



CHALMERS
UNIVERSITY OF TECHNOLOGY



Surface Functionalization of Screen-Printed Carbon Electrodes for Electrochemical Biosensing in Equine Saliva

A study on linker-based surface chemistry for biosensor functionalization

Master's thesis in Materials Chemistry

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DEPARTMENT OF PHYSICS AND ASTRONOMY

CHALMERS UNIVERSITY OF TECHNOLOGY

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Department of Physics and Astronomy

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Abstract

Osteoarthritis (OA) is a progressive, long-term inflammatory condition that damages joint function and causes significant pain in both humans and animals. Many imaging techniques struggle to detect OA in its early stages, making early diagnosis challenging. A promising alternative is biomarker monitoring using electrochemical biosensors in saliva. The aim of this thesis is to investigate surface modification on screen-printed carbon electrodes as a model system for biosensor functionalization. The work focuses on establishing and evaluating a controlled surface chemistry using linker and passivation layers that mimic the immobilization strategy intended for future antibody-based sensing systems. Screen-printed carbon electrodes were functionalized using a PEG-based linker and click-chemistry-based surface modification. The surface immobilization was investigated using cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), field-effect transistor (FET), and chronoamperometry (CA) measurements. Surface modification could be observed using CV, while EIS showed weaker but supporting trends. Transistor measurements showed slight trends, but additional measurements are needed to determine whether the observed changes were caused by surface modification. CA measurements showed no clear evidence of binding. Overall, these findings demonstrate that the investigated surface chemistry can be immobilized on screen-printed carbon electrodes and detected using electrochemical methods, providing a foundation for future biosensor design, where the next step is to introduce Fab' fragments for biomarker binding. However, further optimization of both the surface modification and measurement setup is required.

Keywords: screen-printed carbon electrodes, surface functionalization, electrochemical biosensors, click chemistry, cyclic voltammetry, electrochemical impedance spectroscopy, field-effect transistor, chronoamperometry, equine saliva, osteoarthritis biomarkers.

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1

Introduction

This chapter provides the background for the study, including osteoarthritis in horses, current diagnostic approaches, and the relevance of saliva as a diagnostic medium. It also outlines the aim of the thesis and the scope of the work.

1.1 Background

This project is a first step towards developing a biosensor to measure biomarkers in horse saliva related to osteoarthritis. Due to a limited time frame, antibody fragments could not be included in this study. Therefore, this work presents a more general approach to designing a biosensor on screen-printed carbon electrodes through surface functionalization using linkers and passivation strategies connected via copper-free click chemistry. The antibody fragments are intended to react with the linkers through the same chemistry as the passivation layer; thus, the surface modification strategy can still be investigated without their direct inclusion. However, as this is an initial study, there are still several aspects that need to be examined in future work, for example whether the binding of biomarkers occurs sufficiently close to the surface for detection, as well as optimization of the passivation chain length.

1.1.1 Osteoarthritis in Horses

Osteoarthritis (OA) is a progressive, long-term inflammatory condition that damages joint function and causes significant pain in both humans and animals[1]. Many imaging techniques struggle to detect OA in its early stages[2]. Instead, monitoring biochemical changes can enable earlier detection, allowing treatment to be applied before irreversible joint damage occurs.

Recent studies have identified BGN262 and COMP156 as biomarkers associated with OA progression[1, 3]. Tracking these biomarkers has shown decreased levels after treatment, suggesting a relationship between biomarker concentration and disease activity. This indicates that BGN262 and COMP156 may be useful for monitoring both disease progression and treatment response.

1.1.2 Diagnostic Methods and Biosensing

Current diagnostic approaches include enzyme-linked immunosorbent assay (ELISA)[2]. ELISA uses specific antibodies to detect enzyme- and cartilage-related biomarkers associated with OA. While widely used, ELISA is relatively time-consuming, requires laboratory infrastructure, and involves substantial consumable use[4, 5].

Electrochemical biosensors have emerged as a promising alternative, as they generate an electrical response upon target binding events[6]. This response can be correlated to analyte concentration, enabling potential real-time and on-site monitoring, for example in stable environments.

1.1.3 Saliva as a Diagnostic Medium

BGN262 and COMP156 can be found in saliva[3]. Saliva is readily available, making it a suitable medium for non-invasive biosensing. This enables repeated measurements over time for disease monitoring in a safe and non-destructive manner, without the need for specialized laboratory equipment or trained personnel. Based on these considerations, saliva is selected as the target biological matrix in this work.

However, saliva is a complex biological medium. Although it is composed of approximately 99% water, it also contains proteins and other components that can interfere with electrochemical measurements[7]. The detection of analytes in saliva is therefore influenced by factors such as pH, viscosity, and ionic strength. These parameters have been shown to vary between individual horses[8, 9]. This variability makes saliva a challenging medium for electrochemical sensing and therefore highlights the need for effective surface passivation strategies to reduce non-specific interactions.

1.2 Aim of Thesis

The aim of this thesis is to investigate surface modification on screen-printed carbon electrodes, as a model system for biosensor functionalization. The work focuses on establishing and evaluating a controlled surface chemistry using linker and passivation layers that mimic the immobilization strategy intended for future antibody-based sensing systems.

Electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) are used to verify and characterize the surface modification process, while field-effect transistor (FET) measurements are used as a complementary method to evaluate the electrical response of the modified surfaces, including time-dependent responses.

1.2.1 Research objectives

The specific objectives of this work are to:

- investigate electrochemical activation and surface preparation of screen-printed carbon electrodes,

- develop and apply a stepwise surface modification protocol using linker and passivation chemistry,
- verify each modification step using CV and EIS,
- evaluate the electrical response of modified surfaces using FET measurements,
- perform preliminary tests in relevant electrolyte and biological environments to assess system behavior.

1.2.2 Delimitations

This thesis does not aim to develop or validate a complete biosensor, but instead focuses on surface functionalization and electrical characterization of model systems. Full antibody-based biomarker detection, clinical validation, and optimization of sensing performance, such as sensitivity, selectivity, and long-term stability, are therefore outside the scope of this work.

2

Theory

This chapter provides the theoretical background underlying the methods used in this study, including field-effect transistor operation, electrochemical impedance spectroscopy, and key principles of surface functionalization.

2.1 Biosensors

Biosensors are widely used in many fields to investigate concentrations of a substance in a sample[10]. There are multiple applications and design strategies for biosensors.

2.1.1 Definition of Biosensors

Biosensors are widely used in areas such as soil and food quality monitoring, disease detection and drug discovery[10]. They are used to determine the concentration of an analyte in a sample by generating signals proportional to the analyte concentration. Important performance characteristics of biosensors include selectivity, reproducibility, stability, sensitivity, and linearity. Sensitivity can be quantified using the limit of detection (LOD), which is defined as the lowest detectable concentration of an analyte. There are multiple types of biosensors, such as electrochemical, optical, and acoustic biosensors.

2.1.2 Electrochemical biosensors

Electrochemical biosensors often exhibit low detection limits and high specificity, making them suitable for many analytical applications[6]. They typically contain biological recognition elements such as antibody fragments or other biomolecules. These elements selectively interact with the target analyte, producing an electrical signal proportional to the analyte concentration.

There are two main types of electrochemical biosensors: biocatalytic sensors and affinity biosensors[6]. Biocatalytic sensors use enzymes as recognition elements, whereas affinity biosensors use biomolecules such as antibodies or membrane receptors. In cases where no suitable enzymatic reaction exists, affinity-based approaches are typically preferred.

Two commonly used techniques for electrochemical biosensing are electrochemical

impedance spectroscopy (EIS) and field-effect transistor (FET) based sensing[11, 12].

2.1.3 Biosensor system investigated in this study

The aim of this project is to initiate the development of an electrochemical affinity biosensor for detection of biomarkers in horse saliva. As a first step, screen-printed carbon electrodes are used, and their surfaces are chemically modified. The surface is first activated, followed by attachment of linker molecules and PEG-based passivation layers. These surface modifications are designed to enable future immobilization of antibody fragments. Finally, bovine serum albumin (BSA) is used as a blocking agent. The surface modification process is monitored using electrochemical impedance spectroscopy and field-effect transistor measurements.

2.2 Field Effect Transistor Biosensors

Field-effect transistors (FETs) are often used in sensor applications due to their high sensitivity and low detection limits[12]. They are capable of label-free detection of charged species such as ions, DNA, proteins, and viruses. Furthermore, FET-based sensors show strong potential for use in hand-held diagnostic devices due to their compact electronics and compatibility with established manufacturing techniques[12].

FETs have a source, a drain and a gate[13]. A voltage applied to the gate creates an electric field that controls the charge carriers in the channel. This changes the channel's conductivity and thereby regulates the current between the source and the drain.

FET-based biosensors detect biomolecular interactions by converting charge changes at the sensing surface into an electrical signal[14]. When charged species bind to the surface, they change the interfacial potential, which modifies the electric field acting on the transistor and thereby affects the drain current. However, in electrolyte solutions, ionic screening (Debye screening) reduces how effectively distant charges influence the sensor signal.

How much of the interactions can be measured is dependent on the Debye screening length[14]. The Debye length is defined as the distance where the signal is $1/e$ relative to its original value. This means that if the interaction occurs too far from the surface, the FET will not be able to detect it.

There are two main types of field effect transistors: metal-oxide semiconductor FET (MOSFET) and junction FET (JFET)[15]. Both consist of a gate-source-drain combination. The active region beneath the gate differs between the two types, where MOSFETs are based on an oxide-semiconductor interface, while JFETs consist of a semiconductor structure formed by multiple doped layers.

These FETs can operate as either n-channel or p-channel devices[16]. In n-channel

devices, electrons act as the dominant charge carriers, whereas in p-channel devices, conduction occurs via holes. As a result, the electrical response depends on the charge of the analyte. In n-channel FETs, negatively charged species typically decrease the conductance, while positively charged species increase it. In contrast, p-channel FETs exhibit the opposite behavior, where negative charges increase conductance and positive charges decrease it.

The long-term goal of this work is to enable transistor-based readout of biomarker binding. FET-based biosensors are therefore of particular interest due to their high sensitivity and potential for integration into compact hand-held diagnostic devices.

2.2.1 Chronoamperometry

Chronoamperometry (CA) is a method commonly used for measurements on sensor surfaces[17]. During CA measurements, a constant potential is applied to the electrode surface while the resulting current response is recorded as a function of time. CA is therefore often used to monitor time-dependent electrochemical interactions occurring at the electrode surface. CA measurements can be performed using a FET setup to monitor time-dependent charge changes at the electrode surface during incubation in reagent and analyte.

2.3 Cyclic Voltammetry

Cyclic voltammetry (CV) is a common method in biosensing and surface modification verification[6, 11, 18, 19, 20]. In CV, the potential of an electrochemical cell is swept over a defined range and then reversed while the resulting current is measured[21]. This produces a cyclic current–potential response.

In the resulting voltammogram, oxidation and reduction reactions appear as characteristic peaks in the current response. The oxidation peak corresponds to anodic (positive) current, while the reduction peak corresponds to cathodic (negative) current[21].

To probe electron transfer at the electrode surface, a redox-active species is typically required[22]. One commonly used redox probe is the ferri/ferrocyanide couple, $\text{Fe}(\text{CN})_6^{3-/4-}$, which undergoes reversible oxidation and reduction during the measurement. The resulting current response provides information about electron transfer processes at the electrode interface.

CV can be used to verify surface modification[11]. As the surface is functionalized, the redox peaks become less pronounced, indicating that the surface is increasingly blocked. This is commonly evaluated by comparing CV measurements before and after each fabrication step, where successful formation of a functional layer typically leads to a decrease in redox peak currents due to increased steric hindrance and electrostatic repulsion, which hinder electron transfer[22]. In contrast, a negligible change in peak current may indicate incomplete or unsuccessful surface modification.

2.4 Electrochemical Impedance Spectroscopy

Electrochemical impedance spectroscopy (EIS) is a widely used technique for the characterization of electrochemical systems in areas such as biosensing, corrosion studies and energy storage[18]. It provides information about processes occurring at the electrode–electrolyte interface and is particularly useful for studying surface modifications in biosensor applications.

2.4.1 Principle of Impedance

EIS is based on applying a sinusoidal perturbation, either an alternating current or voltage, to an electrochemical system that is in equilibrium or steady state[18]. The resulting sinusoidal response, measured as current or voltage, is monitored over a range of frequencies. By analyzing the relationship between the applied signal and the system response, information about the electrochemical properties of the system can be obtained. Three main processes affect the response to the perturbation: the diffusion of the redox species from the bulk to the electrode surface, the faradaic reaction kinetics and the electric double layer charge and discharge at the electrode–electrolyte interface.

Impedance consists of resistor, capacitor and inductor components and is defined as the system’s opposition to the flow of current. The impedance response is commonly represented using Nyquist or Bode plots, which allow the identification of different processes occurring at the electrode surface.

EIS measurements can be interpreted using equivalent circuits consisting of components such as resistances and capacitors, enabling the extraction of parameters such as charge transfer resistance, solution resistance and double layer capacitance. Changes in these parameters can be used to monitor surface modifications and interactions at the electrode interface.

2.4.2 Equivalent Circuits

For a three-electrode system, there are two common Randles circuits[18]. If the system is not diffusion limited, the resulting Nyquist plot will be a semicircle, as seen in Figure 2.1, and the equivalent circuit, which can be seen in Figure 2.2, consists of a resistance in series with a resistance in parallel with a capacitor. Here, R_s represents the solution resistance, R_{ct} represents the charge transfer resistance and C_{dl} represents the double layer capacitance. In the Nyquist plot, the high frequencies are represented on the left side. The high frequency intercept is defined as R_s . At the low-frequencies intercept, the real value in the Nyquist plot represents $R_s + R_{ct}$, which is the sum of the solution resistance and the charge transfer resistance. At intermediate frequencies, all elements affect the impedance response. By fitting this circuit using software, the values of the resistances and the capacitance can be extracted.

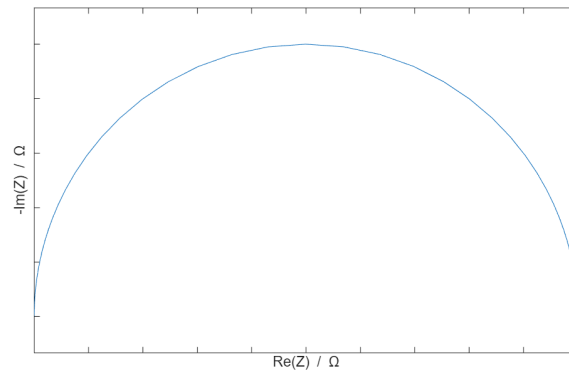


Figure 2.1: Nyquist plot for a non-diffusion limited, three electrode system. Data for the simulation were obtained from CircuitFitting (<https://app.circuitfitting.net/tools/simulation>).

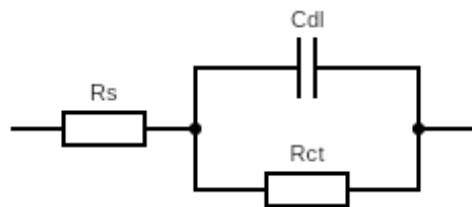


Figure 2.2: Randles circuit for a non-diffusion limited, three electrode system.

If the system is diffusion limited, a 45° line appears at low frequencies, as seen in Figure 2.3. In the equivalent circuit, this is represented as a Warburg element, Z_W , which accounts for diffusion processes. This Randles circuit is shown in Figure 2.4.

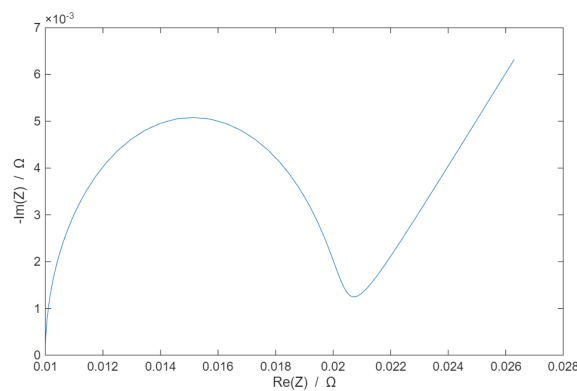


Figure 2.3: Nyquist plot for a diffusion limited, three electrode system. Data for the simulation were obtained from CircuitFitting (<https://app.circuitfitting.net/tools/simulation>).

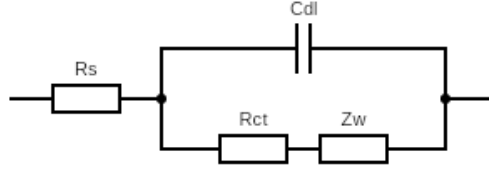


Figure 2.4: Randles circuit for a diffusion limited, three electrode system.

C_{dl} is considered an ideal capacitor, which often is true for flat and homogeneous electrodes[18]. However, often in biosensing applications, the impedance will not act as an ideal capacitor. Therefore, a constant phase element (CPE) is often used instead. This accounts for deviations from ideal capacitive behavior, which commonly arise from surface roughness, heterogeneity, and the presence of biomolecular layers. The behavior of the CPE is described by the exponent α , which ranges from 0 to 1, where $\alpha = 1$ corresponds to an ideal capacitor and lower values indicate increasing deviation from ideal behavior. Using a CPE in the equivalent circuit therefore often results in a better fit to the experimental impedance data.

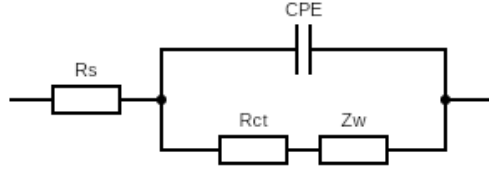


Figure 2.5: Randles circuit for a diffusion limited, non-ideal capacitor, three electrode system.

2.4.3 Equivalent Circuit Model and Parameter Extraction

For a resistor, the impedance is given as Equation 2.1, where R is the resistance [Ω][18].

$$Z_R = R \quad (2.1)$$

The Warburg impedance can be expressed using Equation 2.2, where σ is the Warburg coefficient [$\Omega s^{-1/2}$] and ω is the angular frequency [rad/s][18].

$$Z_W = \frac{\sigma}{\sqrt{j\omega}} \quad (2.2)$$

For systems where the diffusion is limited by a finite diffusion length, the classical Warburg expression is not sufficient to describe the low-frequency behavior[18]. In

this case, a finite-length Warburg impedance is used, which accounts for bounded diffusion effects at low frequencies. This impedance can be expressed using Equation 2.3, where Y_0 is the diffusion related parameter [$\Omega^{-1} s^{1/2}$], and B is a characteristic diffusion parameter [$s^{1/2}$] related to the diffusion length and diffusion coefficient.

$$Z_{W,f} = \frac{1}{Y_0 \sqrt{j\omega}} \tanh \left(B \sqrt{j\omega} \right) \quad (2.3)$$

The parameter B is defined in Equation 2.4, where δ is the diffusion-layer thickness [m] and D is the diffusion coefficient [m^2/s], giving B the unit [$s^{1/2}$] [18].

$$B = \frac{\delta}{\sqrt{D}} \quad (2.4)$$

For numerical fitting in MATLAB, it is convenient to define $\tau = B^2$ [s] and $\sigma = 1/Y_0$ [$\Omega s^{-1/2}$], which allows the finite Warburg impedance to be rewritten as Equation 2.5.

$$Z_W = \frac{\sigma}{\sqrt{j\omega}} \tanh \left(\sqrt{j\omega\tau} \right) \quad (2.5)$$

The constant phase element (CPE) is defined in Equation 2.6, where Q is the scaling factor [$\Omega^{-1} s^\alpha$] and α is a dimensionless parameter describing the deviation from ideal capacitive behavior[18]. If $\alpha = 1$, the CPE behaves as an ideal capacitor.

$$Z_{CPE} = \frac{1}{Q(j\omega)^\alpha} \quad (2.6)$$

For the total Randles circuit shown in Figure 2.5, the impedance can be expressed as Equation 2.7.

$$Z = R_s + \frac{1}{\frac{1}{R_{ct} + \frac{\sigma}{\sqrt{j\omega}} \tanh(\sqrt{j\omega\tau})} + Q(j\omega)^\alpha} \quad (2.7)$$

2.4.4 EIS for Biosensor Characterization

EIS is often used in biosensing[6, 7, 11, 18, 19, 23]. One application of EIS in biosensing is the verification of surface modifications[11]. As the electrode surface is modified, the impedance values will be affected, thus working as a type of verification that the surface modification has occurred. Since charge transfer can be hindered by the thickness and insulating properties of the biolayer, an increase in surface modification leading to increased biolayer thickness will result in an increase in the charge transfer resistance, R_{ct} . In addition to biolayer thickness, the charge of the functionalized surface can also influence charge transfer resistance[23]. Surface charge affects the electrostatic interaction between the redox probe and the interface, which can either facilitate or hinder charge transfer. Therefore, a change in R_{ct} during surface

engineering can be used as an indication of successful surface modification. From a bare electrode to a fully functionalized surface, the charge transfer resistance has been reported to increase by 713% in a previous study[11]. This study found significant differences between individual electrodes. Therefore, EIS measurements were performed on the same electrode after each surface modification step, allowing each electrode to act as its own baseline and enabling reliable comparison of changes in R_{ct} . This approach minimizes the effect of electrode-to-electrode variability.

2.5 Electrodes for Electrochemical Biosensors

Electrochemical biosensors rely on electrode systems to transduce biochemical interactions into measurable electrical signals. The choice of electrode material and configuration is therefore critical for sensor performance, sensitivity, and reproducibility. There are multiple electrode systems that can be used for biosensing, among which screen printed electrodes are widely used in research due to their simplicity and low cost[24]. However, it is important to ensure that they are clean and properly activated before use.

2.5.1 Screen Printed Carbon Electrodes

Screen printed electrodes (SPEs) are single-use, three-electrode systems[24, 25]. Due to their low cost, they are suitable for mass production of single-use sensors, and in projects during early-stages. They are currently used in many fields such as research, food analysis and clinical environments[24]. Screen printed carbon electrodes (SPCEs) are inexpensive and have a wide potential window as well as low background currents, making them well suited for sensing applications. For early-stage development, CTI Phase Zero SPCEs are a good option, as they are high quality and compatible with common potentiostats[25]. These SPCEs have a working electrode (WE) with a diameter of 4 mm and a counter electrode (CE) in carbon and a reference electrode (RE) in silver/silver chloride. The three-electrode system is printed on a PET base layer. The SPCE used in this work is shown in Figure 2.6. In this project, the surface modification will mainly be on the working electrode, so the rest have to be masked during immobilization.



Figure 2.6: Screen printed carbon electrode (SPCE) used in this study, showing the working, counter and reference electrodes.

2.5.2 Electrode Surface Activation

It is important to make sure the electrode surfaces are clean and activated for the surface modification to be effective and reproducible. A common method to clean and activate carbon surfaces is O_2 -plasma[26]. Plasma cleans the surface in an efficient and controllable way, and introduces carboxyl groups on the surface, enabling effective subsequent surface modification. However, since the SPCEs are partially made from PET, O_2 -plasma is not ideal as it can etch or roughen the polymer, potentially contaminating the plasma machine[27, 28]. Chemical oxidation is also problematic, since many strong oxidizing chemicals that clean effectively are harmful to PET[28]. Therefore, electrochemical oxidation through cyclic voltammetry, CV, is commonly used for SPCEs, allowing the active electrodes to be cleaned and activated while leaving the supporting PET material intact[24]. During CV cleaning, the potential of the electrode is cycled over a defined range, oxidizing and reducing the surface to remove contaminants and generate active functional groups.

There are multiple chemicals and settings that can be used for electrochemical cleaning. In a study from 2019, different electrochemical oxidation procedures were tested on SPCEs and the results were evaluated[24]. The conditions which showed the greatest promise were 0.5 M H_2SO_4 , 10 cycles between +2 and -0.3V at a scan rate of $100mVs^{-1}$, and 0.01 M H_2O_2 , 25 cycles between +1 and -0.7V at a scan rate of $10mVs^{-1}$, as well as a combination of both.

The LOD was shown to be the lowest for the H_2O_2 cleaning, closely followed by the combinations of H_2O_2 and H_2SO_4 cleaning. These LODs were evaluated for electroanalysis of H_2O_2 , and these three superior cleaning steps gave LODs of 2.2, 2.3 and $2.7\mu M$ respectively. The H_2SO_4 cleaning alone gave a LOD of $10.8\mu M$, which is higher than the ones including hydrogen peroxide, but still a significant improvement over the uncleaned electrode, which gave a LOD of $82.6\mu M$. The H_2O_2 cleaning alone takes approximately 2.3 h per electrode while the H_2SO_4 cleaning takes approximately 8 min per electrode. Due to time constraints, the H_2SO_4 cleaning was chosen for this project.

The electrochemical cleaning of 0.5M H_2SO_4 , 10 cycles between +2 and -0.3V at a scan rate of $100mVs^{-1}$ was evaluated in the article using X-ray photoelectron spectroscopy[24]. This showed that the amount of O-C=O groups on the surface was approximately 8.5% of the carbon, and 6% of the total surface. For oxygen plasma, the amount of carboxyl groups is highly dependent on the plasma settings[26]. In another article, X-ray photoelectron spectroscopy was used to evaluate the amount of carboxyl groups on plasma-treated carbon. The O-C=O content of the carbons was reported between 4–12%[26], which gives a total amount of 3.5–8.5% O-C=O groups. Therefore, the amount of carboxyl groups for electrochemically oxidized carbon using H_2SO_4 is comparable to that obtained via oxygen plasma.

2.6 Surface Functionalization of Biosensors

To create a biosensor, the carbon surface must be functionalized so that the antibody can be attached. The surface functionalization can also help prevent nonspecific binding. The choice of surface modification is therefore vital for an effective biosensor. This section explains the strategy used in this project.

2.6.1 Importance of Surface Functionalization

Surface functionalization is crucial for an effective biosensor. Specific binding of the antibodies is important, but nonspecific binding needs to be avoided, all while making sure the binding is close enough to the surface for the FET to successfully measure it. There are multiple ways to balance this trade-off and modify surfaces for biosensing applications.

In a study from 2019, they used a FET to measure biomarkers in serum with an antibody biosensor[12]. Their surface modification of the active sensor consisted of a linker, an antibody, longer polyethylene glycol chains (PEGs) and bovine serum albumin (BSA). They also had a control sensor that was identical except for the absence of antibodies. The difference between the active and control signals was used to distinguish specific from nonspecific binding.

The linker was used to link the surface to the antibodies[12]. The longer PEGs were used as a way to reduce nonspecific binding. These longer chains were seen to enhance the signal by threefold compared to only short linkers in a previous article, which was due to improved signal conditions related to Debye screening[29]. BSA is used as a blocking agent, further reducing nonspecific binding[12]. This article uses $F(ab')_2$ fragments, which reduce the distance between the binding event and the surface. The distance can be minimized further by the use of Fab' fragments[30].

How the surface modification is oriented also heavily affects the signal of the biosensors[31]. One of the most promising types of surface modifications for sensor applications is the use of azide linkers that couple to dibenzocyclooctyne (DBCO) through copper-free strain promoted azide-alkyne cycloaddition (SPAAC). DBCO reacts with the azide part of the linker and can be functionalized on antibody fragments and passivating PEGs. This SPAAC chemistry has been shown to be efficient in biosensors and provides stable surface functionalization under mild conditions, without the need for additional catalysts, extreme pH, or elevated temperatures, while also avoiding the formation of toxic byproducts.

The antibody fragments can be functionalized randomly through multiple reactive sites, or controlled with only one active group. The latter showed an 800-fold increase in biosensor sensitivity[32]. This is because the binding sites of randomly functionalized antibodies can become partially blocked upon immobilization, while controlled orientation ensures that the binding sites remain exposed and accessible.

In this project, the surface modification consists of an azide linker and DBCO functionalized antibodies and passivating PEGs. The linker was chosen to be as short

as possible to minimize the distance between the binding event and the surface, and the passivating PEG length was originally chosen to be Dibenzocyclooctyne-PEG12-alcohol, which was believed to be long enough to passivate the surface without hindering biomarkers from binding to the antibodies[33]. However, in this project Dibenzocyclooctyne-PEG4-alcohol was used as a proof of concept of the surface modification, and no antibody fragments were used.

2.6.2 Copper-free Strain Promoted Azide-Alkyne Cycloaddition

As mentioned, the azide group of the linker reacts with DBCO through copper-free strain promoted azide-alkyne cycloaddition (SPAAC), which is a widely used reaction for bioconjugation and surface functionalization in biosensing[31]. This type of reaction is part of click chemistry, which was awarded the Nobel Prize in Chemistry in 2022 due to its importance in bioorthogonal chemistry[34].

This reaction occurs rapidly and under mild conditions, making it suitable for many different applications[35]. The azide group reacts with the triple bond in DBCO to form a stable 1,2,3-triazole linkage, which enables efficient and specific coupling between molecules. This makes SPAAC particularly suitable for biosensor surface functionalization, where stable, selective, and reproducible attachment of biomolecules is required.

2.6.3 Linker Molecule

The linker used in this project is Azido-PEG1-amine, which can be seen in Figure 2.7. This linker was chosen because the amine part reacts with the carbon surface, the azide part can react through SPAAC with the antibody fragments and passivating PEGs, and the PEG1 is the shortest chain, which is important for FET sensors where the Debye length can be an issue.

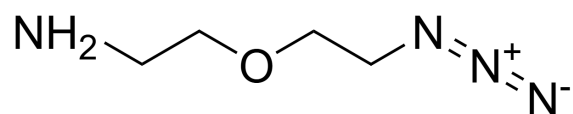


Figure 2.7: Chemical structure for the linker molecule, azido-PEG1-amine.

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The surface is cleaned through electrochemical oxidation, explained in Subsection 2.5.2, which introduces carboxyl groups on the carbon surface[24]. These carboxylic groups can then react with the amine group of the linker after EDC/NHS activation[36]. EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride, is a water-soluble carbodiimide that activates carboxyl groups to form reactive ester intermediates. NHS, N-hydroxysulfosuccinimide, is often used in combination with EDC to form a more stable ester intermediate, which is less susceptible to hydrolysis and increases the lifetime of the activated species. This activated ester then reacts with the amine group of the linker, forming an amide bond.

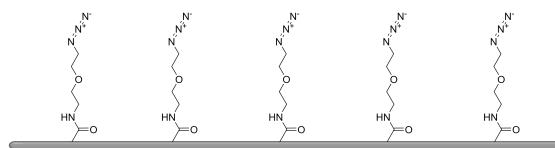


Figure 2.8: Linkers on the carbon surface.

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2.6.4 Antibody and Passivating PEG Molecules

The DBCO-coupled antibodies have a DBCO-PEG4-amide-lipoic acid group coupled to the Fab' fragments, enabling attachment through SPAAC while maintaining a short distance to the surface, as seen in Figure 2.9.

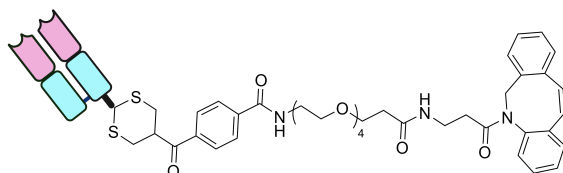


Figure 2.9: Chemical structure of the Fab' coupled to DBCO-PEG4-amide-lipoic acid.

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The passivating PEG, Dibenzocyclooctyne-PEG_n-alcohol, can be seen in Figure 2.10. The chosen length of this was DBCO-PEG12-alcohol, as it was believed to provide sufficient surface passivation without hindering biomarker binding to the Fab' fragments. However, in this project Dibenzocyclooctyne-PEG4-alcohol was used as a proof of principle. The surfaces with Fab' and passivating PEGs can be seen in Figure 2.11, and the surface without the Fab' can be found in Figure 2.12.

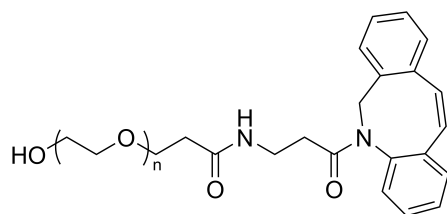


Figure 2.10: Chemical structure of the DBCO coupled PEG chain, Dibenzocyclooctyne-PEGn-alcohol.

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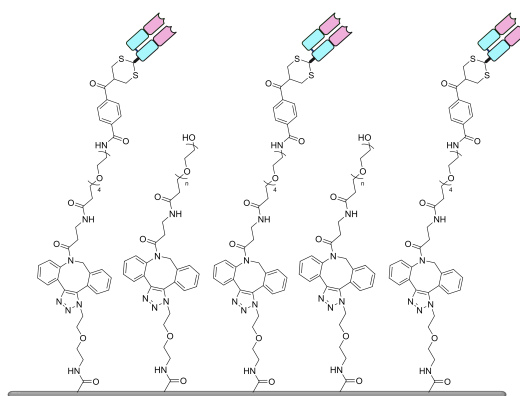


Figure 2.11: The Fab' and passivating PEGs on the carbon surface.

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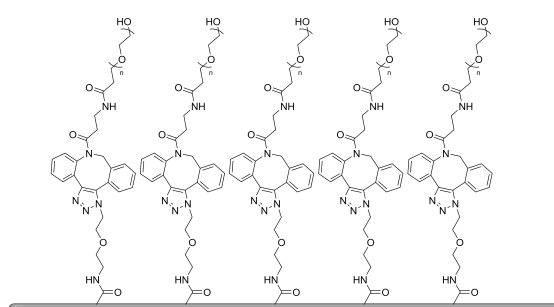


Figure 2.12: The passivating PEGs on the carbon surface.

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2.6.5 Surface Blocking

Finally, blocking to reduce nonspecific binding was done by adding BSA to the surface. The fully modified surfaces for the active and control electrodes can be

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2.8 Surface characteristics

The behavior of biosensors at the electrode interface is strongly influenced by solution properties and surface binding characteristics. Parameters such as ionic strength, Debye length, and binding kinetics determine signal sensitivity and sensor performance.

2.8.1 Ion strength

The ionic strength of a solution will affect how it interacts with the surface. It is therefore an important parameter to assess for a biosensor. Equation 2.8 describes the ionic strength, I , of a solution as a function of the charge of the ion, z , and the concentration, c [38].

$$I = \frac{1}{2} \sum_{i=1}^N z_i^2 c_i \quad (2.8)$$

2.8.2 Debye Length

The debye length is an important metric for biosensors[14]. If the Debye length is too short, binding events will not be detected. Debye length is defined as Equation 2.9, where ε_0 is the permittivity of free space, ε_r is the relative permittivity of the solvent, R is the gas constant, T is the absolute temperature, F is Faraday's constant and I is the ionic strength[38].

$$\lambda_D = \left(\frac{\varepsilon_0 \varepsilon_r R T}{2 F^2 I} \right) \quad (2.9)$$

Equation 2.9 can be rewritten as Equation 2.10 for water at standard conditions[38].

$$\lambda_D = \left(\frac{0.304}{\sqrt{I}} \right) \quad (2.10)$$

The ionic strength and Debye length for PBS, as well as horse saliva samples at different pH, before eating and after 50 min of forage chewing, were calculated from Equation 2.8 and 2.10, and are documented in Table 2.1[39][9]. The chosen pH for horse saliva was 6, 8.3 and 9, since horse saliva pH can range between 6 and 9 with the mean of 8.3[8].

Table 2.1: Ionic strength and Debye length for different solutions.

Solution	pH	I [M]	λ_D [nm]
PBS	7.4	0.167	0.74
Horse saliva 0min	6	0.030	1.74
Horse saliva 50min	6	0.042	1.49
Horse saliva 0min	8.3	0.031	1.73
Horse saliva 50min	8.3	0.042	1.48
Horse saliva 0min	9	0.031	1.73
Horse saliva 50min	9	0.042	1.48

2.8.3 Kinetics

Binding kinetics can be analyzed by the 1:1 Langmuir interaction model through Equation 2.11-2.13[40]. $[A]$ is the analyte concentration, $[B]$ is the concentration of unbound receptor on the surface, $[AB]$ is the concentration of the bound complex, k_a is the association rate constant, k_d is the dissociation rate constant, KD is the ratio of k_d and k_a which represents the analyte concentration at which half of the surface binding sites are bound.



$$k_a[A][B] = k_d[AB] \quad (2.12)$$

$$KD = \frac{[A][B]}{[AB]} = \frac{k_d}{k_a} \quad (2.13)$$

The degree of binding can be labeled θ and is defined as Equation 2.14.

$$\theta = \frac{[AB]}{[B]_{tot}} = \frac{[A]}{KD + [A]} \quad (2.14)$$

The signal from the biosensor will be proportional to θ , which gives rise to Equations 2.15 and 2.16, where S is the equilibrium signal at given analyte concentration and S_{max} is the signal at which all sites are bound.

$$S = S_{max}\theta \quad (2.15)$$

$$S = \frac{S_{max}[A]}{KD + [A]} \quad (2.16)$$

By measuring the equilibrium signal, S , for different analyte concentrations, $[A]$, KD and S_{max} can be estimated. This will give important information about the surface kinetics and also the upper limit of detection of the system. Once surface binding sites are saturated, increasing analyte concentration does not increase the signal, defining an upper practical detection limit.

3

Methods

This chapter describes the materials, fabrication procedures, and experimental techniques used in this work. It includes the design and fabrication of the measurement platform, the preparation of the electrode system, and the electrochemical and electronic characterization methods used to evaluate surface modification.

3.1 Materials

All chemicals were used as received without further purification. The suppliers and chemical information are listed in Table 3.1. Screen-printed carbon electrodes were obtained from Conductive Technologies via PalmSens.

Table 3.1: Chemicals used in this study, including CAS and supplier.

Name	CAS	Supplier
Sulfuric acid	7664-93-9	Sigma-Aldrich
EDAC	25952-53-8	Sigma-Aldrich
NHS	6066-82-6	Sigma-Aldrich
MES buffer	-	Thermo Fisher
Azido-PEG1-amine	464190-91-8	MedChemExpress
PBS	-	Sigma-Aldrich
DBCO-PEG4-OH	-	Sigma-Aldrich
BSA	9048-46-8	Sigma-Aldrich
Potassium hexacyanoferrate(III)	13746-66-2	Sigma-Aldrich
Potassium hexacyanoferrate(II) trihydrate	14459-95-1	Thermo Fisher
Potassium chloride	7447-40-7	Sigma-Aldrich

3.2 3D Printing of Components

To build the setup used for surface modification of only the working electrode, several parts were 3D-printed in polylactic acid (PLA). This setup was developed to enable selective surface functionalization of the working electrode while minimizing modification of the counter and reference electrodes. The components were designed to simplify electrode positioning, improve alignment between layers, and control which parts of the electrode are exposed during experiments.

A base plate was printed to hold 36 electrodes. The plate has fixed positions with walls around each slot so the electrodes stay in place during handling. This part mainly functions as a holder and does not need to be liquid-tight. The design of the base plate can be found in Figure 3.1.

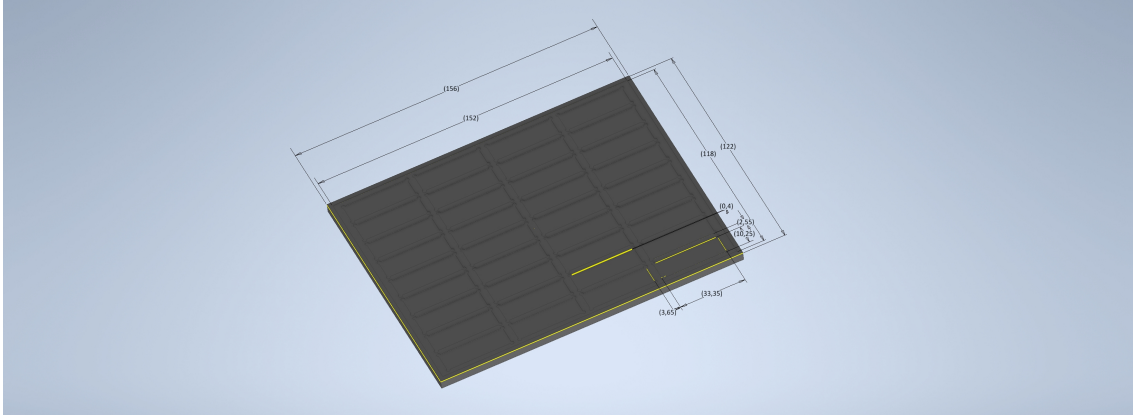


Figure 3.1: Design of the 3D-printed base plate.

In addition to the base plate, a PDMS mold was 3D-printed for casting the PDMS structure. Several versions were printed to optimize the design and ensure a smooth surface for easy removal of the cured PDMS. The ironing setting was used during 3D printing to improve surface quality and reduce adhesion between the PDMS and the mold. The mold includes cylindrical features that define the working electrode exposure area. Around these features, circular indentations were included to form an O-ring structure in the PDMS. The design of the mold can be found in Figure 3.2.

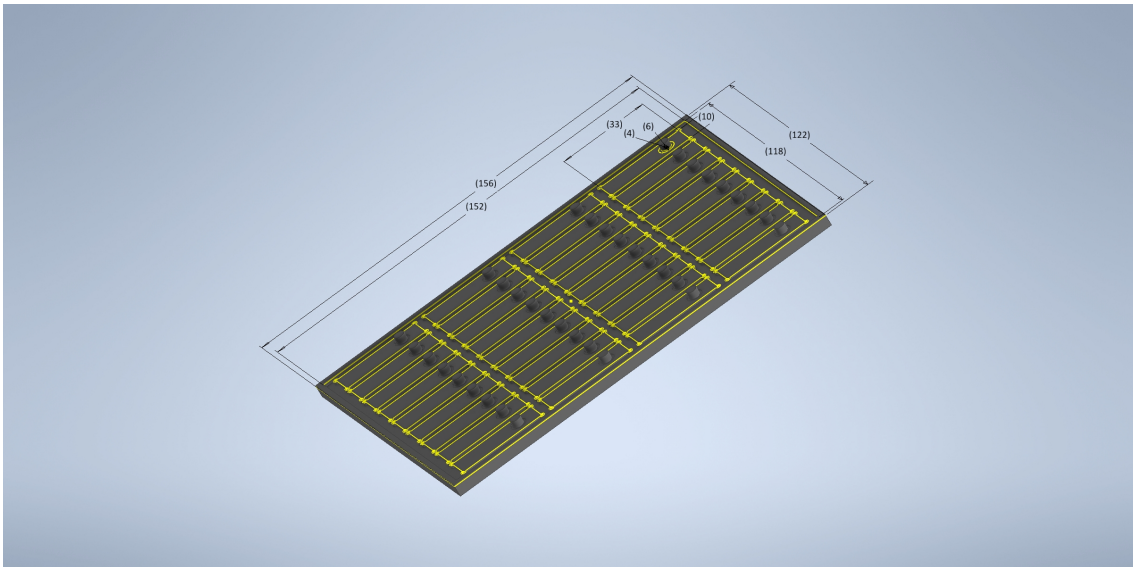


Figure 3.2: Design of the 3D-printed PDMS mold.

To further simplify liquid handling during experiments, a top plate was also designed

and printed. It contains 36 funnel-shaped openings, each with a volume of up to 300 μL , which also helps reduce evaporation during longer incubation steps. The top plate was designed to compress the PDMS layer to keep the system aligned and ensure a tight seal during incubation. The top plate was also printed with ironing to improve surface smoothness. The design of the top plate can be found in Figure 3.3.

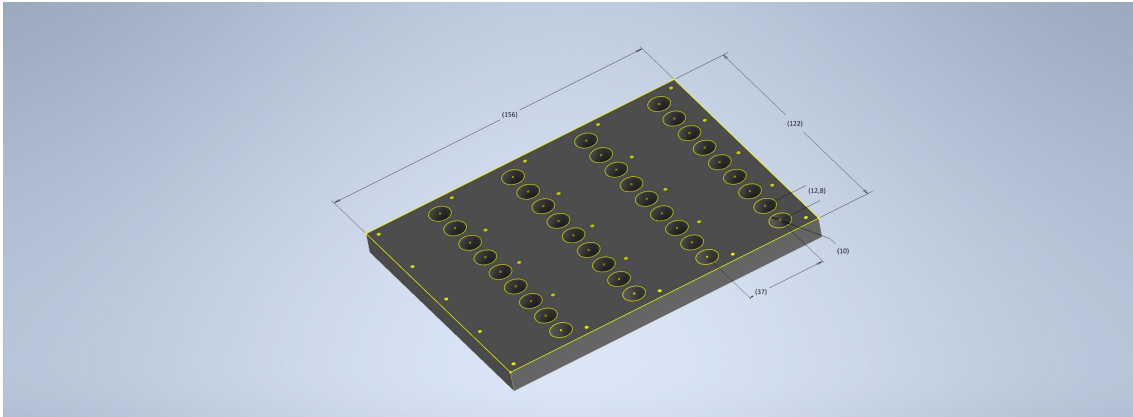


Figure 3.3: Design of the 3D-printed top plate.

A frame was printed to keep the base plate, PDMS layer, and top plate aligned. The entire setup is compressed using a threaded rod and nut system, which applies uniform pressure across the structure and keeps all layers in contact during experiments. The design of the frame can be found in Figure 3.4.

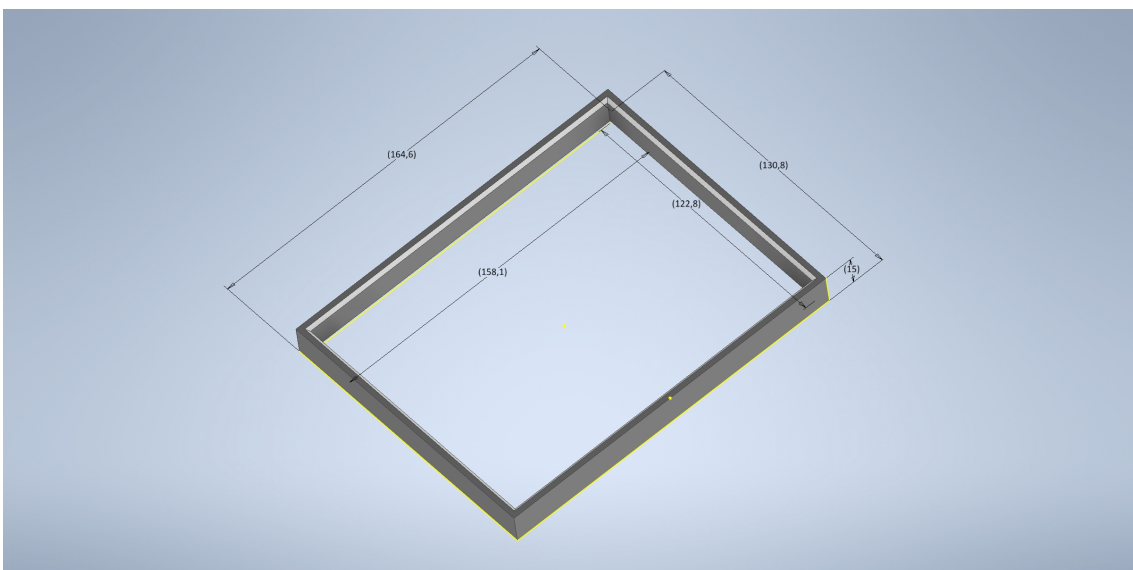


Figure 3.4: Design of the 3D-printed frame.

3.3 PDMS Casting

The PDMS mixture was prepared using a 10:1 ratio of base to curing agent[41]. Approximately 50 g base and 5 g curing agent were used for two PDMS molds. The mixture was carefully mixed and then placed in a vacuum chamber until all air bubbles were removed. The degassed PDMS was pipetted into the mold, which was subsequently placed in a vacuum chamber to ensure removal of any remaining air bubbles. The mixture was left to cure at room temperature for at least 4 days, due to the fact that the PLA mold cannot withstand high temperatures. It was ensured that the mold was evenly leveled during curing, as non-uniform leveling can lead to variations in the PDMS thickness, as observed in preliminary tests. This was achieved by placing the setup on a metal plate and adjusting the height of the corners using paper shims until a spirit level indicated a flat surface. After curing, the PDMS was removed from the mold using a scalpel to cut along the outer edges. The PDMS was then carefully peeled off the PLA mold.

3.4 Electrode Cleaning

A PlamSens3 potentiostat was used for electrochemical cleaning of the electrodes. The SPCEs were immersed in 0.5 M H_2SO_4 in a 10 ml glass beaker and subjected to cyclic voltammetry between -0.3 V and 2.0 V at a scan rate of 100 mV/s for 10 cycles[24]. Prior to the measurements, an equilibrium time of 10 s was applied. After cleaning, the electrodes were briefly dipped three times in Milli-Q water and stored in Milli-Q water until further use. The effect of the cleaning was investigated by measuring electrodes with CV and EIS before and after the electrochemical cleaning.

3.5 Cyclic Voltammetry Measurements

A PalmSens3 potentiostat was used to perform the CV measurements. The SPCEs were immersed in a deaerated solution containing 5 mM $\text{K}_3[\text{Fe}(\text{CN})_6]/\text{K}_4[\text{Fe}(\text{CN})_6]$ in 0.1 M KCl in a cuvette. Cyclic voltammetry was performed between -0.5 V and 0.5 V at a scan rate of 100 mV/s for 3 cycles. Prior to the measurements, an equilibrium time of 20 s was applied. For data analysis only the third cycle will be examined. The code for plotting CV measurements can be found in Appendix A.

3.6 EIS Measurements

A PalmSens3 potentiostat was used to perform the impedance measurements. The SPCEs were immersed in a deaerated solution containing 5 mM $\text{K}_3[\text{Fe}(\text{CN})_6]/\text{K}_4[\text{Fe}(\text{CN})_6]$ in 0.1 M KCl in a cuvette. Impedance measurements were carried out over a frequency range from 20 kHz to 0.05 Hz using 30 frequency points. A DC potential of 0.28 V was applied, with a sinusoidal AC perturbation amplitude of 10 mV. Prior to each measurement, an equilibration time of 45 s was used.

3.6.1 Impedance Data Analysis

As derived in the theory section, the total impedance of the Randles circuit shown in Figure 2.5 can be expressed as Equation 3.1.

$$Z = R_s + \frac{1}{\frac{1}{R_{ct} + \frac{\sigma}{\sqrt{j\omega}} \tanh(\sqrt{j\omega\tau})} + Q(j\omega)^\alpha} \quad (3.1)$$

In this project, this equation is used for nonlinear numerical fitting of the experimental impedance spectra, and the values of R_s [Ω], R_{ct} [Ω], Q [$\Omega^{-1} s^\alpha$], α , σ [$\Omega s^{-1/2}$], and τ [s] are extracted. As R_{ct} is considered the most important parameter, $\Delta R_{ct, step}$, defined as the percentage difference in R_{ct} from the previous measurement step, is also calculated. To evaluate the quality of the fitting procedure, the root mean square error (RMSE) is also calculated. The RMSE has units of ohms [Ω] and provides a quantitative measure of the deviation between the fitted model and the experimental impedance data. The full code can be found in Appendix B.

3.7 Field-Effect Transistor Measurements

Measurements were performed using the EmStat Pico together with a J113 transistor, which is an N-type JFET. The system was operated as an extended-gate FET, where the gate was connected to a screen-printed carbon electrode immersed in the electrolyte, while the JFET was kept electrically isolated from the solution.

Depletion measurements were carried out by sweeping the gate-source voltage while keeping the drain-source voltage constant. The sweep measurements were conducted with a fixed $V_{DS} = 0.4$ V, and the gate voltage was varied over a sweep window from -0.39 V to 0 V. The resulting drain current (I_D) was recorded as a function of V_{GS} . These measurements were performed in phosphate-buffered saline (PBS) in a cuvette. The code for plotting the transistor measurements can be found in Appendix C.

In addition, chronoamperometric (CA) measurements were performed by holding the voltages constant at $V_{DS} = 0.4$ V and $V_{GS} = -0.4$ V, while monitoring the drain current over time. The current was recorded at intervals of 0.1 s. The code for plotting CA measurements can be found in Appendix D.

3.8 Surface Modification Protocol

The overall surface modification workflow is summarized in Figure 3.5, while a detailed overview of the experimental conditions used for the different measurement methods is given in Table 3.2. Together, these provide an overview of the experimental design, including the chemical steps, measurement timing, and method-specific variations.

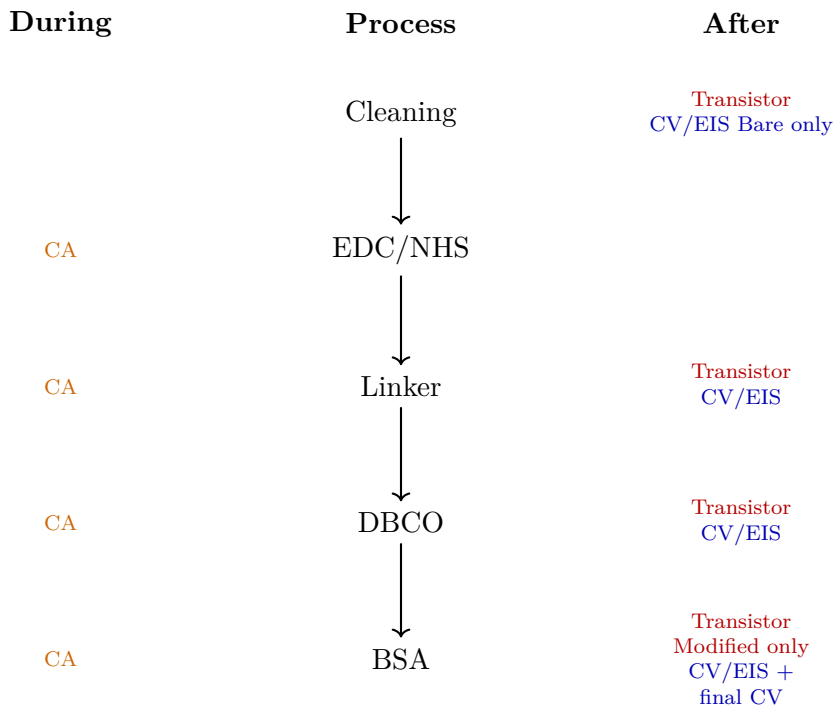


Figure 3.5: Overview of the surface modification workflow and measurement strategy. The diagram shows the chemical modification steps and the stages at which the different measurement techniques are performed.

Table 3.2: Surface modification conditions used for the different measurement systems.

Parameter	CV/EIS	Transistor	CA
EDC/NHS + linker setup	Cuvette	PDMS	Cuvette
EDC concentration	40 mM	10 mM	10 mM
NHS concentration	100 mM	25 mM	25 mM
Linker concentration	15 μ L in 10 mL PBS	3 μ L in 10 mL PBS	3 μ L in 10 mL PBS
DBCO concentration	1.6 mM	760 μ M	760 μ M
BSA concentration	10 mg/mL	10 mg/mL	10 mg/mL
Controls	Bare + linker	Bare	None

The general protocol is written below. Differences in PDMS usage, concentrations, and measurement configurations are summarized in Figure 3.5 and Table 3.2, while detailed method-specific descriptions are provided in subsections 3.8.1–3.8.3.

Prior to surface modification, all electrodes were cleaned and baseline measurements were performed where applicable. After each step, the electrodes were rinsed three times with Milli-Q water unless otherwise stated. Electrodes processed in PDMS were carefully dried without touching the active surface and placed in a pre-cleaned PDMS mold, which was subsequently sealed. In cuvette-based experiments, electrodes were directly immersed in solution.

Surface activation was performed using EDC/NHS chemistry in 0.1 M MES buffer (pH 4.7) for 20 minutes, followed by three rinsing steps with MES buffer to remove residual reagents. Following activation, Azido-PEG1-amine in PBS was added and incubated overnight under light-protected conditions to enable surface functionalization of the linker chemistry. After the linker attachment, DBCO-PEG4-alcohol in PBS was incubated overnight in cuvettes to enable click chemistry coupling. Finally, BSA (10 mg/mL in PBS) was applied as a blocking step and incubated for 60 minutes in cuvettes to reduce nonspecific binding.

3.8.1 CV and EIS Measurement

CV and EIS measurements were performed in cuvettes, exposing all electrodes to identical conditions. Five electrodes were used: one bare electrode, one linker-only electrode, and three fully modified electrodes. The bare electrode was incubated in PBS during all active modification steps (EDC/NHS, linker, DBCO, and BSA). The linker-only electrode underwent EDC/NHS activation and linker coupling but was incubated in PBS during the subsequent DBCO and BSA steps, while the fully modified electrodes followed the complete surface modification sequence. This ensured that observed differences were attributable to surface chemistry rather than incubation conditions.

To minimize variability in the EDC/NHS activation step, fresh EDC/NHS solution was prepared approximately every two electrodes and used within 10 minutes of preparation before incubation. This was done to reduce the impact of hydrolysis and ensure consistent activation efficiency across electrodes.

Only the bare electrode was measured directly after cleaning, since the remaining electrodes proceeded immediately to EDC/NHS activation to minimize surface variation prior to modification. For CV analysis, this measurement served as the baseline for all electrodes, while EIS did not use a baseline due to its higher sensitivity to electrode variation. Measurements were performed after the linker, DBCO, and BSA steps. After the final EIS measurements, the same electrodes were additionally analyzed using CV. This order was chosen to avoid potential surface effects from repeated CV scans influencing the EIS measurements.

3.8.2 Transistor Measurements

For transistor measurements, EDC/NHS activation and linker coupling were performed in PDMS, while all subsequent steps were performed in cuvettes. Five electrodes were analyzed: one bare electrode and four fully modified electrodes. Measurements were performed after cleaning, after linker incubation, after DBCO incubation and after BSA incubation. The bare electrode was incubated in PBS and measured at the same time points, except after the final BSA incubation. To support interpretation, five additional electrodes prepared using the same protocol were characterized using EIS.

3.8.3 CA Measurements

CA measurements were performed on a single fully modified electrode, with real-time signal recording during EDC/NHS activation, linker coupling, DBCO incubation, and BSA blocking. The EDC/NHS step was monitored for 15 minutes, while the subsequent steps were monitored for up to 1 hour. In all cases, incubation was continued overnight when required, even if the measurement was stopped earlier.

3.9 CA Measurements on Horse Saliva and Synthetic Biomarkers

CA measurements were also performed in horse saliva and in synthetic solutions of the two relevant biomarkers, BGN262 and COMP156, dissolved in Milli-Q water.

The horse saliva samples were obtained pre-characterized, where multiple samples had been pooled and analyzed using ELISA prior to this study. The measured concentrations were reported as 425 ng/mL for BGN262 and 80 ng/mL for COMP156. Three electrodes were incubated in the saliva samples for 30 minutes: one immediately after cleaning, one after BSA incubation, and one fully modified electrode that had undergone all surface modification steps (this electrode was also used in the transistor measurements).

Synthetic biomarker solutions were prepared in Milli-Q water at concentrations of 10 µg/mL for both BGN262 and COMP156. The solutions were analyzed individually using cleaned electrodes, and CA measurements were recorded for one hour.

3.10 PDMS Test

The effect of PDMS on the surface modification was investigated by comparing electrodes incubated in PDMS and in a cuvette. Two electrodes were cleaned and characterized using EIS. Both electrodes were then incubated in BSA solution for one hour, one inside a PDMS setup and the other in a cuvette. After incubation, the electrodes were briefly dipped three times in Milli-Q water before being analyzed again using EIS.

3.11 Use of AI

Artificial intelligence tools, such as ChatGPT, were used during the preparation of this thesis as a supportive tool in the writing and development process. The main use cases included improving clarity, grammar, and flow of the written text, as well as helping to rephrase sections to improve scientific readability. In addition, AI was used to help find relevant sources and material during the writing process, mainly as a starting point for literature search and topic understanding.

AI tools were also used to support coding-related tasks in MATLAB. The main

use was to help translate initial ideas and analysis approaches into functional code structures, as well as to support the development and refinement of scripts during implementation. This included assistance with debugging and improving the overall clarity and efficiency of the code.

Furthermore, AI-based image generation tools were used to create the thesis cover illustration. The image was generated using an OpenAI image generation model based on a text prompt describing a screen-printed carbon electrode with surface functionalization and biosensor-related surface chemistry.

4

Results

This chapter presents the results obtained from the experimental work. It includes the electrochemical and electronic characterization of the electrode surfaces, with a focus on evaluating the effects of cleaning, activation, and surface modification. The results from cyclic voltammetry, electrochemical impedance spectroscopy, transistor and chronoamperometry are presented.

4.1 PDMS setup

The PDMS setup was designed to enable controlled and reproducible masking of the electrode surface, ensuring selective exposure of the working electrode during surface modification. The complete PDMS setup is shown in Figure 4.1. The assembly steps from the base plate to the final sealed device are shown in Figures 4.2–4.6.

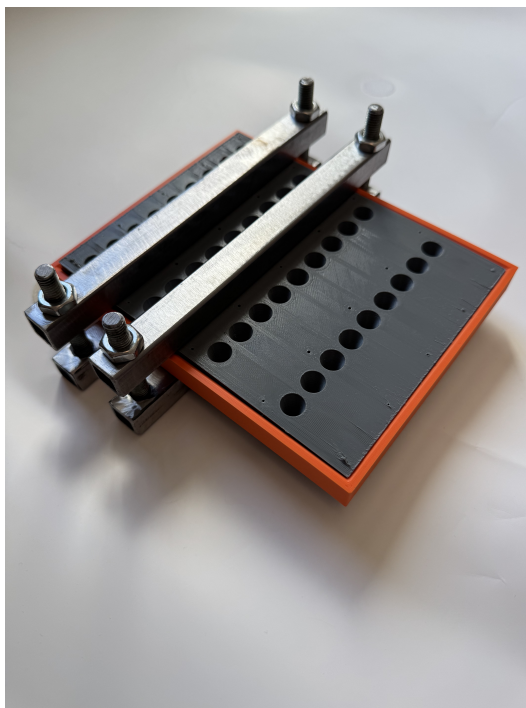


Figure 4.1: Complete sealed PDMS setup from the side.

4. Results

Figure 4.2 shows the electrodes in the base plate, where the base plate guides and secures the electrodes in place. This alignment is important to ensure that only the working electrode is exposed to solution, while the reference and counter electrodes remain physically isolated.



Figure 4.2: 3D printed base plate with carbon electrodes.

Figure 4.3 shows the frame that fits around the base plate allowing alignment of the next components.



Figure 4.3: 3D printed base plate and frame with carbon electrodes.

4. Results

Figure 4.4 shows the setup after the addition of PDMS. The frame and base plate allow for good alignment of the PDMS with the working electrode.



Figure 4.4: 3D printed base plate and frame with carbon electrodes in PDMS.

Figure 4.5 shows the setup when the top plate is aligned with the frame. This shows good alignment of all components.



Figure 4.5: 3D printed base plate, frame and top plate with carbon electrodes in PDMS.

Figure 4.6 shows how the setup is sealed and how the PDMS is compressed, ensuring no leakage.



Figure 4.6: Complete sealed PDMS setup from above.

Overall, the assembled PDMS system provides a stable and sealed configuration that enables controlled alignment and selective exposure of the working electrode, while reducing the risk of leakage between electrodes.

4.2 Electrode Cleaning

The cleaning of the electrodes was verified using both cyclic voltammetry and electrochemical impedance spectroscopy. CV was used to assess the redox behavior, while EIS was used to investigate interfacial characteristics and changes in charge transfer resistance resulting from the cleaning procedure.

4.2.1 CV

The cyclic voltammetry measurements related to electrode cleaning are presented in Figures 4.7–4.9. Figure 4.7 shows the cyclic voltammetry of the electrode cleaning. An increase in peak current for the anodic and cathodic peak per cycle can be seen, indicating an increase in electrochemical activity during the cleaning process. Cycles 9 and 10 are close to identical, suggesting that the cleaning is stable and more cycles would only slightly affect the electrode. Figure 4.8 shows the cyclic voltammogram before and after cleaning. The redox peaks become more defined after cleaning, resulting in a more well-defined redox peak shape. Figure 4.9 shows the cyclic voltammogram for three different electrode after cleaning. There is very little difference between the three electrodes, indicating high reproducibility between electrodes.

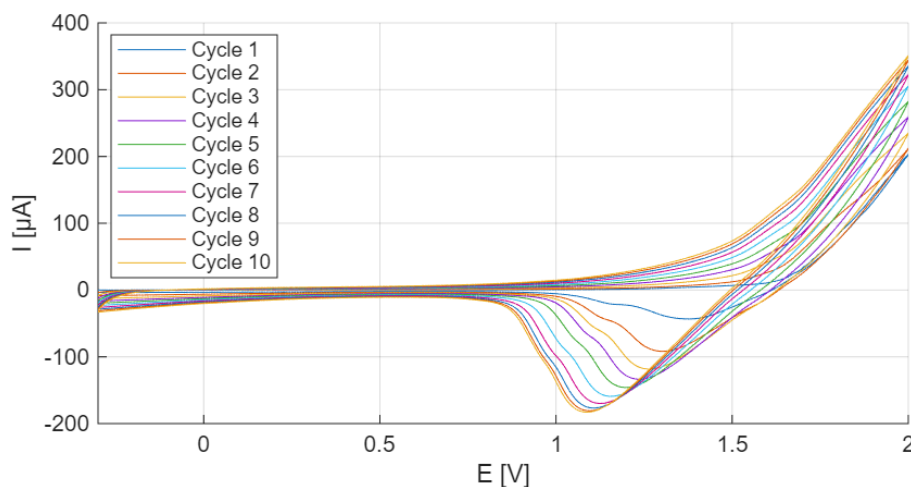


Figure 4.7: Cyclic voltammogram recorded during electrochemical cleaning and activation of the electrode in H_2SO_4 .

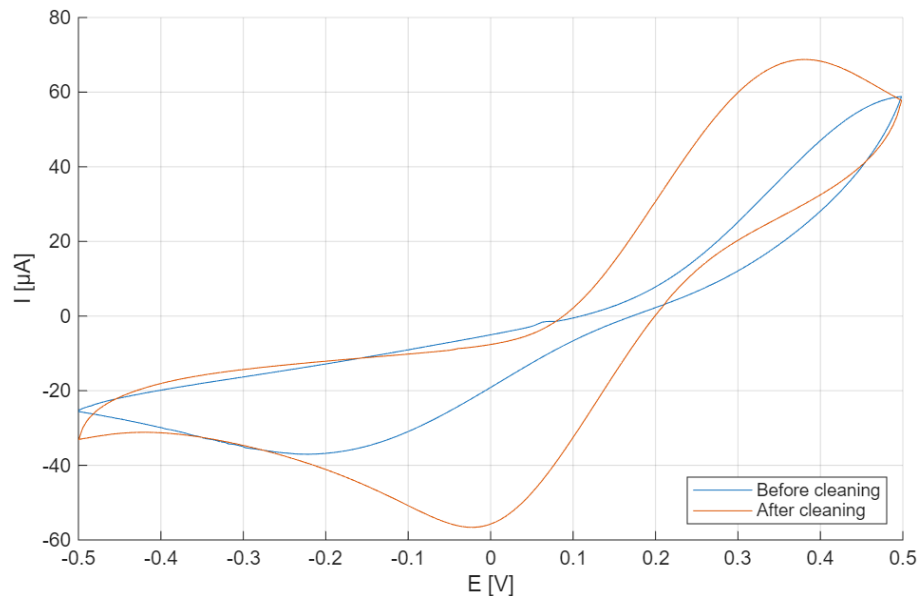


Figure 4.8: Cyclic voltammograms before and after electrode cleaning, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

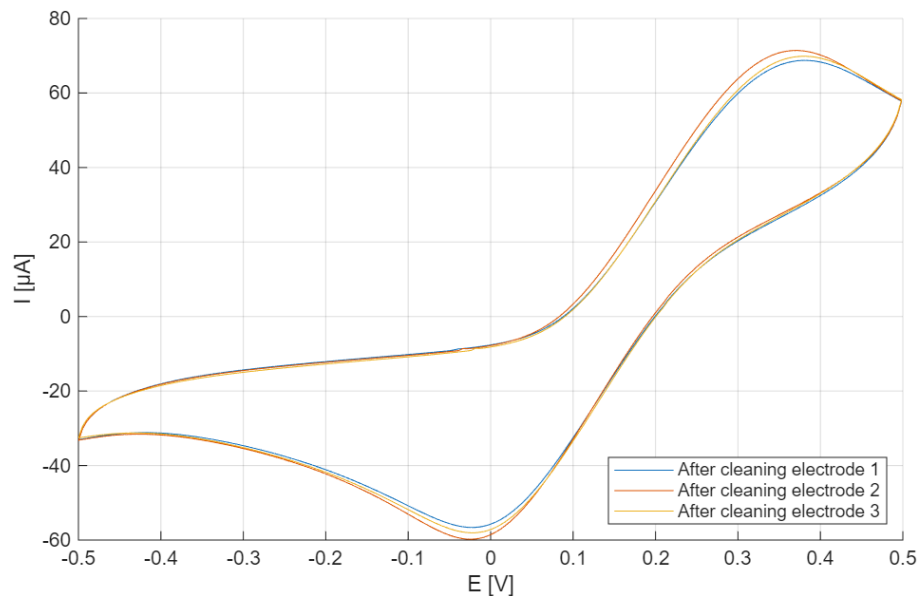


Figure 4.9: Cyclic voltammograms of three electrodes after cleaning, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

4.2.2 EIS

The Nyquist plot showing the impedance measurements before and after cleaning can be found in Figure 4.10. The numerical fit and extracted parameters can be found in Appendix E.1. The spectra before cleaning show a more defined semicircle, indicating high charge transfer resistance. After cleaning, the semicircle becomes less defined and the low-frequency region shows a more linear response, indicating reduced charge transfer resistance.

The fitted R_{ct} step change was 12%, indicating an increase after cleaning. However, this does not fully agree with the qualitative trend observed in the Nyquist plot, where a reduced semicircle is visually indicated.

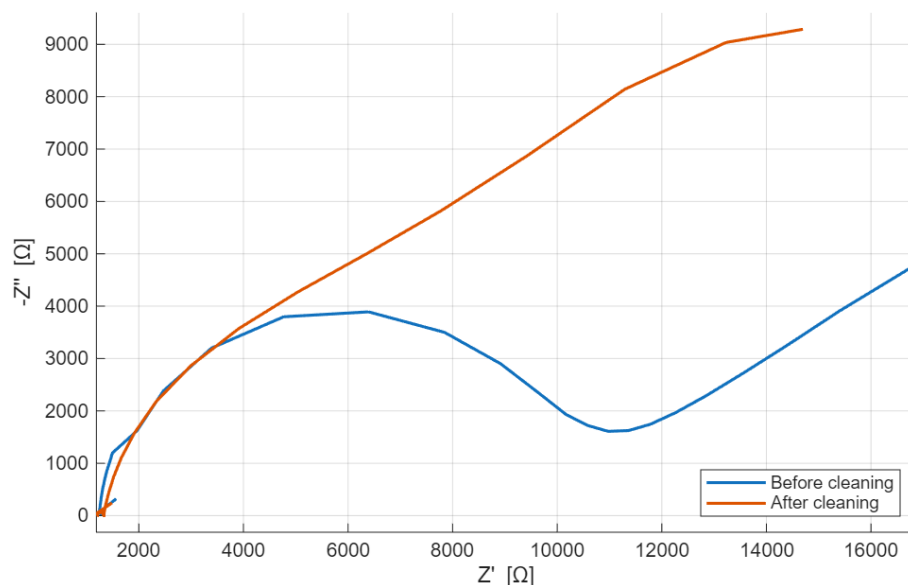


Figure 4.10: Nyquist plot before and after electrode cleaning, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

4.3 Surface Modification Verification

The surface modification of the electrodes was evaluated using CV, EIS, transistor measurements, and CA. These techniques were used to monitor changes in the electrochemical and electrical response of the electrodes after each modification step, providing information on the effect of the surface functionalization.

4.3.1 CV

The cyclic voltammograms for the different electrode conditions are shown in Figures 4.11–4.13, including the bare, linker-modified, and fully functionalized electrodes. Overall, a progressive decrease in anodic and cathodic peak currents is observed with increasing surface modification, indicating systematic changes in the electrochemical properties of the electrode surface during functionalization. Additional measurements are provided in Appendix F.

The bare electrode (Figure 4.11) shows only minor differences between measurements, with a slight decrease in anodic and cathodic peak currents over time, indicating small but limited surface changes during repeated cycling. The linker-modified electrode (Figure 4.12) exhibits a clear decrease in both anodic and cathodic peak currents compared to the bare state, while subsequent measurements remain stable, suggesting the formation of a consistent and stable surface layer after linker immobilization. For the fully modified electrode (Figure 4.13), the peak currents decrease

further with each incubation step, confirming progressive surface modification and increasing blockage of electron transfer.

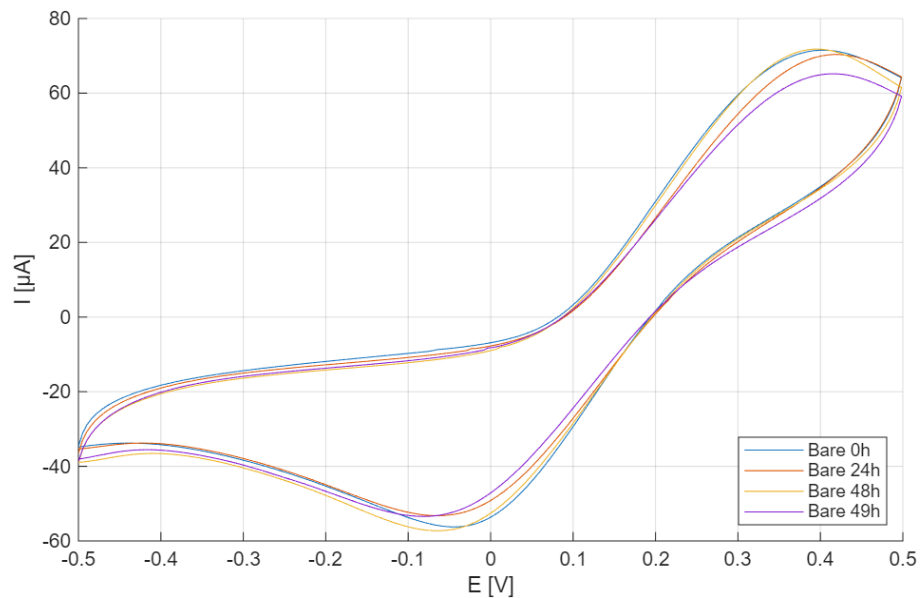


Figure 4.11: Cyclic voltammograms for the bare control electrode, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

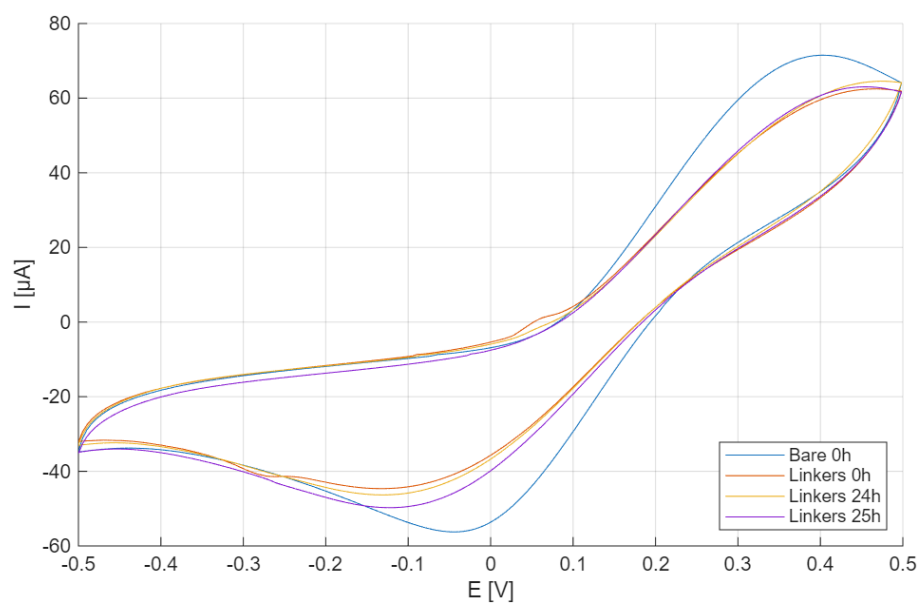


Figure 4.12: Cyclic voltammograms for the linker control electrode, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

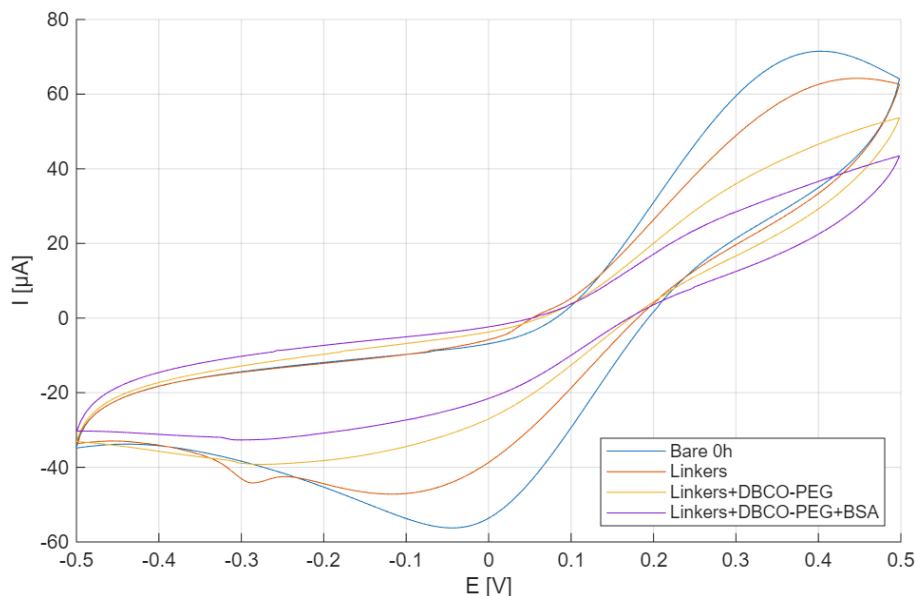


Figure 4.13: Cyclic voltammograms for the functionalized electrode, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

4.3.2 EIS

The Nyquist plots for the different electrode conditions are shown in Figures 4.14–4.16. Overall, the measurements show increasing impedance with successive surface modification steps, although substantial variability is observed in the fitted R_{ct} parameters between electrodes. Additional measurements for fully functionalized electrodes, together with fitting information for all measurements, are provided in Appendix E.2.

The bare electrode exhibits variability between repeated measurements, where one spectrum shows a more defined semicircle compared to the remaining scans, which are more diffusion-dominated. After linker modification, the measurements become more consistent, indicating improved reproducibility of the interfacial response. For the fully functionalized electrode, an overall increase in impedance is observed after each incubation step, consistent with progressive modification of the electrode surface.

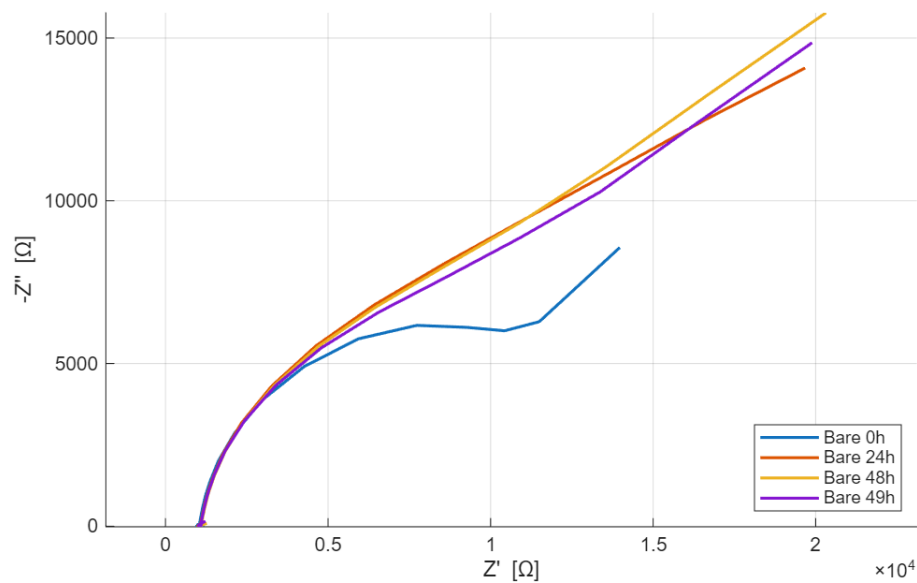


Figure 4.14: Nyquist plot for the bare control electrode, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

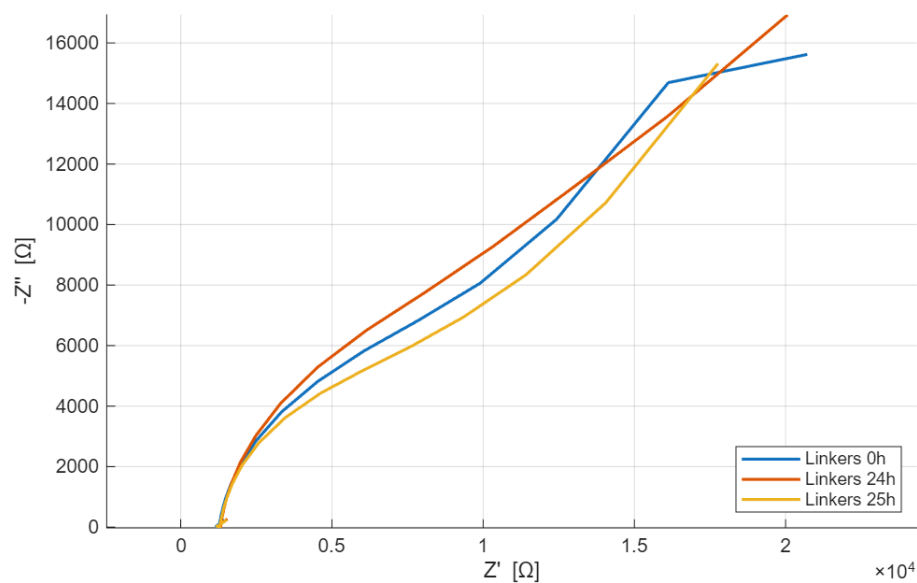


Figure 4.15: Nyquist plot for the linker control electrode, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

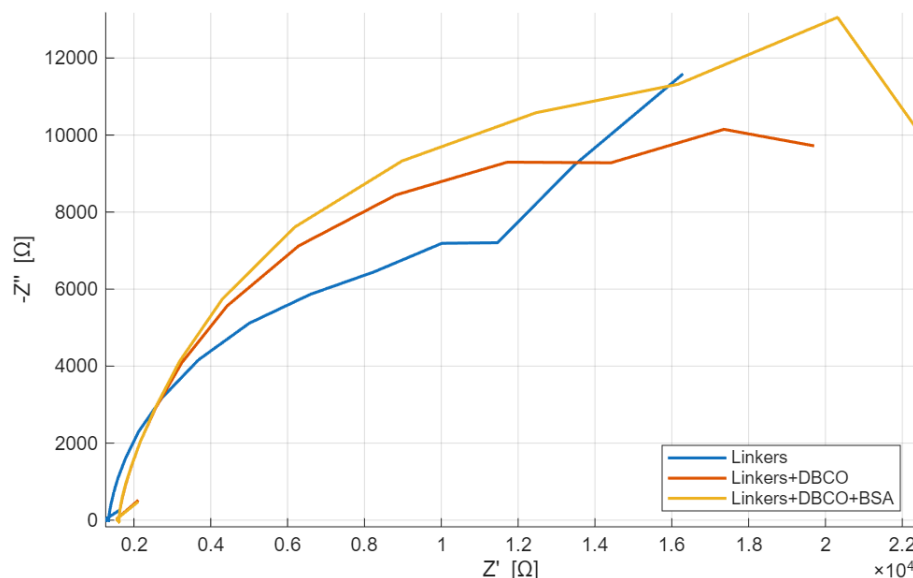


Figure 4.16: Nyquist plot for the functionalized electrode, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

Comparison of R_{ct} step change can be found in Table 4.1. The extracted values vary significantly between the three fully modified electrodes, and no consistent quantitative trend is observed across the dataset, indicating high variability in the fitted parameters. However, the Nyquist plots consistently show an overall increase in impedance with surface modification, suggesting a reproducible qualitative trend.

Table 4.1: Comparison of R_{ct} step change (%) across different electrode systems and time points.

System	24h	48h	49h
Bare electrode	175	-38	33
Linker electrode	-	211	-72
Electrode 1	-	140	36
Electrode 2	-	-5	0.3
Electrode 3	-	-54	336

To further validate the observed trends, the corresponding CV measurements for the EIS electrodes were analyzed. The cyclic voltammograms are shown in Figures 4.17–4.19, and show good agreement with the corresponding CV measurements for each modification state, confirming consistency between the two electrochemical techniques.

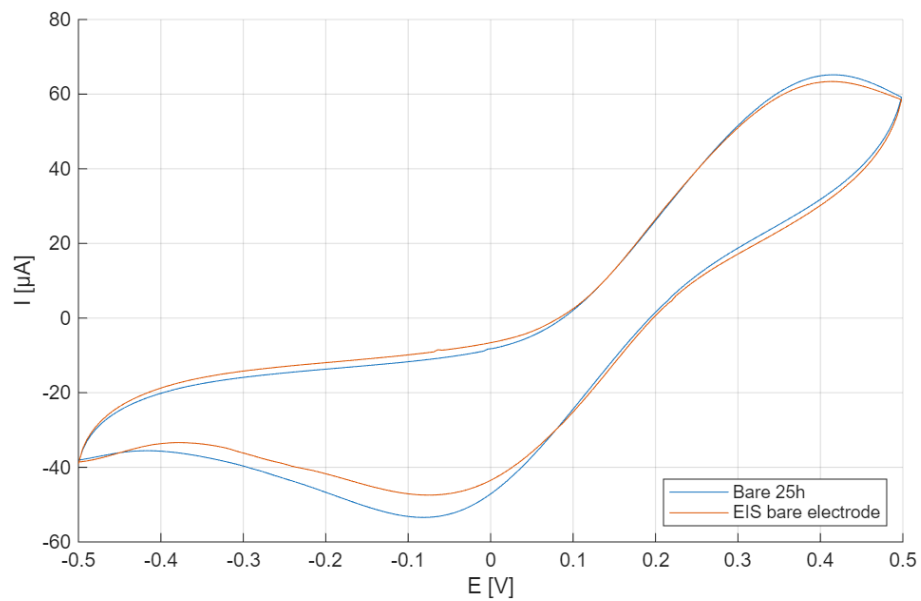


Figure 4.17: Cyclic voltammogram for the bare EIS control electrode, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

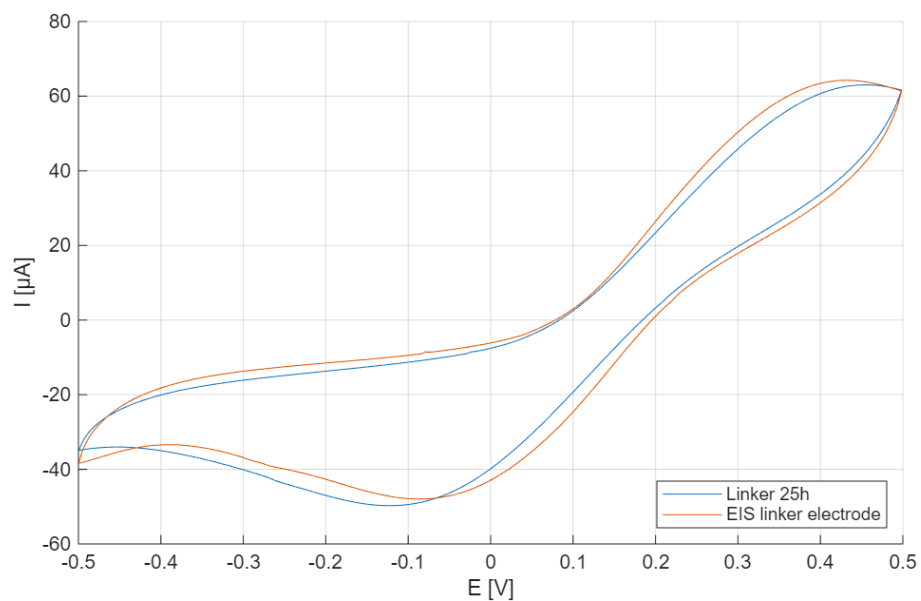


Figure 4.18: Cyclic voltammogram for the linker EIS control electrode, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

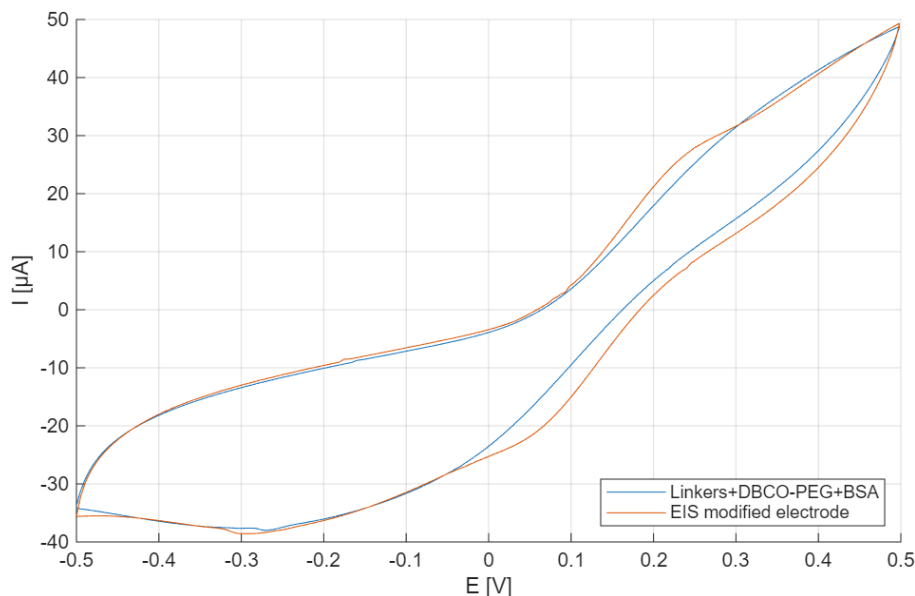


Figure 4.19: Cyclic voltammogram for the functionalized EIS electrode, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

4.3.3 Transistor Measurements

The transistor measurements for the different electrode conditions are shown in Figures 4.20 and 4.21. Overall, the measurements show only minor transistor response trends. The supporting EIS measurements show substantial electrode-to-electrode variability in the fitted EIS parameters. Additional measurements for fully functionalized electrodes are provided in Appendix G.

Figure 4.20 shows the transistor measurements for the bare electrode. The current responses show a slight increase over time. The transfer curves showed a non-ideal, partially parabolic shape rather than a typical linear JFET response, suggesting that the measured behavior may be influenced by extended-gate effects and hysteresis phenomena. Figure 4.21 shows the transistor measurements for the fully modified electrode. The measurement after the linker incubation shows lower current values than the bare state. This trend was not observed for the bare electrode, which showed a gradual increase in current over time. After the DBCO and BSA steps, the current responses increase relative to the previous step.

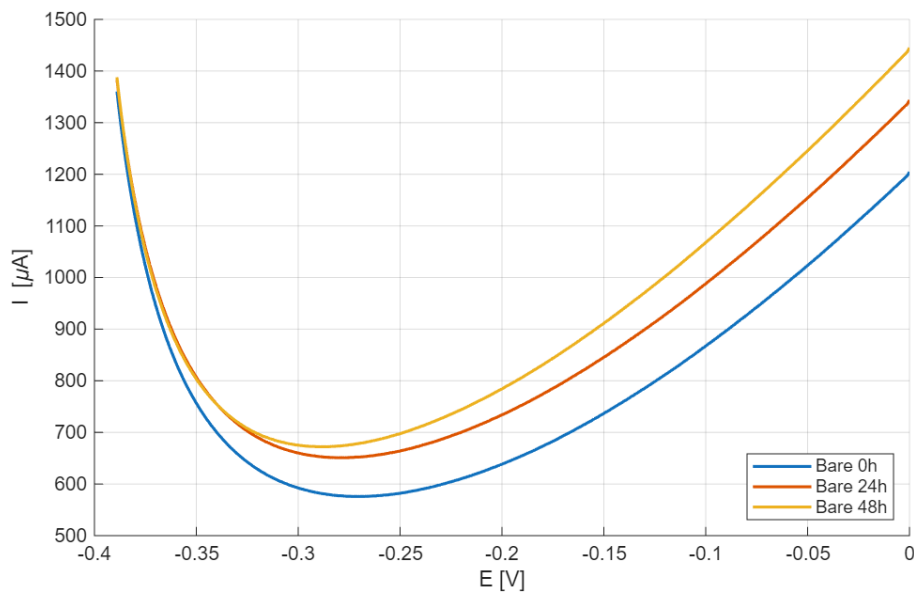


Figure 4.20: Transistor measurement for the bare electrode, measured in PBS.

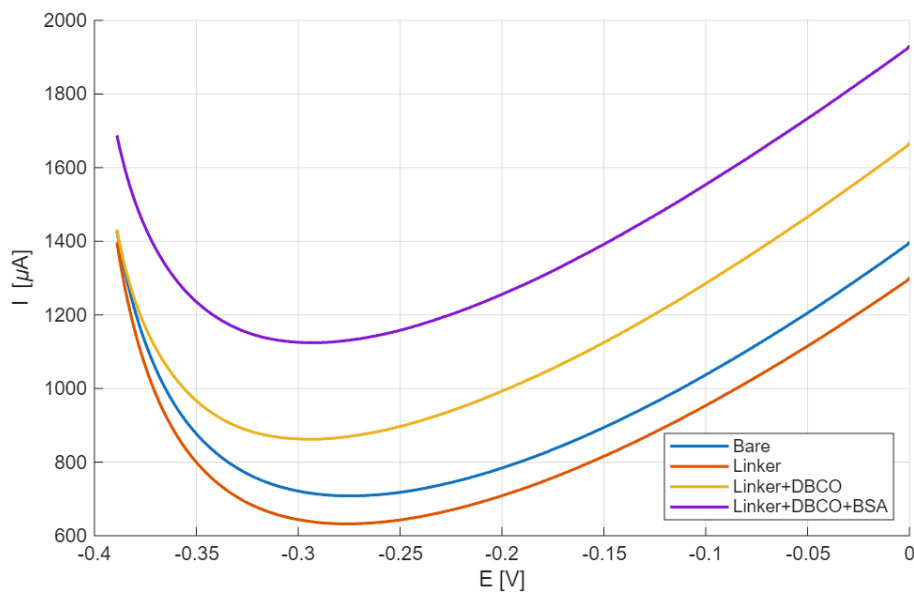


Figure 4.21: Transistor measurement for the fully modified electrode, measured in PBS.

Additional EIS measurements were performed on electrodes prepared using the transistor measurement protocol to investigate the electrochemical response of the PDMS-based surface modification.

Figure 4.22 shows the Nyquist plot for the bare electrode, where the spectra are similar across measurements, suggesting minimal changes in impedance. Figure 4.23 shows the Nyquist plot for the fully modified electrode, where an overall increase in impedance is observed with successive modification steps.

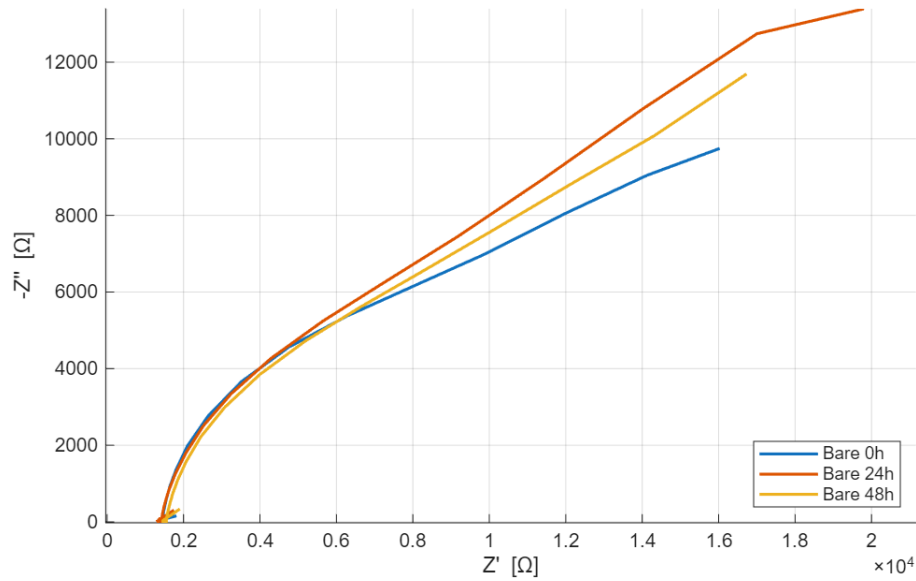


Figure 4.22: Nyquist plot for the bare control electrode prepared using the transistor measurement protocol, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

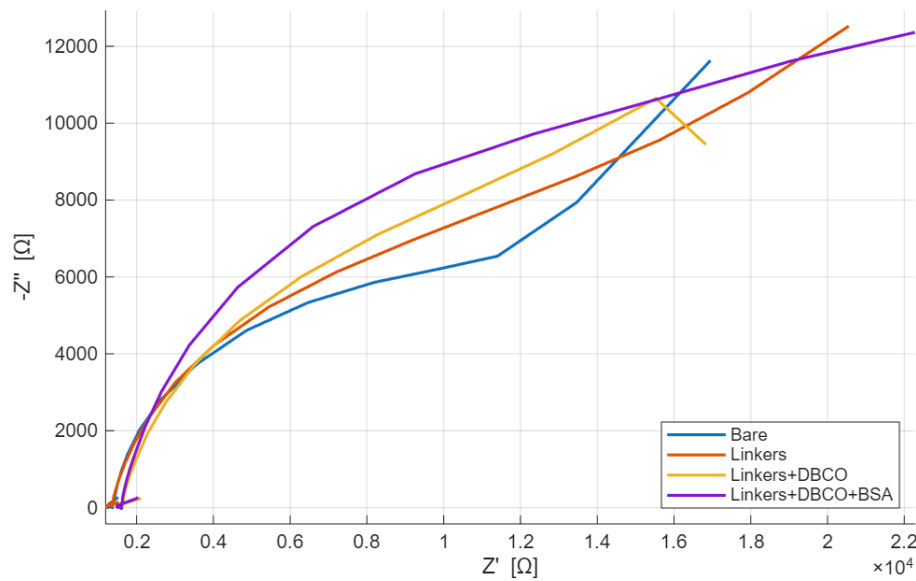


Figure 4.23: Nyquist plot for the modified electrode prepared using the transistor measurement protocol, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

Comparison of R_{ct} step change can be found in Table 4.2. The R_{ct} step change values for the incubation steps vary between electrodes, and no consistent trend is observed across the dataset. However, the Nyquist plots generally indicate an increase in impedance with successive modification steps, although the magnitude and consistency of this trend vary between electrodes.

Table 4.2: Comparison of R_{ct} step change (%) across different electrode systems and time points for the transistor protocol electrodes.

System	24h	48h	49h
Bare electrode	90	-55	-
Electrode 1	76	11	34
Electrode 2	-13	5	-21
Electrode 3	81	-28	68
Electrode 4	77	-27	30

4.3.4 Chronoamperometry

The chronoamperometry measurements during surface modification are shown in Figure 4.24. All curves remain stable over time, with no clear time-dependent changes in current observed after addition of the different reagents.

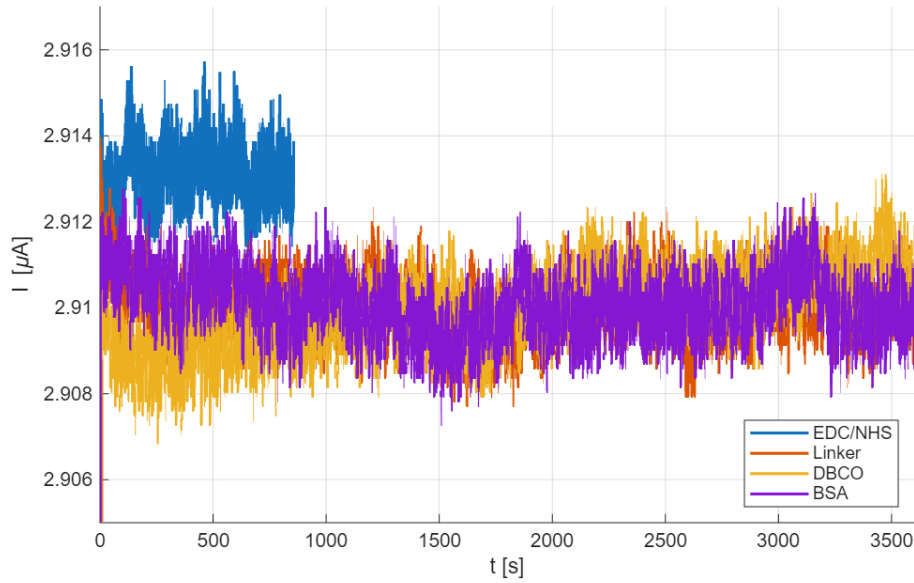


Figure 4.24: Chronoamperometry for the modification steps.

4.4 CA Measurements on Horse Saliva and Synthetic Biomarkers

The chronoamperometry measurements performed in horse saliva are presented in Figure 4.25. All three electrodes showed noisy current responses that remained approximately constant over time. The modified electrodes were stable at around 2.91 μA , while the bare electrode showed a slight decrease in current during the measurement period.

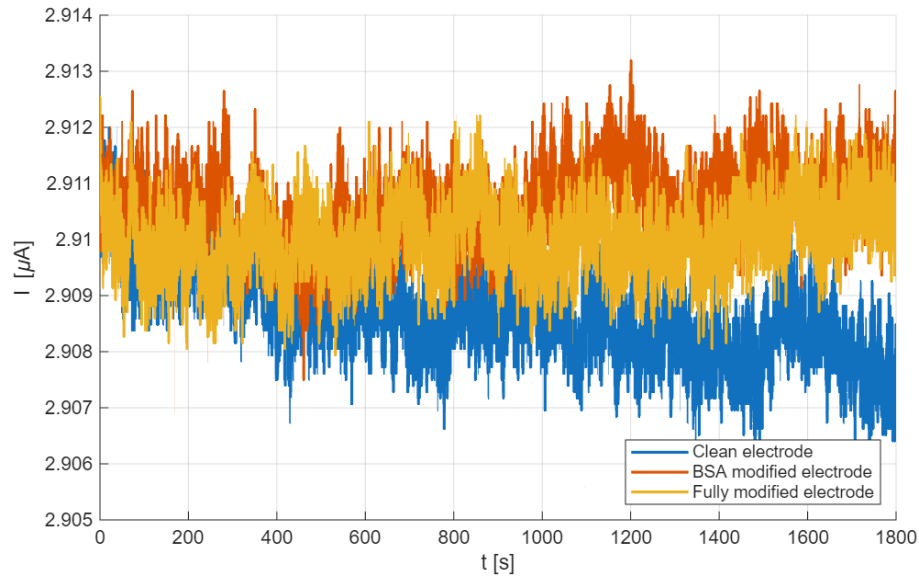


Figure 4.25: Chronoamperometry in saliva.

The chronoamperometry measurements performed in synthetic biomarker solutions are presented in Figure 4.26. Similar to the saliva measurements, all signals were noisy and remained stable over time, with no clear time-dependent current changes observed for either biomarker.

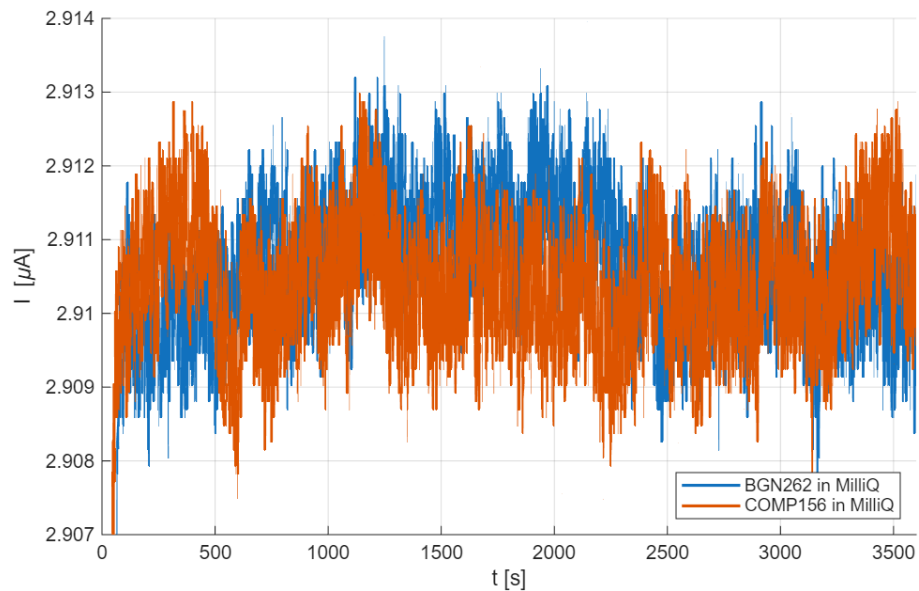


Figure 4.26: Chronoamperometry in synthetic biomarkers.

4.5 PDMS Test

Figure 4.27 shows the electrochemical impedance measurements for the different electrode conditions. From the Nyquist plots, the cuvette setup shows a larger

increase in impedance compared to the PDMS setup after BSA exposure. The numerical fit and extracted parameters can be found in Appendix E.3.

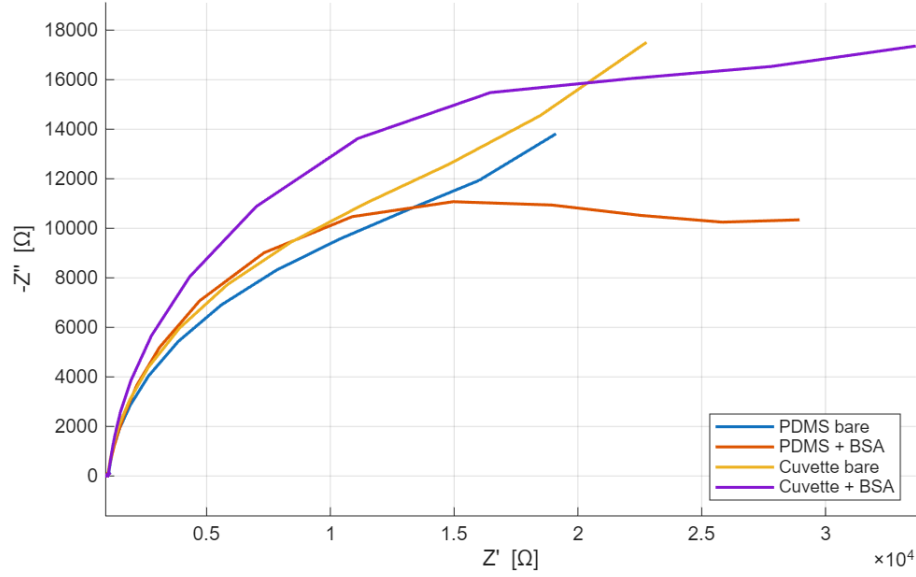


Figure 4.27: Nyquist plot comparing electrodes before and after BSA modification in PDMS and cuvette setups, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl. In the PDMS setup only the working electrode was modified, whereas in the cuvette setup all electrodes were exposed.

Comparison of R_{ct} step change can be found in Table 4.3. The extracted R_{ct} step change shows an increase of 67% for the PDMS-modified electrodes, while a value of -0.3% is observed for the cuvette-modified electrodes. This differs from the qualitative trend in the Nyquist plots, where both systems show an increase in impedance after BSA exposure.

Table 4.3: R_{ct} step change after BSA exposure for PDMS and cuvette electrodes.

System	R_{ct} step change [%]
PDMS	67
Cuvette	-0.3

5

Discussion

This chapter discusses the obtained results in relation to the expected surface chemistry and sensor behavior. The electrochemical and transistor measurements are evaluated, together with limitations of the methods and possible sources of error. Finally, suggestions for future improvements and further studies are presented.

5.1 Numerical Fitting of EIS Data

The numerical fitting of the EIS data often showed good visual agreement, as the fit was usually close to the original curve. However, when looking at the RMSE values, these range from $61\ \Omega$ to $1631\ \Omega$, which is a wide range. It is therefore likely that the code sometimes struggles to fit the Nyquist plot to the Randles circuit.

The extracted parameters do at times not correlate with the chemically or visually expected values. Since R_{ct} is the main parameter used, this is what will be discussed. When EIS was measured before and after cleaning (Figure 4.10), the fit suggested that R_{ct} was increased by 12%. However, this does not agree with the expected electrochemical behavior, as a cleaner surface should allow for more effective charge transfer, lowering the R_{ct} value. In the Nyquist plot, the spectra before cleaning showed a more defined semicircle, indicating higher charge transfer resistance, while this was not the case after cleaning, suggesting a decrease in R_{ct} .

Similarly, for the PDMS test (Figure 4.27), the Nyquist plot showed that the impedance values were increased more for the cuvette modified electrode than for the PDMS electrode. But according to the extracted parameters, R_{ct} decreased 0.3% for the cuvette electrode and increased by 67% for the PDMS electrode. This is contradictory to the theory since more coverage should lead to higher charge transfer resistance.

This could be due to the system's poor ability to numerically fit diffusion-dominated measurements, and more defined semicircles may be easier to fit. This could potentially be improved by changing the EIS measurement settings so that the semicircle becomes more defined even for low resistance systems.

Overall, the numerical fitting of the EIS spectra often gave good visual agreement with the measured data, but the extracted parameters frequently disagreed with the visual interpretation of the Nyquist plots. This suggests that the fitting procedure

requires further improvement to provide more reliable fitted parameters, particularly for diffusion-dominated systems. Therefore, the discussion primarily focuses on the visual interpretation of the Nyquist plots rather than the extracted fitting parameters.

5.2 Electrode Cleaning

The electrode cleaning resulted in a CV response with more defined cathodic and anodic peaks (Figure 4.8). This indicates more efficient redox reactions due to a cleaner and more electrochemically active electrode surface. Moreover, the three cleaned electrodes showed almost identical CV spectra (Figure 4.9), supporting the use of a common baseline for the CV electrodes during the surface modification experiments.

The EIS Nyquist plot (Figure 4.10) showed a more defined semicircle before cleaning and a more diffusion-dominated response after cleaning, suggesting a decrease in charge transfer resistance, which is expected for a cleaner electrode surface.

5.3 Surface Modification Verification

This section evaluates the surface modification using CV, EIS, and transistor measurements, and compares how well each technique reflects the expected changes in surface chemistry. The consistency and reliability of the observed trends are discussed across the different methods.

5.3.1 CV

The bare electrode (Figure 4.11) was consistent over time, with a slight decrease in anodic and cathodic peak current suggesting that the surface was slightly affected by the incubation in PBS. The linker electrode (Figure 4.12) showed a large difference compared to the bare electrode after the linker step. The anodic and cathodic peak current decreased, showing that the oxidation reaction was more hindered. The curves after the linker step were all similar, suggesting stable linker chemistry. The fully modified electrodes (Figure 4.13) all showed similar trends, differing from the linker electrode. The anodic and cathodic peak current continued to decrease with all surface modification steps, suggesting a successful immobilization.

In general, there was a clear difference between the bare, linker, and fully modified electrodes, with decreasing anodic and cathodic peak currents observed after each modification step. This suggests progressively hindered redox reactions due to increased surface coverage, indicating successful surface functionalization.

5.3.2 EIS

The bare electrodes first measurement right after cleaning (Figure 4.14), differed from the remaining measurements. This could be due to carbonyl groups and other

surface groups introduced during the electrochemical cleaning affecting the EIS spectra, but which disappear over time and incubation in PBS. The other measurements were similar and showed minimal variation. The linker molecule had no baseline measurement, therefore the linker step cannot be directly compared to the cleaned electrode. However, the linker chemistry is believed to be successful due to the CV measurement similar to the linker CV electrode (Figure 4.18). The three measurements after incubation in linker were similar (Figure 4.15), suggesting stable surface linker chemistry, with no clear rearrangement or degradation over time.

The three modified electrodes all showed similar CV spectra to the modified CV electrodes suggesting successful surface modification (Figure 4.19). These Nyquist plots showed more variation than the bare and the linker electrode (Figure 4.16). For all modification steps the impedance values increased, suggesting that the immobilization can also be observed in the EIS measurements. However, this did not result in large changes in the spectra, probably due to the small size of the chemicals, therefore EIS alone was not as clear as the CV measurements. No clear trends could be identified from the R_{ct} step values.

Overall, the EIS measurements showed slight differences between the bare, linker and fully modified electrodes, as the modified electrodes showed increased impedance after surface modification. This indicates more hindered redox reactions, suggesting blockage of the electrode surface due to successful immobilization of the surface layers. CV measurements on the same electrodes after full modification also showed voltammograms similar to the corresponding modified CV electrodes, further supporting successful surface functionalization. However, the impedance differences remained relatively small, likely due to the thin molecular surface layers, and the modification trends were therefore clearer in the CV measurements.

5.3.3 Transistor measurements

The transistor transfer curves showed a non-ideal, partially parabolic shape rather than the expected continuous increase in current typically observed for an ideal N-type depletion JFET. This behavior may be related to extended-gate effects, where charge storage, hysteresis, ionic drift, or leakage at the electrode/electrolyte interface influence the effective gate voltage during the sweep. Such effects may contribute to the observed drift and electrode-to-electrode variability in the measurements.

The bare electrode showed a slight increase across all overnight incubations in PBS (Figure 4.20). For the modified electrodes, the bare and linker measurements were generally similar, with the linker signal sometimes slightly below the bare electrode (Figure 4.21). This was followed by an increase from linker to DBCO, and a further increase after BSA for all four electrodes.

Since the bare electrode increased after overnight incubation in PBS, while all modified electrodes either decreased or remained similar after the linker step, this may indicate successful surface modification and sensitivity to the modification steps.

However, it should be noted that a different protocol was used for these measure-

ments compared to the CV and EIS experiments (lower chemical concentrations, initial steps performed in PDMS, and a longer delay between cleaning and EDC/NHS activation of approximately 2 hours). Therefore, the results cannot be directly compared to the CV and EIS measurements, and it is not possible to conclude with certainty that the same modification efficiency was achieved. Nevertheless, similar trends can still be observed across the electrodes.

The additional EIS-type electrodes show similar trends, particularly in Figure 4.23, although these are less pronounced than in the main EIS electrodes. This may be attributed to the fact that only the working electrode was exposed during the linker step in PDMS, and consequently also during DBCO functionalization. As a result, the surface modification may have been only partially achieved, leading to weaker and less distinct signal changes.

Overall, the measurements show some consistent trends across the different modification steps, suggesting that surface modification may be occurring. However, the transistor response also showed non-ideal behavior together with relatively small and not fully consistent signal changes across the electrodes, meaning that the results should be interpreted with caution. For future studies, the protocol should be made more consistent with the CV and EIS experiments, particularly in terms of handling time and chemical concentrations. Additionally, optimization of the transistor measurement conditions, including the applied sweep window, may improve the interpretation of the transistor response and reduce non-ideal behavior. Performing the full modification sequence in cuvettes rather than PDMS may also improve signal quality.

5.3.4 Chronoamperometry

No clear binding signal could be observed from the CA measurements in Figure 4.24, which could be due to several factors. One possible explanation is that the binding events do not significantly alter the surface charge, meaning that the electrochemical response is not sufficiently affected to produce a measurable signal. Another contributing factor may be the measurement environment, as all experiments were performed in PBS. The relatively high ionic strength of PBS leads to a reduced Debye length, which can limit the ability to detect charge changes occurring at the surface.

Overall, no clear signal associated with binding was detected under the current conditions. To investigate this further, the measurements should be repeated using a system known to induce stronger surface polarization, preferably during the SPAAC step. In addition, different linker lengths should be tested in order to systematically evaluate the influence of Debye length on signal detection.

5.4 CA Measurements on Horse Saliva and Synthetic Biomarkers

CA measurements in horse saliva (Figure 4.25) showed overall stable but noisy current responses for all electrodes. The clean electrode exhibited a slight decrease in current over time, whereas the modified electrodes remained approximately constant around 2.91 μA . Due to the high noise level, the observed trends are small and should be interpreted with caution. If related to binding, this would be consistent with negatively charged species leading to a reduced signal in the n-type JFET response, while the BSA and fully modified surfaces may act as passivation layers.

The synthetic biomarker measurements (Figure 4.26) showed no clear response associated with binding or surface modification. All signals remained relatively constant over time, with fluctuations comparable to the noise level.

Overall, the CA measurements in both saliva and synthetic biomarker systems showed weak and inconsistent signal responses. This suggests that either the binding events do not produce a sufficiently strong electrical effect under the present conditions, or that the measurement environment is not optimal for detecting such changes.

5.5 PDMS test

Looking at the Nyquist plot (Figure 4.27), the cuvette-modified electrode showed a larger increase in impedance compared to the PDMS setup, suggesting a difference in the extent of surface modification between the two configurations. This indicates that the PDMS configuration likely limited the effective surface exposure during modification. However, the exact extent of this effect cannot be determined from these measurements alone.

To investigate this further, fluorescent molecules could be bound in both the cuvette and PDMS setups, followed by microscopy analysis to determine the spatial distribution of surface binding. SEM imaging could also potentially be used for similar analysis, although this would require detectable surface chemistry.

5.6 Future research

This section discusses future research needed in this area, based on the limitations and observations from this study. Surface immobilization, measurement optimization, biomarker binding, sensor design, and electrode preparation are addressed as key areas for further improvement.

5.6.1 Surface Chemistry and Immobilization Optimization

There are multiple things that can be optimized and investigated further. A good starting point would be to introduce Fab' fragments and create the active electrodes, since the surface chemistry and reaction mechanisms are believed to have been successful. To do this, a similar protocol to the one in this study would be used, but in the DBCO step a 1:1 molar ratio of the DBCO-PEG12-alcohol and the Fab' coupled DBCO-PEG4-amide-lipoic acid would be mixed and incubated overnight. This immobilization should be investigated using CV and EIS.

When this surface chemistry is successful, there are multiple things that can be optimized. Reaction conditions such as concentration, incubation time and temperature can be optimized to make sure the surface chemistry is effective in terms of both performance and time efficiency. The ratio between Fab' fragments and passivation PEGs also needs to be examined, as well as whether this surface immobilization should be done in one or two steps. The PEG lengths also need to be optimized. The linker molecule was made as short as possible, which is beneficial for the Debye length, but the passivation PEGs should be optimized to ensure sufficient passivation without sterically hindering biomarker binding to the Fab' fragments.

The pH and solvent conditions for the EDC/NHS step can be optimized. Organic solvents such as Dichloromethane could potentially reduce hydrolysis during activation, increasing the lifetime of the esters [33].

To increase the amount of EDC-activated esters on the surface, two approaches can be considered. Carboxymethyldextran can be used to treat the surface between cleaning and EDC/NHS activation, creating more carboxyl groups on the carbon surface [20]. To remove the EDC/NHS step completely, pre-treated electrodes can also be used, such as amine- or carboxylated surfaces [33]. This can be done by using functionalized c-dots and depositing them onto the electrode surface. If carboxylated c-dots are used, the same chemistry as in this study can be used, but for amine-functionalized c-dots a different linker chemistry must be used. To evaluate the effect of surface modification, CV and EIS can be used for comparative analysis of changes in electrochemical behavior, while techniques such as fluorescence microscopy, SEM, or XPS are more suitable for quantitative analysis of surface chemistry and functional group presence.

5.6.2 Measurement Optimization

The measurements used in this study can also be optimized further. The measurement that showed the clearest proof of surface modification was CV. However, it must be noted that the surface functionalization in this project was smaller in scale (due to shorter PEG chains and lower molecular size of the surface chemistry) than what will be used in future studies. Therefore, it is a good idea to use both EIS and CV in the future since, as the electrode biolayer is increased, there is a risk that the CV spectra will be completely flat, or show only small differences between surface modification steps due to blocking of the redox reaction. EIS will probably be more reliable and will likely show larger differences when a thicker biolayer is formed.

Therefore, the EIS settings and numerical fitting procedure should be improved to better handle all Nyquist plots, and to allow discussion of R_{ct} instead of only the shape of the curves.

The transistor measurements and the overall measurement system also require further optimization. Future studies should investigate which transistor is most suitable for this type of sensing system, as well as how the transistor responds to chemical systems known to generate surface charge or polarization differences. To evaluate the transistor response independently of the biomarker system, click reactions known to induce charge changes could be investigated using both transistor and chronoamperometry (CA) measurements. Different linker lengths could then be tested to determine which are sufficiently short to allow signal detection, thereby investigating the relationship between binding events and the Debye length. Furthermore, if biomarker binding to Fab' fragments can be detected using EIS measurements, both CA and transistor responses should also be evaluated. If no measurable CA or transistor response is observed, the Fab' fragments may need to be modified to generate a larger polarization change upon binding.

5.6.3 Biomarker Detection and Binding Studies

The binding time between the biomarker and the Fab' fragments needs to be studied. Ideally, this should be done with CA measurements if the binding produces a detectable signal. If no detectable signal is produced, the best way to do this is probably with EIS. To investigate this, electrodes could be incubated for different time periods and analyzed to determine at which point no further changes occur with increased incubation time. These experiments should preferably be performed in low-concentration saliva samples, as these conditions are expected to produce the slowest binding.

Once the binding time has been studied, the temperature of this should be investigated. In the ELISA protocol, BGN262 binding occurs at 37°C, while COMP156 binding occurs at 25°C [42, 43]. This should be investigated using transistor, CV and EIS measurements to determine how much this influences the system, in order to know if it needs to be taken into account for the biosensor design. The biomarker binding kinetics can be evaluated using Equation 2.16, to determine K_D and S_{max} , to obtain valuable information about the system's kinetics.

5.6.4 Sensor Design and Measurement Strategy

Future sensor designs may benefit from the use of three simultaneously measured electrodes: one control electrode similar to the fully modified electrodes used in this study (linker + DBCO-PEG + BSA with the same PEG length as the passivation PEG used for the active electrodes) and two active electrodes, one for each biomarker. This could make it easier to distinguish specific biomarker binding from background signal changes.

During measurements of real samples, it should also be investigated whether baseline measurements are required, to determine whether electrode-to-electrode variations

significantly affect the results. If this is the case, a baseline measurement in for example PBS before biomarker incubation may be necessary, similarly to the R_{ct} step analysis used in this study.

Furthermore, if excessive unspecific binding is observed after the addition of antibodies, additional sample preparation steps may be required. Possible approaches could include centrifugation or filtration steps integrated with the electrode system, dilution using a supplied buffer, or incubation followed by washing to remove unspecific binding while retaining the biomarker on the surface.

5.6.5 Electrode Preparation and Cleaning

Alternative electrode cleaning methods should also be investigated. In this study, electrochemical cleaning using H_2SO_4 was used, however H_2O_2 cleaning has previously shown lower limits of detection [24]. This method was not feasible within this project due to the longer cleaning time and the need to clean the electrodes individually. Oxygen plasma treatment could also be investigated if the electrodes are fabricated on compatible substrates such as glass. Furthermore, the electrochemical cleaning procedure used in this study was time-consuming and increased the delay between cleaning and EDC/NHS activation unless the activation solution was prepared multiple times. The cleaning process should therefore be optimized, either through alternative cleaning methods or by improving the current setup. One possible improvement could be the use of a multichannel potentiostat, allowing several electrodes to be cleaned simultaneously. This would also enable multiple EIS and CV measurements to be performed in parallel, thereby reducing the total experimental time.

6

Conclusion

The aim of the thesis was to investigate surface modification on screen-printed carbon electrodes as a model system for biosensor functionalization. The work focused on establishing and evaluating a controlled surface chemistry using linker and passivation layers. The overall goal was to contribute to the development of an antibody-based biosensor for detection of osteoarthritis-related biomarkers in horse saliva.

Cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), transistor measurements, and chronoamperometry (CA) were used to monitor surface binding and modification. CA measurements were also performed in horse saliva and synthetic biomarkers to investigate whether any unspecific binding could be detected using this technique.

The surface functionalization showed clear electrochemical changes. CV indicated differences between modified and bare electrodes, suggesting successful surface modification. EIS measurements showed similar trends, with a slight increase in impedance after functionalization. Transistor measurements showed minor trends that may be related to surface modification. However, these results require further investigation. CA measurements did not show a clear indication of binding during surface modification, nor in saliva or synthetic biomarker experiments.

Furthermore, a PDMS-based setup was developed to enable selective modification of the working electrode. EIS measurements suggest that the increase in impedance was reduced when electrodes were functionalized within the PDMS configuration using BSA, indicating improved control over surface modification.

Overall, this work represents a first step toward the development of an antibody-based biosensor for detection of osteoarthritis-related biomarkers in equine saliva. It demonstrates that controlled surface functionalization of screen-printed carbon electrodes is feasible and produces measurable changes in electrochemical response.

Further optimization of the surface chemistry is still required, including reaction conditions and PEG linker length, in order to improve stability and binding efficiency. In addition, the immobilization strategy should be extended to include functional Fab' antibody fragments to enable specific biomarker recognition on the electrode surface. The interaction between antibody fragments and biomarkers should also be further investigated to determine optimal binding conditions.

6. Conclusion

Finally, the sensing performance should be evaluated more extensively using both transistor measurements and chronoamperometry. Future work should focus on improving signal and sensor sensitivity to enable reliable detection in complex biological samples such as saliva.

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A

MATLAB Code for CV Measurements

```
1  %% CV plot script
2
3  files = { % choose which files
4      '...'
5      '...'
6      '...'
7      '...'
8  };
9
10 names = { % edit legend here
11     '...'
12     '...'
13     '...'
14     '...'
15 };
16
17 % Create figure
18 figure; hold on; grid on;
19 xlabel('E [V]');
20 ylabel('I [ $\mu$ A]');
21
22 for k = 1:length(files)
23
24     % Read data
25     A = readmatrix(files{k});
26
27     % Extract potential and current (3rd cycle)
28     E = A(:,5);
29     I = A(:,6);
30
31     % Remove NaN values
32     valid = ~isnan(E) & ~isnan(I);
33     E = E(valid);
```

A. MATLAB Code for CV Measurements

```
34     I = I(valid);
35
36     % Plot CV curve
37     plot(E, I, 'DisplayName', files{k});
38
39 end
40
41 % Add legend
42 legend(names, 'Location', 'southeast')
```

B

MATLAB Code for EIS Measurements

```
1  %% EIS plot and fitting script
2
3  files = { % choose which files
4      '...'
5      '...'
6      '...'
7      '...'
8  };
9
10 names = { % edit legend here
11     '...'
12     '...'
13     '...'
14     '...'
15 };
16
17 % Settings
18 rowRange = 1:30; % rows used from data file
19
20 results = [];
21 Rct_all = zeros(length(files),1); % store Rct values
22
23 % fixed color set
24 colors = lines(length(files));
25
26 % Global Nyquist plot (data only)
27 figAll = figure;
28 hold on;
29 xlabel('Z' '\Omega')
30 ylabel('-Z' '\Omega')
31 grid on;
32 axis equal;
33
```

```
34 % Global Nyquist plot (data + fit)
35 figFitAll = figure;
36 hold on;
37 xlabel('Z' '\Omega')
38 ylabel('-Z' '\Omega')
39 grid on;
40 axis equal;
41
42 h_lines = gobjects(length(files),1);
43 h_lines_fit = gobjects(length(files),1);
44
45 for i = 1:length(files)
46
47     % Read data file
48     data = readmatrix(files{i});
49
50     % Extract frequency and impedance
51     freq = data(rowRange,1);
52     Zre = data(rowRange,5);
53     Zim = -data(rowRange,6);
54
55     % Remove NaN values
56     valid = ~isnan(freq) & ~isnan(Zre) & ~isnan(Zim);
57     freq = freq(valid);
58     Zre = Zre(valid);
59     Zim = Zim(valid);
60
61     % Sort by descending frequency
62     [freq, idx] = sort(freq, 'descend');
63     Zre = Zre(idx);
64     Zim = Zim(idx);
65
66     Z = Zre + 1i*Zim; % complex impedance
67
68     % Equivalent circuit model (Randles + finite Warburg)
69     model = @(p,f) p(1) + ...
70         1 ./ ( ...
71         1 ./ ( p(2) + ...
72         p(5).*tanh(sqrt(1i*2*pi*f*p(6))) ./ sqrt(1i*2*pi*f*p(6)) ) + ...
73         (1i*2*pi*f).^p(4).*p(3) );
74
75     % Cost function (real + imaginary)
76     costfun = @(p) [ ...
77         real(model(p,freq)) - real(Z); ...
78         imag(model(p,freq)) - imag(Z)];
79
80     % Initial guess
```

```

81     p0 = [min(real(Z)), max(real(Z))-min(real(Z)), 1e-5, 0.8, 100, 1];
82
83     % Parameter bounds
84     lb = [0 0 0 0 0 0];
85     ub = [Inf Inf Inf 1 Inf Inf];
86
87     options = optimoptions('lsqnonlin','Display','off');
88
89     % Fit model
90     p_fit = lsqnonlin(costfun, p0, lb, ub, options);
91
92     Zfit = model(p_fit,freq);
93     rmse = sqrt(mean(abs(Z - Zfit).^2));
94
95     % Save results
96     Rct_all(i) = p_fit(2);
97     results = [results; i-1, p_fit, rmse];
98
99     % Plot data only
100    figure(figAll)
101    h_lines(i) = plot(real(Z), -imag(Z), ...
102        'LineWidth',1.5, 'Color', colors(i,:));
103
104    % Plot data + fit (same color)
105    figure(figFitAll)
106    h_lines_fit(i) = plot(real(Z), -imag(Z), ...
107        'LineWidth',1.5, 'Color', colors(i,:));
108
109    hold on
110    plot(real(Zfit), -imag(Zfit), 'o', ...
111        'Color', colors(i,:), 'MarkerSize',4)
112
113    % Individual plot
114    figure;
115    plot(real(Z), -imag(Z), 'bo'); hold on;
116    plot(real(Zfit), -imag(Zfit), 'k-', 'LineWidth',1.5);
117    xlabel('Z' '\Omega');
118    ylabel('-Z' '\Omega');
119    grid on; axis equal;
120
121 end
122
123 % Legends
124 figure(figAll)
125 legend(h_lines, names, 'Location','southeast')
126
127 figure(figFitAll)

```

```
128 legend(h_lines_fit, names, 'Location','southeast')
129
130 % Results table
131 T = array2table(results, ...
132     'VariableNames', {'File','Rs','Rct','Q','alpha','sigma','tau','RMSE'});
133
134 % Step-to-step change in Rct
135 Rct_step = zeros(size(Rct_all));
136 for k = 2:length(Rct_all)
137     Rct_step(k) = (Rct_all(k) - Rct_all(k-1)) / Rct_all(k-1) * 100;
138 end
139
140 T.Rct_step_change_percent = Rct_step;
141
142 disp(T)
```

C

MATLAB Code for Transistor Measurements

```
1  %% Transistor measurement plot script
2
3  files = { % choose which files
4      '...'
5      '...'
6      '...'
7      '...'
8  };
9
10 names = { % edit legend here
11     '...'
12     '...'
13     '...'
14     '...'
15 };
16
17 % Global plot
18 figAll = figure; hold on; grid on;
19 xlabel('E [V]');
20 ylabel('I [\mu A]');
21
22 for i = 1:length(files)
23
24     % Read data
25     A = readmatrix(files{i});
26
27     % Extract potential and current
28     V = A(:,1);
29     I = A(:,2);
30
31     % Remove NaN values
32     valid = ~isnan(V) & ~isnan(I);
33     V = V(valid);
```

```
34     I = I(valid);
35
36     % Add to global plot
37     figure(figAll)
38     plot(V, I, 'LineWidth',1.5)
39
40     % Individual plot
41     figure;
42     plot(V, I, 'LineWidth',1.5)
43     grid on;
44     xlabel('E [V]');
45     ylabel('I [\mu A]');
46
47     title(names{i});
48
49 end
50
51 % Add legend
52 figure(figAll)
53 legend(names, 'Location','southeast')
```

D

MATLAB Code for CA Measurement

```
1  %% CA plot script
2
3  files = { % choose which files
4      '...'
5      '...'
6      '...'
7      '...'
8  };
9
10 names = { % edit legend here
11     '...'
12     '...'
13     '...'
14     '...'
15 };
16
17 % Global plot
18 figAll = figure; hold on; grid on;
19 xlabel('t [s]');
20 ylabel('I [\mu A]');
21
22 for k = 1:length(files)
23
24     % Read data
25     data = readmatrix(files{k});
26
27     % Extract time and current (Vds)
28     t = data(:,1);
29     I = data(:,2);
30
31     % Remove NaN values
32     valid = ~isnan(t) & ~isnan(I);
33     t = t(valid);
```

```
34     I = I(valid);
35
36     % Add to global plot
37     figure(figAll)
38     plot(t, I, 'LineWidth',1.5)
39
40     % Individual plot
41     figure;
42     plot(t, I, 'LineWidth',1.5)
43     grid on;
44     xlabel('t [s]');
45     ylabel('I [\mu A]');
46
47 end
48
49 % Add legend to correct figure
50 figure(figAll)
51 legend(names, 'Location','southeast')
```

E

Additional EIS Results

This appendix contains additional electrochemical measurements, equivalent circuit fitting results, and supporting data related to the experiments presented in the Results chapter.

E.1 Electrode Cleaning

This section contains additional EIS measurements and equivalent circuit fitting parameters related to the electrode cleaning experiments presented in Subsection 4.2.2. The measurements were used to evaluate the effect of electrochemical cleaning on the electrode impedance response.

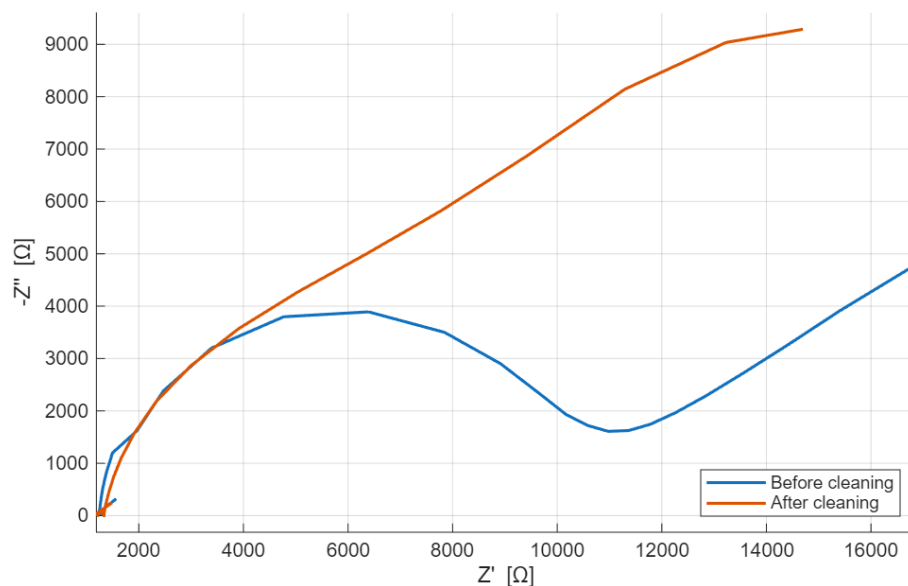


Figure E.1: Nyquist plot before and after electrode cleaning, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

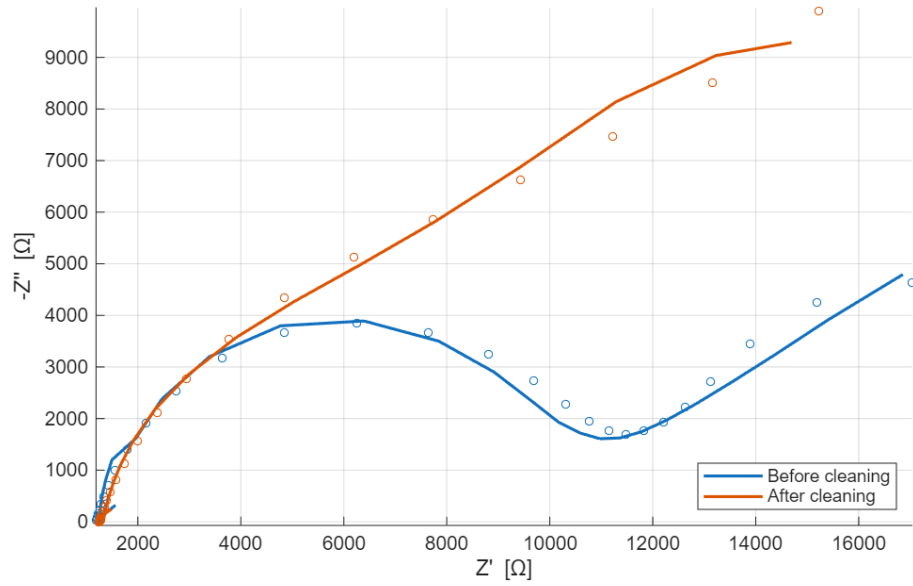


Figure E.2: Nyquist plot before and after electrode cleaning with the numerical fit, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

Table E.1: Equivalent circuit fitting parameters before and after cleaning.

Parameter	Before cleaning	After cleaning
R_s [Ω]	1189.3	1249.5
R_{ct} [Ω]	9666.5	10820
Q [$\Omega^{-1} s^\alpha$]	1.3515×10^{-6}	3.8464×10^{-5}
α	0.84032	0.80432
σ [$\Omega s^{-1/2}$]	10884	45857
τ [s]	8.1765	44.87
RMSE [Ω]	271.79	242.1
R_{ct} change [%]	-	11.935

E.2 Surface Modification Verification

This section contains additional EIS measurements, fitting parameters, and supporting CV measurements related to the surface modification experiments presented in Subsection 4.3.2. The datasets are included to support the observed trends during linker immobilization and subsequent surface functionalization.

E.2.1 Bare Electrode

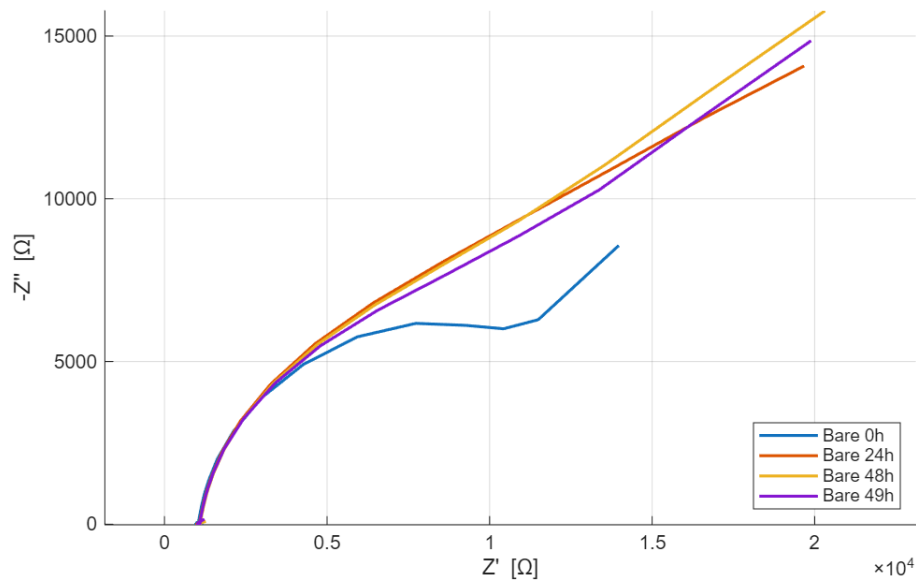


Figure E.3: Nyquist plot for the bare control electrode, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

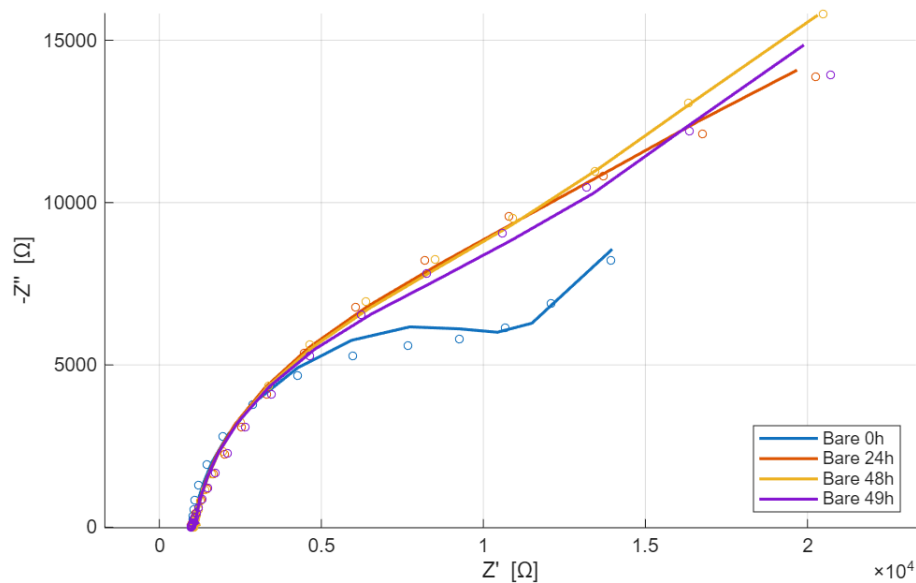


Figure E.4: Nyquist plot for the bare control electrode with the numerical fit, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

Table E.2: Equivalent circuit fitting parameters for the bare electrode measurements.

Parameter	Bare 0h	Bare 24h	Bare 48h	Bare 49h
R_s [Ω]	1020.4	974.04	1060.7	988.18
R_{ct} [Ω]	7980.7	21965	13664	18173
Q [$\Omega^{-1} s^\alpha$]	2.7902×10^{-5}	4.1149×10^{-5}	3.1723×10^{-5}	3.9141×10^{-5}
α	1	0.78446	0.86769	0.79015
σ [$\Omega s^{-1/2}$]	22546	28826	36355	23945
τ [s]	19.014	16.03	12.961	8.4884
RMSE [Ω]	248.86	220.89	138.11	292.84
R_{ct} step change [%]	-	175.22	-37.792	33.003

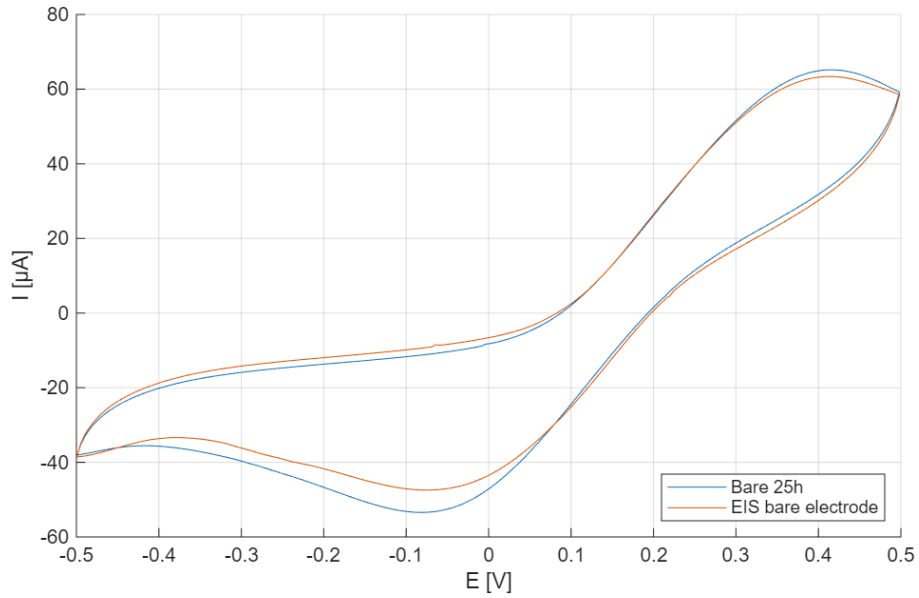


Figure E.5: Cyclic voltammogram for the bare EIS control electrode, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

E.2.2 Linker Electrode

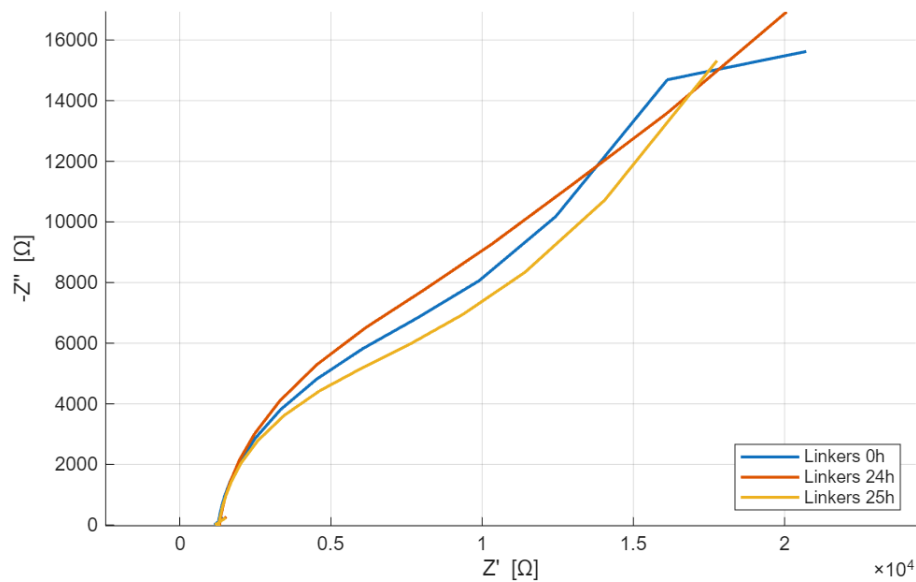


Figure E.6: Nyquist plot for the linker control electrode, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

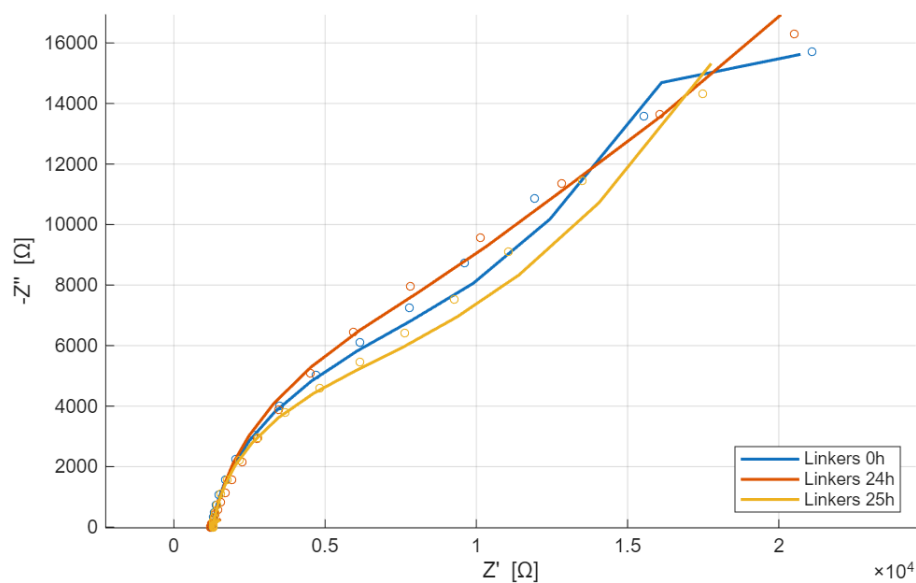


Figure E.7: Nyquist plot for the linker control electrode with the numerical fit, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

Table E.3: Equivalent circuit fitting parameters for the linker-modified electrode measurements.

Parameter	Linkers 0h	Linkers 24h	Linkers 25h
R_s [Ω]	1263.2	1211.4	1286.2
R_{ct} [Ω]	6725.5	20926	5806.3
Q [$\Omega^{-1} s^\alpha$]	2.5339×10^{-5}	4.4211×10^{-5}	2.4247×10^{-5}
α	0.94696	0.77193	0.95116
σ [$\Omega s^{-1/2}$]	32859	32607	35391
τ [s]	8.1401	10.054	12.629
RMSE [Ω]	351.1	236.28	353.52
R_{ct} step change [%]	-	211.15	-72.254

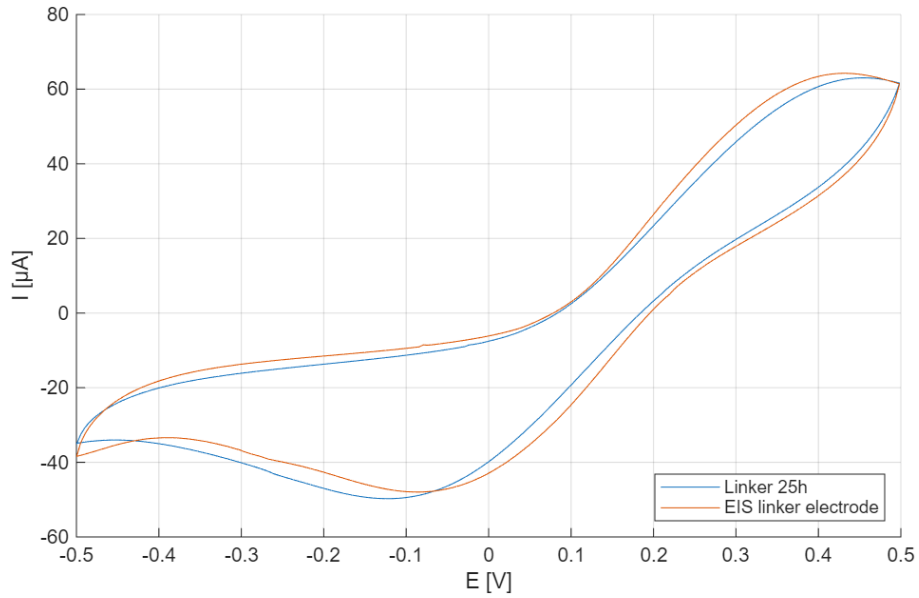


Figure E.8: Cyclic voltammogram for the linker EIS control electrode, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

E.2.3 Fully Modified Electrode 1

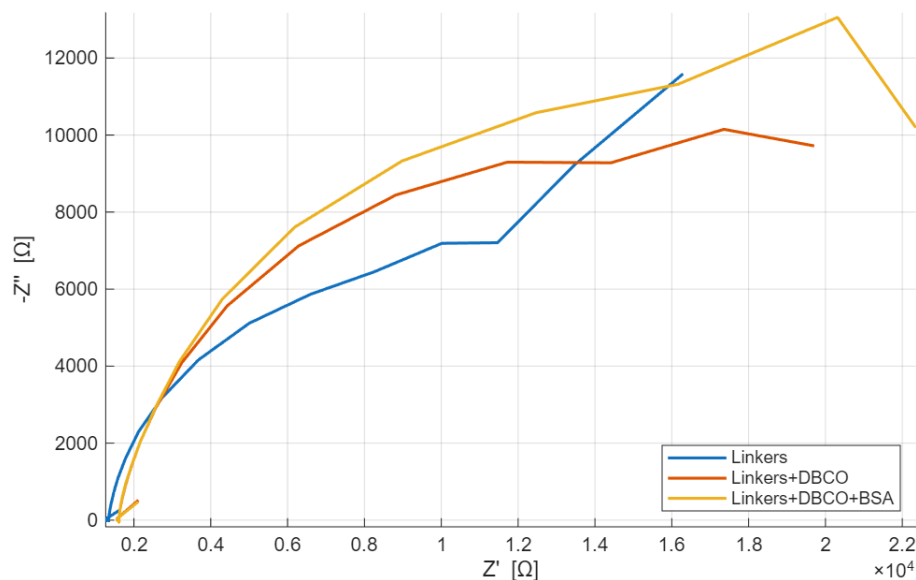


Figure E.9: Nyquist plot for the functionalized electrode 1, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

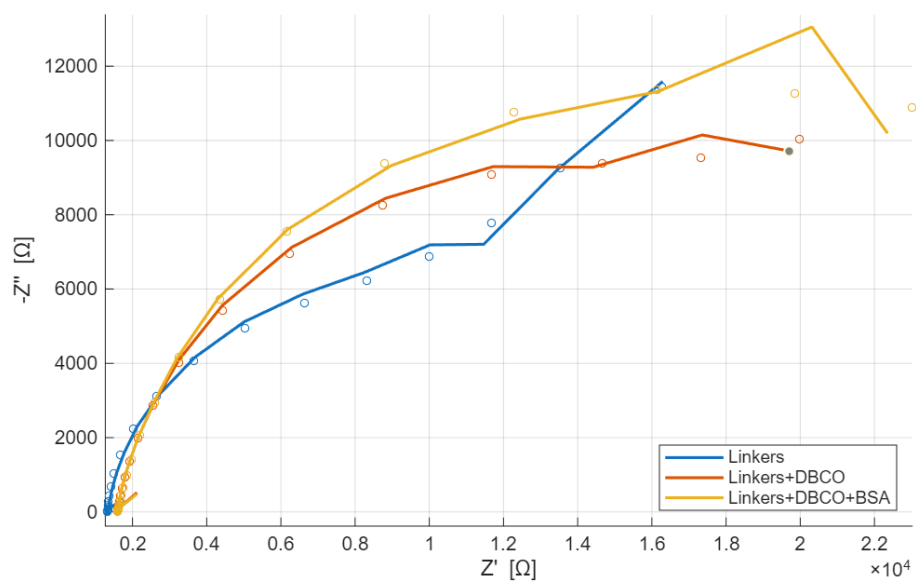


Figure E.10: Nyquist plot for the functionalized electrode 1 with the numerical fit, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

Table E.4: Equivalent circuit fitting parameters for electrode 1 during the surface modification steps.

Parameter	Linkers	DBCO	BSA
R_s [Ω]	1330	1588.9	1596
R_{ct} [Ω]	7521.3	18020	24474
Q [$\Omega^{-1} s^\alpha$]	2.4141×10^{-5}	3.7258×10^{-5}	3.8413×10^{-5}
α	0.9789	0.89096	0.86904
σ [$\Omega s^{-1/2}$]	30853	17728	19246
τ [s]	16.635	17.216	33.768
RMSE [Ω]	174.22	209.7	409.98
R_{ct} step change [%]	0	139.58	35.817

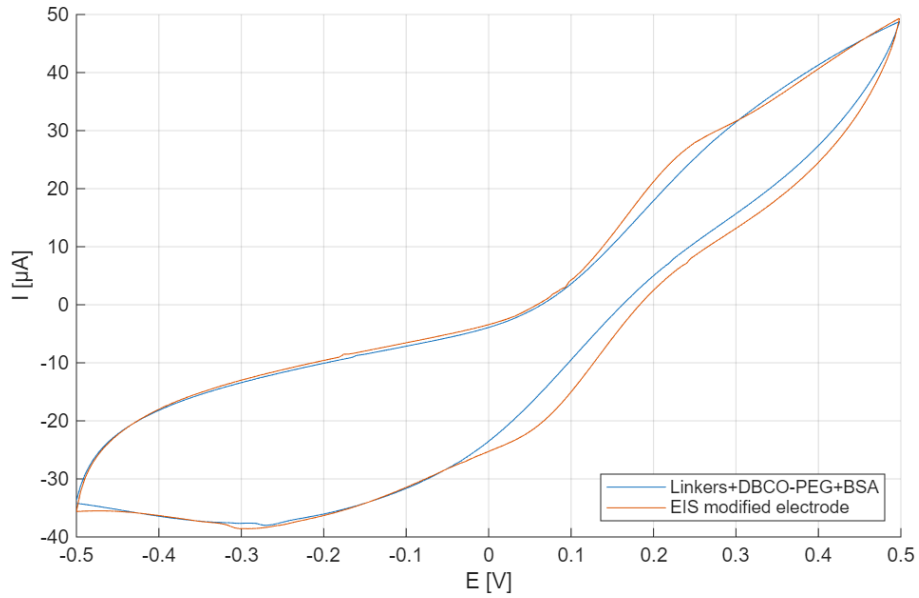


Figure E.11: Cyclic voltammogram for the functionalized EIS electrode 2, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

E.2.4 Fully Modified Electrode 2

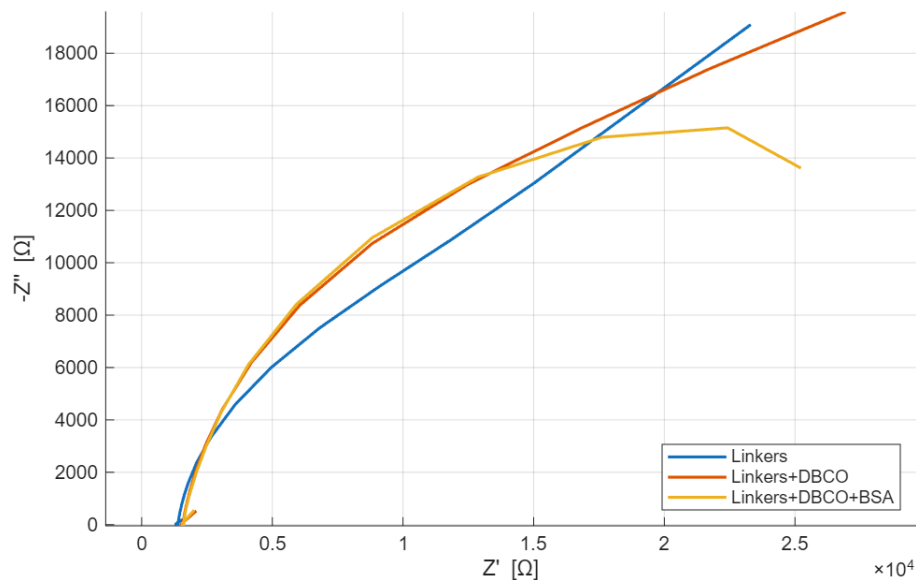


Figure E.12: Nyquist plot for the functionalized electrode 2, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

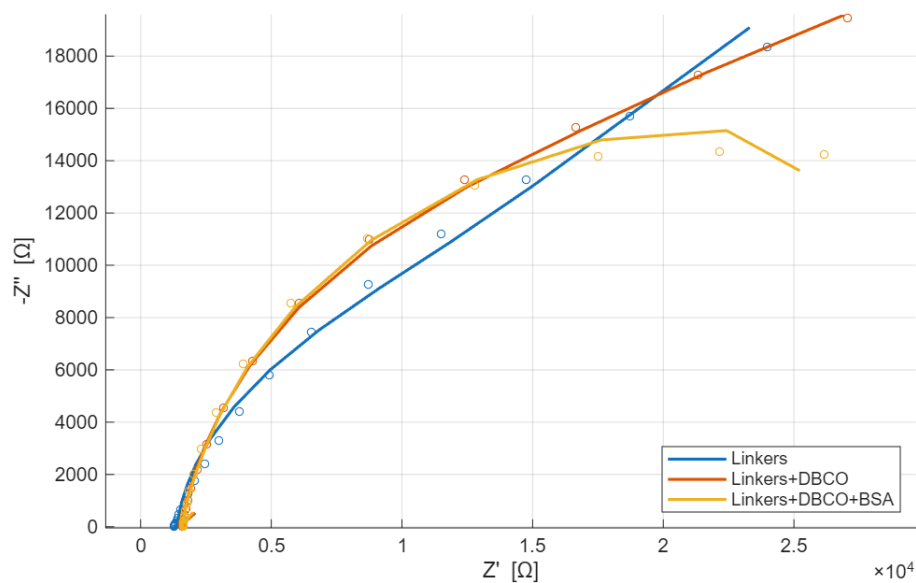


Figure E.13: Nyquist plot for the functionalized electrode 2 with the numerical fit, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

Table E.5: Equivalent circuit fitting parameters for electrode 2 during the surface modification steps.

Parameter	Linkers	DBCO	BSA
R_s [Ω]	1274.4	1590.1	1626.9
R_{ct} [Ω]	28053	26584	26666
Q [$\Omega^{-1} s^\alpha$]	4.0501×10^{-5}	3.4548×10^{-5}	3.4775×10^{-5}
α	0.76566	0.8892	0.9223
σ [$\Omega s^{-1/2}$]	31152	30527	31602
τ [s]	9.0329	11.148	36.809
RMSE [Ω]	290.7	192.69	325.09
R_{ct} step change [%]	-	-5.2377	0.30679

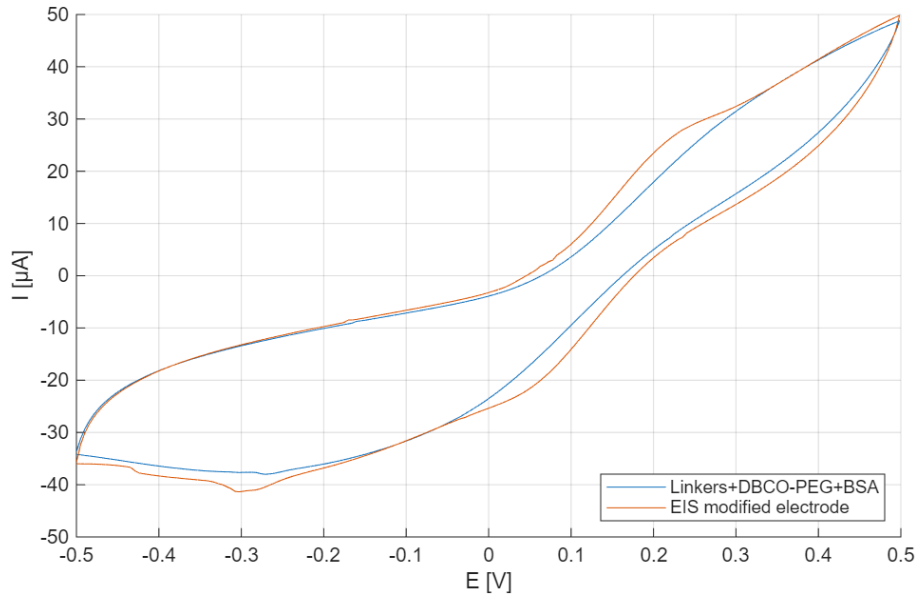


Figure E.14: Cyclic voltammogram for the functionalized EIS electrode 2, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

E.2.5 Fully Modified Electrode 3

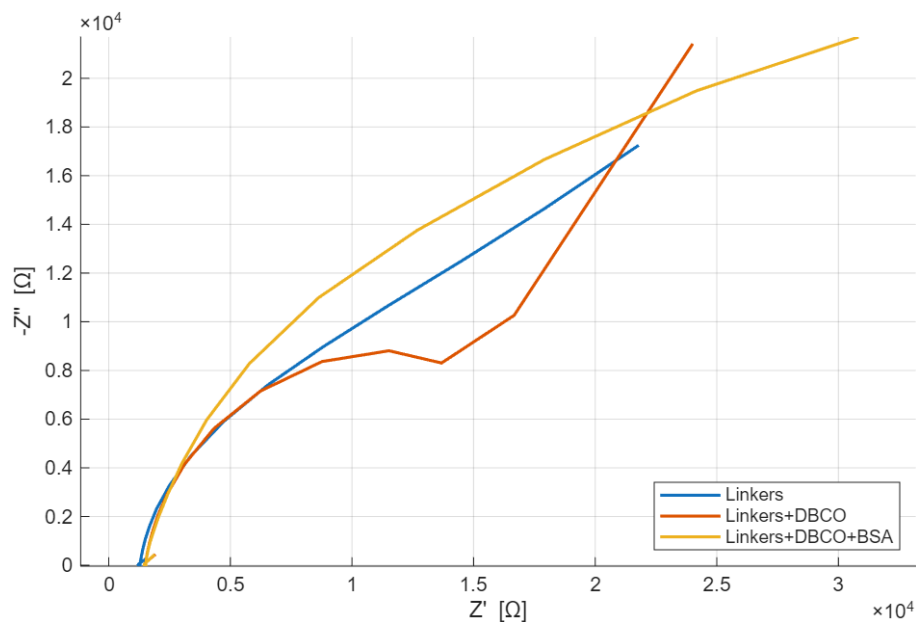


Figure E.15: Nyquist plot for the functionalized electrode 3, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

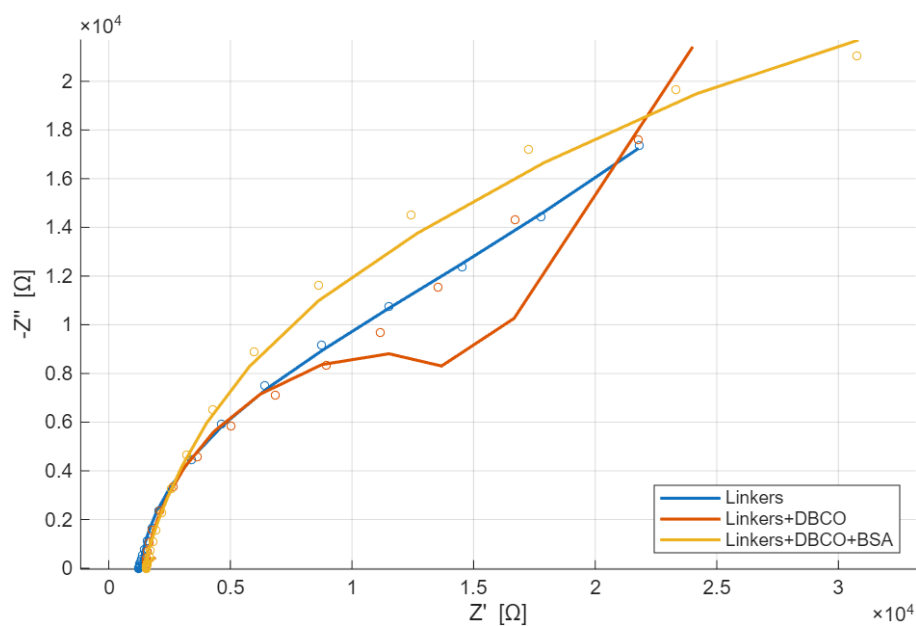


Figure E.16: Nyquist plot for the functionalized electrode 3 with the numerical fit, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

Table E.6: Equivalent circuit fitting parameters for electrode 3 during the surface modification steps.

Parameter	Linkers	DBCO	BSA
R_s [Ω]	1238.3	1538.2	1536.7
R_{ct} [Ω]	17378	8069.5	35218
Q [$\Omega^{-1} s^\alpha$]	3.3284×10^{-5}	2.2327×10^{-5}	3.5838×10^{-5}
α	0.85911	1	0.86218
σ [$\Omega s^{-1/2}$]	44735	40862	20606
τ [s]	16.988	11.98	6.2157
RMSE [Ω]	120.37	1285.2	394.8
R_{ct} step change [%]	0	-53.566	336.44

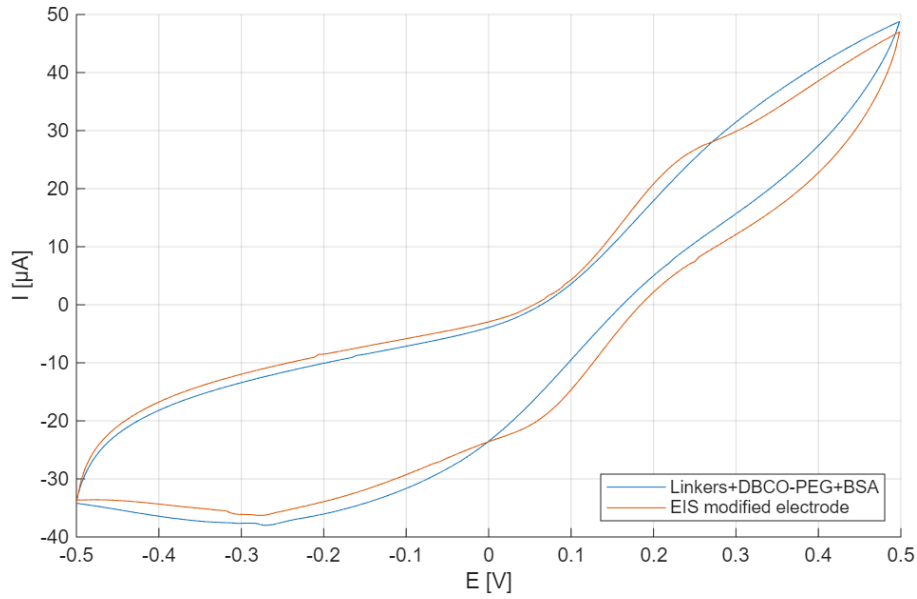


Figure E.17: Cyclic voltammogram for the functionalized EIS electrode 3, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

E.3 PDMS Test

This section contains additional EIS measurements and fitting parameters for the PDMS and cuvette comparison experiments discussed in Section 4.5. The measurements were used to evaluate how the modification environment influenced the electrochemical response.

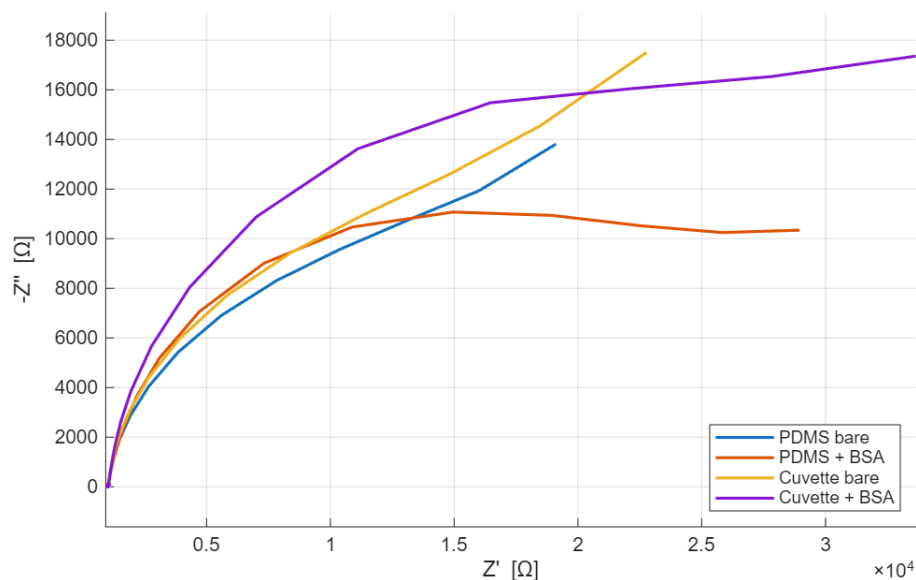


Figure E.18: Nyquist plot comparing electrodes before and after BSA modification in PDMS and cuvette setups, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl. In the PDMS setup only the working electrode was modified, whereas in the cuvette setup all electrodes were exposed.

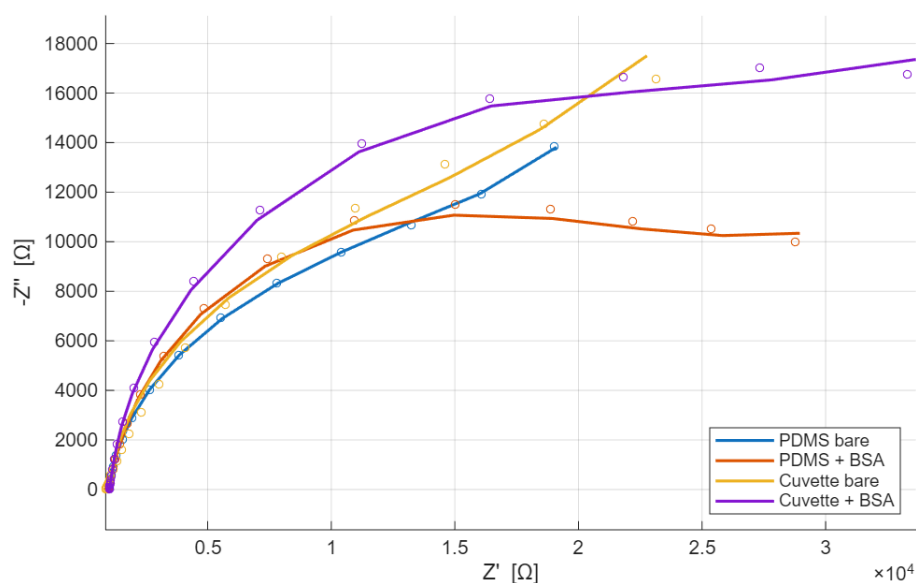


Figure E.19: Nyquist plot comparing electrodes before and after BSA modification in PDMS and cuvette setups with the numerical fit, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl. In the PDMS setup only the working electrode was modified, whereas in the cuvette setup all electrodes were exposed.

Table E.7: Equivalent circuit fitting parameters for PDMS and cuvette BSA modification experiments.

Parameter	PDMS bare	PDMS+BSA	Cuvette bare	Cuvette+BSA
R_s [Ω]	995.38	999.38	873.43	1029.7
R_{ct} [Ω]	14528	24286	30230	30136
Q [$\Omega^{-1} s^\alpha$]	3.4713×10^{-5}	1.8467×10^{-5}	4.1791×10^{-5}	2.4604×10^{-5}
α	0.91087	0.89673	0.79042	0.93386
σ [$\Omega s^{-1/2}$]	42472	12285	27094	18247
τ [s]	25.323	7.8039	14.57	7.7462
RMSE [Ω]	61.15	230.78	314.6	270.74
R_{ct} step change [%]	-	67.167	-	-0.31001

F

Additional CV Measurements

This appendix contains additional cyclic voltammetry measurements for fully functionalized electrodes related to the surface modification experiments presented in Subsection 4.3.1. The measurements are included to support the observed trends in anodic and cathodic peak current changes during electrode functionalization.

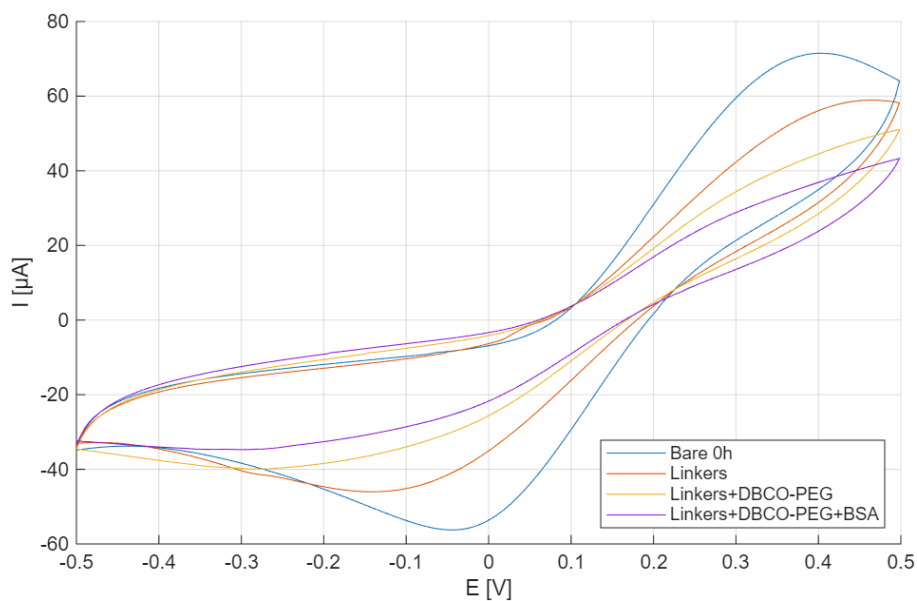


Figure F.1: Cyclic voltammograms for the functionalized electrode 2, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

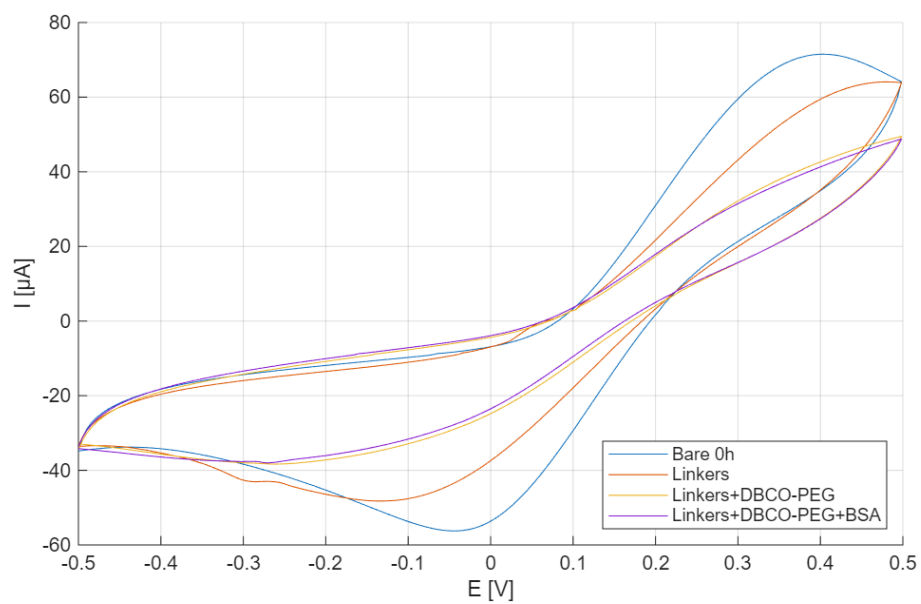


Figure F.2: Cyclic voltammograms for the functionalized electrode 3, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

G

Additional Transistor Measurements

This appendix contains additional transistor measurements together with supplementary EIS measurements and equivalent circuit fitting parameters for electrodes prepared using the transistor measurement protocol, related to the results presented in Subsection 4.3.3. The measurements are included to support the observed transistor signal trends and the electrode-to-electrode variability in the electrochemical response.

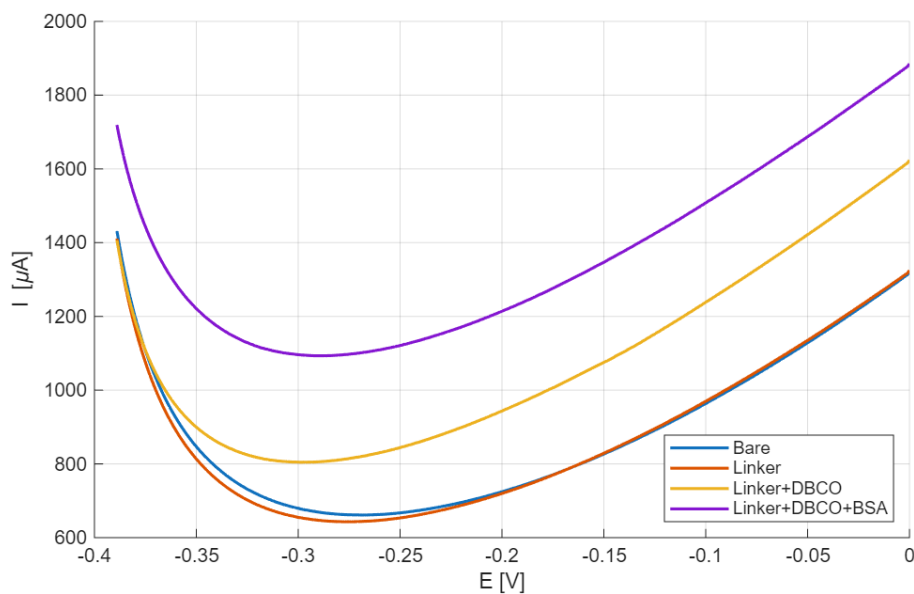


Figure G.1: Transistor measurement for the fully modified electrode 2, measured in PBS.

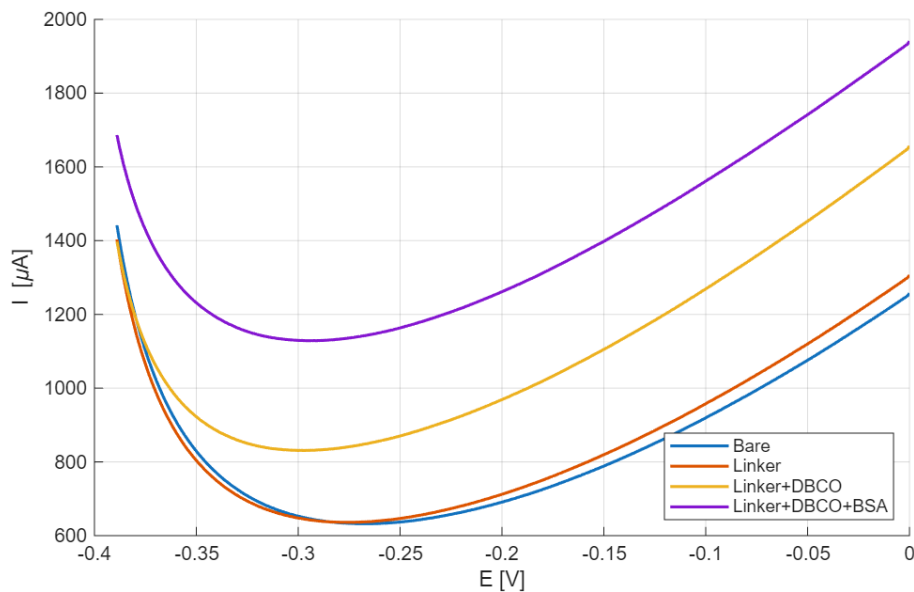


Figure G.2: Transistor measurement for the fully modified electrode 3, measured in PBS.

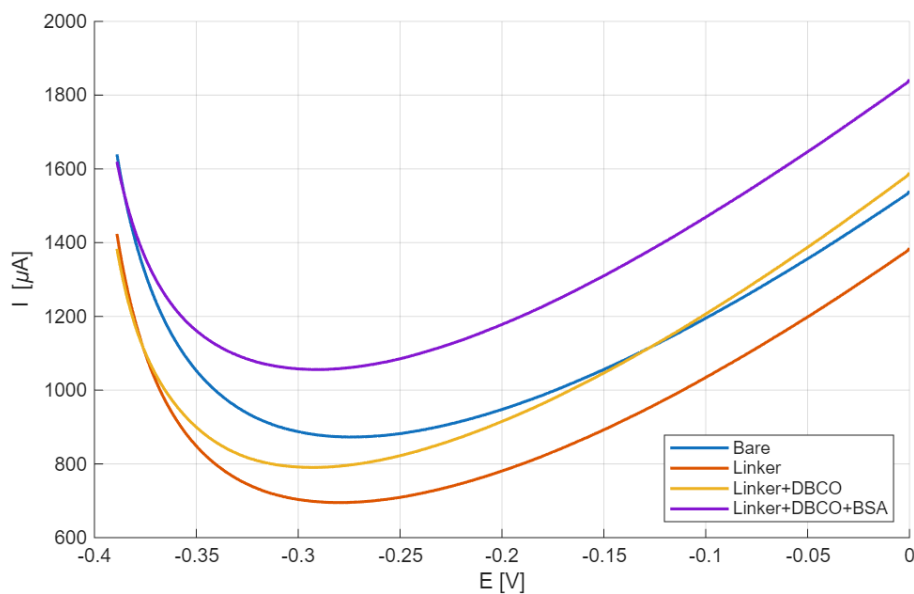


Figure G.3: Transistor measurement for the fully modified electrode 4, measured in PBS.

G.1 EIS Characterization of Electrodes Prepared Using the Transistor Protocol

G.1.1 Bare Electrode

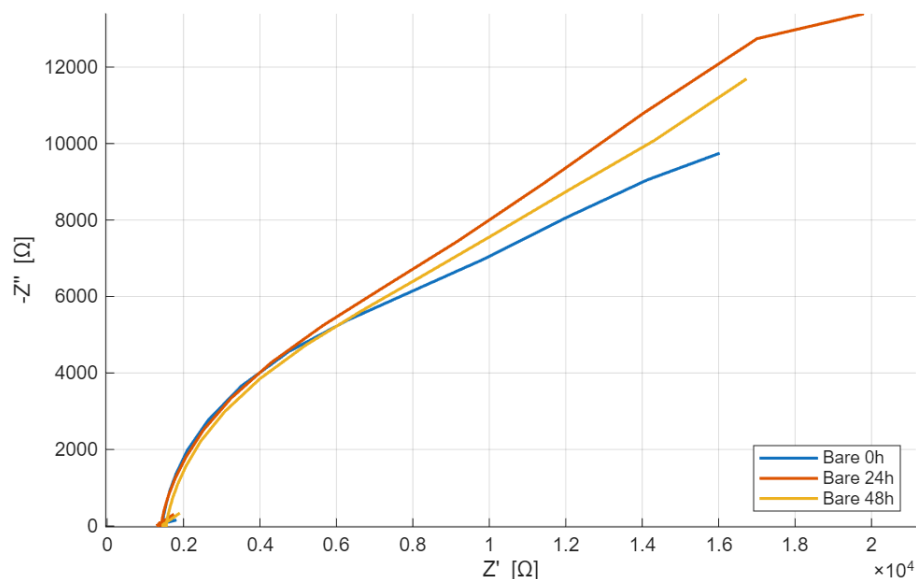


Figure G.4: Nyquist plot for the bare control electrode prepared using the transistor measurement protocol, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

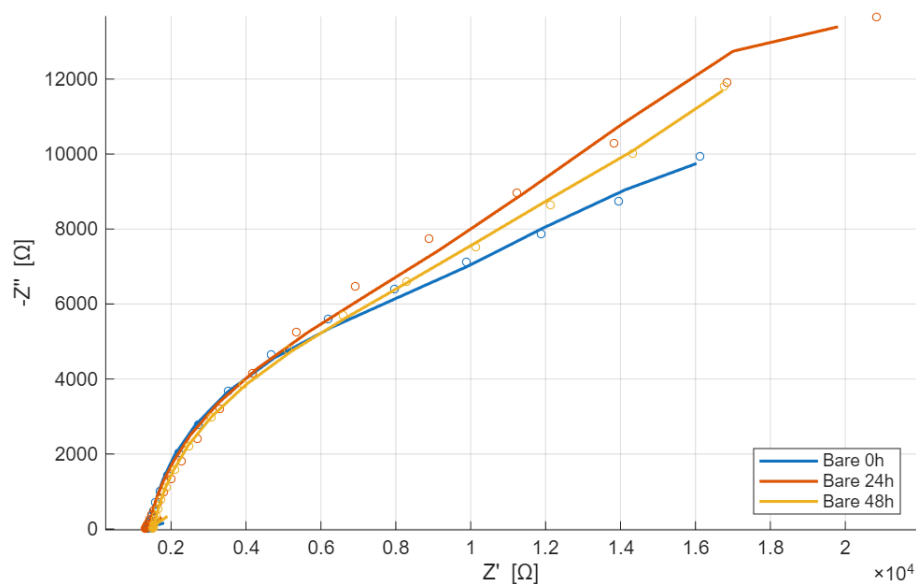


Figure G.5: Nyquist plot and equivalent circuit fit for the bare control electrode prepared using the transistor measurement protocol, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

Table G.1: Equivalent circuit fitting parameters for the bare electrode measurements prepared using the transistor measurement protocol.

Parameter	Bare 0h	Bare 24h	Bare 48h
R_s [Ω]	1392.6	1310.7	1512.4
R_{ct} [Ω]	11408	21657	9791.7
Q [$\Omega^{-1} s^\alpha$]	3.6799×10^{-5}	4.1538×10^{-5}	3.2220×10^{-5}
α	0.86152	0.73525	0.86514
σ [$\Omega s^{-1/2}$]	46860	23758	60871
τ [s]	52.712	9.5201	51.442
RMSE [Ω]	150.15	332.04	116.66
R_{ct} step change [%]	-	89.833	-54.788

G.1.2 Electrode 1

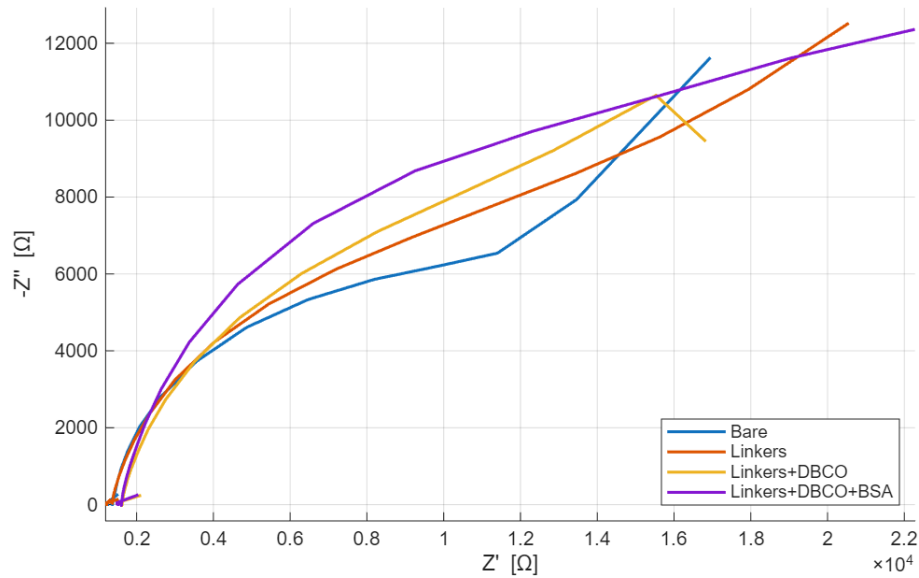


Figure G.6: Nyquist plot for electrode 1 prepared using the transistor measurement protocol, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

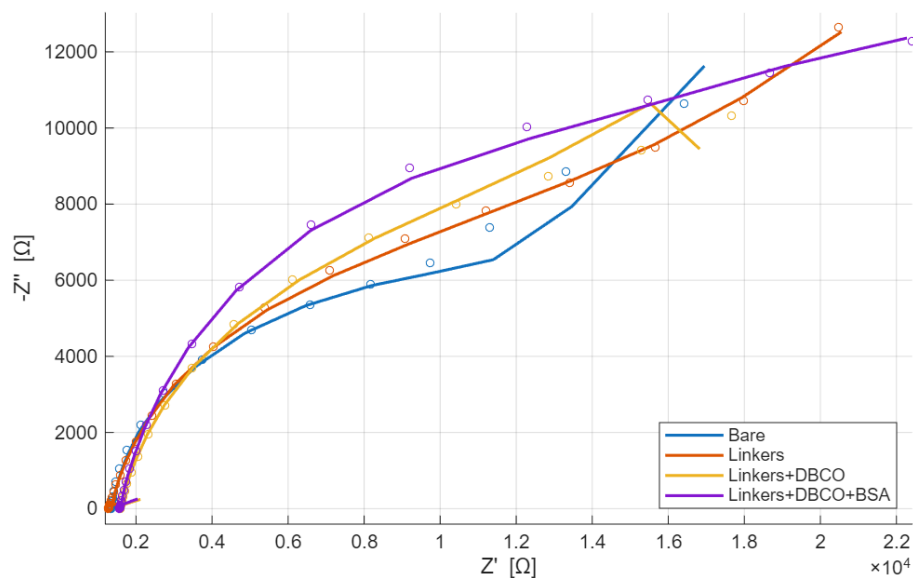


Figure G.7: Nyquist plot and equivalent circuit fit for electrode 1 prepared using the transistor measurement protocol, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

Table G.2: Equivalent circuit fitting parameters for Electrode 1 prepared using the transistor measurement protocol across the surface modification steps.

Parameter	Bare	Linkers	DBCO	BSA
R_s [Ω]	1344.5	1276.6	1580.7	1572.3
R_{ct} [Ω]	7696.2	13541	14998	20095
Q [$\Omega^{-1} s^\alpha$]	2.6062×10^{-5}	2.2227×10^{-5}	4.0721×10^{-5}	3.5335×10^{-5}
α	0.95083	0.83168	0.85074	0.87124
σ [$\Omega s^{-1/2}$]	23969	47972	37693	17967
τ [s]	11.893	31.242	41.721	10.873
RMSE [Ω]	337.28	103.48	355.85	169.72
R_{ct} step change [%]	-	75.951	10.754	33.986

G.1.3 Electrode 2

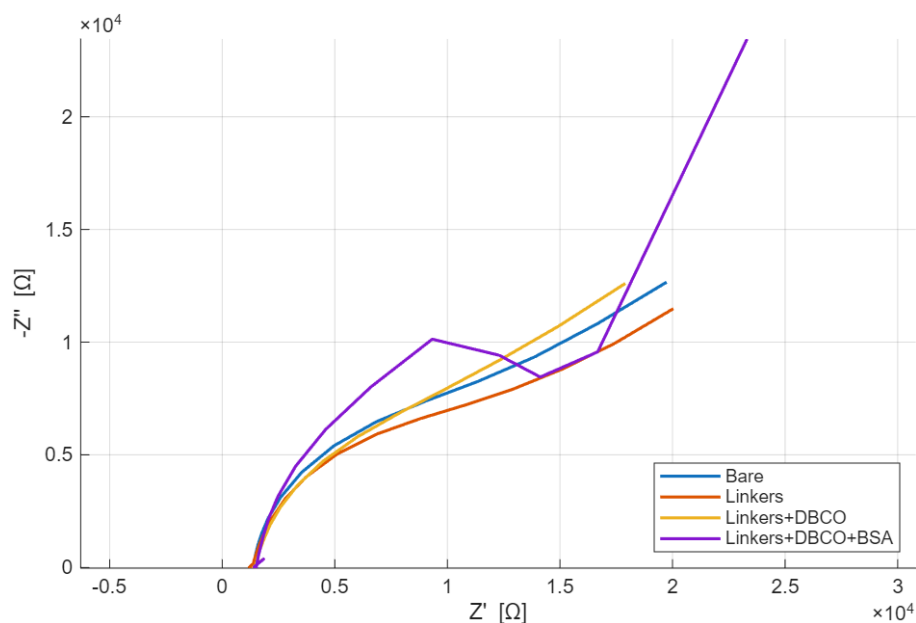


Figure G.8: Nyquist plot for electrode 2 prepared using the transistor measurement protocol, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

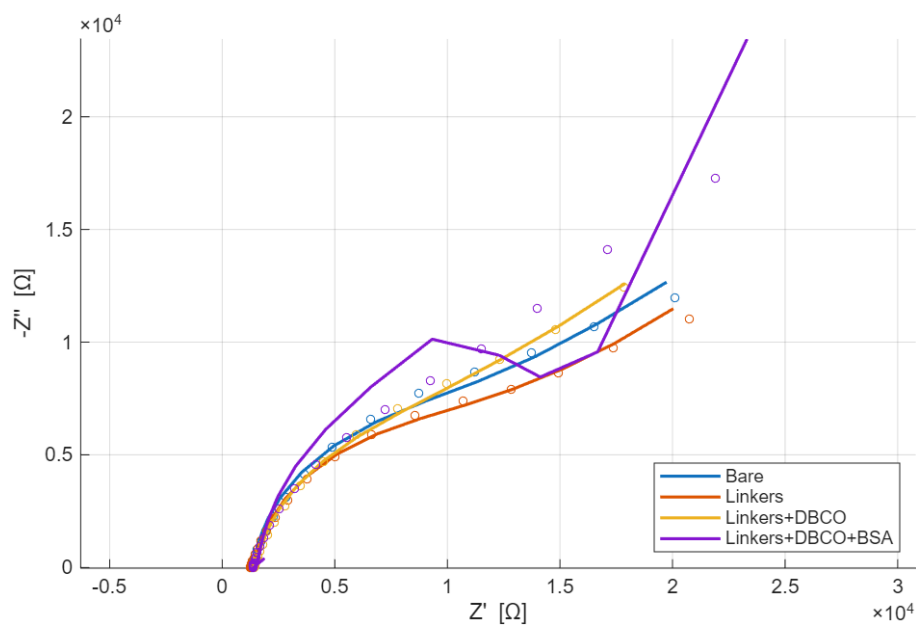
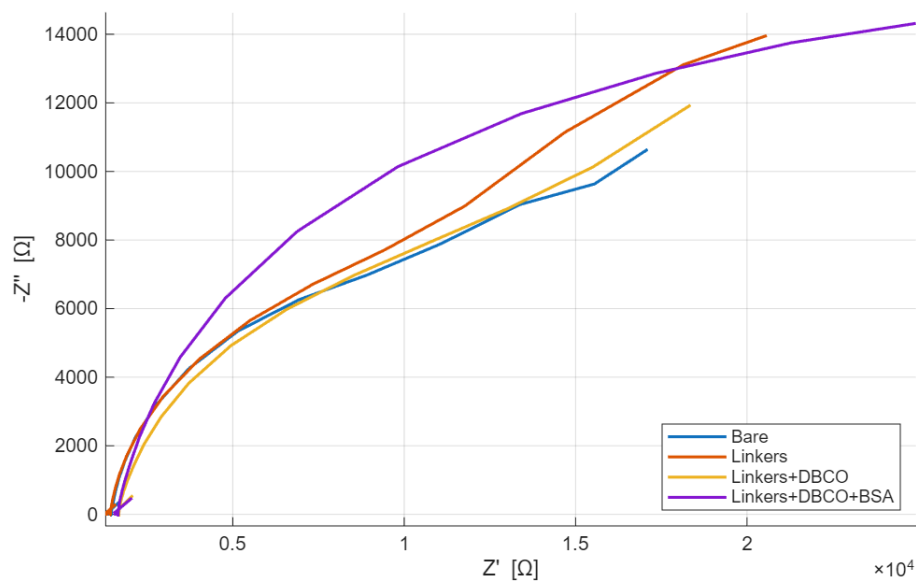


Figure G.9: Nyquist plot and equivalent circuit fit for electrode 2 prepared using the transistor measurement protocol, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

Table G.3: Equivalent circuit fitting parameters for Electrode 2 prepared using the transistor measurement protocol across the surface modification steps.

Parameter	Bare	Linkers	DBCO	BSA
R_s [Ω]	1286.2	1250.4	1416.9	1351.4
R_{ct} [Ω]	17380	15124	15908	12614
Q [$\Omega^{-1} s^\alpha$]	3.7262×10^{-5}	2.6674×10^{-5}	4.3611×10^{-5}	2.8956×10^{-5}
α	0.81937	0.80382	0.80822	0.84338
σ [$\Omega s^{-1/2}$]	20283	20854	28843	39966
τ [s]	10.476	10.365	15.798	11.438
RMSE [Ω]	234.5	203.45	150.19	1631.4
R_{ct} step change [%]	-	-12.976	5.1793	-20.706

G.1.4 Electrode 3

**Figure G.10:** Nyquist plot for electrode 3 prepared using the transistor measurement protocol, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

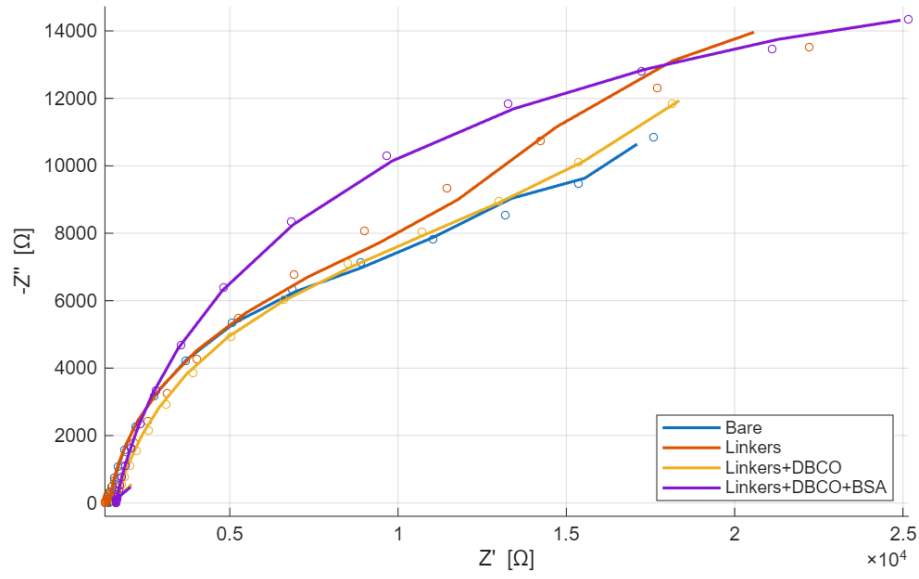


Figure G.11: Nyquist plot and equivalent circuit fit for electrode 3 prepared using the transistor measurement protocol, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

Table G.4: Equivalent circuit fitting parameters for Electrode 3 prepared using the transistor measurement protocol across the surface modification steps.

Parameter	Bare	Linkers	DBCO	BSA
R_s [Ω]	1409.1	1292.6	1607.2	1615.2
R_{ct} [Ω]	11503	20834	14986	25180
Q [$\Omega^{-1} \text{s}^\alpha$]	2.9697×10^{-5}	3.8883×10^{-5}	3.9197×10^{-5}	3.3757×10^{-5}
α	0.90549	0.76621	0.81515	0.86628
σ [$\Omega \text{s}^{-1/2}$]	44217	19978	29147	21934
τ [s]	38.754	6.9121	17.469	14.379
RMSE [Ω]	180.62	431.42	174.71	169.44
R_{ct} step change [%]	-	81.121	-28.069	68.022

G.1.5 Electrode 4

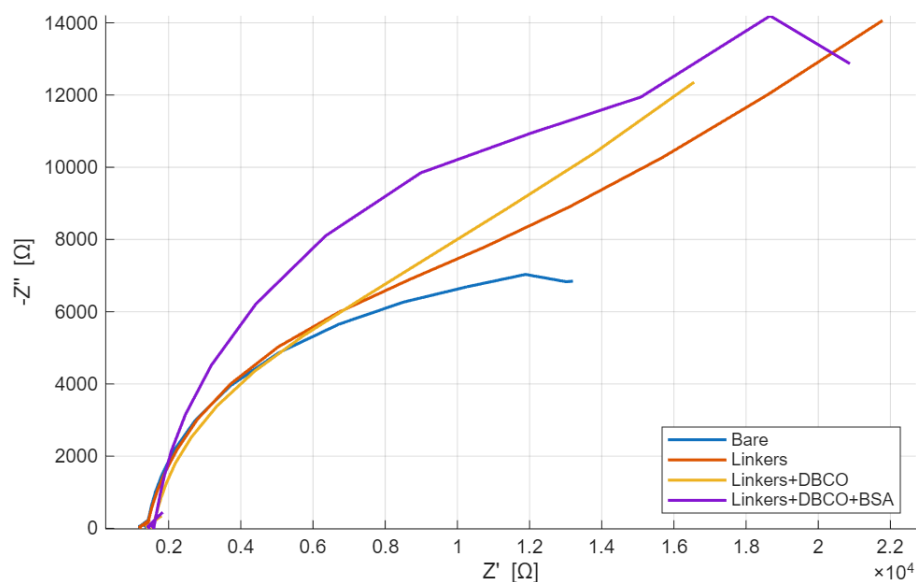


Figure G.12: Nyquist plot for electrode 4 prepared using the transistor measurement protocol, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

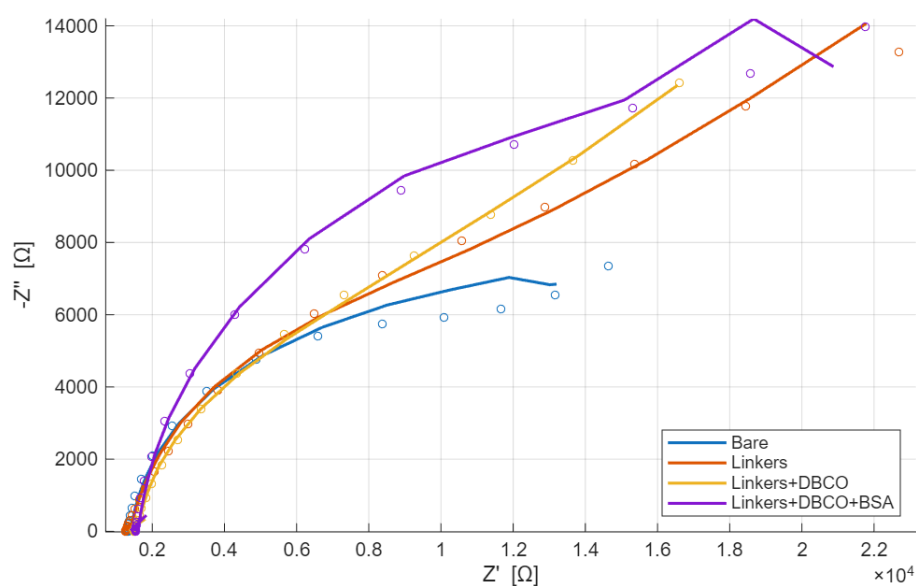


Figure G.13: Nyquist plot and equivalent circuit fit for electrode 4 prepared using the transistor measurement protocol, measured in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

Table G.5: Equivalent circuit fitting parameters for Electrode 4 prepared using the transistor measurement protocol across the surface modification steps.

Parameter	Bare	Linkers	DBCO	BSA
R_s [Ω]	1321.1	1257.9	1494.5	1537.6
R_{ct} [Ω]	9789.2	17279	12696	16556
Q [$\Omega^{-1} s^\alpha$]	3.0433×10^{-5}	2.9321×10^{-5}	4.4274×10^{-5}	3.1593×10^{-5}
α	0.92974	0.77087	0.83114	0.94175
σ [$\Omega s^{-1/2}$]	27557	24083	31217	55574
τ [s]	36.014	8.6459	15.738	45.989
RMSE [Ω]	392.28	284.45	123.33	418.43
R_{ct} step change [%]	-	76.512	-26.526	30.404

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