# GOLD NANOPARTICLE-BASED STRATEGIES FOR IMPROVING SARS-COV-2 ANTI-RBD IMMUNOSENSOR SENSITIVITY

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Although global management of COVID-19 has shifted into a post pandemic phase, the continued circulation of SARS-CoV-2 and the long-term consequences of infection highlight the need for ongoing monitoring of immune responses. Antibodies targeting the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein remain key biomarkers for assessing immunity acquired through infection or vaccination. Accurate quantification of anti-RBD provides valuable insight into both naturally acquired and vaccine-induced immunity, enabling evaluation of an individual's immune status.

Surface plasmon resonance (SPR) immunosensors offer real-time, label-free detection of antigen–antibody interactions and show strong potential for sensitive serological analysis. However, further improvements in analytical performance are required to achieve lower detection limits in complex biological samples. Gold nanoparticles are widely recognized for their ability to enhance optical signals and amplify biosensor responses. Therefore, the aim of this work was to develop SPR-based immunosensors for quantitative anti-RBD detection by employing gold nanoparticles to strengthen the analytical signal and improve overall immunosensor sensitivity.

In this work, two SPR immunosensor variants based on an indirect sandwich-type detection format were developed. In the first design, commercially available streptavidin-modified gold nanoparticles were conjugated with biotinylated anti-IgG antibodies (anti-IgG$_{biot}$–SAv–AuNPs). In the second approach, gold nanoparticles synthesized via the Turkevich method were functionalized with a mixed self-assembled monolayer of 11-mercaptoundecanoic acid and 2-mercaptoethanol, activated using EDC/NHS chemistry, and subsequently modified with 3-aminoboronic acid (AuNPs-11-MUR/2-MEA-3-ABR). In both cases, the resulting nanoparticle conjugates were used to amplify the analytical signal during the second biorecognition step.

Both immunosensors enabled quantitative detection of anti-RBD antibodies over distinct concentration ranges and exhibited strong linear responses. The immunosensor employing anti-IgG$_{biot}$–SAv–AuNPs demonstrated sensitive detection within the picomolar to nanomolar range, while the immunosensor employing AuNPs-11-MUR/2-MEA-3-ABR achieved enhanced sensitivity in the low picomolar range. The latter approach provided a notably lower detection limit, highlighting the effectiveness of nanoparticle surface functionalization in improving analytical performance. Both immunosensors were successfully applied for anti-RBD antibody quantification in human serum samples, demonstrating high accuracy and excellent recovery values. These results confirm the suitability of the developed SPR immunosensors for sensitive and reliable estimation of SARS-CoV-2 anti-RBD antibody levels in biological samples.