FRM II
Forschungs-Neutronenquelle
Heinz Maier-Leibnitz

MLZ
Heinz Maier-Leibnitz Zentrum

Macromolecular crystallography at the FRM II
The new neutron single crystal diffractometer BIODIFF

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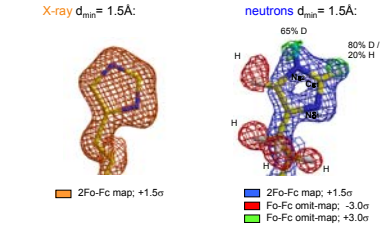
Neutron structure determination:

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 (<x²>) of the hydrogen atoms can be compared with data of other techniques
- no radiation damage compared to measurements at synchrotrons

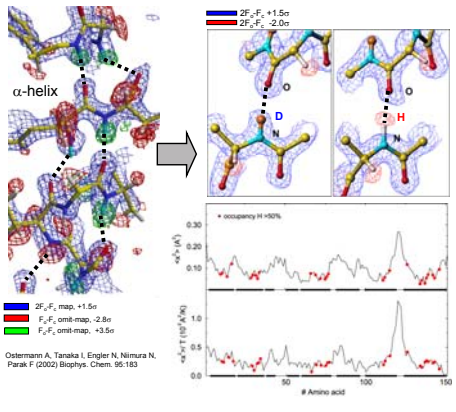
Amino acid protonation states:

example: histidine 97 in myoglobin



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

Analysis of H/D-exchange:

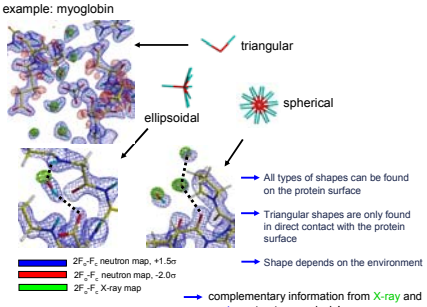


Ostermann A, Tanaka I, Engler N, Niimura N, Parak F (2002) Biophys. Chem. 95:183

- 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

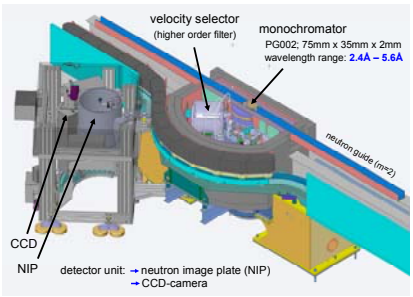
Hydration structure analysis:

example: myoglobin

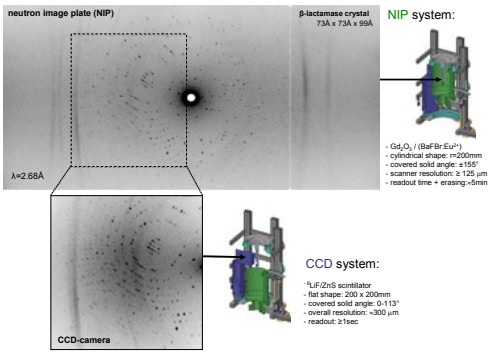


Chatake T, Ostermann A, Kurihara K, Parak F, Niimura N (2003) Proteins 50:516

The diffractometer BIODIFF:

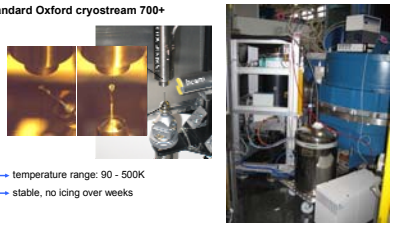


NIP and CCD detector system:



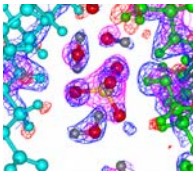
Sample environment:

Standard Oxford cryostream 700+



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

First instrument test with myoglobin:



X-ray data taken from: Ostermann, A., Tanaka, I., Engler, N., Niimura, N.& Parak, F. G. (2002). *Biophys. Chem.*, **95**, 183

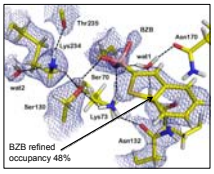
Neutron data(from map calculation using the pdb-file 1L2K.pdb and the BioDiff data set):
R=20.19 %
R_{free}=21.13 %

Water network in the contact region between two myoglobin molecules in the crystal. In the centre of picture a sulfate ion SO_4^- is seen with the sulfur atom shown in yellow, the oxygen atoms shown in red. The deuterium atoms of the water molecules are coloured grey. The x-ray map is shown in magenta at a contour level of +2.7σ. The nuclear map is displayed at a contour level of -1.75σ in red and at +2.3σ in blue.

First "user data-sets":

β-lactamase with bound BZB inhibitor

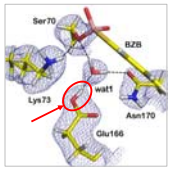
S.J. Tomanicek, R.F. Standaert, K.L. Weiss, J.D. Ng, **L. Coates** (Group of P. Langan)

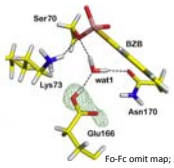


d_{min}	I/σ(I)	N_{meas}	mult.	compl. in shell %	R_{merge} %
4.31	27.8	12685	5.6	97.6	4.9
3.42	19.0	11941	5.5	98.0	8.0
2.99	10.3	10378	4.9	96.9	14.6
2.71	7.6	8757	4.3	95.5	18.7
2.52	5.9	7620	3.9	92.8	21.2
2.37	5.4	7099	3.8	89.2	21.6
2.25	5.0	6095	3.5	84.6	23.0
2.15	4.5	5908	3.4	82.9	24.7
2.07	4.1	5673	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_{sym} = 7.9% (17.9%)

- unit cell: 73.4Å, 73.4Å, 99.1Å P3$_2$21
- fully deuterated protein
- crystal size: 2.7mm³
- Collection time: 9d





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

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

Summary of user experiments after 4 reactor cycles:

protein	unit cell (Å) space group	cell volume (Å³)	crystal size (mm³)	time (d)	d_{min} (Å)	compl. (%)	R_{merge} (%)
β-lactamase (no ligand) L. Coates et al.	73.3, 73.3, 98.7 P3$_2$21	453,000	4.0	8	2.0	89.0 (82.7)	9.8 (22.3)
β-lactamase-BZB-inhibitor L. Coates et al.	73.4, 73.4, 99.1 P3$_2$21	453,000	2,7	9	2.0	90.2 (81.2)	14.7 (27.9)
Inorganic pyrophosphatase J. Ng et al.	105.3 95.3 113.8 C2	1.120,000	3		2.6	not completed	
Xylanase II A. Kovalevsky et al.	49.5 69.9 70.4 P2$_1$2$_1$2$_1$	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$2	685,000	1.0	18	2.5	84.8 (88.7)	11.7 (40.0)
apo human carbonic anhydrase II Z. Fischer et al.	42.8 41.7 72.8 P2$_1$	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	392,000	2.8	25	2.7	97.1 (94.9)	9.8 (47.8)

- 2 proposals (14d) "BIODIFF as low resolution powder machine": CO2 uptake in clay as F(pressure);
- 2 proposals (10d) small compound structures (large magnetic superstructures);

Next proposal deadline: May 2nd, 2014
user.frm2.tum.de
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http://www.mlz-garching.de/biodiff