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

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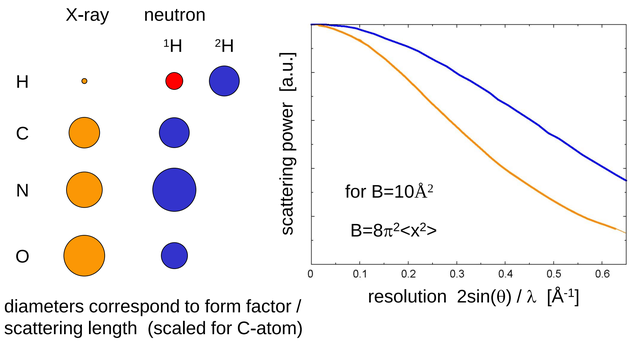
Neutron structure determination:

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 ($<x^2>$) of the hydrogen atoms can be compared with data of other techniques
- no radiation damage compared to measurements at synchrotrons

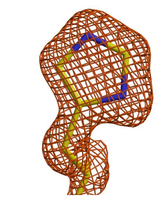
Comparison of form factors (X-ray) and scattering lengths (neutrons):



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

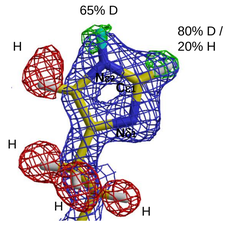
Amino acid protonation states:

X-ray d_{min}= 1.5Å:



2Fo-Fc map; +1.5σ

neutrons d_{min}= 1.5Å:

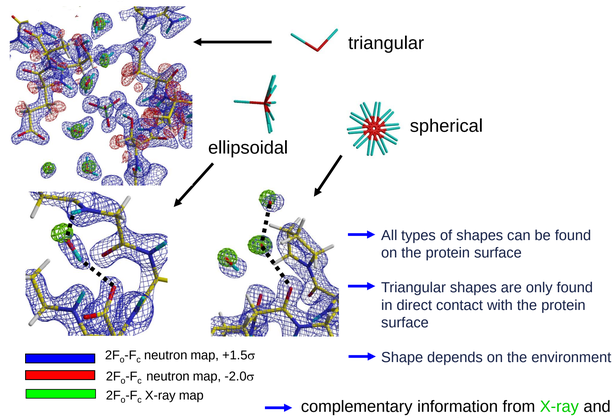


2Fo-Fc map; +1.5σ
Fo-Fc omit-map; -3.0σ
Fo-Fc omit-map; +3.0σ

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

Hydration structure analysis:

example: myoglobin



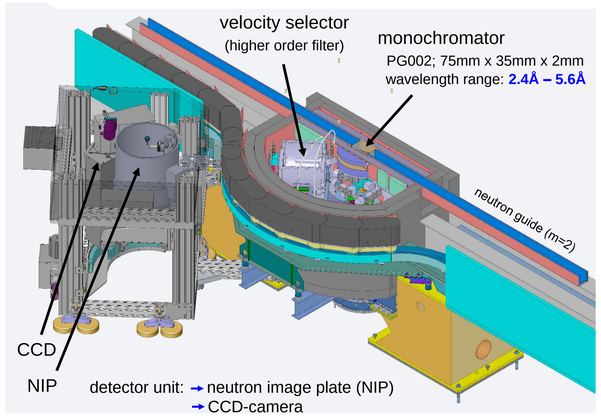
- All types of shapes can be found on the protein surface
- Triangular shapes are only found in direct contact with the protein surface
- Shape depends on the environment

$2F_o$-F_c neutron map, +1.5σ
$2F_o$-F_c neutron map, -2.0σ
$2F_o$-F_c X-ray map

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

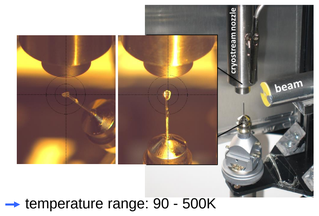
Chatake T, Ostermann A, Kurihara K, Parak F, Niimura N (2003) Proteins 50:516

The diffractometer BIODIFF:



Sample environment:

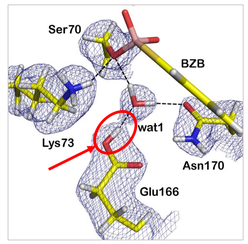
Standard Oxford cryostream 700+



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

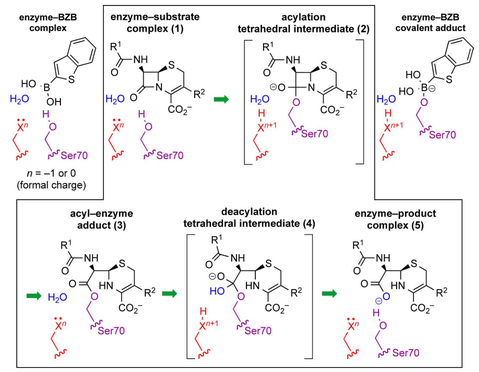
First "user data-sets": β-lactamase with bound BZB inhibitor

Publication: *Tomanicek et al., J. Biol. Chem., 288, 4715 (2013).*
Experimental Team: **S.J. Tomanicek**, R.F. Standaert, K.L. Weiss, J.D. Ng, **L. Coates** (Group of P. Langan)



- 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

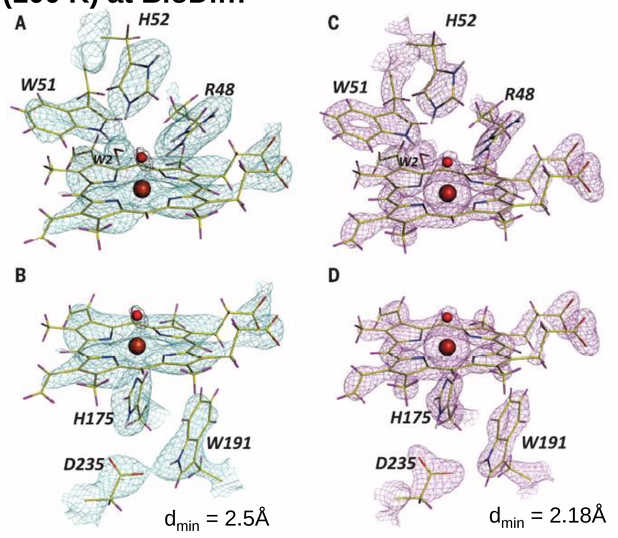


The catalytic cycle of a class A-lactamase illustrated for a cephalosporin substrate (*inside box*) and the mode of inhibition by BZB (*outside box*).

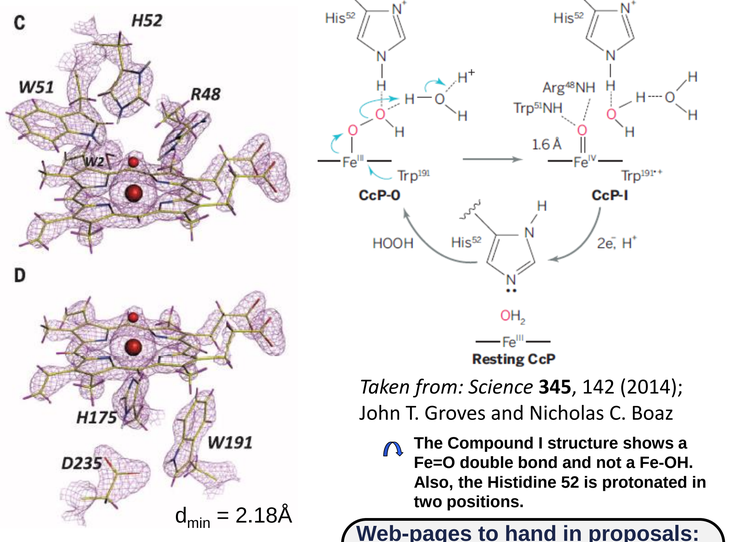
Structure of Compound I of Cytochrome C Peroxidase:

Publication: *Science 11 July 2014: Vol. 345 no. 6193 pp. 142-143 DOI: 10.1126/science.1256754*
Experimental Team: 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, and Peter C. E. Moody

Neutron diffraction (100 K) at BioDiff:



X-ray diffraction:



Taken from: Science **345**, 142 (2014); John T. Groves and Nicholas C. Boaz

The Compound I structure shows a Fe=O double bond and not a Fe-OH. Also, the Histidine 52 is protonated in two positions.

The structure of compound I of CcP in the region of the heme. Nuclear scattering density (2Fo-Fc contoured at 2.2 RMS) in the (A) distal and (B) proximal heme pocket. Electron density (2Fo-Fc contoured at 2.6 RMS) in (C) and (D).

Web-pages to hand in proposals:
user.frm2.tum.de
fzj.frm2.tum.de
Next Proposal Deadline:
January, 16th 2015!

Contact: t.schrader@fz-juelich.de, Andreas.Ostermann@frm2.tum.de

http://www.mlz-garching.de/biodiff