Asymmetrical-Flow Field-Flow Fractionation (AF4) and the Determination of Molar Mass and Size

AF4 is an advanced separation analytical technique similar to liquid chromatography. Unlike liquid chromatography, the AF4 method has no stationary phase and the separation is achieved solely by a flow in an empty channel, where a perpendicular flow force is applied. Recent development of the instrumentation for AF4 brings new possibilities for the characterization of various macromolecules with several advantages over size-exclusion chromatography (SEC), i.e., separation takes place in an entirely empty channel, which eliminates undesirable SEC effects such as shearing degradation of polymers with ultra high molar mass, anchoring of branched macromolecules in SEC column packing, and enthalpic interactions of macromolecules with stationary phase.

Originally, AF4 was intended to be the method yielding the diffusion coefficient and consequently, the hydrodynamic radius calculated on the basis of retention data. That means it served as a separation and characterization method simultaneously. However, light-scattering detectors allow direct online measurement of not only hydrodynamic radius using dynamic light-scattering methodology, but also the direct determination of molar mass and radius of gyration using multi-angle light-scattering method. The concentration of eluted macromolecules is measured by a concentration-sensitive detector, which could be refractive index (IR) or UV detector.

The seminar will comprise the basics of AF4 separation and light-scattering detection as well as various examples dealing with protein conjugate analysis, aggregation studies, liposomes, membrane proteins in native environment, nanoparticles etc.