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Keywords: metaljet, saxs, home-lab, tabletop, x-ray source, x-ray tube, brightness, flux

MS1-P2 In-line purification systems for structural analysis

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Once the average protein sample is subjected to structural studies such as small-angle X-ray scattering (SAXS), it has undergone a number of procedures to ensure that it is of sufficient quantity as well as quality. Most purification protocols typically include an affinity purification step and/or ion-exchange chromatography step as well as size-exclusion chromatography (SEC). A number of beamlines now offer in-line SEC systems to generate a monodisperse sample stream from challenging samples. The SAXS data quality can thereby be improved by removing aggregates and/or separating individual species comprised within the sample. Three big challenges that arise with this approach are i) dealing with radiation damage, ii) strong (8-10 fold) dilution of the sample as well as iii) identification of suitable scattering frames for background subtraction. We could show that the parallel collection of biophysical data such as UV-Vis, differential refractometry and static light scattering has helped in the challenge of finding optimal regions for buffer subtraction (Graewert et al. 2015). In addition, we have now explored the advantage of this set-up for using other commonly applied purification processes such as affinity chromatography. Importantly, the data collected with differential refractometer detector helps follow the scattering behavior of ligand during the elution step which alters especially when a gradient is applied. Alternatively, the use of an on-column tag removal system has emerged as a very promising approach. The clear advantage of such an in-line purification procedure compared to SEC-SAXS is given with the prevention of the strong sample dilution.

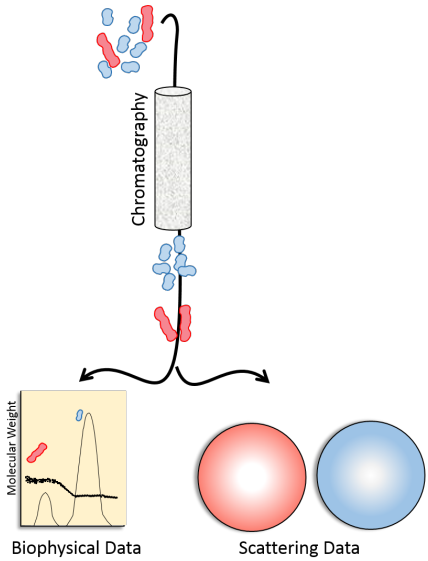

Figure 1.

Keywords: in-line purification, alternative methods, biophysical characterization

MS1-P3 Small-Angle-X ray-Scattering (SAXS) studies of the low-resolution structure of the ribosomal GTPase EFL1, the SBDS protein and their complex

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Ribosome biogenesis is closely linked to the cell growth and proliferation. Dysregulation of this process causes several diseases collectively known as ribosomopathies. One of them is the Shwachman-Diamond Syndrome, and the SBDS protein mutated in this disease participates with EFL1 in the cytoplasmic maturation of the 60S subunit. Recently, we have shown that the interaction of EFL1 with SBDS resulted in a decrease of the Michaelis-Menten constant (K_M) for GTP and thus SBDS acts as a GEF for EFL1 (1). Subsequent studies demonstrated that SBDS debilitates the interaction of EFL1 with GDP without altering that for GTP (2). The interaction of EFL1 alone or in complex with SBDS to guanine nucleotides is followed by a conformational rearrangement. Understanding the molecular strategy used by SBDS to disrupt the binding of EFL1 for GDP and the associated conformational changes will be key to understand their mode of action and alterations occurring in the disease. In this study, we aim to show the conformational changes resulting from the interactions between EFL1 and its binding partners, the SBDS protein and the guanine nucleotides using SAXS technique (3). SAXS provided structural information of the proteins (Fig.1) and their conformational changes (4,5).

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