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In-line SEC-SAXS and MALS/DLS/RI for the Analysis of Polydisperse Macromolecules

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Small Angle X-ray Scattering (SAXS) is a powerful tool for the structural analysis of biological macromolecules in solution and has seen a surge in popularity amongst structural biologists in the past decade. In part, this is because SAXS benefits greatly from the sensitivity and throughput that can be achieved at modern high brightness synchrotron sources. However, the critical need for highly monodisperse samples in SAXS analysis can be a challenge, and as such a number of labs have moved to develop in-line Size Exclusion Chromatography (SEC) at the beamline. Real-time SAXS on elution profiles not only improves monodispersity of samples and provides information on possible oligomeric states, but it also offers new modes of data analysis that can take advantage of the inherent concentration profiles underlying elution peaks and distributions of partially resolved species. Efforts to extend the synergy between SEC and SAXS to other biophysical methods are ongoing. The newly commissioned G1 BioSAXS facility at MacCHESS now offers the option of combining real-time SEC-SAXS with multi-angle static (MALS) and dynamic (DLS) light scattering along with refractive index (RI) detection. In this talk we give a brief overview of the performance and capabilities of the new BioSAXS station at MacCHESS with emphasis on detection limits and signal quality. We then discuss how the complementary light scattering techniques can be combined to offer new insights for complex inhomogeneous samples in terms of biological information and data quality assessment. We also discuss the limitations and possible future developments of these approaches as biologists seek to investigate more dynamic systems as well as shorter time scales.

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