Neutron protein crystallography at the Heinz Maier-Leibnitz Zentrum (MLZ): New developments and recent application examples

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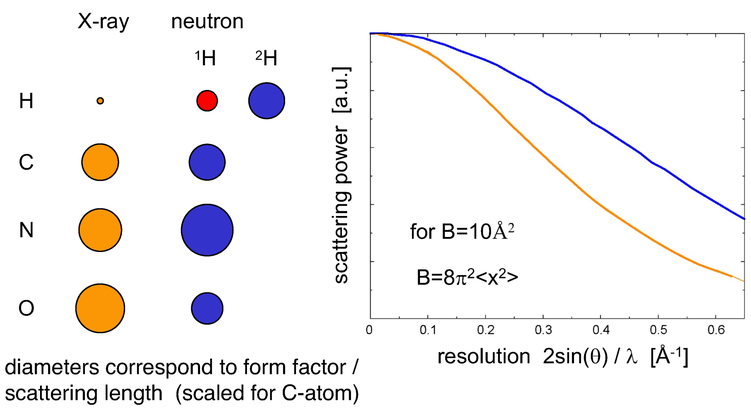
Neutron structure determination:

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

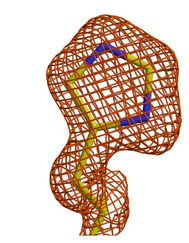
Comparison of form factors (X-ray) and scattering lengths (neutrons):



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

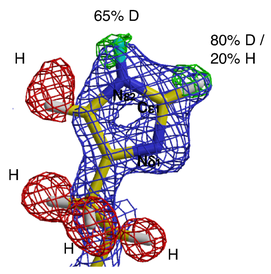
Amino acid protonation states:

X-ray d_{min}= 1.5Å:



2Fo-Fc map; +1.5σ

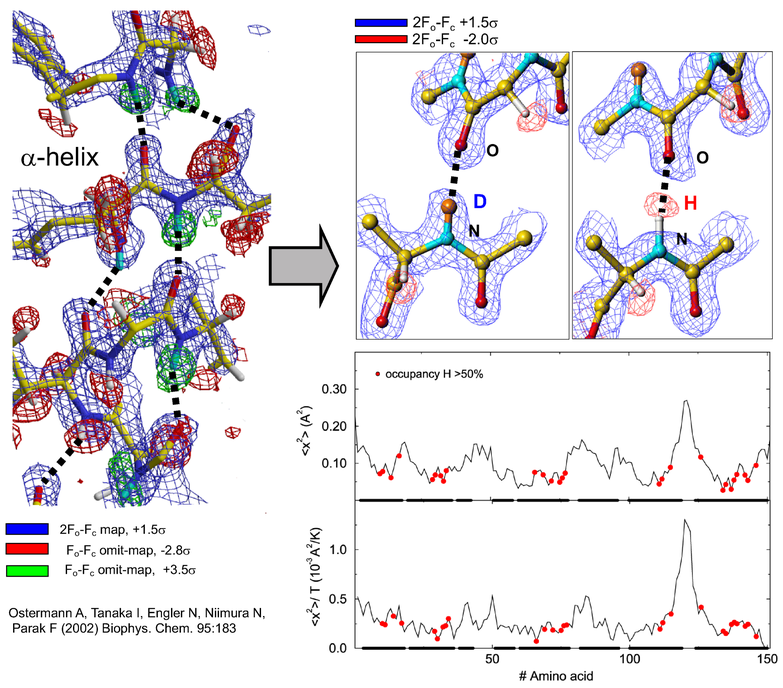
neutrons d_{min}= 1.5Å:



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:

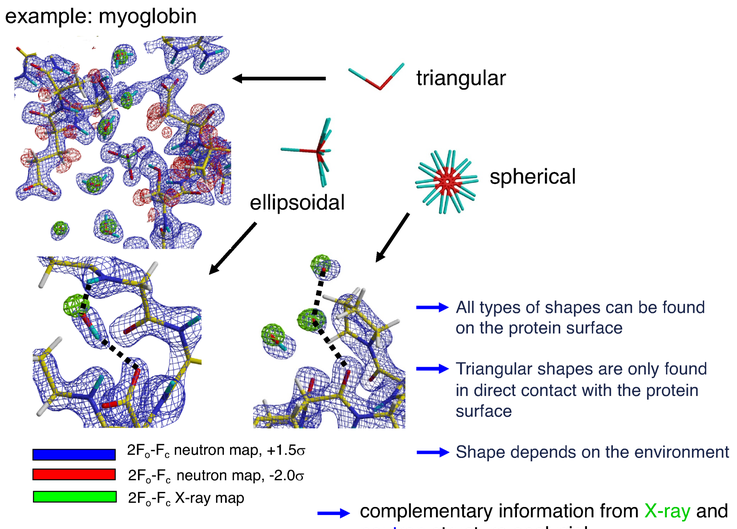


Ostermann A, Tanaka I, Engler N, Niimura N, Parak F (2002) Biophys. Chem. 95:183

- 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:

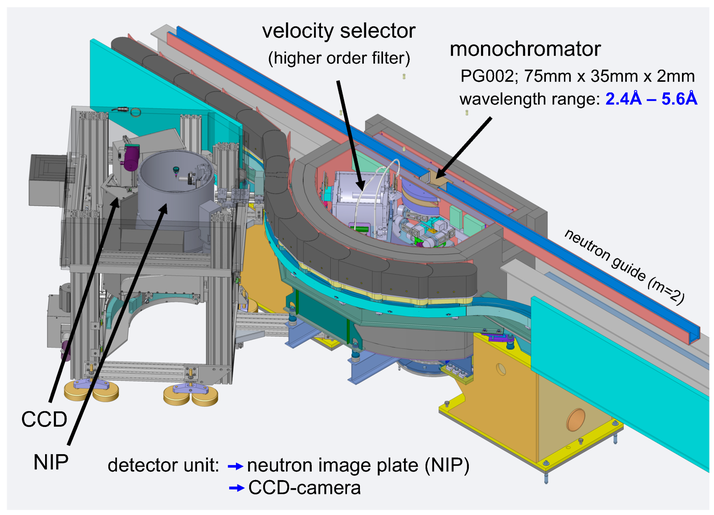
example: myoglobin



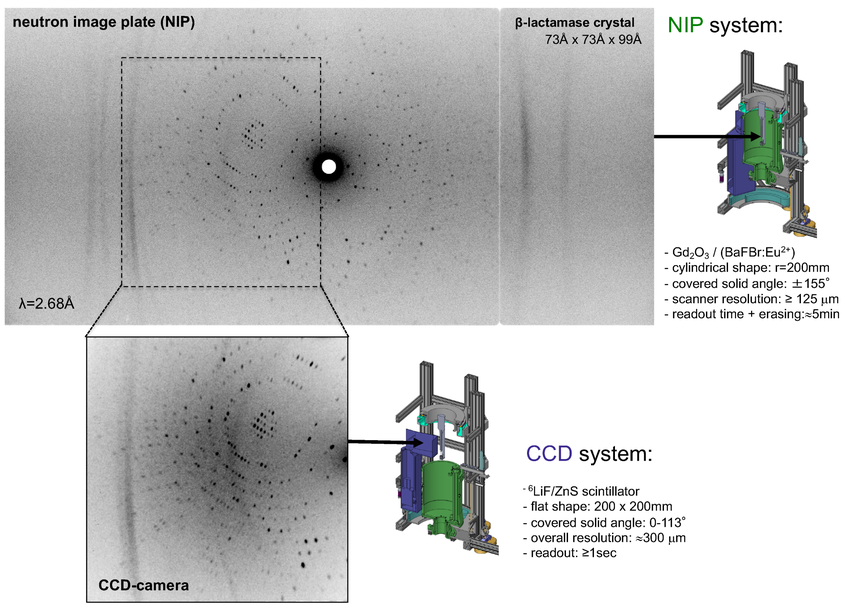
- All types of shapes can be found on the protein surface
- Triangular shapes are only found in direct contact with the protein surface
- Shape depends on the environment
- complementary information from X-ray and neutron structure analysis!

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

The diffractometer BIODIFF:



NIP and CCD detector system:



NIP system:

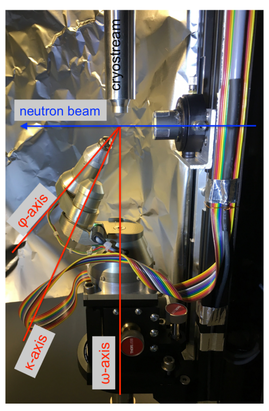
- Gd_2O_3 / ($BaFBr:Eu^{2+}$)
- cylindrical shape: r=200mm
- covered solid angle: ±155°
- scanner resolution: ≥ 125 µm
- readout time + erasing:≈5min

CCD system:

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

Sample environment:

Cryostream & mini-kappa-goniometer

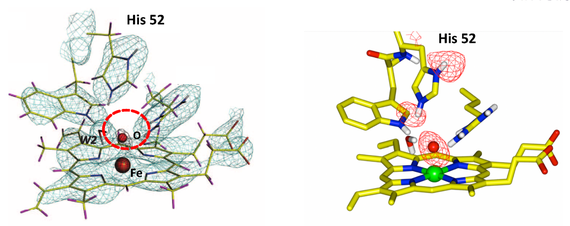


- optimizing datacollection strategy
  - save precious beam time / increase data set completeness
- no manual re-mounting of crystal necessary for changing the orientation under cryo-condition

Example user data-sets:

Compound I of cytochrome c peroxidase @100K

Casadei et al. (2014) Science 345: 193

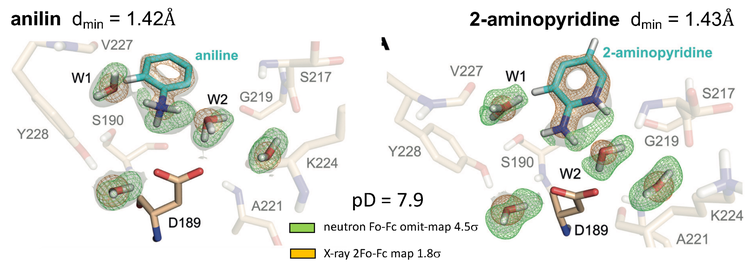


- **The oxygen atom bound to iron (IV) is not protonated!**
- **but His 52 is double protonated!**
- **Reaction mechanism needs to be reconsidered!**

Charges shift protonation: inhibitor binding to trypsin

Schiebel J. et al. (2017) Angewandte Chemie Int. Ed. 56: 4887

- Trypsin as model system for the important family of serine proteases
- **Question**: do inhibitors with less basic properties become protonated upon binding ?

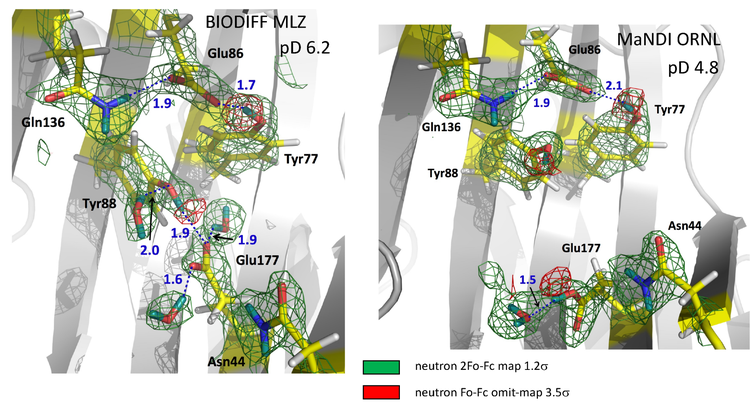


- Despite its low pK_a of 4.6 the amino group of aniline becomes protonated; Asp189 induces a K_a shift of four orders of magnitude
- Whereas in aminopyridine (pK_a of 6.9), the pyridine nitrogen picks up the proton although its amino group is 1.6Å closer to Asp189
- **Therefore, apart from charge–charge distances, tautomer stability is essential for the resulting protonation pattern**
- **Correct prediction of such properties is key in drug development !**

Facilitating processing of biomass

Wan Q. et al., PNAS (2015) 112(40): 12384

- Plant biomass is pre-treated in a very alkaline environment. The goal is to alter the enzymes xylanase to allow it to function effectively in a basic environment.
- This requires detailed knowledge of the reaction sequence of the enzyme!



- The catalytic glutamate residue alternates between two conformations bearing different basicities, first to obtain a proton from the bulk solvent, and then to deliver it to the glycosidic oxygen to initiate the hydrolysis reaction
- **Using this knowledge, work on altering the enzyme in a way that allows efficient biomass decomposition even in high pH environments can begin**

Next proposal deadline: September 13th, 2019

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