Application Note # 180

MP-SPR Characterization of Graphene-Based Biosensors for Virus and Immune Cell Detection

This application note demonstrates the use of Multi-Parametric Surface Plasmon Resonance (MP-SPR) to characterize functionalized graphene surfaces for biosensing applications. Graphene-coated SPR sensors provide an alternative surface for ligand immobilization and can support enhanced signal responses in selected applications. MP-SPR enables real-time, label-free detection of virus–antibody and cell interactions, supporting the analysis of immune receptor binding and cell-based biosensing.

Introduction

Biosensors are designed to selectively and specifically generate a measurable signal upon analyte binding. Signal enhancement in SPR-based biosensing can be achieved either by increasing analyte concentration or by improving the sensor substrate and surface chemistry. Various nanomaterial coatings have been explored to enhance SPR performance, among which graphene has emerged as a particularly promising substrate.

Graphene is well suited for SPR-based biosensing because it:

- enhances the effective plasmonic field interaction at the metal–dielectric interface
- enables high-density and reproducible ligand immobilization via π–π linker chemistry, utilizing non-covalent aromatic interactions between the linker and surface
- can improve signal-to-noise ratio by reducing nonspecific contributions (assay dependent)
- stabilizes the sensor surface

Multi-Parametric Surface Plasmon Resonance (MP-SPR) enables label-free, real-time monitoring of biomolecular interactions and also provides detailed characterization of adsorbed layer properties. By recording the complete SPR curve, including the Total Internal Reflection (TIR) angle and parameters such as the Peak Minimum Angle, MP-SPR allows modelling of layer thickness, optical mass, and refractive index changes.

While conventional gold MP-SPR sensor slides provide robust and well-established performance, enhanced sensitivity remains highly desirable for applications involving whole-cell detection or low-abundance receptors, where refractive index changes can be subtle due to the large size and complexity of cellular analytes. MP-SPR sensor slides and instrumentation readily support the use of coated sensor surfaces on gold, such as monolayer graphene, which can significantly enhance the sensor response. Graphene-coated gold sensors provide enhanced signal intensity and improved ligand immobilization efficiency, thereby increasing sensitivity for challenging biosensing applications, including cell-based receptor detection.

Recent studies demonstrate enhanced sensitivity in graphene-coated MP-SPR sensor slides, enabling clear discrimination of virus interactions (1) and receptor-specific detection of FPR2 on transfected cells and primary human neutrophils (2).

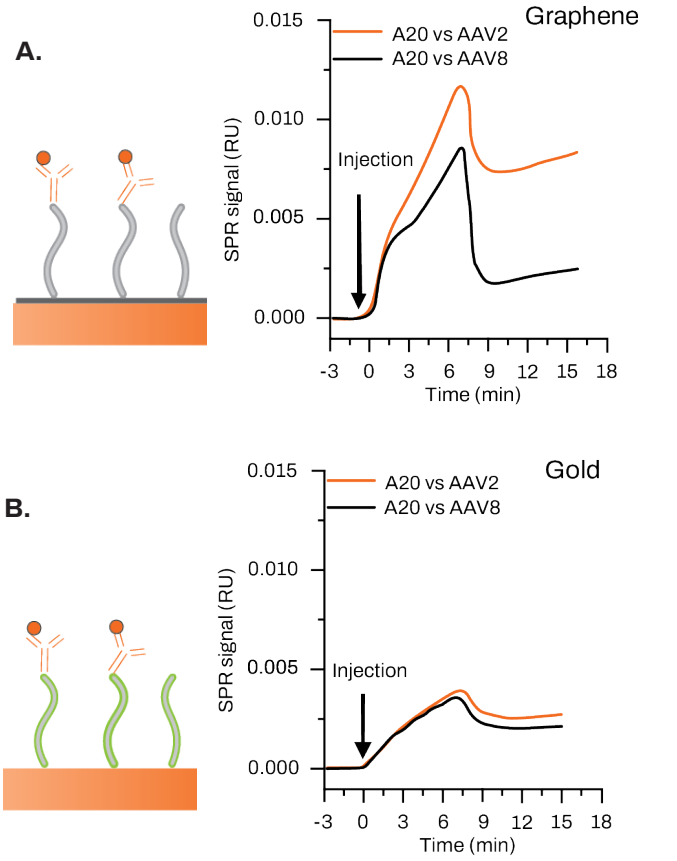

Figure 1. AAV interaction on MP-SPR sensor slide. MP-SPR sensograms showing the interaction of AAV2 (orange) and AAV8 (black) with A20 antibody immobilized on (a) graphene-coated sensor slide and (b) gold sensor slide. *Adapted from Hasnain et al. 2025*

Materials and Methods

Gold MP-SPR sensor slides were coated with CVD-grown monolayer graphene transferred using a PMMA support layer. After air-drying, the chip was cured at 150 °C for 1 h and kept under vacuum overnight to ensure good graphene–gold contact. The PMMA layer was removed using acetone and isopropanol, followed by rinsing and nitrogen drying. The surface was then incubated with 1-pyrenebutyric acid (60 min), and ligands (peptides or antibodies) were covalently immobilized using amine coupling chemistry (1,2).

Measurements were performed using BioNavis MP-SPR Navi™ instrument, equipped with dual-channels (target and control) with real-time angular shift monitoring. Data were analyzed using TraceDrawer™ for MP-SPR Navi™ software. Surface layer properties and surface coverage were further analyzed using LayerSolver™ for MP-SPR Navi™.

BioNavis

email: info@bionavis.com
www.bionavis.com

Results and discussion

Enhanced Sensitivity on Graphene-Coated Sensors
MP-SPR sensograms showed significantly higher responses on graphene-coated sensor surfaces compared with conventional bare gold sensors (1,2). This signal enhancement was observed across several biological systems, including virus detection and peptide-cell interaction assays.

In antibody-virus experiments, graphene-coated MP-SPR sensor slides functionalized with monoclonal antibodies produced significantly stronger responses for AAV2 virus binding, while control virus AAV8 showed minimal interaction (Figure 1A). In contrast, bare gold sensors generated weaker signals and did not clearly distinguish between the two virus serotype (Figure 1B). These results demonstrate that graphene can improve detection sensitivity and enables more reliable discrimination of specific virus-antibody interactions (1).

The improved signal-to-noise ratio observed on graphene surfaces is attributed to enhanced ligand immobilization and stronger plasmonic field interaction at the graphene-gold interface. As a result, graphene-enhanced SPR sensors can provide improved sensitivity for detecting biomolecular interactions involving viruses.

Detection of Cells
FPR2-transfected HEK293T cells were injected at low concentration (~100 cells/mL). MP-SPR measurements demonstrated strong association on the target peptide channel with minimal binding on the control peptide channel. Approximately four-fold higher responses were observed for the specific interaction, and the signal was eliminated when receptors were blocked prior to the measurement (Figure 2B).
To further evaluate the system under physiological conditions, primary human neutrophils from donor samples were analyzed to detect FPR2 at native receptor expression levels. MP-SPR measurements showed strong binding to the target peptide surface and a significant reduction in signal following receptor-blocking treatment (Figure 2C).

The responses were reproducible across multiple donors, and the stable post-rinse signal confirmed specific peptide-FPR2 interactions on living immune cells (2).

Conclusions

MP-SPR is a powerful and versatile tool for biosensor development, enabling real-time, label-free analysis of biomolecular interactions. Its flexible platform allows the integration of advanced surface coatings, such as graphene, which can significantly enhance performance. In the demonstrated application, graphene integration improved ligand immobilization, increased sensitivity, enabled more specific discrimination of biomolecular interactions, and enabled the detection of viruses and cells at low analyte concentrations.. Notably, such graphene integration is uniquely supported within the BioNavis MP-SPR platform, providing a distinct advantage for advanced biosensing applications.

Sensor surfaces can be readily functionalized using established methods such as CVD graphene growth, ALD coatings, spin coating, and self-assembled monolayers (SAMs), enabling flexible and efficient biosensor development. Furthermore, the availability of an electrochemical (EC) flow cell enables combined electrochemical and SPR measurements, further expanding the capabilities of MP-SPR for advanced biosensing and surface analysis applications.

Original publication:

1. Hasnain *et al.*, Adv. Healthcare Mater. 2025, 14, e01723.
2. Hasnain *et al.*, Adv. Sci. 2026, e19436

Recommended instrumentation for reference assay experiments

MP-SPR Navi™ 210A VASA & 220A NAALI

Sensor surface: Au & Graphene

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

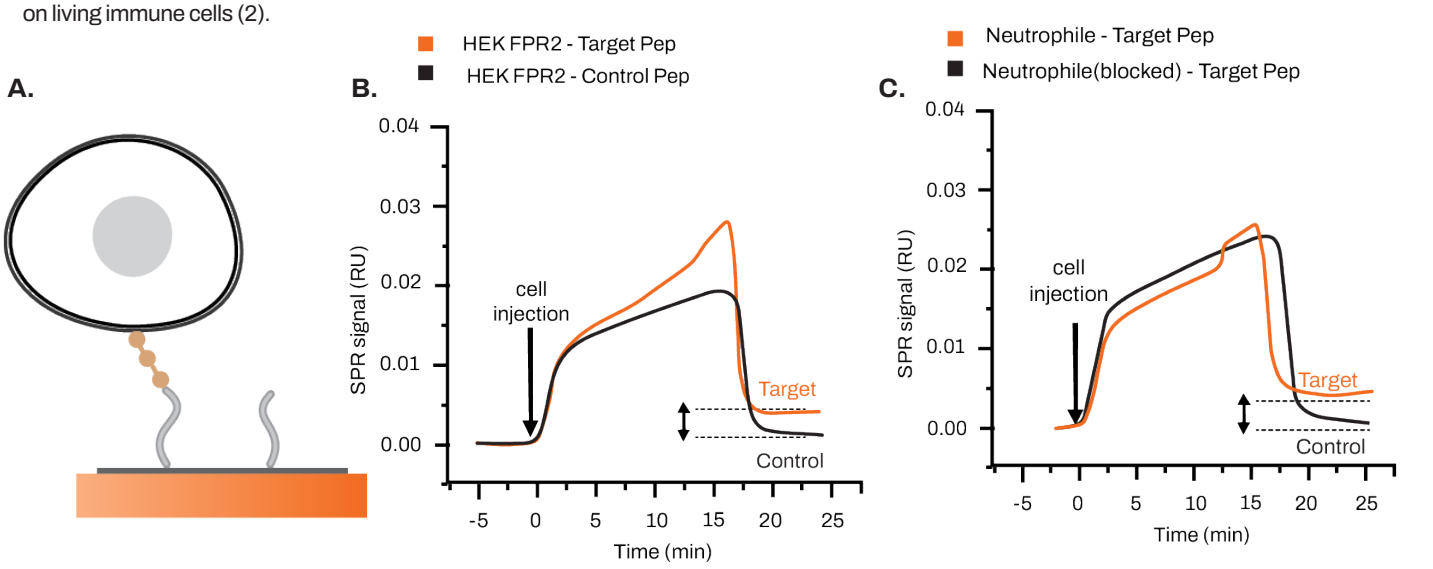

Figure 2. Cell interaction on a sensor slide. (a) Schematic illustration of cell capture on graphene coated MP-SPR sensor slides. (b) MP-SPR sensograms showing the interaction of FPR2-expressing HEK293T cells (orange) and control HEK293T cells (black) with the immobilized peptide WKYMVm-NH_2. (c) MP-SPR sensograms showing the interaction of human donor–derived neutrophils (orange) with the peptide surface, compared with receptor-blocked neutrophils (black). *Adapted from Hasnain et al. 2026*

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BioNavis
email: info@bionavis.com
www.bionavis.com