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Innovative Cell-Driven SPR Competition Frameworks to Quantitatively Characterize Monoclonal Antibody Activity

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ABSTRACT

Antibody-derived therapeutics represent a major and rapidly expanding segment of modern biopharmaceuticals, with established clinical utility across oncology, inflammatory diseases, and immune-mediated disorders. The therapeutic performance of monoclonal antibodies (mAbs) arises from multiple functional mechanisms, including antigen neutralization, antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and modulation of immune signaling pathways. As a result, successful mAb development requires detailed functional and biophysical characterization to fully understand their mechanism of action. Central to both pharmacodynamic behavior and in vivo efficacy is the specific interaction between the antibody and its target antigen, necessitating robust analytical strategies capable of accurately interrogating binding properties. Surface Plasmon Resonance (SPR) is extensively utilized during mAb development as a label-free technique for evaluating binding kinetics, affinity, and functional activity in vitro. SPR-based methods enable quantitative assessment of receptor–antibody interactions and determination of active antibody concentrations required for target engagement. In particular, competition assays performed using SPR provide valuable insight into epitope overlap and neutralizing potential. Nevertheless, traditional SPR formats that depend on purified recombinant proteins frequently fail to recapitulate the complexity of antigen presentation and molecular context encountered on living cell membranes. To overcome these limitations, this study presents an advanced SPR methodology incorporating intact, live cells as the binding substrate, thereby offering a more biologically relevant platform for potency evaluation. The approach enables interrogation of two key competitive interaction modes: functional neutralization, measured through inhibition of ligand–receptor engagement on cell surfaces, and competitive epitope binding between antibodies directed against the same membrane-associated antigen. By integrating cellular systems into SPR analysis, this strategy enhances the physiological relevance and interpretability of potency measurements, supporting improved monoclonal antibody characterization during early-stage therapeutic development.

Keywords

Surface plasmon resonance
Cell-integrated SPR assays
Antibody potency evaluation
Monoclonal antibody characterization

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INTRODUCTION

Antibody engineering and development represent foundational activities in modern biotechnology and therapeutic innovation, centered on the generation of highly selective antibodies—most commonly monoclonal antibodies (mAbs)—capable of recognizing and interacting with defined molecular targets [1]. These biologic agents exert their therapeutic effects through a broad range of mechanisms, including direct neutralization of pathogenic entities, activation or modulation of immune effector pathways, and regulation of cellular signaling processes. Given the molecular complexity and inherent heterogeneity of antibody-based products, extensive characterization is indispensable to ensure product safety, clinical efficacy, and manufacturing robustness [2]. Such characterization provides critical insight into antibody structure, functional behavior, and immunogenic potential, thereby directly influencing development strategies, regulatory evaluation, and clinical performance [2].

A detailed understanding of the mechanism of action (MoA) by which an antibody engages its intended antigen is essential for predicting therapeutic effectiveness. In vitro potency assays play a central role in this process, serving as analytical tools to quantify the functional activity of biologics, including therapeutic mAbs, throughout development, regulatory submission, and routine quality control [3]. To serve as reliable indicators of clinical performance, potency assays must closely reflect the anticipated MoA and provide conclusive evidence of the antibody's capacity to elicit its intended biological effect. Accordingly, multiple assay formats may be employed depending on the antibody class and therapeutic objective, including cell-based functional assays, binding assays, and competitive interaction assays [4].

Among these approaches, cell-based functional assays offer the most biologically relevant evaluation for antibodies whose activity relies on Fc-mediated effector functions, such as antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and antibody-dependent cellular phagocytosis (ADCP). These assays approximate in vivo biological processes while accounting for the intrinsic variability associated with cellular systems. Historically, traditional cell-based assays assessed antibody activity by measuring phenotypic cellular responses, including proliferation, apoptosis, differentiation, or migration [5]. More recently, reporter gene–based cellular assays have gained prominence, providing improved assay robustness and operational simplicity by indirectly quantifying potency through activation of receptors, intracellular signaling cascades, or downstream effector molecules [6-10].

Binding assays constitute another key component of antibody characterization, focusing on verification of correct target engagement. These assays are commonly performed using recombinant antigens or receptors to quantify active antibody fractions through enzyme-linked immunosorbent assays (ELISA) or to characterize binding kinetics and affinity using Surface Plasmon Resonance (SPR) [11]. Although highly informative from a biophysical perspective, binding assays generally capture only a limited aspect of biological function, primarily restricted to antigen recognition, and do not fully represent therapeutic activity [12]. Additionally, removal of biomolecules from their native biological context may substantially alter interaction dynamics. This limitation is particularly evident for membrane-associated proteins, which may adopt distinct conformations when evaluated as isolated extracellular domains rather than as full-length proteins embedded within a cellular membrane [13].

Competitive binding assays further support antibody characterization by addressing two principal questions: the capacity of an antibody to inhibit ligand–receptor interactions, characteristic of neutralizing antibodies, and the potential for epitope overlap among antibodies targeting the same antigen. Conventionally, such competition studies are conducted using recombinant targets in ELISA-based formats or SPR kinetic assays [13]. However, these simplified systems frequently underestimate the influence of the cellular microenvironment on molecular interactions [14]. To mitigate this limitation, cell-based competition assays employing technologies such as flow cytometry (FACS) or electrochemiluminescence (ECL) have been developed. Despite their improved biological relevance, these methods are often labor-intensive, time-consuming, and dependent on differential labeling of interacting molecules, which may inadvertently perturb native protein–protein interactions [15].

A notable advancement in overcoming these methodological constraints is the integration of cell-based assay formats with SPR kinetic analysis. This combined approach enables real-time, quantitative assessment of biomolecular interactions within a biologically relevant context while preserving the kinetic resolution intrinsic to SPR technology. Importantly, SPR is inherently label-free, eliminating the need for secondary reporters such as fluorescent dyes or radioactive tags that can interfere with molecular binding events [16].

Despite its promise, the application of SPR to live cell systems has been limited, largely due to technical challenges associated with incorporating intact, viable cells into SPR platforms [17]. Cell-based SPR assays are susceptible to issues including signal instability, non-specific interactions, and complex response profiles that complicate data interpretation. Successful implementation requires careful optimization of experimental parameters, such as cell density, buffer composition, surface chemistry, and regeneration conditions.

One of the most critical challenges lies in balancing sufficient interaction between analytes and target-expressing cells with preservation of cellular integrity and physiological relevance throughout the assay duration [18,19].

In the present study, we demonstrate the practical feasibility of employing live, whole cells within an SPR framework to enable comprehensive characterization of monoclonal antibody mechanisms of action. Furthermore, we introduce two alternative experimental workflows that are broadly adaptable and potentially applicable to a wide range of therapeutic modalities and target cell types.

MATERIALS AND METHODS

SPR Multi-Cycle Kinetic (MCK) Competition Assays

In Surface Plasmon Resonance (SPR) binding experiments, interaction kinetics are derived from real-time variations in response signals recorded as a function of time. Sensorgrams generated across a range of analyte concentrations are analyzed collectively as a single dataset, and appropriate kinetic models are applied to calculate association and dissociation rate constants, as well as overall binding affinity. In the multi-cycle kinetic (MCK) format, individual analyte concentrations are injected sequentially in discrete cycles, with surface regeneration performed between injections to restore baseline conditions [20].

For SPR-based competition studies, the MCK strategy is typically implemented by immobilizing one of the interacting or potentially competing molecules onto the sensor surface, followed by injection of mixtures containing the target and a second competing species at increasing concentrations. Evidence of competitive interaction is inferred from a concentration-dependent reduction in target binding to the surface-captured molecule.

In this work, the conventional MCK competition assay configuration was modified by immobilizing intact cells onto a CM5 sensor chip, as described above, thereby enabling competition analysis directly at the cellular interface.

All SPR measurements were conducted using a Biacore™ T200 instrument (Cytiva, catalog #28975001). Experimental parameters, including running buffer composition, association times, and dissociation phases, are specified in the corresponding tables within the Results section.

SPR MCK Assay on Protein A Sensor Chip for Non-Competition Verification

Control experiments designed to confirm the absence of competitive interactions were performed using a Series S Protein A sensor chip (Cytiva, #29127556) on a Biacore™ T200 platform. The running buffer consisted of HBS-P+ 10× (Cytiva, #BR100671) diluted 1:10 with ultrapure water. Detailed conditions for analyte injection, association, dissociation, and concentration ranges are provided in Table 1.

SPR Cell-Based Competition Assay to Assess Antibody Competition on Target-Expressing Cells

To evaluate antibody competition at the cellular level, a recombinant human IgG1 antibody produced internally by Menarini Biotech S.r.l. was employed and designated as mAb1. A recombinant mouse IgG1 recognizing the same antigen (mAb2) was obtained from Invitrogen and used as the competing antibody. The A549 human lung carcinoma cell line, which endogenously expresses the target antigen, was sourced from the American Type Culture Collection (ATCC, #CCL-185).

A549 cells were maintained under adherent culture conditions at 37°C in a humidified atmosphere containing 5% CO_2 and 80% relative humidity. Cells were expanded in Corning culture flasks (T25, T75, and T175) using complete growth medium consisting of Gibco™ DMEM (high glucose, with pyruvate; Thermo Fisher Scientific, #41966029) supplemented with 10% fetal bovine serum (FBS; Thermo Fisher Scientific, #16250-078).

Cells were passaged based on confluency, and at passage 5, cryopreservation was performed at a density of 3×10^6 cells per vial in freezing medium composed of 45% fresh medium, 45% conditioned medium, and 10% dimethyl sulfoxide (DMSO; Sigma, #D2438-10 mL).

SPR MCK Assay Using Recombinant Antigen on Protein a Sensor Chip

SPR kinetic analyses using recombinant antigen were carried out on a Biacore™ T200 instrument employing a

Table 1: Optimization of Cell Acidification Conditions for CM5 Sensor Chip Immobilization.

Immobilization Buffer Composition	Buffer pH	Final Cell Suspension pH	Cell Viability (%)	Immobilization Outcome
DPBS only	7.4	7.2	98	No immobilization
DPBS : NaOAc (1:1)	3.5	5.6	96	Low coupling efficiency
DPBS : NaOAc (1:1)	3.0	5.2	95	Moderate immobilization
DPBS : NaOAc (1:1)	2.5	4.9	94	Optimal immobilization

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Series S Protein A sensor chip. The target antigen consisted of a recombinant human protein supplied by R&D Systems [21]. Protein A–mediated capture ensured consistent antibody orientation through selective Fc-region binding, thereby facilitating accurate assessment of antigen–antibody interactions. HBS-P+ running buffer was diluted 1:10 with water prior to use. Experimental conditions and analyte concentrations are detailed in Table 2 of the Results section.

SPR Cell-Based MCK Competition Experiments

For cell-based competition measurements, the MCK format was applied by injecting mixtures containing target-expressing cells and one competing antibody at a fixed concentration, while progressively increasing the concentration of the second antibody in successive cycles.

An AffiniPure™ goat anti-human IgG F(ab′)$_2$ fragment–specific antibody (Jackson ImmunoResearch, #109-005-006) was immobilized onto a Series S CM5 sensor chip (Cytiva, #BR-1005-30) using standard amine coupling chemistry with the Amine Coupling Kit (Cytiva, #BR-1000-50). Bovine serum albumin (BSA; Thermo Fisher Scientific, #23209) immobilized on the reference flow cell served as a control for non-specific interactions.

Buffer composition, injection parameters, and association and dissociation conditions for each experiment are reported in the relevant tables in the Results section [22].

RESULTS

One of the most common mechanisms of action (MoA) of therapeutic monoclonal antibodies involves the partial or complete inhibition of ligand–receptor interactions. This mechanism is central to the activity of antiviral antibodies, where the ligand corresponds to a viral component and antibody binding prevents viral attachment to host cell receptors, thereby blocking cellular infection [22].

In this study, a novel SPR-based strategy employing live receptor-expressing cells was developed using a Biacore™ platform to directly assess the neutralizing capability of monoclonal antibodies under biologically relevant conditions.

The conceptual framework of the assay is illustrated in Figure 1. Briefly, intact host cells expressing the receptor of interest were immobilized on a CM5 sensor chip, followed by injection of mixtures containing a constant concentration of ligand and progressively increasing concentrations of an anti-ligand monoclonal antibody. A concentration-dependent reduction in ligand binding to immobilized cells was interpreted as evidence of neutralization. As depicted in Figure 1, this experimental configuration closely mimics the MoA of antiviral neutralizing antibodies.

Table 2: Live Cell Immobilization Performance on CM5 Sensor Chip.

Flow Cell	Immobilized Material	Immobilization Method	Immobilization Level (RU)	Surface Stability
Fc1	BSA (Reference)	Amine coupling	~8,000	Stable
Fc2	Receptor-expressing CHO cells	Amine coupling	~17,000	Stable

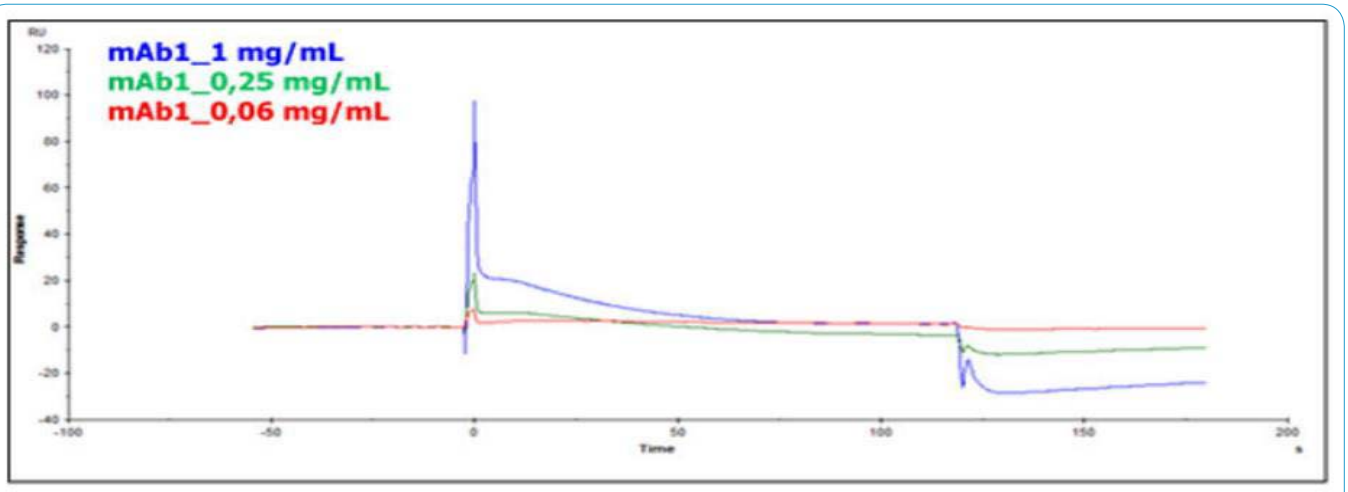

Figure 1: Dose response curve of mAb1 on immobilized target cells. Low binding affinity observed. mAb1 preparations were injected for 120 s, but the signal drops to 0 before the end association indicating a very weak mAb1-cells binding.

Beyond the specific case study presented here, the assay development workflow and troubleshooting strategies for common challenges associated with live-cell SPR experiments are provided as a general guideline. With minor adjustments related to cell type or molecular target, this workflow is broadly applicable to the evaluation of neutralizing antibodies across different therapeutic areas.

Optimization of Cell Immobilization on CM5 Sensor Chip

Effective assessment of ligand–receptor blocking critically depends on the accessibility and functional integrity of cell-surface receptors following immobilization. Cell attachment to the CM5 sensor surface occurs through random orientation via covalent amine coupling between cellular amine groups and the dextran matrix. To maximize detectable ligand binding, the use of engineered cell lines overexpressing the receptor of interest is recommended whenever feasible [23].

A prerequisite for efficient amine coupling is acidification of the cell suspension to approximately pH 5.0, which facilitates imine bond formation between cells and the activated sensor surface. Because factors such as cell passage number and medium age influence buffering capacity, optimal acidification conditions were empirically determined.

In the present study, high-density cell pellets (3×10^6 cells) were resuspended in sodium acetate buffer diluted 1:1 with DPBS to minimize osmotic and pH shock. Multiple buffer pH conditions were evaluated (Table 3), and a sodium acetate solution at pH 2.5—yielding a final cell suspension pH of approximately 4.9—was selected for subsequent experiments.

Using a modified amine coupling protocol and a reduced flow rate to promote efficient surface interaction, live cells were successfully immobilized on the active flow cell, achieving immobilization levels of approximately 17,000 response units (RUs).

Assessment of Cell Viability and Functional Integrity

Maintaining cell viability and receptor functionality following immobilization is essential for reliable SPR measurements. Cell viability was therefore monitored at three critical time points: immediately after acidification, after two hours (corresponding to the immobilization procedure) [24], and after four hours (corresponding to the duration of the multi-cycle kinetic experiment).

Viability measurements performed using the Vi-Cell™ system revealed no statistically meaningful reduction in cell viability over the four-hour period (Table 4). These results confirmed that the immobilization process and experimental conditions did not impair cellular integrity or ligand-binding capability.

To correct for bulk refractive index changes and non-specific binding, one flow cell of the CM5 chip was used as a reference surface. In addition to standard activation procedures, bovine serum albumin (BSA) was immobilized on the reference flow cell (Fc1) via amine coupling to saturate the surface and further minimize non-specific interactions from ligand–antibody mixtures.

Following sensor chip preparation, antibody neutralization was evaluated using a multi-cycle kinetic (MCK) competition format. Mixtures consisting of a fixed ligand concentration and increasing concentrations of the anti-ligand monoclonal antibody were injected over immobilized cells.

Table 3: Viability of Acidified Cells During SPR Experimental Timeline.

Time Point	Experimental Phase	Viability (%)	Observations
0 h	Post-acidification	96	Normal morphology
2 h	Post-immobilization	95	No aggregation
4 h	End of MCK assay	93	Functional binding preserved

Table 4: SPR MCK Parameters for Cell-Based Neutralization Assay.

Parameter	Value
Instrument	Biacore™ T200
Running Buffer	HBS-P+
Flow Rate	5 µL/min
Association Time	180 s
Dissociation Time	300 s
Ligand Concentration	Fixed
mAb Concentration Range	Increasing

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Experimental parameters, including ligand-to-antibody ratios, association time, dissociation phase, and flow rate, were systematically optimized to ensure detectable binding signals and adequate discrimination of potential neutralization effects. The final assay configuration selected for this case study is reported in Table 4. Representative sensorgrams are shown in Figure 2. As expected, no measurable binding was observed for the anti-ligand antibody alone, while robust binding was detected for the ligand control, confirming that immobilized cells retained functional receptor activity and that ligand–receptor interactions could be monitored directly on live cells [25].

Under the optimized conditions, the SPR cell-based competition assay revealed no evidence of ligand neutralization by the tested monoclonal antibody. Specifically, ligand–antibody mixtures produced binding responses comparable to—or exceeding—those observed for ligand alone, even when the antibody was present in substantial molar excess.

These findings indicate that the antibody does not interfere with ligand–receptor interaction and likely recognizes a ligand epitope that is not involved in receptor binding. The results shown in Figure 2 are representative of three independent experimental runs performed with varying dissociation times and antibody concentrations, all yielding consistent outcomes.

To corroborate these observations, an alternative SPR assay format was implemented. The anti-ligand antibody was immobilized in an oriented fashion on a Protein A sensor chip, and mixtures containing ligand and increasing numbers of host cells were injected (Table 5). As illustrated in Figure 3, a characteristic "sandwich" binding profile was observed, indicative of non-competitive interactions [26-28]. In this configuration, ligand simultaneously engaged both the immobilized antibody and cell-surface receptor, resulting in additive binding signals proportional to cell concentration.

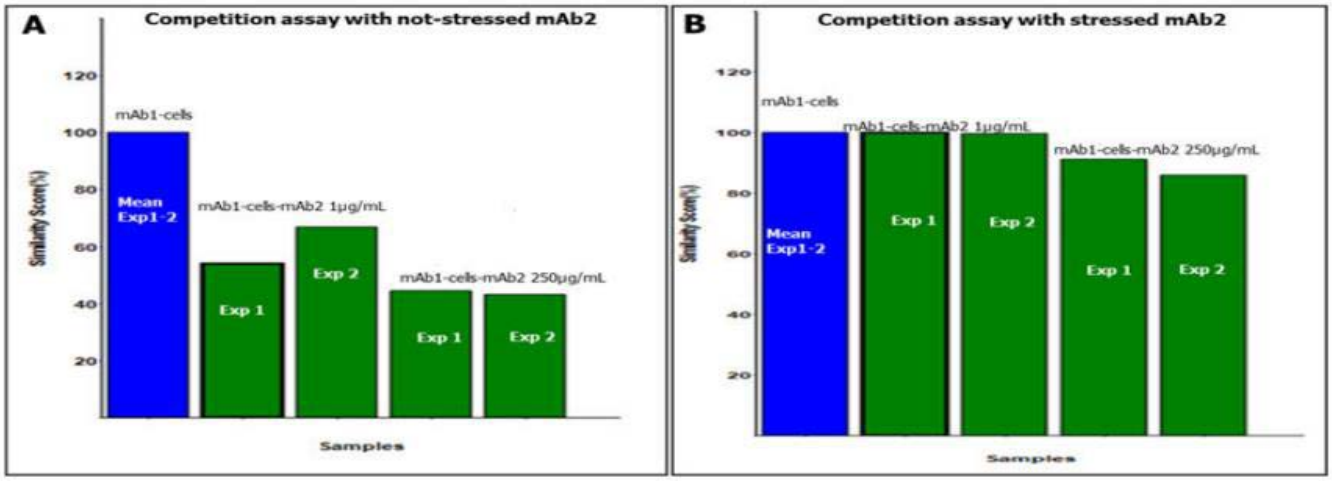

Figure 2: Sensorgrams comparison analysis performed to evaluate method reproducibility and selectivity.

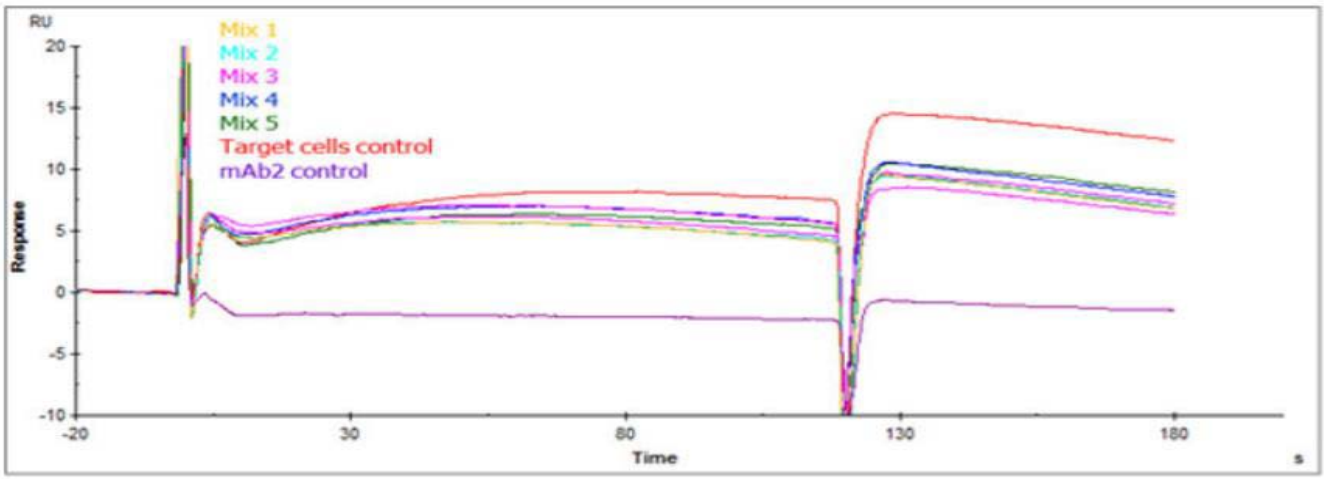

Figure 3: MCK cell-based competition assay results. Each sensorgram represents the binding signal obtained by injecting controls and target cells-mAb2 mixes at different ratios on the immobilized mAb1.

Table 5: Interpretation Criteria for Cell-Based SPR Neutralization Assay.		
Experimental Condition	**Expected SPR Signal**	**Interpretation**
Ligand only	High response	Functional receptor binding
mAb only	No response	No cell interaction
Ligand + neutralizing mAb	Reduced response	Ligand blocking confirmed
Ligand + non-neutralizing mAb	Comparable to ligand control	No neutralization

Table 6: SPR Competition Assay Using Recombinant Antigen (Protein A Chip).			
Immobilized Molecule	**Injected Analyte Mix**	**Observed Binding Profile**	**Competition Outcome**
mAb1 (Human IgG1)	Antigen + mAb2	Reduced signal	Competition confirmed
mAb1 (Human IgG1)	Antigen only	High signal	Baseline binding

Table 7: Comparison of SPR Competition Assay Configurations.			
Assay Format	**Biological Relevance**	**Signal Strength**	**Key Limitation**
Recombinant antigen SPR	Low	High	Lacks cellular context
Injected cell analyte	Moderate	Low–moderate	Cell debris risk
Immobilized cell SPR	High	Moderate–high	Requires optimization
Selective mAb capture SPR	Very high	High	Qualitative output

Table 8: Summary of Applications Enabled by Cell-Based SPR Assays.		
Application	**Information Obtained**	**Development Stage**
Potency ranking	Functional binding strength	Early development
Epitope competition	Shared vs distinct binding sites	Lead optimization
Neutralization screening	Ligand-receptor blocking	Candidate selection
Combination therapy design	Non-overlapping epitopes	Preclinical phase

Together, these complementary SPR approaches conclusively demonstrated the absence of ligand neutralization by the antibody and highlighted the flexibility of the proposed cell-based SPR strategy for MoA characterization.

SPR Cell-Based Competition Assay for Evaluation of Antibody Epitope Competition on Target Cells

SPR-based competition assays are widely used to assess whether monoclonal antibodies bind overlapping or distinct epitopes on a common target antigen. Traditionally, such assays involve immobilization of one antibody followed by injection of antigen mixed with a potential competitor antibody. A reduction in antigen binding signal confirms epitope competition.

In the present work, this principle was adapted by substituting recombinant antigen with live target-expressing cells to enable competition analysis in a physiologically relevant context. Two model antibodies—a human IgG1 (mAb1) and a mouse IgG1 (mAb2)—and the A549 target-expressing cell line were used as a case study.

Confirmation of Antibody Competition Using Recombinant Antigen

As an initial validation step, epitope competition between mAb1 and mAb2 was confirmed using a conventional SPR MCK assay with recombinant human antigen. mAb1 was immobilized on a Protein A chip, and mixtures containing a fixed antigen concentration and increasing concentrations of mAb2 were injected. The observed reduction in antigen binding confirmed competitive interaction (Table 6). Following confirmation with recombinant antigen, the assay was transitioned to live A549 cells. A CM5 sensor chip was prepared with BSA immobilized on the reference flow cell and mAb1 immobilized on the active flow cell.

Initial experiments involved pre-incubation of fixed cell numbers with increasing concentrations of mAb2 for two hours at 37°C to promote binding prior to SPR injection. Cells were maintained in complete culture medium during pre-incubation to preserve viability, which remained high throughout the experiment (Table 7).

Despite extensive optimization of cell-to-antibody ratios, flow rates, and association times (Table 8), binding signals remained low, limiting the ability to clearly discriminate competition effects. This limitation was attributed to the inherently low expression of the target antigen on A549 cells.

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Development of an Extended Pre-Incubation and Selective Detection Strategy

To overcome low signal intensity and viability constraints, a revised assay format was implemented. Instead of prolonging on-instrument interaction times, mixtures of target cells, mAb1, and increasing concentrations of mAb2 were pre-incubated off-instrument for 5 ± 0.5 hours under standard cell culture conditions (37°C, 5% CO_2, 80% humidity), allowing binding equilibrium to be reached without compromising cell health.

To selectively detect one antibody in the presence of the other, an SPR CM5 chip was functionalized with an anti-human IgG F(ab′)$_2$–specific antibody. This approach exploited the species difference between mAb1 (human IgG1) and mAb2 (mouse IgG1). After screening multiple capture antibodies, the selected anti-human IgG F(ab′)$_2$ demonstrated high specificity for mAb1 and no cross-reactivity with mAb2.

If mAb1 and mAb2 compete for the same epitope, increasing concentrations of mAb2 bind more target cells, leaving more free mAb1 available to bind the sensor chip, resulting in a dose-dependent increase in SPR signal. Conversely, absence of competition yields stable signals regardless of mAb2 concentration. Using this refined strategy, a clear and concentration-dependent competition effect was observed. Increasing levels of mAb2 led to progressively higher SPR signals corresponding to free mAb1 binding to the capture surface, confirming effective competition for target binding on live cells [29].

Reproducibility and Selectivity Assessment

Assay robustness was evaluated across three independent experimental sessions, yielding consistent results. Selectivity was further assessed using stressed preparations of mAb2. Preliminary binding studies using recombinant antigen indicated only minor kinetic changes following stress treatment.

Sensorgram comparison analysis performed with Biacore™ T200 Evaluation Software generated similarity scores reflecting overlap with the reference condition (mAb1–cell mixture without competitor). Samples containing both antibodies displayed significant divergence from the reference condition in a dose-dependent manner (similarity score ≤ 50% at 250 µg/mL mAb2), confirming reproducibility and sensitivity despite biological variability.

Importantly, stressed mAb2 samples did not significantly alter mAb1 binding behavior (similarity scores ≥ 90%), demonstrating method selectivity.

Findings

These results highlight the substantial advantage of integrating live cells into SPR competition assays. Minor differences in binding behavior observed using recombinant antigen translated into pronounced functional effects when assessed in the native cellular context. The developed cell-based SPR competition workflows provide a robust, selective, and biologically meaningful platform for evaluating antibody neutralization and epitope competition, offering broad applicability for therapeutic antibody characterization and development.

DISCUSSION

Surface Plasmon Resonance (SPR) enables the real-time, label-free analysis of biomolecular interactions by detecting changes in refractive index at a functionalized sensor surface. This technology allows simultaneous evaluation of binding affinity, association and dissociation kinetics, and equilibrium parameters, making it a well-established analytical platform for protein–protein interaction studies.

The application of SPR to live-cell systems represents a significant methodological evolution, combining the analytical sensitivity of plasmonic detection with the biological relevance of intact cellular environments [30]. Cell-based SPR assays permit direct investigation of receptor–ligand interactions at the plasma membrane, providing insight into binding phenomena that more closely resemble physiological conditions than assays based solely on recombinant proteins.

Currently, two main SPR strategies have been developed to study interactions involving living cells using conventional instrumentation. In the first approach, known as the Injected Cell Analyte (ICA) method, cells are flowed over a surface-immobilized interaction partner. In the second approach, referred to as the Immobilized Target Cell (ITC) method, cells are attached directly to the SPR sensor surface, and soluble ligands or antibodies are injected [31,32].

In the ICA format, standard SPR protocols for ligand immobilization and surface regeneration can be largely maintained. Cells are introduced as analytes, allowing qualitative evaluation of binding behavior. However, because cell concentration cannot be expressed in molar units, kinetic parameters such as association rate constants cannot be accurately calculated. In addition, repeated regeneration cycles may progressively impair surface performance, and incomplete cell removal can introduce signal artifacts due to residual debris [31].

The ITC approach offers the advantage of injecting analytes at defined concentrations, enabling calculation of equilibrium dissociation constants and kinetic parameters. Importantly, the measured interactions reflect the native presentation of receptors within the cell membrane, including the effects of membrane composition, receptor clustering, and non-specific interactions. Nonetheless, this format presents technical challenges. The limited penetration depth of the SPR evanescent field restricts signal detection to events occurring near the sensor surface, and immobilized cells are inherently less stable than covalently attached proteins. Maintaining cell viability and receptor functionality while achieving reproducible immobilization requires careful optimization of experimental conditions, including flow rates and surface chemistry [31-34].

To date, competitive binding studies using cell-based SPR formats have been scarcely reported. Antibody competition is traditionally evaluated using ELISA- or SPR/BLI-based assays with recombinant antigens, where reduced binding of one antibody in the presence of another is interpreted as evidence of epitope overlap [21,22]. While informative, these assays lack key biological determinants such as antigen density, membrane topology, and steric constraints that can critically influence antibody behavior in vivo.

Flow cytometry-based competition assays partially address this limitation by employing live cells, but they require fluorescent labeling of at least one antibody. Such labeling can alter binding characteristics or introduce steric effects that influence competition outcomes, thereby limiting interpretability [35].

In the present work, two distinct cell-based SPR applications were developed using standard Biacore™ instrumentation, demonstrating the versatility of this technology for antibody potency characterization. The first application focused on assessing the ability of an anti-ligand monoclonal antibody to inhibit ligand–receptor binding, a defining mechanism of action for neutralizing antibodies. The most technically demanding aspect of assay development was the immobilization of viable cells on the sensor surface. By implementing a controlled acidification protocol followed by amine coupling, stable attachment of live cells was achieved without compromising receptor functionality.

Subsequent injection of ligand–antibody mixtures at varying ratios revealed no inhibition of ligand binding, even at high antibody concentrations. This outcome was independently confirmed using an alternative assay format in which the antibody was immobilized on a Protein A sensor surface and ligand–cell mixtures were injected. The resulting sandwich-type binding profile indicated simultaneous engagement of distinct ligand epitopes, confirming the absence of neutralizing activity. Together, these approaches establish a generalizable SPR-based workflow applicable to antibodies targeting ligand–receptor interactions and potentially extendable to more complex systems, such as viral or pseudoviral particles.

The second application addressed monoclonal antibody competition directly on target cells. During assay development, low antigen expression on the cell surface emerged as a critical limitation, resulting in weak and transient binding signals. Real-time kinetic monitoring enabled rapid identification of this issue and guided optimization of incubation conditions. A prolonged off-instrument incubation strategy was introduced to allow antibodies and cells to reach equilibrium under physiologically favorable conditions [36-41].

To enable selective detection of competition, a customized SPR sensor surface was prepared to capture only one of the competing antibodies. This design exploited species-specific recognition to distinguish free antibody from cell-bound complexes. Using this configuration, a clear dose-dependent competition effect was observed, with increasing concentrations of the competitor antibody leading to higher levels of free antibody binding to the sensor surface.

Reproducibility and selectivity were confirmed across independent experimental runs and through the inclusion of stressed antibody preparations. Despite the intrinsic variability associated with live-cell assays, the method demonstrated robust performance, highlighting the analytical strength of SPR when combined with appropriate assay design.

Overall, the incorporation of live cells into SPR binding assays significantly enhances the biological relevance of antibody characterization. Unlike recombinant systems, cell-based SPR captures the influence of native antigen presentation, steric hindrance, target accessibility, and expression density—factors that are critical to understanding antibody mechanisms of action but are often overlooked in simplified binding assays.

The flexibility of SPR further strengthens its applicability. Sensor surfaces can be readily customized to immobilize antibodies, ligands, receptors, or whole cells, allowing assay formats to be adapted to specific experimental objectives. A wide variety of cell types, including primary cells, immortalized lines, and engineered models, can be employed provided they are compatible with instrument constraints.

CONCLUSION

Cell-based SPR methodologies inherently present technical complexities, including signal instability, non-specific

interactions, and challenges associated with interpreting multifactorial cellular responses. Achieving reliable and reproducible data therefore requires careful control of experimental variables, particularly cell density, buffer composition, flow conditions, and regeneration strategies. When these parameters are systematically optimized, live-cell SPR assays can be executed with a level of robustness comparable to conventional protein-based SPR experiments. The strategy described in this work integrates established SPR operational principles with the added biological relevance of intact cellular systems. By doing so, it enables the quantitative evaluation of competitive binding and functional potency directly within a native-like biological context. This hybrid approach overcomes key limitations of recombinant-target assays, capturing the influence of membrane organization, antigen accessibility, and cellular architecture on antibody behavior. Overall, the results demonstrate that cell-based SPR assays represent a powerful and adaptable analytical platform for monoclonal antibody characterization. Their application provides deeper mechanistic insight into antibody–target interactions and supports more informed decision-making during therapeutic antibody development, ultimately facilitating the design and selection of more precise and effective biologic treatments.

CONFLICT OF INTEREST

Authors declare there is no conflict of interest.

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