Team Results Document

BiosensUM

University:
Université de Montréal

Team members:

Co-captains:
Kathleen Lupien
Mohamed-Salah Haddoune

Members:
Anthony Ryckman
Carl-Éric Gélinas
Jeanine Looman
Kelvin Tresol Migisha Rugira
Mehdi-Ilyes Haddoune
Melissa Saadane
Sharan Balasundram

Supervisor :
Pr. Jean-François Masson

Coach:
Ryma Boudries

August 9th 2024

SensUs

Abstract

Acute kidney injury (AKI) is a growing global health concern. Rising cases of heart disease and diabetes, medical conditions that put a strain on a patient's kidneys, are linked to kidney failure. AKI-related hospitalizations are therefore increasing (*GlobalNewswire*, 2024), putting additional strain on the healthcare system. BioSensUM is proud to present CreaSensUM, a continuous monitoring creatinine device with aims to not only increase hospital turnover rates, but help nephrologists make decisions grounded in reliable, real-time data, as well as increase patient wellness and satisfaction.

BioSensUM's electrochemical aptamer-based sensor (E-AB) combines the continuous monitoring capabilities of aptamers with square-wave voltammetry, a highly sensitive electrochemical method. Our detection approach holds significant potential for miniaturization, aiming to evolve into a wearable device. Additionally, the biosensor incorporates a Peltier module heating element to enhance signal amplification. It features a touch screen and graphical user interface (GUI) for intuitive operation, and a companion app provides the collection and access to performed analyses.

Contents

1. Biosensor

Our device is an electrochemical aptamer-based (E-AB) sensor, a method enabling continuous monitoring of creatinine (Downs & Plaxco, 2022). These next sections will describe the chemical and technological basis of our biosensor, from molecular recognition to cartridge technology.

1.1 Molecular recognition

Specific binding to creatinine is achieved by using an aptamer whose sequence was taken from (Ganguly et al., 2024) and synthesized using solid-phase oligonucleotide synthesis with the phosphoramidite method. The aptamer, functionnalized at the 3' end with a dithiol-C6 moiety, is functionalized on the working electrode of a CTI sensor (Palmsens, Netherlands). To avoid non-specific adsorption in a complex matrix like interstitial fluid (ISF), 6-mercapto-hexanol (Thermo Fisher Scientific, USA) was used to passivate the gold surface. Before synthesis, UNAFold (Markham et al., 2008) simulations were conducted to evaluate the energetic plausibility of folding/unfolding in physiological states. The predicted thermodynamic values for the stem-loop structure of the ss-DNA aptamer at 20°C and 37°C renders unfolding energies of of −10,69 kcal/mol and −5,12 kcal/mol, indicating that the aptamer is available for binding at physiologically-relevant temperatures.

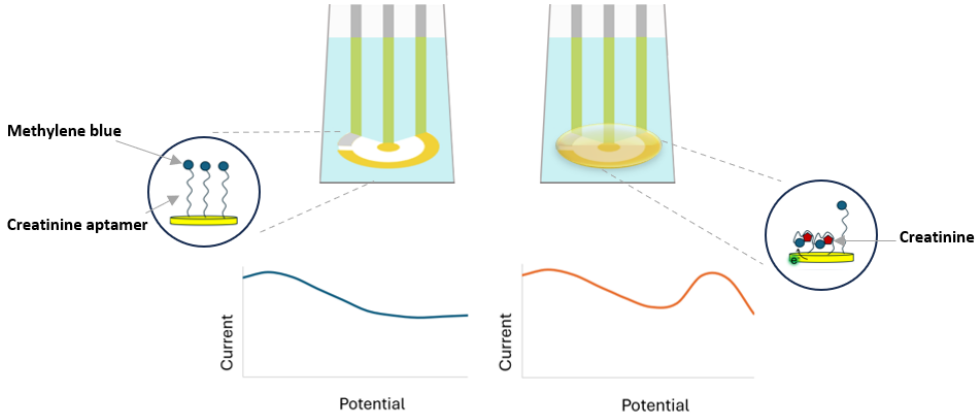


Figure 1. Target-induced conformational change of the aptamer and its effect on the electron exchange of the redox label MB with the gold surface of CTI gold electrodes.

1.2 Physical transduction

The 5' end of the aptamer was modified using a methylene blue redox reporter, commonly used in the literature . Conformational changes of the aptamer, induced by the binding of the analyte creatinine, results in a decreased distance between the MB and the gold electrode, favoring the reduction of MB by the work electrode (see Fig. 1). The electrode is interrogated using square wave voltammetry (SWV) with an Sensit potentiostat (Palmsens, Netherlands), which is a **rapid, sensitive** method (Mirceski & Gulaboski, 2014) . The current peak is measured around the theoretical reduction potential of methylene blue (-0.35V) and compared with a blank measurement to calculate the signal gain relative to the background noise with the following equation:

$$100 * (I_{creat} - I_0)/I_0.$$

To amplify the signal gain, the heating of the solution is operated. The solution is heated to 40 °C.

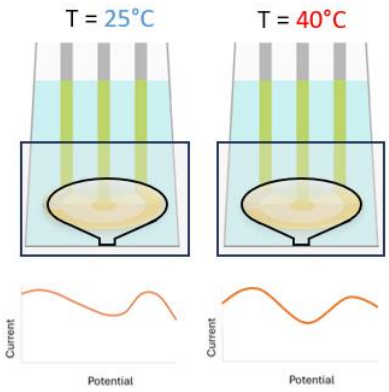


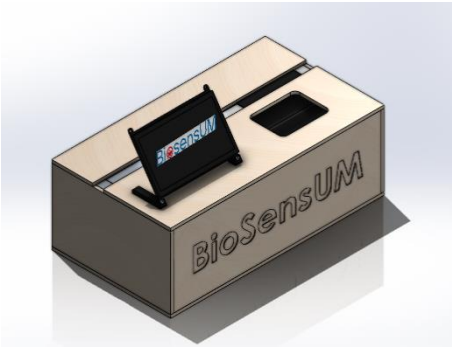
Figure 2. Amplification of signal gain when increasing the temperature.

1.3 Cartridge technology

Our current prototype consists of a double syringe pump for controlling fluid flow and a Peltier module as the heating element. All the components are housed in a box made of plywood boards and T-rail extrusions. The overall dimensions of the device are 45 × 25 × 27 cm. The Peltier module is mounted on a T-rail extrusion, allowing it to slide over the microfluidic system during the heating phase. The syringe pump is assembled using a lead screw, a screw nut, a NEMA 17 stepper motor, a bearing, and three 3D-printed parts that hold everything together. The 3D-printed parts are designed to accommodate two 1 mL syringes. ISF samples are pipetted and then inserted inside the inlet of our microfluidic that is over our sensor for the SWV. Then, using the syringe pump, the fluid will be slowly brought over to the captor. While a Peltier module heating to 40 Degrees Celsius, the SWV will be performed at 10Hz and 10mV, resulting in concentration measurement for that sample. Finally, using the syringe pump, the ISF can be expulsed drawn out from the microfluidic chamber and is ready for another measurement.

1.4 Reader instrument and user interaction

To operate our current prototype, the process (Fig. 6 in Appendix 8.1) begins by introducing the ISF sample into the microfluidic system. The next step involves using the user interface to activate the syringe pump, allowing the liquid to flow through the microfluidic channel. Following this, the Peltier module is positioned over the holder, and heating is initiated via the interface. Once these steps are completed, the Python code is executed, displaying the concentration on the computer. The user primarily interacts with the GUI on the SmartPi Touch 2 screen, designed to be as simple and intuitive as possible.



In the future, the sensor will be integrated into a wearable patch, significantly simplifying its use. The redesigned GUI will focus on data analysis, enhancing usability and providing more intuitive insights. The development process aims to minimize user interaction, making the biosensor almost entirely autonomous. The user will simply need to apply the patch and use the software or the application (Fig. 7 in Appendix 8.2), making the process seamless and effortless.

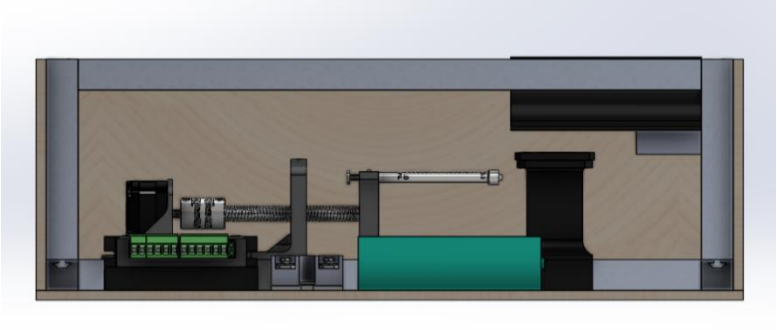


Figure 3. Biosensor assembly and outer casing

2. Technological feasibility

To ensure the specificity of the aptamer, fluorimetry experiments were conducted. A modified aptamer was functionalized at the 5' end with 6-Carboxyfluorescein and at the 3' end with Black Hole Quencher 1. The results demonstrated a clear change in fluorescence upon the addition of creatinine (Fig. 8 Appendix 8.3). Following these aptamer-validation experiments, SWV experiments were performed.

In SWV, both the amplitude and frequency of the square wave significantly impact measurement outcomes, particularly regarding signal gain and electron transfer kinetics (Dauphin-Ducharme & Plaxco, 2016). To maximize signal gain, we performed a grid search to optimize these parameters. This process identified the frequency and amplitude values that were most suitable for our aptamer. By applying the gain equation to each current value and comparing it to the corresponding blanks from the same manufacturing batch, we assessed the linearity and sensitivity of the measurements relative to each parameter.

Some data points shown in the Fig. 4, were considered outliers, mostly due to very low current values for their respective blank measurements. This results in very high gain values, leading to weak correlations and high sensitivity. After careful consideration, the relatively optimal conditions were determined to be a measurement frequency of 10 Hz and an amplitude of 10 mV.

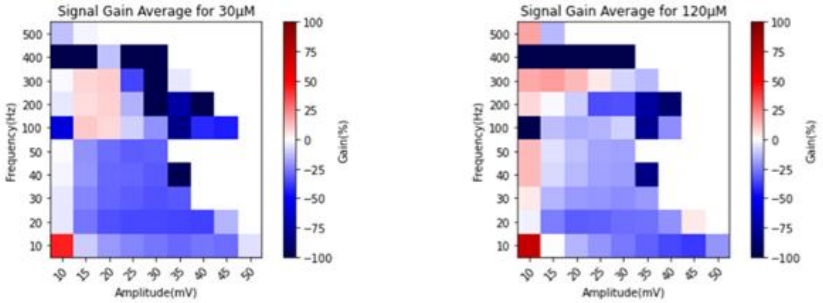


Figure 4. Signal gain heatmap for 30 µM and 120 µM. The average was computed by calculating the average gain values for sensors subjected to the same creatinine concentration for the same frequency and amplitude values. The resulting matrices were rendered as heatmaps.

Further, with a Peltier module, we can achieve heating and cooling for full temperature control. Chen et al. (2021) demonstrate that alternating temperatures can enhance measurement sensitivity and accuracy. Additionally, Downs et al. (2022) state that matching the temperature during calibration with that during measurement reduces variations in gain and binding curve midpoints, leading to more precise quantification. Likely, modulating the temperature increases specificity in some ranges of concentrations, as stated by Pan et al (2022). Therefore, based on the success of Chen et al. (2021), continuous measurements can be performed by first cooling and then heating to favor binding and subsequently "quick-start" aptamer release. Unfortunately, due to a hardware constraint, the cooling step was not completed in time for the TRD. However, the heating step was retained.

Initially, heating was introduced to facilitate the release of creatinine. Indeed, Chen et al, 2021 demonstrated that heating favors conformational changes in aptamers. However, in our case, it was observed that the signal was amplified when the solution was heated. Consequently, we decided to use heating as a signal-amplification method.

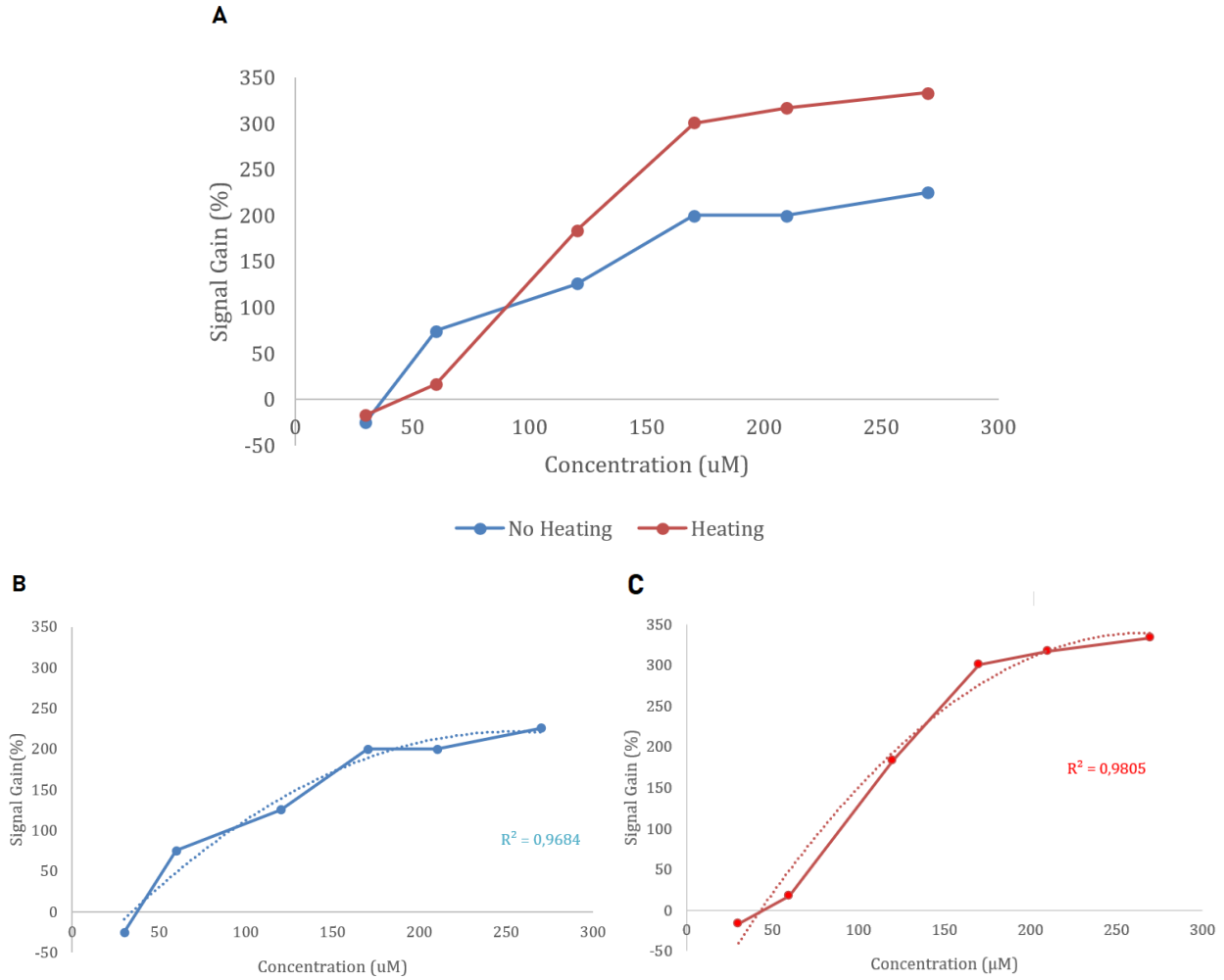


Figure 5. Standard curves with and without heating, including polynomial predicted trendlines for both. A: Standard curves with and without heating. B: Standard curve without heating with a polynomial predicted trendline. C: Standard curve with heating and a predicted polynomial trendline. The standard curve was obtained in ISF with different concentrations of creatinine. Measurements were performed with a frequence of 10Hz and an amplitude of 10mV. The equation of the polynomial curves without heating and with heating are respectively: y = -0,0046x2 + 2,3312x - 74,822 and y = -0,0068x2 + 3,6152x - 143,44.

Figure 5 presents two standard curves: one with sample heating during measurement and one without. After approximately 80 µM, a significant increase in signal gain is observed with heating. According to Pan et al. (2022), heating can enhance sensitivity in higher concentration ranges. For the trendline, a polynomial model was used to fit the curves, and a higher R^2 value is noted when heating is applied.

It is important to mention that applying heat typically results in a logical increase in current, which may be a baseline effect. Also, the literature generally suggests that signal gain is usually amplified by cooling rather than heating. However, factors such as the type of aptamer, its stability or even the tested concentrations range can influence signal gain. Consequently, differences from the literature are possible but further experiments are needed to confirm our results.

3. Originality

3.1 Written by team captains

Initial stages of biosensor development included an extensive literature review of techniques and methods both for continuous monitoring and quantitative creatinine detection. Several methods were pinpointed in literature, including enzymes (Dasgupta et al., 2020) and antibodies (Thompson et al., 2023). However, use of an electrochemical detection using an aptamer (E-AB) was targeted, for both its relative simplicity and miniaturization potential. Our EAB was based off of Kevin Plaxco's pioneering work in the field of continuous detection (Downs & Plaxco, 2022), whereas creatinine-specific detection, was based off of (Ganguly et al., 2023)'s article exploring the detection of creatinine using electro-impedance spectroscopy (EIS) and an aptamer. However, to the best of our knowledge, a continuous biosensor using electrochemical methods for creatinine is not reported in the literature.

The protocol for functionalization of the working electrode was developed based on (Xiao et al., 2007) and validated by Dominic Lauzon, PhD from University of Montreal's Biosensors and Nanomachines laboratory.

Our team developed an integrated electrochemical aptasensor and microfluidic chip, complete with a signal amplification module through heating. The microfluidic mold was 3D printed based on an original idea, after several attempts to optimize the electrochemical signal while maintaining laminar flow. The Peltier module integration, software and data analysis program were original contributions. The syringe pump's electronic circuit was adapted from an open source model (*Wikifactory, 2023*), but the double-syringe t-rail assembly was designed and 3D printed by the team. The electronics and code for precise temperature control using a Peltier module were devised by the team. The GUI and the app were also created by BioSensUM, as well as the final assembly.

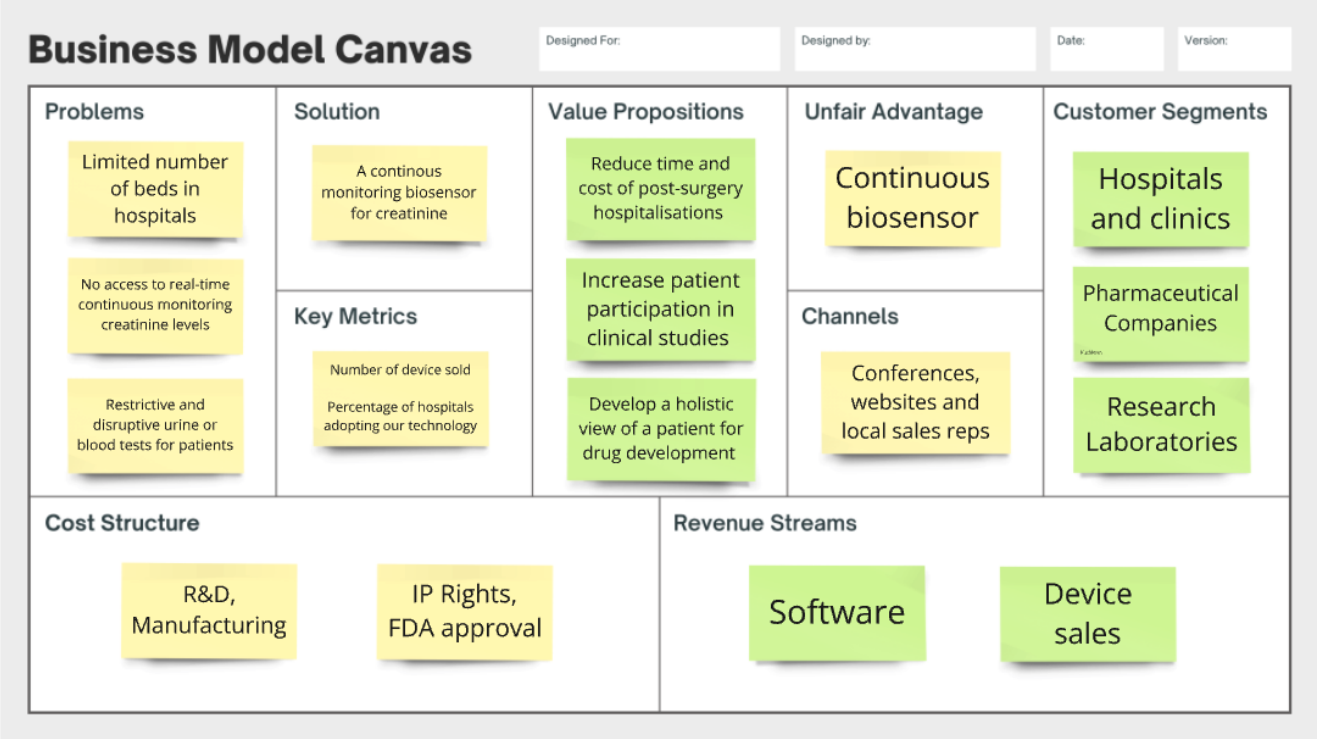
3.2 Written by team's supervisor

The team worked independently with some supervision from scientists in my group and the one of Alexis Vallée-Belisle. The aspects that were accessible to the team or provided by these scientists are the electrode material and the potentiostat, the synthesis of the aptamer and some advices and training were provided to the team on the electrochemical and aptamer aspects. The main aspects of originality are in the microfluidic design, construction of the syringe pump, user interface and in the introduction of the Peltier stage. Those are all new and were not accessible to the student from outside sources. The integration of these aspects into a working device was done independently.
-Jean-Francois Masson

Supervisor	Co-captains	
Pr. Jean-François Masson	Kathleen Lupien	Mohamed-Salah Haddoune

4. Translation potential

4.1 Business model canvas



4.2 Stakeholder desirability

Acute kidney injury (AKI), defined as a sudden drop in kidney function, is a rising global health concern. Indeed, hospitalization rates increased 34% in the US among Medicare Beneficiaries between 2010 and 2019, while the market size reached 5,766M USD$ in 2023 with an expected growth to 9,904 M USD$ in 2034 (*GlobalNewswire*, 2024). AKI is caused by multiple factors, but often occurs when a disease or event puts extra strain on the kidneys, such as diabetes, heart failure, surgery or medication use. An increase in diabetes and heart conditions is therefore a driving industry factor (*MarketInsights,* 2024).

Unfortunately, kidney disease is often termed the "silent killer" (Boyance K., 2023), because symptoms may only become apparent after significant damage to the kidneys has occurred. Dr. Beaubien-Souligny, a nephrologist and researcher at the CHUM, highlights that to prevent renal complications, healthcare practitioners typically schedule longer inpatient stays following kidney-straining events like heart surgeries or kidney transplants to monitor patients' glomerular filtration rates. This approach is costly, with the average daily bed cost in the US totalling $2,883 (Statista, 2022) and contributes to inefficiencies in the healthcare system. With 900,000 heart surgeries in the US in 2023, and estimated to increase to 1.3 million by 2029, there is a reliable and steady demand (*iData Research*, 2023).

Various key stakeholders will be involved, with Hospitals identified as our main customers. Patients will benefit from continuous monitoring that, while invasive, provides critical data, improving their overall health outcomes. Nephrologists and cardiologists can gain access to this data for better patient management, particularly post-surgery. Pharmaceutical companies and laboratories will use our reliable data for drug development and clinical research, improving the efficacy and safety of treatments. Regulatory bodies like the FDA ensure our device meets stringent standards, adding credibility, while organizations like the National Kidney Foundation benefit from advanced tools for kidney health management. Local sales representatives and conferences play crucial roles in promoting and demonstrating our technology to the medical

community, ensuring widespread adoption and integration into healthcare practices. See Figures 6 and 7 in the Appendix for a full stakeholder relationship diagram, as well as a viability analysis of our main three customer segments.

In our Competitive Analysis Framework (Appendix, Fig. 13), we evaluated CreaSenUM against the current standard and other market competitors in terms of portability and measurement continuity. Traditional lab equipment, the gold standard for creatinine detection, though highly accurate, is not portable and requires multiple patient visits for single-time measurements. Portable devices like StatSensor Creatinine and i-STAT offer mobility but only provide point measurements, lacking continuous monitoring capabilities. Other competitors, such as Everlywell and Minuteful, also offer portability but are limited to point measurements. In contrast, BioSensUM distinguishes itself as a portable, continuous monitoring solution, offering real-time data and improving patient outcomes, thus setting a new standard in creatinine monitoring.

Our CreaSensUM will distinguish itself by offering real-time data through a wearable device, enabling continuous monitoring without frequent manual interventions. In comparison, our device minimizes patient discomfort and reduces the need for hospital stays solely for monitoring purposes, addressing both patient and hospital needs more effectively. As we utilize a unique aptamer-based detection method integrated into a wearable format, we will be protected by our own set of patents, ensuring that our innovation remains proprietary.

Our current prototype features a SensIT potentiostat linked to a microfluidic system, a syringe pump, a Raspberry Pi 4, a Smartpi Touch 2 Screen, and a Peltier device. While this setup effectively demonstrates continuous creatinine monitoring, it remains bulky and lacks our proprietary software, making it a step away from the wearable patch we intend to develop. The next phase of development will focus on miniaturizing the potentiostat into a chip capable of performing Square Wave Voltammetry (SWV) with our specific parameters. This chip will be integrated into a small microneedle, eliminating the need for the microfluidic system and syringe pump.

4.3 Business feasibility

During the first year, our activities will mainly be centered at University of Montréal's laboratories. Participation in our University's incubator will help us accelerate our technological development, provide funding and give us access to personalized mentorship and a network of entrepreneurs and advisors. Other sources of funding will include research grants and small business loans, while we aim to gain traction by participating in pitching competitions like the Montreal StartupFest. This first phase will focus on research and development, in order to propel our biosensor from a proof-of-concept prototype to a wearable, user friendly, pain-free device. Since our chosen transduction method, square-wave voltammetry, relies on chemistry that isn't dependent on surface area, our technology is translatable to a miniature, micro-needle-based device.

Our current potentiostat is bulky and designed for research and development, rather than a streamlined biosensor. As such, a microelectronics expert will be needed to miniaturize and simplify the circuits into a microchip. Fabrication will be outsourced to manufacturers like Silex Microsystems or STMicroeletronics. Accounting for mass production discounts, we estimate that the final unit cost of our biosensor will be $47 (details in Appendix Fig 17). Microneedles and electrode fabrication will also be outsourced.

Key in-house production will center around software and mobile application development, micro-needle aptamer functionalization and device assembly. Indeed, our team currently possesses substantial expertise in the chemical and software aspects of our biosensor. Our strategy includes leveraging these resources effectively to maintain a balance between cost and quality while meeting production demands.

With regards to IP rights and licensing, first contact has been made with Brieuc Guillerm, a patent specialist at University of Montreal's *Bureau de Recherche-Développement-Valorisation* (BRDV). The BRDV would provide key insight into submitting an international patent (PCT) as well as accelerate the submission process. A Patent licensing agreement will be negotiated for use of our intellectual property in our startup. We estimate that a 5% revenue agreement can be agreed upon and mutually beneficial for both parties.

One of the most crucial aspects of our technology will be gaining regulatory approval for our biosensor. We plan to gain pre-market approval through a 510(k) submission using an equivalent device as a predicate. To do so, one of our team members will be appointed regulatory approval and quality management officer, ensuring proper documentation, traceability and reproducibility throughout our biosensors. Quality management and regulatory approval tools like Greenlight Guru will help coordinate and streamline the development process from pinpointing user needs to associating design outputs that are part of the verification and validation process. Strong manufacturer relationships, based on quality assurance protocols, will ensure strict quality control guidelines. We plan also to hire a consulting agency to help us navigate the FDA's guidelines and policies. We aim to gain FDA certification in 2027 in order to begin sales in 2028.

Further regulations are applicable to our device, namely the Health Insurance Portability and Accountability Act (HIPAA). Indeed, sensitive medical information will be shared via cloud from patients to healthcare providers and must be protected.

Once we our medical device is certified, we will launch our product in the United States, since the market size is much greater than Canada's, and health guidelines are less stringent. After a few years, we will expand into all of North America, then eventually launch internationally.

Hospitals can be a difficult customer segment to market to, because the acquisition of new medical devices is a lengthy process involving several parties, including doctors, hospital administration and biomedical engineers. Further, different hospitals have different acquisition policies and protocols. Dr. Louise Roy, a nephrologist at the CHUM with more than 40 years of experience identified this as an important barrier to entry, saying that even if nephrologists recommend our device to their associated hospital, the device must be further approved before being acquired. Therefore, our aim is to market our product through local sales representatives, who can help us navigate local hospital policies and capitalize on well-established customer relationships. In return, 10% of the sale revenues will go to the sales representatives. Establishing these partnerships with local sales representatives will be key to our sales strategy. Secondary channels include presenting at medical device conferences, such as the JPMorgan Healthcare Conference, where we can build a network of industry professionals, reach potential investors and gain credibility. Our website will also achieve these goals, as well as fuel company recruitment in order to build a world-class team.

One of our top priorities will be customer satisfaction, which might give us an edge with regards to more well-established companies like Abbott and Merck. By working closely with nephrologists and patients, our device and software will be tailored to their needs and drive iteratively improve our device and software. Customer satisfaction will be ensured through our support service, while seamless user adoption will be achieved through training sessions and videos.

4.4 Financial viability

Our CreaSensUM production cost per use is estimated at $47, primarily due to the specialized chip with Bluetooth capabilities designed for Square Wave Voltammetry (SWV) at 10mV and 10Hz. This cost also includes the microneedles coated with gold particles and the aptamer used for functionalization, as well as the overall assembly into a wearable patch. Initial development and

manufacturing involve a higher R&D budget of around $300,000 annually and a total investment of approximately $1,000,000 over three years.
Our pricing strategy includes selling the CreaSensUM patches at $250 each and licensing the software for $20,000 per hospital. Each hospital will need about 100 patches annually, totaling $45,000 per hospital per year. Despite the significant initial investment, hospitals can save $2,883 per patient by releasing them one day earlier, translating to total net savings of $243,300 for 100 patients. This high potential for savings makes the investment attractive for both hospitals and our company.

Last year, there was more than 900,000 heart surgeries during 2023 in the United States. This number is increasing each year due to the rising incidence of cardiovascular conditions, advancements in surgical techniques, and an aging population requiring more complex cardiac interventions. Considering the number of cardiac surgeries in the past year, if we reach 5% of the market, it would be used in 45,000 cases. Assuming we sell 100 patches per hospital, it would be 450 hospitals that would each pay $45000. The profit would be $20,250,000, which represents a substantial revenue potential. Given that our estimated annual development and production costs, including R&D and manufacturing, are approximately $1,000,000, this revenue significantly exceeds our cost base. Reaching 5% of the market would not only cover our initial development and implementation costs but also provide a robust profit margin. This analysis indicates that the chosen market is indeed large enough to justify the costs of development and has the potential to generate substantial revenues.

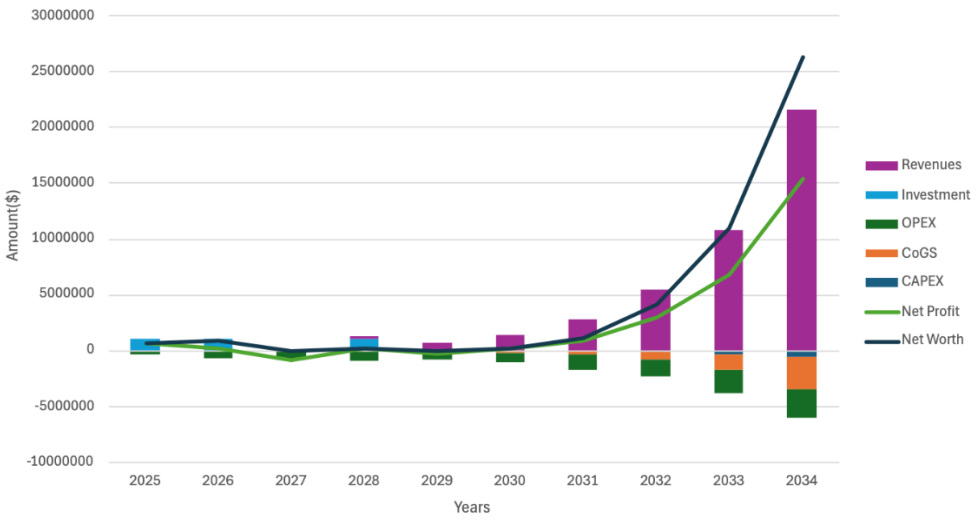


Figure 6. Financial projections of our company over the upcoming 9 years

According to our financial summary, to sustain operations until we achieve sufficient market share for profitability, we will require an initial investment of $1 million from venture capital groups and angel investors to adapt our current prototype. This will be followed by an additional $1 million after we develop the second version of the prototype, approximately one year later, and a final $1 million after our market entry in 2028. These investments are projected to be recouped by reaching the break-even point by the end of 2031, with returns expected to be tenfold by 2035.

5. Team and support

5.1 Contributions of the team members

Kathleen Lupien (Co-captain): Led the technological team through prototyping and integration of chemistry and engineering components, especially microfluidics. Managed team operations and

fueled funding initiatives. **Mohamed-Salah Haddoune (Co-captain):** Led the assay team through development, data analysis and optimization. Coordinated team meetings and schedule, developed overall vision of the sensor and fueled funding intiatives. **Anthony Ryckman:** Worked on market analysis, contributed to biosensor ideation and data analysis. **Carl-Éric Gélinas :** Pivotal in electrochemistry measurement protocol development and optimization. **Jeanine Looman:** Developed an algorithm for current measurement. Contributed to microfluidic prototyping and ideation. **Kelvin Tresol Migisha Rugira:** Contributed to validation of our aptamer, as well as protocol development and wet lab work. **Mehdi Ilyes Haddoune:** Created the GUI, coded our device pipeline, responsible for electronics, including temperature and fluid control. **Melissa Saadane:** Helped characterize the aptamer through fluorescence and SPR measurements. **Sharan Balasundram:** Modelized and built coherent prototype able to fit our needs. 3D printed multiple parts for the team. Responsible for final assembly and design.

5.2 People who have given support

BioSensUM would like to thank **Pr. Jean-François Masson** for his continued involvement as our supervisor. We are grateful for his unparalleled time and dedication towards breaking barriers to our success. Many thanks to **Ryma Boudries**, our team coach, for her help managing lab ressources and answering our unending questions. Thank you to **Joanne X, Bruno Soullam (PharmD), Salma Abdelaziz (PharmD), Dr. William Beaubien-Souligny, Dre. Louise Roy, Dre Isabelle Éthier, Dr. René Wittmer, Dr. Félix Rinfret and Dr. Jean-Maxime Côté** for agreeing to be interviewed. Much appreciation for **Malama Chisanga** and **Maryam Hojjat Jodaylami for volunteering to be our Data Official Manager and Sample Preparation Officers during the DTE.** Thanks to **Thibaut Babin** for his enthusiasm and electrochemical advice and to **Dominic Lauzon** for his guidance and for agreeing to synthesize our aptamer.

5.3 Sponsors and partners

We are thankful to **Millénium Québecor**, **ASEQ**, **Forces Avenir** and **LOJIQ** for their support and faith in our team. BioSensUM thrives because of their help. Special thanks also to **Anasens** for letting us use their equipment and laboratory spaces.

6. Final Remarks

As we look to the future, several key improvements and steps are planned for our biosensor. One of our primary goals is to incorporate cooling with the Peltier module and conduct further experiments to achieve more precise and consistent measurements. Consistency and reproducibility have been challenges, and addressing these issues will be a key focus.

Additionally, we plan to automate the syringe pump in conjunction with the Peltier module. This enhancement will make the system more efficient and reduce human variability. We are also considering integrating an improved algorithm for current calculation to minimize measurement variability. By optimizing this aspect, we aim to enhance the accuracy and reliability of our biosensor.

With more stable technology, we will be better positioned to advance towards miniaturizing the biosensor, with the goal of developing it into a wearable patch format. This would make the biosensor more practical for real-world applications, providing a convenient and portable solution for monitoring various biomarkers.

7. References

1. Dauphin-Ducharme, P. & Plaxco K. (2016). Maximizing the Signal Gain of Electrochemical-DNA Sensors, Analytical Chemistry, 88(23), 11654-11662 https://doi.org/10.1021/acs.analchem.6b03227
2. Zhang, Y., & Plaxco, K. W. (2012). Methylene blue as a reporter in DNA electrochemistry measurements. Langmuir, 28(17), 6938-6944. https://doi.org/10.1021/la300566x
3. Verrinder, E., Ransom, T., & Plaxco, K. (2024). Comparison of voltammetric methods used in the interrogation of electrochemical aptamer-based sensors. Sensors & Diagnostics, 3(1), 95-103. https://doi.org/10.1039/d3sd00083d
4. Ganguly, A., Paul, A., & Prasad, S. (2023). Pysanka-inspired electrode modification with aptamer encapsulation in ZIF-8 for urine creatinine electrochemical biosensing. Chemosensors, 11(11), 557. https://doi.org/10.3390/chemosensors11110557
5. Ganguly, A., Gunda, V., & Prasad, S. (2024). CreCENT: Creatinine and chloride based electrochemical non-faradaic renal health mapping technology. URINE, 6, 1-7. https://doi.org/10.1016/j.urine.2024.01.001
6. Markham, N. R., & Zuker, M. (2008). UNAFold: Software for nucleic acid folding and hybridization. Methods in Molecular Biology (Clifton, N.J.), 453, 3–31. https://doi.org/10.1007/978-1-60327-165-0_1
7. Gulaboski, R., Lovrić, M., Mirceski, V., Bogeski, I., & Hoth, M. (2008). A new rapid and simple method to determine the kinetics of electrode reactions of biologically relevant compounds from the half-peak width of the square-wave voltammograms. Biophysical Chemistry, 138(3), 130-136. https://doi.org/10.1016/j.bpc.2008.06.001
8. Chen, Z.-m., Wang, Y., Du, X.-y., Sun, J.-j., & Yang, S. (2021). Temperature-alternated electrochemical aptamer-based biosensor for calibration-free and sensitive molecular measurements in an unprocessed actual sample. Analytical Chemistry, 93(22), 7843-7850. https://doi.org/10.1021/acs.analchem.1c01540
9. Pan, J., Xu, W., Li, W., Chen, S., Dai, Y., Yu, S., Zhou, Q., & Xia, F. (2023). Electrochemical aptamer-based sensors with tunable detection range. Analytical Chemistry, 95(1), 420-432. https://doi.org/10.1021/acs.analchem.2c04147
10. Downs, A. M., Gerson, J., Leung, K. K., & others. (2022). Improved calibration of electrochemical aptamer-based sensors. Scientific Reports, 12, 5535. https://doi.org/10.1038/s41598-022-09589-5
11. Boyance, K. (2023). Why is kidney disease called the "silent killer"? Balboa Care. Retrieved from https://balboacare.com/why-is-kidney-disease-called-the-silent-killer/
12. Statista. (2022). Inpatient day hospital costs in the U.S. by nonprofit or profit. Retrieved from https://www.statista.com/statistics/630443/inpatient-day-hospital-costs-in-us-by-nonprofit-or-profit/
13. Prophecy Market Insights. (n.d.). Creatinine test market, size, trends, analysis and forecast till 2034. Retrieved from https://prophecymarketinsights.com/market_analysis/creatinine-test-market/

14. Data Research. (n.d.). Over 900,000 cardiac surgeries performed every year in the United States. Retrieved from https://idataresearch.com/over-900000-cardiac-surgeries-performed-every-year-in-the-united-states/
15. Downs, A. M., & Plaxco, K. W. (2022). Real-Time, In Vivo Molecular Monitoring Using Electrochemical Aptamer Based Sensors: Opportunities and Challenges. ACS sensors, 7(10), 2823–2832. https://doi.org/10.1021/acssensors.2c014281
16. Zhang, Y., & Plaxco, K. W. (2019). Electrochemical aptamer-based sensor for real-time monitoring of insulin. *ACS Sensors*, 4(2), 498–503. https://doi.org/10.1021/acssensors.8b01573
17. Pundir, C. S., Kumar, P., & Jaiwal, R. (2019). Biosensing methods for determination of creatinine: A review. *Biosensors and Bioelectronics, 126*, 707–724. https://doi.org/10.1016/j.bios.2018.11.031
18. Takeda, K., Kusuoka, R., Inukai, M., Igarashi, K., Ohno, H., & Nakamura, N. (2021). An amperometric biosensor of L-fucose in urine for the first screening test of cancer. *Biosensors and Bioelectronics, 174*, 112831. https://doi.org/10.1016/j.bios.2020.112831
19. Dong, Y., Liu, B., Hou, Z., Yang, J., Yin, X., Shi, Z., & Liu, H. (2022). A disposable printed amperometric biosensor for clinical evaluation of creatinine in renal function detection. *Talanta, 248*, 123592. https://doi.org/10.1016/j.talanta.2022.123592
20. Tombach, B., Schneider, J., Matzkies, F., Schaefer, R. M., & Chemnitius, G. C. (2001). Amperometric creatinine biosensor for hemodialysis patients. *Clinica Chimica Acta, 312*(1–2), 129–134.
21. Farid, S., Ghosh, S., Dutta, M., & Stroscio, M. A. (2023). Aptamer-based optical and electrochemical sensors: A review. *Chemosensors, 11*(12), 569. https://doi.org/10.3390/chemosensors11120569
22. Zahra, Q. U. A., Khan, Q. A., & Luo, Z. (2021). Advances in optical aptasensors for early detection and diagnosis of various cancer types. *Frontiers in Oncology, 11*, 632165. https://doi.org/10.3389/fonc.2021.632165
23. Divya, D., Dkhar, D. S., Kumari, R., Mahapatra, S., Kumar, R., & Chandra, P. (2022). Ultrasensitive aptasensors for the detection of viruses based on opto-electrochemical readout systems. *Biosensors, 12*(2), 81. https://doi.org/10.3390/bios12020081
24. Hernández, R., Vallés, C., Benito, A. M., Maser, W. K., Rius, F. X., & Riu, J. (2014). Graphene-based potentiometric biosensor for the immediate detection of living bacteria. *Biosensors and Bioelectronics, 54*, 553–557. https://doi.org/10.1016/j.bios.2013.11.053
25. Sung, D., & Koo, J. (2021). A review of BioFET's basic principles and materials for biomedical applications. *Biomedical Engineering Letters, 11*(2), 85–96. https://doi.org/10.1007/s13534-021-00187-8
26. Luo, L., Ye, F., Xu, Q., & Liu, H. (2009). Ion-sensitive field-effect transistor for biological sensing. *Sensors, 9*(9), 7111–7131. https://doi.org/10.3390/s90907111
27. Lasseter, T. L., Gifford, R., & Clark, H. A. (2019). Electrochemical aptamer-based sensor for real-time monitoring of insulin. *ACS Sensors, 4*(2), 498–503. https://doi.org/10.1021/acssensors.8b01573
28. Chen, H., & Chen, Q. (2021). Recent advances in potentiometric biosensing. *Current Opinion in Electrochemistry, 29*, 100735. https://doi.org/10.1016/j.coelec.2021.100735

29. Decher, G., Hong, S., & Schmitt, J. (2021). Biologically coupled gate field-effect transistors meet in vitro diagnostics. *ACS Omega, 6*(4), 2101–2110. https://doi.org/10.1021/acsomega.0c06229
30. Saddique, Z., Faheem, M., Habib, A., UlHasan, I., Mujahid, A., & Afzal, A. (2023). Electrochemical Creatinine (Bio)Sensors for Point-of-Care Diagnosis of Renal Malfunction and Chronic Kidney Disorders. *Diagnostics (Basel, Switzerland)*, *13*(10), 1737. https://doi.org/10.3390/diagnostics13101737
31. Chun-Yueh Huang (2015). Design of a Portable Potentiostat with Dual-microprocessors for Electrochemical Biosensors. Universal Journal of Electrical and Electronic Engineering, 3(6), 159 - 164. DOI: 10.13189/ujeee.2015.030601.
32. A. K. P. Souza, C. A. de Moraes Cruz, G. C. Marques, L. S. O. de Castro and T. B. Bezerra, "A Compact Current Conveyor CMOS Potentiostat Circuit for Electrochemical Sensors," *2019 4th International Symposium on Instrumentation Systems, Circuits and Transducers (INSCIT)*, Sao Paulo, Brazil, 2019, pp. 1-6
33. Liu, Y., Wang, H., Zhao, W., Zhang, M., Qin, H., & Xie, Y. (2018). Flexible, stretchable sensors for wearable health monitoring: Sensing mechanisms, materials, fabrication strategies and features. *Sensors, 18*(2), 645. https://doi.org/10.3390/s18020645

8. Appendix

8.1 Workflow diagram

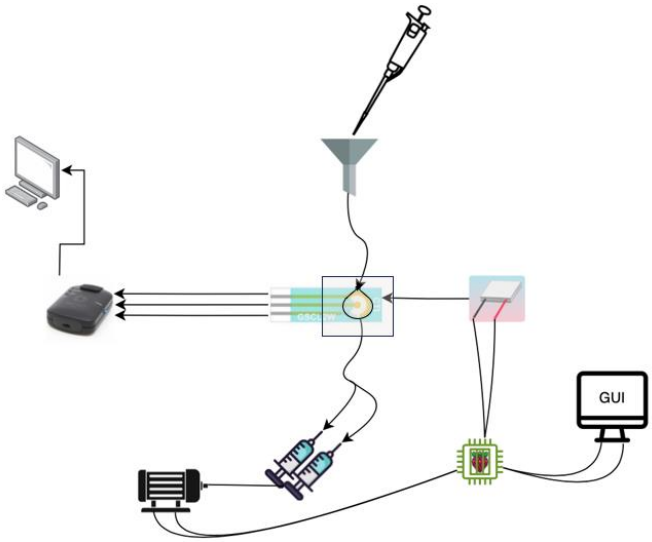
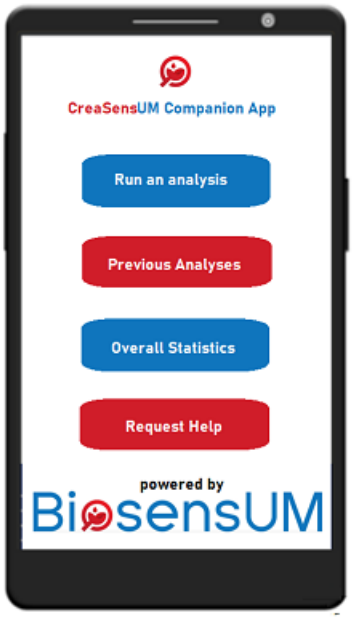


Figure 6. Biosensor workflow diagram, exposing relationships between the user, microfluidics and electronic components

8.2 User interface for companion App



.

Figure 7 Companion application graphical user interface prototype

8.3 Aptamer validation through fluorescence

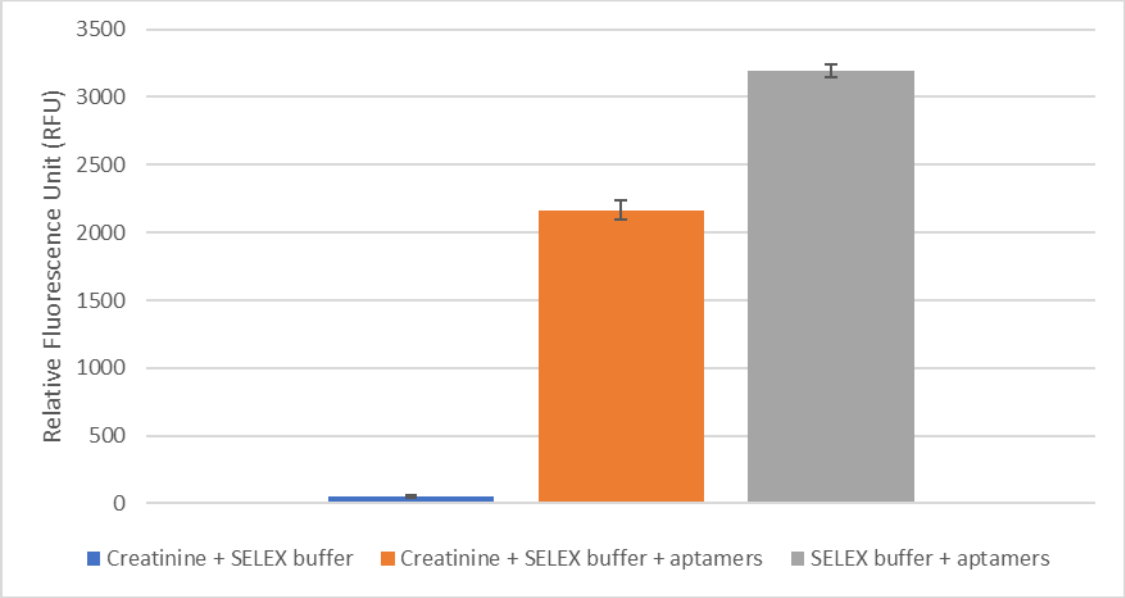


Figure 8. Fluorescence results for the BHQ 3' end and 6-FAM 5'end aptamer in SELEX buffer with and without creatinine.

8.2 Market segment viability analysis

Criteria	Market segment 1: Hospitals	Market segment 2: Pharmaceutical Companies	Market segment 3: Research
1) Is this segment economically attractive?	3	3	1
2) Is this segment accessible to your sales capacities(industry network, etc...)?	1	1	2
3) Does the target market have a convincing reason to buy your product/service?	3	2	2
4) Can you deliver a complete product for this segment?	3	3	3
5) Is there any competitors that could block you?	2	3	3
6) If you manage to « conquer » this segment, would you be able to use it to explore the other ones?	3	1	2
7) Is this segment aligned with you: your values, you personal objectives, etc...?	3	1	3
TOTAL	19	15	17

Which market segment will be your priority("Beachhead Market")? Hospitals

Figure 9. Market Segmentation Analysis

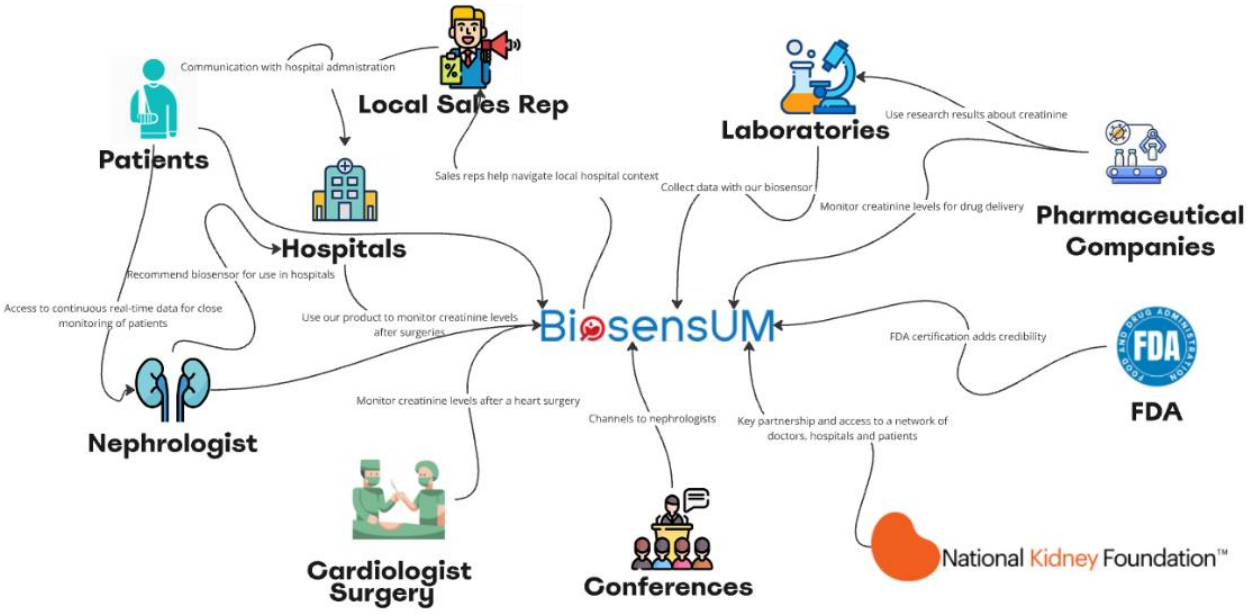


Figure 10: Key Stakeholder relationships and interactions

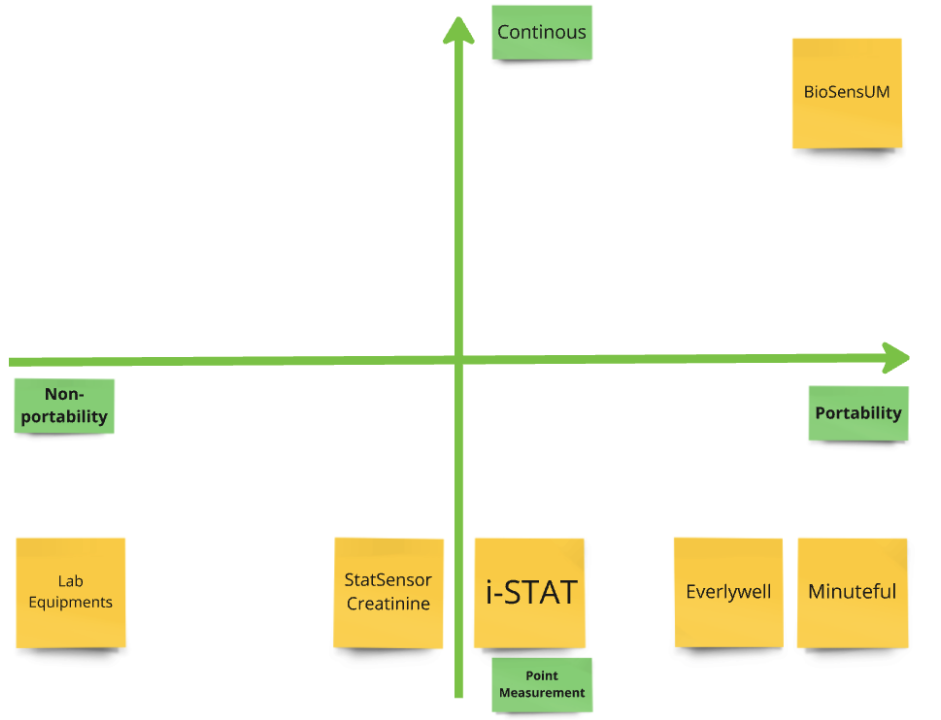


Figure 11: Competitive Analysis Framework

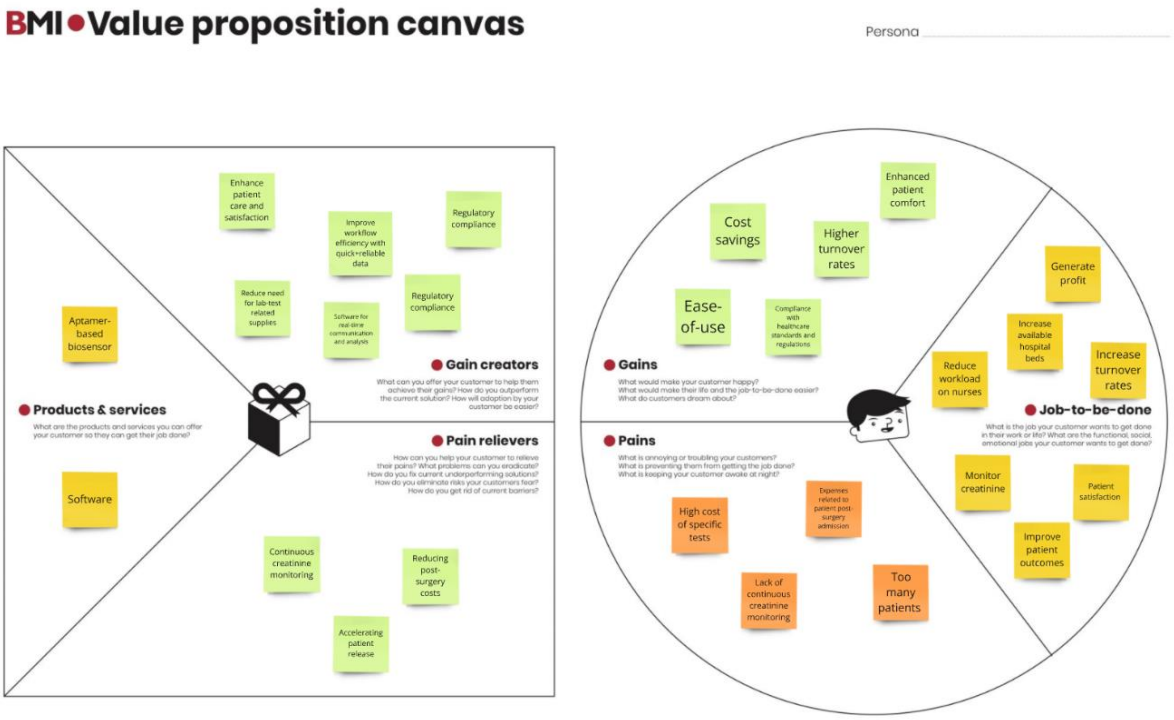


Figure 12: Value Proposition Canvas

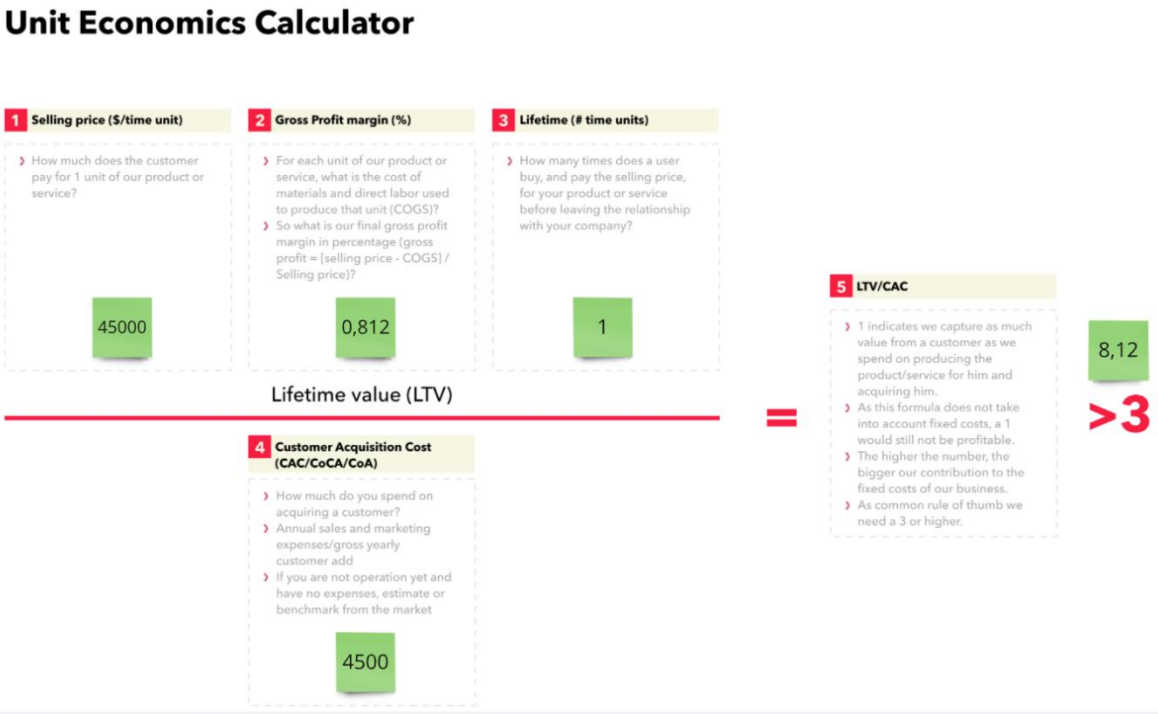


Fig 6:

Figure **13**: Unit Economic Calculator

Figure 14: Profit & Loss Statement

BioSensUM		Market Entry									
Profit and Loss (P&L) Statement		**2025**	**2026**	**2027**	**2028**	**2029**	**2030**	**2031**	**2032**	**2033**	**2034**
Expenses		365000	758000	770360	829360	964080	1014160	1357320	2008640	3186280	5470560
Expenses (+3% for uncertainty)		375950	780740	793470,8	854240,8	993002,4	1044584,8	1398039,6	2068899,2	3281868,4	5634676,8
CAPEX (Capital Expenses)		0	0	0	6750	20250	40500	81000	162000	324000	648000
	IP Rights(estimated 3% of sales)	0	0	0	6750	20250	40500	81000	162000	324000	648000
CoGS (Cost of Goods Sold)		0	0	360	29610	88830	177660	355320	710640	1421280	2842560
	Biosensor components	47	47	6	47	47	47	47	47	47	47
	# of biosensors (5% loss)	0	0	50	525	1575	3150	6300	12600	25200	50400
	Total components cost	0	0	300	24675	74025	148050	296100	592200	1184400	2368800
	Cost insurance and freight (CIF 20%)	0	0	60	4935	14805	29610	59220	118440	236880	473760
	Harmonized Tariff Schedule(HTF)	TBD	TBD	TBD	TBD	TBD	TBD	TBD	TBD	TBD	TBD
OPEX (Operating Expenses)		365000	758000	770000	793000	855000	796000	921000	1136000	1441000	1980000
	Licensing Fees	30000	0	0	0	0	0	0	0	0	0
	Contract manufacture	0	0	50000	0	0	0	0	0	0	0
	Sales Representative Margin(10%)	0	0	0	10000	30000	60000	120000	240000	480000	960000
	R&D budget	300000	300000	250000	250000	200000	50000	50000	50000	50000	50000
	Payroll	0	420000	420000	480000	540000	600000	660000	720000	780000	840000
	Marketing	5000	5000	10000	10000	30000	30000	30000	60000	60000	60000
	Number of employees	5	6	7	8	9	10	11	12	13	14
	Infrastructures per employee	5000	5000	5000	5000	5000	5000	5000	5000	5000	5000
	Infrastructures	25000	30000	35000	40000	45000	50000	55000	60000	65000	70000
	Consultancy	5000	0	5000	0	10000	0	0	0	0	0
	Audit	0	3000	0	3000	0	6000	6000	6000	6000	0
Profits		1018000	1000000	0	1225000	675000	1350000	2700000	5400000	10800000	21600000
Investors		1018000	1000000	0	1000000	0	0	0	0	0	0
	Millenium Quebecor	0	0	0	0	0	0	0	0	0	0
	Propolys	18000	0	0	0	0	0	0	0	0	0
	Venture Capitals	500000	1000000	0	0	0	0	0	0	0	0
	Angel investors	500000	0	0	0	0	0	0	0	0	0
Revenues		0	0	0	225000	675000	1350000	2700000	5400000	10800000	21600000
	# Device Sold	0	0	0	500	1500	3000	6000	12000	24000	48000
	Device Price	250	250	250	250	250	250	250	250	250	250
	# Software Suscription	0	0	0	5	15	30	60	120	240	480
	Software Fee	20000	20000	20000	20000	20000	20000	20000	20000	20000	20000
NET PROFIT ($)		642050	219260	-793470,8	370759,2	-318002,4	305415,2	1301960,4	3331100,8	7518131,6	15965323,2

CreaSensUM costs	
Massproduced chip with bluetooth	15
Microneedles with gold particles	20
Aptamer for Functionalization	1
Patch Assembly	11
Total Cost	**47**

Chip Costs	
ASIC Chip	2
Bluetooth module	5
Power Management	2
Antenna	1
Power Supply	5
Total Cost	**15**

Patch Materials & Assembly	
Flexible Sustrate	1
Adhesive Layer	1
Encapsulation	1
Automated SMT	8
Total Cost	**11**

Figure 15: Costs per Units in $US

8.3 Prototype and user information

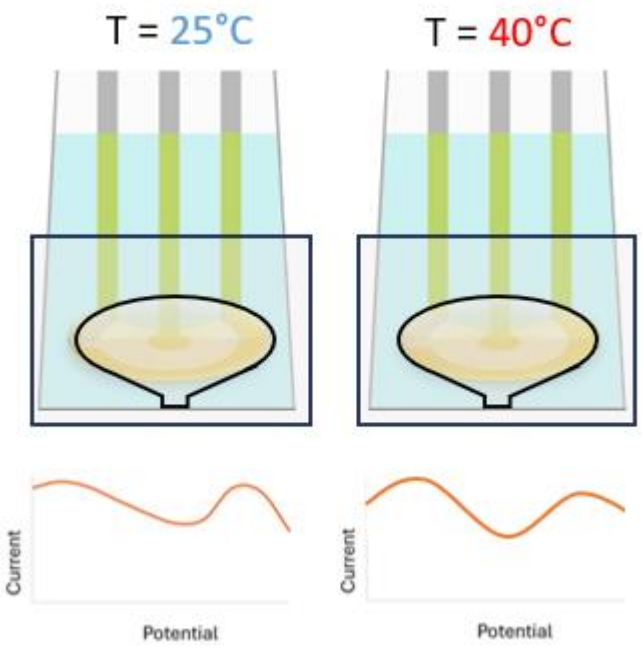


Figure 16. Electrochemical detection

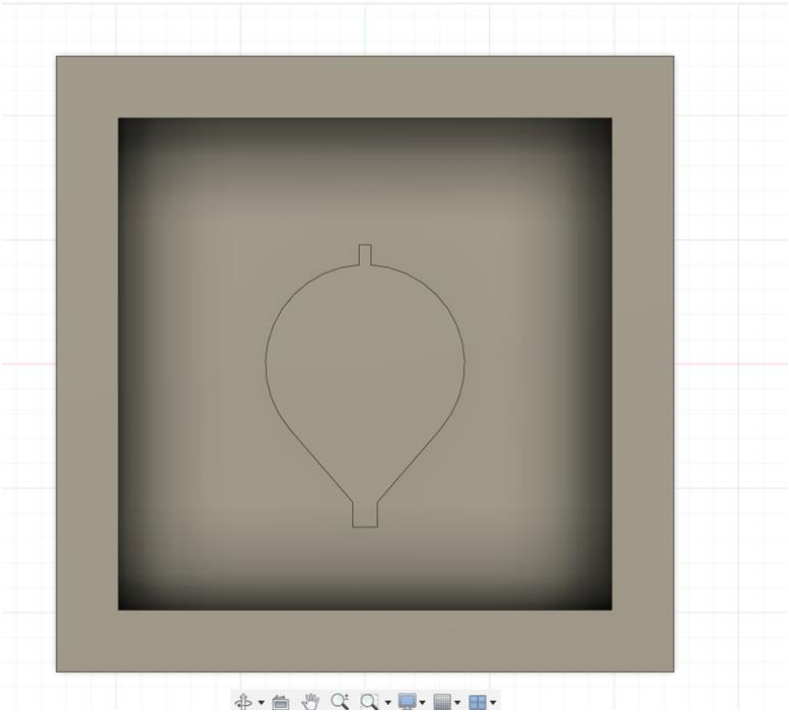


Figure 17. Microfluidic design currently used in our prototype

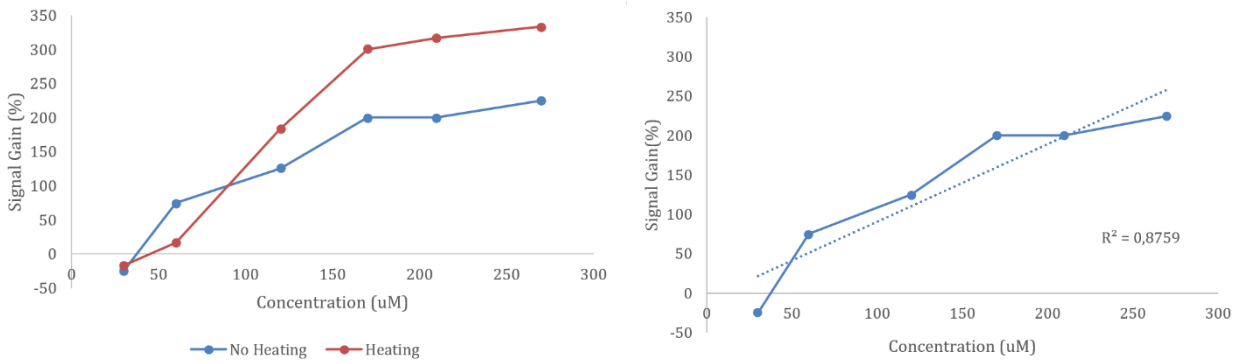
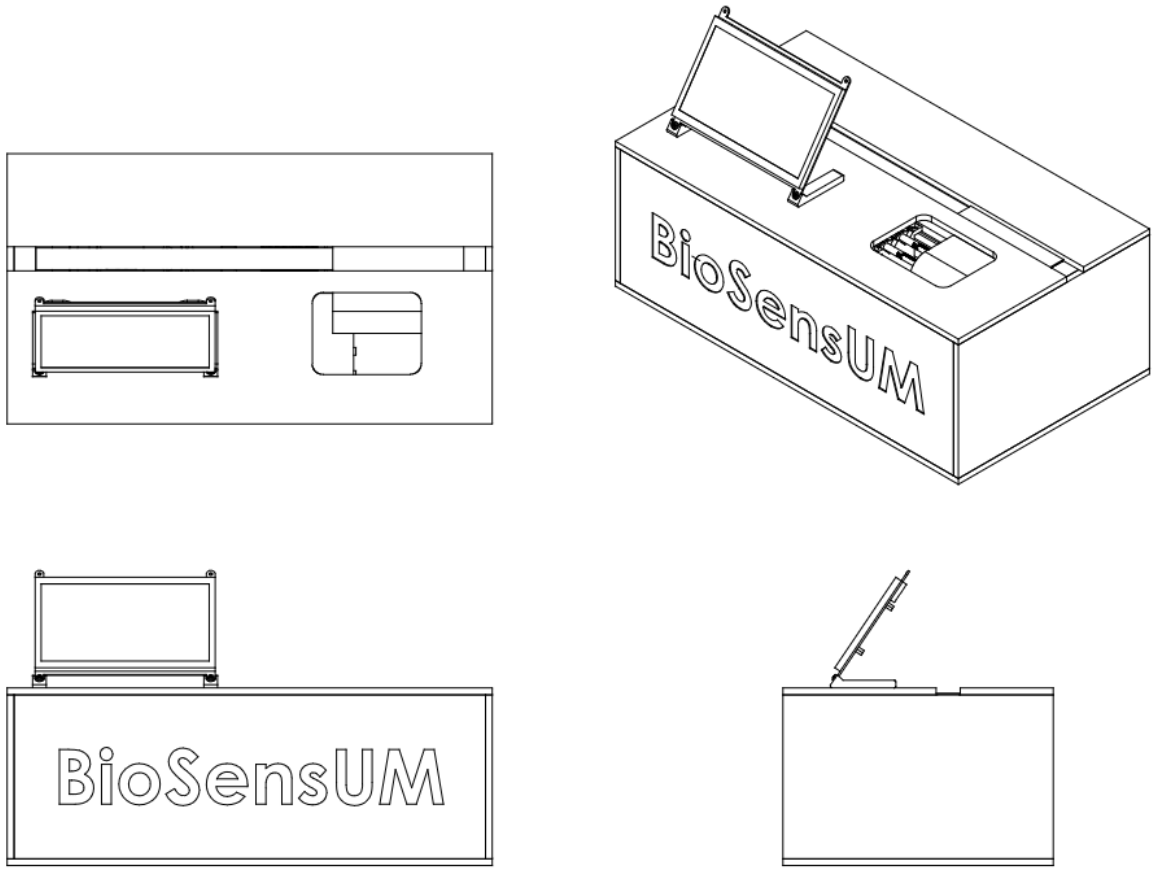


Figure 18. Curve of the concentration compaired to the signal gain with room temperature and 40 Degrees Celsius



SOLIDWORKS Educational Product. For Instructional Use Only.

Figure 19: Assembly drawing of the device with the box closed

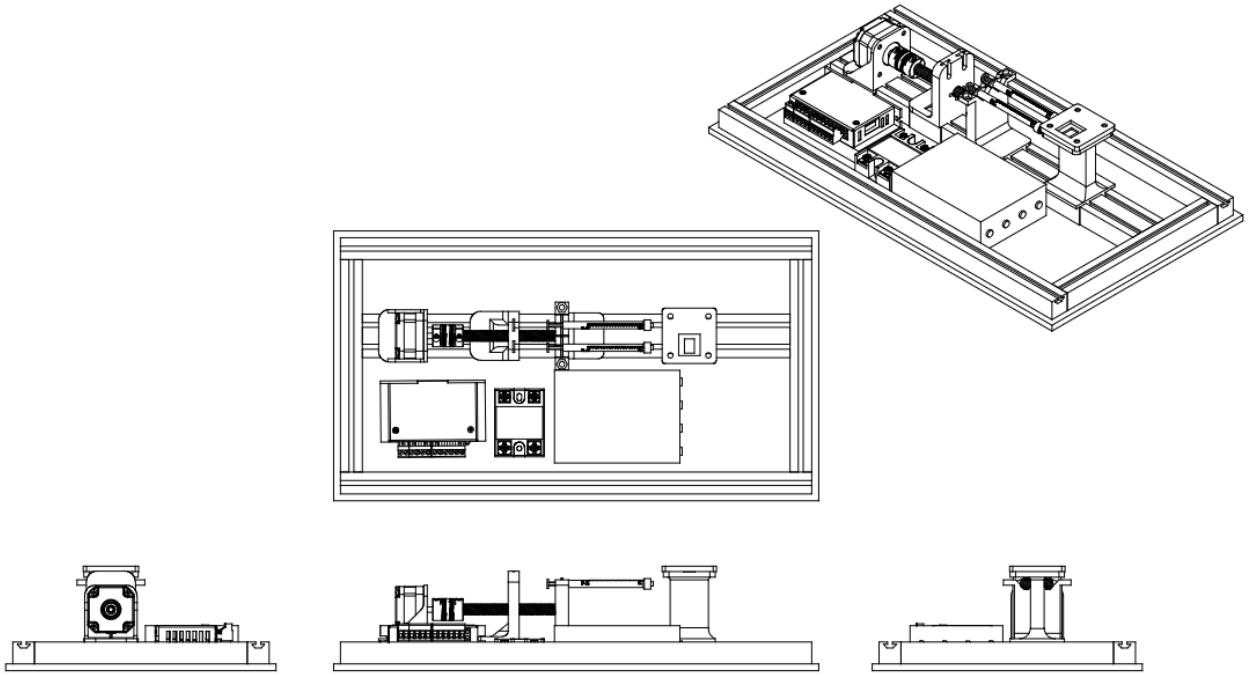


Figure 20: Assembly drawing of the device with the box open