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Integrating SEC-SAXS with MALLS/QELS/RI at the EMBL-P12 bioSAXS beamline.

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The separation of sample components using size exclusion chromatography (SEC) prior to small-angle X-ray scattering (SAXS) has been implemented at a number of beamlines for the low-resolution structural studies of biomacromolecules in solution . At the EMBL-P12 bioSAXS beam line (PETRAIII, DESY, Hamburg) approximately 20% of beam line users elect to perform SEC-SAXS since its commissioning in 2012. More recently, the automated control of the P12 SEC-SAXS system has been integrated into beam line control software BECQUEREL [1] which greatly simplifies SEC-SAXS operations. The main advantage of SEC-SAXS is the potential to isolate components from 'difficult' biological samples, for example those that are unstable over time or exist as mixtures of species in solution such as detergent solubilised membrane proteins. However, SEC-SAXS has a number of disadvantages. Obtaining accurate molecular weight estimates directly from the SAXS profiles is not trivial, yet is required for SAXS data interpretation and structural modelling. We have shown that a continuous split-flow system which incorporates right-angle laser light scattering and refractive index (RI) measurements in parallel with SAXS is extremely useful for molecular weight validation [2]. The P12 SEC-SAXS setup has been recently upgraded to a multi-angle laser light scattering (MALLS)/RI/UV system with in-built quasi-elastic laser light scattering (QELS). The hydrodynamic radius (R_h) can be calculated from the QELS data and combined with the radius of gyration (R_g) from SAXS to obtain the shape factor, R_g/R_h. Along with the MW from MALLS/RI, the R_g/R_h acts as an additional experimentally-determined parameter to interpret SAXS data in terms of overall biomolecular structure. Another complication of SEC-SAXS is processing the often large number of data frames and identifying which frames correspond to the correct buffer for background-scattering subtraction. An intuitive and easy-to-use analysis tool, SEC-PLOT, has been developed to identify the most appropriate buffer for background subtraction and to rapidly extract the subtracted and averaged SAXS profiles from the SEC-elution peaks. SEC-PLOT is part of the new ATSAS 2.8 release of SAXS data analysis package [3].

[1] Franke et al., (2012) Nuclear Instruments and Methods A, 689, 52-5.
[2] Graewert et al., (2015) Scientific Reports, 5:10734.
[3] Franke et al., (2016) in preparation: https://www.embl-hamburg.de/biosaxs/software.html

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