	687	645	582	548	517	488
473	484	537	577	670	754	852	884	934	966	857	738	634	577	516
493	515	593	656	830	1051	1511	1932	2284	2337	1847	1128	764	622	547
502	529	617	740	1168	2605	5824	10432	14677	14750	8939	3090	1039	655	563
513	521	636	868	2304	9173	21188	35982	44400	38837	20638	5866	1285	692	564
491	533	646	957	2829	12021	26401	38395	41797	31614	15800	4664	1200	681	573
504	543	649	878	2074	6871	12827	16143	15621	11003	5464	2072	911	655	568
493	546	611	749	1202	2287	3357	3725	3356	2413	1560	991	717	583	514
487	513	578	632	783	933	1094	1141	1114	998	868	724	608	525	489
484	488	533	559	632	689	737	747	750	709	667	603	562	511	468
462	484	486	509	535	574	595	592	608	587	552	524	506	480	449
451	455	465	474	486	506	524	517	514	509	489	479	470	455	433

The Structure Factor Equation





The Structure Factor Equation

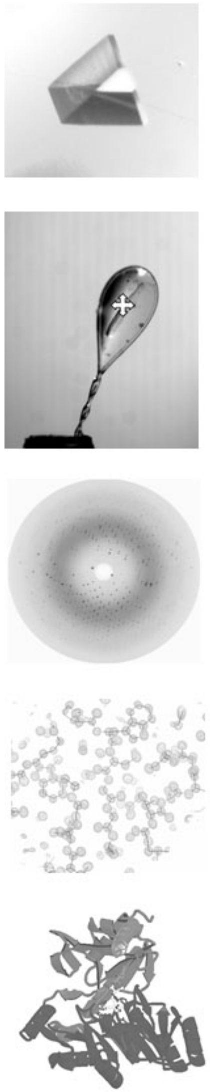


The calculation of F(hkl) from a structure (x_j,y_j,z_j) is nothing but a summation of the waves originating from each atom (j) in the direction defined by (hkl).

$$F(hkl) = \sum_j f_j \, e^{2\pi i(hx_j+ky_j+lz_j)}$$

It is important to note that every atom of the structure contributes to each and every reflection of the diffraction pattern.

The Structure Factor Equation

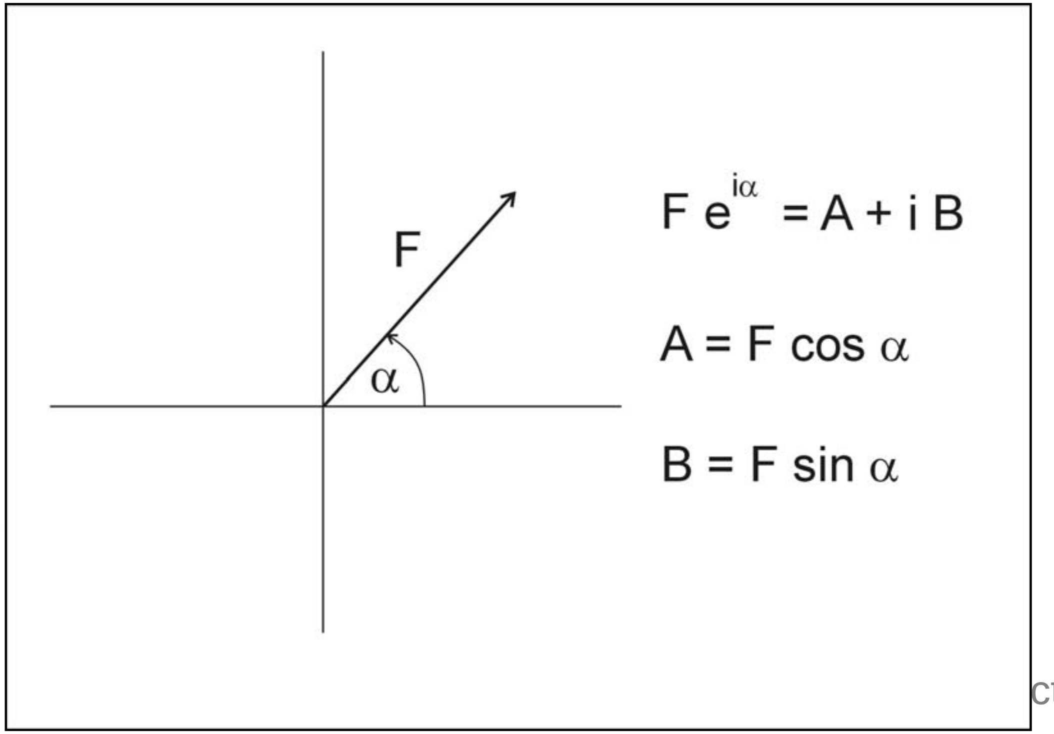


$$F(hkl) = |F(hkl)|\, e^{i\alpha(hkl)} = \sum_j f_j\, e^{2\pi i(hx_j+ky_j+lz_j)}$$

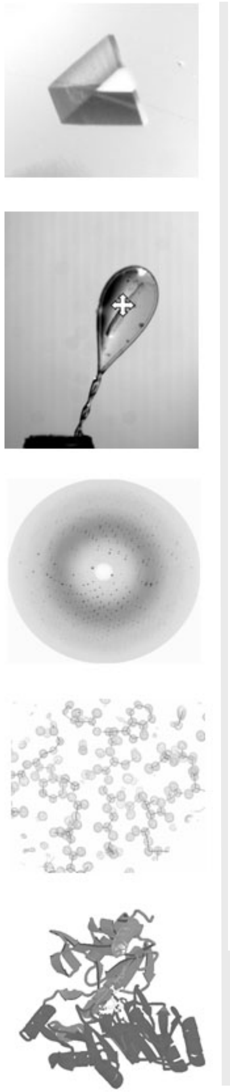
Structure factor amplitude
$|F(hkl)| \propto I(hkl)^{1/2}$

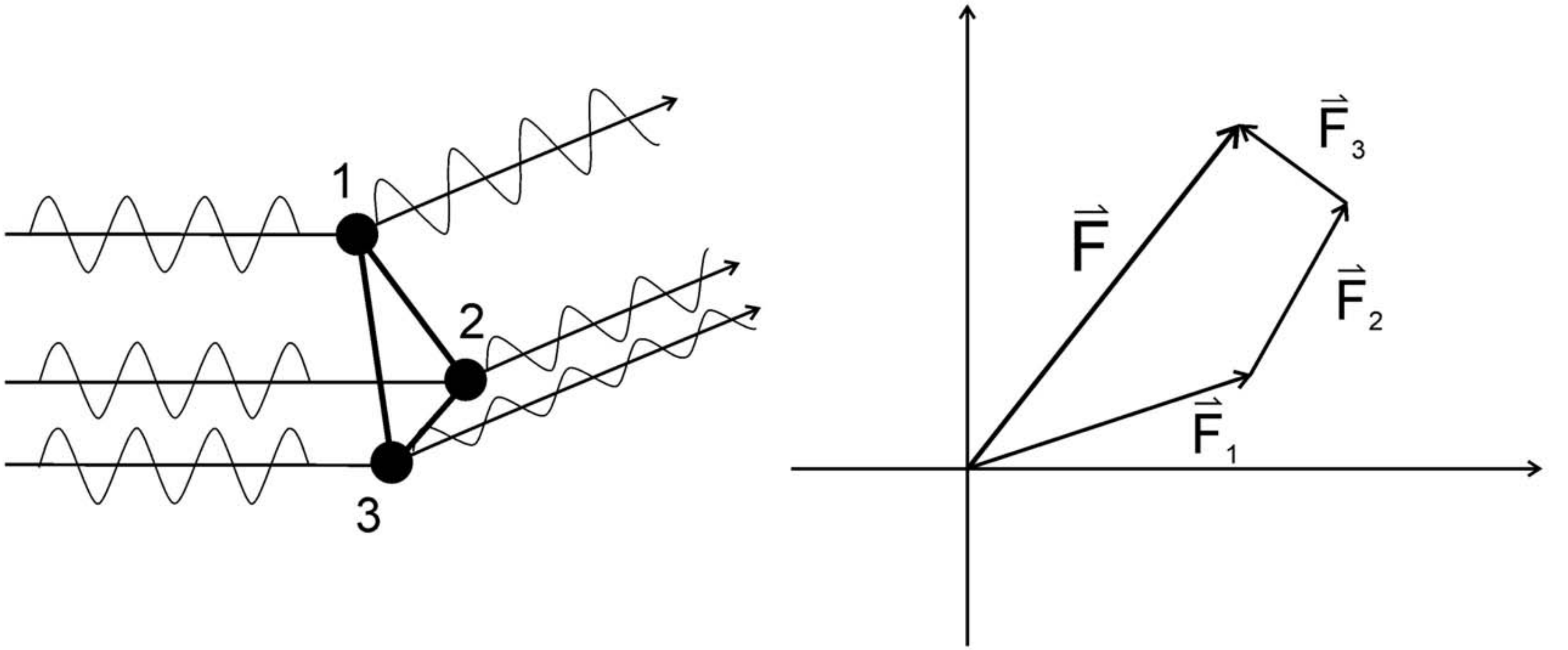
Atomic form factor f_j

Phase $\alpha(hkl)$

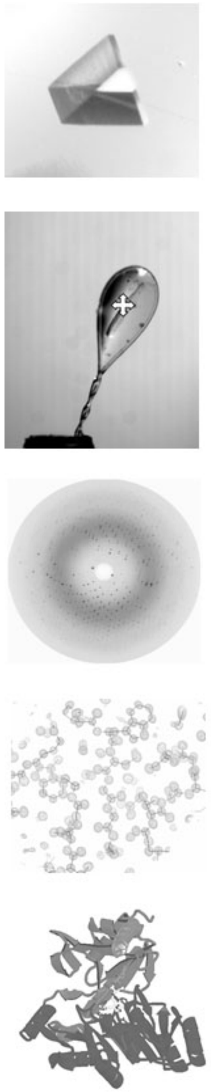


The Structure Factor Equation





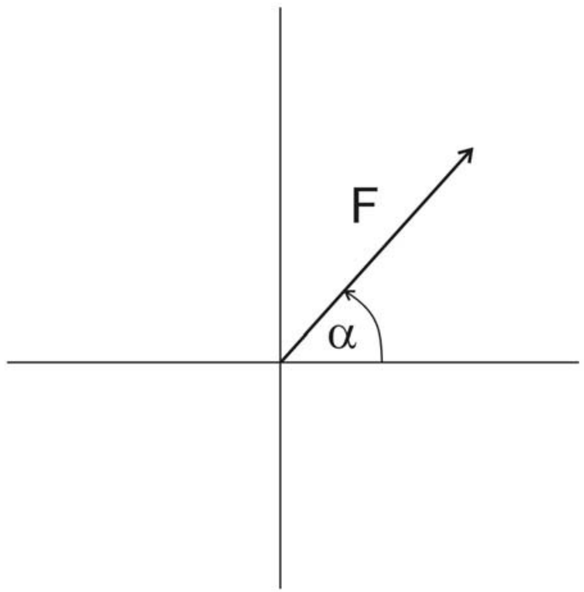
The Electron Density Equation



$$\rho(x,y,z) = 1/V\ \Sigma_{hkl}\ |F(hkl)|\ e^{i\alpha(hkl)}\ e^{-2\pi i(hx+ky+lz)}$$

Structure factor amplitude
$|F(hkl)| \propto I(hkl)^{1/2}$

Phase α(hkl)

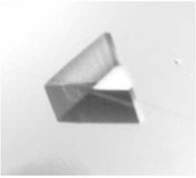


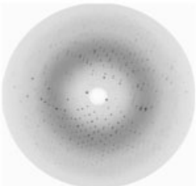
$F e^{i\alpha} = A + i B$

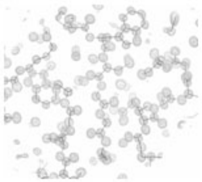
$A = F \cos \alpha$

$B = F \sin \alpha$

The Electron Density Equation





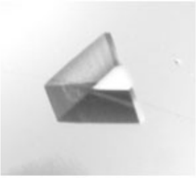


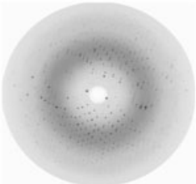
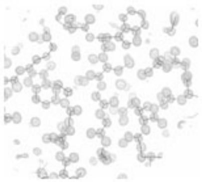
The electron density $\rho(x,y,z)$ is a three-dimensional function (with the unit e/Å^3), which describes where in the unit cell of the crystal the electrons (and therefore the atoms) are. It is basically the image of the structure we want to determine.

$$\rho(x,y,z) = 1/V \sum_{hkl} |F(hkl)|\, e^{i\alpha(hkl)}\, e^{-2\pi i(hx+ky+lz)}$$

It is important to note that every reflection (hkl) of the diffraction pattern contributes to the electron density ρ at each and every position (xyz) in the unit cell of the crystal.

The Phase Problem




From the diffraction pattern, we can only obtain the intensities I(hkl) of the reflections (hkl).

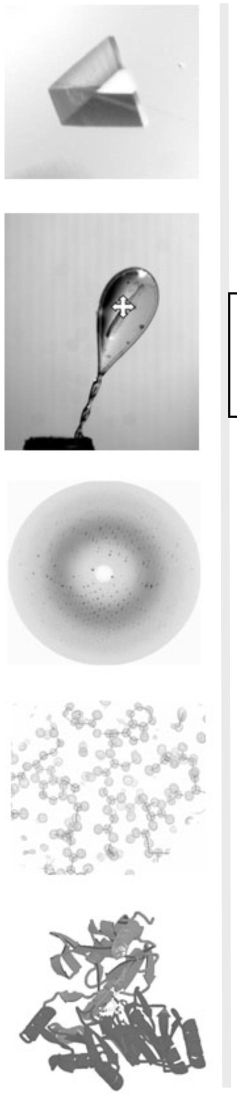
$$I(hkl) \propto F(hkl) \cdot F^*(hkl)$$

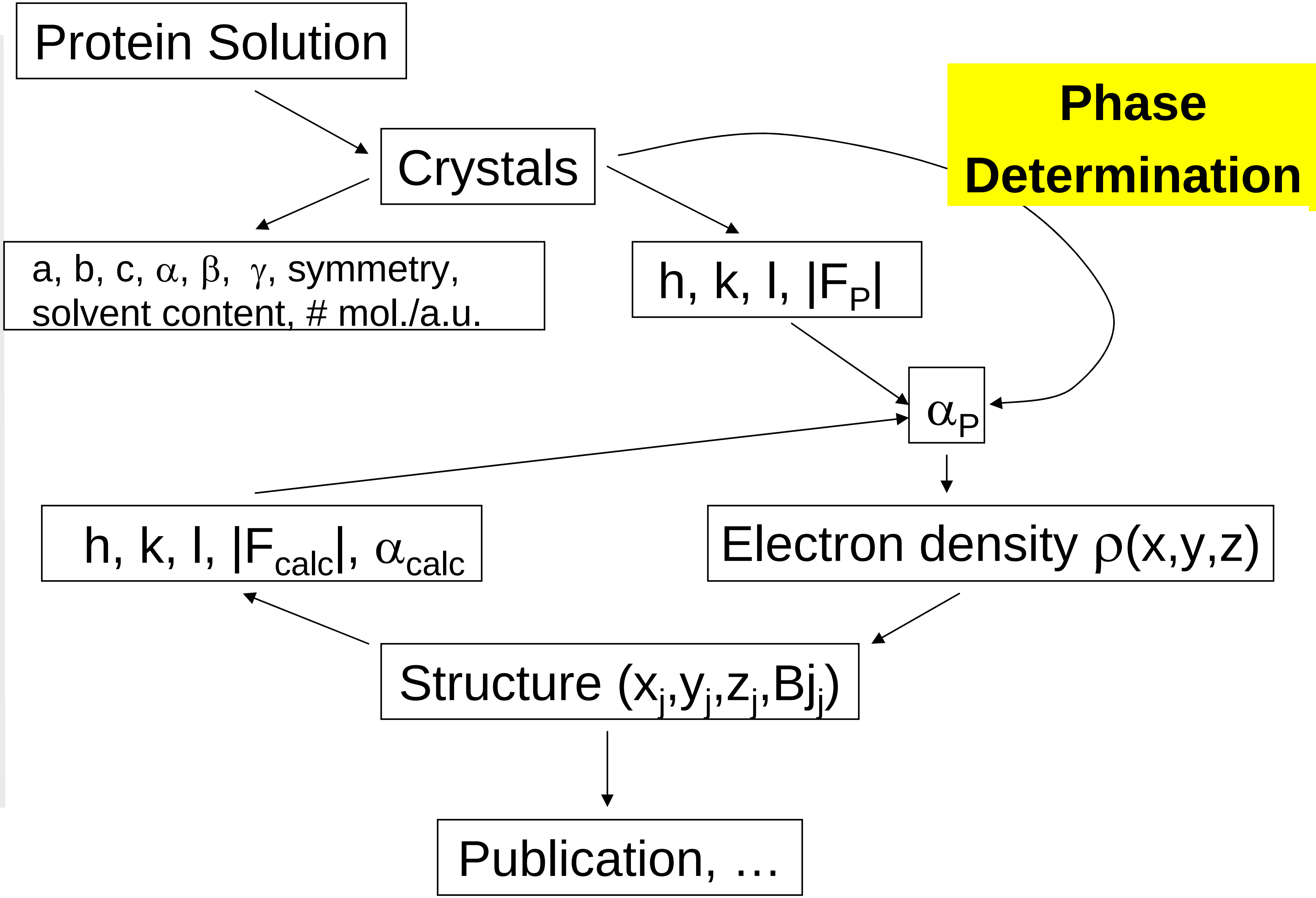
$$= |F(hkl)|\, e^{i\alpha(hkl)} \cdot |F(hkl)|\, e^{-i\alpha(hkl)}$$

$$= |F(hkl)|^2$$

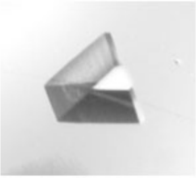
The phase $\alpha(hkl)$ cannot be measured.

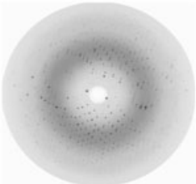
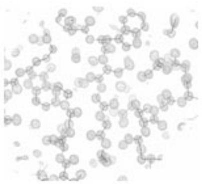
A Flowchart of a Crystal Structure Analysis





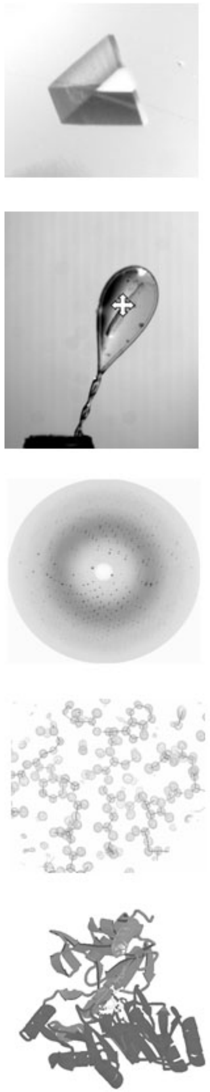
Phase Determination Methods

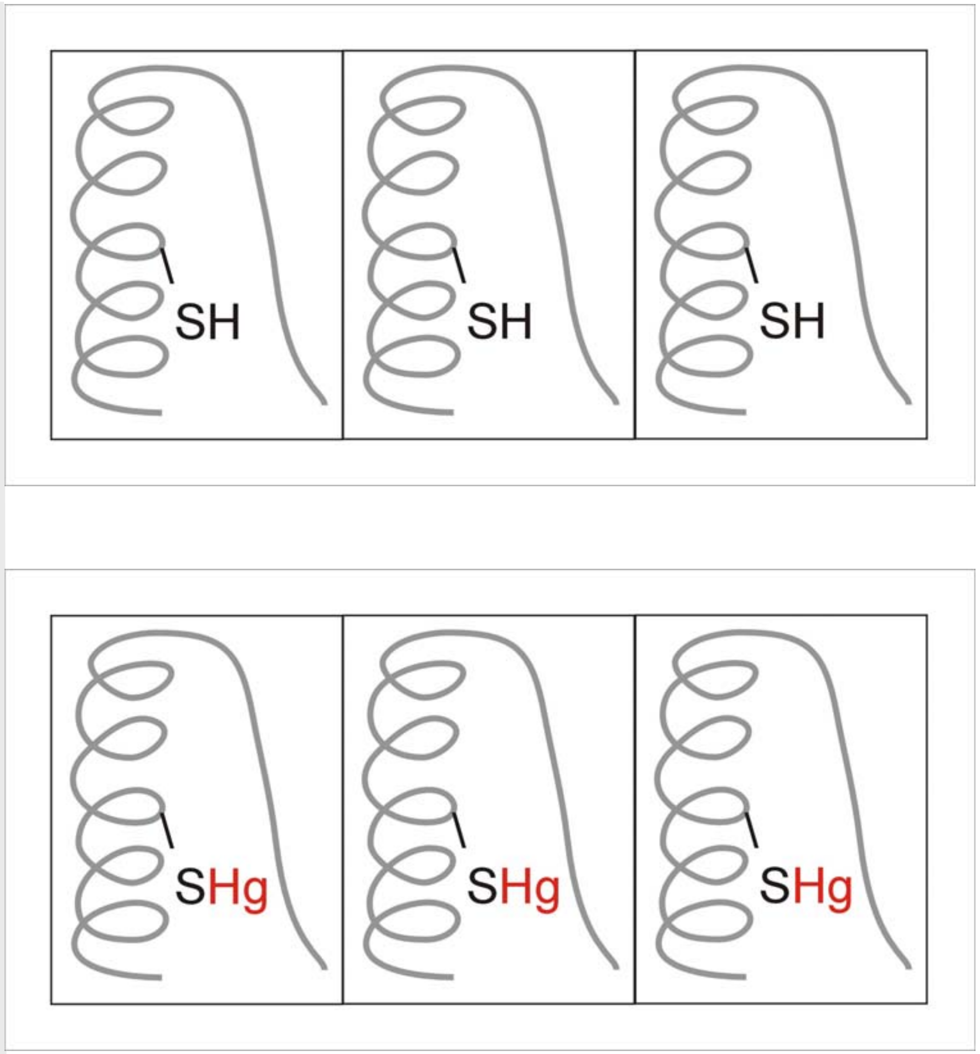



1. SIR, SIRAS, MIR, MIRAS
   (single/multiple isomorphous replacement with anomalous scattering)
2. MAD
   (multiple wavelength anomalous diffraction)
3. SAD (SAS)
   (single wavelength anomalous diffraction/scattering)
4. RIP, RIPAS
   (radiation damage induced phasing with anomalous scattering)
5. MR
   (molecular replacement)
6. Direct Methods

Isomorphous Replacement



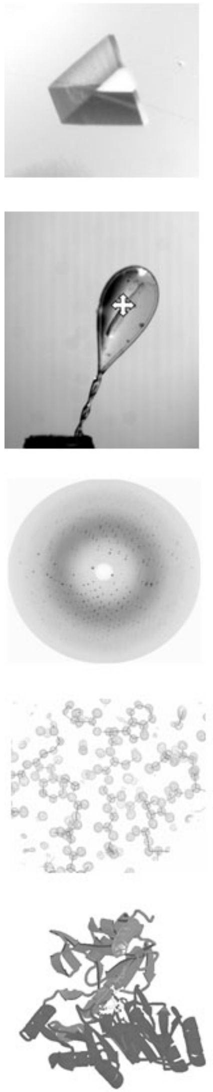


$\rightarrow$ h, k, l, $|F_P(hkl)|$

$$\alpha_P(hkl)$$

$\rightarrow$ h, k, l, $|F_{PH}(hkl)|$

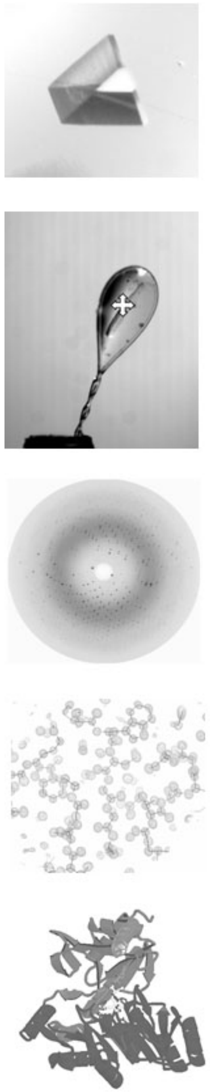
Isomorphous Replacement



<u>Step 1</u>: put $|F_P(hkl)|$ and $|F_{PH}(hkl)|$ on the same scale

$$(R = 100 \cdot \Sigma_{hkl} \, | \, |F_{PH}| - |F_P| \, | \, / \, \Sigma_{hkl} \, |F_P| \,)$$

Isomorphous Replacement



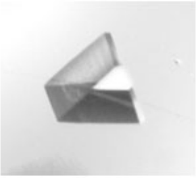
Step 1: put $|F_P(hkl)|$ and $|F_{PH}(hkl)|$ on the same scale

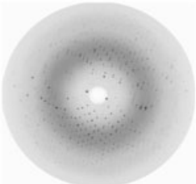
$$(R = 100 \bullet \Sigma_{hkl} \mid |F_{PH}| - |F_P| \mid / \Sigma_{hkl} |F_P|)$$

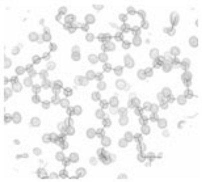
Step 2 : determine the positions of the heavy atoms (F_H)

from the differences ($|F_{PH}(hkl)| - |F_P(hkl)|$)

Isomorphous Replacement







Step 1: put $|F_P(hkl)|$ and $|F_{PH}(hkl)|$ on the same scale

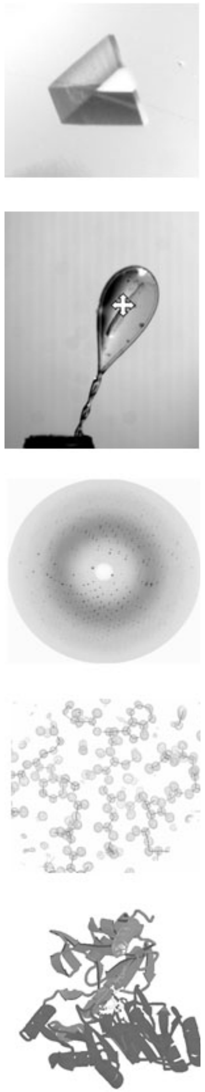
$$(R = 100 \bullet \Sigma_{hkl} \, | \, |F_{PH}| - |F_P| \, | \, / \, \Sigma_{hkl} \, |F_P|)$$

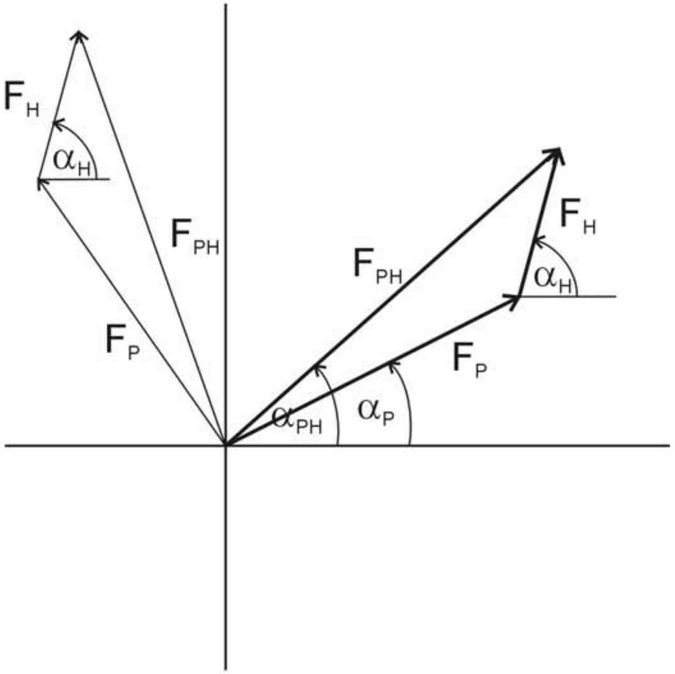
Step 2 : determine the positions of the heavy atoms (F_H)

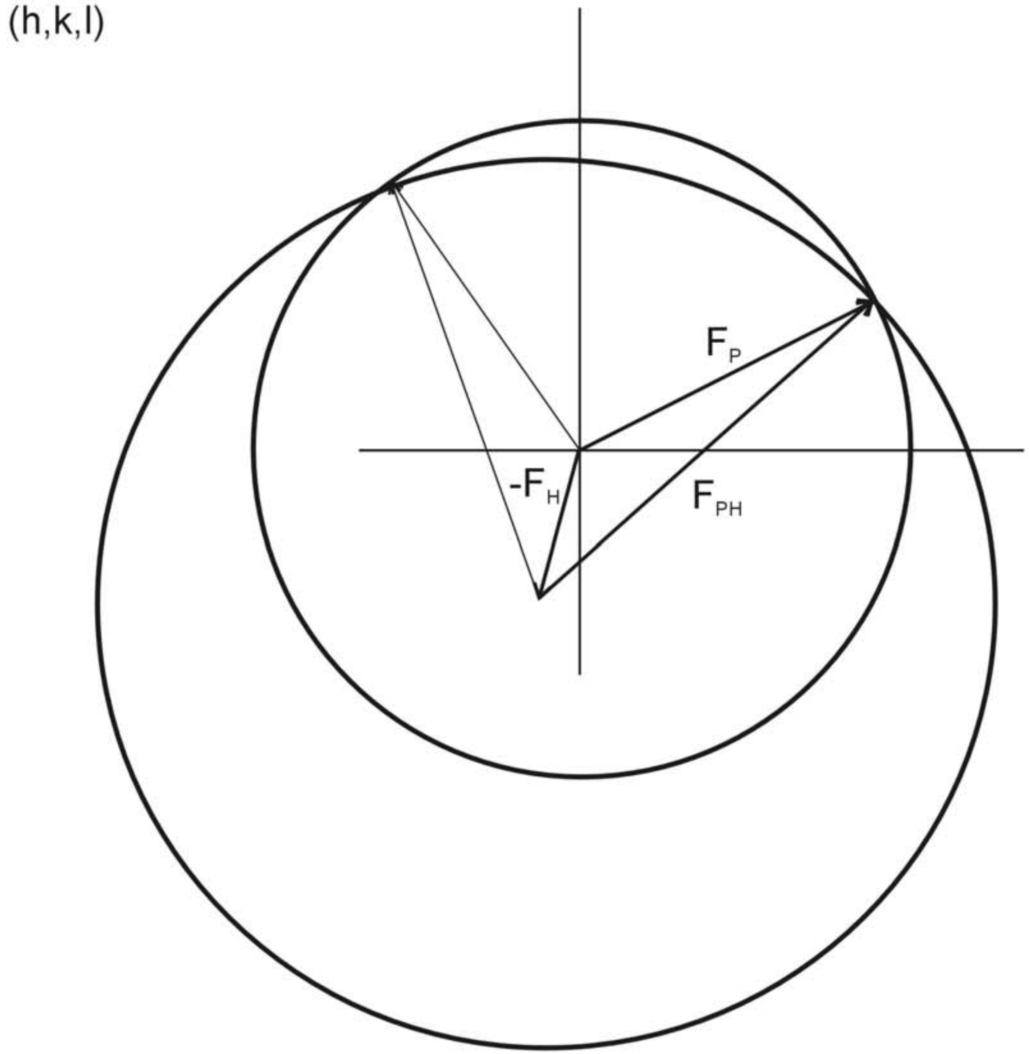
from the differences ($|F_{PH}(hkl)| - |F_P(hkl)|$)

Step 3 : calculate $\alpha_P(hkl)$ from $|F_P(hkl)|$, $|F_{PH}(hkl)|$ and F_H

The Harker Construction - SIR



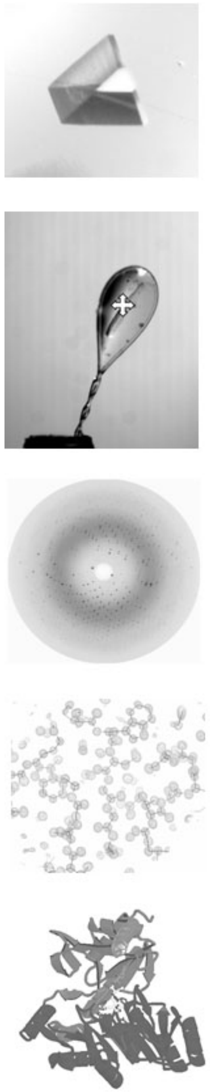




$$F_P + F_H = F_{PH}$$

$$F_P = -F_H + F_{PH}$$

The Harker Construction - SIR

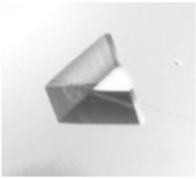


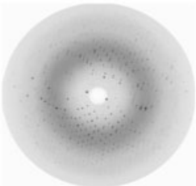
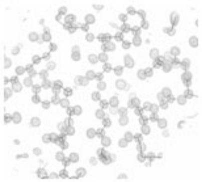
Instead of one value for α_P(hkl) we obtain two possibilities

→ *phase ambiguity*

How can this be made unambiguous ?

The Harker Construction - SIR



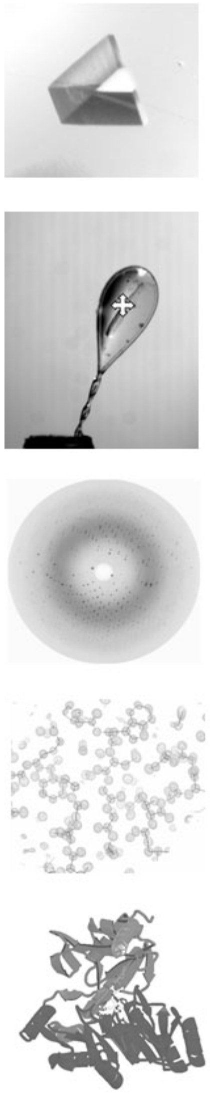

Instead of one value for α_P(hkl) we obtain two possibilities

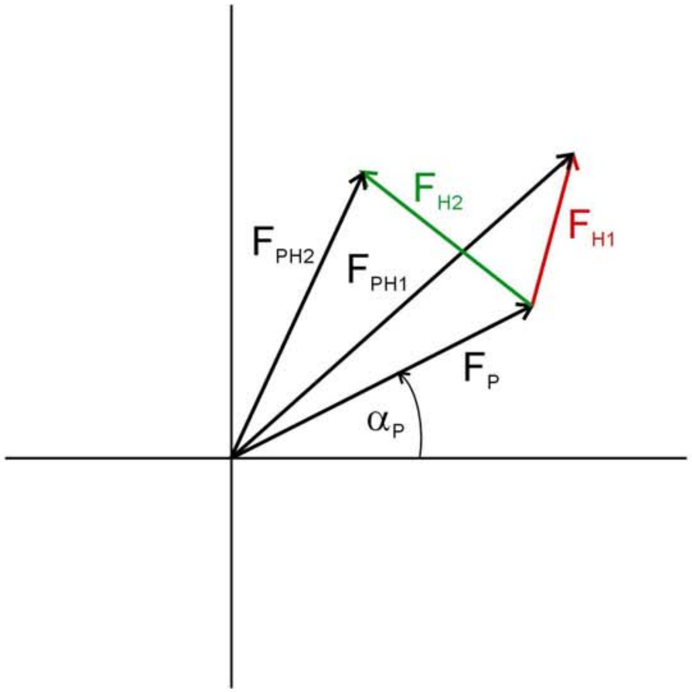
→ *phase ambiguity*

How can this be made unambiguous ?

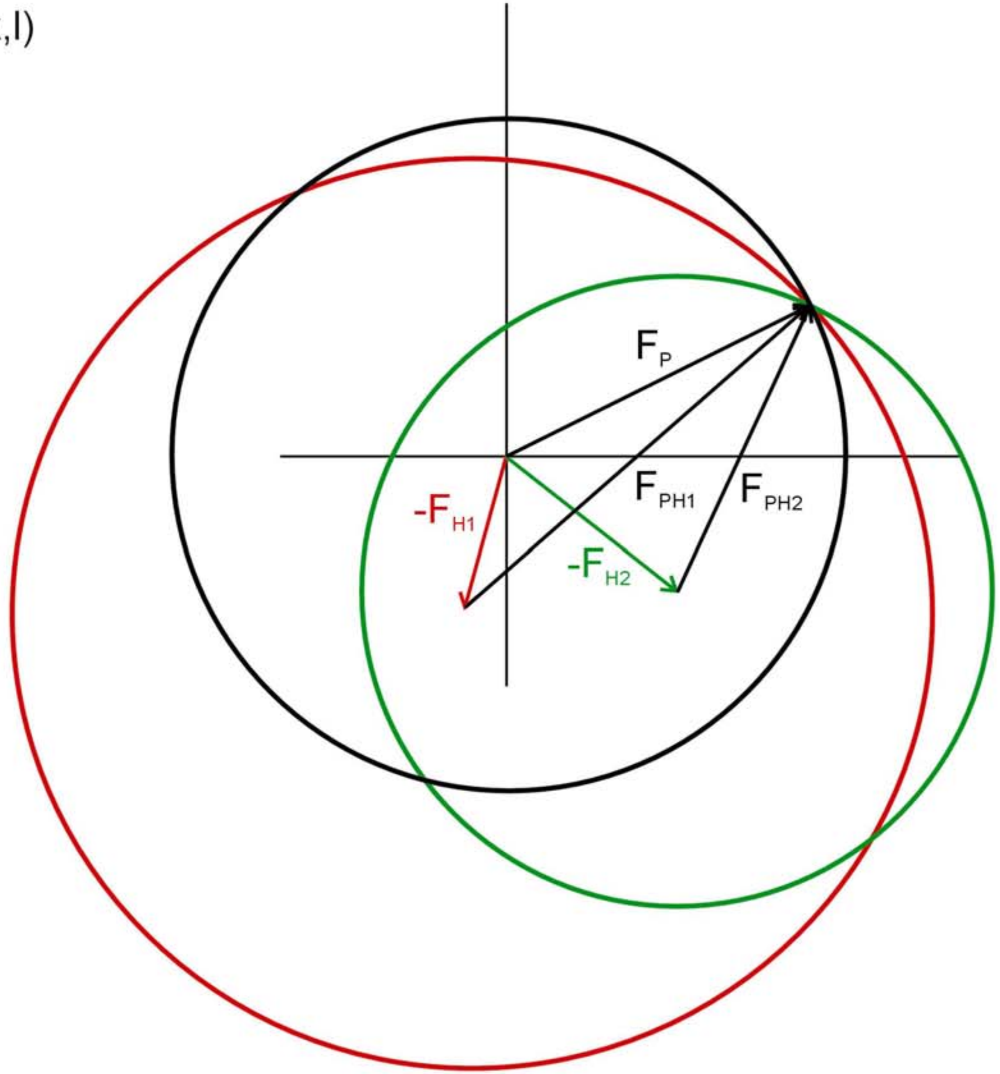
→ a second heavy atom derivative (MIR)

The Harker Construction - MIR

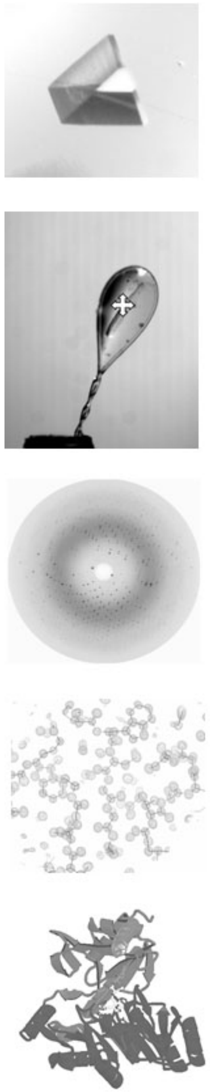




(h,k,l)



The Harker Construction - SIR



Instead of one value for α_P(hkl) we obtain two possibilities

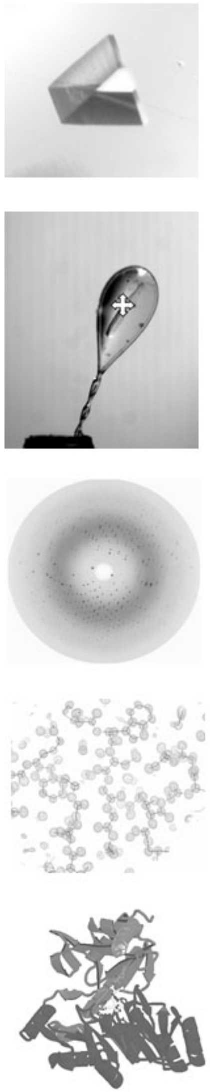
→ *phase ambiguity*

How can this be made unambiguous ?

→ a second heavy atom derivative (MIR)

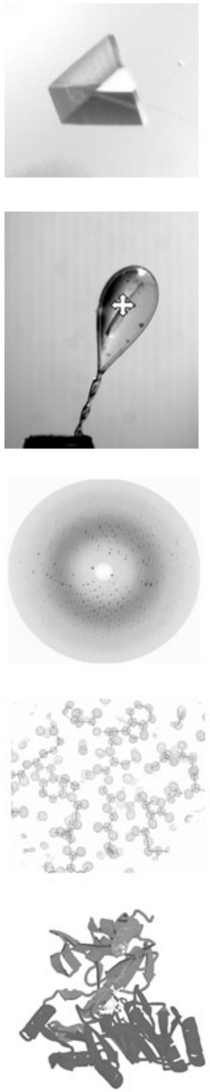
→ density modification

Density Modification



Density Modification is a technique that uses additional information to improve an electron density

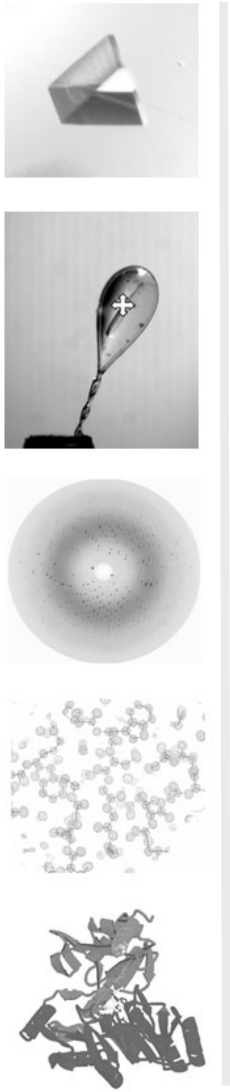
Density Modification

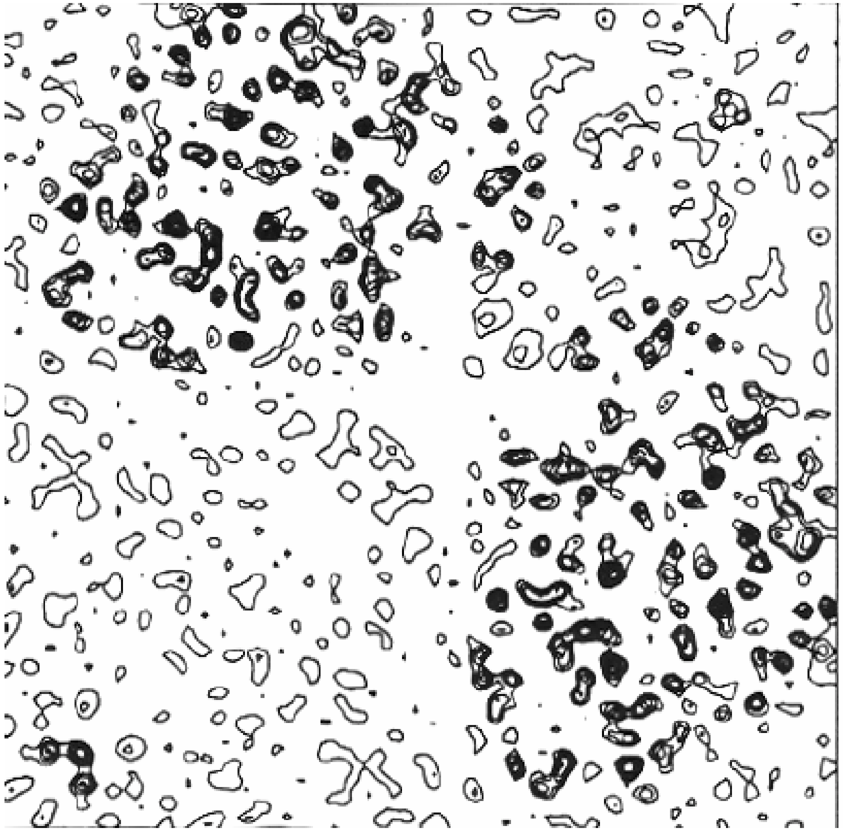


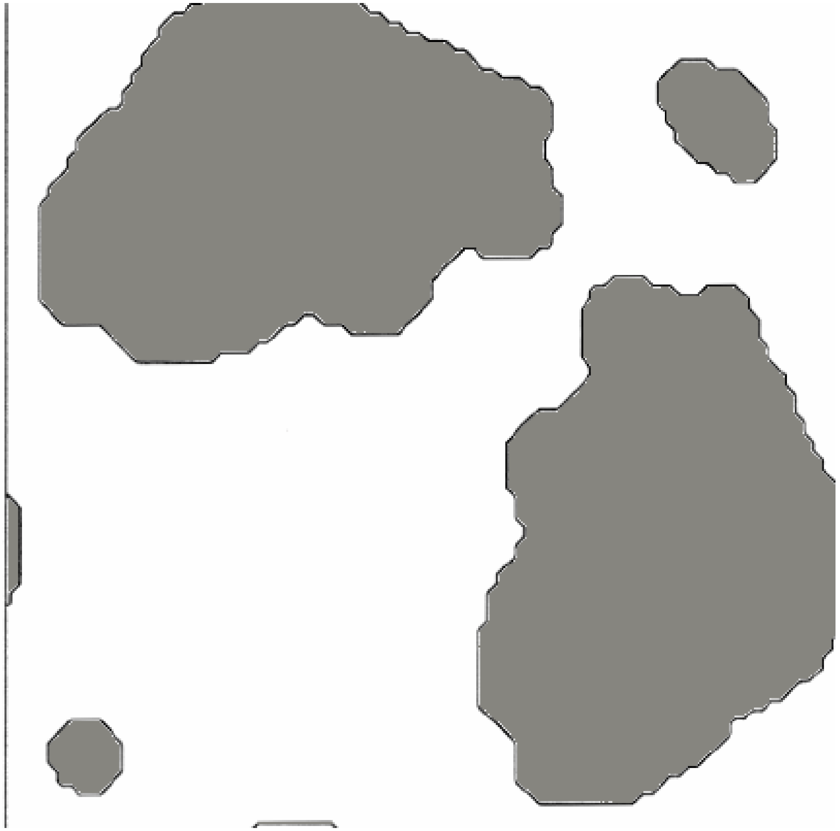
Density Modification is a technique that uses additional information to improve an electron density

- ❑ solvent flattening

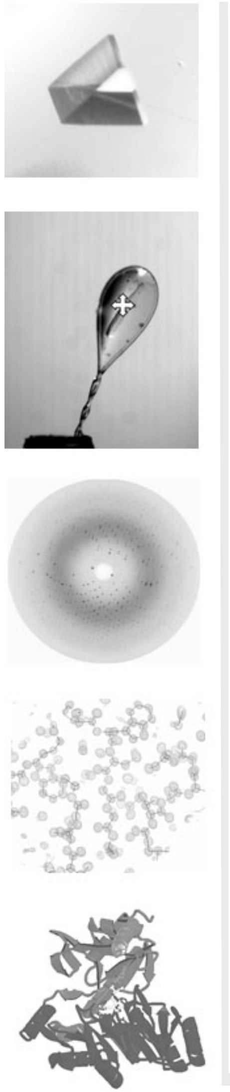
Solvent Flattening

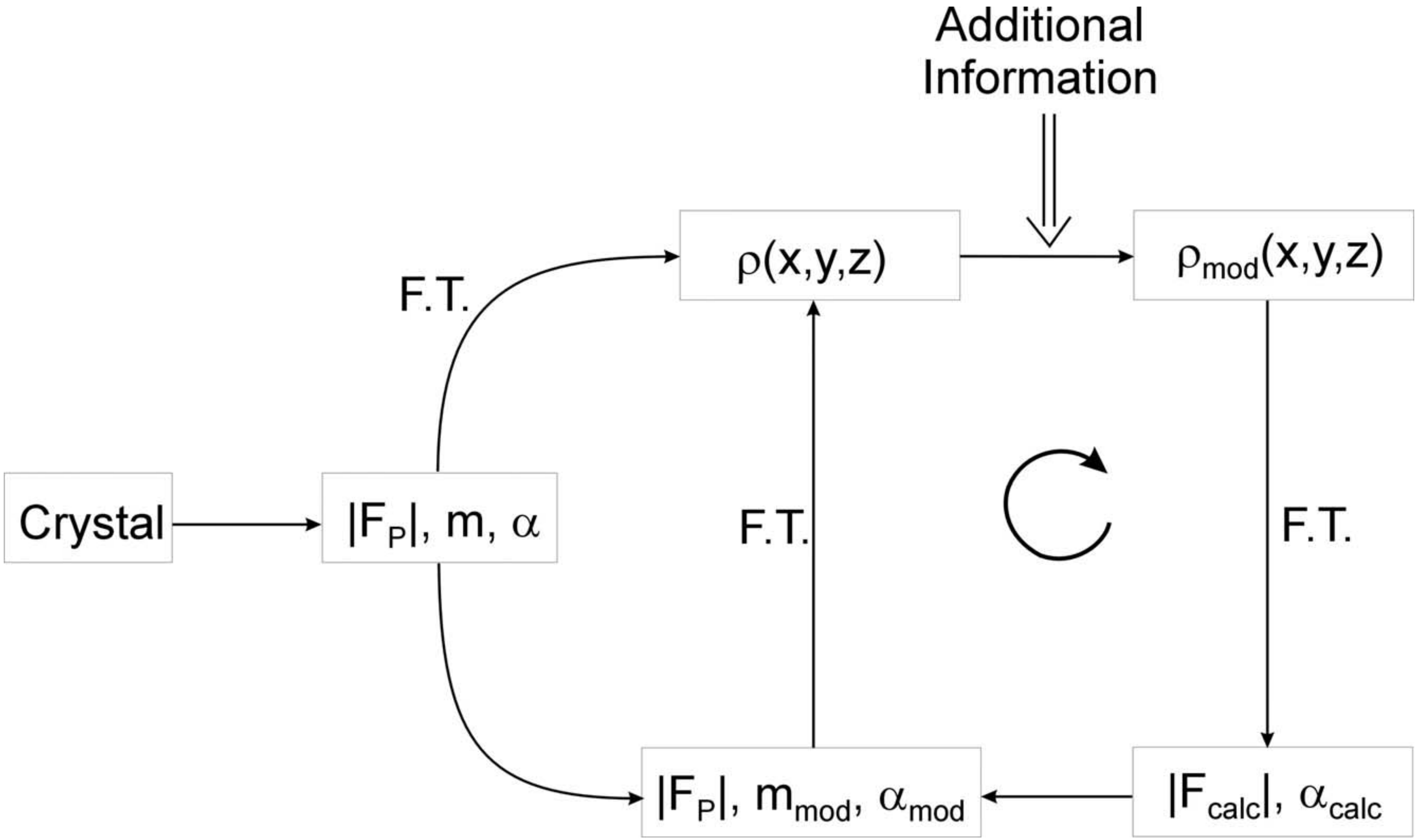




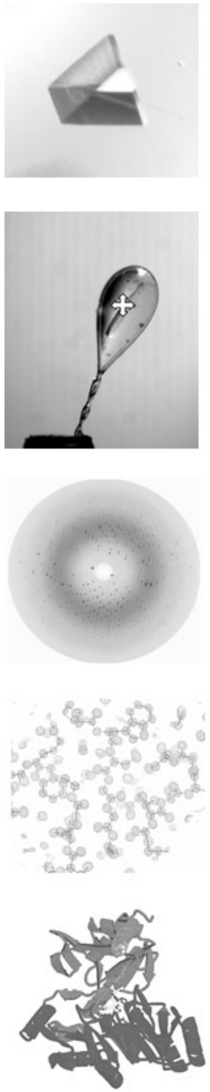


Density Modification





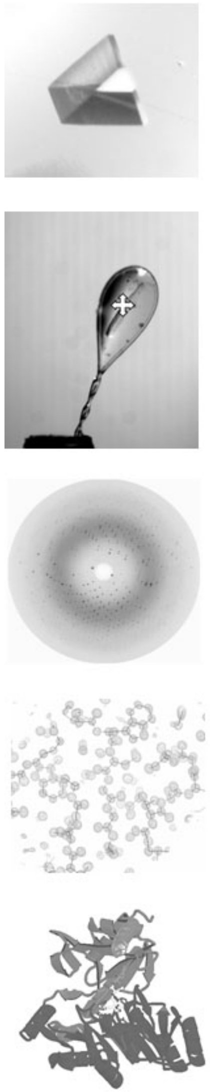
Density Modification



Density Modification is a technique that uses additional information to improve an electron density

- ❑ solvent flattening
- ❑ non-crystallographic symmetry averaging
- ❑ histogram matching
- ❑ map interpretation and refinement

The Harker Construction - SIR



Instead of one value for α_P(hkl) we obtain two possibilities

→ *phase ambiguity*

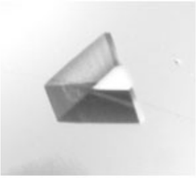
How can this be made unambiguous ?

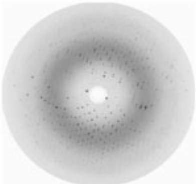
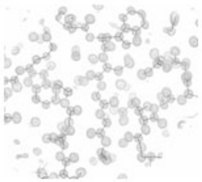
→ a second heavy atom derivative (MIR)

→ density modification

→ incorporation of anomalous scattering (SIRAS)

The Harker Construction - SIR




Instead of one value for α_P(hkl) we obtain two possibilities

→ *phase ambiguity*

How can this be made unambiguous ?

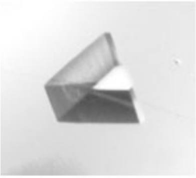
→ a second heavy atom derivative (MIR)

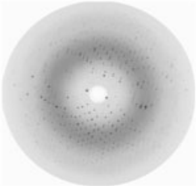
→ density modification

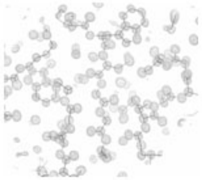
→ incorporation of anomalous scattering (SIRAS)

→ second derivative plus anomalous scattering (MIRAS)

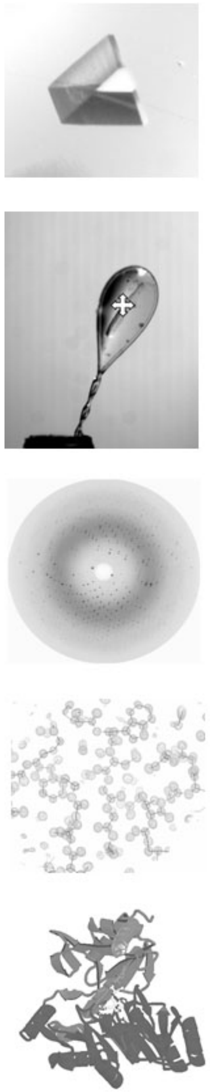
Anomalous Scattering



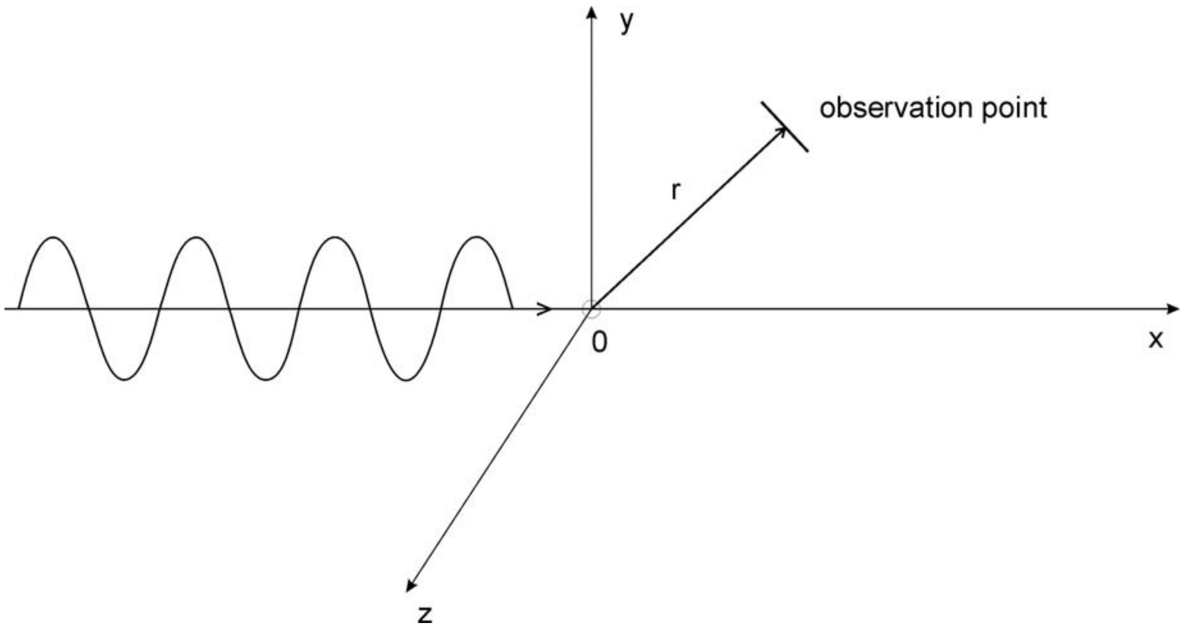




Normal Scattering - 1

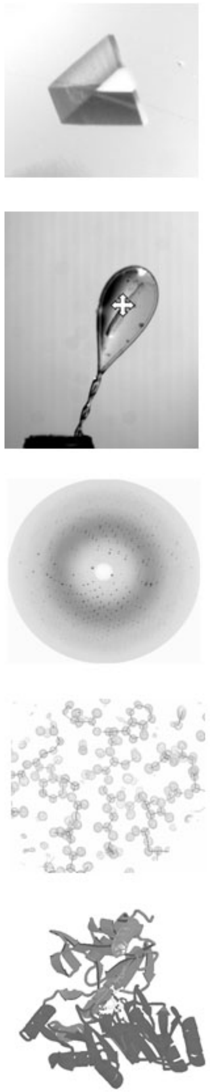


aka Elastic Scattering or Thomson Scattering or Scattering without Loss of Energy

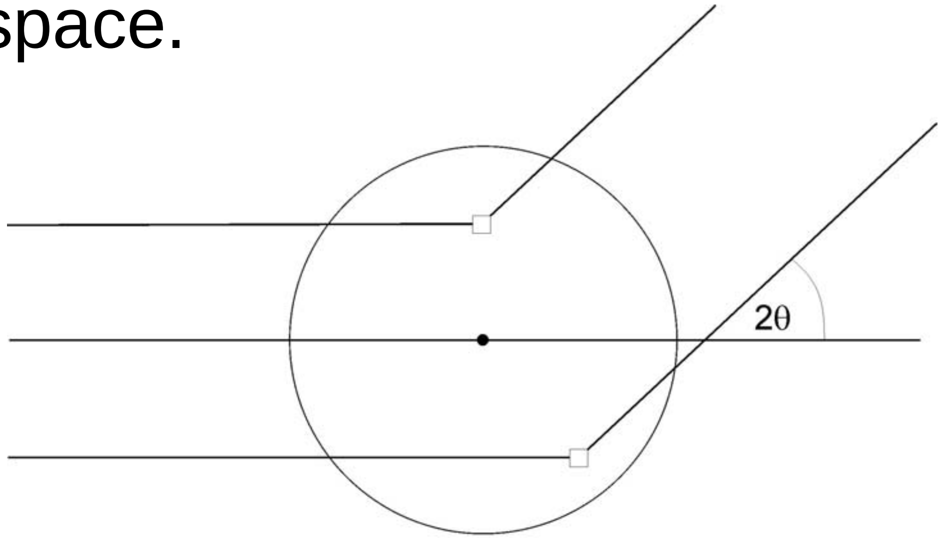


An X-ray wave impinges on an atom and causes the electrons to oscillate in the same frequency as the incoming X-ray wave.

Normal Scattering - 2



The moving electrons emanate X-rays in all directions of space.



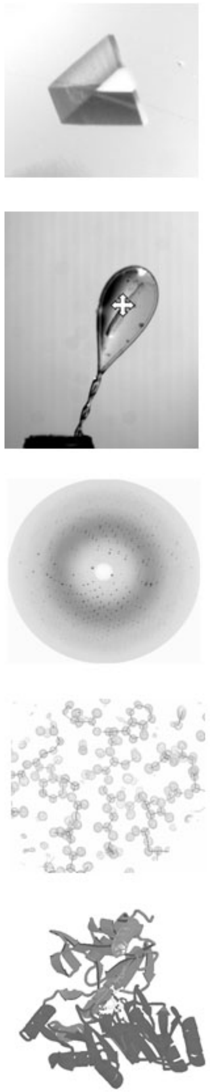
The amplitude of the scattered wave depends on the number of electrons and the scattering angle.

$$f(\vec{s}) = \int_V \rho(\vec{r}) \cdot e^{2\pi i \vec{r}\vec{s}}$$

f(s) is the atomic form factor

f(s) is a scalar function, dependent only on the atom type and 2θ.

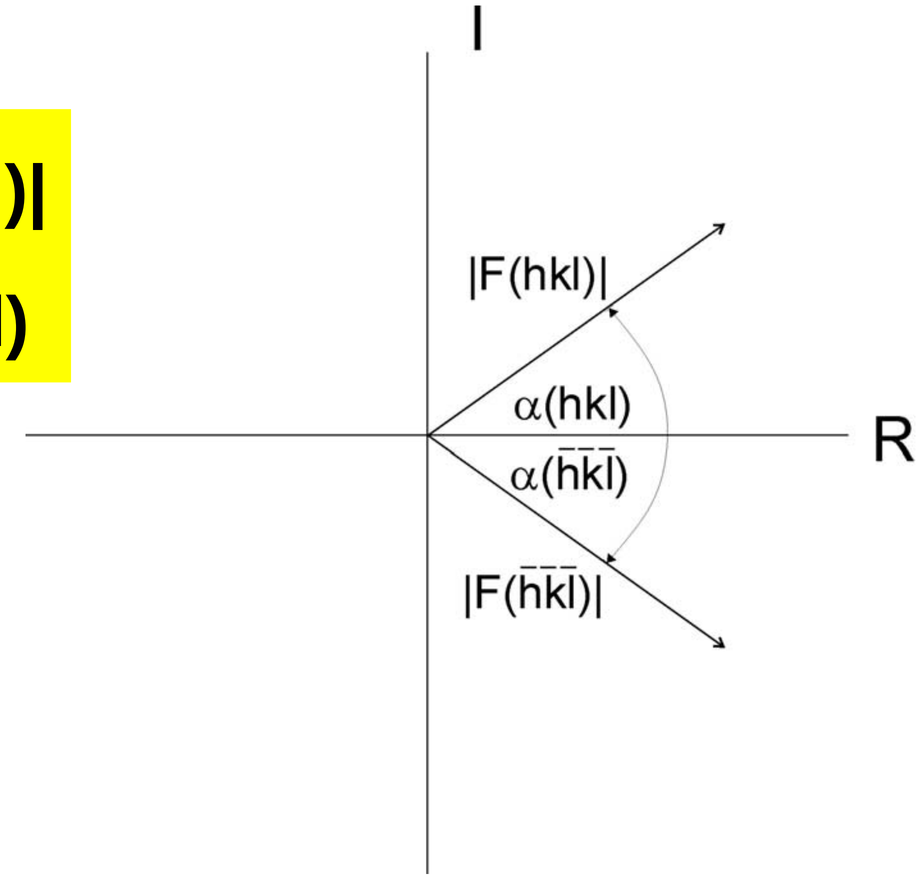
Normal Scattering - 3



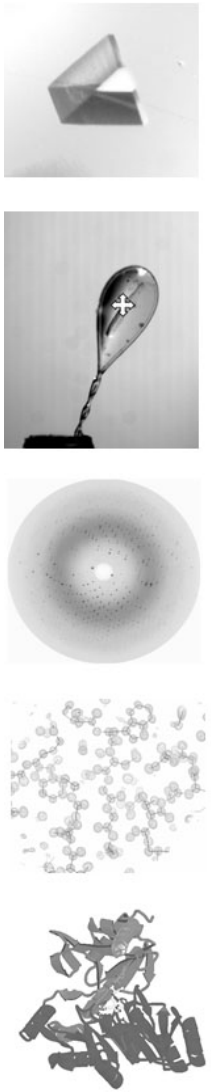
since $\rho(r) = \rho(-r)$ → f(s) = f(-s), this results eventually in Friedel's Law

|F(hkl)| = |F(-h-k-l)|

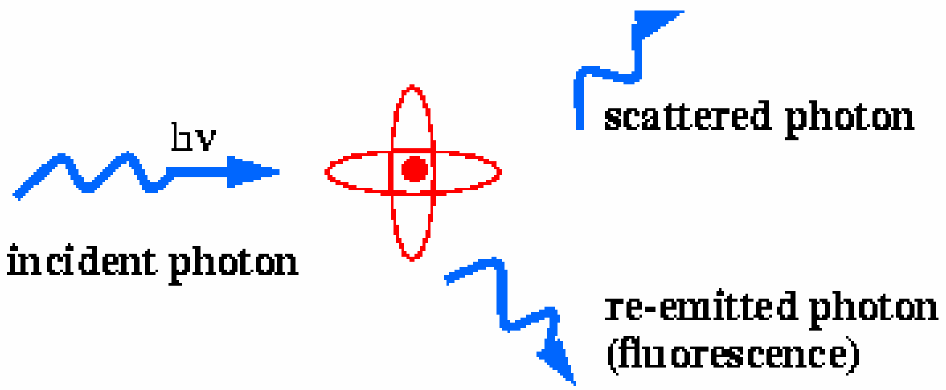
α(hkl) = - α(-h-k-l)



Anomalous Scattering - 1

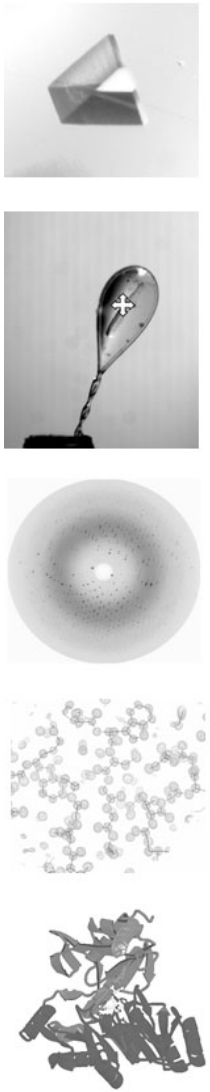


When the energy of the incident X-ray wave is close to an elemental absorption edge, something else happens in addition.

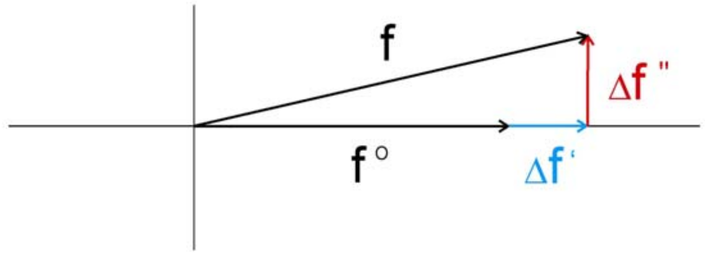


In addition to being scattered elastically, the incident X-ray wave is absorbed and ejects a core electron. A higher-shell electron then falls back to a lower shell, emitting X-radiation.

Anomalous Scattering - 2



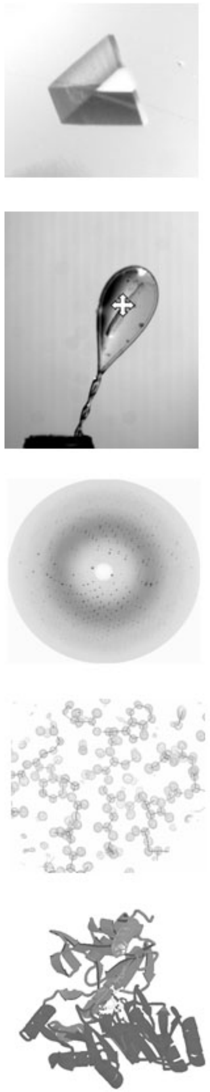
As a net result of absorption, the scattered wave is retarded and the atomic form factor becomes a vectorial quantity.



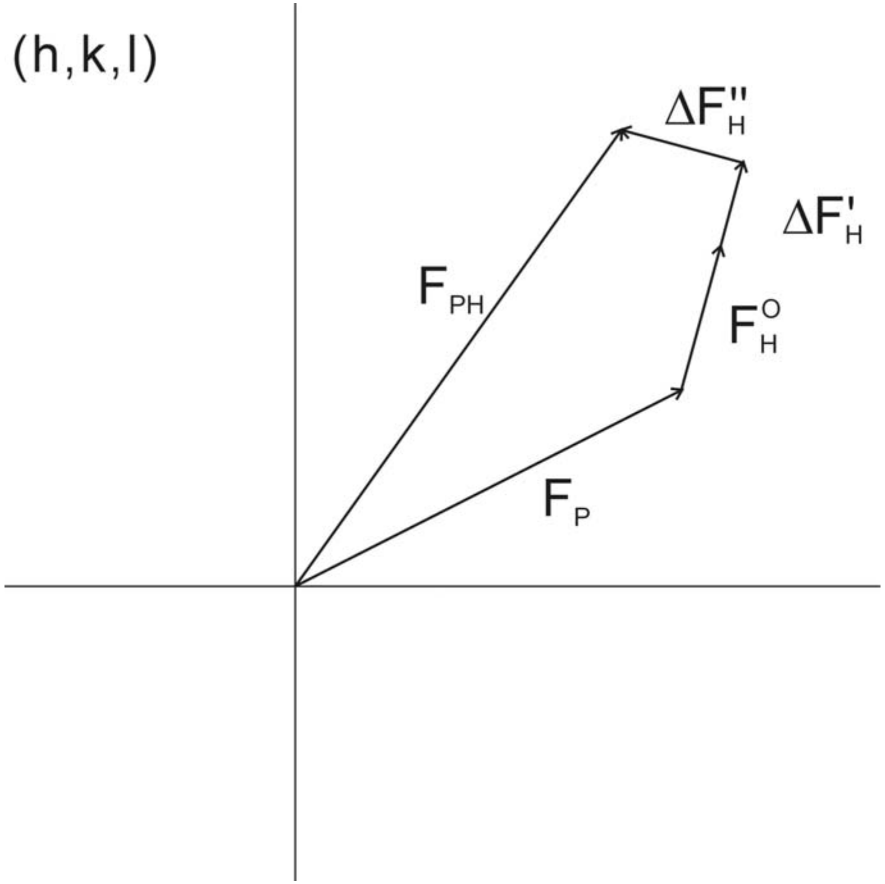
$$f = f^{\circ} + \Delta f' + i\Delta f''$$

f^{o} is the normal atomic form factor, $\Delta f'$ is called the ***dispersive component*** and $\Delta f''$ the ***anomalous component***.

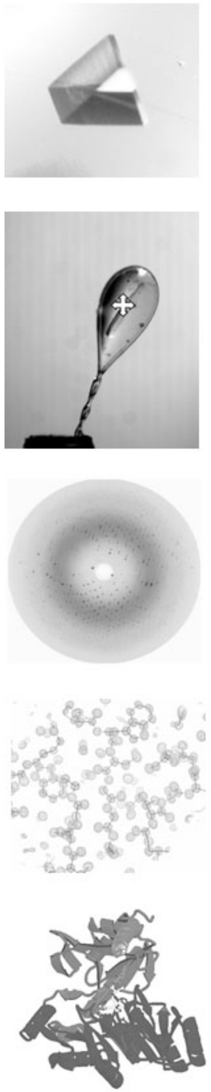
Anomalous Scattering - 3

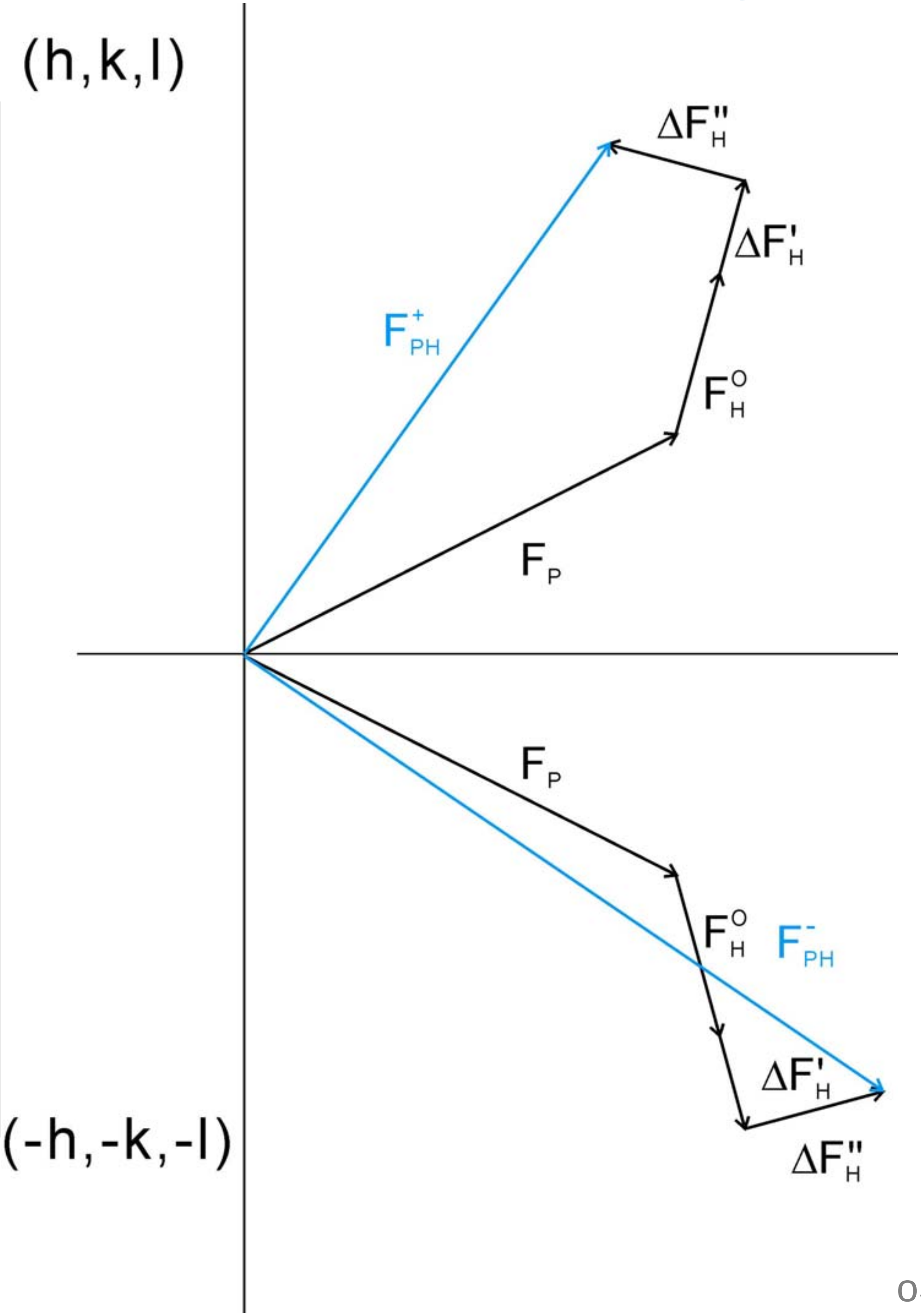


Thus, the total structure factor F_{PH} contains the ***normal scattering*** of all atoms $F_P+F_H^0$, the ***dispersive component*** of the anomalously scattering atoms $\Delta F_H'$ and the ***anomalous component*** of the anomalously scattering atoms $\Delta F_H''$.



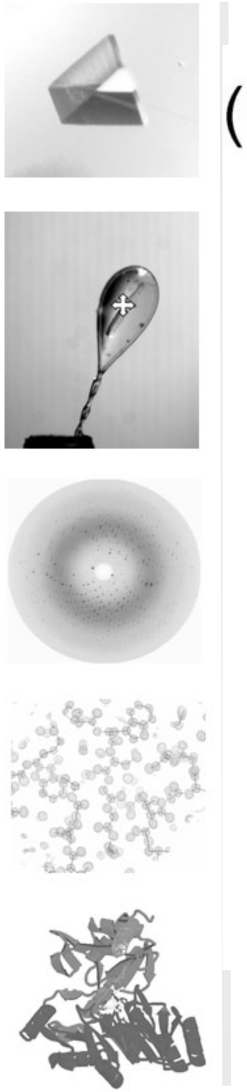
Anomalous Scattering - 4

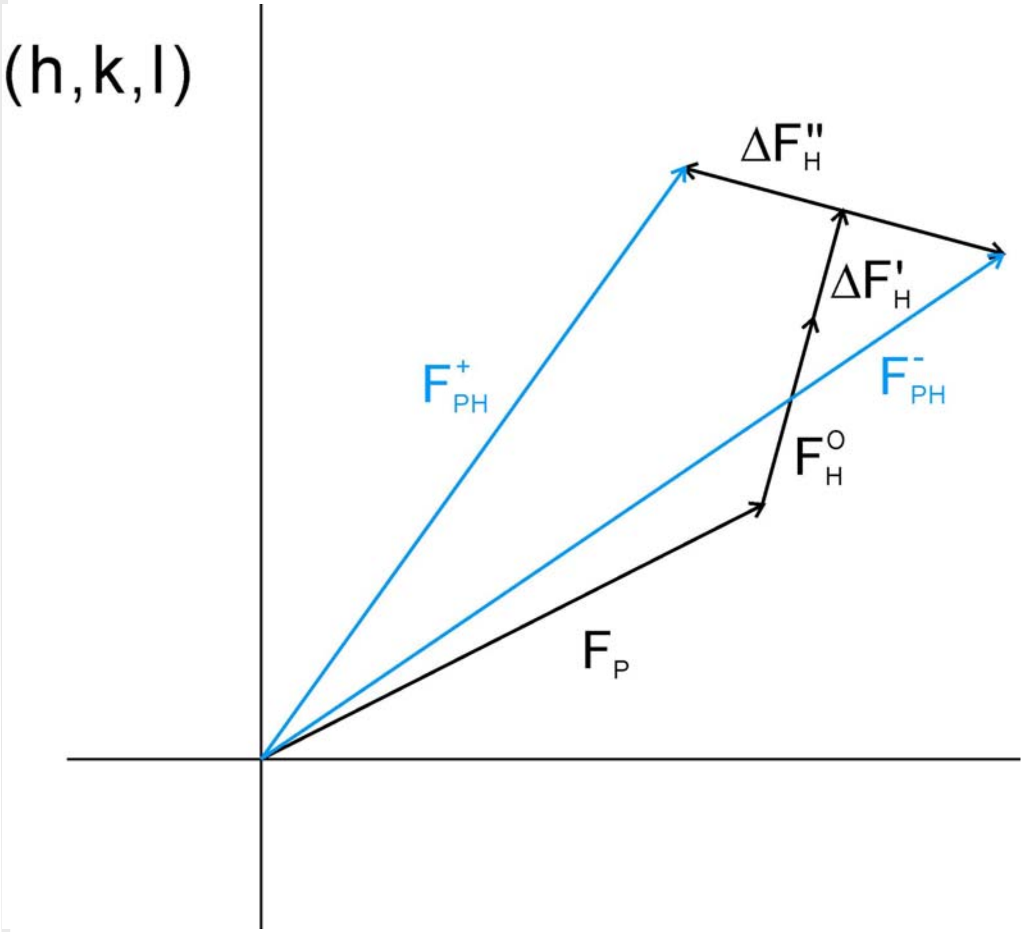




As consequence of anomalous scattering, Friedel’s Law is not valid anymore.

Anomalous Scattering - 5



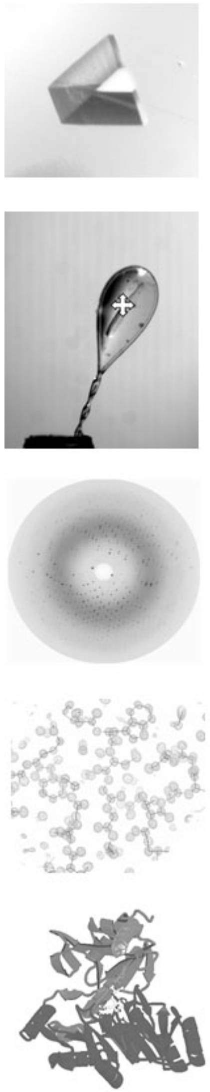


As consequence of anomalous scattering, Friedel’s Law is not valid anymore.

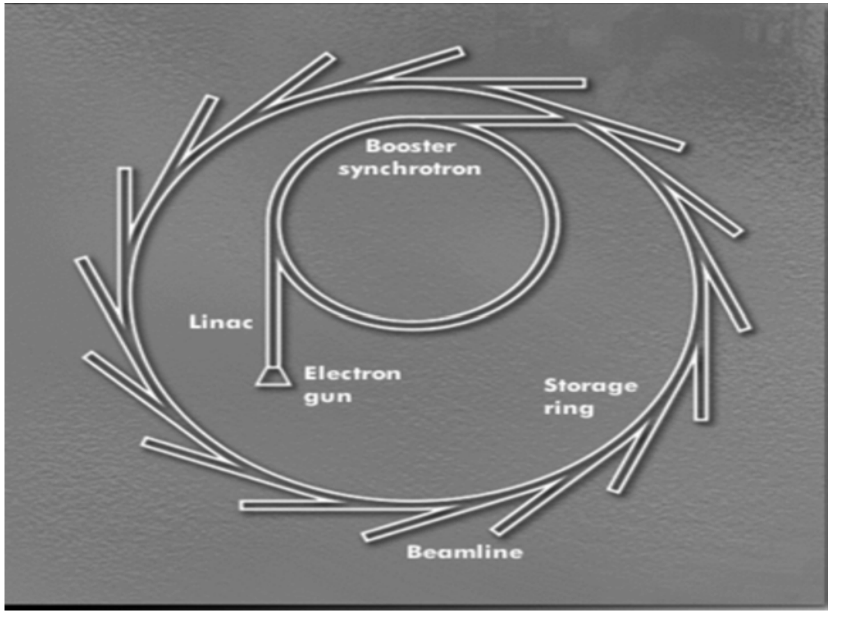
$$|F(hkl)| \neq |F(-h-k-l)|$$

$$\alpha(hkl) \neq -\alpha(-h-k-l)$$

Synchrotron Radiation

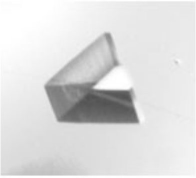


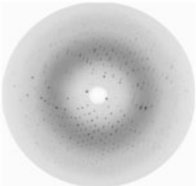
Synchrotron Radiation occurs when a charge moves at *relativistic speed* following a *curved* trajectory.

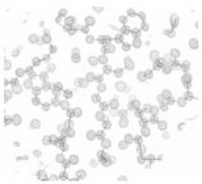


1. high brilliance
2. large spectral range
3. time structure

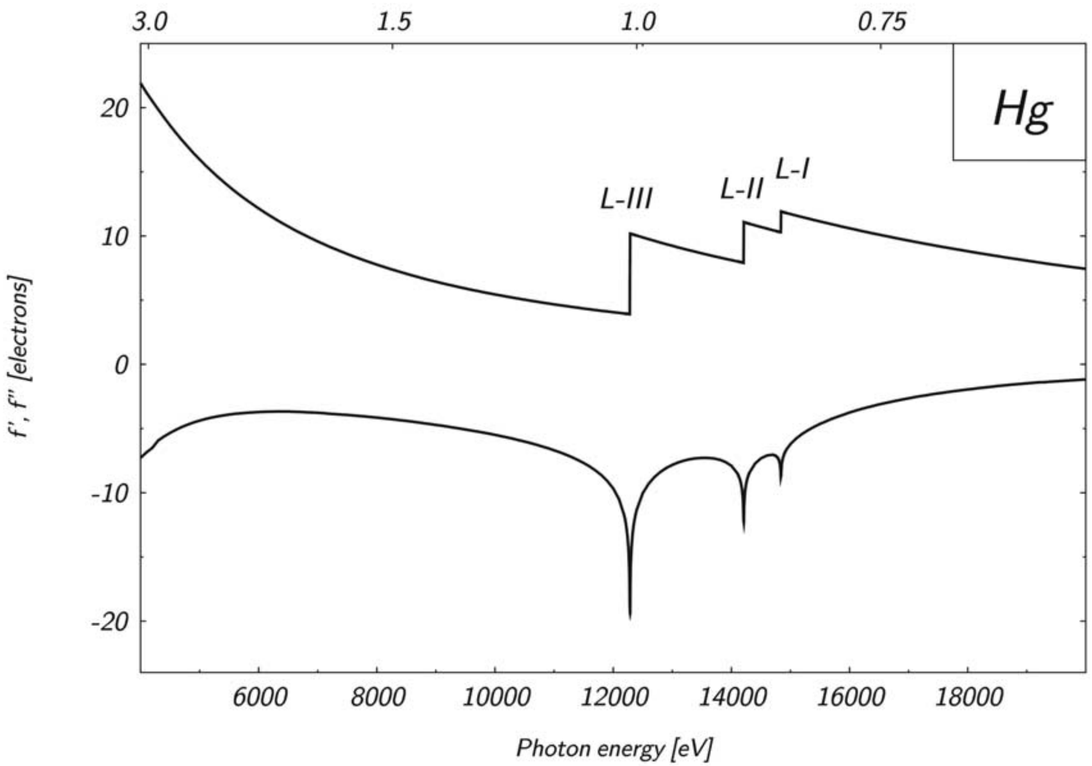
Anomalous Scattering - 6





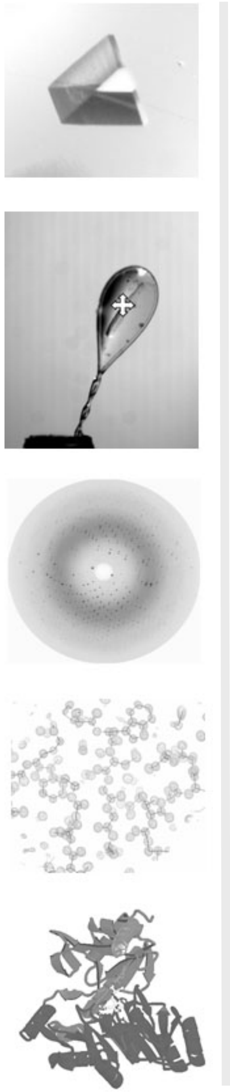


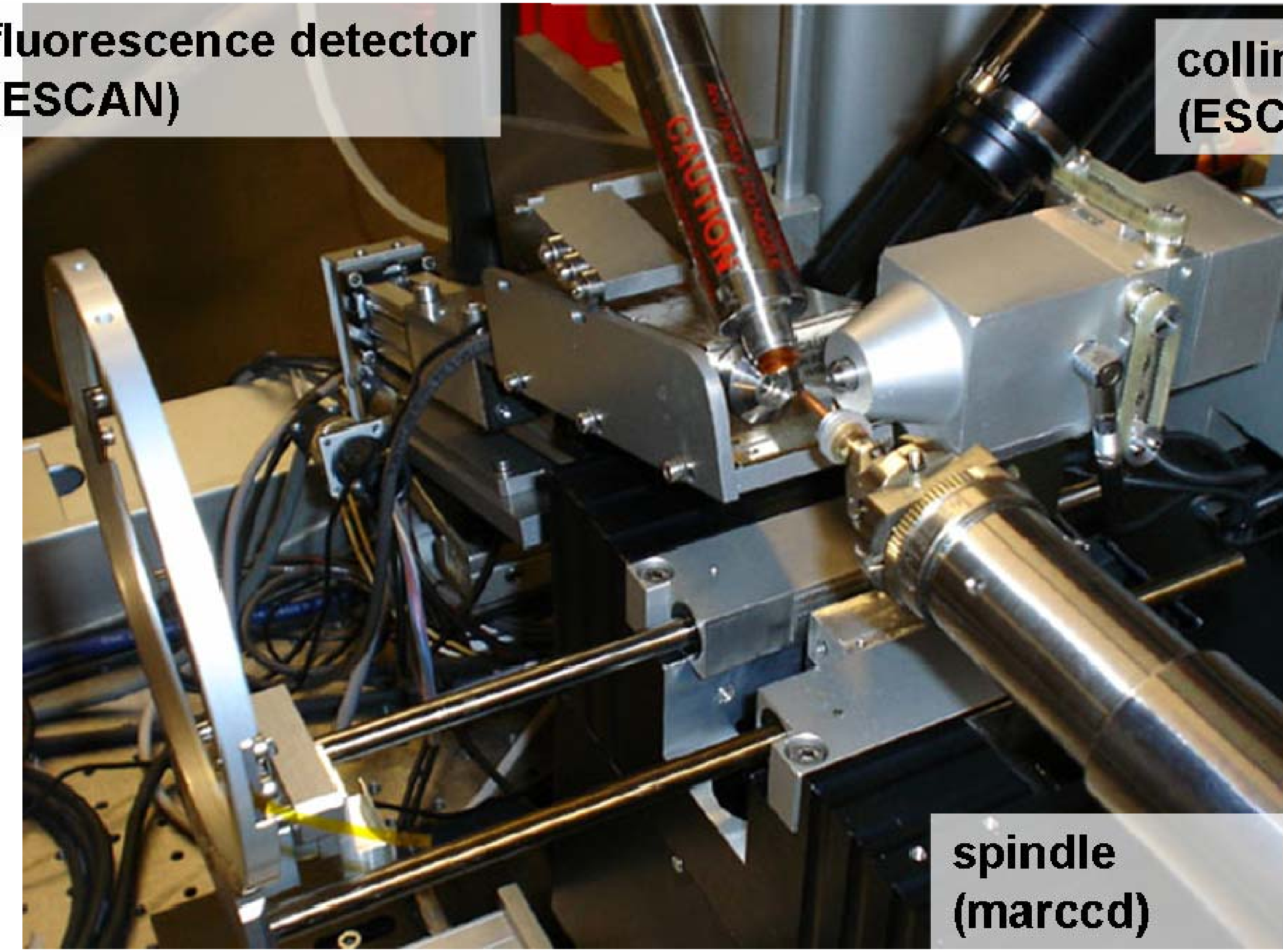
The tunable synchrotron radiation can be used to maximize the effect of anomalous scattering.



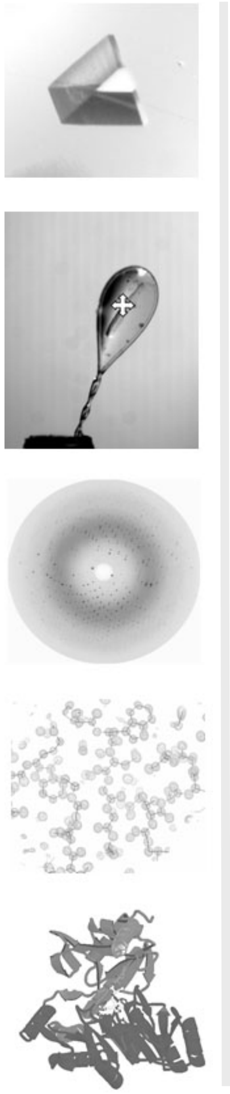
How Can This Be Measured?

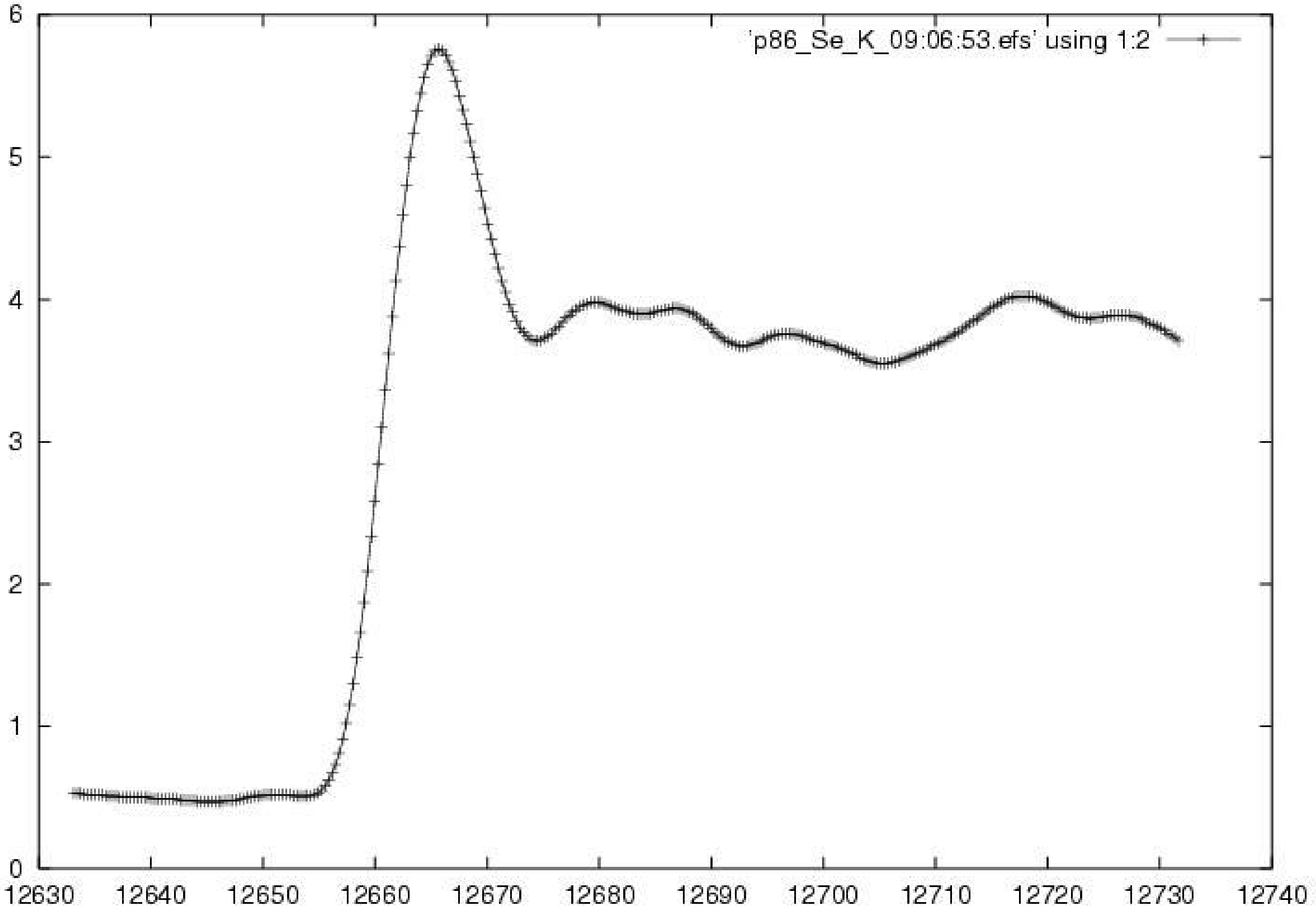
HZB Helmholtz Zentrum Berlin



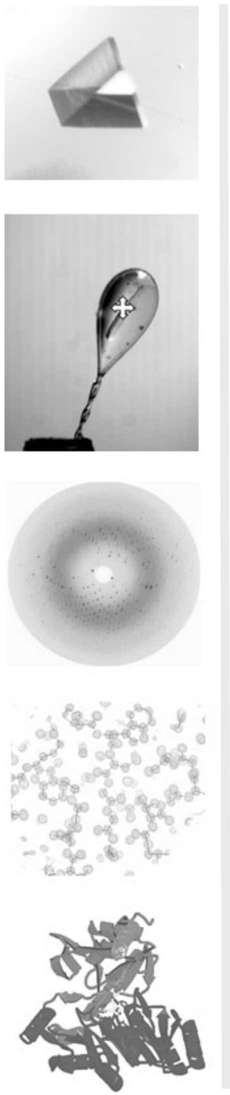


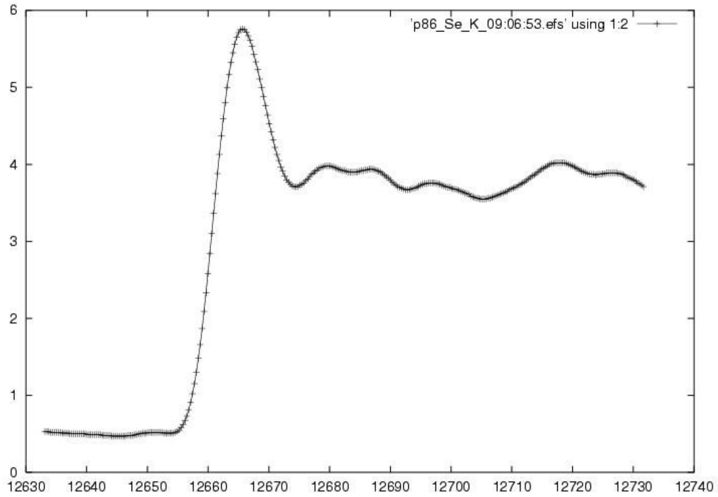
How Can This Be Measured?



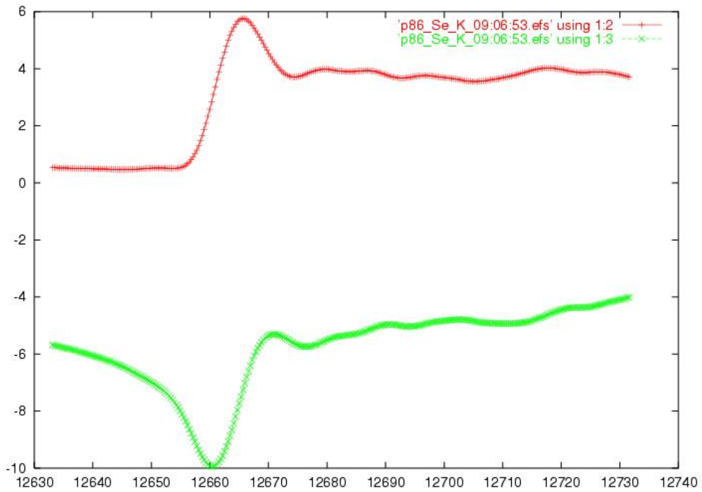


How Can This Be Measured?





Measured fluorescence

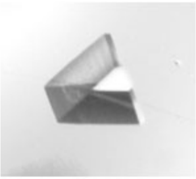


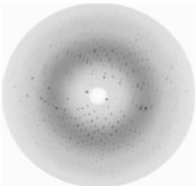
Derived anomalous scattering factors

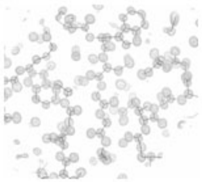
$$f''(\omega) = \left(m\omega c / 4\pi N e^2 \right) \mu(\omega)$$

$$f'(\omega) = (2/\pi) \int_0^{\infty} \left[\omega' f''(\omega') / \left(\omega^2 - \omega'^2 \right) \right] d\omega'$$

What is this Good for?

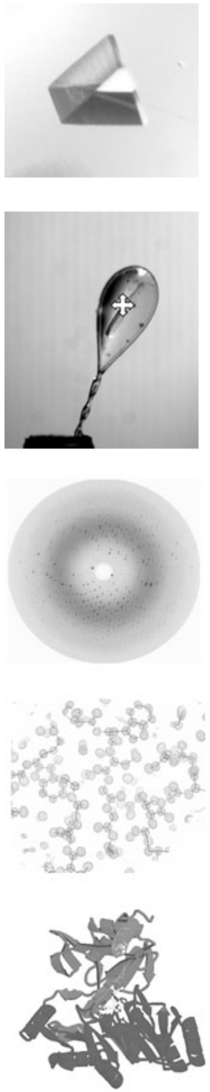


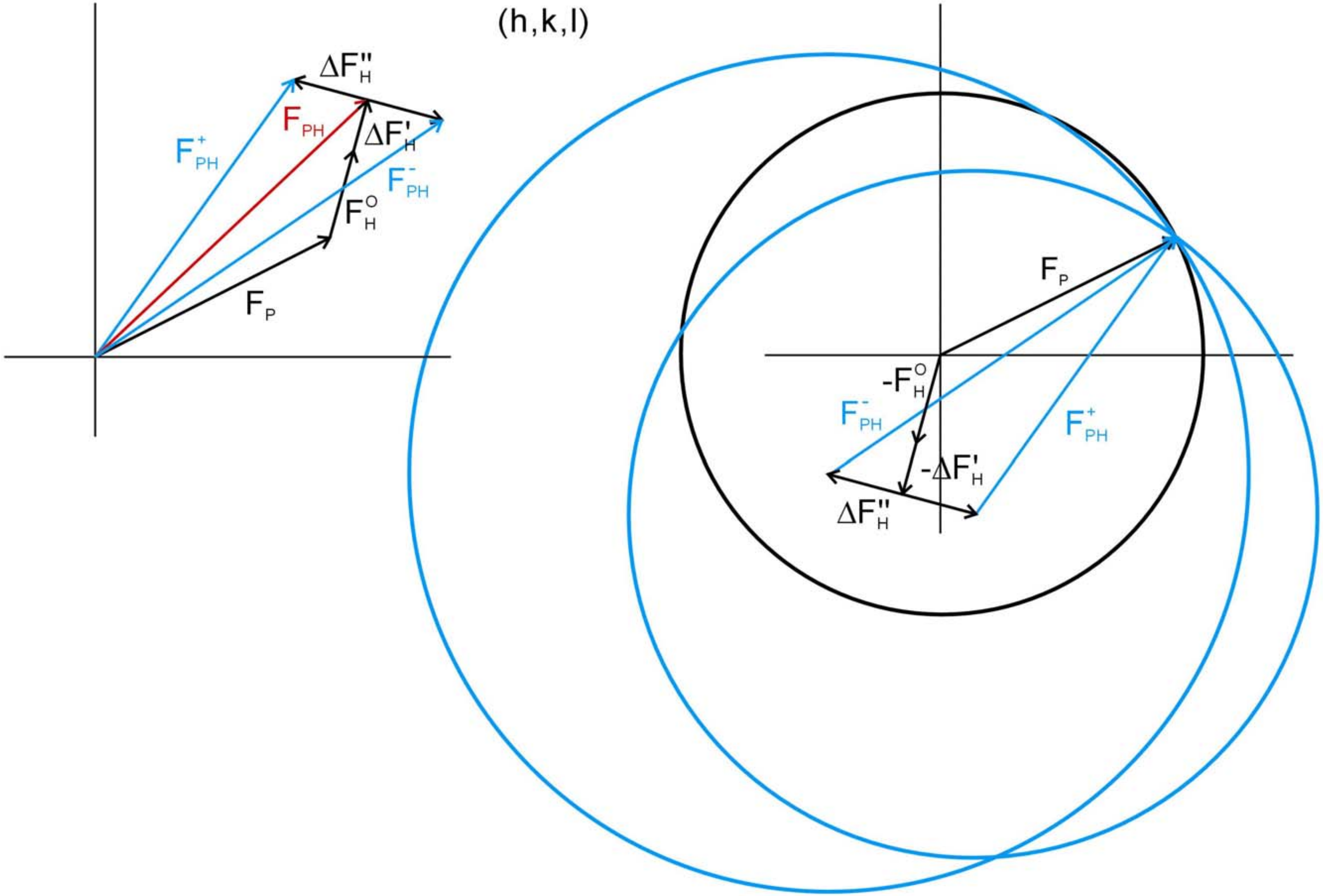




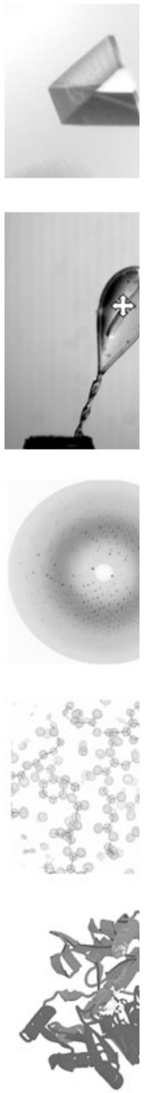
1. Phase Determination
2. Structure Validation
3. Element Identification

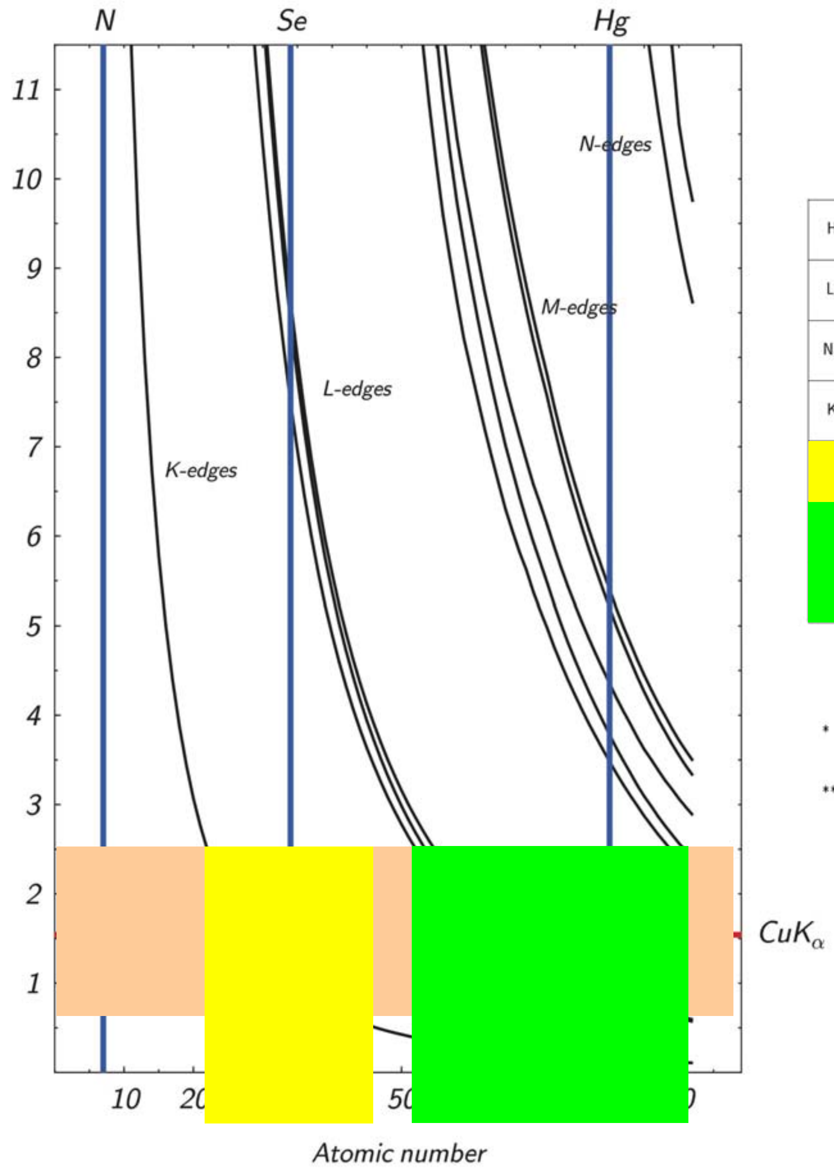
The Harker Construction - SIRAS

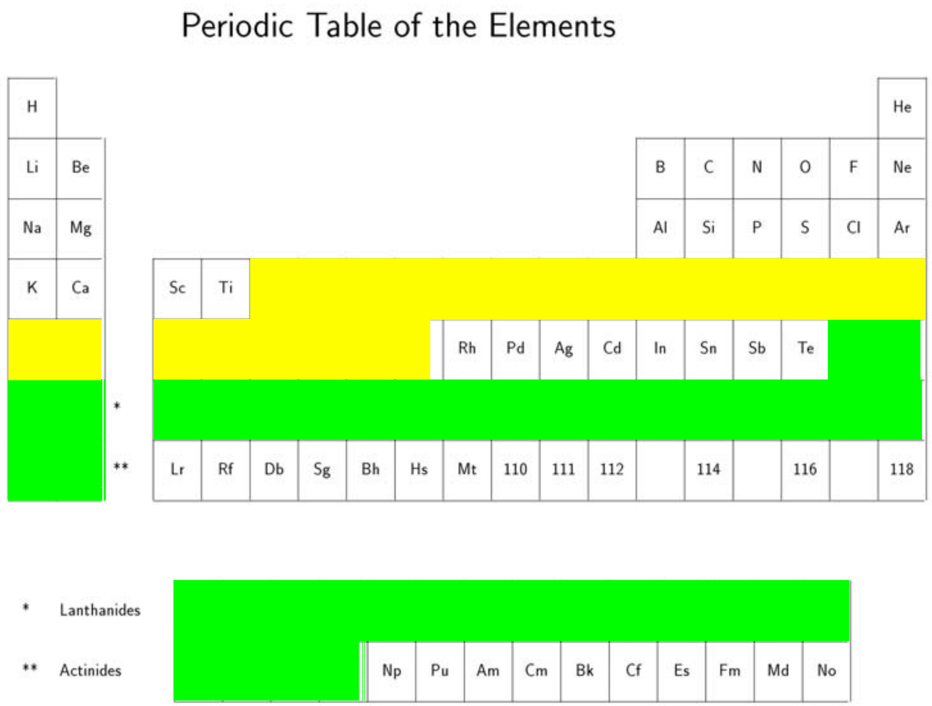




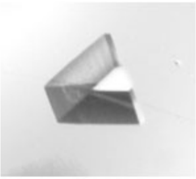
Which Elements Can Be Used?

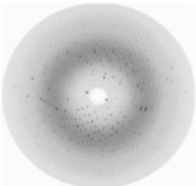


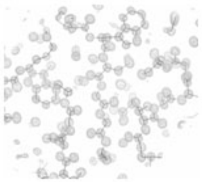




Experiments



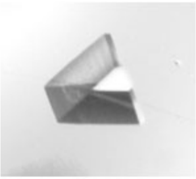


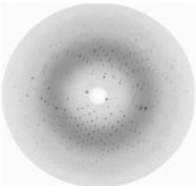


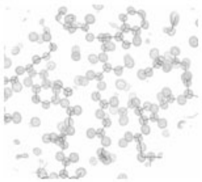
1. conventional heavy-atom derivatization by soaking or co-crystallization

Experiments

HZB Helmholtz Zentrum Berlin



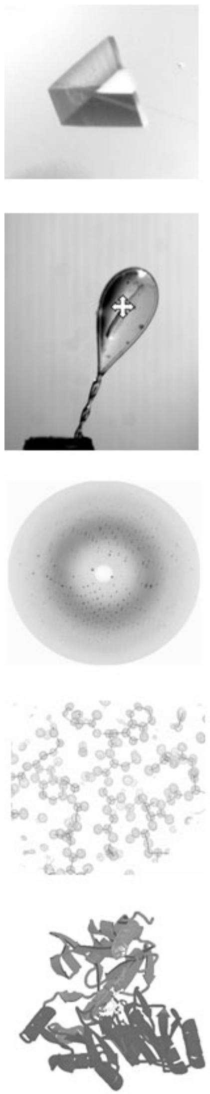




1. conventional heavy-atom derivatization by soaking or co-crystallization

2. quick-soaking

Experiments

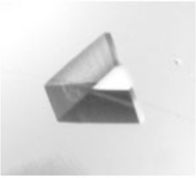


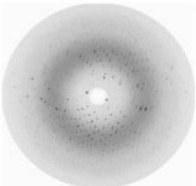
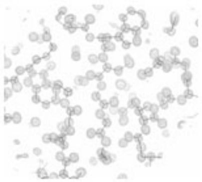
1. conventional heavy-atom derivatization by soaking or co-crystallization

2. quick-soaking

3. quick-soaking using anions (Br^-, I^-, I^-/I_2, ...)

Experiments



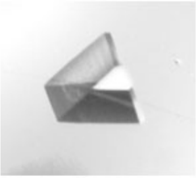

1. conventional heavy-atom derivatization by soaking or co-crystallization

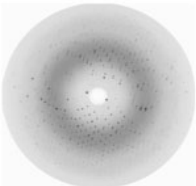
2. quick-soaking

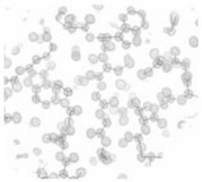
3. quick-soaking using anions (Br^-, I^-, I^-/I_2, ...)

4. pressurization using noble gases (Xe, Kr)

Experiments

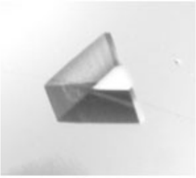


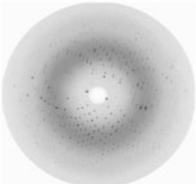
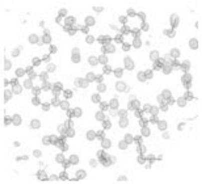




1. conventional heavy-atom derivatization by soaking or co-crystallization
2. quick-soaking
3. quick-soaking using anions (Br^-, I^-, I^-/I_2, ...)
4. pressurization using noble gases (Xe, Kr)
5. covalent modification of the protein (e.g. Met → Se-Met) or of DNA (T → Br-U)

Phase Determination Methods




1. SIR, SIRAS, MIR, MIRAS

   (single/multiple isomorphous replacement with anomalous scattering)

2. MAD

   (multiple wavelength anomalous diffraction)

3. SAD (SAS)

   (single wavelength anomalous diffraction/scattering)

4. RIP, RIPAS

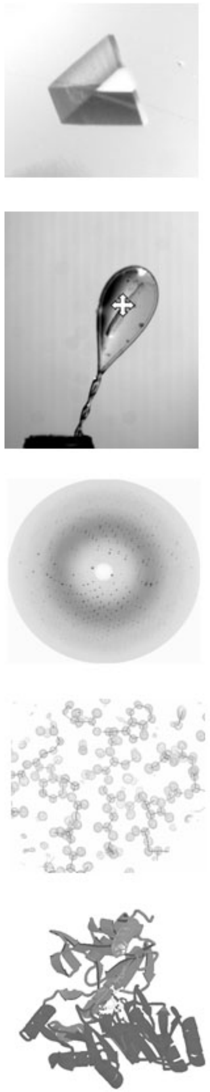
   (radiation damage induced phasing with anomalous scattering)

5. MR

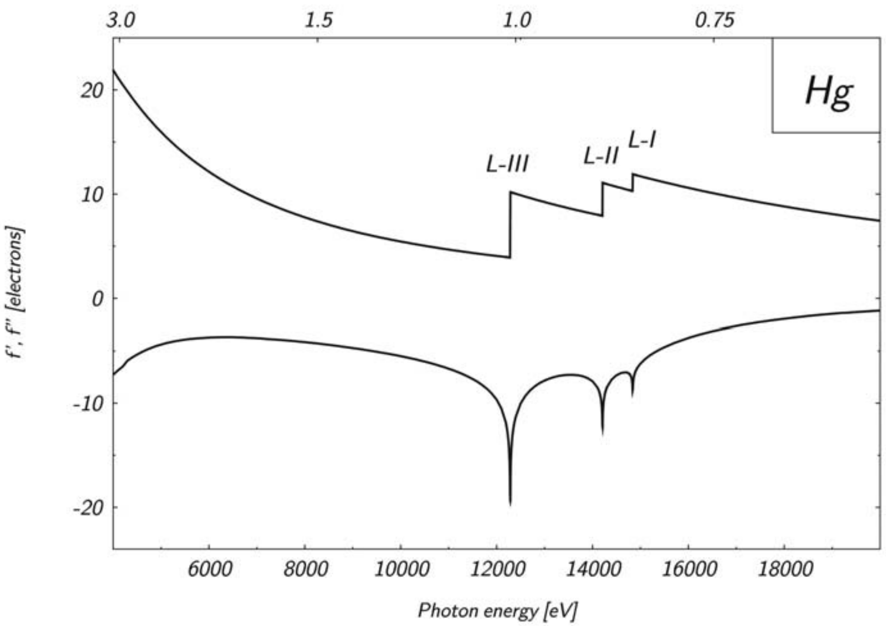
   (molecular replacement)

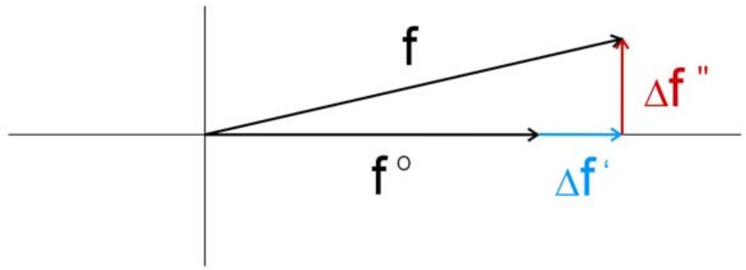
6. Direct Methods

The MAD Method



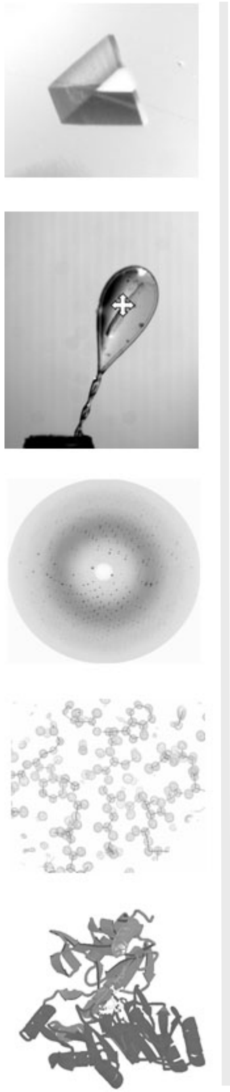
Instead of using two different crystals, we can also collect data from one crystal at two (or more) different wavelengths.

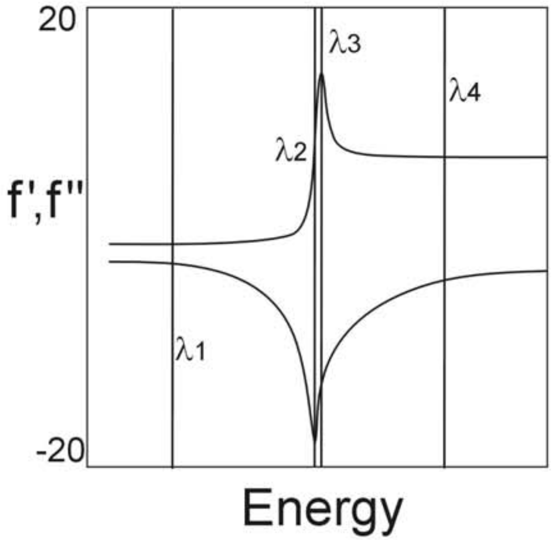


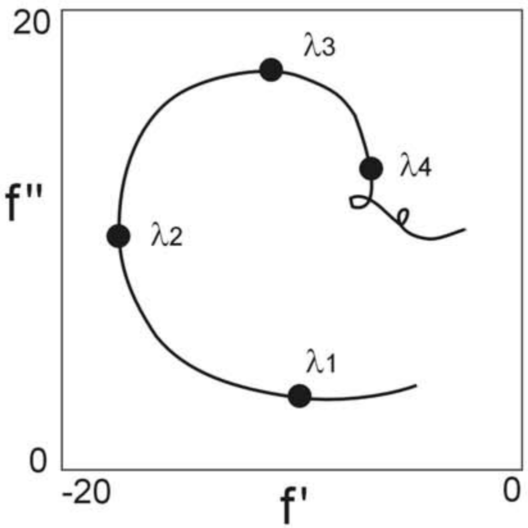


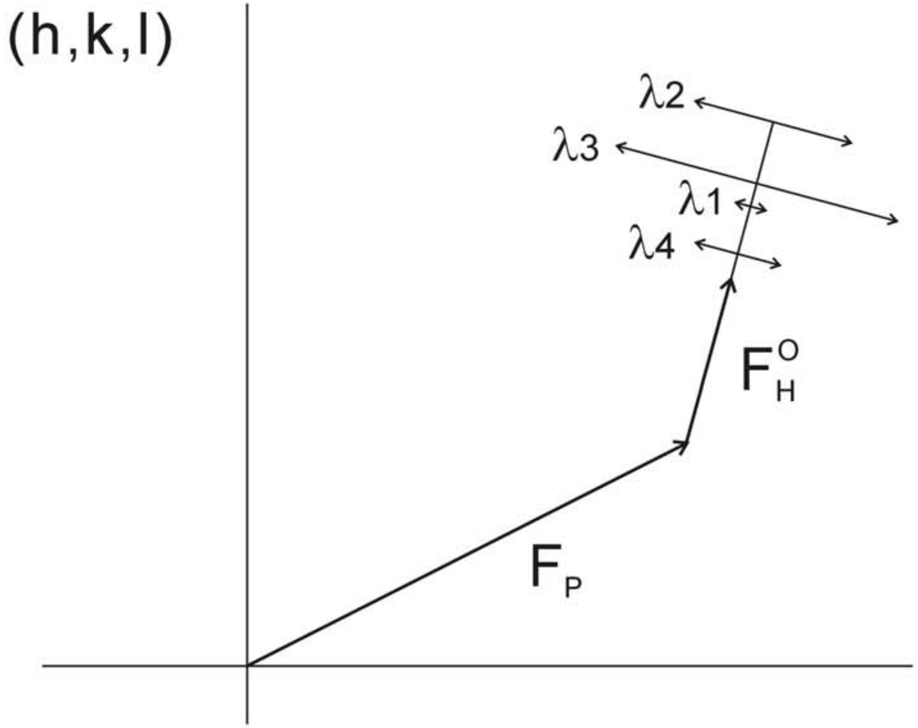
$$f = f^{\circ} + \Delta f' + i\Delta f''$$

The MAD Method

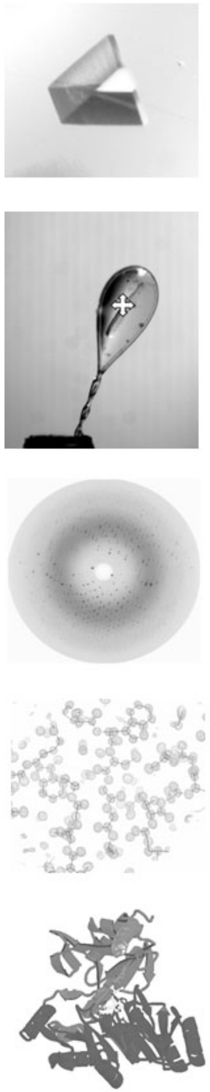








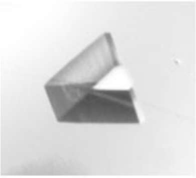
Experiments

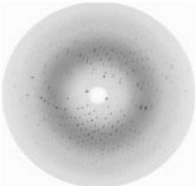
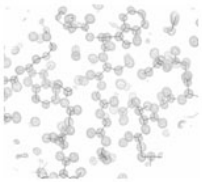


1. in proteins: replacement of Met with Se-Met

Experiments

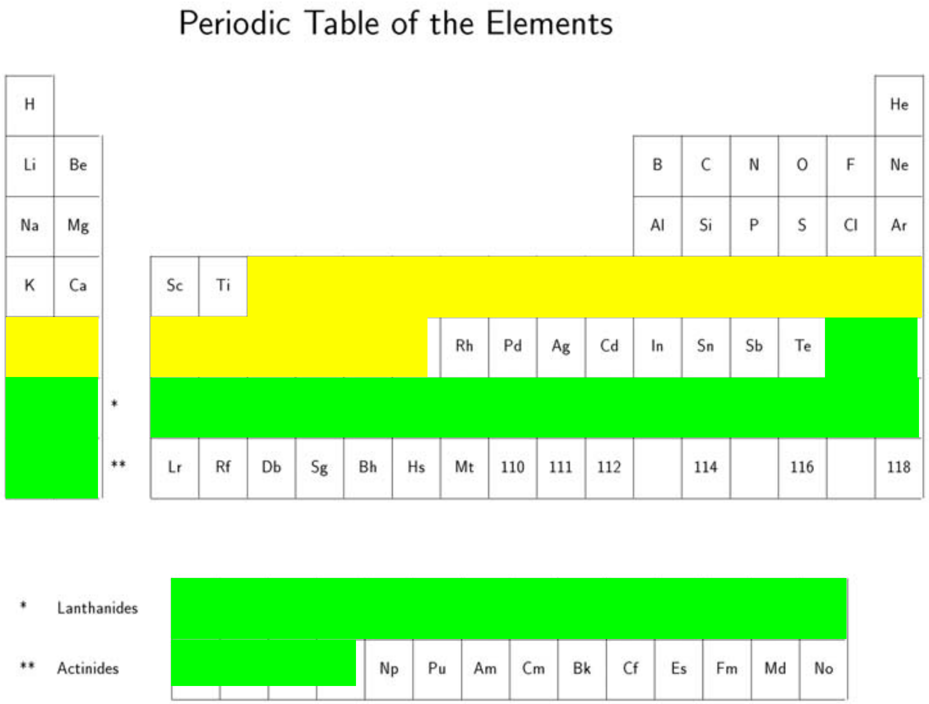
HZB Helmholtz Zentrum Berlin



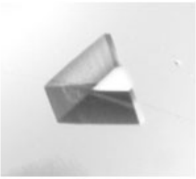



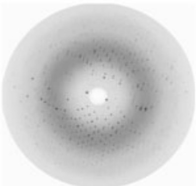
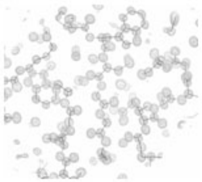

1. in proteins: replacement of Met with Se-Met

2. almost any other heavy atom derivative



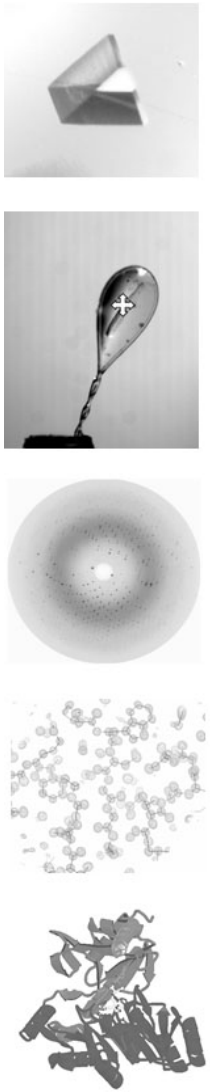
Experiments



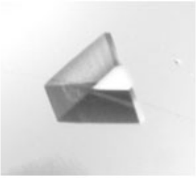

1. in proteins: replacement of Met with Se-Met
2. almost any other heavy atom derivative
3. naturally present Fe, Cu, Zn, etc.

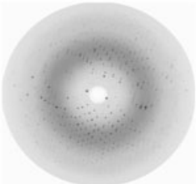
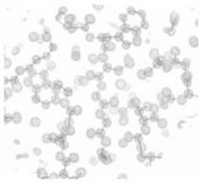
Experiments



1. in proteins: replacement of Met with Se-Met
2. almost any other heavy atom derivative
3. naturally present Fe, Cu, Zn, etc.
4. in DNA: replacement of T with Br-U

Phase Determination Methods




1. SIR, SIRAS, MIR, MIRAS

   (single/multiple isomorphous replacement with anomalous scattering)

2. MAD

   (multiple wavelength anomalous diffraction)

3. SAD (SAS)

   (single wavelength anomalous diffraction/scattering)

4. RIP, RIPAS

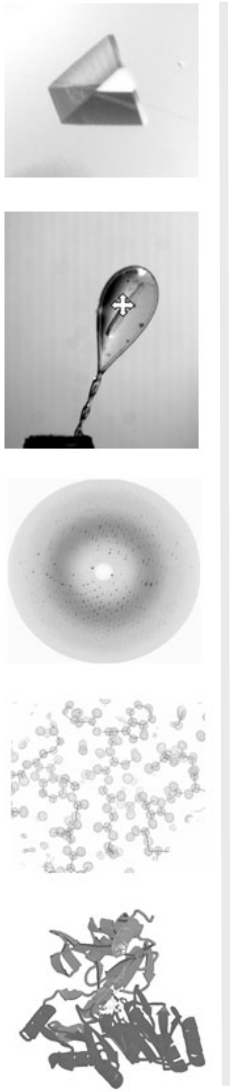
   (radiation damage induced phasing with anomalous scattering)

5. MR

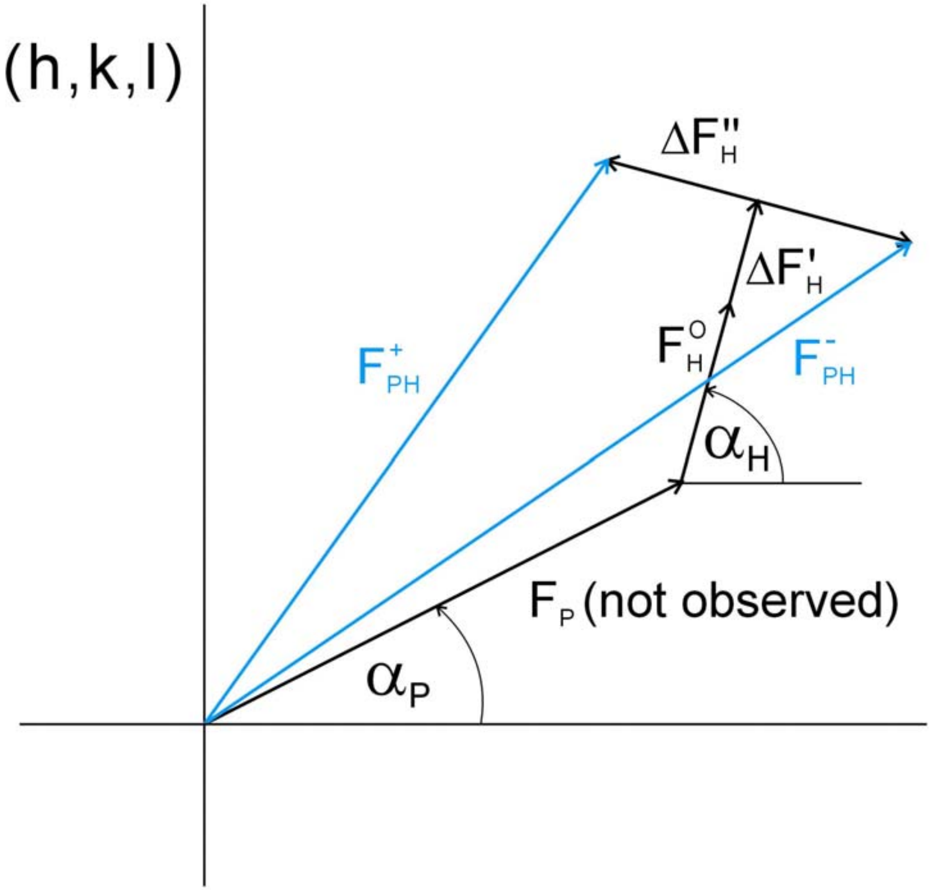
   (molecular replacement)

6. Direct Methods

The SAD/SAS Method



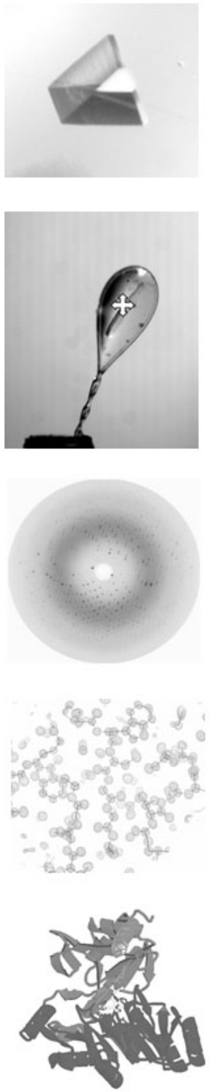
In principle, two, three or four wavelengths are not needed. One should be enough if other information is available, e.g.

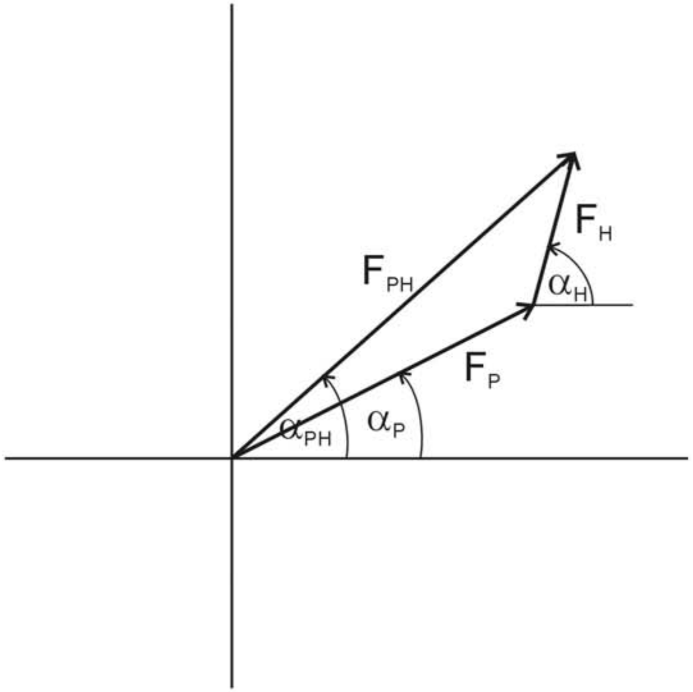


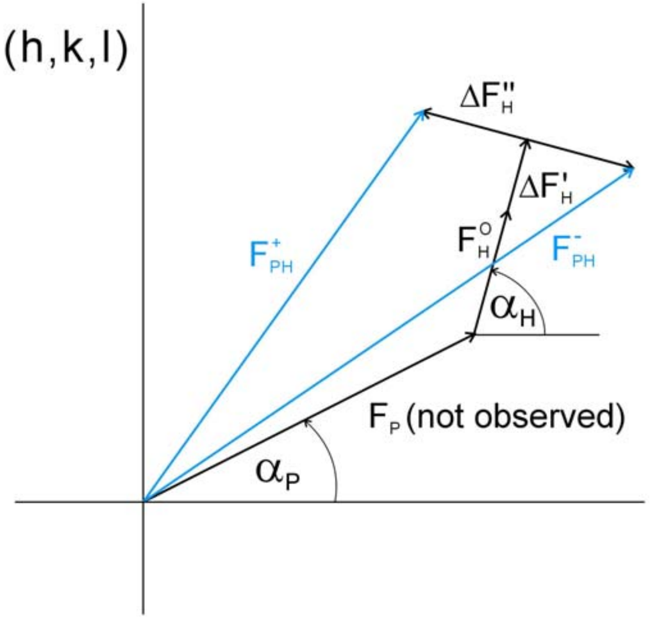
possibility for solvent flattening.

The situation is exactly analogous to SIR.

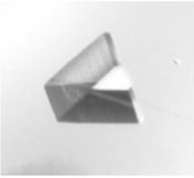
SIR and SAD

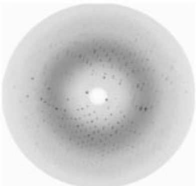
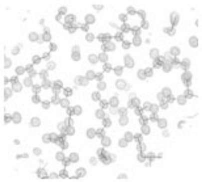






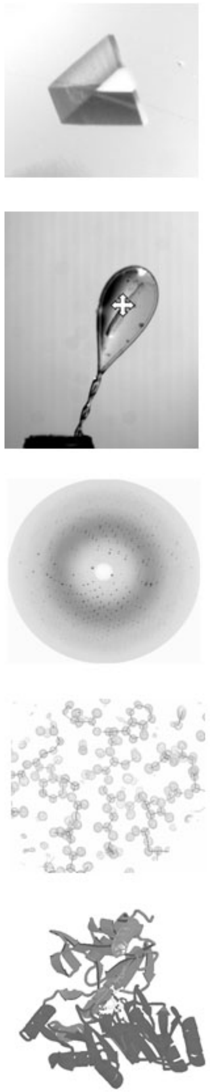
Experiments




1. Se-Met-protein

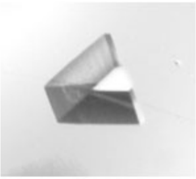
Experiments

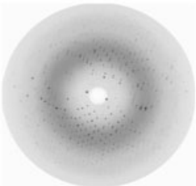
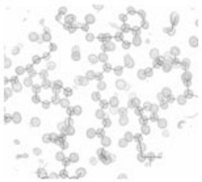


1. Se-Met-protein

2. almost any other heavy atom derivative

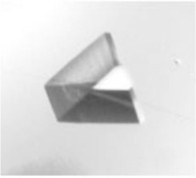
Experiments

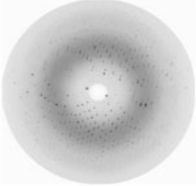
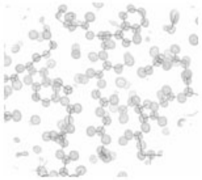



1. Se-Met-protein
2. almost any other heavy atom derivative
3. naturally present Fe, Cu, Zn, etc.

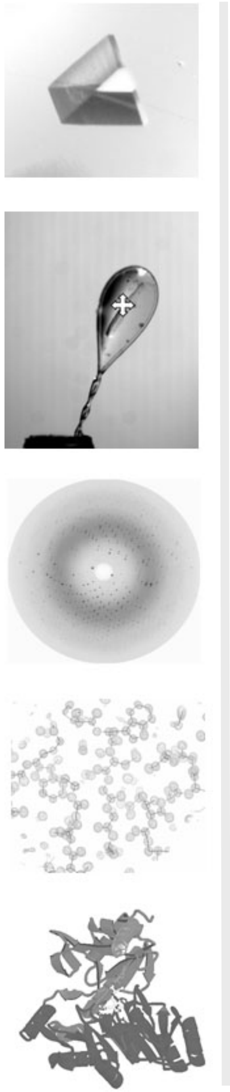
Experiments

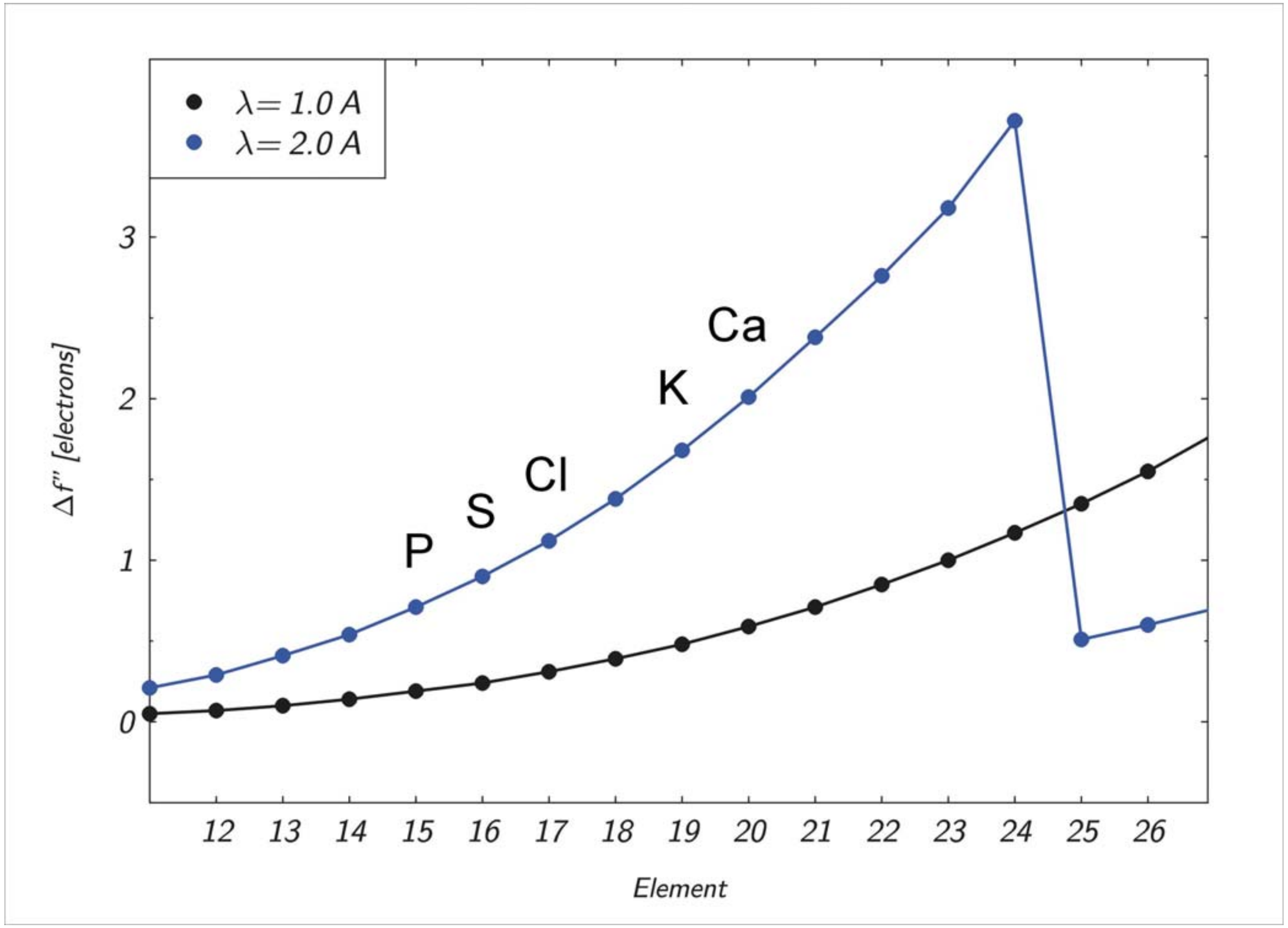


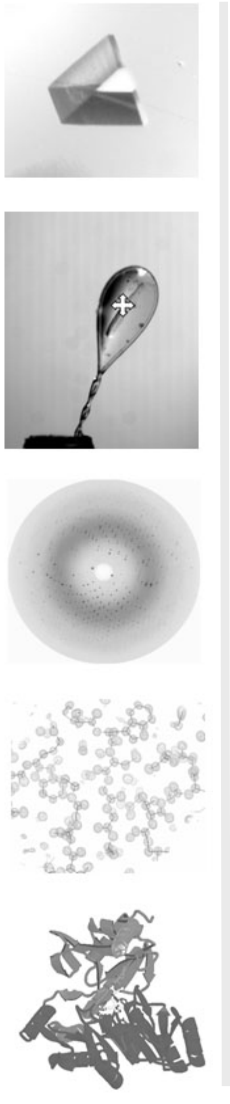

1. Se-Met-protein
2. almost any other heavy atom derivative
3. naturally present Fe, Cu, Zn, etc.
4. light atoms (P, S, Cl, Ca, …), the absorption edges of which are not accessible

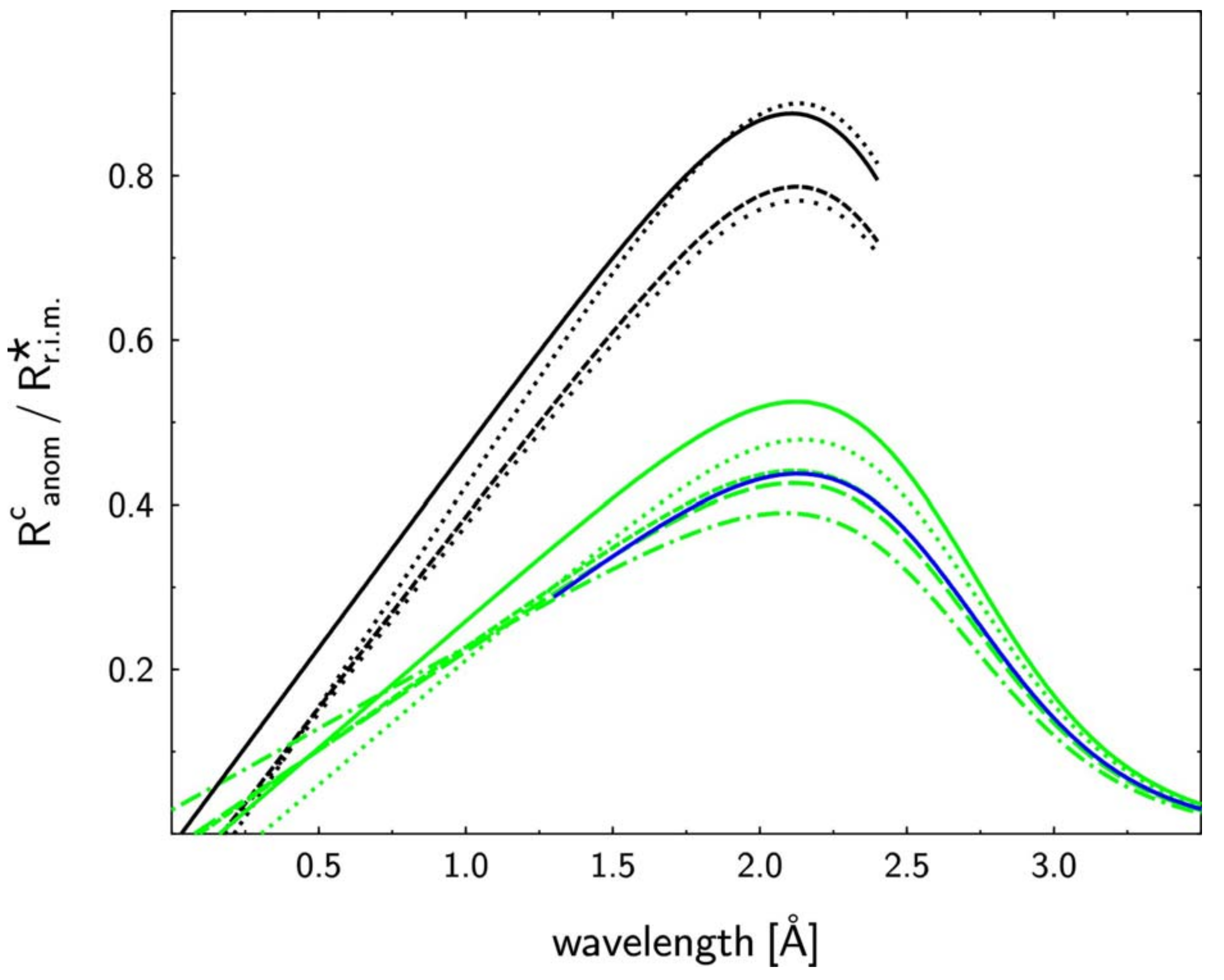
Long Wavelength S-SAD



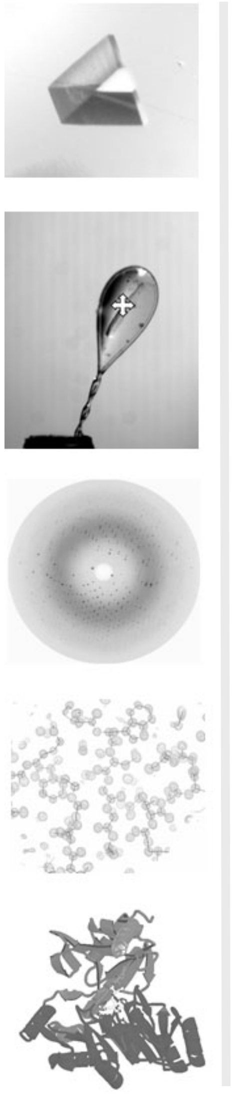


Long Wavelength S-SAD





Long Wavelength S-SAD

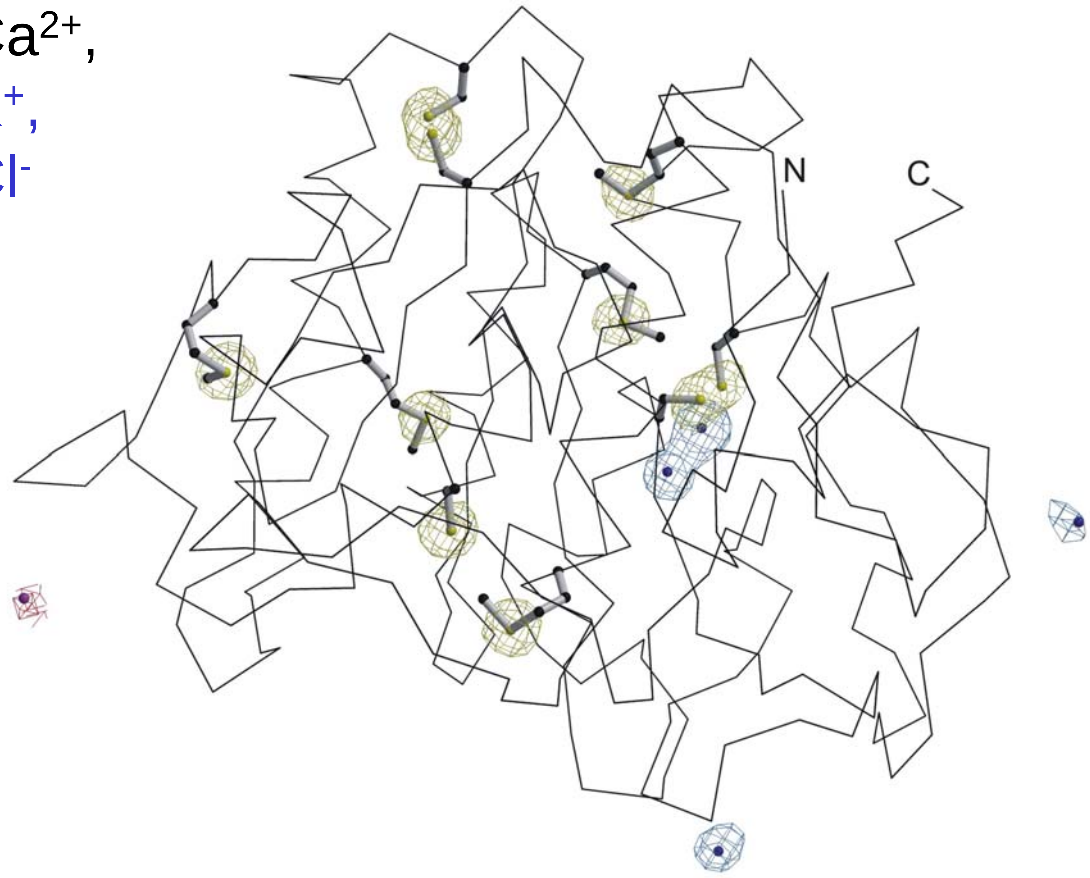


Proteinase K:

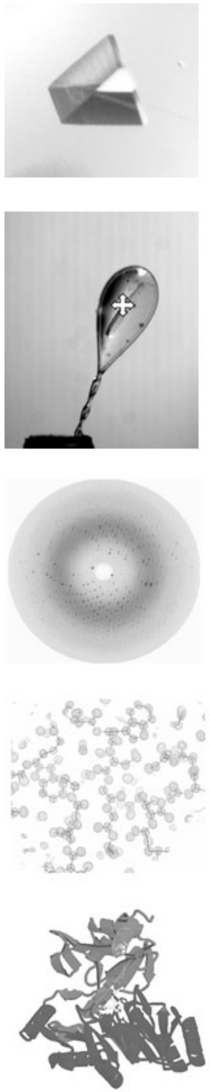
2 Ca^{2+},

2 K^{+},

1 Cl^{-}

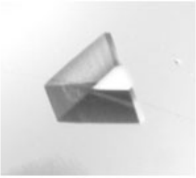


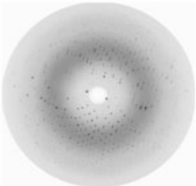
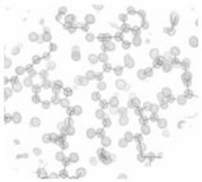
Phase Determination Methods



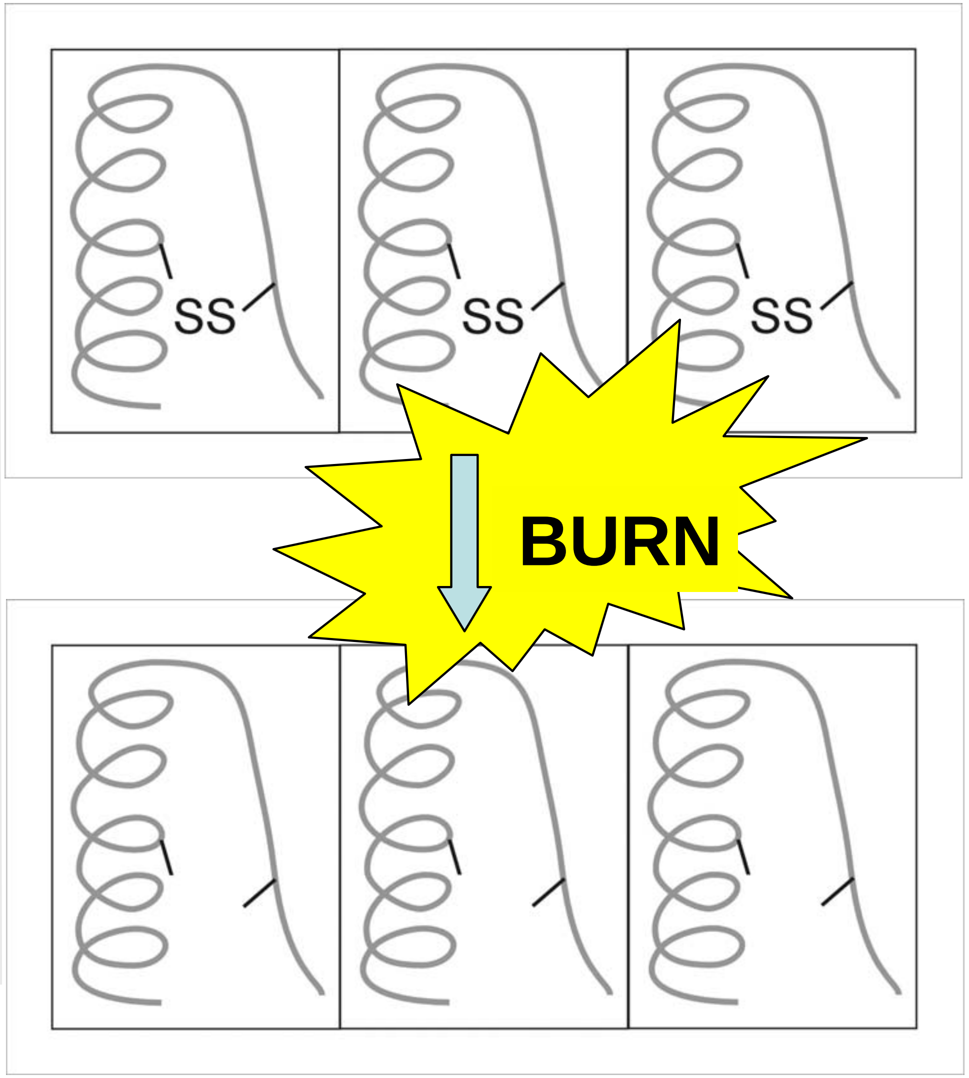
1. SIR, SIRAS, MIR, MIRAS
   (single/multiple isomorphous replacement with anomalous scattering)
2. MAD
   (multiple wavelength anomalous diffraction)
3. SAD (SAS)
   (single wavelength anomalous diffraction/scattering)
4. RIP, RIPAS
   (radiation damage induced phasing with anomalous scattering)
5. MR
   (molecular replacement)
6. Direct Methods

The RIP Method




Radiation damage induced phasing is a relatively new method.

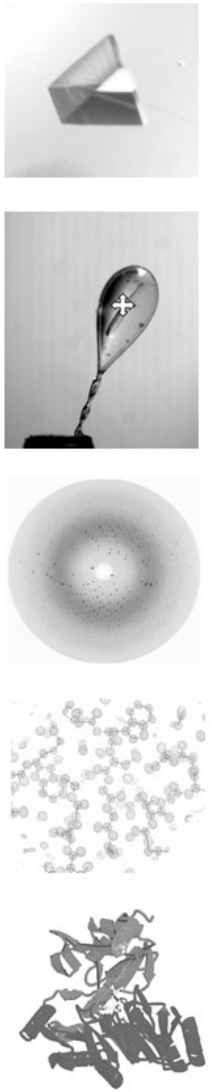


→ h, k, l, $|F_{before}(hkl)|$

$\alpha(hkl)$

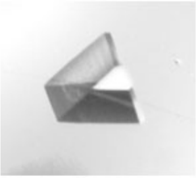
→ h, k, l, $|F_{after}(hkl)|$

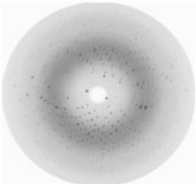
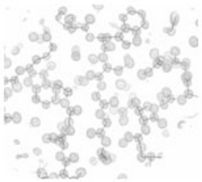
Experiments



The RIP method is in principle applicable to all crystals, which are damaged by X-rays (or UV).

Phase Determination Methods




1. SIR, SIRAS, MIR, MIRAS

   (single/multiple isomorphous replacement with anomalous scattering)

2. MAD

   (multiple wavelength anomalous diffraction)

3. SAD (SAS)

   (single wavelength anomalous diffraction/scattering)

4. RIP, RIPAS

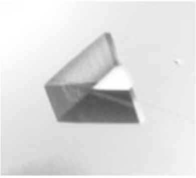
   (radiation damage induced phasing with anomalous scattering)

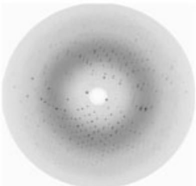
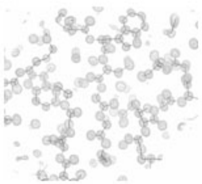
5. MR

   (molecular replacement)

6. Direct Methods

The MR Method



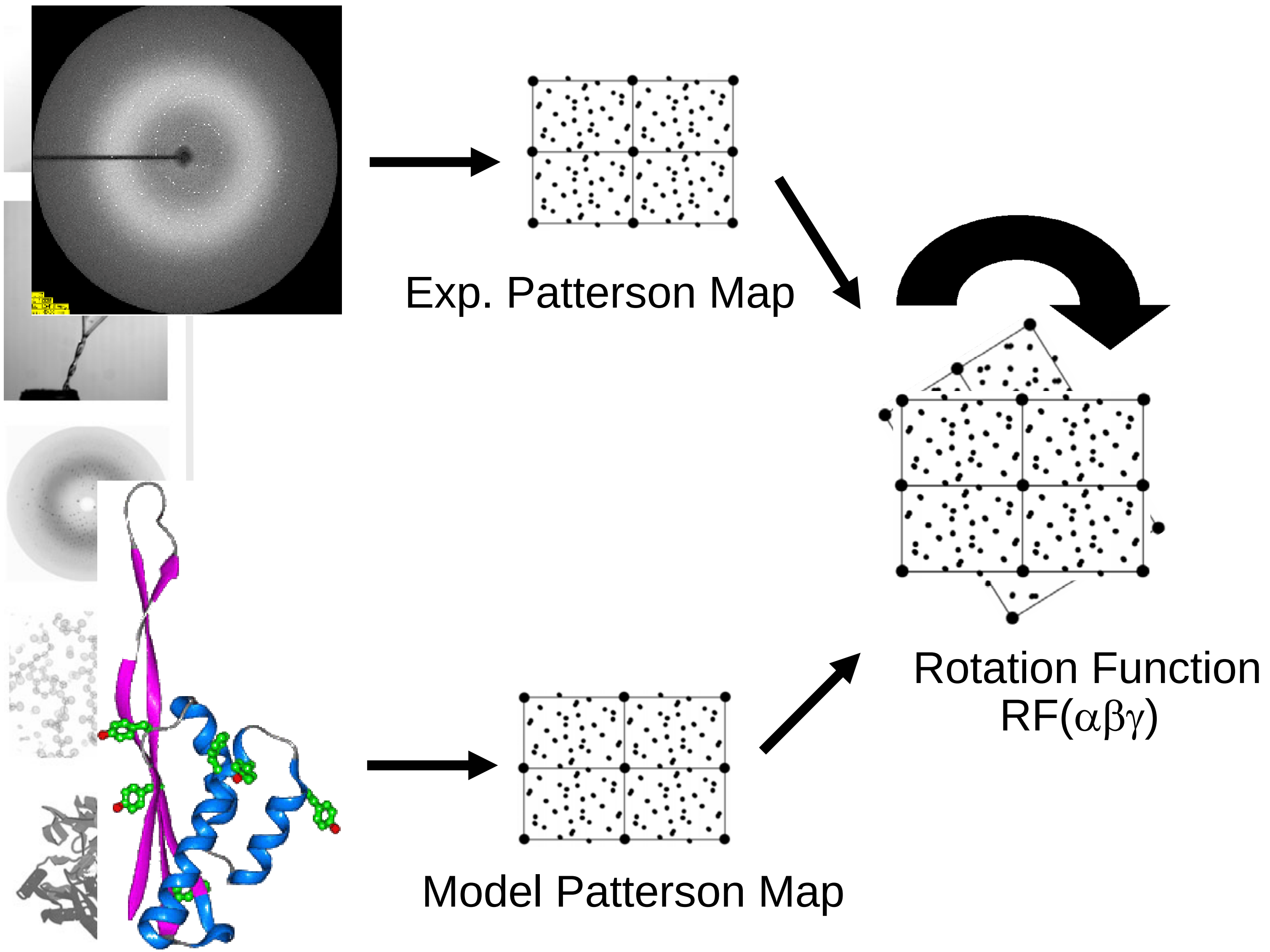




Molecular replacement can be used to solve a structure when a homologous structure is available.

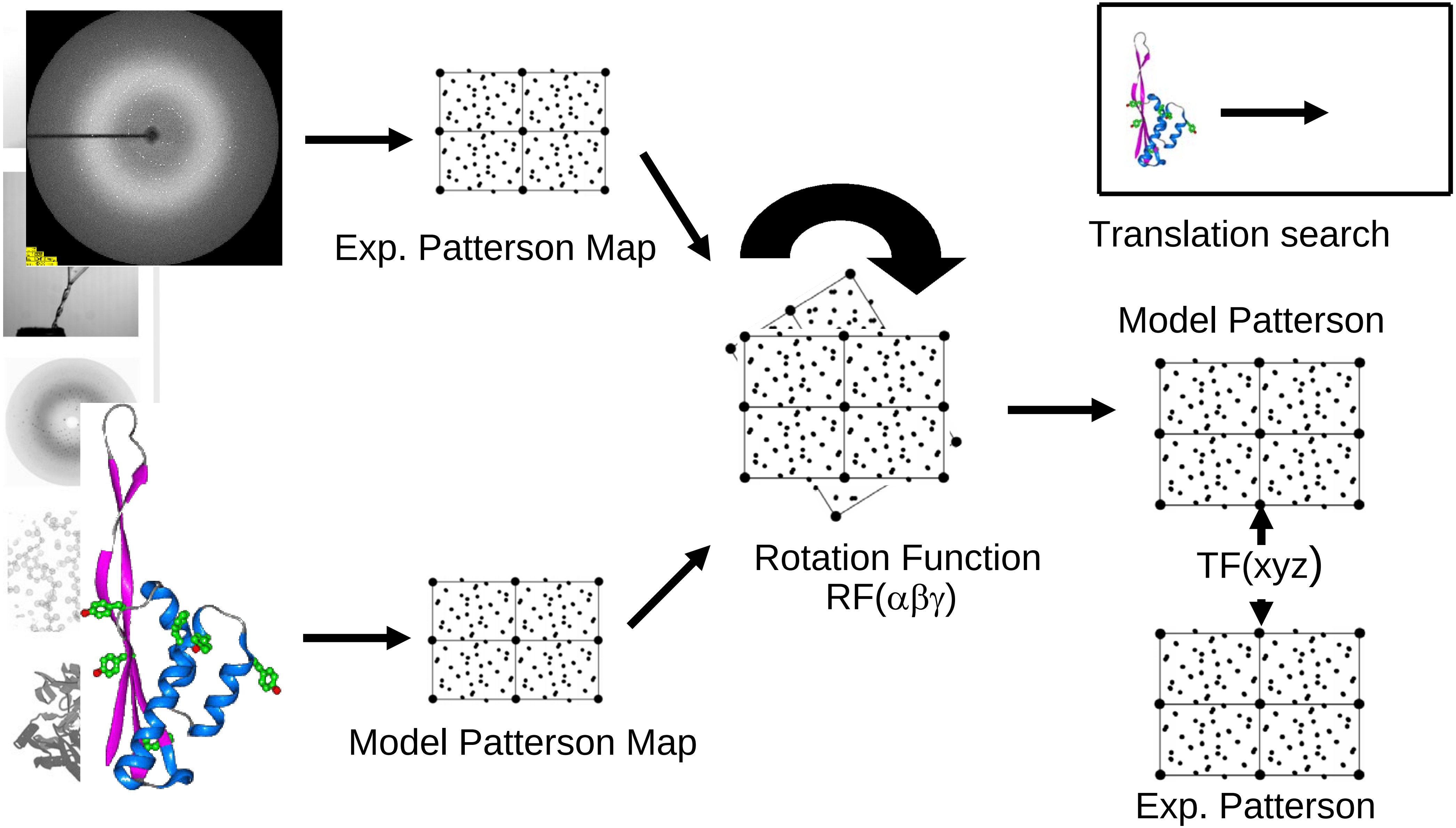
When the homologous structure can be correctly oriented and positioned (6 parameters), it can be used as a starting point for phase calculation.

MR does not necessarily need synchrotron radiation, but MR phases can be validated by ***anomalous scattering***.

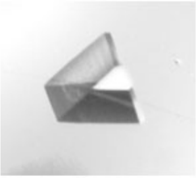
Molecular Replacement

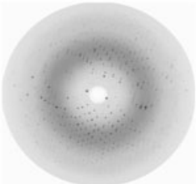
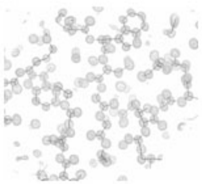


Molecular Replacement



Phase Determination Methods




1. SIR, SIRAS, MIR, MIRAS

   (single/multiple isomorphous replacement with anomalous scattering)

2. MAD

   (multiple wavelength anomalous diffraction)

3. SAD (SAS)

   (single wavelength anomalous diffraction/scattering)

4. RIP, RIPAS

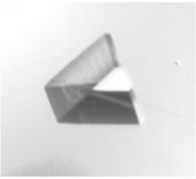
   (radiation damage induced phasing with anomalous scattering)

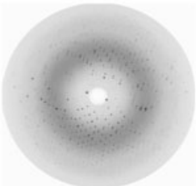
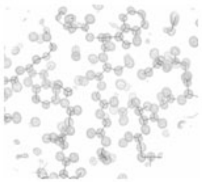
5. MR

   (molecular replacement)

6. Direct Methods

Direct Methods



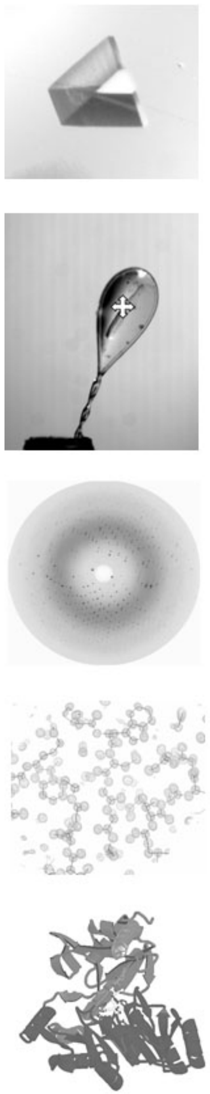

This is the method of choice in small molecule crystallography.

It usually needs a resolution better than ***1.2 Å.***

For proteins, it works only in exceptional cases:

- < 1000 non-hydrogen atoms,
- heavy atoms present.

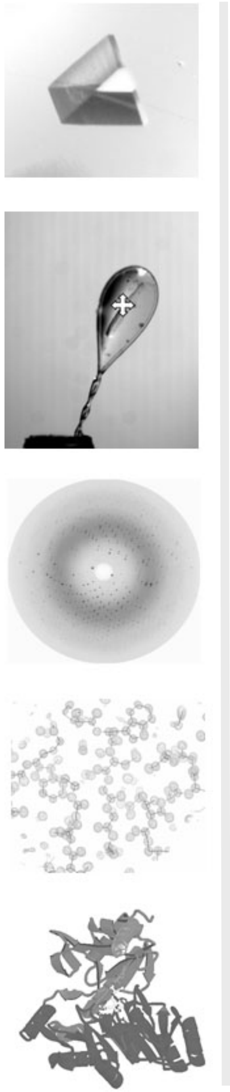
The Electron Density Equation



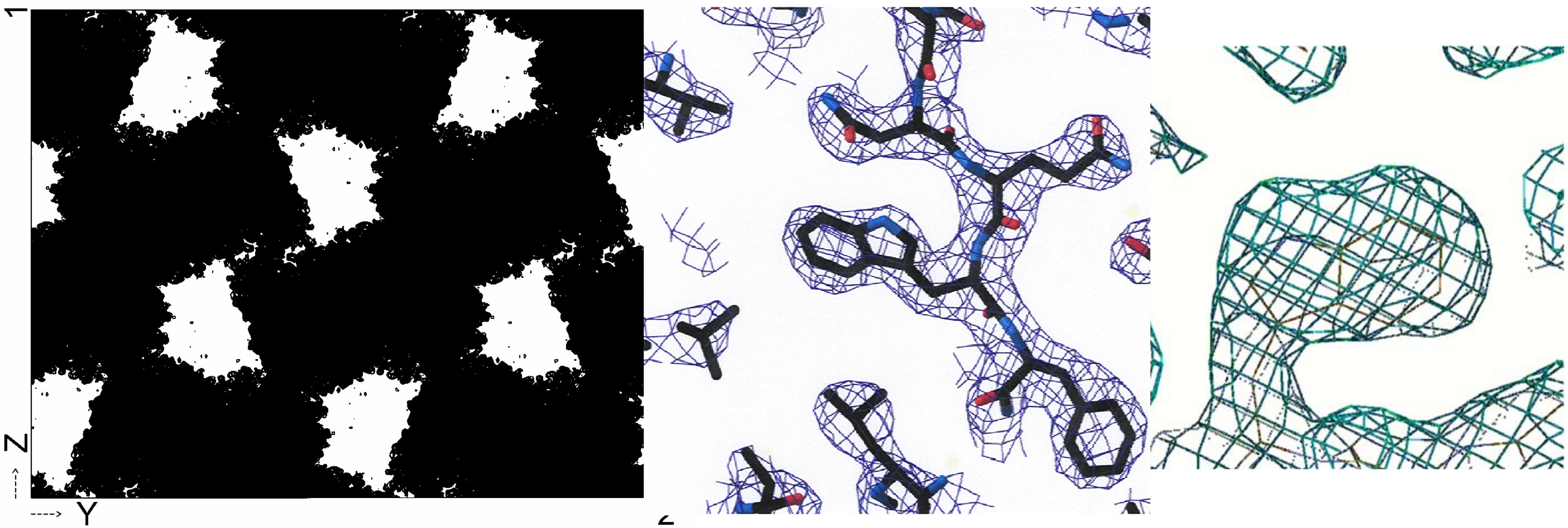
Once the phases $\alpha(hkl)$ have been determined, the three-dimensional electron density function $\rho(x,y,z)$ can be calculated.

$$\rho(x,y,z) = 1/V \sum_{hkl} |F(hkl)| \, e^{i\alpha(hkl)} \, e^{-2\pi i(hx+ky+lz)}$$

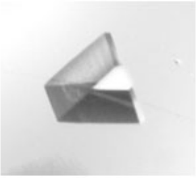
Electron Density

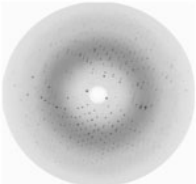


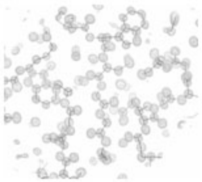
All that is left to do now is to interpret the electron density in terms of the three-dimensional structure.



Signal Strength and Data Quality





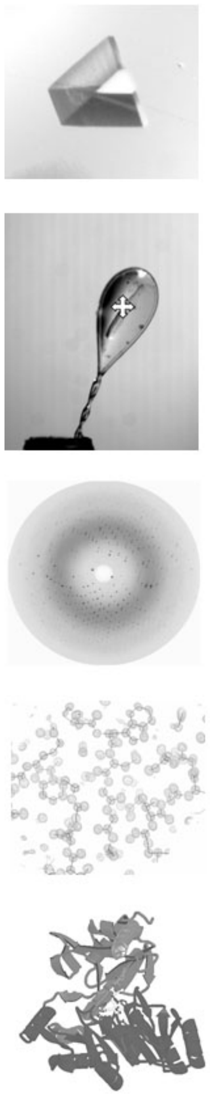


SIR: $R = 100 \bullet \Sigma_{hkl} \mid |F_{PH}| - |F_P| \mid / \Sigma_{hkl} |F_P|$ 15-30%

SAD: $R_{anom} = 200 \bullet \Sigma_{hkl} \mid I^+ - I^- \mid / \Sigma_{hkl} \mid I^+ + I^- \mid$ ~5%

S-SAD: ~1%

Signal Strength and Data Quality



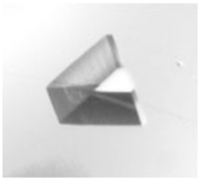
$$R_{merge} = \frac{\Sigma_{hkl}\,\Sigma_i\,|\,I_{i,hkl} - \bar{I}_{hkl}\,|}{\Sigma_{hkl}\,\Sigma_i\,|\,I_{i,hkl}|}$$

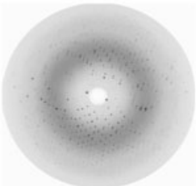
$$R_{r.i.m.} = \frac{\Sigma_{hkl}\,(N/(N-1))^{1/2}\,\Sigma_i\,|\,I_{i,hkl} - \bar{I}_{hkl}\,|}{\Sigma_{hkl}\,\Sigma_i\,|\,I_{i,hkl}|} = R_{meas}$$

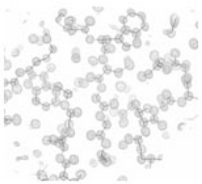
$$R_{p.i.m.} = \frac{\Sigma_{hkl}\,(1/(N-1))^{1/2}\,\Sigma_i\,|\,I_{i,hkl} - \bar{I}_{hkl}\,|}{\Sigma_{hkl}\,\Sigma_i\,|\,I_{i,hkl}|}$$

$$R_{anom} = \frac{\Sigma_{hkl}\,|\,I_{hkl} - I_{\bar{h}\bar{k}\bar{l}}\,|}{1/2\,\Sigma_{hkl}\,|\,I_{hkl} + I_{\bar{h}\bar{k}\bar{l}}\,|}$$

Summary

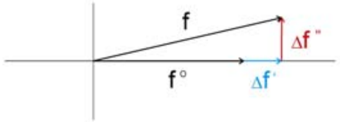






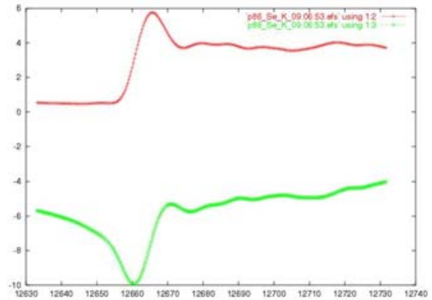
- Two Fundamental Equations in Crystallography,

F(hkl) and ρ(x,y,z)

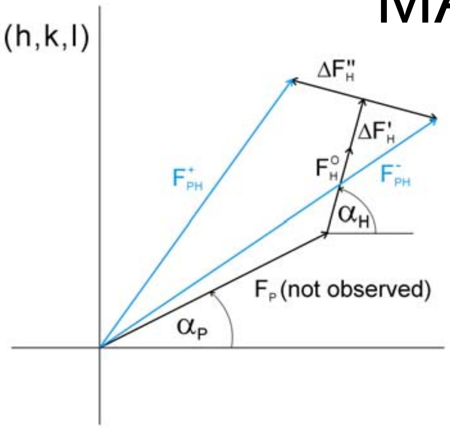


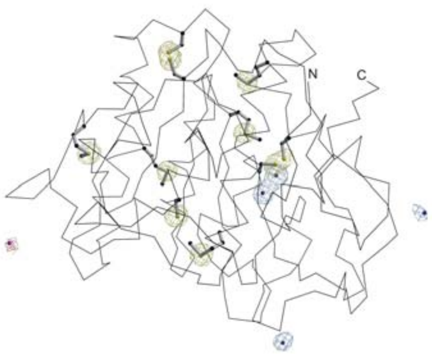
$f = f° + \Delta f' + i\Delta f''$

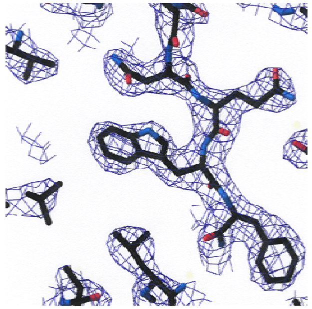
- The Phase Problem
- Normal and Anomalous Scattering

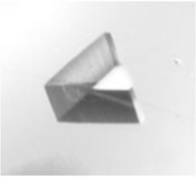


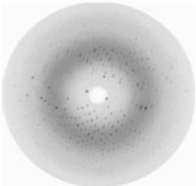
- Determination of Δf ' and Δf ''
- Phase Determination Methods (SIR(AS), MIR(AS), MAD, SAD, RIP(AS), MR, Direct Methods)

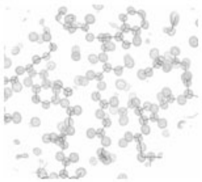












Thank you for your attention