



Isolation of neuron-specific extracellular vesicles from human plasma using FO-SPR

White paper 9 | Version 2 | Tobias Zbik, Filip Delpont

Summary

Extracellular vesicles (EVs) are an increasingly popular target in the search for novel biomarkers in the field of neuronal disease diagnostics. Investigating the composition and cargo of EVs is still challenging because it is difficult to specifically isolate these highly heterogeneous biomolecules from crude samples. Fiber-optic surface plasmon resonance (FO-SPR) is a powerful tool that harnesses the performance of surface plasmon resonance (SPR) in an easy-to-use dip-in fiber-optic configuration. This application note describes a FO-SPR assay for the isolation of neuron-derived EVs from human plasma samples.

In this approach, the neuron-specific L1CAM transmembrane protein was used as a biomarker and target for EV isolation. Multiple capture and release cycles were tested to enrich the EV yield while using as little plasma sample as possible. Detection and signal amplification with gold nanoparticles was used to verify isolation specificity and full release of the EVs into the elution buffer.

We discuss how this FO-SPR assay can provide a fast, specific, and easy EV isolation method that is directly applicable in complex matrices. In contrast to other available isolation techniques, this method combines immune-affinity isolation with real-time monitoring in a single run. FO-SPR can simplify and improve EV isolation for analysis in this fast-developing field.

Introduction

Extracellular vesicles are becoming increasingly prominent in the field of healthcare because of their potential to offer a non-invasive approach to diagnose a diverse array of diseases, such as cancer, cardiovascular conditions, neurological disorders, and diabetes. These nanometer-scale particles are released by cells and they transport lipids, proteins, nucleic acids, and metabolic compounds in both the intercellular space and the bloodstream. This unique capability facilitates the discovery of disease-specific proteomic and genomic indicators using a straightforward blood sample.

Characterizing EVs, however, presents a range of challenges. They are highly heterogeneous in their size, origin, and molecular composition, and the fluids in which they are found are complex and, therefore, difficult to analyze reliably. Isolating EVs from complex solutions still relies on time-consuming, expensive and complex purification methods that have low yield or purity, and result in high variability in terms of numbers, conditions, and mix of EV subpopulations.

SPR is a technique that has demonstrated great potential for EV analysis and isolation, with reported limits of detection of $10^7 - 10^8$ particles/ml in purified samples. However, most commonly used instruments rely on microfluidics which makes them prone to clogging when using complex matrices like human plasma. Furthermore, the microfluidics on these platforms pose a risk of cross-contamination and impede signal amplification techniques using gold nanoparticles (AuNPs) that can improve sensitivity or specificity via a secondary detection.



FO-SPR offers a valuable alternative to conventional SPR and other EV isolation methods. WHITE FOx is a convenient benchtop FO-SPR instrument that provides the performance of SPR with much reduced complexity. The gold layer used in SPR is applied to fiber-optic probes which can simply be dipped into the sample of interest. This approach eliminates the contamination and clogging issues often encountered when analyzing a series of crude samples like serum, plasma or whole blood. Additionally, the increased surface area of the FO-SPR probes makes them highly suitable for the quantitative isolation of EVs.

Here, we present the results of a feasibility study, showcasing that FO-SPR is well suited for the specific isolation of EVs from complex matrix solutions. We demonstrate proof-of-concept for a simple, highly specific method of preparing a high yield of EVs to be tested in hybrid assays. Using a low pH glycine elution buffer, as in this example, proves highly efficient but can affect the structural integrity of the isolated EVs. Still, a glycine elution buffer may provide EVs for downstream assays, such as mass spectrometry or PCR, to study their membrane protein composition and cargo. To this end, we will demonstrate that EVs can be isolated and enriched through multiple capture and release cycles from human plasma samples, which proves to be a fast and easy method to yield isolated EVs while using only minimal volumes of precious samples.

The target molecule in this study is the L1CAM protein. L1CAM is a transmembrane protein that is found in neuronal cells where it is involved in cell adhesion, and is frequently used as a biomarker for neuronal EVs. We will be using a commercially available anti-L1CAM antibody to specifically capture and isolate neuron-derived EVs. Isolation will be performed from human plasma samples from patients that have been diagnosed with Alzheimer's disease.

Materials and methods

Fiber Optic SPR

FOx BIOSYSTEMS turned an optical fiber into a mass sensitive sensor using the well-established surface plasmon resonance (SPR) principle for biomolecular interaction studies. The SPR effect is achieved by coupling a white light source to the fiber optic sensor probe.

In the probe, the light interacts with a gold layer and senses the refractive index up to 200 nm away from the outer surface. At the tip of the probe, light is reflected back through the bifurcated fiber to a spectrometer. The resonance condition is monitored by tracking the wavelength at which the least light is reflected. The fiber optic surface plasmon resonance (FO-SPR) sensor is coated with bioreceptor molecules which can bind to the target molecules of interest. This binding changes the refractive index, resulting in a wavelength shift that produces a sensor signal as shown in Figure 1.3. The sensor probe is simply dipped into the liquid sample to measure biomolecular interactions directly.

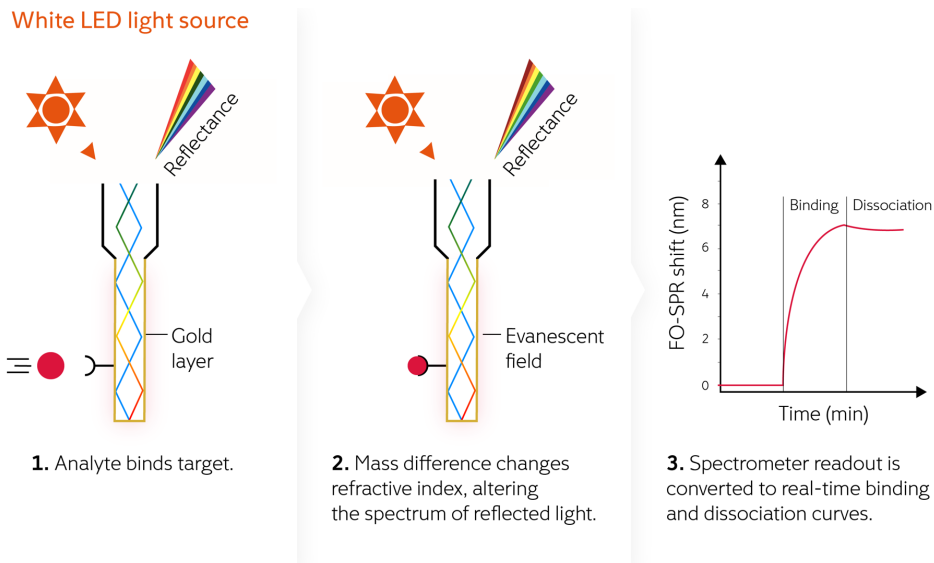


Figure 1: The FO-SPR principle



Tools and reagents

Buffers / reagents	Concentration
Anti-L1CAM	10 µg/ml in MES
Anti-CD81	10 µg/ml in MES
hIgG	10 µg/ml in MES
Biotin-CD9	10 µg/ml in PBST
Anti-biotin AuNP (gold nanoparticles)	20 OD
WHITE FOx carboxyl sensor probes	-
PBS(T)	1X, 0.01% Tween-20, pH 7.4
MES	10 mM MES, pH 6
MES(T)	10 mM MES, 0.01% Tween-20, pH 5.7
Ethanolamine	0.05 M, pH 8.5
Superblock	1X
EDC: 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide	0.4 M in EDC/NHS
NHS: N-hydroxysuccinimide	0.1 M in EDC/NHS
Glycine	10 mM, pH 1.5
Patient plasma: healthy (HP)	NA
Patient plasma: diseased (DP)	NA

Table 1: Buffers and reagents for EV isolation targeting L1CAM.

Detection of neuron-derived EVs

To determine that we can specifically capture neuron-derived EVs from human plasma, we used an amplification setup with gold nanoparticles. To this end, FO-SPR carboxyl probes were functionalized* with three different antibodies: anti-L1CAM, anti-CD81 and a hIgG (10 µg/ml in MES). Anti-CD81 was used as a positive control to capture total EVs since CD81 is a general EV marker, while hIgG served as a negative control since it cannot specifically bind EVs. The functionalized probes were dipped into plasma samples from patients diagnosed with Alzheimer's disease (DP) for 30 minutes. As a negative control, one additional probe was immobilized with anti-L1CAM-antibody and dipped into plasma from a patient that was not diseased (HP). The probes were then transferred to a well containing biotin anti-CD9 antibody (10 µg/ml in PBST) for 10 minutes.

Amplification was achieved by dipping the probes in streptavidin-AuNP (1.5 OD in BPST) for 10 minutes (Figure 2).

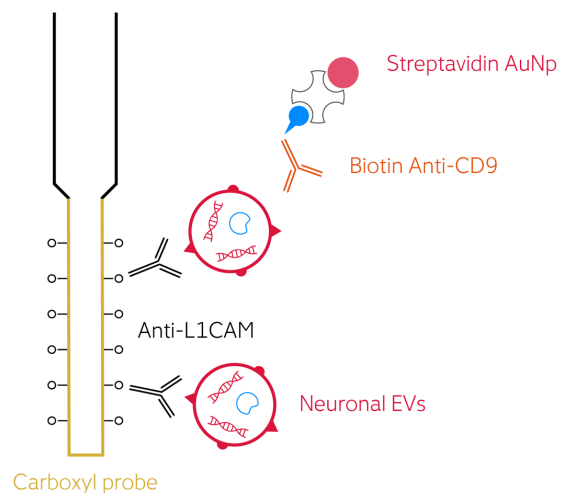


Figure 2: Specific EV signal amplification with gold nanoparticles



EV isolation through multiple capture and release cycles

The quick and scalable isolation of specific EVs from valuable plasma samples is a crucial first step for further downstream analysis of their protein content and cargo. Here we show how FO-SPR can isolate EVs through multiple capture and release cycles from a single plasma sample. This increases the total EV yield with very low sample volume requirements. Figure 3 shows the workflow of this setup.

First, anti-L1CAM antibody is immobilized on carboxyl probes and dipped into plasma samples to capture neuronal EVs.

The success of this capture step is verified by detection via biotin anti-CD9 antibody and amplification with streptavidin-AuNPs. The captured EVs are subsequently released in elution buffer (10 mM glycine, pH 1.5). To verify that release was successful, the detection and amplification steps are repeated on the probe. The probe is then dipped into plasma again to repeat the EV capture. Further subsequent detection and amplification show that the capture and release steps are repeatable without damaging the probe or the anti-L1CAM antibody.

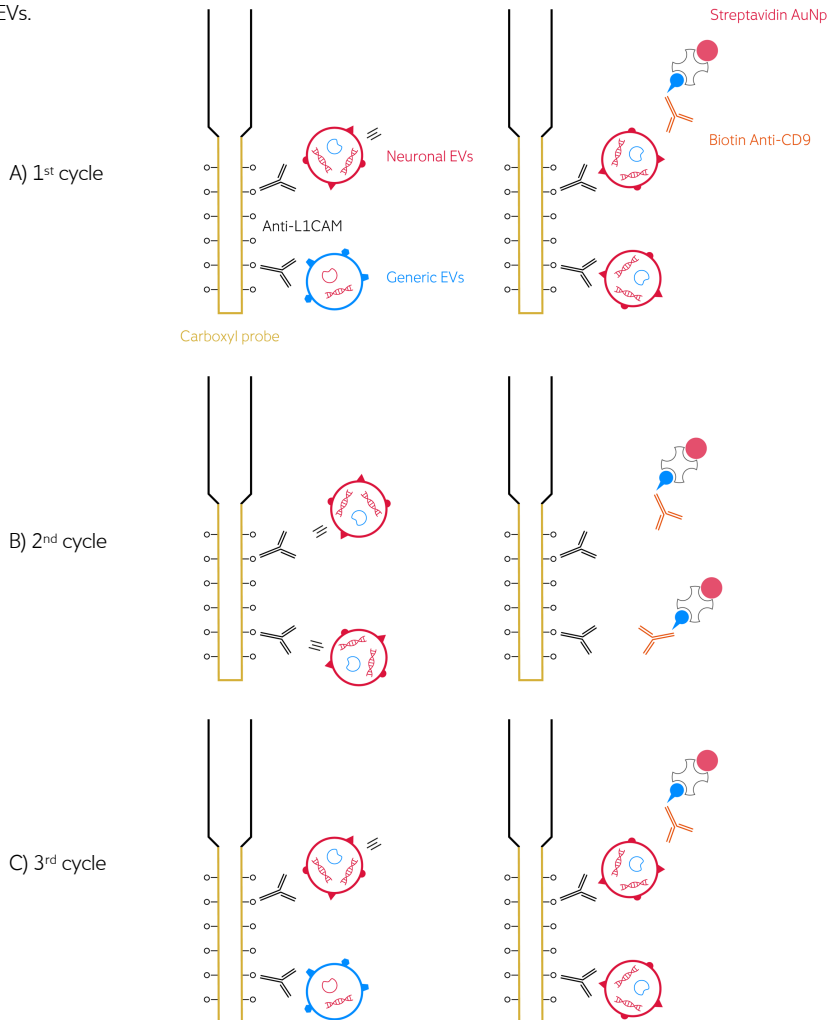


Figure 3: Workflow of multiple capture and release cycles for neuronal EV isolation with verification via gold nanoparticle amplification.

A) The anti-L1CAM antibody is immobilized on the carboxyl probe surface and specifically captures neuronal EVs expressing the L1CAM protein. A biotin anti-CD9 antibody is used to detect bound EVs with an additional signal amplification step using streptavidin-AuNP.

B) Bound EVs are released into elution buffer. The detection and signal amplification with anti-CD9 is repeated to verify that no EVs remain on the probe.

C) The capture and detection is repeated as in the first cycle. This shows that the capture and release can be repeated from the same plasma sample to accumulate EVs in the elution buffer.



Results

The specific capture of neuron-derived EVs from patient plasma samples was successfully demonstrated using a gold amplification step and several control samples. The amplified signal of EVs captured with the anti-L1CAM antibody from diseased patients shows a high signal shift (Figure 4). The control plasma sample from healthy patients (HP) shows around a 2.5-fold lower signal shift. This indicates that the neuron biomarker L1CAM is more commonly found in EV samples that originate from diseased patients than EV samples from healthy patients.

The hIgG negative control shows a very small shift that can be attributed to non-specific binding. In contrast, the anti-CD81 antibody captures the total EV population and shows a high shift relative to the hIgG negative control. The signal increase obtained using the anti-CD81 probe is not as high as with the anti-L1CAM probes, although it should have a higher number of EVs that it can capture from the serum. However, this observation agrees with other findings from our lab (unpublished data) in which CD81 shows a rather slow association rate.

The findings in this run confirm that we specifically capture and detect neuron-derived EVs from plasma samples with immobilized anti-L1CAM antibody.

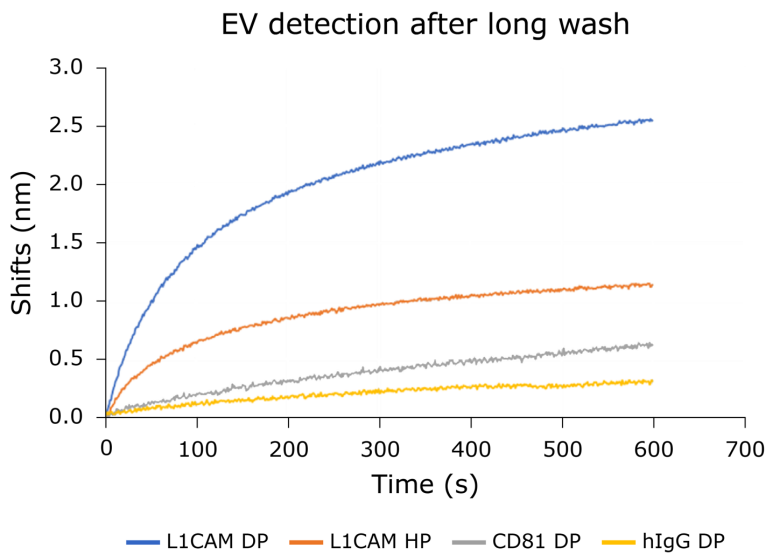


Figure 4: EV detection from plasma samples with gold amplification.

EVs are captured from plasma samples by the anti-L1CAM, anti-CD81 or hIgG immobilized on carboxyl probes. A biotin anti-CD9 antibody is used to detect bound EVs. The graph shows the subsequent signal amplification step using streptavidin-AuNP.



In a second setup, we repeated the capture and release cycle from plasma samples with the anti-L1CAM antibody and verified the EV isolation by repeated gold amplification. In the first step, we can see a high shift from the diseased plasma sample and a much lower shift from the healthy plasma sample (Figure 5A). Of note is that the plasma in this experiment originated from different patients than in the previous experiment, hence the differences in total shift. After the first release, gold amplification does not further increase the shift, demonstrating that the EVs had been released into the elution buffer (Figure 5B). In the third cycle, the capture from plasma was repeated using the same probe and the same plasma sample. In this cycle, the gold amplification again shows a high signal in the diseased sample, and much lower in the healthy sample (Figure 5C). This confirms that repeated capture and release cycles can be applied and used to enrich the neuronal EVs in the elution buffer.

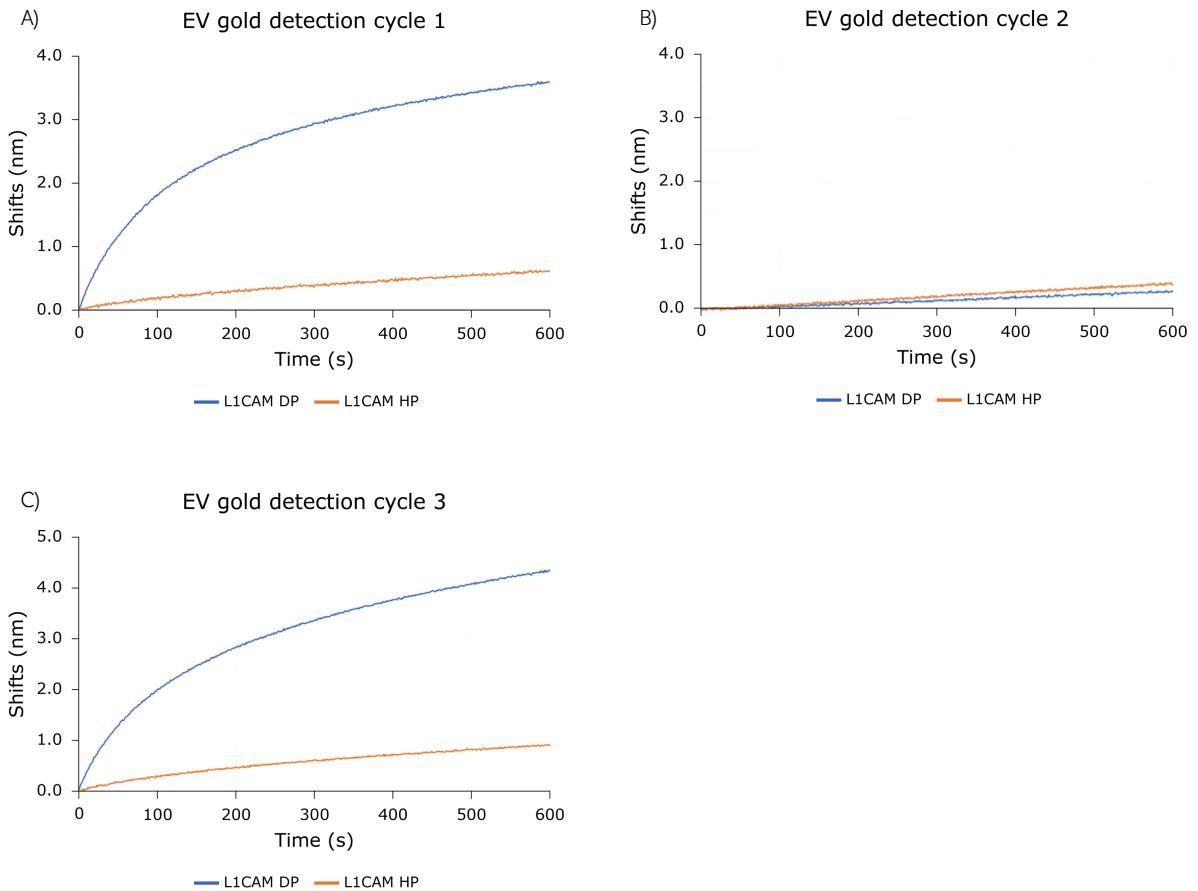


Figure 5: EV capture, release and recapture from patient plasma, detected with biotin anti-CD9 and amplified with strep-AuNP. L1CAM DP is measured in a patient sample, L1CAM HP is measured in a healthy control sample.

- A) The first EV capture shows a higher signal in the DP compared to the HP.
- B) After EV release in the elution buffer, the amplified signal is nearly flat showing that EVs were successfully eluted.
- C) Repeated capture of EVs from plasma samples leads to signal shifts that are similar to the first cycle.



Conclusions

The isolation of EVs from complex samples like plasma or serum is a challenging task. Difficult and expensive methods that offer low yield and high non-specific binding are still widely used. Here we demonstrate how FO-SPR can be applied to the specific isolation of EVs from patient plasma samples.

We present a proof-of-concept, in which WHITE FOx was used to capture and release neuronal EVs twice from a single human plasma sample. With this approach, the yield of the EV isolation can be scaled as required by simply increasing the number of cycles performed. This makes the fast method potentially applicable for a broad range of commonly used hybrid assays in downstream content analysis methods, such as mass spectrometry or PCR, to analyze the composition and cargo of the EVs. Additionally, the ability to reuse the same probe for multiple isolation cycles makes it very cost-efficient.

In this study, we worked with the anti-L1CAM antibody which specifically binds the L1CAM transmembrane protein that is a frequently used neuronal biomarker in EV research. However, this is not only limited to EVs as biomarkers. The flexibility of the FO-SPR probes allows the implementation of a variety of different antibodies, aptamers, or other capture biomolecules.

Another interesting finding was that different patients showed different levels of neuronal EVs in their plasma samples. These results reveal another strength of the FO-SPR approach: Not only does it give immediate feedback about your samples it also does not blindly pick its targets. This provides much more insight into what is happening in the sample than when using other common methods like immunoprecipitation. Furthermore, this technique makes it much easier to control the quality of your samples and to adapt and optimize isolation protocols.

flexible, and easy-to-use tool to the field of EV isolation that can be applied in both crude and purified samples. The yield can be scaled to match the desired downstream application and it gives additional insights into the content of the samples with minimal cost requirements. This makes FO-SPR a versatile technology with huge potential for EV research where other methods have proven sub-optimal.

Acknowledgements

The authors would like to acknowledge Aida Montserrat Pagès and Gesa Rascher-Eggstein for helping to organize and plan the work, and Sarah Parmentier, Laura Marin, and Kris Ver Donck for discussions on the paper.

Bibliography

1. Y. Yildizhan et al. (2021) FO-SPR biosensor calibrated with recombinant extracellular vesicles enables specific and sensitive detection directly in complex matrices. *Journal of Extracellular Vesicles*. 10 (4), e12059; DOI: 10.1002/jev2.12059

Related white papers

* White paper 1: Carboxyl probe immobilization for label-free protein quantification

White paper 5: Extracellular vesicle quantification in complex matrices

In conclusion, the FO-SPR technology introduces a fast,

Contact

info@foxbiosystems.com

+32 11 28 69 73



FOx BIOSYSTEMS NV
BioVille, Agoralaan Abis
3590 Diepenbeek, Belgium
www.foxbiosystems.com

WHITE FOx is for research use only,
not for diagnostic purposes.