Jülich Centre for Neutron Science

JÜLICH FORSCHUNGSZENTRUM

Neutron Protein Crystallography - introducing the method and showing some application examples

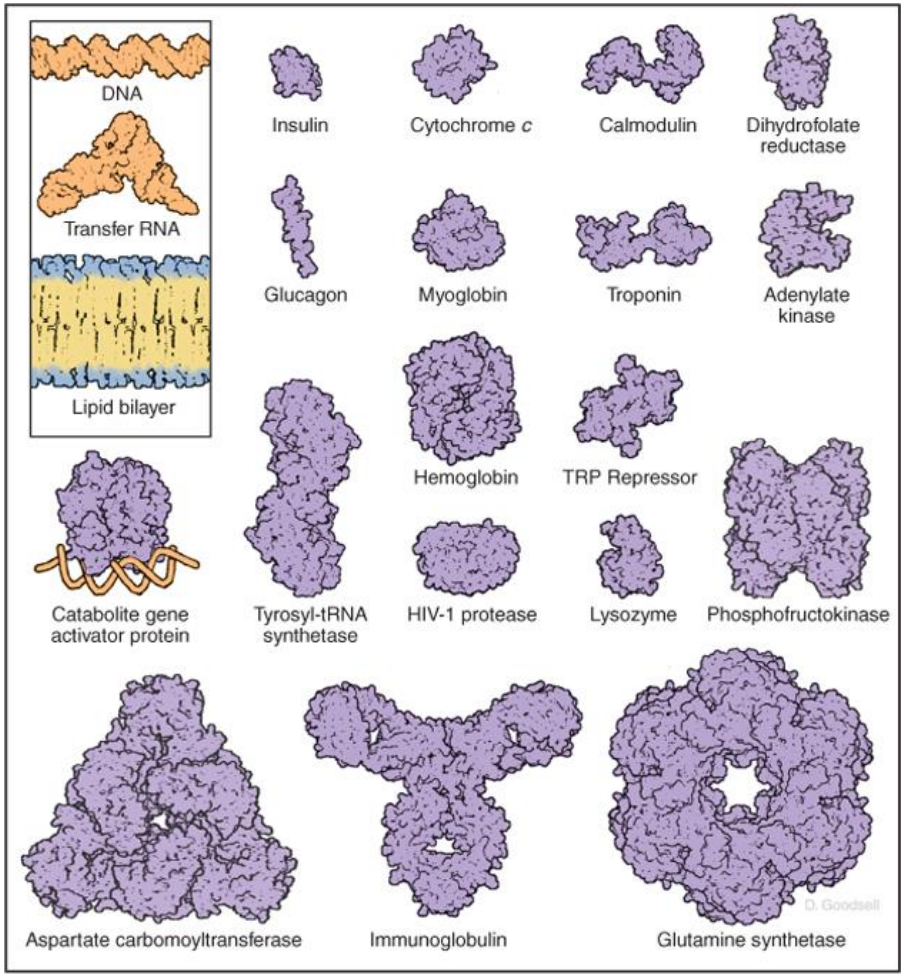
Seminar, IfK, RWTH Aachen

November 3rd 2020 | Tobias E. Schrader

Outline

- Motivation: Why do we need protein structures at atomic resolution?
- x-ray protein crystallography
- neutron protein crystallography
- Two application examples: From Structure to function…

Proteins are structured macromolecules and come in different sizes and shapes



The structure is crucial for the protein‘s function

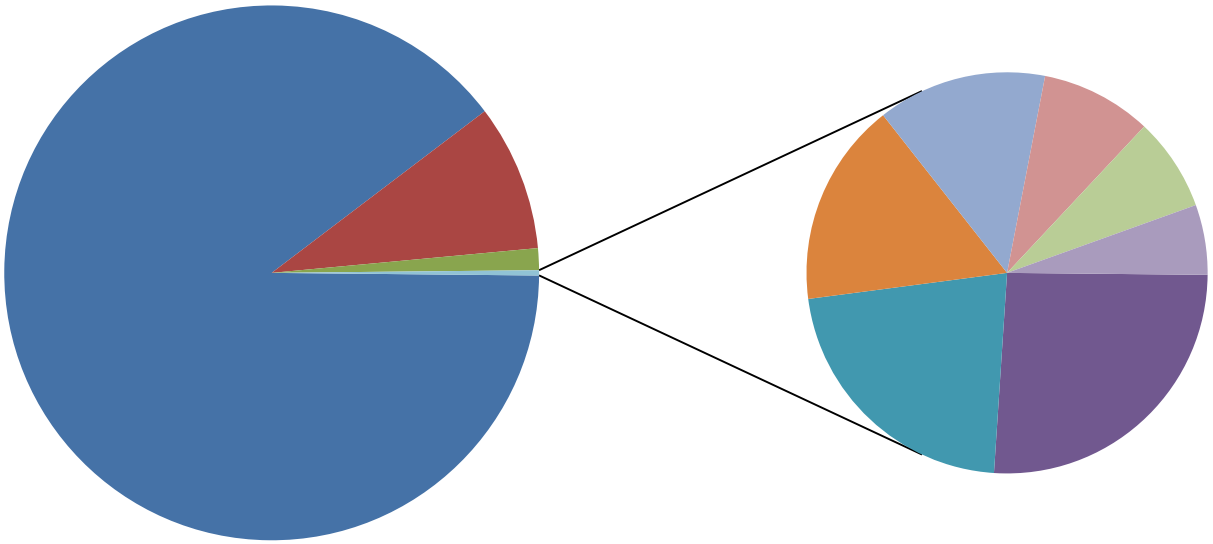
How do we find out about protein structures?

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)

Most structures are obtained by x-ray crystallography, available neutron structures in protein data bank: ca. 150

As of beginning of 2019:



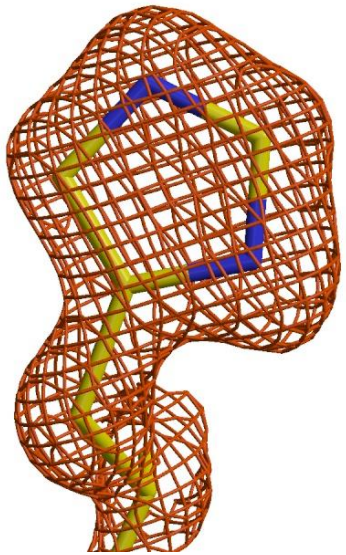
- x-ray (120534)
- solution NMR (11921)
- Electron Microscopy (1776)
- Hybrid (110)
- Solid State NMR (93)
- Electron Crystallography (70)
- Neutron Diffraction (58)
- Fiber Diffraction (38)

http://www.rcsb.org/

Total number of structures: 134656

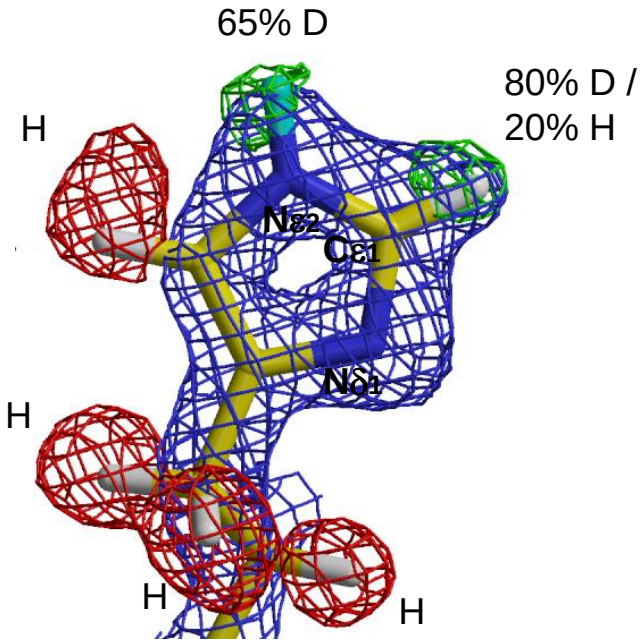
X-ray data versus neutron data on the same protein:

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

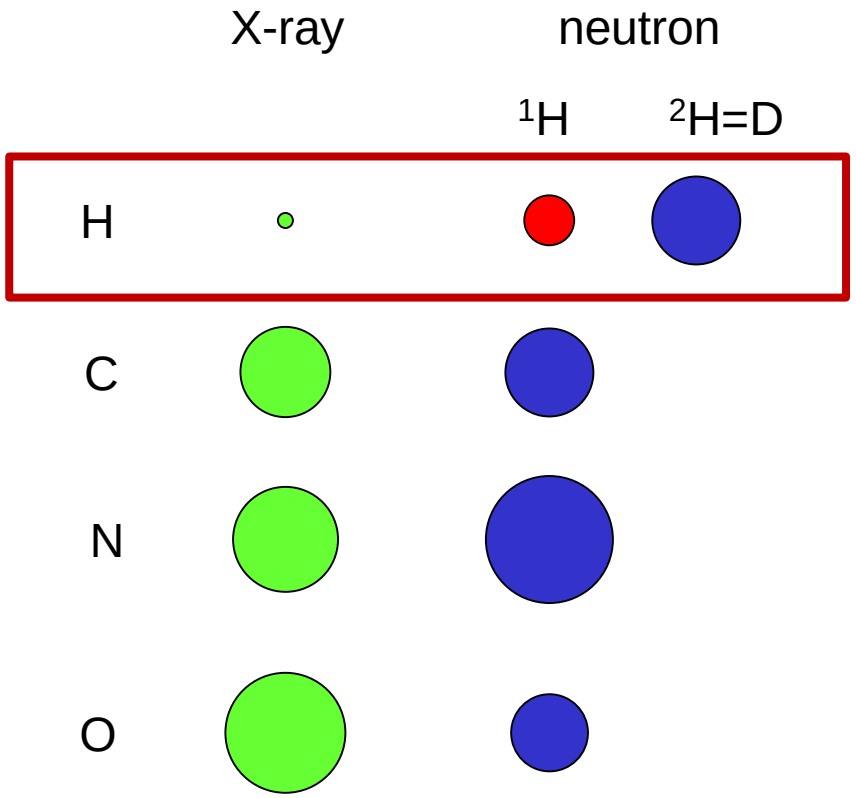
Fo-Fc omit-map; +3.0σ

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):

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**



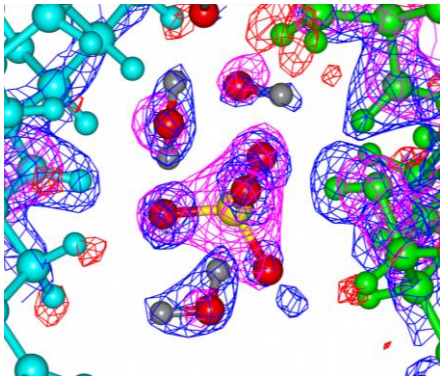
diameters correspond to:
form factor / scattering length
(scaled for C-atom)

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 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



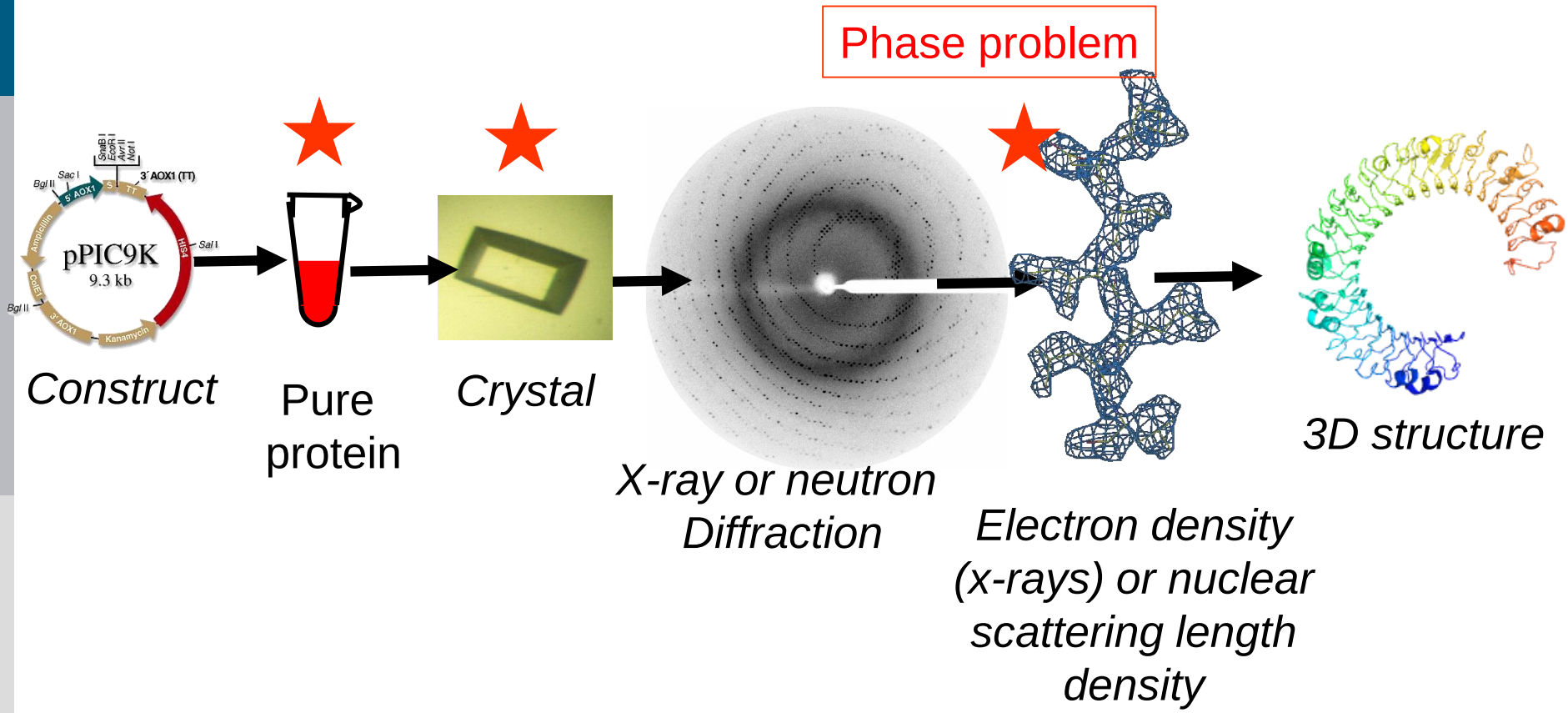
Water network in the contact region between two myoglobin molecules in the crystal.

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

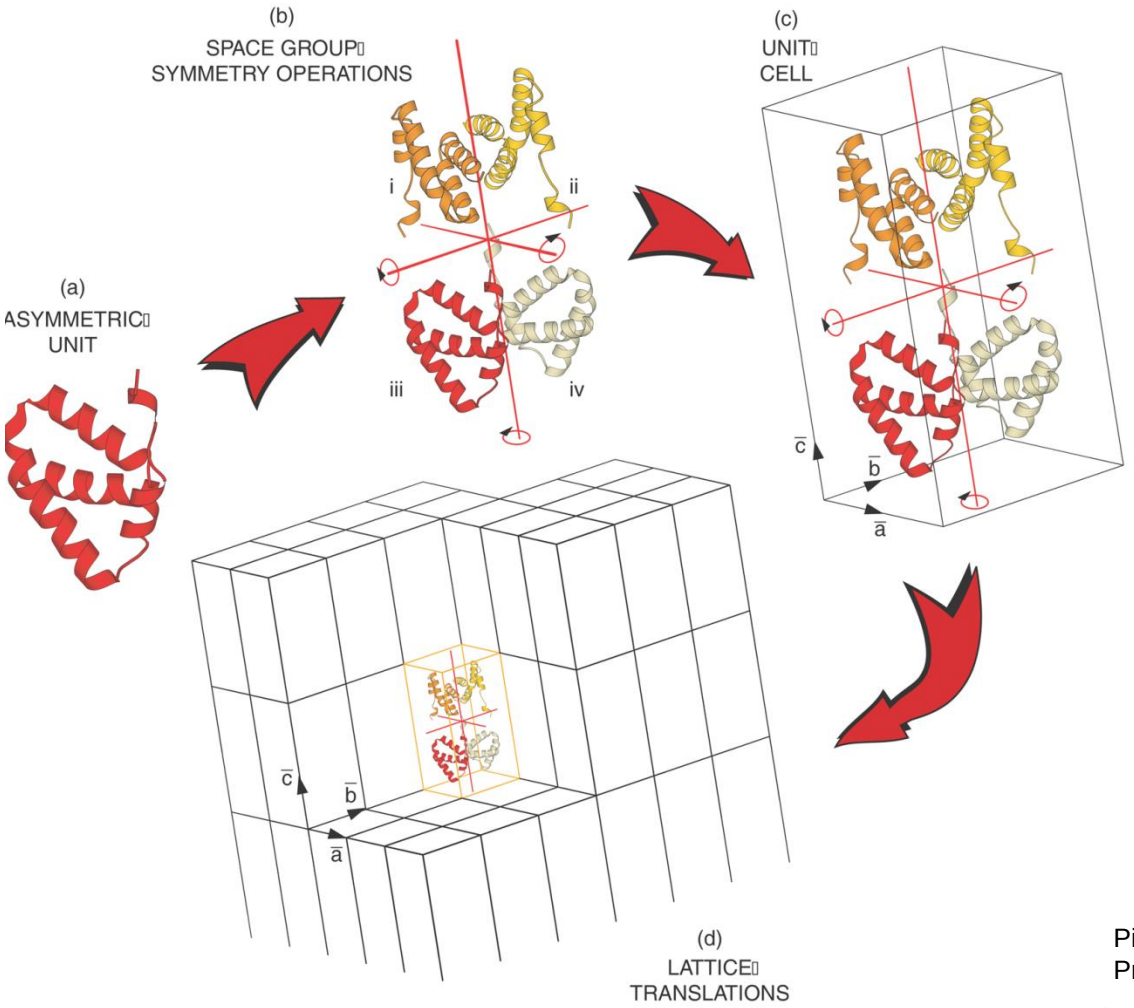
Protein crystallography in general, valid for both x-rays and neutrons as probes

Crystallography: Overiew over the process



Harma Brondijk, Crystal and Structural chemistry, Utrecht University

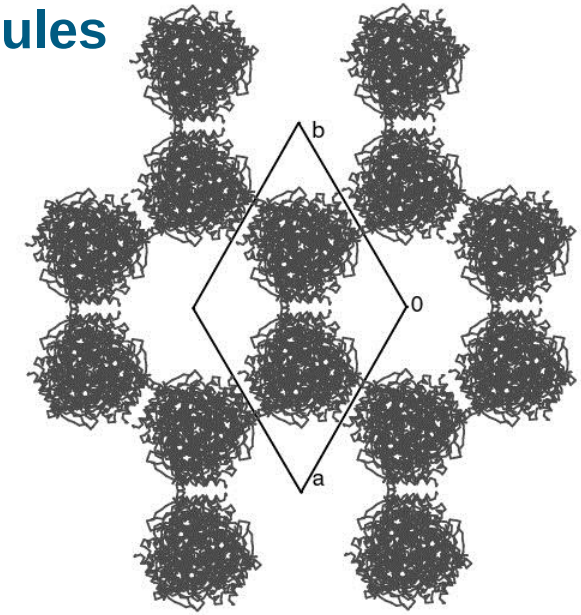
How a typical protein crystal looks like…

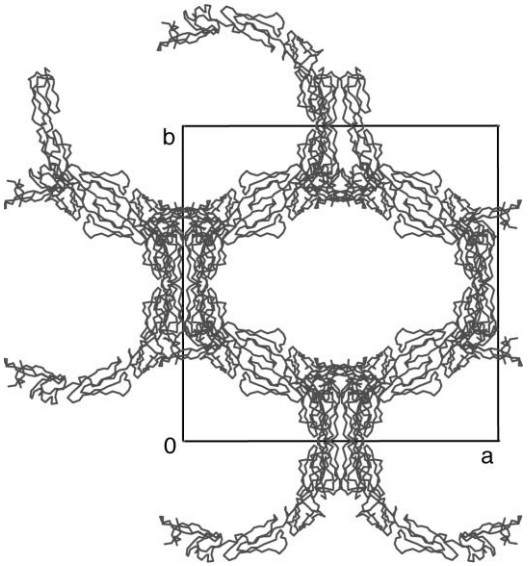


Picture taken from Lecture of Prof. Locher at ETH Zürich

fig 2.2

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

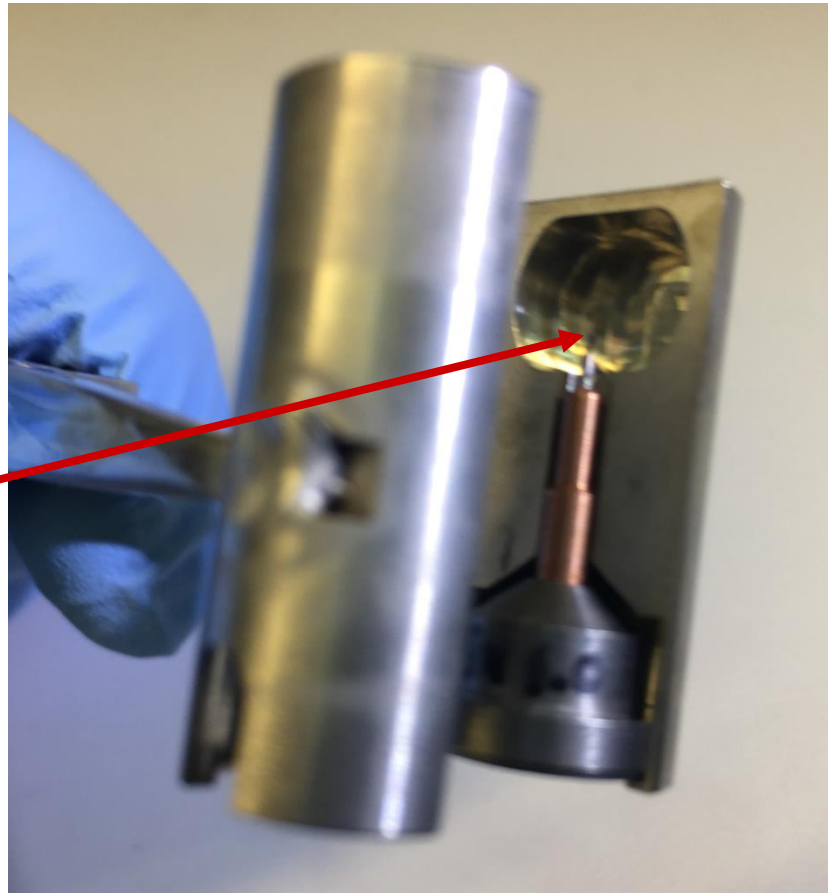
Size considerations of protein crystals

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 0.5 mm x 0.5 mm (depending on the protein/space group)

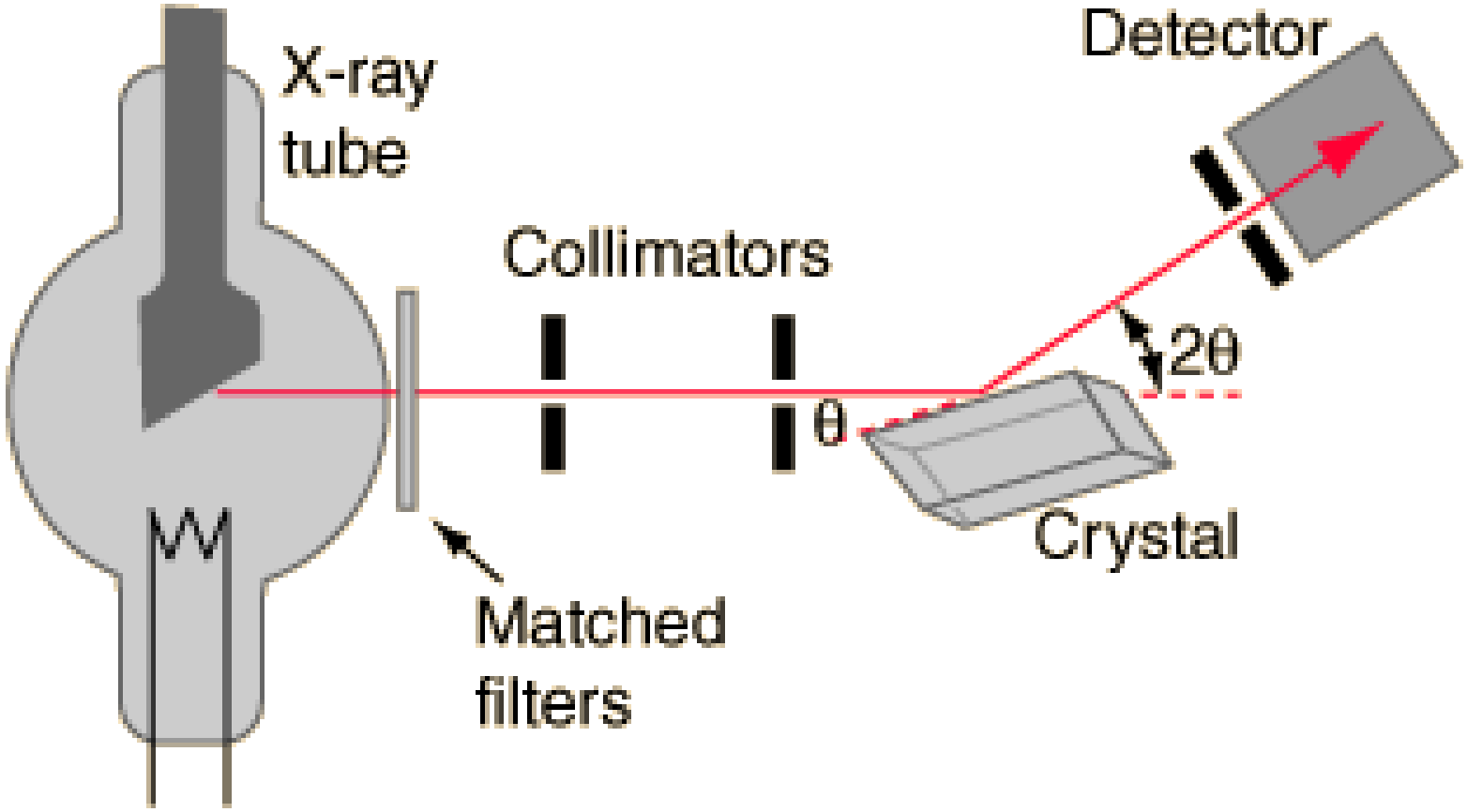
Outer diamter of the glas tube: 5 mm

Cryo-mounting of large crystals

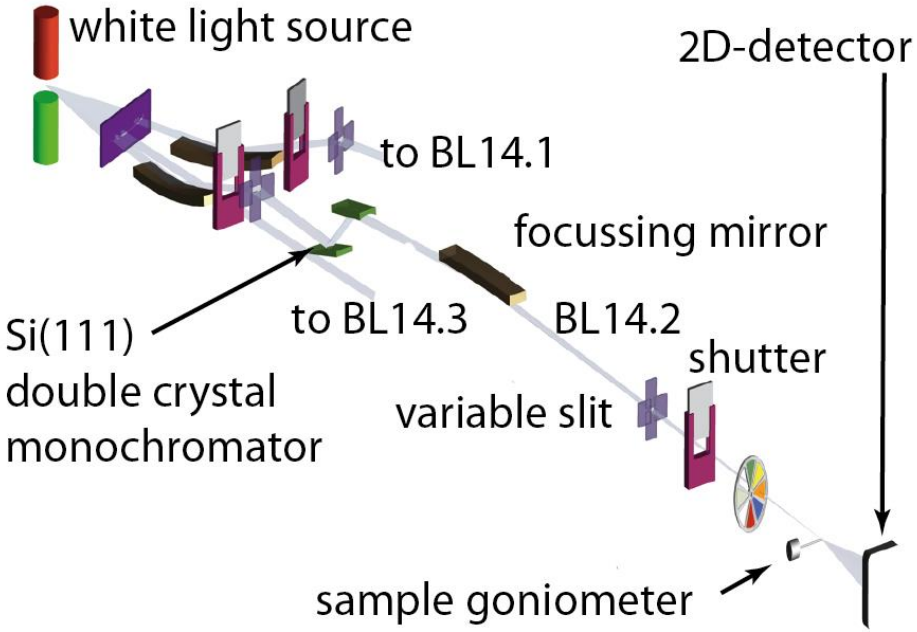


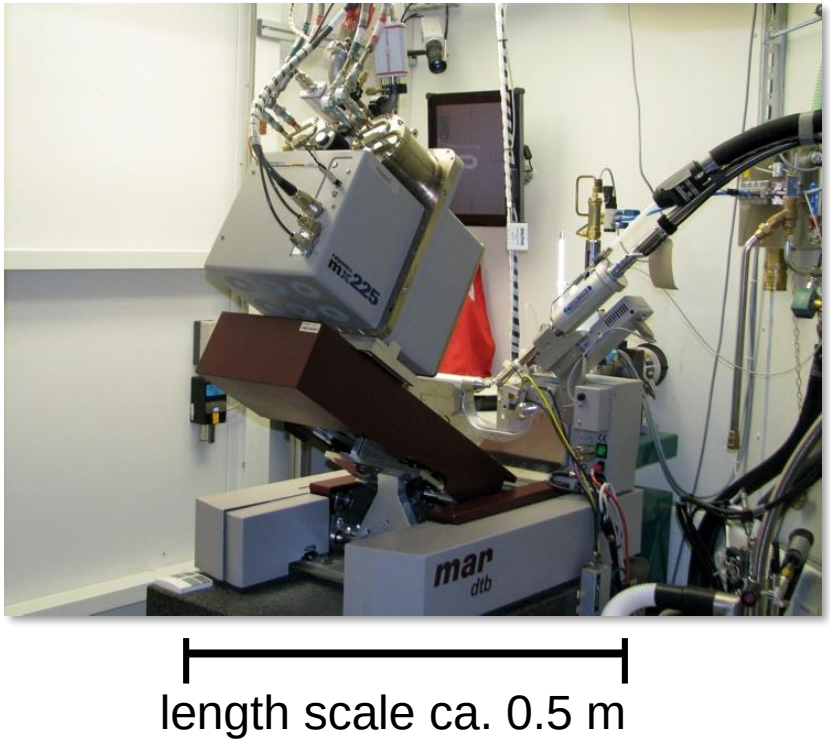
- Avoid hydrogenated polymers in the loop, use capton (Mitigen) or carbon meshes instead (especially when you have a fully deuterated protein)
- Make sure that your crystal fits into the cryoTong: We prefer the 18 mm one.

Experimental set up (in case of x-rays but similar in the case of neutrons):

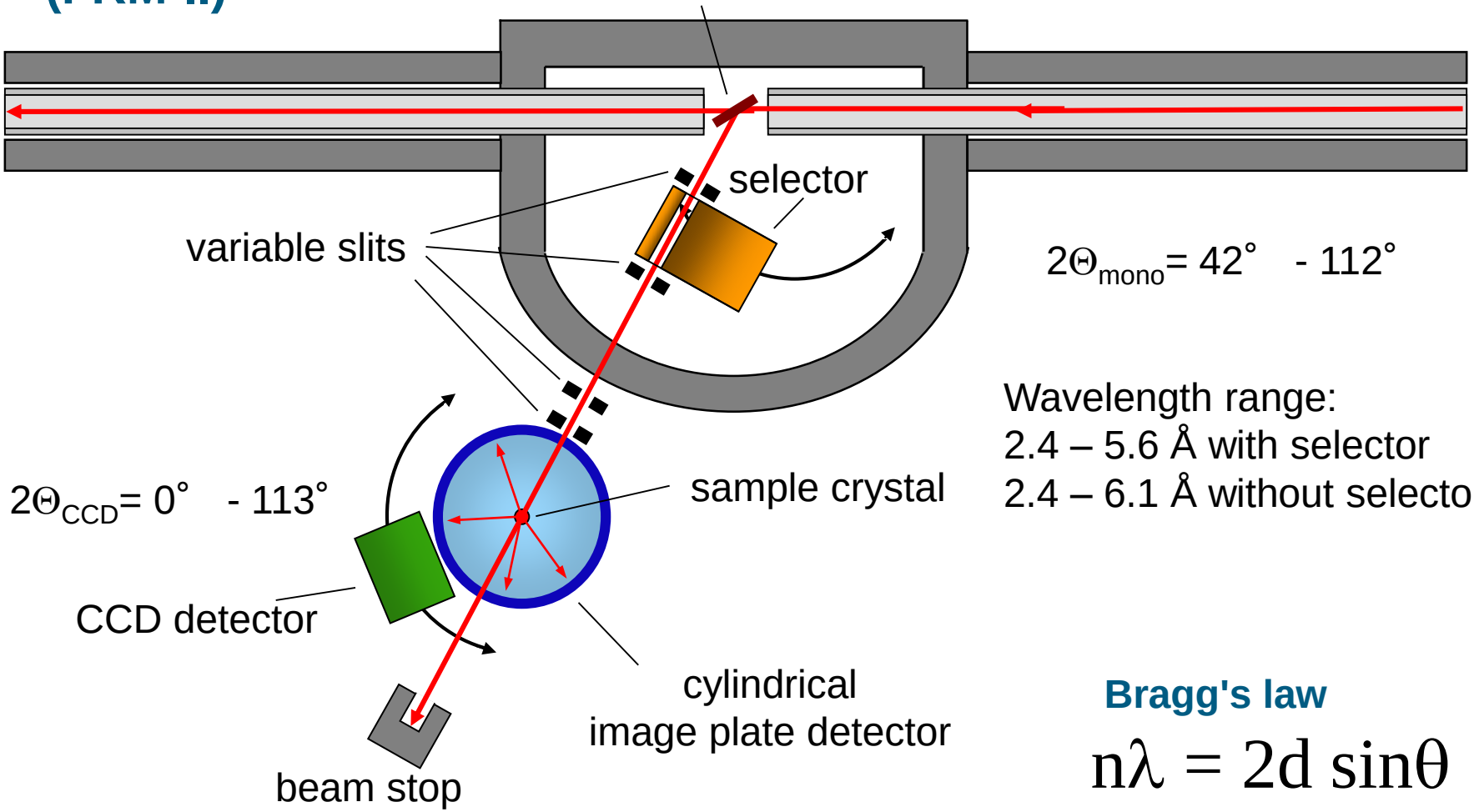


Typical x-ray protein crystallography beamline: BL 14.2 at Bessy (Berlin) run by Manfred Weiss





Schematic overview over BioDiff: A neutron protein diffractometer: collaboration between JCNS and TUM (FRM-II)

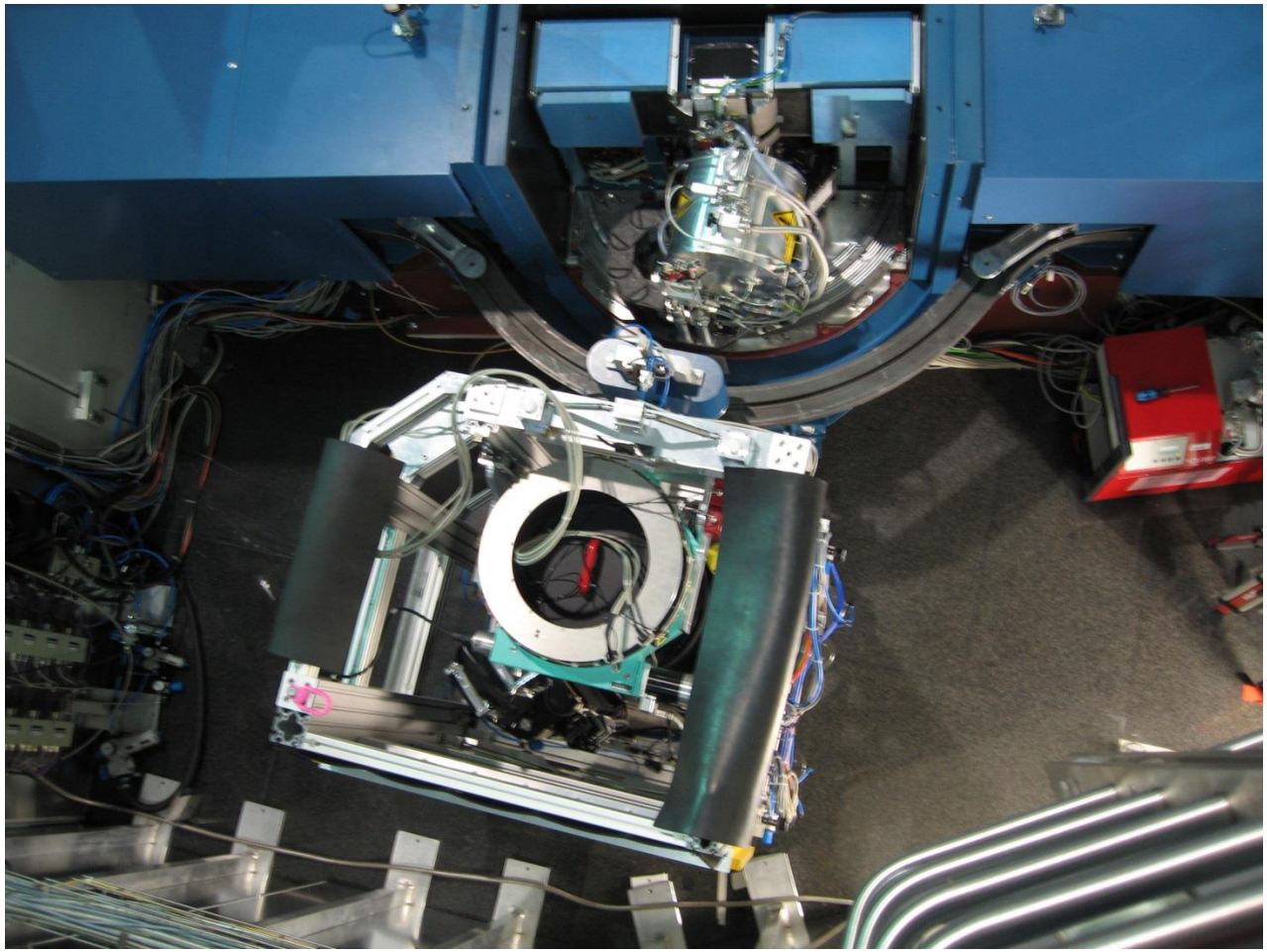


Wavelength range:
2.4 – 5.6 Å with selector
2.4 – 6.1 Å without selector

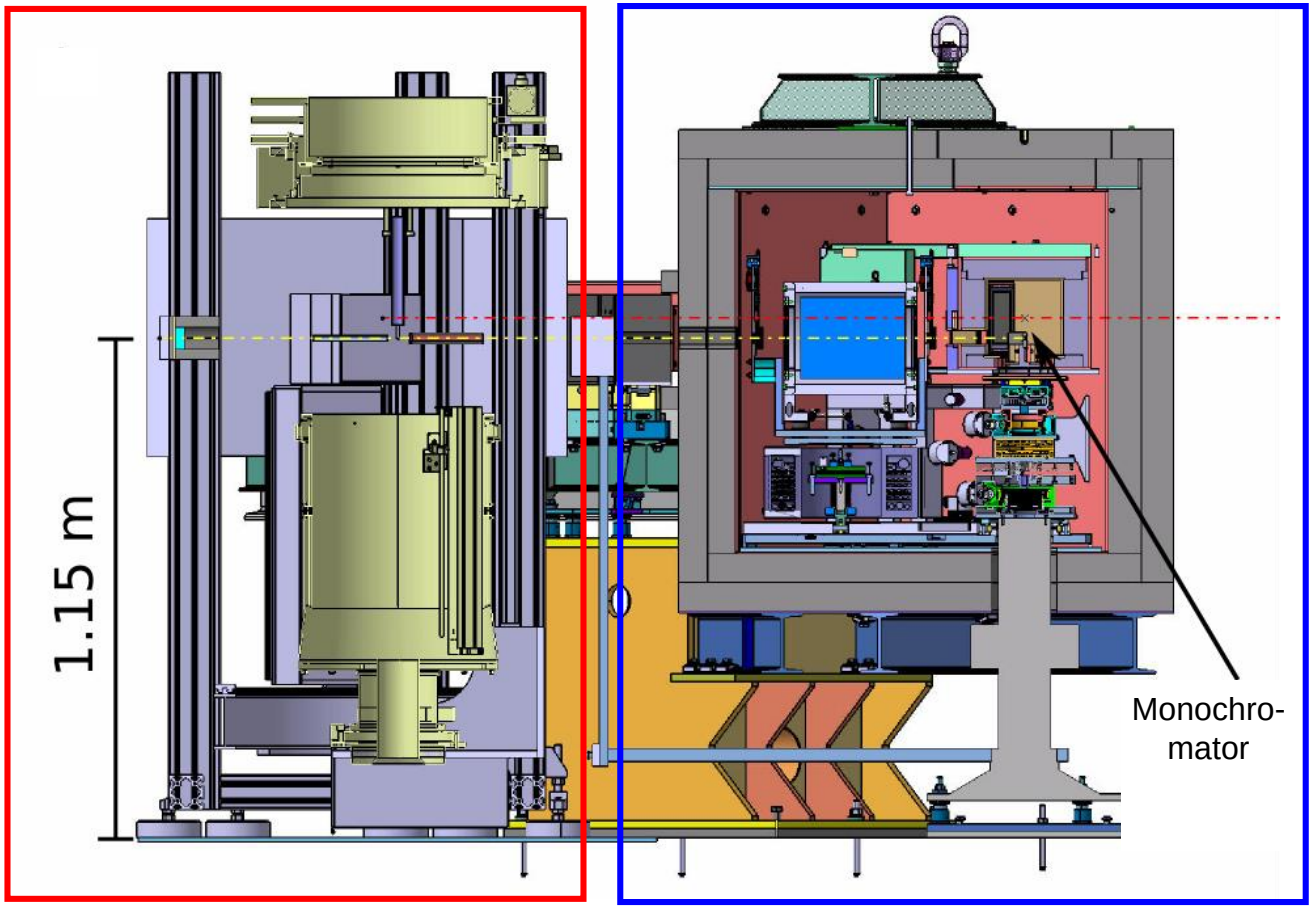
Bragg's law

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

BioDiff, the corresponding view in reality:



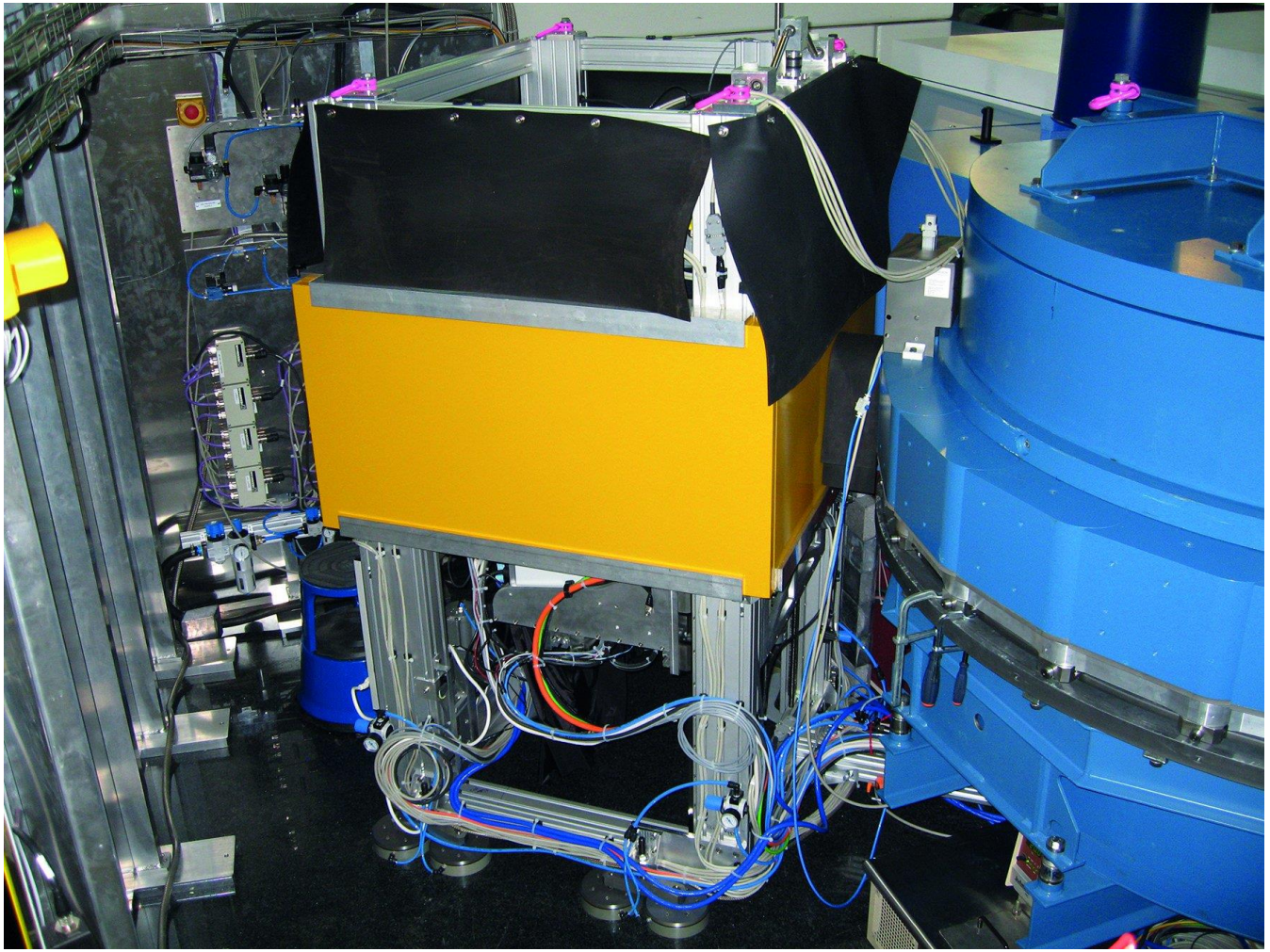
The Simultaneous Construction-phase in Garching and Jülich



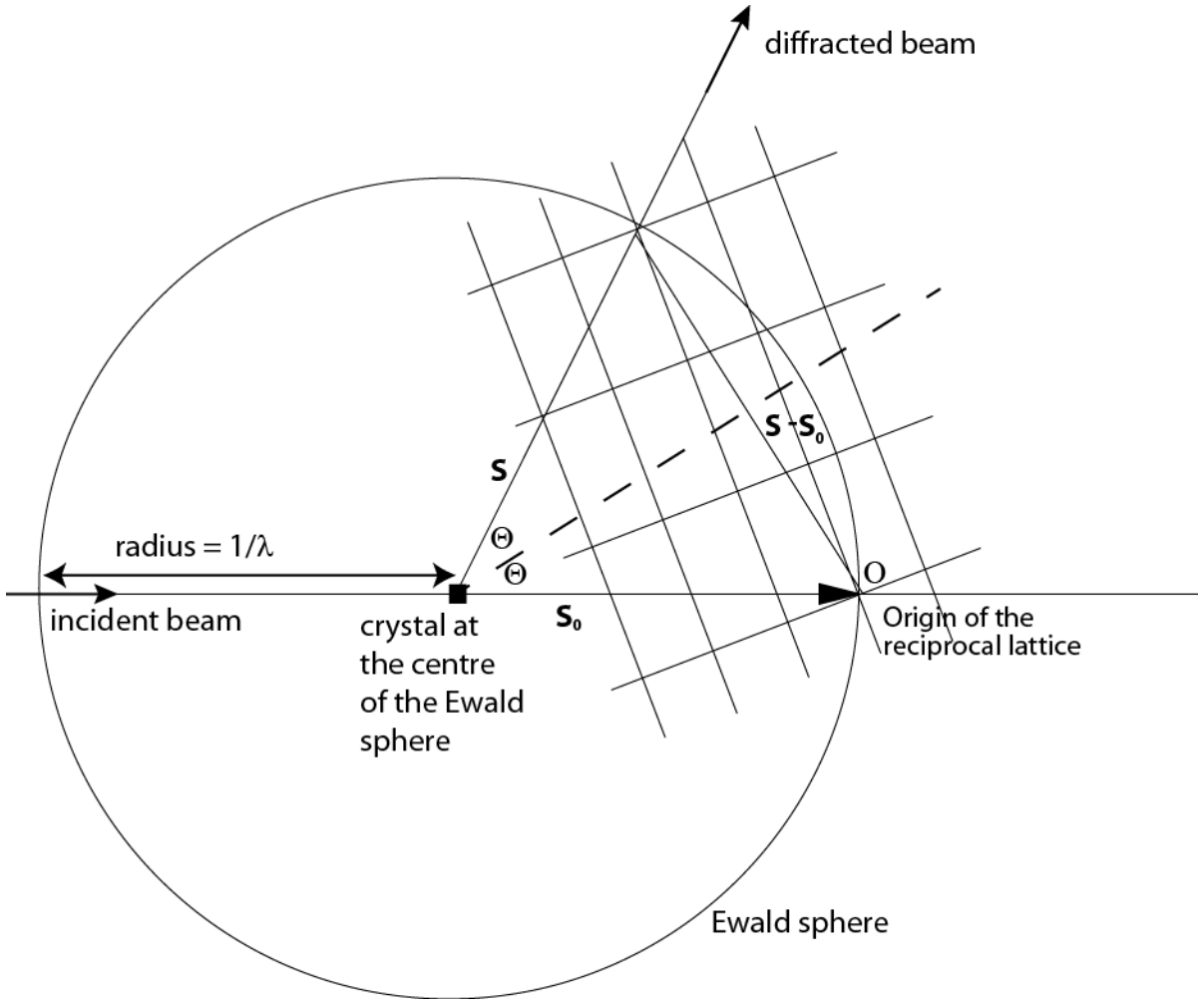
Detector unit, constructed and built in Garching (Ph. Jüttner, MLZ)

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

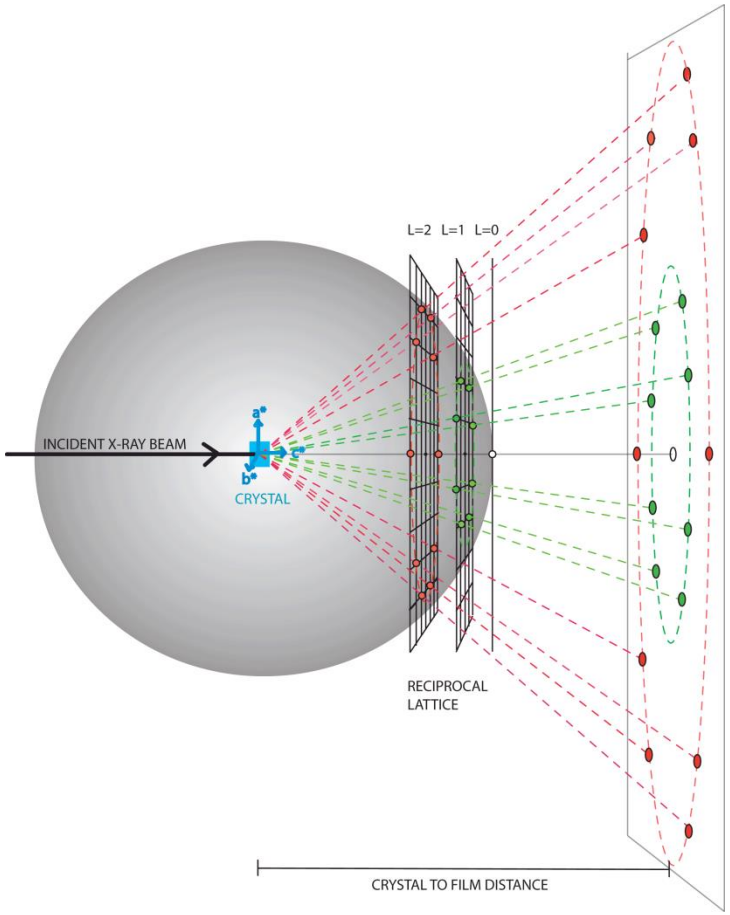
A Most Recent View of the Instrument BioDiff

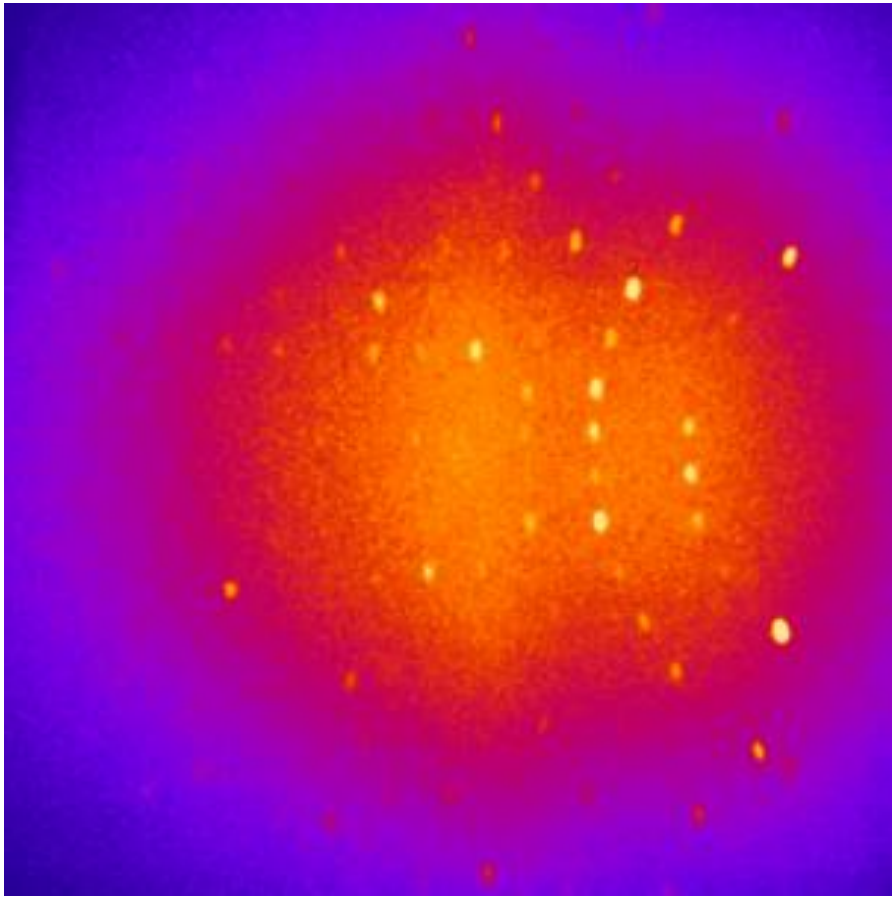


Ewald construction and Bragg‘s Law



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

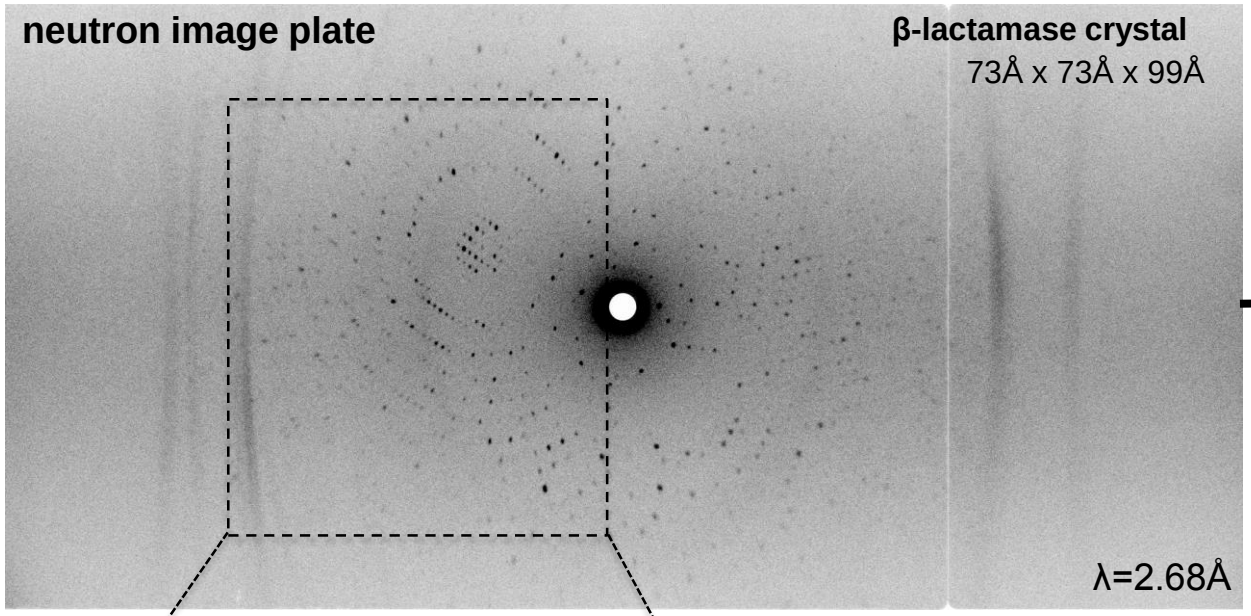


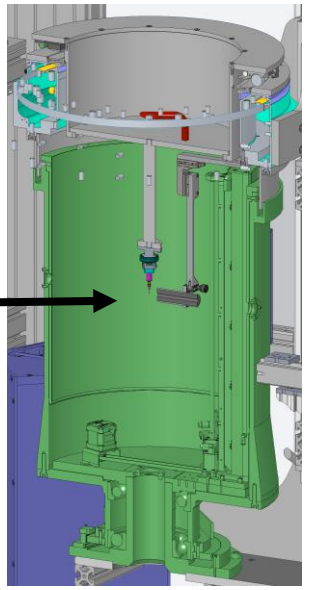


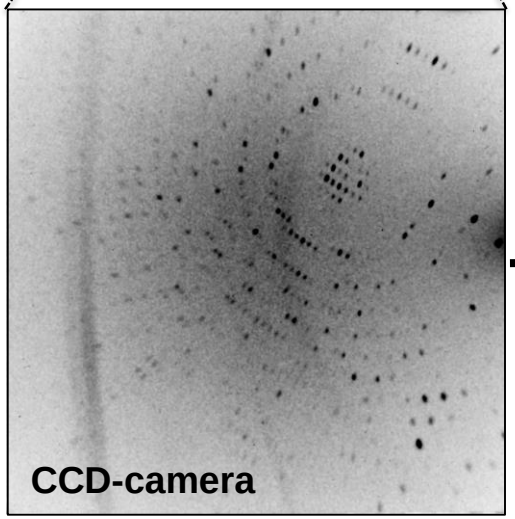
prim. beam

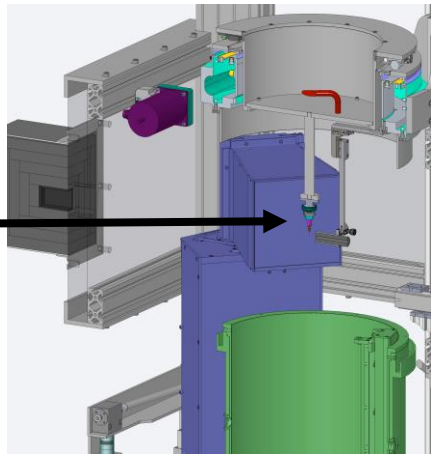
BioDiff: exposure time per frame: 20 minutes, sample: Myoglobin in deuterated mother liquor

The Two Detectors of BIODIFF









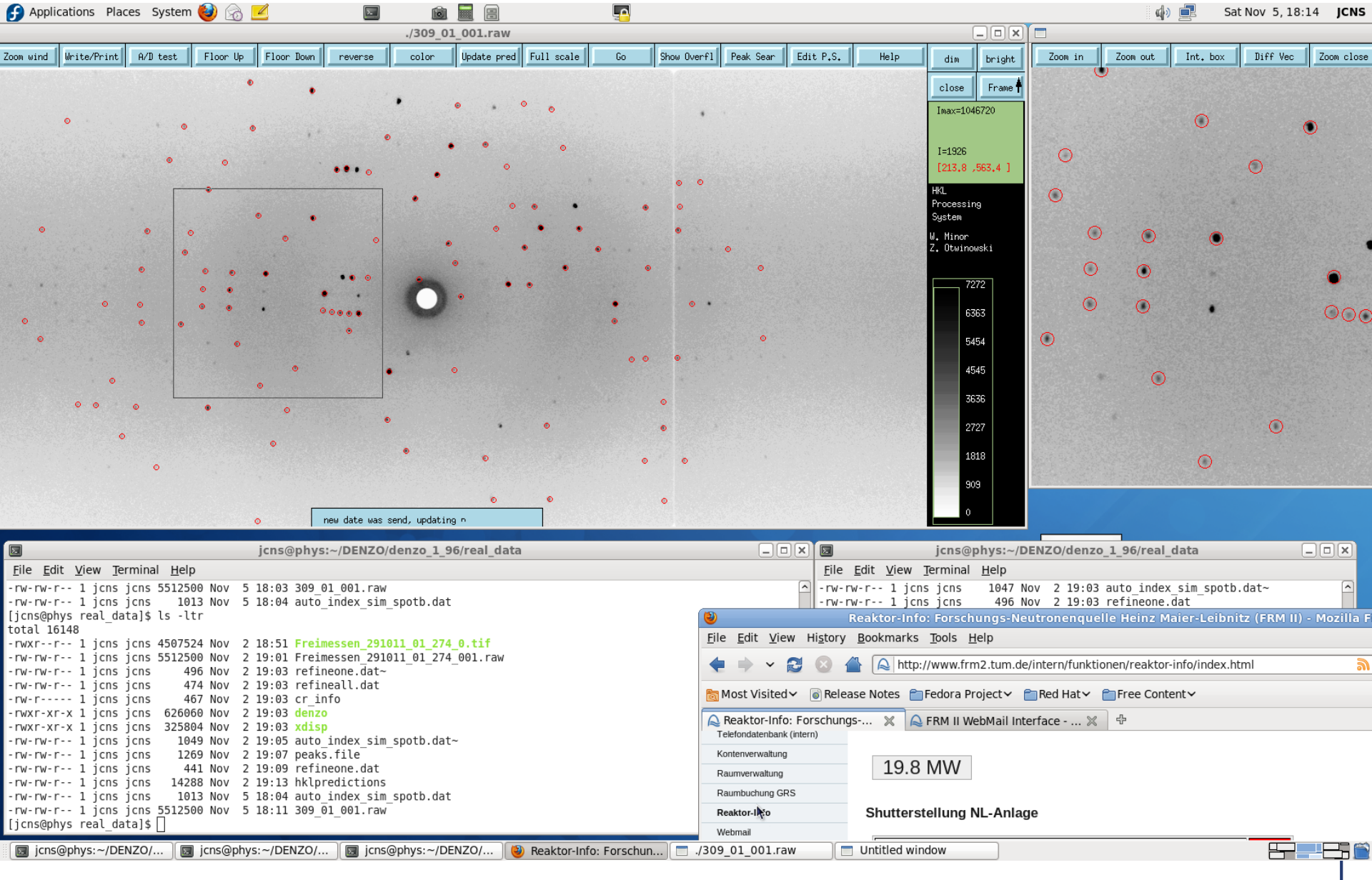
NIP-scanner

- larger solid angle
- readout time ≥ 4 min

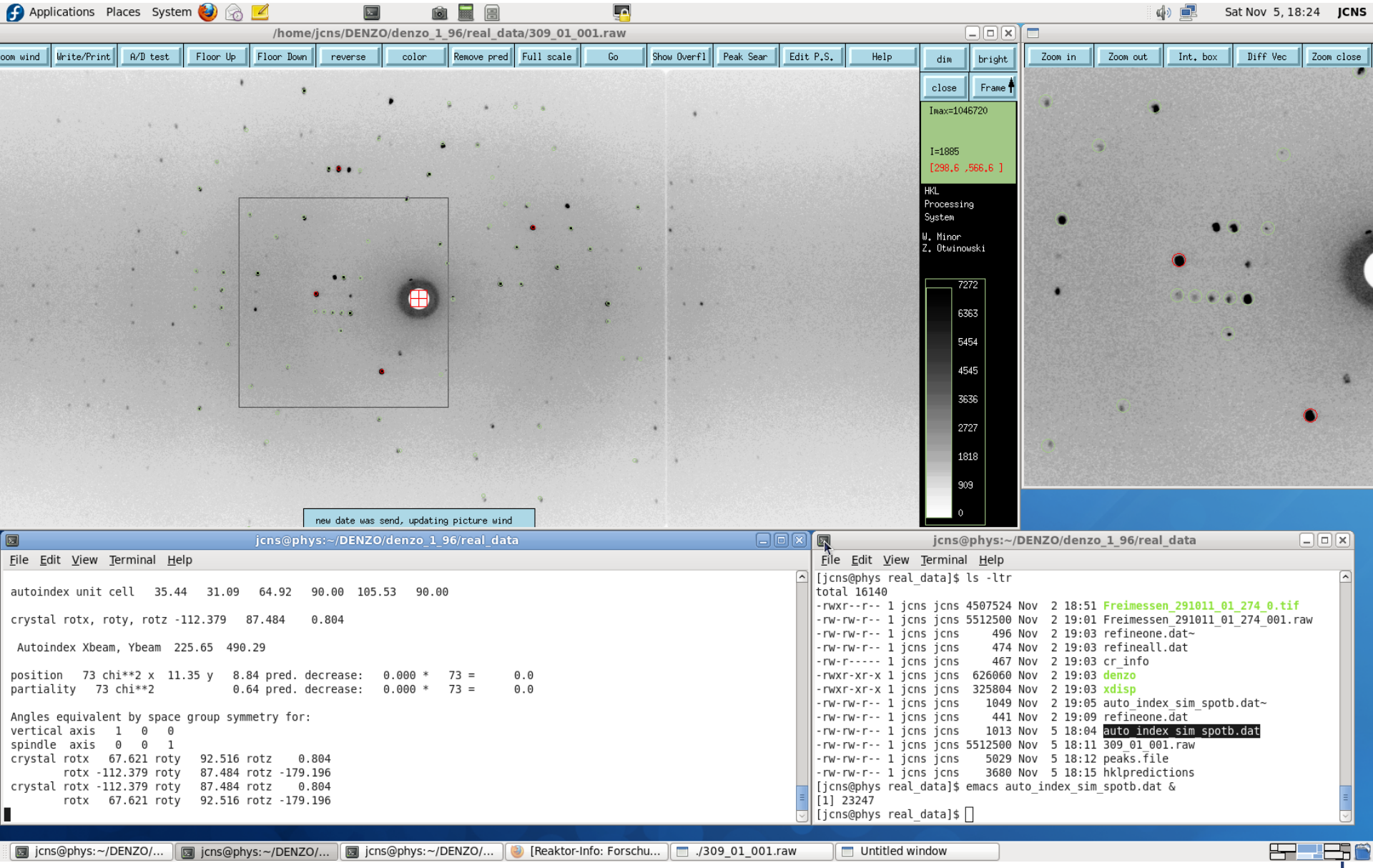
CCD-camera

- smaller solid angle
- readout time ≥ 1 sec

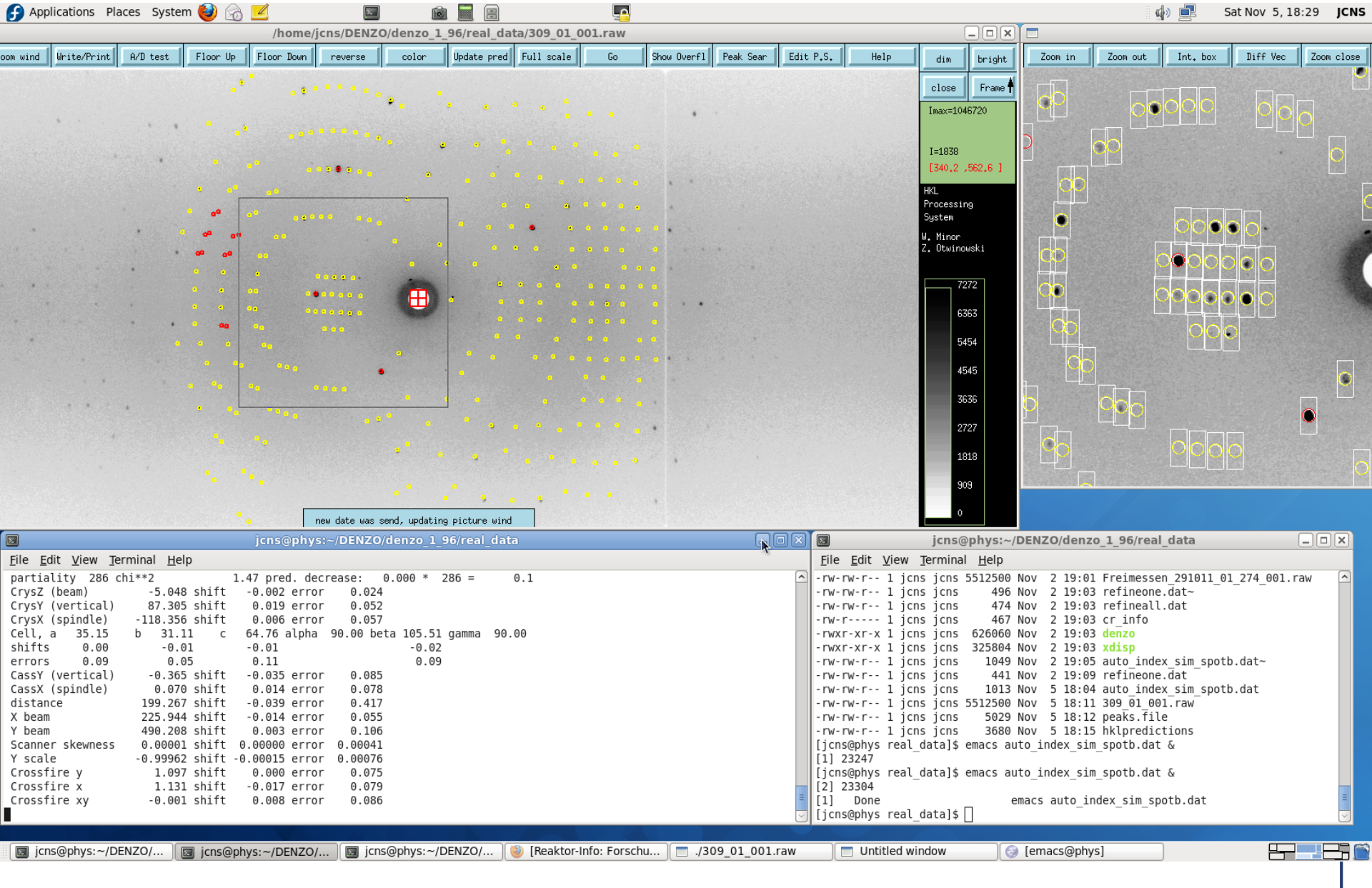
Peak search with hkl DENZO (now we use HKL2000)



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-r--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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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
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Zoom in
Zoom out
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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
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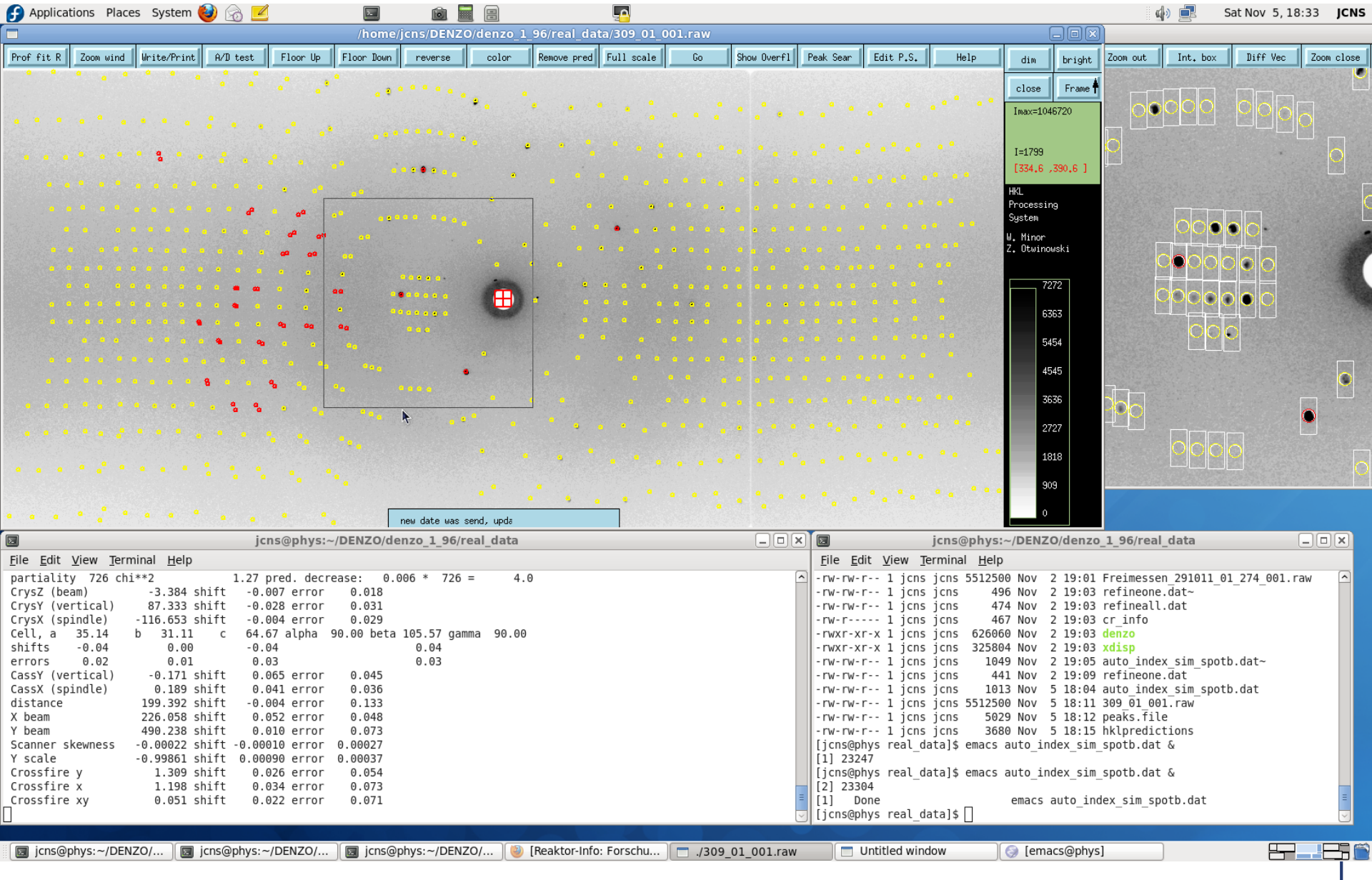
-rw-rw-r-- 1 jcns jcns 1049 Nov 2 19:05 auto_index_sim_spotb.dat~
-rw-rw-r-- 1 jcns jcns 441 Nov 2 19:09 refineone.dat
-rw-rw-r-- 1 jcns jcns 1013 Nov 5 18:04 auto_index_sim_spotb.dat
-rw-rw-r-- 1 jcns jcns 5512500 Nov 5 18:11 309_01_001.raw
-rw-rw-r-- 1 jcns jcns 5029 Nov 5 18:12 peaks.file
-rw-rw-r-- 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]

d_min=1.5 Å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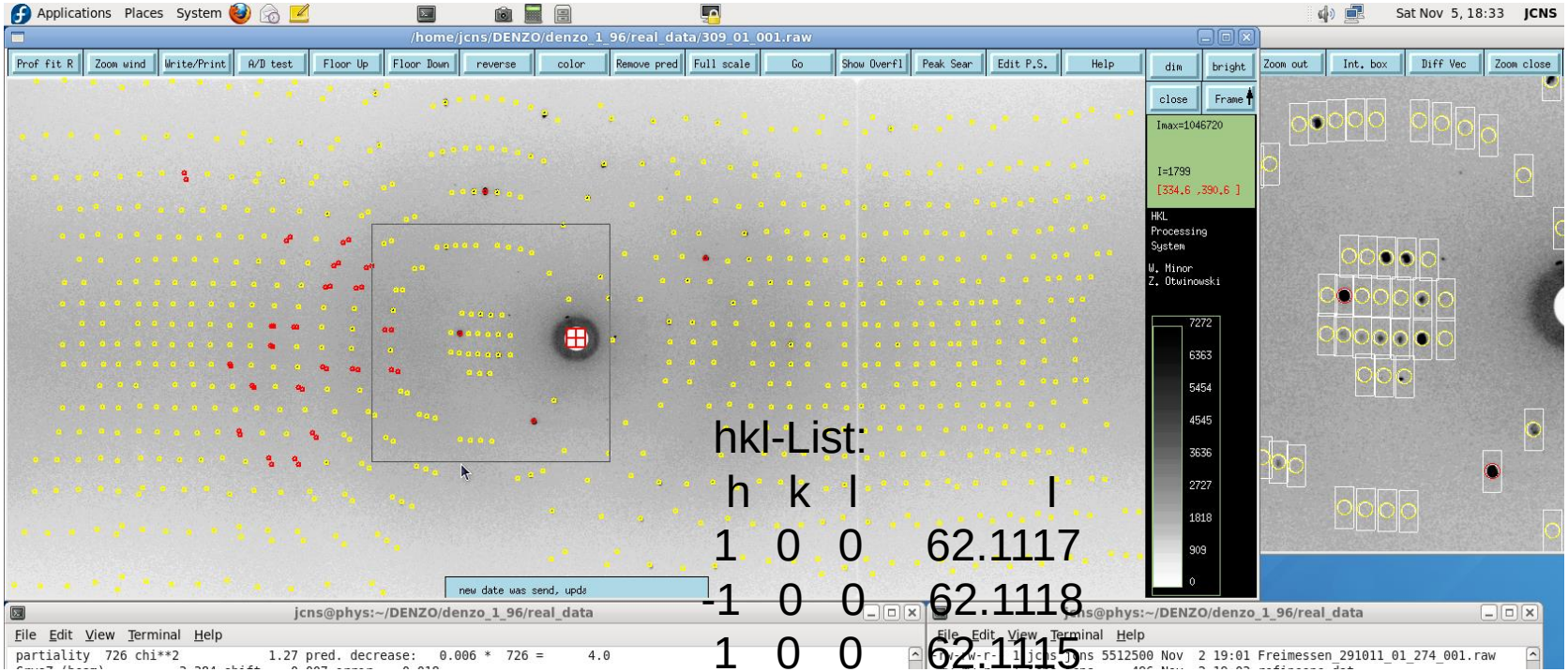
/home/jcns/DENZO/denzo_1_96/real_data/309_01_001.raw
Prof fit R
Zoom wind
Write/Print
A/D test
Floor Up
Floor Down
reverse
color
Remove pred
Full scale
Go
Show Overfl
Peak Sear
Edit P.S.
Help
dim
bright
close
Frame
Imax=1046720
I=1799
[334.6 ,390.6]
HKL
Processing
System
W. Minor
Z. Otwinowski
7272
6363
5454
4545
3636
2727
1818
909
0
Zoom out
Int. box
Diff Vec
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
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
-r--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



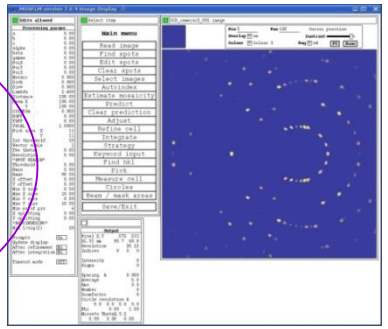
hkl-List:

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

Flow chart of data treatment and model building

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



-MOSFLM
-HKL-denzo
- HKL2000
(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

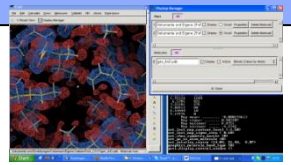
Structure refinement

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

Map-plotting

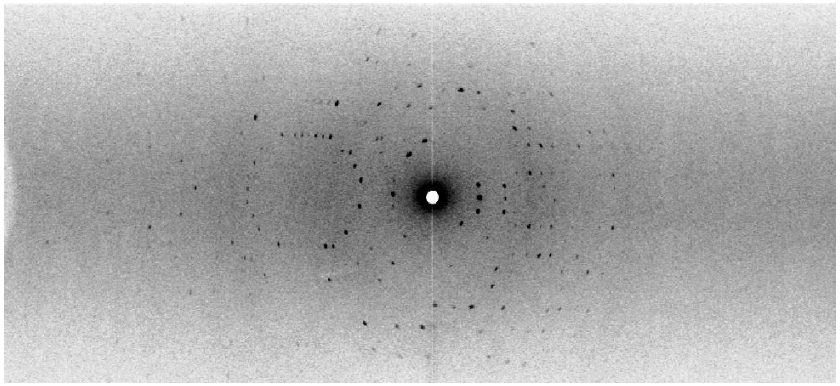
- inspection of model to fit the map)
- real space changes and refinement to the model

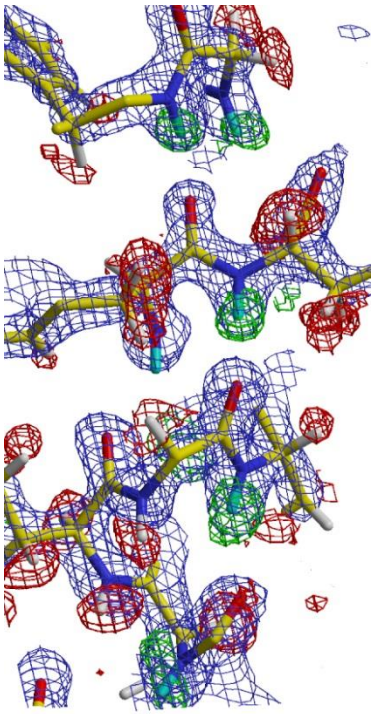
-nCNS
-PHENIX



-XtalView
-Coot

The phase problem:





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

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: $I = \|F_{hkl}\|^2$

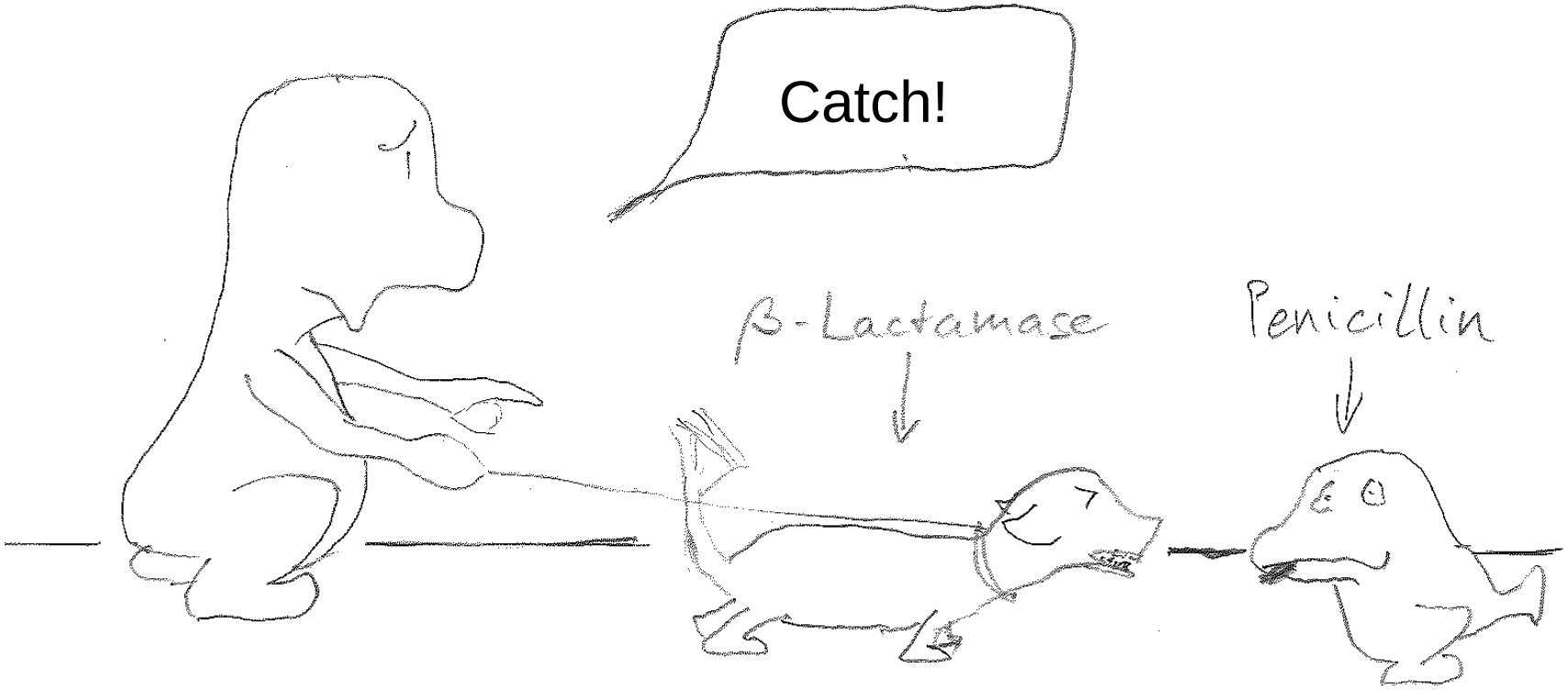
Neutron protein crystallography

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

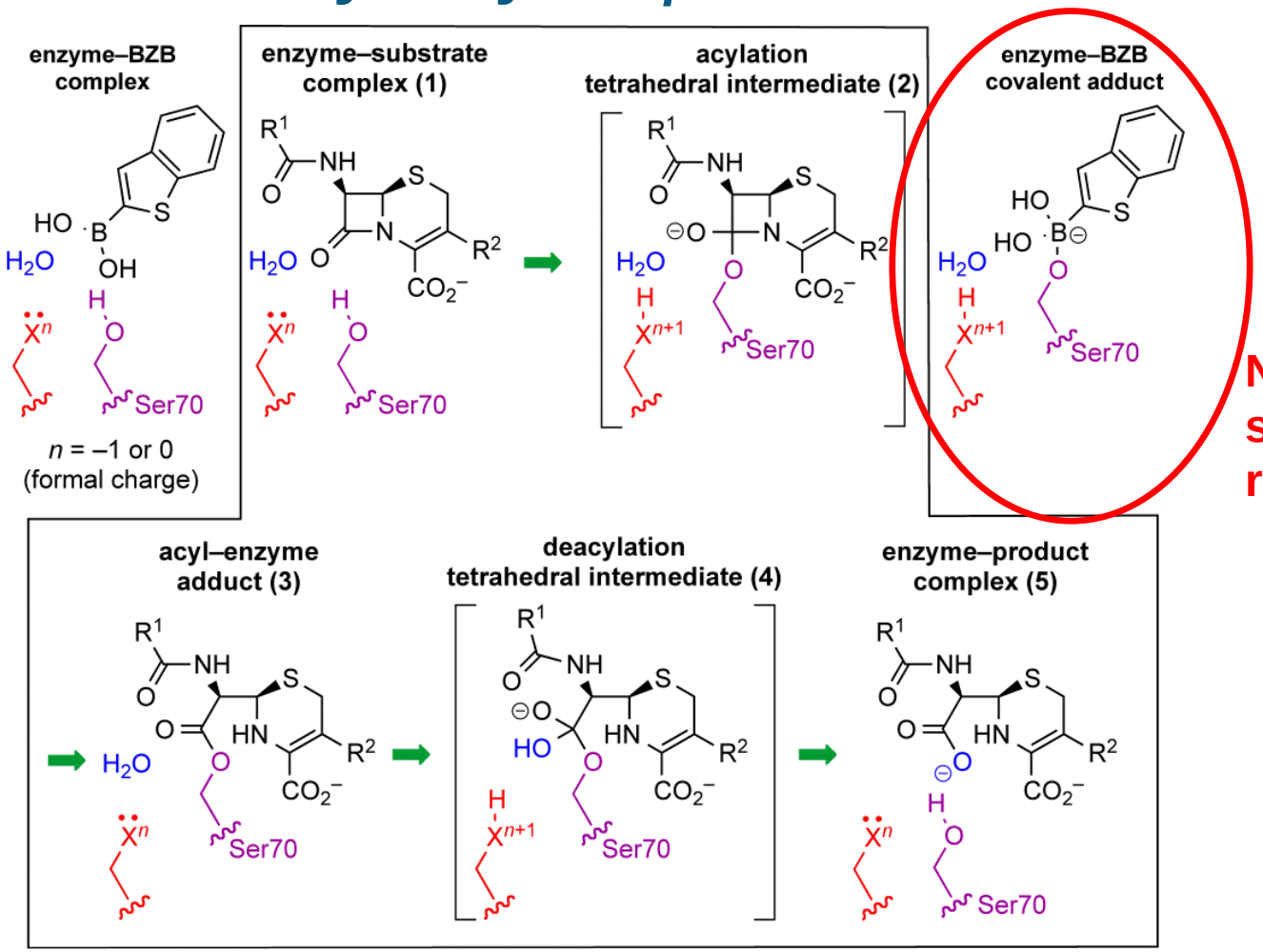
=> x-ray crystallography is a prerequisite of neutron protein crystallography.

Application Example I: Protonation state of amino acid residues

The protein β-lactamase



β-lactamase: hydrolyses β-lactam antibiotics

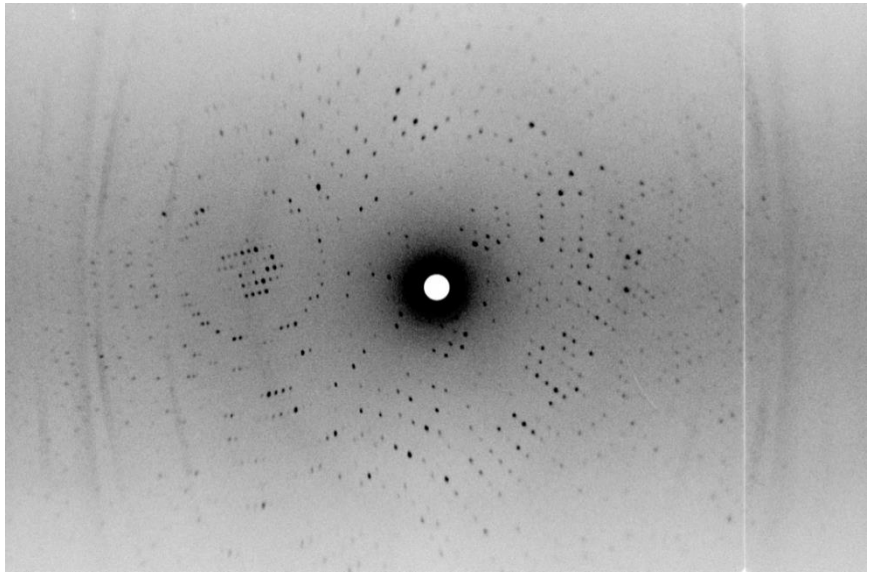


Neutron structure recorded

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



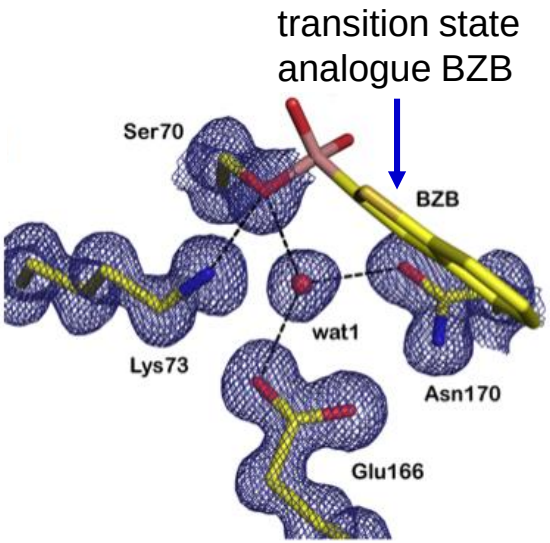
- unit cell: 73.4Å, 73.4Å, 99.1Å $P3_221$
- fully deuterated protein
- crystal size: 2.7mm^3
- Collection time: 9d

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**

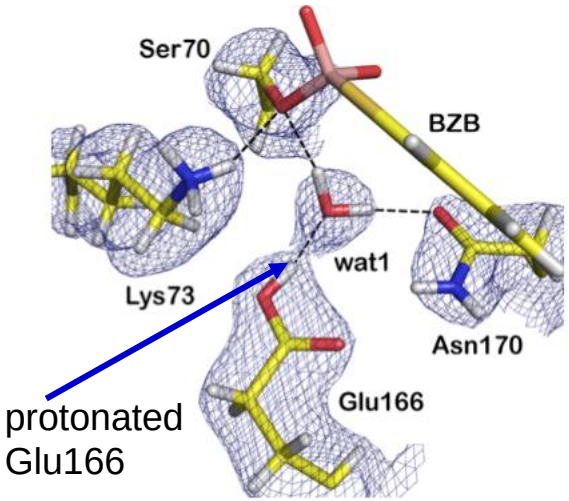
R_{pim} = 7.9% (17.9%)

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

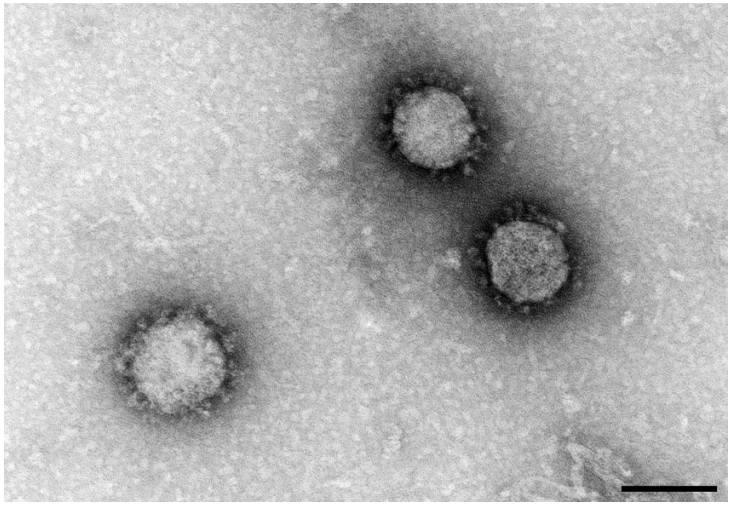
Glu166 acts as the general base during the catalytic action of the enzyme.

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

Application Example II: Research on Corona Virus proteins from SARS-COV-2 with x-ray and neutron crystallography

How does the virus look like?

The size is 60-160 nm



Scale bar = 100 nm,
Source: RKI web-site,
Source: *Hans R. Gelderblom, Freya Kaulbars/RKI*

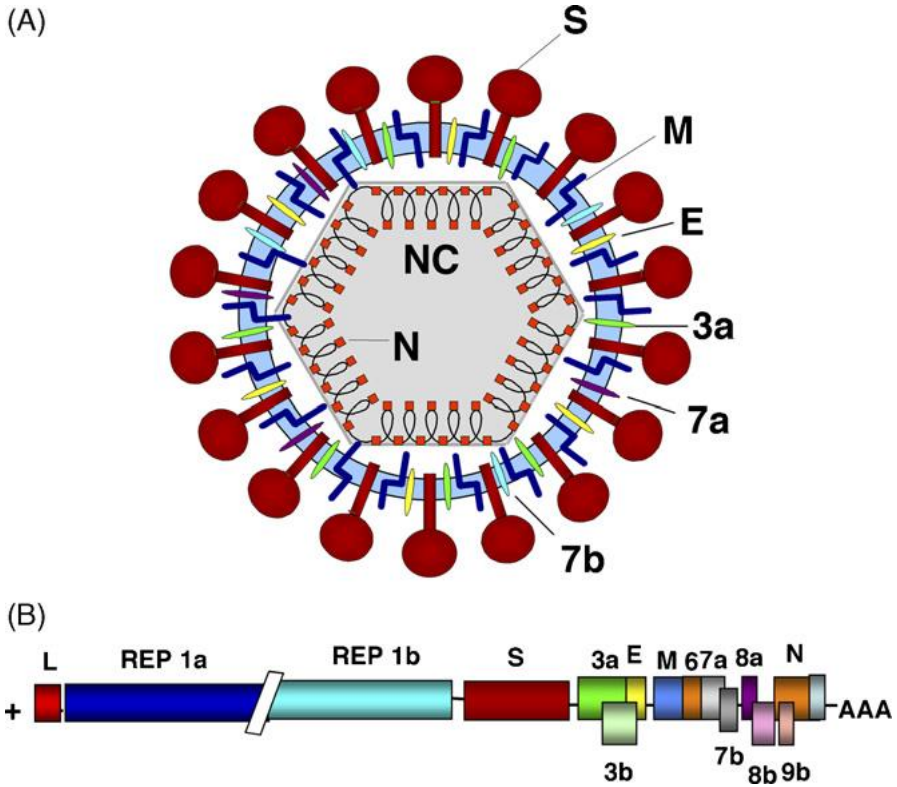
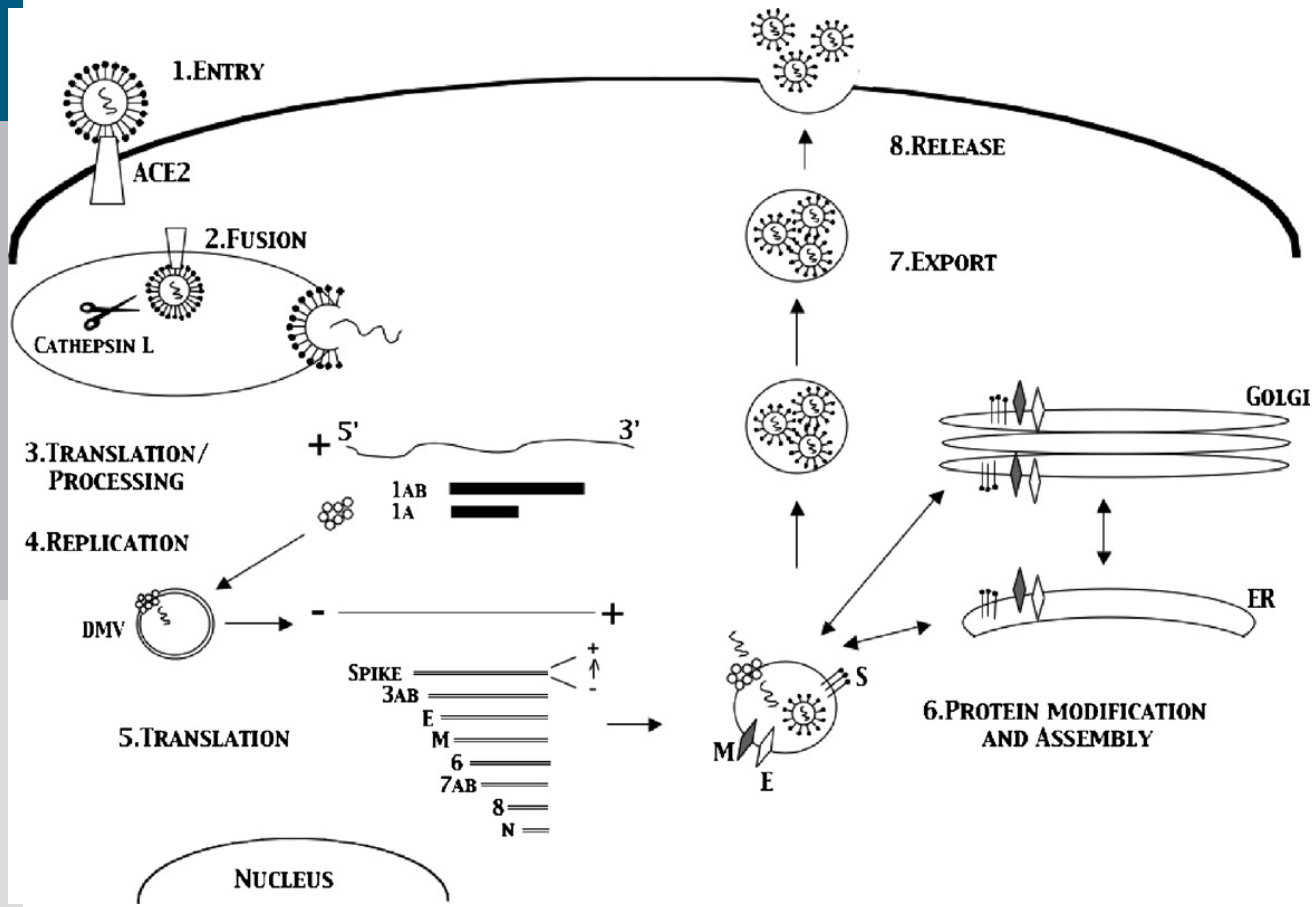


Fig. 2. Structure and genome organization of SARS-CoV. (A). Schematic diagram of SARS-CoV structure. S, spike protein; M, membrane protein; E, envelope protein; N, nucleoprotein; 3a, 7a, and 7b, structural proteins of SARSCoV. (B). Representation of a prototype SARS-CoV genome. Poly(A) tail is indicated by AAA. Numbers and letters indicate viral genes.: Virus Research 133 (2008) 45–62

How does it work?

It programms a human cell to produce replicas of it.



Virus Research 133 (2008) 101–112

Fig. 6. The coronavirus life cycle. Coronavirus entry is mediated by binding of S glycoprotein to the ACE2 receptor, cleavage by cathepsin L and activation of a fusion peptide in S2 that mediates entry via fusion through endocytic compartments [1]. Following fusion with the endosomal compartment the viral genome release into the cytosol where it is translated into the viral replicase proteins ORF1a and 1b [2]. These polyproteins are then cleaved by 2 proteases, Main Protease (Mpro) and Papain like protease, PLP, into the individual proteins necessary for replication [3]. Subgenomic RNA synthesis occurs from discontinuous transcription which joins leader RNA sequences encoded at the 5 end of the genome to the body sequences of each subgenomic RNA. The eight different subgenomic negative strands serve as template for the synthesis of like sized subgenomic mRNA [4]. Subgenomic RNAs are then translated into viral proteins which localize to their relevant compartments [5]. Assembly of virions occurs in an ERGIC like compartment in the cell. Here S, E,Mand N bound to genomic viral RNA are assembled into virions in vesicles [6]. The vesicles are then exported to the cell surface where fusion occurs with release of virions into the exterior environment [7,8].

The genome and the encoded proteins of SARS-CoV-2

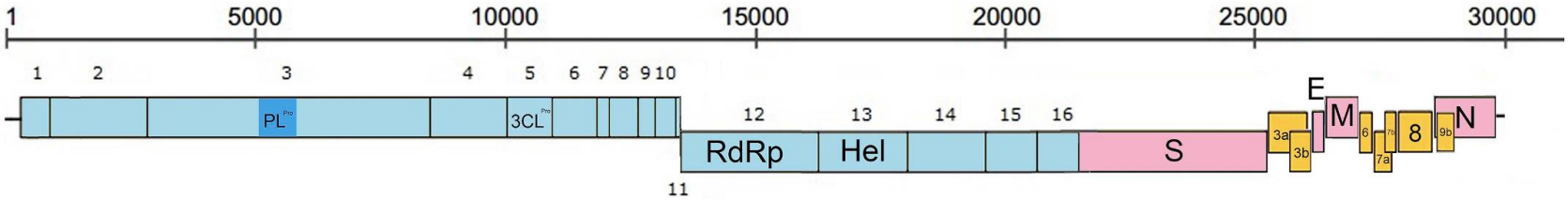


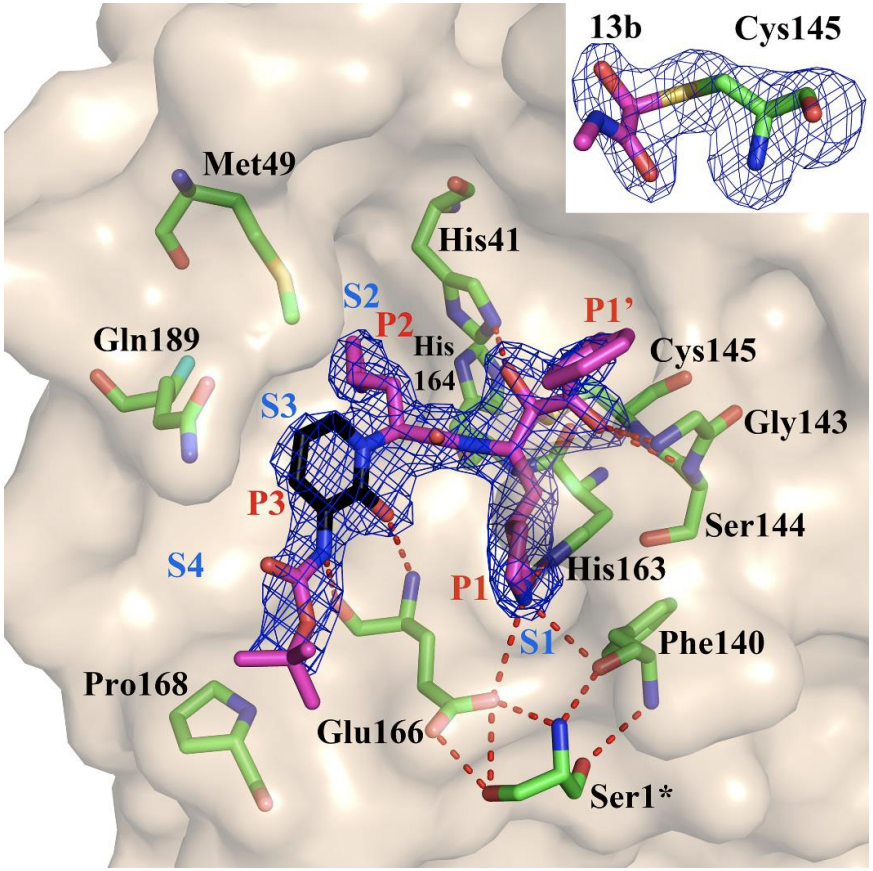
Fig. 1. Genome organization of SARS-CoV. ORF1ab with nsp1–16 are colored in blue. Structural proteins including S, E, M and N are in pink. Accessory proteins were
numbered and in yellow. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Main protease cleaves viral proteins to the right length: This is why it is an important drug target.

From: Infection, Genetics and Evolution 71 (2019) 21–30

The main protease of the corona virus

Here, an inhibitor is bound to the protein



L. Zhang *et al*., *Science* 10.1126/science.abb3405 (2020).

Fig. 3. Compound 13b in the substrate-binding cleft located between domains I and II of the Mpro, in the monoclinic crystal form (space group C2). Fo-Fc density is shown for the inhibitor (contouring level: 3σ). Carbon atoms of the inhibitor are magenta, except in the pyridone ring, which is black; oxygen atoms are red, nitrogens blue, and sulfur yellow. Light-blue symbols S1, S2, S3, S4 indicate the canonical binding pockets for moieties P1, P2, P3, P4 (red symbols) of the peptidomimetic inhibitor. Hydrogen bonds are indicated by dashed red lines. Note the interaction between the N-terminal residue of chain B, Ser1*, and Glu166 of chain A, which is essential for keeping the S1 pocket in the right shape and the enzyme in the active conformation. Inset: Thiohemiketal formed by the nucleophilic attack of the catalytic cysteine onto the α-carbon of the inhibitor in its Fo-Fc density (contoured at 3σ). The stereochemistry of the α-carbon is *S*. See fig. S8 for more details.

The first neutron structure solved for the main protease in its apo state (empty binding pocket)

A: X-ray data

B: neutron data

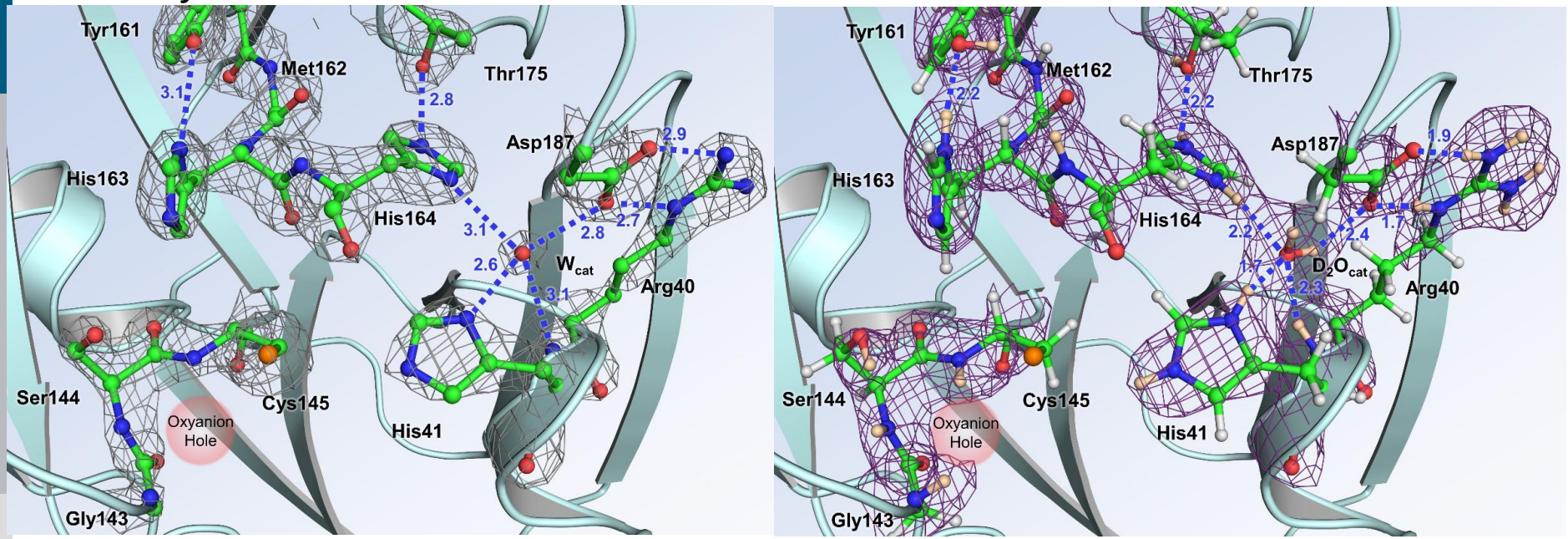


Figure 3. The catalytic site of SARS-CoV-2 3CL Mpro. (**A**) The 2FO-FC electron density map contoured at 2.0 σ level (grey mesh) with no hydrogen atoms visible. Distances between the heavy atoms in Ångstroms illustrate possible hydrogen bonds. (**B**) The 2FO-FC nuclear density map contoured at 2.0 σ level (violet mesh), allowing visualization of the actual protonation states and hydrogen bonding interactions (D…O distances are shown in Ångstroms).

From: https://www.biorxiv.org/content/10.1101/2020.09.22.308668v1, data measured at MANDI and IMAGINE at Oak Ridge National Lab, Oak Ridge, Tennessee, USA

His41: doubly protonated
Cys145: deprotonated

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
- Marialucia Longo
- Livia Balacescu
- Zamaan Raza
- Tobias Weber
- Jonathan Fisher
- Leighton Coates
- Andrey Kovalevsky
- Stephan Förster

and you for your attention!

New Home source X-ray diffractometer at the MLZ in Garching

The XtaLAB Synergy-S gives great data, fast.Whether dataquality or highthroughput is your focus, theXtaLAB Synergy-S is designed to meet your needs.

Benefits include:

- Extremely high performance PhotonJet-S sources
- A robust reliable hardware platform and goniometer that just keeps on going
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Features:

Cu and Mo Source, HiPix Arc detector covering 150° of 2 θ range

The end