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

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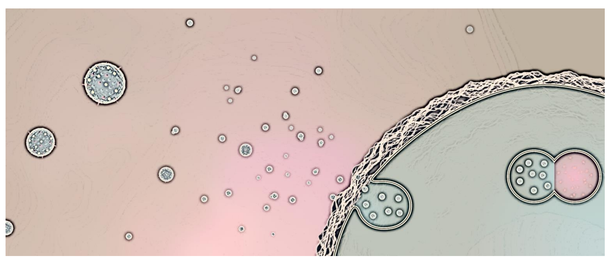
Introduction

What can be obtained from an FFF-MALS-DLS analysis of EVs?

Common techniques for isolating EVs do not discriminate between different classes, while particle sizing methods such as DLS or NTA are biased against the smaller size range.

AF4-MALS-DLS combines size-based isolation with on-line analysis for comprehensive characterization:

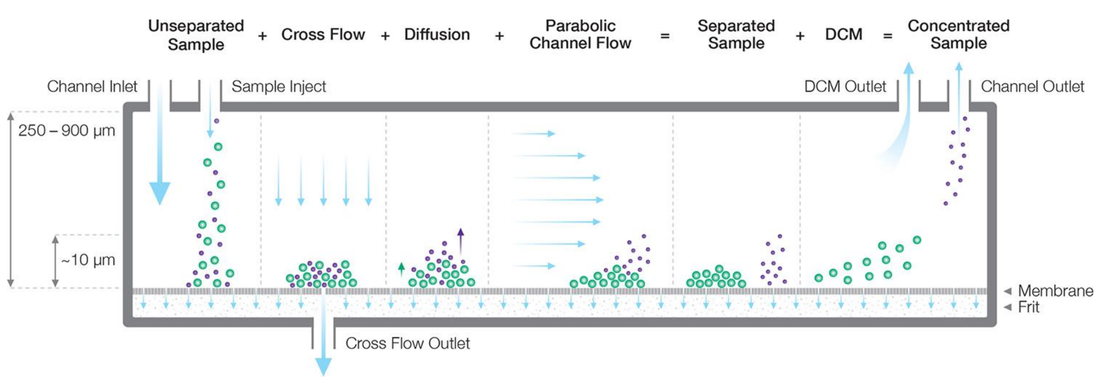
- Size & size distributions (R_g, R_h)
- Concentration (particles/mL) at each size
- M_W and relative composition (lipids/proteins/oligonucleotides)
- Isolation of different-sized EVs such as exo-S, exo-L and exomeres



AF4-MALS-DLS system

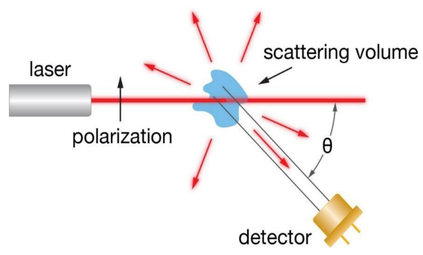
- Standard HPLC including UV detector and fraction collector
- Eclipse™ controller and separation channel
- DAWN® multi-angle light scattering (MALS) instrument with WyattQELS™ embedded DLS module
- Optilab dRI concentration detector

How AF4 with DCM works



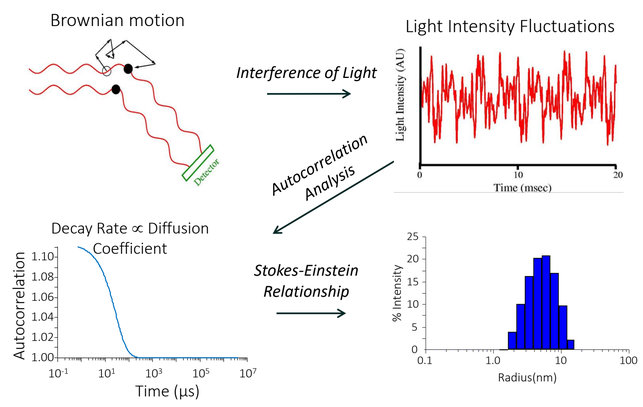
AF4 separates particles from 1 – 1000 nm by hydrodynamic size in an open channel. The sample layer occupies only a small amount of space in the channel. Excess fluid is removed through the DCM port to reduce sample dilution at the end of the separation. This increases detector signals as well as sample concentration in collected fractions.

How MALS and DLS work



$$I_{scatt}(\theta) \propto 1 - KR_g^2 sin^2(\theta/2) + \cdots$$

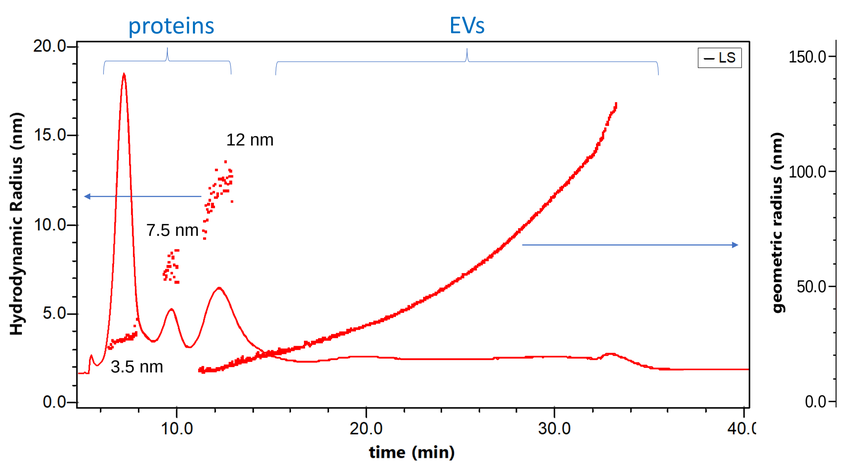
$$I_{scatt} \propto N\left(n_{particle}^2 - n_{solvent}^2\right)^2 V_{particle}^2$$



MALS measures R_g from 10 – 500 nm via the angular dependence of the scattered light, and the particle concentration N via the scattered intensity. Both analyses are based on fundamental physics.

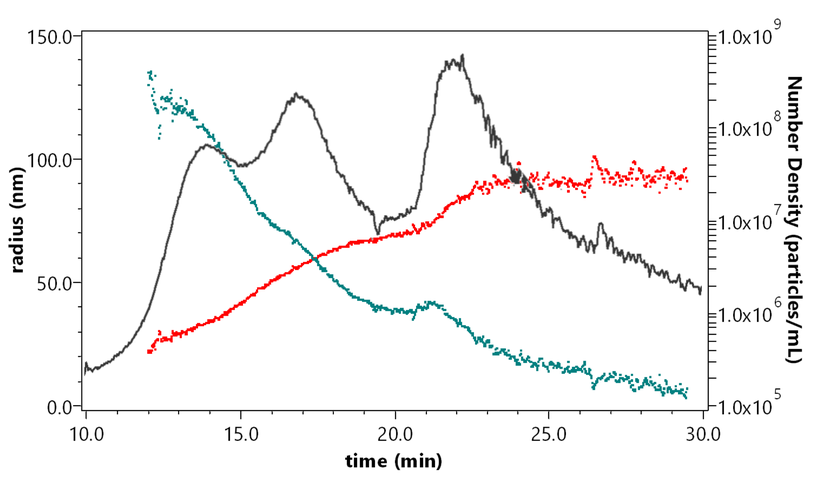
DLS measures the hydrodynamic radius R_h via the rate of fluctuation of the scattered light. The analysis is based on the Stokes-Einstein equation for Brownian motion $R_h \sim 1/D_t$.

Separation from serum and online sizing



The EVs are clearly separated from serum proteins such as HSA, IgGs and lipoproteins that elute in the smaller size range (as determined by online DLS). Since the EVs occur with much lower abundance, MALS is needed to determine their size.

Radius and number density versus elution time



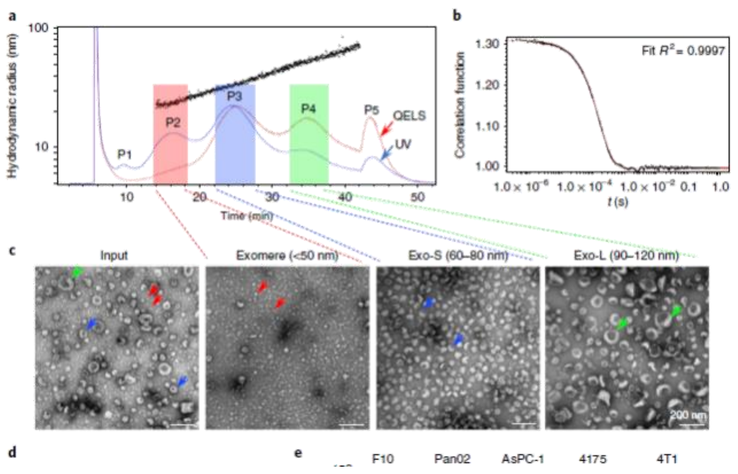
LS intensity - exhibits 3 distinct populations

Geometric radius

Number density – integrate each peak for total EV concentration

The elution time in FFF is directly proportional to R_h, the hydrodynamic radius. For these exosomes, radius by MALS is mostly – but not perfectly – correlated with R_h, indicating structural differences between smaller and larger EVs.

Isolation of size-based populations identifies three types



Zhang, Haiying, et al. "Identification of distinct nanoparticles and subsets of extracellular vesicles by asymmetric flow field-flow fractionation." *Nature cell biology* (2018): 1. doi:10.1038/s41556-018-0040-4

Fractions were collected and analyzed to determine proteomic, genomic and lipidomic content, plus imaging studies.

Zhang et al. identified two distinct types of exosomes, Exo-S and Exo-L, which differ in structure and function, as well as a new type of EV, exomeres, which does not have a lipid membrane. These groups are conserved across many models and cell types.

Conclusions

- AF4-MALS-DLS with Eclipse and DAWN is an ideal system for isolating and characterizing extracellular vesicles.
- Isolation is based purely on physical size so no need to develop complex immunity-capture or precipitation techniques. AF4 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 (right panel).
- Offline characterization of the fractions can provide additional, high-data-content analyses such as gene sequencing, proteomics by mass spectrometry, etc.