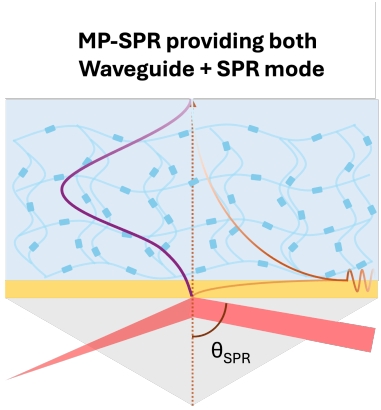
Application Note # 178

# MP-SPR for Continuous and Label-Free Small Molecule Monitoring Using Micrometer-Thick Hydrogels

*Surface plasmon resonance (SPR) has long been used for label-free detection of molecular interactions, especially at surfaces. However, when applied to soft and thick materials such as hydrogels or functional polymer films, traditional SPR alone may not fully capture changes occurring deeper within the material. This is where the waveguiding effect becomes a powerful asset in plasmonic biosensing. This application note describes how MP-SPR featuring waveguide detection enables sensitive and real-time characterization of a novel aptamer-crosslinked hydrogel (aptagel) designed for continuous monitoring of the antibiotic vancomycin.*




## Introduction

In Multi-Parametric Surface Plasmon Resonance (MP-SPR), when a thick dielectric layer (typically >200 nm) is present on the metal surface, it can support waveguide modes in addition to surface plasmons. These modes arise because the thick layer behaves like an optical waveguide, trapping light and producing additional resonance features in the MP-SPR signal. By analyzing the number and positions of these resonance dips, **MP-SPR with its built-in waveguide capability** becomes a powerful tool for characterizing thicknesses and refractive indexes of multilayer films or thick coatings, sometimes up to **tens of micrometers** [1]. In contrast, traditional SPR is confined to near-surface regions, typically within ~200-300 nm of the gold interface. In this application note by continuous monitoring of the complete SPR curve, it was possible to measure the aptamer hydrogel layer and vancomycin interaction beyond the standard SPR sensing range.

## Experimental Overview

Au-coated MP-SPR sensors were modified with a thin hydrogel layer composed of 8-arm PEG-norbornene (10 kDa) crosslinked by thiolated split-aptamer sequences in different configurations (monothiolated split-aptamer pairs (MM), a combination of monothiolated and dithiolated splitaptamer pairs (MD), dithiolated split-aptamer pairs (DD), and the dithiolated full parent aptamer sequence (Full)). Upon UV-induced thiol-ene coupling, the hydrogel was covalently attached to the thiol-functionalized gold surface, forming a stable and responsive matrix. The split-aptamers were engineered to recognize vancomycin and simultaneously act as dynamic cross-linkers. Measurements were performed at physiological temperature (37 °C) under continuous flow (50 µL/min) in PBS (pH = 7.4) containing 2 mM $MgCl_2$, using the four-channel MP-SPR Navi™ 400 KONTIO system equipped with 670nm and 785 nm laser wavelengths. The parameters of the hydrogel - thickness (d) and refractive index (n) - were extracted by fitting the waveguiding mode reflectivity spectra (with 670 nm laser wavelength) with Fresnel models. These were estimated to be 2.41 µm and 1.3510, respectively (Figure 1). Further information can be found in the original publication Park, *et al*., 2025.

## Results and discussion

When vancomycin was introduced into the microfluidic chamber, a clear optical response was observed. The hydrogel exhibited a decrease in thickness (Δd), attributed to analyte-induced contraction, and a simultaneous increase in refractive index (Δn), reflecting the local densification and molecular binding events (Figure 1 & 2). These structural changes were detected simultaneously using both SPR and waveguide modes. The waveguide mode, in particular, demonstrated higher sensitivity. This is largely due to its narrow resonance width, quantified as the Full Width at Half Maximum (FWHM) of the reflectivity dip. A narrower FWHM results in a sharper and more distinct signal, which allows for higher angular resolution and lower baseline noise during real-time sensing. This ultimately enhances the limit of detection (LOD).

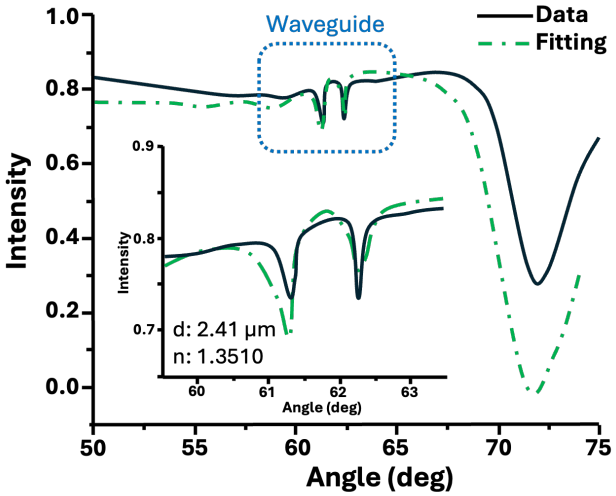



**Figure 1.** Representative example of experimental data fitting used to extract the refractive index and optical thickness of the hydrogel from the waveguide modes as seen from the full reflectivity (recorded with 670 nm laser wavelength) spectrum and highlighted by the blue box.

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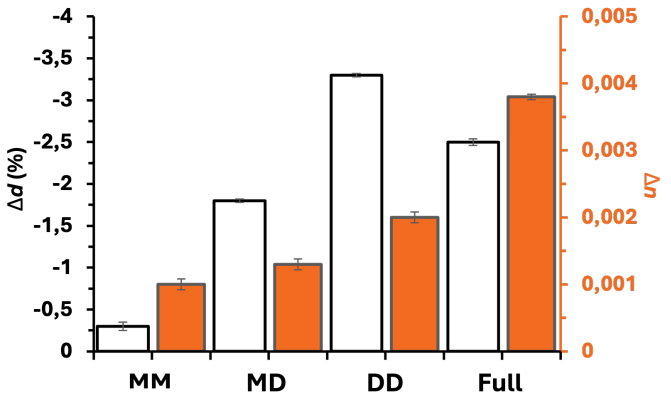


**Figure 2.** The relative change in thickness (Δd%) and refractive index (Δn) was calculated to assess the impact of 1 mM vancomycin binding across different cross-linking configurations.

Indeed, comparative analysis (Figure 3) revealed that the aptagel achieved a LOD of 0.25 µM when tracked by minimum of SPR incident angle shift ($\Delta\theta_{min}$), while the waveguide mode reflectivity change (ΔR) monitored at a specific angle yielded an improved LOD of 0.16 µM. For reference, an aptamer monolayer sensor, lacking the hydrogel volume and waveguide amplification, exhibited a substantially higher LOD of 1.36 µM.

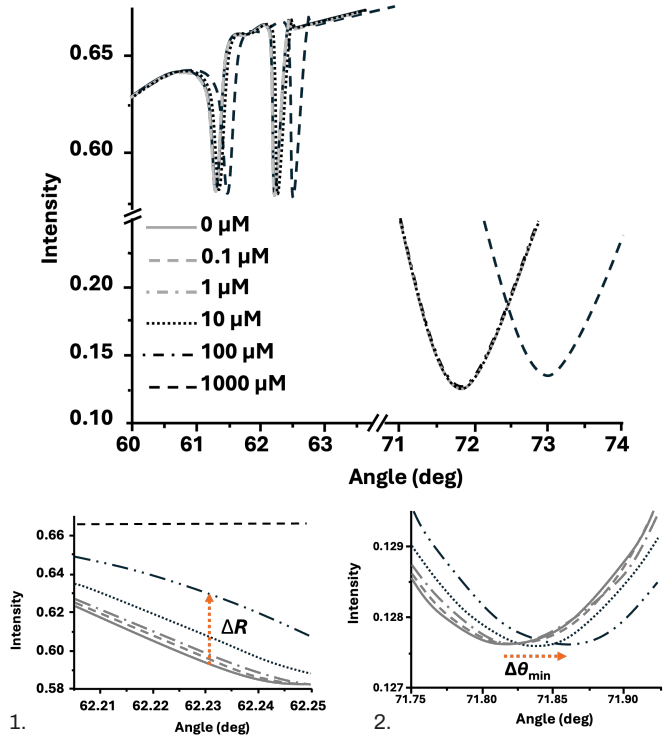


**Figure 3.** Full angular reflectivity (R) spectra of the aptagel recorded at different vancomycin concentrations, illustrating two analysis modes: (1) waveguide reflectivity changes (ΔR) and (2) SPR angle shifts ($\Delta\theta_{min}$, shown in insets) recorded with 670 nm laser wavelength.

In addition to performance in buffer, the aptagel was tested in physiologically relevant media (data not shown here). In 200x diluted rat plasma, simulating interstitial fluid, the sensor maintained a LOD of 0.32 µM. In undiluted horse serum and 5x diluted human plasma, LODs of 0.27 µM and 0.68 µM were achieved, respectively. These values are based on $\Delta\theta_{min}$ monitoring.

The benefits of the waveguide mode could extend beyond improved sensitivity. The authors of the article emphasize that due to its deeper sensing volume, it can be capable of capturing volumetric binding responses (Park, *et al.*, 2025). However, they attribute that this is not the case with their proposed aptagels, because of the homogeneous structure of the system.

## Conclusions

The integration of optical waveguide mode analysis within the MP-SPR framework enables powerful new capabilities for soft matter biosensing. In this study, the waveguide mode provided superior resolution, lower LOD, and thickness and refractive index parameter evaluation, which would not be as accurate with traditional SPR peak minimum angle monitoring alone.

**Original publication:**

Park, *et al.*, J. Am. Chem. Soc. 2025, 147, 13, 11485–11500

**References**

1. Casteleijn *et al.*, Colloids and surfaces, 2018, 539.

### Recommended instrumentation for reference assay experiments

MP-SPR Navi™ 400 KONTIO, 410A KAURIS & 420A ILVES

Sensor surface: SPR102-AU

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



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