BioDiff - a neutron diffractometer optimized for crystals with large unit cell dimensions

or: What can neutrons do for you?

T.E. Schrader[a], A. Ostermann[b], M. Monkenbusch[c], B. Laatsch[d], Ph. Jüttner[b], W. Petry[b], D. Richter[a,c]

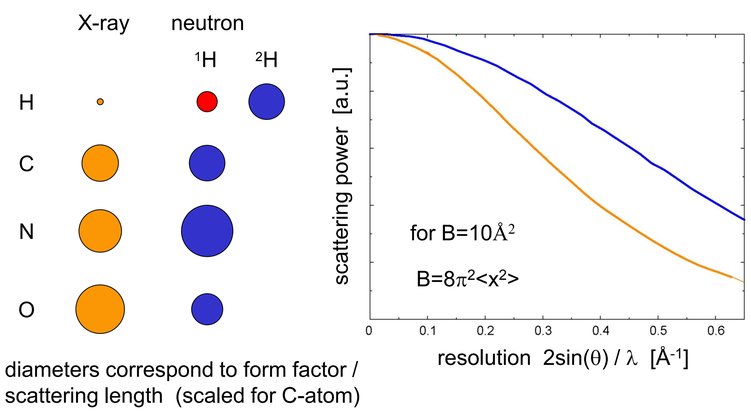
[a]Jülich Centre for Neutron Science (JCNS) at Heinz Maier-Leibnitz Zentrum (MLZ), Forschungszentrum Jülich GmbH, Lichtenbergstr.1, 85747 Garching, Germany
[b]Heinz Maier-Leibnitz Zentrum (MLZ), Technische Universität München, Lichtenbergstr. 1, 85748 Garching, Germany
[c]Forschungszentrum Jülich GmbH, Institute for Complex Systems, D-52425 Jülich,
[d]Forschungszentrum Jülich GmbH, Engineering and Technology (ZEA-1), D-52425 Jülich

Neutron structure determination:

hydrogen atoms can be resolved even at a resolution of $d_{min} \approx 2.5$Å

- 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 can be analysed
- discrimination between neighbors in the periodic table is possible: e.g. **N** and **O**, **Fe** and **Mn**
- B-factors ($<x^2>$) of the hydrogen atoms can be compared with data of other techniques
- no radiation damage compared to measurements at synchrotrons

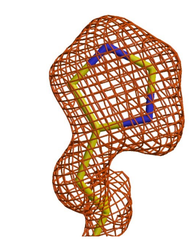
Comparison of form factors (X-ray) and scattering lengths (neutrons):



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

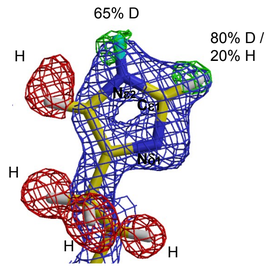
Amino acid protonation states:

X-ray d_{min}= 1.5Å:



2Fo-Fc map; +1.5σ

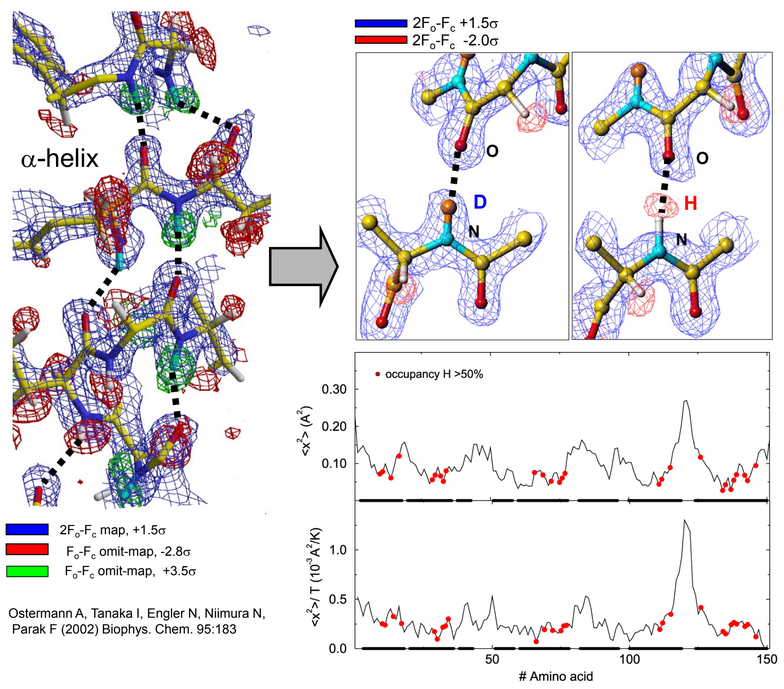
neutrons d_{min}= 1.5Å:



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

Analysis of H/D-exchange:

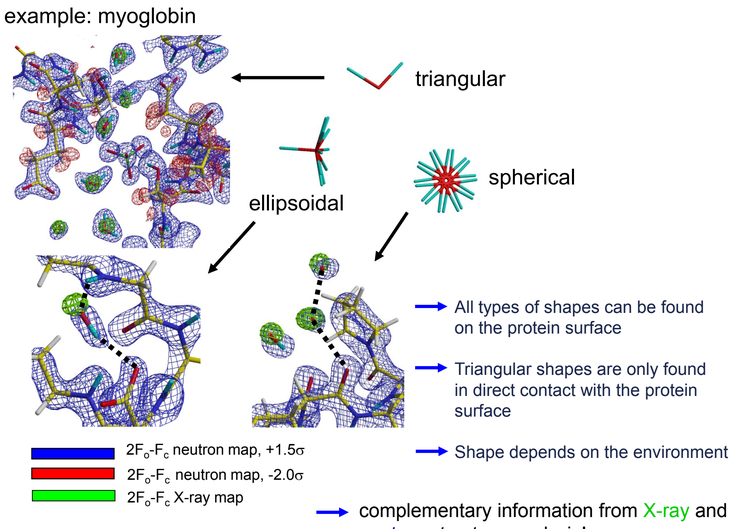


Ostermann A, Tanaka I, Engler N, Niimura N, Parak F (2002) Biophys. Chem. 95:183

- H / D exchange correlates with the flexibility
- protons show higher protection in the interior of the protein
- tells you where water can migrate and which protons can take part in proton transfer reactions

Hydration structure analysis:

example: myoglobin

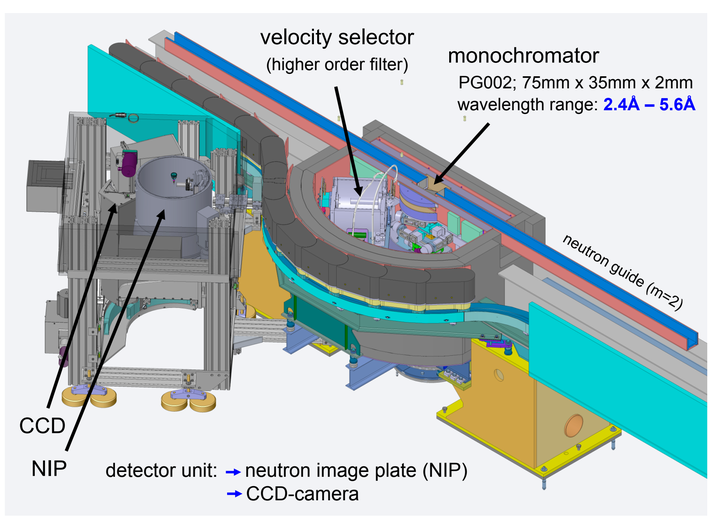


- All types of shapes can be found on the protein surface
- Triangular shapes are only found in direct contact with the protein surface
- Shape depends on the environment

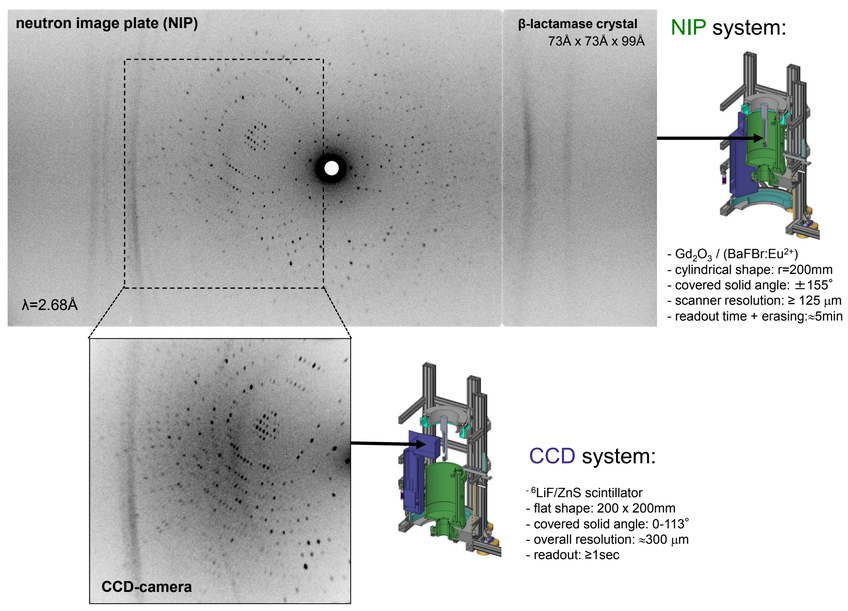
→ complementary information from X-ray and neutron structure analysis!

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

The diffractometer BIODIFF:



NIP and CCD detector system:



NIP system:

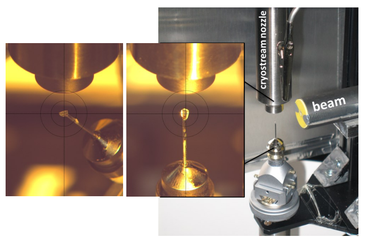
- Gd_2O_3 / ($BaFBr:Eu^{2+}$)
- cylindrical shape: r=200mm
- covered solid angle: ±155°
- scanner resolution: ≥ 125 μm
- readout time + erasing:≈5min

CCD system:

- $^6LiF/ZnS$ scintillator
- flat shape: 200 x 200mm
- covered solid angle: 0-113°
- overall resolution: ≈300 μm
- readout: ≥1sec

Sample environment:

Standard Oxford cryostream 700+

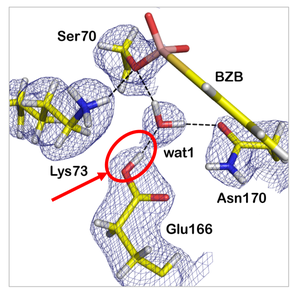


- temperature range: 90 - 500K
- stable, no icing over weeks

First "user data-sets":

β-lactamase with bound BZB inhibitor

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



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

pdb: 4bd1

R_{pim} = 7.9% (17.9%)

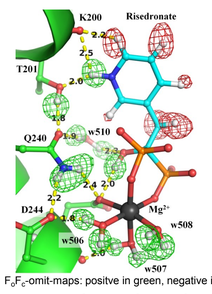
- unit cell: 73.4Å, 73.4Å, 99.1Å P3$_2$21
- fully deuterated protein
- crystal size: 2.7mm^3
- collection time: 9d

The hydrogen-bonding network strongly suggests Glu166 acts as the general base

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

Human farnesyl pyrophosphate synthase with risedronate

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



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

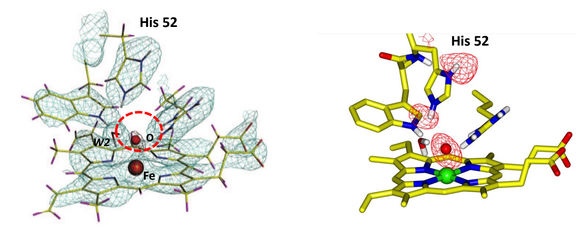
d_{min}	I/σ(I)	N_{meas}	mult.	compl. in shell %	R_{merge} %
140.0 – 5.17	29.1	12784	6.6	94.9	4.3
5.17 – 4.10	14.5	9490	5.0	99.3	9.8
4.10 – 3.59	10.6	8045	4.3	99.5	12.3
3.59 – 3.26	7.0	5833	3.2	98.5	15.5
3.26 – 3.02	6.1	6443	3.5	99.3	19.5
3.02 – 2.85	4.5	6181	3.4	98.6	24.9
2.85 – 2.70	3.3	5772	3.2	98.6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

R_{pim} = 5.8% (37.1%)

- unit cell: 111.9Å, 111.9Å, 72.6Å P4$_1$2$_1$2
- crystal size: 3.5mm^3
- collection time: 25d (5d, 6d, 14d)

Compound I of cytochrome c peroxidase @100K

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



- **The oxygen atom bound to iron (IV) is not protonated!**
- **but His 52 is double protonated!**
- **Reaction mechanism needs to be reconsidered!**

Examples of user experiments:

protein	unit cell (Å) space group	cell volume (Å^3)	crystal size (mm^3)	time (d)	d_{min} (Å)	compl. (%)	R_{merge} (%)
β-lactamase (no ligand) L. Coates et al.	73.3, 73.3, 98.7 P3$_2$21 λ=2.7Å	453,000	4.0	8	2.0	89.0 (82.7)	9.8 (22.3)
β-lactamase-BZB-inhibitor L. Coates et al.	73.4, 73.4, 99.1 P3$_2$21 λ=2.7Å	453,000	2,7	9	2.0	90.2 (81.2)	14.7 (27.9)
Inorganic pyrophosphatase J. Ng et al.	101.0 101.0 100.5 R32 λ=3.4Å, 4.0Å	887,700	1	24	2.0	97.9 (90.5)	13.6 (52.6)
Xylanase II A. Kovalevsky et al.	49.5 59.9 70.4 P2$_1$2$_1$2$_1$ λ=2.7Å	208,000	2.8	17	2.0	96.2 (91.0)	9.7 (32.7)
KDN9P phosphatase Z. Fischer et al.	83.1 108.9 75.8 P2$_1$2$_1$2 λ=2.7Å	685,000	1.0	18	2.5	94.8 (88.7)	11.7 (40.0)
apo human carbonic anhydrase II Z. Fischer et al.	42.8 41.7 72.8 P2$_1$ λ=2.7Å	125,000	2,5	8	1.8	89.9 (76.8)	11.9 (33.0)
Nucleosidase (MTAN) A. Kovalevsky et al.	83.0 83.0 67.4 P3$_2$21 λ=2.7Å	392,000	2.8	25	2.7	97.1 (94.9)	9.8 (47.8)
Cytochrome c peroxidase P. Moody, M. Blakeley, C. Casadei et al.	51.2 75.8 107.6 P2$_1$2$_1$2$_1$ λ=3.4Å, 4.0Å	417,000	0.65	23	2.5	90.7 (71.8)	17.3 (42.8)
Farnesyl pyrophosphate synthase T. Yokoyama et al.	111.9 111.9 72.6 P4$_1$2$_1$2 λ=4.0Å	909,000	3.5	25 (11)	2.4	98.4 (98.0)	10.7 (61.2)
DNA drug complex S. Arai, R. Kuroki et al.	27.9 27.9 52.0 P4$_1$2$_1$2 λ=2.7Å	40,500	3.0	3	1.7	92.7 (83.3)	10.8 (21.5)

- 4 proposals "BIODIFF as low resolution powder machine": - CO2 uptake in clay as F(pressure); - Stratum corneum lipid model membranes;;
- 6 proposals small compound structures (large magnetic superstructures or diffuse scattering);

Next proposal deadline: May 6th, 2016 !!

user.frm2.tum.de
fzj.frm2.tum.de

Journal of large-scale research facilities, 1, A2 (2015) http://dx.doi.org/10.17815/jlsrf-1-19

Contact: Andreas Ostermann
E-mail: Andreas.Ostermann@frm2.tum.de
Phone: +49.89.289.14702

Tobias E. Schrader
E-mail: t.schrader@fz-juelich.de
Phone: +49.89.289.10743

Jülich Centre for Neutron Science

FRM II
Forschungs-Neutronenquelle
Heinz Maier-Leibnitz