Chapter 4

Microfluidics for Small-Angle X-ray Scattering

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Abstract

Small-angle X-ray scattering is a well-established biophysical technique, whilst microfluidics is proving to be a convenient technology for creating miniaturised multifunctional devices. Both fields are highly versatile and find use in multiple scientific disciplines. Together, they offer the potential to obtain structural information on biomacromolecules, nanoparticles and condensed matter, in a high-throughput manner and with enhanced time-resolution capabilities. This chapter provides practical design considerations for X-ray-based microfluidic systems and examines some of the existing microfluidic platforms used in conjunction with small-angle X-ray scattering. As the exclusive advantages of microfluidics become recognised and accessible, the prevalence of microfluidic sample environments in X-ray scattering measurements will hopefully increase.

Keywords: microfluidics, high throughput, time-resolved SAXS, continuous flow, laminar flow, hydrodynamic focusing, turbulent mixing, structural biology

1. Introduction

1.1. Principles and potential of microfluidics

The interdisciplinary field of microfluidics encompasses the science and technology underlying the development of devices that process and manipulate small volumes of fluids within micron-scale channels. A microfluidic 'chip', so-called because its fabrication method was adapted from that used to manufacture computer microchips [1], can be designed with interconnected networks of channels and chambers. These designs can integrate a range of

functions on a single, microsized 'lab-on-a-chip' device, also known as a 'micro total analysis system' (μTAS). Along with the ready possibility of automation and potential for high-throughput screening that microfluidics presents, other general benefits of scaling down include lower sample consumption and consequently lower cost. In addition, microfluidic devices offer precise control over fluid flow and mixing, shorter processing times and hence more rapid results. Accordingly, the technology of microfluidics is influencing many areas of science, from materials to microbiology.

1.1.1. Physics of fluid flow on the microscale

In contrast to fluids moving in large channels, which mix turbulently, a key property of fluid flow in microchannels is that it is laminar. Laminar flow occurs in smooth parallel streams with no significant mixing between streams other than by diffusion [2]. This is a result of the ratio of inertial to viscous forces, described by the Reynolds number (*Re*), which for small channel dimensions is low, meaning that viscous forces dominate. The Reynolds number is defined by

$$Re = \frac{\rho v w}{\mu} \tag{1}$$

where ρ is the fluid density, v is the flow velocity, w is the characteristic dimension of the flow geometry and μ is the fluid viscosity [3]. A *Re* of <1–100 corresponds to pure laminar flow, whilst a Re of >1000–2000 is approaching turbulent flow. The low Reynolds number flow property of microchannels can be exploited, but is not ideally suited to all applications.

1.2. The combination of microfluidics and small-angle X-ray scattering

Small-angle X-ray scattering (SAXS) is a valuable and versatile technique. SAXS is utilised by biochemists and material physicists alike, providing both quantitative and qualitative structural information about biomacromolecules, nanoparticles and condensed matter [4–13]. In structural biology, although SAXS is unable to afford atomic resolution, it serves as a complementary technique to X-ray crystallography and nuclear magnetic resonance (NMR) and compensates for limitations of these techniques, by enabling structural parameters to be determined in biologically relevant solutions, without the size restriction typically imposed by NMR and with no requirement for extrinsic biomolecule labelling or crystals. Further, SAXS allows the characterisation of conformational changes, dynamics and interactions of biomolecules in response to different experimental conditions, which makes it ideally suited to monitoring important biochemical events, such as protein folding, ligand binding and association/dissociation reactions.

1.2.1. Why incorporate microfluidics into SAXS?

SAXS already involves the manipulation of relatively small volumes of fluid; however, the typical solution sample environment for SAXS is based on thin-walled quartz capillaries. These offer a convenient method to acquire SAXS data on solutions, but they do have a number of

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restrictions. Microfluidic devices offer the opportunity to develop customised systems with the flexibility to enable advanced sample handling, such as concentrating, diluting, mixing and filtering, in line with the SAXS measurement. Unsurprisingly, given its broadly applicable advantages, microfluidics has been incorporated into several other techniques in assorted scientific fields and within structural biology, including for screening of protein crystallisation conditions, for a closely related technique, X-ray crystallography [14], and with few perceivable disadvantages, the question really becomes, why not SAXS? Indeed, several groups have already recognised the potential benefits and taken advantage of microfluidics, successfully demonstrating its use with SAXS for studying a diverse variety of specialised systems [12], including the assembly of biomacromolecules such as intermediate filaments [15] and silk fibres [16]; the growth of gold nanoparticles [17]; the rheology of complex fluids, such as liquid crystals [18, 19] and wormlike micelles [20]; as well as numerous studies on protein [21–29] and RNA folding [30, 31]. Perhaps the most obvious, more general advantage that microfluidics can offer SAXS, as it has offered many other techniques, is the potential to provide automated, high-throughput platforms which minimise sample consumption and shorten measurement times whilst maintaining a high level of accuracy and reproducibility, and researchers have begun to lay the groundwork for devices of this type [32–34].

1.2.2. What makes microfluidics particularly well-suited to SAXS?

Several fundamental aspects of these technologies are exceptionally compatible. First, the size of the X-ray beam relative to the size of the channels within a microfluidic chip is quite comparable. Thus beam is not being used to measure extraneous material, and conversely, all of the sample molecules are in the beam interaction volume. Therefore the sample is providing the optimal amount of signal, without being wasted. Second, the continuous flow typical within these systems mitigates radiation damage to samples, improving the reliability and data quality obtainable from SAXS measurements. Moreover, the high-throughput potential mentioned above is especially important for synchrotron-based techniques such as SAXS, where the experiment time is often limited, as a microfluidic sample-handling platform could help to ensure maximum use of the allocated beam time [33, 35]. This is what prompted Lafleur et al. and, very recently, Schwemmer et al., to develop their respective microfluidic high-throughput sample-handling environments [33, 34], which will be discussed. Microfluidics also offers the opportunity to extend the time-resolution capability of SAXS. Various groups have demonstrated the possibility to study reaction kinetics of protein and RNA folding events or complex fluids, using alternative microfluidic approaches to prevail over commercially available stopped-flow devices which fall short of the requisite microsecond time range, and this will be covered in the latter part of this chapter.

2. Incorporating microfluidics into SAXS

In the first part of this section, some general design considerations for microfluidic systems in the context of SAXS will be discussed. In subsequent sections, existing examples of microfluidic chips and how they have been applied using SAXS in order to increase either the throughput

or the time resolution will be examined. As well as providing some background and giving an overview of the current status of the field, it is hoped that this chapter will contain practical information for those looking to realise the benefits of incorporating microfluidic technology into SAXS.

2.1. Design considerations for SAXS microfluidic systems

Whilst the specifications of the system will differ depending on the particular application, some generally applicable guidelines are given here, supplemented with specific examples. As for any analytical microfluidic setup, there are three main aspects of the system which need to be considered: (i) the design and fabrication of the microfluidic chip itself, (ii) the sample delivery system and method of fluid control and (iii) integration with the experimental apparatus, in this case, the SAXS beamline (although the possibility of an on-chip X-ray source has been envisaged [36]). Each aspect will be discussed separately here whilst bearing in mind that these aspects are all interrelated and will have certain SAXS-specific requirements that differ from other light-based detection methods.

2.1.1. Chip design and fabrication

The first key consideration regarding the microfluidic chip is the choice of material, the properties of which, in this case, must be compatible with X-rays. The possibilities will be further narrowed down by practical considerations specific to the particular application, e.g. if high flow rates are required for mixing or time-resolution purposes, the chip material and bonding must be robust enough to withstand the subsequent high pressure generated, without deformation of the microchannels [20, 27]. Cost, both in terms of fabrication time and resources, will also likely factor into material choice, and the type of material will dictate the method of fabrication, which may be limited by available facilities. Microfabrication does require some special equipment and sometimes a clean room, commonplace in a nanofabrication facility, but not found in the average biochemistry lab. If access to the necessary facilities is not available, there are several microfluidic manufacturing companies offering custom microfabrication services.

Microfluidic chips were originally manufactured from silicon using standard photolithography and often bonded to glass, which is not an ideal window material for SAXS [36]. The first microfluidic device used in conjunction with SAXS comprised a silicon chip and silicon nitride windows [21], but in more recent times, silicon has been exchanged for synthetic polymer materials, such as thermoplastics, e.g. polymethyl methacrylate (PMMA), or the silicone elastomer, polydimethylsiloxane (PDMS). The latter is cast from a replication master mould, which can be fabricated using similar lithographic techniques, whilst the former can be fabricated by a range of micromachining methods (milling, electric discharge, laser ablation) and templating techniques (thermoforming, transfer moulding). Both options are cheaper than silicon chips and highly flexible in terms of the possible channel geometries.

Unfortunately, there are two major limitations of PDMS for microfluidic SAXS devices: its low X-ray transparency [36] and its low X-ray endurance—continuous X-ray exposure rapidly

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deteriorates this material [20]. Nevertheless, PDMS can be utilised for channel structures, as long as appropriate X-ray compatible material is chosen for sample observation windows, as Dootz et al. demonstrated [19]. As another example, Stehle et al. used a PDMS chip for a microfluidic droplet-forming module, separate from the thin-walled glass capillary used for SAXS detection [12]. The large dead volumes that are introduced *via* the connecting tubing in this system are the disadvantage of this approach. PDMS also finds use in other constituents of a microfluidic system, as its elasticity and adhesive properties render it useful for valves and seals [33].

Kapton (polyimide) has commonly been used for X-ray-related applications due to its high X-ray resistance and low X-ray absorption [19]. However, in a qualitative assessment of the suitability of various polymer materials for SAXS microfluidic chips by Toft et al., the SAXS profile obtained from a 100 μm thick Kapton foil revealed a diffuse diffraction peak that occurs at in the middle of biologically relevant q-ranges [32]. Given that biological scattering is relatively weak, this feature was suggested to perhaps cause issues with background subtraction [32]. In spite of this, Barrett et al. have fabricated a microfluidic device out of two 150 μm thick Kapton films bonded together, using laser ablation techniques to produce the channels, whilst Graceffa et al. used Kapton windows for their stainless steel turbulent-flow mixing chip [27], as did Dootz et al. for their PDMS chip [19].

Greaves and Manz deemed polycarbonate (PC) to be the best chip-building material in terms of X-ray transmission, with PMMA a close second [36]; however, PC and PMMA were rated poorly by Toft et al., in terms of X-ray endurance and cleaning, and PC was also judged as poor for bonding and machinability, although both materials display low small-angle X-ray scattering [32]. Ultimately, polystyrene (PS) was the polymer of choice for Toft et al., meeting all the criteria for their 'BioXTAS' chip [32, 33], whilst Lafleur et al. selected PMMA for a mixing attachment containing rotary valves by virtue of its superior machinability [33]. Combinations of different plastics have also been demonstrated successfully by Møller et al., who used a PMMA substrate with PS windows, developing a strong PS to PMMA bonding procedure for this design [37].

A second, crucial consideration relating to SAXS chip design concerns the size of the sample detection compartments versus the size of the X-ray beam. For maximum signal, the depth of the sample compartment should be as close to the optimal pathlength as practically possible. The scattering volume and the beam energy both factor into determining the optimum pathlength. Conversely, using smaller compartments results in lower sample consumption. A finite constraint is that the dimensions of the beam must be smaller than the dimensions of the channel or chamber to minimise background intensity resulting from X-ray interaction with the substrate wall, and the X-ray focusing or slitting down ability and resultant flux of individual beamlines will set a lower limit on these dimensions. It is important to note that the beam dimension under consideration is the total beam width, as opposed to the commonly defined full width at half maximum, as the weak outer beam background will still cause parasitic scattering from the chip which will affect data quality. Thus the design must be optimised to the expected beam dimensions and balance signal requirements against sample consumption.

2.1.2. Sample input and fluidic control

For continuous flow systems, fluid delivery *via* pumps would ideally be controlled remotely by software, so as to facilitate fast sample turnover time and minimise the need to access the beamline hutch, thereby increasing the efficiency of the experimental procedure. This is essential for time-resolved measurements, where data acquisition must be initiated immediately after sample mixing. Toft et al. developed software to couple control of commercial syringe pumps delivering samples and buffer to the chip with the beamline shutter system, thereby coordinating sample preparation and data collection [32], whilst Lafleur et al. developed both the software and hardware, including the individually controlled syringe pumps used in conjunction with integrated rotary valves, for their next-generation setup [33]. For stopped-flow systems, sample introduction to the chip and any on-chip processes such as mixing, diluting could be done prior to chip introduction to the beamline, provided that time-resolved data are not required. Alternatively, Schwemmer et al. eliminate the need for pumps and tubing altogether (along with any associated plumbing problems) by depositing samples directly into reservoirs on their 'LabDisk' using regular pipettes. This keeps all fluids confined entirely to the chip and relies on centrifugal forces to transport fluids through the mixing channels, though this does require a custom-built processing device to spin the disk at preset rotational frequencies [34].

2.1.3. Interfacing to a SAXS beamline

Once the chip is fabricated and fluidic control is established, the final considerations to complete the microfluidic SAXS experimental setup and ensure optimal ease of use involve appropriate interfacing to the beamline. This may require some adjustments to the conventional beamline setup to accommodate the microfluidic system. At a minimum, a chip holder, which allows for any necessary inlet and outlet tubing connecting to the fluid delivery system, is needed for stable attachment to an adjustable stage (ideally motor-controlled) to facilitate precise alignment with the X-ray beam. More advanced chip holder designs have included a borescope or camera for sample visualisation and to assist with chip positioning [34, 37]. For the best-quality data, the beamline setup should ensure a vacuum along the complete X-ray flight path, from synchrotron to microfluidic sample chip to detector to avoid air scatter, and contain a minimum of extra window components to reduce background intensity [34]. Temperature control is another important consideration, particularly for unstable biological samples, and has been achieved *via* a channel in the chip holder through which a cooled solution can be passed [37].

2.2. Microfluidics for high-throughput SAXS sample handling

Prior to the development of sample-changing robots at synchrotron SAXS beamlines, loading samples by hand was a tedious and time-consuming process, especially with the necessary sample cell cleaning and drying steps between each sample [35]. This motivated Toft et al. to develop a microfluidic front end, the BioXTAS chip, along with software for external control of the syringe pumps regulating fluid flow through the chip [32]. At the time, this was a considerable improvement upon manual sample handling, with the added benefit of elimi-

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nating the inherent human error associated with sample preparation, such as that of a dilution series, which is generally still a manual task, at present. The first-generation BioXTAS chip (**Figure 1A**) contained four fluidic inlets, providing the option to utilise up to three different solutions that are premixed prior to merging with the sample solution, an extended serpentine mixing channel in which homogenous mixing of sample and buffer solutions occurs *via* diffusion, with the mixing ratio determined by the flow rate, and a 200 nl sample chamber, where the sample is exposed to the X-ray beam and scattering data is collected whilst flow is stopped [32]. In this setup, samples are stored in syringes connected to the chip by a 30 cm length of tubing which requires 150 μl of solution to fill, and following a 15 min pressure-equilibration period, 6 min is required to fill the sample chamber, at a flow rate of 6 μl/min [32]. Consequently, whilst only 36 μl of sample is consumed per measurement, and reasonable quality data has been shown from a mere 200 nl sample volume (of 1–10 mg/ml protein), unfortunately this sample economy is compromised by the dead volume contained in the syringe and tubing, as well as by the pressure-equilibration time. Although the stopped flow during data collection strategy contributes to low sample consumption, it does increase the susceptibility of the sample to radiation damage, particularly with long exposure times. Nevertheless, this BioXTAS chip represents a respectable first proof of concept of a microfluidic alternative for SAXS sample handling and has subsequently been utilised for a study on the oligomeric state of cancer-related protein, as further demonstration of its practicality and promise [37].

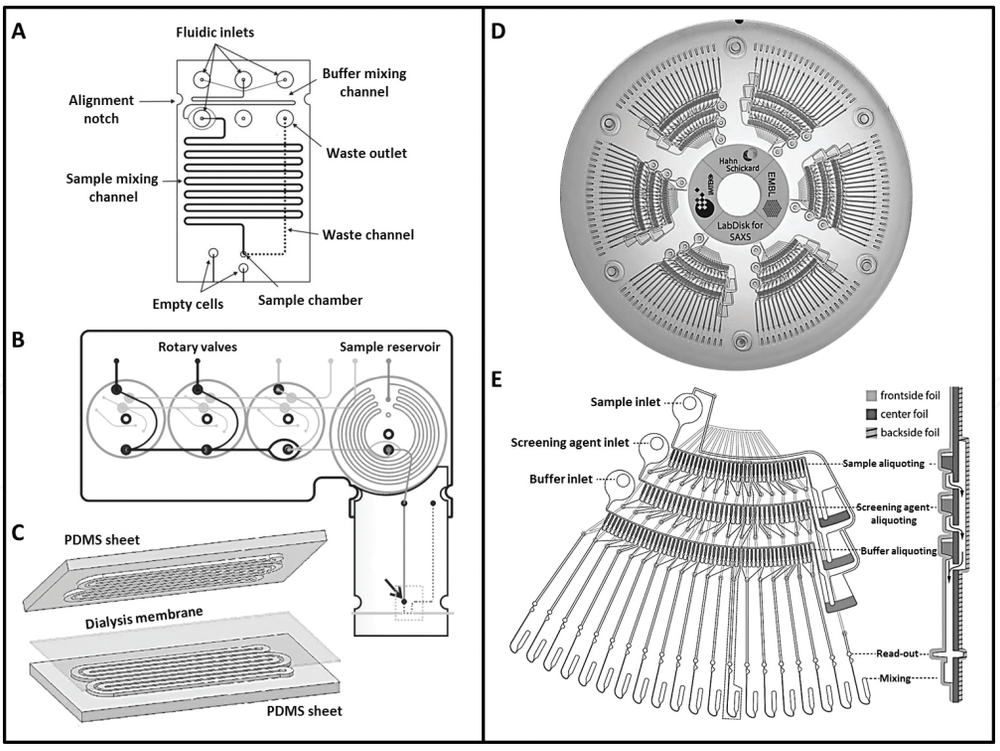

Figure 1. Microfluidic answers to the demand for automated, high-throughput, low consumption sample-handling systems for SAXS. (A–C) The BioXTAS chip by Toft et al. [32] and additions by Lafleur et al. [33] and Skou et al. (2014) [38]. (D and E) The LabDisk for SAXS by Schwemmer et al. [34]. See text for details.

The first-generation BioXTAS chip was subsequently developed to incorporate an additional mixing module (**Figure 1B**), which contains an on-chip sample reservoir and motor-controlled rotary valves to enable more precise fluidic control, in addition to integrated UV absorbance detection capabilities by means of optical fibres built into the separate sample detection chip, enabling UV absorbance measurements to be acquired concurrently with scattering data [33]. The option for data acquisition in continuous flow mode, which would reduce radiation damage, albeit at the expense of sample consumption, is made available in the custom-developed control software [33]. With much shorter exposure times on a more powerful X-ray source and a faster flow rate, the complete experimental cycle time (including cleaning) was considerably reduced from 28 to 3 min whilst consuming slightly less sample than for the first-generation chip [32, 33]. The on-chip sample reservoir does enable a reduction in the dead volume of the system, although external syringe pumps and associated tubing are still required to supply the buffers and pressure on the protein sample, so in order to take full advantage of the on-chip reservoir to minimise the sample volume, the suggestion is made to use an immiscible fluid to supply the necessary volume in the tubing syringe for injecting the sample [33].

Related to this idea of introducing a second fluid phase is the droplet-based microfluidic approach, in which discrete droplets of one liquid are generated, dispersed in and carried by a second, immiscible fluid within a microchannel [12]. These microdroplets can be used to compartmentalise samples, which can then be manipulated as individual vessels: for sorting, splitting, merging and even for performing chemical or biological assays [12]. The usefulness of this approach in association with SAXS was demonstrated by Stehle et al., who used it to analyse gold nanoparticles encapsulated by water droplets dispersed in oil, with each SAXS measurement comprising data averaged from ~ 9000 droplets, over a 15 min period [12]. In view of the available sample-changing robots, which have similar sample requirements and low dead volumes and perform all of the necessary cleaning in a fully automated fashion, distinct advantages of microfluidic devices for SAXS may have thus far been unclear, whereas discrete droplet formation is an example of an exclusive capability of microfluidic approaches which highlights the versatile possibilities created by this technology and which the standard SAXS setups lack. Another example is provided by Skou et al., who adjusted the second-generation BioXTAS design to incorporate a sample dialysis chip, comprising two PDMS sheets: one which contains channels for a polyethylene glycol (PEG) solution and the other which contains channels for the protein sample solution and a dialysis membrane sandwiched in-between (**Figure 1C**) [38]. When utilised with SAXS, this additional microfluidic module offers the unique ability to gradually concentrate protein samples as structural information is continually acquired, to reveal changes in protein oligomeric state and detect concentration-induced protein aggregation early in the experiment. Alternatively, if a different buffer is used instead of a PEG solution, the device can be used to monitor the effect of different experimental conditions, such as a change in ionic strength or pH, during buffer exchange [38]. Further advanced sample-handling features envisaged for microfluidic SAXS include on-chip size exclusion chromatography [32].

Although the BioXTAS chip readily facilitates mixing of multiple solutions with small volumes of a single sample and prepares dilutions with ease (simply by varying the flow rate), in order to properly meet the high-throughput objective, the BioXTAS system would require further development to increase its sample capacity or the means to enable easy and rapid sample changing, to bring it up to par with current autosampler robots. Schwemmer et al. presented an innovative centrifugal microfluidic platform, 'LabDisk for SAXS', which overcomes some of the shortcomings of the BioXTAS system by having six sample modules on a single chip (**Figure 1D**), each containing on-chip reservoirs for sample, buffer and screening agents and requiring only 2.5–3.5 μl of solution, with dead volumes of just over 1 μl, which is the allowance for pipetting errors and guaranteeing complete filling of channels and chambers [34]. The sophisticated fluidic geometry (**Figure 1E**) enables aliquoting and mixing of fixed combinations of protein, buffer and screening agents, simultaneously generating 15 different sample conditions and the five corresponding buffer solutions for background subtraction, with a high degree of accuracy and precision, in under 5 min [34]. Short (20 × 50 ms) exposures make for fast readouts, with the experimental time currently limited by manual positioning of the measurement chambers in the X-ray beam, although this is expected to be automated in due course [34]. Higher noise in the data collected on the LabDisk than the conventional sample changer is attributed to factors relating to the air gap in which the LabDisk is operated, which results in higher background, as well as the shorter pathlength of the measurement chamber and smaller X-ray beam, which result in lower signal [34]. Owing to the relatively low signal of biomolecules, composed of light, weakly scattering atoms, the background subtraction, which accounts for both the solvent and the instrumentation, needs to be carried out as precisely as practically possible. As such, the use of the same sample compartment for both sample and background measurements is considered important, to avoid introducing anomalies due to subtle differences in sample cell [35]. However, the LabDisk has separate readout chambers for samples and buffers, which has potential to cause issues with background subtraction.

2.3. Microfluidics for time-resolved SAXS

The suitability of microfluidic devices for time-resolved studies was recognised early on in the rise of microfluidics, and it has played a key role in increasing the time resolution attainable by SAXS. Whilst stopped-flow techniques have been extensively used for time-resolved SAXS studies, predominantly of protein folding [39–45], since Moody et al. reported the first device used in conjunction with SAXS in 1980 [46] and stopped-flow apparatus specifically for SAXS are commercially available [13], these have only been able to achieve dead times of 0.5 ms at best. Microfluidics offers the potential to extend the accessible time resolution of SAXS to the microsecond time range; however, rapid mixing, which is a crucial requirement, is the challenge, due to the low Reynolds number flow in microchannels.

The two main microfluidic approaches are both continuous flow-based techniques but differ in their mixing strategy; the turbulent-flow approach aims to generate high Reynolds number flow in microchannels, whilst the laminar-flow approach utilises the inherent low Reynolds number flow. The common underlying principle is that data collected at different distance

points along an observation channel after mixing correspond to different time points over the course of the reaction, with the flow velocity determining the temporal resolution. This approach has been coupled with various techniques, such as circular dichroism and fluorescence spectroscopy [29], but with SAXS, the radius of gyration represents an easily determined parameter which can be monitored, whilst the data also provide low-resolution structural information, making it useful for monitoring structural changes such as folding, oligomerization and ligand-induced rearrangements. As previously mentioned, continuous flow offers the SAXS-specific advantage of lower radiation damage but disadvantage of higher sample consumption, particularly at the high flow rates required for the turbulent mixing approach.

Rapid turbulent mixing in a multicapillary device was pioneered by Regenfuss et al. [47], who credits Moskowitz and Bowman [48], and this idea has since been developed by several groups, with various devices emerging [49, 50], including microfabricated versions of the original capillary-based mixer [51, 52]. The 'T-mixer' produced by Takahashi et al. [51] (**Figure 2A**) was subsequently utilised with SAXS to study the folding dynamics of various proteins [23–26, 31], achieving dead times as low as 160 μs [23]. The T-shaped geometry of this microfluidic device forces two solutions flowing at high velocities to meet at a junction and change direction, resulting in rapid mixing, before being transported through to the observation channel, where measurements are obtained [51]. Bilsel et al. advanced this design by altering the angle of the input channels such that they form an arrow-shaped junction (**Figure 2A** inset), which forces the two fluids to undergo a greater change in momentum, theoretically giving rise to more efficient mixing [52]. Using a device of this type, with channel widths of 75 μm, at a flow rate of 10 ml/min, the Reynolds number is calculated to be 2000, and the mixing time was demonstrated to be ~50 μs [52]. For time-resolved SAXS studies of protein folding, the dimensions of the observation channel were increased to 100 μm wide by 400 μm deep to increase the signal to noise, whilst the width of the input channels and mixing region was 30 μm, resulting in a dead time of ~100 μs at flow rates of 10–20 ml/min [27] (**Figure 2B**). Whilst these microfluidic setups are impressive in terms of the time resolution they can achieve, their major drawback is the large sample quantities necessitated by the high flow rates.

The laminar-flow-based rapid-mixing approach, introduced by Knight et al. in 1998, is referred to as 'hydrodynamic focusing' [53]. This approach relies solely on diffusive mixing, without introducing turbulence, taking advantage of the natural mixing process in microfluidic chips that function at low Reynolds numbers. For the hydrodynamic focusing geometry, two input fluids approach a central input fluid from either side, squeezing the central fluid into a thin, focused stream, across which diffusion occurs on a relatively short timescale [21, 53]. SAXS investigations of protein and RNA folding have subsequently been carried out using this type of device [21, 22, 30], achieving a minimum dead time of 240 μs [22]. This is a significant improvement on stopped-flow devices, but has not been improved further to match turbulent-flow mixers, at least, not in combination with SAXS. The hydrodynamic focusing approach for time-resolved SAXS is substantially limited by the size of the X-ray beam, as in order to access shorter timescales, the focused stream must be made even narrower; however, without the matching ability to adequately focus the X-ray beam, the system will suffer from low signal to noise. To complicate matters further, even if the beamline optics allow adequate microfocus-

sing, radiation damage to the slowly flowing sample becomes a particularly severe issue. Hydrodynamic focusing has been used with alternative detection methods [54], which have been able to accomplish mixing times as short as 10 μs [55], demonstrating the potential of this microfluidic approach for time-resolved measurements and providing incentive to work towards optimising optical designs of future SAXS beamline setups to overcome current technical barriers and take full advantage of it.

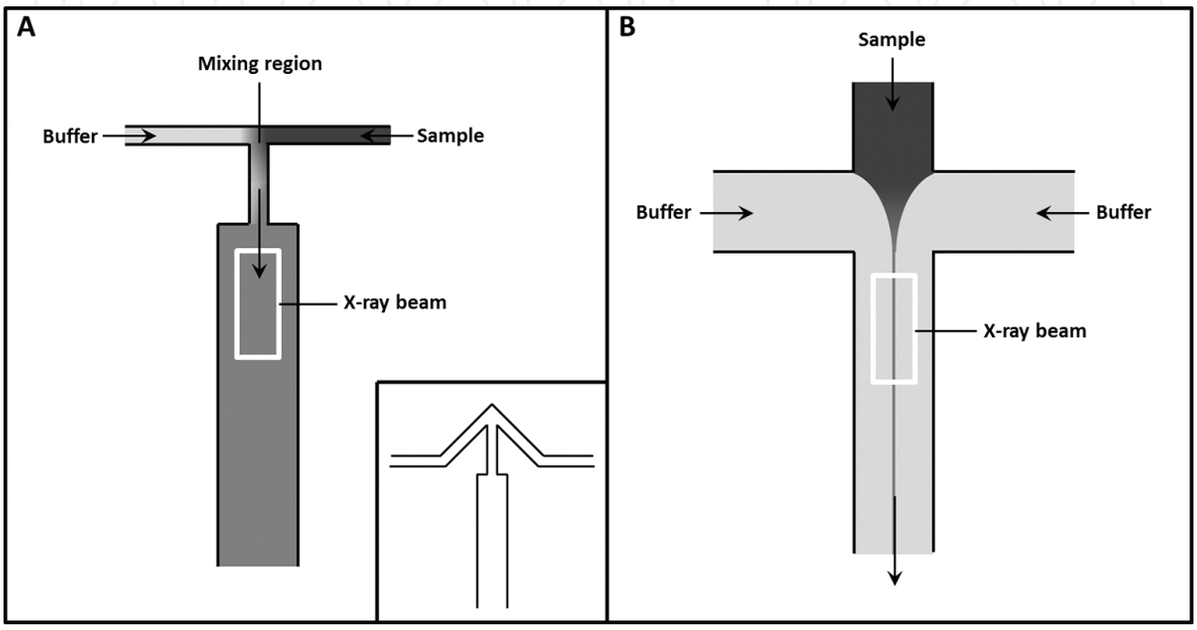

Figure 2. Two alternative rapid-mixing approaches in microfluidic channels for time-resolved SAXS. (A) Turbulent-flow mixing using the T-mixer [23] and arrow-shaped geometry [27] (inset). (B) Laminar-flow hydrodynamic focusing [21].

3. Conclusions and outlook

Over the last two to three decades, since microfluidics arose, various groups have recognised the advantage offered by this miniaturised technology and have successfully demonstrated its incorporation with SAXS. In particular, its high-throughput sample-handling potential and time-resolution–enhancing abilities have begun to be exploited. However this is still an emerging field, and microfluidic technology continues to push the boundaries and open up new possibilities, some likely yet to be conceived. With the current technology, 100 μs time resolution for SAXS studies on protein folding has been achieved. Although the turbulent- and laminar-flow mixers discussed above have been the most popular microfluidic approaches thus far, others have been looking to develop new mixing strategies, which could reduce dead times further, whilst the development of smaller, more highly focussed X-ray beams will be key to accessing faster timescales.

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