# Crystallographic methods: Historical perspectives

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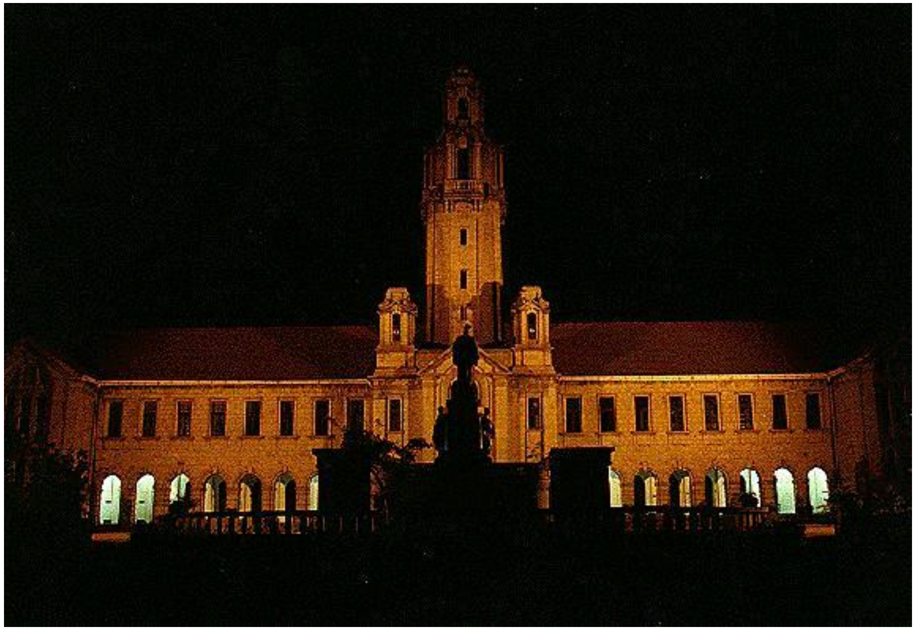
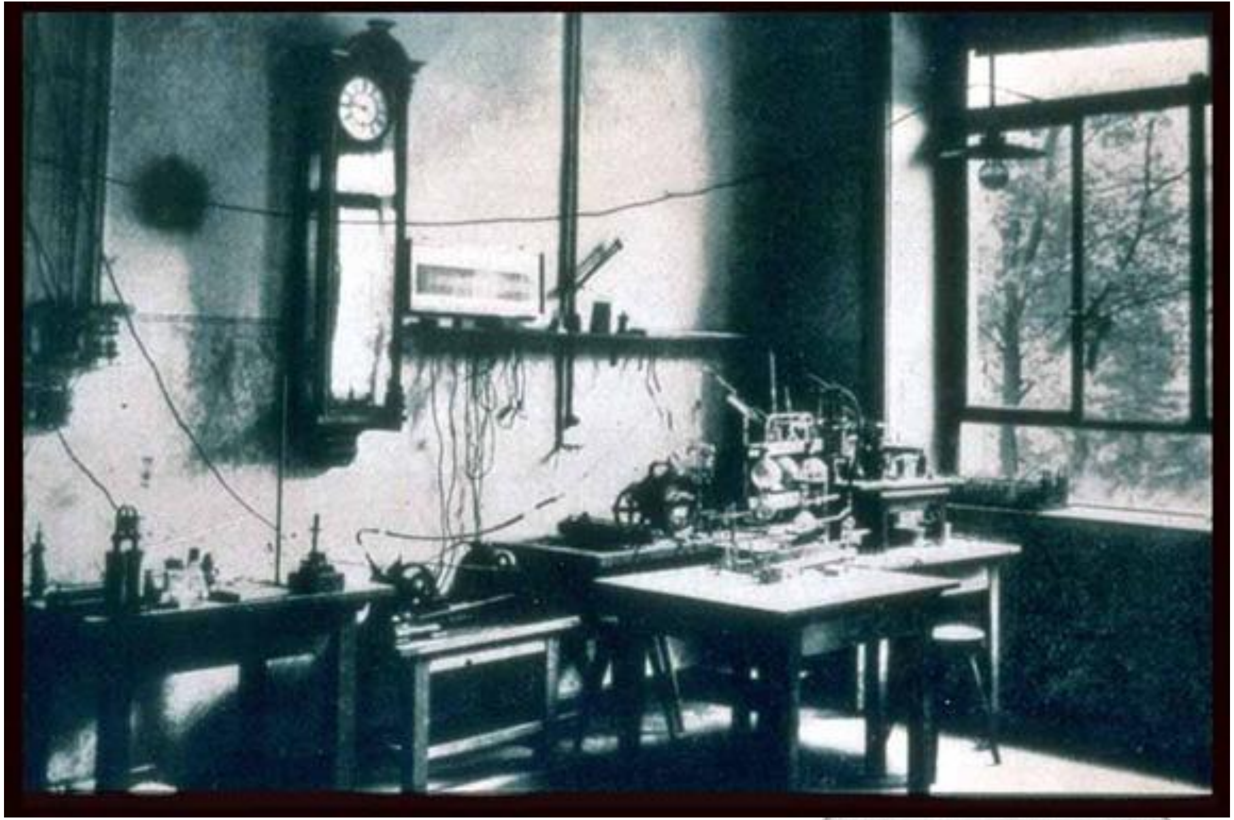
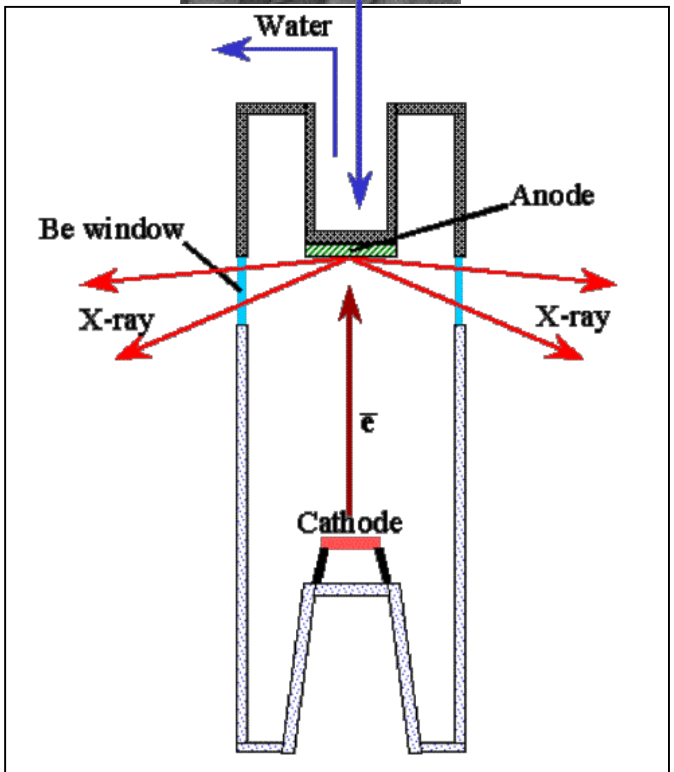


15 August 2017

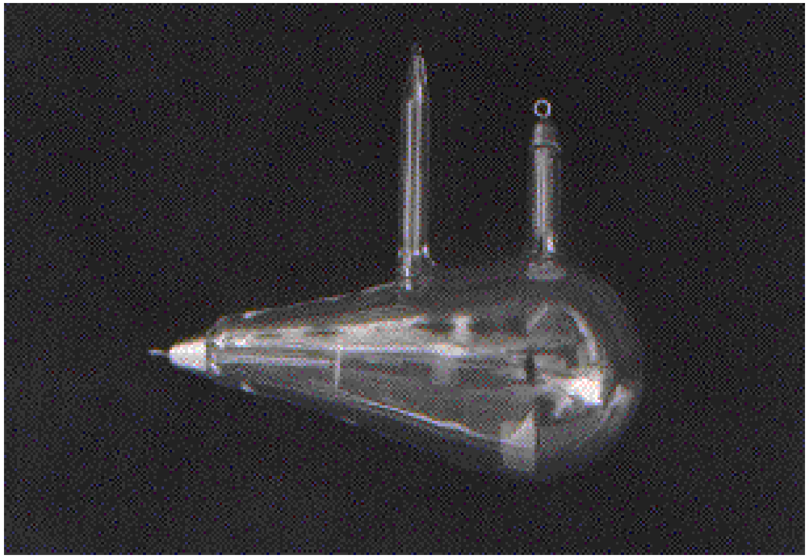
Happy independence day!

# History began in 1895 with Wilhelm Conrad Röntgen






Crooke's tube



*First X ray made in public. Hand of the famed anatomist, Albert von Kölliker, made during Roentgen's initial lecture before the Würzburg Physical Medical Society on January 23, 1896.*

# Wave or particle?

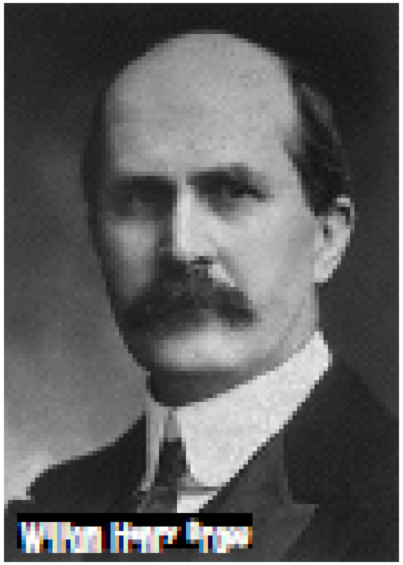
*Physic Nobel price, 1906*

**X-ray : waves or particules**

Theoretical evidence of radiation emission related to the slowing down of charged particles (J.J. Thomson , 1896)

→ « Theoretical discovery of X-rays »

*Physic Nobel price, 1915*



P. Lenard, B. Walter, J.J.Thomson

*waves or particles ?*

W.H. Bragg

# Particles or waves?

## W.L Bragg and W.H. Bragg

**When a wave hits an object, the region immediately behind that object is protected from the wave (shadow). Shadows are sharper close to an object than they are further from it. The wave that just missed the object spreads in a circle or sphere, into the space behind the object. This is why shadows become more blurred further away from the object that casts them.**

## Wilhelm Wien

**wavelength of X-rays should be around one hundredth of a nanometre**

# Lovely quartz crystals in Nature's kitchen

# Crystals: symbols of beauty as well as vanity



**In ancient India, crystallography ws called**

**“Ratnashastra”**

# Origin of gems

भूस्वभावाद्धि रत्नानि जातानि विविधानि तु ।
उपला रत्नरुपत्वं प्राप्ताः कालान्तरेण वा ॥

**Due to earth's nature, various forms of gems are formed over long incubation periods**

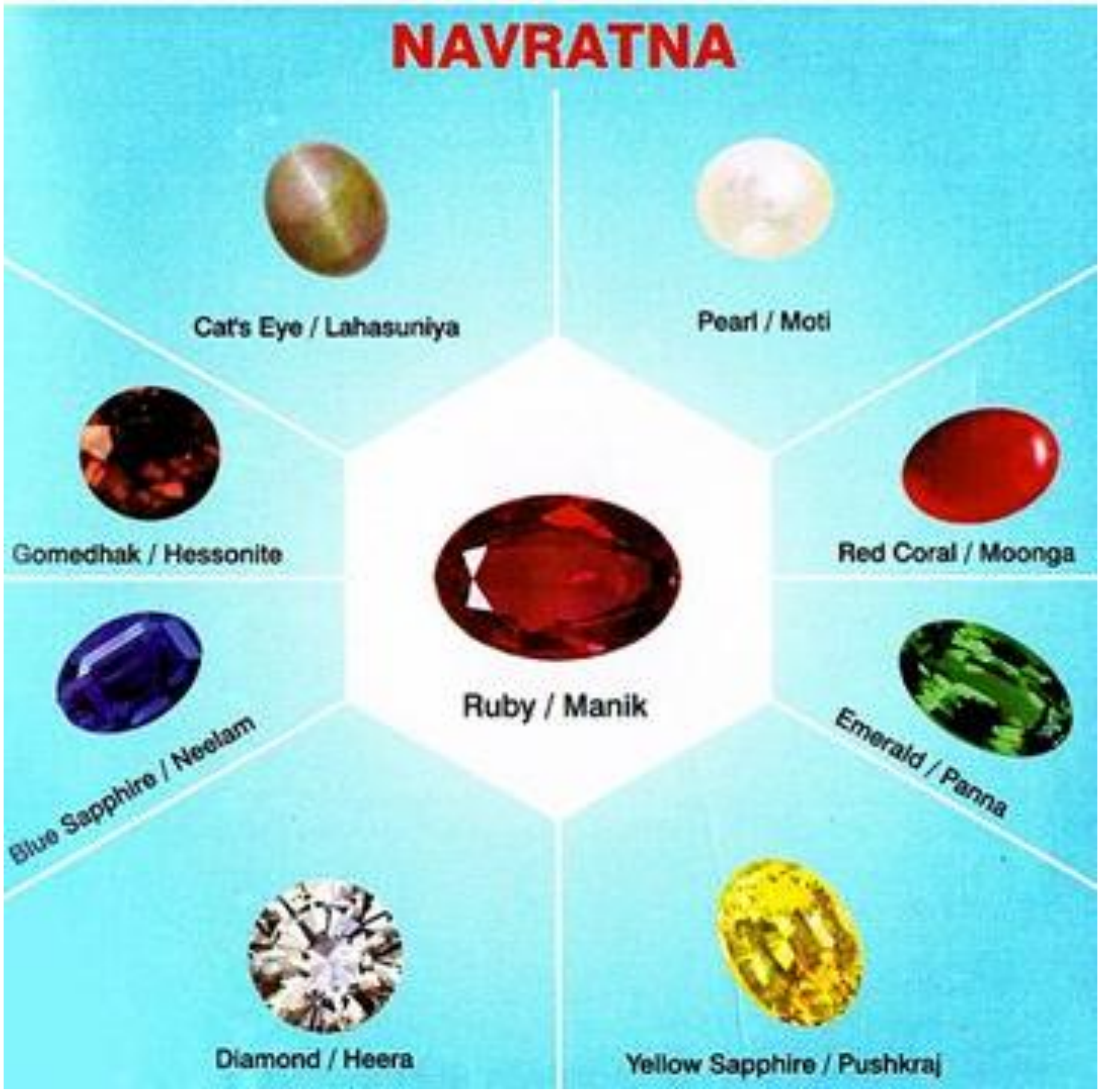
पुरा पृथिव्या रत्नानि गर्भे त्वासन्हि सर्वशः ।
रत्नगर्भा इति सा भूमिः ख्याताभूद् भुवनत्रये ॥

**Because of the presence of gems in its bosom Earth has earned the name "RATNAGARBHA"**

# Classification of gems

| Maharatna (precious stones) | Uparatna (semi precious stones) |
|---|---|
| Vajra (diamond) | Marakata (Emarald) |
| Mukta (pearl) | Vidruma (Coral) |
| Manikya (Ruby) | Gomeda (Jacinth) |
| Indranila (Sapphire) | Pushparaga (Topaz) |
| | Vaidurya (Lapis Lazuli) |

# One possessed of all desirable qualities



# VAJRA (Diamond)

It was well recognized that diamond is the hardest substance

षट्कोणत्वं लघुत्वं च समाष्टदलता तथा
तीक्ष्णाग्रहा निर्मलत्वं इति पञ्च गुणाः स्मृताः ॥

Diamond has six vertices and eight facets

Octahedral symmetry

Five properties of diamond

Vajrakaya

VARAHAMIHIRA studied crystal defects

# Diamond is the hardest gems

पृथिव्यां यानि रत्नानि ये चान्ये लोहधातवः ।
सर्व तान्विलिखेद्वज्रं वज्रं तैर्न विलिख्यते ॥

Halasya Mahatyam

**Of all the gems on earth, and all materials made of metals, the hardest is the diamond**

**Diamond cannot be scratched by any other object**

# Experimental examination of gems

**Diamond**
Pounding

**Emarald**
Green ray scattering when crystal faces sun

**Sapphire**
Blue when placed in milk

# Imagination unlimited

| | |
|---|---|
| **Sun** | **Ruby** |
| **Venus** | **Diamond** |
| **Mars** | **Coral** |
| **Jupiter** | **Yellow Sapphire** |
| **Saturn** | **Blue Sapphire** |
| **Mercury** | **Emerald** |
| **Moon** | **Pearl** |
| **Rahu** | **Hessonite Garnet** |
| **Ketu** | **Cat’s Eye Chrysoberyl** |

# Crystals : periodic arrangement of molecules

# Magic numbers of crystallography

**7 crystal systems**

**14 Bravias lattices**

**11 Laue groups**

**32 Point groups**

**17 Plane groups**

**230 Space groups**

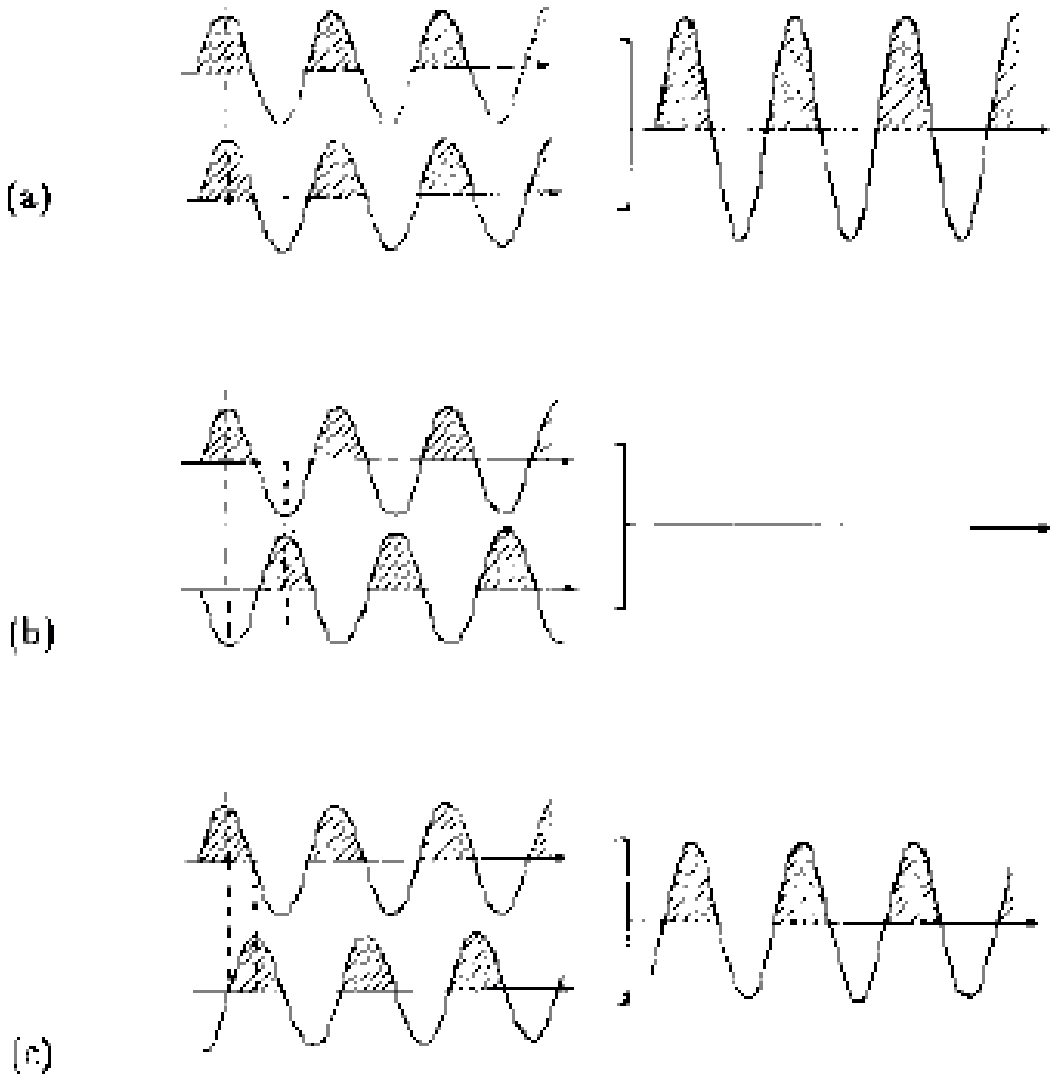
**65 Non-centric space groups**

**The mathematical principles that underlie these numbers were understood before the birth of X-ray crystallography**

# Laue, great synthesis of ideas

*Physic Nobel price, 1914*

# What matters in adding two waves is the phase difference

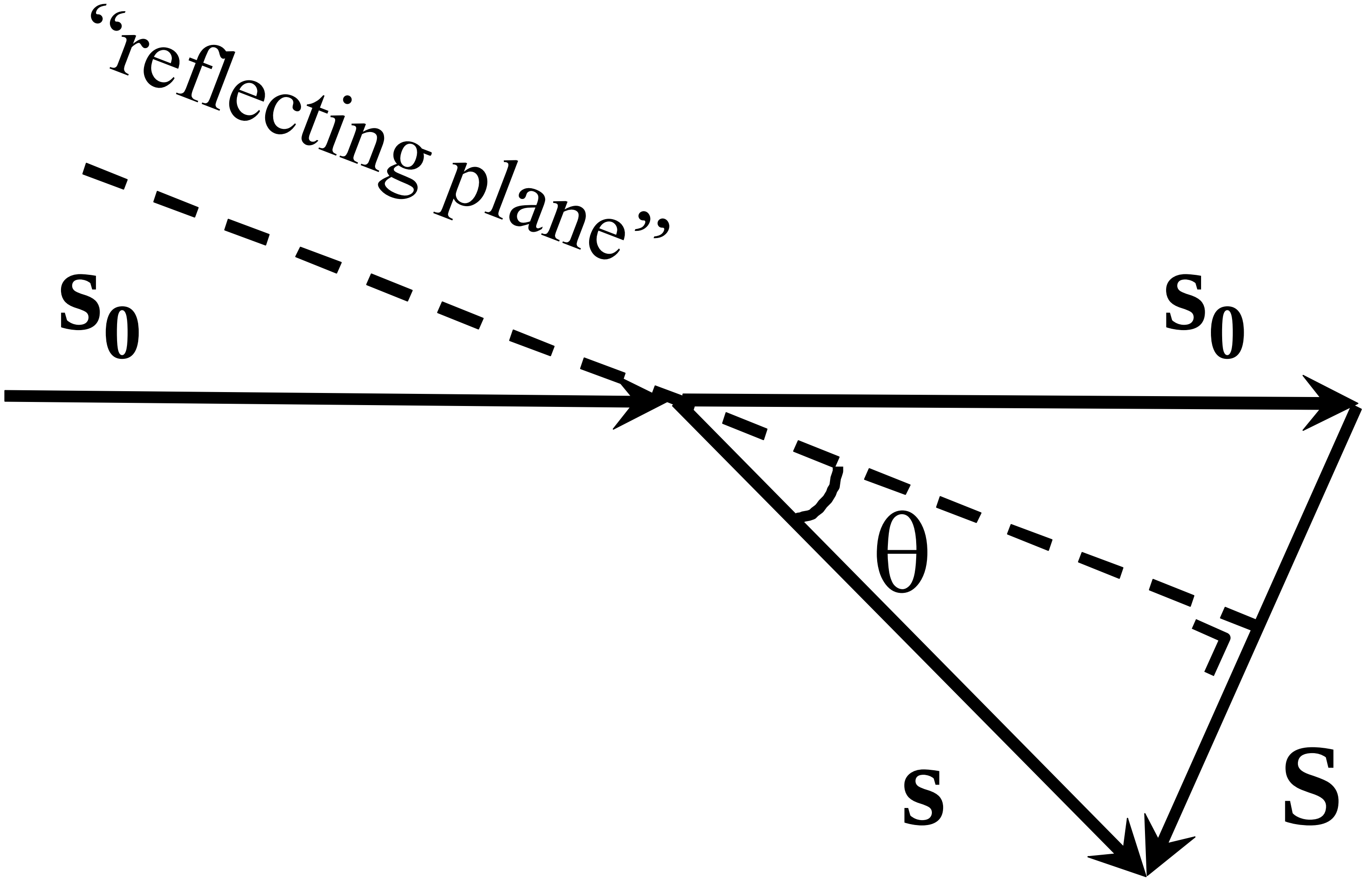



**In phase**
**constructive**

**Out of Phase**
**Destructive**

**Partially out of phase**
**Intermediate amplitude**

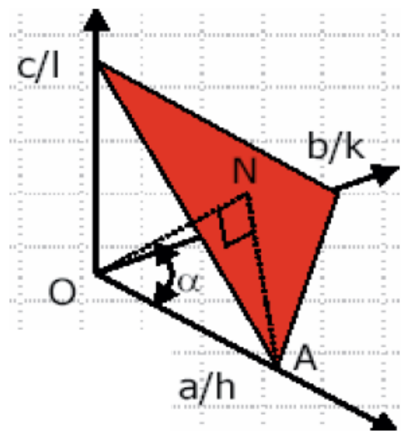
# Scattering vector

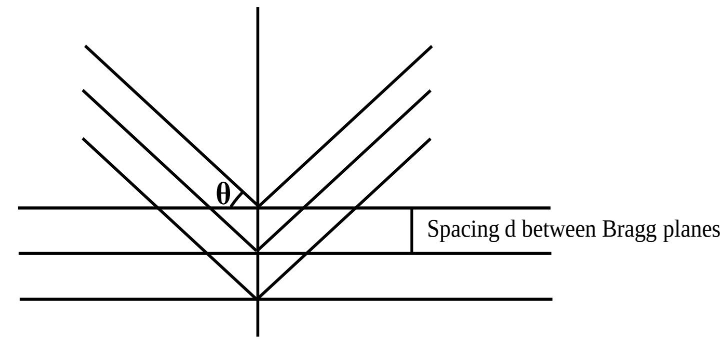


# Three ways of describing interaction of crystals with X-rays

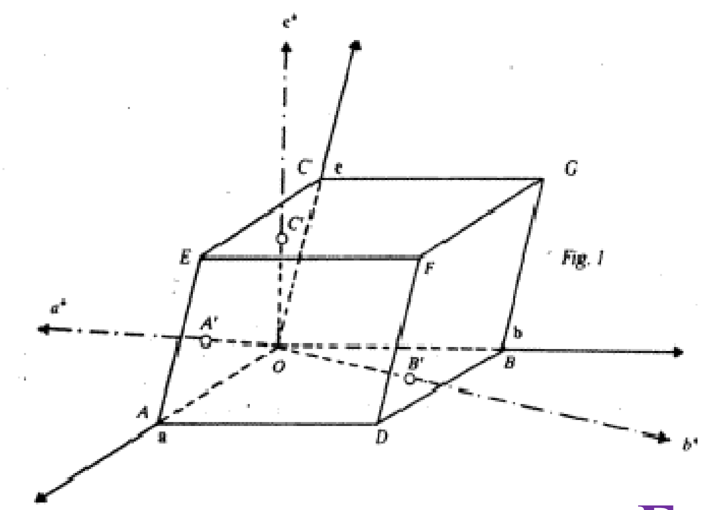
$$\mathbf{a}\cdot\mathbf{S} = h$$
$$\mathbf{b}\cdot\mathbf{S} = k$$
$$\mathbf{c}\cdot\mathbf{S} = l$$

**Laue conditions**

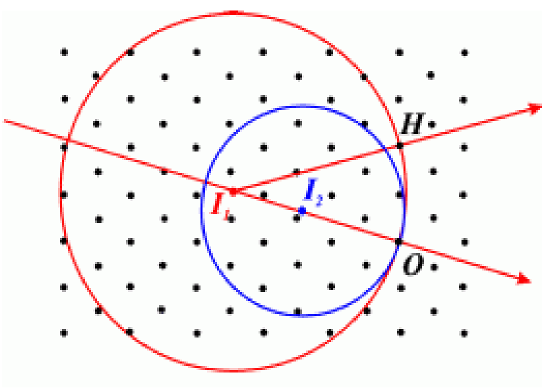



**Bragg's law: 2 d sin θ = λ**



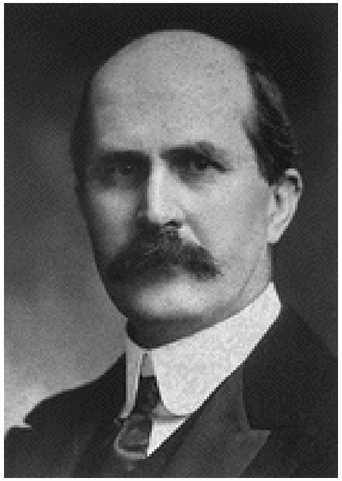

**a* = b x c / ( a. b x c)**
**b* = c x a / ( a. b x c)**
**c* = a x b / ( a. b x c)**

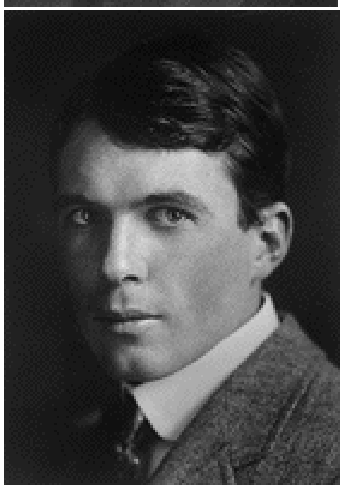


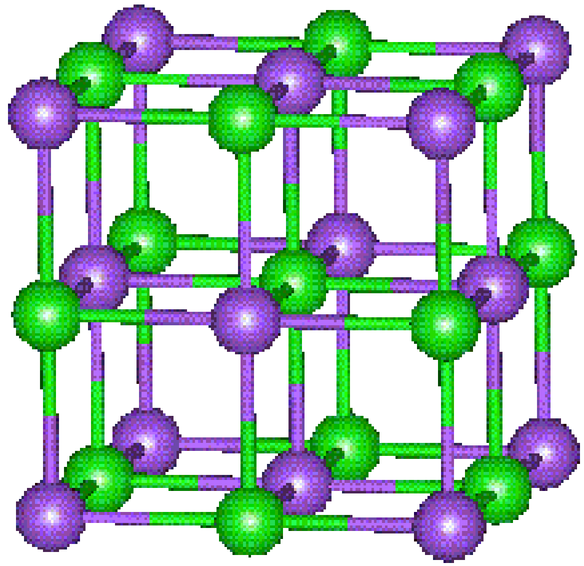

**Ewald's construction**

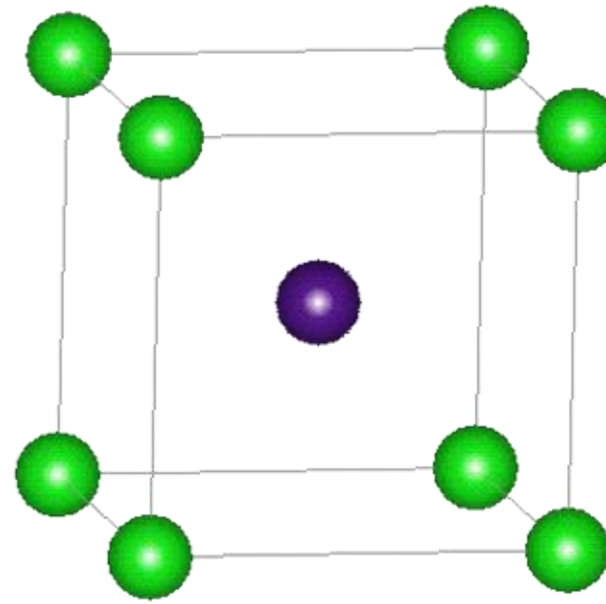
Sir William
Henry Bragg
(1862-1942)

# A humble beginning





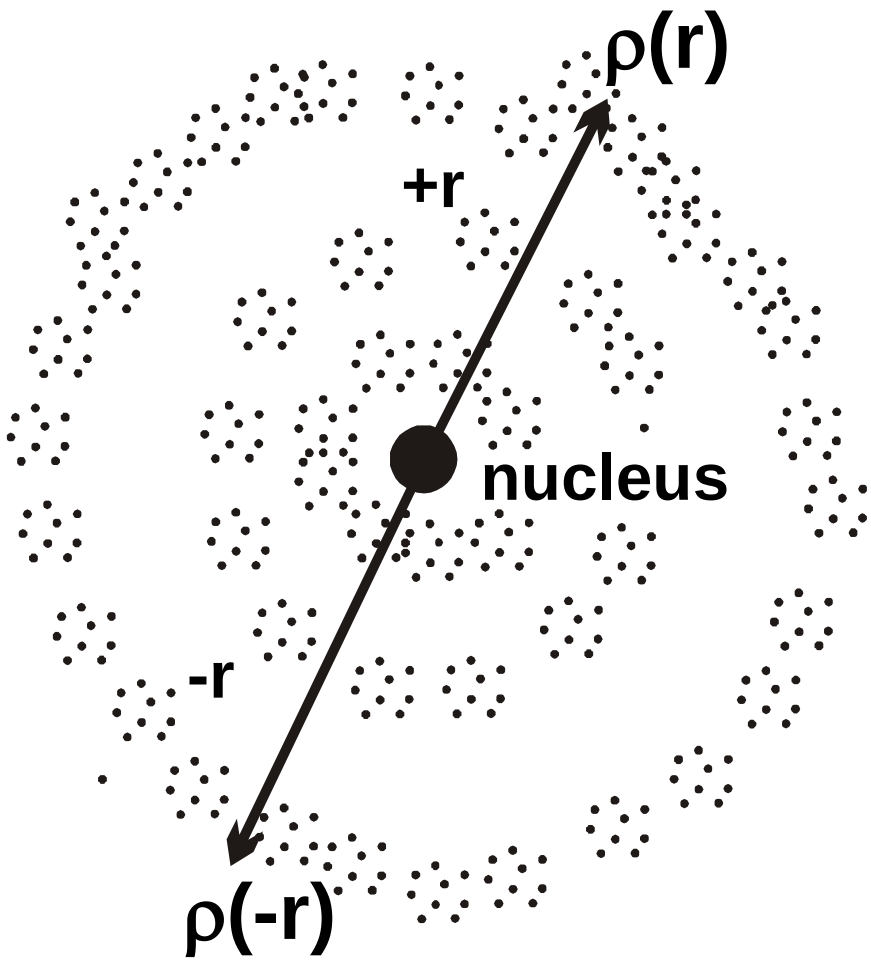




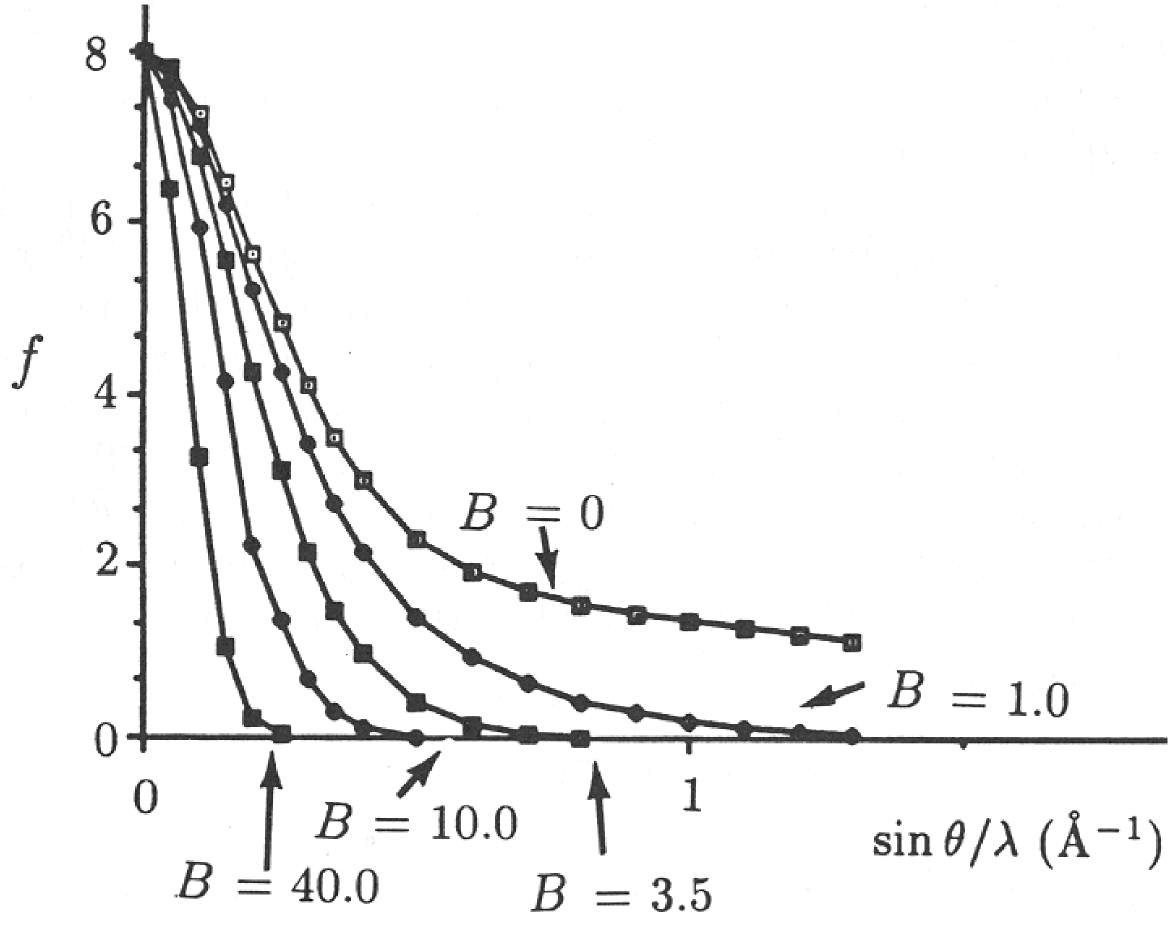
Structure of sodium chloride

Sir William
Lawrence Bragg
(1890-1971)

# Scattering by an Atom



# Atomic scattering factor




$$f = f_o \exp(-B\sin^2\theta/\lambda)$$ :**phase factor**

# Scattering by a Unit Cell

$$\mathbf{f}_j = f_j e^{2\pi i \mathbf{r}_j \cdot \mathbf{S}}$$

$$\mathbf{F}(\mathbf{S}) = \sum_{j=1}^{n} f_j e^{2\pi i \mathbf{r}_j \cdot \mathbf{S}}$$

**This expression assumes X-ray beam scattered by a factitious atom placed at the origin of the unit cell as the reference beam**

# X-ray diffraction yields the unit cell transform sampled at integral reciprocal lattice points

# Fundamental Equations of Crystallography

## Structure Factor

$$F(hkl) = \iiint \rho(xyz)\, e^{2\pi i (hx + ky + lz)}\, dx\, dy\, dz$$

## Electron density

$$\rho(xyz) = \sum\sum\sum F(hkl)\, e^{i\alpha}\, e^{-2\pi i (hx + ky + lz)}$$

# History of first structures

**Dorothy and Bernal took first pictures of protein crystals in the 30's**

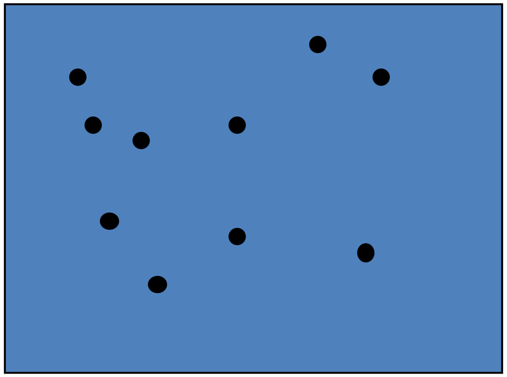
**Max Perutz became a graduate student in the 20's**

**He demonstrated the way of determining phases in Green, Ingram and Perutz, JMB (1954)**

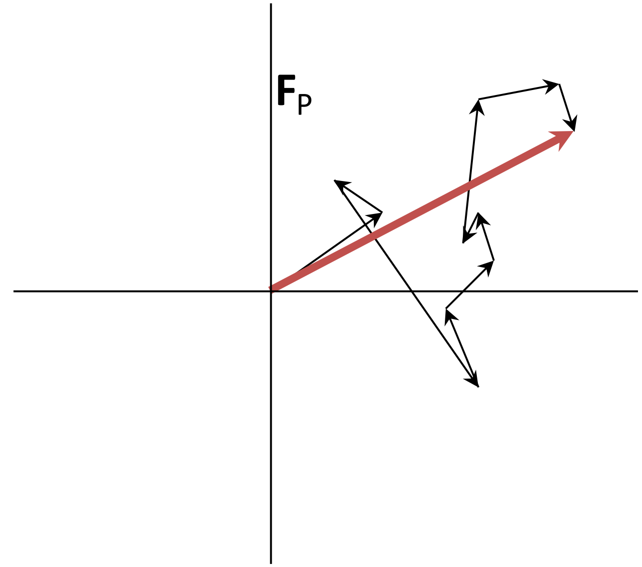
**Structure of myoglobin (1960), haemoglobin(1962)**

**A true revolution**
**Today, we know more than 125,000 protein structures**

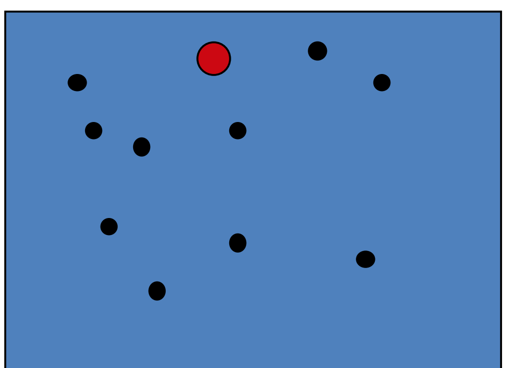
# Crystal structure: Mild perturbation



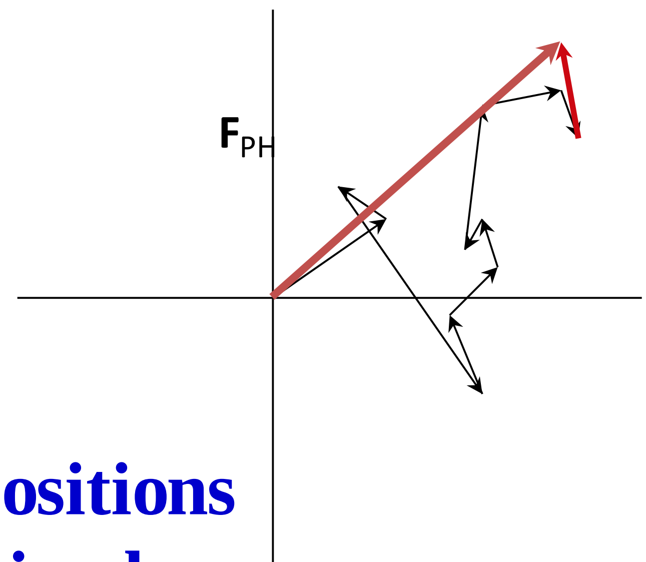
Native




**Adding one (or more) atoms in known positions changes the structure factor in a known way**



Derivative



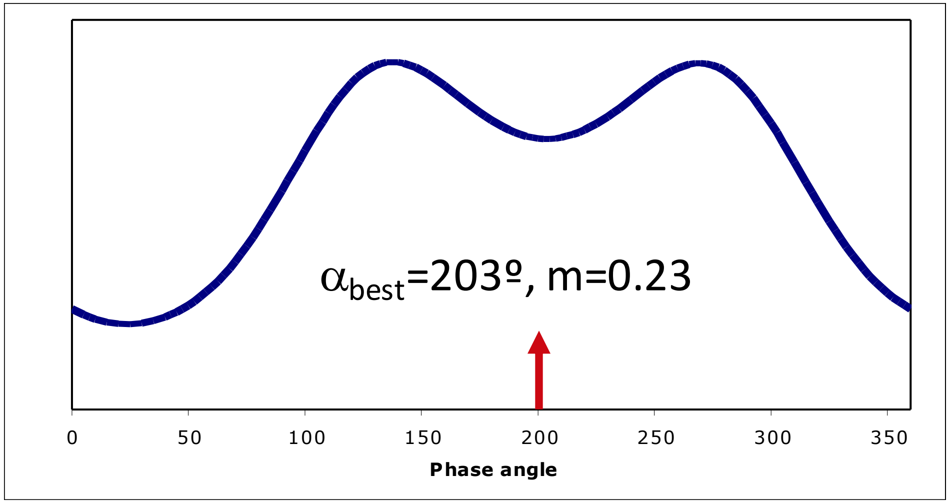

**Heavy atom positions overdetermined**

# Resolving phase ambiguity

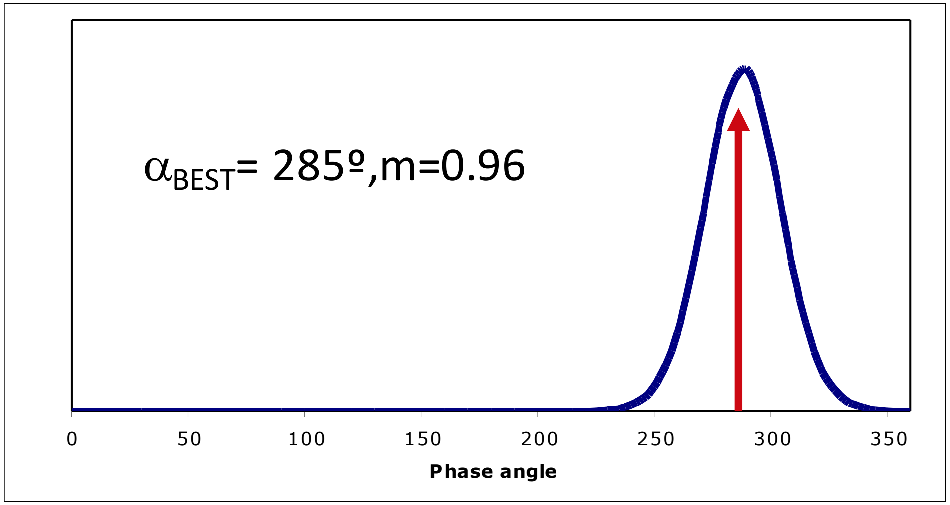
1 1 9 reflection

## Blow and Crick

## One derivative



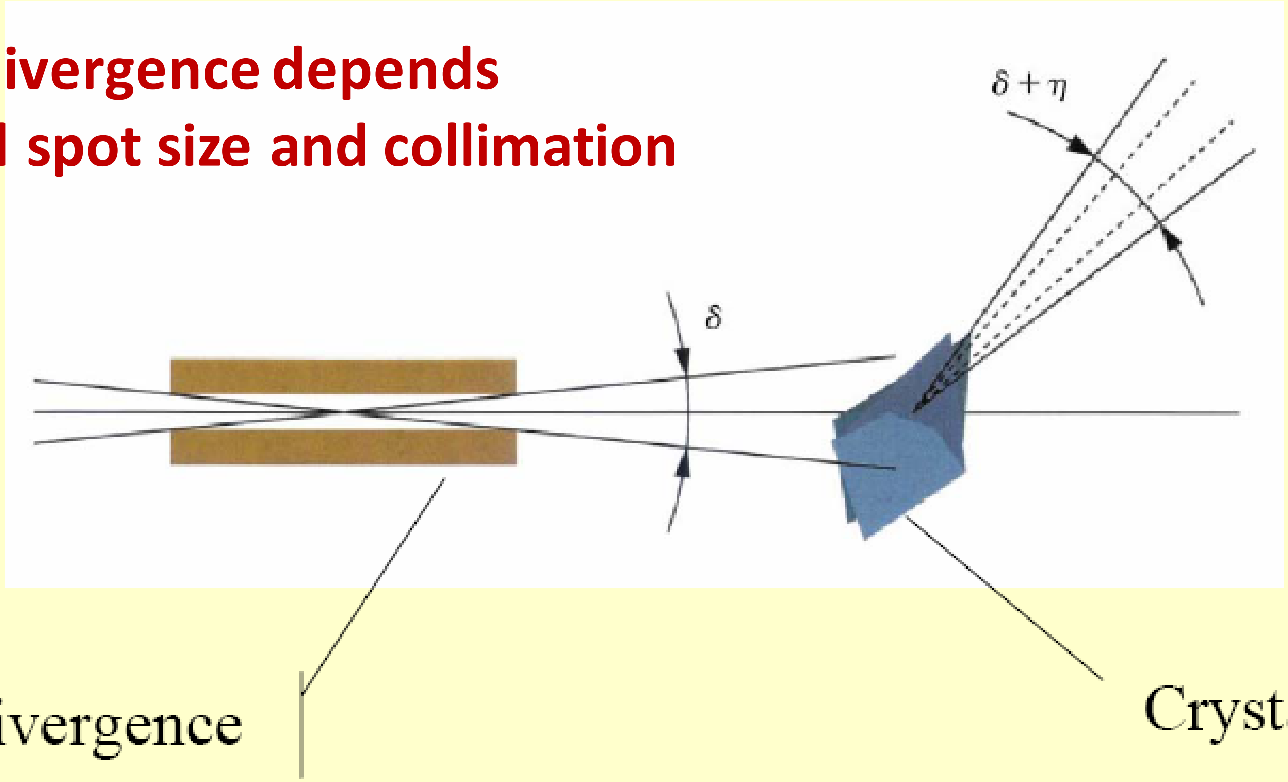

## Three derivatives

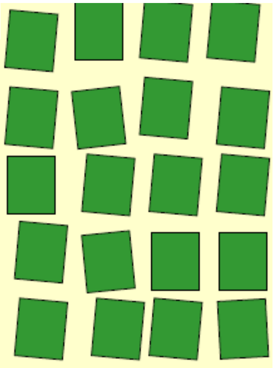



$$P(\alpha_P) \propto \prod_{i=1} \exp\left(-\varepsilon_i^2/2E_i^2\right)$$

# Crystal mosaicity and beam divergence determine the rocking curve

Beam divergence depends
on focal spot size and collimation



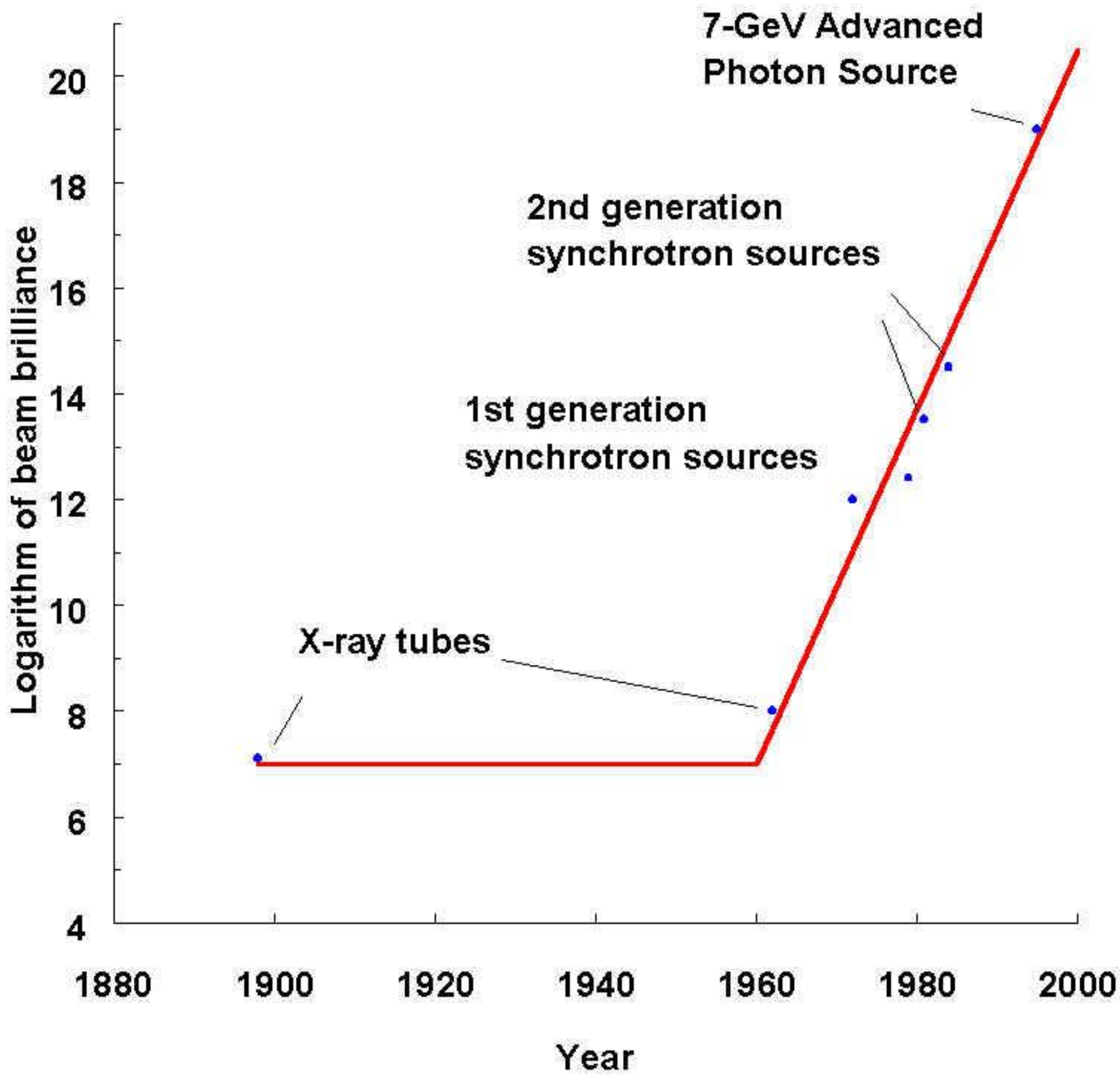



Alas! No control (usually)

# Spot resolution problem: 1970's Home source

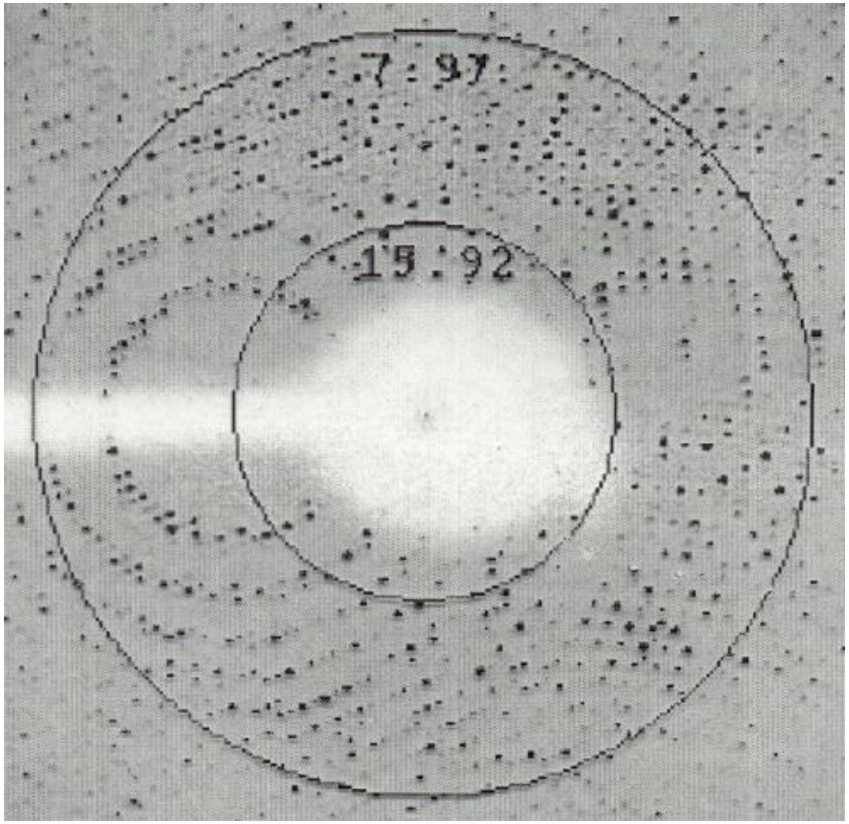
# 1980's synchrotrons

# Unbelivable power

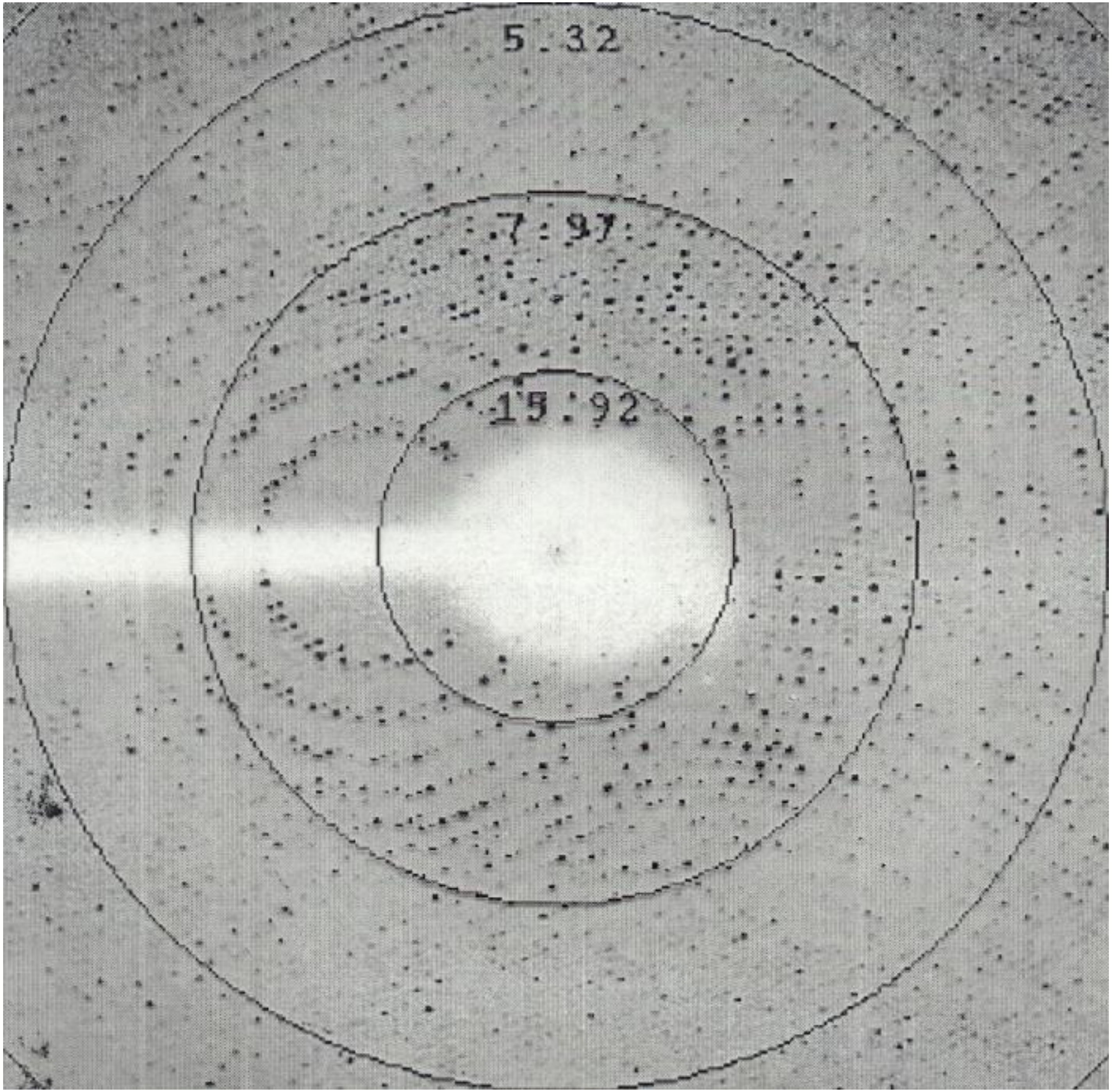


# Recording intensities associated with three-dimensional reciprocal lattice on two-dimensional detectors

## Weisenberg
## Precession
## Screenless oscillation photography



Twinkle twinkle
Little star
How I wonder
What you are




Where do you come from?

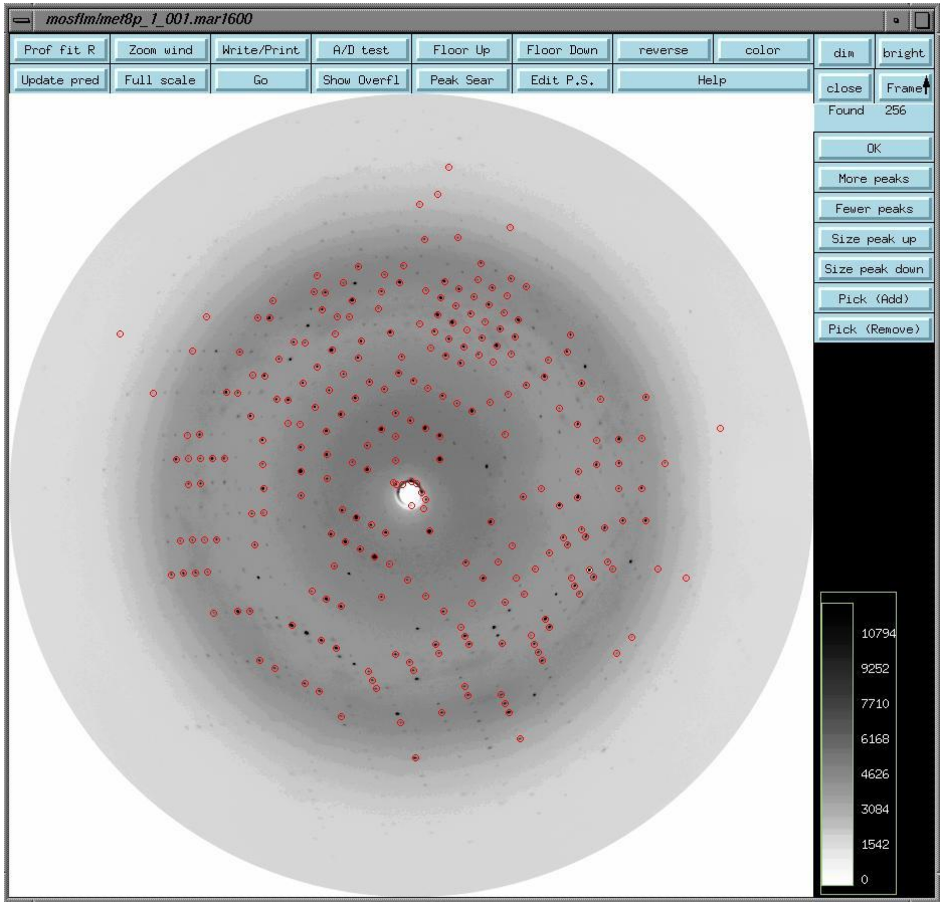
How strong are you?

How much do I trust you?

# Auto-indexing

**Difference vector method**

**Projection of reciprocal lattice points**




No need to pre-orient the crystal

# Indexing

## Refining orientation

## Post-refinement

**Bravais Lattice Table**

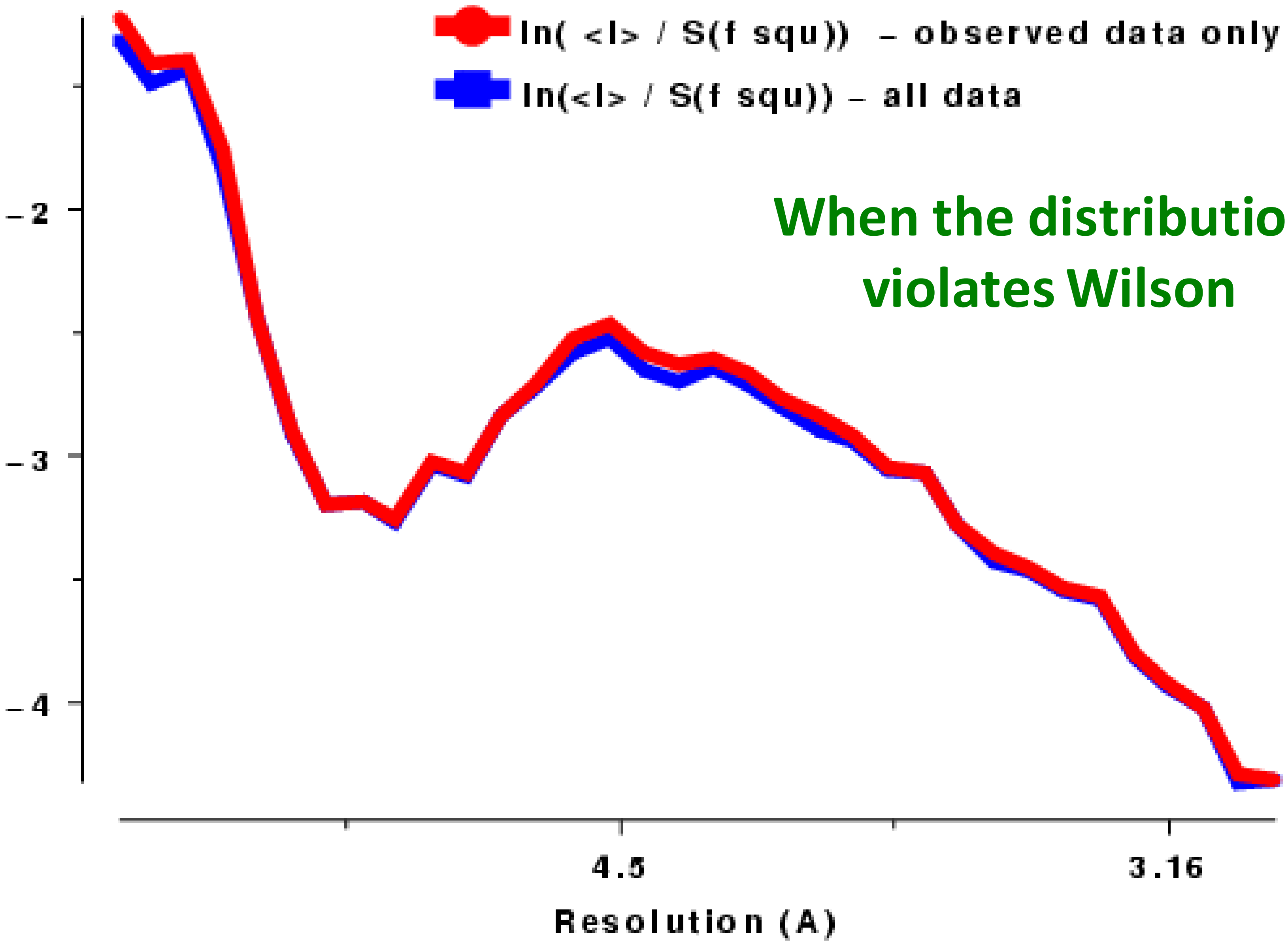
*Autoindexing preformed for unit cell between 11.8 to 315 Angstroms*

| Lattice | Distortion | | | | | | |
|---|---|---|---|---|---|---|---|
| primitive cubic | 22.68% | 45.48 | 90.68 | 90.77 | 60.16 | 90.06 | 90.05 |
| | | 75.64 | 75.64 | 75.64 | 90.00 | 90.00 | 90.00 |
| I centred cubic | 25.95% | 101.67 | 90.77 | 101.41 | 63.61 | 78.46 | 116.65 |
| | | 97.95 | 97.95 | 97.95 | 90.00 | 90.00 | 90.00 |
| F centred cubic | 26.84% | 101.67 | 101.69 | 163.42 | 97.07 | 97.18 | 126.85 |
| | | 122.26 | 122.26 | 122.26 | 90.00 | 90.00 | 90.00 |
| primitive rhombohedral | 7.82% | 90.68 | 90.94 | 101.48 | 116.67 | 116.41 | 120.03 |
| | | 94.37 | 94.37 | 94.37 | 117.70 | 117.70 | 117.70 |
| | | 160.36 | 160.36 | 45.48 | 90.00 | 90.00 | 120.00 |
| primitive hexagonal | 0.08% | 90.68 | 90.94 | 45.48 | 90.02 | 90.05 | 120.03 |
| | | 90.81 | 90.81 | 45.48 | 90.00 | 90.00 | 120.00 |
| primitive tetragonal | 13.52% | 90.68 | 90.77 | 45.48 | 89.94 | 90.05 | 119.84 |
| | | 90.72 | 90.72 | 45.48 | 90.00 | 90.00 | 90.00 |
| I centred tetragonal | 15.44% | 163.42 | 90.94 | 45.48 | 89.98 | 73.90 | 90.06 |
| | | 127.18 | 127.18 | 45.48 | 90.00 | 90.00 | 90.00 |
| primitive orthorhombic | 13.52% | 45.48 | 90.68 | 90.77 | 60.16 | 90.06 | 90.05 |
| | | 45.48 | 90.68 | 90.77 | 90.00 | 90.00 | 90.00 |
| C centred orthorhombic | 0.04% | 90.94 | 157.02 | 45.48 | 89.94 | 89.98 | 89.93 |
| | | 90.94 | 157.02 | 45.48 | 90.00 | 90.00 | 90.00 |
| I centred orthorhombic | 6.79% | 45.48 | 90.68 | 163.87 | 90.13 | 73.93 | 90.05 |
| | | 45.48 | 90.68 | 163.87 | 90.00 | 90.00 | 90.00 |
| F centred orthorhombic | 15.88% | 45.48 | 186.94 | 187.10 | 58.19 | 75.99 | 75.96 |
| | | 45.48 | 186.94 | 187.10 | 90.00 | 90.00 | 90.00 |
| primitive monoclinic | 0.03% | 90.68 | 45.48 | 90.77 | 90.06 | 119.84 | 89.95 |
| | | 90.68 | 45.48 | 90.77 | 90.00 | 119.84 | 90.00 |
| C centred monoclinic | 0.03% | 157.02 | 90.94 | 45.48 | 89.98 | 90.06 | 90.07 |
| | | 157.02 | 90.94 | 45.48 | 90.00 | 90.06 | 90.00 |
| primitive triclinic | 0.00% | 45.48 | 90.68 | 90.77 | 60.16 | 89.94 | 89.95 |

*If you would like to change the crystal lattice: select desired bravais lattice, press Apply button and close window, otherwise just close window.*

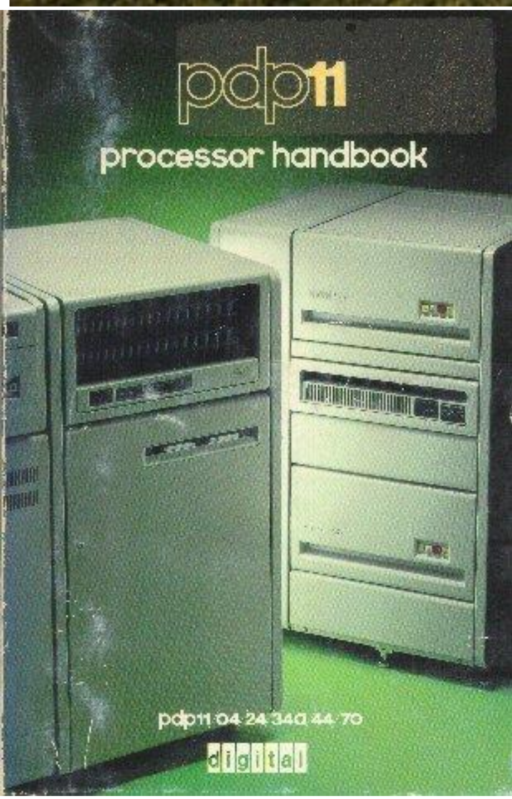
Apply | Apply & Close

# Wilson 1949: Statistics of intensities

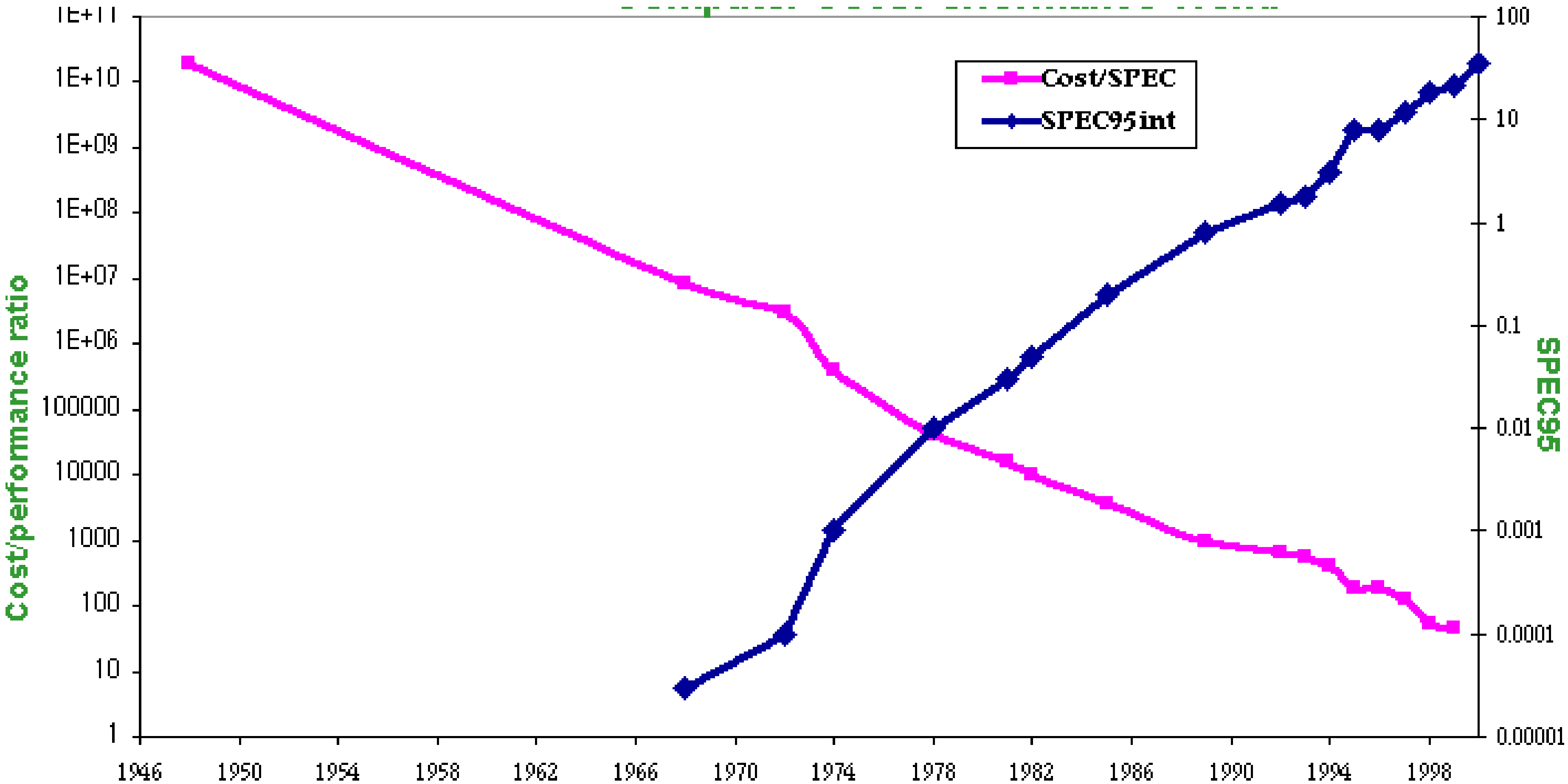



When the distribution violates Wilson

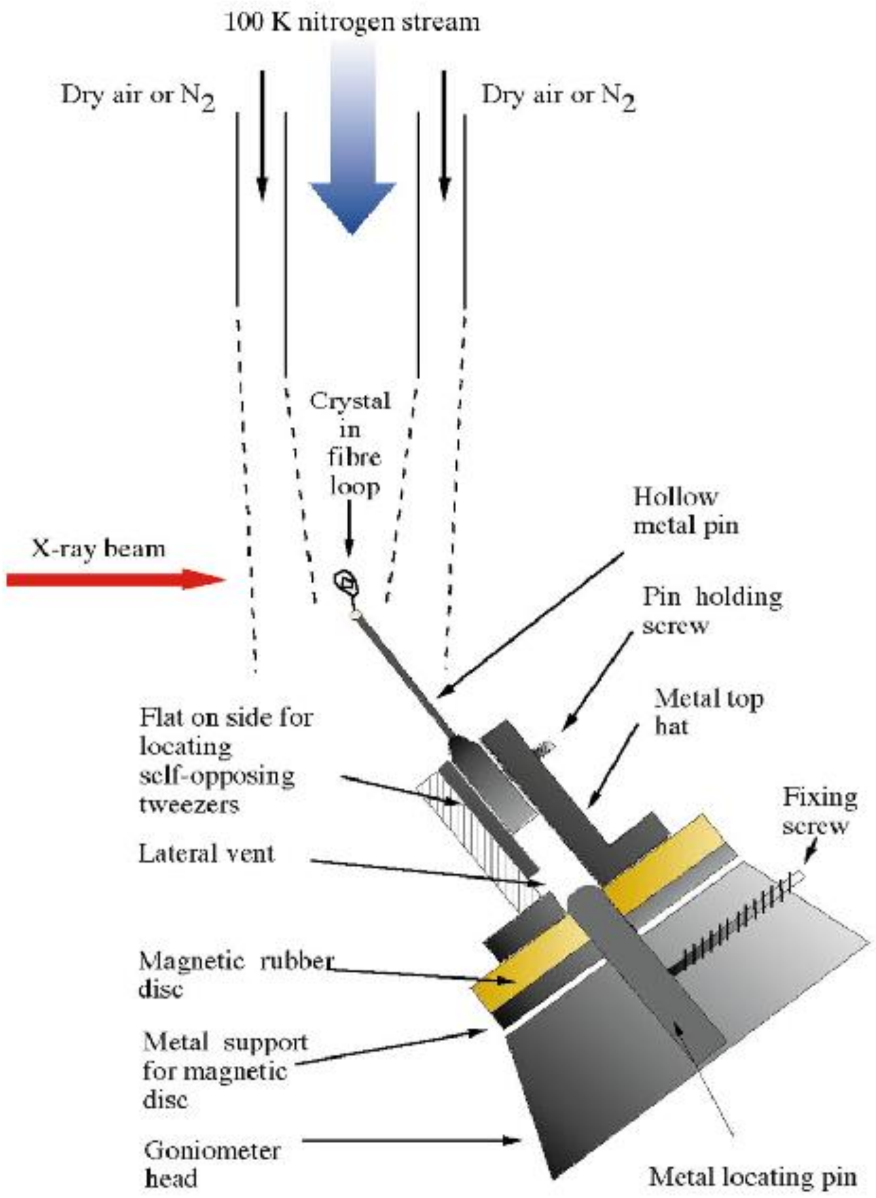
# PDP 11/44: Dinosaurs size Walnut sized brain of DEC10




# Computer cost and performance



# Flash freezing: 1990



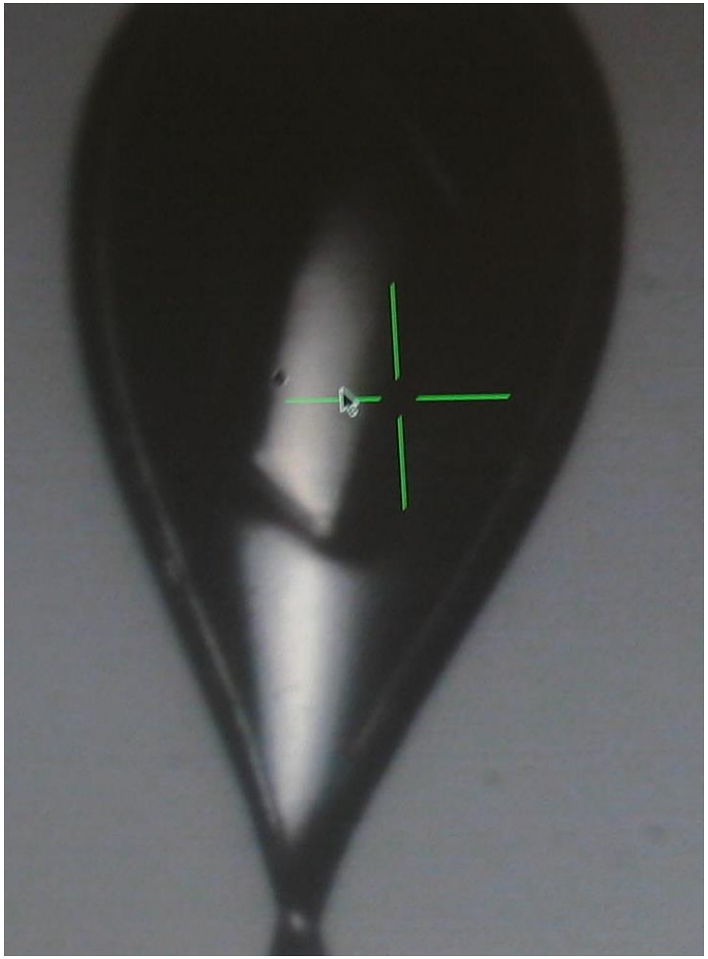
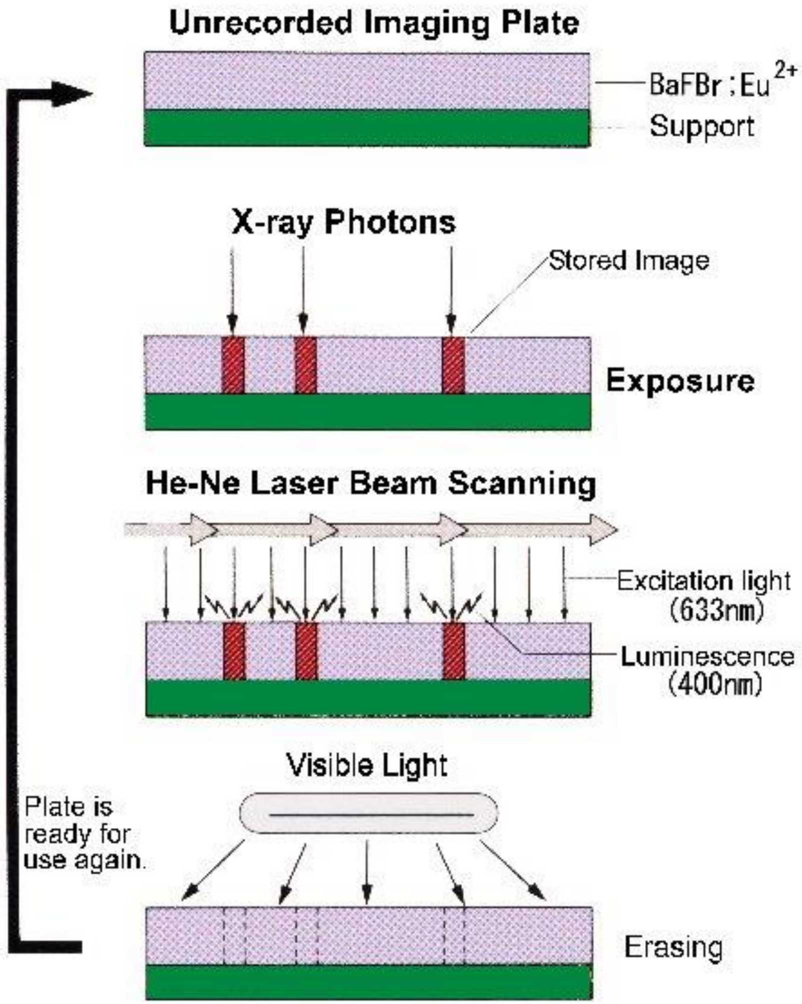
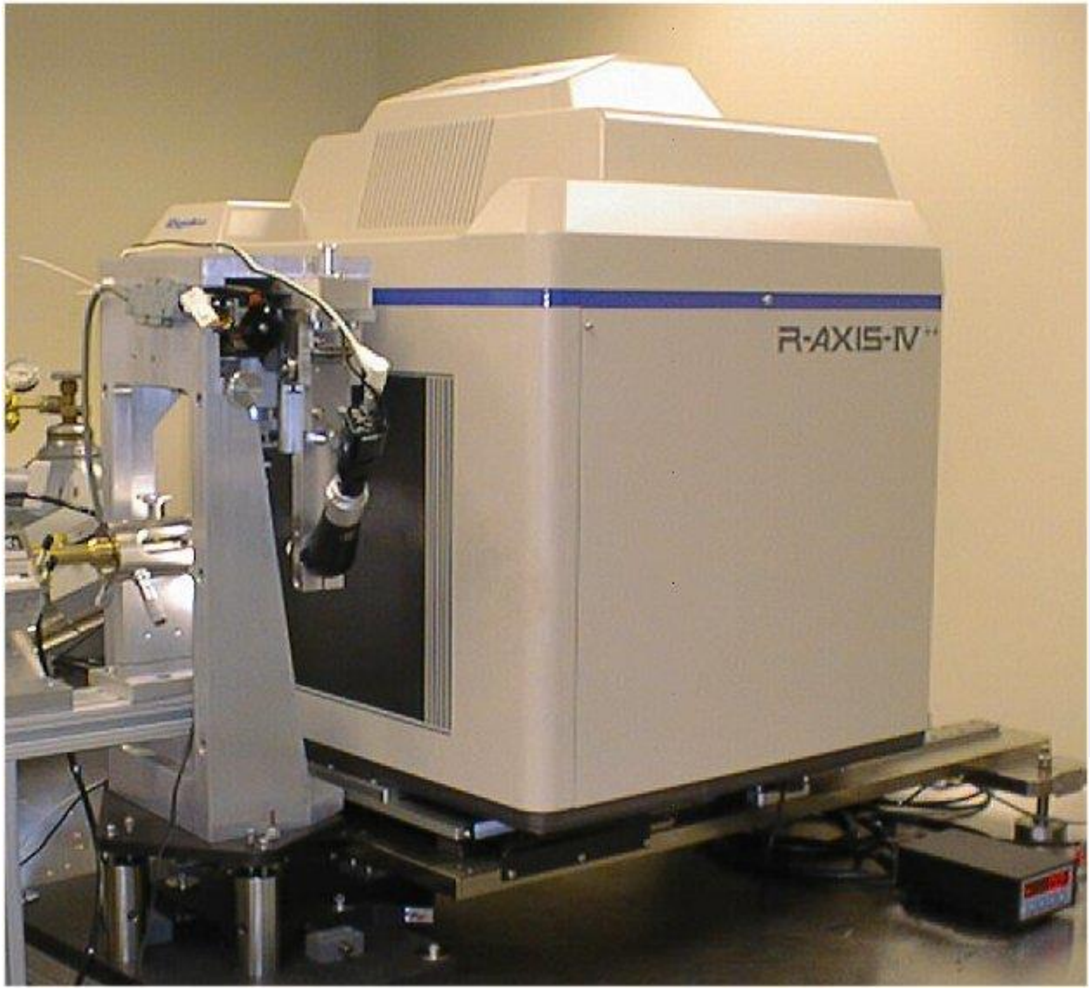
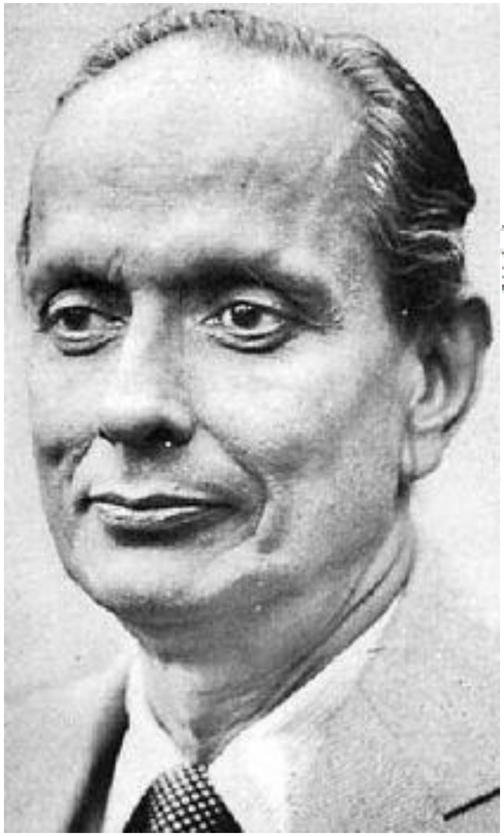



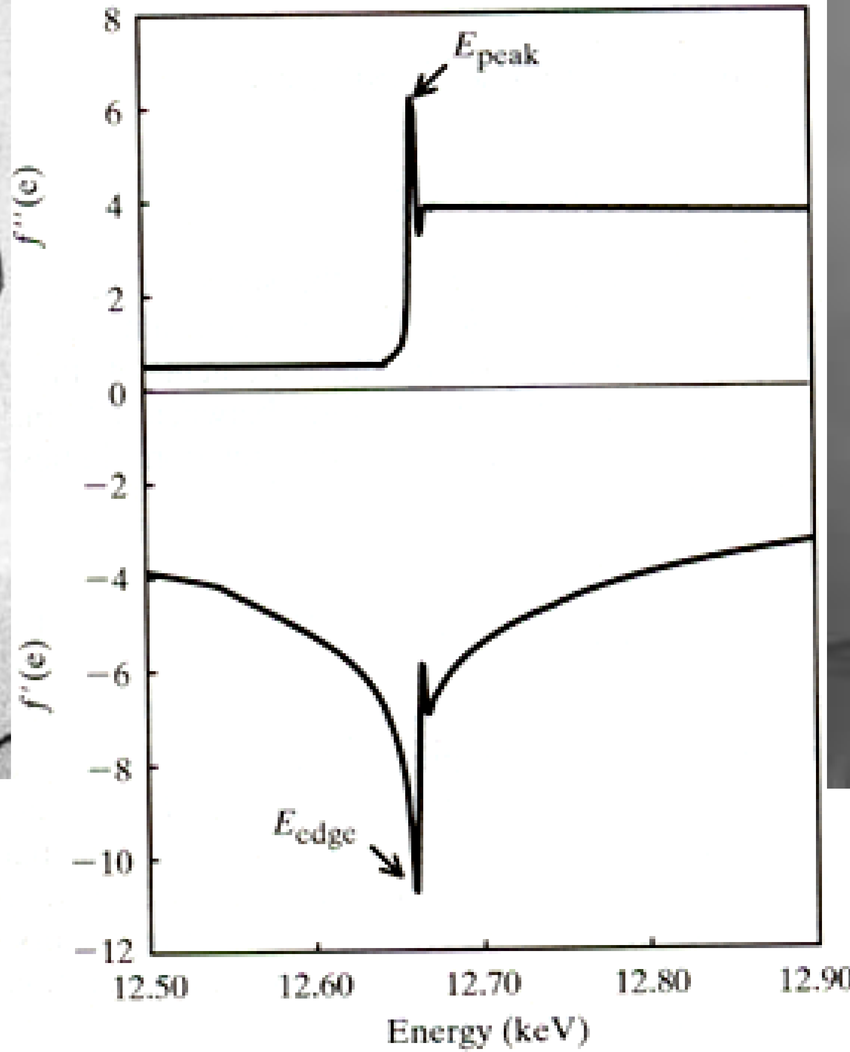
**Figure 2**
A typical experimental arrangement for a cryocrystallographic data collection. A magnetic rubber disc is pierced with a stainless steel pin, the top of which is rounded and the bottom of which fits into the hole in the goniometer head. The magnet must be strong enough to make a rigid connection, but weak enough to allow the experimenter fine control of the top hat, which is fabricated from a magnetic metal such as stainless steel or nickel. (Figure reproduced from Garman & Schneider, 1997.)

# Counters, Films, multiwire, imaging plates, CCD








**Selenium K-edge and peak for *E. coli* thioredoxin *(Hendrickson et al)***

# Breakdown of Friedel's Law

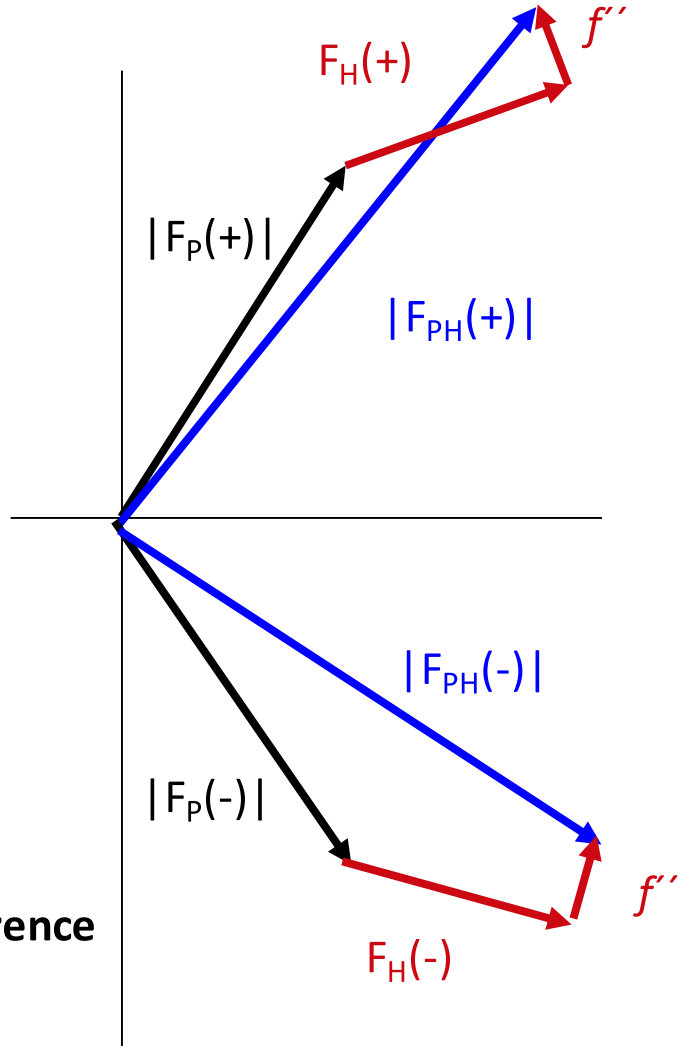
**When an anomalous scatterer is present**

$$f(\theta,\lambda) = f_0(\theta) + f'(\lambda) + if''(\lambda)$$

$$|F_{hkl}| \neq |F_{-h-k-l}|$$

**We can measure this difference of amplitudes**

**$\Delta F\pm = |F_{PH}(+)| - |F_{PH}(-)|$ is the Bijvoet difference**




**this can be used as an approximation to $|F_H|$ to locate sites**

**1980s-1990s**

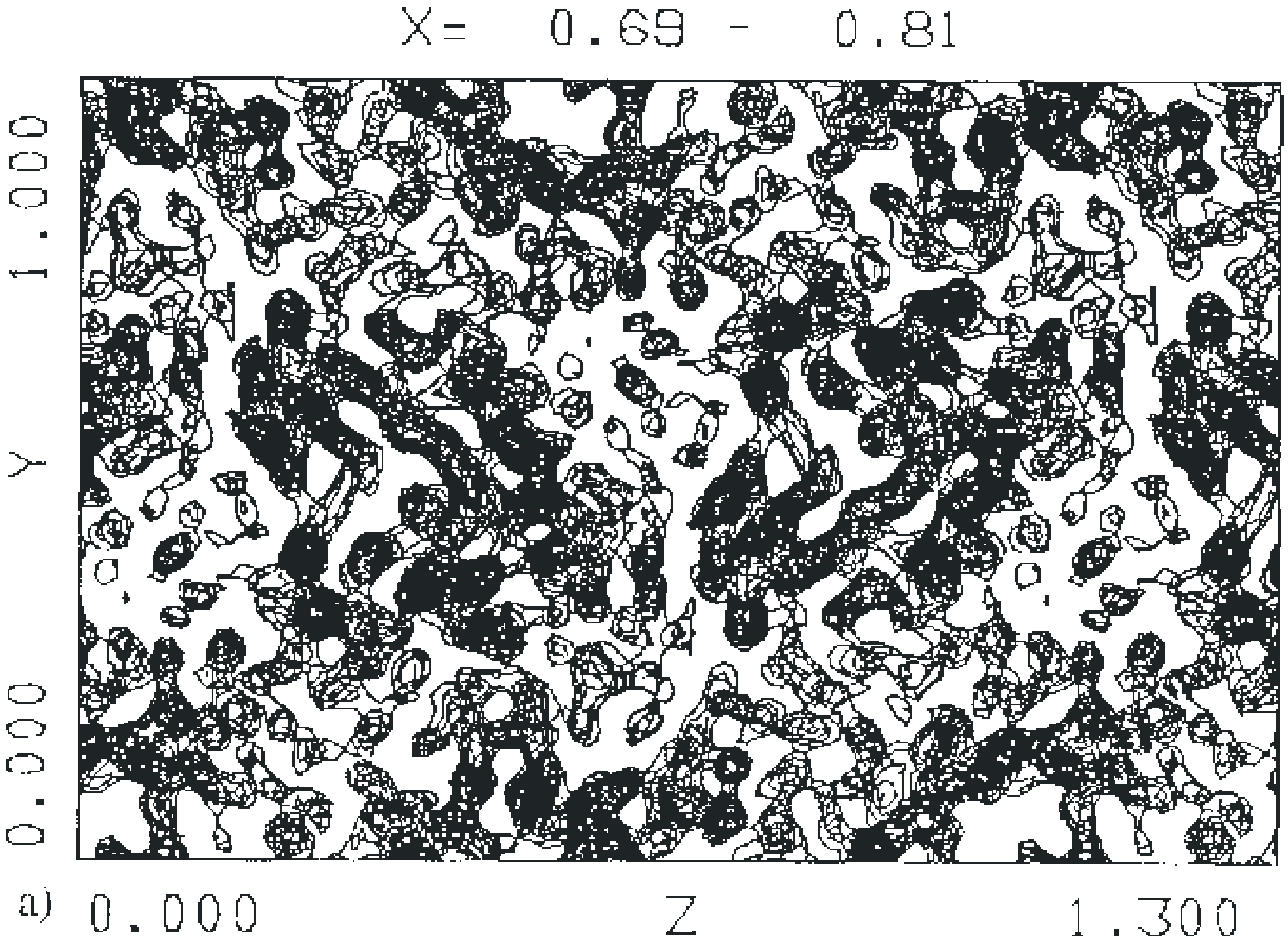
# Molecular replacement

**Can you recognize the similarity?**


$$R(\kappa,\phi,\psi)=\int_{r_{\min}}^{r_{\max}} P_{nat}(\mathbf{u})P_{\mathrm{mod}}(\kappa,\phi,\psi,\mathbf{u})d\mathbf{u}$$

$$\mathbf{T}(\mathbf{t})=\int_{cell} P_{2\rightarrow 1}(\mathbf{u}-\mathbf{t})P_{nat}(\mathbf{u})d\mathbf{u}$$

# Density Modification




**Double sorting algorithm!**

# Perfecting the structure

$$f = \sum \frac{1}{\sigma^2}\left(|F_o| - |F_c|\right)^2$$

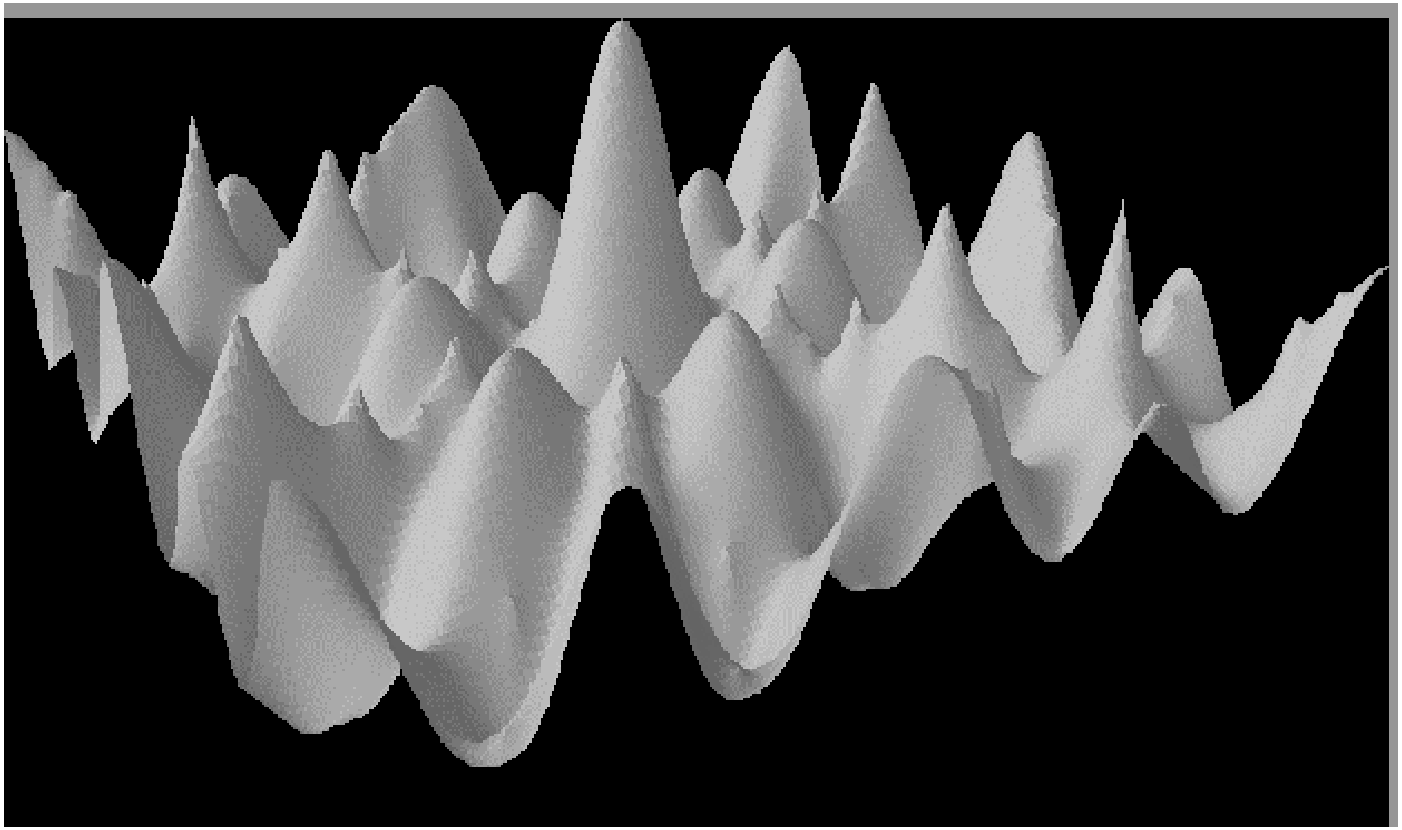
$F_c$s are calculated structure factors

$F\,(hkl) = \iiint \rho\,(xyz)\,[\exp 2\pi i\,(hx + ky + lz)]\,dx\,dy\,dz$

$F\,(hkl) = \sum\sum\sum f\,(xyz)\,\exp 2\pi i\,(hx + ky + lz)$

# Importance of observations to parameters ratio

$$f = \sum \frac{1}{\sigma^2}\left(|F_o| - |F_c|\right)^2$$



NP problems: scary local minima

# RESTRAINTS ARE THE SOLUTION

also...

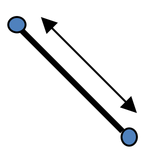
Planar groups

Hydrogen bonds

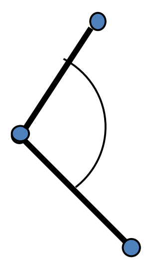
Energy

B's

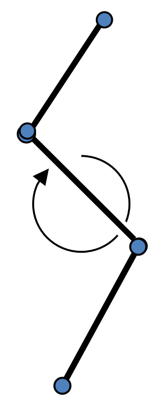
**Stereochemical restraints**



bond lengths



bond angles



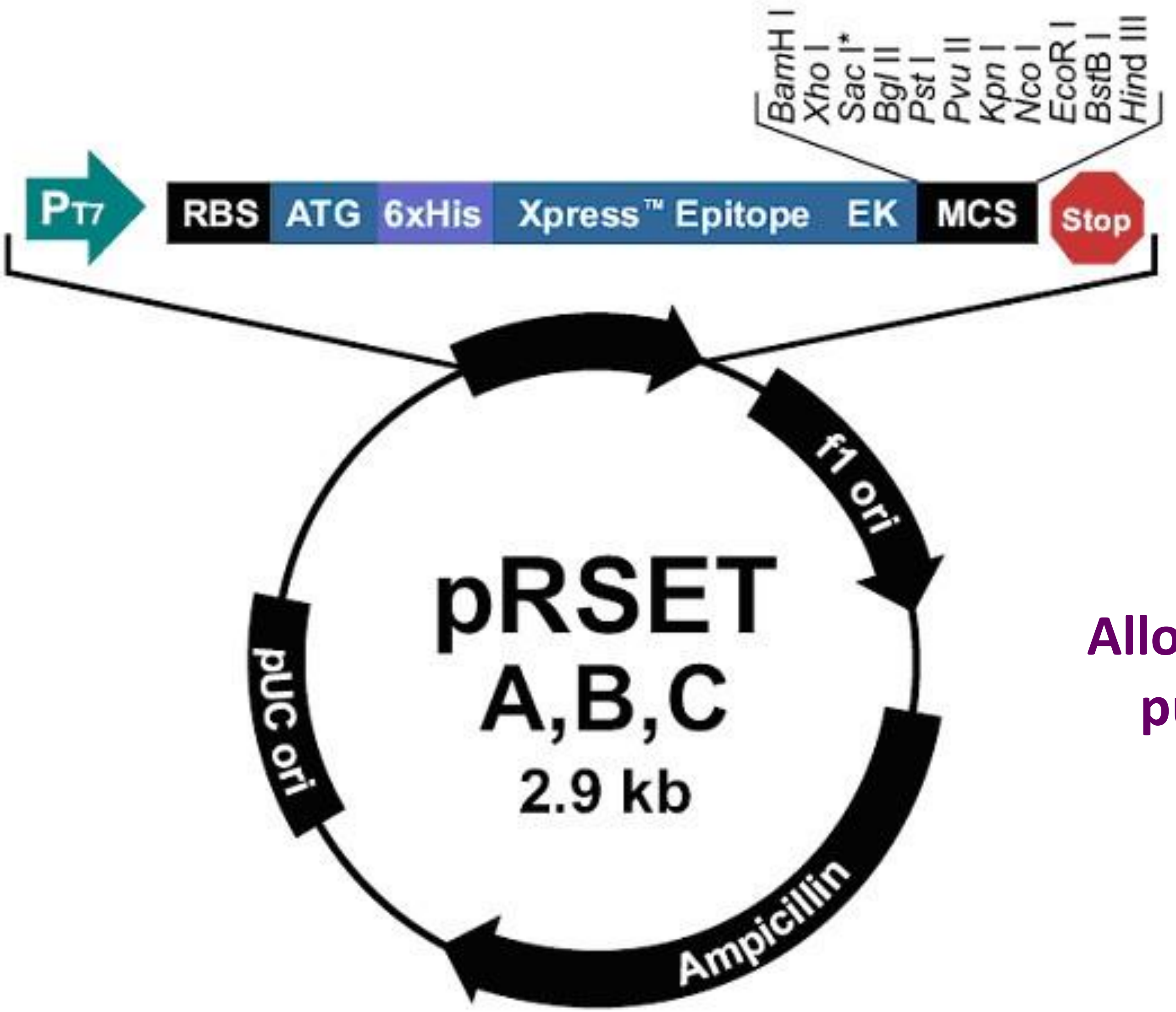
torsion angles

# Validation

**Ramachandran plot**
**Bond lengths/angles**
**Homolog structure comparison**
**Independent structural solutions**


**Robots for setting up crystallization and examining results**

# pRSET vector




Allows one step purification

Most proteins are now obtained by bacterial or cell expression

# Thank you

# 32 point groups (crystal classes)

| | | | | | |
|---|---|---|---|---|---|
| Rotation axis only | 1 | 2 | 3 | 4 | 6 |
| Dihedral | 222 | 32 | 422 | 622 | |
| Isometric | | | | 23 | 432 |
| Rotoinversion axis | $\bar{1}$ | $\bar{2}$ | $\bar{3}$ | $\bar{4}$ | $\bar{6}$ |
| Rotation, ⊥ m | 2/m | 3/m | 4/m | 6/m | |
| Rotation, ll m | 2mm | 3mm | 4mm | 6mm | |
| Rotoinversion | | $\bar{3}2m$ | $\bar{4}2m$ | $\bar{6}2m$ | |
| Dihedral, m | 2/m2/m2/m | | 4/m2/m2/m | | 6/m2/m2/m |
| Isometric, m | $2/m\bar{3}$ | | $\bar{4}/m3m$ | | |