SEC-SAXS @ SSRL

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The small-angle x-ray scattering/diffraction (SAXS/D) station beamline 4-2 at the Stanford Synchrotron Radiation Lightsource (SSRL) is a permanent experimental station dedicated to structural biology and biophysics. As such we provide state-of-the-art facilities for solution scattering experiments on biological samples such as proteins, nucleic acids, protein complexes, and virus particles. A highly monomodal and monodispersed sample is required for a successful SAXS data collection. However, in many cases of BioSAXS, that is obstructed by innate sample properties like structural dispersity, interparticle interaction, and aggregation. Even small amounts of those effects can interfere with the data analysis and make interpretation of the resulting scattering curves difficult, leading to potentially misleading to wrong conclusions. Online size-exclusion chromatography coupled with SAXS (SEC-SAXS) is a powerful tool to overcome and/or mitigate those difficulties of such problematic samples.

Here I will talk about the latest status of the SEC-SAXS setup at SSRL beamline 4-2: The combination of new UHPLC system with smaller inner volume and the small volume column, which maximizes the resolution of SEC separation and sample concentration at the beam position, and the tandem column setup using two columns makes data collection faster. Together with automatic sample storage/injection device, beamline control software (*Blu-Ice*) synchronized with the UHPLC is capable of continuous SEC-SAXS data collections. Implemented into the *Blu-Ice* and our instruments are a number of strategies for SEC-SAXS data collection to overcome those sample difficulties as well as technical problems like radiation damages, cleanliness of sample cell and sample dilution/separation during SEC. A real-time SEC-SAXS analysis pipeline (*SECPipe*) and additional analysis programs for further processing of the data are available. Besides practical considerations, current challenges and future perspectives on SEC-SAXS data collection and analysis will be discussed.