X-Ray Crystallography

Oct 14, 2013

X-Ray Crystallography

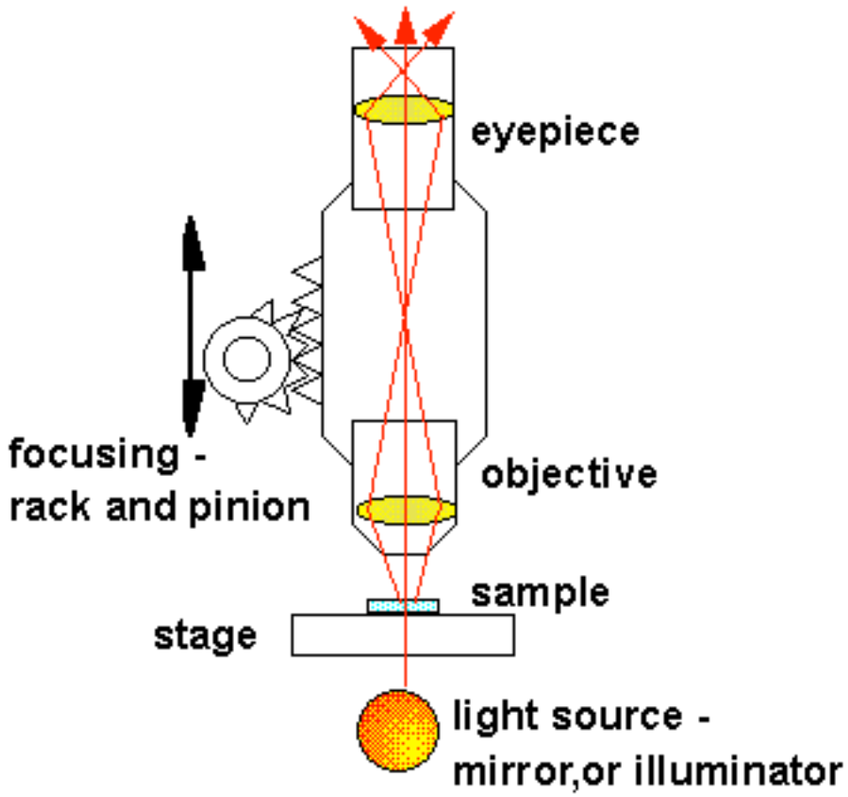
- Method to determine the ATOMIC structure of a molecule
- The most widely used technique for determine structures of biological molecules
- Can also be used to determine small molecules
- Most entries in the PDB were determined by X-ray crystallography
    - X-Rays just under 42,000 entries - numbers from 2009
    - NMR 7,115 entries
    - other techniques 256 entries
- NMR depends on interactions between nuclei X-Rays interacts with electrons.

X-Ray

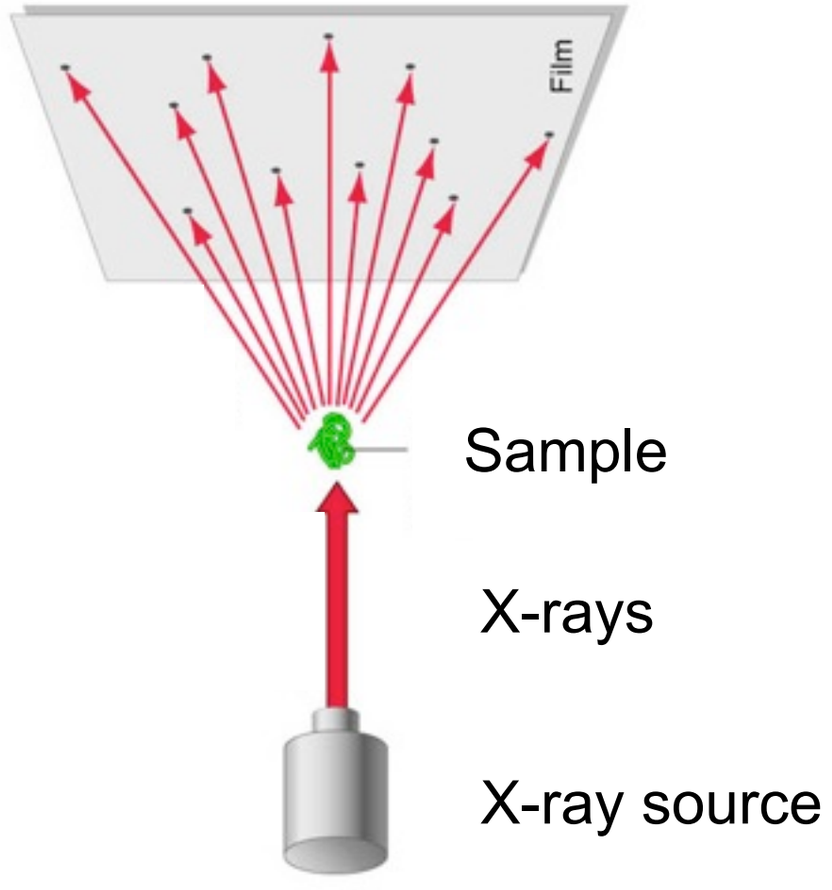
- Why X-rays??
- X-rays are part of the EM spectrum
- Atomic resolution - the position of two bonded atoms can be distinguished
- Typical covalent bond is about 0.12 nm or $1.2x10^{-10}m$
  - $1x10^{-10}$ m is called an Angstrom (Å)
- Limit of resolution of any optical method is defined by half the wavelength of the incident radiation
  - This is a consequence of the wave like properties of light
  - wavelength of visible light (400 to 800nm) therefore it can resolve objects to about 200nm (many cellular organelles)
  - For atomic resolution 0.12nm x2=0.24nm
  - This falls into the range of X-rays.
- NO LENS for X-rays

Light Microscope vs X-Ray Diffraction

Light Microscopy



X-ray Diffraction



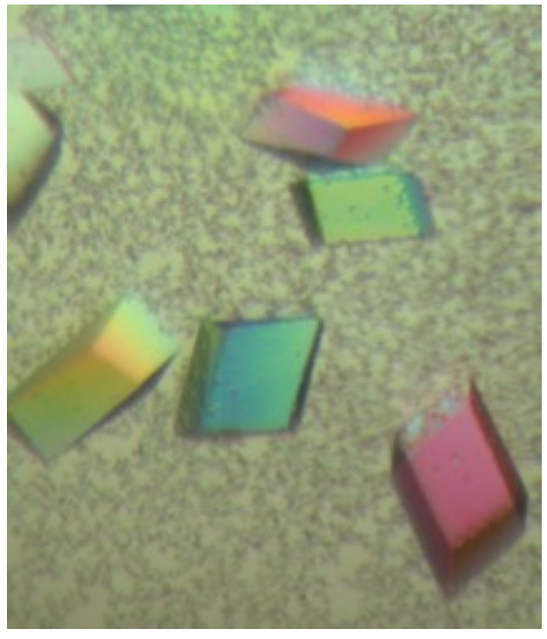
There is no lens for X-rays

Crystals

- X-rays weakly interact with matter
- Crystals serve two purposes
  - High concentration
  - Order
    - Crystals are solids that are exact repeats
- Comparison of crystalline quartz and glass - same molecule but quartz is an ordered crystal and glass is amorphous solid
- Crystals are composed of repeating units - translational and rotational symmetry operators relate molecules to each other
- Biological molecules are composed of chiral molecules.
- Chirality limits the types of symmetry that can be used to describe a crystal
  - No mirror planes or points of symmetry

Growing Crystals

- There is no straightforward way to grow crystals
  - Protein crystals are a solid but do contain a lot of solvent - Most have 30-70% solvent
- Large amounts of purified material
- Empirical process that requires careful observation



Growing Crystals II

- Molecules come out of solution when its concentration exceeds it intrinsic solubility
    - Intrinsic solubility is related to the properties of the molecule (ie basic, acidic, hydrophobic, etc) and conditions (ie salt concentrations, pH, temperature)
- Make a supersaturated solution and slowly precipitate the molecule in an orderly fashion
- Methods
    - Remove excess solvent
        - This method is done for many small molecules
        - Not so useful for proteins since there may be other additives (salt, detergent, etc)
    - Decrease the solubility of molecule
        - This is accomplished by increasing or decreasing ionic strength of the solution or the addition of a molecule to dehydrate the protein (PEG)

Crystal Growth in Theory

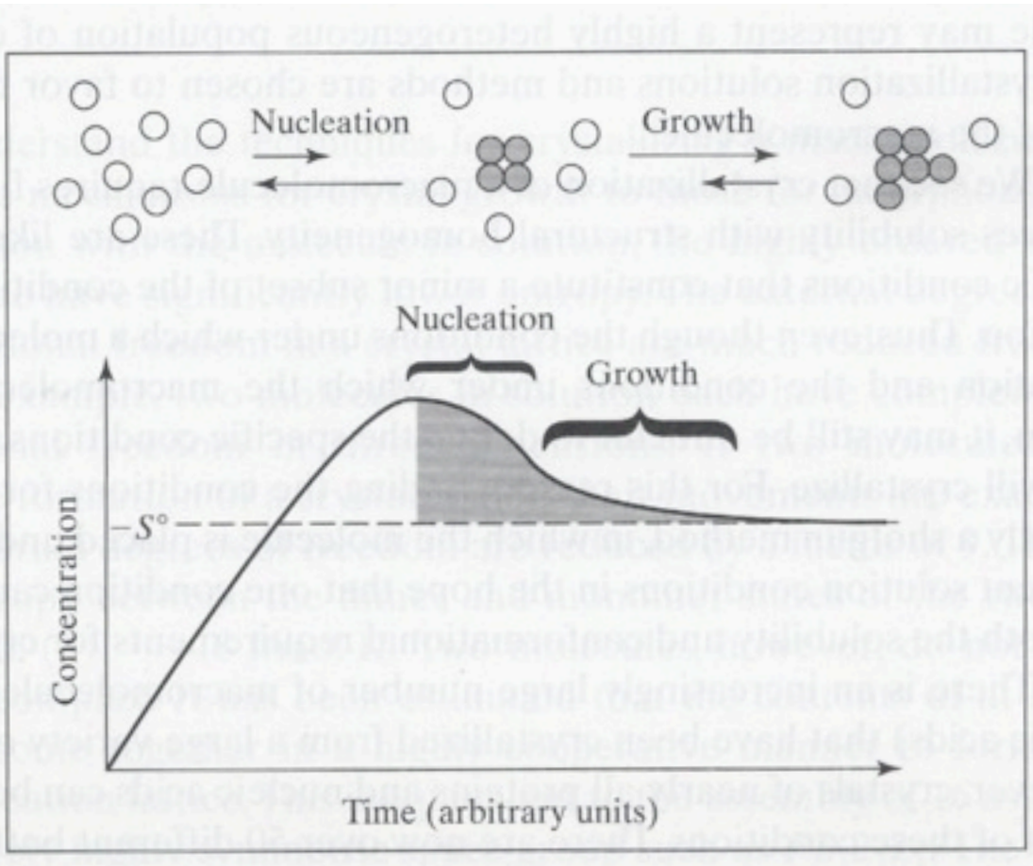


Figure 6.6 Mechanism of crystallization. The initial step in crystallization is the nucleation of a minimum crystal lattice. This is a low probability step that occurs in a supersaturated solution. The crystal grows by adding molecules to the surface of the seed, and occurs at concentrations close to the intrinsic solubility S^0 of the molecule.

Crystal Growth in Practice

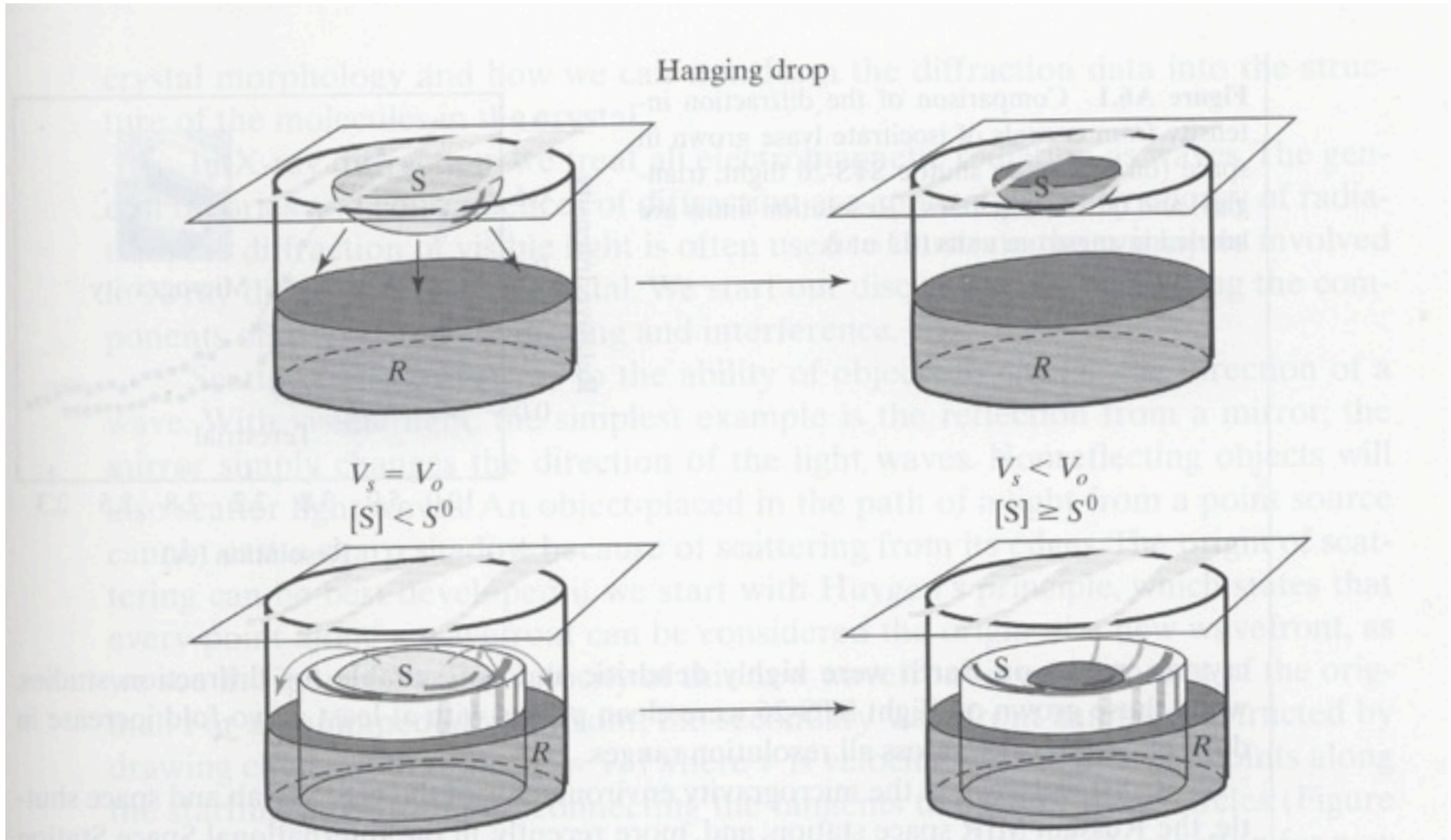
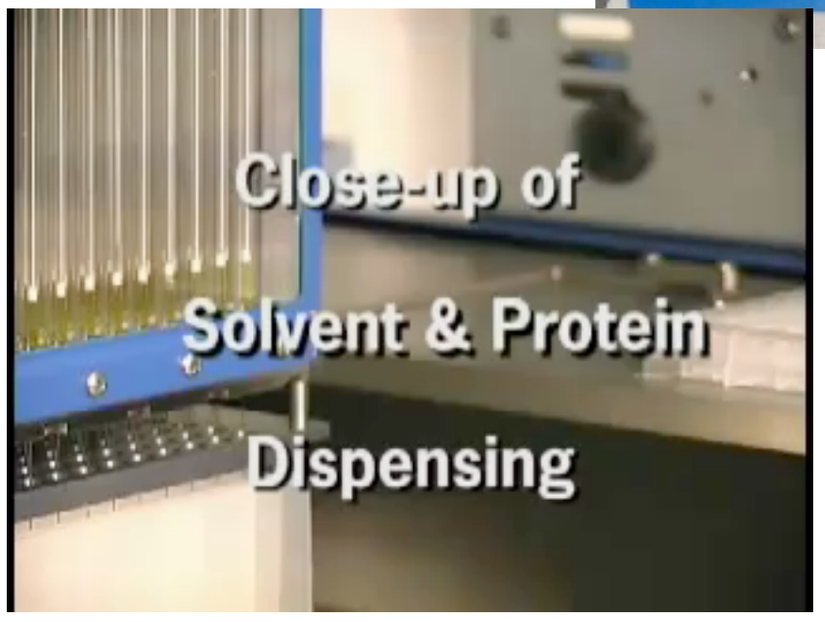


Figure 6.7 Vapor diffusion methods of crystallization. In the hanging drop method of vapor diffusion, a sample in solution is suspended above a reservoir, *R*, that contains a high concentration of a precipitant. The lower vapor pressure of the reservoir draws water from the sample solution, *S*, to reduce the volume of the sample, V_S, below its initial volume, V_o. Consequently, the concentration of molecules in the sample solution, [S], increases to above the intrinsic solubility S^0 of the molecule, resulting in precipitation or crystallization. In the sitting drop method, the sample solution sits in a well rather than hanging suspended, but otherwise the two methods are the same.

Past and Present Technology



Crystals in Space

- Crystal growth in a microgravity
- The microgravity causes a reduction in convection flow and a more uniform concentration within the drop
- First crystals grown in space were on the Columbia Space Shuttle in 1992
- Crystals grown in space can be 50% larger than those grown on Earth.
- DeLucs et al. *Science* Vol 246, 651-4

Bravais Lattice

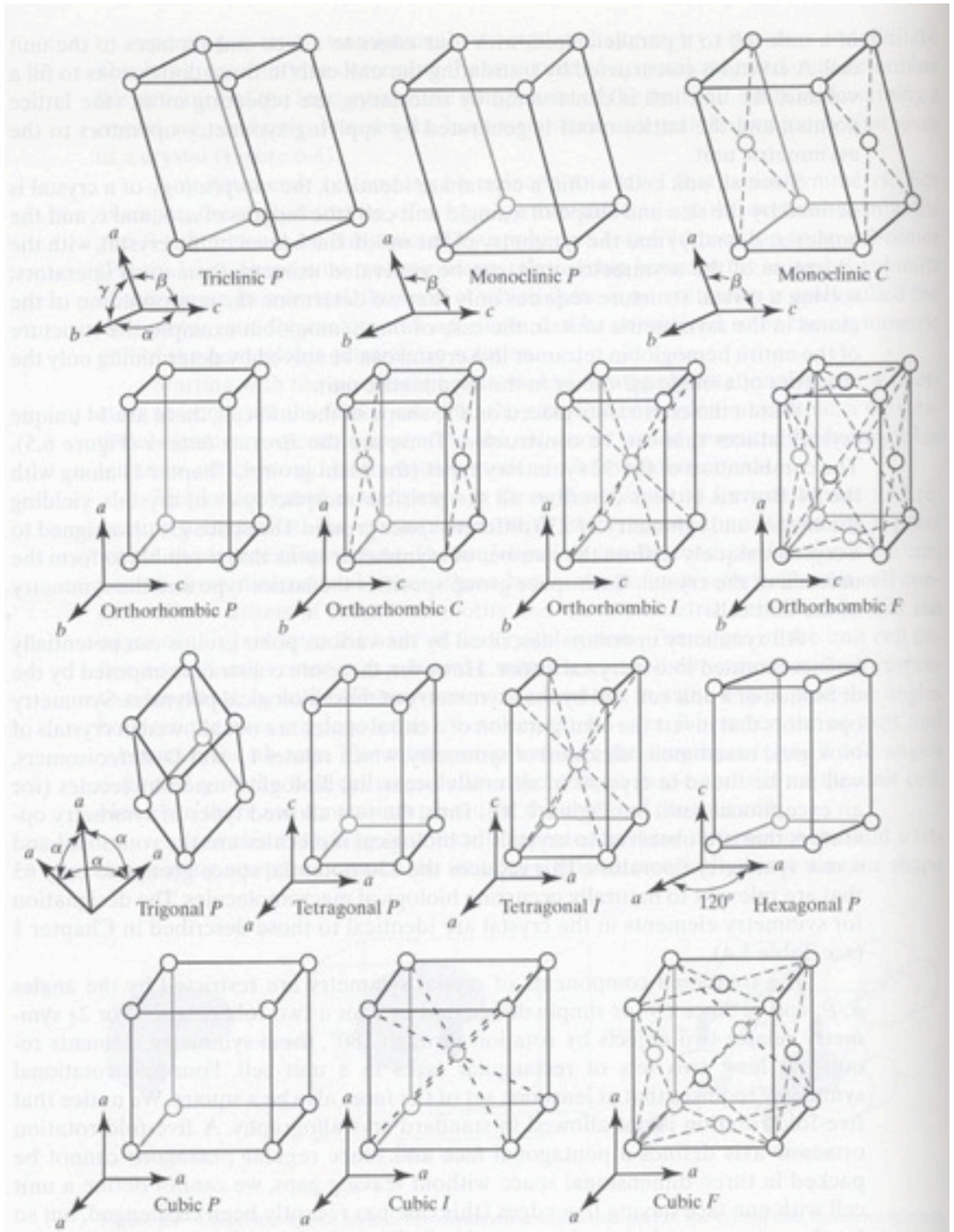


Figure 6.5 The 14 Bravais lattices in crystallography. [Adapted from G. H. Stout and L. H. Jensen (1989), *X-Ray Structure Determination, a Practical Guide,* 2nd ed., 50. John Wiley & Sons, New York.]

Space Group

Table 6.1 Sixty-Five Possible Space Groups in Macromolecular Crystals

Lattice Type	Possible Bravais Lattices	Crystal Shape	Possible Space Groups
Triclinic	P	$a \neq b \neq c$ $\alpha \neq \beta \neq \gamma \neq 90°$	$P1$
Monoclinic	P, C	$a \neq b \neq c$ $\alpha = \gamma = 90°, \beta \neq 90°$ or $120°$	$P2, P2_1, C2$
Orthorhombic	P, C, I, F	$a \neq b \neq c$ $\alpha = \beta = \gamma = 90°$	$P222, P2_12_12_1, P2_12_12, P222_1,$ $C222, C222_1, F222, I222, I2_12_12_1$
Tetragonal	P, I	$a = b \neq c$ $\alpha = \beta = \gamma = 90°$	$P4, P4_1, P4_2, P4_3, I4, I4_1, P422,$ $P42_12_1, P4_122, P4_12_12, P4_222,$ $P4_22_12, P4_32_12, P4_322, I422, I4_122$
Trigonal	P R (Rhombohedral)	$a = b \neq c$ $\alpha = \beta = 90°, \gamma = 120°$ $a = b = c$ $\alpha = \beta = \gamma < 120° (\neq 90°)$	$P3, P3_1, P3_2, P321, P312,$ $P3_112, P3_121, P3_212, P3_221,$ $R3, R32$
Hexagonal	P	$a = c \neq b$ $\alpha = \gamma = 90°, \beta = 120°$	$P6, P6_1, P6_2, P6_3, P6_4, P6_5, P622,$ $P6_122, P6_322, P6_322, P6_422, P6_522$
Cubic	P, I, F	$a = b = c$ $\alpha = \beta = \gamma = 90°$	$P432, P4_132, P4_232, P4_332, F432,$ $F4_132, I432, I4_132$

Two-Fold Axes of Rotation

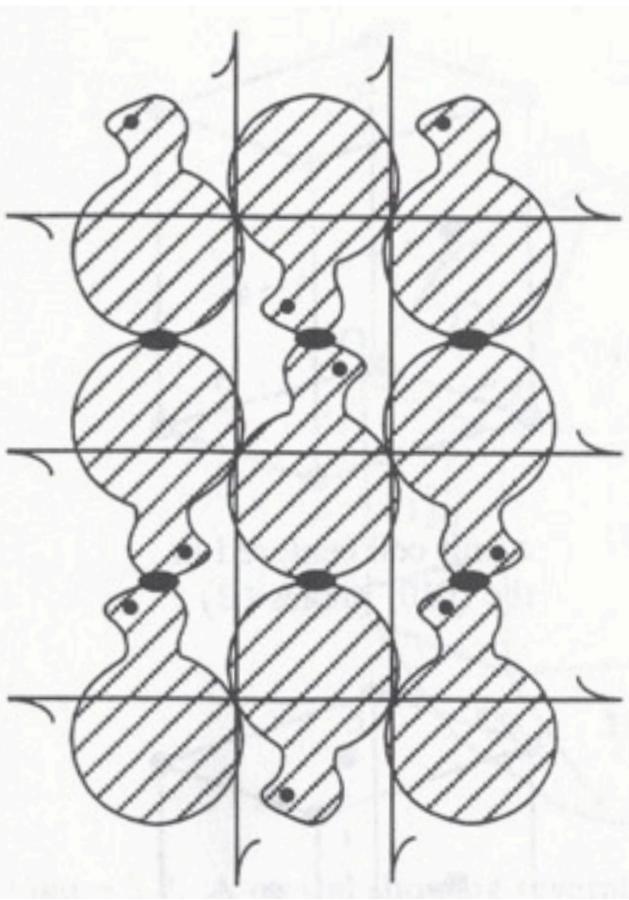


Figure 3.10. A two-dimensional lattice with 2-fold symmetry axes perpendicular to the plane of the figure and 2-fold screw axes in the plane.

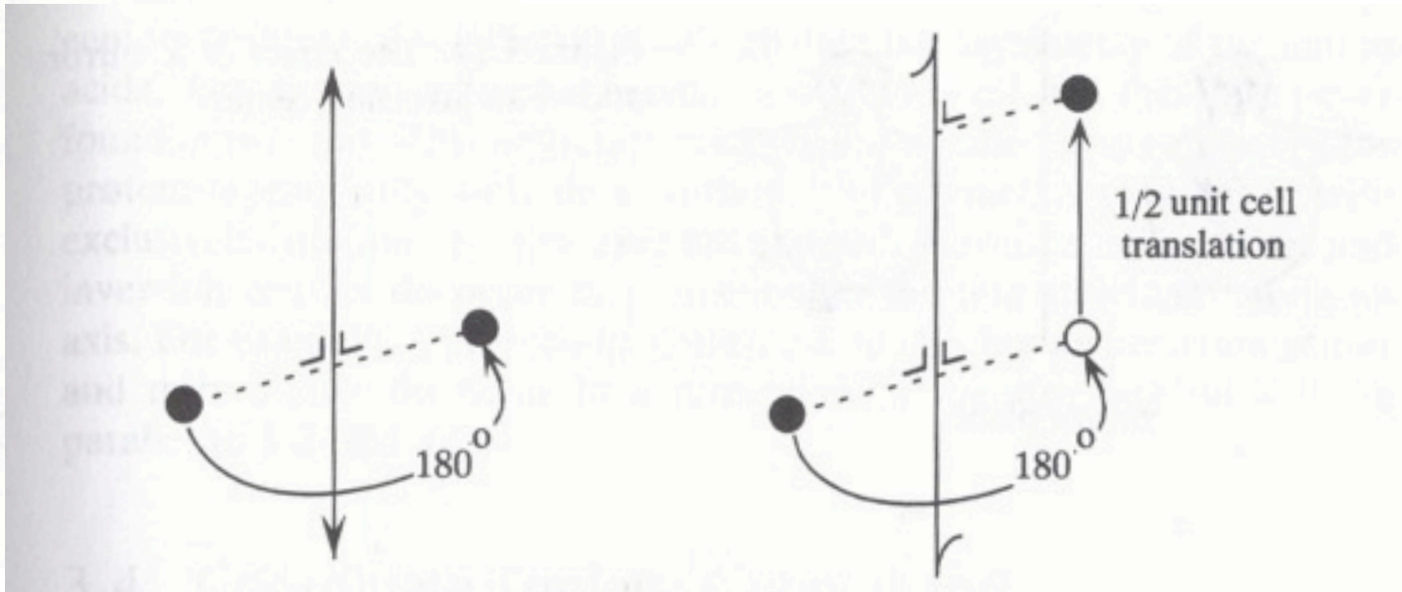


Figure 3.12. A 2-fold axis (left) and a 2-fold screw axis (right); the latter relates one molecule to another by a 180° rotation plus a translation over half of the unit cell.

Three-Fold Axes of Rotation

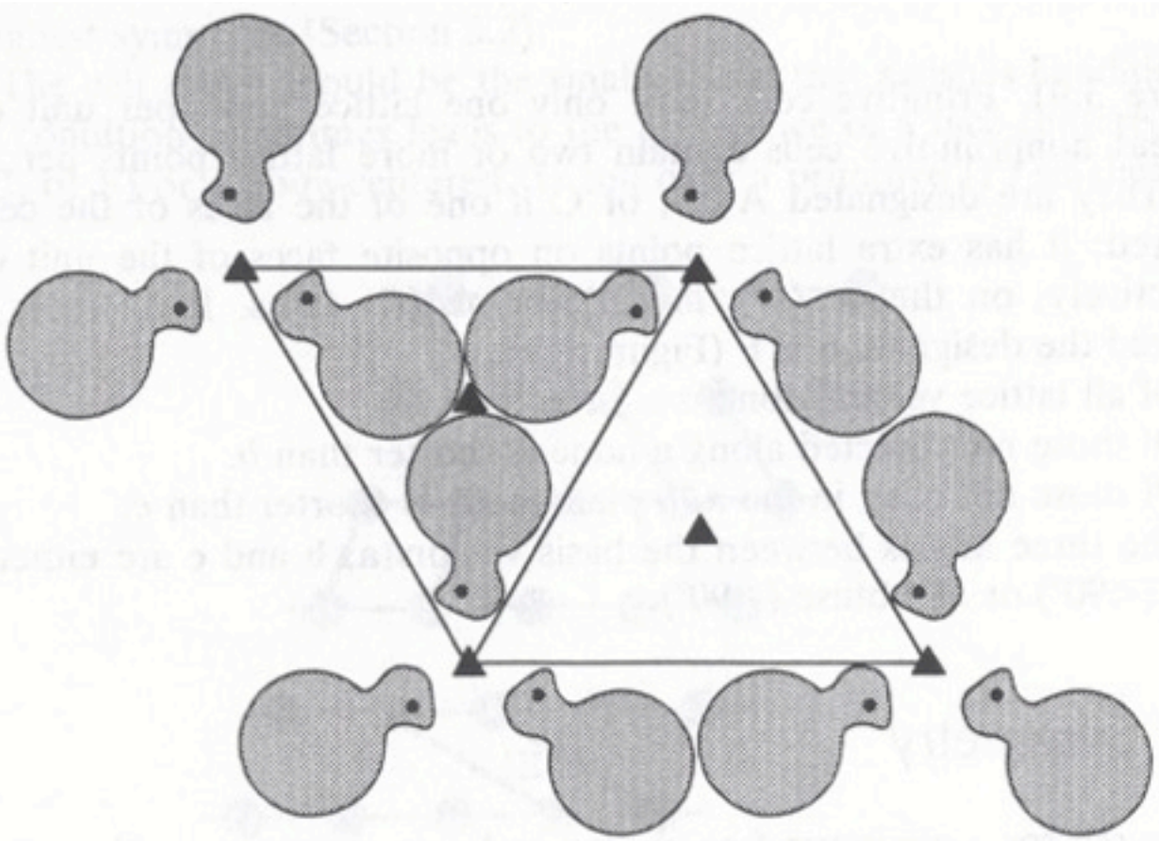
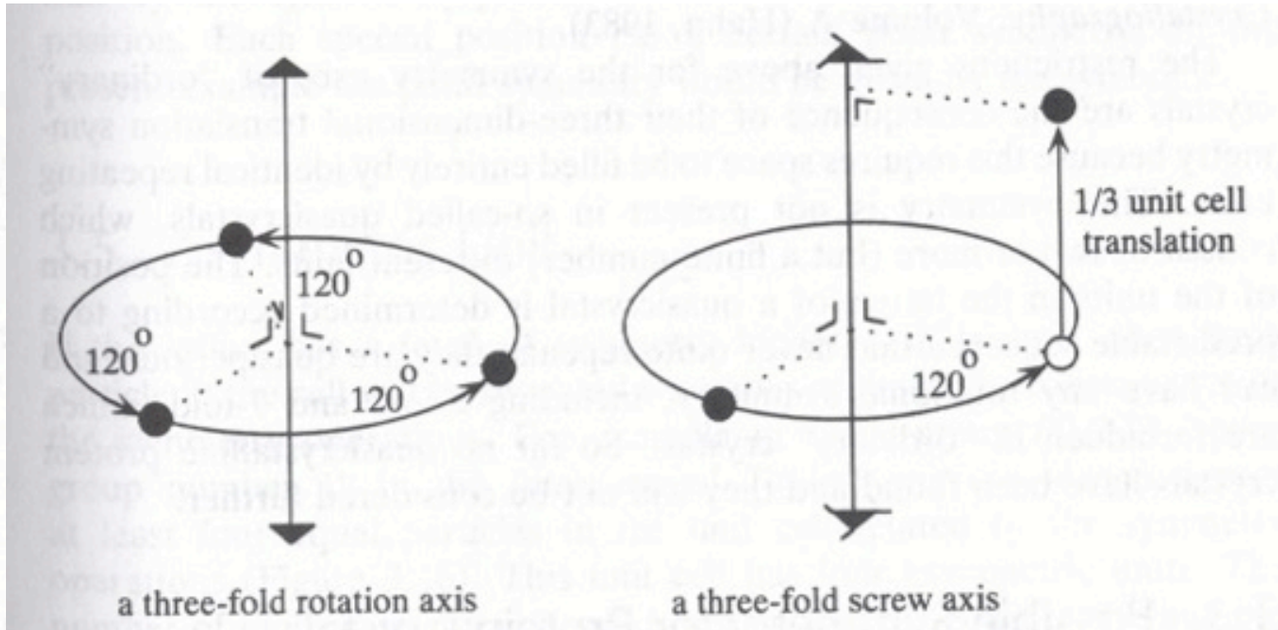


Figure 3.11. A two-dimensional lattice with 3-fold symmetry axes perpendicular to the plane of the figure.



a three-fold rotation axis a three-fold screw axis

Figure 3.13. A 3-fold axis (left) and a 3-fold screw axis (right); the latter relates one molecule to another by a 120° rotation and a translation over one-third of the unit cell.

Mirror Planes and Inversion Center

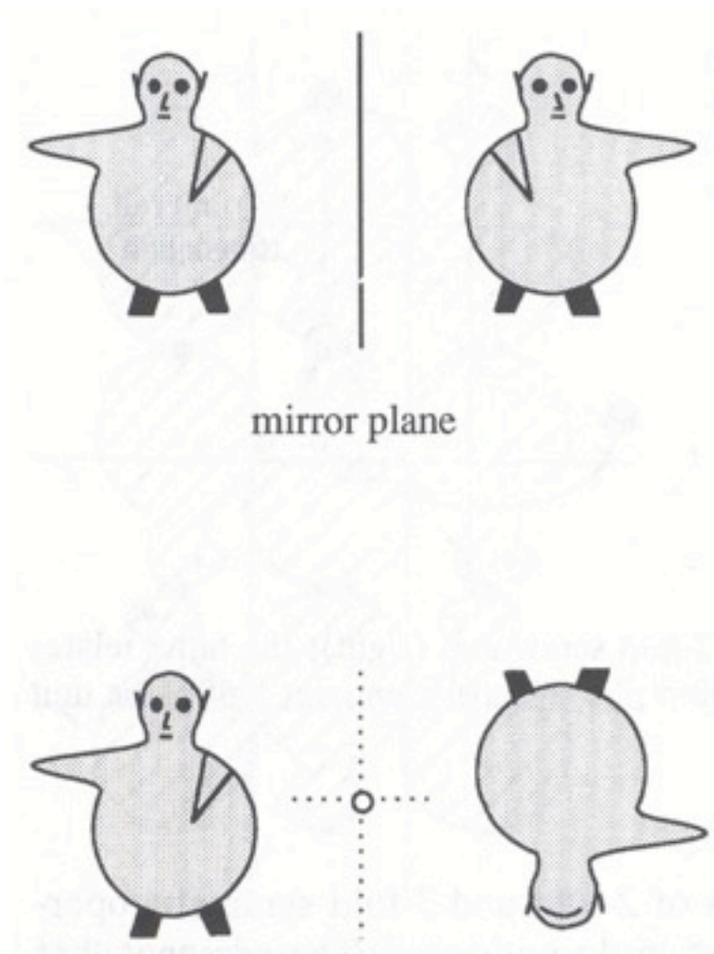


Figure 3.14. The effect of a mirror and of an inversion center.

Projections

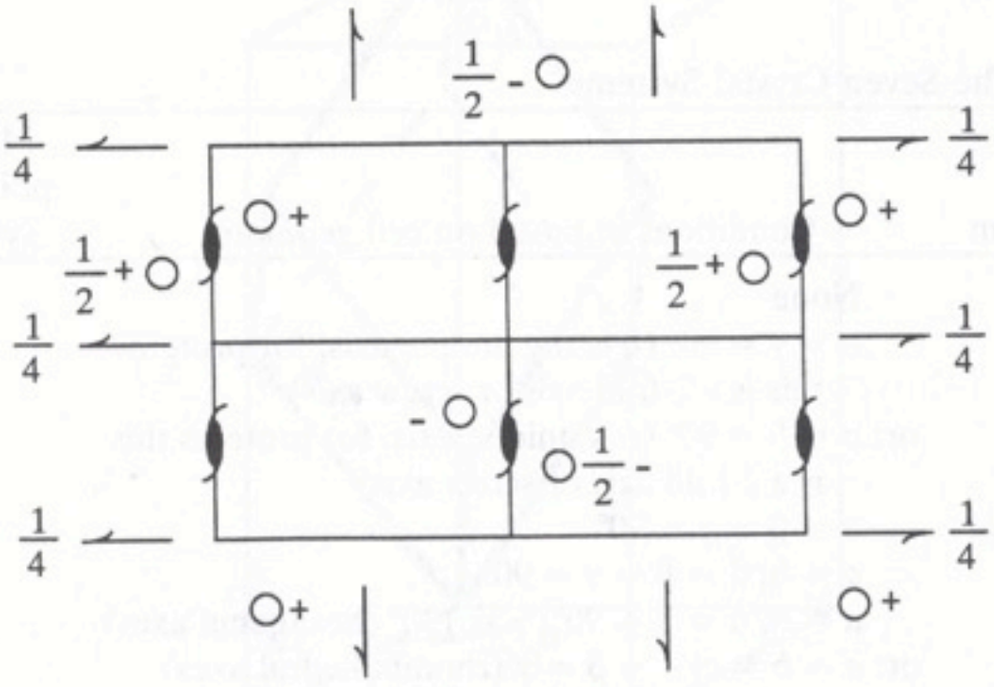


Figure 3.16. The projection of a $P2_12_12_1$ unit cell; it contains four asymmetric units.