[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

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

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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.

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[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.

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The role of conformational change in HIV maturation revealed by SAXS

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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

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High pressure examination of salicylaldoximes complexes for metal extraction

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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.