| ESRF | **Experiment title:** **Attempt to demonstrate collection of biological solution SAXS data suitable for 3D structure determination by spatial correlation averaging** | **Experiment number**: SC-2694 |
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| **Beamline**: ID10C | **Date of experiment**: from: 20/07/2009 to: 26/07/2009 | **Date of report**: 01/09/2009 |
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## Report:

This is a preliminary report on the experiment SC-2694 carried out at beamline ID10C in July 2009. The experiment aimed at demonstrating that SAXS data suitable for 3d structure determination via correlation analysis [1] can be obtained at 3rd generation synchrotrons. The bio-molecule chosen for a test of principle was the *tobacco mosaic virus* (TMV) which exhibits a cylindrical shape 300nm long and 18nm in diameter.

**Preparation of the experiment**

Based on the numbers of a previous test experiment at ID10C, we estimated the best intensity available at ID10C in pink-beam mode to be $2\times10^{10}$ ph/sec/$\mu m^2$. With this intensity available, we calculated that the viscosity of our water-based buffer needed to be increased by a factor 160 to ´freeze´ the rotational diffusion of the TMV particles for a 1ms snapshot. Viscosity enhancement could be achieved by water plus 60 wt% of glycerol at –24°C [2] or water plus 68 wt% dimethyl sulphoxide (DMSO) at –50°C [3] (see Fig. 1a)). The viscosity enhancement (and with it the appearance of spatial correlations) could thus be switched on and off by changing the temperature. On the other hand, the TMV-to-solvent scattering contrast is reduced compared to pure water, especially in the case of glycerol. We calculated that with the above numbers we would have about three scatters per particle per snapshot and could measure the desired correlation coefficients with a signal to noise ratio of 10 in about $10^4$ snapshots. Moreover, our chosen resolution of 4.5 nm would be compromised by radiation damage after about 20 snapshots [4], experimentally confirmed by a test at ID14-3 by scaling the much lower flux at this beamline to the expected flux at ID10C. We tried to introduce radical scavengers into our solutions but their very low solubility even in pure water made them virtually insoluble at the water percentages of our proposed solvents (above). Moreover, even in pure water no significant increase of the tolerated radiation dose was observed in a radiation damage test. Preliminary standard measurements for both glycerol and DMSO and, later, for water plus 70 wt% of 1,2 propanediol (PD) were carried out at the bio-SAXS beamline ID14-3. TMV scattering was almost invisible in glycerol and reduced compared to pure water in DMSO, so we planned to use DMSO. However, we later found near the end of the experiment that PD was much better, having the best scattering contrast and almost no loss of TMV SAXS signal compared to that with pure water (see Fig. 1b)). We take this to mean that PD produces no adverse effects on the sample. This test also showed that we needed about a million particles in the illuminated volume for the

TMV SAXS signal to exceed background under ID14-3 conditions (contrary to a standard SAXS experiment, taking the data in thousands of different spots does not enable a sensible background subtraction).

**Experimental run at ID10C**

We used ID10C in pink beam mode at E=10keV (without monochromator, higher order harmonics from the undulators were cut by a white beam mirror) which is a little-used mode which requires use of a mirror with thermally controlled shape. The incident beam on the sample was defined by a first slit acting as a pinhole and a consecutive slit was used as a guard slit to cut unwanted Fraunhofer fringes. Data collection used the fast maxipix detector in combination with a fast shutter, to protect the sample between acquisitions. Unfortunately, the white beam mirror has a history of producing beam instabilities and it did so on this occasion. We lost more than half of our time due to this hardware problem and for optimization of the shutter opening time. When we finally reached a situation in which we could attempt to take data we ran into what we believe to be a radiation damage problem. We were running the beam in a raster-scan pattern within a small area ($1\times 2\,\mathrm{mm}^2$) of the capillary holding the sample. This was required due to the design of the standard temperature-controlled sample holder we were using. Thus we chose to run about 20 snapshots in the same place with waiting times in between to allow random changes in the particle orientations and then moved on. We now believe that this radiation exposure was tolerated by the virus but not by the solvent and some kind of bubbling or other change lead to SAXS patterns with streaks (not circularly symmetric). We believe that this can be fixed by inexpensive changes to the sample holder to allow a much larger raster extending over the entire filled area of the capillary. This will allow the sample to be moved after every snapshot, which will eliminate waiting for random orientation changes and avoid concentration of the radiation dose on to small regions of solvent.

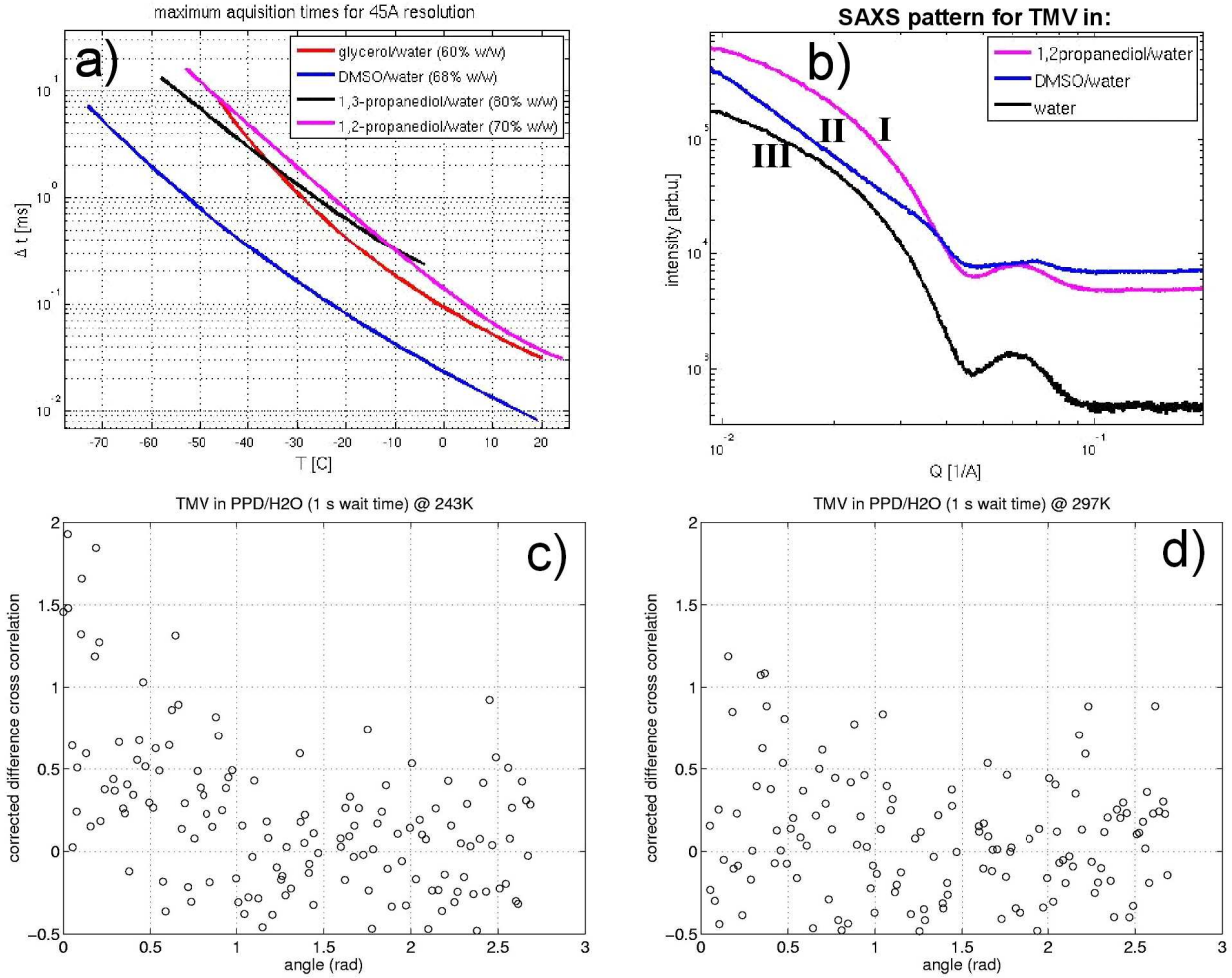


Fig.1. a) maximum acquisition times for one snapshot as a function of temperature for the different viscosity enhancing solutions and a given resolution goal of 4.5nm. b) SAXS curves obtained at ID14-3 for TMV (c=2.42mg/ml) in 1,2propanediol/water (I), c=1.15mg/ml in DMSO/water (II) and c=0.26mg/ml in water buffer (III, background subtracted). c),d) difference cross correlations for TMV in 1,2propanediol/water at T=243K (-30°C) (c) and T=297K (24°C) (d).

With little time remaining our reaction to these problems was to try a different type of sample consisting of Goethite mineral "needles" with similar dimensions as the TMV in water plus 80 wt% propanediol (made

available courtesy of Dr. P. Davidson, Laboratoire de Physique des Solides, Université de Paris-Sud). This gave good looking data, which, with minimal processing, using only differences between nearest neighbour images, showed difference cross correlations with a marked difference between hot and cold solvent, indicating that the expected correlated fluctuations were being seen in the cold (high viscosity) case, but disappeared at ambient temperature where the rotational diffusion smears out the correlations (see figure 1 in our current proposal). The next obvious thing was to do a run with TMV in propanediol. This gave results that were similar but noisier due to the weaker scattering of TMV. The simplest presentation of this data does not immediately show a convincing hot-to-cold difference (see figures 1c and 1d) like in the goethite case. However, it is possible that we may be able see such a difference with more serious attention to data analysis which we plan to give before any further experiments. In any case, the analysis will profit from an improved data quality which we expect to achieve by an optimization of the experimental setup (e.g. minimizing the number of windows in the beam) and by an advanced measuring scheme. By taking only one snapshot in a given spot and then moving to a different location, not only the randomization of the scatterers is assured without any waiting time, but also longer acquisition times at even lower temperatures become feasible and would improve the counting statistics within the individual snapshots.

**References**

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