Label-Free Electrochemical Biosensor for SARS-CoV-2 Spike Protein Detection Using ACE2-Functionalized Graphene Oxide Electrodes

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Introduction

The ongoing global challenge posed by coronaviruses has highlighted the critical need for a deeper understanding of the molecular interactions that govern viral entry into host cells. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), responsible for the COVID-19 pandemic, utilizes its spike (S) protein to bind to the human angiotensin-converting enzyme 2 (ACE2) receptor, a crucial step in the viral infection process (Lu & Sun, 2020). Electrochemical biosensors have emerged as promising alternatives due to their inherent advantages of rapid response, miniaturization potential, and cost-effectiveness (Mao et al., 2022). This work also involves advanced materials handling through the preparation, modification, and functionalization of graphene oxide and other nanomaterials, which are essential for biosensor fabrication and performance.

The spike protein is a large, highly glycosylated, type I transmembrane protein composed of two subunits, S1 and S2. The molecular interaction between SARS-CoV-2 and host cells is mediated by the binding of the viral spike protein to the human angiotensin-converting enzyme 2 (ACE2) receptor, which serves as the primary cellular entry point for the virus (Zhou et al., 2020). This high-affinity protein-protein interaction, with reported dissociation constants (Kd) ranging from

1-74 nM depending on experimental conditions (Wrapp et al., 2020), (Barton et al., 2021), provides a foundation for biosensor development. Recent studies have demonstrated the feasibility of ACE2-based electrochemical detection platforms, with various research groups achieving detection limits ranging from picogram to nanogram levels (Zhao et al., 2024, (Meškinis et al., 2023).

The binding of the SARS-CoV-2 spike protein has been shown to enhance the enzymatic activity of ACE2, which may contribute to the vascular complications associated with COVID-19 (Lu & Sun, 2020b). Therefore, this sensitive and specific method for detecting the ACE2-spike protein interaction is useful for understanding the pathology of the disease but additionally for monitoring the efficacy of potential therapeutic compounds.

Graphene oxide (GO)-based electrochemical platforms have gained recent attention in biosensor applications due to their electrical conductivity, large surface area, and abundant functional groups that facilitate biomolecule immobilization (Sengupta & Hussain, 2023b). Several recent studies have employed GO-modified electrodes for SARS-CoV-2 detection, demonstrating the material's potential for viral protein sensing applications (Chen et al., 2023), (You et al., 2023). However, most existing electrochemical biosensors for COVID-19 detection rely on redox mediators or complex labeling strategies, which increase cost and complexity while potentially compromising the stability and reproducibility of the sensing platform.

The development of biosensors capable of accurate binding affinity determination is important for understanding viral pathogenesis, evaluating therapeutic interventions, and monitoring in real time viral mutations that alter receptor binding characteristics (Korber et al., 2020). The current surface plasmon resonance (SPR) techniques, while highly accurate for Kd determination,

require expensive instrumentation and are not suitable for point-of-care applications (Huo et al., 2020).

This study presents preliminary results from the development of a novel label-free electrochemical biosensor based on ACE2-functionalized graphene oxide electrodes for SARS-CoV-2 spike protein detection. It aims to achieve several key objectives: (1) demonstrate proof-of-concept for ultra-sensitive, label-free electrochemical detection, (2) establish a platform capable of binding affinity determination, (3) evaluate the potential for whole virus detection, and (4) lay the groundwork for a cost-effective, portable diagnostic tool suitable for widespread deployment.

Materials and Methods

1. *Reagents and Buffers*

Recombinant human ACE2 protein (amino acids 18–740) and SARS-CoV-2 S1 spike protein subunit were purchased from Prospect Biosystems. ACE2 was supplied at 0.5 μg/μL in pH 7.4 phosphate-buffered saline (PBS) with 10% glycerol and diluted 1:100 in PBS prior to use. The spike protein was supplied at 0.25 μg/μL and similarly diluted 1:100 in PBS. Bovine serum albumin (BSA) was prepared as a 1:1000 dilution in PBS as a blocking agent. Non-fat dry milk was dissolved in water and further diluted to a final protein concentration of 1.328 fM. MES buffer (pH 4.55), deionized water, and additional buffers at pH 7.0 and pH 9.3 were used throughout the protocol for rinsing, immobilization, and stabilization.

2. *Electrochemical Setup*

Electrochemical measurements were performed with a potentiostat (GalvanoPlot model GX102) using GalvanoPlot software for data acquisition and Microsoft Excel for analysis. A three-

electrode system was employed: a glassy carbon working electrode (GCE, BASi), an Ag/AgCl reference electrode, and a counter electrode platinum wire. Cyclic voltammetry (CV) was used with a scan rate of 100 mV/s and a potential window of –400 mV to +400 mV over 7 cycles. Experiments were conducted at room temperature in a 2 mL electrochemical cell, without the use of external redox mediators.

3. *Electrode Pretreatment and Functionalization*

The working electrode was modified with a graphene oxide (GO) layer to enhance conductivity and facilitate biomolecule attachment. A 10 µL aliquot of GO solution was applied to the electrode surface and oven-dried at 100 °C for 5 minutes. The surface was rinsed three times with MES buffer (pH 4.55).

For carboxyl group activation, 17 mg of EDC (Thermo Fisher Scientific) was dissolved in 1mL of MES buffer to make 100mM solution. 10µL and applied to the GO-coated electrode. After 5 minutes at room temperature, the surface was rinsed with 4.55 MES buffer. Next, 1 µL of 1:100 diluted ACE2 stock solution was mixed with 9 µL of MES buffer and applied to the electrode. After 5 minutes of incubation, the electrode was rinsed with deionized water, pH 9.3 buffer, and again with deionized water. The modified electrode was stored in pH 7.4 buffer prior to use.

4. *Spike Protein Incubation*

Initial CV scans were recorded on the GO/ACE2-functionalized electrode in PBS (pH 7.4) to establish a baseline. The electrode was then exposed to SARS-CoV-2 S1 spike protein diluted to 9550 pM by adding 2 µL of 1:100 stock into 2 mL PBS (pH 7.4). Serial dilutions were performed by replacing 1 mL of solution with 1 mL of fresh PBS, resulting in concentrations of approximately 4775, 2387, 1193, 596, 298, 149, 74, and 37 pM. On the following day, this

37 pM solution was further diluted down to 18, 9, 4.5, 2.25, 1.1 pM, and finally to 550, 275, and 137 fM.

Each dilution was tested using CV, and current responses were extracted from the 6th cycle. Electrodes showing no clear signal were excluded. All data were tabulated in Excel and used to construct calibration curves.

5. *Blocking Studies*

To assess nonspecific binding, blocking experiments were performed using BSA and non-fat dry milk on separate days. The electrode was stored in PBS (pH 7.4) between trials. For BSA blocking, a 1:1000 BSA stock in PBS was further diluted (635 µL stock + 1365 µL PBS). For milk blocking, a 1 g/mL milk solution in water was diluted into 2 mL PBS and tested with a range of concentrations from 1.34 fM to 1.34 µM. Three BSA and one milk trials were tested using CV. All trials were analyzed and added to the calibration dataset.

Results

3.1 *Baseline CV Behavior*

Baseline CV measurements of the GO/ACE2-modified electrode in PBS (pH 7.4) exhibited stable capacitive currents over seven cycles with no distinct faradaic peaks.

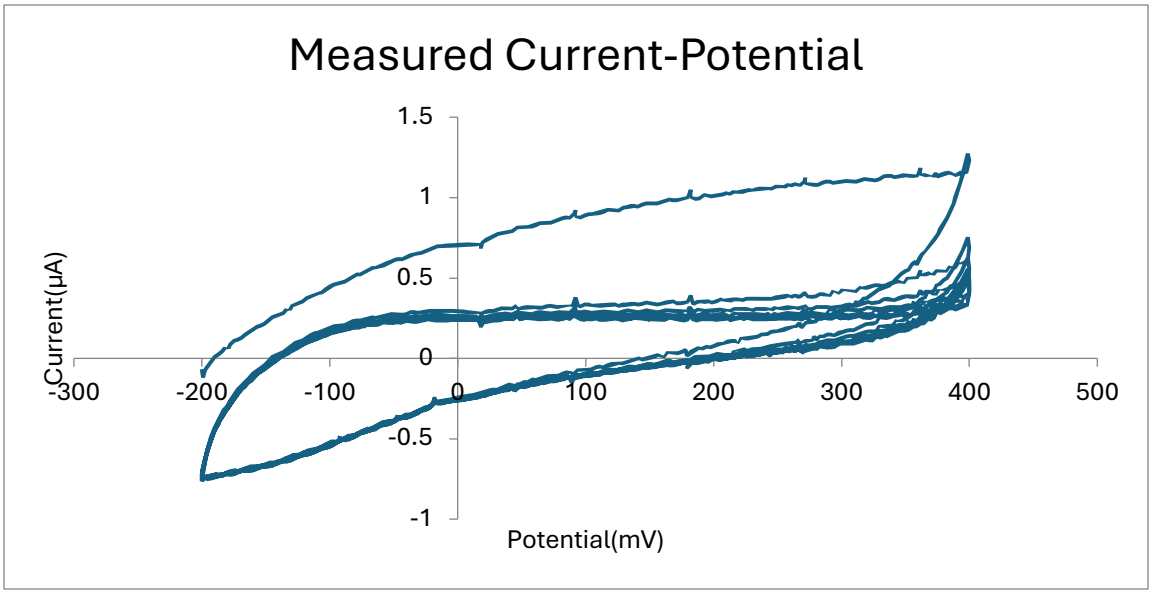
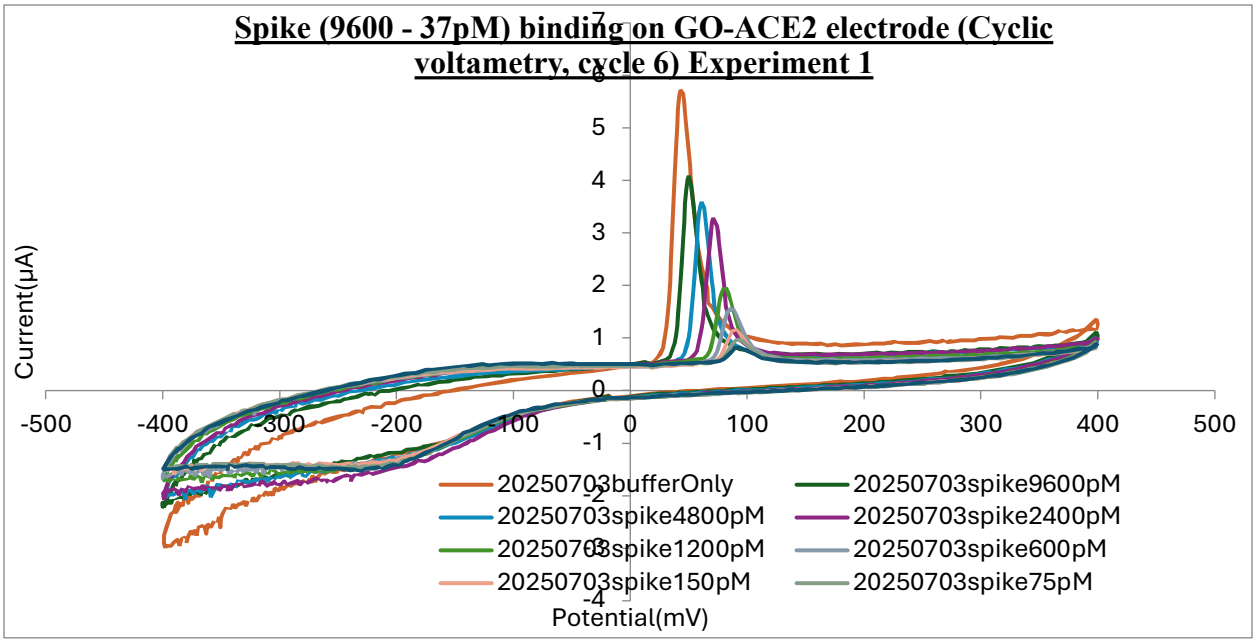


Figure 3.1: Cyclic voltammograms from the baseline scan showing reproducible surface behavior.

3.2 *Spike Protein Detection*

3.2a *CV Overlay Across Dilutions*

Electrodes exposed to serial dilutions of spike protein (9.55 nM to 37 pM) demonstrated concentration-dependent changes in oxidation peak current. The 6th cycle from each dilution was overlaid (Figure 3.2a), showing clear signal decreases with each dilution step. Current response was detected in PBS alone.



3.3 *Detection at Lower Concentrations*

Further dilutions from 37 pM down to 37 aM were analyzed prior to blocking experiments. CV scans showed measurable current responses in the femtomolar range, supporting the sensor's capability for ultra-low concentration detection. These values were incorporated into the full calibration curve (blue circles).

3.4 *BSA Blocking Results*

Following BSA treatment, CV signals remained near baseline across all spike concentrations (37 pM–9.55 nM), with only a small curve, indicating effective surface blocking (light blue "x" markers with connecting blue line). No Kd could be determined due to the limiting binding response. These results confirm the specificity of unblocked spike binding.

3.5 *Milk Blocking Results*

Milk-blocked electrodes showed intermediate current responses, lower than the unblocked control but higher than BSA-blocked samples, suggesting partial blocking (green triangles). Milk-only controls yielded negligible current, confirming milk itself did not produce a signal.

3.6 *Spike Protein Detection After Milk Blocking (37 pM–9.55 nM)*

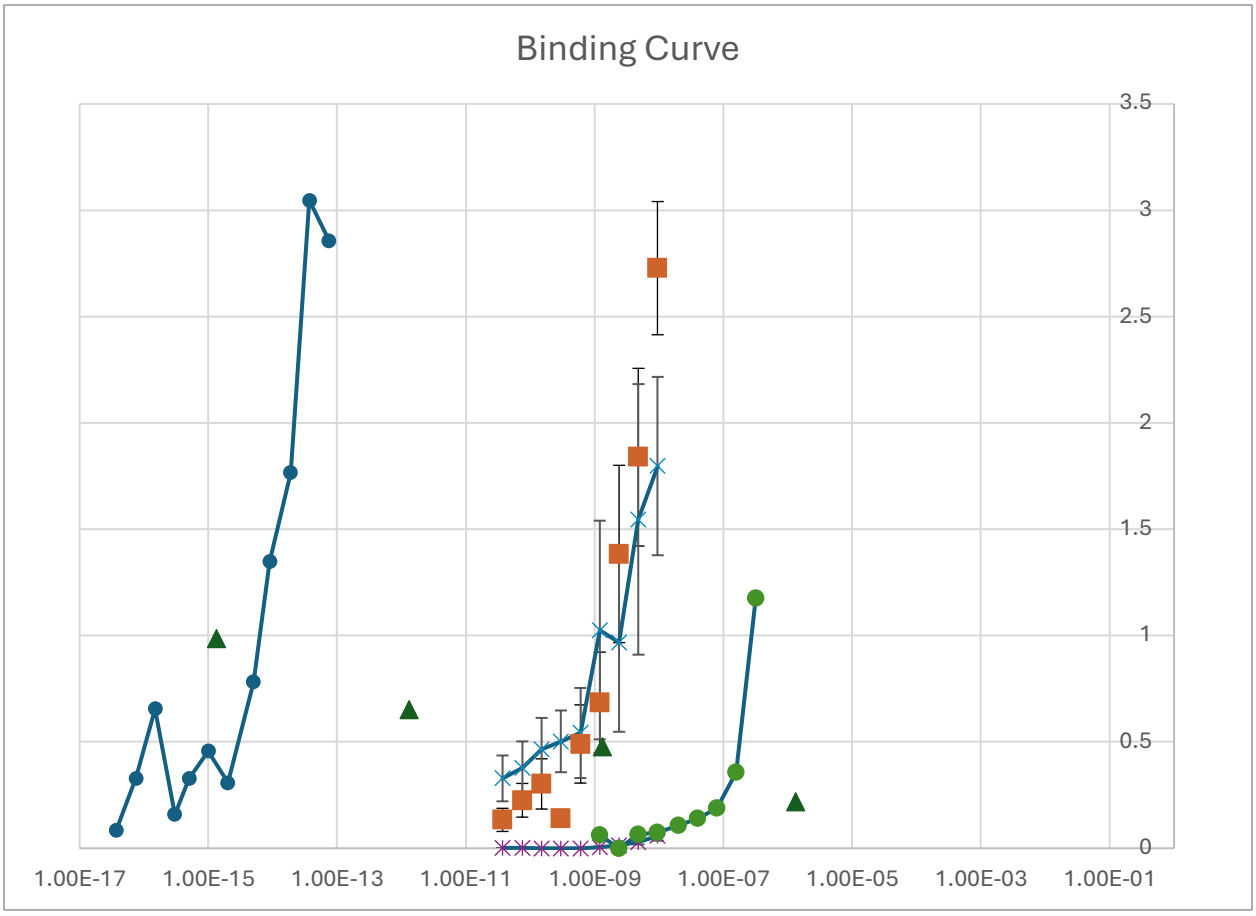
To evaluate sensor performance following milk blocking, spike protein was reintroduced in the range of 37 pM to 9.55 nM. The resulting current responses (light blue "x" markers) were lower than the unblocked control but higher than the BSA-blocked condition.

3.7 *Higher Concentration Testing After Milk Blocking*

To extend the sensor's range, spike concentrations were increased to 318 nM. The binding curve followed a sigmoidal shape, suggesting strong affinity (Green Circles). A preliminary Kd of 1.2–2.5 nM was estimated based on saturation behavior.

Figure 3.2b: *3.3 -3.7 Binding Curves*

Peak currents from a llreplicate experiments were averaged and plotted against concentration. Currents ranged from 2.878 μA at 9.55 nM to 0.133 μA at 37 pM, consistent with high-affinity ACE2–spike interaction. Data are presented in Figure 3.2b, with standard deviations ranging from 0.03 to 0.42 μA. Control samples without spike protein remained near baseline across all concentrations.



Conclusion

This study presents preliminary results demonstrating the feasibility of a label-free electrochemical biosensor for SARS-CoV-2 spike protein detection using ACE2-functionalized graphene oxide electrodes. The findings indicate that the developed platform can detect spike protein across a wide concentration range, from nanomolar to femtomolar levels, with concentration-dependent electrochemical responses that suggest specific ACE2-spike protein interactions. Beyond its biomedical implications, the project also addresses key aspects of materials handling, including the precise preparation, modification, and storage of advanced nanomaterials such as graphene oxide, as well as controlled immobilization of biomolecules. These skills and methods directly align with the materials handling theme of the award.

The blocking studies using BSA and milk proteins provided evidence for the specificity of the binding interaction, with BSA effectively eliminating the electrochemical response while maintaining baseline currents. The intermediate response observed with milk blocking suggests partial surface coverage, indicating that optimization of blocking conditions may further enhance sensor selectivity.

Key preliminary findings include: (1) successful immobilization of ACE2 on graphene oxide-modified electrodes as evidenced by stable baseline cyclic voltammetry profiles, (2) concentration-dependent current responses spanning several orders of magnitude, (3) preliminary binding affinity estimates in the nanomolar range (Kd ~ 1.2-2.5 nM), consistent with literature values for ACE2-spike protein interactions (Wrapp et al., 2020c), (Barton et al., 2021b) and (4) demonstration of sensor specificity through comprehensive blocking studies.

There are some aspects which require further validation and optimization. The reported detection limits in the attomolar range, while promising, exceed the sensitivity typically achieved by label-free electrochemical methods and warrant rigorous statistical validation including proper limit of detection calculations. The electrochemical mechanism responsible for the current changes in the absence of redox mediators therefore these requires further investigation to ensure reproducibility and reliability of the sensing platform.

Future research directions should focus on developing standardized protocols for accurate binding affinity determination (Kd) using electrochemical methods, potentially incorporating kinetic measurements and Langmuir isotherm fitting. Comprehensive validation of the technique through comparison with established methods such as surface plasmon resonance is also essential to establish credibility and accuracy. The platform should be extended for whole virus detection using patient samples or viral lysates to demonstrate clinical relevance. Additionally,

investigating the binding affinity of different viral variants will provide valuable insights into mutation effects on ACE2 interaction, which is crucial for monitoring viral evolution. Finally, optimizing sensor fabrication protocols will enhance reproducibility and support commercialization potential.

The successful development of this biosensor platform could significantly impact COVID-19 diagnostics and surveillance efforts, with broader applications extending to all viral pathogens rather than being limited to COVID-19 alone. A portable, cost-effective device capable of rapid spike protein quantification and binding affinity determination would be invaluable for monitoring viral evolution, evaluating therapeutic interventions, and providing decentralized diagnostic capabilities. Furthermore, the platform's ability to measure binding kinetics could contribute to the understanding of viral pathogenesis and support the development of more effective antiviral strategies. In the context of materials handling, this research contributes novel approaches for manipulating nanoscale materials and integrating them into functional sensing platforms. The potential for multiplexed detection of multiple viral proteins or simultaneous detection of viral antigens and antibodies represents additional avenues for platform enhancement.

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