

Using FFF-MALS-DLS to Isolate, Quantitate and Characterize Extracellular Vesicles

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Common techniques for isolating extracellular vesicles (EVs) do not discriminate between different classes, while particle sizing methods such as DLS or NTA are biased against the smaller size range. FFF-MALS-DLS combines size-based isolation of different-sized EVs such as exo-S, exo-L and exomeres with on-line analysis for comprehensive characterization of size & size distributions, particle concentration at each size, molar mass and relative composition of lipids, proteins, and oligonucleotides. Isolation with FFF is based purely on physical size. It does not induce shear or high pressure and is not likely to damage the EVs, yet it fractionates with high resolution over a large range. Online characterization and quantification yield well-resolved size distributions and concentrations as well as estimates of protein/nucleic acid ratios. Fraction collection for offline characterization of EVs can be improved with the Dilution Control Module (DCM) for FFF. DCM is a breakthrough technology to increase detector signals by a factor of up to 10, and increase sample concentration in collected fractions, as well as improving reproducibility by precise control of the detector flow. Achieving higher levels of sample concentration improves analysis of EV sample components with low S/N-ratio. The ability to reduce detector flow can increase the upper limit of online DLS measurements.