**Combining field flow fractionation (FFF) with SERS for bio-analytical detection**

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Asymmetrical flow field-flow fractionation (AF4) was evaluated as a potential analytical method fordetection of a protective antigen (PA), an Anthrax biomarker. The scheme was based on the recognition of altered AF4 retention through the generation of the size-increased Au nanoparticle probes as aresult of PA binding, in which a PA-selective peptide was conjugated on the probe surface. In the visible absorption-based AF4 fractograms, the band position shifted to a longer retention time as the PA concentration increased due to the presence of probe bound with PAs. The shift was insignificant when the concentration was relatively low at 84.3 pM. To improve sensitivity, two separate probes conjugated with two different peptides able to bind on different PA epitopes were used together. The band shift then became distinguishable even at 84.3 pM of PA sample. In the off-line SERS fractogram constructed using fractions collected during AF4 separation, a band shift was also observed for the 84.3 pM PA sample, and the band intensity was higher when using the dual-probe system.

An analytical scheme, incorporating field-flow fractionation (FFF)-based separation of target-specific polystyrene (PS) particle probes of different sizes and amplified SERS tagging, was demonstrated for simultaneous and sensitive detection of multiple microRNAs (miRNAs). For multiplexed detection, PS particles of three different diameters (15, 10, 5 μm) were used for the size-coding, and a probe single stranded DNA (ssDNA) complementary to a target miRNA was conjugated on an intended PS particle. After binding of a target miRNA on PS probe, polyadenylation reaction was executed to generate a long tail composed of adenine (A) serving as a binding site to thymine (T) conjugated Au nanoparticles (T-AuNPs) to increase SERS intensity. The three size-coded PS probes bound with T-AuNPs were then separated in a FFF channel. With the observation of extinction-based fractograms, separation of three size-coded PS probes was clearly confirmed, thereby enabling of measuring three miRNAs simultaneously. Raman intensities of FFF fractions collected at the peak maximum of 15, 10 and 5 μm PS probes varied fairy quantitatively with the change of miRNA concentrations, and the reproducibility of measurement was acceptable.