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New sample environment options at the neutron diffractometer “BioDiff”

Cryostream - closed cycle cryostat - high pressure cell for powder diffraction

March 22nd 2013 | Tobias E. Schrader

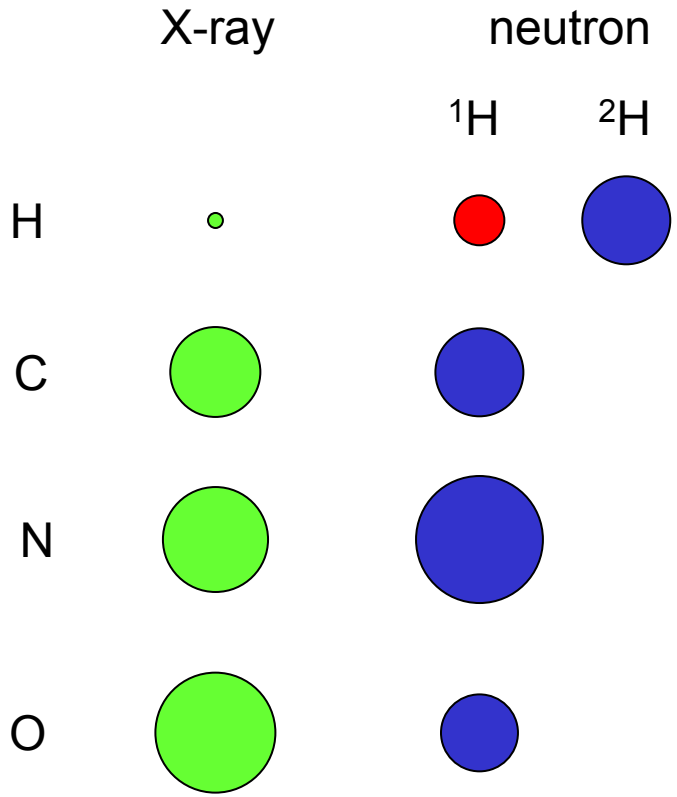
Outline of this talk

- Advantages and draw backs of neutron diffraction as compared to x-ray diffraction
- Short introduction to the instrument BioDiff
- First published results on a β-lactamase crystal
- Sample environment options at the instrument BioDiff
- Summary

Advantages / disadvantages of structure determination with neutrons I

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

Nucleus	atomic number	scattering length [10^{-12} cm]
^{1}H	1	-0.378
^{2}H	1	0.667
^{12}C	6	0.665
^{15}N	7	0.921
^{16}O	8	0.581



diameters correspond to:
form factor / scattering length
(scaled for C-atom)

σ_{coh} of ^{1}H is 1.8×10^{-28} m^2 but
σ_{incoh} of ^{1}H is 80.2×10^{-28} m^2
Large background from hydrogen atoms!

Advantages of structure determination with neutrons II

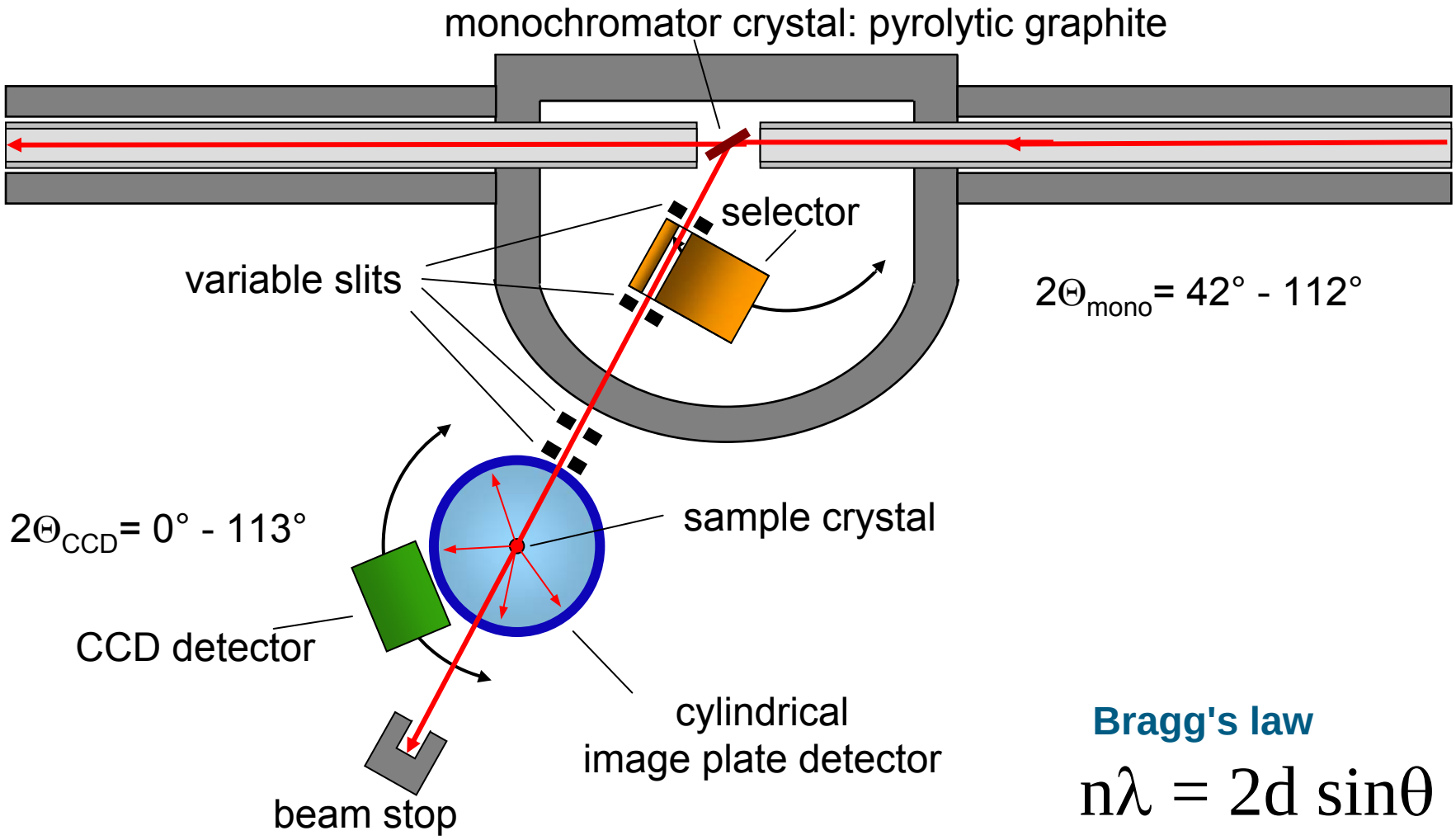
hydrogen atoms can be resolved even at a resolution of $d_{min} \approx 2$Å (for ^2H)
Therefore one can determine:

- → 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

Radiation damage (hydrogen abstraction, disulfide bond cleavage) observed when measuring an x-ray crystallography data set can be avoided.

Especially, the reduction of metals in metalo-proteins can be avoided.

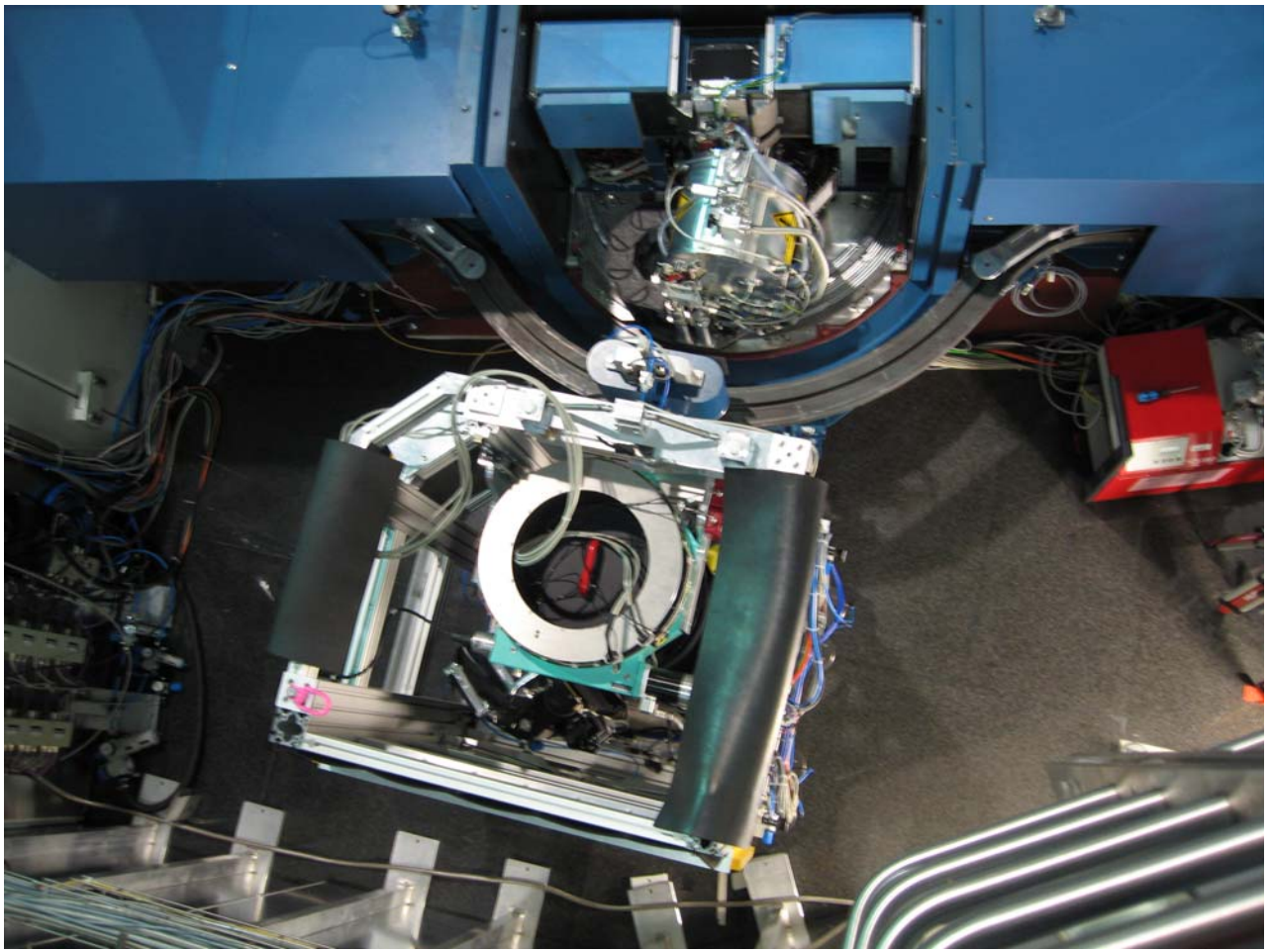
Schematic overview over BioDiff: A neutron protein diffractometer: collaboration between JCNS and FRMII



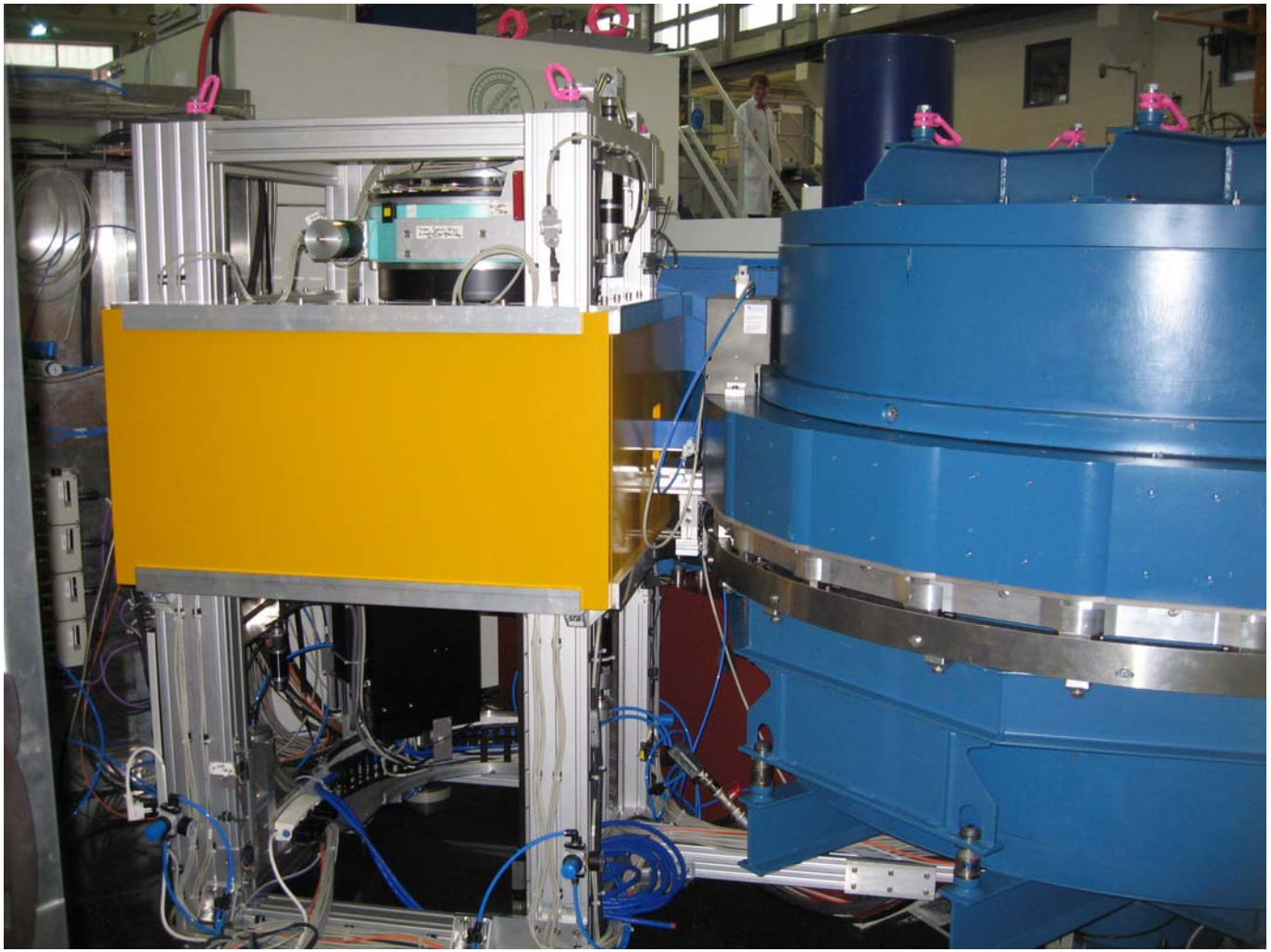
Bragg's law

$$n\lambda = 2d \sin\theta$$

BioDiff, the corresponding view in reality:



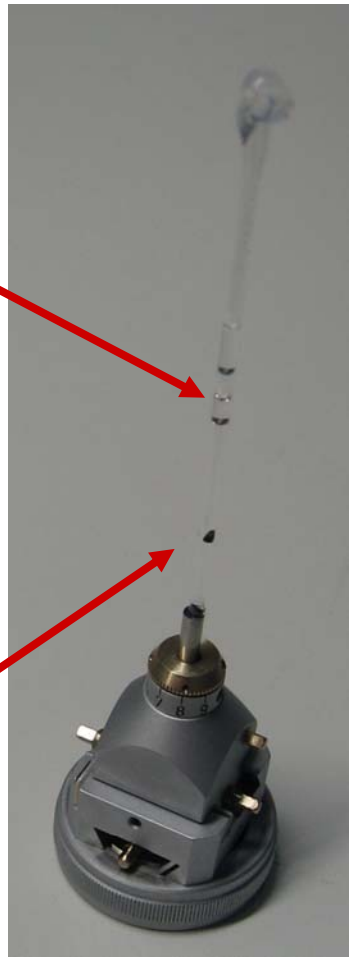
Side view...



Size considerations of protein crystals

deuterated mother liquor

sample crystal



size:

x-ray-crystallography:

ca. 10 µmx 10 µmx 10 µm

typically cryoprotectants needed to facilitate measurements at low (80 K) temperatures

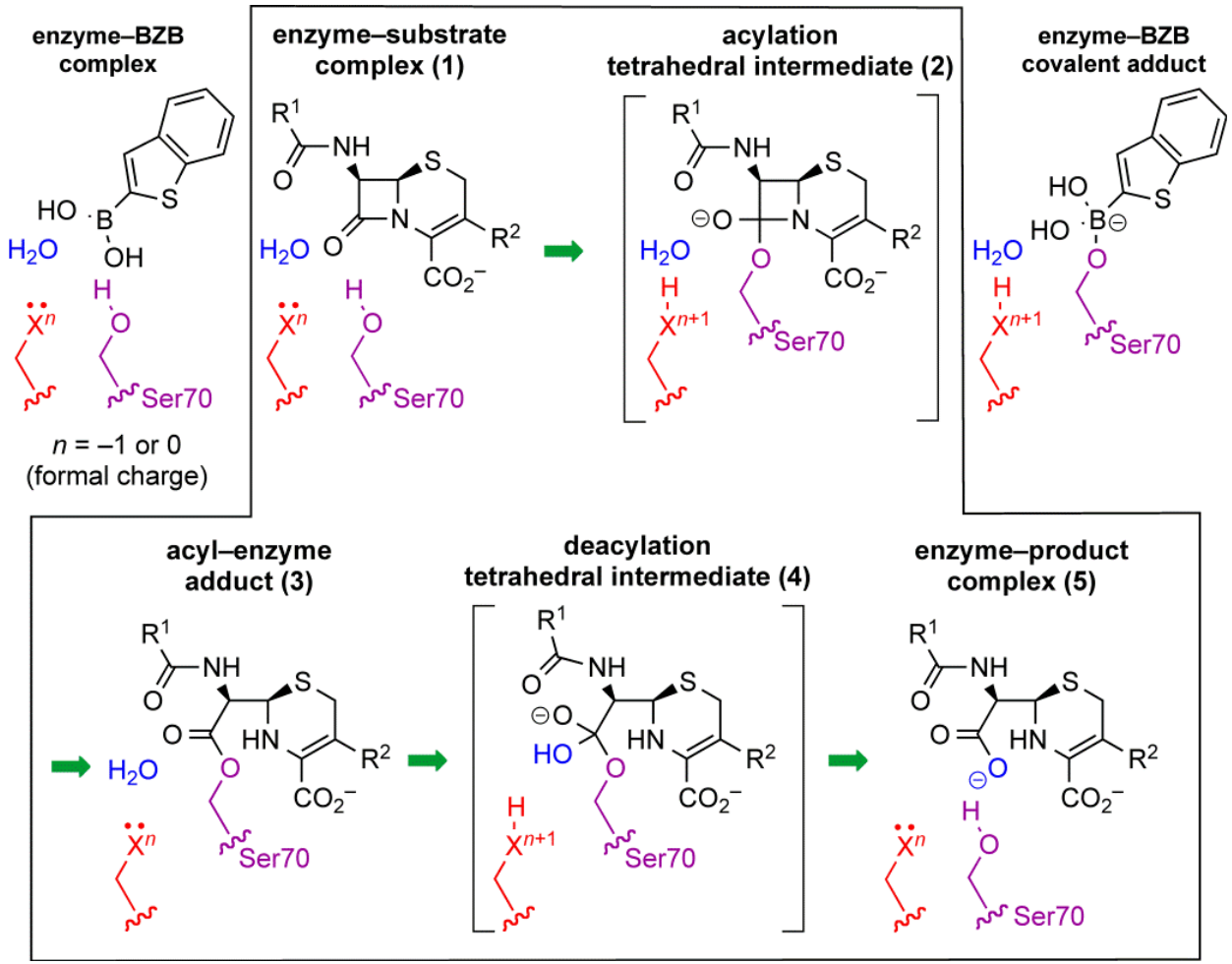
neutron protein crystallography:

The desirable size should be around 1mmx1mmx1mm (depending on the protein/space group)

It is desirable to exchange as many hydrogen atoms by deuterium as possible to reduce the incoherent scattering background caused by hydrogen atoms in the protein.

Outer diamter of the glas tube: 2 mm

β-lactamase: hydrolyses β-lactam antibiotics

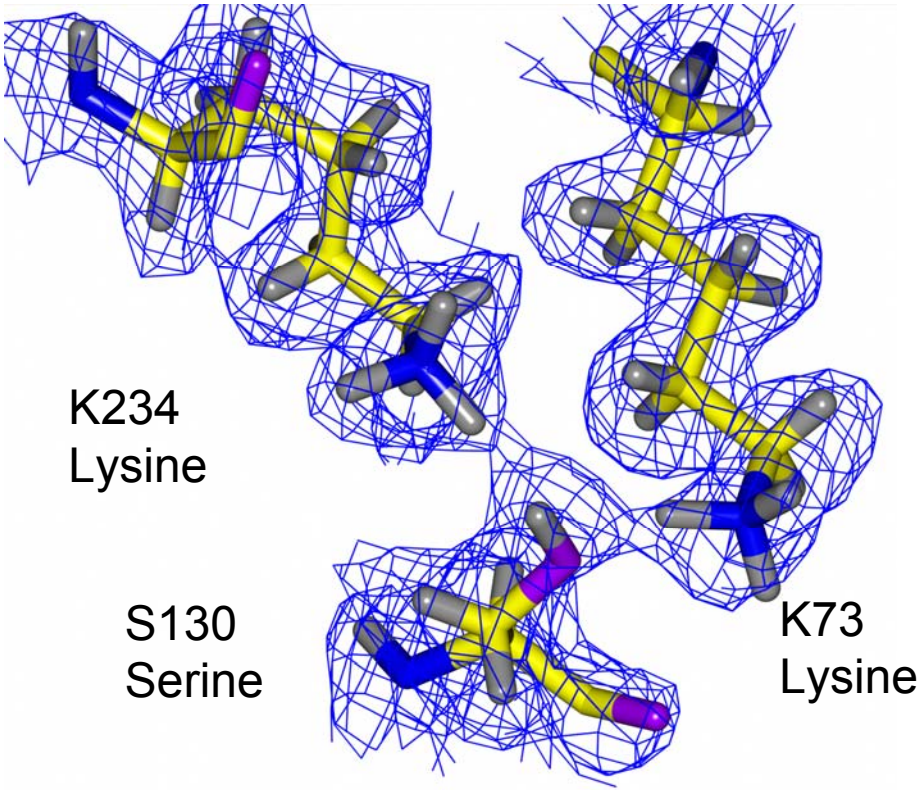


The catalytic cycle of a class A β-lactamase illustrated for a cephalosporin substrate (inside box) and the mode of inhibition by BZB (outside box). The general base employed is not necessarily the same for acylation and deacylation. The overall reaction pathway for β-lactam hydrolysis of a cephalosporin-like substrate by the class A β-lactamase enzymes.

Figures from: Tomanicek et al., doi: 10.1074/jbc.M112.436238

Comparing the first user data set on β-lactamase with the structure recorded on LADI III @ ILL

BioDiff



LADI III @ ILL

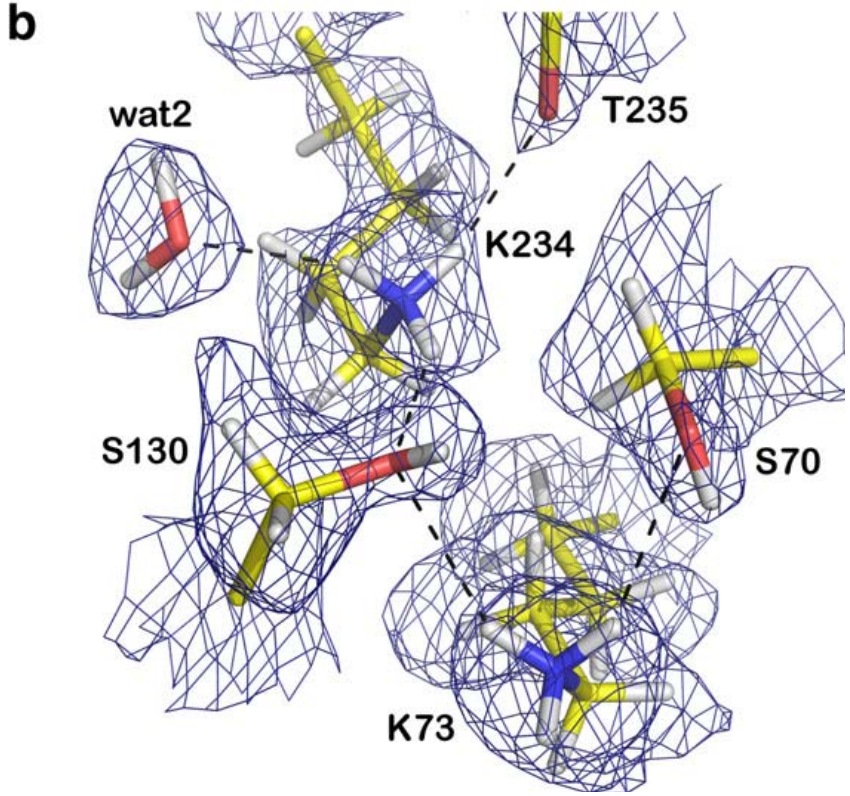


Fig. 2. Protonation states of the active site residues adjacent to Glu166 (a) and Lys234 (b) identified by σ_A-weighted $2F_o - F_c$ positive nuclear density maps colored in blue and contoured at positive 1.2σ. Nuclear density corresponding to the orientation of the catalytic water molecule (wat1) is also shown.

Fig. 2b of S. J. Tomanicek, et al. and Leighton Coates: FEBS Letters 585 (2011) 364

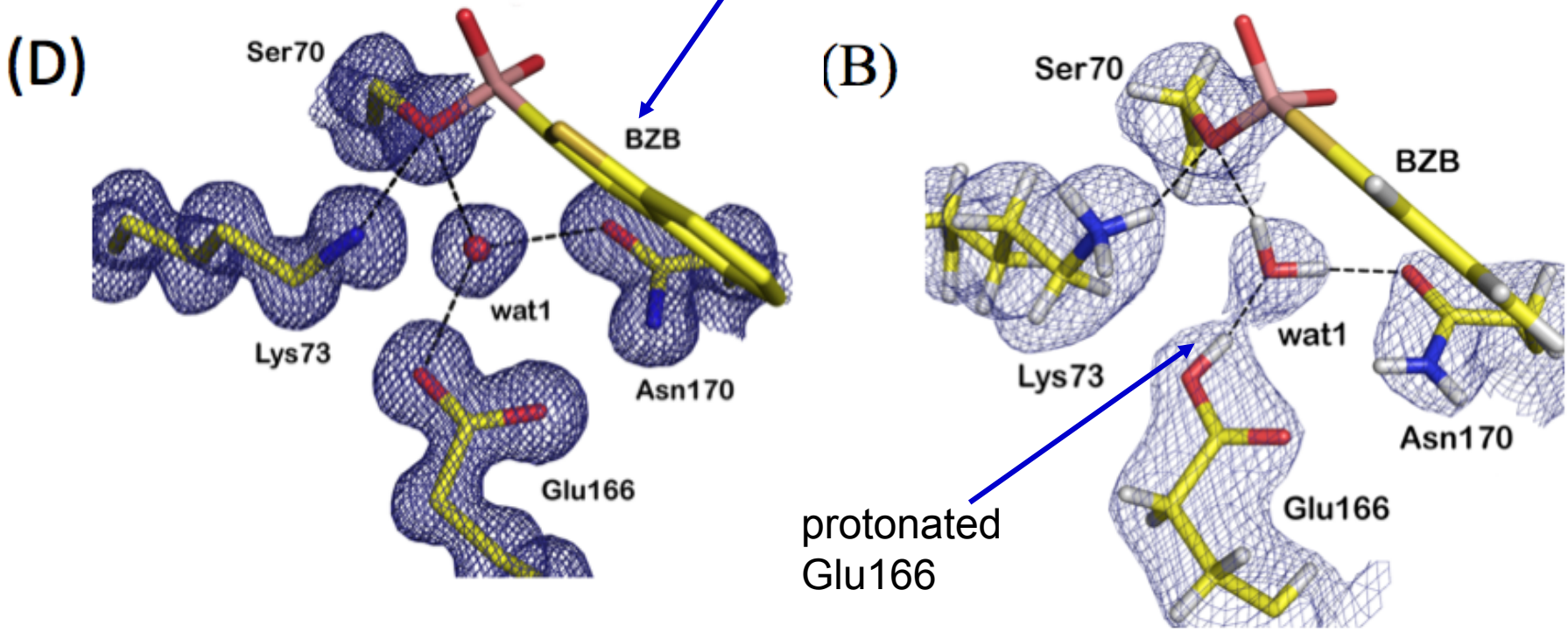
The active center of β-**lactamase** as seen by the BioDiff data set

Data collection at room temperature.

Catalytic proton network of the Toho-1 β-lactamase complexed with BZB

BZB=benzothiophene-2-boronic acid (BZB)

transition state analogue BZB



(D) electron density map

(B) nuclear density map from BioDiff, recorded with cryostream at 100 K

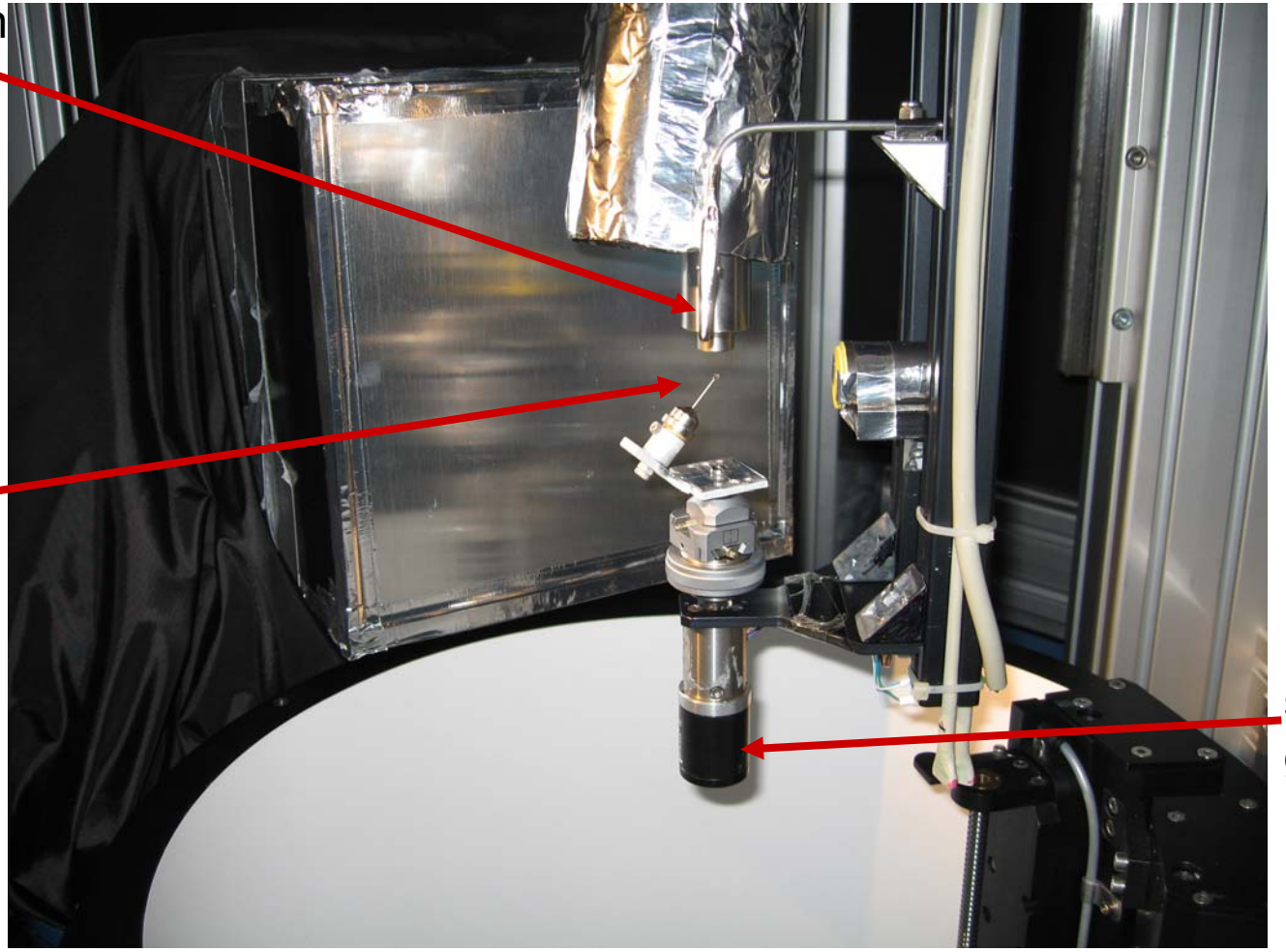
Figures from: Tomanicek et al., doi: 10.1074/jbc.M112.436238

Sample Environment options besides room temperature operation

Cryostream with or without κ-goemetry

Oxford Cryosystems cryostream nozzle

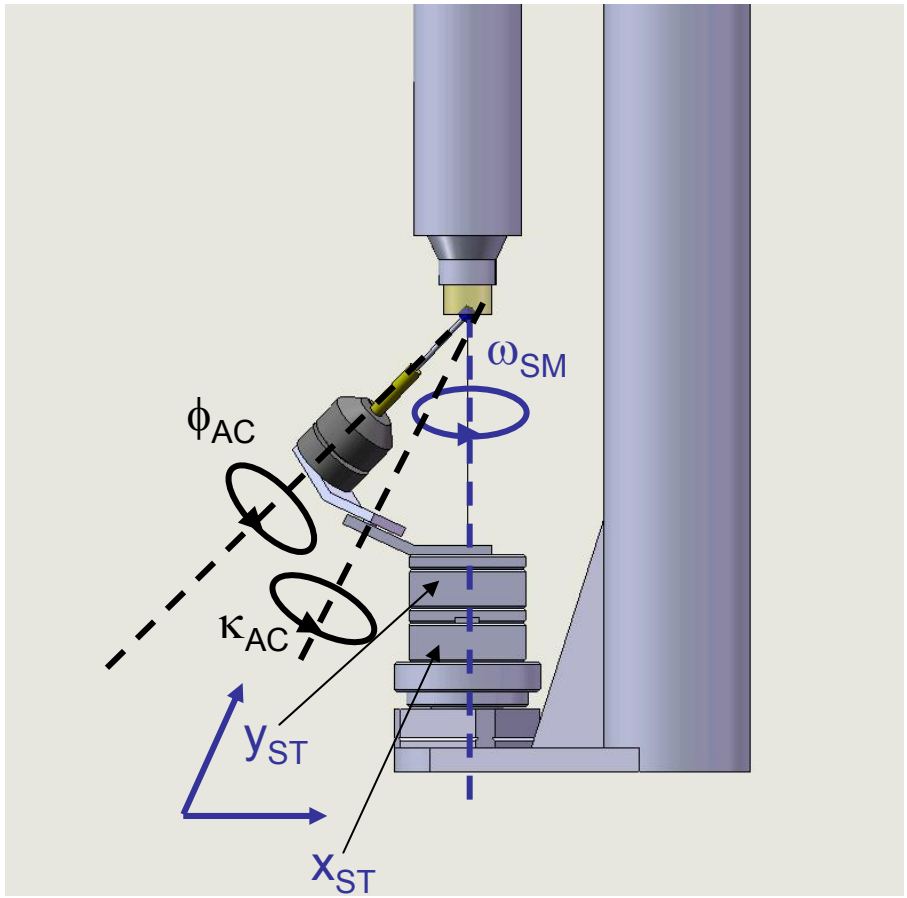
sample crystal



stepper motor goniometer

Nitrogen gas stream temperature range: 80 K to 500 K

Improved κ-geometry sample holder

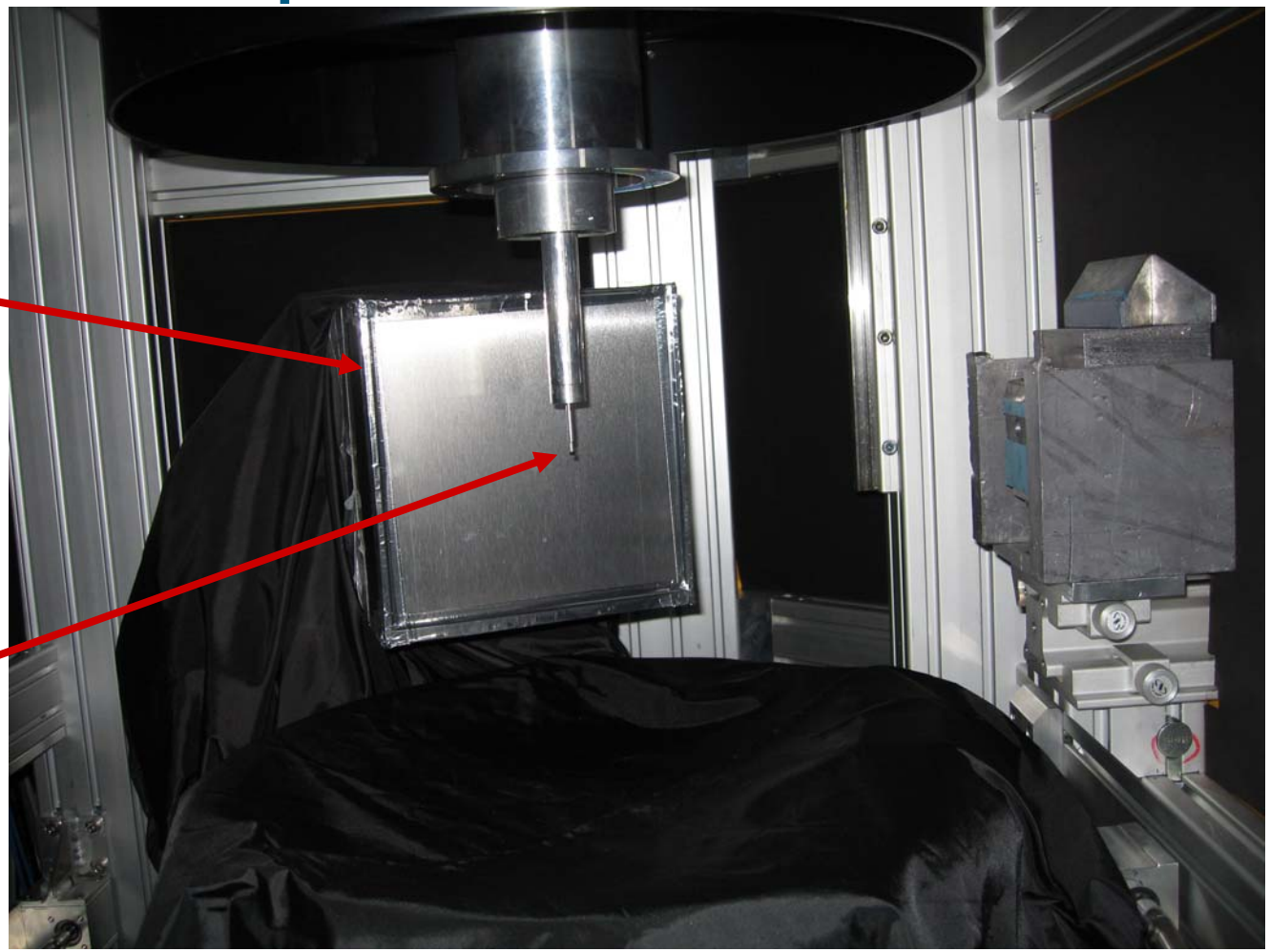


ϕ_{AC} and κ_{AC} moved manually, keeping the crystal in the cryostream

Closed Cycle Cryostat for temperatures down to 4 K

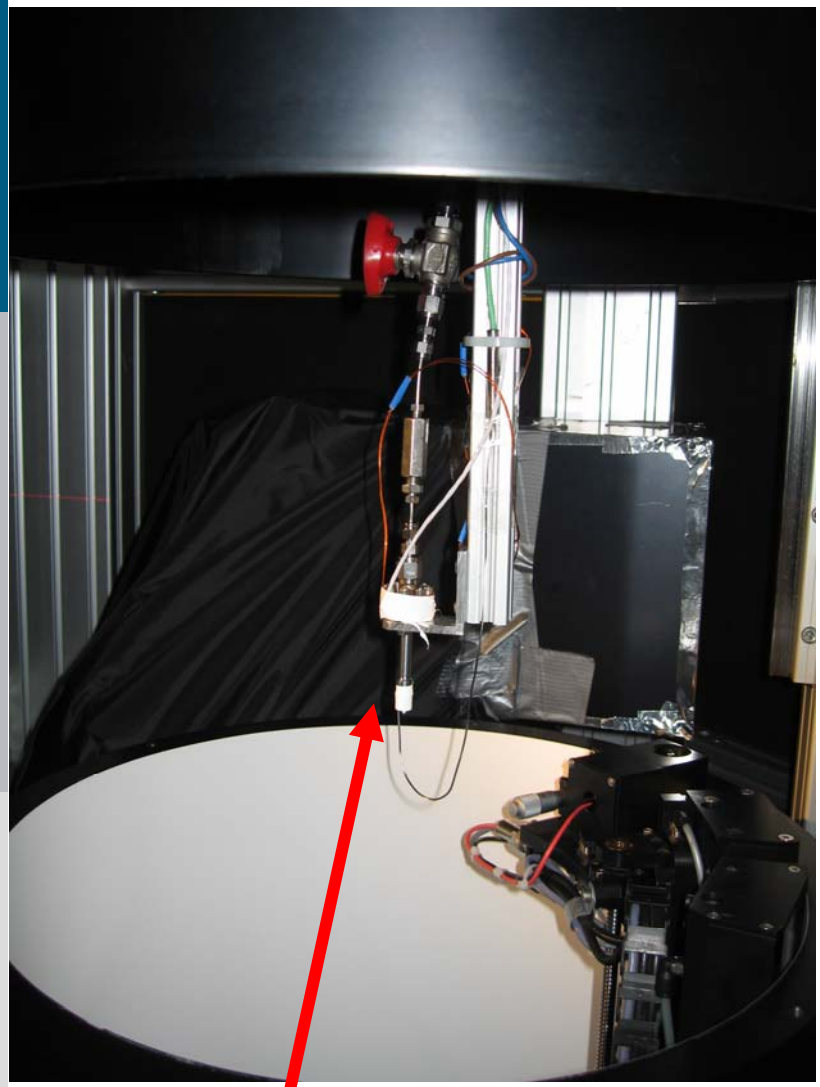
CCD-auxiliary detector can be used to align the crystal in the beam

sample crystal glued with GE-varnish

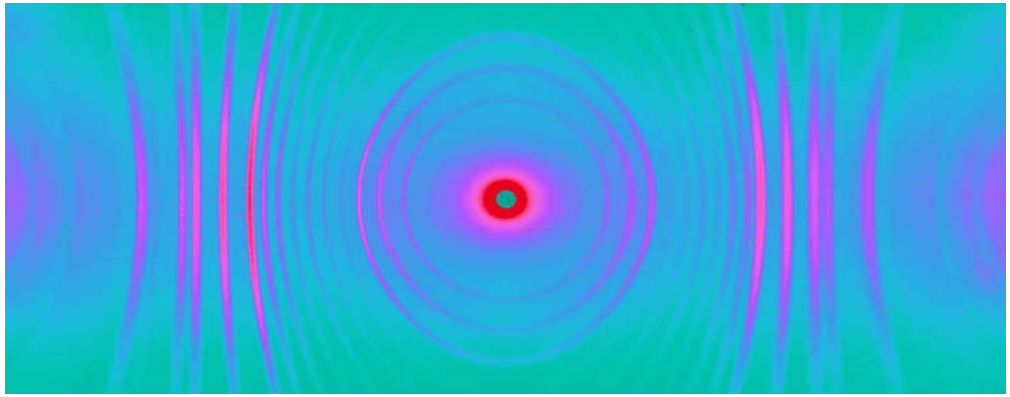


Cryostat temperature range: 4 K to 300 K (in vacuum)

BioDiff as a powder diffractometer



high pressure cell



NAG powder sample at 2.7 Å

Summary

- The instrument BioDiff is a monochromatic neutron diffractometer optimised for crystals with large unit cells.

- Besides room temperature BioDiff can offer a Cryostream which allows for temperatures between 90 K and 500 K.

- A closed cycle cryostat is available for temperatures down to 4 K.

- BioDiff can also be used as a powder diffractometer for crystal powders with large unit cell dimensions.

Thanks to... ... the BioDiff-Team:

- Philipp Jüttner
- Andreas Ostermann
- Reinhard Schätzler
- Bernhard Laatsch
- Frank Suxdorf
- Manfred Bednarek
- Matthias Drochner
- Harald Kleines
- Kevin Körrentz
- Karl-Heinz Mertens
- Michael Monkenbusch
- Michael Wagener
- Heinrich Pohl
- Vladimir Ossovyi
- Andreas Nebel
- Simon Staringer
- Winfried Petry
- Severin Denk
- Dieter Richter

and you for your attention!

The End

Comparison between a data set recorded at BioDiff and LADI III

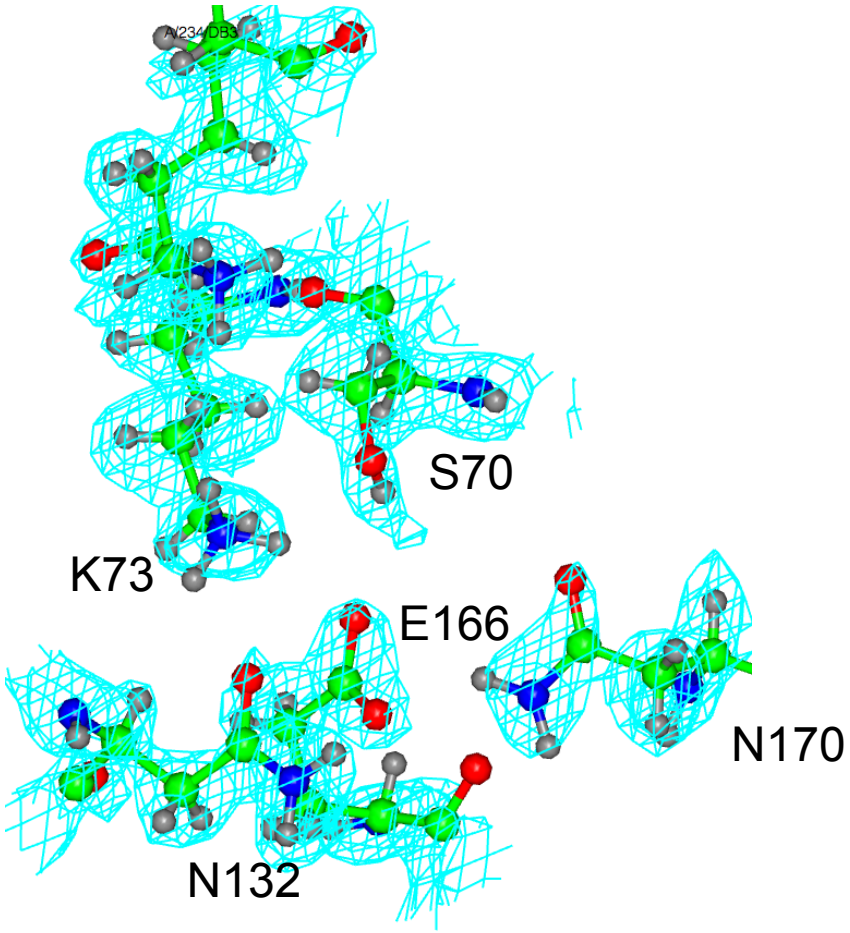
β-lactamase	LADI III @ ILL[1]	BioDiff @ FRM II
used beam time	?	8.3 days
Unit-cell parameters (Å)	a=72.92, b=72.92, c=98.53; α=β=90°, γ=120°	a=73.26, b=73.26, c=98.66; α=β=90°, γ=120°
Space group	$P3_221$	$P3_221$
No. of unique reflections	14 991	18 914
Resolution range (Å)	63.15-2.10 (2.21-2.10)	50-2.0 (2.07-2.0)
Multiplicity	7.2 (6.2)	2.0 (1.5)
Mean ((I)/sd(I))	10.1(8.2)	6.5(3.2)
Data completeness (%)	83.7(59.4)	93.6(89.6)

Crystallographic refinement	LADI III @ ILL	BioDiff @ FRM II[2]
R_{factor} (%)	22.5	23.3
R_{free} (%)	25.9	25.2

[1]S. J. Tomanicek et al., FEBS Letters (2011) 585, 368

[2]ridig body refinement and 1x minimize, no water

β-lactamase: binding pocket without substrate



water was not included in the refinement

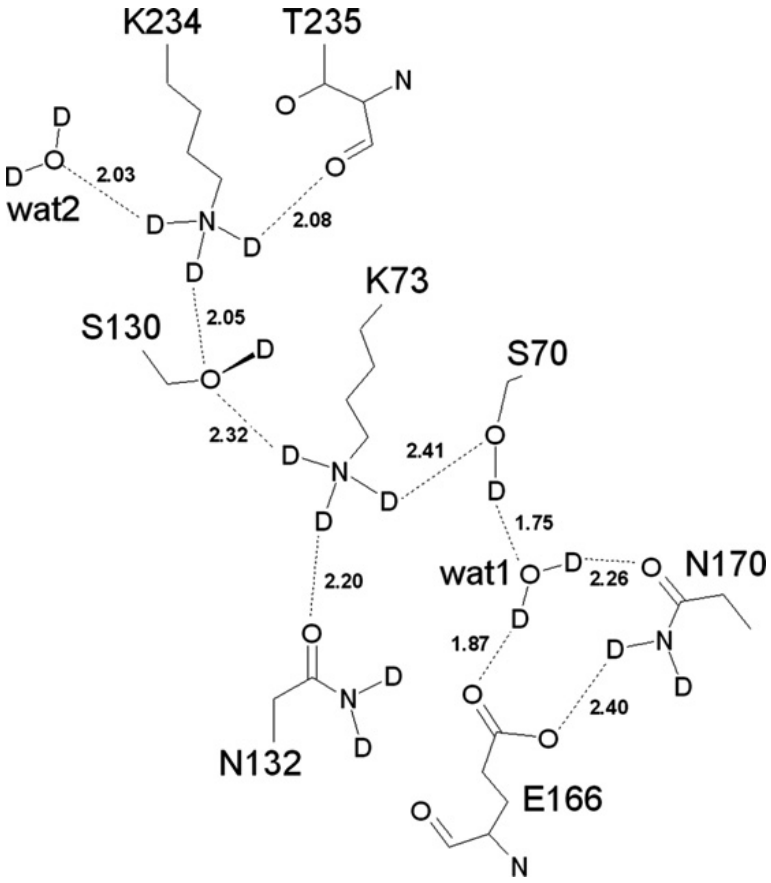
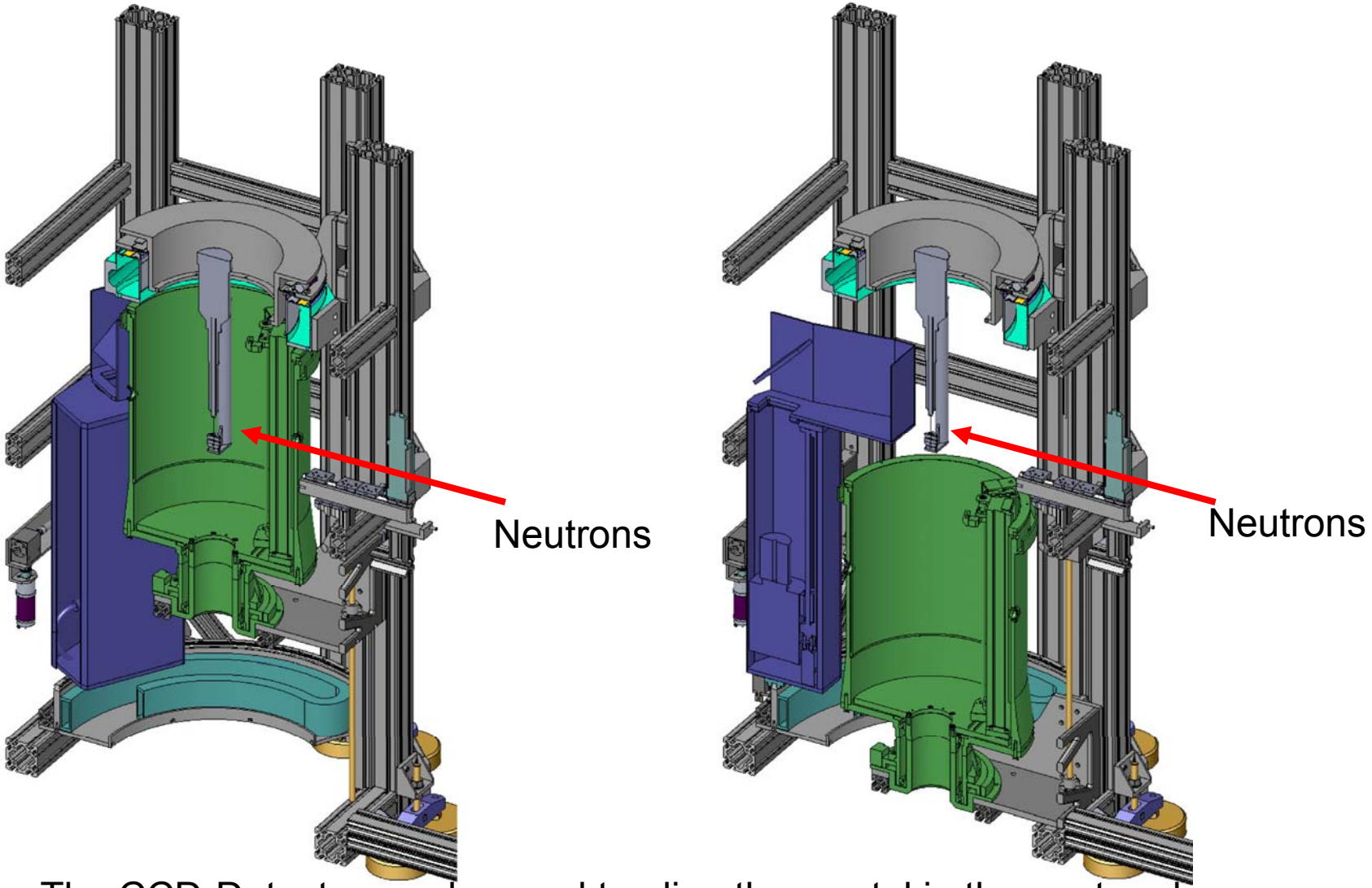


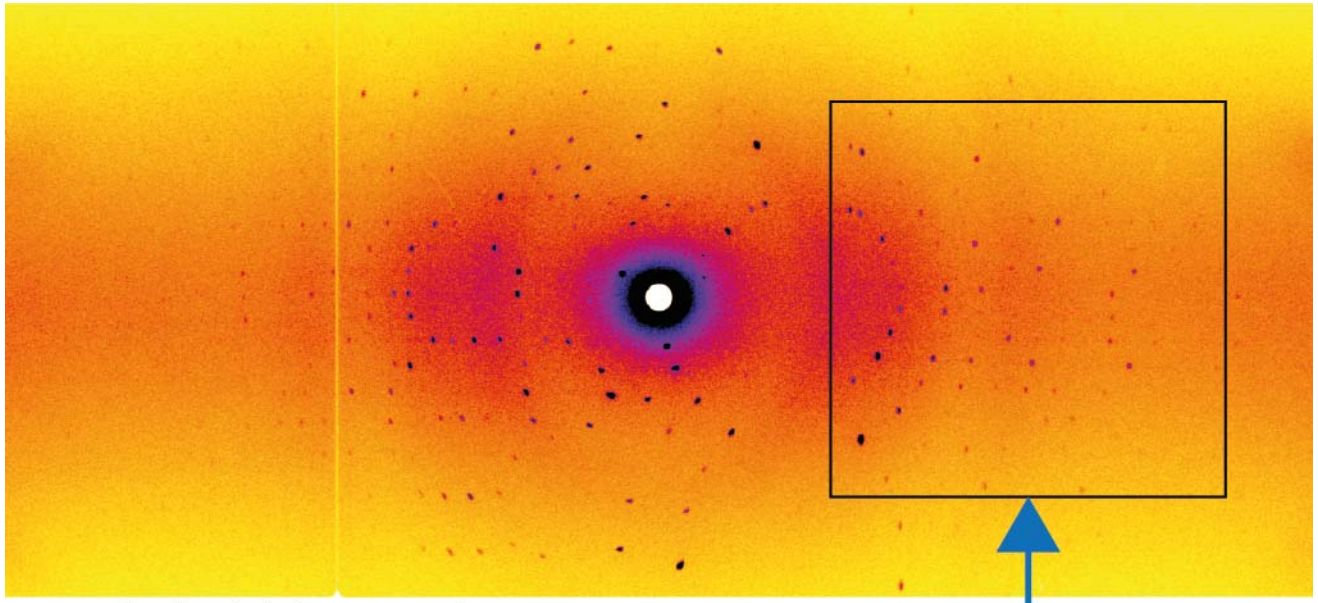
Fig. 3. Summary of the hydrogen-bonding network (shown with dashed lines) and bond distances (Å) in the active site region of the pd-Toho-1 R274N/R276N.

Switching between imageplate and CCD detector



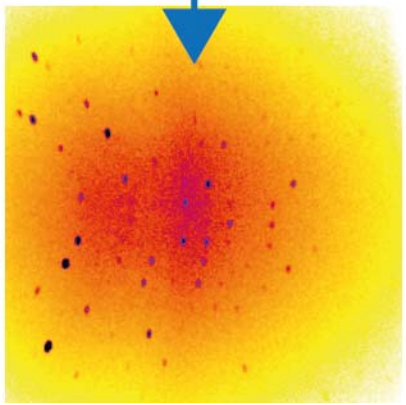
The CCD-Detector can be used to align the crystal in the neutron beam.

Instrument Characterization: The two detectors



recorded with image plate detector

region of interest shown above recorded with CCD-detector



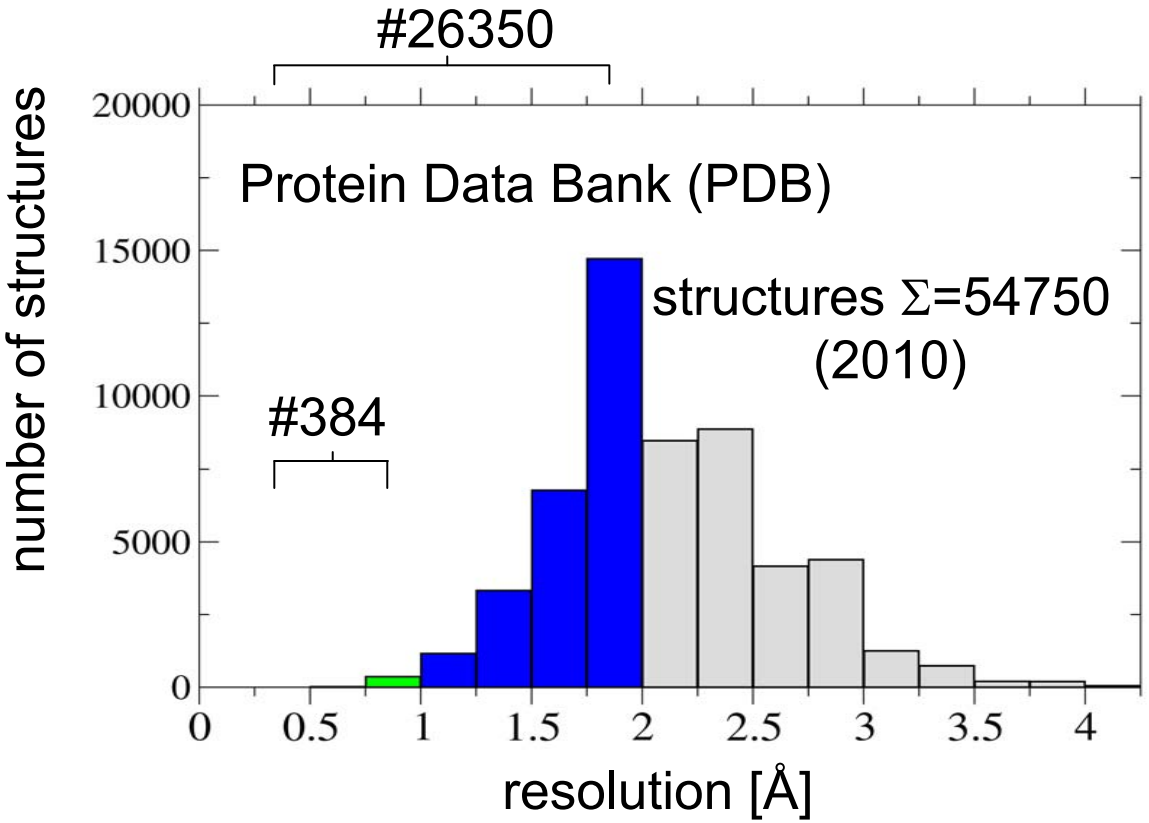
Very little x-ray structural studies give access to hydrogen positions

X-ray:

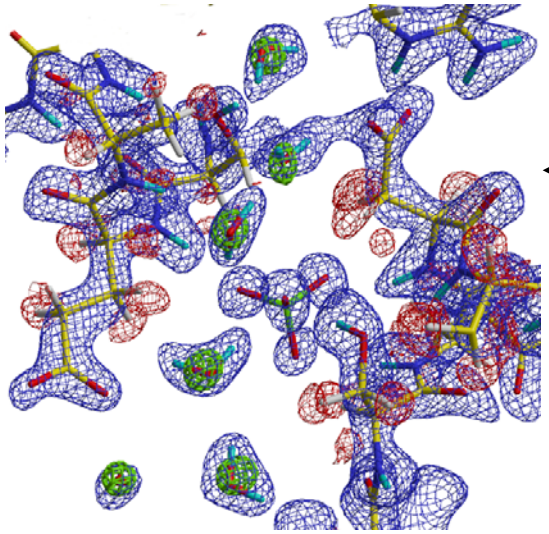
hydrogens visible at resolution of $d_{min} \leq 1$Å

neutrons:

hydrogens visible at resolution of $d_{min} \approx 2$Å (for ^{2}H)



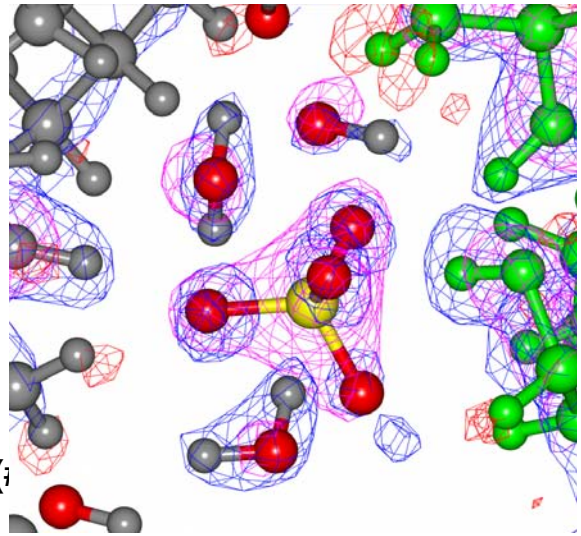
HYDRATION STRUCTURE:

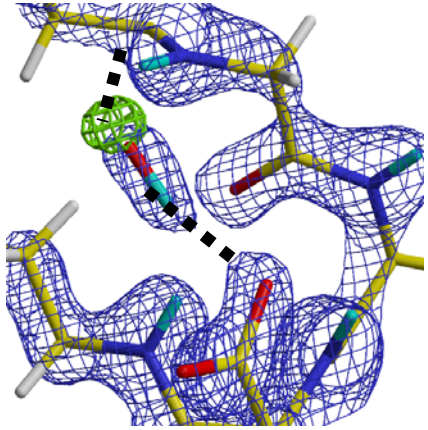


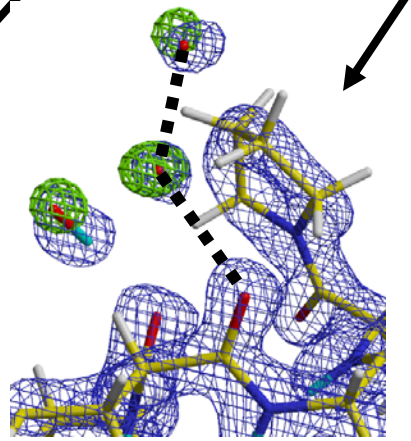
TRIANGULAR

ELLIPSOIDAL (#10)

SPHERICAL (







- All types of shapes can be found on the protein surface
- Triangular shapes are only found in direct contact with the protein surface
- Shape depends on the environment

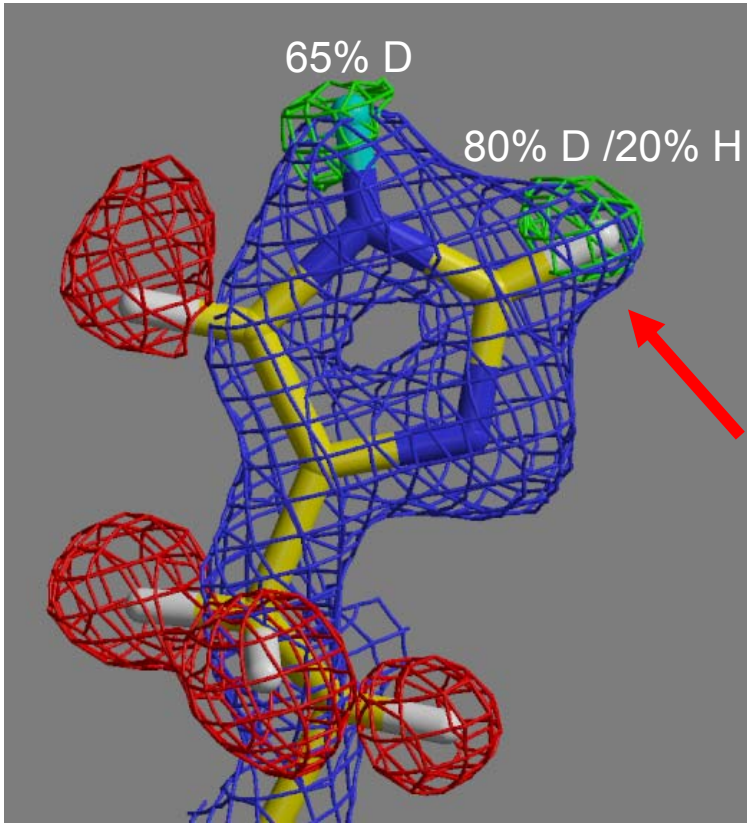
$2F_o$-F_c neutron map, +1.5σ

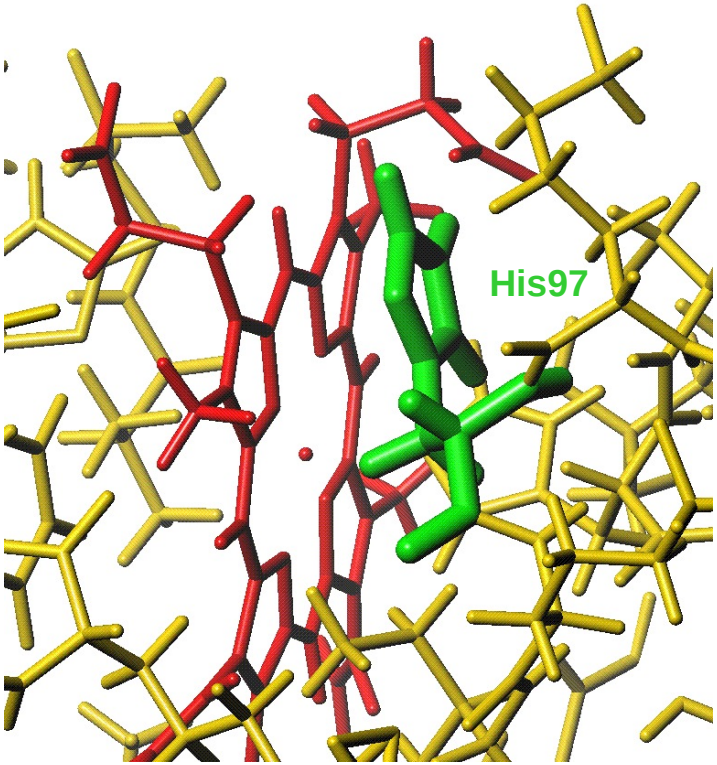
$2F_o$-F_c neutron map, -2.0σ

$2F_o$-F_c X-ray map

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

PROTONATION STATES OF AMINO ACIDS:





EXAMPLE HIS-97:

Fo-Fc OMIT-map; +3.0σ

Fo-Fc OMIT-map; -3.0σ

2Fo-Fc map; +1.5σ

OMIT: H, D

→ $C_{\varepsilon 1}$-H group shows acidic behavior

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

Other neutron protein diffractometers in the world:

Table 2

Neutron diffractometers available for protein crystallography

Diffractometer	Method	Flux at sample position (n/(cm^2 s))	Typical crystal size (mm^3)	Cell parameters (Å)	Necessary days to collect data (days)	Resolution (Å)	Power target (spallation source)	Power (reactor)
BIX-3 (JAEA)	Monochromatized 2.9 Å	2.9×10^6	>1	<100	>20	>1.4		20 MW
BIX-4 (JAEA)	Monochromatized 2.6 Å	4.5×10^6	>1	<100	>15	>1.4		20 MW
iBIX[a] (J-PARC)	Pulsed white (TOF) 25 Hz (0.7–3.8 Å)	2.1×10^8 at 0.20° divergence	>0.1	<150	>3	>1.2	1 MW Hg target	
LADI-III (ILL)	Continuous source quasi-Laue (multilayer band-pass filters with $\delta\lambda/\lambda$ from 5 to 25%)	3×10^7 using $\delta\lambda/\lambda = 20\%$ centered at $\lambda = 3.5$ Å	>0.1 (if perdeuterated) >0.5 otherwise	<160	>3	>1.4		58 MW
PCS (LANSCE) [46,47]	Pulsed white (TOF Laue) 20 Hz (0.6–6 Å)	9.7×10^6 at 0.12° divergence	>0.3	<180	>4	>1	0.1 MW W target	
MaNDi[a] (SNS)	Pulsed white (TOF Laue) 60 Hz, adjustable wavelength range with $\Delta\lambda \sim 2.7$ Å	1.2×10^7 at 0.23° divergence and 6.9×10^7 at 0.56° divergence	>0.1	<150	>1	>1.5	2 MW Hg target	

[a] If under construction.

Blakeley et al. Current Opinion in Structural Biology 18 (2008) 593-600

Comparison with the BioDiff instrument:

	monochro-mator	λ	Δλ/λ	hor. divergence	vert. divergence	flux n / cm² s
FRM II BioDiff	PG002	2.4Å	2.9%	0.82°	0.7°	$9.0 \cdot 10^6$
		4.0Å	2.5%	1.2°	0.7°	$7.6 \cdot 10^6$
	Ge311	2.4Å	1.4%	0.83°	0.7°	$2.6 \cdot 10^6$
		2.7Å	1.2%	0.95°	0.7°	$2.3 \cdot 10^6$
		3.0Å	0.9%	1.0°	0.7°	$1.7 \cdot 10^6$
BIX-4	Si111	2.6Å	2.1%	0.8°	1.75°	$4.5 \cdot 10^6$
LADI(III)		3.2Å	≈20%			$3.0 \cdot 10^7$