Anomalous Solution Scattering for Protein Structure Modeling

Small-angle solution scattering is widely used for ensemble structural modeling of intrinsically unstructured proteins, which will greatly benefit from anomalous scattering. We showed that the transition metal ions bound to polyhistidine tags of small proteins could be used for obtaining K-edge anomalous SAXS data, which can be useful in a number of ways for structural modeling and validations.

With recent spectacular advances in artificial intelligence-based structure prediction methods and the "resolution revolution" in cryo-electron microscopy, determination of the structure of proteins, including large macromolecular complexes, has entered a brave new era. However, challenges remain for determining the structure of those proteins or parts of proteins that are intrinsically unstructured. These intrinsically unstructured or disordered proteins (IDP), or disordered regions of proteins, are not amenable to conventional structure determination methods that rely on multiple copies of the same protein having more or less the same structure. An ensemble of structures is required to adequately describe these IDPs [1]. Solution-based methods, such as NMR and single-molecule FRET, in combination with computational methods such as molecular dynamics simulation, can address the problem of structural modeling of these IDPs [1]. Such integrative structural biology approaches to the modeling of structural ensembles of IDPs have recently gained traction.

Small-angle X-ray scattering (SAXS) has emerged as a key component of such integrative structural biology approaches [1]. Unlike crystallography and cryo-EM, SAXS data is obtained from an ensemble of structures that are free to move in solution. However, SAXS data does not provide location or site-specific information. The anomalous scattering from special label atoms attached to proteins can be used to obtain additional location-specific information to supplement SAXS-based structural modeling. Anomalous scattering at the characteristic absorption edge of the label element, which is due to electrons that are knocked out from its inner orbitals (such as K shell), can alter the SAXS signal [2]. Changes in SAXS intensity due to anomalous scattering depend upon the number, locations and nature of the anomalous scattering label atoms.

Anomalous SAXS can be a significant addition to the information content of SAXS data and can aid structural modeling in various ways. However, biological samples pose a special challenge to the collection of anomalous scattering data as the anomalous signal will be a very small percent of the total scattering in a typical natural metalloprotein. Biological samples are also prone to aggregation induced by radiation damage, making it difficult to collect anomalous SAXS data from the same sample at multiple wavelengths. In addition, obtaining anomalous SAXS data for those proteins that are devoid of metal centers and those that are unstructured poses a special problem due to the lack of a generic way to label them.

We labeled polyhistidine tags attached to a small, partially unstructured, dimeric protein segment Myo10 with cupric ions and measured the anomalous signal from copper-labeled Myo10 at beamline BL-15A2 (**Fig. 1**) [3]. Resultant scattering profiles, collected at multiple wavelengths at the K absorption edge of copper, showed the presence of a small anomalous signal. The metal-protein part of the anomalous signal, which contains information about all the pair-wise metal-protein distances in the protein-metal complex, can be obtained from these SAXS profiles.

This result opens up the possibility of obtaining an anomalous signal from any small protein with an attached metal-labeled polyhistidine tag. Further protein engineering for grafting multivalent metal-labeling sites in proteins will lead to the more widespread application of anomalous SAXS in structural biology. Similarly, seleno-methionine labeling of proteins that are routinely used for phase determination in crystallography can be exploited for anomalous SAXS studies of folded proteins. The new information gained may help in detecting protein-protein interactions and conformational changes in proteins, as well as for validating structural models obtained from integrative structural biology approaches.
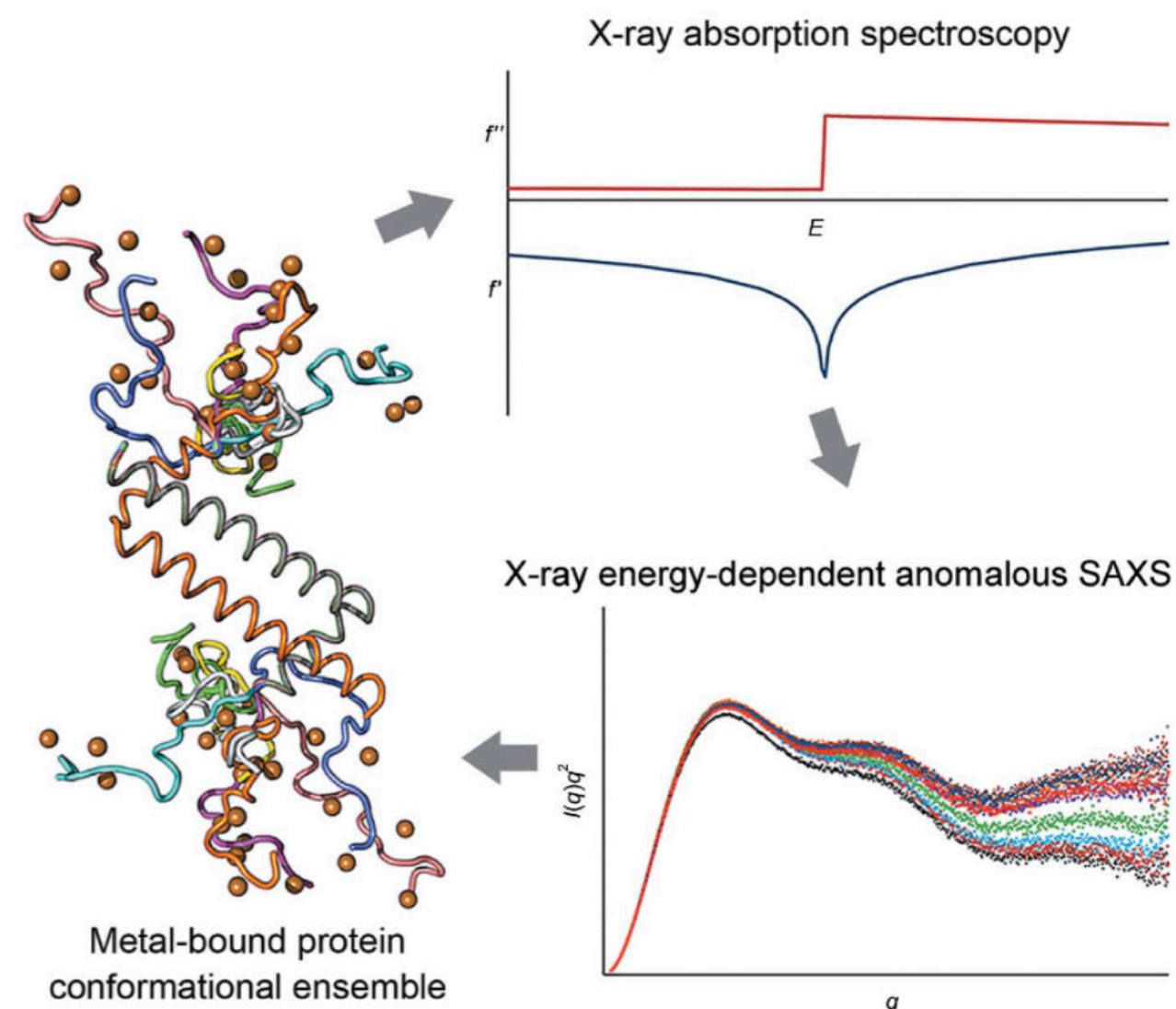

Figure 1: Schematic representation of our work (https://doi.org/10.1107/S205979832101247X, reproduced with permission).

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