Exosome Characterization in Biological Matrices Using Asymmetrical Flow Field-Flow Fractionation and Nanoparticle Tracking Analysis

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Introduction

For therapeutics´ development a comprehensive investigation of formulations during the optimization process is essential for later clinical trials. Often the *in vivo* behavior differs significantly from the previous *in vitro* activity. Therefore a characterization under conditions mimicking the *in vivo* environment is gaining an interest. [1]
Both Field-Flow Fractionation and Nanoparticle Tracking Analysis (NTA) gained increasing popularity for the analysis of complex biological samples including extracellular vesicles such as exosomes over the past years. [2-4]
Here we present the online hyphenation of Asymmetrical Flow Field-Flow Fractionation (AF4), Multi-Angle Light Scattering (MALS) and NTA to analyze exosomes spiked into rabbit serum.

Experimental

The exosome pellet (exosome standard, which was extracted from human urine) was recovered according to the manufacturer´s guideline. Prior to injection the exosomes were diluted to the final concentration of 1.64 x 10^{10} particles mL^{-1} in phosphate buffered saline respectively in a 1:10 dilution of rabbit serum in ultrapure water.
The experimental setup is illustrated in Figure 1. The samples were fractionated in the AF4 channel according to their hydrodynamic size. To reduce the flow rate for NTA measurement and to increase the sample concentration after the dilution in the channel the Slot Outlet technique (also known as Smart Stream Splitting) was used: the upper sample-free solvent stream was split away at the end of the fractionation channel. The AF4 system was also coupled with a MALS detector to derive the size (i.e. radius of gyration, R_g) of the fractionated sample constituents. To deliver the sample at an appropriate flow rate (here approx. 12 µL min^{-1}) for the NTA measurement an additional flow splitter was used for coupling both systems. The NTA determined the hydrodynamic diameter D_h and number concentration of the separated sample constituents.

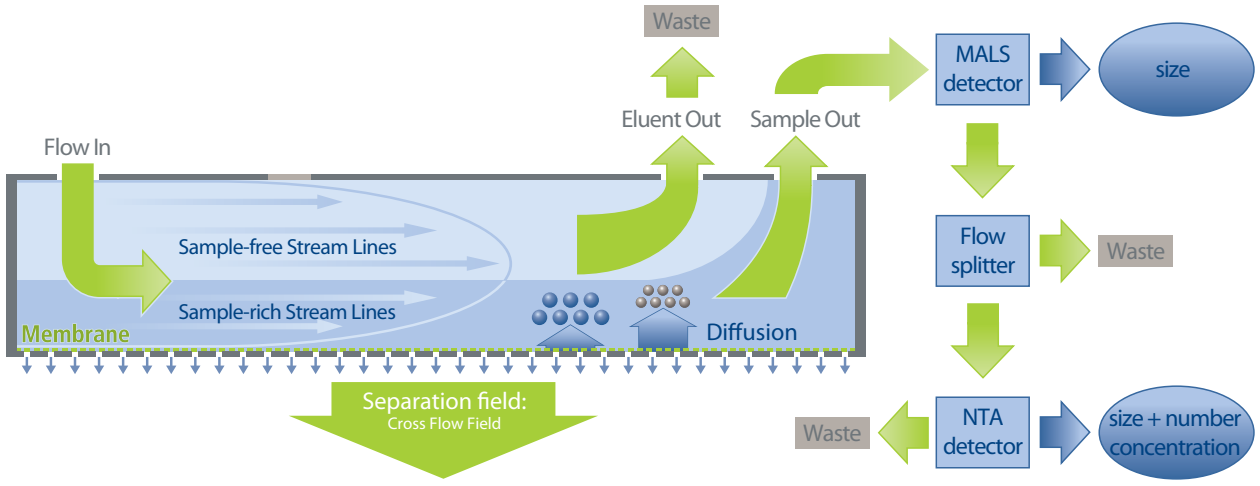

Figure 1: Schematic principle of the AF4-MALS-NTA setup. [1]

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Results

The rabbit serum represents a highly complex mixture containing a variety of proteins and electrolytes. To exclude matrix-induced interferences a comprehensive fractionation step is advised prior to the analysis. Otherwise, various interactions between the matrix components and the incident laser light such as e.g. fluorescence would overlap with the scattered light and may render an accurate size determination by MALS and NTA virtually impossible. In addition, in NTA, the significantly lower intensity of scattered light from small particles may also result in a size distribution that is skewed towards the bigger particles.

Figure 2 shows the obtained AF4-MALS fractograms overlaying the exosome standard, the serum blank and the exosomes in serum including the obtained R_g distributions. The data confirms the successful separation of rabbit serum constituents from the exosomes. However, the serum blank shows also particles in the same size range as the exosomes indicating that there may also be exosomes present already in the serum itself. The R_g distribution of the exosomes ranged from 23 nm to 100 nm and was not affected by the spiking into rabbit serum.

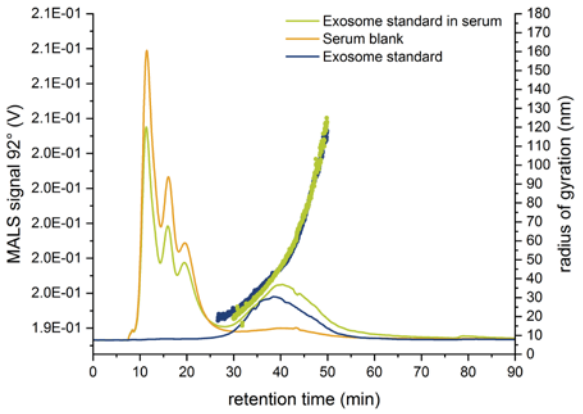

Figure 2: AF4-MALS fractograms overlaying different measurements comparing MALS signals and R_g's (dots) from the exosome-serum sample (green line) with the exosome standard (blue line) and the serum blank (orange line). [1]

In Figure 3, AF4-NTA fractograms with an overlay of D_h and the particle number concentration are displayed for the exosome standard (a) and the exosomes in serum (b). The D_h distribution of the exosomes ranged from around 43 nm up to a maximum of 150 nm. The D_h distribution of the exosome-serum sample ranged from roughly 35 nm up to 90 nm with increasing variation to the exosome standard. The particle concentration and the relationship of R_g and D_h in a blank medium and in serum provides several information on the morphology of the exosomes or indications of a corona formation. The hyphenation of AF4-MALS-NTA delivers this information within one single measurement, which reduces time, costs and used sample material.

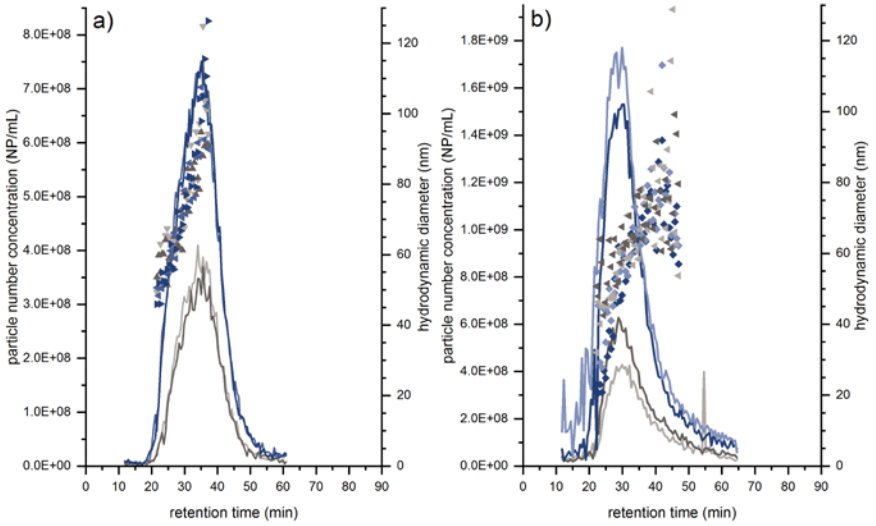

Figure 3: AF4-NTA fractograms of an exosome standard overlaying particle number concentration (lines) and D_h (dots) of (a) an exosome standard and (b) of an exosome-serum sample. [1]

Conclusion

Asymmetrical Flow Field-Flow Fractionation hyphenated to Multi-Angle Light Scattering detection and Nanoparticle Tracking Analysis represents a powerful analytical platform to study the behavior of promising drug delivery vehicles under *in vivo* like conditions. Both techniques complement each other perfectly. The online matrix removal and sample purification capability of AF4 supports the NTA to overcome its limitations while NTA acts as a true particle counting detector for AF4. Together with MALS, AF4-NTA may also enable particle shape analysis.

References

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