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Focusing Capillary Optics for Use in Solution Small-Angle X-Ray Scattering

Abstract
Measurements of the global conformation of macromolecules can be carried out using small-angle X-ray scattering (SAXS). Glass focusing capillaries, manufactured at the Cornell High Energy Synchrotron Source (CHESS), have been successfully employed for SAXS measurements on the heme protein cytochrome c. These capillaries provide high X-ray flux into a spot size of tens of micrometres, permitting short exposures of small-volume samples. Such a capability is ideal for use in conjunction with microfluidic mixers, where time resolution may be determined by beam size and sample volumes are kept small to facilitate mixing and conserve material.

Keywords
X-ray scattering (SAXS), capillary optics

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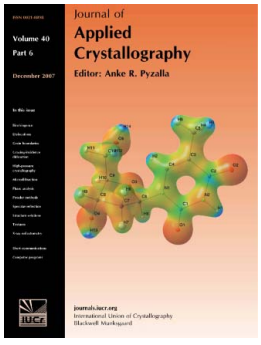

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Focusing capillary optics for use in solution small-angle X-ray scattering

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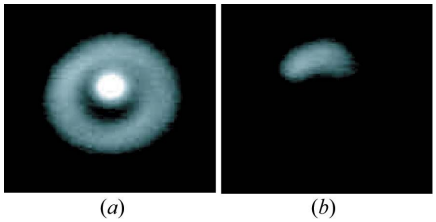
Measurements of the global conformation of macromolecules can be carried out using small-angle X-ray scattering (SAXS). Glass focusing capillaries, manufactured at the Cornell High Energy Synchrotron Source (CHESS), have been successfully employed for SAXS measurements on the heme protein cytochrome *c*. These capillaries provide high X-ray flux into a spot size of tens of micrometres, permitting short exposures of small-volume samples. Such a capability is ideal for use in conjunction with microfluidic mixers, where time resolution may be determined by beam size and sample volumes are kept small to facilitate mixing and conserve material.

1. Introduction

Borosilicate glass capillaries have been used to focus X-ray beams into high flux spots as small as micrometres across (Engstrom *et al.*, 1991) at the expense of beam collimation. Recently, continued development and widespread use of these focusing capillaries has been pursued at the Cornell High Energy Synchrotron Source (CHESS) (Bilderback *et al.*, 1994; Huang & Bilderback, 2006) in applications such as Laue diffraction, X-ray imaging (Bilderback *et al.*, 1994), and crystallography (Bilderback & Huang, 2001). Capillaries have also been tested in scattering studies involving wide-angle X-ray scattering and small-angle X-ray scattering (SAXS) (Riekel *et al.*, 2000; Riekel, 2003).

SAXS measures the low angles of the X-ray scattering profile to characterize the size and shape of a collection of randomly oriented molecules, and can be adapted for a wide variety of targets. A focused microbeam has proven useful on small and spatially resolved samples (Riekel, 2003; Zafeiropoulos *et al.*, 2005) and microfluidic devices (Otten *et al.*, 2005). Macromolecular folding can be induced using stopped-flow or continuous-flow mixers, allowing time-resolved compaction studies (Semisotnov *et al.*, 1996; Pollack *et al.*, 1999). In the latter, time is a function of position, so a large beam may limit time resolution. If the beam flux is low, long exposure times are necessary, requiring increased sample consumption. The high flux microbeam produced by a single-bounce focusing capillary is an ideal tool for these experiments, provided that the divergence of the beam does not limit the measurement, either by smearing the SAXS profile or restricting access to the small angles needed for the measurement.

We present here SAXS measurements of the protein cytochrome *c* taken with focusing capillaries at the CHESS D1 station. Scattering profiles from a calibrant and cytochrome *c* demonstrate no great loss of data or resolution when compared with profiles acquired with an unfocused beam. By using single-bounce capillaries and limiting the accepted beam, a microbeam with sufficiently low divergence to allow SAXS on moderately sized proteins can be generated. These capillaries are readily incorporated into existing SAXS setups.



(*a*) (*b*)

Figure 1
Images of the beam incident on a fluorescent screen approximately 1 m from the focus. Image (*a*) shows the entire beam, including the unfocused beam through the capillary. Image (*b*) shows the portion of the beam used in the measurements. The unfocused beam has been blocked, as well as all but one quadrant of the focused beam, to minimize divergence.

2. Implementation

2.1. Sample

Cytochrome *c* was obtained from Sigma and used without further purification. Cytochrome *c* was dissolved at 30 mg ml^{-1} in a citrate–phosphate buffer, pH 7. The sample was pumped at a rate of 0.1 ml min^{-1} through a small tube consisting of a 2 mm diameter thin-walled polyester (PET) tube (Advanced Polymers, Salem, NH) (Kalinin *et al.*, 2005). Silver stearate [*d* spacing 48.68 Å (Vand *et al.*, 1949)] powder held in a 1 mm PET tube was used as a calibrant.

2.2. Focusing capillary

The single-bounce focusing capillary, identified as BSG644, was used for these measurements. The inner diameters of the base and the tip of the capillary are 400 µm and 266 µm, respectively, and the length is 11 mm. Using the D1 beam source, we expect the capillary to produce a 15 µm spot (Huang, 2005) located 52 mm from the tip. The angular divergence with the optic fully illuminated is 4.3 mrad; guard slits restricted the beam incident on the capillary (as shown in Fig. 1) to limit the divergence to approximately 2 mrad. To estimate the size of a molecule which might scatter below 2 mrad, we note the *D* spacing of a lattice that would scatter to 2 mrad is given by $D =$

wavelength/angle (Guinier & Fournet, 1955). At 9 keV, spatial variations on length scales larger than 690 Å cannot be probed. Fully unfolded cytochrome *c* has a radius of gyration (R_g) of only 32 Å (Kataoka *et al.*, 1993), which is much smaller than the limiting value. Thus, we do not expect the divergence of the beam to be an issue for measurements on small proteins.

2.3. Beamline

Measurements were conducted at the D1 bending magnet station. A beam energy of 9 keV with 1.5% resolution was achieved through double bounce multilayers. Fig. 2 depicts the station setup, typical for SAXS, with the exception of the focusing capillary and an additional 1 mm pinhole. This guard aperture was attached to the sample cell, and eliminated most parasitic scatter from the capillary tip as well as the scatter from the air around the capillary. A small amount of parasitic scatter and small-angle scatter from the glass capillary tip had to be masked in the analysis process. The sample was placed at the focal spot of the capillary.

The capillary was mounted in a groove on a motorized stage, which controlled the position and the tilt angle. These were carefully adjusted until a far-field image produced on a fluorescent screen showed a centered beam. The upstream slits were then set to block the unfocused beam and further restrict the divergence of the focused beam (Fig. 1). The incident beam was positioned on the lower part of the capillary. To explore the effect of focusing on the SAXS profiles, we acquired data without the capillary. For these measurements, the beam was defined to be 0.1 mm × 0.1 mm with slits, and the capillary was moved out of the beam path. The setup was not otherwise altered.

3. Results

A pin diode monitor was placed in the beamstop to monitor the X-ray flux, both with and without the capillary. We measured 30% higher flux with the capillary in place. However, the slit-defined beam had an area of 100 µm × 100 µm, while the calculated focal spot for the capillary is much smaller, at about 180 µm^2. Thus the beam from the capillary is about 60 times more intense.

This intensity gain is easily seen by comparing 10 s exposures of silver stearate, taken with and without the capillary (Fig. 3). The relative heights of the calibrant peak at q = 0.129 Å^{-1} (where $q = 4\pi \sin\theta/\lambda$, with θ = half the scattering angle and λ = X-ray wavelength) reflects the gain in intensity. Furthermore, the full width half-maximum of the peaks goes from 0.0053 Å^{-1} with the capillary to 0.0046 Å^{-1} without it, showing only a small amount of smearing.

The scattering profile of cytochrome *c* was also measured with and without the capillary (Fig. 4). Four 60 s exposures were acquired under each condition, two with protein, and two without for subtracting the scattering background. When the curves are scaled to account for the higher intensity of the capillary beam, the curve shapes are seen to agree within the noise present in the data. Thus, information about the size and shape of the protein in solution are not altered by the focusing optic.

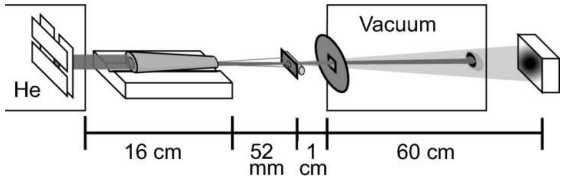


Figure 2
Downstream beamline setup (not to scale): depicted are the slits (in He) the focusing capillary, pinhole and sample (in air), the downstream flight path with a pin diode incorporated into the beamstop, and finally the CCD.

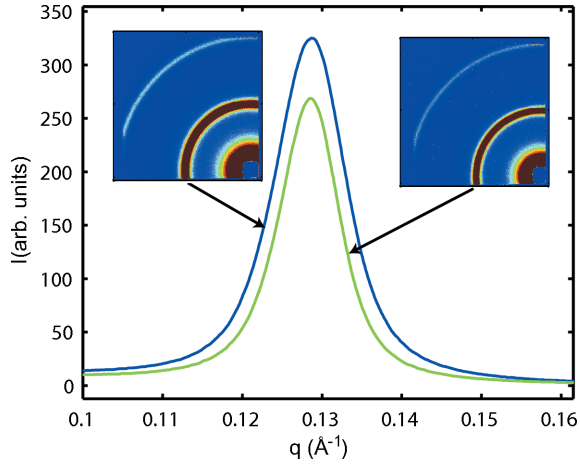


Figure 3
Comparison of the silver stearate image taken with a capillary focused beam, and with the beam defined by slits. The main plot is the radially integrated SAXS profile of the first ring for both measurements; the insets show the CCD images. The line in blue (upper curve) represents the image taken with the focusing capillary, the one in green with slits. The peaks are 324 and 267 intensity units, respectively.

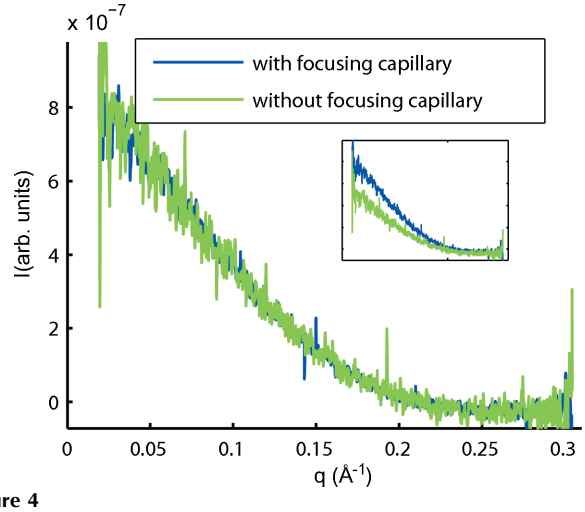


Figure 4
Radially integrated SAXS intensity profiles of cytochrome *c*; the curve taken without the capillary has been adjusted to account for the difference and beam intensity and a slight detector offset. The profiles without adjustments are shown in the inset. The lowest q value reflects the size of the beamstop, the highest value the end of the detector range.

4. Conclusion

We have demonstrated that focusing capillaries with mrad of divergence can be useful in solution SAXS studies on small molecules. Many biological molecules are small enough so that the low divergence from these capillaries will not block important scattering lengths. The use of a small, highly intense beam enables acquisition of good quality scattering data from small sample volumes. In conjunction with continuous flow mixers, sharper time resolution can be achieved compared with slit-defined beams. In the future we intend to use this setup to take time-resolved data.

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