Jülich Centre for Neutron Science

JÜLICH FORSCHUNGSZENTRUM

The instrument BIODIFF

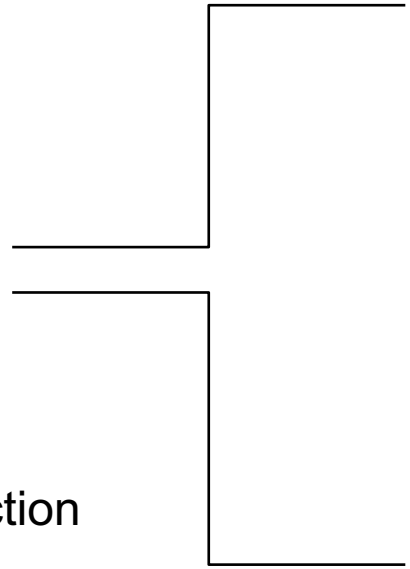
Fachkunde Schichtpersonal

November 28st 2019 | Tobias E. Schrader

Outline

- Motivation
- Introduction into proteins/crystals
- Introduction into the instrument BIODIFF
- Theory of scattering from crystals
- neutron protein crystallography: how it works in reality!
- One application example: From Structure to function…

What to do in Science as a Physicist?



Astronomy
Particle Physics (GUT)
Nuclear Physics

Physics

Fundamental Forces:
- Gravity
- Electromagnetic interaction
- Weak Force
- Strong Force

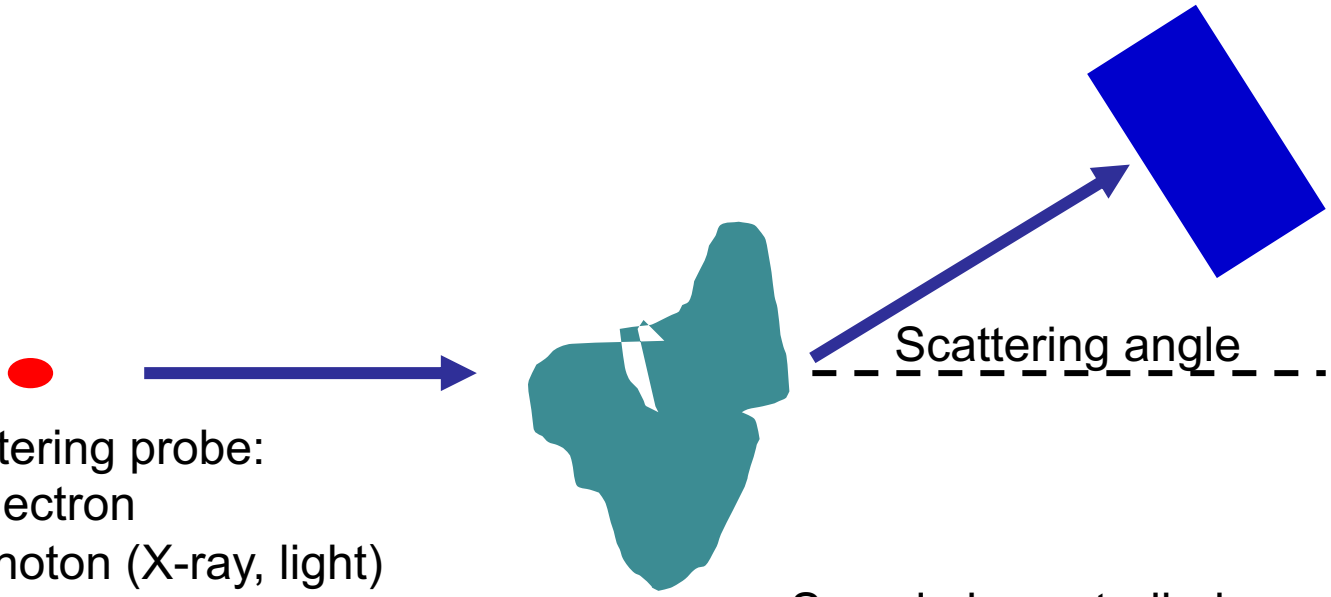
Physics of complex Systems
- Magnetism
- Softmatter
- Biological Samples (Proteins, lipid bilayers=membranes)
- Complex samples of technical relevance: Batteries, turbine blades

Why do we need experimental studies on proteins?

- **MD-Simulations** suffer from non-perfect force fields: Especially the long range electrostatics is not reproduced very well. But proteins use defined and structure related electrostatics to move the acidity constants of side chains in order to make them fullfill their tasks. MD-simulations cannot model bond breaking and forming very well since the quantum chemistry nature of this process is not included in the theoretical foundation of MD.
- **Ab initio quantum chemical calculations** are still too demanding to model the complete active centre of a protein (including its substrate)

Standard Scattering process

- The probe in use determines the information you get…



Scattering probe:
- Electron
- Photon (X-ray, light)
- Neutron
- Alpha particle
- ^{3}He
- Proton
- (Heavy) Ion
- Myon
- Positron

Sample in controlled condition:
- Temperature
- Humidity
- Magnetic field
- Electric field
- Pressure

All instruments can be sorted according to their energy resolution and type of scattering

Coherent (Contains information on relative distances in the sample and has a distinct angular dependence)

Incoherent (Contains no information on relative distances in the sample and has no angular dependence)

Scattering

„Structure“

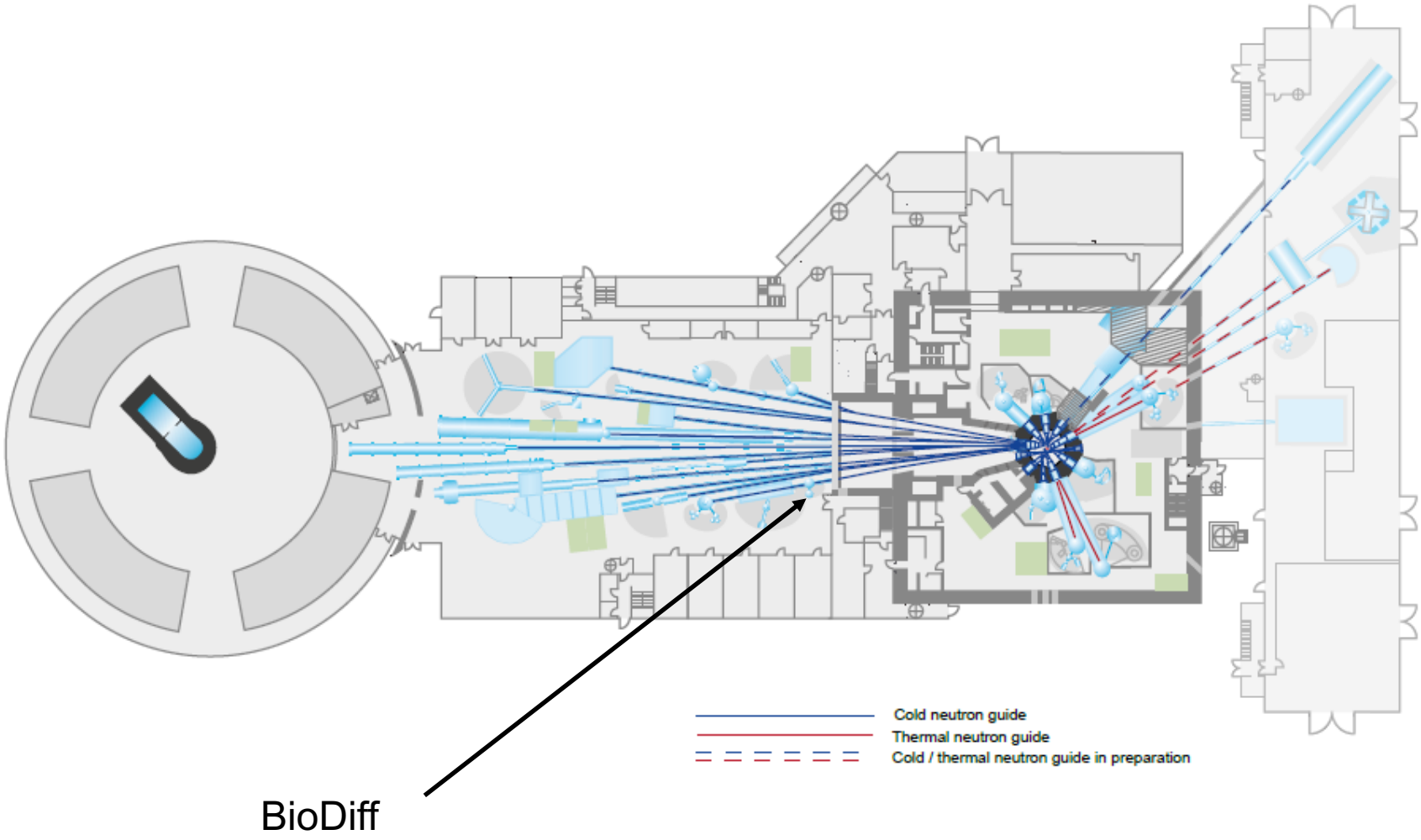
Elastic (no energy transfer from or to the sample)

„Dynamics“

Inelastic (Scattering probe transfers to the sample or receives energy from it)

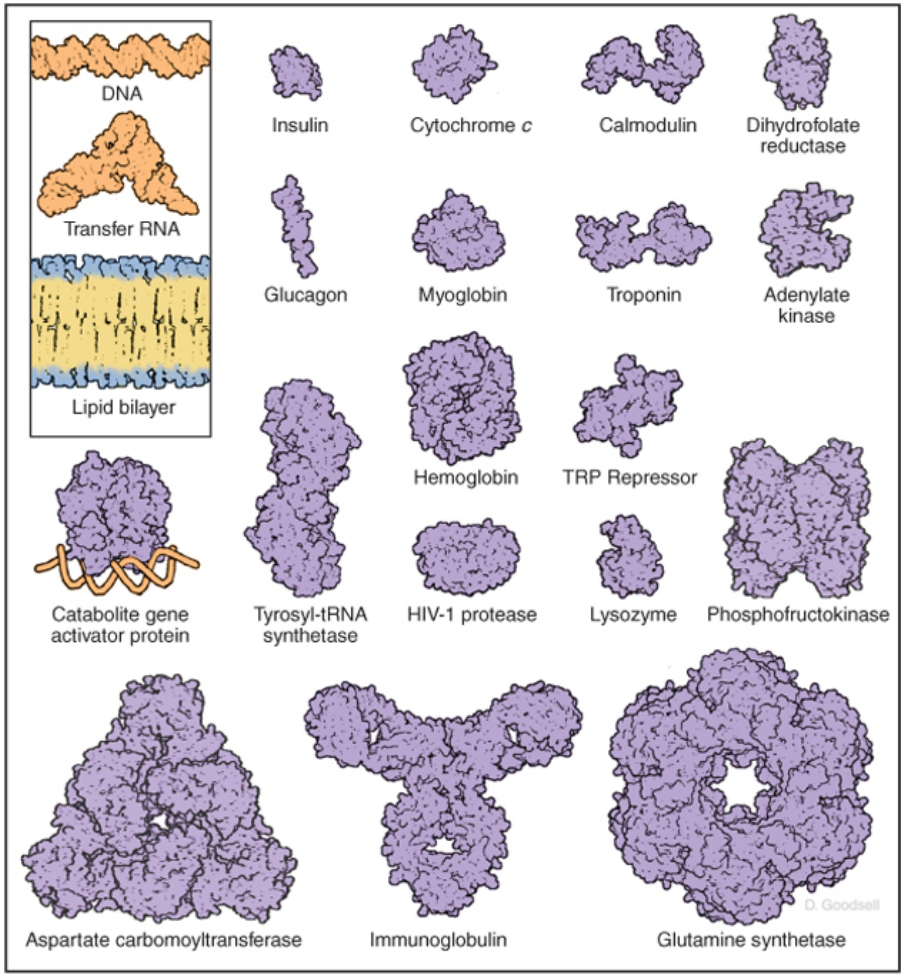
- Only elastic incoherent scattering is of no interest to the scientists

BioDiff at FRM II in the neutron guide hall west



Introduction into structured macromolecules and a simple model for proteins

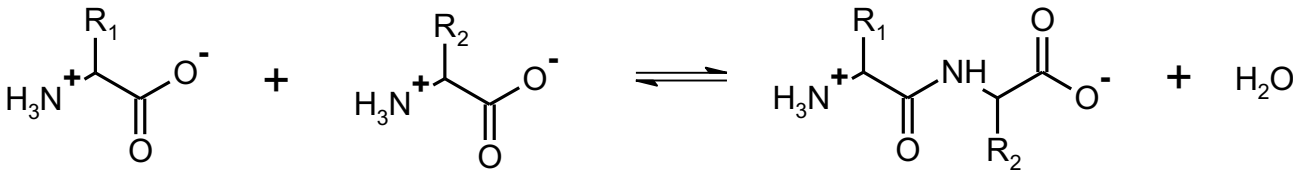
Structured macromolecules come in different shapes and sizes



Definition of "protein"

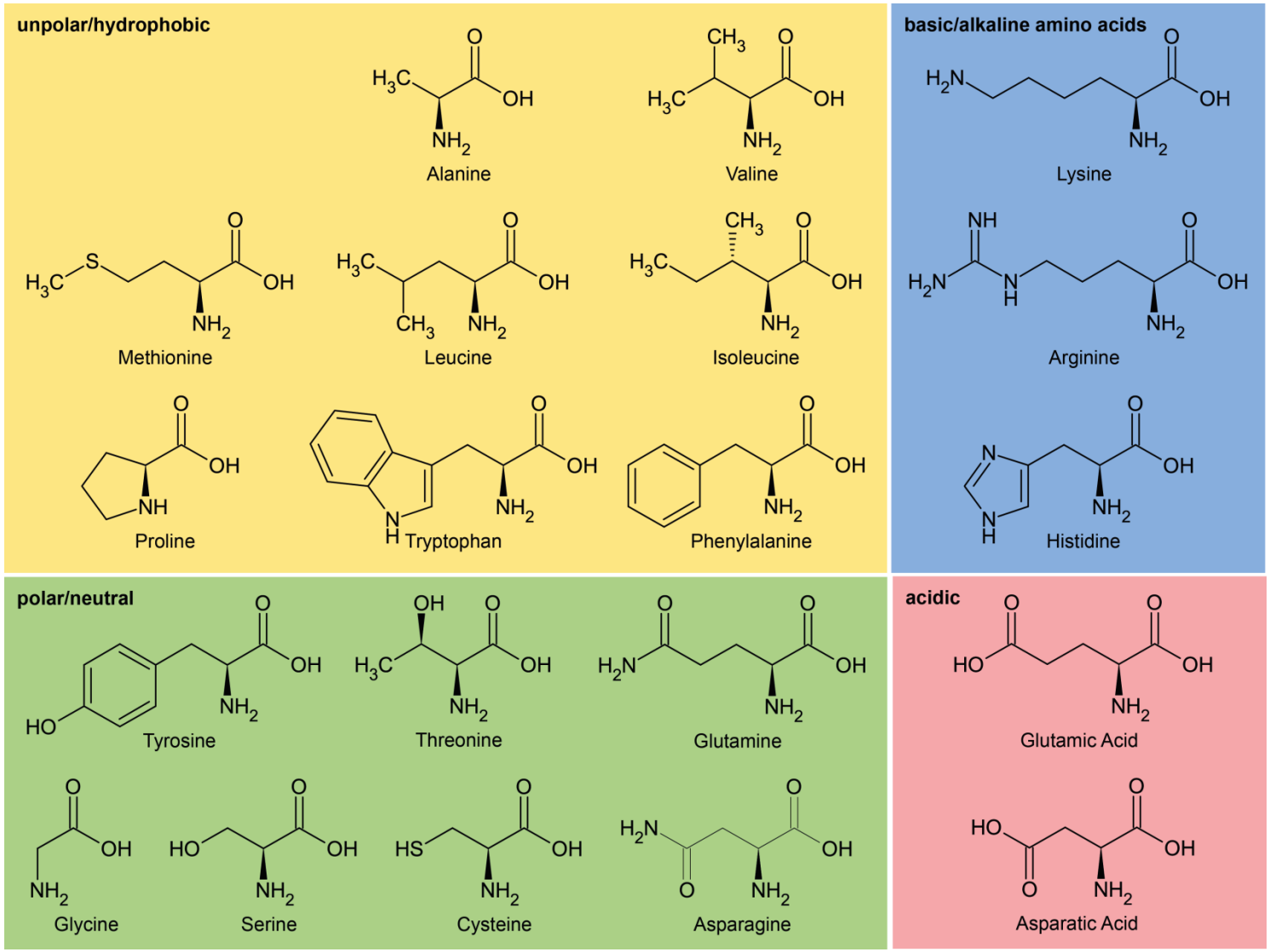
• Proteins are the most abundant class of biological macromolecules in all cellular organisms.

• They are polymers of α-amino acids (2-amino carboxylic acids), formed by condensation of carboxyl and amino functions of adjacent units. There are 20 different amino acids in nature.



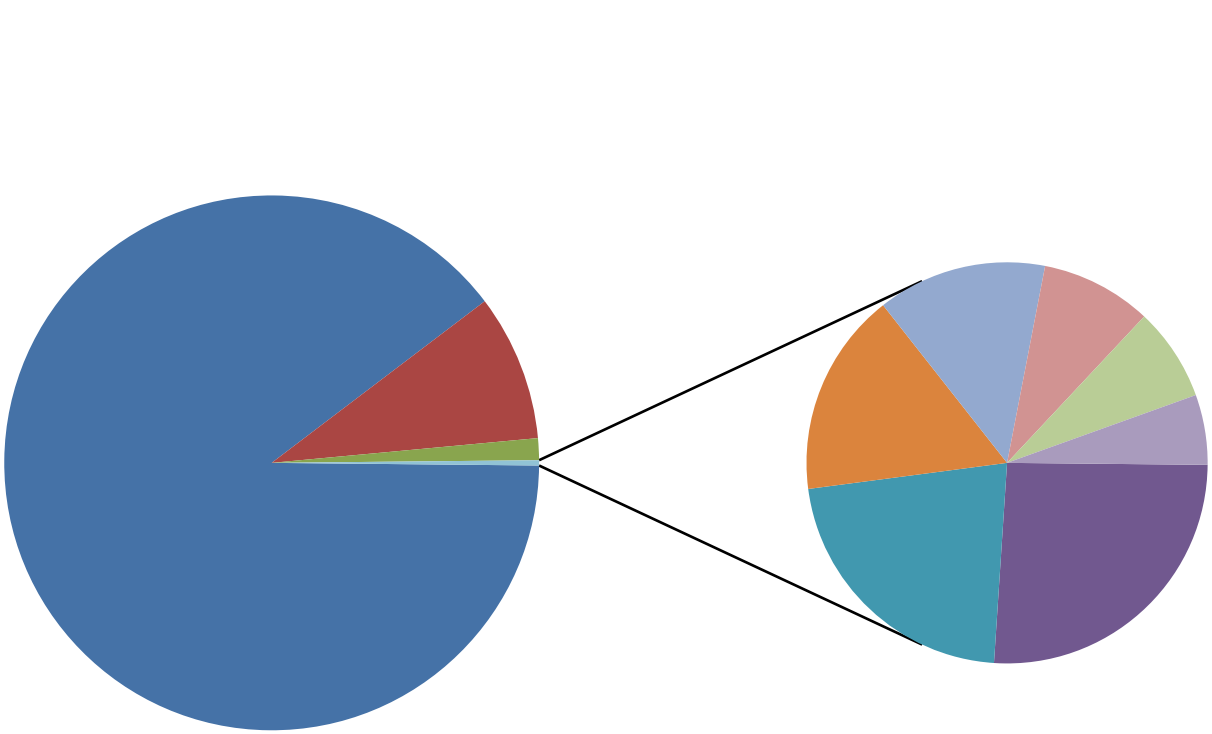
• Historical nomenclature (n=number of amino acids):
n < 10: oligopeptide
10 < n < 100: polypeptide
n > 100: protein

The 20 amino acids occuring in nature



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)

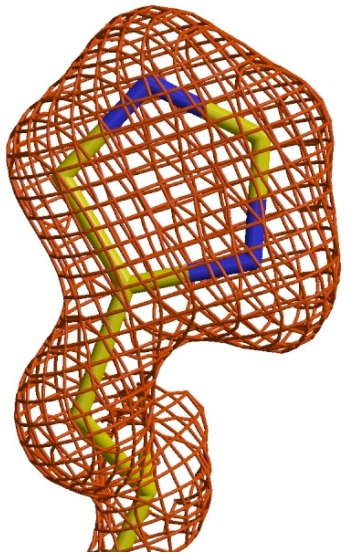
```
ATOM     25  N   ASP A 928      19.062   9.157  35.067  1.00  4.73           N
ATOM     26  CA  ASP A 928      19.770  10.123  34.232  1.00  4.58           C
ATOM     27  C   ASP A 928      19.075   9.938  32.899  1.00  4.56           C
ATOM     28  O   ASP A 928      19.074   8.824  32.351  1.00  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

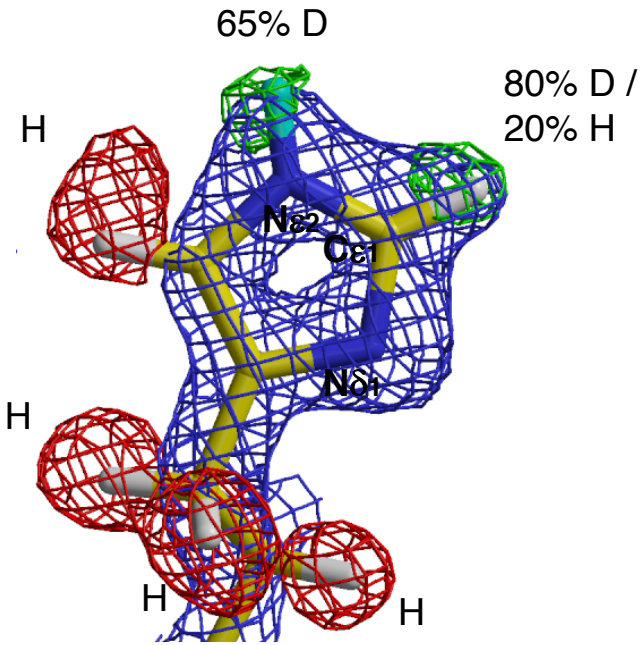
Protonation states of amino acids:

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

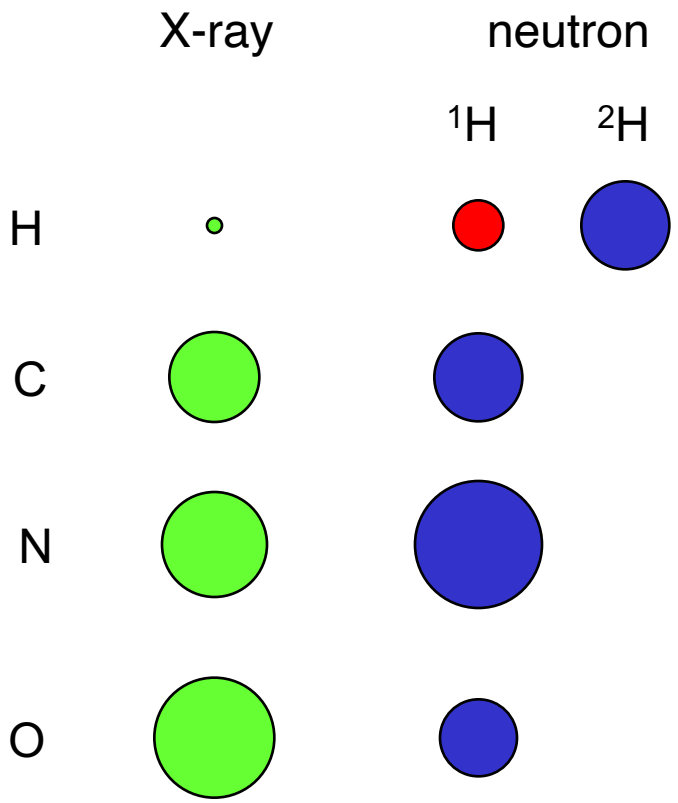
Niimura N, Chatake T, Ostermann A, Kurihara K, Tanaka T. (2003) Z. Kristallogr. 218:96

Advantages of structure determination with neutrons:

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

X-rays scatter from electrons,
neutrons from nuclei.

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

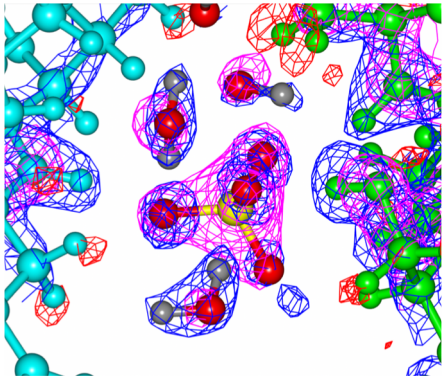


diameters correspond to: form factor / scattering length (scaled for C-atom)

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



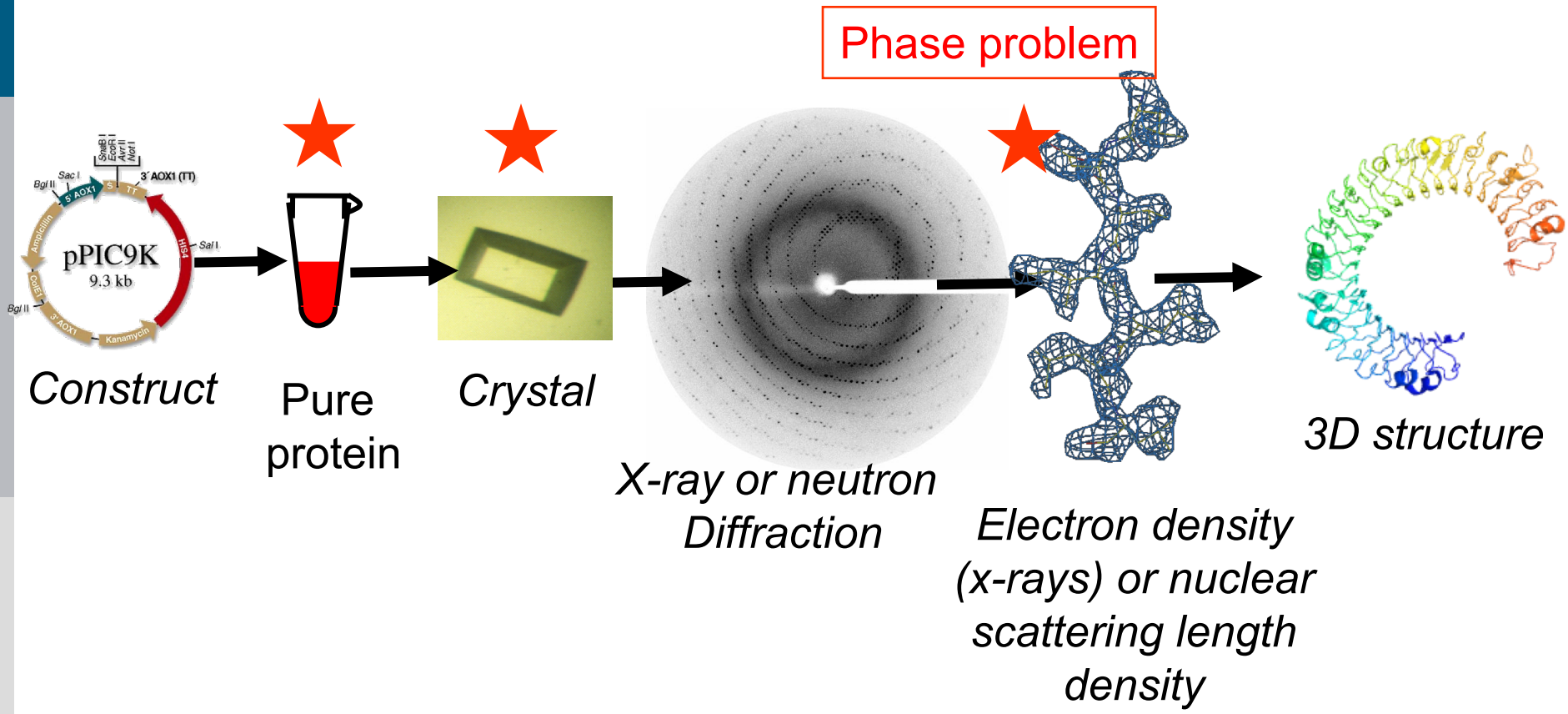
Water network in the contact region between two myoglobin molecules in the crystal.

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

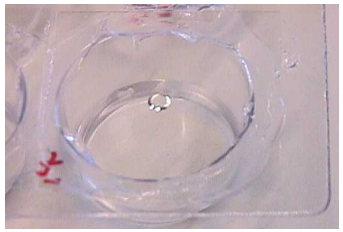
Protein crystallography in general, valid for both x-rays and neutrons as probes

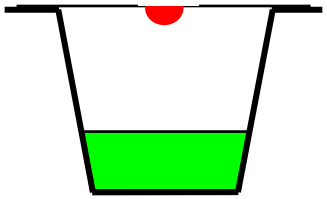
Crystallography: Overiew over the process

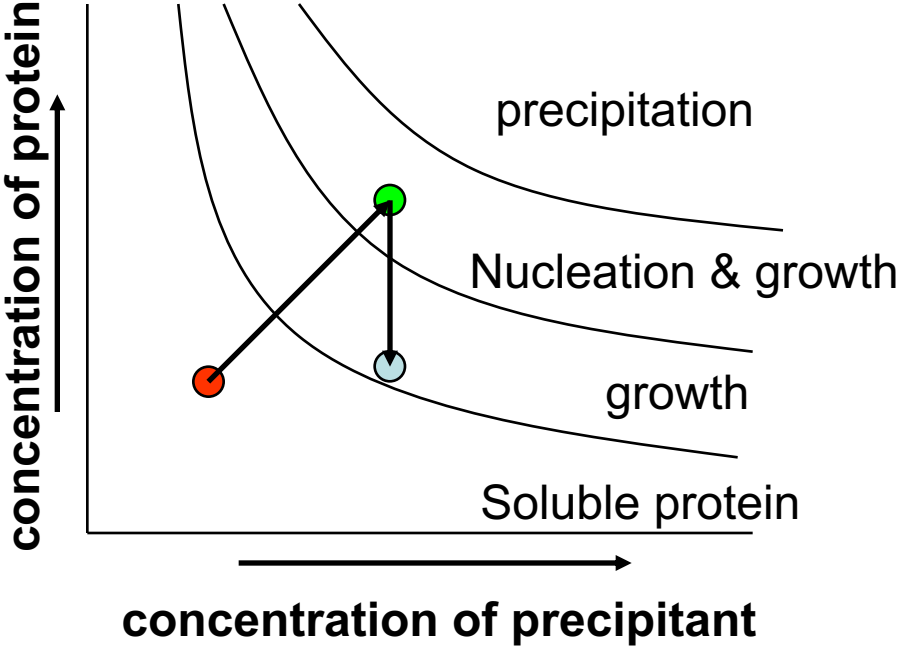


Harma Brondijk, Crystal and Structural chemistry, Utrecht University

Growing crystals



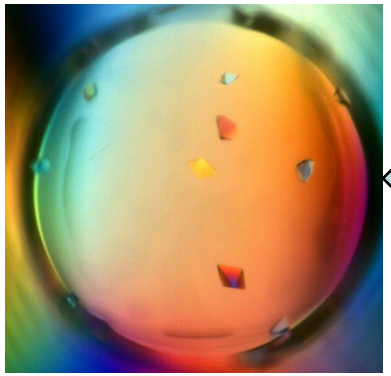


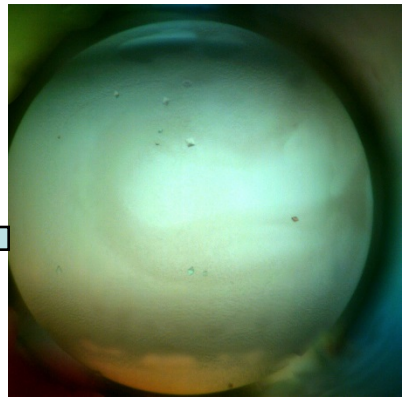


Hanging drop: 1 µl protein solution+ 1 µl reservoir solution

Reservoir: precipitant solution e.g. 1 M NaCl

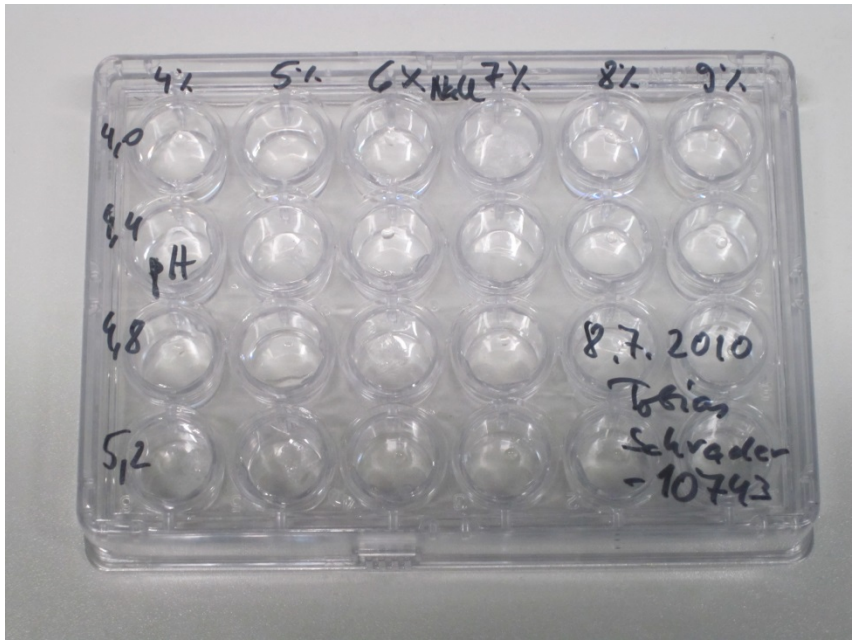
Concentrations of precipitant and of protein slowly rise as drop equilibrates with reservoir





Harma Brondijk, Crystal and Structural chemistry, Utrecht University

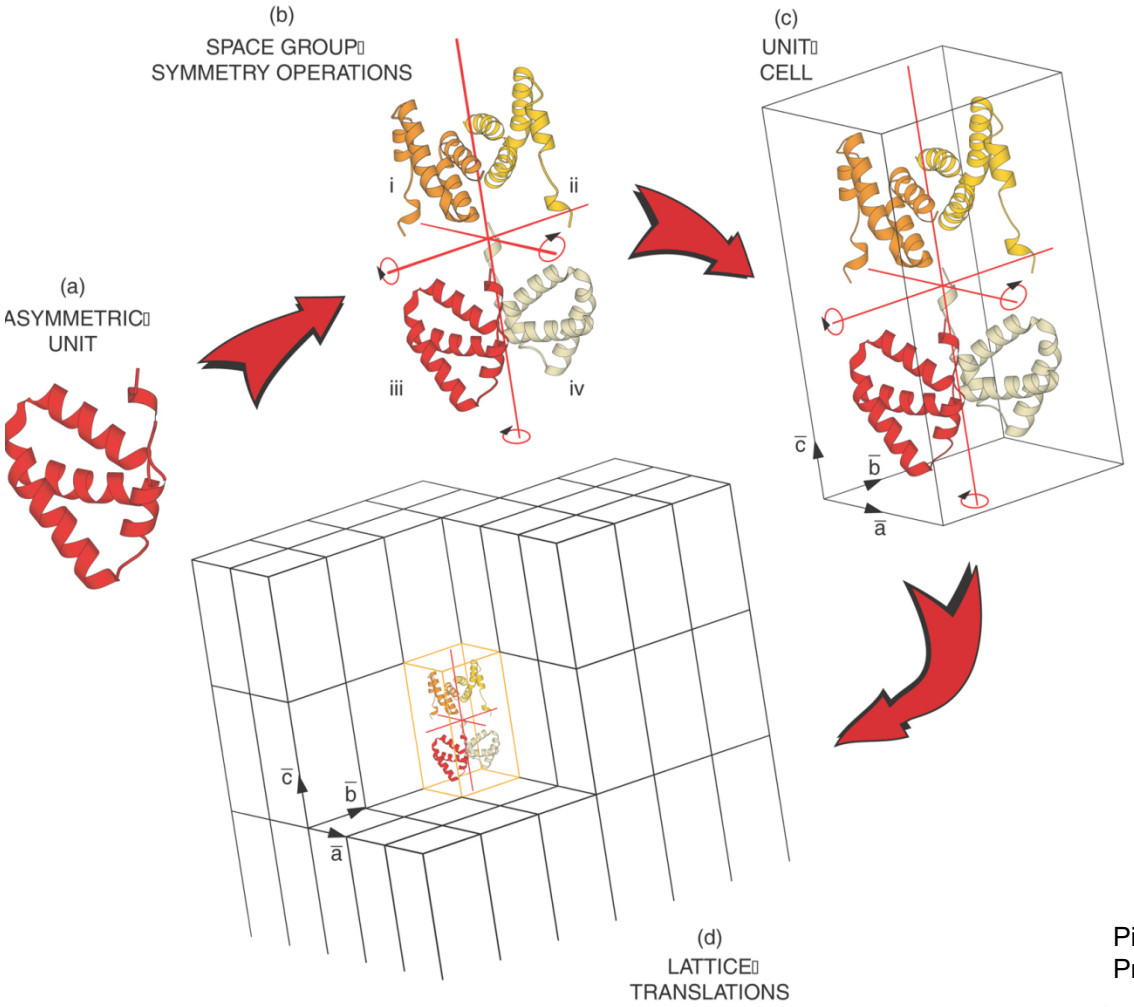
Hanging drop with variable solvent conditions (lyosozyme) and batch crystallization of myoglobin crystals



Hanging drop crystallization

Batch crystallization

How a typical protein crystal looks like…



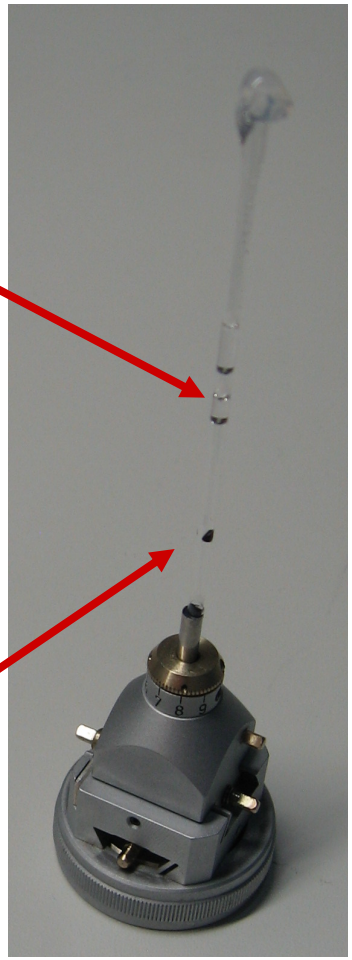
Picture taken from Lecture of Prof. Locher at ETH Zürich

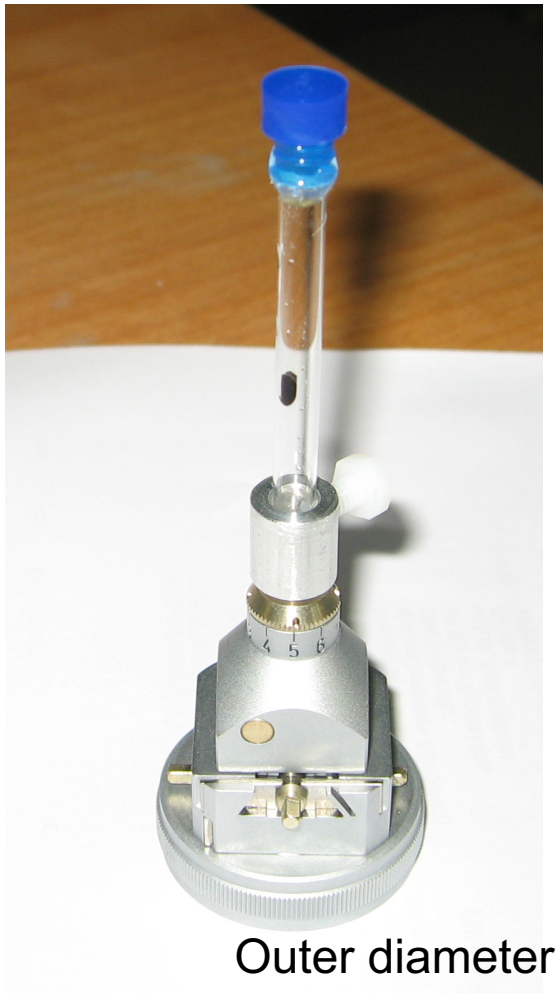
fig 2.2

Room temperature packaging of crystals

deuterated mother liquor

sample crystal



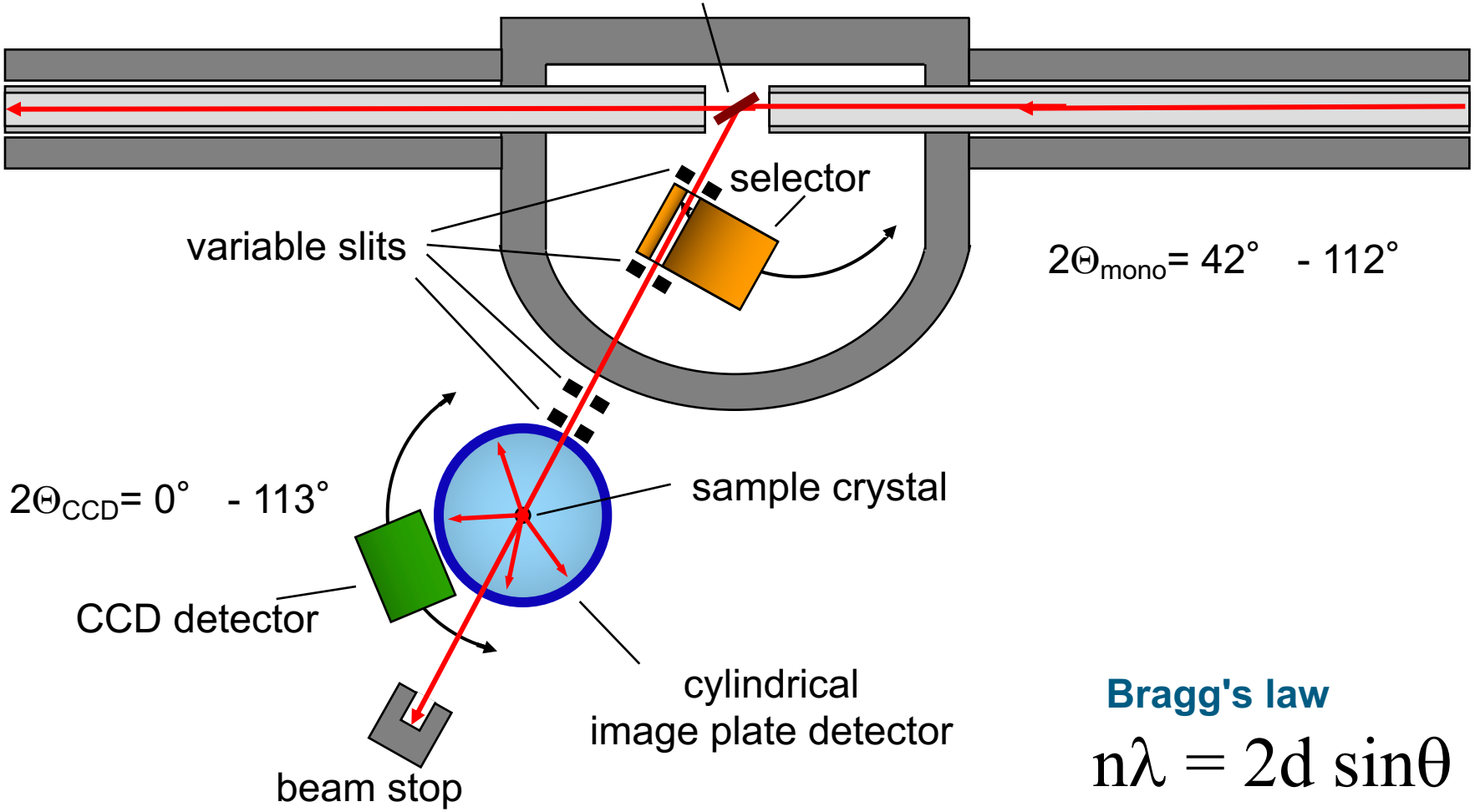


- Avoid boron glas, since boron absorbs neutrons, use quartz glas instead
- Leave as little mother liquor around the crystal as possible, put a droplet of mother liquor at one end of the capillary instead.

Outer diameter of the glas tube: 5 mm

Outer diameter of the glas tube: 2 mm

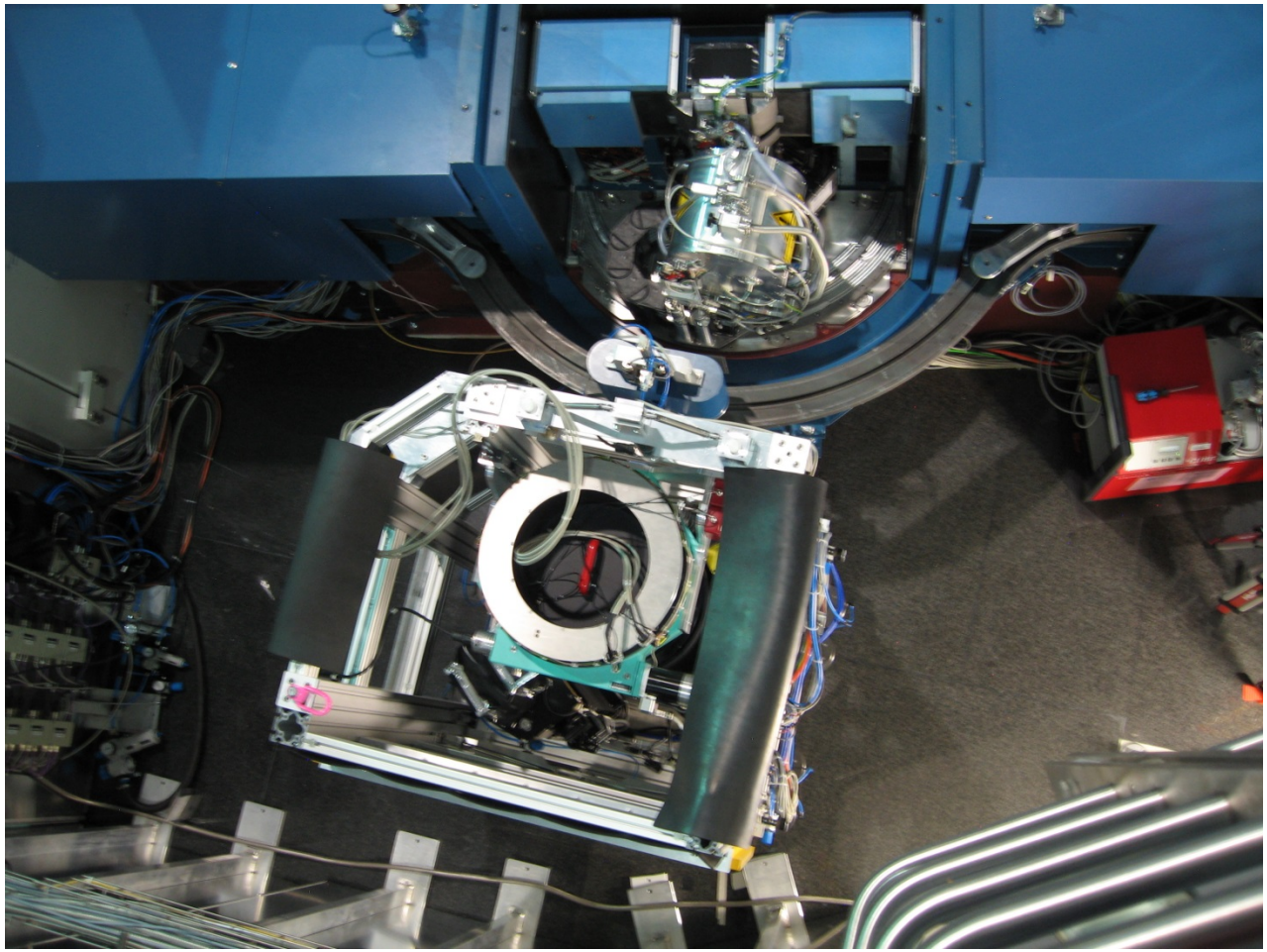
Schematic overview over BioDiff: A neutron protein diffractometer: collaboration between JCNS and FRMII



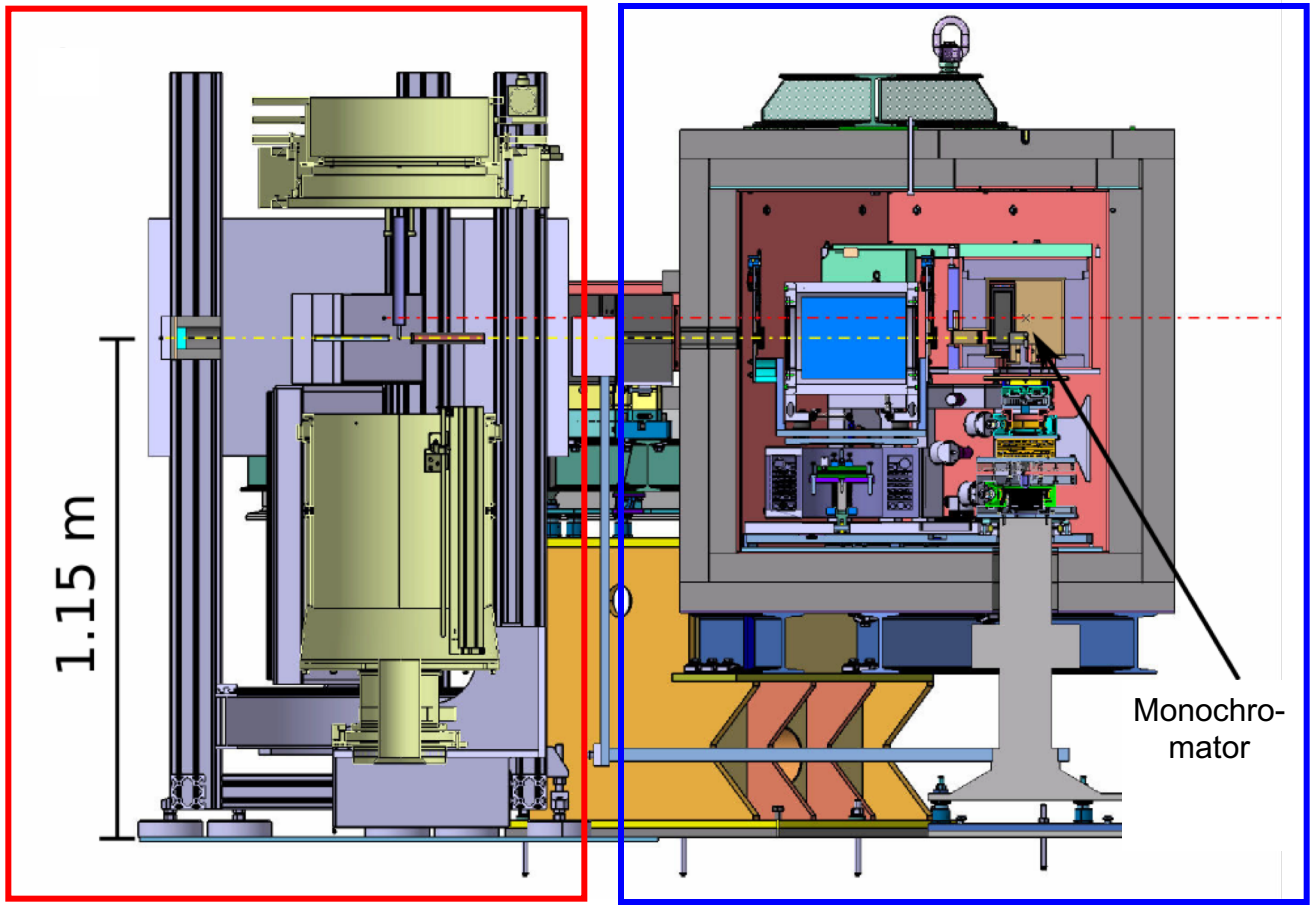
Bragg's law

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

BioDiff, the corresponding view in reality:



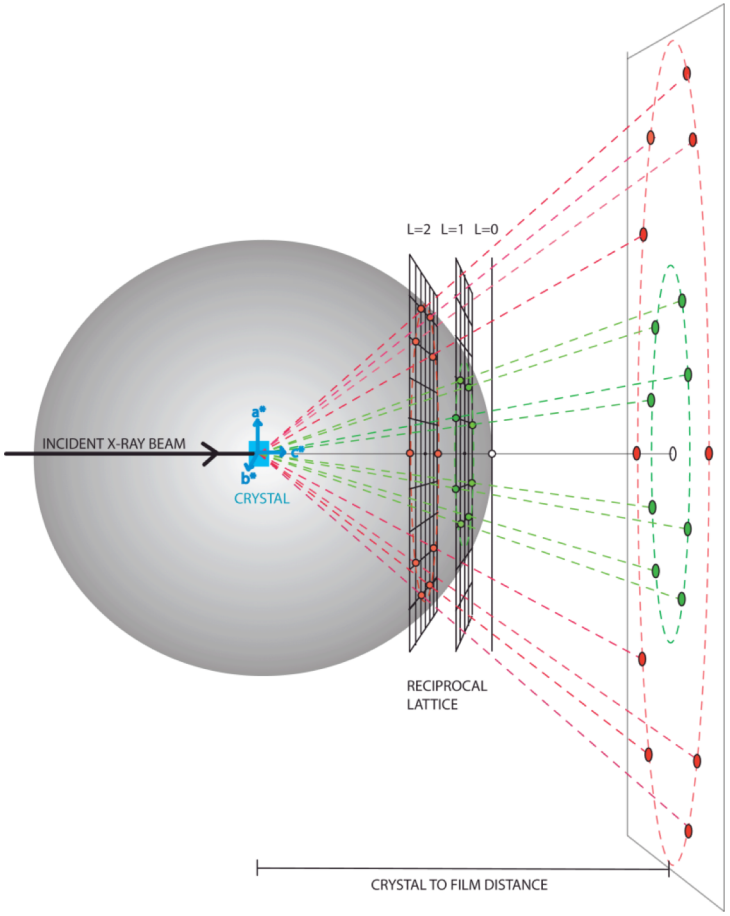
The Simultaneous Construction Phase in Garching and Jülich

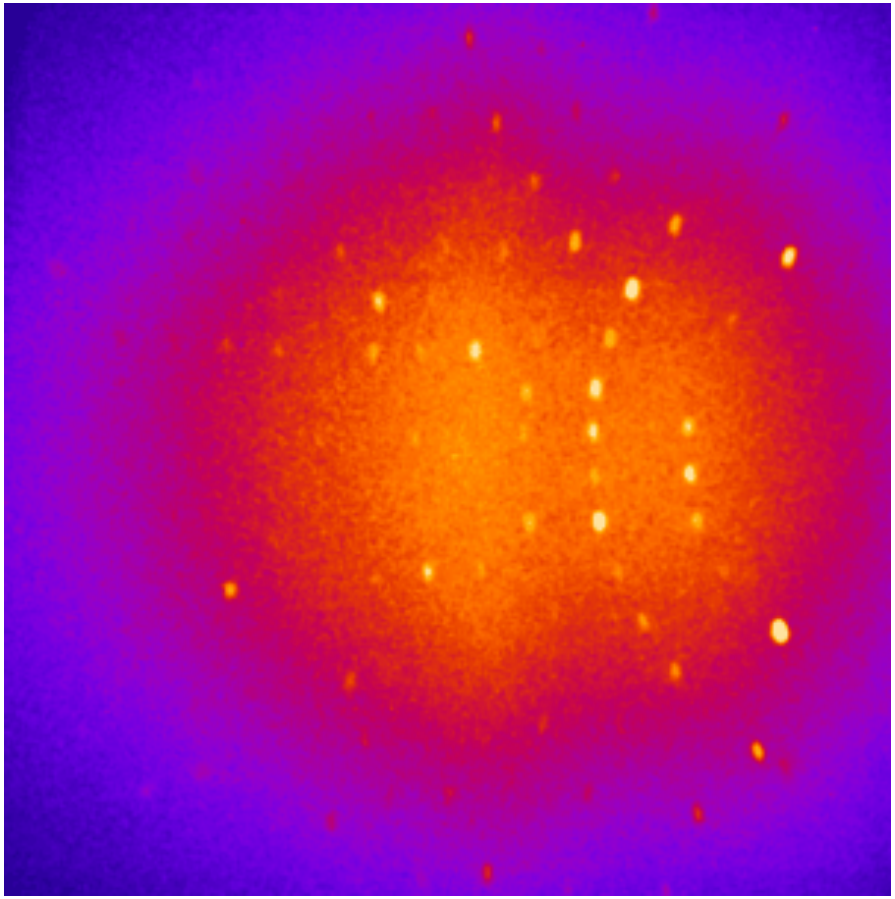


Detector unit, constructed and built in Garching (Ph. Jüttner, MLZ)

Monochromator-shielding, constructed and built in Juelich (B. Laatsch, ZEA-1 Engineering)

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

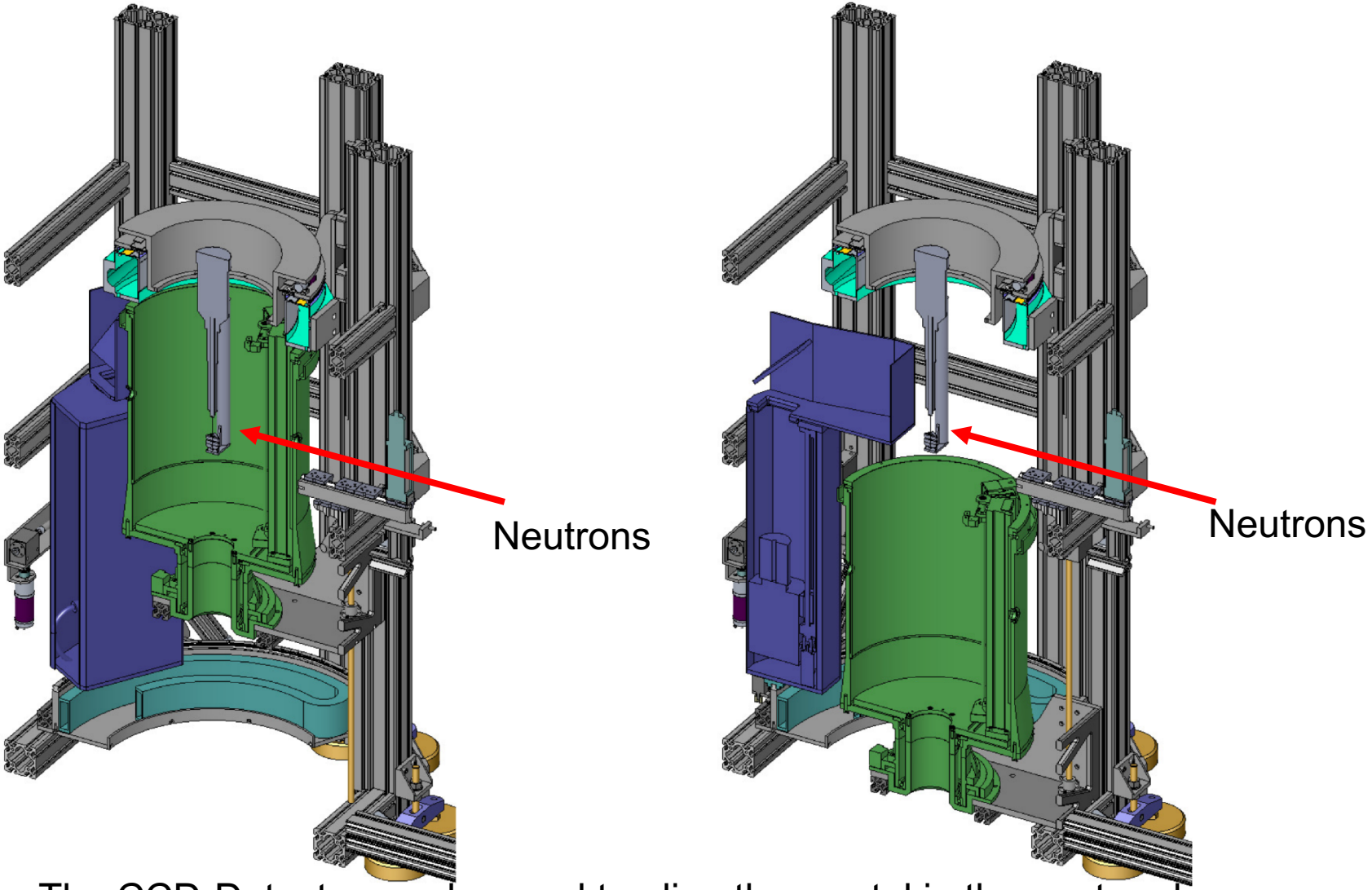




prim. beam

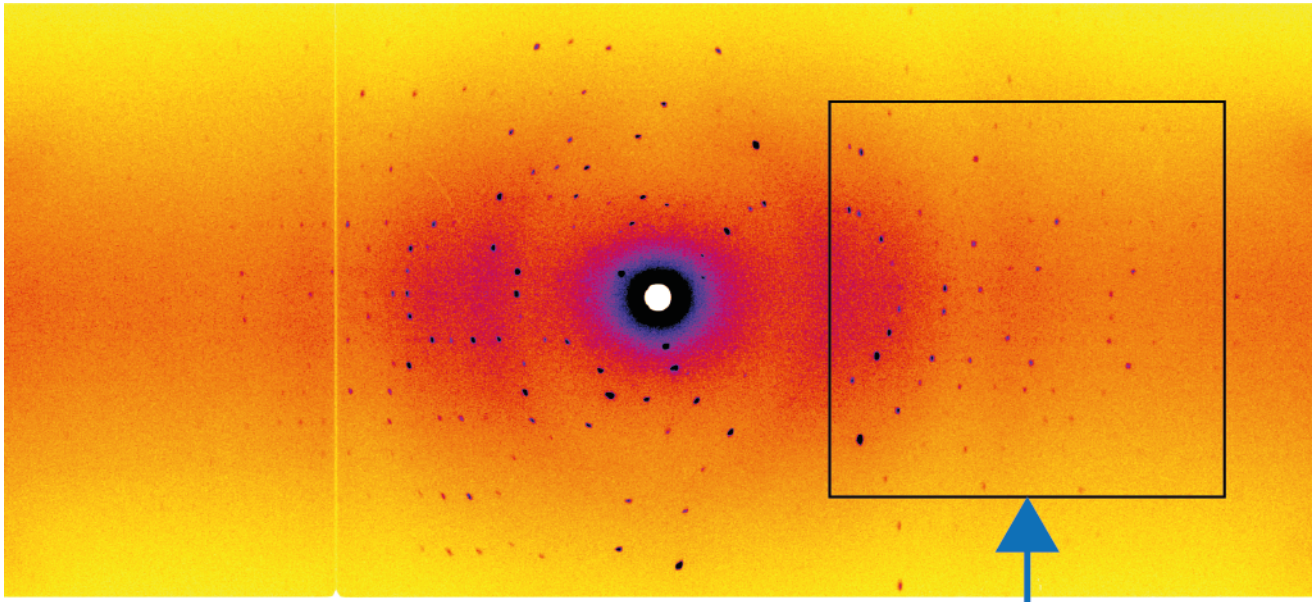
BioDiff: exposure time per frame: 20 minutes, sample: Myoglobin in deuterated mother liquor

Switching between imageplate and CCD detector



The CCD-Detector can be used to align the crystal in the neutron beam.

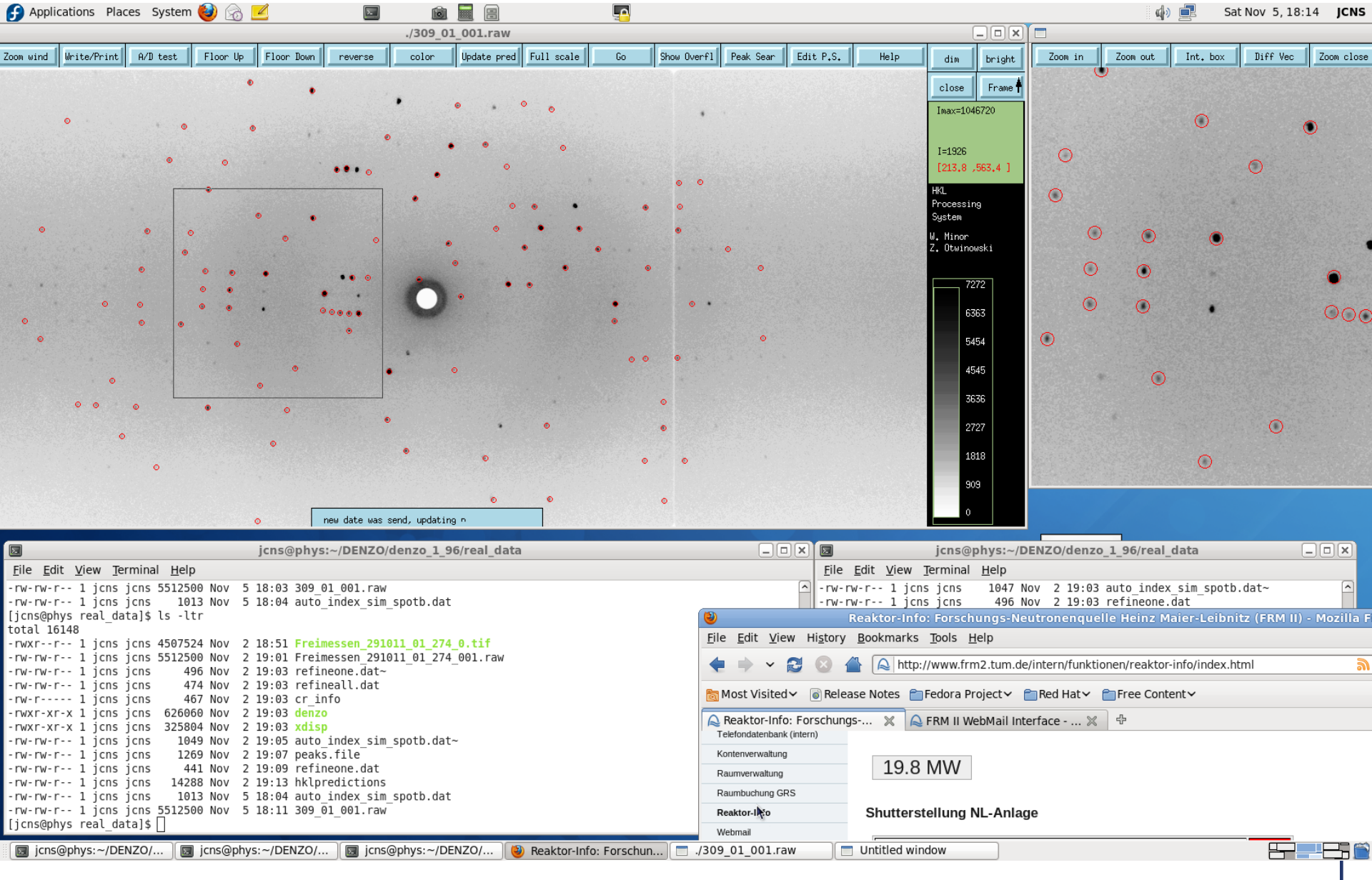
Instrument Characterization: The two detectors



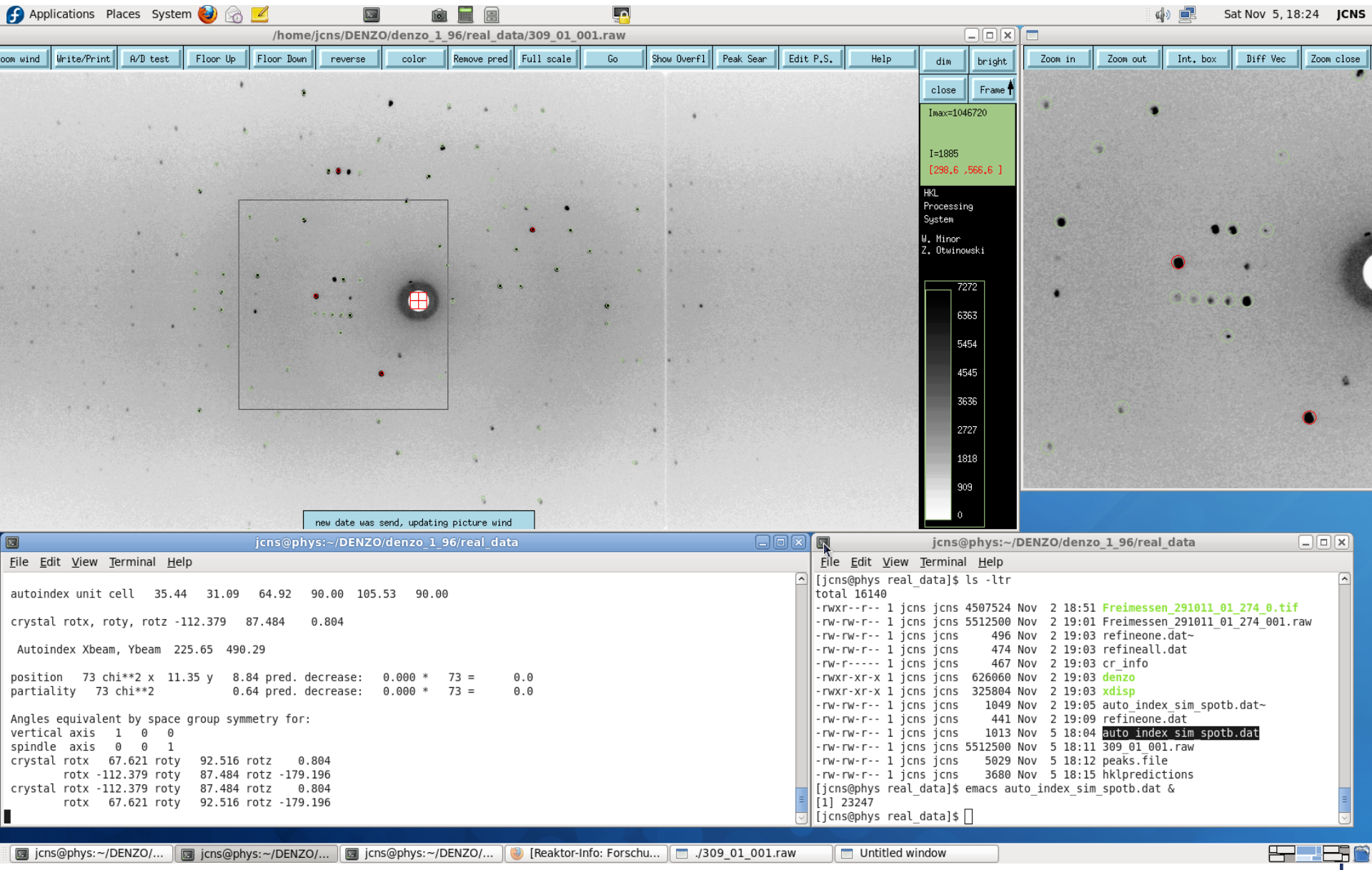
recorded with image plate detector

region of interest shown above recorded with CCD-detector

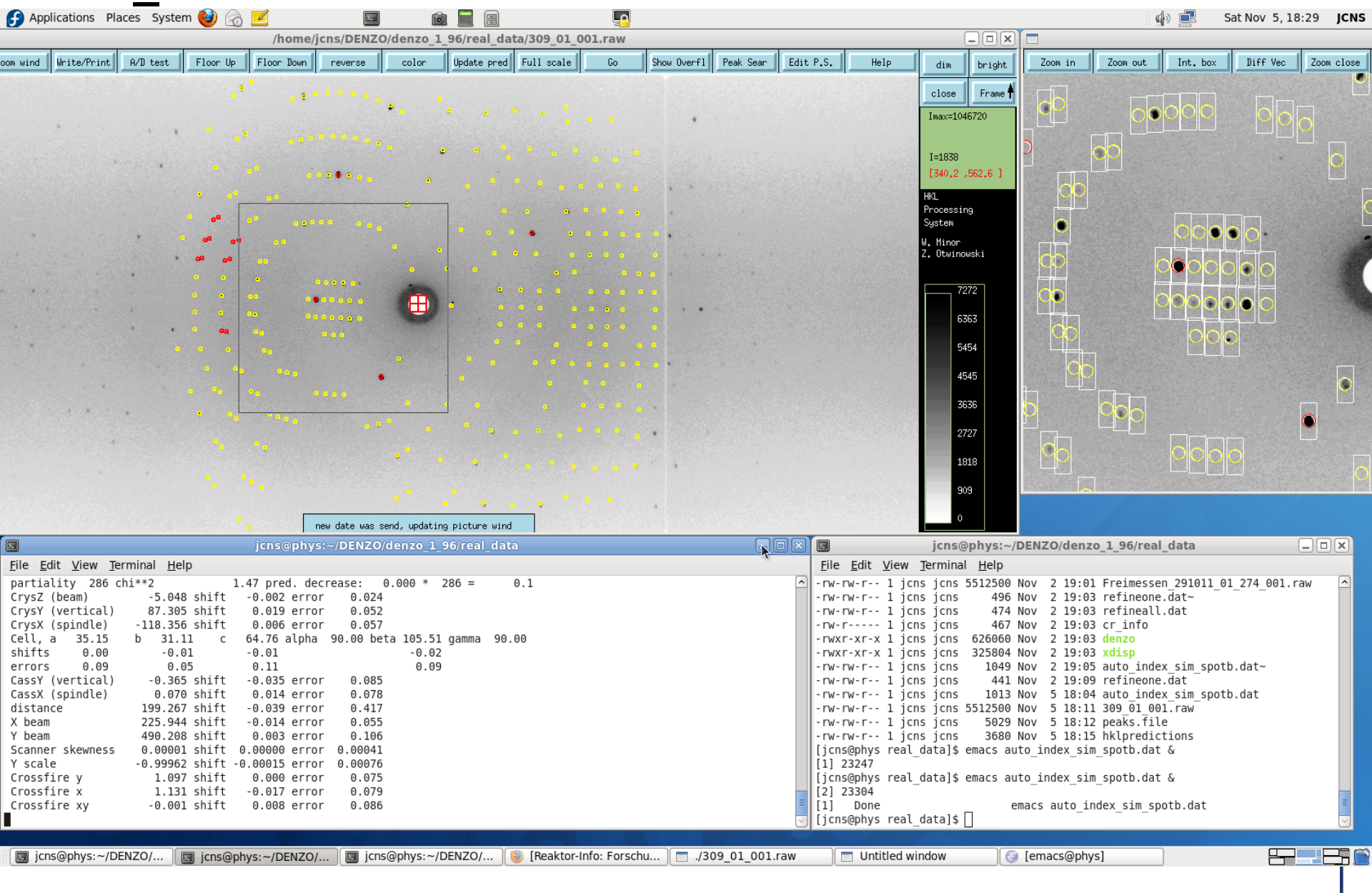
Peak search with hkl DENZO



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
Processing
System
W. Minor
Z. Otwinowski
7272
6363
5454
4545
3636
2727
1818
909
0
Zoom in
Zoom out
Int. box
Diff Vec
Zoom close
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=2.5 Å


Applications Places System
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/home/jcns/DENZO/denzo_1_96/real_data/309_01_001.raw
Write/Print
A/D test
Floor Up
Floor Down
reverse
color
Update pred
Full scale
Go
Show Overfl
Peak Sear
Edit P.S.
Help
dim
bright
close
Frame
Imax=1046720
I=1838
[340.2 ,562.6]
HKL
Processing
System
W. Minor
Z. Otwinowski
7272
6363
5454
4545
3636
2727
1818
909
0
Zoom in
Zoom out
Int. box
Diff Vec
Zoom close
new date was send, updating picture wind
jcns@phys:~/DENZO/denzo_1_96/real_data
File Edit View Terminal Help
partiality 286 chi**2 1.47 pred. decrease: 0.000 * 286 = 0.1
CrysZ (beam) -5.048 shift -0.002 error 0.024
CrysY (vertical) 87.305 shift 0.019 error 0.052
CrysX (spindle) -118.356 shift 0.006 error 0.057
Cell, a 35.15 b 31.11 c 64.76 alpha 90.00 beta 105.51 gamma 90.00
shifts 0.00 -0.01 -0.01 -0.02
errors 0.09 0.05 0.11 0.09
CassY (vertical) -0.365 shift -0.035 error 0.085
CassX (spindle) 0.070 shift 0.014 error 0.078
distance 199.267 shift -0.039 error 0.417
X beam 225.944 shift -0.014 error 0.055
Y beam 490.208 shift 0.003 error 0.106
Scanner skewness 0.00001 shift 0.00000 error 0.00041
Y scale -0.99962 shift -0.00015 error 0.00076
Crossfire y 1.097 shift 0.000 error 0.075
Crossfire x 1.131 shift -0.017 error 0.079
Crossfire xy -0.001 shift 0.008 error 0.086
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
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[2] 23304
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jcns@phys:~/DENZO/...
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jcns@phys:~/DENZO/...
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Untitled window
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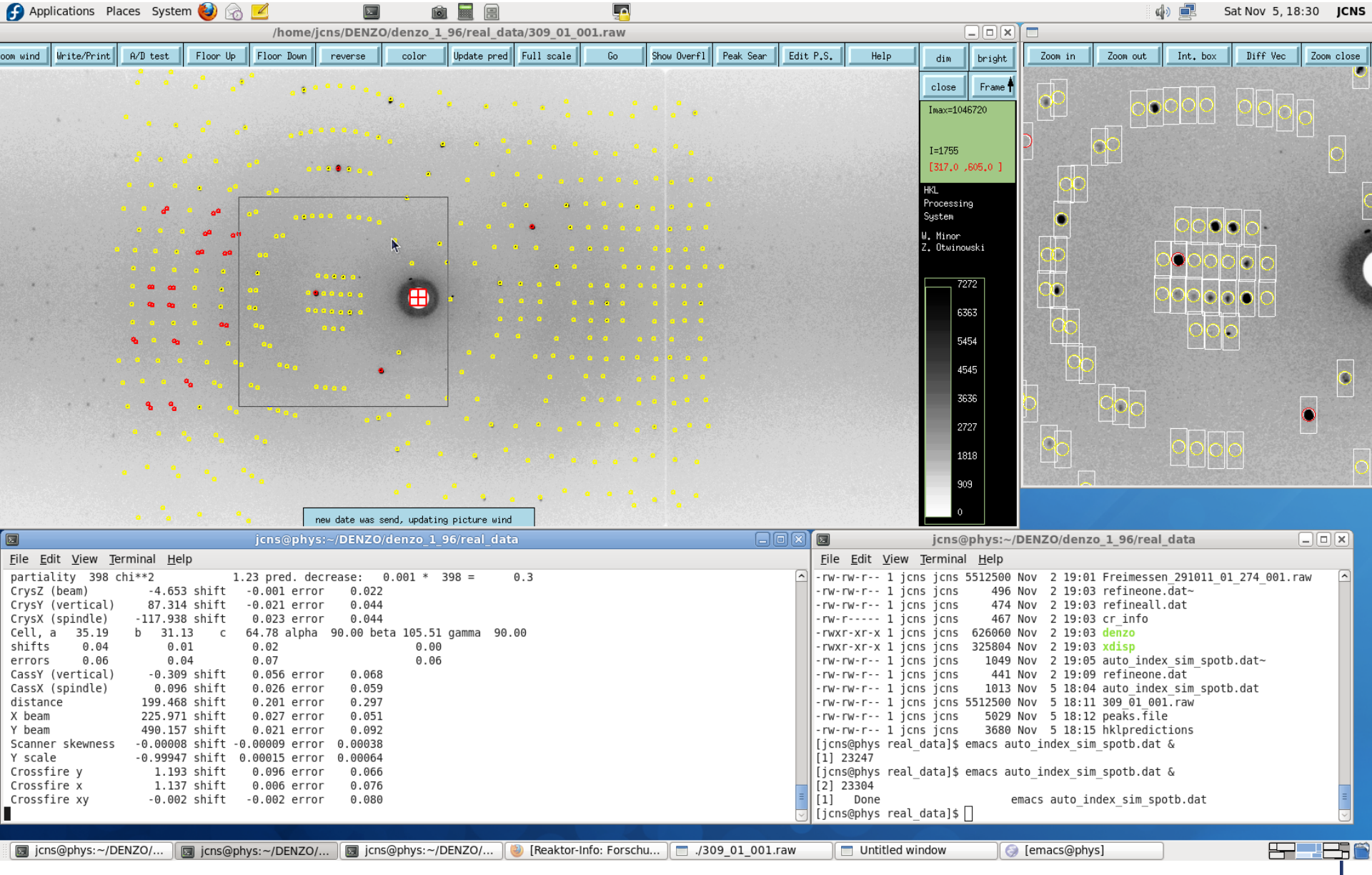
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oom wind
Write/Print
A/D test
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reverse
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Update pred
Full scale
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Show Overfl
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Help
dim
bright
close
Frame
Imax=1046720
I=1755
[317.0 ,605.0]
HKL
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System
W. Minor
Z. Otwinowski
7272
6363
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new date was send, updating picture wind
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Zoom close
jcns@phys:~/DENZO/denzo_1_96/real_data
File Edit View Terminal Help
partiality 398 chi**2 1.23 pred. decrease: 0.001 * 398 = 0.3
CrysZ (beam) -4.653 shift -0.001 error 0.022
CrysY (vertical) 87.314 shift -0.021 error 0.044
CrysX (spindle) -117.938 shift 0.023 error 0.044
Cell, a 35.19 b 31.13 c 64.78 alpha 90.00 beta 105.51 gamma 90.00
shifts 0.04 0.01 0.02 0.00
errors 0.06 0.04 0.07 0.06
CassY (vertical) -0.309 shift 0.056 error 0.068
CassX (spindle) 0.096 shift 0.026 error 0.059
distance 199.468 shift 0.201 error 0.297
X beam 225.971 shift 0.027 error 0.051
Y beam 490.157 shift 0.021 error 0.092
Scanner skewness -0.00008 shift -0.00009 error 0.00038
Y scale -0.99947 shift 0.00015 error 0.00064
Crossfire y 1.193 shift 0.096 error 0.066
Crossfire x 1.137 shift 0.006 error 0.076
Crossfire xy -0.002 shift -0.002 error 0.080
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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
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-rw-rw-r-- 1 jcns jcns 1013 Nov 5 18:04 auto_index_sim_spotb.dat
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-rw-rw-r-- 1 jcns jcns 3680 Nov 5 18:15 hklpredictions
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[2] 23304
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jcns@phys:~/DENZO/...
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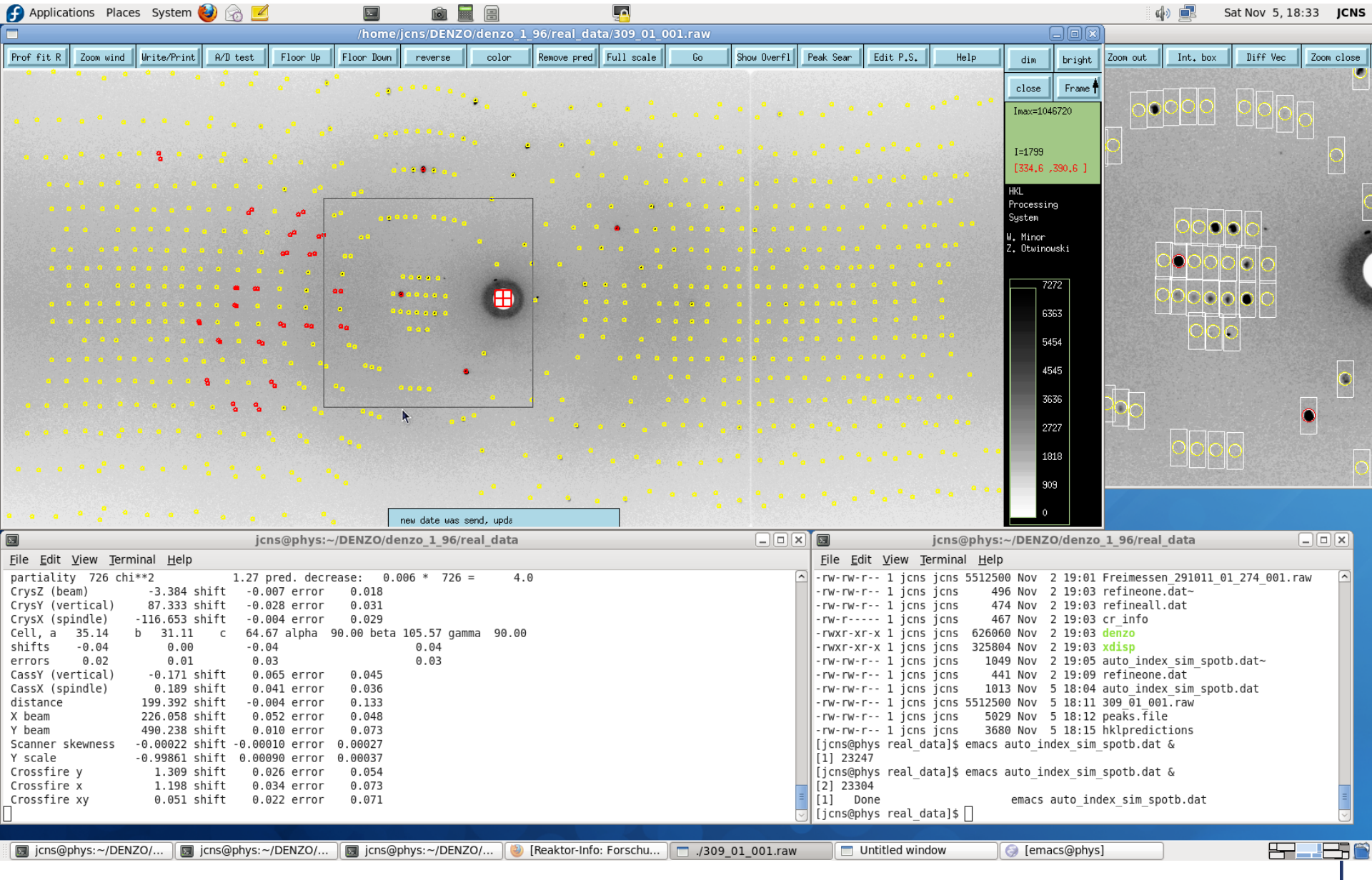
[emacs@phys]

d_min=1.5 Å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Zoom wind
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A/D test
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Floor Down
reverse
color
Remove pred
Full scale
Go
Show Overfl
Peak Sear
Edit P.S.
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
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Zoom close
new date was send, upds
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
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File Edit View Terminal Help
-rw-rw-r-- 1 jcns jcns 5512500 Nov 2 19:01 Freimessen_291011_01_274_001.raw
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-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
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-rw-rw-r-- 1 jcns jcns 441 Nov 2 19:09 refineone.dat
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[1] Done emacs auto_index_sim_spotb.dat
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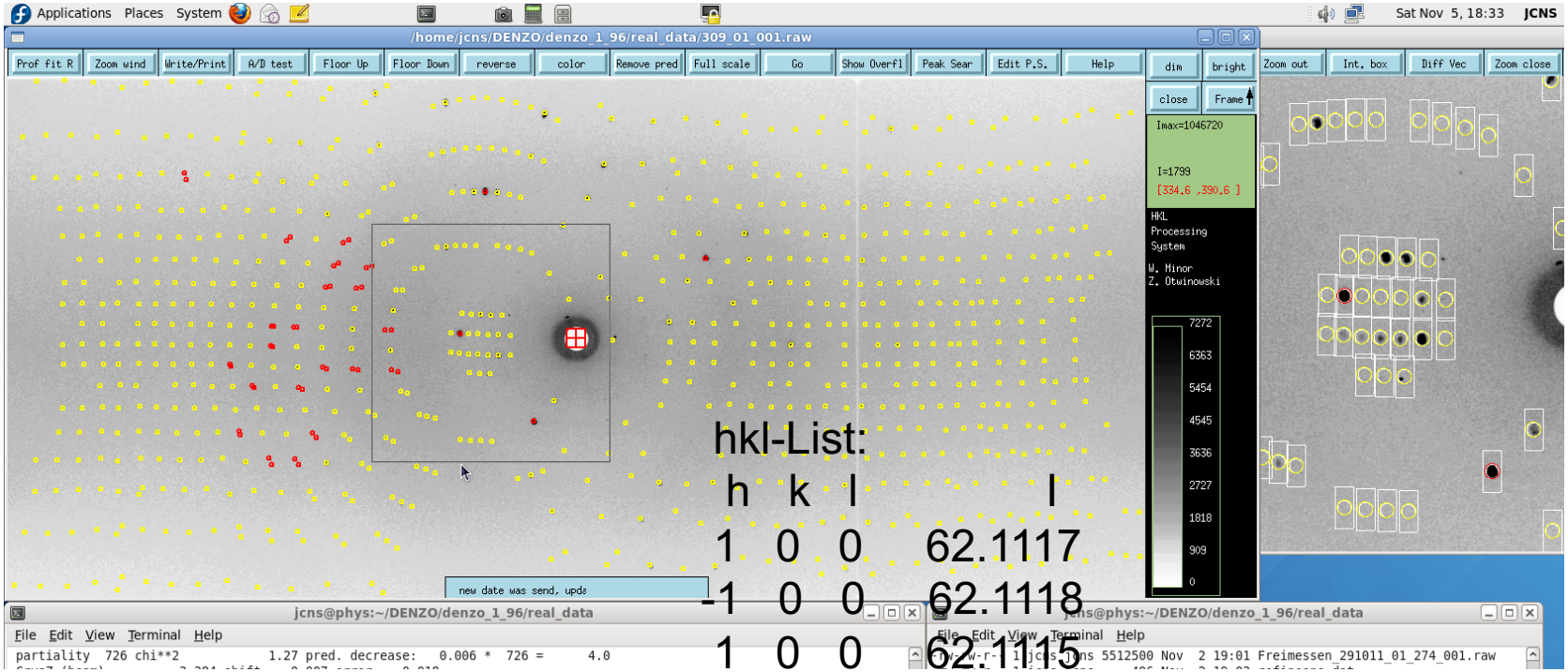
jcns@phys:~/DENZO/...
jcns@phys:~/DENZO/...
[Reaktor-Info: Forschu...
./309_01_001.raw
Untitled window
[emacs@phys]

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

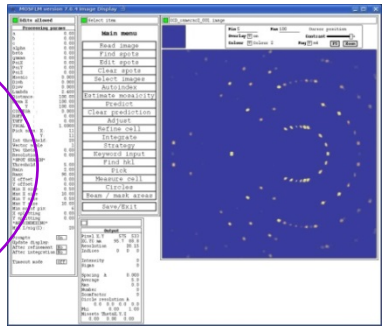


hkl-List:

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

Flow 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

Structure refinement

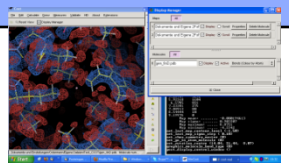
-Refinement of atom coordinates displacements
- Calculation of scattering density maps (netrons) or electron density maps (x-rays)

↔

Map-plotting

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

-nCNS
-PHENIX

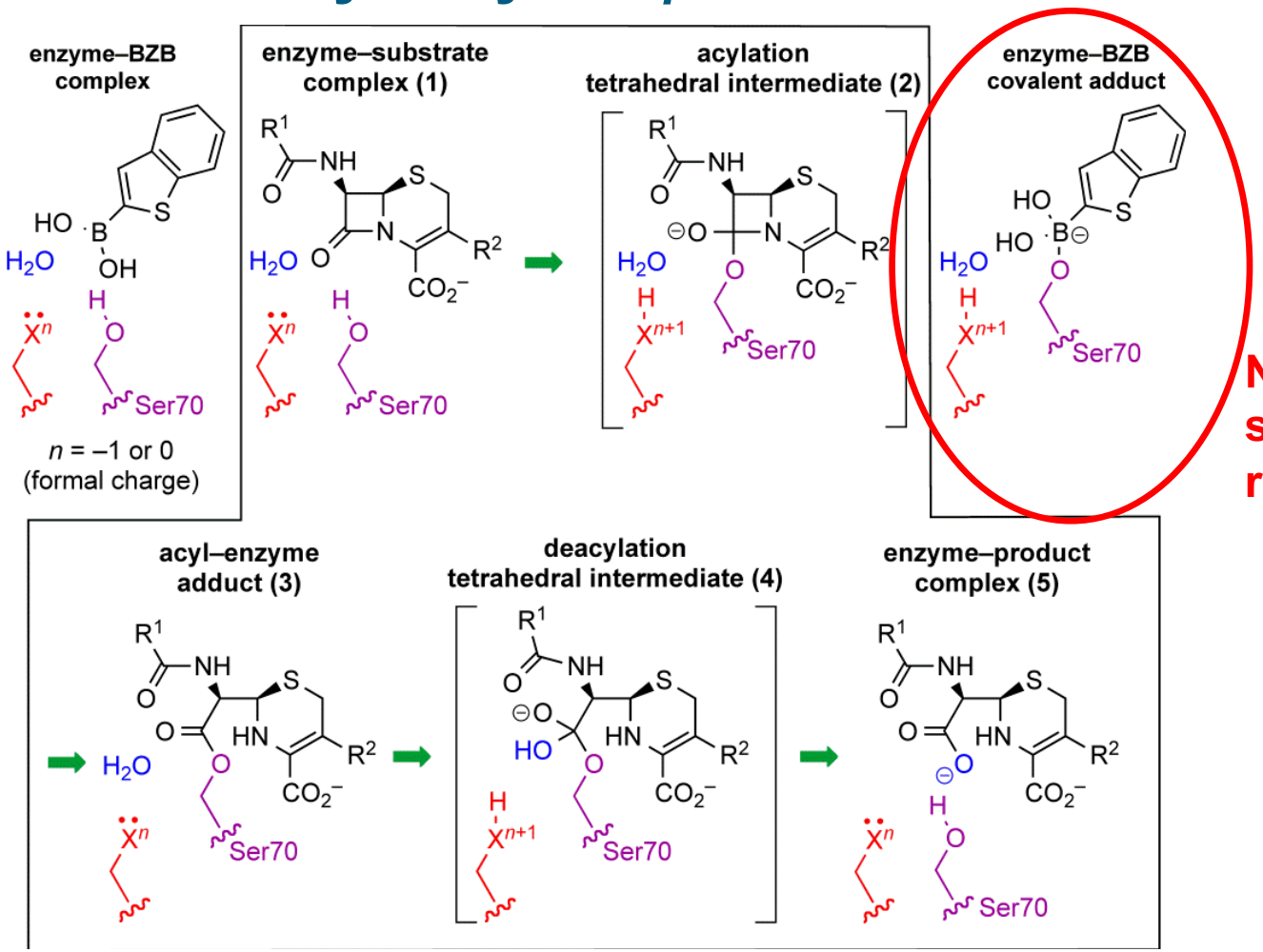


-XtalView
-Coot

Application Example: Protonation state of amino acid residues

The protein β-lactamase

β-lactamase: hydrolyses β-lactam antibiotics

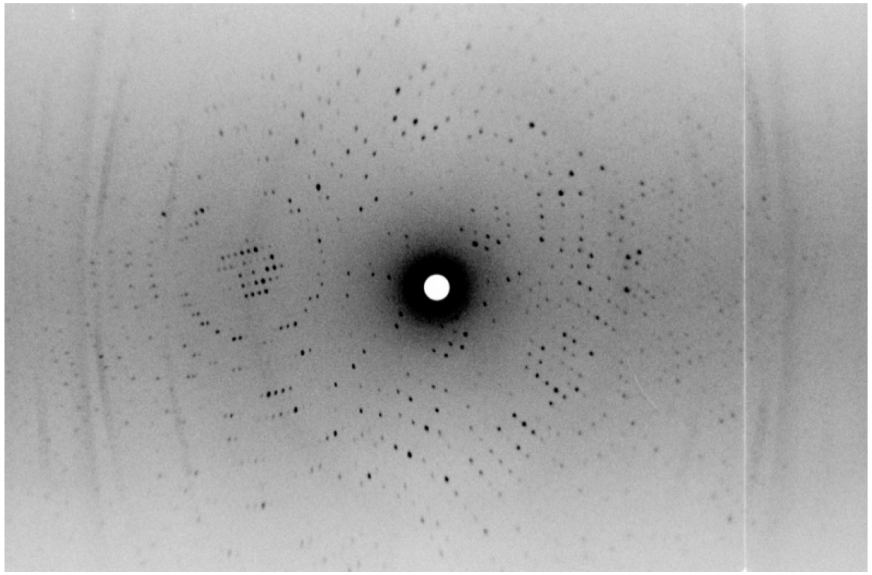


Neutron structure recorded

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

Data-set: β-lactamase with bound inhibitor



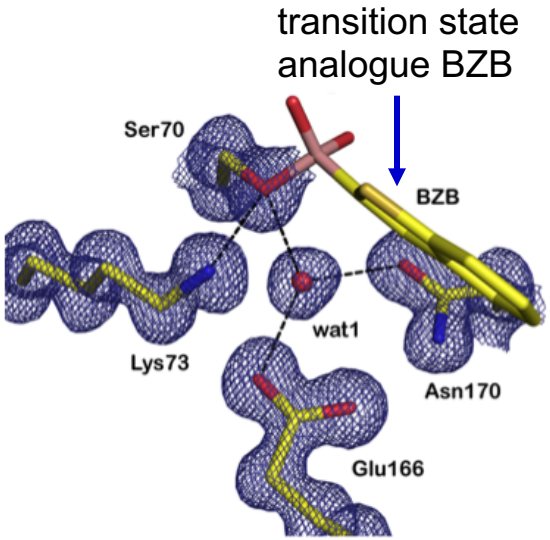
- unit cell: 73.4Å, 73.4Å, 99.1Å $P3_221$
- fully deuterated protein
- crystal size: 2.7mm^3
- Collection time: 9d

d_{min}	I/σ(I)	N_{meas}	mult.	compl. in shell %	R_{merge} %
4.31	27.8	12685	5.6	97.6	4.9
3.42	19.0	11941	5.5	98.0	8.0
2.99	10.3	10378	4.9	96.9	14.6
2.71	7.6	8757	4.3	95.5	18.7
2.52	5.9	7820	3.9	92.8	21.2
2.37	5.4	7099	3.8	89.2	21.6
2.25	5.0	6095	3.5	84.6	23.0
2.15	4.5	5906	3.4	82.9	24.7
2.07	4.1	5673	3.2	82.0	27.2
2.0	3.7	5059	2.9	81.2	27.9
overall	**7.4**	**81413**	**4.0**	**90.2**	**14.7**

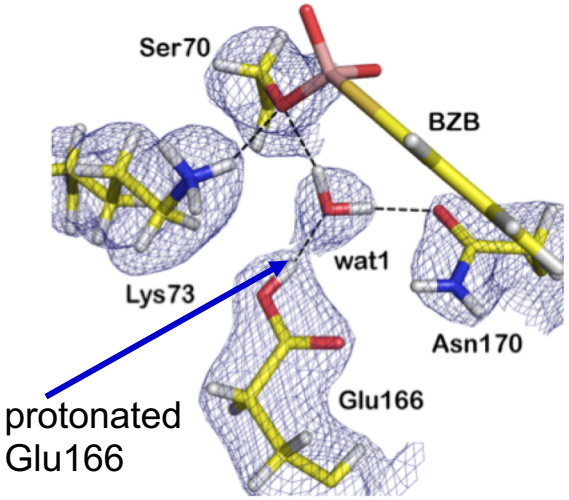
R_{pim} = 7.9% (17.9%)

Tomanicek et al., J. Biol. Chem., 288, 4715 (2013).

Catalytic Proton Network of the Toho-1 β-Lactamase



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

Thanks to our users and the BioDiff-Team:

- Philipp Jüttner
- Andreas Ostermann
- Reinhard Schätzler
- Bernhard Laatsch
- Frank Suxdorf
- Manfred Bednarek
- Matthias Drochner
- Harald Kleines
- Kevin Körrentz
- Karl-Heinz Mertens
- Michael Monkenbusch
- Michael Wagener
- Heinrich Pohl
- Vladimir Ossovyi
- Andreas Nebel
- Simon Staringer
- Winfried Petry
- Severin Denk
- Dieter Richter

Marialucia Longo
Johannes Hermann
Philipp Nowotny

…and you for your attention!

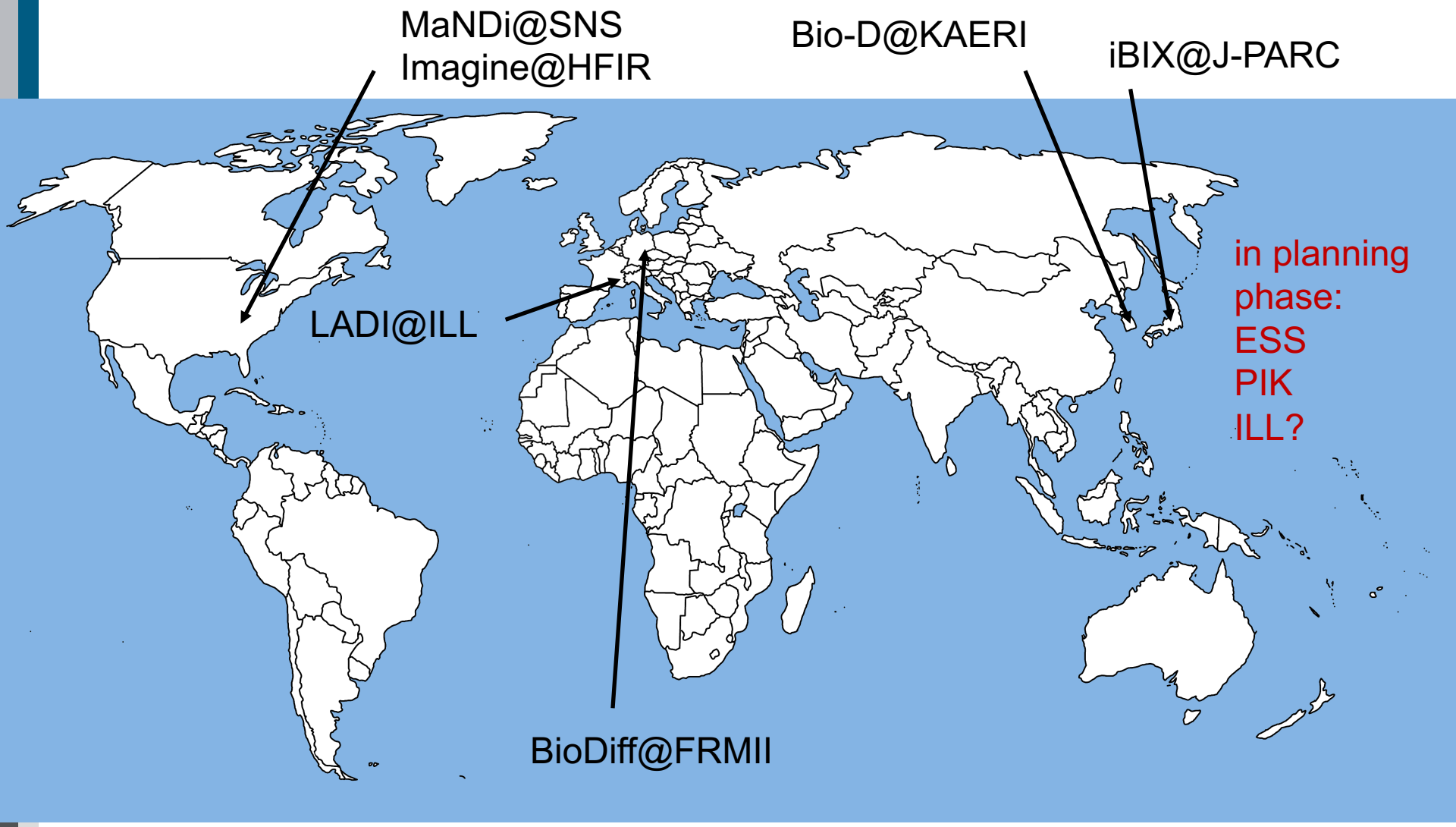
Funding by:

JÜLICH FORSCHUNGSZENTRUM

Technische Universität München

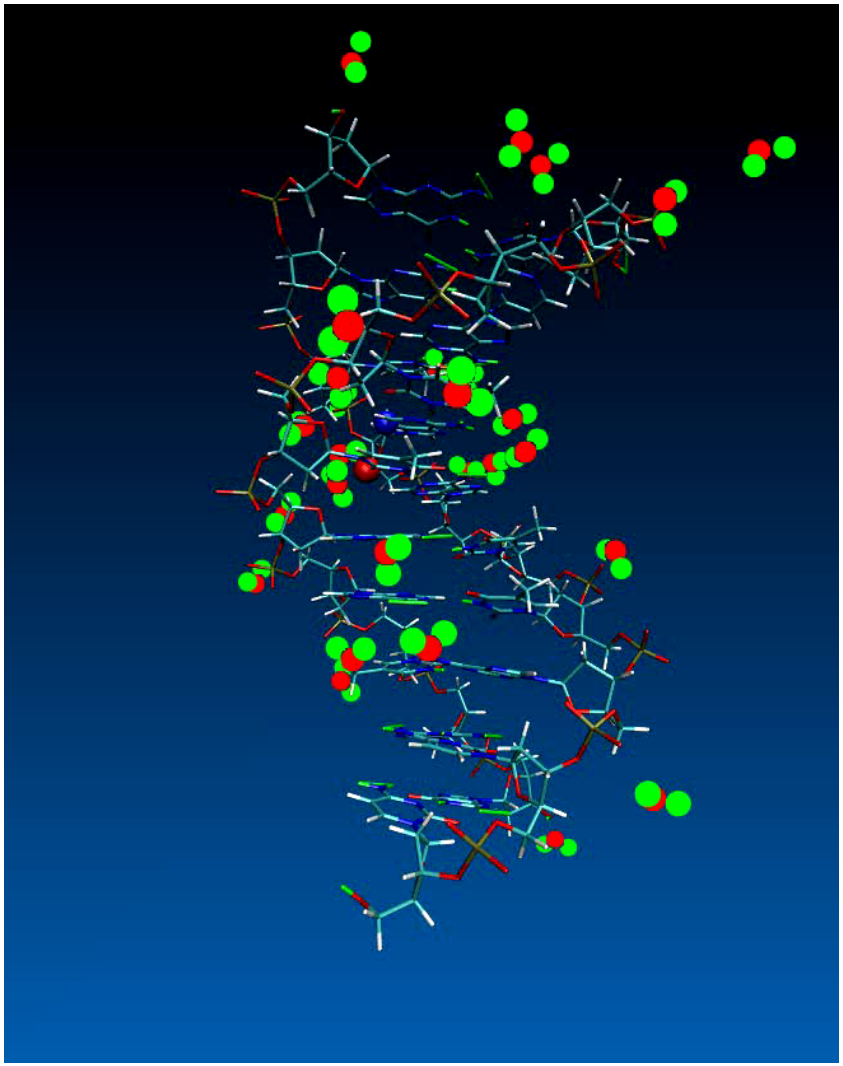
Danke für die Neutronen!

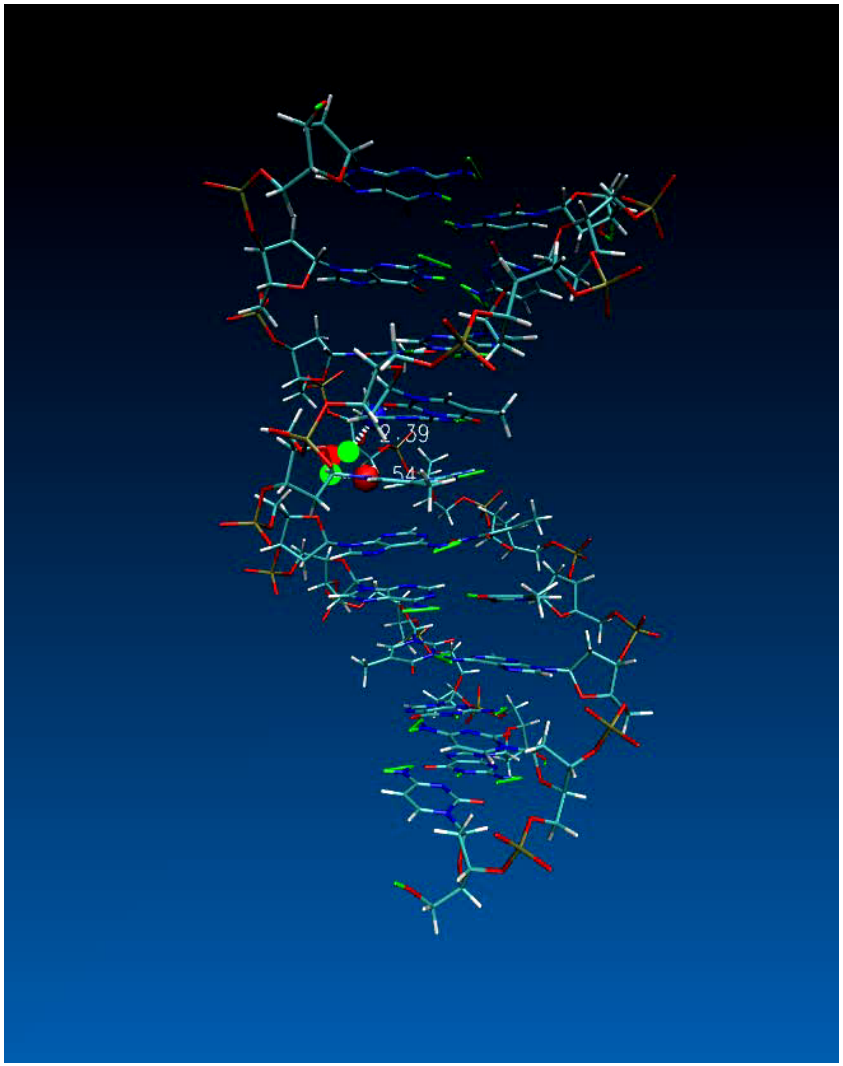
World map of neutron diffractometers optimized for protein crystals



Other structured macromolecules to be investigated by crystallography… mostly DNA

DNA stabilized by water bridge in minor groove





Summary

- Proteins show a special 3-D structure which is specific to their function
- 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. deuterium exchange as a measure of flexibility and accessibility (discrimination between **H** / **D**)
  3. solvent structure including hydrogen atoms

Thanks to...

- Andreas Ostermann
- Reiner Zorn
- Marialucia Longo
- Livia Balacescu

and you for your attention!

The hierarchy of protein architecture

- Primary structure

The sequence of amino acid residues
(N to C terminus)

- Secondary structure

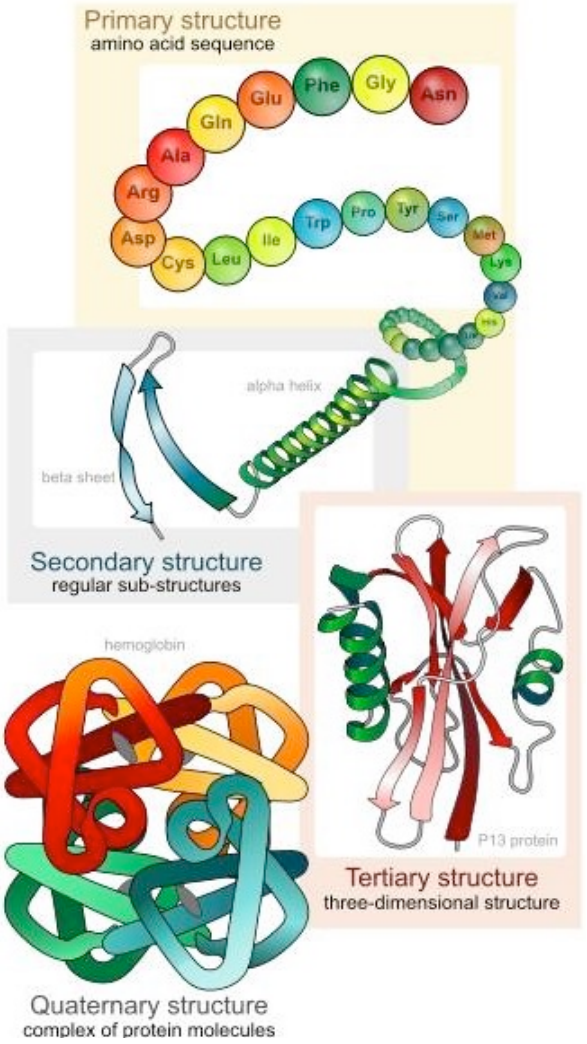
The local arrangement of main-chain atoms, often with specific hydrogen bonding pattern

- Tertiary structure

The packing of non-contiguous segments into a compact fold

- Quaternary structure

The association of individual chains into oligomeric structures



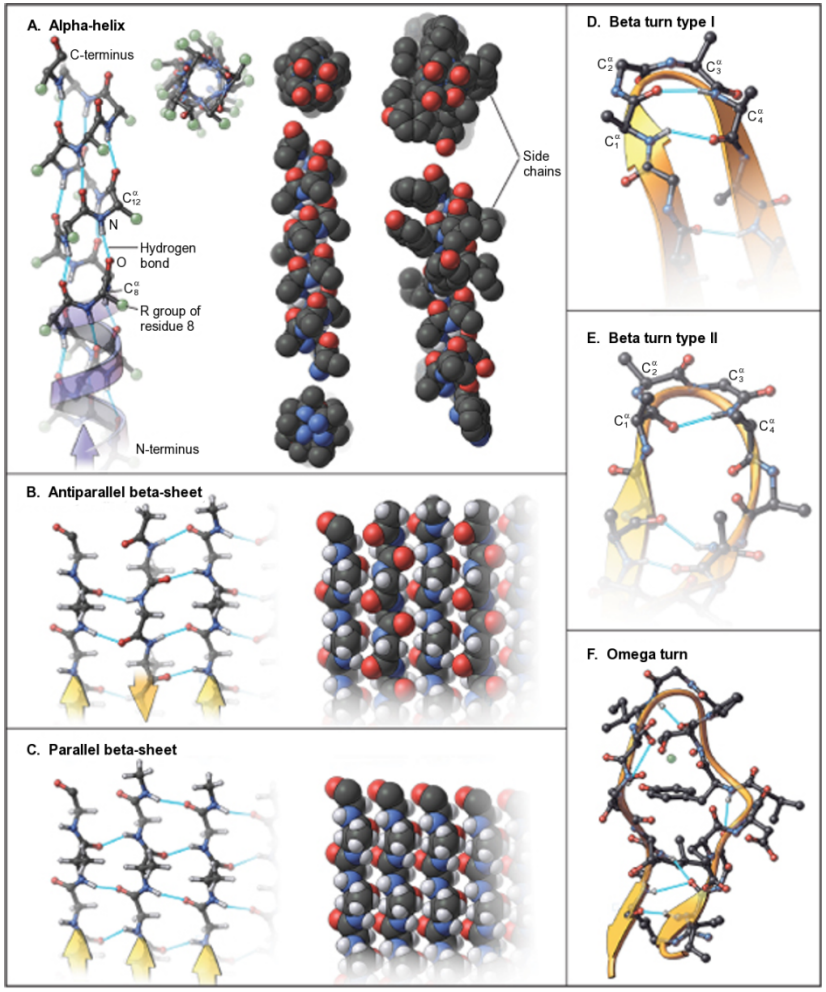
Secondary structural elements

α-helix
spiral conformation of a
continuous chain

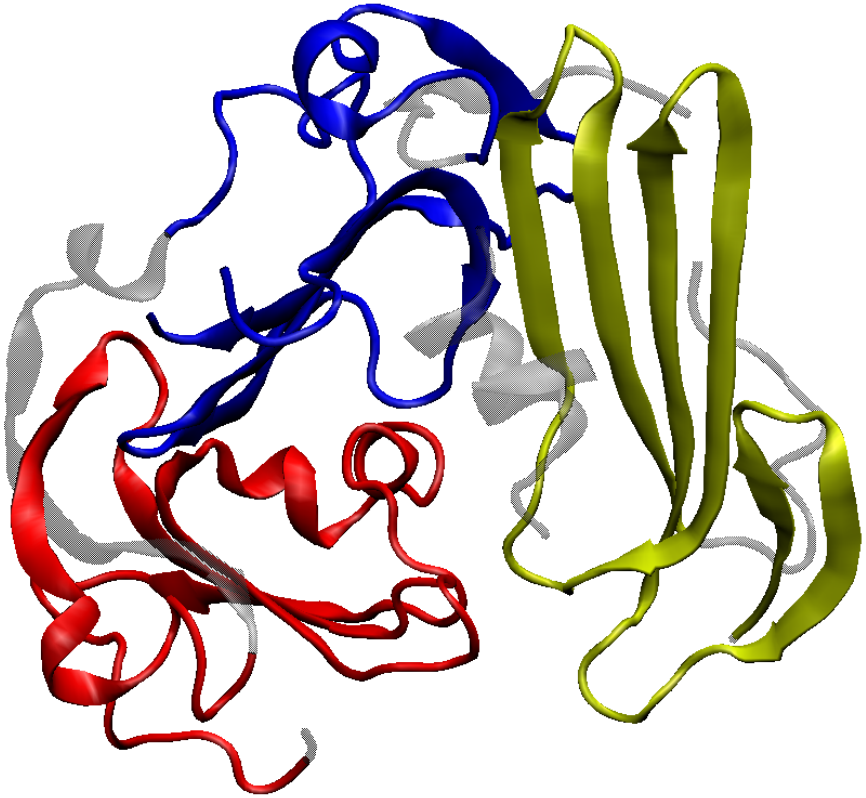
β-sheet
extended strands
aligned side-by-side

turns / loops
changing direction of
chain

Secondary structure elements are generally stabilized by hydrogen bonds between different parts of the backbone of the protein.



Tertiary structure: sub-dividing the protein into (functional) domains

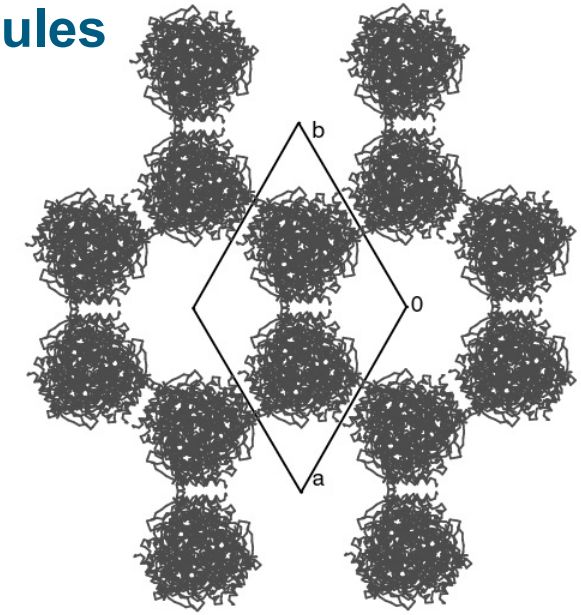


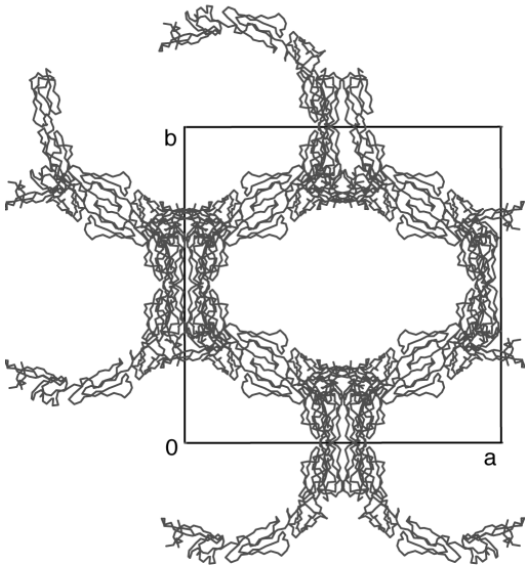
Secondary structure plot of human urokinase plasminogen activator receptor, a complex protein (pdb code 1YWH). The domains DI (yellow), DII (blue) and DIII (red) are shown. Amino acids not belonging to any domain are depicted in grey.

What stabilizes the 3-dimensional structure:

Non-covalent bonds	Covalent bonds
• Hydrogen bonds • Electrostatic forces • V. d. Waals contacts	• Disulfides • Isopeptide bonds

Protein crystals contain a lot of solvent and are held together by a limited number of weak contacts between protein molecules



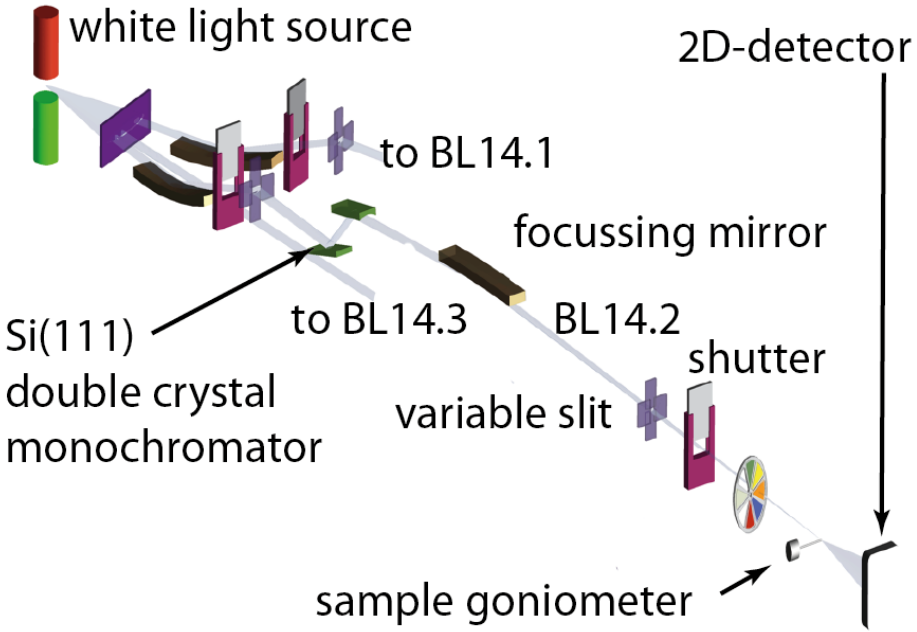


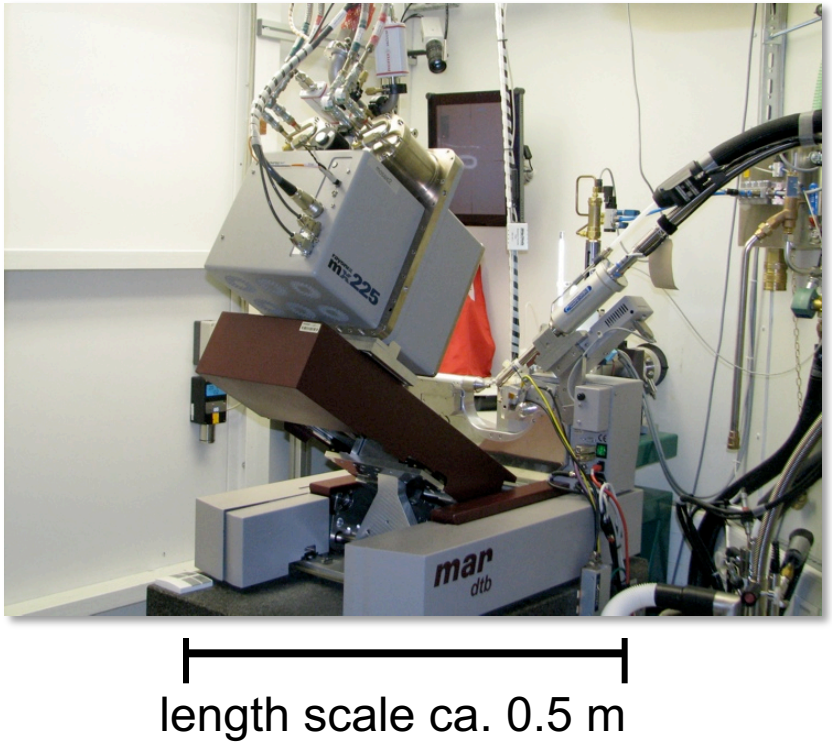
Acetylcholinesterase
~68% solvent

β2 Glycoprotein I
~90% solvent
(extremely high!)

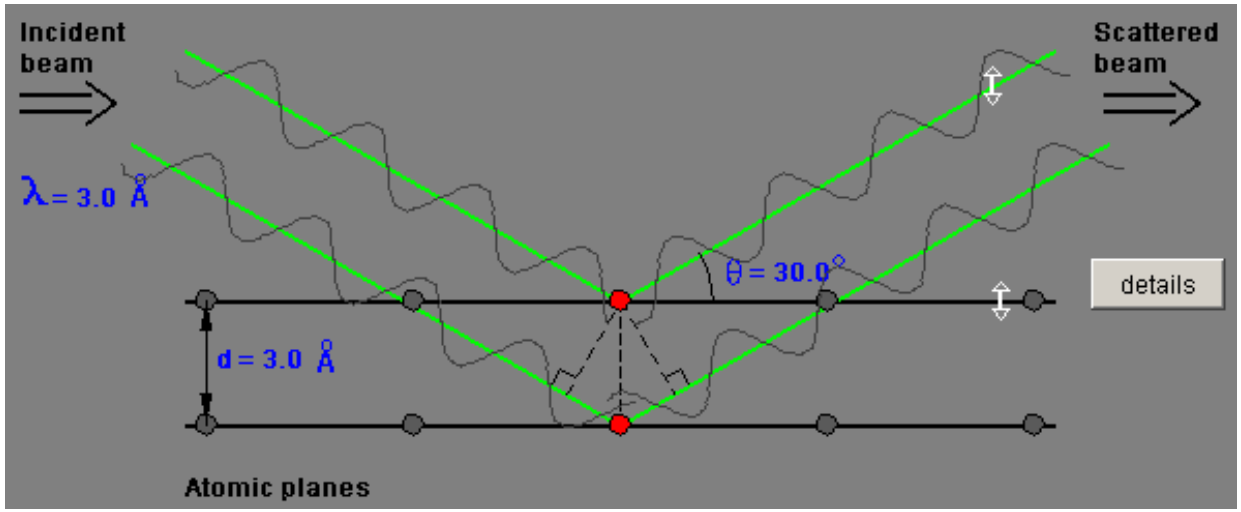
Typical solvent content 40-60%
Solvent channels allow diffusion of compounds into crystal
Often these compounds can reach the active or binding site
Often enzymes are active in crystalline state

Typical x-ray protein crystallography beamline: BL 14.2 at Bessy (Berlin) run by Uwe Müller





Bragg's Law

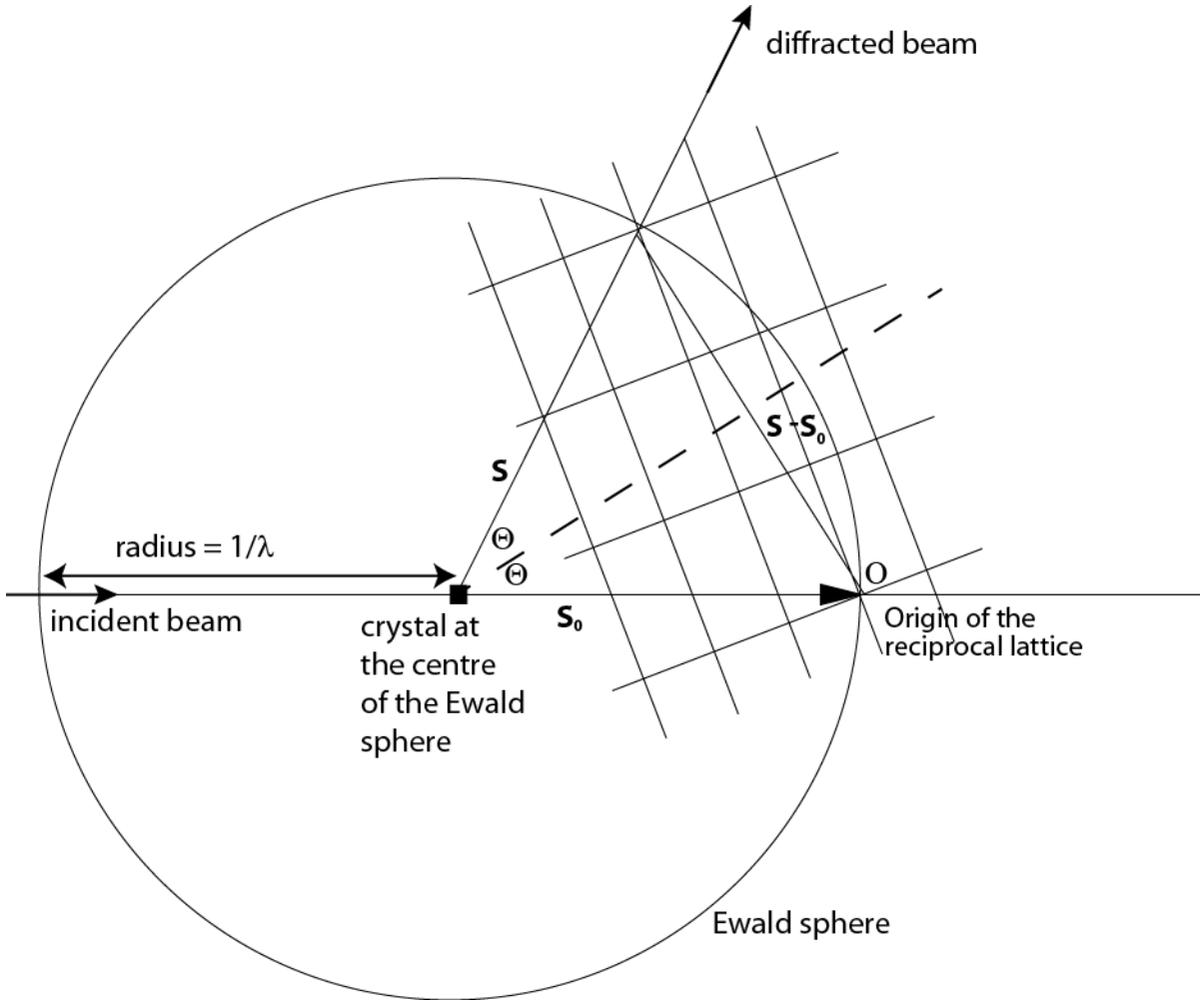


If scattered beams are in phase, they add up

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

http://www.eserc.stonybrook.edu/ProjectJava/Bragg/

Ewald construction and Bragg‘s Law



Solving the phase problem

• Structures of <1000 atoms can be solved by brute force statistical *Direct Methods*, given data to 1.2 Å or better. Most small molecule structures are solved this way.

• A closely related known structure can be used as a search fragment for MR = *Molecular Replacement*.

• Heavy atoms can be introduced and the small changes in the reflection intensities exploited (SIR / MIR = Single / Multiple *Isomorphous Replacement*).

• Heavier atoms exhibit wavelength-dependent *Anomalous Scattering*, with the result that F_{hkl} and F_{-h-k-l} are not exactly equal. These small differences can be exploited in the Single Anomalous Diffraction and Multiple Anomalous diffraction (MAD) methods. For MAD, either metal atoms such as Fe present in the protein, or Se in selenomethionine (genetically modified methionine) are suitable anomalous scatterers.

Neutron protein crystallography

Phase problem is solved by molecular replacement method using the structure obtained from the x-ray data.

=> x-ray crystallography is a prerequisite of neutron protein crystallography.

Advantages of structure determination with neutrons:

- Hydrogen atoms can be resolved even at a resolution of d_{min} ≈2Å (for ^2H) Therefore one can determine:

→ protonation states of amino acid side chains

→ deuterium exchange as a measure of flexibility and accessibility (discrimination between **H** / **D**)

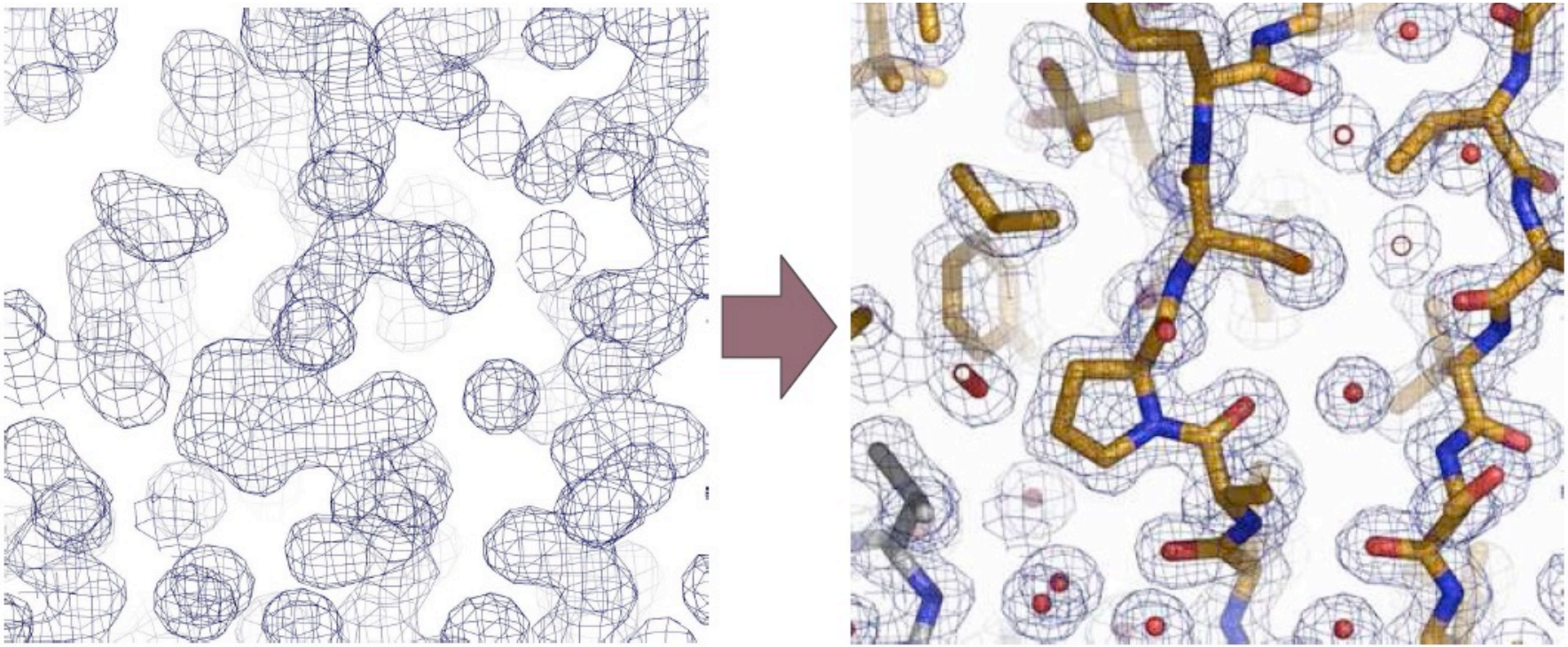
→ solvent structure including hydrogen atoms

Radiation damage (hydrogen abstraction, disulfide bond cleavage)
observed when measuring an x-ray crystallography data set can be avoided.

Especially the reduction of metals in metalo-proteins can be avoided.

Structural Refinement (similar problems apply to both x-ray and neutron protein crystallography)

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



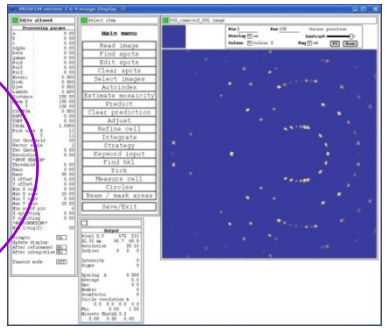
Electron density (x-ray) or nuclear density map (neutrons)

Amino acid chain is fitted into electron densities via dedicated software

from Armstrong.pdf: Qiagen

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)

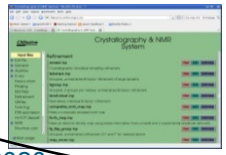
Unified hkl-list of measurement := complete data set

Calculation of a first map

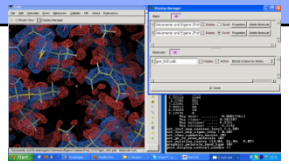
Additional information from the solution of the phase problem

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

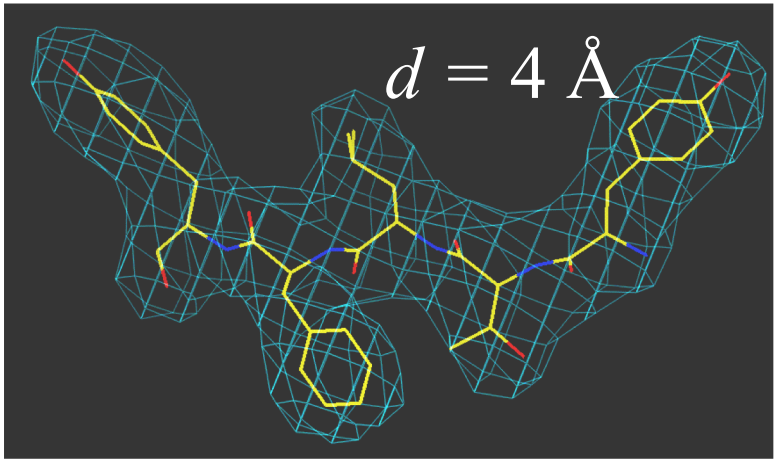


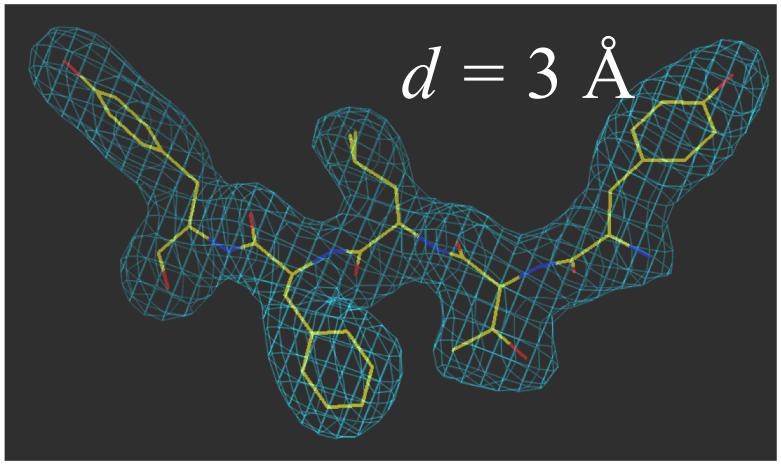
-nCNS
-PHENIX

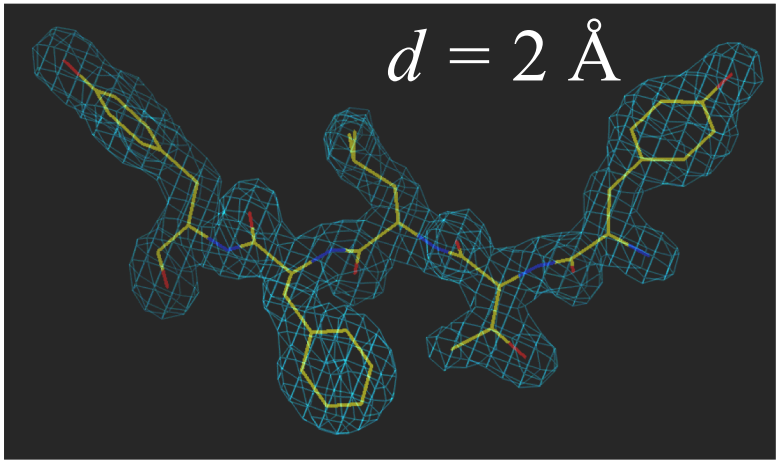


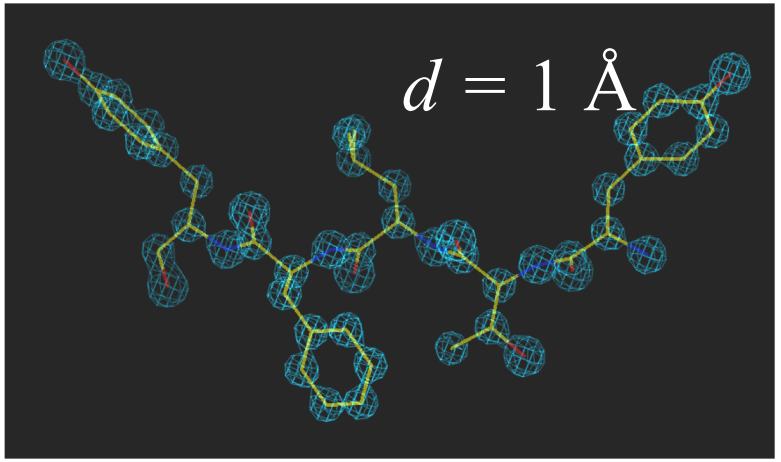
-XtalView
-Coot

The precision of the atomic model is mainly determined by the maximal resolution to which the crystal diffracts X-rays









Atomic resolution

The R-Factors: Judging Convergence between model and data

To compare the generated electron density map and your model, you have to use the R-factor.

The R-factor is a measure of convergence between the intensities calculated from your model and the observed intensities.

$$R_{work} = \frac{\sum_{\mathbf{h}} \left| \left| F_{obs}(\mathbf{h}) \right| - \left| F_{calc}(\mathbf{h}) \right| \right|}{\sum_{\mathbf{h}} \left| F_{obs}(\mathbf{h}) \right|}$$

R:

0.6-VERY BAD

0.5 -BAD

0.4-Recoverable

0.2-Good for Protein

0.05-Good for small organic models

0-PERFECT FIT

However R_{work} can be reduced artificially by refining more parameters, so now it is usual to reserve 5 to 10% of the reflections to calculate an index R_{free} (same formula).

Table 1. Data collection statistics

Data collection	TAFI	TAFI-GEMSA	TAFI-IIYQ
Space group	P31 2 1	P31 2 1	P31 2 1
Cell dimensions			
a, *b*, *c*, Å	161.7, 161.7, 139.5	161.1, 161.1, 139.0	159.5, 159.5, 139.5
α, β, γ, °	90, 90, 120	90, 90, 120	90, 90, 120
Resolution, Å	49.51-3.10 (3.27-3.10)	49.27-3.40 (3.58-3.40)	49.03-2.80 (2.95-2.80)
R_{merge}	0.105 (0.833)	0.091 (0.630)	0.067 (0.600)
$I / \sigma I$	13.9 (2.6)	11.4 (2.2)	13.8 (2.1)
Completeness, %	100.0 (100.0)	99.6 (100.0)	100.0 (100.0)
Redundancy	8.6 (8.7)	4.9 (5.0)	4.3 (4.4)

Values in parentheses are for highest-resolution shell. A single crystal was used for each structure.

Table 2. Refinement statistics

Refinement	TAFI	TAFI-GEMSA	TAFI-IIYQ
Resolution, Å	3.1	3.4	2.8
No. reflections	36638	27424	48058
R_{work} / R_{free}	0.204 / 0.240	0.203 / 0.258	0.188 / 0.232
No. atoms			
Protein	9861	9892	9901
Ligand/ion	0 / 3	45 / 3	36 / 3
B-factors, Å²			
Protein	94.8	113.3	67.5
Ligand/ion	— / 102.9	111.9 / 117.5	72.4 / 71.3
R.m.s. deviations			
Bond lengths, Å	0.018	0.013	0.018
Bond angles, °	1.751	1.453	1.871

— Indicates not applicable.

Judging the quality of the data:

- I/σ = signal/noise >2.0
- Completeness > 90%
- R/R_{free}: difference < 0.05,
- Deviations of known geometry: weird angles

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

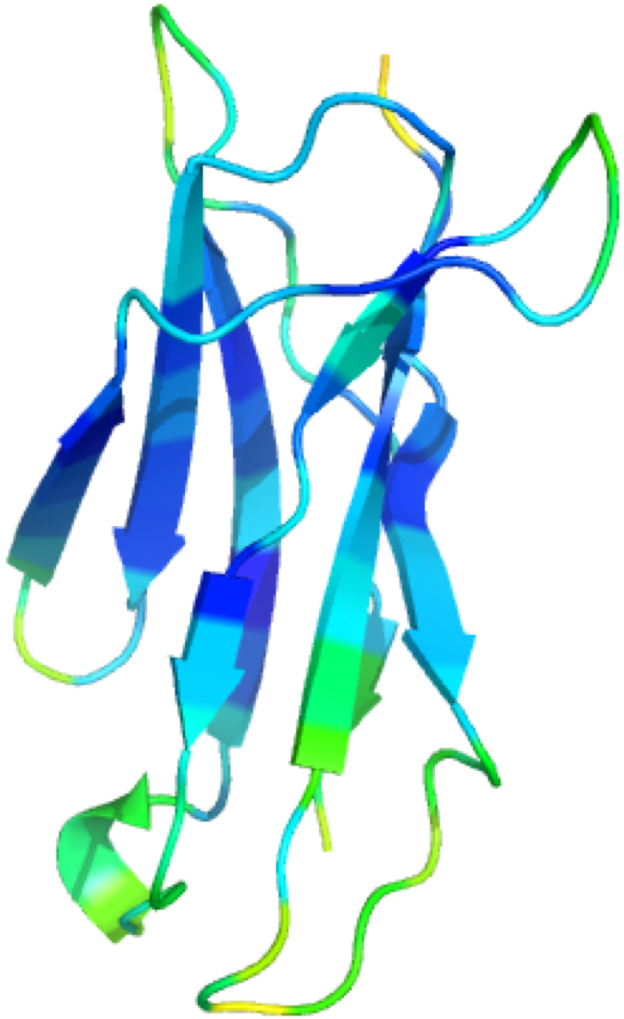
occupancy

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

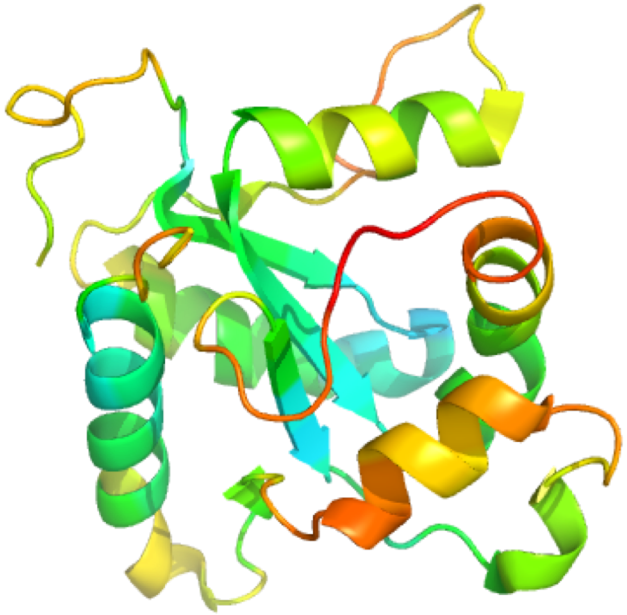
Look at B-factor distribution!



Protein coloured by B-factor:

Well defined regions have low B-factors (blue/green)

Poorly defined/more mobile regions have high B-factors (yellow/orange/red)



A protein molecule is dynamic

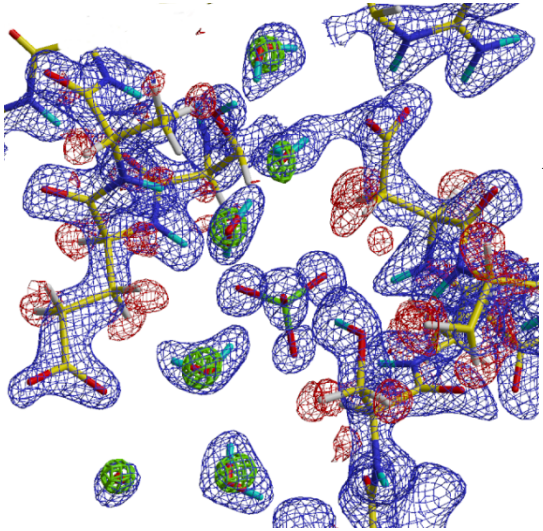
- The electron density is a spatial average over all molecules in the crystal and a time average over the duration of the X-ray data measurement

=> Multiple discrete conformations of a residue in different molecules are superimposed.

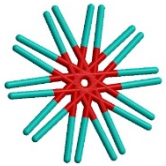
- A crude description of dynamics is provided in the pdb file as the isotropic B-factor
- Some dynamical aspects evident in the electron density are lost in the pdb file

From structure to function: Some case studies

Example A) HYDRATION STRUCTURE:

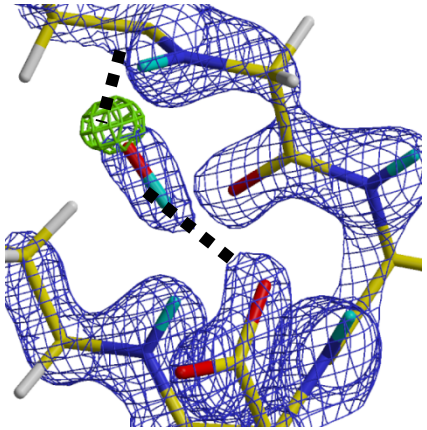


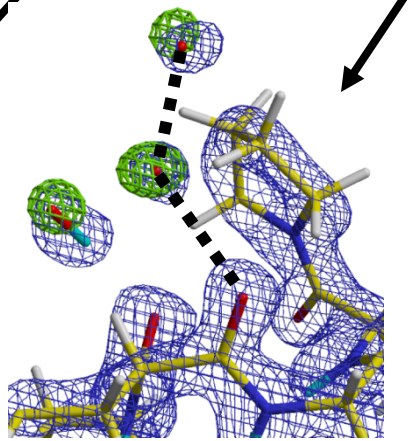
TRIANGULAR (#17)



ELLIPSOIDAL (#10)

SPHERICAL (#47)





- All types of shapes can be found on the protein surface
- Triangular shapes are only found in direct contact with the protein surface
- Shape depends on the environment

$2F_o$-F_c neutron map, +1.5σ

$2F_o$-F_c neutron map, -2.0σ

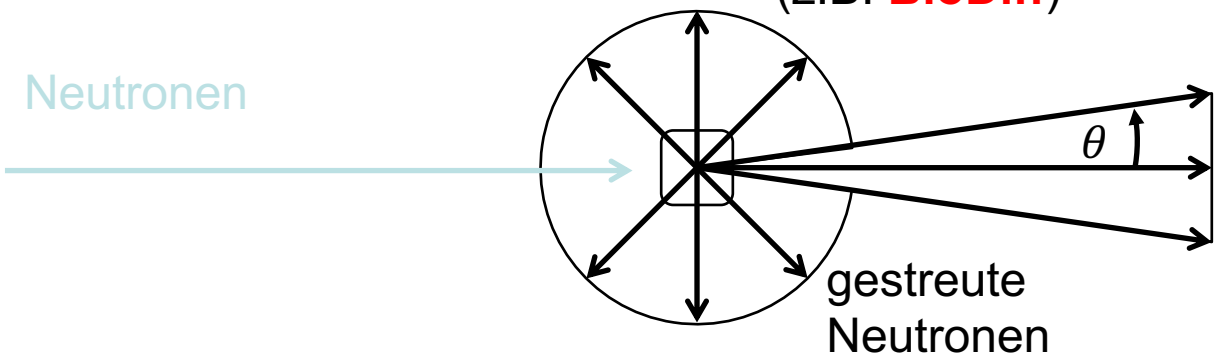
$2F_o$-F_c X-ray map

Chatake T, Ostermann A, Kurihara K, Parak F, Niimura N (2003) Proteins 50:516

Methoden & Instrumente

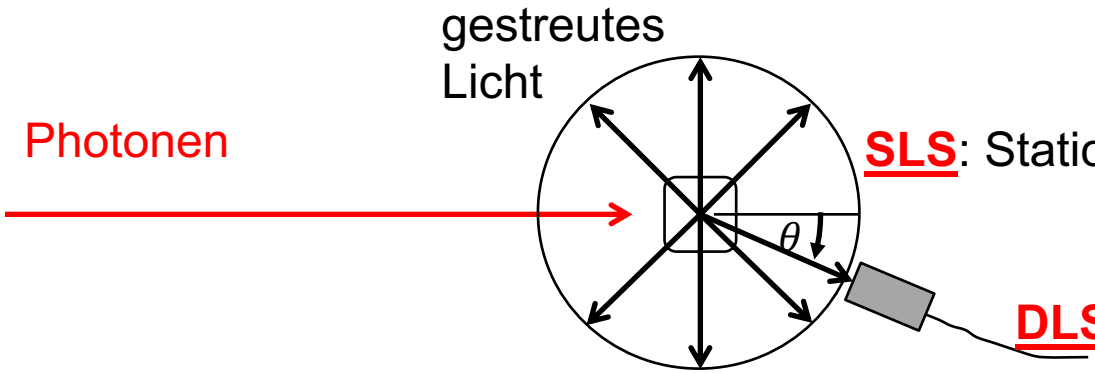
Kristallisationsprozess läuft auf verschiedenen Zeit- und Größenskalen ab

Einkristall Diffraktometer
(z.B. **BioDiff**)



SANS:
Small Angle Neutron Scattering
(z.B. KWS-2, D11)

$$q = \frac{4\pi n}{\lambda} * \sin\frac{\theta}{2}$$



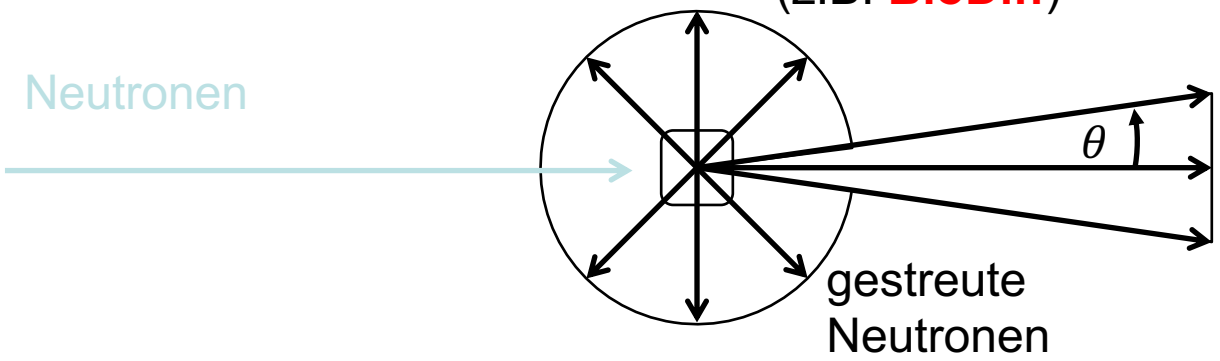
SLS: Static Light Scattering

DLS: Dynamic Light Scattering

Methoden & Instrumente

Kristallisationsprozess läuft auf verschiedenen Zeit- und Größenskalen ab

Einkristall Diffraktometer
(z.B. **BioDiff**)



SANS:
Small Angle Neutron Scattering
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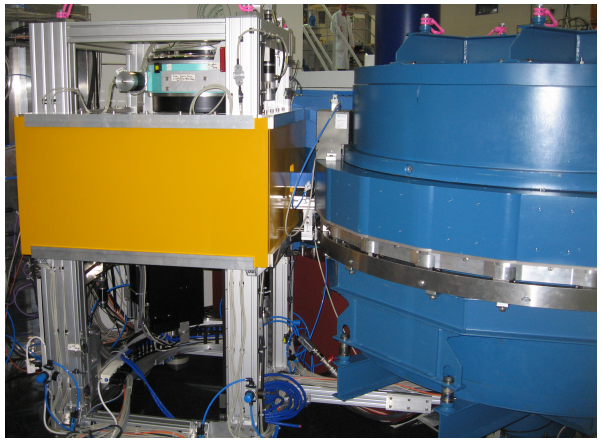
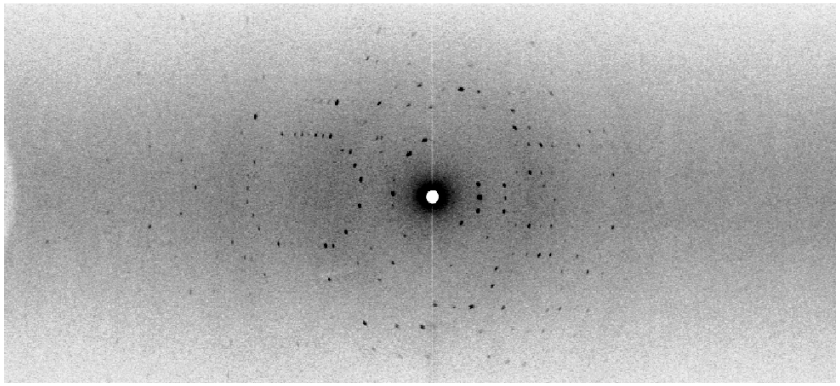
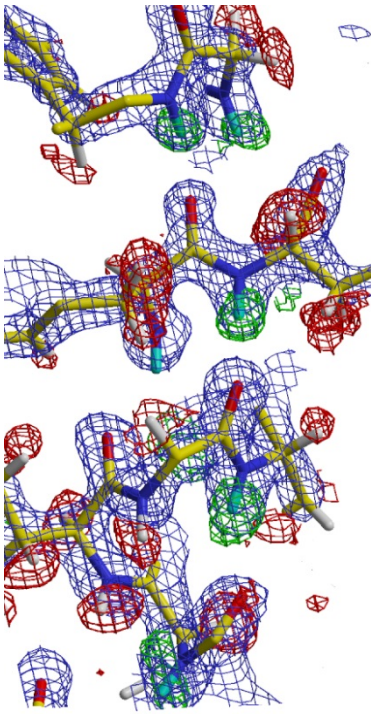


Theory on scattering from a crystal

3D structural analysis:





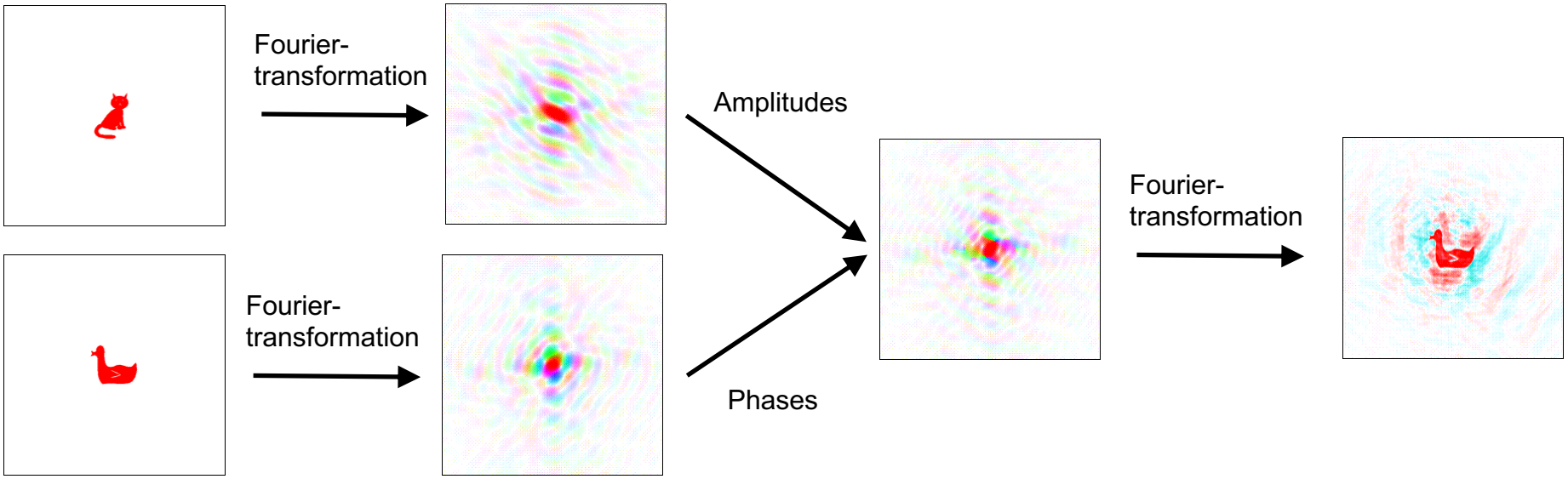
$$\rho(x,y,z) = \frac{1}{V_E} \sum_{h,k,l} F_{hkl} \cdot e^{-2\pi i(h\cdot x + k\cdot y + l\cdot z)}$$

Structure factors are complex numbers: $F_{hkl} = \|F_{hkl}\| e^{-2\pi i \alpha_{hkl}}$

with amplitudes $\|F_{hkl}\|$ and phases α_{hkl}

→ Phase Problem, because we only record intensities: $I = \|F_{hkl}\|^2$

The phases are stronger than the intensities



http://www.ysbl.york.ac.uk/~cowtan/fourier/magic.html