

BioDiff - a neutron diffractometer optimized for crystals with large unit cell dimensions

or: What can neutrons do for you?

T.E. Schrader^a, A. Ostermann^b, M. Monkenbusch^c, B. Laatsch^d, Ph. Jüttner^b, W. Petry^b, D. Richter^{a,c}

^aJülich Centre for Neutron Science (JCNS) at Heinz Maier-Leibnitz Zentrum (MLZ), Forschungszentrum Jülich GmbH, Lichtenbergstr.1, 85747 Garching, Germany

^bHeinz Maier-Leibnitz Zentrum (MLZ), Technische Universität München, Lichtenbergstr. 1, 85748 Garching, Germany

^cForschungszentrum Jülich GmbH, Institute for Complex Systems, D-52425 Jülich,

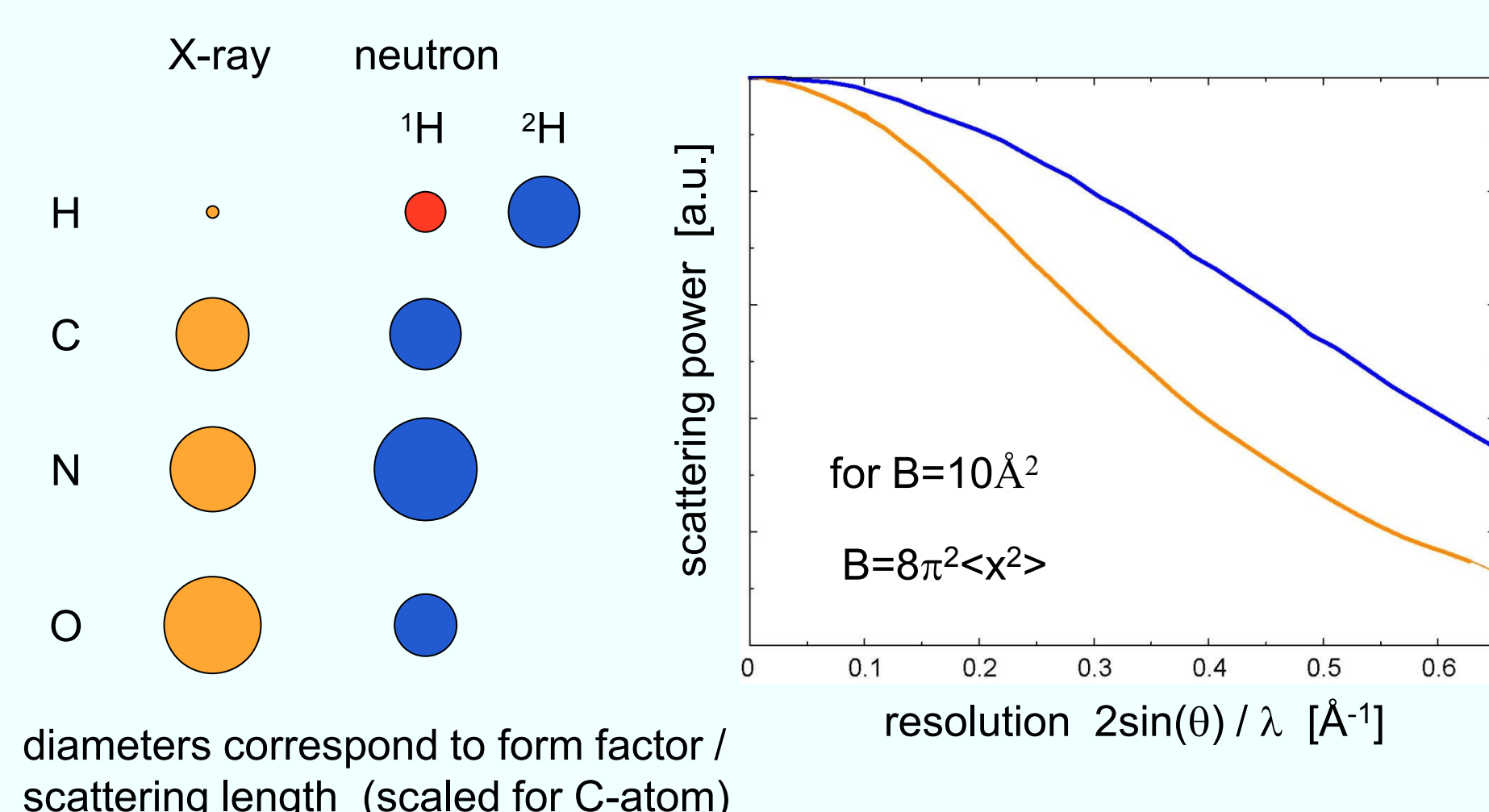
^dForschungszentrum Jülich GmbH, Engineering and Technology (ZEA-1), D-52425 Jülich

Neutron structure determination:

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

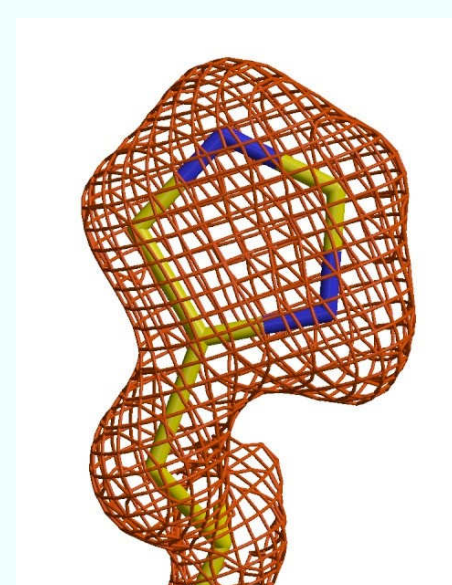
- protonation states of amino acid side chains
- deuterium exchange as a measure of flexibility and accessibility (discrimination between H / D)
- solvent structure including hydrogen atoms can be analysed
- discrimination between neighbors in the periodic table is possible: e.g. N and O, Fe and Mn
- B-factors ($\langle x^2 \rangle$) 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):

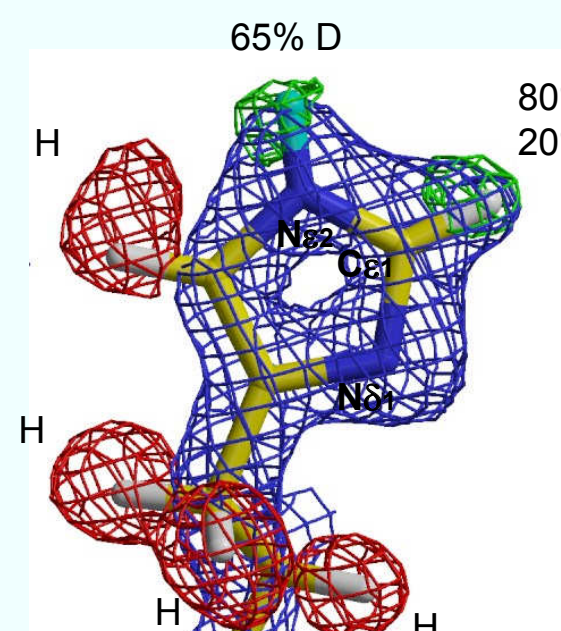


Amino acid protonation states:

X-ray $d_{\min} = 1.5 \text{ \AA}$:



neutrons $d_{\min} = 1.5 \text{ \AA}$:



2Fo-Fc map; +1.5σ

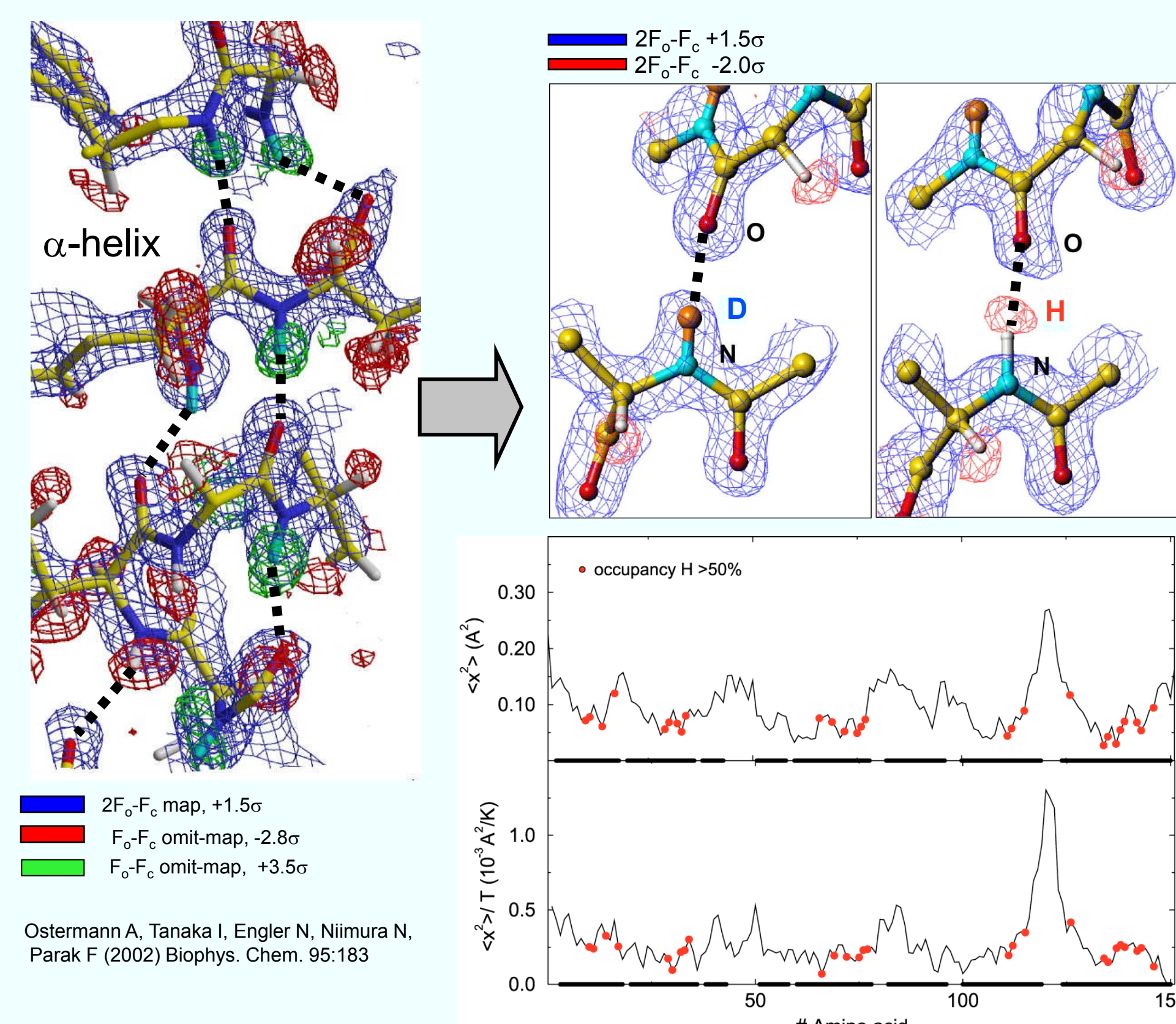
2Fo-Fc map; +1.5σ

Fo-Fc omit-map; -3.0σ

Fo-Fc omit-map; +3.0σ

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

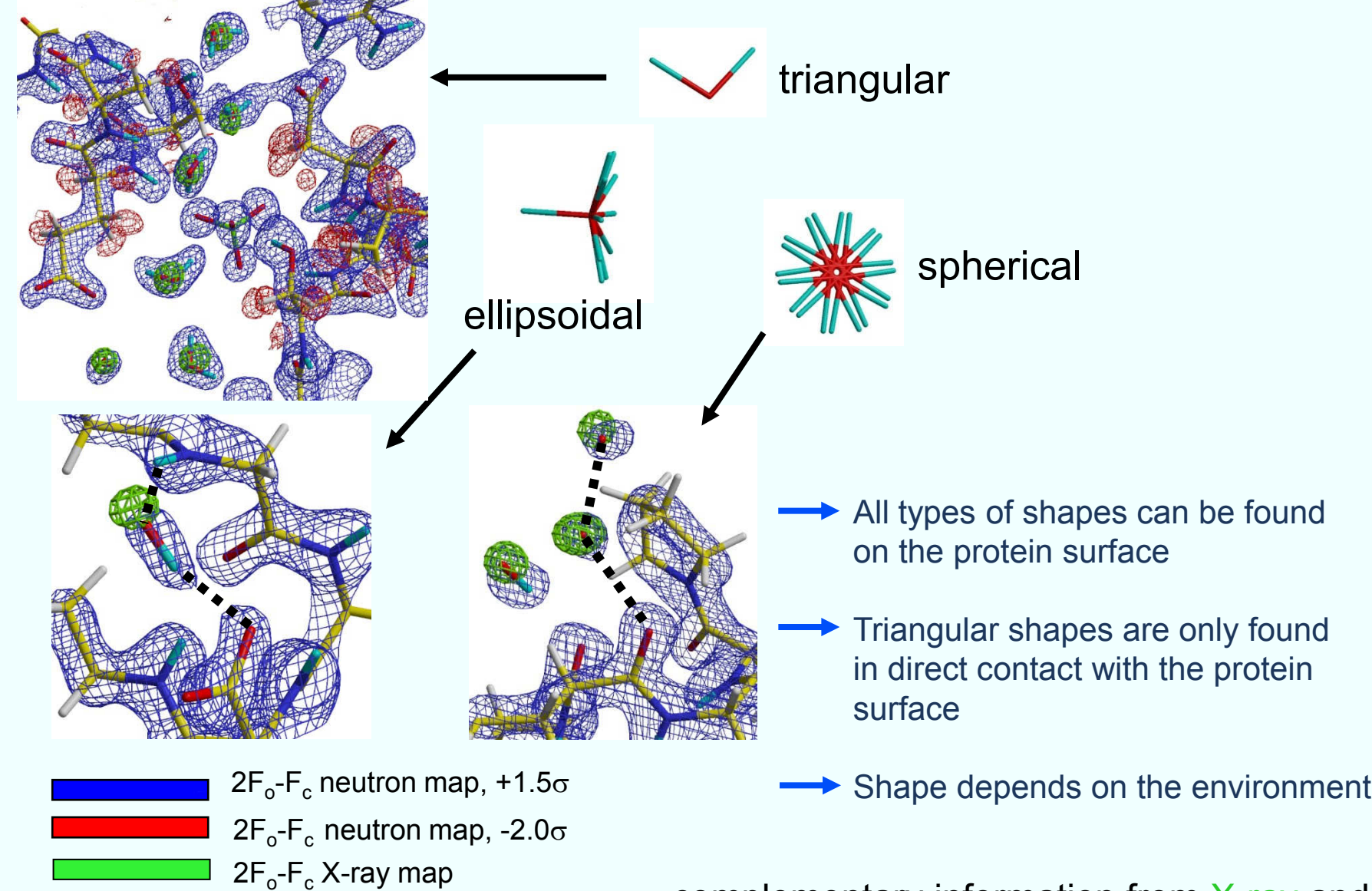
Analysis of H/D-exchange:



- 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

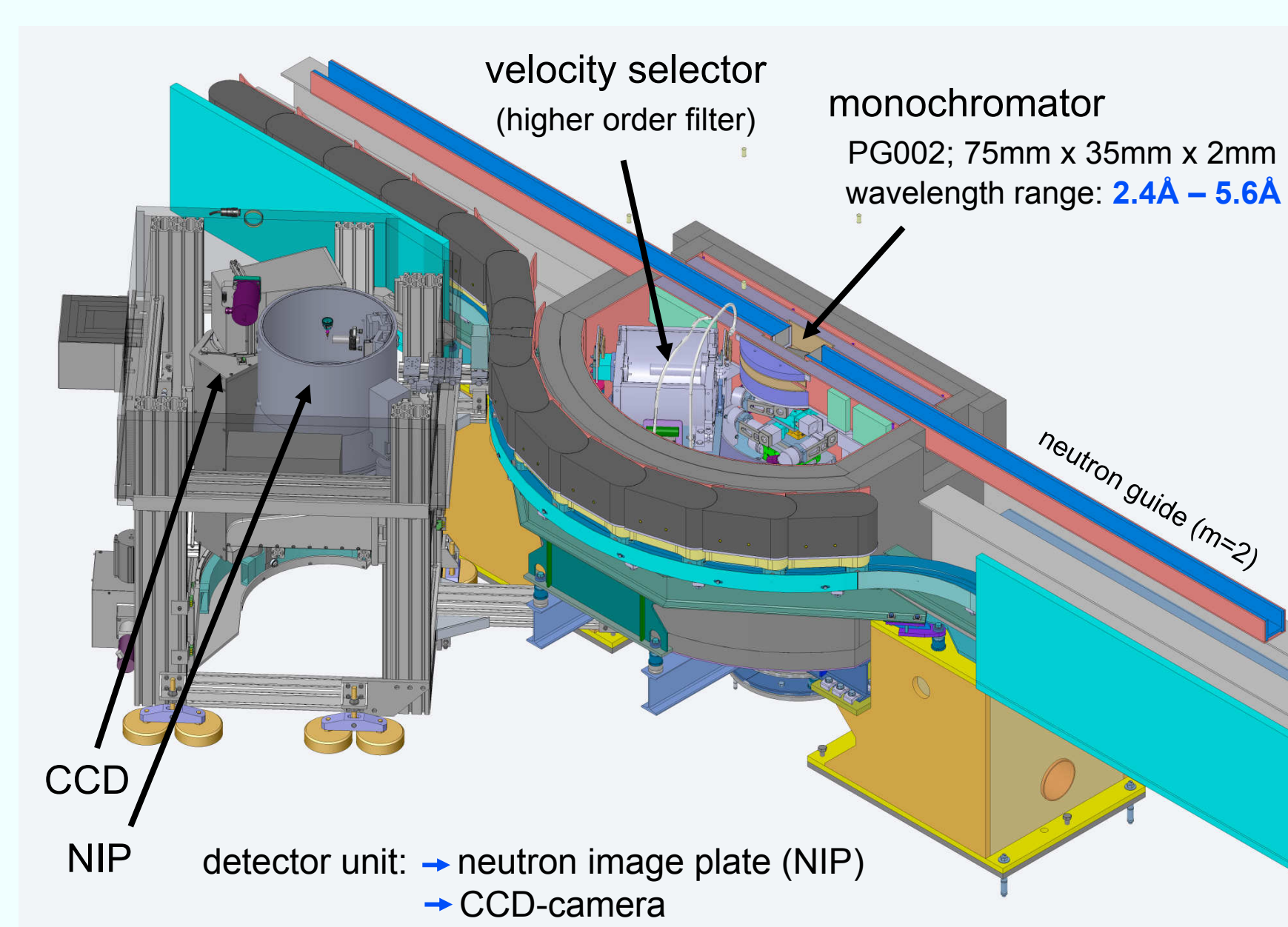
Hydration structure analysis:

example: myoglobin

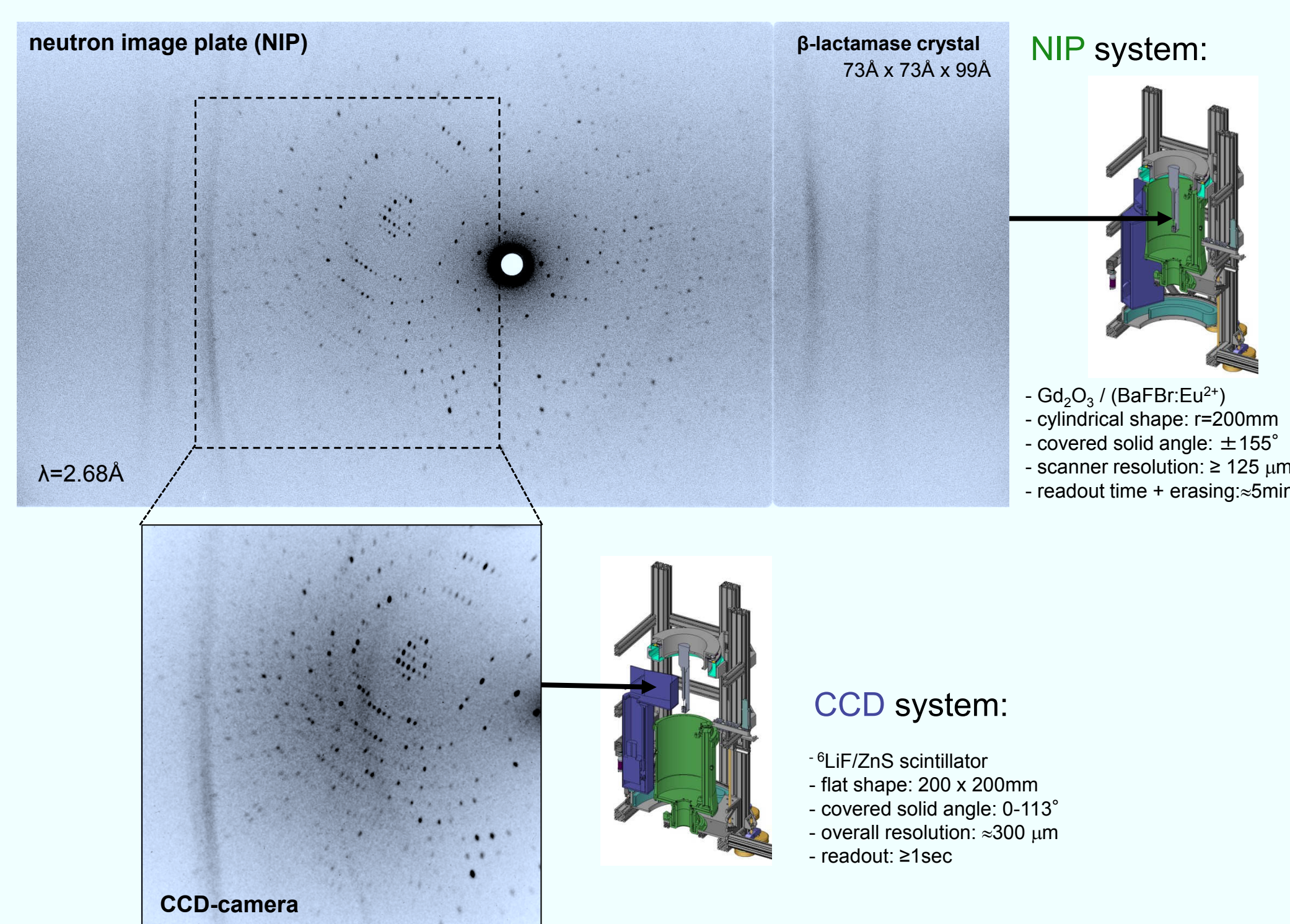


Chatake T, Ostermann A, Kurihara K, Parak F, Niimura N (2003) Proteins 50:516

The diffractometer BIODIFF:

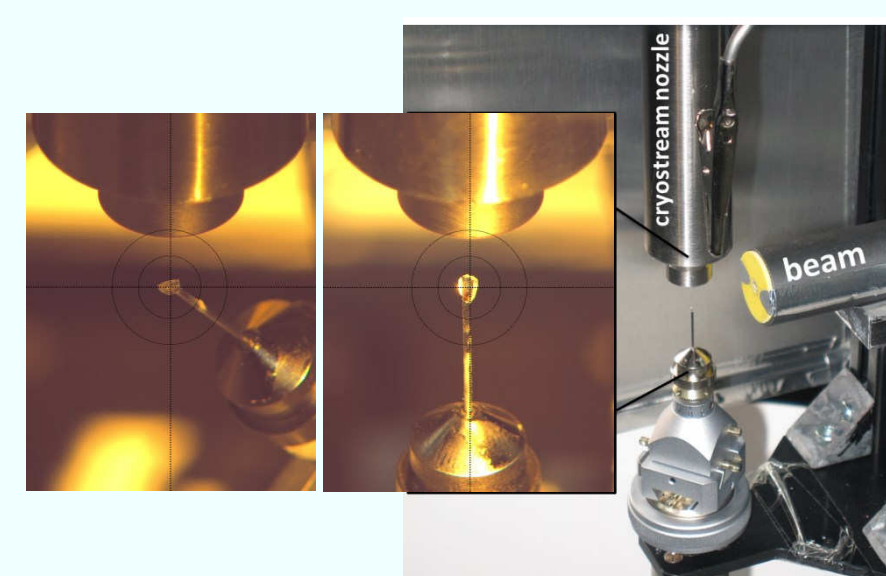


NIP and CCD detector system:



Sample environment:

Standard Oxford cryostream 700+



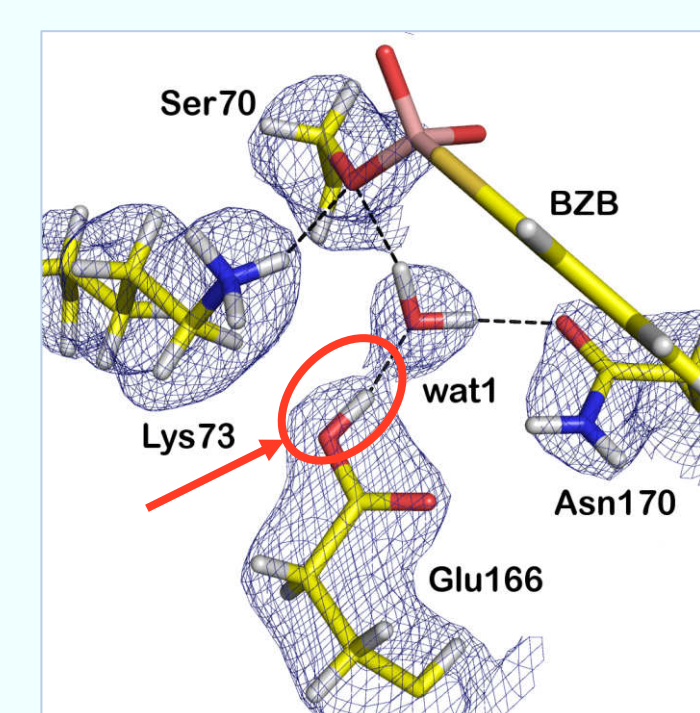
- temperature range: 90 - 500K
- stable, no icing over weeks



First “user data-sets”:

β-lactamase with bound BZB inhibitor

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



$d_{\min}$	$I/\sigma(I)$	N_{meas}	mult.	compl. in shell %	R_{merge} %
4.31	27.8	12685	5.6	97.6	4.9
3.42	19.0	11941	5.5	98.0	8.0
2.99	10.3	10378	4.9	96.9	14.6
2.71	7.6	8757	4.3	95.5	18.7
2.62	5.9	7820	3.9	92.8	21.2
2.37	5.4	7099	3.8	89.2	21.6
2.25	5.0	6095	3.5	84.6	23.0
2.15	4.5	5906	3.4	82.9	24.7
2.07	4.1	5673	3.2	82.0	27.2
2.0	3.7	5059	2.9	81.2	27.9
overall	7.4	81413	4.0	90.2	14.7

pd: 4bd1 $R_{\text{sym}} = 7.9\%$ (17.9%)

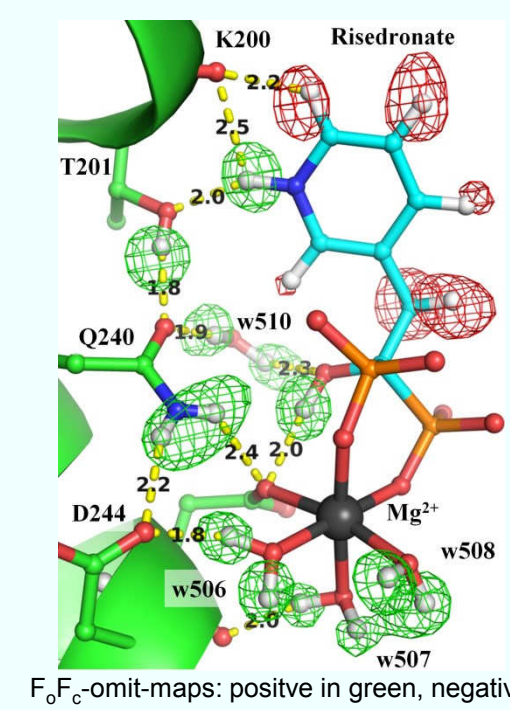
- unit cell: 73.4 Å, 73.4 Å, 99.1 Å P3₂21
- fully deuterated protein
- crystal size: 2.7 mm³
- collection time: 9d

The hydrogen-bonding network strongly suggests Glu166 acts as the general base

Tomanicek et al., J. Biol. Chem., 288, 4715 (2013).

Human farnesyl pyrophosphate synthase with risedronate

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

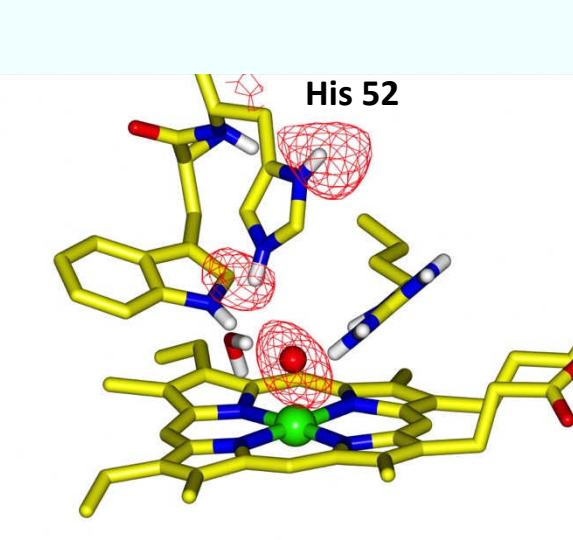
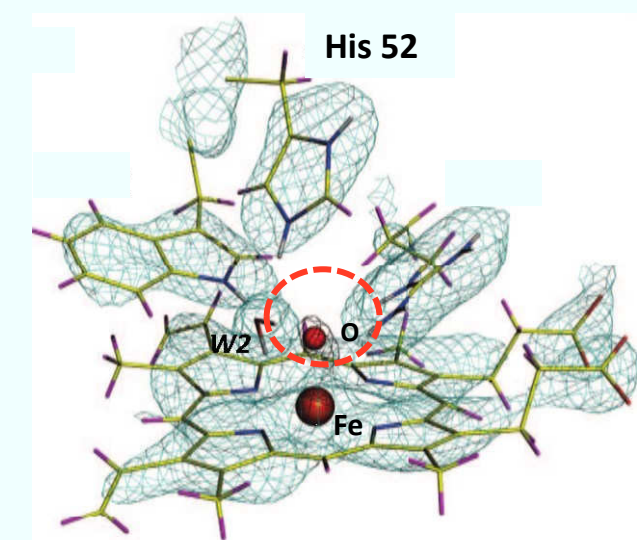


$d_{\min}$	$I/\sigma(I)$	N_{meas}	mult.	compl. in shell %	R_{merge} %
140.0 – 5.17	29.1	12784	6.6	94.9	4.3
5.17 – 4.10	14.5	9490	5.0	99.3	9.8
4.10 – 3.59	10.6	8045	4.3	99.5	12.3
3.59 – 3.26	7.0	5833	3.2	98.5	15.5
3.26 – 3.02	6.1	6443	3.5	99.3	19.5
3.02 – 2.85	4.5	6181	3.4	98.6	24.9
2.85 – 2.70	3.3	5772	3.2	98.6	31.2
2.70 – 2.59	2.5	5442	3.0	98.5	39.8
2.59 – 2.49	2.1	5260	2.9	99.0	46.2
2.49 – 2.40	1.8	4846	2.7	98.0	61.2
overall	8.2	69977	3.8	98.4	10.7

$R_{\text{sym}} = 5.8\%$ (37.1%)

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!

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	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	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	887,700	1	24	2.0	97.9 (90.5)	13.6 (32.6)
Xylanase II A. Kovalevsky et al.	49.5 59.9 70.4 P2 ₁ 2 ₁ 2 ₁	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 ₁	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 2 ₁ 2 ₁	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	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	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 ₂ 2 ₂	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 ₂ 2 ₂	40,500	3.0	3	1.7	82.7 (63.3)	10.8 (21.5)

- 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: May 6th, 2016 !!

user.frm2.tum.de
fzj.frm2.tum.de

