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

or: What can neutrons do for you?

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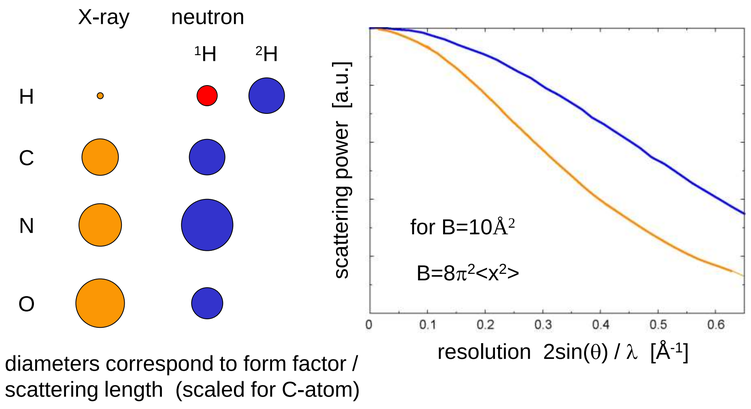
Neutron structure determination:

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

- 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

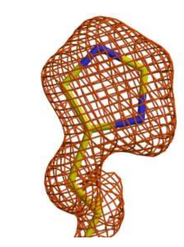
Comparison of form factors (X-ray) and scattering lengths (neutrons):



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

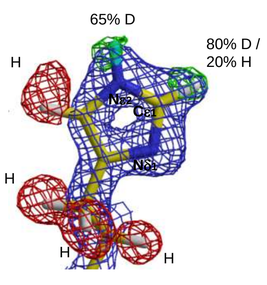
Amino acid protonation states:

X-ray d_{min}= 1.5Å:



2Fo-Fc map; +1.5σ

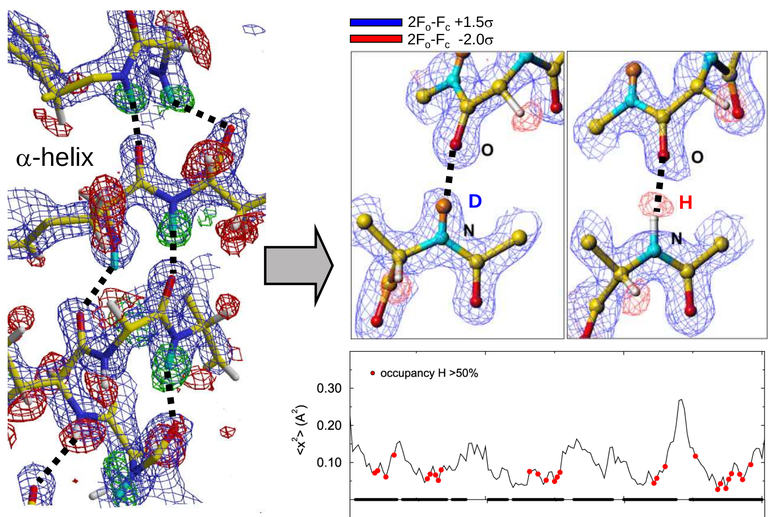
neutrons d_{min}= 1.5Å:



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:



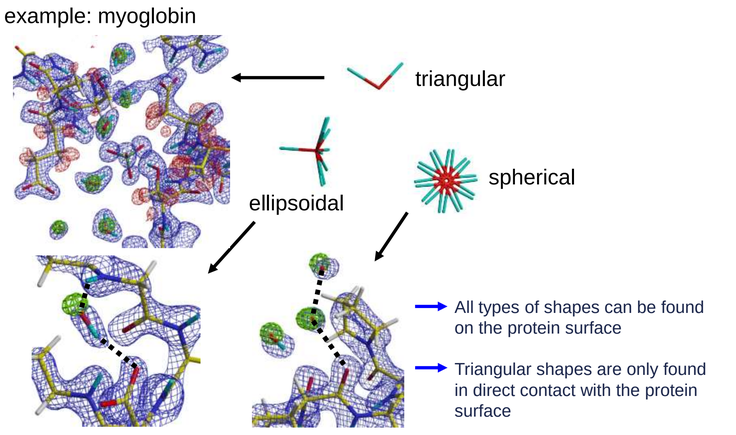
2Fo-Fc map, +1.5σ
Fo-Fc omit-map, -2.8σ
Fo-Fc omit-map, +3.5σ

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:

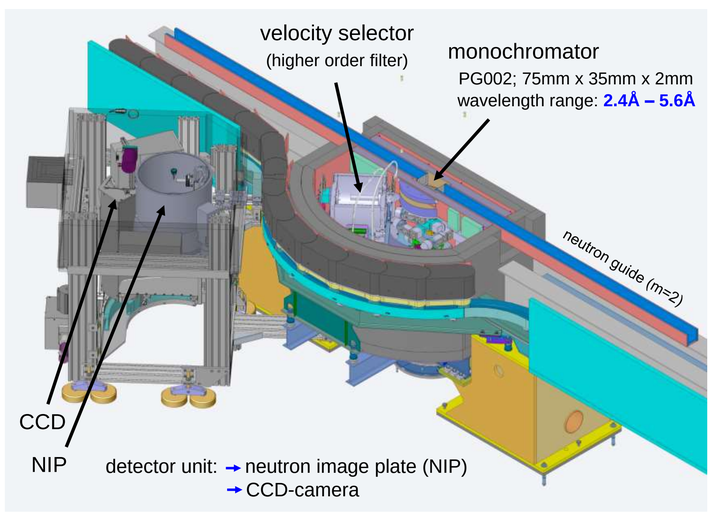
example: myoglobin



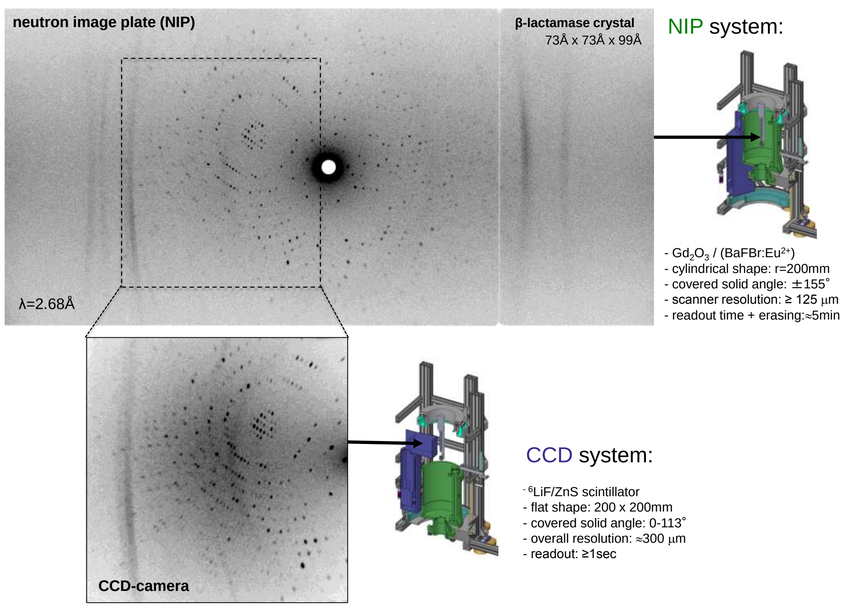
- All types of shapes can be found on the protein surface
- Triangular shapes are only found in direct contact with the protein surface
- Shape depends on the environment
- complementary information from X-ray and neutron structure analysis!

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

The diffractometer BIODIFF:

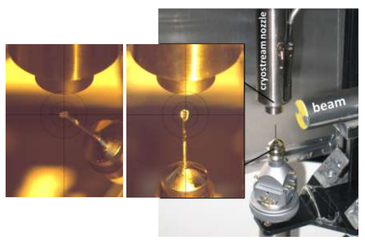


NIP and CCD detector system:



Sample environment:

Standard Oxford cryostream 700+

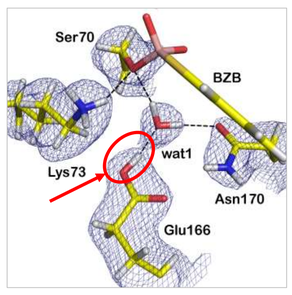


- temperature range: 90 - 500K
- stable, no icing over weeks

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%)

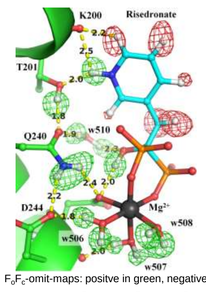
- unit cell: 73.4Å, 73.4Å, 99.1Å P3₂21
- fully deuterated protein
- crystal size: 2.7mm³
- collection time: 9d

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

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

Human farnesyl pyrophosphate synthase with risedronate

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



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**

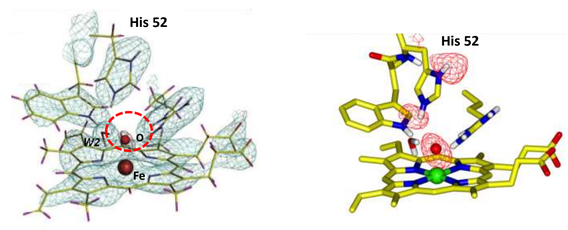
F_oF_c-omit-maps: positve in green, negative in red;

R_{pim} = 5.8% (37.1%)

- unit cell: 111.9Å, 111.9Å, 72.6Å P4₁2₁2
- crystal size: 3.5mm³
- 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 (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 P3₂21 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 P3₂21 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 P2₁2₁2₁ 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 P2₁2₁2 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 P2₁ 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 P3₂21 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 P2₁2₁2₁ 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 P4₁2₁2 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 P4₁2₁2 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: Sep 11th, 2015 !!

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