Application Note # 176

Size and Concentration Measurement of Extracellular Vesicles Using MP-SPR

This Application Note demonstrates the use of Multi-Parametric Surface Plasmon Resonance (MP-SPR) for simultaneous size and concentration measurements of extracellular vesicles (EVs) derived from COLO-1 cells. MP-SPR quantifies EV capture at the sensor surface and extracts both size and bulk concentration through dual-wavelength operation. The approach builds upon validated methodologies where dual-wavelength detection resolves vesicle deformation effects and improves quantification accuracy. The regenerable sensor surface allows repeated measurements, enhancing throughput and reducing assay costs.

Introduction

Extracellular vesicles (EVs) are lipid-bound nanoparticles secreted by cells, involved in intercellular communication, immune signaling, and disease progression. They hold promise as diagnostic biomarkers and therapeutic carriers, and accurate determination of their size and concentration is crucial for both basic research and translational applications. Conventional nanoparticle characterization techniques, such as nanoparticle tracking analysis (NTA) and dynamic light scattering (DLS), provide bulk size distributions but have several limitations. DLS tends to overestimate vesicle size in polydisperse samples because the signal scales with the sixth power of particle diameter, making large aggregates dominate the readout. Both NTA and DLS usually assume spherical particles, which can bias results if vesicles are deformed, elongated, or irregular. Neither technique measures surface binding interactions or real-time kinetics, as they only provide size and concentration in suspension rather than functional capture properties.

In contrast, MP-SPR provides a label-free, real-time, and regenerable approach. By operating at two wavelengths (670 and 785 nm), MP-SPR exploits different sensing depths of the evanescent field to simultaneously determine vesicle diameter and bulk concentration. This dual-wavelength method corrects for potential EV deformation upon surface capture and differences between subpopulations, leading to improved accuracy, and the integration of regenerable capture chemistries allows sensor surfaces to be reused for multiple EV analyses.

Materials and Methods

Capture strategy:

The BioNavis EV Size and Concentration Measurement Kit (SPR102-EV) streamline the workflow. Biotin-functionalized sensor slides are first activated with regeneration solution, then coated with regenerable avidin (AVD) in PBS. After functionalization with regenerable avidin, the sensor can be further functionalized with biotinylated CD9 (or with an antibody of choice). This creates a highly specific, regenerable surface for EV capture.

Detection principle:

- Dual-wavelength operation (670 nm and 785 nm) provides independent sensing depths. The ratio of shifts between 670 nm and 785 nm directly reveals vesicle diameter.
- Real-time binding slopes under diffusion-limited conditions are analyzed to determine bulk concentration.

Results

Vesicle size determination:

Dual-wavelength MP-SPR measured vesicle diameters in excellent agreement with the manufacturer's specification (HansaBiomed (Product Code: HBM-COLO-30/2)), while also correcting for surface-induced deformation.

- Our measurement (dual-wavelength MP-SPR): 100 ± 15 nm
- Manufacturer specification: 95 ± 7 nm

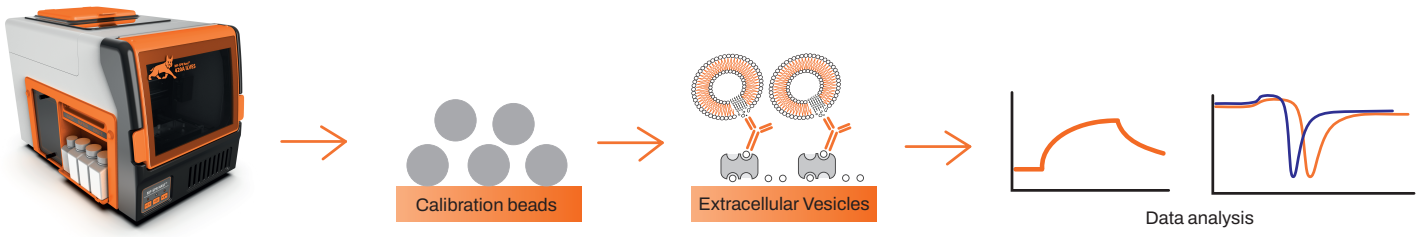

Figure 1. Schematic of the size and concentration measurement methodology using the MP-SPR instrument in combination with the BioNavis EV Size and Concentration Measurement Kit (SPR102-EV).

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Concentration measurement:

Real-time binding slopes showed a strong linear correlation with bulk concentration until saturation, matching previous validations for EV subpopulations.

- Our measurement (dual-wavelength MP-SPR): $(1.5 \pm 0.7) \times 10^{11}$ particles/mL
- Manufacturer specification: $(4.80 \pm 0.48) \times 10^{11}$ particles/mL

Regenerability:

Sensor surfaces were regenerated using the regeneration solution provided in the kit, maintaining consistent performance across multiple capture and release cycles. Each sensor can be reused for up to 30 regeneration cycles without compromising functionality, offering both reliability for scientific applications and cost-effectiveness for routine use.

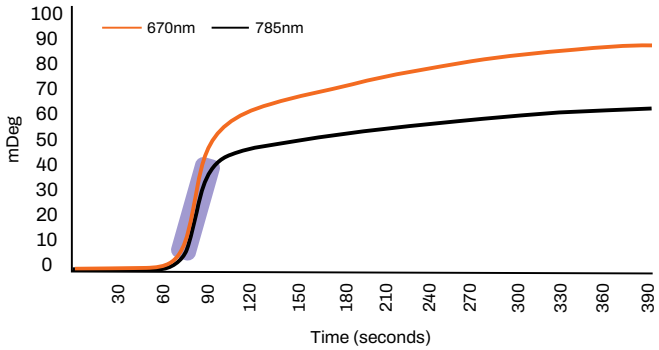

Figure 2. EV concentration measurement derived from sensogram data obtained with the MP-SPR instrument. The highlighted region indicates the signal used for calculating EV concentration.

Recommended instrumentation for reference assay experiments

MP-SPR Navi™ 420A ILVES or 410A KAURIS

Sensor surface: EV Size and Concentration Measurement Kit SPR102-EV

Software: MP-SPR Navi™ Control, DataViewer and LayerSolver for MP-SPR Navi™

Conclusions

MP-SPR delivers a powerful, affordable, and label-free solution for EV characterization. Dual-wavelength measurements provide size determination, while real-time kinetics not only reveal bulk concentrations under diffusion-limited conditions but can also be used to calculate binding interaction kinetics. Regenerable surfaces make repeated analysis practical and cost-effective. This methodology extends beyond EVs, it is equally valuable for nanoparticle characterization, therapeutic quality control, and diagnostics development.

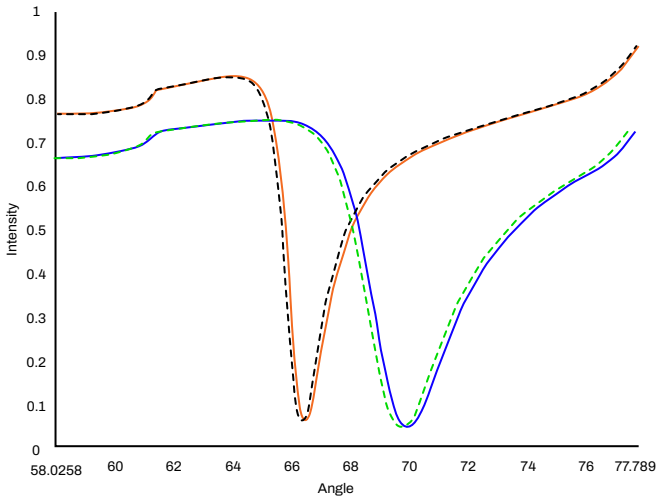

Figure 3. Changes in the complete SPR curve, used to determine the size of extracellular vesicles (EVs).

Read also:

- How Protein Corona Formation on Extracellular Vesicles is Monitored Using MP-SPR (Application Note #175)
- How affinity and kinetics of extracellular vesicles – protein interaction is measured (Application Note #164)
- How extracellular vesicle uptake by cells is measured using MP-SPR (Application Note# 156)

References:

Parkkila et al. Colloids Surf A Physicochem Eng Asp, 2022, 654, 5, 130015

Rupert et al. Anal. Chem., 2014, 86, 12, 5929-5936

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