

Neutron macromolecular crystallography at the FRM II

The neutron single crystal diffractometer BIODIFF

or: What can neutrons do for you?

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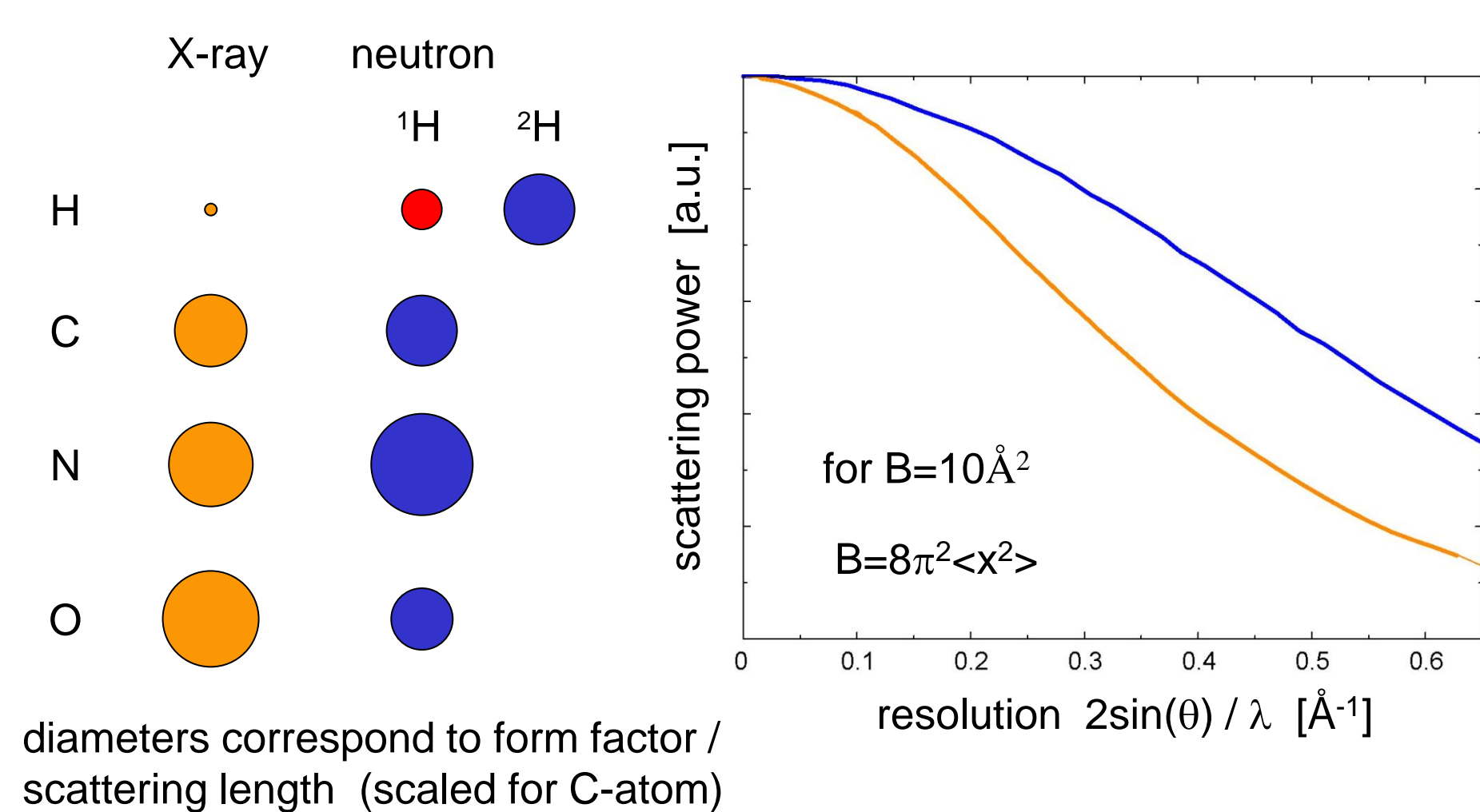
^dForschungszentrum Jülich GmbH, Zentralabteilung Technologie, D-52425 Jülich

Neutron structure determination:

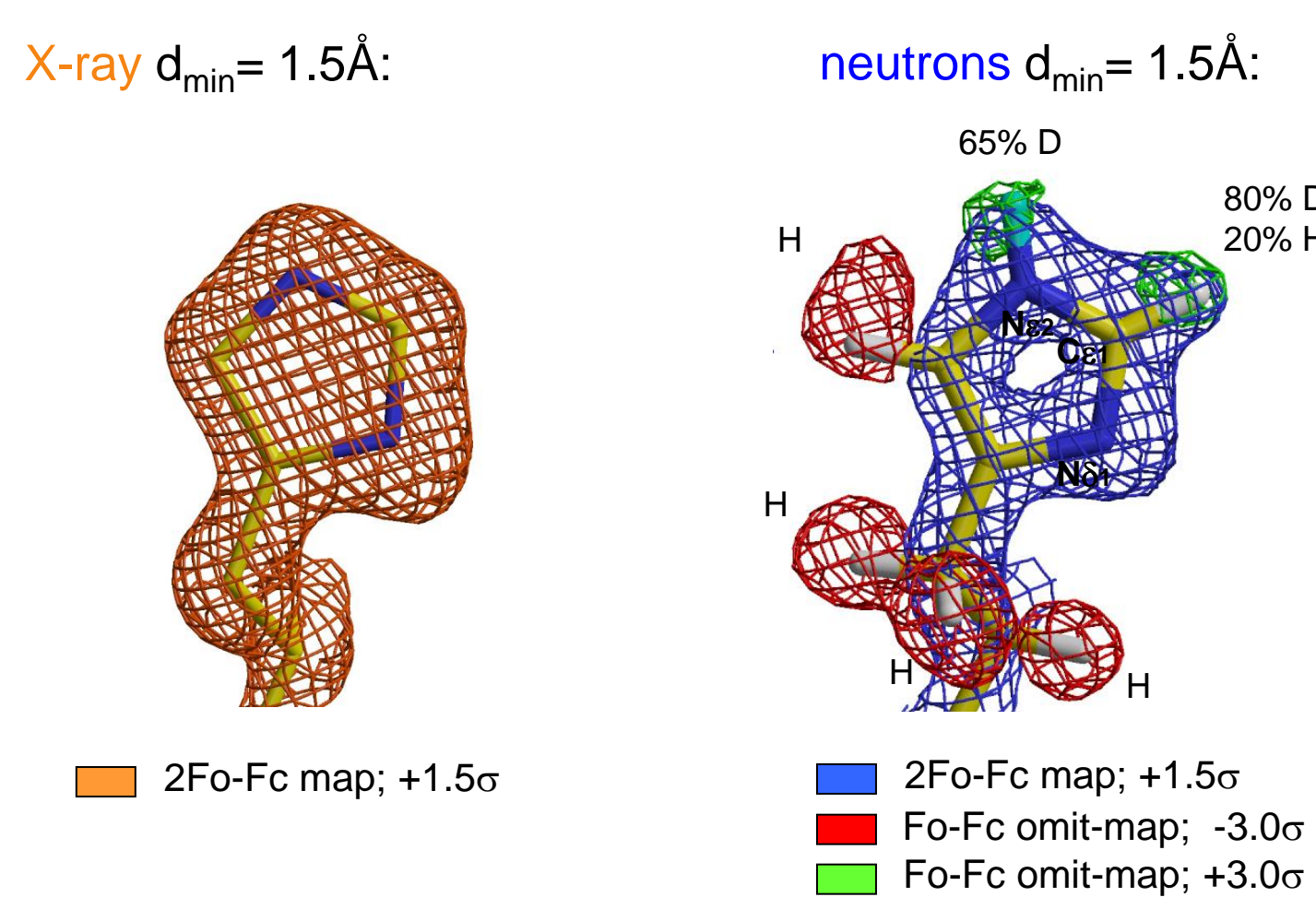
hydrogen atoms can be resolved even at a resolution of $d_{min} \approx 2.5 \text{ \AA}$

- 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

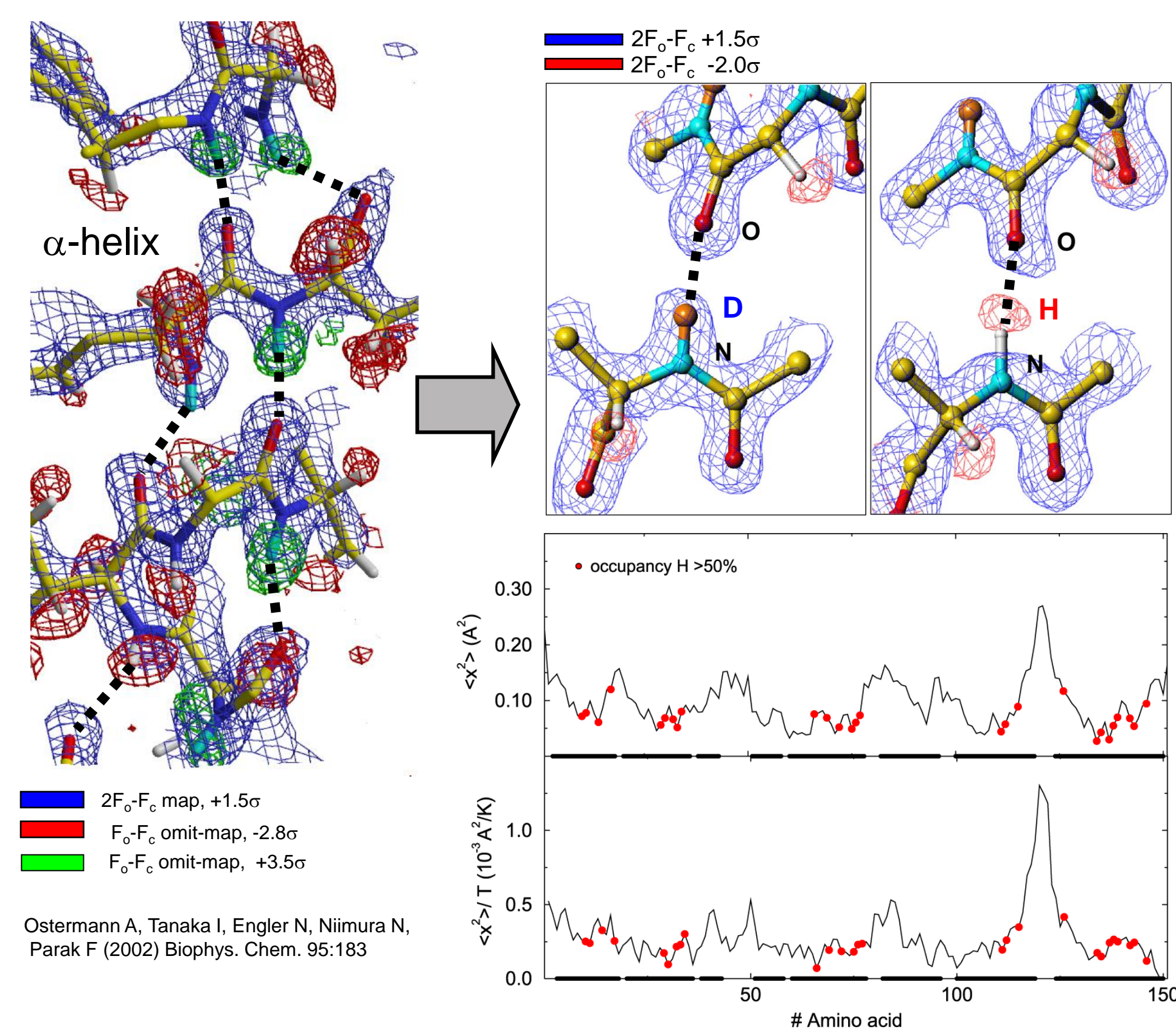
Comparison of form factors (X-ray) and scattering lengths (neutrons):



Amino acid protonation states:

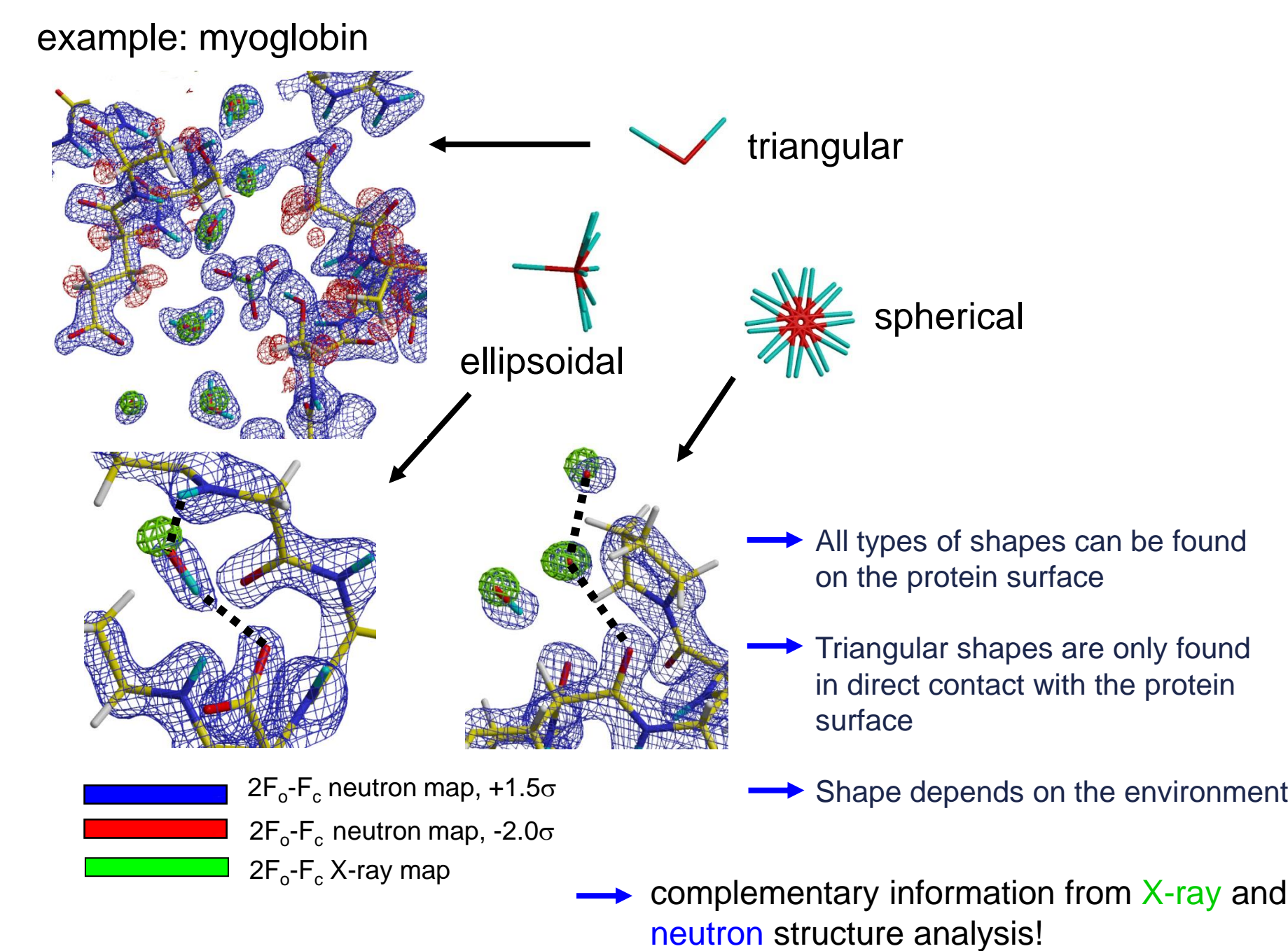


Analysis of H/D-exchange:

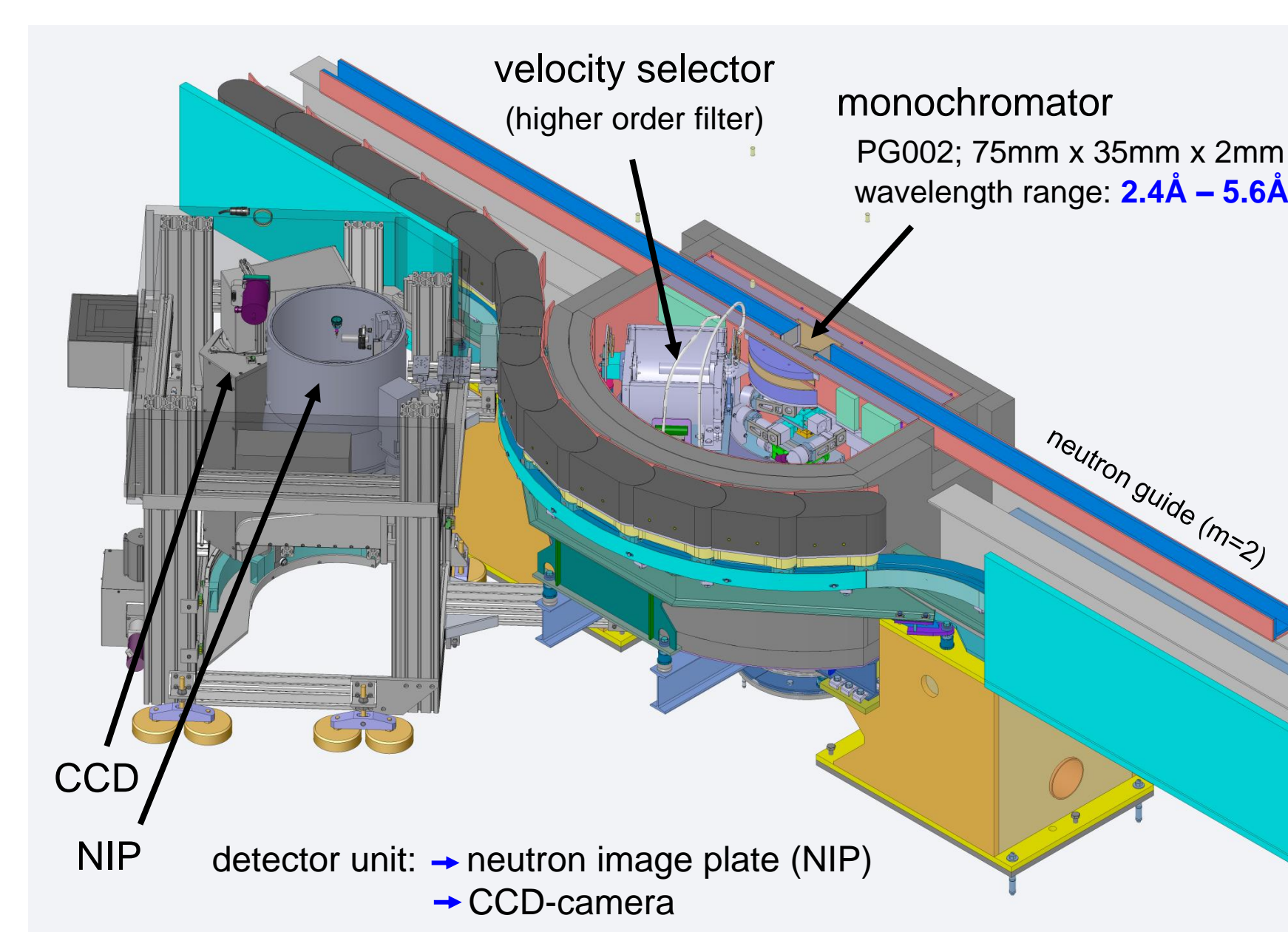


- H / D exchange correlates with the flexibility
- protons show higher protection in the interior of the protein
- tells you where water can migrate and which protons can take part in proton transfer reactions

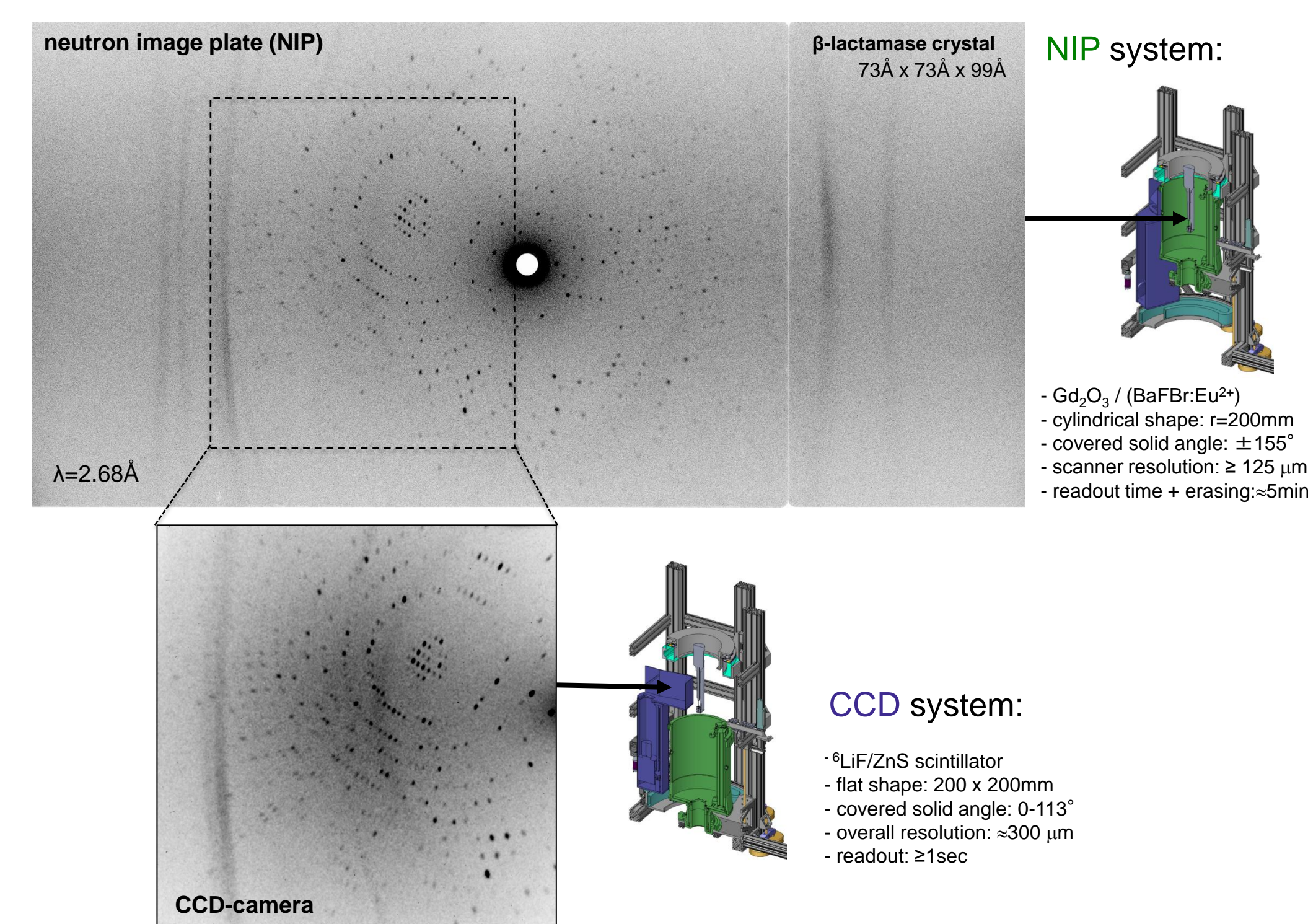
Hydration structure analysis:



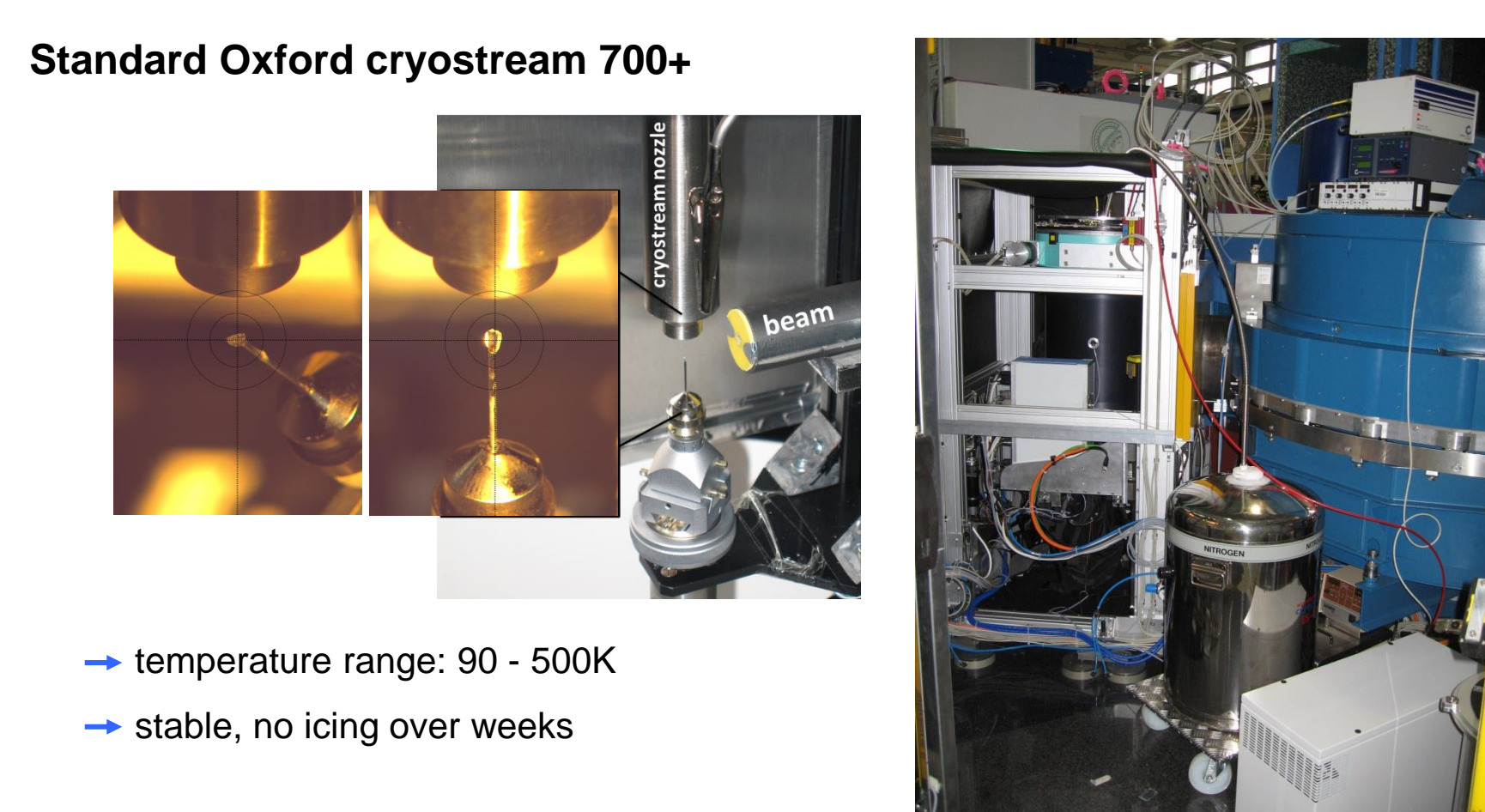
The diffractometer BIODIFF:



NIP and CCD detector system:



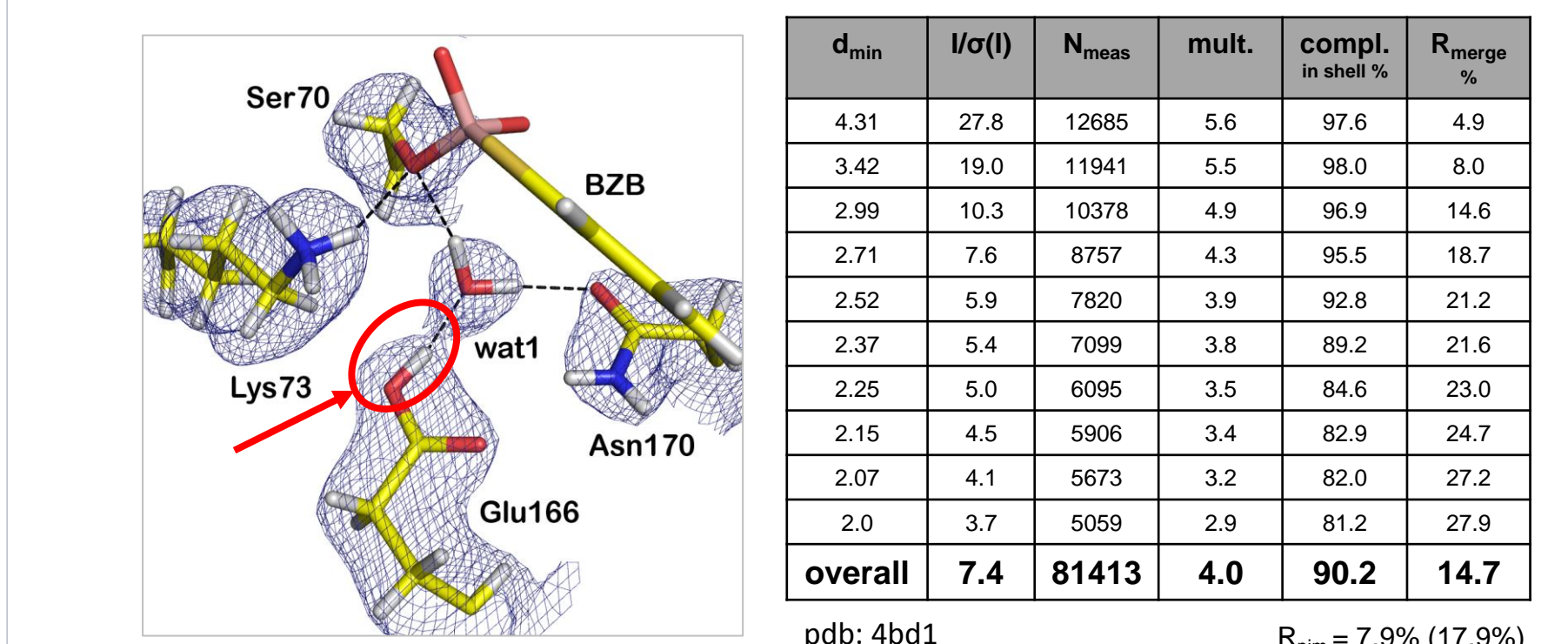
Sample environment:



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)

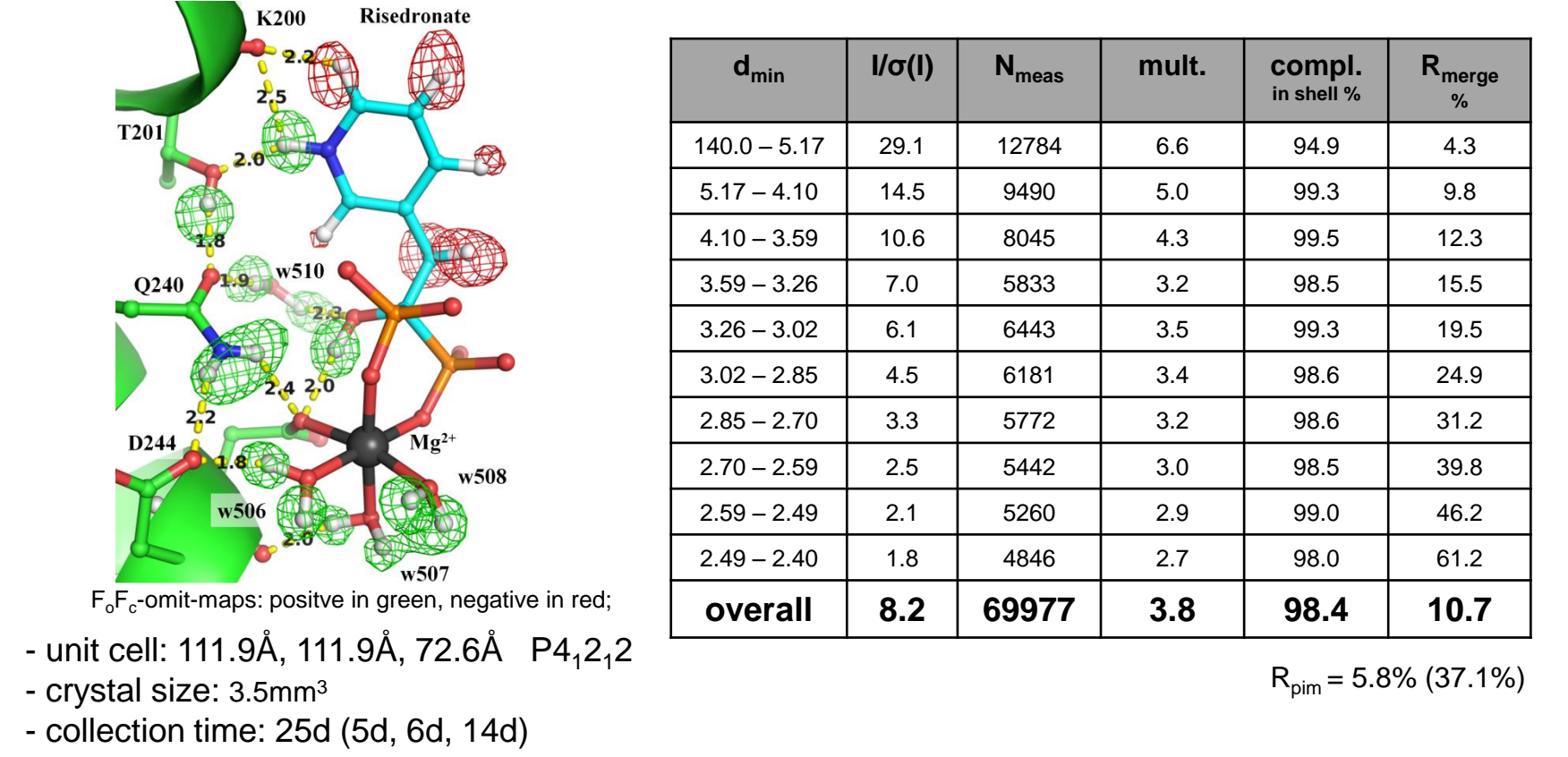


The hydrogen-bonding network strongly suggests Glu166 acts as the general base

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

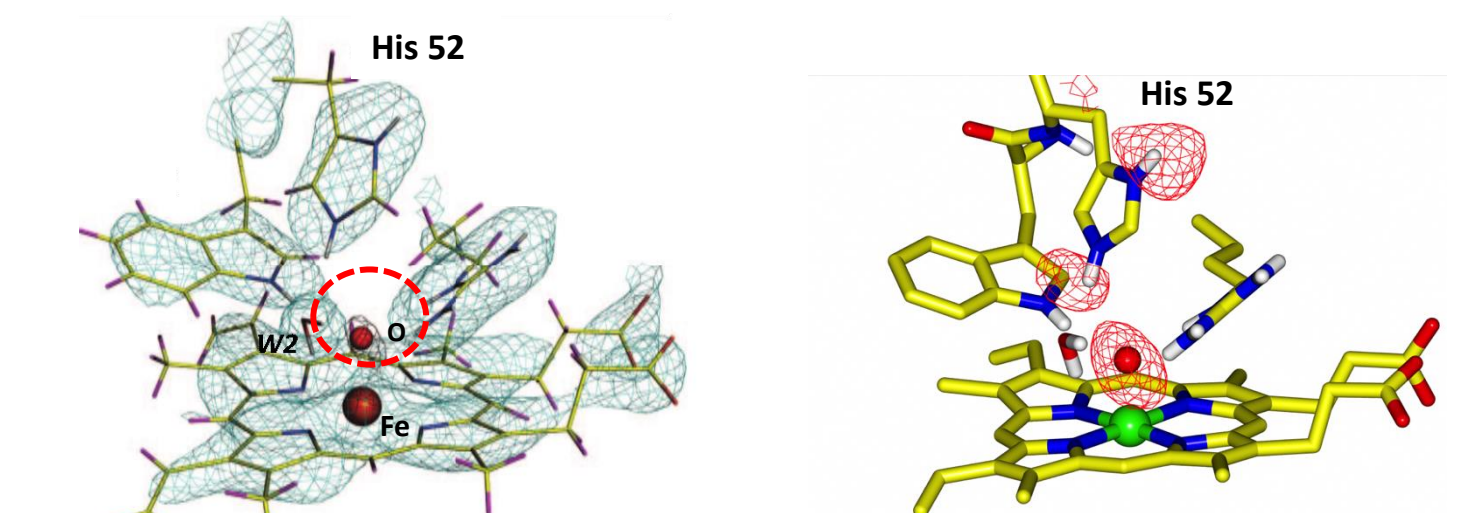
Human farnesyl pyrophosphate synthase with risedronate

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



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 (\AA) space group | cell volume (\AA ³) | crystal size (mm ³) | time (d) | d_{min} (\AA) | compl. (%) | R_{merge} (%) |
|----------------------------------|--|---------------------------------|---------------------------------|----------|-----------------|-------------|-----------------|
| β -lactamase (no ligand) | 73.3, 73.3, 98.7 P3 ₂ 2 ₁ | 453,000 | 4.0 | 8 | 2.0 | 89.0 (82.7) | 9.8 (22.3) |
| β -lactamase-BZB-inhibitor | 73.4, 73.4, 99.1 P3 ₂ 2 ₁ | 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 (52.6) |
| Xylanase II | 49.5, 59.9, 70.4 P2 ₁ , 2 ₁ | 208,000 | 2.8 | 17 | 2.0 | 96.2 (91.0) | 9.7 (32.7) |
| KDNPP phosphatase | 83.1, 108.9, 75.8 P2 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 2 ₁ | 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 | 417,000 | 0.65 | 23 | 2.5 | 90.7 (71.9) | 17.3 (42.8) |
| Farnesyl pyrophosphate synthase | 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 | 27.9, 27.9, 52.0 P4 ₂ 2 ₂ | 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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