

Neutron macromolecular crystallography at the FRM II

The neutron single crystal diffractometer BIODIFF

or: What can neutrons do for you?

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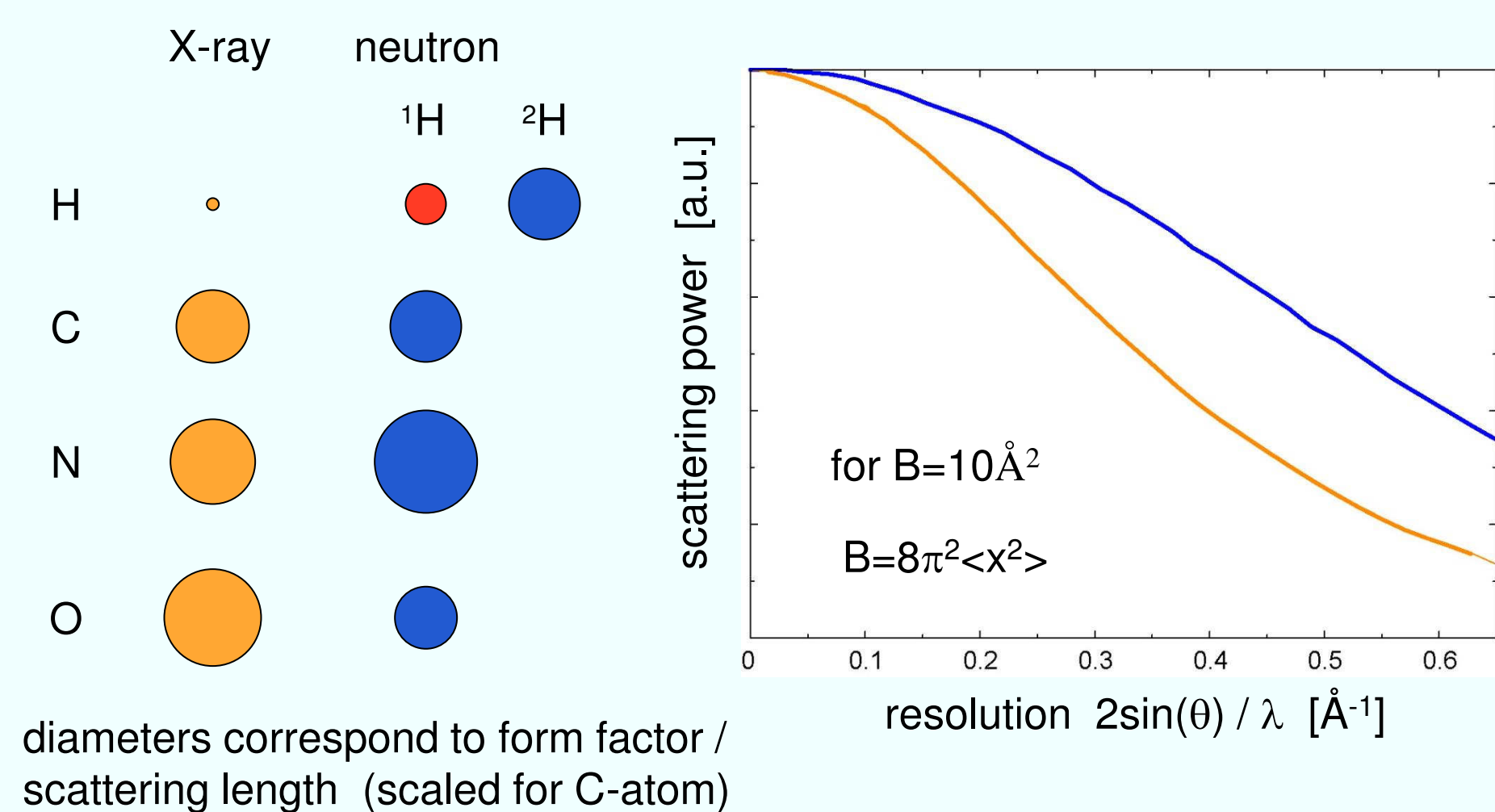
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Neutron structure determination:

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

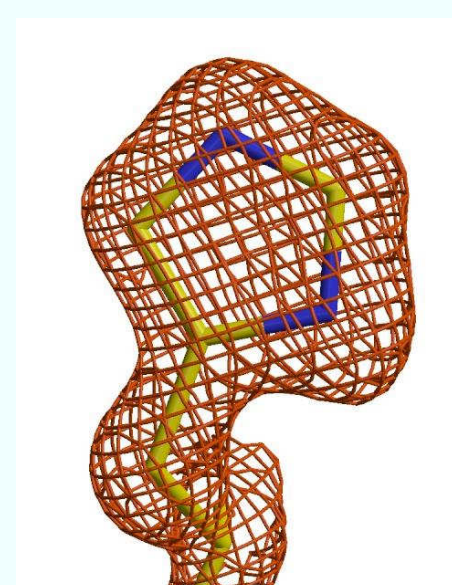
- protonation states of amino acid side chains
- deuterium exchange as a measure of flexibility and accessibility (discrimination between H / D)
- solvent structure including hydrogen atoms can be analysed
- discrimination between neighbors in the periodic table is possible: e.g. N and O, Fe and Mn
-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

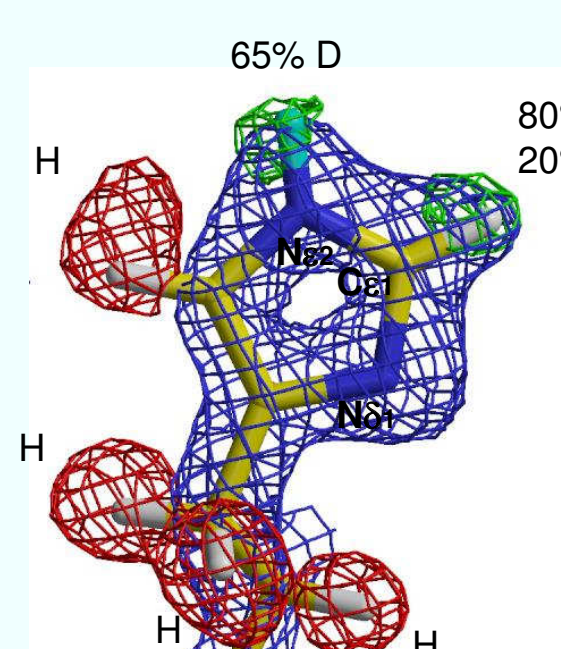


Amino acid protonation states:

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



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



2Fo-Fc map; +1.5σ

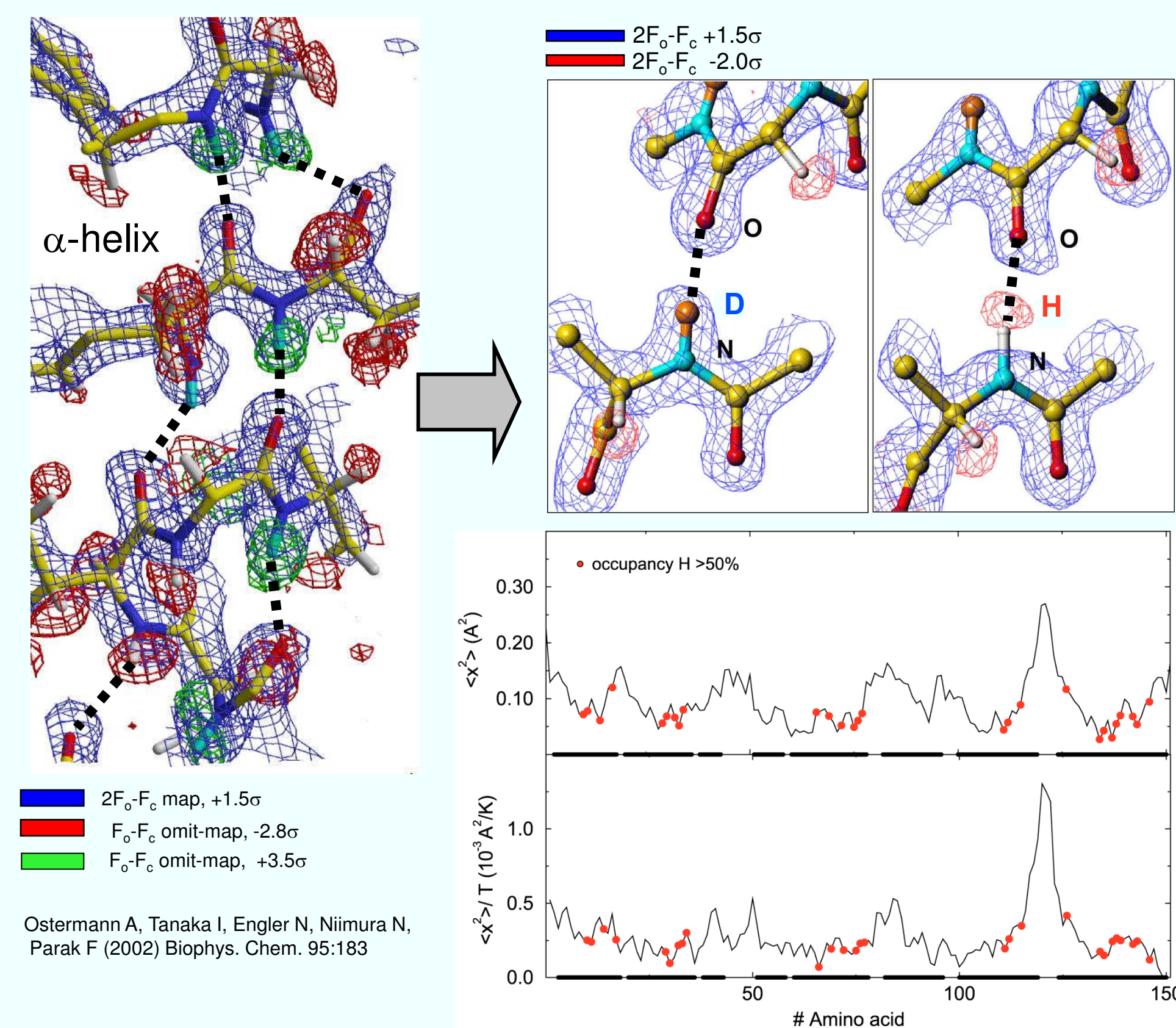
2Fo-Fc map; +1.5σ

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

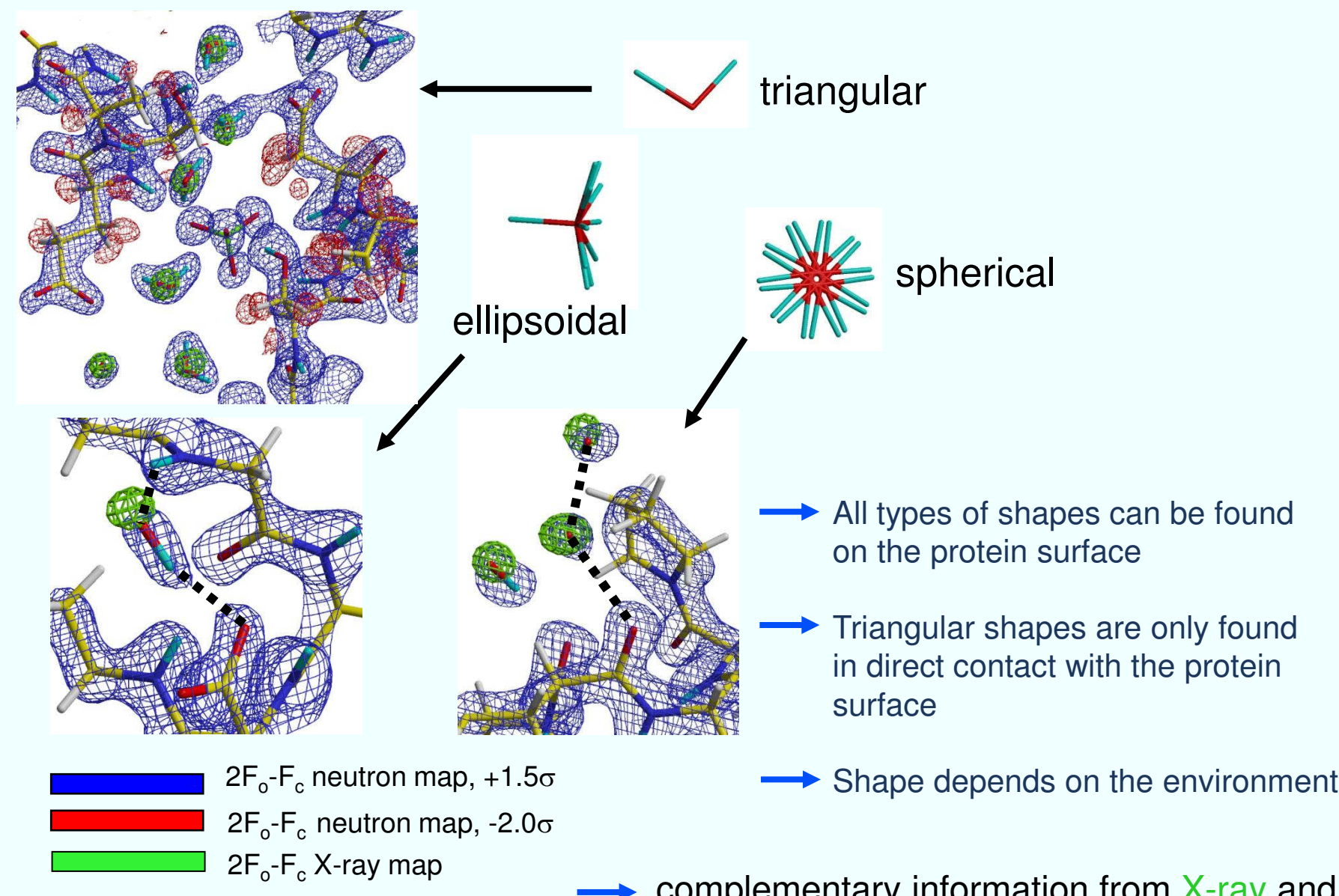
Analysis of H/D-exchange:



- 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

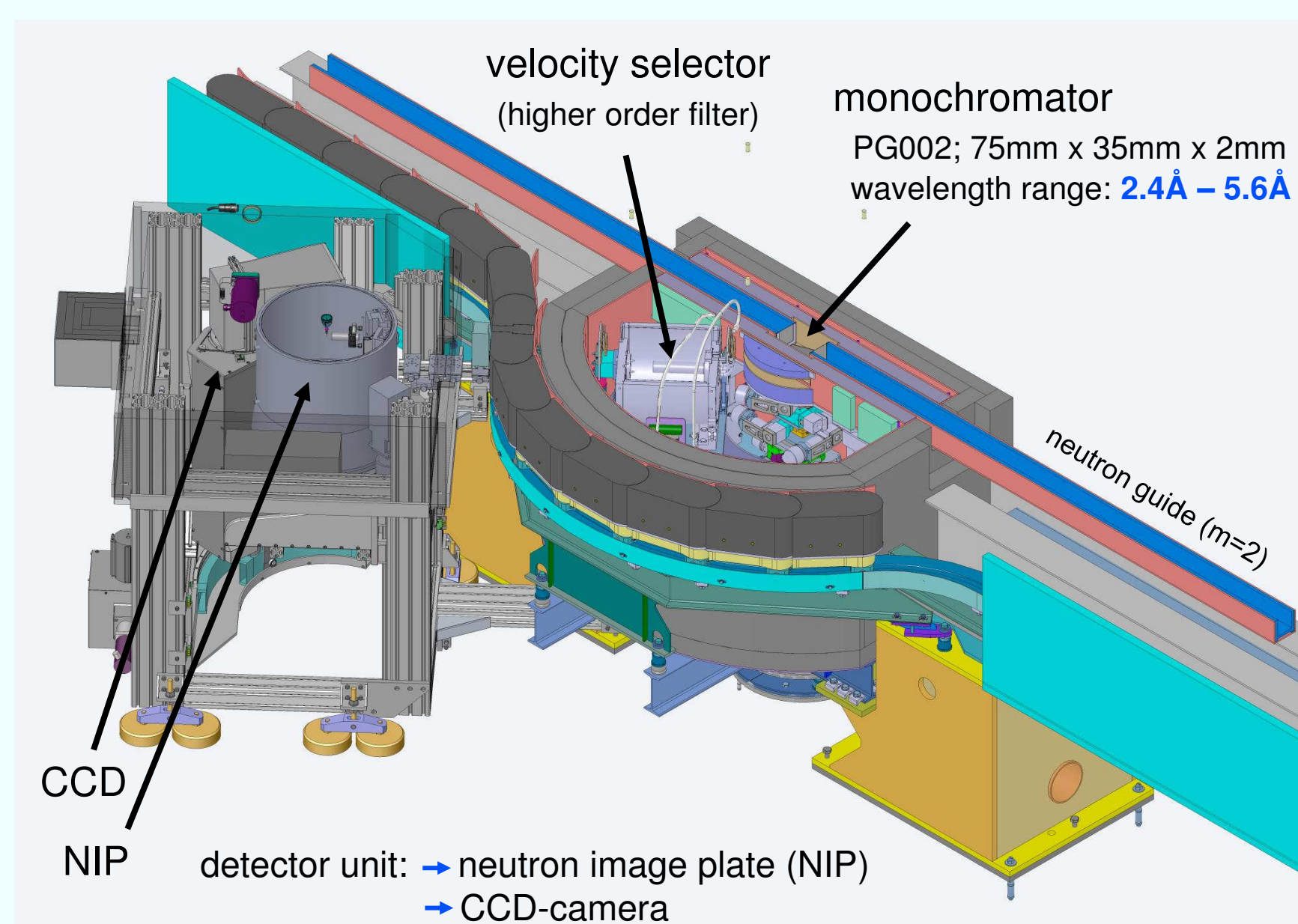
Hydration structure analysis:

example: myoglobin

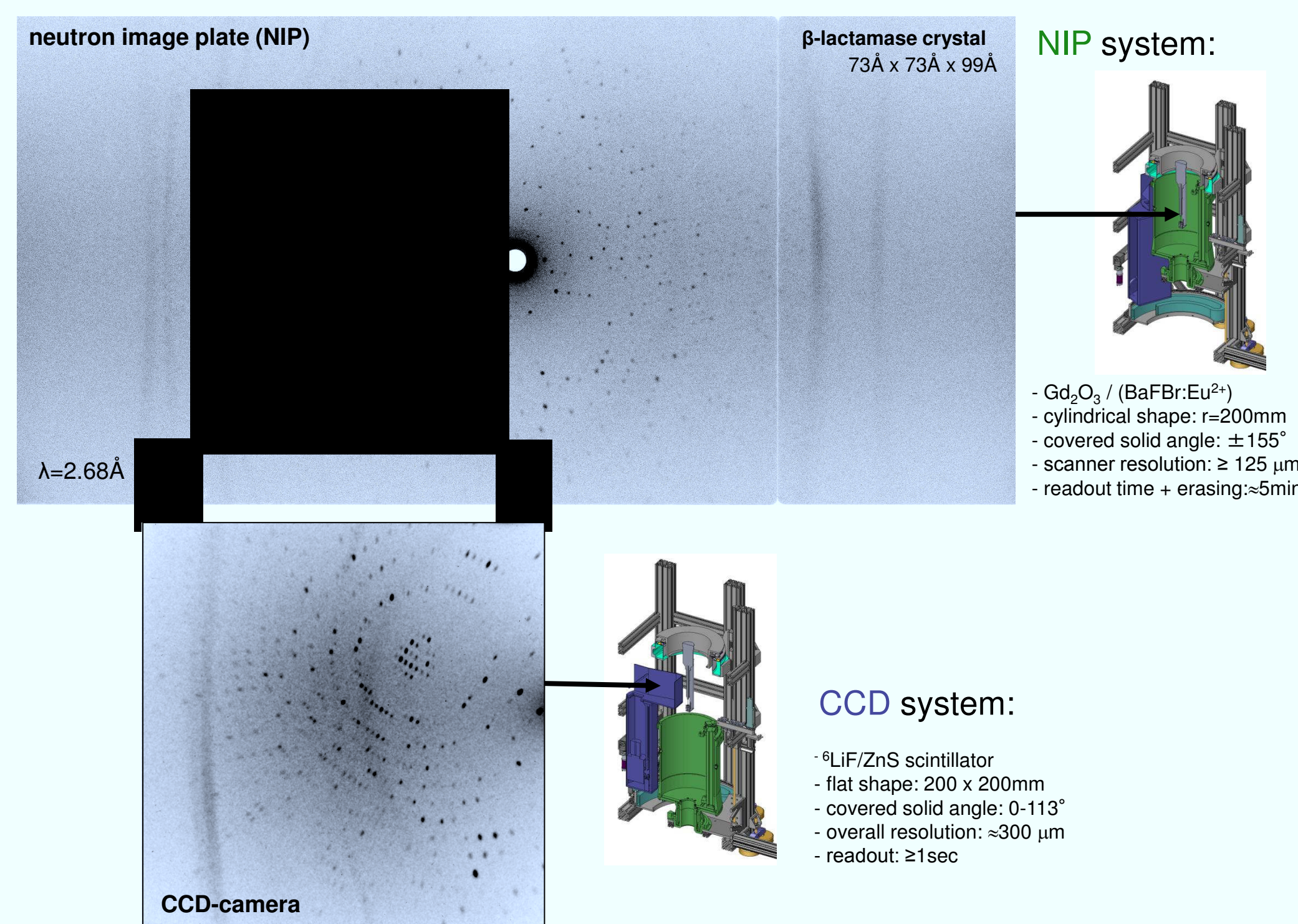


Chatake T, Ostermann A, Kurihara K, Parak F, Niimura N (2003) Proteins 50:516

The diffractometer BIODIFF:

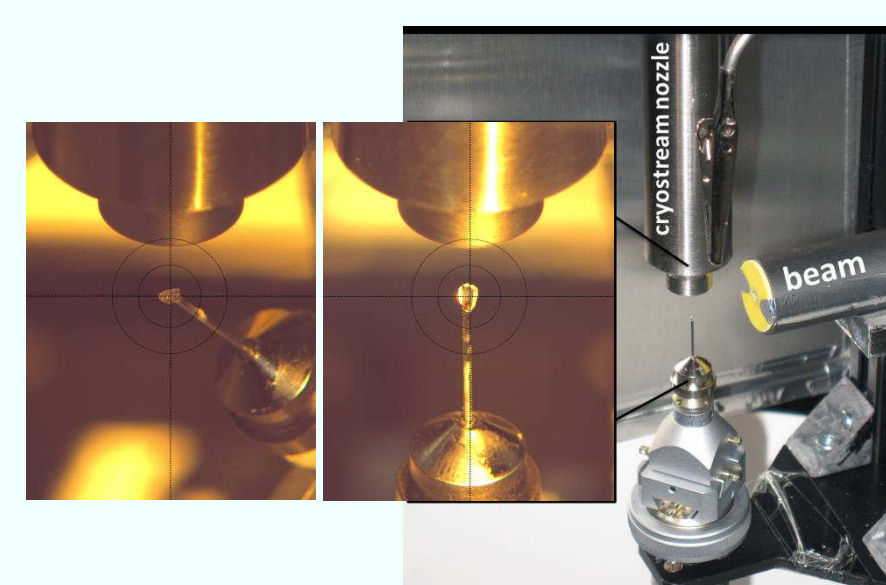


NIP and CCD detector system:



Sample environment:

Standard Oxford cryostream 700+



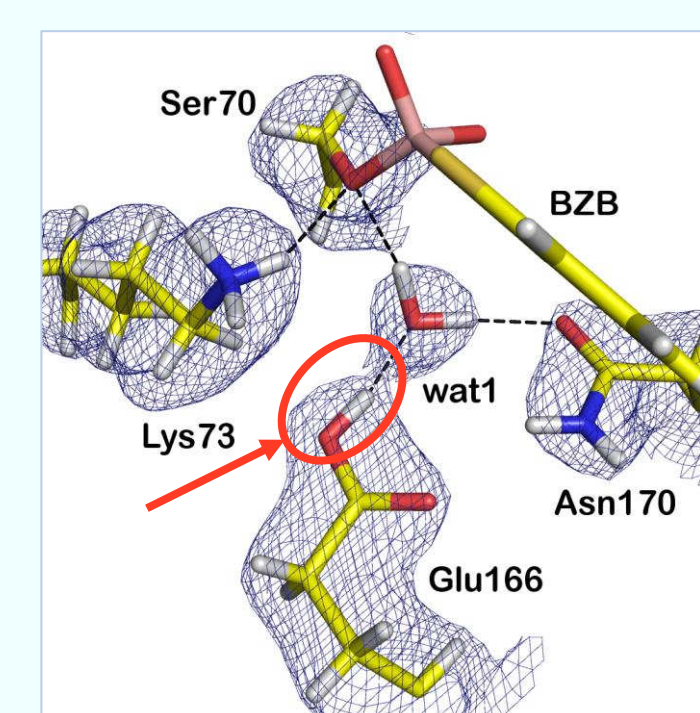
- temperature range: 90 - 500K
- stable, no icing over weeks



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)



$d_{\min}$	$I(\sigma)$	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
2.52	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

pdbs: 4bd1 $R_{\text{pim}} = 7.9\%$ (17.9%)

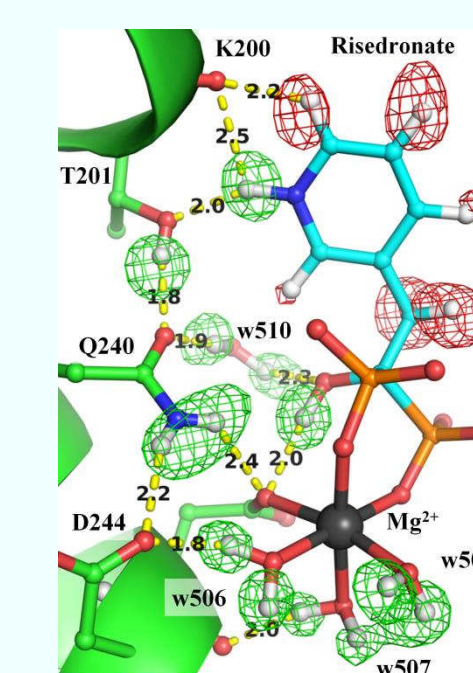
- unit cell: 73.4 Å, 73.4 Å, 99.1 Å P3₂21
- fully deuterated protein
- crystal size: 2.7mm³
- collection time: 9d

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



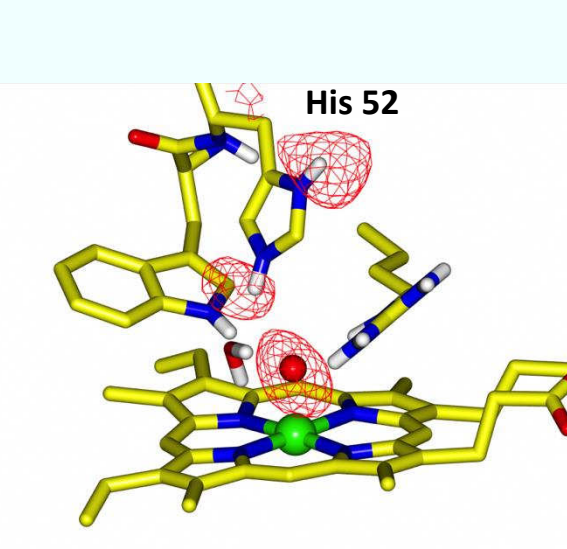
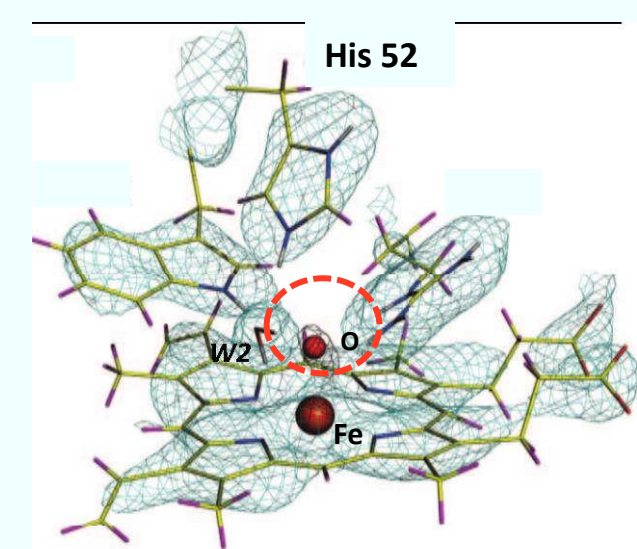
$d_{\min}$	$I(\sigma)$	N_{meas}	mult.	compl. in shell %	R_{merge} %
140.0 – 5.17	29.1	12784	6.6	94.9	4.3
5.17 – 4.10	14.5	9490	5.0	99.3	9.8
4.10 – 3.59	10.6	8045	4.3	99.5	12.3
3.59 – 3.26	7.0	5833	3.2	98.5	15.5
3.26 – 3.02	6.1	6443	3.5	99.3	19.5
3.02 – 2.85	4.5	6181	3.4	98.6	24.9
2.85 – 2.70	3.3	5772	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	4946	2.7	98.0	61.2
overall	8.2	69977	3.8	98.4	10.7

- unit cell: 111.9 Å, 111.9 Å, 72.6 Å P4₂2₁
- crystal size: 3.5mm³
- collection time: 25d (5d, 6d, 14d)

$R_{\text{pim}} = 5.8\%$ (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)	73.3, 73.3, 98.7 P3 ₂ 21	453,000	4.0	8	2.0	99.0 (82.7)	9.8 (22.3)
β-lactamase-BZB-inhibitor	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	101.0 101.0 100.5 R32	887,700	1	24	2.0	97.9 (90.5)	13.6 (32.6)
Xylanase II	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	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	42.8 41.7 72.8 P2 ₁	125,000	2.5	8	1.8	89.9 (76.8)	11.9 (33.0)
Nucleosidase (MTAN)	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	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	111.9 111.9 72.6 P4 ₂ 2 ₁	909,000	3.5	25 (11)	2.4	98.4 (98.0)	10.7 (61.2)
DNA drug complex	27.9 27.9 52.0 P4 ₂ 2 ₁	40,500	3.0	3	1.7	92.7 (83.3)	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: Sep 11th, 2015 !!

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