Macromolecular Neutron Diffraction at the Heinz Maier-Leibnitz Zentrum MLZ

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Neutron structure determination:

H/D atoms can be resolved even at a resolution of dmin=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
- no radiation damage compared to measurements at synchrotrons

Amino acid protonation states:

X-ray dmin=1.5 Å:
- 80% E, 16% D, 4% H

neutrons dmin=1.5 Å:
- 80% D, 20% H

Fig. 2. The structure of compound I of CcP in the region of the heme.

Analysis of H/D-exchange:

- H/D exchange correlates with the flexibility of protons
- protons show higher protection in the interior of the protein
- pH-dependent spectroscopy
- hydrogen bonds to water molecules
- direct evidence for hydrogen bond formation

The diffractometer BIODIFF:

Scheme:

NIP and CCD detector system:

Sample environment:

Cryostream & mini-kappa-goniometer
- optimizing data collection 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

Facilitating processing of biomass

Charges shift protonation: inhibitor binding to trypsin

Scheidler 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?

NIP system:
- cryo-scintillator
- read out: ≥1 sec
- overall resolution: ≥300 µ
- cylindrical shape: r=200 mm
- Hilbert filter: ±155 µmin
- monochromator P0252: 75 mm x 35 mm x 2 mm
- wavelength range: 2.4 Å – 6.4 Å

MLZ 5.6 Å, h=24, d=19 Å, λ=2.68 Å, centre, N/P

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 glycosyl 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: to be announced

user.frm2.tum.de

Funding: to be announced

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