Protein Crystallography on the Max-Planck Beamline BW6

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The wiggler beamline BW6 at DORIS is dedicated to protein crystallography. The facility is financed by the Max-Planck Society (MPG). It may be used also by other academic or industrial groups on the basis of research proposals or for proprietary research. The webpage **www.mpasmb-hamburg.mpg.de/bw6/** provides further information on the access to the beamline. For academic usage, an interactive online booking system has been implemented.

The beamline offers excellent conditions for most applications in protein crystallography. BW6 covers a wide wavelength range in the hard and soft X-ray regimes, and it has a strong record in anomalous phasing using SAD or MAD methods. The instrumentation offers a high degree of automation and high throughput rates. Infrastructure is available for all steps of sample preparation including protein expression, automated crystallization, and control of the sample environment.

Applications

Anomalous phasing applications constituted an important part of the experiments carried out on BW6. One example is provided by the study of structure and function of the Tim14-Tim16 complex (Fig. 1) by Michael Groll and coworkers (Mokranjac et al., 2006). Translocation of preproteins carrying an N-terminal matrix targeting signal from the cytosol into the mitochondrial matrix involves the concerted action of two translocases, the translocase of the outer membrane (TOM) complex and the translocase of the inner membrane (Tim23). The J cochaperon Tim14 and the J-like protein Tim16 form an essential part of the import motor of Tim23. Based on the structural results, the authors suggested a mechanism for the individual steps of the mitochondrial import motor.

Studies of restriction endonucleases represent further examples of anomalous structure solution. Matthias Bochtler and colleagues solved the crystal structure of the complex of *Ecl*18kI with DNA by SAD methods and identified the enzyme as the first restriction endonuclease that uses nucleotide flipping for DNA recognition (Bochtler et al., 2006). Like most Type II restriction endonucleases, *Ecl*18kI is active as a dimer and cuts within the boundaries of the recognition

sequence. The enzyme is one of the few structurally characterized pseudopalindromic cutters.

The crystal structure of N.BspD6I (nickase), which was solved at 1.8 Å resolution by MAD phasing (Kachalova et al., 2006), represents the first known structure of a site-specific DNA-nicking endonuclease. Nickase belongs to a recently discovered class of enzymes that recognize a short specific pseudosymmetric sequence on double-stranded DNA and cleave one predetermined DNA strand at a defined distance from the recognition site. Nickase acts as a monomer. A model of the nickase-DNA docking complex shows for the first time how a single polypeptide chain can cut one DNA strand at a defined distance from the recognition site.

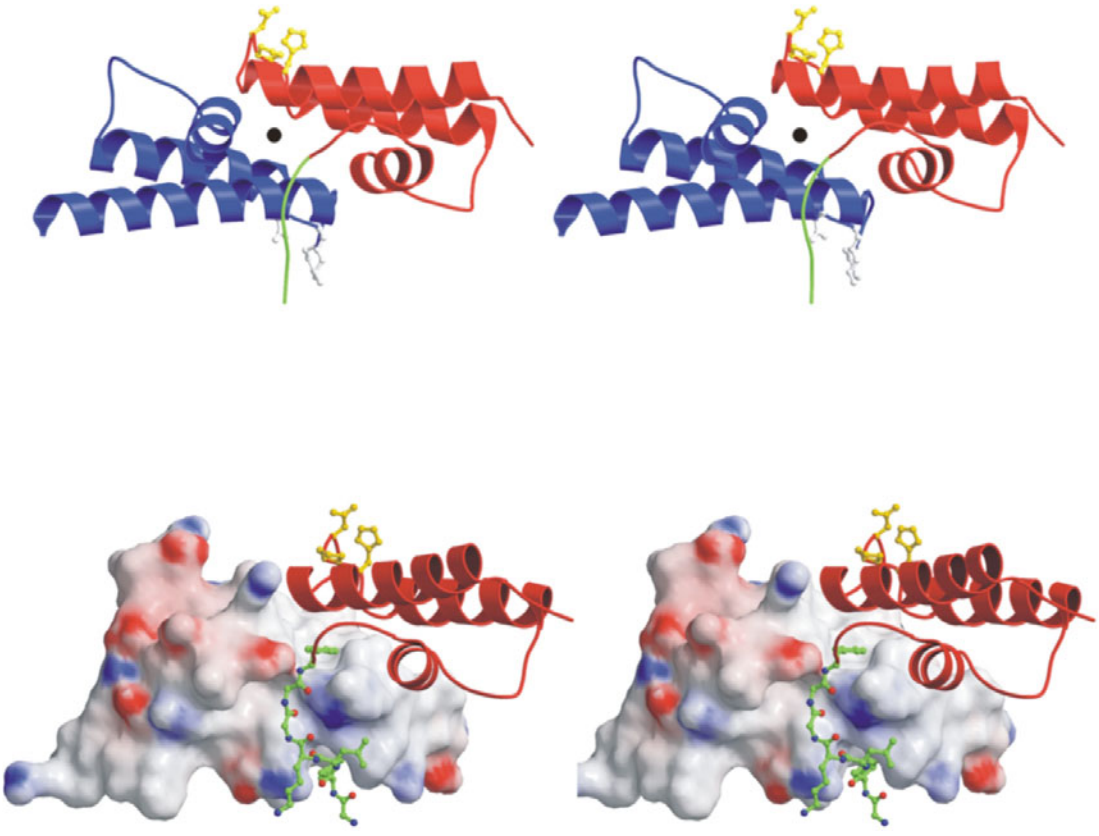

Fig. 1:
Tim14-Tim16 complex from yeast (Mokranjac et al., 2006). The crystal structure was solved by SAD phasing of an Os derivative on BW6. The stereoviews show Tim14 as ribbon model (red), Tim16 as ribbon model (blue) and surface model, respectively.

The beamline BW6 has been used for **structural proteomics** within the framework of the XMTB Consortium funded by the BMBF. Examples of such applications are described in a number of the following reports.

Anomalous phasing

The beamline BW6 has been optimized for rapid experimental phasing of protein structures using anomalous diffraction methods. BW6 is tunable over an unusually broad wavelength range $0.6\ \text{Å} < \lambda < 3.1$. In particular, BW6 is one of very few beamlines worldwide that provide suitable conditions for diffraction studies in the soft X-ray regime. An integrated system for measuring and evaluating X-ray fluorescence permits rapid identification of anomalous scatterers and experimental determination of dispersion terms. SAD and MAD experiments may be carried out in a fully automatic mode, including the measurement of diffraction data at a number of different wavelengths. The high degree of beamline automation and the powerful data handling and processing environment on BW6 support rapid on-line solution of new protein structures. Two different devices (MSC CryoXeSiter; Hampton Research Xenon Chamber) are available for Xe derivatization.

Automation of beamline alignment and data collection

Essentially all experimental parameters that are of relevance to beam alignment and protein data collection on BW6 are under computer control via a graphical user interface. The X-ray optics and the diffractometer setup are automatically realigned during data collection. The diffraction data collection has been automated to great extent. A sequence of complete data sets may be measured in automatic mode without the need for supervision. This mode includes automatic changes in the wavelength, the rotation range, and possibly other parameters like the crystal-to-detector distance or the collimator apertures in between data sets. Thus, unattended data collection is feasible. A new version of BEST (Bourenkov and Popov, 2006) has been implemented for an automatic choice of the optimum strategy of normal and anomalous data collection taking X-radiation damage into account.

Samples are viewed with a high-performance long-distance microscope (QUESTAR) in combination with a high-resolution camera. Samples may be aligned and centered semi-automatically under remote control.

Online data processing and evaluation

A 64 bit file server system is available on BW6. A high-speed link connects the front-end PC, a Quad Opteron (2.4 GHz) cluster, and further processors, all running under LINUX, for very fast data processing, storage and evaluation. Data are stored on a 1 TB raid system with continuous backup on an SDLT tape library (3 TB). Users may chose a number of different media (DVD, CD-ROM, DDS, DLT, FireWire- or USB disks) or connect their notebook for data output.

Data processing and backup may proceed in an automatic way during ongoing data collection. Powerful LINUX workstations are available for data reduction and all steps of data evaluation and structure solution. The network includes a Xeon cluster with 16 processors, which may be used for parallel data processing. A stereographic workstation is available on BW6 for immediate interpretation of electron density maps.

Beamline characteristics

BW6 is a wiggler beamline with double-focussing X-ray optics in 3.3:1 demagnifying geometry. A third mirror has recently been installed and may be used for a dynamical optimization of vertical focusing conditions. The beamline can be operated under monochromatic conditions with rapid tunability (0.6 Å < λ < 3.1 Å) or under polychromatic conditions (using a „white beam“ with λ > 0.55 Å or a “pink beam” with a bandpass $\Delta\lambda/\lambda \sim 3\%$). A chopper system is available for nanosecond time-resolved Laue diffraction studies.

References

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