



Neutron macromolecular crystallography at the FRM II The neutron single crystal diffractometer BIODIFF or: What can neutrons do for you?

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Neutron structure determination:

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

Hydration structure analysis:

example: myoglobin First "user data-sets":

β-lactamase with bound BZB inhibitor

- 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 ($<x^2>$) of the hydrogen atoms can \rightarrow 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):



Amino acid protonation states:



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

The diffractometer BIODIFF:



S.J. Tomanicek, R.F. Standaert, K.L. Weiss, J.D. Ng, **L. Coates** (Group of P. Langan)



Human farnesyl pyrophosphate synthase with risedronate

T. Yokoyama, M. Mizuguchi, N. Niimura, I. Tanaka

K200 Risedronate						
2.2	d _{min}	l/σ(l)	N _{meas}	mult.	compl. in shell %	R _{merge} %
T201	140.0 – 5.17	29.1	12784	6.6	94.9	4.3
	5.17 – 4.10	14.5	9490	5.0	99.3	9.8
	4.10 - 3.59	10.6	8045	4.3	99.5	12.3
Q240 1.9 w510	3.59 – 3.26	7.0	5833	3.2	98.5	15.5
	3.26 - 3.02	6.1	6443	3.5	99.3	19.5
2,4 2,0	3.02 – 2.85	4.5	6181	3.4	98.6	24.9
2,2 D244	2.85 – 2.70	3.3	5772	3.2	98.6	31.2
w508	2.70 – 2.59	2.5	5442	3.0	98.5	39.8
w506	2.59 – 2.49	2.1	5260	2.9	99.0	46.2
w507	2.49 - 2.40	1.8	4846	2.7	98.0	61.2
F_oF_c -omit-maps: positve in green, negative in red;	overall	8.2	69977	3.8	98.4	10.7
- unit cell: 111.9Å, 111.9Å, 72.6Å P4 ₁ 2 ₁ 2 - crystal size: 3.5mm ³ - collection time: 25d (5d, 6d, 14d)					R _{pim} = 5.8	% (37.1%)



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

Analysis of H/D-exchange:



NIP and CCD detector system:





CCD system:
⁻⁶ LiF/ZnS scintillator
- flat shape: 200 x 200m
- covered solid angle: 0-

covered solid angle: 0-113 - overall resolution: ≈300 µm readout: ≥1sec

Compound I of cytochrome c peroxidase @100K

Casadei et al. (2014) Science **345**: 193



- → The oxygen atom bound to iron (IV) is <u>not</u> protonated!
- - Reaction mechanism needs to be reconsidered!

Examples of user experiments:

protein	unit cell (Å) space group	cell volume (Å ³)	crystal size (mm ³)	time (d)	d _{min} (Å)	compl. (%)	R _{merge} (%)
β-lactamase (no ligand) L. Coates et al.	73.3, 73.3, 98.7 P3 ₂ 21 λ=2.7Å	453,000	4.0	8	2.0	89.0 (82.7)	9.8 (22.3)
β-lactamase-BZB-inhibitor L. Coates et al.	73.4, 73.4, 99.1 P3 ₂ 21 λ=2.7Å	453,000	2,7	9	2.0	90.2 (81.2)	14.7 (27.9)
Inorganic pyrophosphatase J. Ng et al.	101.0 101.0 100.5 R32 λ=3.4Å, 4.0Å	887,700	1	24	2.0	97.9 (90.5)	13.6 (52.6)
Xylanase II A. Kovalevsky et al.	49.5 59.9 70.4 P2 ₁ 2 ₁ 2 ₁ λ=2.7Å	208,000	2.8	17	2.0	96.2 (91.0)	9.7 (32.7)
KDN9P phosphatase Z. Fischer et al.	83.1 108.9 75.8 P2 ₁ 2 ₁ 2 λ=2.7Å	685,000	1.0	18	2.5	94.8 (88.7)	11.7 (40.0)
apo human carbonic anhydrase II Z. Fischer et al.	42.8 41.7 72.8 P2 ₁ λ=2.7Å	125,000	2,5	8	1.8	89.9 (76.8)	11.9 (33.0)
Nucleosidase (MTAN) A. Kovalevsky et al.	83.0 83.0 67.4 P3 ₂ 21 λ=2.7Å	392,000	2.8	25	2.7	97.1 (94.9)	9.8 (47.8)
Cytochrome c peroxidase P. Moody, M. Blakeley, C. Casadei et al.	51.2 75.8 107.6 P2 ₁ 2 ₁ 2 ₁ λ=3.4Å, 4.0Å	417,000	0.65	23	2.5	90.7 (71.8)	17.3 (42.8)
Farnesyl pyrophosphate synthase T. Yokoyama et al.	111.9 111.9 72.6 P4 ₁ 2 ₁ 2 λ=4.0Å	909,000	3.5	25 (11)	2.4	98.4 (98.0)	10.7 (61.2)
DNA drug complex S. Arai, R. Kuroki et al.	27.9 27.9 52.0 P4 ₁ 2 ₁ 2 λ=2.7Å	40,500	3.0	3	1.7	92.7 (83.3)	10.8 (21.5)



- 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

Sample environment:

Standard Oxford cryostream 700+



→ temperature range: 90 - 500K → stable, no icing over weeks



• 4 proposals "BIODIFF as low resolution powder machine": - CO2 uptake in clay as F(pressure) - Stratum corneum lipid model membranes;;

6 proposals small compound structures (large magnetic superstructures or diffuse scattering);

Next proposal deadline: Sep 11 th , 2015 !!	
user.frm2.tum.de	Here Made-Lebotz Zertum
fzj.frm2.tum.de	

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