BIODIFF - a neutron diffractometer optimized for crystals with large unit cells

New developments and recent application examples

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MLZ is a cooperation between:

JÜLICH FORSCHUNGSZENTRUM

Helmholtz-Zentrum Geesthacht
Zentrum für Material- und Küstenforschung

Technische Universität München

Outline of this talk

1. The case of Cytochrome C Peroxidase
2. Introduction to the instrument BioDiff
3. Some more application examples
4. Summary

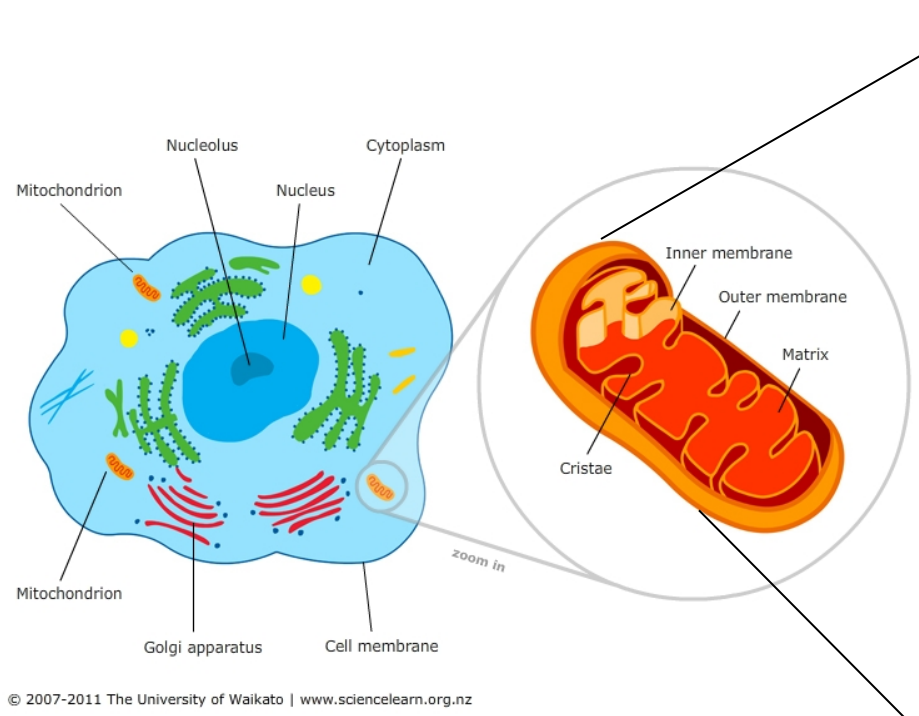
An example for a metallo-protein:

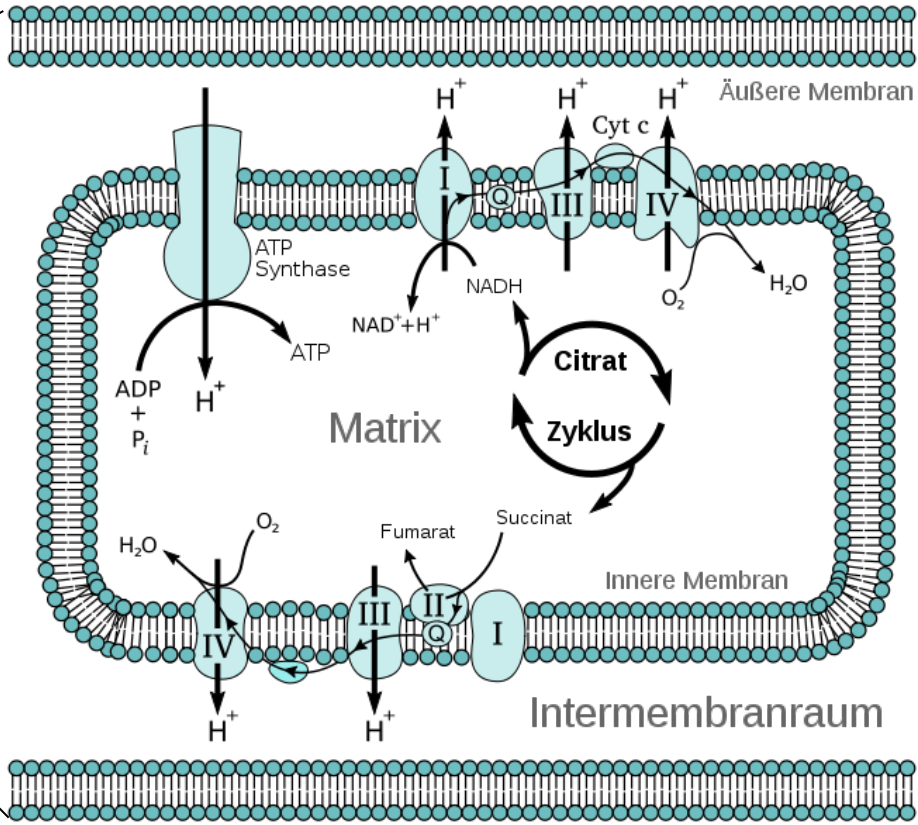
Cytochrome *c* peroxidase, or CcP is a water-soluble heme-containing enzyme of the peroxidase family that takes reducing equivalents from cytochrome *c* and reduces hydrogen peroxide to water:

$$CcP + H_2O_2 + 2\ \text{ferrocytochrome } c\ (Fe^{2+}) + 2H^+ \rightarrow CcP + 2H_2O + 2\ \text{ferricytochrome } c\ (Fe^{3+})$$

(taken from http://en.wikipedia.org/wiki/Cytochrome_c_peroxidase)

Mitochondria are the power plant of a cell (production of ATP):

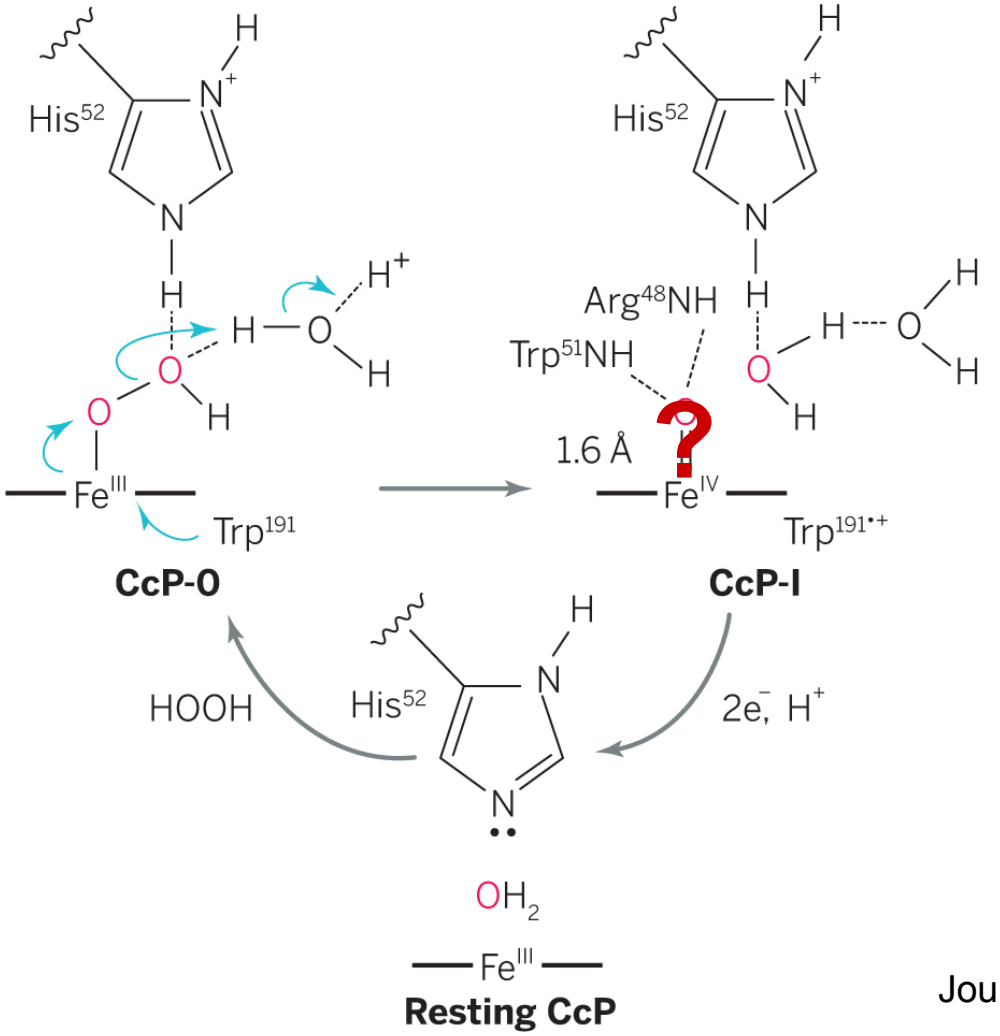




http://de.wikipedia.org/wiki/Atmungskette

- Cytochrome C serves as an electron transporter in the respiratory chain.
- Cytochrome c Peroxidase uses two ferro-cytochrome C proteins to reduce H_2O_2 to water and two ferricytochrome C molecules

Proton-mediated mechanism.Reaction of ferric CcP with H_2O_2 first gives CcP-0, followed by O-O bond scission driven by external protonation to afford CcP-I.



J T Groves, and N C Boaz Science 2014;345:142-143

Alternative Hypothesis:

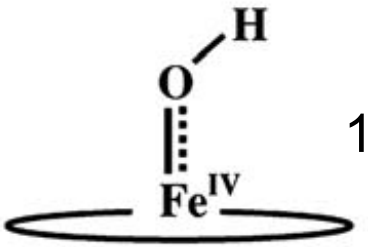
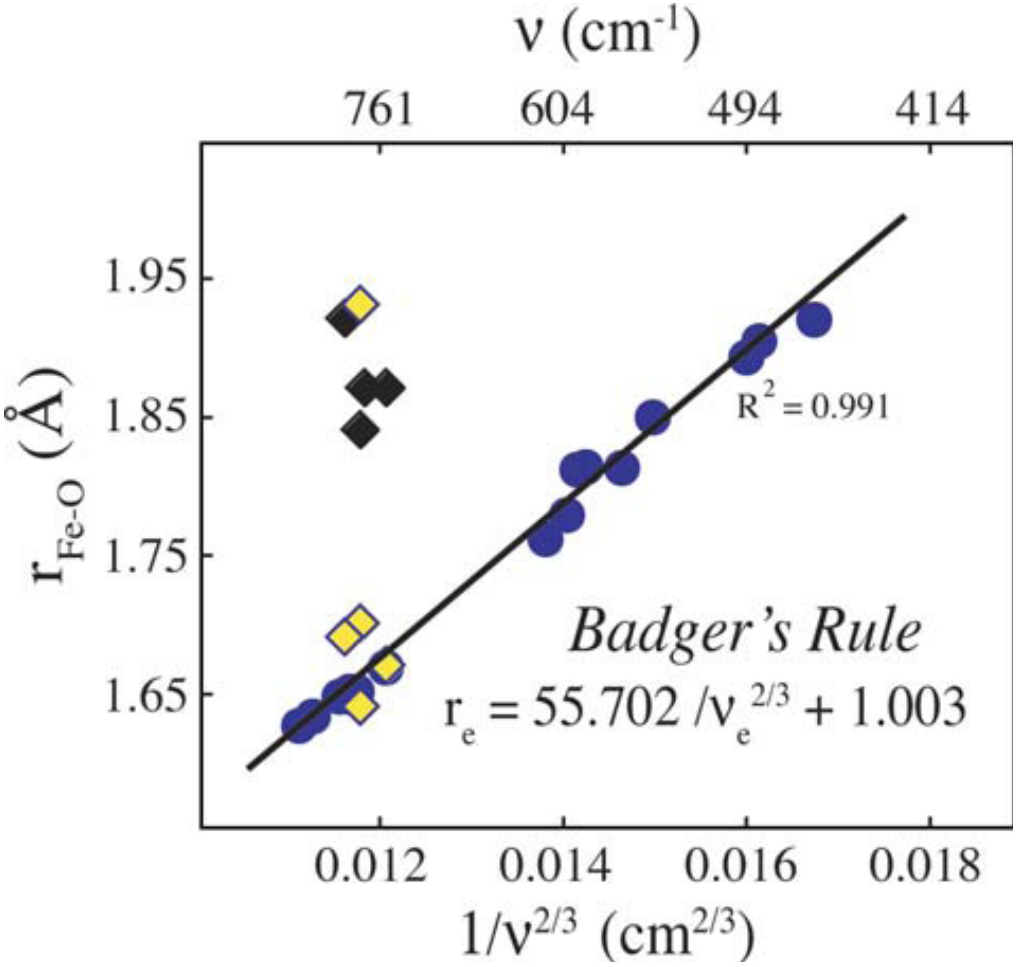


Fig. 3. Compound I with an O–H bond and a bond length of Fe-O of ca. 1.85 Å.

Journal of Inorganic Biochemistry 100 (2006) 448–459

No method so far could unambigiously show the nature of the iron-oxide bond



Plot of computed stretching frequency vs Fe–O bond distance. **Yellow diamonds** are from resonance Raman or EXAFS and the solid diamonds from X-ray crystal structures. The **blue circles** are from calculations.

Figure taken from: Journal of Inorganic Biochemistry 100 (2006) 448–459

Excitation laser power affects resonane raman spectra

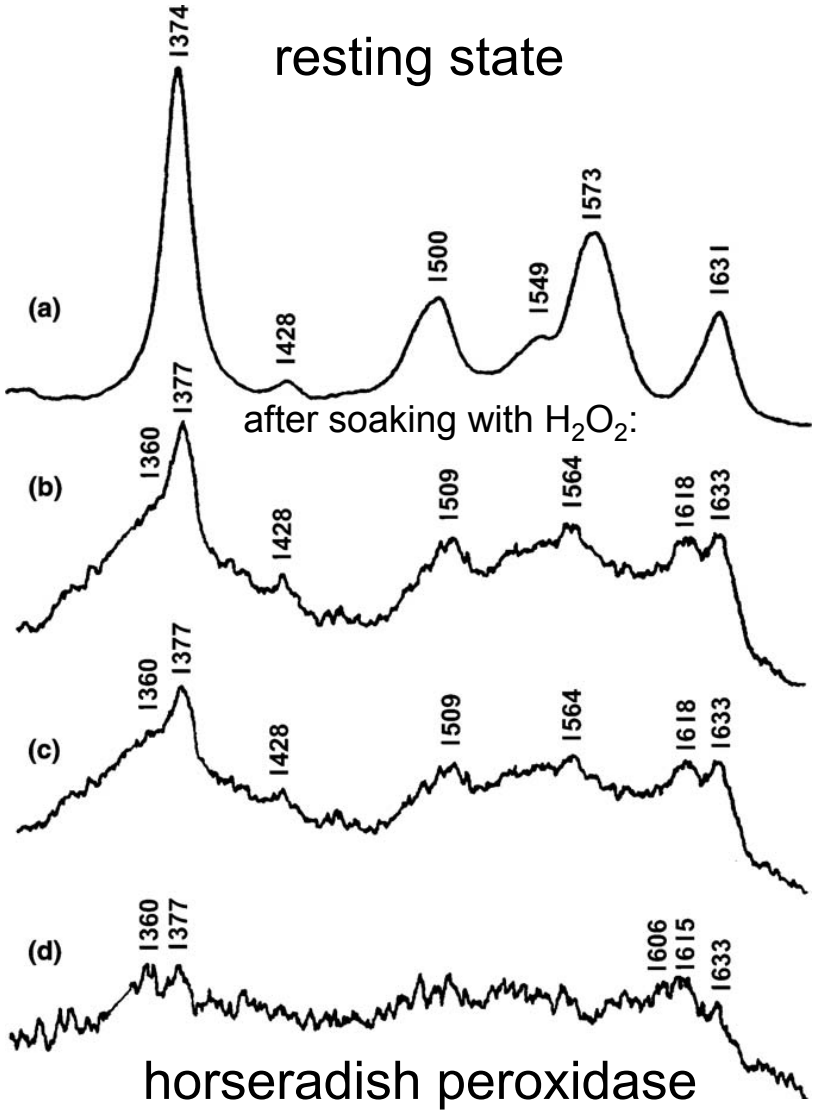


Fig. 2. Resonance Raman spectra of flowing stream of horseradish peroxidase (isozymes B and C, 2 mM, 0.1 M sodium phosphate, pH 6.8) with 406.7 nm excitation: (a) before mixing with equimolar H2O2, 5 mw laser power; (b) after mixing with H2O2, 3 mw laser power; (c) as in (b) but with laser power lowered to 1 mw; (d) as in (b) and (c) but with laser power reduced to 0.3 mw. Bands from an HRP-II type photoproduct predominate in traces (b) and (c). Bands at 1360, 1606 and 1615 cm-1in trace (d) arise from HRP-I.

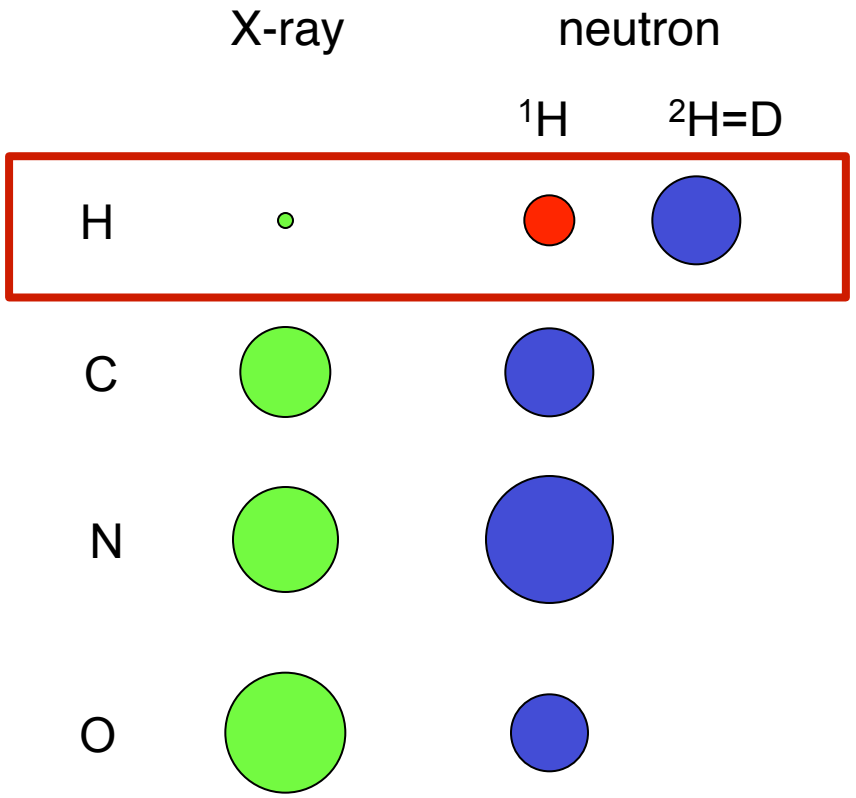
Only here, compound I bands are visible

Journal of Inorganic Biochemistry 100 (2006) 480–501

Advantages of structure determination with neutrons:

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

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)

σ_{coh} of ^{1}H is 1.8×10^{-28} m^2 but
σ_{incoh} of ^{1}H is 80.2×10^{-28} m^2
Large background from hydrogen atoms!

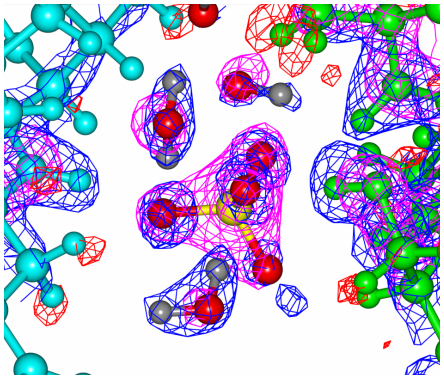
Scientific questions to be adressed:

Hydrogen/deuterium atoms can be resolved even at a resolution of $d_{min} \approx 2.5$ Å (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 +2.7σ
nuclear map (red): contour level of -1.75σ
nuclear map (blue): contour level of +2.3σ

Much less radiation damage as compared to x-rays: Metallo-proteins can be measured without reducing the metal centres

The neutron data is from the instrument BioDiff

X-ray structure needed to solve the phase problem

Table S2 *Cc*P Compound I Data Collection and refinement statistics
*values in parenthesis are for the outer resolution bin

Space group	$P2_12_12_1$
Cell dimensions	
a, b, c (Å)	51.19 75.83 107.59
Data collection (Neutron λ=3.39Å, λ=3.98Å)	
Resolution (Å)	50 -2.5 (2.59-2.5)*
R_{merge}	0.173 (0.428)
I / σI	4.6 (1.5)
Completeness (%)	90.7 (71.8)
Redundancy	2.3 (1.7)

Data collection (Xray λ=1.5418Å)	
Resolution (Å)	17-2.18 (2.25-2.18)*
R_{merge}	0.074 (0.164)
I / σI	16.5 (5.9)
Completeness (%)	99.3 (94.9)
Redundancy	4.7 (2.6)

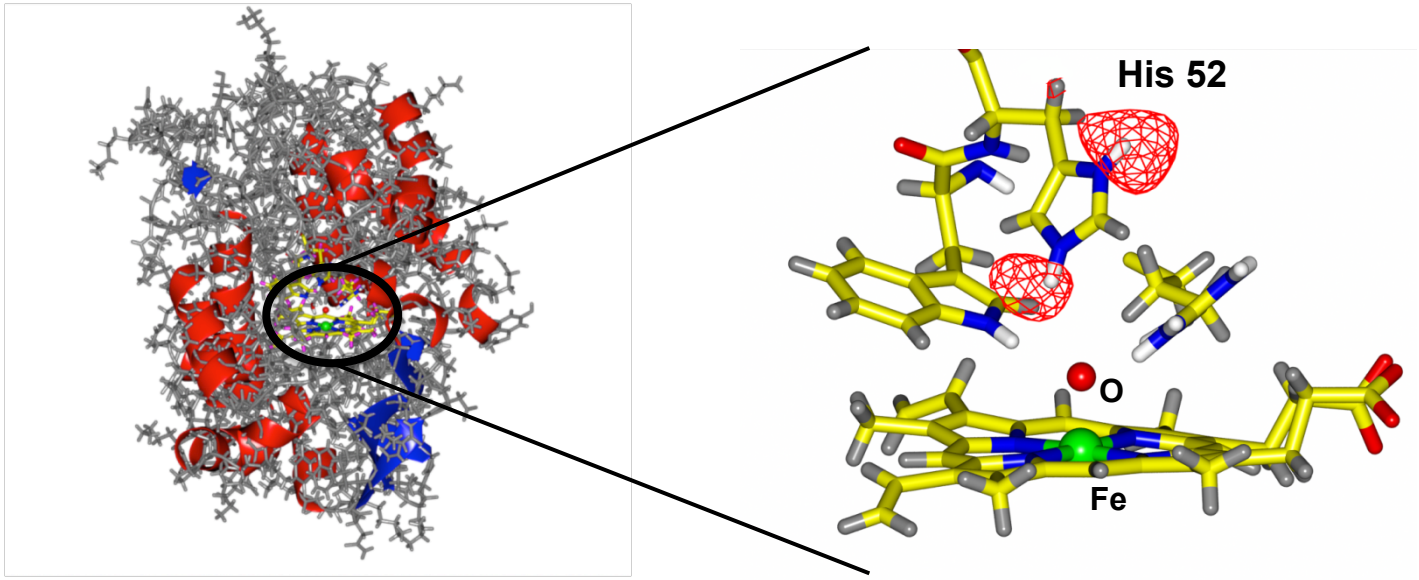
Joint Refinement cycle75	
d_{min} (Neutron)	2.5(Å)
d_{min} (Xray)	2.18(Å)
Number of reflections (Neutron)	13661
Number of reflections (X-ray)	22053
Rwork/ Rfree (Neutron)	0.1916/0.2720
Rwork/ Rfree (X-ray)	0.1488/0.2056
R.m.s deviations	
Bond lengths (Å)	0.011
Bond angles (°)	1.255

CcP CI structure at 100 K solved and refined.

Time needed for recording the data set: 23 days, crystal size: 0.65 mm^3

Omit-Map for the two exchangable hydrogen atoms at His52

Cytochrome-c-Peroxidase, Compound I at 100 K:

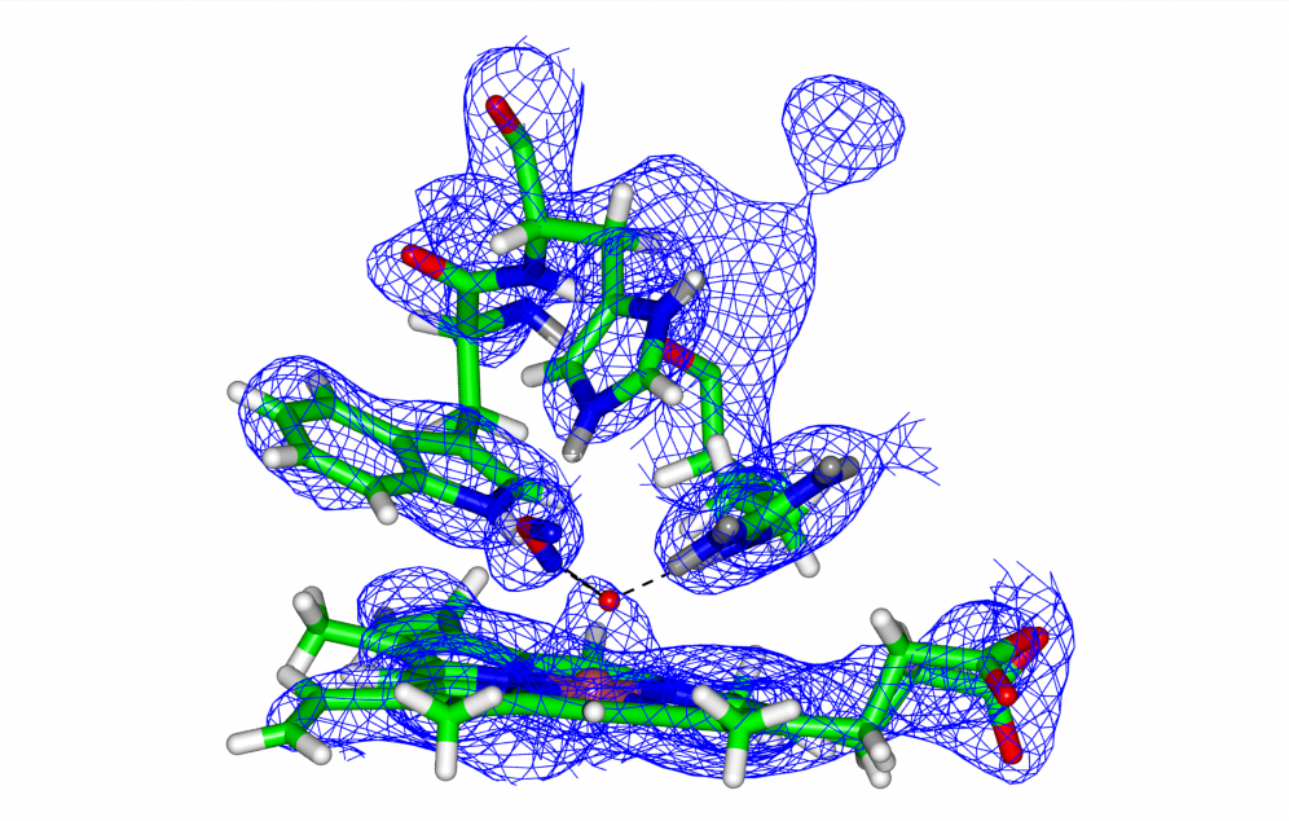


- The oxygen atom bound to iron is not protonated.
- The amino-acid His52 is doubly protonated

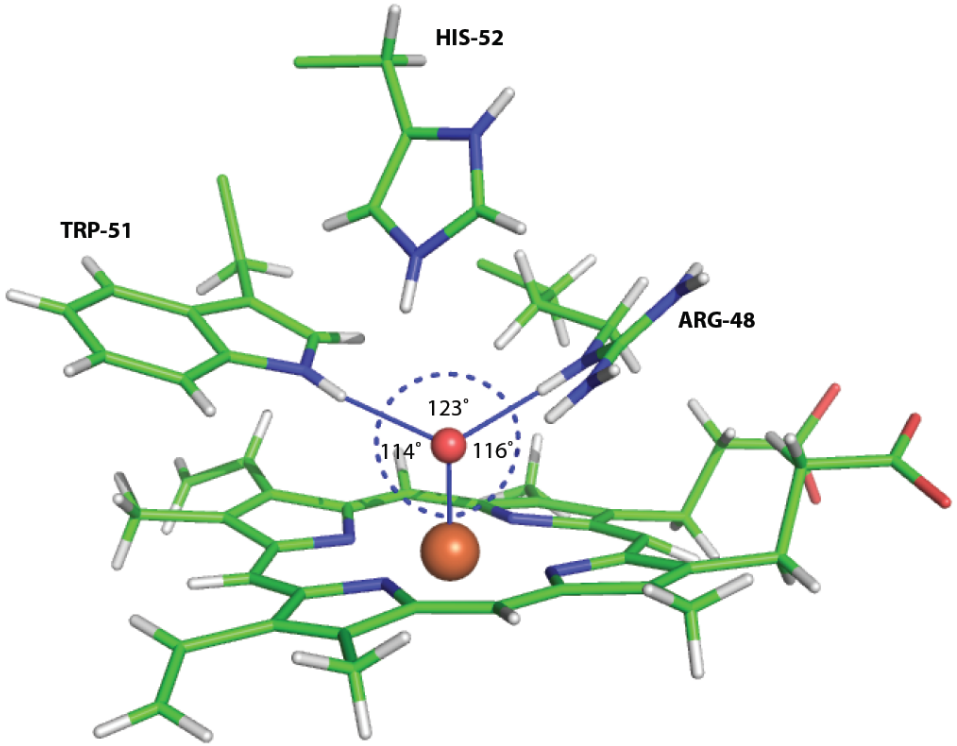
- The reacton mechanism has to be thought over again!

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

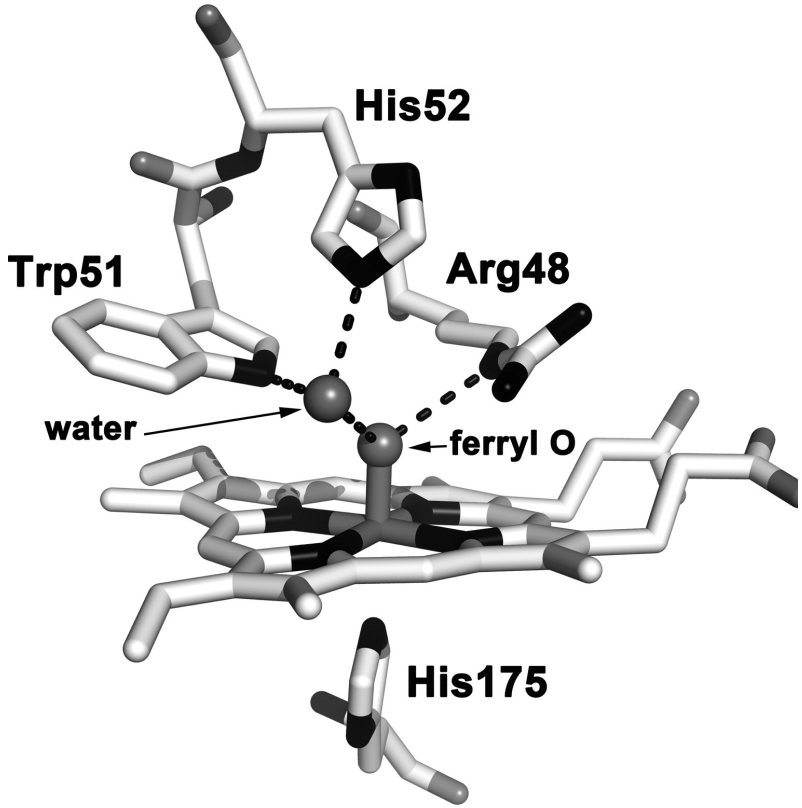
Compound I of Cytochrome c Peroxidase



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, Peter C. E. Moody, Science 2014;345:193-197

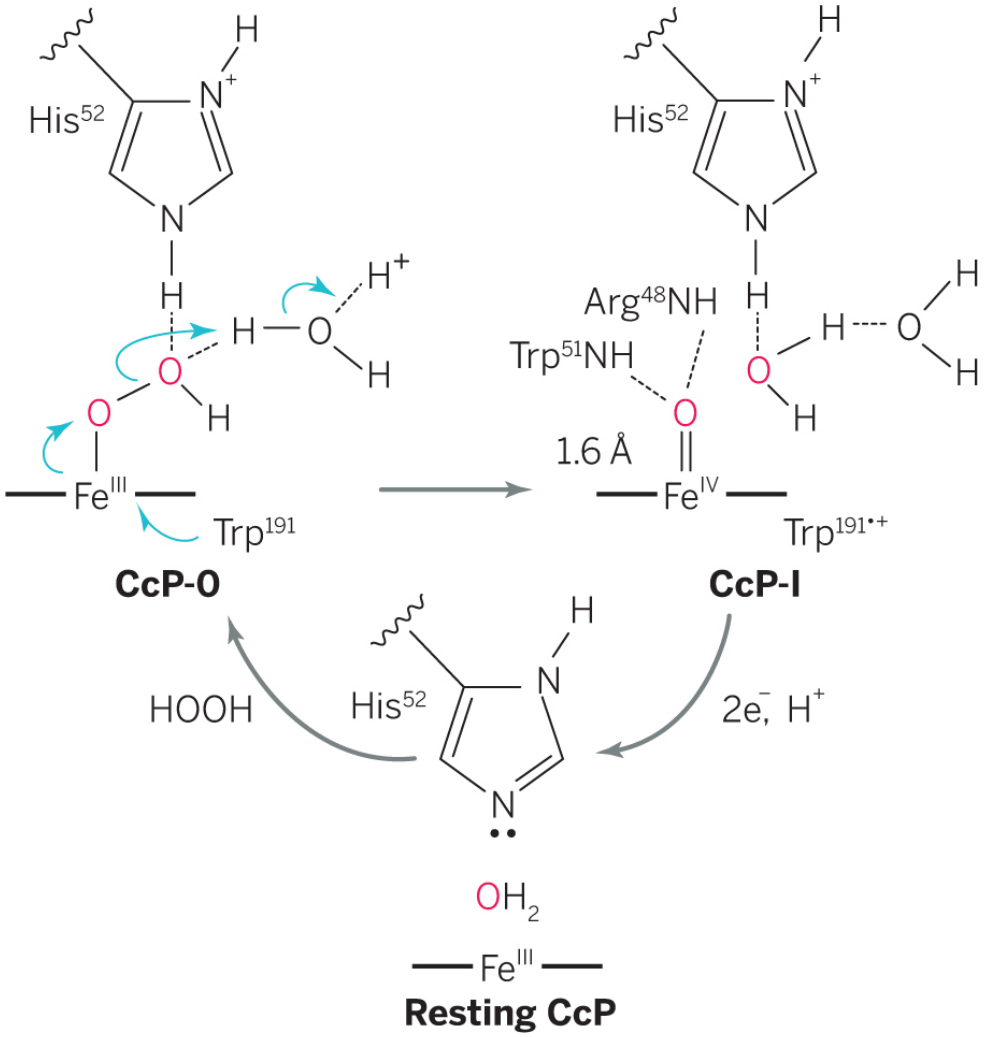


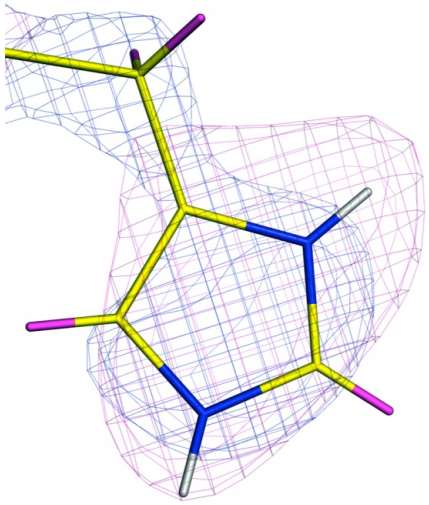
Neutron structure of CcP compound I .The water molecule H-bonded His 52 does not hydrogen bond to the ferryl O atom. Trp 51 interacts directly with the ferryl O.



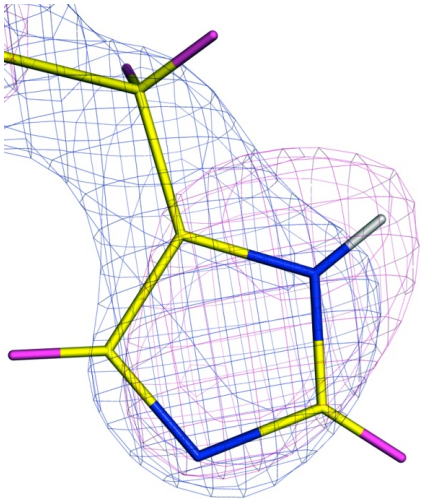
Crystal structure of CCP compound I(38) which is basically the same as the HRP compound I structure.(37) The water molecule H-bonded to the ferryl O atom is ideally positioned to assist His52 in acid–base catalysis as suggested.(39)

Proton-mediated mechanism: Reaction of ferric CcP with H_2O_2 first gives CcP-0, followed by O-O bond scission driven by external protonation to afford CcP-I.





His 52
Compound I

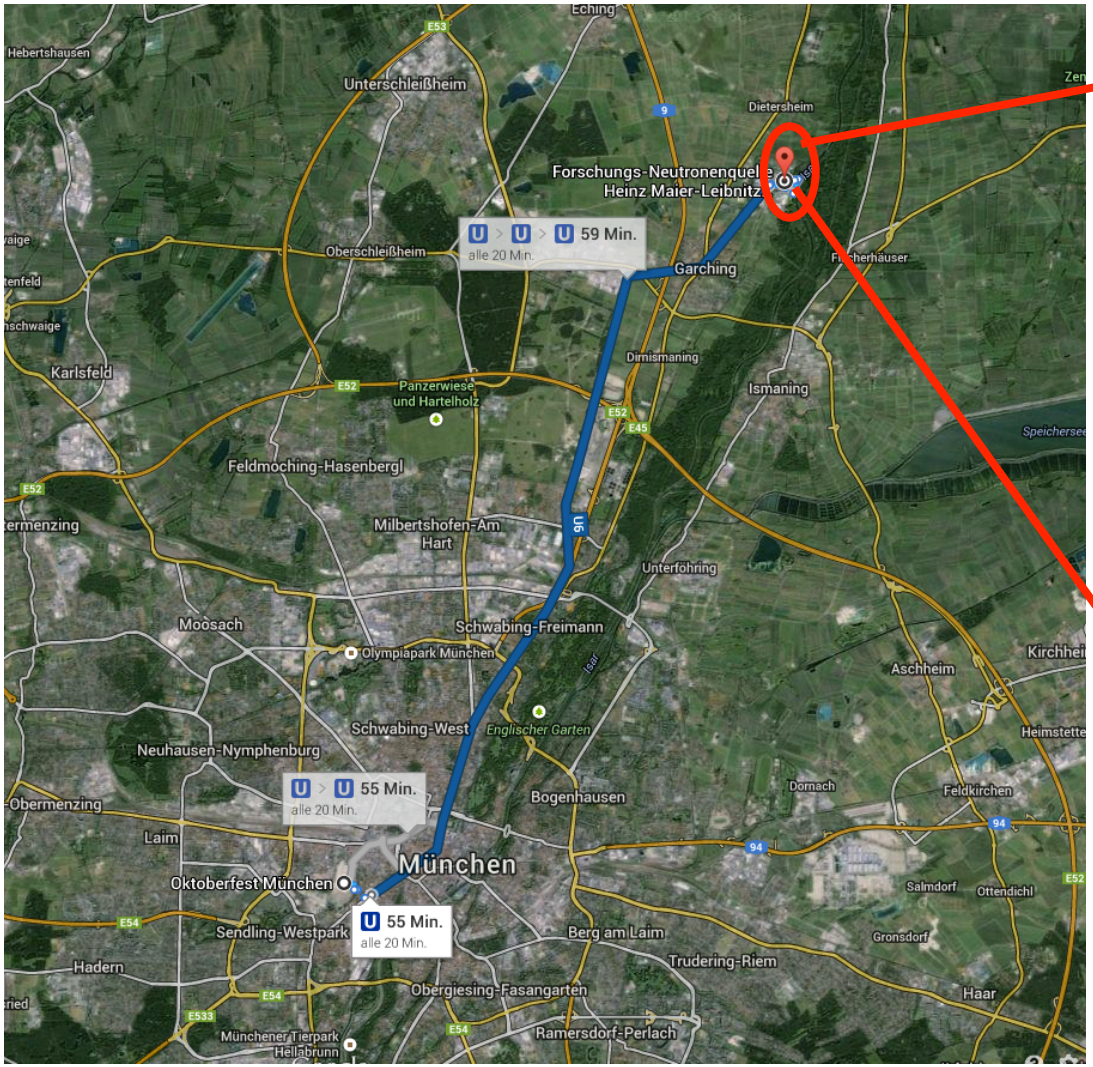


His 52 ferric
(resting)

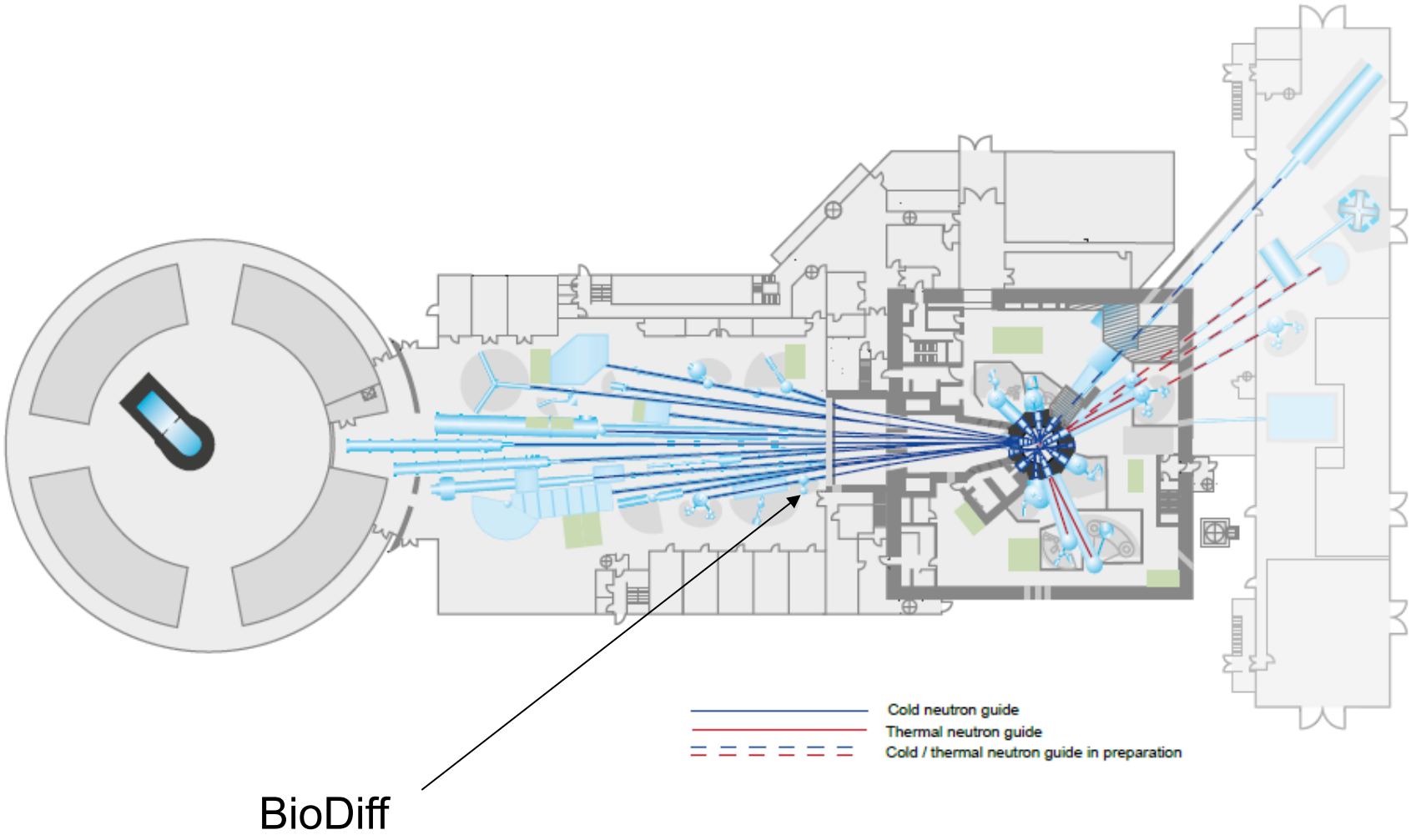
Published by AAAS

J T Groves, and N C Boaz Science 2014;345:142-143

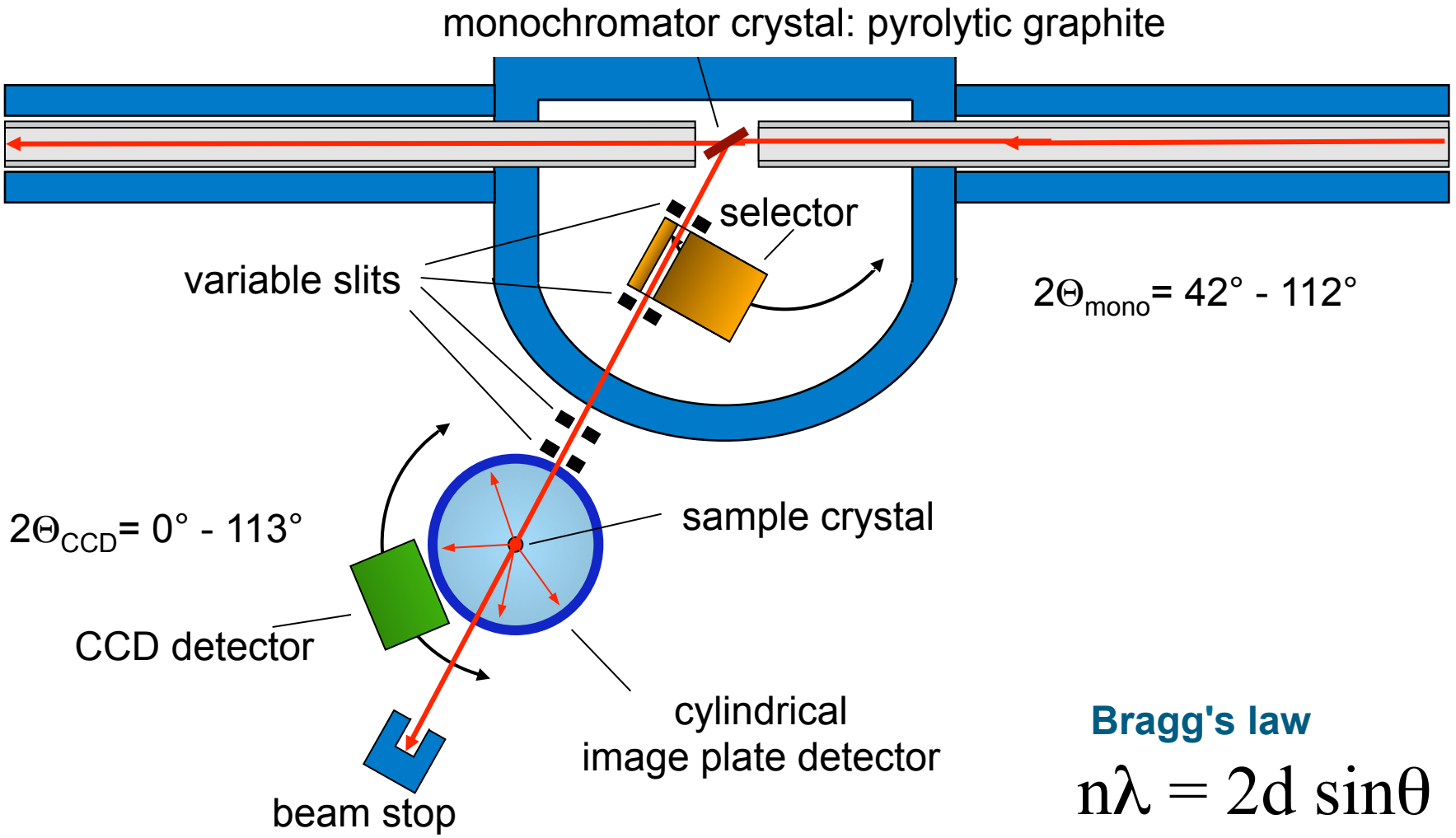
BioDiff at FRM II in Garching (close to Munich)



BioDiff at FRM II in the neutron guide hall west



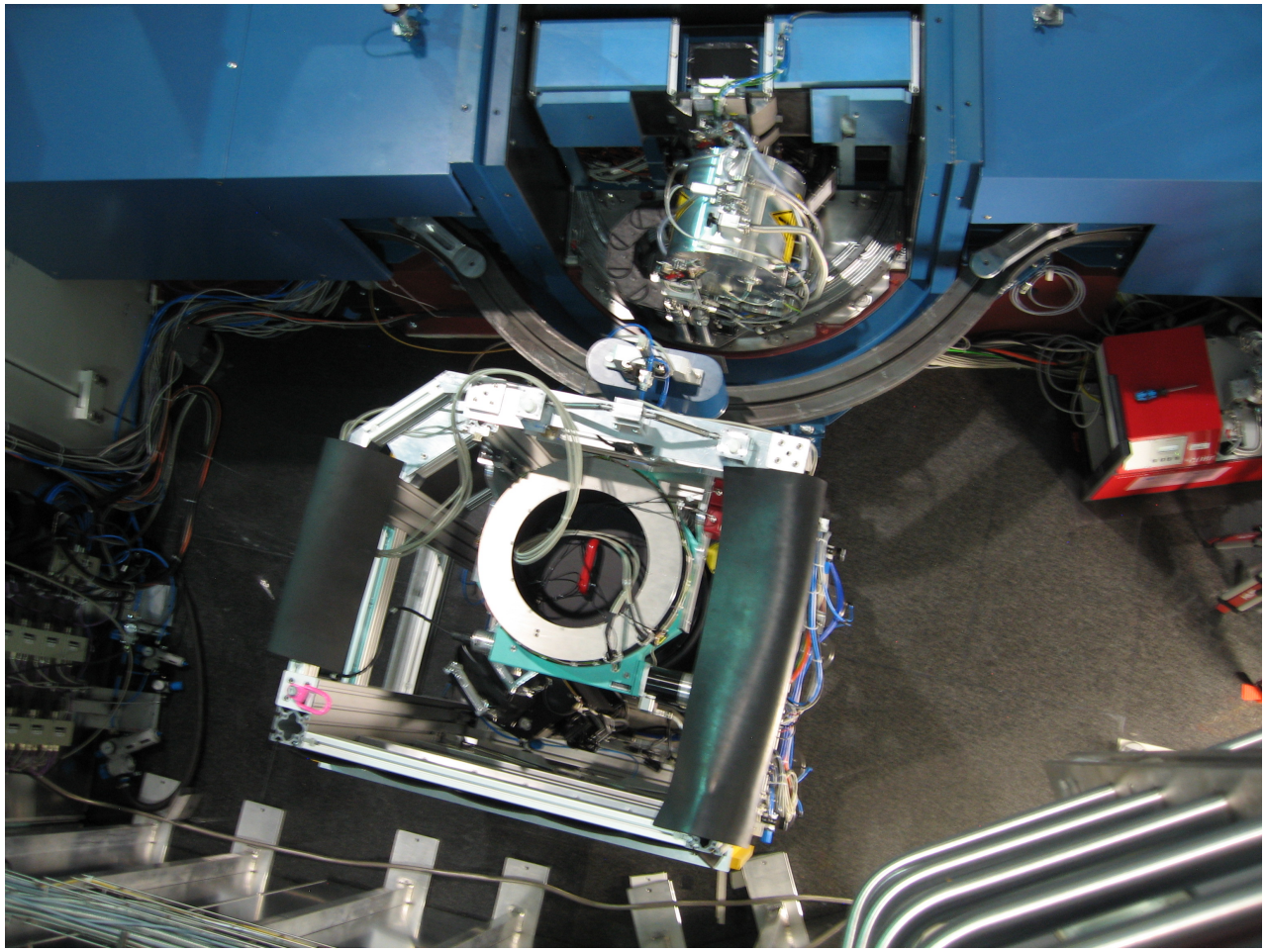
Schematic overview over BioDiff: A neutron protein diffractometer: collaboration between JCNS and FRMII



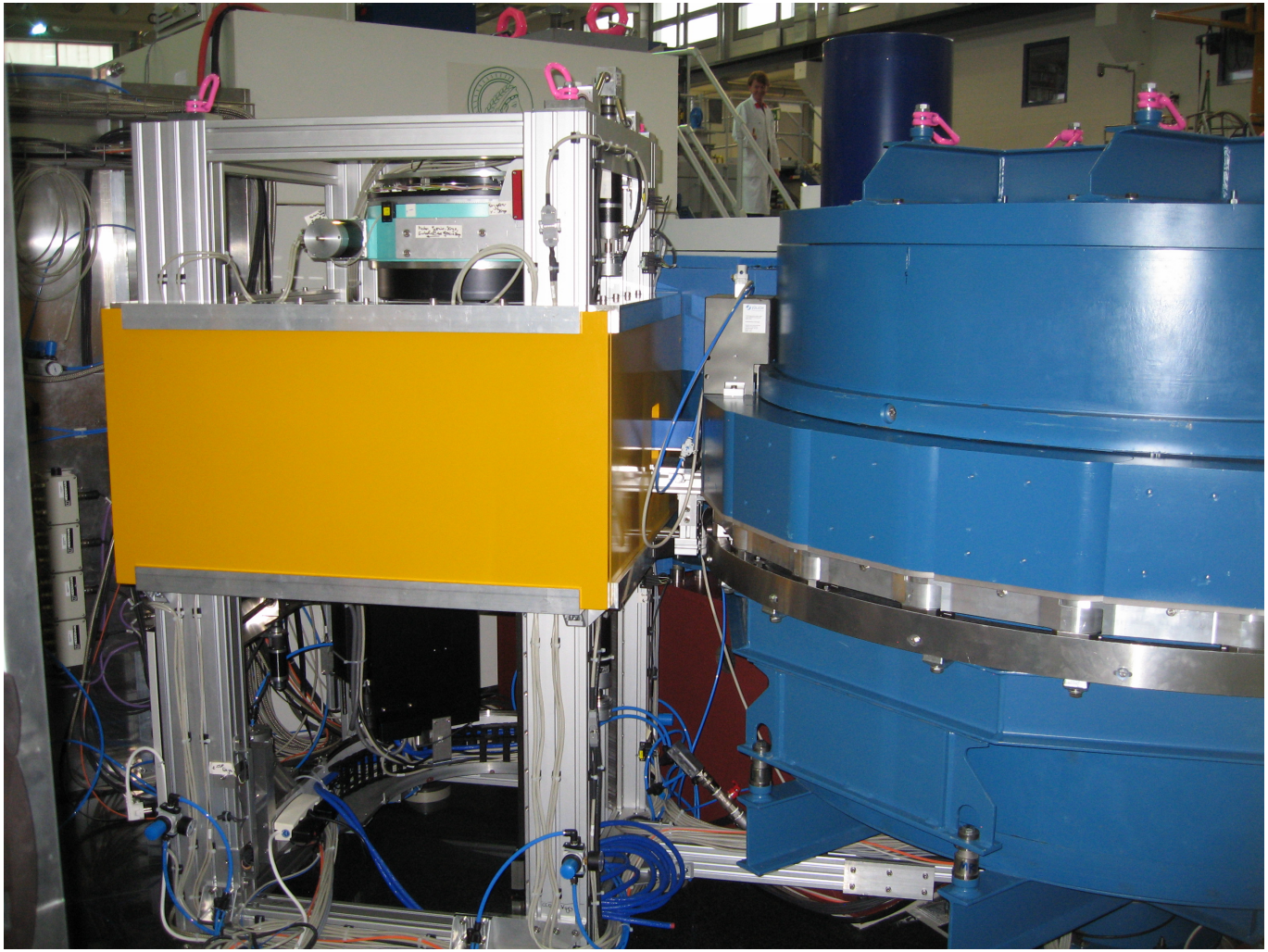
Bragg's law

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

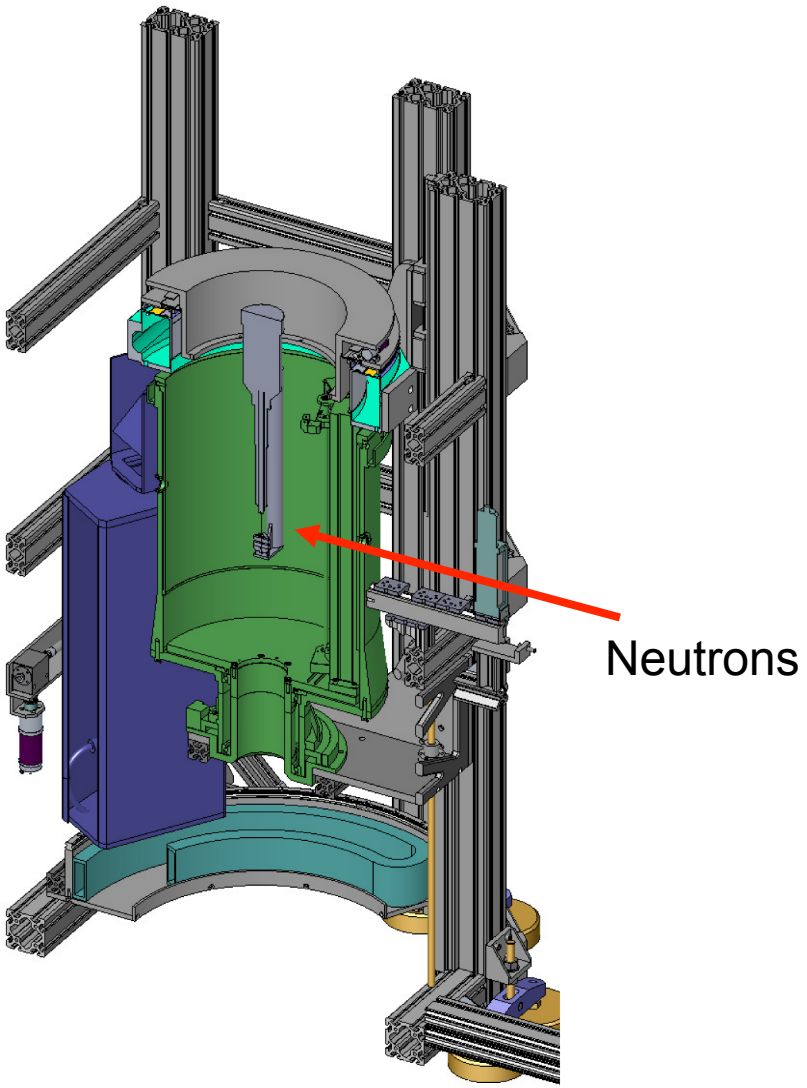
BioDiff, the corresponding view in reality:

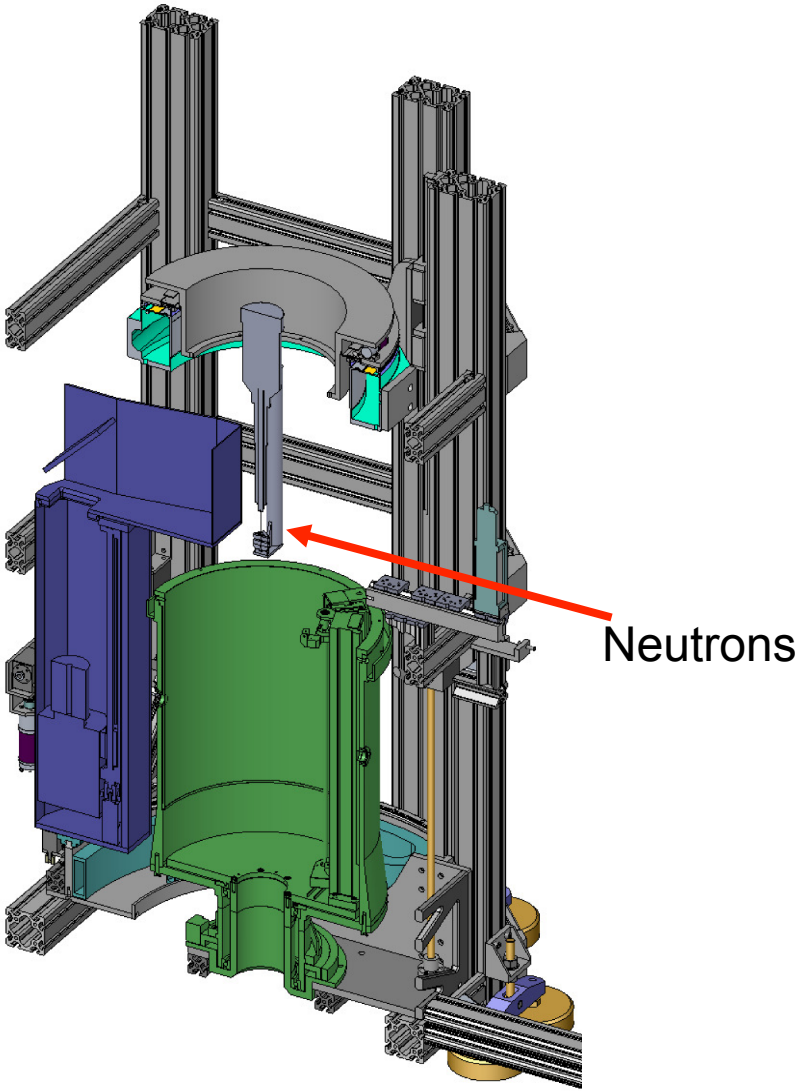


Side view...



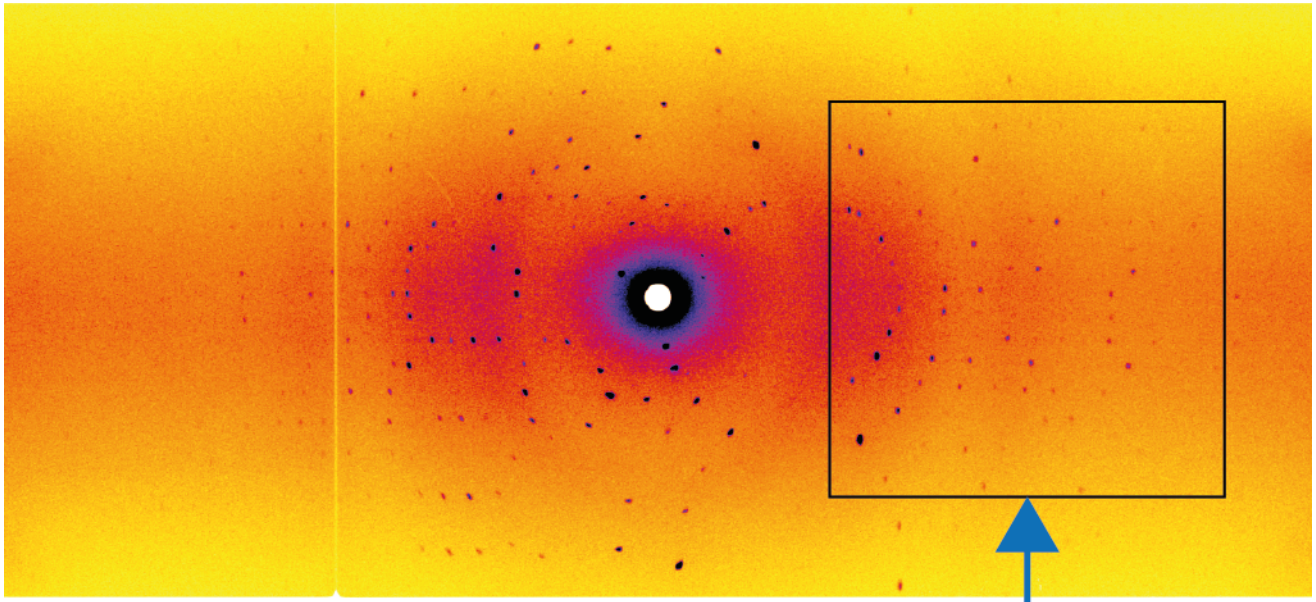
Switching between imageplate and CCD detector





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

Instrument Characterization:
The two detectors

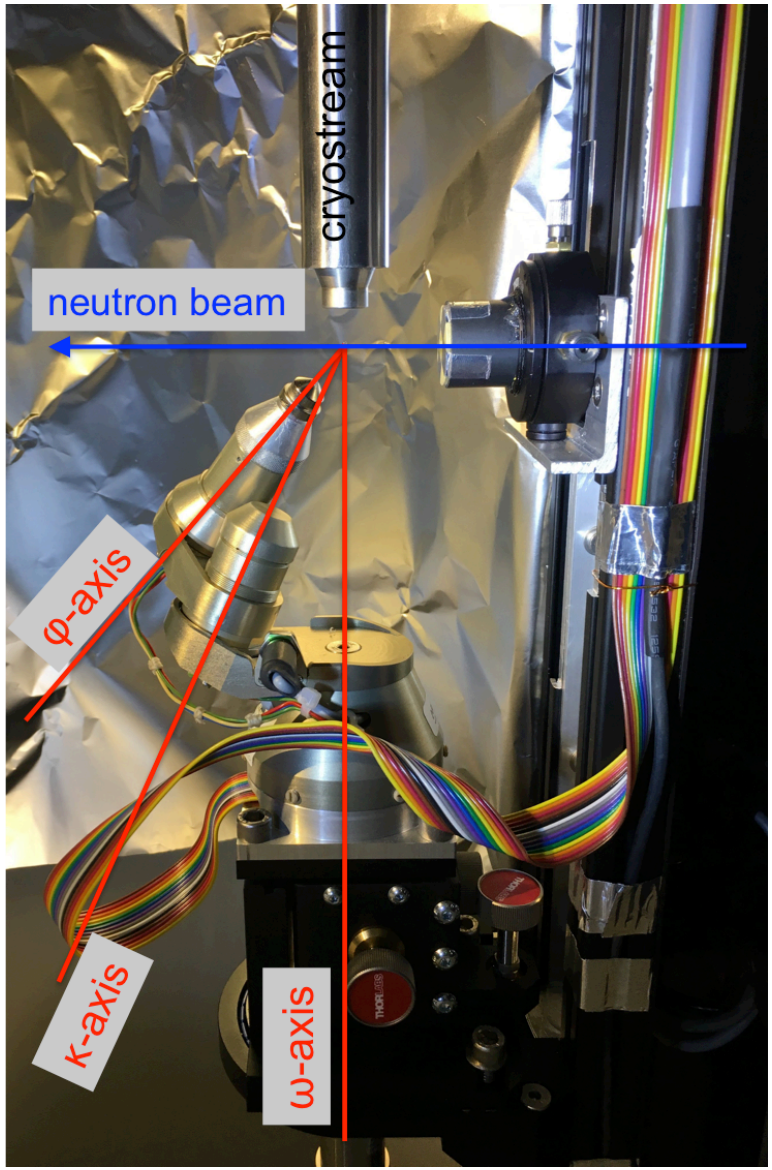


recorded with image plate detector

region of interest shown above recorded with CCD-detector

BioDiff Upgrade:

mini-kappa-goniometer
with standard Oxford
instruments cryostream

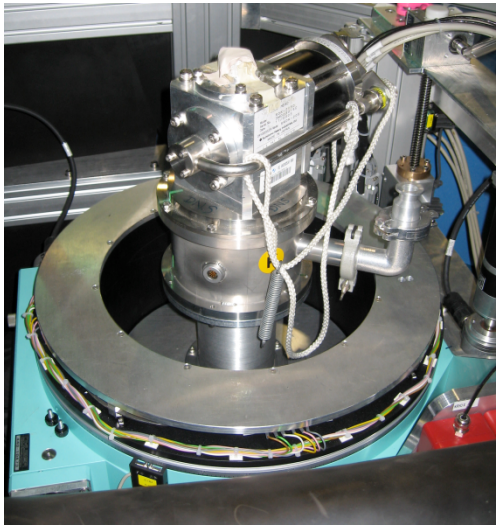


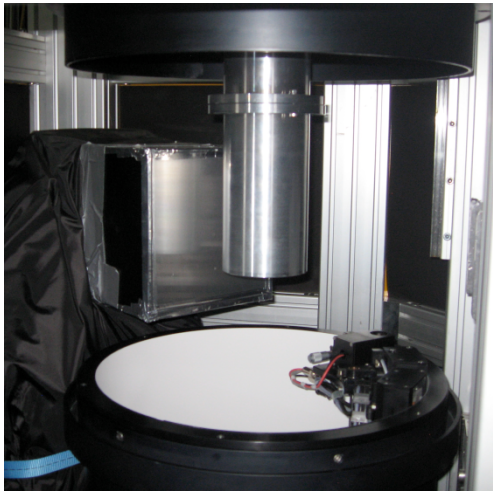
➡ optimizing data collection strategy

↷ save precious beam time /
increase data set complete

➡ no manual crystal re-mounting
necessary for changing the crystal
orientation under cryo conditions

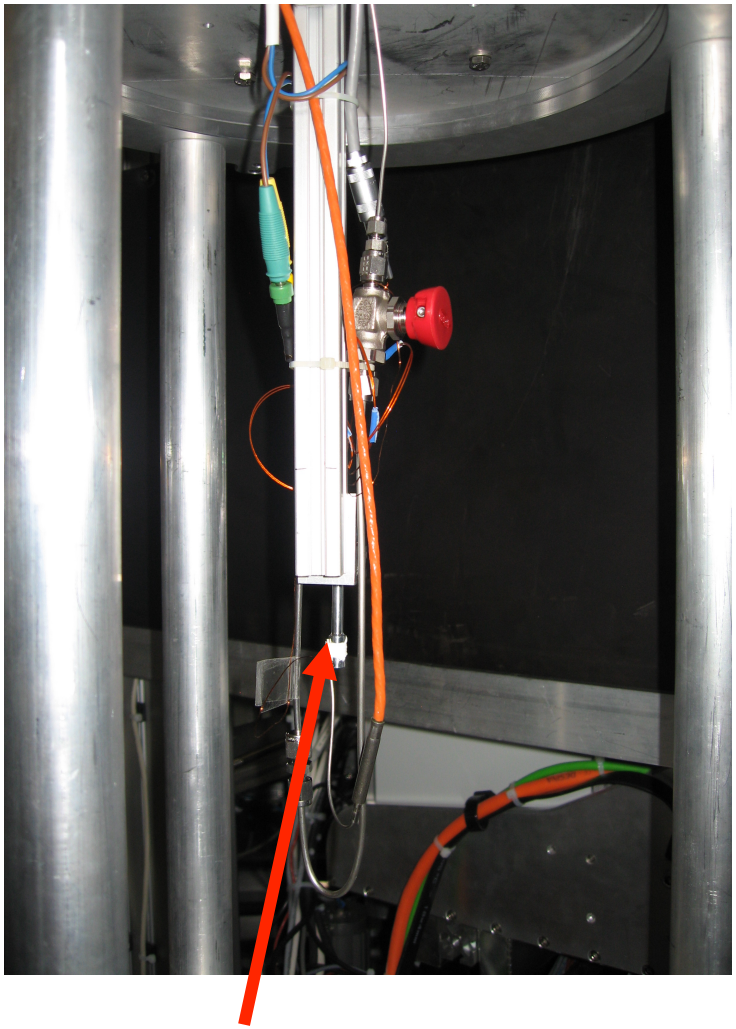
Closed cycle cryostat: T ≥ 4K



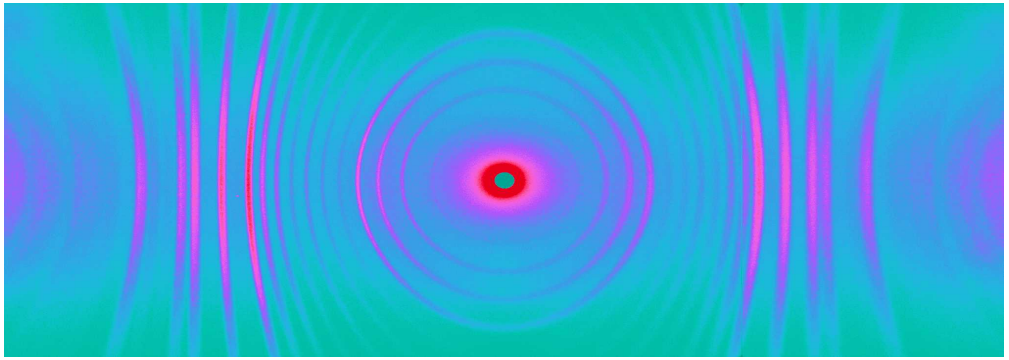


- for small compound crystals (one unit dell dimension larger than 25 Å)
- sample alignment easy with neutron CCD camera

BioDiff as a clay powder diffractometer



high pressure cell



NAG powder sample at 2.7 Å

Summary

- Neutrons can be helpful to find hydrogen atom positions
- There is virtually no radiation damage associated with neutron scattering on proteins. So, metallo-proteins can be investigated without the risk of changing the oxidation state of the metal centre.
- Neutron cryo-crystallography can trap intermediate states in the catalytic process of proteins
- If you have a protein crystal which is large enough (0.5 mm^3) you can apply for beam time at BioDiff

Thanks to our users and the BioDiff-Team:

- Philipp Jüttner
- Andreas Ostermann
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- Andreas Nebel
- Simon Staringer
- Winfried Petry
- Severin Denk
- Dieter Richter

…and you for your attention!

Funding by:

JÜLICH FORSCHUNGSZENTRUM

Technische Universität München

Upcoming deadline for proposals: July 21st, 2017

all users are welcome!

user.frm2.tum.de

fzj.frm2.tum.de

We are open for suggestions on sample environments and support labs for BioDiff!