Absolute structure and molecular chirality
(From anomalous scattering to absolute structure)

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(with a great help from H. Flack & P. García)

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Outline

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2.- Absolute structure and absolute configuration
3.- Some useful terms
4.- On the origin …
5.- Anomalous scattering
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   Flack parameter
7.- Determination of the abs. structure → abs. configuration
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1.- Definition of chirality

The IUPAC defines the term **chirality** as the geometric property of a rigid object (or spatial arrangement of points or atoms) of being non-superposable on its mirror image.

No symmetry elements of the second kind; a mirror plane ($\sigma=S_1$), centre of inversion ($i=S_2$), rotation-reflection axis ($S_{2n}$).

If the object is superposable on its mirror image the object is described as being achiral.

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Are they chiral?
1.- Definition of chirality

IUPAC (Crystallographic): The geometric property of a rigid object (or spatial arrangement of points or atoms) of being non-superposable by pure rotation and translation on its image formed by inversion through a point.

The symmetry group of the object does not contain symmetry elements of the second kind: \( \bar{1}, m, 3, 4, 6 \)

When the object is superposable by pure rotation and translation on its inverted image, the object is described as being achiral.

Are these molecules chiral?
When a molecule is chiral?

We should search for chiral centers/atoms..., when a central atom is bounded to four different atoms (or atomic groups) with a tetrahedral geometry.

1 chiral center $\rightarrow$ chiral molecule

What happens with 2 (or even number) chiral centers?

Meso compounds:
Chiral center + symmetry plane = No quirality

2,3-dichloro-butane

When a molecule is chiral?

We should search for chiral centers/atoms..., when a central atom is bounded to four different atoms (or atomic groups) with a tetrahedral geometry.

1 chiral center $\rightarrow$ chiral molecule

What happens in molecules without chiral centers?

Molecule can be chiral

1,1’-bi-2-naphthol (BINOL)
Is the chirality an important molecular characteristic?

Definitely yes. Thalidomide

Thalidomide was found to act as an effective tranquiliser, painkiller and have an inhibition effect on morning sickness in pregnant women. It was sold from 1957 to 1961.

More than 10,000 children of 46 countries were born with deformities.

2.-Absolute structure and absolute configuration

Absolute structure:
The spatial arrangement of the atoms of a physically-identified non-centrosymmetric crystal and its description by way of unit cell, space group and coordinates of the atoms.

Crystallographer’s term
* Entity: Non-centrosymmetric crystal structure
* Symmetry restrictions:
  Non centrosymmetric

Absolute configuration:
The spatial arrangement of the atoms of a physically-identified chiral molecular entity and its stereochemical description (R or S, D or L, etc).

Chemist’s term
* Entity: Chiral molecules
* Symmetry restrictions:
  lack of mirror reflection

Complete specification of the spatial arrangement of atoms. In some cases, both require characterization by other physical measurements.

X-ray diffraction determines the absolute structure in the solid state (crystal).
3.- Some useful terms

**Enantiomorph**: Either of a pair of crystals, molecules or compounds that are mirror images of each other.

**Enantiomer**: one of a pair of molecular entities which are mirror images of each other and non-superposable (R vs S, D vs L).

*All the diastereoisomers are enantiomers??*

**Diastereoisomer**: each of the molecular entities that differ at one or more of the chiral centers.

\[
\begin{align*}
&\text{COOH} \\
&\text{(R)} \quad H \quad \overrightarrow{\text{C}} \quad \overrightarrow{\text{OH}} \\
&\text{COOH} \\
&\text{(S)} \quad H \quad \overrightarrow{\text{C}} \quad \overrightarrow{\text{OH}} \\
&\text{COOH} \\
&\text{COOH} \\
&\text{HO} \quad \overrightarrow{\text{C}} \quad \overrightarrow{\text{H}} \\
&\text{(S)} \quad H \quad \overrightarrow{\text{C}} \quad \overrightarrow{\text{OH}} \\
&\text{COOH}
\end{align*}
\]
3.- Some useful terms

- **Enantiomorph**: Either of a pair of crystals, molecules or compounds that are mirror images of each other.

- **Enantiomer**: one of a pair of molecular entities which are mirror images of each other and non-superposable (R vs S, D vs L).

- **Diastereoisomer**: each of the molecular entities that differ at one or more of the chiral centers.

- **Racemate**: Chemical compound that contains equal quantities of a pair of enantiomers.

Basic terminology of Stereochemistry: IUPAC recommendations: http://www.chem.qmul.ac.uk/iupac/stereo/

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**Naming conventions**

- By configuration: **R** and **S**. This notation labels each chiral center according to a system by which its substituents are each assigned a priority.

- By configuration: **D** and **L** denote chirality as defined by Emil Fischer, i.e., in reference to D- and L- glucose.

- By optical activity. (+) and (–), denote the direction (dextro- and levo-, respectively) in which polarized light is rotated by the compound. Historically it is the oldest convention used to identified the optically active stereoisomers.

The optical activity is the ability of a chiral molecule to rotate the plane of polarized light.
4.- On the origin ...

Tartaric acid (2,3-dihydroxybutanedioic acid):

2 asymmetric carbon atoms

L-tartaric acid
(2R,3R)-(+)-tartaric acid

D-tartaric acid
(2S,3S)-(-)-tartaric acid

mesotartaric acid
(2R,3S)-tartaric acid

Another form:
50/50 L- and D

'Racemic acid'

‘Natural’
‘Enantiomer’
‘Optically inactive’

Optical activity of tartaric acid: Biott (1774-1862)

4.- On the origin ...

“... La dissolution laisse déposer, après quelques jours, des cristaux qui ont tous exactement les mêmes angles, le même aspect; et pourtant, a coup sûr, l’arrangement moléculaire dans les uns et les autres est tout à fait différent’

(Pasteur, 1848)

In 1930, Coster, Knol and Prins demonstrate the possibility to use anomalous scattering (in a X-ray diffraction experiment) to distinguish a crystal structure from its inverted image, using zinc sulphide and gold L_\text{\alpha}2 radiation ...

‘111 and $\bar{1}$1$\bar{1}$ reflections are not equivalent!’

Bijvoet: X-ray diffraction determines the absolute structure in the solid state (crystal).

Point classes vs Laue group
From absolute structure to absolute configuration …

Absolute structure leads to absolute configuration.

There are 3 types of space groups:

**Type 1:** centrosymmetric, and then achiral. CA
    every motif + its inverse

**Type 2:** non-centrosymmetric and achiral. NA.
    crystal has a ‘sense’, but the motif and its inverse are present in the unit cell

**Type 3:** non-centrosymmetric and chiral. NC.

32 geometric crystal classes …

<table>
<thead>
<tr>
<th>Centro</th>
<th>Non-centro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Achiral</td>
<td>1.- CA</td>
</tr>
<tr>
<td></td>
<td>2.- NA</td>
</tr>
<tr>
<td>Chiral</td>
<td>3.- NC</td>
</tr>
</tbody>
</table>

222 422 32 622 23 432 1 2 4 3 6
5.- Anomalous scattering (AS) . The concept

Atomic contribution to the structure factor (atomic scattering factor) is considered in first approximation to be a real number. (Its value depends on resolution, but it is always in phase with incident beam).

The ‘anomalous’ dispersion corrections take into account the effect of absorption in scattering of phonons by electrons. If the frequency of the incident beam is close to a natural frequency of some atom type an anomalous phase change occurs on scattering by atoms of that type. The scattering factor of the electron is then complex and the atomic scattering factor is given by:

\[
f = f_0 + \Delta f' + i \Delta f''
\]

Real Imaginary

Anomalous dispersion correction

Resonance scattering of radiation

5.- Vector representation of anomalous scattering …

\[
f = f_0 + \Delta f' + i \Delta f''
\]

\(f_0\) and \(f'\) are collinear (\(f'\) is usually negative)
and \(f''\) is the imaginary component

Anomalous component depends on:

<table>
<thead>
<tr>
<th></th>
<th>Cr Kα</th>
<th>CuKα</th>
<th>Mo Kα</th>
</tr>
</thead>
<tbody>
<tr>
<td>(f')</td>
<td>(f'')</td>
<td>(f')</td>
<td>(f'')</td>
</tr>
<tr>
<td>K</td>
<td>0.1</td>
<td>2.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Br</td>
<td>-0.2</td>
<td>2.6</td>
<td>-0.8</td>
</tr>
<tr>
<td>Ag</td>
<td>-0.9</td>
<td>8.2</td>
<td>-0.1</td>
</tr>
<tr>
<td>T</td>
<td>-5.9</td>
<td>12.8</td>
<td>-0.6</td>
</tr>
<tr>
<td>Hg</td>
<td>-4.8</td>
<td>14.1</td>
<td>-5.0</td>
</tr>
</tbody>
</table>

Whereas \(f_0\) decreases drastically with the scattering angle, anomalous component of the atomic scattering factor is independent of this angle. This occurs because the effect involves mainly the inner electron of the atom. Therefore for a given atom, the effects of dispersion are relatively greater at high resolution.
5.- Effect of AS: the centrosymmetric case

The effects of dispersion on structure factor can be illustrated by Argand diagrams.

1st case: centrosymmetric structure with a number of atoms without anomalous dispersion and 2 atoms which exhibit anomalous scattering: $\Delta f'$ and $\Delta f''$.

\[
\begin{align*}
F_w & : \text{Resultant of the scattering from the atoms without dispersion} \\
F_{hkl} & = F_{\pi\pi} \\
|F_{hkl}| = |F_{\pi\pi}| & \iff \text{Friedel’s law holds.} \\
\alpha_{hkl} = \alpha_{\pi\pi} &
\end{align*}
\]

Phases are equal, but \textbf{neither 0 nor } \pi, so the structure factors are complex. The contribution of the anomalous scattering atoms should be taken into account in $F_c$.

5.- Conclusion I: centrosymmetric case

Friedel’s law always hold in centrosymmetric structures.

Talking about absolute structure or absolute configuration has no sense.
5. - Effect of AS: the noncentrosymmetric case

2nd case: non centrosymmetric structure with a number of atoms without anomalous dispersion ($F_w$) and 1 atom which exhibit anomalous scattering: $\Delta f'$ and $\Delta f''$.

$$|F_{hkl}| \neq |F^{-}_{hkl}|$$

Differences in intensity and phase: Friedel’s law does not hold

$$\alpha_{hkl} \neq -\alpha^{-}_{hkl}$$

5. - Conclusion II: Non-centrosymmetric case

Friedel’s law is not fulfilled in non centrosymmetric structures if the contribution of the anomalous scattering is observable.

This effect allows to determine the absolute structure (of the crystal) and therefore the absolute configuration (of the chiral molecules).
5.- Anomalous scattering depends on…

\[ f = f_0 + \Delta f' + i \Delta f'' \]

Anomalous component depends on:

1.- The atom type

2.- The incident radiation

<table>
<thead>
<tr>
<th></th>
<th>Cr Kα</th>
<th>Ca Kα</th>
<th>Mo Kα</th>
</tr>
</thead>
<tbody>
<tr>
<td>f'</td>
<td>0.1</td>
<td>2.1</td>
<td>0.4</td>
</tr>
<tr>
<td>f''</td>
<td>0.4</td>
<td>1.1</td>
<td>0.2</td>
</tr>
<tr>
<td>f'''</td>
<td>0.2</td>
<td>0.3</td>
<td></td>
</tr>
</tbody>
</table>

In the past, visualization of the anomalous scattering effects required the presence of very heavy atoms. Nowadays, modern detectors allow to accurately measure smaller intensity differences. Moreover, synchrotron radiation combines tuneable \( \lambda \) and bigger intensity.

6.- Enhancing inversion-distinguish power

What conditions control the inversion-distinguish power in an X-ray diffraction experiment?

Simple model: Contribution to the structure factor \( \tilde{F}(\tilde{h}) \) of:

1.- Non dispersive light atoms: \( \tilde{F}_{i}(\tilde{h}) = L[ \cos(\phi_l) + i \sin(\phi_l) ] \)

2.- Dispersive heavy atoms: \( \tilde{F}_{ii}(\tilde{h}) = (H + iH'')[L\cos(\phi_{hi}) + i\sin(\phi_{hi})] \)

The structure factor: \( \tilde{F}(\tilde{h}) = \tilde{F}_{i}(\tilde{h}) + \tilde{F}_{ii}(\tilde{h}) \)

Difference in intensity between Friedel opposites:

\[
\Delta(\tilde{h}) = |\tilde{F}(\tilde{h})| - |\tilde{F}(\tilde{-h})| = 4LH'' \sin(\phi_l - \phi_{hi})
\]
6.- Enhancing inversion-distinguish power

\[ \Delta(\tilde{h}) = |\tilde{F}(\tilde{h})| - |\tilde{F}(-\tilde{h})| = 4LH'' \sin(\phi_L - \phi_H) \]

The power to distinguish between a model and its inverted depends on \( \Delta \) of all reflection pairs. A rough approximation:

\[ \langle |\Delta| \rangle \approx 4 \langle L \rangle \langle H'' \rangle \langle \sin(\phi_L - \phi_H) \rangle \]

\[ \langle |\Delta| \rangle \to 0 \]

- a) \(<L> \) increases. Important contribution of non-dispersive atoms
- b) \(<H'> \) increases. Important contribution of the dispersive atoms.
- c) In general the sinusoidal term is not zero (except for strong correlations, or centrosymmetric or pseudo-centrosymmetric contributions of \( L \) and \( H \) substructures)

Inclusion of dispersive scatterers, with few restrictions on their positions.

Simple way: cocristallize the molecule with CCl\(_4\) (achiral dispersive).
6.- In practice … how can we determine absolute structure / configuration?

**Bijvoet** was the first who determine the absolute configuration by measuring anomalous dispersion in 1951.

Bijvoet determined the absolute configuration of the sodium rubidium salt of tartaric acid, using Zr radiation.

Rb absorption edge: 0.8157 Å  
Zr Kα radiation: 0.7859 Å

6.- In practice … Bijvoet method

**Bijvoet** method compares differences of intensity between Friedel pairs structure factors.

\[
\Delta F_c = \sum \left| F_i(hkl) - F_i(\bar{h}\bar{k}\bar{l}) \right|
\]

\[
\Delta F_o = \sum \left| F_o(hkl) - F_o(\bar{h}\bar{k}\bar{l}) \right|
\]

Bijvoet parameter:

\[ B = \frac{\Delta F_c}{\Delta F_o} \]

\[ \begin{cases} >0: \text{ Correct configuration} \\ <0: \text{ Inverse configuration} \end{cases} \]

We have to inverse the configuration of our model (crystal structure), i.e., change our model by its mirror image.

\[ x_i \rightarrow -x_i \]

\[ y_i \rightarrow -y_i \]

\[ z_i \rightarrow -z_i \]
6.- In practice … other methods: Flack parameter.

Physical model: the macroscopic crystal (C) is twinned, formed by two domain types, related by inversion.

Oriented crystal, with domains:
- $X$: model crystal structure (cell dimensions, space group, atomic coordinates).
- $\overline{X}$: its mirror image model (inverse atomic coordinates; $-x, -y, -z$)

Macroscopic crystal: $C = (1-x).X + x.\overline{X}$

Flack parameter measures the mole fraction of the domains.

In the practice, SHELX (and another programs) estimate the Flack parameter and its eds. Please note that this parameter should be calculate with the other parameters (positions…) to reduce the effect of correlation.

These effects tend to be greater for example for polar groups and for poor data to parameter ratio (less that 8:1). $x$ can be refined as all the other parameters using the TWIN instruction with the default matrix $R$ and BASF instruction.

TWIN -1 0 0 0 -1 0 0 0 -1
BASF 0.50 Racemic twin
6.- Interpretation of Flack parameter.

Macroscopic crystal: \[ C = (1 - x) \cdot X + x \cdot \overline{X} \rightarrow x \text{(esd)} \]

Physically meaningful: \(0 < x < 1\)

- \(x \approx 0\) Only one domain in the crystal. Model \(X\) → correct absolute structure
- \(x = 1\) Only one domain in the crystal. Model \(\overline{X}\) → inverted absolute structure
- \(x \approx 0.5\) Racemic twin.
- \(x \approx 0.3\) Two domains in the crystal in 30% - 70% proportion.

For a statistically valid determination of the absolute structure:
- \(\text{esd} < 0.04\) and \(x < 2 \cdot \text{esd}\)

For a compound with known enantiopurity: \(\text{esd} < 0.1\) and \(x < 2 \cdot \text{esd}\)

7.- Determination of the absolute structure.

Macroscopic crystal: \[ C = (1 - x) \cdot X + x \cdot \overline{X} \rightarrow x \text{(esd)} \]

**Absolute structure:**
The spatial arrangement of the atoms of a physically-identified **non-centrosymmetric crystal** and its description by way of unit cell, space group and coordinates of the atoms.

**Absolute configuration:**
The spatial arrangement of the atoms of a physically-identified **chiral molecular entity** and its stereochemical description (R or S, D or L, etc).

With the contribution of the anomalous scattering, the absolute structure can be determined, and if the **molecular motif is chiral** the absolute configuration can be established.
7.- Determination of the absolute configuration.

Macroscopic crystal: \[ C = (1 - x)X + x\overline{X} \]

With the contribution of the anomalous scattering, the absolute structure can be determined, and if the molecular motif is chiral the absolute configuration can be established.

Don’t forget we need the \((h \, k \, l)\) and \((\overline{h} \, \overline{k} \, \overline{l})\) reflections (or symmetry equivalent ones)

Data completeness !!!

One question:

*When the space group is non-centrosymmetric and the anomalous scattering effects are observable, can the absolute configuration be always established??*

No, not all valid determination of the absolute structure leads can necessary lead to the assignment of an absolute configuration.

---

7.- Determination of the absolute configuration.

**Restrictions to the absolute configuration from the absolute structure:**

1.- **Space group restrictions:**

1.1. Space group contains symmetry operations of the second kind (i.e. rotoinversions or rotoreflections, glide reflections).

These symmetry operations can operate:

Intramolecularly → molecules are forced to be achiral

Intermolecularly → forcing an arrangement of pairs of opposite enantiomers: racemate

1.2. Space group contains symmetry operations of the first kind (i.e. pure or proper rotations, screw rotations). → absolute configuration can be determinated.

Geometrical crystal classes:
1,2,22,4,42,23,32,6,622,23 and 432
7.- Determination of the absolute configuration.

Restrictions to the absolute configuration from the absolute structure:

2.- Chiral molecular entity restriction:

To determine the absolute configuration, one needs to identify a chiral molecular entity and its spatial arrangement (definition of absolute configuration).

If the candidate chiral molecule contains rotoinversion or rotoreflection operations (even if they are not imposed by the space-group crystal symmetry), the molecule is achiral.

For example: if the molecule is planar, it has mirror symmetry and it’s achiral, (even if the mirror plane is not part of the space-group symmetry).

7.- Determination of the absolute configuration.

Restrictions to the absolute configuration from the absolute structure:

3.- Solid-state enantiomeric purity restriction:

Are all the molecular entities in the crystal structure the same enantiomer? One should verify for an absolute structure assignment to be valid.

This is very important when the asymmetric unit contains more than one molecule!!
7.- Conclusions: Determination of the absolute configuration from the absolute structure

1.- Space group restrictions: without 2nd order symmetry operation

2.- Chiral molecular entity restriction: without molecular symmetry

3.- Solid-state enantiomeric purity restriction: one only enantiomer in the crystal

Third restriction is the strongest and it is sufficient in itself.

8.- Transferability of the results.

Is the determined crystal/molecule representative of the sample? :

Bulk samples which are a mixture of diastereoisomers (each of the molecular entities differs at one or more of the chiral centers; A and B) can lead to crystal containing …

… only one of the diastereoisomers (A or B): Pasteur’s case!!

… achiral centrosymmetric crystals (with the same number of A and B)

… a inversion twin (NC). Non-centrosymmetric, but with different zones with opposite orientation (A and B)
Once we have determined the structure

We should verify the next points:

✓ The X-ray data collection should have a good completeness. Within the scattering limit of the material, almost the 100% the Friedel pairs should have been measured.

✓ Crystallographic / Chemical model seems OK.

✓ The weight and unweight R factors are small

✓ The value of the Flack parameter is close to zero.

✓ The standard uncertainty of Flack parameter is adequately small.

✓ The checkcif/PLATON do not generate any serious alert.

Summary

The term chirality reflects the lack of symmetry through inversion.

The absolute structure is the spatial order of atoms in a non-centrosymmetric crystal. We describe it by the unit cell, the space group and the atomic coordinates.

We can determine the absolute structure in a non-centrosymmetric crystal, using the differences between Friedel opposites reflections (caused by the anomalous scattering). In the practice we should judge our results with the Flack parameter.

From the absolute structure, we can establish the absolute configuration when the space group has no operation of second order, the molecule is chiral, and we have only 1 enantiomer in the crystal.

The absolute configuration is the spatial order of atoms in a chiral molecule. We describe it by its stereochemical description (R or S; D or L ...).

Even if we have establish the absolute configuration, we should ask ourselves about the transferability of our results.
How would you like to live in Looking-glass House?
I wonder if they’d give you milk there?
Perhaps looking-glass milk isn’t good to drink ....

http://www.youtube.com/watch?v=KkYevBx99mY

Some references

**Question 1:**

*Can the absolute configuration of an organic compound (C, H, N and O) be obtained with molybdenum radiation?*

Yes, in these cases......

- With an internal reference
- Co-crystallized with an enantiomeric pure compound
- Co-crystallized with a solvent with heavy atoms

**Question 2:**

*Someone asks the diffraction service for a data collection up to a resolution of $2\theta=60^\circ$, as he wants to determine the absolute configuration...*

The possibility of distinguish a structure of its inverted image does not depend on the data collection resolution.
Absolute structure and molecular chirality

...any question ...any curiosity ...
... any doubt!

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