

PROPOSAL FOR A MACROMOLECULAR CRYSTALLOGRAPHY (MX) BEAMLINE IN ALBA

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Summary

It is proposed to establish a dedicated macromolecular crystallography beamline (MX) in ALBA for the analysis of single crystals with large unit cells, at high resolution. The primary requirements for the beamline can be summarised as high flux, very low beam divergence, and rapid tunability over the wide range of energies. The beamline should be equipped with state of the art detectors of large size, and be capable of fully automated operation.

I. Introduction and general overview.

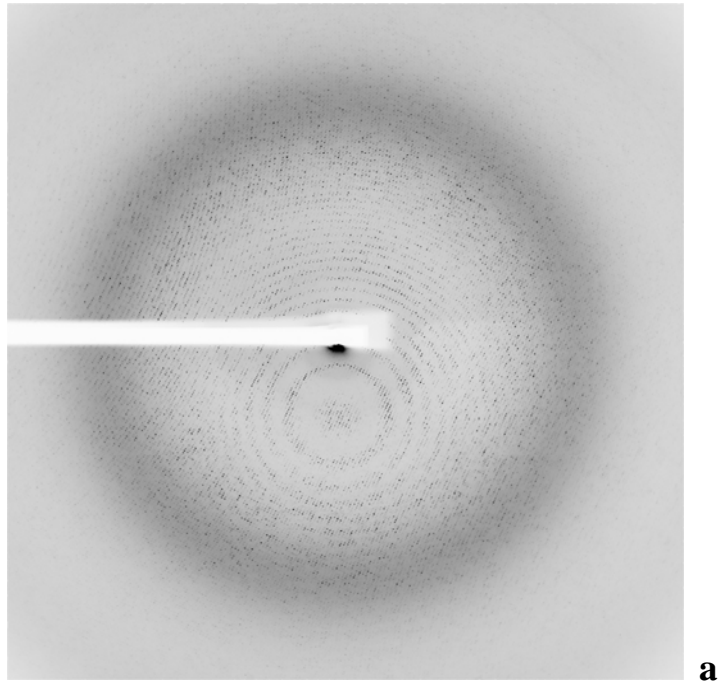
Present day biology and biomedicine have succeeded in providing massive amounts of information, and offer now a very real potential for a spectacular increase in new therapies and other biologically active approaches based on the rational input derived from well founded biological knowledge. A key element of this knowledge leading into medical and biotechnological applications is the structural and architectural understanding of the biological machinery at atomic resolution, a knowledge that is mainly produced by macromolecular X-ray crystallography. The growth in biocrystallography has been spectacular in the recent past and is expected to accelerate even further in the foreseeable future. Thus, we can be optimistic on the possibility that we will be able to get a more or less complete structural and architectural understanding of living beings and of their function by the middle of the XXI century. This means the understanding not only of individual macromolecules and of their interactions with small ligands, but also the understanding of inter-macromolecular interactions and of complex multi-molecular architectures. X-ray crystallography is not the only player into the generation of this knowledge, but it is and it is expected to continue to be the central player. Spanish science must be well prepared for coping with this challenge.

ALBA has to provide the present and future Spanish structural biology groups with the necessary elements to facilitate their work at a state-of-the-art level, and within national reach. Spain is investing great efforts into the creation of pioneering centres for the promotion of biomedical research that need the biocrystallographic support easy and at hand to change the present situation in which the access to synchrotron radiation is a bottleneck. The new centres include the Spanish Oncological Research (CNIO) and Cardiovascular Research (CNIIC) Centers in Madrid, the Biomedical Research (IRB-PC) and Cardiovascular Research (IBBV) Institutes and the Centre for Genomic Regulation (CRG) in Barcelona, the Biomedicine Institute (IBV) and the Higher Center for Transplant and Regenerative Medicine (CSAT) in Valencia, the Cancer Research Centre in Salamanca (CRC), the new CIC-Biogune Centre and the Unidad de Biofísica CSIC/EHU in Bilbao, as well as the building of new and expanded premises for the pre-existing Center for

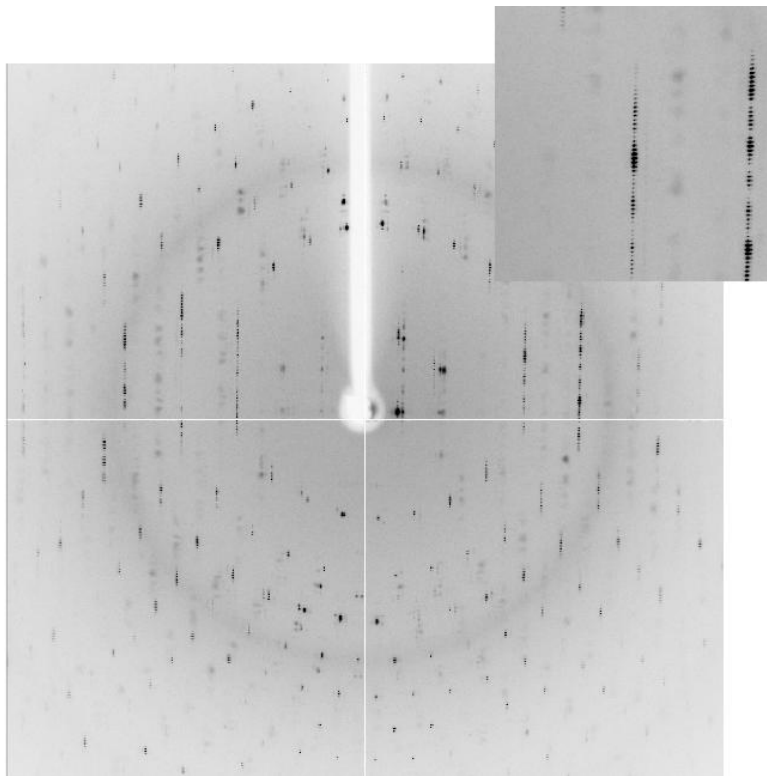
Molecular Biology Severo Ochoa (CBM) and Center of Biological Research (CIB) in Madrid, all of which are involved and are expected to increase their involvement into macromolecular crystallography. Furthermore, the research branch of the Health Ministry of Spain, Instituto de Salud Carlos III, has made a great investment in funding research networks that link a large number of centres and groups. A number of these networks would benefit from having macromolecular crystallography at ALBA. The need for a large leap forward in macromolecular crystallography in Spain is so widely recognized as a must among Spanish bioscientists, that the Spanish Society of Biochemistry and Molecular Biology (SEBBM), the largest scientific society in Spain, having more than 3000 members and representing all the Spanish biochemists and molecular biologists, has expressed officially its interest into having a dedicated line for biocrystallography at ALBA (see annexe III). Furthermore, the regional aspect is also relevant since Barcelona concentrates an important fraction of the academic groups involved in macromolecular crystallography in Spain, and also because Catalonia has been officially designated a Bioregion by the Catalanian Government, and therefore should be provided with the means to implement such status, a central one of which is obviously the highest level of structural biology.

Spain owns and operates since late 2003 line BM16 on the ESRF synchrotron at Grenoble. However, the fact that this line derives from a bending magnet device represents an insurmountable limitation (see below) that is already critical and that will get more acute with time. ALBA, by dedicating an undulator beam line, as proposed here, providing high flux over a small area, and tunability over a large energy range, will make possible the successful approach of a large number of problems that would otherwise be intractable with BM16 (indeed, with any bending magnet synchrotron line) such as diffraction of nanocrystals, of crystals having very large cells, of solving the phase problem using very weak anomalous signals, and of working with multi-macromolecular complexes.

Indeed, we foresee that a key scientific challenge in the coming years, in order to finally understand how living beings are functioning, is to collate all the partial information and know how the different components or macromolecules interact within the cell. From the viewpoint of structural biology we believe that crucial innovative research in the forthcoming years will involve the study of complex assemblies and subcellular machines (some examples have already been reported such as the ribosome, the proteasome, the nucleosome etc.). Spanish protein crystallography groups are currently working in or expect to enter this field of research soon (Fig. 1). Examples of research into large or complex structures carried out in Spain include: the complex for arginine metabolism (V. Rubio, IBV); the thermosome (J. Hermoso, IQFR); chromatin restructuring complexes (G. Montoya, CNIO); Lumazine synthetase/riboflavin synthetase complex (F. Otálora, IACT); TRV virus (D. Guerin, UPV); Calicivirus virus PCV (N. Verdager, IBMB); Picornavirus (I. Fita, IBMB); viral receptors (J.M. Casasnovas, CNB); DNA translocation complexes, transcriptional complexes (M. Coll, IBMB); DNA superhelices (J.A. Subirana, UPC); multiprotein complexes involved in signalling transduction (A. Albert, IQFR); integral-membrane metalloproteins (F.X. Gomis-Rüth, IBMB); T4 filamentous phages (M.J. van Raaij, US); and large multimodular protein (J. Sanz-Aparicio, IQFR).



a



b

Figure 1. Example of a large unit cell projects: **a)** the triatoma virus, unit cell parameters: $a=336$, $b=351$ and $c=332\text{\AA}$ (Dr. Guerin group, Bilbao), **b)** bacteriophage T4 short tail fibre, unit cell parameters $a=b=50.78\text{\AA}$, $c=435.6\text{\AA}$ (Dr. van Raaij group, Santiago de Compostela). Data collected at ESRF undulator beamlines ID14 and ID29.

Moreover, spectacular advances in genomics and the possibility of producing massive amounts of protein predict a huge growth in both the number of protein crystallography groups and the number of samples to be analysed. Protein crystallography groups have been growing exponentially in Spain and this tendency is expected to continue as has happened in other countries. Nowadays, we have listed 25 research groups (with projects financed by the Spanish Ministry of Education and Science, formerly of Science and Technology) (Fig. 2) in a total of 12 institutes spread all over the country, which implies that the number of direct synchrotron users exceeds a hundred (see Annexe II). On the other hand, the intrinsic character of the research in structural biology, usually carried out in collaboration with biochemistry and molecular biology groups and medical centres, implies an extraordinary amplification of the benefits of this kind of research. Thus, a large number of collaborating groups would be beneficiaries of the protein crystallography line in ALBA. An initial estimate shows that approximately 244 national and international research groups would be indirect users/beneficiaries of this beamline. Furthermore, the currently operative research groups working in the field of macromolecular X-ray crystallography in Portugal (Dra. Sandra Ribeiro, Coimbra; Prof. Ana Margarida Damas, Porto; Prof. Maria Armenia Carrondo, ITQB (Lisbon); and Prof. Maria João Romão, UNL (Lisbon)) have expressed their full support and interest in participating and benefiting from such a pioneering facility in the Iberian Peninsula. Other protein crystallography groups in southern France (Marseille, Toulouse) are also expected users of a MX facility at short driving distance.

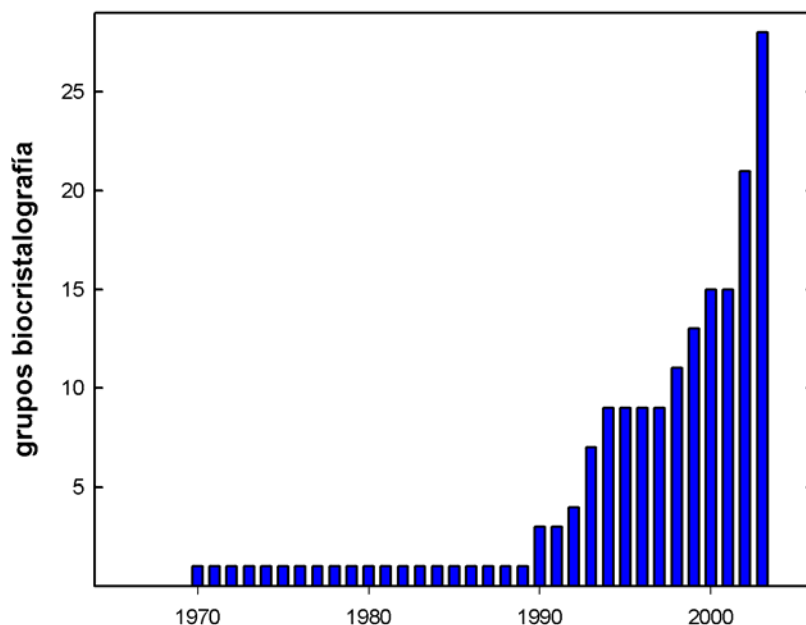


Figure 2. Evolution of the total number of (main) senior researchers in biocrystallography in Spain (including Ramón-y-Cajal Programme Researchers with awarded research grants)

All these structural biology crystallographers are regular users of synchrotron radiation facilities (ESRF, DESY, BESSY, ALS ELETTRA, LURE, SRS), and in all cases the research is strongly dependent of the availability of this radiation source. Moreover, congestion at the European Synchrotron Radiation Facility (ESRF), with requests for beam time approximately doubling the effective time available, together with intrinsic limitations of the Spanish beam line (BM16), makes it impossible to use such a facility as often as required in such a competitive research field.

Pharmaceutical and biotech companies are becoming world-wide the main industrial users of synchrotron facilities. The need to know the structure of protein-ligand complexes at atomic resolution for a rational approach in the development and optimization of new drug has fostered the pharma and biotech industry to extensively use X-ray crystallography. Spanish main pharmaceutical companies such as Almirall Prodesfarma, Laboratorios Esteve and Uriach are already engaged in macromolecular crystallography projects and would greatly benefit from a synchrotron facility located nearby. New biotech companies such as CrystaX Pharmaceuticals and Oryzon Genomics are also using macromolecular crystallography and are potential users of a MX beamline in ALBA.

II. Scientific Case

II.1 General characteristics of the beamline

Research on large macromolecules, assemblies and complexes by X ray diffraction raises some difficulties apart from those usually associated with sample preparation and growth of crystals as such crystals diffract weakly and contain very large unit cells (500-1000 Å). In this proposal, we suggest the use of an appropriate fully dedicated beamline for these samples that would allow the collection of high-resolution data. In such a line, the flux must be high, the beam divergence low and the size of the beam in the sample should be able to be reduced to close to 50µm.

The beamline should be tuneable within a range of energies that includes the most common anomalous scatterers employed for determination of structures by MAD or SAD.

The proposed line should also have a microdiffractometer and a large surface detector allowing the collection of high-resolution data. With regard to the cryogenic system, the installation of a robotic sample exchanger is important because apart from accelerating one of the most tedious steps of the experiment it allows the rapid analysis of a group of crystals and selection of the one with the best diffraction pattern.

Obviously, this beamline is also suitable for smaller unit cells and would be particularly useful for small or weak diffracting crystals. New robotized nanodrop crystallisation techniques are being implemented in protein crystallography laboratories, and we expect them to be of general use in a couple of year. These techniques drastically reduce the amount of protein needed for crystallisation trials, thus accelerating the process

of structural determination. However, nanodrop crystallization implies, in many cases, a reduced size of the crystal. Therefore, it is expected in the near future a large increase of projects where only very small crystals are available, correlating with a decrease in the amount of sample required.

II.2 No overlap with other beamlines

This proposal involves a large range of experiments that are impossible to carry out within BM16 (Spanish CRG beamline at the ESRF) because of the general design and the fact that it is installed on a bending magnet. For various Spanish groups, the majority of their projects cannot be tackled with this facility (Fig. 1), which is optimised for relatively large and rather well diffracting crystals. Moreover, the large aforementioned unit cells, of several hundred Å, can not be resolved either in this line. Besides, tuning to large wavelengths (more than 1.3 Å) results in a dramatic reduction of the intensity in BM16 which, taken together with the limited size of the detector, produces a drastic reduction of the measurable resolution limits.

It is important to stress that the proposed beamline is not a microfocus line in strict sense (ID13 ESRF type). Microfocus beamlines can be useful in particular cases (very small crystals or needles) but the beam divergence makes it impossible to collect data from crystals with large unit cells. Furthermore, the crystal damage is dramatic in this type of lines, making it necessary to move the crystals in order to successively expose different zones, or to use various crystals. This makes it practically impossible to properly carry out MAD experiments and on most occasions, the data after merging are of very poor quality.

III. Beamline technical specifications

III.1 Optical arrangement

Calculations based on wigglers / undulators published material on the CELLS web site provide a curve similar to the one shown in Fig. 3. But in the above, magnet gaps for ID's (Insertion device) are limited to 20mm which limits to use high power wigglers. Every other modern MX line under construction is built on an undulator. The plot provides evidence that a short undulator (ALBA), will significantly out-perform a bending magnet (BM16, ESRF). An estimate of the performance of such a facility is given below. These performance figures need to be verified during the next (feasibility) stage.

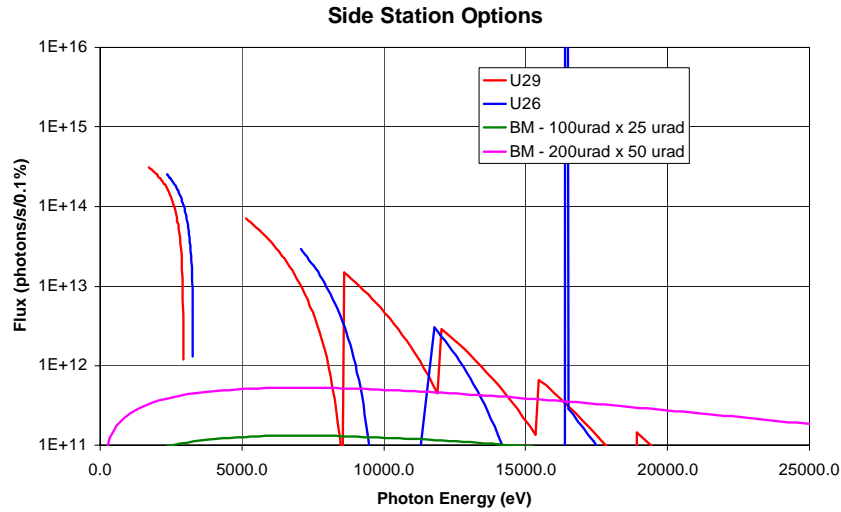


Figure 3. Representation of flux against energy for different undulators and bending magnets. The figure highlights the differences. An undulator offers the possibility of a high flux at different range of energies.

Table I. Optical characteristics

Beam at Source	
□ x,y [mm]	0.08,0.008
□' x, y [mrad]	0.035,0.003
Flux in to 0.1x0.025 mrad aperture (10^{-3} bandpass)	6×10^{13}
Collection efficiency	0.8
Beam at Experiment	
Focal Spot Size (FWHM h,v) /mm	0.1, 0.05
Divergence (h,v) /mrad	0.2, 0.014
Flux at 0.98\AA /ps ⁻¹	5×10^{12}
	Calculated

Optics – The recommended energy range should cover from 5.5 KeV (~2.25 λ) to 14.5 KeV (0.85 λ). This includes all common absorption edges (Se L2 12.658 KeV, Fe L2 7.112 KeV, Hg L3 12.284 KeV, Pt L3 11.564 KeV, Cu K 8.979 KeV, Zn K 9.659 KeV etc etc). The limits are chosen:

- a) To include the Kr K edge at the hard energy end (14.326) and
- b) to allow S SAD at a fairly long wavelength

Especially the last possibility is probably essential to tackle the phasing problem in small crystals and large macromolecular complexes that are usually very sensitive to radiation damage.

Bandpass - 1.5×10^{-4} for MAD measurements. This is needed for reasonable sampling of the absorption edge.

Convergence – If a variable beam size optics is employed (KB or sagittal focussing monochromator plus mirror), this parameter can vary. 0.1 mrad is needed for big (1000 Å) cell constants, 0.5 mrad is adequate for normal usage. 0.3 mrad is suggested for the present proposal as a compromise.

Energy stability – A reasonable value is 0.25 eV, which gives an f' stability of about 10% at the point of inflexion of the Se edge. This value is essential for structure solution using anomalous signals

Beamsize at sample – Our suggestion propose 50 – 250 μm . Upon receipt of more recent information on the characteristics of ALBA, these values, together with the convergence, will be reassessed. 50 μm would be a desirable beamsize.

Beam stability and intensity – [valor beam stability] and a flux of $> \sim 10^{12}$ ph/s into the 1.5×10^{-4} bandpass over the whole energy range.

Design Issues

Associated with this beamline are several design issues that need to be investigated in due course. These principally concern the use of undulators on Alba and what precise optical components are required for the beamline to meet the required specification.

Focusing of the undulator beam can be achieved in several ways :

- A single toroidal mirror to focus horizontally and vertically
- A sagittal bent monochromator to focus horizontally followed by a mirror bent to a large radius to focus vertically
- Two orthogonal mirrors to provide separate focusing in the horizontal and vertical directions (Kirkpatrick-Baez arrangement).

All of these arrangements have been successfully used on undulator sources (Fig. 4). The final choice of systems depends on factors such as cost and any requirement for flexibility in focusing. A full assessment of these options will be done in the next (feasibility) stage The protein crystallography beamline working group felt that the requirement for small

(10 μm) beams could be met by slitting down the beam dimensions and that microfocussing was probably unnecessary and possibly more problematic. This issue will be further investigated in due course.

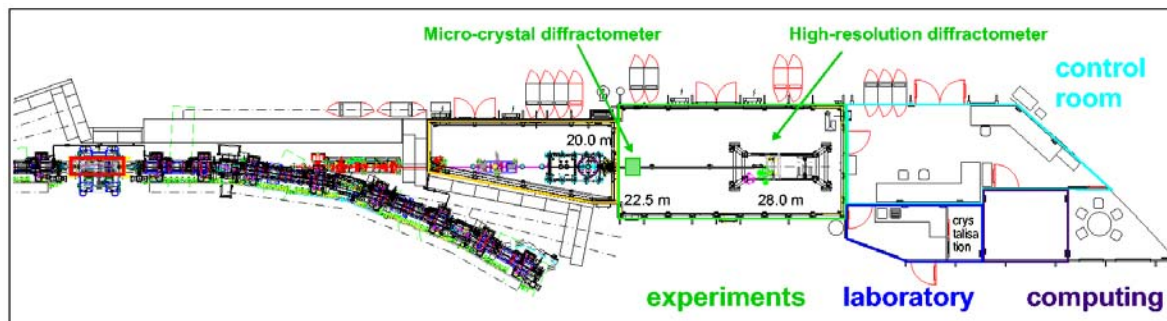


Figure 4. Scheme of the macromolecular single-crystal diffraction beam line X06SA from Swiss Light Source (Villigen, Switzerland).

III.2 Goniostat and diffractometer

This subchapter deals with the direct environment of the sample, the interface between the out coming X-ray beam and the detector on which diffraction data will be collected. As it is not possible to foresee which will be the state of the arts of the technology in 2010, it is not advisable to make a detailed proposal. The sample, single crystals mounted either on a cryo-loop (or, exceptionally, in sealed thin-walled glass-capillaries) will be attached to a goniometer head by means of a magnetic base. State-of-the-art (as of 2004) would include a goniostat to position and rotate the sample and detector for measurements, with either multiple axes and 3 μm sphere of confusion or a single rotation axis with less than 1 μm . In the first case, the sample could be better aligned in the beam as there are more axis of rotation to position it, but the second could be more appropriate if we consider that there will be a series of other devices around the sample, like an automated sample exchanger (see below), a cryostream, and a fluorescence detector, besides several other. A decision on the number of circles has to be made in due time. In any case, the system installed should enable auto-loading and auto-changing of samples, as well as auto-alignment in the beam. The micro or mini-diffractometers, as currently available at beamline ID23-1 and ID14-4 with the camera along the beam path and a 3-click centering software, obviously further developed as of 2010, is conceivable and adequate. One micro/mini-diffractometer model, the MD2, is sold by EMBL/ESRF MAATEL based on technology developed at ESRF in Grenoble.

III.3 Detector

A further issue is the collection of X-ray diffraction data. To collect the data that shall enable solution of a three-dimensional structure it has to be considered that a certain number of degrees rotating around one (or several) axis have to be measured. In general, frames of 0.1 to 1.5 degrees are taken, with exposure times in the range of few seconds. Depending on the

cell parameters, space group of the crystal, and the type of experiment (e.g. multiple-anomalous diffraction with collection of Friedel mates), more or less degrees of data rotating around an axis must be collected, typically between 30 and 180 degrees.

The design of end-station instrumentation and data acquisition systems is currently under steady evolution. As of 2010, they will be based upon those that are currently state-of-the-art, but they will be subjected to the emergence of improvements and new technologies. Now and in the future, in particular taking into account the kind of samples that are intended to be measured in the proposed beamline, the requirements for a detector will be high readout speed, a large detector area, and low background/noise. The first to permit collection of complete datasets in as short times as possible and a fast, more versatile data acquisition; the second to enable to collect data to higher resolution under separation of the diffraction spots; and the third to get data as neat as possible for posterior integration.

Currently, practically all MX beamlines operate with CCD-technology based detectors of large area, such as the ADSC Quantum system, MarCCD and MarMosaic from MarResearch, or Proteum 300 from Bruker, some of them combining several CCDs (2x2 or 3x3). These devices enable fast read out speeds (reaching less than a second per frame of data collected) and it is conceivable that the technological development will continue on this path for the next years, with optimised and even faster systems, with steadily larger areas. Two glimpses of new technologies, that could represent real alternatives by the time under consideration, are the Flat Panel technology, currently under development by MarResearch, and the pixel array technology.

III.4 Fluorescence detector

The beamline must be provided with a fluorescence detector. This is required to accurately determine the absorption edge of metals during experiments to measure anomalous diffraction, like SAD or MAD. This device is introduced automatically (controlled from the users area) in vicinity of the sample, this requires a careful design of the direct environment of the sample to avoid clashes. The detector must be able to perform scans around the theoretical absorption edges of the elements intended to be employed for phasing strategies and cover the same range of wavelengths as the beamline. Currently, examples of employed detectors are the EURISYS detector or a Rontec Silicon Drift Diode (SDD) detector.

III. 5 Cryogenic System

Crystals of biological macromolecules at near to room temperature are sensitive to X-rays and frequently suffer from radiation damage, especially when X-ray experiments are carried out on highly intense synchrotron beamlines. Performing such experiments at cryogenic temperatures greatly reduce radiation damage and thus produce higher quality diffraction data.

Radiation damage of biocrystals appears to be related to the formation of free radicals. But performing diffraction experiments on protein crystals cooled to near liquid-N₂ temperature (80-120 K) leads to significant reduction in radiation damage. In a typical experimental setup of diffraction experiment at cryogenics temperature the crystal is mounted in a thin nylon fiber loop and rapidly cooled, either in a cold gas stream, or by immersion in a cryogen such as liquid

nitrogen. The temperature of the crystal is maintained by a stream of nitrogen gas during the diffraction measurement. To avoid the formation of ice around the crystal, the nitrogen stream is shielded against humidity by a coaxial stream of warm dry air. This kind of systems is currently available as a Cryoshutter that has been designed to work via a foot pedal, leaving you both hands free to manipulate your crystal.

A humidity control chamber device, as the one presently commercialized by Proteros, would be an interesting equipment to install in order to check optimal diffracting conditions prior to cryo-cooling.

III. 6 Automation

The growing demand for beam-time at synchrotron radiation sources all around the world has made it a necessity to increase the efficiency with which users can perform their experiments. To achieve this goal, automation at all levels of diffraction measurements is necessary in order to realise a fully automated beamline. Several steps in the data collection process have, up to now, been automated in the different protein crystallography beamlines. In this sense similar levels of automation can be envisaged in the MX beamline at ALBA. First, the beamline alignment for different wavelengths and the control of the beam intensity should be automated. An automated accurate calibration of the monochromator near the absorption edge can now be realised for anomalous experiments in most of beamlines. This point is very important considering the increasing relevance of anomalous diffraction experiments in structure determination. The percentage of structures solved using anomalous dispersion measurements, usually from SeMet proteins, is increasing dramatically, and this approach is rapidly becoming the method of first choice. Second, users can remotely centre their crystals by a simple mouse click or alternatively can use the automated procedure. Third, fully automated data collection can also be implemented. The data collection can be run completely unattended; the beamline program should automatically take care of both the beam optimization and the wavelength modification. Fourth, automation of reduction of diffraction data can also be envisaged with different software and, depending on the data quality, the analysis can also lead to automatic model building. Fifth, the automation of sample mounting and dismounting is nowadays been developed in most of synchrotron radiation sources (SSRL, ESRF, Photon Factory...) and consequently it appeared mandatory to introduce this improvement in a Protein Crystallography devoted beamline. Indeed, this automation would present four main advantages:

- First, automation of the crystal mounting would allow users to save time and thus increase the efficiency of their experiments.

- Second, crystal samples are usually pre-frozen in a Hampton Research CrystalCap pin loop and stored in liquid nitrogen. Automation of crystal mounting would prevent any improper transfer from the cryogenic storage location to the diffractometer head and therefore prevents from ice formation and warming.

- Third, in most cases, users come to synchrotron beamlines with different crystals but without having tested all of them. As soon as they find a suitable crystal with regard to the diffraction quality, they start data collection. The automated process would allow users to decrease the screening time, to test many more crystals and finally to choose the best one.

- Fourth, users have to work in the heavy environment of the diffractometer and mistakes (especially during the night) may result in wasted beam-time and beamline-component damage. Automation of crystal sample mounting would prevent these potential drawbacks.

A natural way to automate this mounting/dismounting process is to use robots. Several crystal sample mounting/dismounting robots are commercially available or have been developed in-house at different synchrotron sources. Especially interesting are the initiatives from the SSRL (Stanford Synchrotron Radiation Laboratory) and ESRF.

The setup adopted by SSRL consists of a multi-axis robot that loads the metal base cryopins stored in a liquid-nitrogen cryostat. The SSRL robot is a four-axis robot and the crystals are stored in a 96-sample cassette placed in a Dewar, which can hold up to three cassettes. This system employs a robotic sample changer and an automated crystal centering algorithm to complete the crystal screening process without human intervention and will be available to the general user community on the four protein crystallography beamlines in the SPEAR3 storage ring. On the other hand, two different initiatives are now being developed at the ESRF, the first one done at the ID14 beamline and the second one developed at the BM30A beamline.

In BM30A it has been developed CATS (a Cryogenic Automated Transfer System) that is a highly reliable, versatile and powerful system to mount and dismount crystals on the diffractometer head. The crystal samples are stored in the pin loop of Hampton Research CrystalCaps and placed in a carousel of a liquid-nitrogen Dewar. CATS allows the use of the two CrystalCap standards (the screw and the magnetic caps) and always leaves the crystals in liquid nitrogen during the transportation between the Dewar and the diffractometer head. CATS has increased the efficiency of recording data. Indeed, CATS has removed the dead time of entering the experimental hutch. CATS has also reduced improper mounting/dismounting of crystal samples. Finally, CATS has allowed users to screen more crystals. As we mentioned earlier, the different steps of diffraction measurements have been successively automated on FIP. The CATS integration in the automated control of the beamline allowed the FIP team to plug this gap and now FIP can be considered as a fully automated beamline for protein crystallography.

III. 7 Computing and software

The computer system to control detector and data collection, as well as to take care of the massive amount of data produced, will be a current state-of-the-art system with commensurate enhancements of all data acquisition software with large data storage capacity (in the Tb range). Mechanisms to permit rapid transfer of the measured data to transportable storage devices by the users must be present, too. Backup through Firewire and USB disks, and also by ftp, should be available at the ALBA source.

The MX beamline should also have facilities to allow data processing and analysis. Support facilities to continue computation (including data backup) after scheduled user session has finished (i.e. while others are using the beamline). This could be achieved via a central facility or by the provision of sufficient computational facilities at the beamline.

III. 8 Ancillary laboratory

Support facilities to allow sample storage, inspection and manipulation are absolutely necessary for the PX beamline. This includes provision of basic equipment, like stereo-microscope with polarising lenses, crystal mounting and manipulation tools, liquid N₂ dewars for temporary storage of samples, pipettes, glassware etc. Especially important is the presence of thermostable cupboards/chambers at constant temperature (20°C and 4°C) for crystallisation plates that could maintain the extremely fragile crystal samples during the scheduled time.

III. 8 Personnel

It has to be considered that a beamline is intended for providing service to users travelling to the synchrotron to perform diffraction experiments or to users that choose to send their samples adequately prepared to be handled by synchrotron staff (“FedEx crystallography”). Taking into account the development within the field, it is conceivable that the latter choice will be more and more employed, in particular by pharmaceutical companies working on rational drug design approaches. Accordingly, both kind of experiments should be made possible, the one of the dedicated scientist with a particular highly difficult project and the one of the company as the latter probably will provide additional funding to contribute to the maintenance of the synchrotron.

The beamline must be taken care of by responsible staff scientists, professionals with a potent background in the mechanical and optical aspects of the beamline as well as in macromolecular single-crystal diffraction data collection and processing. Two or three staff scientists with a Ph.D. in a related discipline would be the main responsables. It must be taken into account that allocated beamtime runs 24 h per day and 7 days per week during the periods of full operativity of a synchrotron. Accordingly, staff in charge of coaching users or performing “FedEx crystallography” tasks should be enough in number. In the time where no beamtime is allocated to users, these scientists will be in charge of the maintenance, improvement and development of the beamline or even carrying out their own diffraction experiments (inhouse research).

Besides these staff scientists, a technical engineer (taking care of the optics and mechanics) and one or two technicians would be required to assist in the tasks related to user coaching, provision of the necessary consumables to furnish an ancillary user lab and the maintenance of the latter. The assistance of an additional System Manager to take care of the computing will also probably be a must.

Annexe I. Estimated costs (includes undulator)

Undulator	350 KEu
Optics - monochromator	250 KEu (more expensive if you use sagittal focussing)
Optics – mono crystals	30 KEu
Optics – mono cryocooling	150 KEu
Optics - mirrors	300 KEu
Diffractometer	350 KEu
Alignment table	40 KEu
Sample changer	240 KEu
Cryo for sample	40 KEu
Slits 2 sets both V and H	130 KEu
Vacuum components	100 KEu
Beam position monitors	50 KEu
Hutches	175 KEu
Cabling and fluids	70 KEu
PSS	36 KEu
Computation	25 KEu
Control electronics	40 KEu
Data acquisition	25 KEu
Racks	25 KEu
Networking	5 KEu
MCA + Fluorescence detector	25 KEu
Control cabins	25 KEu
Tools	15 KEu
Air conditioning	15 KEu
Additional shielding	10 KEu
2 – D Detector	400 KEu
Detector support	30 KEu
Additional components	25 KEu
Lab (microscopes etc)	25 KEu
Furniture	10 KEu
Shutter	30 KEu
Be windows	5 Keu
External design effort	50 KEu
Exposure box	25 KEu
General electronics	15 KEu
Beam pipes, crosses, bellows etc	25 KEu
Contingency 10 %	316 KEu
TOTAL	3477 KEu

Annexe II. Spanish MX users

Group leader:	Armando Albert
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Department:	Grupo de Cristalografía Macromolecular y Biología Estructural. Instituto de Química-Física "Rocasolano"(CSIC)
Address:	Serrano 119, 28006 Madrid.
Group members:	María José Sánchez-Barrena, Marta Jimenez, Sandra Moreno.

5 relevant publications, including synchrotron-beamline (if any):

Albert, A., Yenush, L., Gil-Mascarell, R., Rodriguez, P.L., Patel, J., Martinez-Ripoll, M., Blundell, T.L. and Serrano, R.

Structure of yeast Hal2p, a major target of lithium and sodium toxicity. Identification of a framework of interactions determining cation sensitivity.

Journal of Molecular Biology (2000) 295, 927-938. SRS DARESBURY

Albert, A., Martínez Ripoll, M., Espinosa-Ruiz, A., Rodriguez, P.L., Culiñez-Macia, F. and Serrano, R. The X-ray structure of the FMN binding protein atHal3 provides the structural basis for the activity of a regulatory domain involved in signal transduction.

Structure (2000), 8, 961-969 ESRF GRENOBLE

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Structural Enzymology of Li⁺-sensitive/Mg²⁺-dependent Phosphates

Journal of Molecular Biology (2002) 320, 1087-1094. ESRF GRENOBLE

Sanchez-Barrena MJ, Martinez-Ripoll M, Galvez A, Valdivia E, Maqueda M, Cruz V, Albert A.

Structure of bacteriocin AS-48: from soluble state to membrane bound state

J Mol Biol. (2003), 334, 541-549. ESRF GRENOBLE

Sánchez-Barrena, M.J., Martínez-Ripoll, M., Zhu, J.K. and Albert, A.

The structure of the *Arabidopsis thaliana* SOS3: molecular mechanism of sensing calcium for salt stress response

J. Mol. Biol. (2004) in press. ESRF GRENOBLE

Number of Synchrotron related publications in the last 5 years: About 10

Collaborators:

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5 relevant publications, including synchrotron-beamline (if any):

Gomis-Rüth, F.X., Moncalián, G., Pérez-Luque, R., González, A., Cabezón, E., de la Cruz, F. & Coll, M. (2001). The bacterial conjugation protein TrwB resembles ring helicases and F1-ATPase. *Nature* **409**, 637-641.

Pereira, P.J.B., Macedo-Ribeiro, S., Párraga, A., Pérez-Luque, R., Cunningham, O., Darcy, K., Mantle, T.J. & Coll, M. (2001) Three-dimensional structure of human biliverdin-IX beta reductase, an early fetal bilirubin-IX beta producing enzyme. *Nature Structural Biology* **8**, 215-220.

Pereira, P.J.B., Vega, M.C., González-Rey, E., Fernández-Carazo, R., Macedo-Ribeiro, S., Gomis-Rüth, F.X., González, A. & Coll, M. (2002). Trypanosoma cruzi macrophage infectivity potentiator has a rotamase core and a highly exposed alpha-helix. *EMBO Reports* **3**, 88-94

Lisgarten, J.N., Coll, M., Portugal, J., Wright, C.W. & Aymamí, J. (2002) The antimalarial and cytotoxic drug cryptolepine intercalates into DNA at cytosine-cytosine sites. *Nature Structural Biology* **9**, 57-60.

Guasch A., Lucas, M., Moncalián, G., Cabezas, M., Pérez-Luque, R., Gomis-Rüth, F.X., de la Cruz, F. & Coll, M. (2003). Recognition and processing of the origin of transfer DNA by conjugative relaxase TrwC. *Nature Structural Biology* **10**, 1002-1010.

Number of Synchrotron related publications in the last 5 years: 49

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5 relevant publications, including synchrotron-beamline (if any):

Structural Analysis of a Series of Antiviral Agents Complexed with Human Rhinovirus 14
J. Badger, I. Minor, M.J. Kremer, M.A. Olivera, T.J. Smith, J.P. Griffith, D.M.A. Guérin, S. Krishnaswamy, M. Luo, M.G. Rossmann, M. McKinlay, G. Diana, F.J. Dukto, M. Fancher, R.R. Rueckert and B.A. Heinz.
Proceeding of the National Academy of Sciences. USA, Vol. 85, pp 3304-3308 (1988).

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J.V.N. Vara Prasada, Kimberly S. Para, E. Lunney D.F. Ortwine, J. B. Dunbar, Jr., D. Ferguson, P.J. Tumino, D. Hupe, B.D. Tait, J.M. Domaglia, C. Humblet, T.N. Bhat, B. Liu, D.M.A. Guérin, E.T. Baldwin, J.W. Erickson and T. Sawyer.
Journal of the American Chemical Society, Vol. 116, pp 6989-6990 (1994).

Structure-Based Design of Achiral, Nonpeptidic Hydroxybenzamide as a Novel P2/P2' Replacement for the Symmetry-Based HIV Protease Inhibitors.
R.S. Randad, L. Lubrkwoska, A.M. Silva, D.M.A. Guérin, S.V. Gulnik, B. Yu and J.W. Erickson.
Bioorganic & Medicinal Chemistry, Vol. 4, No. 9, pp 1471-1480 (1996).

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G.A. Tavares, H. Souchon, D.M.A. Guérin, M.B. Lascombe and P.M. Alzari
in "Carbohydrates from *Trichoderma Reesei* and other Microorganisms. Structures, Biochemistry, Genetics and Applications", edited by M. Claeysens, W. Nerinckx and K. Piens. The Royal Society of Chemistry, London, pp 174-181 (1998).

Number of Synchrotron related publications in the last 5 years: 2

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5 relevant publications, including synchrotron-beamline (if any):

González, B., Pajares, M.A., Martínez-Ripoll, M., Blundell, T.L. and Sanz-Aparicio, J.
Crystal structure of rat liver betaine-homocysteine S-methyltransferase reveals new oligomer features and conformational changes upon substrate binding.
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González, B. Pajares, M.A., Hermoso, J. and Sanz-Aparicio, J.
Crystal structure of methionine adenosyl-transferase complexes gives new insights into the catalytic mechanism.
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J. Mol. Biol. (2000), 300, 363-375. (D2AM, ESRF)

Sanz-Aparicio, J. Hermoso, J., Martínez-Ripoll, M., Lequerica, J.L. and Polaina, J.
Crystal Structure of α -glucosidase A from *Bacillus polymyxa*: Insights into the Recognition Mechanisms of Family 1 Glycosyl-hydrolases:
J. Mol. Biol. (1998), 275, 491-502. (D2AM, ESRF)

Number of Synchrotron related publications in the last 5 years: 14

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5 relevant publications, including synchrotron-beamline (if any):

Verdaguer N, Fita I, Reithmayer M, Moser R, Blaas D.
X-ray structure of a minor group human rhinovirus bound to a fragment of its cellular receptor protein.
Nat Struct Mol Biol. 2004 May;11(5):429-34.
Beamlines: ESRF ID14.4, ID29

Ferrer-Orta C, Arias A, Perez-Luque R, Escarmis C, Domingo E, Verdaguer N.
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template-primer RNA.
J Biol Chem. 2004 Nov 5;279(45):47212-21.
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coxsackie B5 virus to infect swine. J Virol. (2003) 77:9780-9.
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N.Verdaguer, S. Corbalan-Garcia, W.F. Ochoa, I. Fita, J.C. Gomez-Fernandez. "Ca²⁺ bridges the
membrane-binding domain C2 of Protein Kinase Ca directly to phosphatidylserine EMBO J. 18,
(1999), 6329-6338. Beamline: DESY X31

Number of Synchrotron related publications in the last 5 years: 13

Collaborators:

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5 relevant publications, including synchrotron-beamline (if any):

Karathanassis D, Stahelin RV, Bravo J, Perisic O, Pacold CM, Cho W, Williams RL.
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EMBO J 2002 Oct 1;21(19):5057-5068.
Sincrotrón: ESRF, Grenoble beamlines: ID29, ID14-4

Bravo J, Karathanassis D, Pacold CM, Pacold ME, Ellson CD, Anderson KE, Butler PJ, Lavenir I, Perisic O, Hawkins PT, Stephens L, Williams RL.
The Crystal Structure of the PX Domain from p40(phox) Bound to Phosphatidylinositol 3-Phosphate
Mol Cell. 2001 Oct;8(4):829-39.
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J. Bravo, Z. Li, N. A. Speck & A. J. Warren.
The leukemia-associated AML1 (Runx1)-CBF complex functions as a DNA-induced molecular clamp
Nature Structural Biology, 2001, 8 (4) 371 - 378
Sincrotrón: ESRF beamline ID14-3

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Receptor recognition by gp130 cytokines
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J. Bravo, D. Staunton, J.K. Heath & E.Y. Jones.
Crystal Structure of a cytokine binding region of gp130
EMBO J, (1998) 6: 1665-1674
Sincrotrón: ESRF beamline BM14

Number of Synchrotron related publications in the last 5 years: 13

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Group members: **Luis Alfonso Martinez de la Cruz, Olatz Sabas Garcia Borreguero, Marta Sanz Martinez, Mercedes Vazquez Chantada.**

5 relevant publications, including synchrotron-beamline (if any):

Martinez-Cruz LA, Dreyer MK, Boisvert DC, Yokota H, Martinez-Chantar ML, Kim R, Kim SH.
Crystal structure of MJ1247 protein from *M. jannaschii* at 2.0 Å resolution infers a molecular function of 3-hexulose-6-phosphate isomerase.
Structure (Camb). 2002 Feb;10(2):195-204.

Chi YI, Martinez-Cruz LA, Jancarik J, Swanson RV, Robertson DE, Kim SH.
Crystal structure of the beta-glycosidase from the hyperthermophile *Thermosphaera aggregans*: insights into its activity and thermostability.
FEBS Lett. 1999 Feb 26;445(2-3):375-83.

Gallwitz H, Bonse S, Martinez-Cruz A, Schlichting I, Schumacher K, Krauth-Siegel RL.
Ajoene is an inhibitor and subversive substrate of human glutathione reductase and *Trypanosoma cruzi* trypanothione reductase: crystallographic, kinetic, and spectroscopic studies.
J Med Chem. 1999 Feb 11;42(3):364-72.

Martinez-Cruz LA, Rubio A, Martinez-Chantar ML, Labarga A, Barrio I, Podhorski A, Segura V, Sevilla Campo JL, Avila MA, Mato JM.
GARBAN: genomic analysis and rapid biological annotation of cDNA microarray and proteomic data.
Bioinformatics. 2003 Nov 1;19(16):2158-60.

Martinez-Chantar ML, Corrales FJ, Martinez-Cruz LA, Garcia-Trevijano ER, Huang ZZ, Chen L, Kanel G, Avila MA, Mato JM, Lu SC.
Spontaneous oxidative stress and liver tumors in mice lacking methionine adenosyltransferase 1A.
FASEB J. 2002 Aug;16(10):1292-4. Epub 2002 Jun 07.

Number of Synchrotron related publications in the last 5 years: 3

Collaborators:

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5 relevant publications, including synchrotron-beamline (if any):

Albert, A., Martínez Ripoll, M., Espinosa-Ruiz, A., Rodriguez, P.L., Culiañez-Macia, F. and Serrano, R. The X-ray structure of the FMN binding protein atHal3 provides the structural basis for the activity of a regulatory domain involved in signal transduction.

Structure (2000), 8, 961-969 ESRF GRENOBLE

Patel, S., Martínez-Ripoll, M., Blundell, T.L. and Albert, A.

Structural Enzymology of Li⁺-sensitive/Mg²⁺-dependent Phosphates

Journal of Molecular Biology (2002) 320, 1087-1094. ESRF GRENOBLE

Sanchez-Barrena MJ, Martínez-Ripoll M, Galvez A, Valdivia E, Maqueda M, Cruz V, Albert A.

Structure of bacteriocin AS-48: from soluble state to membrane bound state

J Mol Biol. (2003), 334, 541-549. ESRF GRENOBLE

Juan A. Hermoso, Begoña Monterroso, Armando Albert, Beatriz Galán, Oussama Ahrazem, Pedro García, Martín Martínez-Ripoll, José Luis García, Margarita Menéndez.

Structural Basis for Selective Recognition of Pneumococcal Cell Wall by Modular Endolysin from Phage Cp-1.

Structure, (2003). Vol. 11, 1239-1249. (Portada de la revista) ESRF-BM14.

Mancheño, J.M., Martín-Benito, J., Martínez-Ripoll, M., Gavilanes, J.G. and Hermoso, J.A.

Crystal and electron microscopy structures of Sticholysin II actinoporin reveal insights into the mechanism of membrane pore-formation

Structure (2003). Vol. 11, 1319-1328. ESRF-BM14.

Number of Synchrotron related publications in the last 5 years: About 15

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5 relevant publications, including synchrotron-beamline (if any):

Garcia-Alvarez B, Bobkov A, Sonnenberg A, de Pereda JM (2003)
Structural and functional analysis of the actin binding domain of plectin suggests alternative mechanisms for binding to F-actin and integrin beta4
Structure, 11(6):615-625

De Pereda JM, Waas WF, Jan Y, Ruoslahti E, Schimmel P, Pascual J (2004)
Crystal structure of a human peptidyl-tRNA hydrolase reveals a new fold and suggests basis for a bifunctional activity
J Biol Chem., 279(9):8111-8115.
Stanford Synchrotron Radiation Source (USA), beamline 1.5

García-Álvarez B, de Pereda JM, Calderwood DA, Ulmer TS, Critchley D, Campbell ID, Ginsberg MH, Liddington RC (2003)
Structural determinants of integrin recognition by talin
Mol. Cell 11(1): 49-58
Brookhaven NSLS (USA), beamline X12b

Bakolitsa C, de Pereda JM, Bagshaw CR, Critchley DR, Liddington RC. (1999)
Crystal structure of the vinculin tail suggests a pathway for activation
Cell. 99(6):603-613.
SRS Daresbury (UK), beamline 7.2

de Pereda JM, Wiche G, Liddington RC. (1999)
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EMBO J. 18(15):4087-4095.
Advanced Photon Source, Argonne Nacional Laboratory (USA), beamlines BM-14C and BM-14D

Number of Synchrotron related publications in the last 5 years: 4

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5 relevant publications, including synchrotron-beamline (if any):

K. Papanikolopoulou, S. Teixeira, H. Belrhali, V.T. Forsyth, A. Mitraki & M.J. van Raaij (2004)
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M.J. van Raaij, G. Schoehn, M.R. Burda & S. Miller (2001)
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M.J. van Raaij, A. Mitraki, G. Lavigne & S. Cusack (1999)
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Nature 401, 395-398 - ESRF ID14-3

Number of Synchrotron related publications in the last 5 years: 7

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5 relevant publications, including synchrotron-beamline (if any):

The crystal structure of the DNA binding domain of yeast RAP1 in complex with a telomeric DNA site.
P. König, R. Giraldo, R., L. Chapman, D. Rhodes.
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D. Hargreaves, R. Giraldo, S. Santos-Sierra, R. Boelens, D.W. Rice, R. Díaz-Orejas, J.B. Rafferty.
Acta Crystal. 2002; **D58**: 355-358.

3. Structural and functional analysis of the Kid toxin protein from *E. coli* plasmid R1.
D. Hargreaves, S. Santos-Sierra, R. Giraldo, R. Sabariego-Jareño, G. de la Cueva-Méndez, R. Boelens, R. Díaz-Orejas, J.B. Rafferty.
Structure 2002; **10**: 1425-1433.

4. A conformational switch between transcriptional repression and replication initiation in the RepA dimerization domain.
R. Giraldo, C. Fernández-Tornero, P.R. Evans, R. Díaz-Orejas, A. Romero.
Nature Struct. Biol. 2003; **10**: 565-571.

Number of Synchrotron related publications in the last 5 years: 3

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Group members: **Eva Valencia, Jordi Querol, Xavie Carpena, Barbara Calisto, Rosa Perez, Francisco Martin, Diana Hervás**

5 relevant publications, including synchrotron-beamline (if any):

Verdaguer N, Fita I, Reithmayer M, Moser R, Blaas D.
X-ray structure of a minor group human rhinovirus bound to a fragment of its cellular receptor protein.
Nature Structural and Molecular Biology. 2004. **11**(5):429-34.

Rosell A, Valencia E, Ochoa WF, Fita I, Pares X, Farres J.
Complete reversal of coenzyme specificity by concerted mutation of three consecutive residues in alcohol dehydrogenase
J Biol Chem. 2003 ;278:40573-80.

Gil-Ortiz F, Ramon-Maiques S, Fita I, Rubio V.
The course of phosphorus in the reaction of N-acetyl-L-glutamate kinase, determined from the structures of crystalline complexes, including a complex with an AIF(4)(-) transition state mimic
J Mol Biol. 2003 ;331:231-44.

Donald LJ, Krokhin OV, Duckworth HW, Wiseman B, Deemagarn T, Singh R, Switala J, Carpena X, Fita I, Loewen PC.
Characterization of the catalase-peroxidase KatG from Burkholderia pseudomallei by mass spectrometry
J Biol Chem. 2003 ;278:35687-92.

Chelikani P, Carpena X, **Fita I**, Loewen P.C.
An electrical potential in the access channel of catalases enhances catalysis
J Biol Chem. 2003 ;278:31290-6..

Number of Synchrotron related publications in the last 5 years: About 35

Collaborators:

Group Leader: Joan Guinovart (Univ. Barcelona, Spain)
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Group members: **1 staff scientist, 1 Ramón y Cajal fellow , 2 technical assistants, 2 predocs, 1 EMBO fellow.**

5 relevant publications, including synchrotron-beamline (if any):

Crystal structure of the sulphur metabolism enzyme PAPS-reductase at 2.0 Å resolution.
H. Savage, G. Montoya, C. Svensson, D. Schween & I. Sinning.
Structure (1997) 5, pp 895-906.

Expression purification and crystallization of FtsY the signal recognition particle receptor of *E. coli*.
G. Montoya, C. Svensson, J. Lührink & I. Sinning.
Proteins (1997) 28 pp 285-288.

Crystal structure of the NG domain of the signal recognition particle receptor of *E. coli* (FtsY).
G. Montoya, C. Svensson, J. Lührink & I. Sinning.
Nature (1997) 385, pp 365-368.

Crystal Structure of the NG-Ffh from *A. ambivalens*. A Comparison with other SRP-GTPases and nucleotide binding proteins suggests a model for a SRP/SRP-receptor complex. G. Montoya, K. tee Kaat, R. Moll Scheffer G. & I. Sinning.
Structure (2000) (8), pp 515-525.

Crystal Structure of the complete core of the archaeal SRP. Implications for interdomain communication.
K. Rosendal, K. Wild, I. Sinning & G. Montoya.
Proc. Natl. Acad. Sci. USA. (2003) 100(25): 14701-14706

Number of Synchrotron related publications in the last 5 years: 7

Collaborators:

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Group leader:	F.Xavier Gomis-Rüth
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5 relevant publications, including synchrotron-beamline (if any):

F. X. Gomis-Rüth, M. Gómez, W. Bode, R. Huber & F. X. Avilés (1995).
The three-dimensional structure of the native ternary complex of bovine pancreatic procarboxypeptidase A with proproteinase E and chymotrypsinogen C.
EMBO J., 14, 4387-4394 (Impact Index 2003: 10.46).

F.X. Gomis-Rüth, K. Maskos, M. Betz, A. Bergner, R. Huber, K. Suzuki, N. Yoshida, H. Nagase, K. Brew, G.P. Bourenkov, H. Bartunik & W. Bode (1997).
Mechanism of inhibition of the human matrix metalloproteinase stromelysin-1 by TIMP-1.
Nature, 389, 77-81 (II: 30.98)

F.X. Gomis-Rüth, M. Solà, P. Acebo, A. Párraga, A. Guasch, R. Eritja, A. González, M. Espinosa, G. del Solar & M. Coll (1998).
The structure of plasmid-encoded transcriptional repressor CopG, unliganded and bound to its operator.
EMBO J., 17, 7404-7415 (II: 10.46)

F.X. Gomis-Rüth, V. Companys, Y. Qian, L.D. Fricker, J. Vendrell, F.X. Avilés & M. Coll (1999).
Crystal structure of avian carboxypeptidase D domain II : a prototype for the regulatory metallocarboxypeptidase family.
EMBO J., 18, 5817-5826 (II: 10.46).

F.X. Gomis-Rüth, G. Moncalián, R. Pérez-Luque, A. González, E. Cabezón, F. De la Cruz & M. Coll (2001).
Structure of a membrane DNA transfer protein essential for bacterial conjugation.
Nature, 409, 637-641 (II: 30.98).

Number of Synchrotron related publications in the last 5 years: 22

Collaborators:

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Miquel Coll, Institut de Biologia Molecular de Barcelona (IBMB, CSIC), Barcelona (Spain)	mcoll@ibmb.csic.es DNA-binding proteins
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Elena Hidalgo, Universitat Pompeu Fabra, Barcelona (Spain)	elena.hidalgo@cexs.upf.es Oxidative stress regulation in bacteria
Stephen H. Leppla, National Institute of Allergy and Infectious Diseases, NIH, Bethesda (USA)	sleppla@niaid.nih.gov Proteases in Bacillus anthracis
Amadeu Llebaria, Institut d'Investigacions Químiques i Ambientals de Barcelona, CSIC, Barcelona (Spain)	allebaria@iiqab.csic.es Glycolytic enzymes
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Isabel Usón, IBMB, Barcelona (Spain)	iufcri@ibmb.csic.es Crystallographic methods
Winfried Weissenhorn, European Molecular Biology Laboratory, Outstation Grenoble, Grenoble (France)	weissen@embl-grenoble.fr Large protein complexes
Kornelius Zeth, Max-Planck-Institute for Biochemistry, Matrinsried/Munich (Germany)	zeth@biochem.mpg.de Membrane proteins

Group leader: **Vicente Rubio**
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Group members: **Fernando Gil-Ortiz, Clara Marco-Marín, Leonor Fernandez-Murga, Sandra Tavárez, Benito Alarcón, Jose Luis Llácer, Patricia Tortosa, Santiago Ramón-Maiques.**

5 relevant publications, including synchrotron-beamline (if any):

Marina, A., Alzari, P.M., Bravo, J., Uriarte, M., Barcelona, B., Fita, I. y Rubio, V. (1999)
Carbamate kinase: New structural machinery for making carbamoyl phosphate, the common precursor of pyrimidines and arginine.

Protein Sci. 8, 934-940.

Beamlines X11, X31, BW7A and BW7B, DESY (Hamburg)

Ramón-Maiques S, Marina A, Uriarte M, Fita I, Rubio V (2000)

The 1.5 Å resolution crystal structure of the carbamate kinase-like carbamoyl phosphate synthetase from the hyperthermophilic archaeon *Pyrococcus furiosus*, bound to ADP, confirms that this thermostable enzyme is a carbamate kinase, and provides insight into substrate binding and stability in carbamate kinases.

J. Mol. Biol. 299, 463-476.

Beamline ID14-1, ESRF (Grenoble)

Ramón-Maiques, S., Marina, A., Gil-Ortiz, F., Fita, I. y Rubio, V. (2002)

Structure of acetylglutamate kinase, a key enzyme for arginine biosynthesis and a prototype for the amino acid kinase enzyme family, during catalysis

Structure 10, 329-342.

Beamline ID14-1, ESRF (Grenoble); Beamline X31 at DESY (Hamburg)

Gil-Ortiz, F, Ramón-Maiques, S., Fita, I. y Rubio, V (2003)

The course of phosphorus in the reaction of N-acetyl-L-glutamate kinase, determined from the structures of crystalline complexes, including a complex with an AlF_4^- transition state mimic

J. Mol. Biol., 331:231-44.

Beamline BW7B, DESY (Hamburg); Beamline X11, DESY (Hamburg); Beamline ID14-2, ESRF (Grenoble)

Pérez-Arellano, I., Gil-Ortiz, F., Cervera, J. y Rubio, V. (2004)

Glutamate-5-kinase from *Escherichia coli*: gene cloning, overexpression, purification and crystallization of the recombinant enzyme, and preliminary X-ray studies.

Acta Crystallog D 60:2091-209448.

Beamline BM16, ESRF (Grenoble)

Number of Synchrotron related publications in the last 5 years: 13

Collaborators:

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Fernández Cereijo**

5 relevant publications, including synchrotron-beamline (if any):

A.L.Llamas-Saiz, M.Agbandje-McKenna, J.S.L.Parker, A.T.M.Wahid, C.R.Parrish, M.G.Rossmann.
Structural Analysis of a Mutation in Canine Parvovirus which Controls Antigenicity and Host Range.
Virology, 225, (1996) 65-71.

SYNCHROTRON: CHESS, *BEAMLINE*: F1

A.L.Llamas-Saiz, M.Agbandje-McKenna, W.R.Wickoff, J.Bratton, P.Tattersall, M.G.Rossmann.
Structure Determination of Minute Virus of Mice.

Acta Crystallographica, Section D, 53, (1997) 93-102.

SYNCHROTRON: CHESS, *BEAMLINE*: F1

M.Agbandje-McKenna, A.L.Llamas-Saiz, F.Wang, P.Tattersall, M.G.Rossmann.
Functional Implications of the Structure of the Murine Parvovirus, Minute Virus of Mice.
Structure, 6, (1998) 1369-1381.

SYNCHROTRON: CHESS, *BEAMLINE*: F1

E.Hernando, A.L.Llamas-Saiz, C.Foces-Foces, R.McKenna, I.Portman, M.Agbandje-McKenna,
J.M.Almendral.

Biochemical and Physical Characterization of Parvovirus Minute Virus of Mice Virus-like Particles.
Virology, 267-2 (2000) 299-309.

SYNCHROTRON: Daresbury (SRS), Hamburg (DESY), *BEAMLINES*: 9.6 (SRS), X11 (DESY)

G.M.Grotenbreg, M.S.M.Timmer, A.L.Llamas-Saiz, M.Verdoes, G.A. van der Marel, M.J. van Raaij,
H.S.Overkleeft, M.Overhand.

Synthesis and Structures of Furanoid Sugar Amino Acids Incorporated in Gramicidin S.
PNAS (2004) Invited paper in preparation.

SYNCHROTRON: Grenoble (ESRF), *BEAMLINE*: ID23.1

Number of Synchrotron related publications in the last 5 years: 2

Group leader:	MANCHEÑO GÓMEZ, JOSÉ MIGUEL
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Group members:	1

5 relevant publications, including synchrotron-beamline (if any):

Mancheño JM, Jayne, S, Kerfelec, B., Crenon, I., Chapus, C., Hermoso, J.A.
Crystallization of a proteolyzed form of the horse pancreatic lipase related protein 2: structural basis for the specific detergent requirements
Acta Crystallographica (2004) D60, 2107-2109 (ID14-2; ESRF)

Mancheño JM, Tateno, H., Goldstein, I.J., Hermoso, J.A. Crystallization and preliminary X-ray diffraction analysis of a novel haemolytic lectin from the mushroom *Laetiporus sulphureus*
Acta Crystallographica (2004) D60, 1139-1141 (ID14-2; ESRF)

Mancheño JM, Martín-Benito, J., Martínez-Ripoll, M., Gavilanes, J.G., Hermoso, J.A. Crystal and Electron Microscopy Structures of Sticholysin II Actinoporin reveal insights into the Mechanism of Membrane Pore-formation. Structure (2003) 11, 1319-28 (BM14; ESRF)

Mancheño JM, Pernas, N.A., Jiménez, M.J., Ochoa, B. Rúa, M.L., Hermoso, J.A.
Structural insights into the lipase/esterase behavior in the *Candida rugosa* family: crystal structure of the lipase 2 isoenzyme at 1.97 Å resolution. J. Mol. Biol. (2003) 332, 1059-1069

Mancheño, JM, Martínez-Ripoll, M, Gavilanes JG., Hermoso, J.A. Crystallization and preliminary X-ray diffraction studies of the water-soluble state of the pore-forming toxin Sticholysin II from the sea anemone *Stichodactyla helianthus*.
Acta Crystallographica (2002) D58, 1229-1231 (BM14; ESRF)

Number of Synchrotron related publications in the last 5 years: 6

Collaborators:

Group Leader: Dr. C. Chapus (Institute National de la Sante et Recherche Medicale, , Marseille, France)
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Group Leader: Dr. E. Zlotkin (The hebrew Univ. of Jerusalem, Israel)
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Group leader:	Juan A. Hermoso Domínguez
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5 relevant publications, including synchrotron-beamline (if any):

Juan A. Hermoso, Begoña Monterroso, Armando Albert, Beatriz Galán, Oussama Ahrazem, Pedro García, Martín Martínez-Ripoll, José Luis García, Margarita Menéndez.

Structural Basis for Selective Recognition of Pneumococcal Cell Wall by Modular Endolysin from Phage Cp-1.

Structure, (2003). Vol. 11, 1239-1249. (Portada de la revista) ESRF-BM14.

Mancheño, J.M., Martín-Benito, J., Martínez-Ripoll, M., Gavilanes, J.G. and Hermoso, J.A.
Crystal and electron microscopy structures of Sticholysin II actinoporin reveal insights into the mechanism of membrane pore-formation

Structure (2003). Vol. 11, 1319-1328. ESRF-BM14.

Jesús Tejero, Marta Martínez-Júlvez, Tomás Mayoral, Alejandra Luquita, J. Sanz-Aparicio, Juan A. Hermoso, John K. Hurley, Gordon Tollin, Carlos Gómez-Moreno and. Milagros Medina.

Involvement of the Pyrophosphate and the 2'-Phosphate Binding Regions of Ferredoxin-NADP+ Reductase In Coenzyme Specificity.

Journal of Biological Chemistry. (2003), Vol.278 (49), 49203-49214. ESRF-ID14

Milagros Medina, Alejandra Luquita, Jesus Tejero, Juan A. Hermoso, Tomás Mayoral, J. Sanz-Aparicio, Koert Grever and Carlos Gómez-Moreno.

Probing the Determinants of Coenzyme Specificity in Ferredoxin-NADP+ Reductase by Site-Directed Mutagenesis.

Journal of Biological Chemistry. (2001), 15, 11902-11912. ESRF-BM30A

J. Hermoso, D. Pignol, S. Penel, M. Roth, C. Chapus and J.C. Fontecilla-Camps.

Neutron Crystallographic Evidence of Lipase/Colipase Complex activation by a Micelle.

EMBO Journal. (1997) Vol. 16, 18, 5531-5536. ESRF-D2AM, LURE

Number of Synchrotron related publications in the last 5 years: 30

Collaborators:

Group Leader: José Luis Garcia (Centrode Investigaciones Biológicas, CSIC, Spain)

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Group Leader: José G. Gavilanes Franco (Universidad Complutense de Madrid, Spain)

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5 relevant publications, including synchrotron-beamline (if any):

Crystal structure of the first dissimilatory nitrate reductase at 1.9 Å solved by MAD methods
Dias J.M., Than M.E., Humm A., Huber R., Bourenkov G.P., Bartunik H.D., Bursakov S.,
Calvete J., Caldeira J., Carneiro C., Moura J.J.G., Moura I. & Romão M.J.
Structure 7 (1999) 65-79

Crystal structure of native and Cd/Cd-substituted *Dioclea guianensis* seed lectin. A novel
manganese binding site and structural basis of dimer-tetramer association
Wah,D.A., Romero,A., Gallego del Sol,F., Cavada,B.S., Ramos,M.V., Grangeiro,T.B.,
Sampaio,A.H. & Calvete,J.J.
J. Mol. Biol. 310 (2001) 885-894

Sperm Coating Mechanism from the 1.8 Crystal Structure of PDC-109 with Bound 0-
Phosphorycholine
Wah, D.A., Fernández-Tornero, C., Sanz, L., Romero, A. & Calvete, J.J.
Structure 10 (2002) 505-514

Crystal structure of a prostate kallikrein isolated from stallion seminal plasma: a homologue
of human PSA
Carvalho, A.L., Sanz, L., Baretino, D., Romero, A., Calvete, J.J. & Romão, M.J.
J. Mol Biol. 322 (2002) 325-337

Crystallization and preliminary X-ray diffraction analysis of the lectin from *Canavalia gladiata*
seeds
Moreno, F.B.M.B. , Delatorre, P., Freitas, B.T., Rocha, B.A.M. , Souza, E.P. , Facó, F.,
Canduri, F., Cardoso, A.L. H. , Freire, V.N. , Lima-Filho, J.L., Sampaio, A.H., Calvete, J.J.,
De Azevedo, W.F. & Cavada, B.S.
Acta Crystallogr. D 60 (2004) 1493-1495

Number of Synchrotron related publications in the last 5 years: 7

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Annexe III.

SEBBM
SEBBM
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de Bioquímica y
Biología Molecular

Madrid, December 10, 2004

To whom it may concern:

This is to express the support of the Spanish Society of Biochemistry and Molecular Biology (SEBBM) to the installation of a dedicated state-of-the-art macromolecular crystallography facility within the Vallés synchrotron ALBA. The SEBBM is the largest scientific society in Spain, having more than 3000 members and representing all the Spanish biochemists and molecular biologists, and is a founding member of FEBS (the Federation of European Biochemical Societies), as well as the society sending the Spanish representatives to the IUBMB (the International Union of Biochemistry and Molecular Biology).

SEBBM has witnessed the important growth of the field of X-ray biocrystallography in Spain in the last few years. A very active group on Protein Structure and Function was formed within the SEBBM some eight years ago, and since then the number of group members has escalated to over one hundred and fifty. Virtually every yearly general meeting of the SEBBM has a symposium (out of a total of nine) directly related to macromolecular structure. In addition, in every yearly congress the group on Protein Structure and Function meets for a whole afternoon to present scientific communications. In this Group Session SEBBM together with Bruker gives the Josep Tormo award to the best structural paper of the year, and generally the award is given to X-ray crystallography papers. In 2003 the SEBBM held a joint 3-day Workshop with the Portuguese Biochemical Society on the Structure and Function of Proteins. The SEBBM is supporting one of the first interuniversity PhD training programmes on Protein Structure which has been awarded the highly coveted "Mención de Excelencia" of the Spanish Ministry of Education and Science. The SEBBM is a sponsor of the next European meeting of the Protein Society, to be held in Barcelona in 2005.

The support given by SEBBM to X-ray biocrystallography stems not only from the rapid growth of the crystallographic community in Spain, but also because of the demand of the SEBBM members, and by the shared and well founded belief that biocrystallography is still underrepresented in Spain and that it must be promoted at a very fast pace to catch up with Europe, if Spanish biology is to reach the level that it should. Spain has lagged behind too long in this field. Thus, the opening of a really good and dedicated X-ray crystallography line in 2010 at a reasonably short distance from the then expectedly (if grow continues at the present rate) large Spanish biocrystallographic community would be an incredibly important boon for Spanish biology, and would actually help to get the best return from the large investments either made or planned to be made on biomedical science in Spain in the next few years, which includes the opening by the CSIC of at least 15 Institutes on biomedical science during the 2000-2010 period, the large investment by the Farmaindustria consortium of drug companies in the granting of public biomedical science through the Spanish

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IUBMB
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Ministry of Health, and the initiative of the Catalanian Government to make of Catalonia the center of a Bioregion, with its initiatives of Biomedical Institutes, Center for Genomic Research, and Biomedical Alliances. In fact, placing ALBA in Catalonia makes sense if it homes state of the art biomedical technologies at the center of the Bioregion. X-Ray crystallography is, and surely will continue to be in 2010, a key biomedical technology for getting to the very deepest level of biological understanding, and for providing insight to develop new treatments. Thus, the SEBBM supports with total conviction the installation of such a crystallographic facility in ALBA.

Yours sincerely



Vicente Rubio
President of SEBBM