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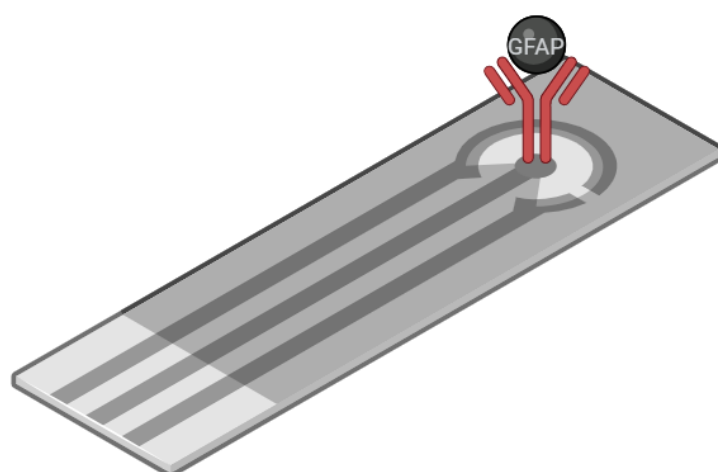
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## Summary for SensUs

Glial fibrillary acidic protein (GFAP) is a type of glial cell in the central nervous system known as an astrocyte which is important for sustaining neuronal health, preserving brain homeostasis, and offering structural support. GFAP has attracted the interest of scientists and medical professionals in recent years as a promising biomarker for the quick and accurate detection of brain injuries. In this paper, we present a novel biosensor based on electrochemistry, which is capable of functionalizing and optimizing the biosensor. The biosensor is designed to be easy to use, reliable, and accessible in clinical settings, so the hospitals can quickly get a positive or negative result for the patient for quick treatment.

## Introduction

Brain injuries are a serious public health issue that has wide-ranging effects on both people and society. Numerous factors, such as accidents, sports-related occurrences, falls, and medical disorders, can lead to these injuries. Brain injuries can have serious aftereffects, including cognitive, emotional, and physical impairments that lower the patient's quality of life. Because of this, successful medical intervention and care depend on the early and precise detection of brain injuries. (Lei et al., 2015)

Glial fibrillary acidic protein (GFAP) has attracted the interest of scientists and medical professionals in recent years as a promising biomarker for the quick and accurate detection of brain damage. GFAP is a type of glial cell in the central nervous system known as an astrocyte which is important for sustaining neuronal health, preserving brain homeostasis, and offering structural support. When the brain experiences trauma or injury, astrocytes often undergo changes that lead to an increased release of GFAP into the cerebrospinal fluid and bloodstream. (Okonkwo et al., 2013)

The use of GFAP as a biomarker offers several advantages for the timely and accurate detection of brain injuries. Traditional diagnostic methods, such as imaging techniques, can be time-consuming and may not always capture the subtle changes that occur immediately after an injury. In contrast, GFAP levels can rise rapidly following brain trauma, providing a potential window of opportunity for swift identification. This has led to the development of GFAP-based biosensors, that allow healthcare providers to assess the severity of brain injuries and guide treatment decisions more effectively. (Bhalla et al., 2016)

## Biosensor system and assay

### Molecular recognition

In molecular recognition, molecules interact only when their complementary chemical or structural characteristics are present. It supports crucial biological processes such as antibody-antigen binding, cell signalling, and interactions. Beyond biology, this idea has been cleverly used, notably in materials science and electrochemistry. (Bhalla et al., 2016; Fernández-la-Villa et al., 2019)

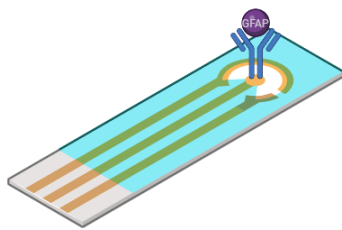
To make the sensor work we need to create an electrode that contains a biological recognition site on the surface. This is shown in Figure 1. The sensor should work by first having the antibody immobilized on the surface. Second the addition of the sample which contains the analyte GFAP and measuring the correct concentration. Then a washing step is required to remove unbound molecules from the electrode, before using the PalmSens4 along with PSTrace to measure the impedance. (Pruna et al., 2017)

To make this work, we need to:

1. Be able to see on the sensor, that the functionalization of DPS has been done correctly.
2. Attach the antibodies to the electrode.
3. Successfully remove unwanted molecules.

x`

Figure  
GFAP



1: the antibody is immobilized on the surface of the electrode, and the binds to the antibody.

The  
We

### Electrodes

used a Drop sense gold electrode as the basis for our biosensor as a base, as it creates a good conductor for the test. This biosensor when optimized offers a combination of sensitivity and ease of use which makes it suitable for testing for GFAP. The Drop sense electrodes have low noise levels which is crucial when testing for trace levels in plasma. The biosensor offers a wide and uniform surface area for functionalization and binding of the biomarker, which allows for efficient immobilization of antibodies specific to GFAP enhancing the chances of successful biomarker capture. (Mincu et al., 2020) The biosensor must be easy to use, reliable, and accessible in clinical settings, so the hospitals can quickly get a positive or negative result for the patient for quick treatment.

### Functionalization

We chose the method of layer-by-layer, as there was already some knowledge about this method in the group, and as it has been seen working, it was a good starting point for the biosensor. When making a biosensor work for another biomarker, there will be some things that need to be changed, as the antibody will react differently based on concentration and ability. Therefore, the protocol was taken, and different optimization steps were done to find the optimal biosensor for the identification of GFAP. (Mehmandoust et al., 2023)

### The cartridge technology

Now we are testing the best cartridge technology for the sensor, to make it user-friendly and Point-of-care (POC). One of the areas we are working on is a holder for the electrode, which makes it easier for the user to place the sample correctly and quickly on the electrode.

The present design is shown in Figure 2: Design for the holder.. the principle of the holder is to control the sample when it is placed on the electrode. The electrode is slid into the cartridge and then it is closed, so the working electrode can be seen through the opening in the cartridge, and the sample can easily be placed on it and controlled. The three connection electrodes are then available to be connected to the PalmSens4 for reading.

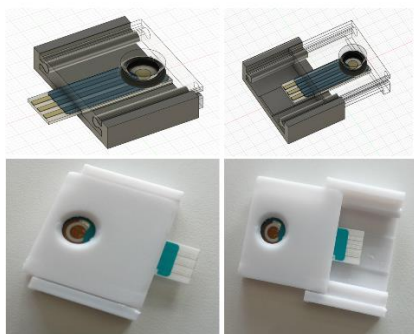


Figure 2: Design for the holder.

By using a holder, this allows the microfluids to only be where they should and not all over the electrode, which makes it more user friendly, and less likely for mistakes in the addition of the sample.

### Impedance spectroscopy as an instrument reader

Impedance Spectroscopy (IS) stands as a powerful and versatile method. Offering a straightforward way for measuring the electrical properties of materials and systems. We used the PalmSens4 to read the electrode along with the computer program PSTrace.(PalmSens, n.d.) The reason for this is, that it is easy to use, takes up little space, and most labs, hospitals, and private practices already have a computer where they can add this concept. Figure 3 shows the reader used.



Figure 3: The PalmSens4 features a huge current range, an enormous potential range, and low noise levels. The affordable PalmSens4 is a full-featured laboratory tool, but because of its small size and robust construction.

This gives a curve on the screen, and from a calibration curve, it will be possible to compare the sample to this to compare and conclude the amount of GFAP in the plasma sample.

## Technological feasibility

As is known, the group has undergone a major change in participants and were in May changed to people with limited time for the project, because the original team were unable to continue. This section will therefore hold some initial testing, but the sensor is undergoing optimization for a more specific and repeatable result.

We started testing the different Anti-GFAP antibodies, and had most success with GFAP94cc, and choose to continue with that based on time.

The protocol for the first setup is shown in Appendix 1. The following graph shows the impedance measurements for the electrodes before they were used, as we have seen that not all electrodes have the same IS before use, and we, therefore, need to take this into account when measuring.

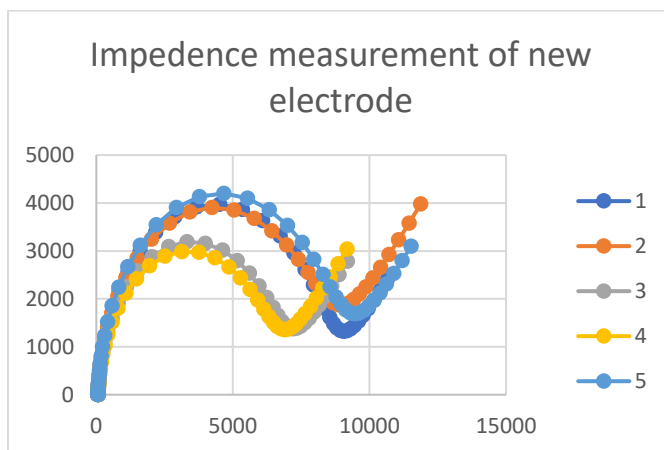


Figure 4: Measurements of the electrodes before use

As seen on the graph, it is noticeably clear that there is a difference between the start measurement between the different electrodes, which is why we are measuring the electrodes before use, so we can remove this variable to get a more specific result.

The next step was then to measure the electrodes after the functionalization step with  $1\mu\text{g}/\text{mL}$  of GFAP in PBS and incubated for 15 minutes. Again here, we see a difference in the impedance measurements, but we see a bit of a correlation between where the different measurements are compared to each other.

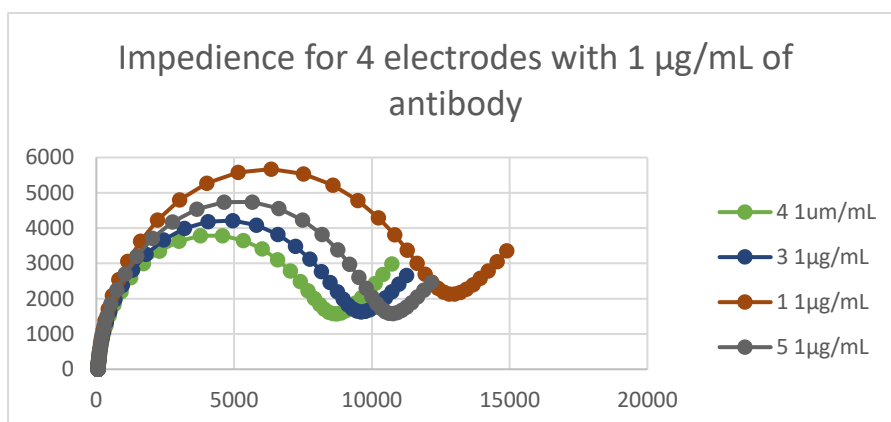


Figure 5: Impedance measurement with the addition of GFAP antibodies

The following graph shows the impedance measurements after the functionalization with DPS and Abs. This showed that there is a correlation between the measurements, and the biosensor has a good starting point but needs optimization, which is planned for next week.

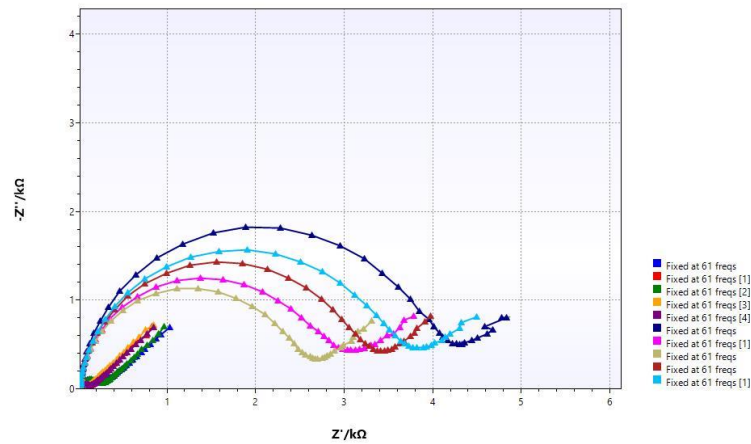


Figure 6: The measured impedance after the functionalization with DPS (the 4 lower signals measured) and Abs (the 5 stronger signals).

The last measurement is the complete biosensor assay. This means that the DPS, ABS, and blocking steps with BSA have been done. As we can see the grey graph looks different compared to the other measurements, and this is therefore some of what we are looking at for the optimization, so the final sensor does not give false positive or false negative. It is evident from the graph showing the measurements between the various phases that each step is performing as it should, but not to its full potential.

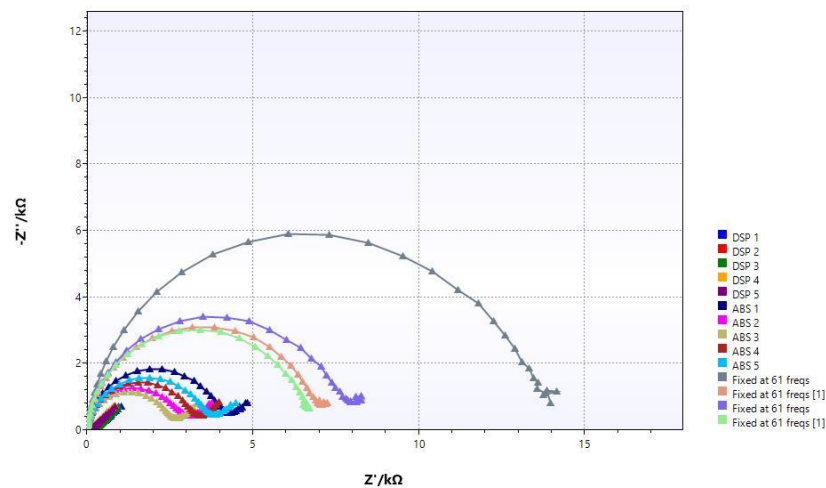


Figure 7: The measurement for the complete assay, with the impedance or the different steps included. The last four measurements correspond to the data acquired after the final BSA blocking step of the functionalization.

We still need a lot of optimization and testing, but the biosensor we have is already showing promise, and with the optimization steps and the holder, we are sure we can get something that can show the concentration of GFAP in plasma.



## Novelty and creativity

### Captains

The precise affixing of specific identification components to the electrode surfaces is one of the technology's most innovative features. These recognition components are designed to bind with GFAP molecules only when they are present in plasma samples, allowing for the precise collection and subsequent measurement of this important biomarker. The foundation of a highly specialized and sensitive biosensor is formed by the incorporation of such complex biofunctionalization procedures with electrode platforms.

The novelty of this method is further enhanced by the creation of a special electrode holder. To reduce outside interference and improve signal capture, the holder offers a controlled environment for sample interactions. This customized technology guarantees measurement accuracy and repeatability, which is essential for attaining accurate GFAP identification. Another novel aspect of the biosensor design is the creation of a trustworthy baseline for each electrode. The baseline acts as a standard against which variations in the electrochemical signal brought on by contact with GFAP molecules are measured. The biosensor may consider any innate variability by calibrating each electrode separately, resulting in accurate and consistent results.

The dynamics of the project significantly changed, and just before the event, the crew was completely reorganized. Despite the limitations of the limited time, all students, mentors, and collaborators shared their knowledge and support to get a biosensor that works. The group discussed which method to use based on the group's ability and from there by talking with supervisors a protocol was found, which has already been used, but with optimizations and changes, so they worked for this setup.

### Supervisor

Mrs., Maria has been supervising the DTU SensUs team during this year. There were several issues with the team collaboration, and we had to restart the process with a new team quite late in the process, with significantly fewer students. Despite this setback, the new team has worked hard on two parallel tracks, until it was decided to focus on an electrochemical biosensor, due to time constraints related to the ordering of parts required for their preferred option of SPR (Surface Plasmon Resonance).

Although electrochemical sensing is not in itself a novel approach, I believe that the students have utilized the technology in an innovative way. As they noticed the significant differences between signals coming from samples of the same concentration, they decided to investigate ways to improve reproducibility by getting measurement and baseline on the same chip sequentially. Moreover, they have worked with designing a novel, easy to use holder for the electrodes, that includes a chamber where the sample can be inserted at a controlled volume. In the future they are looking to include a pipette-free sample introduction to the sensor.

The students have been working independently, only receiving instructions on how to use the equipment (potentiostat and 3D printer) and links to relevant articles, although they were themselves very proactive in finding and reading literature. The design of the basic holder was already developed in our group, but the team is working on changing part of the design, e.g., to allow for the correct volume, and the pipette free sample addition to the sensor. The students have conducted all functionalization, measurements, and data analysis without any external help.

## Translational potential

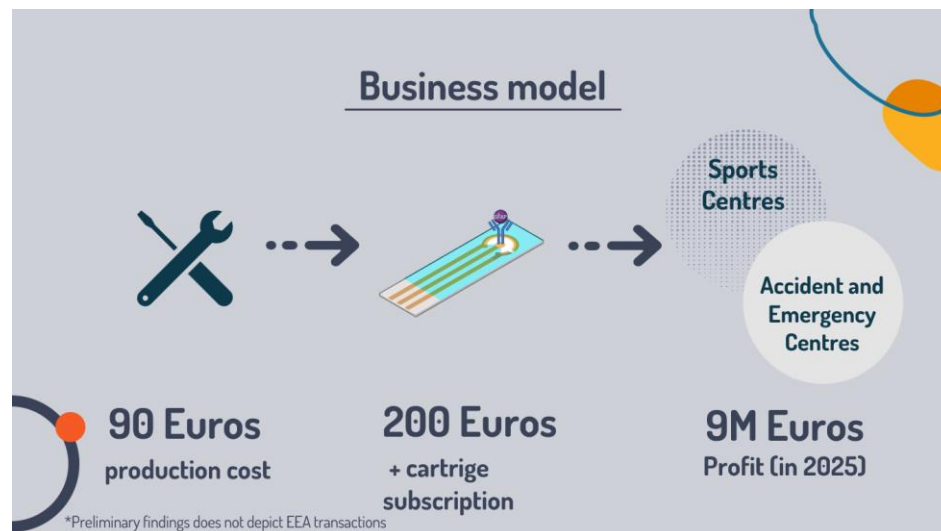
Having a successful business model translates to understanding good backing power and help in realising the potential of the product. The biosensor produced by DetectUs will be sold in 2 different kind of business models. In the first phase, the biosensor comes with cartridges, and then the consumer will have an option of buying an extra subscription set of cartridges.

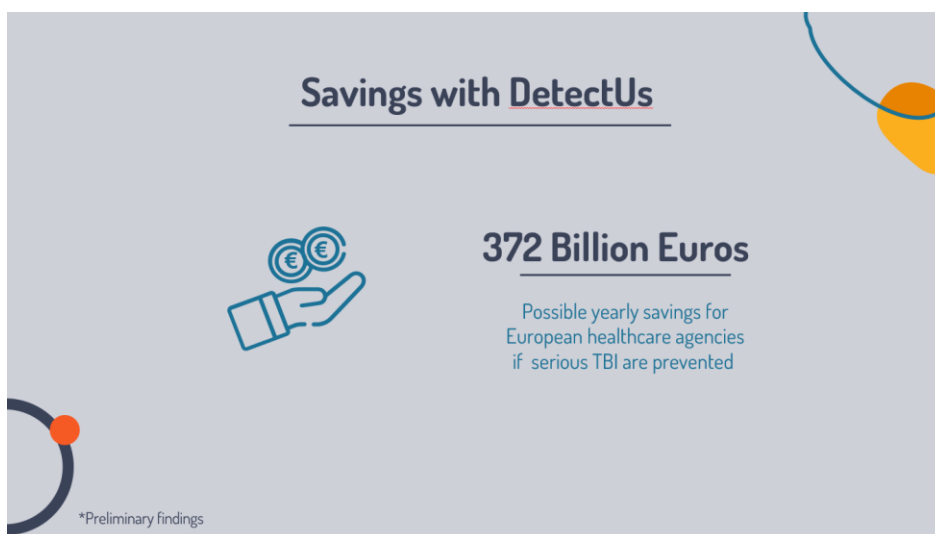
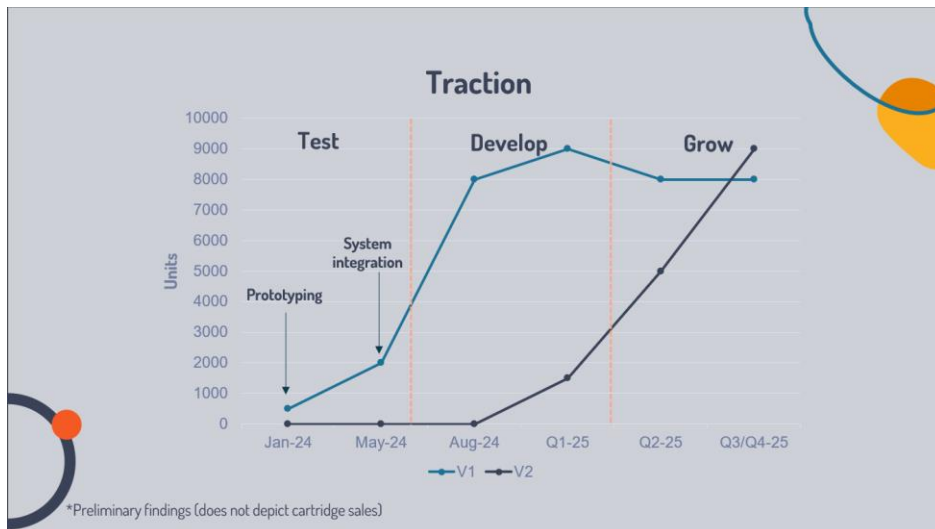
There are two iterations of the production. In the first iteration, the prototyping and the development of the product is accordance with the cartridge technology. But the second iteration (V2) will feature the use of QCM and nano-chip technology which will be the MVP (Minimal Viable Product) for further development case. The second iteration will lead to a compact and yet comprehensive biosensor which will save customers from changing their cartridges frequently and have one sensor integrated with their daily fitness devices.

The end goal of this product would be to make it sustainable and technologically developed at the same instance.

Considering the production and development costs, Poland was chosen as the front-runner for the manufacturing and R&D for the biosensor technology. The reasons for this are that Poland has one of the highest growth curve in technology and cheap costs of manufacturing compliment this decision.

## Business model canvas





## Stakeholder desirability

With **Blue Dot** as our partner, the goal was to make this project sustainable for development, and at the same time render it on the right stages of growth.

The current TBI (Traumatic Brain Injury) tests include diagnosis based on symptoms which is often seen once the TBI reaches the mild urgency level, or performing CT and MRI tests when the person undergoes a cranial injury. These methods might be effective but often lead to overlooking and negligence due to human ignorance.

DetectUs' TBI sensor skips all this and provides a comprehensive understanding about the person's injury. Detecting TBI allows us to tap into the European market where the annual health cost of TBI treatment is 2.9 billion Euros where the estimated average cost of a patient hospitalised with TBI is 2,883 Euros ranging from a cost of 987 Euros per patient with concussion, and 6,362 Euros per patient with severe ABI. Using the biosensor paves path in detecting and treating 75% of the cases before they become mild or severe TBI casualties. And in this way, we have the potential of a market worth at least 1.6 billion Euros every year, starting from 2023. Adding this to the 160 billion

Euros market for brain activity testing, the stakeholders have a wonderful opportunity of turning this into a profitable venture by being one of the frontrunners in the market.

### Business feasibility

The marketing strategy that DetectUs' TBI sensor follows the gap in the market. This is that there is no early detection method for brain trauma injuries. This segment does not get that much exposure. To accelerate the process and reach that segment we first will go to the nursing homes where our solution will be optimised and developed the final model of the solution with more actual case patients that will add more real time value to our testing and improve the accuracy of the sensor. After that, the target will be **Sports Centres** and the **Accident and Emergency Healthcare Centre**. The plan is to establish partnership with the health organisations of the respective countries.

### Team and support

#### Contribution of the team members

Sub teams	Responsibilities	Team members
Biotechnical team	Laboratory testing	Andrea Alberdi Hidalgo Karoline Valentin Jensen Leila Alex Botansen Oscar Erik Begazo (Beate Ramshøj Knudsen)
Entrepreneurship team	Business plan	Vishal Vidyadhar Angadi
Report preparation	Gathering the information from everyone and writing them to a coherent text	Beate Ramshøj Knudsen

#### People who have provided support.

- Maria Dimaki: Academic supervisor, [madi@dtu.dk](mailto:madi@dtu.dk)
- Winnie Svendsen: Supervisor and contact person for the SensUs organization, [wisv@dtu.dk](mailto:wisv@dtu.dk)
- Neeti Kalyani – post.doc helping with the lab and buying.
- Pulkit Saluja – 3D printer support

## Final remarks

It is important to find a quick way to identify traumatic brain injuries in patients, as a late diagnosis can have serious or fatal consequences. Therefore, it is critical to have a quick and accurate monitoring system in place to gauge the level of GFAP in the plasma. To do a proper analysis, it is important to have antibodies that can identify the analyte even in low concentrations. By using electrochemistry, we have the ability with functionalization and optimization.

The biosensor is not finished at the time of the handing in of this report, but we have a plan for a final sprint to get a biosensor to where we get reliable results on every electrode and holder which makes it easy for the end-user to use. As a team, we are trying to find a solution to avoid some electrodes giving a different result compared to what we expect to see. We might not be as far as we had hoped, but we are optimistic and believe that this sensor could help with the identification of GFAP in plasma to quick identification of a traumatic brain injury to give patients the best treatment possible.

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## Appendix 1 – protocol for the functionalization of the electrode

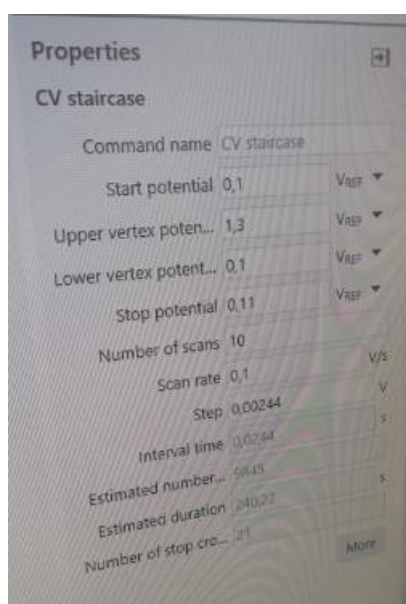
### Materials and Chemicals:

- Drop sense gold electrodes.
- 0.5 M H<sub>2</sub>SO<sub>4</sub>
- Milli-Q water
- 10 mM DSP in DMSO
- 100 µg/mL anti-GFAP
- 1x PBS
- 1% BSA solution in 1x PBS
- 200 µm high PMMA chambers
- GFAP in 1x PBS
- Potassium ferri-/ferrocyanide
- Potassium chloride (KCl)

### Procedure:

Electrode cleaning: Over ALL the electrodes surfaces

Drop sense electrodes were cleaned using H<sub>2</sub>SO<sub>4</sub> **cyclic voltammetry**. 80 µl of **0.5 M H<sub>2</sub>SO<sub>4</sub>** was deposited **on the 3-electrode system** and 10 CV scans were performed between -0.1 and 1.2 V with a 0.1 V/s scan rate. After cleaning the electrodes were **rinsed with Milli-Q water and dried** using **nitrogen gas**.



This is from the other software but should look something like this.

### Functionalization with DSP: Only on the WE (Working electrode)

- The DSP powder is equilibrated at **room temperature** for **30 minutes**. (**Take it out of the freezer**, it is stored in a box at the lowest shelf in freezer 2)
- 10 mg of DSP are dissolved in 2.5 mL anhydrous DMSO to obtain a 10 mM DSP solution.
- Cleaned electrodes (WE) were incubated with **4 µL** of 10 mM DSP solution in DMSO for **1.5h**.
- The electrodes are rinsed with **Milli-Q water (count 10 seconds because it is quite sticky and helps with reproducibility. Also try not to impact directly on the working electrode)** and then dried with nitrogen gas.

ABS attachment: Look at the concentration of the antibody first!

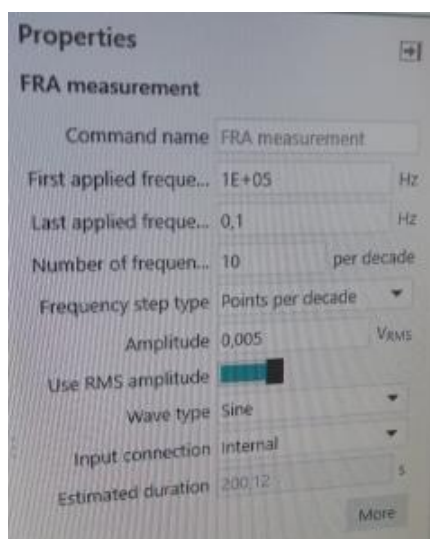
- An antibody dilution of **100 ug/mL** is prepared in 1xPBS.
- V of antibody needed =  $(100 * 150) / \text{Concentration of Abs in ug/mL}$  --> For GFAP-94 it is **2,17uL IN 150µm pbs**
- First add 150uL of PBS buffer (glass bottle with blue cap in Fridge 2) and then the resulting V of antibody needed.
- **Only the WE area is incubated** with **15 µL** AB suspension for **1.5h**.
- The electrodes are rinsed with **Milli-Q water (10 sec.)** and dried using nitrogen gas.

BSA blocking:

- A 1% BSA solution was prepared in a 1x PBS buffer.
- The WE area was incubated (use **15uL** like with the Abs) with the solution for **45 minutes** to react the unreacted NHS reactive groups on the DSP SAM.
- The electrodes were rinsed with **Milli-Q water (10 sec.)** and dried with nitrogen gas.

Impedance Spectroscopy measurements

- Impedance spectroscopy in the software and the parameters should look something like this:



- EIS (Electrochemical Impedance Spectroscopy) measurements were performed using a 5 mM potassium ferri-/ferrocyanide solution with **200 mM KCl** supporting electrolyte.
- I put **80uL** covering the entire electrode (Same as cleaning step)