structural biology. Furthermore, unstructured proteins, RNA or DNA components provide functionally important flexibility that is key to many macromolecular assemblies throughout cell biology. As objective, quantitative experimental measures of flexibility and disorder in solution are limited, small angle scattering (SAS), and in particular small angle X-ray scattering (SAXS), provides a critical technology to assess macromolecular flexibility as well as shape and assembly at the proteomic scale [1], [2], [3]. The accurate modeling of such SAS data requires not only well-characterized homogeneous samples but also analytical tools for objective, high-throughput assessments of mass, models and resolution derived from the SAS experiment scale [4], [5]. We apply the Porod-Debye law as a powerful tool for detecting biopolymer flexibility in SAS experiments. We find that the Porod-Debye region fundamentally describes the nature of the scattering intensity decay, which captures information needed for distinguishing between folded and flexible particles. Particularly for comparative SAS experiments, proper analyses can distinguish between discrete conformational changes and localized flexibility relevant to molecular recognition and interaction networks. This approach aids insightful analyses of fully and partly flexible macromolecules that is more robust and conclusive than traditional Kratky analyses. Furthermore, we demonstrate for prototypic SAXS data that the ability to calculate particle density by the Porod-Debye criteria provides an objective quality assurance parameter suitable for SAXS modeling and validation. We have moreover defined a novel SAS invariant called the volume-of-correlation, Vc, that reflects the information contained within the entire scattering curve. Vc is specific to the structural state of the particle, yet independent of concentration and the requirements of a compact folded particle. Together with radius-of-gyration, Vc provides the means to accurately determine the molecular mass of proteins or RNA and to define a statistical measure, $R_{SAS}$, for improved evaluation of structural models.

[1] C.D. Putnam, M. Hammel, G.L. Hura, J.A. Tainer, *Quarterly Reviews in Biophysics* **2007,** *40,* 191-285. [2] G.L. Hura, A.L. Menon, M. Hammel, R.P. Rambo, F.L. Poole, S.E. Tsutakawa, F.E. Jenney, K.A. Frankel, R.C. Hopkins, J.O. Scott, B.D. Dillard, S. Classen, M.W.W. Adams, J.A. Tainer, *Nature Methods* **2009**, *6,* 606-612. [3] R.P. Rambo, J.A. Tainer, *RNA* **2010**, *16,* 638-646. [4] S. Classen, I. Rodic, J. Holton, G.L. Hura, M. Hammel, J.A. Tainer. *J Synchrotron Radiat* **2010**, *17,* 774-81. [5] R.P. Rambo, J.A. Tainer, *Current Opinion Structural Biol*. **2010**, *20,* 128-1376.

**Keywords: SAXS, crystallography, macromolecular**

## MS.11.2

*Acta Cryst.* (2011) A**67,** C42

**A system for automated data analysis and interpretation for biological solution SAXS**

Maxim V. Petoukhov,[a] Daniel Franke,[a] Alexey .G. Kikhney,[a] Peter V. Konarev,[a] Dmitri I. Svergun,[a] [a]*European Molecular Biology Laboratory, Hamburg Outstation, (Germany).* E-mail: petoukhov@embl-hamburg.de

Small angle X-ray scattering (SAXS) is a structural method providing information about the overall structure of dissolved macromolecules and functional complexes at low resolution. The technique is increasingly demanded by the biological community thanks to the development of both cutting edge instrumentation and novel data analysis approaches enhancing the reliability of the reconstructed models. We have designed a system for automated high throughput solution SAXS encompassing both data acquisition and analysis. The system includes a Beamline Meta Server allowing for queuing of multiple samples for subsequent measurement by a robotic sample changer and remote experiment control. The primary data reduction and primary processing are done on-the-fly by a pipeline style performing radial averaging, check for radiation damages, buffer subtraction, calculation of the invariants, fast shape evaluation and reporting in convenient tabular form using XML format. The pipeline is already running on several synchrotron SAXS beamlines at the storage rings in Hamburg and Grenoble.

An integrated system DANESSA reads in the processed data from the pipeline to perform automated data analysis and model building. DANESSA includes modules for *ab initio* shape determination, high resolution structure validation, rigid body modeling, mixture analysis. The system requires a rather simple input containing *(i)* primary sequences of the objects, *(ii)* scattering profiles of the measured construct(s), and *(iii)* a table with molarities of the objects for each curve to describe the sample stoichiometry. DANESSA makes an initial assessment of the data quality and queries external databases and servers to gather available bioinformatic knowledge on the object(s). Depending on the scenario identified by the system (e.g. complex formation / deletion mutants study / dissociation / model validation), it makes a decision what are the appropriate modules to launch. Given that the full modeling cycle consists of multiple runs of multiple programs, the system employs 140-processor cluster at EMBL Hamburg. The system may run in a standalone mode from a Web interface. The details on the modules and the data flow in DANESSA as well as practical examples of its usage will be presented.

**Keywords: SAXS, automation, biomolecule**

## MS.11.3

*Acta Cryst.* (2011) A**67,** C42-C43

**Quality Control of Protein Standards for Molecular Mass Determinations by SAXS**

Shuji Akiyama,*Division of Biological Science, Graduate School of Science, Nagoya University, Furo-cho, Chikusaku, Nagoya 464-8602, (Japan). PRESTO, (Japan). Science and Technology Agency, 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, (Japan). RIKEN SPring-8 Center, Harima Institute, 1-1-1 Kouto, Sayo, Hyogo 679-5148, (Japan).* E-mail: akiyama@bio.nagoya-u.ac.jp

Forward scattering intensity, $I(0)$, is one of the important model-independent parameters that can be determined with small-angle scattering experiments. $I(0)$ is proportional to the number of particles and also to the squared particle volume, thus $I(0)$ normalized relative to the particle concentration ($I(0)/c$) is often used as a measure of molecular mass of biological macromolecules.

There are two major procedures upon estimating the particle mass from $I(0)$. One is to use known scattering length of water as a standard [1], and the other is to refer $I(0)$ of a biological standard with known molecular mass [2]. In the former case, molecular mass of protein samples can be determined mostly within ~ 10 % deviation by using 'effective' particle specific volume [3]. On the other hand, the latter procedure requires reproducible and monodispersed preparations of the biological standard and its exact concentration.

In this presentation, I will report my effort to establish a reproducible protocol of preparing a series of protein standards suited for solution scattering experiments. The size distribution of the prepared standards was checked with dynamic light scattering to be as narrow as monodispersed system. Furthermore, hydrodynamic properties of the prepared standards were carefully examined with analytical ultracentrifuge. Several factors limiting the accuracy of molecular mass determination will be discussed through a direct comparison of molecular mass from solution scattering with that from hydrodynamic measurements.

[1] D. Orthaber, A. Bergmann, O. Glatter, *Journal of Applied Crystallography* **2000**, *33*, 218–225. [2] L.A. Feigin, D.I. Svergun, *Structure Analysis by Small-Angle X-ray & Neutron Scattering* **1987**. [3] E. Mylonas, D.I. Svergun, *Journal of Applied Crystallography* **2007**, *40*, s245–s249.

**Keywords: saxs, scattering, purification**

## MS.11.4

*Acta Cryst.* (2011) A**67,** C43

**The molecular weight of proteins from a single SAXS measurement on a relative scale**

Mario de Oliveira Neto,[a] Hannes Fischer,[b] Hamilton B. Napolitano,[c] Igor Polikarpov,[a] Aldo F. Craievich,[b] *[a]Institute of Physics of São Carlos, IFSC, São Carlos (Brazil). [b]Institute of Physics, IF/USP, São Paulo. [c]Exact Sciences and Technology, Goiás (Brazil).* E-mail: mario@if.sc.usp.br

An important step in the characterization of proteins is the determination of their molecular weights and their multimeric state in solution. Accuracies of classical methodologies for the determination of the molecular weight of proteins in dilute solution were recently evaluated by Mylonas & Svergun [1]. These authors demostrate that the molecular weight of a protein can be obtained by comparing the experimental SAXS curve produced by the protein in dilute solution *(i)* to another experimental SAXS curve corresponding to a standard protein with known molecular weight, or *(ii)* to a SAXS curve corresponding to pure water leading in this case to the determination of SAXS intensity of the studied protein in an absolute scale. Both of these procedures require the determination of at least two SAXS curves. In addition, the first procedure requires the precise knowledge of the protein concentration, which is frequently not known with high accuracy, and the second method needs the determination of the SAXS intensity by water with a considerable precision, which implies in rather long counting times. Both methodologies yield the molecular weight of proteins with an error of about 10% provided the solute concentration is measured with an accuracy of 5 – 10 %, which might not always be straightforward. A novel procedure for the determination of the molecular weight of proteins in diluted solution from a single SAXS curve measured on a relative scale is avaiable, which uses experimental data of a single small angle X-ray scattering (SAXS) curve measured on a relative scale [2]. This procedure does not require the measurement of SAXS intensity on an absolute scale and does not involve a comparison with another SAXS curve determined from a known standard protein. The proposed procedure can be applied to monodisperse systems of proteins in dilute solution, either in monomeric or multimeric state, and it was successfully tested by applying it to SAXS data measured for 22 proteins with known molecular weights. The molecular weights determined by using this novel method of all the measured set deviate from their known values by less than 10 % and the average discrepancy was 5.6 %. Importantly, this method allows for a simple and unambiguous determination of the multimeric state of proteins with known monomeric molecular weight.

Work supported by FAPESP and LNLS, Brazil.

[1] E. Mylonas, D.I. Svergun *J. Appl. Cryst*. **2007,** *40,* 245–249. [2] H. Fischer, M. Oliveira Neto, H.B. Napolitano, I. Polikarpov, A.F. Craievich *J. Appl. Cryst.* **2010***, 43,* 101-109.

**Keywords: SAXS, protein, molecular weight**

## MS.11.5

*Acta Cryst.* (2011) A**67,** C43

**The role of conformational change in HIV maturation revealed by SAXS**

Cowieson N.P.,[a] Hijnen M.,[b] Wilce J.,[b] Wilce M.,[b] Mak J.,[c] Kirby N.,[a] *[a]Australian Synchrotron, 800 Blackburn Rd., Clayton, VIC 3168 (Australia). [b]Department of Biochemistry and Molecular Biology, Monash University, Clayton, VIC 3800 (Australia). [c]Burnet Institute, 85 Commercial Rd, Melbourne, VIC 3004 (Australia).* E-mail: nathan.cowieson@synchrotron.org.au

The HIV Gag poly-protein is the main structural component of the viral capsid and is sufficient to form virus-like particles *in vitro*. During viral maturation the Gag poly-protein undergoes a series of specific proteolysis events catalysed by the viral protease. Conformational change in the polyprotein is suggested by the specificity of the proteolytic sequence and by gross structural rearrangement of the viral capsid. However, conformational change in the Gag polyprotein has not previously been measured due to the inherent flexibility of Gag and its tendency to aggregate.

We have used SAXS together with CD and a combination of biochemical methods to analyse the orientation of the four domains of Gag in solution and to track how their relative orientation changes during the proteolytic processing that is part of this proteins normal life cycle.

To control for aggregation and multimerisation we have used absolute calibration of scattering intensity, protein dilution series, size exclusion chromatography with in-line SAXS, Gag mutants deficient in dimerisation, SAXS measurements of bands in native gels and complementary methods such as MALS and DLS. The work reveals an important aspect of HIV biology and has broader application to the study of aggregation prone proteins by SAXS.

**Keywords: hiv, saxs, flexibility**

## MS.12.1

*Acta Cryst.* (2011) A**67,** C43-C44

**High pressure examination of salicylaldoximes complexes for metal extraction**

Peter J. Byrne, John Chang, Simon Parsons, Peter Tasker, *Department of Chemistry, University of Edinburgh (Scotland).* E-mail: pbyrne@staffmail.ed.ac.uk

The application of pressure can be shown to have an effect on small molecules by changing their properties either chemically or physically, providing an extremely interesting and novel way to fine tune materials, such as the use of high pressure to alter the cavity size within porous materials [1].

Metal oxides are extracted using either pyrometallurgical or hydrometallurgical techniques, with the later being more economical. Salicylaldoxime based ligands are heavily used in the copper industry with some 20% of the world's copper extracted in this way. The selectivity arises from the goodness of fit of the cavity with the $Cu^{2+}$ ion. It has been shown that high pressure can be used to alter the size of the cavity [2], [3] with an increase in pressure resulting in a decrease in cavity size. Can we use high pressure to tune this cavity so that we can actively select specific metals?

This work has involved the use of high pressure crystallography to examine various salicylaldoxime ligands to investigate their properties and their possible involvement in improving extraction processes.