

Proposal by EMBL-Hamburg for an Integrated Life Science Centre at PETRA-III

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Summary

In this document, the EMBL proposes to extend the collaborative partnership of its Hamburg Unit with DESY by developing an integrated concept for applications in the life sciences that utilises synchrotron radiation produced by the PETRA-III storage ring. As such, PETRA-III is expected to become one of the leading synchrotron sources worldwide in terms of the available energy, emittance, infrastructure, and scientific environment. We plan to construct and operate integrated facilities for biological applications in Macromolecular X-ray crystallography, Small Angle X-ray Scattering and X-ray Absorption Spectroscopy. All of these facilities will be linked through a joint sample preparation facility. The proposed beamlines at PETRA-III will provide upgraded infrastructures and equipment for both established techniques and novel applications, eventually replacing existing beamlines at DORIS-III. The planned life science centre will be operated in close collaboration with DESY, and will be open to other research organisations. For the initial construction of three beamlines and one endstation, we are seeking 9.800.000 Euros in funding. The planned life science centre will constitute a unique infrastructure available to the international scientific community, with the capacity to unravel the most challenging structures of the molecules of life, thereby enabling important contributions to the quantitative investigation of the functions and structures of biological systems.

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Abbreviations

Bio	Biological
HTP	High throughput
LS	Life sciences
MX	Macromolecular X-ray Crystallography
SAXS	Small Angle X-ray Scattering
SG	Structural genomics
SR	Synchrotron radiation
XAS	X-ray Absorption Spectroscopy

1. Introduction and background

The life sciences have been revolutionised by the availability of synchrotron radiation (SR). Over 30 years ago, when X-ray crystallography could only be applied to a small proportion of biological macromolecules, the potential of SR for the acquisition of diffraction and scattering data in biology was recognised by pioneering scientists at DESY, in Hamburg, Germany. However, only during the last few years, with the advent of state-of-the-art third-generation SR facilities, atomic structures of some of the most complex macromolecules of life have been unravelled, including large protein-protein complexes, complete virus particles, and some integral membrane proteins. An outstanding example is the determination of the molecular structure of the ribosome, which was accomplished with the use of numerous SR beamlines around the world over a period of twenty years (Ban et al., 2000; Wimberly et al., 2000; Schlutzen et al., 2000; Yusupov et al., 2001). Several Nobel Prizes have been awarded to projects that, for their time, were of comparable complexity, including the determination of the structures of the photosynthetic reaction centre (1985), ATPase (1997), and the potassium ion channel (2003). In addition, SR has become a key technological tool for the discovery of novel drugs by the pharmaceutical and biotechnology industries, with the potential to improve treatments for a wide range of human diseases and to extend the human life span.

The Hamburg Unit of the European Molecular Biology Laboratory (EMBL) was founded in 1974 in response to the emerging capabilities of SR in the life sciences. Over the last three decades, a unique relationship has developed between DESY and EMBL-Hamburg, fostering the evolution of one of the largest facilities for the provision of SR in the life sciences well before the availability of third-generation synchrotron sources. At present, the EMBL-Hamburg SR beamline facilities in Macromolecular X-ray Crystallography (MX), Small Angle X-ray Scattering (SAXS), and X-ray Absorption Spectroscopy (XAS) are used for approximately 250 to 300 biological projects per year by researchers from more than 100 different research institutes across Europe (Figure 1, Annexes 1-3). Recent scientific highlights demonstrate that the EMBL-Hamburg SR facilities are still in high demand for the most challenging structural biology projects, such as that of the calcium pump (Sorensen et al., 2004), that of the outer membrane channel from mycobacteria (Faller et al., 2004), and for targets of highest biomedical relevance (Anand et al., 2003). The track record of EMBL-Hamburg in the provision of SR

beamlines for life science applications has been complemented by key technological advances associated with these facilities. Seminal examples are the construction of the first Imaging Plate Scanners (subsequently commercialised by MarResearch GmbH, Norderstedt/Hamburg) and software developments for the automatic interpretation of X-ray data, both in X-ray crystallography (Morris et al., 2004) and Small Angle X-ray Scattering (Svergun & Koch, 2003). Over the last few years, the EMBL-Hamburg SR beamline facilities have been enhanced by the extension and upgrading of sample preparation facilities with infrastructures for heterologous protein expression, purification, and characterisation. As part of a structural proteomics project, a high-throughput (HTP) crystallisation facility with the capacity for 10000 trays is currently being established and will be made available to the scientific community as part of a continuing commitment to provide scientific resources to external laboratories. The EMBL-Hamburg Unit has been integrated into the multidisciplinary framework encompassing research activities across the different research units of the EMBL and the recently established centres for specific fields of research (<http://www.embl.org/research/centres/index.html>). For example, the Centre for Cellular and Molecular Imaging (CCMI) provides a unique opportunity to combine SR-associated structural biology methods with other leading methods, such as NMR spectroscopy and electron microscopy, in addition to imaging approaches that use advanced light microscopy techniques.

Based on its track record of provision of services at DORIS-III and on research and development activities, EMBL-Hamburg proposes a centre for integrated life science applications using SR at PETRA-III. The development of this centre will ensure a synergistic interaction of leading expertise at EMBL in the life sciences and will take advantage of the unique source properties of the PETRA-III storage ring. Such an integrated facility will provide a world-renowned infrastructure for present and future challenges in structural biology.

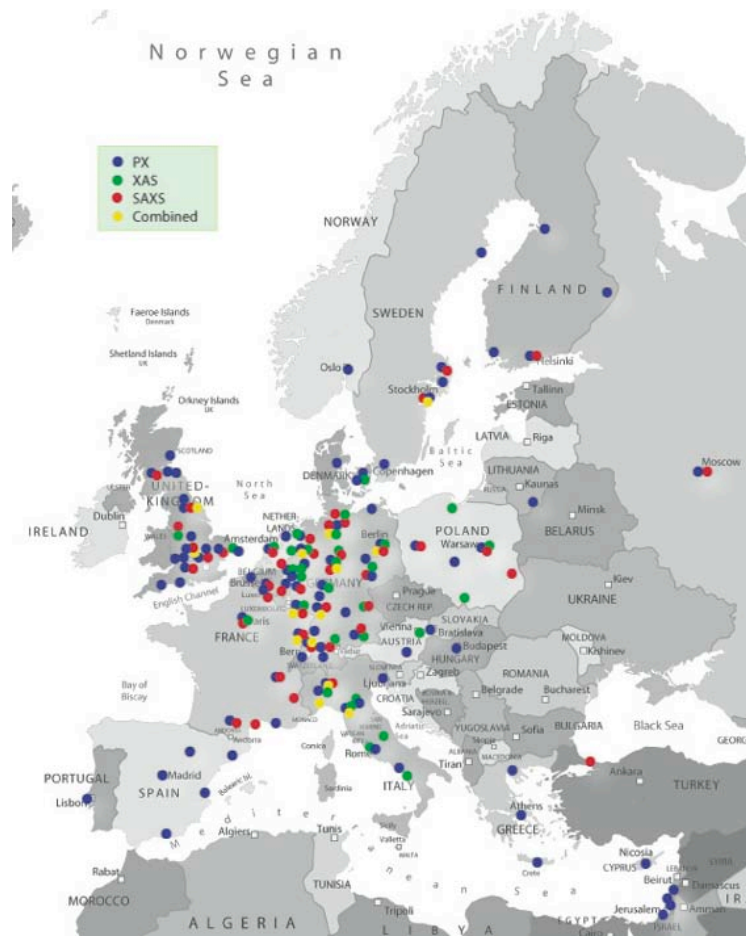


Figure 1: Geographic distribution of the EMBL-Hamburg user community (2000-2003). See also Annexes 1-3.

2. Future needs for state-of-the-art SR beamline facilities

Experiments using SR have become key tools for a wide spectrum of applications in the life sciences, exploiting the spectroscopic, scattering, and diffraction properties of biological samples in order to attain detailed structural information. At the EMBL-Hamburg Unit, three different experimental categories have emerged over the last few decades: X-ray Crystallography of Biological Macromolecules (MX), Small Angle X-ray Scattering (SAXS), and X-ray Absorption Spectroscopy (XAS). Recently, in MX applications, there has been a considerable shift from the use of in-house sources towards the use of SR. In fact, about one decade ago, only a small proportion of deposited X-ray structures of biological macromolecules were actually determined from SR data, while at present, the proportion is in the order of 90% (Jiang & Sweet, 2004). In addition, ongoing technological developments and proof-of-principle experiments demonstrate that SR may emerge as a key tool in future imaging techniques of large cellular structures (Schroer et al., 2003). Free Electron X-ray Laser (XFEL) facilities, which are planned in Stanford (USA) and Hamburg, may also provide unprecedented opportunities through the exploitation of their expected unique properties such as high photon flux, coherence, and a femtosecond time structure of the radiation.

The following assessment of needs for SR will focus on the three established experimental categories - MX, SAXS and XAS - in which EMBL-Hamburg has already demonstrated expertise and intends to offer future facilities at PETRA-III. Our analysis is based on the following informational resources:

- *US Biosynch report 2002* (<http://biosync.sdsc.edu/>). In this report, the third in a series of reports which appeared in 1991, 1997, and 2002, the needs for synchrotron radiation facilities with life science applications in North America were assessed.
- *ESF-Report "Needs for European Synchrotron and Related Beam-Lines for Biological and Biomedical Research"* (1998) and the follow-up report "*Protein Structure and Function in the Post Genomic Era*" (2001). These reports have assessed the need for SR beamline facilities for life science applications in Europe.
- *Strategic Forward Look, 2006-2015* (2002), EMBL document.
- EMBL-Hamburg user statistics (2000-2003), part of this proposal.

- EMBL-Hamburg Survey for future application needs in the life sciences using SR at PETRA-III, part of this proposal.

2.1. User community

Over the last few years, SR beamline facilities at EMBL-Hamburg have been used by a total of 300 different research groups (Annexes 1-3). The vast majority of these groups are from countries in Europe and Israel; about one third comes from the host country, Germany. Despite the advent of new state-of-the-art SR facilities in Europe (Annex 2), EMBL-Hamburg has been able to maintain its research-based community as well as to attract researchers from different institutions, as evidenced, for example, by an increased interest from researchers in neighbouring countries, such as the Benelux states, Denmark, and Poland. There has also been a substantial increase in the number of user groups from Central Eastern European countries and Russia. Although the proportion of users from other continents has decreased to below 10% over the last few years, we have very recently (including 2004) observed substantial interest from Asian countries, such as India, China, and Singapore.

Historically, the EMBL-Hamburg MX user community generally comprised structural biology groups who possessed their own in-house expertise in relevant methods. Although similar types of groups are also present in the SAXS and XAS user communities, a large proportion of these have been highly dependent on the scientific expertise for data acquisition and interpretation available at EMBL-Hamburg. We have recently observed similar trends in MX as well. The needs for specialised training are reflected in large oversubscriptions to the advanced training courses and training fellowships, offered by the EMBL-Hamburg Unit (<http://www.embl-hamburg.de/workshops2004.html>). In parallel, we have implemented several new benchmarks to ensure scientific excellence of the EMBL-Hamburg user community. The members of the Priorities Committee (current chair: Prof. Dino Moras, Illkirch, France) play a pivotal role in selecting and prioritising SR experiments by assessing experimental proposals on the sole basis of scientific quality.

With the proposed SR beamline facilities at PETRA-III, we plan to provide state-of-the-art integrated facilities to the structural biology-oriented scientific community. In addition, we aim

to expand our user community by reaching out to newly established research groups or to those that have not previously used our SR beamline facilities. Although the main objective will remain to continue to provide SR to research projects from academic research groups, we are also planning to allow access to groups from industry. This sector has expressed a strong interest in the proposed beamlines at PETRA-III, particularly in automated and HTP facilities with the options of remote operation. Our expectations for the future user community of the proposed PETRA-III facilities are summarised below.

2.1.1. Demands for future life science-oriented SR facilities by the present scientific community.

Over the last decade, the number of X-ray structures of biological macromolecules deposited in the Protein Data Bank (PDB) has increased by more than five-fold (1993: 628 structures; 2003: 3529 structures). During the same period, the average molecular mass per asymmetric unit of all the X-ray structures submitted to the PDB, which is an indicator of the complexity of the structures investigated, has more than doubled (1993, 37 kDa; 2003, 80 kDa). Therefore, even by conservative estimates, it can be projected that, at the time of the opening of PETRA-III, there will be over 10000 structures deposited annually, with European countries and Israel contributing to about 35-40% of the total. In parallel, we expect that the average molecular mass per asymmetric unit will be substantially greater than 100 kDa, reflecting present trends towards the structure determination of more complex biological assemblies. Although we anticipate that improvements in future SR beamline facilities (see Annex 4) and their associated infrastructures will allow a further increase in throughput, in the absence of additional new facilities the existing ones will not provide sufficient capacity to meet the expected demand for beamline experiments.

We have attempted to determine the projected needs of SR life science experiments by surveying the demands of the current EMBL-Hamburg user community (Annex 5). Of the 159 research groups that completed the survey, 70%, 33% and 18% have made use of our MX, SAXS, and XAS facilities at DORIS-III, respectively (Table 1). Once the SR beamlines at PETRA-III will become available, 81% (+11%), 50% (+17%) and 25% (+7%) of these surveyed research groups, respectively, would like to make use of the corresponding SR beamline facilities, representing an increase in interest in all three areas of our proposed operation. We have observed that a large

proportion of the user community is interested in the combined use of two of the three applications: MX/SAXS (39%); MX/XAS (17%); SAXS/XAS (11 %); or a combination of all three (8%). Additionally, the majority (66%) of the user community has stated that they would like to gain access to future sample preparation facilities, and most of them (81%) are interested in local data processing and interpretation infrastructures. In essence, the survey data demonstrate a clear and strong demand for the use of combined and integrated SR facilities. The data, of course, do not include demands from research groups that have, to date, not made use of the facilities at EMBL-Hamburg.

Table 1: Survey of EMBL-Hamburg user community			
Access category	Specific applications	Past/present applications	Future interest
MX		112 (70%)	128 (81%)
	Energy tuneability		111 (70%)
	Microfocusing		117 (74%)
	High throughput		49 (31%)
SAXS		53 (33%)	79 (50%)
XAS		31 (19%)	40 (25%)
Sample preparation		17 (10%)	120 (75%)
	Expression, purification		19 (12%)
	Sample characterisation		53 (33%)
	HTP crystallisation		52 (32%)
Data processing and interpretation			128 (81%)
	On-line facilities at EMBL-Hamburg		119 (75%)
Remote accessibility			97 (61%)
Advanced training			134 (84%)
	Training courses		112 (70%)
	Training fellowships		80 (50%)
	Remote training		69 (43%)

Table 1: Survey of EMBL-Hamburg user community for present applications and future demands. The complete questionnaire is in **Annex 5**. The questionnaire was completed by 159 (100%) external research groups.

2.1.2. Projected demands for PETRA-III SR facilities by new research groups

The underlying methods and techniques of present SR applications in the life sciences, particularly in MX, have advanced sufficiently to allow their application by research groups with core expertise in methods other than structural biology, provided that appropriate external infrastructures are in place. Recently, genomic and proteomic data from an increasing number of completely sequenced genomes have created unprecedented opportunities to investigate the structure and function of an inordinate number of novel biological targets, in turn, imposing enormous challenges on their production, separation, and purification from biological systems. We expect that the provision of integrated sample preparation, crystallisation, and SR beamline facilities will enable many new life science research groups to make use of SR.

Structural genomics consortia. While research in structural biology is largely driven by scientific hypotheses, an increasing number of structures of biological macromolecules originate from structural genomics (SG) consortia. Furthermore, there is a clear trend towards the establishment of large-scale national structural biology centres such as the Oxford Protein Production Facility (<http://www.oppf.ox.ac.uk/>), the Swiss National Structural Biology Centre (<http://www.structuralbiology.unizh.ch>), and the Israel Structural Proteomics Centre (<http://www.weizmann.ac.il/ISPC>). EMBL-Hamburg is already involved in a number of SG initiatives and once the SR beamline facilities at PETRA-III are in place, the research unit will be ideally positioned to make them available to these initiatives. We expect that the EC-funded project BIOXHIT (<http://www.embl-hamburg.de/BIOXHIT/>), coordinated by EMBL-Hamburg, will play a catalysing role in developing and providing relevant SR beamline and endstation technologies.

'Remote' user community. While in the past, life science experiments at SR facilities have inevitably required the physical presence of the experimenters, recent advances in automation and electronic communication have begun to provide tools for the remote control of beamline experiments (McPhillips et al., 2002). Protocols for the remote monitoring of SR beamlines, such as the *Virtual Collaboratory System* from Elettra (<http://www.elettra.trieste.it/science/highlights/2002-2003>), are currently being implemented at a number of SR facilities worldwide. Although we anticipate that a large proportion of future experiments at EMBL-Hamburg will still be carried out by external visiting scientists, for

advanced training in the relevant methods and due to specific experiment requirements, we do expect an increasing demand for remote monitoring and control. This will apply to experiments in all three categories (MX, SAXS, XAS) in which screening of experimental conditions will be required. Similarly fervent requests for remote monitoring and control have been expressed by the industrial user community.

2.2. Samples for SR experiments

2.2.1. Large multi-component assemblies and integral membrane proteins

Although the number of structures of integral membrane proteins and large multi-subunit assemblies determined to date has been modest, there are several factors that indicate a projected increased emphasis to characterise the most complex molecular structures:

- Recently, experimental methods have been established which systematically identify complexes of biological targets (proteins) bound to ligands such as other proteins, DNA, RNA, etc., in living cells. Some of these methods, such as the TAP technology (Gavin et al., 2002), also provide conditions for purification of such assemblies.
- While there is clear evidence that the number of folds of protein domains is limited, assembling these ‘fold units’ into larger oligomeric complexes permits a virtually unlimited number of combinatorial assemblies. Naturally, the scientific community is highly interested in novel protein-protein or protein-ligand interactions found in large complexes and assemblies.
- Advances in heterologous expression systems permit the preparation of many novel large protein assemblies. These techniques are complemented by modern fermentation facilities, which are required for the purification of complexes from natural sources that cannot be assembled using heterologous expression.

It is expected that efforts to purify such complex structures will be severely taxed, hampering the provision of samples in adequate yields for structural analysis. Sample stability and maintenance of composition with respect to subunit assembly will be of particular concern, requiring specific sample monitoring tools. Furthermore, in many cases, it will not be possible to achieve

sufficiently high sample concentrations (mM range). Therefore, the analysis of samples at low concentrations will become paramount in SR applications such as XAS and SAXS. For MX applications, the resulting crystal properties of such complex systems (i.e., smaller crystal size and weaker diffraction properties) need to be considered to assess the feasibility of X-ray structure determination. Given the current trends, we expect that it will be essential to provide extensive testing facilities that are suitable for very small crystals and allow rapid evaluation of their diffraction properties.

2.2.2. Biological samples for structural chemistry analysis

Over the last decade, data mining of new sequences from an increasing number of genomes has been at the forefront of research in the life sciences. However, it is expected that the quantitative investigation of their properties in the context of living biological systems will become a main focus of future research, requiring a broad spectrum of experimental tools appropriate for the characterisation of single molecules as well as that of large cellular structures. These investigations are not expected to be limited to descriptive analyses of effects observed upon system alterations and will require appropriate techniques to elucidate the underlying chemical and physical mechanisms.

Chemistry at atomic resolution: In the field of structural analysis, we expect an increased demand for methods with the capacity to provide sufficient atomic detail to unravel the underlying chemistry of major biological processes. In MX, for instance, methods for atomic resolution studies (resolution $< 1.2 \text{ \AA}$), and automated analyses, have been pioneered by members from EMBL-Hamburg (Schmidt & Lamzin, 2002) amongst others. In addition, XAS is a superb tool to reveal precise information on the coordination chemistry of metal centres in biological systems.

Structural dynamics: Although MX is unequalled by any other structural biology method in investigating biological structures at the atomic detail, most of the resultant structures present only time-averaged snapshots of a specific conformational state. Although some dynamic processes in protein crystals have been monitored by the use of polychromatic SR (Stoddard, 1998; Schotte et al., 2003) such experiments have been restricted to a limited set of biological

model systems, and it remains unclear whether they will become applicable to a broader range of biological macromolecular samples. Future X-ray Free Electron Laser (XFEL) facilities, such as those planned at DESY (<http://www-hasylab.desy.de/>), may provide an appropriate time structure to unravel dynamic processes in biological systems by pump-and-probe experiments. With the high level of accuracy in determining structures by X-ray crystallography, there is a great potential to combine these data with those that emerge from other experimental methods, such as NMR spectroscopy and biocomputational methods. SAXS and, to a lesser extent, XAS are ideal for time-resolved studies, allowing insight into structural dynamics within the inherent limitations of the method.

Compound screening: There is an increasing interest in combining screening approaches of large compound libraries with structural investigations. The biotechnology and pharmaceutical industries have a long-standing tradition and successful track record in structure-based drug design approaches, coupled with compound screening, and/or combinatorial chemistry efforts (Kuhn et al., 2002; Noble et al., 2004). More recently, the systematic use of small-molecule ligands has begun to shape the analysis of entire biological systems (Koh and Crews, 2002; Mayer, 2003), complementing established methods such as genetic knock-out technologies and RNAi approaches. Since throughput is a key parameter in screening approaches, third-generation SR facilities with a high photon flux in a small focal spot are ideally suited for this purpose. In addition, these types of experiments will critically depend on HTP sample preparation, crystallisation, mounting, and data acquisition as well as on automated processing and interpretation of the collected data.

2.2.3. Structural genomics

The availability of completely sequenced genomes has created novel opportunities for the quantitative analysis of the translated protein products (proteome) and their associated functions. Although structural biology techniques, such as X-ray crystallography, historically have not been designed for parallel HTP approaches, major efforts have been undertaken to advance X-ray crystallography to a level that enables the determination of most of the structures of entire proteomes (Burley & Bonanno, 2003). In European initiatives, such as SPINE, 3D-

REPERTOIRE, and E-MEP, a trend has emerged to specifically focus on the most challenging targets, such as multi-protein complexes and integral membrane proteins from higher vertebrates like *Homo sapiens* and the mouse. For most initiatives, the method of choice will remain X-ray crystallography, and it is apparent that an immense need for SR beamline facilities will emerge. In addition, our own experience and that of others demonstrate that SAXS has already become a key tool for biophysical sample characterisation and low resolution shape determination for many complex targets from SG consortia. Although SG-oriented sample requirements are not different from those mentioned previously, given the central emphasis of genomics/proteomics efforts on throughput, it will become necessary to optimise infrastructures for automated sample preparation and mounting, as well as for processing and interpretation of SR data.

2.2.4. Towards supramolecular and cellular structures

Current trends in the life sciences point to a phase of integrative systems biology, with the aim of understanding living systems across the many different levels of biological organisation. Due to the recent availability of submicron size X-ray probes, it is now possible to carry out two-dimensional mapping of biological samples at the cellular level by various X-ray analytical methods. In this manner, information has been obtained on the elemental distribution (X-ray fluorescence), local oxidation states (XAS), and distribution of supramolecular or subcellular structures (SAXS) (Bohic et al., 2001; Fernandez et al., 2004). In some cases, it has even been possible to generate three-dimensional maps by applying tomographic reconstruction algorithms (Ortega et al., 2004). However, the acquisition of new information is generally accompanied by an exponential increase in the scanning time. In contrast to other available scanning techniques, many of the X-ray imaging methods work in a full-field mode, reducing the scan duration and, in turn, allowing the tomographic study of a wide spectrum of biological objects, ranging from cells to tissues to even micro-organisms (Larabell et al., 2004). Sources such as PETRA-III with a high transverse coherence could be ideal to explore these methods for biomedical applications. Although there are no immediate plans to implement such applications in Hamburg, we will monitor developments and respond to any emerging needs in this area.

3. Proposal for an integrated life science SR centre at PETRA-III

The recent decision by DESY to convert the PETRA ring into a dedicated world-renowned storage ring provides a unique opportunity for EMBL to upgrade its current research and service facilities. Our proposal is driven by the demands expressed by our user community (Table 1) and by the projected SR-related challenges in the life sciences.

We are proposing an integrated centre for life science applications using SR at PETRA-III (Figure 2).

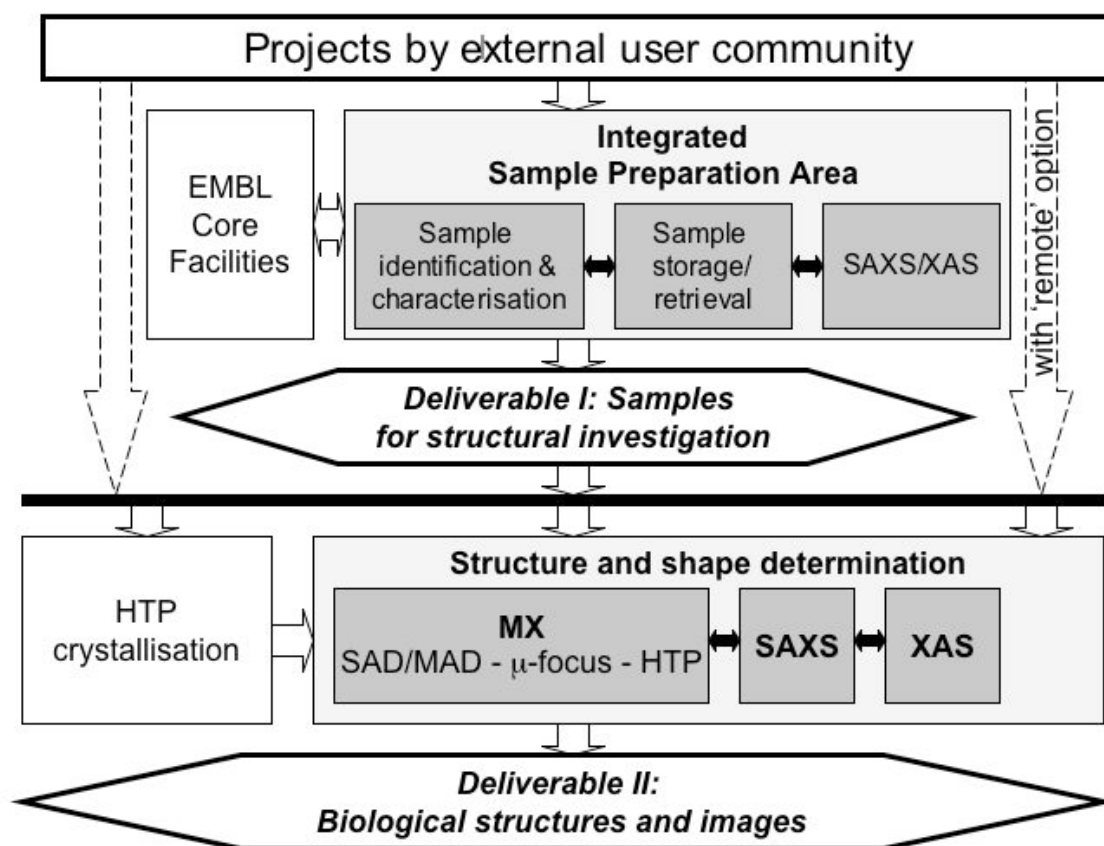


Figure 2: Flow chart of the planned for the integrated PETRA-III for life science applications.

The centre will offer state-of-the-art beamline facilities for three established structural biology methods:

- Macromolecular X-ray Crystallography (MX) (3.1.)
- Small Angle X-ray Scattering (SAXS) (3.2.)
- X-ray Absorption Spectroscopy (XAS) (3.3.)

The facilities will have a uniform experimental design for most beamline components and, where possible, also for the experimental endstations. This common concept will enhance the combined and integrated use of the planned PETRA-III beamlines. They will be constructed such that the outstanding beam properties offered by PETRA-III with respect to beam focus, beam divergence, and energy dispersion will be optimally used for specific experiments. Furthermore, we will ensure that the experiments will be highly automated and options for remote control will be included wherever possible. The beamlines will be operated and supervised by a sufficient number of scientific and technical staff in cooperation with the local instrumentation group (see Chapter 4). The combined beamline facilities will be complemented by and directly associated with a common sample preparation area (3.4.), which will provide facilities for sample characterisation, crystallisation, and storage. Thus, the integrated future PETRA-III facilities will essentially provide two types of deliveries: (a) the preparation and characterisation of samples optimised for structural investigation, including their crystallisation; (b) the determination of structures and images of biological samples by the SR beamline facilities.

With the establishment of the proposed facilities, the centre will offer experimental solutions to provide structural characterisation into a wide range of biological molecules and assemblies, both in solution and in crystalline forms. Proposed applications in biological XAS and SAXS will extend towards the interface with tomographic images of cellular structures, beyond the level of individual biological macromolecules. The future facilities at PETRA-III will be complemented by other structural biology facilities across the EMBL units, particularly in Heidelberg (Biological Structures and Computational Biology), the Grenoble Unit, and the EMBL Unit in Hinxton, Cambridge (EBI, Computational Biology).

We would like to construct and operate the centre in close cooperation with DESY, building on more than 30 years of successful partnership. An important step towards further joint

cooperation has recently been signalled by the signing of the *Framework Institutional Partnership Agreement* by DESY and EMBL on May 19th, 2004. This agreement stipulates that EMBL Hamburg will continue its role in fostering and providing synchrotron radiation facilities to the European structural biology community using photons provided by the Hamburg Synchrotron laboratory (HASYLAB) at DESY. In addition, EMBL and DESY remain open to enlarging the partnership to incorporate additional partner research organisations. We would also like to arrange the facilities with a level of flexibility that will allow us to adopt novel applications, such as X-ray tomography (section 2.2.4.).

Each of the following proposals comprises a justification for one of the proposed facilities, a description of the proposed facilities, and a summary of possible applications. Further technical details of the proposed facilities are described in the PETRA-III Technical Design Report (2003) by DESY, pages 404-448. Web site: http://www-hasylab.desy.de/facility/upgrade/petra_tdr.htm

3.1. Macromolecular X-ray Crystallography (MX)

3.1.1. Justification

EMBL-Hamburg has been a leading provider of SR beamlines for Macromolecular X-ray Crystallography (MX) for over two decades. At present, about 200 PDB depositions per annum (about 25% of all PDB depositions from European synchrotrons and 8% world-wide) are based on experiments performed at the DORIS-III beamlines. Reflecting our expectations of future requirements in MX applications (Chapter 2), the strongest demands of the present EMBL-Hamburg user community are for wide range energy tuneability (for experimental determination of phases, using a wide spectrum of elements) and for microfocusing (for testing and data collection from small crystals). In addition, there are strong needs for automation and remote control/monitoring of experiments, particularly for those which originate from structural proteomics projects and from the industrial-based user community. To optimally respond to these needs, we are proposing an integrated concept of three MX beamlines, each optimised for one of the three specific demands. We are seeking funding for beamlines MX1 (wide range energy tuneability) and MX2 (microfocusing and micro collimation), and we are offering our cooperation to operate MX3 (high-throughput structure production) with other research

organisations. The MX beamlines will be integrated into a highly automated pipeline for sample preparation (see Chapter 2) and automated data interpretation using in-house packages such as ARP/wARP. The HTP crystallisation facility will be directly associated with the future MX beamlines at PETRA-III, and the development, implementation, and provision of automated tools for transfer of crystals, either for test purposes or data acquisition, will be of critical importance. We will also ensure a user-friendly environment for combined MX, SAXS, and XAS applications, reflecting the demands expressed by the user community, by providing compatible hardware and software user interfaces. We are confident that the planned setup of the MX beamlines at PETRA-III will provide an excellent infrastructure to meet the most demanding challenges in structural biology using X-ray crystallography.

3.1.2. Proposal

We suggest that the three proposed MX beamlines (MX1, MX2, MX3) should be located on two neighbouring canted undulator pairs. Following the demands expressed by the EMBL-Hamburg user community (Table 1), MX1 will be characterised by its tuneability over the widest possible energy range, MX2 by microfocusing optics, and MX3 by its specialisation for HTP applications. Our aim is to provide adequate facilities that can meet the challenges of both standard crystallographic applications and new, cutting-edge experiments that have not been attempted previously at the facility.

- State-of-the-art tuneability over a broad energy range, energy band pass and excellent beam stability;
- High brilliance coupled with small focus size;
- A high degree of automation, user friendliness and parallelisation;
- Experimental conditions to allow for measurements of extremely high resolution (< 0.8 Å);
- An experimental environment which allows time-resolved measurements matched to the time structure of the PETRA ring, with an option to use wide energy band pass radiation;

- Adaptations to meet specific user demands (lighting, data collection temperature, use of lasers, etc.)

Table 2 summarises the principal characteristics of the proposed MX beamlines. Figure 3 shows the proposed generic layout of all stations. The optics of all MX beamlines will be similar in design, allowing the standardisation of components and straightforward knowledge transfer, particularly during the commissioning stage. The instrumental solutions proposed here are based on the current state-of-the-art technology.

Table 2: Design parameters of the proposed MX beamlines at PETRA-III			
Beamline	MX1	MX2	MX3
Main purpose	Wide range tuneability	Microfocus and special applications	High-throughput structure production
Energy range, keV	5.0–35.0	6.0–18.0	6.0–16.5
Bandpass (after monochromator) $\Delta E/E$	Si(111): $1.4\text{--}2.3 \cdot 10^{-4}$ Si(311): $4\text{--}9 \cdot 10^{-5}$	Si(111): $<1.7 \cdot 10^{-4}$	Si(111): $<1.6 \cdot 10^{-4}$
Minimum focus size, μm^2 (horizontal x vertical)	25 x 6	14 x 4	24 x 6
Divergence of the focused beam, mrad^2 (horizontal x vertical)	0.3 x 0.2	0.5 x 0.25	0.3 x 0.2
Intensity, ph/s	$3 \cdot 10^{13} - 1 \cdot 10^{12}$	$2 \cdot 10^{13}$	$2 \cdot 10^{13}$
Pink beam option ($\Delta E/E = 1.5 \cdot 10^{-2}$), Intensity, ph/s	Yes $\sim 10^{15}$	No	No

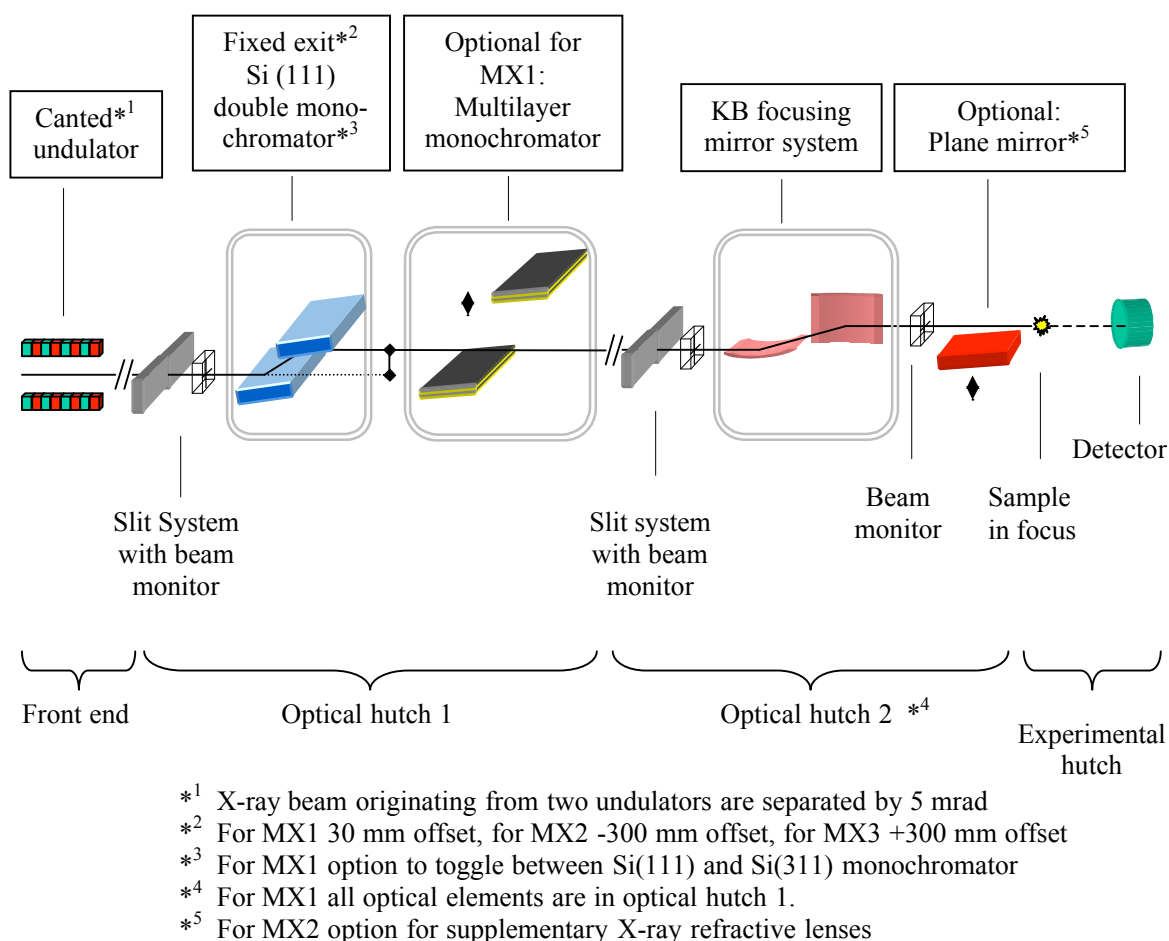


Figure 3: Generic layout of the optical components for beamlines MX1, MX2, and MX3

3.1.3. Possible applications

The proposed MX beamlines will be ideally suited for all established crystallographic applications. With the high brilliance and degree of automation they will ensure that future data collections can be carried out at a comparable to or higher efficiency than that possible at the presently leading SR facilities. In addition, their high brilliance and small focal spot sizes will make it possible to carry out cutting-edge MX experiments. Although most of the applications will be feasible on more than one of the three planned MX beamlines, each of these beamlines will be specifically designed for one of the major projected directions: (i) experimental phasing, using a wide range of elements, requiring broad energy tuneability; (ii) microfocusing and

microcollimation for small crystals; (iii) HTP for rapid screening of conditions, followed by X-ray data collection. Some of the planned applications are listed below; the feasibility of the planned MX beamlines for these applications is summarised in Table 3.

Table 3: Feasibility of applications at planned MX beamlines			
Application	MX1	MX2	MX3
	Wide range tuneability	Microfocus	HTP
(a) Small crystals	+	+++	+
(b) Large unit cells	+	+++	+
(c) Phase determination (incl. novel approaches)	+++	+++	+
(d) Ultra high resolution	+++	-	-
(e) High throughput applications	+	+	+++
(f) Time-resolved measurements	+++	-	-
(g) Remote data collection	+	+	+++

Table 3: Feasibility of applications at the three MX beamlines

+++ tuned to specified application.
+ feasible for specified application.
- unsuitable for specified application.

- a) *Testing of and Data Collection from Small Crystals.* Many of the macromolecular crystals, particularly those of integral membrane proteins, generally tend to be small in size and their diffraction properties are often inadequate to allow successful structure determination. The planned beamline MX2 will be ideally suited to test and collect X-ray data from this type of crystals. Its focal spot and options for fine micro collimation will allow the testing of crystals smaller than 10 μm in size. A state-of-the-art infrastructure for automated mounting and centring will be provided as well. In addition, we plan to implement a pipeline for the automated transfer of crystals from the HTP crystallisation facility.
- b) *Crystals with Large Unit Cells.* Crystals of large macromolecular assemblies often have large cell dimensions in the range of several hundred \AA , and, in some cases, even exceeding 1000

Å. The superior spectral parameters of PETRA-III will allow efficient resolution of the resulting dense diffraction patterns in reciprocal space. An important parameter to consider in choosing future detectors at the MX beamlines is the spatial resolution for recording single diffraction spots.

- c) *Novel Approaches to Phase Determination.* The wide energy tuneability of the MX1 beamline will allow experiments to be performed at the K- or L- absorption edges of all elements from Ti to U, thereby encompassing most of the periodic table (Figure 4). This will be coupled with a small focal spot size to allow anomalous diffraction experiments to be conducted on small crystals in the tens of microns range. Current techniques involve the measurement of datasets at 1-3 different wavelengths, imposing a risk of radiation damage during data collection in the exposed volume, thus degrading the quality of the final signal. The small focus of PETRA-III will translate to lower collection times from small illuminated volumes ($\sim 1 \mu\text{m}^3$) of a given crystal, allowing X-ray data to be collected at different, unexposed portions of the same crystal. This, in conjunction with a narrow energy band pass and robust scaling protocols, will lead to an increase in the quality of the anomalous signal and a radical improvement in the probability of a successful structure determination. The high brilliance available from the PETRA-III source will allow experiments to be conducted that utilise the special properties of both long and short wavelengths. The planned setup for MX1, which will be tuneable in the range of 5 to 35 keV, will allow the use of the anomalous signal of sulphur (or phosphorus), naturally present in most biological macromolecules, to obtain phase information from native crystals (Dauter, 2002). Recently, it has been demonstrated that radiation damage may, in and of itself, be used as a means of solving the “phase problem” (Ravelli *et al.*, 2003; Weiss *et al.*, 2004); the high brightness of the PETRA-III beamlines will allow this feature to be used constructively. For instance, by taking advantage of the small beam size, it will be possible to collect complete datasets from a large number of unexposed portions of the crystal, with angular offsets between the start of each dataset. It will also be possible to combine data in such a way as to mimic data sets actually recorded with different absorbed doses. Furthermore, from a comparison of these datasets, phase information could then be extracted, making use of native crystal(s) for both phasing and structure refinement.

- d) *Ultra-High Resolution Data Collection.* A considerable fraction of crystals of biological samples display diffraction properties, allowing structure determinations at true atomic resolution ($< 1.2 \text{ \AA}$) and investigations of chemical properties, such as those, for example, associated with enzymatic reactions. These opportunities have been only partially exploited in the past due to the lack of SR beamline facilities with an appropriate experimental environment. The high energy spectral end of MX1 is ideal to provide an environment for these kinds of applications, allowing Bragg angles of ultra-high resolution diffraction spots to be reduced sufficiently to fit onto commercially available detectors without the need for complicated detector movements. The detector efficiency at high energies, however, remains a critical parameter for this application.
- e) *High-throughput (HTP) applications.* The infrastructure of the MX beamlines at PETRA-III will allow rapid screening of experimental conditions. One major focus will be the testing of the diffraction properties of new crystals. Automated equipment for crystal transfer, mounting, and diffraction image acquisition/processing will substantially reduce the enormous time requirements for screening. A second area of focus will be to test a large number of ligands, soaked or co-crystallised with crystals of known 3D structure. Most of the SR beamtime used by commercial enterprises from the biotechnology and pharmaceutical sectors will be requested for this latter application. Although we plan to furnish each MX beamline with appropriate equipment for automated sample mounting and centring, we are planning to dedicate MX3 specifically to this kind of HTP application. Taking into consideration the expected user community for HTP screening applications (particularly commercial enterprises), we are proposing to construct and operate this beamline jointly with additional external partners.
- f) *Time-Resolved Measurements.* Dynamic processes in protein crystals can be monitored with the use of polychromatic SR even though current applications remain limited (Stoddard, 1998). One possible and attractive alternative involves the use of a narrow-band polychromatic beam ($\Delta E/E \approx 0.01-0.02$), which has been proven to considerably reduce X-ray data acquisition time (Bourenkow & Popov, EMBL-Hamburg, unpublished). This could allow the synchronisation of experimental schedules and the study of the dynamics of some processes in biological macromolecules. In addition, the use of a narrow-band polychromatic

beam is expected to reduce, or even eliminate, some of the largest sources of errors in X-ray diffraction data collection. The implementation of an appropriate experimental setup for time-resolved experiments at PETRA-III MX beamlines may provide the foundation to consider time-resolved experiments at the future X-FEL facility at DESY, Hamburg, which will create opportunities for experiments to be performed at an unprecedented time range of femto seconds.

- g) *Remote Data Collection.* The PETRA-III MX beamlines are also expected to provide a highly valuable and widely anticipated “FedEx” service for the European structural biology community, while still maintaining the philosophy of readily available access to synchrotron users who would wish to collect their own datasets. The following options are envisaged: (a) remote monitoring of experiments, which will be carried out by a representative of the user laboratory; (b) remote monitoring of experiments, which will be carried out by either EMBL staff or by a third party; (c) remote control of, at least, part of the equipment that is involved in data collection.

1	1 H																	2 He
2	3 Li	4 Be											5 B	6 C	7 N	8 O	9 F	10 Ne
3	11 Na	12 Mg											13 Al	14 Si	15 P	16 S	17 Cl	18 Ar
4	19 K	20 Ca	21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	28 Ni	29 Cu	30 Zn	31 Ga	32 Ge	33 As	34 Se	35 Br	36 Kr
5	37 Rb	38 Sr	39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe
6	55 Cs	56 Ba	57 La	72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn
7	87 Fr	88 Ra	89 Ac															
Lanthanide Series			58 Ce	59 Pr	60 Nd	61 Pm	62 Sm	63 Eu	64 Gd	65 Tb	66 Dy	67 Ho	68 Er	69 Tm	70 Yb	71 Lu		
Actinide Series			90 Th	91 Pa	92 U													

Figure 4: Periodic table for phasing experiments at beamline MX1.

Blue, MAD/SAD at K-edge; green, MAD/SAD at L-edge; red, MAD/SAD at K- end L-edges; cyan, SAD.

3.2. Small Angle X-ray Scattering (SAXS)

3.2.1. Justification

SAXS is a unique method for studying low resolution structure and structural transitions of individual proteins and large macromolecular complexes in solution. In the past, the efficiency of biological SAXS studies has been severely limited by the lack of adequate analysis methods. The recent novel approaches, in particular, developed at EMBL-Hamburg (for review see Svergun & Koch, 2003), tremendously improved resolution and reliability of the technique and significantly enlarged its range of applications and user community. Currently, SAXS beamlines are available (and in high demand) or planned at all major synchrotrons in Europe (Annex 4) and worldwide. Most of these, however, are general-purpose stations with mixed applications in physics, chemistry, and biology. A rapidly increasing number of European users perform solution scattering experiments at EMBL-Hamburg, largely due to an accumulated expertise in biological SAXS and novel analysis methods. However, the most interesting and challenging experiments are currently being carried out at the limit of sensitivity of the existing beamline at DORIS-III. In response to a clear need of the European biological user community for high-quality SAXS experiments (Table 1), the presence of a biologically-oriented SAXS beamline at PETRA-III capable of HTP and cutting-edge experiments will be of crucial importance. In the HTP mode, the SAXS experiments performed on ultra-small sample volumes in parallel with MX applications will provide valuable complementary information on the structure of targets in solution. The beamline will further provide a means to characterise complex, potentially flexible macromolecules and mixtures of different oligomeric and functional states, which may not be easily amenable to study by high resolution methods. Cutting-edge experiments, such as kinetic experiments on an ultra-short time scale, anomalous SAXS on biologically relevant atoms/ions, and pilot experiments for future X-FEL applications will become possible. To cope with the large data flow, the expertise of the EMBL SAXS group will also be developed towards an automated data analysis system for on-line construction of structural models, leading to a unique synergy of hardware and software development for SAXS experiments.

3.2.2. Proposal

The proposed BioSAXS beamline will be built in the framework of the general EMBL concept of life science applications at PETRA-III. The modular design of the proposed BioSAXS beamline will facilitate the straightforward switching between different modes of operation (Figure 5):

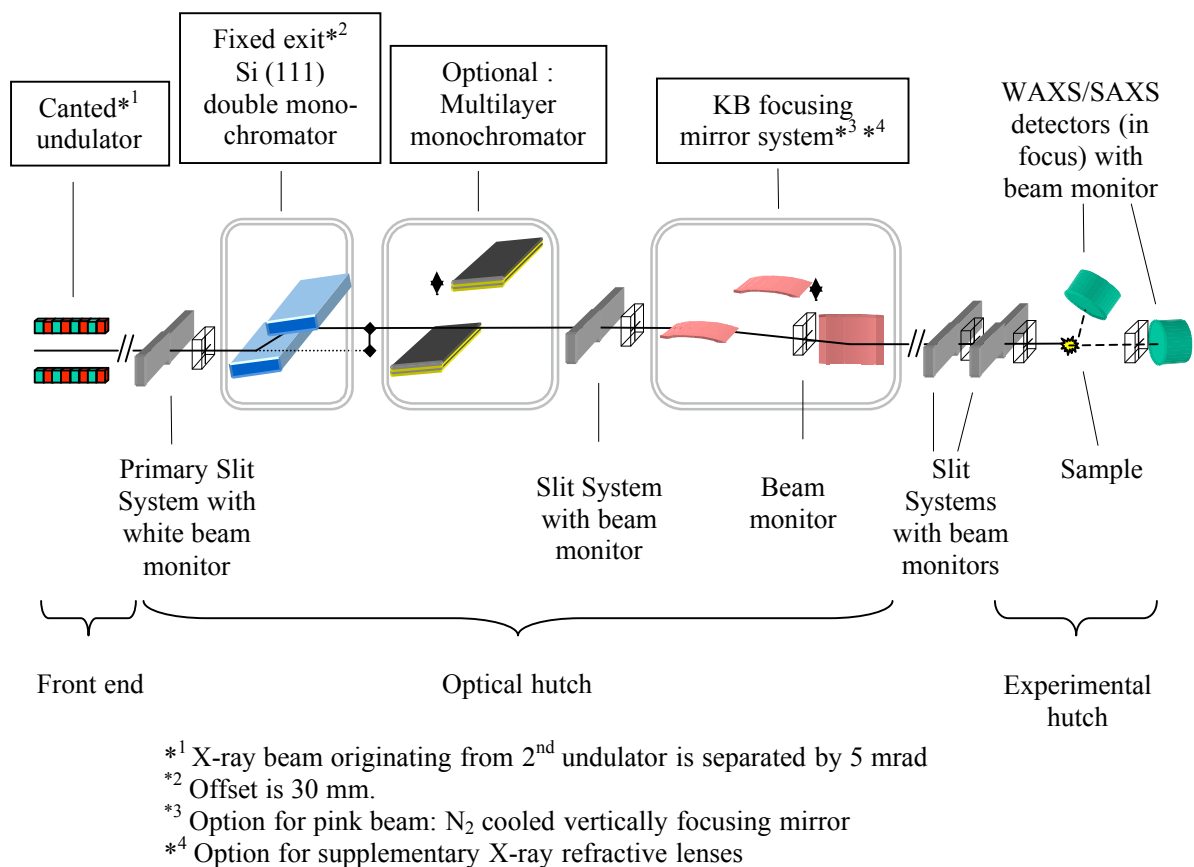


Figure 5: Schematic design of the SAXS beamline for biological applications.

- High-precision scattering experiments using the high energy resolution of a double crystal monochromator system, with its ability to tune the energy in a large energy range from 4 to 20 keV ($\Delta\lambda/\lambda = 1 \times 10^{-4}$ and 2×10^{13} ph/s at 8 keV);
- Increased flux with a lower energy resolution of a multi-layer monochromator system for fast kinetic experiments ($\Delta\lambda/\lambda = 1 \times 10^{-2}$ and 3×10^{15} ph/s at 8 keV);

- The pink beam mode for experiments that requires the ultra-high flux of the sharp undulator line. This mode is achieved by simply moving the monochromators out of the optical path ($\Delta\lambda/\lambda=2 \times 10^{-2}$ and 9×10^{15} ph/s at 8 keV).

The beam focusing will be carried out by a KB mirror system, yielding a beam size of about $0.2 \times 0.06 \text{ mm}^2$ with unsurpassed beam divergence of $40 \times 10 \text{ } \mu\text{rad}$ (three-fold better than that of any existing SAXS station worldwide). The broad dynamic range of the scattering vectors will yield resolutions from 2000 to 0.1 nm to study large macromolecular machines and small conformational changes. Moreover, implementation of nanofocusing techniques such as parabolic X-ray lenses or two-dimensional waveguides (currently tested at third-generation sources like ESRF), will produce focal spots of about 100 nm.

Using a modular approach, the BioSAXS beamline will provide a tuneable stable beam of high flux with small size and low divergence to encompass a wide range of applications. The normal working mode with a double crystal monochromator will be implemented for most of the static, HTP, and anomalous scattering experiments. In the high flux mode, kinetic SAXS experiments on ultra-fast biological reactions will be possible. The pink beam mode will allow the utilisation of the high coherence of the PETRA beam and will enable the design of test experiments for future X-FEL applications.

3.2.3. Possible applications

The outstanding properties of PETRA-III will create new opportunities to perform quantitatively and qualitatively novel SAXS experiments on biological objects, reducing the sample volume requirements to the nanolitre range. This downscaling will significantly enlarge the types of samples amenable to SAXS to those that can be purified only in limited yields or to those that are soluble only at low ($< 1 \text{ mg/ml}$) concentrations. On the qualitative level, completely new concepts are emerging, which can only be implemented on a high brilliance source like PETRA-III. In addition to high-quality studies of static systems, anomalous SAXS experiments on biologically relevant atoms or ions, and conventional stopped-flow analysis of kinetic processes, novel possibilities will include the following:

- a) *A micro-screening combinatorial biology approach.* The use of micro-screening plates,

which have a maximal sample volume of only a few nanolitres, will be an innovative technique for rapid, large-scale biomacromolecular screening. The applications range from automated shape analysis and studies of protein-protein interactions or complex formation to quantitative investigations of the protein crystallisation process.

- b) *Ultra-fast continuous flow kinetics with micro mixers.* This approach will allow the millisecond barrier imposed by the time resolution of the detection systems in time-resolved studies to be surpassed. In the continuous flow geometry, reaction time as a function of the focused X-ray beam position can be adjusted by the flow velocity and the distance from the mixing point. Sub-millisecond reaction times can be explored, whereby modern micro mixers and micro channels will allow sample consumption of as low as 100 nl per experiment.
- c) *Combinatorial kinetics by micro fabricated Lab-on-a-chip systems.* The combination of the two above mentioned approaches will lead to a system for assessing combinatorial kinetics on a microfluidic chip. This technique will permit large-scale studies of fast kinetics, which are dependent on different conditions (pH, ionic strength, ligand concentration), to be conducted in a single fluidic device. Reaction time points and conditions can be probed by the X-ray beam. Further developments could lead to yet more complex devices emulating a complete biological machinery. This “biomimetics” approach should allow SAXS investigation of a cascade of biochemical reactions and interactions—a very important step towards an integrated systems biology.
- d) *Three-dimensional visualisation of structural transitions.* At present, most time-resolved experiments are analysed with respect to integral system parameters, such as the radius of gyration. On BioSAXS, kinetic experiments will be performed by recording a series of high-precision scattering patterns corresponding to different conformational states (e.g. assembly or quaternary structure snapshots). These can be analysed simultaneously by three-dimensional model–building methods to visualise transitions and kinetic processes at low resolution with respect to “real-time” movements of structural elements.
- e) *Flash freezing of micro droplets and cryo-SAXS.* The cryogenic freezing method is widely used in MX and EM but not in SAXS. Cryo-cooling of the sample will drastically reduce radiation damage, and a quenched freezing technique in combination with micro-fabricated fluidic devices will allow the trapping of reaction intermediates in the micro- to

millisecond range. Combined with nanofocusing optics, scanning experiments on thin-frozen solutions should allow the observation of single or few particle scattering, which is an essential step for future developments of single molecule diffraction imaging on forthcoming X-FELs.

All these experimental techniques will take advantage of the proposed infrastructure for biological sample preparation and handling of the EMBL beamlines and endstations. In particular, a pipeline is envisaged that will link the BioSAXS station to the HTP crystallisation facility, enabling the following: (i) determination of low resolution shapes of proteins, which cannot be crystallised (or of those, which have been crystallised, in which case the shapes can be used as masks for low resolution phasing); (ii) on-line validation of MX models in solution, and construction of biologically active oligomers; (iii) fast screening of protein-protein interactions with respect to the quaternary structure of the complexes; and (iv) determination of oligomeric content in equilibrium mixtures. Apart from the scattering studies in aqueous solutions, the BioSAXS beamline will also be suitable for the microprobing of biocomposites, fibres and tissues, advanced analysis of membrane proteins in various environments, phase transformations of lipid systems, and for wide range of condensed matter applications. The hardware developments will be accompanied by the creation of an expert data analysis system, which will in particular allow automated model building in the large-scale SAXS studies.

3.3. X-ray absorption spectroscopy

3.3.1. Justification

Metals play a key role in many biological processes, such as electron transfer, catalytic reactions, transport processes, and stabilisation of protein folds or assemblies. Complex mechanisms control the metal uptake and metal storage in all organisms. The ability of XAS to gather local structural information on the metal environment and electronic configuration of metalloproteins of any biochemical state is instrumental in enabling the technique to elucidate the various roles of these proteins. The synergism between NMR and MX has recently been exploited, as evidenced by an increasing number of joint applications (Pohl *et al.*, 2003, Svetlitchnyi *et al.*, 2004). Moreover, organism tissues have been targeted to elucidate processes such as metal

uptake (Küpper *et al.*, 2004). XAS beamlines are available or planned at major SR facilities. Most of them are, however, general-purpose XAS beamlines.

A limiting factor in biological XAS is the amount and concentration of available sample. A PETRA-III undulator is expected to boost the beam intensity by several orders of magnitude, in comparison with the present XAS beamline at DORIS-III; the small focal size will lower the illuminated sample volume. Thus, the amount of sample required is expected to be reduced by approximately two orders of magnitude, which may allow us to redefine the role of XAS in life science applications. In addition, the source characteristics of PETRA-III allow for unprecedented focussing of the X-ray beam, which should permit spatially resolved XAS. With a resolution of less than 100 nm, the technique will be able to locate metal positions in various tissues and simultaneously provide insight into the function of metals in biological environments. As such, it is expected to play an important role in providing novel data on metal trafficking at several cellular levels.

3.3.2. Proposal

The design of the proposed XAS station at PETRA-III is optimised for high throughput (HTP) XAS of biological samples as well as for spatially resolved XAS in two and three dimensions (Figure 6). To achieve this, 25-100 samples will be mounted in a closed cycle cryostat system to ensure optimum use of the beamtime, and data acquisition will be performed in a fully automated mode. The presence of metals will be analysed and, if appropriate, EXAFS and XANES spectra will be measured. For specialised experiments (e.g. time-resolved studies) special equipment can be installed on demand.

Advanced focussing of the X-ray beam and accurate sample positioning will be required for two- and three-dimensional XAS. This proposal suggests a KB system for a wide energy range and X-ray lenses for selected elements such as zinc. It is expected that most samples for spatially resolved XAS will be freshly prepared at the joint sample preparation area to prevent ice formation, drying of the surface or the creation of other artefacts during storage. The main energy range from 5 keV – 35 keV will cover the K-edges of the biologically important 3d metals (Cr, Mn, Fe, Ni, Cu, and Zn) as well as 4d elements (Mo, Cd). The study of additional

probe atoms, such as iodine and bromine in substrates or tungsten, will also be possible within this energy range. The lower limit of 5 keV is defined by the need for a flexible set-up, which requires components separated by Polyimid or Beryllium windows.

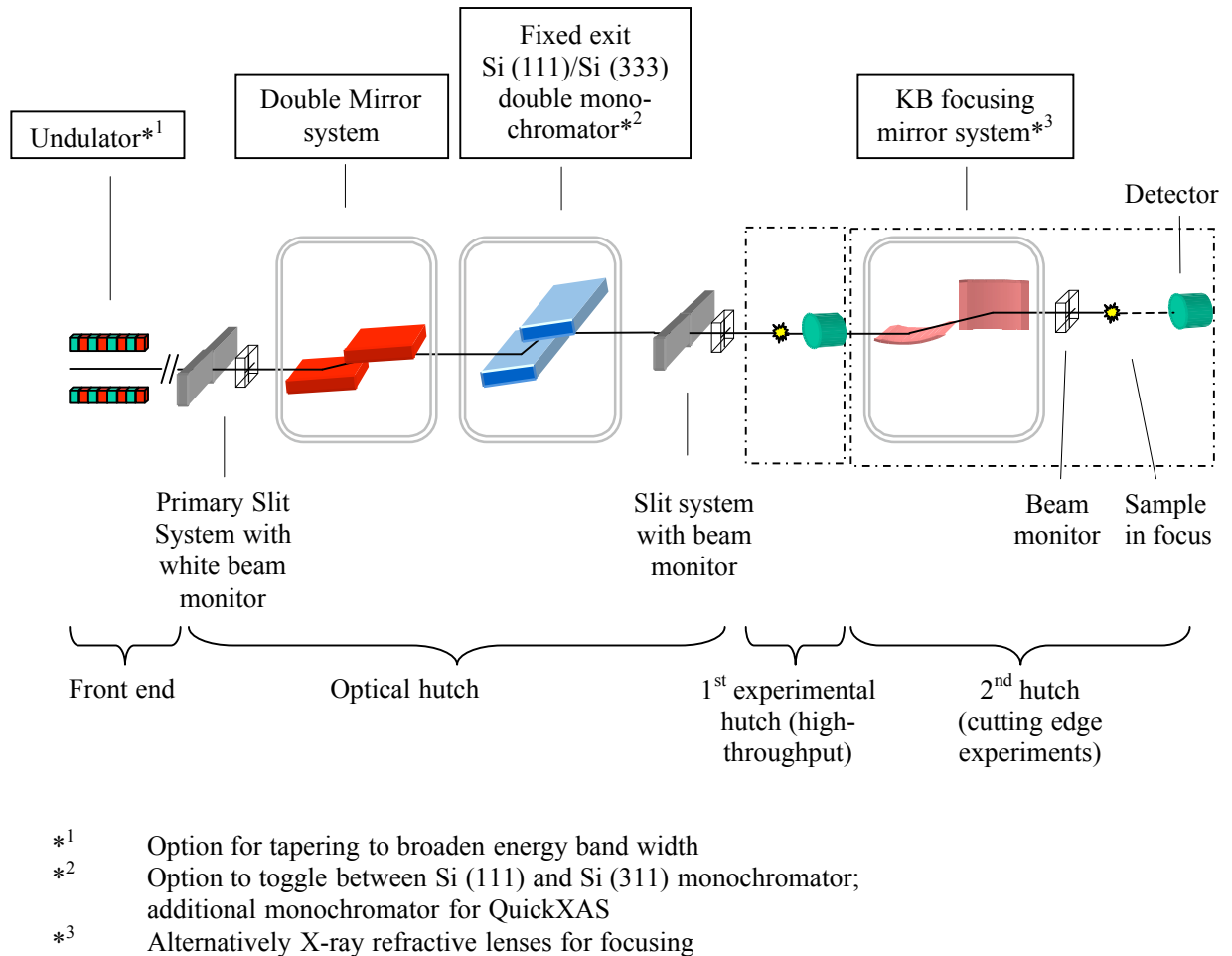


Figure 6: Schematic design of the XAS beamline for biological applications.

Based on these considerations the station is proposed to comprise:

- An undulator on a low β -section of the storage ring for optimised focussing;
- A double mirror system with a bendable second mirror to adopt the number of photons per area to the sample's sensitivity for photoreduction;

- A fixed exit monochromator covering the energy range from 5 to 35 keV [Si(111) and Si(311)];
- An undulator and a monochromator to simultaneously scan an energy range of up to 2 keV within less than 5 seconds;
- An independent hutch for biological XAS with exchangeable setup components.

Specific requirements and design criteria are based on the radiation sensitivity of the biological samples. The potential photoreduction of proteins and metals will be minimised through four methods. Firstly, samples will be cooled to 5°K in a closed cycle cryostat. Secondly, due to the smaller spot size the solid angle for gathering the X-ray fluorescence radiation will be increased over that of current setups. An optimised ratio between incoming photons and collected fluorescence radiation will lower the number of photons on the sample that are required for a defined data quality. Thirdly, the data collection strategy will be optimised for each individual sample. In order to achieve a constant signal-to-noise ratio over the entire energy range of about 1.5 keV, the acquisition times at higher energies will be increased, depending on the type of metal ligands. This will require control over the undulator gap in order to continuously match the monochromator energy. Fourthly, the photon flux can be spread over an area of 2 - 5 mm² by a bending mirror, depending on the sensitivity of the sample.

3.3.4. Possible applications

A XAS undulator station for biological samples at PETRA-III will create new opportunities in XAS research. Many experiments, like those hosted at the present EMBL EXAFS beamline, will be performed under vastly optimised conditions and will strengthen the role of XAS in initial protein characterisation, determination of element specific metal binding geometries, inhibitor binding studies / drug screening, metal affinity determination, and identification of metal binding residues.

- a) *High-throughput XAS*. Compared with the amount of protein needed for present data collection, that needed for high-throughput XAS will be reduced by a factor of 100 to 1000, lowering the requirements to as little as 5 µg of sample. This will provide new opportunities for the characterisation of metal binding sites in systematic approaches and

metal trafficking, as well as ligand / compound screening for active sites. High-throughput XAS will also respond to the increasing demand for synergistic applications in structural biology. Together with the extracted fine-structure and its Fourier transform, the resulting data may be forwarded to external users electronically, in an attempt to allow remote operation of HTP-XAS applications.

- b) *Spatially resolved XAS*. This technique will facilitate spatially resolved speciation of sample oxidation state and ligand environment (Pickering *et al.*, 2000; Schroer *et al.*, 2003). Most experiments on tissues will require reference spectra collected under similar conditions. In order to keep radiation damage at a minimum, rapid scans covering several edges will be performed. Three-dimensional maps on metal oxidation state and environment will allow us to visualise metal accumulation and distribution, providing new insights into detoxification processes and metal utilisation.
- c) *Time-resolved XAS studies*. Experiments on different time scales can be performed with the help of specialised equipment: (i) Continuous flow-and-mixing; such setups continuously provide fresh sample at the beam spot, permitting the measurements of highly radiation sensitive proteins. In combination with rapid mixing, intermediate states can be identified and characterised on a ms time scale, providing insight into catalytic mechanisms (Kleinfeld *et al.*, 2003; Penner-Hahn, 2003); (ii) Triggered reactions with $\Delta t > 10 \text{ ms} - 100 \text{ s}$; such reactions will be initiated by a light pulse (phototrigger, release of caged substances), temperature pulse or jump, potential jump, or stopped-flow techniques. A time series of spectra will be collected either by piezo-QXAFS ($10 \text{ ms} < \Delta t < 1 \text{ s}$) or by rapid scanning of monochromator and undulator gap ($\Delta t > 1 \text{ s}$); (iii) Laser-triggered reactions with a time resolutions of $\Delta t > 1 \text{ } \mu\text{s}$; they will employ either the photochemical reactions of the investigated metalloprotein or those of suitable caged compounds. The process under investigation will be initiated by a ns Laser pulse. Complete XANES, or even EXAFS spectra of high quality, can be constructed by carrying out time scan experiments at different excitation energies.
- d) *XAS on protein crystals*: This application will bridge MX and XAS, elucidating the metal environment at sub-atomic resolution within a selected plane and will also provide insight into the electronic metal states within a protein crystal.

- e) High-resolution fluorescence analysis: This application will provide greatly improved experimental resolution for studies on the electronic structure of metal centres (de Groot, 2002; Kivimaki *et al.*, 1998; Hamalainen *et al.*, 1991). High-resolution fluorescence analysis will offer the possibility of revealing ligand features at metal K-edge energies. In this manner, strategies successful for small molecules may be applied to biological material without destructive sample treatment such as lyophilization.

3.4. Joint sample preparation area

3.4.1. Justification

State-of-the-art sample preparation facilities are essential for the most efficient use of the proposed beamlines at PETRA-III, irrespective of what type of SR experiments are under consideration. This is particularly true for difficult samples such as multi-component protein-protein complexes and integral membrane proteins. For the optimised sample delivery to the experimental SR beamline facilities, we are planning to locate the facilities for sample characterisation, crystallisation, and sample storage and transfer in direct association with the proposed PETRA-III beamlines. Along with additional sample preparation facilities at the current EMBL-Building (25A) at DESY, and further backup from EMBL core facilities (mostly at EMBL-Heidelberg), the PETRA-III facilities are designed to provide a state-of-the-art infrastructure for sample preparation by the general external user community. Limited additional facilities will be available for user groups, which lack some necessary equipment at their own research infrastructures. The latter will particularly apply to the on-site HTP crystallisation user facility that will present a type of large infrastructure that is nonexistent in Europe and rare in other parts of the world. With the proposed sample preparation facility, along with the leading expertise of the EMBL-Hamburg in molecular biology methods, the laboratory will pioneer a unique interface in life science methodologies suited for demanding SR beamline projects for the benefit of the international scientific community.

3.4.2. Proposal

We are proposing an integrated state-of-the-art sample preparation facility for applications in the three proposed SR beamline categories: MX, SAXS, and XAS. The facility will be in direct association with these beamlines and may be combined with other associated facilities for general services (safety instructions, administration) as well as for on-line data processing and interpretation. For this, an area of 250 m² of laboratory and office space will be required. While some of the facilities for HTP crystallisation and sample storage/retrieval need to be in direct association with the proposed PETRA-III beamlines, the remaining facilities (sample preparation, data processing and interpretation, administration and safety) could be either located on the ground floor (level of beamlines) or on the first floor of the future PETRA-III building. The sample preparation laboratory will be operated at the P1 safety level. It will offer full-scale facilities in the areas of biophysical and biochemical sample characterisation, crystallisation, and sample storage and transfer to the PETRA-III beamlines. It will be complemented by limited facilities for cloning, heterologous expression (prokaryotic and eukaryotic, including a High Cell Density Fermentation facility), and protein purification. The latter infrastructures are expected to remain in the current EMBL Building (25A). Most of these facilities are already operating at the P2 safety level, allowing the expression of potentially pathogenic targets in non-pathogenic hosts. The following specific proposal focuses on the planned full-scale facilities at PETRA-III.

Biophysical and Biochemical Sample Characterisation: We are planning to provide the following relevant equipment (excluding minor equipment) for biophysical and biochemical sample characterisation. For the sake of completeness, we have listed all such equipment currently available at EMBL-Hamburg (Table 4). These items need to be upgraded or replaced by state-of-the-art equipment at the onset of operations at the sample preparation facilities at PETRA-III.

Table 4: Sample characterisation equipment for SR applications	
Type of equipment	Current equipment
Dynamic light scattering	DynaPro99
Circular dichroism spectral photometer	(Heidelberg laboratory)
Spectrometers, based on absorbance and fluorescence	Uvikon Spectrophotometer 922 (Kontron) Ultraspec 3000 (Amersham Biosciences)
Size exclusion chromatography	Aekta 3D Purifier
Isotope labelling facility	License available
Isothermal microcalorimetry	MicroCal VP-ITC
MALDI-TOF mass spectrometry	(Heidelberg laboratory)

HTP Crystallisation: The high-throughput crystallisation facility will consist of two highly integrated modules. One module will be in charge of all liquid handling steps while the other will store and image the crystallisation experiments. The liquid handling module was designed to minimise manual intervention. It will be built around the Lissy 2002 pipetting platform from Zinsser Analytic, which consists of an 8-channel pipetting head and a robotic arm. Its primary purpose will be in the preparation of crystallisation screens from stock solutions into 96-well plates (1.5-2 ml volume). Its secondary purpose will be to provide the hardware and software control of a sealer from HJ-Bioanalytik (RoboSeal, and a crystallisation robot from Matrix (Hydra plusOne). The camera and storage hotel will be purchased from RoboDesign. The storage hotel will host a capacity of 10000 microtitre plates and is directly linked to a dual head colour camera system. The camera can be operated in different modes (e.g. auto-focus, slicing, etc.). Every crystallisation plate can be imaged with an individual schedule. The system software will integrate all relevant parts of the pipetting robot's database and produce user-friendly output.

Sample Storage: The sample storage area will provide a complete, dual infrastructure to either receive samples that are already prepared for SR beamline experiments or to prepare samples locally for these experiments. We are planning to make use of the standardised European sample transport cassette, which is currently been developed by EMBL-Hamburg and collaborating synchrotron facilities for MX applications.

Automated Sample Transfer: Although the vast majority of MX experiments are carried out under cryogenic conditions, the transfer of MX crystals into appropriate conditions that prevent ice formation remains a delicate process. The situation becomes more precarious for small crystals, for which crystal mounting difficulties often make it impossible to characterise their diffractions properties at non-cryogenic conditions (as a reference to identify the crystal-specific diffraction limits). We are planning to provide user-friendly solutions to allow rapid testing of many different experimental conditions. First, we will provide cryogenic storage facilities allowing the mounting of crystals into loops that will be stored in cassettes that, in turn, can be transferred into sample changers, which are attached to the PETRA-III MX beamlines. Secondly, we are planning to develop, implement, and provide a system that will be allow an *in situ* characterisation of X-ray diffraction properties of crystals in crystallisation plates. Preliminary proof-of-principle experiments have already demonstrated the feasibility of such an approach (J. Mueller-Dieckmann, personal communication). This facility will be directly interfaced with the HTP crystallisation facility (see above).

3.4.3. Possible applications

The joint sample preparation facility is expected to be primarily used for SR beamline experiments in the MX, SAXS, and XAS analysis of highly purified samples of biological macromolecules. As the quality of the samples critically influences the crystallisability of the proteins, all proteins intended for experimentation at the EMBL HTP crystallisation facility will be tested using a series of biophysical characterisation methods. Key applications for the planned sample preparation facility are summarised in Table 5.

Table 5: Methods for joint sample preparation area			
Objective	Method (Equipment)	Operation mode	
		User*	EMBL-staff
Sample Purity	SDS-PAGE, Size exclusion chromatography	X	
Sample association / aggregation	Dynamic light scattering (DLS), Analytical ultracentrifugation (AUC), Size exclusion chromatography.	X	X
Sample fold	Circular dichroism (CD)	X	X
Sample identity (including modifications)	Mass spectrometry, N-terminal sequencing		X
Sample stability	Differential scanning calorimetry (DSC), Circular dichroism (CD), Gel mobility techniques.	X	X
Sample function	UV/VIS spectrometry, Fluorescence, Isotope labelling	X	
Screening of crystallisation conditions	Automated setup of crystal drops, storage, scheduled inspections, using HTP crystallisation facility.		X
Refinement of crystallisation conditions	Semi-automated procedures for crystallisation, using elements of the HTP crystallisation facility.	X	X
Sample storage / retrieval	Transfer of frozen crystals in standardised cassettes.	X	X

*Including supervision by EMBL-staff. In specific cases, the experiments may be carried out by EMBL-staff.

4. Resource requirements and schedule

The present proposal outlines the development of an integrated centre for life science applications at PETRA-III, including three MX beamlines, one SAXS beamline, and one XAS endstation, integrated with a common sample preparation area. We are seeking funding for two MX beamlines, one SAXS beamline, and one XAS endstation (Table 6). In addition, we are offering our expertise and infrastructures, in collaboration with DESY, to help construct and operate a third MX beamline with funds received from other research organisations or different external resources.

The planned sample preparation area will serve as the core for the future PETRA-III beamline facilities. However, we are not seeking additional resources at the moment. The planned HTP crystallisation facility at PETRA-III is already largely funded (from a research & technology grant from the German Ministry for Science and Education, BMBF) and we are planning to seek additional resources from other external funding agencies.

Table 6: Funding requests for EMBL-facilities at PETRA-III		
PETRA-III	Proposal	Request for Funding
MX beamlines	3	2
SAXS beamline	1	1
XAS beamline	0.5	0.5
HTP crystallisation	Yes	No
Sample Preparation	Yes	No

4.1. Strategies and resource requirements for the construction of PETRA-III beamlines

Our estimates for the resources needed for the construction of PETRA-III beamlines are mainly based on previous experiences with the construction of life-science oriented beamlines at 3rd

generation SR sources. At the ESRF, beamline construction within three years typically required 9 person-years of staff directly attached to the beamline, with an additional 15 person-years from support groups and for outsourced tasks. The estimates have translated into overall costs per beamline of the order of 1.2 M€ for personnel and 2 - 2.5 M€ for hardware, excluding insertion devices and front-ends. These numbers assume the provision of a large number of standard components developed by the central support groups and the availability of an efficient support structure for mechanical engineering, software engineering, electronics, vacuum, cryogenics, crystal engineering and drafting.

Our requirements for resources and the planning for PETRA-III beamlines will be guided by the following considerations:

In-house expertise: EMBL-Hamburg's instrumentation group (group leader: Christoph Hermes; staff scientist: Stefan Fiedler, since June 2004, previously working at ESRF) has had a long-standing record on the construction and maintenance of SR beamlines and endstations with applications in MX, SAXS, and XAS. Recent activities have been centred on the rebuilding, modification, and transformation of three beamlines located on a DORIS-III bending magnet and the implementation of key optical elements on the DORIS-III wiggler beam lines BW7A/B (Pohl et al., 2001; Hermes et al., 2004). This has involved the development of beamline concepts and the construction, manufacturing, installation, and commissioning of various beamline components. Additional X-ray optical components, special endstations, and associated equipment for MX experiments were designed and built (Pohl et al., 2004). The expertise of the instrumentation group will provide the core knowledge and backbone for the construction of PETRA-III beamlines. It is planned that the current staff of the instrumentation group (nine posts) will take over a major portion of the work, in particular, the central support tasks such as drafting, electronics, vacuum, and cryogenics. However, in order to set up the planned PETRA-III beamlines while maintaining the operation of some of the present DORIS-III beamlines, additional dedicated staff needs to become involved into the task.

Internal collaboration with other EMBL Units. The two EMBL units in Grenoble and Hamburg, both located on the grounds of leading SR facilities in Europe (ESRF, DESY) share complementary expertise in SR beamline and endstation instrumentation, operation of facilities, and SR-based research experiments. In addition, the Grenoble unit has already gained substantial

experience with MX applications on 3rd generation SR beamlines. Additional know-how is generated by the recently established *Partnership for Structural Biology* (PSB) in Grenoble, in which the EMBL-Grenoble unit participates. For the PETRA-III project we are planning to make maximum use of joint knowledge and expertise in the construction and operation of the new beamline facilities. As a first step to this end, we have established a joint working group, comprising staff from both EMBL units (the initial meeting of this group was held recently in Hamburg, and further bilateral meetings are planned on an annual basis). In addition, a number of project-oriented collaborations are either already in place (e.g. high throughput crystallography, automated crystal recognition and centring) or envisaged for the near future (e.g. search for fast, high resolution area detector system, standardisation of automatic sample changers).

External collaborations with SR facilities: For the construction of PETRA-III beamlines, collaborations with leading SR facilities across Europe will be essential to efficiently use available resources and to comply with the time constraints. These collaborations will focus on X-ray optical components such as beam position monitors, monochromators and mirrors, as well as specialized endstation designs and beamline control electronics. A framework for joint developments is given by the EC-supported Integrated Project BIOXHIT (<http://www.embl-hamburg.de/BIOXHIT/>) that focuses on automation and general beamline design for MX applications. In addition, there are already ongoing collaborations and possibilities for future joint research projects with SR facilities via the EC funded network of SR and FEL facilities (<http://www.elettra.trieste.it/i3/>). This network promotes improvements in the infrastructures of the facilities, networking, and access to these facilities by external users. However, a strong collaboration with DESY/HASYLAB is of the highest importance for the coordinated construction of the PETRA-III beamlines. Their guidelines for beamline infrastructure and standard sub-systems, including the beamline control system, specific optical elements, monitoring devices, and vacuum elements, will be mandatory for the construction of the PETRA-III beamlines by EMBL-Hamburg. A suitable format for the future cooperation with DESY/HASYLAB has recently been reconfirmed and extended for the next ten years by the EMBL/DESY partnership agreement.

Commercial solutions. There are a number of enterprises in Europe that are specialised in commercial solutions for either single components or integrated systems for SR beamlines and endstations. Commercial solutions will be considered, provided they meet our requirements and can be acquired at reasonable cost levels. In-house developments by the instrumentation group of EMBL-Hamburg will complement industrial solutions, and will play a critical role in the overall beamline assembly and standardisation. They will be particularly important for the automation of beamline alignment as well as for automated sample transfer, mounting, and alignment. Ongoing developments (e.g. in automatic crystal mounting, centring, and data collection) will be integrated into the concepts for construction of the PETRA-III beamlines.

Taking the concepts described above into account, our estimates for personnel requirements for the planning, construction, and setup of a single beamline are made on the assumption of three person-years for scientific staff (0.1 Mio € per person-year) and nine person-years for technical staff (0.07 Mio € per person-year). Further details are summarised in **Table 7**.

Table 7: Estimated requirements of the person-power for the planning, construction, and setup of a single beamline		
Task	Workload per beamline (person-years)	Costs per beamline (M €)
Scientific & project management	3	0.3
Mechanical engineering	2	0.14
Machining & Installation	3	0.21
Software engineering & control	3	0.21
Commissioning	1	0.07
Total	12	0.93

Considering 0.93 M€ per beamline for personnel costs, the overall requirement for the present proposal will be in the order of 3.26 M€. We are seeking 50% funding of personnel costs while

the remaining part will be covered by external sources as well as the re-organisation of staff at the EMBL-Hamburg Unit during the PETRA-III beamline construction phase. For the three sub-projects in MX, SAXS, and XAS, we are seeking a total of 8.2 M€ of investment costs (estimation based on two MX beamlines, one SAXS beamline, and an XAS endstation; for a breakdown see Table 8). These estimates do not include any contingency margin and should be regarded as an absolute lower limit for the construction of a state-of-the-art facility.

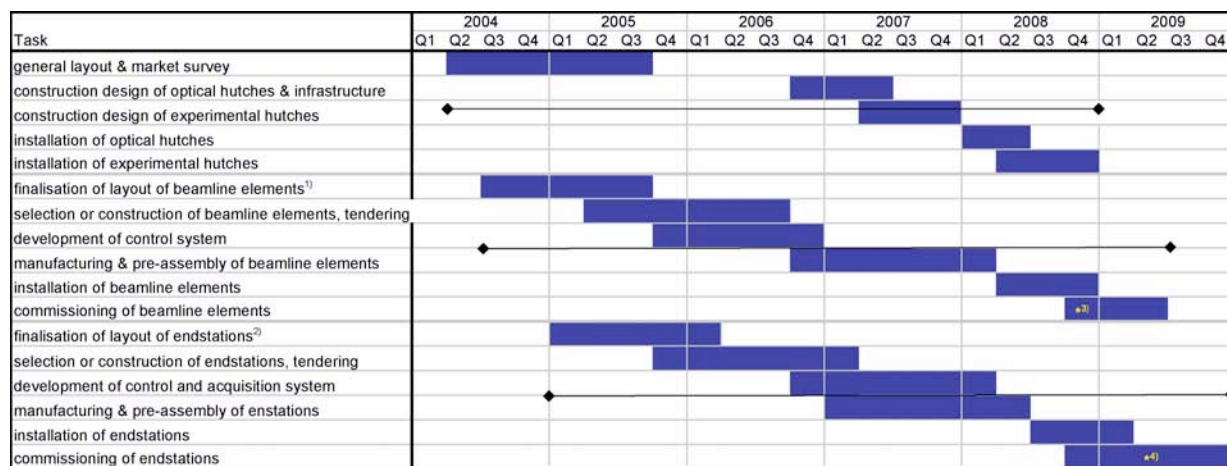
Table 8: The breakdown of the costs for which funding is requested			
	Sub-project costs in M€		
Budget item	MX	SAXS	EXAFS
X-ray optics (monochromator, mirror)	1,4	0,9	0,4
X-ray detector system	1,1	0,75	0,4
Endstations and sample environment	0,7	0,4	0,1
Computer, electronics, and networking	0,5	0,35	0,2
Infrastructure, vacuum equipment & supports	0,4	0,3	0,3
Sub total	4,1	2,7	1,4
Total investment costs	8,2		
Total person-power costs	1.6		
Grand total costs	9.8		

4.2. Preliminary timetable for PETRA-III beamline construction

The project will be split into three main tasks (**Figure 7**):

1. General layout and construction of hutches with infrastructure (water, gases, etc.);
2. Construction of beamline elements;
3. Construction of the endstations

The EMBL-Hamburg Unit is planning to set up the proposed beamlines within a limited time frame for onset of user operation in parallel, but with some staggering. However, key beamline components can be pre-assembled and sequentially installed and commissioned from spring 2008 onwards, after the installation of the crane in the new experimental hall in January 2008. By the projected onset in user mode in spring 2009, two beamlines will be initially operational, with the remaining ones following rapidly thereafter. We expect the first experiments to be performed on all planned experimental stations by the end of 2009.



- 1) beamline elements : monochromators, mirrors, slits, montiors, vacuum system etc.
- 2) endstations comprise: detectors, diffractometers, sample changers etc.
- 3) first beam : November 2008
- 4) first users (not all endstations) : June 2009

Figure 7: Tentative schedule for the construction of beamlines

4.3. Resource requirements for PETRA-III beamline operation

After completion of the construction of the PETRA-III beamlines for LS applications, EMBL-Hamburg is planning to dedicate its present technical and scientific support staff (25 of 38 positions, excluding ancillaries, and research fellows) entirely to the operation of these beamlines, eventually reducing the number of endstations from eight to four. As a result, we anticipate that, on average, we will have at least six staff members for each PETRA-III endstation available. Depending on the plans of DESY for the future operation of DORIS-III and the performance of the EMBL-Hamburg beamlines at DORIS-III, we may consider seeking external funding, possibly in conjunction with external partner organisations, in order to temporarily continue the operation of some of the DORIS-III beamlines.

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7. List of Annexes

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Annex 1:

Experiments by external user groups at SR beamlines of EMBL-Hamburg (2000-2003)*

Year	2000	2001	2002	2003
Number of user visits / individual users				
PX	500 / 358	384 / 278	483 / 316	371 / 249
XAS	30 / 22	21 / 18	62 / 43	14 / 13
SAXS	44 / 33	48 / 43	86 / 64	53 / 49
Total	598 / 423	460 / 341	621 / 409	432 / 302
Number of projects proposed / carried out				
PX	377 / 281	401 / 216	447 / 259	409 / 190
XAS	23 / 10	32 / 16	37 / 32	26 / 9
SAXS	26 / 23	34 / 12	42 / 39	42 / 33
Total	426 / 314	467 / 244	526 / 330	477 / 232
Number of research groups applying / visiting				
PX	127 / 104	116 / 84	104 / 80	93 / 73
XAS	17 / 11	27 / 9	26 / 20	21 / 8
SAXS	24 / 15	30 / 16	38 / 31	40 / 27
Total	168 / 130	173 / 109	168 / 131	154 / 108

*The data have been taken from the current statistical database at EMBL-Hamburg, which leans on the data, required for the last EU access contract (HPRI 1999 CT-00017).

Annex 2:

Geographic distribution of projects carried out at EMBL SR beamlines (2000-2003)

Country	2000	2001	2002	2003	Total
European States					
Austria	10	6	8	2	26
Belgium	27	24	22	22	95
Cyprus	0	0	0	1	1
Denmark	13	9	29	20	71
Finland	8	11	30	23	72
France	13	5	14	4	36
Germany	96	75	97	67	335
Greece	11	4	6	10	31
Hungary	0	0	2	3	5
Israel	3	0	3	0	6
Italy	29	27	27	18	101
Lithuania	0	0	4	2	6
Norway	0	4	0	0	4
Poland	1	6	13	5	25
Portugal	0	2	3	1	6
Russia	3	3	7	6	19
Slovakia	3	3	3	2	11
Spain	22	11	6	2	41
Sweden	22	16	12	11	61
Switzerland	6	9	3	5	23
The Netherlands	12	10	11	3	36
Turkey	0	0	2	0	2
United Kingdom	34	25	32	32	123
TOTAL	313	250	334	239	1136
Non-European States					
Canada	1	1	1	1	4
India	2	0	0	0	2
Singapore	0	0	3	2	5
USA	5	2	0	1	8
TOTAL	8	3	4	4	19
GRAND TOTAL	321	253	338	243	1155

Annex 3:

List of research groups using EMBL-Hamburg facilities during 2000-2003

European States											
Country	City	Project leader	Research Organization	MX	SAXS	XAS	Visit 2000	Visit 2001	Visit 2002	Visit 2003	
Austria	Graz	Kratky, C.	University of Graz	X			X	X	X	X	
	Graz	Prassl, R.	University of Graz	X			X				
Belgium	Brussel	Ivanov, D.	Vrije Universiteit Brussel		X				X		
	Ghent	Beeumen, J.	University of Ghent	X			X	X		X	
	Heverlee	Goderis, B.	K. U. Leuven		X		X	X	X		
	Heverlee	Reynaers, H.	K. U. Leuven		X		X				
	Leuven	Declercq, J.-P.	K. U. Leuven	X			X	X	X	X	
	Leuven	Deranter, C.	K. U. Leuven	X			X				
	Leuven	Meervelt, L. van	K. U. Leuven	X			X	X	X		
	Leuven	Rabijns, A.	K. U. Leuven	X				X	X	X	
	Mons	Dosiere, M.	Université de Mons-Hainaut		X			X	X		
	Sint-Genesius-Rode	Wyns, L.	Vrije Universiteit Brussel	X			X	X	X	X	
Cyprus	Nicosia	Zographos, S.	University of Cyprus	X						X	
Denmark	Aarhus	Nyborg, J.	University of Aarhus	X			X	X	X	X	
	Copenhagen	Bendix, J.	University of Copenhagen			X				X	
	Copenhagen	Gajhede, M.	Danish University of Pharmaceutical Sciences	X						X	
	Copenhagen	Larsen, I.	Royal Danish School of Pharmacy	X			X	X	X		
	Copenhagen	Larsen, S.	University of Copenhagen	X			X	X	X	X	
	Valby	Henriksen, A.	Carlsberg Laboratory	X						X	
Finland	Helsinki	Goldman, A.	University of Helsinki	X			X		X	X	
	Helsinki	Lappalainen, P.	University of Helsinki	X				X			
	Helsinki	Stenberg, K.	University of Helsinki	X			X				
	Helsinki	Tuma, R.	University of Helsinki		X		X	X			
	Joensuu	Rouvinen, J.	University of Joensuu	X					X	X	
	Oulu	Glumoff, T.	University of Oulu	X			X	X	X		
	Oulu	Hiltunen, K.	University of Oulu	X			X				
	Oulu	Wierenga, R.	University of Oulu	X			X	X	X	X	
	Oulu	Yläanne, J.	University of Oulu	X					X	X	
	Turku	Papageorgiou, T.	Turku Centre for Biotechnology	X				X	X	X	
Turku	Salminen, T.	Åbo Akademi University	X						X		
France	Gif-sur-Yvette	Rey, F.	LGV-CNRS	X			X	X	X		
	Grenoble	Kozielski, F.	IBS/LMES		X		X				
	Illkirch	Moras, D.	IGBMC	X	X		X	X	X		
	Lyon	Haser, R.	CNRS	X			X				
	Lyon	Hulmes, D.	CNRS		X			X			
	Marseille	Cambillau, C.	AFMB UMR6098 CNRS-UI-UII	X					X		
	Nancy	Aubry, A.	LCM3B	X			X	X	X	X	
	Orsay cedex	Doucet, J.	LURE		X				X		
	Toulouse	Mourey, L.	IPBS-CNRS		X				X		
	Toulouse	Samama, J.-P.	IPBS-CNRS	X	X		X				
Germany	Aachen	Hoffmann, K.	TU Aachen	X			X				
	Bayreuth	Meyer, O.	University of Bayreuth			X		X			
	Berlin	Dau, H.	Free University of Berlin			X		X	X		
	Berlin	Haumann, M.	Free University of Berlin			X				X	
	Berlin	Heinemann, U.	Max-Delbrück-Centre	X			X	X	X		
	Berlin	Hoehne, W.	Humboldt University Berlin	X			X	X	X	X	
	Berlin	Koellner, G.	Free University of Berlin	X			X				
	Berlin	Orth, P.	Free University of Berlin	X			X				
	Berlin	Saenger, W.	Free University of Berlin	X	X			X	X		
	Berlin	Straeter, N.	Free University of Berlin	X	X			X	X		
	Bielefeld	Müller, A.	University of Bielefeld		X	X			X		
	Borstel	Brandenburg, K.	Research Centre Borstel		X				X		
	Braunschweig	Heinz, D.	German Research Centre for Biotechnology	X	X	X	X		X		
	Braunschweig	Müller-Goymann, C.	TU Braunschweig		X			X			
	Cologne	Schomburg, D.	University of Cologne	X			X	X	X	X	
	Dortmund	Schlichting, I.	MPI for Molecular Physiology	X			X	X	X		

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Country	City	Project leader	Research Organization	MX	SAXS	XAS	Visit 2000	Visit 2001	Visit 2002	Visit 2003
Germany	Duisburg	Henkel, G.	University of Duisburg			X			X	
	Düsseldorf	Groth, G.	University of Düsseldorf	X			X	X	X	X
	Frankfurt	Ermler, U.	MPI for Biophysics	X			X	X		
	Frankfurt	Fritzsche, G.	MPI for Biophysics	X			X	X	X	X
	Frankfurt	Hunte, C.	MPI for Biophysics	X			X	X	X	
	Frankfurt/Main	Kuehlbrandt, W.	MPI for Biophysics	X			X		X	X
	Frankfurt/Main	Lancaster, R.	MPI for Biophysics	X				X	X	
	Freiburg	Baumstark, M.	University of Freiburg	X			X			
	Freiburg	Schulz, G.	University of Freiburg	X			X	X	X	X
	Freising	Komdoerfer, I.	TU München	X					X	
	Garching	Parak, F.	TU München			X		X	X	X
	Garching	Unruh, T.	TU München		X		X	X	X	
	Geesthacht	Willumeit, R.	GKSS Research Center		X				X	
	Göttingen	Becker, S.	MPI for Biophysical Chemistry	X						X
	Göttingen	Einsle, O.	University of Göttingen	X						X
	Göttingen	Ficner, R.	University of Göttingen	X					X	X
	Göttingen	Rudolph, M.	University of Göttingen	X						X
	Göttingen	Sheldrick, G.	University of Göttingen	X		X	X	X	X	
	Greifswald	Hinrichs, W.	University of Greifswald	X						X
	Halle/Saale	Koenig, S.	University of Halle-Wittenberg	X					X	X
	Halle/Saale	König, S.	University of Halle-Wittenberg		X		X	X	X	
	Hamburg	Betzel, C.	University of Hamburg	X	X	X	X	X	X	X
	Hamburg	Yonath, A.	MPG-ASMB	X				X		
	Heidelberg	Kull, J.	MPI for Medical Sciences	X			X	X		
	Heidelberg	Madden, D.	MPI for Medical Sciences	X			X	X		
	Homburg	Scheidig, A.	Saarland University	X			X	X	X	
	Homburg (Saar)	Grüber, G.	Saarland University		X				X	
	Jena	Bunjes, H.	University of Jena		X				X	
	Jena	Hilgenfeld, R.	Institute of Molecular Biotechnology	X			X	X		
	Jena	Pohle, W.	Institute of Molecular Biotechnology		X		X			
	Jena	Westesen, K.	University of Jena		X		X	X		
	Jülich	Bueldt, G.	Research Centre Jülich	X			X			
	Jülich	Sager, W.	Research Centre Jülich		X				X	
	Konstanz	Diederichs, K.	University of Konstanz	X			X	X		
	Konstanz	Küpper, H.	University of Konstanz			X			X	
	Leipzig	Morawski, M.	University of Leipzig			X			X	X
	Lübeck	Trautwein, A.	Medical University of Lübeck			X		X	X	
	Marburg	Essen, L.-O.	University of Marburg	X	X			X		X
	Martinsried	Brandstetter, H.	MPI for Biochemistry		X				X	
	Martinsried	Clausen, T.	MPI for Biochemistry	X			X	X		
	Martinsried	Goettig, P.	MPI for Biochemistry		X				X	
	Martinsried	Kim, J.-S.	MPI for Biochemistry		X				X	
	Mülheim/Ruhr	Weyhermüller, T.	MPI for Radiation Chemistry			X			X	
	Münster	Krebs, B.	University of Münster	X		X	X	X	X	X
	Münster	Rompel, A.	University of Münster			X			X	X
	Saarbruecken	Adolph, H.-W.	Saarland University	X			X			
	Würzburg	Mueller, T.	University of Würzburg	X						X
Greece	Athens	Mavridis, I.	NCSR Demokritos	X					X	X
	Athens	Oikonomakos, N.	The National Hellenic Research Foundation	X			X	X	X	X
	Athens	Panagiotis, K.	Biomed. Sci. Research Centre	X			X			
	Athens	Vorgias, C.	Athens University	X			X	X		
	Athens	Zographos, S.	The National Hellenic Research Foundation	X						X
	Heraklion	Kokkinidis, M.	University of Crete & IMBB-FORTH	X			X		X	X
	Heraklion	Petratos, K.	University of Crete & IMBB-FORTH	X			X			X
	Thessaloniki	Kavounis, C.	Aristoteles University of Thessaloniki	X				X		
Hungary	Budapest	Vertessy, B.	Hungarian Academy of Sciences	X					X	X
Israel	Haifa	Adir, N.	Technion	X					X	
	Jerusalem	Shoham, G.	The Hebrew University of Jerusalem	X			X			
Italy	Bologna	Ciurli, S.	University of Bologna	X			X	X		
	Bologna	Roveri, N.	University of Bologna		X		X			
	Genova	Bolognesi, M.	University of Genova	X	X		X	X	X	X
	L' Aquila	Della-Longa, S.	University of Aquila			X			X	

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Country	City	Project leader	Research Organization	MX	SAXS	XAS	Visit 2000	Visit 2001	Visit 2002	Visit 2003
Italy	Milano	Degano, M.	Fondazione Centro S. Raffaele del Monte Tabor	X			X	X		
	Milano	Musacchio, A.	European Institute of Oncology	X	X		X			
	Milano	Vanoni, M. A.	University of Milano		X				X	
	Napoli	Berisio, R.	University of Naples	X						X
	Napoli	Pedone, C.	University of Naples	X			X	X		
	Pavia	Mattevi, A.	University of Pavia	X			X	X		X
	Pomezia, Rome	Sollazzo, M.	IRBM "P. Angeletti"	X			X			
	Rome	Morante, S.	University of Rome "Tor Vergata"			X		X	X	
	Rome	Cesareni, G.	University of Rome "La Sapienza"	X			X		X	X
	Rome	Ilari, D. T.	CNR	X				X	X	X
	Siena	Mangani, S.	University of Siena	X		X	X	X	X	X
	Trieste	Benini, S.	Elettra	X				X	X	
	Trieste	Djinovic, K.	Elettra	X			X			
Lithuania	Vilnius	Grazulis, S.	Institute of Biotechnology	X					X	X
Norway	Oslo	Andersson, K.	University of Oslo	X				X		
Poland	Cracow	Korbas, M.	Jagiellonian University			X			X	
	Lodz	Bujacz, G.	Institute of Technical Biochemistry	X					X	
	Lublin	Tchorzewski, M.	Maria Curie-Sklodowska University		X				X	
	Poznan	Jaskolski, M.	Polish Academy of Natural Sciences	X			X	X	X	X
	Poznan	Kozak, M.	Adam Mickiewicz University		X			X	X	
	Poznan	Rypniewski, W.	Polish Academy of Natural Sciences	X					X	X
	Poznan	Tykarska, E. M.	Adam Mickiewicz University	X				X		
	Warsaw	Bal, W.	IBB PAS			X			X	
	Warsaw	Bochtler, M.	Internat. Institute of Molecular & Cell Biology	X					X	
	Portugal	Oeiras	Carrondo, M. A.	Instituto de Tecnologia Quimica e Biologica	X				X	X
Russia	Moscow	Melikadamyam, W.	Russian Academy of Sciences	X			X	X		
	Moscow	Nikonov, S.	Russian Academy of Sciences	X			X	X	X	X
	Moscow	Popov, V.	Russian Academy of Sciences	X						X
	Moscow	Serdyuk, I.	Russian Academy of Sciences		X		X			
Slovakia	Bratislava	Sevcik, J.	Slovak Academy of Sciences	X			X	X	X	X
Spain	Barcelona	Coll, M.	CSIC	X			X	X		
	Barcelona	Fita, I.	CSIC	X					X	
	Barcelona	Subirana, J.	TU of Catalonia	X				X		
	Granada	Otalora, F.	University of Granada	X			X	X	X	
	Madrid	Romero, A.	CIB-CSIC	X			X	X	X	X
	Valencia	Rubio, V.	CSIC	X			X			
	Zaragoza	Peleato, M.	University of Zaragoza	X			X			
	Sweden	Huddinge	Casanovas, J.	Karolinska Institute	X			X	X	X
Huddinge		Ladenstein, R.	Karolinska Institute	X	X		X	X	X	X
Lund		Alkaradaghi, S.	Lund University	X			X	X		
Lund		Logan, D.	Lund University	X					X	X
Stockholm		Nordlund, P.	Stockholm University	X						X
Stockholm		Schneider, G.	Karolinska Institutet	X			X	X	X	X
Stockholm		Tryggvason, K.	Karolinska Institute	X			X			
Umea		Sauer, U.	Umea University	X			X	X	X	X
Uppsala		Ehrenberg, M.	Uppsala University		X				X	
Uppsala		Eklund, H.	Swedish University of Agricultural Sciences	X			X			
Switzerland	Basel	Aebi, U.	University of Basel	X			X	X		
	Basel	Engel, J.	University of Basel	X			X			
	Basel	Mayans, O.	University of Basel	X	X	X			X	
	Basel	Schirmer, T.	University of Basel	X			X			
	Basel	Stetefeld, J.	University of Basel	X				X		
	Basel	Strelkov, S.	University of Basel		X			X	X	
	Bern	Baumann, U.	University of Bern	X				X		X
	Zurich	Gruetter, M.	University of Zurich	X				X		
	Zurich	Perriard, J.-C.	ETH		X				X	
	Zurich	Piontek, K.	ETH	X				X		
The Netherlands	Amsterdam	Crielaard, W.	University of Amsterdam	X					X	X
	Amsterdam	Jeu, W. de	FOM-Institute for Atomic and Molecular Physics		X				X	

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Country	City	Groupleader	Institute	MX	SAXS	XAS	Visit 2000	Visit 2001	Visit 2002	Visit 2003
The Netherlands	Amsterdam	Sixma, T.	Netherlands Cancer Institute	X			X	X	X	
	Eindhoven	Kleppinger, R.	TU Eindhoven		X				X	
	Groningen	Dijkstra, B.	University of Groningen	X			X	X	X	X
	Nijmegen	Feiters, M. C.	University of Nijmegen			X		X	X	X
	Utrecht	Gros, P.	University of Utrecht	X				X	X	
Turkey	Istanbul	Sayers, Z.	Sabanci University		X				X	
United Kingdom	Bath	Acharya, R.	University of Bath	X					X	X
	Bath	Crennell, S.	University of Bath		X				X	
	Brighton	Muller, Y.	University of Sussex	X			X		X	
	Cambridge	Gherardi, E.	MRC		X				X	
	Cambridge	Hirshberg, M.	University of Cambridge		X			X		
	Colney, Norwich	Bornemann, S.	John Innes Centre			X		X		
	Coventry	Fulop, V.	University of Warwick	X					X	X
	Edinburgh	Walkinshaw, M.	University of Edinburgh	X				X	X	X
	Exeter	Littlechild, J.	University of Exeter	X				X	X	X
	Glasgow	Byron, O.	I.B.L.S.		X				X	
	Glasgow	Laphorn, A.	University of Glasgow	X			X			
	Heslington, York	Main P.	University of York	X			X			
	Leeds	Jaeger, J.	University of Leeds	X	X		X			
	Leicester	Haris, P.	De Montfort University		X		X			
	Leicester	Leys, D.	University of Leicester	X	X				X	X
	London	Brown, K.	Imperial College		X		X	X	X	
	London	Curry, S.	Imperial College	X	X		X	X	X	X
	London	Freemont, P.	Imperial Cancer Research Fund	X			X			
	London	Keep, N.	Birkbeck College	X			X			
	London	Pastore, A.	National Institute for Medical Research		X				X	
London	Pickersgill, R.	Queen Mary University of London	X				X	X		
London	Slingsby, C.	Birkbeck College	X			X				
London	Sutton, B.	Randall Centre, King's College London	X			X				
Norwich, Norfolk	Hemmings, A.	University of East Anglia	X			X				
Oxford	Doyle, D.	University of Oxford	X				X			
Reading	Cardin, C.	University of Reading	X			X	X	X	X	
Sheffield	Barynin, V.	University of Sheffield	X			X	X			
Southampton	Coles, S.	University of Southampton	X			X				
Southampton	Wood, S.	University of Southampton	X			X				
St Andrews Fife	Taylor, G.	University of St Andrews	X			X				
York	Wilson, K.	University of York	X				X			

Non-European States										
Country	City	Groupleader	Institute	MX	SAXS	XAS	Visit 2000	Visit 2001	Visit 2002	Visit 2003
Canada	London, Ontario	Shilton, B.	University of Western Ontario		X		X		X	
India	New Delhi	Singh, T.	All India Institute of Medical Sciences	X			X			
Singapore	Singapore	Dokland, T.	Institute of Molecular and Cell Biology	X					X	
Singapore	Singapore	Song, H.	Institute of Molecular and Cell Biology	X						X
USA	Princeton NJ	Jacobson, B.	Bristol-Myers Squibb PRI	X			X	X		

Annex 4:

European SR facilities with LS-oriented beamlines

Source	Energy [GeV]	MX	MX (proj. ³)	SAXS	SAXS (proj. ³)	XAS	XAS (proj. ³)
ESRF	6	ID14-1 ID14-2 ID14-3 ID14-4 ID23-1 (GEMINI) ID29 BM14 BM30A (FIP) ID9 ID13 BM1 (SNBL) BM8 (GILDA) BM25	ID23-2 (GEMINI) BM26 (DUBBLE)	ID1 ID2 ID13 BM25 BM26 (DUBBLE)		ID24 ID26 BM8 (GILDA) BM30B (FAME)	BM26 (DUBBLE)
DORIS	4.45	EMBL - X11 EMBL - X12 EMBL - X13 EMBL - X31 EMBL - BW7A EMBL - BW7B MPG - BW6		EMBL - X33 B1 (JUSIFA) BW4		EMBL - XAS	
SLS	2.4	X06SA (PX)	X10SA (PXII)				
ELETTRA	2/2,4	BL5.2R		BL5.2L			BL11.1
SRS	2	BL9.5 BL9.6 BL14.2 BL14.1	BL10.1	BL2.1 BL16.1		BL7.1 BL16.5	
BESSY	1.7	PSF - BL1 PSF - BL2 PSF - BL3					
MAX II	1.5	I711	I911-1 (CASSIOPEIA) I911-2 I911-3 I911-4 I911-5				
SOLEIL	2.75		PROXIMA 1		SWING		SAMBA
DIAMOND	3		I02 I03 I04		I22		

- 1) Beamlines marked in red are only partially devoted to biological applications or multi-purpose beamlines;
- 2) All information has been extracted from web-sites of respective facilities;
- 3) Proj.; planned, or under construction or in commissioning.

Annex 5:

Questionnaire for future needs of SR beamlines at PETRA-III

Questionnaire on past/present applications at DORIS-III, and possible future applications at PETRA-III.

1. Project Leader identification

Name of Project Leader:

Name of Research Organisation, Institute:

Town, City:

Country:

E-mail address:

2. Past/present applications at EMBL/DESY beamline facilities at DORIS-III during the last five years:

- Protein crystallography (PX)
- Small angle scattering (SAXS, NCS)
- X-ray absorption spectroscopy (EXAFS)
- Sample preparation

Comments:

3. Future interest in applications at PETRA-III synchrotron radiation beamlines

- Protein crystallography (PX)
 - Energy tuneability (phasing)
 - Micro focussing (small crystals)
 - High-throughput
- Small angle scattering of biological material (SAXS)
- X-ray absorption spectroscopy of biological material (EXAFS)
- Remote data acquisition
- Other interests or applications (please specify under 'comments')

Comments:

4. Future interest in applications at PETRA-III associated facilities & infrastructures

- Sample preparation**
 - Sample expression, purification
 - Sample characterisation with non-SR equipment (DLS, CD, Mass spec etc)
 - Automated high-throughput crystallisation
 - Sample preparation
 - Sample storage / retrieval (for remote experiments)
- Data processing and interpretation**
 - On-line facilities at EMBL/DESY, Hamburg
 - Remote accessibility
- Advanced training**
 - Advanced training courses
 - Training fellowship for work at EMBL/DESY, Hamburg
 - Remote, Internet based training

Comments:

Signature :

Thank you very much!

EMBL-Hamburg