Abstract

This is the first of two seminars on method development for high throughput analysis of protein complexes using proteomics. Knowledge about protein complex composition and dynamics is valuable because it can reveal how cells respond to signals or adapt to different physiological conditions, and carry out complicated biochemical tasks. For example, enzyme complexes can form to influence substrate selectivity, catalytic efficiency, or the flux of metabolites into specific pools. Sequentially assembled protein complexes precisely execute mechanical tasks like chromosome segregation, vesicle budding, and long distance intracellular transport. A given eukaryotic cell contains hundreds if not thousands of protein complexes; therefore, there is a strong need to develop new methods to discover and analyze this complexity and how systems of protein complexes operate in the cell. One approach is to analyze stable complexes that persist in extracts of homogenized cells. Tandem affinity purification-mass spectrometry (AP-MS) is the most widely used method for high throughput analysis of protein complex composition. However, AP-MS requires thousands gene transfer experiments with affinity tagged "bait" proteins and is sensitive to the expression level and functionality of the tagged protein. As an alternative approach, we recently developed a new method that combines size exclusion chromatography (SEC) with quantitative MS to analyze hundreds of endogenous protein complexes in Arabidopsis leaves. The profiling based technique can be used to analyze protein complex dynamics under different physiological conditions. If the cell extracts are fractioned using orthogonal methods, co-elution of proteins that bind to one another under all conditions can be used to predict the composition of protein complexes and reduce the confounding effects of chance co-elution. Our strategies to validate the method will be discussed.