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 \( \Delta_{\text{H}} = 2.5 \AA \)

- deuteration states of amino acid side chains
- deuteration 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 (\( \sigma^2 \)) 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):

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<th>X-ray</th>
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Amino acid protonation states:

- X-ray: \( \Delta_{\text{H/D}} = 1.5 \AA \)
- Neutron: \( \Delta_{\text{H/D}} = 1\) Å

The diffractometer BIODIFF:

- Neutron detector unit: neutron image plate (NIP) + CCD
- X-ray detector unit: neutron image plate (NIP) + CCD

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

- X-ray: H, C, N, O
- Neutron: H, O

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 protein transfer reactions

Sample environment:
- Cryostream & mini-kappa-goniometer
  - optimising data collection strategy
  - saving precious beam time / increasing 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 @10K
  - The oxygen atom bound to iron (90) is not protonated!
  - but His 52 is double protonated!
  - Reaction mechanism needs to be reconsidered!

Charges shift protonation: inhibitor binding to trypsin

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

Facilitating processing of biomass

- Plant biomass is pre-treated in a very alkaline environment. The goal is to alter the enzymes xylose isomerase to allow it to function effectively in a basic environment.
- This requires detailed knowledge of the reaction sequence of the enzyme.
- Using this knowledge, work on altering the enzyme in a way that allows efficient biomass decomposition even in high pH environments can begin

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Next proposal deadline: September 13th, 2019
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