Neutron Protein Crystallography

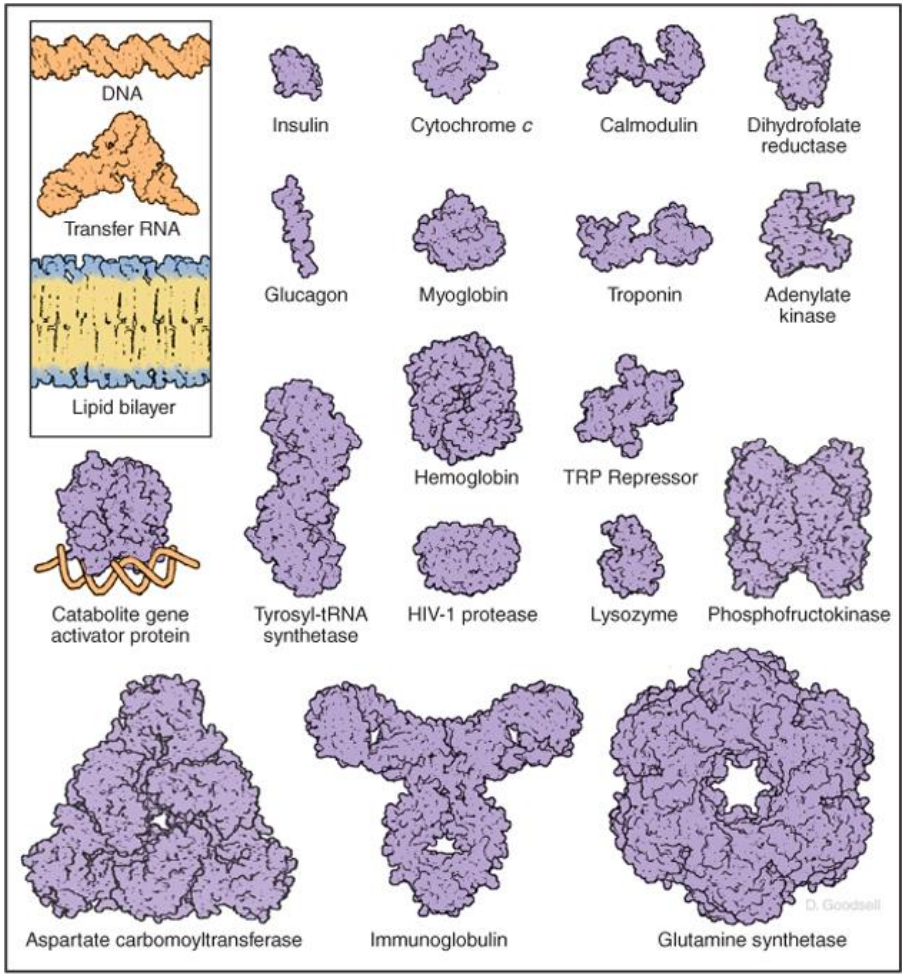
ECM satellite, Vienna, Austria

August 16th 2019 | Tobias E. Schrader

Outline

- Motivation: Why do we need protein structures at atomic resolution?
- x-ray protein crystallography
- neutron protein crystallography
- Theory of scattering from crystals
- One or two application examples: From Structure to function…

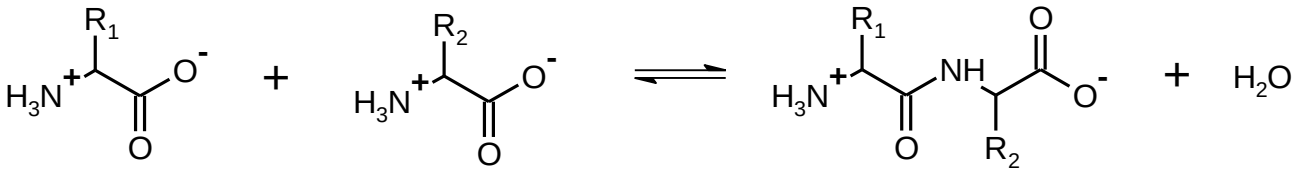
Proteins or structured macromolecules come in different shapes and sizes



Definition of the name "protein"

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

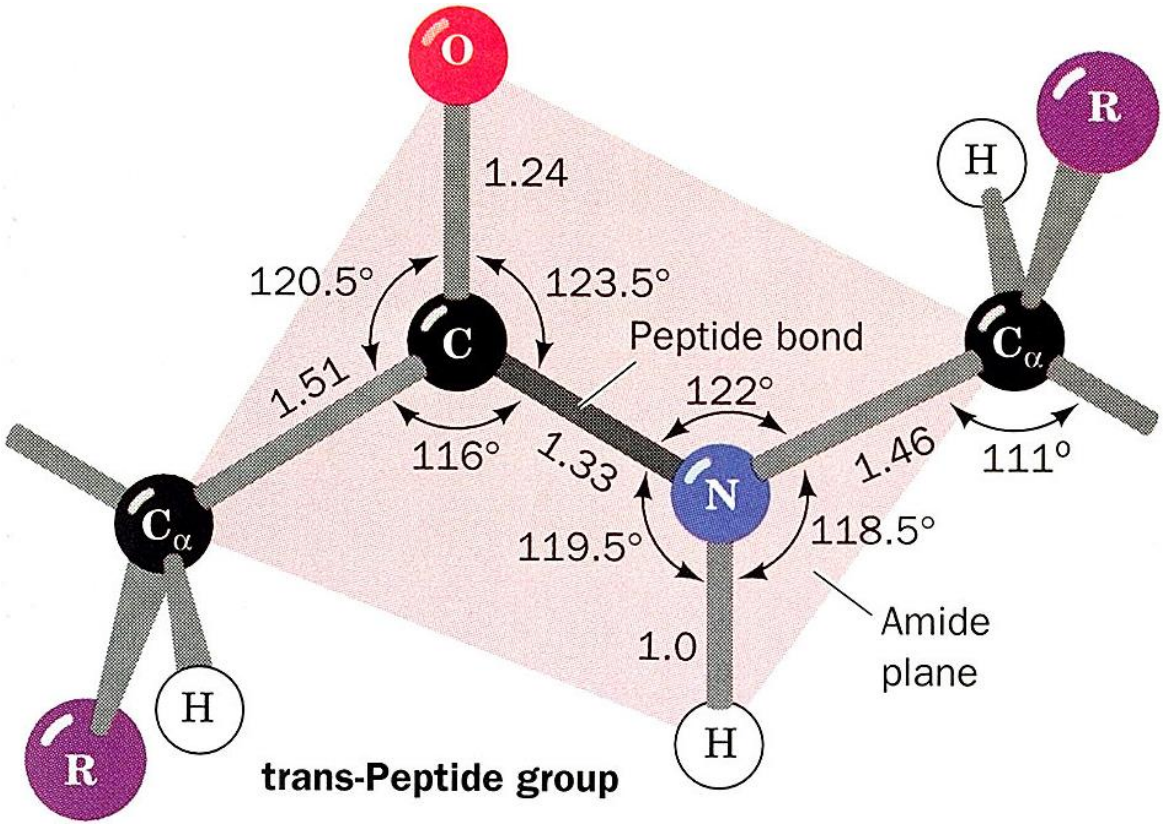
• They are polymers of α-amino acids (2-amino carboxylic acids), formed by condensation of carboxyl and amino functions of adjacent units.



• Historical nomenclature:
n < 10: oligopeptide
10 < n < 100: polypeptide
n > 100: protein

• Preferable: A protein is a polypeptide with a defined three-dimensional fold.

Character of N-C bond is partially a double bond: H-N-C=O form a plane



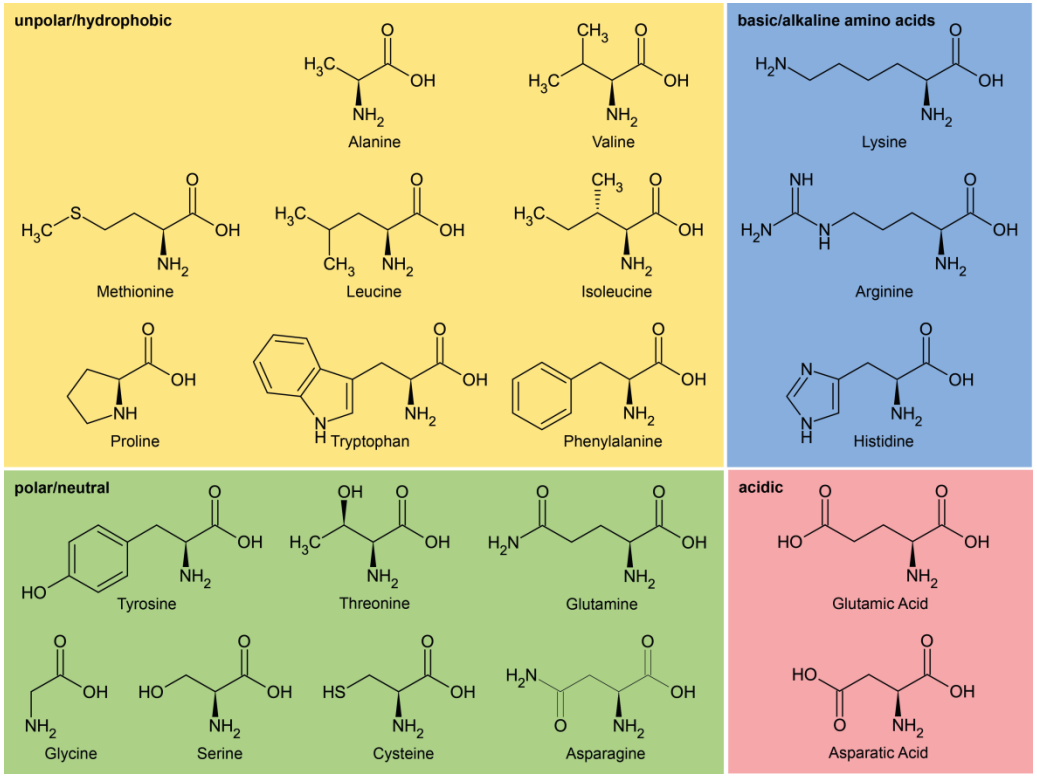
The 20 amino acids occuring in nature

- Side chains have different length and character
- α-Carbon is a chiral centre (exception: glycine)

=> D- and L-forms possible

- Cellular protein synthesis uses L-enantiomers only.

Proteins are chiral molecules!



The hierarchy of protein architecture

- Primary structure

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

- Secondary structure

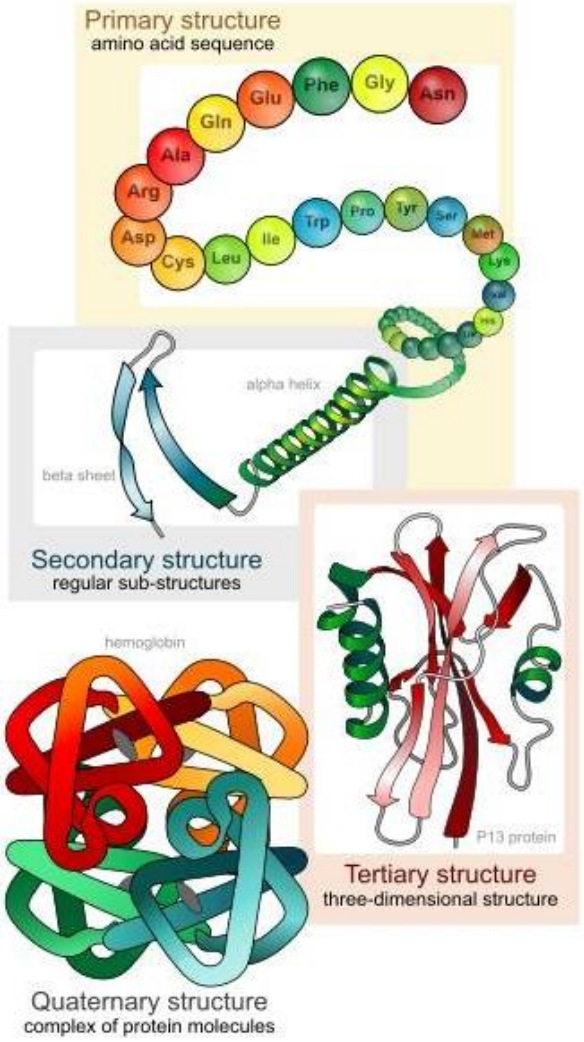
The local arrangement of main-chain atoms, often with specific hydrogen bonding pattern

- Tertiary structure

The packing of non-contiguous segments into a compact fold

- Quaternary structure

The association of individual chains into oligomeric structures



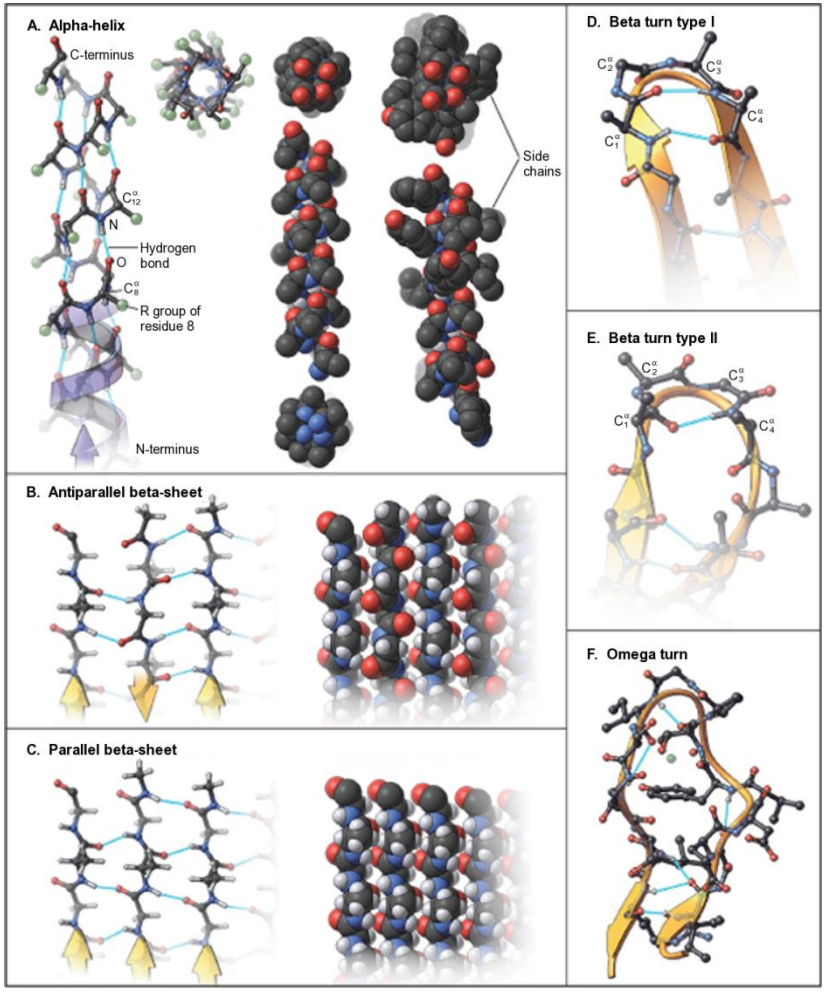
Secondary structural elements

α-helix
spiral conformation of a continuous chain

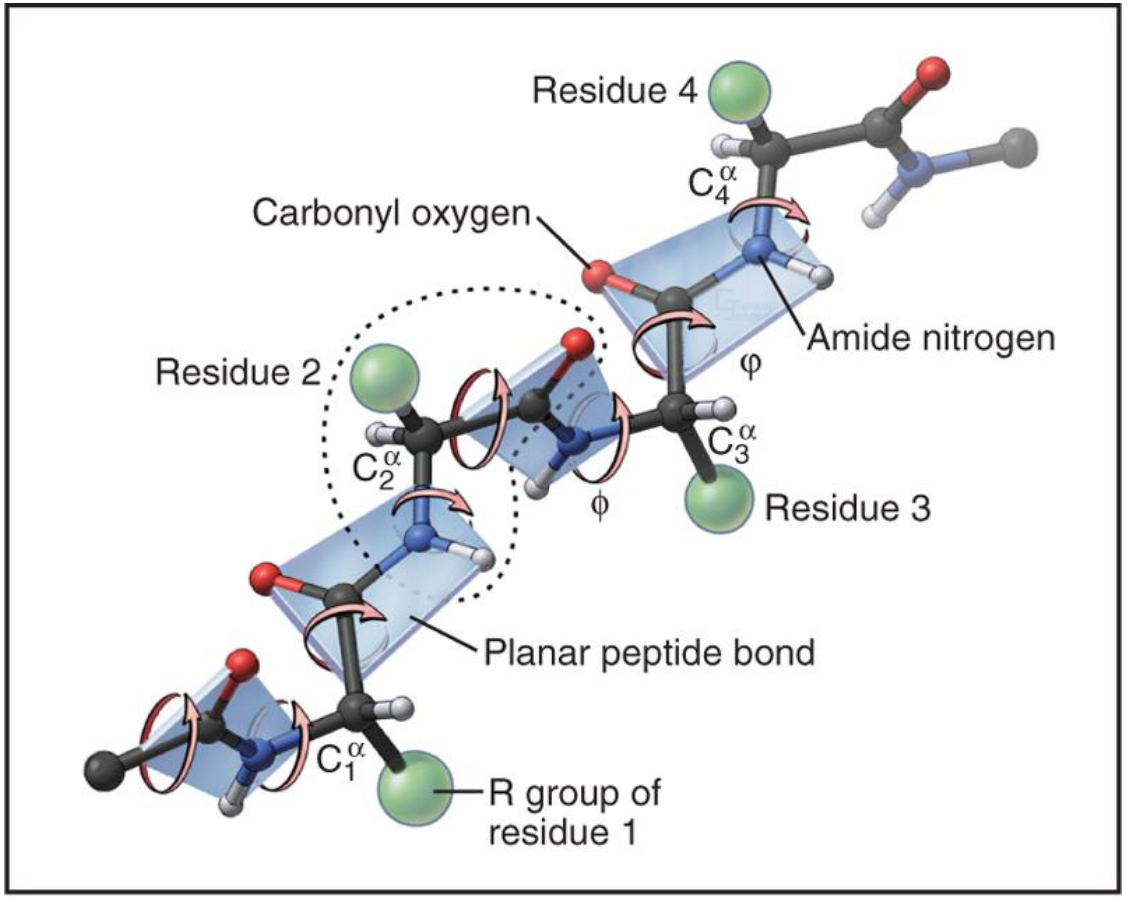
β-sheet
extended strands
aligned side-by-side

turns / loops
changing direction of chain

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



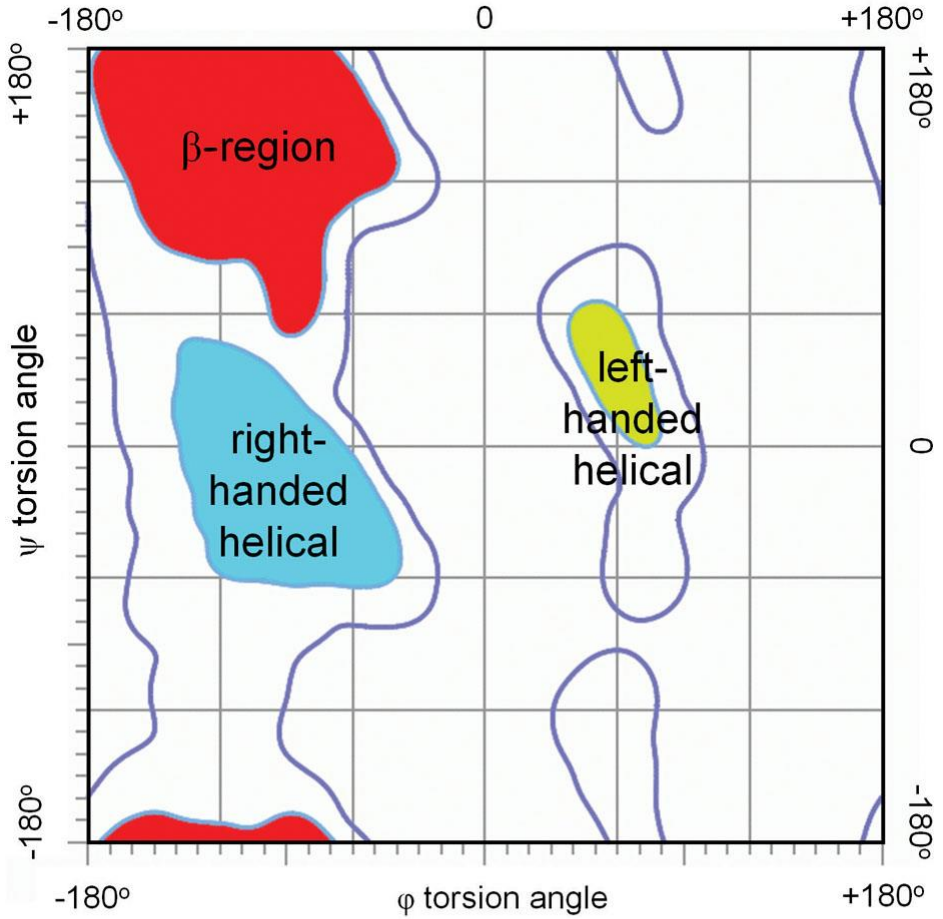
Definition of dihedral angles



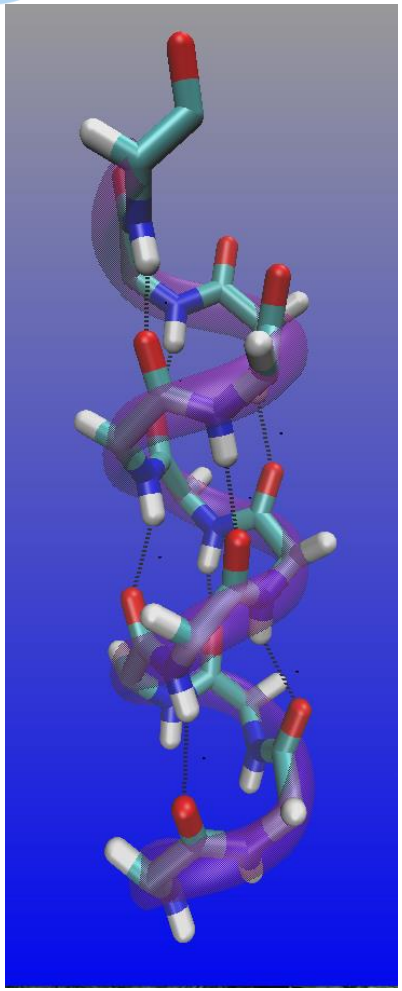
color code:
blue: nitrogen
red: oxygen
white: hydrogen
grey: carbon
green: symbol for side chains

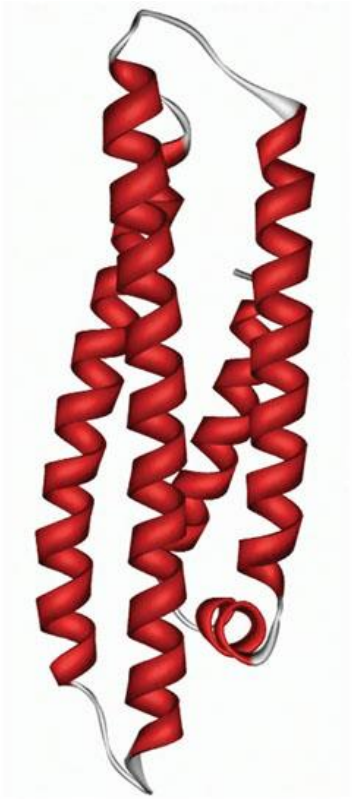
Per amino acid the peptide chain has these two dihedral angles as degrees o freedom.

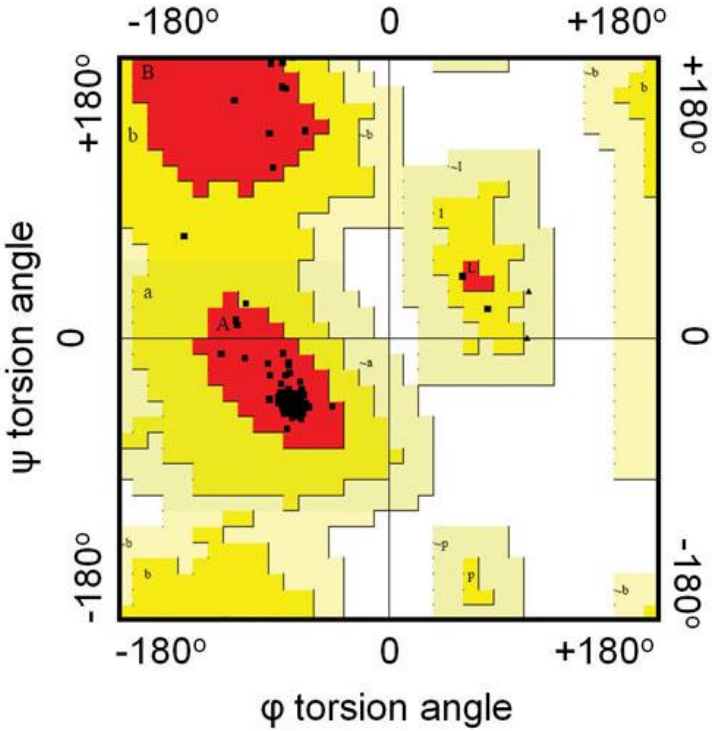
Not all dihedral angles a sterically allowed or favourable: The Ramachandran plot



α-Helix

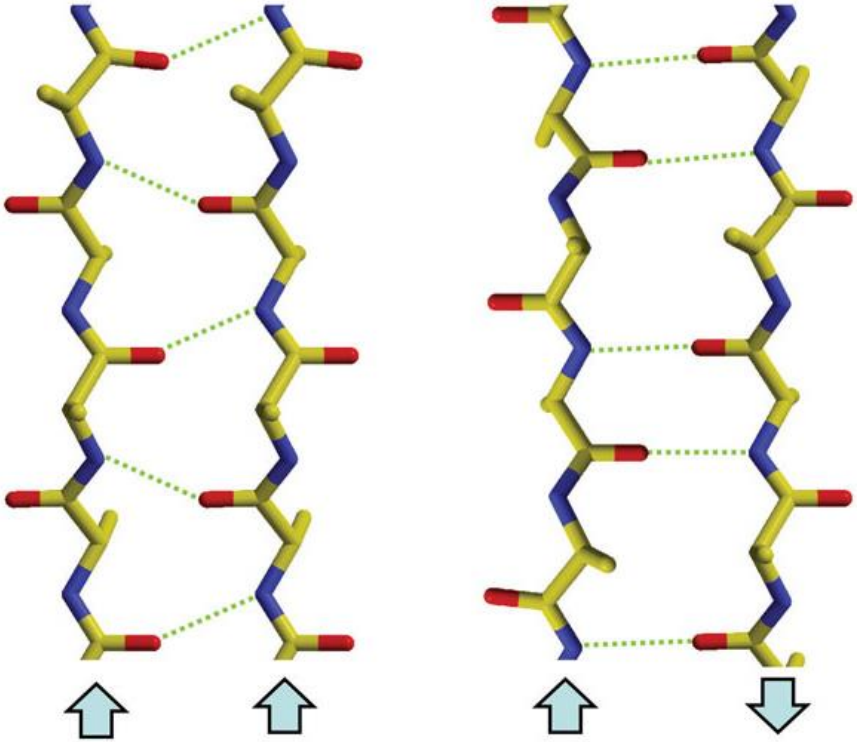


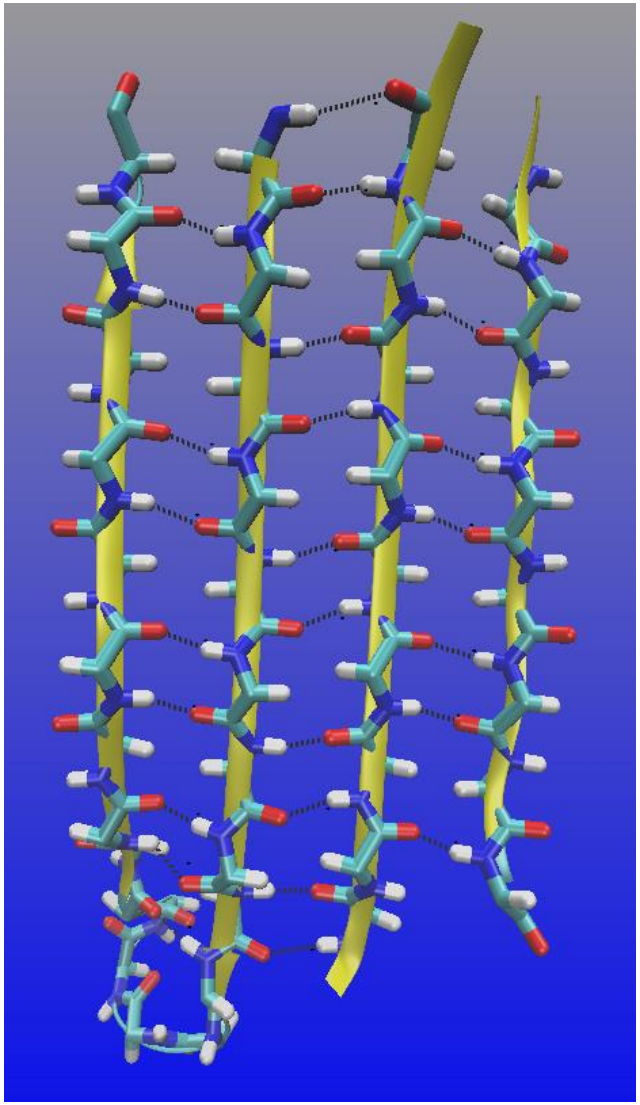




- Most common element of secondary structure in proteins
- BKP symbol 3.6_{13}: 3.6 residues per turn, 13 atom ring closed by hydrogen bond (n··n+4)

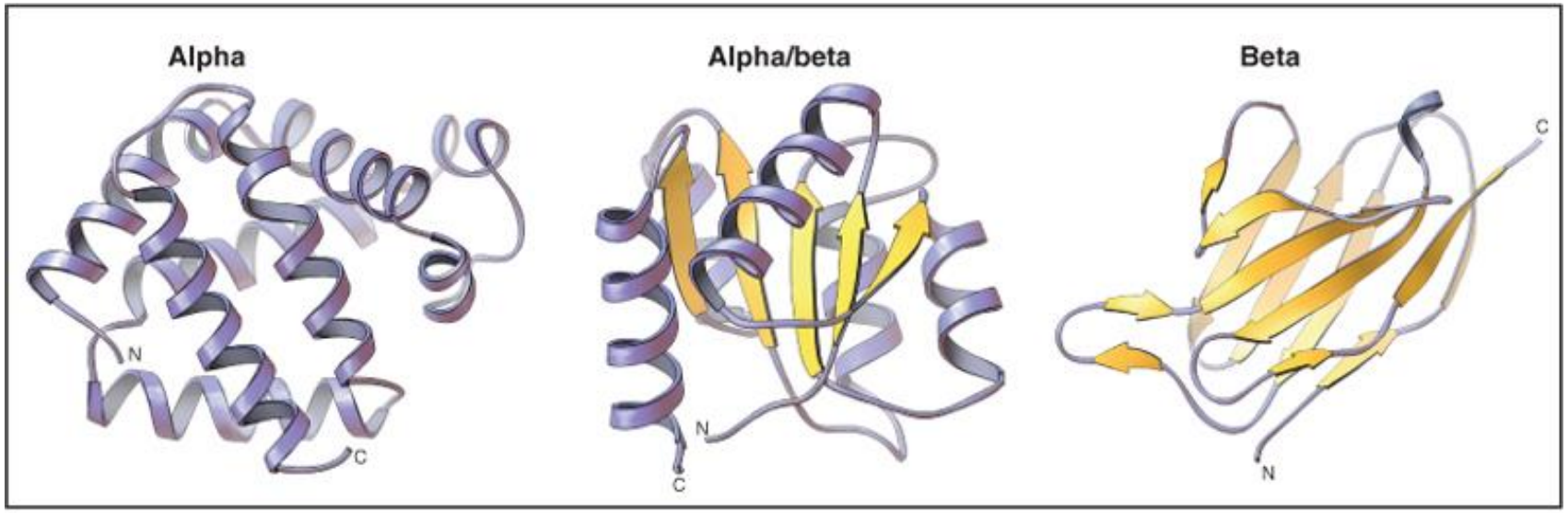
β-sheet





- parallel b-sheets: "wide pair" hydrogen bonds (less stable)
- antiparallel b-sheets: "close pair" hydrogen bonds (more fabourable)

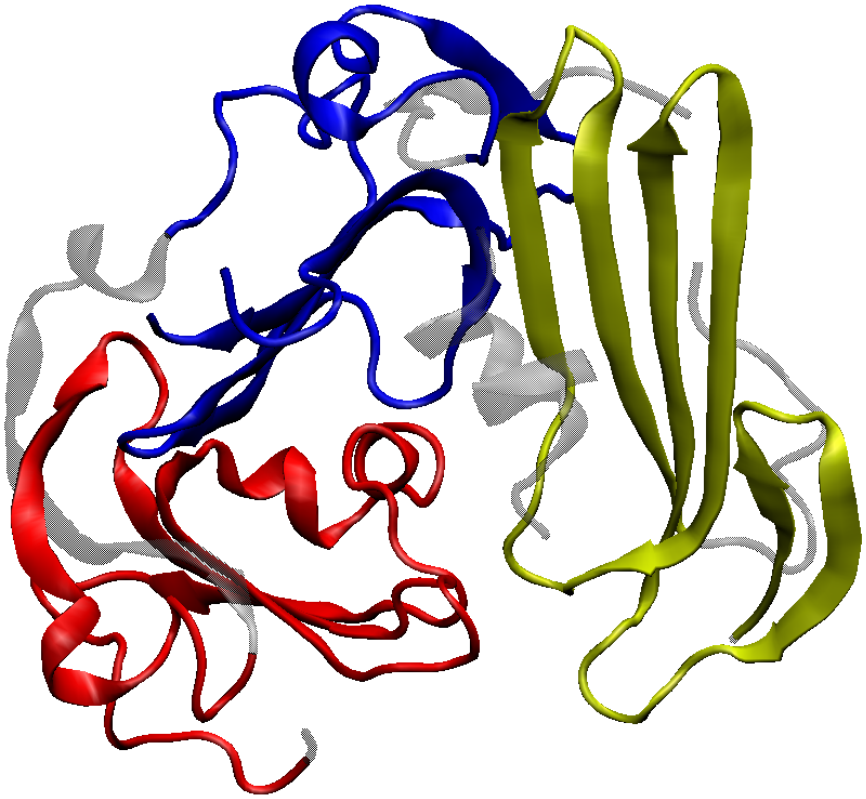
Tertiary structure: Fold classes



•Tertiary structure results from the arrangement of secondary structure elements self assembling into the native fold.

• This arrangement critically depends on side-chain interactions, the hydrophobic effect being a major driving force.

Tertiary structure: sub-dividing the protein into 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 fold: The protein folding problem

Unfolded states ⇌ Intermediate states ⇌ Native state(s)

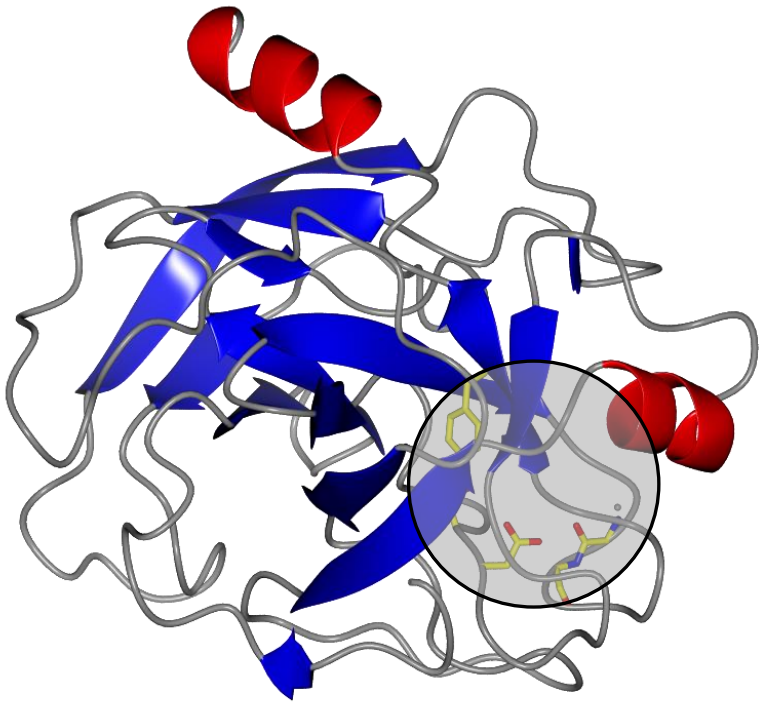
- Polypeptide chains tend to collapse into compact structures.
- Major driving force is the **hydrophobic effect** (entropic contribution of the solvent).
- Forces stabilizing folded proteins:

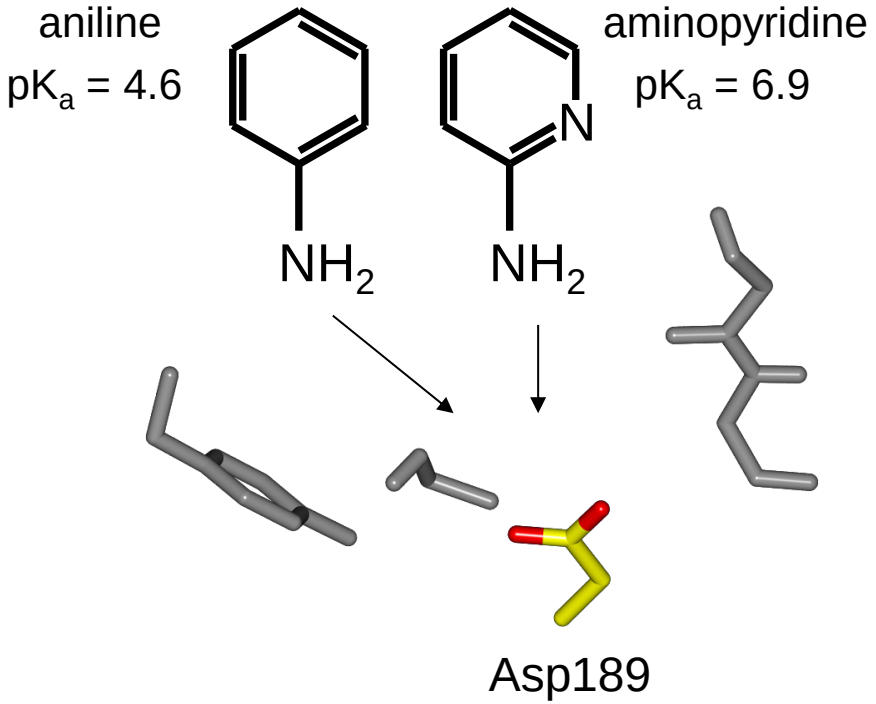
Non-covalent bonds	Covalent bonds
• Hydrogen bonds	• Disulfides
• Electrostatic forces	• Isopeptide bonds
• V. d. Waals contacts	

Summary of the protein part

- Proteins consist of a chain of amino acids.
- There are 20 amino acids occuring in nature.
- Proteins show a special 3-D structure which is specific to their function

Tutorial: Inhibitor binding to trypsin: charges shift protonation





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

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

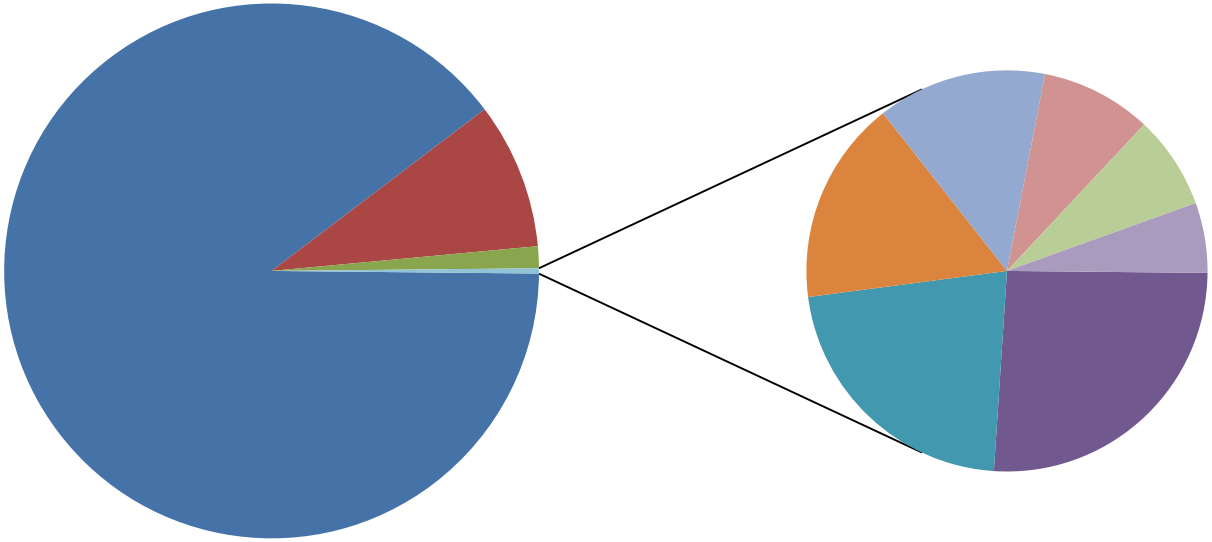
Schiebel J. et al., Angewandte Chemie Int. Edition (2017), 56 (17):4887-4890

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. 100



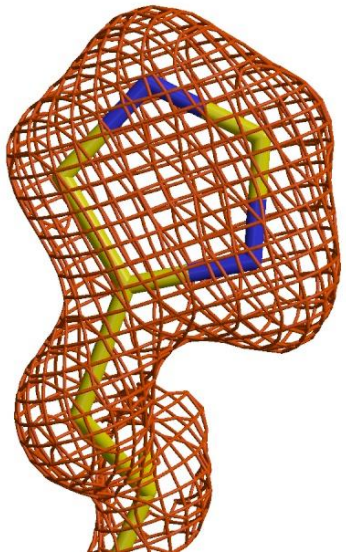
- x-ray (120534)
- solution NMR (11921)
- Electron Microscopy (1776)
- Hybrid (110)
- Solid State NMR (93)
- Electron Crystallography (70)
- Neutron Diffraction (58)
- Fiber Diffraction (38)

http://www.rcsb.org/

Total number of structures: 134656

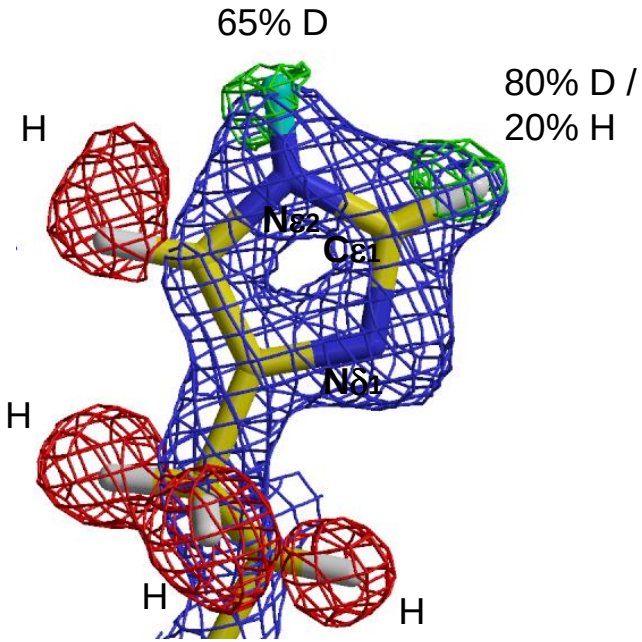
Protonation states of amino acids:

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

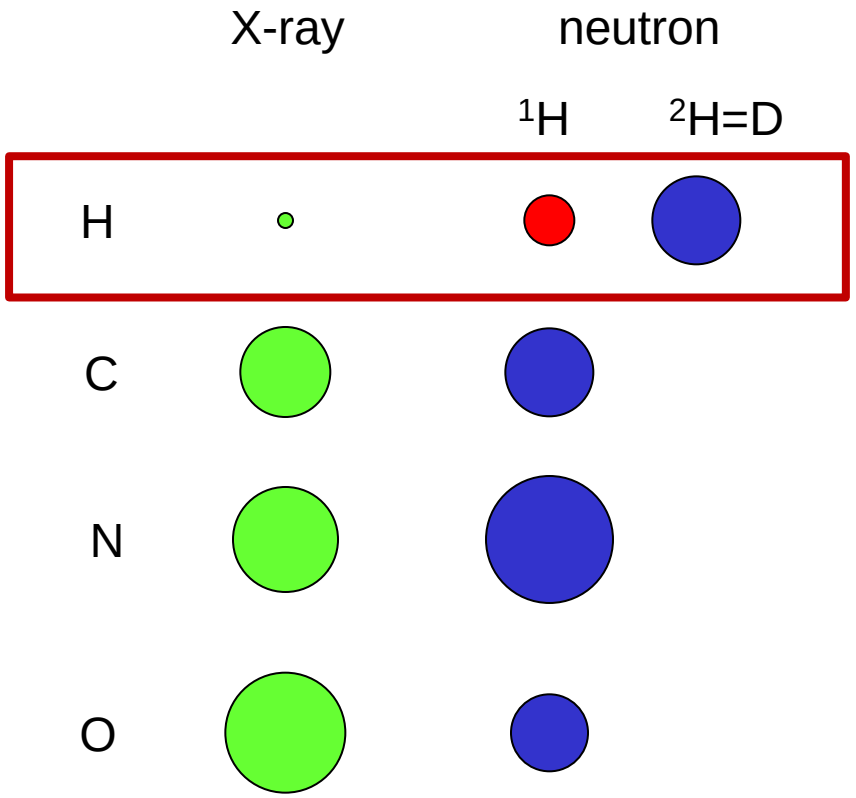
Fo-Fc omit-map; +3.0σ

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):

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!

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

x,y,z coordinates (Å)

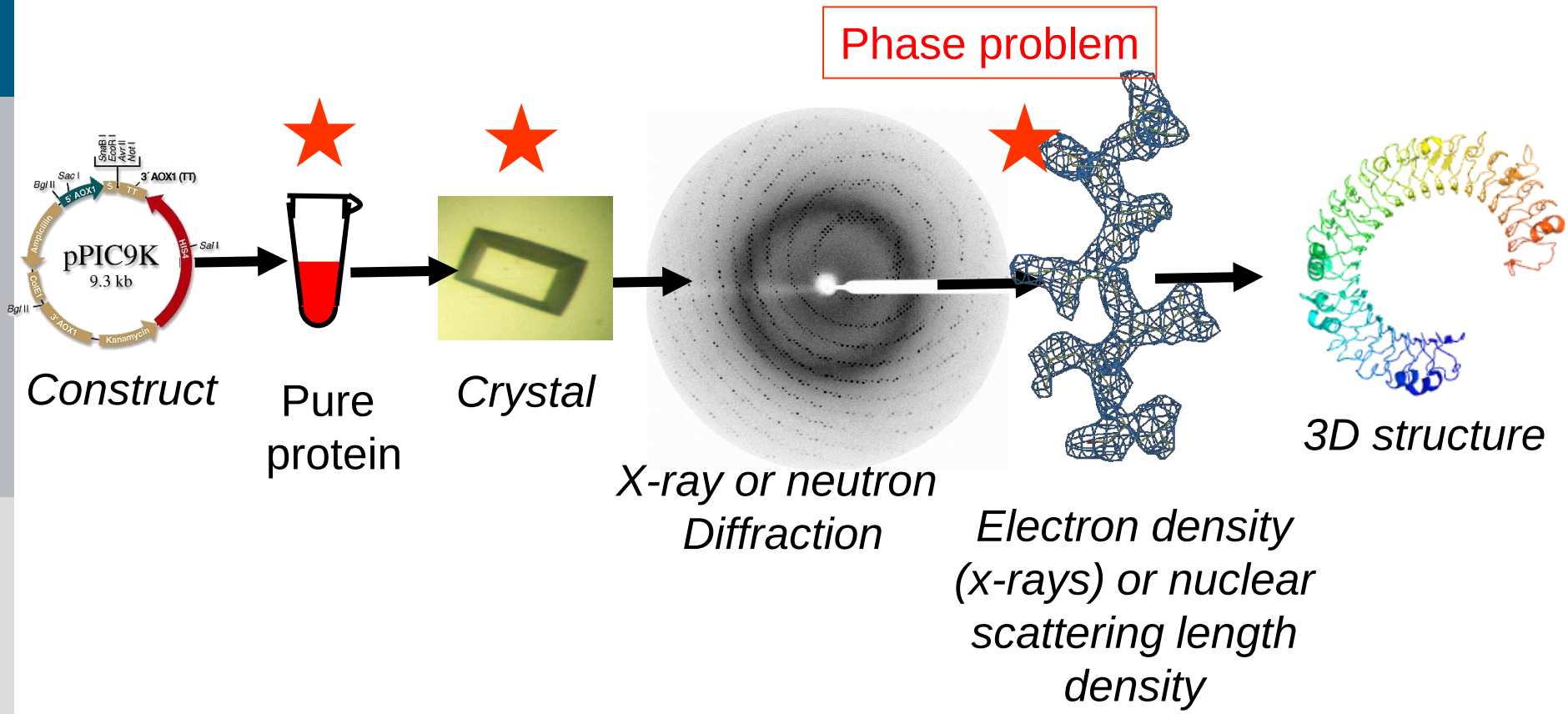
ATOM	25	N	ASP	A	928	19.062	9.157	35.067	1.00	4.73	N
ATOM	26	CA	ASP	A	928	19.770	10.123	34.232	1.00	4.58	C
ATOM	27	C	ASP	A	928	19.075	9.938	32.899	1.00	4.56	C
ATOM	28	O	ASP	A	928	19.074	8.824	32.351	1.00	5.39	O
ATOM	29	CB	ASP	A	928	21.259	9.776	34.071	1.00	3.13	C
ATOM	30	CG	ASP	A	928	22.112	10.245	35.233	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

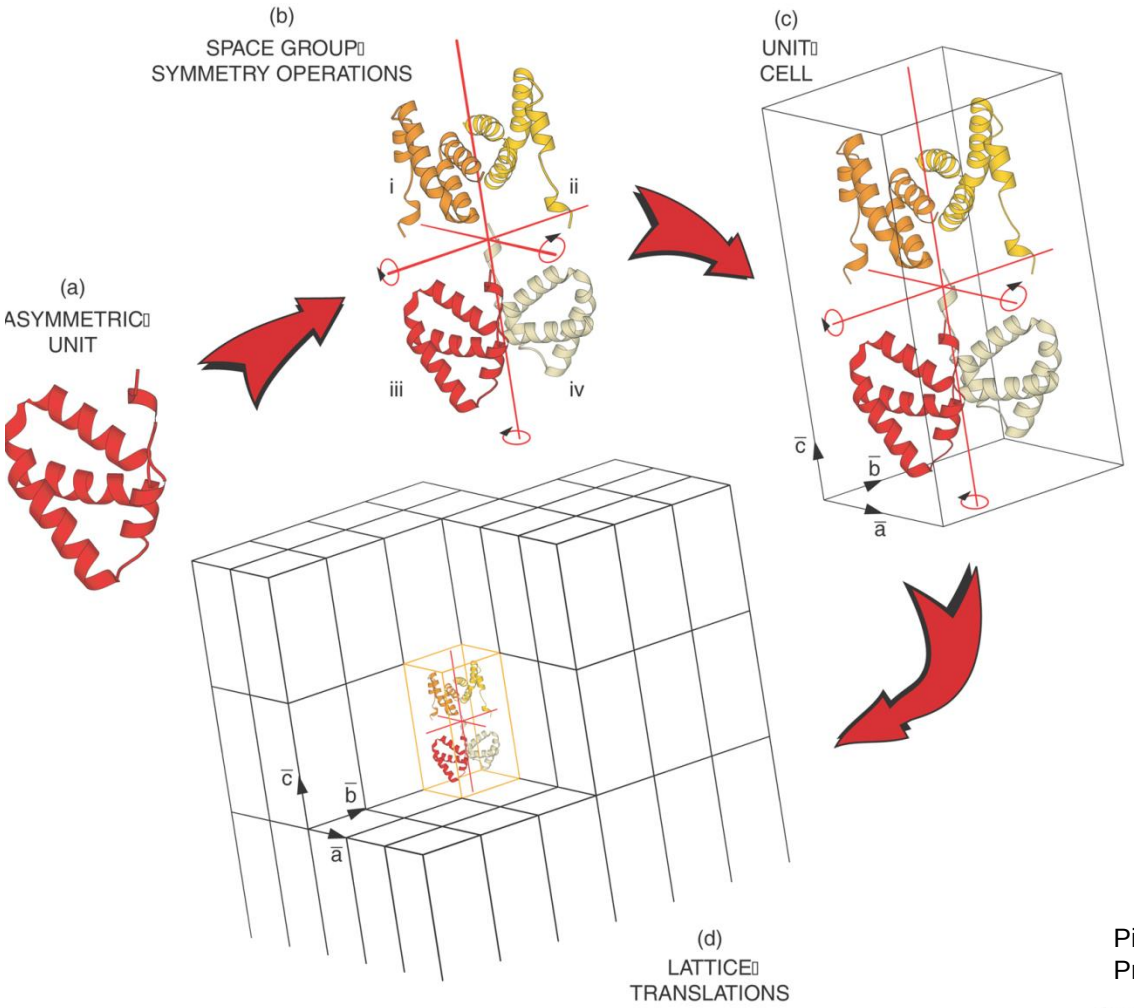
Protein crystallography in general, valid for both x-rays and neutrons as probes

Crystallography: Overiew over the process



Harma Brondijk, Crystal and Structural chemistry, Utrecht University

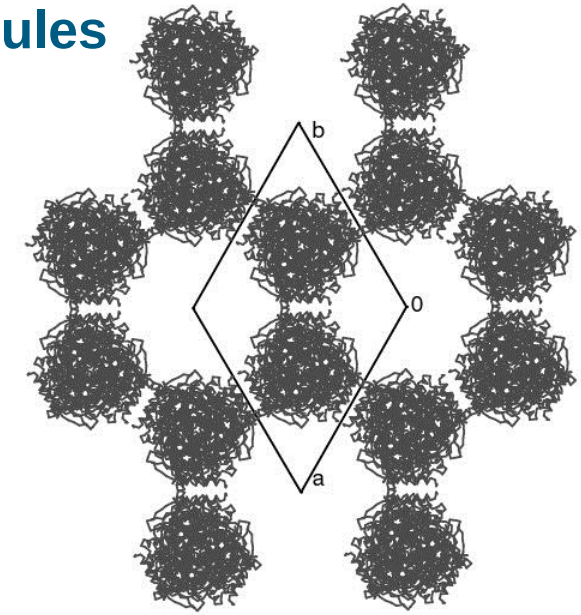
How a typical protein crystal looks like…

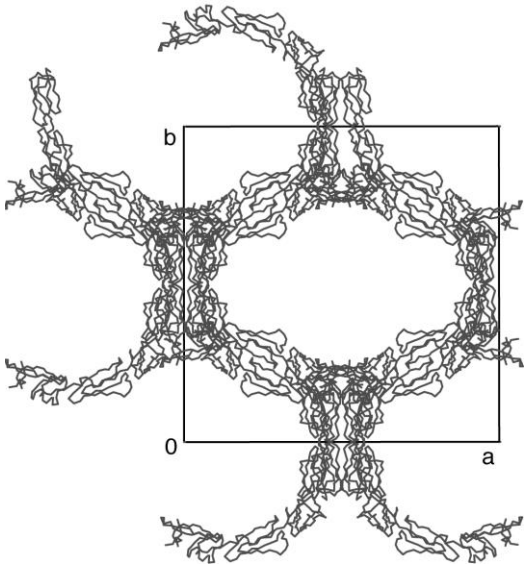


Picture taken from Lecture of Prof. Locher at ETH Zürich

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

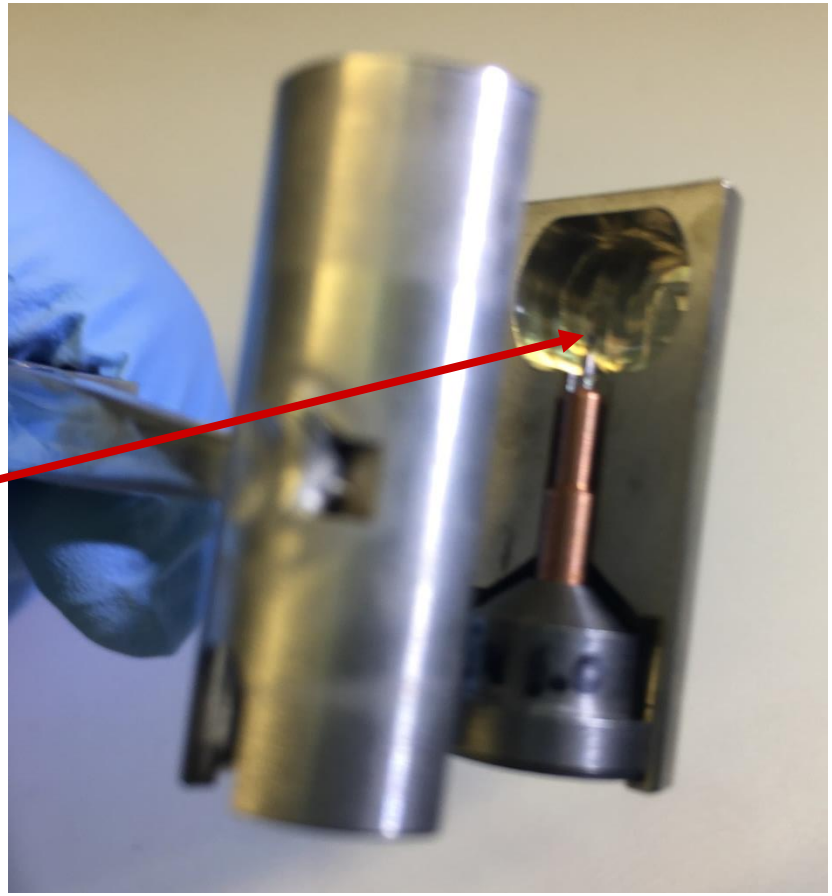
Size considerations of protein crystals

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 1 mm x 1 mm (depending on the protein/space group)

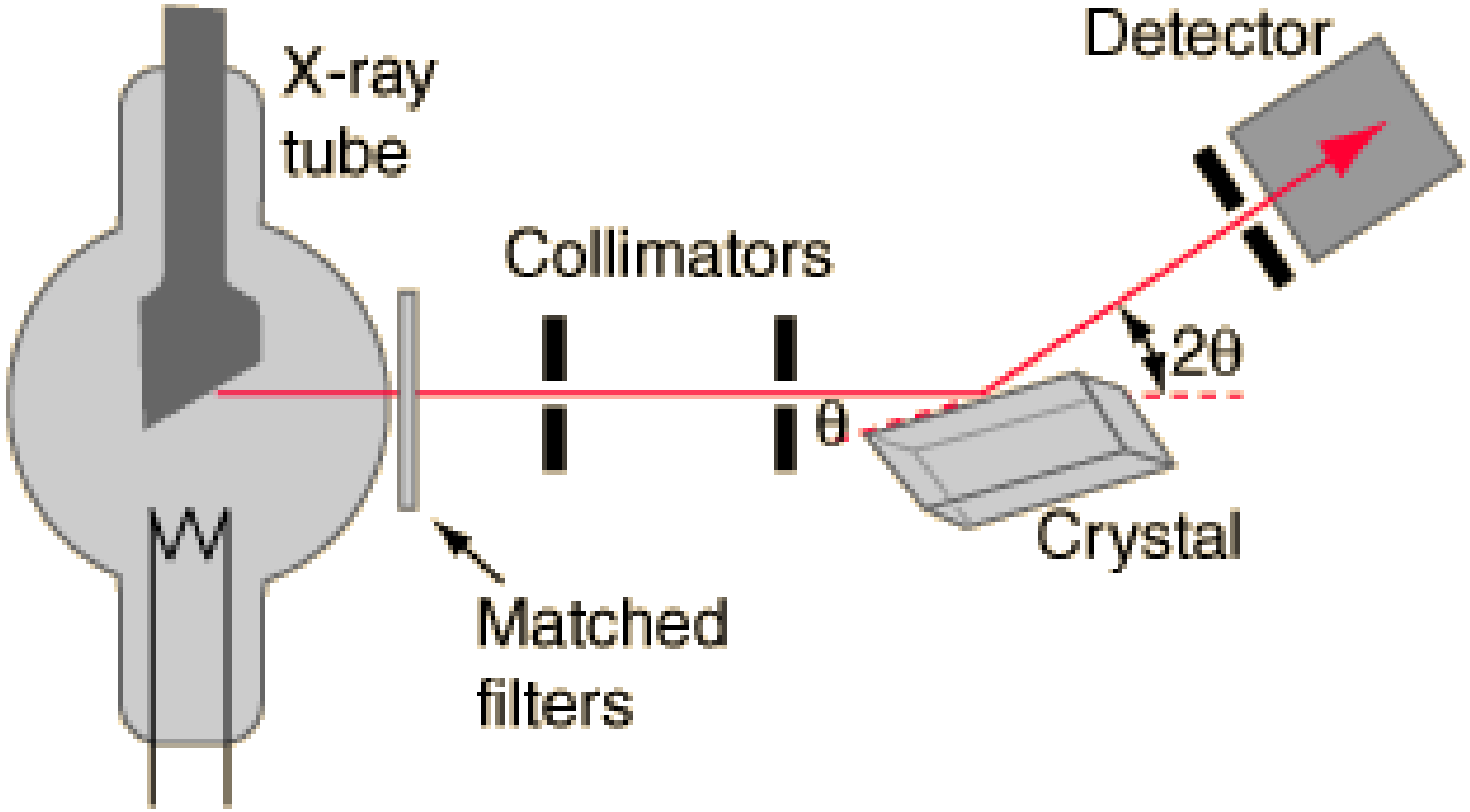
Outer diamter of the glas tube: 5 mm

Cryo-mounting of large crystals

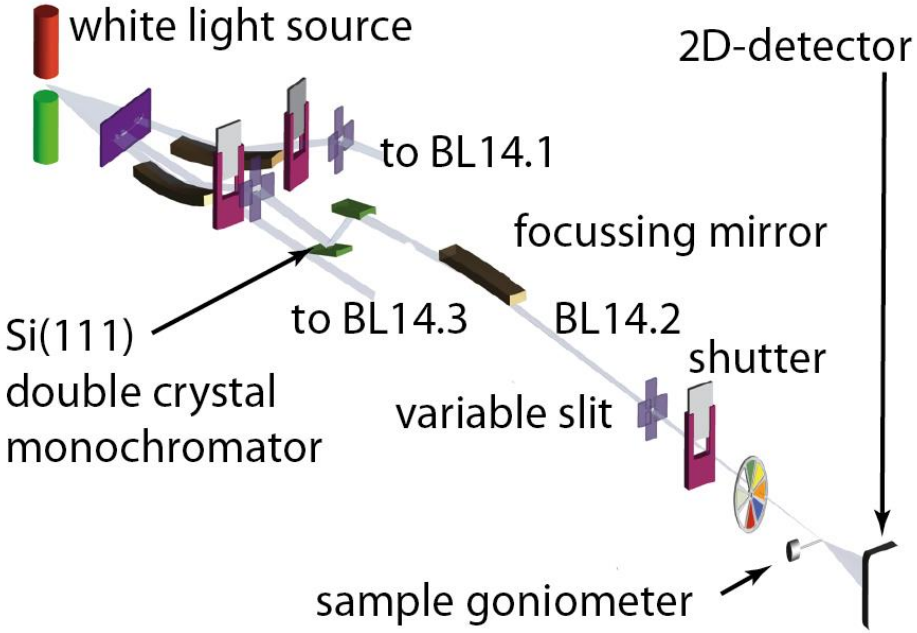


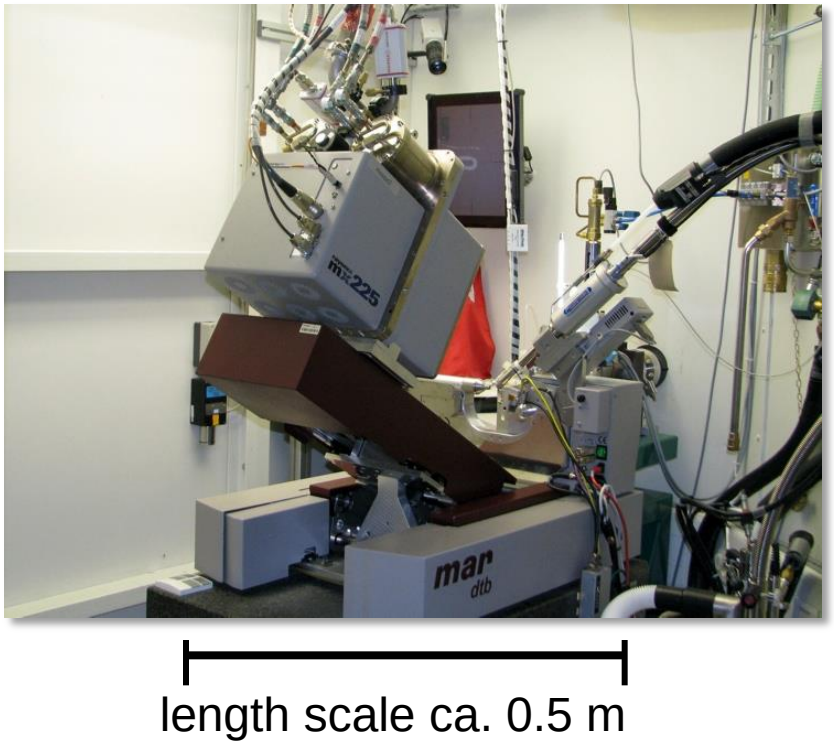
- Avoid hydrogenated polymers in the loop, use capton (Mitigen) or carbon meshes instead (especially when you have a fully deuterated protein)
- Make sure that your crystal fits into the cryoTong: We prefer the 18 mm one.

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

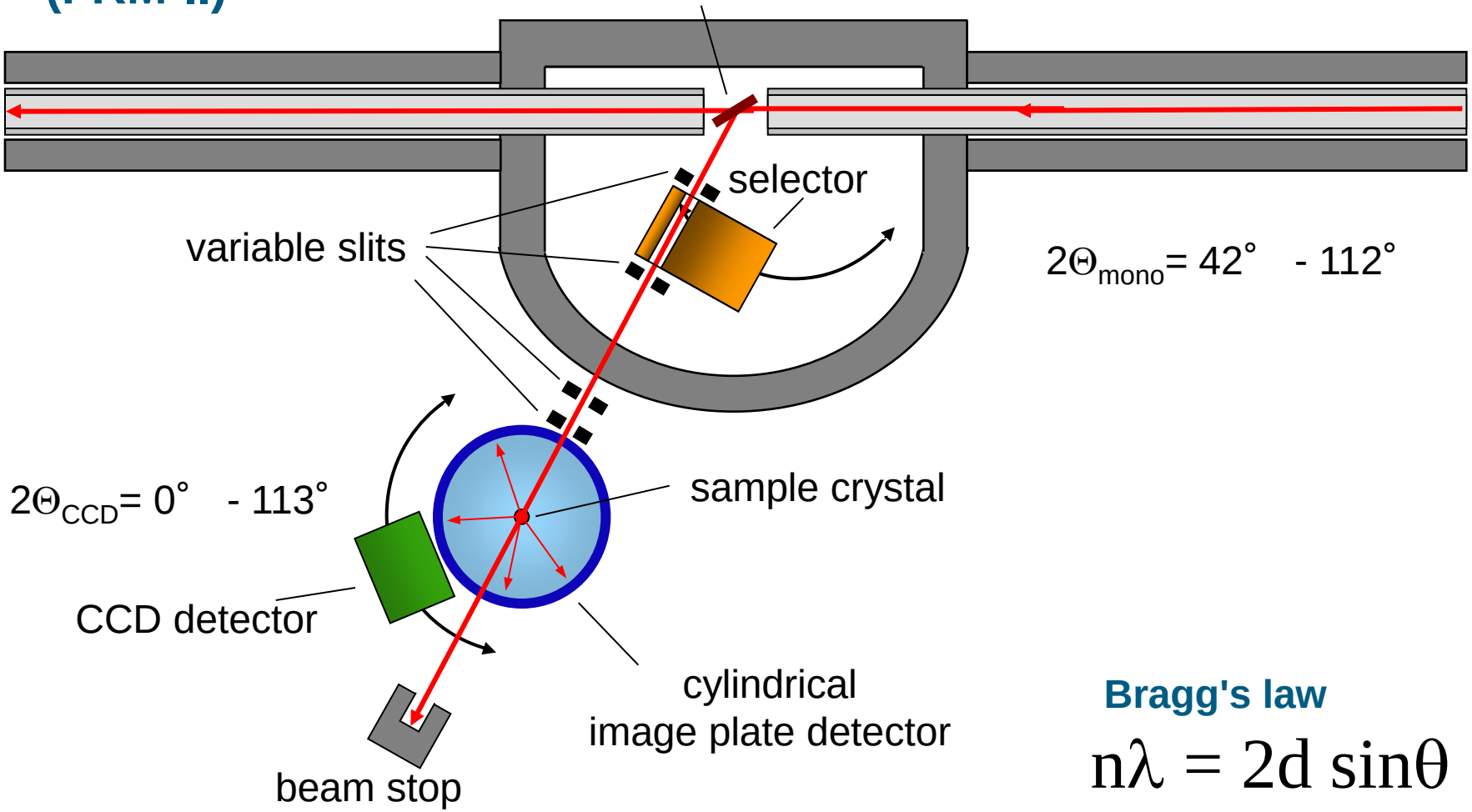


Typical x-ray protein crystallography beamline: BL 14.2 at Bessy (Berlin) run by Manfred Weiss





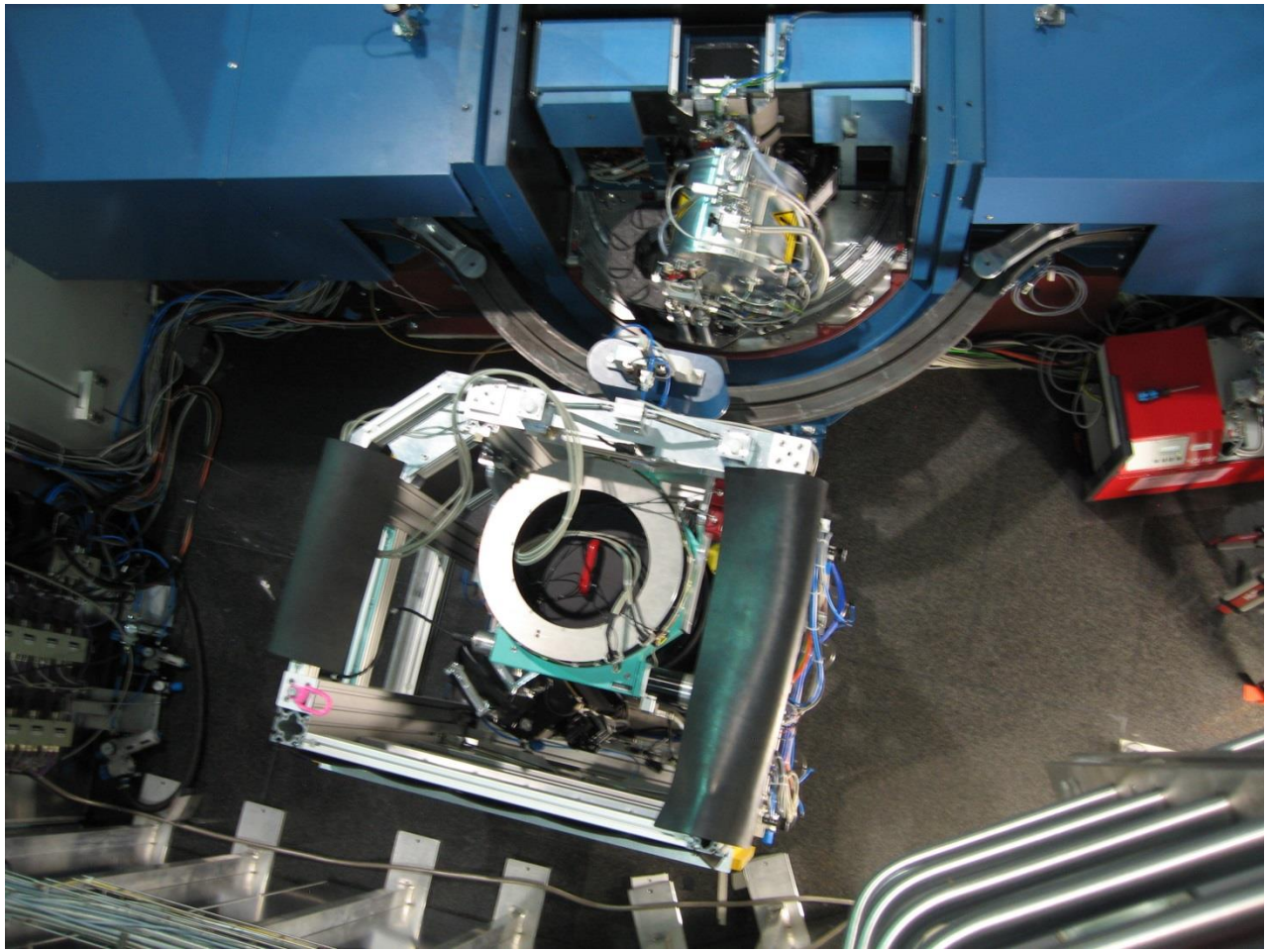
Schematic overview over BioDiff: A neutron protein diffractometer: collaboration between JCNS and TUM (FRM-II)



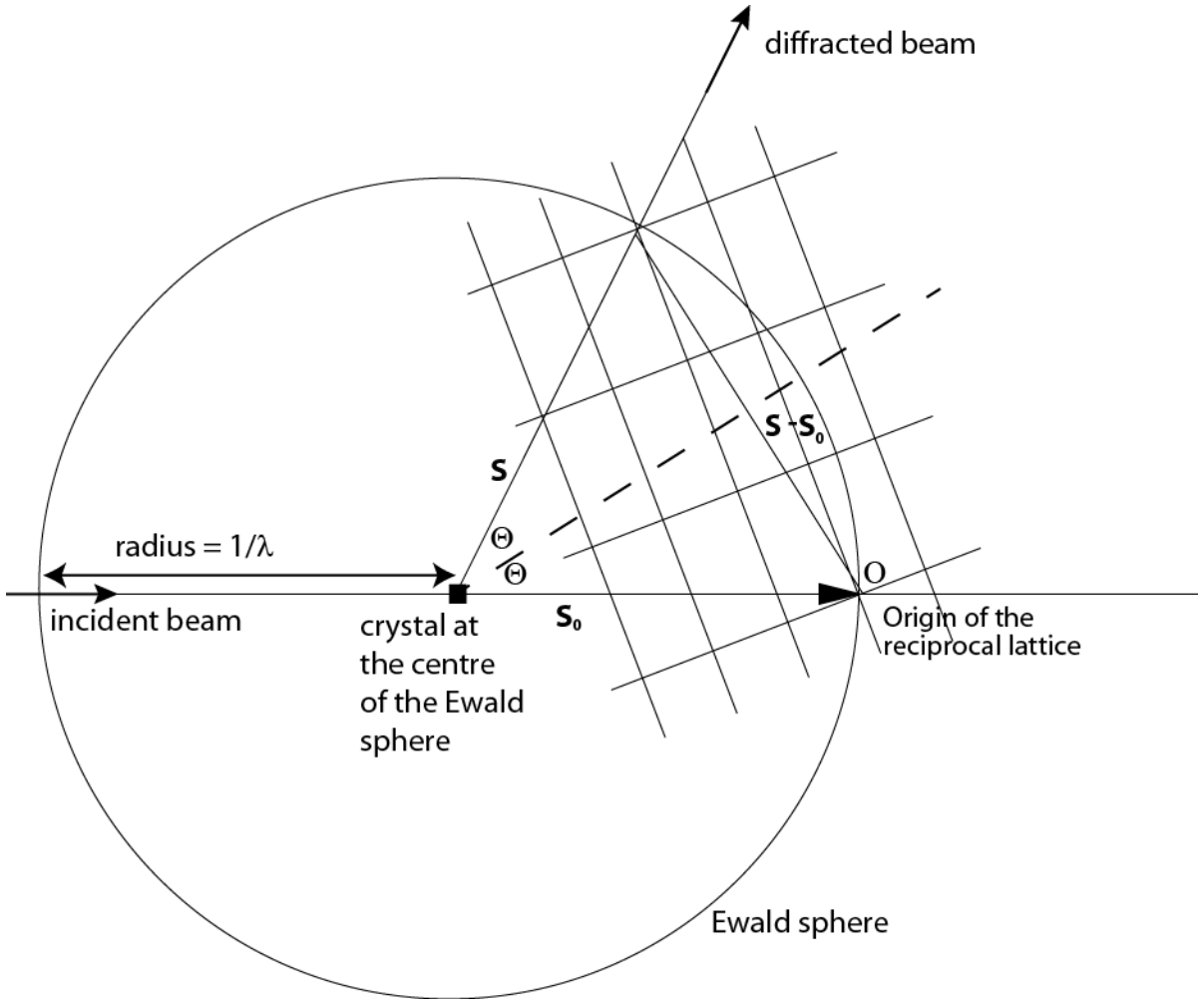
Bragg's law

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

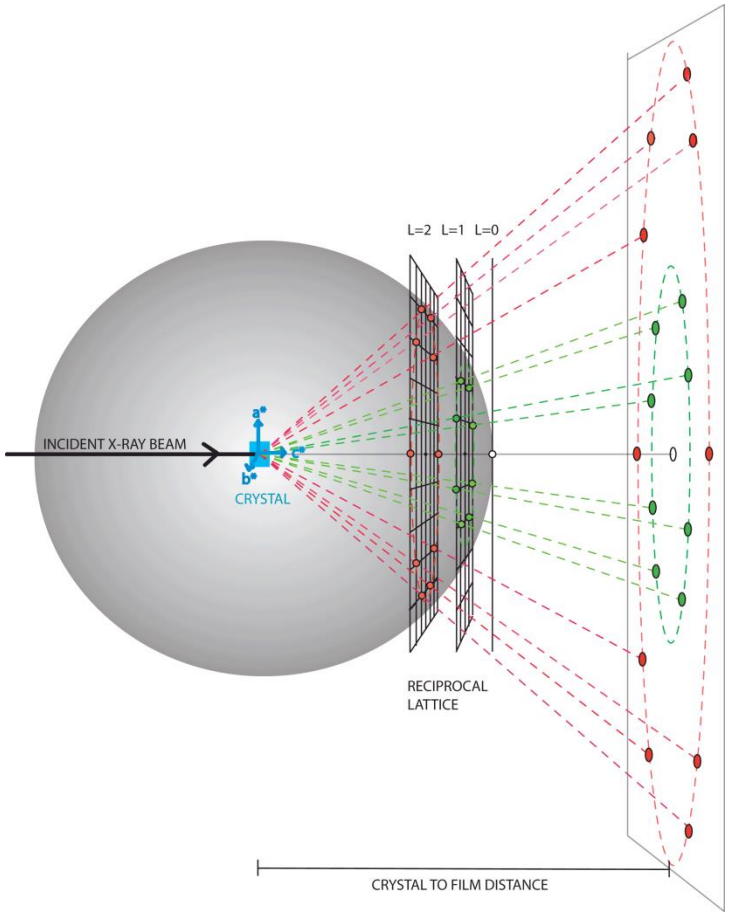
BioDiff, the corresponding view in reality:

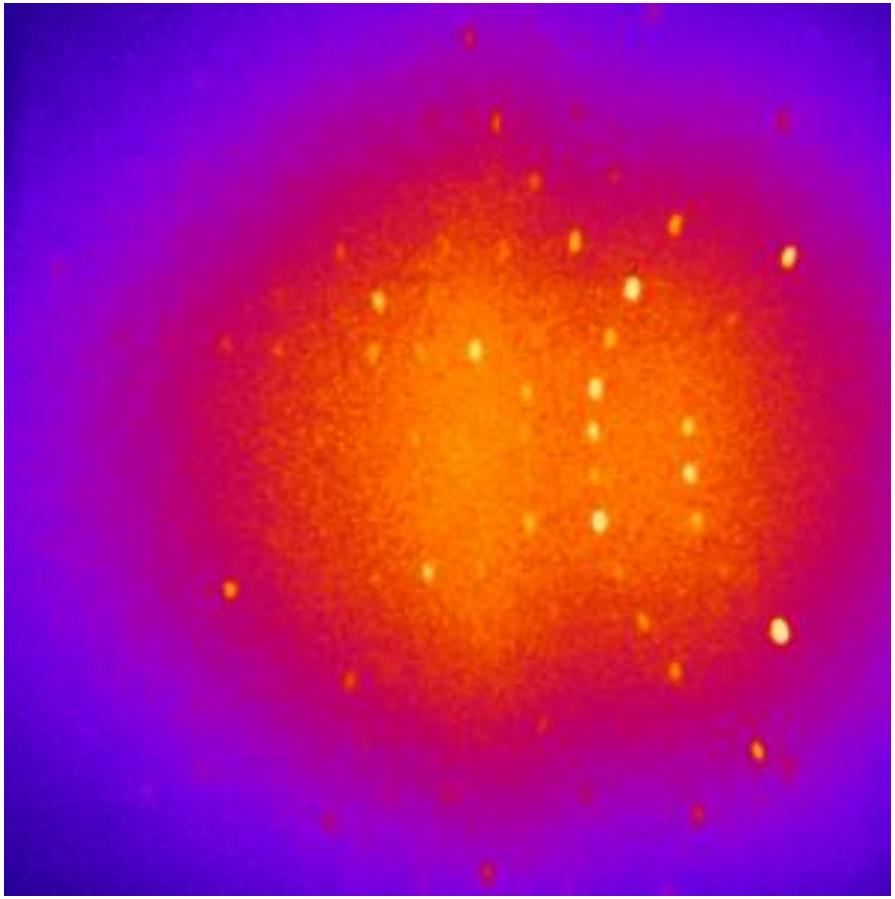


Ewald construction and Bragg‘s Law



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

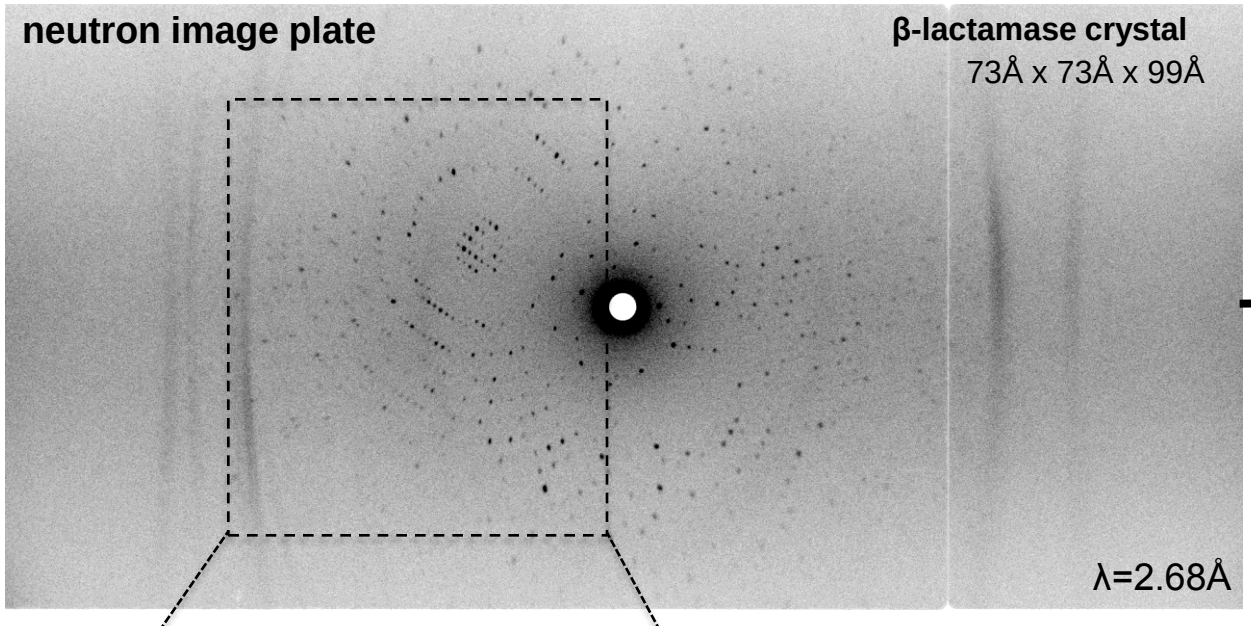


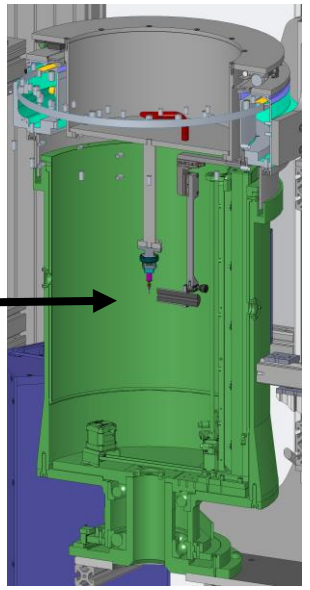


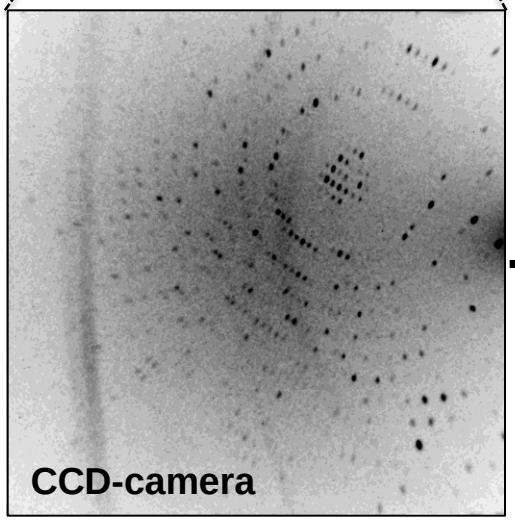
prim. beam

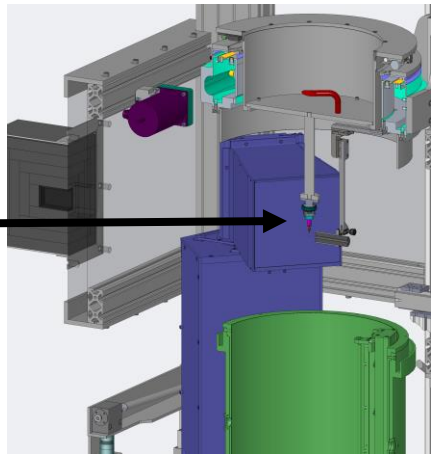
BioDiff: exposure time per frame: 20 minutes, sample: Myoglobin in deuterated mother liquor

The Two Detectors of BIODIFF









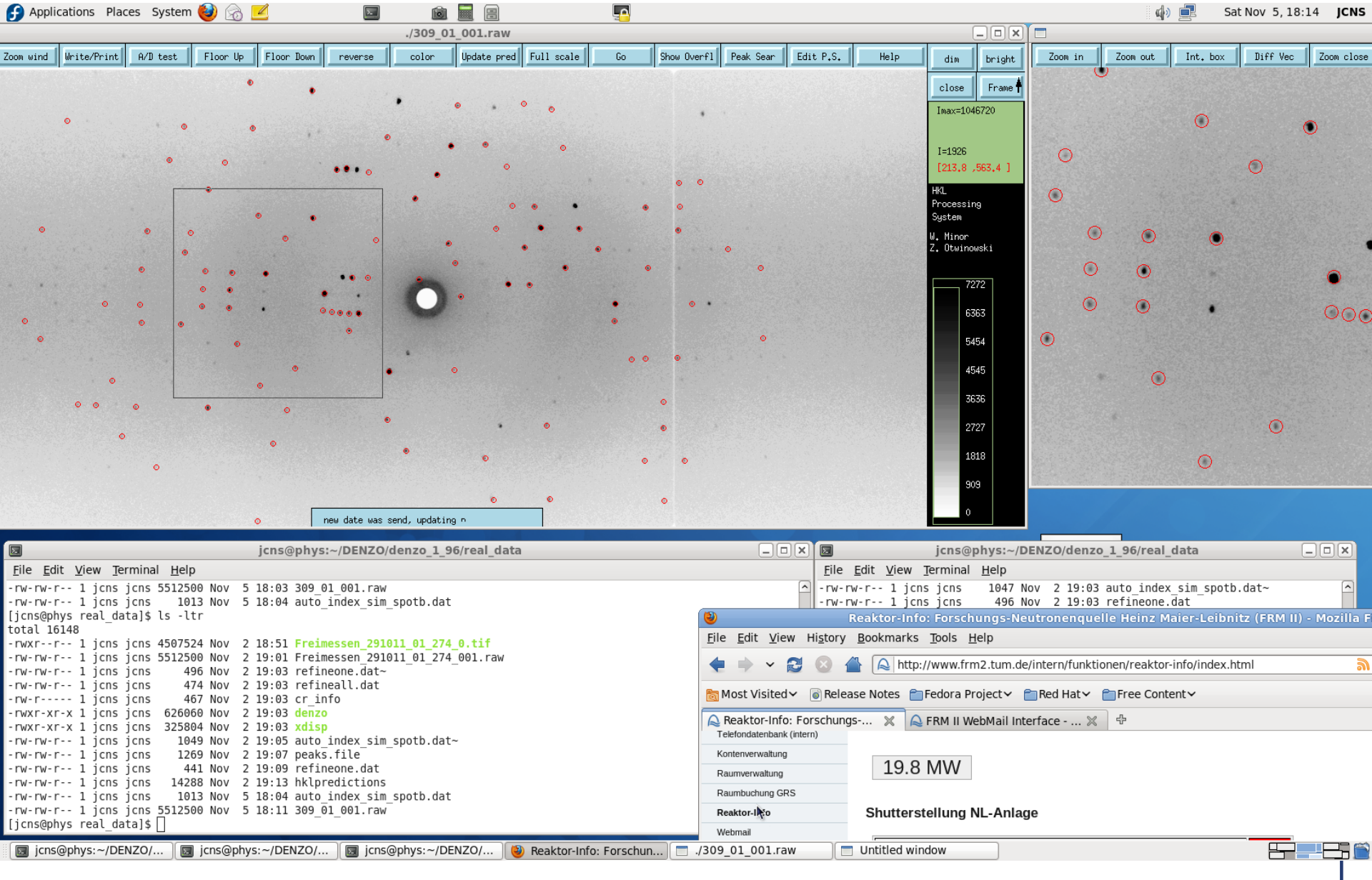
NIP-scanner

- larger solid angle
- readout time ≥ 4 min

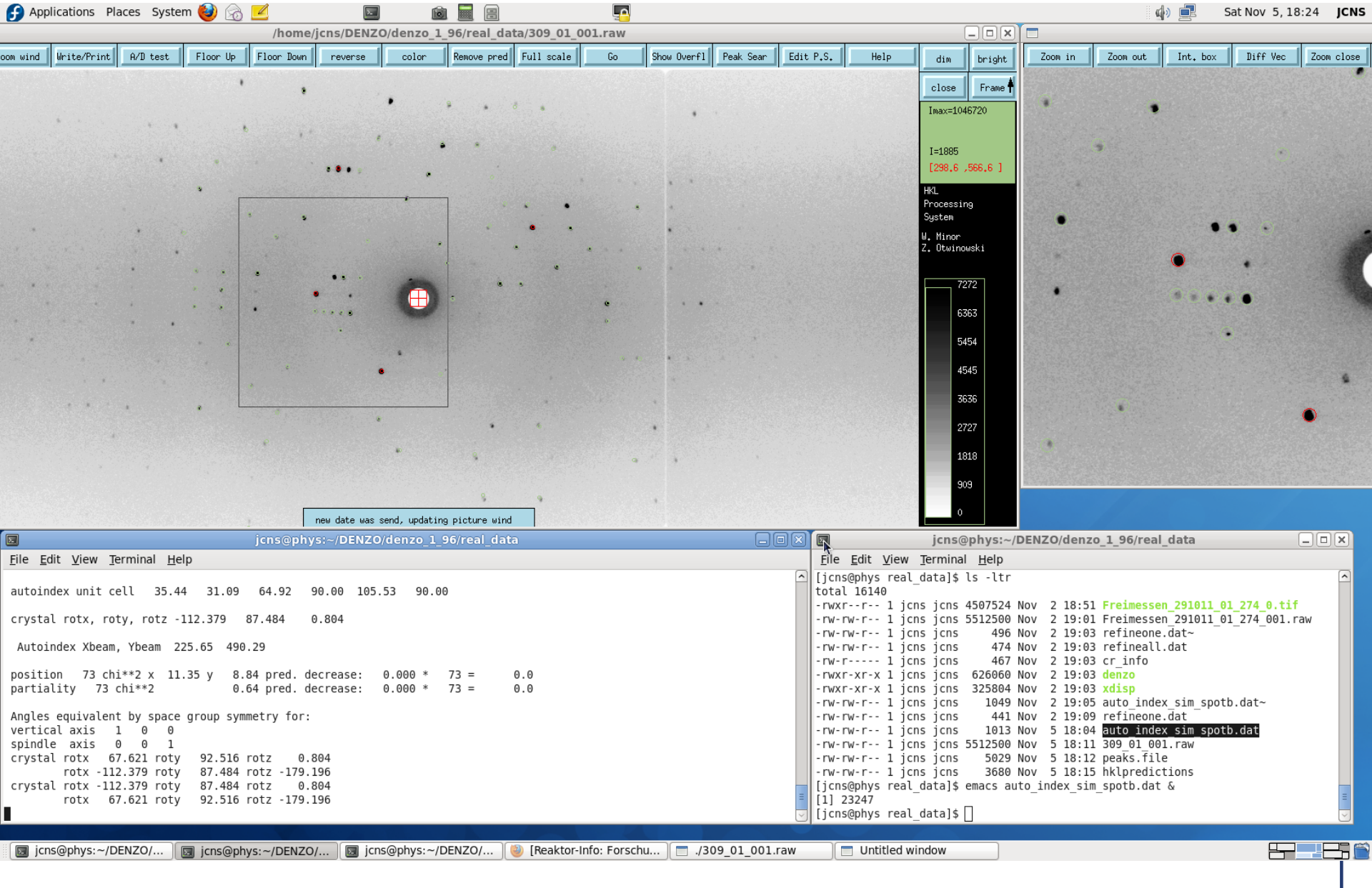
CCD-camera

- smaller solid angle
- readout time ≥ 1 sec

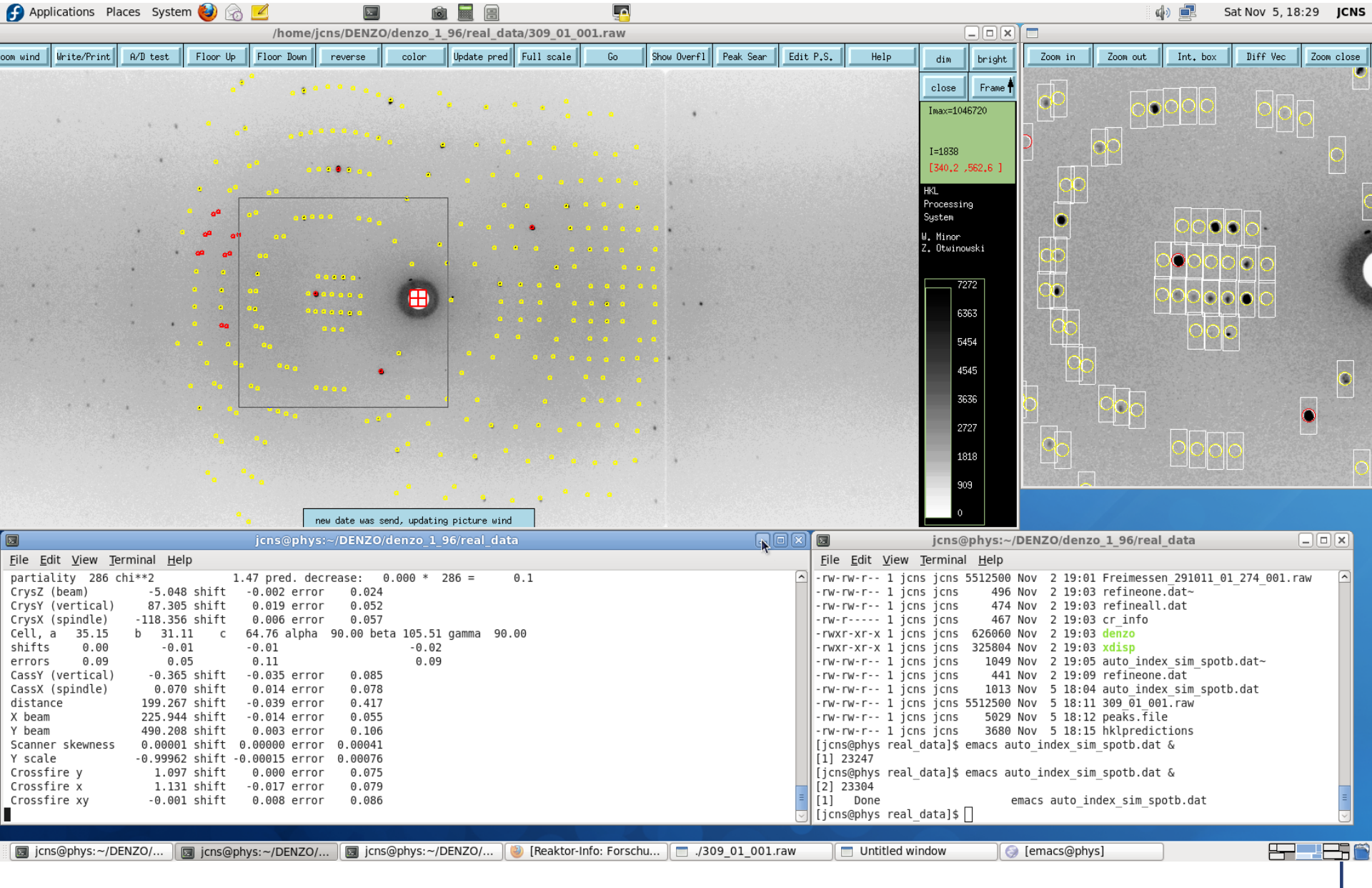
Peak search with hkl DENZO



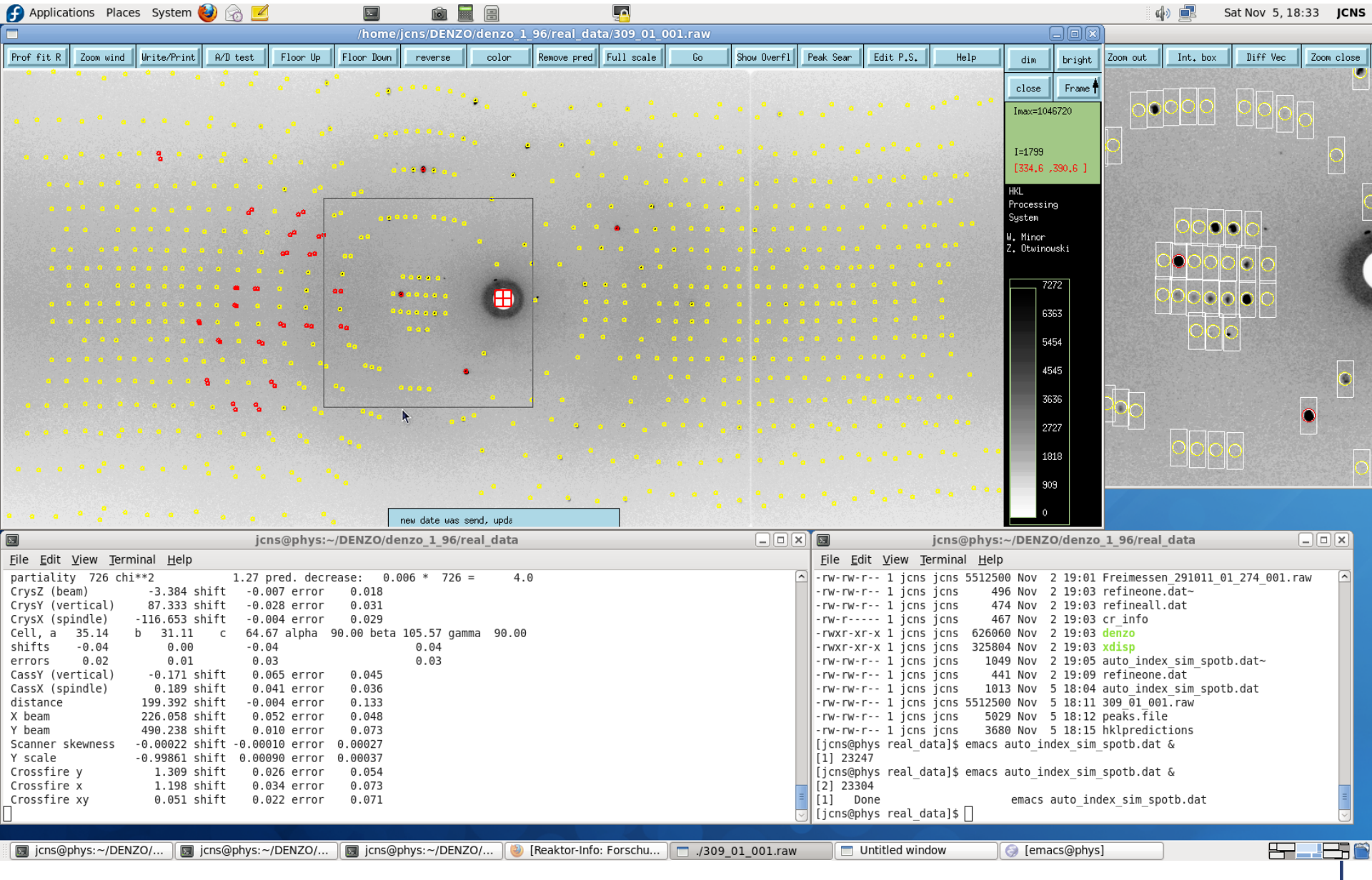
auto-index


Applications Places System
Sat Nov 5, 18:24 JCNS
/home/jcns/DENZO/denzo_1_96/real_data/309_01_001.raw
Write/Print
A/D test
Floor Up
Floor Down
reverse
color
Remove pred
Full scale
Go
Show Overfl
Peak Sear
Edit P.S.
Help
dim
bright
close
Frame
Imax=1046720
I=1885
[298.6 ,566.6]
HKL
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

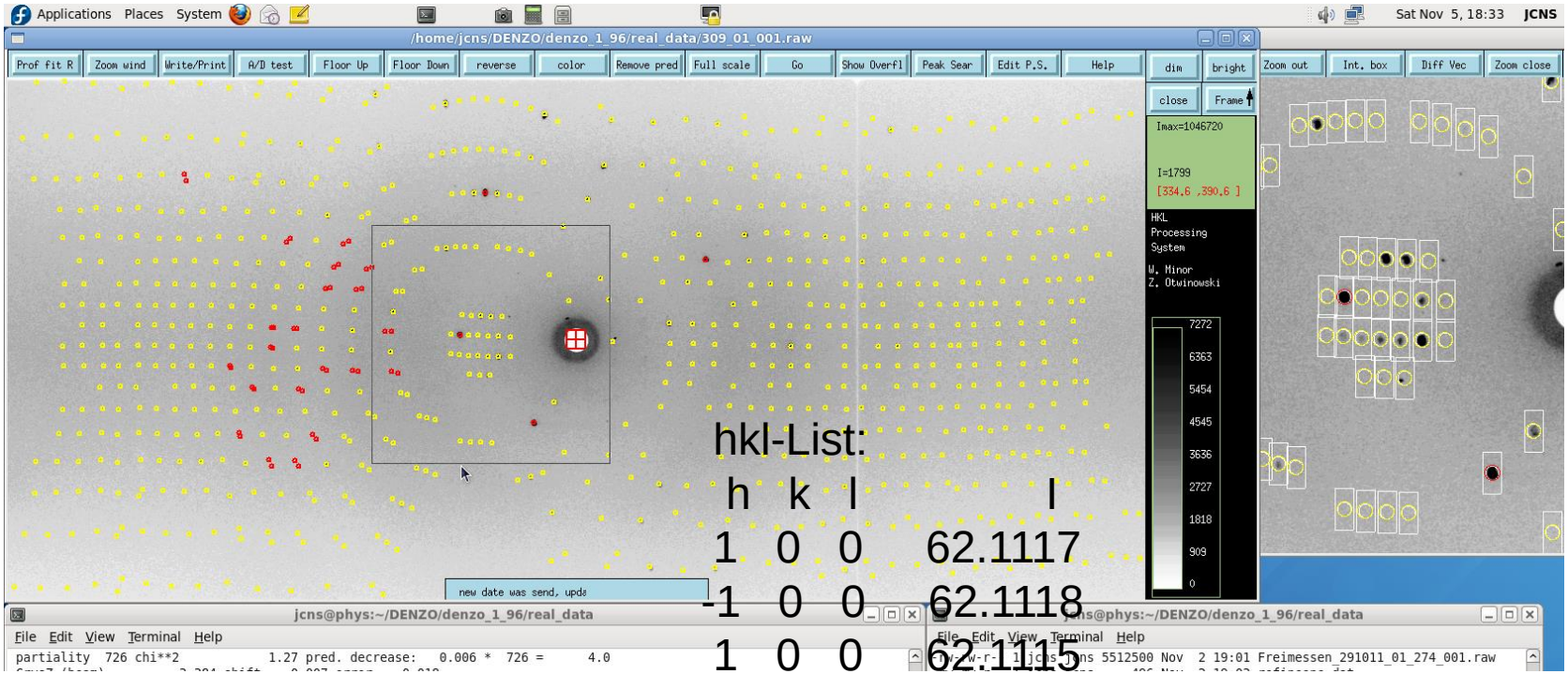
d_min=2.5 Å


Applications Places System
Sat Nov 5, 18:29 JCNS
/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
-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


Applications Places System
Sat Nov 5, 18:33 JCNS
/home/jcns/DENZO/denzo_1_96/real_data/309_01_001.raw
Prof fit R
Zoom wind
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
-rw-r----- 1 jcns jcns 467 Nov 2 19:03 cr_info
-rwxr-xr-x 1 jcns jcns 626060 Nov 2 19:03 denzo
-rwxr-xr-x 1 jcns jcns 325804 Nov 2 19:03 xdisp
-rw-rw-r-- 1 jcns jcns 1049 Nov 2 19:05 auto_index_sim_spotb.dat~
-rw-rw-r-- 1 jcns jcns 441 Nov 2 19:09 refineone.dat
-rw-rw-r-- 1 jcns jcns 1013 Nov 5 18:04 auto_index_sim_spotb.dat
-rw-rw-r-- 1 jcns jcns 5512500 Nov 5 18:11 309_01_001.raw
-rw-rw-r-- 1 jcns jcns 5029 Nov 5 18:12 peaks.file
-rw-rw-r-- 1 jcns jcns 3680 Nov 5 18:15 hklpredictions
[jcns@phys real_data]$ emacs auto_index_sim_spotb.dat &
[1] 23247
[jcns@phys real_data]$ emacs auto_index_sim_spotb.dat &
[2] 23304
[1] Done emacs auto_index_sim_spotb.dat
[jcns@phys real_data]$
jcns@phys:~/DENZO/...
jcns@phys:~/DENZO/...
jcns@phys:~/DENZO/...
[Reaktor-Info: Forschu...
./309_01_001.raw
Untitled window
[emacs@phys]

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



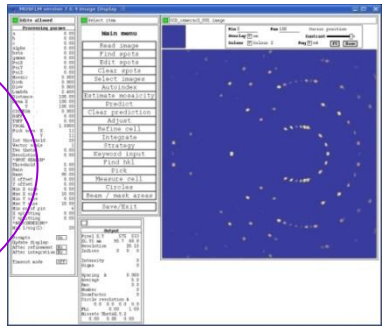
ca. 300 images

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

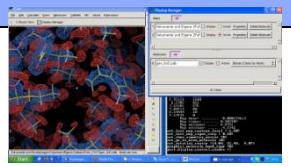
Structure refinement

-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

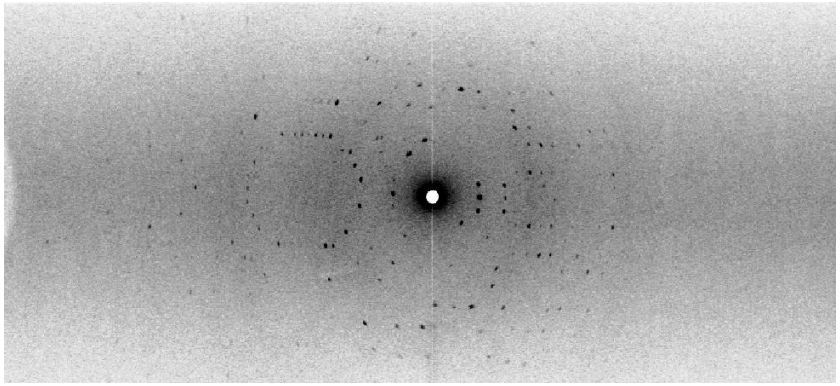
-nCNS
-PHENIX

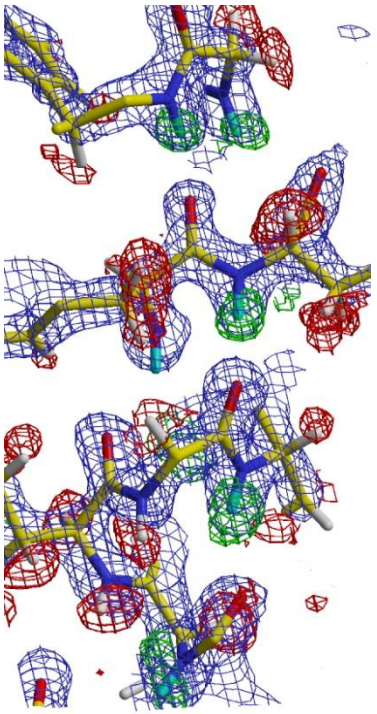


-XtalView
-Coot

Theory on scattering from a crystal

3D structural analysis:





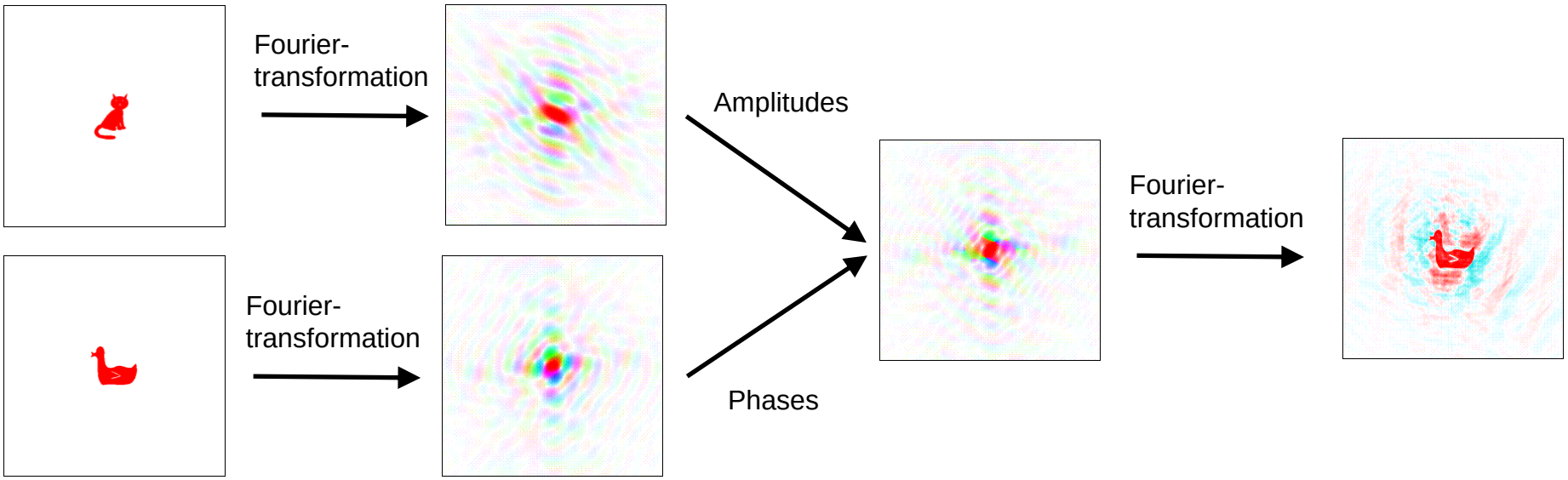
$$\rho(x,y,z) = \frac{1}{V_E} \sum_{h,k,l} F_{hkl} \cdot e^{-2\pi i(h\cdot x + k\cdot y + l\cdot z)}$$

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

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.

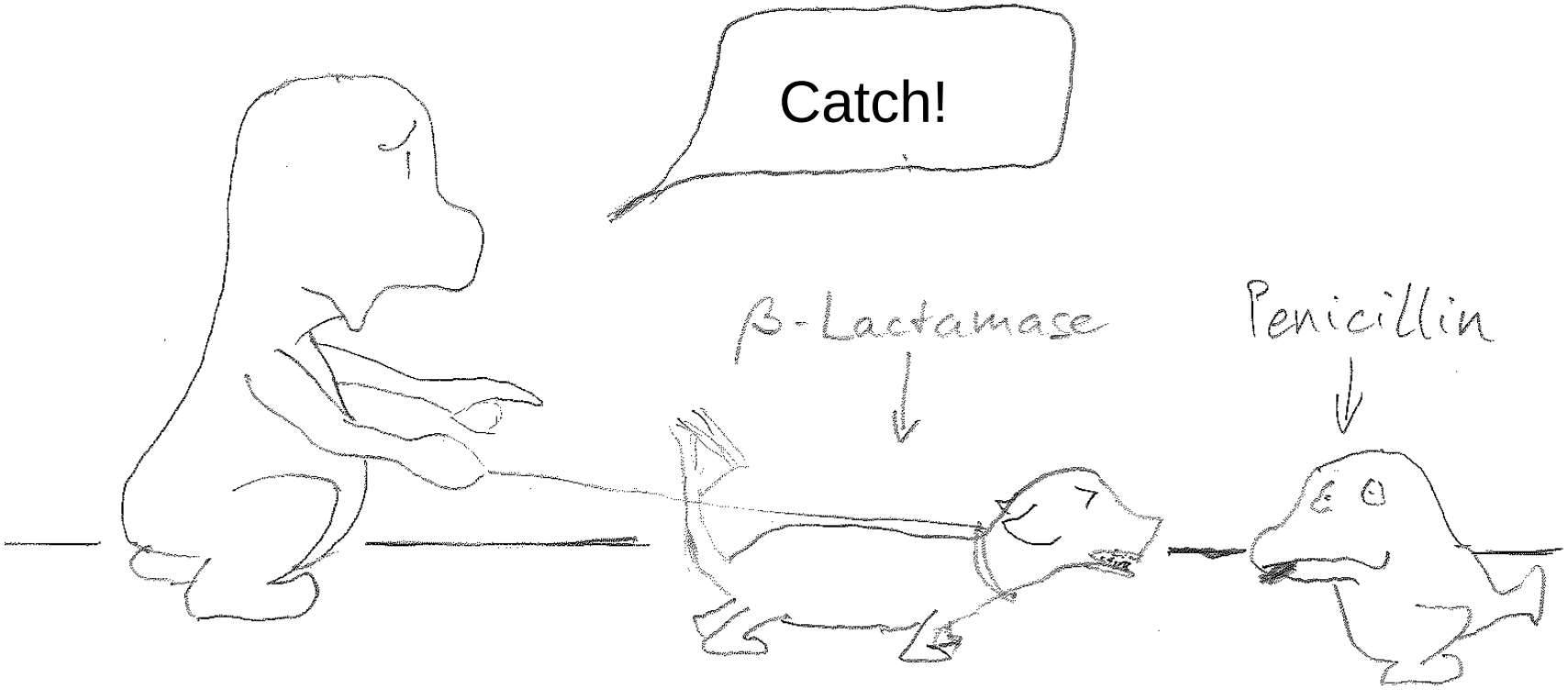
Neutron protein crystallography

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

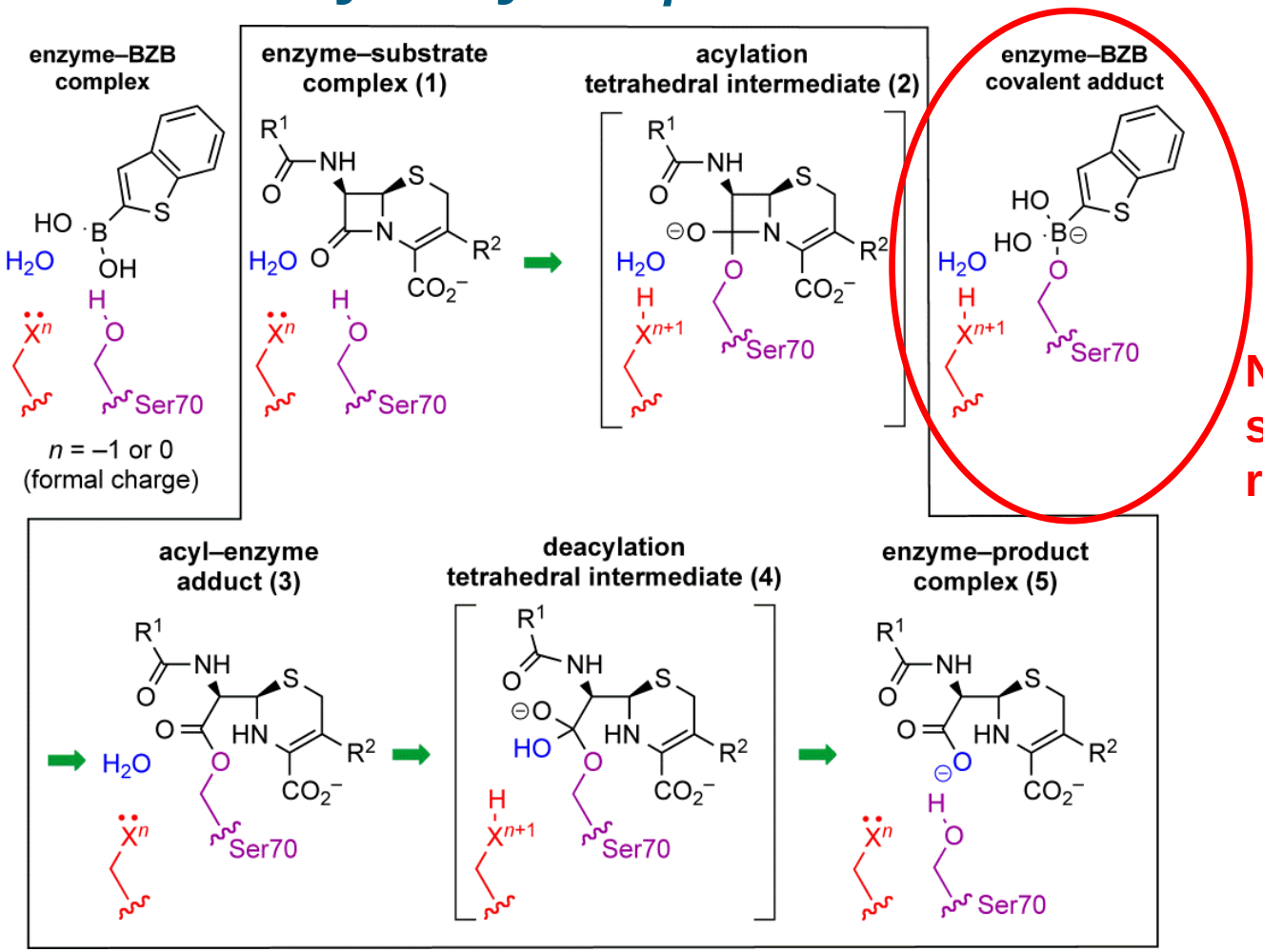
=> x-ray crystallography is a prerequisite of neutron protein crystallography.

Application Example: Protonation state of amino acid residues

The protein β-lactamase



β-lactamase: hydrolyses β-lactam antibiotics

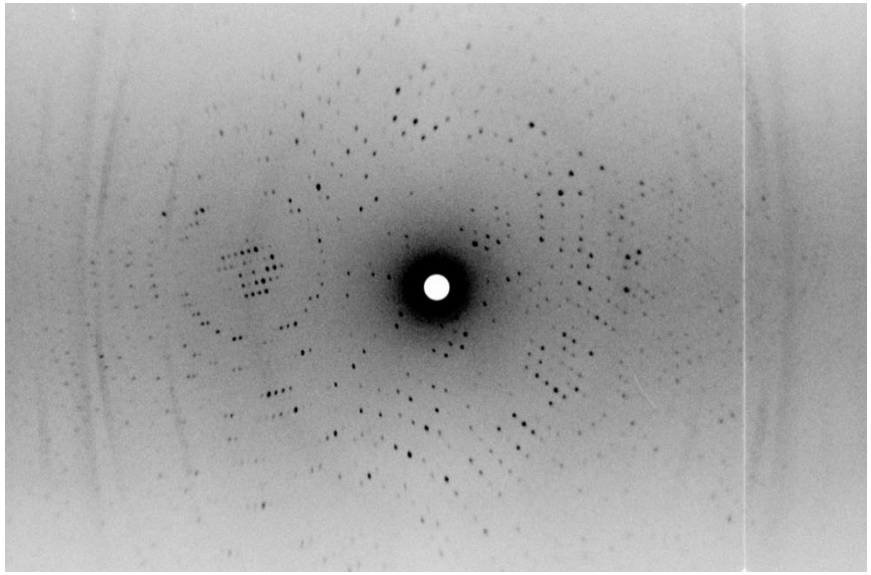


Neutron structure recorded

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



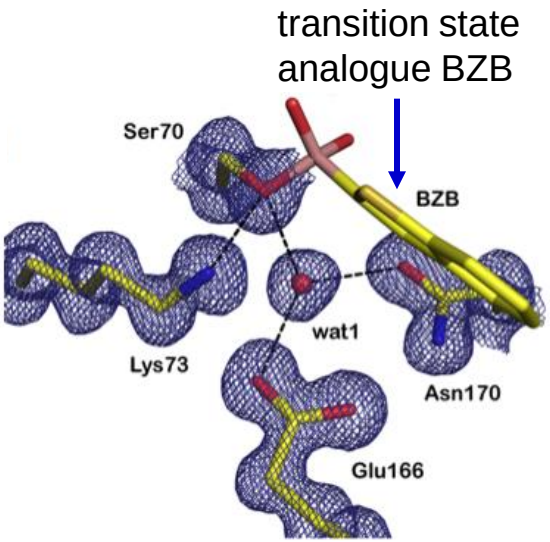
- unit cell: 73.4Å, 73.4Å, 99.1Å $P3_221$
- fully deuterated protein
- crystal size: 2.7mm^3
- Collection time: 9d

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**

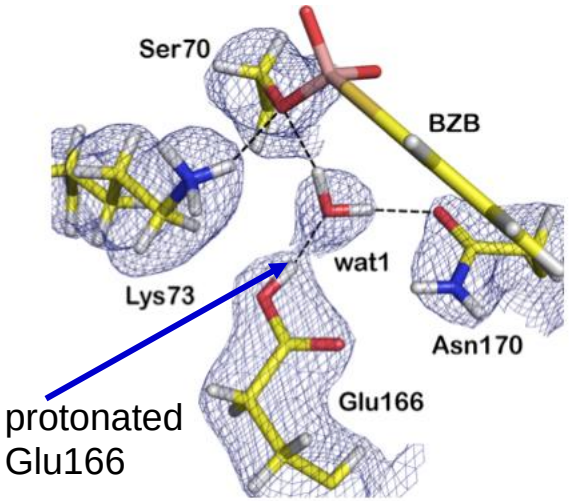
R_{pim} = 7.9% (17.9%)

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

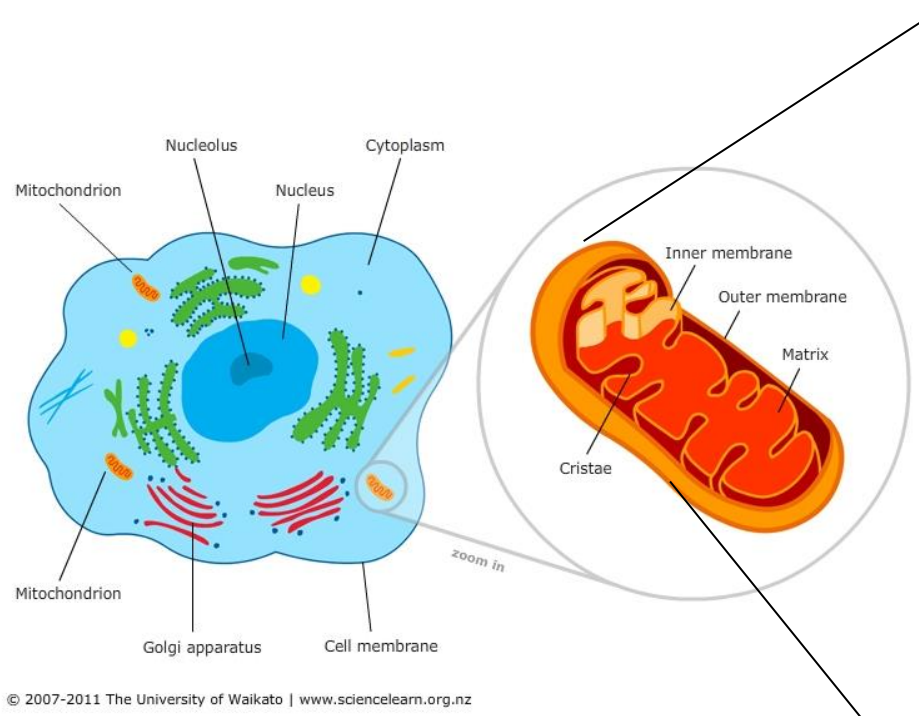
An example for a metallo-protein:

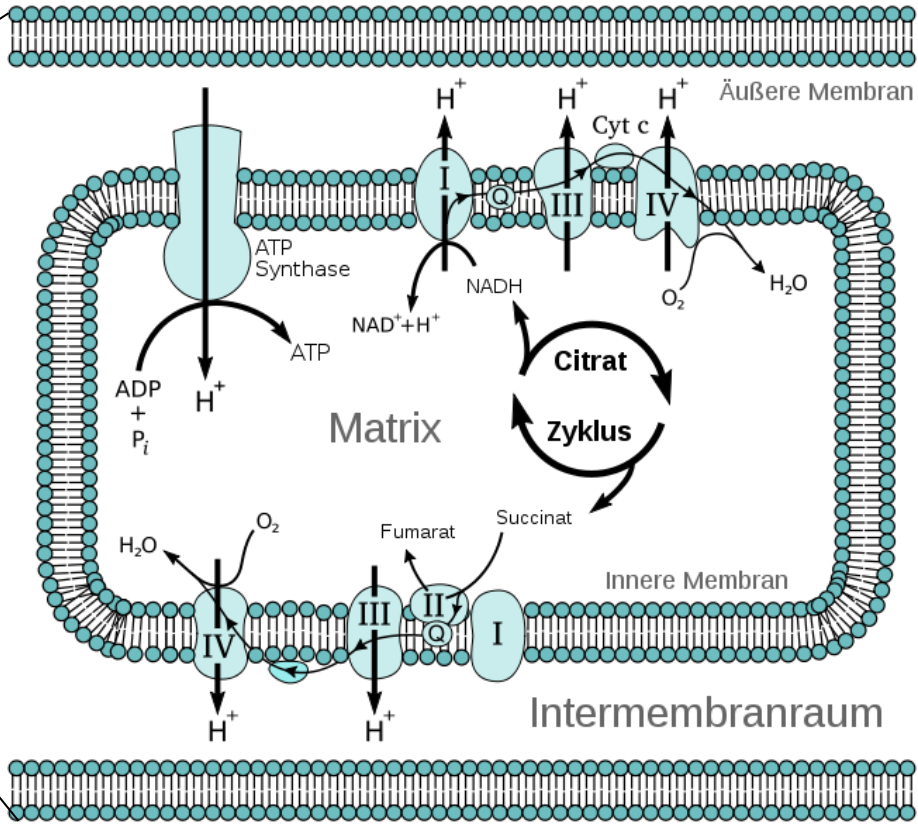
Cytochrome *c* peroxidase, or CcP is a water-soluble heme-containing enzyme of the peroxidase family that takes reducing equivalents from cytochrome *c* and reduces hydrogen peroxide to water:

$$CcP + H_2O_2 + 2\ \text{ferrocytochrome } c\ (Fe^{2+}) + 2H^+ \rightarrow CcP + 2H_2O + 2\ \text{ferricytochrome } c\ (Fe^{3+})$$

(taken from http://en.wikipedia.org/wiki/Cytochrome_c_peroxidase)

Mitochondria are the power plant of a cell (production of ATP):

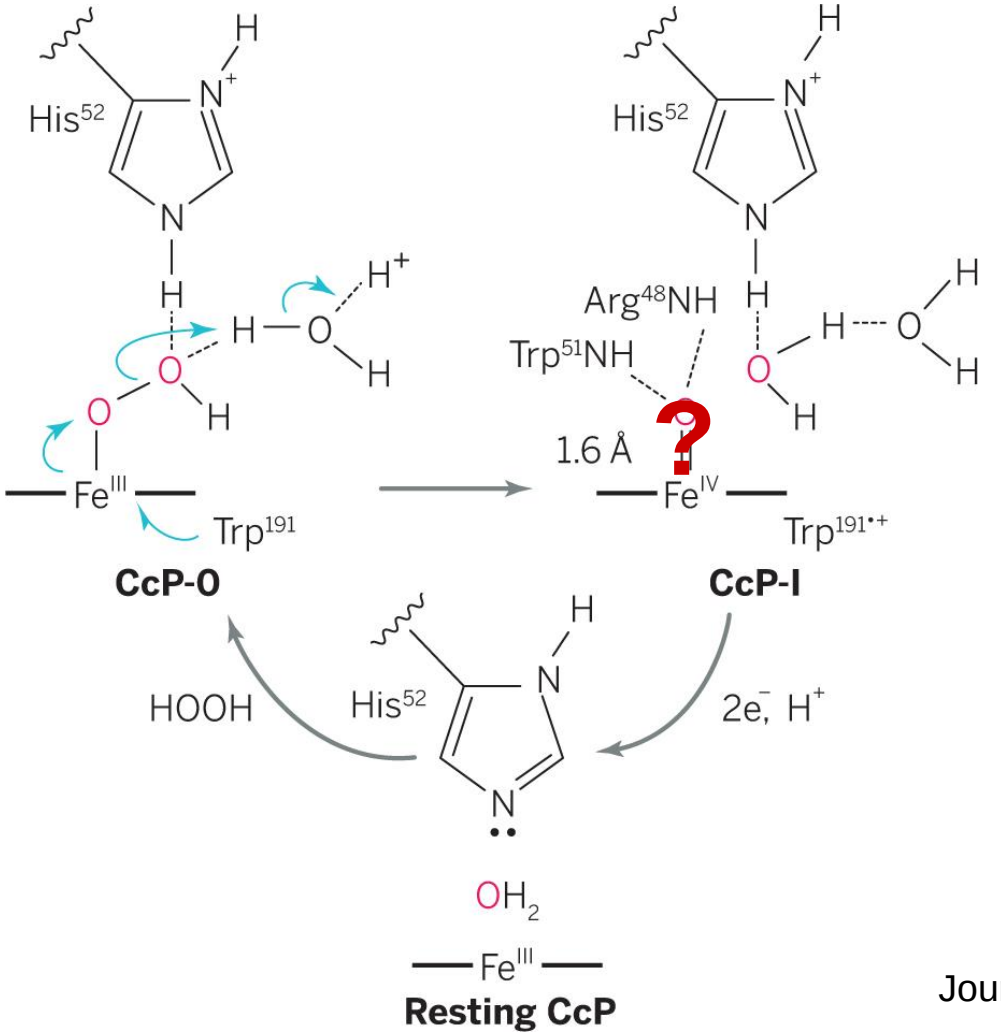




http://de.wikipedia.org/wiki/Atmungskette

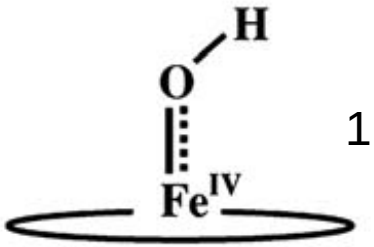
- Cytochrome C serves as an electron transporter in the respiratory chain.
- Cytochrome c Peroxidase uses two ferro-cytochrome C proteins to reduce H_2O_2 to water and two ferricytochrome C molecules

Proton-mediated mechanism.Reaction of ferric CcP with H_2O_2 first gives CcP-0, followed by O-O bond scission driven by external protonation to afford CcP-I.



J T Groves, and N C Boaz Science 2014;345:142-143

Alternative Hypothesis:

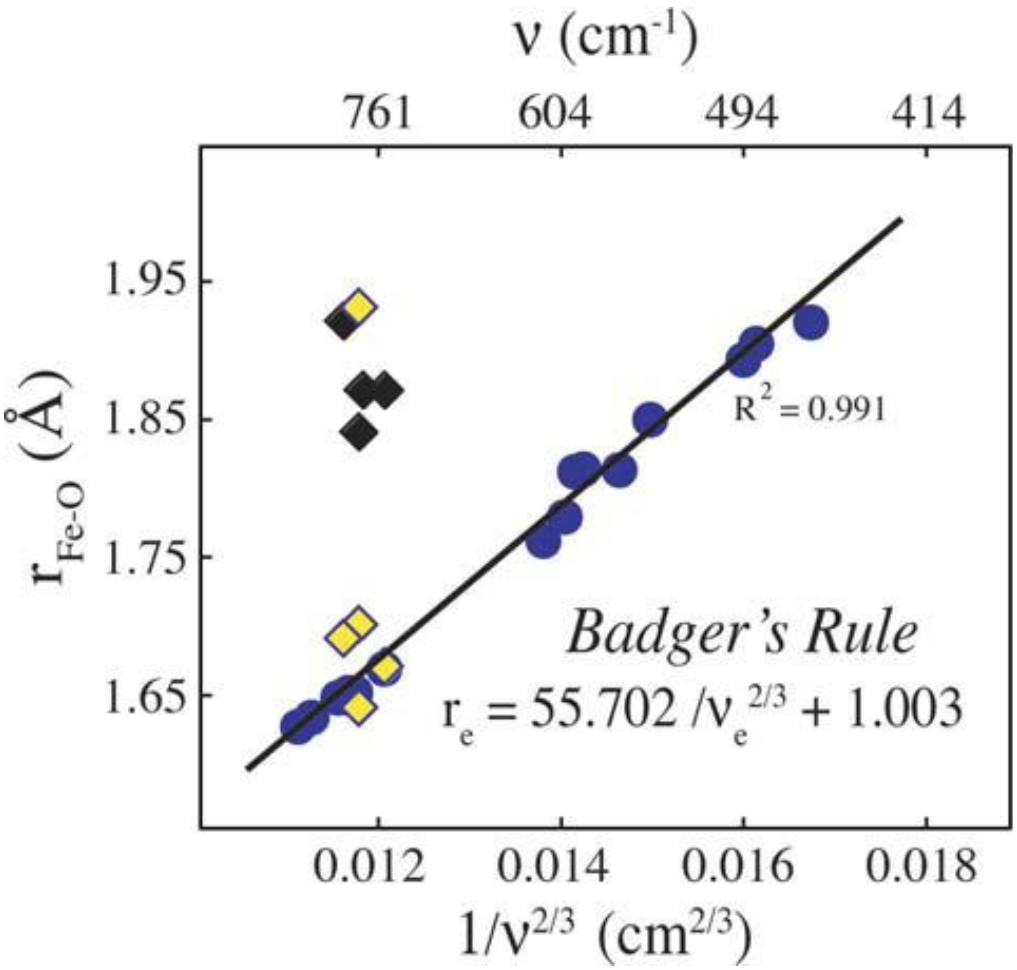


1.85 Å

Fig. 3. Compound I with an O–H bond and a bond length of Fe-O of ca. 1.85 Å.

Journal of Inorganic Biochemistry 100 (2006) 448–459

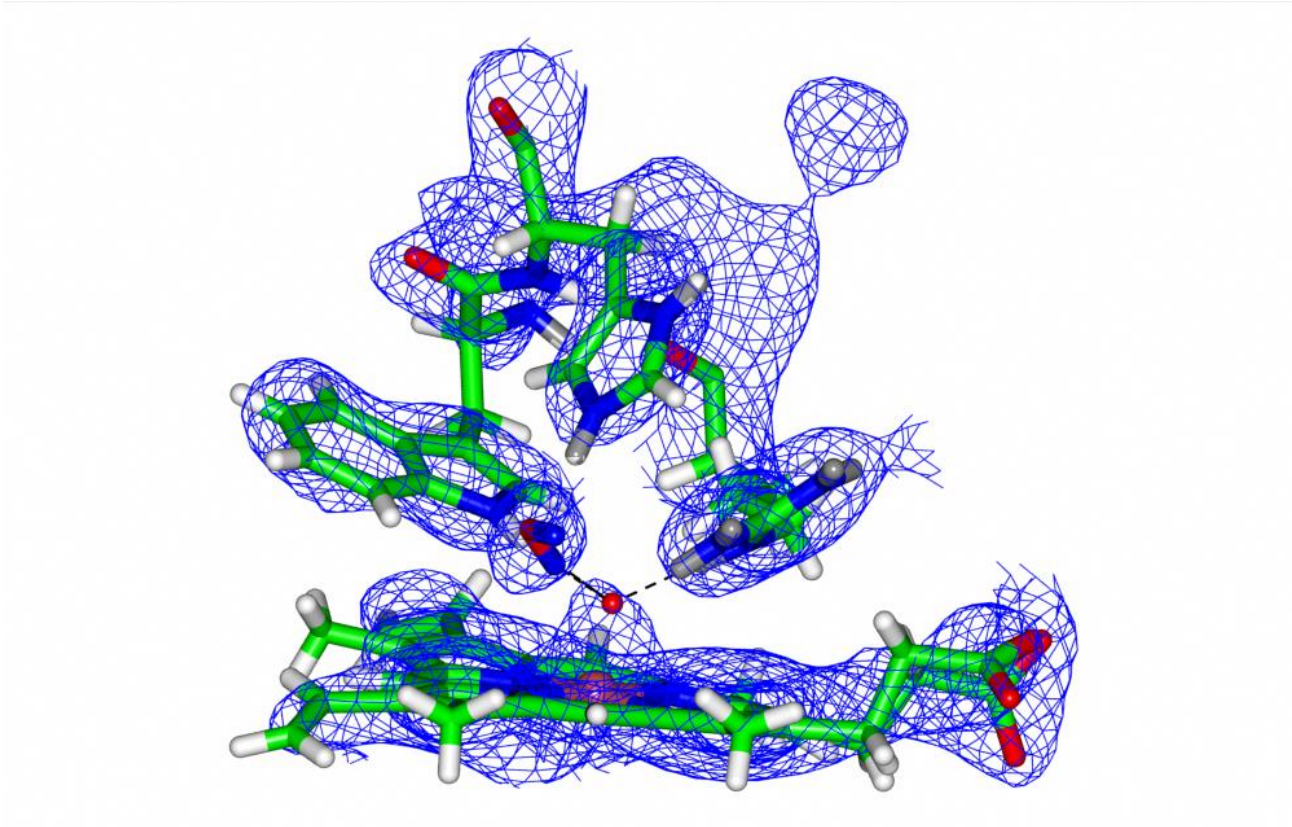
No method so far could unambigiously show the nature of the iron-oxide bond



Plot of computed stretching frequency vs Fe–O bond distance. **Yellow diamonds** are from resonance Raman or EXAFS and the solid diamonds from X-ray crystal structures. The **blue circles** are from calculations.

Figure taken from: Journal of Inorganic Biochemistry 100 (2006) 448–459

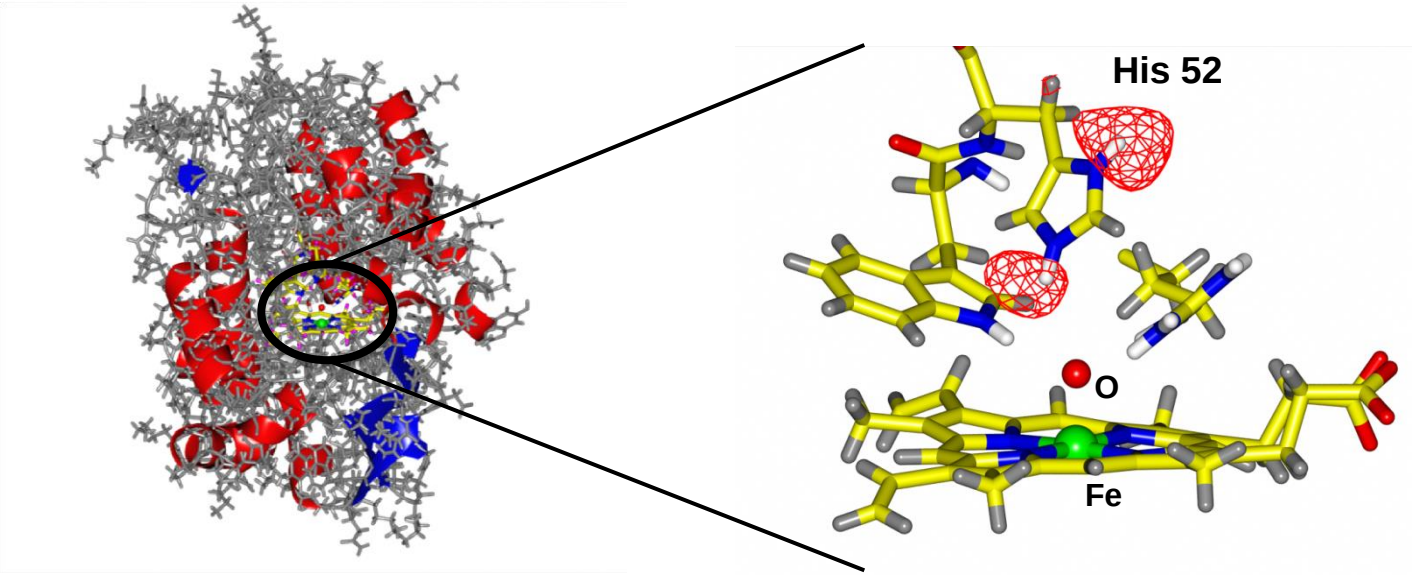
Compound I of Cytochrome c Peroxidase



Cecilia M. Casadei, Andrea Gumiero, Clive L. Metcalfe, Emma J. Murphy, Jaswir Basran, Maria Grazia Concilio, Susana C. M. Teixeira, Tobias E. Schrader, Alistair J. Fielding, Andreas Ostermann, Matthew P. Blakeley, Emma L. Raven, Peter C. E. Moody, Science 2014;345:193-197

Omit-Map for the two exchangable hydrogen atoms at His52

Cytochrome-c-Peroxidase, Compound I at 100 K:



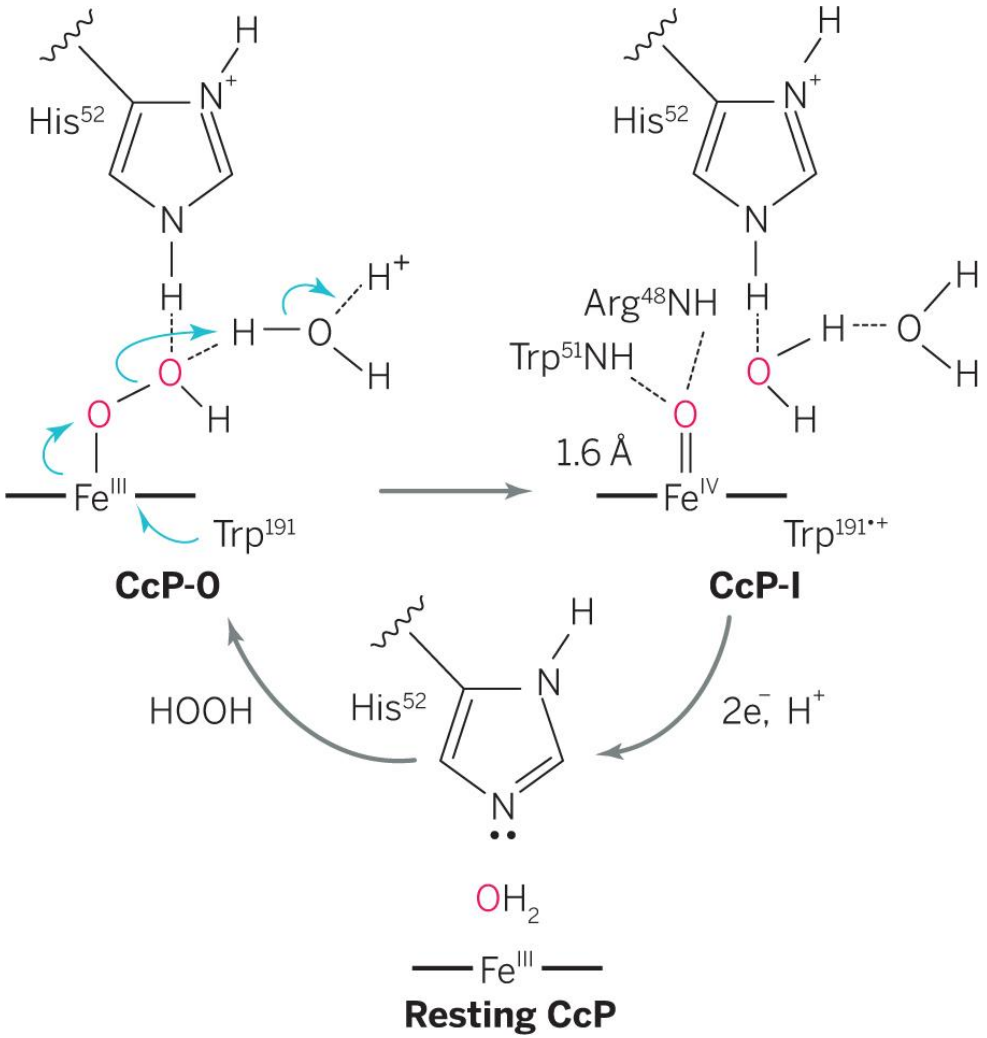
→ The oxygen atom bound to iron is not protonated.

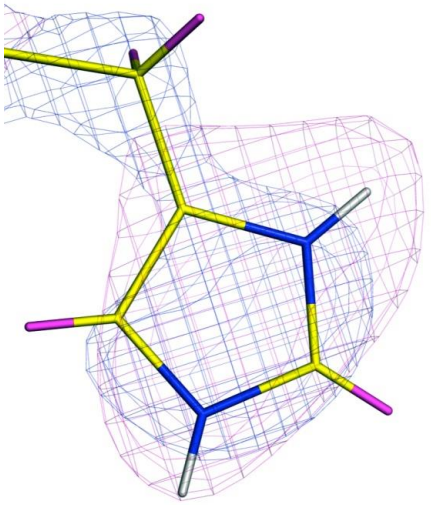
→ The amino-acid His52 is doubly protonated

↷ The reacton mechanism has to be thought over again!

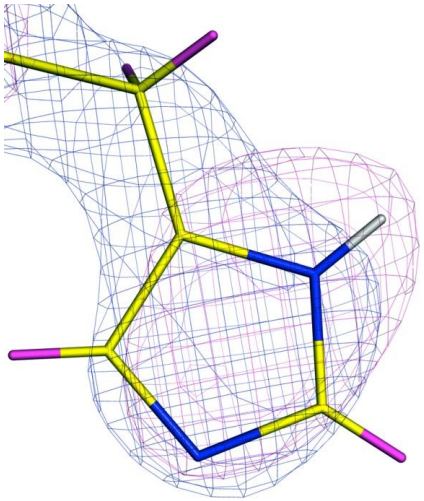
Casadei et al. Science **345**, 193 (2014)

Proton-mediated mechanism.Reaction of ferric CcP with H2O2 first gives CcP-0, followed by O-O bond scission driven by external protonation to afford CcP-I.





His 52
Compound I



His 52 ferric
(resting)

J T Groves, and N C Boaz Science 2014;345:142-143

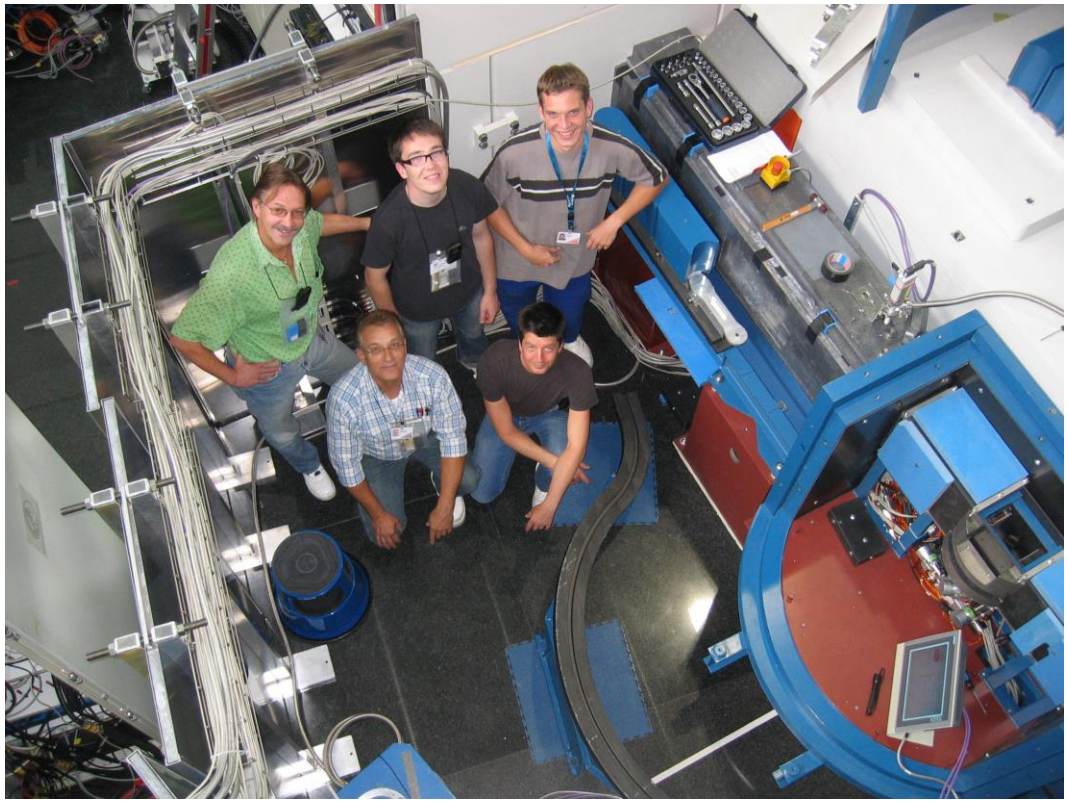
Summary

- 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... ... the BioDiff-Team:

Andreas Ostermann
- Philipp Jüttner
- Reinhard Schätzler
- Bernhard Laatsch
- Frank Suxdorf
- Manfred Bednarek
- Matthias Drochner
- Harald Kleines
- Kevin Körrentz
- Karl-Heinz Mertens
- Michael Monkenbusch
- Nikolas Arendt
- Christian Felder
- Michael Wagener
- Lydia Fleischhauer-Fuss
- Vladimir Ossovyi
- Andreas Nebel
- Simon Staringer
- Harald Kusche
- Winfried Petry
- Dieter Richter



... and you for your attention!

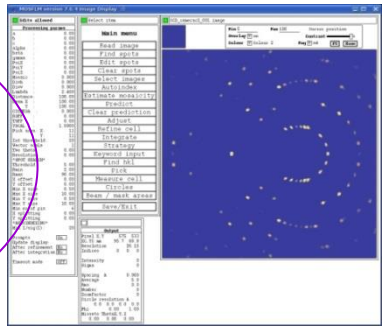
Post Doc position on SANS + IR spectroscopy

Deadline for application is 9. September 2019

Tutorial: Drug development based on Fragment screening: Trypsin + Aminopyridin

Fow chart of data treatment and model building

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



-MOSFLM
-HKL-denzo
(comercial)

Data reduction

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

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

Scaling of each hkl list to match each other

-SCALA (CCP4-program package)

Unified hkl-list of measurement := complete data set

Calculation of a first map

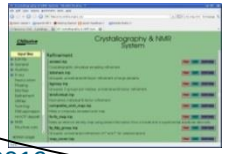
Additional information from the solution of the phase problem

Struktur refinement

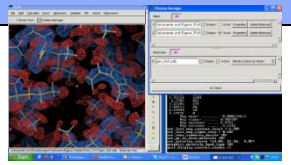
-Refinement of atom coordinates displacements
- Calculation of scattering density maps (netrons) or electron density maps (x-rays)

Map-plotting

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

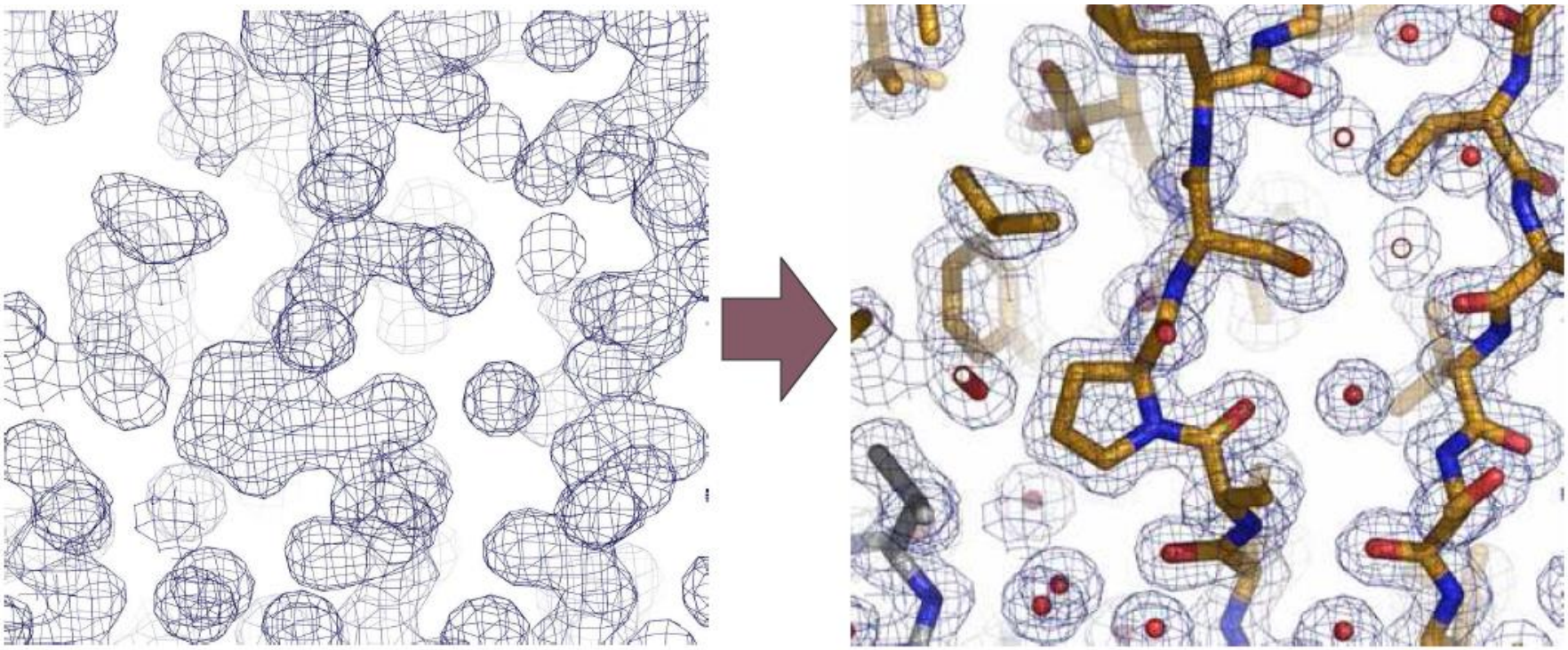


-nCNS
-PHENIX



-XtalView
-Coot

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

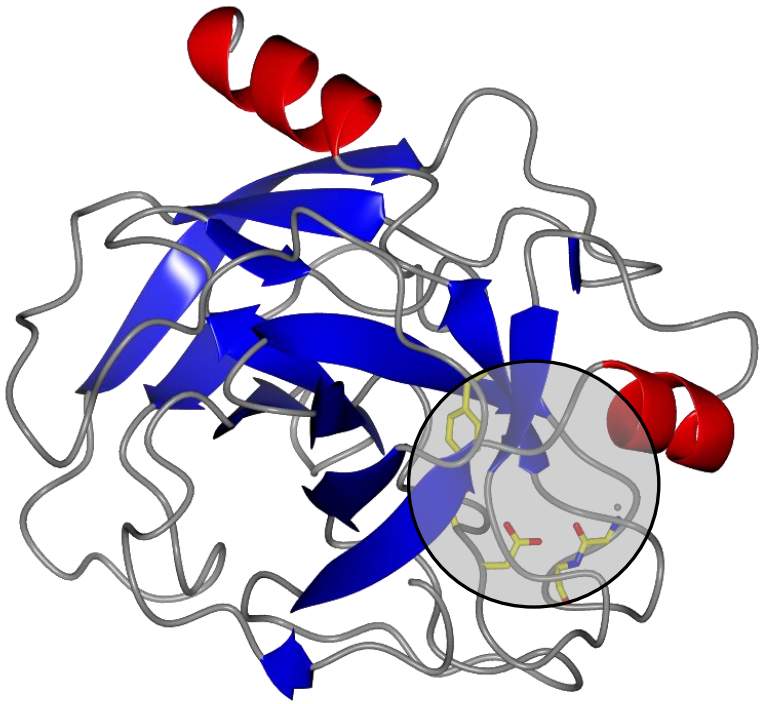


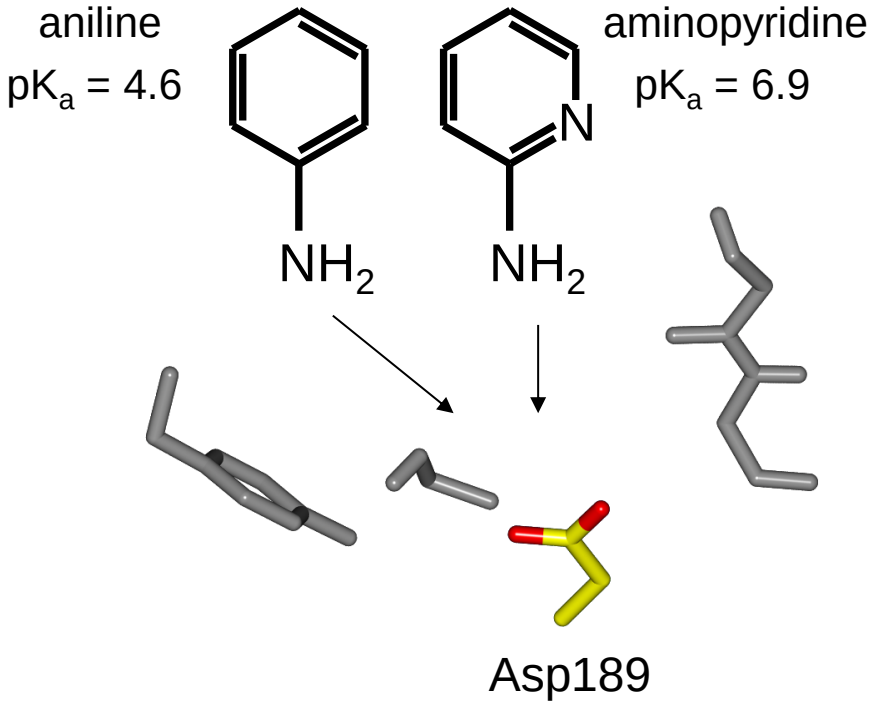
Electron density (x-ray) or nuclear density map (neutrons)

Amino acid chain is fitted into electron densities via dedicated software

from Armstrong.pdf: Qiagen

Inhibitor binding to trypsin: charges shift protonation



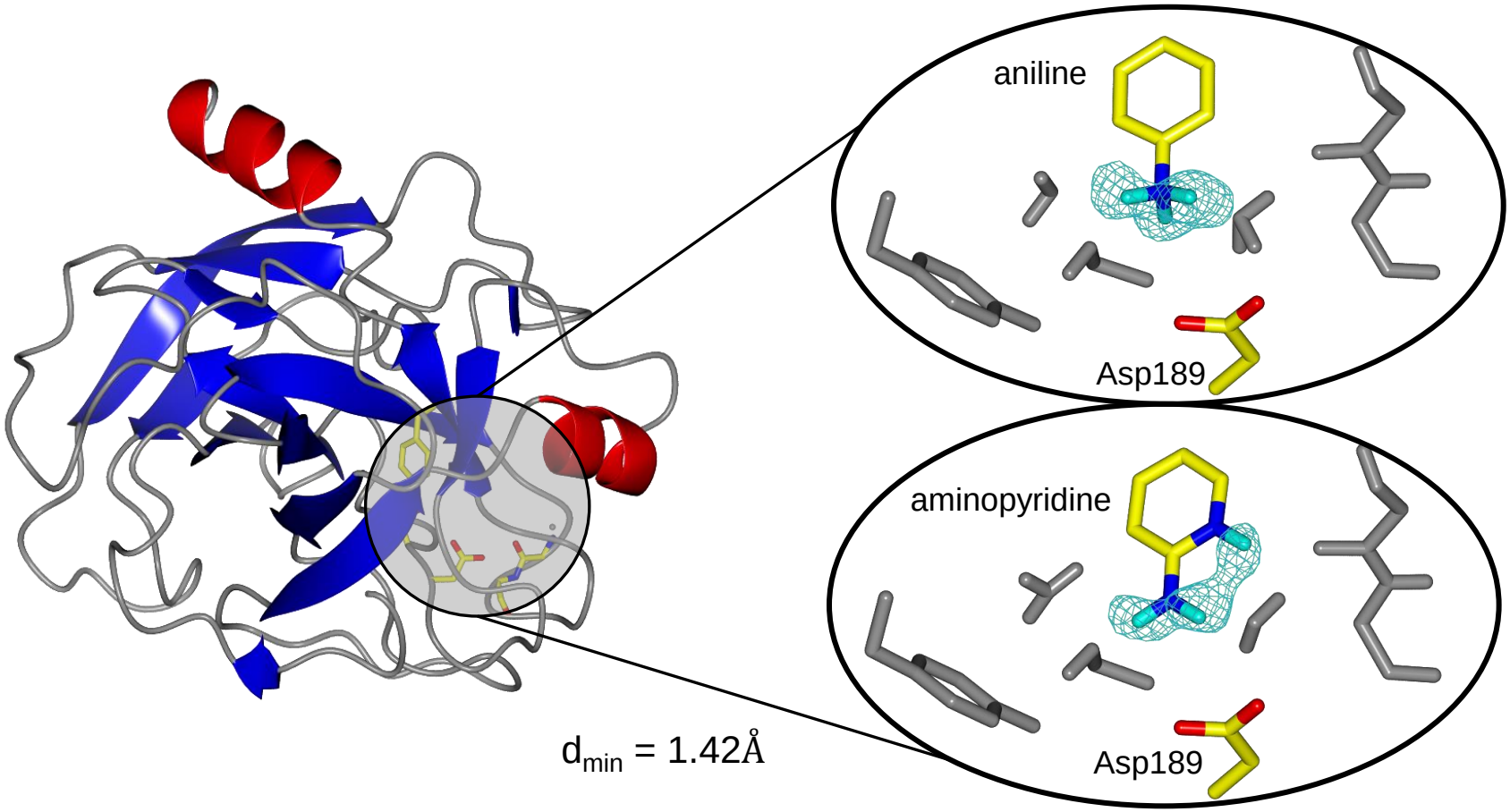


➡ question: inhibitors with less basic properties become protonated upon binding ?

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

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

Inhibitor binding to trypsin: charges shift protonation



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

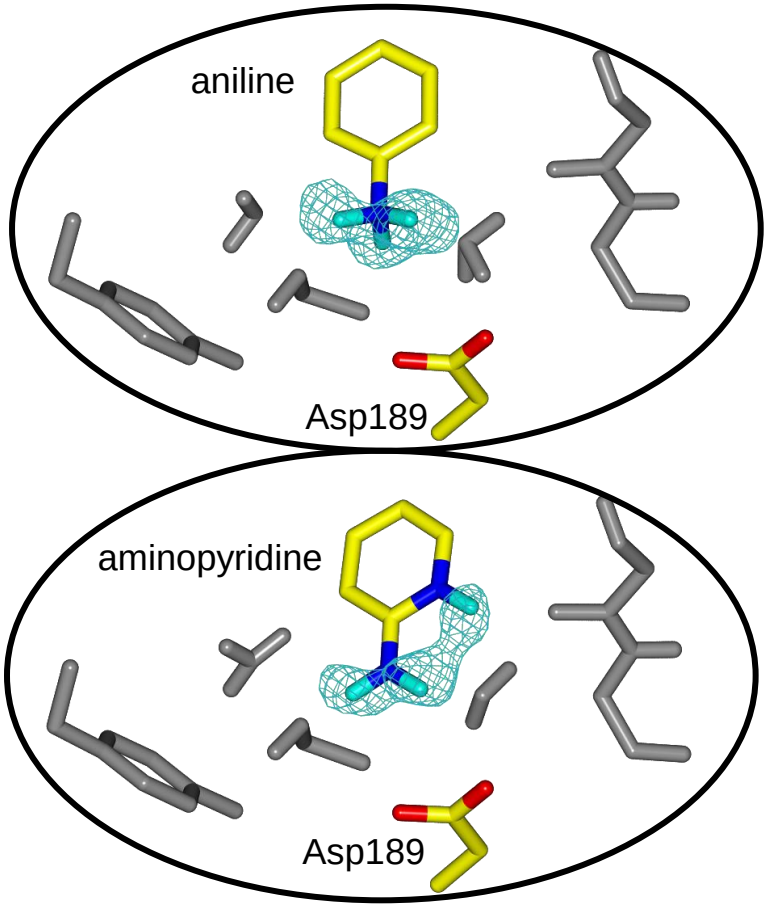
Fo-Fc omit-map; +3.5σ

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

Inhibitor binding to trypsin: charges shift protonation

- 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 !



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

Fo-Fc omit-map; +3.5σ

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

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.

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

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)

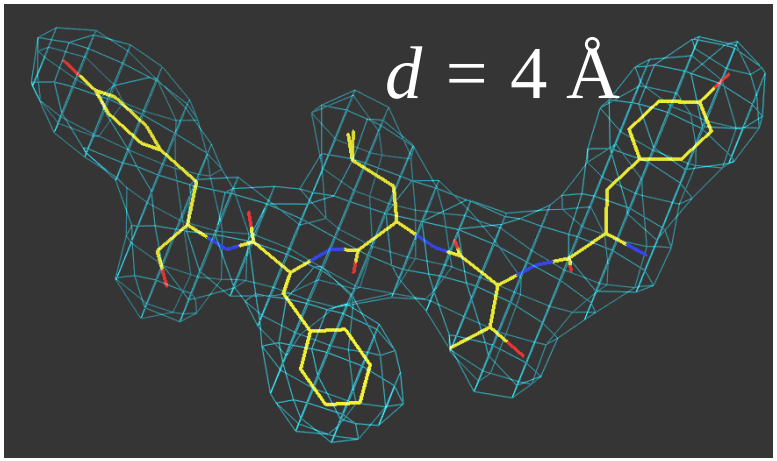
occupancy

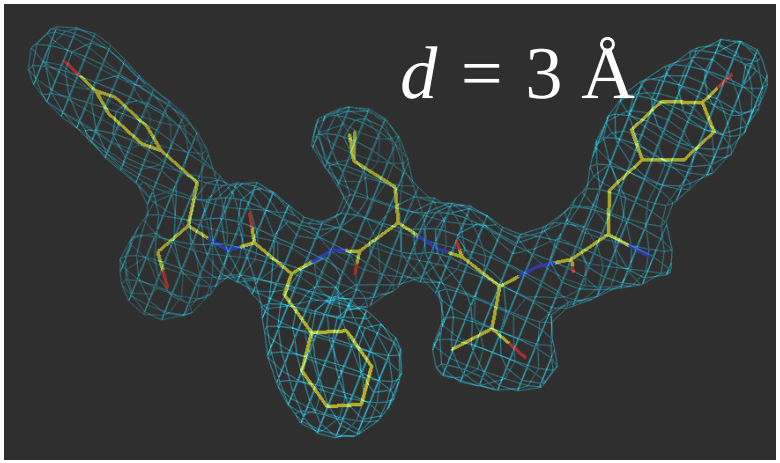
x,y,z coordinates (Å)

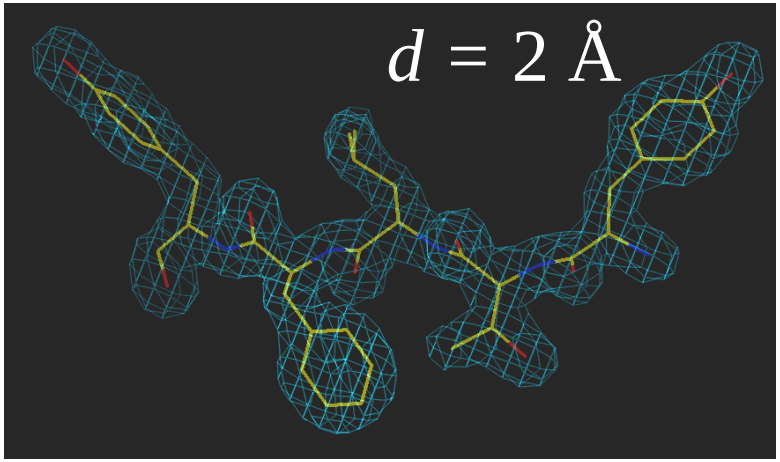
```
ATOM     25  N   ASP A 928      19.062   9.157  35.067  1.00  4.73           N
ATOM     26  CA  ASP A 928      19.770  10.123  34.232  1.00  4.58           C
ATOM     27  C   ASP A 928      19.075   9.938  32.899  1.00  4.56           C
ATOM     28  O   ASP A 928      19.074   8.824  32.351  1.00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

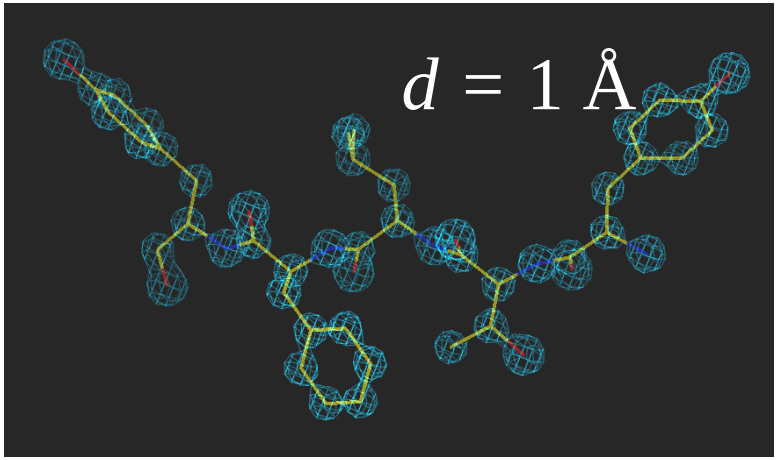
Isotropic B-factor or temperature factor is a measure of the mobility of an atom

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





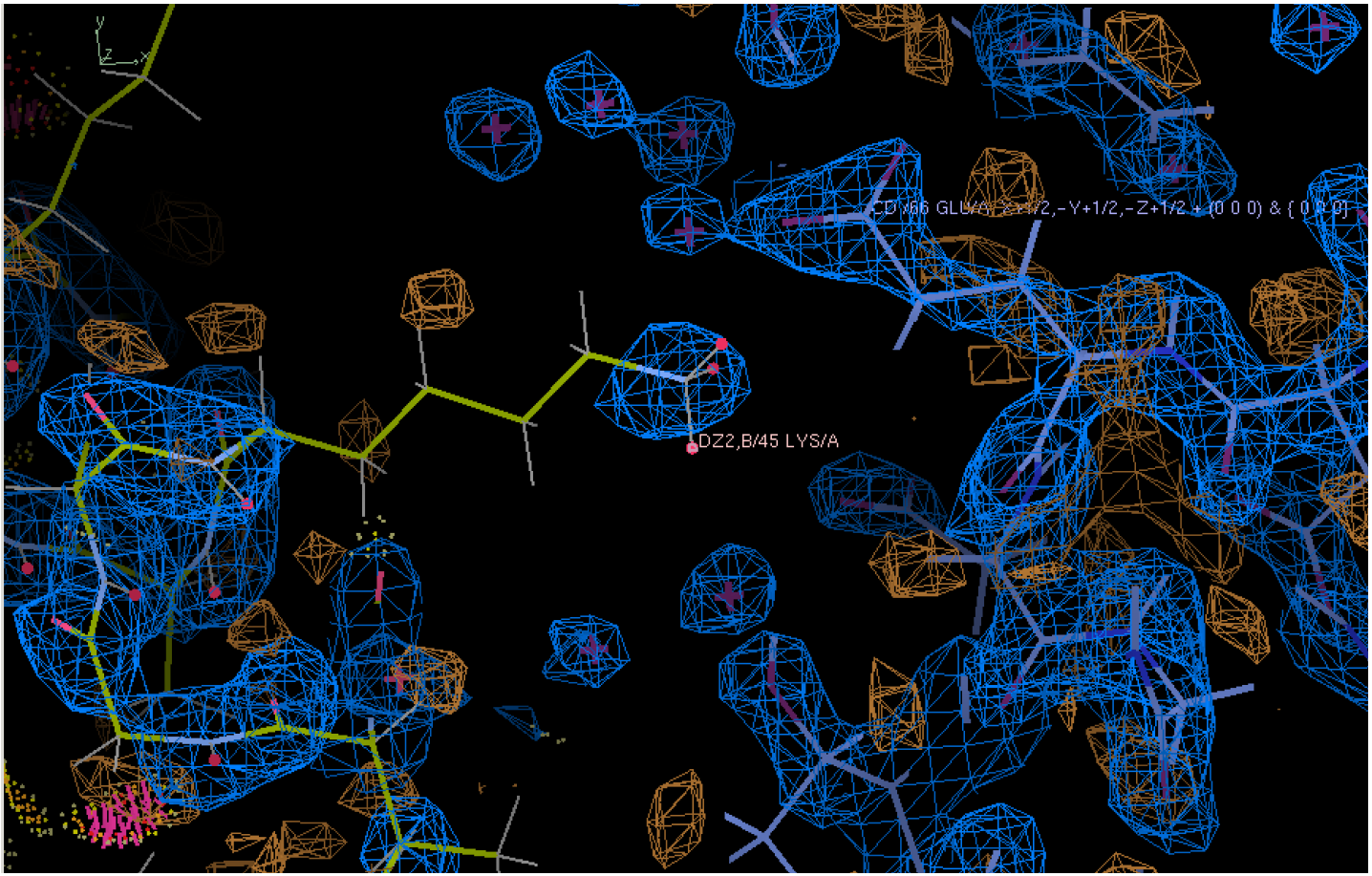




Atomic resolution

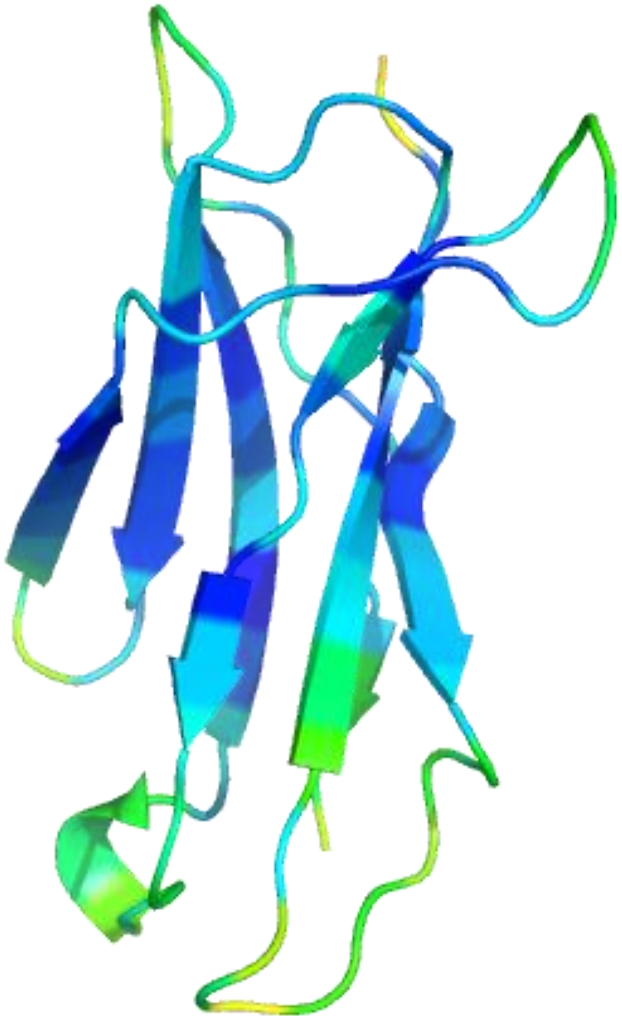
Lysine side chain on the surface of Alcoholdehydrogenase

Johannes Hermann et al. Acta Cryst. (2018). F74, 754–764



Please look at the scattering (length) density not only at coordinates…

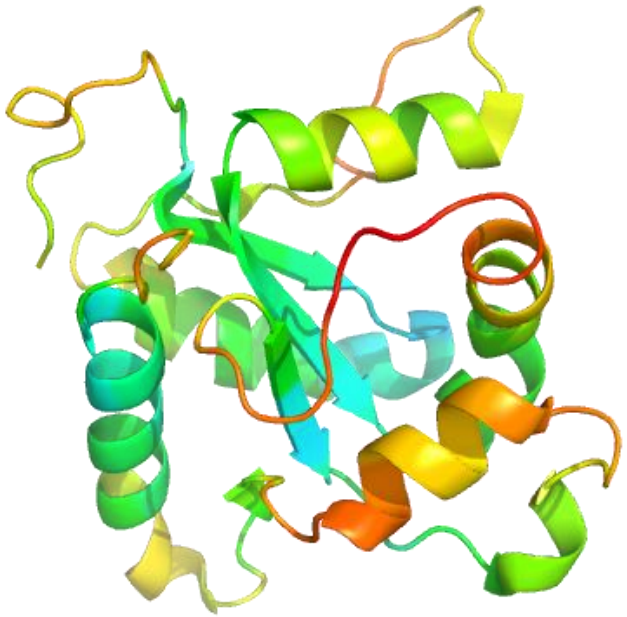
Look at B-factor distribution!



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

Starting situation: You would like to do the neutron data refinement of Trypsin with the ligand Aminopyridin

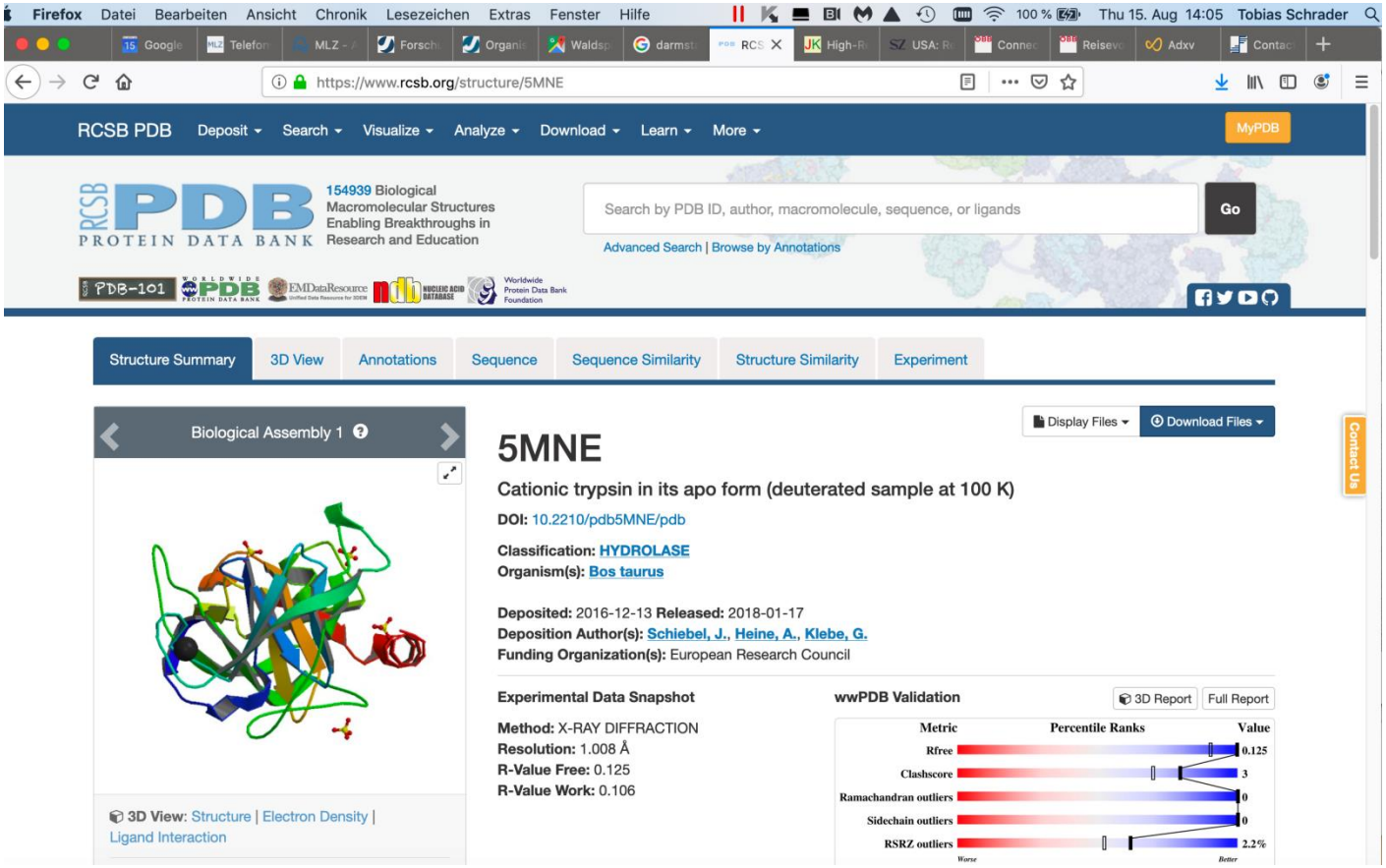
What you have:

A .sca file from the instrument responsible with the data (hkl, intensities, errors of intensities, the unit cell and the space group)

A starting model from the pdb (may be a close by x-ray model)

Starting model without the ligand

Protein data bank: www.rcsb.org



What do you need?

- The Software phenix.refine in its latest version:

https://www.phenix-online.org/download/

- The Software Coot:

https://www2.mrc-lmb.cam.ac.uk/personal/pemsley/coot/

- A two mouse button wheel mouse
- A good editor for text
- Some patience

A tribute to the software programmers…

Citation for the PHENIX suite:

Adams P.D., Afonine P.V., Bunkoczi G., Chen V.B., Davis I.W., Echols N., Headd J.J., Hung L.W., Kapral G.J., Grosse-Kunstleve R.W., McCoy A.J., Moriarty N.W., Oeffner R., Read R.J., Richardson D.C., Richardson J.S., Terwilliger T.C., & Zwart P.H. (2010). Acta Cryst. D66, 213-221.

Validation:

Williams C.J., Hintze B.J., Headd J.J., Moriarty N.W., Chen V.B., Jain S., Prisant MG Lewis S.M., Videau L.L., Keedy D.A., Deis L.N., Arendall WB I.I.I., Verma V., Snoeyink J.S., Adams P.D., Lovell S.C., Richardson J.S., & Richardson D.C. (2018). Protein Science 27, 293-315.

phenix.refine:

Afonine P.V., Grosse-Kunstleve R.W., Echols N., Headd J.J., Moriarty N.W., Mustyakimov M., Terwilliger T.C., Urzhumtsev A., Zwart P.H., & Adams P.D. (2012). Acta Crystallogr D Biol Crystallogr 68, 352-67.

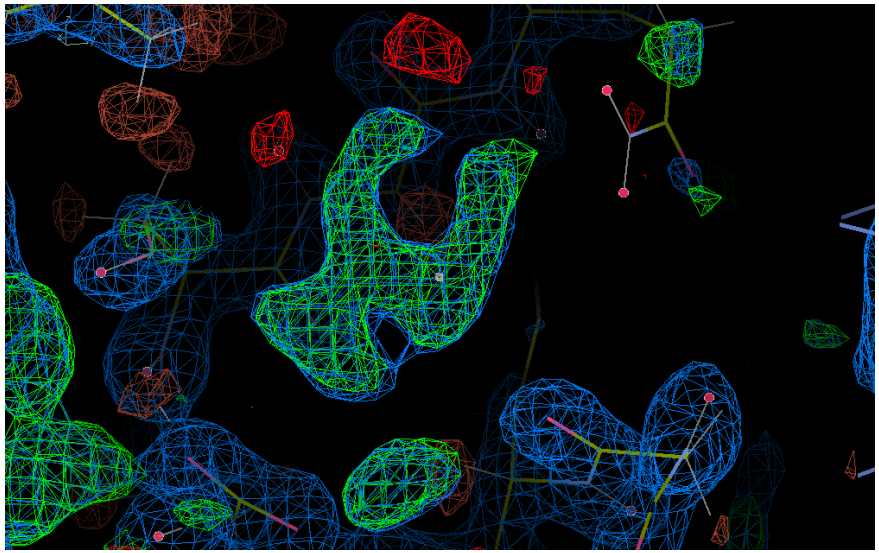
My advice

1. Always look at the maps and difference maps produced by each refinement step
2. Disregard refinement steps when r- factors increase or the difference between r-work and r-free gets too large (more than 2.5%)
3. Always look at the output pdb model with an editor of your own choice
4. Use the pdb checking tools by phenix and later from the web-site www.rcsb.org

Final results

Refinement is never finished!

Result without ligand aminopyridin



Result with the published structure in