Affinity and kinetics of extracellular vesicles – protein interaction

Extracellular vesicles (EVs) are extensively studied as potential therapeutics, drug delivery systems and for non-invasive diagnostics of diseases, such as cancer. Early detection of ovarian cancer was explored using Multi-Parametric Surface Plasmon Resonance (MP-SPR) instrument. Tumor EVs (SKOV-3) were loaded onto surface-immobilized LXY30 peptide. Affinity and kinetic constants of the interaction, bound mass and layer thickness were determined based on MP-SPR measurement. LXY30 peptide exhibits a high affinity to EVs expressing α3β1 integrin and offers possibility for diagnostic applications.

Introduction

Extracellular vesicles (EVs) are nano-sized membrane vesicles that are released by human cells into body fluids. They are known to have an important role in delivery of signals to surrounding cells and facilitate intercellular communication even with distant cells. EVs reflect composition and state of the parent cell, and thus can be potentially used as markers for a variety of diseases, such as cancer. The membrane signature of EVs can be represented by integrins (transmembrane glycoproteins) which affect for example cell adhesion, inflammation, thrombosis and metastasis. Specific integrins are overexpressed in certain tumor cells creating characteristic combinations, and thus are interesting targets for cancer diagnostic (detection) and therapeutic applications (i.e. blocking of integrins to reduce metastasis). Ovarian tumor cells and their secreted extracellular vesicles express α3β1 integrin. This particular integrin can be targeted with a cyclic nonapeptide LXY30 which has been developed for detection of the EVs.

Typically, EVs are characterized by various techniques requiring labels and using endpoint analysis methods which do not provide real-time information. However, introducing a label may alter the biochemical structure of particles, and thus affect the results. MP-SPR is a real-time, label-free and extremely sensitive method, measuring physicochemical phenomena under dynamic flow conditions. Therefore, it can be considered as an excellent method to characterize interactions of extracellular vesicles.

The proven Surface Plasmon Resonance (SPR) detection method is widely used in different biosensor studies, such as evaluating affinity and kinetics of molecular interactions and in determination of biomolecule concentration. Multi-Parametric Surface Plasmon Resonance (MP-SPR) is an excellent platform to study a greatly extended variety of targets. Unique optical configuration of MP-SPR enables assays from small molecules and antibodies to nanoparticles and even whole living cells. Special optics combined with robust fluidic set-up allows MP-SPR to assess not only purified samples but also crude samples, such as milk, sea water, plasma, serum and saliva. These features are highly relevant for biosensor development and applicable for research in Life Sciences domain.

Distinctively, MP-SPR provides not only affinity and kinetics of the interaction but also information about layer properties and adsorbed mass from the same experiment. Due to measurements being performed in an exceptionally wide angular range (40-78 degrees) and at more than one wavelength of light, thickness and refractive index of the deposited layer can be evaluated.

Materials and methods

Prior to MP-SPR experiment, BioNavis standard gold sensor slides were coated *ex situ* with a self-assembled monolayer (SAM) of biotinylatedalkanethiol (11-mercapto-1-undecanol, MuOH). Once in the instrument, the baseline signal was set in the running buffer TRIS-EDTA (25 mM TRIS, 1 mM EDTA, pH 8.0), followed by injection of streptavidin into both channels. Biotinylated LXY30 peptide was introduced only in one channel, leaving the second channel as a reference without peptide. Extracellular vesicles SKOV-3 from ovarian cancer cells were loaded in both channels within sequential injections from lowest to highest concentration (3.4 × 109, 5.7 × 109, and 1.7 × 1010 particles/ml). The data was analyzed using TraceDrawer™ for MP-SPR Navi™ and MP-SPR Navi™ LayerSolver.

Further details of the measurement can be found in the original publication of Carney *et al*. 2017.

Results and discussion

Extracellular vesicles were successfully extracted from cancer cell culture supernatant and characterized to confirm success of the isolation procedure (Carney *et al*. 2017). Based on nanoparticle tracking analysis (NTA), the concentration of extracellular vesicles was 1.70 ± 0.11 × 1013 particles/ml and the size distribution was 177 ± 87 nm.

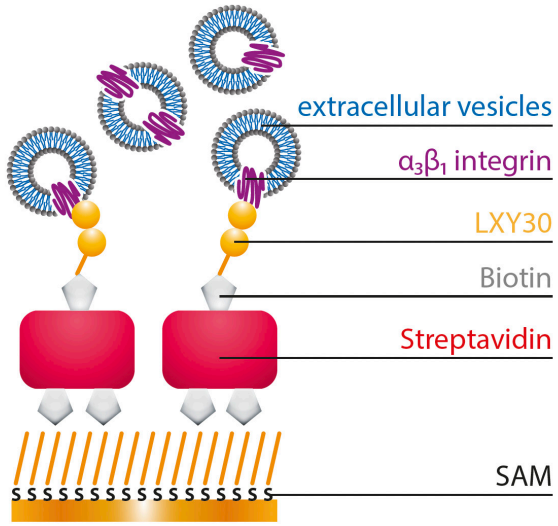

Figure 1. Binding of extracellular vesicles on nonapeptide determined for early detection of ovarian cancer.

Interaction of surface bound peptide (LXY30) with flowing extracellular vesicles (SKOV-3) was determined by MP-SPR (Figure 1). The "two-site" binding model of TraceDrawer™ software was used to analyze the interaction interpreted as two sets of peptide molecules binding to one extracellular vesicle. Three hypothetical cases of EVs were assessed here, where the α3β1 integrin amount on the surface was evaluated to either 1%, 5%, or 10% of the total protein per particle. Affinity of all three cases indicated a strong

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binding of SKOV-3 particles to the surface-bound LXY30 ligands (Figure 2, Table 1). In this study, only three concentrations of analyte were measured, however, it is generally recommended to have five or more concentrations to evaluate affinity and kinetics.

Thickness and refractive index (RI) of the adsorbed EV layer were evaluated by fitting the full SPR curves in the LayerSolver™ software (Figure 3). Analysis was based on multi-wavelength measurement of MP-SPR, provided by 670 and 758 nm laser sources. The surface mass density of surface bound EVs was estimated to be 90 ng/cm2. Refractive index of the EV layer was 1.341, which is slightly higher than the RI of aqueous buffer solution like PBS. The calculated thickness of the exosome layer was only 34 nm based on MP-SPR, even if average diameter of the particles was 177 ± 87 nm based on NTA. This might be explained by the fact that MP-SPR provides average thickness from the measurement area, while the EVs were unevenly distributed over the sensor. The low thickness value was most likely caused by empty spaces between the exosomes bound onto the surface. Also, the deformation of the EVs shape due to binding may explain the observed lower value of thickness. Furthermore, size and concentration of the extracellular vesicles can be assessed with high accuracy when using the obtained MP-SPR data with a modelling protocol published by Rupert *et al*. 2016.

Conclusions

MP-SPR has proven to be an excellent platform to characterize interactions of extracellular vesicles, as shown in this example. A single MP-SPR measurement provided several numerical values, such as affinity and kinetic constants, thickness, refractive index and adsorbed mass, while the experiment was performed in a label-free manner. The approach presented here is not limited to extracellular vesicles but is applicable to other types of nanoparticles as well, including polymeric and metallic nanoparticles. The possibility to work with various samples, surfaces and matrices make MP-SPR an outstanding platform for biosensor development and life science research.

See also how MP-SPR can measure the formation of protein corona on liposome in 100% serum Application Note #151 and how exosomes and other nanoparticles uptake by living cells was measured Application Note #156.

Original publication:

Carney *et al.*, Advanced Biosystems Vol. 1, Issue 5, 2017

Reference:

Rupert *et al*., Analytical Chemistry Vol. 88 (20), 2016

Recommended instrumentation for reference assay experiments

MP-SPR Navi™ 200 OTSO, 400 KONTIO, 210A VASA, 410A KAURS, 220A NAALI and 420A ILVES with additional wavelenghts -L

Sensors surfaces: CMD, Au or inorganic coating

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

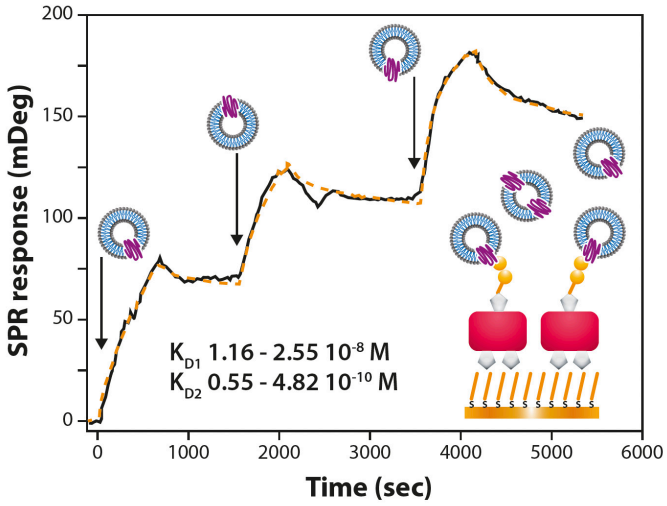

Figure 2. Affinity and kinetic constants of peptide – extracellular vesicle interaction was determined using TraceDrawer™ for MP-SPR Navi™. The solid black line shows measured data, and the dashed orange line represents the fit to sensogram from sequential injections of three different populations of EVs having A) 1%, B) 5%, and C) 10% coverage of α3β1 integrin.

Estimated α3β1	k_{a1} (1/M*s)	k_{d1} (1/s)	K_{D1} (M)	k_{a2} (1/M*s)	k_{d2} (1/s)	K_{D2} (M)
1%	5.14×10^{6} ($\pm 6.97 \times 10^{5}$)	9.35×10^{-3} ($\pm 9.11 \times 10^{-5}$)	**1.82×10^{-8} ($\pm 4.23 \times 10^{-10}$)**	1.28×10^{6} ($\pm 7.28 \times 10^{2}$)	7.00×10^{-5} ($\pm 1.88 \times 10^{-5}$)	**5.46×10^{-11} ($\pm 1.47 \times 10^{-11}$)**
5%	6.32×10^{6} ($\pm 1.85 \times 10^{4}$)	7.30×10^{-3} ($\pm 1.54 \times 10^{-4}$)	**1.16×10^{-8} ($\pm 5.83 \times 10^{-10}$)**	2.52×10^{6} ($\pm 5.93 \times 10^{2}$)	6.05×10^{-5} ($\pm 2.53 \times 10^{-5}$)	**2.40×10^{-10} ($\pm 1.01 \times 10^{-10}$)**
10%	2.90×10^{5} ($\pm 1.86 \times 10^{4}$)	7.40×10^{-3} ($\pm 1.26 \times 10^{-4}$)	**2.55×10^{-8} ($\pm 2.08 \times 10^{-9}$)**	1.26×10^{6} ($\pm 6.30 \times 10^{2}$)	6.07×10^{-5} ($\pm 2.13 \times 10^{-5}$)	**4.82×10^{-10} ($\pm 1.71 \times 10^{-10}$)**

Table 1. Affinity and kinetic constants of SKOV-3 particles binding to LXY30 peptide.

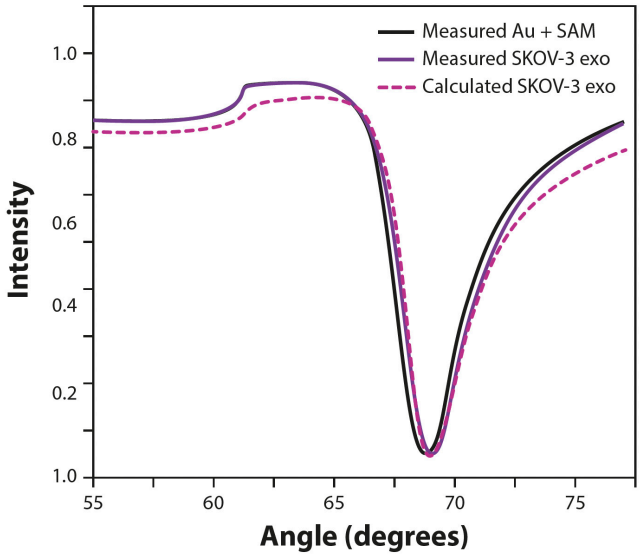

Figure 3. Full MP-SPR curves before and after adsorption of SKOV-3 extracellular vesicles were analyzed using LayerSolver software to solve thickness and refractive index of the particle layer. Multi-wavelength analysis was performed but for presentation clarity only 670 nm measurement curves are presented here.

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