Micro Sample Environments for High Brilliance Small Angle X-ray Scattering
SC-2266

The objective of this Long term project is the development of micro environments for small angle X-ray scattering. This includes cryogenically cooled sample mounts and SAXS measurements in microfluidic generated small droplets. In the reporting period 11/2007 to 01/2008 the following achievements can be reported:

1. CryoSAXS sample environment

The construction of a cold finger cryostat and vacuum translation stage parts of the CryoSAXS setup was finished in the first half of 2007. This included the final assembly of the mechanical parts and the support electronics (stepper motors and drivers, encoders and temperature sensors) and programming the control software which unifies the system under a single user interface. The software communicates with the electronics via CAN and Ethernet buses and runs under Linux in an ultraportable laptop computer.

After first tests at EMBL beamline X33, it became evident that more work on the transfer of samples from storage to the sample holder and in prevention of sample frosting was needed. It was also decided to have an independent method of sample inspection and alignment in the form of an on-axis optical camera.

Figure. 1 CryoSAXS setup being tested at EMBL X33 beamline.

The requirement of being able to do experiments at other SAXS measurement stations independently of the beamline instrumentation at EMBL Hamburg necessitated the construction of a new sample vacuum chamber. The improvements in sample transfer and moisture control were implemented in this new chamber, which was designed and constructed at EMBL Hamburg. The chamber interfaces to the vacuum system of a beamline with two standard KF-40 flanges and has two compressed air operated gate valves and a vacuum pump outlet for independent flushing and pumping of the sample vacuum. An integrated on-beam-axis camera optics and lighting system is built into the chamber, allowing images of the sample to be taken during X-ray exposure. The moisture control problems were solved by interfacing the sample chamber door to a portable glove-box, which is filled with dry air during sample mounting.

Figure 2: Cryo-setup mounted on ID02 beamline at ESRF

The efforts in sample preparation concentrated on high-pressure freezing, a method used commonly in electron cryomicroscopy of unstained vitreous sections. The studies on the applicability of this standard EM sample preparation method to dilute protein solutions were conducted in collaboration with the EMBL Heidelberg electron microscopy core facility.

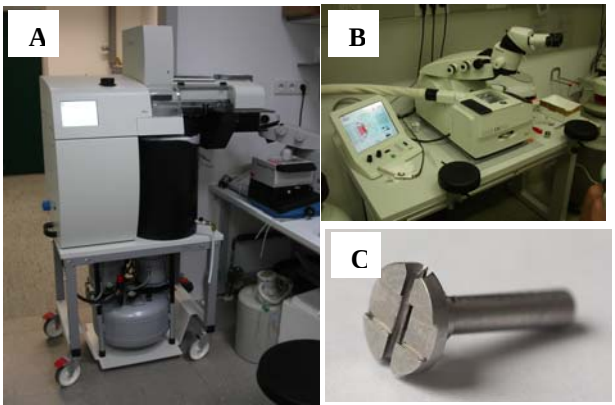


Figure 3: (A) Leica EM-PACT2 high pressure freezing machine and (B) Leica Ultracut ultra-cryomicrotome at

EMBL Heidelberg. (C) Custom specimen holder for a Leica ultra-cryomicrotome

The Leica high-pressure freezing machine EM-PACT2 (figure 3 (A)) was used to freeze samples containing an inexpensive test protein (bovine serum albumin) in standard buffer and varying amount of cryoprotectant (ethylene glycol or glycerol). During freezing, the sample solution is held on a copper tube with 0.3 mm inner diameter. The machine applies a pressure of around 2500 bars to the sample, and quickly freezes it with a jet of liquid nitrogen. After freezing the sample, which much be retained in temperatures below 130 K, is deposited to a vessel containing liquid nitrogen. The copper tube holding the sample inside are then cut to a length of around 1 mm with a Leica Ultracut ultracryomicrotome, which allows specimens to be cut and manipulated in low temperature (down to 110K) open gas phase environment (figure 3 (B)). A new microtome specimen holder, allowing accurate measurement and cutting of 1 mm tube pieces was designed and made together with the mechanical workshop at EMBL Heidelberg (figure 3 (C)). The resulting 0.7 mm outer diameter, 1mm long piece has a volume of about 100 nl.

The improved sample environment and sample preparation methods were tested at the ESRF beamline ID02. The size of the X-ray beam was reduced by slits to 85 um by 40 um, and the 300 um diameter samples were aligned to the beam position with the help of the on-axis camera. Although some samples suffered from a considerable background intensity in the very small angles, in some cases scattering intensity arising from the protein solute could be observed. The results (Figure 4) demonstrate that scattering from a protein sample can be measured from a 100 nl volume in solid amorphous solvent at sub-100 K temperature.

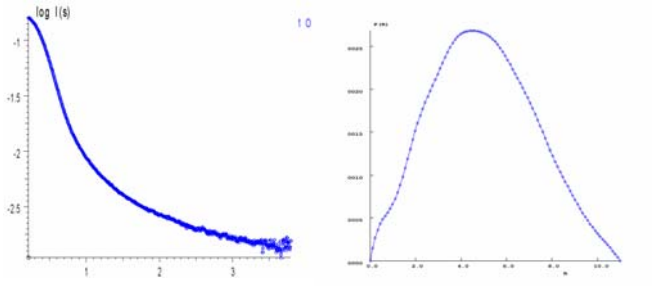


Figure 4. Small-angle X-ray scattering intensity and p(r) function measured at cryogenic temperature from a 100 nl bovine serum albumin sample in a buffer containing 33% v/v ethylene glycol.

Scattering originating from protein could be measured, albeit with low repeatability and large amount background in the very small-angle region originating from the amorphous water matrix. During these measurements it was observed that the scattering contrast of the protein was increasing during repeated exposures of the same sample (Figure 5). One possible explanation for this phenomenon is a transition of the aqueous matrix from high-density amorphous (HDA) state to low-density amorphous (LDA) during the measurement. This transition is caused by elevated temperature, indicating that *in situ* measurements with better temperature measurement and control in the sample stage are needed in order to understand this phenomenon.

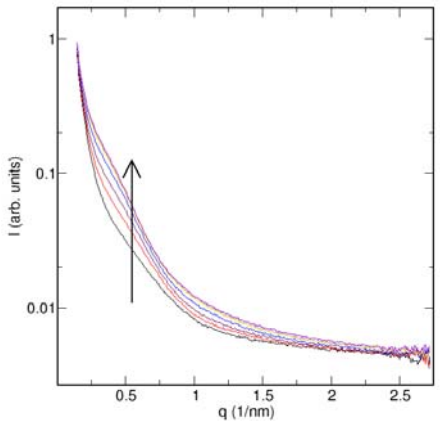


Figure 5: Increasing scattering contrast during measurement observed in a high-pressure frozen solution of bovine serum albumin in water / ethylene glycol mixture (raw data, background not subtracted).

An experiment involving the CryoSAXS setup was made at the microfocus beamline ID13 at the ESRF. The objective was to check the micrometer scale homogeneity of the high-pressure frozen samples by scanning a 10 micrometer (μm) X-ray beam on the 300 um diameter sample. This objective could not be achieved due to high radiation background, which made it difficult to disentangle the sample scattering intensity from the background radiation originating from other beamline components. Figure 2 shows the X-ray scattering intensity from a 20 nm diameter gold colloid sample in amorphous water / ethylene glycol solution at 90 K measured at ID13 compared with the same sample in aqueous solution at room temperature.

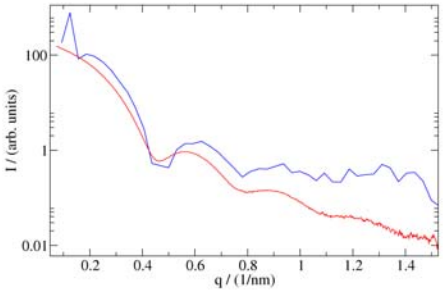


Figure 6: Colloidal gold (20 nm diameter) measured at approx. 90 K temperature in amorphous water / ethylene glycol matrix at ESRF ID13 (upper curve, blue) and at RT in aqueous solution at EMBL Hamburg, X33 (lower curve, red).

Despite for the semi-optimal conditions the results on gold nano particles are comparable with high statistic data recorded at X33 EMBL Hamburg.The data shown in fig. 2 were recorded using a PILATUS 500 K pixel detector. The detector owned by EMBL Hamburg was brought during the long shutdown of the DORIS synchrotron for tests to ESRF.

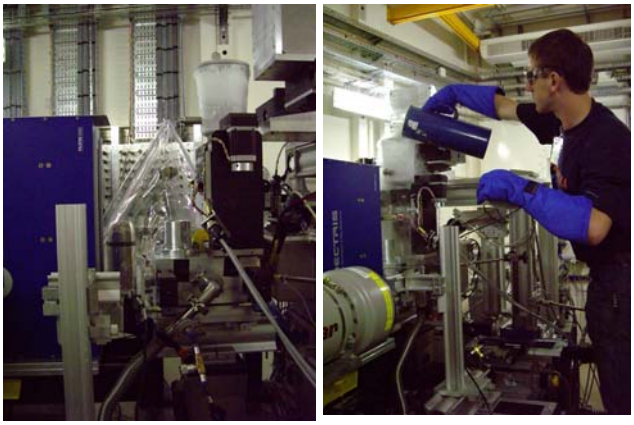


Figure 7: Cryo-SAXS setup and PILATUS 500K detector at the ESRF ID13 microfocus beamline. Micro focusing mirrors in Kirk-Patrick-Baez geometry produced a small X-ray beam of ~ 1μm.

The experimental setup was facilitating scanning experiments and the resolution of the implemented stepper motor is high enough for future applications. The icing problem was resolved by further improving the dry nitrogen environment.

In order to better investigate the contrast modification phenomenon observed previously (Figure 5), plans for the improving the temperature measurement, temperature control and thermal shielding in the CryoSAXS sample holder were made and they will be implemented in late 2008 and early 2009.

Figure 7: CAD drawing of an improved cryo-SAXS sample holder. The thermal shielding is facilitated by a metal cylinder with beam entrance and exit windows. The cylinder is moveable by a cryogenic adapted motor.

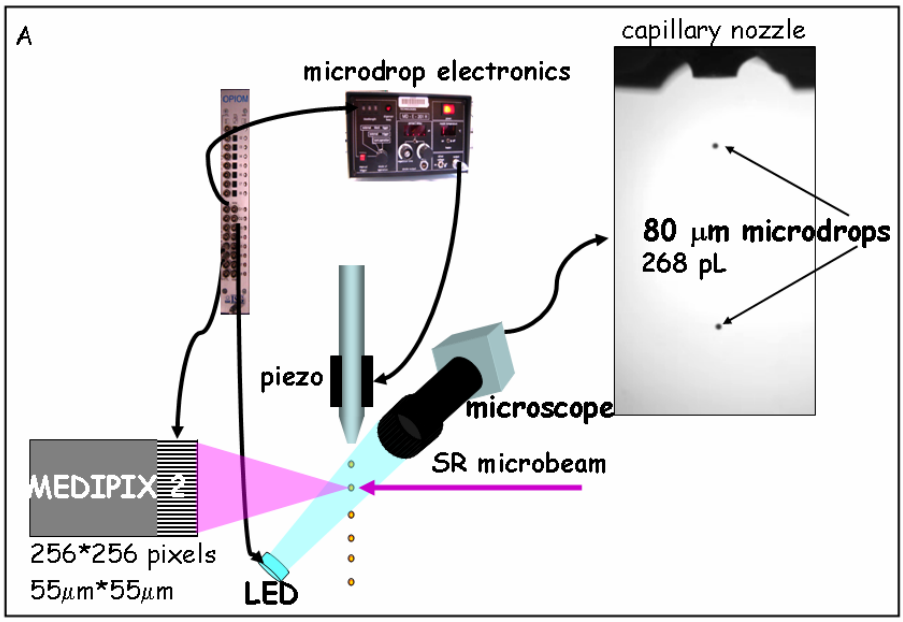
This new setup was successfully used in late December 2008 at SOLEIL and be operational for the next ID02 experiments end of January 2009.

2. Microdrop setup

Microdrops generated by drop-on-demand inkjet systems have been studied at the ID13 beamline by microdiffraction techniques. Ballistic microdrops are free of physical boundaries and are therefore of interest for the study of aggregation, phase transformation or chemical reactions without the wall effects encountered in microfluidic cells.[1] The experiments reported below have been performed in the context of the PhD project by R. Graceffa (ID13).

Wide-angle X-ray scattering patterns (WAXS) were observed stroboscopically from paraffin wax microdrops, generated by a high temperature inkjet system, travelling through a 1 μm synchrotron radiation beam with a speed of about 1.4 m/s.[2] WAXS patterns were recorded in-flight by a FReLon CCD with a microchannel plate image intensifier stage, which was activated with the microdrop generation frequency of 1KHz during 2 μs. The data show liquid microdrops with a constant temperature up to 8 mm from the inkjet system capillary exit.[2]

Small-angle X-ray scattering patterns (SAXS) have been collected stroboscopically from microdrops of cytochrome C protein solution.[3] The microdrops, measuring around 80 μm diameter (~ 268 pL) travelled at approximately 1.7 m/s through a 3 μm synchrotron radiation beam. The SAXS patterns were accumulated on a noise free Medipix pixel detector[4], which was activated for a few μsec during the transit time of each microdrop through the microbeam. (Figure 1A,B) The stability of the microdrop sequence allowed observing interface scattering from HCl buffer microdrops. The SAXS data (Figure 1C) have been analyzed in terms of a Kratky plot and a radius of gyration.[3] Future stroboscopic SAXS experiments will explore transient states of cytochrome C and other biological macromolecules during in-flight microdrop mixing. We also plan studying drop deposition on surfaces, which would provide the possibility of studying protein array deposition in biochips.



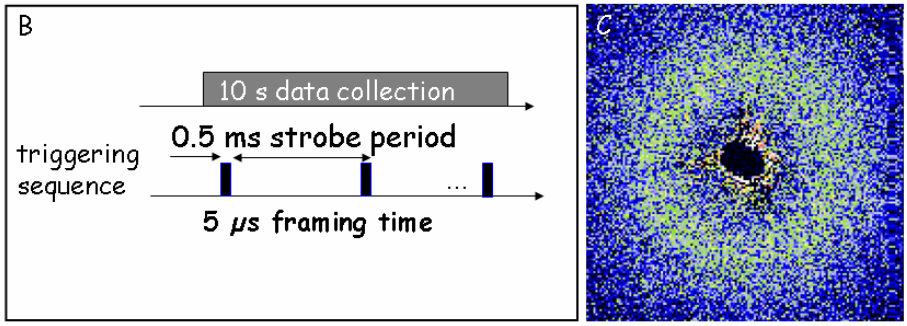


Fig.1 A: Schematic picture of the inkjet system and triggering electronics.[3] Microdrops are observed under stroboscopic illumination by the LED. For SAXS experiments the pixel detector is triggered. B: triggering sequence for SAXS experiments. The pixel detector is activated for 5 μs framing time during every 0.5 ms strobe period; C: stroboscopicallly collected cytochrome C SAXS pattern after subtraction of the 15 mM HCl buffer scattering.[3]

References

1 E.R. Lee, *Microdrop Generation*. (CRC Press, Boca Raton, 2003).

2 R. Graceffa, M. Burghammer, R. Davies and C. Riekel, Rev. Sci. Instrum. **79**, 086106-086101 (2008).

3 R. Graceffa, M. Burghammer, R. Davies, C. Ponchut and C. Riekel, Appl. Phys. Let. submitted (2009).

4 C. Ponchut, J. L. Visschers, A. Fornaini, H. Graafsma, M. Maiorino, G. Mettivier and D. Calvet, NIM A **484** (1-3), 396-406 (2002).