PROPOSAL FOR A BIOSAXS BEAMLINE (CALIMA) AT ALBA SYNCHROTRON

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1. SUMMARY

Small Angle X-Ray Scattering on biological material (BioSAXS) is a powerful technique to elucidate the shape and, ultimately, the function of macromolecules at the micro- and nanoscales. Over the past decade, the technique has consistently proven to be extremely useful in investigating macromolecular structures in its native form under physiological conditions without complex sample preparation methods such as crystallization or cryopreservation. [2, 3]. Samples studies include soluble proteins, membrane proteins, DNA, RNA, lipids, virus particles, disordered systems, and macromolecular complexes.

BioSAXS also offers new opportunities. In this regard, life sciences studies are quickly moving towards an integrated vision to tackle societal challenges on health. Several techniques are combined, or even correlated using the same sample, to study at different resolution scales, spanning from macromolecular level (*e.g.*, macromolecular crystallography and electron microscopy) to cell and tissue levels (*e.g.*, soft and hard X-ray tomography, nano-fluorescence, spectroscopy, and Fourier-transform infrared spectroscopy (FTIR)). In this scenario, structural information from SAXS has been successfully combined with data from macromolecular crystallography (MX), nuclear magnetic resonance (NMR), cryo-electron microscopy (Cryo-EM), cryo-electron tomography (Cryo-ET,) FRET, mass spectrometry (MS), and computational methods. SAXS appears nowadays to be central in this integrative approach due to an easy sample preparation, wide range of applications, and a resolution range between the atomic and the organelle levels [4, 5].

BioSAXS is also a key player in the "era of structural dynamics" as it can reveal structural changes of macromolecules and macromolecular complexes in response to physical and chemical stimuli in real time. SAXS also permits the visualization of the structure evolution during the formation of a complex and, the behaviour during oligomerization, assembly or folding. Therefore, SAXS presents a tremendous insight for investigating the response towards external influences, either chemical (e.g., pH variation, ionic strength, denaturants) or physical perturbations (e.g., temperature, pressure) resulting in changes on particle size distribution or interparticle interactions. This also allows to investigate polymers and nanostructured material.

Currently, the BL11-NCD-SWEET beamline at ALBA is dedicated to SAXS, being used in a wide range of disciplines from nanomaterials, catalysis, and polymer sciences to life sciences, to biophysics and medicine, as well as environment and cultural heritage. In the past years, BL11-NCD-SWEET has experienced a rapid increase of the beamtime oversubscription rate, in parallel with an increasing focus of the beamline towards material sciences field, including GISAXS. Nevertheless, the demand for biological applications is rapidly growing, highlighting the urgent need for a fully dedicated BioSAXS end-station at ALBA-II synchrotron. SAXS studies on biological samples is not highly demanding in terms of beam features but requires a very well-equipped end-station to be able to fully exploit this technique. The present proposal features a **bending magnet beamline devoted to performing BioSAXS** experiments, where we aim to

include a wide range of current state-of-the-art SAXS applications including fully automated high-throughput screening SAXS (HTS-SAXS), size exclusion chromatography SAXS (SEC-SAXS), time-resolved SAXS (TR-SAXS), and 3D scanning SAXS (3DsSAXS). In addition, this proposal gives a step forward towards novel and highly pushing applications using microfluidics, as well as the simultaneous data acquisition of full SAXS and WAXS (wide-angle X-ray scattering) spectra in the entire quadrants. The acquisition of the full WAXS quadrant is not currently feasible at BL11-NCD-SWEET, and to include them in the portfolio is important for experiments on polymers in solution and nanostructured materials.

The BioSAXS-CALIMA beamline will be of extreme interest for the local and global Structural Biology and Molecular Biology communities, as well as for the Soft Matter and Polymeric Matter scientific communities. Considering that most synchrotrons count with only a beamline fully or partly dedicated to these disciplines, the inclusion of BioSAXS-CALIMA at ALBA-II will considerably increase the beamtime availability dedicated to these studies, positioning ALBA-II at the forefront of the structural biology facilities in Europe.

2. SCIENTIFIC CASE

SAXS for biological macromolecules (BioSAXS) has become an important component in structural biology since the early days of the technique and has played in biology an ever-increasing role in structural evaluation since the advent of synchrotron radiation facilities. With SAXS, it is possible to acquire valuable structural information (size and shape) by analysing the way that all the components of the samples scatter X-rays, allowing to study how the macromolecules interact with each other in our cells. SAXS provides low resolution structural and biophysical parameters and is widely applied to confirm high-resolution crystal structures in solution, and information from difficult-to-measure protein samples that are not suitable for high-resolution structure determination techniques such as MX and EM. Increasingly, synchrotron-based SAXS has advanced through the development of new and more friendly data analysis tools, beamline instrumentation, and X-ray detector technology, as well as advances in sample delivery and preparation [6, 7].

The **BioSAXS-CALIMA beamline** will be developed to facilitate the measurement of scattering data converging the possibilities offered by other SAXS synchrotron beamlines. We also have considered implementations that will be key for future approaches considering our knowledge about the state-of-the-art of this technique in other synchrotrons (Diamond Light Source, beamline B21 in UK; PETRA III (DESY), beamline P12 in Germany; European Synchrotron Radiation Facility, beamline BM29 in France, etc.), and the planned future upgrades of those (improvements and new capabilities that they will acquire in the future). Importantly, the first thing we have considered for the development of the beamline is the limitation step experienced by many SAXS users, i.e., the production of suitable samples (high yield, purity and correctly folded) for SAXS experiments, which is one of the biggest challenges in structural studies on biological macromolecules. Thus, to overcome this limitation, the BioSAXS-CALIMA beamline

will be equipped with the instrumentation that minimizes the sample volume and concentration necessary to obtain high-quality results.

The high success and rapid growth of the SAXS technique in the past decade, has been mainly due to the possibility of coupling other biophysical techniques such as size exclusion chromatography (SEC), multi-angle laser light scattering (MALLS), UV absorption spectroscopy, refractive index (RI), and quasi-elastic light scattering (QELS), to the scattering experiment itself. In this proposal, we suggest the use of an appropriate and fully dedicated BioSAXS-CALIMA beamline for:

2.1. Batch Mode SAXS

Background: This measurement mode is for highly pure samples, free of aggregates and large species, since samples will be directly loaded into the continuous-flow sample cell by an autosampler robotic system from PCR strips, centrifuge tubes or 96-well plates. This method significantly reduces the sample volume to ~10-30 µL and the concentration of protein required for the experiments without compromising the yield of usable SAXS data. Importantly, the continuous-flow of the sample in the cell helps to minimize radiation damage even if using a small beam-size.

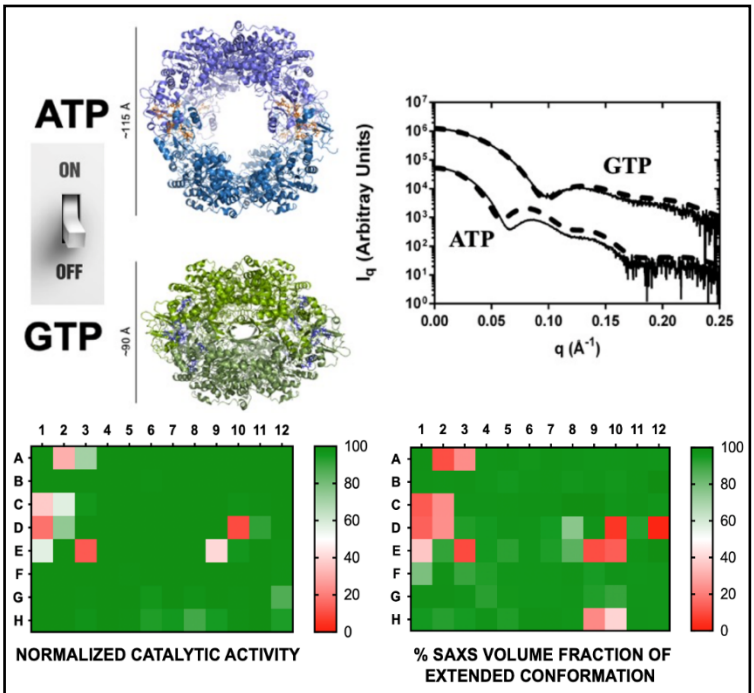


Figure 1: Top. IMPDH MX high-resolution structures of ATP and GTP-bound state on the left (PDB codes: 5MCP and 4Z87, respectively). IMPDH ATP and GTP-bound profiles by SAXS (right panel), where continuous lines are the experimental SAXS profiles and, the discontinuous lines are the theoretical profiles estimated from the MX high-resolution structures. **Bottom.** Heatmap of the normalized initial velocities (V_o / $V_{o\text{-ligand}}$) and the % of SAXS volume fraction for the extended (active) conformation, for IMPDH in the presence of an *in house*-built guanine nucleotide library. Wells in red represent IMPDH inhibitors. It can be clearly observed the higher sensitivity of the SAXS structural assay with respect to the functional assay.

Scientific case 1: Our understanding on the *allosteric modulation of biological macromolecules* has increased dramatically during the last decades, attracting considerable attention in drug discovery. Ligands that target allosteric sites offer significant advantages over the corresponding orthosteric ligands due to unprecedent selectivity and improved physicochemical properties, while minimizing toxicity and other side effects. Therefore, allosteric ligands offer excellent opportunities for the development of novel therapeutic strategies spanning a broad spectrum of human disease and is at present widely

used in drug discovery. Successful examples of allosteric modulators used as inhibitor drugs that have already entered the market include ligands targeting G-protein coupled receptors (GPCRs), kinases, HIV-1 reverse transcriptase, or AMPA, GABA and adrenergic receptors, among others. Inosine-5'-phosphate dehydrogenase (IMPDH, EC 1.1.1.205) catalyses the rate-limiting step in guanine nucleotide biosynthesis and, thus, is a key player of the global cellular metabolism and cell proliferation. A combination of MX and SAXS data has recently reported a molecular mechanism by which ATP and GTP nucleotides allosterically control a conformational switch that modulates the catalytic activity of the enzyme, by shifting from an expanded to a compacted conformation of IMPDH octamers (**Fig. 1,** top [8]). The relevance of this mechanism is stressed by the fact that missense mutations within the human IMPDH coding sequences, which map the allosteric binding sites, have been associated to severe retinopathies and dystonia [9, 10]. IMPDH is the cellular target of a diverse group of drugs with antiviral and immune-depressive activities, including CellCept®, Bredinin®, Virazole® and Rebetol®. All known IMPDH inhibitors described so far, bind to the active site, but still there is an urgent need to investigate new targeting approaches. The group of Dr. Buey has identified molecules that bind to the allosteric sites of IMPDH and have different mechanisms of enzyme inhibition than the known inhibitors. They have performed high-throughput screening assays by monitoring the ligand-induced conformational changes of IMPDH, obtained by SAXS, since allosteric inhibitors change the conformation of the enzyme from extended (active) to compact (inhibited) and both conformations can be perfectly distinguished by SAXS experiments (**Fig. 1**, top). The results using this dual approach with a small chemical library of 96 guanosine nucleotide analogues have demonstrated the convenience and advantages of using SAXS as primary high-throughput screening assay to identify allosteric inhibitors of IMPDH: (i) allow to specifically identify allosteric inhibitors, while the functional assay will not differentiate between competitive and allosteric inhibitors, (ii) chemical libraries contain Pan Assay INterference (PAIN) compounds that will result in a significant number of false positives in the functional, but not in the structural approach and, (iii) SAXS has resulted to be significantly more sensitive than the functional assay (**Fig. 1**, bottom) because IMPDH adopts the inhibited conformation at a lower inhibitor concentration than that needed for catalytic activity inhibition.

In this case, the initial pilot experiment with the 96 compounds took about 30 minutes for the functional assay and 3h 20 min for the SAXS structural assay, at the B21 beamline at Diamond Light Source (DLS). The long experimental time required for this type of SAXS structural assays is a clear drawback of this technique and a clear indication that a competitive BioSAXS beamline need to be further optimized for high-throughput approaches. Indeed, almost 80% of the experimental time was used for sample loading and capillary washing. Another drawback of this methodology is the amount of sample required, which will lie in the milligram scale per 96-well plate. Again, this is also a clear indication that volume reduction is also prerequisite for viable high throughput BioSAXS experiments.

Scientific case 2: Intrinsically disordered proteins often perform important functions in regulation of gene expression and in the cell cycle. These proteins do not have a fixed tertiary structure [11] and thus crystalline structure is not available or not showing all the physiological conformations. Although NMR, the only high-resolution method applicable, yields valuable information about their local structure [12-14], there is a clear need for the development of approaches for the quantitative characterization of flexible and intrinsically disordered proteins in solution. Thus, obtaining structural information from flexible macromolecular systems such as intrinsically disordered or multidomain proteins with flexible linkers is a difficult task as high-resolution techniques are barely applicable. To this end, the SAXS based ensemble optimization method (EOM), was proposed to quantitatively characterize flexible proteins in solution using SAXS [15].

Functional specifications: A fully automated BioSAXS-CALIMA beamline will be key for high-throughput screening SAXS. This involves the combination of a robot sample changer and automated data analysis. Considering the samples to be used, in the robot, the storage and exposure cell should be independently thermoregulated (4-40ºC and 4-60ºC respectively). Sample cleaning and loading should take about 30 seconds and a full cycle time should be ~1 min, which is ideal, for example, for screening compounds on a specific target protein. A plate compatible data-collection system will allow to minimize actual limitations due to time-consuming inter-samples cell cleaning, which is the bottleneck for real high-throughput screening.

2.2. SAXS coupled to chromatography methods

Background: To improve biological solution scattering experiments, current synchrotron beamlines specifically dedicated to BioSAXS have adapted chromatography methods, which allow to further purify samples immediately before being loaded into the capillary for SAXS data acquisition [16-19]. The most popular chromatographic method that is on-line coupled with SAXS is the size-exclusion chromatography (SEC), SEC-SAXS, in which the sample runs first through a size exclusion column to separate potential aggregates or different oligomeric states, before being immediately loaded into a capillary for X-ray scattering measurements. This setup helps to discriminate between different-size populations during data collection, as well as mitigate the buffer matching issue, resulting in an overall quality improvement of the collected data. The sample volume typically ranges between 5-20 μl and concentrations in the range of 1-10 mg/ml, depending on the size of the particles under study and the size-exclusion chromatography column choice.

Ion exchange chromatography (IEC) is an alternative chromatographic method, where instead of a SEC column the user can load the sample in an IEC column (IEC-SAXS). Unlike SEC, where particles are separated by size, IEC allows separation of particles by charge, making it very useful in certain cases where SEC cannot resolve particles of similar dimension. Because IEC does a buffer exchange (with a variation of salt or pH) during elution, either in a linear or in a step gradient, the experiment often requires optimization and discussion of the potential issues that

could affect the IEC-SAXS experiment with the beamline personnel. Besides, IEC-SAXS data analysis tends to be more challenging than that for SEC-SAXS due to plausible buffer subtraction problems; however, data analysis algorithms are now widely available and more user friendly, making IEC-SAXS a routine technique.

Scientific case 3: Membrane proteins are crucial for the normal function of an organism as they are involved in essential processes such as signalling, nutrient and ion transport, maintaining biological membrane structure and integrity [20-23]. Membrane proteins encode about 30% of the human genome [24] and represent >60% of all drug targets, being also the key players in the pathogenesis of infectious diseases. This makes structural studies on membrane protein solutions extremely important both scientifically and medically [25-27]. A critical step for the elucidation of the complex processes that are catalysed by membrane proteins is the understanding of their structure, dynamics, and function. However, membrane proteins have traditionally presented many limitations that make them difficult to study, namely, partially hydrophobic surfaces, high flexibility, and lack of stability [28]. In addition, crystallization of membrane proteins has always been a challenge in comparison to their soluble counterparts. This is why the application of SAXS to the structural characterization of membrane proteins has become increasingly popular and a routine at the BioSAXS beamlines around the world in recent years [29-32]. The success is due to the possibility of doing SAXS coupled with online size-exclusion chromatography (SEC-SAXS).

Functional specifications: Considering the experimental setups above described, the HPLC system at BioSAXS-CALIMA might include: (1) autosampler (allowing small sample quantity loading without loses, 5-20 uL; (2) buffer gradient (up to four buffer components) with conductivity measurements; and (3) short tubing between the column and the quartz capillary to reduce sample consumption. It should be able to be used in parallel with the sample changer robot, and the switch between the two modes (robot and SEC) should be quick and fully automatized. Users will have the option to use different types of columns provided by the beamline that could be attached to an HPLC system for small scale SEC-SAXS and IEC-SAXS. The columns available at the beamline will be renovated as soon as possible when clogged, so it is crucial to count with a stock. Alternatively, users will be encouraged to bring their own columns to avoid potential cross contamination and reproducibility issues.

At BioSAXS-CALIMA beamline, a variety of data acquisition modes will be available besides the standard SAXS flow-cell. Multi-angle laser light scattering (MALLS) detectors will be available, which will allow for: (*i*) the absolute molecular weight determination, which is hard to determine via SAXS itself; and (*ii*) the hydrodynamic radius that in combination with the radius of gyration (Rg) obtained by SAXS, will give a more accurate determination of the particle size. In addition to MALLS detectors, UV absorption, UV-vis fluorescence, mass spectrometry MS, and refractometric (RI) detectors will be also available to contribute to determine the monodispersity and conformational heterogeneity of the samples. Importantly, all these experimental setups will be available upon sample elution from the SEC column, which will

subsequently pass through the MALLS, UV, and RI detectors in a sequential mode before it is loaded into the flow-cell for SAXS inspection. This approach will provide users with information regarding different possibilities of sample preparation and eliminate ambiguities arising from the differences between non-identical separate SAXS and MALLS measurements. Sample quality prerequisites for this system are considerably stricter than the simpler SEC-SAXS setup and the suitability of the sample must be determined through discussion.

2.3. Time-resolved SAXS

Background: Time-resolved X-ray crystallography is the technique currently able to provide the highest spatial and temporal resolution over the whole structure of a protein. However, it is limited by the requirement for well-diffracting protein crystals. Although great advances have been made in this field, crystallization remains an art rather than an exact science. Indeed, crucial protein classes such as membrane proteins, large proteins or protein complexes, and intrinsically disordered proteins, which are key players in the life of the cell, are difficult or impossible to crystallize. An alternative approach is to use time-resolved SAXS (TR-SAXS) [33], which is a powerful in-solution technique to pursue conformational changes of biological samples during reactions that occur on timescales on the order of millisecond to minutes. TR-SAXS at synchrotron light sources has experienced a tremendous increase in popularity in the past decade. The recent advances in high-brilliance synchrotron radiation sources and detector technology, along with the synergistic advances in hardware and software have been crucial in this explosive growth. In this regard, the higher flux achievable at ALBA-II and the efficient fast readout detectors will enable TR-SAXS experiments at BioSAXS-CALIMA beamline. The quality of the data produced at 3rd generation synchrotrons has allowed unprecedented signal-to-noise- ratios, which has in turn, motivated efforts for *ab initio* modelling of macromolecular structures [15, 34]. All these developments are entering TR-SAXS in an era that brings us increasingly closer toward our goal of obtaining structural snapshots of macromolecular processes such as folding, ligand binding, and association/oligomerization reactions on timescales from microseconds to hundreds of seconds.

TR-SAXS offers the possibility of analysis under native-like conditions, (sub)nanometer spatial resolution and an extremely broad range of time resolution from femtoseconds to days [35] the main aspects considered in the design of TR-SAXS experiments include the need: **(i)** to collect interpretable signals in short times and, **(ii)** of a rapid trigger to initiate the reaction of interest over a statistical ensemble of molecules. Fast detectors together with X-ray pulses of as short as 100 ps and fluxes of up to 10^{15} photons s^{-1} provided by 3rd generation synchrotrons offer the possibility of very efficient data collection. A key element in TR-SAXS experiments is the triggering mechanism to start and synchronize dynamic processes. The pump-probe method (in which an excitation signal is followed by a single probing pulse or a train of pulses) takes further advantage of the high brilliance of 3rd generation synchrotrons. Short and intense X-ray pulses can be isolated by mechanical choppers or fast-gated detectors [36, 37]

Rapid changes in temperature generate a plethora of biological reactions. Temperature jumps can be achieved by mixing solutions at different temperatures with dead times in the millisecond range, using infrared laser-induced heating or by employing inert absorbing dyes with fast internal energy conversion [38]. Rapid mixing experiments enable fast perturbations using a relatively simple technology; mixing is an ideal method to change solvent conditions such as pH, salt, or ion content and to induce protein–ligand or protein–protein interactions. Devices employing continuous flow mixing inside micro-fabricated channels achieve a time resolution of around 100 μs and low sample consumption [39]. Different time points of the reaction can be achieved by modifying the distance between the mixing point and the interaction region. Light-triggered experiments push the time resolution of TR-SAXS further [40]. The difference in absorption between X-rays and visible or UV light must be considered to ensure uniform excitation in the volume probed. Thin samples are usually required, and liquid jets represent a possible technical solution for both synchrotron and free-electron laser sources [41]. Fast laser pulses (with a duration of picoseconds to femtoseconds) are used to initiate structural changes and intramolecular reactions. The potential of this approach has been shown in the study of the quaternary transition in carbonmonoxy haemoglobin [40], the study of the photoactive yellow protein (PYP) photocycle [42], and the study on signal amplification and transduction in phytochrome photosensors [43]. To increase the time resolution of the experiment, the diameter of the capillaries is reduced to tens of microns to minimize the limiting factor, i.e. the diffusion time of the reactants. Therefore, the beam size delivered by the beamline at sample is required to be in the same order of magnitude to optimize the experimental setup[44].

Traditionally, SAXS was used as a routine technique to examine molecular structures; however, when used in a time-resolved manner, it can also enable the observation of functionally related structural changes and dynamics over a broad range of spatial and temporal resolution. The timescales of biologically relevant processes range from femtoseconds to hours and so the design of a time-resolved experiment must be matched to the timescale of the process of interests. TR-SAXS has been successfully applied to study numerous biological processes like protein folding [39, 45-47], DNA compaction [48]; RNA folding [33, 49-51] protein conformational dynamics [52] and enzyme catalysis. Some of them are briefly described below.

Scientific case 4: Amyloid proteins are found in a wide range of organisms owing to the high stability of the β-sheet core of the amyloid fibrils. There are both pathological amyloids involved in various diseases such as: **(i)** Alzheimer's disease involving tau protein [53, 54] and amyloid-β [55, 56] proteins, **(ii)** Parkinson's disease involving α-synuclein protein [57] and, **(iii)** Huntington disease with polyglutamine expansions [58] as well as functional amyloids that play a beneficial role for the organism [59]. The aggregation process is complex and often involves many different species. The supramolecular structure of the amyloid fibrils makes structural characterization by MX methods impractical because fibril forming proteins tend to not crystallize. Full understanding of fibril formation process requires parallel acquisition of data by complementary techniques monitoring the time course of aggregation, which is not an easy task, given the often-

stochastic nature of the aggregation driven process, which can lead to significant variations in lag time.

The formation of protein aggregates is naturally and easily detectable in the SAXS region of the scattering pattern. There exist a few cases in the literature reporting on protein aggregation studies by TR-SAXS experiments [60-62], being one of the most recent ones the work presented by Rasmussen and co-workers on FapC protein (**Fig. 2**) [63].

From this data, the initial state in the fibrillation mechanism can be described by the form factor of a flexible polymer, this points to the initial state not being a folded protein with a defined tertiary structure but predominantly, if not completely, in the random-coil state. The scattering curve 2.5 h later (end of lag phase) can also be described with the simple polymer model, giving a mass of 236 kDa corresponding to 10 monomers. Still, the scattering curve has not completely converged to a flat line at low-q, and therefore, the mass could be even larger.

At BioSAXS-CALIMA it will be possible to carry out TR measurements to follow amyloid fibril formation combining several complement in-solution techniques "simultaneously". Combination of CD to monitors the change in a secondary structure and, MALLS and SAXS to report on changes in overall size and shape will make possible to follow the whole aggregation process and benefit from the insight provided by each technique while avoiding ambiguities from possible differences in assay conditions.

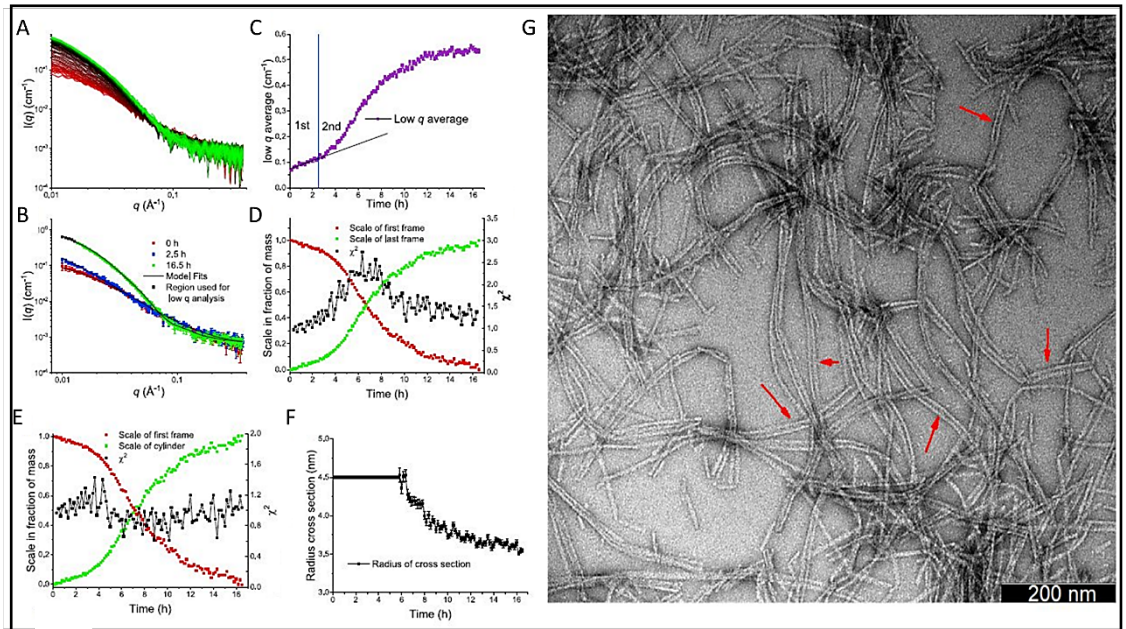


Figure 2. TR-SAXS and TEM data. (A) All SAXS data collected every 10 min over 16.5h. The curves are colored as a function of time from red to black to green. (B) SAXS data at 0, 2.5, and 16.5 h with model fits as described in the text. (C) Low-q average as a function of time using the part of the curved indicated in (B). The blue vertical line shows the lag time of 2.5 h. (D) Two-state transition analysis of SAXS data using the first and last frame. The sum of the scales is set to unity. (E) Two-state transition analysis using the first frame as the initial state and a cylinder with an ellipsoidal cross section as the final state. (F) The radius of the short axis of the cross section of the cylinder used as the final state in (E). The AR was fixed at 3.2. G) TEM image of final fibrils of FapC. Red arrows show the places where fibrils consist of two protofilaments.

Scientific case 5: Domain swapping phenomenon has been associated with the development of conformational diseases like neurodegenerative diseases. Domain swapping is closely related to the susceptibility of proteins to oligomerize, thus leading to the formation of amyloid fibril deposits. Overall, the domain swapping mechanism typically involves the partial unfolding of one of the protomer molecules followed by subsequent association with another protomer molecule. So far, domain swapping has been observed to occur in numerous proteins with flexible conformations. Despite the structural information available in the literature, the mechanism by which domain swapping take place and how it further evolves into amyloid fibrils is still poorly understood. This is because the proteins capable of swapping domains are rather flexible, being, overall, not suitable for studies with MX approaches. This limitation becomes SAXS an ideal technique for understanding the mechanism by which domain swapping is triggered and how further oligomerization leads to amyloid fibrils[64].

Scientific case 6: The structures of complex biological assemblies demand considerable attention, since critical cellular activities are more often carried out by such assemblies rather than by a single molecular component. A high-resolution structural model of an assembly is often crucial for understanding its function, and biological mechanisms can be deduced from a detailed view of the structure and interactions of components in an assembly. Because the limiting step for successful high-resolution structural studies of such large assemblies by X-ray diffraction is the production of well-diffracting crystals, SAXS technique is an attractive alternative. Furthermore, by using SAXS one can access both well-defined macromolecular architecture and flexible dynamics simultaneously, revealing functional conformations and dynamics often invisible to static approaches, such as X-ray crystallography and cryoEM. Good and representative examples of studying macromolecular assemblies with SAXS are the works recently published for the penicillin-binding protein-1 from *Staphylococcus aureus* (**Fig. 3**) [65] and a tumour-targeted trimeric 4-1BB-agonistic antibody [66]**.**

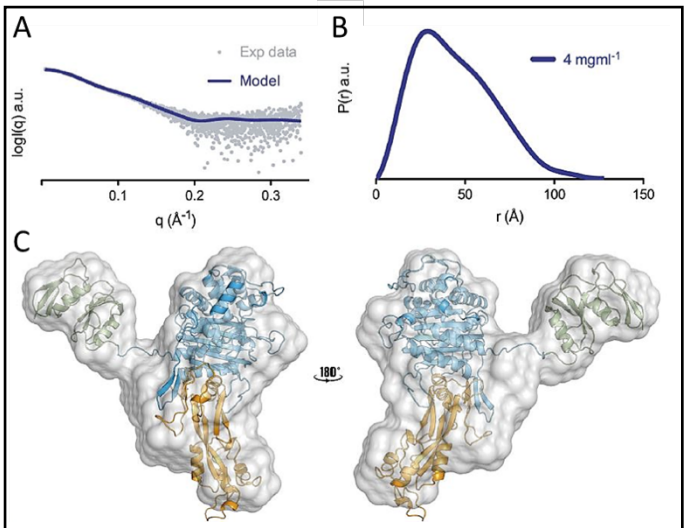


Figure 3. Analysis by SAXS of saPBP1 in solution. A) Fit of the experimental scattering curve (gray dots) and theoretical scattering (blue line) computed for the model of saPBP1 at 4 mg/ml. B) Plot showing the normalized pair-distance distribution function P(r) for saPBP1 (blue graph) reflecting the distance distribution. C) Superimposition on the ab initio-determined SAXS envelope (pale gray) for saPBP1 with its crystal structure-based model.

Scientific case 7: RNA folding into specific three-dimensional structures enables RNA involvement in processes including splicing [67], gene regulation [68], or the assembly of ribo-nucleoprotein machines[69, 70]. The application of time-resolved SAXS to this problem offers the unique opportunity to monitor both the large- and small-scale structural changes that

accompany RNA tertiary structure acquisition. One of the most recent examples of TR-SAXS applied to RNA folding was reported by Plumridge *et al.* for a small RNA domain, the tP5abc helix junction [71]. Here, a microfluidic assembly that addresses the shortcomings of previous mixers for TR-SAXS utilizes mixing technology developed for time-resolved crystallographic experiments, delivering high signal-to-noise data with low sample consumption on time scales that span the folding reaction. The tP5abc, initially in a low salt state, was rapidly combined with Mg^{2+} to induce folding and subsequently probed at time-points ranging from 10ms to 3s after mixing (**Fig. 4**). TR-SAXS data reveal two distinct collapse phases, on short (10ms) and medium (300ms) timescales. Subtler conformational re-arrangements were observed throughout the remaining time-course (cartoon summary of the timescales, kinetic phases, key intermediates and (inferred) Mg associations in **Fig. 4D**).

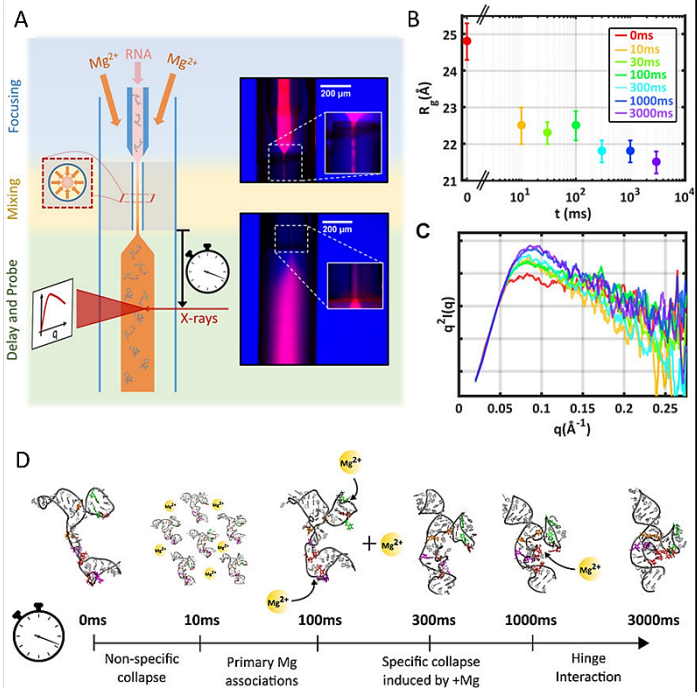


Figure 4. TR-SAXS experiments to trigger Mg-induced folding of tP5abc and the full-time course acquired. A) Cartoon schematic of the microfluidic device used in these experiments (left). Fluorescent images of each device region are shown (right). B) The measured *Rg*'s report on the mean global size of the RNA ensemble. C) Kratky plots highlight subtler structural details. These curves have been smoothed for presentation purposes. The color scheme is identical to panel B. D) Summary of the distinct phases of collapse observed on the route to the native state. The key phases of collapse are named and illustrated, along with a cartoon representation of the inferred Mg associations triggering each phase. Mg ions are schematically depicted as orange spheres; arrows suggest their suspected interaction regions with RNA

In addition to RNA folding, it is important to note that TR-SAXS studies on RNA (and DNA) macromolecules can be extended to their interaction with proteins giving place to large macromolecular RNA/protein complexes that cannot be examined by MX technique due to the limitation of crystallizing such huge complexes. Conventional mixing approaches in crystallography are also impractical due to the large size of the ligand, RNA or DNA, which cannot diffuse into previously grown protein crystals.

Scientific case 8: The study of conformational changes, allosteric processes, and kinetics of proteins over a reaction course. TR-SAXS requires a trigger in the form of either photoexcitation, temperature and pH jumps or, mixing of ligands to initiate the reaction as uniformly as possible in the biomolecule of interest. TR-SAXS studies have predominantly focused on photoactive proteins (*e.g.*, PYP protein [72], hemoglobin [73], and myoglobin [42]), or proteins amenable to either pH-induced changes such as ferritin [74]. There are also some examples of conformational changes of proteins monitored by TR-SAXS, upon combining them with substrates [75]. However, light triggering offers some of the fastest and homogeneous reaction-initiation conditions. Considering that only a small fraction of proteins are naturally photoactivatable, non-natural triggering methods must be employed. Since many protein processes are mediated by small molecules, synthetic analogues of the molecules bearing photocleavable protecting groups can be prepared and used to initiate protein function by photocleaving with a light pulse. Thus, photocaged-initiated TR-SAXS is an emerging approach that allows tracking of time-resolved structural transitions initiated by small-molecule binding using SAXS. The ligand is rapidly released into the protein solution by photocleavage and SAXS profiles are collected at appropriate time delays after decaging. In this regard, adenosine triphosphate (ATP) hydrolysis drives numerous enzymatic reactions and biological processes from the translocation of substances across cell membranes, signalling cascades, protein folding and chaperoning to protein degradation. These enzymes, ATPases, utilize the binding and breakdown of ATP as a source of

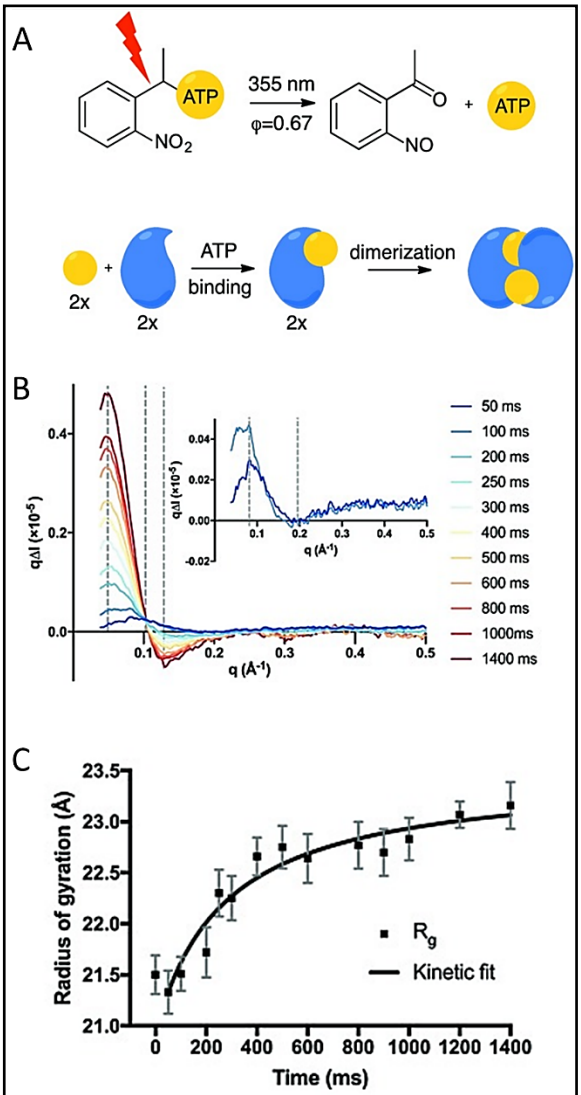


Figure 5. TR-SAXS using photocagable compounds. A) Photoexcited decaging of the NPE group from NPE-ATP using laser irradiation at 355 nm releases free ATP into solution (*top*). Mechanism of ATP-dependent (yellow circle) dimerization of NBDs (blue) (*bottom*). B) Time-resolved SAXS data following photo-decaging of NPE-ATP. Plot of the scattering difference curves ($q\Delta I$ *versus* q) calculated by subtracting the protein scattered with a negative laser offset (~100 µs) from all the subsequent time-point measurements. Inset shows the difference curves of the first two time points (50 and 100 ms). C) Radius of gyration representing NBD dimerization and kinetic fit with an increase in R_g over time (black squares with corresponding standard deviations). The time points after laser excitation were fitted with a second-order kinetic function (black line). The calculated rate constant for dimerization (k_{dim}) from the fit is 6200 M^{-1} s^{-1}.

energy to undergo changes in their tertiary or quaternary structure during their functional cycle. A recent study by Josts and *co-workers* has combined laser-flash photolysis of a caged ATP with TR-SAXS measurements to investigate the ATP-dependent dimerization of soluble nucleotide-binding domains (NBDs) from a bacterial lipid flippase, MsbA [76]. Using this novel approach, they observed the formation of a dimerization competent conformation of NBDs (present in the very early time-point of the experiment) and the subsequent NBD dimer formation (**Fig. 5**) that is a metastable intermediate which decays upon hydrolysis of the ATP molecule (~10 s) and cannot be imaged using the standard static SAXS data-collection strategies.

Functional specifications: For a successful TR-SAXS experiment, there are two requirements that must be considered to initiate the reaction in solution. First, the process must be triggered in a significant fraction of the sample and second, the triggering event must be faster than the process of interest. To this end, at BioSAXS-CALIMA we have envision two modes of TR-SAXS: **(i)** rapid mixing and, **(ii)** direct and indirect light activation. In the case of rapid mixing, classical stopped-flow, and continuous-flow setups (see below) will be available to study reactions triggered by the rapid mixing of different solutions in the ms [75, 77] or even µs timescales if a continuous-flow apparatus with T-shaped micromixers, [39] or jet-in-jet microfluidic devices are employed [78]. To achieve higher time-resolutions, reactions must be triggered with a short laser light pulse, which can be used to either directly photoexcite a light sensitive protein or to create a temperature jump that initiate, for example, a folding or enzymatic reaction. The simplest biological processes to investigate with time-resolved methods are those occurring in inherently light sensitive proteins, many of which can be activated efficiently with short (fs–ns) laser pulses. For non-naturally photoactivatable systems, either photo-labile protecting groups can be chemically added to the natural substrate, or photocaged unnatural amino acids can be site-specifically incorporated into the protein itself.

2.3.1 ***Stopped-Flow TR-SAXS***. The BioSAXS-CALIMA stopped-flow setup will require a stopped-flow mixer with multiple syringes (~4) with 2-10mL reservoirs and a micro-volume mixer, which is essential for triggering the reaction by fast mixing allowing 0.5 ms dead time, and an X-ray observation cell. A schematic view of the experimental setup for the mix-and-inject stopped-flow TR-SAXS experiments is shown in **Fig. 6**.

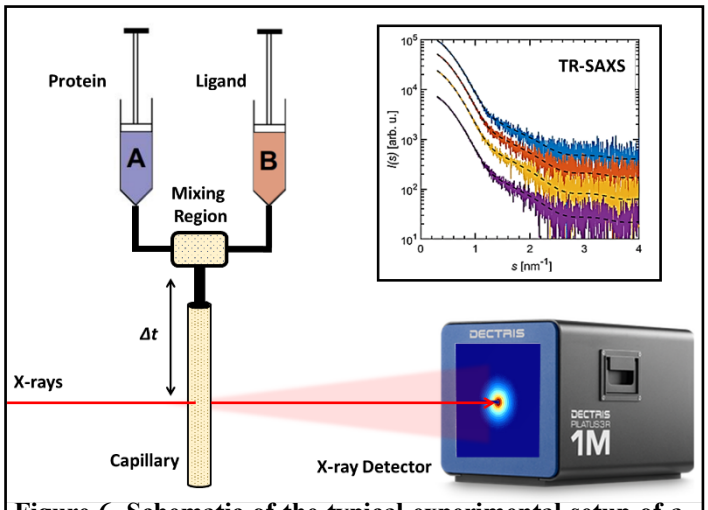


Figure 6. Schematic of the typical experimental setup of a stopped-flow mixer TR-SAXS. Protein and ligand samples loaded into syringes are pushed through a mixing region where the reaction starts, and then through a capillary for measuring. Scattered X-rays are recorded on the detector. By varying the capillary length (Δt) and/or the mixing speed one can collect TR-SAXS at various timescales as shown in the inset image.

2.3.2 *Continuous flow TR-SAXS*. In order to explore faster reactions, reduce sample consumption, and mitigate radiation damage, the BioSAXS-CALIMA will work in conjunction with **Prof. Alexandra Ros** (Arizona State University), to design and develop advanced continuous flow microfluidic mixers, including a chaotic/turbulent mixer and a laminar flow mixer, to collect SAXS data on reactions on the sub-millisecond time resolution range. Dr. Ros has a wide expertise developing microfluidic devices for delivering crystal samples for serial femtosecond crystallography experiments at XFELs [78]. We have planned fully integrated 3D printed or PDMS rapid mixing devices for SAXS to facilitate fast and efficient mixing events between multiple fluid streams containing the biological macromolecule of interest and small molecules (*e.g.,* antibiotics, substrates, inhibitors) that trigger structural changes in the macromolecule. Because one of the main limitations of SAXS experiments is the high sample consumption, we have envisioned a novel prototype that will further reduce the amount of sample in SAXS experiments. This novel device will be a segmented flow generator based on the generation of sub-nL sized droplets of sample suspension intersected by a continuous an immiscible oil phase, allowing injection with a traditional gas dynamic virtual nozzle (GDVN), typically used in Serial Femtosecond Crystallography (SFX) experiments at XFELs. The principle consists of compartmentalizing liquid sample suspension in droplets segmented through an immiscible oil phase (**Fig. 7**). This liquid injection method will be based on creating fast jets in continuous mode injection suitable for pulsed X-ray beams. Our novel device will also be compatible with currently available mix-and-inject and pump-probe TR-SAXS approaches.

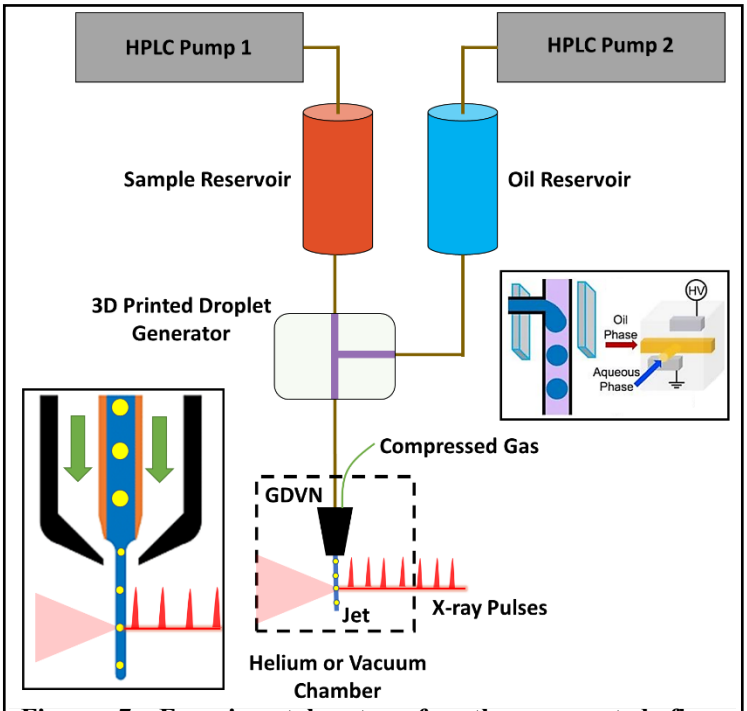


Figure 7. Experimental setup for the segmented flow generator device. Two HPLC pumps will be used to drive two stainless steel reservoirs containing the oil phase and the sample. The reservoirs will be connected to the T-junction droplet generator using fused silica capillaries. The outlet of the droplet generator will be connected to a droplet detector and subsequently to the GDVN using a fused silica capillary. Helium gas will be used in the GDVN to form a stable jet.

2.4. Fibre Diffraction

Background: Many essential functions in the cell are exerted by polymers (microtubules, intermediate filaments, actin filaments, collagen, fibronectin, etc.). The investigation of the structural details of these polymers is challenging to their large size, thus compromising the

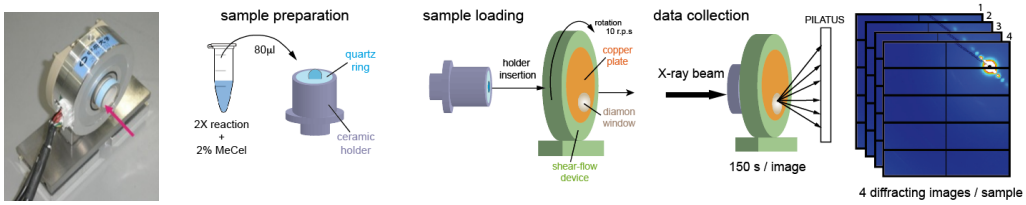


Figure 8: shear-flow device and working pipeline on fiber diffraction of aligned filaments.

obtention of crystals or even the use of NMR approaches; and flexibility, which is also a handicap for EM studies due to preference views and asymmetries). Alternatively, fibre diffraction analysis using high-energy synchrotron X-rays is one of the most powerful approaches for studying the molecular architecture of these polymers due to these are built on a large number of identical repeating subunits that are arranged in specific periodicities. Since these fibres are randomly oriented in solution, the resulting diffuse scattering is not suitable for structural analysis. Instead, the alignment of the polymers in a uniform orientation increases the signal-to-noise ratio by producing a diffraction pattern that can be treated as those from naturally oriented biological and synthetic polymers. Thanks to the combine effort of the main developer, **Dr. Shinji Kamimura** ([79]), and the **NCD-SWEET beamline staff**, ALBA synchrotron holds a device used for the shear-flow alignment of polymers that can has a Peltier attached for setting temperature and fully controlled from the working station. This apparatus is a parallel rotary rheometer (**Fig. 8**), where the specimen suspension is placed in the narrow space between two discs and one of the discs is rotated, giving a stable gradient of low velocity to the suspension [80]. With this technique it is possible to measure structural parameters with sub-Å precision and, in the near future, the plan is to further develop the device to modify the suspension during the experiment and follow polymers structural changes in real-time.

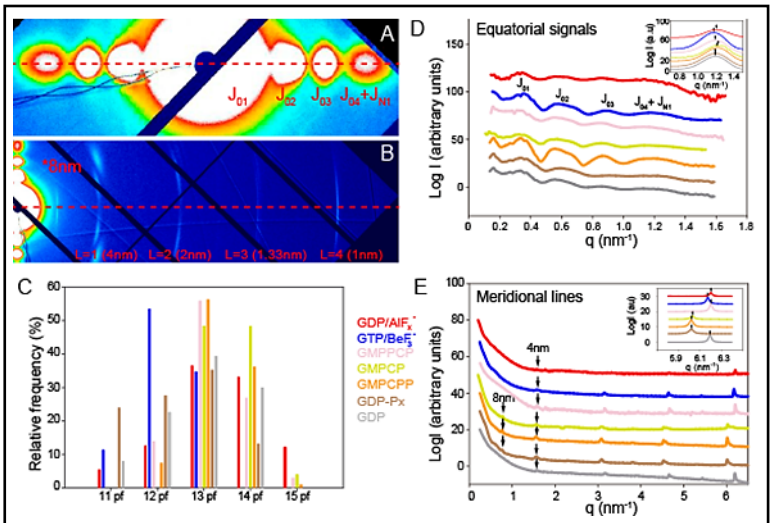


Figure 9: Fiber diffraction of microtubules model systems. GDP-BeF3--MT (blue), GDP-AlFx-MT (red), GMPPCP-MT(salmon), GMPCP-MT (yellow), GMPCPP-MT (orange), GDP-Tx-MT (brown) and GDP-MT (gray). (A) *Top*; representative image of meridional diffraction displaying the meridional plane from l = 1 (4nm) layer line and related harmonics (l = 2 to 4) for longitudinal metric calculations. *Bottom*; meridional intensity. patterns, where arrows indicate the 4 nm and 8 nm peaks. The inset shows the best fit of 1 nm band experimental intensities in a Lorentzian normal distribution, highlighting positional differences between all tested microtubule growing conditions (peaks maxima, arrows). (B) *Top*; representative image of equatorial diffraction highlighting the equatorial plane (l = 0) for lateral metric calculations. *Bottom*; equatorial intensity patterns showing the corresponding Bessel functions from J01 to J04+JN1. The inset shows the JN1 displaying the differences in peak maxima (arrows) that occur under various nucleotide polymerization conditions. The red dashed line in (A) and (B) top images shows planes used for intensity line plotting in qx space and further metric calculations. (C) Estimation of the number of PFs per MT and percentage of each subpopulation within the solution from fiber diffraction experiments (left) and cryo-EM images (right).

Scientific case 9: Microtubules (MTs) are cellular dynamic

polymers made of tubulin heterodimers, a GTPase, and hence, able to generate forces. GTP hydrolysis triggers structural changes in the lattice, which are responsible for the interaction with regulatory factors. The stabilizing GTP-cap is a hallmark of MTs and the mechanism of the chemical-structural link between the GTP hydrolysis site and the MT lattices is a matter of debate. Using X-ray fibre diffraction Estévez-Gallego *et al.* [81] found key differences on MTs lattice depending on the nucleotide bound state (**Fig. 9**), which also argued against some well stablished nucleotide analogues because they compromise the wild-type state of the MT lattice. Besides, this technique has allowed to determine the effect of drugs on the MT lattice [82].

Functional specifications: a shearing aligning device as described in [80] which has been already implemented in NCD_SWEET. For time resolved, the cell should be couple to the Stopped-flow mixer device described above.

2.5. 3D Scanning SAXS

Background: 3D scanning SAXS (3DsSAXS), will enable the study of biological nanostructures in tissues, thus allowing to map small areas to obtain a detailed analysis of structural changes (**Fig. 10**). The arrangement and orientation of the ultrastructure plays an important role for the mechanical properties of inhomogeneous and anisotropic materials, such as polymers, wood, brain, or bone. 3DsSAXS can spatially resolve and quantify the material's ultrastructure orientation in a macroscopic context. This knowledge will benefit a range of medical investigations since understanding the formation of biomaterials could be used in the attempt of bioengineering new systems. As in the case of TR-SAXS, this scientific case requires a small beam at sample position, typically 10-20 µm FWHM, as seen in scientific cases below.

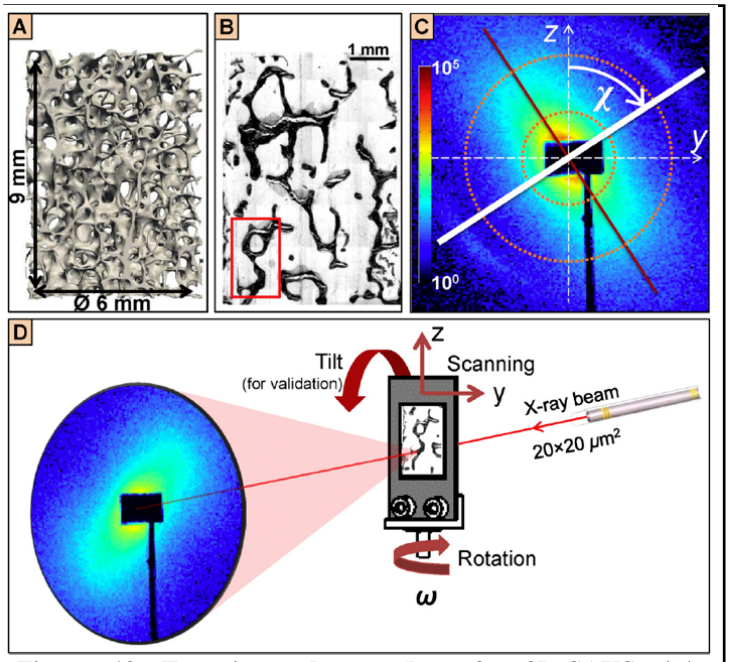


Figure 10. Experimental procedure for 3DsSAXS. (A) Extracted vertebral trabecular bone specimen, imaged using X-ray micro-computed tomography (µCT) at 12.1 µm voxel size. (B) Bright-field image of the bone section, where the selected region of interest (ROI) is indicated (red rectangle). (C) A typical X-ray scattering pattern of the investigated trabecular bone sample. The color-coded bar indicates the number of photons per pixel for an exposure time of 50 ms in a logarithmic scale. (D) Schematic representation of the experimental setup for 3D scanning SAXS. The X-ray beam was focused down to 20 x 20 µm^2 at the sample position. For each rotation angle**ω**, the selected ROI was raster-scanned in the **y**–**z** plane (plane of the detector and plane of the sample for **ω**=0). The transmitted direct beam was recorded by a photodiode, whereas the diffraction data were collected by a PILATUS detector (pixel size= 0.172 mm, sample-to-detector distance= 7.149 m). The ROI used in the proof-of-principle experiment (1.2x1.9 mm^2 or 60x95 raster scan points) was scanned for 30 different rotation angles with an exposure time of 50 ms and a readout time of 3 ms for a single diffraction pattern. The total experimental time for this ROI added up to 4.5 h. The sample tilt around the **y**-axis was only used in the validation experiments.

Scientific case 10: Bone is a composite material, mainly composed of collagen fibrils and mineral platelets. Together, they form the structural units of bone tissue, the

mineralized collagen fibrils. Platelets are located in between and around collagen fibrils. However, it has been shown repeatedly that the main orientation of both components lies along the same direction, the direction of the mineralized collagen fibrils. Mechanical experiments have demonstrated a close relationship between bone ultrastructure orientation and mechanical competence. Knowledge of the ultrastructure orientation can thus provide information that can help improve the biomechanical simulations of bone, and at the same time, advance the understanding of bone mechanics at the local level. Georgiadis and co-workers helped to quantify and understand structure-function relationships between ultrastructure and bone mechanics through the investigation of the trabecular bone tissue, **Fig. 10** [83]. This tissue plays a pivotal role for the biomechanics of vertebral bodies since it carries up to 90% of the load in lumbar vertebrae, where osteoporosis-related fractures represent a major health problem.

Scientific case 11: Understanding brain function requires knowledge of its connectivity both at the microscopic and at the whole organ scale. The anatomical substrate for connectivity is a highly structured network of fibres consisting of myelinated axons ensuring efficient propagation of information between neurons even when located a distant location within the central nervous system (CNS). Hence, methods providing accurate information of cerebral fibre architecture (connectome) have become important tools for neuroscientists. In the case of myelin, which is of relevance for studying brain microstructure, the interaction of X-ray photons with the repeated structure of the myelin sheath produces characteristic scattering maxima, as illustrated in **Fig. 11**. Thus, SAXS signal at the values corresponding to the known periodicity of myelin sheath is heavily weighted by the ordered myelin present in myelinated axons. This has allowed investigations of the myelin sheath structure

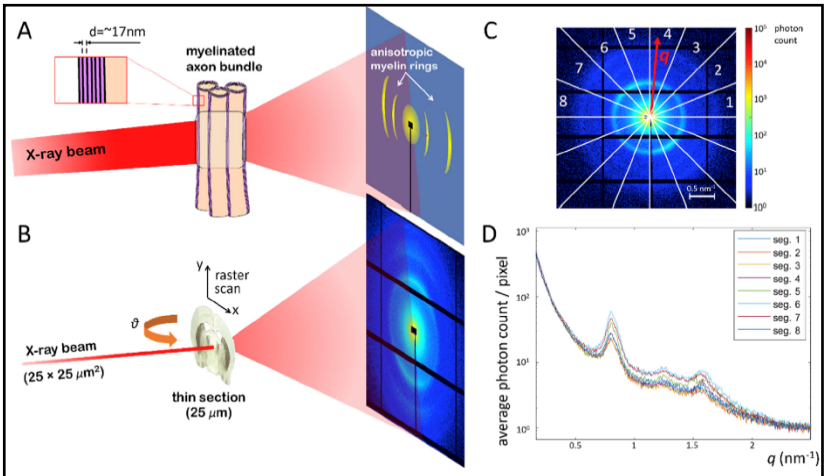


Figure 11: A) The X-ray photons scatter elastically on the layered structure of the myelin sheath, and produce characteristic maxima on the photon-counting detector, at a distance from the center, i.e. momentum transfer value **q**, corresponding to the myelin sheath's repeat distance **d**. B) The thin section is raster-scanned through a pencil beam, moving at a plane perpendicular to the beam and parallel to the detector, for a series of sample rotation angles **θ**. Black horizontal and vertical lines correspond to "blank" detector areas that accommodate the detector's electronics. Signal in these areas is filled in considering the center-symmetry of the SAXS diffraction pattern. The black rectangle in the center and the black line connected to it are caused by the beamstop and its support, respectively. The beamstop is used to block the main unscattered beam and thereby protect the detector. C) For each scan point, a diffraction pattern is recorded and analyzed. It is divided in 16 azimuthal segments to capture the signal anisotropy, and then each segment is integrated as a function of momentum transfer **q**. In practice, values of diagonal segments are averaged weighted by the corresponding number of pixels, thereby exploiting the center symmetry of the pattern, resulting in analysis for 8 segments. D) Plot of the signal for the 8 segments, as a function of the momentum transfer **q**, whose values correspond to real space features as a function of d 2π=q

in different types of human and animal myelinated axons [84]. Also, since the myelin sheath periodicity occurs primarily radially to the axon's main axis, the angle in which the myelin-specific peak appears can be related directly to the axon orientation. Hence, feasibility of probing the anisotropy of the myelin-related SAXS scattering signal and axonal orientation was demonstrated.

Scientific case 12: The skin is designed to protect the organism against injuries, working as an external barrier against external environment like sun-like damages resulting of the formation of free radicals (FRs), responsible for erythema/edema, inflammation, photoaging and skin cancer. This negative effect is usually associated with the ultraviolet radiation (UV). Also, the skin is exposed to infrared (IR) radiation, which is responsible for the generation of FRs. Depending on the dose, can initiate a cascade of different signalling pathways including pathologic or therapeutical effects. It penetrates the epidermal and dermal layers of the skin and reaches deeper than UV, damaging both compartments. Collagen is one of the more damaged proteins by IR radiation, since induces the overexpression of matrix metalloproteinases (MPP). Fernandez *et al.* studied the protective effect of bicosomes (formed by lipid molecules) as discoidal structures or spherical vesicles because they are capable of absorbing the IR radiation, and how they interact with the skin [85]. The state of the skin collagen was evaluated by studying the height of the Bragg peaks obtained using SAXS (**Fig. 12**) and they showed how the biscosomes reduce the formation of FRs in skin subjected to this treatment, preserving the structure of collagen.

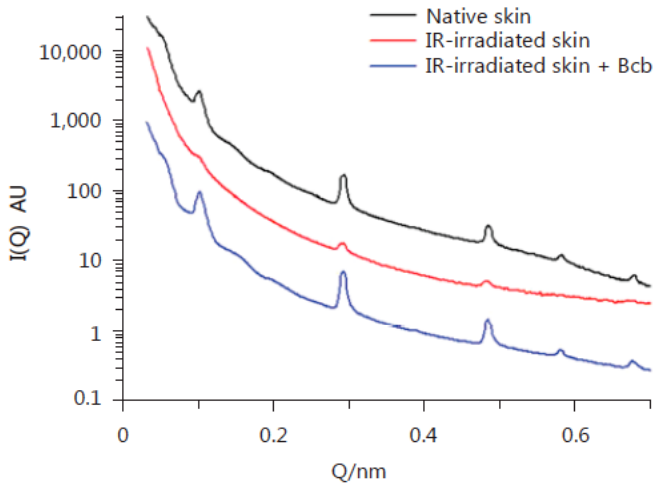


Figure 12: SAXS profiles of different skin samples with the corresponding reflections of collagen. Native skin (black), IR-irradiated skin (red), and skin treated with Bcb (blue). The spectra were shifted on the vertical axis to allow visual comparation IR: 800-1500 nm.

Functional specifications: There are several limitations of the presented method that need to be mentioned. Although the acquisition of SAXS patterns for two rotation angles should theoretically be sufficient to determine the 3D orientation, the errors introduced in practice increase the number of measurements needed for a reliable fit in each sub-volume. Such errors arise experimentally from small uncertainties when calculating 2D orientations, presumably due to the neighbouring volume effect and to the registration based on a nearest neighbour scheme. The effect of these errors becomes more pronounced for cases where the polar angle is 90º or very close to it, since it can lead to "phase wrapping" of the measured 2D orientation angle, which can result in an erroneous determination of the azimuthal orientation angle. Although considering the azimuthal angle from the degree of orientation (DO) fit leaves only a few problematic cases, a

further enhancement could include measuring the sample at different tilt angles with respect to the rotation axis or, alternatively, taking advantage of the information on the azimuthal angle provided by the X-ray diffraction rings. The latter would require less effort since no additional experimental step would be involved. Finally, further validation of the technique concerning accuracy, precision, and sensitivity is needed to make 3DsSAXS a standard method to study the ultrastructure orientation of inhomogeneous anisotropic materials.

2.6. nLC/MS-SAXS

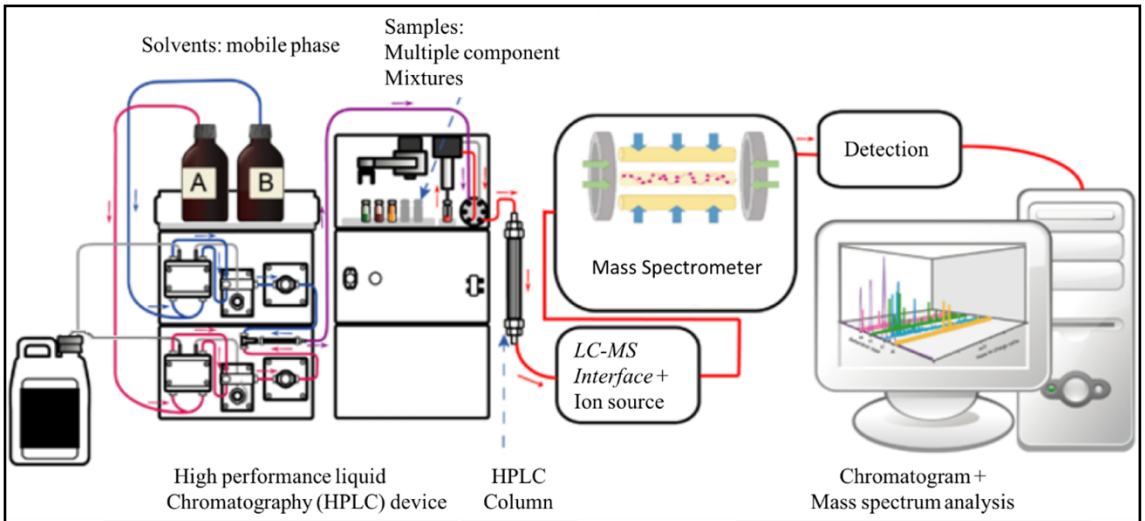


Figure 13: Example schematic diagram of an LC/MS setup. *Credit: Cwszot, Dagui1929, CasJu, and YassineMrabet*

Background: Native liquid chromatography/mass spectrometry (nLC/MS) brings a powerful analytical capability for the determination of the mass and composition of macromolecular samples corresponding to the different sample populations separated by SEC before being exposed to the X-rays. A typical schematic set-up of an LC/MS experiment is shown in **Fig. 13**. By coupling these two techniques one can use them to analyse proteins and macromolecular complexes, as well as to determine the presence of compounds of environmental and biological origin (**Fig. 14**).

Scientific case 13: Nowadays, LC/MS is considered the dominant analytical technique for proteomics and pharmaceutical laboratories. It has been extensively used in proteomics for the detection and identification of the components forming a certain mix and frequently used in projects involving drug development since gives an accurate molecular weight and identification of the populations present in a solution. This also

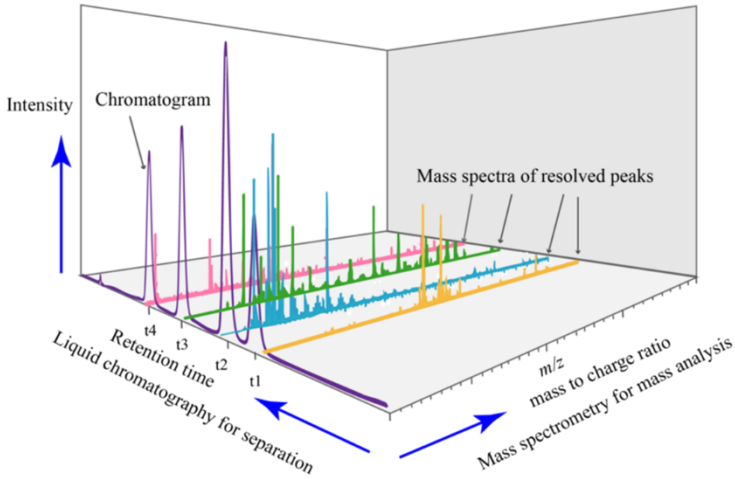


Figure 14: Example output plot from LC-MS analysis. Credit: Daniel Norena-Caro.

includes the possibility to score and select the biological relevant solutions from a pool of generated conformations [86].

Functional specifications*:* The nLC/MS platform needs to tolerate different types of salts, pH range, flow rates and high salt concentrations.

2.7. WAXS

Background: The self-assembly of soft matter involves, in many occasions, crystallization that is very relevant to pharmacy, biological science as well as soft matter in general. Projects on these topics have greatly benefitted from the various attempts of combining time resolving SAXS and WAXS into a single instrument and experiment. In the past, technology has led to compromised geometries, and it is certainly the case that the data has not been available in a single calibrated dataset.

Scientific case 14*:* Elucidate the internal structure of semiconducting polymer nanoparticles due to interaction with surfactants. Nanoparticles (NPs) of semiconducting polymers are being the subject of many investigations due to the advantages of using conjugated polymers dispersed in water or to the possibility of generating nanostructures with controlled morphology previous deposition and implementation in a certain device. Polymeric colloids in water may be an alternative to halogenated solutions in which conjugated polymers are usually dissolved during the deposition step of the process of fabrication of the device. Sometimes surfactants are needed to stabilize semiconducting polymer nanoparticles. An example of these situation has been revealed by combining simultaneous wide and small angle X ray scattering. By SAXS, the long-range assembling of the semiconducting polymer crystals is revealed, while WAXS revealed the crystallization of the polymer in a specific phase that confers it with interesting optical properties of relevance in photovoltaics (**Fig. 15**) [1]

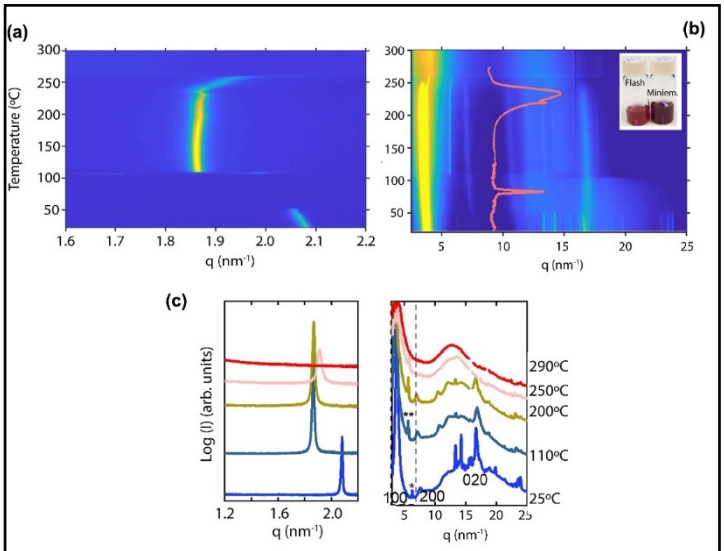


Figure 15. Two-dimensional map of the temperature dependent SAXS (a) and WAXS (b) data of P3HT NPs prepared by miniemulsion. (c) SAXS and WAXS intensities as a function of the module of the scattering vector q from miniemulsion NPs at selected temperatures during heating. (d) Different colour of the semiconducting polymer colloids prepared with and without surfactant. Adapted from [1].

Functional specifications: A WAXS detector mounted at short distance from the sample, above the beam and organize at different azimuthal angle so that it will cover at least one quadrant of the diffractogram and the *q*-range. The synchronization of these WAXS detectors with the SAXS

one would allow to obtain in a single shot at least one quadrant of a continuous SAXS-WAXS q range or larger to be coupled to the full azimuthal range SAXS. This will involve a simple and efficient method to place all data on the same absolute scale of intensity to offer maximum benefit to users and the interpretation of the data.

3. USER COMMUNITY

3.1. Existing BioSAXS community

Life sciences is a large and high-impact area in which synchrotron facilities play an essential role. ALBA synchrotron is no exception: we estimate that about 31% of all ALBA publications are related to life sciences, using only about 27% of the granted beamtimes from all the operating beamlines. In Europe, the growth of the structural biology scientific community has pushed several synchrotrons to build fully dedicated beamlines for BioSAXS (Diamond and ESRF) or adapt generic SAXS beamlines to be compatible with the specific instrumentation required for

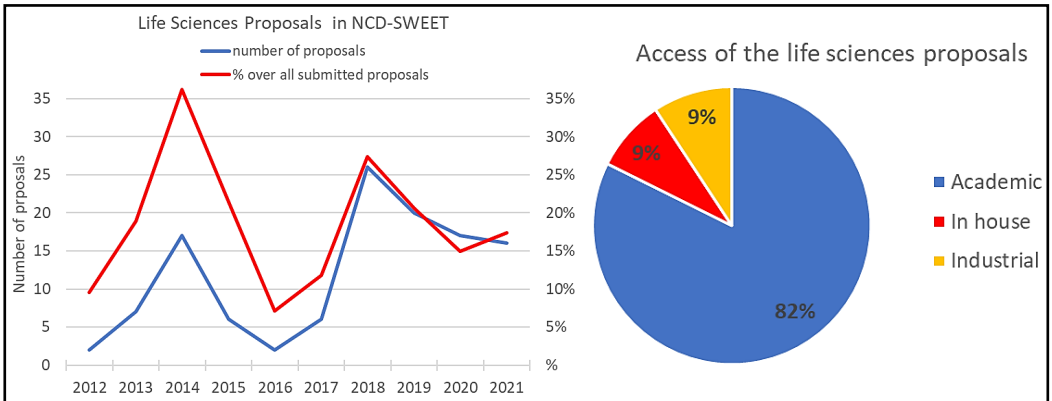


Figure 16. (***left***) evolution of the life science proposals in NCD-SWEET. The descent of the proposals in 2015-17 is due to the refurbishment of the beamline. (***right***) type of access of the life science proposals.

BioSAXS (PETRA III and Soleil). The current SAXS beamline at ALBA, BL11-NCD-SWEET has also felt an increasing pressure from life sciences users from the start of operations (**Fig. 16**, *right*). However, the refurbishment in 2015-17 strongly oriented the beamline towards materials science field. The initial user pressure after the refurbishment (2018) faded in views of the new focus of the beamline, so the structural biology user community is more and more submitting beamtime proposals to other dedicated beamlines in Europe since then, with the result that the percentage of the life sciences proposals in BL11-NCD-SWEET has decreased from 27% in 2018 to 17% in 2021.

Still, the user community is strong and the interest for SAXS experiments is always present, as shown in the number of submitted proposals in last years despite the non-bio-orientation of BL11-NCD-SWEET. A total number of 119 life sciences proposals have been submitted (85 in last 5 years). Remarkably, the life sciences proposals have been granted access to BL11-NCD-SWEET beamline through all possible ways, that is, in-house, industrial, and academic (**Fig. 16**, *left*). While BioSAXS-CALIMA beamline is not meant to cope with all the life sciences experiments

carried out at BL11-NCD-SWEET, the trends in this beamline are one of the best estimators of the current user demand.

The large number of the letters of support and the number of unique life sciences academic research groups that have used the ALBA SAXS beamline are also showing the strong support of the community for the construction of a BioSAXS beamline at ALBA (**Table 1**). On top, we can add the MX research groups that are users of BL13-XALOC beamline, whose majority are also users of other European BioSAXS beamlines. In conclusion, the potential BioSAXS scientific community at ALBA is large, mature, and highly productive.

Concept	**Number**	**Comments**
Letters of support for this proposal	25	A particular institution may host several research groups
Number of Life sciences research groups using BL11-NCD-SWEET	54	Only academic proposals accounted
Number of MX research groups at BL13-XALOC in last 5 years (2017-2021)	49	Potential users of a BioSAXS beamline
Number of soft condensed matter research groups using BL11-NCD-SWEET in last 5 years (2017-2021)	25	Standard measurements of these groups can be compatible with BioSAXS-CALIMA

Table 1. Estimators of the BioSAXS-CALIMA beamline user community. Research groups can be accounted in more than one category.

The research groups employing BL11-NCD-SWEET on biological samples are spread geographically throughout many *Comunidades Autónomas* in Spain, with two main focuses in Barcelona and Madrid (**Fig. 17**). Thus, the construction of the BioSAXS-CALIMA beamline will benefit many local communities, facilitating the dissemination of the enabled techniques and science. The international community of the BL11-NCD-SWEET on biological samples counts for the 42% of the total groups. However, it is foreseen that the BioSAXS-CALIMA beamline

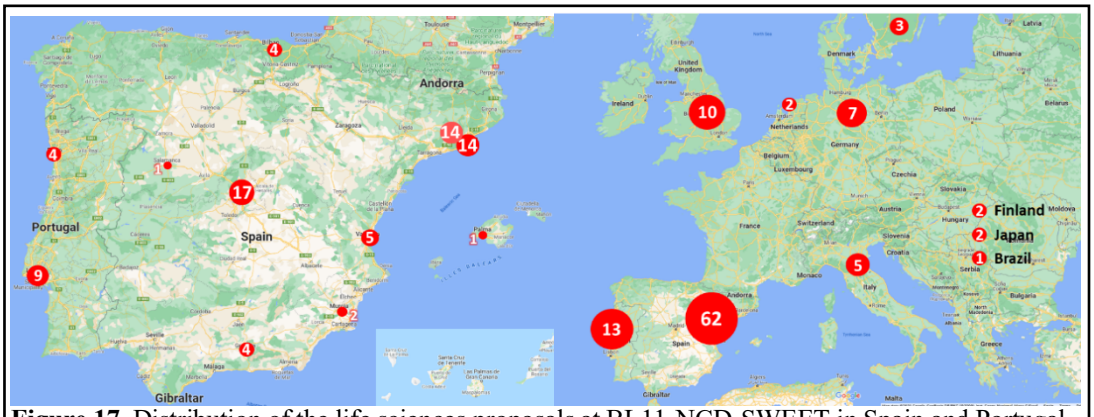


Figure 17. Distribution of the life sciences proposals at BL11-NCD-SWEET in Spain and Portugal, (*left*) and in Europe (*right*). The proposals of the ALBA staff and the user groups in Barcelona area are shown separately. Interestingly, the geographical distribution of the BioSAXS groups in Spain follows very closely that of the MX groups.

will attract more local community, as the scientific case is also covered by beamlines in other synchrotron facilities. The ratio national/international proposals can therefore be kept in healthy levels.

3.2. User community attracted by new scientific cases.

The BioSAXS-CALIMA beamline not only aims at attracting both the preserved and the lost BL11-NCD-SWEET user community for standard experiments on biological samples. The beamline also aims at offering to the user community new experiments not previously covered at ALBA and, most importantly, link them to techniques already included in the ALBA user program.

Probably, the most important use case is the *standardized* BioSAXS experiment applied to *high throughput* BioSAXS (HT-BioSAXS). The number of important biological targets is staggering, moreover, novel societal threats and challenges to human health are constantly emerging. Structure-based drug discovery and fragment-based screening are major strategies used by all pharmaceutical companies to bring new drugs to market. And yet, most new drugs fail because they lack efficacy. These strategies often rely upon ground-state high-resolution crystal structures. An emerging alternative, that de-risks new therapeutic avenues, is to study the whole dynamic reaction cycle, which will reveal and mediate changes that are focused upon a particular function. Indeed, BioSAXS-CALIMA will expand capabilities to room-temperature complexes or reaction mechanisms inspired by serial crystallography approaches. To meet these challenges, there is strong consensus in life science community to widen the applications of SAXS technique in life sciences in the mid-term by:

- Making BioSAXS and time-resolved SAXS methods more accessible to smaller groups through automation in sample delivery and data processing
- Building expertise and engagement by training the user community and facilitating the computational and wet-lab tools to make best use of the future BioSAXS-CALIMA beamline at ALBA-II.
- Increasing industry involvement: dynamics of enzymes and protein-ligand interactions will have major implications in drug discovery.

Most of the scientific cases above go in this direction. In addition, new ones which may appear would eventually benefit from this approach. A paramount example is the new opportunity that the availability of Alphafold2 [87, 88], which can predict *in silico* the folding of proteins. This computational tool coupled with experimental verification by BioSAXS measurements could increase dramatically the prediction of new protein structures without the need of crystallizing proteins.

In summary, it is expected that the new user-oriented beamline, BioSAXS-CALIMA, at ALBA-II will lead to novel results of fundamental importance in areas such as structural biology and biochemistry, among others. Also, actions can be taken within the user community to stimulate

the R&D of academic and industrial groups to achieve a leading position in the science and technology transfer based on the BioSAXS-CALIMA capabilities.

3.3. Soft condensed matter user community

In addition to life sciences, the BioSAXS-CALIMA beamline could be used to perform routine experiments and measurements on soft condensed matter and polymeric science. Some scientific cases fall between biology and soft condensed matter (*e.g.*, micelles, biopolymers) and some links exist between two scientific communities. From the BL11-NCD-SWEET, it is estimated that around 25 research groups could benefit from the access to BioSAXS-CALIMA beamline, that could successfully substitute BL11-NCD-SWEET beamline. This ID beamline would deviate the more standard experiments/measurements to the bending magnet beamline. As a result, the user pressure on BL11-NCD-SWEET could be reduced. The beam properties of BioSAXS-CALIMA are fully compatible with this type of experiments. while the end-station may require a relatively small change consisting in a rearrangement of the end-station instrumentation. The full geometrical compatibility needs to be assessed in an early stage of the end-station design.

4. BEAMLINE DESCRIPTION

4.1. Optical layout

BioSAXS-CALIMA is conceived as a bending magnet beamline at the current ALBA facility but is designed to be fully compatible and greatly benefit from the ALBA-II upgrade. As a conceptual principle, the sample-to-detector (camera length) and the beamstop positions will be fixed to reduce the complexity and time required for operating the beamline between experiments, so that maximum throughput can be achieved. Experience by other European BioSAXS beamlines clearly points to this design principle: the two dedicated BioSAXS beamlines always operate at a fixed camera length, even though the distances are adjustable. The scattering vector q range can be easily tuned by changing the photon energy.

At BioSAXS-CALIMA, two optical setups will be required to accomplish all the scientific cases exposed in section 2:

- **Large beam** at sample: the beam is focused at the beamstop/detector to maximize the resolution in q. The beam should be ~200-500 μm in size at sample position to fully expose the sample and reduce radiation dose per sample volume. This setup is considered as standard, and will be used in batch mode, SEC/IEC-SAXS, fibre diffraction, nLC/MS-SAXS and WAXS experiments.
- **Microfocus beam** at sample: The beam is focused down to ~10 μm at the sample position to obtain spatial resolution at the expense of degrading the resolution in q. This setup will be used in TR-SAXS and 3DsSAXS experiments.

Importantly, the optical design presented here is fully compatible with standard, non-challenging soft matter, and polymer science experiments. Moreover, the requirements on the end-station setup may be different, but compatible after a detailed 3D design.

The beamline optics for the large beam mode consists in a double multilayer monochromator and a KB system as a focusing optics (**Fig. 18**). The microfocus mode is attained by inserting a set of Compound Refractive Lenses (CRLs) in the beam path, close to the sample, and focusing the beam to a secondary source slit (SSS) upstream the focusing lenses. As a design feature, the sample-to-detector distance is fixed and set to 3m. The layout has been modelled in 3D to verify the geometrical constraints (**Fig. 19**).

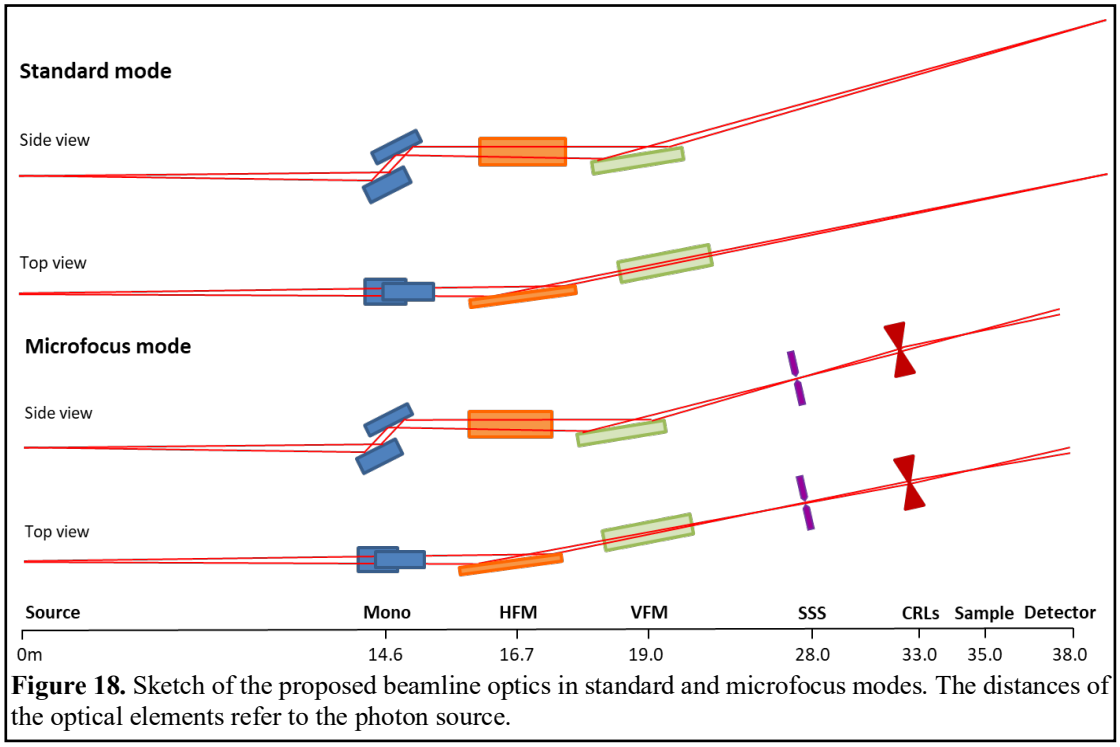


Figure 18. Sketch of the proposed beamline optics in standard and microfocus modes. The distances of the optical elements refer to the photon source.

The main optical elements are described in the sections below. The diagnostics of the beamline are based on fluorescence screens, which provide an easy-to-interpret monitoring of the beam at a moderate, but still sufficient, spatial, and temporal resolution. Measurement of the flux will be done using PIN diodes.

4.2. Photon source

The beamline is proposed to be built on a bending magnet port, as the beam properties allowed by undulator sources, such as a sub-micron focus or a coherent beam, are not strictly required by BioSAXS. Nevertheless, the technique is photon hungry, especially in time-resolved experiments

with exposure times in the ms range. Therefore, it is proposed to install a super-bend source in the ALBA-II storage ring. The port proposed is BL07, for the following reasons:

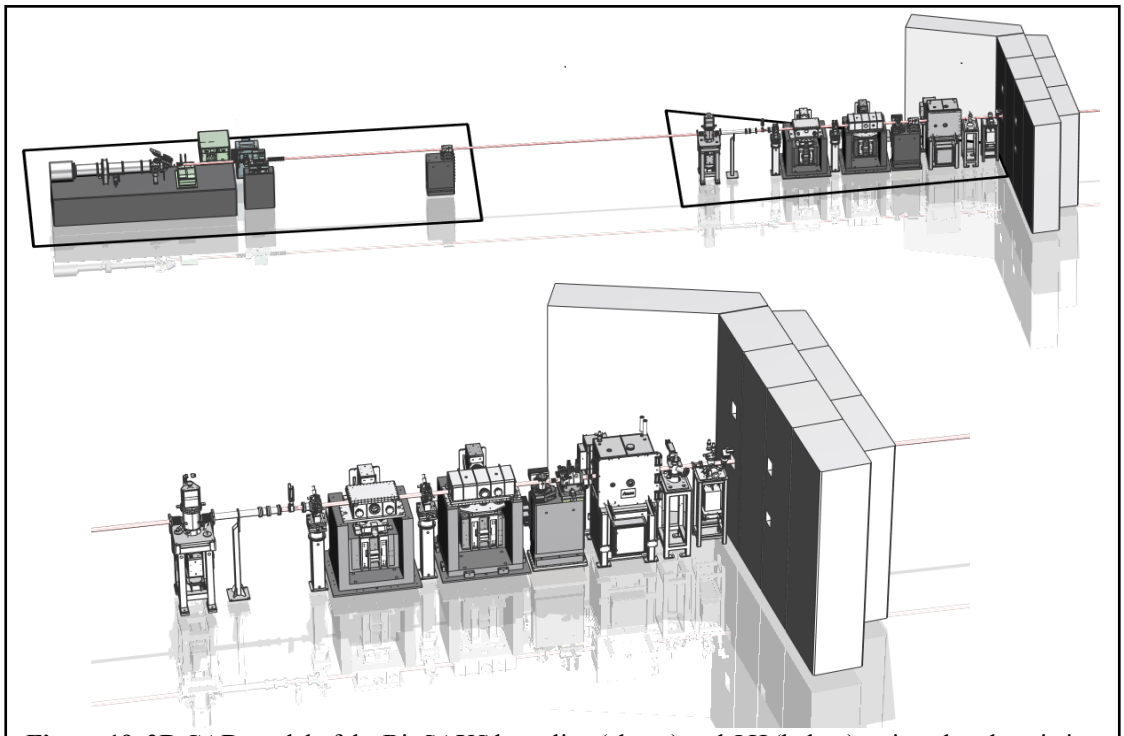


Figure 19. 3D CAD model of the BioSAXS beamline (above) and OH (below), using already existing optical elements at ALBA from different beamlines. Distances to source and between elements can be derived from Fig. 4.1 (Montse Prieto, Engineering Division-Transversal).

- Distance to source: BL07 is the bending magnet port which has the shortest source-to-wall distance in the current ALBA-II design (11.219m), and the second shortest distance in the current storage ring (12.841m). A short distance is convenient to accept a larger horizontal beam divergence and increase the flux.
- Convenient location: the port will be located in the life sciences area, between BL06-XAIRA and BL09-MISTRAL beamlines, and is very close to perimetral biological laboratory.

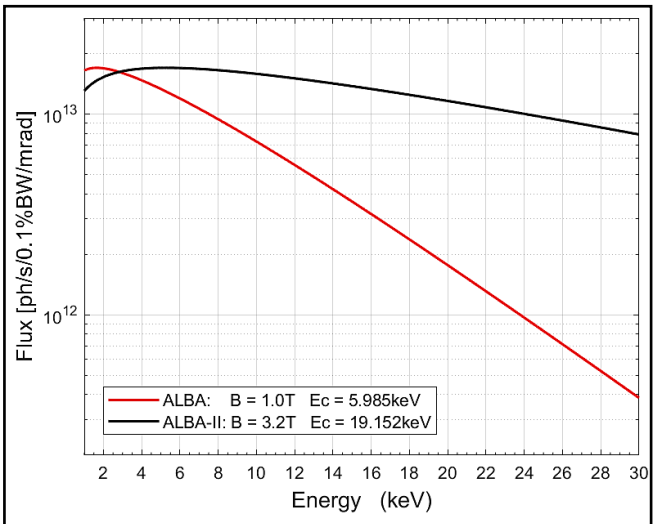


Figure 20. Photon flux delivered by the proposed sources for ALBA (bending magnet) and ALBA-II (Superbend) storage rings.

Second best option is port BL03, which has a source-to-wall distance in the ALBA-II design and the current storage ring of 11.978m and 11.839m, respectively.

The photon flux delivered by the ALBA-II bending magnet (which is very similar to that of ALBA) and the ALBA-II superbend is shown in Figure 20. Importantly, the cooling system of the monochromator must be dimensioned to withstand the increased power delivered by the superbend.

The front-end proposed for the BioSAXS beamline is the ALBA standard, already installed in other BM beamlines. It includes a set of fixed slits, a four-blade XBPM used for diagnostic purposes, a set of moveable slits to shape the beam before the beamline optics, a photon shutter and a bremsstrahlung stop.

4.3. Double Multilayer Monochromator (DMM)

The monochromator will be a water-cooled Double Multilayer Monochromator (DMM). Only one pair of multilayers will be enough to cover the whole energy range of the beamline (6-17 keV), although a second pair cannot be excluded if shown to increase the flux at high energies. The technical specifications of the monochromator are listed in **Table 2**.

DMM (Double Multilayer Monochromator) Material: Mo/B_4C; Energy bandpass (BW): 1%; d-spacing ~2.5nm; number of bilayers ~150; gamma ratio Γ=0.5; possible second set of multilayers: W/C.
HFM (Horizontal Focusing Mirror) Material: Silicon; Coating: Rh; Optical length 1m; angle of incidence 3.8mrad.
VFM (Vertical Focusing Mirror) Material: Silicon; Coating: Rh; Optical length 1m; angle of incidence 3.8mrad.
CRLs (Compound Refractive Lenses) – only used in microfocus mode. Material: Be; biconcave 2D lenses; radius R=0.5mm; Acceptance: 1mm, circular; Number of lenses: 19 @6keV, 80 @12.4keV, (154 @17keV); Transmission of the lenses is 18.2% (6keV), 23.9% (12.4keV) and 22.4% (at 17keV); focal length at 12.4 keV: 1.425m

Table 2. Technical specification of the main optical elements.

Accepted horizontal divergence by the optics is ~0.2mrad. The gamma ratio must be set to 0.5 to suppress the second harmonic of the multilayer, as the KB mirrors do not offer enough harmonic suppression at low energies (**Fig. 21**).

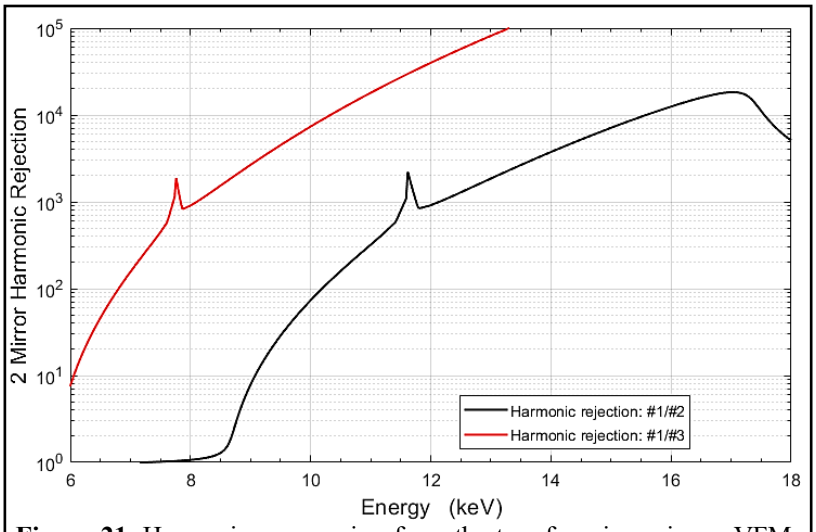


Figure 21. Harmonic suppression from the two focusing mirrors VFM and HFM. The second harmonic needs to be suppressed by the multilayer.

Horizontal bounce is strongly recommended in DMMs for stability reasons, especially in an automated beamline such as BioSAXS-CALIMA. However, the energy resolution, which depends on the beam divergence and the energy distribution in the dispersive direction, is degraded. A careful analysis needs to be

done in a further stage of the project. For the purpose of the study, a vertical bounce has been assumed, although the study and conclusions do not differ otherwise, since the accepted beam divergence is similar in both directions.

The monochromator can operate as a nearly fixed exit beam, without adjustment of the gap between multilayers, since the beam excursion in the dispersive direction $\Delta h = 2g \sin\theta \, \Delta\theta$, where g is the gap between optical surfaces and θ is the Bragg angle, is of the order of few tens of microns for the whole energy range. We note that the optics behind the DMM, including diagnostics and slits, could require water cooling as the wide photon energy bandpass of the multilayers may lead to a significant amount of power or power density on the in-vacuum optics behind.

4.4. KB focusing optics (HFM and VFM mirrors)

The proposed focusing optics is based on a pair of reflective mirrors in a typical KB configuration, which is the optimal choice for an automated BioSAXS beamline since:

- The KB configuration separates optically the properties of the beam in the horizontal and vertical directions, making the beamline commissioning and operation easier than toroids.
- Mirrors are non-dispersive elements, thus making the properties of the beam independent from energy. The photon energy for the experiment is set by adjusting the DMM only. This is important as the photon energy determines the *q*-range of the experiment.
- There is a strong in-house know-how at ALBA, with excellent results.

The main factor preventing CRLs to be used as a main focusing optics is the very limited beam acceptance, which will reduce dramatically the collected flux in a divergent beam from a bending magnet. Toroidal mirrors can collect a larger horizontal beam divergence than the HFM mirror, but this is not offering any advantage to the BioSAXS experiments, as a larger divergence implies a larger beam at sample. The beam falls out of the sample area to be exposed and is not used for the experiment. As a matter of fact, typical beam divergence of the European BioSAXS beamlines is ~0.2 mrad, which is that accepted by the proposed KB system. On top, toroidal mirrors cannot change the focal point, as required in the microfocus mode, without changing the beam path.

The technical specifications of the HFM and VFM are listed in Table 2.

4.5. Microfocus setup: CRLs refocusing optics

Beam focusing at sample position, as required in the microfocus mode, will be achieved by inserting a series of CRLs in the beam path. The proposed technical specifications, which assume conservative manufacturing parameters of the lenses, are listed in Table 2. The CRLs are required to be mounted in a transfocator setup to adjust the number of lenses upon photon energy to keep the focus always at the sample position. As expected, the number of lenses increases with energy, to the point that 154 biconcave lenses are needed to focus the beam at 17keV. The number of

lenses could be reduced by careful design of the transfocator, with different types of lenses designed for the high energy range.

4.6. Beam characteristics in Standard Mode

	ALBA (BM)	ALBA-II (superbend)
Standard mode (focus at detector)		
Spot at sample position	532×520 μm^2 176×198 μrad^2	532×590 μm^2 175×175 μrad^2
Spot at detector position	174×76 μm^2	23×58 μm^2
Microfocus mode (focus at sample)		
Spot at sample position	37×15 μm^2 425×403 μrad^2	5.3×11 μm^2 428×425 μrad^2
Spot at detector position	1.30×1.24 mm^2	1.30×1.28 mm^2

Table 3. Beam sizes and divergences (FWHM) at sample and detector positions for the ALBA and the ALBA-II sources, modelled at 12.4 keV. The focal spot sizes are limited by the source. The divergences and the defocused sizes are limited by the acceptance of the mirrors (3.8mm) and the CRLs (1mm) for the standard and the microfocus modes, respectively.

The optics described above are delivering a beam in the 6-17 keV range with beam sizes and divergences listed in **Table 3**. Essentially a beam of ~0.2 μrad divergence in both dimensions is efficiently transported to the end-station. This acceptance is similar with respect to the dedicated BioSAXS European beamlines despite the toroidal mirrors (with slit acceptances of 4mm at 20m from source). The beam size at sample is suitable for all the capillary diameters. The current size of the focused beam at the detector at ALBA is similar to the pixel size of the Dectris-PILATUS3 detectors (172μm). In ALBA-II storage ring, the size of the focused beam will be significantly smaller, opening the possibility to focus the beam on an intermediate distance between sample and detector, to reduce the beam size at sample, if deemed convenient.

The raytracing simulation has assumed a photon energy of 12.4 keV (**Fig. 22**) but is valid in the whole energy range as the setup is non-dispersive.

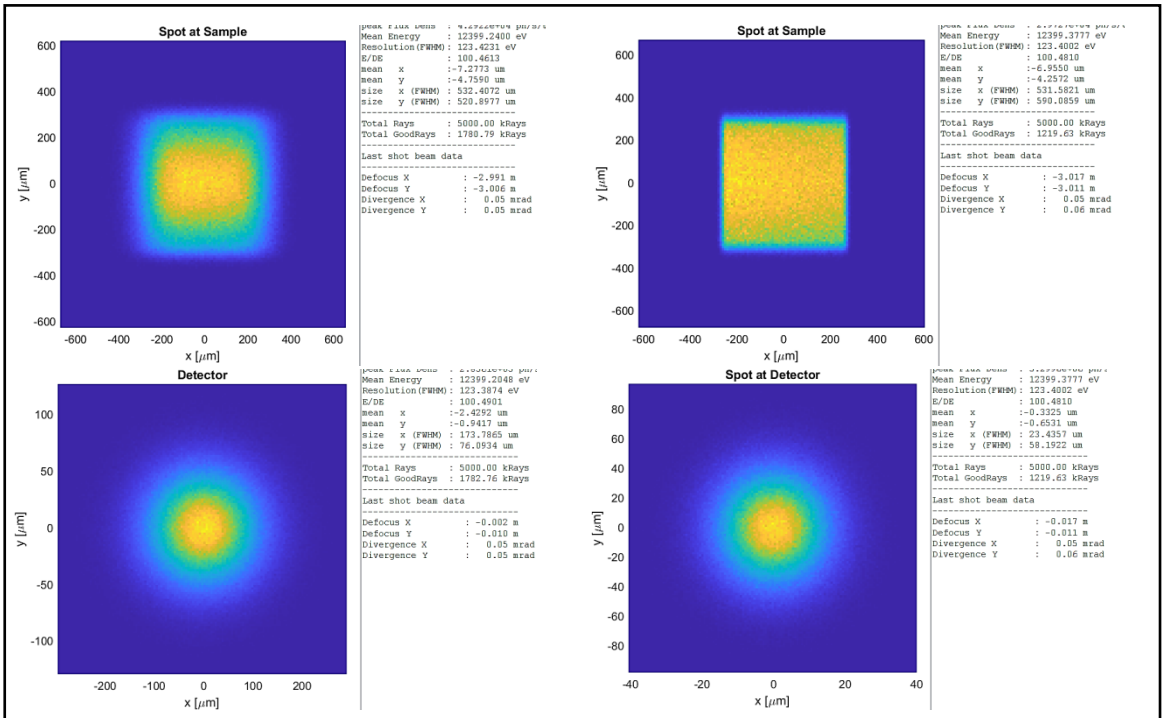


Figure 22. Beam spot in Standard Mode at sample (above) and detector (below) positions, for the current ALBA bending magnet source (left) and the Superbend source available with ALBA-II (right). The beam is focused on the detector plane, which lies 3m from sample position.

Flux delivered by the beamline is above 2×10^{12} ph/s at sample in the whole 6-17 keV range (Figure 23). The value compares well with respect to other beamlines and allows very high-quality data collected at 100 ms/frame, and even good data when collecting in the few ms frame rate (minimum flux required for such experiments is 10^{11}–10^{12} ph/s).

Overall, the modelled beam dimensions and fluxes of the standard mode of the BioSAXS-CALIMA beamline compare very well with the dedicated beamlines in other synchrotron sources, which makes the beamline very competitive. Moreover, the beamline offers a beam at higher photon energies than the rest of the BioSAXS beamlines, which will make BioSAXS-CALIMA one of the most competitive beamlines in Europe for experiments with high-density or thick samples.

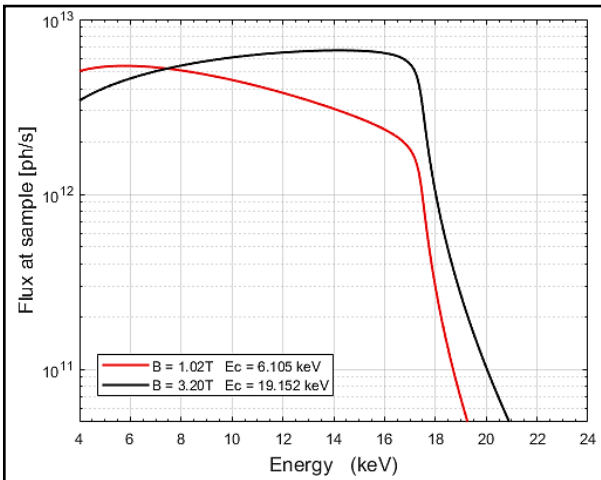


Figure 23. Flux at sample for the BM (ALBA and ALBA-II) and superbend (ALBA-II sources). The calculation includes reflectivity of multilayers (lineally increasing with E, 1% BW); aperture and reflectivity (with roughness) of the mirrors; dependence of the source vertical divergence on the photon energy, and the critical energy of the source. The flux at sample in the superbend source is much reduced due to increased critical energy. Optimum magnetic field B of the superbend source for BioSAXS is 1.8-2.2 T (data can be sent upon request).

4.7. Beam characteristics in Microfocus Mode

The microfocus mode will be greatly benefitted by the ALBA-II upgrade, as the horizontal beam size at sample is reduced a factor of 6, while the vertical is also reduced by 30% (**Table 3** and **Fig.**

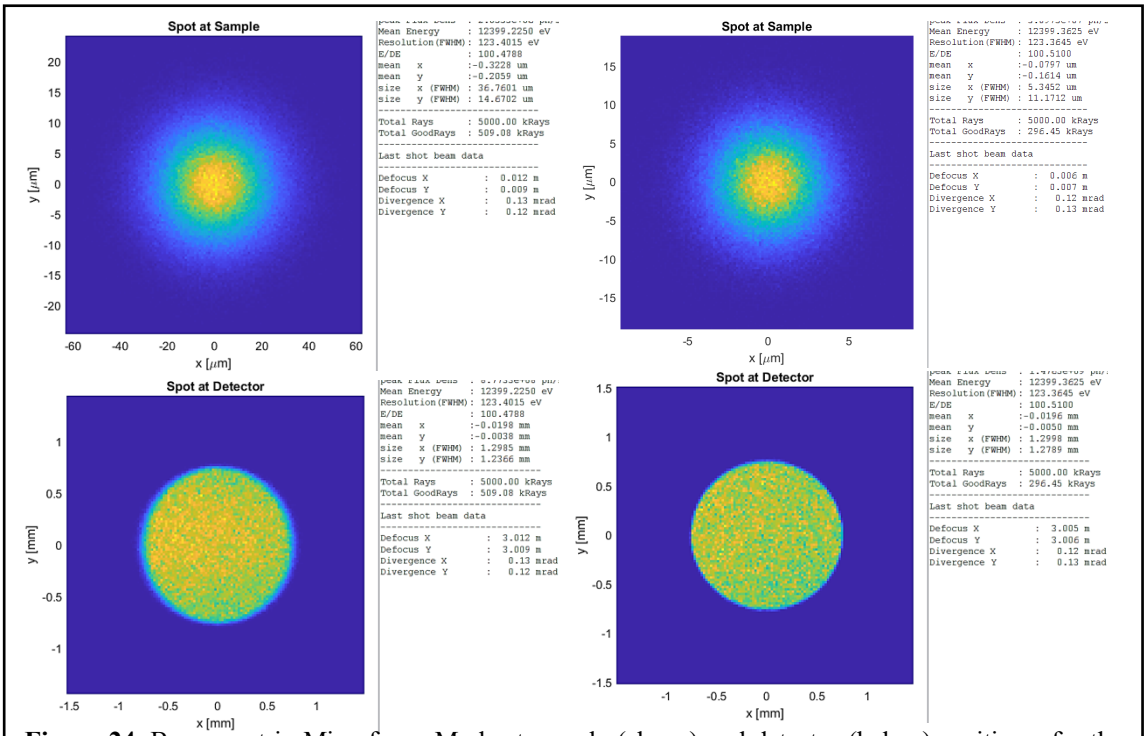


Figure 24. Beam spot in Microfocus Mode at sample (above) and detector (below) positions, for the current ALBA bending magnet source (left) and the superbend source available with ALBA-II (right). The beam is focused on the detector plane, which lies 3m from sample position.

24). This makes BioSAXS-CALIMA a beamline with unique capabilities for TR-SAXS and 3DsSAXS experiments.

Inevitably, the defocused beam at the detector position (~1.2mm) is much larger than the pixel size (172µm) and will reduce the resolution in q. Two strategies can be implemented to mitigate this effect. First strategy consists in simply reducing the accepted divergence, at the expense of reducing flux. Second is to focus on an intermediate position between the sample and the detector, at the prize to enlarge the beam at sample and the beam stop size, preventing to detect the scattering pattern in the low q-range. The optimum balance will depend ultimately on the sample and the goals of the experiment.

5. EXPERIMENTAL END-STATION

The experimental station will be housed in a conventional hutch with sufficient space to accommodate all experimental components, including the secondary source slits, and to allow easy access (**Fig. 25**). It will include as well the focusing system to spare beam path length. The temperature regulation within ±0.5K is essential.

5.1 Instrumentation

The instrumentation of the end station is also described in detail in the Scientific case. Importantly, all the end-station equipment is commercially available, and can be readily implemented. The new BioSAXS-CALIMA beamline should be compatible with in-air and in vacuum sample environments and will provide with optimized experimental conditions for different SAXS approaches:

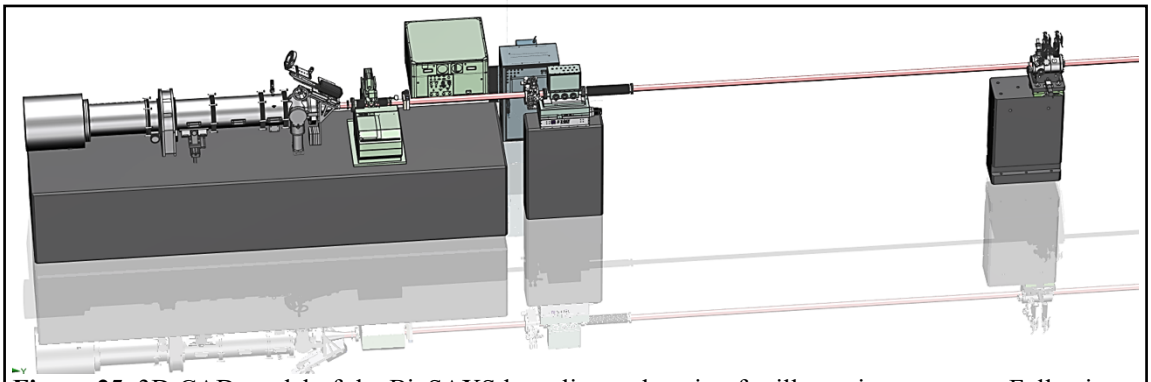


Figure 25. 3D CAD model of the BioSAXS beamline end-station for illustration purposes. Following the beam direction, depicted elements are: secondary source slits, CRL transfocator, beam defining and guard slits, automatic sample changer, WAXS detector and vacuum chamber with SAXS detector inside. (Montse Prieto, Engineering Division-Transversal).

- **Sample mounting robot** as described in High-Throughput screening section (HT-SAXS). The most used commercial product is supplied by Arinax, although other products are to be considered.
- **Sample holders**: capillaries (solution), gels/solid (polymer slice, tissues, pellet), liquids/gels holder, high pressure cell (pressure induce preparation of super crystals) and shear aligning cell (fibre diffraction).
- **Capillary holders** containing several slots for capillaries of 1.8 mm, 1 mm, 0.5 mm and 0.3 mm diameters. The sample capillary will be kept in a vacuum environment to remove the background due to atmospheric scattering.
- **Integrated syringe pumps** controlled remotely for injection into the sample cell, including a Hamilton dispenser. Several pumps may be required for the different diameters.
- **Sample lightning**: cold light source with filters to cover the range 275 nm – 1800 nm.
- **HPLC**: A variety of SEC and IEC columns will be available, who may also have the option to use their own set of columns.
- **Lasers for photoactivation**: Picosecond laser system composed of two lasers. Preferred choice is: 1) Spectra Physics, Ti:Sapphire Spitfire Pro 5; 780nm; 2ps; 1kHz; 5mJ/pulse; and 2) TOPAS OPA; tuneable range: 350nm-2µm; pulses typically stretched to 30ps. Nanosecond laser system: OPOTEK OPOLETTE 355 II HE; 7ns pulse duration; 20Hz; 410-600nm: ~3-6mJ; 230-400nm:0.5-2mJ.
- **Temperature-controlled flow cell** will be placed in a vacuum chamber. Best commercial choice is the Linkam TMS600 Hostage, with a temperature and environmental control stage. The minimum temperature range should be 5ºC – 60ºC.
- **Stopped-flow cell**: As for now, Biologic SM400 seems to be the obvious choice. If any other solution arises, it will be considered. The cell could also be built in-house.
- **Novel continuous flow cell for TR**-SAXS designed in collaboration with Dr. Ros
- **Microfluidic cell** will be used in TR-SAXS and 3D-sSAXS. Different channel diameters may be required to optimize the time resolution and sample availability.

- **A refractometer** to measure the concentration of the sample for an accurate data analysis. A thoughtful selection will be done on the different companies supplying such a device. It can be included in HPLC set up.
- **Other cells** will also be considered: rheometer, traction cell.
- **Vacuum chamber** between sample and detector: A 3m, fixed-length vacuum cylindric chamber eliminates the air scattering. The beamstop with motorized adjustments and a photodiode in the pellet will be installed inside the chamber, close to the detector.
- **Other detector systems:** Multi-angle laser light scattering (MALLS) detectors, UV absorption, UV-vis fluorescence, mass spectrometry (MS) and refractometric (RI).

5.2. Detectors

Two large area detectors, photon, or hybrid-counting detectors, for SAXS and WAXS, are required to cope with all the covered scientific cases. The general consensus is that pixel array detectors are, currently, the best X-ray detector for BioSAXS experiments, main reasons being the minimal readout times, high dynamic range, and negligible background. Current generation of Dectris Pilatus3 X detectors offer readout frame rates in the 250-500 Hz range, a large dynamic range of 2^{20} and virtually no intrinsic noise. These characteristics make this type of detectors idea for time resolved and diluted samples experiments.

The challenge of interpreting the WAXS signal has traditionally limited its biological applications, but efforts are made to use the WAXS signal, which is highly sensitive to dynamic structural fluctuations and hydration effects. WAXS experiments, which are very important for soft matter experiments, can easily be adapted to BioSAXS-CALIMA beamline without compromising the BioSAXS setup by adding a WAXS detector in an appropriate azimuthal angle not shadowing the SAXS signal.

In summary, the proposed detectors for BioSAXS-CALIMA beamline will be:

- **SAXS detector**: the accessed q range should span from 0.01 nm^{-1} to 7 nm^{-1}. This can be achieved at a 17keV by a Pilatus3 X 2M due to the large area of 253.7×288.8. Pixel size is 172 μm, which corresponds to a Δq resolution of 5×10^{-3} nm^{-1} and 3×10^{-3} nm^{-1} for 17 and 10 keV, respectively. Frame rate is 250 Hz in full area, and 500 Hz in central ROI. The detector should be adapted to work in in-vacuum condition (already done by the company).
- **WAXS detector**: The proposed detector is a Pilatus3 X 1M, as it has an active area of 168.7×179.4 mm^2 suitable to cover one full quadrant of the WAXS profile. To avoid shadowing the SAXS detector while fully covering the vertical and horizontal directions simultaneously, one or two modules of a corner should be removed (feasible, already offered by the company). Frame rate is 500 Hz.

5.3. Data acquisition and Analysis

An experiment control and data acquisition systems are required in an automated beamline such as BioSAXS-CALIMA is required. This goes well beyond a basic central beamline control

system. Fortunately, collaborative European projects have already tackled this requirement, and made available solutions such as ISPyB, SASFLOW and the equivalent of MXCuBE for SAXS. BioSAXS-CALIMA beamline is expected to join these projects for an efficient implementation of these services. Pre-processing and processing data analysis pipelines (ATSAS, Scåtter-www.bioisis.net, and others) must also be available to users by default. Final delivery of the beamline must be the modelled 3D envelopes, integrated scattering profiles and raw images.

6. BEAMLINE PROJECT MANAGEMENT

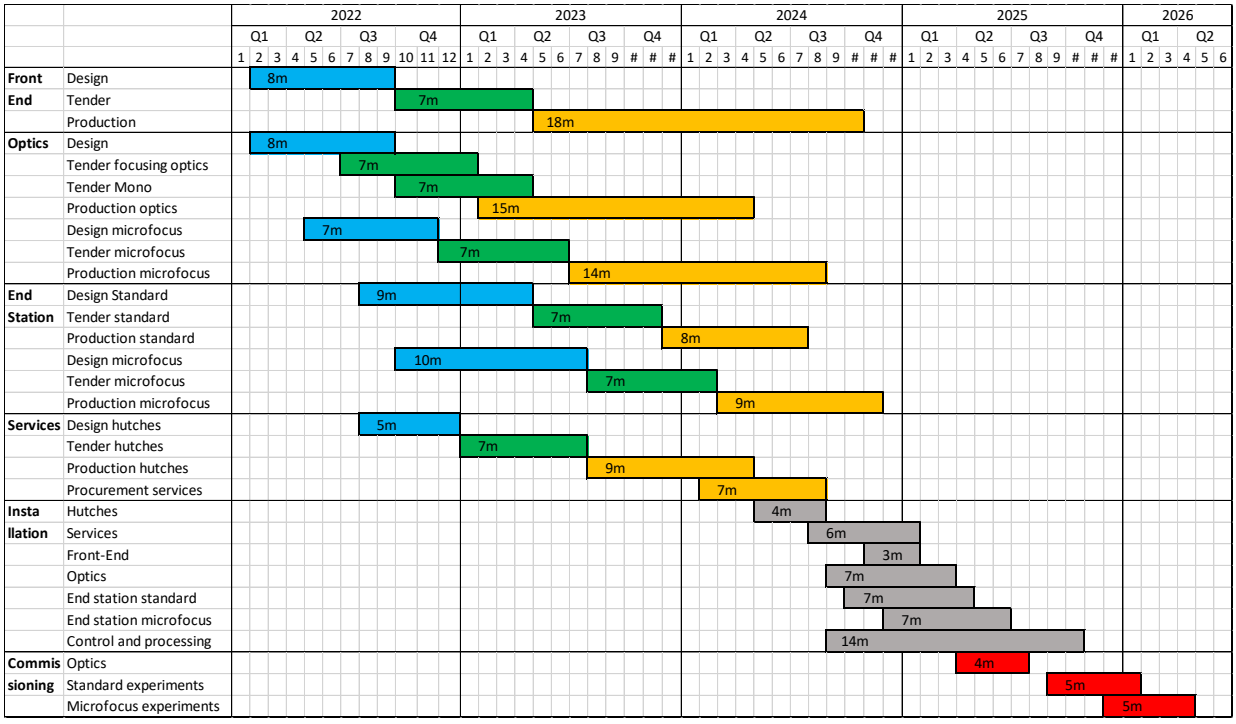
6.1. Budget

The estimated budget is given in the table below. Note that contingency budget and human resources are not included.

Work package	**Item**	**Cost (k€)** Item	Workpackage	**Comments**
Front-end		200	200	
Optics	White beam fluo screen	40	1685	
	Double Multilayer Mono	350		
	Multilayers	80		Assuming only one pair
	Mirror positioning system	400		
	Mirror optics	120		plane optics, 1m-long
	Nanobenders	100		
	Pink beam OH diagnostics	90		2 fluo screens w/ PIN diodes
	OH apertures	70		
	Water chillers	30		Pink beam optics included
	Photon shutter	30		
	Secondary source slit	75		Used in microfocus mode
	Transfocator	250		Includes Be lenses
	Supports, others	50		
Services	Radiation Safety Hutches	450	605	
	Control Hutch	40		
	Fluids	65		
	Air conditioning	20		
	Radiological permits	30		
Vacuum	Vacuum Hardware	250	250	
Electronics	Hardware	150	350	
	Infrastructure	90		
	EPS, PSS	110		
Systems	Network, computers	90	90	
End-station	Supports and structures	90	1846	includes granite
	Beam conditioning elements	200		
	Slits	90		With beam defining & guard slits
	Visualization systems	50		includes sample lightning
	Sample mounting robot	350		Includes sample storage

	Sample Holders	30		
	HPLC system	461		Includes LC/MS TOF, column system, 1260 GPC/SEC GPC MDS Dual Angle LSD
	Capillary holders	20		
	Syringe pumps	10		
	Lasers	20		Photoactivation experiments
	Flow cell	30		Temperature controlled
	Stopped flow cell	10		
	Microfluidic cell	15		
	Traction (stretcher)	30		
	Rheometer	100		
	Mass spectrometer	50		
	UV cell	30		
	MALLS	30		
	Refractometer	30		
	Soft Condensed matter stage	100		Motorized stages for soft condensed matter experiments
	Vacuum chamber	100		Includes beam stop
Detectors	SAXS detector	900	1650	Pilatus3 X 2M, in vacuum
	WAXS detector	750		Pilatus3 X 1M, edgeless
TOTAL			**6676**	

6.2. Schedule



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Barcelona Supercomputing Center
Centro Nacional de Supercomputación

Barcelona, November 29th 2021

To whom it may concern,

The Life Sciences Department of the Barcelona Supercomputing Center (BSC) hereby gives its support to the proposal for a new BioSAXS beamline at the Spanish Synchrotron ALBA. We feel that the inclusion of a BioSAXS beamline in the portfolio of available beamlines at ALBA would provide a state-of-the-art system crucial for successful performance of life science research, and particularly in structural biology. The possibility of analysing macromolecules in solution and its time resolved response to chemical and physical perturbations of the system as well as the versatility of approaches allowed by SAXS is crucial on the insight obtained on the tertiary/quaternary state of proteins as well as their interactions with other macromolecules or small ligands. In addition, high-throughput approaches as drug screening could be easily covered by the proposed beamline.

We strongly believe that a BioSAXS beamline at ALBA will boost the competitiveness of the structural biology research undertaken at the BSC.

Prof. Alfonso Valencia
ICREA Research Professor
Director, Life Sciences, Barcelona Supercomputing Center (BSC)
Director, Spanish National Bioinformatics Institute (INB-ISCIII)
Head of the Spanish Node of the European Bioinformatics Infrastructure ELIXIR
Former President, International Society for Computational Biology (ISCB)

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

20th November 2021

To whom it may concern,

The Institute for Biocomputation and Physics of Complex Systems, BIFI, hereby gives its support to the proposal for a new BioSAXS beamline at the Spanish Synchrotron ALBA. We feel that the inclusion of a BioSAXS beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art system crucial for successful performance of life science research, and particularly in structural biology. The possibility of analyzing macromolecules in solution and its time resolved response to chemical and physical perturbations of the system as well as the versatility of approaches allowed by SAXS is crucial on the insight obtained on the tertiary/quaternary state of proteins as well as their interactions with other macromolecules or small ligands. In addition, high-throughput approaches as drug screening could be easily covered by the proposed beamline.

We strongly believe that a BioSAXS beamline at ALBA will boost the competitiveness of the structural biology research undertaken in BIFI.

MILAGROS MEDINA
Deputy-Director at Instituto of Biocomputation and Physics of Complex Systems
Full Professor in Biochemistry and Molecular and Cellular Biology
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DE CIENCIA
E INNOVACIÓN

CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS
Margarita Salas
CENTRO DE INVESTIGACIONES BIOLOGICAS MARGARITA SALAS

To whom it may concern

The Margarita Salas Center for Biological Research hereby gives its support to the proposal for a new BioSAXS beamline at the Spanish Synchrotron ALBA. We feel that the inclusion of a BioSAXS beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art system crucial for successful performance of life science research, and particularly in structural biology. The possibility of analyzing macromolecules in solution and its time resolved response to chemical and physical perturbations of the system as well as the versatility of approaches allowed by SAXS is crucial on the insight obtained on the tertiary/quaternary state of proteins as well as their interactions with other macromolecules or small ligands. In addition, high-throughput approaches as drug screening could be easily covered by the proposed beamline.

We strongly believe that a BioSAXS beamline at ALBA will boost the competitiveness of the structural biology research undertaken in our Center.

Enrique J. de la Rosa
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Salamanca, 19th November 2021

To whom it may concern,

The Centro de Investigación del Cáncer (CIC-IBMCC) hereby gives its support to the proposal for a new BioSAXS beamline at the Spanish Synchrotron ALBA. We feel that the inclusion of a BioSAXS beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art system crucial for successful performance of life science research, and particularly in structural biology. The possibility of analyzing macromolecules in solution and its time resolved response to chemical and physical perturbations of the system as well as the versatility of approaches allowed by SAXS is crucial on the insight obtained on the tertiary/quaternary state of proteins as well as their interactions with other macromolecules or small ligands. In addition, high-throughput approaches as drug screening could be easily covered by the proposed beamline.

We strongly believe that a BioSAXS beamline at ALBA will boost the competitiveness of the structural biology research undertaken in the Centro de Investigación del Cáncer.

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MINISTERIO
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CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS
Centro Nacional
de Biotecnología

Madrid, 17th December 2021

To whom it may concern,

The Centro Nacional de Biotecnología (CNB-CSIC) hereby gives its support to the proposal for a new BioSAXS beamline at the Spanish Synchrotron ALBA. We feel that the inclusion of a BioSAXS beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art system crucial for successful performance of life science research, and particularly in structural biology. The possibility of analyzing macromolecules in solution and its time resolved response to chemical and physical perturbations of the system as well as the versatility of approaches allowed by SAXS is crucial on the insight obtained on the tertiary/quaternary state of proteins as well as their interactions with other macromolecules or small ligands. In addition, high-throughput approaches as drug screening could be easily covered by the proposed beamline.

We strongly believe that a BioSAXS beamline at ALBA will boost the competitiveness of the structural biology research undertaken at the CNB.

Prof. José María Valpuesta

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cnio stop cancer

Fundación del Sector
Público Estatal Centro
Nacional
de Investigaciones
Oncológicas Carlos III
(F.S.P CNIO)

November 17th, 2021

To whom it may concern,

The Experimental Therapeutics Programme at the Spanish National Cancer Research Centre (CNIO) gives its support to the proposal for a new BioSAXS beamline at the Spanish Synchrotron ALBA. We feel that the inclusion of a BioSAXS beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art system crucial for successful performance of life science research, and particularly in structural biology. The possibility of analyzing macromolecules in solution and its time resolved response to chemical and physical perturbations of the system as well as the versatility of approaches allowed by SAXS is crucial on the insight obtained on the tertiary/quaternary state of proteins as well as their interactions with other macromolecules or small ligands. In addition, high-throughput approaches as drug screening could be easily covered by the proposed beamline.

We strongly believe that a BioSAXS beamline at ALBA will boost the competitiveness of the structural biology research undertaken at CNIO.

Joaquín Pastor Fernández
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Spanish National
Cancer Research Centre
(CNIO)

Maria A. Blasco, Ph.D.
Director

November 18th, 2021

To whom it may concern,

The Spanish National Cancer Research Centre (CNIO) hereby gives its support to the proposal for a new BioSAXS beamline at the Spanish Synchrotron ALBA. We feel that the inclusion of a BioSAXS beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art system crucial for successful performance of life science research, and particularly in structural biology. The possibility of analyzing macromolecules in solution and its time resolved response to chemical and physical perturbations of the system as well as the versatility of approaches allowed by SAXS is crucial on the insight obtained on the tertiary/quaternary state of proteins as well as their interactions with other macromolecules or small ligands. In addition, high-throughput approaches as drug screening could be easily covered by the proposed beamline.

We strongly believe that a BioSAXS beamline at ALBA will boost the competitiveness of the structural biology research undertaken at CNIO.

Best regards

Maria A. Blasco

cnio stop cancer

Spanish National
Cancer Research Centre
(CNIO

In relation to the proposal for a new BioSAXS beamline at the Spanish Synchrotron ALBA

Madrid, November 15th 2021

To whom it may concern,

The Structural Biology Programme at the Spanish National Cancer Research Centre (CNIO) is mostly devoted to the study of proteins and macromolecular complexes related to cancer. For this, the Groups and Units at the Programme offer expertise and access to a list of structural techniques and biophysical methods. A clear focus has been placed in recent years in the implementation of cryo-EM methods but supporting and encouraging other technologies of general application in Structural Biology and protein science.

Given the complexity of biological processes, access to a wide portfolio of techniques and biophysical methods is important to study proteins and macromolecular complexes. A new BioSAXS beamline at the Spanish Synchrotron ALBA would facilitate the access to Spanish and European scientists to this technology, which can help to address questions that are not always possible or accessible by high-resolution methods such as X-ray crystallography or cryo-EM.

I do not have sufficient expertise in this technique to comment on what the demand for the potential services provided by this new BioSAXS beamline would be, or to assess any other aspect related to the actual implementation of a SAXS beamline at ALBA, in one direction or the other, a task that corresponds to others.

Best regards,

Oscar Llorca
Director Structural Biology Programme, CNIO

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CRG
Centre
de Regulació
Genòmica

November 27th, 2021

To whom it may concern,

The CRG hereby gives its support to the proposal for a new BioSAXS beamline at the Spanish Synchrotron ALBA. We feel that the inclusion of a BioSAXS beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art system crucial for successful performance of life science research, and particularly in structural biology. The possibility of analysing macromolecules in solution and its time resolved response to chemical and physical perturbations of the system as well as the versatility of approaches allowed by SAXS is crucial on the insight obtained on the tertiary/quaternary state of proteins as well as their interactions with other macromolecules or small ligands. In addition, high-throughput approaches as drug screening could be easily covered by the proposed beamline.

We strongly believe that a BioSAXS beamline at ALBA will boost the competitiveness of the structural biology research undertaken at CRG.

Luis Serrano
Director

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prof. PharmDr. Ján Klimas, PhD., MPH
Dean of the Faculty

Bratislava, November, 2021

To whom it may concern,

The Faculty of Pharmacy, Comenius University Bratislava hereby gives its support to the proposal for a new BioSAXS beamline at the Spanish Synchrotron ALBA. We feel that the inclusion of a BioSAXS beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art system crucial for successful performance of life science research, and particularly in structural biology. The possibility of analyzing macromolecules in solution and its time resolved response to chemical and physical perturbations of the system as well as the versatility of approaches allowed by SAXS is crucial on the insight obtained on the tertiary/quaternary state of proteins as well as their interactions with other macromolecules or biologically and pharmaceutically important molecules. In addition, high-throughput approaches as drug screening could be easily covered by the proposed beamline.

We strongly believe that a BioSAXS beamline at ALBA will boost the competitiveness of the structural biology research undertaken in (name of the center).

UNIVERZITA KOMENSKÉHO V BRATISLAVE
Farmaceutická fakulta
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832 32 Bratislava

prof. PharmDr. Ján Klimas, PhD.,MPH
dean
Faculty of Pharmacy, Comenius University Bratislava

prof. PharmDr. Ján Klimas, PhD., MPH

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MINISTERIO DE CIENCIA E INNOVACIÓN
CSIC CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS
GRUPO SODERCAN sociedad para el desarrollo regional de cantabria
UC UNIVERSIDAD DE CANTABRIA
INSTITUTO DE BIOMEDICINA Y BIOTECNOLOGIA DE CANTABRIA

To whom it may concern,

The Instituto de Biomedicina y Biotecnología de Cantabria (IBBTEC) hereby gives its support to the proposal for a new BioSAXS beamline at the Spanish Synchrotron ALBA. We feel that the inclusion of a BioSAXS beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art system crucial for successful performance of life science research, and particularly in structural biology. The possibility of analyzing macromolecules in solution and its time resolved response to chemical and physical perturbations of the system as well as the versatility of approaches allowed by SAXS is crucial on the insight obtained on the tertiary/quaternary state of proteins as well as their interactions with other macromolecules or small ligands. In addition, high-throughput approaches as drug screening could be easily covered by the proposed beamline.

We strongly believe that a BioSAXS beamline at ALBA will boost the competitiveness of the structural biology research undertaken in the IBBTEC.

Piero Crespo
Director

direccion.ibbtec@csic.es

Cl. Albert Einstein, 22
39011 SANTANDER ESPAÑA
TEL.: 942 206856
FAX.: 942 266399

IBMC

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

Porto, 23rd November 2021

To whom it may concern,

The IBMC – Instituto de Biologia Molecular e Celular hereby gives its support to the proposal for a new BioSAXS beamline at the Spanish Synchrotron ALBA. We feel that the inclusion of a BioSAXS experimental setup in the portfolio of available beamlines at ALBA would provide a state-of-the-art system crucial for carrying out research at the highest level in life sciences, and particularly in structural biology. The possibility of analyzing macromolecules in solution and their time-resolved response to chemical and physical perturbations of the system, as well as the versatility of approaches allowed by SAXS, is crucial to obtain insights on the tertiary/quaternary state of proteins and on their interactions with other macromolecules or small ligands. In addition, high-throughput approaches (e.g., drug screening) could be easily covered by the proposed beamline.

We strongly believe that a BioSAXS beamline at ALBA will boost the competitiveness of the structural biology research undertaken at IBMC.

With best regards,

Dr. Mónica M. Sousa
Director

Rua Alfredo Allen, 208
4200-135 Porto
Tel +351 226 074 900
www.ibmc.up.pt

GOBIERNO DE ESPAÑA
MINISTERIO DE CIENCIA E INNOVACIÓN

INSTITUTO DE BIOMEDICINA DE VALENCIA CSIC

To whom it may concern,

The Instituto de Biomedicina de Valencia (IBV-CSIC) hereby gives its support to the proposal for a new BioSAXS beamline at the Spanish Synchrotron ALBA. We feel that the inclusion of a BioSAXS beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art system crucial for successful performance of life science research, and particularly in structural biology. The possibility of analyzing macromolecules in solution and its time resolved response to chemical and physical perturbations of the system as well as the versatility of approaches allowed by SAXS is crucial on the insight obtained on the tertiary/quaternary state of proteins as well as their interactions with other macromolecules or small ligands. In addition, high-throughput approaches as drug screening could be easily covered by the proposed beamline.

We strongly believe that a BioSAXS beamline at ALBA will boost the competitiveness of the structural biology research undertaken in Instituto de Biomedicina de Valencia (IBV-CSIC).

Valencia 29th November 2021

Jerónimo Bravo Sicilia (Director of IBV-CSIC)

4th November 2021

Carlos Martí-Gastaldo, PhD.
Instituto de Ciencia Molecular (ICMol), Universidad de Valencia
C/ Catedrático José Beltrán, 2; 46980 Paterna (Spain)
T: +34 96 354 4421
F: +34 96 354 3273
E: carlos.marti@uv.es
W: www.icmol.es/funimat

To whom it may concern,

The Instituto de Ciencia Molecular hereby gives its support to the proposal for a new BioSAXS beamline at the Spanish Synchrotron ALBA. We feel that the inclusion of a BioSAXS beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art system crucial for successful performance of life science research, and particularly in structural biology. The possibility of analyzing macromolecules in solution and its time resolved response to chemical and physical perturbations of the system as well as the versatility of approaches allowed by SAXS is crucial on the insight obtained on the tertiary/quaternary state of proteins as well as their interactions with other macromolecules, small ligands or porous hosts. In addition, high-throughput approaches as drug screening could be easily covered by the proposed beamline.

We strongly believe that a BioSAXS beamline at ALBA will boost the competitiveness of the structural biology research undertaken the Instituto de Ciencia Molecular de la Universidad de València in the frame of the ongoing María de Maeztu Excellence Research Program.

Yours sincerely,

Carlos Martí-Gastaldo, PhD.
Instituto de Ciencia Molecular (Universidad de Valencia)

MINISTERIO
DE CIENCIA
E INNOVACIÓN

CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

Barcelona, November 17, 2021

To whom it may concern,

The Institute of Advanced Chemistry of Catalonia (IQAC-CSIC) hereby gives its support to the proposal for a new BioSAXS beamline at the Spanish Synchrotron ALBA. We feel that the inclusion of a BioSAXS beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art system crucial for successful performance of life science research. The possibility of analyzing macromolecules in solution and its time resolved response to chemical and physical perturbations of the system as well as the versatility of approaches allowed by SAXS is very important on the insight obtained on the tertiary/quaternary state of proteins as well as their interactions with other macromolecules or small ligands. In addition, high-throughput approaches as drug screening could be easily covered by the proposed beamline.

We strongly believe that a BioSAXS beamline at ALBA will boost the competitiveness of the research undertaken in the IQAC.

Dr Jesús Joglar
Director IQAC-CSIC

iQAC
C/ Jordi Girona, 18-26
08034 Barcelona. Espanya
Tel: 93 400 61 00
Fax: 93 204 59 04

INSTITUT DE QUÍMICA AVANÇADA DE CATALUNYA

GOBIERNO DE ESPAÑA

CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

Madrid,4 de noviembre de 2021

To whom it may concern,

The Departamento de Cristalografía y Biología Estructural del Instituto de Química Física Rocasolano CSIC hereby gives its support to the proposal for a new BioSAXS beamline at the Spanish Synchrotron ALBA. We feel that the inclusion of a BioSAXS beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art system crucial for successful performance of life science research, and particularly in structural biology. The possibility of analyzing macromolecules in solution and its time resolved response to chemical and physical perturbations of the system as well as the versatility of approaches allowed by SAXS is crucial on the insight obtained on the tertiary/quaternary state of proteins as well as their interactions with other macromolecules or small ligands. In addition, high-throughput approaches as drug screening could be easily covered by the proposed beamline.

We strongly believe that a BioSAXS beamline at ALBA will boost the competitiveness of the structural biology research undertaken in Departamento de Cristalografía y Biología Estructural del Instituto de Química Física Rocasolano CSIC.

Armando Albert de la Cruz
Head of Departamento de Cristalografía y Biología Estructural
Instituto de Química Física Rocasolano CSIC
Calle Serrano 119
28006 Madrid, Spain.

C/SERRANO, 119
28006 MADRID ESPAÑA
TEL.: 91 5619400

MINISTERIO
DE CIENCIA
E INNOVACIÓN

CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS
INSTITUTO DE QUÍMICA-FÍSICA "ROCASOLANO"

To whom it may concern,

The Institute of Physical-Chemistry "Rocasolano" of the CSIC hereby gives its support to the proposal for a new BioSAXS beamline at the Spanish Synchrotron ALBA. We feel that the inclusion of a BioSAXS beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art system crucial for successful performance of life science research, and particularly in structural biology. The possibility of analyzing macromolecules in solution and its time resolved response to chemical and physical perturbations of the system as well as the versatility of approaches allowed by SAXS is crucial on the insight obtained on the tertiary/quaternary state of proteins as well as their interactions with other macromolecules or small ligands. In addition, high-throughput approaches as drug screening could be easily covered by the proposed beamline.

We strongly believe that a BioSAXS beamline at ALBA will boost the competitiveness of the structural biology research undertaken in the Institute of Physical-Chemistry "Rocasolano".

Juan A. Hermoso
Director IQFR

Serrano 119, E-28006 Madrid (Spain) Tel.: +34 91 745 95 00 Fax: +34 91 564 24 31

Prof. Eliseo Ruiz
Full Professor of the Inorganic and Organic Chemistry Department
Director of Institut of Theoretical and Computational Chemistry
of the University of Barcelona (IQTCUB)
Facultat de Química, Diagonal 645, Universitat de Barcelona
08028 Barcelona, España
email: eliseo.ruiz@qi.ub.es tel: 34 93 403 7058

In Barcelona, November 19th, 2021

To whom it may concern,

The Institut of Theoretical and Computational Chemistry of University of Barcelona hereby gives its support to the proposal for a new BioSAXS beamline at the Spanish Synchrotron ALBA. We feel that the inclusion of a BioSAXS beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art system crucial for the successful performance of life science research, and particularly in structural biology. The possibility of analyzing macromolecules in solution and its time-resolved response to chemical and physical perturbations of the system as well as the versatility of approaches allowed by SAXS is crucial on the insight obtained on the tertiary/quaternary state of proteins as well as their interactions with other macromolecules or small ligands. In addition, high-throughput approaches as drug screening could be easily covered by the proposed beamline.

We strongly believe that a BioSAXS beamline at ALBA will boost the competitiveness of the structural biology research undertaken in Institut of Theoretical and Computational Chemistry.

Sincerely yours,

Prof. Dr. Eliseo Ruiz
Director of the IQTCUB

CSIC
Consejo Superior de Investigaciones Científicas

IRNASA
Instituto de Recursos Naturales y Agrobiología de Salamanca

To whom it may concern,

The Institute of Natural Resources and Agrobiology of Salamanca (IRNASA-CSIC) hereby gives its support to the proposal for a new BioSAXS beamline at the Spanish Synchrotron ALBA. We feel that the inclusion of a BioSAXS beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art system crucial for successful performance of life science research, and particularly in structural biology. The possibility of analyzing macromolecules in solution and its time resolved response to chemical and physical perturbations of the system as well as the versatility of approaches allowed by SAXS is crucial on the insight obtained on the tertiary/quaternary state of proteins as well as their interactions with other macromolecules or small ligands. In addition, high-throughput approaches as drug screening could be easily covered by the proposed beamline.

We strongly believe that a BioSAXS beamline at ALBA will boost the competitiveness of the structural biology research undertaken in The Institute of Natural Resources and Agrobiology of Salamanca (IRNASA-CSIC).

Mar Siles Lucas

Directora del IRNASA

direccion.irnasa@csic.es

C/CORDEL DE MERINAS, 40-52
37008 SALAMANCA / ESPAÑA
TEL.: 923 219 606
FAX: 923 219 609

UNIVERSIDAD DE ALMERÍA

Almeria 27th November 2021

To whom it may concern,

The research group BIO328 *Protein Structure* from the Almeria University, conducted by Dr. Ana Cámara-Artigas, hereby gives its support to the proposal for a new BioSAXS beamline at the Spanish Synchrotron ALBA. We feel that the inclusion of a BioSAXS beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art system crucial for successful performance of life science research, and particularly in structural biology. The possibility of analyzing macromolecules in solution and its time resolved response to chemical and physical perturbations of the system as well as the versatility of approaches allowed by SAXS is crucial on the insight obtained on the tertiary/quaternary state of proteins as well as their interactions with other macromolecules or small ligands. In addition, high-throughput approaches as drug screening could be easily covered by the proposed beamline.

We strongly believe that a BioSAXS beamline at ALBA will boost the competitiveness of the structural biology research undertaken by the research group BIO328 *Protein Structure* from the Almeria University.

Yours sincerely,

Ana Cámara-Artigas
Dpto. Química y Física
Edificio Científico Técnico I (Quimicas)
Universidad de Almería
Carretera Sacramento s/n 04120
Almería (España)
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School of Molecular Sciences
Arizona State University

BIODESIGN INSTITUTE
ARIZONA STATE UNIVERSITY

Professor Alexandra Ros
School of Molecular Sciences
Center for Applied Structural Discovery, The Biodesign Institute
Arizona State University
Tempe, Arizona 85287-7401

Telephone: +1(480)-965-5323
Fax: +1(480)-965-2747
alexandra.ros@asu.edu

November 28, 2021

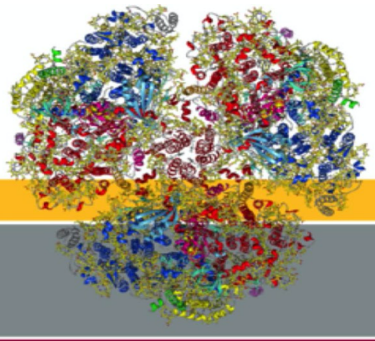
To whom it may concern,

This is to give my full support to the proposal for a new BioSAXS beamline at the Spanish Synchrotron ALBA. I believe that the inclusion of a BioSAXS beamline in the portfolio of available beamlines at ALBA would provide a state-of-the-art system crucial for successful performance of life science research, and particularly in structural biology. The possibility of analyzing macromolecules in solution and their time resolved response to chemical and physical perturbations as well as the versatility of approaches allowed by SAXS is crucial on the insight obtained on the tertiary/quaternary structure of proteins as well as their interactions with other macromolecules or small ligands. In addition, high-throughput approaches for drug screening could be investigated by the proposed beamline.

I strongly believe that a BioSAXS beamline at ALBA will boost the competitiveness of the structural biology research undertaken in my group at the Center for Applied Structural Discovery at the Biodesign Institute at Arizona State University. With several years of experience of designing and applying injectors for structural discovery at Synchrotrons and X-ray free electron lasers, I am happy to support the project in the development of suitable sample delivery tools including such that allow time-resolved studies.

Sincerely,

Alexandra Ros,
Professor



Center for
Applied Structural Discovery
727 East Tyler Street Mail Code 7401
PO Box 877401, Tempe, AZ 85287-7401
Ph: 480.965.3728 Fax: 480.965.2747
biodesign.asu.edu

MINISTERIO
DE CIENCIA, INNOVACIÓN
Y UNIVERSIDADES

CSIC

UNIVERSIDAD
DE GRANADA

IACT
INSTITUTO ANDALUZ DE CIENCIAS DE LA TIERRA

Granada, 4 de Noviembre de 2021

To whom it may concern,

The *Laboratorio de Estudios Cristalográficos* (LEC), Unit of the *Instituto Andaluz de Ciencias de la Tierra* (CSIC-UGR), hereby gives its support to the proposal for a new BioSAXS beamline at the Spanish Synchrotron ALBA. We feel that the inclusion of a BioSAXS beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art system crucial for successful performance of life science research, and particularly in structural biology. The possibility of analyzing macromolecules in solution and its time resolved response to chemical and physical perturbations of the system as well as the versatility of approaches allowed by SAXS is crucial on the insight obtained on the tertiary/quaternary state of proteins as well as their interactions with other macromolecules or small ligands. In addition, high-throughput approaches as drug screening could be easily covered by the proposed beamline.

We strongly believe that a BioSAXS beamline at ALBA will boost the competitiveness of the structural biology research undertaken at LEC.

Sincerely greets you,

Dr. Jose A. Gavira Gallardo
Head of the Laboratorio de Estudios Cristalográficos
Instituto Andaluz de Ciencias de la Tierra (IACT, CSIC-UGR)

j.gavira@csic.es

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UNIVERSITAS Miguel Hernández

Chicago 4 November 2021

To whom it may concern,

Dear Sir/Madam

My name is José Luis Neira and I work in protein structure in aqueous solution in University Miguel Hernández. I would like to give my support to the proposal for a new BioSAXS beamline at the Spanish Synchrotron ALBA. I feel that the inclusion of a BioSAXS beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art system crucial for successful performance of life science research, and particularly in structural biology. The possibility of analyzing macromolecules in solution and its time resolved response to chemical and physical perturbations of the system as well as the versatility of approaches allowed by SAXS is crucial on the insight obtained on the tertiary/quaternary state of proteins as well as their interactions with other macromolecules or small ligands. In addition, high-throughput approaches as drug screening could be easily covered by the proposed beamline.

I strongly believe that a BioSAXS beamline at ALBA will boost the competitiveness of the structural biology research undertaken in (name of the center).

Please, if you need further details, just let me know it.Thanks a lot for your help and time.

Yours faithfully,

José Luis Neira
IDIBE, Edificio Torregaitán, Universidad Miguel Hernández. Avda del ferrocarril s/n; 03202 Elche (Alicante), Spain. Tel: +34 96 6658459; Tel: + 34 96 6658758.
Email: jlneira@umh.es

VICERRECTORADO DE INVESTIGACIÓN
Y TRANSFERENCIA
JOSÉ MIGUEL MATEOS ROCO
VICERRECTOR (EN FUNCIONES)

To whom it may concern,

The University of Salamanca hereby gives its support to the proposal for a new BioSAXS beamline at the Spanish Synchrotron ALBA. We feel that the inclusion of a BioSAXS beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art system crucial for successful performance of life science research, and particularly in structural biology. The possibility of analyzing macromolecules in solution and its time resolved response to chemical and physical perturbations of the system as well as the versatility of approaches allowed by SAXS is crucial on the insight obtained on the tertiary/quaternary state of proteins as well as their interactions with other macromolecules or small ligands. In addition, high-throughput approaches as drug screening could be easily covered by the proposed beamline.

We strongly believe that a BioSAXS beamline at ALBA will boost the competitiveness of the structural biology research undertaken in the University of Salamanca.

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Tel.: +34 923 29 44 30
www.usal.es
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European XFEL GmbH, Holzkoppel 4, 22869 Schenefeld, Germany

Dr. Sakura Pascarelli

Scientific Director
Phone +49 40 8998-2524
Secretary +49 40 8998-6858
Fax +49 40 8998-1905
sakura.pascarelli@xfel.eu

To whom it may concern

28 November 2021

RE: Letter in support of BioSAXS beamline for the ALBA upgrade

I hereby give my support to the proposal for a new BioSAXS beamline at the Spanish Synchrotron ALBA. The inclusion of a BioSAXS beamline in the port-folio of available beamlines at ALBA would provide a state-of-the-art system crucial for successful performance of life science research, and particularly in structural biology. The possibility of analyzing macromolecules in solution and its time resolved response to chemical and physical perturbations of the system as well as the versatility of approaches allowed by SAXS is crucial on the insight obtained on the tertiary/quaternary state of proteins as well as their interactions with other macromolecules or small ligands. In addition, high-throughput approaches as drug screening could be easily covered by the proposed beamline.

I strongly believe that a BioSAXS beamline at ALBA will contribute to the creation of a stronger, more vibrant SAXS user community in Europe and in the world, and thereby also boost the competitiveness and the impact of the structural biology research undertaken at the European XFEL.

Sincerely,

Sakura Pascarelli

Sakura Pascarelli
Scientific Director