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The macromolecular crystallography beamlines at BESSY II of the Helmholtz-Zentrum Berlin: Current status and perspectives*

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Abstract. For a little over a decade now, the Macromolecular Crystallography (MX) group at the Helmholtz-Zentrum Berlin (HZB) has been operating three state-of-the-art synchrotron beamlines for MX at the BESSY II storage ring in Berlin. The three HZB-MX beamlines, BL14.1, BL14.2 and BL14.3, serve a stable and growing user community of currently more than 100 independent research groups from Berlin, Germany and Europe. Every year, the beamlines provide close to 200 days of MX-beamtime. Over time, the HZB-MX beamlines and endstations, in particular BL14.1, have been continually developed and upgraded and, since 2010, they operate as the most productive MX beamlines in Germany. The environment of the beamlines includes various ancillary equipment as well as additional facilities, such as office space adjacent to the beamlines, a sample preparation laboratory, a safety level 1 biology laboratory (HZB-MX BioLab) and all necessary computing resources. In this paper, the current status of the beamlines as well as the ongoing developments are described.

1 Introduction

No other technique has contributed more to our understanding of the molecular principles of life than Macromolecular Crystallography (MX). Since the first three-dimensional structures of proteins (myoglobin and haemoglobin) have been elucidated in the late 50s of the last century [1,2], the structures of more than 100000 further molecules of life have been determined. As of March 17, 2015, the depositions in the Protein Data Bank PDB [3] amount to 107436. Around 90% of these (95557) have been determined by X-ray crystallography (see `http://www.rcsb.org/pdb`). As indicated by the statistics of the BioSync web server (`http://biosync.sbkb.org/`), close to 90% of all deposited X-ray structures presently rely on the access to synchrotron radiation.

Worldwide, more than 100 synchrotron beamlines are currently being operated, at which MX experiments can be carried out. Just counting the ones, which cater primarily to the needs of the MX community (a beamline is counted here when it produced at least 10 PDB depositions during the year 2013) yields 85 beamlines. Of these, there are 59 beamlines, each of which produced a scientific output of 50 structures and more during 2013. For Europe, the total number of MX beamlines amounts to 28, 18 of them generating 50 or more PDB depositions per year. These latter beamlines are located at BESSY II (Berlin, Germany), DIAMOND (Didcot, UK), ESRF (Grenoble, France), MAXLAB (Lund, Sweden), SLS (Villigen, Switzerland) and SOLEIL (Saint-Aubin, France).

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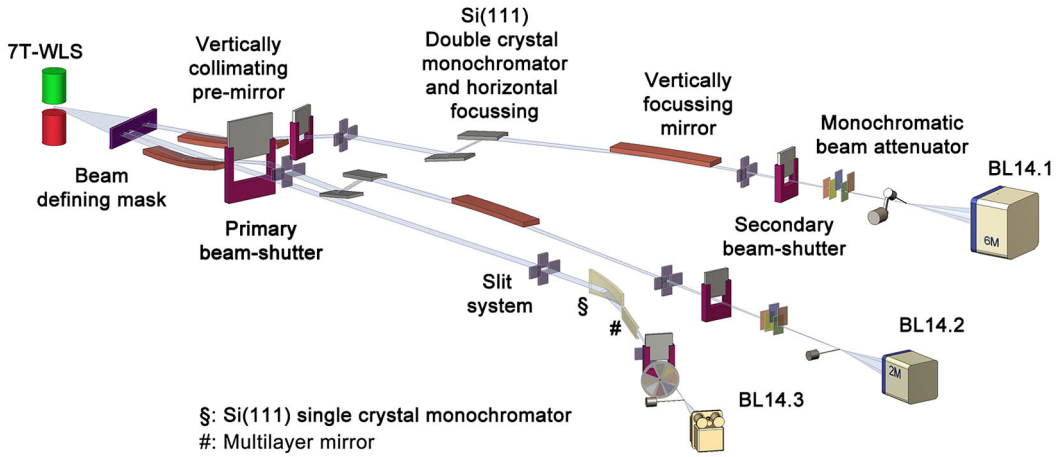

Fig. 1. Optical layout of the HZB-MX beamlines BL14.1, BL14.2 and BL14.3. The horizontal beam separation is 40 mrad between BL14.1 and BL14.2 and 4 mrad between BL14.2 and BL14.3.

2 The HZB MX beamlines

2.1 The BESSY II storage ring

The third-generation synchrotron radiation facility BESSY II of the Helmholtz-Zentrum Berlin für Materialien und Energie (HZB) is in operation since 1998. The storage ring runs at an energy of 1.7 GeV. Various operation modes such as multi-bunch, single-bunch and low alpha are in effect, tailored to the needs of the user communities. Until October 2012, the BESSY II ring was operated in decay mode with three injections per day. Then, the injection scheme was switched to the topping-up mode at a ring current of 300 mA and a mini-injection every 20 or 30 seconds. Most of the close to 50 beamlines make use of the XUV part of the synchrotron radiation, although a number of hard-X-ray beamlines are in operation as well. These include the three MX-beamlines BL14.1-BL14.3.

2.2 The 7 T wavelength shifter at sector 14

In 2002, a super-conducting 7 T wavelength shifter (7T-WLS), which was developed and built at the Budker Institute of Nuclear Physics (Novosibirsk, Russia), was installed in the low-beta section sector 14 of the BESSY II ring lattice. Due to its rather wide horizontal radiation fan of 40 mrad, and very small vertical divergence of 0.1 mrad at a moderate source size of $50 \times 20\,\mu\text{m}^2$ ($h \times v$), the 7T-WLS was designed to be the source for all of the three HZB-MX beamlines [4]. This device has proven to be extremely reliable with no unexpected downtime during its more than 12 years of operation now.

2.3 The three MX beamlines

The three HZB-MX beamlines have been built in the early 2000s within the frame of one of the first Structural Genomics projects, called Protein Structure Factory [5] by ACCEL Instruments (today Bruker ASC). Shortly after the end of this project, the beamlines have been made available to the general user community. Despite the fact that at that time, other established sites (EMBL-Hamburg at the DORIS II storage ring at DESY in Hamburg, Germany, ESRF in Grenoble (France), SLS in Villingen (Switzerland)) were all operating an extensive user program, the MX-beamlines in Berlin managed to draw an ever growing local, national and international user community. In the following paragraph, the three beamlines will be described in more detail (see also table 1, fig. 1).

2.3.1 BL14.1

BL14.1 can be operated at a tunable energy range between 5.5–16 keV. The whole energy range is accessible for the users without any assistance of the beamline staff. The longest time required for an energy change is less then 5 minutes. The 26 m long beamline contains the following optical elements in downstream direction: a Rh-coated 1200 mm long and upwards deflecting, vertically collimating, cylindrical Si-mirror, a water-cooled Si(111) double-crystal monochromator with a sagitally bent second crystal for horizontal focusing and a Rh-coated 1200 mm downwards

Table 1. Properties of the MX beamlines BL14.1-BL14.3.

	BL14.1	BL14.2	BL14.3
Wavelength range [Å]	0.8–2.25	0.8–2.25	0.89
Wavelength at highest intensity [Å]	0.92	0.92	–
Photon Flux at Sample (uncollimated) [Phot/s/0.1 A/0.05% BW]	1.3×10^{11} at 0.92 Å	1.9×10^{11} at 0.92 Å	4×10^{10} at 0.89 Å
Energy resolution [eV]	< 2	< 2	< 5
Sample automation	CATS sample mounting robot (handling of up to 90 SPINE-standard sample and 96-well plates)	G-Rob sample changer (handling of up to 150 SPINE and 144 Unipuck samples)	–
Goniometry	MD2 microdiffractometer with MK3 mini-kappa	Nanodiffractometer	mardtb
X-ray detector	Pilatus2 6M	Pilatus3S 2M	Rayonix MX-225
Beam size FWHM (collimated) [μm]	30–100 (diameter)	30–100 (diameter)	200×100 ($h \times v$)
Achievable resolution [Å]	0.9	0.7	0.7
Maximum unit cell length [Å] (at 2.0 Å max. resolution)	600	500	250
Typical exposure time per frame [s]	0.1–2	0.1–2	3–30
Special equipment and operations	UV-RIP. 3-axis goniometer. On-axis zoom microscope. Crystal annealing. High throughput crystal screening. *In situ* crystal screening.	Very short detector-sample distance (50 mm). On-axis zoom microscope. Crystal annealing. High throughput crystal screening. Ultra-high resolution data collection. Long-wavelength data collection.	Crystal diffraction improvement by controlled dehydration with HC1c. Crystal annealing. Crystalscreening. Ultra-high resolution data collection.

deflecting vertically focusing, cylindrical glass mirror. The chosen design optimally supports the large horizontal divergence of the synchrotron beam with the aim to match the beam cross-section at the sample position as well as possible to crystal of 20–100 μm diameter. Behind each of the optical components, beam monitors are installed to determine the beam form as well as its position and intensity [6].

The experimental endstation consists of an MD2 microdiffractometer with a minikappa goniometer MK3 (Arinax, France), which has a small sphere-of-confusion (SOC) of 1 μm and 3 μm for kappa angles of 0° and 180°, respectively. A Pilatus2 6M hybrid photon counting area detector (Dectris, Switzerland) enables a large 2Θ-acceptance range. Standard exposure times are 0.1 s–0.5 s for one frame. A CATS sample changer in combination with a 90 sample containing LN2 dewar (Irelec, France) (fig. 2) is completing the setup. The sample changer is fully SPINE compatible and uses ESRF/SPINE sample pucks and a rotating gripper for wet sample transfer. Over the past 7 years of operation, this device has provided an extraordinary high sample handling success rate of greater than 99%. At BL14.1 a Amptek

Fig. 2. Experimental station of BL14.1 with Pilatus2 6M detector, MD2-microdiffractometer and CATS sample changer (from left to right).

XR-123SDD Si drift-diode detector is used to perform absorption edge energy scans, which typically require about 5 minutes of experimental overhead time per scan. Energy dispersive fluorescence spectra analysis is possible as well, to identify the metal content of unknown proteins. Using this environment it is possible to screen up to 100 crystals within an 8 h user shift. BL14.1 as well as all other beamlines are controlled by VME hardware controller using SPEC and TANGO [4].

2.3.2 BL14.2

The 28 m long beamline BL14.2 has exactly the same optical components as BL14.1. It is shifted by 40 mrad in clock-wise direction from BL14.1 and shares partially the vacuum system with BL14.3.

Until the end of 2014, the experimental end station contained a mardtb goniometer (marXperts, Germany) and a Rayonix MX225 CCD-detector [4]. At present it is being completely rebuilt, and will be set back into operation in 2015. It will comprise a single axis nanodiffractometer with a SOC of less then 1 μm, which has been built in-house in a collaboration with the research group of Alke Meents at DESY [7], an on-axis sample microscope (Arinax, France), a G-Rob sample changer for the storage and delivery of 296 samples using SPINE pucks or universal pucks (Nat-Xray, France) and a Pilatus3S 2M detector (Dectris, Switzerland). Just like BL14.1 (see previous paragraph) BL14.2 is equipped with am Amptek XR-123SDD Si drift-diode detector for X-ray fluorescence applications. It is also foreseen to have the possibility to mount a microspectrophotometer for special experiments onto the nanodiffractometer (Nat-Xray, France) or to operate this in off-line mode within the MX-SpectroLab (see fig. 3 and sect. 2.3.4).

2.3.3 BL14.3

Beamline BL14.3 is 25 m long and is operated at a static focus and constant energy of 13.8 keV. It shares partially the same vacuum system as BL14.2 and has a 5 mrad counter-clockwise horizontal offset to BL14.2. The optical elements are an asymmetrically cut single crystal Si(111)-monochromator with direct water cooling, which can be meridionally bent for horizontal focusing. Vertical focusing is achieved by a horizontally deflecting, laterally gradient and cylindrically shaped Si/Mo multi-layer mirror. Both optical elements are arranged at Kirkpatrick-Baez geometry.

The endstation consists of a mardtb goniometer (marXperts, Germany) which supports an MX225 CCD-detector (Rayonix, USA) (see fig. 4). Furthermore it is possible to mount an HC1c crystal humidity controller to change the hydration level inside protein crystals (Arinax, France) [8].

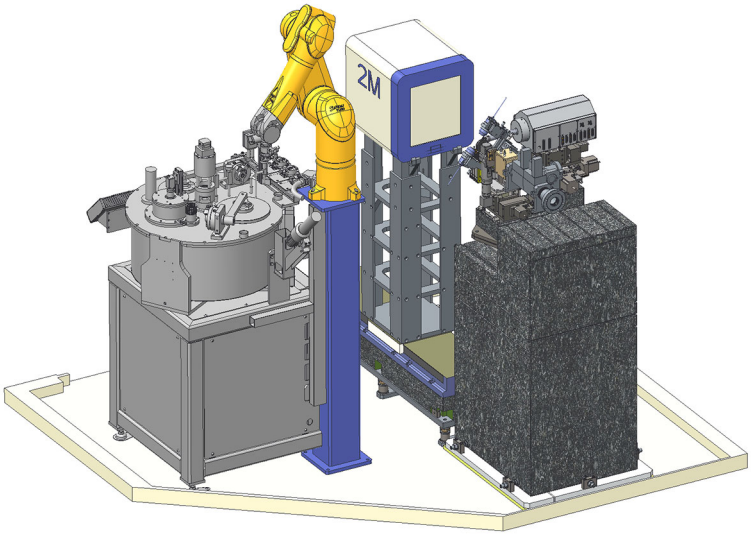

Fig. 3. Layout design of the new experimental station BL14.2 with the G-Rob sample changer, Pilatus3S 2M detector and the nanodiffractometer with mounted microspectrophotometer (from left to right).

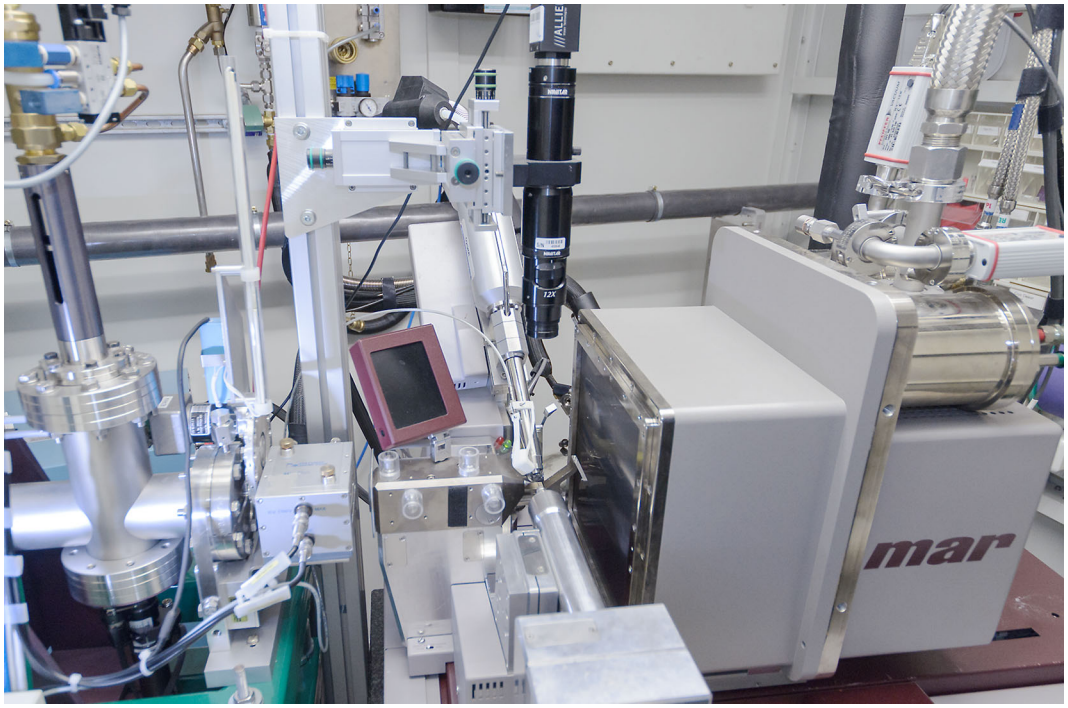

Fig. 4. BL14.3 experimental station with the mardtb goniometer and the MX225 CCD detector (from left to right).

2.3.4 Ancillary facilities and Biolab

In addition to the experimental endstations, the user community has access to ancillary experimental infrastructures supporting special experiments. A 266 nm UV-radiation damage induced phasing (UVRIP) setup installed at BL14.1 can be used for *de novo* structure determination using the specific signals from the conformational rearrangement of cysteine side chains after the photoreduction of the corresponding disulphide bridges [9].

At BL14.3 a HC1c crystal humidifier (Arinax, France) [10] has been installed and is used with a constantly growing demand up to 2 times a week. The device provides experimental evidences of the impact of the alteration of the hydration level inside crystals during diffraction experiments. It can also be used for room temperature experiments and for 100 K experiments after the complete removal of the surrounding mother liquor and without any addition of

cryo-agents without compromising the diffraction quality [11]. The beamline can be switched between normal operation at cryo-conditions and operation using the HC1c in minutes and therefore provides a lot of flexibility to the users.

A gas pressure cell (Hampton research, USA) for the incubation of crystals with noble gases xenon and krypton is also available. Besides their known *de novo* phasing capabilities, these gases can be used to probe the presence and access of potential binding sides of molecules, while diffusing the gas under high pressure into the solvent channels of the incubated protein crystals [12].

Each experimental station has a crystal annealing device mounted to the nozzle of the cryosystem. This device blocks the cryo-stream for 1–10 s and can be controlled remotely [13].

After the BL14.2 upgrade the diffractometer can be equipped with an UV-vis spectrophotometer to follow spectral shifts of chromophores within crystals under redox-conditions. This set-up can also be used for *in situ* monitoring the radiation damage during data collection as well. Alternatively, the spectrometer can be operated offline in a separate room (MX-SpectroLab) adjacent to the beamlines. This mode of operation is already available.

A S1 biological safety level laboratory is located and operated by the MX-group in the vicinity of the beamlines. In the HZB-BioLab, facilities for protein expression, fermentation, purification, protein chemistry, biophysical characterization and automated crystallization are available.

2.3.5 Computational facilities

The MX group operates 5 high performance-computing servers with a total of 204 Xeon cpus, 30 TB of centralized SAN storage array and 1.2 TB of CPU memory. This infrastructure is dedicated to the X-ray diffraction data processing and structure solution during the user beamtime. The diffraction data can be backed up using a dedicated rsync-script and remain on tape storage for months. All relevant scientific program packages for MX like CCP4 [14], PHENIX [15], SHELX/C/D/E [16] have been installed and are maintained by the MX-group.

An on going development is the expert GUI XDSAPP, which enables experimenters to process their data on the fly using XDS as well as some auxiliary programs [17]. This software has also been made available for external usage and is currently used by 563 registered users from 40 countries. For the set-up, strategy calculation, beamline optimization and execution of the diffraction experiments at BL14.1 and BL14.2, the software MXCuBE [18] is used. The development of this software has been initiated at the ESRF and is currently carried out jointly by the MXCuBE Collaboration consisting of seven synchrotron sites across Europe.

2.4 Access to the HZB-MX beamlines

Access to the HZB-MX beamlines is organized via the HZB's digital user office portal GATE. The acronym GATE stands for *G*eneral *A*ccess *T*ool to the *E*xperimental infrastructures of HZB. Within GATE all user communication is managed, from user registration to safety training, to applications for beamtime, to managing of allocated beamtimes, including scheduling, all the way up to reporting on the used beamtime shifts.

User beamtime is available at 200 days per year at 24 h per day from Tuesday 09:00 am until Sunday 09:00 am. Depending on the booked beamline, the user setup starts between 09:00–11:00 am at each day of user beamtime. On-site user support is maintained during the normal working hours. Additionally, a continuous 24 h user call service is offered to all of our users, to secure stable and reliable beamline operations.

Users can apply for beamtime twice per year. Deadlines for the submission of proposals are typically in early February (for the second half the current year) and early September (for the first half of the following year). Beamtime proposals are then evaluated by an international scientific selection panel and shifts are allocated for each of the approved proposals. Currently the HZB MX-beamlines are operating 220 scientific proposals per year, which amount to 20% of all photon proposals at HZB. A speciality of the HZB-MX user operation compared to other synchrotrons is that users can book and manage their beamtime themselves using the MX calendar which is also accessible via GATE. This feature has always been highly regarded by the user community, since it allows the necessary flexibility. Users can book their beamtime when they need it or when it fits their experimental plan.

For transnational user access (TNA) users also have the possibility to submit a proposal to the European project BioStruct-X (`http://www.biostructx.eu`). With a positively evaluated BioStruct-X project and a project within GATE, European users can get reimbursed for their travel to and from BESSY and for subsistence. This mode of access is of prime importance to guarantee fair and easy access to large-scale facilities in Europe. During the duration of the BioStruct-X project from September 2011 until February 2016 (54 months), HZB is offering a total of 4880 h of TNA to its three MX-beamlines.

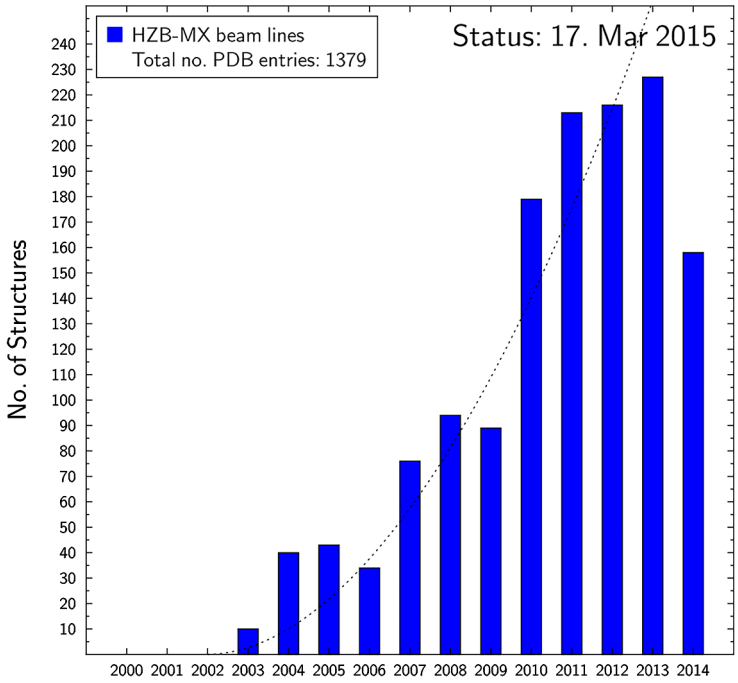

Fig. 5. HZB-MX beam lines deposition statistics from the Protein Data Bank. (2014-depositions will be complete 12/2015.)

2.5 The HZB-MX user community

As has been mentioned, the beamlines in Berlin serve a stable and growing user community. Currently, there are about 100 independent research groups, which are frequently using the three MX-beamlines. A large fraction of the user community is recruited from the Berlin area, where about 15 independent research groups benefit from access to MX facilities. Another large fraction of the HZB-MX user community is from the rest of Germany (about 40 research groups), Scandinavia (about 20 groups) and Eastern Europe (also about 20 groups). Approximately 5% of the beamtime is currently allocated to industrial users. Customized access schemes are offered in order to meet the high demands of this user community. Apart from macromolecular crystallography there is also a small but growing fraction of users, which comprises approximately 5% of all user groups, who appreciate the MX beamlines for small organic and inorganic molecule projects. In particular if those compounds crystallize in large unit cells and have properties reminiscent of protein crystals, access to the synchrotron beamlines is crucial in order to obtain reliable structural information.

3 Scientific output

The scientific output of the HZB-MX beamlines is best evidenced by looking at the PDB depositions statistics. In 2003, the first structure based on data collected at BESSY was published (PDB code 1NLF) [19]. In 2010, the 500th structure was published by scientists from Bayer Healthcare (PDB code 2XIZ) [20] and in 2013 the mark of the 1000th structure was reached with the elucidation of the sirtuin-3 structure by scientists from the University of Bayreuth (PDB code 4BVH) [21]. The development of the number of PDB depositions is shown in fig. 5. It clearly demonstrates that the growth is still exponential.

In terms of publications and theses, the last reliable figures are available for the period 2008–2011. During this period 338 scientific publications including 101 PhD dissertations and 55 diploma or master theses, are fully or partly based on data collected at one of the HZB-MX beamlines. These numbers clearly demonstrate the importance of these beamlines for the local, national and international user community.

3.1 In-house research program

3.1.1 Synchrotron based fragment screening

Fragment based ligand discovery is a relatively new approach to probe the functional surface of proteins using a limited number of small molecules with a molecular mass range from 80–300 Da [22]. At the HZB, a new fragment library

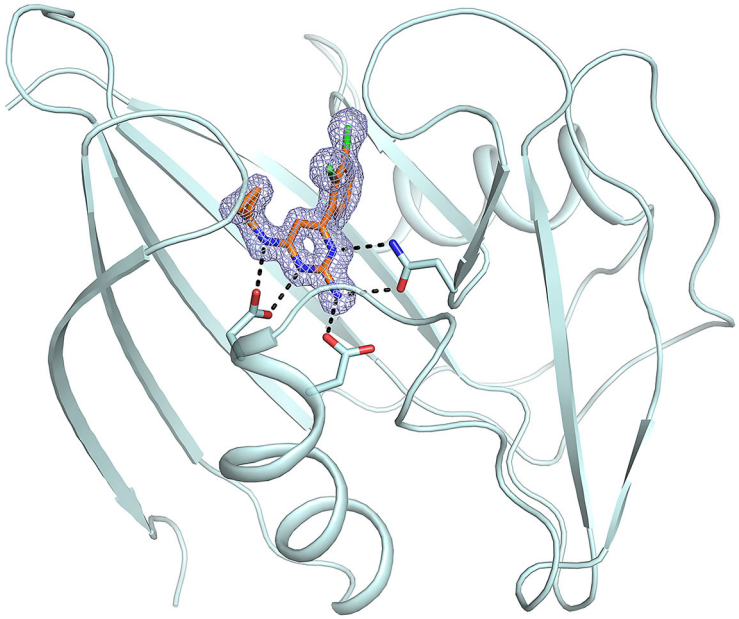

Fig. 6. Ribbon diagram of MTH1 with bound ligand [25].

has been developed, which currently consists of 96 substances and methods for a fast and efficient application of these substances to protein crystals. The newly upgraded experimental station of BL14.2 will serve as the first dedicated fragment screening facility at a synchrotron. This will be achieved by an anticipated elevated sample throughput level, which will enable the collection of more then 100 diffraction data sets per day. In order to support this, a fast sample changer with a high sample storage capability was chosen. The required software tools to execute the data collections automatically are being developed at the HZB. The major project goal is to provide the whole fragment screening facility to our user community. A fragment screening workshop, very recently held at BESSY II with more then 100 participants, demonstrated the strong interest and need for this kind of service. This project is performed in strong collaboration with the research group of Gerhard Klebe from the Philipps-Universität Marburg.

3.1.2 Soft X-rays

A second major research branch is the application of longer wavelengths in MX. At both beamlines BL14.1 and BL14.2 wavelengths up to 2.1 or 2.2 Å can be reliably reached. Applications of such wavelengths range from using the anomalous signal of light atoms such as S, P, Cl, etc., for phase determination [23] to the complete identification of the anomalously scattering substructures [24]. In particular BL14.2 was particularly suited to carry out such experiments, because it featured a very small minimum crystal-to-detector distance of 50 mm, so scattering angles of more than 60 ° could be observed [25–27]. This will also be the case after the beamline upgrade, so that it can be envisaged that such experiments will also be important in the future.

3.2 User highlights

3.2.1 BL14.1 — Structure of MTH1 a new cancer target

MTH1 is a protein, which removes oxidized dNTP pools and thus prevents the incorporation of those damaged bases during DNA replication and cell death. It has been shown that the MTH1 is an anti-cancer target since cancers have non-functional redox-regulations and require the MTH1 activity. The 1.9 Å resolution apo-structure of MTH1 (PDB code 3ZR1) as well as MTH1-inhibitor structures (PDB code 4N1T, 4N1U) were solved on the basis of diffraction data, which have been collected at BL14.1 (see fig. 6) [28,29].

3.2.2 BL14.2 Structure of an O_2-tolerant [NiFe] hydrogenase

Hydrogenases constitute an important class of enzymes, which are able to split water into hydrogen and oxygen. Although most hydrogenases are oxygen intolerant, a small subgroup of hydrogenases, *e.g.*, the membrane bound

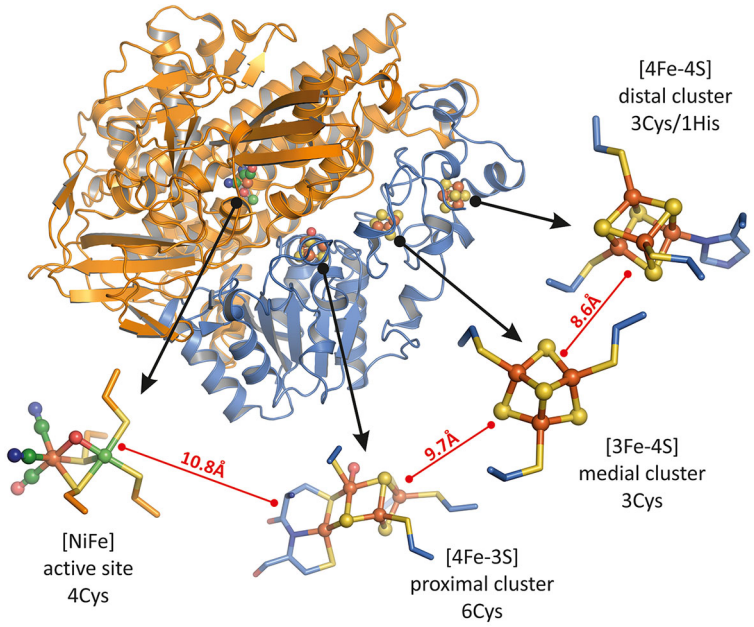

Fig. 7. Oxygen-tolerant (NiFe) hydrogenase from *Ralstonia eutropha* H16 with highlighted (NiFe) active site and 3 Fe-S clusters [26].

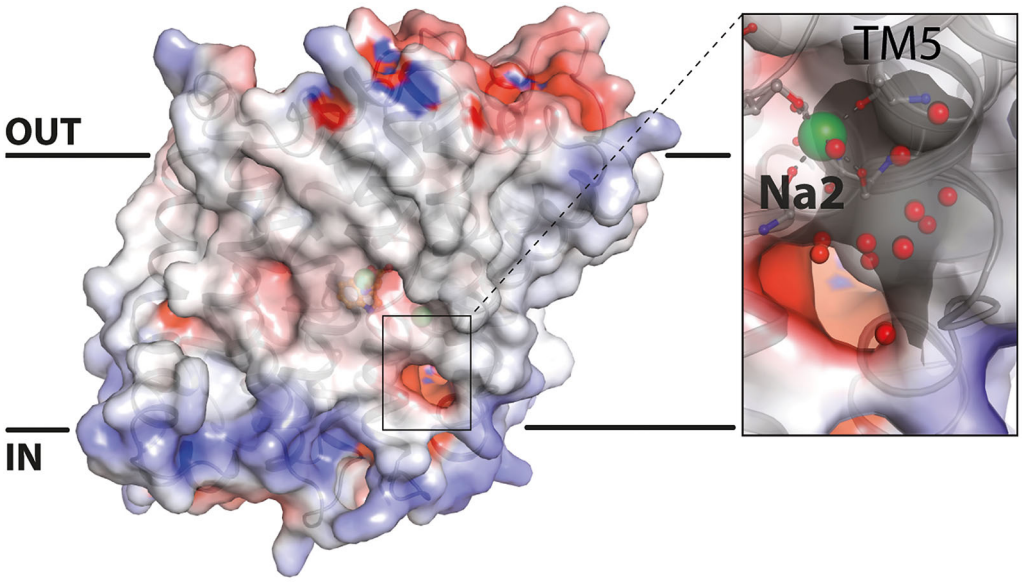

Fig. 8. MhsT crystal structure electrostatic surface representation with the close-up of the cytoplasmic cavity that reaches the Na2 site formed be transmembrane helix 5 (TM5) unwinding. Na^+ ions are shown as green spheres [11].

[NiFe] hydrogenase from *Ralstonia eutropha* pertains its activity also in the presence of O_2. With this property this enzyme is an interesting target for biotechnology applications of H_2 generation as a future source of the production of this clean fuel. The precise knowledge of highly resolved structures at different oxidation states is a prerequisite for the further understanding of this biotechnologically important enzyme. Various forms of the hydrogenase (pdb code 4IUB, 4IUC, 4IUD) have been solved with data obtained from BL14.2 (see fig. 7) [30,31].

3.2.3 BL14.3 Crystal dehydration of MhsT, a neurotransmitter/sodium symporter

MhsT is a membrane bound neurotransmitter / sodium symporter, which terminates synaptic signal transmission by Na^+-dependent reuptake of released neurotransmitters. To improve the weakly diffracting initial crystals, MhsT crystals have been dry mounted and subsequently dehydrated using the HC1c device installed at BL14.3. This procedure improved the diffraction limit of the crystals considerably. Diffraction data collected elsewhere revealed a 2.1 Å resolution data set, from which the structure could be solved (see fig. 8) [11].

The authors thank the HZB scientific, technical and administrative staff for the constant support over the 13 years of operation. We thank the users of our beamlines for the long-term collaborations and interactions during the operation and the development of the beamlines. Especially we thank Patrick Scheerer (Charitè Berlin), Lina Malinauskaite (University Aarhus) and Pål Stenmark (University Stockholm) for contributing figures to this paper.

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