

Chromatography-coupled SAXS is a recent addition to the scattering toolkit. In-line size exclusion chromatography (SEC) complements routine SAXS measurements by providing independent verification of sample purity and a high-quality buffer match. Additionally, the technique allows the individual components of a mixture to be studied in a single SAXS experiment. However, a major limitation of the technique is that the resolution of the column is often insufficient to separate species of interest. To take full advantage of the technique, we have developed a new method to “computationally purify” scattering components using an extension of singular value decomposition (SVD) known as evolving factor analysis (EFA). After introducing the method, I will discuss the application of SEC-SAXS and EFA to allosteric enzymes. These enzymes are found in key points in metabolic pathways, where they change their activity in response to an effector. In particular, we have used SEC-SAXS to visualize the domain motions of mammalian phenylalanine hydroxylase (PheH), an enzyme that converts phenylalanine to tyrosine in the liver. We find that the tetrameric PheH is activated by dimerization of the regulatory domains, which bind phenylalanine in the active conformation.