

X-Ray Crystallography

“If a picture is worth a thousand words, then a macromolecular structure is priceless to a physical biochemist.” – van Holde

Topics:

1. Protein Data Bank (PDB)

Data mining and Protein Structure Analysis Tools

2. Image Formation

Resolution / Wavelength (Amplitude, Phase) / Light Microscopy / EM / X-ray / (NMR)

3. X-Ray Crystallography (after NMR)

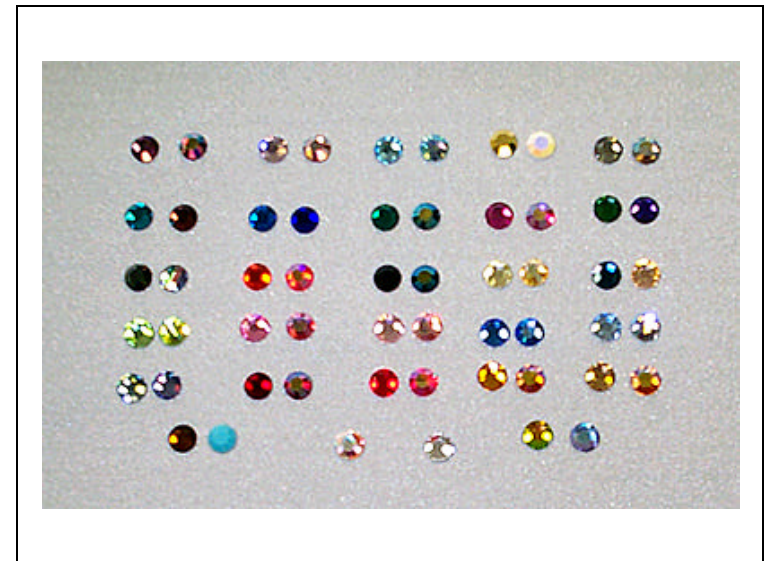
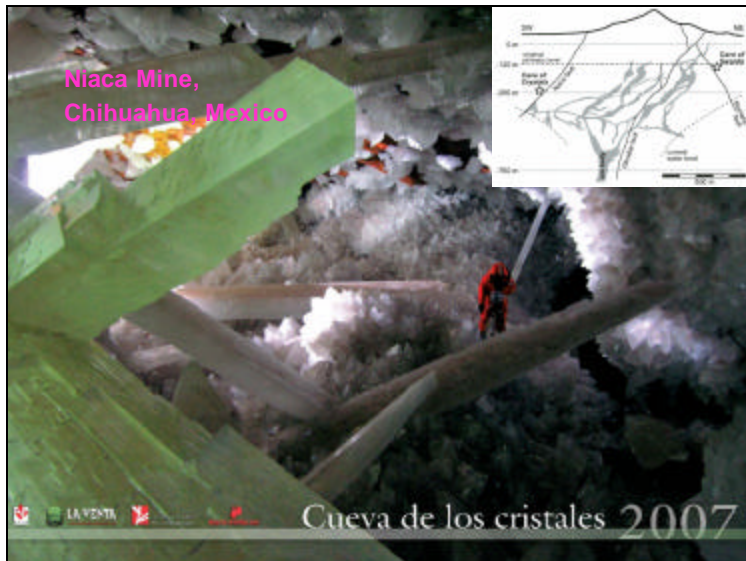



e) Data Collection – Methods / Detectors / Structure Factors

f) Structure Solution – Phase Problem: MIR / MR / MAD

h) Refinements and Models

i) Analysis and presentation of results





Glucose Isomerase Crystals

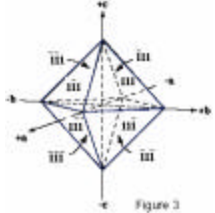
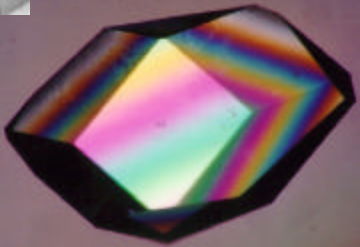
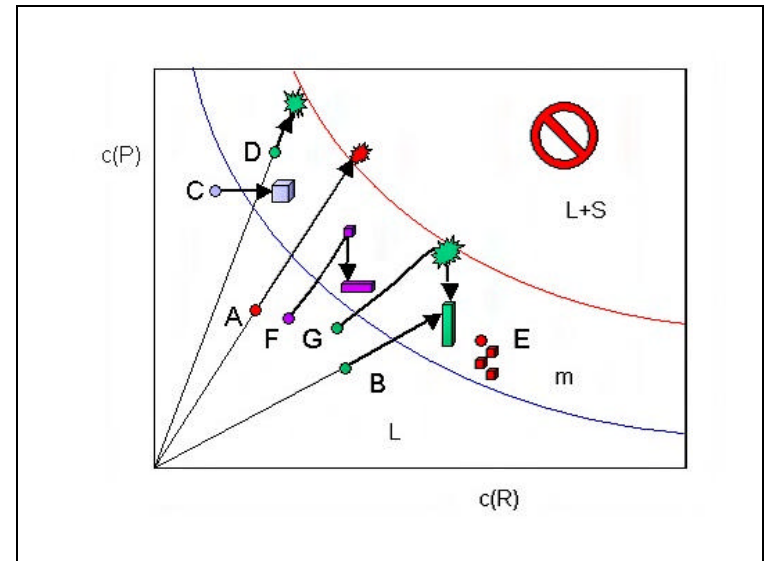


Figure 3



Lysozyme Crystals



Types of Crystals
 Welcome to the Home Page of Terese Bergfors
 "The Protein Crystallization Page" <http://xray.bmc.uu.se/~terese/>

Spheres: These often begin as spherulites (see picture below) or grow from a spherulite. They are extremely flat needles clustered around a single nucleation site. Once they appear "fuzzy" the whole sphere is like paper (see three-dimensional crystals growing in the adjacent nucleation site in the same drop).

Needles: Extremely thin needles growing from a single nucleation site. Since they nucleate on each length, I would not call that a "one needle" way longer. Watch the large 3-D crystal growing in the same drop.

Needles: Still too many but at least they are single needles. The nucleation site is too high which is why you have too many, and too thin. Try reducing the protein or precipitant concentrations on both. Another method is to put a layer of oil over the reservoir in the vapor drop setup. See Chapter, just adjacent box. See also the Tutorial 4 on seeding.

Plates: Two-dimensional. Plates are usually obtained as improvement over needles. Those are growing from a single nucleation site and overlapping each other, which is the best option. Optimize to grow them as regularly and thicker.

A three dimensional crystal: that sticks the diffusion before you get out the changeover.

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Tutorial 1. Appearances can be deceiving!

The last thing you should know is that the appearance (color, morphology, etc.) of your crystal is NOT what it is or what it should be. Beware! Looking crystals may not diffract, and really ugly crystals might diffract beautifully. The only definitive proof of a "GSGG" crystal is its diffraction pattern in the 2- θ scan. Therefore, do not be misled by the appearance of your crystals, except at such times as in the box.

Beauty is only skin deep.
 Don't be fooled by appearance. These crystals may look nice, but they don't diffract.

These are ugly. In fact they are so ugly you probably wouldn't even notice or avoid them, but they diffract! HA.

Most of the time, don't always go by the appearance of your crystals. It is the 2- θ scan diffraction pattern that counts.

With that said, you can now continue the tutorial on adapting your crystallization drop.

Variables that influence crystal growth

1. Nature of macromolecule – Purity and concentration of macromolecule
2. Nature and concentration of precipitant
3. pH / Temperature / Pressure
4. Level of reducing agent or oxidant
5. Substrates, coenzymes, and ligands / Metal ions
6. Preparation and storage of macromolecule / Proteolysis and fragmentation
7. Age of macromolecule / Degree of denaturation
8. Vibration and sound
9. Volume of crystallization sample
10. Seeding
11. Amorphous precipitate
12. Buffers
13. Cleanliness
14. Organism or species from which the macromolecule was isolated
15. Gravity, gradients and convection

Common Compounds used in Crystallization

Ammonium or sodium sulfate
 Sodium or ammonium citrate
 Sodium or ammonium acetate
 Magnesium sulfate
 Cetyltrimethyl ammonium salts
 Polyethylene glycol 400, 1000, 4000, 6000, 15,000 (now also 2,000, 8,000, etc.)

Methods for protein crystallization

Batch crystallization (simply dump reagents together)
 Liquid-liquid diffusion in a capillary tube
 Vapor diffusion-the most successful method (hanging drop, sitting drop), typically using a Limbro plate. Equilibration occurs between the liquid and vapor phase.
 Dialysis

Hampton Crystal Screen Solutions



Note :

A mini-screen can be set up from the most successful conditions. Those are indicated in the column labeled Miniconcen.

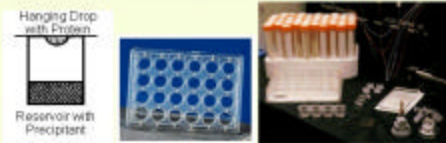
- [Special list of ingredients](#) commonly used in crystallization experiments
- [Recipe database](#)
- [CRYSTALOG](#), efficient random screen made for you here on the WEB.

| Tube # | SALT | BUFFER | Precipitant | Miniconcen | Tube # |
|--------|-------------------------|---------------------------|------------------------------------|--------------|--------|
| 1 | 0.02M Calcium Chloride | 0.1M Na Acetate pH4.0 | 20% w/v 2-methyl 2,4-pyridinediol | Y | 1 |
| 2 | None | None | 0.4M LiCl Na Tartrate dihydrate | | 2 |
| 3 | None | None | 0.4M Ammonium dihydrogen phosphate | | 3 |
| 4 | None | 0.1M Tris-HCl pH 8.5 | 0.2M Ammonium Sulfate | Y | 4 |
| 5 | 0.2M Tri-sodium citrate | 0.1M Na HEPES pH 7.5 | 30% w/v 2-methyl 2,4-pyridinediol | | 5 |
| 6 | 0.2M Magnesium chloride | 0.1M Tris-HCl pH 8.5 | 30% w/v PEG 4000 | | 6 |
| 7 | None | 0.1M Na Cacodylate pH 8.5 | 1.4M Sodium acetate trihydrate | | 7 |
| 8 | 0.2M Tri-sodium citrate | 0.1M Na Cacodylate pH 8.5 | 30% w/v 2-pyridinol | | 8 |
| 9 | 0.2M Ammonium acetate | 0.1M Na Citrate pH 5.8 | 30% w/v PEG 4000 | Y | 9 |
| 10 | 0.2M Ammonium acetate | 0.1M Na Acetate pH4.0 | 30% w/v PEG 4000 | Y | 10 |
| 11 | None | 0.1M Na Citrate pH 5.8 | 7.3M Ammonium dihydrogen phosphate | | 11 |
| 12 | 0.2M Magnesium chloride | 0.1M Na HEPES pH 7.5 | 30% w/v 2-pyridinol | | 12 |
| 13 | 0.2M Tri-sodium citrate | 0.1M Tris-HCl pH 8.5 | 30% w/v PEG 4000 | | 13 |
| 14 | 0.2M Calcium Chloride | 0.1M Na HEPES pH 7.5 | 20% w/v PEG 4000 | Y (weak) | 14 |
| 15 | 0.2M Ammonium acetate | 0.1M Na Cacodylate pH 8.5 | 30% w/v PEG 3000 | | 15 |
| 16 | None | 0.1M Na HEPES pH 7.5 | 1.5M Lithium sulfate monohydrate | Y | 16 |
| 17 | 0.2M Lithium sulfate | 0.1M Tris-HCl pH 8.5 | 30% w/v PEG 4000 | Y (2nd best) | 17 |

Hanging Drop Method - Crystal Screening

The Experimental Setup

In order to obtain a crystal, the protein molecules must assemble into a periodic lattice. One starts with a solution of the protein with a fairly high concentration (2-30 mg/ml) and adds reagents that reduce the solubility either by spontaneous precipitation. By slow further concentration, and under conditions suitable for the formation of a few nucleation sites, small crystals may start to grow. Often, very strong conditions have to be used to succeed. This is usually done by [batch screening](#), followed by a systematic optimization of conditions. [Crystalogs](#) should be a few trials of runs in each direction to be useful for the diffusion experiments.



Right: The hanging-drop technique. Center: 20 such hanging-drop experiments are set up in a Laskin plate. Right: A lot of different screening solutions, a set-up Laskin plate, slides, reservoir and a main batch plate loaded a glassware load.

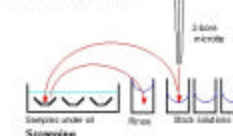
The most common setup to grow protein crystals is by the [hanging drop technique](#). A few microliters of protein solution are mixed with an almost equal amount of reservoir solution containing the precipitant. A drop of this mixture is put on a glass slide which covers the reservoir. As the protein/precipitant mixture in the drop is less concentrated than the reservoir solution (remember the size of the protein solution with the reservoir solution about 1:1), water evaporates from the drop into the reservoir. As a result the concentration of both protein and precipitant in the drop slowly increases, and crystals may form. There is a variety of other techniques available such as sitting drops, dialysis baskets, and gel and microbatch techniques. Various are useful for automatic [screening](#) and optimization of crystallization conditions. We have implemented a web computing service of [Brian Douglas's CRYSTALOG](#), an inherently efficient random screen for crystallization conditions that you can customize. The main information is the small sample size a [crystallization robot](#) can handle reproducibly, but it needs a new effort to set it up. Click here to learn more about the [IMPaX](#) robot.

Using Oryx 6 for Crystallization with Microbatch

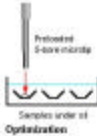
Microbatch operation is identical to [MFA X 1-5](#)

<http://www.douglas.co.uk/oryx.htm>

Very Fast: 240 wells/scan
 now (but 50 wells/scan for screening)



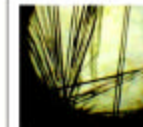
Uses little protein
 0.1 - 1 µg per well



Microbatch screening finds more leads
 50% YD in a given time

| | Automated MB | Manual YD |
|----------------------------------|--------------|-----------|
| Proteins screened | 8 | 5 |
| Screening solutions | 40 | 40 |
| Cost | 2 | 1 |
| Volume per well | 1 + 3 µl | 4 + 4 µl |
| Final protein conc. | 300 µM | 115 µM |
| Crystal size | 2 to 24 µm | |
| Crystallization conditions found | 40 | 40 |
| Unique conditions | 17 | 17 |

Large diffracting crystals



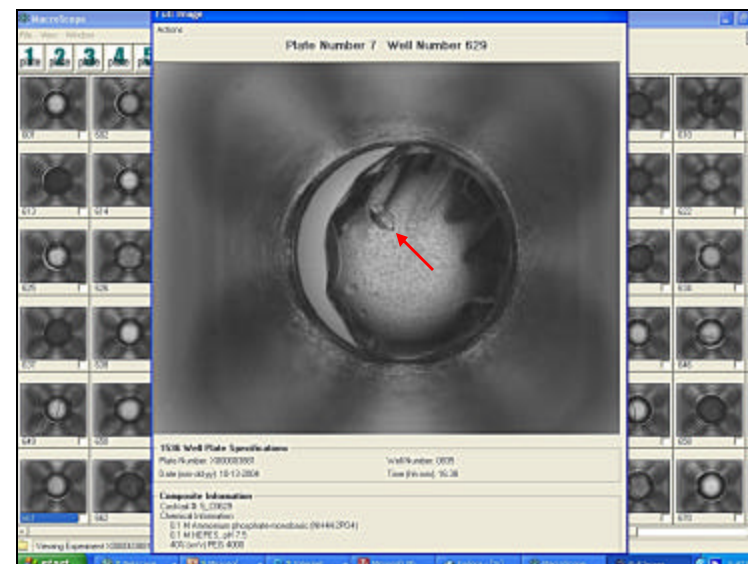
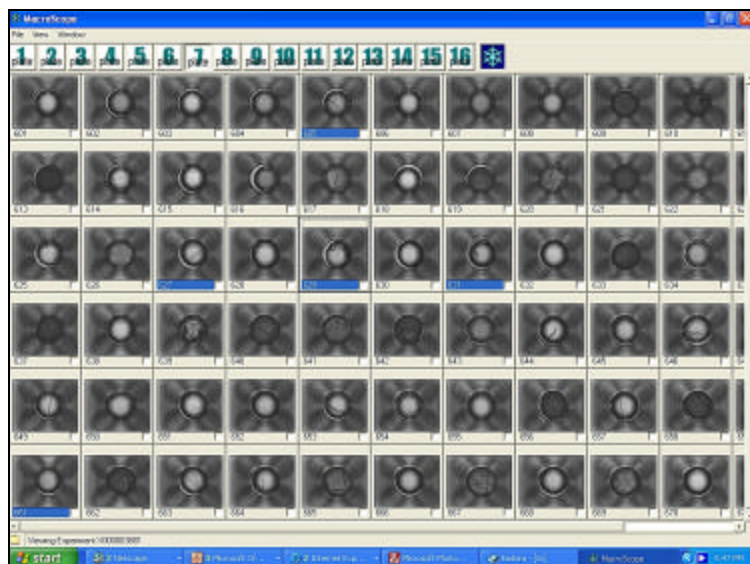
30 crystals diffract to 2.0 Å resolution. One suitable for X-ray diffraction. See [http://www.douglas.co.uk/oryx.htm](#) for more information.

crystals



30 crystals diffract to 2.0 Å resolution. One suitable for X-ray diffraction. See [http://www.douglas.co.uk/oryx.htm](#) for more information.

1 Crystal Screen, 96 (1999) 24-29. at <http://www.douglas.co.uk/oryx.htm>



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3. X-Ray Crystallography (after NMR)

a) Crystal Growth – Materials / Methods

c) X-ray Sources – Sealed Tube / Rotation Anode / Synchrotron

d) Theory of Diffraction – Bragg's Law / Reciprocal Space

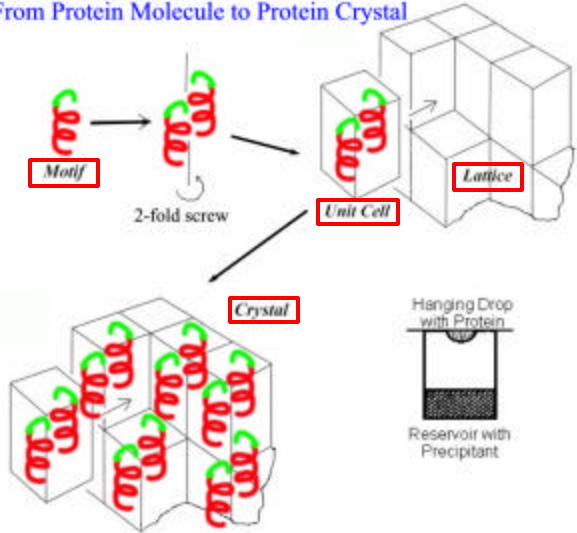
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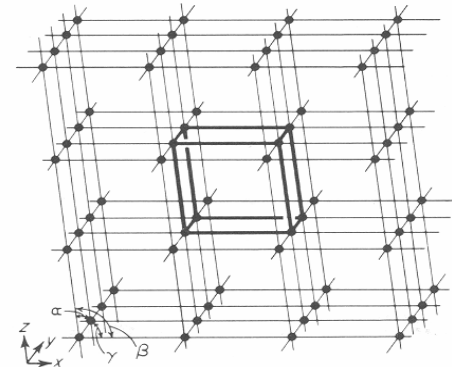
i) Analysis and presentation of results

From Protein Molecule to Protein Crystal



A **unit cell** is defined by its lattice constants:

a, b, c and **α, β, γ**

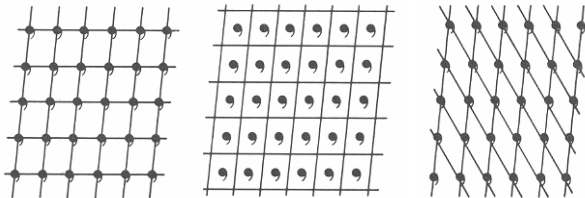


Three-dimensional lattice, showing unit cell (heavy lines).

How to identify “the” **unit cell** ?



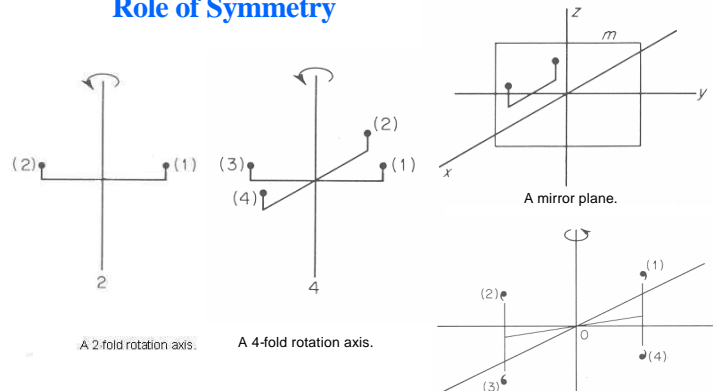
Regular two-dimensional array.



Three different grid systems referred to the array same array.

How to identify “the” **unit cell** ?

Role of Symmetry

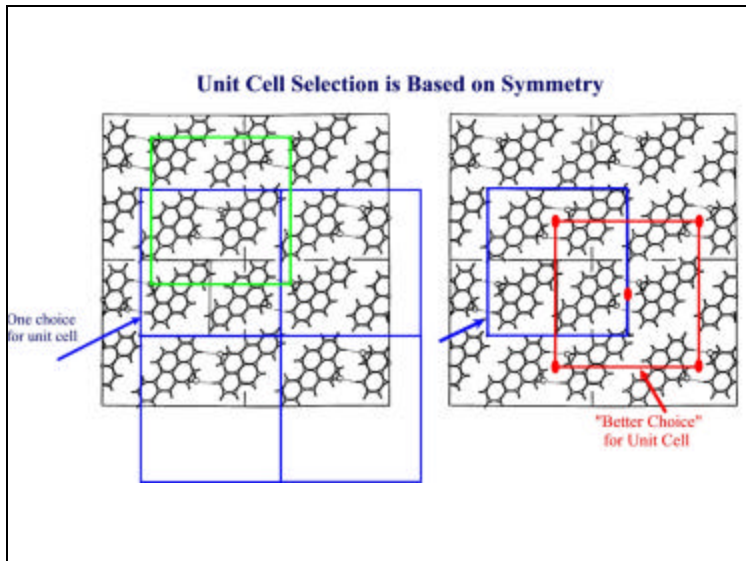


A 2-fold rotation axis.

A 4-fold rotation axis.


A mirror plane.

Center of symmetry produced by a 2-fold axis combined with reflection.



The Fourteen Bravais Lattices

There are fourteen distinct space groups that a Bravais lattice can have. Thus, from the point of view of symmetry, there are fourteen different kinds of Bravais lattices. Auguste Bravais (1811-1863) was the first to count the categories correctly.



Crystal Systems

| Crystal System | Bravais Type(s) | Essential Maximal Symmetry | Unit Cell Properties |
|----------------|-----------------|---|---|
| Triclinic | P | None | $a \neq b \neq c$, $\alpha \neq \beta \neq \gamma$ |
| Monoclinic | P, C | One 2-fold axis, parallel to b (unique) | $a \neq b \neq c$, $90^\circ, \beta, 90^\circ$ |
| Orthorhombic | P, I, F | Three perpendicular 2-fold | $a \neq b \neq c$, $90^\circ, 90^\circ, 90^\circ$ |
| Tetragonal | P, I | One 4-fold axis, parallel c | $a = a \neq c$, $90^\circ, 90^\circ, 90^\circ$ |
| Trigonal | P, R | One 3-fold axis | $a = a \neq c$, $90^\circ, 90^\circ, 120^\circ$ |
| Hexagonal | P | One 6-fold axis | $a = a \neq c$, $90^\circ, 90^\circ, 120^\circ$ |
| Cubic | P, F, I | Four 3-fold along space diagonal | $a = a = a$, $90^\circ, 90^\circ, 90^\circ$ |

Symmetry operations: 1, 2, 3, 4, 6, ∞

The 14 Bravais Lattices

| Crystal System | Bravais groups | Lattice Class | Point Group Symmetry |
|----------------|---|---------------------------|--|
| Triclinic | L, $\bar{1}$ | L | C_1 |
| Monoclinic | 2, m , $2/m$ | 2M | C_2 , C_2h |
| Orthorhombic | 222, $m\bar{2}2$, $m\bar{2}m$ | mmm | C_2 , C_2v , C_2h , C_2h |
| Tetragonal | 4, $\bar{4}$, $4/m$, C_{4v} , $4/m\bar{2}2$, $4/m\bar{2}m$ | 4M, $\bar{4}M$ | C_4 , C_4v , C_4h , C_4h , C_4h |
| Trigonal | 3, $\bar{3}$, 32 , $\bar{3}2$, $\bar{3}2/m$ | 3, $\bar{3}2$ | C_3 , C_3v , C_3h , C_3h , C_3h |
| Hexagonal | 6, $\bar{6}$, $6/m$, C_{3v} , $6/m\bar{2}2$, $6/m\bar{2}m$ | 6M, $\bar{6}M$ | C_6 , C_6h |
| Cubic | 23, $m\bar{3}$, 432 , $\bar{4}3m$, $m\bar{3}m$ | $m\bar{3}m$, $\bar{4}3m$ | C_2 , C_3 , C_4 , C_6 , C_2h , C_3h , C_3h , C_3h , C_3h |

Note

- Less than corresponds to symmetry of crystal space (diffraction pattern)
- Pattern symmetry in Lattice class allows Bravais stacking, i.e. centrosymmetric and acentric

$x, 1+y, z$

$y = 1.0$

$-x, 1/2+y, -z$

$y = 0.0$

x, y, z

$-x, -1/2+y, -z$

(c) two-fold screw axis through the origin

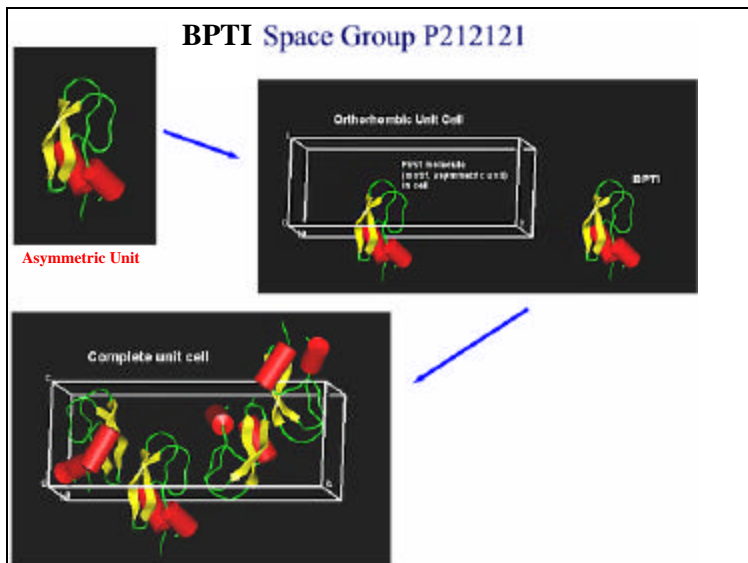
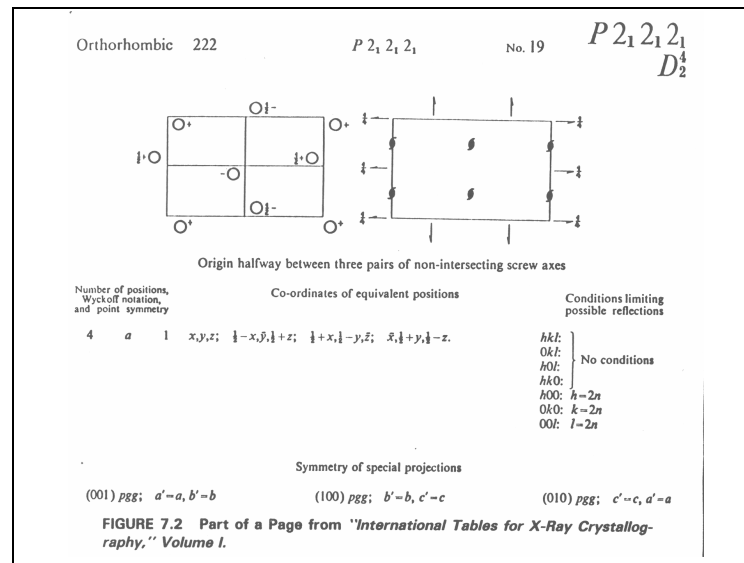
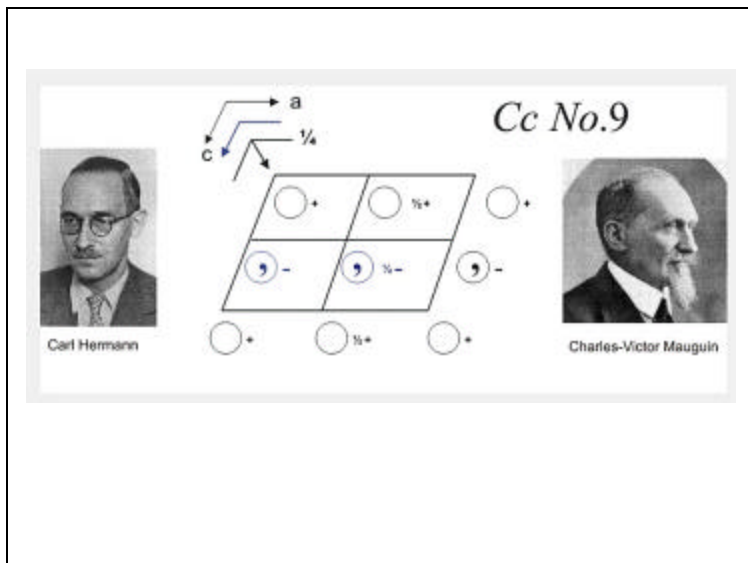


TABLE 3.4 Space Groups in Standard Orientations*

| System | Point Group | Space Group | Fraction |
|-----------------------|-------------|---|----------|
| Triclinic | 1 | <i>P1</i> | 1/2 |
| Monoclinic | 2 | <i>P2</i> , <i>C2</i> | 1/4 |
| | <i>m</i> | <i>Pm</i> , <i>Cm</i> | |
| | <i>2/m</i> | <i>P2/m</i> , <i>C2/m</i> | |
| Orthorhombic | 222 | <i>P222</i> , <i>F222</i> , <i>I222</i> | 1/8 |
| | <i>mm2</i> | <i>Pmm2</i> , <i>Pmc21</i> , <i>Pma2</i> , <i>Cmc21</i> , <i>Ccc2</i> , <i>Amc2</i> , <i>Fdd2</i> | |
| | <i>mmm</i> | <i>Pmmm</i> , <i>Pnma</i> , <i>Pmma</i> , <i>Cmnm</i> , <i>Cmmm</i> , <i>Pmnm</i> , <i>Pncm</i> , <i>Pbcm</i> , <i>Cmca</i> , <i>Icma</i> | |
| Tetragonal | 4 | <i>P4</i> , <i>C4</i> | 1/8 |
| | $\bar{4}$ | <i>I4</i> | |
| | $4/m$ | <i>P4/m</i> , <i>F4/m</i> | |
| | 422 | <i>P422</i> , <i>F422</i> | 1/16 |
| | 4mm | <i>P4mm</i> , <i>F4mm</i> | |
| | 42m | <i>P42m</i> , <i>F42m</i> | |
| | 4/mmm | <i>P4/mmm</i> , <i>F4/mmm</i> | |
| Trigonal/rhombohedral | 3 | <i>P3</i> , <i>R3</i> | 1/6 |
| | $\bar{3}$ | <i>R3</i> | |
| | 32 | <i>P312</i> , <i>P321</i> | 1/12 |
| | 3m | <i>P3m1</i> , <i>P31m</i> | |
| | $\bar{3}m$ | <i>P31c</i> , <i>P3c1</i> | |
| Hexagonal | 6 | <i>P6</i> , <i>C6</i> | 1/12 |
| | $\bar{6}$ | <i>P6/m</i> | |
| | 622 | <i>P622</i> , <i>C622</i> | 1/24 |
| | 6mm | <i>P6mm</i> , <i>C6mm</i> | |
| | 6m2 | <i>P6m2</i> , <i>C6m2</i> | |
| | 6/mmm | <i>P6/mmm</i> , <i>C6/mmm</i> | |
| Cubic | 23 | <i>P23</i> , <i>F23</i> | 1/24 |
| | $\bar{m}3$ | <i>Pm3</i> , <i>Fm3</i> | |
| | 432 | <i>P432</i> , <i>F432</i> | 1/48 |
| | $\bar{4}3m$ | <i>P43m</i> , <i>F43m</i> | |
| | $\bar{m}3m$ | <i>Pm3m</i> , <i>Fm3m</i> | |
| | $\bar{4}32$ | <i>P432</i> , <i>F432</i> | |

*The 11 Laue symmetries are separated by horizontal lines.

TABLE 16-5 The 65 "Biological" Space Groups

| CRYSTAL SYSTEM | LAT-TICE | MINIMUM SYMMETRY OF UNIT CELL | UNIT CELL EDGES AND ANGLES* | DIFFRACTION PAT-TERN SYM-METRY* | SPACE GROUPS† |
|-----------------------|----------------------|--------------------------------------|--|---------------------------------|--|
| Triclinic | <i>P</i> | None | $a \neq b \neq c$ $\alpha \neq \beta \neq \gamma$ | $\bar{1}$ | <i>P1</i> |
| Monoclinic | <i>P</i> | 2-fold axis parallel to <i>b</i> | $a \neq b \neq c$ $\alpha = \gamma = 90^\circ$ $\beta \neq 90^\circ$ | <i>2m</i> | <i>P2, P2₁</i> |
| | <i>C</i> | | | | <i>C2</i> |
| Orthorhombic | <i>P</i> | 3 mutually perpendicular 2-fold axes | $a \neq b \neq c$ $\alpha = \beta = \gamma = 90^\circ$ | <i>mmm</i> | <i>P222, P2₁2₁2₁, P222₁, P2₁2₁2</i> |
| | <i>C</i> | | | | <i>C222, C222₁</i> |
| | <i>I</i> | | | | <i>[I222, I2₁2₁2₁]</i> |
| | <i>F</i> | | | | <i>F222</i> |
| Tetragonal | <i>P</i> | 4-fold axis parallel to <i>c</i> | $a = b \neq c$ $\alpha = \beta = \gamma = 90^\circ$ | <i>4m</i> | <i>P4, (P4₁, P4₂), P4₃</i> |
| | <i>I</i> | | | | <i>I4, I4₁</i> |
| | <i>F</i> | | | | <i>F432, (P4₃22, P4₂22), P4₂22</i> |
| Trigonal/rhombohedral | <i>R^h</i> | 3-fold axis parallel to <i>c</i> | $a = b = c$ $\alpha = \beta = \gamma \neq 90^\circ$ | $\bar{3}$ | <i>R3</i> |
| | <i>pd</i> | | | | <i>P3, (P3₁, P3₂)</i> |
| | <i>F</i> | | | | <i>R32</i> <i>[P321, P312]</i> <i>[(P3₁21, P3₂21), (P3₁12, P3₂12)]</i> |
| Hexagonal | <i>P</i> | 6-fold axis parallel to <i>c</i> | $a = b \neq c$ $\alpha = \beta = 90^\circ$ $\gamma = 120^\circ$ | <i>6m</i> | <i>P6, (P6₁, P6₂)</i> |
| | <i>I</i> | | | | <i>P6₃, (P6₃, P6₃)</i> |
| | <i>F</i> | | | | <i>P622, (P6₂22, P6₂22)</i> |
| Cubic | <i>P</i> | 3-fold axes along cube diagonals | $a = b = c$ $\alpha = \beta = \gamma = 90^\circ$ | <i>m3</i> | <i>P23</i> |
| | <i>I</i> | | | | <i>P2₁3</i> <i>[I23, I2₁3]</i> |
| | <i>F</i> | | | | <i>F23</i> |
| | <i>F</i> | | | | <i>P432, (P4₃32, P4₃32)</i> <i>P4₂22</i> <i>I432, I4₁32</i> <i>F432, F4₃32</i> |

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b) Crystal Lattices - Lattice Constants / Space Groups / Asymmetric Unit

d) Theory of Diffraction – Bragg's Law / Reciprocal Space

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X-ray tubes: the "sealed" tube

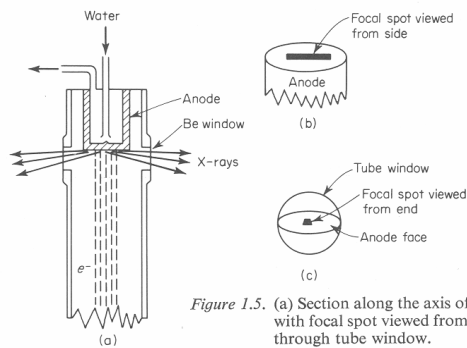
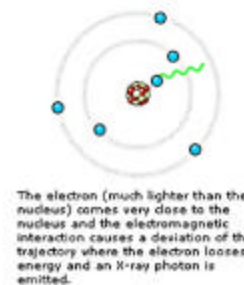


Figure 1.5. (a) Section along the axis of an X-ray tube. (b) Anode with focal spot viewed from side. (c) Focal spot viewed through tube window.

Origin of Non-characteristic X-rays

Bremsstrahlung X-rays

In an X-ray tube the electrons emitted from the anode are accelerated towards the metal target cathode by an accelerating voltage of typically 50 kV. The high energy electrons interact with the atoms in the metal target. Sometimes the electron comes very close to a nucleus in the target and is deviated by the electromagnetic interaction. In this process, which is called bremsstrahlung (braking radiation), the electron loses much energy and a photon (X-ray) is emitted. The energy of the emitted photon can take any value up to a maximum corresponding to the energy of the incident electron.



The electron (much lighter than the nucleus) comes very close to the nucleus and the electromagnetic interaction causes a deviation of the trajectory where the electron loses energy and an X-ray photon is emitted.



Origin of characteristic X-rays

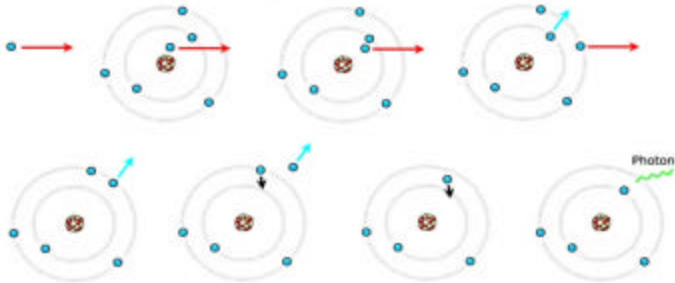
Related Laureate



The Nobel Prize in Physics 1917 - Charles Glover Barkla >

Characteristic X-ray Lines

The high energy electron can also cause an electron close to the nucleus in a metal atom to be knocked out from its place. This vacancy is filled by an electron further out from the nucleus. The well defined difference in binding energy, characteristic of the material, is emitted as a monoenergetic photon. When detected this X-ray photon gives rise to a characteristic X-ray line in the energy spectrum. C. Barkla observed these lines in 1908-09 and was given the 1917 Nobel Prize for this discovery. He also made the first experiments suggesting that the X-rays are electromagnetic waves.



Characteristic X-rays arise from electronic transitions

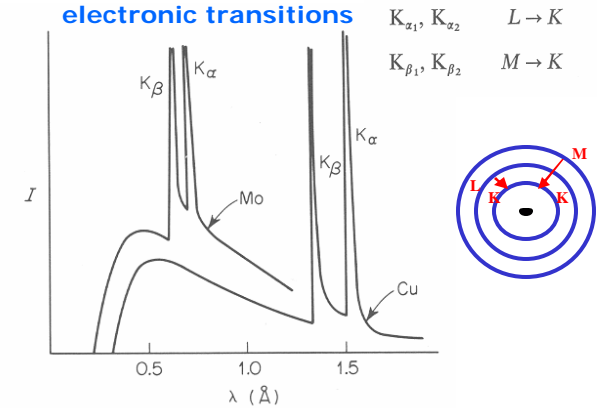


Figure 1.2. X-ray spectra with characteristic peaks: MoK α , 50 Kv; CuK α , 35 Kv.

Characteristic X-rays have defined λ

Table 1.1. Target Materials and Associated Constants

| | Cr | Fe | Cu | Mo |
|----------------------------|-------------|-------------|-------------|------------|
| Z | 24 | 26 | 29 | 42 |
| $\alpha_1, \text{Å}$ | 2.2896 | 1.9360 | 1.5405 | 0.70926 |
| $\alpha_2, \text{Å}$ | 2.2935 | 1.9399 | 1.5443 | 0.71354 |
| $\bar{\alpha},^* \text{Å}$ | 2.2909 | 1.9373 | 1.5418 | 0.71069 |
| $\beta_1, \text{Å}$ | 2.0848 | 1.7565 | 1.3922 | 0.63225 |
| β_2 filt. | V, 0.4 mil† | Mn, 0.4 mil | Ni, 0.6 mil | Nb, 3 mils |
| α filt. | Ti | Cr | Co | Y |
| Resolution, Å | 1.15 | 0.95 | 0.75 | 0.35 |
| Critical potential, kV | 5.99 | 7.11 | 8.98 | 20.0 |
| Operating conditions, kV: | 30-40 | 35-45 | 35-45 | 50-55 |
| half- or full-wave- | 10 | 10 | 20 | 20 |
| rectified, mA | | | | |
| constant potential, mA | 7 | 7 | 14 | 14 |

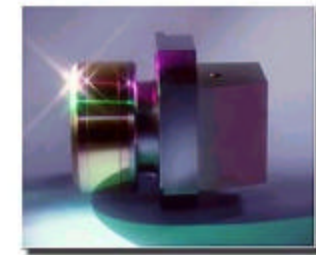
* $\bar{\alpha}$ is the intensity-weighted average of α_1 and α_2 and is the figure usually used for the wavelength when the two lines are not resolved.

† 1 mil = 0.001 inch = 0.025 mm.

X-ray Generators



FR591



FR591 Rotating Anode X-ray Generator

The Human FR591 rotating anode X-ray generator now has dramatically improved the performance of the anode, by a complete redesign. We now have a static shaft and a rotating anode, instead of rotating both. The rotating water flow has also been redesigned to give much higher throughput, higher flow and higher turbulence, which results in better heat transfer and hence better cooling capacity.

Now with the new ULTRA mode you can get 615W on a 3.2mm focal!



Another Source of "X-rays"

Synchrotron Radiation

X-ray photons can also be created under different conditions. When physicists were operating the first particle accelerators, they discovered that electrons can produce photons without colliding at all. This was possible because the magnetic field in the accelerators was causing the electrons to move in large spirals around magnetic field lines of force. This process is called synchrotron radiation.

In the cosmos particles such as electrons can be accelerated to high energies—near the speed of light—by electric and magnetic fields. These high-energy particles can produce synchrotron photons with wavelengths ranging from radio up through X-ray and gamma-ray energies.



Synchrotron Radiation: Electrons moving in magnetic field radiate photons.

"X-ray" Sources: Beyond X-ray tubes

The **brilliance** of a light source is defined as the number of photons emitted per second, per unit source size, per unit space angle and for a bandwidth of 1% of the photon energy.

The Comparison between various sources of X-rays shows large differences in their brilliance.

X-ray tubes

Wilhelm Conrad Roentgen discovered X-rays in 1895 whilst working with cathode-ray tubes. Using the principle of fast electrons hitting a metallic target, a first substantial gain in brilliance was not obtained until the introduction of rotating anode sources (~1960).

Synchrotron Radiation Facilities:

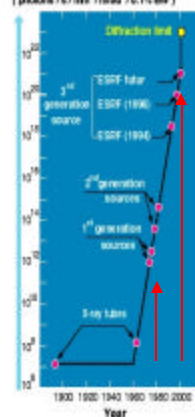
The progress of high energy physics, with the construction of powerful particle accelerators gave birth to what we now call first generation synchrotron sources (~1970). Using the deflection of high energy electrons by a magnetic field for the production of X-rays proved so promising that a number of dedicated second generation sources were built (~1980). Relying on the combination of steady state electron beams and insertion Devices, third generation synchrotron sources (~1990) are now emitting synchrotron X-ray beams that are a billion (10^9) times more brilliant than those produced by X-ray tubes.

Free Electron X-ray Lasers:

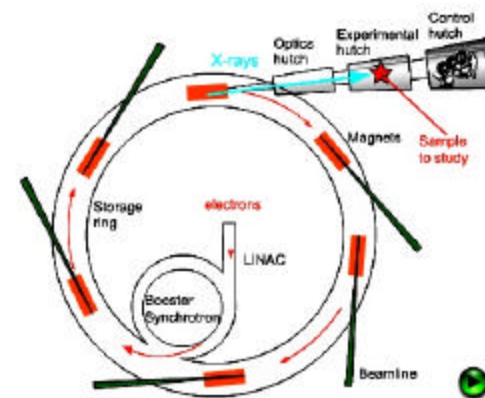
Coupling electron and X-ray beams together, the Free Electron X-ray Laser currently on the drawing boards could be the next generation of X-ray sources. While they promise to achieve an increase in peak brilliance by another factor of a billion, the first prototypes may be operational around the year 2010.

Brilliance of the X-ray beams

(photons / s / mm² / mrad² / 0.1% BW)



How synchrotron light is produced?





X-Ray Crystallography

“If a picture is worth a thousand words, then a macromolecular structure is priceless to a physical biochemist.” – van Holde

Topics:

1. Protein Data Bank (PDB)

Data mining and Protein Structure Analysis Tools

2. Image Formation

Resolution / Wavelength (Amplitude, Phase) / Light Microscopy / EM / X-ray / (NMR)

3. X-Ray Crystallography (after NMR)

a) Crystal Growth – Materials / Methods

b) Crystal Lattices – Lattice Constants / Space Groups / Asymmetric Unit

c) X-ray Sources – Sealed Tube / Rotation Anode / Synchrotron

e) Data Collection – Methods / Detectors / Structure Factors

f) Structure Solution – Phase Problem: MIR / MR / MAD

h) Refinements and Models

i) Analysis and presentation of results

X-RAYS
X-rays: What Are They? 4.7

More About the Bragg Formula

X-rays scattered from different layers of atoms can interfere with each other. The interference depends on the wavelength of the X-ray and on the distance between the atom layers. An X-ray with well-known wavelength can be used to explore the structure of the crystal. For a well-known crystal, the X-ray properties can be examined.

$n\lambda = 2d \sin\theta$

$d = 0.3 \text{ nm}$

Crystal planes, in NaCl, ordinary salt. Other planes are also possible.

X-ray scattering from three crystal planes, separated by the distance d . For constructive interference in a direction θ the path difference must be an even number of wavelengths.

Related Laureates

The Nobel Prize in Physics 1935 - Sir William Henry Bragg >

The Nobel Prize in Physics 1939 - William Lawrence Bragg >

Related Laureates

| | | |
|---|--|---|
| The Nobel Prize in Physics 1901 - Wilhelm Conrad Röntgen > | The Nobel Prize in Physics 1924 - Max von Laue > | The Nobel Prize in Physics 1955 - Sir William Henry Bragg > |
| The Nobel Prize in Physics 1925 - William Lawrence Bragg > | The Nobel Prize in Physics 1927 - Charles Glover Barkla > | The Nobel Prize in Physics 1928 - Karl Manne Georg Siegbahn > |
| The Nobel Prize in Physics 1927 - Arthur Holly Compton > | The Nobel Prize in Chemistry 1936 - Petrus (Peter) Josephus Wilhelmus Debye > | The Nobel Prize in Chemistry 1962 - Max Ferdinand Perutz > |
| The Nobel Prize in Chemistry 1962 - John Cowdery Kendrew > | The Nobel Prize in Physiology or Medicine 1962 - Francis Harry Compton Crick > | The Nobel Prize in Physiology or Medicine 1962 - James Dewey Watson > |
| The Nobel Prize in Physiology or Medicine 1962 - Maurice Hugh Frederick Wilkins > | The Nobel Prize in Chemistry 1964 - Dorothy Crowfoot Hodgkin > | The Nobel Prize in Chemistry 1976 - William N. Lipscomb > |
| The Nobel Prize in Physiology or Medicine 1979 - Alan M. Cormack > | The Nobel Prize in Physiology or Medicine 1979 - Godfrey H. Hounsfield > | The Nobel Prize in Physics 1981 - Kai M. Siegbahn > |
| The Nobel Prize in Chemistry 1985 - Herbert A. Hauptman > | The Nobel Prize in Chemistry 1985 - Jerome Karle > | The Nobel Prize in Chemistry 1988 - Johann Deisenhofer > |
| The Nobel Prize in Chemistry 1988 - Robert Huber > | The Nobel Prize in Chemistry 1988 - Hartmut Michel > | |