

Macromolecular Neutron Diffraction at the Heinz Maier-Leibnitz Zentrum MLZ

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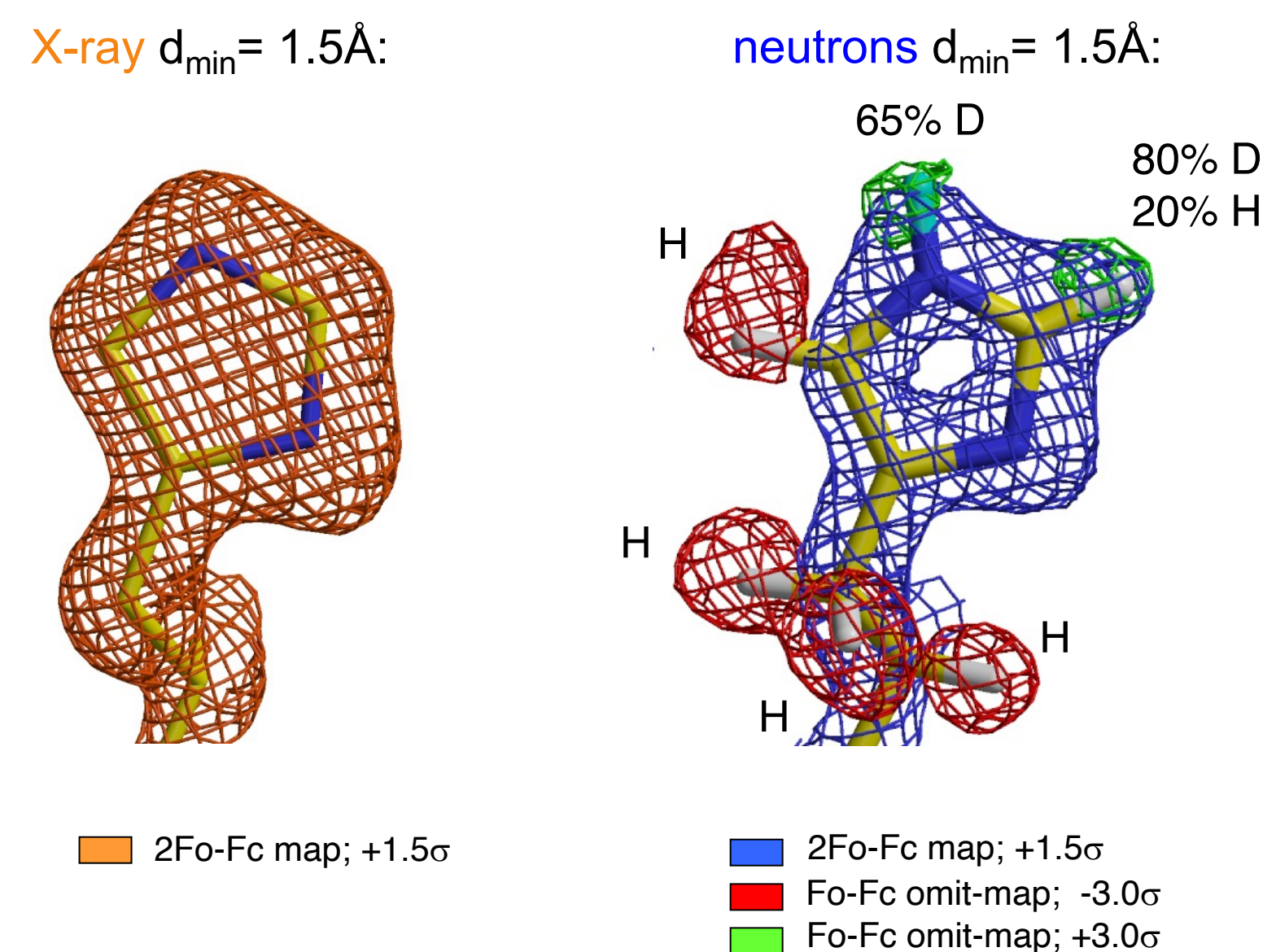
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Neutron structure determination:

H/D atoms can be resolved even at a resolution of $d_{\min} \approx 2.5 \text{ \AA}$

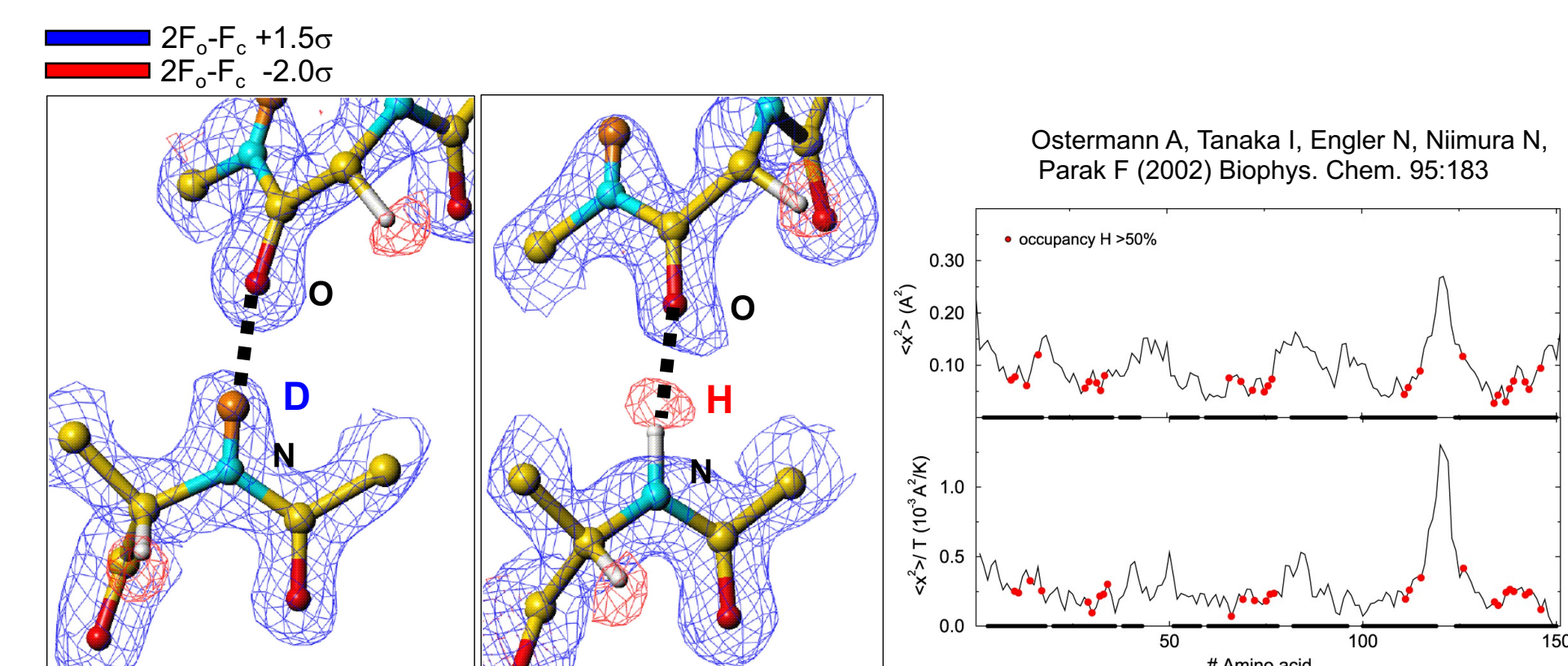
- protonation states of amino acid side chains
- deuterium exchange as a measure of flexibility and accessibility (discrimination between H / D)
- solvent structure including hydrogen atoms can be analysed
- discrimination between neighbors in the periodic table is possible: e.g. N and O, Fe and Mn
- no radiation damage compared to measurements at synchrotrons

Amino acid protonation states:



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

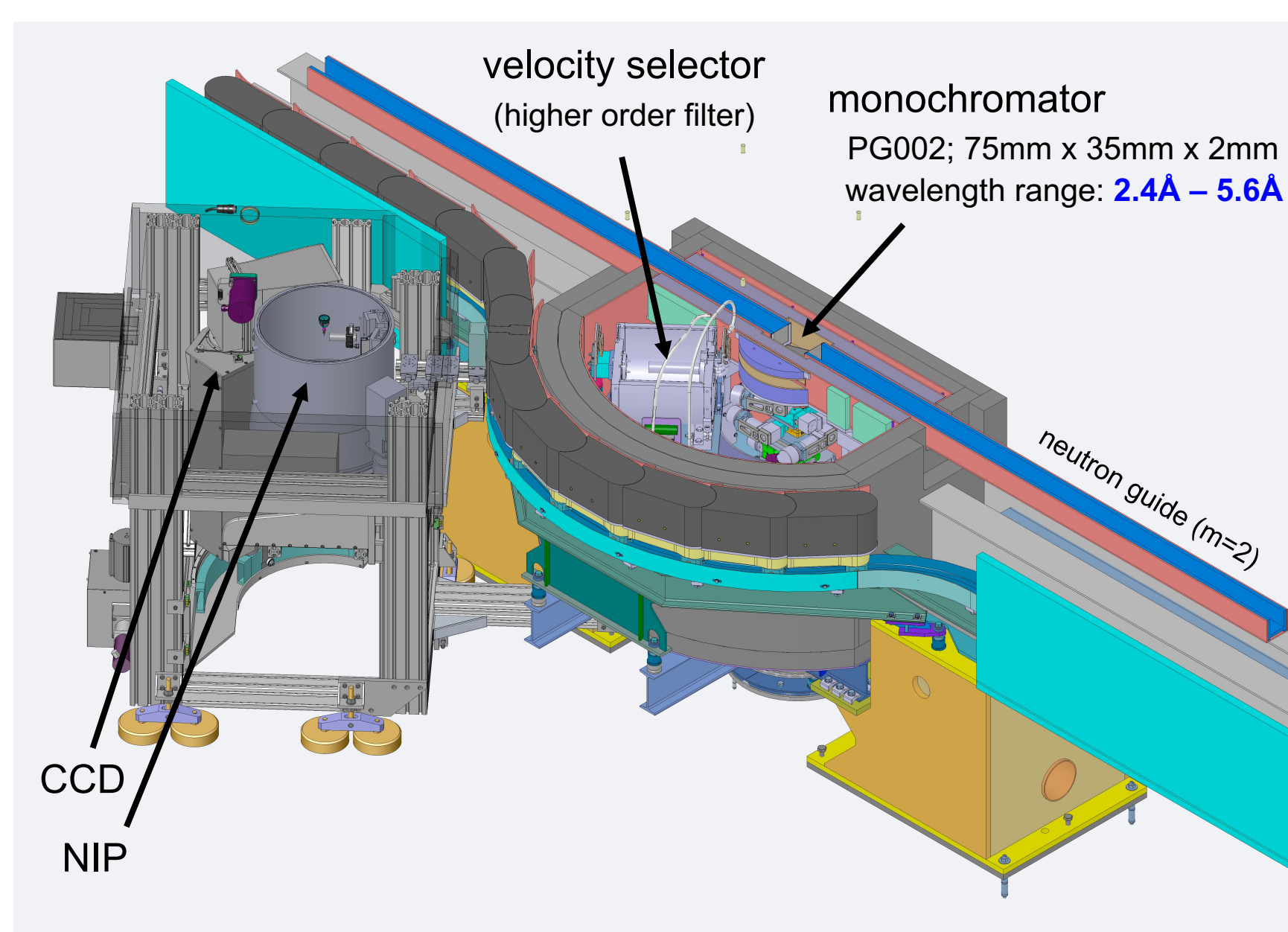
Analysis of H/D-exchange:



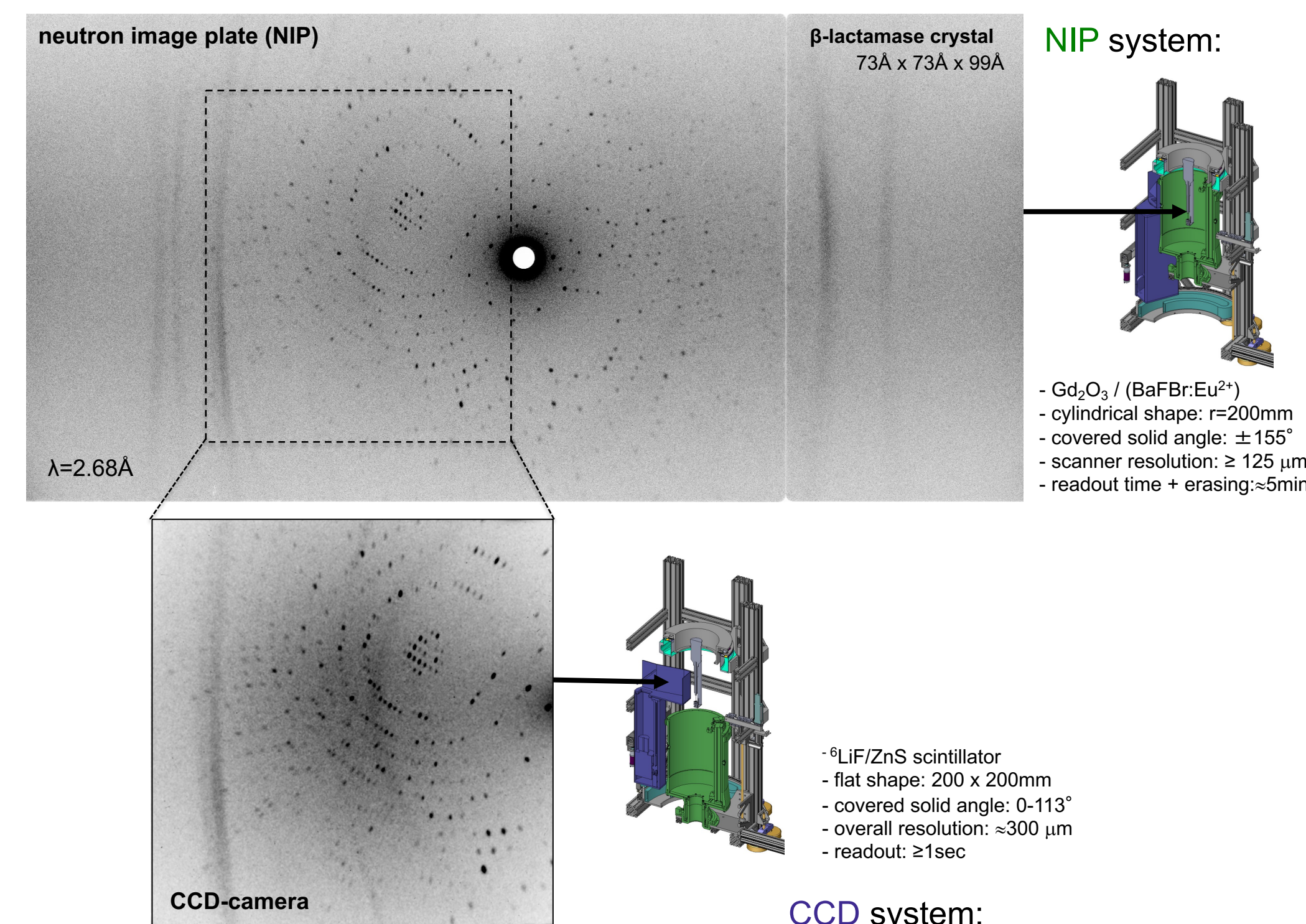
- H / D exchange correlates with the flexibility
- protons show higher protection in the interior of the protein
- tells you where water can migrate and which protons can take part in proton transfer reactions

The diffractometer BIODIFF:

Scheme:

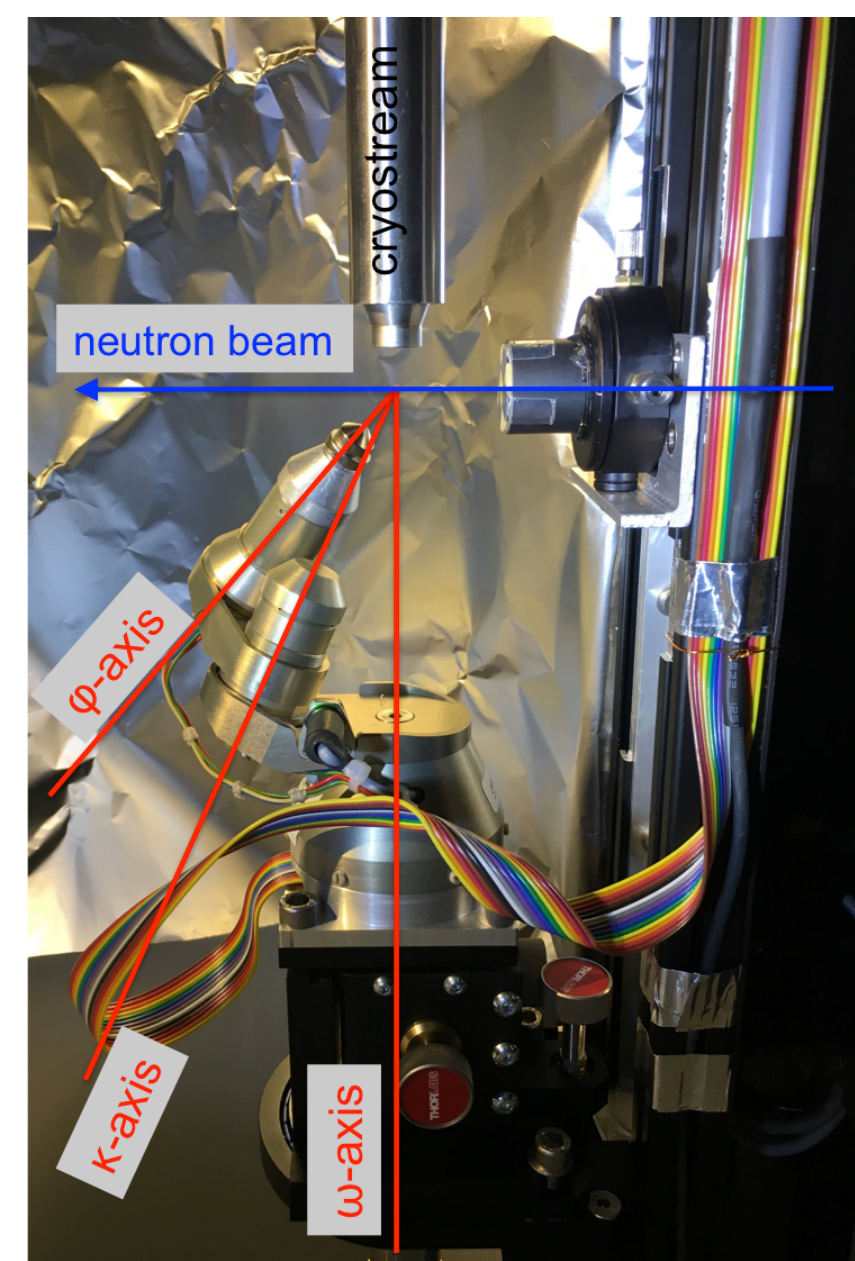


NIP and CCD detector system:



Sample environment:

Cryostream & mini-kappa-goniometer

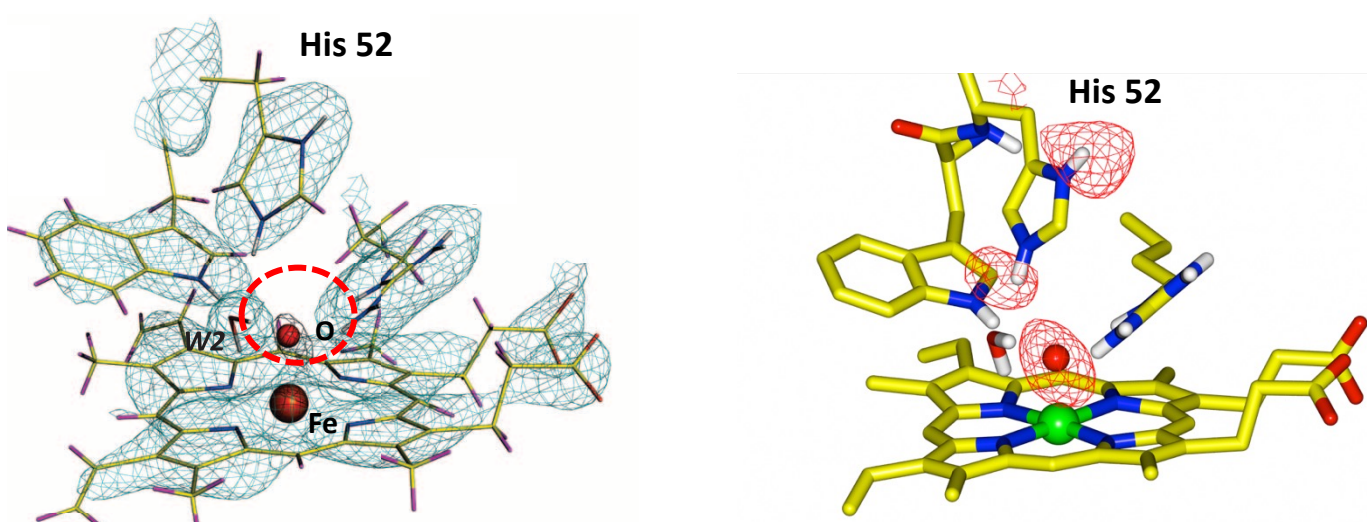


- optimizing datacollection strategy
- save precious beam time / increase data set completeness
- no manual re-mounting of crystal necessary for changing the orientation under cryo-condition

Example user data-sets:

Compound I of cytochrome c peroxidase @100K

Casadei et al. (2014) Science 345: 193



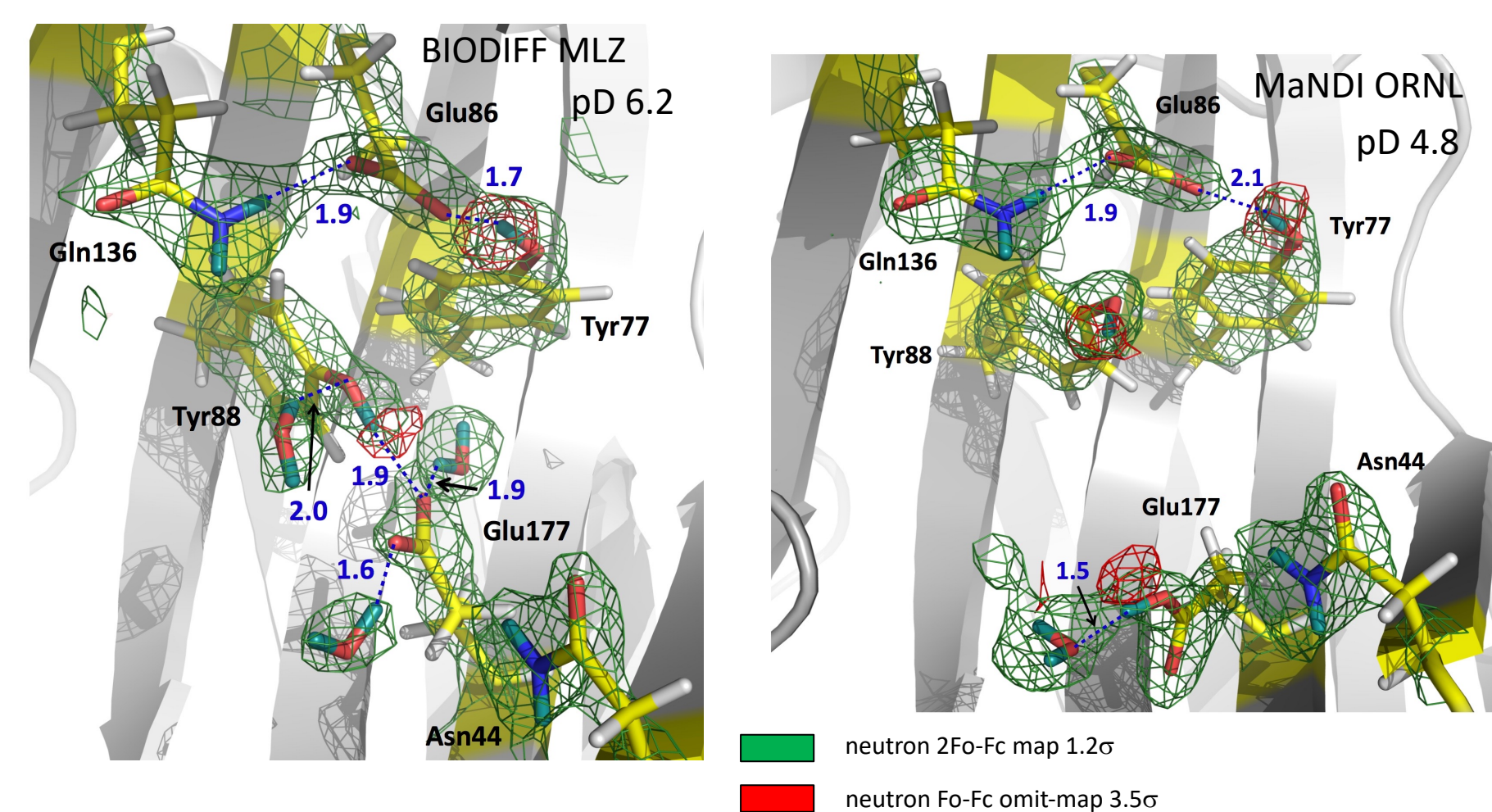
- The oxygen atom bound to iron (IV) is **not** protonated!
- but His 52 is double protonated!
- Reaction mechanism needs to be reconsidered!

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!



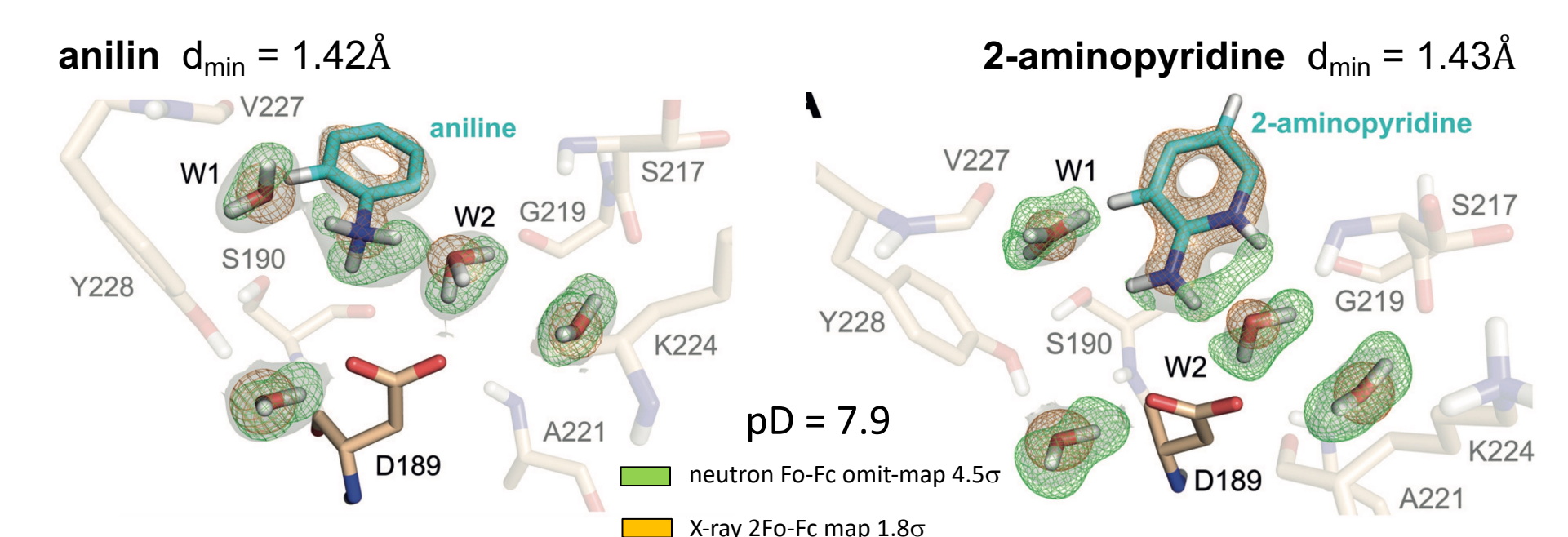
- The catalytic glutamate residue alternates between two conformations bearing different basicities, first to obtain a proton from the bulk solvent, and then to deliver it to the glycosidic oxygen to initiate the hydrolysis reaction
- Using this knowledge, work on altering the enzyme in a way that allows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

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!

Next proposal deadline: to be announced
 user.frm2.tum.de
 fzj.frm2.tum.de

