Biological Small-Angle X-Ray Scattering Beamline (BioSAXS)

Report submitted Wednesday 31 Aug, 2011

Executive summary

Design Objective.

The bioSAXS beamline outlined in this proposal is designed to cater for at least 95% of biological small-angle scattering experiments. The beamline will record high quality scattering data from dilute aqueous solutions of biological macromolecules and will be integrated with state-of-the-art biological liquid handling and sample characterisation systems to maximise the application and capabilities in biological studies.

Target Scientific Community.

The target user group is the structural biology community, which is a large and highly productive sector of Australian and New Zealand science. Small-angle scattering offers biologists structural and dynamic information complimentary to mainstay structural biology techniques such as crystallography and Nuclear Magnetic Resonance (NMR). This target community is experienced in synchrotron use, and is currently creating a substantial oversubscription on the existing SAXS/WAXS beamline. The research outcomes from the bioSAXS beamline will span national research priorities from the development of advanced biomaterials and biotechnologies to breakthroughs in medical research.

Technical Specifications.

Compared with the existing SAXS/WAXS beamline, that is utilised to perform a diverse range of measurements for researchers from disciplines, such as chemistry, geology, physics and biology, the bioSAXS beamline will be a specialised instrument. The bioSAXS beamline will encompass an engineering and design philosophy that incorporates optical systems tailored specifically for biological sample investigations in combination with a state-of-the-art detector and sample handling (including a fully integrated liquid-handling system with in-built sample monitoring) that will improve overall efficiency, output and quality assurance over and above the existing SAXS/WAXS beamline. The optical configuration will consist of a channel-cut Si111 monochromator typically fixed at 12 keV, a high performance Kirkpatrick and Baez (KB) mirror pair at 20 m, and the sample position at 34.5 m. A 2.6-3.0 m SAXS camera will feature a Pilatus-2M detector (or equivalent) mounted in vacuum. The source will be a 22 mm period NdFeB in-vacuum undulator (IVU). To ensure world leading beamline performance it is crucial that the undulator be a 3 m device. Sample handling/monitoring will be performed using an in-line multi-angle laser light scattering (MALLS) system and a 'near beam' ultraviolet (UV) spectrophotometer.

This design will support high quality solution scattering with a q-range of 0.005-0.5 $Å^{-1}$ and will service the vast majority of biological solution scattering proposals. The instrument will also be capable of routine measurements on other types of solutions. The liquid handling system on bioSAXS will be able to perform in several specific modes of operation (manual injection, high-throughput and liquid chromatography modes) and be fully integrated with the instrument control systems. The data output streams from the various in-line detectors incorporated in the liquid handling system will be integrated with the SAXS detector output. Those highly specialised biological scattering measurements not suited to this instrument will then have a good scientific case to be run on the existing SAXS/WAXS beamline.

Introduction

Constructing a dedicated solution bioSAXS beamline at AS is necessary because:

- the existing SAXS/WAXS beamline is highly oversubscribed (~300%), and is highly productive with the highest rate of AS beamline publications (double the facility average) and the highest fraction of A and A* papers of all AS beamlines (85%)
- the fraction of SAXS proposals for proteins is high (approx 50%) and growing steadily. There is a great deal of scope for further community growth as more and more labs learn how SAXS adds to their research.
- the flexible capabilities of the existing SAXS/WAXS will support all the experiments outside bioSAXS' scope. A dedicated bioSAXS beamline will use a tailored optical design and instrument/detector configuration to achieve optimal performance for biological solution scattering.

The application of small-angle X-ray scattering (SAXS) to the study of biological macromolecules in solution (bioSAXS) is an expanding field of research. The rate of publication of bioSAXS data has grown steadily from around 5 per month in the year 2000 to almost 25 per month in 2011 (Figure 1). In line with this trend bioSAXS has accounted for approximately 50% the total proposals for time on the SAXS/WAXS beamline at the Australian Synchrotron in 2011. This figure has grown from less than 10% when the user program began two years ago.

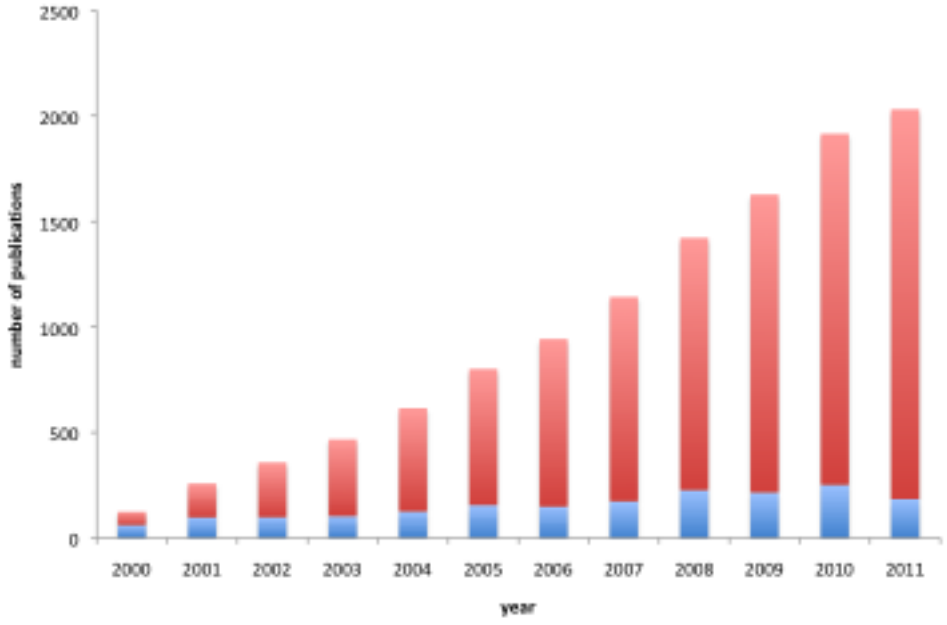

Figure 1: growth in protein SAXS publications in the last decade. Red bars display cumulative and blue bars per year.

This growth in bioSAXS data acquisition and publication has principally come from the large and productive structural/molecular biology community among whom there is a fast-growing realisation that bioSAXS can provide information that is complimentary to and extends beyond what is accessible by more traditional X-ray diffraction methods. Four sessions relating to bioSAXS were featured in the last International Union of Crystallography Congress meeting in Madrid (2011), further indicating the relationship of bioSAXS to protein crystallography. The importance of this is that the structural biology community in Australia provides a pool of new users that is large enough to allow the bioSAXS community to increase substantially. Current growth of the biology-focussed SAXS community in Australia is becoming limited by over-subscription of the current SAXS/WAXS beamline. Further demand for bioSAXS will come from continued development of data collection and analysis strategies that lead to new bioSAXS applications. Also, other disciplines such as biochemistry, proteomics, protein NMR and single particle electron microscopy represent large and virtually untapped communities of potential users whose current research would be well complemented by bioSAXS studies.

A digest of the biological applications

There are currently several dozen labs in Australia and New Zealand who regularly perform bioSAXS measurements at the Australian Synchrotron, several examples of these are listed below. The breadth and productivity of this community is indicative of the outcomes of a dedicated bioSAXS beamline and warrants further consideration.

Understanding HIV assembly

Prof. Johnson Mak — Deakin University, VIC
Dr. Nathan Cowieson — Australian Synchrotron, VIC
Dr. Marcel Hijnen — Monash University, VIC

The HIV Gag polyprotein is expressed in the cytoplasm of an infected cell. Here it interacts with the viral genome and hijacks the protein export machinery of the host cell to relocate to the plasma membrane. At the cell surface Gag interacts with the plasma membrane, oligomerises, induces membrane curvature, packages the viral genome and a variety of host and viral factors into the nascent virion. Finally, Gag is cleaved into six discrete parts by the viral protease leading to viral maturation. It has been postulated that the diverse range of functions shown by Gag are mediated by conformational change however such changes have not been shown due the difficulty of studying flexible, multi-domain proteins by high resolution methods. JM has used a combination of SAXS and CD to demonstrate conformational change in both tertiary and secondary structure during viral maturation. The nature of these changes point to a previously unreported role of a small C-terminal domain of Gag in regulating conformational change.

Real World Outcomes: HIV and retroviral therapy

Glucose transport defects in type-2 diabetes

Prof. Jennifer Martin — University of Queensland, QLD

Insulin triggers glucose uptake into muscle and fat cells by relocating vesicles containing the glucose transporter-4 protein (GLUT4) from the cytoplasm to the surface. Defects in this process lead to type-2 diabetes (T2D), a chronic disease that is now the fifth leading cause of death worldwide. The goal of this research is to investigate the structure and function of molecular elements critical to insulin-regulated

delivery of GLUT4 vesicles to the plasma membrane (PM). The use of SAXS to study these protein complexes, their dynamics and conformational changes is a crucial component of this project. A detailed knowledge of the fundamental molecular processes involved in glucose homeostasis will underpin a better understanding of the molecular pathology of T2D. This in turn will lead to the validation of new therapeutic targets and the development of new treatments for diabetes.
Real World Outcomes: Diabetes therapy

The biology of protein export

Dr. Brett Collins — University of Queensland, QLD
Dr. Nathan Cowieson — Australian Synchrotron, VIC

The retromer is a three protein complex that plays a crucial role in the trafficking of protein through the cell. Protein trafficking is a fundamental function in all eukaryotic cells involved in functions such as nutrient uptake and cell signalling. While much work has been done to try and understand the structure and function of the retromer the complex remains poorly understood. BC has used biochemical studies to define the relationship between retromer subunits and SAXS analysis to confirm this model. The SAXS additionally revealed that the retromer complex is a dimer of trimers, a detail that had not previously been recognised.
Real World Outcomes: Diseases of protein transport

Harnessing bacterial toxins as therapeutic agents

Prof. Michael Parker — St. Vincent's Institute of Medical Research, VIC
Dr. Terry Mulhern — University of Melbourne, VIC

The cholesterol-dependent cytolysins (CDCs) are a superfamily of pore-forming toxins that punch holes in target cell membranes through a highly regulated process. *Streptococcus mitis* lectinolysin (LLY) is an un usual member of the CDCs, as it exhibits another layer of regulation with a carbohydrate-binding "lectin" domain located at its N-terminus that has previously been shown to enhance the pore-forming activity of the toxin on platelets. MP is combining X-ray crystallography, electron microscopy and SAXS to understanding how LLY assembles into ring-shaped "pre-pores" and these how these pre-pores insert into the cell membranes. The ability of LLY to bind to certain carbohydrate antigens expressed on tumour cells suggest that it may be able to be employed as an anti-cancer therapeutic agent.
Real World Outcomes: Cancer therapy

Anti-cancer applications of insulin-like growth factor binding proteins

Dr. Briony Forbes, — University of Adelaide, SA
Prof. Raymond Norton — Monash University, VIC
Dr. Terry Mulhern — University of Melbourne, VIC

Insulin-like growth factors (IGFs) are important for pre- and post-natal growth and development. Understanding the IGF system at a molecular level has important implications for biotechnology, agriculture and pharmaceutical development. IGF actions are regulated by a family of high affinity IGF binding proteins (IGFBPs 1-6). BF and RN are currently focusing on gaining a detailed understanding the interaction between IGF-I and IGFBP-2 through SAXS and nuclear magnetic resonance (NMR) spectroscopy. The structural information gained will complement their animal studies of mutant forms of IGFBP-2 as cancer therapeutics.
Real World Outcomes: Cancer therapy

Molecular mechanisms of cardiac-related sudden death

Prof. Jill Trewhella University of Sydney, NSW
Prof. Chris Semsarian Centenary Institute of Cancer Medicine and Cell Biology, NSW
Dr. Cy Jeffries University of Sydney, NSW

Genetic mutations in cardiac myosin binding protein-C (cMyBP-C) and the β-myosin heavy chain (βHC) are the most common causes of familial hypertrophic cardiomyopathy (HCM), a cardiac disorder that affects thousands of people annually. Individuals with HCM exhibit diverse pathophysiologies that cause morphological dysfunction, heart failure and sudden death. Intensive structural investigations have begun to probe the molecular mechanisms underpinning the disorder. JT, CS and CJ have shown, using a combination of SAXS, SANS and NMR, that human cMyBP-C is an extended protein in solution and that the modular domain arrangement is commensurate with mediating interactions across the distances between the thick and thin filaments in cardiac muscle sarcomeres. Further, regions of structural flexibility in key cardiac-specific regions of the protein indicate how cMyBP-C can 'flip' between myosin and actin during contraction and that the consequent binding to actin can fine-tune calcium signals which are responsible for triggering the contractile cycle. Their continuing structural investigations incorporating clinically relevant mutations in cMyBP-C and βHC will provide information for evaluating clinical risk and inform the design of therapeutic strategies to ameliorate the effects of ongoing HCM. Synchrotron based SAXS investigations will be crucial for accessing very low-*q* to investigate very large (>300 Å) cMyBP-C/βHC complexes in solution.

Real World Outcomes: Heart Disease Therapy

Paraspeckle proteins

Prof. Charles Bond University of Western Australia, WA

PSP1, p54nrb and PSF are proteins that have been recently identified as interacting with long non-coding RNA molecules to form the solid core of a subnuclear structure called the paraspeckle. CB is using SAXS and other structural biology techniques to understand how these proteins coordinate RNA to promote functional interactions with other molecules, and whether the homo- and heterodimers are globally similar or radically different to each other and whether interaction with RNA alters this global structure. The results of this work contribute to understanding of DNA compaction in the nucleus and cell differentiation and inputs into the development of adult stem cells as theraputic agents.

Real World Outcomes: Development of stem cell therapies

Protein-RNA interactions in innate immunity

Prof. Bryan Williams Monash Institute for Medical Research, VIC
A/Prof. Matthew Wilce Monash University, VIC
A/Prof. Jackie Wilce Monash University, VIC

Interactions between proteins and double-stranded RNAs underpin the ability of organisms to silence expression of a targeted gene via siRNA. While siRNA offers fantastic potential in genetic validation and future therapeutics, of great significance is a recent observation that siRNA also activates the innate immune system, induces cytokine production and activates the interferon system(5). Before the full potential of siRNA can be developed the actions of siRNA other than gene silencing need to be addressed. Retinoic acid Inducible Gene I (Rig-I) has been shown to contribute to virus induced IFN production in response to viral RNA and dsRNA. BW, MW and JW are using SAXS to characterize the conformational rearrangments of the multi-domain protein Rig-I in the presence of a range of siRNAs.

Real World Outcomes: Development of gene therapy for genetic diseases such as cystic fibrosis

Regulation of a bacterial exoglucanase

A/Prof. John Cutfield — Otago University, NZ

Exo-glucanase enzymes with specificity for particular glycosyl linkages are powerful tools for engineering novel oligosaccharide materials. After identifying the first bacterial exo-1,3/1,4-β-glucanase (ExoP) with specificity for 1-3 glycosyl, JC has used biochemistry and protein crystallography to characterise the protein. A mobile domain was identified that plays a role in regulation of the enzyme. JC has used SAXS to study the dynamics and flexibility of this regulatory domain to better understand the action of this enzyme.

Real World Outcomes: Creation of novel nano materials

The effects of phytic acid on monogastric nutrition

A/Prof. Aaron Cowieson — University of Sydney, NSW
Adjunct A/Prof Peter Selle — University of Sydney, NSW
Dr. Nathan Cowieson — Australian Synchrotron, VIC

Phytic acid is a phosphate storage molecule that is found at high levels in a range of plant materials including important food crops such as corn, wheat and soy. Phytic acid has been described as an anti-nutrient due to its effect in reducing absorption of minerals and amino acids during digestion. AC, PS and NC are using SAXS to characterise the effect of phytate on protein solubility and the formation of insoluble complexes of phytate and divalent cations. In addition, these researchers are using dynamic SAXS to study the activity of digestive enzymes in the gastric phase of digestion in the presence and absence of phytate. This research has implications for feeding strategies of monogastric livestock such as pigs and poultry and also to human nutrition.

Real World Outcomes: Improving efficiency in agriculture and improving human nutrition

Structure and mechanism of the pre-T-cell receptor

Prof. James Rossjohn — Monash University, VIC

The pre-T-cell receptor functions during the maturation of T-cells in the immune system. T-cells have receptors on their surface that have two function, firstly to recognise foreign material such as bacteria or viruses and secondly to signal to the T-cell that a foreign object has been encountered leading to an immune response. Reflecting these functions, T-cells are also matured in two stages. In the first stage the signalling function of the T-cell receptor is assessed and this is the role of the pre-T-cell receptor. JR has determined the high-resolution structure of the pre-T-cell receptor using crystallography. The structure shows how the pre-T-cell receptor can bind to a T-cell receptor, checking the integrity of those parts of the molecule involved in signalling functions. However, the crystal structure suggests two alternative modes of interaction between the pre-T-cell and T-cell receptor proteins. SAXS was used to differentiate between the two modes of binding and determine the biologically relevant solution form of the proteins. The structure gives insight into a key stage in immune development and has implications for autoimmune disease.

Real World Outcomes: Treatment of autoimmune disease

SAXS beamlines dedicated to biology

The key principle driving the design of the bioSAXS beamline is to meet the increasing demands of the biological research community in Australia and to guarantee that the Australian Synchrotron retains its

position as recognised international leader in the very competitive field of synchrotron-based biological small-angle scattering. This objective will be achieved by striking the right balance between design versatility and simplicity so as to accommodate diverse user requirements while at the same time improving efficiency and output via the application of a world-leading automated sample handling and data collection platforms. Consequently, the bioSAXS beamline will accommodate at least 95% of the requirements and needs of the structural biology user community. The design will allow the beamline to feature a high level of automation in data collection, reduction and analysis and a sophisticated endstation for sample handling and high throughput operation that will be readily accessible and easy to use for end-users, thus avoiding the complications of the existing multi-purpose SAXS/WAXS beamline.

The bioSAXS approach represents a move away from measuring biological samples at general purpose SAXS beamlines that has been, and continues to be, implemented in an ever-increasing number of international facilities. The design and operation of several biological SAXS beamlines are outlined below.

ALS, Berkeley, USA.

The SIBYLS beamline (12.3.1) at the Advanced Light Source, Berkeley, CA is a dual function beamline splitting its time between protein SAXS and X-ray crystallography. Light comes from a superbend source via a double crystal monochromator allowing energy changes. There is a fixed length camera around 1.5 m and energy changes allow for some variation of *q*-range. Samples are measured in small cuvettes (~20 µl volume) via mica windows. While the cuvettes allow data to be measured from small volume samples they do not allow for flow and this increases the time taken to wash cuvettes between samples and also increases the problem of radiation damage. To combat this problem, the beam is defocused at the sample position giving a relatively large (~0.5 mm) spot size, reducing flux density. An 'off-the-shelf' liquid handling platform is used to load samples from 96 well plates.

The main advantage of the SIBYLS beamline is that by being dedicated to protein samples there is little need to alter the configuration of the beamline between experiments. This greatly increases throughput and creates a beamline that is very easy to use. Disadvantages of the beamline are problems of radiation damage inherent to the static sample cuvettes and long washing times between samples. The beamline does not operate down to 0.005 $Å^{-1}$.

DESY, Hamburg, Germany.

The biological SAXS beamline X33 at the DESY synchrotron at Hamburg, Germany is a dedicated biomolecular SAXS beamline. The beamline features a bending magnet source giving $5x10^{11}$ photons per second to a focal spot size of 1.2 mm. Scattered X-rays are detected on a Pilatus 1M detector located 2.7 m from the sample and on a Pilatus 300K WAXS detector located 1.2 m from the sample. The detectors give a *q*-range of 0.006-0.6 $Å^{-1}$ on SAXS and between 38 and 176 $Å^{-1}$ on WAXS.

Samples are loaded to a 30 µl in-vacuum or to a 100 µl in-air, static sample cell by a 96 well plate compatible liquid handling workstation. Filtration, centrifugation and chromatography are offered offline for sample clean up but these services are not automated and are not used to deliver the sample to the beamline.

The highest level of automation on the beamline is seen in the data reduction and analysis software. The software allows a user to upload a spreadsheet containing information relating to their sample. SAXS data from the sample list can then be measured without further user intervention. The software then uses information about oligomerisation state and monodispersity calculated from the SAXS data, together with sequence information provided by the user and structural information from modelling against the protein data bank of structures to run an appropriate modelling strategy in a time period of around 24 hours on a 140 node computing cluster. Features of this software will be implemented on the bioSAXS beamline.

ESRF, Grenoble, France.

ID14-3 is a SAXS beamline at the ESRF synchrotron that has been running since the end of 2008 and is dedicated to biological samples. The beamline features a fixed energy of 13.3 KeV with an undulator source and a spot size of around 100 μm at the sample position. The sample environment is a 1.8 mm quartz capillary and is temperature controlled. A fixed length camera gives a *q*-range of 0.005–0.5 $Å^{-1}$.

ID14-3 offers automated sample changing via a liquid handling platform as well as in-line chromatography and the same automated data reduction and analysis software as X33 at DESY. The beamline is one of the newest bioSAXS beamlines and is similar to the proposed Australian Synchrotron bioSAXS beamline.

Soleil, Paris, France.

Whilst not exclusively a protein beamline, the Soleil SAXS beamline is broadly similar to the proposed AS bioSAXS beamline. The Soleil SAXS beamline uses an undulator source, focussing KB optics, in-vacuum sample handling and an in-vacuum detector. The detector is mounted in a very large vacuum flight tube similar to ESRF and many SANS instruments. This productive beamline is used for many types of solid and liquid samples. All protein SAXS samples are currently run through an FPLC instrument, including static samples. Because this substantially reduces throughput compared to a dedicated static sample setup, the beamline intends to develop one in the near future. Two notable features include in-situ UV-vis analysis and in-situ size exclusion chromatography capability.

Design and features of the proposed bioSAXS beamline

The essential requirements for the bioSAXS beamline are:

1. Routine operation at a *q*-range of 0.005-0.5 $Å^{-1}$ with extremely low levels of instrument background intensity.
2. An X-ray spot size at the sample position of 0.4 mm (±0.1mm), allowing the use capillaries between 1.0-1.5 mm in diameter.
3. Flux levels of 3 x 10^{12} photons/second, at 12 keV, at the useful acceptance of the beamline optics from an in vacuum undulator source.
4. A sophisticated end-station set-up integrating ancillary hardware for sample handling and with the flexibility of several operation modes.

Detailed optical design establishes the overall performance of the beamline via interplay of flux, spot size, slit widths, component placement, component specification etc. The outcome of these variables on the value of q-min is the primary design concern. Spot size is also important because it controls the levels of sample radiation dose (a small a spot size exacerbates radiation damage). The spot size is controlled primarily by horizontal focal size: vertical is less significant due to source asymmetry and due to the direction of liquid sample flow. Other factors that have been considered are mirror aberrations (which are increased by increased focal distances), vibration, and optical stability. All optical geometries have been investigated for maximum useful optical acceptance, which leads to optimal layouts as well as "flux on sample" results.

1) Q-range.

The required working *q*-range of the instrument is 0.005-0.5 $Å^{-1}$. The optical design provides for absolute q_{min} = 0.0035-0.004 $Å^{-1}$ to suit routine data collection to 0.005 $Å^{-1}$. This lower resolution limit will facilitate measurement of macromolecular samples from peptide size and up to a maximum dimension of around 600 Å (π/q_{min}). This resolution should be sufficient for at least 95% of biological macromolecular samples.

A fixed camera length for the instrument can meet the resolution requirements, with experiments requiring an extended resolution range (lower and/or higher) performed on the existing SAXS/WAXS beamline. This set up will: (1) reduce workload on beamline staff and time spent in setup changes; (2) provide the capability for working in a high through-put mode that is suitable for a wide variety of samples; (3) enhance instrument stability; (4) reduce endstation design complexity; and (5) allow for enhanced optimisation of performance.

The camera will be between 2.6-3.0 m in length (exact length to be decided during final design), with a single 254 x 289 mm^2 PILATUS 2M detector (or similar) to provide the required resolution and to maintain adequate collection efficiency (solid angle). Whilst the Pilatus detectors produced by Dectris (Switzerland) are ideal for this beamline, all other relevant vendors and future developments will be considered at the final design stage, to ensure the most appropriate technical solution is chosen and that proper procurement protocols are followed.

The camera length is a balance between factors that affect both low- and high-*q* :

- low-*q* is primarily determined by the storage ring and source properties, the optical layout of the beamline, the performance of its optical components, and the required flux.
- low-*q* is also determined by the size of the beamstop. As has been extremely successful on SAXS/WAXS, the detector in the beamstop will be the primary normalisation signal. The smallest highly quantitative beamstop available to date is 3.5 mm diameter. Rather than rely on an as-yet undeveloped improvements, the optical design is based on the existing beamstop so that performance is certain. Any future beamstop developments are a bonus, and in any case only small improvements are possible.
- The high-*q* end is determined by the available flux and the solid angle of the detector; the longer the camera the worse this property, hence the upper limit on camera length is 3.0 m. The beamline

must deliver very high flux to provide adequate detection statistics because proteins scatter extremely weakly at higher *q*. Such measurements are already flux limited on SAXS/WAXS.

A large area solid-state photon counting detector such as Pilatus 2M is essential because of many key properties, including zero noise, high dynamic range, high efficiency at the working photon energy, fast readout, and gating/timing/controls properties amongst others.

Unlike the Pilatus 1M detector on SAXS/WAXS, the size of the active area of a 2M requires the detector to be mounted inside the vacuum space of the SAXS camera in order for users to access both the central and offset detector positions required. This is because it is unsafe to construct exit windows larger than 320 mm diameter (based on experience at SAXS/WAXS and other facilities) hence an in-air Pilatus 2M can only have a fixed detector position unless user operations includes changing nose-cones and complete camera realignments. Such major mechanical operations and specialist alignments are unfeasible for high throughput in 24-hour user operations and therefore an in-vacuum detector is required. The detector would be mounted on an in-vacuum multi-axis stage, similar to "Cartesius1" engineered in house at the AS and providing excellent service on the SAXS/WAXS beamline. Dectris have confirmed the latest version of the Pilatus 2M is vacuum compatible. The SAXS camera would be broadly similar to those in service at the ESRF and Soleil but shorter, smaller in diameter and therefore easier to implement and to manage. Whilst the design is primarily intended for fixed camera length operations, in-vacuum mounting does allow for automated camera length changes simply by using pre-saved motor positions because the beam position will be static. This ensures flexibility into the future if required.

2) The X-ray spot size at the sample position

Following the proven optical principles of the SAXS/WAXS beamline, the beam will be focussed at the sample position by a high quality KB mirror pair. The mirrors and three sets of slits that follow make up the collimation system, which almost entirely determine the beam properties both at the sample and at the detector.

The real-space beam geometry at the sample requires tight control over 4 key properties (two in each of the horizontal and vertical planes):

- the focal size of the primary beam. The horizontal focal size directly affects radiation damage limits of the samples. The vertical direct beam size matters but to a lesser extent because it will be smaller (due to source asymmetry) and because of the vertical flow direction of samples. Whilst the "spot size" is easy to understand and is to a first approximation trivial to determine, for SAXS it is not the most important beam profile property unless the samples are small, which is not the case for solution scattering.
- the width of the background intensity on the sides of the direct beam. This is the most important property for SAXS as it directly affects the instrument background intensity at low-*q* and determines the lower useful resolution limit. This background intensity extends well out from the main beam. The background width must be constrained to smaller than the diameter of the capillary for solution samples, as even weak background intensity scatter efficiently from the side-walls of capillaries and affects low-*q* data quality. The width of the background is strongly coupled to the focal quality of

the mirrors (hence very high quality mirrors are essential for the same reasons but to an even higher degree than SAXS/WAXS), the geometry of the collimation system and the optical acceptance used.

A key design goal for the bioSAXS beamline was increasing the horizontal width of the direct beam (to decrease radiation damage while maintaining adequate signal) whilst maintaining a small background intensity width to maintain an acceptable minimum capillary diameter. Capillary diameters are an important issue for proteins because the volume of sample required increases with the square of the diameter, and sample volumes are frequently limited by difficulty and expense in preparation. Larger capillaries allow for more sample in the beam and inherently high scattering intensity, whereas smaller capillaries not only scatter more weakly but also require tighter incident beam collimation so less incident flux is available.

The diameter of suitable capillaries is not directly controlled by the focal size of the direct beam but by the width of the background intensity distribution. SAXS/WAXS allows for the use of 1.5-2.0 mm diameter capillaries, but smaller capillaries are avoided because of the need for quite challenging slit settings with very tight tolerances, and large losses in incident flux.

This optical design has been based on a standard capillary diameter of 1.5 mm, whilst still allowing 1.0 mm capillaries to be used with care. Using 1.0 mm capillaries involves exchanging capillaries, centering to small tolerances, setting slit gaps and re-calibrating scattering intensities. Early optical design work suggests it might be worth considering adding a 4th slit to the collimation system specifically for small capillary work, as the ideal slit positions for standard and small capillaries may be in different places. It is not practicable to use less than 1.0 mm capillaries down to q = 0.005 Å^{-1} at the Australian Synchrotron source on a stable, high-throughput style beamline.

Because bioSAXS is not aiming for the q_{min} = 0.0015 Å and small spot size of the existing SAXS/WAXS beamline, the optical design possibilities are less constrained. Reduced source demagnification together with space savings from a shorter camera length allow major improvements in the collimation system for bioSAXS. A summary of the beam properties of three main layout options is shown in Table 1.

Table 1: key beam properties for the three main layout options for bioSAXS

KB position (m)	Sample Position (m)	Comment	Slit 2 position (m)	Optimal Slit 3 position (m)	Slit 4 position (m)	Slit 2 gap (max) (mm)	FW of background function (mm)	Horizontal focal size (FWHM, µm)	q_{min} (Å^{-1})
23	30	same as SAXS/ WAXS	24	27.5	29.5	0.5	1.2	210	0.0045
23	34.5		24	32	34.5	0.65	0.95	370	0.0035
20	34.5		21	31.5	34.5	0.65	1.06	520	0.0035

Analysis of key layout parameters show that compared to the existing SAXS/WAXS beamline:

- significant improvements in collimation performance (for measurements at or above 0.005 $Å^{-1}$) can be gained by moving the sample position downstream. The 40 m rear hutch wall dictates the furthest downstream sample position. Allowing 3 m for the internal camera length, 1 m for the length of the detector and vacuum vessel, and ~1.5 m for access space, the maximum sample position is 34.5 m. This distance should be adequate to achieve the instrument specifications, hence, there is no need to extend the beamline beyond the standard 40 m length of the experiment floor.

- moving the mirror position slightly upstream will help signal levels by increasing the horizontal focal size, whilst still allowing adequate widths of the background intensity distribution at the sample position and at the beamstop (i.e. meeting q-min and capillary diameter requirements).

The detailed optical configuration is outlined in Table 2.

Table 2: the complete optical configuration of the bioSAXS beamline

Component	**Distance from Source (m)**	**Specification details**
Fixed mask	13.5	4 mm x 2 mm aperture is adequate
Bremsstrahlung collimator	15	
White beam slits	16	
Fluorescent screen	16.5	
Si111 monochromator	17	Si111, channel cut. 25mm vertical offset. 6 – 20 keV capability, but will routinely run at 12 keV.
White beam stop	17.5	Can be incorporated within monochromator vessel
Bremsstrahlung stop	18.0	
QBPM1 (in-flange)	18.6	In-flange type, 2+ foils
Fluorescent screen	18.8	
Monochromatic slit 1	19	Highly polished radiussed edge type
HFM	19.5	- 750 mm working length - bimorph mirror substrate with tangential slope error < 0.6 µrad RMS and < 2.5 µrad peak-peak - Si and Rh stripes each >15mm wide, both with applied voltages - Focal range 32-38 m from source.
VFM	20.2	- 400 mm working length - bimorph mirror substrate with tangential slope error < 0.6 µrad RMS and < 2.5 µrad peak-peak, both with applied voltages - Si and Rh stripes each >15mm wide - Focal range 32-38 m from source.
Be window	20.6	100 µm thick, highly polished

Fluorescent screen	20.8	
Monochromatic slit 2	21	Highly polished radiussed edge type
QBPM2 (in-flange)	21.3	In-flange type, 2+ foils
Attenuator assembly	21.5	XIA type or equivalent
Mono shutter	22	Safety shutter for entry of experiment hutch
Monochromatic slit 3	30.0	Highly polished radiussed edge type
Monochromatic slit 4	31.5	Possible additional slit in position better optimised for small capillaries. To be decided during final design. Highly polished radiussed edge type.
QBPM3 (in-flange)	32	In-flange type, 2+ foils
Monochromatic slit 5	33.8	Highly polished radiussed edge type
Beam position feedback detector	34.0	Video-based system using scintillator image. Same approach as SAXS/WAXS and MX1&2.
Fast shutter	34.2	<50ms opening/closing time including jitter
Sample camera	34.35	Same design as SAXS/WAXS.
Sample position	34.5	
Maximum SAXS detector position	37.5	
Downstream end of SAXS camera	38.5	Provide safe access space (~1.5m) around end of SAXS camera to both sides of hutch
Downstream hutch wall	40	

3) Components and Flux levels

The two key optical components are the KB pair, and the monochromator.

The optical specifications on the KB pair are necessarily stringent. The mirrors form the core of the collimation system: this is a focussing SAXS beamline. The critical requirement for the mirrors is not solely related to the quality of the direct beam itself (usually described by simple parameters such as the FWHM). Instead the critical factor is achieving very low levels of background on the edges (tails) of the main beam at the position of the last two slits in the beamline because this directly controls the low-*q* background intensity. Whilst achieving the latter characteristically assists the former and results in a nicely focussed main beam, the mirror quality is a critical driver of low-*q* signal : noise. The quality of focus achieved on the SAXS/WAXS beamline has been instrumental to the scientific mission of the beamline.

BioSAXS will be even more dependent on mirror quality than SAXS/WAXS because the increase in the distance from the mirrors to the final slit doubles the effect of mirror aberrations. The substrate specification directly reflects the improvement required in the RMS slope, and contains an additional requirement of the maximum peak-peak error against the prospect of any local step-functions in the mirror profile, which has been seen on Phase-2 beamlines, in particular MX2. The mirror specifications represent the current standard of bimorph mirrors, which have improved since SAXS/WAXS was built.

The monochromator can be a channel cut design. A double crystal monochromator (DCM) is not required as the beam will typically operate at a fixed 12 keV energy, and because harmonic rejection at

the monochromator is not required. Beam steering can be accomplished by piezo motors on the KB mirrors. Not only will a channel cut design reduce the cost of the monochromator, it will aid optical stability by reducing the effects of angular and translational vibrations from sources such as ground inputs and cooling systems. The monochromator will be positioned as far downstream as the collimation optics allow. Heatload modelling during detailed SAXS/WAXS design showed Si111 is suitable as close as 14 m to the source, so will not have heatload issues at 17 m. The monochromator performance will easily exceed that of the current SAXS/WAXS beamline.

The useful acceptance of the beamline optics relates to the angular spread of the incident monochromatic beam that is suitable for use at the sample position, and is strongly coupled to the q-minimum required. Increasing the beamline's angular acceptance potentially allows more flux on sample, however beyond a limit dependent (shown in Table 1) additional acceptance destroys the ability to take usable data at low q because intense background intensity can no longer be contained within the beamstop diameter. Increases above "useful acceptance/useful flux" are highly counterproductive. Such requirements are intrinsic to small angle scattering in a way that does not apply to other techniques such as diffraction and spectroscopy. The optical design reported here provides detailed information on the maximum useable flux that can be extracted from the AS storage ring to meet the scientific requirements.

The choice of slit blades for the collimation system is also important. The highly polished radiussed blades currently in use are working well, but further experiments using the SAXS/WAXS beamline are intended to assist final design for bioSAXS.

Fixed wavelength is at 12 keV (1.033 Å). This has several advantages: (1) the detector efficiency of silicon photon counting detectors is high: ~80%; (2) radiation damage is minimized (compared to longer wavelengths); (3) the specified *q*-ranges are attainable at this wavelength; (4) the photon energy is not too high to obtain reasonable flux using an in vacuum undulator on a 3 GeV storage ring, and; (5) longer camera lengths that would be needed to use higher energies are not well matched to the horizontal source properties of the Australian Synchrotron.

Analysis has shown that a bioSAXS beamline operating below 0.01 $Å^{-1}$ with sufficient performance can only be successfully built on the AS storage ring using an undulator. As for SAXS/WAXS, the only relevant X-ray source for bioSAXS is an in-vacuum undulator. The primary requirement of this source is to deliver sufficient flux, at a fixed energy of 12 keV, down the limited angular acceptance of a relatively low-*q* SAXS beamline.

Because the required photon energy is already known, and because a wide range of energies is not required, more optimised tuning curves (i.e. steeper) than the existing U22 IVUs can be considered. Seeking the correct source properties is particularly important because of the prospect of using AS-straight #2, which in its current state not only reduces the available straight length from 3m to approximately 2m but also places the IVU away from the mid-point of the straight section and therefore increases the minimum gap from 6.6 mm to 7.0 mm. Both factors compromise the source properties compared to full length straights.

The positions of harmonics of candidate period lengths of conventional NdFeB hybrid in-vacuum undulators was calculated using the 'Gap Tool' utility of the 'xurgent' module of XOP v2.11. The properties of suitable NdFeB magnets were derived from the accurately measured X-ray tuning curves of 13ID. Table 2 shows that the relevant undulator periods are 16.5, 20 and 22 mm; these place the required 12 keV energy close to the bottom of odd harmonics close to minimum gap. The tuning curves of each of these undulators are shown in Figure 2. The 16.5 mm undulator has such a steep tuning curve that large gaps in flux open up away from minimum gap, which allows no flexibility in energy selection and would require much higher useful flux at 12 keV to justify this disadvantage. The tuning curves of the 20 and 22 mm IVUs are significantly flatter, whilst still maintaining high brilliance at minimum gap.

Table 3: Minimum energy achievable at each of 4 harmonics for a range of periods

7mm gap	Kmax	E1 (keV)	E3 (keV)	E5 (keV)	E7 (keV)
15	0.644	4.72	14.16	23.60	33.03
16	0.760	4.15	12.44	20.73	29.02
16.5	0.820	3.88	**11.63**	19.38	27.13
17	0.796	3.82	11.46	19.09	26.73
18	1.011	3.14	9.43	15.71	22.00
19	1.145	2.72	8.15	13.59	19.02
20	1.284	2.34	7.03	**11.71**	16.39
21	1.428	2.01	6.04	10.07	14.10
22	1.577	1.73	5.20	8.66	**12.12**

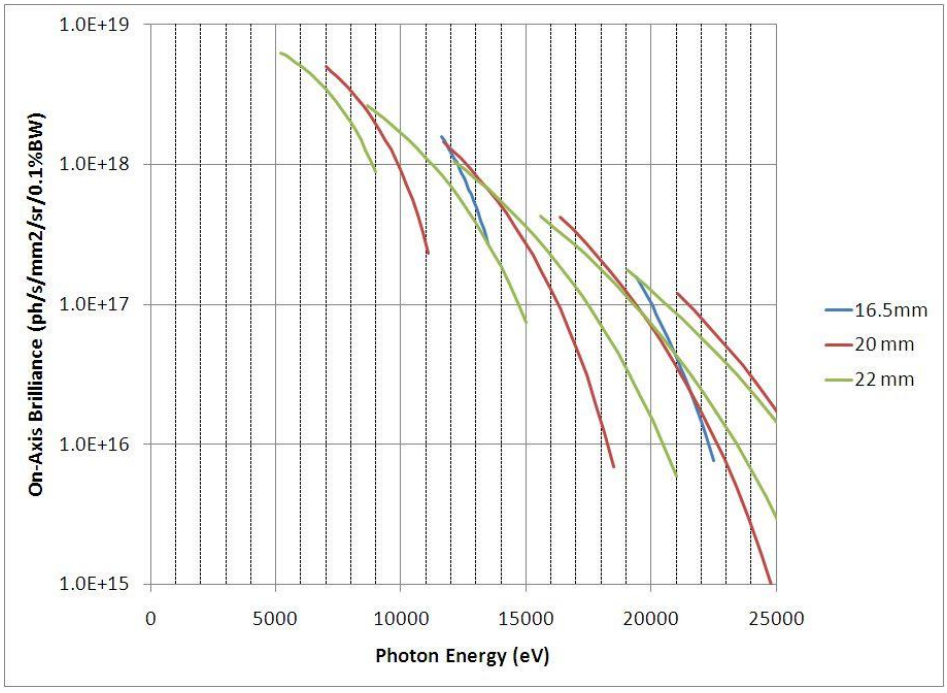

Figure 2: Undulator tuning curves, brilliance as a function of energy with a gap of 7 mm, of in vacuum undulators from the 3rd harmonic and with periods of 16.5, 20 and 22 mm.

The final choice of IVU period is actually straightforward because the optical design of the beamline is already fixed by geometric constraints coming from q_{min} and spot size characteristics: aside from the shapes of tuning curves, the best undulator is simply the one which provides maximum flux into the limited useful acceptance of the beamline optics. This is easily modelled in the Xus module of XOP v2.11, by setting the undulator properties and the real space size and position of the limiting aperture of the beamline which is monochromatic slit 2, then correcting for optical efficiency of components. The results of this calculation are shown below in Table 3 and match the measured fluxes on 13ID using a calibrated photodiode (International Radiation Detectors AXUV-100).

Table 4: comparing selected source properties of the SAXS/WAXS beamline with the proposed bioSAXS beamline

IVU period (mm)	harmonic number	1st harmonic wavelength (Å)	K required for 12 keV	K Max at 7mm gap	Device length (mm)	Number of magnet periods	White beam into beamline acceptance (ph/s/0.1% BW)	Monochromatic flux at Sample (ph/s)	Relative % of 2m U22
16.5	3	3.099	0.7681	0.820	2200	133	3.09E+13	1.19E+12	79
20	5	5.166	1.249	1.284	2200	110	3.80E+13	1.46E+12	97
22	**7**	**7.232**	**1.591**	**1.576**	**2200**	**100**	**3.91E+13**	**1.50E+12**	**100**
13ID (3m U22 into existing SAXS/WAXS optic setup for proteins)	7	7.232	1.591	1.576	3000	136	3.90E+13	1.50E+12	100
13ID (3m U22 into bioSAXS optic)	**7**	**7.2324**	**1.59140**	**1.57674**	**3000**	**136**	**6.33E+13**	**2.44E+12**	**162**

Table 3 shows that the most appropriate undulator period for bioSAXS is 22mm as it emits the highest flux into the acceptance limits of the bioSAXS optic, with an added flexibility of having a gentle tuning curve allowing different energies to be used in the future if required.

Table 3 also shows a problem: the benefits of optimising bioSAXS optics have been removed almost completely by the inferior straight section currently proposed for this beamline. BioSAXS would not provide the significantly improved flux over SAXS/WAXS originally expected, because of the shorter device length (2m rather than 3) and the higher gap (7 rather than 6.6 mm) because of the displacement of the device from the optical centre of the straight. The facility is therefore requested to relocate the diagnostics that currently partly occupy this straight section to elsewhere in the ring if at all possible. This would allow a full 3m U22 to be used resulting in a 60% increase in flux, putting the beamline at the world leading level intended. An alternative is to relocate BioSAXS onto a full length straight.

An equally effective but more expensive alternative would be to use a cryocooled permanent magnet IVU, which should deliver a 50% increase in flux but with significantly higher capital and operating costs. We can definitely rule out requiring a superconducting undulator.

4) Experimental hutch design.

SAXS data in structural biology is most commonly interpreted with reference to high-resolution protein structures solved by crystallography or NMR. Experiments are generally designed to answer questions such as 'does this protein adopt the same conformation in solution as seen in the crystal structure?', 'which of the crystal contacts represent the true dimerisation interface?', 'what does a complex of these proteins look like?' or 'what can we say about these disordered loops or domains?'. The major challenge facing an experimenter is preparing a suitable sample devoid of non-specific aggregates and convincingly validating that the measured entity is the same as the modelled entity i.e. if monomeric protein was modelled that the sample contained only monomeric protein with no trace of contaminants or higher-order oligomerisation states. Of measured SAXS data that is of insufficient quality for publication, a proportion fails due to poor sample quality or for lack of validation controls. The endstation setup is largely designed to address this issue and thereby increase both the throughput and, more importantly, the output of the beamline.

To cover a range of common experimental designs and to afford a user the ability to alter their approach in response to emerging information on sample quality, the beamline will run in one of three distinct modes without altering the basic beamline setup.

The operation modes will be underpinned by:

a) A sample environment featuring temperature control, low background scatter, accurate normalisation and automated data reduction and analysis.

The three operation modes are:

b) basic operation mode with manual sample loading.

c) online chromatography mode featuring sample delivery via HPLC with optical measurements.

d) high-throughput mode with robotics handling 96 well plates.

These 4 aspects of the endstation design are summarised below.

a) Sample environment, normalisation and data reduction

An 'in-vacuum' capillary sample chamber is currently in development on the SAXS/WAXS beamline and will be adopted on the bioSAXS beamline. The chamber features a composite metal body that allows a quartz capillary to transect the vacuum section. This removes the requirement for an air path around the capillary and the need for windows on either side dramatically reducing background scatter and improving performance when measuring dilute and weakly scattering protein samples. In addition, should the capillary become fouled, the chamber allows the capillary to be changed without breaking vacuum reducing down time and the need for staff intervention. Finally, close contact between the metal chamber and the capillary allows for excellent temperature control. Standard fittings above and below the capillary allow the chamber to be connected to a variety of sample delivery systems.

Beam stability and measurements of transmitted flux allow for accurate buffer blank subtraction and for normalisation of the data to known scattering standards. Transmitted flux on the SAXS/WAXS beamline is measured by an integrating backstop and achieves performance at ± 0.05% overall; ± 0.02 % on standard experimental timescale (30 minutes). The bioSAXS beamline will in addition feature active beam position feedback and increased stability from a channel cut monochromator leading to a beamline with exceptionally accurate normalisation.

The experiment hutch will be temperature controlled due to the temperature sensitivity of many samples. This has been a longstanding concern amongst Users of the existing SAXS/WAXS beamline. In line with other facilities such as 12.3.1 at the ALS and beamlines at Soleil, Diamond etc., the experiment hutch will operate at 20 ºC.

The "ScatterBrain" software package is currently in late-stage development for use on the SAXS/WAXS beamline. It is based on the saxs15ID platform, but has been heavily updated and has an expanded interface with many new features and easier operation, including improved automated data acquisition. E.g. Many- dimensional parameter space direct from a Microsoft Excel spreadsheet. Automated data reduction and analysis is required, due to the large amounts of data that will be generated rapidly, especially in high-throughput mode. Automated data reduction software has been developed at the biological SAXS beamline at DESY, Hamburg, Germany. The software allows a user to upload data relating to their samples and features automated blanking and averaging of SAXS curves, calculations of principle components and appropriate modelling strategies. The software meets many of the needs of the bioSAXS beamline and will be customised to suit.

b) Basic operation mode

In basic operation a user will manually present their sample to the lower end of the in vacuum capillary and control a syringe either manually or via a pump to draw their sample into the beam path. High quality sample cameras will allow the user to visualise the capillary and sample position. Data collection will be triggered via the 'Scatterbrain' software and will give total control over the number of acquisitions, the shutter speed, movement of the sample, sample temperature etc. In this mode the sample contacts the quartz capillary and an absolute minimum of other tubing, this is important for sensitive samples to avoid contact with other components where introduction of contaminants and/or degradative enzymes is possible.

To facilitate manual mode a "mini experimental hutch" design is planned. The beamline will be located off-centre in the endstation hutch with a small hatch giving access to the sample environment. This will allow a user to change samples without entering the hutch through a small, interlocked hatch cover.

c) Online chromatography mode

A high-pressure liquid chromatography (HPLC) system can be used to load a sample onto the in vacuum quartz capillary via a size exclusion chromatography column. This method has the great advantage of separating different sized protein species in a polydispersed sample, removing aggregate and

exchanging buffer allowing more accurate blanking. This option is currently available on the SAXS/WAXS beamline and is being developed to increase its capabilities.

The HPLC system will feature an automated sample changer allowing samples to be queued and loaded from a 96 well plate potentially faciliting remote access. In addition, the system will have detectors for measuring protein concentration by absorbance at 280 nm and by measurement of refractive index and for measuring protein size and weight by multi-angle laser scattering (MALS). This additional information will be automatically integrated with the SAXS data. The measured protein concentration together with the normalised SAXS data will allow for calculation of the molecular weight of the scattering particles. This can be cross-validated against the molecular weight from the MALS instrument giving a high degree of confidence in the oligomerisation state and monodispersity of the scattering particles. The radius of gyration from SAXS can also be cross-validated with the MALS data. Integrating these ancillary measurements will allow a very high degree of automation on the beamline and will make measured data very much easier to validate and publish.

d) High-throughput mode

In high-throughput mode samples will be loaded into the capillary from 96 well plates situated in temperature-controlled stages below and close to the sample environment. Sample flow and cleaning of the capillary will be controlled via switching valves and syringe pumps. A system like this is in use on the existing SAXS/WAXS beamline and is being developed to increase performance and capability.

In addition, 96 well plates will be loaded to the sample changing system from a commercially available liquid handling workstation. The workstation will allow centrifugation of the plates, aspiration of titration series, storage of multiple plates in temperature controlled hotels, sample de-gassing etc. They system will allow for measurement of between 700 and 1000 samples per day and could allow remote access of the beamline for users who send samples to the synchrotron in a standardised 96-well plate format.