Neutron protein crystallography at the Heinz Maier-Leibnitz Zentrum (MLZ)

New developments and recent application examples

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Joint Polish-German Crystallographic Meeting, Wroclaw 2020

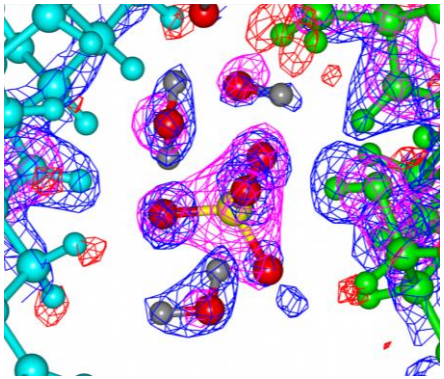
Advantages of Structure Determination with Neutrons

Hydrogen/deuterium atoms can be resolved even at a resolution of $d_{min} \approx 2.5$ Å (for ^2H). Therefore one can determine:

→ protonation states of amino acid side chains and ligands

→ deuterium exchange as a measure of flexibility and accessibility (discrimination between **H** / **D**)

→ solvent structure including hydrogen atoms



Water network in the contact region between two myoglobin molecules in the crystal.

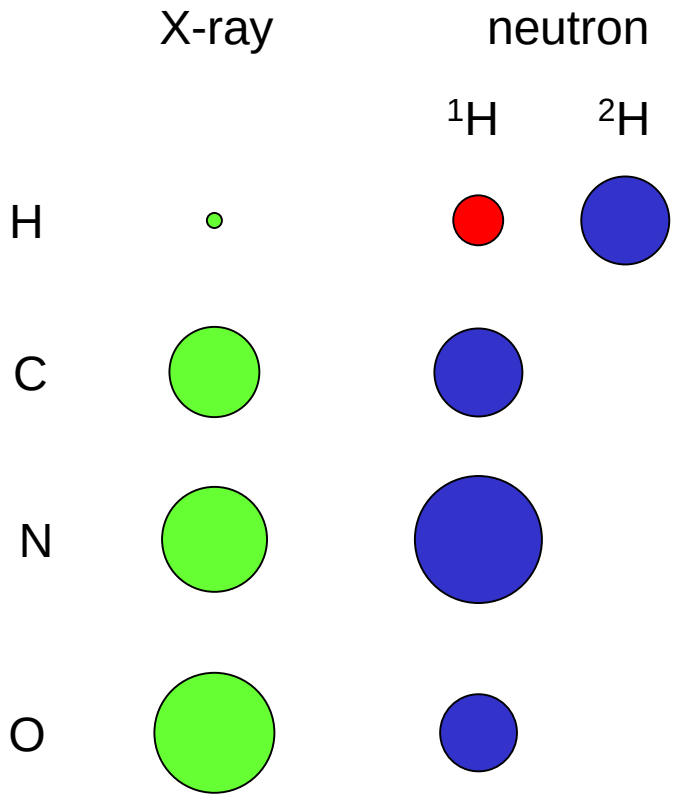
x-ray map (magenta): contour level of +2.7σ
nuclear map (red): contour level of -1.75σ
nuclear map (blue): contour level of +2.3σ

Much less radiation damage as compared to x-rays: Metallo-proteins can be measured without reducing the metal centres

Advantages of structure determination with neutrons:

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

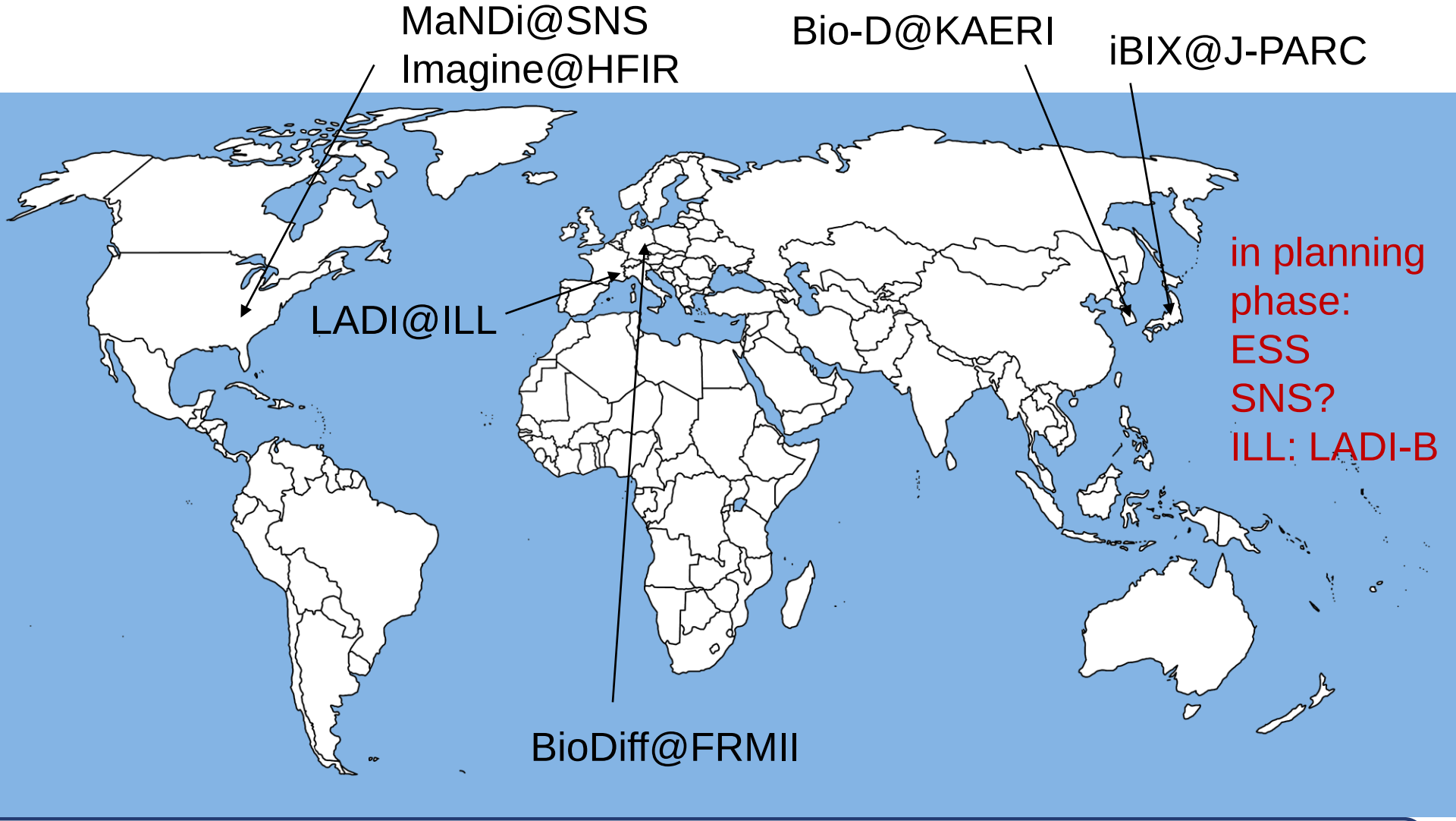
Nucleus	atomic number	scattering length [10^{-12} cm]
^{1}H	1	**-0.378**
^{2}H	1	**0.667**
^{12}C	6	**0.665**
^{15}N	7	**0.921**
^{16}O	8	**0.581**



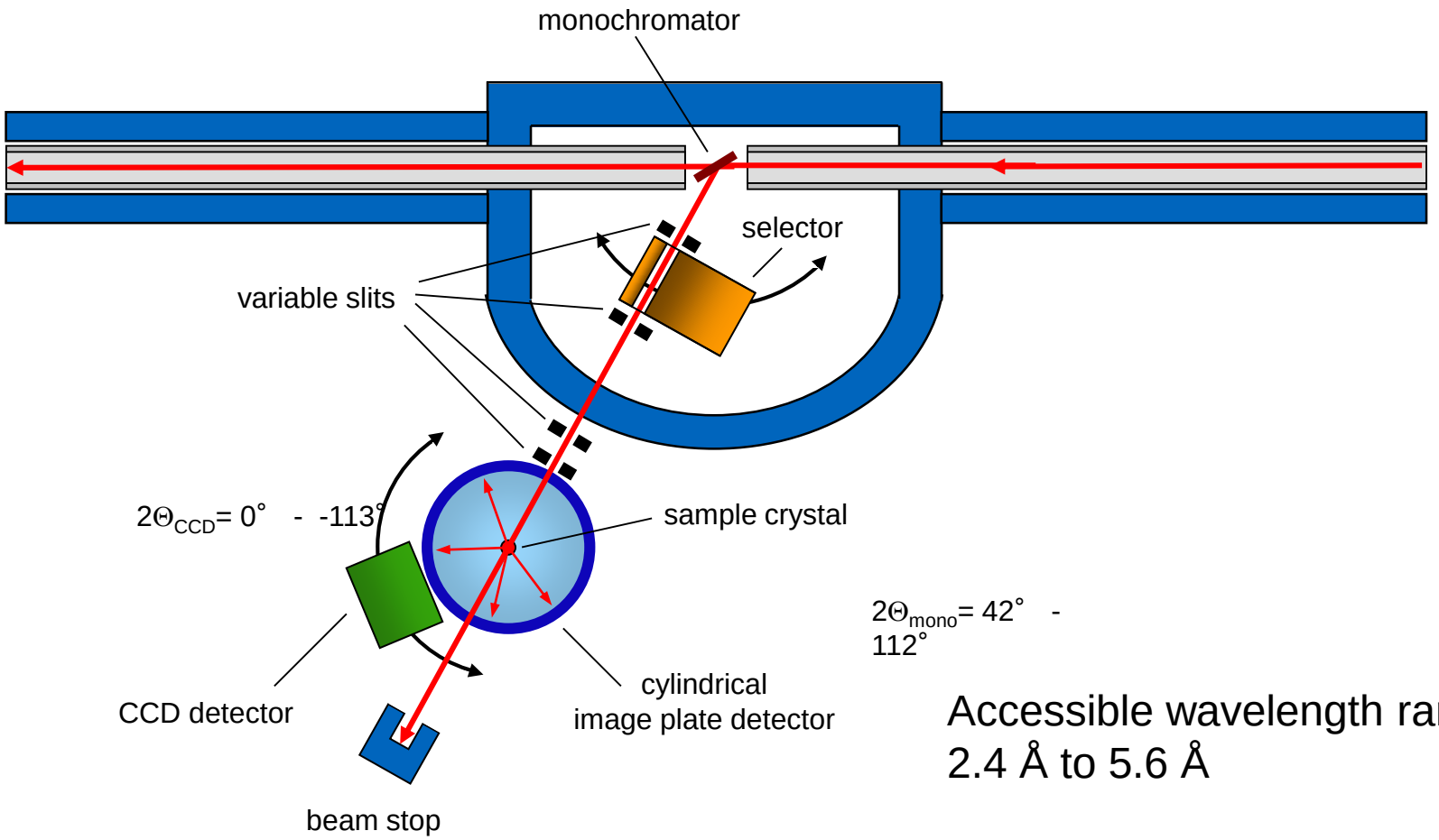
diameters correspond to:
form factor / scattering length
(scaled for C-atom)

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!

World map of neutron diffractometers optimized for protein crystals

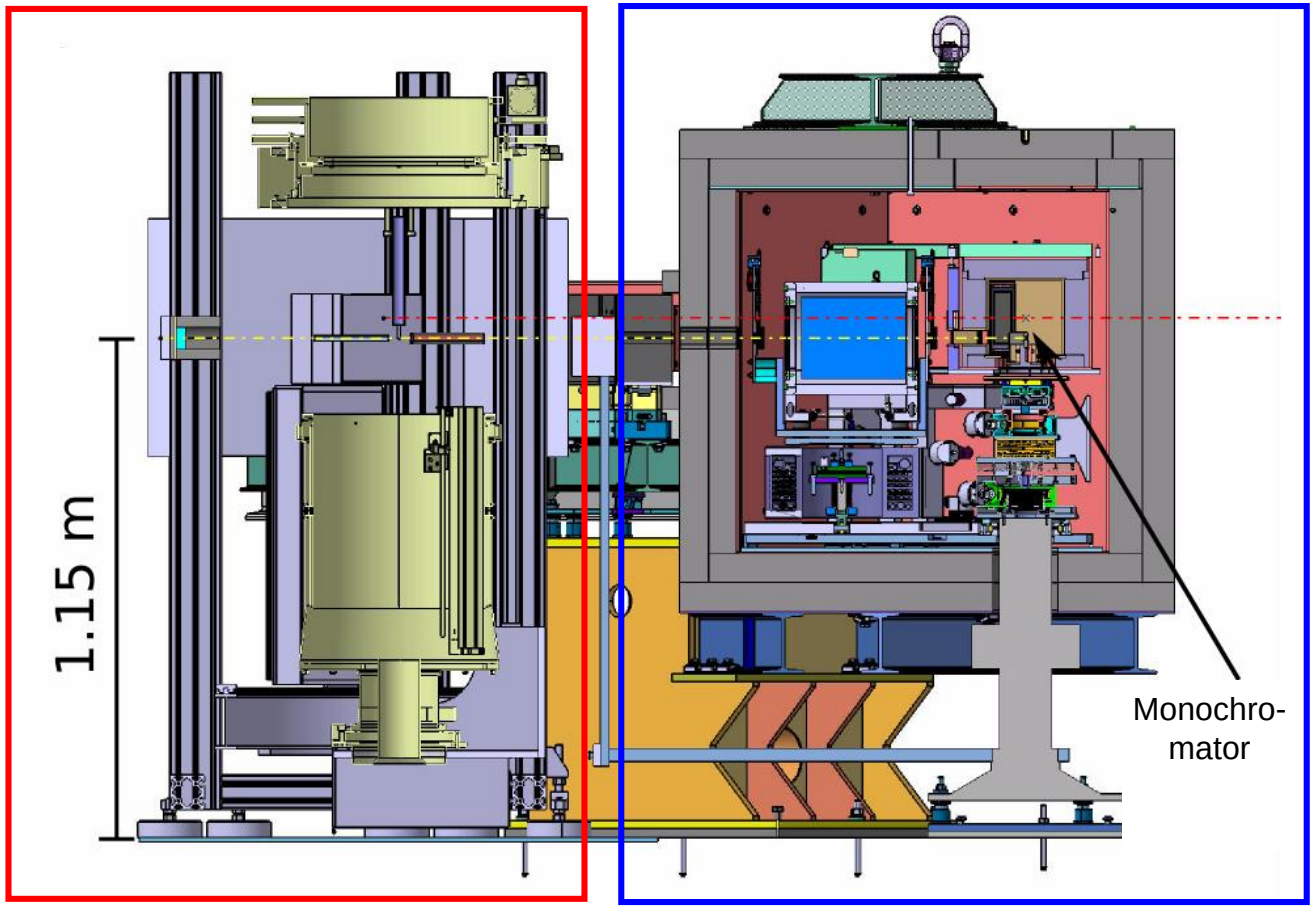


Schematic Overview over the Instrument BioDiff



Accessible wavelength range:
2.4 Å to 5.6 Å

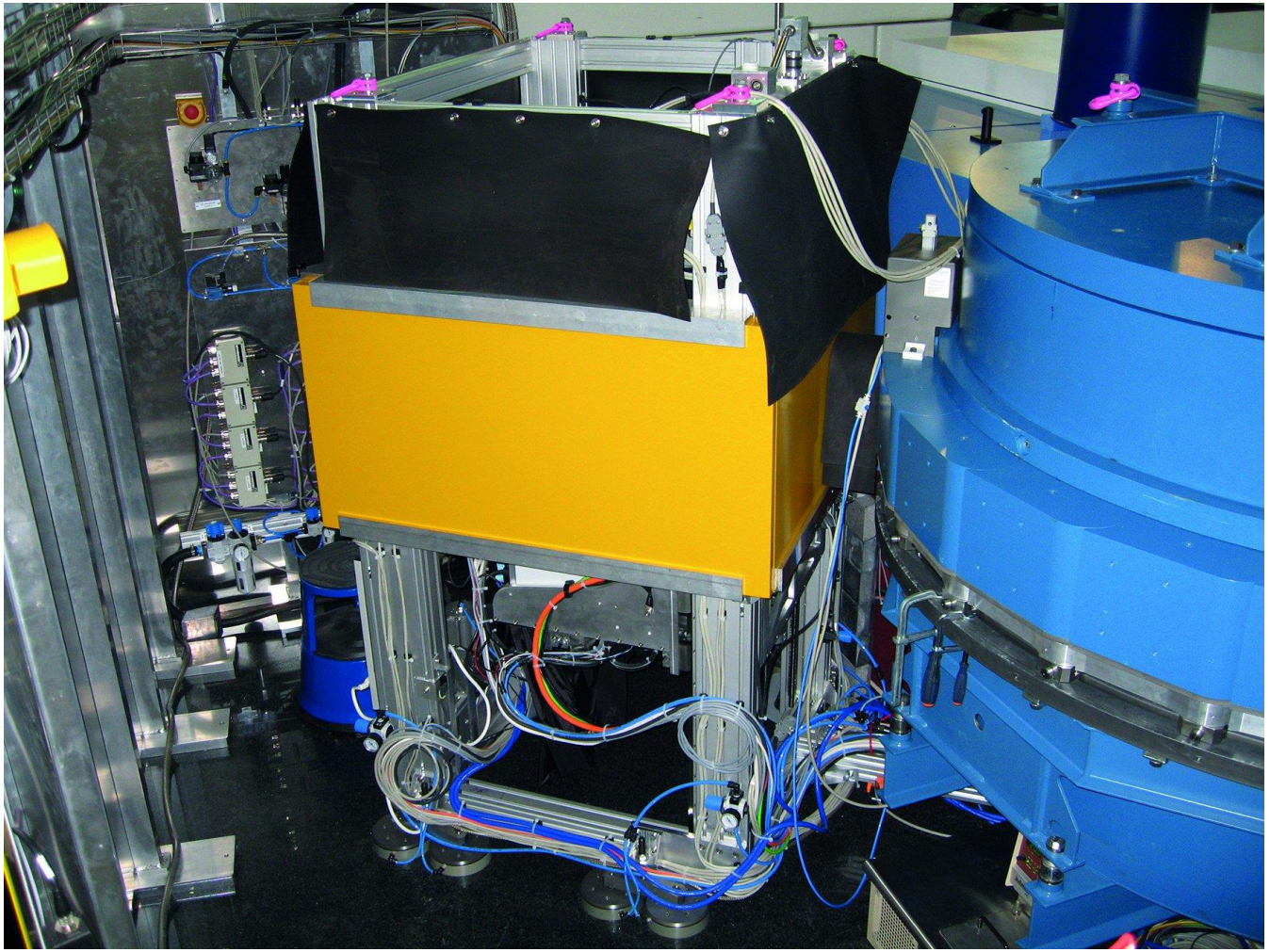
The Simultaneous Construction-phase in Garching and Jülich



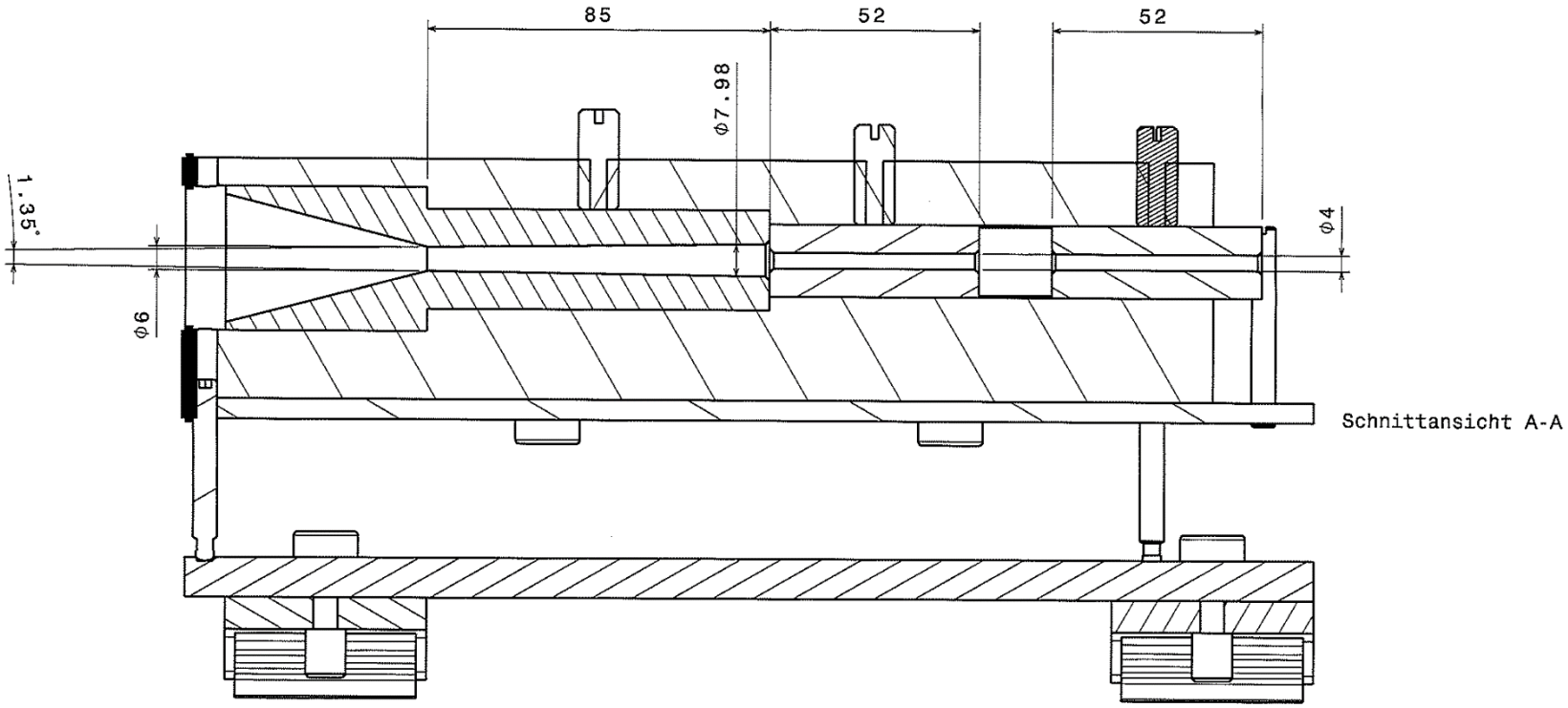
Detector unit, constructed and built in Garching (Ph. Jüttner, MLZ)

Monochromator-shielding, constructed and built in Juelich (B. Laatsch, ZEA-1 Engineering)

A Most Recent View of the Instrument BioDiff

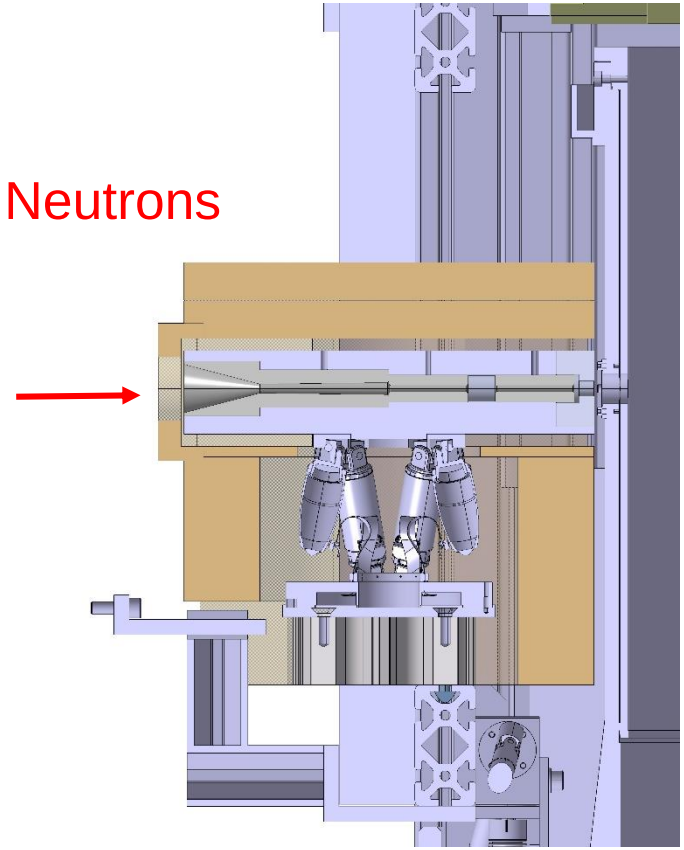


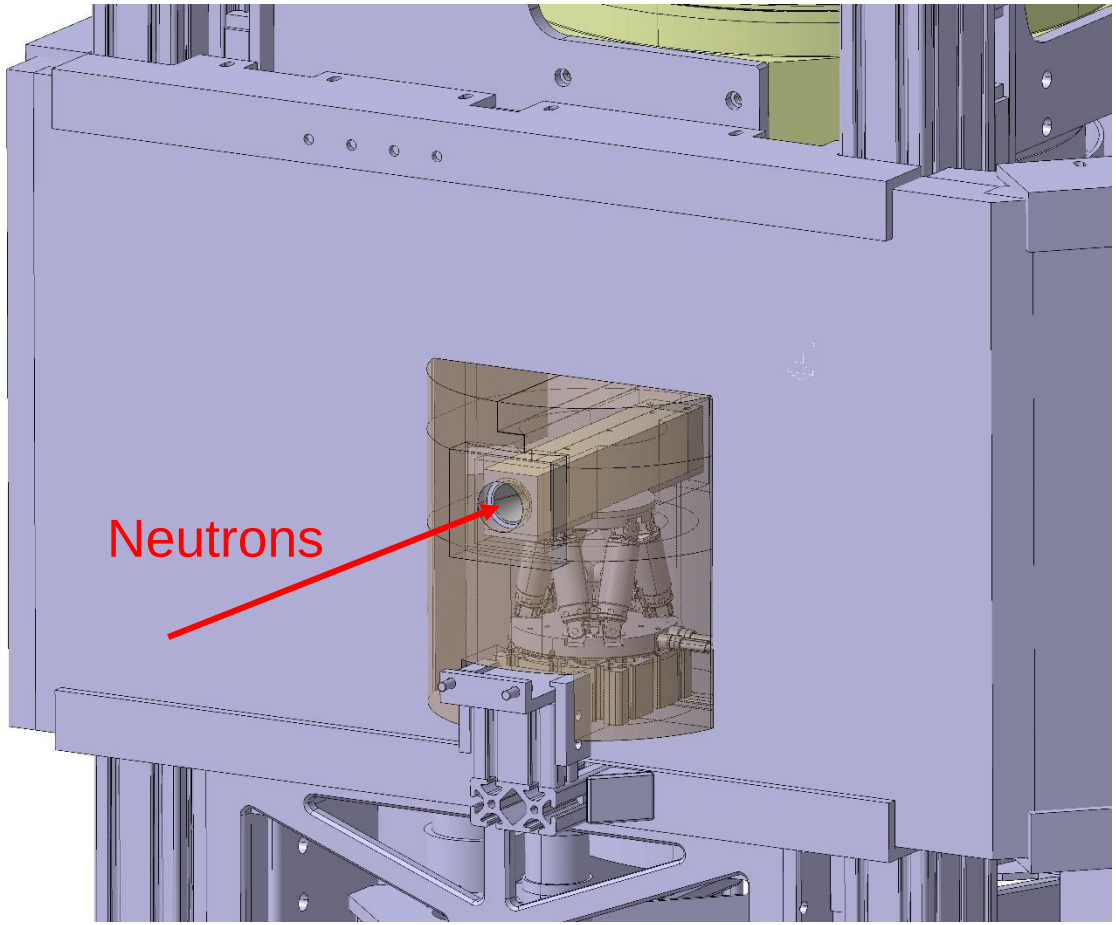
Cross section through the existing collimator



Monochromator housing → Neutrons → Detector housing

New Beram collimation between monochromator and detector housing

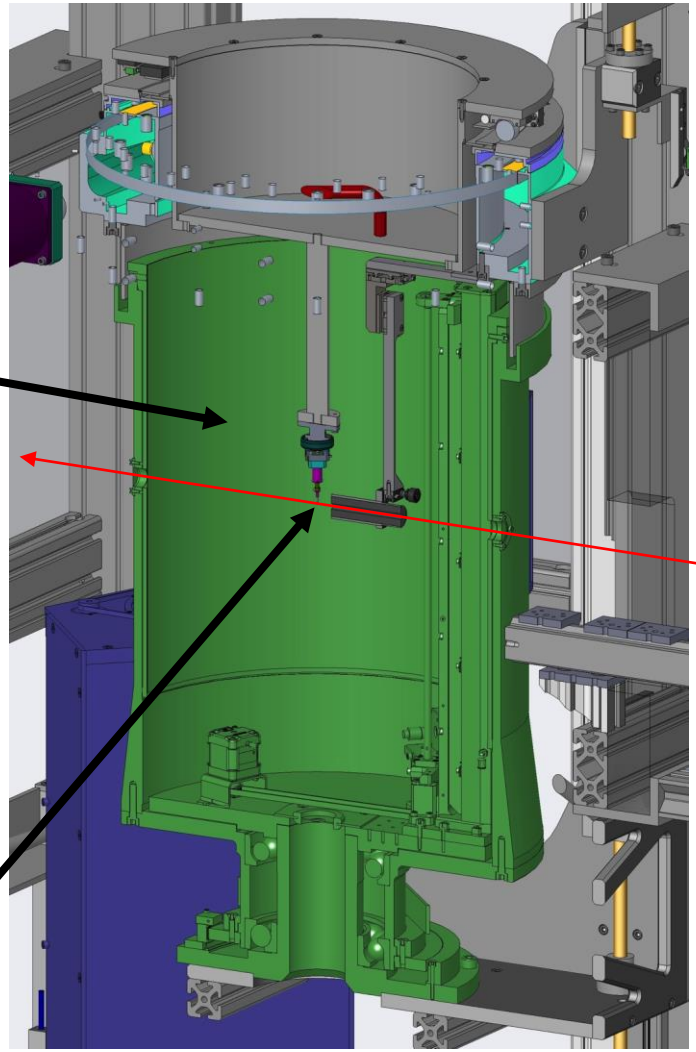




Detector Unit: Neutron Image Plate

neutron image plate

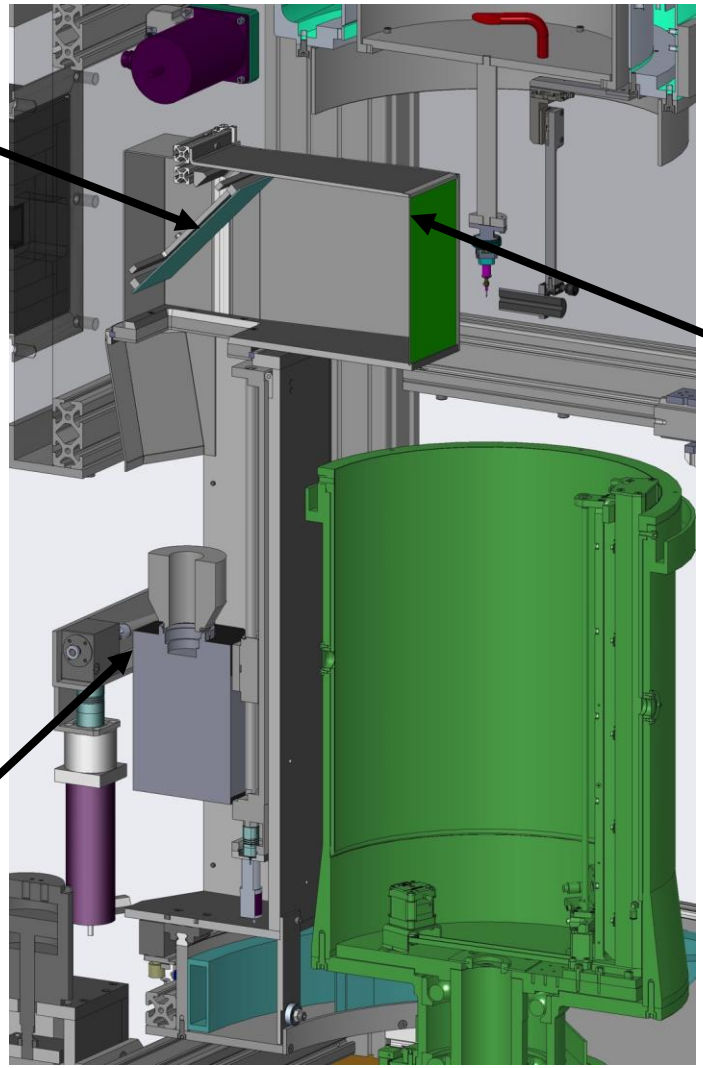
- Gd_2O_3 / $BaFBr:Eu^{2+}$ (white Niimura-type)
- cylindrical shape: r = 200mm; h = 450mm
- scanner resolution: 125, 250, 500 μm
- readout time + erasing: ≈ 4min (500 μm)

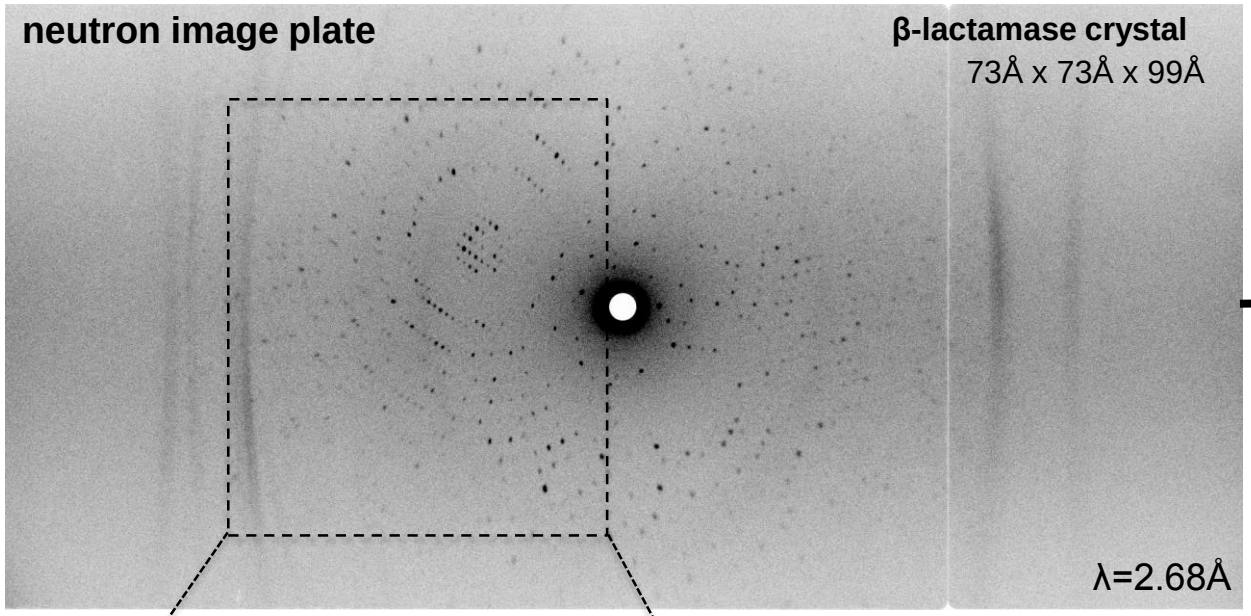


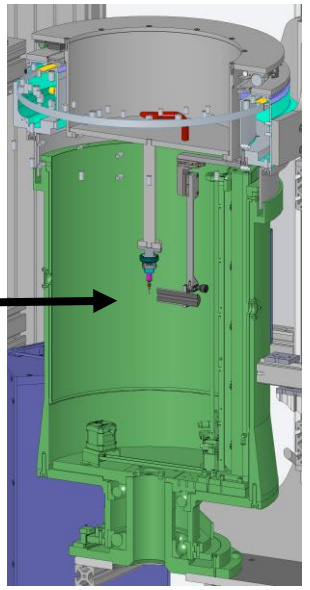
Detector Unit: CCD-camera

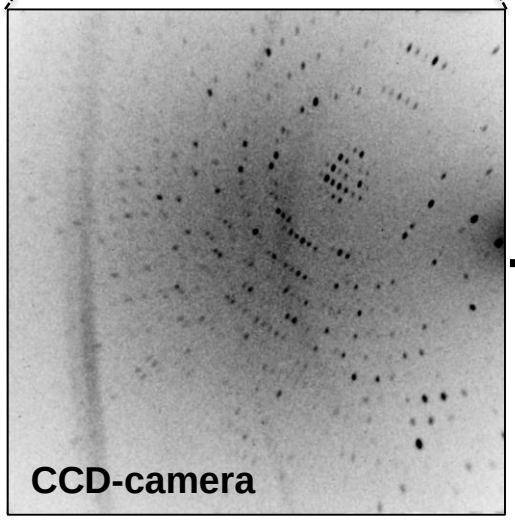
Scintillator based CCD-camera

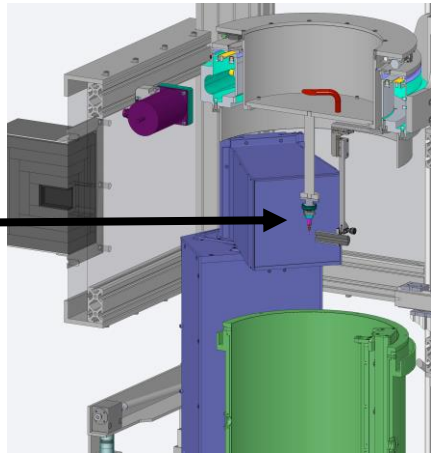
- ^{6}LiF/ZnS scintillator
- flat shape: 200 x 200mm
- overall resolution: ≈300 μm
- Andor iKon-L CCD camera
- readout time: ≥1sec











NIP-scanner

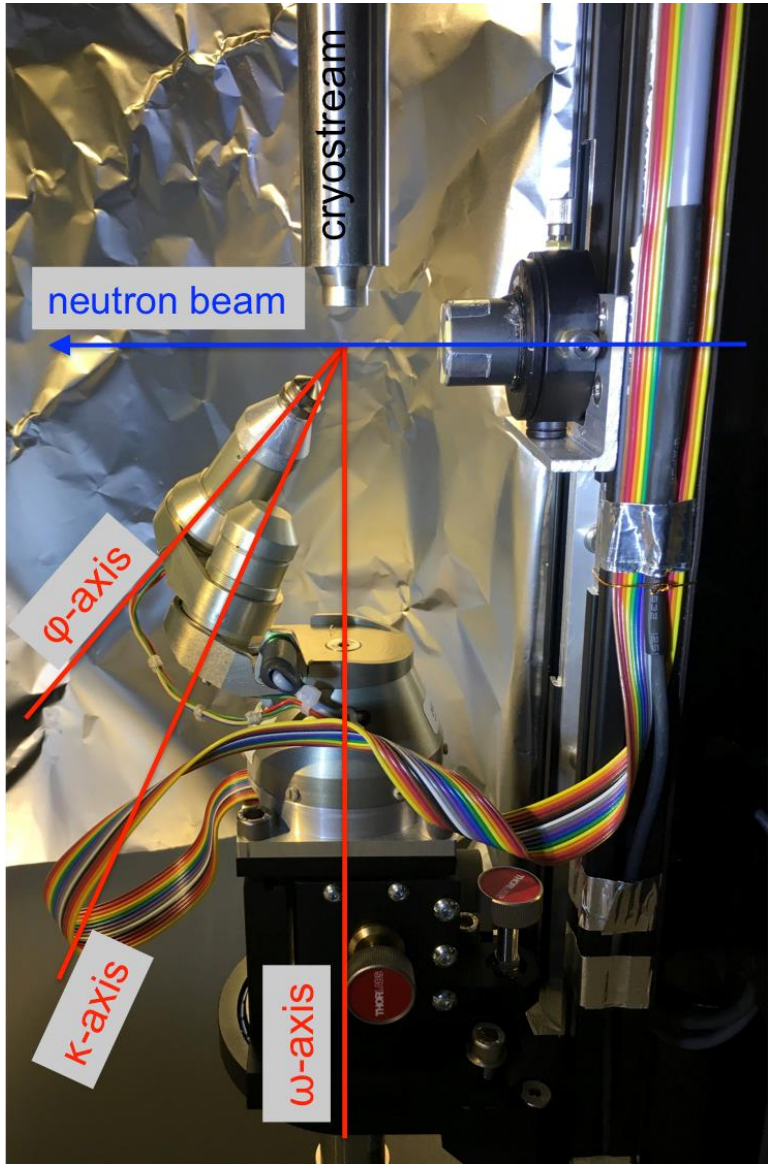
- larger covered solid angle
- readout time ≥ 4min

CCD-camera

- smaller covered solid angle
- readout time ≥ 1sec

BioDiff Upgrade:

mini-kappa-goniometer
with standard Oxford instruments cryostream

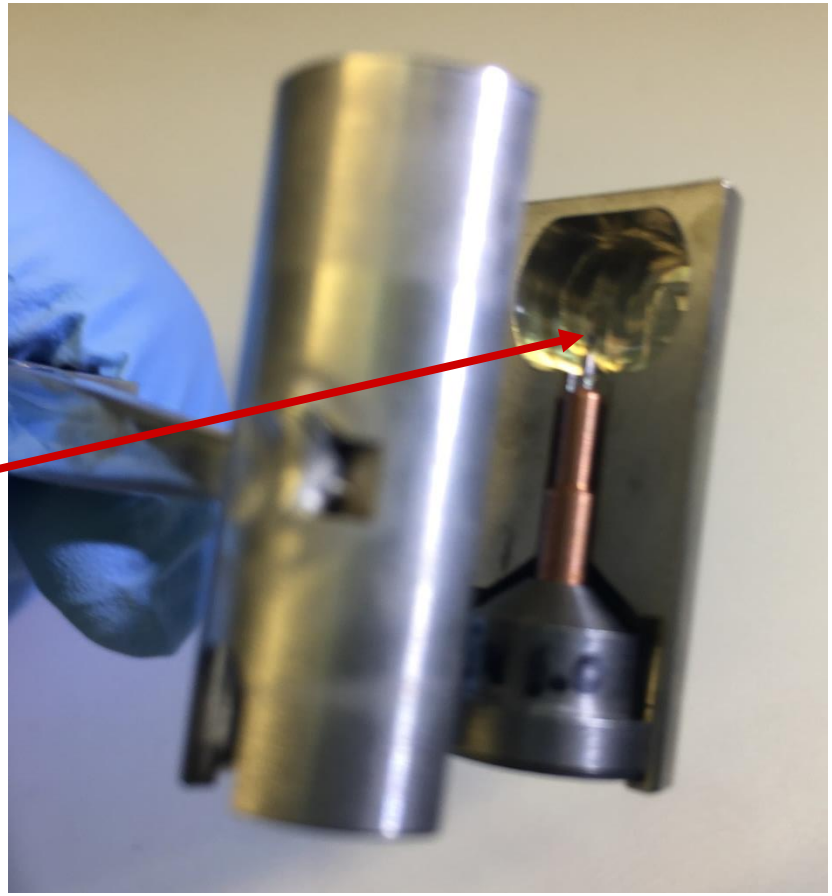


➡ optimizing data collection strategy

↷ save precious beam time / increase data set completeness

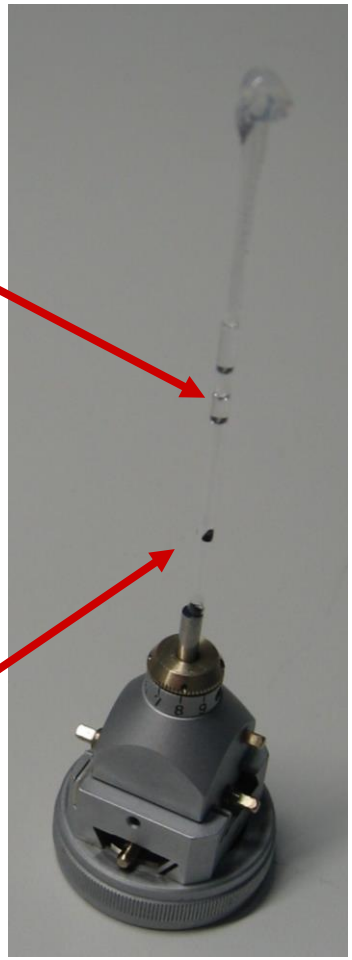
➡ no manual crystal re-mounting necessary for changing the crystal orientation under cryo conditions

Cryo-mounting of large crystals

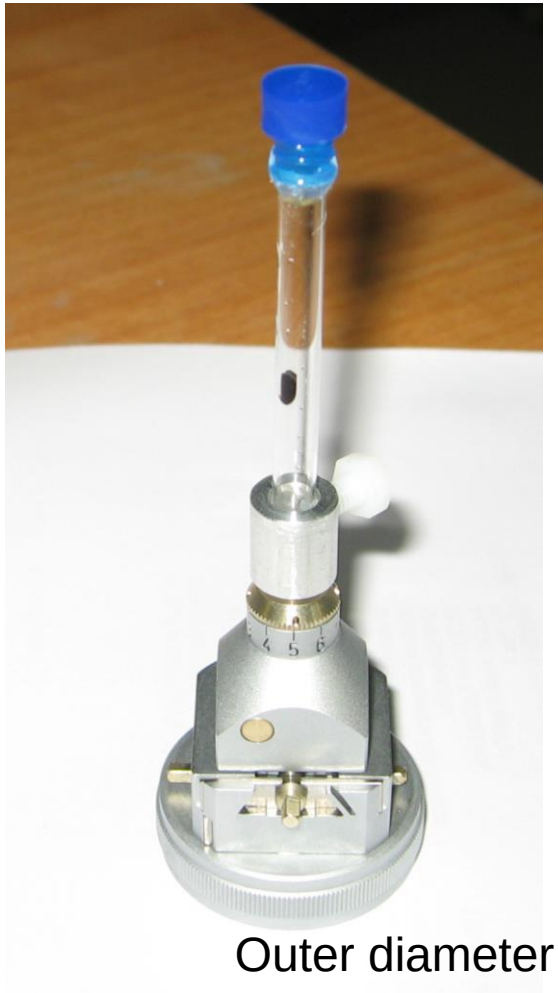


- Avoid hydrogenated polymers in the loop, use capton (Mitigen) or carbon meshes instead (especially when you have a fully deuterated protein)
- Make sure that your crystal fits into the cryoTong: We prefer the 18 mm one.

Room temperature packaging of crystals



Outer diameter of the glas tube: 2 mm

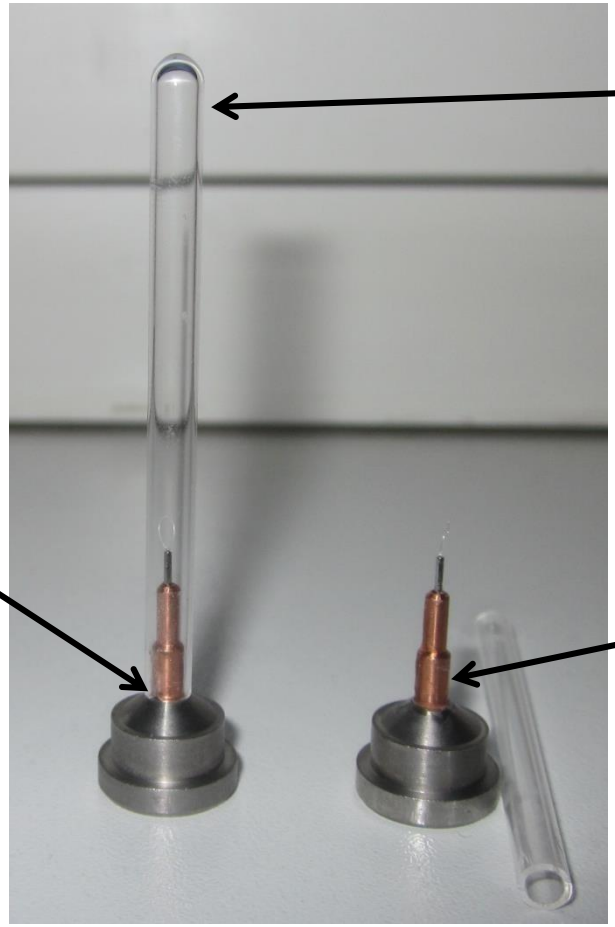


Outer diameter of the glas tube: 5 mm

- Avoid boron glas, since boron absorbs neutrons, use quartz glas instead
- Leave as little mother liquor around the crystal as possible, put a droplet of mother liquor at one end of the capillary instead.

New room temperature mounting scheme: Fish and Seal

Using standard equipment from Mitigen and Sigma Aldrich (capillary).



Droplet of mother liquor

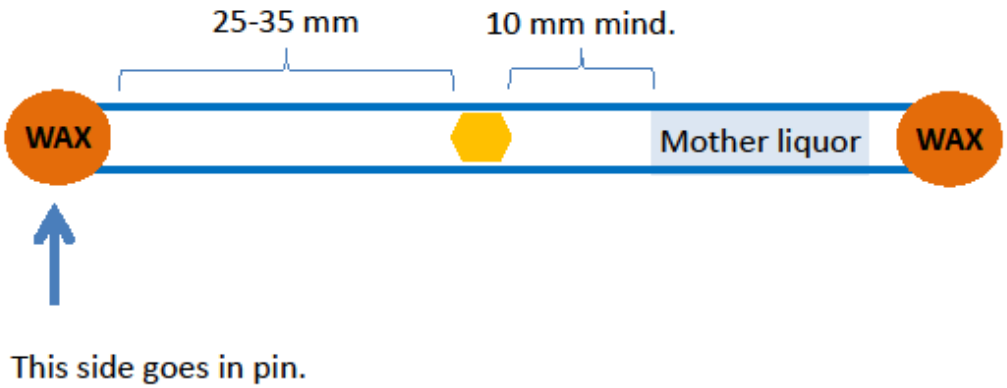
Seal with Epoxy-glue

Vacuum grease as diffusion barrier

All order information published in:
Hermann et. al.
Acta Cryst. (2018). F74, 754–764

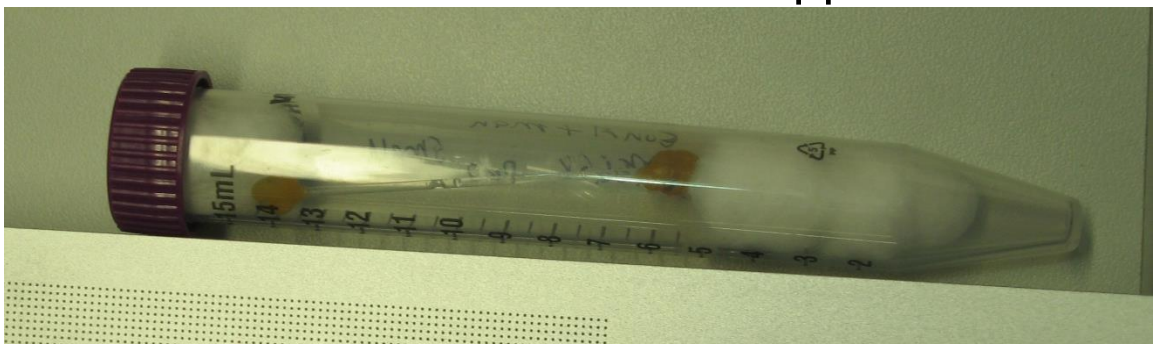
How to send crystals to us by mail?

Biodiff (Jülich) mounting scheme

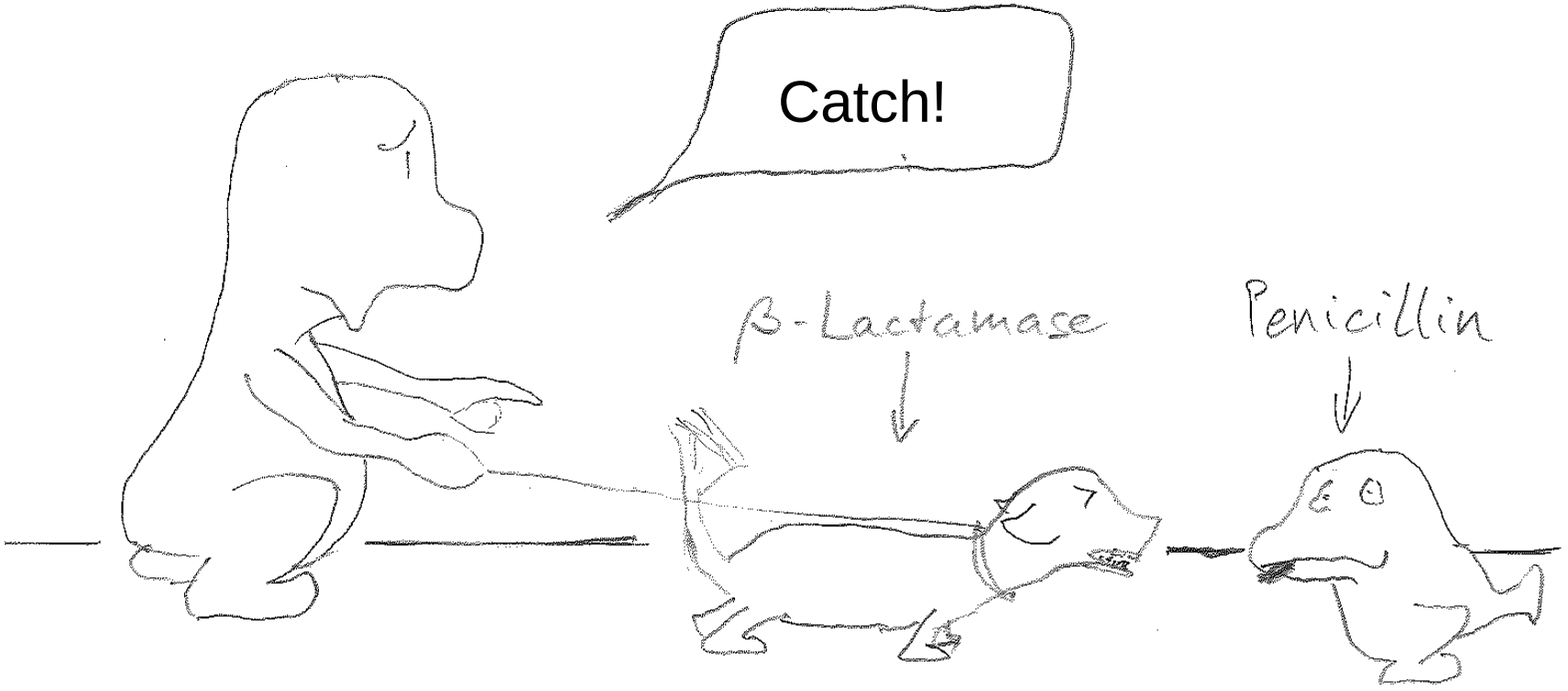


Crystal mounted on the instrument

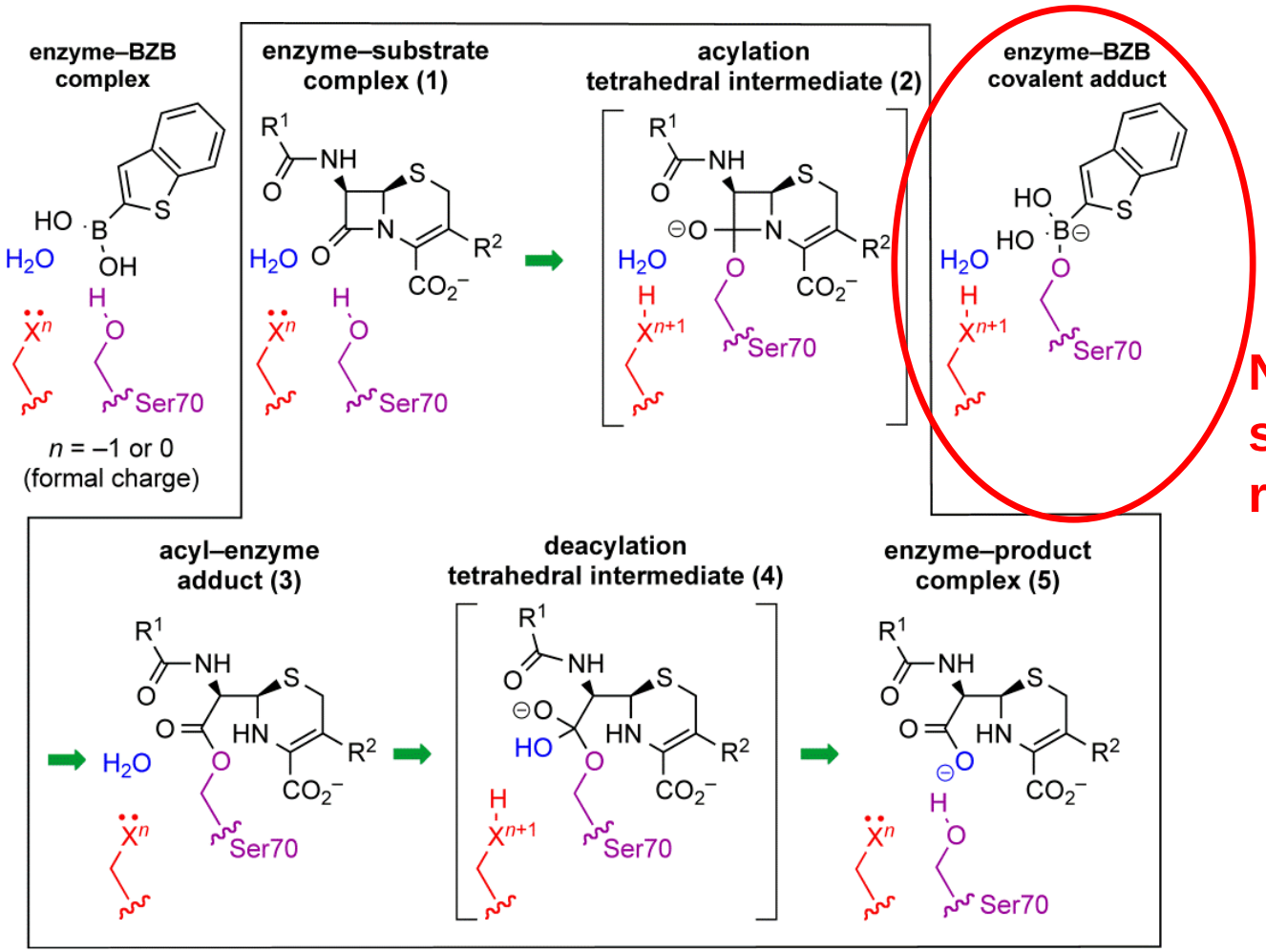
Falcon tubes with cotton wool stoppers:



The protein β-lactamase



β-lactamase: hydrolyses β-lactam antibiotics

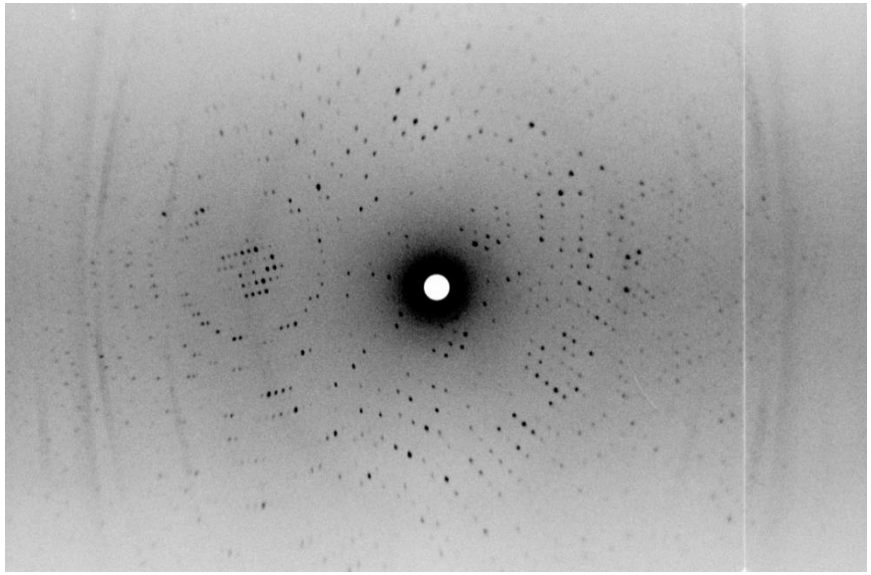


Neutron structure recorded

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

Data-set: β-lactamase with bound inhibitor



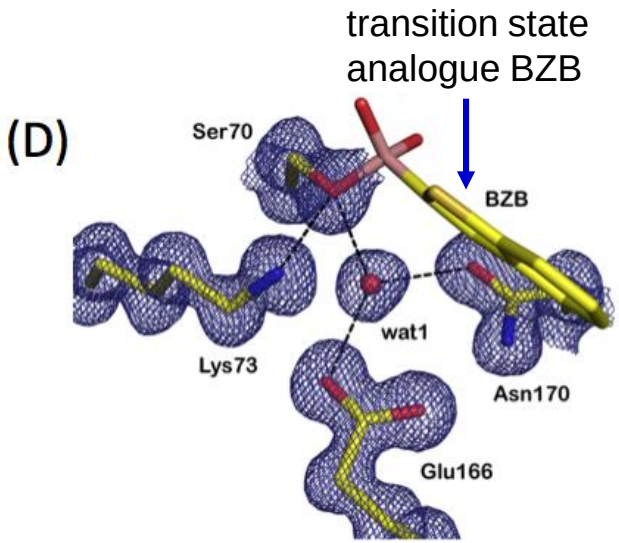
- unit cell: 73.4Å, 73.4Å, 99.1Å $P3_221$
- fully deuterated protein
- crystal size: 2.7mm^3
- Collection time: 9d

d_{min}	I/σ(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
2.52	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**

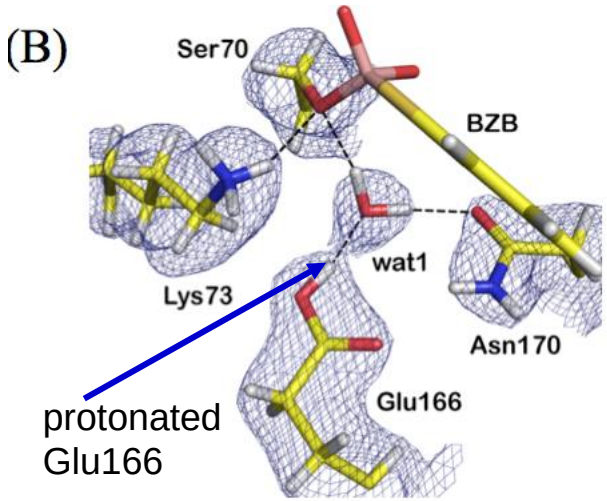
R_{pim} = 7.9% (17.9%)

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

Application Example:
Catalytic Proton Network of the Toho-1 β-Lactamase



(D) electron density map



(B) nuclear density map from BioDiff

Glu166 acts as the general base during the catalytic action of the enzyme.

Stephen J. Tomanicek, Robert F. Standaert, Kevin L. Weiss,
Andreas Ostermann, Tobias E. Schrader, Joseph D. Ng, and Leighton Coates
J. Biol. Chem. 2013, 288:4715-4722

Summary

- The x-ray structure is a pre-requisite of the neutron structure. It is used to do moleculare replacement and solves thereby the phase poblem.
- Neutron fluxes will always be lower than x-ray fluxes: There is a need for large (>0.2… 0.5 mm^3 in volume) protein crystals
- The mother liquor should be exchanged by deuterated mother liquor in a step by step mannor (or crystallization directly in D_2O should be considered)
- Expression of perdeuterated proteins is nice but not necessary
- Room temperature data colletion is possible (but 100 K also available)
- Virtually no radiation damage observed (esp. Important for metallo-proteins)
- Hydrogen (Deuterium) atom positions can be determined at a dmin of better than 2.5 Angstroems already

Upcoming deadline for proposals: 27.03.2020

Thanks to our users and 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

Marialucia Longo
Johannes Hermann
Philipp Nowotny

…and you for your attention!

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JÜLICH
FORSCHUNGSZENTRUM

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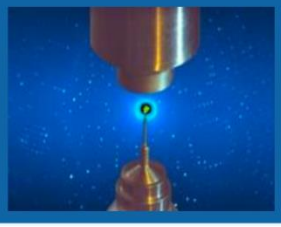

MLZ Conference 2020: Neutrons for Life Sciences

16-19 June 2020
Lenggries
Europe/Berlin timezone

MLZ Conference 2020 Support
mlz-conference-2020@...

Sessions and confirmed speakers

Sessions and confirmed speakers:

- Protein structure, function and dynamics - **Peter Moody,** Leicester Institute for Structural & Chemical Biology, University of Leicester, UK
- Membrane structure, function and dynamics - **Valentin Gordeliy**, Institut de Biologie Structurale (IBS) Grenoble & Institute of Complex Systems (ICS) Jülich
- Drug design and delivery - **Tommy Nylander**, Lund University, Physical Chemistry, Sweden
- Biological surface and interfaces - **Frank Schreiber**, Universität Tübingen and **Bruno Demé,** Institute Laue Langevin, France
- Neutron methods in biology - **Martin Weik,** Institut de Biologie Structurale (IBS) Grenoble
- Life Sciences with neutrons in Russia - **Andrey Konevega,** Petersburg Nuclear Physics Institute (PNPI), NRC, Russia
- **New:** Complementary methods - **Michael Sattler,** Biomolecular NMR-Spectroscopy, Technical University of Munich

Abstract deadline 28th of February 2020!
(Will be most likely extended to 13th of March)