

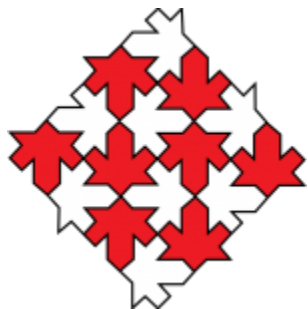
Absolute Configuration and Absolute Structure Determination

CCCW23

June 3, 2023

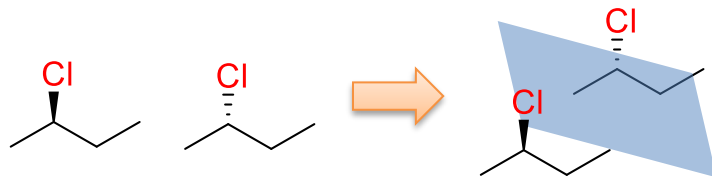
Kate M. Marczenko

University of Guelph

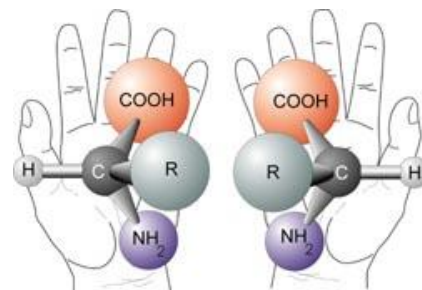
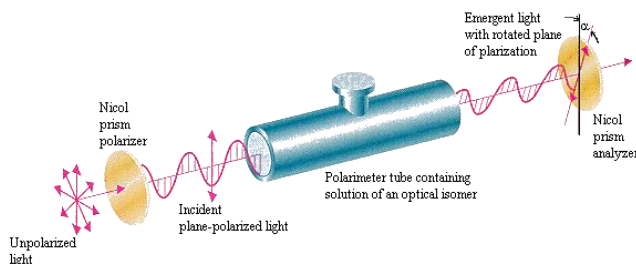


Absolute Configuration

- Absolute Configuration → spatial arrangement of the atoms for a chiral molecule (R/S, P/M or D/L assignment)



- Determination of absolute configuration → handedness of the molecule.
- Two non superimposable mirror images of a chiral molecule are called *enantiomers* – they are optical isomers.



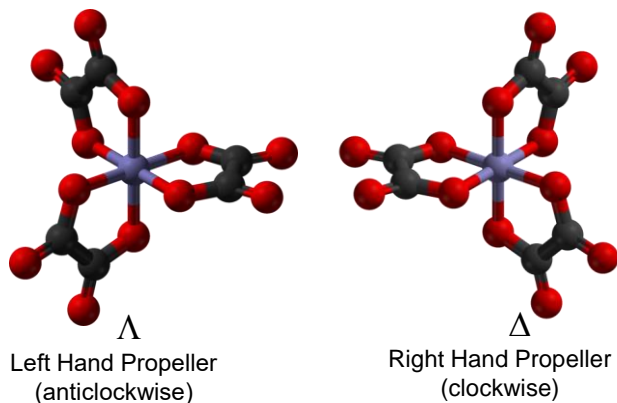
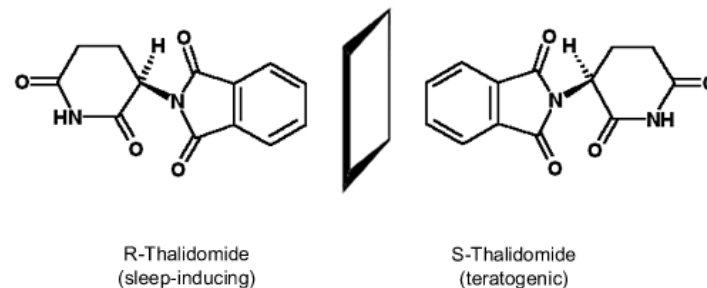
Absolute Structure

- Absolute Structure → spatial arrangement of atoms in a non-centrosymmetric crystal structure (unit-cell, space group).
- For a chiral crystal structure, absolute structure distinguishes between and specifies enantiomorphs of the crystal structure.

| | Absolute Structure | Absolute Configuration |
|-----------------|---------------------|------------------------|
| Property | Spatial Arrangement | Spatial Arrangement |
| Content | Atoms | Atoms |
| Object | Crystal | Molecule |
| Symmetry | Non-centrosymmetric | Chiral |

Chirality

- Chirality plays an important role in the binding affinity and interactions between a drug and its target.



- Experimental techniques include optical rotation, circular dichroism spectroscopy, enantioselective chromatography, etc.
- X-ray diffraction of single crystals has the ability to distinguish between enantiomorphs of a chiral structure and enantiomers of a chiral molecule.

Crystallization

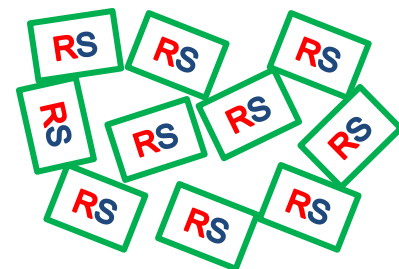
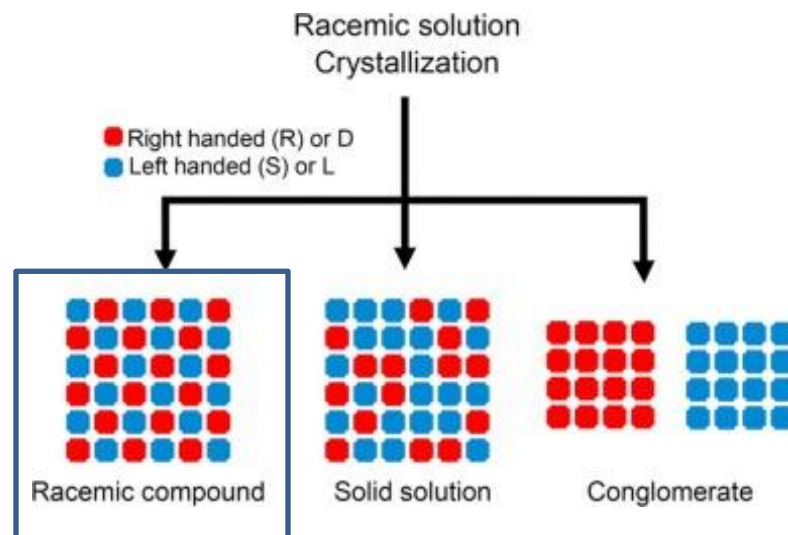
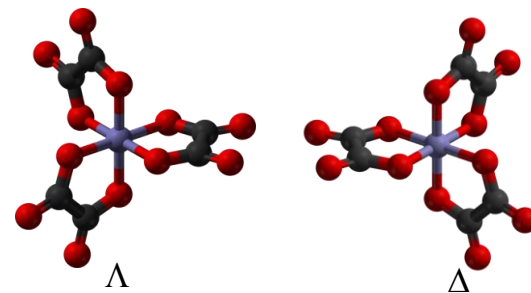
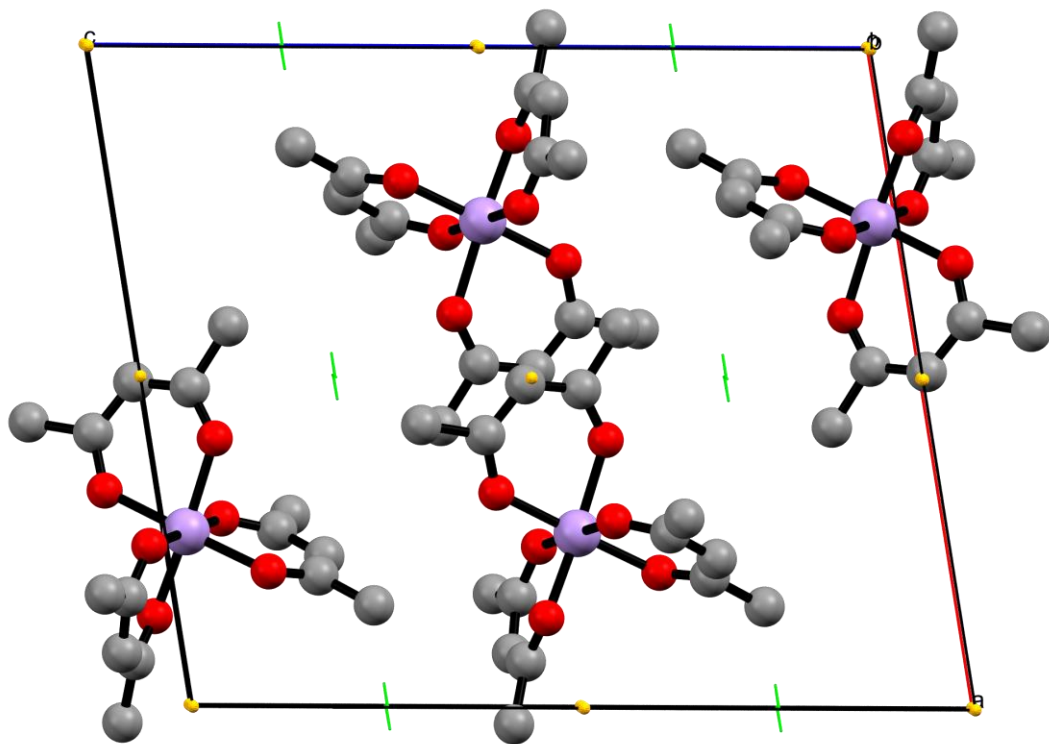


Image from: Ultrasonics Sonochemistry, 2018, 43, 184-192.

- The solid phase formed from a racemic solution can be a **racemic compound**.
 - Crystal containing an even ratio of both enantiomers in a regularly structured array.
 - No optical activity.
 - Usually centrosymmetric space group.

Racemic Compound



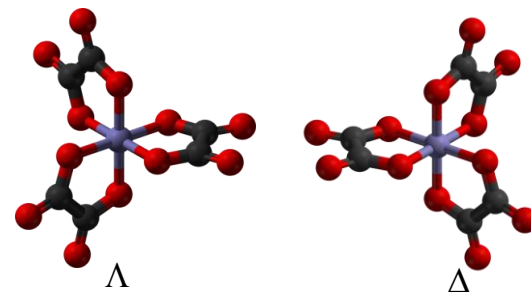
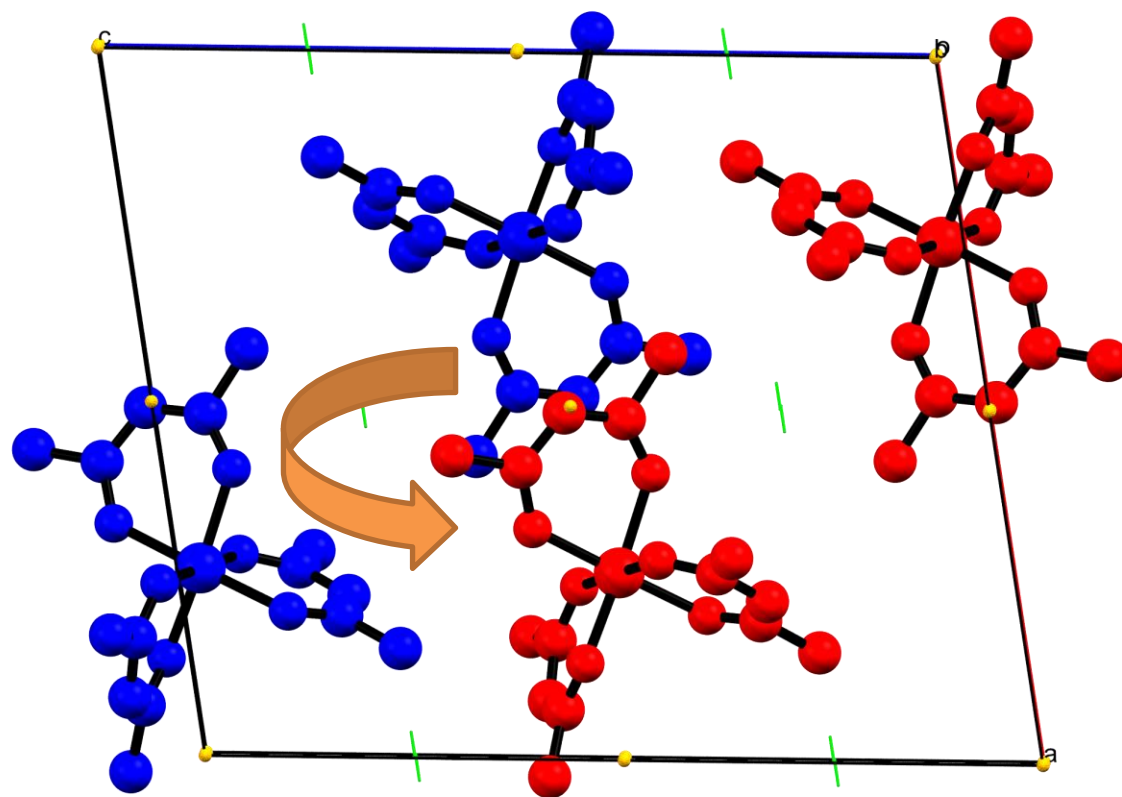
Legend

- | 2-fold screw axis
- Inversion center

ACACMN31 - $P2_1/c$

*Glide plane omitted for visual clarity

Racemic Compound



Legend

Right hand red
Left hand blue

ACACMN31 - $P2_1/c$

Crystallization

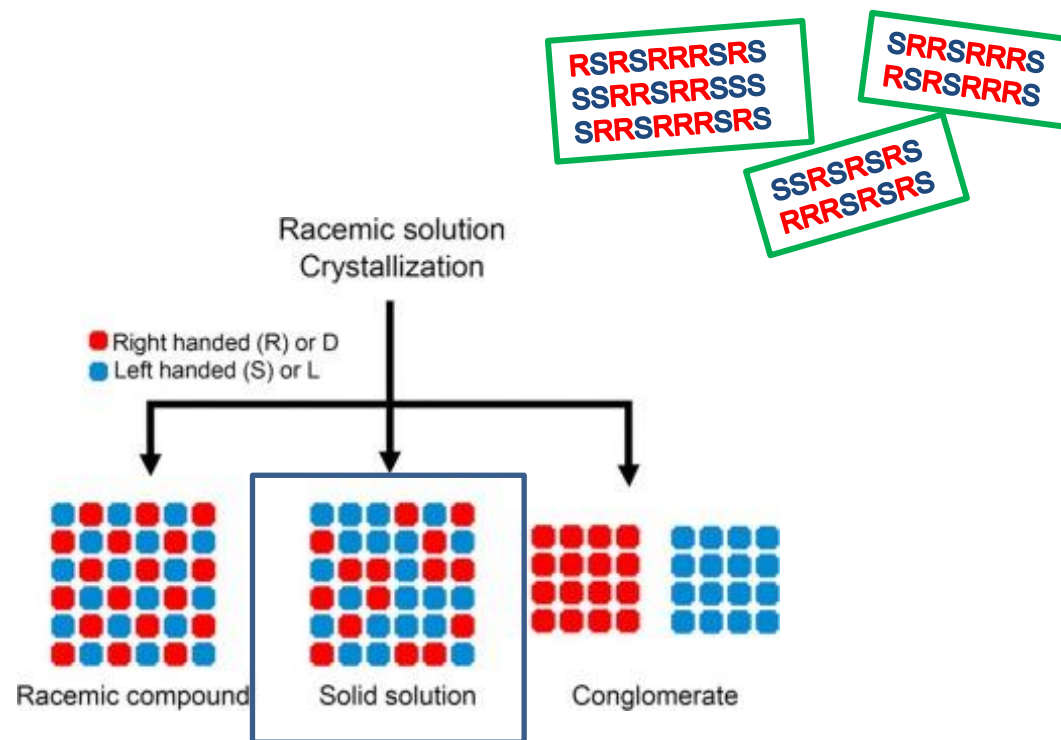


Image from: Ultrasonics Sonochemistry, 2018, 43, 184-192.

- **(Disordered) Solid Solution** is a crystal containing the two enantiomers in a disordered arrangement.
 - Usually centrosymmetric space group.

Crystallization

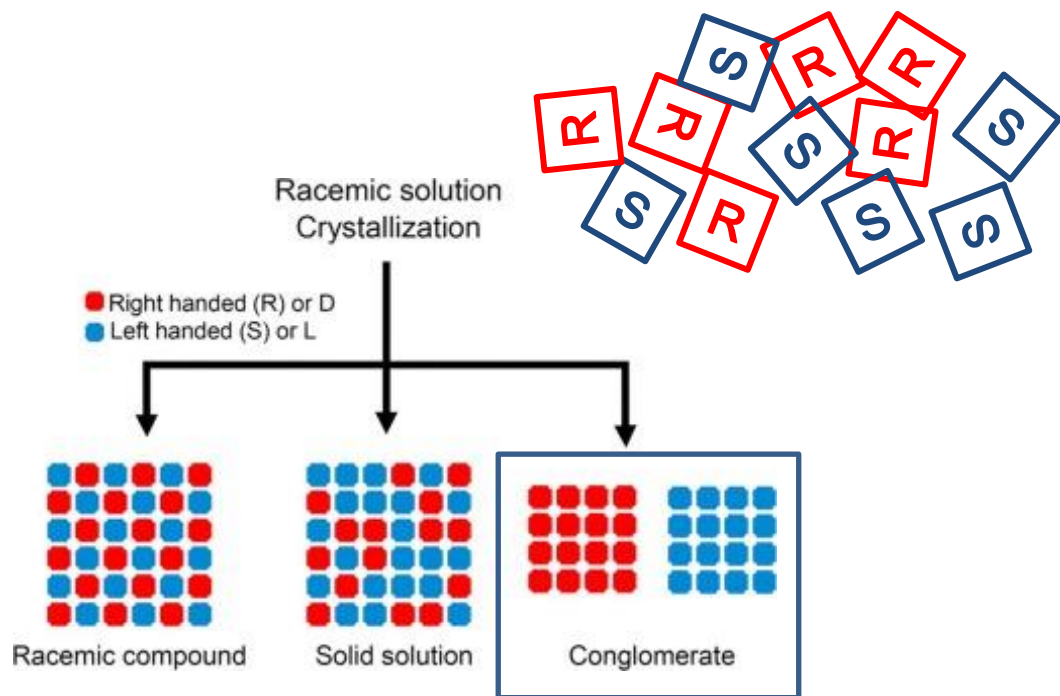


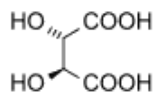
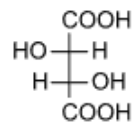
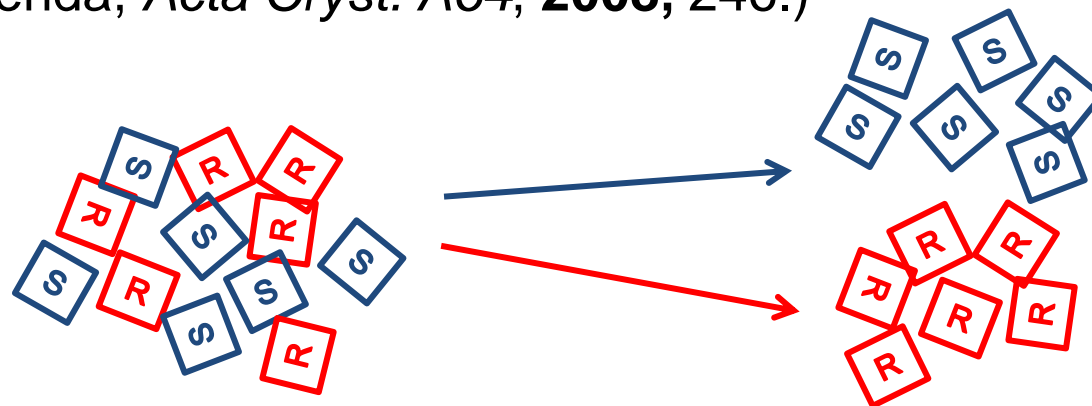
Image from: [Ultrasonics Sonochemistry](#), 2018, 43, 184-192.

- Crystallization of a racemic solution may give a **conglomerate** with homochiral resolution of each enantiomer.
 - i.e. a mixture of well-resolved crystals of both enantiomers
 - Individual crystals have optical activity
 - Chiral space group

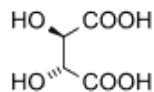
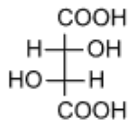
Enantiomorphous Crystals

Louis Pasteur Experiment (1848): Separation of the two enantiomers by the visual sorting of crystals of a conglomerate

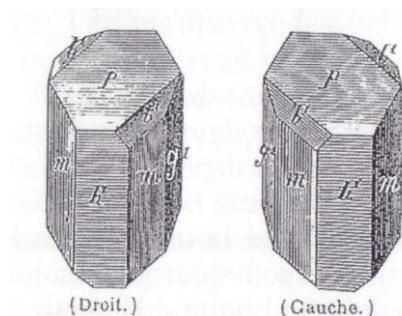
(Z. S. Derewenda, *Acta Cryst. A*64, **2008**, 246.)



D-(-) levotartaric acid



L-(+) dextrotartaric acid



Sodium ammonium tartrate crystals

feature articles

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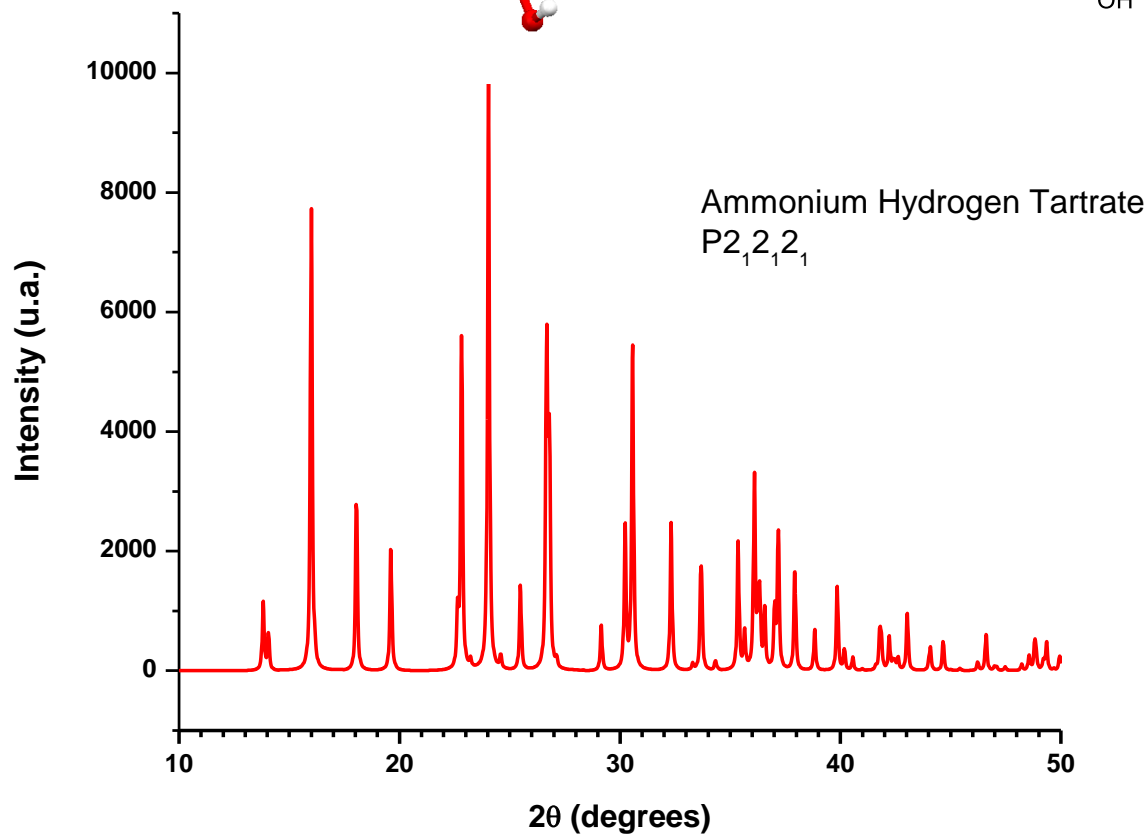
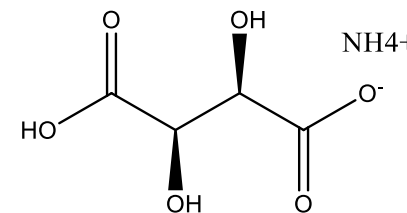
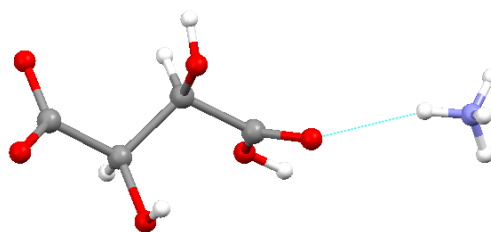
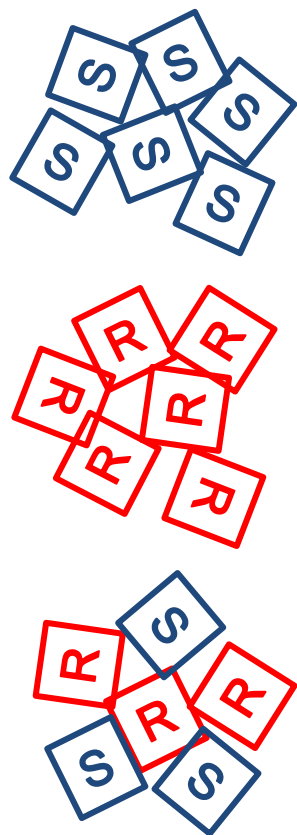
On wine, chirality and crystallography

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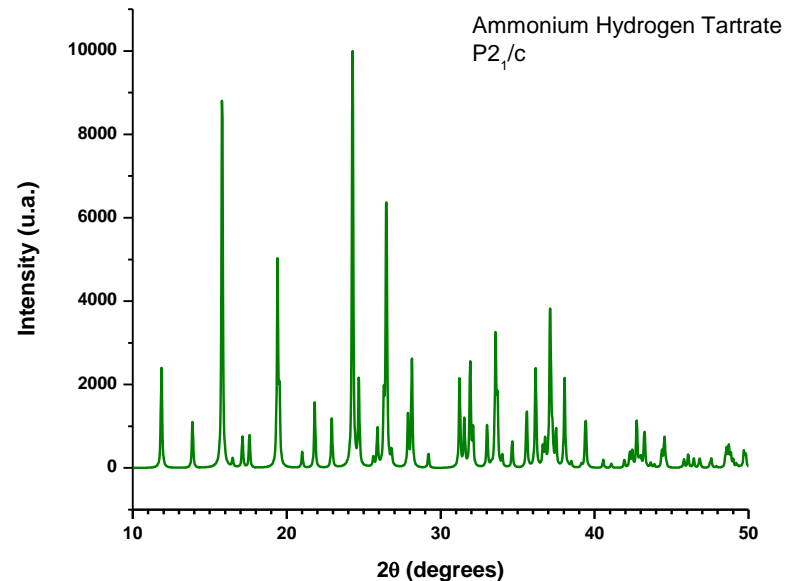
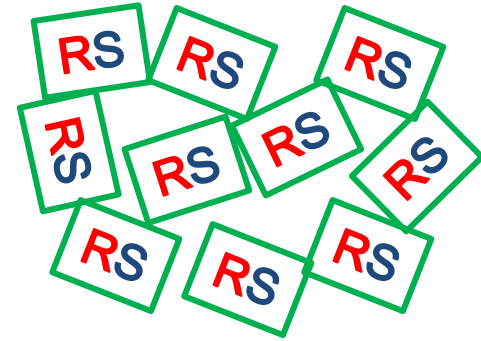
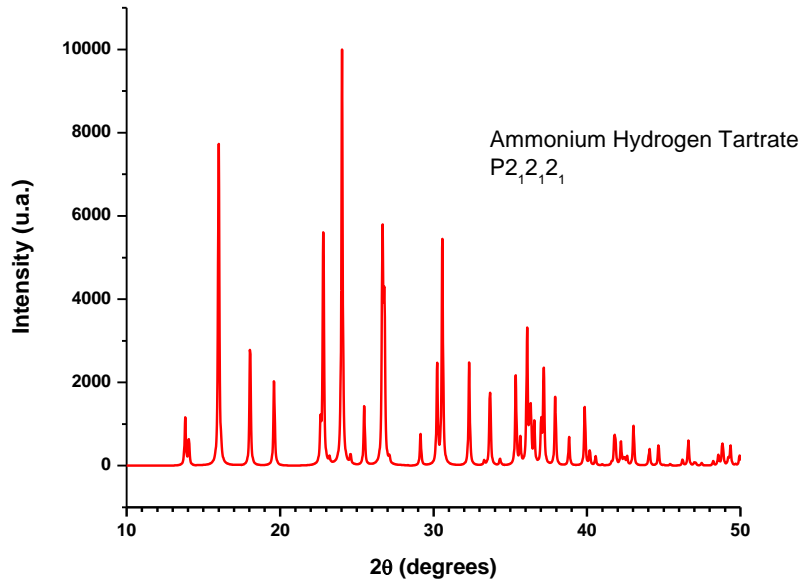
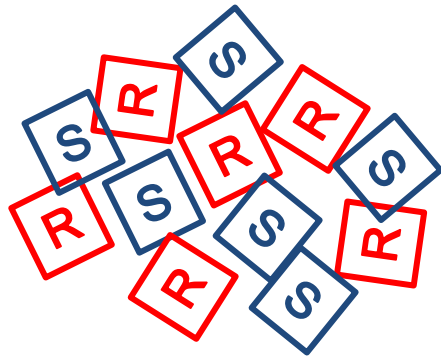
Powder Diffraction

The two enantiopure samples and the conglomerate give the same powder X-ray diffraction pattern.



Powder Diffraction

The conglomerate and the racemate have different space groups, so the powder diffraction pattern is different.



In the Solid-State

- Chiral molecules can crystallize as an enantiopure bulk sample or as a racemic mixture.
- For enantiopure crystals → Space group restriction:
 - Only 65 space groups allowed for enantiopure chiral molecules: No Inversion Center / No Mirror / No Glide Plane (Sohncke groups)
 - These include 11 pairs of enantiomorphic space groups (screw axes of opposite handedness) eg: $P4_1/P4_3$ -or $P6_1/P6_5$
 - Forty-three other space groups allow for chiral crystal structures, making a complete set of sixty-five space groups known as the Sohncke groups.

In the Solid-State

Triclinic: $P1$

Monoclinic: $P2$ $P2_1$ $C2$

Orthorhombic: $P222$ $P222_1$ $P2_12_12$ $P2_12_12_1$ $C222_1$ $C222$ $F222$ $I222$
 $I2_12_12_1$

Tetragonal: $P4$ $P4_1$ | $P4_3$ $P4_2$ $I4$ $I4_1$ $P422$ $P42_12$ $P4_122$ | $P4_322$
 $P4_12_12$ | $P4_32_12$ $P4_222$ $P4_22_12$ $I422$ $I4_122$

Trigonal: $P3$ $P3_1$ | $P3_2$ $R3$ $P3_12$ $P321$ $P3_112$ | $P3_212$ $P3_121$ | $P3_221$
 $R32$

Hexagonal: $P6$ $P6_1$ | $P6_5$ $P6_2$ | $P6_4$ $P6_3$ $P622$ $P6_122$ | $P6_522$ $P6_222$ |
 $P6_422$ $P6_322$

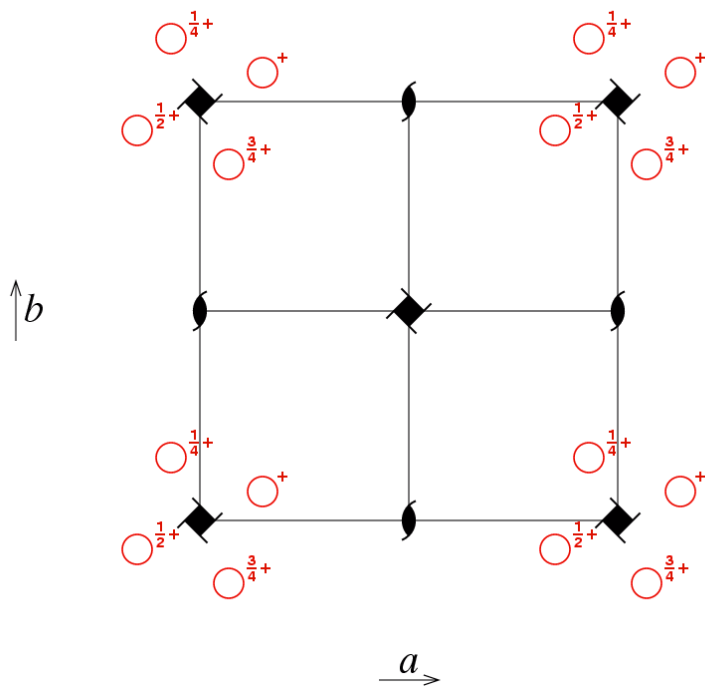
Cubic: $P23$ $F23$ $I23$ $P2_13$ $I2_13$ $P432$ $P4_232$ $F432$ $F4_132$ $I432$
 $P4_132$ | $P4_332$ $I4_132$

Enantiomorphic Space Groups

$P4_1$

$P4_1$

4



1 x, y, z

2 $\bar{x}, \bar{y}, \frac{1}{2} + z$

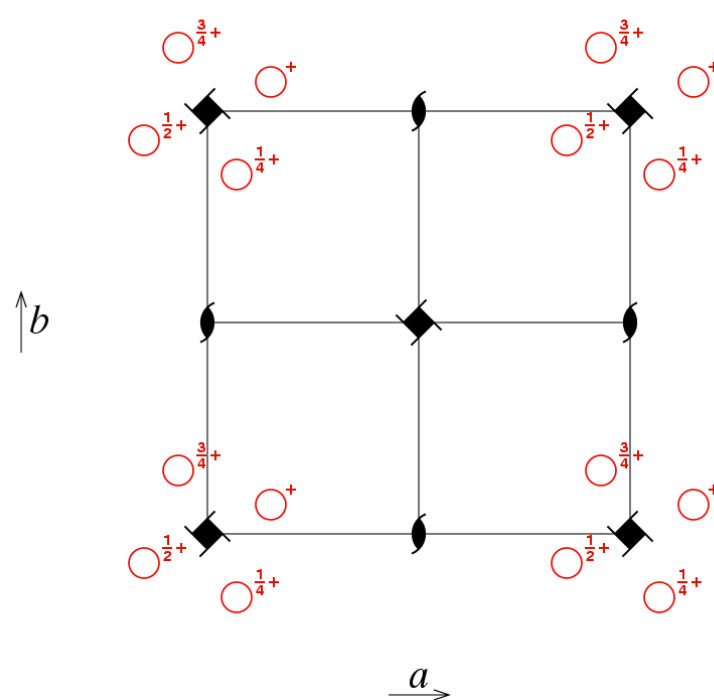
3 $\bar{y}, x, \frac{1}{4} + z$

4 $y, \bar{x}, \frac{3}{4} + z$

$P4_3$

$P4_3$

4



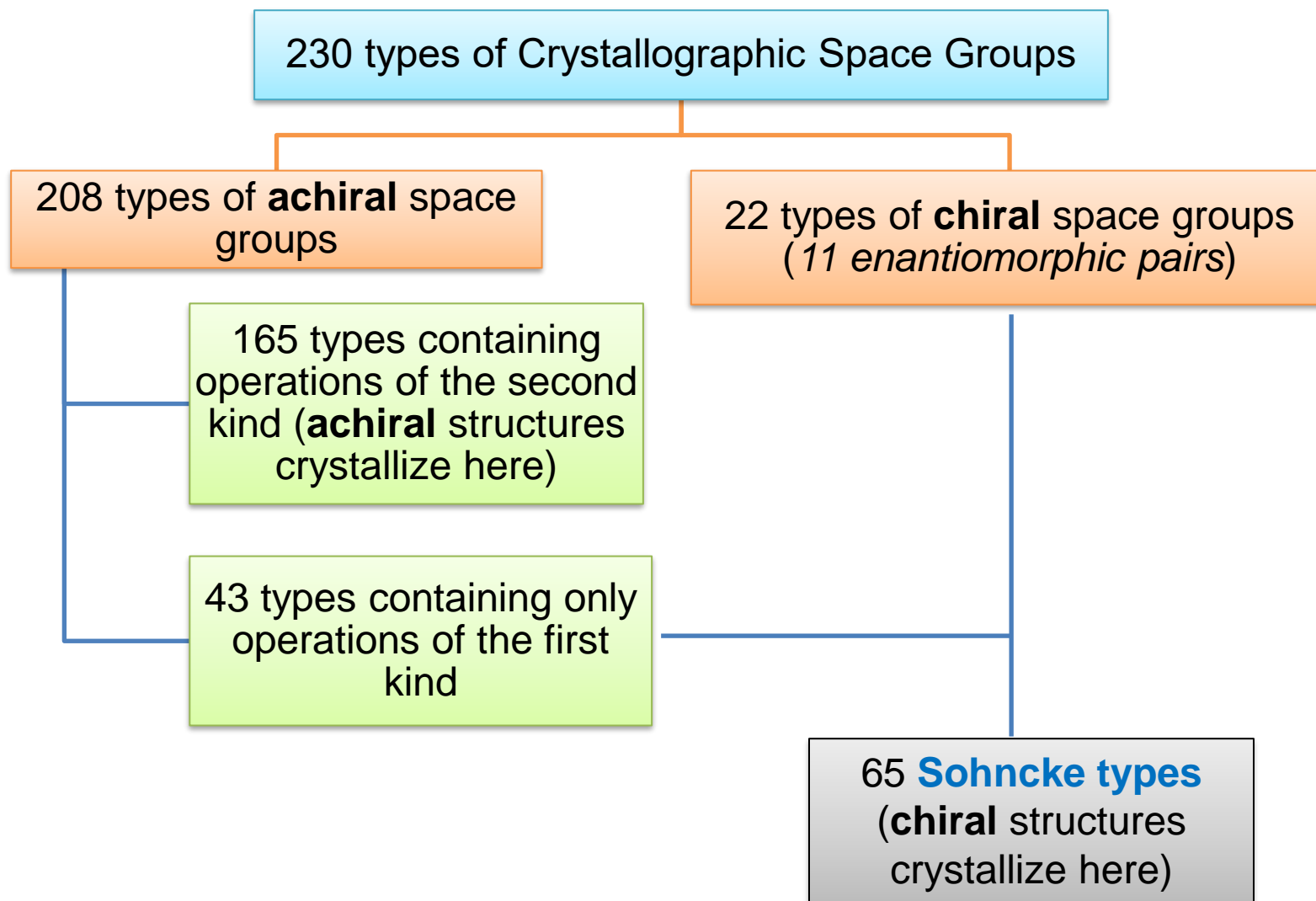
1 x, y, z

2 $\bar{x}, \bar{y}, \frac{1}{2} + z$

3 $\bar{y}, x, \frac{3}{4} + z$

4 $y, \bar{x}, \frac{1}{4} + z$

Space Group Framework



How can we determine the Absolute Configuration?

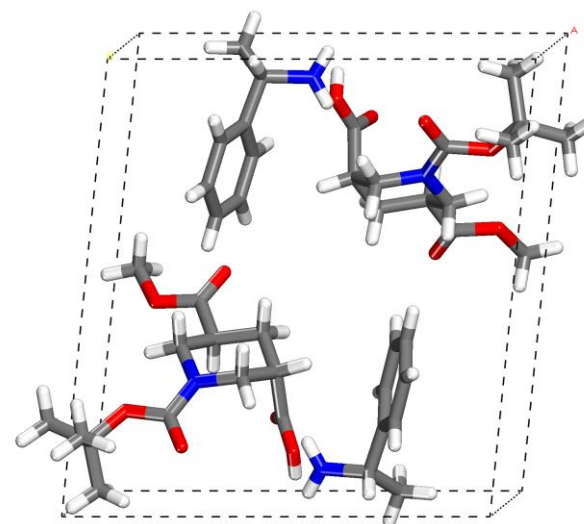
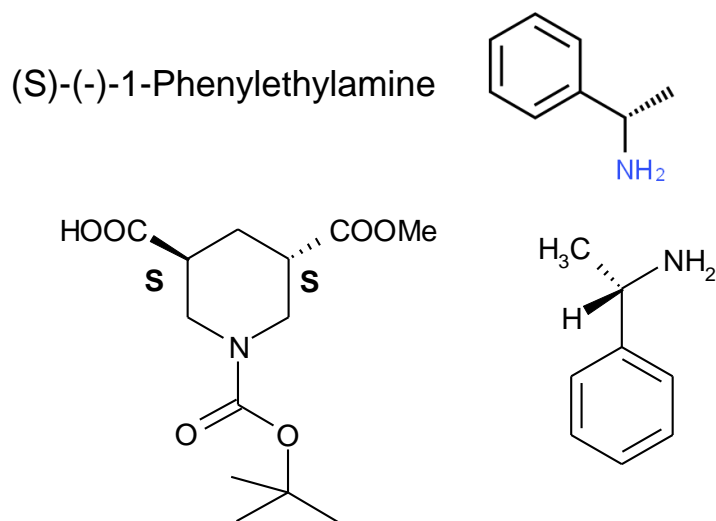
- Method 1: Internal Chiral Reference
- Method 2: Absolute configuration established by anomalous-dispersion effects in diffraction measurements on the crystal.

How can we determine the Absolute Configuration?

Method 1: Absolute configuration from an internal chiral reference

- Incorporation of a compound with known absolute configuration into the crystal structure (chemical reaction, cocrystallization), and using that as an internal reference.

You can not always assume that chemical reaction, crystallization, or operations of mechanochemistry will conserve the chirality of the reference material.

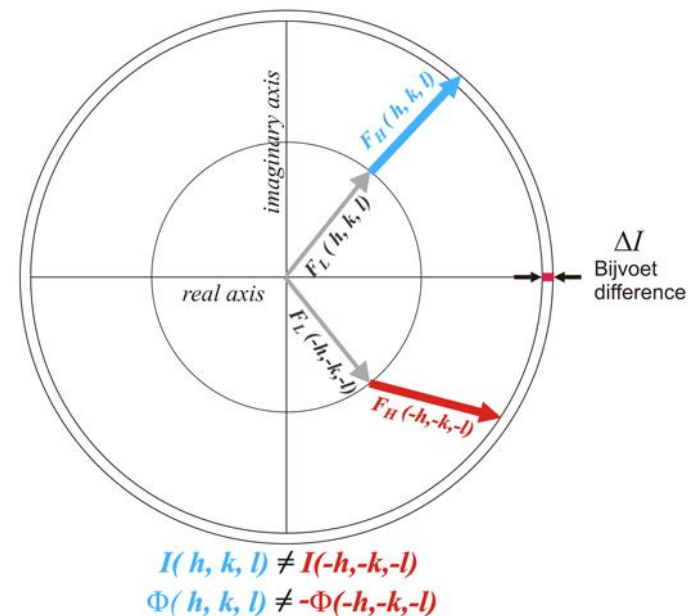
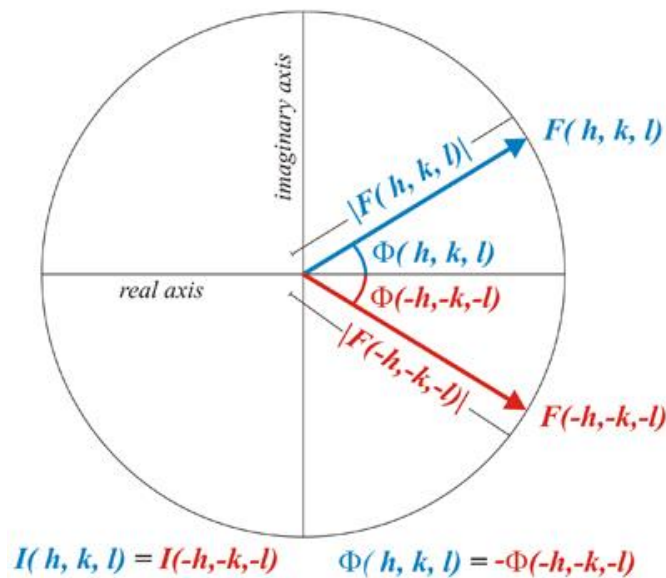


FOCXAX P₂₁
Hans Iding, Beat Wirz, Rosa-María Rodríguez Sarmiento,
Tetrahedron Asymmetry 14 (2003) 1541-1545

How can we determine the Absolute Configuration?

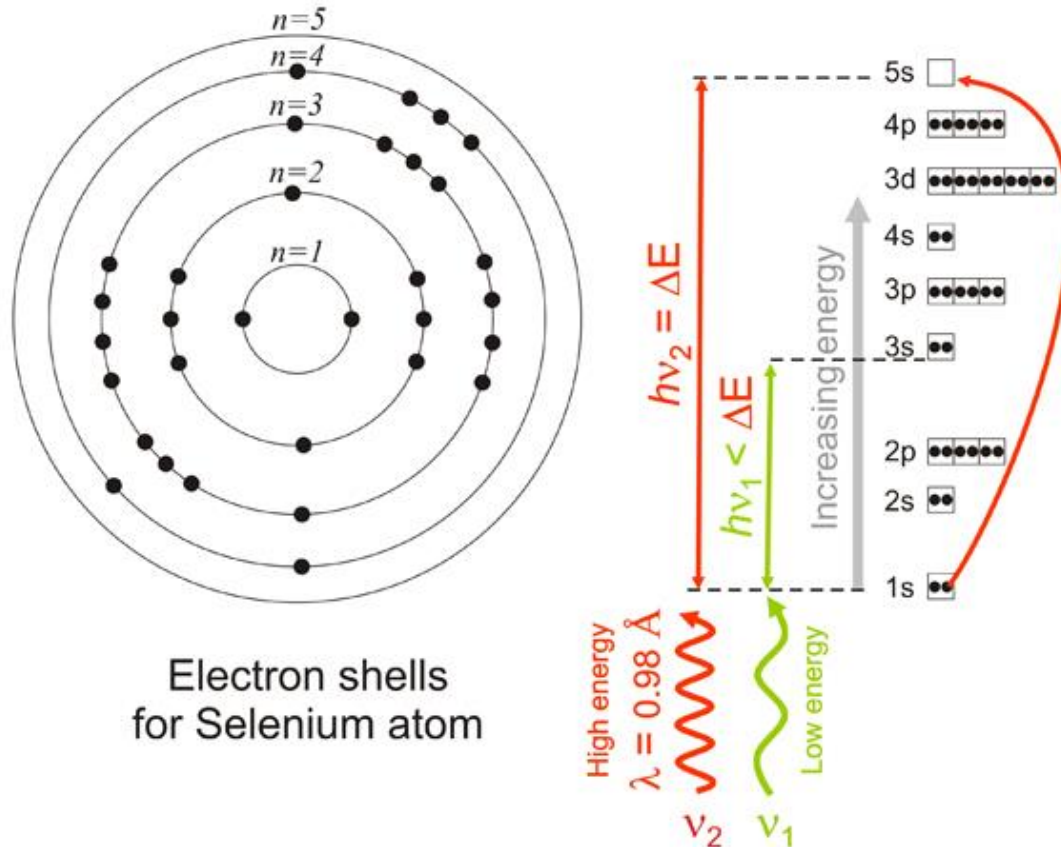
Method 2: From the diffraction data via the absolute structure

- Method based on the ***anomalous scattering (dispersion)***.
 - Friedel pairs (hkl) and $(-h-k-l)$ will show a small difference in intensity ... “breaking” of Friedel’s law.



Anomalous Scattering

- When the incident radiation has sufficient energy to promote an electronic transition in atoms contained in the crystal, we observe anomalous scattering in non-centrosymmetric crystals.



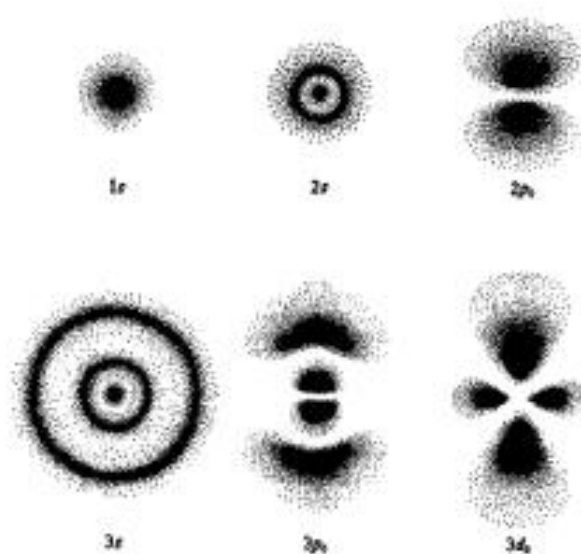
ΔE is near 8keV for most heavy and some light elements.

- Anomalous scattering will be observed if the X-Ray energy is slightly higher than the absorption edge for the element.

Anomalous Scattering

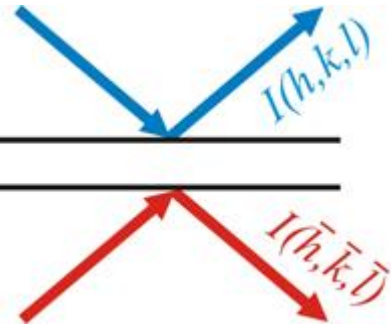
- Anomalous scattering causes small but measurable differences in intensity between the reflections (h,k,l) and $(-h,-k,-l)$.
- In non-centrosymmetric crystals in the presence of atoms heavier than sulfur, Friedel's law is not strictly true!

Under normal conditions, electron distributions within atoms are centrosymmetric.



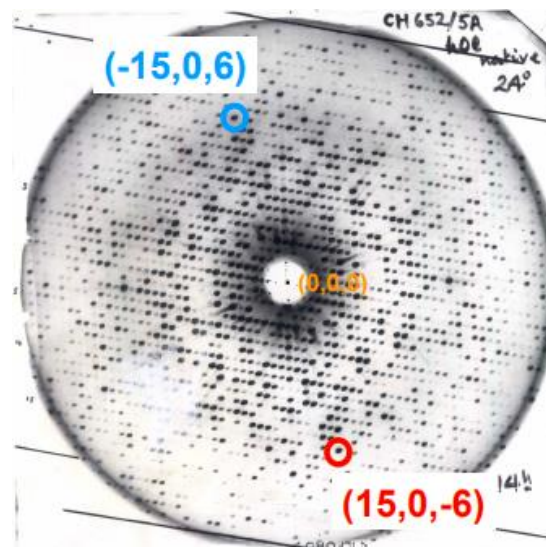
Anomalous Scattering

- The centrosymmetry in the scattering atoms is reflected in the centrosymmetry in the pattern of scattered X-ray intensities.
- The positions of the reflections (hkl) and $(-h-k-l)$ on the reciprocal lattice are related by a center of symmetry through the reciprocal lattice origin $(0,0,0)$.
- The reflections (h,k,l) and $(-h,-k,-l)$ are called Friedel pairs.



The diagram illustrates the principle of Friedel's Law. It shows two horizontal lines representing crystal planes. An incident blue beam strikes the top plane, and a scattered blue beam is labeled $I(h,k,l)$. A red beam strikes the bottom plane, and a scattered red beam is labeled $I(\bar{h},\bar{k},\bar{l})$. Below the diagram, a yellow box contains the equation:

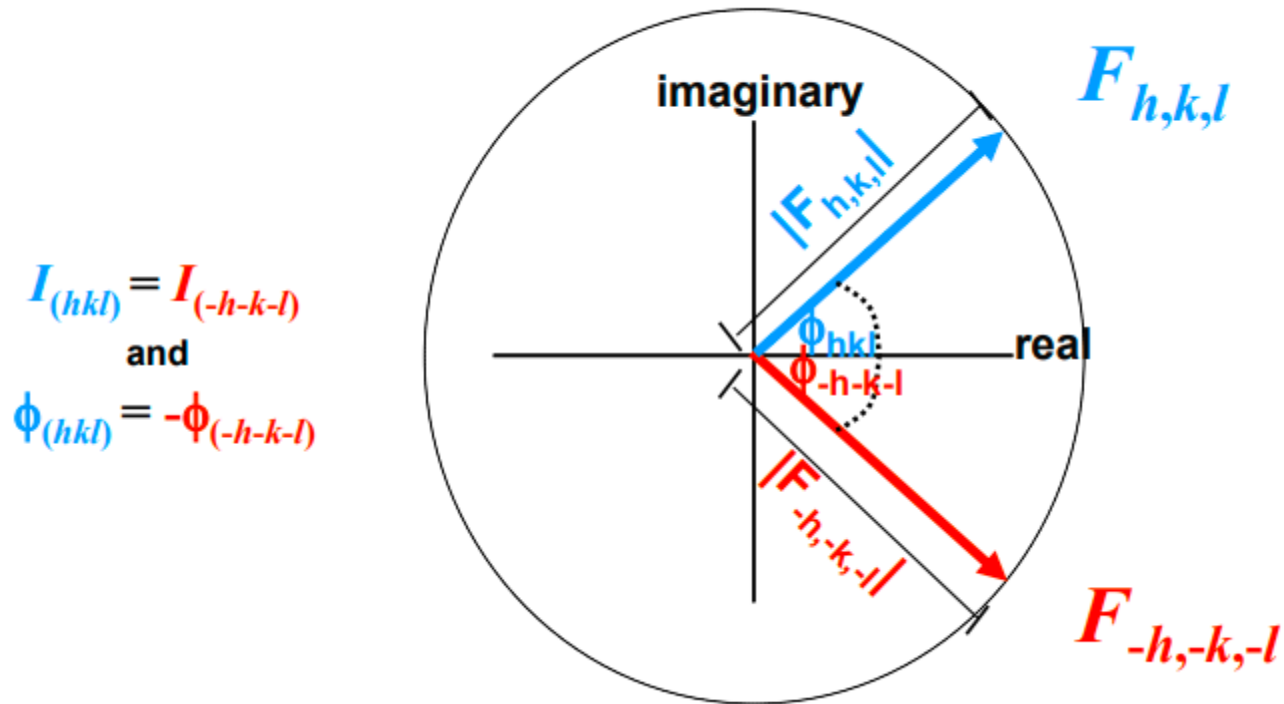
$$|F_{hkl}|^2 = |F_{\bar{h}\bar{k}\bar{l}}|^2$$



Friedel's Law is fulfilled in centrosymmetric crystals or in absence of anomalous dispersion.

Anomalous Scattering

On an Argand diagram, $F_{(hkl)}$ and $F_{(-h-k-l)}$ appear to be reflected across the real axis.

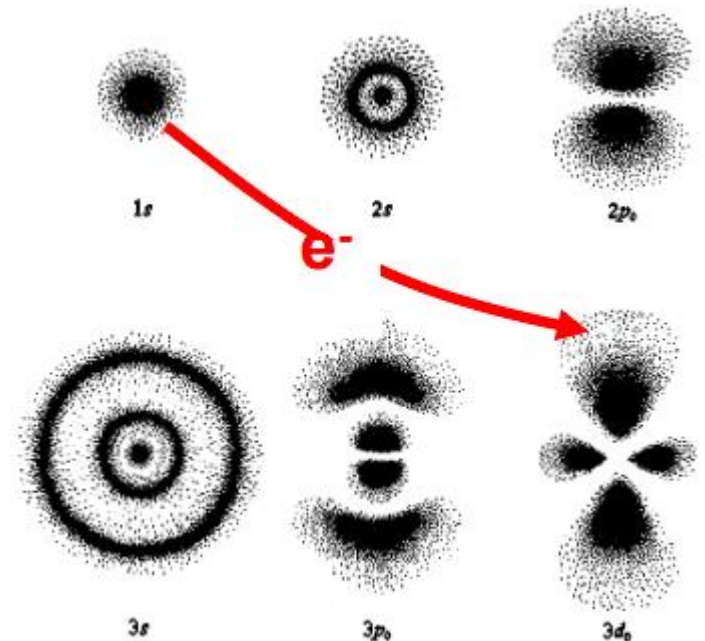


True for any crystal in the absence of anomalous scattering. Normally, $I_{(hkl)}$ and $I_{(-h-k-l)}$ are merged together.

Anomalous Scattering

- Anomalous scattering causes small but measurable differences in the intensity between the reflections (h,k,l) and $(-h,-k,-l)$.
- Under conditions of anomalous scattering, electrons are perturbed from their centrosymmetric distributions; they are jumping between orbitals.
- The breakdown of centrosymmetry in the scattering atoms is reflected in a loss of centrosymmetry in the pattern of scattered X-ray intensities.

Thus, $I(h, k, l) = I(-h, -k, -l)$ is no longer true.



Structure Factors

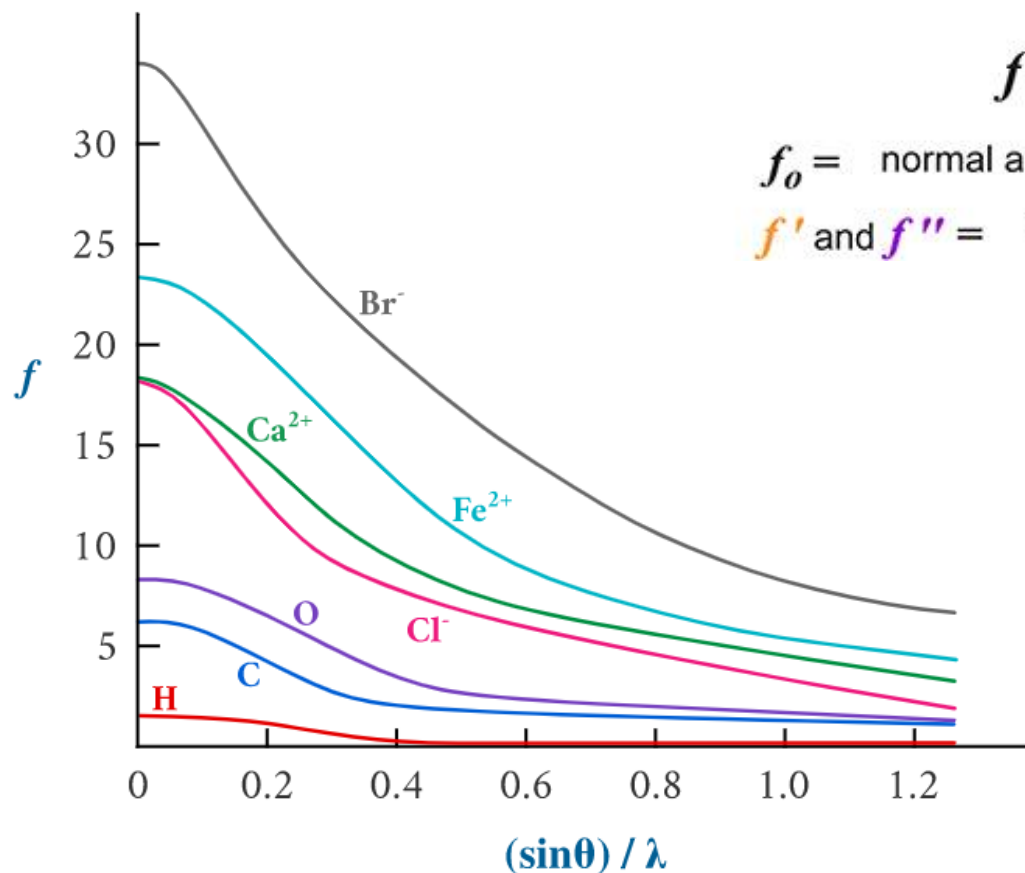
- **Structure factor:** represent the diffracted waves...a mathematical description of how a material scatters incident radiation.

$$F(hkl) = \sum_{j=1}^n f_j e^{2\pi i[hx+ky+lz]}$$

- ...a structure factor, $F(hkl)$, is the resultant of all waves scattered in the direction of the hkl reflection by the n atoms contained in the unit cell.
- Each of these waves shows an amplitude proportional to the atomic scattering factor, f_j , that measures the X-ray scattering power of each atom.

Resonant Scattering (Anomalous Dispersion)

- **Atomic scattering factor:** measure of the scattering power of an isolated atom. The scattering factor depends on the amplitude (*intensity*) of an individual atom and the Bragg angle of scattering.



$$f = f_o + f' + i f''$$

f_o = normal atomic scattering factor (real number)

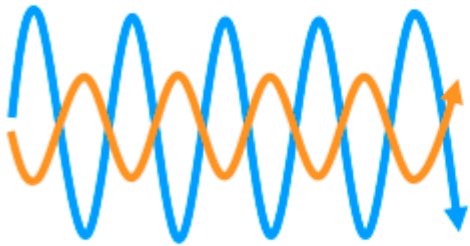
f' and f'' = real and imaginary correction factors

If anomalous dispersion occurs, the atomic scattering factor (f) behaves as a complex number.

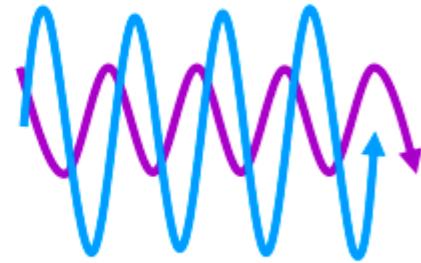
Resonant Scattering

- The scattering factor is a complex number.

$$f = f_o + \Delta f' + i\Delta f''$$



real component, $\Delta f'$

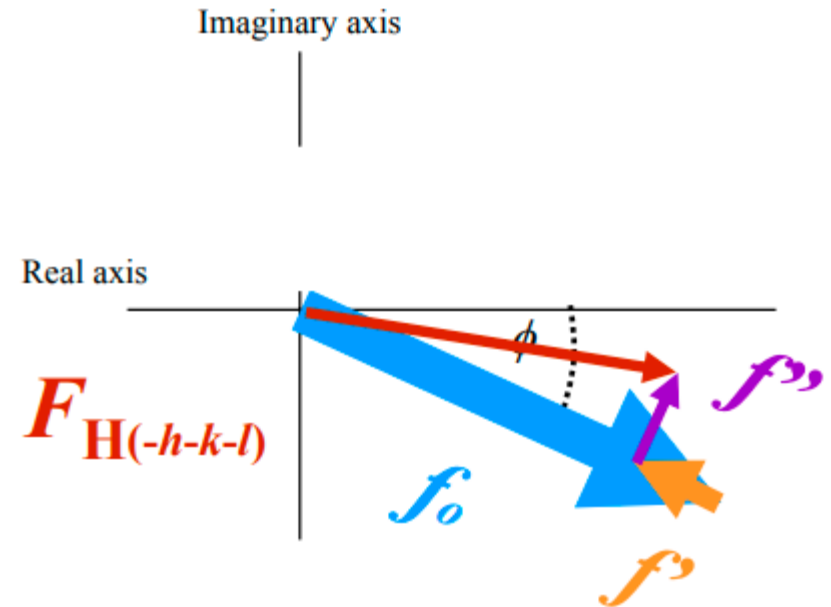
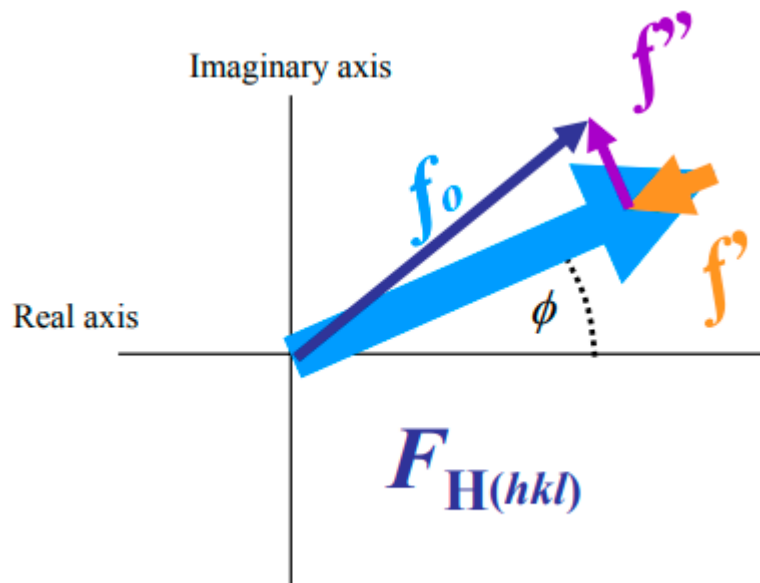


imaginary component, $\Delta f''$

- Real component:** a small component of the scattered radiation is 180° out of phase with the normally scattered radiation given by f_o .
- Imaginary component:** a small component of the scattered radiation is 90° out of phase with the normally scattered radiation given by f_o .

Resonant Scattering

- Argan diagrams for $F_{H(-h-k-l)}$ is constructed in a similar way as $F_{H(hkl)}$ except Φ is negative.

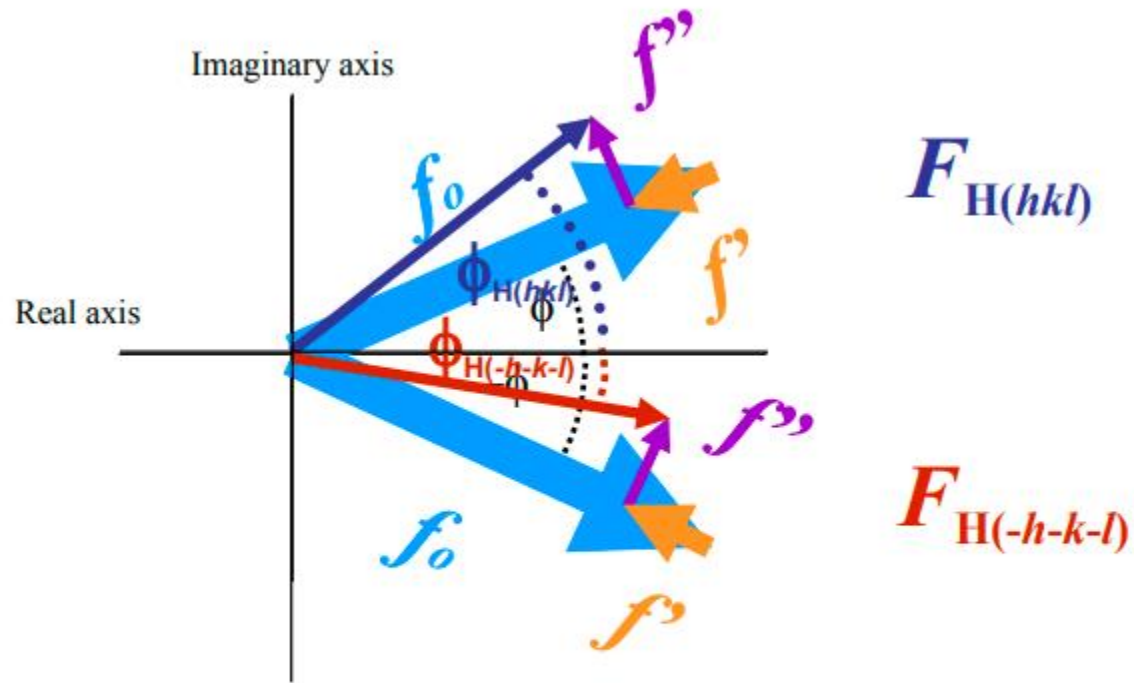


$$F_{H(hkl)} = [f_o + \Delta f'(\lambda) + i\Delta f''(\lambda)] e^{2\pi i(hx + ky + lz)}$$

$$F_{H(-h-k-l)} = [f_o + \Delta f'(\lambda) + i\Delta f''(\lambda)] e^{2\pi i(-hx - ky - lz)}$$

Resonant Scattering

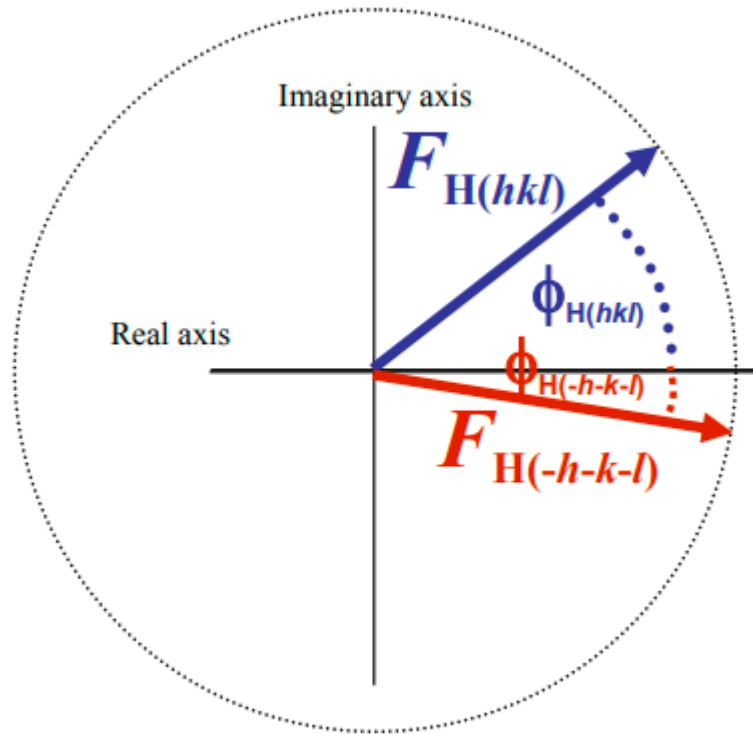
Friedel's law is broken!



$$\phi_{H(hkl)} \neq \phi_{H(-h-k-l)}$$

Resonant Scattering

- For only the contribution of one type of heavy atom: $I_{H(hkl)} = I_{H(-h-k-l)}$
- This changes in the presence of other atoms (i.e. lighter atoms that do not show anomalous scattering).



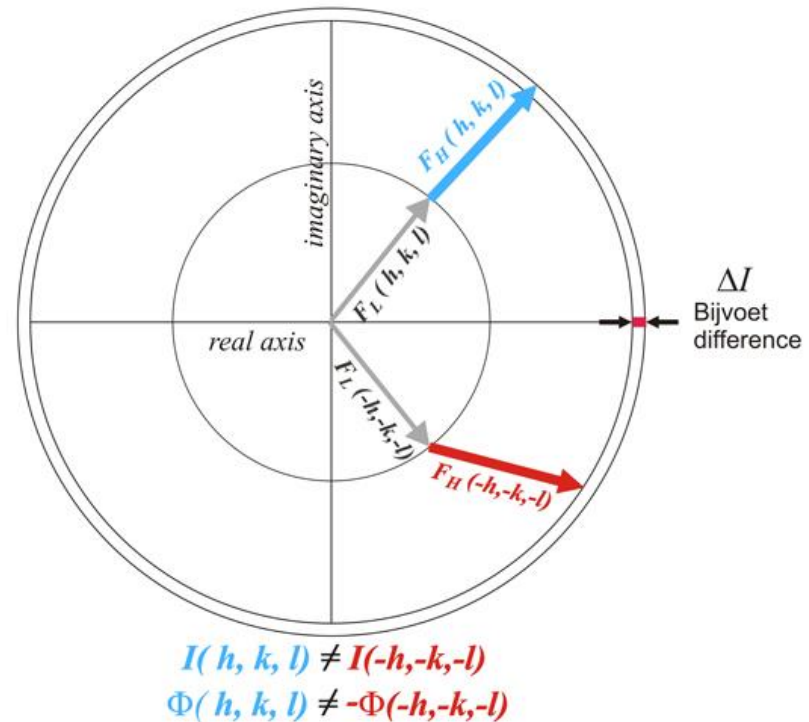
$$I_{H(hkl)} = I_{H(-h-k-l)}$$

$$\phi_{H(hkl)} \neq \phi_{H(-h-k-l)}$$

Resonant Scattering

- In the presence of other atoms, there is an observable difference between $|F_{(-h-k-l)}|$ and $|F_{(hkl)}|$ called a Bijvoet or Friedel difference, or an anomalous difference.
- This weak signals, ΔI , typically low (<3%) and not always easy to measure, can be used to solve the phase problem, and to determine the absolute configuration of molecules.

When both normal and anomalous dispersors are present, Friedel's Law is totally broken.



Check-in...

- Anomalous scattering of X-ray photons occurs with atoms that absorb X-rays near their absorption edge, resulting in a breakdown of _____.

Check-in...

- Anomalous scattering of X-ray photons occurs with atoms that absorb X-rays near their absorption edge, resulting in a breakdown of Friedel's law.
- The atomic structure factor becomes a _____.

Check-in...

- Anomalous scattering of X-ray photons occurs with atoms that absorb X-rays near their absorption edge, resulting in a breakdown of Friedel's law.
- The atomic structure factor becomes a complex number.
- There is now a difference in between $|F_{(hkl)}|^2$ and $|F_{(-h-k-l)}|^2$.
 - Result is a measurable difference between _____.

Check-in...

- Anomalous scattering of X-ray photons occurs with atoms that absorb X-rays near their absorption edge, resulting in a breakdown of Friedel's law.
- The atomic structure factor becomes a complex number.
- There is now a difference in between $|F_{(hkl)}|^2$ and $|F_{(-h-k-l)}|^2$.
 - Result is a measurable difference between $I_{(hkl)}$ and $I_{(-h-k-l)}$.
- We can compare the original and inverted diffraction patterns calculated from the original and inverted structure models with the one that is measured experimentally.

The calculated pattern that agrees better with the experimental data defines which absolute structure is the correct one.

Metric Parameters

- We can compare the intensities of Bijvoet pairs to determine the correct absolute structure.
- The Flack parameter is a single parameter that clearly indicates the absolute structure.

$$|F(hkl, x)|^2 = (1 - x)|F(hkl)|^2 + x|F(\bar{h}\bar{k}\bar{l})|^2$$

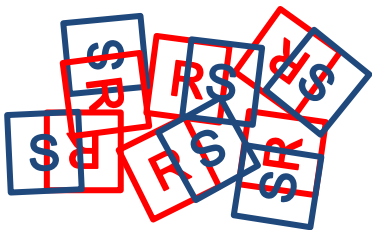
x is the absolute structure parameter

Howard Flack, *Acta Cryst.* **1983**, A39, 876.

$x = 0$: correct absolute configuration

$x = 1$: inverted absolute configuration

$0 < x < 1$: racemic twin



*racemic
twin*

- Racemic twinning is also called inversion twinning.
- Occurs in non-centrosymmetric space groups.
- Easy to identify using the Flack test.

$$|F_{hkl}|^2 \neq |F_{\bar{h}\bar{k}\bar{l}}|^2$$

Flack Parameter

$$|F(hkl, x)|^2 = (1 - x)|F(hkl)|^2 + x|F(\bar{h}\bar{k}\bar{l})|^2$$

Chiral space groups (P_1 , $P2_12_12_1$, ...) :

- Either no symmetry elements or only rotations
- May contain optically pure compounds
- Flack parameter has to be calculated

Polar space groups (Pm , $Pna2_1$, ...) :

- Contain mirror planes, but no inversion center
- May contain only achiral molecules or racemic mixtures
- Flack parameter has to be calculated

Centrosymmetric space groups:

- Flack parameter is not defined, since $|F(H)|^2 = |F(-H)|^2$

Parsons Quotients

- It was later shown that the Flack parameter can be improved using of quotients of **Friedel Pairs differences (Parsons method)**.
- Reduces the systematic errors and usually gives lower standard deviation.
Flack x is still defined from:

$$\begin{aligned} |F(H, x)|^2 &= (1-x)|F(H)|^2 + x|F(-H)|^2 \\ \Leftrightarrow I_{obs, hkl} &= (1-x)I_{hkl} + xI_{\overline{hkl}} \end{aligned}$$

- The new method use the following **quotients of Friedel Pairs differences**.

$$\frac{I_{hkl}(x) - I_{\overline{hkl}}(x)}{I_{hkl}(x) + I_{\overline{hkl}}(x)} = (1 - 2x) \frac{I_{hkl} - I_{\overline{hkl}}}{I_{hkl} + I_{\overline{hkl}}}$$

Parsons, S., Flack, H. D. & Wagner, T. *Acta Cryst.* **2013**, B69, 249.

Parsons, S. & Flack, H. D., *Acta Cryst.* **2004**, A60, s61.

The Hooft Parameter, y

- Hooft parameter calculates the probability to have the right absolute structure using Bijvoet pairs.
- **Friedel pair**: pair of reflections related by inversion symmetry: I_{hkl} and I_{-h-k-l} .
- **Bijvoet pair**: pair of reflections that are equivalent by symmetry and are a Friedel Pair.

$$I_{hkl} = I_{h-k-l} \quad \& \quad I_{-h-k-l} = I_{-h-k-l}$$

- E.g. Two-fold related reflections in P2 or P2₁. The true symmetry equivalents of a Bragg reflection have equal amplitude even in the presence of anomalous scattering. So true symmetry equivalents of one member of a Friedel pair form a set whose members have the same amplitude $|F^+|$, while all symmetry equivalents of the other member of the Friedel pair form a second set whose members share a different amplitude $|F^-|$.

$$|F^+| \equiv |F_{hkl}| = |F_{\bar{h}\bar{k}\bar{l}}| \quad |F^-| \equiv |F_{\bar{h}kl}| = |F_{h\bar{k}\bar{l}}|$$

R. W. W. Hooft, *et. al. J. Appl. Cryst.* **2008**, 41, 96.

J. Appl. Cryst. **2010**, 43, 665.

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

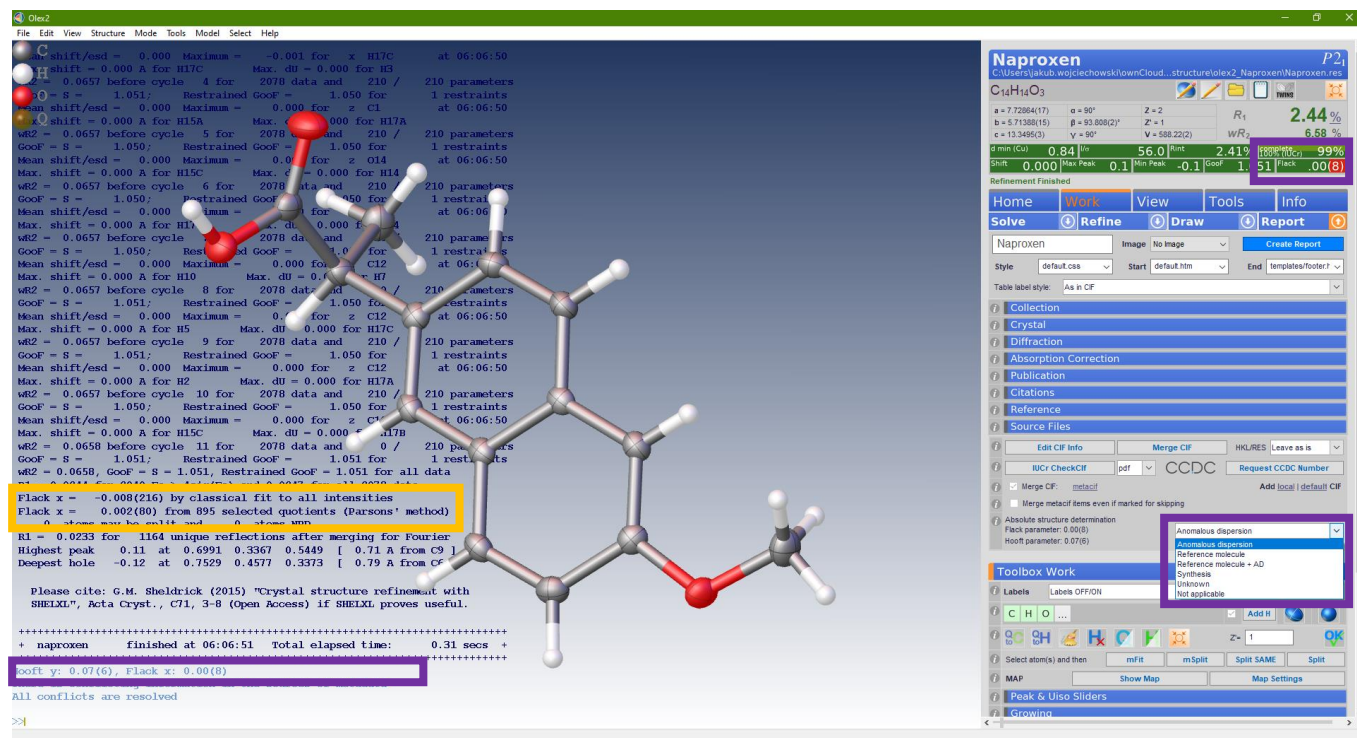
USEFUL OLEX2 OPTIONS FOR ABSOLUTE STRUCTURE REFINEMENT

Automatic Hooft and Flack parameter calculations.

Completeness is reported for both anomalous and merge data

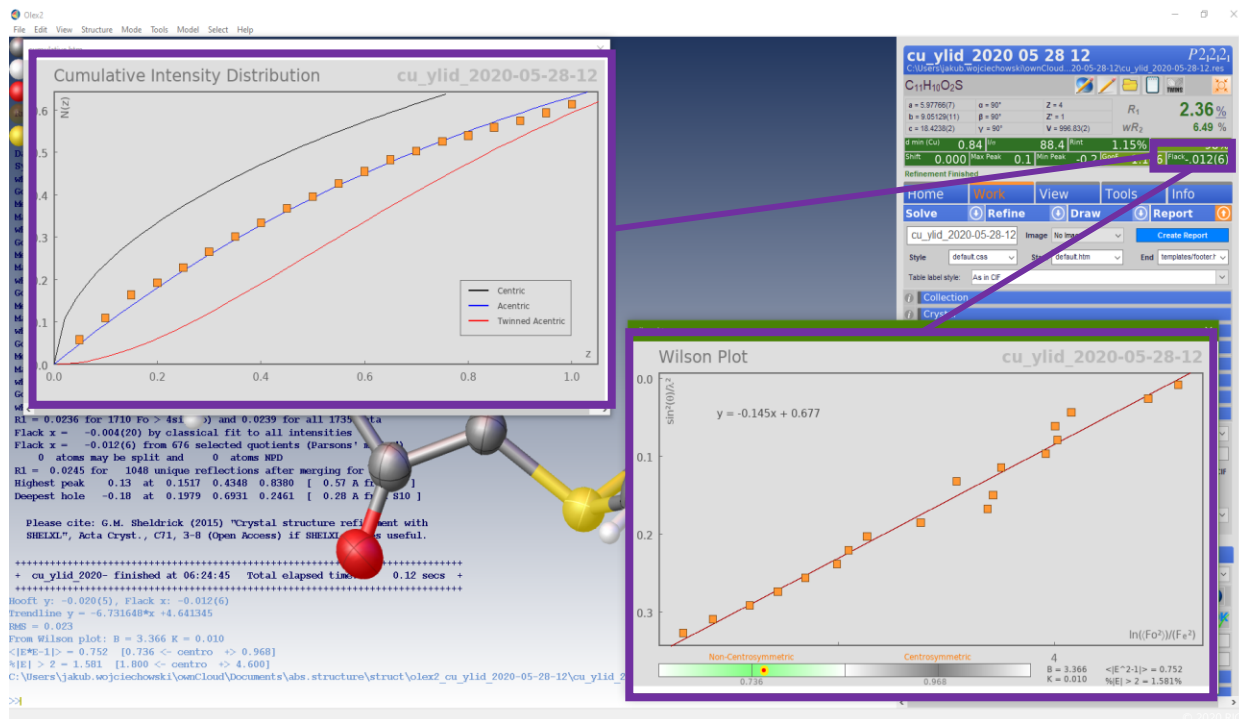
Useful CIF auditing options.

- Anomalous dispersion
- Reference molecule
- Reference molecule +AD
- Synthesis
- Unknown



USEFUL OLEX2 OPTIONS FOR ABSOLUTE STRUCTURE REFINEMENT

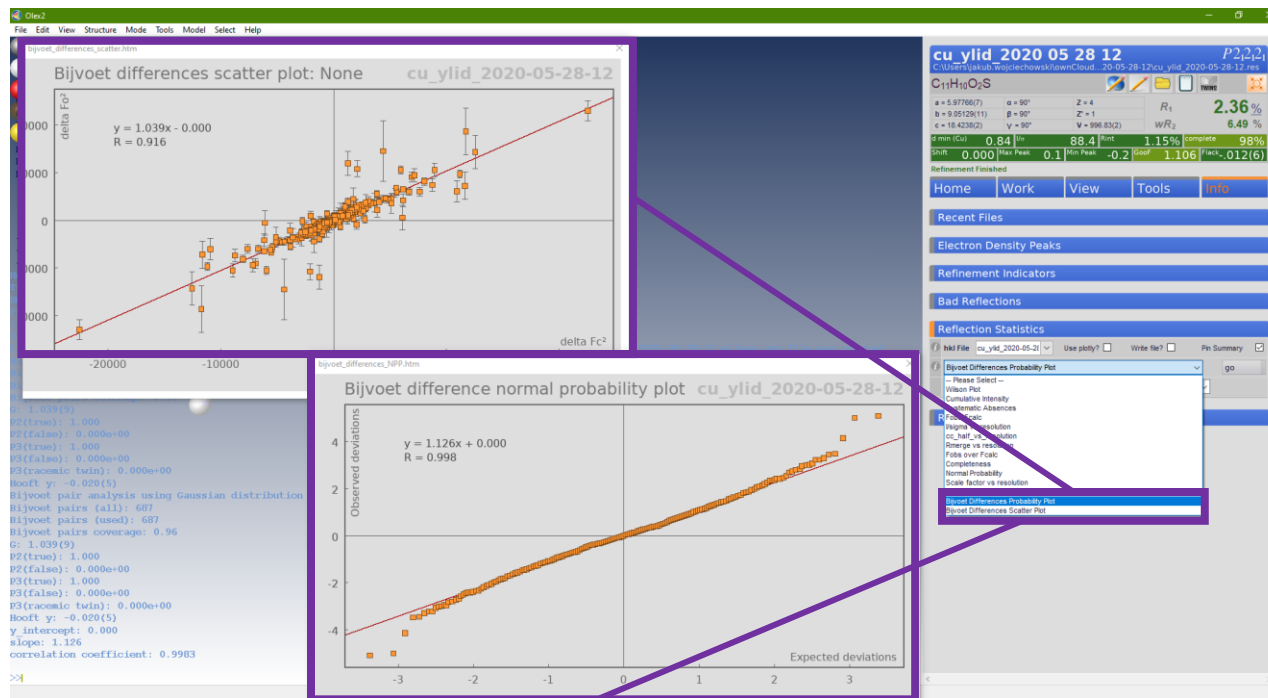
A statistical comparison of the observed intensity data with the theoretical distribution for a random atomic arrangement, since the atomic scattering decreases with increasing 2Θ . Establishes an overall displacement parameter for the structure, B and scale factor for the data, K.



TIP: Right click on Flack parameter box will open useful plots (CID and Wilson).

USEFUL OLEX2 OPTIONS FOR ABSOLUTE STRUCTURE REFINEMENT

Bijvoet Differences Scatter Plot: This plots the calculated Bijvoet differences, $F^2_{\text{calc}}(+)-F^2_{\text{calc}}(-)$, against the observed Bijvoet differences, $F^2_{\text{obs}}(+)-F^2_{\text{obs}}(-)$, along with error bars indicating the uncertainty in the measurement of the Bijvoet differences. For a correct, strongly determined absolute structure, this plot should form a positive slope, with gradient close to 1.



Bijvoet Differences Probability Plot: Similarly to the 'Normal probability' plot above, this plots the ordered deviations between the observed and calculated Bijvoet differences. Frequently it is observed that this plot can deviate from linearity, suggesting that the errors are not normally distributed.

TIP: Bijvoet scatter plot and normal probability plot are accessible from Info-Reflection statistics

Determination of the Absolute Structure

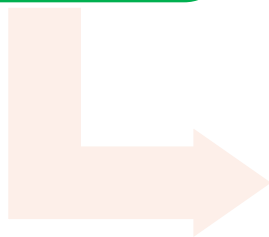
Determine the best possible crystal structure

- Screen your sample extensively.
- Mount and center properly.
- Careful with absorption correction.



Take care for Bijvoet Differences

- Collect high redundant data.
- Check the observability.
- Check amount of Friedel pairs and anomalous signal.
- Select proper X-ray source.



Assign proper absolute Configuration

- Make sure that **descriptor** values and its standard deviation make sense.



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