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Sample holder for small-angle x-ray scattering static and flow cell measurements

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We present the design of a sample holder for small-angle x-ray scattering (SAXS) that can be used for both static and flow cell measurements, allowing to switch between these two types of measurement without having to realign the detector and camera geometry. The device makes possible high signal-to-noise experiments with sample volumes as small as 16 μl and can be thermocontrolled using a standard circulating water bath. The setup has been used successfully for a range of biological SAXS measurements, including peptides, detergent micelles, membrane proteins, and nucleic acids. As a performance test, we present scattering data for horse heart cytochrome *c*, collected at the BESSRC CAT beam line 12-ID of the Advanced Photon Source. The design drawings are provided in the supplementary material. © *2006 American Institute of Physics.* [DOI: 10.1063/1.2194484]

Small-angle x-ray scattering (SAXS) is an important technique to study the size, shape, and interactions of biological macromolecules and other polymer or detergent systems.[1–3] It allows to investigate proteins, nucleic acids, and protein-detergent complexes under (near) physiological conditions in solution, without the need to crystallize the sample. SAXS can be used to study unfolded and/or denatured conformations[1] and to follow conformational changes as a function of time. Anomalous SAXS (ASAXS) is employed to probe the position of metal ions specifically bound to macromolecules[4,5] or the distribution of ions around a charged polymer.[6,7] In particular, third generation synchrotron sources such as the Advanced Photon Source at Argonne National Laboratory with their high brightness and great beam stability have made possible high quality (low signal-to-noise) measurements.[8] In order to position the solution containing the biological macromolecule in the x-ray beam, a sample cell and holder design are required, which should offer (i) low background (parasitic) scattering, (ii) high scattering signal from the sample, (iii) the ability to thermocontrol the sample, and (iv) convenient handling. Here we present a system that meets the above requirements improving on previous sample setups (which often use capillaries[9,10]) and allows for dual use for two different commonly encountered experimental setups: Static cell measurements, where a sample volume is loaded and exposed to the x-ray beam for a certain amount of time, and flow cell measurements, where the sample volume exposed to the beam is exchanged throughout the measurement by pumping fresh solution through the observation volume. The former approach allows for measurements on relatively small sample volumes, and is preferred if the sample scatters strongly and is difficult and expensive to obtain, the latter type of measurements allows for much longer integration times without incurring radiation damage and allows for precise measurements of weak scatterers, at the expense of requiring a relative large amount of sample. The sample holder design described here allows for both static and flow cell measurements using the same holder mount and different, easily exchangeable sample cells. This offers the possibility to switch between the two types of measurements without having to realign and recalibrate the detector and camera geometry. The design has recently been used by our group and collaborators in SAXS studies carried out at the BESSRC CAT beam line 12-ID of the Advanced Photon Source. The flow cell setup was employed in several studies on peptides, such as a $\approx$0.9 kDa, 11 residue alanine peptide,[11] a series of alanine and lysine rich peptides,[12] and a denaturation study on a $\approx$7 kDa artificially designed protein fragment.[13] For these low molecular weight samples long integration times are required to obtain a good scattering signal, as the SAXS intensity is proportional to the square of the molecular weight.[14] Static cell measurements have been used in a study on membrane proteins solubilized by micelle forming detergents, where it is difficult to obtain large sample quantities due to the difficulty of overexpressing

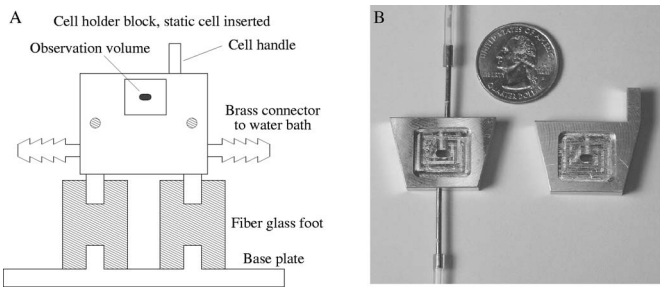

FIG. 1. Overview drawing of the sample holder, with the static cell inserted (A) (left), and photograph of the sample cells (B) (right). In the photograph, the flow cell with tubing is shown on the left and the static cell on the right. A quarter is shown for comparison.

membrane proteins.[15] Furthermore, the static cell setup was employed in several studies of nucleic acid samples: A construct of tethered DNA duplexes was used to probe their interactions under different salt conditions,[16] a RNA riboswitch was studied as a function of salt and ligand concentration, and gold labeled DNA was utilized to probe molecular distances (work in preparation). Generally, nucleic acids scatter strongly (the electron contrast density is about a factor of 5 higher than for proteins[17]) and are relatively insensitive to radiation damage, which make them suited for static cell work. In the following, we discuss details of the the sample holder design and then present data collected at the beam line 12-ID at the Advanced Photon Source on horse heart cytochrome *c*, which is a readily available protein that we employ routinely as a molecular weight standard.

In order to allow for dual use in a flow and static cell setup, we have designed a sample cell mount that holds both flow and static cells [see Fig. 1(a)]. The cells [see Fig. 1(b)] are easy to handle, as both kinds of cells simply slide into the mount with angled sides providing accurate positioning without the need for further adjustment. The observation volumes for both cell types are identical, such that once the beam and camera geometry are aligned with respect to the sample holder mount, one can quickly switch between static and flow cell measurements without the need for realignment, which greatly facilitates measurements of many different samples in a short period of time.

In order to minimize parasitic scattering and to maximize the scattering signal from the sample, it is advantageous if the entire beam cross section traverses through sample volume, without intersecting the window or holder edges. Therefore, we designed the cross section of the observation volume (4×2 mm) to be larger than the typical beam dimensions [0.5×0.1 mm^2 at beam line 12-ID of the Advanced Photon Source (APS)]. In this fashion, a correct alignment without scattering of the cell walls is ensured even in the presence of slight uncertainties and drifts in the cell position and beam geometry. We note that for synchrotron sources with less beam stability and/or a larger beam size, it would be very straight forward to change our design to have a larger observation volume cross section. On the contrary, if extremely small sample volumes are the primary concern, one could similarly reduce the size of the observation volume, which sets directly the amount of sample required for static cell work.

The windows of the sample cell are made from 25 μm thick potassium aluminosilicate (muscovite mica) sheets

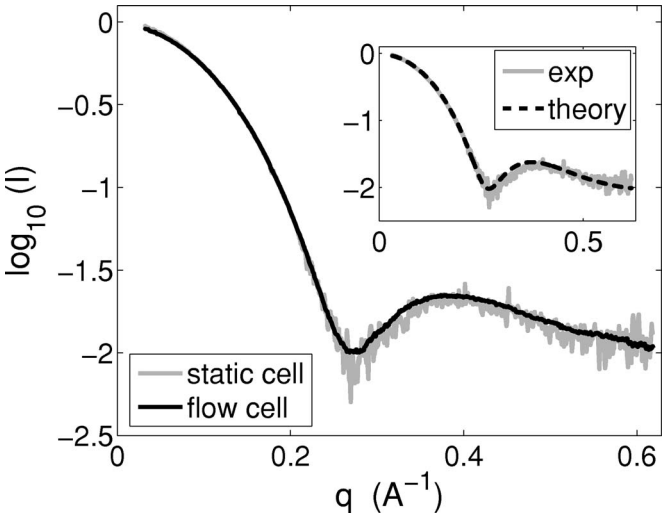

FIG. 2. Scattering profiles of horse heart cytochrome *c*. Average over 5 profiles with 0.1 s exposure time for static cell measurement (red, solid line) and over 40 profiles with 1 s exposure time for a flow cell measurement (black, solid line). Inset: Experimental (static cell) scattering profile (red, solid line) and theoretical profiles obtained from the crystal structure (PDB accession code 1CRC) using the software CRYSOL (black, dashed line). Scattering intensity is shown as a function of q, $q=4\pi\sin(\theta)/\lambda$, where 2θ is the total scattering angle, and λ is the x-ray wavelength.

(Goodfellows) which scatter only very weakly. The mica is glued to the cell with 302-3M epoxy (Epoxy Technology) and provides a reliable seal. We have found that a total of 50 μm of mica introduces negligible attenuation and scattering over a very wide scattering angle. The thickness of the observation volume, i.e., the x-ray path length in solution is 2 mm, which we found to give a strong scattering signal without significant angular smearing: The $d\theta$ introduced by the finite path length is $\lesssim3.5\times10^{-5}$ for a 2 m camera, which is still smaller than the width of the angular bins typically used in our SAXS measurements, which employ ca. 1000 bins. The cells as well as the base plate and cell mount of the cell holder are made from 6061-T6 aluminum, which is inexpensive, highly resistant to corrosion, easy to machine, and ensures adequate thermal coupling of the sample cell to the cell mount. The foot of the cell holder is made from G-10 glass fiber, which provides high strength, high dimensional stability, and thermal insulation of the cell block, which can be thermocontrolled in the temperature range of −25 to +150 °C using a circulating bath (RTE-111, Neslab) connected to the flanges of the holder mount by nylon reinforced silicone tubing.

The static cells are loaded using a Hamilton syringe (Hamilton Company), the required sample volume is about 16 μl. The flow cell is connected to a peristaltic pump (Model Rabbit, Rainin) using biological inert Tygon or silicone peristaltic pump tubing (Fisher Scientific), see Fig. 2. The total volume of the flow cell setup depends on the details of the tubing, typically 0.7–1 ml solution, recirculated at a flow rate of about 1 ml/min. Details of the design and construction drawings are provided in the supplementary material.

Data on horse heart cytochrome *c* were taken on undulator beam line BESSRC CAT 12-ID at the Advanced Photon Source, Argonne, IL, USA, employing a 2 m camera and a x-ray phosphor detector optically coupled to 3×3 mosaic

charge-coupled device (CCD) read out. The data were collected at 25 °C at an x-ray energy of 12 keV, corresponding to an x-ray wavelength of 1 Å. Details of the beam line are essentially as described.[8,10] An improvement to the setup was made by implementing a new beam stop to block the direct x-ray beam. The design is similar to that described in Ref. 10 with a diode in the center of the beam stop for on-line measurement of the transmitted beam intensity. Instead of lead, however, we employ iridium, which has a higher stopping power for x rays. Tantalum, another frequently used metal for shielding, exhibits similar x-ray absorption strength around 12 keV photon energy, however, considering the the entire range of photon energies relevant for ASAXS work, iridium is preferable (see supplementary material). Furthermore, we found that using stepper motor controlled vertical and horizontal slits as in-vacuum guard slits in the beam path directly upstream of the sample, instead of a round pinhole (item 9 in Fig. 1 of Seifert *et al.*[10]), decreased parasitic scattering.

Horse heart cytochrome *c* is a 104 residue, 11.6 kDa protein that was studied by SAXS previously as a function of denaturant concentration[18] and in the presence of imidazole.[19] We employ it routinely as in inexpensive and reliable spectroscopic standard. Cytochrome *c* was purchased from Sigma and used without any further purification. Measurements were taken at a protein concentration of 8 mg/ml in 100 mM acetate buffer, *p*H 4.6, with 0.5*M* guanidinium hydrochloride added. Prior to measurement, the solution was filtered through a 0.22 μm syringe filter (Millipore) and centrifuged at 11 000 g for 10 min in a tabletop centrifuge (Eppendorf). Two sets of data were recorded: One using the static cell setup and one employing the flow cell, with otherwise identical settings and samples. In the case of the static cell experiment, 16 μl protein solution was loaded and 5 exposures of 0.1 s each taken. For the flow cell measurement, 1 ml of protein solution was recirculated at a flow rate of ca. 0.02 ml/s and 40 exposures of 1 s each were collected. Data were image corrected and circularly averaged, the 5 and 40, respectively, profiles for each condition were averaged to improve signal quality. Appropriate buffer profiles were collected using identical procedures and subtracted for background correction.

Figure 2 shows scattering profiles obtained from static cell exposures (gray, solid lines) and from flow cell measurements (black, solid line). The profiles are superimposable, however, the difference in signal to noise is apparent. We determine the radius if gyration (R_g) by Guinier analysis[20] and find it to be 13.7±0.3 Å, in good agreement with the previously measured native radius of gyration[18] of 13.8±0.3 Å and the theoretically predicted R_g of 14.0 Å (see below). As a further test, we use the known crystal structure of cytochrome *c* [protein data bank (PDB) accession code 1CRC (Ref. 21)] and the program CRYSOL by Svergun *et al.*[22] to compute a theoretical scattering profile and radius of gyration. The theoretically predicted scattering profile (black, dashed line) is shown together with the experimental static cell profile in the inset of Fig. 2. There is good agreement between the experimental and calculated scattering profiles.

In conclusion, we have described a new SAXS sample holder design that allows high signal-to-noise measurements on biological and other samples in solution. The setup can be used for static cell experiments with sample volumes as low as 16 μl, as well as for flow cell measurements where the sample volume is continuously exchanged without the need to change the beam or detector geometry. The design has been used successfully in several studies on peptides,[11–13] membrane proteins,[15] and nucleic acids.[16] The design drawings are provided in the supplementary material and may be used freely, in which case the authors kindly request citation of this work.

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