Physics and Biology: applications of synchrotron radiation in biology

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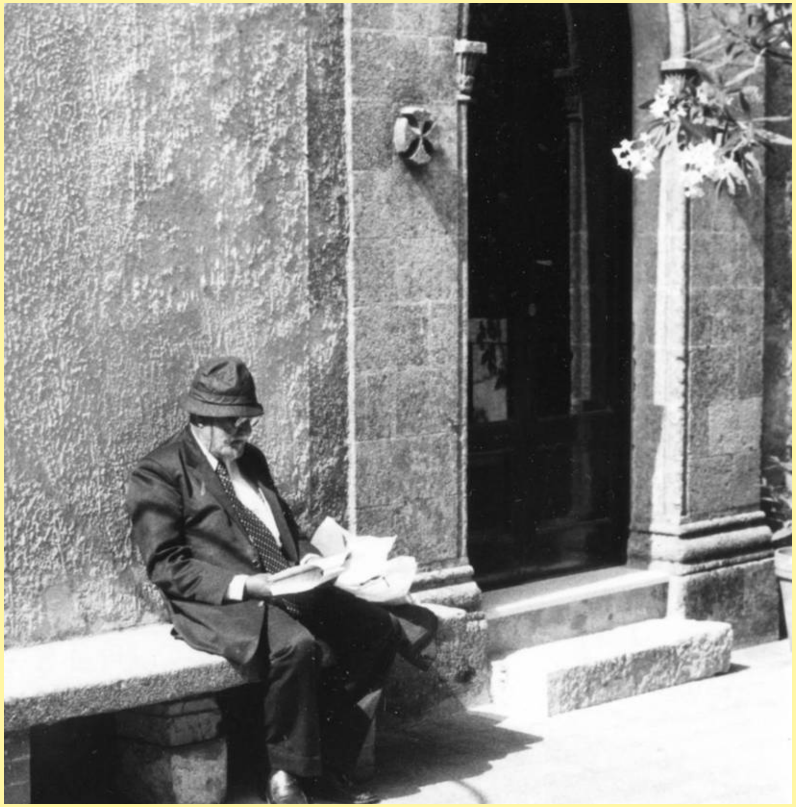
Islamabad , March 2007

Abdus Salam 1926-2006

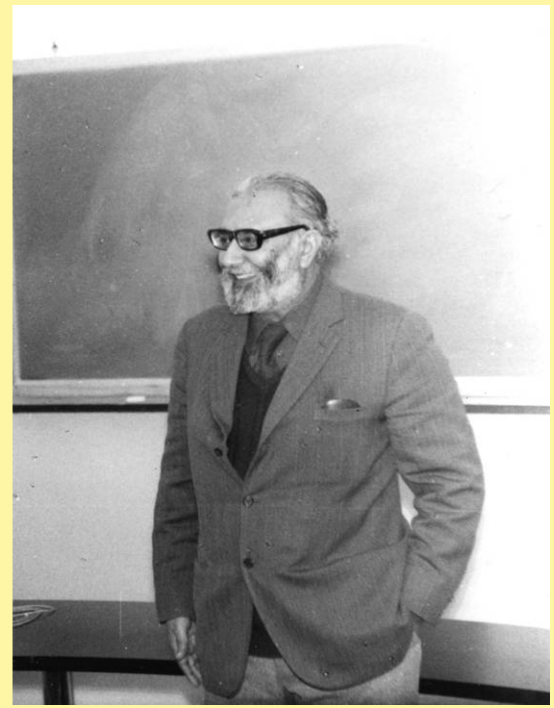
Nobel ceremony Stockholm 1979



Erice 1980

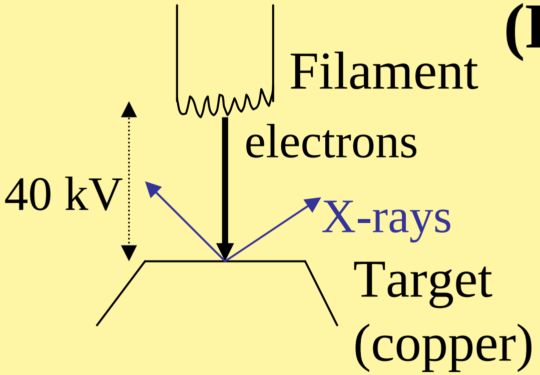
Abdus Salam

Enthusiasm for Physics and for Science in the Third World

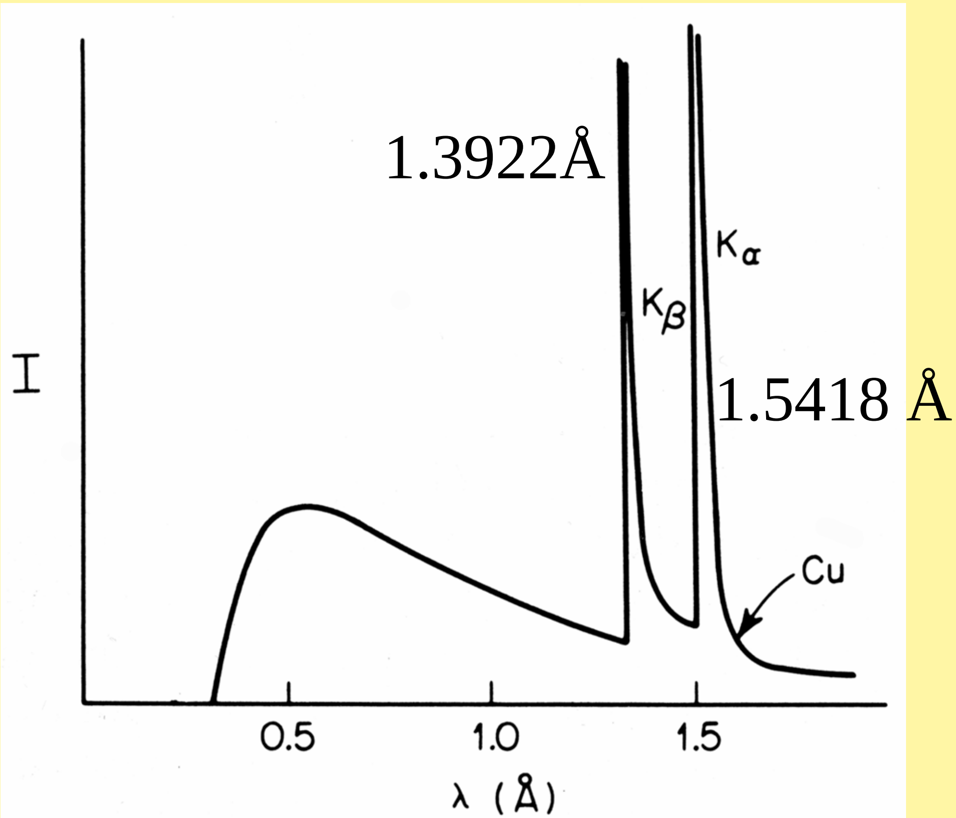


- Scientific thought is the common heritage of all mankind

Discovery of X-rays
(Roentgen 1896 in Wurzburg)

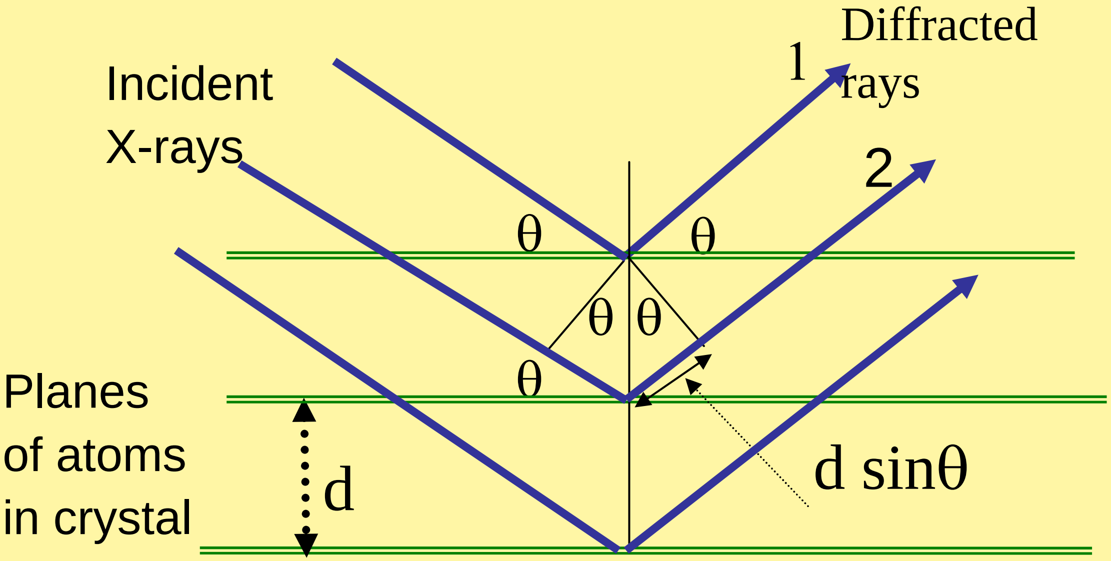


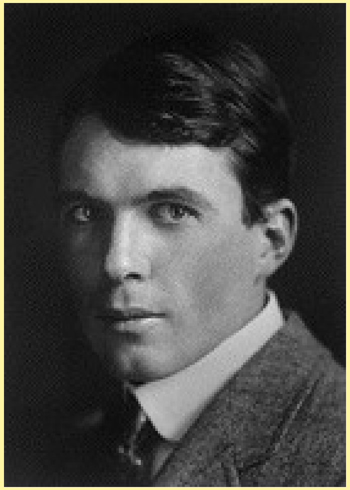
X-rays penetrate most materials.
Only those containing heavy elements
absorb X-rays significantly



Spectrum from an X-ray tube with a copper anode

Bragg's Law (W. L. Bragg 1913)



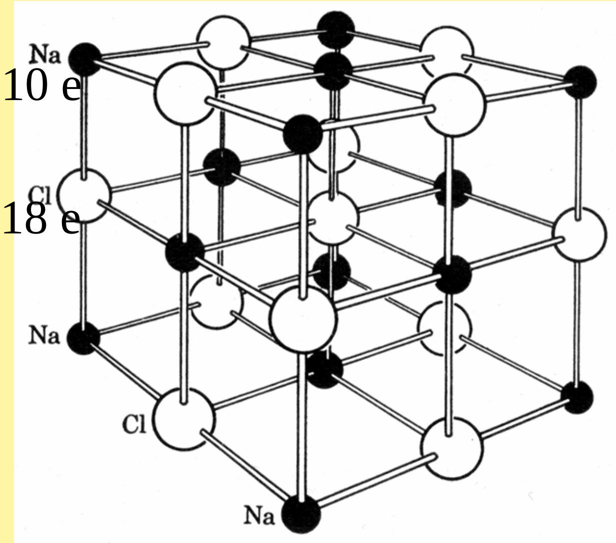


$$2\,d\,\sin\theta = n\,\lambda$$

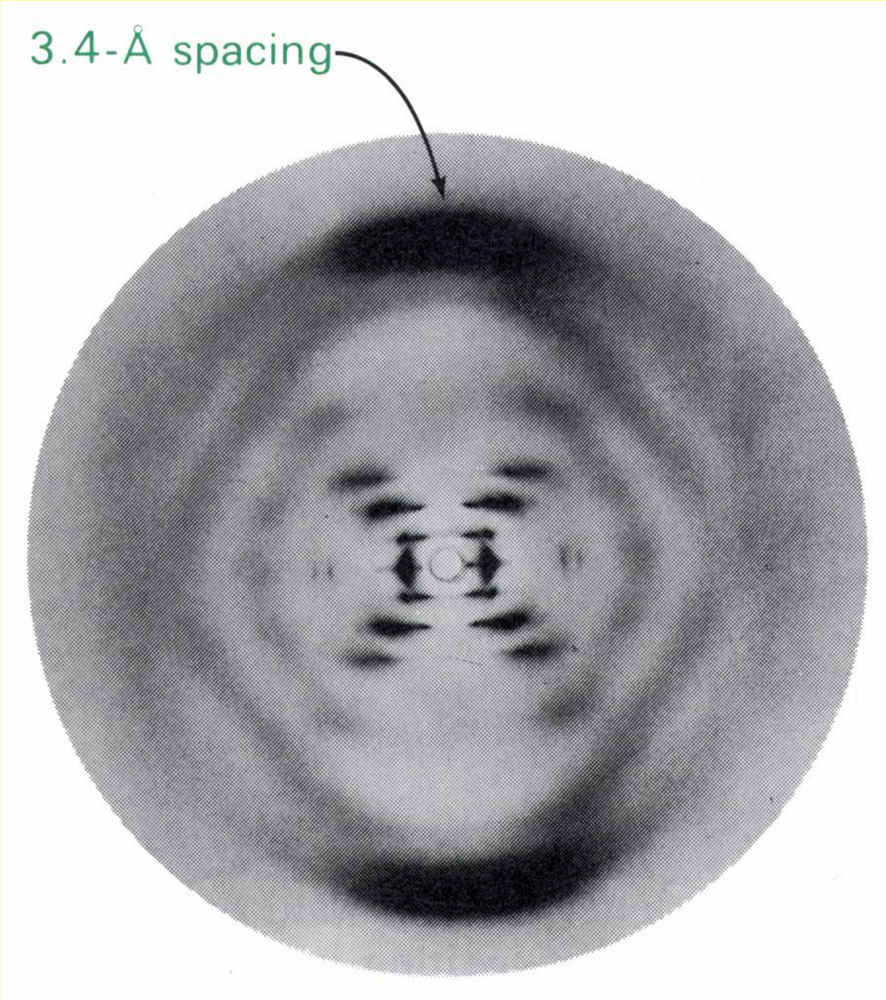
where d is interplanar spacing
θ is angle of reflection
(Bragg angle)
n is an integer
λ is wavelength

Z-1 = 10 e

Z+1 =18 e



DNA diffraction pattern (Franklin & Wilkins 1952

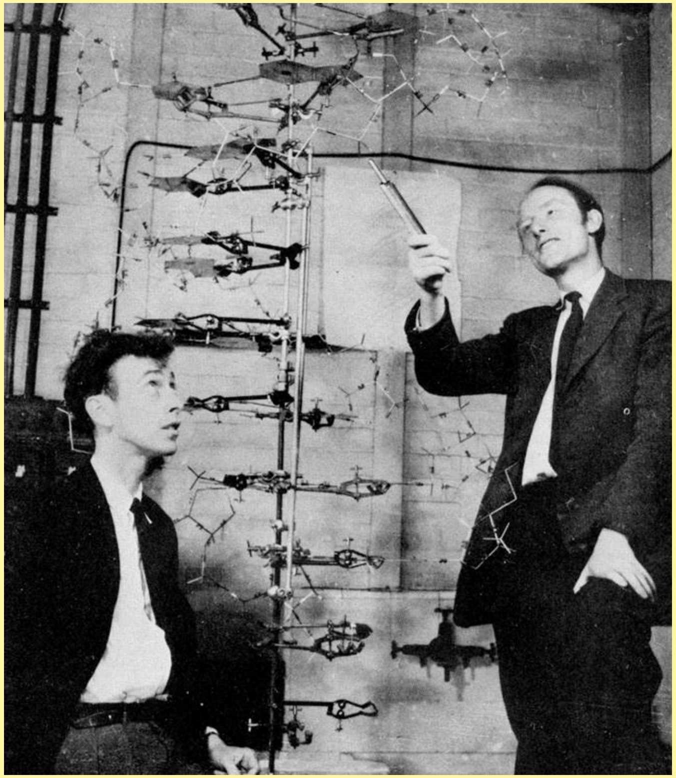


Layer lines

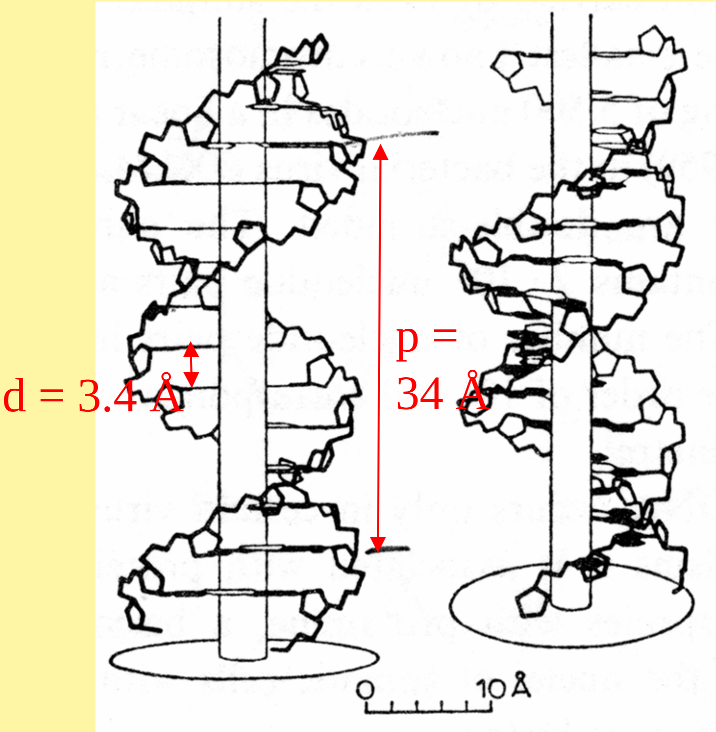
Rosalind Franklin

Maurice Wilkins

Watson Crick model for DNA 1953

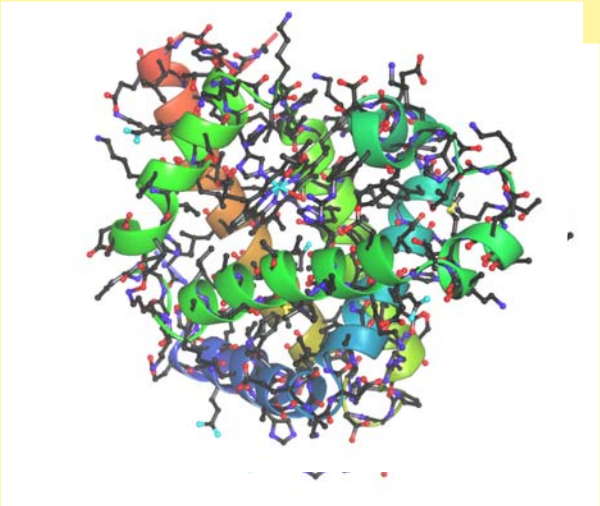


James Watson & Francis Crick

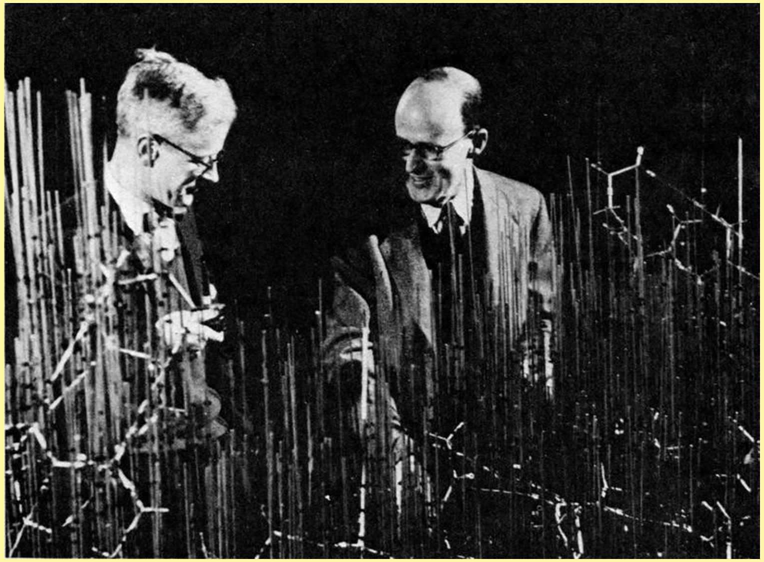


10 base pairs per turn of helix
d is spacing between nucleotides;
p is pitch of helix

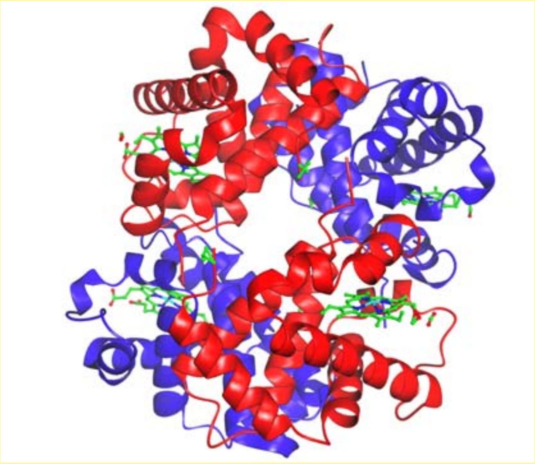
The first protein crystal structure; Myoglobin (1959)



myoglobin

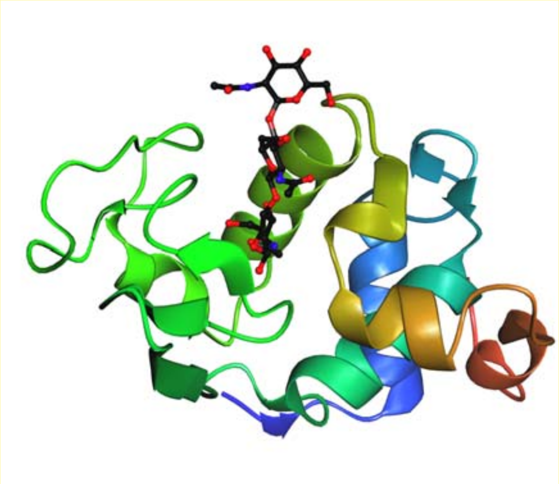


John Kendrew & Max Perutz



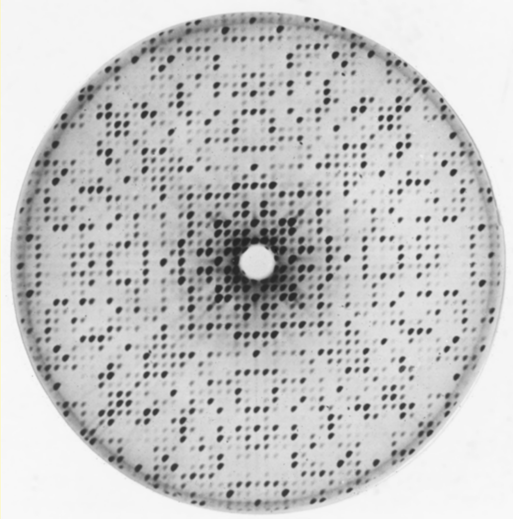
Hemoglobin1968

Lysozyme; the second protein structure and the first enzyme (1965)



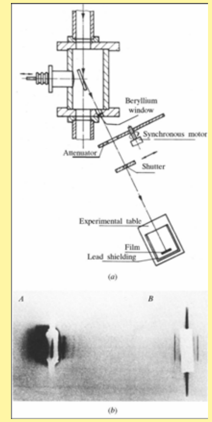
David Phillips

David Phillips: The Royal Institution, London, 1965



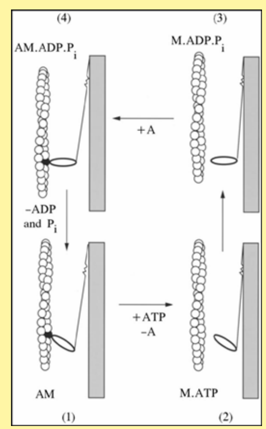
Synchrotron radiation

- Building on work of A. Lienard (1898), G. A. Schott (1912), D. W. Kerst (1942), I. Pomeranchuk & Ivanenko (1944), synchrotron radiation was first observed indirectly in 1946 (J. Blewit) and a 70 MeV synchrotron was produced in 1947 (Pollock, H. C. et al).
- 1949 J. Schwinger 'On the classical radiation of accelerated electrons' Phys Rev. 75, 1912-1925. -definitive theoretical work.
- **1971 First biological experiment at DESY, Hamburg (G. Rosenbaum, K. C. Holmes & J. Witz Nature 230, 434-437).**

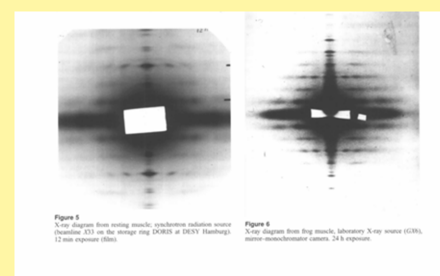


First synchrotron photo of muscle (1971)

H. Schopper & J. C. Kendrew agree the EMBL outstation at DESY 1975



Muscle



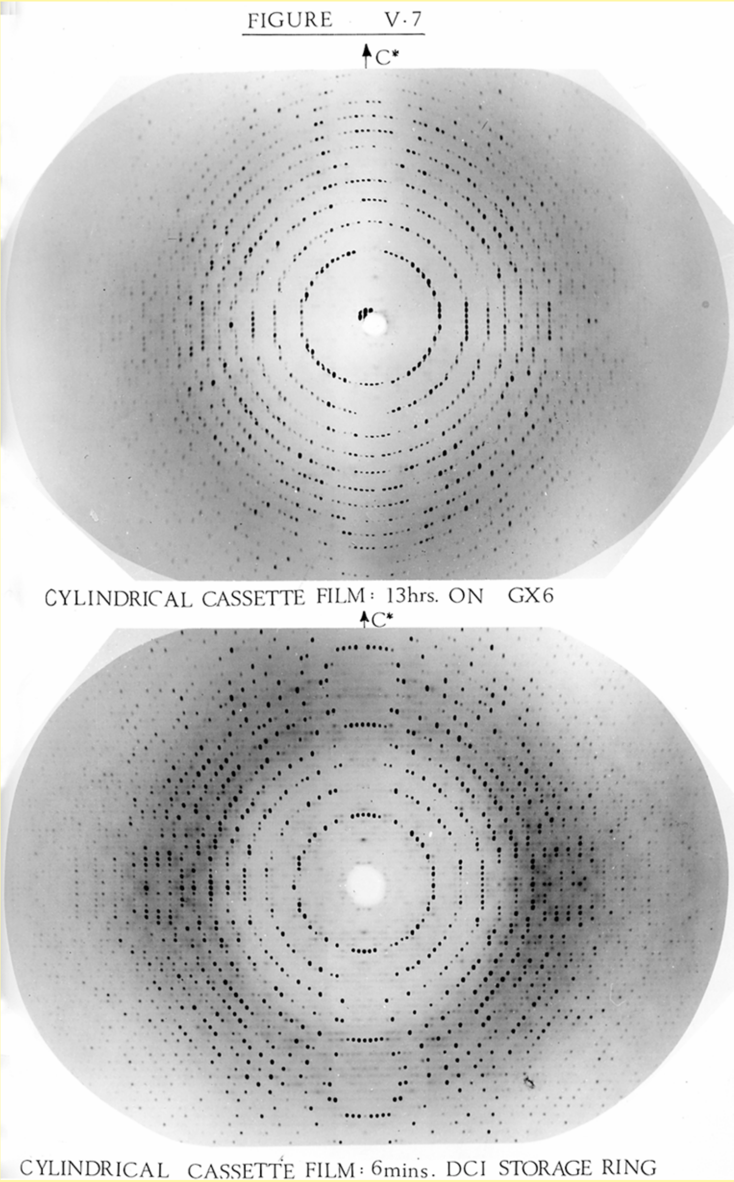
12 min SR   24 h Lab

Protein crystal diffraction at a synchrotron source

1979 At LURE (Paris)

Diffraction photographs of glycogen phosphorylase crystal

Enrico Stura



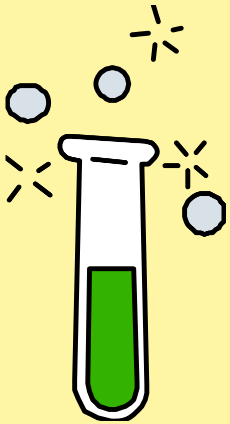
Lab
13 h

LURE SR
6 mins

Applications in Biology

Macromolecular crystallography

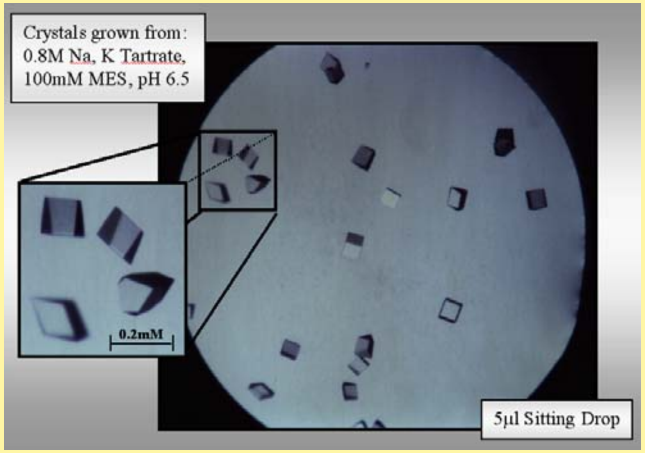
Processes in protein crystallography



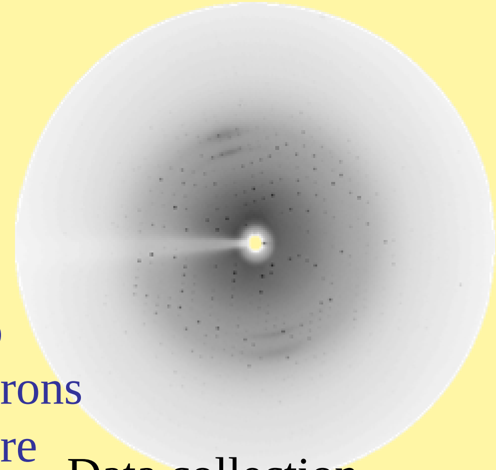
Purification

Screens and Robots

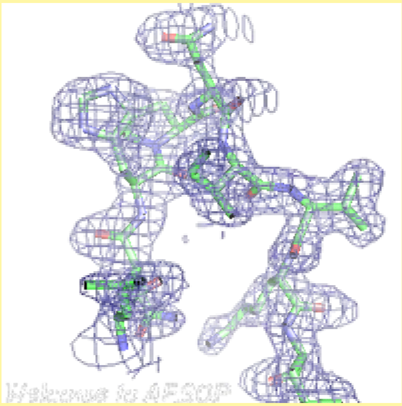
Crystallization



Cryo
Synchrotrons
software



Data collection



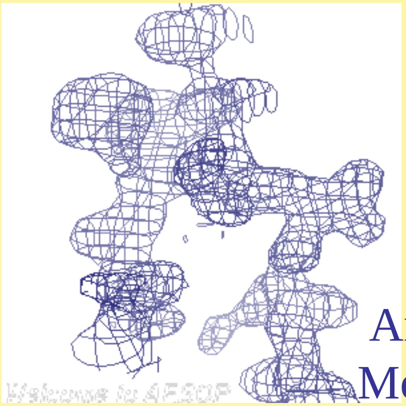
Map interpretation

nent

$$\rho(x,y,z)=\frac{1}{V}\sum_{h}\sum_{k}\sum_{l}|F(h,k,l)|\exp[-2\pi i(hx+ky+lz)+i\alpha(h,k,l)]$$

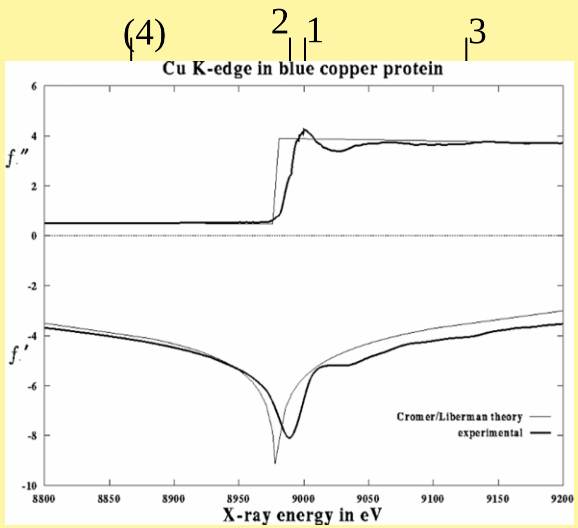
10 days to
10 years

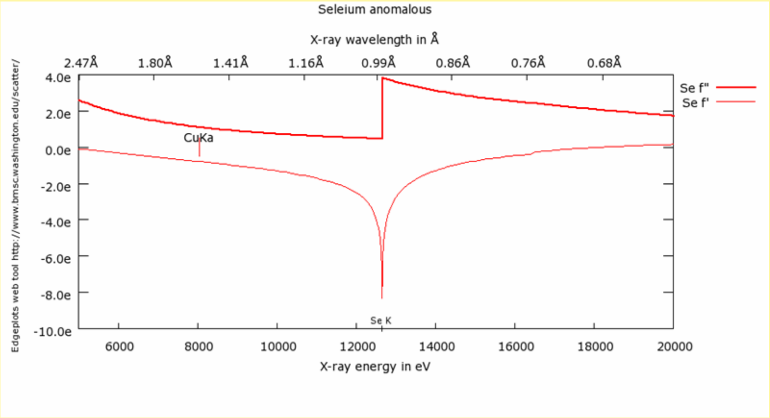
Phasing
Anomalous scattering
Molecular replacement



Electron density map calculation

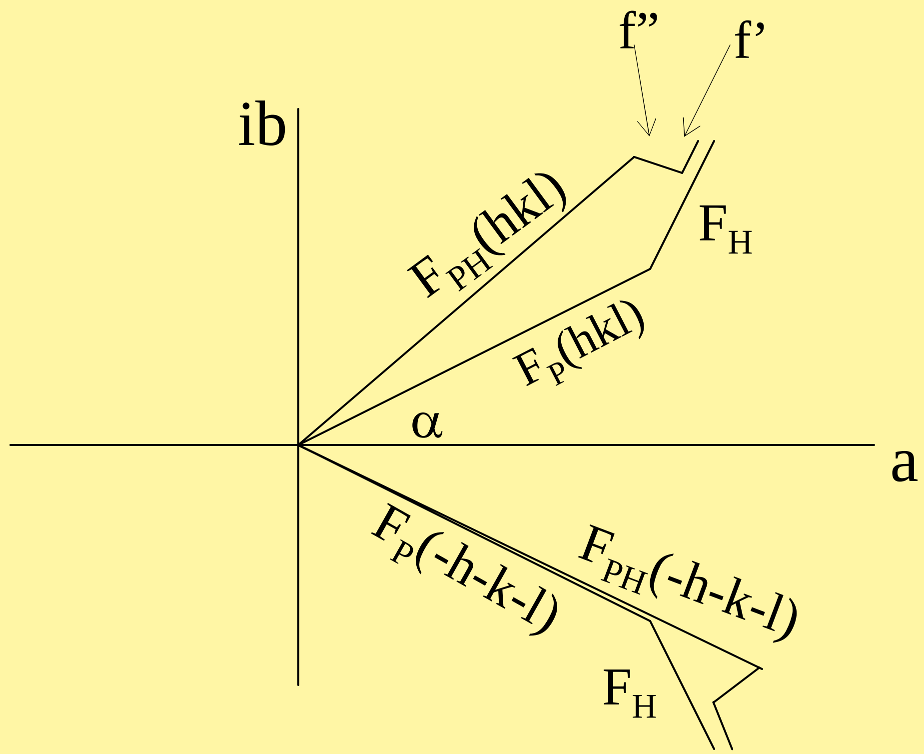
Choice of wavelength for anomalous scattering measurements





- Wavelengths are chosen and used as shown
- 1) Peak wavelength: maximum Bijvoet difference
- 2) Inflexion Point: minimum of f'
- 3) High Energy Remote: maximum of f'
- 4) Low Energy Remote: alternative maximum of f', and also easiest dataset to use in scaling due to lack of Bijvoet differences.

- Most frequently, wavelengths 1-3 are collected, in that order. 4 is generally held to be optional.
- Strategy may vary depending on specific characteristics of the heavy atom
- E.g. Mercury has such a large f' (> 10 electrons at LIII edge), and such a poor white line, that wavelengths 1 and 2 generally suffice)



$$F_{PH}^{+} - F_{PH}^{-} \approx 2F_H'' \sin(\alpha_{PH} - \alpha_H)$$

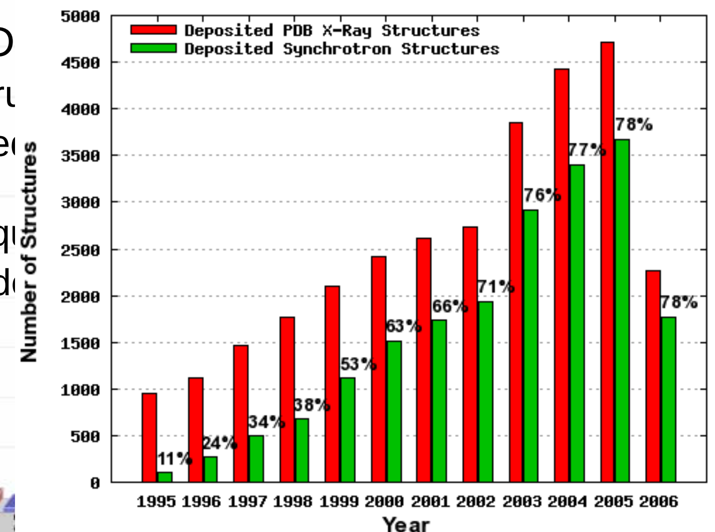
Phenomenal success of Macromolecular Crystallography

Number of X-ray structures solved per year from 1976 - 2006

•**Bright non-divergent beam**: hence able to work with small samples (> 10 μm);
•improved precision of the data; improved resolution

•**Tunable wavelength**: ability to optimise anomalous scattering and hence
•exploit for phase determination

- February 2007 PD
  - 35361 X-ray stru
  - 15803 <95% se
    chain)
  - 8448 <30% seq
  - 1055 folds as id

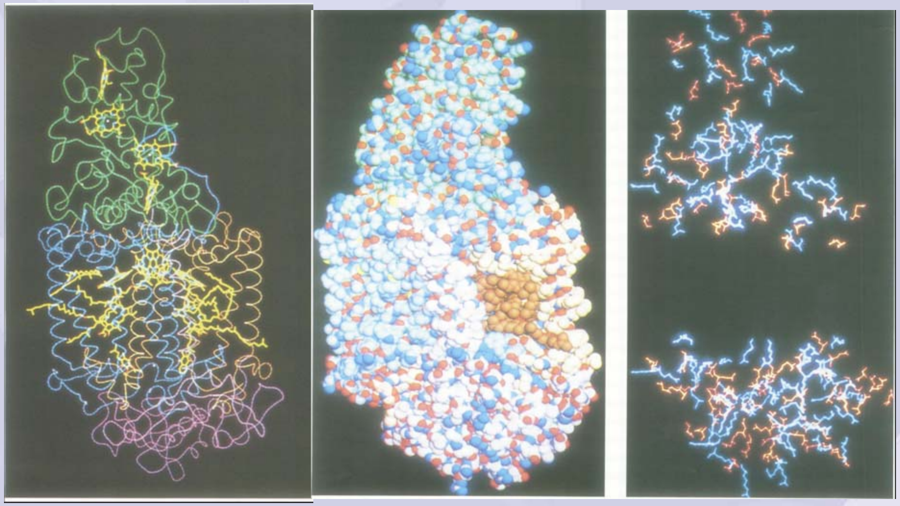


Rosenbaum, Holmes & Witz
DESY, Hamburg 1971

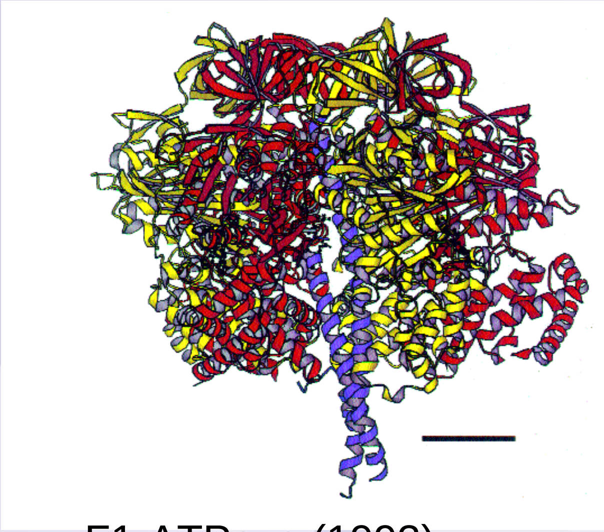
SRS 1981, Elettra 1993, APS 1994, ESRF 1994, Spring 8 1997, Diamond 2007

diamond

Nobel prizes in synchrotron structural biology

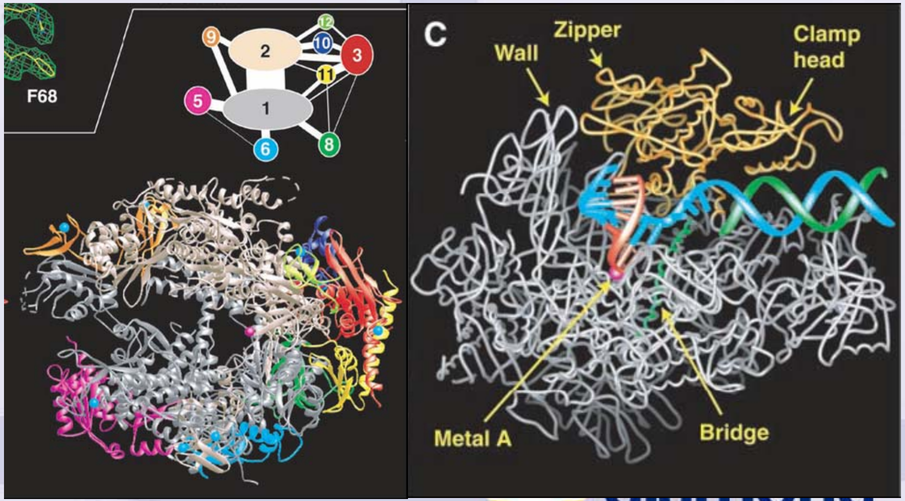


Bacterial photoreaction centre (1985)
J. Deisenhofer, R. Huber & H. Michel (Nobel 1989)



F1-ATPase (1993)
J. Walker (Nobel 1997)

KcsA potassium channel (1998)
R. MacKinnon (Nobel 2004)

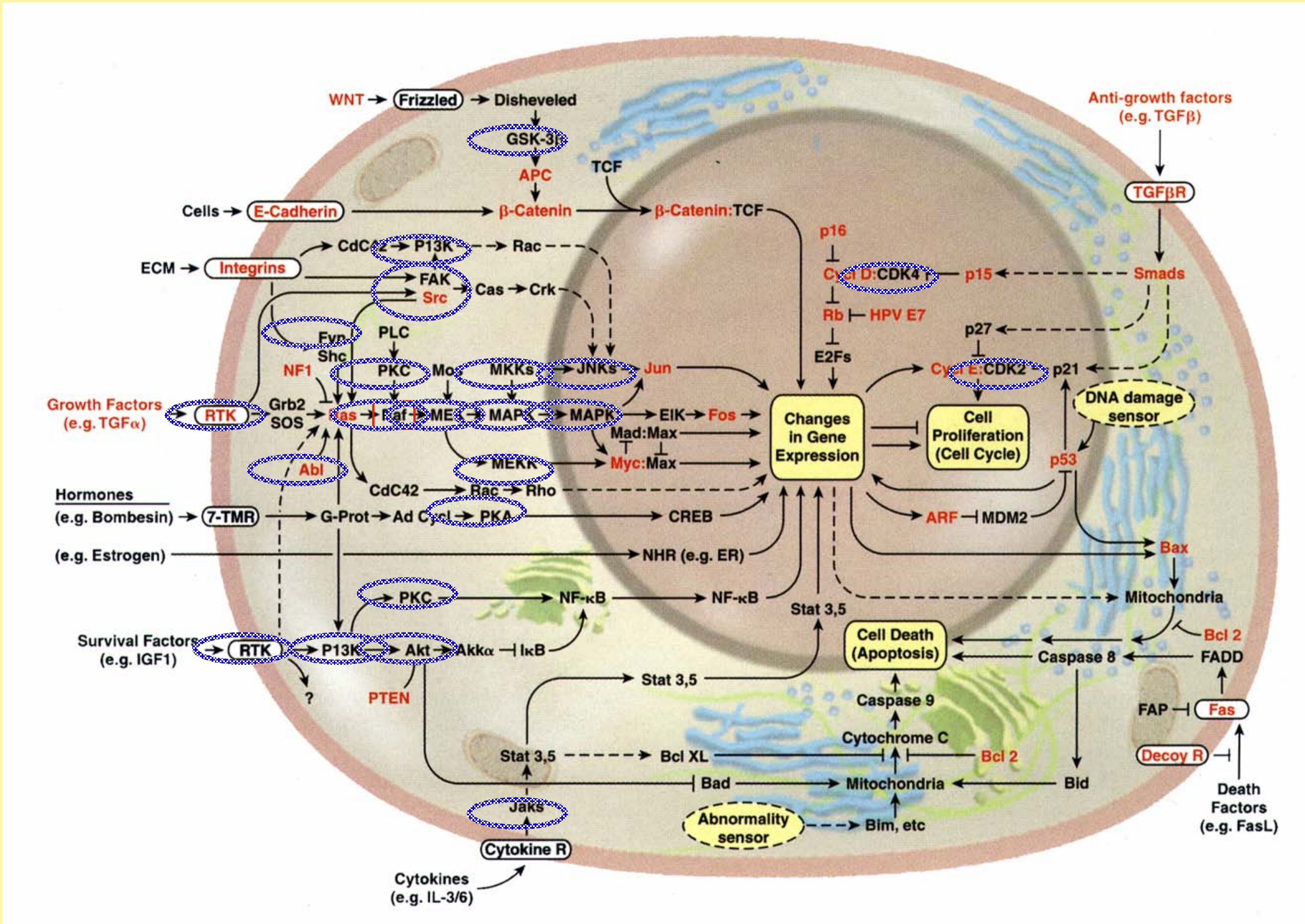


RNA Polymerase II (2001)
R. Kornberg (Nobel 2006)

Marketed drugs for which structural biology has contributed information on the target protein structure

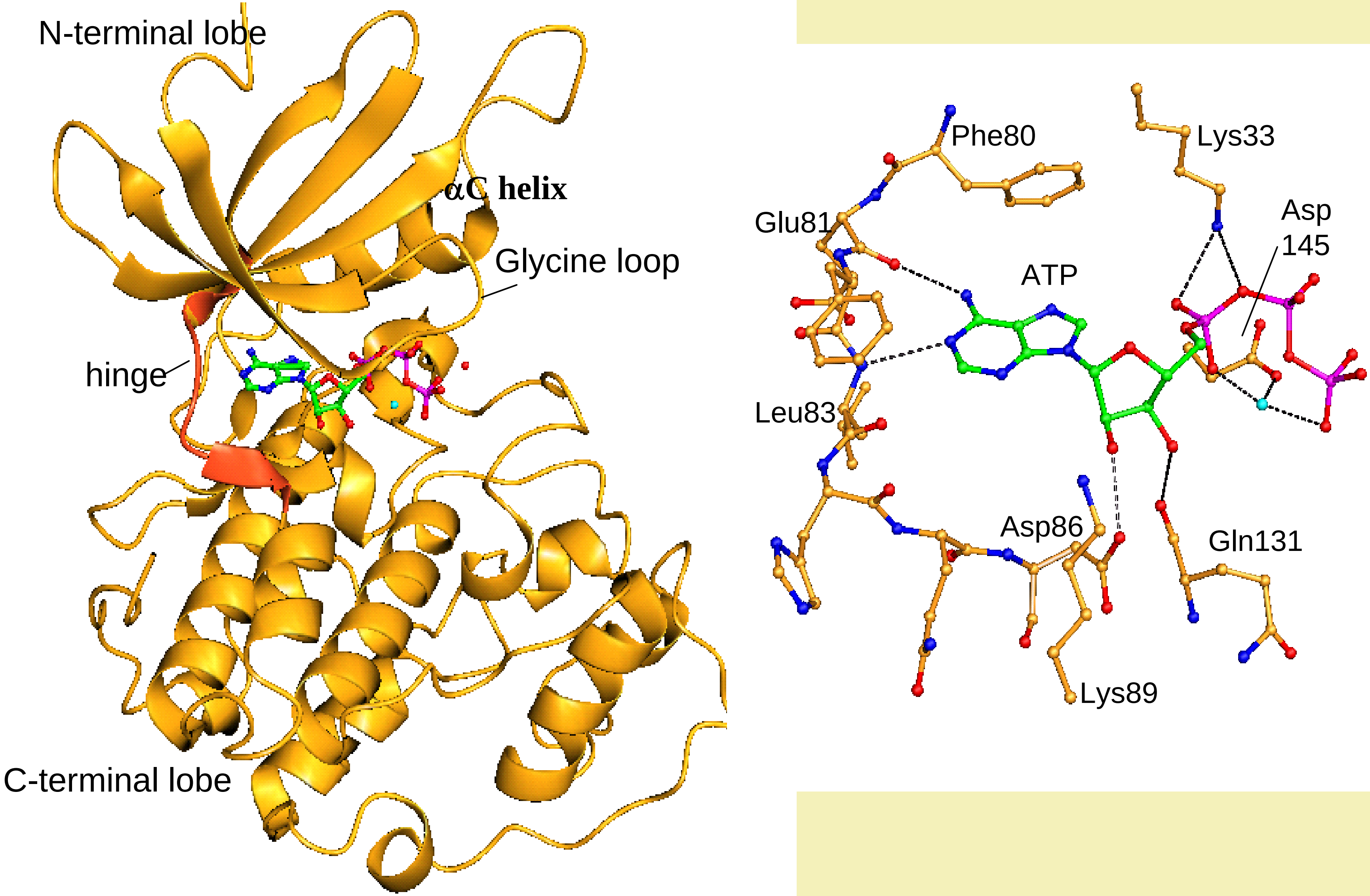
Drug	Compound	Company	Disease target	Protein target	PDB entry
Gleevec	Imatinib	Novartis	Chronic myeloid leukemia Gastrointestinal stroma tumours	Abl Tyrosine kinase C-Kit PDGFR	1XBB
Herceptin	Trastuzumab	Genentech	Breast cancer	Her2 receptor	1N8Z
Lipitor	Atorvastatin	Pfizer	High cholesterol	HMG (3-hydroxyl-3-methylglutaryl) CoA reductase	1HWK
Avandia	Rosiglitazone	GSK	Type 2 diabetes	Peroxisome proliferator-activated receptor (PPARγ)	2PRG
Actonel	Risedronate	Proctor& Gamble	Osteoporosis	Farnesyl diphosphate synthase	1YV5
Casodex	Bicalutamide	AstraZeneca	Prostate cancer	Androgen receptor	1E3G
Norvir	Ritonavir	Abbott	HIV	HIV protease	1HXW
Relenza	Zanamivir	GSK	Influenza	Influenza neuraminidase	1A4G

Protein kinases as targets for drugs



Hanahan & Weinberg (2000) Cell 100, 57 The hallmarks of cancer

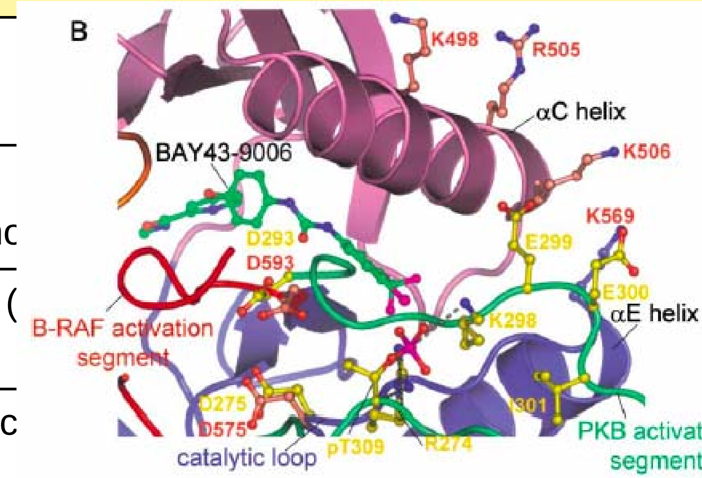
Most protein kinase inhibitors target the ATP site
518 proteinkinases encoded in the human genome

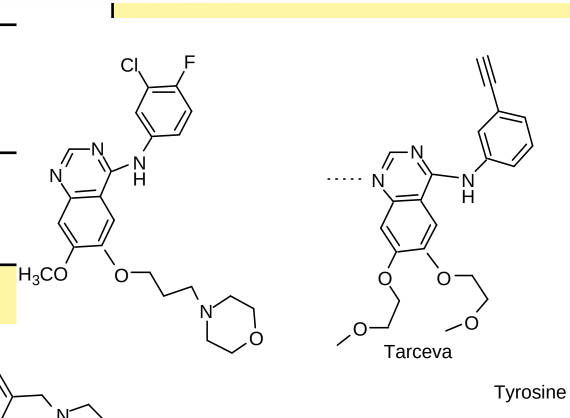


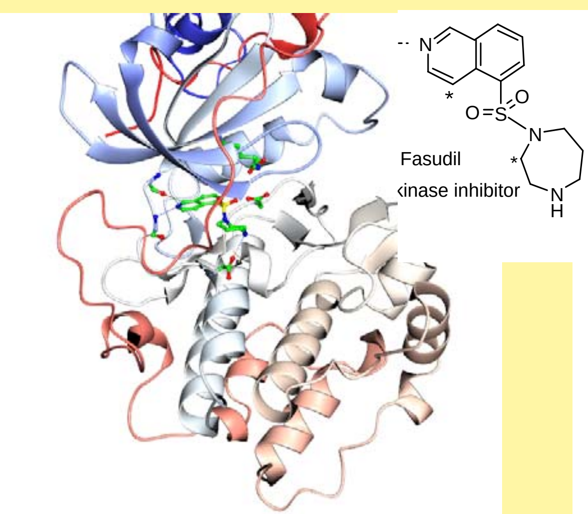
ATP bound to pCDK2/cyclin A

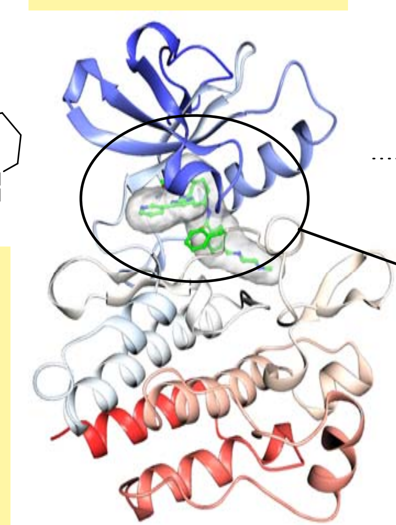
Protein kinase inhibitors: Target specific (patient specific) drugs.

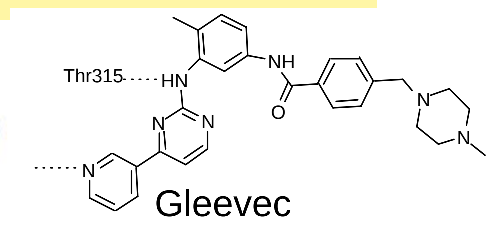
Fasudil (1999)	Rho-kinase	Cerebral vasospasm
Gleevec STI-571 (2001)	Abl tyrosine kinase c-Kit, PDGFR	Chronic myeloid leukemia Gastrointestinal stromal tumc
Iressa ZD-1839 (2004)	EGFR tyrosine kinase	Non-small cell lung cancers (adenocarcinomas)
Tarceva OSI 774 (2004)	EGFR tyrosine kinase	Non-small cell lung and panc carcinomas
Sorafenib BA 43-9006 (2006)	B-RAF, VEGFR, PDGFR, FLT3	Renal cell carcinoma
Sunitinib SU11248 (2006)	VEGFR, PDGFR, FLT3, c-Kit	Renal cell carcinoma, GIST

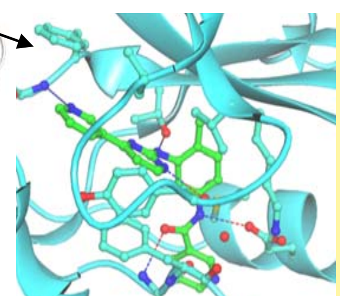


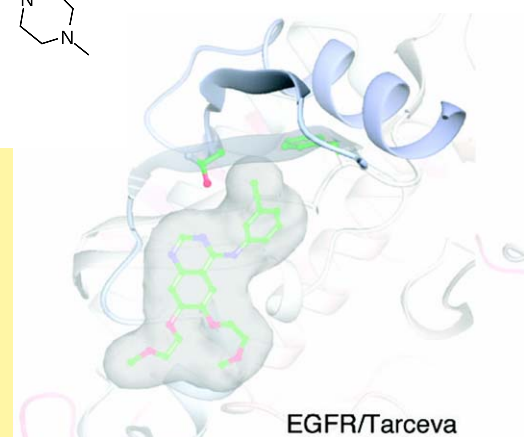












Receptor protein kinase antibodies: target specific (patient specific) drugs.

Herceptin (Trastuzumab) (2002)	Her2 EGF receptor	Breast Cancer
Erbitux (Cetuximab) (2004)	EGFR	Metastatic colorectal cancer
Avastin (Bevacizumab) (2004)	VEGF	Metastatic colorectal cancer

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

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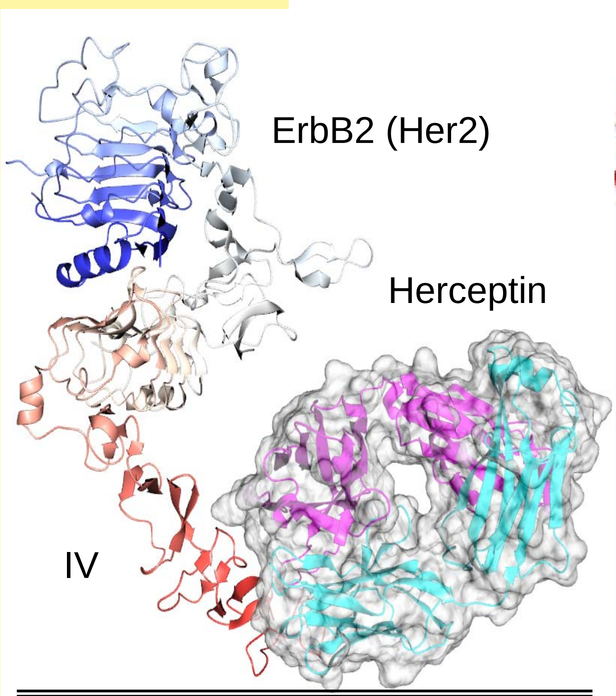
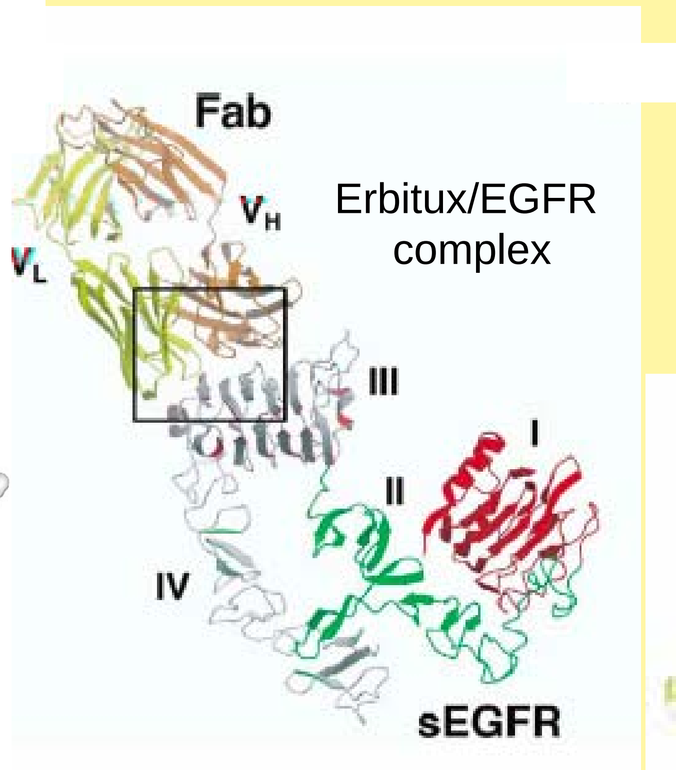
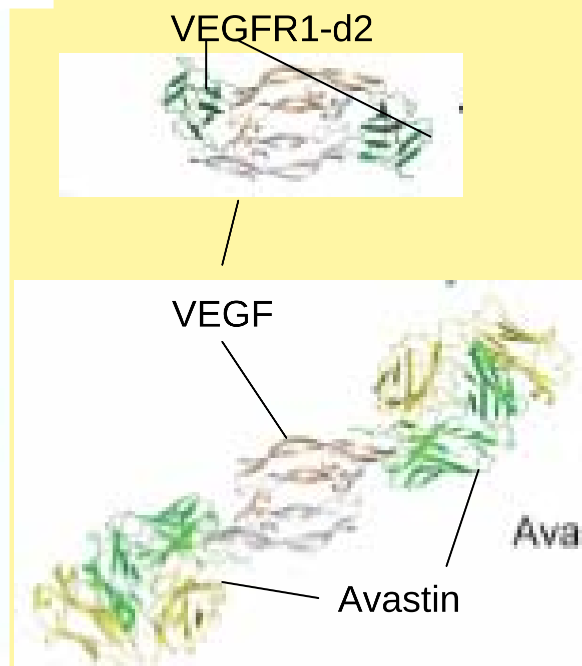


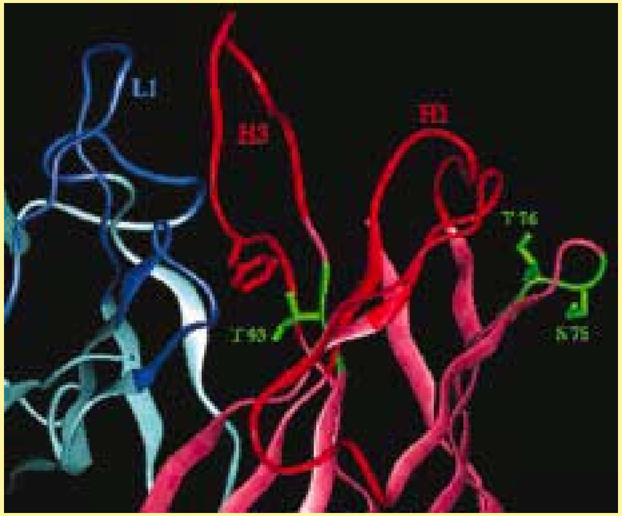
Membrane





CIMAher (TheraCIM, Nimotuzumab)

Humanised monoclonal antibody anti epidermal growth factor receptor (EGFR3)
Center for Molecular Immunology, Havana



Model of variable region of murine Mab for EGF/r3
VL & VH in blue & red, respectively. S75, T76 & T93 in green

-Humanised mAb h-R3, isotype IgG1 was obtained by transplanting the complementarity determining regions (CDRs) of the murine antibody for EGF/r3 to a human framework assisted by computer modeling

-Used in the treatment of tumours of epithelial origin overexpressing EGF-R in combination with standard cancer treatments (chemotherapy and radiotherapy)

So what's left to be done ?

- Bigger and more complex: Macromolecular assemblies and machines: connection with electron microscopy and cell biology

- Smaller crystals (e.g. <10 μm).
  E.g. membrane proteins

- More: Complete dictionary of protein folds

(Kuhlman et al & Baker D. Science (2003) 302, 1364)

~12% of new protein structures (for proteins with <30% identity to existing structures) have a new fold.

- Medical: Structure based drug design and structural genomics

- Faster: time resolved studies to observe chemical reactions

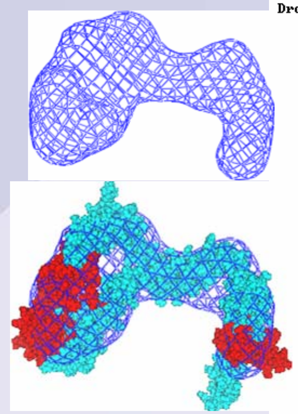
- More complex: transient protein-protein complexes which govern cellular processes

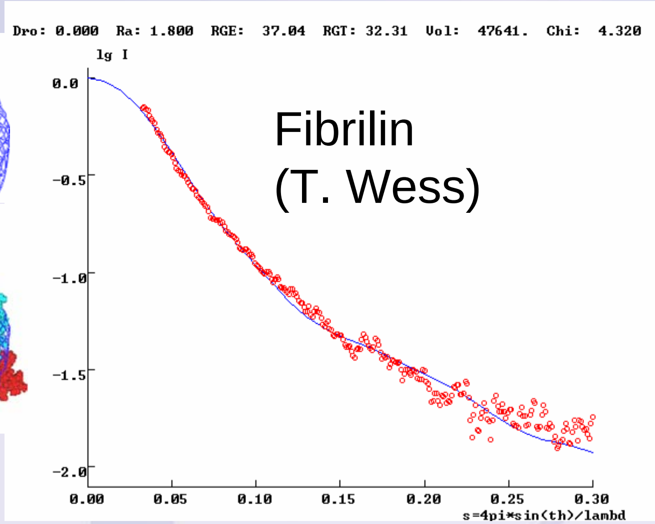
Applications in Biology

2. Non-crystalline diffraction: size and shape of molecules and particles: geometric parameters of natural fibres (DNA, muscle, collagen)

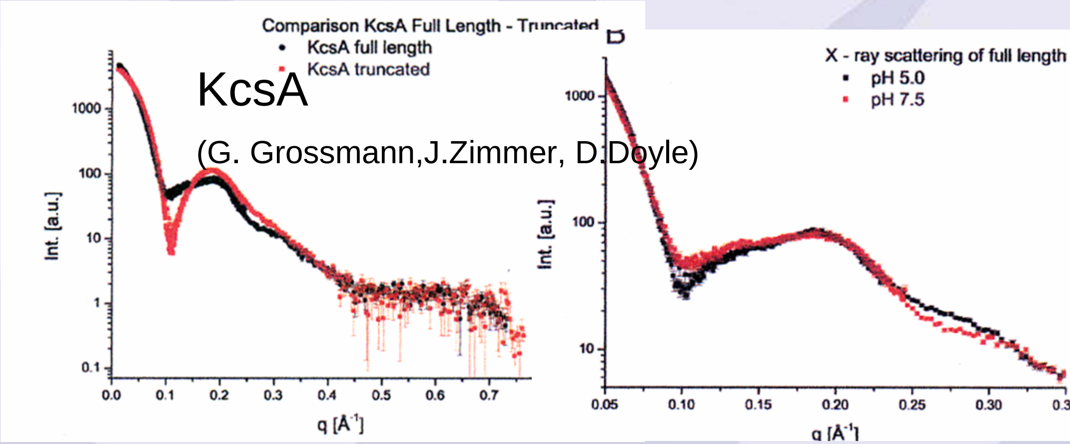
Non-Crystalline diffraction

- **Fibre diffraction and X-ray solution scattering**
  Natural fibres e.g. muscle, cornea, membranes, amyloid fibres etc
  Macromolecules and complexes in solution: overall shape and dimensions
- **Spot size: 70µ x 300µ**
- **With Micro-focus: 1µ x 1 µ**
- **Energy range 4-20 keV**
- **Small angle and wide angle X-ray cameras with area and linear detectors for static and time resolved measurements**
- **Detector Development Programme**
- **Sub-millisecond time resolved studies**





Fibrilin
(T. Wess)



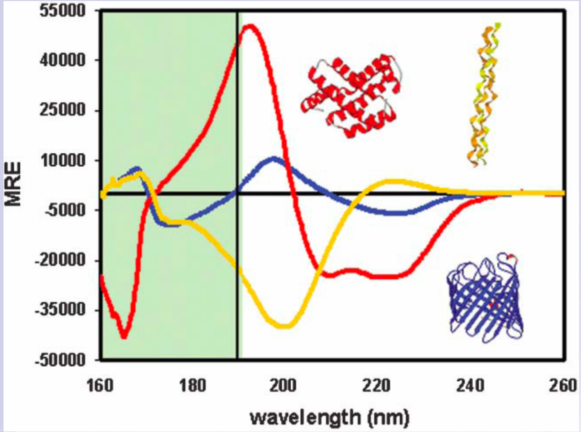
KcsA

(G. Grossmann,J.Zimmer, D.Doyle)

Applications in Biology

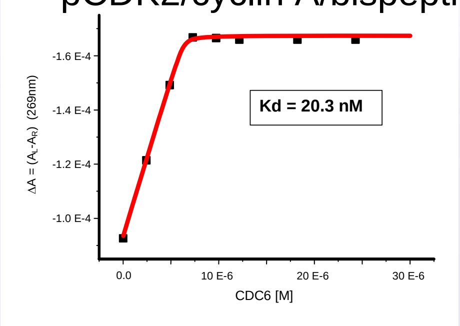
3. Circular dichroism

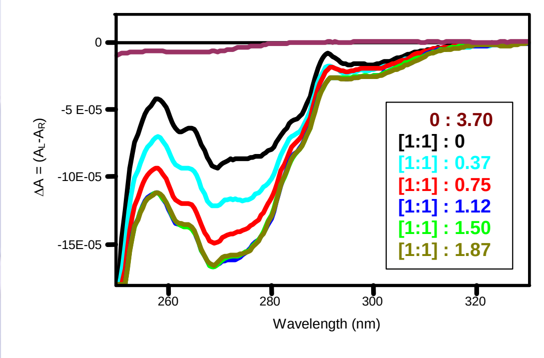
Circular Dichroism



- A bending magnet beam line to allow the measurement of circular dichroism from solution of chiral molecules in the wavelength range of 150-1000 nm
- The small size of the beam, its brightness and the possibility of using light down to 150 nm allows small or more dilute samples to be used and experimental times reduced from days to hours.
- Measurements are used to monitor secondary structure composition of proteins, protein folding in time resolved experiments, and protein/protein or protein/ligand interactions.

pCDK2/cyclin A/bispeptide complex



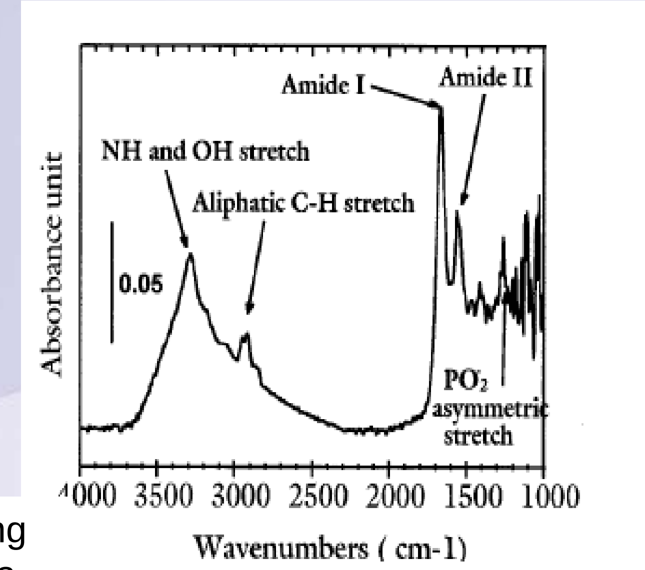


Applications in Biology

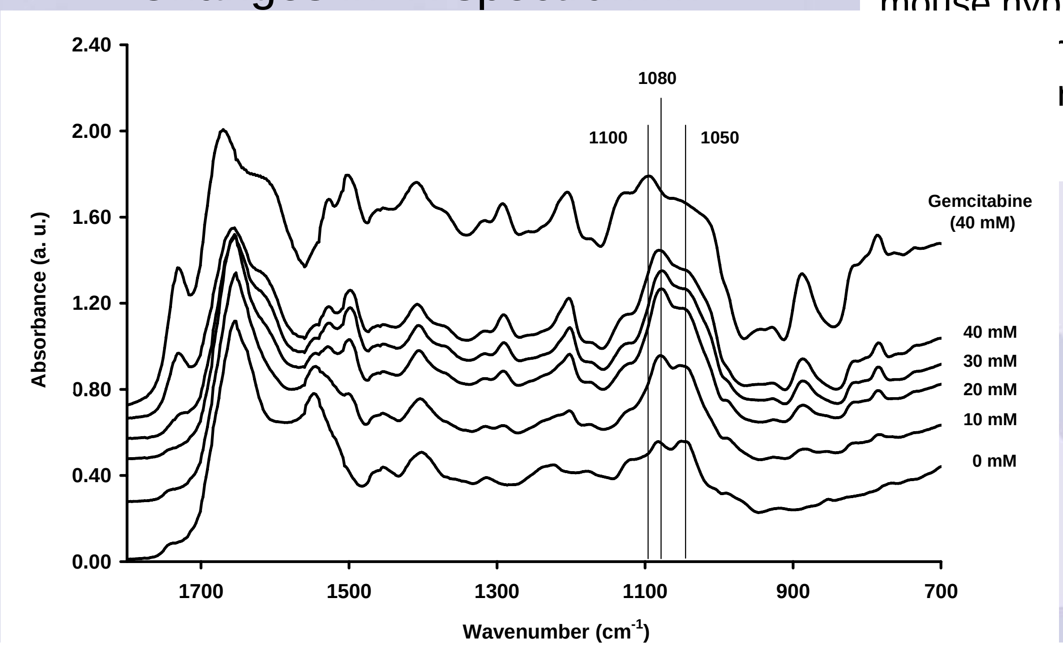
4. Infrared microspectroscopy

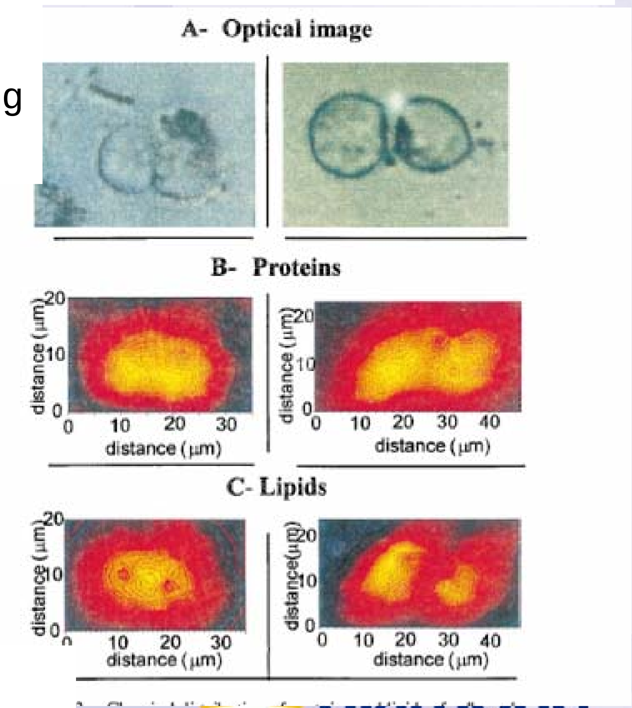
Infra-red Microspectroscopy

- Different molecules give different IR spectra - spectra can be used as diagnostic footprint
- Changes in IR spectrum in



IR spectra of living
mouse hybridoma
e size
recording





Micro-FTIR spectra of A549 lung cancer cell line following different doses of gemcitabine. Top gemcitabine alone In culture medium. (J. Sule-Seso (Keele & N. Staffordshire Hospital))

Jamin, N. et al PNAS (1998)

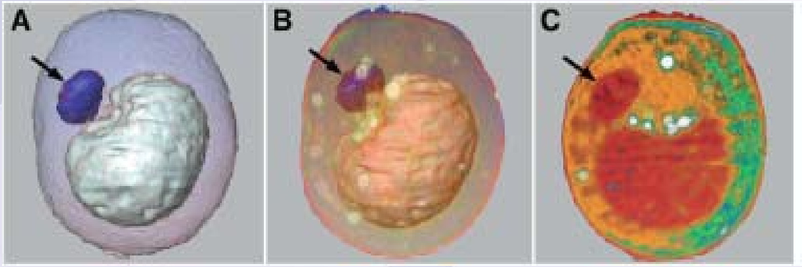
Applications in Biology

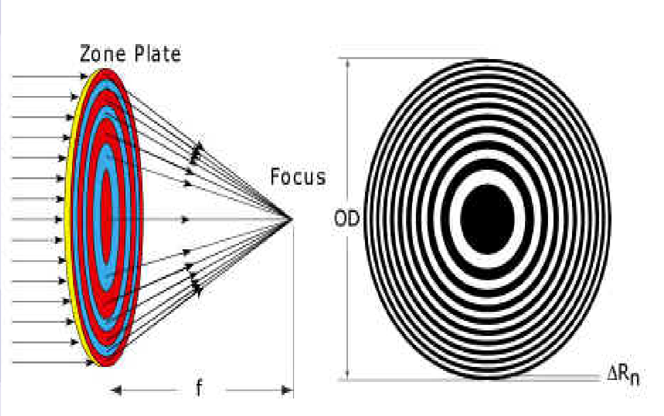
5. X-ray imaging

Soft X-ray Microscopy beamline

C. Larabell & M. Le Gross. Yeast cell 60 nm resoln.
Imaged with X-rays λ=2.4 nm & Fresnel zone plate

- **Imaging beamline**
- **Energy 90 - 2500 eV (137 - 4.96 Å)**
- **Fresnel zone plates used for focusing**
- **Spatial resolution in range 20-60 nm**
- **Penetration depth of X-rays would allow intact cells to be examined but specimens would need to be frozen to alleviate radiation damage**
- **3D tomography of whole cells up to 10 μ thick achieved at ALS, BESSY and ESRF**





Cryo-electron tomography of a *Dictostelium* cell; resolution ~ 5-6 nm

Medalia, O et al & Baumeister W. (2002) Science, 298, 1209-1213

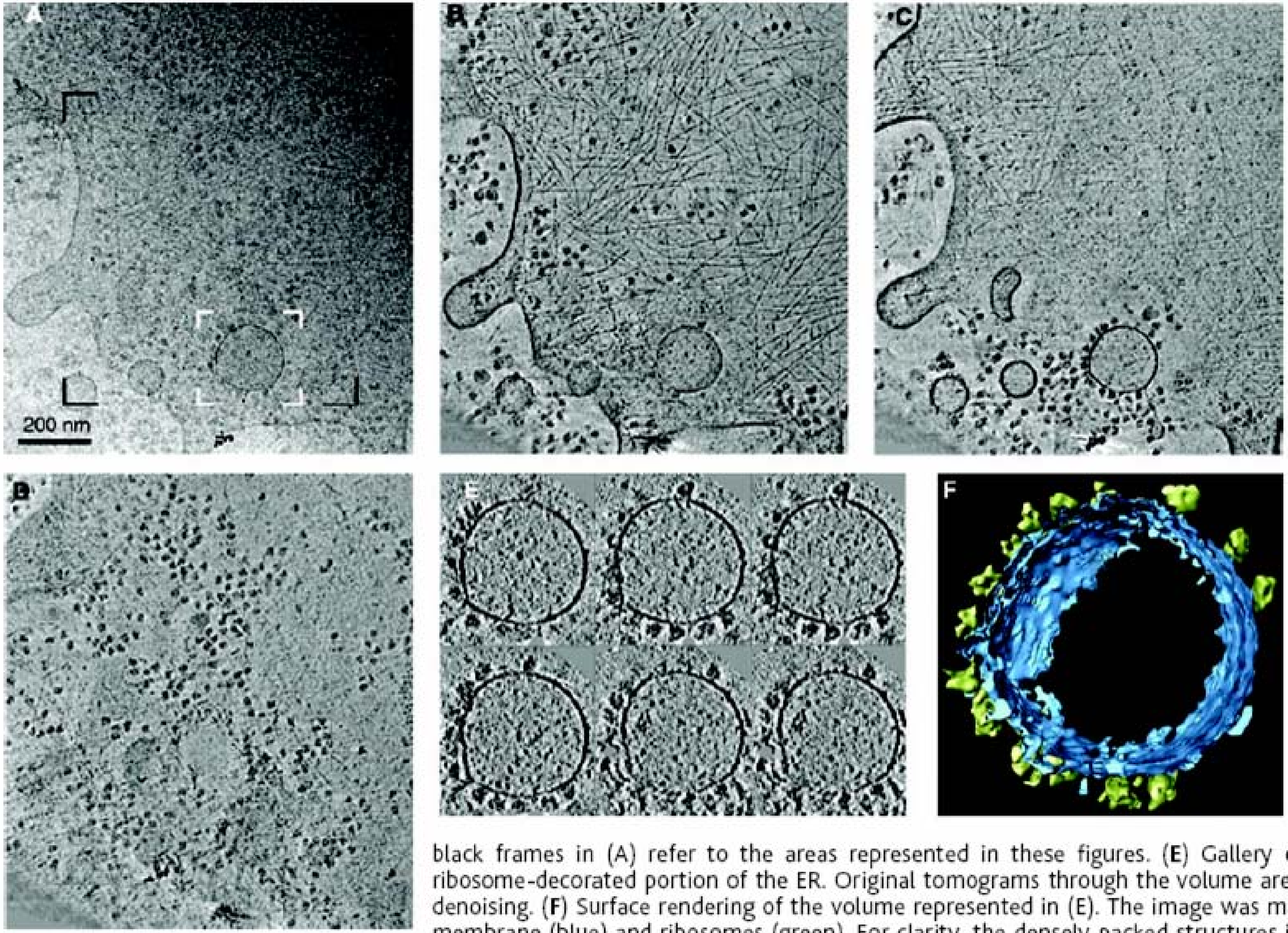


Fig. 1. Peripheral region of a vitrified *Dictyostelium* cell analyzed by cryo-ET. (**A**) Conventional transmission electron micrograph (2D projection) of the region used for recording a tilt series. Thickness of the cell in this region varied from 200 to 350 nm. (**B** to **D**) Series of successive tomographic *x-y* slices of 60-nm thickness from bottom to top of the substrate-attached region of the cell. In (B) and (C), portions of the plasma membrane that are perpendicular to the *x-y* plane can be recognized. In (B), the cortical actin network is viewed. Detailed images of the molecular complexes and vesicular structures are shown in (E) and in Fig. 3; white and black frames in (A) refer to the areas represented in these figures. (**E**) Gallery of *x-y* slices through a ribosome-decorated portion of the ER. Original tomograms through the volume are displayed without any denoising. (**F**) Surface rendering of the volume represented in (E). The image was manually segmented into membrane (blue) and ribosomes (green). For clarity, the densely packed structures in the lumen have been omitted.

E. Coli imaged from coherent scattering 30 nm resoln. and λ = 2 Å

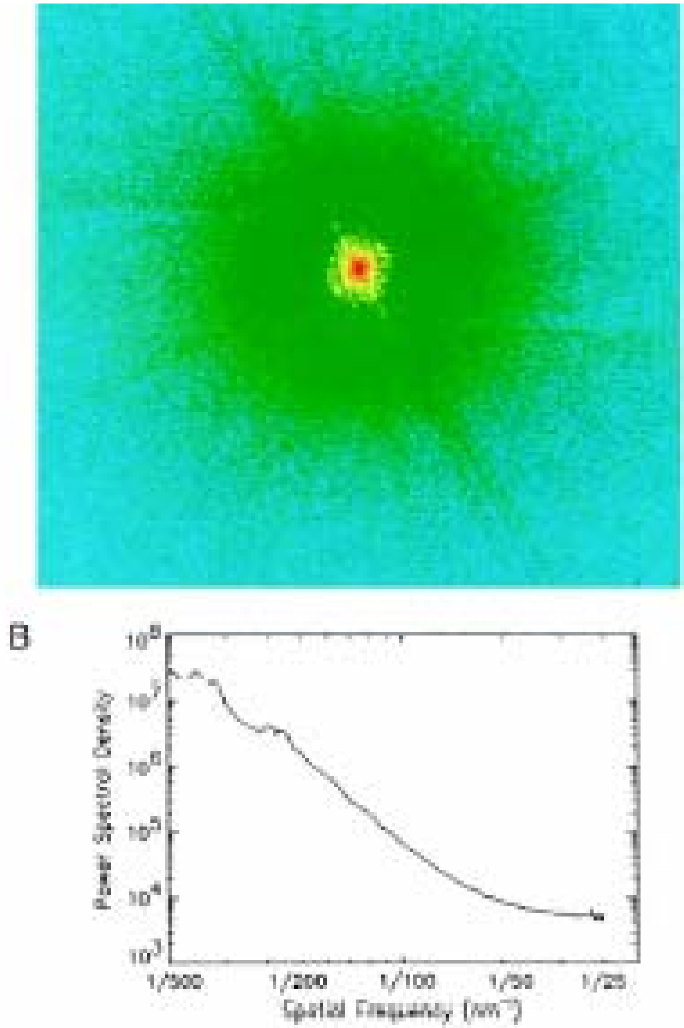


Fig. 1. (A) A diffraction pattern from *E. coli* bacteria displayed in a logarithmic scale. (B) The power spectral density of the diffraction pattern, which indicates the resolution extends to 30 nm.

Fig. 2. An image reconstructed from Fig. 1. The dense regions inside the bacteria are likely the distribution of proteins labeled with $KMnO_4$. The semitransparent regions are devoid of yellow fluorescent proteins.

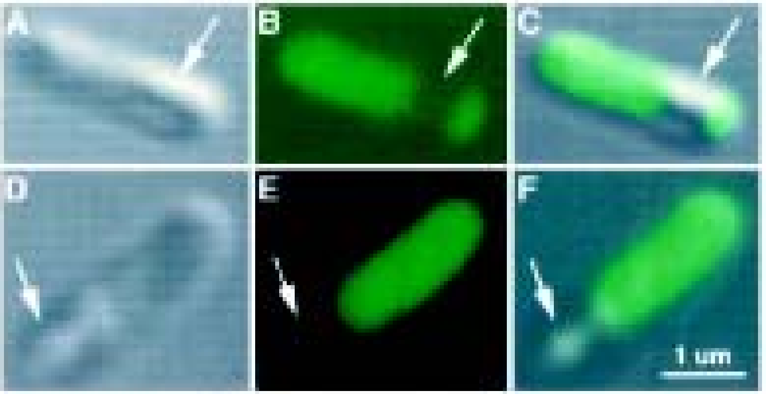


Fig. 3. *E. coli* expressing the indicator protein. Individual bacteria are seen using transmitted light (A and D) and fluorescence (B and E), where the yellow fluorescent protein (green) is seen throughout most of the bacteria except for one small region in each bacterium that is free of fluorescence (arrows), consistent with Fig. 2. C and F show the fluorescent image superimposed on the transmitted light image.

Miao, Hodgson et al (2003) Proc.Natl. Acad. Sci., 100, 110

Applications in Biology

6. Medical beam line e.g. ID17 at ESRF, Grenoble

Medical Beamline at ESRF, Grenoble ID17

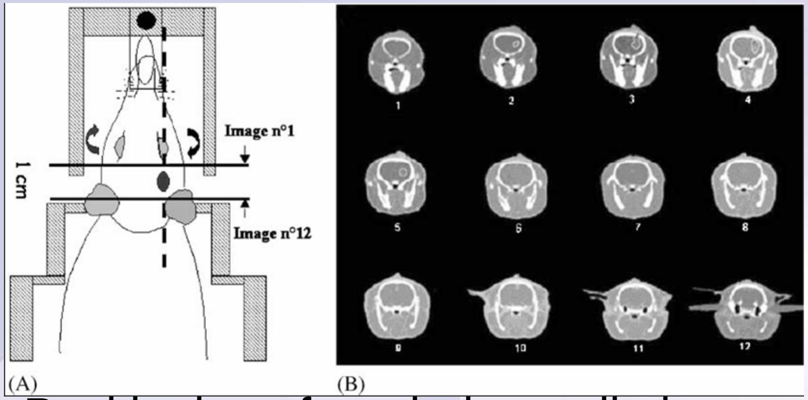
- Angiography, computed tomography
- Distance from source 140 m
- 15-90 keV
- Max beam size at patient 150 x 10 mm^2
- Flux 2 x 10^{14} ph/s 0.1% bw, 0.1 A, mradh.
- max radiation dose 0.2 Gy

- **Coronary angiography** -imaging arteries in human volunteers (~30)- contrast agent (iodine or gadolinium) injected intravenously.Good agreement with conventional angiography.Programme discontinued.
- **Functional lung imaging** with xenon in rabbits
- **Diffraction enhanced imaging** for breast cancer. Soft tissue imaging ; measures regions of different refractive indices using phase contrast. Doses < 0.1 mGy
- **Microbeam radiation therapy.** Beam 10-30 μm wide 200 μm spacing. Parallel array of microbeams used to treat brain tumours.Tested with rats and piglets.
- **Photon activation therapy.** Radiotherapy of tumours loaded with $Pt(NH_2)_2Cl_2$. Irradiation with monochromatic beam results in release of Auger electrons. Tested with rats bearing gliomas.

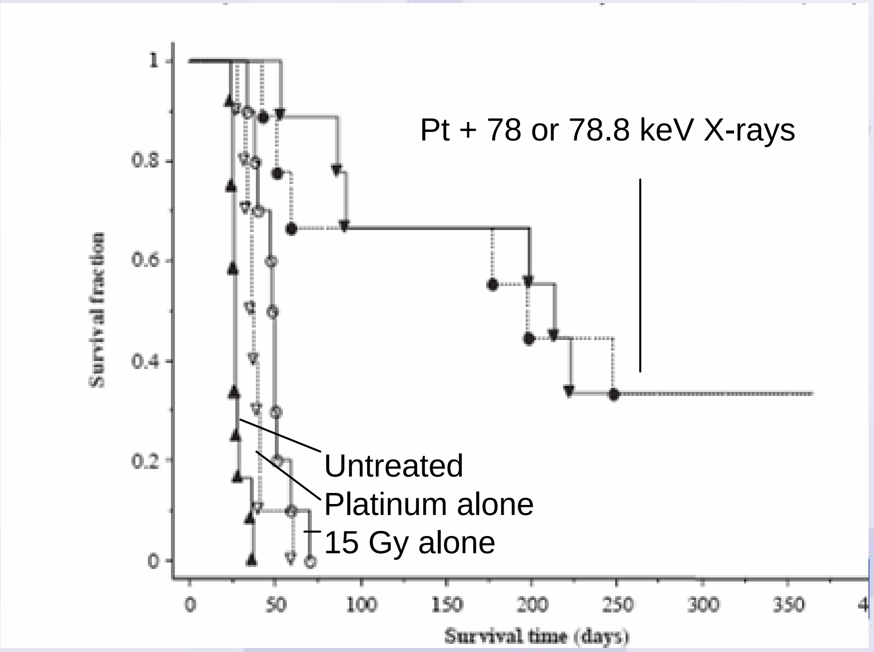
Contrast enhanced synchrotron stereotactic radiotherapy

(Biston, M-C et al. (2004) Cancer Research 64, 2317)

- Cure of rats bearing radioresistant F98 Glioma treated with cis-platinum and irradiated with synchrotron X-rays.
- Following incorporation of platinum agent into cerebral tumour, dose enhancement to tumour was achieved with 78 keV X-rays delivered stereotactically in a tomographic mode.
- Entering Phase I clinical trials 2007



Positioning of rat during radiation treatment



Survival curves after treatment:

Diamond Light Source

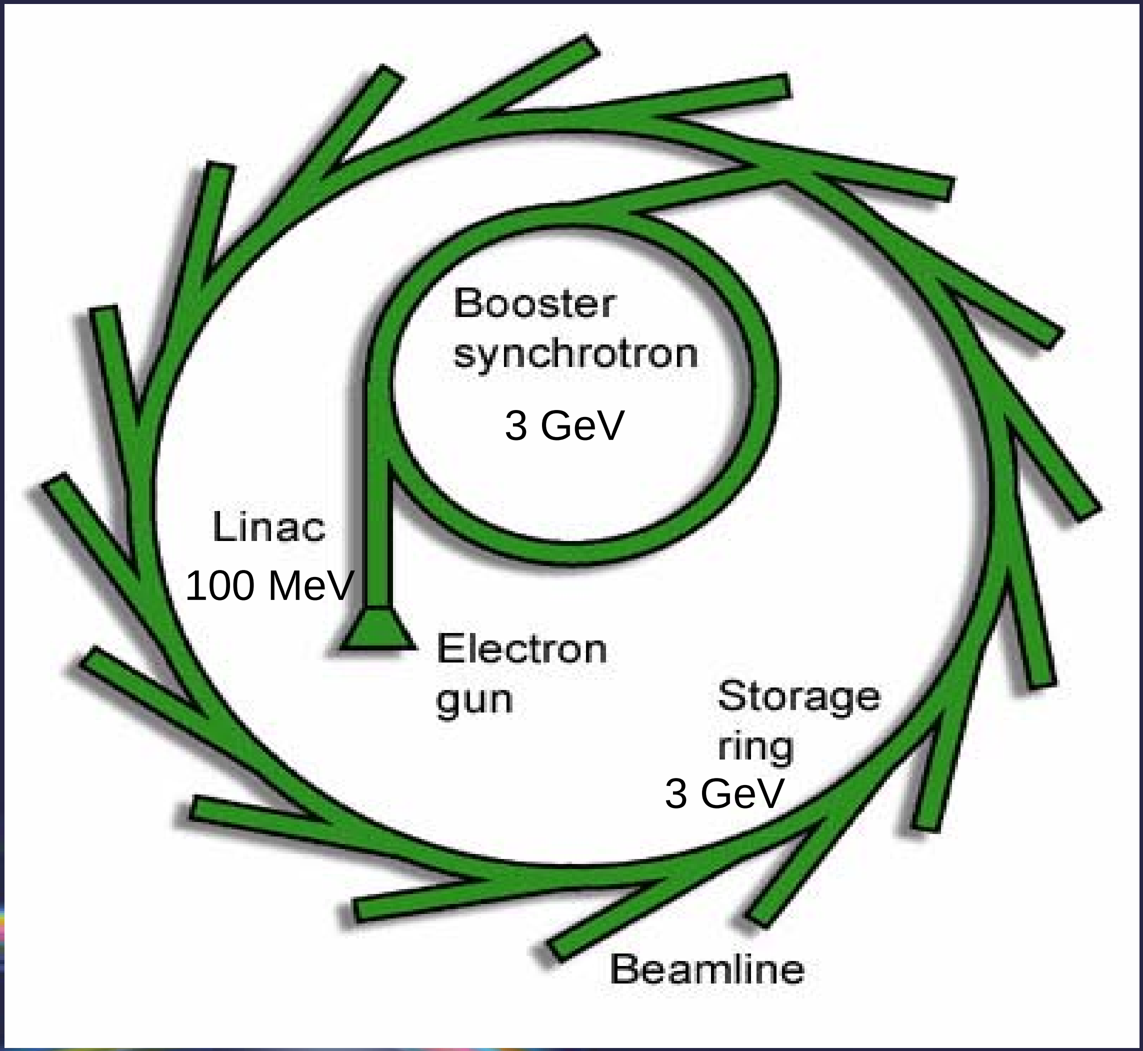
• The UK's new synchrotron X-radiation source

- Diamond is the largest single scientific investment in the UK for 30 years ~ £263 M (Phase I)
- Private Limited Company
  - Funded by UK Government through OSI (STFC) (86%) and the Wellcome Trust (14%)
- A source of extreme intensity light
  - 10^6 brighter than a laboratory X-ray generator -
  - wavelengths range from infra-red to hard X-rays.
- Application in diffraction, spectroscopy and imaging

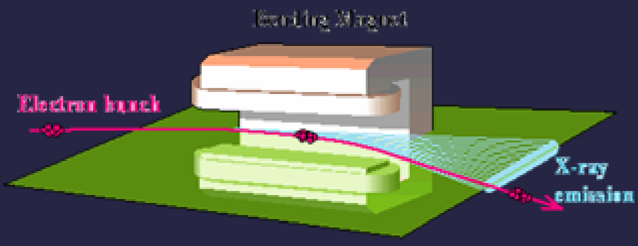
- Structural Biology
- Pharmaceuticals – drug design
- Chemistry - structure; reaction states; corrosion, archaeology; food processing
- Materials – understanding new materials, surface science, thin films, electrochemistry, magnetism, magnetic properties
- Nanoscience -catalysts and chiral structures, nanomagnetism, nanostructures
- Earth & Environmental sciences – investigating pollution levels in the environment (corals);
- extreme conditions : material under high pressure and temperature

diamond

How the light is generated



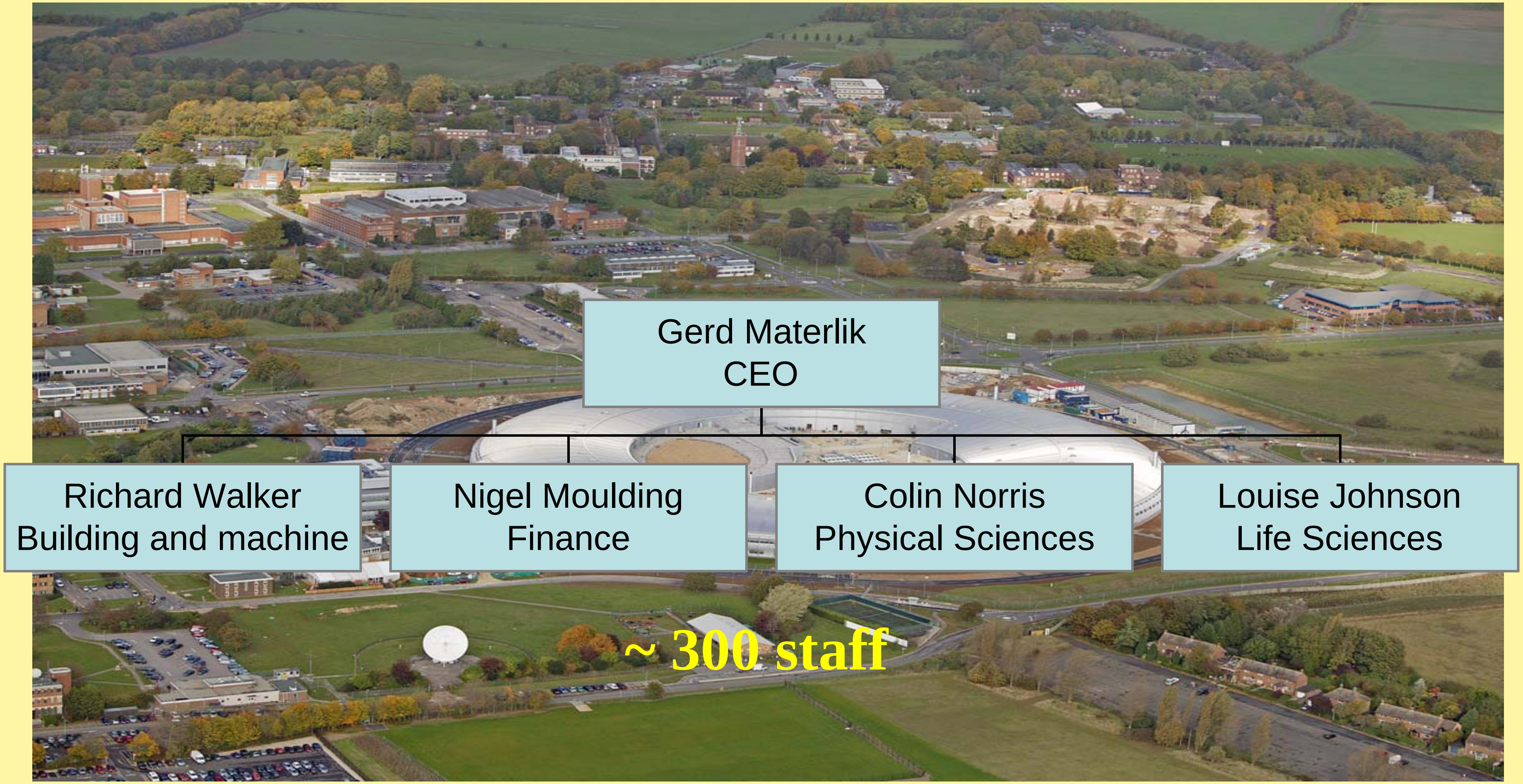
A quick overview of how it works…



Undulator

diamond

Diamond Spring 2006…
(start March 2003: Users February 2007)



24 Cells
Insertion device straights: 4 x 8 m plus 18 x 5m

Electron Beam Energy	3 GeV		
Beam current	300 mA (500 mA)	Beam lifetime: >10h (20h)	
Beam Emittance	2.7 nm rad (horizontal)		
Circumference	561.6 m.	Diameter of outer wall	235 m

Linac (2005)

Installation complete:	Aug. 3rd
1st beam from gun:	Aug. 31st
1st 100 MeV beam:	Sep. 7th
Acceptance tests complete:	Nov. 10th

Booster

- Electrons in Booster December 2005

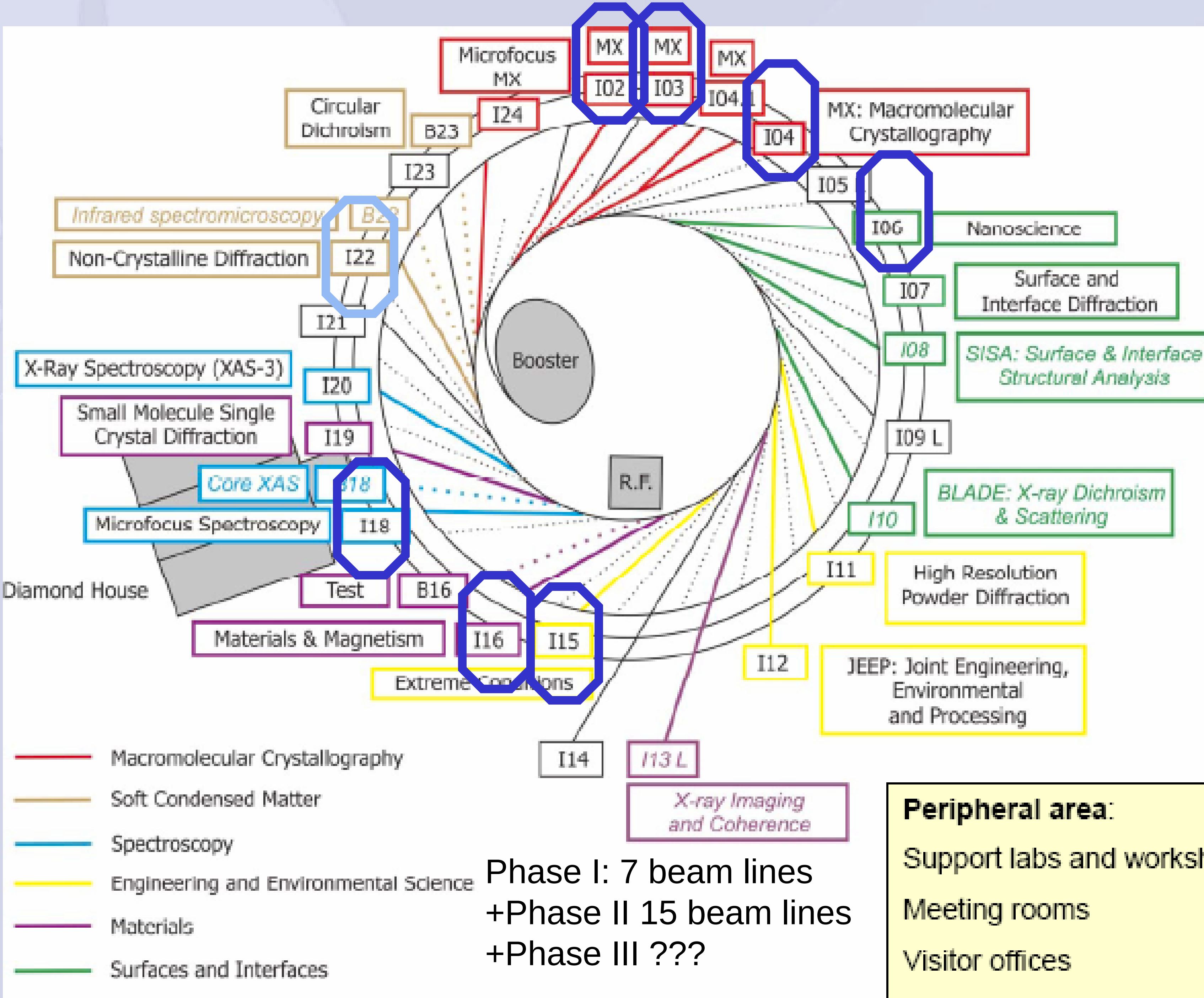
Storage Ring

-May 2006: First electrons in storage ring at 700 MeV

-Installation of 7 insertion devices

-October 2006 first light in beam line I06

-January 2007: 3 GeV, current 120 mA, lifetime 10 h



Phase I: 7 beam lines
+Phase II 15 beam lines
+Phase III ???

Peripheral area:

Support labs and workshops

Meeting rooms

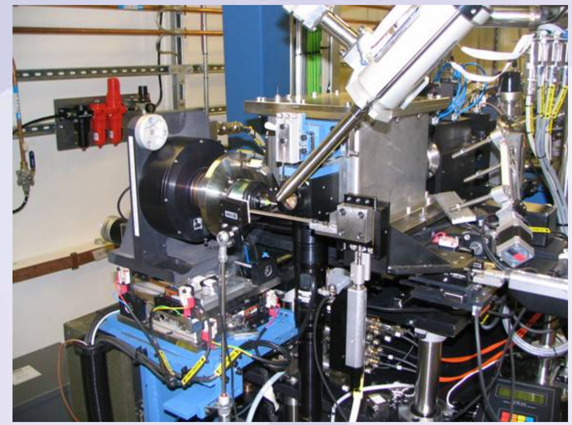
Visitor offices

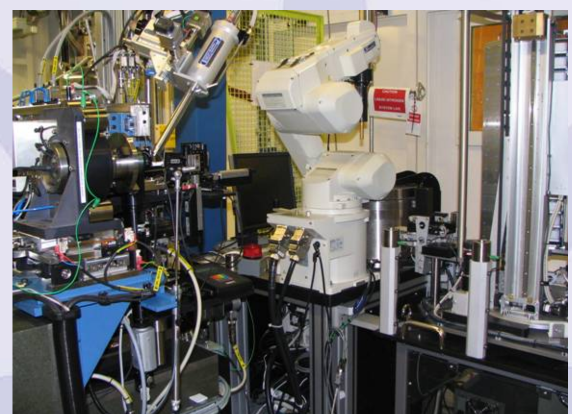
Staff

Macromolecular Crystallography

- **Phase I Liz Duke**
- 3 MX beam lines I02, I03, I04;
  MAD; automated; Cryo; one category 3
- Phase II Gwyndaf Evans
  Microfocus beam line I24
- Fixed wavelength side station I04.1 Jose Brandao-Neto
- Phase III
- Long wavelength MX

- Energy range 0.5 -2.5 Å, optimised for 0.98 Å (12.6 keV), spot size 94 μ(h) x 17 μ(v); flux 3.5 x 10^{12} ph/s.





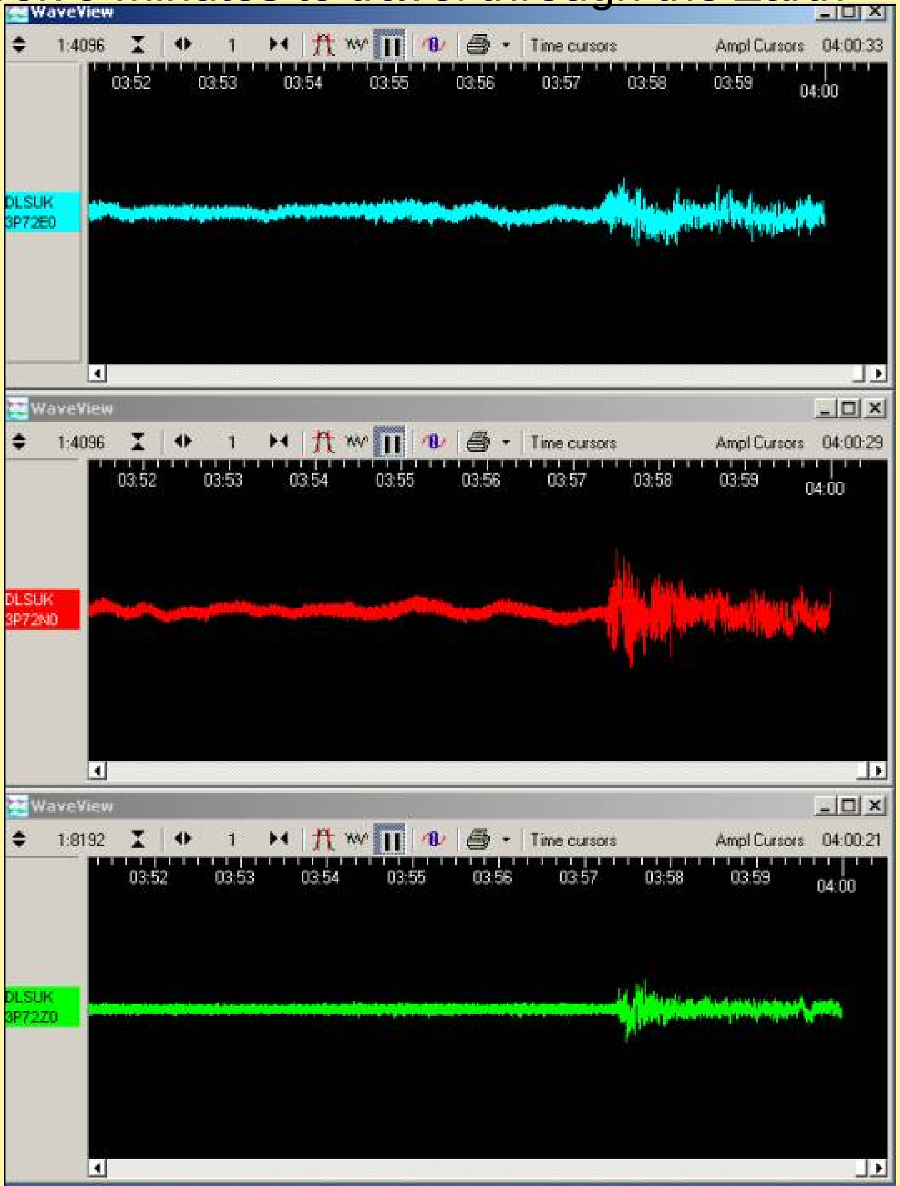
diamond

Foundations – Sept 2003

1500 piles each 15 m deep

Diamond 4am 8th October 2005

DLS Tri-axial seismometer sensors in Diamond House picked the signal of the Earthquake (7.6) in Pakistan 3,800 miles away. The signal took 9 minutes to travel through the Earth



Summary: Biological applications of synchrotron radiation

- **Macromolecular crystallography**
  - Molecular understanding of structural biology
  - Structure based drug design
- **Non-crystalline diffraction**
  - Size and shape of molecules
  - Forensic and archaeological information e.g. bone and collagen
- **Circular dichroism**
  - Solution studies
- **Infrared microspectroscopy**
  - Imaging of cells; element specific imaging
- **X-ray Imaging**
  - Great potential but has to complement optical confocal microscopy and electron microscopy
- **Medical**
  - Contrast enhanced stereotactic radiotherapy
  - Microbeam radiation therapy

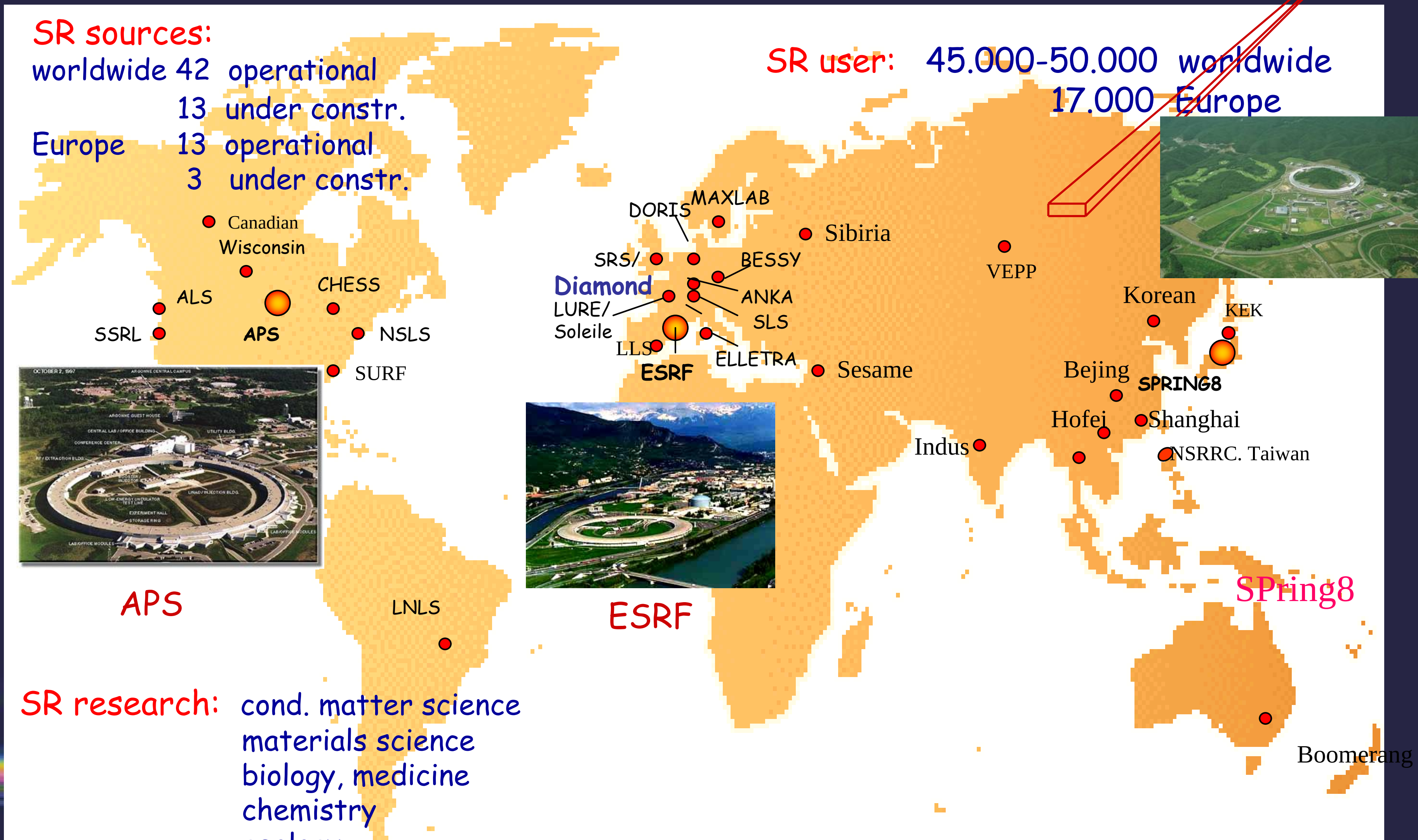
- Light is a messenger, carrying a story about the form of the object …

Lawrence Bragg, 1928

Synchrotron Radiation Sources worldwide

SR sources:
worldwide 42 operational
13 under constr.
Europe 13 operational
3 under constr.

SR user: 45.000-50.000 worldwide
17.000 Europe



APS

ESRF

SPring8

SR research: cond. matter science
materials science
biology, medicine
chemistry
geology

diamond

The structure of the multidrug transporter AcrB:

Murakami, S et al & Yamaguchi, A Nature (2006) 443, 173

Seeger, MA et al & Pos, KM Science (2006) 313, 1295

Use of Br anomalous to locate drug at 5 Å resolution λ = 0.918 Å