Chemical Crystallography and Structural Chemistry

VO 270287

Lecture № 2 — 11th March 2021

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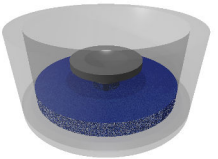
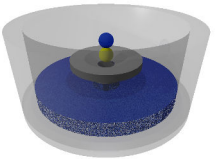
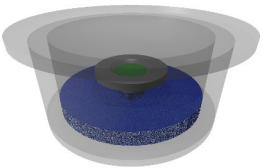
ad Crystallisation with vapour diffusion



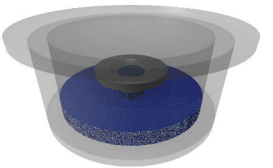
Well with mother liquor (ML) with precipitant



Mix ML with compound solution (V_{drop}=1 µl+1 µl)



Seal well (V_{drop}=2 µl)



After equilibration: V_{drop}=1 µl

ad Crystallisation with vapour diffusion

1. Compound dissolved in 100 % isopropanol, concentration c
2. Mother liquor: 90 % isopropanol, 10 % water

Setup procedure

	Volume	H_2O	i-PrOH	conc^n	soluble?
before	1 µl	0 %	100 %	c	yes
mixing	2 µl	5 %	95 %	$c/2$	yes
equilibration	1 µl	10 %	90 %	c	no

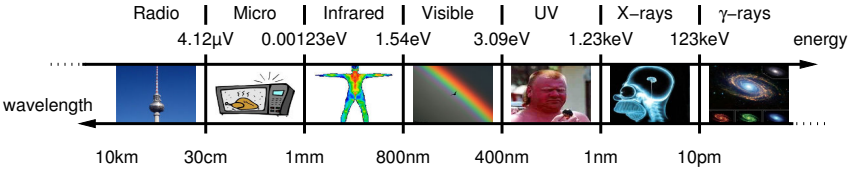
Remarks

- composition of mother liquor fine tunes end state
- insoluble compound precipitates
- slow diffusion enhances crystallization

1 X-rays and Diffraction

X-rays as electromagnetic radiation

X-rays are one type electromagnetic radiation — like visible light, UV-radiation, or radio waves



- Energy E and wavelength λ are equivalent ($E = h\frac{c}{\lambda} = 12.4keV\text{Å}/\lambda$):
- Long wavelength $\lambda \leftrightarrow$ low energy E.
- Short wavelength $\lambda \leftrightarrow$ high Energie E.

X-rays as electromagnetic radiation

- Typical wavelength range for structure determination: 0.5-2 Å (24.8 - 6.2 keV).
- Inhouse X-ray instruments:

  CuK_α: 1.54 Å $\leftrightarrow$ 8.0 keV
  MoK_α: 0.71 Å $\leftrightarrow$ 17.3 keV
  AgK_α: 0.56 Å $\leftrightarrow$ 22.1 keV
  WK_α: 0.21 Å $\leftrightarrow$ 59.3 keV (medical applications)

- 1 Å $= 10^{-10}$ m $=$ 100 pm

Generation of X-rays

1. **X-ray fluorescence** (mainly laboratory sources) electron beam at specific energy hits metal surface (Cu or Mo). This creates
   a) Bremsstrahlung (background)
   b) X-ray fluorescence

   Examples: rotating anode, liquid jet anode
2. **Synchrotron radiation** Acceleration of electron bunches in a magnetic field

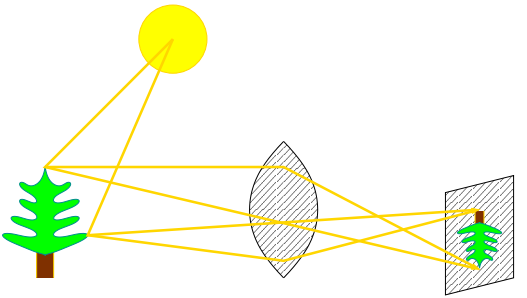
Why X-rays?

Atom distances of molecules about 1–2 Å: required resolution d

Optical instruments are limited in resolution to $d > \lambda/2$ (later: derivation *via* Bragg's law)

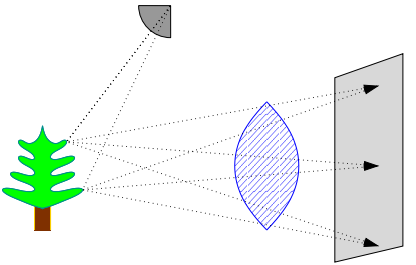
2 Why Crystals?

Optics and Imaging (Microscope / Telescope)



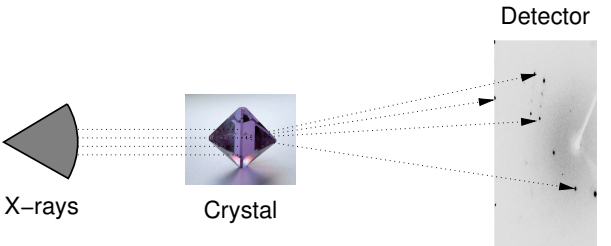
Objects scatter light. In order to see the object, the scattered light must be focused by **at least** one lens

X-ray Scattering



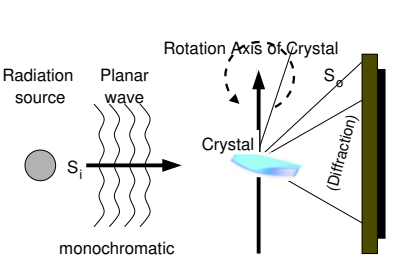
X-ray lenses do not exist: It is not possible to create a direct image of an object with X-rays.

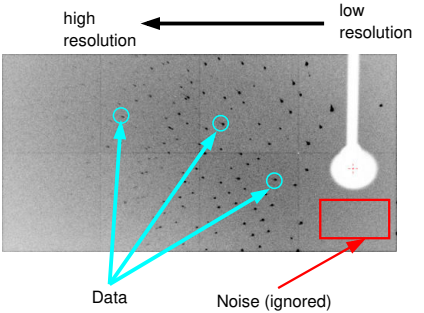
X-ray Scattering by Crystals: Diffraction



The periodicity of the crystal results in a focussing of the scattered X-rays into **discrete spots**. The spots (reflections) can be measured without lenses. The crystal acts like a signal amplifier.

The X-ray Diffraction Experiment

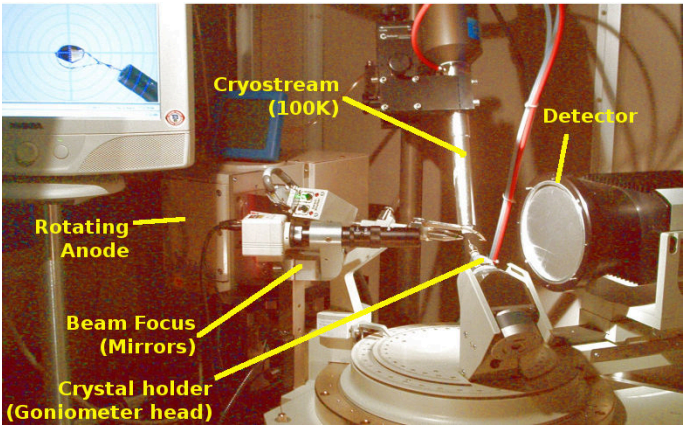




The crystal diffracts X-rays. This creates a **diffraction pattern** recorded by the detector.

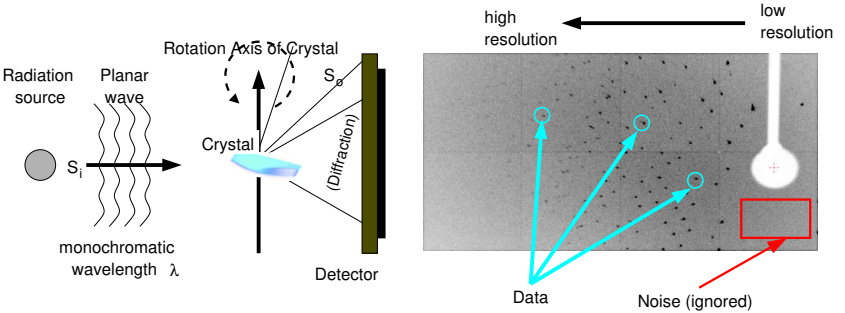
The reflection spots are not images of the atoms

Typical laboratory X-ray Diffractometer



3 Data Collection

Data collection experiment



Reflections are data point. Each one contains different information. In order to collect as many data points as possible:

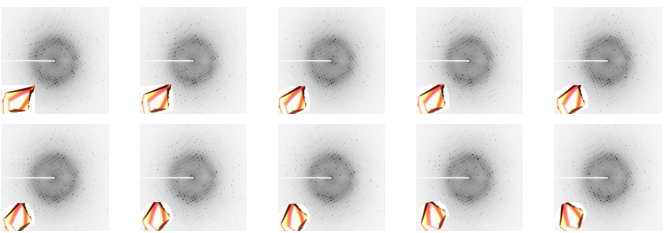
1. Rotation of the crystal (about one of three different axes, called ϕ–, ω–, and χ–circles)).
2. Rotation of the detector around the crystal, called 2θ–circle. This is parallel to the ω–circle).

The Data Set

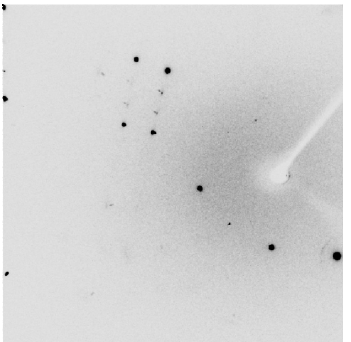
The reflections can be described as three dimensional **reciprocal lattice**. The two dimensional detector records an intersection of the three dimensional lattice.

The full experiment results in a **data set**.

One data set consists of several runs (1–20). One run is the rotation of the crystal about a single axis. Per run, 180–2,000 **frames** are recorded. One frame corresponds typically to 0.1° – 1° rotation of the crystal.

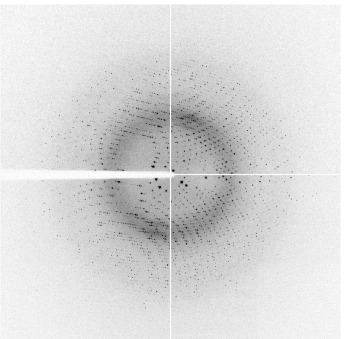


Examples of Data Frames



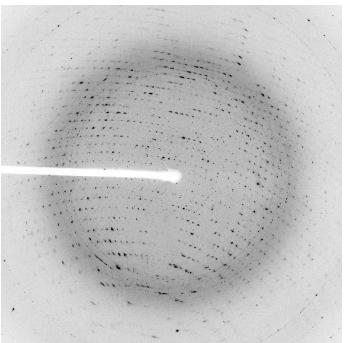
- Small molecule, unit cell dimensions: $a = 10.56$Å, $b = 11.64$Å, $c = 16.14$Å, $\alpha = \beta = \gamma = 90°$
- Small unit cell: $\Rightarrow$ few reflections
- Reflections beyond edge of detector: $\rightarrow 2\theta$ offset of detector necessary
- black reflections = data; grey regions: noise, neglectable

Examples of Data Frames



- Macromolecule. unit cell dimensions: $a = 92.6$Å, $b = 92.6$Å, $c = 128.9$Å, $\alpha = \beta = 90°, \gamma = 120°$
- Many more reflections
- Reflexes form patterns (lunes, "Kugeldreiecke")
- Intensity reduces towards edge of detector

Examples of Data Frames



- Macromolecule. unit cell dimensions: $a = 111.7$Å, $b = 80.5$Å, $c = 70.3$Å, $\alpha = \gamma = 90°, \beta = 94.2°$
- smeared reflexes
- ice rings (formed during measurement, or due to poor shock-freezing conditions)
- Closer look: small spots between "patterns": twinned crystal, not a single crystal.

4 Objectives of a Crystal Structure

Why Crystal Structure Determination?

The Structure provides atom coordinates: arrangement of elements in 3D space

Organic Chemistry:

- Success (or failure) of synthesis
- Determination of absolute structure
- Polymorphism: different crystal packing of the same compound

Inorganic Chemistry:

- Bonding geometry, coordination geometry (of metals ...)
- Polymorphism: different crystal packing of the same compound
- Space group determination: space group symmetry relates to electro-optical properties (thermal expansion, piezo-material, ...)

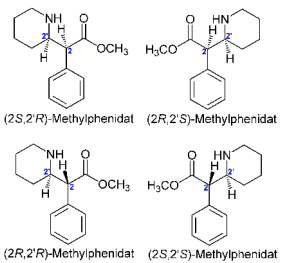
Comparison with other Structural Methods

NMR : chemical environment, sum formula. Not absolute structure

Rotational spectroscopy: (and gas phase electron diffraction): bond distances (**much more precise** than crystal structure)

Crystallography : Virtually no size limit (protein complexes $>$ 1.5 MDa; differentiation of element types

Examples: Absolute structure and degree of purity [1]

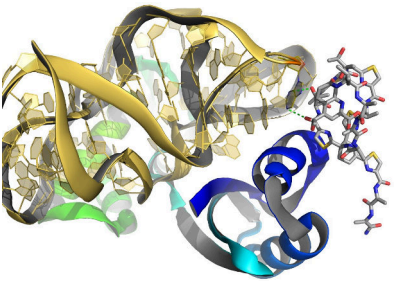


- Methylphenidate (*alias* Ritalin): medication to treat Attention Deficit Hyperactivity Disorder (ADHD).
- Two chiral centres, *four* stereoisomers
- Typical: only one stereoisomer with desired effect.
- Remaining stereoisomers: side effects

`http://de.wikipedia.org/wiki/Methylphenidat`

The crystal structure is the only method to determine the absolute structure and the degree of purity of mixtures.

Structure based Drug Development



The antibiotic *Thiostrepton* together with its target DNA. Dr. K. Pröpper.

Knowledge of structure of ligand and target:

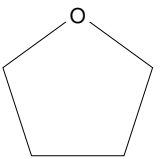
- Improvement of chemical interaction
- Improvement of shape / surface: Functionality and access to cell or nucleus.
- Uptake in body (cf. `http://de.wikipedia.org/wiki/Insulinpräparat`)

5 Crystal Diffraction: Why do crystals produce reflections?

Independent Atom Model (IAM)

Crystal structure determination is based on the **independent atom model** (IAM):

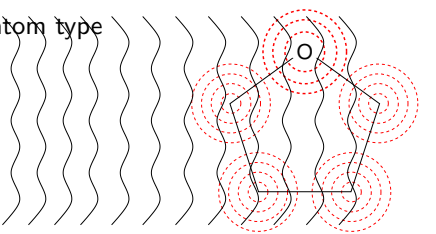
- the molecule consists of spherical atoms



Independent Atom Model (IAM)

Crystal structure determination is based on the **independent atom model** (IAM):

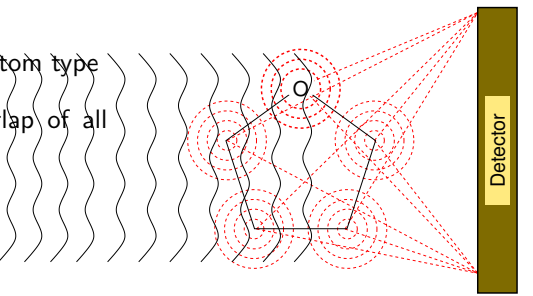
- the molecule consists of spherical atoms
- upon irradiation, each atom re-emits a small spherical wave independently from the others
- the strength depends on the atom type



Independent Atom Model (IAM)

Crystal structure determination is based on the **independent atom model** (IAM):

- the molecule consists of spherical atoms
- upon irradiation, each atom re-emits a small spherical wave independently from the others
- the strength depends on the atom type
- the detector records the overlap of all (tiny) waves

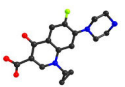


Independent Atom Model (IAM)

- every atom emits a tiny signal
- individual molecules are too weak to detect
- the crystal **amplifies** the signal

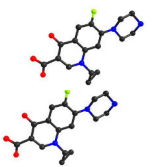
To understand, we introduce the **unit cell** and the **crystal lattice**.

The Unit Cell



"Periodicity of the unit cell"?

The Unit Cell



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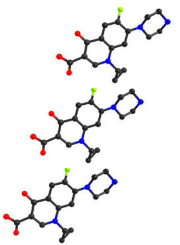

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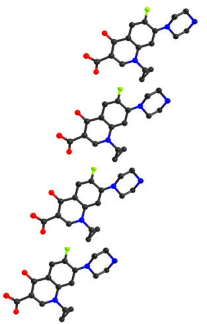

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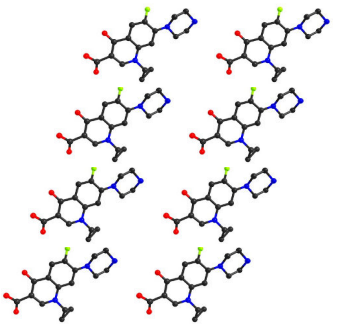

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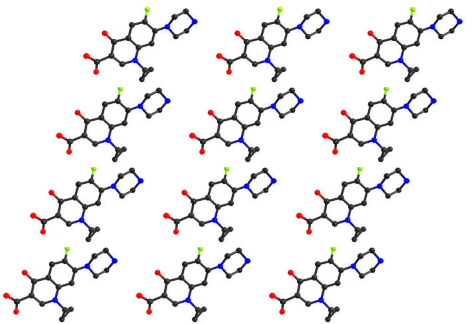

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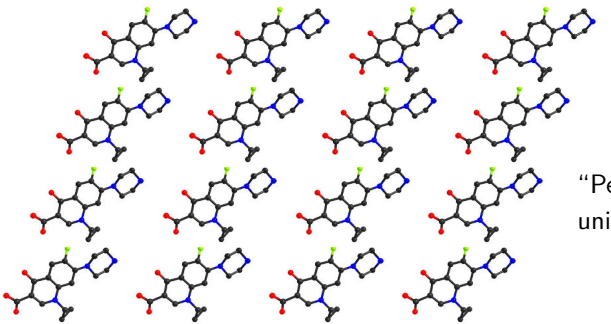
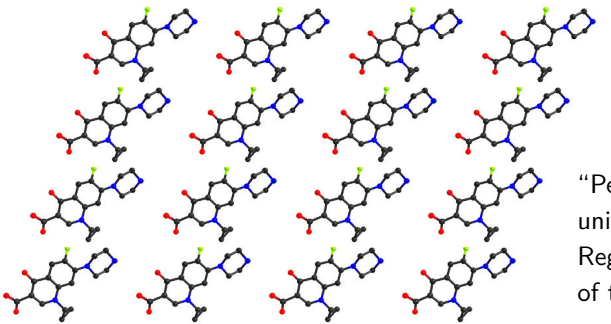


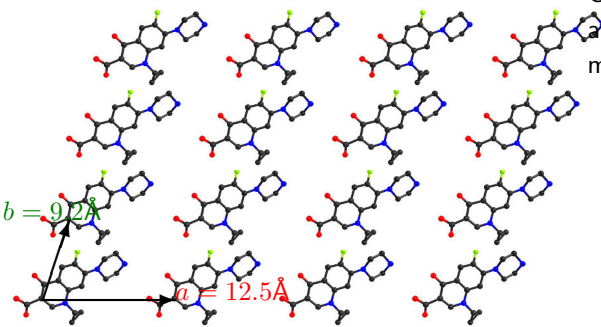
"Periodicity of the unit cell"?

The Unit Cell



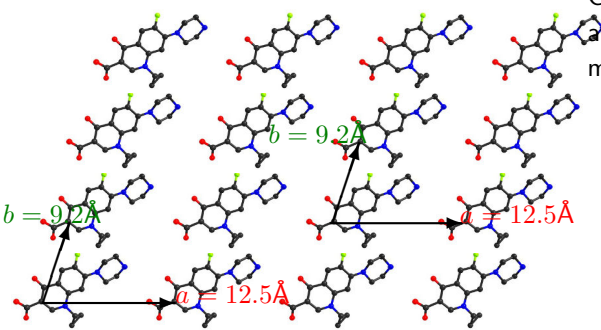
"Periodicity of the unit cell":
Regular repetition of the molecule

The Unit Cell



Periodicity of the unit cell:
Connect two equivalent atoms in two equivalent molecules

The Unit Cell

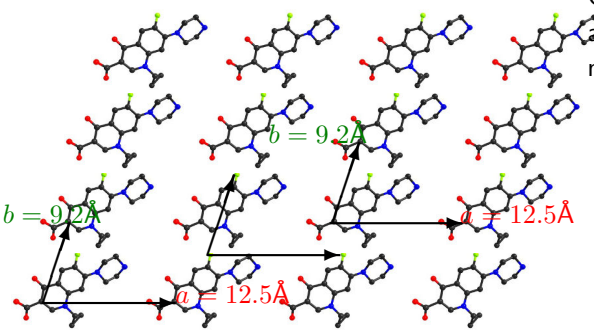


Periodicity of the unit cell:

Connect two equivalent atoms in two equivalent molecules

- connection can be shifted throughout the crystal

The Unit Cell

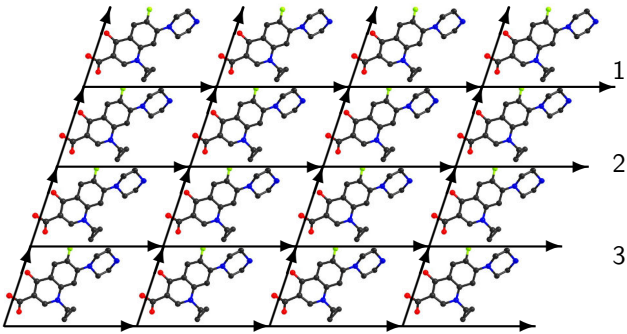


Periodicity of the unit cell:

Connect two equivalent atoms in two equivalent molecules

- connection can be shifted throughout the crystal
- can be any atom

The Unit Cell



1. select arbitrary reference point
2. connect to next equivalent point
3. in all three directions

This results in the **crystal lattice**. The smallest parallelepiped (smallest box) forms the **unit cell** of the crystal.

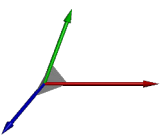
The Unit Cell

A three-dimensional box requires six parameters:

- unit cell constants a, b, c (edge lengths)
- angles between the edges

$$\alpha = \angle(b, c) \qquad \beta = \angle(c, a) \qquad \gamma = \angle(a, b)$$

- constants and angles are independent from the orientation of the crystal
- when written as vectors $\vec{a}$, $\vec{b}$, $\vec{c}$, they also describe the orientation of the crystal with respect to the instrument.



The convention a: red, b: green, c: blue comes from computer graphics, where colours are described as *rgb*.

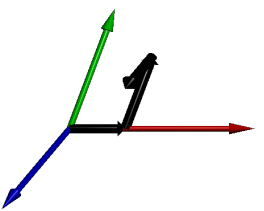
Fractional Coordinates

Atom coordinates are often described with *fractional coordinates*.
Every position in the crystal has unique coordinates (x, y, z)

$$x * \vec{a} + y * \vec{b} + z * \vec{c}$$

(x, y, z) are called the **fractional coordinates** of this position.
For any position *inside* the unit cell:

$$0 \leq x, y, z \leq 1.$$



The position (0.3, 0.6, 0.5).

Fractional Coordinates

Atom coordinates are often described with *fractional coordinates*.
Every position in the crystal has unique coordinates (x, y, z)

$$x * \vec{a} + y * \vec{b} + z * \vec{c}$$

(x, y, z) are called the **fractional coordinates** of this position.
For any position *inside* the unit cell:

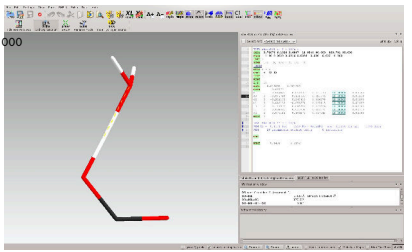
$$0 \leq x, y, z \leq 1.$$

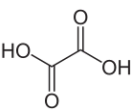
Fraction coordinates facilitate the use of symmetry operators. They are normally used in crystallographic computing.

- SHELXL ins-files always use fractional coordinates.
- Macromolecular PDB-files use orthogonal coordinates.

Example: ins-file for Oxalic Acid

```
TITL Oxalic Acid in P 1 21/n 1
CELL   0.71073 6.1026 3.4867  11.9540 90.000  105.791 90.000
ZERR    4.00 0.0020 0.0016 0.0036  0.000  0.027  0.000
LATT 1
SYMM  1/2 - X, 1/2 + Y, 1/2 - Z
SFAC C H O
UNIT  4  12 12
LIST 6
RIGU
L.S.  10
WGHT  0.0180  1.3244
FVAR   0.09892
C1   1 -0.045033  0.058931  0.051985  11.00000  0.00919
O3   3 -0.048452  0.131974  0.321439  11.00000  0.01180
O2   3 -0.221285  0.243842  0.036277  11.00000  0.01151
O1   3  0.085162 -0.055871  0.150165  11.00000  0.01216
H3   2 -0.142238 -0.045413  0.350385  11.00000  0.02677
H1   2  0.023619  0.022591  0.223012  11.00000  0.02363
H2   2  0.079486  0.197530  0.387391  11.00000  0.02464
HKLF  4
END
```



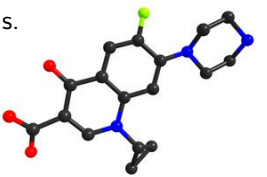


Summary: The Unit Cell

The crystal structure is described by

1. the unit cell parameters $a, b, c, \alpha, \beta, \gamma$
2. positions and element types of the atoms inside the unit cell

The whole crystal is the result of integer translations (= shifts without gaps or overlaps) of the unit cell in all three directions.



References

[1] E. J. Ariëns. 'Stereochemistry, a basis for sophisticated nonsense in pharmacokinetics and clinical pharmacology'. In: *Eur. J. Clin. Pharmacol.* 26 (1984), pp. 663–668.