

The instrument BIODIFF

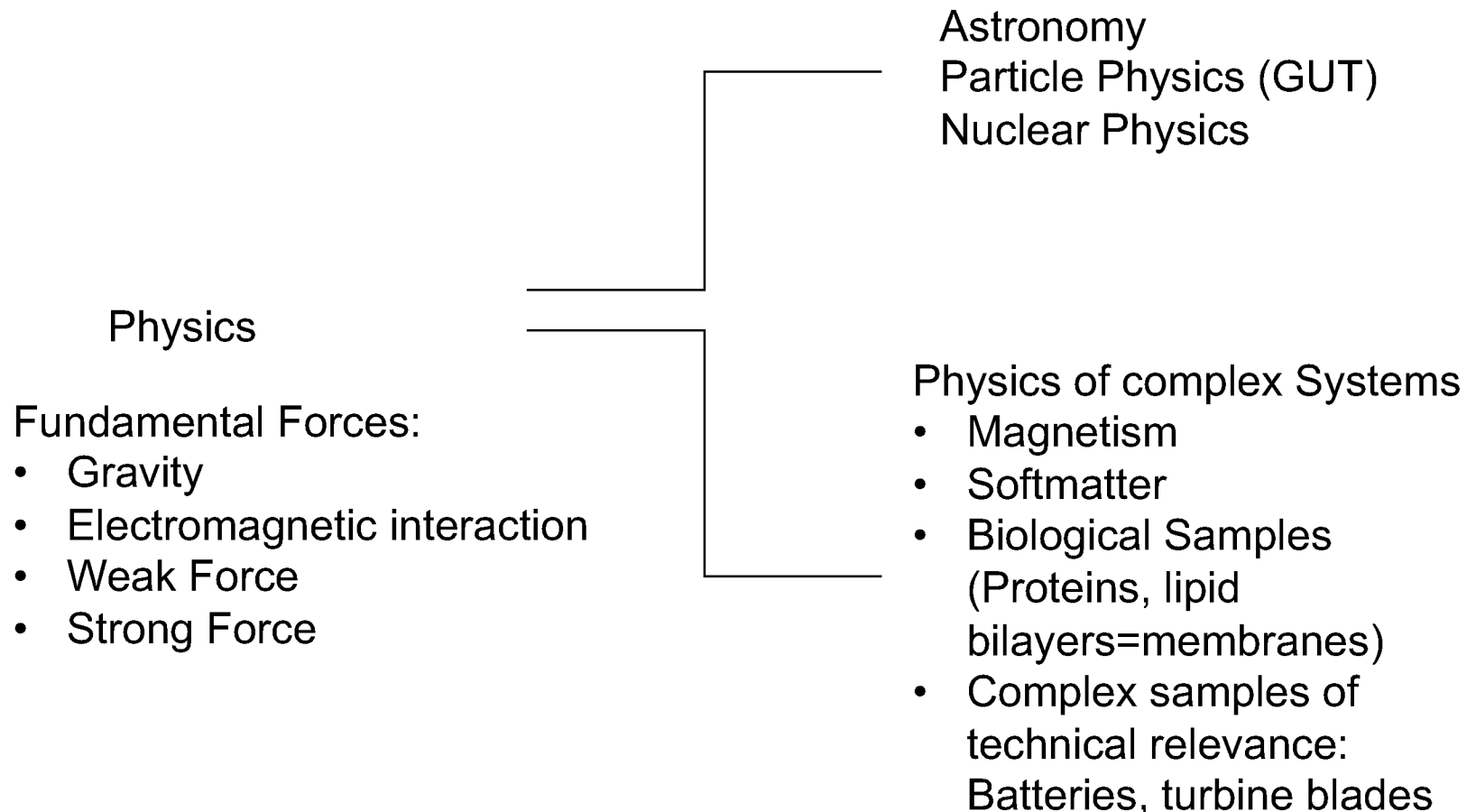
Fachkunde Schichtpersonal

November 28st 2019 | [Tobias E. Schrader](#)

Outline

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

What to do in Science as a Physicist?



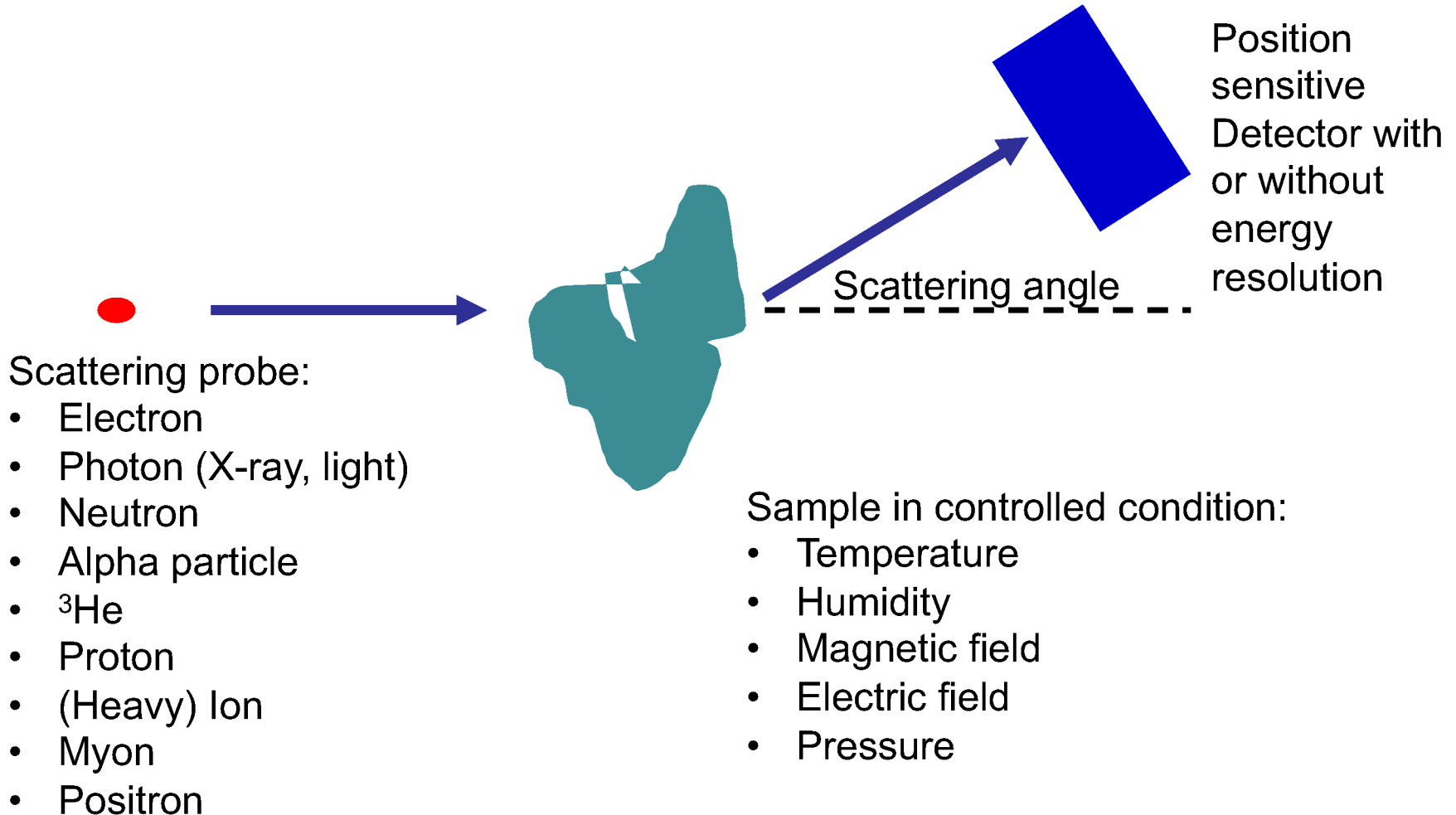
Why do we need experimental studies on proteins?

- **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 fulfill their tasks. MD-simulations cannot model **bond breaking** and forming very well since the quantum chemistry nature of this process is not included in the theoretical foundation of MD.
- **Ab initio quantum chemical calculations** are still **too demanding** to model the complete active centre of a protein (including its substrate)



Standard Scattering process

- The probe in use determines the information you get...



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

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

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

Scattering

„Structure“

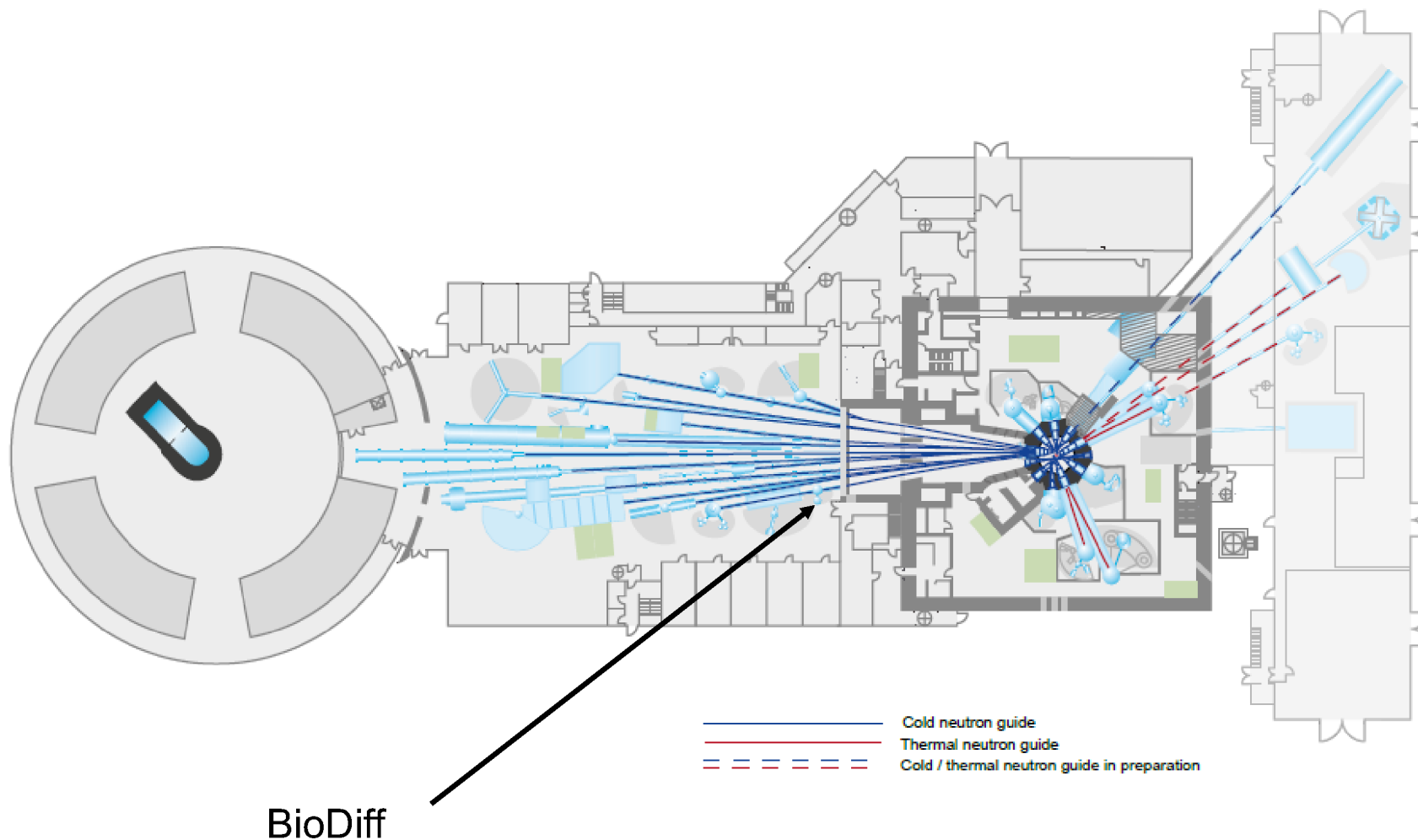
Elastic
(no energy transfer from or to the sample)

„Dynamics“

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

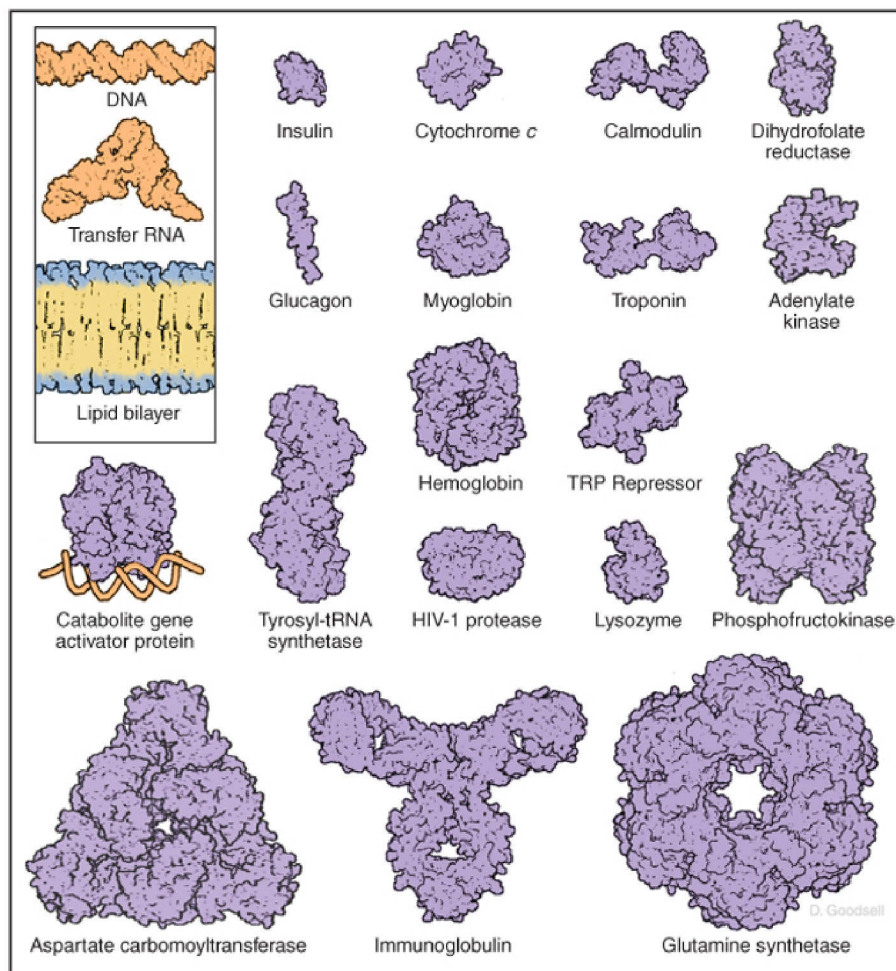
- Only elastic incoherent scattering is of no interest to the scientists

BioDiff at FRM II in the neutron guide hall west



Introduction into structured macromolecules and a simple model for proteins

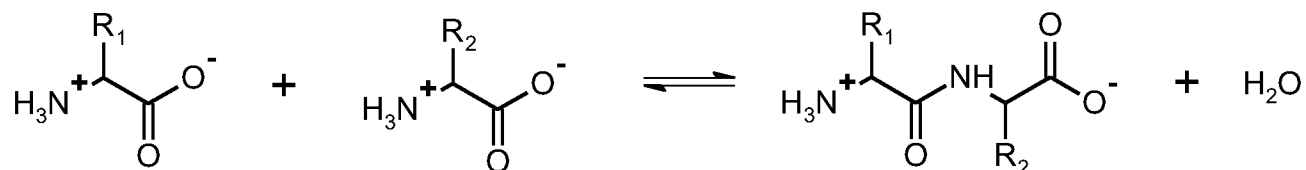
Structured macromolecules come in different shapes and sizes



© Elsevier. Pollard et al: Cell Biology 2e - www.studentconsult.com

Definition of "protein"

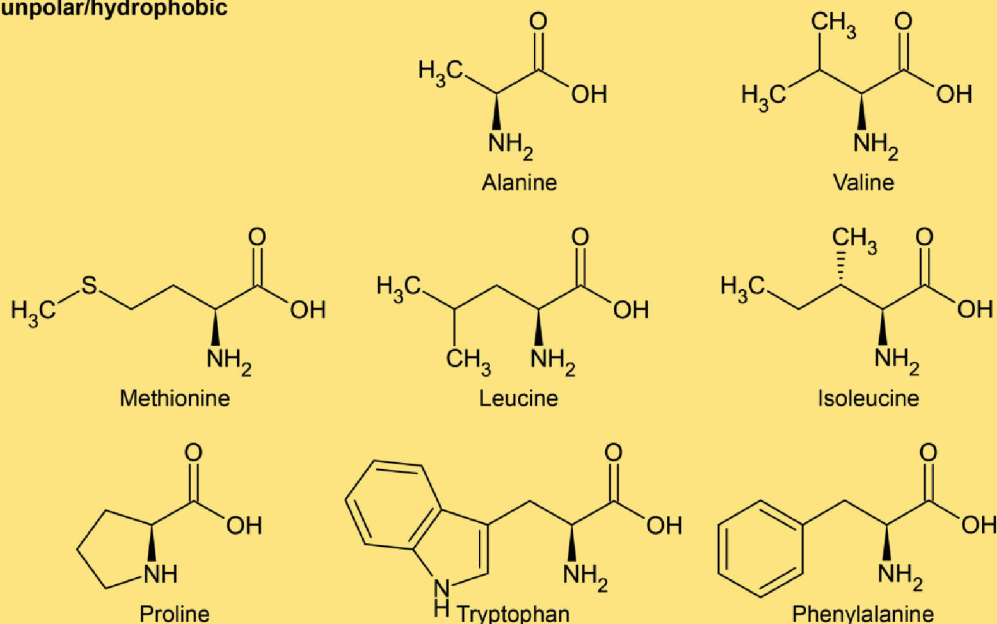
- Proteins are the most abundant class of biological macromolecules in all cellular organisms.
- They are polymers of α -amino acids (2-amino carboxylic acids), formed by condensation of carboxyl and amino functions of adjacent units. There are 20 different amino acids in nature.



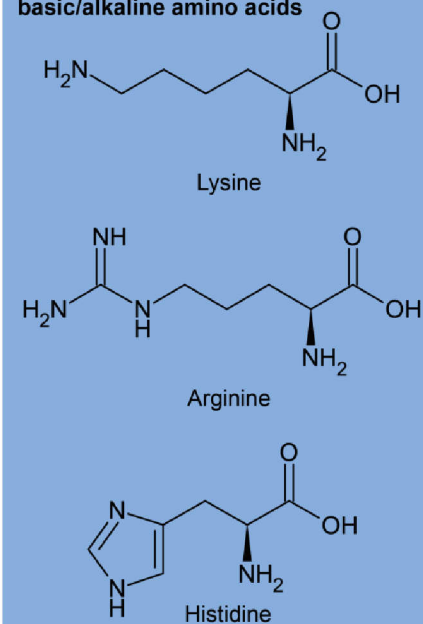
- Historical nomenclature (n=number of amino acids):
 $n < 10$: oligopeptide
 $10 < n < 100$: polypeptide
 $n > 100$: protein

The 20 amino acids occurring in nature

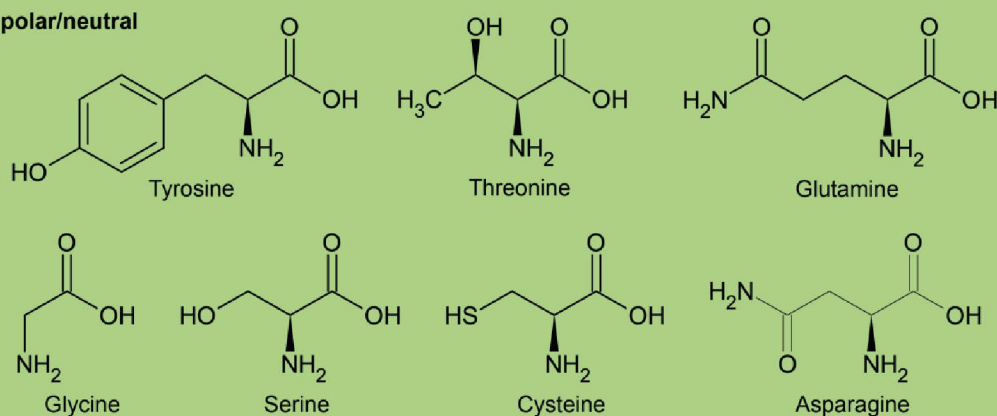
unpolar/hydrophobic



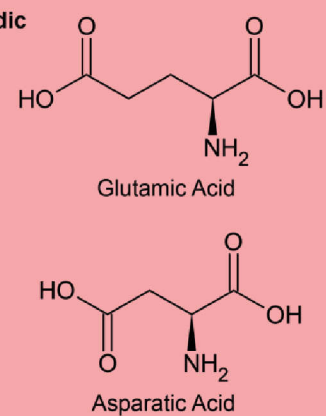
basic/alkaline amino acids



polar/neutral

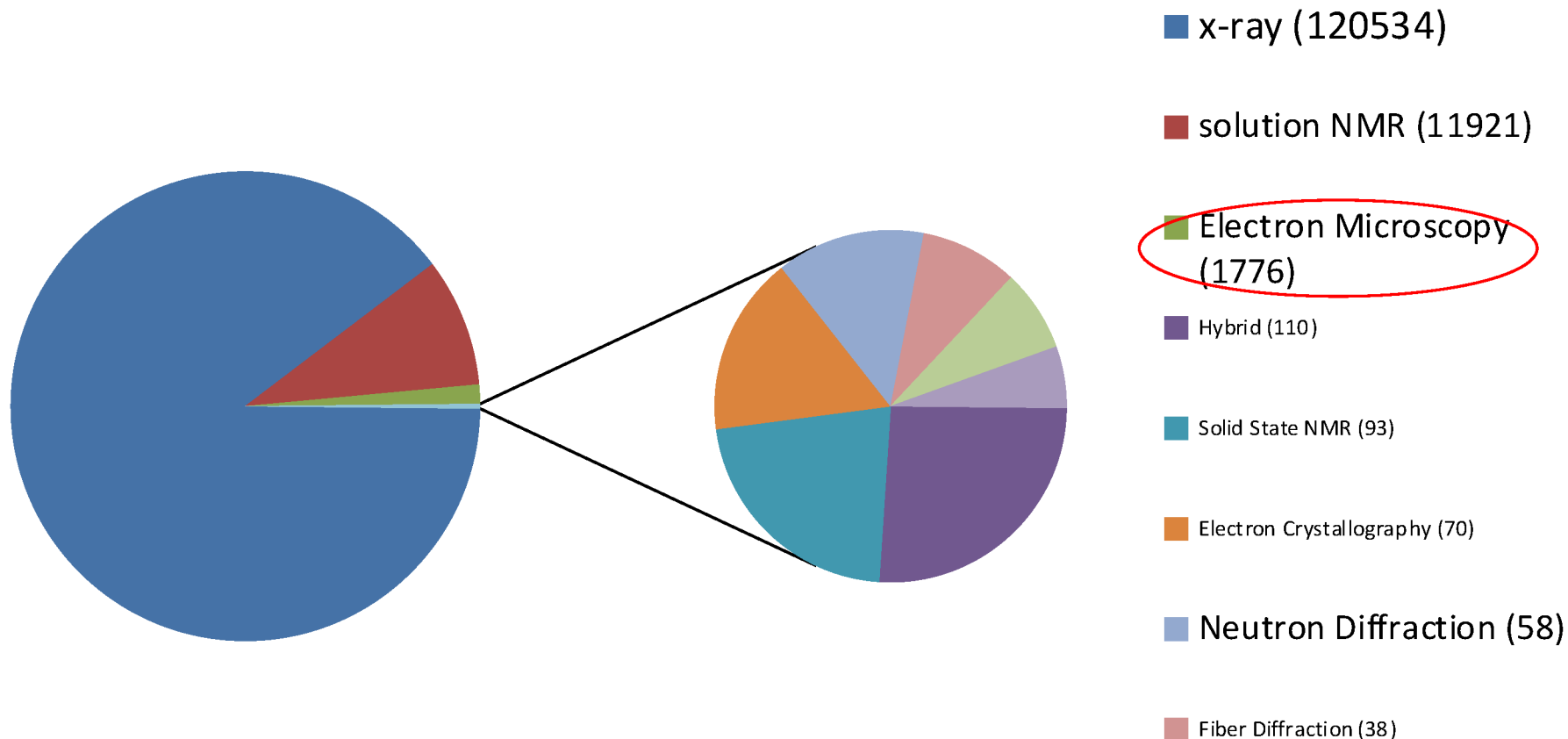


acidic



How do we find out about protein structures?

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



<http://www.rcsb.org/>

Total number of structures: 134656

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

x,y,z coordinates (Å)

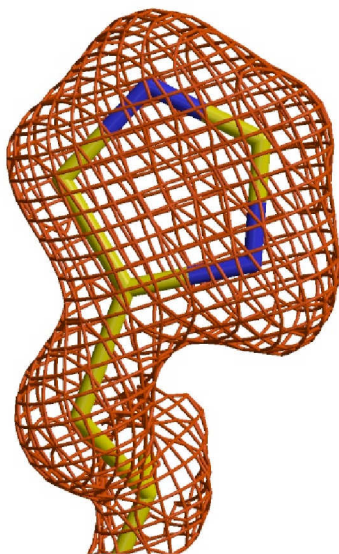
ATOM	25	N	ASP	A	928	19.062	9.157	35.067	1.00	4.73	N
ATOM	26	CA	ASP	A	928	19.770	10.123	34.232	1.00	4.58	C
ATOM	27	C	ASP	A	928	19.075	9.938	32.899	1.00	4.56	C
ATOM	28	O	ASP	A	928	19.074	8.824	32.351	1.00	5.39	O
ATOM	29	CB	ASP	A	928	21.259	9.776	34.071	1.00	3.13	C
ATOM	30	CG	ASP	A	928	22.112	10.245	35.233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

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

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

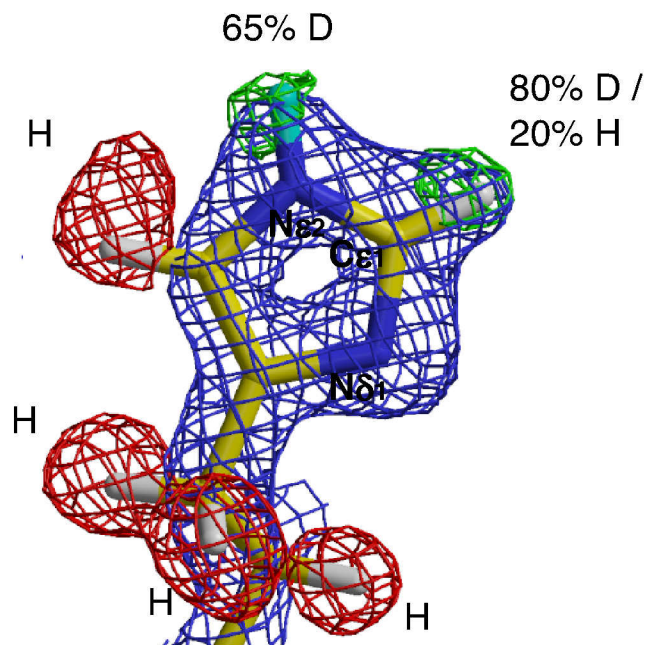
Protonation states of amino acids:

X-ray $d_{\min} = 1.5\text{\AA}$:



2Fo-Fc map; $+1.5\sigma$

neutrons $d_{\min} = 1.5\text{\AA}$:



2Fo-Fc map; $+1.5\sigma$

Fo-Fc omit-map; -3.0σ

Fo-Fc omit-map; $+3.0\sigma$

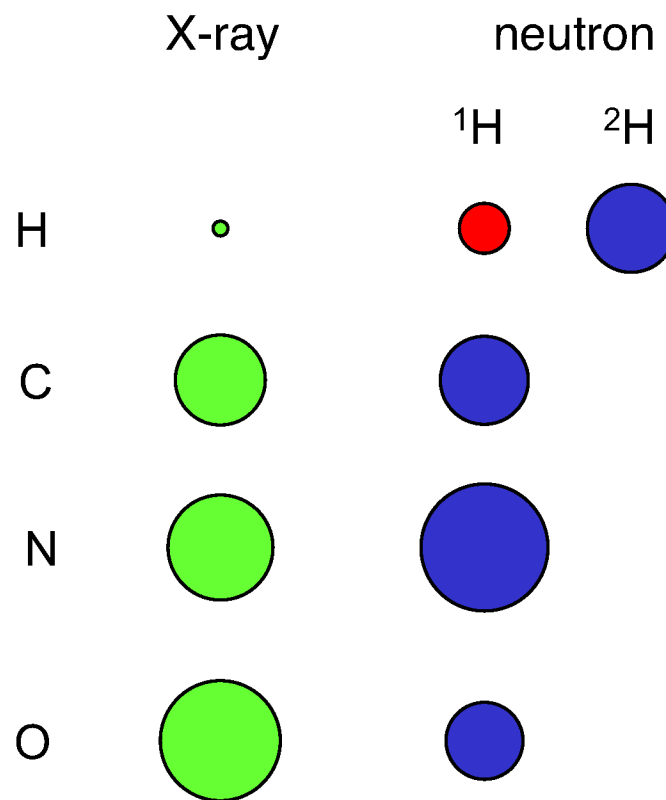
Niimura N, Chatake T, Ostermann A, Kurihara K, Tanaka T. (2003) Z. Kristallogr. 218:96

Advantages of structure determination with neutrons:

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

X-rays scatter from electrons,
neutrons from nuclei.

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

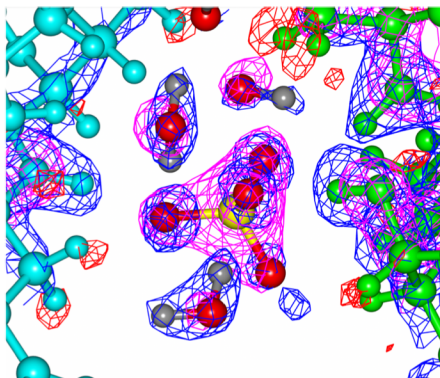


diameters correspond to: form factor / scattering length (scaled for C-atom)

Advantages of Structure Determination with Neutrons

Hydrogen/deuterium atoms can be resolved even at a resolution of $d_{\min} \approx 2.5 \text{ \AA}$ (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



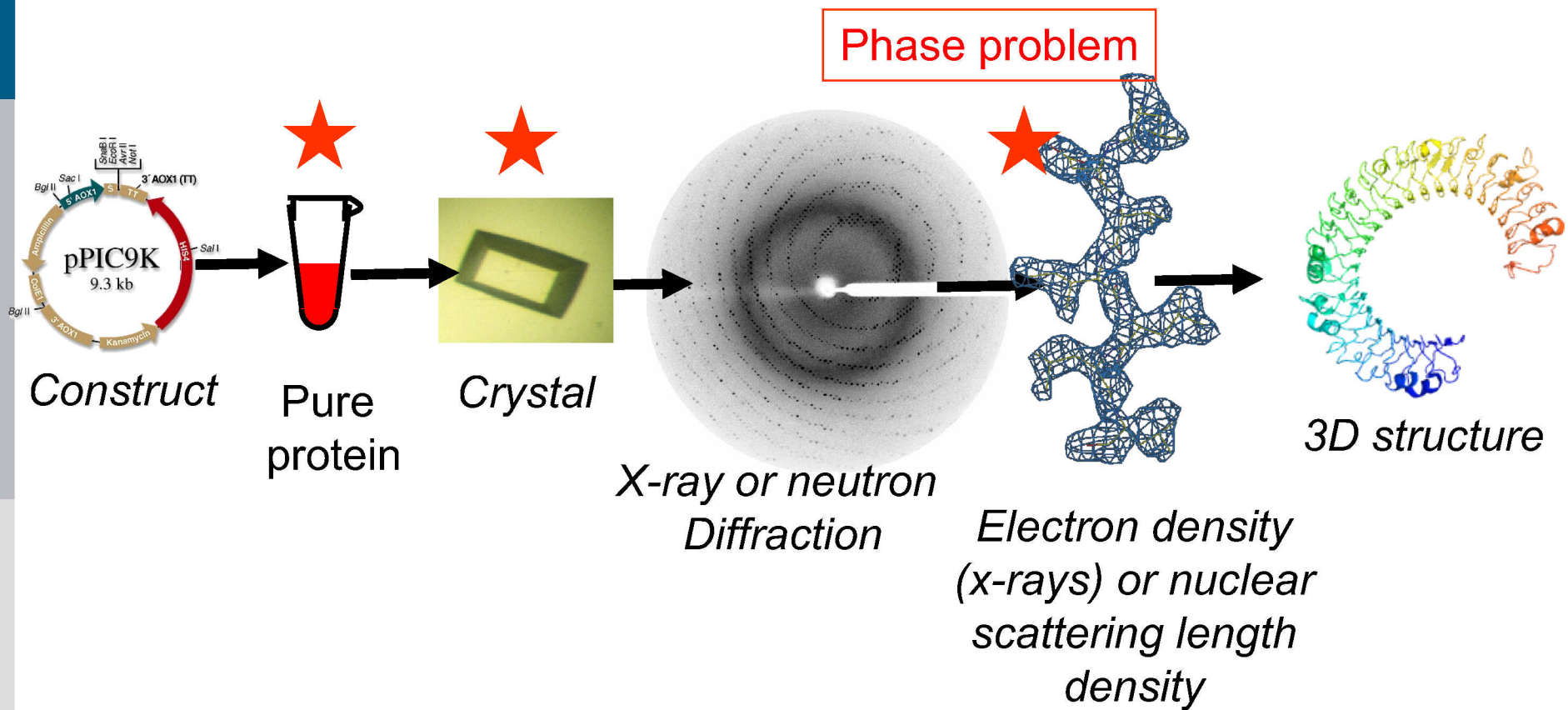
Water network in the contact region between two myoglobin molecules in the crystal.

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

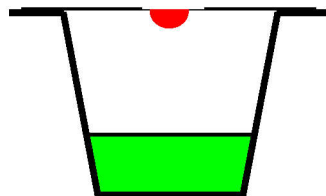
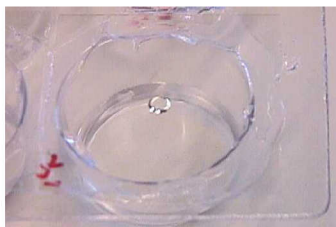
Protein crystallography in general, valid for both x-rays and neutrons as probes

Crystallography: Overview over the process



Harma Brondijk, Crystal and Structural chemistry, Utrecht University

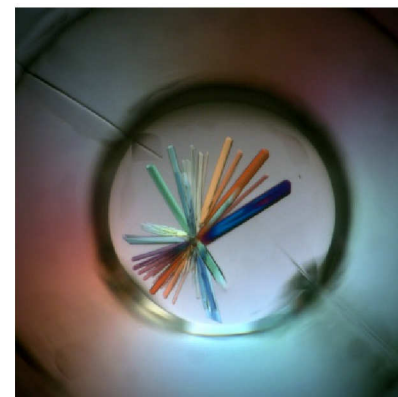
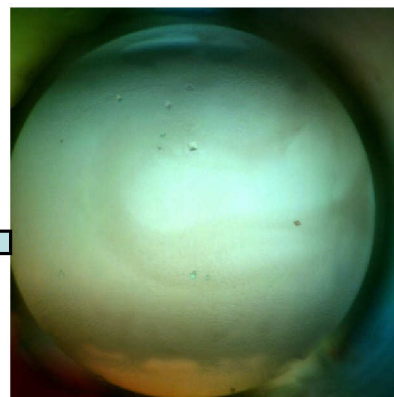
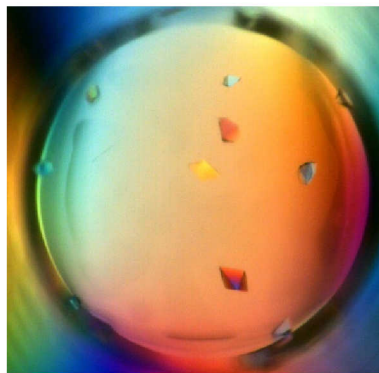
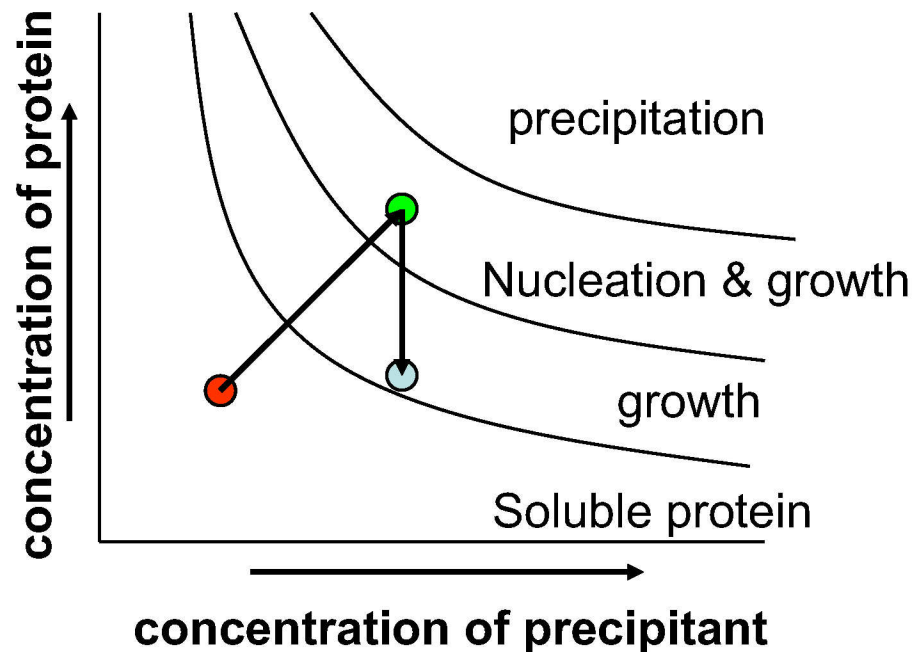
Growing crystals



Hanging drop: 1 μ l protein solution + 1 μ l reservoir solution

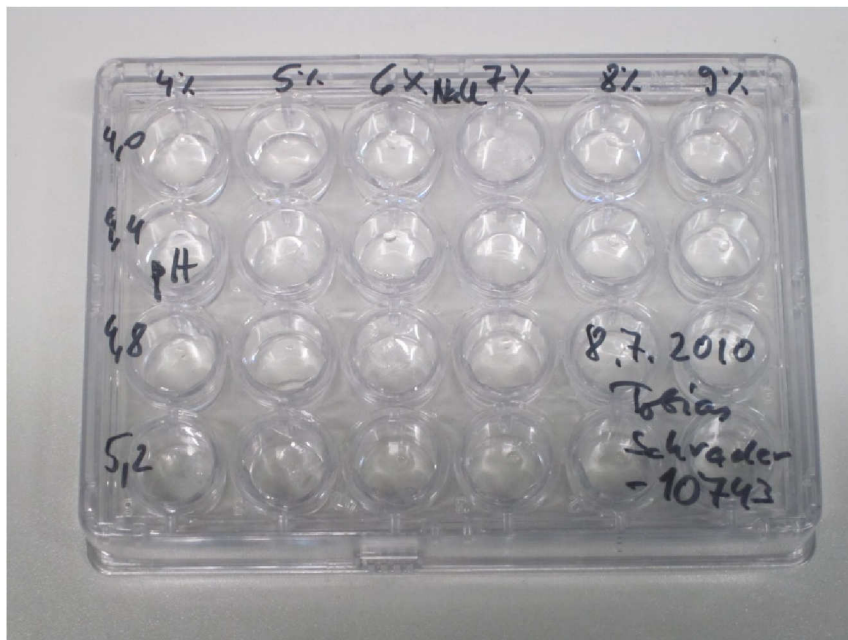
Reservoir: precipitant solution e.g. 1 M NaCl

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



Harma Brondijk, Crystal and Structural chemistry, Utrecht University

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

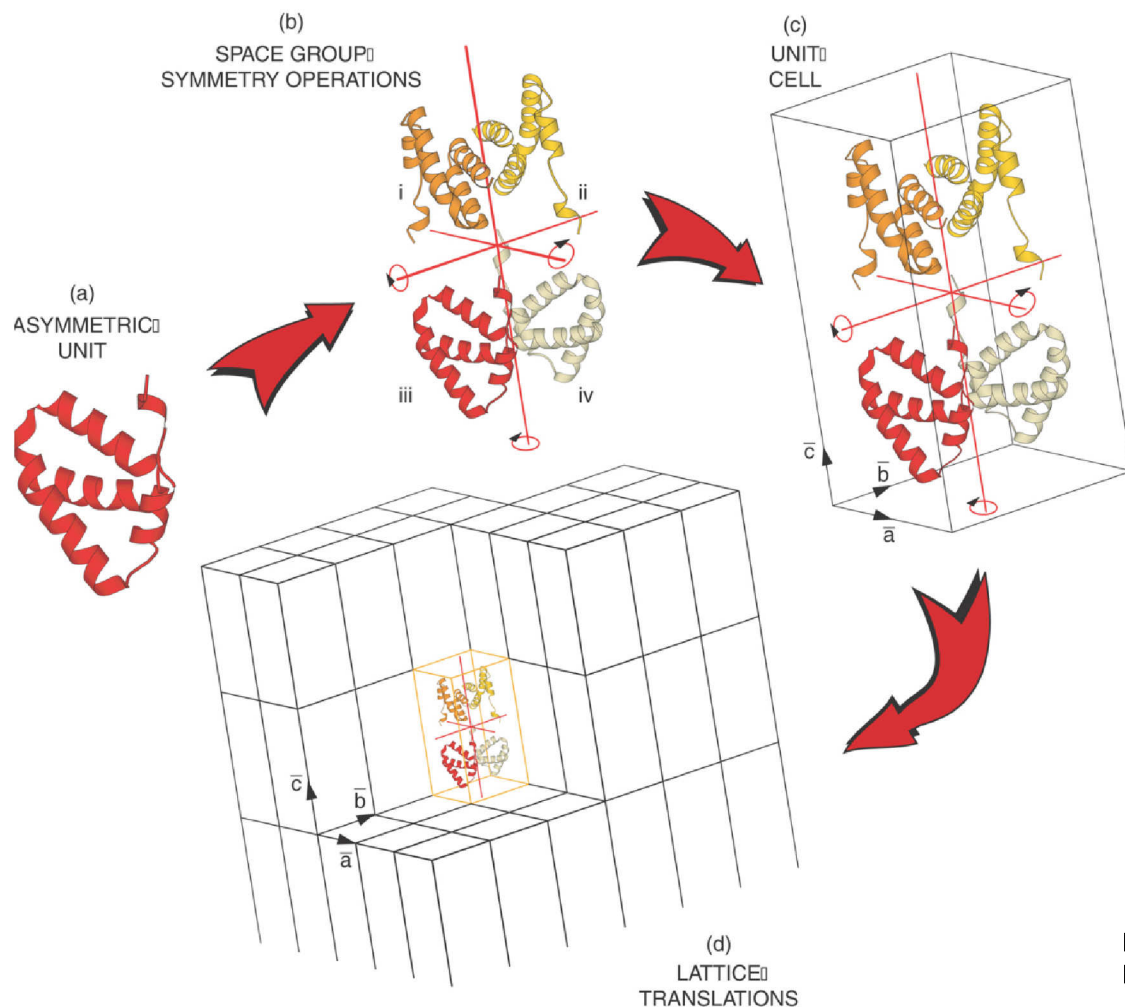


Hanging drop crystallization



Batch crystallization

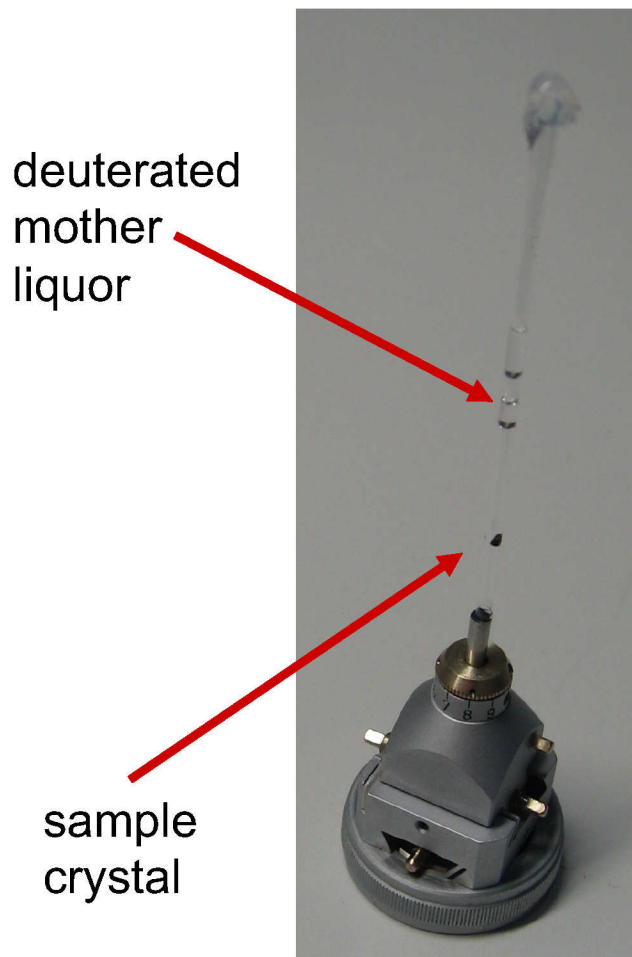
How a typical protein crystal looks like...



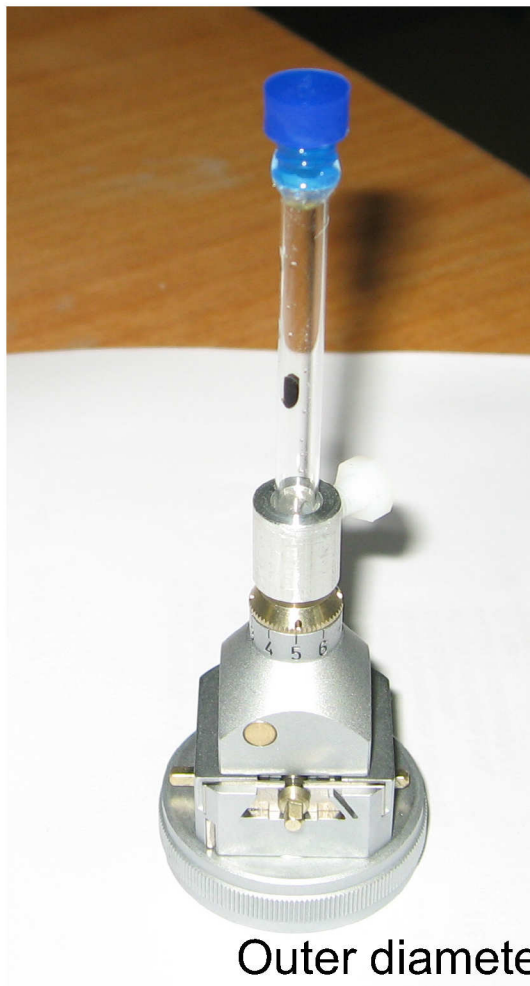
Picture taken from Lecture of
Prof. Locher at ETH Zürich

fig 2.2

Room temperature packaging of crystals



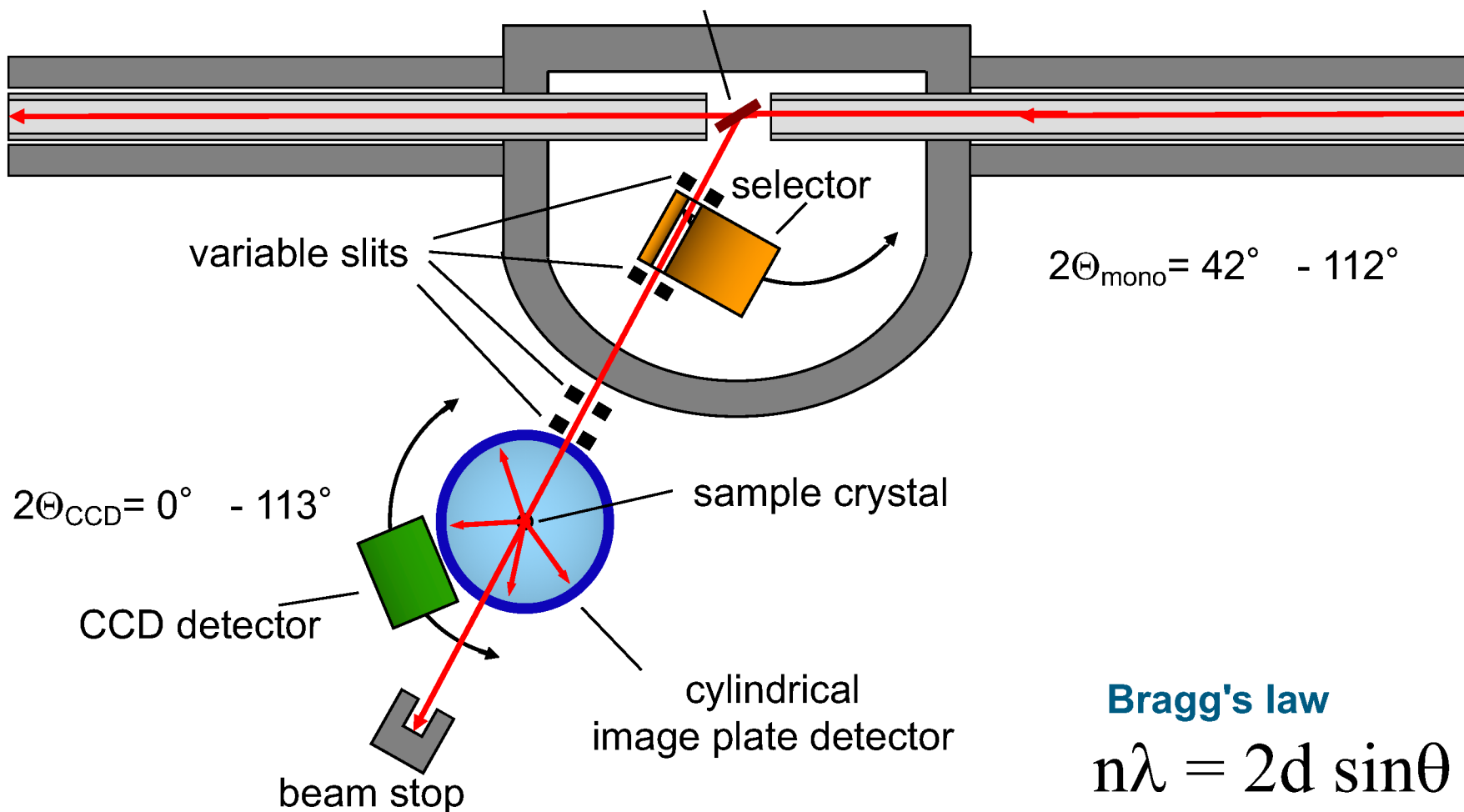
Outer diameter of the glas tube: 2 mm



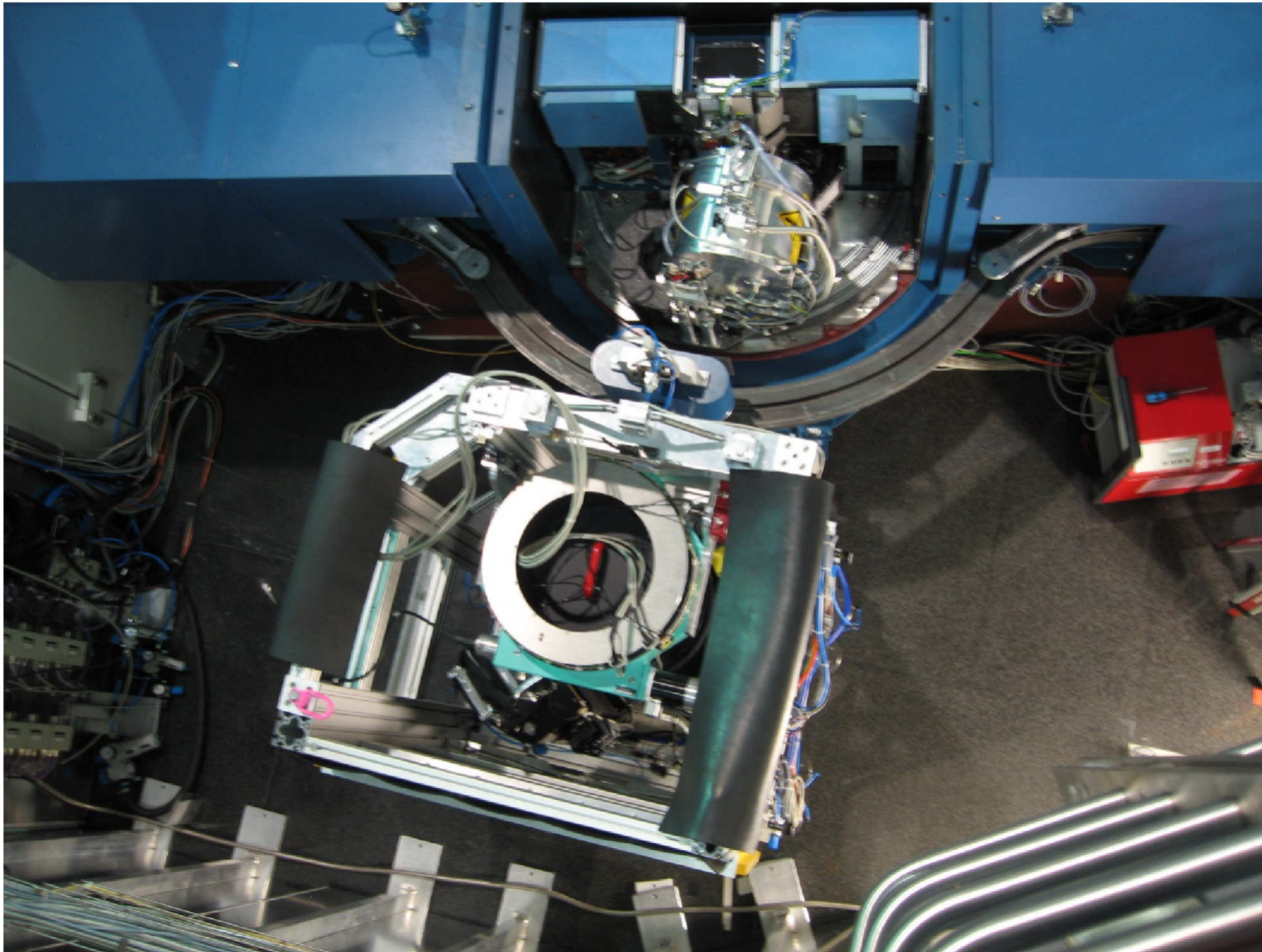
Outer diameter of the glas tube: 5 mm

- 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.

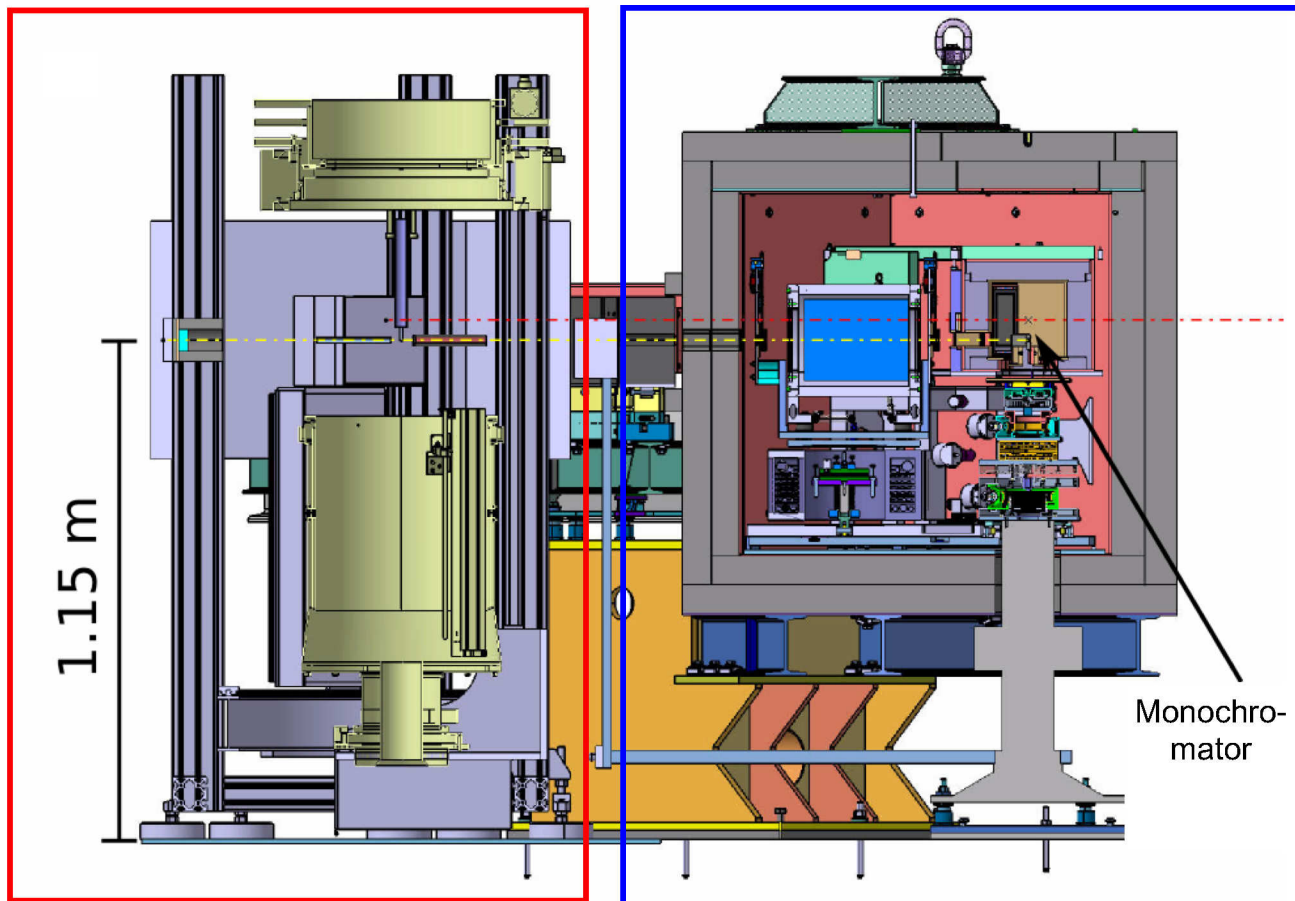
Schematic overview over BioDiff: A neutron protein diffractometer: collaboration between JCNS and FRMII



BioDiff, the corresponding view in reality:



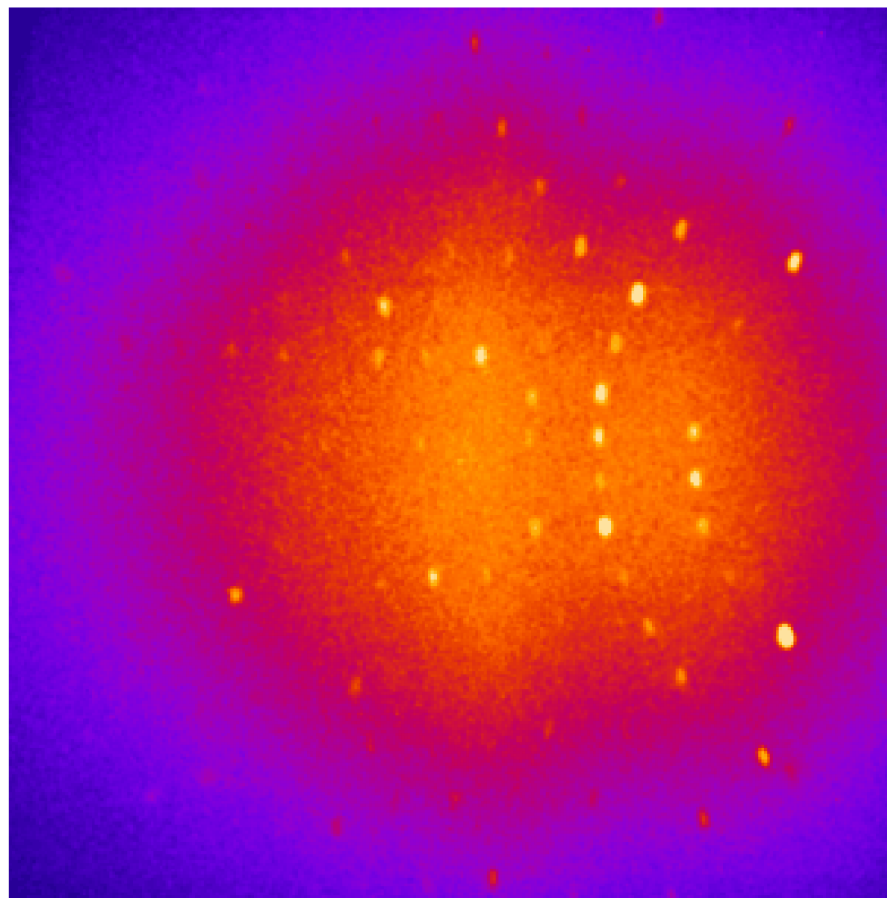
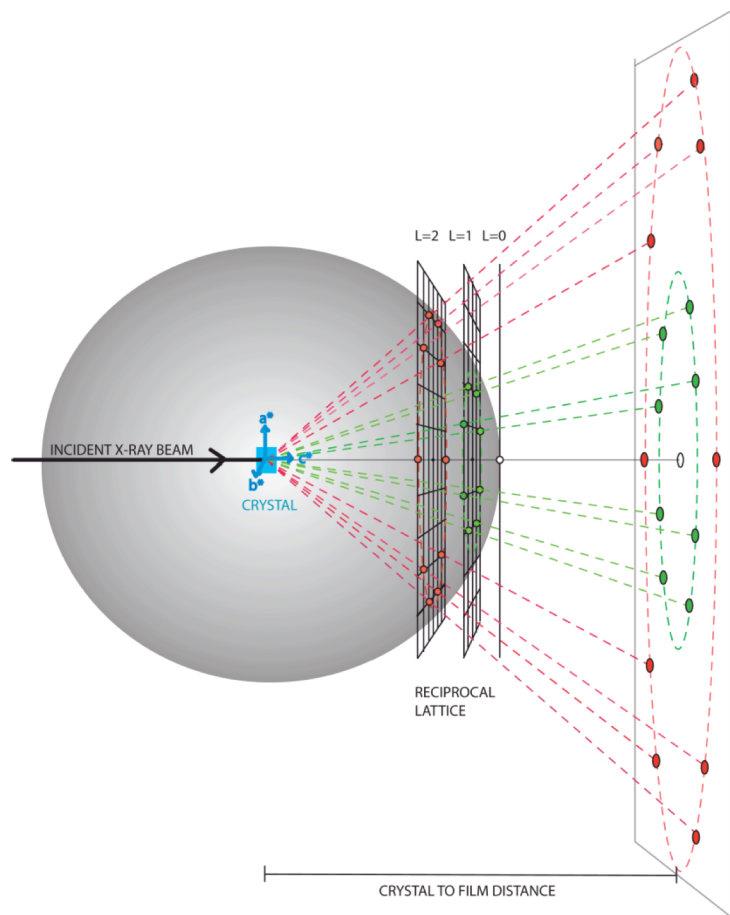
The Simultaneous Construction Phase in Garching and Jülich



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

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

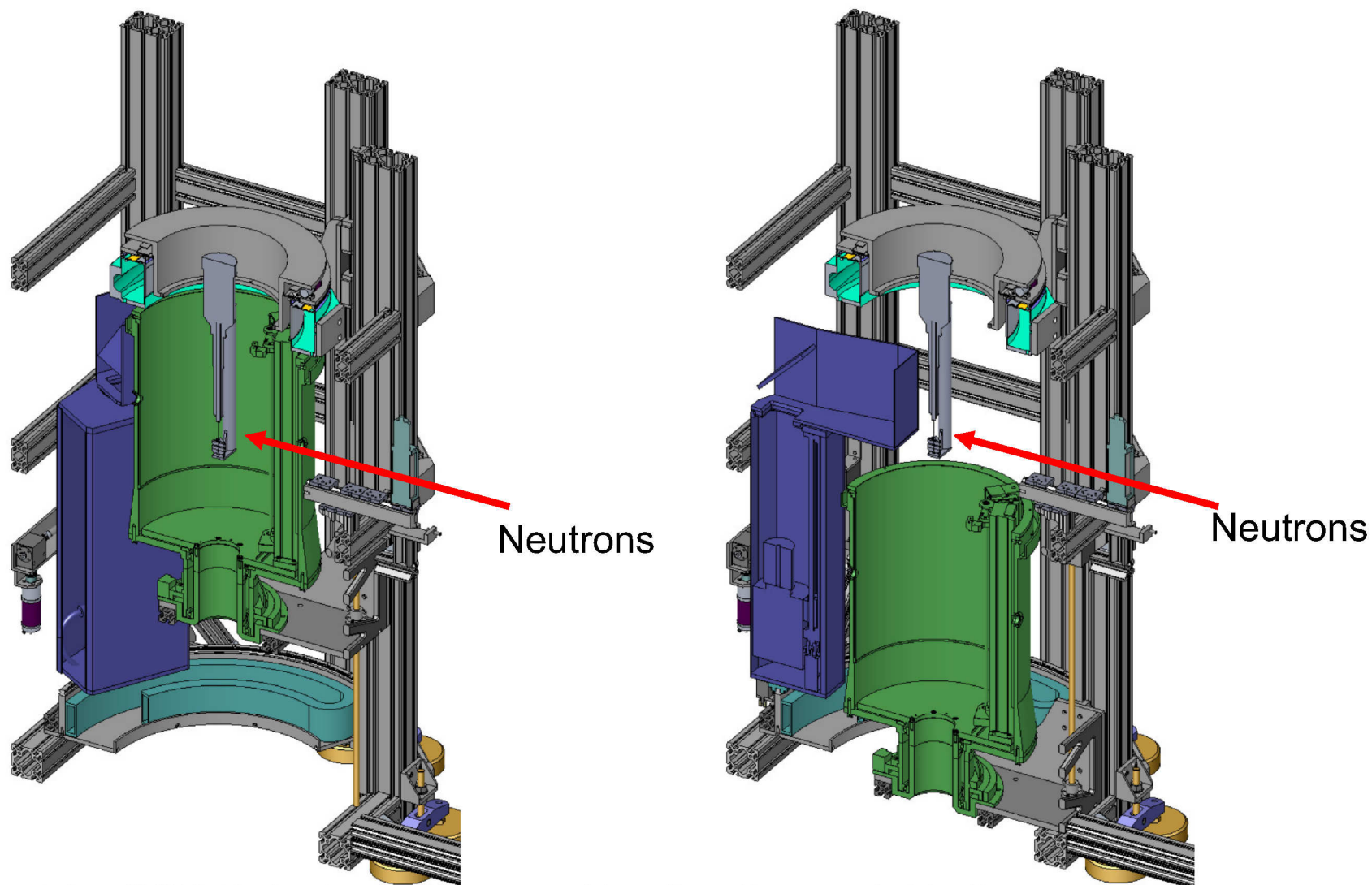
Myoglobin protein crystal (deuterated mother liquor) full data set recorded with CCD



prim.
beam

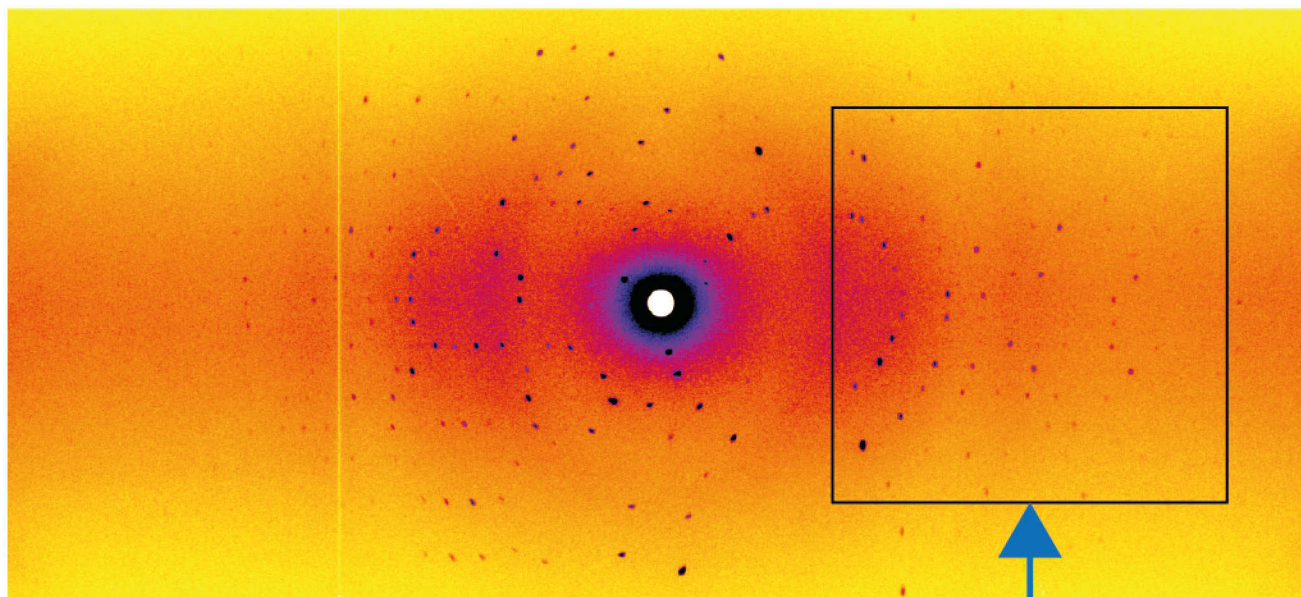
BioDiff: exposure time per frame: 20 minutes,
sample: Myoglobin in deuterated mother liquor

Switching between imageplate and CCD detector



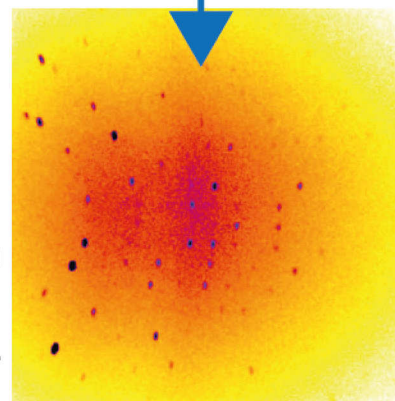
The CCD-Detector can be used to align the crystal in the neutron beam.

Instrument Characterization: The two detectors



recorded with image plate
detector

region of interest shown
above recorded with
CCD-detector



Peak search with hkl DENZO

Applications Places System Sat Nov 5, 18:14 JCMS

./309_01_001.raw

Zoom wind 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 Zoom in Zoom out Int. box Diff Vec Zoom close

close Frame

Imax=1046720
I=1926
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Z. Otwinowski

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jcns@phys:~/DENZO/denzo_1_96/real_data

```
File Edit View Terminal Help
-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]$
```

jcns@phys:~/DENZO/denzo_1_96/real_data

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File Edit View Terminal Help
-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
```

Reaktor-Info: Forschungs-Neutronenquelle Heinz Maier-Leibnitz (FRM II) - Mozilla Firefox

File Edit View History Bookmarks Tools Help

<http://www.frm2.tum.de/intern/funktionen/reaktor-info/index.html>

Most Visited Release Notes Fedora Project Red Hat Free Content

Reaktor-Info: Forschungs-Neutronenquelle Heinz Maier-Leibnitz (FRM II) - Mozilla Firefox

Telefondatenbank (intern)

Kontenverwaltung

Raumverwaltung

Raumbuchung GRS

Reaktor-Info

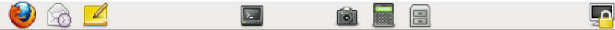
Webmail

19.8 MW

Shutterstellung NL-Anlage

jcns@phys:~/DENZO/... jcns@phys:~/DENZO/... jcns@phys:~/DENZO/... Reaktor-Info: Forschun... ./309_01_001.raw Untitled window

auto-index

Applications Places System  Sat Nov 5, 18:24 JCS

/home/jcns/DENZO/denzo_1_96/real_data/309_01_001.raw

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 Zoom in Zoom out Int. box Diff Vec Zoom close

close Frame

Imax=1046720

I=1885

[298.6 ,566.6]

HKL Processing System

M. Minor

Z. Otwinowski

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new date was send, updating picture wind

jcns@phys:~/DENZO/denzo_1_96/real_data

File Edit View Terminal Help

```

autoindex unit cell  35.44  31.09  64.92  90.00 105.53  90.00
crystal rotx, roty, rotz -112.379  87.484  0.804
Autoindex Xbeam, Ybeam 225.65 490.29
position 73 chi**2 x 11.35 y 8.84 pred. decrease: 0.000 * 73 = 0.0
partiality 73 chi**2 0.64 pred. decrease: 0.000 * 73 = 0.0
Angles equivalent by space group symmetry for:
vertical axis 1 0 0
spindle axis 0 0 1
crystal rotx 67.621 roty 92.516 rotz 0.804
rotz -112.379 roty 87.484 rotz -179.196
crystal rotx -112.379 roty 87.484 rotz 0.804
rotz 67.621 roty 92.516 rotz -179.196
    
```

jcns@phys:~/DENZO/denzo_1_96/real_data

File Edit View Terminal Help

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[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-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]$
    
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Zoom wind 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

Zoom in Zoom out Int. box Diff Vec Zoom close

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I=1838
[340.2, 562.6]

HKL Processing System
W. Minor
Z. Otwinowski

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new date was send, updating picture wind

jcns@phys:~/DENZO/denzo_1_96/real_data

File Edit View Terminal Help

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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 -0.02
errors 0.09 0.05 0.11 0.09 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

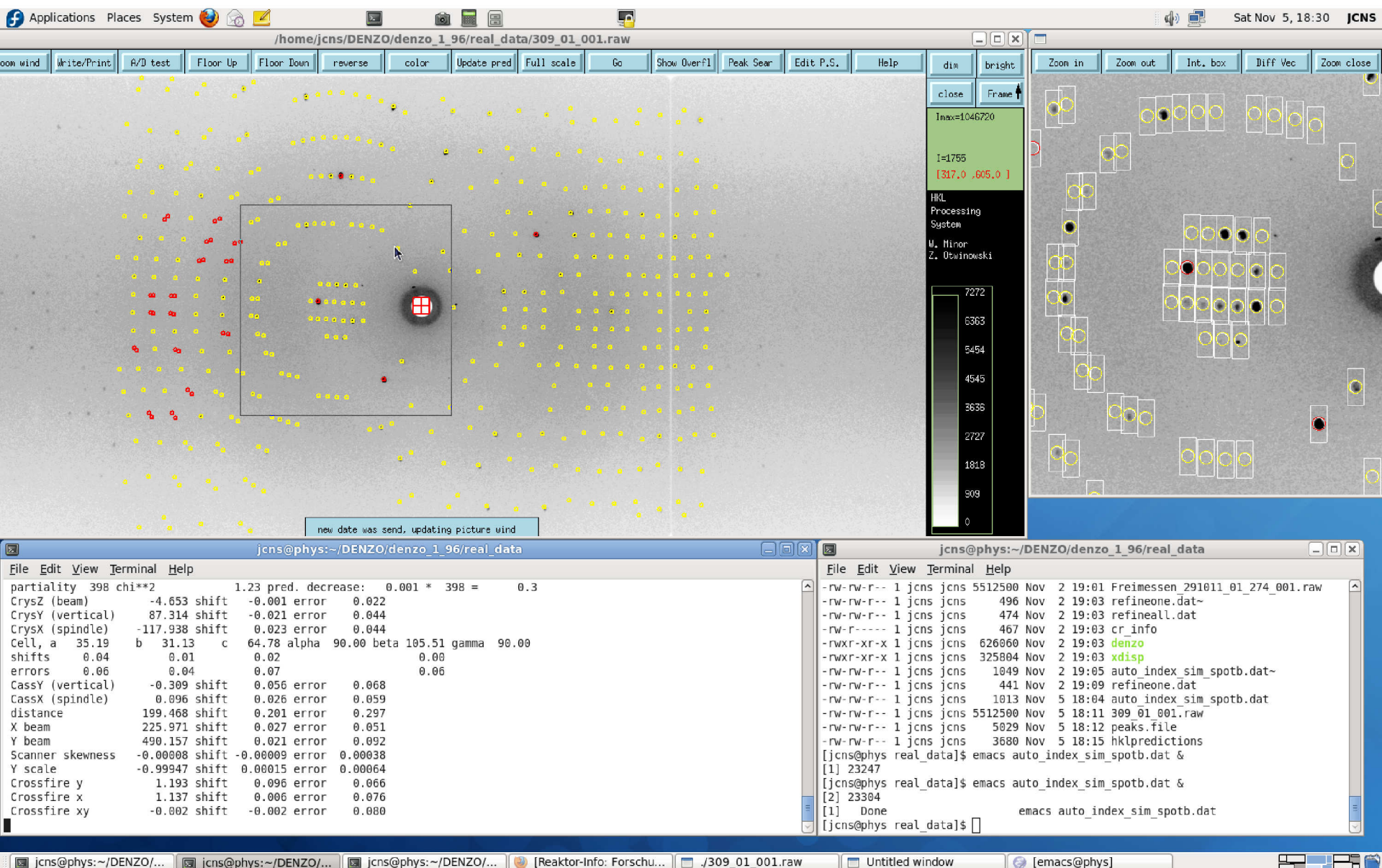
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-rw-rw-r-- 1 jcns jcns 5512500 Nov 2 19:01 Freimessen 291011_01_274_001.raw
-rw-rw-r-- 1 jcns jcns 496 Nov 2 19:03 refineone.dat~
-rw-rw-r-- 1 jcns jcns 474 Nov 2 19:03 refineall.dat
-rw-rw-r-- 1 jcns jcns 467 Nov 2 19:03 cr info
-rwxr-xr-x 1 jcns 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
[jcns@phys real_data]$

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jcns@phys:~/DENZO/... jcns@phys:~/DENZO/... jcns@phys:~/DENZO/... [Reaktor-Info: Forschu... /309_01_001.raw Untitled window [emacs@phys]

d_min=2.0 Å



d_min=1.5 Å

Applications Places System

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dim bright Zoom out Int. box Diff Vec Zoom close

close Frame

I_{max}=1046720

I=1798
[334.6 ,390.6]

HKL
Processing
System
W. Minor
Z. Otwinowski

7272
6363
5454
4545
3636
2727
1818
909
0

new date was send, upde

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

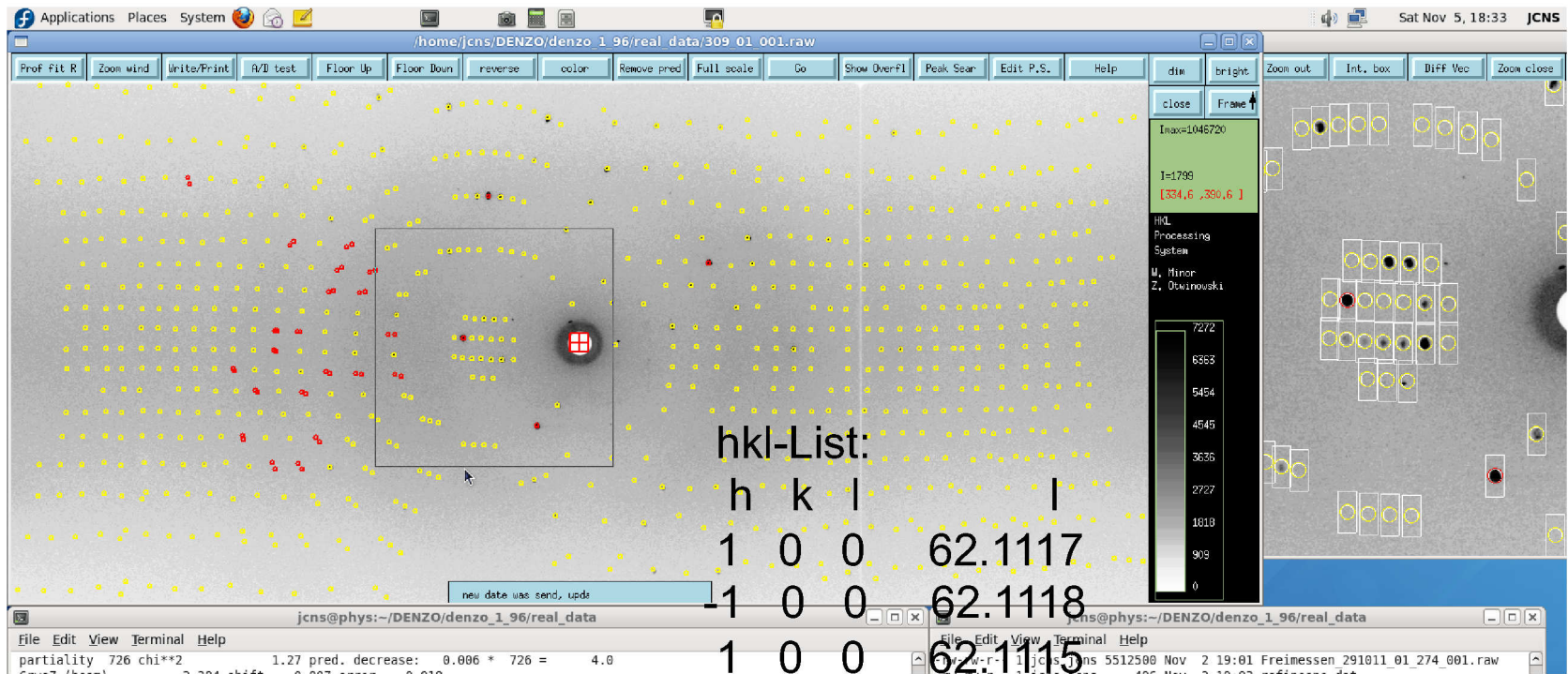
jcns@phys:~/DENZO/denzo_1_96/real_data

File Edit View Terminal Help

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-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-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
[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 $d_{\min}=1.5 \text{ \AA}$



Flow chart of data treatment and model building

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

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

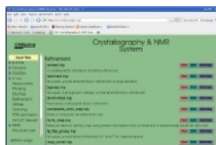
Additional information from the
solution of the phase problem

Structure refinement

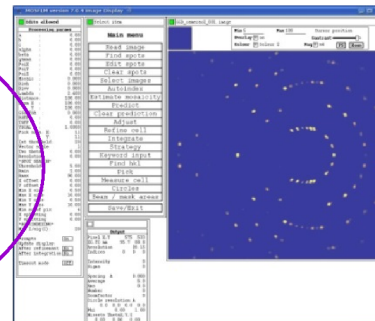
- Refinement of atom coordinates displacements
- Calculation of scattering density maps (neutrons) or electron density maps (x-rays)

Map-plotting

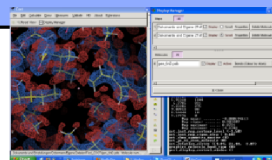
- inspection of model to fit the map)
- real space changes and refinement to the model



-nCNS
-PHENIX



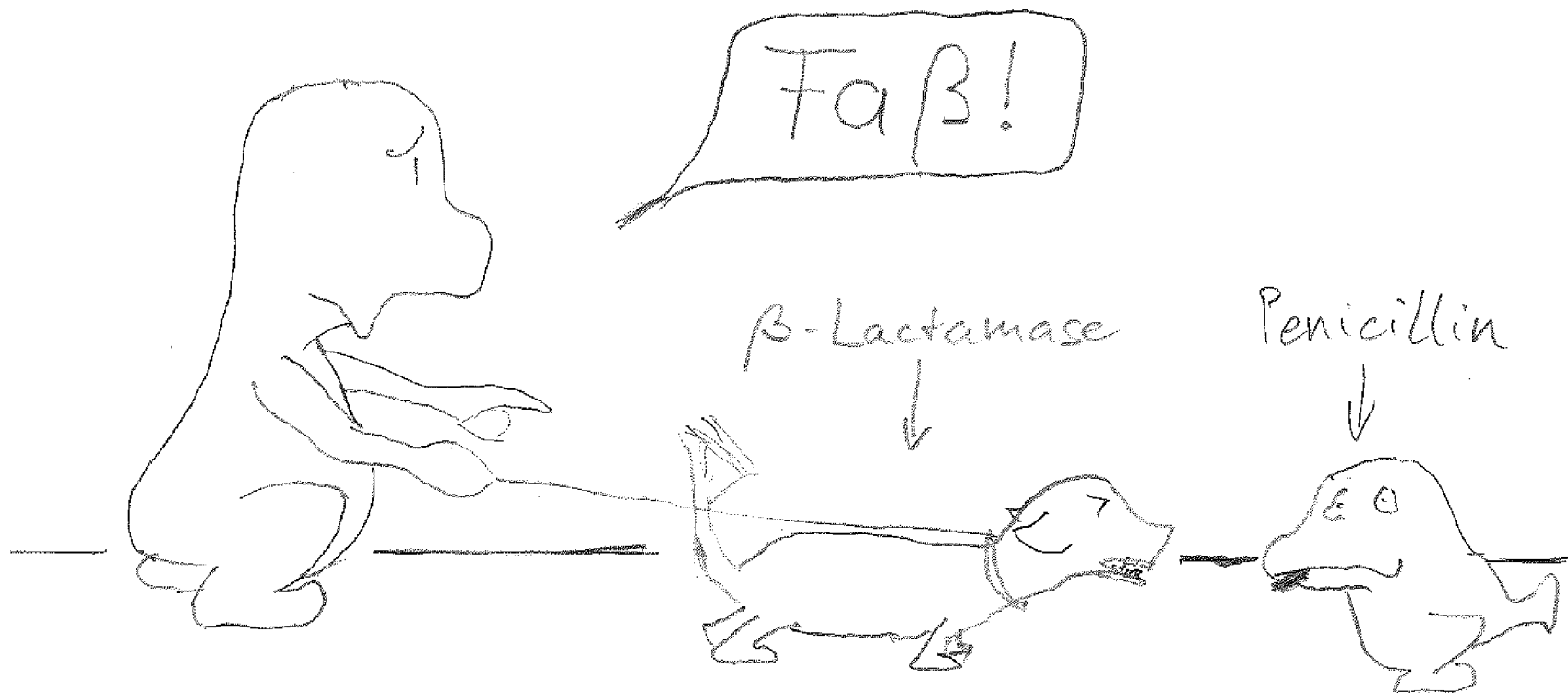
-MOSFLM
-HKL-denzo
(comercial)



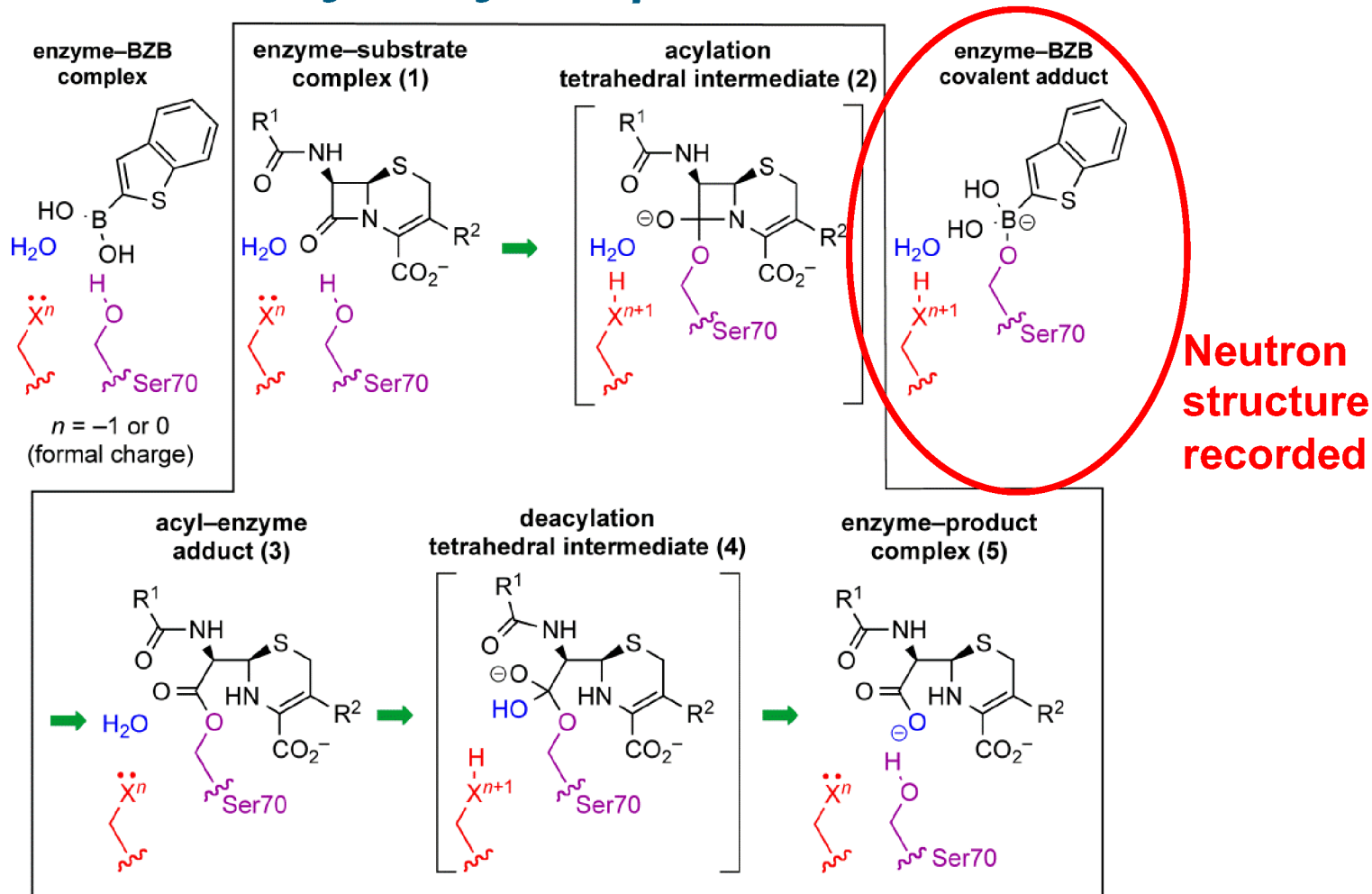
-XtalView
-Coot

Application Example: Protonation state of amino acid residues

The protein β -lactamase

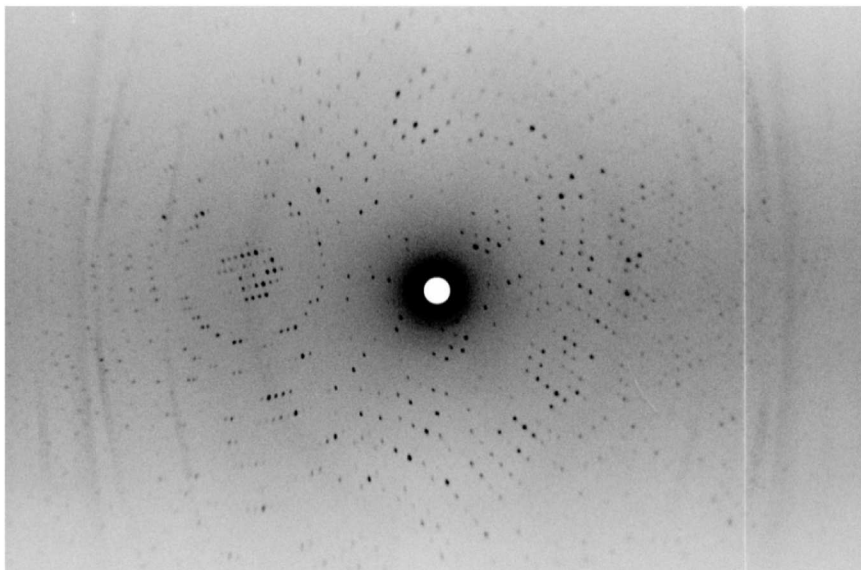


β -lactamase: hydrolyses β -lactam antibiotics



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.

Data-set: β -lactamase with bound inhibitor



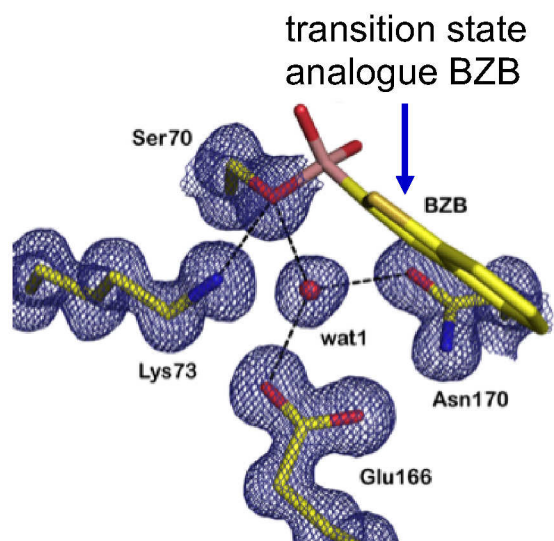
$d_{\min}$	$I/\sigma(I)$	N_{meas}	mult.	compl. in shell %	R_{merge} %
4.31	27.8	12685	5.6	97.6	4.9
3.42	19.0	11941	5.5	98.0	8.0
2.99	10.3	10378	4.9	96.9	14.6
2.71	7.6	8757	4.3	95.5	18.7
2.52	5.9	7820	3.9	92.8	21.2
2.37	5.4	7099	3.8	89.2	21.6
2.25	5.0	6095	3.5	84.6	23.0
2.15	4.5	5906	3.4	82.9	24.7
2.07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

- unit cell: 73.4Å, 73.4Å, 99.1Å $P3_221$
- fully deuterated protein
- crystal size: 2.7mm³
- Collection time: 9d

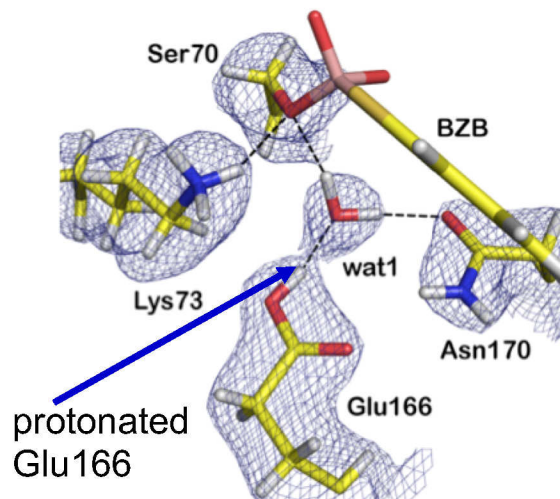
$R_{\text{pim}} = 7.9\%$ (17.9%)

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

Catalytic Proton Network of the Toho-1 β -Lactamase



electron density map



nuclear density map from BioDiff

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

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

Thanks to our users and the BioDiff-Team:

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

Marialucia Longo
Johannes Hermann
Philipp Nowotny

...and you for your attention!

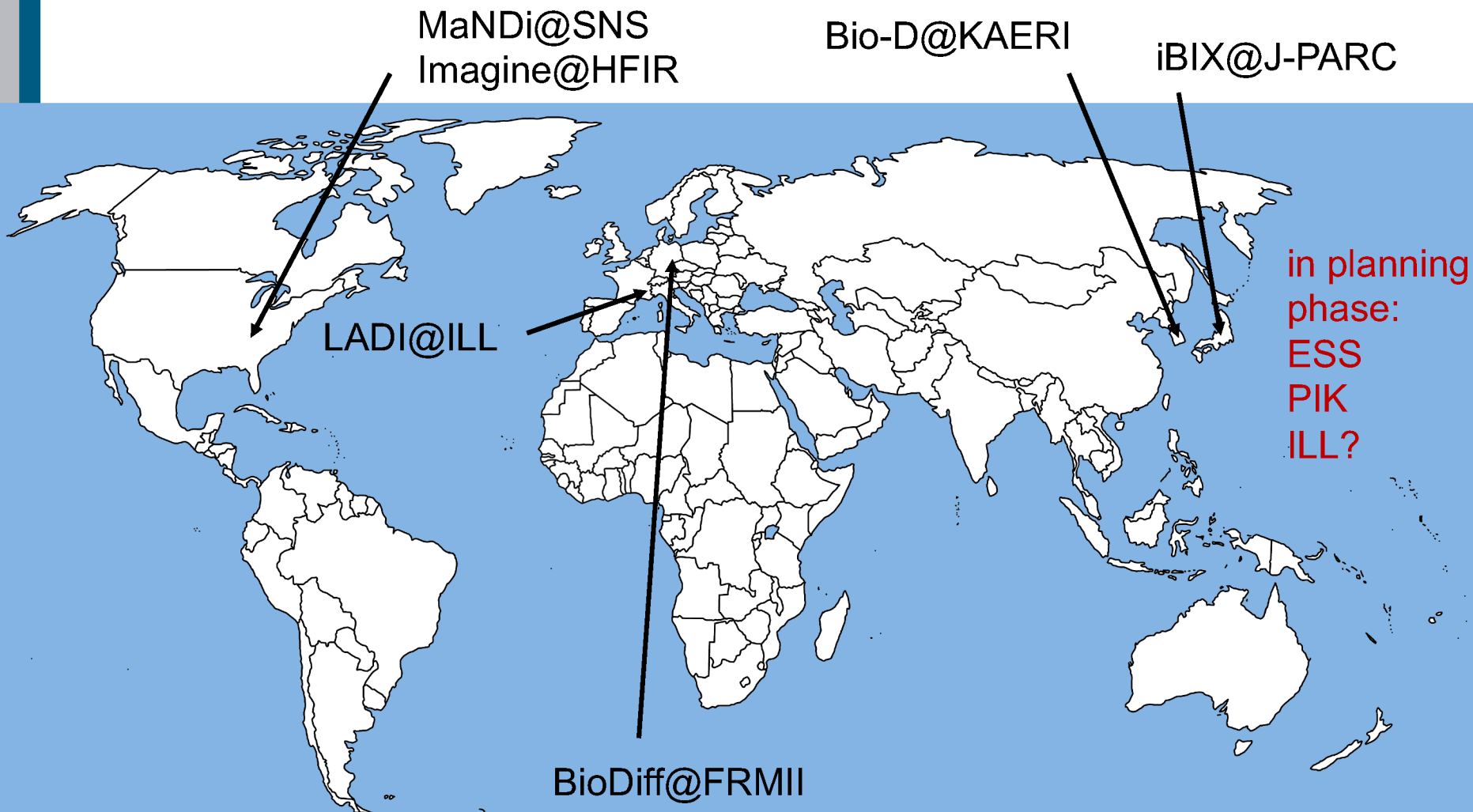
Funding by:



Technische Universität München

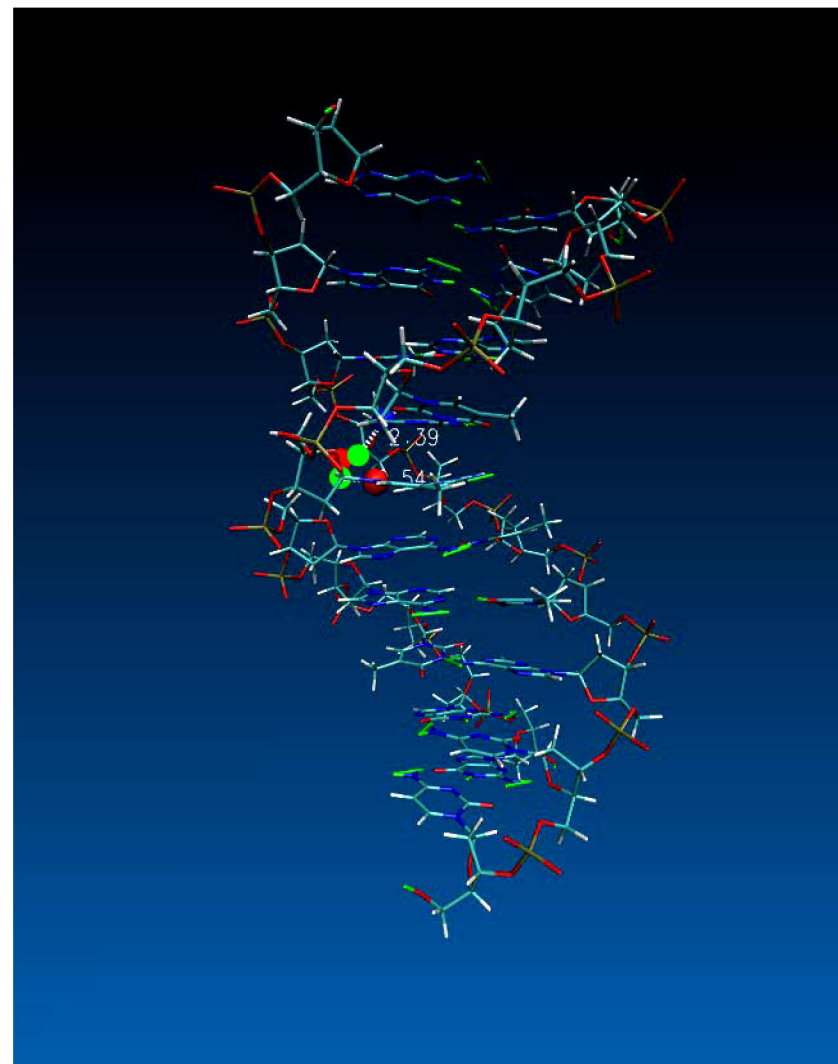
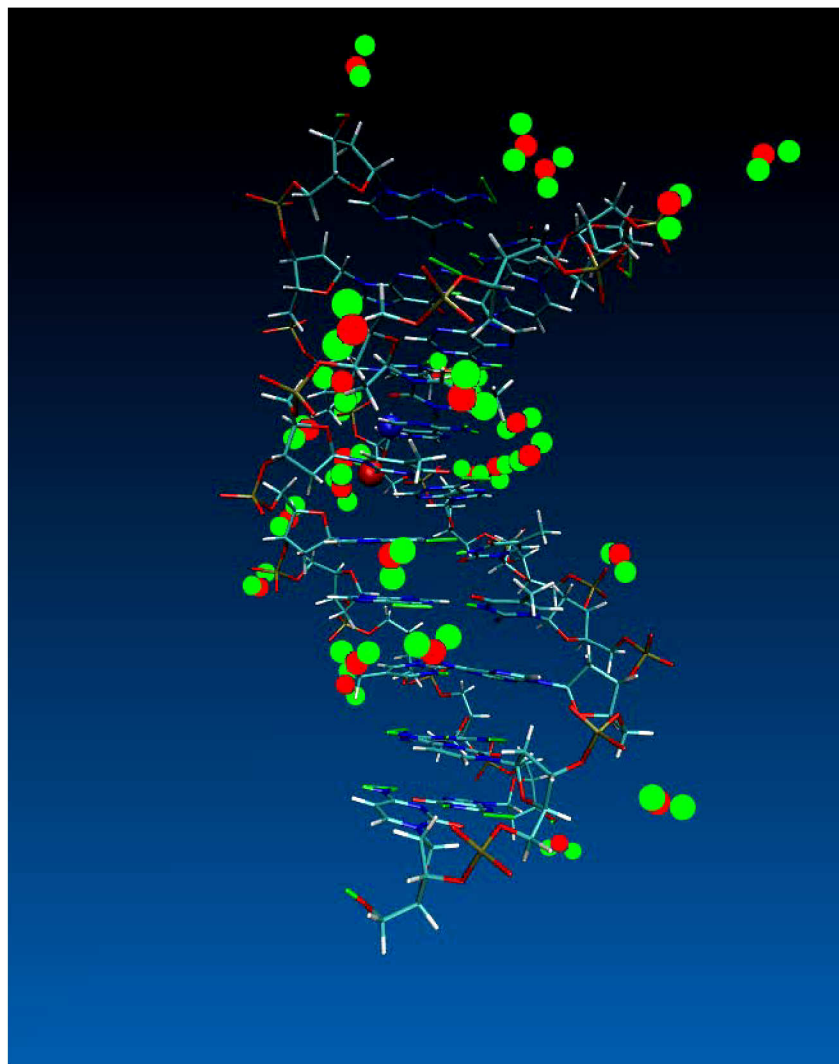
Danke für die Neutronen!

World map of neutron diffractometers optimized for protein crystals



Other structured macromolecules to be investigated by crystallography... mostly DNA

DNA stabilized by water bridge in minor groove



- 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 throughput
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 metallo-proteins, disulfide bond cleavage.
 2. Hydrogen positions can usually not be determined (only at high resolution)
- **Neutron protein crystallography** is a complementary technique as compared to x-ray crystallography. Here one can determine:
 1. protonation states of amino acid side chains (important for the function of the protein)
 2. deuterium exchange as a measure of flexibility and accessibility (discrimination between **H** / **D**)
 3. solvent structure including hydrogen atoms

Thanks to...

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

and you for your attention!

The hierarchy of protein architecture

- **Primary structure**

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

- **Secondary structure**

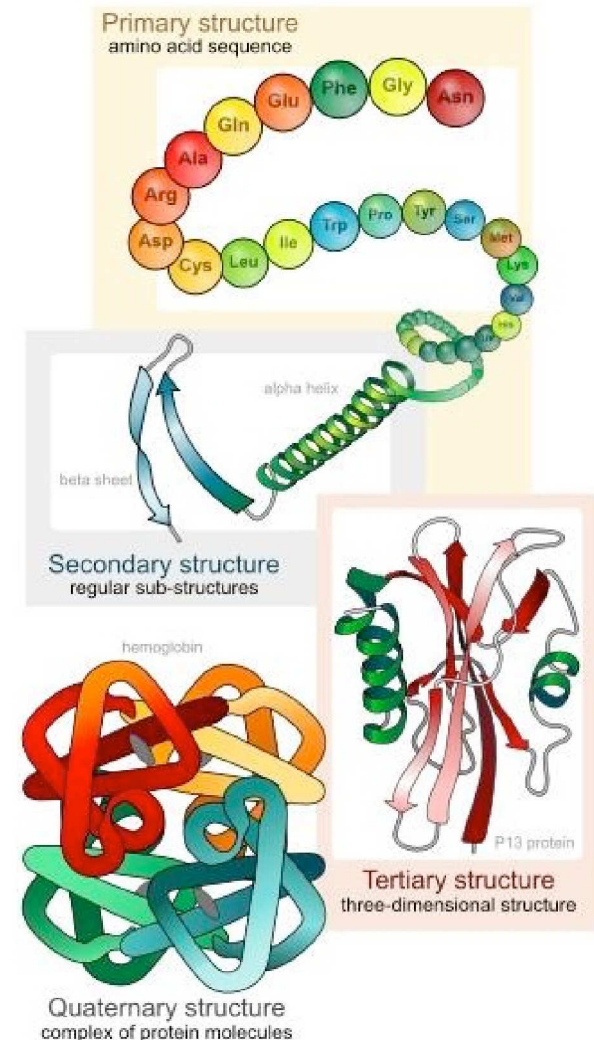
The local arrangement of main-chain atoms, often with specific hydrogen bonding pattern

- **Tertiary structure**

The packing of non-contiguous segments into a compact fold

- **Quaternary structure**

The association of individual chains into oligomeric structures



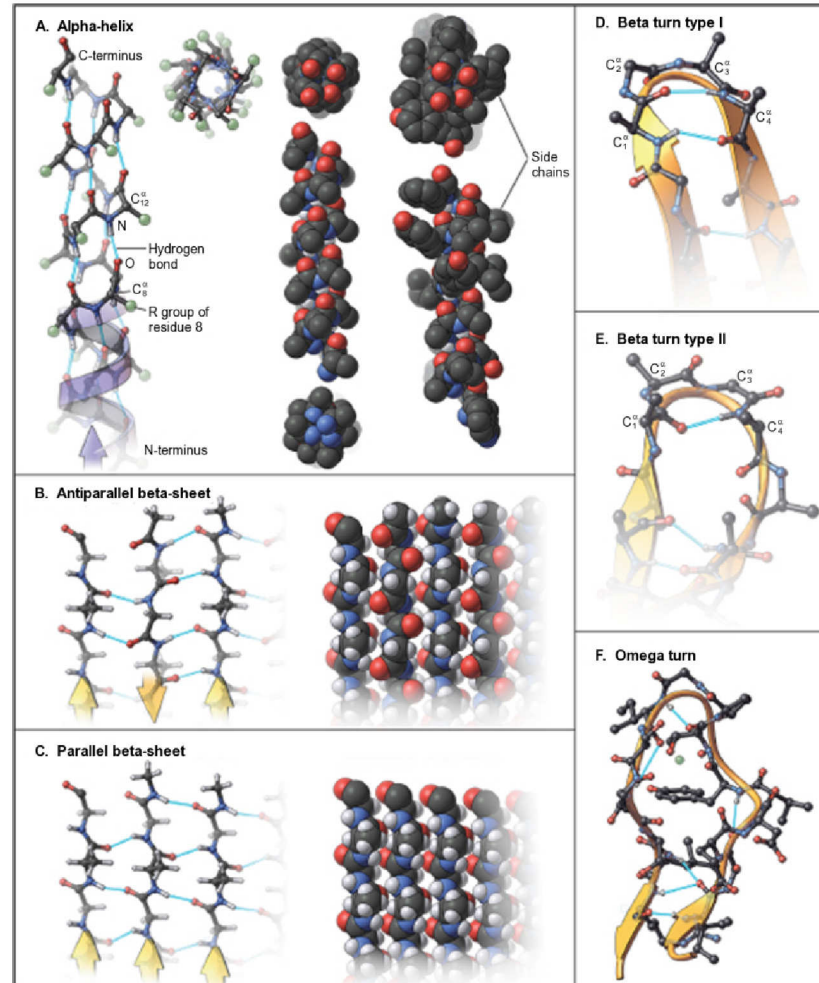
Secondary structural elements

α -helix
spiral conformation of a continuous chain

β -sheet
extended strands
aligned side-by-side

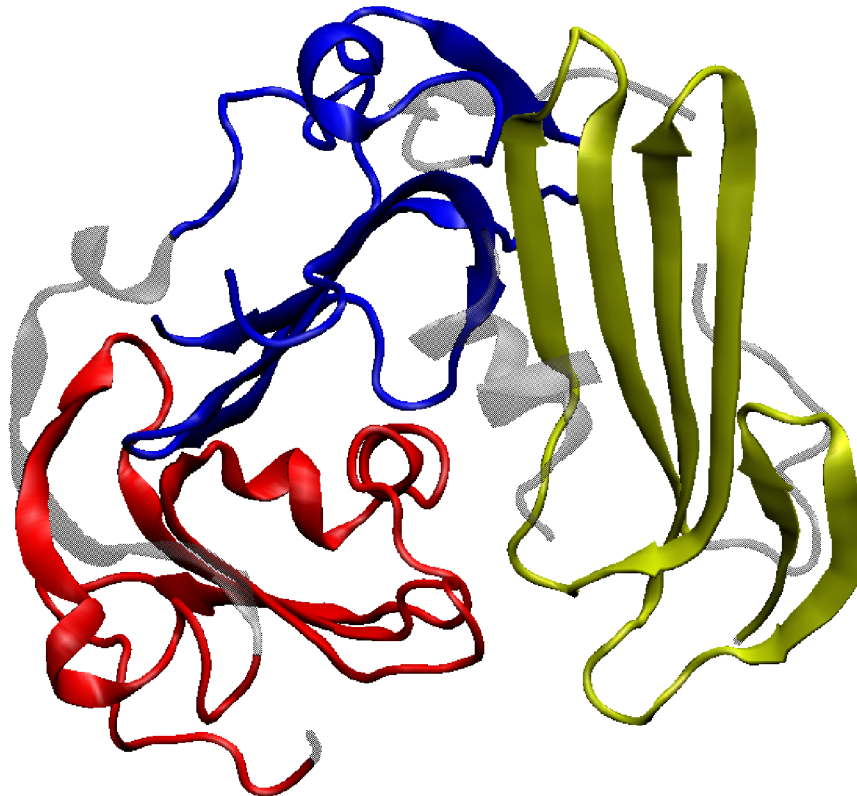
turns / loops
changing direction of chain

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



© Elsevier. Pollard et al: Cell Biology 2e - www.studentconsult.com

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



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

What stabilizes the 3-dimensional structure:

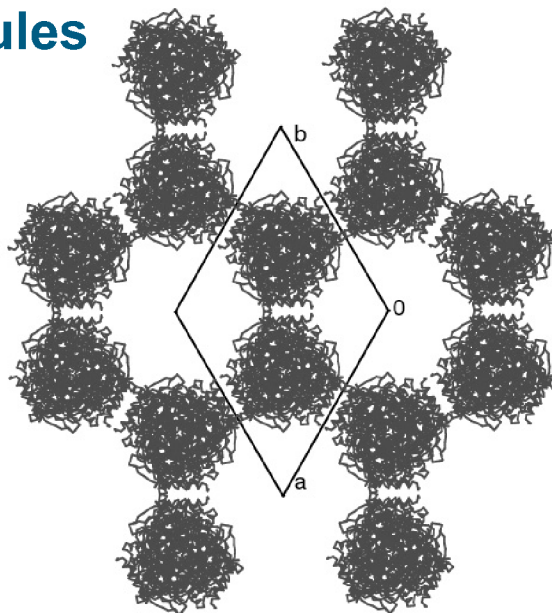
Non-covalent bonds

- Hydrogen bonds
- Electrostatic forces
- V. d. Waals contacts

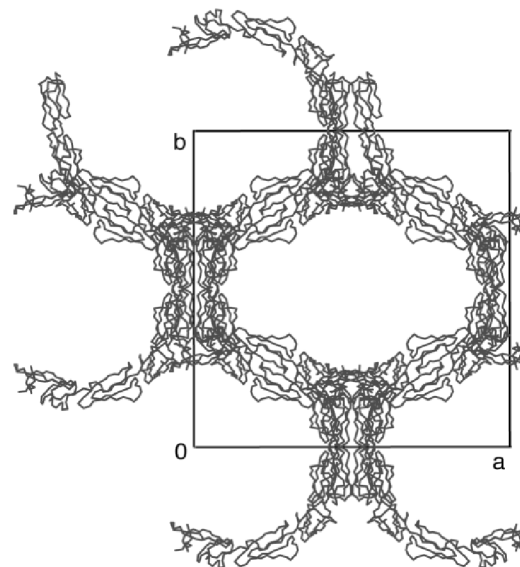
Covalent bonds

- Disulfides
- Isopeptide bonds

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



Acetylcholinesterase
~68% solvent



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

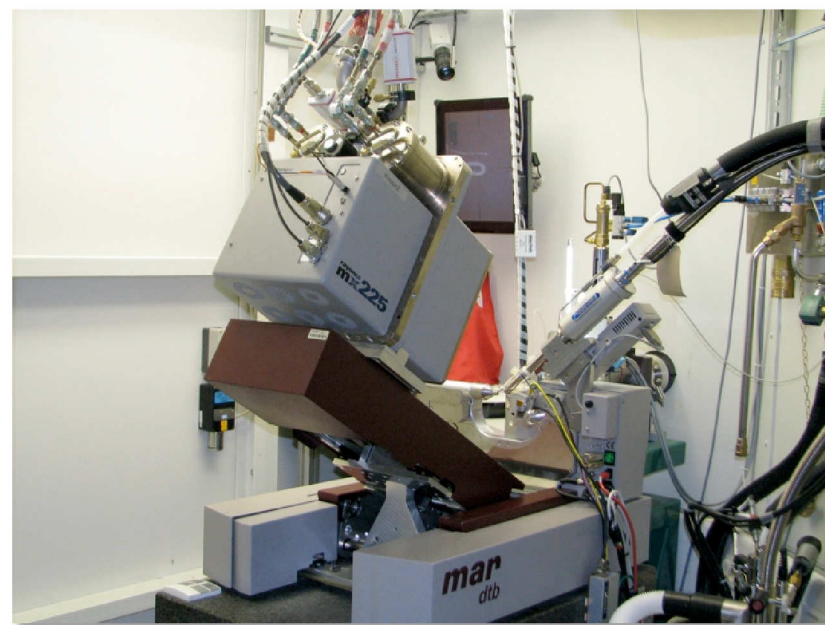
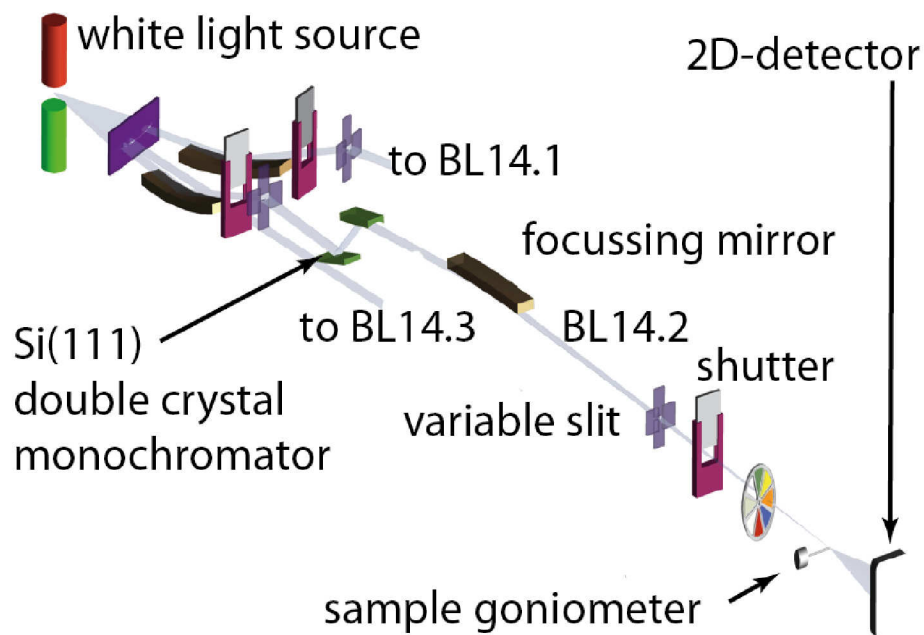
Typical solvent content 40-60%

Solvent channels allow diffusion of compounds into crystal

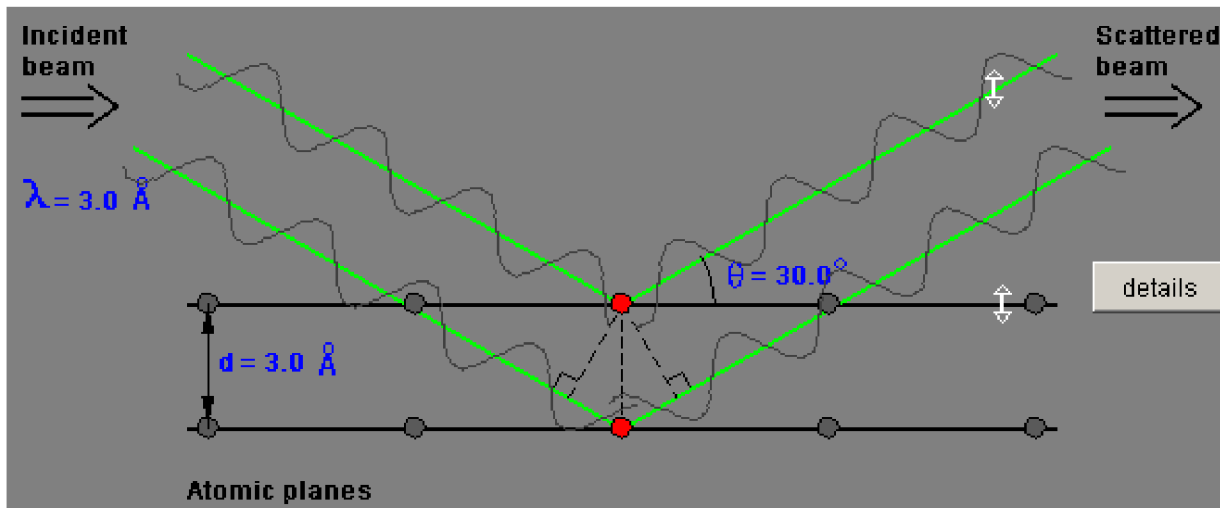
Often these compounds can reach the active or binding site

Often enzymes are active in crystalline state

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



Bragg's Law

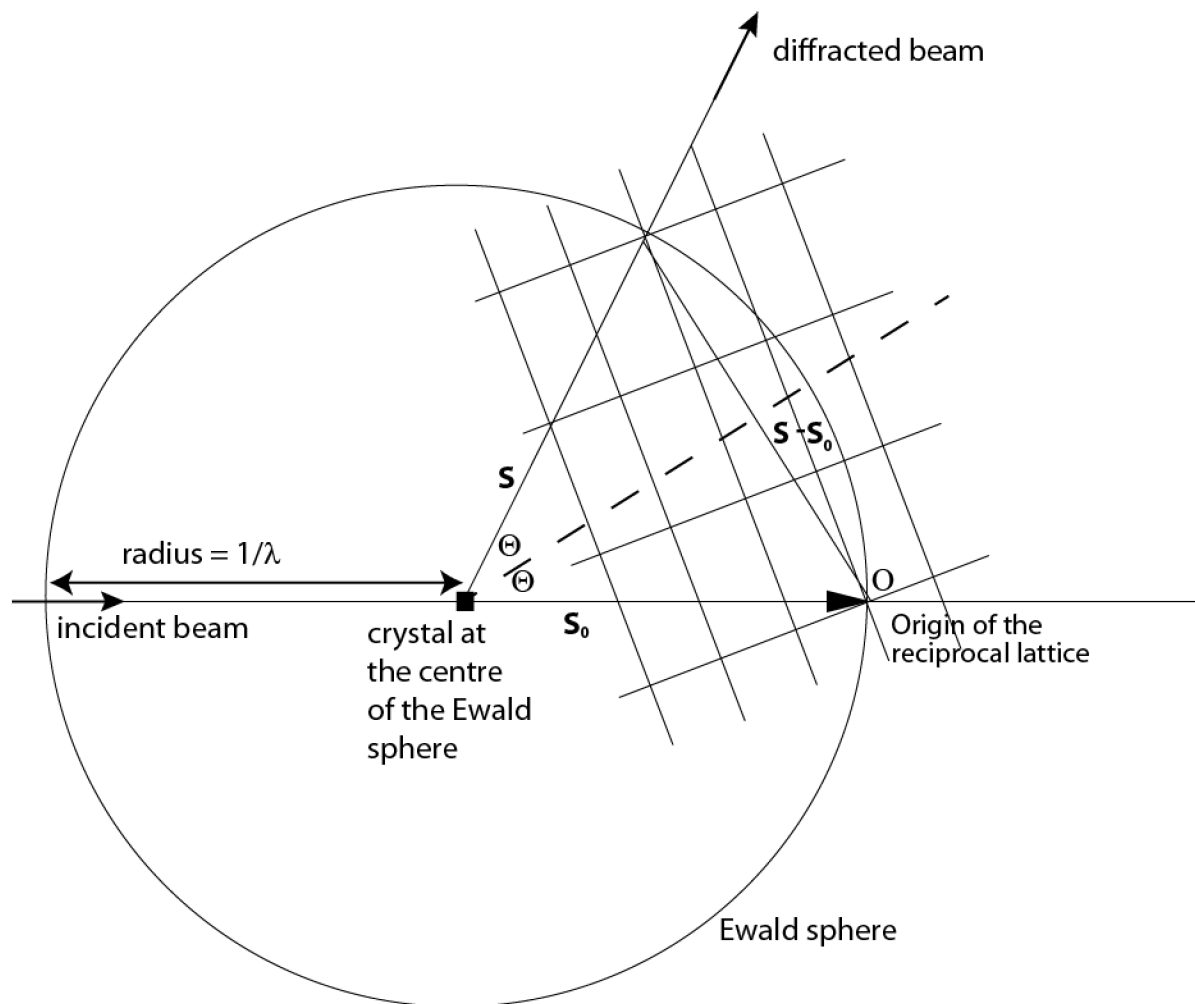


If scattered beams are in phase, they add up

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

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

Ewald construction and Bragg's Law



Solving the phase problem

- Structures of <1000 atoms can be solved by brute force statistical *Direct Methods*, given data to 1.2 Å or better. Most small molecule structures are solved this way.
- A closely related known structure can be used as a search fragment for MR = *Molecular Replacement*.
- Heavy atoms can be introduced and the small changes in the reflection intensities exploited (SIR / MIR = Single / Multiple *Isomorphous Replacement*).
- Heavier atoms exhibit wavelength-dependent *Anomalous Scattering*, with the result that F_{hkl} and F_{-h-k-l} are not exactly equal. These small differences can be exploited in the Single Anomalous Diffraction and Multiple Anomalous diffraction (MAD) methods. For MAD, either metal atoms such as Fe present in the protein, or Se in selenomethionine (genetically modified methionine) are suitable anomalous scatterers.

Neutron protein crystallography

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

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

Advantages of structure determination with neutrons:

- Hydrogen atoms can be resolved even at a resolution of $d_{\min} \approx 2\text{\AA}$ (for ^2H) Therefore one can determine:

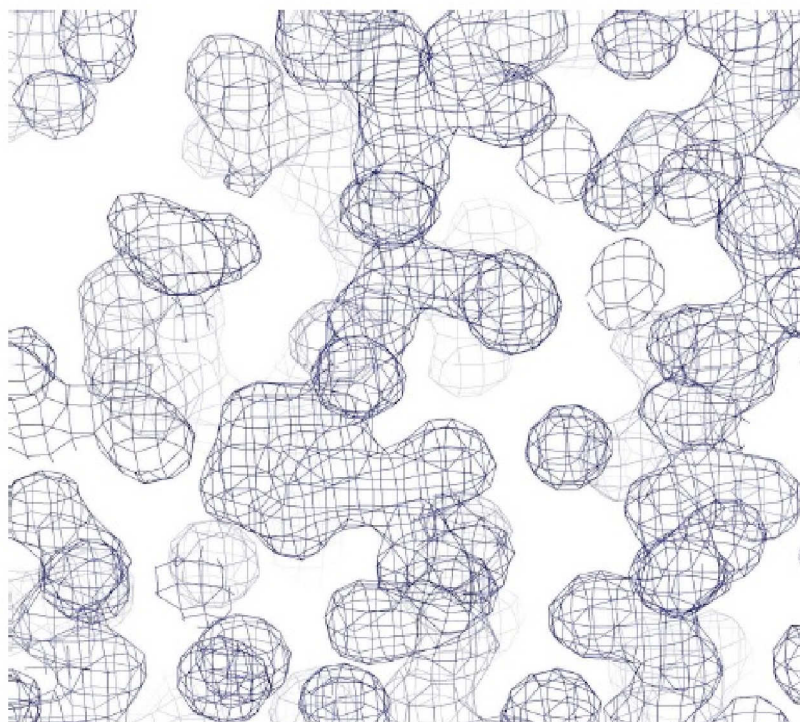
- protonation states of amino acid side chains
- deuterium exchange as a measure of flexibility and accessibility (discrimination between **H** / **D**)
- solvent structure including hydrogen atoms

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

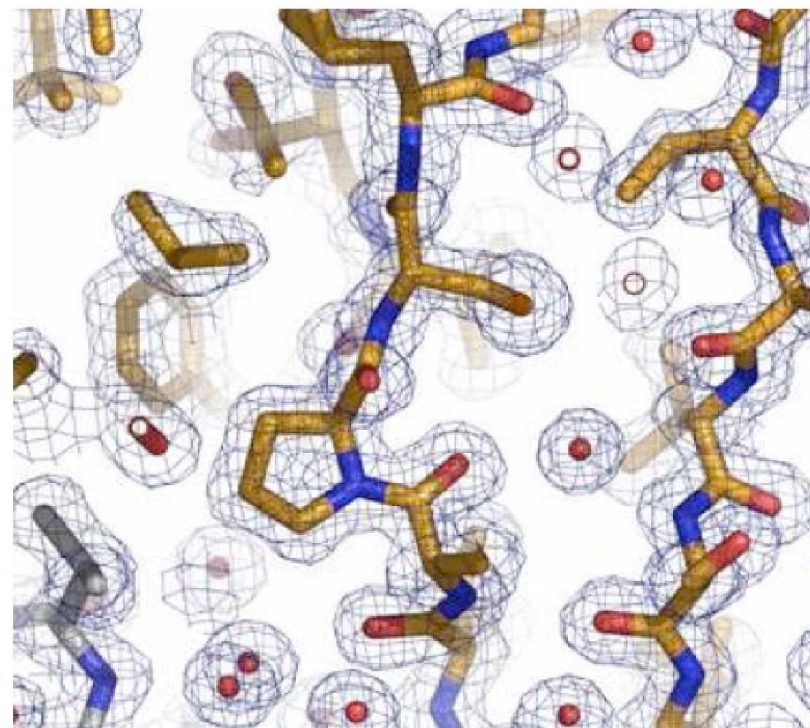
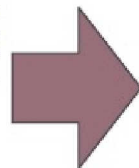
Especially the reduction of metals in metallo-proteins can be avoided.

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

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



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



Amino acid chain is fitted
into electron densities via
dedicated software

Flow chart of data treatment and model building

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

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

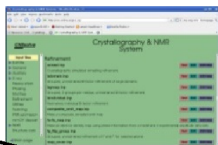
Additional information from the
solution of the phase problem

Struktur refinement

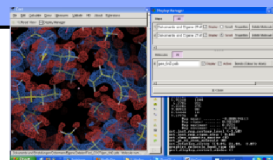
- Refinement of atom coordinates displacements
- Calculation of scattering density maps (neutrons) or electron density maps (x-rays)

Map-plotting

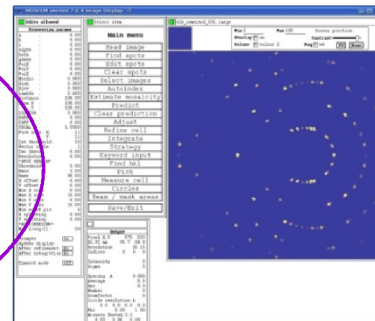
- inspection of model to fit the map)
- real space changes and refinement to the model



-nCNS
-PHENIX

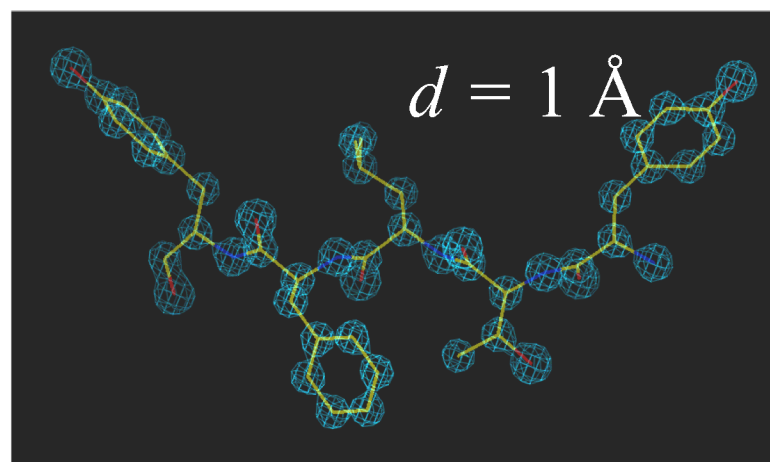
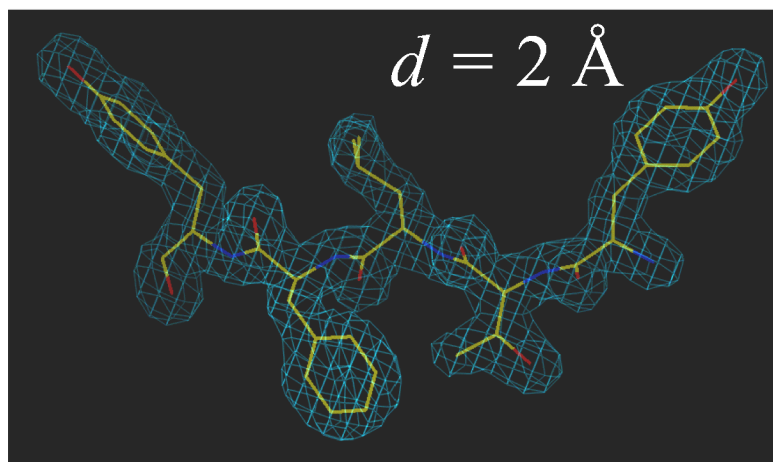
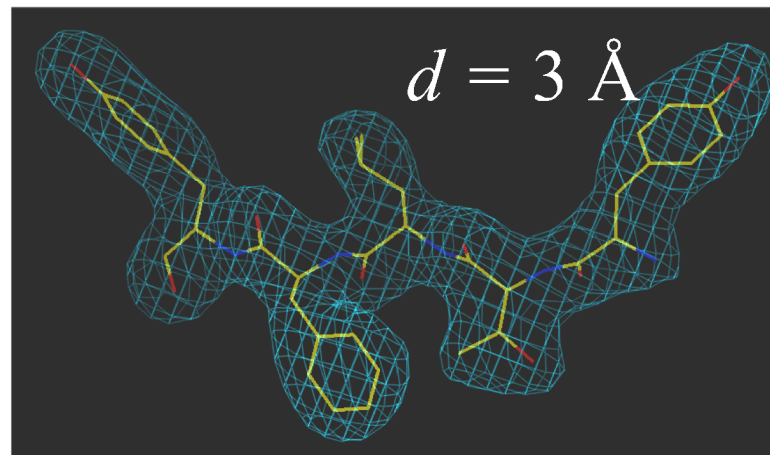
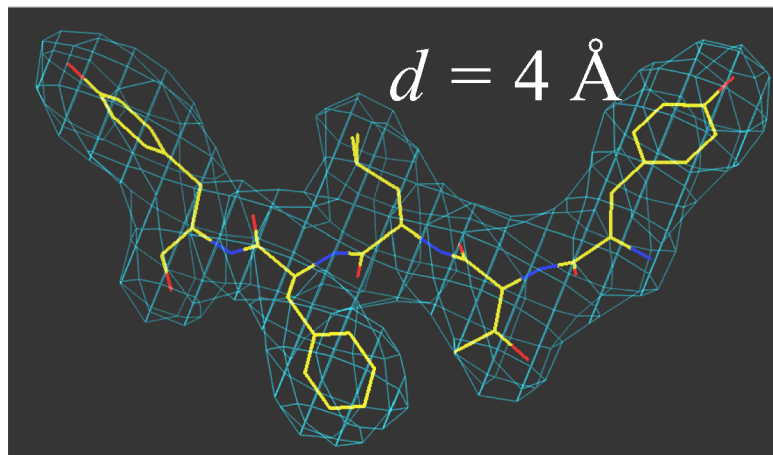


-XtalView
-Coot



-MOSFLM
-HKL-denzo
(comercial)

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



Atomic resolution

The R-Factors: Judging Convergence between model and data

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

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

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

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
$\alpha, \beta, \gamma, ^\circ$	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/σ	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
$R_{\text{work}} / R_{\text{free}}$	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, Å ²			
Protein	94.8	113.3	67.5
Ligand/ion	— / 102.9	111.9 / 117.5	72.4 / 71.3
R.m.s. deviations			
Bond lengths, Å	0.018	0.013	0.018
Bond angles, °	1.751	1.453	1.871

— Indicates not applicable.

Judging the quality of the data:

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

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

occupancy

x,y,z coordinates (Å) ↓

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

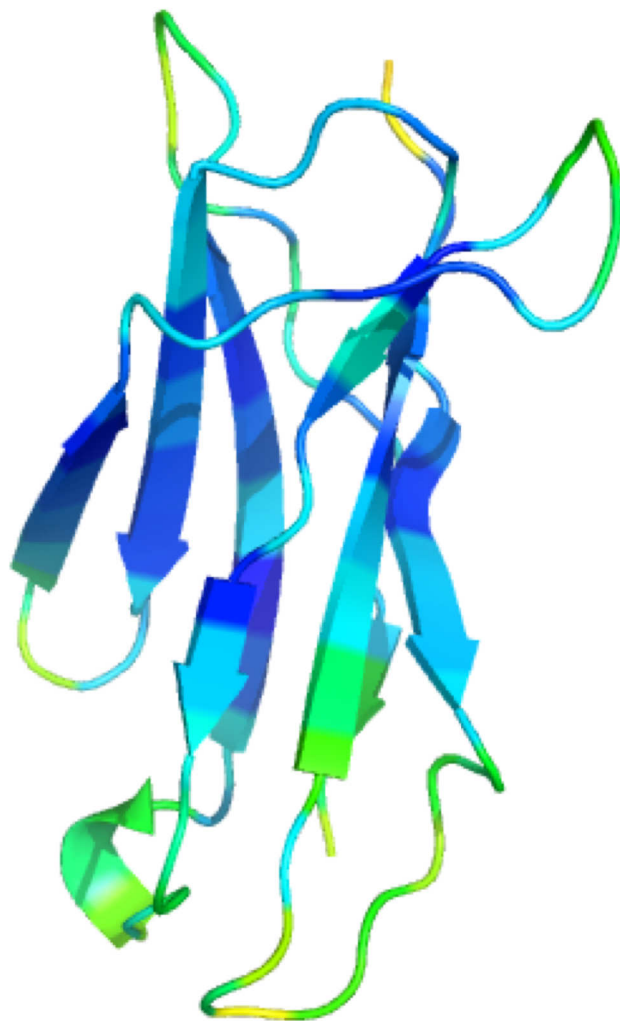
Isotropic B-factor or temperature factor is a measure of the mobility of an atom

Look at B-factor distribution!

Protein coloured by B-factor:

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

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

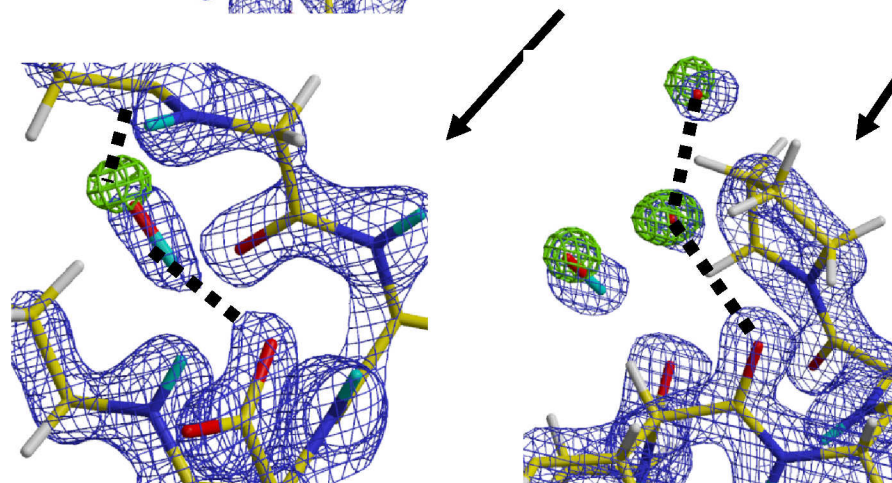
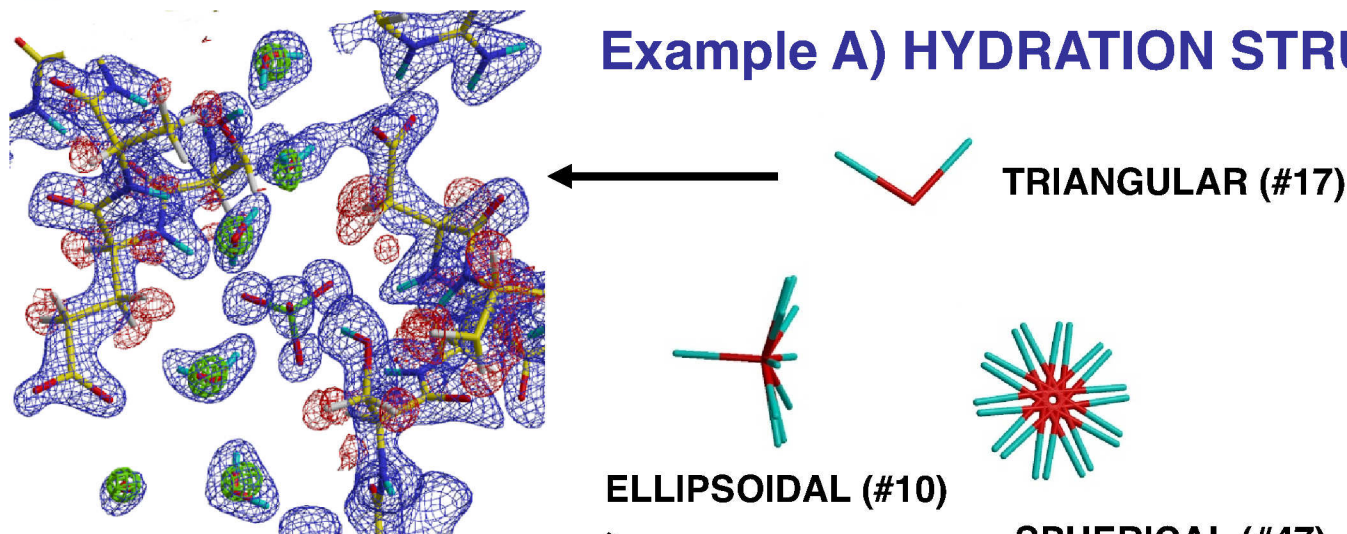


A protein molecule is dynamic

- The electron density is a spatial average over all molecules in the crystal and a time average over the duration of the X-ray data measurement
- => Multiple discrete conformations of a residue in different molecules are superimposed.
- A crude description of dynamics is provided in the pdb file as the isotropic B-factor
 - Some dynamical aspects evident in the electron density are lost in the pdb file

From structure to function: Some case studies

Example A) HYDRATION STRUCTURE:



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

 $2F_o - F_c$ neutron map, $+1.5\sigma$

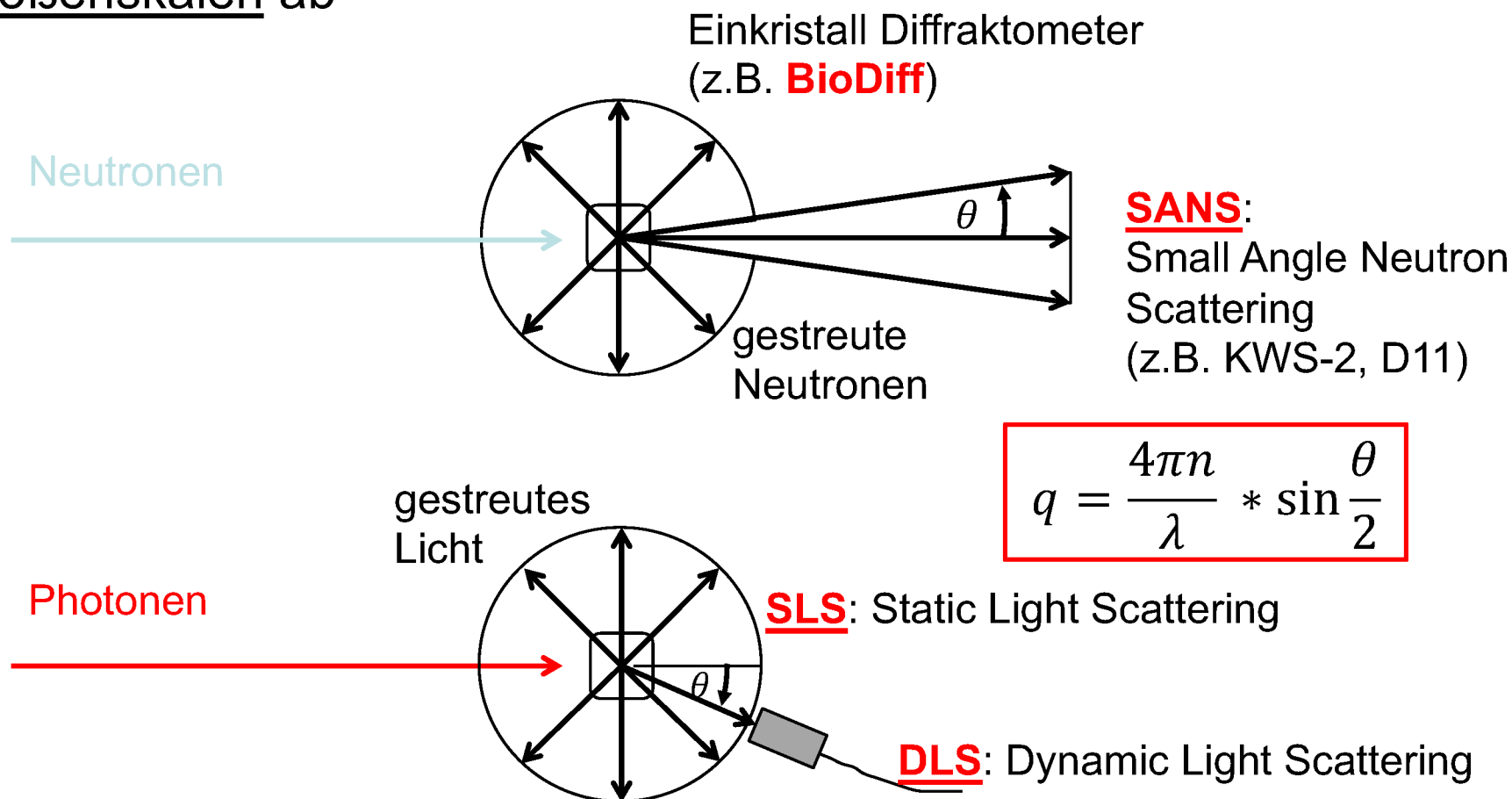
 $2F_o - F_c$ neutron map, -2.0σ

 $2F_o - F_c$ X-ray map

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

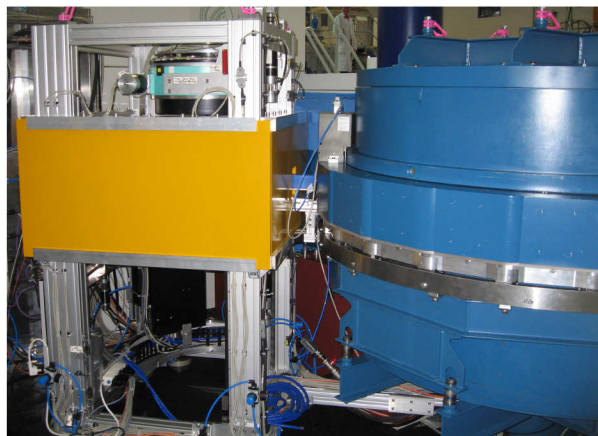
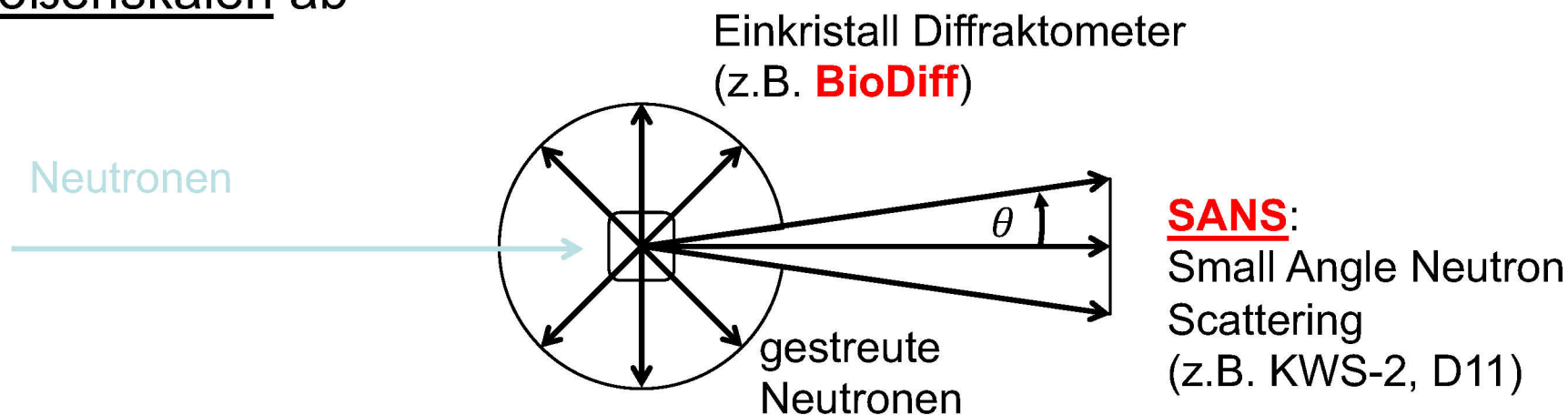
Methoden & Instrumente

Kristallisationsprozess läuft auf verschiedenen Zeit- und Größenskalen ab



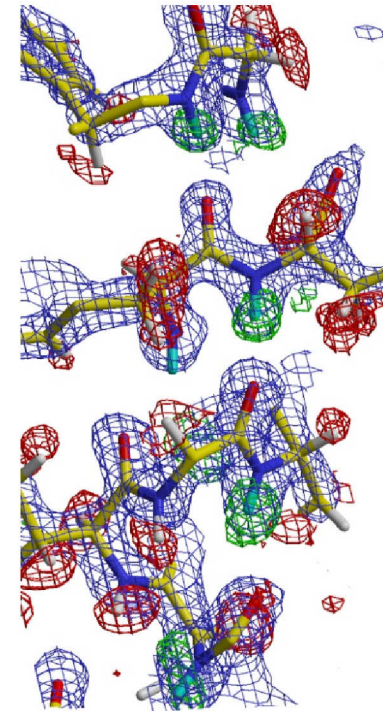
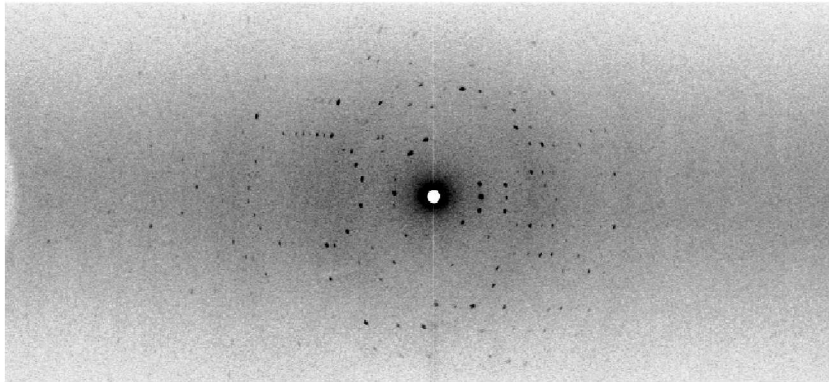
Methoden & Instrumente

Kristallisationsprozess läuft auf verschiedenen Zeit- und Größenskalen ab



Theory on scattering from a crystal

3D structural analysis:

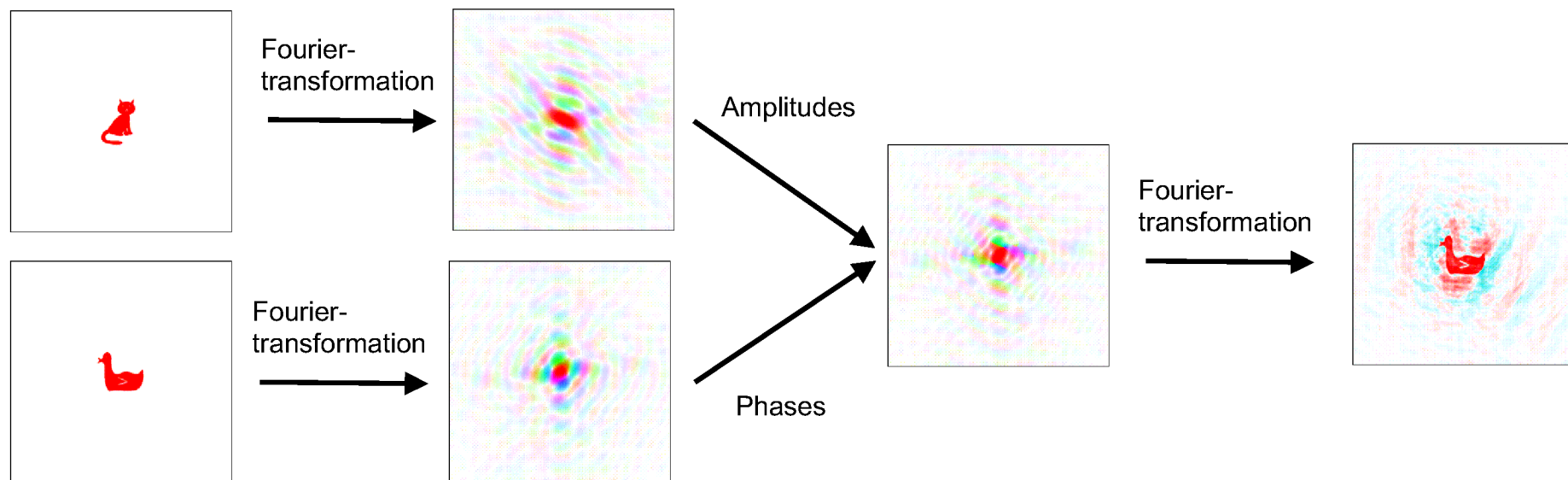


$$\rho(x, y, z) = \frac{1}{V_E} \sum_{h,k,l} F_{hkl} \cdot e^{-2\pi i(h \cdot x + k \cdot y + l \cdot z)}$$

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>