Drug development using information from Neutron protein crystallography

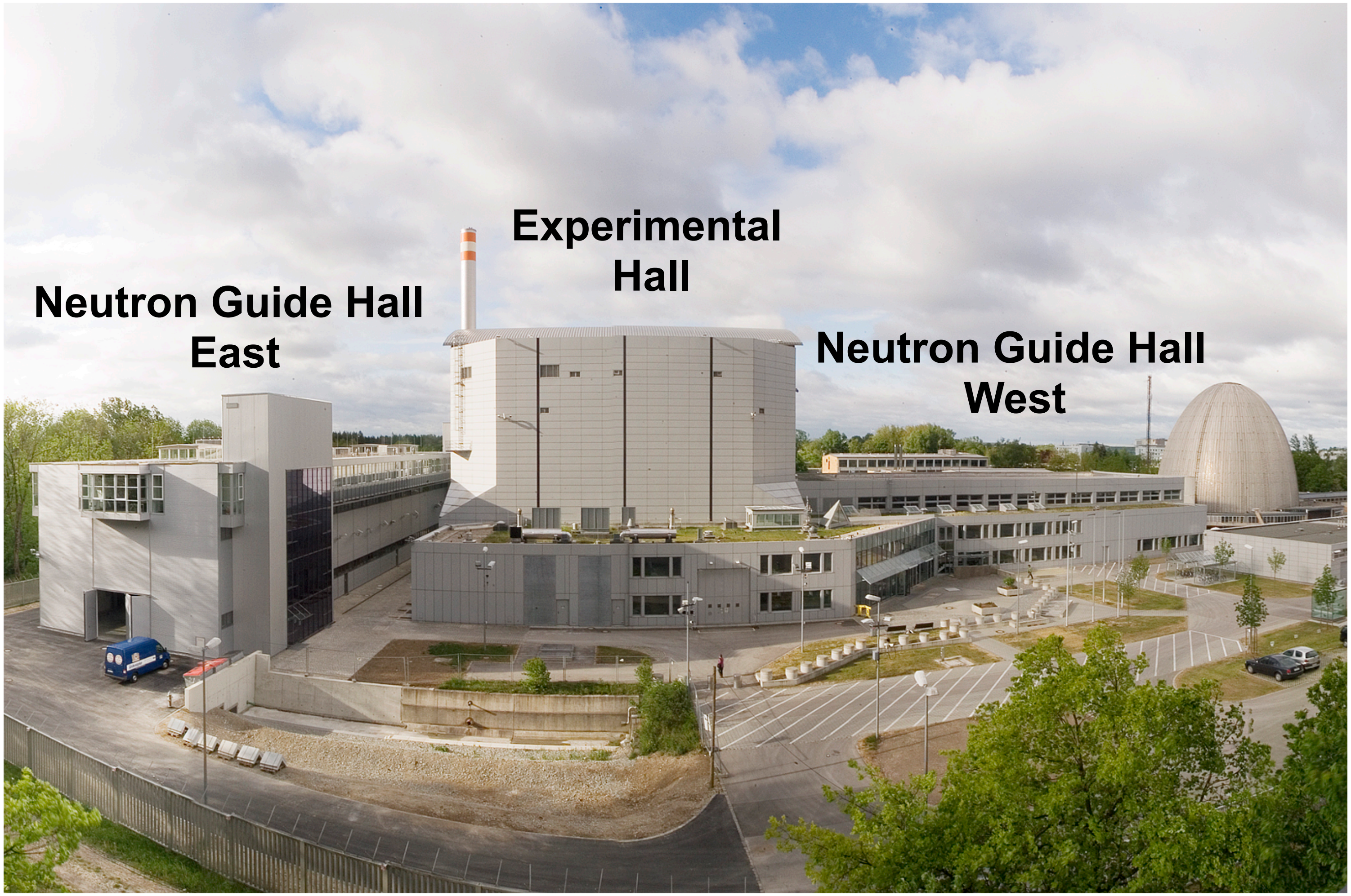
Tobias E. Schrader

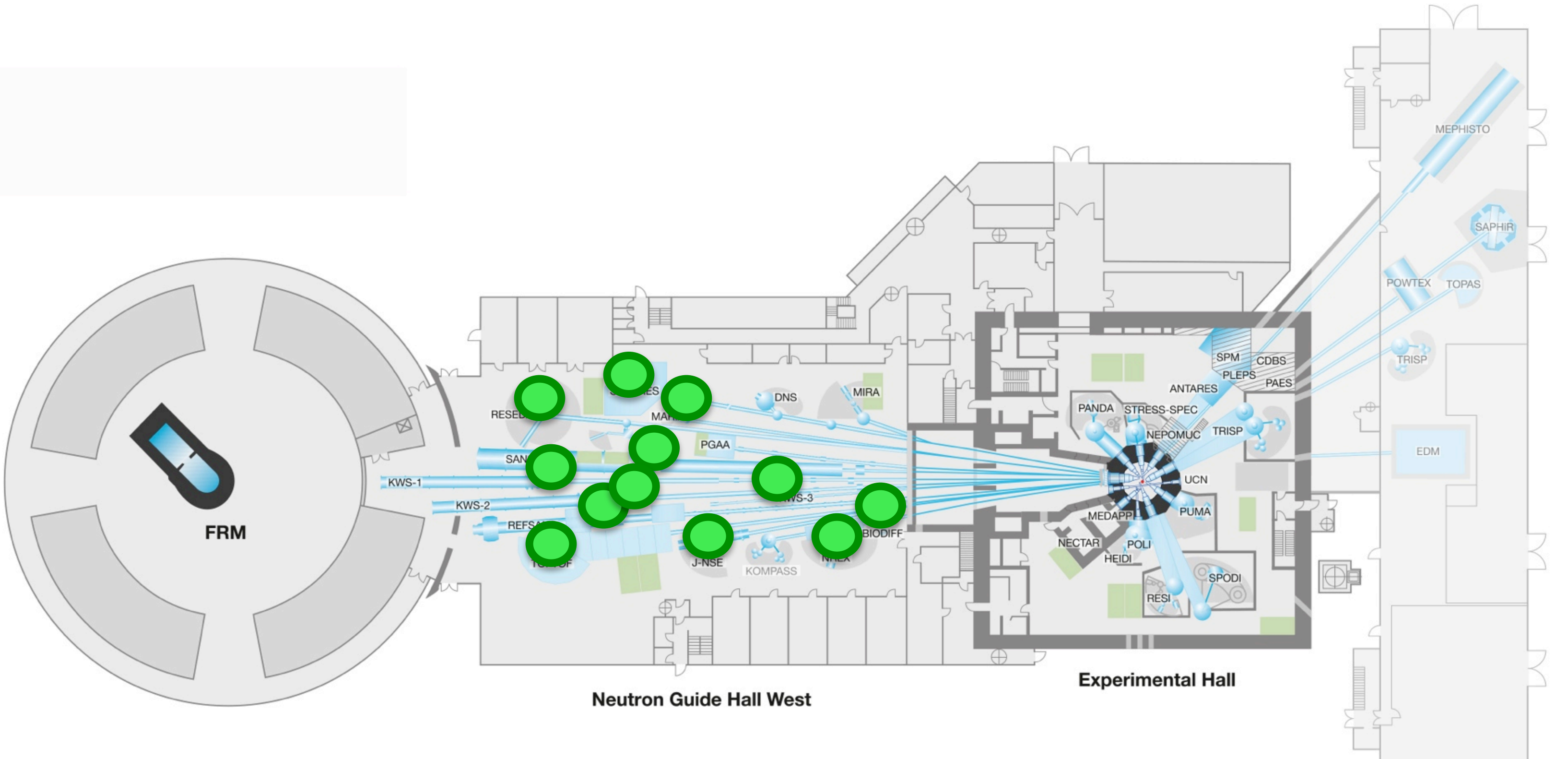
MLZ is a cooperation between:

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)

Research Neutron Source Heinz Maier-Leibnitz (FRM II)

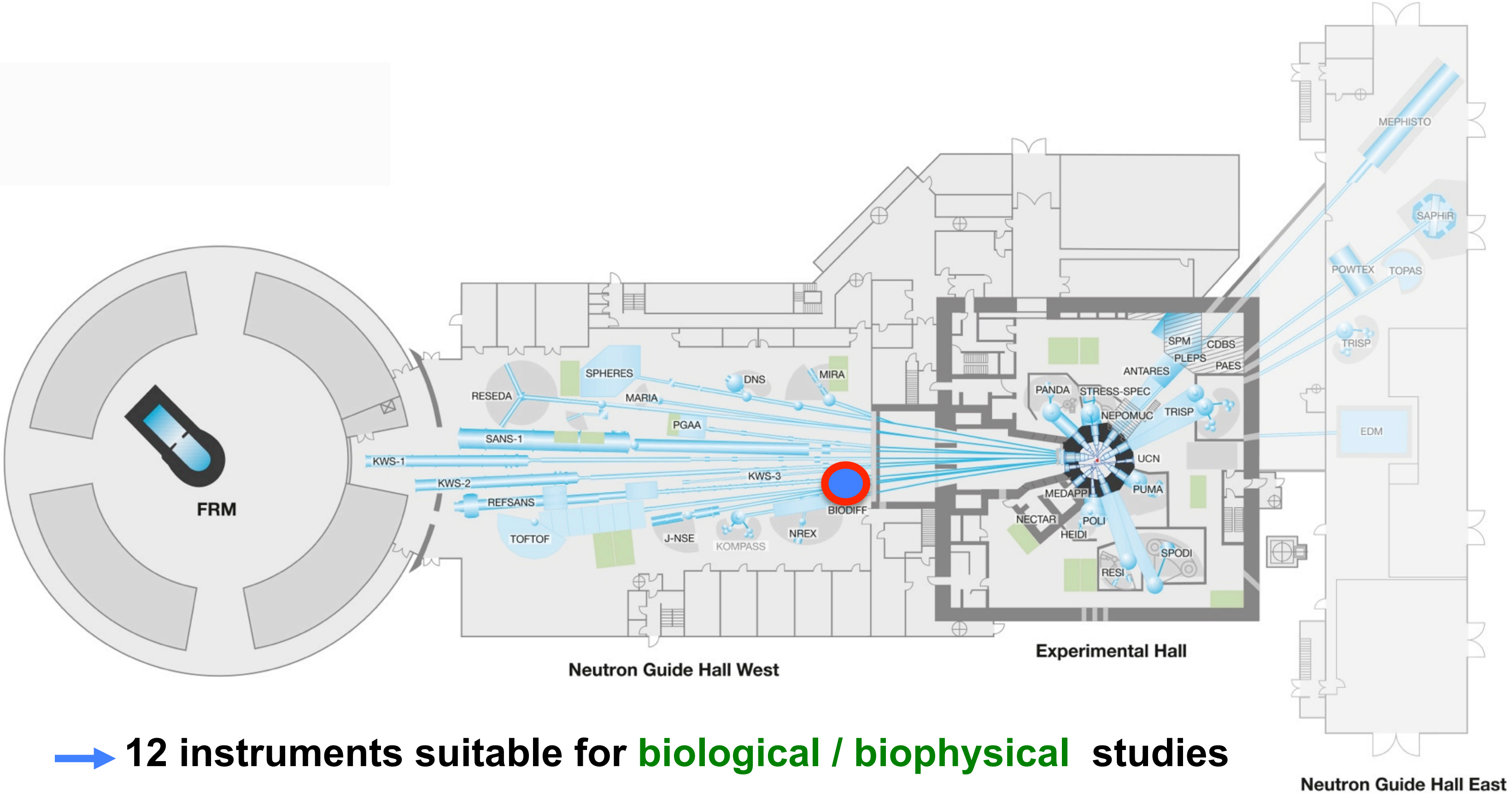




12 instruments suitable for biological / biophysical studies

- 4 spectrometer: TOF, backscattering, spin echo (2);
- 3 reflectometers;
- 4 small angle scattering machines;
- 1 diffractometer for macromolecular diffraction

+ irradiation and tomography facilities…



→ **12 instruments suitable for biological / biophysical studies**

→ 1 diffractometer for macromolecular diffraction

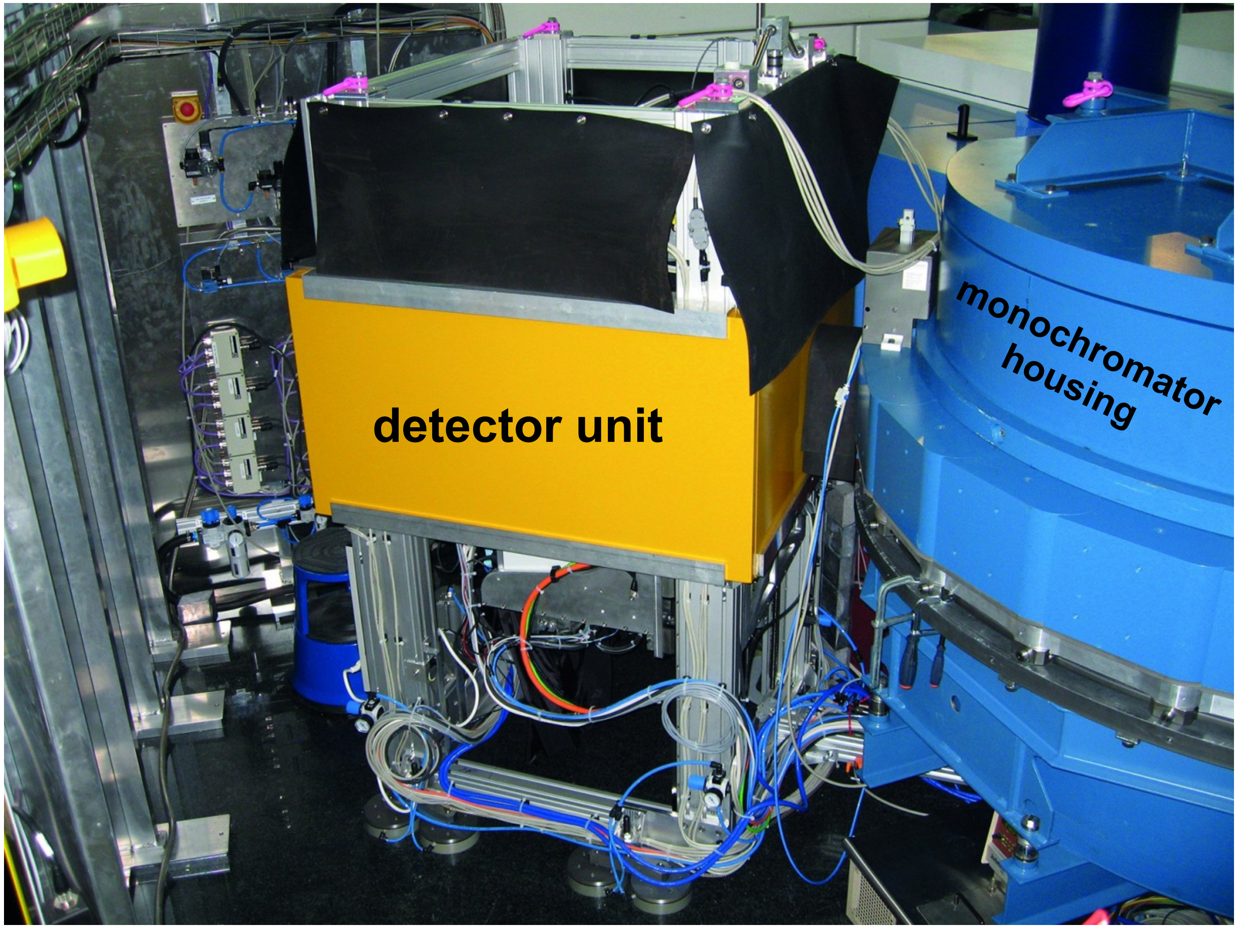
BIODIFF: Diffractometer for macromolecular neutron crystallography

operated by

Technische Universität München

Jülich Centre for Neutron Science

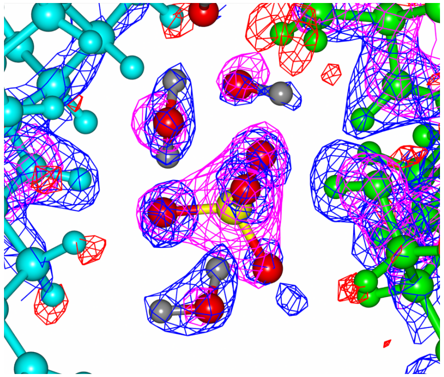
in operation
since April 2012



Scientific questions to be adressed:

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



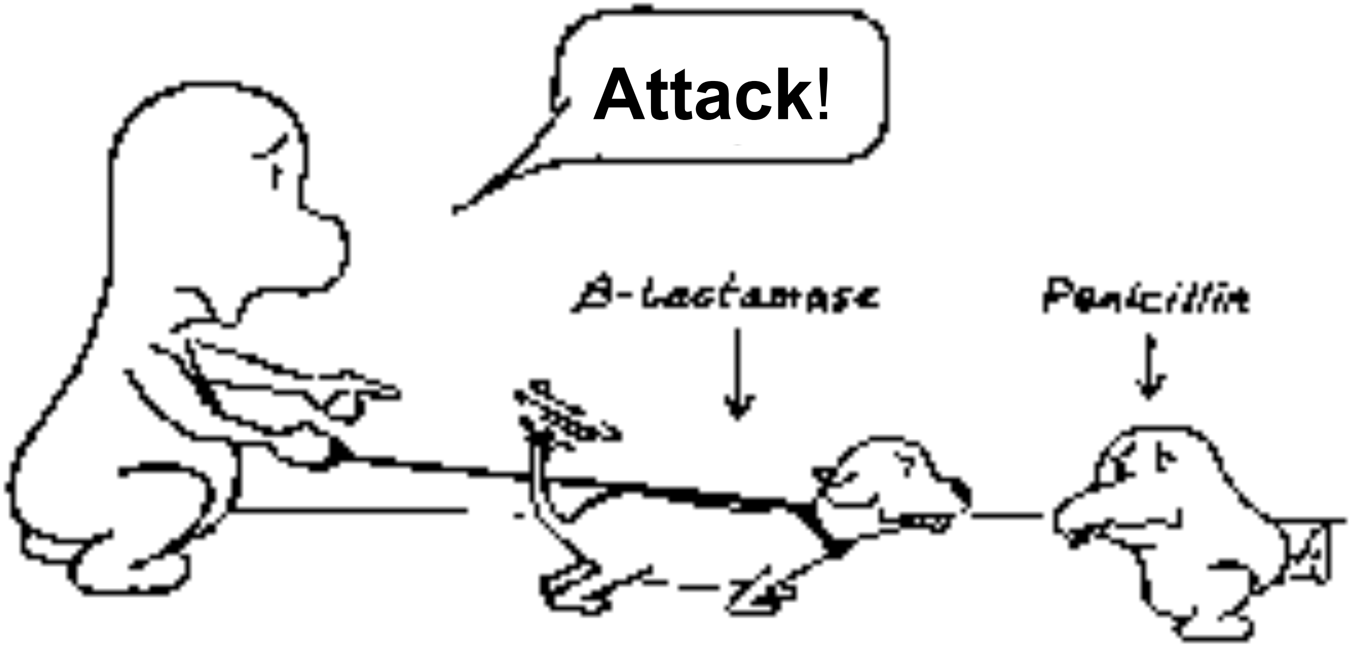
Water network in the contact region between two myoglobin molecules in the crystal.

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

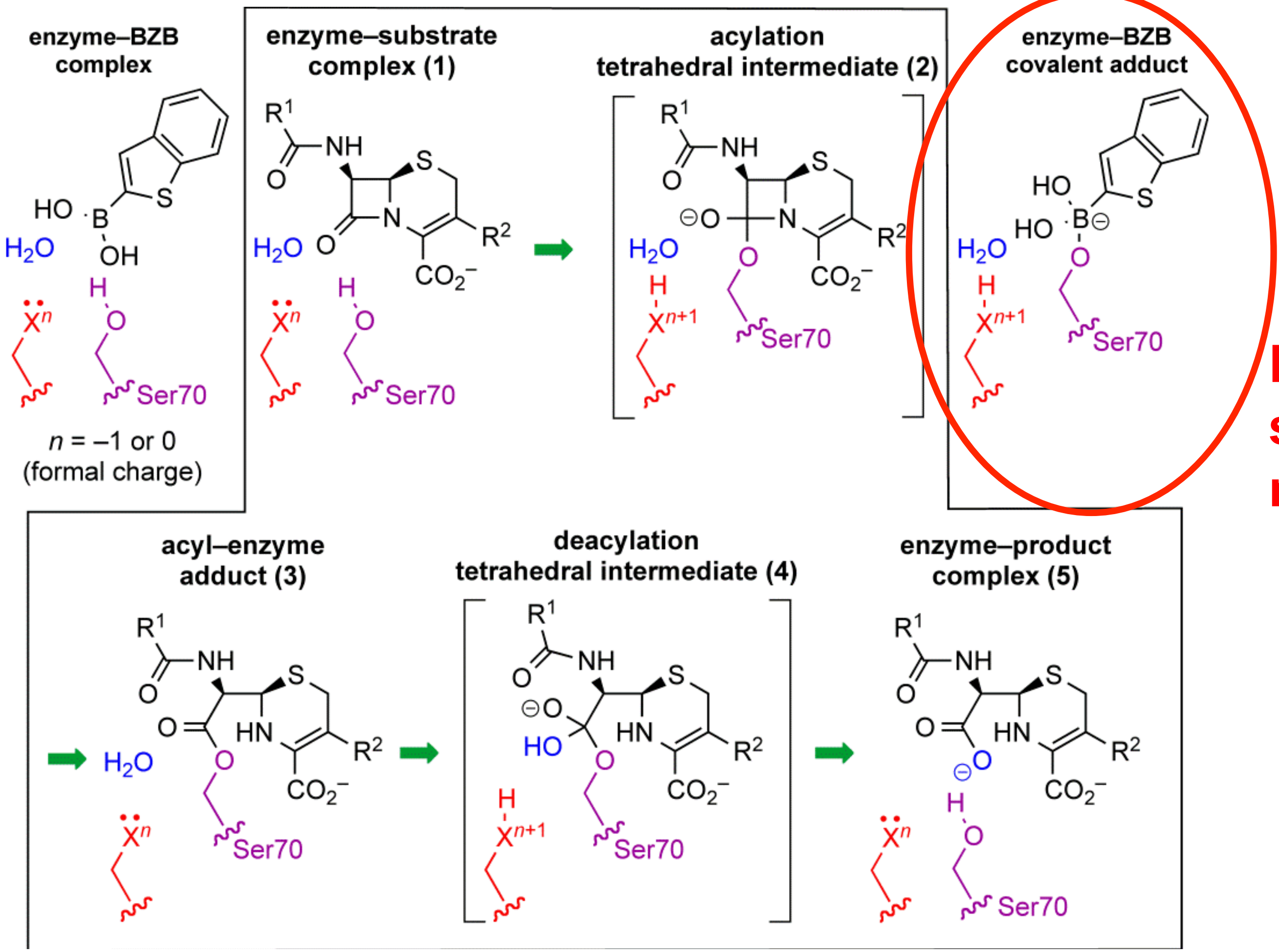
Much less radiation damage as compared to x-rays: Metallo-proteins can be measured without reducing the metal centres

Application examples: Protonation state of amino acid residues

The protein β-lactamase



β-lactamase: hydrolyses β-lactam antibiotics

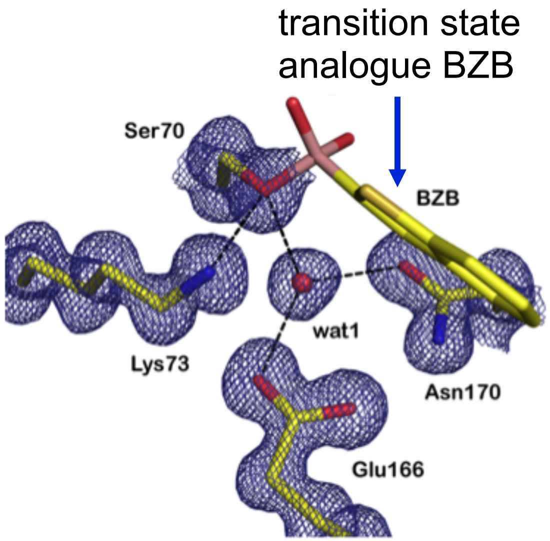


Neutron structure recorded

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.

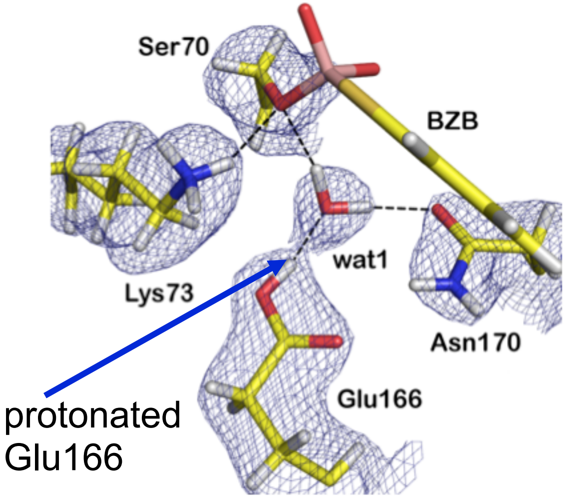
Figures from: Tomanicek et al., doi: 10.1074/jbc.M112.436238

Catalytic Proton Network of the Toho-1 β-Lactamase



transition state analogue BZB

electron density map



protonated Glu166

nuclear density map from BioDiff

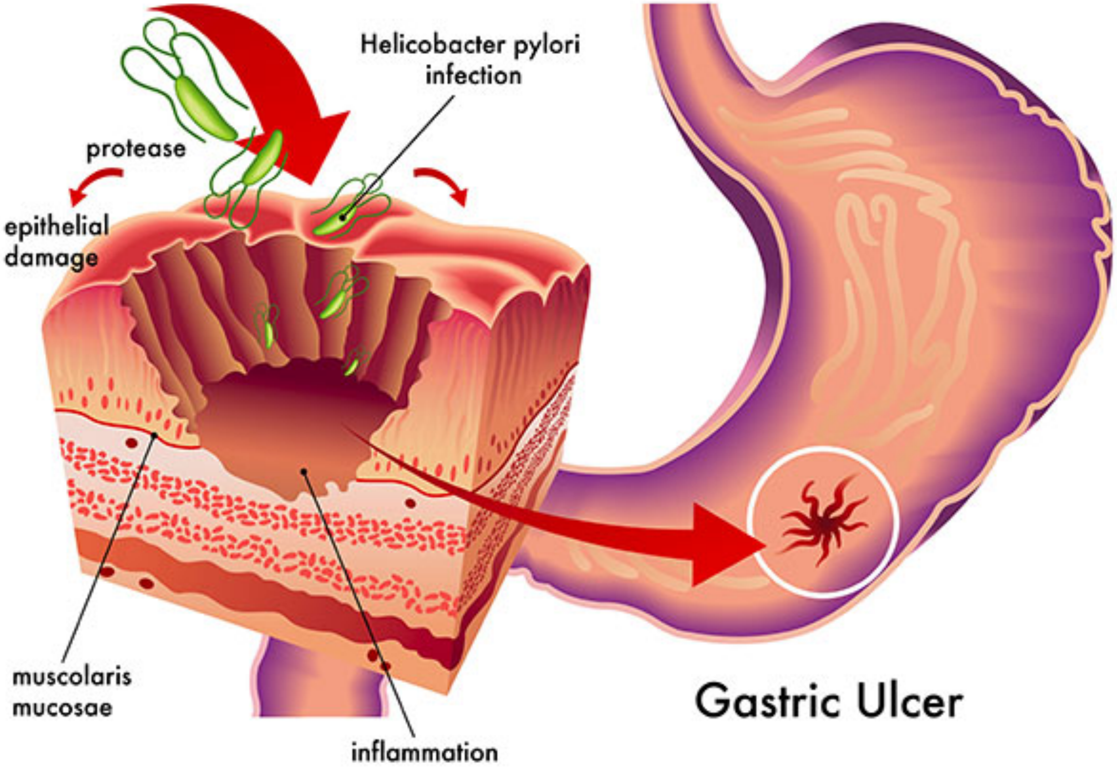
Glu166 acts as the general base during the catalytic action of the enzyme.

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

Yet another drug target example

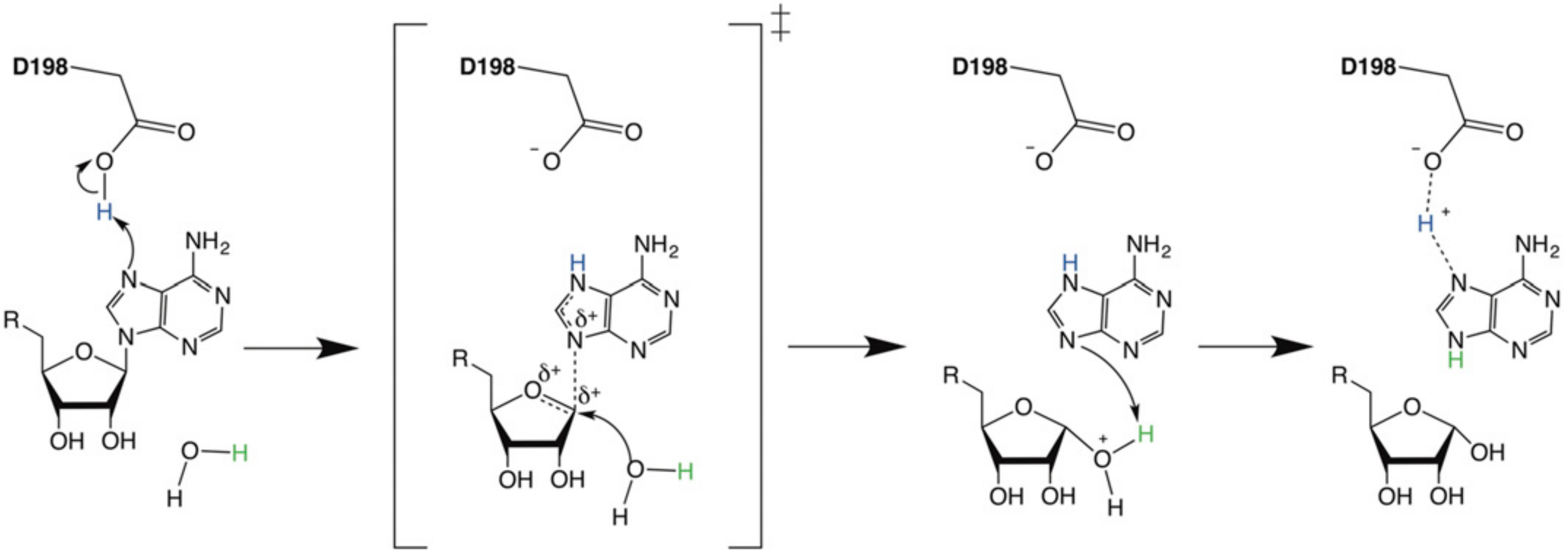
Helicobacter Pylori Infection Makes Your Stomach Wall Thinner:

Too much drinking and eating can easily stress the stomach's wall and irritate it. In this case, the bacterium **Helicobacter Pylori** is capable of breaking the surface cells of the stomach and making the mucous membrane even thinner. This may lead to stomach cancer and gastric ulcer.



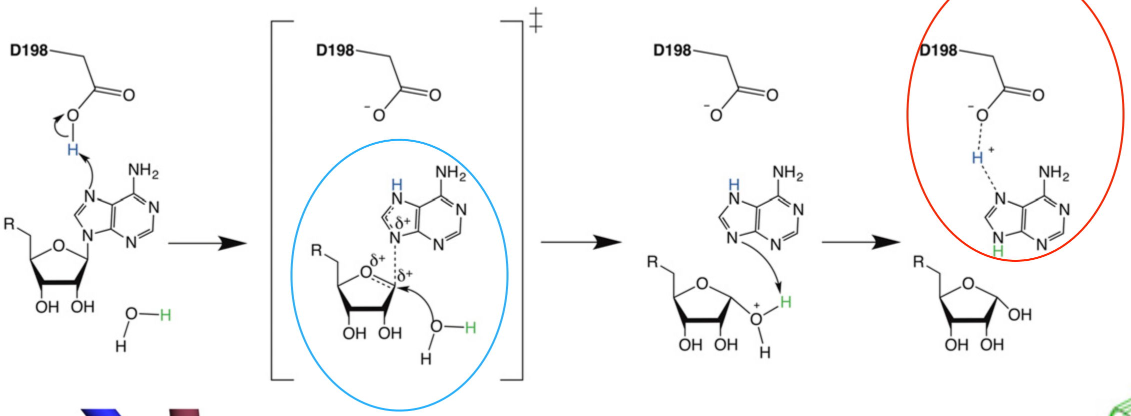
Proteins as drug target: Example of MTAN

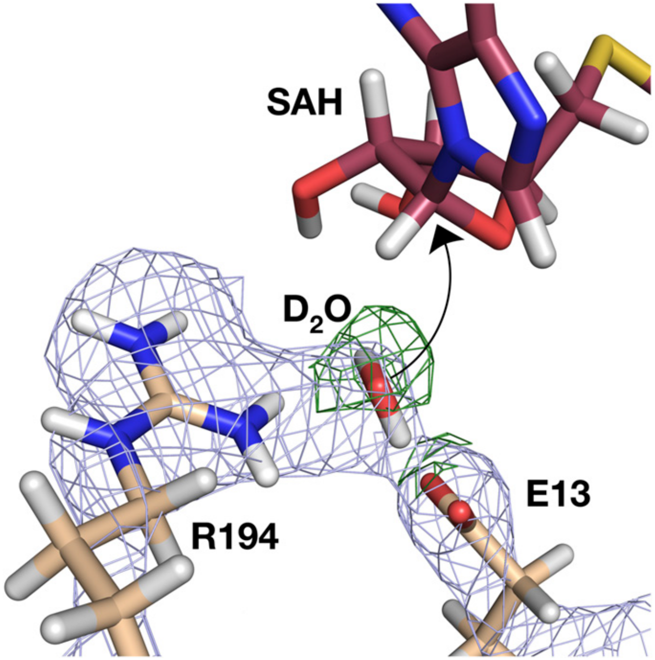
H. pylori 5′-methylthioadenosine nucleosidase (HpMTAN) is an interesting drug target because of its vital role in the production of menaquinone (vitamin K) for the bacterium Helicobacter pylori. It does not play a role in homen beeings.

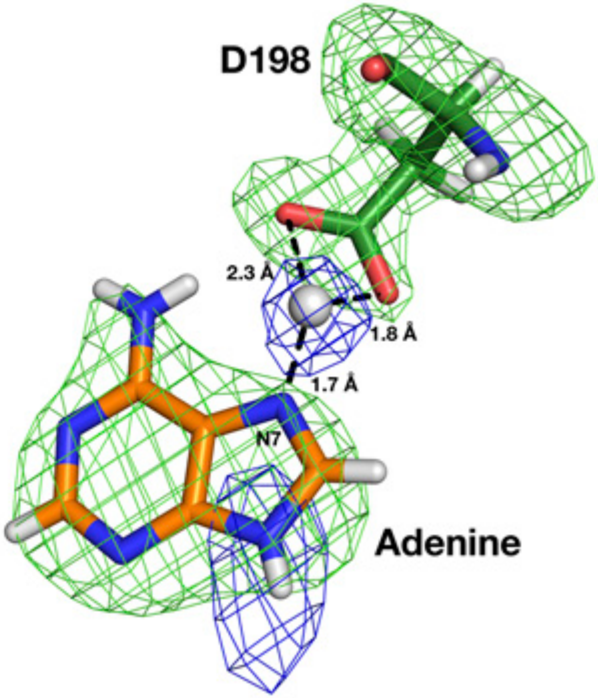


Banco et al. PNAS (2016) 113, pp.13756–13761

The neutron contribution



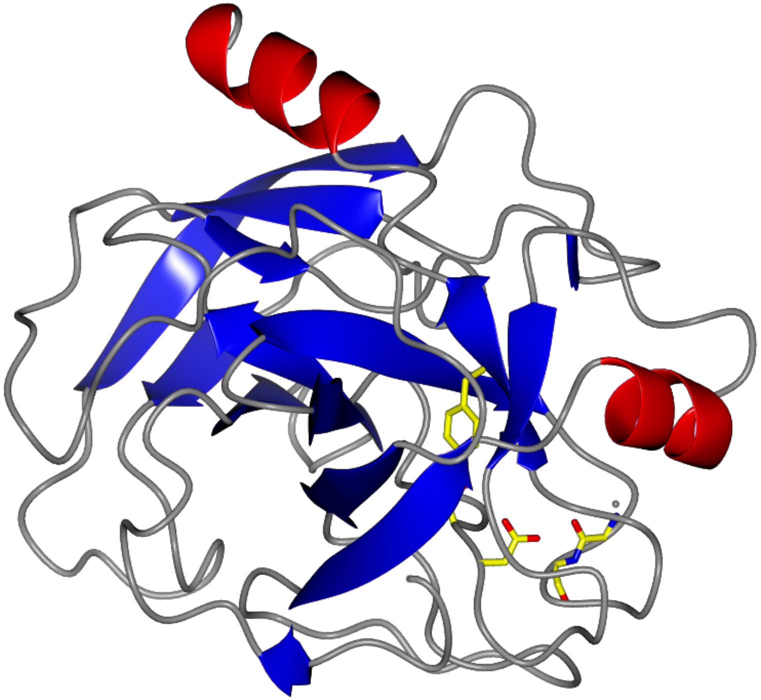




Banco et al. PNAS (2016) 113, pp.13756–13761

Inhibitor binding to Trypsin

Inhibitor binding to trypsin: charges shift protonation



- ➡ trypsin as model system for the family of serine proteases
- ➡ cleaves peptidic substrates that contain basic amino acids such as arginine or lysine

Group of Prof. G. Klebe (Univ. Marburg)

Schiebel J. et al., Angewandte Chemie Int. Edition (2017), 56 (17):4887-4890

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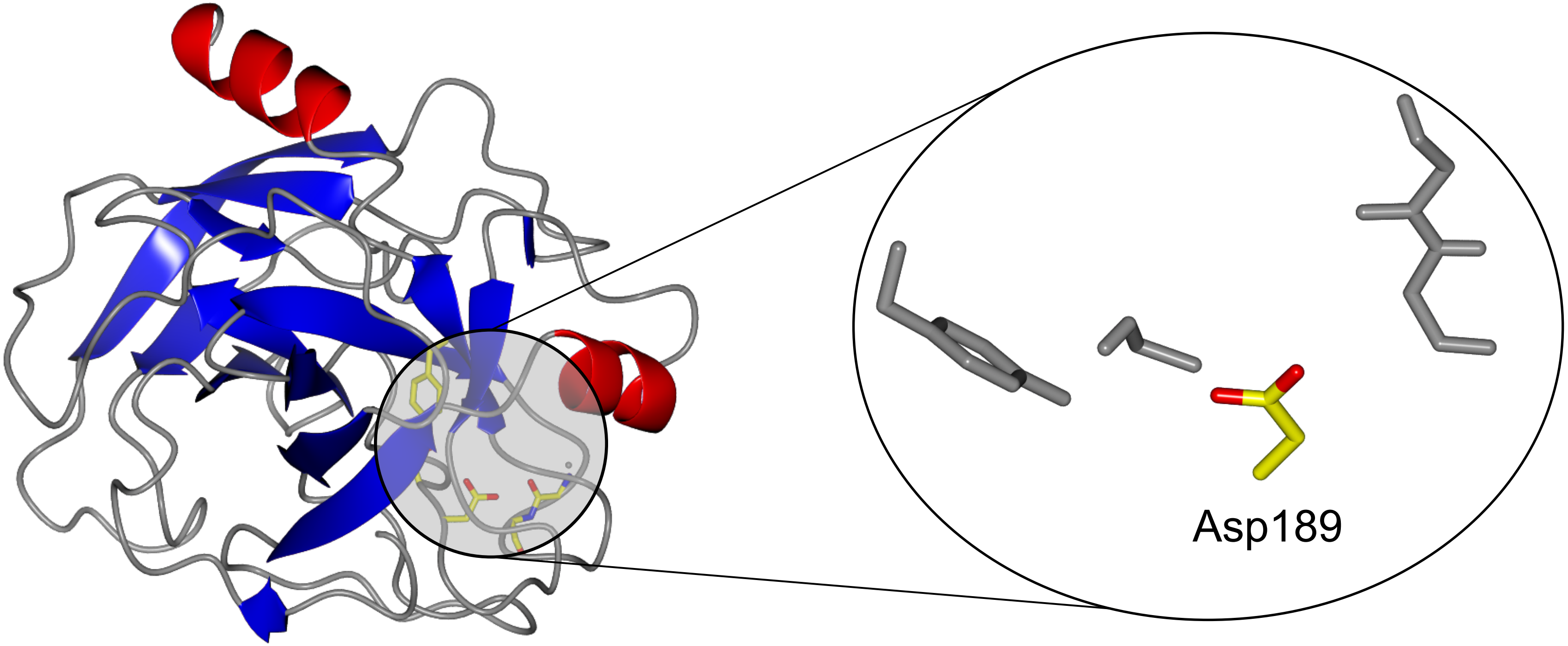

- Asp189 is responsible for the substrate specificity

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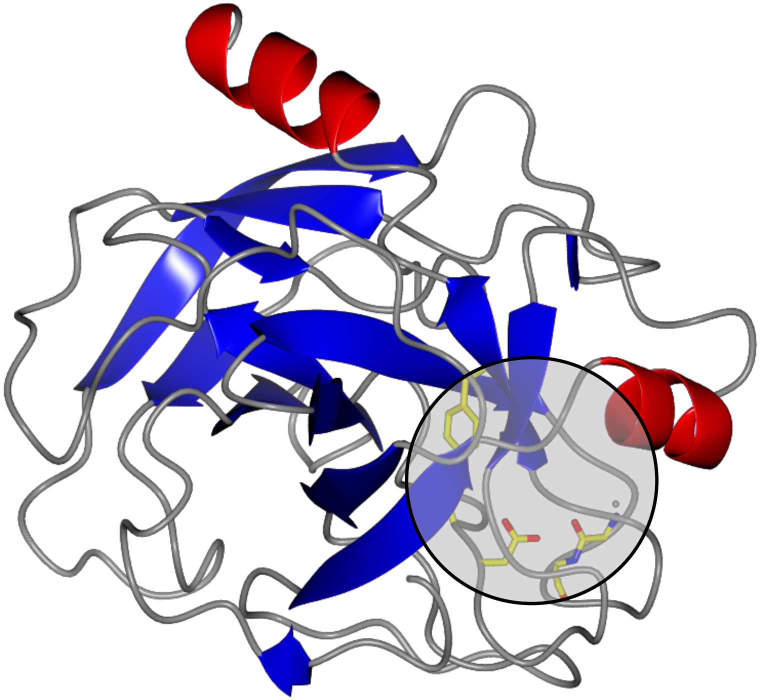
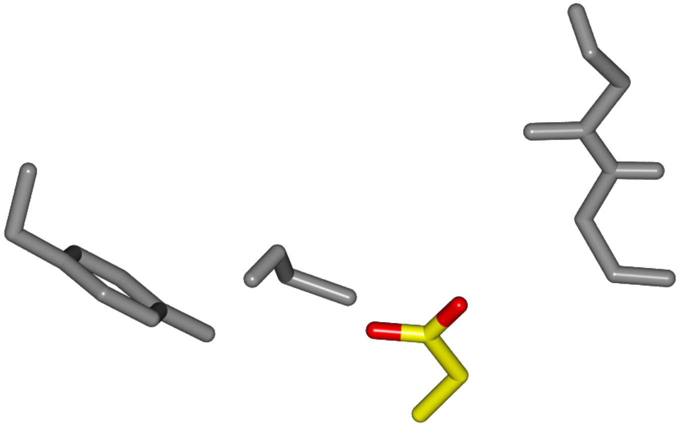
Schiebel J. et al., Angewandte Chemie Int. Edition (2017), 56 (17):4887-4890

Slides courtesy of Andreas Ostermann

Inhibitor binding to trypsin: charges shift protonation





➡ question: Do inhibitors with less basic properties become protonated upon binding ?

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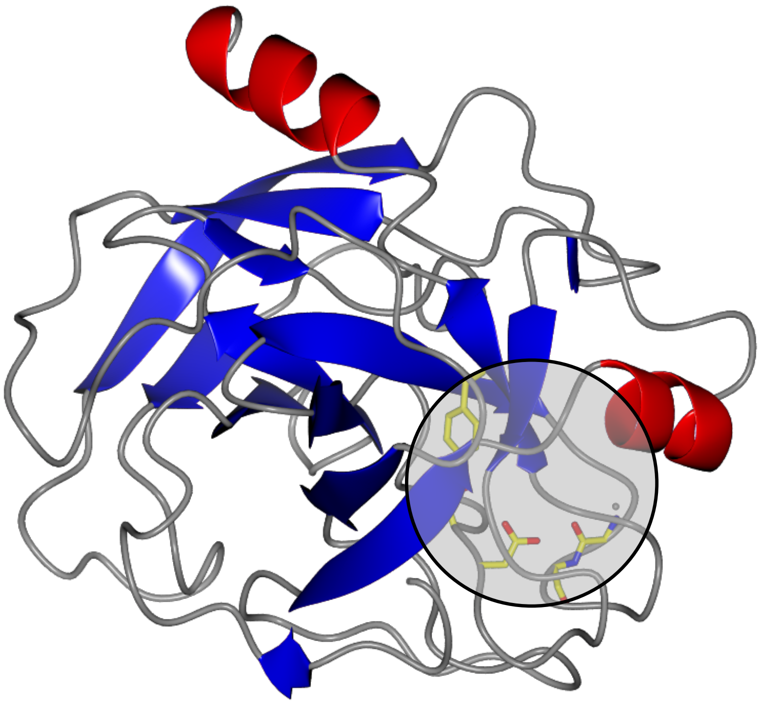
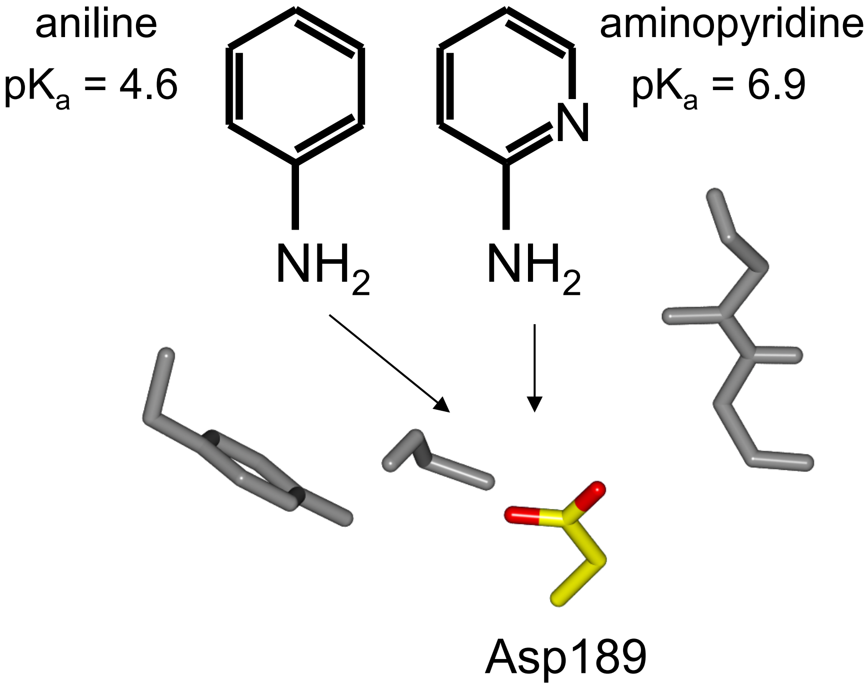
Schiebel J. et al., Angewandte Chemie Int. Edition (2017), 56 (17):4887-4890

Inhibitor binding to trypsin: charges shift protonation





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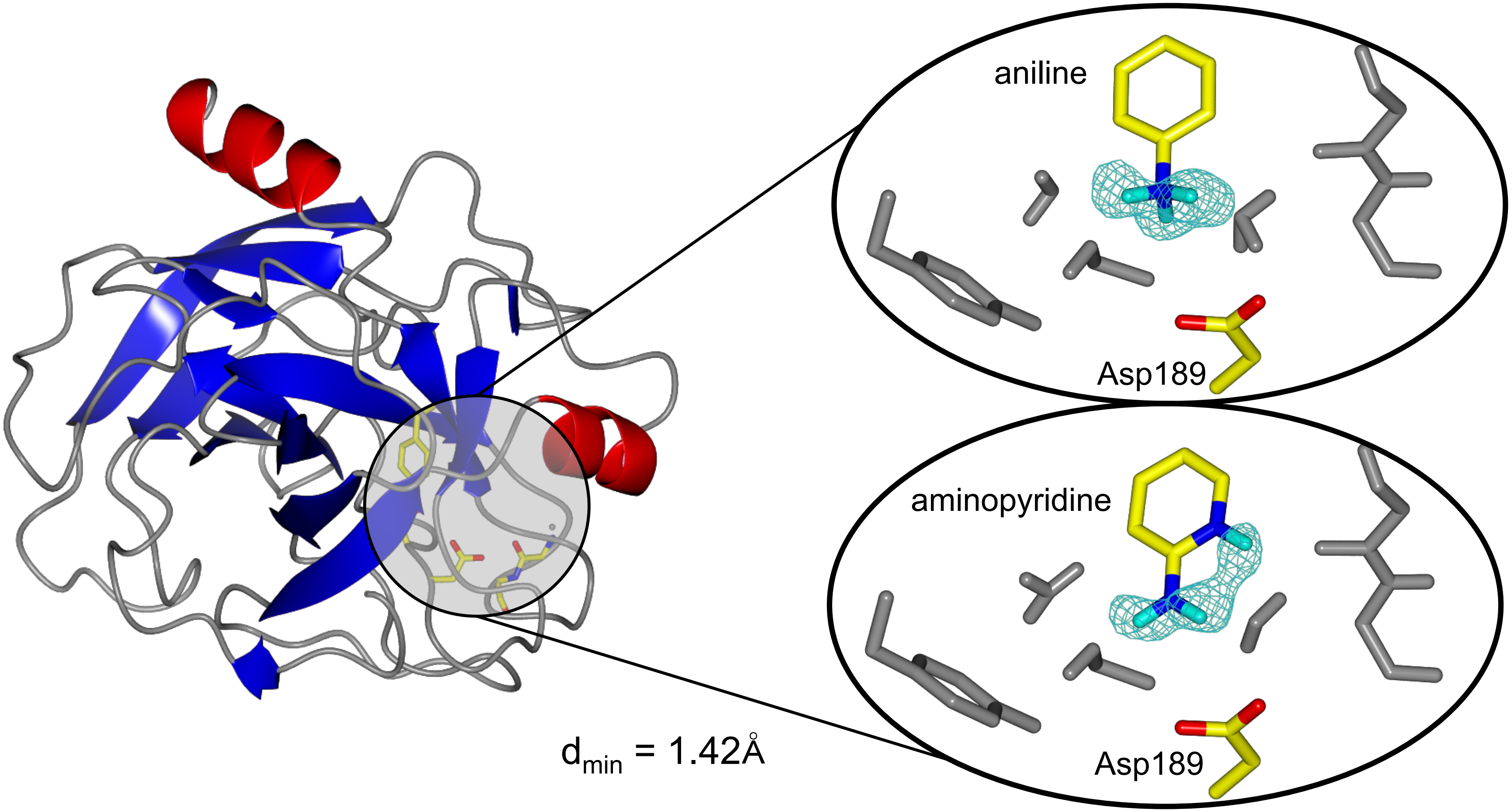

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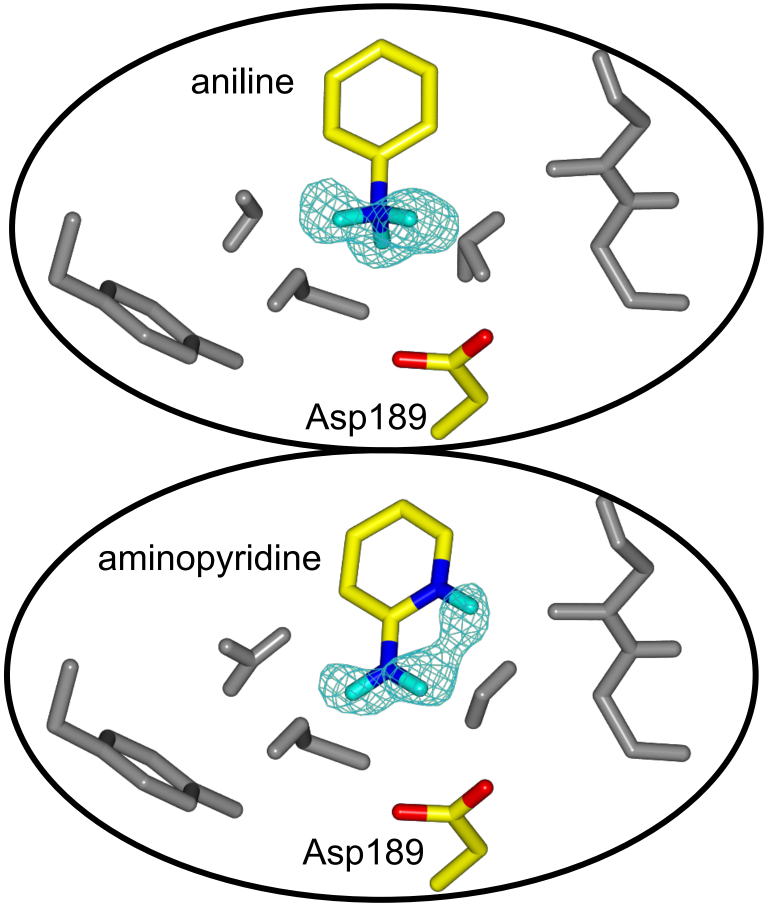
🟩 Fo-Fc omit-map; +3.5σ

Schiebel J. et al., Angewandte Chemie Int. Edition (2017), 56 (17):4887-4890

Inhibitor binding to trypsin: charges shift protonation

- ➡ despite its low pK_a of 4.6 the amino group of aniline becomes protonated
- ➡ Asp189 induce a K_a shift of four orders of magnitude in the amino group of aniline
- ➡ whereas in aminopyridine, the pyridine nitrogen picks up the proton although its amino group is 1.6Å closer to Asp189



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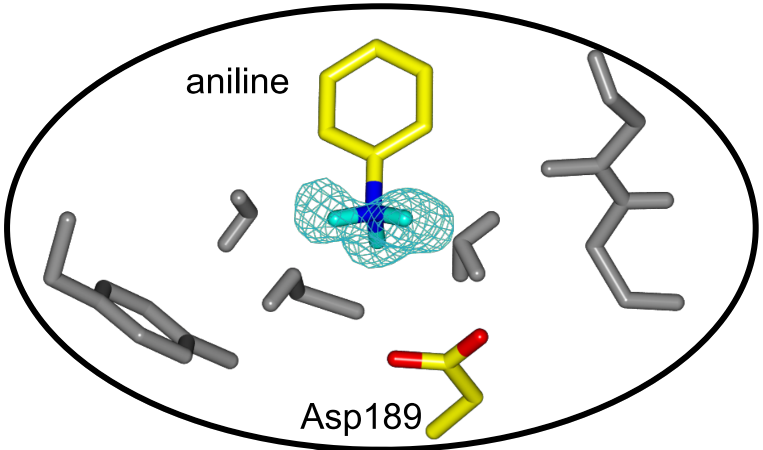
Schiebel J. et al., Angewandte Chemie Int. Edition (2017), 56 (17):4887-4890

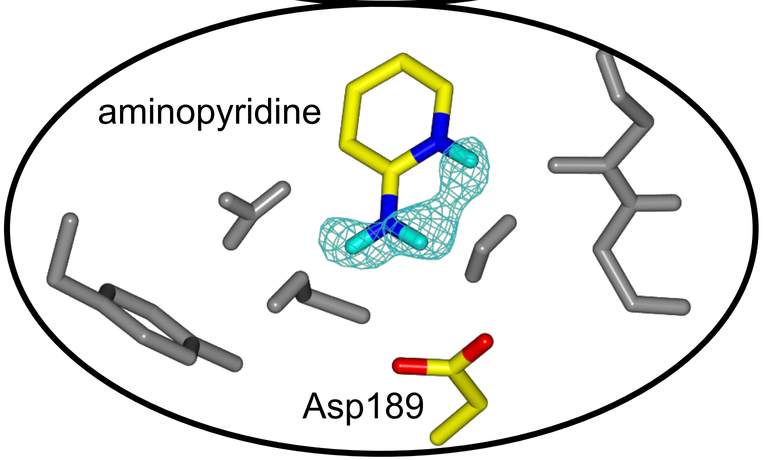
Slides courtesy of Andreas Ostermann

Inhibitor binding to trypsin: charges shift protonation

Therefore, apart from charge–charge distances, tautomer stability is essential for the resulting protonation pattern

➡ correct prediction of such properties is key in drug development !





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Group of Prof. G. Klebe (Univ. Marburg)

Schiebel J. et al., Angewandte Chemie Int. Edition (2017), 56 (17):4887-4890

User data:

Example β-lactamase @ d_{min} = 1.42Å:

Dr. Leighton Coates	Oak Ridge National Laboratory
Stephen J. Tomanicek	Oak Ridge National Laboratory
Dr. Robert F. Standaert	University of Tennessee
Prof. Dr. Joe Ng	University of Alabama

Example Trypsin @ d_{min} = 1.42Å:

Prof. Dr. G. Klebe	University Marburg
Dr. J. Schiebel	University Marburg
Prof. Dr. A. Heine	University Marburg

Example MTAN @ d_{min} = 2.5Å:

Prof. Dr. D.R. Ronning	University of Toledo
Dr. M.T. Banco	University of Toledo
Dr. A. Kovalevsky	Oak Ridge National Laboratory

Typical user data sets collected

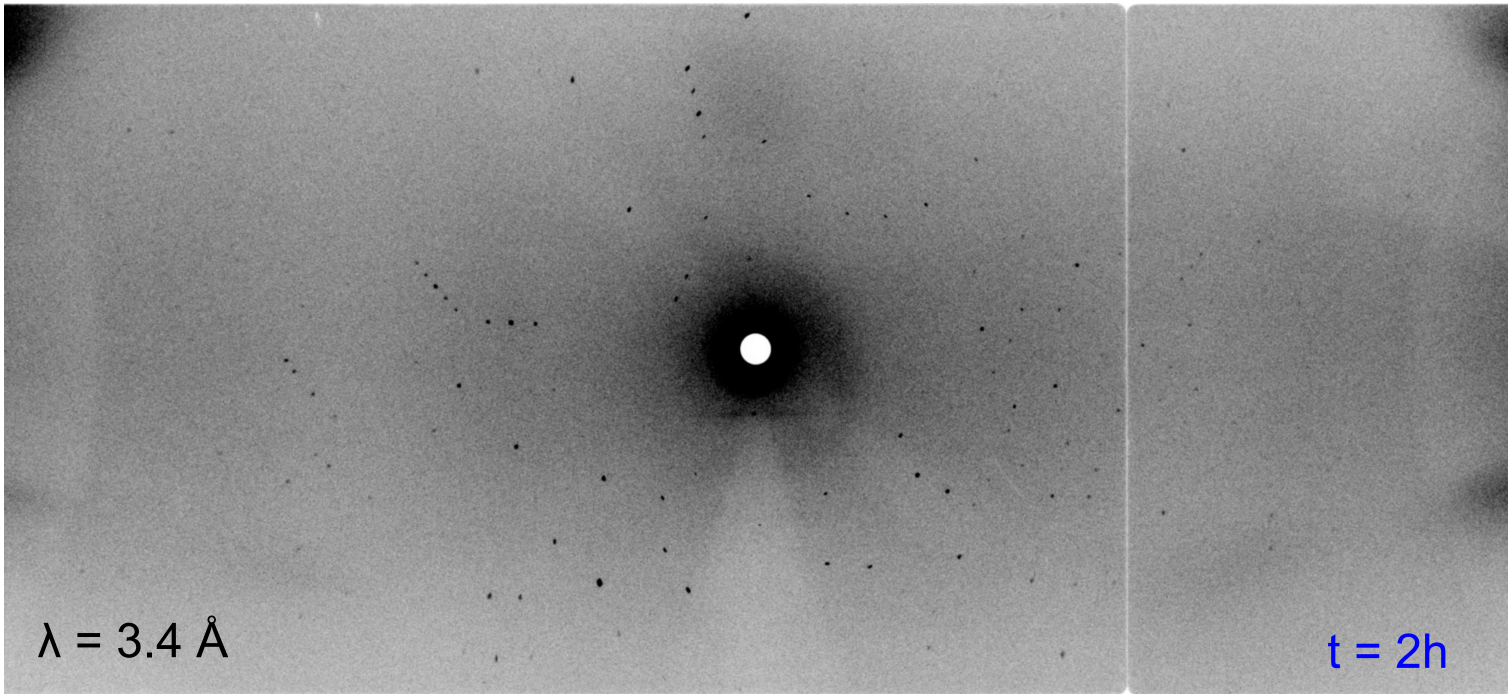
protein	**unit cell** (Å) space group	**cell volume** (Å^3)	**crystal size** (mm^3)	**time** (d)	**d$_{min}$** (Å)	**compl.** (%)	**R$_{merge}$** (%)
β-lactamase (no ligand) L. Coates et al.	73.3, 73.3, 98.7 $P3_221$ λ=2.7Å	453,000	4.0	8	2.0	89.0 (82.7)	9.8 (22.3)
β-lactamase-BZB-inhibitor L. Coates et al.	73.4, 73.4, 99.1 $P3_221$ λ=2.7Å	453,000	2,7	9	2.0	90.2 (81.2)	14.7 (27.9)
Inorganic pyrophosphatase J. Ng et al.	101.0 101.0 100.5 R32 λ=3.4Å, 4.0Å	887,700	1	24	2.0	97.9 (90.5)	13.6 (52.6)
Xylanase II A. Kovalevsky et al.	49.5 59.9 70.4 $P2_12_12_1$ λ=2.7Å	208,000	2.8	17	2.0	96.2 (91.0)	9.7 (32.7)
KDN9P phosphatase Z. Fischer et al.	83.1 108.9 75.8 $P2_12_12$ λ=2.7Å	685,000	1.0	18	2.5	94.8 (88.7)	11.7 (40.0)
apo human carbonic anhydrase II Z. Fischer et al.	42.8 41.7 72.8 $P2_1$ λ=2.7Å	125,000	2,5	8	1.8	89.9 (76.8)	11.9 (33.0)
Nucleosidase (MTAN) A. Kovalevsky et al.	83.0 83.0 67.4 $P3_221$ λ=2.7Å	392,000	2.8	25	2.7	97.1 (94.9)	9.8 (47.8)
Cytochrome c peroxidase P. Moody, M. Blakeley, C. Casadei et al.	51.2 75.8 107.6 $P2_12_12_1$ λ=3.4Å, 4.0Å	417,000	0.65	23	2.5	90.7 (71.8)	17.3 (42.8)
Farnesyl pyrophosphate synthase T. Yokoyama et al.	111.9 111.9 72.6 $P4_12_12$ λ=4.0Å	909,000	3.5	25 (11)	2.4	98.4 (98.0)	10.7 (61.2)
DNA drug complex S. Arai, R. Kuroki et al.	27.9 27.9 52.0 $P4_12_12$ λ=2.7Å	40,500	3.0	3	1.7	92.7 (83.3)	10.8 (21.5)

Wavelength can be adapted to unit cell size.

Fatty acid binding protein:

→ per-deuterated, high diffraction power but small crystals

Alberto Podjarny & Andre Mitschler



unit cell: 33.8Å x 55.0Å x 70.8Å $P2_12_12_1$

crystal size: **0.08 mm³**

$d_{min} \approx 2.1$ Å
data set in 2 – 3 weeks possible

Conclusions

- Neutron Protein Crystallography can contribute to drug development and drug binding studies.

- It is the ratio of crystal volume to unit cell volume which makes an experiment at a neutron protein crysallography beam line feasible or not

MLZ
Heinz Maier-Leibnitz Zentrum

Upcoming deadline for proposals: September 9th, 2017

all users are welcome!

user.frm2.tum.de

fzj.frm2.tum.de

“rapid access” program for BIODIFF: July 28th, 2017

- 12h test beam time for testing crystals
- up to 3 rapid access proposals per cycle accepted

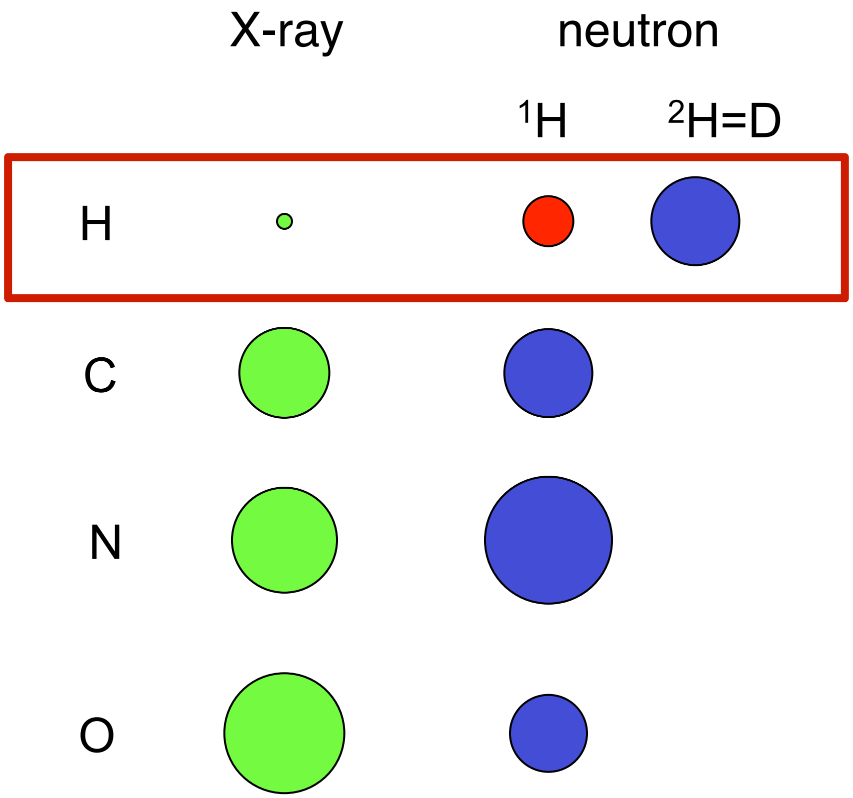
Thank you for your attentenion!

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**

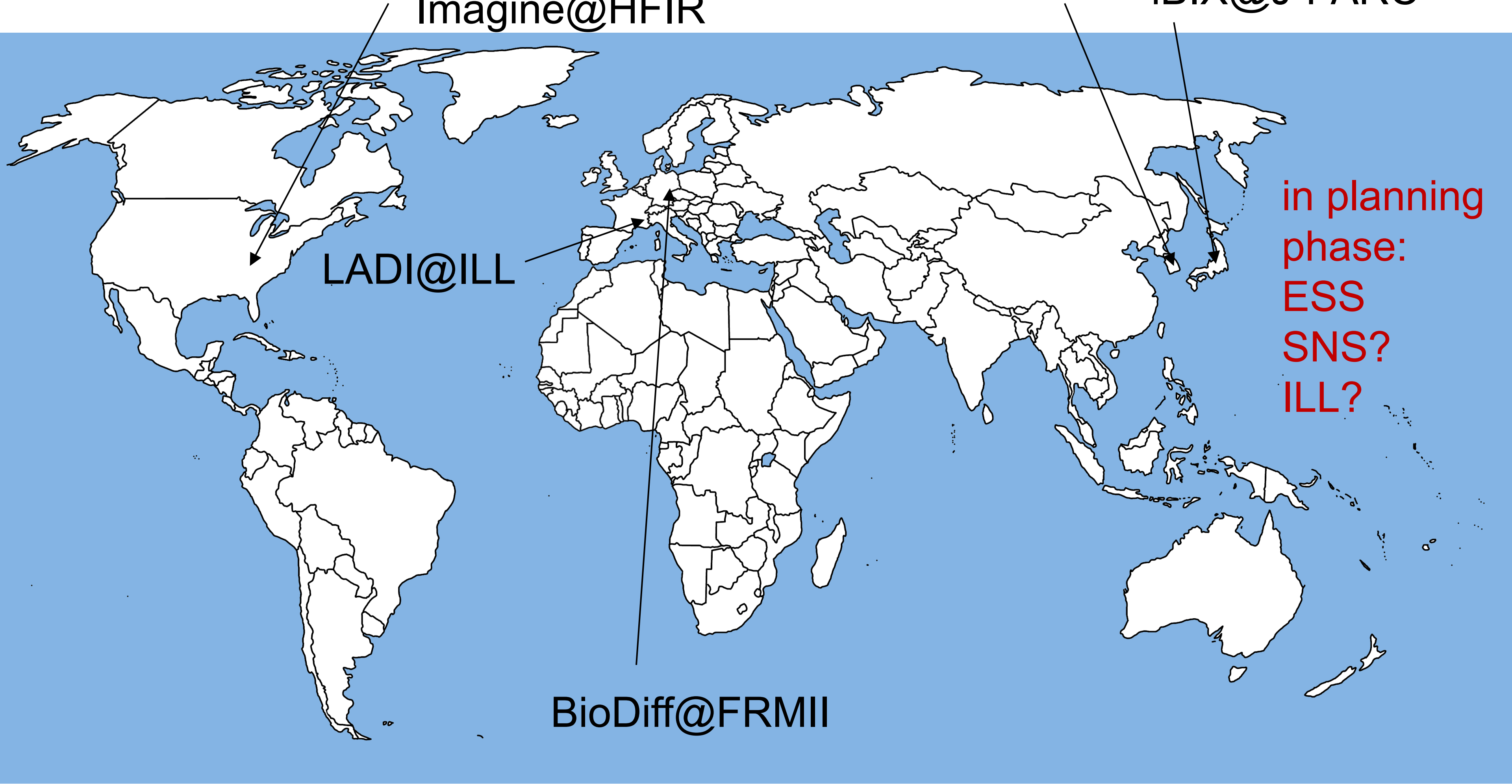
σ_{coh} of ^{1}H is 1.8×10^{-28} m^{2} but
σ_{incoh} of ^{1}H is 80.2×10^{-28} m^{2}
Large background from hydrogen atoms!



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

The Neutron Protein Crystallography beamlines in the world

World map of neutron diffractometers optimized for protein crystals



MaNDi@SNS
Imagine@HFIR

Bio-D@KAERI

iBIX@J-PARC

LADI@ILL

BioDiff@FRMII

in planning phase:
ESS
SNS?
ILL?

Neutron protein diffractometers in the world:

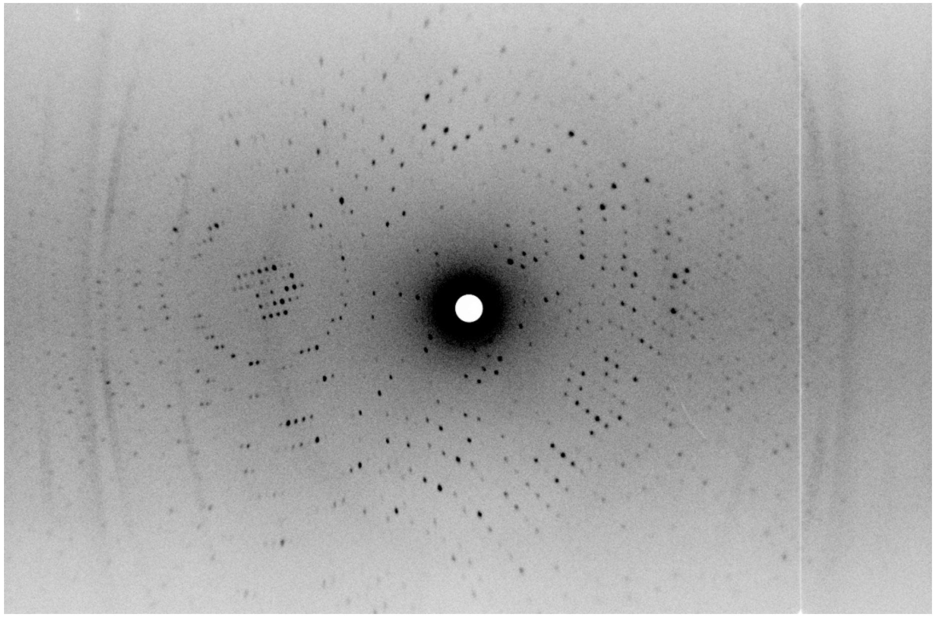
Diffracto-meter	Method used	Flux at sample $n/cm^2/s$	Typical crystal size mm3	max. cell dimension Å	best resolution Å
BioDiff (FRMII)	mono, variable	$9.0\cdot10^6$	> 0.1	< 180	>1.4
Imagine (HIFR)	quasi-Laue	$3.0\cdot10^7$	> 0.1	?	>1.4
Ladi-III (ILL)	quasi-Laue	$3.0\cdot10^7$	> 0.1	< 160	>1.4
Mandi	TOF-Laue	$6.9\cdot10^7$	> 0.1	< 150	>1.5
iBIX	TOF-Laue	$2.1\cdot10^8$	> 0.1	< 150	>1.2

For Bio-D no data is available.

Data partially taken from Blakeley et al. Current Opinion in Structural Biology 18 (2008) 593-600

First “user data set”: β-lactamase with bound inhibitor

S.J. Tomanicek, R.F. Standaert, K.L. Weiss, J.D. Ng, **L. Coates** (Group of P. Langan)



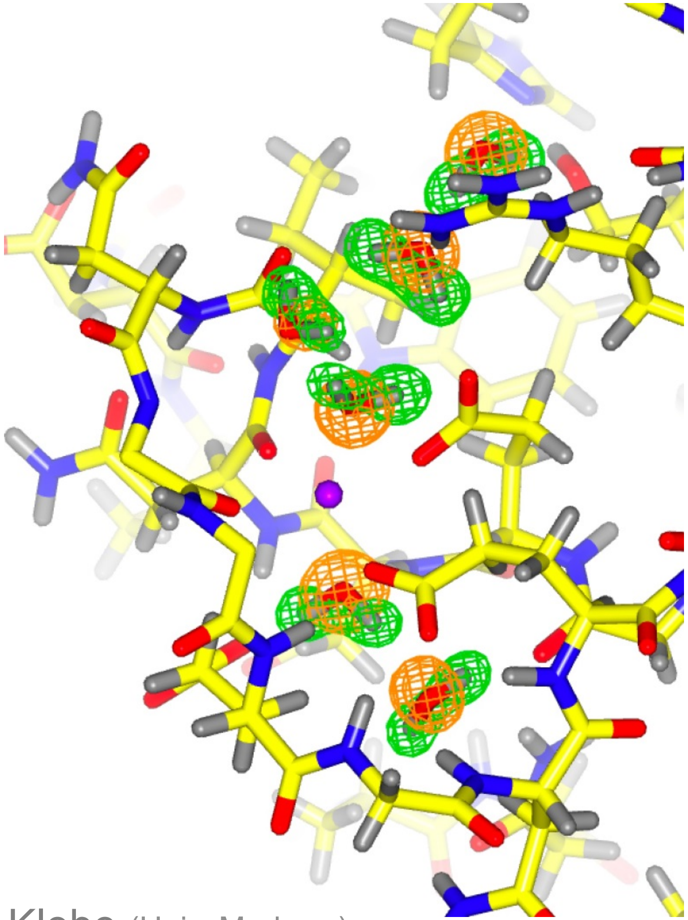
d_{min}	I/σ(I)	N_{meas}	mult.	compl. in shell %	R_{merge} %
4.31	27.8	12685	5.6	97.6	4.9
3.42	19.0	11941	5.5	98.0	8.0
2.99	10.3	10378	4.9	96.9	14.6
2.71	7.6	8757	4.3	95.5	18.7
2.52	5.9	7820	3.9	92.8	21.2
2.37	5.4	7099	3.8	89.2	21.6
2.25	5.0	6095	3.5	84.6	23.0
2.15	4.5	5906	3.4	82.9	24.7
2.07	4.1	5673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

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

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

Ca binding site of trypsin: water molecules



🟩 Fo-Fc neutron omit-map; $+6\sigma$

🟨 Fo-Fc xray omit-map; $+6\sigma$

Group of Prof. G. Klebe (Univ. Marburg)

Schiebel J. et al., Angewandte Chemie Int. Edition (2017), 56 (17):4887-4890

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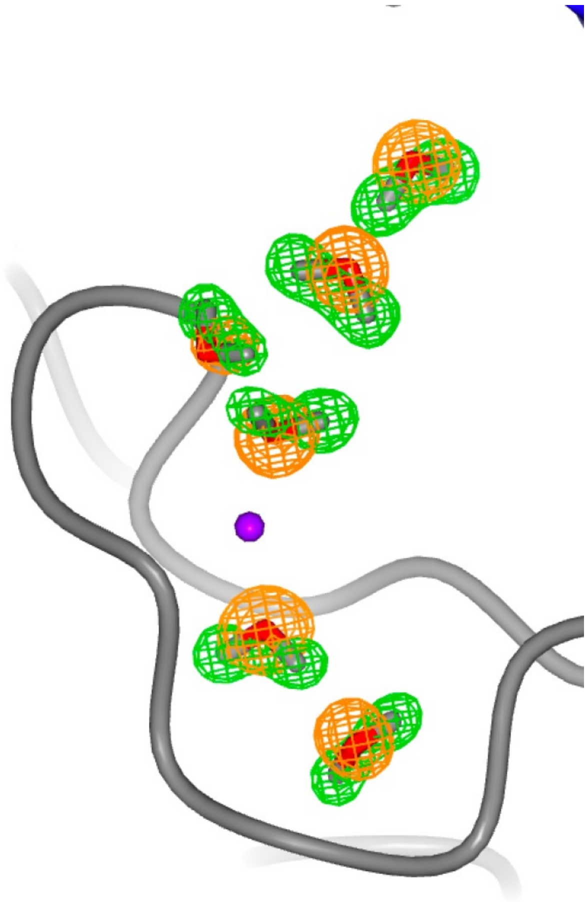

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🟨 Fo-Fc xray omit-map; +6σ

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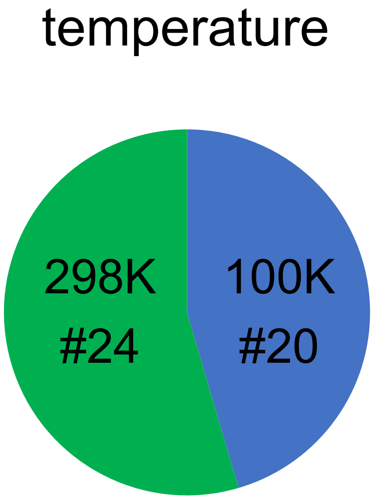
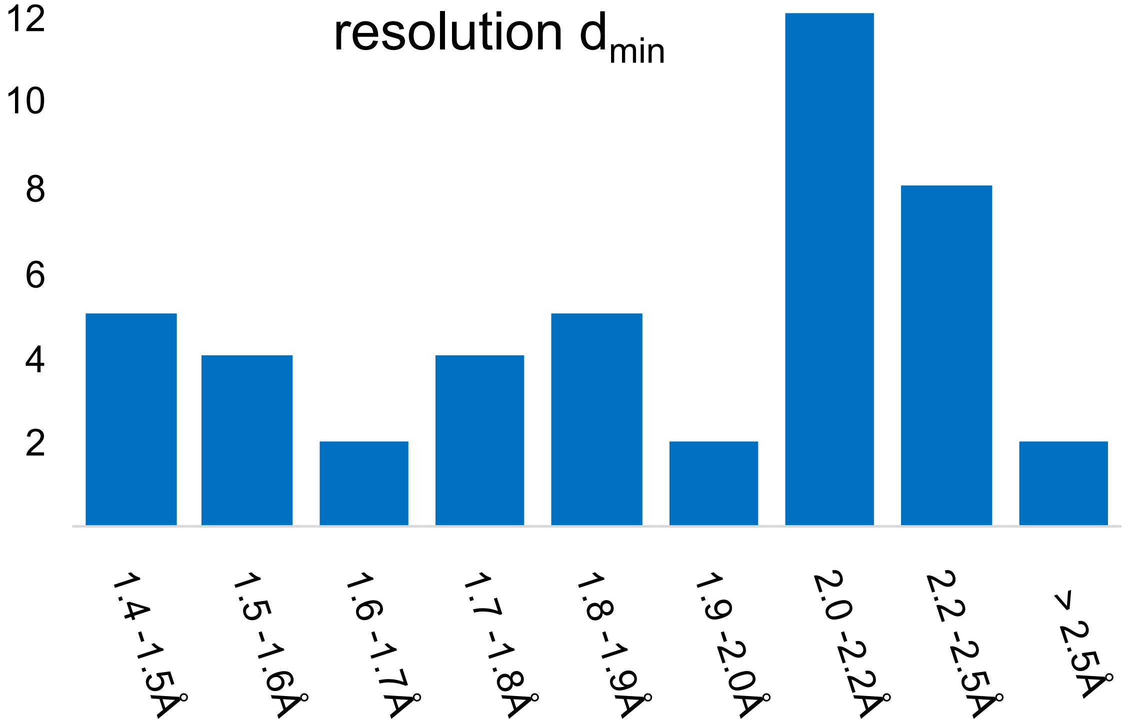
Schiebel J. et al., Angewandte Chemie Int. Edition (2017), 56 (17):4887-4890

Some statistics from BioDiff:

- ⟹ 44 protein structures
- ⟹ 4 magnetic superstructure proposals
- ⟹ 3 multilamellar membrane proposals





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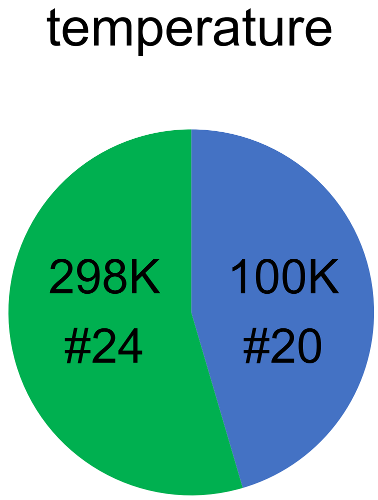
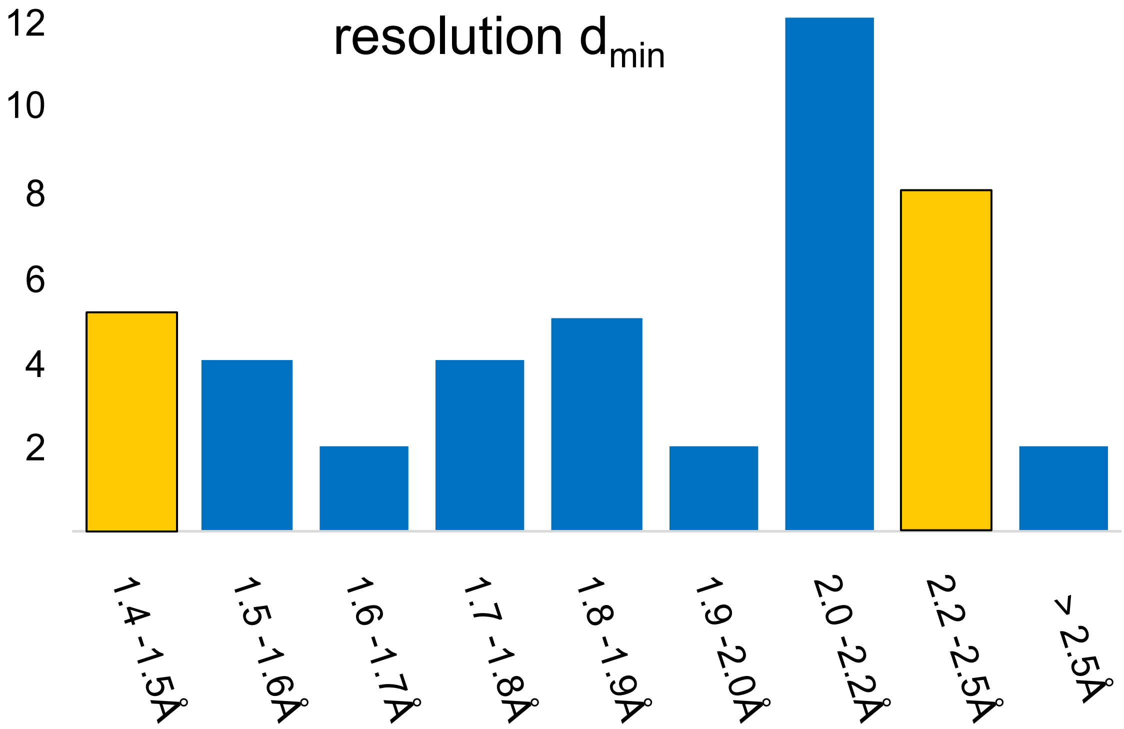
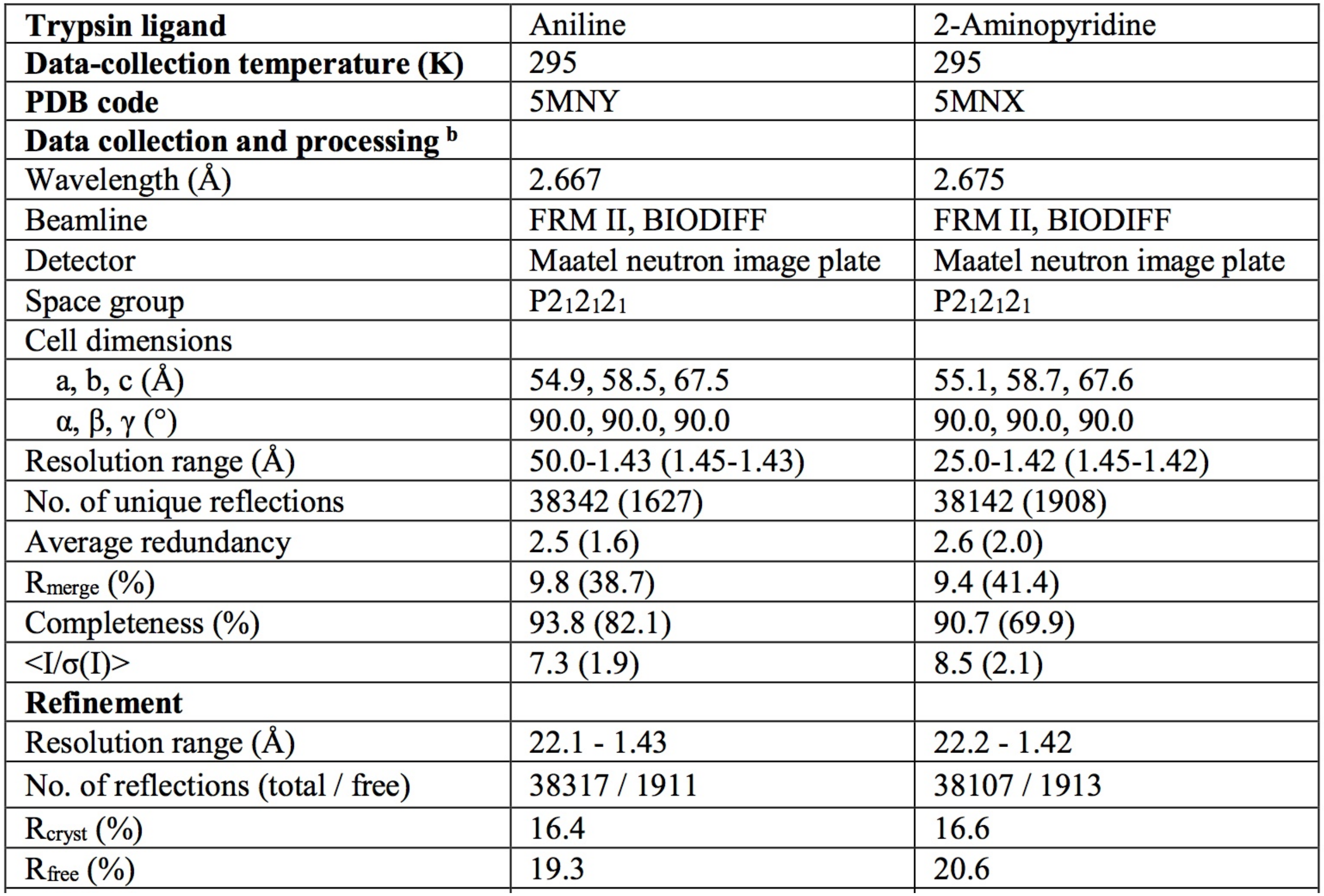




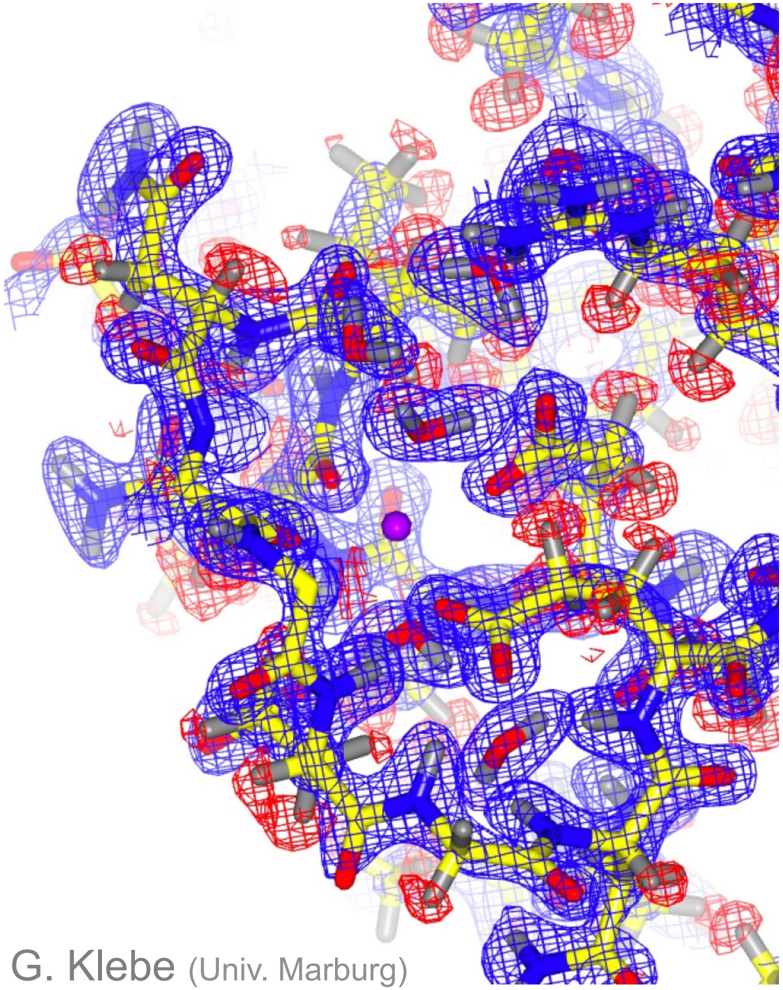
Inhibitor binding to trypsin: charges shift protonation



Trypsin ligand	Aniline	2-Aminopyridine
Data-collection temperature (K)	295	295
PDB code	5MNY	5MNX
Data collection and processing [b]		
Wavelength (Å)	2.667	2.675
Beamline	FRM II, BIODIFF	FRM II, BIODIFF
Detector	Maatel neutron image plate	Maatel neutron image plate
Space group	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions		
a, b, c (Å)	54.9, 58.5, 67.5	55.1, 58.7, 67.6
α, β, γ (°)	90.0, 90.0, 90.0	90.0, 90.0, 90.0
Resolution range (Å)	50.0-1.43 (1.45-1.43)	25.0-1.42 (1.45-1.42)
No. of unique reflections	38342 (1627)	38142 (1908)
Average redundancy	2.5 (1.6)	2.6 (2.0)
R_{merge} (%)	9.8 (38.7)	9.4 (41.4)
Completeness (%)	93.8 (82.1)	90.7 (69.9)
<I/σ(I)>	7.3 (1.9)	8.5 (2.1)
Refinement		
Resolution range (Å)	22.1 - 1.43	22.2 - 1.42
No. of reflections (total / free)	38317 / 1911	38107 / 1913
R_{cryst} (%)	16.4	16.6
R_{free} (%)	19.3	20.6

Schiebel J. et al., Angewandte Chemie Int. Edition (2017), 56 (17):4887-4890

Ca binding site of trypsin: water molecules



🟦 2Fo-Fc neutron map; +1.5σ

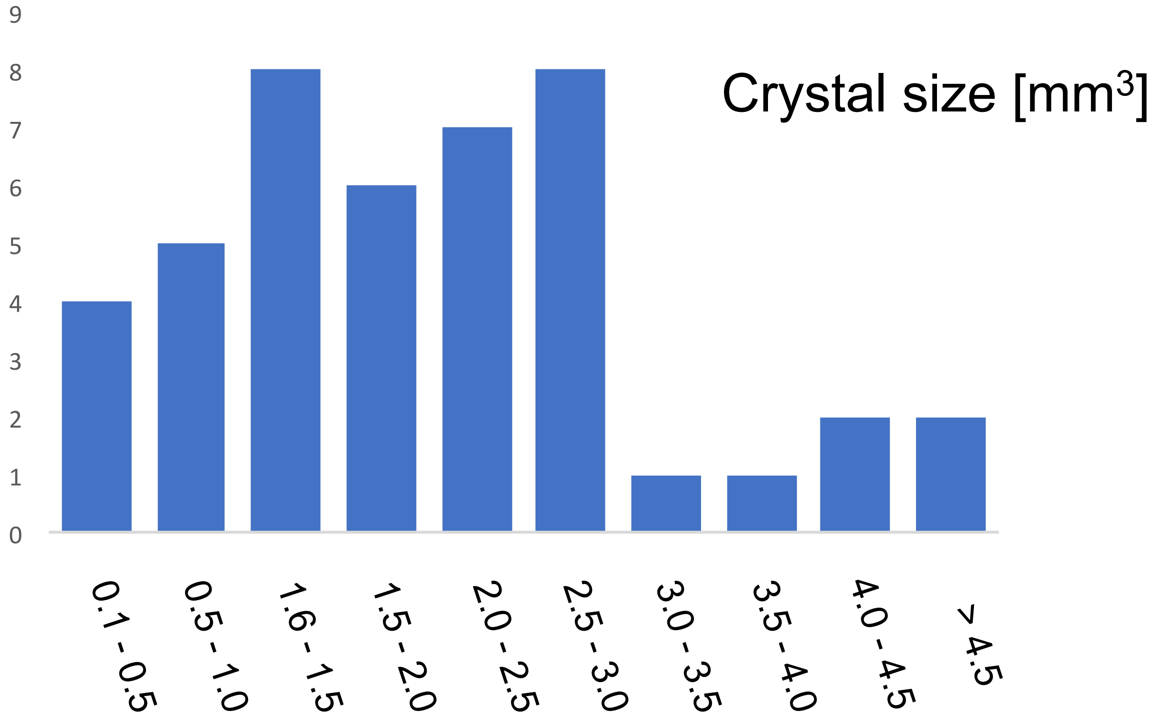
🟥 2Fo-Fc neutron map; -2σ

Group of Prof. G. Klebe (Univ. Marburg)

Schiebel J. et al., Angewandte Chemie Int. Edition (2017), 56 (17):4887-4890

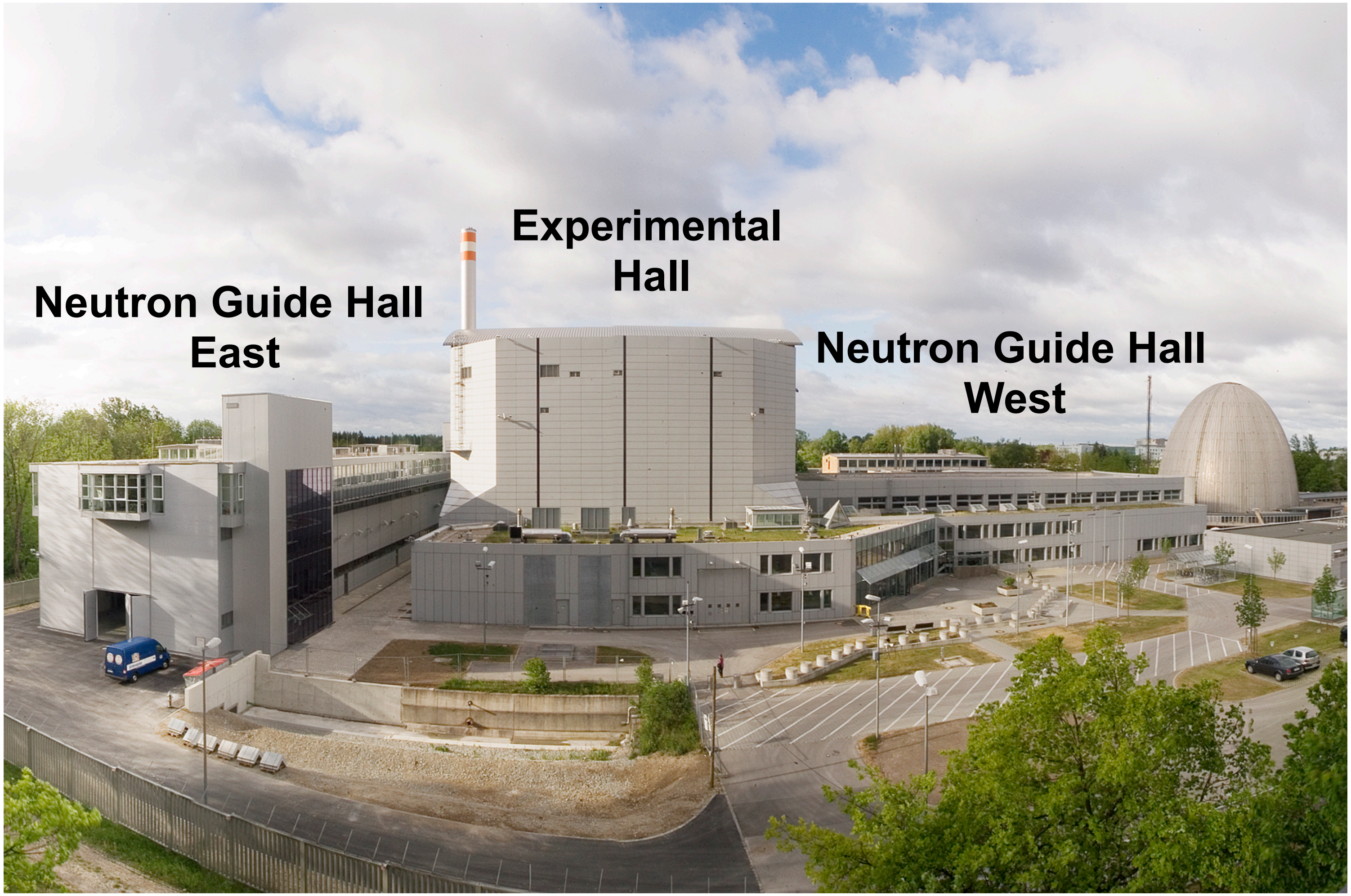
Some statistics from BioDiff:

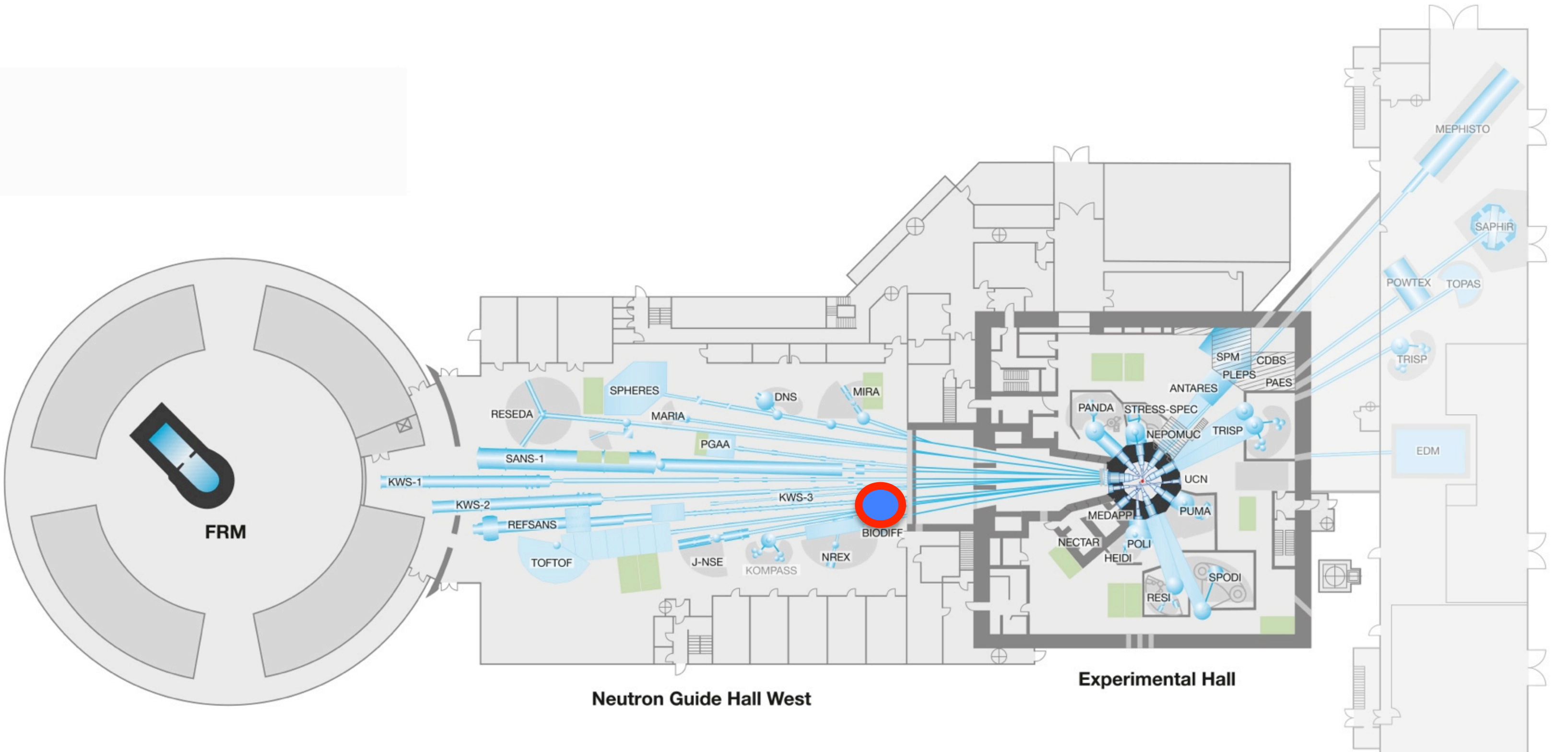
⟹ 44 protein structures
⟹ 4 magnetic superstructure proposals
⟹ 3 multilamellar membrane proposals



The instrument BioDiff at MLZ in Garching, Germany

Research Neutron Source Heinz Maier-Leibnitz (FRM II)





- **12 instruments suitable for biological / biophysical studies**
  - 4 spectrometer: TOF, backscattering, spin echo;
  - 3 reflectometers;
  - 4 small angle scattering machines;
  - 1 diffractometer for macromolecular diffraction

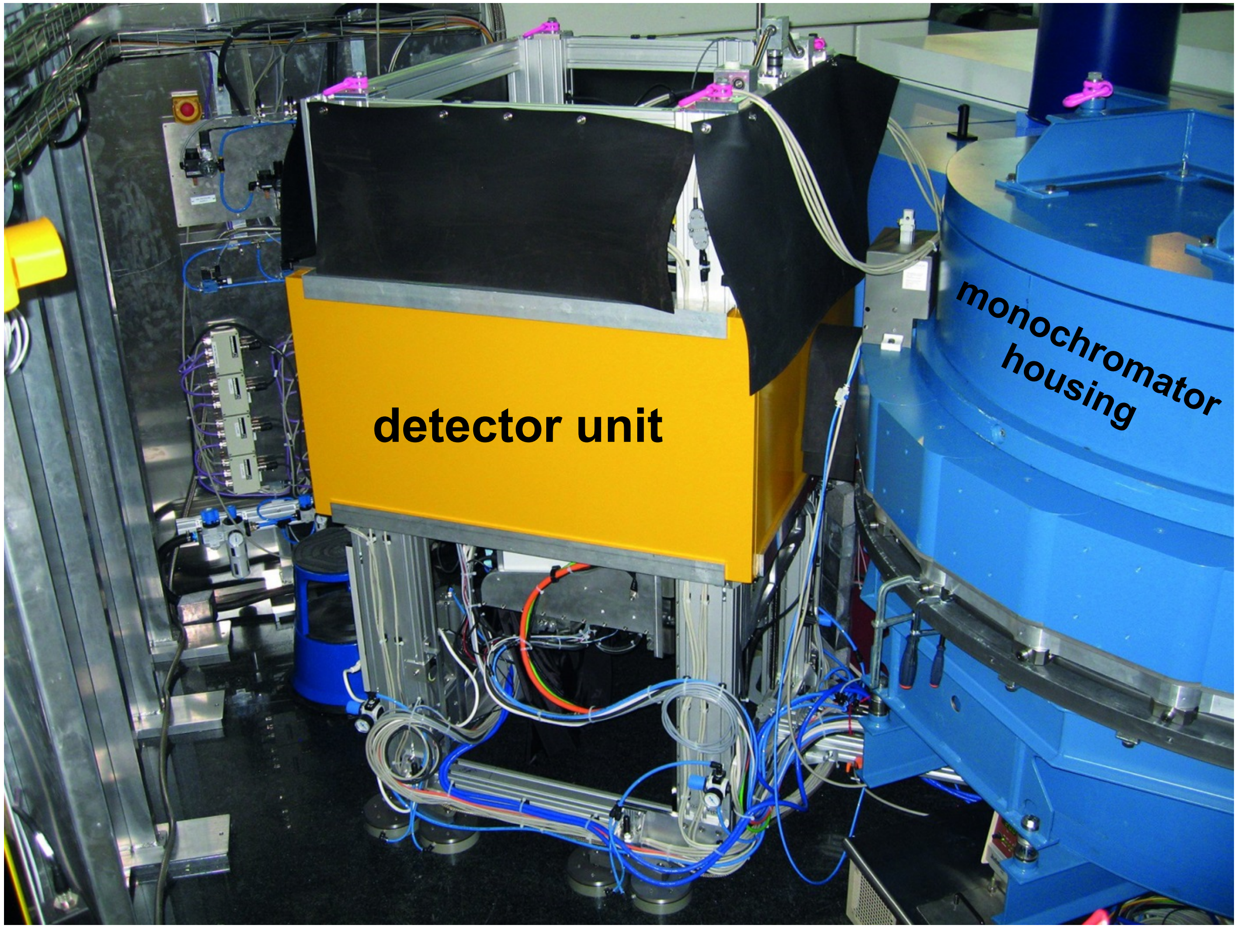
Neutron Guide Hall West:

BIODIFF: Diffractometer for macromolecular neutron crystallography

operated by

Technische Universität München

Jülich Centre for Neutron Science



in operation since April 2012

BIODIFF: Diffractometer for macromolecular neutron crystallography

Beam condition:

wavelength range: 2.4Å - 5.6Å

sample position (2 x 2 mm^2)
@2.4Å; slits open;

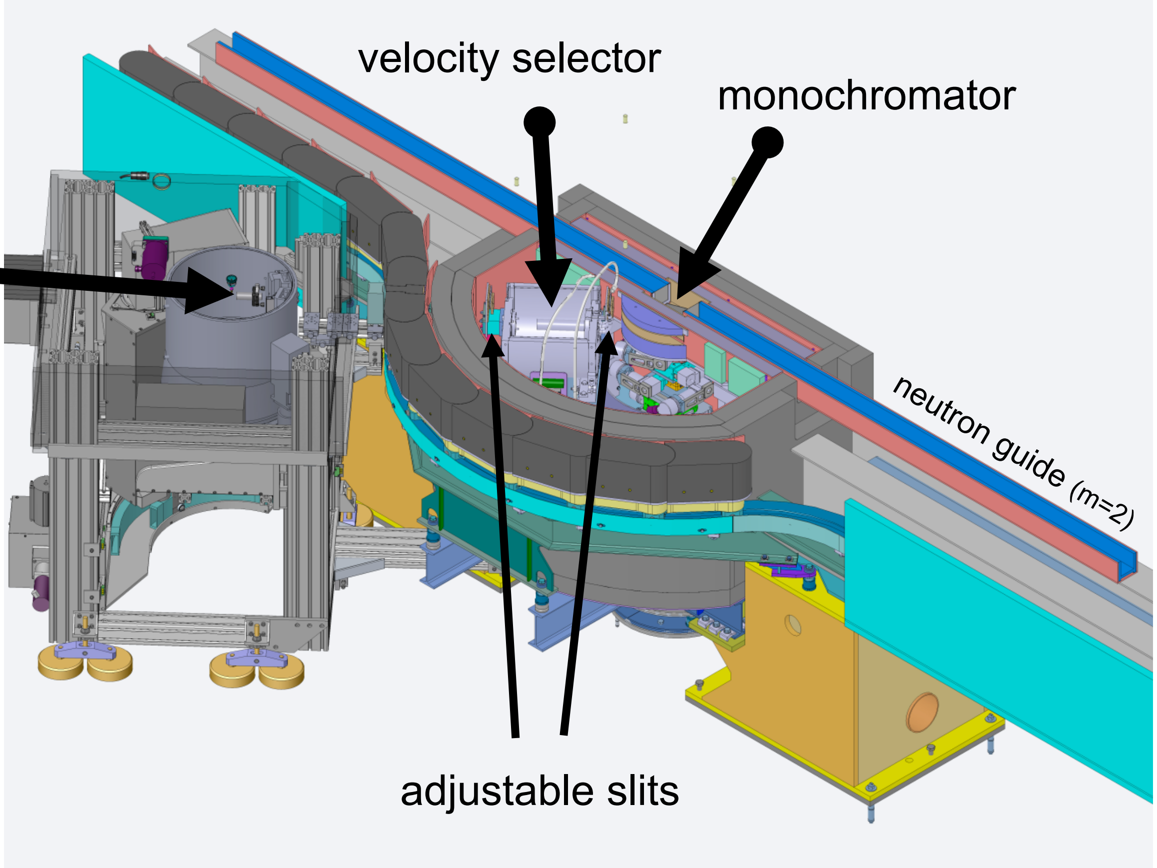
hor. divergence: 0.8° (FWHM)

vert. divergence: 0.7° (FWHM)

$\Delta\lambda/\lambda$ = 2.5% (FWHM)

Flux: 9 x 10^6 n cm^{-2} s^{-1} @2.7Å

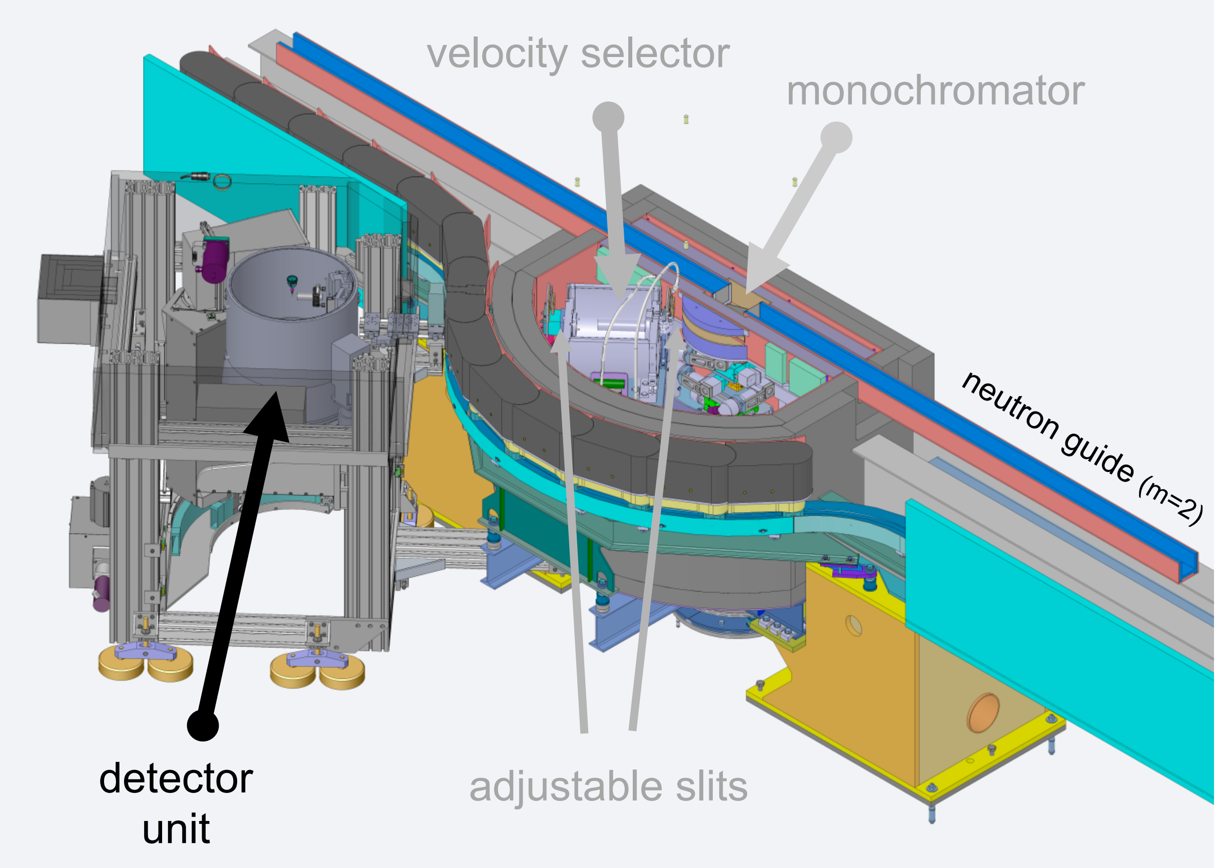
7 x 10^6 n cm^{-2} s^{-1} @4.7Å



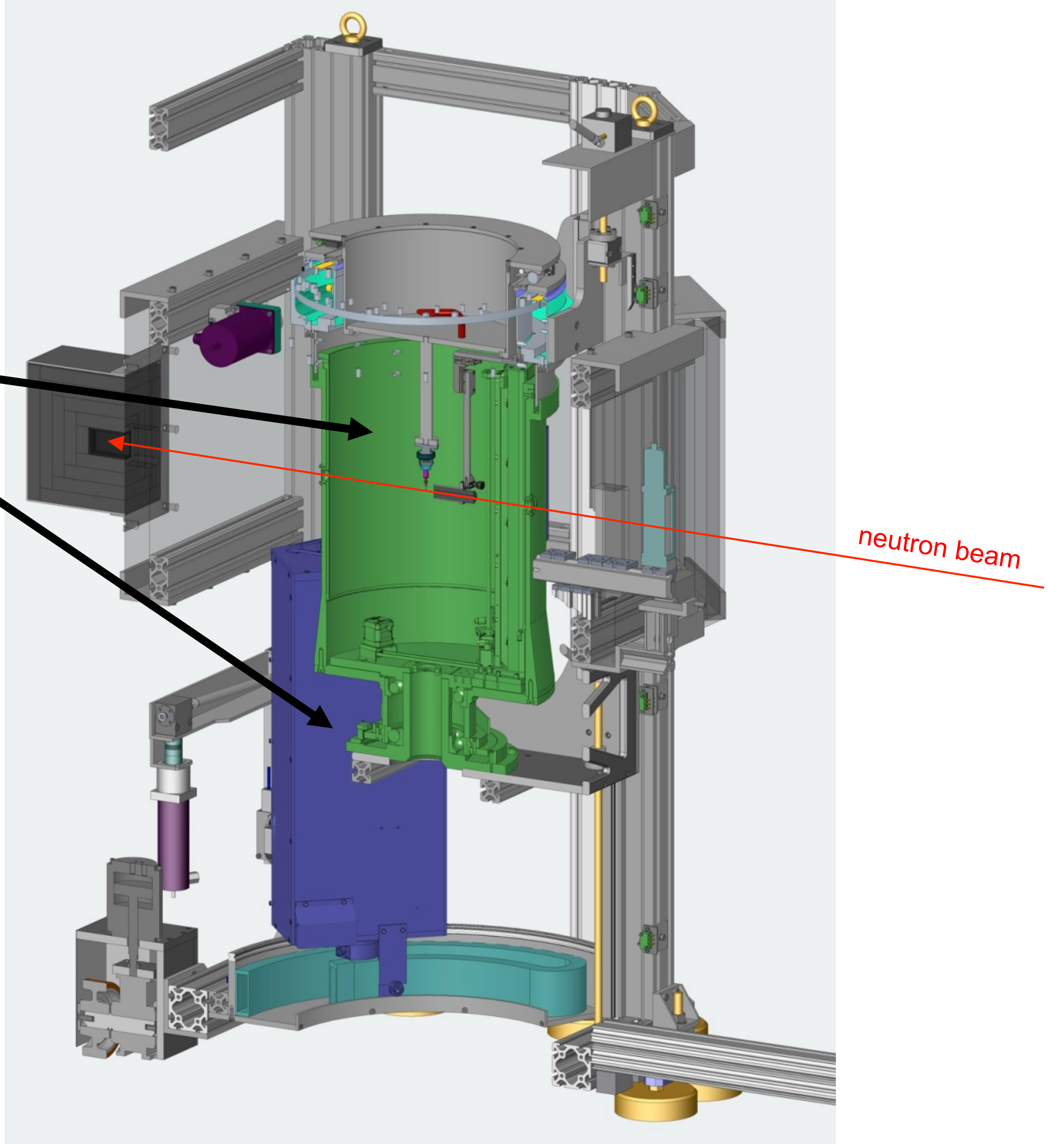
BIODIFF: Diffractometer for macromolecular neutron crystallography

detector unit:

- image plate detector
- scintillator CCD-camera



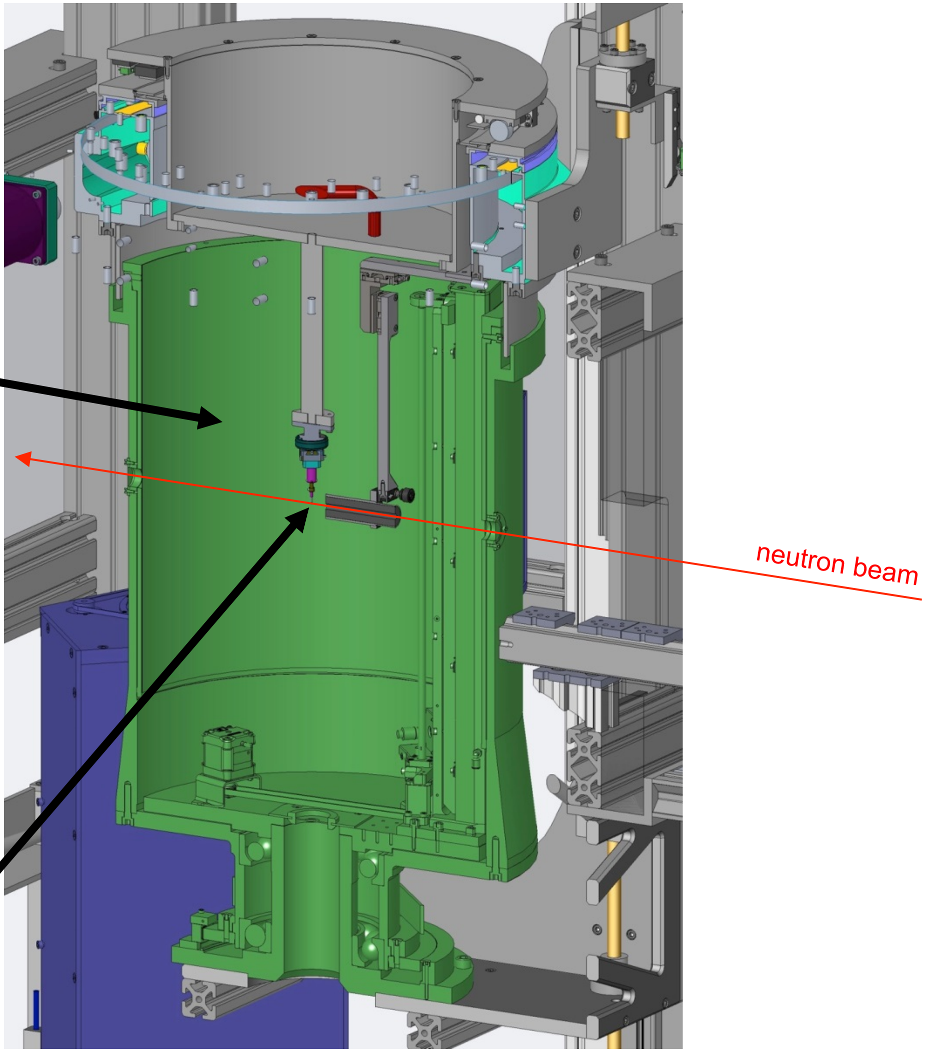
Detector unit



cylindrical image plate detector

scintillator based CCD-camera

Detector unit



neutron image plate

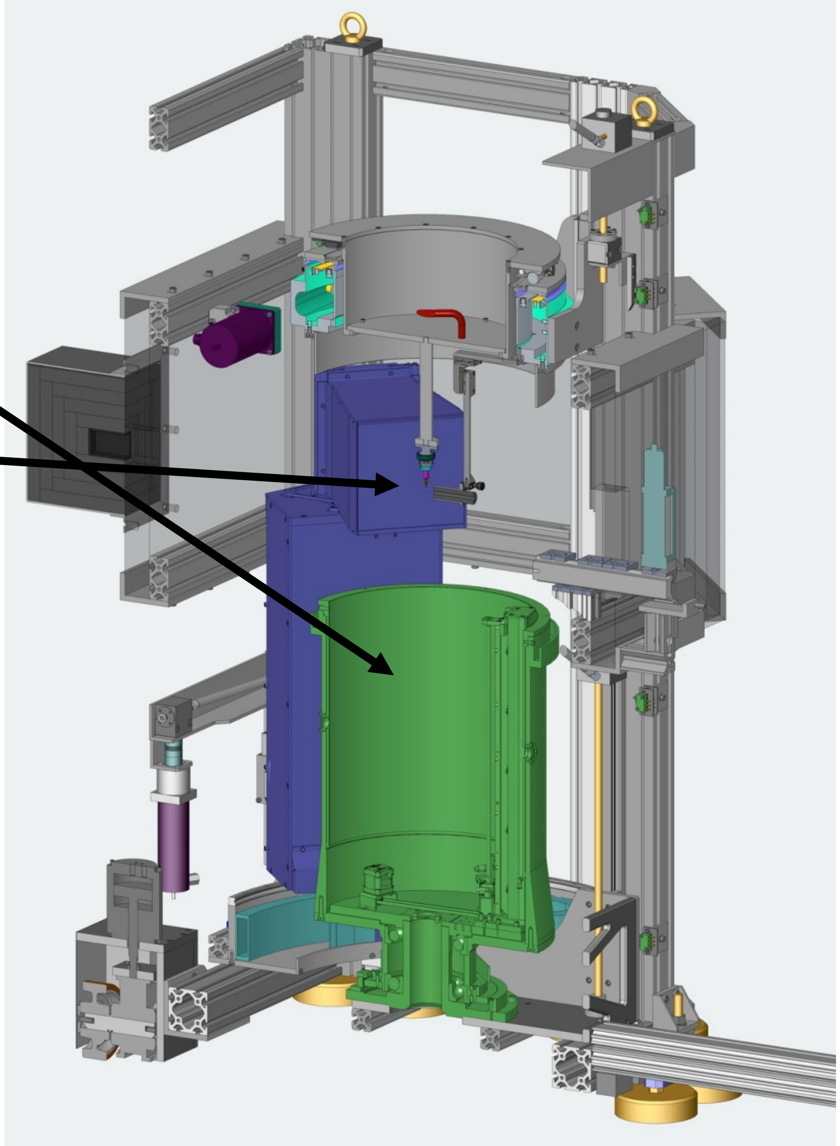
- Gd_2O_3 / $BaFBr{:}Eu^{2+}$ (white Niimura-type)
- cylindrical shape: r = 200mm; h = 450mm
- covered hor. solid angle: ±155°
  vert. solid angle: ±48°
- scanner resolution: 125, 250, 500 μm
- readout time + erasing: ≈ 4min (500 μm)

same scanner type as
LADI-III@ILL and IMAGINE@ORNL

Company: Maatel /France

sample position

Detector unit



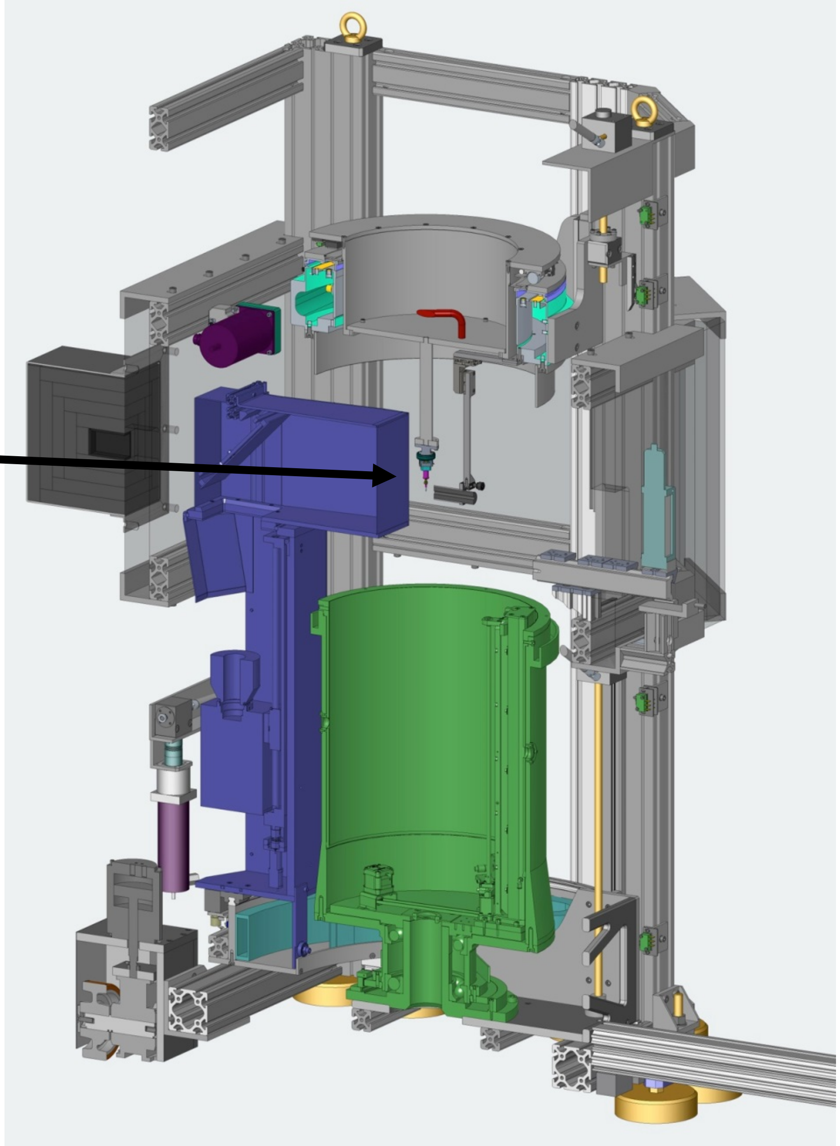
cylindrical image plate detector

scintillator based CCD-camera

Detector unit

scintillator based CCD-camera

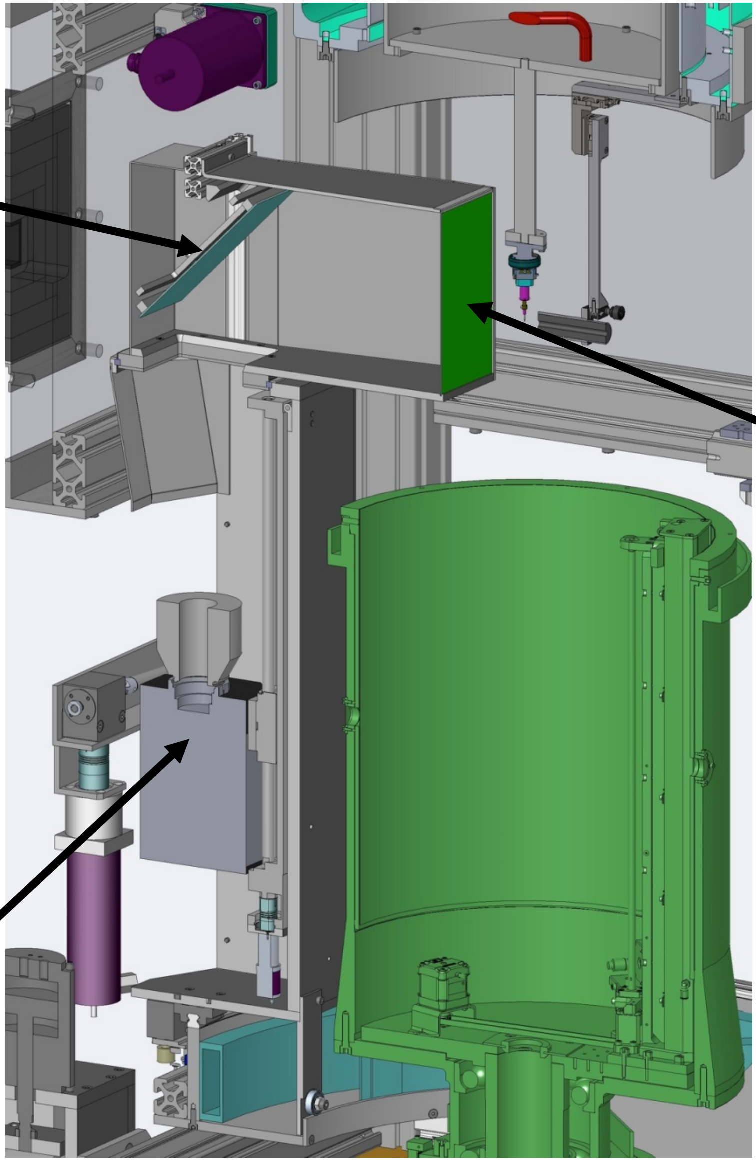
2Θ-range: 0° - 112°



Detector unit

Scintillator based CCD-camera

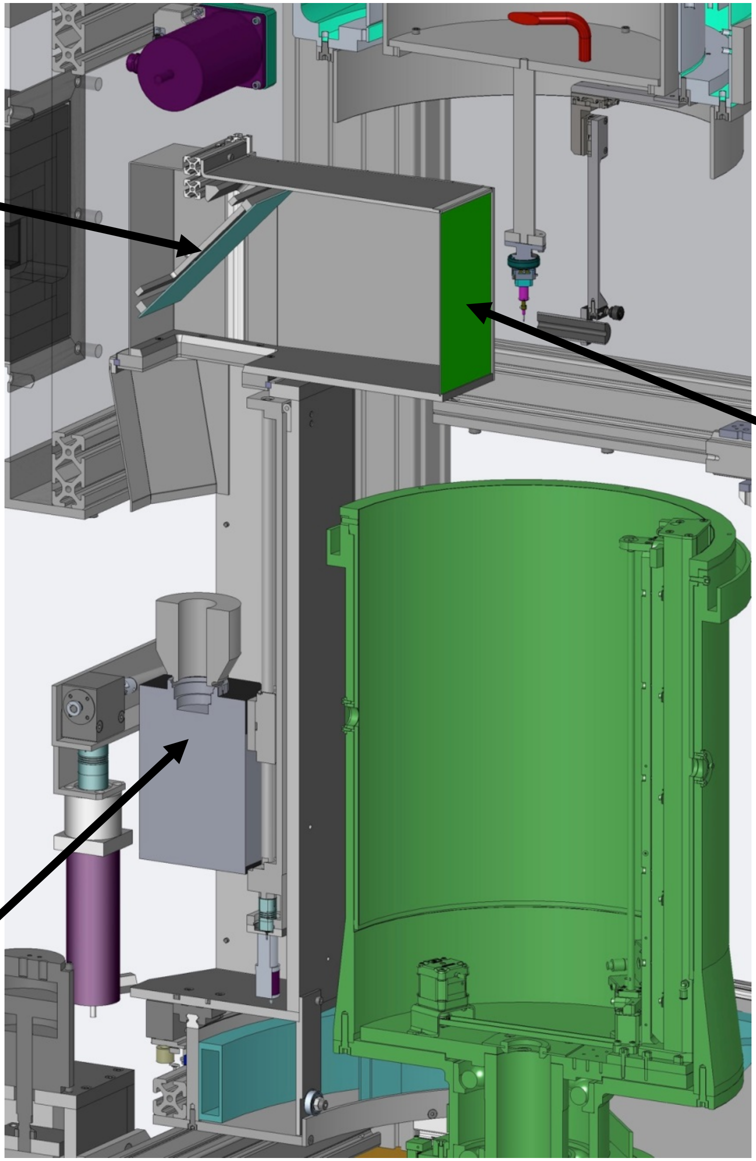
- ^{6}LiF/ZnS scintillator
- flat shape: 200 x 200mm
- overall resolution: ≈300 μm



Detector unit

Scintillator based CCD-camera

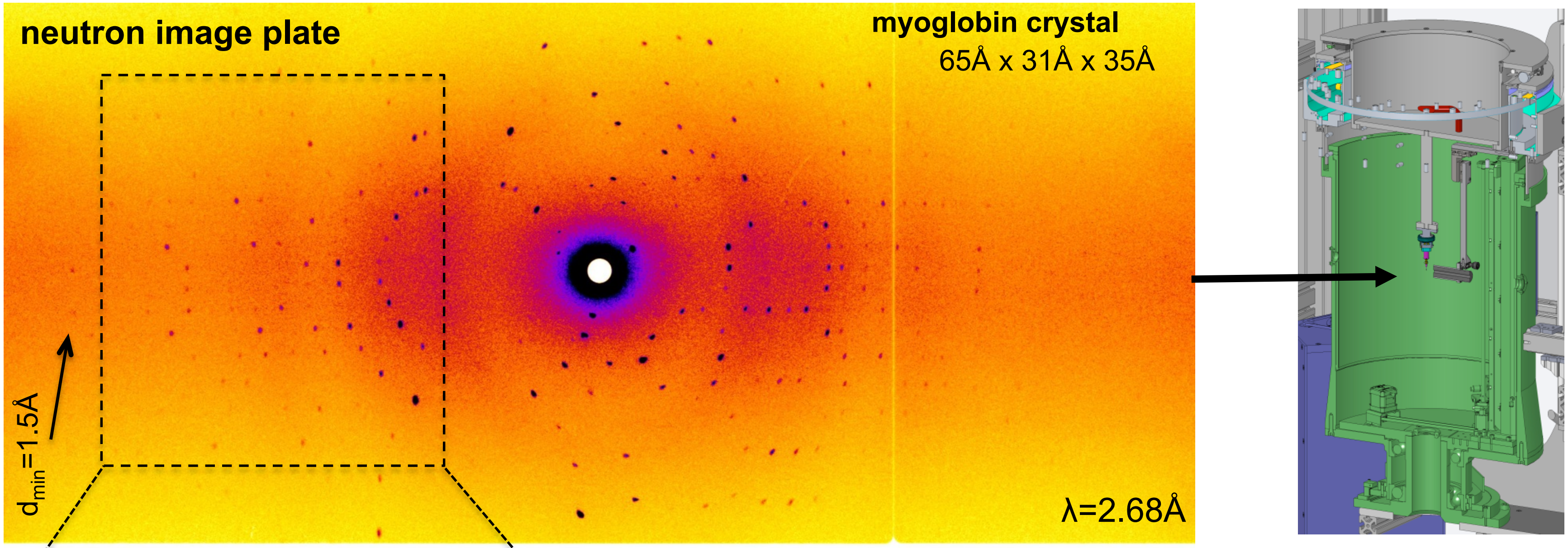
- Andor iKon-L CCD camera
- CCD-chip cooled to -90°C
- very low dark current and readout noise
- readout time: ≥1sec

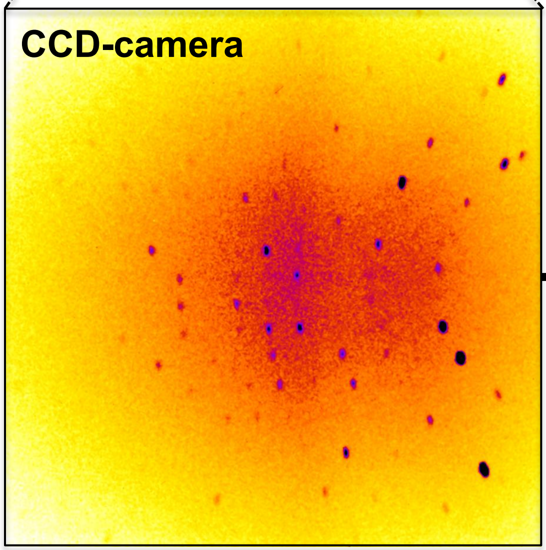


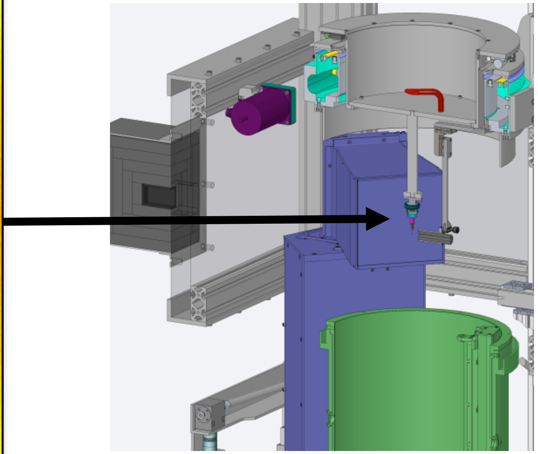
mirror

^{6}LiF/ZnS scintillator

CCD camera





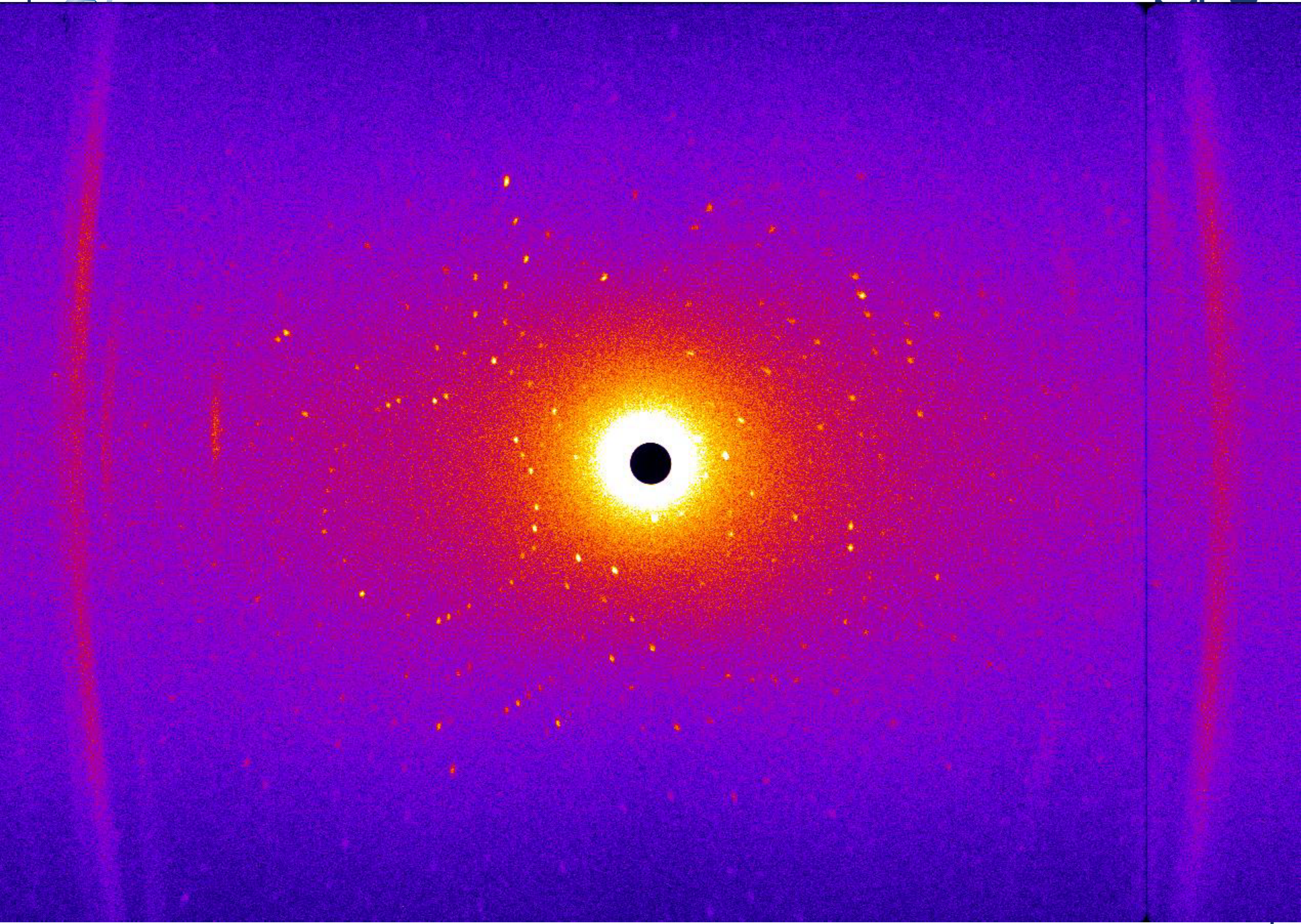


NIP-scanner

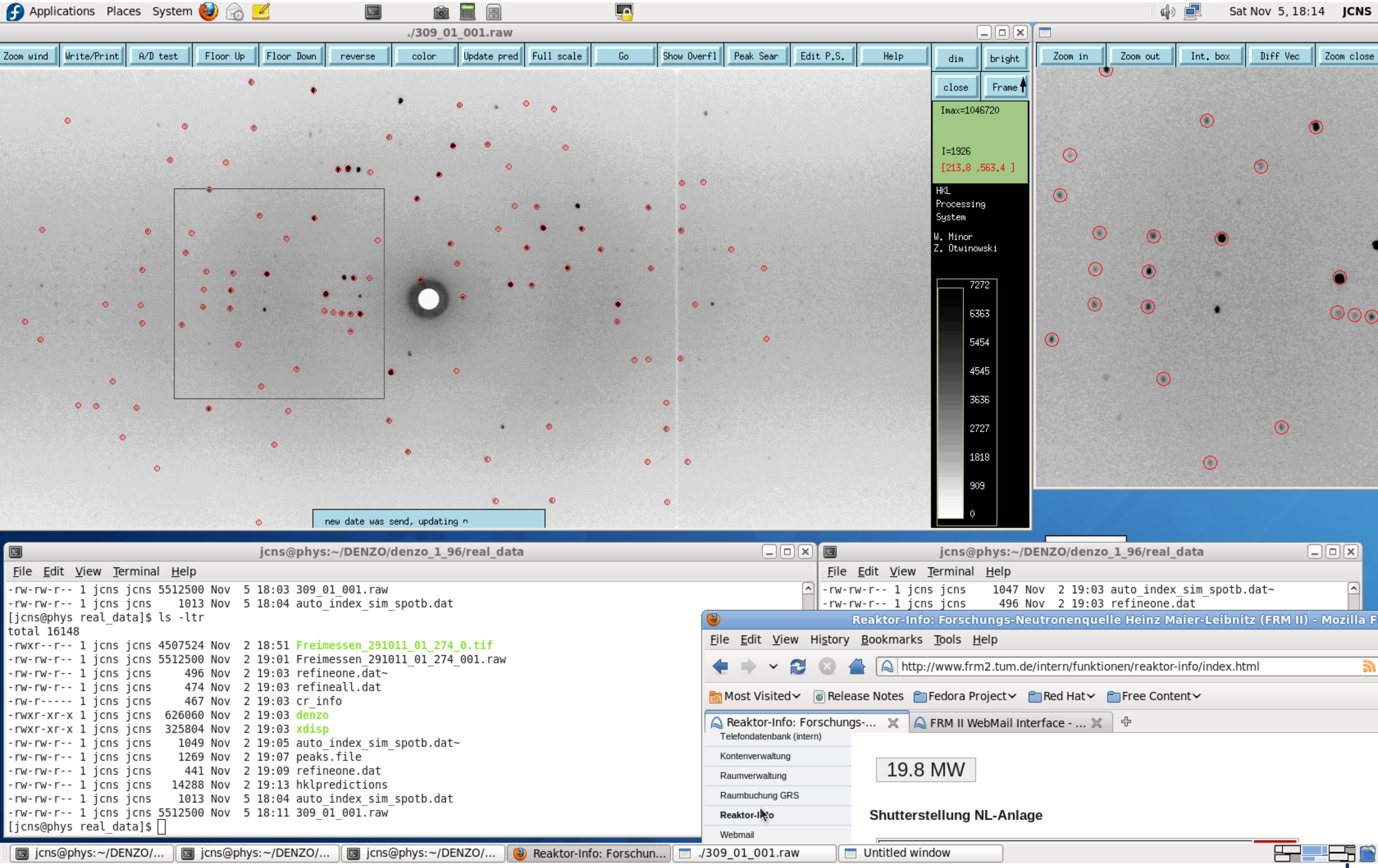
- larger covered solid angle
- readout time ≥ 4min

CCD-camera

- smaller covered solid angle
- readout time ≥ 1sec



peak search with hkl DENZO



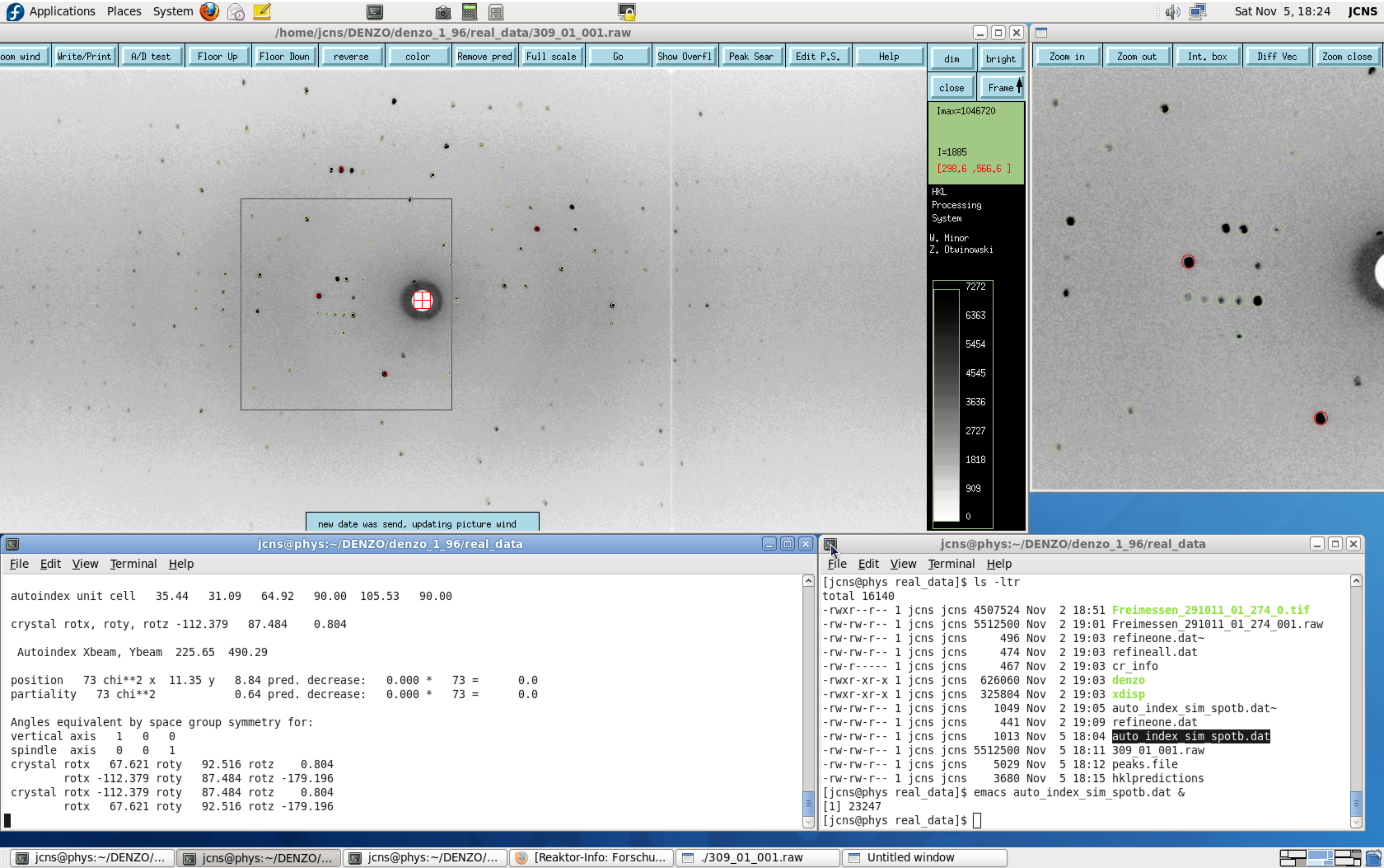
```
-rw-rw-r-- 1 jcns jcns 5512500 Nov  5 18:03 309_01_001.raw
-rw-rw-r-- 1 jcns jcns    1013 Nov  5 18:04 auto_index_sim_spotb.dat
[jcns@phys real_data]$ ls -ltr
total 16148
-rwxr--r-- 1 jcns jcns 4507524 Nov  2 18:51 Freimessen_291011_01_274_0.tif
-rw-rw-r-- 1 jcns jcns 5512500 Nov  2 19:01 Freimessen_291011_01_274_001.raw
-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    1269 Nov  2 19:07 peaks.file
-rw-rw-r-- 1 jcns jcns     441 Nov  2 19:09 refineone.dat
-rw-rw-r-- 1 jcns jcns   14288 Nov  2 19:13 hklpredictions
-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
[jcns@phys real_data]$ 
```

```
-rw-rw-r-- 1 jcns jcns    1047 Nov  2 19:03 auto_index_sim_spotb.dat~
-rw-rw-r-- 1 jcns jcns     496 Nov  2 19:03 refineone.dat
```

19.8 MW

Shutterstellung NL-Anlage

auto-index

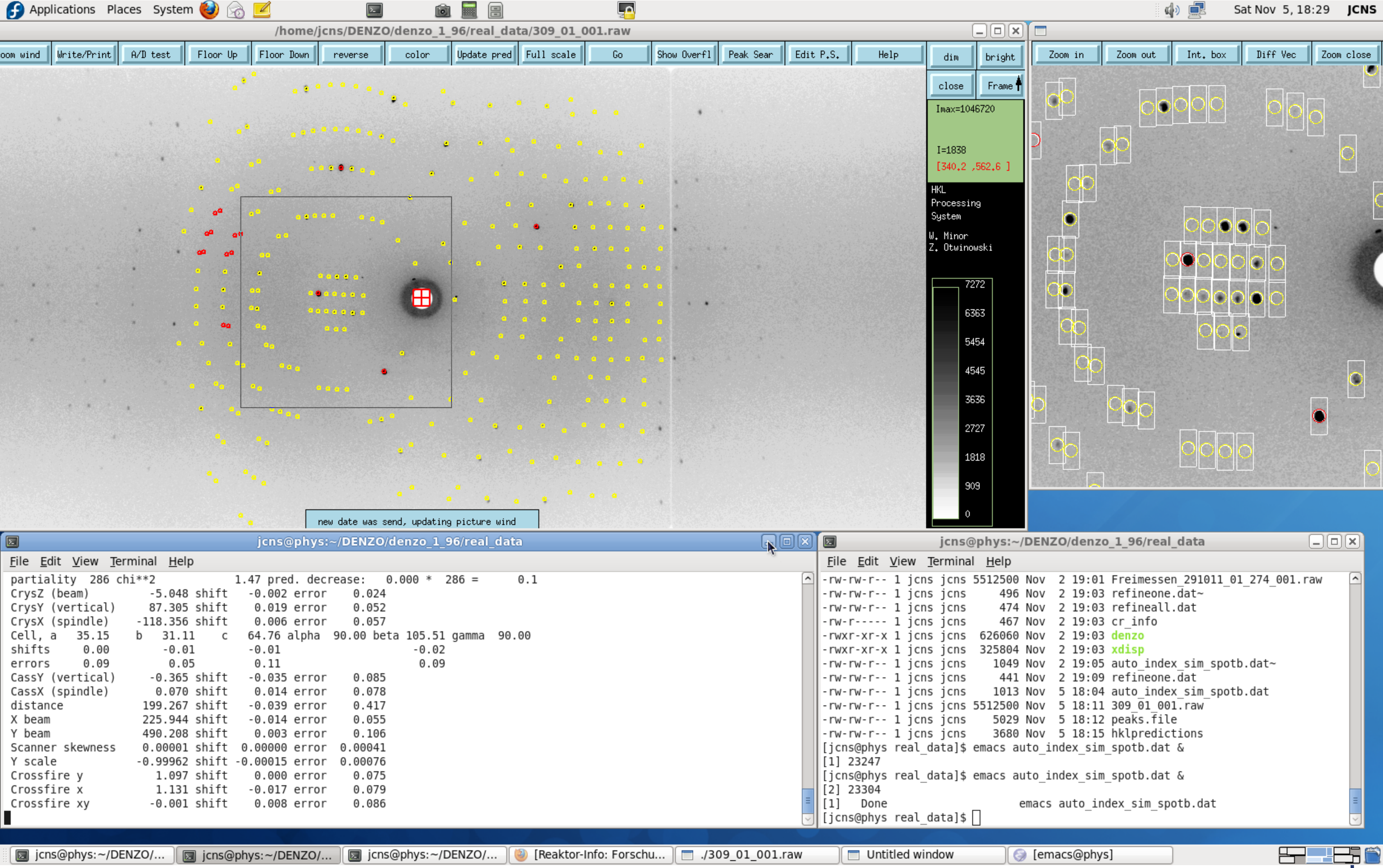


new date was send, updating picture wind

```
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 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]$ 
```

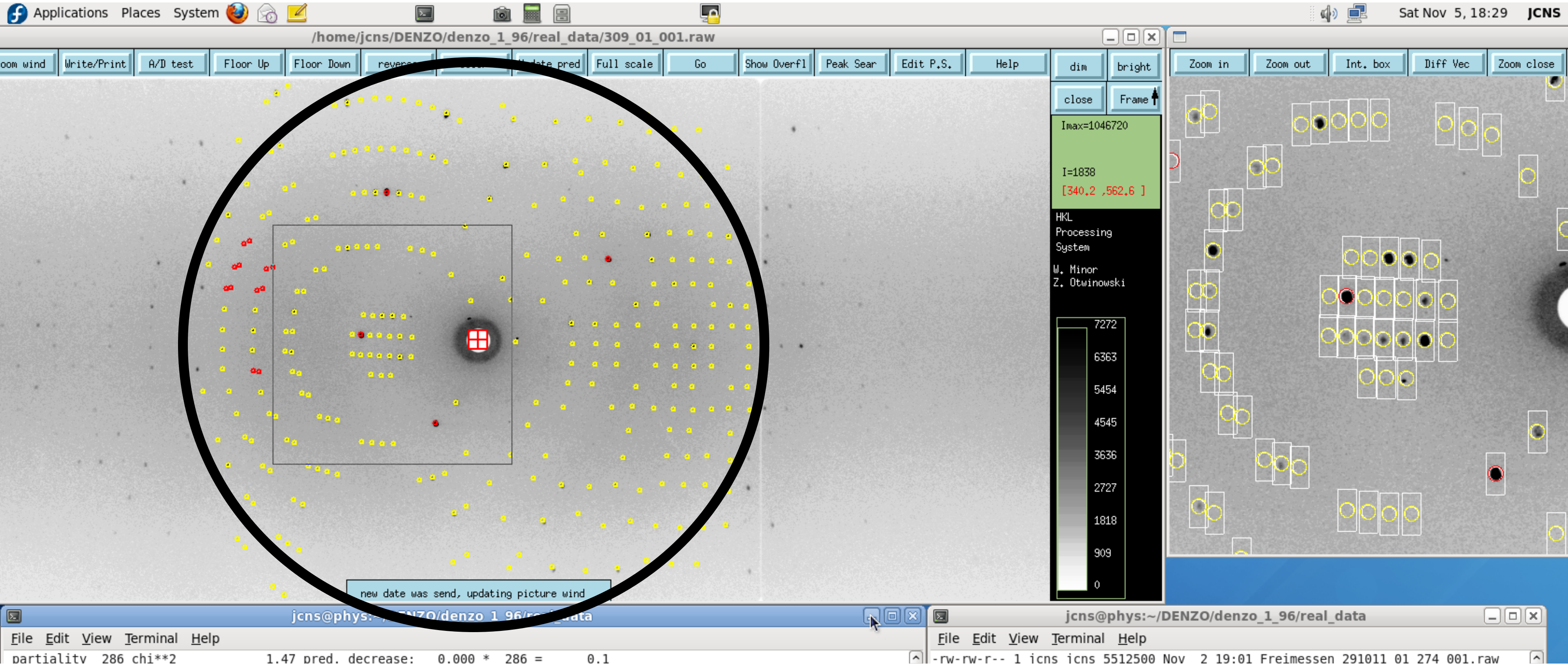
d_min=2.5 A



```
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
```

```
-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]$ 
```

d_{min}=2.5 A



```
new date was send, updating picture wind
```

jcns@phys:~/DENZO/denzo_1_96/real_data

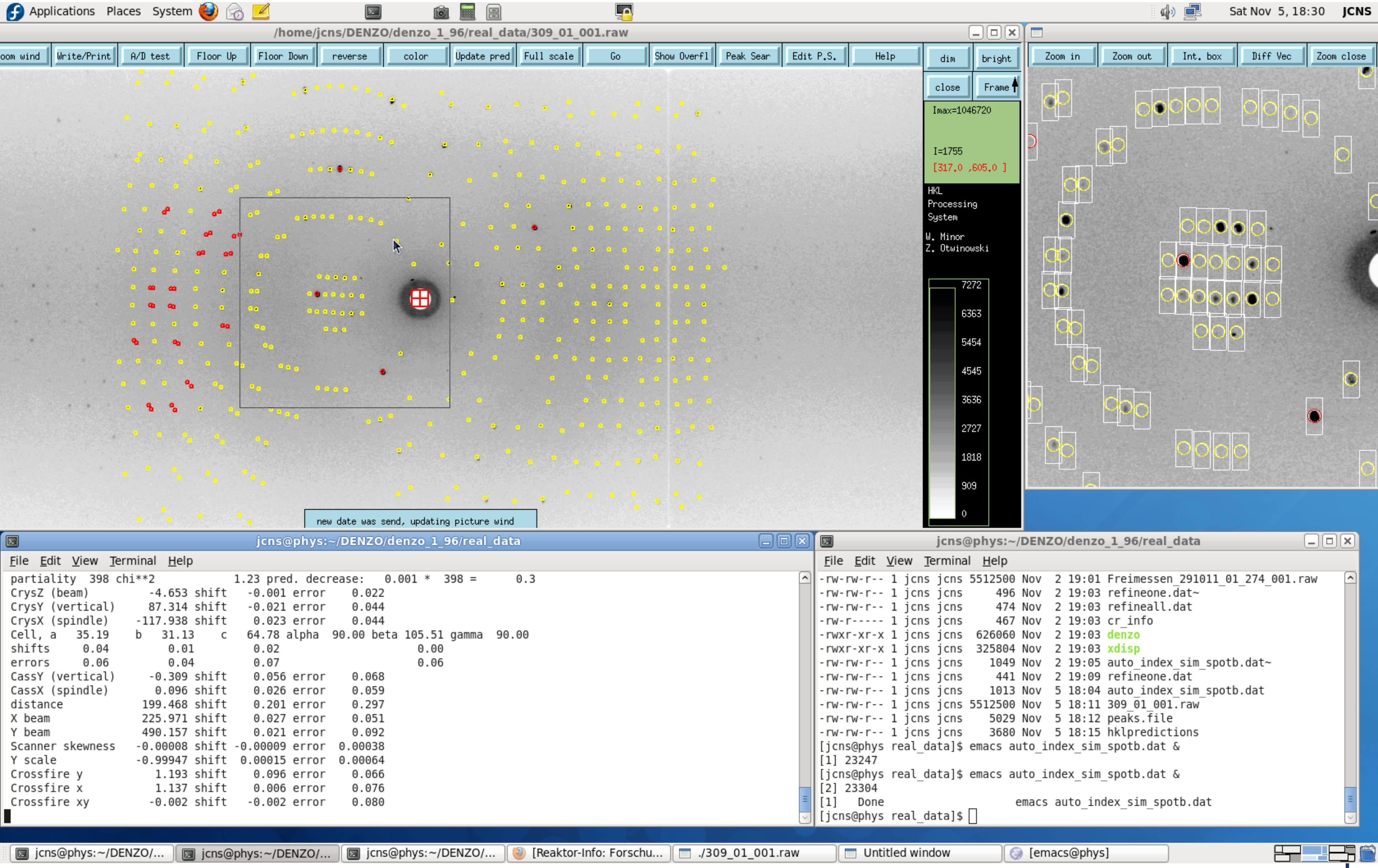
```
partiality  286 chi**2          1.47 pred. decrease:   0.000 *  286 =      0.1
```

jcns@phys:~/DENZO/denzo_1_96/real_data

```
-rw-rw-r-- 1 jcns jcns 5512500 Nov  2 19:01 Freimessen 291011 01 274 001.raw
```

Resolution shell of 2.5 Å

d_min=2.0 A



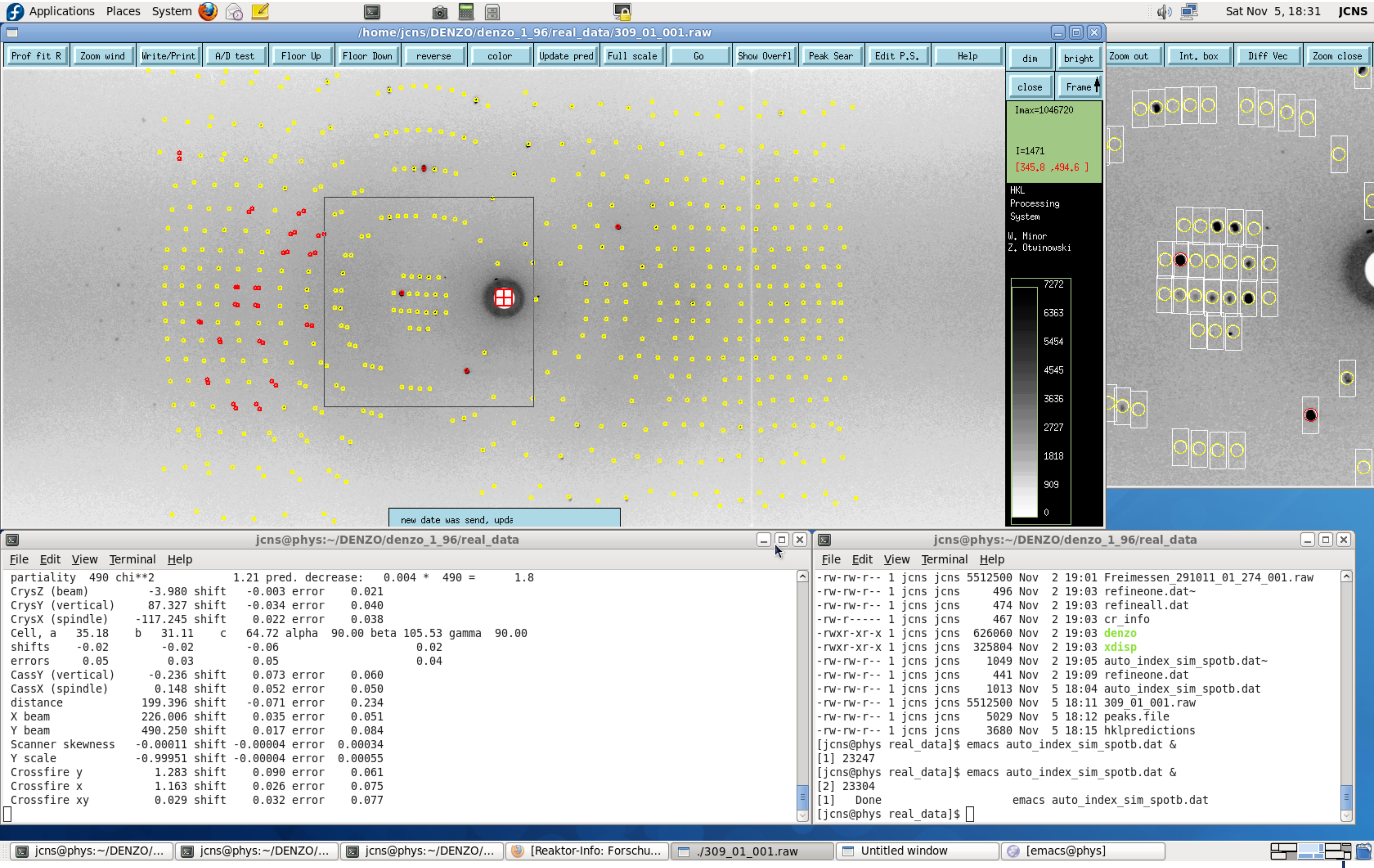
```
Imax=1046720

I=1755
[317.0 ,605.0 ]
HKL
Processing
System

W. Minor
Z. Otwinowski
```

```
new date was send, updating picture wind
```

```
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
```

```
-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]$ 
```

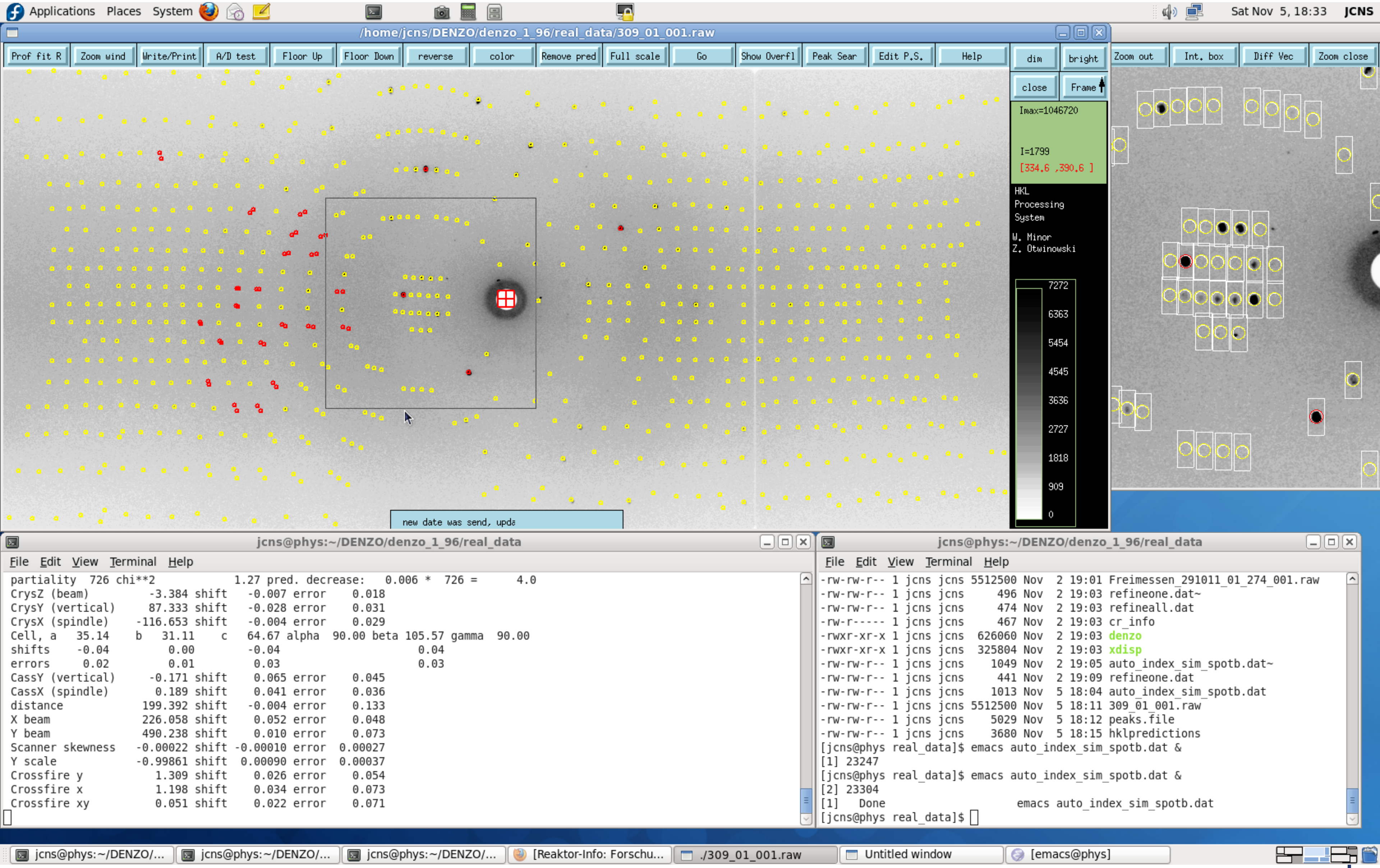
d_min= 1.8 A



```
partiality  490 chi**2           1.21 pred. decrease:   0.004 *  490 =      1.8
CrysZ (beam)         -3.980 shift   -0.003 error    0.021
CrysY (vertical)     87.327 shift   -0.034 error    0.040
CrysX (spindle)    -117.245 shift    0.022 error    0.038
Cell, a    35.18      b    31.11      c    64.72 alpha  90.00 beta 105.53 gamma  90.00
shifts     -0.02          -0.02          -0.06                   0.02
errors      0.05           0.03           0.05                   0.04
CassY (vertical)     -0.236 shift    0.073 error    0.060
CassX (spindle)       0.148 shift    0.052 error    0.050
distance            199.396 shift   -0.071 error    0.234
X beam              226.006 shift    0.035 error    0.051
Y beam              490.250 shift    0.017 error    0.084
Scanner skewness   -0.00011 shift -0.00004 error  0.00034
Y scale            -0.99951 shift -0.00004 error  0.00055
Crossfire y           1.283 shift    0.090 error    0.061
Crossfire x           1.163 shift    0.026 error    0.075
Crossfire xy          0.029 shift    0.032 error    0.077
```

```
-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]$ 
```

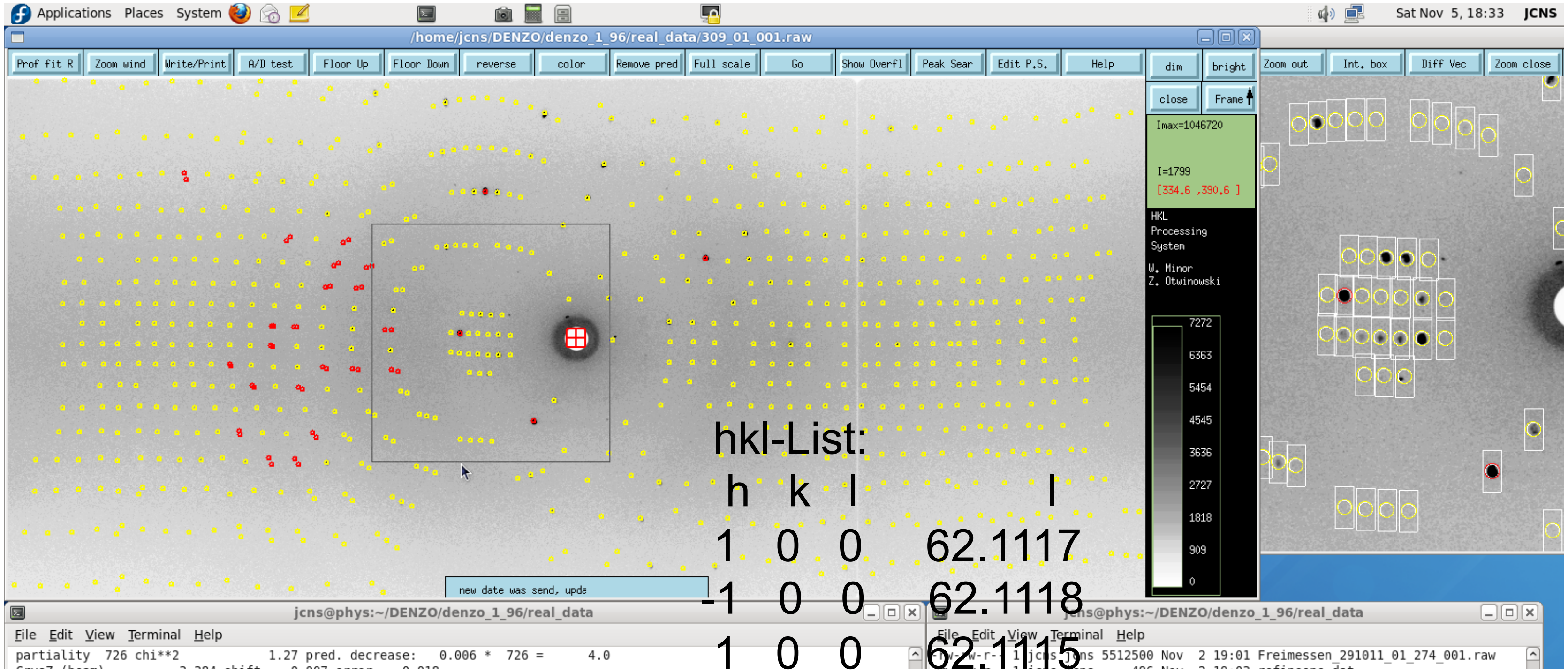
d_min=1.5 A



```
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
```

```
-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]$ 
```

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



```
partiality  726 chi**2             1.27 pred. decrease:    0.006 *  726 =       4.0
```

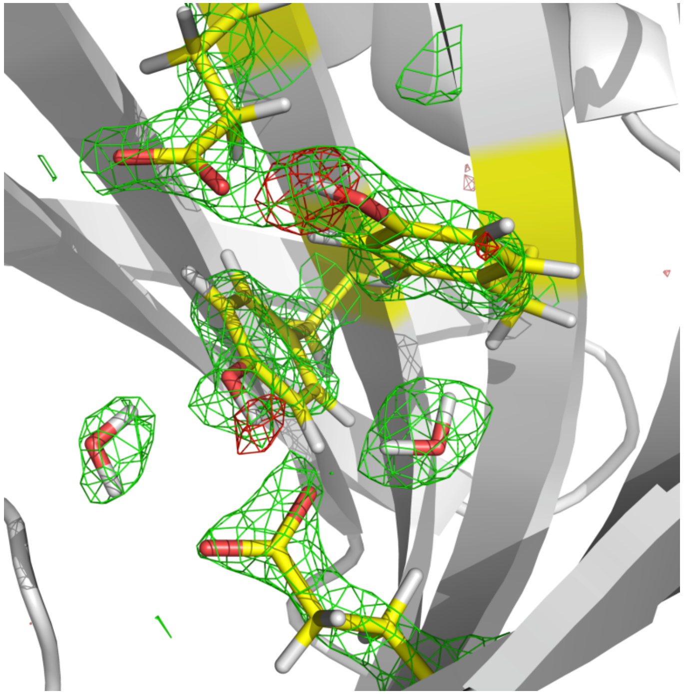
```
-rw-rw-r-- 1 jcns jcns 5512500 Nov  2 19:01 Freimessen_291011_01_274_001.raw
```

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

Xylanase II (pH 5.8):

A. Kovalevsky, **Q. Wan**, L. Coates, D.Graham, Q. Zhang (Group of P. Langan)



d_{min}	I/σ(I)	N_{meas}	mult.	compl. in shell %	R_{merge} %
4.31	52.9	9709	6.2	98.0	3.1
3.42	34.6	9306	6.2	99.6	5.0
2.99	21.0	8456	5.8	99.4	7.9
2.71	11.9	7400	5.1	98.9	12.9
2.52	8.2	6806	4.8	97.1	17.2
2.37	6.0	6142	4.4	96.3	20.6
2.25	4.8	4935	3.7	92.8	23.4
2.15	4.5	5061	3.7	94.9	25.6
2.07	3.6	4998	3.7	93.2	30.8
2.0	3.0	4313	3.3	91.0	32.7
overall	**11.2**	**67126**	**4.7**	**96.2**	**9.7**

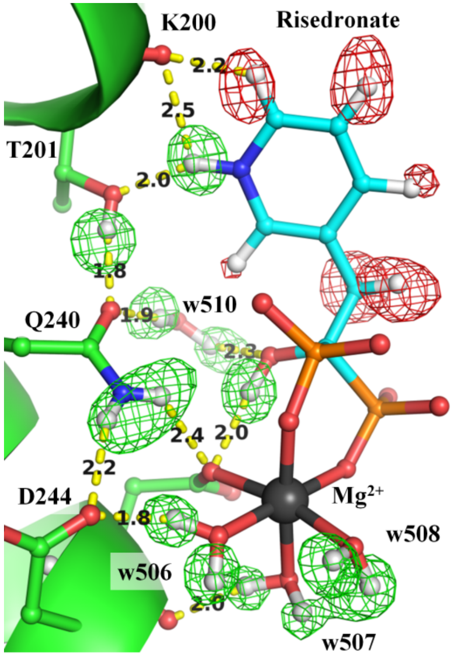
- unit cell: 49.5Å, 59.9Å, 70.41Å $P2_12_12_1$
- crystal size: 2.8mm^3
- Collection time: 17d

Human farnesyl pyrophosphate synthase with risedronate:

⟶ limited resolution but high completeness

T. Yokoyama, M. Mizuguchi, N. Niimura, I. Tanaka

d_{min} = 2.4Å



F_oF_c-omit-maps: positve in green, negative in red;

- unit cell: 111.9Å, 111.9Å, 72.6Å $P4_12_12$
- crystal size: 3.5mm³
- collection time: (5d, 6d, 14d)

d_{min}	I/σ(I)	N_{meas}	mult.	compl. in shell %	R_{merge} %
140.0 – 5.17	29.1	12784	6.6	94.9	4.3
5.17 – 4.10	14.5	9490	5.0	99.3	9.8
4.10 – 3.59	10.6	8045	4.3	99.5	12.3
3.59 – 3.26	7.0	5833	3.2	98.5	15.5
3.26 – 3.02	6.1	6443	3.5	99.3	19.5
3.02 – 2.85	4.5	6181	3.4	98.6	24.9
2.85 – 2.70	3.3	5772	3.2	98.6	31.2
2.70 – 2.59	2.5	5442	3.0	98.5	39.8
2.59 – 2.49	2.1	5260	2.9	99.0	46.2
2.49 – 2.40	1.8	4846	2.7	98.0	61.2
overall	**8.2**	**69977**	**3.8**	**98.4**	**10.7**

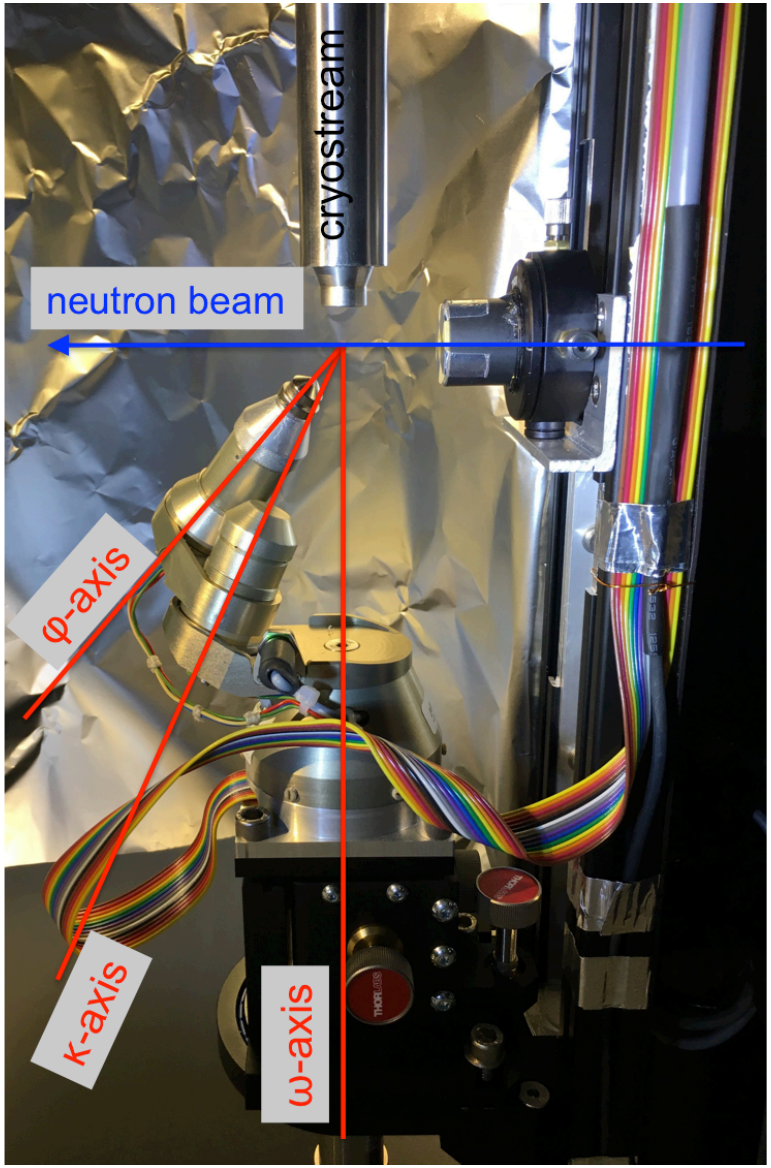
R_{pim} = 5.8% (37.1%)

Sample enviroment @ BioDiff

BioDiff Upgrade:

mini-kappa-goniometer

Standard Oxford cryostream 700plus: T = 90 - 500K



- optimizing data collection strategy
  - save precious beam time / increase data set complete
- no manual crystal re-mounting necessary for changing the crystal orientation under cryo conditions

Why are proteins interesting?

Due to their important roles in all processes of life:

Enzymatic: Enzymatic proteins accelerate metabolic processes in your cells.

Structural: Structural proteins are necessary components of your body.

Defensive: Antibodies, or immunoglobulins, are a core part of your immune system.

Storage: Storage proteins mainly store mineral ions in your body.

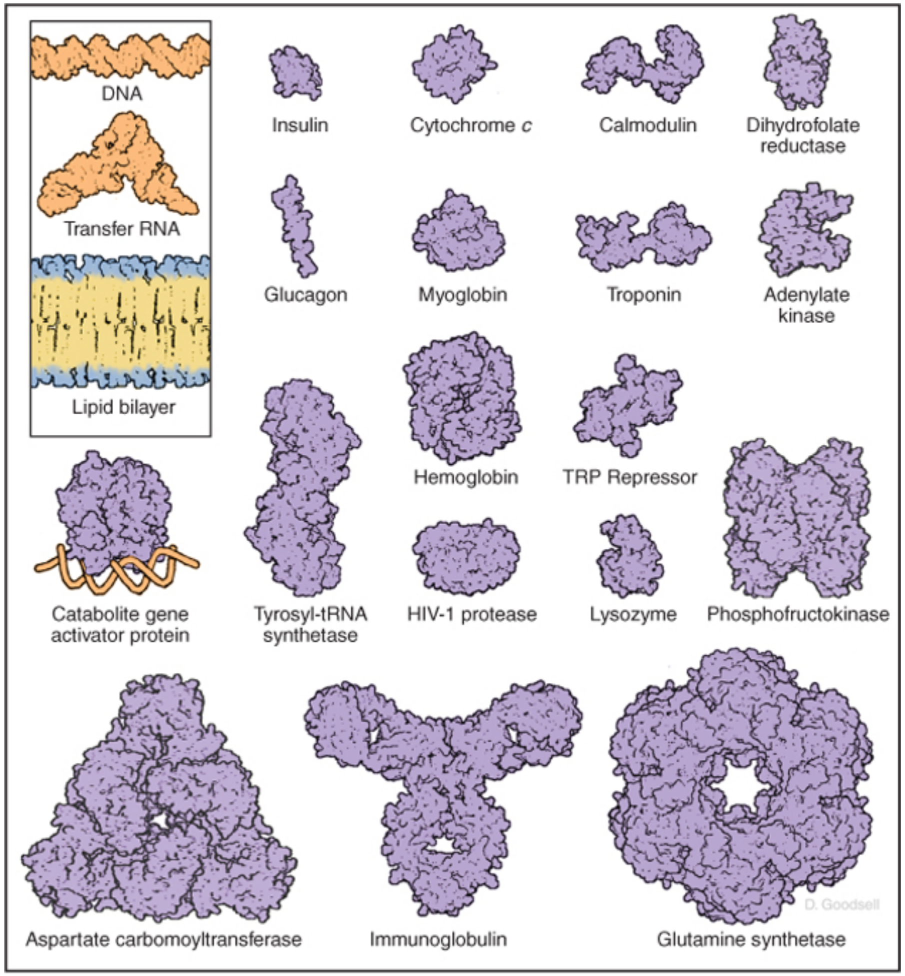
Transport: Transport proteins carry vital materials to the cells.

Receptor: Located on the outer part of the cells, receptor proteins control the substances that enter and leave the cells.

Contractile: Also known as motor proteins, contractile proteins regulate the strength and speed of heart and muscle contractions.

This is why misfolded or genetically altered proteins lead to serious diseases…

Proteins come in different shapes and sizes



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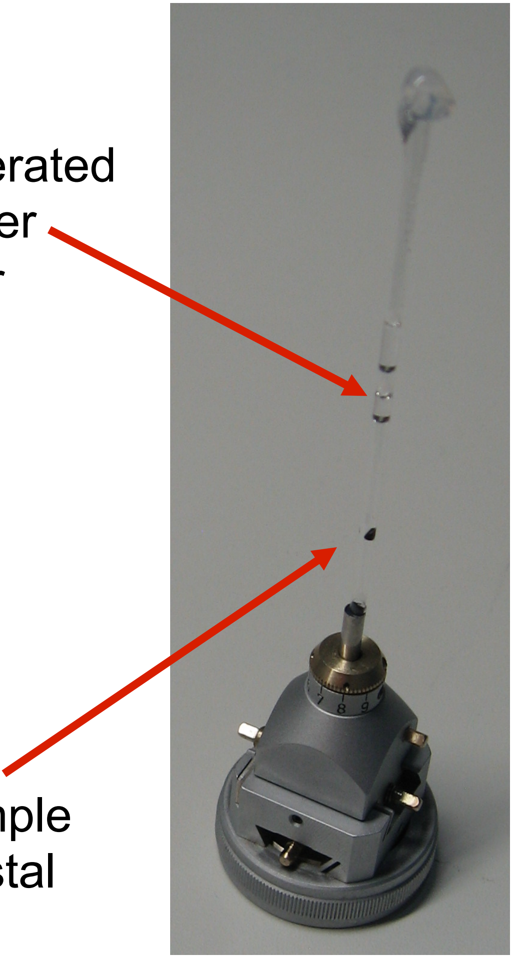
This is where the crystallography comes in…

Some general remarks on crystal mounting

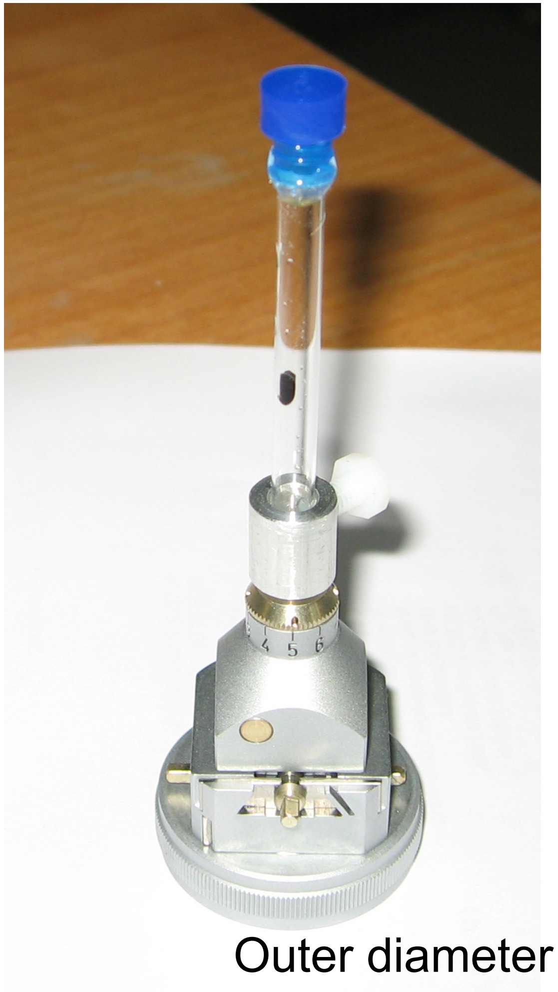
Room temperature packaging of crystals



deuterated mother liquor

sample crystal

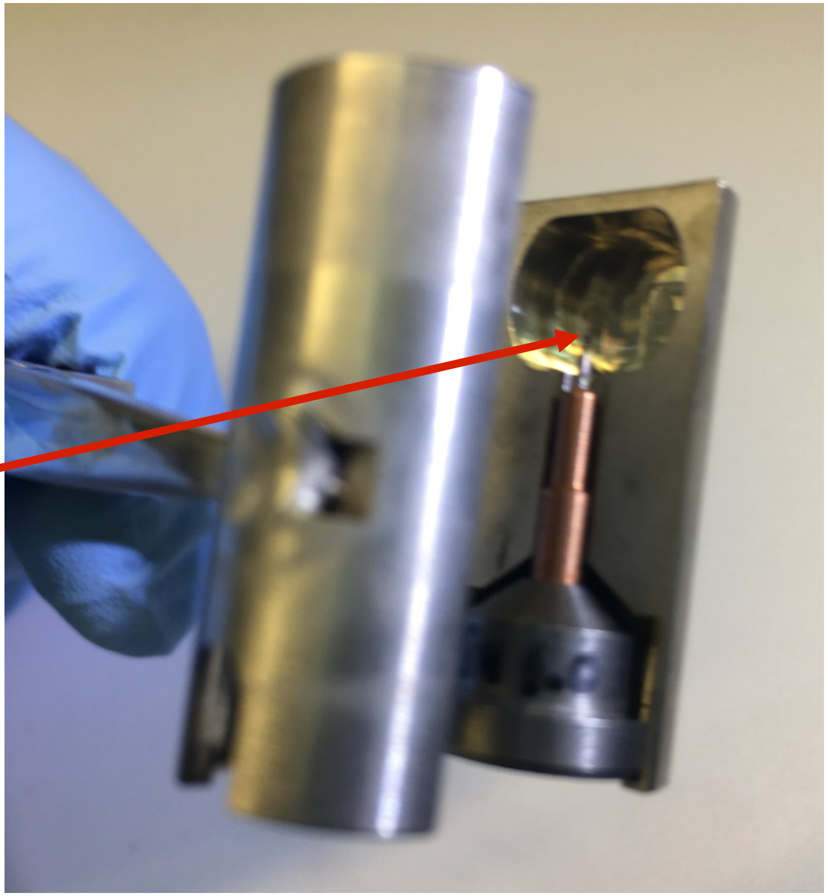
Outer diameter of the glas tube: 2 mm



- 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

Cryo-mounting 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.

If your crystal is still too small: Consider a proposal to the D-Lab

http://www.ill.eu/sites/deuteration/

- Fill in a two page word form as an application
- receive decision on it within a few weeks
- Send a post-Doc or a PhD student to the D-Lab in Grenoble, France
- Use the expertise of the D-Lab to grow cells on minimal fully deuterated media
- make the cells express your protein
- purify the protein from the cells
- grow the fully deuterated crystals at your home lab

Short summary:

→ Neutrons can contribute to the understanding of the catalytic processes of proteins

→ BIODIFF is a monochromatic instrument
possible wavelength range 2.4 – 5.6Å

→ Standard cryostream system in operation, with mini-kappa gioniometer head

→ Users are welcome!

Upcoming deadline for proposals: July 21st, 2017

all users are welcome!

user.frm2.tum.de

fzj.frm2.tum.de

We are open for suggestions on sample environments and support labs for BioDiff!

Collaborators:

Project leaders:

Prof. Dr. W. Petry	FRM II
Prof. Dr. D. Richter	JCNS
Prof. Dr. F. Parak	TUM (started project)

Instrument co-responsible:

Dr. A. Ostermann	TUM

Beamline Post-doc:

Dr. M. Longo	JCNS

Engineers :

B. Laatsch	FZ-Jülich
P. Jüttner	FRM II

Thank You!