Methods 59 (2013) 363–371

Contents lists available at SciVerse ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

Developing advanced X-ray scattering methods combined with crystallography and computation

J. Jefferson P. Perry [a,b,*], John A. Tainer [a,c]

[a] Department of Integrative Structural and Computational Biology and Skaggs, Institute for Chemical Biology, The Scripps Research Institute, La Jolla, CA, USA
[b] School of Biotechnology, Amrita University at Amritapuri, Kollam, Kerala, India
[c] Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA, USA

ARTICLE INFO

Article history:
Available online 29 January 2013

Communicated by Peter Schuck

Keywords:
SAXS
Crystallography
High-throughput
In-solution
Screening
Protein interaction

ABSTRACT

The extensive use of small angle X-ray scattering (SAXS) over the last few years is rapidly providing new insights into protein interactions, complex formation and conformational states in solution. This SAXS methodology allows for detailed biophysical quantification of samples of interest. Initial analyses provide a judgment of sample quality, revealing the potential presence of aggregation, the overall extent of folding or disorder, the radius of gyration, maximum particle dimensions and oligomerization state. Structural characterizations include *ab initio* approaches from SAXS data alone, and when combined with previously determined crystal/NMR, atomistic modeling can further enhance structural solutions and assess validity. This combination can provide definitions of architectures, spatial organizations of protein domains within a complex, including those not determined by crystallography or NMR, as well as defining key conformational states of a protein interaction. SAXS is not generally constrained by macromolecule size, and the rapid collection of data in a 96-well plate format provides methods to screen sample conditions. This includes screening for co-factors, substrates, differing protein or nucleotide partners or small molecule inhibitors, to more fully characterize the variations within assembly states and key conformational changes. Such analyses may be useful for screening constructs and conditions to determine those most likely to promote crystal growth of a complex under study. Moreover, these high throughput structural determinations can be leveraged to define how polymorphisms affect assembly formations and activities. This is in addition to potentially providing architectural characterizations of complexes and interactions for systems biology-based research, and distinctions in assemblies and interactions in comparative genomics. Thus, SAXS combined with crystallography/NMR and computation provides a unique set of tools that should be considered as being part of one's repertoire of biophysical analyses, when conducting characterizations of protein and other macromolecular interactions.

1. Introduction

The importance of studying protein interactions to gain a coherent understanding of complex biological systems has been highlighted by the plethora of tools that have been created to analyze such interactions. This is because the progression of cellular pathways, and often catalysis, is largely controlled through such interactions. Tools critical to defining these interactions may characterize proteins at atomic resolution or function at the level of studying the entire interactomics system of a cell. At higher-resolution, detailed crystallographic, nuclear magnetic resonance (NMR) and electron microscopy (EM) studies have revealed profound insights into the molecular mechanisms of cellular machines functioning to control fundamental biological processes. Examples include analyses of the molecular machines involved in the many different DNA metabolism steps of DNA replication, recombination or repair [1,2]. These studies have often been supported by biophysical techniques providing values to binding affinities, on–off rates of protein interactions and complex formations, and helping to identify allosteric control mechanisms. Yet, much still remains to be defined due to the inherent difficulties in studying large, complex molecular machines and their interactions, and the inherent limitations of the structural methods being used.

One technique whose beginnings date back to the 1930s, and is now very much coming to the fore for studying protein interactions, is in-solution small angle X-ray scattering (SAXS). The interest being generated in this powerful, complementary and robust technique is that there is a lack of size constraints that can hinder other methods such as NMR or EM. Also, there is no requirement for diffraction-quality crystals as needed for macromolecular

* Corresponding authors. Fax: +1 858 784 2277.
E-mail addresses: jjperry@scripps.edu (J. Jefferson P. Perry), jat@scripps.edu (J.A. Tainer).

1046-2023/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ymeth.2013.01.005

crystallography. Disadvantages of SAXS are that rotational averaging of the data means that enantiomorphs cannot be distinguished, and that the structural models produced contain lower information content akin to low-resolution EM images, with data in the 10–30 Å range. Importantly however, SAXS has very rapid data collection and processing times relative to other structural techniques. Also, SAXS analyses are conducted in solution that can include near physiological conditions including ambient temperatures, and with minimal sample preparation. Thus, SAXS readily lends itself to charactering protein interactions, flexibility, conformational changes and formations or disruptions of higher order complexes, and has an added advantage of being a higher throughput method than the other major structural techniques.

The ability of SAXS to characterize flexibility in larger proteins and complexes is a noticeable asset when studying protein interactions [3]. Interestingly, eukaryotic proteins contain significant regions of flexibility and disorder [4], more than typically observed proteins in bacteria or archaea [5,6]. The increased flexibility likely reflects more complex regulatory roles for eukaryotic proteins. This could be through the occurrence of post-translational modifications within the regions of disorder that are more accessible by the modification machinery, and through conformational controls and switches regulating enzymatic activities or pathway progression through protein partner handoffs. However, in crystallography these disordered regions are often removed to aid crystallization or are not clearly visible in the electron density maps unless they are involved in a crystal-packing interface. Moreover, certain flexible regions are known to have disorder-to-order transitions, upon partner interactions that can promote catalytic activities or cellular signaling. SAXS studies can reveal these key switches within global architectures in solution, albeit at lower resolution. Additionally, SAXS is a very sensitive technique for defining assemblies, including transient complexes, as the scattering power in SAXS is related to the square of the number of electrons in the protein/complex, and as such, the formation of larger complexes can be readily observed. These mixtures of the individual proteins and their higher order assemblies can be deconvoluted, providing that the initial protein constituents are known.

Combining high-resolution information from crystallography or NMR to SAXS data generates an effective hybrid method to reveal key biological insights into protein interactions. This combinatorial approach has been aided and developed in recent years through the advances in computing power and new SAXS algorithms and software to provide detailed analyses, and due to the ease of sample preparation and speed of data collection. As an example, it can be difficult to identify the correct biological oligomer within a crystal from crystal packing interfaces alone, but SAXS analyses can define the in solution biological oligomer and hence reveal the best match within the crystal (e.g. [7,8]). Similarly, SAXS data can distinguish cases in which the solution behavior of a sample does not perfectly match the crystalline assembly, perhaps due to conformational relaxation from forced crystal contacts. Another application is to study larger, inherently flexible molecules, which high-resolution techniques have difficulties in analyzing due to being out of the typical range of NMR or being a notable challenge to crystallize. Here, SAXS analyses can reveal *holo*-architectures that can be fitted with individual domains that have been determined by NMR and/or crystallography. Advantageously, the use of 96-well plate technology and data collection time of seconds, allows for hundreds of samples to be analyzed within a typical allocation of 8-h beamtime, enabling the *holo*-architectures to be characterized in detail. This includes defining interactions with multiple partners, substrates, co-factors, altering buffer conditions etc., to define overall architectural structural states.

Here, we highlight these recent developments in SAXS for studying protein interactions, provide methods and examples of results used to gain such information on flexible and reversible molecular complexes. In particular, we discuss applications and provide our insights that have been gained from SAXS studies from our own research and that of collaborators, which have been conducted at the Structurally Integrated BiologY for Life Sciences (SIBYLS) beamline, Advanced Light Source (ALS), Berkeley National Laboratory, Berkeley, California. Despite the growth of SAXS studies that are markedly helping define key biomolecular complexes and interactions, these methods are under-utilized and underexplored. For example, SAXS could be utilized as a tool in the early states of drug discovery. Here, much of the low-hanging fruit in drug discovery has been plucked, leaving more challenging structural-targets, requiring new approaches characterizing protein interactions and assemblies, and disrupting or stabilizing these via small molecules. SAXS clearly falls within this this category, being of potential benefit due to an ability to both screen and produce structure and conformation-based outputs rapidly and facilely. Thus, through this review we hope to provide the reader with an understanding of the latest methods and practical uses of SAXS, encouraging those interested to explore and further evolve the uses SAXS methods to define protein interactions to uncover new insights into biological processes.

2. Methods

In addition to home sources, SAXS data is routinely collected at multiple synchrotron beamlines across the world, with a list of current SAXS capable beamlines at Wikipedia (http://en.wikipedia.org/wiki/Small-angle_X-ray_scattering). Our data is collected at the 'Structurally Integrated Biology for Life Sciences' (SIBYLS) beamline 12.3.1 at the ALS. A user can request SIBYLS SAXS time by visiting the RAPIDD access link on the SIBYLS beamline homepage at http://bl1231.als.lbl.gov. SIBYLS is a dual function end station for SAXS (schematic of SAXS setup depicted in Fig. 1) and crystallography. Switching between the SAXS and crystallographic data collection modes takes approximately 1 h, enabling a user to collect both SAXS and crystallographic data on a single visit to the beamline. Data is generally collected on 15–25 μl sample volumes at 1–5 mg/ml range, with at least 3 serial dilutions preferred. Data is collected in the order of buffer, lowest concentration, medium concentration(s) and highest concentration, and lastly a second buffer is measured. The sample cell is washed both between the highest-concentration sample and subsequent second buffer, as well as between differing protein samples by a mild detergent for 1 min, followed by 3 rinses in buffer solution. The data collection occurs in a high-throughput fashion, using a 96-well plate and pipetting robot that loads samples into the sample cell, which is situated in a positive helium pressure to reduce air scatter and oxidative damage [9]. The sample plate is typically maintained at 15 °C prior to chamber dispensing, and temperature can be altered for increased user-control. Control software for this high throughput data collection has been developed from the Blu-Ice/DCS control systems, which is used from crystallography data collection at certain synchrotron beamlines [10].

Data for each sample is typically collected by four exposures of 0.5, 1, 2 and 4 s, although longer exposures, such as up to 40 s, are collected if there is an interest collecting at the highest resolutions. The scattering profiles of the buffers collected before and after the molecule samples are compared, to ensure to no significant errors have occurred from the instrumentation or from bubbles occurring from loading the buffer blank. Similarly, a photographic image of the sample cell captured for each sample ensures proper loading and lack of bubbles.