10th Oxford School on Neutron Scattering

Mansfield College, Oxford

Life Science Research and Development: from Structures to Systems

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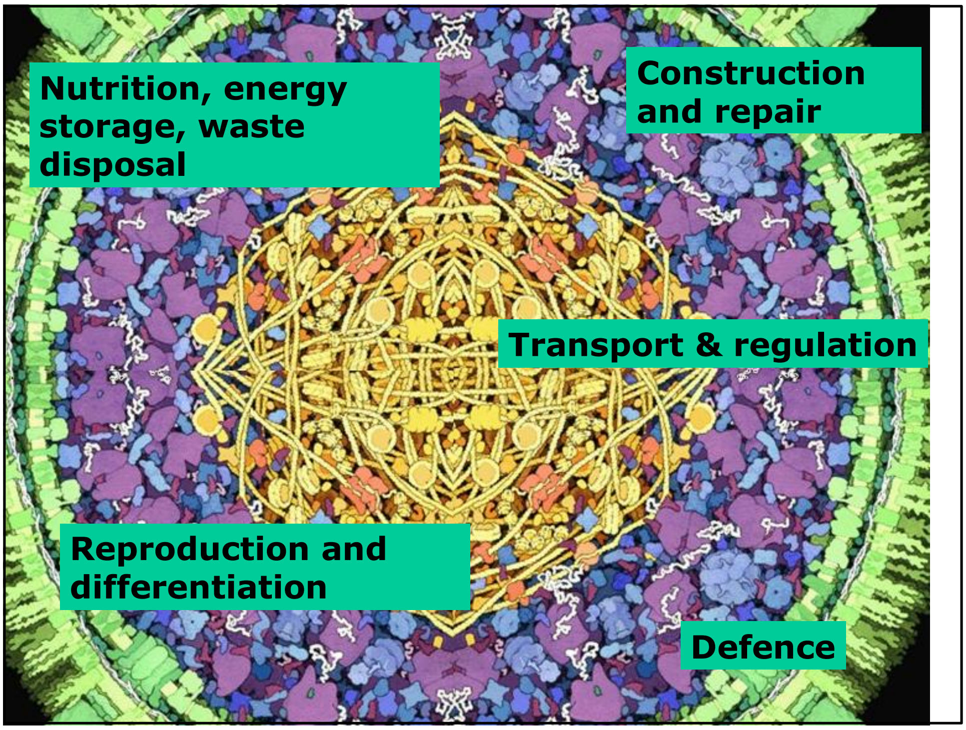
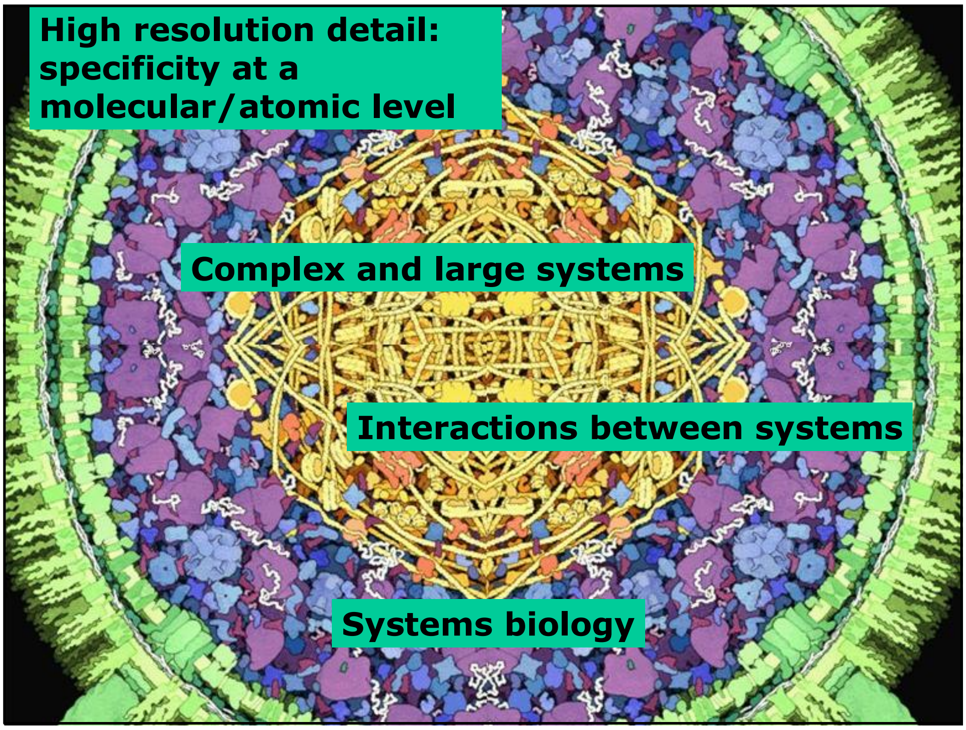
13/14th September 2007

The action-packed, crowded cell

D. Goodsell: the *E. Coli* cell

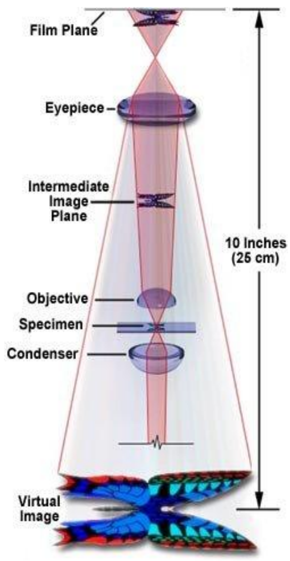
D. Goodsell: HIV under attack

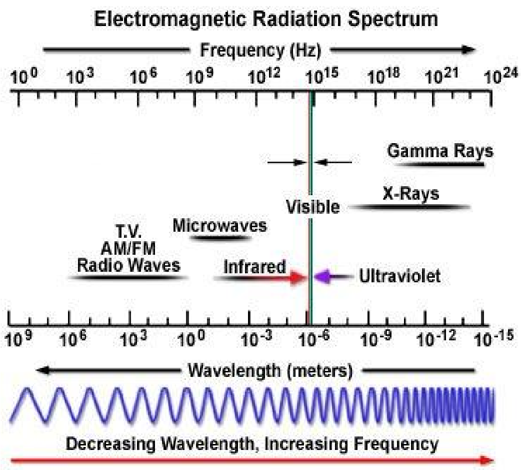

Nutrition, energy storage, waste disposal
Construction and repair
Transport & regulation
Reproduction and differentiation
Defence


High resolution detail: specificity at a molecular/atomic level
Complex and large systems
Interactions between systems
Systems biology

The Grenoble Campus

Seeing small things



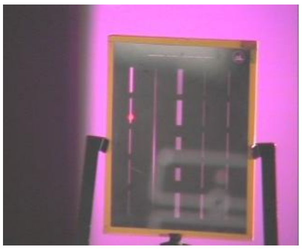


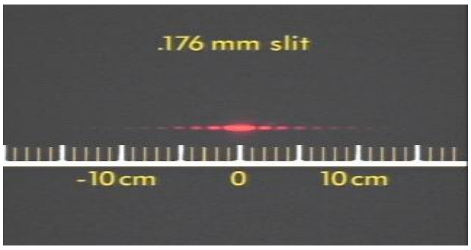
From microscopy to diffraction

Diffraction by water waves

Diffraction by light

Diffraction of light by a single slit





From microscopy to diffraction

Diffraction by water waves

Diffraction by light

Diffraction by x-rays

Diffraction by neutrons

X-rays and neutrons

X-rays

- Definitive molecular structures
- Small samples (~1μm)
- Time-resolved data
- Sample damage issues

Neutrons

- Low resolution - contrast variation using mixtures of D_2O and H_2O
- High resolution crystallographic experiments, particularly for location of hydrogen atoms
- Fibre diffraction - location of water and hydrogen atoms in filamentous molecules eg nucleic acids, cellulose, filamentous viruses
- Dynamics
- No sample damage

Scattering lengths

Element	Neutrons	X Rays
	$b \times 10^{13}$ (cm)	$b \times 10^{13}$ (cm)
H	-3.74	3.8
D	6.67	2.8
C	6.65	16.9
N	9.40	19.7
O	5.80	22.5
P	5.10	42.3
S	2.85	45.0
Mn	-3.60	70.0
Fe	9.51	73.0
Pt	9.50	220.0

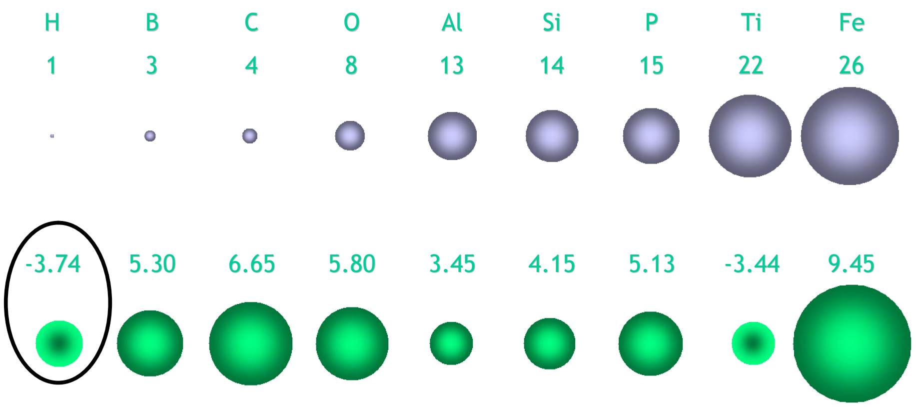
X-ray and neutron scattering

X-ray

Scattering proportional to Z

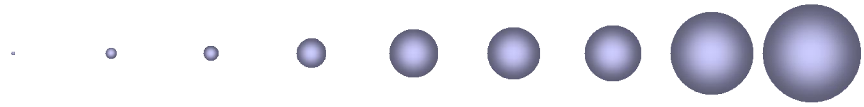
Neutron

Scattering not proportional to Z

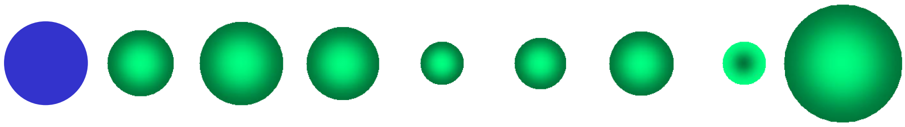


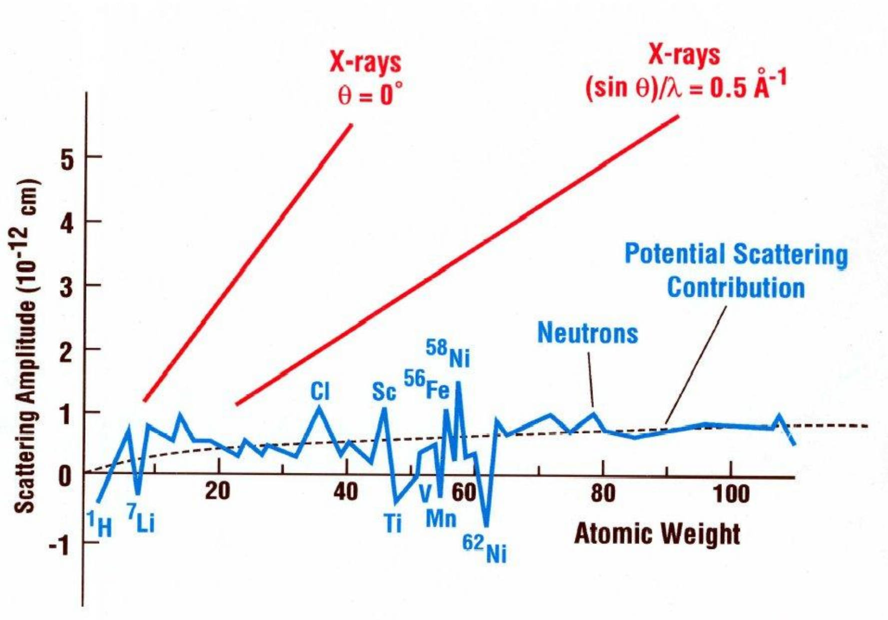
X-ray and neutron scattering

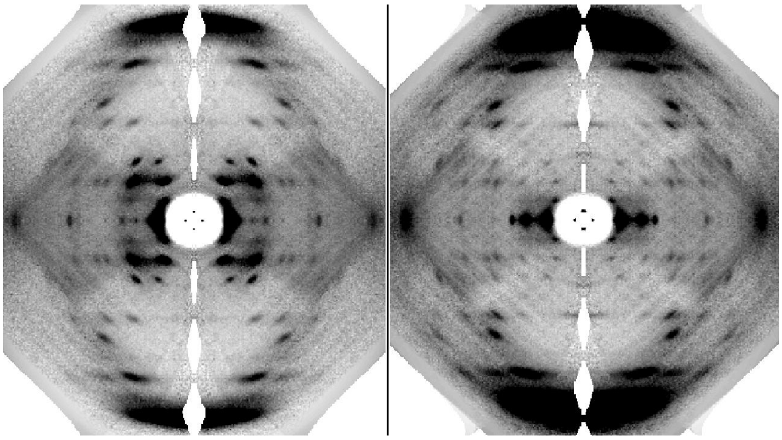
X-ray					Neutron			
Scattering proportional to Z					Scattering not proportional to Z			
H	B	C	O	Al	Si	P	Ti	Fe
1	3	4	8	13	14	15	22	26



6.67	5.30	6.65	5.80	3.45	4.15	5.13	-3.44	9.45

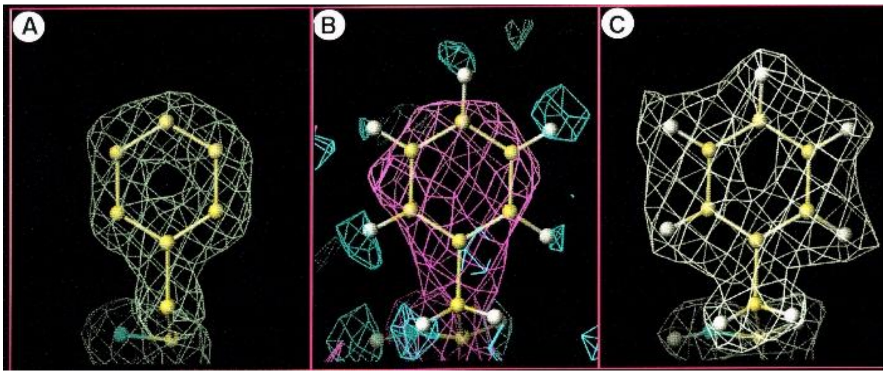






DNA Hydrated with H_2O　　DNA Hydrated with D_2O

Deuterated myoglobin



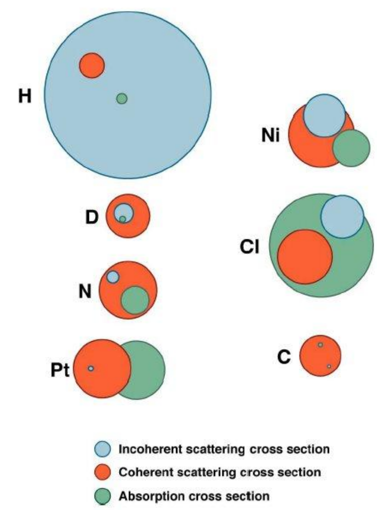
$2F_o - F_c$ x-ray map (6A → 1.5A data)

F_c neutron map, unlabelled protein (6A → 2A data)

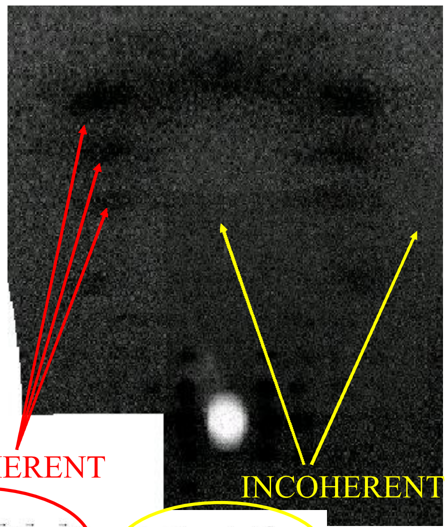
$2F_o - F_c$ neutron map, deuterated protein (6A → 2A data)

Shu, Ramakrishnan & Schoenborn, *PNAS* 97(8), 3872 (2000)

Neutron Incoherent Cross-Sections

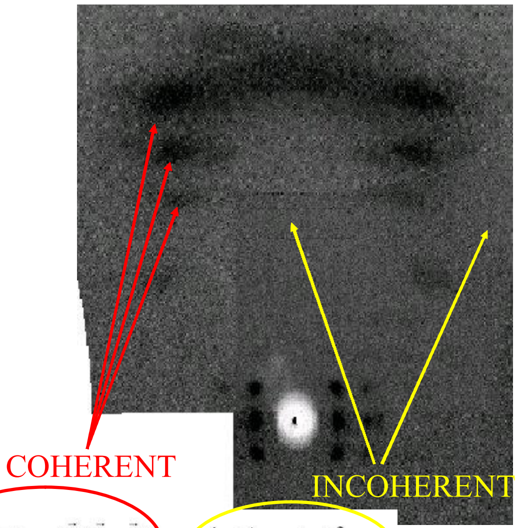


Coherent and incoherent scattering



$$\frac{d\sigma}{d\Omega} = \langle b\rangle^2 \sum_{i,j} e^{-i\vec{Q}.(\vec{R}_i - \vec{R}_j)} + (\langle b^2\rangle - \langle b\rangle^2)N$$

Coherent and incoherent scattering



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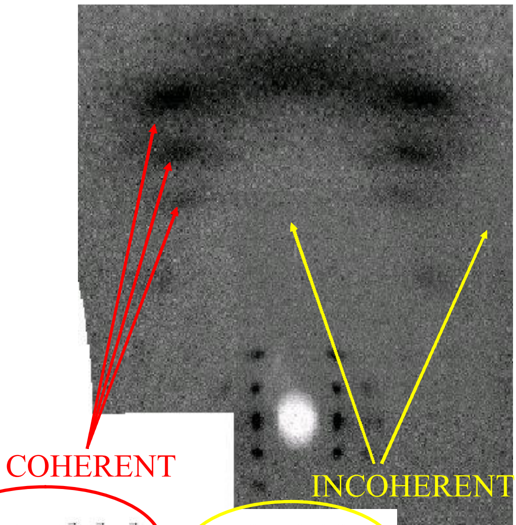

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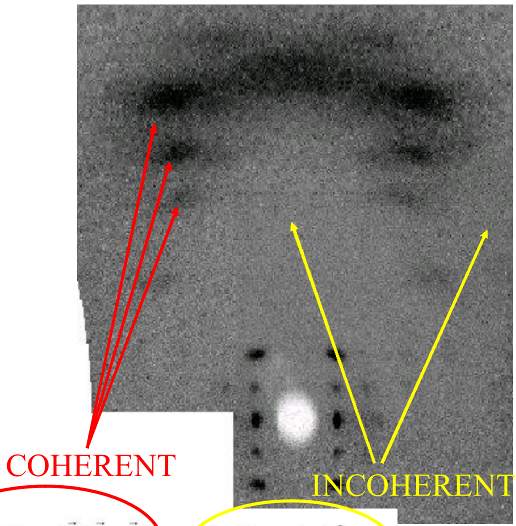
Coherent and incoherent scattering



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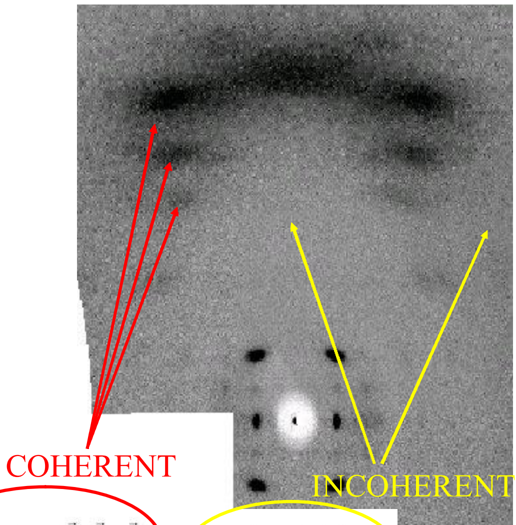
Coherent and incoherent scattering



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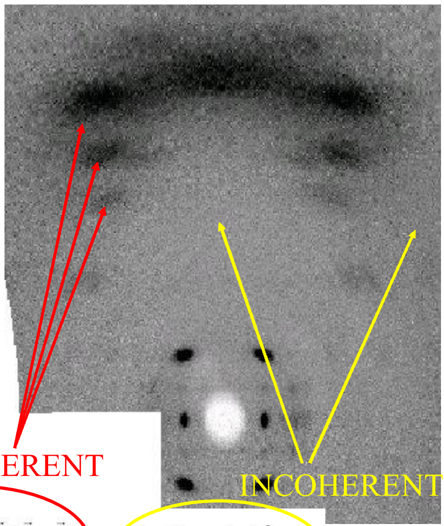
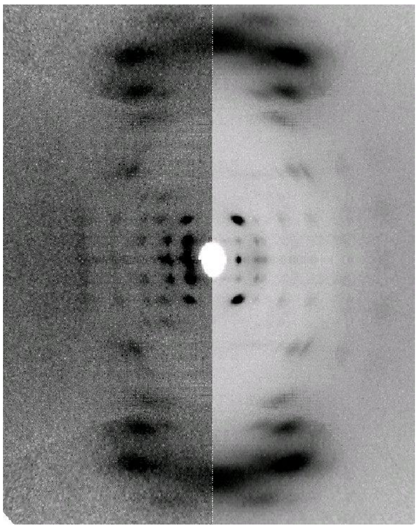
Coherent and incoherent scattering

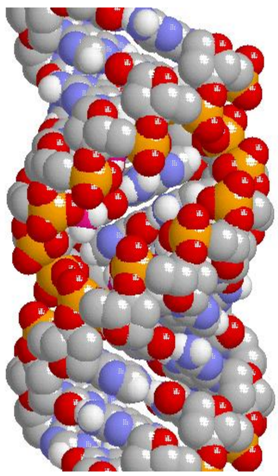


$$\frac{d\sigma}{d\Omega} = \langle b \rangle^2 \sum_{i,j} e^{-i\vec{Q}.(\vec{R}_i - \vec{R}_j)} + (\langle b^2 \rangle - \langle b \rangle^2)N$$

Coherent and incoherent scattering

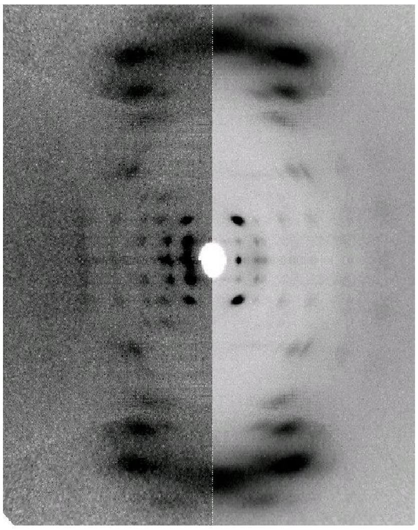


A-DNA/H2O A-DNA/D2O

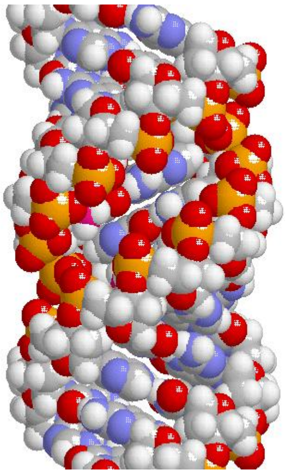


A-DNA without hydrogen

Coherent and incoherent scattering

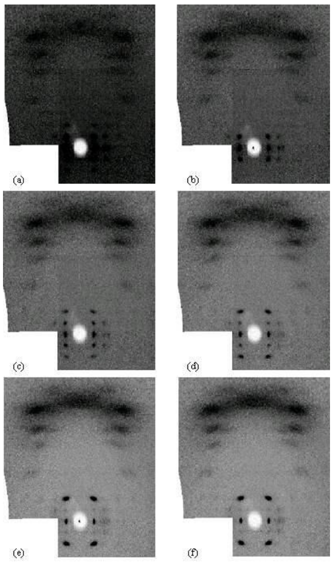


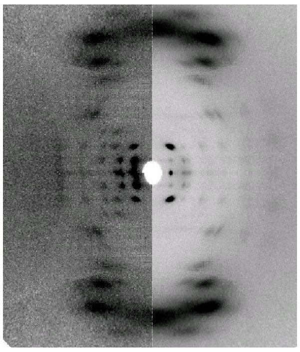
A-DNA/H2O A-DNA/D2O



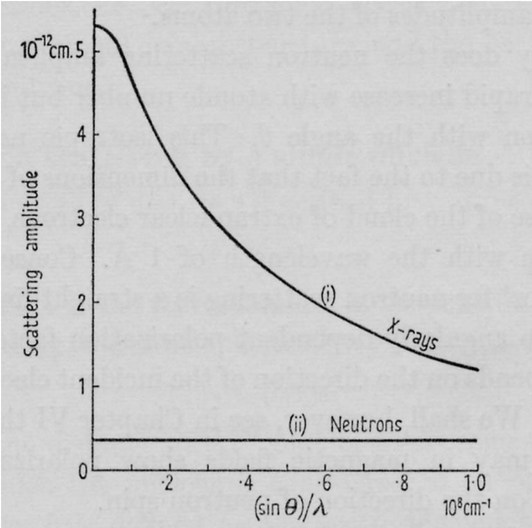
A-DNA with hydrogen

Coherent and incoherent scattering

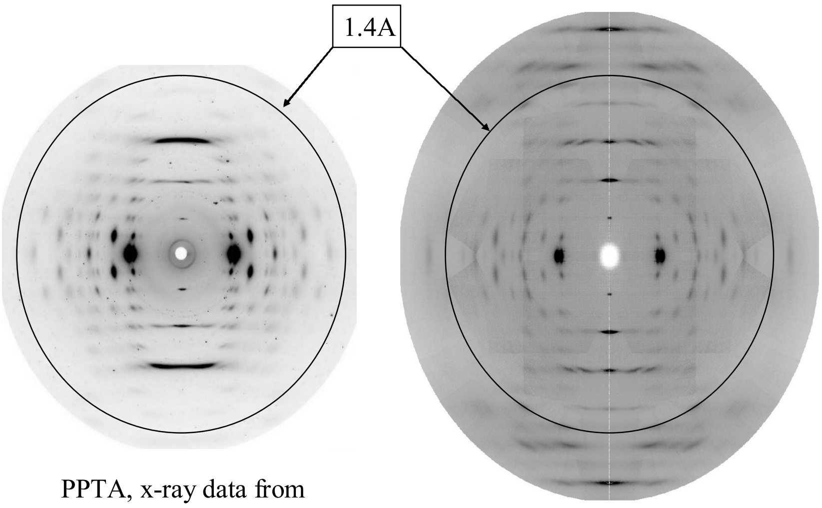




X-ray and neutron scattering amplitudes for a potassium atom



High resolution



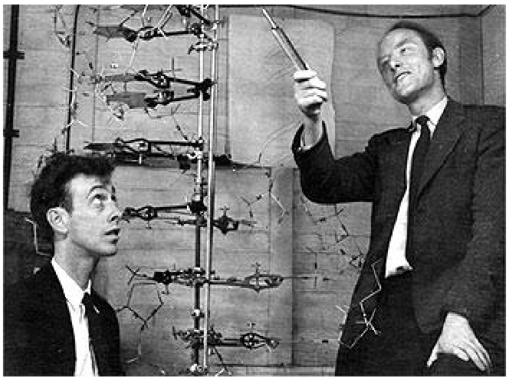
PPTA, x-ray data from APS
λ=0.8A

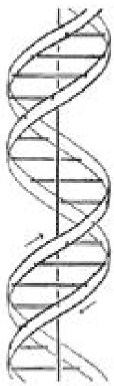
PPTA, neutron data from ILL Grenoble
λ=1.31A

Techniques for *structure* using neutron scattering

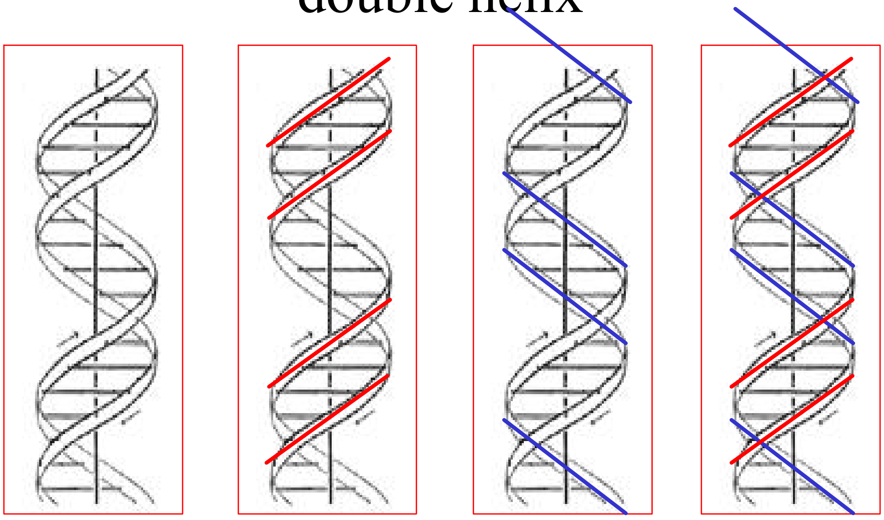
- High resolution crystallography
- Fibre diffraction
- Membrane diffraction
- Reflectometry
- Low resolution crystallography
- Small angle neutron scattering

The DNA Double Helix

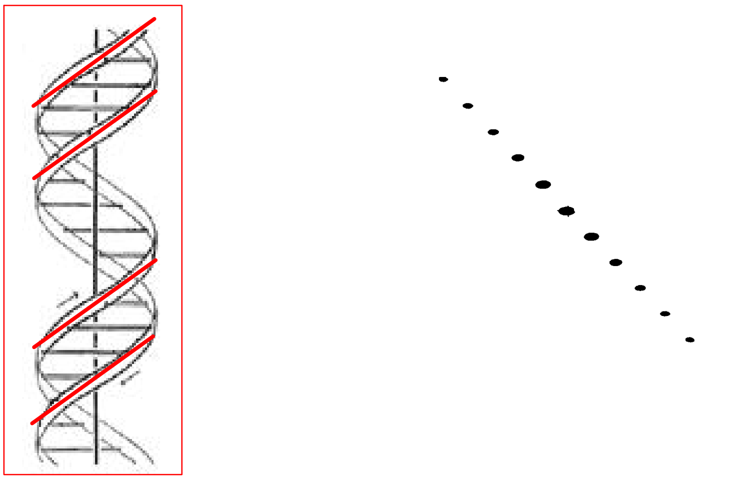




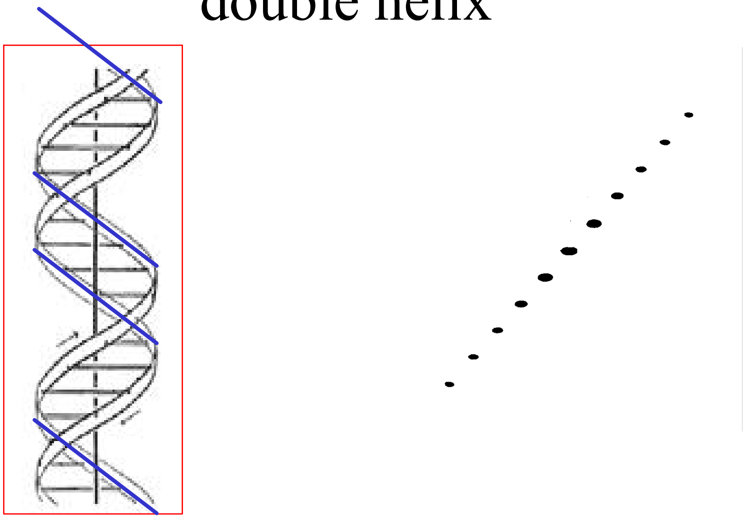
A simple representation of a double helix



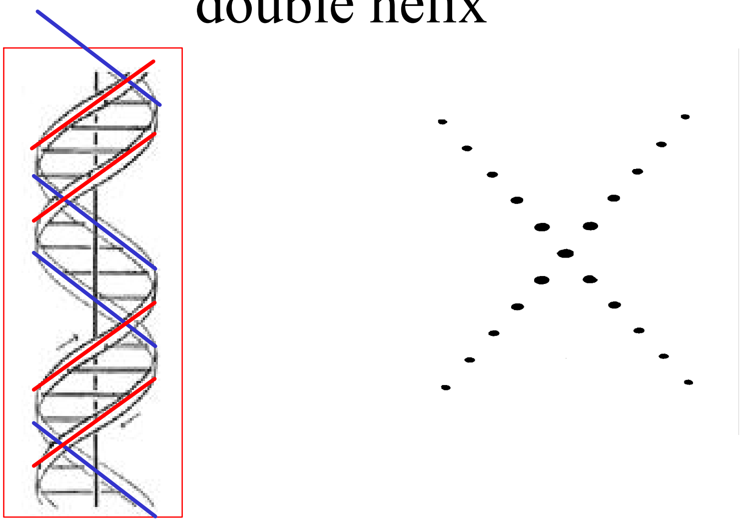
A simple representation of a double helix



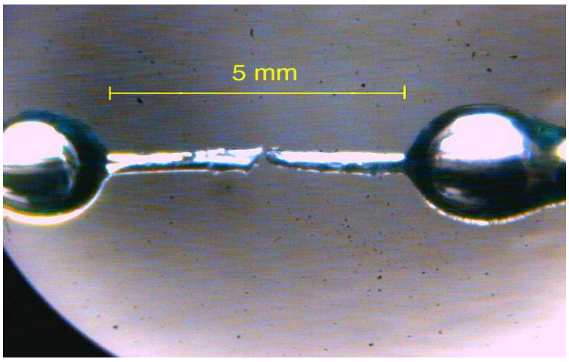
A simple representation of a double helix



A simple representation of a double helix

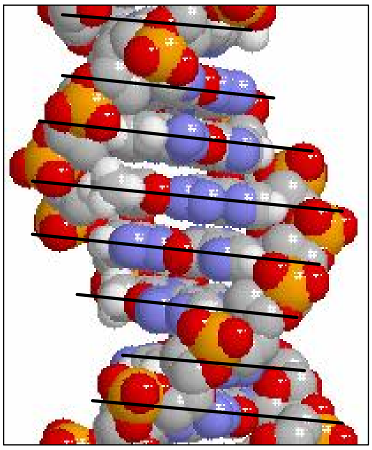


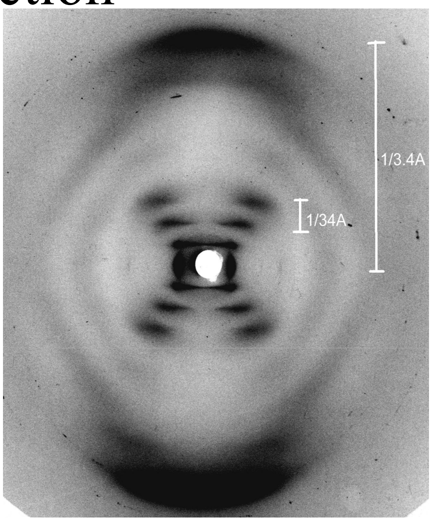
A fibre of DNA



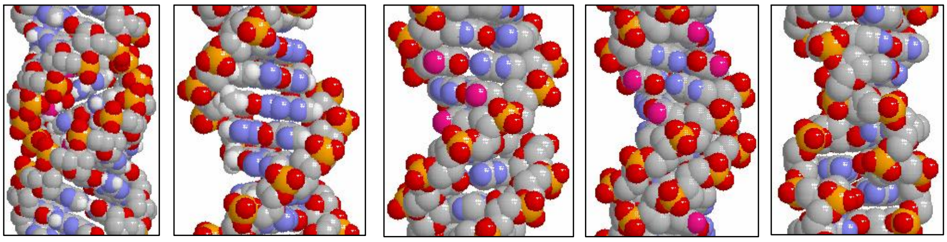
DNA fibre drawn between two glass rods

A simple view of DNA diffraction

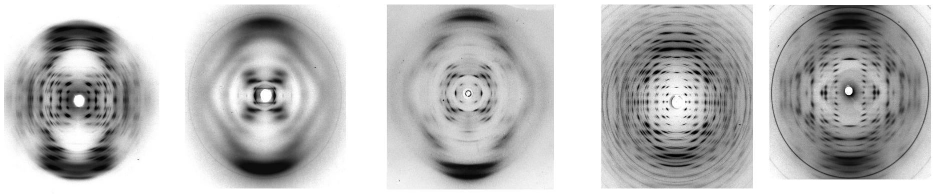




DNA polymorphism



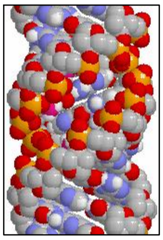
A-DNA	B-DNA	C-DNA	D-DNA	Z-DNA
RH	RH	RH	RH	LH
11 bp/turn	10 bp/turn	9.33 bp/turn	8 bp/turn	12 bp/turn
pitch=28.2A	pitch=34A	pitch=31A	pitch=24.2A	pitch=43A

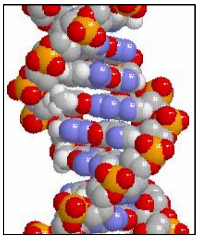


DNA structure & base-pair sequence

NATURAL DNA	ALTERNATING A-T	ALTERNATING G-C	ALTERNATING G-m^5C	HOMOPOLYMER G-C	HOMOPOLYMER A-T
"Random" sequence	A - T T - A A - T T - A A - T T - A A - T T - A A - T T – A	G - C C - G G - C C - G G - C C - G G - C C - G G - C C - G	G – m^5C m^5C - G G – m^5C m^5C - G G – m^5C m^5C - G G – m^5C m^5C - G G – m^5C m^5C - G	G - C G - C G - C G - C G - C G - C G - C G - C G - C G - C	A - T A - T A - T A - T A - T A - T A - T A - T A - T A - T
C↔A↔B	**D ↔ B**	**Z ↔ B**	**A ↔ Z**	**A**	**B**

A-DNA changing to B-DNA

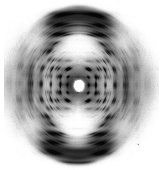


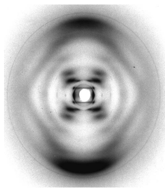


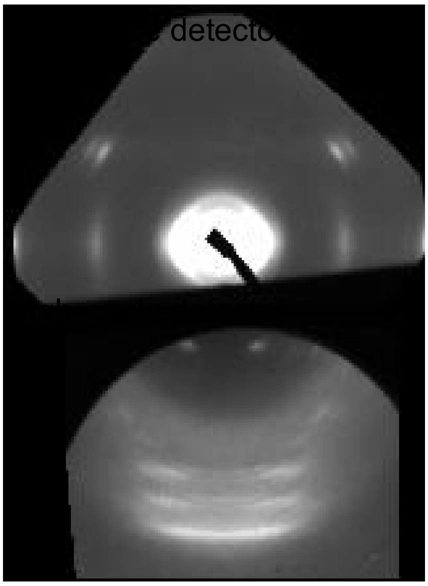
A-DNA
RH
11 bp/turn
pitch=28.2A

Humidity in sample environment

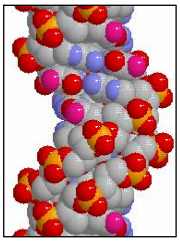
B-DNA
RH
10 bp/turn
pitch=34A

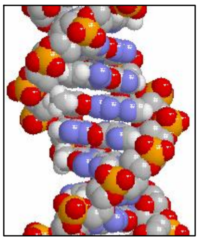






D-DNA changing to B-DNA

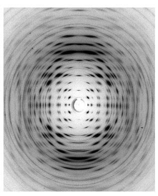


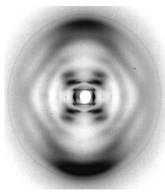


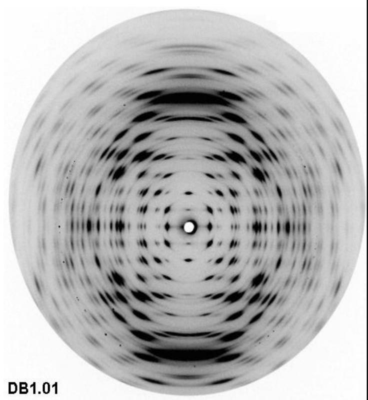
D-DNA
RH
8 bp/turn
pitch=24.2A

Humidity in sample environment

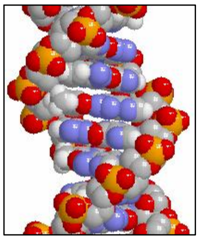
B-DNA
RH
10 bp/turn
pitch=34A

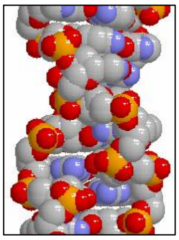


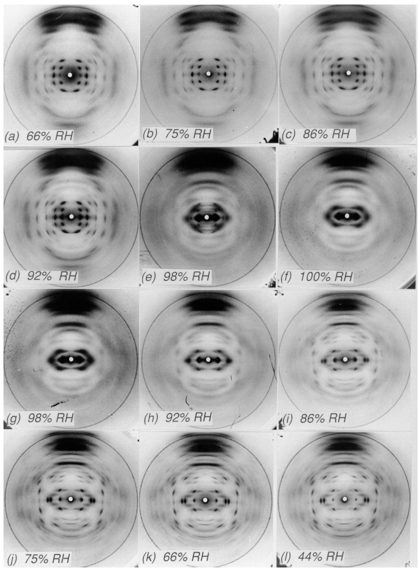




B-DNA changing to Z-DNA



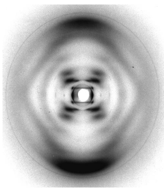


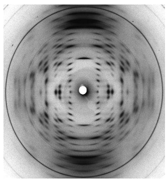


B-DNA
RH
10 bp/turn
pitch=34A

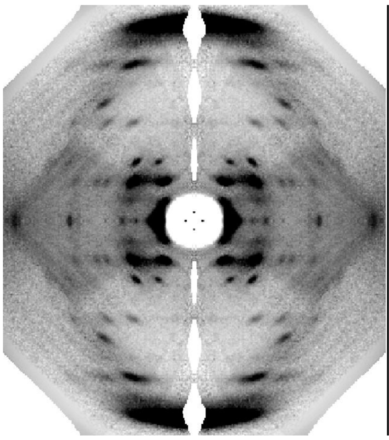
Humidity in sample environment

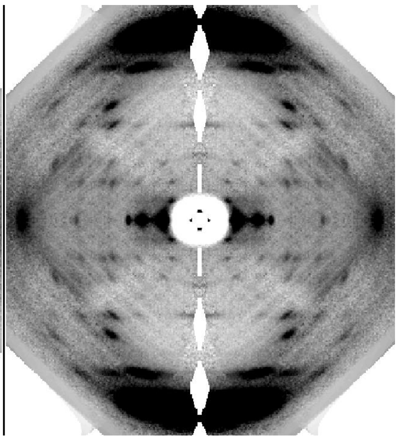
Z-DNA
LH
12 bp/turn
pitch=43A





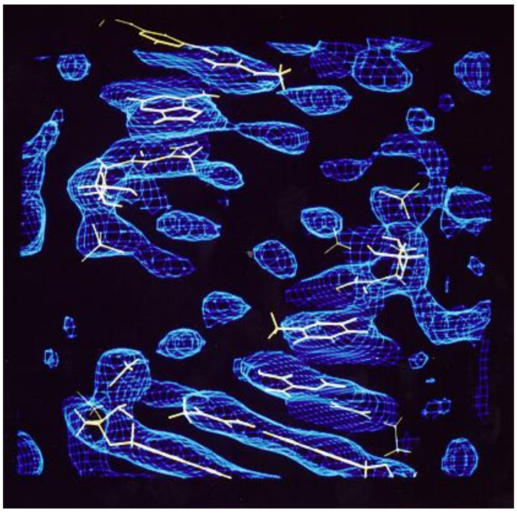
Neutrons see water in DNA



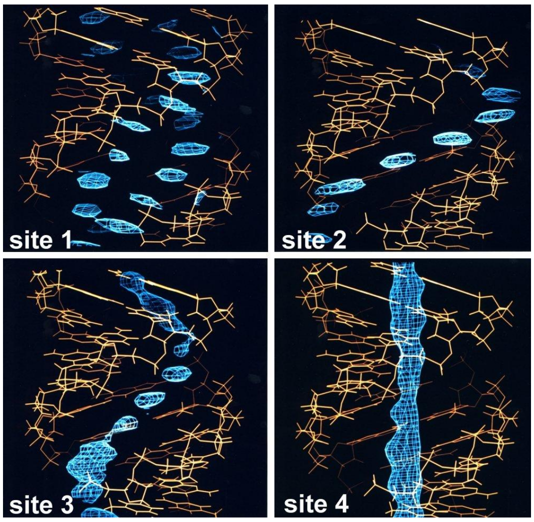


Hydrated with H_2O Hydrated with D_2O

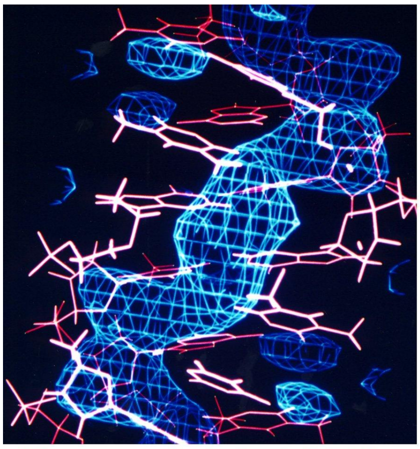
Water linking DNA phosphates



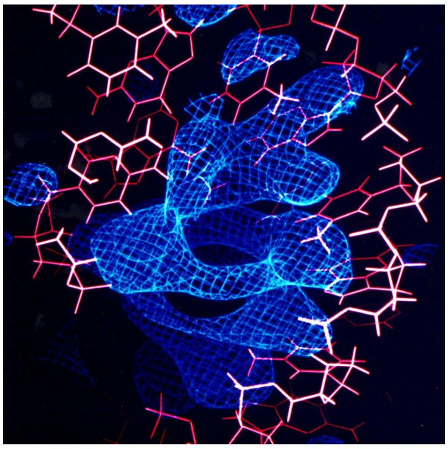
Water in DNA



Water in DNA

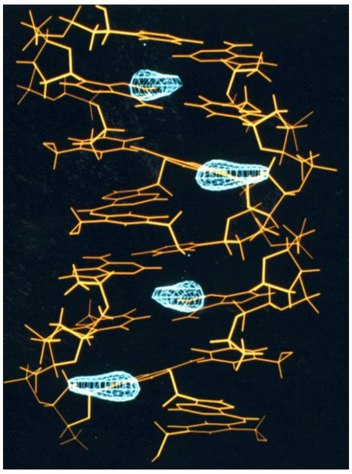


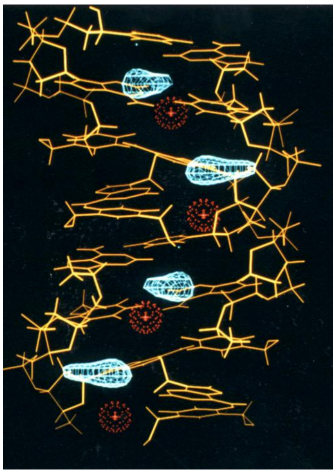
Minor groove hydration in D-DNA



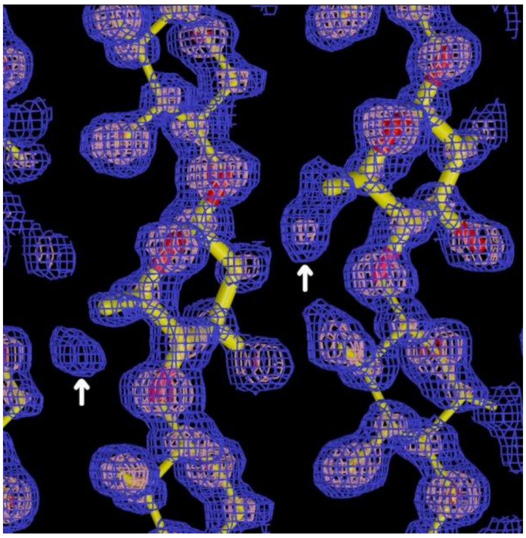
Major groove hydration in A-DNA

Water and ions in DNA



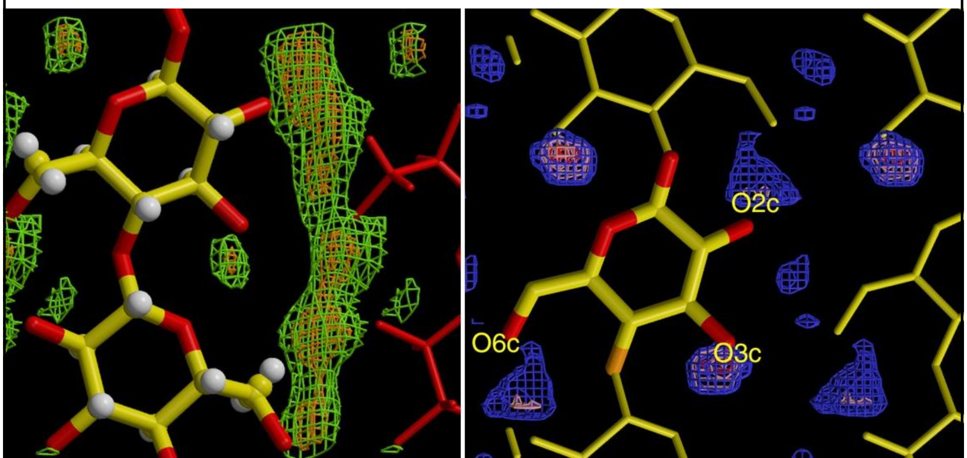


Cellulose: the most abundant polymer on Earth



Nishiyama *et al*; Langan *et al*, *J. Am. Chem Soc.*

Cellulose: the most abundant polymer on Earth



Nishiyama *et al*; Langan *et al*, *J. Am. Chem Soc.*

Techniques for *structure* using neutron scattering

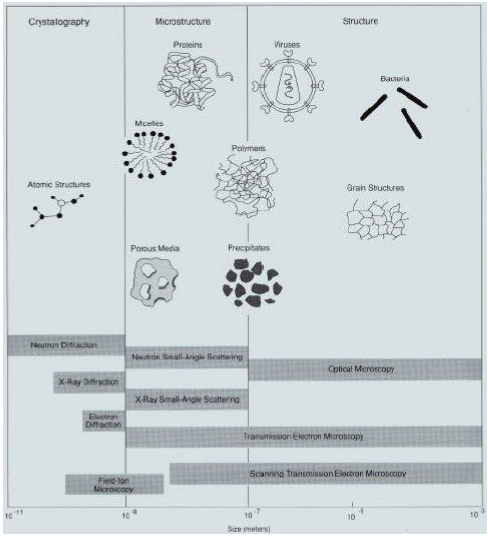
- High resolution crystallography
- Fibre diffraction
- Membrane diffraction
- Reflectometry
- Low resolution crystallography
- Small angle neutron scattering

Small-angle neutron scattering (SANS)

Scattering angles from ~0.2 degrees
Large length scales: 10A – thousands of A,
Information: yields low resolution information on shape.

Contrast variation and labelling can be used to provide important information on structure in multicomponent systems

Small-angle neutron scattering

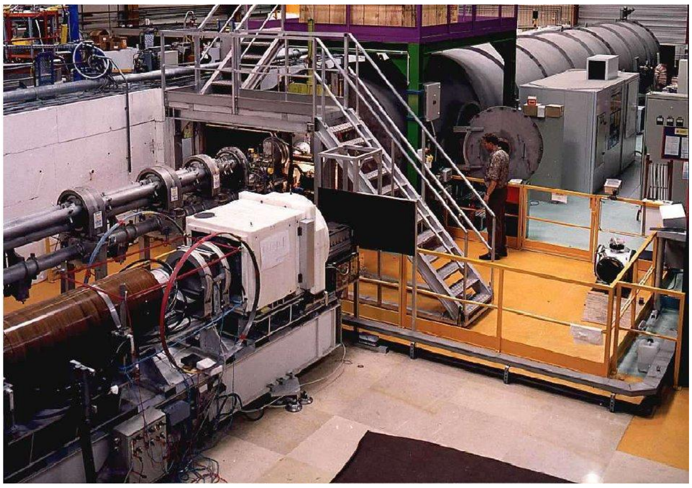


SANS in the study of biological systems

<u>Extremely</u> important for the study of complex macromolecules in solution, interactions between molecules, changes in structure.

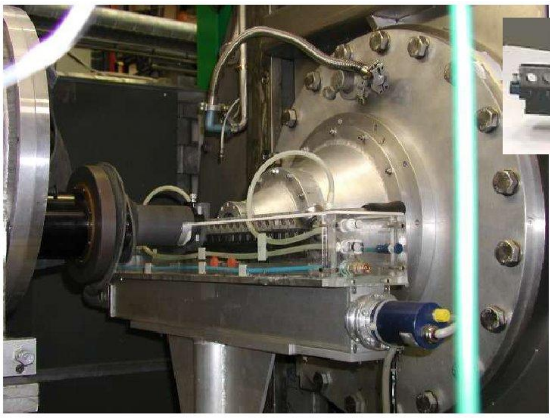
Examples:
- Protein-nucleic acid complexes
- Multicomponent protein systems
- Lipids and protein-lipid complexes

Small-angle neutron scattering



D22 at ILL: monochromatic (velocity selector) with bandpass of ~10%

Small-angle neutron scattering



computer-controlled thermostatable sample changer for various rack plates taking 9 to 22 sample cells/holders

Contrast variation in small-angle neutron scattering

• *Contrast variation* can be used to study the location of various components in a system, at low resolution

• Relies on:

(a) differing scattering lengths of H and D

(b) exploitation of the *difference* in scattering length density between a macromolecule and its surrounding environment.

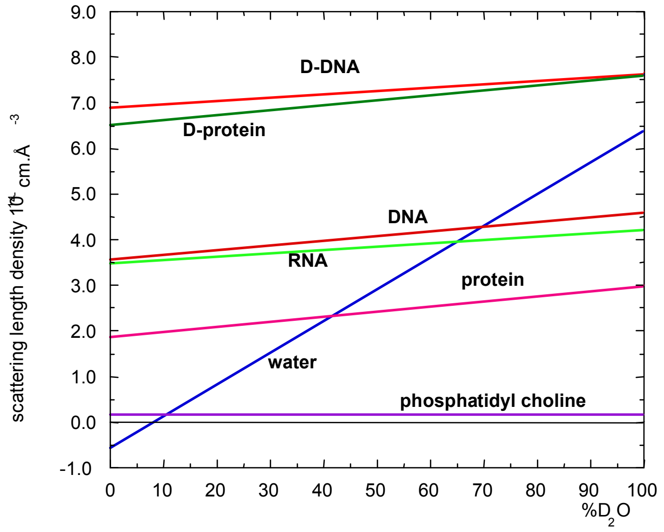
Scattering length density

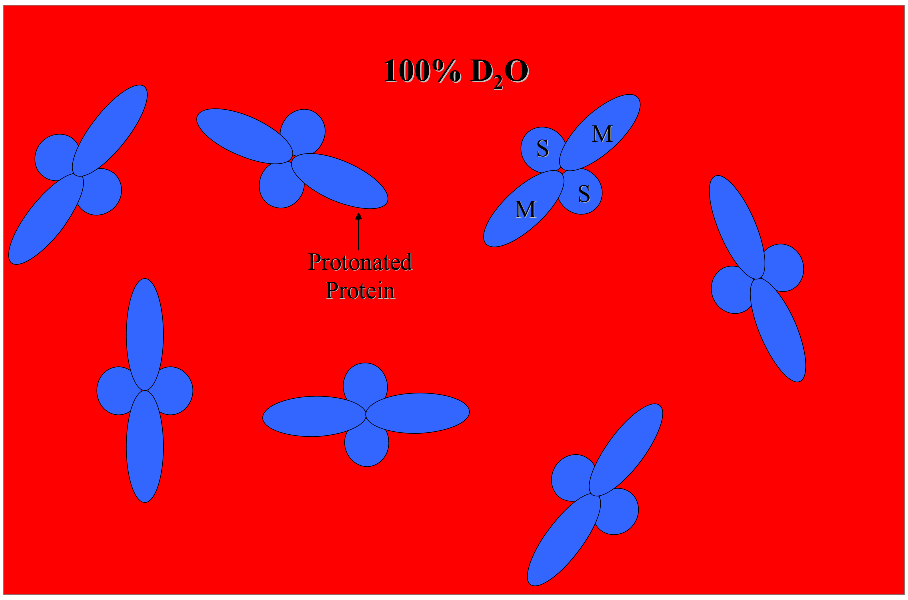
At small angles, it is usually reasonable to define a scattering length density

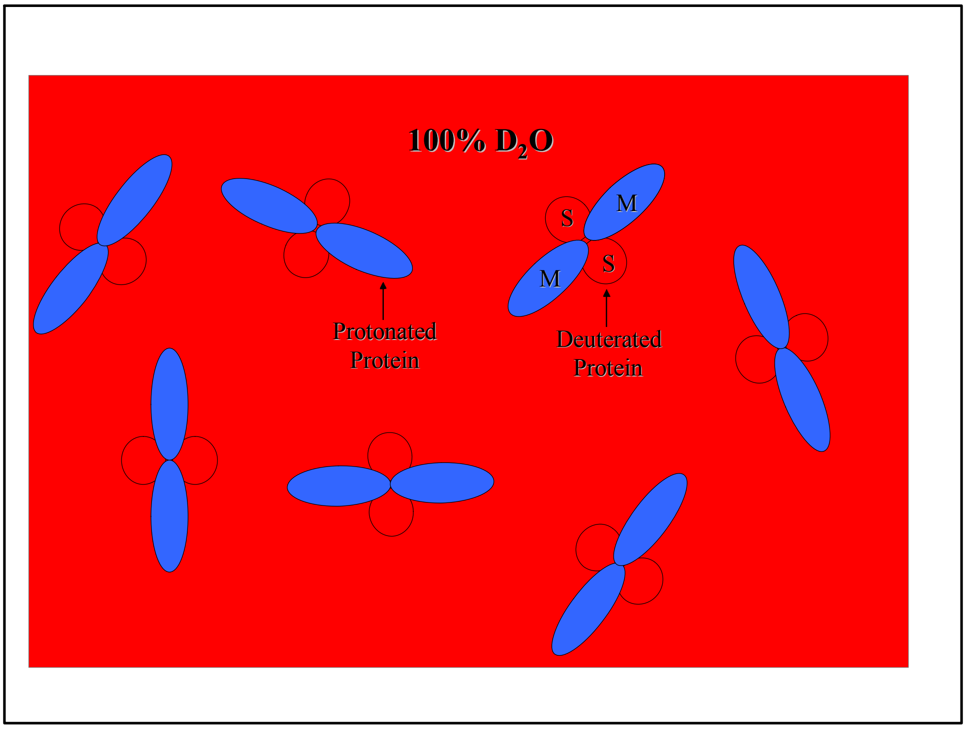
For D_2O: Density = 1.11gm/cc, MW= 20 gm/mole,
Number of molecules per cubic angstrom=
$(1.11/20) \times 10^{-24} \times 6 \times 10^{23} = 0.0333$ mols/A^3
SLD = $0.0333 \times (6.67 *2 +5.8) \times 10^{-13} \times 10^{-8}$
$=6.4 \times 10^{-6}$ A^{-2} or **6.4×10^{-14} cm/A^3**

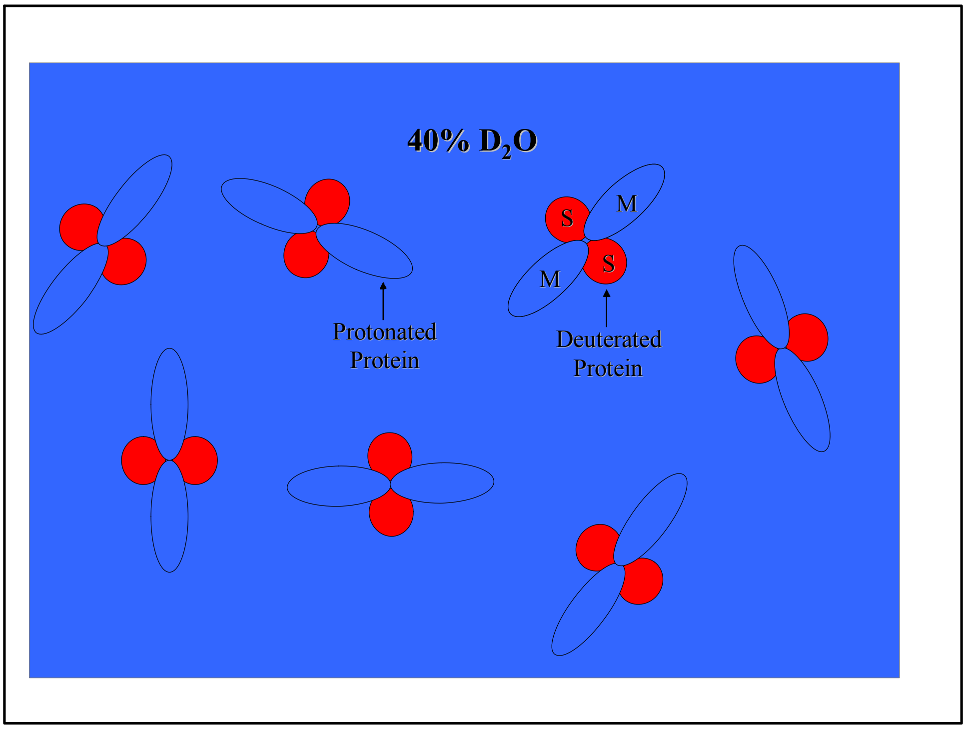
For H_2O: Density = 1.0gm/cc, MW= 18 gm/mole,
Number of molecules per cubic angstrom=
$(1.0/18) \times 10^{-24} \times 6 \times 10^{23} = 0.0333$ mols/A^3
SLD = $0.0333 \times (-3.74 *2 +5.8) \times 10^{-13} \times 10^{-8}$
$=-0.559 \times 10^{-6}$ A^{-2} or **-0.559×10^{-14} cm/A^3**

Contrast variation



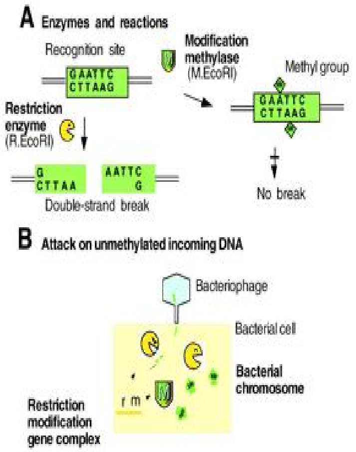



100% D_2O
S
M
M
S
Protonated
Protein
Deuterated
Protein


40% D_2O
S
M
M
S
Protonated
Protein
Deuterated
Protein

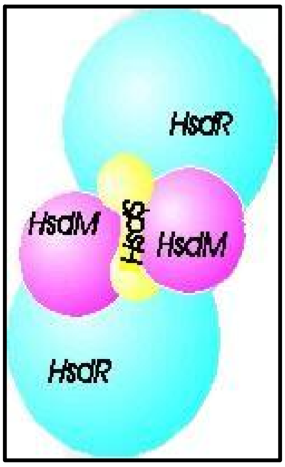
Restriction-modification (R-M) systems

- Bacterial "immune system"
- Endonuclease (ENase)
  Methyltransferase (MTase)
- Both enzymes recognise same DNA sequence
- MTase adds $-CH_3$ group to sequence, preventing restriction
- ENase cuts DNA if recognition site not methylated
- Invading DNA is cleaved by ENase (restriction)



(Callow, Sukhodub , Kneale, J. Mol. Biol 2007)

Type I restriction-modification enzymes

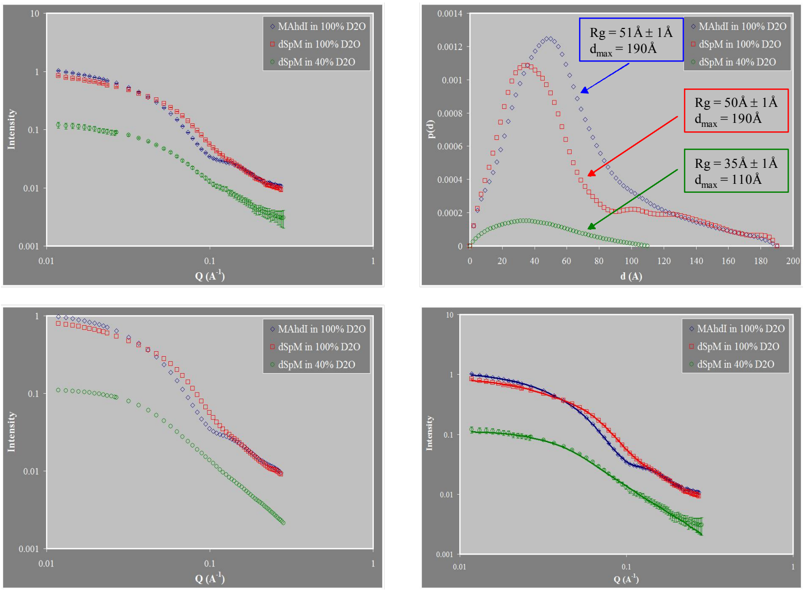


3 genes one for each of the subunits (**S**, **M** & **R**) that are responsible for **s**pecificity, **m**ethylation and **r**estriction respectively

Two subunits (**M** & **S**) form the trimeric 160 kDa methyltransferase (MTase), $\mathbf{M_2S}$, that methylates a specific base within the recognition sequence and protects the DNA from cleavage by the endonuclease

The 400 kDa endonuclease is a pentameric enzyme formed from the MTase by the addition of two **R** subunits to form the complex $\mathbf{R_2M_2S}$

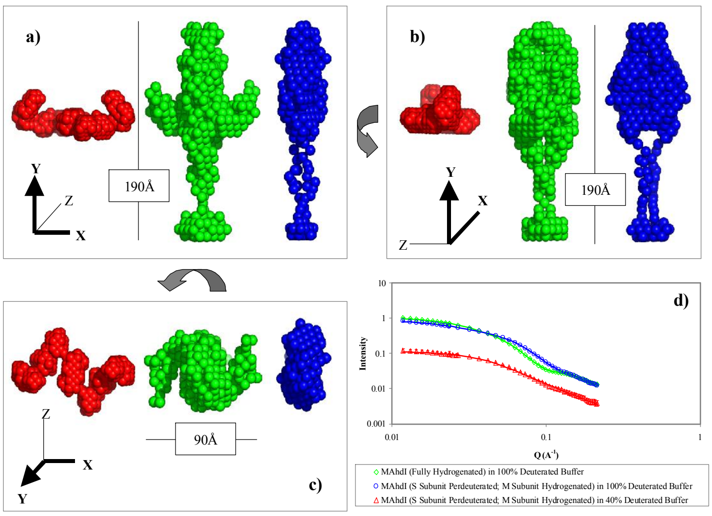
(Callow, Sukhodub , Kneale, J. Mol. Biol 2007)



(Callow, Sukhodub , Kneale, J. Mol. Biol 2007)

Modelling the subunit structure of M.AhdI

(DAMMIN)



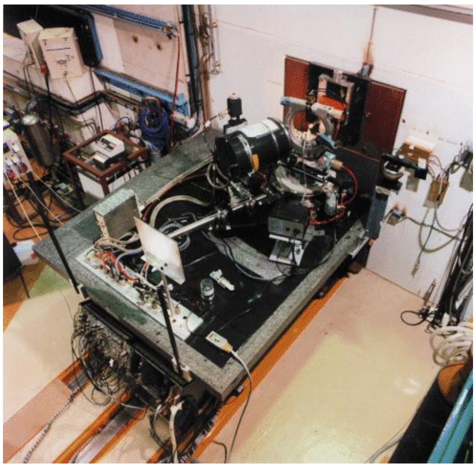
(Callow, Sukhodub , Kneale, J. Mol. Biol 2007)

Techniques for *structure* using neutron scattering

- High resolution crystallography
- Fibre diffraction
- Membrane diffraction
- Reflectometry
- Low resolution crystallography
- Small angle neutron scattering

ILL instrument DB21

- Cold neutron guide
- Graphite monochromator, pinhole collimation
- Huber 4-circle goniostat



Porins

• integral membrane proteins from the outer membrane of Gram-negative bacteria, e.g. *E. Coli*

• allow selective diffusion of small hydrophilic molecules across the membrane

• selectivity based on size with some specificity for charge and/or chemical nature

• regulated by temperature, osmotic pressure, pH....

• structures - 16-strand anti-parallel beta-barrel, 8 short loops on the cytoplasmic rim, 8 longer loops on the periplasmic rim.

• active pore - trimer ~75 x 55Å

Low resolution neutron crystallography

Ompf porin - an integral membrane protein from *E. Coli*

Pebay-Peroula *et al*, Structure 3 (10), 1051

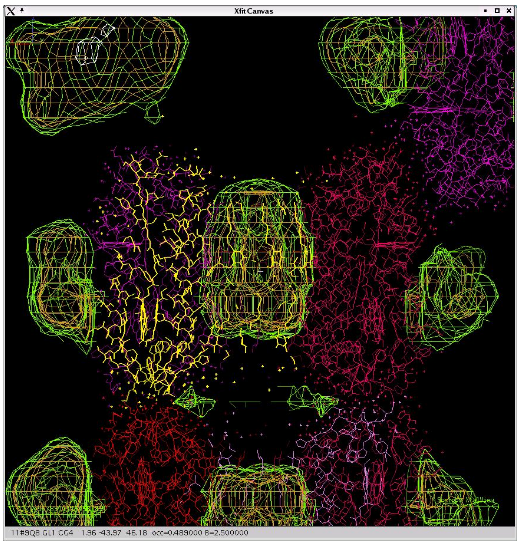
Investigation of protein-detergent interactions - significant for understanding protein lipid interactions and also crystallisation of membrane proteins

40% D_2O - protein is contrast matched (x-ray structure shown in yellow). Deuterated detergent molecules

View parallel to the threefold axis of the trimer.

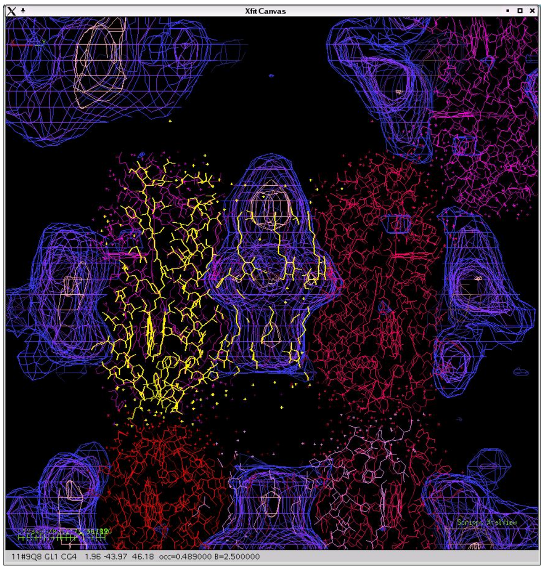
Map shows details of detergent molecules bound to ‘ hydrophobic zone ’ surrounding the trimer and which is exposed to lipid *in vivo*

Hydrogenated detergent map



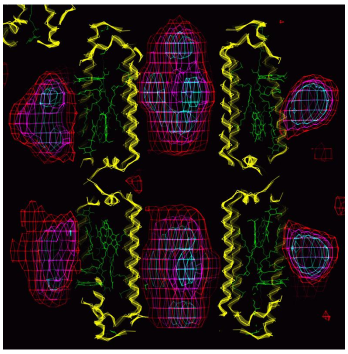
Prince S.M., *et al*, *J. Mol. Biol.* 326 307-315 (2003)

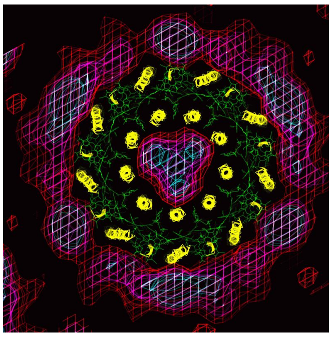
Deuterated detergent map



Prince S.M., *et al*, *J. Mol. Biol.* 326 307-315 (2003)

Final detergent model





- Chickenwire representations of the final tails model (16%, 8% & 2.5% volume contours)

Prince S.M., *et al*, *J. Mol. Biol.* 326 307-315 (2003)

Techniques for *structure* using neutron scattering

- High resolution crystallography
- Fibre diffraction
- Membrane diffraction
- Reflectometry
- Low resolution crystallography
- Small angle neutron scattering

High Resolution Crystallography

•Location of hydrogen atoms

•Location and orientation of water molecules

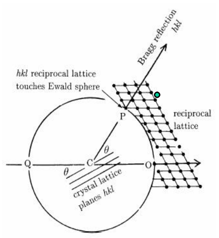
•Orientation of amide groups

•H/D exchange

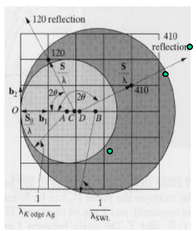
The LADI diffractometer at ILL

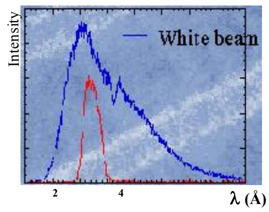
Diffractometer designed to maximise flux on sample and detector solid angle

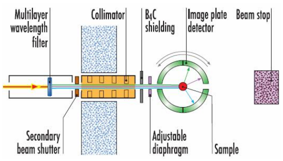
Monochromatic crystallography



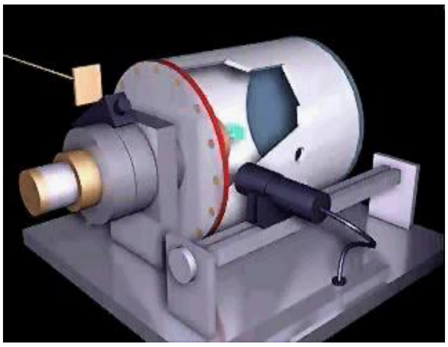
Polychromatic (Laue) method







The LADI Diffractometer at ILL



• Radius	159.19 mm
• Length	400 mm
• Active area	800 x 400 mm²
• Angle subtended	144° in T, 52° in v
• Pixel size	200 x 200 μm²

Monochromators
Laue White beam
Quasi-Laue (δλ\λ < 30%)

Ti/Ni multilayer bandpass filters

Collimation
Pinholes 0.5 to 4mm

Detector
Cylinder covered with image plates

NIP — Gd_2O_3 doped $BaF(Br.I){:}Eu^{2+}$

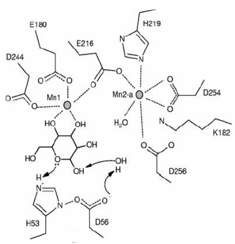
Sample
Flux at specimen =3 x 10 ^{7} n cm^{-2} s^{-1}
(λ=3.5Å, δλ\λ = 20%)

Sample environment
Displex cryostat under vaccum

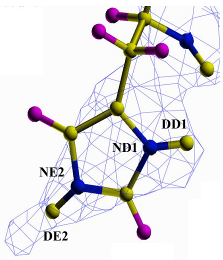
going down to approx. 12K

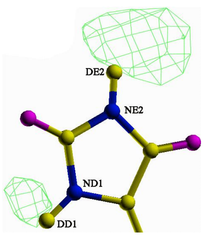
D-Xylose Isomerase

June 2004: 0.95 Å X-ray structure (Fenn, T. D., Ringe, D., Petsko, G. A; *Biochemistry* (2004) **43**(21):6464-74)



No direct observation of hydrogen but the refined model suggested that "...**the site of ring opening (O5) is left positioned appropriately for proton donation by His53**..."



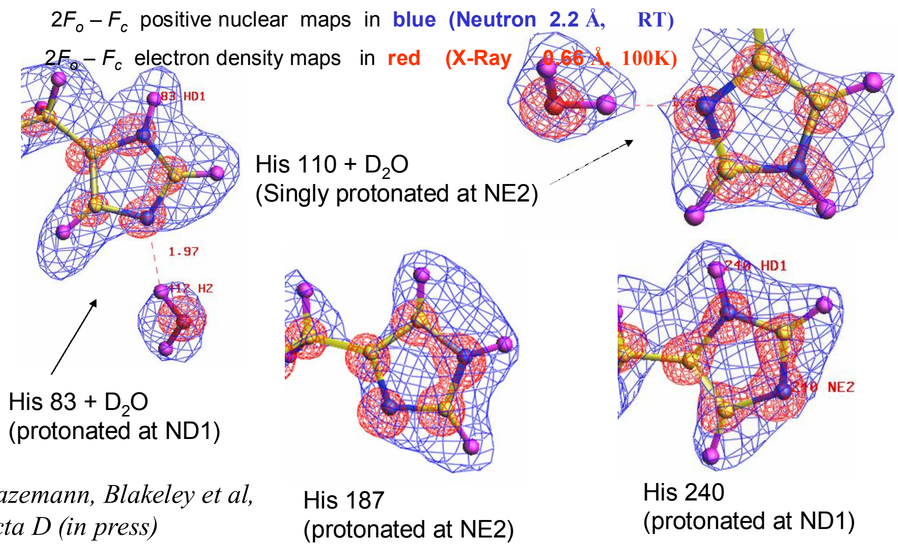


F^D_{obs}-F^H_{obs} Fourier difference map contoured at 2.5σ

Neutron analysis @ 2.2 Å: direct observation of His53 NE2 protonation state supporting the proposed mechanism.

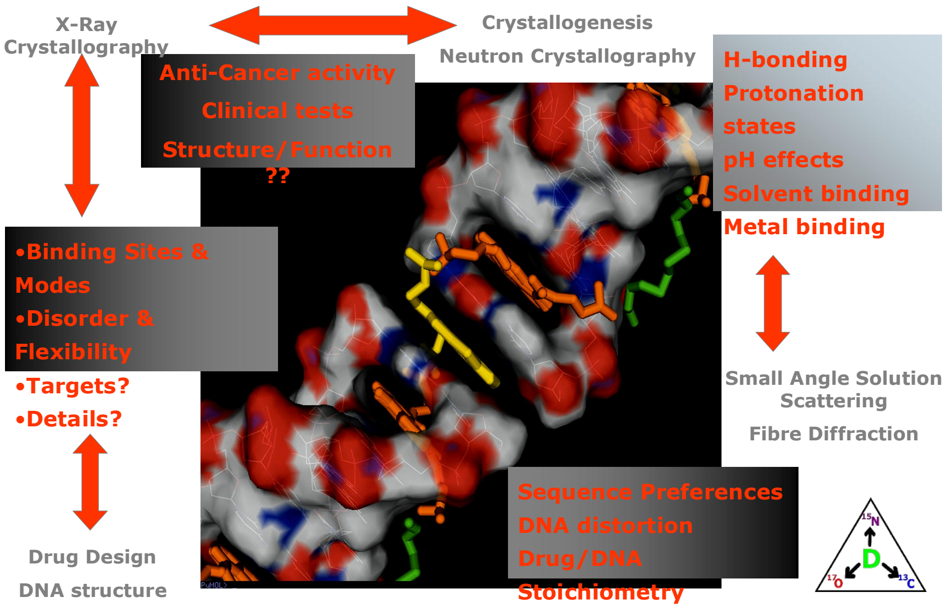
(Meilleur et al)

Aldose reductase - protonation state of His residues.

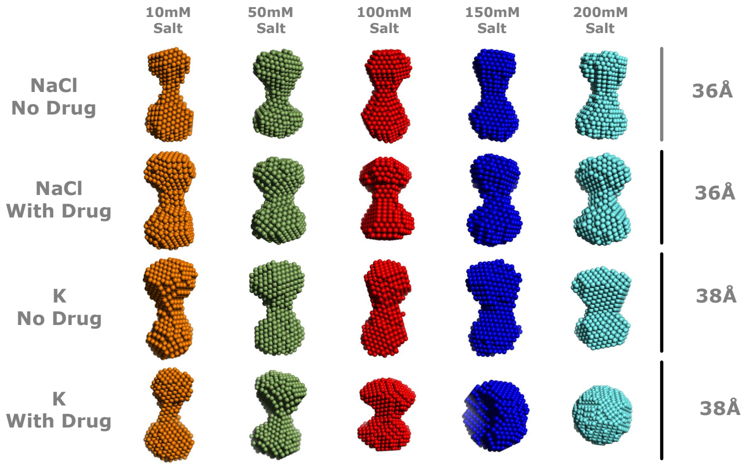


Hazemann, Blakeley et al, Acta D (in press)

DNA/anti-cancer drug interactions

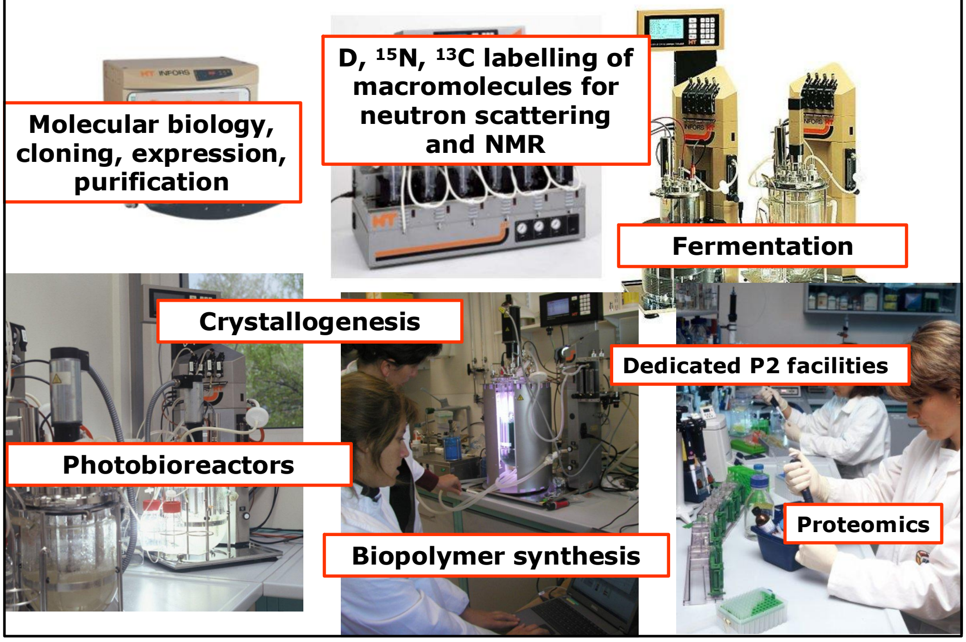


DNA/anti-cancer drug interactions



Miles, Callow, Teixeira, Forsyth, in preparation

The ILL-EMBL Deuteration Laboratory



Feeding biology to neutron instruments