Methods in Chemistry III – Part 1
Modul M.Che.1101 WS 2010/11 – 4

Modern Methods of Inorganic Chemistry

Mi 10:15-12:00, Hörsaal II

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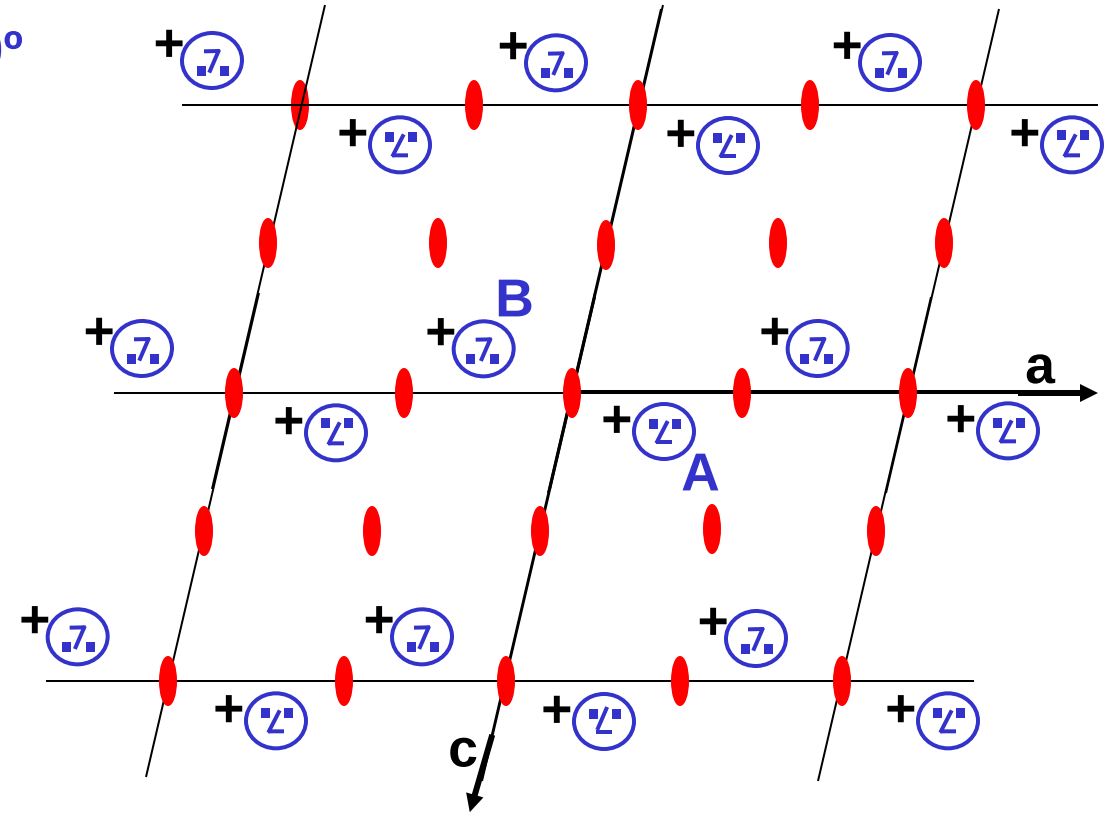
The space group *P*2 (monoclinic): 4 cells

$a \neq b \neq c$, $\alpha = \gamma = 90^o$, $\beta \neq 90^o$

Atoms at: *x*, *y*, *z* [A]

–x, *y*, *–z* [B]

The *asymmetric unit* corresponds to one half of a unit-cell



Special positions on twofold axes at:

0, *y*, 0; ½, *y*, 0; 0, *y*, ½; ½, *y*, ½

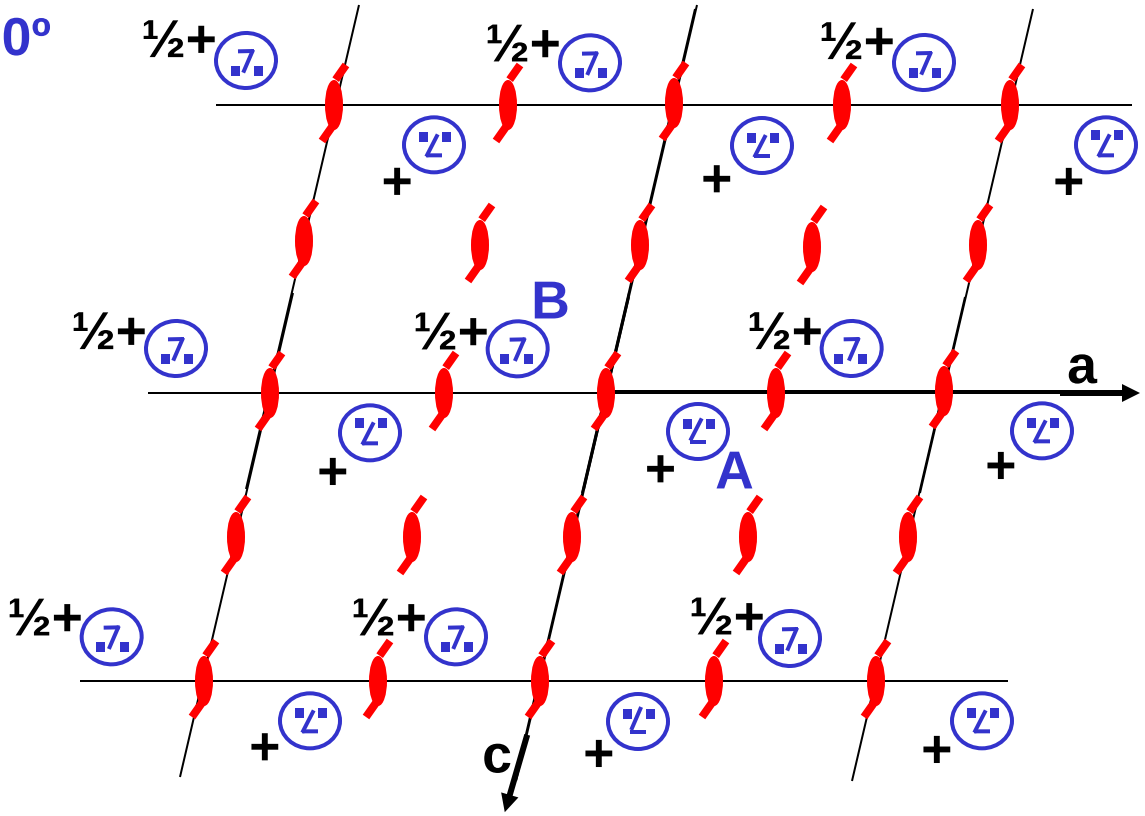
The space group $P2_1$ (monoclinic): 4 cells

$a \neq b \neq c$, $\alpha = \gamma = 90^o$, $\beta \neq 90^o$

Atoms at: *x*, *y*, *z* [A]

–x, ½+*y*, *–z* [B]

The *asymmetric unit* corresponds to one half of a unit-cell



No special positions!

The space group *C*2 (monoclinic): 4 cells

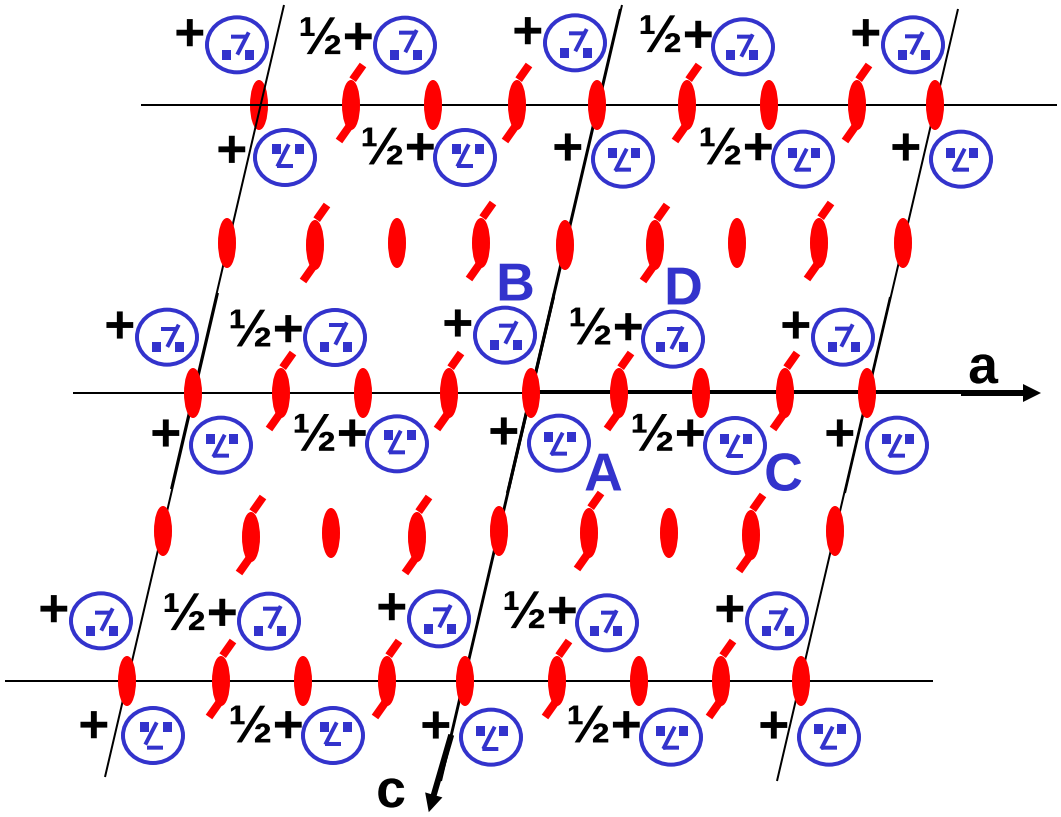
$a \neq b \neq c$, $\alpha = \gamma = 90°$, $\beta \neq 90°$

Atoms at: *x*, *y*, *z* [A]

–*x*, *y*, –*z* [B]

½+*x*, ½+*y*, *z* [C]

½–*x*, ½+*y*, –*z* [D]



The *asymmetric unit* corresponds to one quarter of a unit-cell

Special positions on twofold axes at:

0, *y*, 0; ½, *y*, 0; 0, *y*, ½; ½, *y*, ½

The space group $P2_1/c$ (monoclinic): 4 cells

$a \neq b \neq c$, $\alpha = \gamma = 90°$, $\beta \neq 90°$

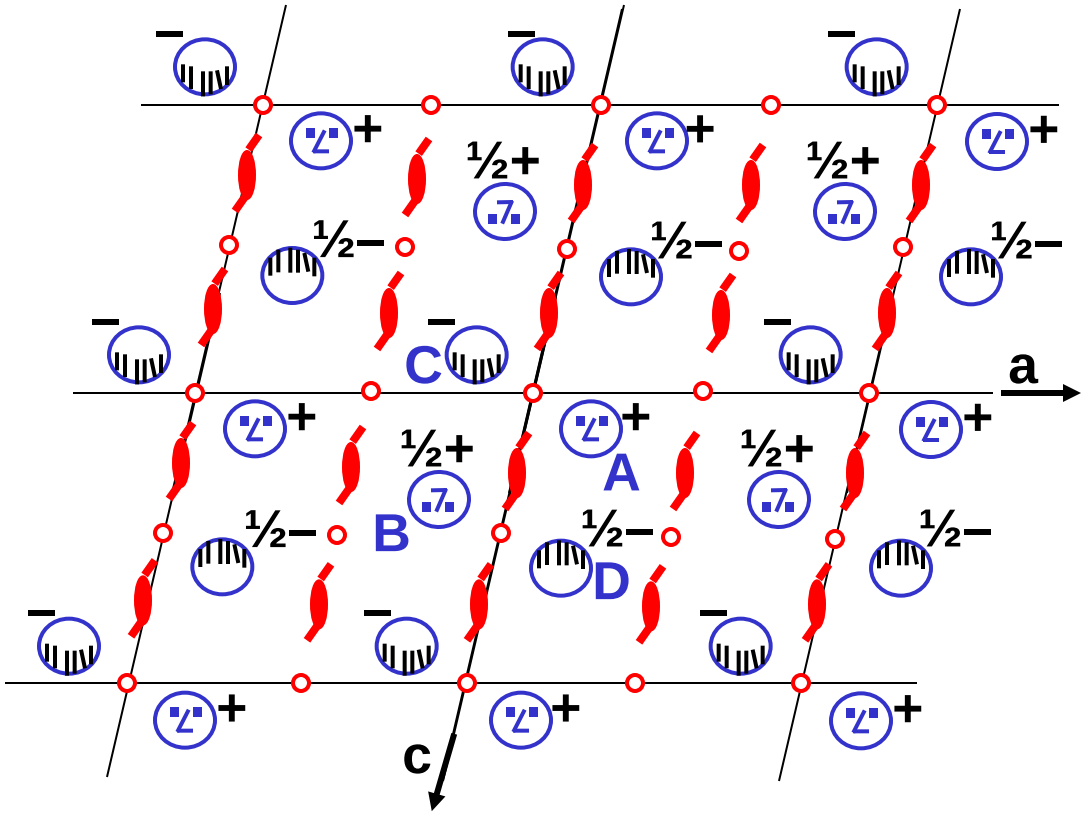
Atoms at: x, y, z [A]

$-x, \frac{1}{2}+y, \frac{1}{2}-z$ [B]

$-x, -y, -z$ [C]

$x, \frac{1}{2}-y, \frac{1}{2}+z$ [D]

The *asymmetric unit* corresponds to one quarter of a unit-cell



A→D: c glide-plane perpendicular to b (at y=¼)

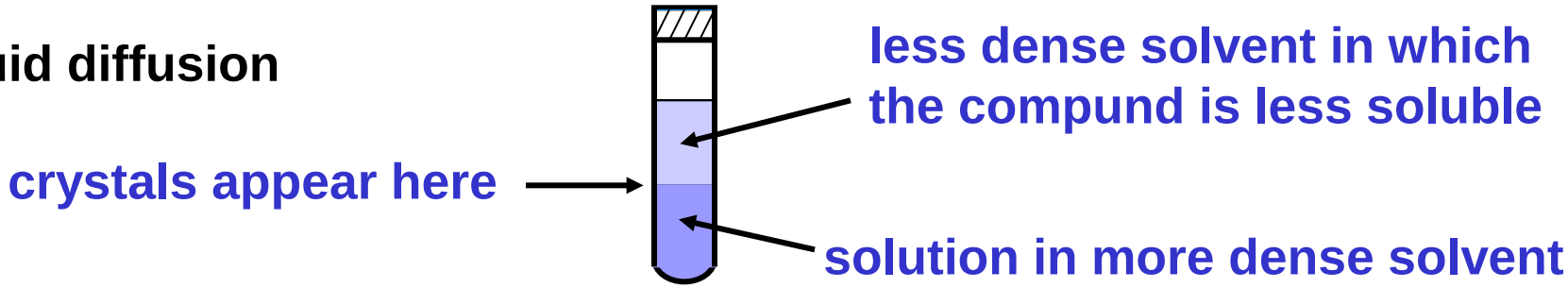
Special positions on inversion centers at:

0, 0, 0; ½, 0, 0; 0, ½, 0; 0, 0, ½; 0, ½, ½; ½, 0, ½; ½, ½, 0; ½, ½, ½

Growing small molecule crystals

Good crystals grow slowly – about two weeks appears to be optimal. The most common beginner's blunder is to evaporate off all the mother liquor!

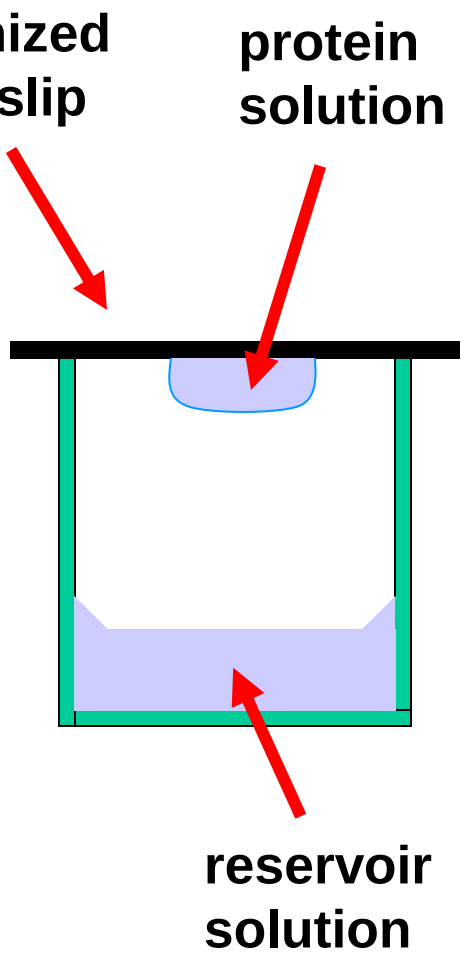
Liquid diffusion



One can also make an almost saturated solution and allow the solvent to evaporate very slowly or (better) cool it slowly. Examples are not quite air-tight NMR-tubes and THF solutions in a –80 ºC refridgerator.

Very small crystals can sometimes be used successfully as seed crystals to grow bigger crystals!

Growing protein crystals: the *hanging drop* method



Protein crystals may be grown from aqueous solutions (usually 10 – 20 mg/ml) by varying the pH, the temperature and the concentrations of PEG and other additives. First a course *screening* with many trials (possibly with the help of a robot) is used to find potential crystallization conditions, which are then improved by fine tuning. Seeding may be required to obtain crystals that are large enough. Extremely pure protein and adequate amounts (5 – 20 mg) are essential.

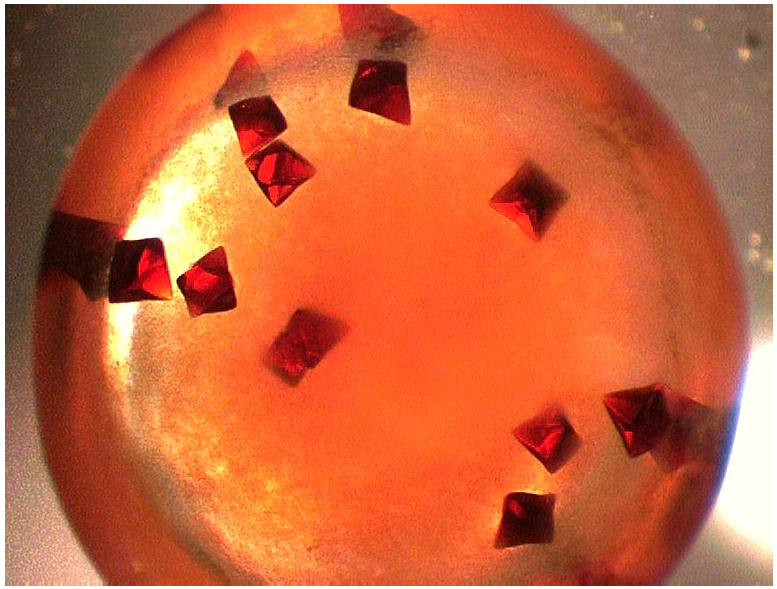
If all crystallization attempts fail, expressed proteins may be manipulated; for example the N and C terminii may be truncated.

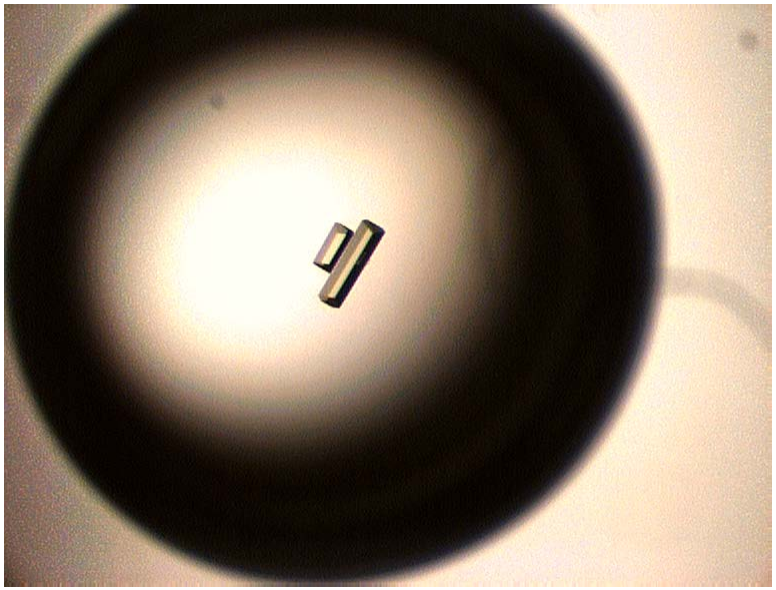
Growing lysozyme crystals

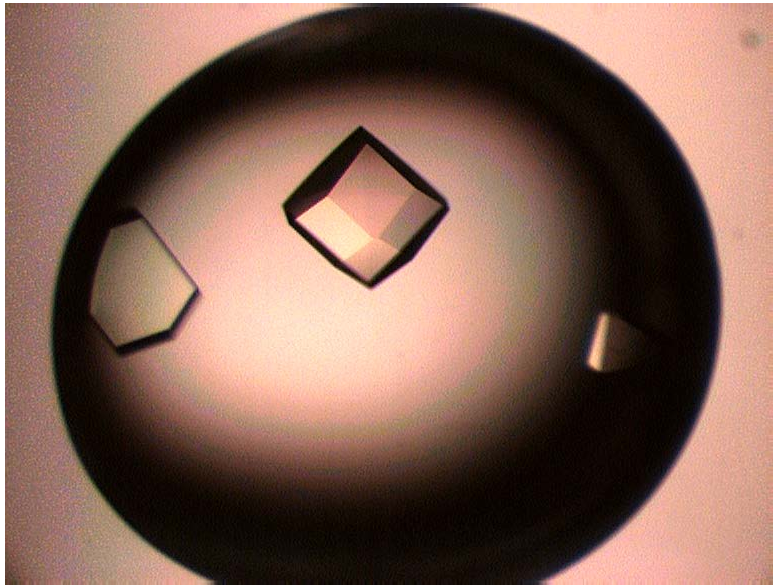
Kirsten Böttcher and Thomas Pape

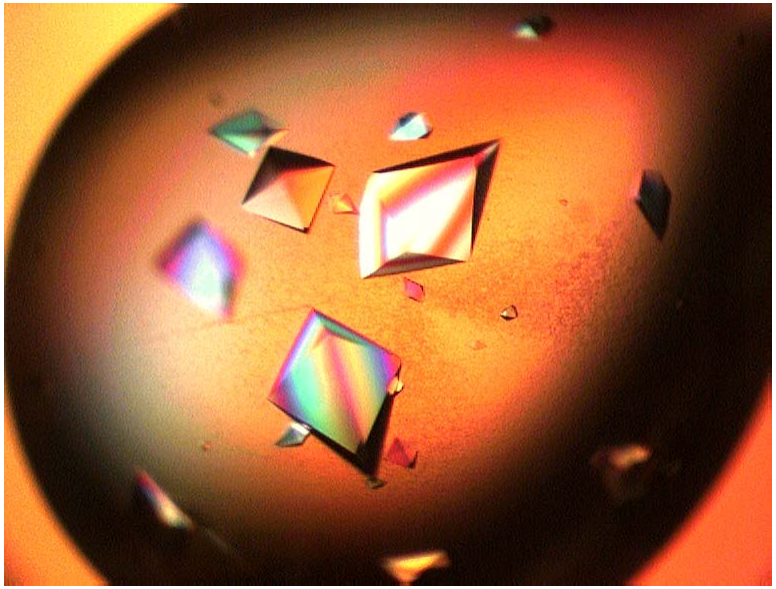
Protein crystals

Kirsten Böttcher

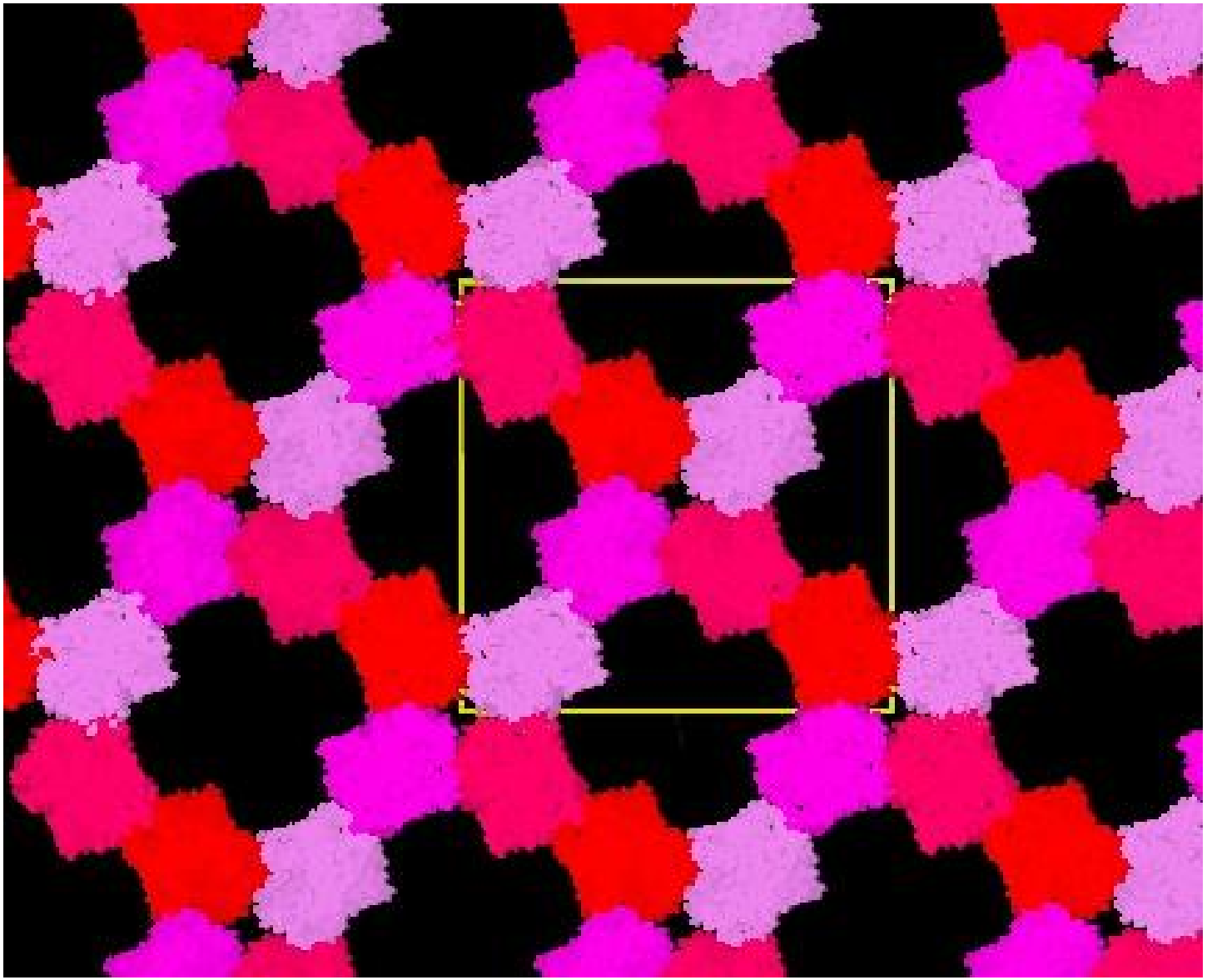








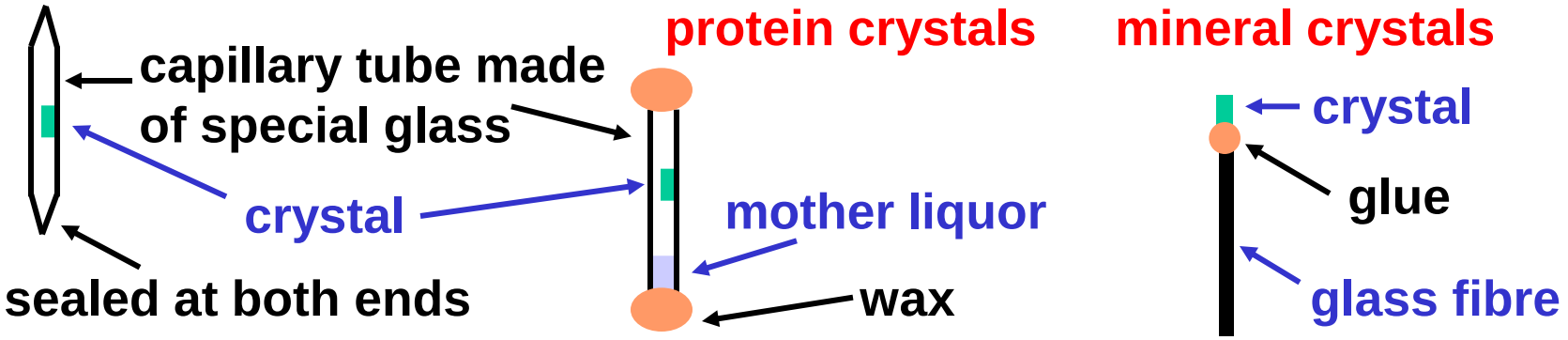
Why do proteins crystallize?



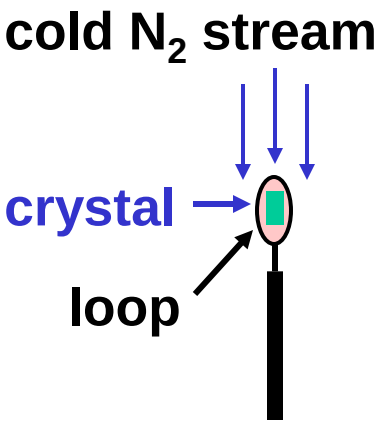
Ralph Krätzner

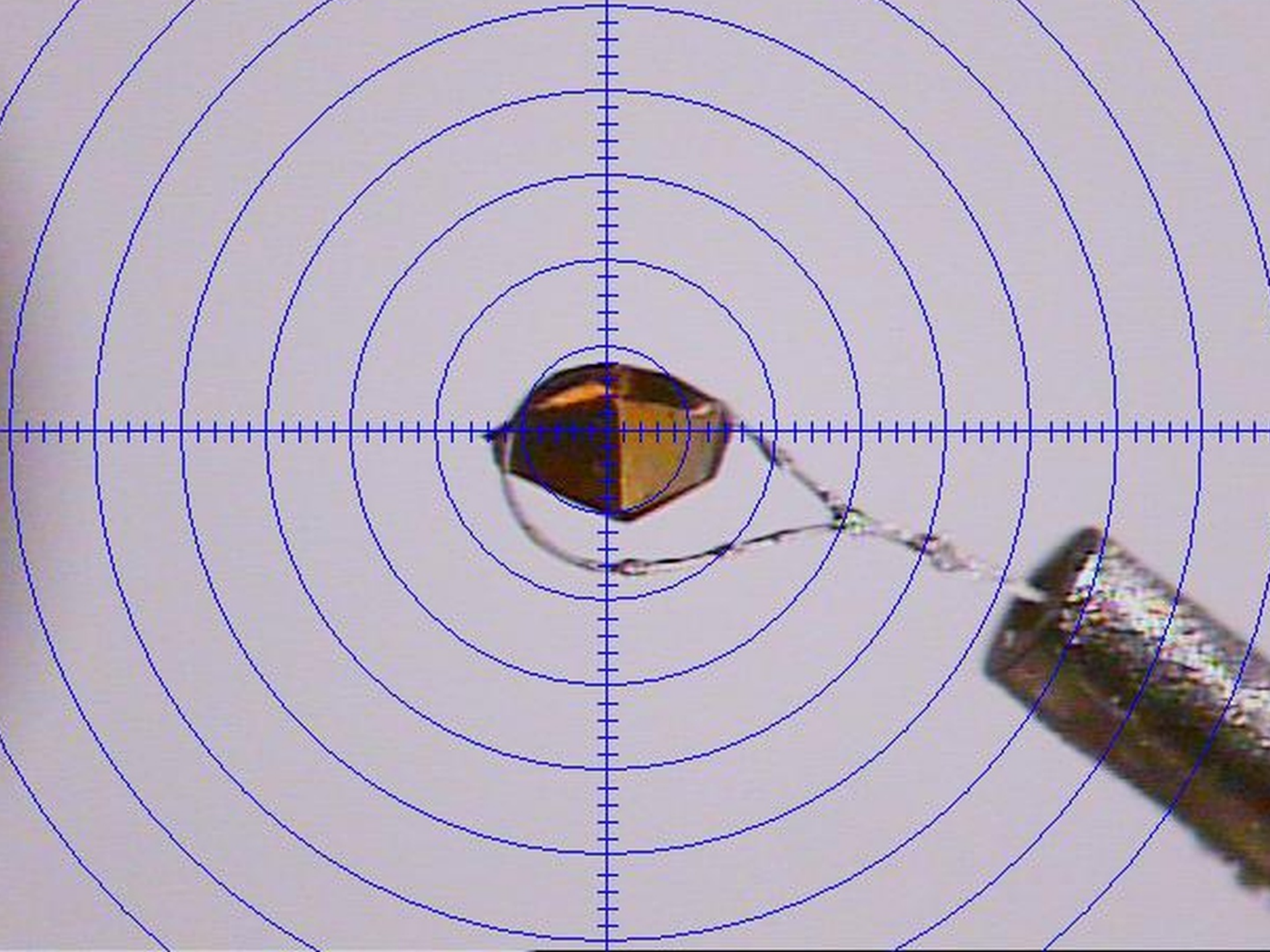
The diffraction experiment

The crystal must be mounted in such a way that nothing gets in the way of the incident and diffracted X-ray beams:

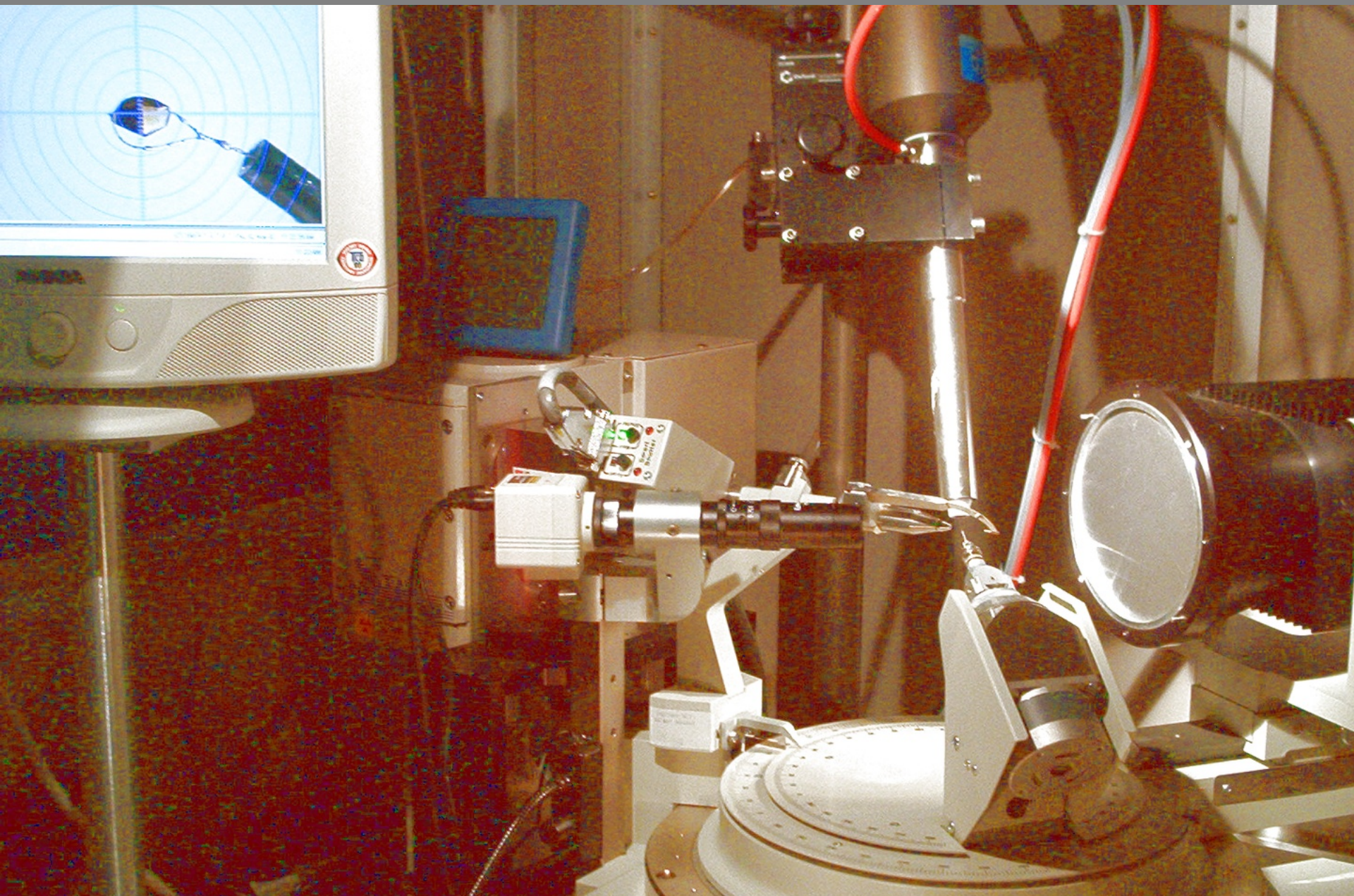


Capillary tubes protect the crystal from the air, but are not recommended because (a) they absorb X-rays, (b) the crystal is not held very securely and (c) they can cause turbulence and ice formation in the cold gas stream. It is better to mount the crystal in a drop of oil that freezes to a glass on cooling. Protein crystals are held in a *cryoprotectant* film (e.g. a 30% glycerol solution) in a small loop which is cooled to 100 K. The glass-like frozen oil or cryoprotectant holds the crystal firmly and protects it from air and moisture, and works well for very reactive or sensitive crystals.



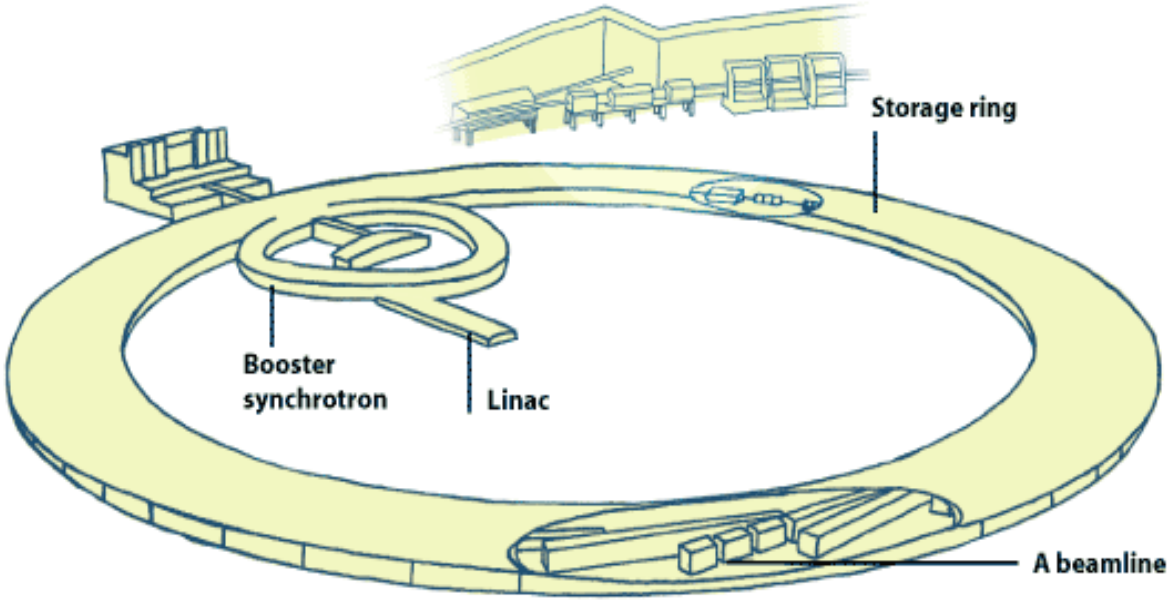


SMART 6000 CCD in Göttingen



X-ray data collection at synchrotrons

X-ray radiation from a synchrotron is much brighter than from a laboratory X-ray source and has the advantage that the wavelength can be varied. These days most protein structures and some small molecule structures are determined using synchrotron radiation.



ESRF Grenoble

The number of molecules in the cell

The density of a crystal is given by:

$$\rho = 10^{24} ZM / N_a V$$

where ρ = density in Mg m^{-3}, Z = number of molecules in one unit-cell, M = molecular weight in Da, N_a = Avogadro's number = 6.0226×10^{23} and V = volume of the unit-cell in Å^3.

It is not always easy to measure the density of very sensitive or reactive crystals. An alternative method is to assume that *the volume of a non-hydrogen atom is about 18 Å^3* (somewhat more when heavier atoms are present and somewhat less for compounds with fused aromatic rings). In this calculation the hydrogen atoms are ignored. It is usually possible to estimate Z (to the nearest whole number) quite reliably by this method.

For proteins one can estimate *Z* similarly by assuming a volume of 140 Å^3 per amino-acid. However it is necessary to allow for a solvent content of between about 25 and 75%!

Exercises

1. **The space group $P2_1/c$ is the most common for inorganic structures, but it has not yet been observed for protein crystals. Is there a reason for this?**

2. **Biphenyl ($C_6H_5–C_6H_5$) crystallizes in the space group $P2_1/c$ with $a = 7.82$, $b = 5.58$, $c = 9.44$ Å, $\alpha = 90$, $\beta = 94.62$, $\gamma = 90^o$. The density is 1.25 Mg m^{-3}. Calculate the volume of the cell and so the number of biphenyl molecules in it. Does it fit the *18 Å³ rule*? How many molecules constitute one *asymmetric unit*? How can this be reconciled with the number of asymmetric units per cell? Is the molecule planar in the crystal? Why are the molecular conformations different in the crystal and in the gas phase?**

Volume of the unit-cell

$$= abc\,[\,1 - \cos^2\alpha - \cos^2\beta - \cos^2\gamma + 2\cos\alpha\cdot\cos\beta\cdot\cos\gamma\,]^{1/2}$$

$$= abc\cdot\sin(\beta) \quad \text{when} \quad \alpha = \gamma = 90^o \text{ (monoclinic)}$$