Neutron Protein Crystallography and equilibrium dynamics

FEBS Practical Course, **Biomolecules in Action II**
DESY, Hamburg,

June 25th 2019 | Tobias E. Schrader, Ralf Biehl

Outline

- Introduction: Why neutrons?
- High resolution neutron structures
- Inelastic neutron scattering: Biomolecules in action: Equilibrium dynamics as key for the function of enzymes

Neutrons are scattered by the nuclei, x-rays by the electrons

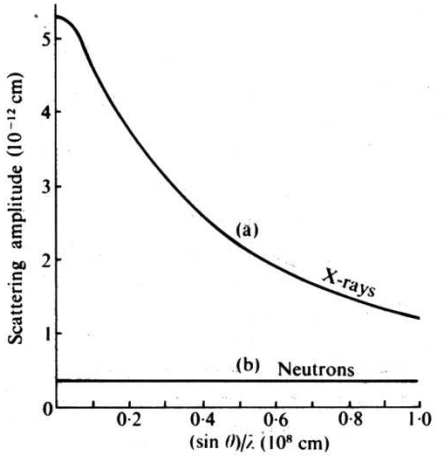


FIG. 16. X-ray and neutron scattering amplitudes for a potassium atom.

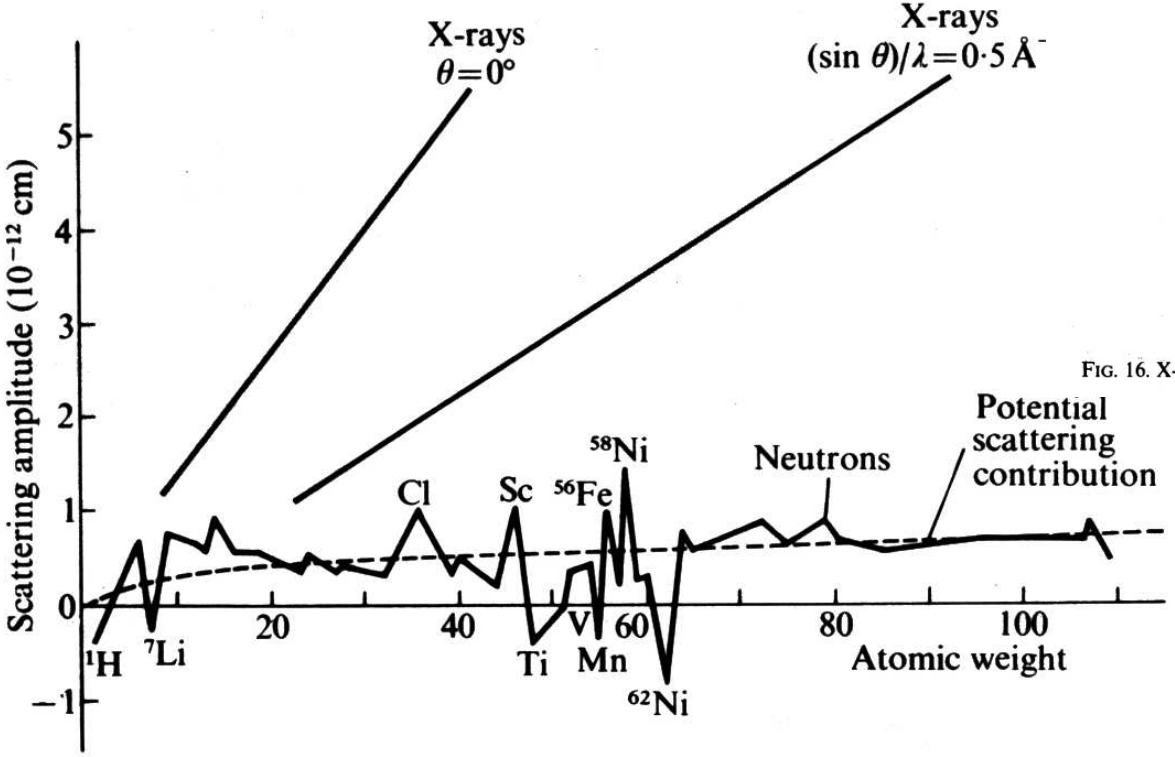


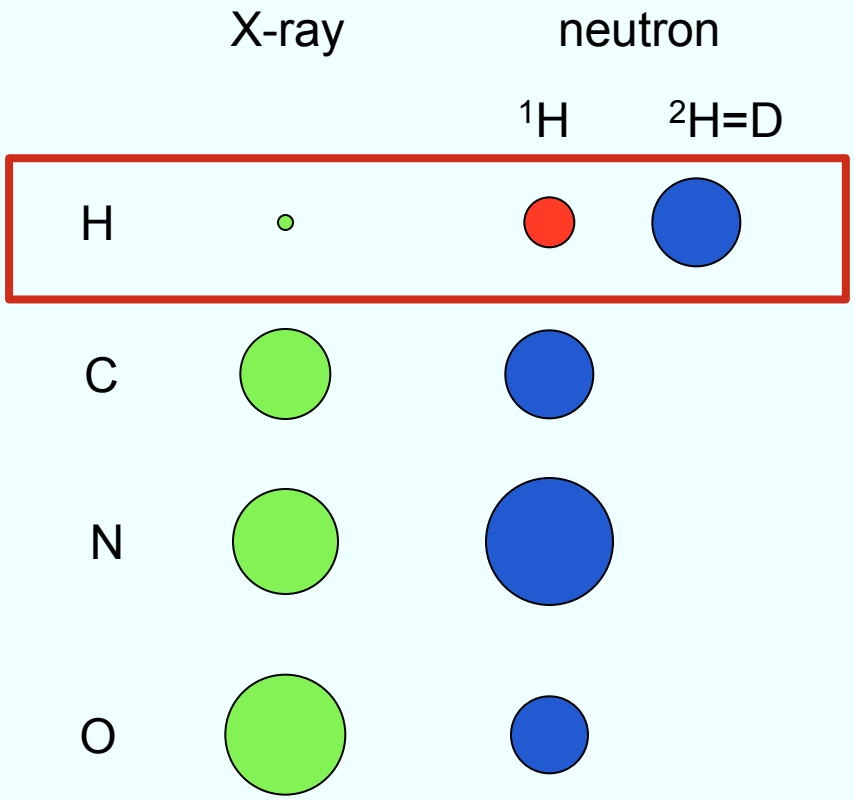
FIG. 22. Irregular variation of neutron scattering amplitude with atomic weight due to superposition of 'resonance scattering' on the slowly increasing 'potential scattering'; for comparison the regular increase for X-rays is shown. (From *Research* (*London*) 7, 257 (1954).)

Atoms are point-like particles for neutrons, but the electron shell has some size on the scale of the atom distances.

Advantages of structure determination with neutrons:

Comparison of form factors (X-ray) and scattering lengths (neutrons):

Nucleus	atomic number	scattering length [10^{-12} cm]
^{1}H	1	-0.378
^{2}H	1	0.667
^{12}C	6	0.665
^{15}N	7	0.921
^{16}O	8	0.581



diameters correspond to:
form factor / scattering length
(scaled to match for C-atom)

σ_{coh} of ^{1}H is 1.8×10^{-28} m^2 but
σ_{incoh} of ^{1}H is 80.2×10^{-28} m^2
Large background from hydrogen atoms!

Advantages of neutrons as compared to x-rays as probes for scattering techniques

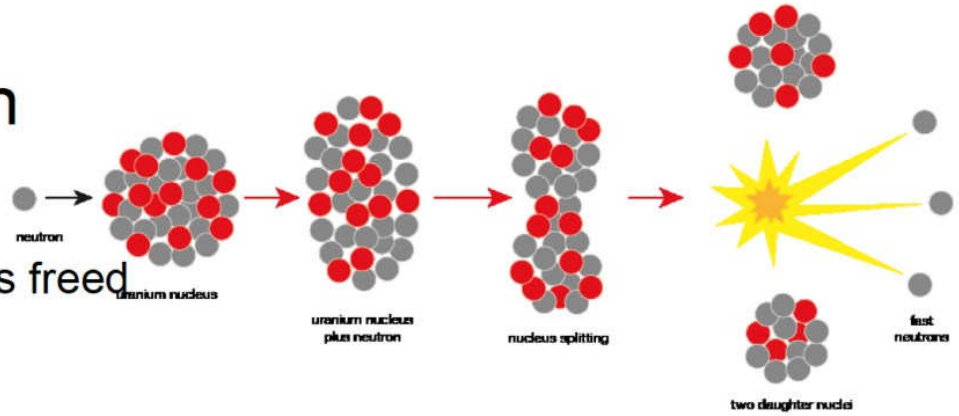
1. Neutrons are neutral particles and have a fairly small scattering cross section as compared to x-rays of similar wavelength.
    a) Large penetration depth can be reached (several mm or cm)
    b) More complex sample environment can be afforded: Cryostats, high pressure cells
2. Neutrons scatter from the nuclei and can therefore „destinguish“ between different isotopes of the same element
    a) Contrast matching can be used in Small Angle Scattering: Frank Gabel‘s talk
    b) In case of high resolution this may be problematic:
3. Neutrons cause much less radiation damage as x-rays
    a) time resolved investigations on one and the same sample are more feasible
4. Neutrons have a magnetic moment (magnetism and superconductivity can be investigated)

The (free) neutron production problem…

Small excursion: Neutron production

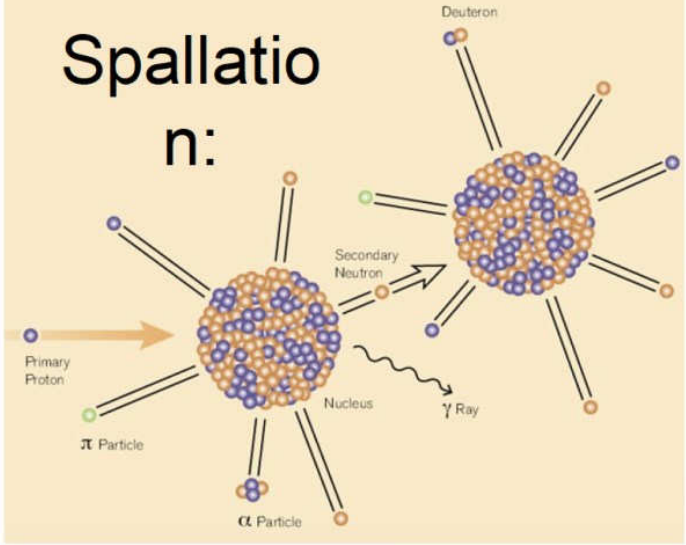
Fission

200 MeV/fission
2.35 – 1 = 1.35 neutrons freed
=> 150 MeV/neutron



usually continous beam

Spallation:



1 GeV proton in:
250 MeV becomes mass (endothermic reaction)
30 neutrons freed
=> 25 MeV/neutron

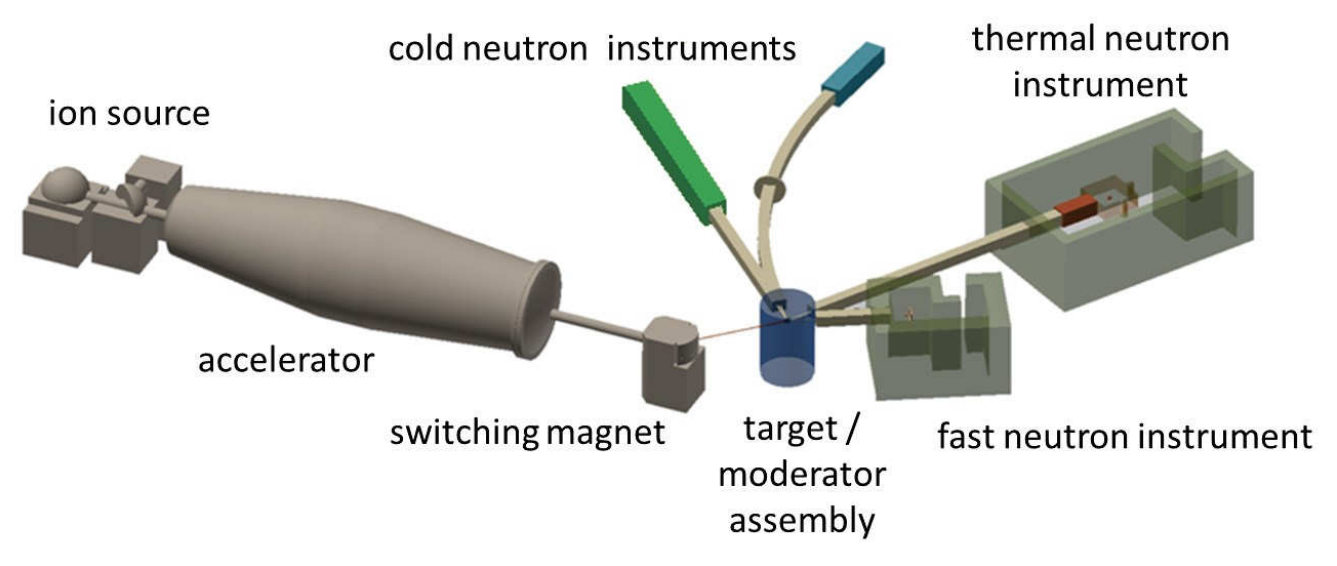
6x more neutrons per unit heat

usually pulsed beam

slide from Ken Andersen

High brilliant sources: The Juelich idea of a new neutron source

- Artistic view of NOVA ERA with four beam-lines and instruments



Neutron production using a Beryllium target and the following process

$^{9}Be(p,n)^{9}B$

Disadvantages of neutrons as probes

1. Neutrons are very expensive and highly brilliant beams are not easy to produce

2. Incoherent scattering (mostly of hydrogen atoms) produces background and limits resolution.

    a) Deuterated samples are needed, sometimes per-deuteration is an advantage.

3. Absorption sometimes plays a role and leads to activation of the sample

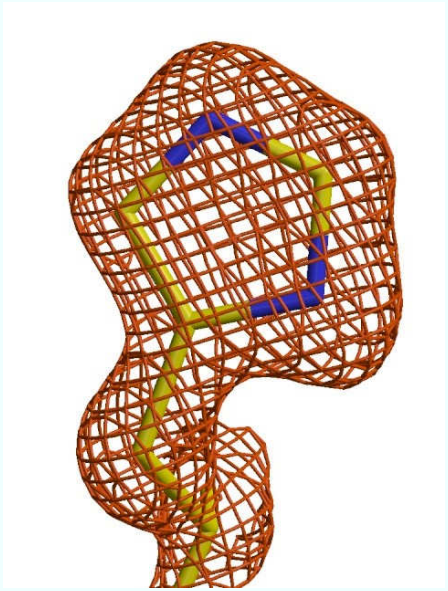
Neutron Protein Crystallography

Elastic scattering recorded, no energy resolution on the detector.

Inelastic scattering neglected because it is much weaker in intensity.

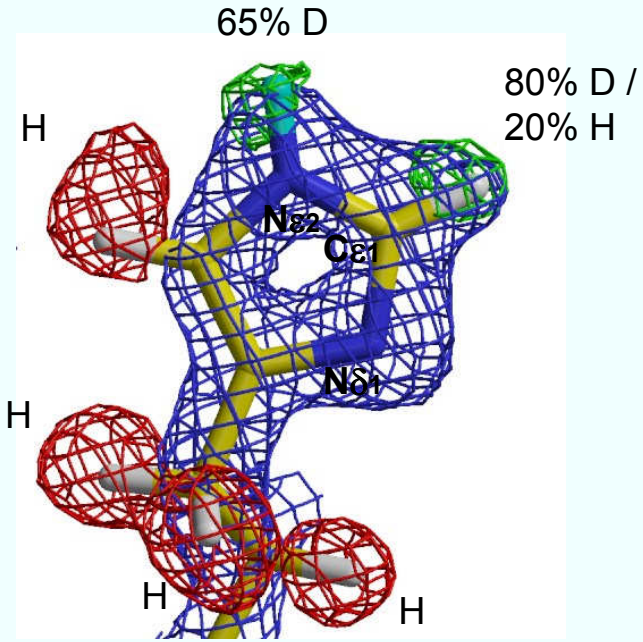
Protonation states of amino acids:

X-ray d_{min}= 1.5Å:



2Fo-Fc map; +1.5σ

neutrons d_{min}= 1.5Å:



2Fo-Fc map; +1.5σ

Fo-Fc omit-map; -3.0σ

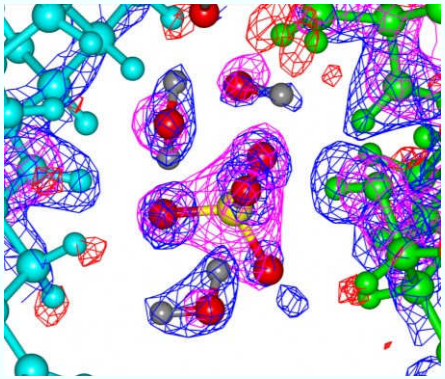
Fo-Fc omit-map; +3.0σ

Niimura N, Chatake T, Ostermann A, Kurihara K, Tanaka T. (2003) Z. Kristallogr. 218:96

Advantages of Structure Determination with Neutrons

Hydrogen/deuterium atoms can be resolved even at a resolution of $d_{min} \approx 2.5$ Å (for ^2H). Therefore one can determine:

- protonation states of amino acid side chains and ligands
- deuterium exchange as a measure of flexibility and accessibility (discrimination between **H** / **D**)
- solvent structure including hydrogen atoms

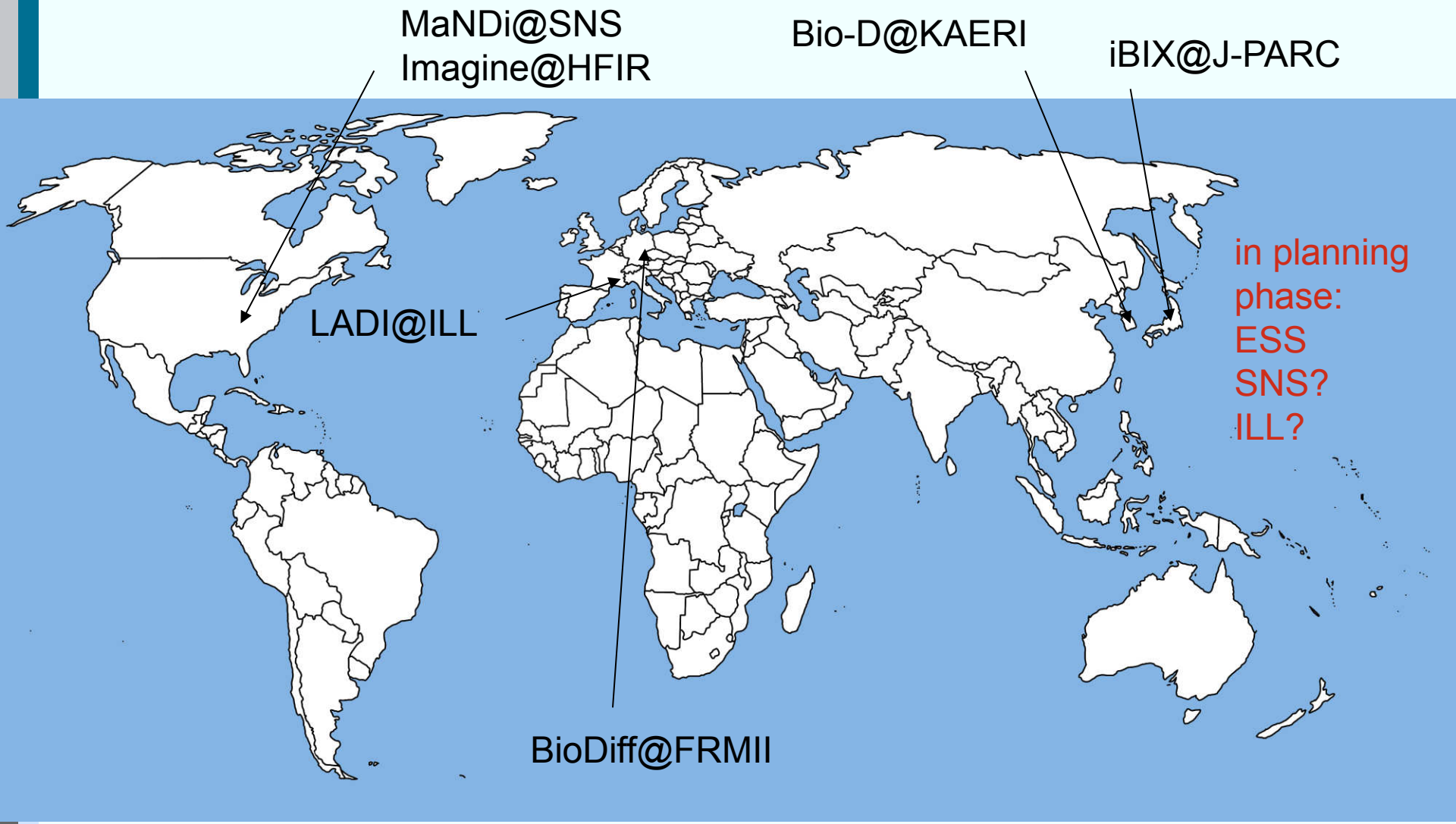


Water network in the contact region between two myoglobin molecules in the crystal.

x-ray map (magenta): contour level of +2.7σ
nuclear map (red): contour level of -1.75σ
nuclear map (blue): contour level of +2.3σ

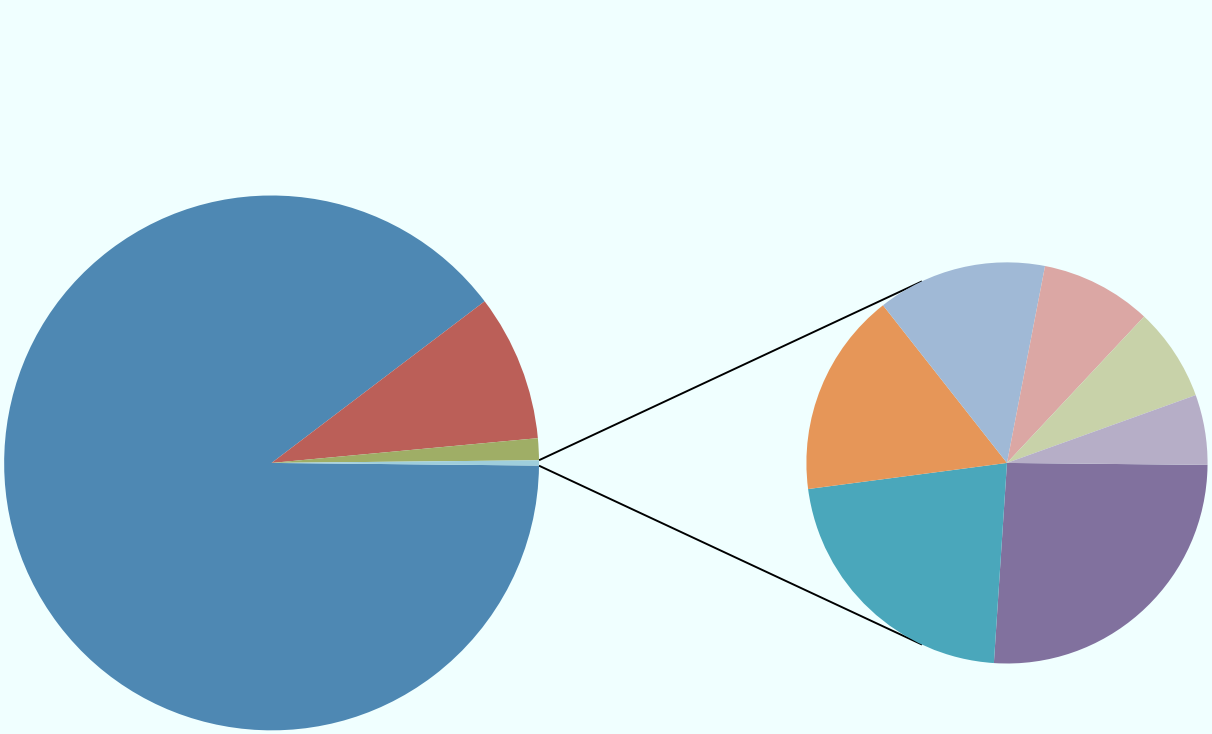
Much less radiation damage as compared to x-rays: Metallo-proteins can be measured without reducing the metal centres

World map of neutron diffractometers optimized for protein crystals



How do we find out about protein structures?

Most structures are obtained by x-ray crystallography, available neutron structures in protein data bank: 58



- x-ray (120534)
- solution NMR (11921)
- Electron Microscopy (1776)
- Hybrid (110)
- Solid State NMR (93)
- Electron Crystallography (70)
- Neutron Diffraction (58)
- Fiber Diffraction (38)

http://www.rcsb.org/

Total number of structures: 134656

A crystal structure according to the protein data bank (PDB)

x,y,z coordinates (Å)

```
ATOM     25  N   ASP A 928      19.062   9.157  35.067  1.00  4.73           N
ATOM     26  CA  ASP A 928      19.770  10.123  34.232  1.00  4.58           C
ATOM     27  C   ASP A 928      19.075   9.938  32.899  1.00  4.56           C
ATOM     28  O   ASP A 928      19.074   8.824  32.351  1.00  5.39           O
ATOM     29  CB  ASP A 928      21.259   9.776  34.071  1.00  3.13           C
ATOM     30  CG  ASP A 928      22.112  10.245  35.233  1.00  5.52           C
ATOM     31  OD1 ASP A 928      21.693  11.114  36.025  1.00  5.42           O
ATOM     32  OD2 ASP A 928      23.239   9.742  35.349  1.00  7.93           O
ATOM     33  N   VAL A 929      18.417  10.985  32.405  1.00  3.68           N
ATOM     34  CA  VAL A 929      17.726  10.864  31.125  1.00  4.63           C
```

Isotropic B-factor or temperature factor is a measure of the mobility of an atom

B ($Å^2$) = $8\pi^2\langle u^2\rangle$, where $\langle u^2\rangle$ is the mean square atomic displacement

Very few x-ray structural studies give access to hydrogen positions

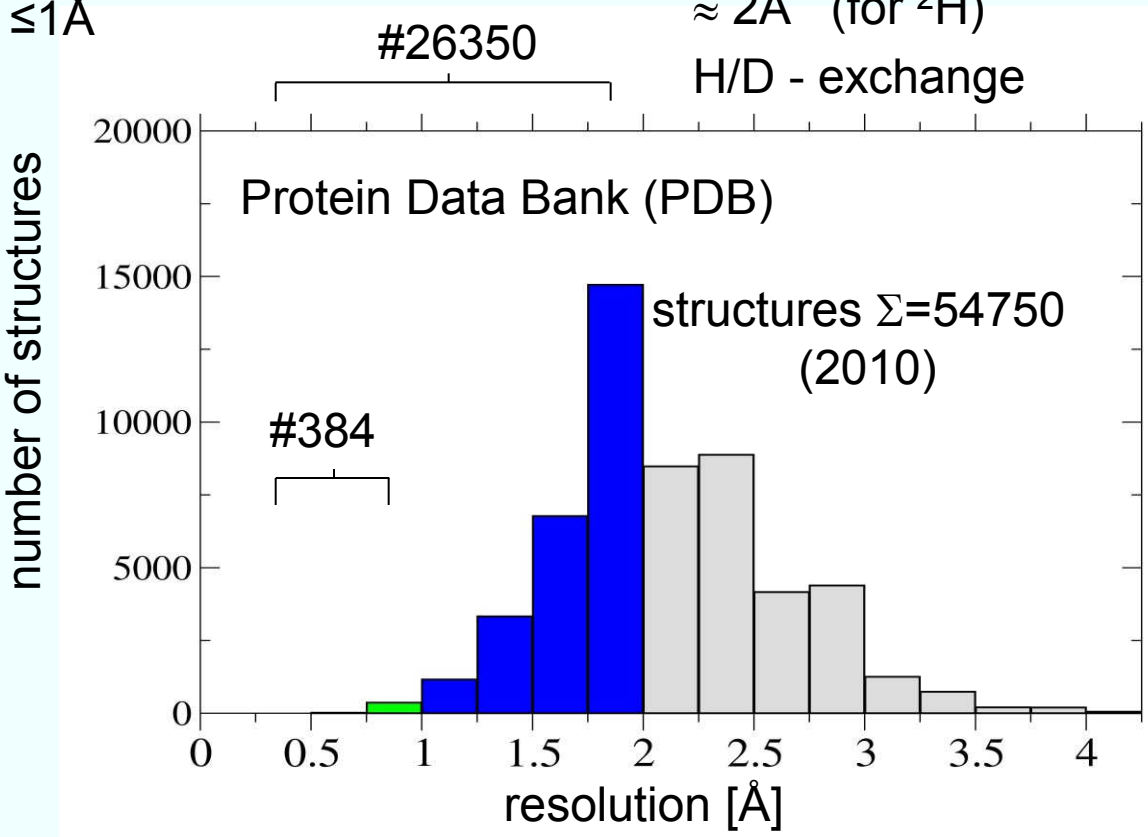
X-ray:

hydrogens visible at resolution of $d_{min} \leq 1$Å

neutrons:

hydrogens visible at resolution of $d_{min} \approx 2$Å (for ^{2}H)

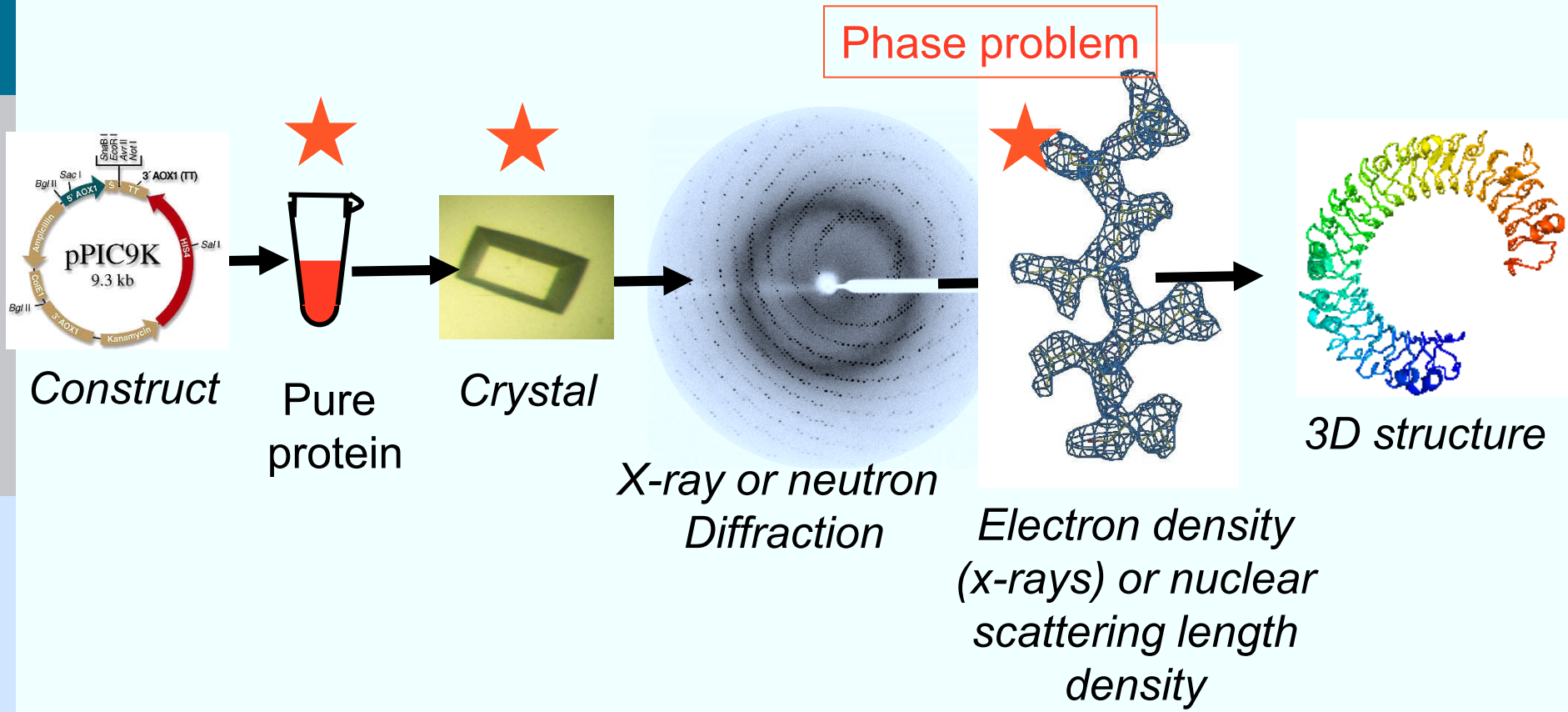
H/D - exchange



But crystals need to have a volume of more than 1 mm^3!

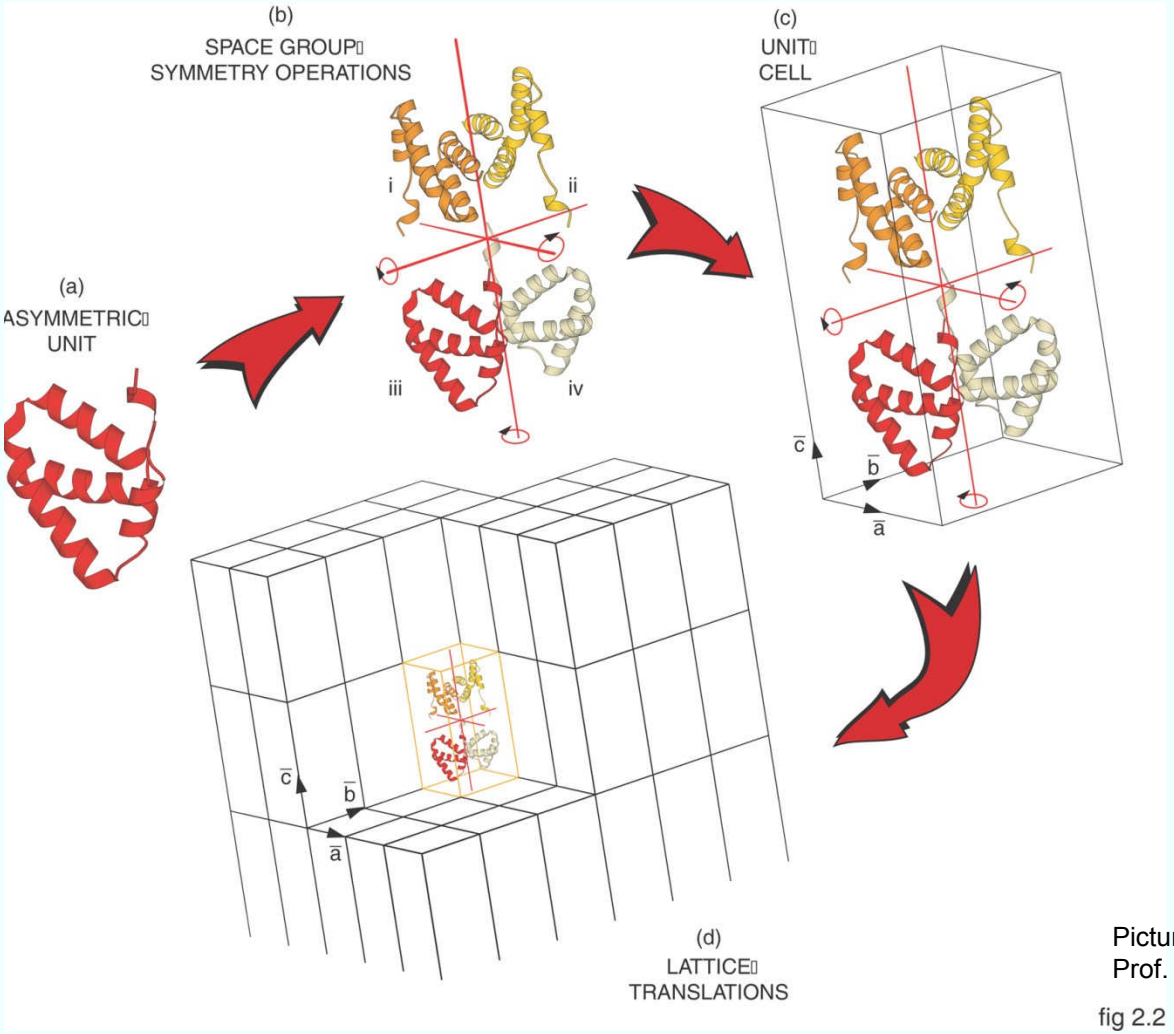
Protein crystallography in general, valid for both x-rays and neutrons as probes

Crystallography: Overiew over the process



Harma Brondijk, Crystal and Structural chemistry, Utrecht University

How a typical protein crystal looks like…



Picture taken from Lecture of Prof. Locher at ETH Zürich

fig 2.2

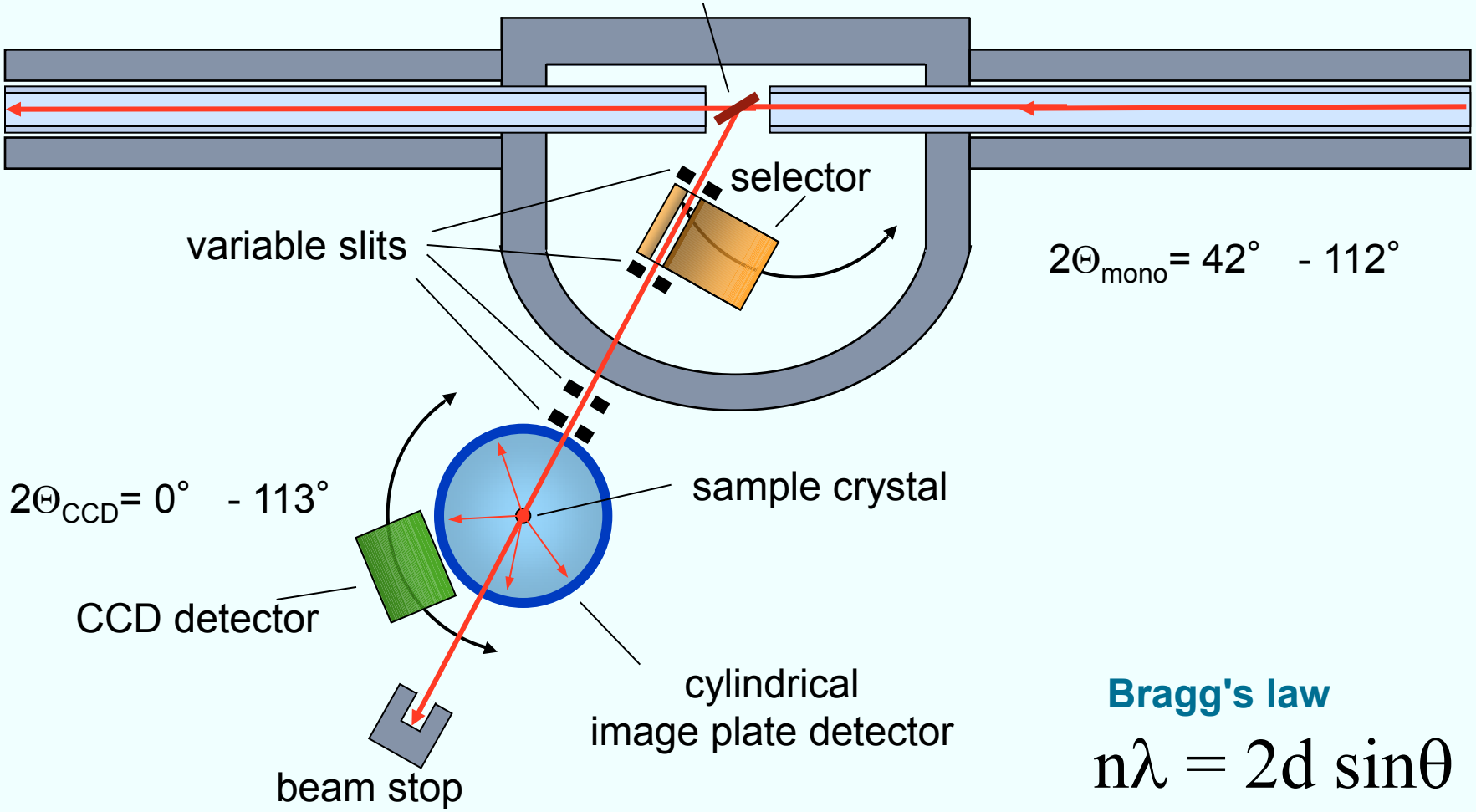
Size considerations of protein crystals

size:
x-ray-crystallography:
ca. 10 µm x 10 µm x 10 µm
typically cryoprotectants needed to facilitate measurements at low (80 K) temperatures

neutron protein crystalography:
The desirable size should be around 1 mm x 1 mm x 1 mm (depending on the protein/space group)

Outer diamter of the glas tube: 5 mm

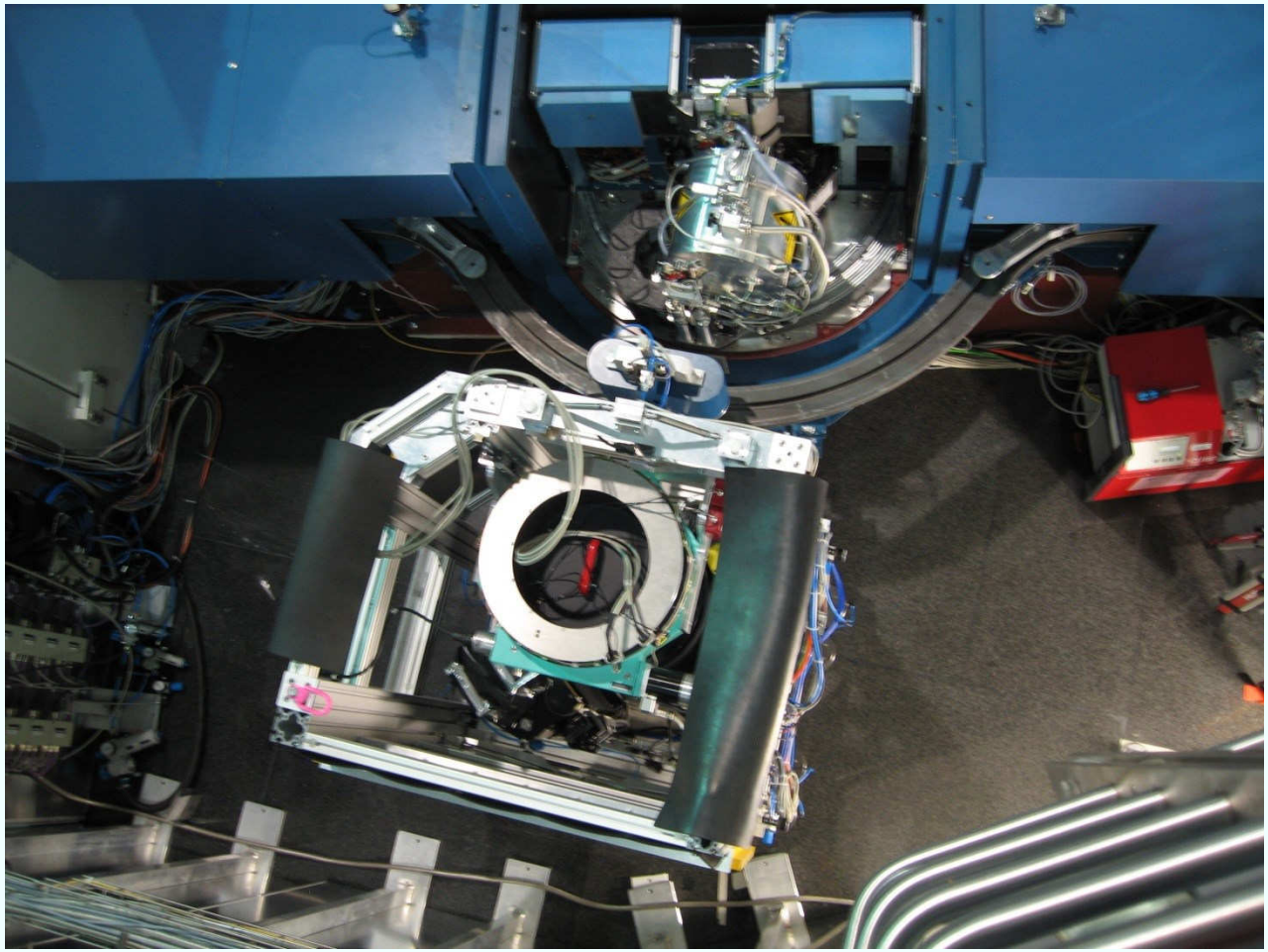
Schematic overview over BioDiff: A neutron protein diffractometer: collaboration between JCNS and FRMII



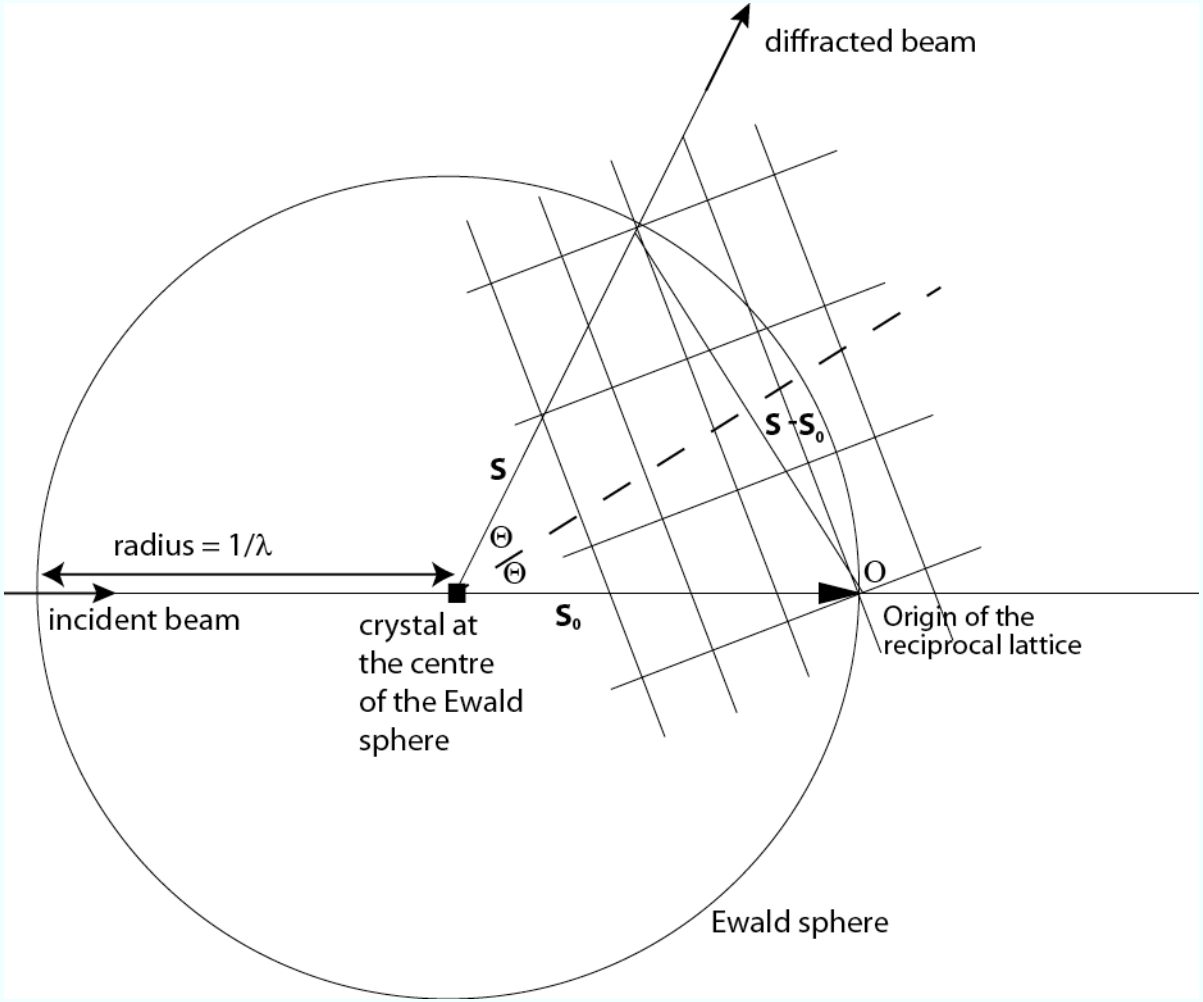
Bragg's law

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

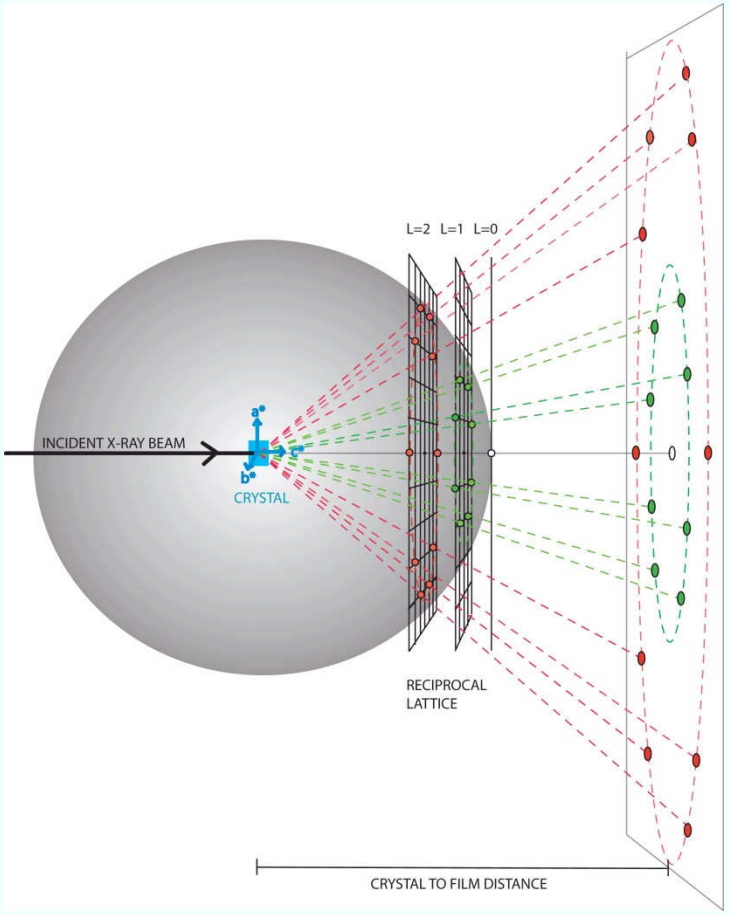
BioDiff, the corresponding view in reality:

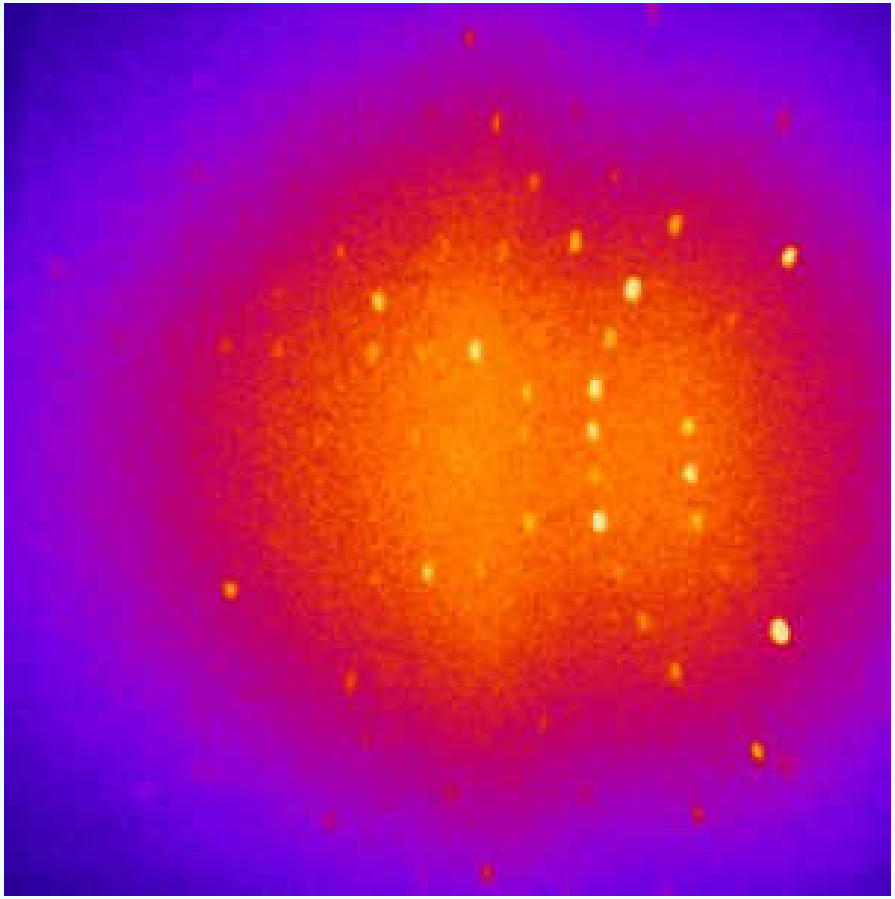


Ewald construction and Bragg‘s Law



Myoglobin protein crystal (deuterated mother liquor) full data set recorded with CCD

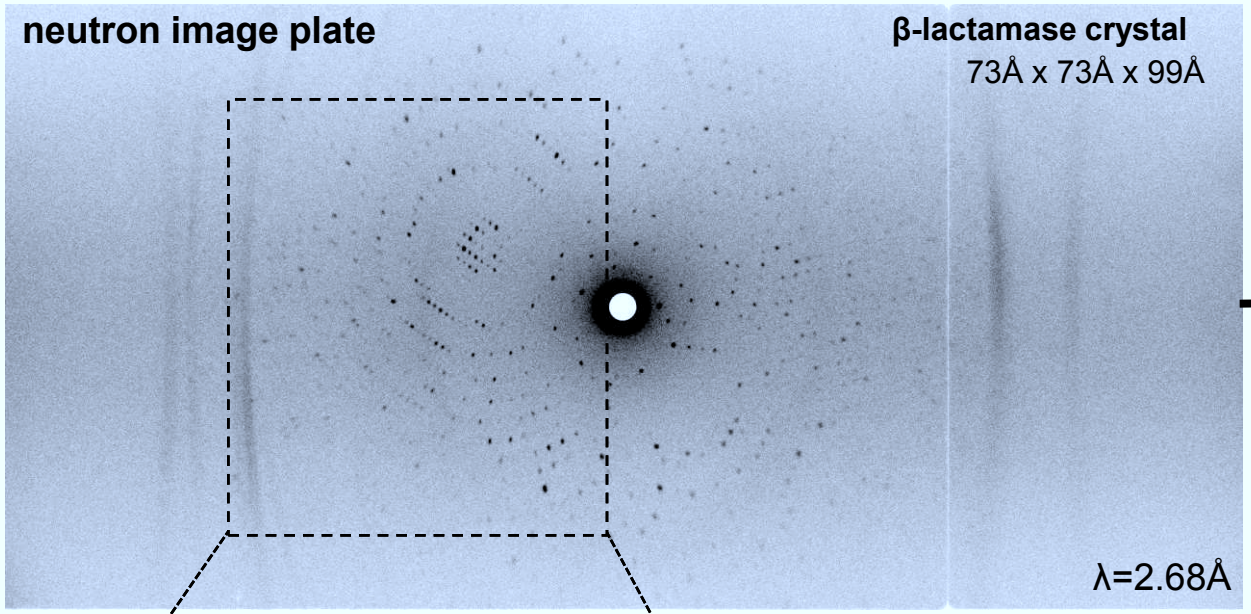


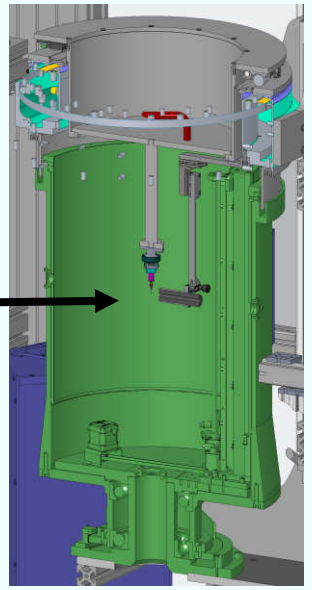


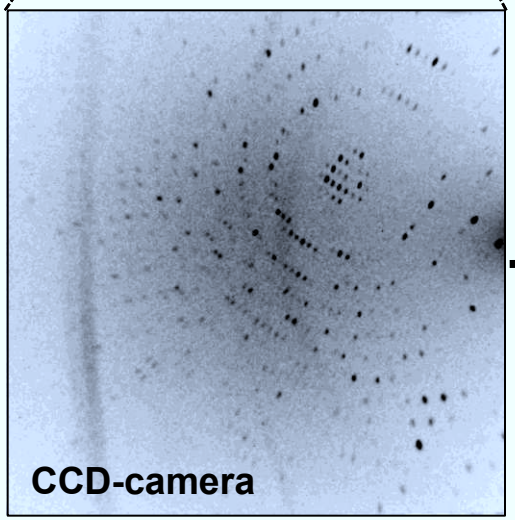
prim. beam

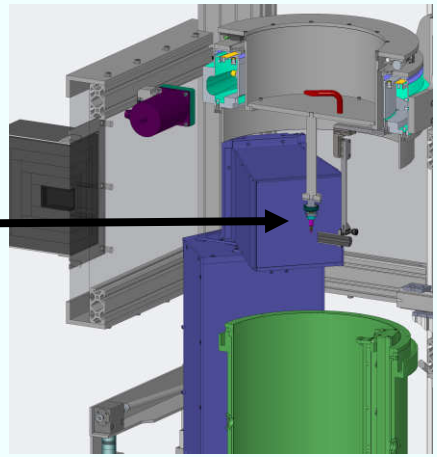
BioDiff: exposure time per frame: 20 minutes, sample: Myoglobin in deuterated mother liquor

The Two Detectors of BIODIFF









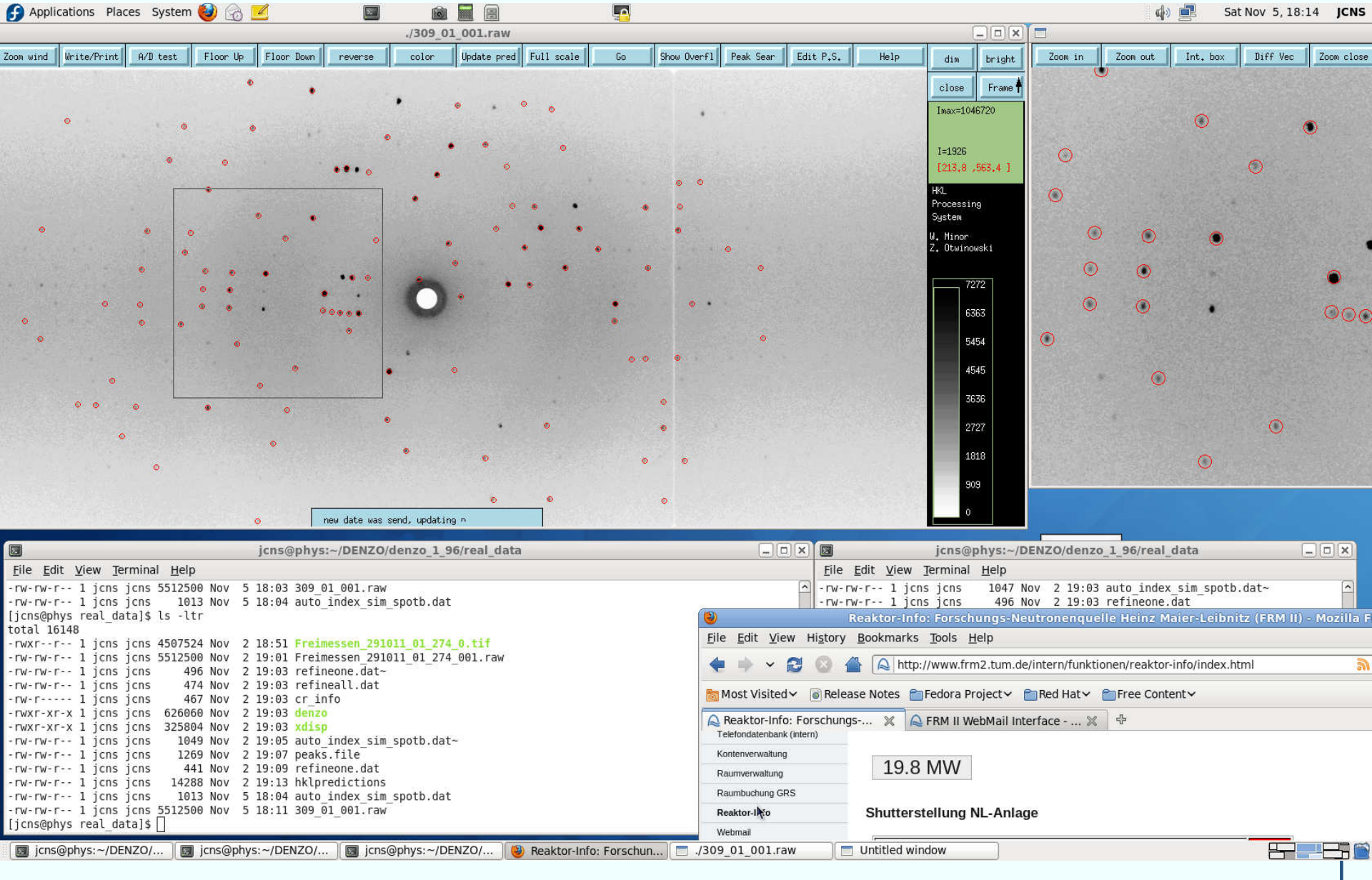
NIP-scanner

- larger solid angle
- readout time ≥ 4 min

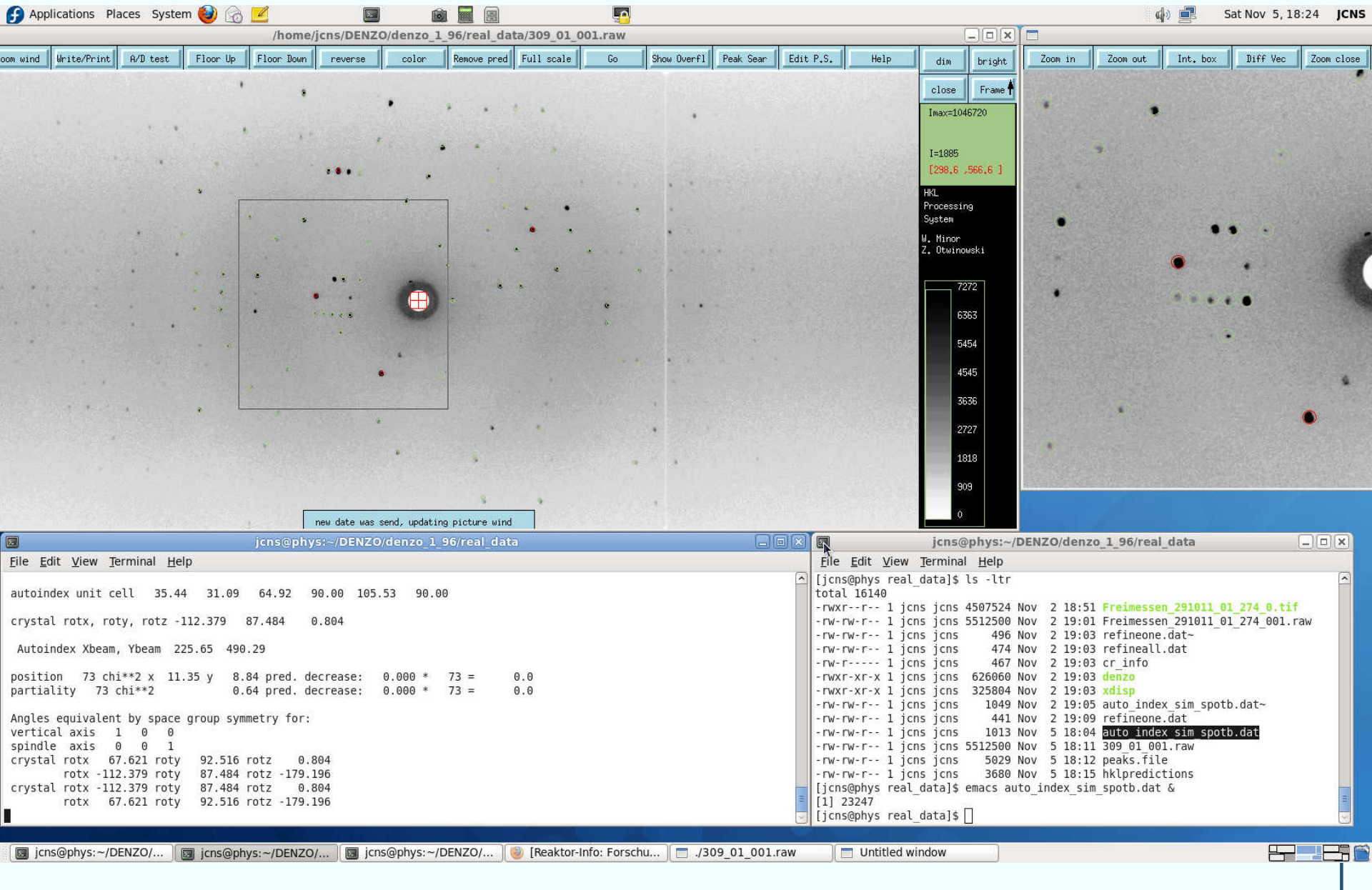
CCD-camera

- smaller solid angle
- readout time ≥ 1 sec

Peak search with hkl DENZO



auto-index


Applications Places System
Sat Nov 5, 18:24 JCNS
/home/jcns/DENZO/denzo_1_96/real_data/309_01_001.raw
Write/Print
A/D test
Floor Up
Floor Down
reverse
color
Remove pred
Full scale
Go
Show Overfl
Peak Sear
Edit P.S.
Help
dim
bright
close
Frame
Imax=1046720
I=1885
[298.6 ,566.6]
HKL
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W. Minor
Z. Otwinowski
7272
6363
5454
4545
3636
2727
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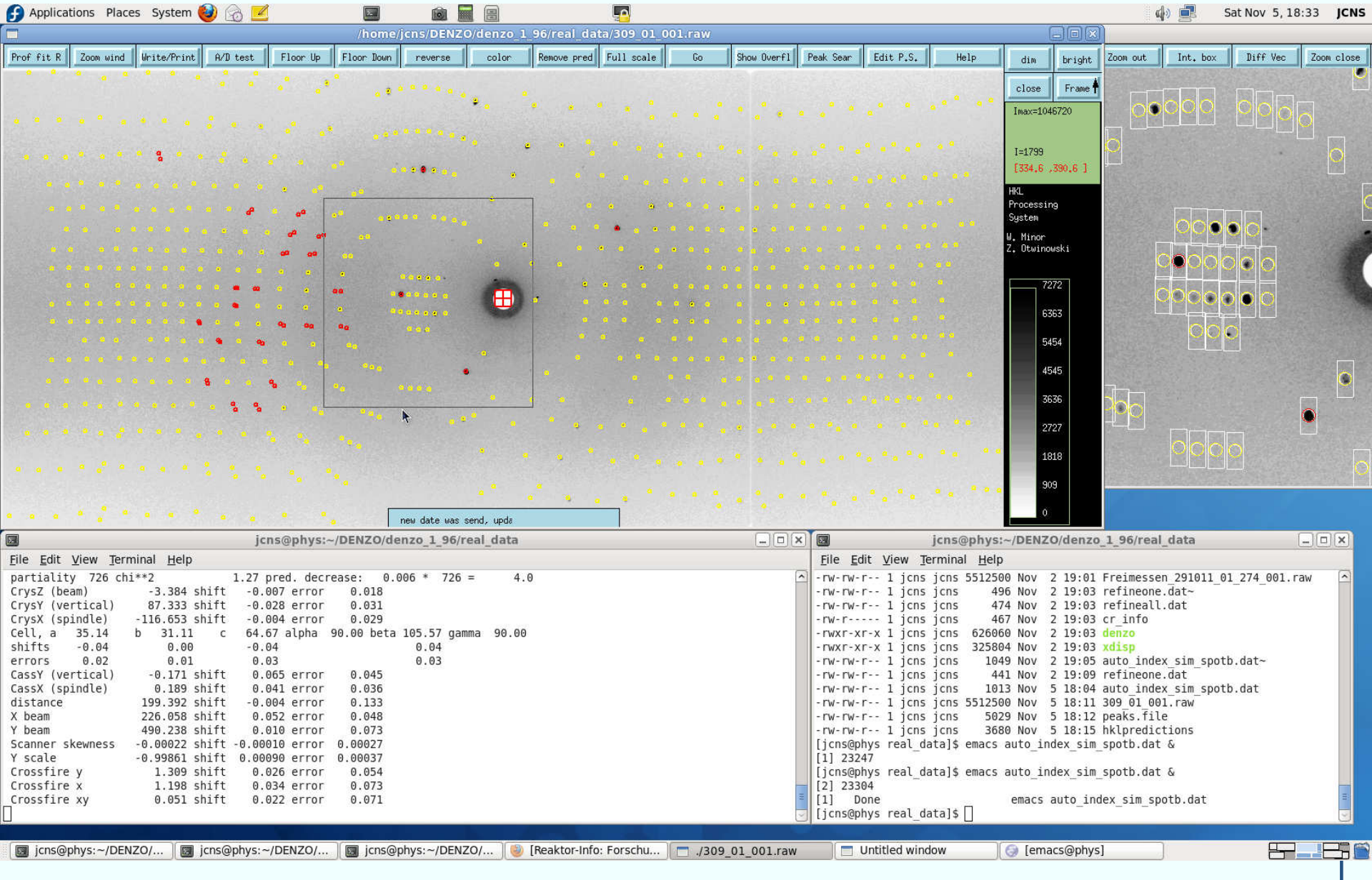
new date was send, updating picture wind
jcns@phys:~/DENZO/denzo_1_96/real_data
File Edit View Terminal Help
autoindex unit cell 35.44 31.09 64.92 90.00 105.53 90.00
crystal rotx, roty, rotz -112.379 87.484 0.804
Autoindex Xbeam, Ybeam 225.65 490.29
position 73 chi**2 x 11.35 y 8.84 pred. decrease: 0.000 * 73 = 0.0
partiality 73 chi**2 0.64 pred. decrease: 0.000 * 73 = 0.0
Angles equivalent by space group symmetry for:
vertical axis 1 0 0
spindle axis 0 0 1
crystal rotx 67.621 roty 92.516 rotz 0.804
rotx -112.379 roty 87.484 rotz -179.196
crystal rotx -112.379 roty 87.484 rotz 0.804
rotx 67.621 roty 92.516 rotz -179.196
jcns@phys:~/DENZO/denzo_1_96/real_data
File Edit View Terminal Help
[jcns@phys real_data]$ ls -ltr
total 16140
-rwxr--r-- 1 jcns jcns 4507524 Nov 2 18:51 Freimessen_291011_01_274_0.tif
-rw-rw-r-- 1 jcns jcns 5512500 Nov 2 19:01 Freimessen_291011_01_274_001.raw
-rw-rw-r-- 1 jcns jcns 496 Nov 2 19:03 refineone.dat~
-rw-rw-r-- 1 jcns jcns 474 Nov 2 19:03 refineall.dat
-rw-r----- 1 jcns jcns 467 Nov 2 19:03 cr_info
-rwxr-xr-x 1 jcns jcns 626060 Nov 2 19:03 denzo
-rwxr-xr-x 1 jcns jcns 325804 Nov 2 19:03 xdisp
-rw-rw-r-- 1 jcns jcns 1049 Nov 2 19:05 auto_index_sim_spotb.dat~
-rw-rw-r-- 1 jcns jcns 441 Nov 2 19:09 refineone.dat
-rw-rw-r-- 1 jcns jcns 1013 Nov 5 18:04 auto_index_sim_spotb.dat
-rw-rw-r-- 1 jcns jcns 5512500 Nov 5 18:11 309_01_001.raw
-rw-rw-r-- 1 jcns jcns 5029 Nov 5 18:12 peaks.file
-rw-rw-r-- 1 jcns jcns 3680 Nov 5 18:15 hklpredictions
[jcns@phys real_data]$ emacs auto_index_sim_spotb.dat &
[1] 23247
[jcns@phys real_data]$
jcns@phys:~/DENZO/...
jcns@phys:~/DENZO/...
jcns@phys:~/DENZO/...
[Reaktor-Info: Forschu...
./309_01_001.raw
Untitled window

d_min=1.5 Å


Applications Places System
Sat Nov 5, 18:33 JCNS
/home/jcns/DENZO/denzo_1_96/real_data/309_01_001.raw
Prof fit R
Zoom wind
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Floor Up
Floor Down
reverse
color
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Full scale
Go
Show Overfl
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Help
dim
bright
close
Frame
Imax=1046720
I=1799
[334.6 ,390.6]
HKL
Processing
System
W. Minor
Z. Otwinowski
7272
6363
5454
4545
3636
2727
1818
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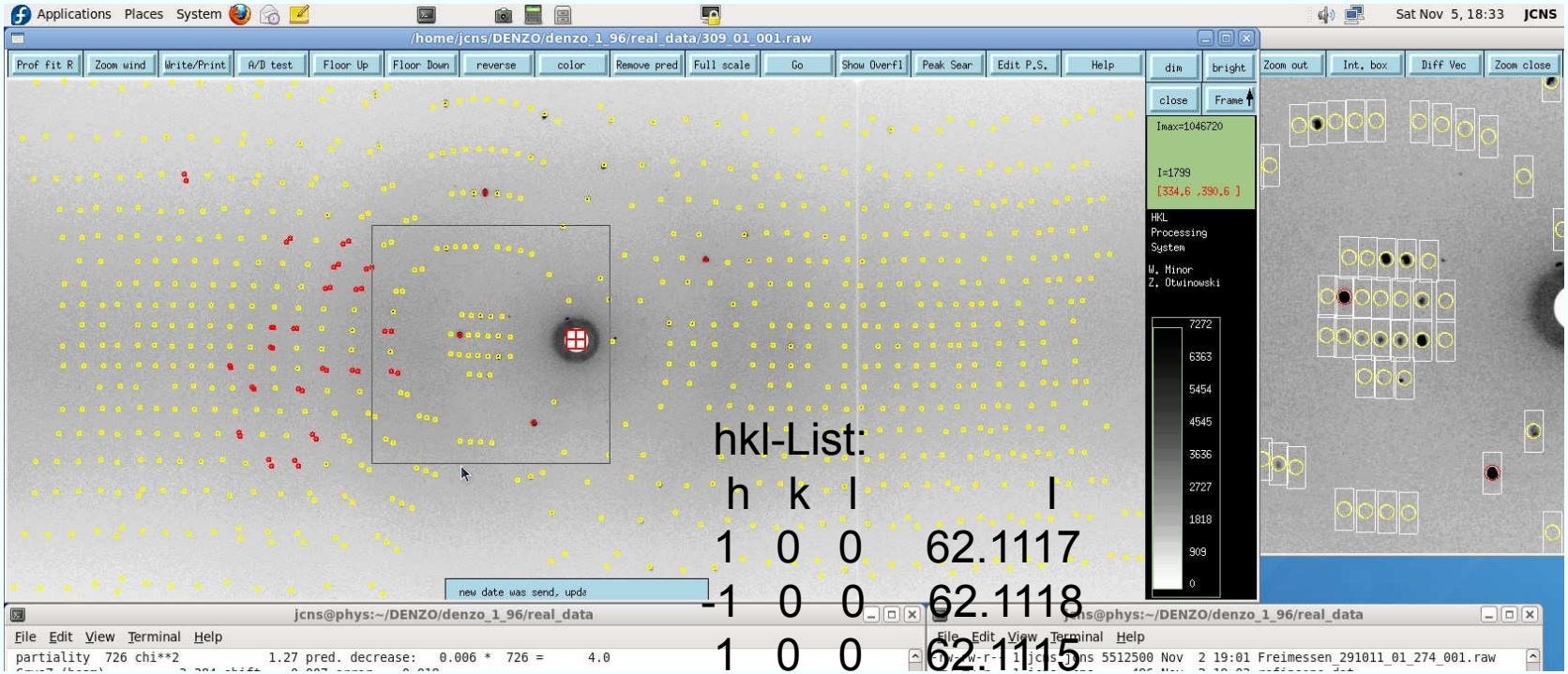
jcns@phys:~/DENZO/denzo_1_96/real_data
File Edit View Terminal Help
partiality 726 chi**2 1.27 pred. decrease: 0.006 * 726 = 4.0
CrysZ (beam) -3.384 shift -0.007 error 0.018
CrysY (vertical) 87.333 shift -0.028 error 0.031
CrysX (spindle) -116.653 shift -0.004 error 0.029
Cell, a 35.14 b 31.11 c 64.67 alpha 90.00 beta 105.57 gamma 90.00
shifts -0.04 0.00 -0.04 0.04
errors 0.02 0.01 0.03 0.03
CassY (vertical) -0.171 shift 0.065 error 0.045
CassX (spindle) 0.189 shift 0.041 error 0.036
distance 199.392 shift -0.004 error 0.133
X beam 226.058 shift 0.052 error 0.048
Y beam 490.238 shift 0.010 error 0.073
Scanner skewness -0.00022 shift -0.00010 error 0.00027
Y scale -0.99861 shift 0.00090 error 0.00037
Crossfire y 1.309 shift 0.026 error 0.054
Crossfire x 1.198 shift 0.034 error 0.073
Crossfire xy 0.051 shift 0.022 error 0.071
jcns@phys:~/DENZO/denzo_1_96/real_data
File Edit View Terminal Help
-rw-rw-r-- 1 jcns jcns 5512500 Nov 2 19:01 Freimessen_291011_01_274_001.raw
-rw-rw-r-- 1 jcns jcns 496 Nov 2 19:03 refineone.dat~
-rw-rw-r-- 1 jcns jcns 474 Nov 2 19:03 refineall.dat
-rw-r----- 1 jcns jcns 467 Nov 2 19:03 cr_info
-rwxr-xr-x 1 jcns jcns 626060 Nov 2 19:03 denzo
-rwxr-xr-x 1 jcns jcns 325804 Nov 2 19:03 xdisp
-rw-rw-r-- 1 jcns jcns 1049 Nov 2 19:05 auto_index_sim_spotb.dat~
-rw-rw-r-- 1 jcns jcns 441 Nov 2 19:09 refineone.dat
-rw-rw-r-- 1 jcns jcns 1013 Nov 5 18:04 auto_index_sim_spotb.dat
-rw-rw-r-- 1 jcns jcns 5512500 Nov 5 18:11 309_01_001.raw
-rw-rw-r-- 1 jcns jcns 5029 Nov 5 18:12 peaks.file
-rw-rw-r-- 1 jcns jcns 3680 Nov 5 18:15 hklpredictions
[jcns@phys real_data]$ emacs auto_index_sim_spotb.dat &
[1] 23247
[jcns@phys real_data]$ emacs auto_index_sim_spotb.dat &
[2] 23304
[1] Done emacs auto_index_sim_spotb.dat
[jcns@phys real_data]$
jcns@phys:~/DENZO/...
jcns@phys:~/DENZO/...
jcns@phys:~/DENZO/...
[Reaktor-Info: Forschu...
./309_01_001.raw
Untitled window
[emacs@phys]

Integration of partial Bragg peaks with the commercial software hkl-denzo up to d_{min}=1.5 Å



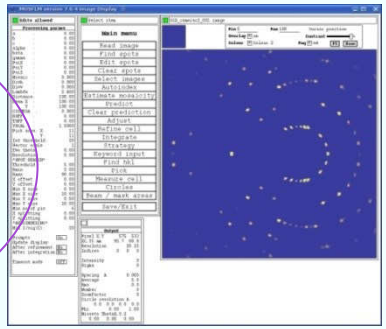
hkl-List:

h	k	l	I
1	0	0	62.1117
-1	0	0	62.1118
1	0	0	62.1115
-1	0	0	62.1120
0	0	-1	33.5555
1	0	-1	33.5589
0	0	1	33.5533
-1	0	1	33.5511

ca. 300 images

Fow chart of data treatment and model building

Scans at varying crystal orientation
Scan := Series of detector images



-MOSFLM
-HKL-denzo
(comercial)

Data reduction

- determination of crystal orientation, unit cell dimensions etc.
- Calculating integral of reflection intensities

hkl-list for each scan:
h k l Intensity Intensity error

Scaling of each hkl list to match each other

-SCALA (CCP4-program package)

Unified hkl-list of measurement := complete data set

Calculation of a first map

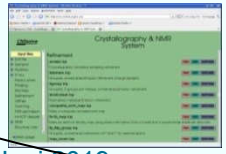
Additional information from the solution of the phase problem

Struktur refinement

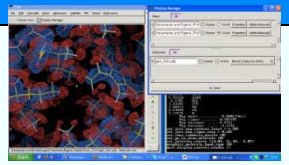
-Refinement of atom coordinates displacements
- Calculation of scattering density maps (netrons) or electron density maps (x-rays)

Map-plotting

- inspection of model to fit the map)
- real space changes and refinement to the model

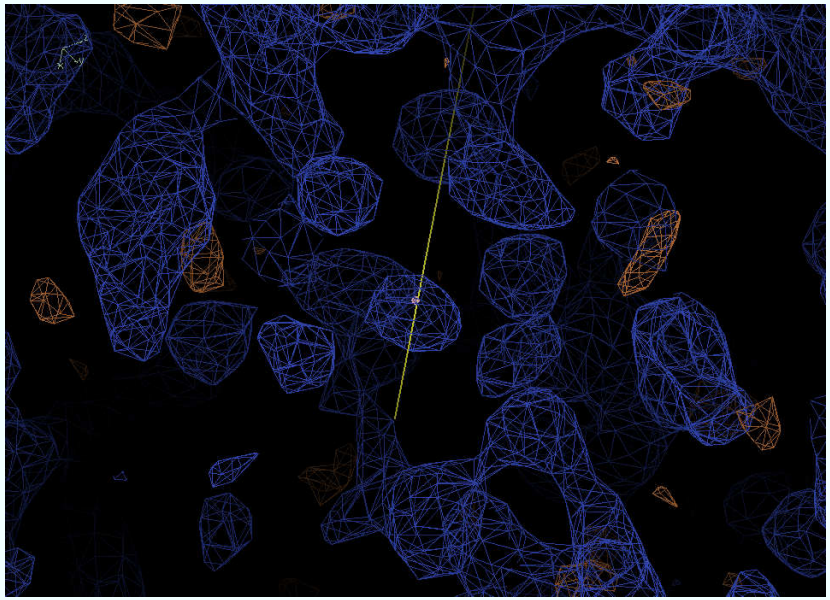


-nCNS
-PHENIX

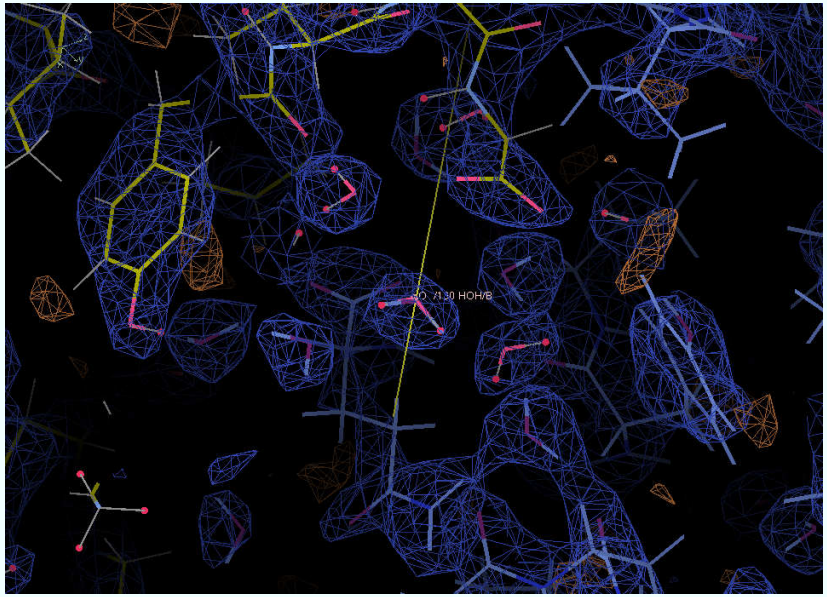


-XtalView
-Coot

Structural Refinement: Putting the model in and applying changes in real space



PHENIX

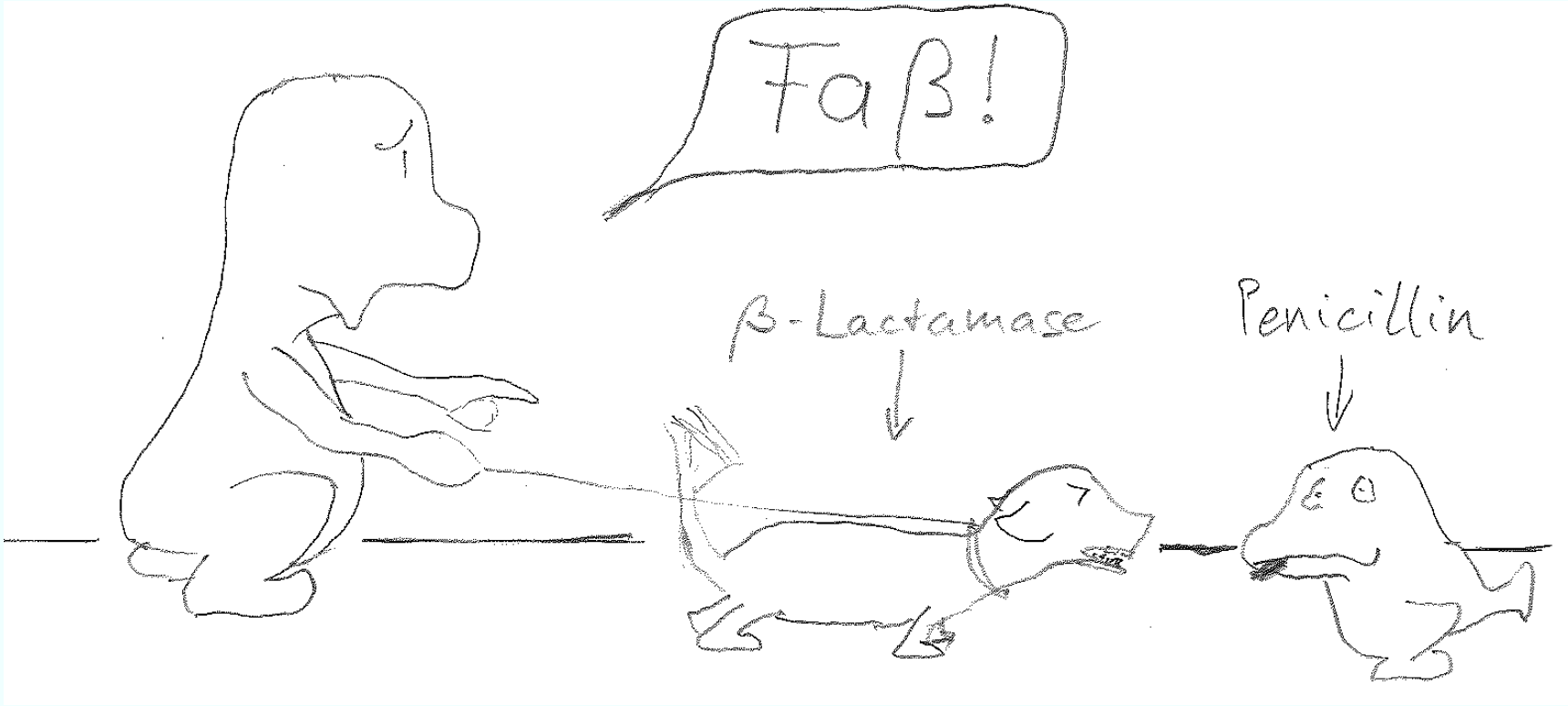


scattering length density map (neutrons)

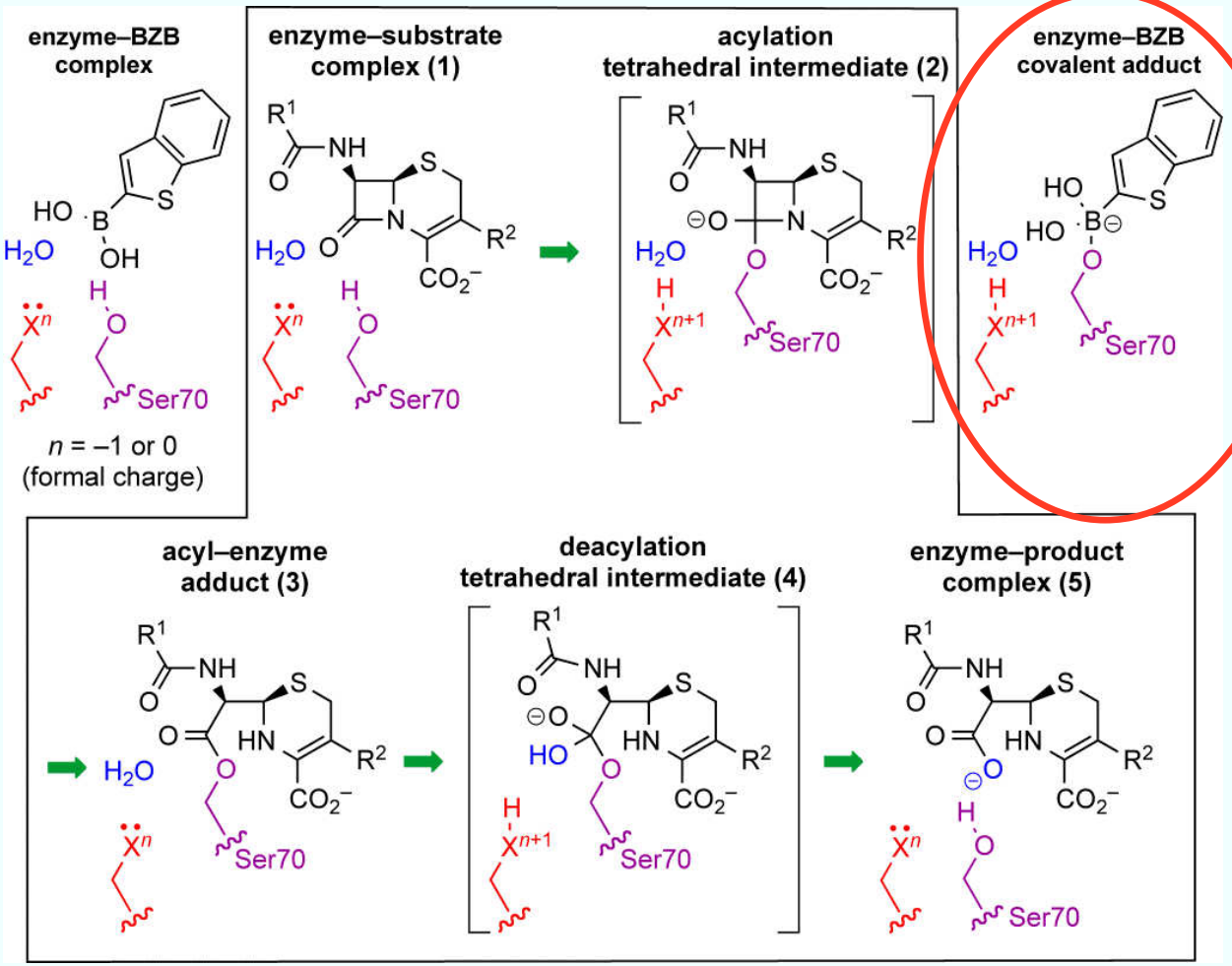
All atoms, the whole amino acid chain is fitted into the scattering length density map

Application Example: Protonation state of amino acid residues

The protein β-lactamase



β-lactamase: hydrolyses β-lactam antibiotics



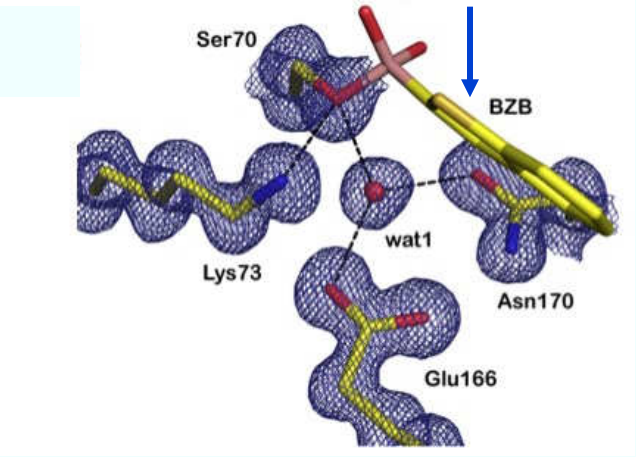
Neutron structure recorded

The catalytic cycle of a class A β-lactamase illustrated for a cephalosporin substrate (inside box) and the mode of inhibition by BZB (outside box). The general base employed is not necessarily the same for acylation and deacylation. The overall reaction pathway for β-lactam hydrolysis of a cephalosporin-like substrate by the class A β-lactamase enzymes.

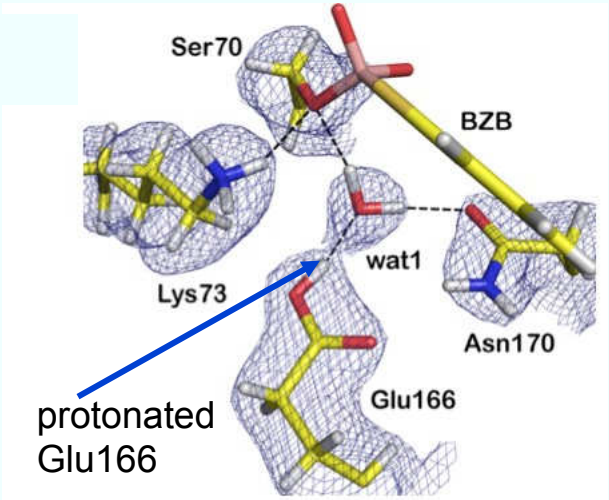
Figures from: Tomanicek et al., doi: 10.1074/jbc.M112.436238

Catalytic Proton Network of the Toho-1 β-Lactamase

transition state
analogue BZB



electron density map



nuclear density map from BioDiff

Glu166 acts as the general base during the catalytic action of the enzyme.

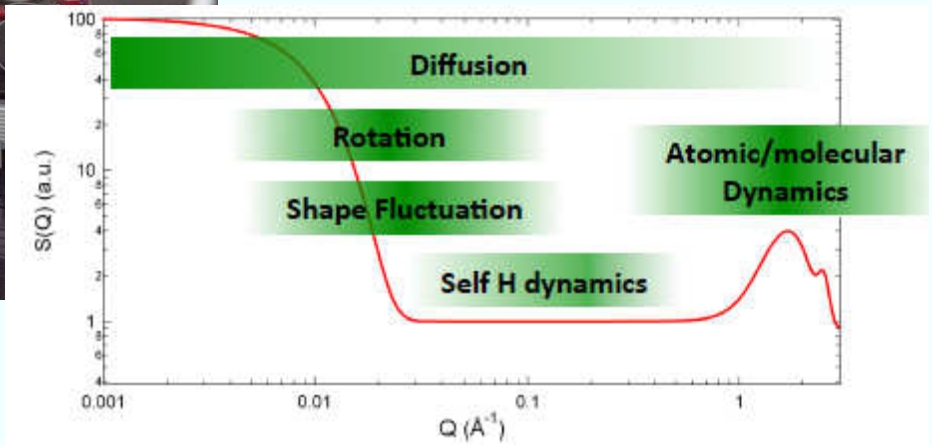
Stephen J. Tomanicek, Robert F. Standaert, Kevin L. Weiss,
Andreas Ostermann, Tobias E. Schrader, Joseph D. Ng, and Leighton Coates
J. Biol. Chem. 2013, 288:4715-4722

Summary

- x-ray crystallography: Most of the beautiful schematic pictures of proteins in textbooks of chemistry and molecular biology represent structures determined by X-ray diffraction. Advantages:
  1. only small crystals needed
  2. short measurement times enable large throuput
  3. phase problem can be solved with more and more sophisticated methods

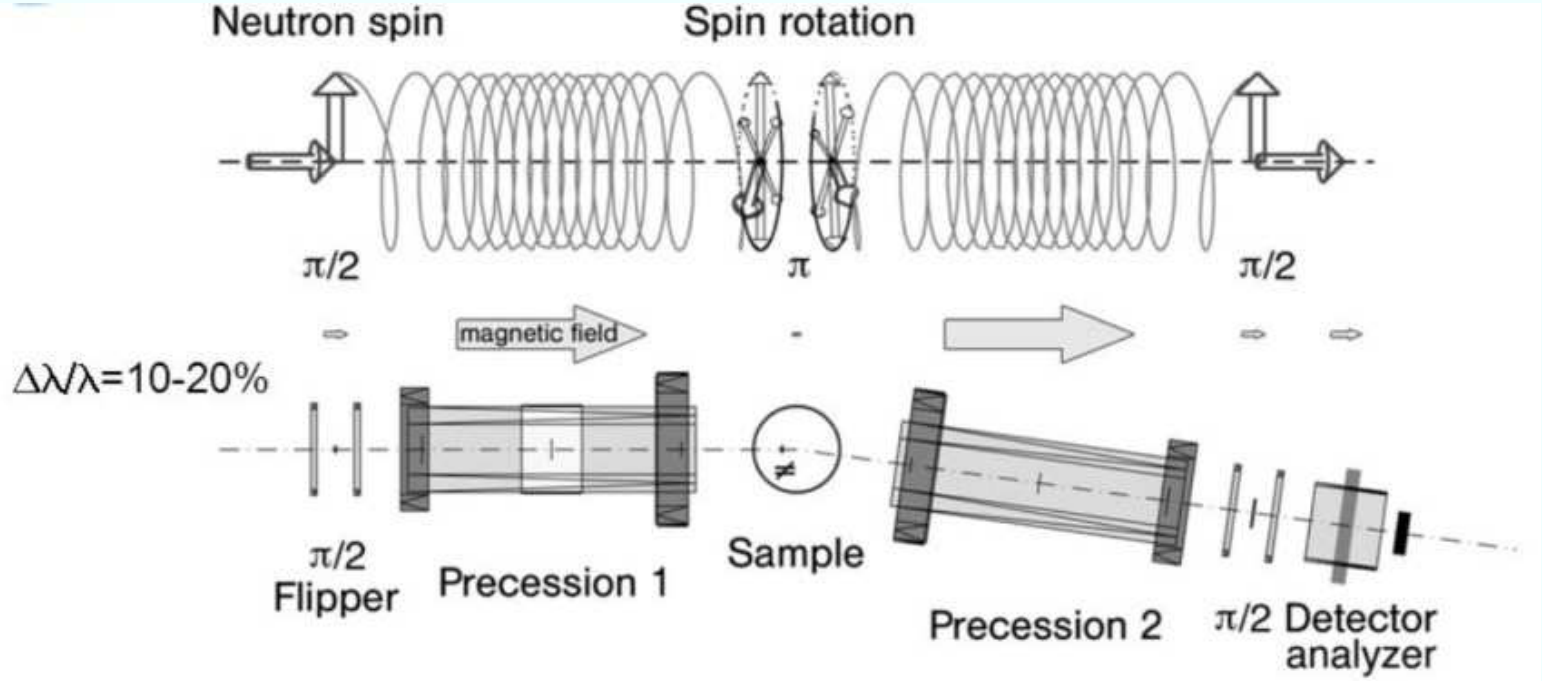
  Disadvantages:
  1. radiation damage often observed: hydrogen abstraction, reduction of metal centres in the metalo-proteins, disulfide bond cleavage.
  2. Hydrogen positions can usually not be determined (only at high resolution)
- Neutron protein crystallography is a complementary technique as compared to x-ray crystallography. Here one can determine:
  1. protonation states of amino acid side chains (important for the function of the protein)
  2. solvent structure including hydrogen atoms

Neutron Spin Echo Spectroscopy



A. Farone: Methods and Applications of SANS, NR, and NSE NCNR, 2012

The Spin Echo Principle

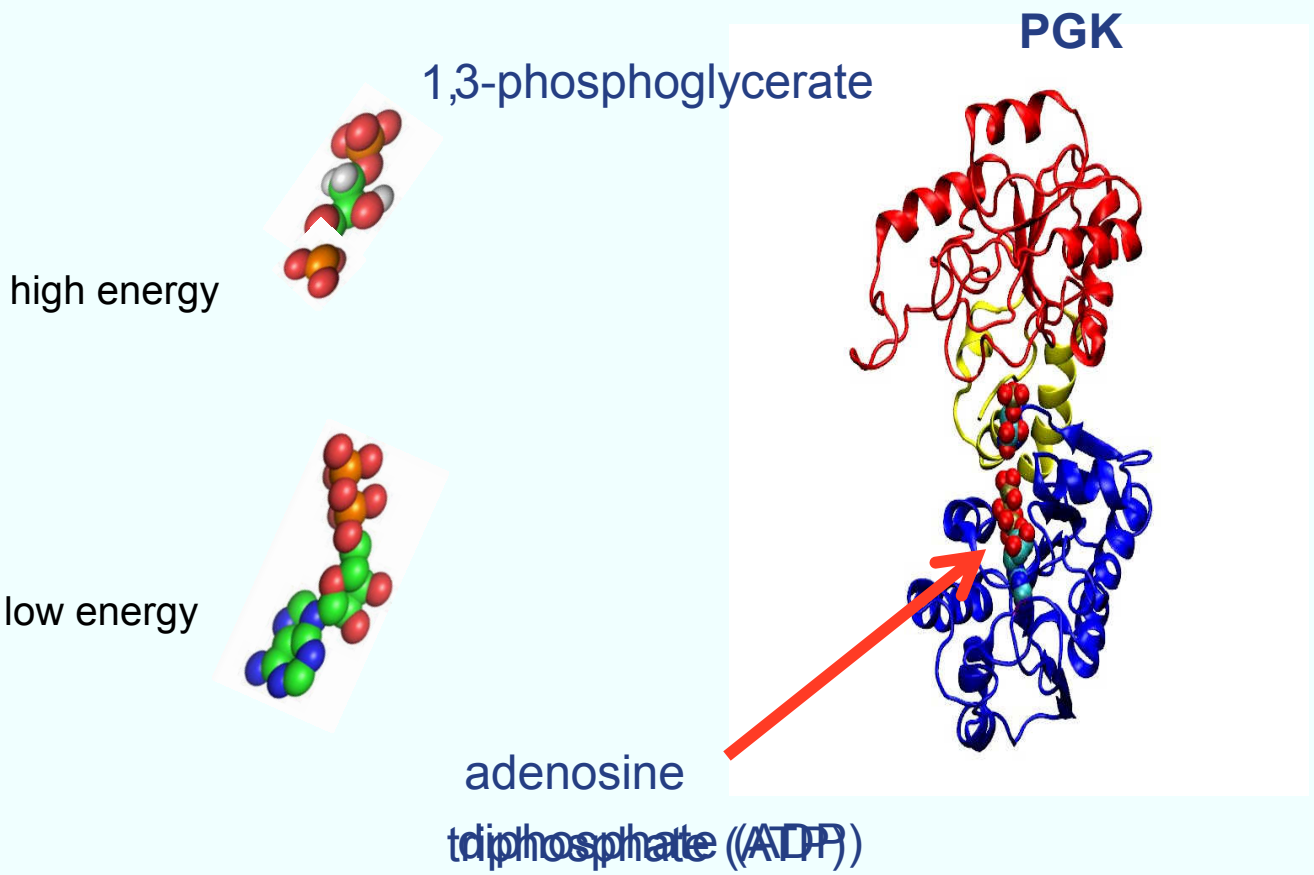


decoupling detectability of tiny velocity changes caused by the scattering process from the width of the incoming velocity distribution

the key is the neutron spin

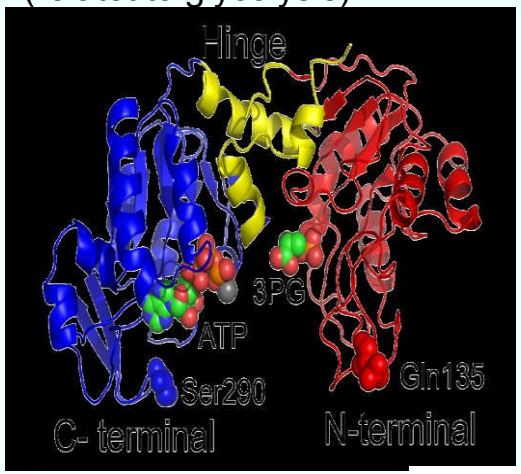
Slide by A. Radulescu and Olaf Holderer

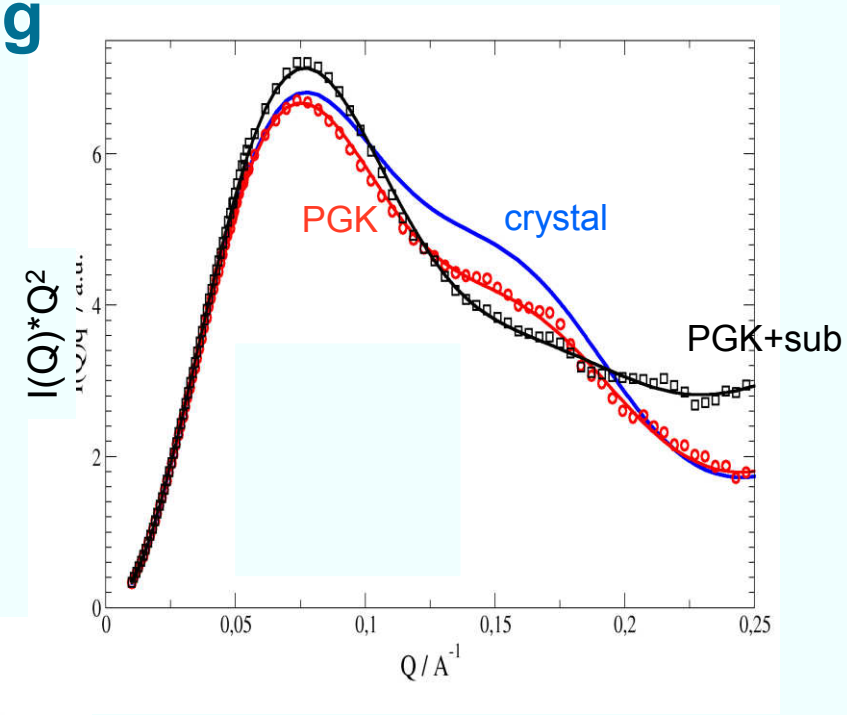
Phosphoglycerate Kinase is sixth step in glycolysis to deliver energy from sugar by phosphate transfer



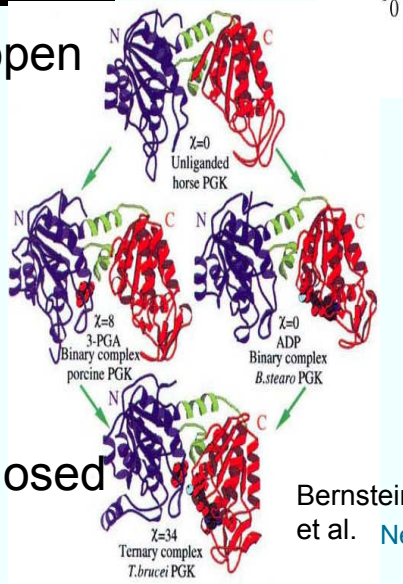
Structural change of Phosphoglycerate Kinase (PGK) due to substrate binding

yeast PGK
(relatet to glycolysis)





open



closed

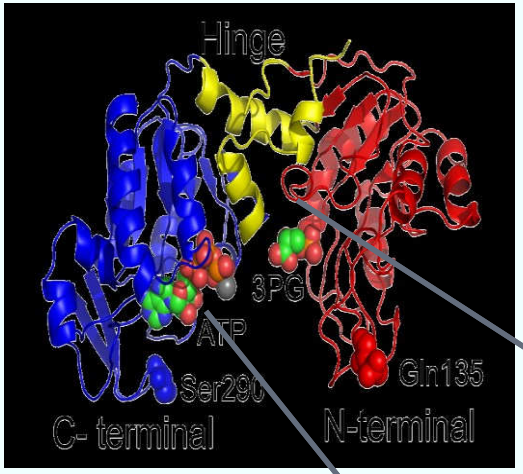
homologes
binding induces closing

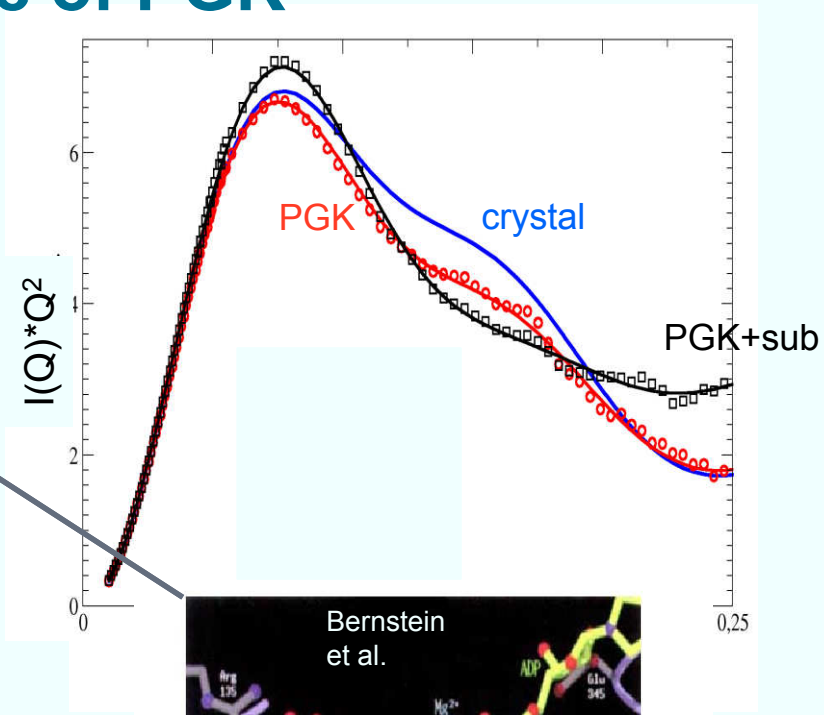
Bernstein
et al.

Inoue et al. BiophysJ 99, 2309 (2010)

Elastic normal modes as templates for the structural change of PGK

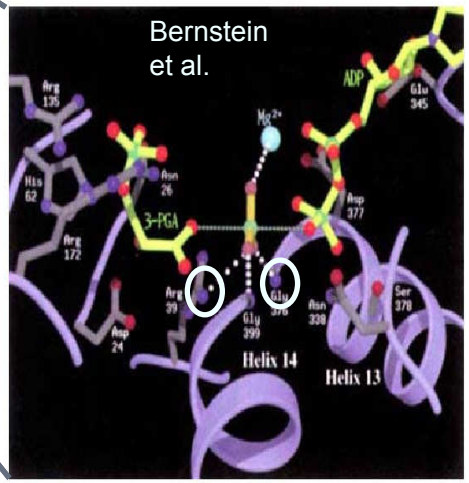
yeast PGK
(relatet to glycolysis)





Arg38-Gly371

crystal	➔ 11.8 A
PGK	➔ 11.4 A
PGK+sub	➔ 8.2 A
active	➔ 3.5 A

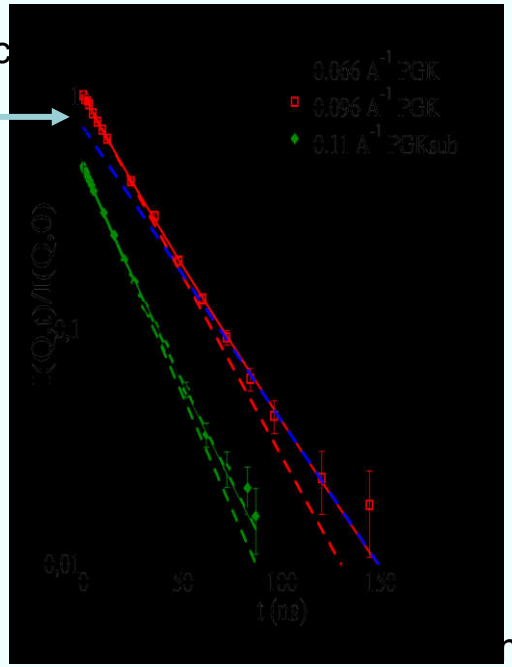


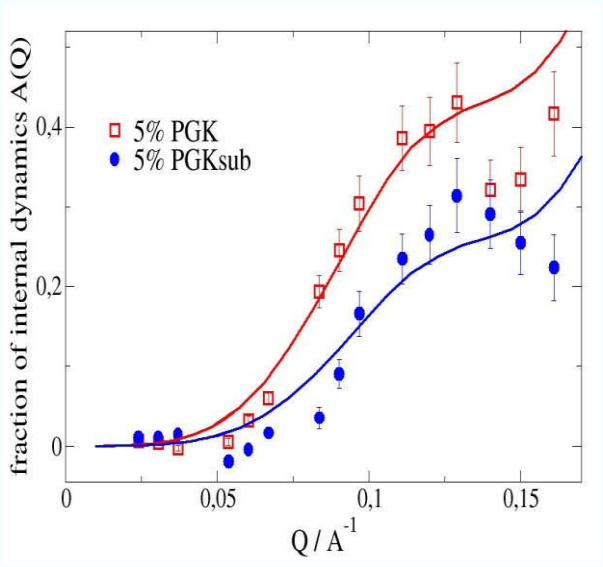
activity not possible in **this** closed configuration with substrate bound

Inoue et al. BiophysJ 99, 2309 (2010)

Substrate binding reduces relaxation time and internal dynamics amplitude for PGK

internal dynamic

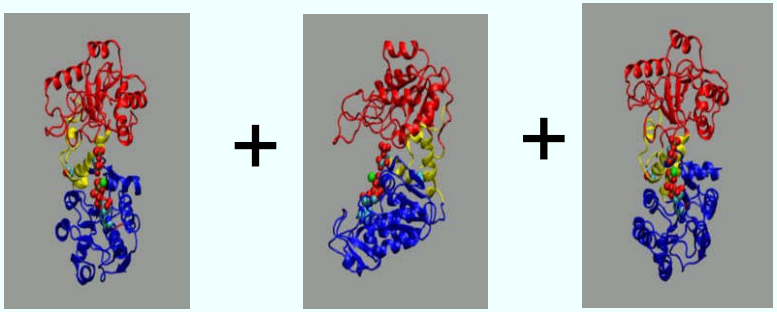




1/Γ=60(±10) ns PGK
1/Γ=45(±10) ns PGKsub

mean atomic displacement
10.5±2A for PGK
7.0±2A for PGKsub

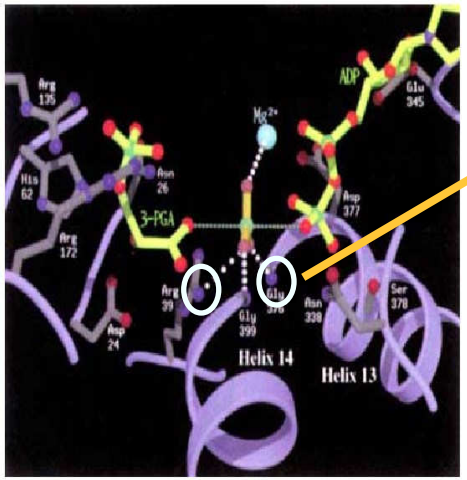
mode 8 mode 9



contribution of normal modes

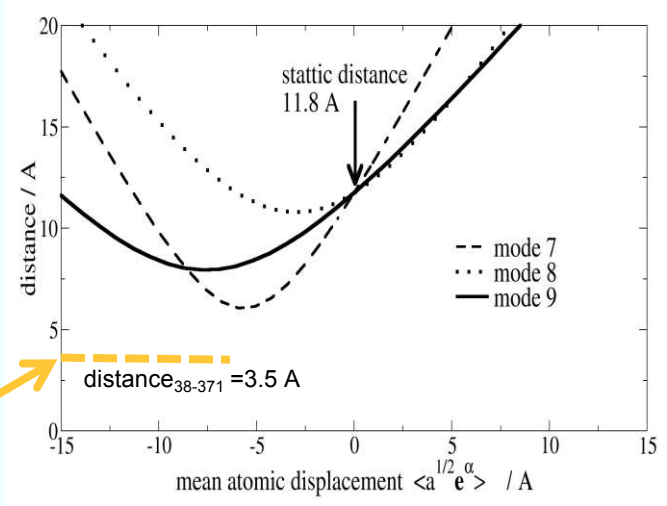
Inoue et al. BiophysJ 99, 2309 (2010)

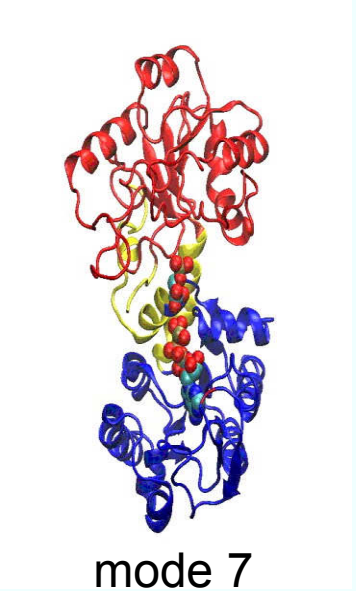
Domain dynamics is essential to reach catalytic configuration



Bernstein et al. Nature 385, 275 (1997)

Figure 5 The active site of *T. brucei* PGK with the transition state modelled as a





mode 7

Arg 38

Gly 371

➔ without dynamics no function

Inoue et al. BiophysJ 99, 2309 (2010)

Spin Echo summary

Dynamics of biological macromolecules in solution can be investigated without the need for (fluorescent) labels

Equilibrium dynamics (normal modes) are essential for the function of some enzymes

Thanks to...

- Ralf Biehl
- Andreas Ostermann
- Marialucia Longo
- Livia Balacescu
- Florian Vögl
- Leo Rothmayer
- Olaf Holderer
- Aurel Radulescu

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and you for your attention!