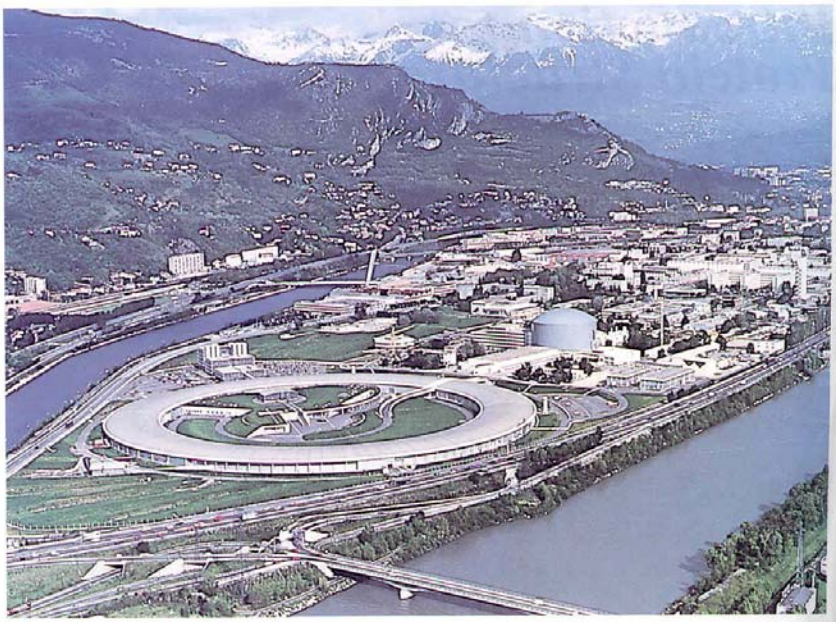
Forum for European Structural Proteomics

# Research Infrastructures in Structural Proteomics: Assessment of Requirements for Synchrotron Radiation in Macromolecular Crystallography



## Table of contents

**Executive summary**

Synchrotrons have played a major role in the dramatic scientific breakthroughs in structural biology that we have witnessed during the last two decades. The availability of adequate synchrotron beam time is an absolute prerequisite for maintaining European structural biology at the forefront of the field. In view of the on-going and planned high-throughput and structural/functional proteomics projects within Europe, the EC found it timely to assess present and future needs of synchrotron radiation for such activities *from a user perspective*. This assessment was carried out within the context of a Specific Support Action (Forum for European Structural Proteomics, FESP) that addresses large-scale infrastructures within Structural Proteomics in general (NMR spectroscopy, electron microscopy, protein production, bioinformatics, synchrotrons). To this purpose, a questionnaire was sent to the heads of macromolecular crystallography research groups throughout Europe, in academia and the pharmaceutical industry. The findings of the survey are summarized in this report. They provide information about the current status of the biological research being done at synchrotron facilities around Europe, and are intended to contribute to the necessary background for formulating policies concerning the infrastructures required for structural proteomics/biology.

On the basis of this survey and of discussions with a reference group and with beam line scientists from a number of synchrotrons, FESP recommends the following actions to the European Commission.

- ***Essential role of synchrotron radiation***: Synchrotron radiation sources are large-scale infrastructures that are crucial for the success and competitiveness of European structural biology. These infrastructures have been used by structural biologists for more than three decades, and macromolecular crystallographers provide the largest community of synchrotron users. Access to these large-scale facilities has been supported by the European Commission and this support has been absolutely vital for the success of European structural biology. FESP strongly recommends continued funding of access to synchrotrons as large-scale infrastructures of vital importance for internationally competitive biological research. Funding under the access scheme for large infrastructures should include direct support to users for travel (including crystal shipment), and support to the synchrotron installations for staff, technological developments and running costs.

- ***Number of beam lines for macromolecular crystallography:*** The number of crystallographic beam lines presently available and under construction appears sufficient to meet the current demands for the immediate future. These together with the additional synchrotrons in Europe either under construction (PETRA & ALBA) or in the planning phase (MAX IV) should satisfy the expected increase in demand for synchrotron radiation over the next decade. Thus, at present there is no need for specific support for the construction of dedicated beam lines for structural proteomics. Beam time requests for structural proteomics projects can be handled within the established beam time review procedures.

- ***Automation:*** Continued support for macromolecular crystallography beam line automation is of the highest priority. Upgrade of beam lines for remote access should be supported, as this would decrease the cost of travel and other expenses for routine experiments. It is also environmentally and user friendly as it decreases the number of users travelling to

synchrotron facilities. However, this requires the appointment of additional technical staff at the synchrotron facilities.

- ***Developing technologies:*** Development and implementation of new technologies for synchrotron-based macromolecular crystallography are of the utmost importance for maintaining the competitiveness of European structural biology in both academia and industry. This includes new beam line technologies, high performance 2D detectors and software for data acquisition and structure solution. These activities should be coordinated and supported at the European level, and FESP strongly recommend that the European Commission funds projects towards this objective.

## Background

Synchrotrons continue to play a major role in the dramatic scientific breakthroughs in structural biology as witnessed during the last two decades. The impact of these large-scale research facilities on structural biology cannot be overstated. The importance of synchrotron radiation (SR) for structural biology is reflected by the fact that the macromolecular crystallography (MX) community is one of the largest user groups (in actual numbers of people involved, the largest) of synchrotron facilities. The availability of sufficient synchrotron beam time on adequate beam lines is an absolute prerequisite for state-of-the-art structural biology. This is even more vital for projects aimed at the high-throughput structure determination of proteins.

In view of the on-going and planned structural proteomics projects within Europe, the EC found it timely to assess present and future needs for SR for structural biology/genomics *from a user perspective*. This assessment was carried out in the framework of a Specific Support Action funded by the EC, named the Forum for European Structural Proteomics (FESP, www.ec-fesp.org), established at the beginning of 2006. Its mission is to evaluate requirements for large-scale infrastructures within Structural Proteomics, including synchrotrons, NMR spectroscopy, electron microscopy, protein production and bioinformatics. The outcome of the activities of FESP is expected to aid the EC in formulating policies concerning large infrastructures and structural genomics.

One major activity of FESP has been the assessment of the existing European synchrotrons for structural biology/proteomics as well as considering future demands for synchrotron radiation. To aid this assessment a questionnaire was sent out to the heads of European MX research groups, in academia as well as in the pharmaceutical industry. A second questionnaire was sent to representatives of the European synchrotron facilities.

The study was designed to address the following questions:

- what beam lines are presently (or in the near future) available at synchrotrons in Europe?
- what is the current demand for beam time from structural biology/proteomics projects, and what are the projected requirements (i.e. is availability of SR a bottle neck)?
- what types of beam lines (and associated infrastructures) do users expect to find (design, software, compatibility, automation, user support etc)?
- is there a need for dedicated beam lines for structural proteomics, or can the expected requests for beam time be handled within the framework of existing beam lines?

The findings of the surveys are summarized in this report (Appendices 1-3).

A reference group assisted both in the compilation of the report, and in the formulation of the conclusions and recommendations to the European Commission. The members of the reference group were M. Bolognesi, University of Milano, Italy, M.A. Carrondo, Institute of Chemical and Biological Technology, Portugal, K. Diederichs, University of Konstanz, Germany, B. Dijkstra, University of Groningen, The Netherlands, E. Garman, University of Oxford, UK, K. Wilson, University of York, UK, A. Perrakis, Netherlands Cancer Institute, The Netherlands, L. Wyns, Free University of Bruxelles, Belgium, N. Oikonomakos, The National Hellenic Research Foundation, Greece, M. Jaskolski, University of Poznan, Poland, R. Wierenga, University of Oulu, Finland, C. Cambillau, CNRS-Universités Aix-Marseille, France, I. Schlichting, Max-Planck Institute Heidelberg, Germany, A. Mattevi, University of

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We thank the members of the reference group for their advice and constructive criticism. The findings and conclusions of this report, including any errors, are however the full responsibility of FESP.

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## Introduction

Macromolecular crystallography has been the primary method for obtaining information about the three-dimensional structures of biological macromolecules at atomic or near-atomic resolution for decades, and the continuous expansion of the field is best illustrated by the almost exponential increase in depositions of protein structures in the Protein Data Bank (46679 structures as of 18-10-2007). Reasons for the accelerating crystallographic output include increased availability of SR, technological advances in hardware (synchrotrons, home sources, computers, crystallization robots), methodology (phasing, crystallographic computing, cryo-protection of crystals, nano-drop technology), and in particular the exploitation of molecular biology techniques to obtain sufficient amounts of soluble purified proteins. The past decade has seen the development of high-throughput methods in protein production, crystallisation and data collection, heralded in particular by the structural genomics programmes that were started in the late 90's in the USA and somewhat later in Europe and Japan. The Structural Genomics Initiatives have, due to these technical advances, quickened the pace of protein structure output, and are at the moment responsible for most of the *de novo* protein structures deposited in the Protein Data Bank. At the same time, the number of structural biology laboratories has increased significantly, and the diffusion of medium- and high-throughput techniques from structural genomics consortia to these smaller, individual laboratories has increased the pace of structure determination also for investigator-driven research projects. The recognition and awareness of the importance of three-dimensional structure information for a deeper understanding of biological processes, in combination with the development of crystallography into a mainstream molecular biology research tool, has further led to structural studies being pursued by biologists whose primary research does not involve crystallography. All these developments have led to an increase in demand for access to infrastructures, in particular synchrotrons, for structural biology.

These trends in structural proteomics/biology are reflected in the answers to the survey conducted by FESP in 2006/2007 of the MX community in Europe. A questionnaire was sent to 350 principal investigators in macromolecular crystallography at universities, research institutes and industry that cover a large part of the community; about 50% of these principal investigators responded in the review and returned the questionnaires. Their answers clearly illustrate that crystallography has advanced to the point where structure determination (i.e. phasing and refinement) is no longer perceived as a major rate-limiting step in understanding the molecular mechanism of a biological function (Fig 12). Protein production and crystallisation (52%) and, not unexpectedly, funding (for 36% of the survey participants) are more often a bottleneck in the progress of structural studies than the actual structure determination, refinement and analysis (8%). In fact, the latter gathers most answers (36%) for never being a rate-limiting step. It is quite clear that production of sufficient amounts of soluble, homogenous and functional protein samples is the major obstacle for most of the responding researchers and requires the greatest effort in the course of a structure analysis.

## The impact of synchrotron radiation

The use of SR for data collection had and still has an enormous impact on the field of macromolecular crystallography, both quantitatively and qualitatively. A current survey of the Protein Data Bank reveals that 65% of the deposited X-ray structures have been determined using SR sources. For those structures deposited in 2006, the ratio is even higher (78%,

Biosync report on PDB homepage), and in the future it will only increase. In addition, atomic resolution structures (< 1.2 Å) are accumulating and count currently 715 (December 2006).

Since the pioneering experiments by Ken Holmes and colleagues the technological advances over the last 30 years have made SR the fastest and most efficient tool for data collection in structural biology. Furthermore the use of SR has made it possible to tackle successfully challenging biological problems such as for example the structures of the ribosome, blue tongue virus, and the nucleosome core particle which would not have yielded to a crystallographic structure analysis otherwise. Key methodological advances have included:

- The adoption of cryogenic techniques diminished radiation damage as the major cause of failure of a synchrotron experiment, and data quality improved substantially as complete or even multiple datasets can be measured from only one crystal. However at the most brilliant beam lines radiation damage re-appeared as a significant problem in data collection and various approaches are being developed at synchrotrons to deal with this issue.

- The development of improved detectors enabled much faster data collection, typically less than one hour at 3$^{rd}$ generation sources. Exciting developments of novel detectors (for instance the PILATUS detector developed at the SLS) increase the speed of data collection even further.

- The availability of tuneable beam lines has made techniques based on anomalous scattering a routine experimental phasing technique.

- The highly brilliant and stable beams at third-generation sources led to the construction of micro-focus beam lines with beam sizes of 5-10 μm that permit data collection from very small crystals, and/or from crystals with very large unit cells.

- Advances in both computer hardware and software have greatly simplified data collection and processing, permit better experiment monitoring, and allow in many cases determination of the crystal structure already at the beam line, or at least greatly reduce the time between synchrotron experiment and structural result.

- Automated sample changers allow screening of large numbers of crystals for the best diffracting specimen, which results in better data quality and structures at higher resolution, thus increasing the biological information that can be extracted.

The vast majority of the survey participants stated that SR is essential for their research (Fig 4). Indeed, most of their publications depended on access to these large-scale infrastructures (Fig 9).

**Current beam line resources and new synchrotrons**

Appendices 2 and 3 provide on overview of European synchrotron facilities presently in operation or under construction, and of operating and planned beam lines for MX. Currently, there are nine storage rings in Europe providing a total of 28 operational MX beam lines (or rather end stations). One of these synchrotrons, the SRS in Daresbury, UK, is being replaced by the 3rd generation Diamond Light Source, located near Didcot, Oxfordshire, UK. Diamond

commenced user operation in January 2007 on three out of an eventual six MX beamlines and is thus the first of the new sources to come "on-line". Soleil started operation shortly thereafter, in spring 2007 and will provide one tunable beam line for structural biology, another one is to be commissioned in 2009. An additional facility, ALBA, is under construction in Spain, and yet another, MAX IV in Sweden, is in the planning stage. One of the phase I beam lines at ALBA will be dedicated to macromolecular crystallography. PETRA, the synchrotron with by far the largest circumference (2.3 km) is currently being converted to provide one of the most brilliant X-ray sources in the world at the DESY-site in Hamburg, Germany. Reconstruction was started in 2007, and PETRA III is expected to commence user operation in 2009. Three of the planned 14 beam lines will be dedicated to the study of biological macromolecules, two operated by the EMBL and one jointly by the Helmholtz and Max-Planck societies. Furthermore upgrade programmes at other synchrotrons, for instance the ESRF, may involve a redistribution of MX beamlines. In view of these and other on-going developments, the landscape of beam lines for MX will change over the next few years.

In view of the anticipated changes in the number of beam lines available for MX in Europe, it is at present difficult to foresee the future need for additional beam lines. A significant increase in the number of applications for beam time at synchrotron facilities had been expected from the diverse European Structural Proteomics initiatives. This increase in structural proteomics related applications was however not as dramatic as anticipated, perhaps partly due to the focus of the European structural proteomics projects (for instance SPINE and SPINE2) on biological impact rather than on sheer numbers of structures. In addition, beam line automation and improvement in detectors has led to an increase in throughput at many beam lines which in part compensated for the increasing number of beam time proposals. This is reflected in the reported use of SR per research group of 8.6 days in 2005, 1.2 days more than in 2003 (Fig 6).

To the question of whether the current synchrotron access is sufficient for their needs, 85% of the researchers responded that it was, and 38% anticipated that it will remain so during the next three years (Fig 5). However, more than half of the respondents (57%) expect an increase in their demand for beam time during the next three years (Fig 7). Thus, at first glance it appears that although the overall demand for synchrotron beam time continues to outpace the supply, a large part of the future demands can be met by the new synchrotrons Diamond (UK) and Soleil (France).

However a cautionary note must be added at this point. While individual data collection times are decreasing due to more intense beams, faster detectors, better data collection software and sample changers, the overall time spent to find the optimal specimen is increasing. The focus on biologically challenging samples (large protein complexes, membrane proteins), often results in difficult crystals that need extensive screening before a suitable sample can be identified. In addition, automated beam lines enable extensive screening for the best specimen in order to collect the best data set. FESP anticipates that on average more time will be spent on crystal screening, potentially leading to an increase in the demand for synchrotron beam time. Radiation damage at high-brilliance beam lines that necessitates data collection on several crystals to obtain a complete data set may further increase the average time per data set. FESP therefore expects that the need for SR will increase and that the number of operating and planned beam lines for MX applications is highly appropriate.

## Methodologies

### *MAD/SAD*

Multi-wavelength anomalous diffraction (MAD) experiments require the high brilliance and tunability of SR, and cannot be performed on a home source. SAD and MAD techniques are widely applicable and rapid phasing methods for *de novo* structure determination, and are likely to gain even greater importance with the increase in the number of tunable experimental stations. Nineteen tunable beam lines are currently available to the user at European synchrotrons, and the construction of another seven is either planned or underway (Appendix 3).

The most widely used and already standardized method of protein modification for MAD/SAD experiments is selenomethionine substitution. Of the 182 survey participants, 50% and 39%, respectively, use MAD/SAD methods often or occasionally (Fig 10). MAD is already a major contributor to macromolecular structure determination and 91 % of the survey participants anticipate using MAD/SAD to a significant extent over the next three years (Fig. 11).

### *Data collection from micro crystals*

In recent years there has been an increasing trend towards data collection from micro- and ultra-tiny crystals. The responses to the survey clearly indicate that this development will continue: more than two thirds of the respondents plan to use microcrystals (Fig. 11). The reasons are several, but most are based on the availability of only limited amounts of pure protein for crystallization experiments (due to very low expression levels, poor protein solubility or stability) or intrinsically low crystallization propensity (for membrane proteins or of large protein complexes). Technological developments within Structural Genomics Initiatives led to the wide-spread use of crystallization robots and nano-drop technologies, which enabled researchers to pursue structural studies even when only sub-milligram amounts of protein are available, by making use of smaller crystals.

The high brilliance of the new synchrotron sources permits data collection from very small crystals. However, specialized beam lines are needed with a microfocus spot size (typically 5-10 μm), which in turn requires a higher precision in crystal and X-ray beam alignment. Presently, there are three micro-focus beam lines for biological studies at European synchrotrons, ID13, ID23-2, and ID29 at the ESRF. All are overbooked, and the level of oversubscription is increasing. Two more micro-focus beam lines are currently under construction (I24 at Diamond and PROXIMA2 at Soleil) and will be available for users in 2008 and 2009, respectively, but whether they will be sufficient to handle the rapidly growing demand remains to be seen. The exciting construction of nano-focus beam lines with focus sizes in the range of 100 nm at the sample may allow data collection from even smaller protein crystals.

### *Very large unit cells*

Of the respondents to the survey, 57% expect to be working to a significant extent during the next three years with crystals having very large cell dimensions (Fig. 11). This trend is caused by the shift from studies of single protein molecules and their complexes with low molecular weight ligands to large protein-protein and protein-nucleic acid complexes. Thus far, only a

few such huge assemblies have been structurally characterized, but in light of their biological importance it is obvious that this is just the beginning of a new era in structural biology.

The crystals of large membrane proteins and macromolecule assemblies will have typical unit cell dimensions of several hundred Å and diffract more weakly. Effective data collection will need the highly collimated and parallel beams produced by third-generation synchrotron sources and may require dedicated beam lines for such difficult projects.

***Conventional monochromatic data collection and high resolution crystallography***

At present, conventional monochromatic data collection is still the dominant kind of experiments performed at synchrotrons. Ten beam lines of fixed wavelength are provided by European synchrotrons, and 91% of the survey respondents visited them during the last three years. A substantial need for such facilities will continue for the next decade (Fig 11). The rapidly growing number of depositions in the Protein Data Bank will allow more and more structures to be solved by molecular replacement, which does not require anomalous diffraction and thus is not dependent on the use of a beam line of tunable wavelength. In addition ligand complexes of proteins of known structure are usually studied using monochromatic radiation, and as the focus shifts from determination of novel protein folds to exploration of protein structure-function relationships, there will be an increasing demand for this experimental set-up. Structure-assisted drug development in the pharmaceutical industry requires a large number of target-inhibitor structures to be determined, and there is thus also a large demand from industrial users for access to SR.

A better understanding of biological processes is coupled to the increasing spatial and temporal resolution of structural information. The superior signal-to-noise ratio obtained with the highly parallel and monochromatic X-ray beam produced by synchrotrons provides the means for collection of ultra-high resolution data. Compared to data collected at home sources, the effective resolution is usually higher.

Ultra-high resolution results in more precise atomic coordinates and allows the refinement of anisotropic displacement parameters and the location of hydrogen atoms. Beneficial consequences are a more detailed description of active sites thus enabling a better modelling of catalytic mechanisms, while individual anisotropic temperature factors provide valuable information about protein flexibility.

## Trends and future developments

In the survey, users were asked for their views on desirable future developments at synchrotrons, and about the types of experiments they would like to perform at synchrotron beam lines that cannot be carried out at present (Figs. 15, 16). The highest priority was given to various improvements of beam lines, and user suggestions are summarized below. Many of these suggestions have already been implemented or are in the process of being implemented and others are under development. It may be appropriate here to emphasize the importance of support of such method developments by the EC, as for instance through the BioXHit project that has a tremendous impact on the development of improved methods towards automated data collection and structure analysis and the standardization of beam lines.

***Beam line automation/Remote data collection/Mail service***

The objective of beam line automation is to reduce user intervention during data collection and analysis to a minimum. At the ESRF, major advances in throughput were achieved by

implementation of a robotic system sample changer, and a semi-automatic system for crystal alignment. Only if beam lines are equipped with more powerful computer hardware and software, can data collection strategies be optimized to achieve maximum efficiency.

Automation projects are underway at most European synchrotrons. The goal is to develop a flexible, but robust and user-friendly system that can be installed at all crystallographic beam lines of one particular synchrotron, and preferably also at other synchrotrons. It should include a crystal mounting robot, a program for (semi-)automated crystal alignment, programs to control and monitor data collection, a system for reducing data in real time, and for data backup. Consideration should be given to the automation of crystal annealing, an automated fluorescence scan at MAD beam lines, and the automated translation of the crystal in the beam at microfocus or very-high brilliance beam lines. Such systems may then be interfaced with semi-automatic analysis and refinement pipelines.

An obvious extension of beam line automation is the possibility of remote submission of samples and control of data collection, which would substantially reduce the number of user visits. Cryo-cooled crystals are sent to the beam lines via courier services, loaded into the robotic sample changers by local staff and data collection is controlled remotely by the user. This mode of data collection has already been successfully implemented at several synchrotrons in the US. For instance, at SSRL in Stanford, already more than 75% of the MX users collect data using remote access. The ESRF is pioneering this approach in Europe and will introduce remote data collection mode for academic users in the near future. An alternative mode of operation is for the beam line staff to carry out data collection ("FEDEX" data collection), a service that is provided already at some synchrotrons for industrial users.

Beam line automation is regarded as an essential/important development by the majority of the user community. The opportunity to mail crystals to the synchrotrons, to screen crystals and collect data remotely is also given high priority. It is vital that efforts to automate beam lines and to allow remote data collection are continued, as a substantial demand for these options can be expected from the structural proteomics/biology community in the near future. This will require a substantial investment in trained technical staff to deal with the transfer of appropriate sample dewars to the beam lines, and to initiate operation of the equipment by the remote user: this investment will be offset by the enormous reduction anticipated in the financial, time and energy saving impacts of reduced travel.

***Standardization***

System standardization should be aspired at all European beam lines. In addition to reducing user burdens, it would reduce the time spent by the support staff on training of new users. In addition to the software, sample mounting procedures should be standardized to simplify the sending of cryo-cooled crystals by express-mailing services in pre-filled sample changer cassettes. This is already underway as most European synchrotrons are introducing the SPINE-standards for crystal mounting pin length and type, which will allow users to more conveniently utilise available synchrotron time at the various infra-structures.

***User support***

Figures 13 and 15 clearly indicate that user support by beam line scientists is a key aspect of success. 60% of the survey respondents stated that local support at the beam lines is essential and guarantees more efficient use of precious beam time. For another 34% of the users it is important. In fact, local support is given higher priority than training at specially organized courses. Increasing numbers of users at synchrotrons lack in-depth training in crystallography

and need additional supervision and advice by beam line scientists during their visit. User support often requires a significant commitment from beam line staff, and it is vital that career paths for beam line scientists are developed where activities such as user support are given sufficient credit. In addition it must be ensured that beam line scientists do have sufficient time for their own research, since development of novel methods and technology is to a large extent based on the research efforts and dedication of beam line scientists.

In contrast to the importance given to travel cost reimbursements and local support by beam line staff, there is a lower demand for improvement of wet lab facilities provided by synchrotrons, i.e. completely equipped laboratories for protein expression, purification, crystallization and crystal handling (Fig. 15).

***Travel support***
Although the availability of remote data collection modes is welcomed by a large part of the user community, it will not completely eliminate the need for structural biologists to travel to synchrotron facilities. For about 80 % of the users of these research infrastructures travel support provided by national and/or European granting agencies is essential/important. In the future travel support must include shipment of cryo-cooled crystals to the synchrotron, as this often is as expensive as user travel. Recently implemented Europe-wide air-line security measures make it impossible to transport crystals directly.

***Detectors and other beam line hardware***
A significant part of the user community expects an increased effort to be devoted to the development of faster detectors. There have been important developments in this area at the SLS where the pixel detector is now in operation, and significantly speeds up data collection. As it is more cost-effective to increase the output of a synchrotron facility by upgrading existing beam lines rather than building new ones, funding should be provided to allow for upgrades of optical and other hardware components. Adequate computer and networking services are equally important and require continuous upgrading to handle the enormous and increasing data volume generated at SR MX beam lines.

## Conclusions and recommendations

The survey of the MX user community with respect to their need for access to synchrotrons as a large-scale infrastructures and discussions with beam line scientists as to the present and future state of beam lines at European synchrotrons has led FESP to the following conclusions and recommendations to the European Commission.

- ***Importance of access to SR***: SR sources are large-scale infrastructures that are of immense importance for the success and competitiveness of European structural biology. These infrastructures have been used by structural biologists for more than three decades, and macromolecular crystallographers provide the largest community of synchrotron users. Access to these large-scale facilities has been supported by the European Commission and this support has been essential for the success of European structural biology. FESP strongly recommends continued funding of access to synchrotrons as large-scale infrastructures of vital importance for internationally competitive biological research. Funding under the access scheme for large infrastructures should include support for user travel (including crystal shipment) and support to the synchrotron for staff and running costs.

- ***Number of MX beam lines:*** The present number of crystallographic beam lines appears adequate to meet the demands for the immediate future, and with the additional synchrotrons in Europe under construction and in the planning phase a further increase in demand can be handled over the next decade. At the moment there is no need for specific support for the construction of designated beam lines for structural proteomics. Beam time requests for such projects can be handled within the established beam time review procedures.

- ***Automation:*** Efforts to automate crystallographic beam lines should be continued and strongly supported. Upgrading of beam lines for remote access will reduce cost for travel and expenses for routine experiments. It is also environmentally and user friendly as it decreases the number of users travelling to the synchrotron facilities.

- ***New technologies:*** Development of beam line technology and new methods for synchrotron-based MX are of great importance for the competitiveness of European structural biology in academia and industry. Such activities should be coordinated and supported at the European level, and FESP therefore strongly recommend that the European Commission funds projects directed towards this objective.

**Appendix 1: FESP survey of synchrotron users in macromolecular crystallography**

## A. Survey group profile

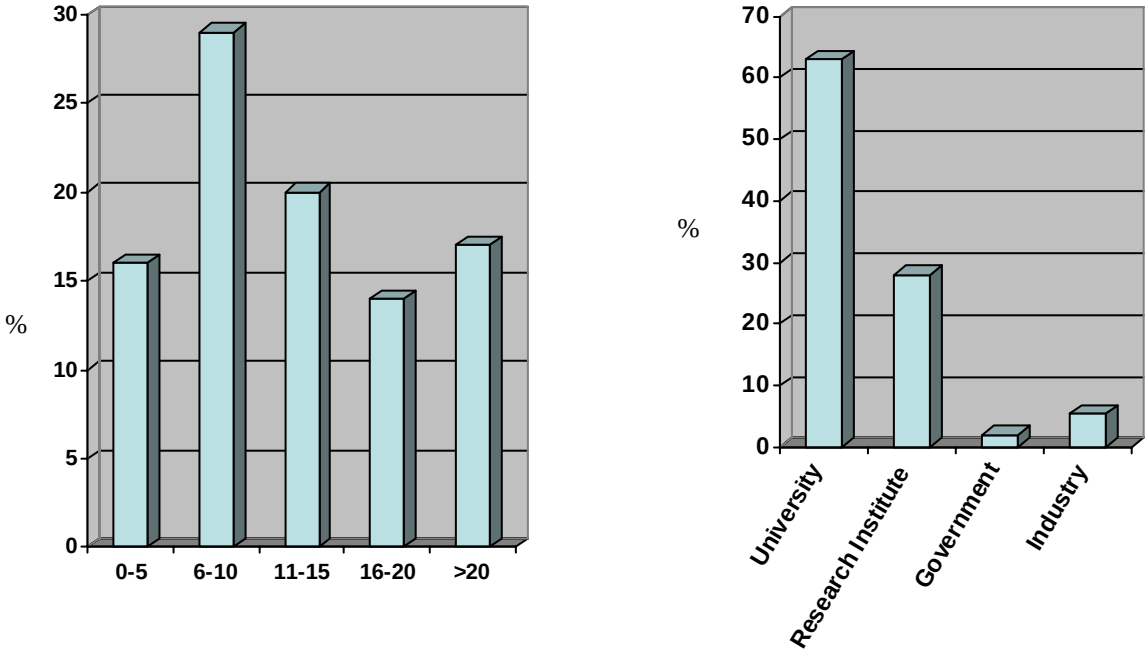



**Figure 1**: Years as principal investigator and research environment of the 182 survey respondents (percentage of answers).

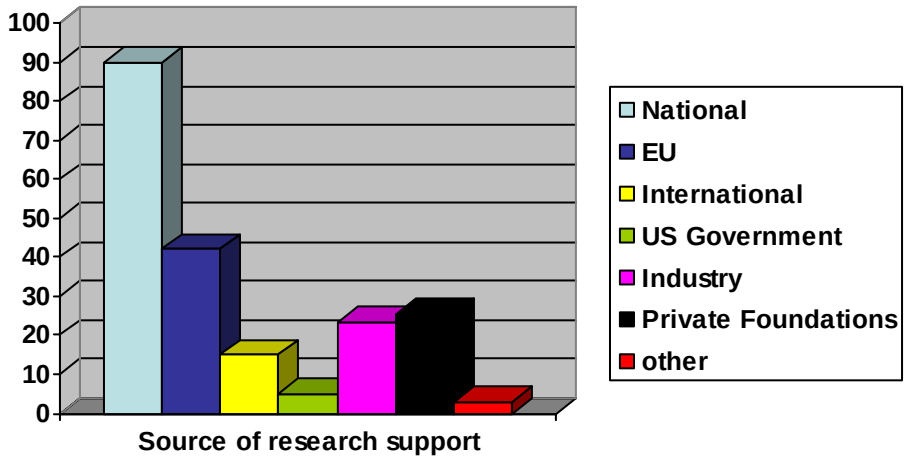



**Figure 2:** Major sources of research grants (% of answers per category) of the survey respondents.

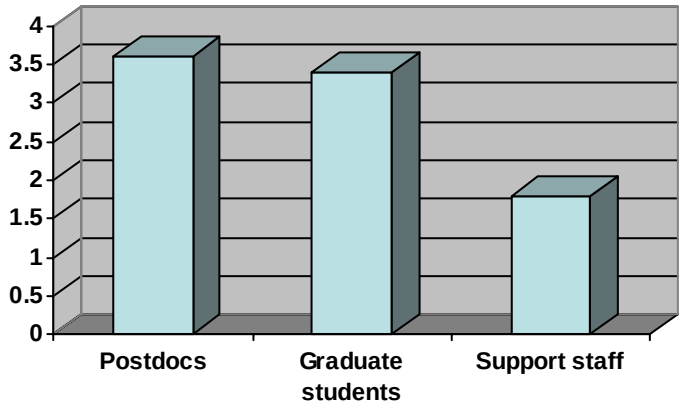



**Figure 3:** Average number of postdoctoral students, doctoral students and support staff per respondent.

## B. Questionnaire responses

How important is access to synchrotron radiation for your research?

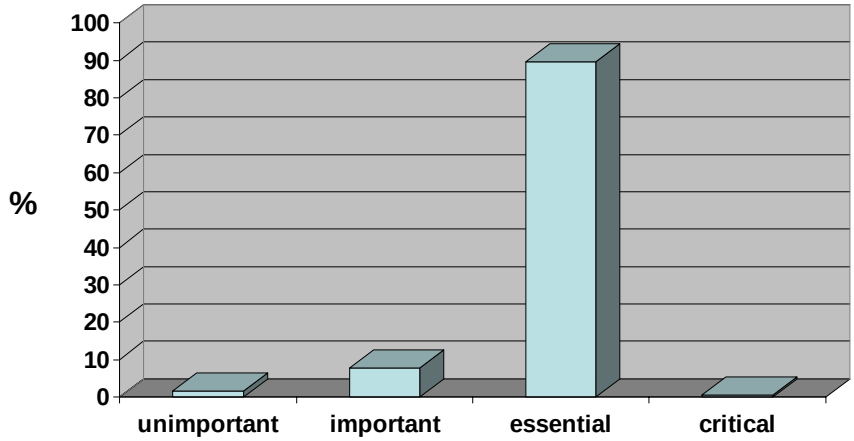



**Figure 4**

Is present access to beam time sufficient for your needs?

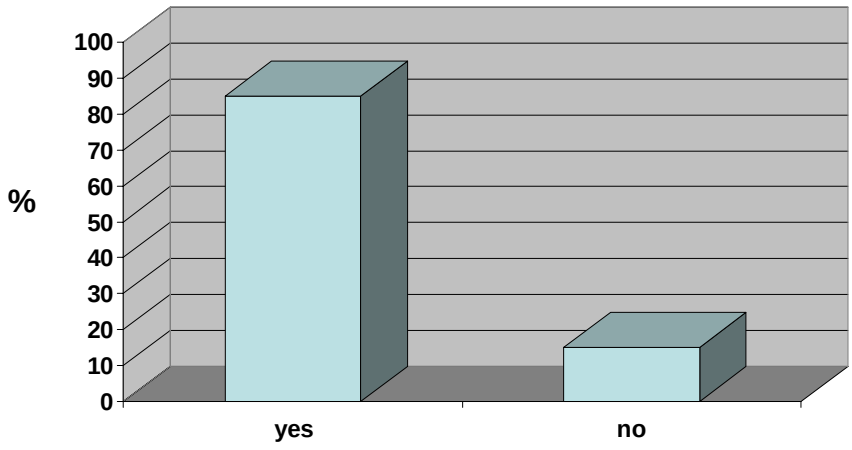
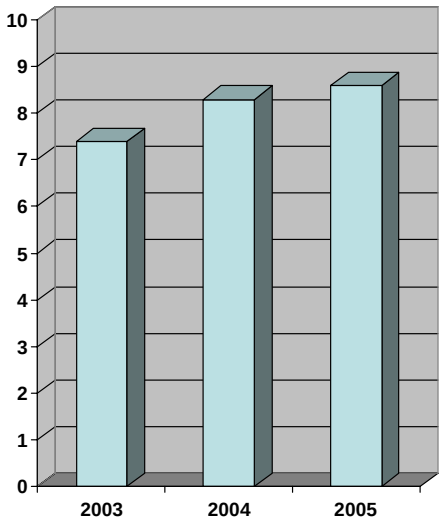
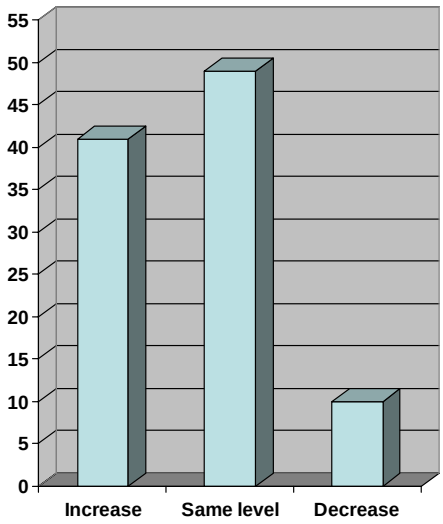



**Figure 5**

Development of beamtime access in the period 2003-2005




Average number of days per PI




Development for individual PIs (in %)

**Figure 6**

How do you anticipate that your needs for access to synchrotron radiation will change during the next 3 years?

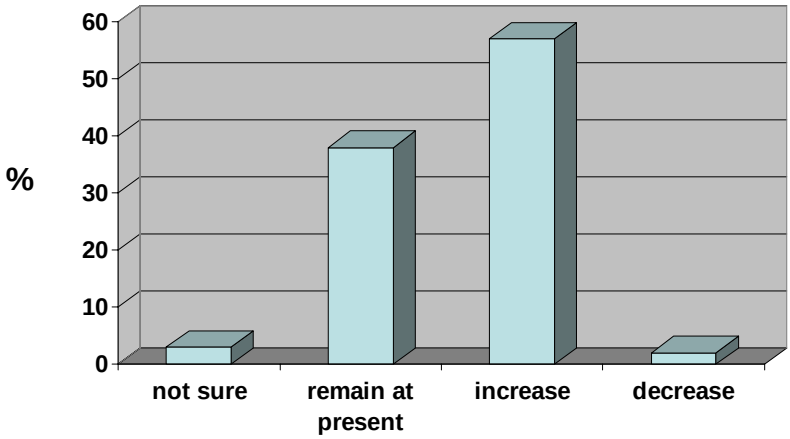



**Figure 7**

Which synchrotrons have you been using during the past 3 years?

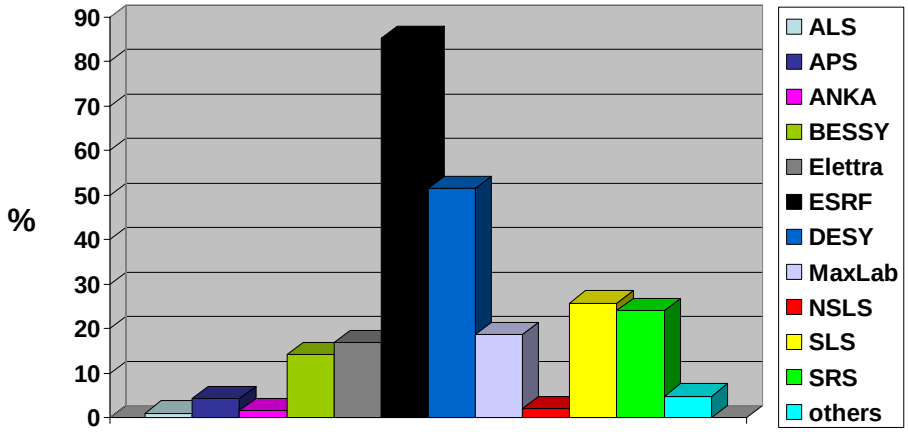



**Figure 8**

What percentage of your publications relies on access to a synchrotron facility?

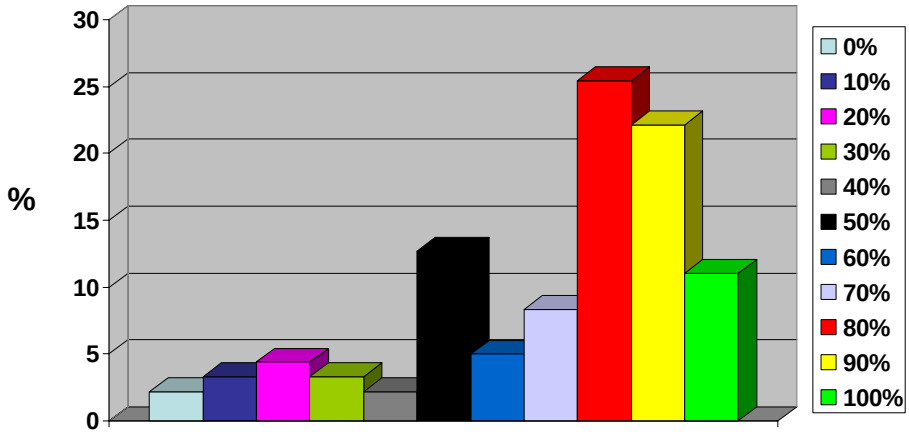



**Figure 9**

What type of experiments have you been doing at synchrotrons in the past?

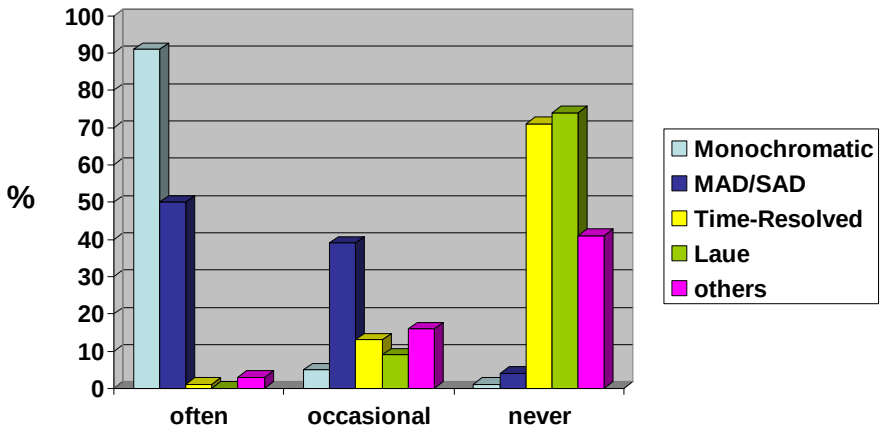



**Figure 10**

Which of these methodologies do you anticipate using to a significant extent in the next 3 years?

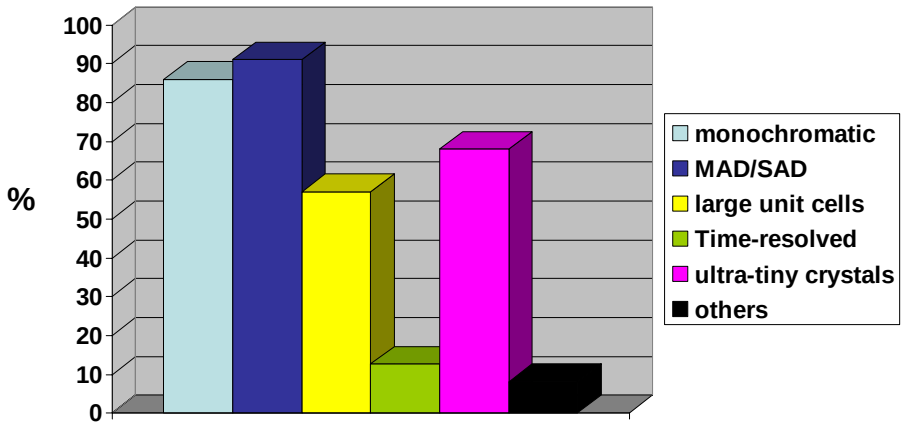



**Figure 11**

What are the rate-limiting steps in your research at present?

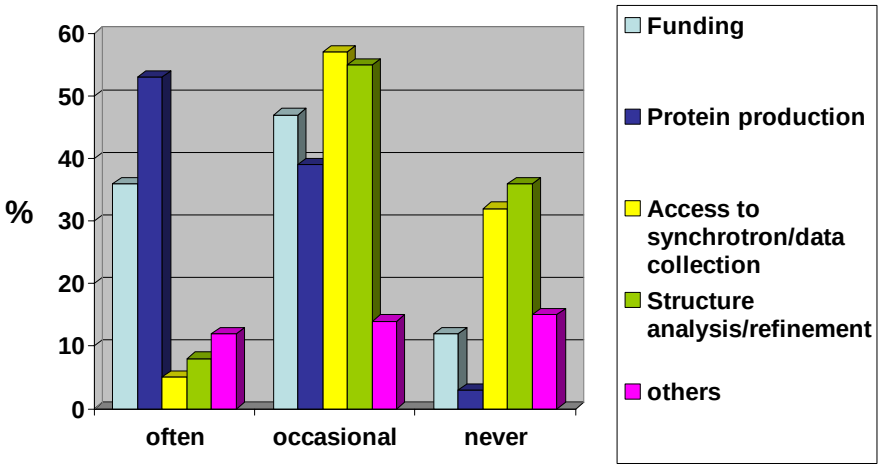



**Figure 12**

Indication of the significance given to the training and education of users

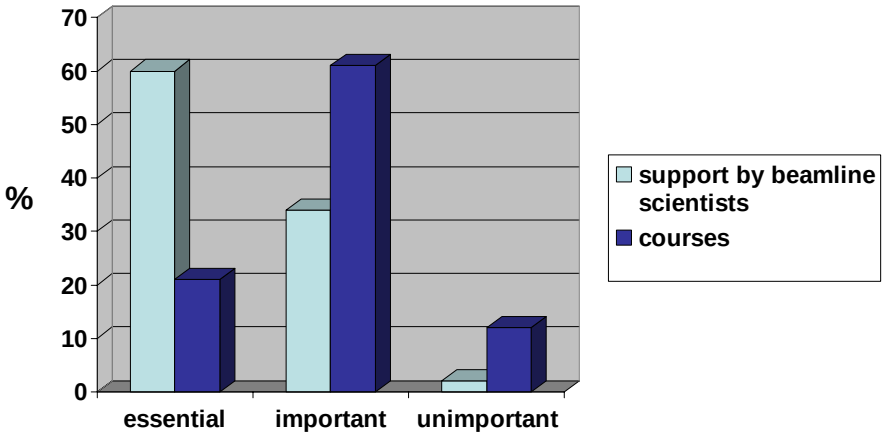



**Figure 13**

How important is travel support for the use of synchrotron radiation in your research?

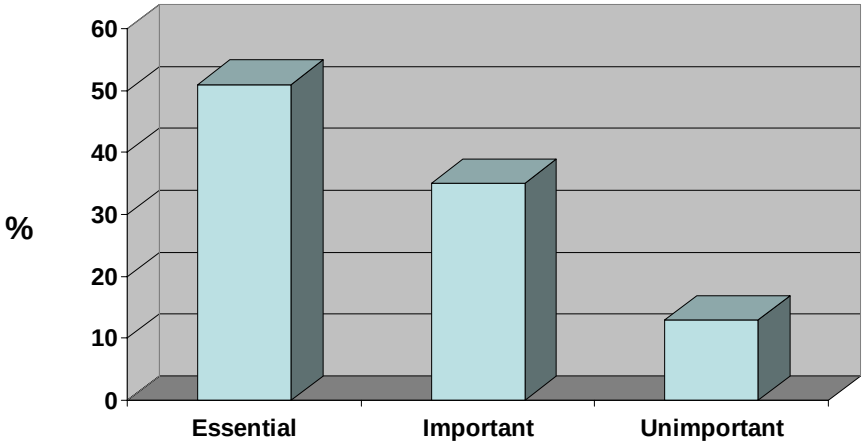



**Figure 14**

What developments would you like to see at synchrotrons in the near future?

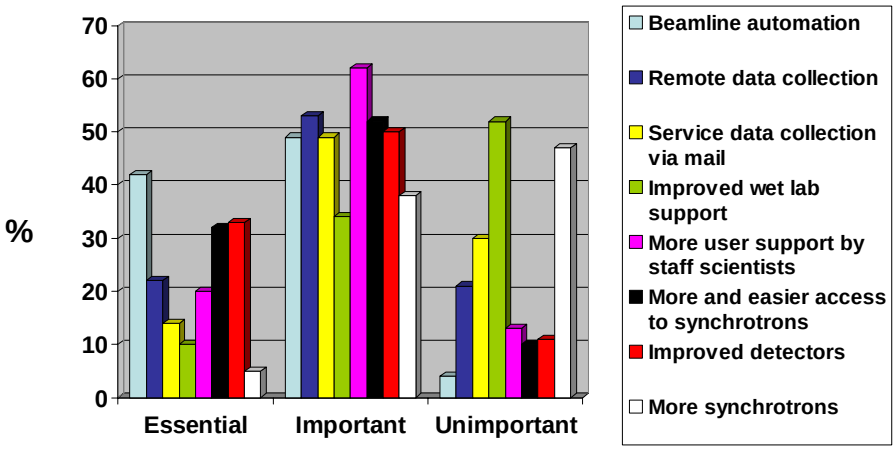



**Figure 15**

What type of experiments would you like to do at synchrotrons that cannot be done at present? (number of answers)

- data collection from thin or very small xtals/microfocus (**14**)
- online microspectrophotometry (UV/Vis, fluorescence, Raman, tomography, CD) (**14**)
- fast automated fluorescence scan/(fragment, derivative)screening/data collection (**13**)
- crystal climatization (high/low temperature, high pressure, humidity control) (**9**)
- better detectors (**6**)
- in situ crystal diffraction tests (**4**)
- time-resolved studies (different protein states, large assemblies) (**3**)
- protein powder/fibre diffraction (**3**)
- long-wavelength MAD (**3**)
- beamlines set up for ”difficult” projects (large unit cells, poor diffraction…) (**3**)
- (narrow-beam) Laue (**3**)
- ultra-high resolution (**2**)
- SAXS for time-resolved studies (**2**)
- obtain beamtime at very short notice (**2**)
- measurement of photon flux (**2**)
- remote data collection (**2**)
- routine-like phasing using S-SAD (**2**)
- flow-cell experiments (**2**)
- mail service for simple check for diffraction (**1**)
- X-ray laser diffraction (**1**)
- automated translation of crystals to avoid radiation damage (**1**)
- EXAFS on proteins (**1**)
- merging data from several microcrystals (**1**)
- improved flash-freezing procedures with minimal manual handling (**1**)

**Figure 16**

## Appendix 2: European synchrotrons in operation and under construction

| Machine and Location | Energy GeV | Storage ring circumference m | Injected current mA | Lifetime h | Horizontal emittance nm•rad |
|---|---|---|---|---|---|
| **Operational** | | | | | |
| BESSY II<br>- Berlin (DE) | 1.7 | 240.0 | 220 | 10 | 5.2 |
| DORIS III at DESY<br>- Hamburg (DE) | 4.45 | 289.2 | 120 | 12 | 404 |
| ELETTRA<br>- Trieste (IT) | 2.0/2.4 | 259.2 | 320/140 | 8.5/26 | 7.0/9.7 |
| ESRF<br>-Grenoble (EU) | 6.0 | 844.0 | 200 | 75 | 4.0 |
| MAX II<br>- Lund (SE) | 1.5 | 90.0 | 200 | >10 | 8.8 |
| Diamond<br>- Didcot (UK) | 3.0 | 561.6 | 300-500 | > 10 (20) | 2.74 |
| SLS<br>- Villingen (CH) | 2.4 | 288.0 | 400 | - | 5 |
| Soleil<br>- Gif-sur-Yvette (FR) | 2.75 | 336.0 | 500 | 15 | 3.7 |
| **To be closed December 2008** | | | | | |
| SRS<br>- Daresbury (UK) | 2.0 | 96.0 | 150/250 | > 24 | 130.0 |
| **Under construction** | | | | | |
| PETRA III at DESY<br>- Hamburg (DE) | 6 | 2300 | 100-200 | 2/24 | 1 |
| ALBA<br>- Barcelona (ES) | 3.0 | 268.8 | 250 | - | 4.3 |

## Appendix 3: Beam lines for macromolecular crystallography in Europe

| Station | λ in Å (E in keV) | % Biology | ΔE/E | B(rilliance) F(lux) * | $d_{min}$ (Å) | Typical exposure time (s/°) | Beam size (µm) ** | Detector | Set-up/ equipment | Special scientific applications, comments *future developments* |
|---|---|---|---|---|---|---|---|---|---|---|
| **1. Essentially fixed wavelength** | | | | | | | | | | |
| **BESSY II** | | | | | | | | | | |
| BL14.3 | 0.89 | 100 | | F 0.4 × $10^{11}$ | 0.8 | 20 | 255×40 | MAR 345 IP | SB | *crystal screening station, crystal diffraction optimization, MAR165 CCD detector* |
| **DORIS III** | | | | | | | | | | |
| X11 | 0.81 | 100 | $1\times10^{-3}$ | F $2.5\times10^{11}$ | 0.8 | 10-180 | 100-2000 | MAR 165 CCD | BM | high resolutions studies and SAD/MIR |
| X13 | 0.81 | 100 | $1\times10^{-3}$ | F $2.8\times10^{11}$ | 0.8 | 10-180 | 100-2000 | MAR 165 CCD | BM | high resolutions studies and SAD/MIR |
| BW7B | 0.84 | 100 | $1\times10^{-3}$ | F $8.1\times10^{11}$ | 0.8 | 10-60 | 100-2000 | MAR 345 IP | W, SC | high resolutions studies and SAD/MIR, crystals with large cell |
| **MAX II** | | | | | | | | | | |
| I911-2 | 1.043 | 100 | $2\times10^{-4}$ | B $3\times10^{15}$ | 0.95 | 60 | 300×300 | MAR 165 CCD | W, SC | |
| I911-5 | 0.907 | 60 | $2\times10^{-4}$ | B $3\times10^{15}$ | 0.83 | 60 | 300×300 | *MAR flatpanel detector* | W | Br-SAD |
| **Diamond** | | | | | | | | | | |
| I04.1 | 0.9163 | 100 | $3.9\times10^{-4}$ | F $3.5\times10^{11}$ | ~1 | To be established | 52×48 | CCD | To be established | **Design phase, to be commissioned 2009** MR, SAD, ligand binding studies |
| **ESRF** | | | | | | | | | | |
| ID14-1 | 0.934 | 100 | $1\times10^{-3}$ | B $5.8\times10^{10}$ | 0.95 | 1-10 | 20-200 | ADSC Q210 CCD | U, SC | fixed wavelength rapid data collection and SAD/MIR |
| ID14-2 | 0.933 | 100 | $1\times10^{-3}$ | B $1.3\times10^{11}$ | 0.97 | 1-10 | 20-200 | ADSC Q4R CCD | U, SC | fixed wavelength rapid data collection and SAD/MIR |
| ID14-3 | 0.931 | 100 | $1\times10^{-3}$ | B $9.4\times10^{10}$ | 1.04 | 1-10 | 20-200 | ADSC Q4R CCD | U, SC | fixed wavelength rapid data collection and SAD/MIR |
| ID23-2 | 0.873 | 100 | $<5\times10^{-4}$ | B $4.0\times10^{11}$ | 0.86 | 1 | 5.5×7.5 | MAR 225 CCD | U, SC, microfocus beam, variable focal length, | very small crystals, fixed wavelength rapid data |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | nanospindle, mini-focus (20 µm) | collection and SAD/MIR |
| BM01 (Swiss-Norwegian) CRG | (6-22) | | | | | | | MAR CCD | BM | |
| **2. Tunable Wavelength** | | | | | | | | | | |
| **BESSY II** | | | | | | | | | | |
| BL14.1 | 0.8-2.48 | 100 | | F $1.3\times10^{11}$ | 0.8 | 5 | 170×210 | MARMosaic 225 CCD | SB, F | *Flatpanel detector* |
| BL14.2 | 0.8-2.48 | 100 | | F $1.9\times10^{11}$ | 0.8 | 5 | 180×70 | MAR 165 CCD | SB, F | *SC, Mini-Kappa goniometer* |
| **DORIS III** | | | | | | | | | | |
| X12 | 0.7-2.1 | 100 | $2\times10^{-4}$ | F $3.3\times10^{11}$ @ SE-edge | 0.6 | 10-180 | 50-4000 | MARMosaic 225 CCD | BM, F, SC | |
| BW7A | 0.7-1.8 | 100 | $2\times10^{-4}$ | F $6.6\times10^{11}$ @ SE-edge | 0.6 | 10-180 | 50-1000 | MAR 165 CCD | W, F, SP | high resolution studies, crystals with large unit cells, long wavelengths |
| **ELETTRA** | | | | | | | | | | |
| BL 5.2R XRD1 | 0.5-3.1 | 60 | $10^{-4}$ | F $10^{12}$-$10^{13}$ | 0.5-0.6 | 30 | 200×700 | MAR 165 CCD & MAR 345 IP | W, F, "Elettra Virtual Collaboratory system", helium purged beam path, pressure cell, cryocooler | Laue diffraction, powder diffraction and high pressure experiments, high resolution studies *SC, R* |
| BL-11.2 XRD2 | ? | ? | ? | ? | ? | ? | ? | ? | ? | **under development** |
| **MAX II** | | | | | | | | | | |
| I911-3 | 0.7-2.0 | 100 | $2\times10^{-4}$ | B $3\times10^{15}$/ $1\times10^{13}$ | 1.36 | 15 | 250×150 | MAR 225 CCD | W,F | |
| **Diamond** | | | | | | | | | | |
| I02 | 0.5-2.5 | 100 | $1.4\times10^{-4}$ | F $10^{12}$ | | | 10-200 | ADSC 315 CCD | U, F, SC, R | |
| I03 | 0.5-2.5 | 100 | $1.4\times10^{-4}$ | F $10^{12}$ | | | 10-200 | ADCS 315 CCD | U, F, SC, R | studies on category 3 pathogens |
| I04 | 0.5-2.5 | 100 | $1.4\times10^{-4}$ | F $10^{12}$ | | | 10-200 | ADSC 315 CCD | U, F, SC, R | |
| I24 | 0.70-1.91 | 100 | $<2\times10^{-4}$ | F $>10^{12}$ | | | 5-30 | CCD | U, Microfocus beam, SC, R | **under construction, scheduled July 2008** very small crystals |
| **ESRF** | | | | | | | | | | |
| ID13 | 0.73-2.48 | | | | | | 0.3-30 ? | MAR CCD ? | U, SC, microfocus beam | MAD/SAD, SAXS, fibre diffraction, X-ray micro-fluorescence scanning, very small crystals |
| ID14-4 | 0.85-1.29 | 100 | $1\times10^{-4}$ | $1.8\times10^{12}$ | 0.88 | 1 | 20-200 | ADSC Q315r CCD | U, F, SC | MAD/SAD, PX projects requiring high intensity X-rays *Monochromator and focusing* |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | *mirrors upgrade* |
| ID23-1 | 0.62-2.48 | 100 | $2.2\times10^{-4}$ | $1.5\times10^{12}$ | 0.64 | 0.1-1 | 50 | ADSC Q315r CCD | U, F, SC | MAD/SAD, high-throughput data collection *R in planning, variable focal length* |
| ID29 | 0.35-2.1 | 100 | $2.2\times10^{-4}$ | $1.8\times10^{12}$ | 0.64 | 0.1-1 | 50 | ADSC Q315r CCD | U, F, SC, microfocus beam | MAD/SAD, high-throughput data collection *µfocus MAD* |
| BM14 (UK) CRG | 0.6-1.95 | 100 | | $1.0\times10^{13}$ | | | | MAR MOSAIC 225 CCD | BM, F, SC | MAD/SAD |
| BM16 (spanish) CRG | 0.73-2.06 | 100 | | | | 1-20 | 100-200 | MAR 165 CCD | BM, SC?, F | MAD/SAD, also SAXS/WAXS, small crystals, large cell |
| BM30A (French) CRG | 0.59-1.77 | 100 | | | | | | MAR 165 CCD | BM, F, pressure cell | MAD/SAD |
| **Soleil** | | | | | | | | | | |
| PROXIMA 1 | 0.83-2.48 | 100 | $7.5\times10^{-5}$ - $2\times10^{-4}$ | B $5\times10^{19}$ | 0.8 | <1 | 100-250 | MAR 555 (flat panel) | U, F, SC, software package "Structure studio" | high resolution, large unit cells |
| PROXIMA 2 | 0.83-2.48 | 100 | $10^{-4}$ | B $1\times10^{19}$ | <1 | <1 | 20 | not yet defined | U, microfocus beam, F, SC | **in design phase, user operation estimated for 2009** very small crystals |
| **SLS** | | | | | | | | | | |
| X06SA | 0.71-2.48 | 100 | 0.02 | F $>2\times10^{12}$ | 0.7 | | 85 × 10 25 × 5 | MAR 165 CCD & MAR 225 CCD | U, F, µ-diffractormeter | MAD/SAD, microcrystals, large unit cells, high resolution |
| X06DA | 0.71-2.07 | 100 | $1.4\times10^{-4}$ | F $1\times10^{12}$ | | | 90 × 90 | MAR 225 CCD | BM, F, SC | **under construction, scheduled May 2008** MAD/SAD production beamline for macromolecular crystallography |
| X10SA | 0.62-1.91 | 100 | <0.02 | F $>2\times10^{12}$ | 0.7 | | 50 × 10 | MAR 225 CCD | U, F, SC | MAD/SAD, large unit cells, small crystals Principal users: MPG, Novartis, Hoffman La Roche, Paul Scherrer Institute |
| **PETRA III** | | | | | | | | | | |
| P13 | 0.35-2.48 | 100 | | | | | | | | **in design phase, user operation estimated for 2009** |
| P14 | 0.35-2.48 | 100 | | | | | | | | **in design phase, user operation estimated for 2009** |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **ALBA** | | | | | | | | | | |
| XALOC | 0.62-2.48 | | $2\times10^{-4}$ | F $>10^{12}$ | | | 50-200× 20-100 | | | **under construction** |

**Abbreviations**

BM bending magnet
F fluorescence detector
SB super bend/wavelength shifter
SC robotic sample changer
SP UV/Vis spectrophotometer
R remote data collection/experiment control
U undulator
W wiggler

* in following units:

BESSY: photons/s/100 mA at 13.87 keV (14.3) or 13.5 keV (14.1, 14.2)
DORIS III: photons/(s 100 mA $mm^2$)
Elettra: photons/sec
MAX II: peak brilliance at source (photons/(s 0.1 % beamwidth $mm^2$ $mrad^2$)/peak brilliance at sample (photons/(s $mm^2$ $mrad^2$)
Diamond: photons/(s 0.1 % beam width) at 13530 eV for I04.1, photons/s in 100 $\mu m^2$ at 1 Å for I02, I03, I04, and photons/(s 0.02 % beam width) into 30×30 $\mu m^2$ for I24
Soleil: photons/(s 0.02 % beam width)
SLS: photons/(s 400 mA) at 1 Å
ALBA: photons/s in 0.1×0.1 $mm^2$
ESRF: photons/s

** if a single number or range is given, it applies to both horizontal and vertical beam size;
if two numbers are given, the first applies to horizontal, the second to vertical beam size