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

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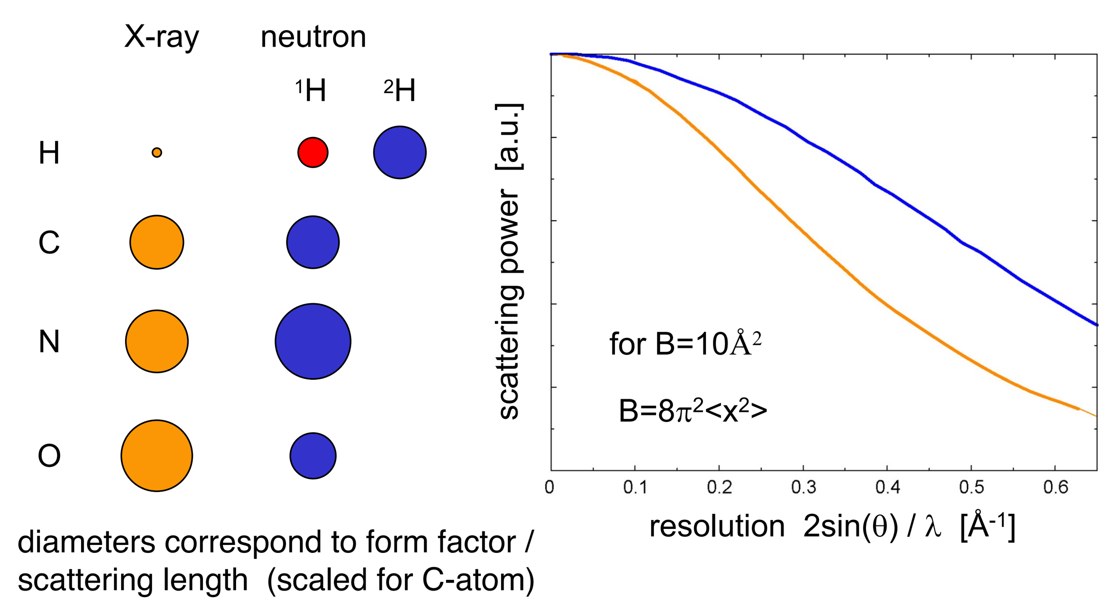
Neutron structure determination:

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

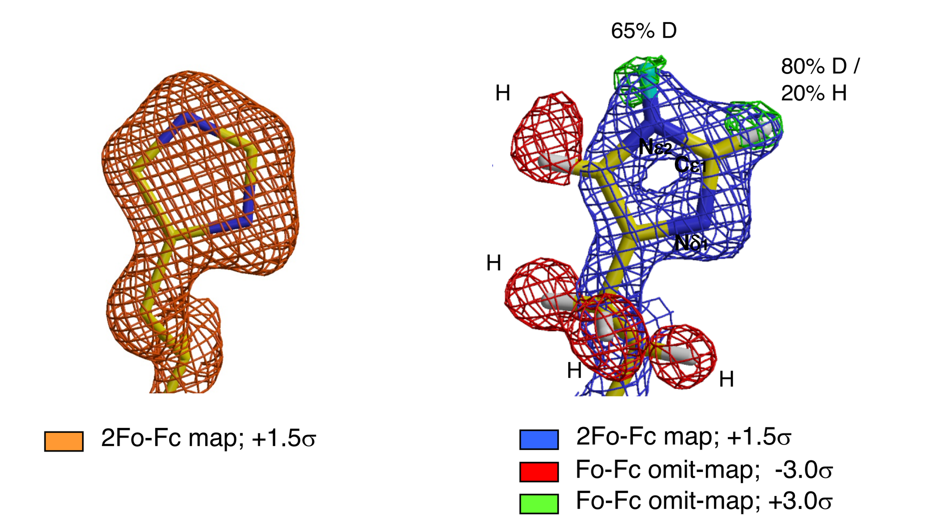
- protonation states of amino acid side chains
- deuterium exchange as a measure of flexibility and accessibility (discrimination between **H** / **D**)
- solvent structure including hydrogen atoms can be analysed
- discrimination between neighbors in the periodic table is possible: e.g. **N** and **O**, **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):



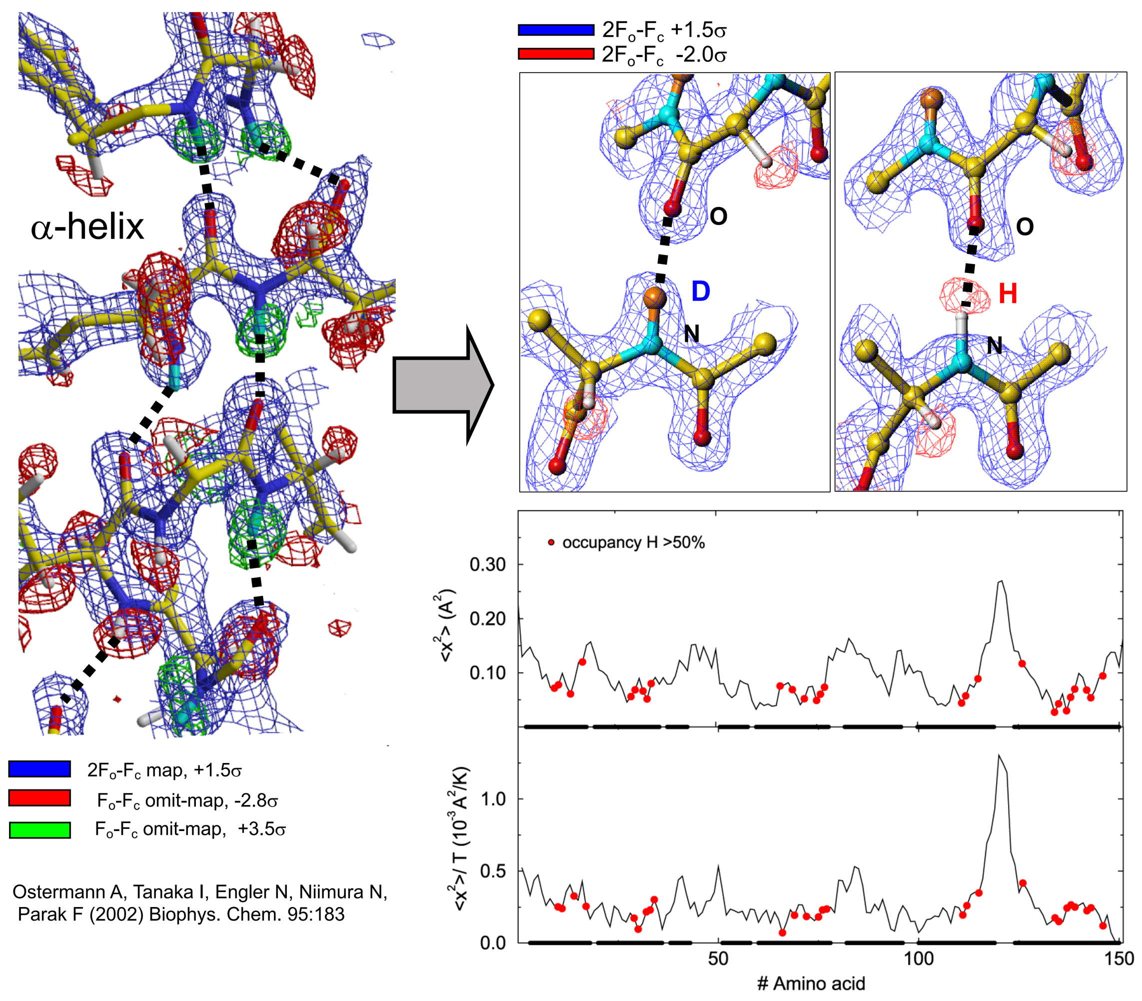
diameters correspond to form factor / scattering length (scaled for C-atom)

Amino acid protonation states:



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

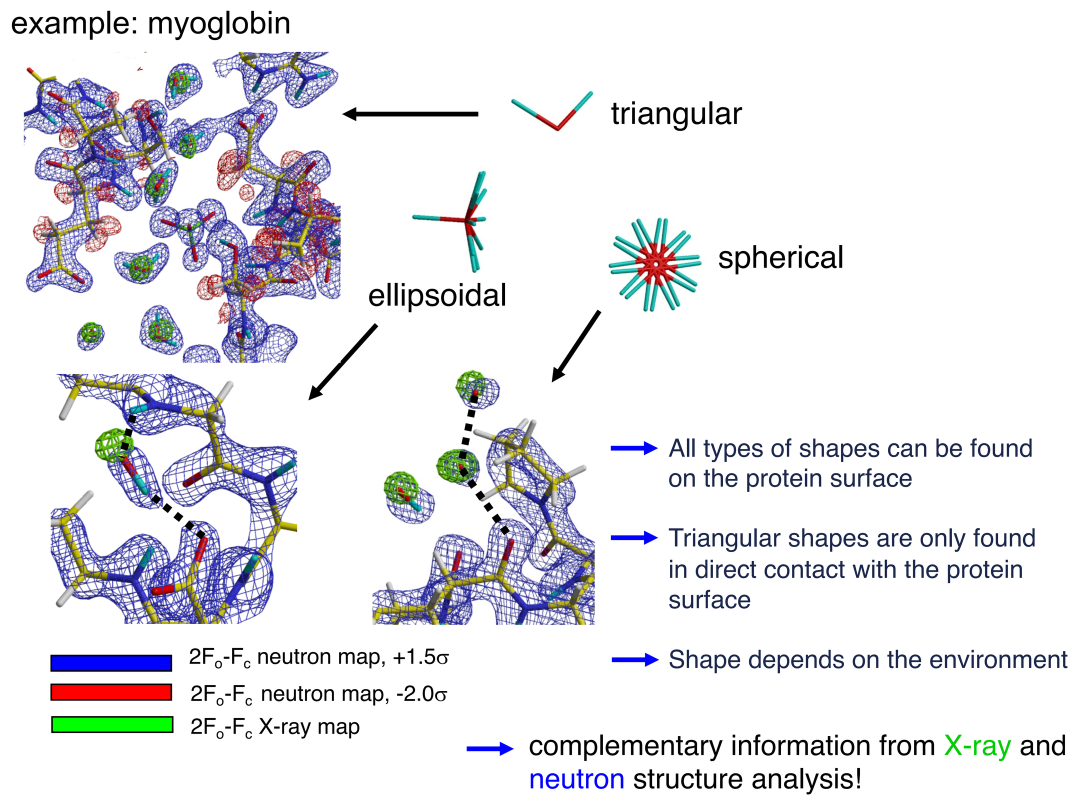
Analysis of H/D-exchange:



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

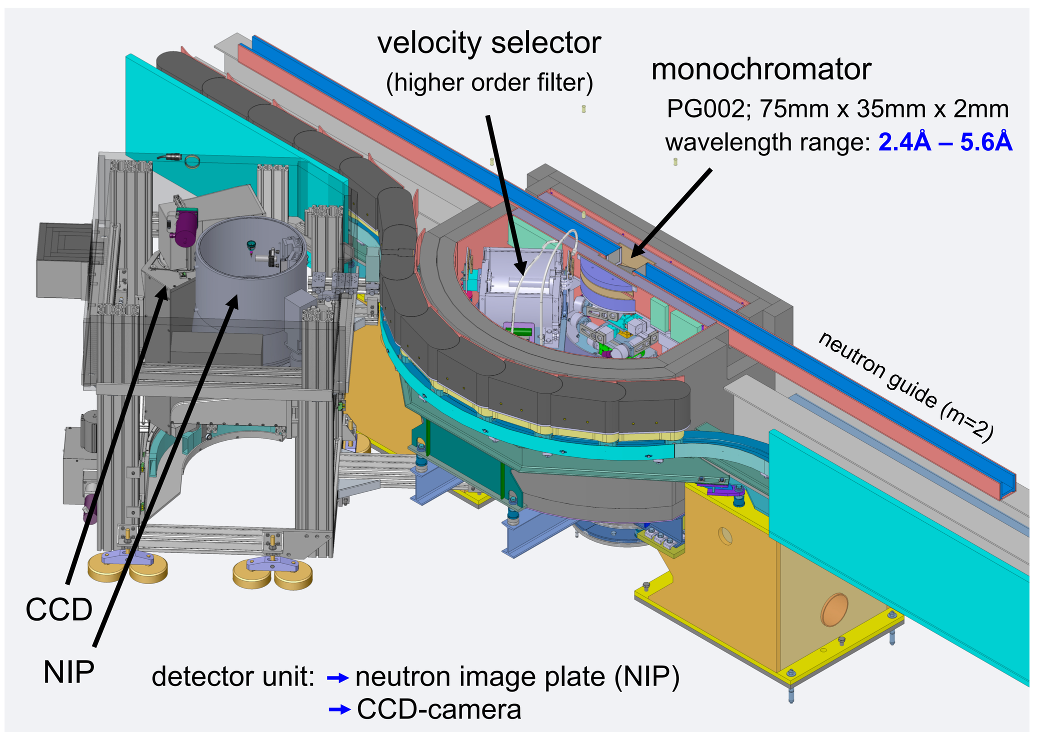
- H / D exchange correlates with the flexibility
- protons show higher protection in the interior of the protein
- tells you where water can migrate and which protons can take part in proton transfer reactions

Hydration structure analysis:

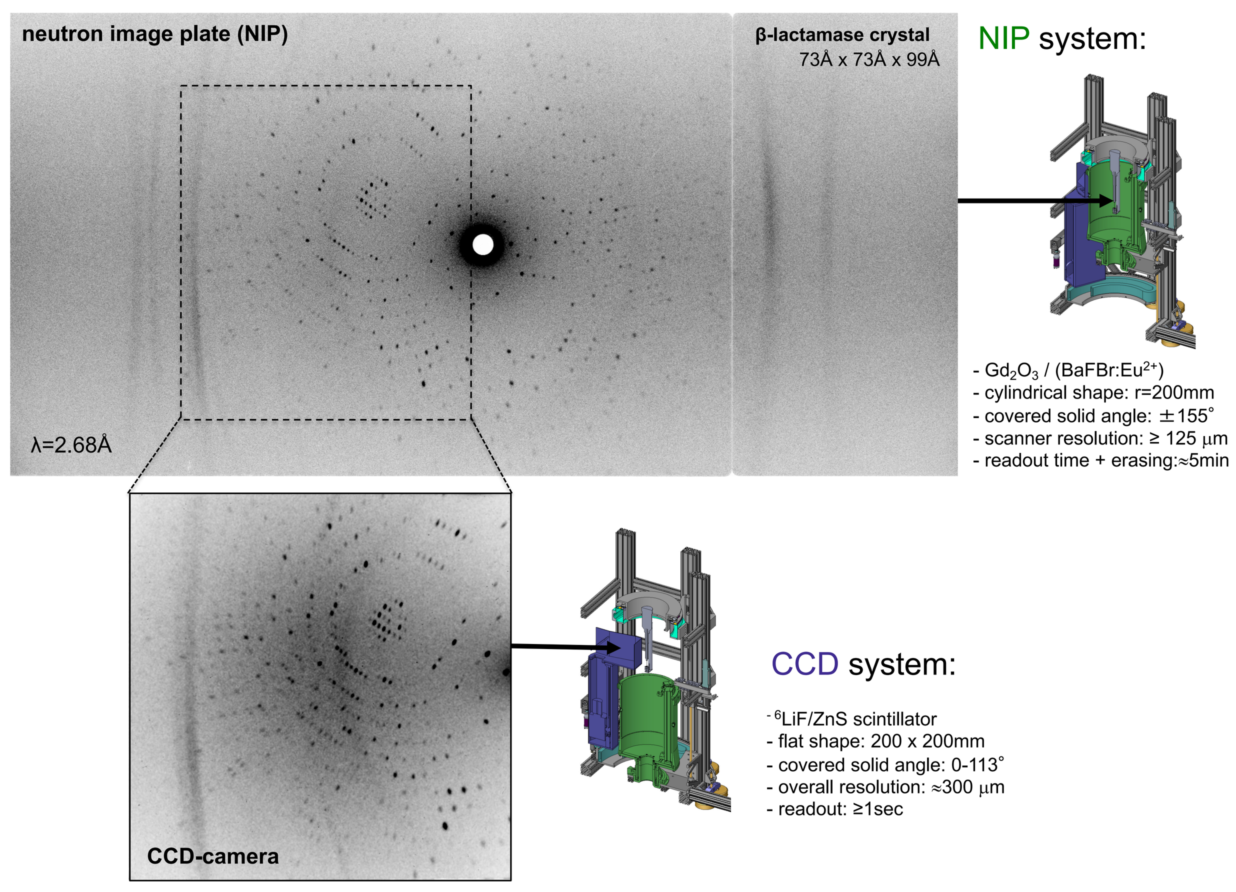


Chatake T, Ostermann A, Kurihara K, Parak F, Niimura N (2003) Proteins 50:516

The diffractometer BIODIFF:

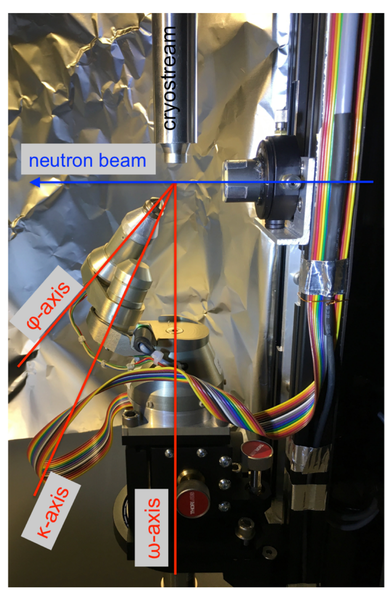


NIP and CCD detector system:



Sample environment:

Cryostream & mini-kappa-goniometer

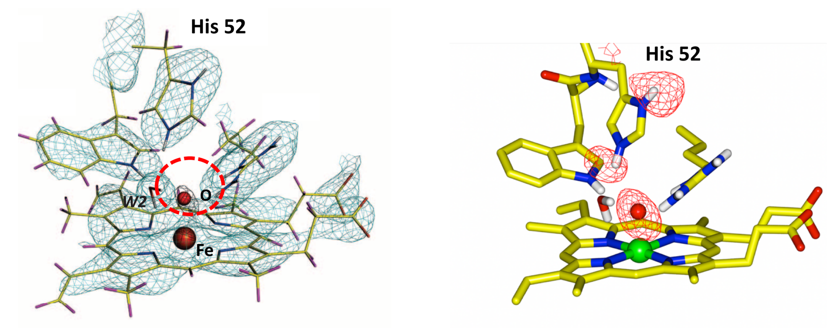


- optimizing datacollection strategy
- save precious beam time / increase data set completeness
- no manual re-mounting of crystal necessary for changing the orientation under cryo-condition

Example user data-sets:

Compound I of cytochrome c peroxidase @100K

Casadei et al. (2014) Science 345: 193

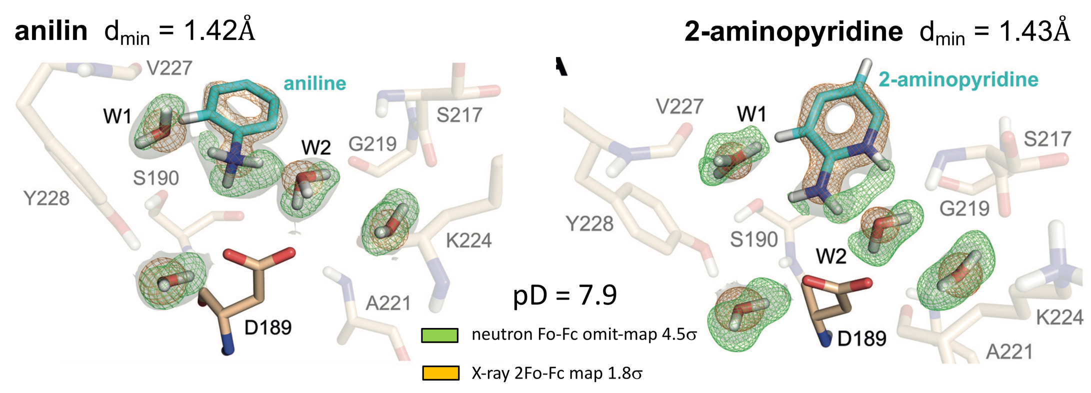


- **The oxygen atom bound to iron (IV) is not protonated!**
- **but His 52 is double protonated!**
- **Reaction mechanism needs to be reconsidered!**

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 ?

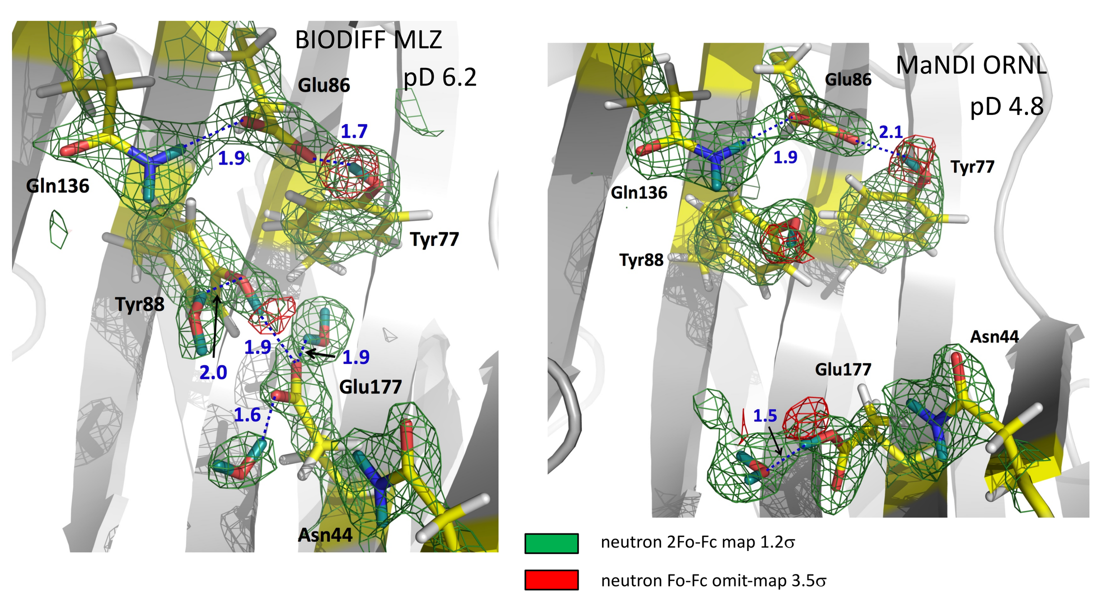


- Despite its low pK_a of 4.6 the amino group of aniline becomes protonated; Asp189 induces a K_a shift of four orders of magnitude
- Whereas in aminopyridine (pK_a of 6.9), the pyridine nitrogen picks up the proton although its amino group is 1.6Å closer to Asp189
- **Therefore, apart from charge–charge 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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