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 \( d_{\text{H}} = 2.5\AA \)
- Protonation states of amino acid side chains
- Deuteration exchange as a measure of flexibility and accessibility (discrimination between H/D)
- So-called structure including hydrogen atoms can be analysed
- Discrimination between neighbors in the periodic table is possible: e.g., N, Fe, and Mn
- B-factors \((\sigma^2=\sigma^2(\text{H})^2)\) 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):

<table>
<thead>
<tr>
<th>Element</th>
<th>X-ray</th>
<th>Neutron</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>0.74</td>
<td>0.75</td>
</tr>
<tr>
<td>C</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>N</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>O</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Amino acid protonation states:

- X-ray: \( d_{\text{H}} = 1.5\AA \)
- Neutron: \( d_{\text{H}} = 1.5\AA \)

Hydration structure analysis:

- Example: myoglobin
- All species of shapes can be found on the protein surface
- Protonated shapes are only found in close contact with the protein surface

The diffractometer BIODIFF:

- Neutron image plate (NIP) - CCD-camera
- Velocity selector (higher order filter)
- Monochromator

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?

- Despite its low pKa, 4.6 the amino group of aniline becomes protonated: Asp188 induces a \( \Delta pK_a \) shift of four orders of magnitude
- Whereas in anilinopyridine (pKa 6.9), the pyridine nitrogen picks up the proton although its amino group is 1.6 closer to Asp188
- Therefore, apart from charge-charge distances, tautomer stability is essential for the resulting protonation pattern

Facilitating processing of biomass

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

- 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 hydrolase 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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