PROPOSAL FOR A MICROFOCUS BEAMLINE FOR MACROMOLECULAR CRYSTALLOGRAPHY AT ALBA SYNCHROTRON

Working Committee:

Núria Verdaguer (IBMB-CSIC, Barcelona)
Isabel Uson (IBMB-CSIC, Barcelona)
Maria Solà (IBMB-CSIC, Barcelona)
Ana Cámara (Univ of Almería, AUSE member)
Mark van Raaij (CNB-CSIC, Madrid)
Jeronimo Bravo (IBV, Valencia)

Contents

1. SUMMARY

Macromolecular crystallography (MX) represents the largest single group of synchrotron users in Europe and, as a community, it is still growing. About 40% of ESRF users are crystallographers, also producing about 40% of the publications appearing in peer review journals. Crystallography has a major applied interest, indeed MX by pharmaceutical industries generate the highest external income at synchrotron radiation facilities. In Spain, the MX community is growing strongly. Our latest survey has identified 48 research groups engaged in macromolecular crystallography in a total of 18 institutes spread all over the country (about 200 direct synchrotron users). Apart from national interest, synchrotron MX beamlines also attract international users. Consulted macromolecular crystallography groups in Portugal, Italy and South America have expressed their full support and interest.

MX aims to provide an understanding at the atomic level of how biological systems function, by determining the three dimensional structures of isolated macromolecules and of the many complexes that account for most of the cellular machinery. Modern medicine and pharmaceutical companies have extensively profited from protein crystallography. Several drugs with a wide impact on human health have been developed by structure-based drug design, an approach which has been fully incorporated to modern drug discovery strategies (e.g.: inhibitors of the HIV aspartic acid protease). In this respect, synchrotron facilities are essential in generating diffraction data, since up to 85% of the structures in the PDB involved their use.

However, MX is becoming increasingly limited by the amount of sample that can be obtained. As today's most challenging targets (membrane proteins, large protein complexes, low-abundance or proteins involved in structural functions) are difficult to isolate in the milligram amounts required for efficient crystallisation, only tiny (5-50 μm in each of the three dimensions) or globally imperfect crystals can be realistically obtained. To extract useful diffraction data from such crystals, crystallographers resort to highly brilliant and micro-sized X-ray sources, the so-called microfocus beamlines. Using these sources, low-background datasets can be obtained from minute crystals or by illuminating specifically well-ordered domains from globally imperfect crystals. Minimal, non-

damaging manipulation of macromolecular fragile crystals can be achieved by in situ X-ray diffraction at room temperature. Besides, data collection with high-flux low-energy X-rays at a microfocus beamline will allow structure determination by native phasing, obviating the bottle-neck of introducing heavy atoms.

The present proposal features a microfocus beamline for macromolecular crystallography, to be built at ALBA, specifically designed to deliver high flux X-rays with a beam size down to 1.7 x 1mm, fully tuneable around all essential K and L edges. Such a beamline would target those highly challenging scientific cases typically leading to small and/or heterogeneous crystals and contemplate native phasing in this scenario. The microfocus beamline would complement without overlap the capabilities provided by the highly successful, general purpose, macromolecular beamline XALOC operating since 2010?. The proposed design would be unique in Europe and attract users with highly challenging projects worldwide.

2. Scientific case

2.1 Background

Progress in the most challenging problems in structural biology is often hindered by the inability to grow crystals that are large enough or sufficiently homogeneous. As a consequence, conventional beamlines fail to produce diffraction data that are suitable for solution of the structure.

Exhaustive efforts, often exceeding many years, do not always lead to the growth of larger or more perfect crystals. However, useful diffraction data can be collected from small crystals when background is reduced by matching the size of the beam to the size of the sample crystal. There are three particular areas in MX where lack of suitable crystals is typically the rate-limiting step in structure determination: membrane proteins, multi-protein complexes and protein fibres. In addition, many projects, especially those relying on integrated high-throughput methods, would be more productive if initial micro-crystals were used for structure determination directly without further optimisation of the crystallisation conditions. Moreover, for many crystals, small beams can dramatically improve the diffraction quality if compared to diffraction patterns obtained using regular beams. Small beams can also be used as probes to identify optimal regions of a crystal for data collection with a larger beam. In fact, current synchrotron beamlines have recently observed a substantial increment in the number of user samples of small sizes (5-10 μm) or with special pathologies (high mosaicity, extreme sensitivity to radiation damage, irregular spot shapes, multiple or cracked crystals) that could benefit from the use of a small beam. Thus, there is a growing need to resort to small X-ray beams to address biologically relevant problems in macromolecular crystallography as well as developing optimal use of such beams. In the paragraph below we summarise several examples of particular cases from the Spanish MX community.

Growing evidence supports the fact that a small X-ray beam can be used to collect useful micro-diffraction data for structural determination of biological macromolecules from very small or imperfect crystals. A number of high-profile publications have described successful data collection experiments in which a

micro-beam, defined as having a diameter of < 10 μm, intersected small volumes of 5-10 μm thick plate shaped crystals or needle crystals with 10 x 10 μm cross-sections (Cusack et al., 1998) (Sanishvili et al., 2008). Furthermore, small beams have also been used with large crystals to address problems derived from non-homogeneous crystals (Renault et al., 2001), radiation damage (Fotinou et al., 2001) (Nave and Hill, 2005) and high mosaicity (Xiao et al., 2003).

2.2 Membrane proteins

In spite of tremendous efforts and some spectacular successes (Cherezov et al., 2007) (Rasmussen et al., 2007) (Palczewski et al., 2000) (Warne et al., 2008) (Lebon et al., 2011), membrane proteins in structural biology are still a largely unconquered area. These proteins represent the ~25 % of the proteome of most organisms and are involved in a wide variety of fundamental biological processes including photosynthesis, respiration, signal transduction, molecular transport and catalysis. In spite of membrane proteins representing more than 40% of all known drug targets, only about 502 unique structures are available (http://blanco.biomol.uci.edu/mpstruc/). From these, eukaryotic membrane proteins are particularly underrepresented.

Membrane proteins have proven to be difficult to study due to their partially hydrophobic surfaces, flexibility and lack of stability (Carpenter et al., 2008). Additional difficulties arise from the fact that many membrane proteins occur with a distinctly low natural abundance and their expression in heterologous systems appears to be extremely difficult. The levels of expression of correctly folded and active membrane proteins are commonly several orders of magnitude lower than for soluble proteins. Consequently it is practically unfeasible to perform extensive crystallisation trials in order to determine conditions that might, ideally, yield large crystals, nor is it doable to simply discard all the small crystals (largest dimension less that 30 μm which invariably form the majority of crystals even under optimised conditions). The ability to collect diffraction data from small (<30 μm) crystals is therefore key to the success of this type of project.

Data collection for membrane protein crystals is often challenging. Most crystals incorporate a high solvent content owing to the detergent micelle, which

covers the hydrophobic part of the protein. Consequently, the crystals are mechanically fragile, difficult to handle, diffract to low resolution and suffer from radiation damage during the diffraction experiment. In addition, crystal quality can vary considerably, even between crystals from the same crystallisation drop. The presence of automatic sample changers at most synchrotron beamlines has helped address this issue, enabling many crystals to be screened quickly and efficiently. The use of microfocus beamlines with low background scatter and small beam size greatly improved the situation. There are several recent examples of structure elucidation of different members of the important families of G Protein-Coupled receptors (Cherezov et al., 2007); (Rasmussen et al., 2007) or the amino acid, polyamine and organocation (APC) transporters (Gao et al., 2009); (Fang et al., 2009; Gao et al., 2010); (Kowalczyk et al., 2011) (Figure 2.2.1) that illustrate the successful use of microfocus beamlines to obtain high quality data of such important proteins. These beamlines can be used to collect datasets from very small crystals and from well diffracting regions in heterogeneous crystals. They can also be used to collect segments of datasets along the length of the crystals, when individual regions suffer radiation damage.

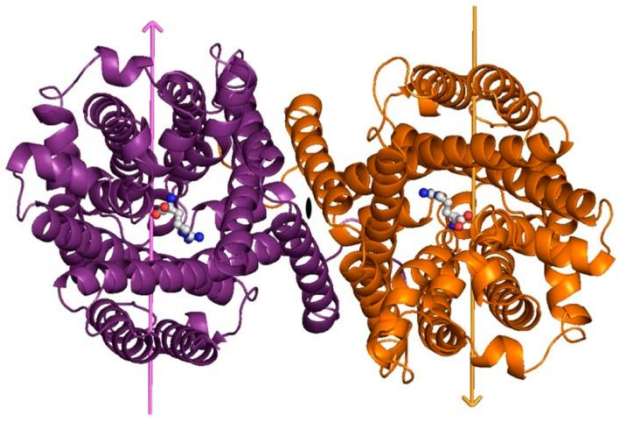


Figure 2.2.1 **Structure of the proton pump AdiC-N101A bound to Arg^+**. Periplasmic view of the complex homodimer with the two protomers shown in purple and orange and the bound substrate depicted as balls and sticks. Data was collected at beamlines X06SA (PXI) in the SLS and ID23-2 in the ESRF. Image taken from (Kowalczyk et al., 2011) (IRB, Barcelona, Spain).

2.3 Large multicomponent macromolecular complexes

An intense focus of current biological research efforts is the elucidation of protein interaction networks (the interactome) and as a result many large macromolecular complexes are discovered. Structural information on these complexes is essential to understand the molecular basis of many of the fundamental aspects of cellular machinery, for example DNA transcription into messenger RNA (Kettenberger et al., 2003);(Kuhn et al., 2007); (Fernandez-Tornero et al., 2013) (Figure 2.3.1), the translation of messenger RNA at the ribosome (Schluenzen et al., 2000);(Harms et al., 2001), the RNA processing and degradation by the exosome (Liu et al., 2006), the structural organization of the nuclear pore complex (Hsia et al., 2007) or of the large eukaryotic ribonucleoprotein particles, called vaults (Tanaka et al., 2009); (Querol-Audi et al., 2009). The limiting step for successful structural studies of many of these complexes is the production of homogeneous and stable specimens for crystallisation. Heterogeneity in these complexes can arise as a result of the absence of one or more constituent proteins of the complex or because some of the components can adopt a number of distinct conformations. In both cases the incorporation of heterogeneous molecules would interfere in crystal growth, blocking the possibility of obtaining large crystals, even when an endless number of crystallisation experiments are set up.

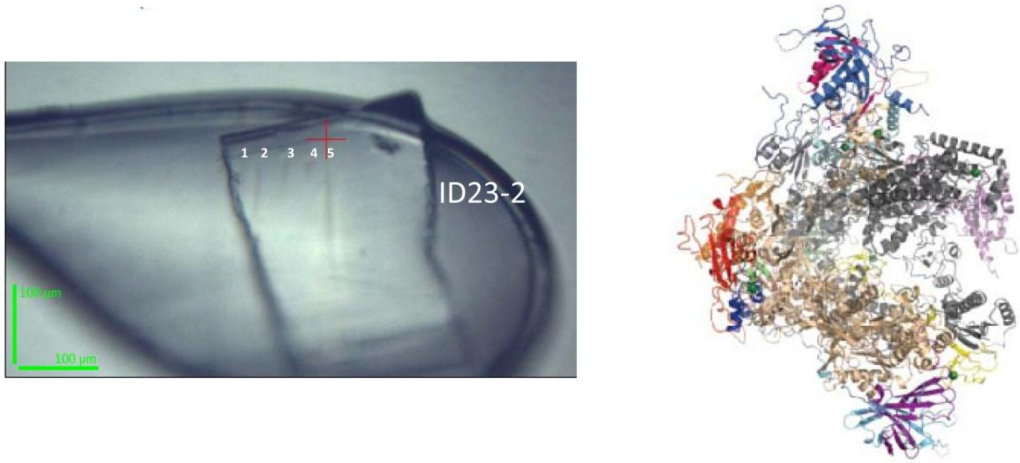


Figure 2.3.1 **Structure of the RNA polymerase I (RNA Pol I)**, a 600 kDa protein complex consisting of 14 subunits. Initial native data sets were collected at 4 Å resolution at the microfocus beamlime ID23-2 from ESRF (France). Small edges of 30° were collected from 5 spots on the same crystal, yielding a complete data set after merging. (B) Overall structure of PolI. Images kindly given by Carlos Fernandez-Tornero (CIB-CSIC, Madrid, Spain).

2.4 Fibril-forming proteins

Improper protein folding (misfolding), leading to fibril formation, underlies the aetiology of many degenerative diseases. This phenomenon is now thought to be a universal property of proteins, as long as appropriate conditions for loosening the native folded structure can be found, which may be outside those of normal physiology. Misfolding can lead to the formation of disordered (amorphous) or ordered (amyloid fibril) aggregates. Amyloid-forming proteins are therefore one example, in which conformational change may lead to fibrils and cause several diseases including cataract formation or neurodegenerative maladies with no known cure, such as Alzheimer's and Parkinson's diseases (Westermark, 2005) (Westermark et al., 2005). Amyloid diseases are accompanied by the deposition of elongated, unbranched protein fibrils. Structural characterisation has been elusive since fibril forming proteins only yield microcrystals, due to the heterogeneous nature of the samples. Several segments from proteins that form amyloid-like fibrils have recently been characterised using microfocus beamlines including segments from the Alzheimer's amyloid-beta and tau proteins, the PrP prion protein, insulin, islet amyloid polypeptide (IAPP), lysozyme, myoglobin, alpha-synuclein and beta(2)-microglobulin (Sawaya et al., 2007).

2.5 Small crystal size is a limitation in many MX projects

Even though membrane proteins, protein complexes and fibril proteins seem to be the most challenging areas in terms of producing crystals of convenient size (> 50μm), most MX laboratories have a large number of cases where only microcrystals can be obtained, in spite of extensive crystallisation experiments. For example, it is extremely common to obtain needle-shaped crystals, with a long dimension around 100μm but less than 10μm in width (Figure 2.5.1). This morphology will arise whenever crystal growth in one direction is highly favoured as a result of the packing arrangement of the molecules in the crystal lattice. Useful X-ray data from such crystals can be obtained in a microfocus beamline by collecting and merging successive partial data sets from different crystal sections along the largest dimension of the crystal (Figure 2.5.2).

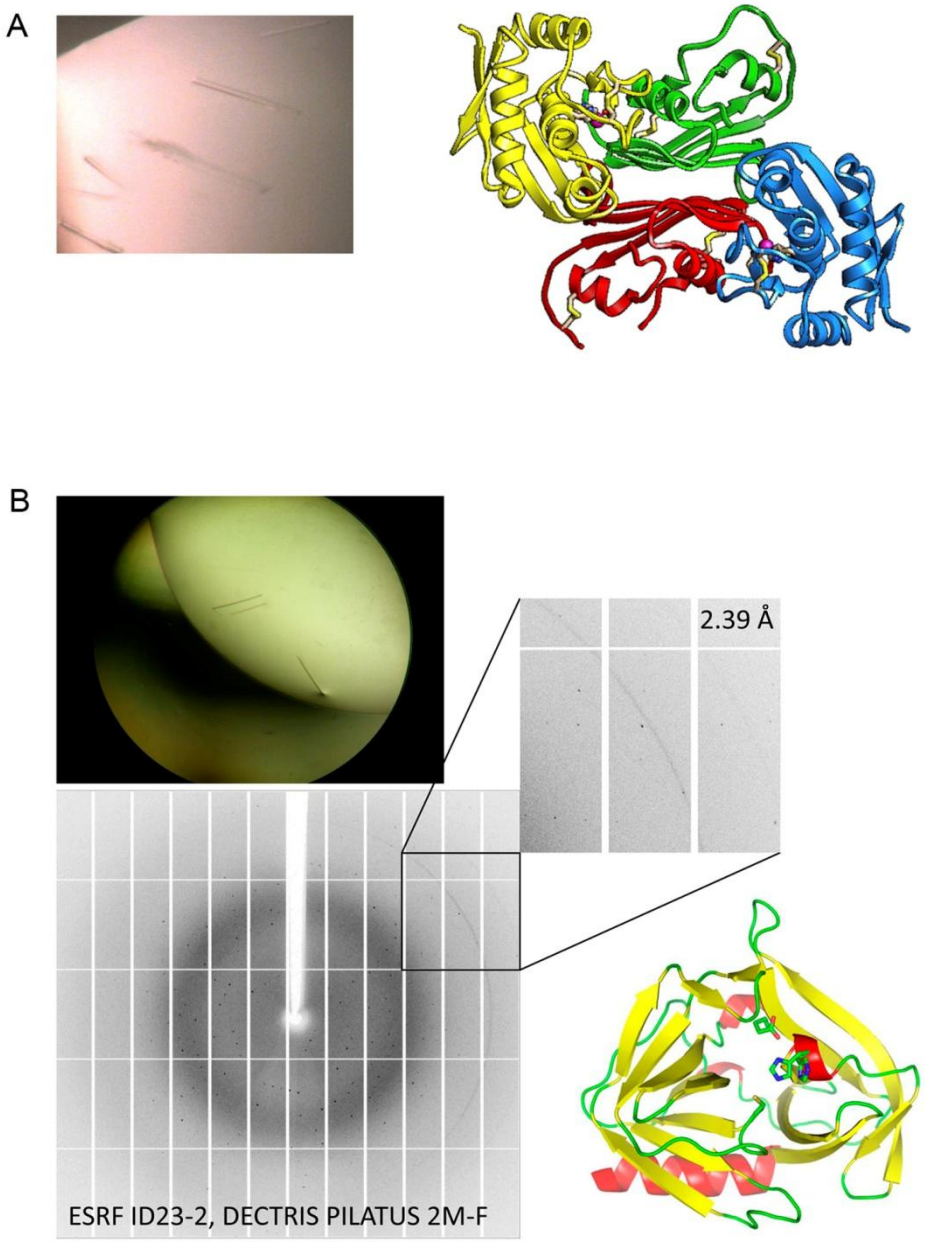
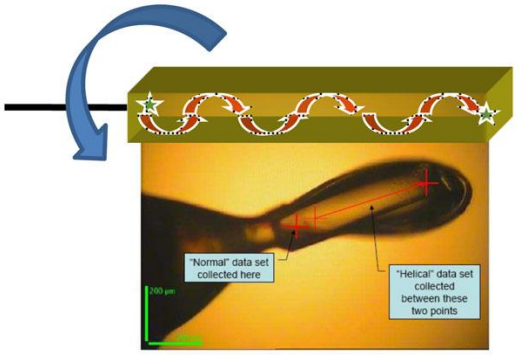


Figure 2.5.1 Classical cases of very thin needle-shaped crystals. (A) Left panel shows crystals of the the metalloprotease Snapalysin in complex with the protein inhibitor Sermetstatin. These crystals were fragile in front of X-rays and required helical data collection at the microfocus beamline ID23-2 (ESRF) (See also Figure 2.5.2). The right panel shows the structure of the complex with the Snapalysin molecules shown in yellow and blue and Sermetstatin in green and red. (From (Trillo-Muyo et al., 2013), IBMB-CSIC; Barcelona, Spain). (B) The Enterovirus 93 3C proteinase crystallizes as very thin plates that are usually too small for attempting to diffract them in a standard beamline (left). A 2.39 A data set collected at ID23-2 (middle) allowed the structure determination of this antiviral target (right) (Images kindly provided by Zuzanna Kaczmarska and Miquel Coll, IBMB-CSIC, Barcelona, Spain).



Data collection for the crystal of xylanase 10B

	Data set	
	Normal	Helical
Wavelength (Å)	0.873	
No. images/oscillation angle	270/1.0°	
Temperature	100 K	
Exposure time per image (s)	1.0	1.5
Space group	$P2_12_12_1$	
Unit-cell parameters (Å)	a = 65.7, b = 108.8, c = 113.6	
Resolution range (Å)	56.89–1.70 (1.74–1.70)	56.89–1.70 (1.74–1.70)
No. unique reflections	89547	89503
Completeness (%)	99.2 (91.8)	99.1 (90.9)
R_{merge} (%)[‡]	7.8 (34.0)	4.9 (14.2)
Multiplicity	10.5 (7.3)	10.7 (7.5)
$\langle I \rangle / \langle \sigma(I) \rangle$	25.2 (5.7)	32.2 (11.5)
Wilson B-factor (Å^2)	11.9	12.3

[‡] $R_{merge} = \Sigma_h \Sigma_i |I(h,i) - \langle I(h) \rangle| / \Sigma_h \Sigma_i I(h,i)$, where $I(h,i)$ is the intensity of the ith measurement of reflection h and $\langle I(h) \rangle$ is the mean value of $I(h,i)$ for all i measurements.

Figure 2.5.2 Helical data collection. (A) Two points on the crystal are defined by the users as the start and end points. One single data set is collected during which the crystal is automatically translated between these two points so as to move fresh sample gradually into the beam. This has the potential to allow a longer exposure per image without significant radiation damage occurring and thereby increasing signal-to-noise ratios. A crystal of xylanase 10B from *Clostridium thermocellum* was used to compare normal and helical data collection methods in ID23-2. The beam size was 7 µm in diameter; the crystal used was approximately 600 µm long by 150 µm wide. (B) Data collection statistics, comparing the two methods (from (Flot et al., 2010).

2.6 High-throughput methods for protein crystallography and *in situ* diffraction

Structural genomics initiatives have developed the necessary technology to clone, express and purify many protein targets in parallel, as well as to perform crystallisation trials at a rate of 100 crystallisation plates per day (Blow, 2008);(Service, 2008). Currently, many of these techniques are being applied to membrane proteins in a number of dedicated initiatives (see as example http://www.diamond.ac.uk/Science/MPL/default.html).

Automation techniques run in parallel with miniaturisation, so that the largest possible range of conditions can be explored from a limited supply of pure protein. In recent years, the volumes required for initial crystallisation screens have been greatly reduced, so that now routine screening for crystals is set-up in 100nl drops, dispensed by crystallisation robots, rather than the 2µl characteristic for manual methods. This fact inevitably influences the size of the crystals that are obtained from such trials. While, in most cases, it is possible to "scale-up" the volumes used for the production of crystals for data collection, it would be very advantageous if initial microcrystals were used directly for

structure determination. In addition, with the commercialisation of nanolitre crystallisation robots, these methods are becoming the standard approach to crystallographic problems in most laboratories. This area is of particular relevance for many pharmaceutical and structural biology-focused biotechnology companies.

The number of crystallographic projects that depend on using very small amounts of protein (and subsequent crystallisation of nanolitre drops) is therefore set to grow, resulting in an increase in the demand for access to a microfocus beamline. To this regard, the new *in situ* diffraction protocols (where crystals are not extracted from their crystallization media prior to data collection) established at SLS X06DA and Diamond I24 beamlines, using beam sizes between 5 and 40 μm (Bingel-Erlenmeyer et al., 2011);(le Maire et al., 2011);(Axford et al., 2012); (Owen et al., 2012);(Owen et al., 2014) have demonstrated important advantages in the identification of good quality crystals for difficult proteins or in rapidly obtaining large numbers of X-ray datasets for protein complexes with different compounds in industrial drug development. The feasibility of the technique, in which the crystals remain in their crystallization solution (Jacquamet et al., 2004), has also been demonstrated for successful data collection of different biological systems for which crystals are too sensitive to either manipulation or standard cryogenic procedures (e.g. viruses and other large macromolecular assemblies) (De Colibus et al., 2014).

2.7 Small beams playing big roles in Macromolecular Crystallography

2.7.1. Improved signal-to-noise ratio for small crystals

X-ray beams at standard synchrotron beamlines for MX are typically ellipsoids with the large dimension in the range of 50 to 300 μm (Pohl et al., 2001). For samples that are much smaller than the incident beam, the intrinsically weak diffracted intensities can be overwhelmed by high backgrounds from the crystal mount. The portion of beam cross-sections that does not intercept the sample crystal contributes only background to the diffraction image and reduces the signal-to-noise ratio. The effective diffraction limit is also reduced by loss of weak diffracted intensities into background noise. The smallest volume samples reported to have produced useful crystallographic data were the 2 x 2 x 2 μm crystals of cypovirus polyhedra (Coulibaly et al.,

2007). Another example of successful data collection of very small crystals is that of the membrane remodelling protein 2B from Hepatitis A virus (Garriga et al., 2011) and Vives-Adrian et al., 2014 submitted), Figure 2.7.1. Despite the small size of the crystals (~ 5 to 10 μm per edge), complete data sets were collected from native and SeMet derivative crystals to 2.5 Å and 2.7 Å resolution respectively on the microfocus beamline ID23-2 (ESRF).

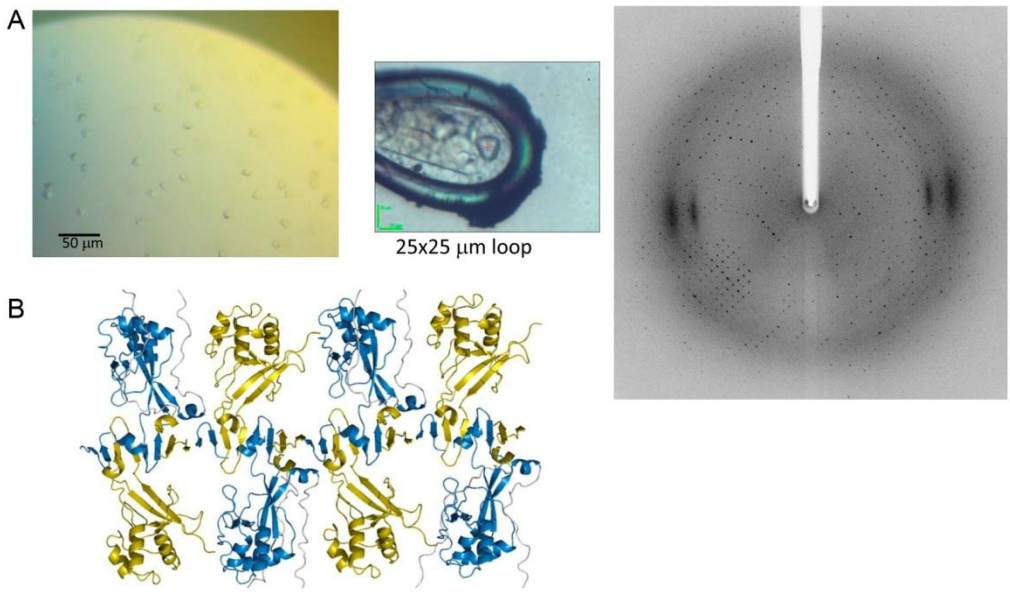


Figure 2.7.1.1 The membrane remodeling protein 2B from Hepatitis A virus. (A) Crystals and X-ray diffraction pattern of the 2B protein. The detector was set to an edge resolution of 2.50 Å. Diffraction spots were observed to 2.25 Å resolution. (B) Overall view of the structure of 2B and crystal packing (from (Garriga et al., 2011) and Vives-Adrian et al., 2014 submitted; IBMB-CSIC, Barcelona, Spain)

Although it is arguable, whether crystals with such characteristics would allow to measure useful data in a conventional beamline coupled to a low noise detector, provided enough data collection time is granted and trusting on the absolute stability of the experiment parameters during a long enough time, the fact remains that, in practice, success has only been achieved resorting to a microfocus beamline.

2.7.2 Scanning well ordered parts of heterogeneous crystals

Diffraction quality is reduced when the X-ray beam intercepts a non-homogeneous crystal volume; these inhomogeneities are common in crystals of biological macromolecules and arise from effects such as split or multiple

crystals, bent crystals, satellite crystals and crystals with damaged or imperfect local regions. Experience gained at microfocus beamlines (Cusack et al., 1998),(Renault et al., 2001),(Xiao et al., 2003), using very small beams (< 10 μm) allows the selection of small crystal volumes by crystal scanning using tiny grids, showing much lower mosaicity than the average of the entire crystal, leading to a significant improvement in the quality of the diffraction data. An example illustrating an extreme case of heterogeneous crystals is shown in figure 2.7.2.1

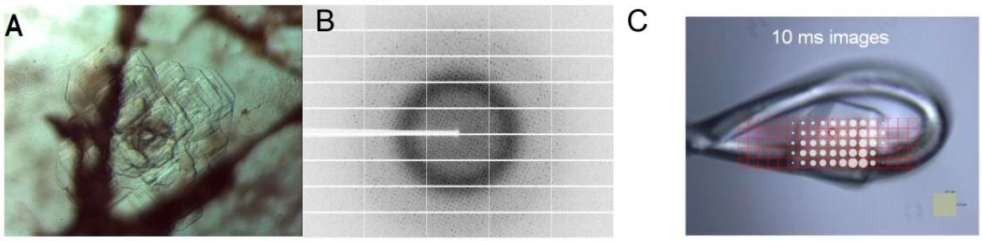


Figure 2.7.2.1 Poly-crystals of the third PDZ domain of the post-synaptic density protein 95, PSD95. These crystals, from a construct lacking a C-terminal a-helix, were obtained after six years trials of different crystallization conditions. (B) Diffraction images collected after selecting a well-diffracting region in the crystal. (Images kindly provided by Ana Cámara, Univ of Almeria, Spain). (C) The diffraction grid scan as implemented in diamond. This function is particularly useful to: i) Locate small crystals in the loop ii) Locate crystals in opaque mother liquors and iii) Identify the optimal diffraction from a large crystal (Bowler et al., 2010).

2.7.3 Radiation Damage

Radiation damage can be viewed as time-dependent inhomogeneity. For several cases of extreme sensitivity to radiation, useful data has been recorded using a microfocus beam in raster fashion to collect data from several sections on a relatively large crystal. The different partial data sets, each collected at a high X-ray dose from a very small crystal volume can be successfully scaled against a low resolution reference and merged to yield a good-quality complete data set (see Figures 2.7.3.1 and 2.7.3.3, or (Rasmussen et al., 2007); (Dimasi et al., 2007);(Coll et al., 2002) as examples). Other experiments documented the feasibility of avoiding radiation damage effects by merging multi-crystal datasets obtained from several micro-crystals using a 7 μm beam (Sanishvili et al., 2008) (Coulibaly et al., 2007). Theoretical calculations (Nave and Hill, 2005)

showed that significant reductions in radiation damage are expected to occur for crystals of a few micrometer in size.

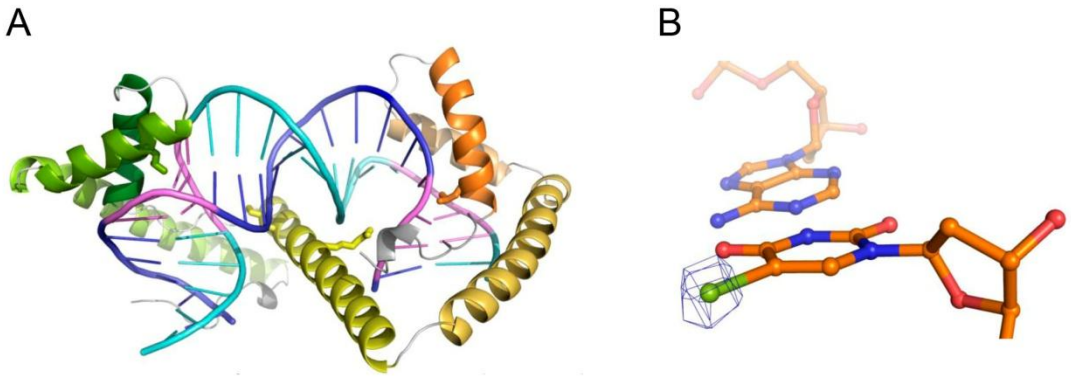


Figure 2.7.3.1. Structure of the Human mitochondrial transcription factor A bound to DNA. A data set was collected with the *helical* data collection from crystals containing a Thymidine replaced by the unstable Br-Uracyl as anomalous scatter. The 2.4 Å resolution data, collected at ID23-2 (λ above Br edge) yielded the anomalous signal that allowed confirming the right DNA sequence assignment (from (Rubio-Cosials et al., 2011); IBMB-CSIC, Barcelona, Spain).

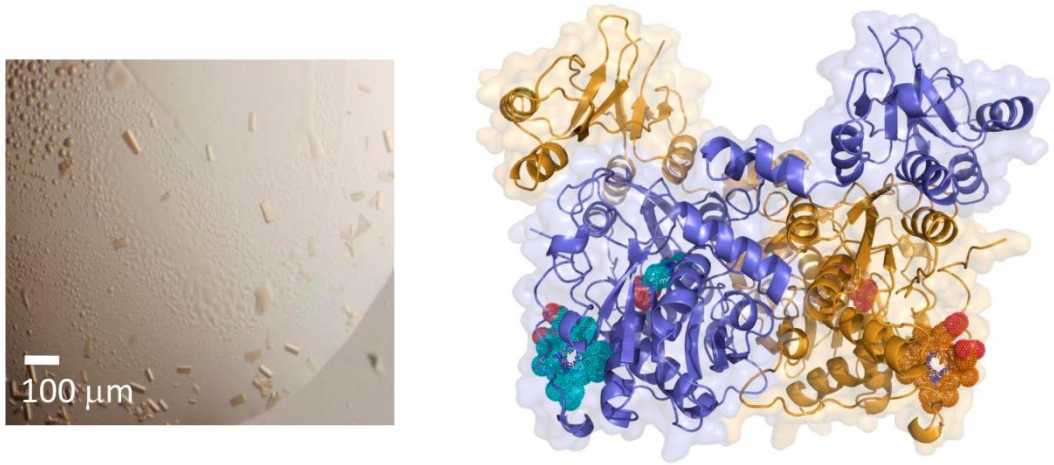


Figure 2.7.3.2 Structure of cystathionine β-synthase, the central enzyme of transsulfuration. This highly unstable protein has a half-life of 27 hours at 4°C. The best diffracting crystals, of average size 15x20x100 μm (A), extremely fragile, were obtained after testing more than 50000 crystallization conditions and more than 1000 optimization solutions. Complete datasets from only 3 crystals served to solve the structure to 3.2 Å resolution (from (Ereno-Orbea et al., 2013); CIC BioGUNE, Derio, Spain).

2.8 Native phasing using a microfocus beamline

Native SAD methods are increasingly used for phasing since no need of heavy atom derivative crystals is required. Anomalous scattering atoms such us Ca, Cl, S, P or Mg can be present in native crystals and be used for light-atom-

only native SAD phasing. However, difficulties in making accurate measurements of the weak anomalous signals from native biological macromolecules have restricted the method only to very well diffracting crystals. Attempts to enhance the signal-to-noise ratio for anomalous diffraction experiments can contemplate increasing the anomalous signal strength in synchrotron beamlines capable of performing low-energy X-ray diffraction and also reducing the signal to noise ratio by averaging of diffraction data. Recently, the measurement of several statistically equivalent crystals has been proposed to increase the multiplicity. A low energy microfocus beamline should reduce the chances of potential anisomorphism among crystals, since several data sets could be collected at several positions of the same crystal, maximizing the statistical equivalences.

3. Current state of macromolecular crystallography in Spain

A thorough analysis of Spanish Macromolecular Crystallography (MX) users is given, including the research interests, that generated all scientific cases exposed above; beamline requirements; beamtime usage; and synergy between the proposed MX microfocus beamline and existing ALBA facilities.

3.1 Macromolecular crystallography groups in Spain

Over the last decades, the Spanish macromolecular crystallography community has grown steadily. The size and number of groups that were the cradle for this activity in Spain centered in Barcelona and Madrid, grew five-fold in as little as ten years during the period before the crisis that started in 2008 and, since then, 10 more groups have been identified (Figure 3.1).

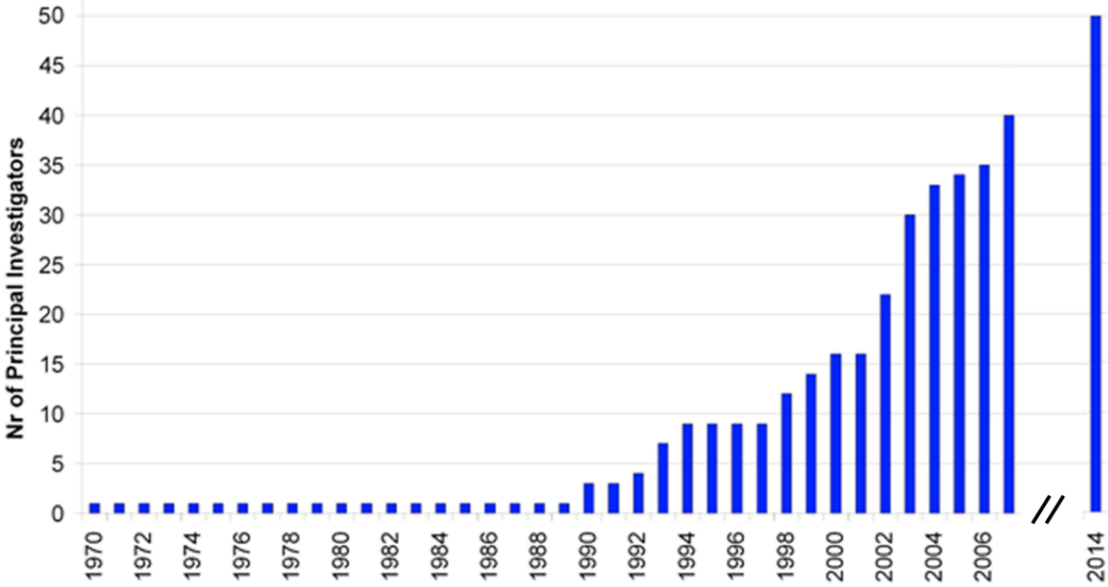


Fig. 3.1: A register of Spanish Principal Investigators in biocrystallography shows a steady increase over the years. This reflects the growth of the field, which became consolidated by important initiatives like the Ramón y Cajal, I3 and ICREA programs and economical support from institutions and governments.

The number of institutes hosting macromolecular crystallography has increased threefold in the same period. These are now not only found in Madrid and Barcelona, but also in Valencia, Santiago de Compostela, Granada, Bilbao, Santander, Zaragoza, Oviedo, Pamplona and Almería (Figure 3.2). The number of PIs employing macromolecular crystallography (MX) continues to grow and

can be expected to do so in the future (see Annex 1 for a list of PIs and research interests).

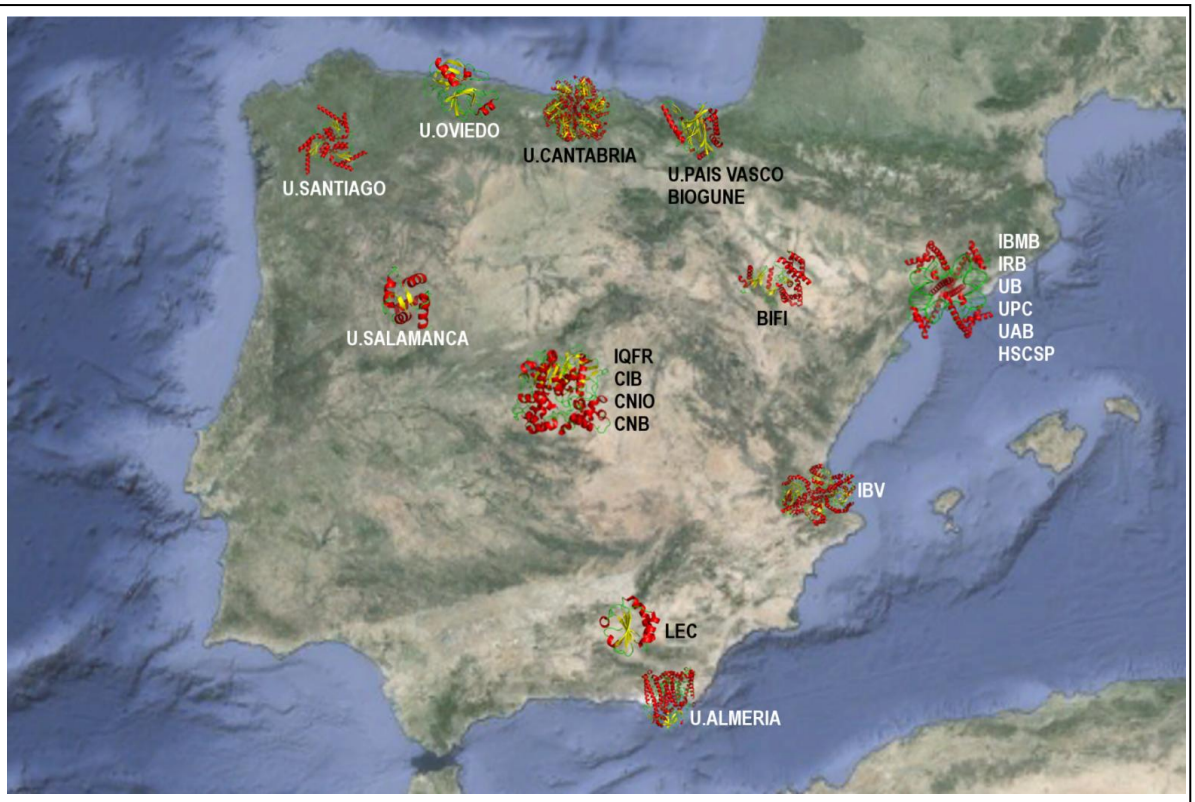


Fig. 3.2: Distribution of Institutes that harbour a macromolecular Crystallography Department or Unit in Spain. U stands for University. IQFR (Institute of Physical Chemistry "Rocasolano"), CIB (Biological Research Centre), CNIO (National Cancer Research Centre) and CNB (National Biotechnology Centre) are located in Madrid. IBMB (Institute of Molecular Biology Barcelona, CSIC), IRB (Institute for Research in Biomedicine), UB (U. Barcelona), UPC (Catalonian Polytechnic U.), UAB (Autonomous U. Barcelona) and HSCSP (Santa Creu i Sant Pau Hospital) are located in Barcelona. IBV (Biomedical Institute of Valencia) is located in Valencia and LEC (Laboratory for Crystallographic Studies) in Granada.

3.2 Research interests of Spanish macromolecular crystallographers

Since its beginning, MX has strongly contributed to the understanding of the fundamental molecular mechanisms underlying biological processes and related diseases. The characterized specimens range from single molecules (proteins, DNA) to macromolecular complexes. The body of knowledge generated enables development of molecules that can alter macromolecular interactions, enzymatic activities or induce conformational changes that underlie pathological processes, and has efficiently contributed and continues contributing to the development of therapeutic molecules. This is reflected by 13 Nobel Prizes in Chemistry in the field of protein crystallography, the three most

recent in 2006, awarded to Roger Kornberg for the first RNA polymerase structure, in 2009, awarded to Ada Yonath, Tom Steitz and Venki Ramakrishnan for the crystal structure determination of the ribosome, the essential nano-machine for protein synthesis in cells, and in 2012 awarded to Brian Kobilka for studies of G-protein-coupled receptors (GPCRs) that conduct the majority of transmembrane responses to hormones and neurotransmitters, and mediate the senses of sight, smell and taste; this included solving the first structures of a ligand-activated G protein-coupled receptor (PDB access codes 2r4r, 2r4s, & 2rh1 in 2007) and the first activated G protein-coupled receptor in complex with its G protein (3sn6 in 2011).

In this respect, Spanish users of beamline XALOC contribute by investigating important societal challenges, such as health and food challenges and microbial biodegradation, bioremediation and biotransformation, which are summarized below and detailed in Annex 1.

3.2.1 Health and food challenges

Health challenges will remain daunting in the decades to come. There is still no effective vaccine or cure for such pandemics as Ebola virus, HIV/AIDs, dengue fever and malaria. Other current and future health concerns include the emergence of new pathogens and the growing resistance of bacteria to existing medical treatments. Related to these challenging issues, several projects of the Spanish MX community are involved in the search of new drugs against emerging and re-emerging viruses or the growing resistance of bacteria to antibiotics (see Table in Annex 1). MX also contributes to the understanding of complex diseases such as heart disease, cancer, ageing-related diseases (rheumatoid arthritis, age-related neuropathologies, etc.), neurological disorders and diabetes. This second type of health challenges is also investigated by Spanish researchers as summarized in Annex 1.

The Spanish MX community also participates in other strategic areas of research, such as food challenges. The world population is expected to grow from currently 7.2 billion to 9.6-12.3 billion by 2100. The combination of rapid population growth and a diet more heavily reliant on meat and dairy products than in the past may increase the demand for food by 70% by 2050. This presents a major challenge for agriculture. Some potential threats include

animal and plant diseases caused by microorganisms, or hydric stress to plants due to the global climate change. State-of-the-art MX techniques may help in the agricultural and food sectors. Structural studies on plant and animal proteins can contribute to the development of cures for plant and animal diseases, including e.g. the development of vaccines against avian or swine flu, to which Spanish MX users contribute. Another field of research currently pursued focuses on the threats of drought and salinity to agricultural productivity, which restrain plant growth. The three-dimensional structures of multiprotein complexes of important plant protein families such as abscisic acid (ABA) receptors and interacting partners will help to design plant varieties with enhanced properties and to develop structure-based novel agrochemicals to improve crop performance.

3.2.2 Microbial biodegradation, bioremediation and biotransformation

The elimination of a wide range of pollutants and waste from the environment is an absolute requirement to promote a sustainable development of our society with low environmental impact. Biological processes play a major role in the removal of contaminants and they take advantage of the astonishing catabolic versatility of microorganisms to degrade or convert such compounds. New breakthroughs in structural characterization of the involved macromolecules and their regulation are producing vast amounts of information to which Spanish MX users also contribute.

All aforementioned research interests of the Spanish MX investigators meet the objectives of the European Research and Innovation Program H2020.

3.3 Interest of Spanish MX researchers in a microfocus beamline at ALBA

With the advent of high-throughput approaches, Spanish groups have invested in robotic techniques that only require minute amounts of material and multi-well plates for nano-crystallization. This has dramatically sped up the process of getting crystals, but has created new challenges for diffraction capacity, tilting the balance towards dealing with very small crystals and with thin needle-shaped crystals.

Overall, the rapid advances in protein production, coupled to the tremendous improvement of crystallization facilities, has resulted in a rapid increase of crystal production, which, together with the growth in the number of investigators, substantiates the overall higher demand of synchrotron beamtime. The vast increase in the demand for synchrotron time was already evident when the XALOC line was granted, as attested by the support given by SEBBM (Spanish Society for Biochemistry and Molecular Biology), which has roughly 3,800 members and is the largest non-medical scientific society of Spain. This society has one of its full sections devoted to structural biology and awards a yearly price, the Josep Tormo Award, to contributions excelling in this field. Already during the support for an MX beamline at ALBA in Phase I in 2005, there was a strong urge by SEBBM members to request two beamlines, one with the XALOC characteristics and another one with the characteristics of a microfocus beamline, although, eventually, it appeared economically and operatively unreasonable to request both at the very start of ALBA. The time is now ripe for the request of this microfocus beamline. In the Phase II call for new beamlines in 2009, both the very strong support of the MX community, which participated actively in the preparation of the Microfocus Beamline Proposal, together with the high quality of the proposal itself, ranked the Macromolecular Crystallography Microfocus beamline proposal to the top “A” group, reflecting its strong interest. However, despite its success, no financial support was given because the difficult period of the economical crisis started. As a consequence, the Spanish funding agencies changed the priorities. In the present period of Phase III, ALBA and AUSE (Spanish Association of Synchrotron Users) launched a Synchrotron Users Survey to better inquire the users interests without any bias from previous years. Based on the results published in December 2013 (see https://www.cells.es/old/NewsAndEvents/News/UsersSurveyResults/?searchterm=survey), ALBA and AUSE concluded:

- Participants clearly considered that the most interesting future beamline for ALBA should be the microfocus MX beamline proposed here (Micro PX, 50%), followed by a Single Microcrystal small-molecule diffraction beamline (15%) and a MIRAS Infrared Microspectroscopy beamline (11%).

- Respondents chose Micro PX (45%), MIRAS Infrared Miscrospectroscopy (11%) and Single Microcrystal diffraction (9%) as the beamlines that they will actively support, expressing a strong interest in them.

Therefore, as an overall conclusion from this survey, the Spanish MX community expressed once again the need of, and full support to, a microfocus MX beamline. Such an enthusiasm was already reflected in Phase II during a users' symposium held on the 6th March 2009 at the Barcelona Science Park (see program in annex 2), culminating the consultation process opened among the Spanish MX community to request their input and feedback specifically for the Phase II proposal. As in the last occasion, the SEBBM, the Spanish Society of Biophysics (SBE) and national and international directors of research centres and structural biology units and departments also formally underpin their commitment with the current proposal by providing letters of support, in which they express their certainty that its success will foster the scientific research in their Institutions (see annex 3).

Likewise, a number of Portuguese, Argentine and Italian groups have also shown high interest and expressed their support for a new microfocus beamline located at ALBA (annex 3 for support letters).

3.4 Return of the beamline investment to society

The beamline investment to society returns in the form of economical development at the local region and beyond, in education and in comercial uses, as decribed below.

Regional development: From a technical point of view, the conception and construction of a new beamline at ALBA includes the design and production of synchrotron-related specific solutions that can be developed by neighbouring companies, in ALBA's case at the industrial Vallès area. This fosters regional economical development. To our knowledge, XALOC is currently the only beamline at ALBA that generates products that are under commercial development (personal communication).

Education potential: Education in crystallography and in high-level technical development will increase the critical mass of MX researchers in Spain in number and quality. In this regard ALBA, and the MX beamlines in

particular, have a strong potential in contributing to the academic formation of future researchers in the field. XALOC was awarded with a PhD fellowship from the Spanish Nuclear Safety Council and participates in master studies from the Autonomous University of Barcelona.

Commercial uses: During 2013, the percentage of MX use of the total industrial beamtime in fully running synchrotrons was of 90% at the SLS, 50 % at the ESRF and 70% at Diamond (Table 3.4.1). ALBA should be interested in attracting national and international MX industrial users, as it is the main source of beamtime sales in these reference synchrotrons.

Synchrotron (contacted person)	**SLS** (Stephan Jansen)	**ESRF** (Ed Mitchell)	**Diamond** (E. Shotton)
Of total beamtime sold to industry, % belonging to MX	90 %	50 %	70 %

Table 3.4.1. Percentage of MX beamtime of the total beamtime sold to industry for three European reference synchrotrons: SLS (Swiss Light Source), ESRF (European Synchrotron Radiation Facility, France) and Diamond (UK).

We are aware that the synchrotrons selected in this comparison profit from local, well-established pharmaceutical industries, while in Spain a similar level still take some time to reach. Nevertheless, we expect that the continued success of ALBA will lead to industrial MX beamtime being requested by companies both in Spain and beyond.

3.5 Beamtime use and beamline requirements of Spanish MX users

Before 2011, the ESRF was the synchrotron most accessed by the Spanish MX community, which used up to 6% of total ESRF beamtime, from 8% requested. From the 6%, 2% of the beamtime corresponded to overuse of Spanish users that was not paid to the ESRF, who requested the payment. However, due to the financial crisis, in 2009 the Spanish Government decided not to pay the 6% of used beamtime but to restrict the access to the ESRF to

4%. In 2010, this reduction affected all disciplines, including Macromolecular Crystallography, imposing a lower access to this synchrotron. Due to this beamtime restriction, and for the sake of optimal beamtime usage, the ESRF allowed Spanish MX groups access only *via* the Block Allocation Group (BAG) system, which joins several groups in a single one that manages the beamtime between partners. Coincident with the ESRF beamtime restriction, the number of BAGS increased in Spain, adding to the two long running Barcelona and Madrid BAGs, the two more recent CIC BioGUNE Bilbao BAG (2009) and South-West Andalusia BAG (2010). In addition, the number of groups inside each BAG increased. The restriction of beamtime at ESRF was partially solved by the increasing beamtime in XALOC since its opening, and by making additional use of SLS (Switzerland), Soleil (France) and Diamond (UK).

The success of the young XALOC beamline has been confirmed during 2013, when 74% of beamtime is dedicated to user experiments, with the remaining time dedicated to inhouse experiments, commissioning or other purposes (source: ALBA User Office). This points to a full use of an additional beamline with a different purpose. The MX community agrees that its most urgent need now is a state-of-the-art microfocus beamline with capabilities that cover the needs that XALOC cannot meet. The combination of low energy phasing and *in situ* diffraction at room temperature with the proposed micro beam (1.7 x 1 µm) will result in a unique beamline, meeting emerging and foreseen challenges in MX crystallography. Therefore, the inclusion at ALBA of such a microfocus MX beamline will intensively boost the European MX research and will ensure the synchrotron to be competitive in the MX field.

3.6. Synergies of the microfocus beamline with techniques and human resources currently available at ALBA

We foresee a high synergy between the proposed MX microfocus and the existing resources at ALBA. The XALOC beamline is pivotal in providing the acquired know-how and experienced personnel for the construction, commissioning and operation of a microfocus beamline for macromolecular crystallography. Both beamlines are technically related, thus they will benefit from the exchange of equipment, personnel, knowledge and expertise, and

together will foster scientific research, technological development, and academic and industrial exploitation.

In addition, the proposed microfocus will be in a synchrotron that provides excellent complementary instrumentation, thus facilitating analysis of biological samples by different techniques and at different observation levels. A microfocus beamline able to analyze structurally heterogeneous crystals of minimal size (at nano level) will complement XALOC, a beamline specialized in homogeneous crystals of small (tens of microns) to medium or larger sizes; these two complementary beamlines will be devoted to structure determination of macromolecules at the atomic level. On the other hand, the future BioSAXS beamline at BL11-NCD will allow the determination of (macro)molecular structures in solution (thus, not trapped in a crystal in a "*frozen"* conformation), a technique that is increasingly used by all laboratories specialized in macromolecular crystallography. Finally, the presence of the soft X-ray microscope MISTRAL, intended for cryonano-tomography for biological applications, among which the determination of the molecular content in cells, will put the isolated macromolecules structures in a biological context. Together, these resources will provide mutual feedback and imprint an excellent, complete and very strong interdisciplinary character to ALBA in the Life Science domain.

4. Technical specifications

The objective of this section is to describe the technical details of the proposed microfocus beamline for macromolecular crystallography at ALBA. Schemes of the optical design and experiment set up are plotted in this section.

4.1 Photon Source: Undulator

Synchrotron beamlines designed to focus the beam down to the 1 micron range, all rely on photon sources having minimal size and divergence. This limits the choice to undulator insertion devices. Moreover, in a 3-GeV storage ring and under an energy requirement in the hard X-ray range, the undulator magnetic array must be in-vacuum set to reduce the magnetic gap between jaws.

Having established this basic choice, a variety of magnetic designs remain suited to fulfil the particular criteria derived from the scientific case.

The scientific case stablishes the priority of maximizing the flux around a wavelength of 1 Å, and in particular at the Se K-edge (0.979 A, 12.658 keV). This feature ensures both the possibility of solving the phase problem exploiting the anomalous and dispersive signal of Selenium incorporated through selenomethionine derivatization and the capability to measure high resolution data whenever crystals permit it. Moreover, beyond this basic requirement, the scientific case presses two more considerations:

- The beamline should be tuneable to cover all common K and L3 edges between 2.06 and 0.82 A (6 and 15 keV).
- The beamline design should not preclude the possibility to reach longer wavelengths. Even though the experiments and experimental configurations in the context of the proposed microfocus beamline are challenging, design in the beamline source and instrumentation should take into account that current development is pushing native phasing using the residual anomalous signal of naturally occurring low-Z elements (S, Cl, K, Ca) (Liu et al., 2014). The choice of keystone components will ponder implications in this direction. If possible, a

flexible layout will be favoured, ensuring necessary experimental optimization would take place at the more external layer.

An ideal undulator, able to optimize all these constraints simultaneously does not exist, and therefore a compromise must be found. In the present proposal we are favouring a robust and conservative approach for the hardware foundation, enhanced through choices selected to enhance performance in the above described aspects.

The favoured undulator, U20, exploits the seventh harmonic at the Se-K edge energy (0.979 A, 12.659 keV) at a minimum gap to deliver the highest possible flux. In the design of the photon source we have incorporated the estimation that the minimum gap of the undulator jaws once installed at the Alba storage ring is going to be set at 4.8 mm. This figure is based upon calculations of the Alba Accelerator division foreseeing to adopt the value quoted rather than the 5.7 mm gap currently set for the in-vacuum undulators in Alba. It is also assumed that the magnetic material is going to be Sm_5Co_{17}. The features of the designed U20 are listed in table 4.1

Electronbeamsize (FWHM)	Σ_x, Σ_y	311, 18	μm
Electronbeam divergence (FWHM)	Σ'_x, Σ'_y	114, 14	μrad
Electron beam energy	E	3	GeV
Magneticperiod	λ_U	2.04	cm
Number of periods	N	98	
Magnetic field	B_0	0,864	T
Undulator constant	K	1.645	
Undulator total power (@400 mA)	*P*	3.4	kW
Photon energy (first harmonic)	ε_1	1.781	keV

Table 4.1 Characteristics of the beam at the medium straight section of the Alba storage ring, and the proposed in-vacuum U20 undulator at the foreseen final minimum gap of 4.8 mm.

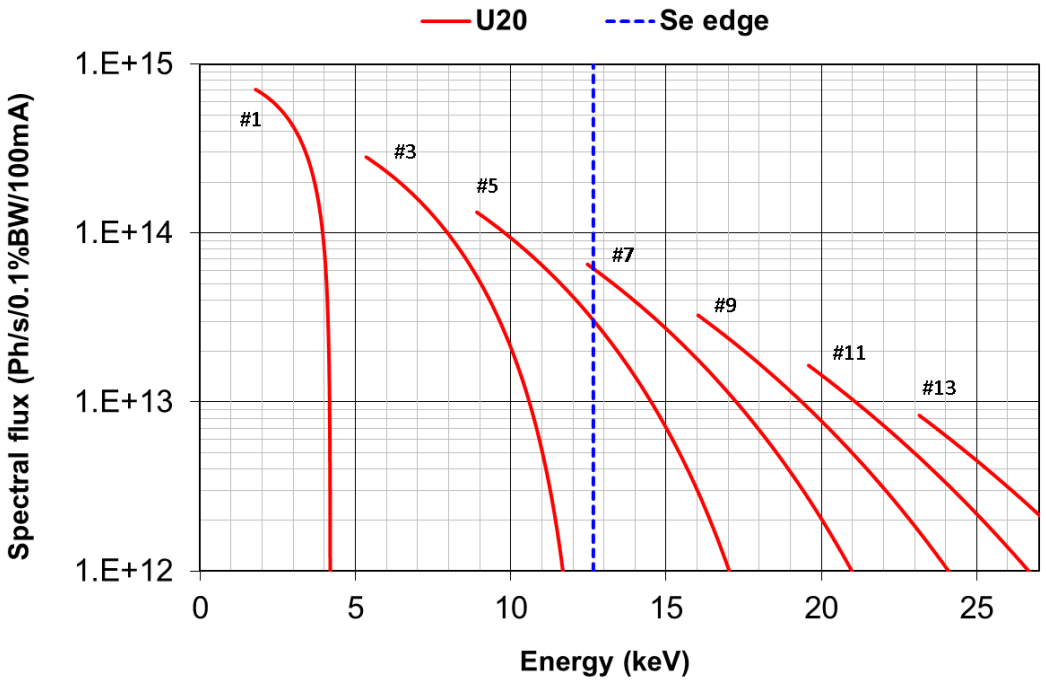


Figure 4.1. Spectral photon flux, in the central emission cone, delivered by undulator U20 as designed following parameters in table 3.1

The photon flux calculated for the U20 undulator exceeds $5 \cdot 10^{13}$ph/s/(0.1%spectral bandwidth) at the Se K-edge energy (figure 4.1), delivering, at a fixed current of 100 mA in the storage ring, an increase of circa 40% with respect to the current IVU21 installed at the XALOC beamline.

The undulator also offers full energy tuneability above 5.3 keV (2.32 A), which ensures the accessibility to all common K and L3 edges used in MX. Regarding the possibility of efficient performance at lower energies (longer wavelengths) the following complementary actions will be evaluated:

- **Use of other magnetic materials**. Pure permanent magnets have been assumed for the undulator. However, the use of a hybrid undulator, which produces a higher magnetic field, must be studied.
- **Reduce the minimum gap achievable**. The current estimation is that a gap of 4.8 mm can be safely adopted. A further reduction of 0.3 mm in the minimum gap will be pondered. This would allow shorter magnetic periods, which would increase the flux at the Se K-edge. Furthermore, the proposed gap reduction would bring about an increase of tuneability

of about 0.4 keV at 5.3 keV, which leads to a significant enhancement in the residual anomalous signal from light-Z elements.

- **Use of cryogenic undulator**: The use of cryogenically refrigerated undulators has been tested successfully in other synchrotrons. The effect of this technology would be similar to the reduction of the minimum gap. A careful evaluation of all the practical costs and constraints this choice would impose in exchange to delivering an evident flux increase needs to be fully assessed.
- **Increase the period**: Increasing the undulator magnetic period in a way that the Se K-edge is covered by the 9^{th} harmonic of the undulator at the minimum gap would increase the tuneability of the undulator at low energies. However, this choice would entail a flux reduction at the Se K-edge by 10%. Consequently, this option is not preferred due to the large number of projects demanding this energy.

Besides, other options such as aperiodic or hybrid undulators should be explored.

The focal spot produced by the U20 undulator at Se K-edge is 291x19 μm FWHM (HxV). Horizontally the beam dimension is determined by the storage ring emittance (currently 4.5 nm rad), whereas the vertical dimension is contributed mainly by the emission cone and the energy spread of the electron beam.

4.2 Front End

The front end, including bremsstrahlung stopper, four-blade beam position monitor used for diagnostic purposes and primary shutter will be similar to the system used at XALOC and other ALBA beam lines. Indeed, in order to minimise overhead and training costs and create synergies between the beamlines it will be advantageous to resort to the same systems and manufacturers implemented in XALOC or other phase III beamlines for as many standard components as possible.

A Beryllium window or a thin diamond window at the end of the front end should separate the vacuum of the front end and the storage ring from that of the beamline. The windows have to be as thin as possible to increase the beam transmission at low energies. To this purpose, the diameter of the window has

to be reduced to the minimum allowed by the beam dimensions and the alignment tolerances, and the front-end masks have to limit the maximum aperture of the beam delivered by the undulator. For instance, a 250-μm Be window of 6-mm diametertransmitting 76% of the photon flux at 4.5 keV (2.75 Å) can absorb a power of ca. 500 W. The diameter of the window can be even smaller to reduce the thickness and increase the beam transmission.

4.3 Beamline optics

White beam optical elements

The first element of the beamline optics is a beam conditioning unit, consisting of a pair of vertical and horizontal slits, a filter unit and possibly a white beam fluorescence screen. Any of these devices could also be placed at the front end as long as the control remains at the beamline.

Monochromator

The first critical optical element is the monochromator. Given the required energy range and the incoming power, the selection of the energy must be based on the diffraction provided by the (111) reflection of a polished Silicon crystal cryogenically cooled. The energy resolution of this reflection is $\Delta E/E \sim 1.4\ 10^{-4}$, which is suitable for MAD experiments while keeping a large integrating power and thus not restricting too much the output flux. A standard double-crystal monochromator (DCM) with fixed exit geometry (Suortti et al., 1995; 2000) can be used successfully for this type of beamline microfocus. The advantage of this instrument is to provide a monochromatic beam at a fixed height. However, the mechanic adjustment is complex, as several motors moving the two crystals are required to maintain the height of the beam fixed as energy changes.

The preferred solution is to use instead a cryogenically cooled channel-cut monochromator, also based on the Si(111) reflection. The mechanics are decidedly simpler in this type of monochromator while providing an output beam of high spectral and spatial stability. The channel-cut monochromator produces

a change upon energy in the height of the output beam. However, the effective beam excursion at the sample can be reduced by several means. The beam vertical excursion h due to the double bounce on the crystals is:

$$h = 2\,g\,\cos\theta$$

Where g is the gap between crystals and θ is the Bragg angle of the reflection used to select the energy. Our own experience at XALOC, in line with the outcome of other beamlines, has proven that this beam excursion is very reproducible and thus relatively easy to correct using the beamline control system. As can be seen from the equation above, the beam excursion increases at low Bragg angles, that is, at low energies, hampering the effective energy range covered by the beamline. If deemed necessary, this may be overcome by using a novel design consisting in a channel-cut monochromator with double gap between crystal surfaces (Figure 4.2). It has been calculated that a step of 0.4 mm between gaps reduces the beam excursion to around 0.7 mm between 4 keV and 20 keV. This excursion is lower than the excursion covered successfully by XALOC beamline. Another complementary solution to reduce the beam excursion would be to further reduce the gap g between crystals to 4 or 5 mm, if safety considerations concerning gas bremsstrahlung allow it.

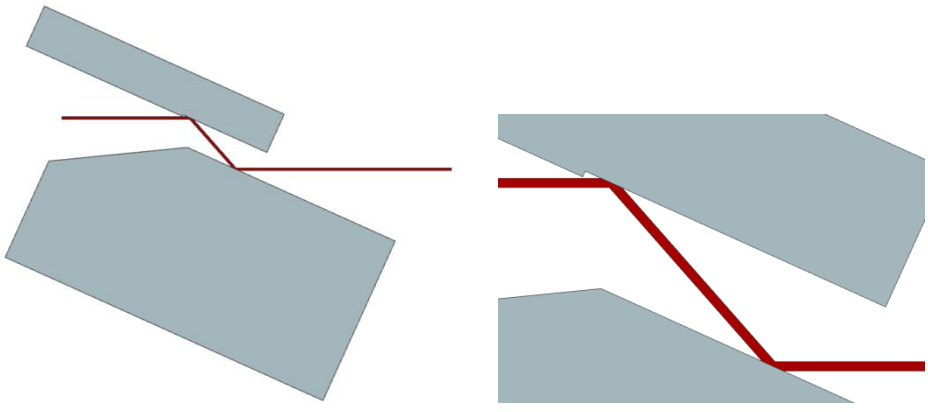


Figure 4.2. (left) Double-step channel cut. Assumed gaps are 6 mm (high energy end) and 6.4 mm (low energy-range). (right) Detail of the double gap. The shadowed energy range shadowed by the gap step can be tailored by the value and the position of the step.

Moreover, the extremely large demagnification in the dispersive direction of this microfocus beamline leads to a small excursion at the sample position, after the focusing optics. In the proposed beamline layout, where D=18.5 and g~6mm, at 12.661 keV the beam excursion is only 82 um between 4 and 20 keV.

Focusing optics

Beam focussing will be achieved in all probability by mechanically bent polished mirrors. Main advantages of this type of optics are: (a) focusing provided by mirrors is non-dispersive, that is, focusing properties do not change essentially within the targeted energy range, (b) effective suppression of undulator harmonics (c) vertical beam excursions can be easily covered by the typical acceptance of the mirror, (d) There is a consolidated know-how on this type of optics and their metrology at Alba, and (e) well proven and very stable system in the long term.

Nevertheless, other focusing systems, such as bimorph mirrors or multilayer mirrors, as well systems based upon compound refractive lenses, have been developed during the last years, and proved useful in similar beamlines in other synchrotrons (Diamond, Soleil, ESRF), and cannot be disregarded.

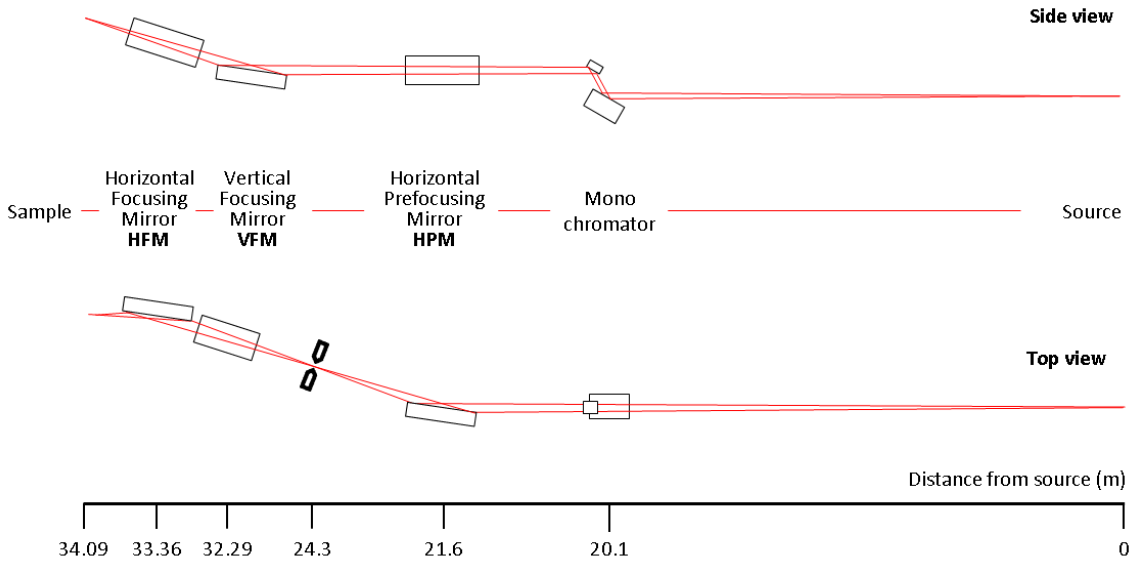


Figure 4.3. Beamline layout

The mirror surfaces will describe the figure of elliptical cylinders. The mirrors will focus the beam meridionally to allow variable focusing without changing the beam path, to suppress aberrations, and to decouple the horizontal and the vertical beam dimensions. The vertical dimension of the photon source is small enough to be reduced to the order of 1 μm at the sample position with only one focusing mirror. The source size is much larger horizontally, and two focusing mirrors are required to reduce the beam to micron range in this dimension. A horizontal slit placed at the focus of the first, prefocusing, mirror (HPM) is required to clean the beam in the horizontal direction. The resulting layout of the beamline is sketched in figure 4.3. Some relevant parameters of the mirrors are listed in table 4.2.

The optical surfaces of the mirrors should be as long as possible while keeping a low enough contribution of the slope errors to the beam size at the sample position. Optical lengths of 300 mm and 600 mm for the vertical and horizontally focusing mirrors have been shown to fulfil this requirement, and have been adopted in this technical review. Increased optical lengths can be chosen if the corresponding slope errors are proved to cope with this requirement. The incidence angle for all mirrors is assumed to be 4.5mrad.Also, several coating stripes of the mirrors are suggested (bare Si, Rh and Pt/Ir) to provide effective suppression of higher undulator harmonics. The resulting reflectivity curves are shown in Fig 4.4.

Horizontal Prefocusing Mirror	Mirror length	600	mm
	Position from source	21.6	m
	Demagnification factor	8	
Horizontal slit	Position from source	24.3	m
Vertical Focusing Mirror	Mirror length	300	mm
	Position from source	32.293	m
	Demagnification factor	8	
Horizontal Focusing Mirror	Mirror length	600	mm
	Position from source	33.362	m
	Demagnification factor	12.5	
Working distance	Distance between end of mirror and sample position	325	mm

Table 4.2. Optical parameters of the focusing optics

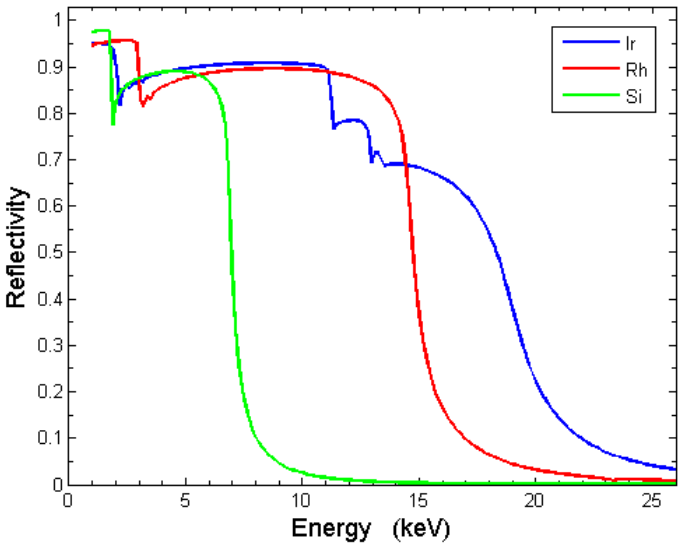


Figure 4.4. Reflectivity of the proposed mirror stripes at 4.5 mrad grazing incidence angle

Beam characteristics at sample position

The X-ray beam is conditioned by the beamline optics described above and propagates to the sample position with excellent characteristics for MX experiments requiring a beam size in the micron range. According to raytracing modelling (Nicolás et al., 2013), the uncut beam will be focused at sample position to a dimension of 2.6×1.1 μm FWHM (h×v). The beam size does not depend significantly upon energy. The calculation includes the reflectivity of the mirrors and their slope errors, which are assumed to be those measured for the BL13-XALOC beamline mirrors at Alba. The meridional RMS slope errors amount to 55 nrad and 85 nrad, for the vertically and horizontally focusing mirrors, respectively. Table 4.3 lists the main beam characteristics at the sample position.

Horizontal slit fully open	Beam size (FWHM)	2.6×1.1	μm×μm
	Flux at 100mA, 12.661 keV	$3\ 10^{12}$	ph/s
Horizontal slit Open 20 μm	Beam size (FWHM)	1.3×1.1	μm×μm
	Flux at 100mA, 12.661 keV	$1.3\ 10^{12}$	ph/s
	Flux density at 100 mA, 12.661 keV	$9.3\ 10^{11}$	ph/(sμm^2)
	Beam divergence(FWHM)	1.2×0.15	rad×mrad
	Energy resolution (ΔE/E)	$1.7\ 10^{-4}$	
	Overall horizontal demagnification factor	100	

Overall vertical demagnification factor	18

Table 4.3. Properties of the X-ray beam at the sample position.

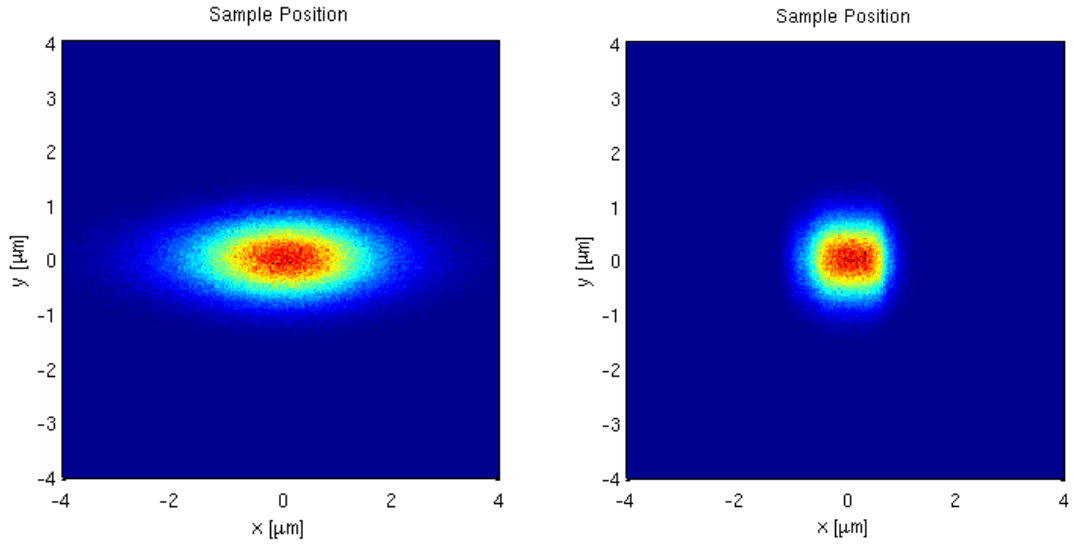


Figure 4.5. Beam spot at sample position modelled using raytracing calculations. (right) Spot at fully open horizontal slits (left) spot with the horizontal slit set at 20 μm opening gap

The ray tracing calculations show that, with the assumed slope errors, beam may effectively be defocused to 5×5 μm FWHM without introducing significant striations on the spot and preserving the flux. The defocusing of the mirrors implies moving the focal point of the horizontal and vertical focusing mirrors (HFM and VFM) by 13.5 and 1.4 mm, respectively (Figure 4.6).

Thermal stability is a key issue as it may compromise the positional stability of such a small beam. This implies the need to minimise temperature fluctuations in the environment (+/- 0.5 K should be feasible) in addition to insulating components such as motors with teflon disks or the mirror vacuum chambers by a perspex box. Continuous monitoring of the beam position within 1 μm and, ideally, an automatic feedback system to correct the optics if required should also contribute to tackle this issue (Sehr et al., 2004).

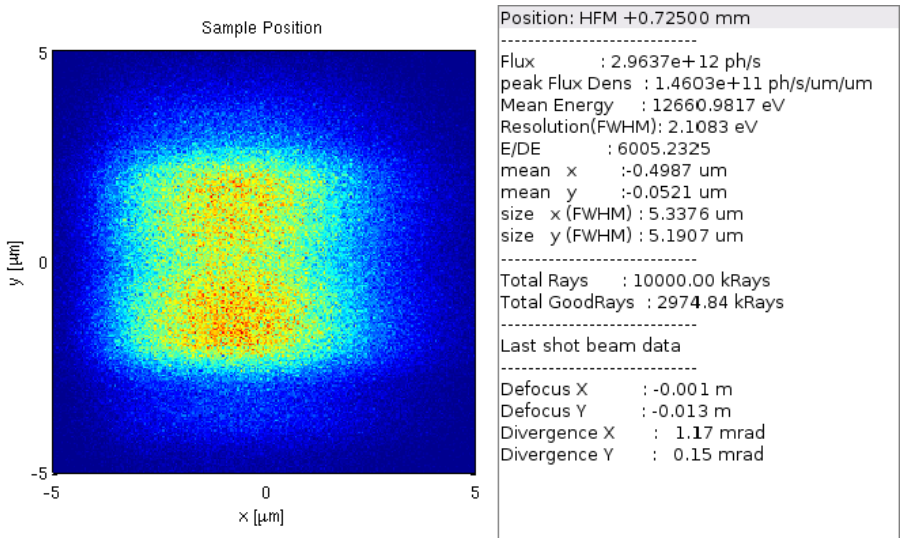


Figure 4.6. Defocused X-ray beam to deliver a spot size of 5×5 μm FWHM at sample position. The considered slope errors are taken from the measured slope errors of the XALOC beamline mirrors.

4.4 Experimental-station

4.4.1. **Hutch design**

The experimental station will be housed in a conventional hutch with sufficient space to accommodate all experimental components and to allow easy access of users and staff. It will include as well the pair of focusing mirrors in order to spare beam path length. The temperature regulation within ±0.5K and the humidity control is essential.

The experimental station consists of the exposure box, the goniometer, the detector and is completed by some auxiliary equipment.

4.4.2. **Beam conditioning elements**

The beam conditioning elements are part of the vacuum system and are located between the last mirror (HFM) and the goniometer holding the sample. This space is very reduced, ca. 300 mm between the end of the mirror and the sample position. Elements comprise a set of high precision slits for further beam cleaning, an X-ray beam position monitor and pin diode for diagnostic purposes, and possibly a fluorescence screen. Other elements, such as a set of filter and metal foils for calibration and a high-speed shutter (Owen et al., 2009;

Gembicky et al., 2005) might be included before the pair of focusing mirrors if deemed necessary due to space constraints.

4.4.3. **Diffractometer**

The requirements imposed to the diffractometer in a microfocus beamline are very pressing and strict due to the small dimension of the beam and the target crystals. However, current developments in single axis diffractometers have reached the extremely high accuracy level required, and sub-micrometric cylinders of confusion have been currently reached. In Europe, the new MD3 diffractometer developed by EMBL-Grenoble has been successfully commissioned at beamline P14 run by EMBL-Hamburg at Petra III, and is now available commercially. The omega axis is oriented vertically to reduce the gravity sag. Also, the new D3 diffractometer developed by the SLS (Fuchs et al., 2014) has recently shown excellent results. The omega axis, with a submicrometric cylinder of confusion, is oriented horizontally in this case. Both diffractometers are able to support and orient diffraction plates to perform in situ diffraction experiments. The first in-situ rastered data collections were made on the MD3 of P14, with batches of 5-20 micron-sized crystals of model proteins [www.embl.fr/research/unit/cipriani].Another successful project has been the horizontal goniometer of the P11 beamline at Petra III beamline in DESY (Meents et al., 2013). In America, the new FMX beamline under construction at the NSLS-II aims to a cylinder of confusion of only 0.1 μm (M. Fuchs, 2014). Huber is also commercializing an air bearing rotary table combined with a XY stage offering submicrometer cylinder of confusion (Huber EZ0675 XY).

There are also projects to reduce the sphere of confusion of multi-axis diffractometers, notably the SmarActSnarGon (formerly known as PRIGo, developed at the SLS (Waltersperger et al., 2012). Possibly some working space at the main axis goniometer axis has to be foreseen, should the evolution of the field in the forthcoming years render these devices compatible with the small beam at sample position in the proposed beamline.

4.4.4. **Sample viewing system**

Sample viewing and alignment is key to successfully perform MX experiments. As the dimensions of the beam and the crystal will be in the micrometre range, the sample camera needs to be a high-magnification video microscope. The sample camera should be placed on the beamline axis so that the crystal is correctly centred on the oscillation axis, whilst not introducing a significant path in air that could hamper the quality of the data taken at long wavelengths. In addition, the system needs to be coupled to an X-ray sensitive screen which will allow beam alignment at the sample position. This microscope can be either procured commercially (e.g. the OAV system by Arinax, Grenoble, France) or develop an in-house program profiting from the current know-how at Alba.

Complementary, a SONICC (second order nonlinear optical imaging of chiral crystals) system should be implemented for rapid protein crystal localization and centring (Kissick et al., 2013). This system has been recently implemented at GM/CA beamline at the APS with much success (Madden et al., 2013), and is already commercialized by Formulatrix (Bedford, MA). Other systems such as the Questar QM 100 telescope, based on UV-excited fluorescence imaging (Gofron et al., 2011) have to be also evaluated.

4.4.5 **Detector**

The general consensus is that pixel array detectors are currently the best X-ray detector for MX experiments, main reasons being the minimal readout times, high dynamic range and negligible background (Broennimann et al., 2006). Current generation of Dectris Pilatus3X detectors offers readout times down to 0.95 milliseconds, a counter depth of 2^{20} and virtually no intrinsic noise [www.dectris.com].

The fast readout time of this type of detector (for example, 100 Hz for the Pilatus3 X 6M) has a growing interest in MX, besides the obvious advantage of enabling the continuous mode data collection and removing any potential shutter errors. A new strategy for the collection of complete diffraction data close to the diffraction limit from micrometre-sized crystals using synchrotron radiation has been demonstrated (Gati et al., 2014).This method is based on the serial illumination of a cryogenically vitrified suspension of microcrystals mounted in a standard nylon loop, which is rotated “classically” during

exposure. The X-ray dose received by an exposed subvolume is chosen to fully exploit the crystal lifetime during the exposure time. This requires a combination of a high flux beam in a spot size in the one micrometre-range, as offered by this beamline, combined by a high frame rate as offered by the Pilatus3 X detectors. For example, the P14 beamline used by Gati et al. delivered a flux 1.2 10^{12}ph/s at 10 keV in a spot size of 4×5 μm, to crystals with average dimensions of 0.9×0.9×11 μm, exposed at a frame rate of 240 Hz (Figure 4.7).The conclusion from their experiment is that "reducing the beam size to 1×1 μm will reduce the background scattering 20-fold. Increasing the flux density of the X-ray beam will reduce the data-collection time, which is currently several hours for a single loop mounted drop of crystalline suspension".

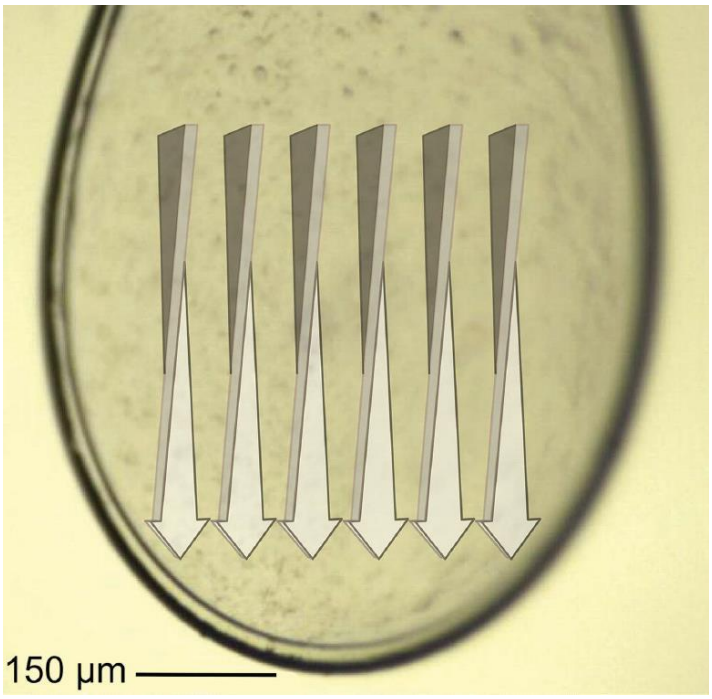


Figure 4.7. Schematic illustration of the serial helical line-scan approach using a standard cryogenic loop (From Gati et al., 2014).

The fast readout time of the detector also allows minimizing the radiation damage when collecting in-situ diffraction data at room temperature from crystallization plates. As shown in recent experiments at Diamond, there is a lag phase of few hundreds of millisecond in which the diffraction pattern does not show the exponential decay expected by radiation damage (Owen et al, 2014).The ability to rapidly collect diffraction data from room-temperature

crystals within this lag phase opens the possibility of significantly reducing the number of crystals required for room-temperature structure solution.

Moreover, the recent availability of Dectris Eiger detectors, with even higher framer rates (for example, 238 Hz for the Eiger X 9M) raises the possibility of fully exploiting these new data collection strategies. A careful analysis of the available state-of-the-art pixel array detectors is required at the moment of the procurement of this essential piece of equipment.

It has to be noted that the availability of a high frame rate detector demands considerable computer resources to be able to deal with the bulk of data generated and process them at a reasonable speed. The specifications of these resources should be selected among the high end limit of the available computer resources at the time of building the beamline, and based on previous experience on these detectors.

The detector will be mounted on a positioning table and will thus remain decoupled from other components to be flexible to position it without affecting the position of the diffractometer and the beam conditioning elements. Finally, a removable vacuum or helium-filled cone should be placed between the sample/beam stop and the detector when the beamline is set at an energy below 6 or 7 keV to reduce the background noise of the detector images produced by the photons scattered by the air.

4.4.6. **Automated sample changer**

An automated sample changer to increase the productivity of the beamline and mount/unmount reliably and reproducibly the crystals is mandatory. In addition, a sample changer is essential to ensure remote data collection, which is proven extremely efficient and successful at a number of synchrotrons including Diamond, ESRF, SSRL or ALS, among others.

A six-axis robot is preferred due to the flexibility to be coupled to virtually any setup, including cryogenic pins, capillars, and crystallization plates. A number of different systems are made at the sites such as DESY P11 beamline Automatic Sample Changer (Meents et al., 2013) or SAM (SSRL), or are currently available commercially such as CATS (Irelec, St. Martin d'Heres, France), ACTOR (Rigaku), among others. The commissioning of the automated sample changer is proven complex, but is not a fundamental issue for the beamline.

The automatic sample changer should be able to adapt to the ever changing sample holder (pin) and crystallization plate standards.

4.4.7. **Auxiliary equipment**

A cryocooling system based on liquid nitrogen is absolutely required to cool down the sample. A solid state X-ray detector is needed to collect the emission fluorescence spectrum required to choose the optimal wavelength for any MAD experiment. Spectroscopy measurements combining *in cristallo* UV-Vis or Raman spectroscopy with data collection would be of use for some experiments (Pearson et al., 2009; McGeehan et al., 2009; Katona et al., 2007; Vernede et al., 2006). Consequently, the installation of a photo spectrophotometer should be considered in case the tight packing of the sample environment instrumentation allowed it. Even though it is questionable whether enough signal for spectroscopy is to be measured on a microcrystal, there would be a strong case for monitoring radiation damage. Having to combine data from multiple crystals, knowing exactly how much to collect from each would be extremely efficient and difficult to establish by any other means as not every crystal behaves in the same way, even if they look identical. Open flow Helium cryostat has proven useful in some cases to lower radiation damage and reduce background (Meents et al., 2007; Moukhametzianov et al., 2008).

A large variety of relevant tools and instrumentation implemented at the beamline should be at the disposal for the user community to increase the success of the facility for macromolecular crystallography, as experienced successfully at BESSY (Mueller et al., 2012) or Diamond, among others. These techniques include Xe/Kr derivatization, crystal dehydration, crystal annealing and UV-RIP phasing. Although these techniques are used in a limited number of projects, they are instrumental when needed. A biological laboratory in the vicinity of the beamline (which can be shared with the existing life sciences beamlines) should also be available to user community.

4.5 Human resources

Experience shows that the amount of dedicated manpower to the beamline staff directly reflects on the productivity of the beamline. There should be a minimum of 4 scientific staff dedicated to the beamline and to research associated with it.

More scientific staff would be beneficial for a tailored user support and would increase the reactivity of the beamline to the changing needs of the scientific community. Mechanical, electrical, IT and control engineer support are required in sufficient amount to face the major challenges that a state-of-the-art microfocus beamline imposes. It is of particular importance to provide a very large amount of control engineer support to tackle the large and growing demand for automation, complex data collection strategies, beamline optical flexibility, big data management and data processing pipelines. Besides, support from mechanical and electrical technicians are required, especially at the construction and commissioning phases.

It is important to note that the beamline imperatively requires a continuous evolution to follow up the pace of the field, most particularly regarding end station instrumentation, beamline control tools and widgets, and data processing. The manpower required to face this evolution has to be appointed.

Finally, the microfocus beamline and the existing XALOC beamline will benefit from a strong synergic effect, as much of the control and instrumentation will be similar.

4.6 Estimated construction costs

In-vacuum Undulator U20	**650**
Front-end	300
Safety hutches (optics, experimental)	450
Personal Safety System	100
Cabling	120
Fluids	100
White beam attenuator	40
White beam fluorescence screen	40
Optics - monochromator	280
Optics – mono cryocooling	120
Optics – HPM, VFM and HFM mirrors	460
Optics - Horizontal secondary source slit	80
Optics - Beam apertures	100
Monochromatic beam diagnostics	100
Vacuum elements (pipes, bellows, ion pumps)	100
Vacuum electronics (gauges, controllers)	80
Vacuum windows	20
End station - Beam attenuator	10
End station - diagnostics	80
End station - beam apertures	20
End station - shutter	30
Diffractometer alignment table	70
Diffractometer	350
Sample viewing system	30
Automatic sample changer	300
Sample cryogenic system	40
Detector alignment table	60
Pixel Detector	1500
Vacuum cone	10
Fluorescence detector+MCA	20
Racks	25
Motor drivers	50
Electronic boards	100
Networking	25
Air conditioning	50
Temperature/Humidity conditioners and sensors	30
Tools	15
Control hutch	35
Lab equipment	20
Microscopes	10
Furniture	10
Contingency 10 %	603
TOTAL (kEUR)	**6633**

Acknowledgments

Dr.Ehmke Pohl, University of Durham UK

Dr. Peter Zwart, ALS, USA

Dr. Clemens Schulze-Briese, Beamline Scientist at SLS

Dr. Stefan Janssen, SLS User Office

Dr. Joanne McCarthy. Head, ESRF User Office

Dr. Gerlind Sulzenbacher, president of the ESRF User Organization.

Ms. Katherine Fletcher. Industrial and Commercial Unit (ICU), ESRF

Dr. Andy Thomson, SOLEIL

Dr. Timm Maier, ETH

Dr. Vicente Rubio, IBV

Dr. José Miguel Mancheño, IQFR-CSIC

Dr. Jordi Quintana, PCB

Red Temática en Radiación Sincrotrón, CSIC

Dr. Jesús Purroy, dirección Científica del PCB

Dr. Guillermo Montoya, CNIO

Abbreviations:

AFMB Architecture et Fonction des Macromolécules Biologiques dep. at CNRS

BAG, Block Allocation Group

ESRF, European Synchrotron Radiation Facility

DESY, Deutsches Elektronen S*y*nchrotron

ICU, Industrial and Commercial Unit of the ESRF

MX, Macromolecular Crystallography

PI, Principal Investigator

PX, Protein Crystallography

SLS, Swiss Light Source

REFERENCES

Axford, D., Owen, R.L., Aishima, J., Foadi, J., Morgan, A.W., Robinson, J.I., Nettleship, J.E., Owens, R.J., Moraes, I., Fry, E.E.*, et al.* (2012). In situ macromolecular crystallography using microbeams. Acta Crystallogr D Biol Crystallogr *68*, 592-600.

Bingel-Erlenmeyer, R., Olieric, V., Grimshaw, J.P.A., Gabadinho, J., Wang, X., Ebner, S.G., Isenegger, A., Schneider, R., Schneider, J., Glettig, W.*, et al.* (2011). SLS Crystallization Platform at Beamline X06DA—A Fully Automated Pipeline Enabling in Situ X-ray Diffraction Screening. Cryst. Growth Des. *11*, 916-923.

Blow, N. (2008). Structural genomics: inside a protein structure initiative center. Nat Methods *5*, 203-207.

Bowler, M.W., Guijarro, M., Petitdemange, S., Baker, I., Svensson, O., Burghammer, M., Mueller-Dieckmann, C., Gordon, E.J., Flot, D., McSweeney, S.M., and Leonard, G.A. (2010). Diffraction cartography: applying microbeams to macromolecular crystallography sample evaluation and data collection. Acta Crystallogr D Biol Crystallogr *66*, 855-864.

Broennimann Ch, Eikenberry EF, Henrich B, Horisberger R, Huelsen G, Pohl E, Schmitt B, Schulze-Briese C, Suzuki M, Tomizaki T, Toyokawa H, Wagner A. (2006) The PILATUS 1M detector. J Synchrotron Radiat. 13:120-30

Carpenter, E.P., Beis, K., Cameron, A.D., and Iwata, S. (2008). Overcoming the challenges of membrane protein crystallography. Current opinion in structural biology *18*, 581-586.

Coll, M., Seidman, J.G., and Muller, C.W. (2002). Structure of the DNA-bound T-box domain of human TBX3, a transcription factor responsible for ulnar-mammary syndrome. Structure *10*, 343-356.

Coulibaly, F., Chiu, E., Ikeda, K., Gutmann, S., Haebel, P.W., Schulze-Briese, C., Mori, H., and Metcalf, P. (2007). The molecular organization of cypovirus polyhedra. Nature *446*, 97-101.

Cusack, S., Belrhali, H., Bram, A., Burghammer, M., Perrakis, A., and Riekel, C. (1998). Small is beautiful: protein micro-crystallography. Nat Struct Biol *5 Suppl*, 634-637.

Cherezov, V., Rosenbaum, D.M., Hanson, M.A., Rasmussen, S.G., Thian, F.S., Kobilka, T.S., Choi, H.J., Kuhn, P., Weis, W.I., Kobilka, B.K., and Stevens, R.C. (2007). High-resolution crystal structure of an engineered human beta2-adrenergic G protein-coupled receptor. Science *318*, 1258-1265.

De Colibus, L., Wang, X., Spyrou, J.A., Kelly, J., Ren, J., Grimes, J., Puerstinger, G., Stonehouse, N., Walter, T.S., Hu, Z.*, et al.* (2014). More-powerful virus inhibitors from structure-based analysis of HEV71 capsid-binding molecules. Nature structural & molecular biology *21*, 282-288.

Dimasi, N., Flot, D., Dupeux, F., and Marquez, J.A. (2007). Expression, crystallization and X-ray data collection from microcrystals of the extracellular domain of the human inhibitory receptor expressed on myeloid cells IREM-1. Acta Crystallogr Sect F Struct Biol Cryst Commun *63*, 204-208.

Ereno-Orbea, J., Majtan, T., Oyenarte, I., Kraus, J.P., and Martinez-Cruz, L.A. (2013). Structural basis of regulation and oligomerization of human cystathionine beta-synthase, the central enzyme of transsulfuration. Proc Natl Acad Sci U S A *110*, E3790-3799.

Fang, Y., Jayaram, H., Shane, T., Kolmakova-Partensky, L., Wu, F., Williams, C., Xiong, Y., and Miller, C. (2009). Structure of a prokaryotic virtual proton pump at 3.2 A resolution. Nature *460*, 1040-1043.

Fernandez-Tornero, C., Moreno-Morcillo, M., Rashid, U.J., Taylor, N.M., Ruiz, F.M., Gruene, T., Legrand, P., Steuerwald, U., and Muller, C.W. (2013). Crystal structure of the 14-subunit RNA polymerase I. Nature *502*, 644-649.

Flot, D., Mairs, T., Giraud, T., Guijarro, M., Lesourd, M., Rey, V., van Brussel, D., Morawe, C., Borel, C., Hignette, O.*, et al.* (2010). The ID23-2 structural biology microfocus beamline at the ESRF. Journal of synchrotron radiation *17*, 107-118.

Fotinou, C., Emsley, P., Black, I., Ando, H., Ishida, H., Kiso, M., Sinha, K.A., Fairweather, N.F., and Isaacs, N.W. (2001). The crystal structure of tetanus toxin Hc fragment complexed with a synthetic GT1b analogue suggests cross-linking between ganglioside receptors and the toxin. The Journal of biological chemistry *276*, 32274-32281.

Fuchs, M.R., Pradervand, C., Thominet, V., Schneider, R., Panepucci, E., Grunder, M., Gabadinho, J., Dworkowski, F.S.N., Tomizaki, T., Schneider, J. Mayer, A., Curtin, A., Olieric, V., Frommherz, U., Kotrle, G., Welte, J., Wang, X., Maag, S. Schulze-Briese, C. and Wang. M. D3, the new diffractometer for the macromolecularcrystallography beamlines of the Swiss Light Source.J. Synchrotron Rad. (2014). **21**:340–351.

Fuchs, M., Sweet, R., Berman, L., Bhogadi, D., Hendrickson, W., Chubar, O., Yang, L., McSweeney S. and Schneider, D. NSLS-II MX beamlines FMX for micro-crystallography & AMX for highly automated MX. ActaCryst. (2014). A**70**, C1733.

Gao, X., Lu, F., Zhou, L., Dang, S., Sun, L., Li, X., Wang, J., and Shi, Y. (2009). Structure and mechanism of an amino acid antiporter. Science *324*, 1565-1568.

Gao, X., Zhou, L., Jiao, X., Lu, F., Yan, C., Zeng, X., Wang, J., and Shi, Y. (2010). Mechanism of substrate recognition and transport by an amino acid antiporter. Nature *463*, 828-832.

Garriga, D., Vives-Adrian, L., Buxaderas, M., Ferreira-da-Silva, F., Almeida, B., Macedo-Ribeiro, S., Pereira, P.J., and Verdaguer, N. (2011). Cloning, purification and preliminary crystallographic studies of the 2AB protein from hepatitis A virus. Acta Crystallogr Sect F Struct Biol Cryst Commun *67*, 1224-1227.

Gati, C., Bourenkov, G., Klinge, M., Rehders, D., Stellato, F., Oberthür, D., Yefanov, O., Sommer, B.P., Mogk, S., Duszenko, M., Betzel, C., Schneider, T.R., Chapmanand, H.N. and Redecke, L. IUCrJ (2014).**1**:87–94.

Gembicky M, Oss D, Fuchs R, Coppens P. (2005) A fast mechanical shutter for submicrosecond time-resolved synchrotron experiments. J Synchrotron Radiat. 12:665-669

Gofron K.J. and Duke. N.E.C. Nucl. Instr. and Meth. A (2011), **649**:216-218.

Harms, J., Schluenzen, F., Zarivach, R., Bashan, A., Gat, S., Agmon, I., Bartels, H., Franceschi, F., and Yonath, A. (2001). High resolution structure of the large ribosomal subunit from a mesophilic eubacterium. Cell *107*, 679-688.

Hsia, K.C., Stavropoulos, P., Blobel, G., and Hoelz, A. (2007). Architecture of a coat for the nuclear pore membrane. Cell *131*, 1313-1326.

Jacquamet, L., Ohana, J., Joly, J., Borel, F., Pirocchi, M., Charrault, P., Bertoni, A., Israel-Gouy, P., Carpentier, P., Kozielski, F.*, et al.* (2004). Automated analysis of vapor diffusion crystallization drops with an X-ray beam. Structure *12*, 1219-1225.

Katona G, Carpentier P, Nivière V, Amara P, Adam V, Ohana J, Tsanov N, Bourgeois D. (2007). Raman-assisted crystallography reveals end-on peroxide intermediates in a nonheme iron enzyme. Science 316:449-453.

Kettenberger, H., Armache, K.J., and Cramer, P. (2003). Architecture of the RNA polymerase II-TFIIS complex and implications for mRNA cleavage. Cell *114*, 347-357.

Kissick, D.J., Becker, M., Mulichak, A.M., Cherezov, V., Ginell, S.L., Battailed, K.P., Keefe, L.J., Fishetti R.F. and Simpson, G.J. ActaCryst. (2013) D**69**:843-851

Kowalczyk, L., Ratera, M., Paladino, A., Bartoccioni, P., Errasti-Murugarren, E., Valencia, E., Portella, G., Bial, S., Zorzano, A., Fita, I.*, et al.* (2011). Molecular basis of substrate-induced permeation by an amino acid antiporter. Proc Natl Acad Sci U S A *108*, 3935-3940.

Kuhn, C.D., Geiger, S.R., Baumli, S., Gartmann, M., Gerber, J., Jennebach, S., Mielke, T., Tschochner, H., Beckmann, R., and Cramer, P. (2007). Functional architecture of RNA polymerase I. Cell *131*, 1260-1272.

le Maire, A., Gelin, M., Pochet, S., Hoh, F., Pirocchi, M., Guichou, J.F., Ferrer, J.L., and Labesse, G. (2011). In-plate protein crystallization, in situ ligand soaking and X-ray diffraction. Acta Crystallogr D Biol Crystallogr *67*, 747-755.

Lebon, G., Warne, T., Edwards, P.C., Bennett, K., Langmead, C.J., Leslie, A.G., and Tate, C.G. (2011). Agonist-bound adenosine A2A receptor structures reveal common features of GPCR activation. Nature *474*, 521-525.

Liu, Q., Greimann, J.C., and Lima, C.D. (2006). Reconstitution, activities, and structure of the eukaryotic RNA exosome. Cell *127*, 1223-1237.

Liu, Q., Guo, Y., Chang, Y., Cai, Z., Assur, F., Mancia, M.I. Greened and W.A.Hendrickson. Multi-crystal native SAD analysis at 6 keV.ActaCryst.(2014) D**70**, 2544–2557.

Madden, J.T., Toth, S.J., Dettmar, C.M., Newman, J.A., Oglesbee, R.A., Hedderich, H.G., Everly, R.M., Becker, M., Ronau, J.A., Cherezov, V., Morrow, M.E., Xu, S., Ferguson, D., Makarov, O., Das, C., Fishetti R.F. and Simpson. G.J. J. Synchrotron Rad. (2013), **20**:531-540

McGeehan J, Ravelli RB, Murray JW, Owen RL, Cipriani F, McSweeney S, Weik M, Garman EF. (2009). Colouring cryo-cooled crystals: online microspectrophotometry.J Synchrotron Radiat. 2009 16:163-172.

Meents, A., Reime, B., Stuebe, N., Fischer, P., Warmer, M., Goeries, D., Roever, J., Meyer, J., Fischer, J., Burkhardt, A., Vartiainen, I., Karvinen, P., David. C. Development of an in-vacuum x-ray microscope with cryogenic sample cooling for beamline P11 at PETRA III Proceedings of SPIE 8851, 88510K (2013).

Meents A, Wagner A, Schneider R, Pradervand C, Pohl E, Schulze-Briese C. (2007). Reduction of X-ray-induced radiation damage of macromolecular crystals by data collection at 15 K: a systematic study. Acta Crystallogr D63:302-309.

Moukhametzianov R, Burghammer M, Edwards PC, Petitdemange S, Popov D, Fransen M, McMullan G, Schertler G F X, Riekel C. (2008). Protein crystallography with a micrometre-sized synchrotron-radiation beam. Acta Crystallogr. D64:158-166.

Mueller, U., Darowski, N., Fuchs, M. R., Förster, R., Hellmig, M., Paithankar, K. S., Pühringer, S., Steffien, M., Zocher G. and Weiss, M. S. Facilities for macromolecular crystallography at the Helmholtz-Zentrum Berlin.J. Synchrotron Rad. (2012). **19**, 442-449.

Nave, C., and Hill, M.A. (2005). Will reduced radiation damage occur with very small crystals? Journal of synchrotron radiation *12*, 299-303.

Nicolas, J., Barla, A., Juanhuix. J. The Alba ray tracing code: ART. Proc. SPIE 8848, 88480Z.

Owen, R.L., Axford, D., Nettleship, J.E., Owens, R.J., Robinson, J.I., Morgan, A.W., Dore, A.S., Lebon, G., Tate, C.G., Fry, E.E.*, et al.* (2012). Outrunning free radicals in room-temperature macromolecular crystallography. Acta Crystallogr D Biol Crystallogr *68*, 810-818.

Owen RL, Holton JM, Schulze-Briese C, Garman EF. (2009) Determination of X-ray flux using silicon pin diodes. J Synchrotron Radiat. 16:143-151.

Owen, R.L., Paterson, N., Axford, D., Aishima, J., Schulze-Briese, C., Ren, J., Fry, E.E., Stuart, D.I., and Evans, G. (2014). Exploiting fast detectors to enter a new dimension in room-temperature crystallography. Acta Crystallogr D Biol Crystallogr *70*, 1248-1256.

Palczewski, K., Kumasaka, T., Hori, T., Behnke, C.A., Motoshima, H., Fox, B.A., Le Trong, I., Teller, D.C., Okada, T., Stenkamp, R.E*., et al.* (2000). Crystal structure of rhodopsin: A G protein-coupled receptor. Science *289*, 739-745.

Pearson AR, Owen RL. (2009) Combining X-ray crystallography and single-crystal spectroscopy to probe enzyme mechanisms. Biochem Soc Trans. 37:378-381.

Pohl, E., Gonzalez, A., Hermes, C., and van Silfhout, R.G. (2001). Overview of the tunable beamlines for protein crystallography at the EMBL Hamburg Outstation; an analysis of current and future usage and developments. Journal of synchrotron radiation *8*, 1113-1120.

Querol-Audi, J., Casanas, A., Uson, I., Luque, D., Caston, J.R., Fita, I., and Verdaguer, N. (2009). The mechanism of vault opening from the high resolution structure of the N-terminal repeats of MVP. EMBO J *28*, 3450-3457.

Rasmussen, S.G., Choi, H.J., Rosenbaum, D.M., Kobilka, T.S., Thian, F.S., Edwards, P.C., Burghammer, M., Ratnala, V.R., Sanishvili, R., Fischetti, R.F*., et al.* (2007). Crystal structure of the human beta2 adrenergic G-protein-coupled receptor. Nature *450*, 383-387.

Renault, L., Hanzal-Bayer, M., and Hillig, R.C. (2001). Coexpression, copurification, crystallization and preliminary X-ray analysis of a complex of ARL2-GTP and PDE delta. Acta Crystallogr D Biol Crystallogr *57*, 1167-1170.

Rubio-Cosials, A., Sidow, J.F., Jimenez-Menendez, N., Fernandez-Millan, P., Montoya, J., Jacobs, H.T., Coll, M., Bernado, P., and Sola, M. (2011). Human mitochondrial transcription factor A induces a U-turn structure in the light strand promoter. Nature structural & molecular biology *18*, 1281-1289.

Sanishvili, R., Nagarajan, V., Yoder, D., Becker, M., Xu, S., Corcoran, S., Akey, D.L., Smith, J.L., and Fischetti, R.F. (2008). A 7mum mini-beam improves diffraction data from small or imperfect crystals of macromolecules. Acta Crystallogr D Biol Crystallogr *64*, 425-435.

Sawaya, M.R., Sambashivan, S., Nelson, R., Ivanova, M.I., Sievers, S.A., Apostol, M.I., Thompson, M.J., Balbirnie, M., Wiltzius, J.J., McFarlane, H.T*., et al.* (2007). Atomic structures of amyloid cross-beta spines reveal varied steric zippers. Nature *447*, 453-457.

Schluenzen, F., Tocilj, A., Zarivach, R., Harms, J., Gluehmann, M., Janell, D., Bashan, A., Bartels, H., Agmon, I., Franceschi, F., and Yonath, A. (2000). Structure of functionally activated small ribosomal subunit at 3.3 angstroms resolution. Cell *102*, 615-623.

Sehr, H., Schulze-Briese, C., Pradervand, C., Schift, H., Gobrecht, J. *A CVD-diamond based beam position monitor for synchrotron radiation*, Technical Digest of Eurosensors XVIII, 212-215 (2004).

Service, R.F. (2008). Structural biology. Protein structure initiative: phase 3 or phase out. Science *319*, 1610-1613.

Suortti P, Schulze C. (1995) Fixed-exit monochromators for high-energy synchrotron radiation. J Synchrotron Radiat. 2:6-12.

Suortti P, Fiedler S, Bravin A, Brochard T, Mattenet M, Renier M, Spanne P, Thomlinson W, Charvet AM, Elleaume H, Schulze-Briese C, Thompson AC. (2000). Fixed-exit monochromator

for computed tomography with synchrotron radiation at energies 18-90 keV. J Synchrotron Radiat. 7:340-347

Tanaka, H., Kato, K., Yamashita, E., Sumizawa, T., Zhou, Y., Yao, M., Iwasaki, K., Yoshimura, M., and Tsukihara, T. (2009). The structure of rat liver vault at 3.5 angstrom resolution. Science *323*, 384-388.

Trillo-Muyo, S., Martínez-Rodríguez, S., Arolas, J.L., and Gomis-Rüth, F.X. (2013). Mechanism of action of a Janus-faced single-domain protein inhibitor simultaneously targeting two peptidase classes. Chemical Science *4*, 791-797.

Vernede X, Lavault B, Ohana J, Nurizzo D, Joly J, Jacquamet L, Felisaz F, Cipriani F, Bourgeois D. (2006). UV laser-excited fluorescence as a tool for the visualization of protein crystals mounted in loops. Acta Crystallogr D62:253-261.

Waltersperger, S., Olieric, V., Salathe, M., Pradervand, C., Panepucci, E., Glettig, W., Schulze-Briese, C., Wang. M. PRIGo: a novel multi-axis goniometer for macromolecular crystallography at the Swiss Light. Synchrotron Radiation Instrumentation Conference, Lyon, France.

Warne, T., Serrano-Vega, M.J., Baker, J.G., Moukhametzianov, R., Edwards, P.C., Henderson, R., Leslie, A.G., Tate, C.G., and Schertler, G.F. (2008). Structure of a beta1-adrenergic G-protein-coupled receptor. Nature *454*, 486-491.

Westermark, P. (2005). Aspects on human amyloid forms and their fibril polypeptides. FEBS J *272*, 5942-5949.

Westermark, P., Benson, M.D., Buxbaum, J.N., Cohen, A.S., Frangione, B., Ikeda, S., Masters, C.L., Merlini, G., Saraiva, M.J., and Sipe, J.D. (2005). Amyloid: toward terminology clarification. Report from the Nomenclature Committee of the International Society of Amyloidosis. Amyloid *12*, 1-4.

Xiao, B., Spencer, J., Clements, A., Ali-Khan, N., Mittnacht, S., Broceno, C., Burghammer, M., Perrakis, A., Marmorstein, R., and Gamblin, S.J. (2003). Crystal structure of the retinoblastoma tumor suppressor protein bound to E2F and the molecular basis of its regulation. Proc Natl Acad Sci U S A *100*, 2363-2368.

Annex 1

List of Principal Investigators

Principal Investigator	Disease	Action against disease
1. Nicola G. A. Abrescia (CIC bioGUNE)	Pathogenic viruses in humans and animals	X-ray structural studies of virus particles and viral proteins
2. Joan Aymami (UPC)	Cancer Malaria	Structural characterization of complexes between DNA with 100% AT and drugs, peptides and proteins
3. Ester Boix (UAB)	Human RNAses	Innate inmmunity
4. Jeronimo Bravo IBV–CSIC	Chronic lymphocytic leukemia and breast cancer	Structurally driven design of proapoptotic peptides
5. Elena Cabezon (Univ. Cantabria)	Antibiotic resistance *Brucellosis pneumoniae*	Molecular and evolutionary mechanism in molecular motors of the DNA and protein translocation.
6. Ana Cámara (Univ. Almería)	Breast cancer, leukemia, AIDS, Ebola, muscular distrophy, Alzheimer's disease.	structure-based design of antitumoral and antiviral drugs
7. Lourdes Campos (UPC)	Malaria Cancer	Molecular motors. Structural characterization of complexes between DNA , drugs, peptides and proteins
8. José Mª Casasnovas (CNB)	Coronavirus, measles virus receptor-binding	structure-based design of antiviral drugs
9. Miquel Coll (IBMB-CSIC)	-Human herpesvirus-derived diseases -pathogenic RNA viruses, -Cancer by attacking specific DNA structures or sequences.	-Design of drugs against proteins responsible for DNA packaging into the capsid - Design of drugs against replication enzymes and ancillary proteins - novel DNA-binding drugs against specific DNA sequences or unique DNA structures
10. Eva Estébanez (IBUB)	Prostate and breast cancers, androgen insensitivity syndromes and the rare neurodegenerative Kennedy´s disease.	structure-based drug design of novel antiandrogens and allosteric modulators to control NR function in disease.
11. Ignacio Fita (IBMB-CSIC)	- The Functional and Structural Study of Mycoplasmas Terminal Organelle - The Structure and Interactions of Peroxisomal Proteins	- Study of macromolecular aggregates and membrane-associated or integral membrane proteins - oxidative-stress processes and pathologies.
12. Carlos Fernandez-Tornero (CIB)	Cancer	RNA polymerase

13. Paola Fucini (CIC bioGUNE)	Bacterial infections (including respiratory infections, metritis, and acute mastitis in cattle; mastitis in sheep and goats; enteritis, pneumonia, erysipelas, and infectious arthritis in swine)	- Structure-based design of novel (tylosin-related) antibiotic derivatives that inhibit bacterial protein synthesis. - Hygromycin A Elucidating the mode of action of an antibiotic (hygromycin A) that inhibits peptide bond formation on the bacterial ribosome.
14. Pablo Fuentes Prior (HSPSC)	- thrombosis - Spinal muscular atrophy, SMA - Parkinson's disease	-Design of novel thrombin inhibitors (potential antithrombotic drugs) -Design of compounds to boost inclusion of exon 7 in SMN2 transcripts - Structural characterization of PINK1 and design of compounds that enhance its activity
15. J. A. Gavira (LEC)	Bacterial diseases	Antibiotic resistance studies
16. F. Xavier Gomis-Rüth (IBMB-CSIC)	- Bacterial infections that are the causing agents of respiratory tract and skin infections, and infections of the gums - several types of cancer, cardiovascular diseases and neurodegenerative disorders	- Study bacterial infections via the analysis of virulence or antibiotic resistance factors, such as *S. aureus and P. gingivalis* - design of new drugs against proteins involved in different types of cancer, cardiovascular diseases and neurodegenerative disorders.
17. Beatriz González Pérez (IQFR)	Cancer and apoptosis	- discovery of dual inhibitors of tyrosine and phosphoinositide kinases
18. Diego M. Guérin Aguilar (U. País Vasco/EHU)	American and African Trypanosomiasis; Leishmaniasis, Bovine paratuberculosis, Porcine and human Cysticercosis;	- X-tal resolution of antigens and VLP-bases vaccines
19. Marcelo E. Guerín (CSIC- U. País Vasco/EHU)	Host-pathogen interactions in bacterial diseases - *mycobacterium tuberculosis* - *Staphylococcus aureus*	- Glycosyltransferases function, structural and mechanistic properties of glycosyltransferases with a special emphasis in the study of integral and peripheral membrane associated enzymes.
20. Juan A. Hermoso (IQFR-CSIC)	*Streptococcus pneumoniae* infection - Antibiotics Resistance in MRSA (Methicillin resistant *Staphylococcus aureus*) -Antibiotics Resistance in *Pseudomonas aeruginosa*	-Design of new antibiotics targeting topoisomerase I and -Search of new pharmaceutical targets in the pneumococcal surface proteins -Design of new drugs against MRSA -Study of the critical proteins responsible of antibiotics resistance
21. Aitor Hierro (CicBIOGUNE, Leioa)	Alzheimer's disease; *Legionella pneumophila* infection	Design of pharmacological chaperones for protein-protein stabilization; Design of cyclic peptide inhibitors to avoid pathogen proliferation through competitive binding.

22. Ramón Hurtado-Guerrero (BIFI)	Alzheimer's disease; cancer; diabetes.	Structural enzymology of carbohydrate-active enzymes involved in human diseases; Development of inhibitors to develop new treatments
23. Lourdes Infantes (IQFR)	Bacterial diseases	Structural studies in *Pseudomonas aeruginosa* proteins
24. Daniel Lietha (CNIO)	- Cancer	-Structure-based discovery of FAK inhibitors, a central kinase in the integration of cellular growth and adhesion signals.
25. Hartmut Lücke (U. País Vasco/EHU)	- Bacterial and parasite diseases, *Helicobacter pylori*, *Plasmodium falciparum*,*Tritrichomonas foetus*	- Structure-function research of integral membrane proteins
26. Antonio Llamas (USC)	Bacterial diseases	- antibiotic peptides - enzyme-inhibitor complexes from pathogenic bacteria
27. Alberto Marina (IBV– CSIC)	Bacterial infectious diseases	- structure-based drug design of novel antibiotics. Structural characterization of new targets.
28. Luis Alfonso Martínez de la Cruz (CIC bioGUNE)	Homocystinuria	- structure-based drug design of activators/inhibitor of Cystathionine beta-synthase activity.
29. Martin Martinez Ripoll (IQFR)	Streptococcus pneumoniae infection - Antibiotics Resistance in MRSA (Methicillin resistant *Staphylococcus aureus*) -Antibiotics Resistance in *Pseudomonas aeruginosa*	-Design of new antibiotics targeting topoisomerase I and -Search of new pharmaceutical targets in the pneumococcal surface proteins -Design of new drugs against MRSA -Study of the critical proteins responsible of antibiotics resistance
30. Manuel Palacín (IRB)	- Aminoacid heteromeric transporters, LAT1 y xCT, involved in cancer. - Complete eukaryotic HAT transporters involved in aminoaciduria.	- Identify inhibitors of LAT1 and xCT transporters. - Discern the molecular basis to develop modifiers of the activity of aminociaduria's transporters
31. Jose María de Pereda (IC)	Epidermolysis bullosa	- Structural studies on molecular recognition mechanisms responsible for cell adhesion and signal transduction events.
32. Mark van Raaij (CNB, Madrid)	Infective diseases caused by M tuberculosis, H pylori and others	- design of inhibitors for dehydroquinase
33. Santiago Ramón Maiqués (CNIO)	Cancer	-Understanding molecular mechanism underlying pyrimidine synthesis and cellular proliferation
34. David Reverter (UAB)	Transthyretin amiloydoses - ATTR (peripheral neuropathy and	Drug design improvement against transthyretin aggregation in Transthyretin

	cardiomyopathy).	amiloydoses (ATTR).
35. Antonio Romero Garrido (CIB)	Neuroblastoma, erythroleukemia and colon adenocarcinoma	Descubrimiento y validación de dianas terapéuticas
36. Adriana Rojas Cardona (CIC bioGUNE)	Liver and colon cancer	structure based drug design of compounds that regulate the cellular growth in hepatoma and colon cancer.
37. Vicente Rubio (IBV–CSIC)	Urea cycle diseases and allied disorders	Improving genetic counselling on structural bases; structure-function and genotype-phenotype correllations for different mutations; and pharmacological chaperone discovery to treat inborn errors of the urea cycle.
38. Julia Sanz (IQFR-CSIC)	Aspartic proteinases	hypertension (renin), gastric ulcer disease (pepsin), muscular dystrophy and neoplastic diseases (cathepsins D and E), human immunodeficiency virus (HIV), tissue invasion and virulence of fungal pathogen
39. Nuria Saperas (UPC)	Cancer, sepsis, arthritis	- Protein-DNA interactions, chromatin remodelling (especially sperm chromatin remodelling). - DNA remodelling associated proteins: structure and function.
40. Maria Solà (IBMB-CSIC)	mitochondrialDNA regulation–related diseases	Structure determination of mtDNA lack of transcription and replication function
41. Juan Antonio Subirana (UPC)	Cancer	DNA interactions by X-ray crystallography.
42. Lourdes Urpi (UPC)	Cancer	DNA interactions by X-ray crystallography
43. Cristina Vega (CIB)	Bacterial diseases	Host-Pathogen interactions; Production of therapeutic molecules
44. Núria Verdaguer (IBMB-CSIC)	Viral diseases	Drug design against emerging and re-emerging diseases
Principal Investigator	Other challenges	Action against challenge
45. Armando Albert (IQFR-CSIC)	drought and salinity constrain agricultural productivity	Structural studies of multiprotein complexes from plant protein abscisic acid (ABA) receptors PYR family, the interacting protein phosphatase type 2C (PP2C) and a group of protein kinases (SnRK2 and SnRK3)

46. Jose M. Mancheño (IQFR-CSIC)	Protein lipid interaction Structure-function relationships	Structural studies of the catabolism of aromatic compounds by microorganisms
47. Gabriel Moncalian (UC)	Biotechnological application of the relaxases. Nanotechnology. Biodiesel production. Omega-3 production	Structural studies in proteins implied in the production of high added value goods. Drugs administration
48. Isabel Usón (IBMB-CSIC; ICREA)	Detergents desulfonation/desulfatation	Studies on bacterial proteins involved in S-metabolism

Annex 2

Users Symposium

PROGRAM (see http://www.pcb.ub.es/homePCB/live/ct/o193.asp?nid=3623)

6/3/2009 ALBA-MX user's meeting
IBMB-CSIC i Direcció Científica
> Torre D, Auditori 11.00h

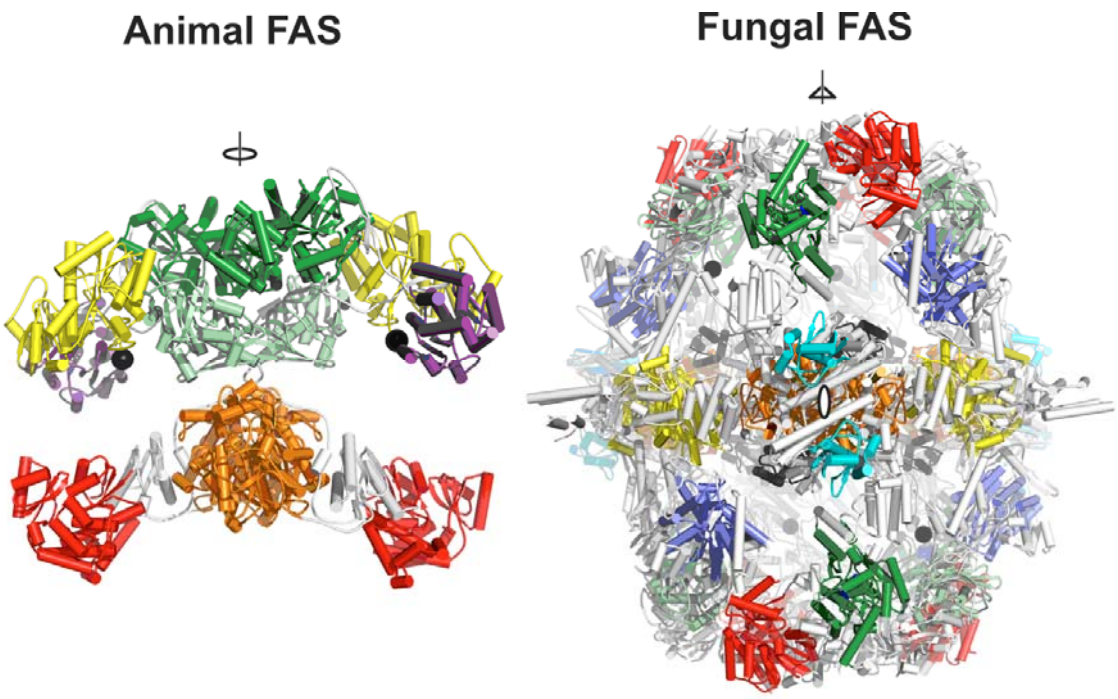
10:30 Registration and coffee
11:00 Joan Pous: Welcome and presentation of the Automated Crystallography Platform at the Barcelona Science Park
11:15 Timm Maier: "Crystallographic Structure Determination of Multifunctional Fatty Acid Synthases"
12:00 Jordi Juanhuix & Jordi Benach: "Status and opportunities at beamline XALOC in ALBA"
13:00 lunch
15:30 Ehmke Pohl: "Microfocus beam lines for protein crystallography in the third millenium"
16:30 Organizing comittee: Presentation of the proposal and open discussion
19:00 End of meeting

ABSTRACT

Crystallographic Structure Determination of Eukaryotic Fatty Acid Synthases

Timm Maier, Marc Leibundgut, Simon Jenni, Nenad Ban; ETH Zurich, Switzerland.

Fatty acids are basic building blocks of cells and their biosynthesis is an essential process in all kingdoms of life. In bacteria, fatty acid synthesis is carried out by sets of individual mono-functional enzymes. In contrast, eukaryotes harbor giant multifunctional fatty acid synthases (FASs), that catalyze all steps of cyclic fatty acid synthesis from malonyl- and acetyl-CoA precursors. Advances in synchrotron radiation data collection have contributed considerably to the recent crystallographic structure determination of the two distinct types of eukaryotic FAS, the 2.6 MDa heterododecameric yeast and the 540 kDa dimeric animal FAS. Although both enzymes catalyze identical overall reactions with closely related chemistries, they have completely dissimilar architectures and are built on different principles of enzyme integration and substrate shuttling.



References:

- T. Maier, M. Leibundgut and N. Ban, The crystal structure of a mammalian fatty acid synthase, Science 321 (2008), 1315.
- S. Jenni, M. Leibundgut, D. Boehringer, C. Frick, B. Mikolasek and N. Ban, Structure of fungal fatty acid synthase and implications for iterative substrate shuttling, Science 316 (2007), 254.
- M. Leibundgut, S. Jenni, C. Frick and N. Ban, Structural basis for substrate delivery by acyl carrier protein in the yeast fatty acid synthase, Science 316 (2007), 288.
- S. Jenni, M. Leibundgut, T. Maier and N. Ban, Architecture of a fungal fatty acid synthase at 5 Å resolution, Science 311 (2006), 1263.
- T. Maier, S. Jenni and N. Ban, Architecture of mammalian fatty acid synthase at 4.5 A resolution, Science 311 (2006), 1258.

ABSTRACT

Status and opportunities of XALOC beamline in Alba

Jordi Juanhuix and Jordi Benach
ALBA Synchrotron-CELLS[†]

Beamline BL13-XALOC in Alba synchrotron is now under construction. The beamline, devoted to macromolecular crystallography, has an intentionally generalist approach: it has to be able to tackle small crystals of a few tens of microns but at the same time it has to cope with large complexes involving many different macromolecules, which frequently crystallize in relatively large crystals. To this aim, a flexible optics including a channel-cut monochromator and a pair of mirrors in KB configuration has been chosen. The experimental station includes all the equipment needed to perform wavelength-selective and wavelength-independent experiments in an automated operation.

The current status of the beamline regarding infrastructure, optics and end-station will be reviewed, as well as the performance and the scientific opportunities that XALOC is expected to offer to the scientific community in the near future.

[†] Currently in address: Ed. Cn Nord, Universitat Autònoma de Barcelona E-08193 Bellaterra, Barcelona

Annex 3

Support letters from Academia

Sociedad Española
de Bioquímica y
Biología Molecular

Madrid, October 17, 2014

To whom it may concern,

The Spanish Society of Biochemistry and Molecular Biology (SEBBM) hereby gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the port-folio of available beamlines at ALBA would provide a crucial state-of-the-art device for structural biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can only be efficiently analyzed using a microfocus beamline. In addition, other new experiments, such as rapid room temperature data collection, would be covered by the proposed beamline.

We strongly believe that a MX microfocus beamline at the ALBA synchrotron will boost the competitiveness of the structural biology research, being highly complementary and synergic to the highly successful general-purpose MX beamline XALOC.

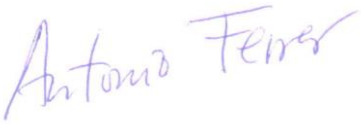
Federico Mayor jr.
Professor of Biochemistry and Molecular Biology
President, Spanish Society for Biochemistry and Molecular Biology (SEBBM)
fmayor@cbm.csic.es

Sociedad de Biofísica de España.

To whom it may concern,

The Spanish Biophysical Society (SEB) hereby gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the portfolio of available beamlines at ALBA would provide a crucial state-of-the-art device for structural biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can only be efficiently analyzed using a microfocus beamline. In addition, other new experiments, such as rapid room temperature data collection, would be covered by the proposed beamline.

We strongly believe that a MX microfocus beamline at the ALBA synchrotron will boost the competitiveness of the structural biology research, being highly complementary and synergic to the highly successful general-purpose MX beamline XALOC.



Signature: A. Ferrer Montiel
Position: SBE, President
Address: Universidad Miguel Hernández, Elche, Spain
e-mail: aferrer@umh.es

Date and place: Elche, 7 October 2014

Parc Científic de Barcelona

Name: Montserrat Vendrell
Position: Executive Director
Name of the Centre: Barcelona Science Park
Address: Baldiri Reixac, 4-8, Barcelona, Spain
e-mail: mvendrell@pcb.ub.cat

To whom it may concern,

The **BARCELONA SCIENCE PARK** hereby gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art device crucial for successful performance in Life Sciences research,and particularly in Structural Biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can only be efficiently analyzed using a microfocus beamline. In addition, other new experiments, such as projects requiring room temperature or possibly native data collection, could be covered by the proposed beamline.

We strongly believe that a MX microfocus beamline at ALBA will boost the competitiveness in the field of Structural Biology.

Signature:

Dr. Montserrat Vendrell
Executive Director

Barcelona, 7th October 2014

Direcció i Gerència
Baldiri Reixac, 4
08028 Barcelona
Tel. +34 93 4034475 / 34826
Fax +34 93 4029063
www.pcb.ub.cat

Universidad de Granada
Vicerrectorado de Política Científica e Investigación

María Dolores Suárez Ortega
Science Policy and Research Vicerector
Centro de Transferencia Tecnológica
Gran Vía 48, 2ª planta
msuarez@ugr.es

To whom it may concern,

The Science Policy and Research Vicerector hereby gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art device crucial for successful performance in life sciences research,and particularly in structural biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can only be efficiently analyzed using a microfocus beamline. In addition, other new experiments, such as projects requiring room temperature or possibly native data collection, could be covered by the proposed beamline.

We stronglybelieve that a MX microfocusbeamline at ALBA will boost the competitiveness of the structural biology research undertaken in our University of Granada, being highly complementary and highly synergic to the very successful general-purpose MX beamline XALOC at ALBA.

Vicerrectora de Política Científica
e Investigación

VNiVERSiDAD D SALAMANCA
CAMPUS DE EXCELENCIA INTERNACIONAL

VICERRECTORADO DE INVESTIGACIÓN Y TRANSFERENCIA
Patio de Escuelas, nº 1 37071 Salamanca
Tel.: +34 923 29 44 30 Fax:+34 923 29 45 02
www.usal.es vic.investigacion@usal.es

Name: Juan Manuel Corchado Rodríguez
Position: Vice Chancellor of Research and Technology Transfer
Name of the Centre/Institute/Department: University of Salamanca
Address: Patio de Escuelas, 1 (37008) Salamanca
e-mail: vic.investigacion@usal.es

To whom it may concern,

The University of Salamanca hereby gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the portfolio of available beamlines at ALBA would provide a state-of-the-art device crucial for successful performance in life sciences research,and particularly in structural biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can only be efficiently analyzed using a microfocus beamline. In addition, other new experiments, such as projects requiring room temperature or possibly native data collection, could be covered by the proposed beamline.

We strongly believe that a MX microfocusbeamline at ALBA will boost the competitiveness of the structural biology research undertaken in our University, being highly complementary and highly synergic to the very successful general-purpose MX beamline XALOC at ALBA.

Signature:

Universidad de Salamanca Vicerrectorado

Date and place: 7th October, Salamanca

Name: Fernando Plazaola Muguruza
Position: Vicerrector de Investigación
Name of the Centre/Institute/Department: **Universidad del País Vasco (EHU)**
Address: Bº Sarriena S/N, 48940, Leioa, Vizcaya
e-mail: vrinvestigacion@ehu.es

To whom it may concern,

The **Universidad del País Vasco (EHU)** hereby gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art device crucial for successful performance in life sciences research,and particularly in structural biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can only be efficiently analyzed using a microfocus beamline. In addition, other new experiments, such as projects requiring room temperature or possibly native data collection, could be covered by the proposed beamline.

We stronglybelieve that a MX microfocusbeamline at ALBA will boost the competitiveness of the structural biology research undertaken in our **Universidad del País Vasco (EHU)**, being highly complementary and highly synergic to the very successful general-purpose MX beamline XALOC at ALBA.

Leioa, 20 October 2014

IKERKETA ERREKTOREORDETZA VICERRECTORADO DE INVESTIGACIÓN UNIVERSIDAD DEL PAÍS VASCO EUSKAL HERRIKO UNIBERTSITATEA

Fernando Plazaola
Vice Chancellor for Research
University of the Basque Country

UNIVERSIDAD DE ALMERÍA
VICERRECTORADO DE INVESTIGACIÓN, DESARROLLO E INNOVACIÓN.

Ctra. Sacramento s/n La Cañada de San Urbano 04120 Almería (España) www.ual.es Telf.: 950015037 - Fax: 950015073

Name: F. Javier de las Nieves
Position: Vicerrector of Research and Innovation
Name of the Centre/Institute/Deparment: University of Almería
Address: La Cañada de S. Urbano s/n, Almería 04120, Spain
e-mail: vinvest@ual.es

To whom it may concern,

The University of Almeria hereby gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the port-folio of available beamlines at ALBA would provide a crucial state-of-the-art device for structural biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can only be efficiently analyzed using a microfocus beamline. In addition, other new experiments, such as rapid room temperature data collection, would be covered by the proposed beamline.

We strongly believe that a MX microfocus beamline at the ALBA synchrotron will boost the competitiveness of the structural biology research undertaken in our University, being highly complementary and synergic to the highly successful general-purpose MX beamline XALOC.

Signature:

F. Javier de las Nieves
Vicerrector of Research and Innovation
University of Almeria

Date and place: Almeria 6th October 2014

Name: Angel Pazos Carro
Position: Vice-rector for Research and Knowledge Transfer
Address: Pabellón de Gobierno, Avda. Los Castros s/n, 39005 Santander, Cantabria
e-mail: vr.investigacion@unican.es

To whom it may concern,

The **UNIVERSITY OF CANTABRIA,** hereby gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art device crucial for successful performance in life sciences research,and particularly in structural biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can only be efficiently analyzed using a microfocus beamline. In addition, other new experiments, such as projects requiring room temperature or possibly native data collection, could be covered by the proposed beamline.

We stronglybelieve that a MX microfocusbeamline at ALBA will boost the competitiveness of the structural biology research undertaken in our University (UC), being highly complementary and highly synergic to the very successful general-purpose MX beamline XALOC at ALBA.

Signature:

Date and place: 10/07/2014 Santander

Name: Dr. F. Azorín
Position: Director
Name of the Centre/Institute/Department: Institute of Molecular Biology of Barcelona, CSIC
Address: Baldiri Reixac, 10. 08028 Barcelona. Spain
e-mail: fambmc@ibmb.csic.es

To whom it may concern,

The Institute of Molecular Biology of Barcelona, CSIC, hereby gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art device crucial for successful performance in life sciences research,and particularly in structural biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can only be efficiently analyzed using a microfocus beamline. In addition, other new experiments, such as projects requiring room temperature or possibly native data collection, could be covered by the proposed beamline.

We stronglybelieve that a MX microfocusbeamline at ALBA will boost the competitiveness of the structural biology research undertaken in our Institute of Molecular Biology of Barcelona, CSIC, being highly complementary and highly synergic to the very successful general-purpose MX beamline XALOC at ALBA.

Signature: Dr. F. Azorín. Director IBMB, CSIC.

Date and place: Barcelona, 8 October 2014.

MINISTERIO
DE ECONOMÍA
Y COMPETITIVIDAD

CONSEJO SUPERIOR
DE INVESTIGACIONES
CIENTÍFICAS
CSIC

INSTITUTO DE QUIMICA FISICA "ROCASOLANO"

Juan de la Figuera Bayón
Director
Instituto de Química-Física "Rocasolano"
direccion.iqfr@csic.es
c/Serrano, 119
Madrid 28006
España

To whom it may concern,

The Instituto de Química Física "Rocasolano" (CSIC) hereby gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the port-folio of available beamlines at ALBA would provide a crucial state-of-the-art device for structural biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can only be efficiently analyzed using a microfocus beamline. In addition, other new experiments, such as rapid room temperature data collection, would be covered by the proposed beamline.

We strongly believe that a MX microfocus beamline at the ALBA synchrotron will boost the competitiveness of the structural biology research undertaken in our Instituto de Química Física "Rocasolano", being highly complementary and synergic to the highly successful general-purpose MX beamline XALOC.

Madrid, 6 de octubre de 2014

Juan De La Figuera

C/ Serrano, 119
28006 MADRID. ESPAÑA
TEL.: (34) 91 561 94 00
FAX.: (34) 91 564 24 31

UNIVERSITAT POLITÈCNICA
DE CATALUNYA
BARCELONATECH

Fernando Orejas Valdés as Vice-rector for Research Policy of the Universitat Politècnica de Catalunya.

To whom it may concern,

The Universitat Politècnica de Catalunya, hereby gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the portfolio of available beamlines at ALBA would provide a state-of-the-art device crucial for successful performance in life sciences research, and particularly in structural biology.
An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can only be efficiently analyzed using a microfocus beamline. In addition, other new experiments, such as projects requiring room temperature or possibly native data collection, could be covered by the proposed beamline.

We stronglybelieve that a MX microfocusbeamline at ALBA will boost the competitiveness of the structural biology research undertaken in our University, being highly complementary and highly synergic to the very successful general-purpose MX beamline XALOC at ALBA.

Fernando Orejas Valdés

Barcelona, 7h october 2014

UNIVERSITAT POLITÈCNICA DE CATALUNYA
BARCELONATECH
Vicerectorat de Política de Recerca

CIC bioGUNE
Biozientzietako Ikerkuntza Kooperatiboko Zentroa
Centro de Investigación Cooperativa en Biociencias

Name: José M Mato
Position: General Director
Name of the Centre/Institute/Department: CIC bioGUNE
Address: Parque Tecnológico de Bizkaia, Edif. 801A- 1º. 48160 Derio- Bizkaia.
e-mail: director@cicbiogune.es

To whom it may concern,

The Asociación Centro de Investigación Cooperativa en Biociencias - CIC bioGUNE, hereby gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art device crucial for successful performance in life sciences research, and particularly in structural biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can only be efficiently analyzed using a microfocus beamline. In addition, other new experiments, such as projects requiring room temperature or possibly native data collection, could be covered by the proposed beamline.

We strongly believe that a MX microfocusbeamline at ALBA will boost the competitiveness of the structural biology research undertaken in our Structural Biology Unit, being highly complementary and highly synergic to the very successful general-purpose MX beamline XALOC at ALBA.

Signature: Prof. José M Mato

CICbioGUNE
G-95229142
Parque Tecnológico de Bizkaia
Edificio 801ª - 1ª Planta
48160 - DERIO (Bizkaia)
Tel.: 94 406 13 00 - Fax: 94 406 13 01

Date and place: Derio, October 8, 2014

GOBIERNO DE ESPAÑA
MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS
INSTITUTO DE BIOMEDICINA
DE VALENCIA (IBV)

Name: Pascual Sanz Bigorra
Position: Director
Name of the Centre/Institute/Department: Instituto de Biomedicina de Valencia
Address: C/ Jaime Roig 11, 46010 Valencia, Spain
e-mail: direccion.ibv@csic.es

To whom it may concern,

The "Instituto de Biomedicina de Valencia" hereby gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art device crucial for successful performance in life sciences research, and particularly in structural biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals that can only be efficiently analysed using a microfocus beamline. In addition, new scientific cases, such as the projects requiring room temperature or possibly native data collection, could be covered by the proposed beamline.

We strongly believe that a MX microfocus beamline at ALBA will boost the competitiveness of the structural biology research undertaken in our "Instituto de Biomedicina de Valencia", being highly complementary and synergic to the general-purpose MX beamline XALOC at ALBA as well as to our local data collection facilities.

Signature:

INSTITUTO DE BIOMEDICINA CSIC VALENCIA

Date and place: Valencia 6 October 2014

PASCUAL SANZ BIGORRA
Director de IBV-CSIC
P.D. (Resolución 12 de julio 2012 B.O.E. 19 de julio)

C/ JAUME ROIG, 11
46010 VALENCIA ESPAÑA
TEL.: 96 339 17 60

FAX: 96 369 08 00

MOLECULAR BIOLOGY
INSTITUTE OF BARCELONA (IBMB)

F.Xavier Gomis-Rüth
Research Professor CSIC

Dept. of Structural Biology, Director
Molecular Biology Institute of Barcelona (IBMB)
Spanish Research Council CSIC
Barcelona Science Park; c/ Baldiri Reixac, 4-6; 08028 Barcelona
e-mail: xgrcri@ibmb.csic.es

To whom it may concern,

The Structural Biology Department of the Molecular Biology Institute of Barcelona hereby gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art device crucial for successful performance in Life Sciences Research,and particularly in Structural Biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can only be efficiently analyzed using a microfocus beamline. In addition, other new experiments, such as projects requiring room temperature or possibly native data collection, could be covered by the proposed beamline.

We strongly believe that a MX microfocus beamline at ALBA will boost the competitiveness of the structural biology research undertaken in our Structural Biology Department, being highly complementary and highly synergic to the very successful general-purpose MX beamline XALOC at ALBA.

Signature:

F.Xavier Gomis-Rüth

Date and place: Barcelona, October 15, 2014

Proteolysis Lab
IBMB-CSIC
Barcelona Science Park; Helix Building
c/ Baldiri Reixac, 15-21
08028-Barcelona (Spain)
e-mail: fxgr@ibmb.csic.es
Lab homepage: www.ibmb.csic.es/home/xgomis

Telf. +34 93 403 4668
Fax: +34-934 034 979
VAT: Q2818002D

IRB BARCELONA

INSTITUTO DE INVESTIGACIÓN BIOMÉDICA

Barcelona, 10th October 2014

To whom it may concern,

As Head of the Structural and Computational Biology Programme at the Institute for Research in Biomedicine (IRB Barcelona) I would like to express my strong support to the proposal for a Microfocus Macromolecular Crystallography beamline in ALBA.

These beamline would allow data collection from very small crystal samples and heterogeneous crystals. Several projects from our laboratories are dealing with large protein complexes, protein-nucleic acid complexes and membrane proteins which are produced in limited amounts and crystallized as very small crystals. These samples are not suitable for standard beamlines. A microfocus beamline in ALBA would be complementary for the existing XALOC beamline allowing the Spanish Synchrotron facility to be competitive in biological studies with other state-of-the-art synchrotrons.

Our research groups will highly benefit of such a beamline.

Prof. Miquel Coll

Head of the Structural and Computational Biology Programme

Joan J. Guinovart
Director

To whom it may concern,

The Institute for Research in Biomedicine (IRB Barcelona) gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA.

The inclusion of a microfocus MX beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art device crucial for successful performance in Life Sciences Research,and particularly in Structural Biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can only be efficiently analyzed using a microfocus beamline. In addition, other new experiments, such as projects requiring room temperature or possibly native data collection, could be covered by the proposed beamline.

We strongly believe that a MX microfocusbeamline at ALBA will boost the competitiveness of the structural biology research undertaken in our Institute for Research in Biomedicine (IRB Barcelona), being highly complementary and highly synergic to the very successful general-purpose MX beamline XALOC at ALBA.

Joan J. Guinovart
Director
Institute for Research in Biomedicine (IRB Barcelona)

Barcelona, 8 October de 2014

Parc Científic de Barcelona
Baldiri Reixac, 10
E-08028 Barcelona
Spain
www.irbbarcelona.org

Instituto Universitario de Investigación
Biocomputación y Física de Sistemas Complejos
Universidad Zaragoza

To whom it may concern,

The Instituto de Biocomputacion y Fisica de Sistemas Complejos de la Universidad de Zaragoza, hereby gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the port-folio of available beamlines at ALBA would provide a crucial state-of-the-art device for structural biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can only be efficiently analyzed using a microfocus beamline. In addition, other new experiments, such as rapid room temperature data collection, would be covered by the proposed beamline.

We strongly believe that a MX microfocus beamline at the ALBA synchrotron will boost the competitiveness of the structural biology research undertaken in our institute, being highly complementary and synergic to the highly successful general-purpose MX beamline XALOC.

Zaragoza, 6th October 2014.

Signature: Alfonso Tarancon

Instituto de Biocomputación y Física de Sistemas Complejos • Universidad de Zaragoza
Edificio I + D • C/ Mariano Esquillor s/nº • 50018 Zaragoza – SPAIN • Tel. + 34 976 762989 • + 34 976 76 29 88
bifi@unizar.es http://bifi.unizar.es

MINISTERIO
DE ECONOMÍA
Y COMPETITIVIDAD

CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS
CENTER FOR BIOLOGICAL RESEARCH
SPANISH NATIONAL RESEARCH COUNCIL

To whom it may concern,

The Centro de Investigaciones Biológicas (CIB-CSIC) hereby gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art device crucial for successful performance in life sciences research,and particularly in structural biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can only be efficiently analyzed using a microfocus beamline. In addition, other new experiments, such as projects requiring room temperature or possibly native data collection, could be covered by the proposed beamline.

We strongly believe that a MX microfocus beamline at ALBA will boost the competitiveness of the structural biology research undertaken in our Centro de Investigaciones Biológicas (CIB-CSIC), being highly complementary and highly synergic to the very successful general-purpose MX beamline XALOC at ALBA.

Madrid, on October 8th, 2014

CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS
CENTRO DE INVESTIGACIONES BIOLÓGICAS
CSIC

Prof. María Jesús Martínez Hernández
Director Centro de Investigaciones Biológicas (CIB-CSIC)
Ramiro de Maeztu 9, 28040 Madrid
Spain
e-mail: direccion.cib@csic.es

CENTRO DE INVESTIGACIÓN
DEL CÁNCER

Universidad de Salamanca - CSIC
CSIC

Campus Miguel de Unamuno
37007 Salamanca - España
Tel. +34 923 294 720
Fax +34 923 294 743
www.cicancer.org

Salamanca, 8th October 2014

To whom it may concern,

The Centro de Investigación del Cáncer (CIC-IBMCC) hereby gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art device crucial for successful performance in life sciences research, and particularly in structural biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can only be efficiently analyzed using a microfocus beamline. In addition, other new experiments, such as projects requiring room temperature or possibly native data collection, could be covered by the proposed beamline.

We strongly believe that a MX microfocus beamline at ALBA will boost the competitiveness of the structural biology research undertaken in our institute, being highly complementary and highly synergic to the very successful general-purpose MX beamline XALOC at ALBA.

Dr Eugenio Santos
Director
Centro de Investigación del Cáncer (CIC - IBMCC)
Universidad de Salamanca - CSIC
Campus Unamuno s/n
37007 Salamanca
esantos@usal.es

MINISTERIO
DE ECONOMÍA
Y COMPETITIVIDAD

CSIC
Consejo Superior de Investigaciones Científicas
Centro Nacional
de Biotecnología

To whom it may concern,

The Centro Nacional de Biotecnologíahereby gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the port-folio of available beamlines at ALBA would provide a crucial state-of-the-art device for structural biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can only be efficiently analyzed using a microfocus beamline. In addition, other new experiments, such as rapid room temperature data collection, would be covered by the proposed beamline.

We strongly believe that a MX microfocus beamline at the ALBA synchrotron will boost the competitiveness of the structural biology research undertaken in our Institute, being highly complementary and synergic to the highly successful general-purpose MX beamline XALOC.

Dra. Castresana Fernández
Directora del Centro Nacional de Biotecnología CNB-CSIC

October 7th, 2014
Madrid, Spain

direccion.cnb@cnb.csic.es

c/ Darwin, 3
Campus de
Cantoblanco
28049 MADRID
TEL.: 91 585 4689
FAX: 91 372 0244

MINISTERIO
DE ECONOMÍA
Y COMPETITIVIDAD

CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

Centro Nacional
de Biotecnología

Madrid, October 6th 2014

To whom it may concern,

The Department of Macromolecular Structure at the Centro Nacional de Biotecnología (CSIC) hereby gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the port-folio of available beamlines at ALBA would provide a crucial state-of-the-art device for structural biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can only be efficiently analyzed using a microfocus beamline. In addition, other new experiments, such as rapid room temperature data collection, would be covered by the proposed beamline.

We strongly believe that a MX microfocus beamline at the ALBA synchrotron will boost the competitiveness of the structural biology research undertaken in our Department, being highly complementary and synergic to the highly successful general-purpose MX beamline XALOC.

José María Valpuesta
Head
Department of Macromolecular Structure
Centro Nacional de Biotecnología
Consejo Superior de Investigaciones Científicas
Darwin, 3
e-mail: jmv@cnb.csic.es

c/ Darwin, 3
Campus Universidad
Autónoma de Madrid
Cantoblanco
28049 MADRID
TEL.: 91 5854690
FAX: 91 5854506

Name: JAVIER LEON
Position: Director
Name of the Centre/Institute/Department: Instituto de Biomedicina y Biotecnología de Cantabria (IBBETC)
Address: C/Albert Einstein, 22. PCTCAN. 39011 Santander (Cantabria)
e-mail: javier.leon@unican.es

To whom it may concern,

The **INSTITUTO DE BIOMEDICINA Y BIOTECNOLOGÍA DE CANTABRIA (IBBTEC),** hereby gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art device crucial for successful performance in life sciences research,and particularly in structural biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can only be efficiently analyzed using a microfocus beamline. In addition, other new experiments, such as projects requiring room temperature or possibly native data collection, could be covered by the proposed beamline.

We stronglybelieve that a MX microfocusbeamline at ALBA will boost the competitiveness of the structural biology research undertaken in our Institute (IBBTEC), being highly complementary and highly synergic to the very successful general-purpose MX beamline XALOC at ALBA.

Signature:

Date and place: October 6, 2014, Santander

CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS
INSTITUTO DE BIOMEDICINA Y BIOTECNOLOGIA DE CANTABRIA
UNIVERSIDAD DE CANTABRIA - IDICAN

IBUB
Institut de Biomedicina
de la Universitat de Barcelona

Name: Francesc Villarroya Gombau
Position: Director
Name of the Centre/Institute/Department: Institut de Biomedicina de la Universitat de Barcelona (IBUB)
Address: Dept. Bioquimica. Facultat de Biologia. Universitat de Barcelona. Av Diagonal 643. 08028 Barcelona. Spain
e-mail: fvillarroya@ub.edu

To whom it may concern,

The Institute of Biomedicine of the University of Barcelona hereby gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art device crucial for successful performance in life sciences research,and particularly in structural biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can only be efficiently analyzed using a microfocus beamline. In addition, other new experiments, such as projects requiring room temperature or possibly native data collection, could be covered by the proposed beamline.

We strongly believe that a MX microfocusbeamline at ALBA will boost the competitiveness of the structural biology research undertaken in our Institute, being highly complementary and highly synergic to the very successful general-purpose MX beamline XALOC at ALBA.

Signature:

Francesc Villarroya

Barcelona, 8 october 2014

INSTITUTO DE BIOTECNOLOGÍA UNIVERSIDAD DE GRANADA

Name: Antonio Osuna
Position: Full Professor Director
Name of the Centre/Institute/Department: Institute Biotechnology University of Granada
Address: Campus Universitario Fuentenueva
e-mail: aosuna@ugr.es

To whom it may concern,

The Institute of Biotechnology University of Granada hereby gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the portfolio of available beamlines at ALBA would provide a state-of-the-art device crucial for successful performance in life sciences research,and particularly in structural biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can only be efficiently analyzed using a microfocus beamline. In addition, other new experiments, such as projects requiring room temperature or possibly native data collection, could be covered by the proposed beamline.

We stronglybelieve that a MX microfocusbeamline at ALBA will boost the competitiveness of the structural biology research undertaken in our Institute of Biotechnology University of Granada, being highly complementary and highly synergic to the very successful general-purpose MX beamline XALOC at ALBA.

Signature:

Date and place: 10/08/2014 Granada, Spain.

MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

CSIC
Consejo Superior de Investigaciones Científicas

ugr Universidad de Granada

IACT
INSTITUTO ANDALUZ DE CIENCIAS DE LA TIERRA

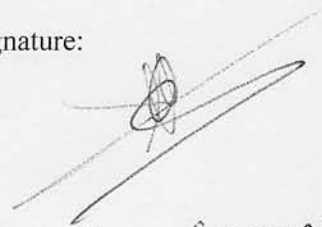
Fermin Otálora Muñoz
Director of the Laboratorio de Estudios Cristalográficos,
Instituto Andaluz de Ciencias de la Tierra (CSIC-U. de Granada)
Avd. De la Palmeras, 4. 18100 Armilla, Granada, Spain
e-mail: otalora@ugr.es

To whom it may concern,

The Laboratorio de Estudios Cristalográficos from the Instituto Andaluz de Ciencias de la Tierra (CSIC-U. Granada), hereby gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art device crucial for successful performance in life sciences research,and particularly in structural biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can only be efficiently analyzed using a microfocus beamline. In addition, other new experiments, such as projects requiring room temperature or possibly native data collection, could be covered by the proposed beamline.

We strongly believe that a MX microfocusbeamline at ALBA will boost the competitiveness of the structural biology research undertaken in our Institute, being highly complementary and highly synergic to the very successful general-purpose MX beamline XALOC at ALBA.

Signature:



Date and place: GRANADA 6/OCT/2014

HOSPITAL DE LA SANTA CREU I SANT PAU
UNIVERSITAT AUTÒNOMA DE BARCELONA

Institut de Recerca
Pav. Sant Frederic, 1a pl.
St. Antoni M. Claret, 167
08025-Barcelona
Tel. 93 553 7613

To whom it may concern,

The INSTITUTE OF BIOMEDICAL RESEARCH (IIB-SANT PAU) hereby gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art device crucial for successful performance in life sciences research, and particularly in structural biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can only be efficiently analyzed using a microfocus beamline. In addition, other new experiments, such as projects requiring room temperature or possibly native data collection, could be covered by the proposed beamline.

We strongly believe that a MX microfocus beamline at ALBA will boost the competitiveness of the structural biology research undertaken in our INSTITUTE OF BIOMEDICAL RESEARCH (IIB-SANT PAU), being highly complementary and highly synergic to the very successful general-purpose MX beamline XALOC at ALBA.

Dr. Jaime Kulisevsky
Director Research Institute
Sant Pau Hospital
Director IIB-Sant Pau
Email: JKulisevsky@santpau.cat

Barcelona, October 8th, 2014

Universitat Autònoma de Barcelona

Departament de Bioquímica i de Biologia Molecular

Unitat de Bioquímica de Ciències

Name: **Ester Boix Borràs**
Position: **Associate Professor. Head of the Biosciences Unity**
Name of the Centre/Institute/Department: **Dpt. of Biochemistry and Molecular Biology. Universitat Autònoma de Barcelona, Spain**
Address: **Faculty of Biosciences. Campus UAB**
e-mail: **Ester.Boix@uab.cat**

To whom it may concern,

The **Unity of Biosciences of the Dpt. of Biochemistry and Molecular Biology, Universitat Autònoma de Barcelona** hereby gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art device crucial for successful performance in life sciences research,and particularly in structural biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can only be efficiently analyzed using a microfocus beamline. In addition, other new experiments, such as projects requiring room temperature or possibly native data collection, could be covered by the proposed beamline.

We stronglybelieve that a MX microfocusbeamline at ALBA will boost the competitiveness of the structural biology research undertaken in our **Unity, at the Dpt. of Biochemistry and Molecular Biology, UAB**, being highly complementary and highly synergic to the very successful general-purpose MX beamline XALOC at ALBA.

Universitat Autònoma de Barcelona

Departament de Bioquímica
i de Biologia Molecular

Unitat de Bioquímica de Ciències

Signature:

Date and place:
Cerdanyola del Vallès, 7th October 2014

eman ta zabal zazu

euskal herriko unibertsitatea

universidad del pais vasco

CSIC-UPV/EHU

Name: Félix M. Goñi Urcelay
Position: Director
Name of the Centre/Institute/Department: **Unidad de Biofísica (CSIC, UPV/EHU)**
Address: Bº Sarriena S/N, 48940, Leioa, Vizcaya
e-mail: felix.goni@ehu.es

To whom it may concern,

The **Unidad de Biofísica (CSIC, UPV/EHU)** hereby gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art device crucial for successful performance in life sciences research,and particularly in structural biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can only be efficiently analyzed using a microfocus beamline. In addition, other new experiments, such as projects requiring room temperature or possibly native data collection, could be covered by the proposed beamline.

We stronglybelieve that a MX microfocusbeamline at ALBA will boost the competitiveness of the structural biology research undertaken in our **Unidad de Biofísica (CSIC, UPV/EHU)**, being highly complementary and highly synergic to the very successful general-purpose MX beamline XALOC at ALBA.

Signature:

Unidad de Biofísica/Biofisika Unitatea

CSIC

Date and place: Leioa, 8 October 2014

VICERREITORADO DE INVESTIGACIÓN E INNOVACIÓN
RIAIDT
Unidade de Raios X

Edificio CACTUS. Campus Vida
15782 Santiago de Compostela
Tel. 881 816 223 Fax: 881 816 203
Correo electrónico: raios_x@usc.es
WWW: http://www.usc.es/gl/investigacion/riaidt/raiosx/

Name: Dr. Antonio L. Llamas-Saiz.
Position: Head of the X-Ray Unit
Name of Centre/Institute/Department: University of Santiago de Compostela/RIAIDT.
Address: Edif. CACTUS. Campus Vida. 15782 Santiago de Compostela
e-mail: raios_x@usc.es

The X-Ray Unit of the University of Santiago de Compostela hereby gives its support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art device crucial for successful performance in life sciences research, and particularly in structural biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can only be efficiently analysed using a microfocus beamline. In addition, other new experiments, such as projects requiring room temperature or possibly native data collection, could be covered by the proposed beamline.

We strongly believe that a MX microfocus beamline at ALBA will boost the competitiveness of the structural biology research undertaken in our University, being highly complementary and highly synergic to the very successful general-purpose MX beamline XALOC at ALBA.

Signature:

Santiago de Compostela, October 9, 2014

INSTITUTO LELOIR
FUNDACIÓN

Fundación Instituto Leloir
Av. Patricias Argentinas 435 (C1405BWE)
Buenos Aires, Argentina
Teléfono: +54-11-5238-7500
Fax: +54-11-5238-7501

Buenos Aires, October 8th, 2014

To whom it may concern,

The **Fundación Instituto Leloir** hereby gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art device crucial for successful performance in life sciences research, and particularly in structural biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can only be efficiently analyzed using a microfocus beamline. In addition, other new experiments, such as projects requiring room temperature or possibly native data collection, could be covered by the proposed beamline.

We strongly believe that a MX microfocus beamline at ALBA will boost the competitiveness of the structural biology research undertaken in our Institute, being highly complementary and highly synergic to the very successful general-purpose MX beamline XALOC at ALBA.

Dr. LUIS IELPI
DIRECTOR
FUNDACION INSTITUTO LELOIR

E-mail: lielpi@leloir.org.ar

UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI BIOSCIENZE

Martino Bolognesi
Professor of Biochemistry
University of Milano, Dept. BioSciences:
e-mail: martino.bolognesi@unimi.it

Milano, 08 October 2014

RE: support of a microfocus X-ray diffraction beam line at ALBA

To whom it may concern

My Structural Biology lab, at the Department of BioSciences, University of Milano (Italy) hereby gives its full support to the proposal for a microfocus beam line dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beam line in the port-folio of available beam lines at ALBA would provide a state-of-the-art device crucial for successful performance in life sciences research, and particularly for the structural biology projects we are interested in. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can be analyzed efficiently using a microfocus beam line that allows selecting the best part of the crystal, or shine radiation properly on a micrometer-size sample. In addition, other new experiments, such as those requiring room temperature or possibly native data collection, could be covered by the proposed beam line.

We strongly believe that a MX microfocus beam line at ALBA will boost the competitiveness of the structural biology research undertaken by our group, being highly complementary and highly synergic to the existing general-purpose MX beam line XALOC at ALBA.

Prof. Martino Bolognesi

Prof. Martino Bolognesi - Dipartimento di Bioscienze
Via Celoria 26 – 20133 Milano, Italy
Tel 02 503 14893 - Fax 02 503 14895 – martino.bolognesi@unimi.it

Università degli Studi di Torino

CrisDi

CENTRO INTERDIPARTIMENTALE PER LO SVILUPPO DELLA CRISTALLOGRAFIA DIFFRATTOMETRICA

Name: Fernando Cámara Artigas
Position: Full professor of Mineralogy – President of the CrisDi
Name of the Centre/Institute/Department: CrisDi - Interdepartmental Center for Crystallography
Address: via P. Giuria 7 – 10125 Turin, Italy
e-mail: fernando.camararartigas@unito.it
website: http://www.crisdi.unito.it/index.php/crisdi

To whom it may concern,

The **Interdepartmental Center for Crystallography** of the **University of Turin** hereby gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art device crucial for successful performance in life sciences research, and particularly in structural biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can only be efficiently analyzed using a microfocus beamline. In addition, other new experiments, such as projects requiring room temperature or possibly native data collection, could be covered by the proposed beamline.

We strongly believe that a MX microfocus beamline at ALBA will boost the competitiveness of the structural biology research undertaken in our **Interdepartmental Center for Crystallography**, being highly complementary and highly synergic to the very successful general-purpose MX beamline XALOC at ALBA.

Signature:

Date and place: 8th October 2014, Torino

University of Torino
DEPARTMENT OF LIFE SCIENCES AND SYSTEMS BIOLOGY

Head of Department *Prof. Gianfranco GILARDI*

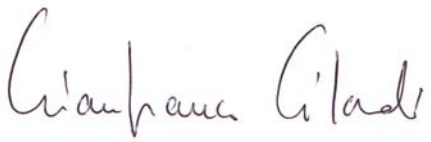
Turin, 6th October 2014

TO WHOM IT MAY CONCERN

The Department of Life Sciences and Systems Biology hereby gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art device crucial for successful performance in life sciences research, and particularly in structural biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can only be efficiently analyzed using a microfocus beamline. In addition, other new experiments, such as projects requiring room temperature or possibly native data collection, could be covered by the proposed beamline.

We strongly believe that a MX microfocus beamline at ALBA will boost the competitiveness of the structural biology research undertaken in our Department, being highly complementary and highly synergic to the very successful general-purpose MX beamline XALOC at ALBA.

Yours sincerely,



Gianfranco Gilardi

Head Office: Via Accademia Albertina, 13 - 10123 TORINO – Italy
Switchboard (+39)-011-670.4617 Fax (+39)-011-670.4508
Laboratories in Viale Mattioli, 25 - 10125 TORINO – Italy – Tel (+39)-011-670.5980 Fax (+39)-011-670.5962
Laboratories in Via Quarello, 15/a - 10135 TORINO – Italy – Tel (+39)-011-670.5967 Fax (+39)-011-670.6364

U N I V E R S I T A ' D I P A V I A
DIPARTIMENTO DI BIOLOGIA E BIOTECNOLOGIE
"L. SPALLANZANI"
Via Ferrata 1,
27100, Pavia, Italy

Letter of Support

October 5th, 2014

To whom it may concern,

The Department of Biology and Biotechnology of the University of Pavia (Italy) hereby gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art device crucial for successful performance in life sciences research,and particularly in structural biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can only be efficiently analyzed using a microfocus beamline. In addition, other new experiments, such as projects requiring room temperature or possibly native data collection, could be covered by the proposed beamline.

We strongly believe that a MX microfocus beamline at ALBA will boost the competitiveness of the structural biology research undertaken in our Structural Biology Division at the Department of Biology and Biotechnology of the University of Pavia (Italy), being highly complementary and highly synergic to the very successful general-purpose MX beamline XALOC at ALBA.

Sincerely Yours,

Prof. Andrea Mattevi
Dept. Biology and Biotechnology, University of Pavia
Via Ferrata 1, 27100 Pavia - Italy
Tel. +39-0382-985525/985534; Fax +39-0382-528496
Web www.unipv.it/biocry

IC|OI
CNR Istituto di Cristallografia

Consiglio Nazionale delle Ricerche

Unità Organizzativa di Supporto di Basovizza (Trieste)

Trieste, October 7th 2014

To whom it may concern

The Istituto di Cristallografia (IC), Consiglio Nazionale delle Ricerche (CNR), Trieste Outstation, Trieste, Italy, hereby gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA.

The inclusion of a microfocus MX beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art device crucial for successful performance in life sciences research, and particularly in structural biology.
An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals.
These crystals can only be efficiently analyzed using a microfocus beamline.
In addition, other new experiments, such as projects requiring room temperature or possibly native data collection, could be covered by the proposed beamline.

We strongly believe that a MX microfocus beamline at ALBA will boost the competitiveness of the structural biology research undertaken in our Istituto di Cristallografia (IC), Consiglio Nazionale delle Ricerche (CNR), Trieste Outstation, Trieste, Italy, being highly complementary and highly synergic to the very successful general-purpose MX beamline XALOC at ALBA.

Dr. Doriano Lamba
Deputy Head of the IC-CNR Trieste Outstation
Scientist in charge, CRG, IC-CNR, ELETTRA-Sincrotrone Trieste
X-Ray Diffraction 1 (XRD1) Beamline

CNR IC c/o Area Science Park – S.S. 14 Km 163.5 – Basovizza – I-34149 Trieste (Italia)
Telefono ++39 040 226881 - Fax ++39 040 9221126
sito web: http://www.ic.cnr.it
P. IVA 02118311006 C.F. 80054330586

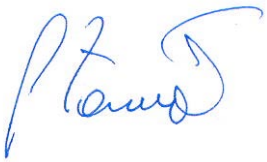
DIPARTIMENTO DI SCIENZE BIOMEDICHE - DSB
DEPARTMENT OF BIOMEDICAL SCIENCES
Via Ugo Bassi 58/B
35131 Padova
Italy
www.biomed.unipd.it

UNIVERSITÀ DEGLI STUDI DI PADOVA

Giuseppe Zanotti
Full Professor of Biochemistry
Department of Biomedical Sciences, University of Padova
Viale G. Colombo 3, 35131 Padova - Italy
giuseppe.zanotti@unipd.it

To whom it may concern

I hereby give my full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art device crucial for successful performance in life sciences research,and particularly in structural biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can only be efficiently analyzed using a microfocus beamline. In addition, other new experiments, such as projects requiring room temperature or possibly native data collection, could be covered by the proposed beamline.
We strongly believe that a MX microfocus beamline at ALBA will boost the competitiveness of the structural biology research undertaken in our Department, being highly complementary and highly synergic to the very successful general-purpose MX beamline XALOC at ALBA.



Padova, October 9th, 2014

IBMC
INSTITUTO DE BIOLOGIA MOLECULAR E CELULAR
INSTITUTE FOR MOLECULAR AND CELL BIOLOGY

To whom it may concern:

Porto, 9th October 2014

The IBMC – Instituto de Biologia Molecular e Celular hereby gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the portfolio of available beamlines at ALBA would provide a crucial state-of-the-art device for Structural Biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with very small or internally heterogeneous crystals that can only be efficiently analyzed using a microfocus beamline. In addition, the proposed beamline would cover other new experimental approaches, such as rapid room temperature data collection.

We strongly believe that a MX microfocus beamline at the ALBA synchrotron will boost the competitiveness of the Structural Biology research undertaken at IBMC – Instituto de Biologia Molecular e Celular, being highly complementary and synergic to the highly successful general-purpose MX beamline XALOC.

Claudio E. Sunkel
Director
cesunkel@ibmc.up.pt

Rua do Campo Alegre, 823
4150-180 Porto
Tel +351 226 074 900
Fax +351 226 099 157
www.ibmc.up.pt

Name: Claúdio Soares
Position: Director
Name of the Centre/Institute/Department:
Instituto de Tecnologia Química e Biológica da Universidade Nova de Lisboa
Address: Av. Da República, Oeiras, Portugal
e-mail: claudio@itqb.unl.pt

INSTITUTO
DE TECNOLOGIA
QUÍMICA E BIOLÓGICA
/UNL

To whom it may concern

The Instituto de Tecnologia Química e Biológica hereby gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art device crucial for successful performance in life sciences research, and particularly in structural biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can only be efficiently analyzed using a microfocus beamline. In addition, other new experiments, such as projects requiring room temperature or possibly native data collection, could be covered by the proposed beamline.

We strongly believe that a MX microfocusbeamline at ALBA will boost the competitiveness of the structural biology research undertaken in our Instituto de Tecnologia Química e Biológica, being highly complementary and highly synergic to the very successful general-purpose MX beamline XALOC at ALBA.

With the coming limitations in the funds available to use European synchrotrons, it is very important for Portugal to have a highly competitive facility such as ALBA in Spain.

Signature: Cláudio Manuel Soares

Date and place: Oeiras, 2014-10-07

Av. da República,
2780-157 Oeiras, Portugal
Tel. (+351) 214 469 100
Fax (+351) 214 411 277
www.itqb.unl.pt

To whom it may concern,

The Institute of Pharmacology and Structural Biology (CNRS / Université Toulouse III – Paul Sabatier) hereby gives its full support to the proposal for a microfocus beamline dedicated to Macromolecular Crystallography (MX) at the Spanish Synchrotron ALBA. The inclusion of a microfocus MX beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art device crucial for successful performance in life sciences research, and particularly in structural biology. An increasing number of challenging MX projects must be performed with limited amounts of biological sample, and have to deal with tiny or internally heterogeneous crystals. These crystals can only be efficiently analyzed using a microfocus beamline. In addition, other new experiments, such as projects requiring room temperature or possibly native data collection, could be covered by the proposed beamline.

We strongly believe that a MX microfocus beamline at ALBA will boost the competitiveness of the structural biology research undertaken in our Institute, being highly complementary and highly synergic to the very successful general-purpose MX beamline XALOC at ALBA.

Toulouse, 17/10/2014

Dr Lionel MOUREY, Senior Research Scientist
Institut de Pharmacologie et de Biologie Structurale (IPBS)
Group leader for the Structural Biophysics team
Deputy Director of the Structural Biology and Biophysics department
Deputy Manager of the PICT platform
E-mail : lionel.mourey@ipbs.fr – Tél : 05 61 17 54 36

iPBS Institut de Pharmacologie et de Biologie Structurale
www.ipbs.fr
205 route de Narbonne, BP64182
F-31077 Toulouse Cedex04 - France
T. 33-5 61 17 59 00
F. 33-5 61 17 59 94
UMR 5089