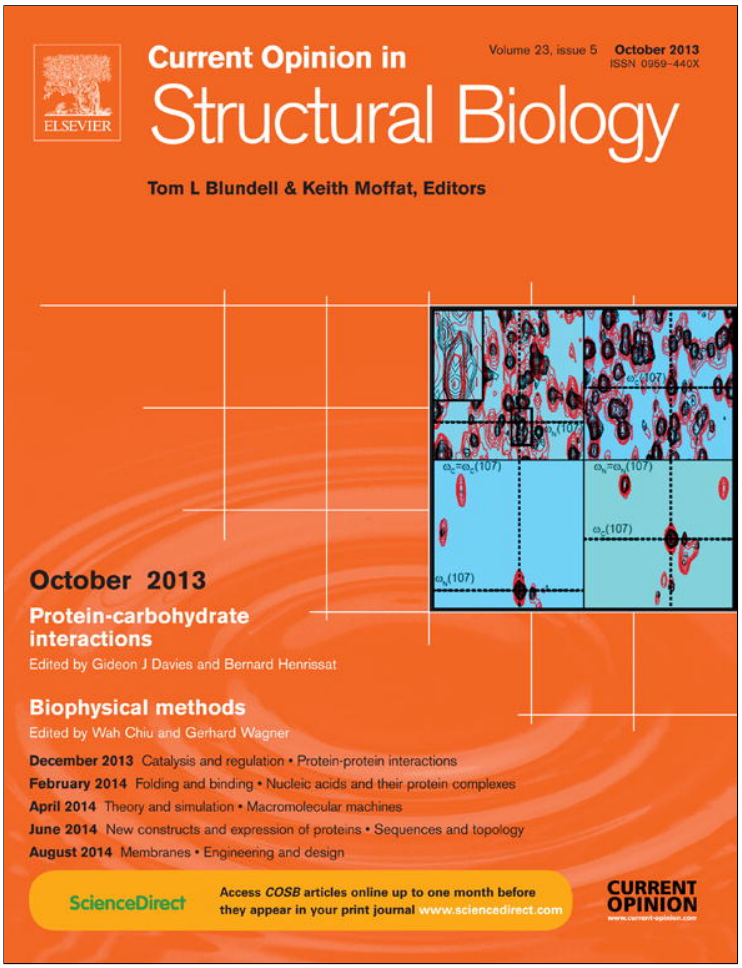

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Current Opinion in Structural Biology

Impact and progress in small and wide angle X-ray scattering (SAXS and WAXS)

Melissa A Graewert and Dmitri I Svergun

The advances made in small and wide angle X-ray scattering over the past decades have had a large impact on structural biology. Many new insights into challenging biological probes including large and transient complexes, flexible macromolecules as well as other exciting objects of various sizes were gained with this low resolution technique. Here, we review the recent developments in the experimental setups and in software for data collection and analysis, specifically for hybrid approaches. These progresses have allowed scientists to address a number of intriguing questions which could not be answered with other structural methods alone.

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Current Opinion in Structural Biology 2013, **23**:748–754

This review comes from a themed issue on **Biophysical methods**

Edited by **Wah Chiu** and **Gerhard Wagner**

For a complete overview see the Issue and the Editorial

Available online 5th July 2013

http://dx.doi.org/10.1016/j.sbi.2013.06.007

Introduction

The progress in structural studies with small-angle X-ray scattering (SAXS) of biological macromolecules in solution is best reflected in the substantial increase of publications based on this technique over the last years (Figure 1a). This gain in popularity can easily be explained by the synergistic improvement in hardware as well as software resulting in an automated data collection and in-depth analysis of the scattering data [1–3]. Thus, many structural biologists profit by adding SAXS to their tool box which includes other techniques such as macromolecular X-ray crystallography (MX), Nuclear magnetic resonance spectroscopy (NMR) and electron microscopy (EM). Especially the ability to apply SAXS (and more recently also wide angle scattering, WAXS) to challenging biological questions including, for example flexible macromolecules and complexes, weakly interacting systems as well as dynamic processes, makes this method a powerful tool. SAXS elegantly complements the high resolution probes that often reach their limitations in respect to these problematic samples. Here, we highlight the progress in SAXS and WAXS over the last two years and the impact these developments have had in gaining exciting biological insights. In advance, we deeply regret that space limitations prevent us from referring to all of the excellent work performed in this field.

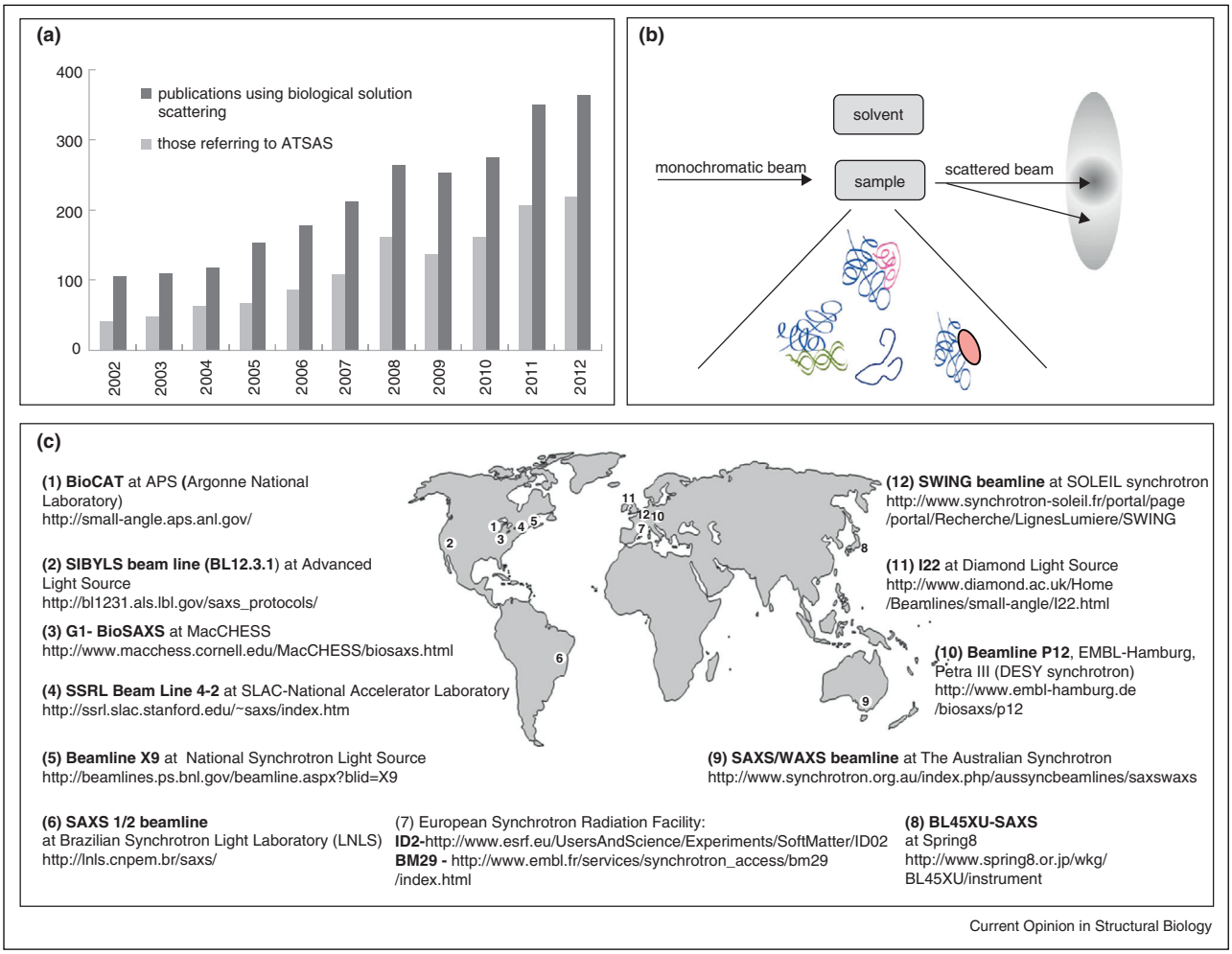
Progress in data collection

In a SAXS experiment, elastically scattered waves are recorded that arise from an X-ray beam impinging on electrons in the sample (Figure 1b, Box 1). As there are hardly any restrictions concerning the sample composition a large variety of conditions for macromolecules in solution can easily be tested including ligand binding [4] or changes of the environment [5].

The rapidly growing demand for SAXS by the structural biology community has fuelled the development of beamlines suitable for and dedicated to biological scattering experiments in solution at almost every major synchrotron facility (Figure 1c). The high brilliance synchrotron sources enable data collection from relatively small sample amounts (a few μl) within a few seconds. The progress in the development of third generation synchrotrons was accompanied by advances in X-ray detectors, and solution SAXS/WAXS was among the X-ray techniques profiting mostly from single-photon-counting pixel detectors such as PILATUS [6]. The future will show how X-ray scattering experiments will benefit from the next-generation pixel detectors (EIGER) with even smaller pixel sizes, higher frame rates and negligible readout dead-times [7]. In addition, data collection has been improved with introduction of automated sample changers that do not only maximize the use of allocated beamtime, but also minimize human errors [8,9]. Pipelines are available for on-line data handling, allowing for high-throughput experiments [10,11]. Here, a number of data analysis programs run in the background during data collection ensuring that users promptly receive a first summary on data quality and even shape models [8]. Future progress in this area is expected to include cross linking the experimental data with information submitted during beamtime application processes, similar to the MX developments [12].

Nevertheless, the biological SAXS beamlines profit tremendously from the creative and intuitive ideas of the user community and, thus, most sample environments have remained flexible in terms of configurable hardware and software, to accommodate the special request of individual projects [8,9]. This has made a number of studies possible using microfluidic devices [13•], set-ups for time resolved

Figure 1



Impact of the developments in SAXS and WAXS. **(a)** Substantial increase in the number of publications using this technique over the last decade. Thereby, about 60% of publications have profited from the use of the ATSAS package for data analysis. **(b)** Schematic drawing of a typical SAXS/WAXS measurement indicating the challenging probes that can be sampled with this method including flexible proteins, (weak) protein–protein interactions, mixed complexes, as well as ligand induced conformational changes. To obtain the intensity profile solely arising from the investigated macromolecules a background subtraction is performed with an independent measurement of the buffer in the same measuring cell. **(c)** World map of SAXS beamlines capable of and routinely used for biological scattering experiments in solution. Note that there are numerous multipurpose SAXS beamlines at virtually all synchrotrons, but not all of them have sufficiently low background and high stability to reliably perform solution experiments on weakly scattering biological samples. This map reflects only the beamlines either dedicated or actively employed for biological solution SAXS.

measurements [14–16] and for cryo-frozen SAXS specimens [17].

Advanced in data analysis methods

Naturally, the advances in data collection have been accompanied by important progress in analysis methods for data interpretation and many algorithmic developments have been initialized (Table 1). The ATSAS suite, available for more than a decade from the Hamburg BioSAXS group, is still the mostly used (Figure 1a, [18••]).

Among the software developments over the last years, one may mention firstly, hybrid approaches accounting for restraints derived from complementary methods; secondly, alternative algorithms for calculation of theoretical SAXS curves at wider angles (Box 2) and thirdly, analysis of the experimental scattering patterns in terms of ensembles.

One of the biggest strengths of SAXS lies in its complementary nature to high resolution methods and thus, its contribution to a greater understanding of the biological system as a whole [19]. Thus, much effort has been put into the further development of tools that allow the integration of various complementary methods especially of MX, EM and NMR [20••]. For this, modeling and visualization programs [21,22]

Box 1 Information revealed at small angles.

Due to radial symmetry, the scattering intensities can be transformed into a function of the momentum transfer $I(q)$, with $q = 4\pi \sin\theta/\lambda$ (θ = half the scattering angle, λ = X-ray wavelength). It is generally not possible to directly derive a 3D structure from this 1D curve that is unique, as a number of models may be compatible with the derived scattering profile. However, an impressive amount of useful information about the macromolecular structure can be derived from the $I(q)$ profile. This includes overall parameters like the molecular mass and the radius of gyration, extracted from a classical Guinier approximation on the low q range. The mid q range can be used to determine the compaction state of the molecule, by analyzing Kratky plots, $q^2I(q)$ versus q. On top of that and most importantly, low resolution particle shapes can be reconstructed ab initio, quaternary structure and oligomeric state can be modeled, and flexibility can be assessed.

have been extended with SAXS profile fitting options. The programs for rigid body modeling were also further developed. Thus, SASREF was extended to account for possible dissociation of oligomers and complexes [18••]; In

Box 2 Extending measurements to higher angles.

Wide angle X-ray scattering patterns ($q > 0.5$ Å^{-1}) are collected, as the name suggests, at higher angles, typically by placing a detector closer to the sample. The WAXS region contains information about the secondary structures and their rearrangements. The extraction of this information is, however, not straight forward and the interpretation of the data without available high resolution models is hardly possible. The strength of WAXS lies in its high sensitivity to small changes, and this can, therefore, be applied to identify structural similarities and characterize structural fluctuations (e.g. [68]).

FoXSDock global search docking, energy based scoring functions and a SAXS fit scores were integrated [23]. A new algorithm to add missing fragments in multi-chain and symmetric oligomeric structures was implemented in CORAL [18••]. In QUAFIT [24], oligomeric models are determined accounting for both point symmetry and screw axis. Zheng and Tekpinar revisited a normal mode analysis approach to propose a refinement algorithm that allows for

Table 1

Overview of advances is data analysis methods over the last years

Program	Features	Website
Hybrid approaches		
Situs [21]	Originally designed for the handling of cryoEM data, has been extended to perform multi-scale modeling and docking from a variety of biophysical sources including SAXS	http://situs.biomachina.org/
IMP [20]	A hybrid approach (integrated modeling platform) that employs heterogeneous data including SAXS scoring functions to evaluate models	http://salilab.org/imp/
SASREF_MX [18]	In this enhanced version of SASREF, the possible dissociation of oligomers and complexes can be taken into account	http://www.embl-hamburg.de/biosaxs/sasref.html
FoXSDOCK [23]	This webserver calculates potential complexes sorted by a score based on an interface energy as well as SAXS profile fit	http://modbase.compbio.ucsf.edu/foxsdock/
CORAL [18]	In the newest upgrade missing fragments in multi-chains as well as symmetry of oligomeric structures are accounted for	http://www.embl-hamburg.de/biosaxs/coral.html
QUAFIT [24]	SAXS data is used to model symmetric oligomers including point-group symmetry as well as possible screw axis	–
Algorithms for the calculation of theoretical SAXS curves at wider angles		
Zernike expansions [27]	Zernike polynomial expansions are used to account of the solvent contributions from molecules with large cavities and complicated surfaces	http://sastbx.als.lbl.gov
AquaSAXS [28]	A Poisson–Boltzmann–Langevin formalism is applied to describe the hydration layer as an assembly of self-orienting dipoles of variable density on a grid.	http://lorentz.immstr.pasteur.fr/aquasaxs.php
HyPred [29]	All-atom, explicit-solvent molecular dynamic simulations and a set of proximal radial distribution functions derived from ~300 different categories of atoms are used to calculated the hydration layer	http://www.godzilla.uchicago.edu/cgi-bin/jouko/waxs.cgi
Analysis of experimental SAXS data in terms of ensembles		
EOM [18,30]	The newest upgrade allows for the assessment of the number of conformers and for the analysis of symmetric oligomers	http://www.embl-hamburg.de/biosaxs/eom.html
EROS [33]	Combines SAXS with the results of coarse-grained computer simulations for multi-domain proteins	–
ENSEMBLE [34]	The weighted ensemble to represent the unfolded states is coupled with NMR data on chemical shifts, RDCs and other information.	http://abragam.med.utoronto.ca/~JFKlab/

large-scale conformational changes while maintaining pseudobonds and secondary structures [25•].

The evaluation of the structural models is only possible with the ability to rapidly compute theoretical scattering profiles from these models. Given an atomic structure, the scattering intensity is, of course, readily calculated by the Fourier transform of the electron density. Yet, this scattering computed in vacuum neglects the contribution of the solvent and thus does not adequately represent solution scattering especially at wider angles. The excluded solvent and the partially ordered, surface-bound solvent have to be accounted for. In the first publicly available program to compute scattering from atomic models, (CRYSOL [26]), which has set the standard for SAXS calculations, the hydration shell is approximated by a single uniform solvent layer of constant thickness. Especially for WAXS computations, more elaborate representation of the shell is required, and numerous alternative programs have been developed (see [1,3] for reviews). The most recent approaches include the use of Zernike polynomial expansions [27], a Poisson–Boltzmann–Langevin formalism (AquaSAXS [28]) and a set of proximal radial distribution functions (HyPred [29]).

SAXS is one of the very few structural tools capable of quantitative characterization of metastable systems such as multi-domain proteins with flexible linkers, unfolded and intrinsically disordered proteins (IDPs). All these objects exhibit a large number of different conformations in solution, and one may account for the conformational flexibility by selecting a subset of conformers that best fit the experimental data from a large (random) pool. This concept of ensemble fitting of the SAS data first implemented by Bernado *et al.* [30] in an ensemble optimization method (EOM) became very useful in solution scattering studies of flexible systems. Several other approaches utilizing the EOM-like strategy have subsequently become available (see e.g. [31,32]). Recent developments include the program EROS (ensemble-refinement of SAXS), [33] making use of an energy function optimized for protein binding constitutes to filter coarse-grained models of molecular assemblies. Often, the SAS-based ensemble analysis is coupled with experimental NMR data on chemical shifts, residual dipolar coupling (RDCs) and other information (e.g. program ENSEMBLE [34]). Also, the original EOM technique was recently enhanced to allow for the assessment of the number of conformers and for the analysis of symmetric oligomers [18••].

Applications to challenging probes

Functional macromolecular complexes shifted to the main focus of the SAXS solution studies, whereby rigid body modeling and docking algorithms have made it easy to gain information on complex formation [35–37]. Thereby, the studies on the formation of weak protein interactions [38,39•], fibrillation processes [40] as well as mixed complexes [41–44] are feasible.

The power of SAXS is especially demonstrated with the study of various types of flexible systems and IDPs [31,32,45,46]. This ensemble approach becomes even more dominant when constraints from other structural biological methods such as NMR are added [20••,47]. In addition, changes in the sample environment or ligand binding can be visualized by a shift of the ensemble [48,49,50]. This has enabled the study of phosphorylation effects [51,52•] temperature induced transitions [53,54] and the consequence of high pressure [55].

SAXS and WAXS have also shown to play an important role in drug development. Especially for formulation studies SAXS can be applied to study the oligomerization state of drugs at high concentrations (>100 mg/ml [56]) or in the presence of different ions [57]. In addition, the use of scattering profiles to gain information on challenging probes should be beneficial for drug development in the near future. For example, IDPs represent a rich and unexplored reservoir of new drugs due to their involvement in many disease-related signaling pathways including cancer, cardiovascular disease, amyloidosis, neurodegenerative diseases, and diabetes [32,58]. In a similar manner, the increasing accessibility to analyze membrane proteins with SAXS [59,60••] should support further drug development, as a high percentage of membrane proteins are the target of therapeutic agents.

Scattering data in the future

The development of SAXS and WAXS over the past years is just the beginning and we are convinced that these techniques will advance even further in the future giving rise to a number of intriguing biological insights. In this sense, it is also from immense importance that the solution scattering community agrees and establishes publication and validation guidelines [61•].

As the rapidly growing demand in biological solution SAXS has led to difficulties in obtaining beamtime at synchrotrons, there is a renewed interest in the laboratory X-ray sources, where the modern cameras are now available providing point collimation (not smeared) data. The quality of the data, although inferior to that from synchrotrons, may be sufficient to gain information about the quaternary static structure, for example [62••]. Still, the high brilliance X-ray sources at large scale facilities will stay the major sites for all cutting edge experiments. Here, future developments may be expected by exploiting the possibilities of measurements with variable wavelength for anomalous scattering experiments [63,64•]. The improved power and brilliance of the experimental sources may also allow one to revisit the classical approaches such as

measuring distances between heavy atoms [65•]. The progress in free electron lasers with its immense brilliance and higher pulse repetition rates may also have a significant impact on the future of solution scattering experiments [2,66,67].

Acknowledgment

The authors like to acknowledge funding received from the European Community's Seventh Framework Program (FP7/2007-2013) under BioStruct-X (grant agreement no. 283570).

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