Final report

Membranes in electrochemistry

Development of a novel ion-selective potentiometry/amperometry

Membranes in electrochemistry have multiple roles. Their most important application in sensors is converting chemical signals to electrical signals. A crucial issue in analytical chemistry is the demand to minimize the effect of interfering components on the signal of the analyte. Electrochemical sensors with selective membranes offer an efficient and inexpensive solution for determining biologically important molecules and ions. Recently, many ionophores with high selectivity for their preferred ion have been developed. With the utilization of these ionophores, selective, sub-nanomolar detection of many ions became possible with ion-selective electrodes (ISEs). [1-3] However, there are some biologically important ions for which there are no good ionophores. We developed new detection principles to tackle this issue. A differential sensor was utilized that could be used to detect ions when significant interference from the matrix is expected under physiological conditions. Two solid-contact ion-selective electrodes (SCISEs) were applied, which were prepared similarly, except for adding an ionophore to one of the membranes. The electrochemical signal – potential or current – was measured between the two electrodes. We showed experimentally and theoretically that a significant signal can only be observed when the analyte ion is present in the sample solution. The paper on the research was published in the Journal of Electrochemical Society (D1 journal in Materials Chemistry). [4]

Following the research plan, our goal was to improve the differential ion-selective sensor developed in our research group. We endeavored to further enhance the analytical performance characteristics of the solid-state differential sensor by reducing sensor resistance. Gold nanopore membranes were used as selective sensors in place of conventional plasticized polymer-based membranes. Consequently, the thickness of the membranes were reduced from 100 µm to 6 µm. We not only expected an improvement in the response time, but also in the amperometric signal by reducing the resistance of the sensor. The surface of gold nanoporous polycarbonate membranes was modified with components responsible for lipophilicity, permselectivity and ion-selectivity. The differential setup consisted of three compartments, which were separated by two membranes (one containing ionophore and the other containing no selective complexing agent). The resulting sensors were subjected to various electrochemical tests. The appropriate permselectivity was investigated by I-V curves and the resistance by electrochemical impedance spectroscopy. The differential gold nanoporous sensor reduced the matrix effect compared to conventional ion-selective sensors. Unfortunately, the detection limit and response time for gold nanoporous membranes were worse than conventional plasticized PVC-based ion-selective membranes.

Coupled experimental/theoretical/machine learning framework for rapid assessment of thermodynamic and kinetic parameters

Discerning different phenomena that govern the dynamic behavior of membranes require the theoretical understanding of the processes (e.g., diffusion, heterogeneous kinetics, etc.) that determine the time-dependent response. Experimental investigation of electrochemical devices (e.g., sensors and batteries) has long relied on laborious experiments, including performing large numbers of electrochemical tests in various combinations. A data-driven approach can drastically reduce the time and effort needed to test various device setups with little prior knowledge about the underlying physical mechanisms. By learning the relevant characteristics from existing experimental data, one can find promising arrangements for future experimental testing. In the past few years, a great number of different methods have been applied to simulate the operation of electrochemical devices. [5-7] On the other hand, machine learning methods were successfully applied to many other scientific areas to analyze large amounts of data and find predictive relations among parameters and the experimental performance. [8-11] Combining experimental and simulation/machine learning methods in a way that helps both, in turn, provides an ideal and fast feedback cycle, allows to explore a variety of experimental scenarios with reduced experimental costs and can also lead to more accurate theoretical models.

When we have a sufficiently sophisticated theoretical model of a system, we can use the device's experimental response to an external stimulus to assess the most essential kinetic and thermodynamic parameters that govern their behavior. In many cases, this can be accomplished by using a simplified model – or even an explicit equation – and fitting it to the experimental data. However, in a large number of situations, simplified models are inadequate. For example, the electrochemical response of many electrochemical devices, such as the complex impedance of a sensor or a battery, is governed by a complex interaction of the electric field and the diffusing-migrating species. The time-dependent response is not well-described by a simple equation. For these situations, we must resort to more sophisticated models, such as finite element numerical simulations. Unfortunately, the simulation time of finite element models is on the order of several minutes, which can make the parameter estimation, where thousands of simulations are required, impractical.

During the project, we developed a method for speeding up the prediction of the electrochemical response in highly nonlinear and time-dependent systems to make parameter estimation feasible. We replaced finite element simulations with supervised machine learning algorithms that could provide accurate results six orders of magnitude faster. Two types of machine learning algorithms were used. Feedforward deep neural networks are black-box machine learning models, so exact knowledge of the internal mechanism and reasons behind the output of the network is not accessible to us. However, it was possible to use white-box machine learning models that provide understandable results for experts. Symbolic regression – an accurate white-box machine learning technique – automates the process of finding human-readable symbolic expressions that match the observed data.

Our research group was the first to devise experiments and data evaluation schemes that could provide the diffusion coefficients of all mobile ionic species in an ion-selective membrane:

1) Conventional liquid-contact ion-selective membranes are known for their reproducible potential response. Fundamentally, two groups of parameters determine the response of ion-selective membranes (ISMs): selectivity coefficients and diffusion coefficients of mobile species in the membrane. It is possible to assess both by performing a single potentiometric ion-breakthrough experiment. Basically, the ISM is placed between two contacting electrolyte solutions that do not contain the ion that the ISM is selective for (primary ion). After the primary ion is added, the potential trace carries valuable information about the thermodynamics and the kinetics of the membrane. So far, extracting parameters

from the experimental results was possible only after unrealistic simplifications (e.g., assuming all of the diffusion are the same). The state-of-the-art simulation technique the Nernst-Planck-Poisson finite element method was utilized to give insight into how the different physico-chemical processes generate the measured potential. Numerical simulations were used to train a feedforward neural network to learn the connection between the physico-chemical parameters (e.g., thickness, diffusion coefficients, selectivity coefficients, coextraction etc.) and the shape of the ion-breakthrough potential trace. Using the trained neural network, it was possible to obtain the diffusion coefficient of all of the mobile species in the ISM for the first time in milliseconds (more than six orders of magnitude speed-up compared to finite element simulations). The paper was published in the Journal of Electrochemical Society (D1 journal in Materials Chemistry). [12]

2) The parameter estimation technique devised in the paper mentioned above can only be used for conventional liquid-contact ion-selective electrodes. Consequently, we wanted to show that the combined approach of experimental interrogation of the sensor, numerical simulation, and machine learning can be used with solid-contact ion-selective electrodes. Solid-contact ion-selective electrodes offer many benefits over traditional liquid-contact ion-selective electrodes. Their small size made them the default choice in many clinical analysis tools. Reproducibility of their production is crucial in achieving calibration-free sensors. Electrochemical impedance spectroscopy (EIS) is a versatile technique that can provide valuable information on many physico-chemical parameters of examined SCISEs and give results in under 1 min. Discerning different phenomena that govern the EIS spectrum require the theoretical understanding of the processes (e.g., diffusion, heterogeneous kinetics etc.) that determine the time-dependent response of SCISEs. Complex impedance simulations of SCISEs with the Nernst-Planck-Poisson finite element method were applied to describe the experimental response of SCISEs. The numerical simulations were used to train a black-box supervised learning algorithm – a deep feedforward neural network – and a white-box symbolic regression algorithm to learn the underlying model of EIS spectra of SCISEs. The neural networks were used to significantly speed up the solution of the inverse problem of obtaining physico-chemical parameters from experimental data. The paper was published in the Journal of Electrochemical Society (D1 journal in Materials Chemistry). [13] We thought the reviewer's opinion is worth citing: "Reading the manuscript of Kovacs and coworkers was a true pleasure. Electrochemical impedance spectroscopy of ion-selective electrode devices offers a lot of insightful information, but unfortunately EIS spectra are not trivial to interpret. This has limited the use of EIS in this field. Also, among the publications that do use EIS for ISE characterization, interpretation is too often minimal and not too infrequently inadequate. Importantly, aside from a better understanding of the origin of artifacts, there has not been significant new insight into EIS of ISE membranes for at least 20 years. It is, therefore, very notable that this manuscript does have the potential to help EIS data to be used more effectively."

Our papers received favorable responses from the chemical sensor community. I was contacted by multiple leading research groups in the field. The first group was from Université de Genève, Switzerland led by one of the most renowned experts: Prof. Dr. Eric Bakker. We started a collaboration on using regenerated cellulose-based dialysis membranes as liquid junction materials to be used in electrochemical reference electrodes. The method developed in our Journal of Electrochemical Society papers was used to understand the processes governing the potential response of the widely used material. We showed that with high bridge electrolyte concentrations (above 100 mM), the potential response of dialysis membranes fitted the predictions based on the Henderson equation. However, an undesirable net flux of ions was observed. Using less concentrated bridge electrolytes (10 mM) resulted in deviations of up to 26 mV between experimental data and simplistic theoretical predictions. Due to the failure to estimate the liquid junction potential arising at the interface with the classical Henderson equation, a simple calculation method, providing a better fit for experimental data, was developed and tested. The proposed model is based on partial Donnan exclusion generated by the negatively charged surface of the membrane in aqueous samples. The validation of the new model was achieved through a comparison with the Nernst-Poisson-Planck (NPP) simulation method used to calculate the potential across permselective membranes. The results were published in the Journal of Electroanalytical Chemistry (Q1 journal in Chemical Engineering) [14]. The paper was featured in the special issue for Prof. Shaojun Dong's 90th birthday.

In collaboration with Ecole Polytechnique Fédérale de Lausanne, we worked on a porous membrane based method for filtering and electrochemically investigating transition metal based homogeneous catalysts. We tested various voltammetric methods (e.g., differential pulse voltammetry, square wave voltammetry, cyclic voltammetry, and differential linear sweep voltammetry) to measure the transition metal content of the filtrate. In both limit of detection and robustness, the differential linear sweep voltammetry (dLSV) was the best. The most significant advantage of using electrochemistry was that we could directly observe the change in the oxidation state of the catalyst, which proved to help us understand the variability in the reaction rates. A BSc dissertation was written on the topic: Kishonti Pál, Höfler Lajos: Fémkomplexek transzport tulajdonságainak vizsgálata, BME Szervetlen és Analitikai Kémia Tanszék, 2020. The results of the dissertation give the basis of the manuscript we are currently working on.

In collaboration with Keele University, United Kingdom, we worked on the theoretical description of ion-selective membranes with isolated conductive particles.

The machine learning-based rapid parameter estimation framework developed during the project has attracted the interest of several industrial partners (Werfen, Spain; PalmSens, The Netherlands; SILEX Ipari Automatizálási Zrt., Hungary), and as a result, the framework has been integrated into an open-source library, becoming the first-ever open-source on-line tool for rapid parameter estimation of electrochemical systems. It is based on our novel deep learning approach using artificial neural networks. The following link points to the EIS prediction of solid-contact ion-selective electrodes: http://152.66.55.61/operon-bokeh-dir

The parameter dependence of the complex impedance of Li-ion batteries can be found here: http://152.66.55.61/cat-an-nyquist_CPEqCdlgauss-p3

Differential detection of biomolecules with chemically modified gold surfaces

Our goal was to develop a sensor design demonstrating a new biomolecule detection principle. We investigated experimentally and theoretically – with molecular dynamics simulations – whether electrochemically detectable, reversible protein binding sites can be fabricated entirely from thiolated molecules in a bottom-up fashion. Gold electrodes were used since their surface can be easily modified with thiol-containing reagents. Two types of gold surfaces were investigated: A) Commercially available gold electrode, B) nanoporous gold membrane that was housed in an electrode body made in our laboratory. It consisted of two concentric polypropylene rings with the gold membrane and Teflon insulation sandwiched between them. A copper cable was passed through the wall of the inner ring to ensure electrical contact with the membrane. The two types of electrodes are shown in Figure 1.

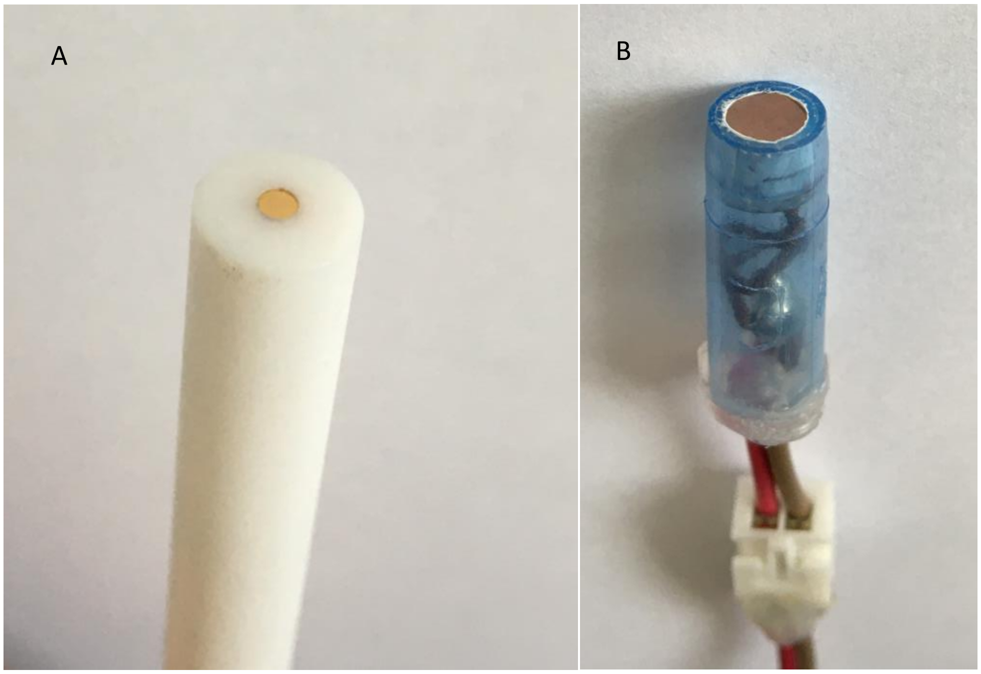

Figure 1: Photos of gold electrodes: A) A commercially available gold electrode. B) Nanoporous gold membrane housed in a polypropylene electrode body made in our laboratory.

Biotin binds very tightly to avidin. It is considered the strongest non-covalent binding between a ligand and a protein, with a dissociation constant of $4\cdot10^{-14}$ M. A short incubation in nonionic aqueous solutions at temperatures above 70 °C can efficiently break the biotin - avidin interaction without denaturing the avidin tetramer. [15] Both biotin and the avidin remain active after dissociation, and both molecules can be reused. In order to establish selective molecular recognition, thiolated biotin was attached to avidin, and the complex was bound to the gold electrode surface. The free surface around the surface-bound protein was blocked with thiolated polyethylene glycol (PEG) blocking agent. After proteins were removed by heat treatment, nanoscopic wells formed containing a bare gold surface. This nanoscopic well's open or closed state served as a signal for analysis. The effect of non-specific adsorption could be eliminated by using a differential sensor. A reference sensor was needed that alternates between blocked and free gold surfaces but does not contain biotin. The differential signal emerged due to the difference between the signals of a biotin-containing and a biotin-free sensor.

Avidin has four equivalent biotin binding sites. Based on our molecular dynamics simulation results (Fig. 2), binding site formation on the gold surface is optimal when the biotin ligand is pre-attached to all four biotin binding sites of avidin. After the biotin-avidin complex reaches the gold surface, all four thiolated biotin chains bind to the gold surface and form a binding site where the protein molecule is stably anchored to the surface. Keeping the protein sufficiently close to the gold surface is essential to prevent the thiolated PEG from blocking the gold surface under the avidin. Thus, the prepared avidin selective sensor's surface was constructed in a way that thiolated biotin chains anchored avidin to the gold, and the free gold surface between them was covered with PEG blocking agent. After removal of the avidin, free gold surface wells were left behind. When avidin was reintroduced, the anchored thiolated biotin chains selectively captured avidin molecules, thus closing the wells.

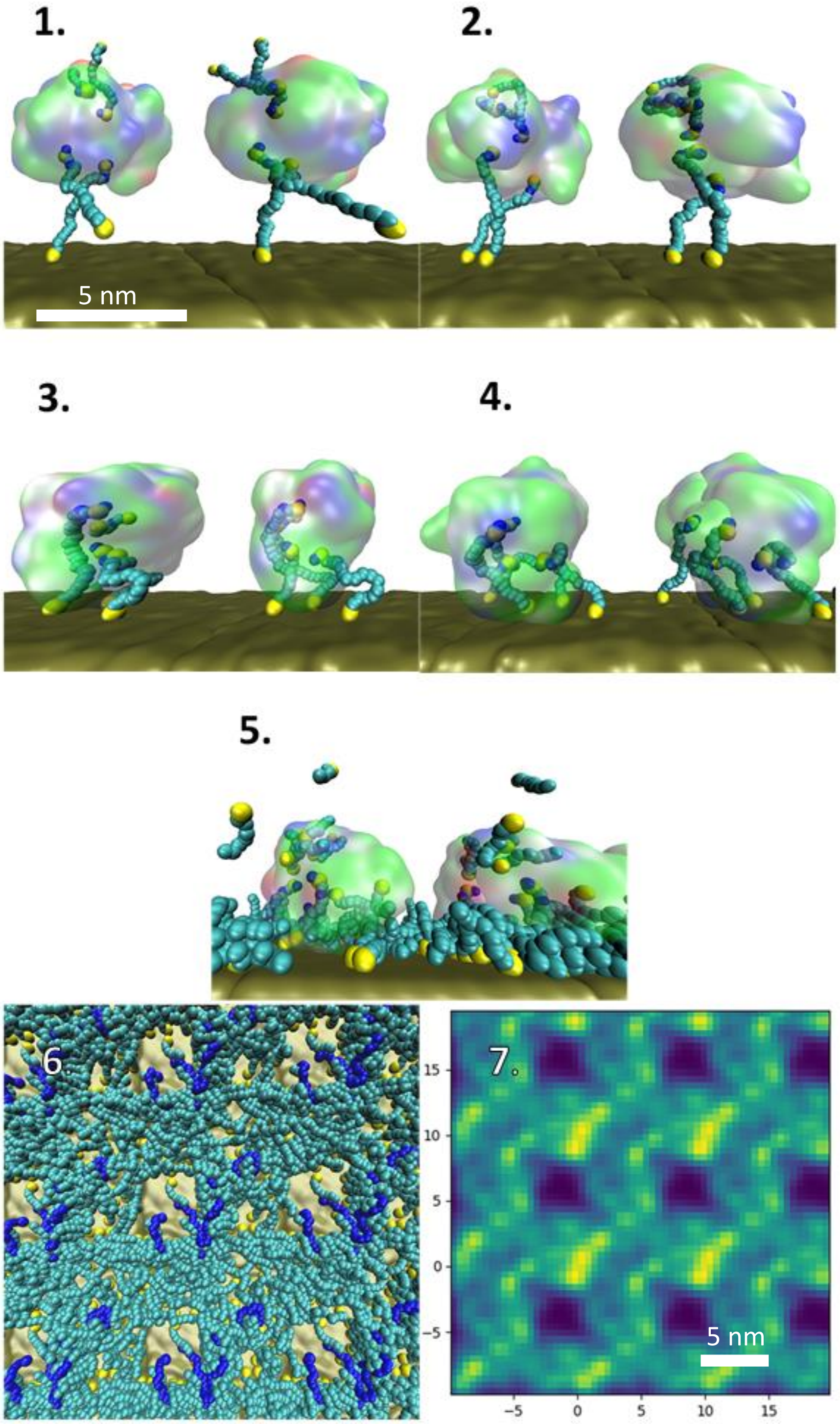

Figure 2: Molecular dynamics simulation of surface modifications: **1** Avidin (semitransparent protein) with the four thiolated biotins (long cyan molecules) arrives at the surface where one of the thiol groups (yellow spheres) binds. **2** Binding of the second thiol group. **3** Binding of the third thiol group. **4** Binding of the fourth thiol group. **5** Blocking with thiolated PEG (short cyan molecules). **6** Top-down view of gold surface nanoscopic wells remaining after the removal of avidin. **7** Density plot of the thiol groups attached to the surface. Density increases from blue to yellow.

Several signal transduction techniques were investigated. When an auxiliary redox system is added to the sample (e.g., $[Fe(CN)_6]^{4-}$ /$[Fe(CN)_6]^{3-}$ redox pair), the complex impedance varies as a function of the amount of free nanoscopic wells. In the case of cyclic voltammetry (CV), we expect lower currents and less reversible behavior when avidin molecules dwell in their respective nanoscopic wells. When avidin is not present, the redox pair can be easily oxidized/reduced, thus we expect reversible behavior. At a given scan rate, the peak separation value depends on the number of free wells, which in turn depends on the avidin concentration. It is also possible to detect molecules utilizing the fact that when the analyte is charged, the binding event is accompanied by charge separation, which results in an electrostatic potential and capacitance change. Complex impedance carries information on both charge transfer resistance and double-layer capacitance changes. Another tested electrochemical technique was amperometry. Here a voltage is applied to the system at which the oxidation - reduction occurs. The magnitude of the Faradaic current registered depends on the effective surface area over time, i.e., if there are more free wells in the system, a larger current is measured.

The most reproducible electrochemical methods to characterize the surface modification were CV and EIS. In the first step of the surface modification protocol, we performed electrochemical measurements of the nanoporous gold membrane electrodes in the unmodified state (Fig. 3). The increase in the separation of CV oxidation and reduction peaks and the complex impedance values show that the gold surface was partially blocked as predicted by molecular dynamics simulations.

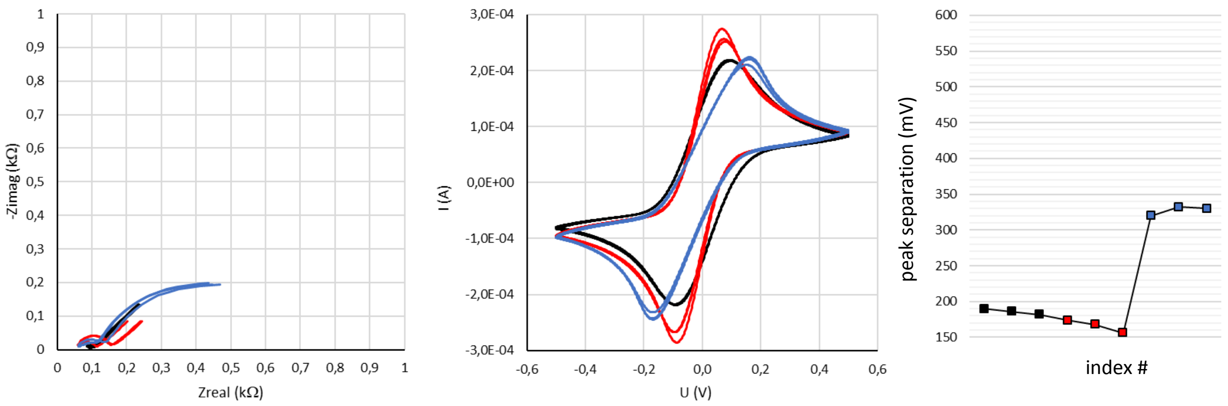

Figure 3: Investigation of the preparation of binding sites on the surface of nanoporous gold membrane electrode with EIS (left) and CV (middle). Black curves show Pt - Pt reference measurements, red curves show the unmodified nanoporous gold membrane electrode, the blue curves show results after the preparation of binding wells. The separation of CV oxidation and reduction peaks is shown in the right.

Avidin rebinding was tested in a control experiment with a differential sensor where none of the electrodes contained avidin binding wells. Here the surface was entirely blocked by thiolated PEG molecules (Fig. 4 top row). The introduction of avidin does not significantly change the complex impedance and the CV peak separation.

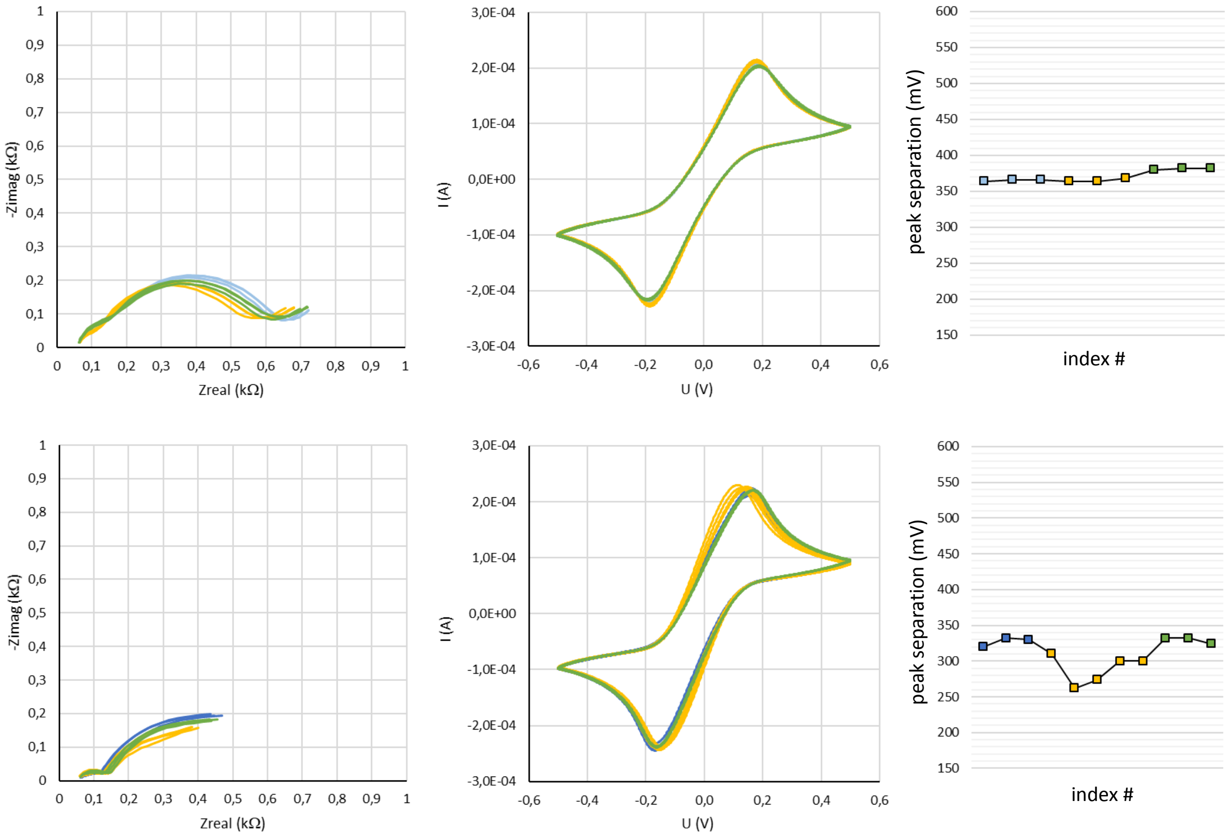

Figure 4: Investigation of protein rebinding with EIS (left) and CV (middle). The top row shows gold nanoporous membrane without avidin binding wells. The bottom row shows gold nanoporous membrane with avidin binding wells. Blue curves show measurements after surface modification, yellow curves show measurements after heat treatment to break biotin – avidin bond, and green curves show results after reintroduction of avidin (10^{-2} mg/ml). The separation of CV oxidation and reduction peaks is shown in the right.

When we tested the differential sensor, where one of the membranes contained avidin binding wells, we could break the avidin – biotin bond with heat treatment, evidenced by the decrease in the peak separation (Fig. 4 bottom row). Increased peak separation was observed after avidin was reintroduced in 10^{-2} mg/ml concentration to the differential sensor.

We showed for the first time experimentally and theoretically that electrochemically detectable, reversible protein binding sites can be fabricated entirely from thiolated molecules in a bottom-up fashion. The binding sites are nanoscopic wells, where the analyte protein can rebind, causing a measurable change in the differential electrochemical signal. An MSc dissertation was written on the topic: Kis Martin: Fehérjék vizsgálata differenciális elrendezésű elektrokémiai szenzorokkal, BME Szervetlen és Analitikai Kémia Tanszék, 2022. The results of the dissertation give the basis of the manuscript we are working on currently. We plan to publish the experimental and theoretical investigation of the proposed method for differential detection of biomolecules after establishing the reproducibility and lower limit of detection.

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