

# Microbeam MX White Paper

Dieter Schneider, Marc Allaire and Lonny Berman  
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This report incorporates conclusions reached at the NSLS and NSLS-II co-sponsored workshop on *MX Frontiers at the One Micron Scale* held at Brookhaven National Laboratory on July 23-24, 2009, as well as concepts developed since then.

## Summary

Scientific interest in microdiffraction is growing rapidly, in part stimulated by the remarkable achievements of a few leading groups. Among them is Eisenberg's group who first derived the bio-medically important atomic structures of amyloid peptides and Schertler's laboratory who elucidated those of adrenergic G-receptor membrane proteins. (References to this work are given in Section 1). Other structural biologists increasingly resort to micro-diffraction in their pursuit of macromolecular structures and functions that must be wrested from small or heterogeneous crystals. To meet this growing demand several new crystallography beamlines at the world's major synchrotron sources are planned or under construction specifically designed to deliver mini- or microbeams, and a few established beamlines are being retrofitted for this use. However, only the ESRF ID13 beamline, where the potential and success of the technique was first demonstrated, actually routinely achieves a one micron size beam focus and is even just partially used for macromolecular crystallography.

The well-attended MX Frontiers at the One Micron Scale Workshop's proceedings (<http://www.nsls.bnl.gov/newsroom/events/workshops/2009/mx/>) and discussions underscored the potential scientific impact and community need for micro-focusing beamlines dedicated to macromolecular crystallography. This outcome validates plans to develop a dedicated macromolecular crystallography beamline at NSLS-II that would achieve a focused beam of one micron diameter or smaller and exceed the flux of competing concepts elsewhere by exploiting the unsurpassed brightness of the new source. An outline of a design for a two-stage focusing scheme involving an intermediate virtual source is described in Section 3 of this paper and compared with the international competition.

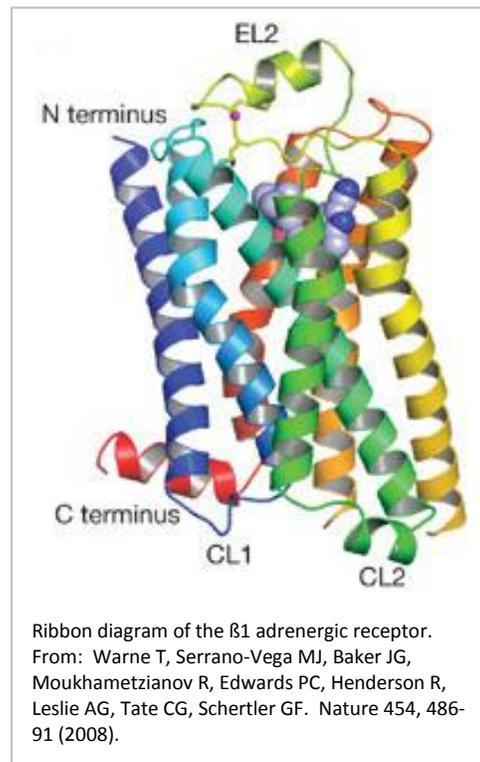
Technical and operational challenges of microdiffraction are summarized in Section 2. A key point is the fact that experimental work at the one micron scale, unlike standard crystallographic data collections, will succeed best when investigators and beamline scientists collaborate closely and when ample beam time is available to develop and refine optimal experimental conditions for the problem at hand. Further, owing to the minute scale of the specimen and its environment and the usual need to search for viable crystals, a high degree of automation and remote control will be needed to make the experimental work accurate, expedient, and people-friendly.

## 1. Scientific Opportunities with Microbeam MX at NSLS-II

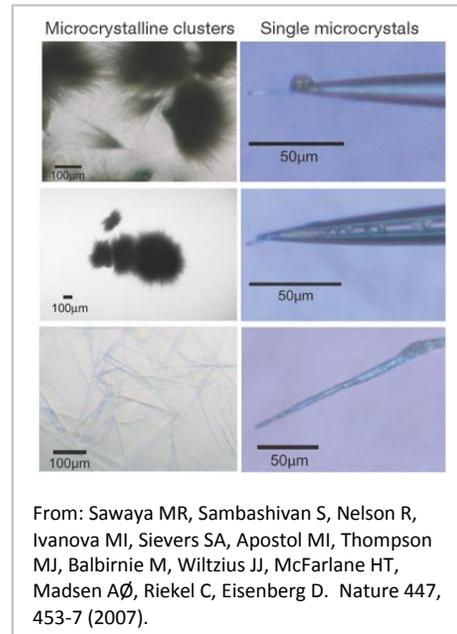
Structural biology has transformed our understanding of biological processes where cellular mechanisms are now enlightened at a molecular level. We are truly in a golden age of macromolecular crystallography (MX), the prominent method in structural biology, and largely because of synchrotron radiation. The intrinsic value of these approaches is recognized by Nobel Prize selection committees with four Nobel Prizes in Chemistry in the last decade. MacKinnon (2003), Kornberg (2006), Tsien (2008), and Ramakrishnan, Steitz, and Yonath (2009) all used MX and synchrotron radiation.

The most difficult problems are often the most interesting, and these large structures represent the pinnacle of the structural biologists. Indeed, the size and complexity of macromolecules that can be studied has increased by an order of magnitude in the last decade. The crystal structures of the prokaryotic and eukaryotic ribosomes, honored with the 2009 Nobel Prize, are the most complex to date providing atomic details of a macromolecular assembly of more than 150,000 atoms. Determination of this structure – the location of every atom! – was an amazing *tour de force*.

In recent years we have seen an increase in the number of MX beamlines capable of attaining beam sizes down to a few microns. Diffraction data collected at micro- and minibeam facilities increasingly are critical to the success of many challenging structure determinations. Among these are the long awaited structures of the  $\beta 1$  and  $\beta 2$  adrenergic receptors, two members of the family of G-protein-coupled receptors. GPCRs play a major role in trans-membrane signaling and many are important drug targets. Only tiny crystals were obtained of both the  $\beta 1$  and  $\beta 2$  receptors and very limited crystal screening could be carried out because only minute amounts of these integral membrane proteins can be produced. The structures determined by Kobilka, Stevens, and Schertler and coworkers (see figure of the  $\beta 1$  adrenergic receptor) reveal the fold of the seven trans-membrane helices and associated loops, and the critical elements defining the receptor's ligand-binding pocket. These insights revolutionized the GPCR field and continue to have a dramatic impact on the development of agonists and antagonists for many members of this important family of receptors.



Needle-like micro crystals that may be 10,000 times smaller than conventional biological samples are another challenge that recently yielded structures by microdiffraction using the smallest currently available beam anywhere, the one micron-sized beam at ESRF's ID13. David Eisenberg and his group first succeeded in obtaining high-resolution structures from 2  $\mu\text{m}$  sized crystals (see figure) containing untwisted amyloid-like fibrils, giving the first glimpse of the atomic arrangements of proteins in the amyloid state. Over the past five years, his group has determined structures for some 60 other amyloid-like microcrystals, many of them are the agents or products of nervous diseases such as Alzheimer's. Presently, working with even smaller specimen, they are demonstrating that useful diffraction can be obtained from one micron or smaller crystals.



## 2. Technical and Operational Challenges with Micro-focused Beams

*Microdiffraction requires experiment optimization:*

Superb examples of structures derived by microdiffraction from macromolecular complexes of medical, pharmacological, and fundamental biological importance are reported in the literature. The elucidation by Eisenberg and coworkers of many members in the 'systematic table' of possible amyloid peptide aggregates, including the agent of Alzheimer's diseases, are examples of structures from crystals that, due to their intrinsically stressed architecture, will not grow beyond micron size. And the insights gained by Schertler's group into the structures and function of G protein coupled receptors are examples of results derived from soft crystals containing but a few tiny well-ordered domains.

The number of results from microdiffraction studies is minute when compared to the steady stream of equally important results obtained with standard synchrotron X-ray crystallographic methods from full-size crystals measuring several tens of microns. However, in the above mentioned examples and in other pioneering work, microdiffraction was the breakthrough method that yielded unique high resolution structures. Investigators advancing these frontier studies readily talk about the sustained experimental effort that was required to achieve breakthroughs. They praise close collaborations with beamline scientists and extol the need for frequent access and ample time at diffraction facilities as key ingredients that helped them set up, optimize, and refine their experiments in ways quite different from routine measurements.

*Beam diameters of one micron or less reduce radiation damage in probe volume:*

Radiation damage in macromolecular crystals is primarily due to energetic photo-electrons that are emitted following absorption of the beam along its path. Monte-Carlo calculations show that if the beam size is sufficiently small, having a diameter of one micron or smaller, many of the photo-electrons escape the interrogated volume and thus extend the acceptable exposure time before radiation damage results in loss of resolution in diffraction images. While early experiments with beam diameters of one micron or more indicate that photo-electrons indeed escape the beam path, the deduced reduction in radiation damage was less than expected. Experts agree that more experimental studies are needed on better defined experimental systems before firm conclusions can be drawn. Currently, several competent groups are advancing this work and now report preliminary results that support the physical expectation and thus underline this potential of microbeams.

*Microbeams afford an opportunity of serial crystallography:*

An undisputed advantage of small beams is the possibility of moving a larger crystal through the incident beam either in steps along nodes of a raster, or continuously, resulting in the sampling of a spiral path during crystal rotation. This is already practiced in experimenter-assisted ways, often involving the manual re-centering of virgin crystal volumes into the beam. Additionally, the increasingly popular crystal mounting grids may hold multiple small crystals that each may yield useful diffraction data. This method too is already under active development here at the NSLS (Soares) and elsewhere. If the serial exposures across a large crystal in a one micron beam are spaced more than about two beam widths apart, photo-electrons can escape from the diffraction region. Thus microbeams would extend the life time of each exposed spot and amplify the benefits of stepping beam into untouched crystal volumes.

*Minimal required crystal size is still falling:*

The diffraction yield of macromolecular crystals is lower than that of inorganic crystals because of their typically large internal solvent content and lower density of atoms arrayed with long-range order. Accordingly, the minimal crystal volume of about  $20 \mu\text{m}^3$  that led to successful structure determinations using standard methods is about twice that of the inorganic record. However, the recent microdiffraction results mentioned before, were derived from crystal volumes of about  $8 \mu\text{m}^3$ . This indicates that the minimum crystal volume – i.e. the minimum number of unit cells - required for macromolecular structure determination is still falling and quite naturally, that well-designed micro-focusing beamlines will be the instruments of choice in exploring this frontier.

*Crystal visualization and handling will require ingenuity:*

Once micron-sized beams are offered, crystals of that size-scale will follow. While it is quite conceivable to deploy a high-quality visible light microscope near the specimen, it may be difficult to provide the desirable in-beam sample observation because of the short length between secondary focusing optics and specimen. UV-light microscopes, may be

needed. Multi-axis observation combined with computational methods may also prove useful to reconstruct the crystal scene.

Crystal handling is a further challenge that undoubtedly will require microscopes equipped with optical tweezers such that crystals can be nudged out of thick mother liquor drops. Ablation of buffer drops with lasers could be considered. However, it is reasonable to assume that experimenters working on micro-systems will be the true innovators of new techniques.

*Micro-diffraction requires high-resolution detectors:*

Currently available pixel-array detectors, when properly calibrated, have zero dark current and under suitable experimental conditions achieve optimal signal-to-noise ratios. However, current detectors have large pixels (i.e.  $172 \times 172 \mu\text{m}^2$ ) that will intercept background intensity from mother liquor and specimen support over the full pixel area. By contrast, Bragg spots from micron sized beams and crystals will deposit signal intensity in just a fraction of that area. This will compromise their signal-to-noise ratio and ultimately the best achievable resolution. For a high-performance microdiffraction setup new detectors will be required that have pixels at least ten times smaller than currently available. An additional challenge will be the acquisition or development of detectors and methods suitable for beam imaging, profile and position measurements.

*Exquisite thermal, vibrational, and mechanical stability will be tantamount:*

Given the smallness of a one micron beam and the expected time-consuming challenge of performing beam alignments and optimizations ahead of experiments, superb mechanical stability will be required so that alignments will hold up for many hours. This time scale should encompass several experiments such that difference studies become feasible. Inevitably, this will lead engineers to construct experimental apparatus on perhaps a monolithic block kept in a temperature-controlled envelope. It should keep final focusing elements, beam conditioning slits and counters as well as goniometry within nanometers of the line of sight.

*Microbeams will require nano-precision goniometry:*

Data collection requires the rotation of crystals in the beam. Therefore, the wobble of the rotation axis must be much smaller than the probing one micron beam. Moreover, to take advantage this fine beam, crystal positioning and orientation on a kappa stage should also approach this target. Clearly, for use in a one micron-sized beam the mechanical precision of the omega spindle and the goniometer stage must be on the nanometer scale. Considerations of the effects of gravity will likely lead to a vertically oriented omega axis. Sample stages developed for electron microscopy may offer a solution. In addition, suitable active feedback systems, perhaps based on interferometry, should be considered to continuously true the point of interest.

*Automation:*

Practitioners of microdiffraction have advised with one voice that a high degree of automation in sample mounting, handling and positioning will be critical to open the path to the extensive experimentation that will be required in project development and later for data collection. This is entirely reasonable considering that current investigator groups already rely on the speed and steady hand of robots when screening hundreds of crystals for the few that yield good diffraction. At a microdiffraction beamline robots will be part of the method to maintain thermal stability in the hutch.

### **3. Facility and Beamline Requirements for Microbeam MX at NSLS-II, Including Comparison with Others**

*Comparing existing and planned MX micro-beamlines:*

The current world-wide landscape of dedicated MX beamlines which are optimized to deliver small beams includes beamlines at ESRF, Diamond, SLS, and APS. Others are under construction at PETRA-III and SPring-8. With the exception of ID13 at ESRF which is partially used for MX, none of the operational beamlines are designed to deliver a beam of 1  $\mu\text{m}$  size. These beamlines target a small beam size limit of about 5  $\mu\text{m}$ . GM/CA-CAT at APS has recently retro-fitted a capability to deliver a beam of 1  $\mu\text{m}$  size, which isn't ideal. An optical system that will be dedicated to delivering a beam of 1  $\mu\text{m}$  size is now being designed by GM/CA-CAT.

The beamline which is now under construction at SPring-8 will eventually deliver a beam size of 1  $\mu\text{m}$ . Like many of the optical systems that are in place to deliver small beams, the SPring-8 beamline will employ a secondary source, for the purposes of stability and facile control of the source dimensions.

MX beamlines that will deliver a beam of about 1  $\mu\text{m}$  size are currently in the planning stage for ALBA and MAX-IV. These beamlines deserve special mention here, owing to the similarity of the properties of ALBA and MAX-IV with those of NSLS-II (in terms of ring energy, emittance, etc.) While details about the beamline being proposed for MAX-IV are forthcoming, a description of the beamline envisioned for ALBA is already available at [http://www.cells.es/Beamlines/SECOND-PHASE/MMC/proposal\\_mmc/](http://www.cells.es/Beamlines/SECOND-PHASE/MMC/proposal_mmc/). To our knowledge, this beamline, if approved, will be the first dedicated MX beamline designed to achieve a day-one operational objective of delivering a beam size of  $\sim 1$   $\mu\text{m}$  (the ones mentioned above, which have a 1  $\mu\text{m}$  beam size objective, seek to attain this objective in a later stage of development). The anticipated FWHM beam size at the sample position, for this beamline, will be 3  $\mu\text{m}$  horizontally and 1  $\mu\text{m}$  vertically, and the anticipated angular divergence will be 3 mrad horizontally and 0.3 mrad vertically. The optical scheme envisioned for this beamline employs two-stage focusing in the horizontal direction and one-stage focusing in the vertical direction. The horizontal beam size at the sample position could be doubled by removing the first horizontal focusing stage, while preserving the angular divergence, in this scheme.

The following table summarizes attributes of MX beamlines, present and future, that are designed to deliver small beams.

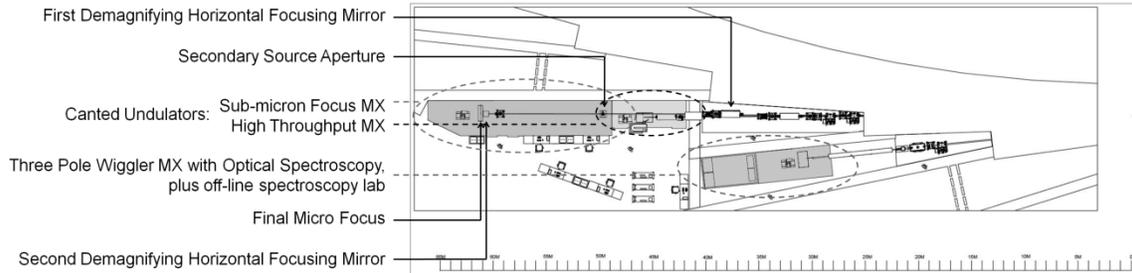
<i>Beamline</i>	<i>Beam Size <math>h \times v</math> [<math>\mu\text{m} \times \mu\text{m}</math>]</i>	<i>Flux [<math>\text{ph/s}</math>]</i>
<b>ESRF ID13</b> not dedicated to MX (Ch. Riekel)	1 x 1	$8 \times 10^{10}$
<b>ESRF ID23-2</b> dedicated to MX, fixed $\lambda$ (D. Flot)	7 x 4 (eventually 1x1)	$4 \times 10^{11}$
<b>SLS X06SA</b> (C. Schulze-Briese)	15 x 5	$10^{12}$
<b>APS GM/CA-CAT</b> (R. Fischetti)	65 x 20 (standard) 5 x 5 (mini) 1 x 1 (micro, under re-design)	$2 \times 10^{13}$ $5 \times 10^{10}$ $3 \times 10^9$
<b>Diamond I24</b> (G. Evans)	9 x 9 (eventually 5x 5)	$10^{12}$
<b>SPring-8 BL32XU</b> under construction (M. Yamamoto)	1 x 1	$6 \times 10^{10}$
<b>Alba MicroFocus</b> proposed	3 x 1	$3 \times 10^{12}$

*Concepts for microfocusing MX beamline at NSLS-II:*

An optical system concept based on use of a secondary source is also envisioned for an NSLS-II beamline that would be designed to deliver a beam size of 1  $\mu\text{m}$ . Unlike the new SPring-8 beamline design which employs one-stage focusing and places the secondary source at a different location from the focal point (thus costing significant flux), the NSLS-II beamline design would employ two-stage focusing in the horizontal direction and place the secondary source at the focal point for the first horizontal focusing stage, thus optimizing the flux throughput while retaining the above-mentioned advantages of employing a secondary source. This is similar to what is proposed for the ALBA microfocus beamline.

This concept is shown in the below figure, which is accompanied by a table showing the horizontal beam properties at various positions along the beamline. It is envisioned that this beamline, viewing a U20 undulator source installed in an NSLS-II low-beta straight section, could deliver as much as  $5 \times 10^{11} - 1 \times 10^{12}$  ph/sec at 12 keV (using a Si(111) double crystal monochromator) into a 1  $\mu\text{m}$  beam size and have a horizontal angular divergence of 1 mrad (3 times less than being proposed for the ALBA microfocus

beamline). This flux is an order of magnitude higher than has been achieved in a 1  $\mu\text{m}$  beam size at the ESRF ID13 beamline or than will be achieved in a 1  $\mu\text{m}$  beam size at the new SPring-8 beamline that is under construction, and comparable to what will be available at the ALBA microfocus beamline but in a less divergent beam.



<i>Optical Component</i>	<i>Location [m]</i>	<i>H-Size [<math>\mu\text{m}</math>]</i>	<i>H-Divergence [<math>\mu\text{rad}</math>]</i>
Source	0	66	45
Front End Slit	20	234	15
Horizontal Focusing Mirror	38	504	15 incident
Secondary Source Aperture	50	27	47
Second Horz Focusing Mirror	61	490	47 incident
Final Focal Point	61.5	$\leq 1.5$	$\leq 1045$

Deserving careful consideration are the possibility and implications of a beamline delivering larger beam sizes (up to  $\sim 100 \mu\text{m}$  if called for) while offering a capability of a  $\sim 1 \mu\text{m}$  beam size in the same endstation. Such capabilities will be offered in the beamlines mentioned already, but these will vary. One way to address such a need is through adjustment of the microfocus beamline optics, via either defocusing them or removal of a focusing stage; the former approach generally doesn't alter the angular divergence of the beam. It would normally be desirable, in selecting a larger size beam, to accommodate a smaller angular divergence in doing so. A different way to address such a need would be to provision the experimental station with two setups, one optimized with final focusing optics to deliver a 1  $\mu\text{m}$  beam size and the other having focusing optics to deliver a more standard (larger) beam size. A deflecting mirror can be used to direct the beam to one or the other setup, in such a way that the more delicate setup for the 1  $\mu\text{m}$  beam size is never disturbed. The two setups could share the same detector and other components. This approach is expected to be used at the APS GM/CA-CAT beamline where a new microfocus setup is now being designed.