Team Results Document

BiosensUM

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SensUs

Abstract

Acute kidney injury (AKI) is a medical condition affecting 13,3 million people per year and leads to 1,7 million deaths annually (Kung & Chou, 2023). This condition happens when the kidneys lose their main function and can't filter anything from the bloodstream causing the build-up of harmful levels of toxic metabolites (*Acute kidney injury*, 2024). To better control the evolution of AKI and prevent deaths, a solution could be to continuously monitor the concentration of creatine which is a small metabolite found in the body that can give precious information about kidney function. Unfortunately, there are currently no biosensors on the market that can accurately and effectively continuously measure creatinine levels (Han et al., 2024).

Our team conducted a market analysis through a series of interviews with a patient suffering with AKI, nephrologists, pharmacists and clinical biochemists. Based on their insights, we developed an electrochemical aptamer-based (E-AB) biosensor that can continuously detect creatinine in the interstitial fluid that can be collect in a non-invasive way. With the use of a novel functionalized gold wire electrode system, a 3D-printed peristaltic pump and an algorithm enabling calibration-free sensing, this soon-to-be wearable biosensor holds great promises for the future.

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1. Biosensor

1.1. Molecular recognition

The molecular recognition ability of our sensor is made possible by using structure-switching aptamers, short single-stranded DNA oligonucleotides that display a change in their conformational state when they bind to a specific molecule. The intensity of the conformational state change can then be detected with a proper method of transduction. Four specific sequences reported in literature to have a high affinity for creatinine were selected and synthesized via solid-phase oligonucleotide synthesis using the phosphoramidite method)(Stojanovic et al., 2017). Modifiers were introduced to enable functionalization at the 3' and the 5' extremities. We first used a fluorescent tag (FAM-3') with a quencher (5'-BHQ-1) for the screening process. In another instance a thioalkane chain (HS-C6-3') with an amino-modifier (5'-C6-NH2) were used for the assay of electrochemical transduction ability using a redox reporter on the 5'-end (Methylene Blue NHS ester). Their potential secondary structures and thermodynamic properties (Gibbs free energy, melting temperature or T_m) of the aptamer sequences were predicted with the mFold software (See Figure 10 in the *Appendix*). Furthermore, their conformational behaviour in the presence and absence of creatinine was investigated through fluorescence assays (see Figure 11 in the *Appendix*).

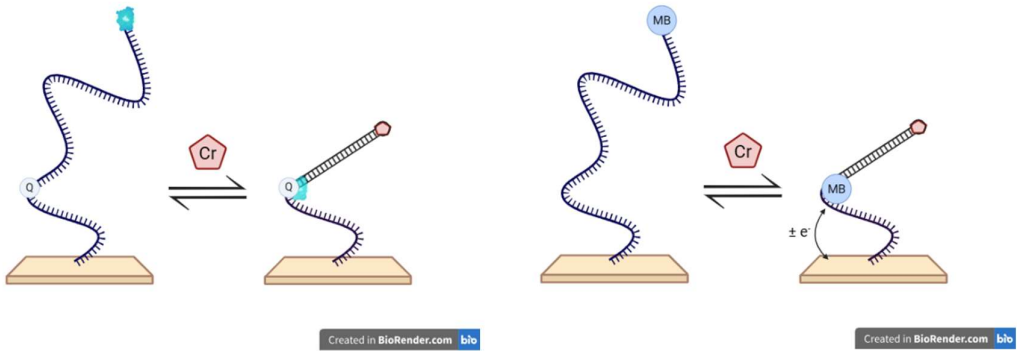


Figure 1. Schematic representation of fluorophore-functionalized and redox-labelled aptamer.

Several factors are related to those characteristics, such as the binding affinity, conformational changes, thermodynamic properties and structural characteristics. An increase of the melting temperature in presence of creatinine was hypothesized to be related to the formation of a complex between the aptamers and creatinine (Sakamoto et al., 2018)(see Figure 10 in Appendix). The sequences 256 and 288 providing both changes in relative fluorescence and increase in melting temperature in the presence of creatinine were considered fit for electrochemical applications and were subsequently synthesized

with a 5'-MB redox tag and 3'-C6-Thiol modification enabling functionalization of gold surfaces with those aptamers.

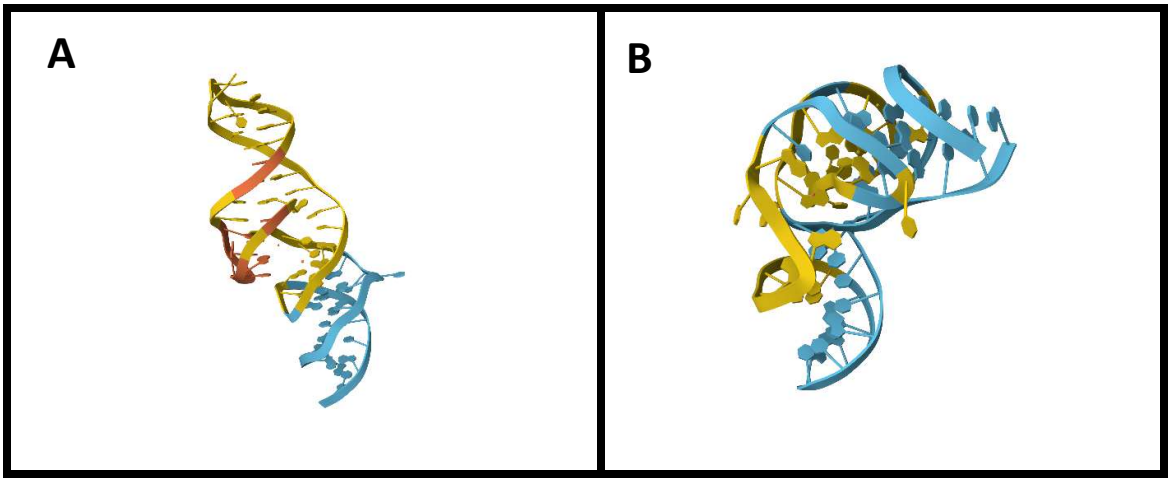


Figure 22. Potential tertiary structures of the aptamers 256 and 288.
The possible tertiary structures of the aptamers (A) 256 and (B) 288 in the presence of common body ions (Na^+, K^+, Mg^{2+}, Cl^-, Ca^{2+}) which were considered fit for electrochemical applications. All 3D structures were generated via the folding simulation of the AlphaFold Server from Google Deep mind.

1.2. Physical transduction

The biosensor relies on electrochemical processes to quantify the amount of creatinine present in the sample. The 5' end of the aptamer is bonded to a molecule of methylene blue (MB) while the 3' end is functionalized on the working electrode via a thiol-gold covalent bond. MB gets reduced when the working electrode is interrogated over a certain range of potential which then induces a current that can be measured. Our goal is to develop a detection method that results in a proportional relationship between the peak current measured at the reduction potential and the concentration of creatinine in the sample. As predicted by the AlphaFold Server, there is most likely a minor stem structure located at the extremities of the final chosen aptamer sequence (Apt#288). This suggests that MB is closer to the surface of the working electrode in the absence of creatinine. When the working electrode is interrogated by square-wave voltammetry (SWV), the absence of creatinine causes the highest peak. As the aptamer binds to creatine, it unfolds and the distance between the working electrode and the MB increases, which in turn decreases the rate of electron transfer associated with the reduction of MB. Consequently, the peak current decreases. This has been confirmed experimentally through electrochemical tests. Further testing was done to optimize the SWV parameters, namely the frequency and amplitude yielding the highest derivative of current variation with respect to creatinine concentration.

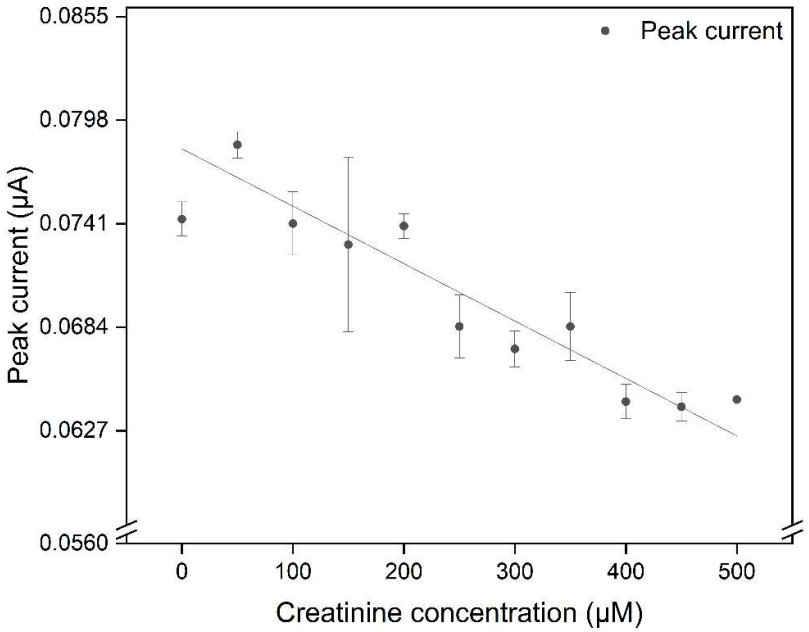


Figure 33. Variation of the peak current with the concentration of creatinine.
The working electrode was interrogated using SWV at a frequency of 290Hz and at an amplitude of 40mV. Each concentration was measured in triplicates. The means and the standard deviations were calculated and plotted against the concentrations of creatinine. The equation of the graph obtained is y = -3.16E-5 + 0.0.0782 with a R2 of 0.8661.

1.3. Cartridge technology

The cartridge is fixed directly on the plate on which the peristaltic pump is located. A silicone tube goes from the sample, through a peristaltic pump and to the microfluidic cell. To make sure proper collection is done, a needle is added at the beginning of the tube, which is then plucked into the sample. In further prototype versions, the tube will end with a microneedle, allowing for direct ISF collection.

The peristaltic pump used in this set-up is based on the design proposed by Ching et al., 2021, albeit some modifications were made to accommodate. The pump was printed using fused deposition modelling (FDM) with PLA filaments, as opposed to the aforementioned paper which used stereolithography (SLA) printing. This ensures low production cost while keeping the necessary sturdiness needed for this usage. Lengthen usage of the PLA-made pump has yet to be tested, but other alternatives have been considered if wear and tear becomes an issue. The pump is powered by a 28BYJ-48 stepper motor by GERRIT Electronics, which was incorporated in the set-up using custom-made 3D printed pieces. This motor offers similar performances for a lower cost compared to the one used in the previous paper. The microfluidic cell is also customized and made from PDMS (1:10 cross-linker to bulk). By switching between samples in the sample holder, different ISF samples can be inserted without any washing needed into the microfluidic cell, ensuring monitoring in a consecutive fashion. In the same vein, as the sample are introduced one after the other, the sensor can take measurements without having to stop at a given rate; 30 seconds in this current set-up. This ensures a continuous monitoring of the flow coming into the cell.

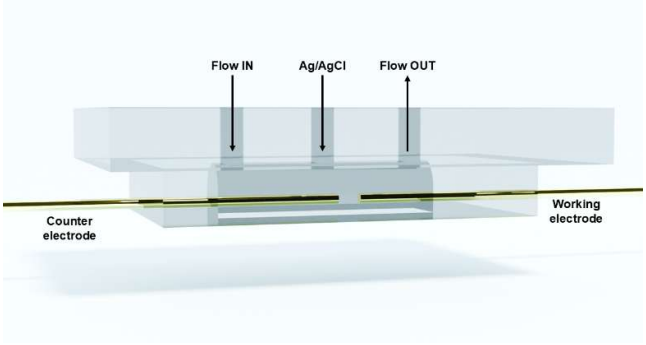


Figure 44. Schematic of the PDMS microfluidic cell and gold wires connections.

1.4. Reader instrument and user interaction

Our current prototype detection chain is composed of an electrochemical sensor allowing square wave voltammetry, we are for now using a *Sensit BT* device from *PalmSens*, and of a *Raspberry Pi* 3B+ chip allowing customisable data query, analysis, graphical display and pump control. Once ready, they must then activate the peristaltic pump by pressing the button directly on the interface of the device. Once the liquid has reached the microfluidic chamber, the pump is stopped, and the data query is sent to the Sensit device. All interactions with the *Raspberry Pi* system are made using a simple GUI displayed on our *SmartPi Touch* screen. Since the necessary queries are already written, the measurement process is straightforward. The data analysis is performed automatically by a custom python script which has been adapted iteratively to handle all voltametric reading we encountered (see *section 3.2* for more details). Therefore, the user is able to read the results directly on their device.

Concerning the dimensions, the main set-up including the pump, microfluidic, electrodes and the motor control board are on a support of 154 x 105mm. Connected to this main board, we have our *RaspberryPi* and touch screen interface as well as the *Sensit BT* device.

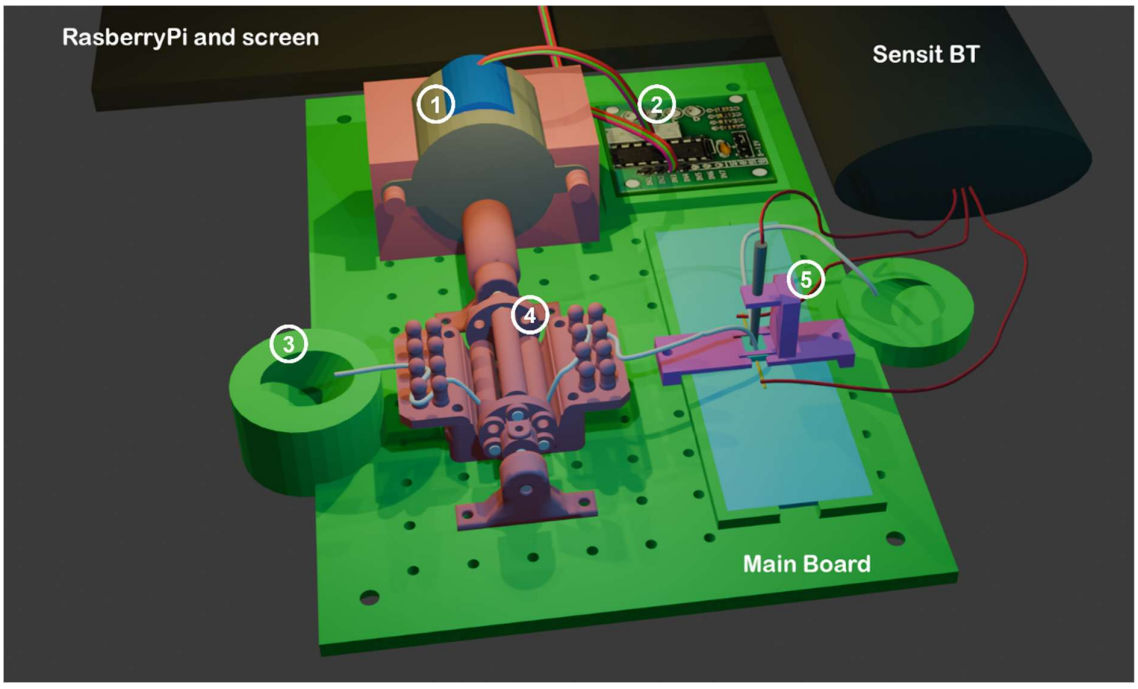


Figure 55. Schematic of all components put together.
(1) Stepper motor; (2) motor control board; (3) sample holder; (4) peristaltic pump; (5) cartridge holder and Ag/AgCl reference electrode.

2. Innovation

2.1. Wearable sensor

The key concept to achieve the wearability of a sensor is the miniaturisation of its technology. Our current prototype is equipped with a few innovative concepts that, once optimized could allow our technology to become wearable. First, the pump and data acquisition are controlled exclusively by the *RasberryPi*, which could be controlled by Bluetooth, hence allowing a potential wireless sensor. Given enough time, a simple cell phone application could control the entire process, allowing to get rid of the screen currently used that take a lot of space.

The *Sensit BT* device from *Palmsens* could also be reworked to take less space; the connections themselves in the shell of the device do not take a lot of space and could easily be fitted on top of the motor mother board. In further iterations, the implementation of a proprietary potentiostat chip, tailored to our needs, will accelerate miniaturization, reduce energy consumption and improve usability. Having this settled, it is also possible to produce all electronic components on the same board and using sample wire bonding to remove all excess crocodile clips and connectors.

From here, the only major thing we would have to reduce in size would be the peristaltic pump and a question needs to be asked: Should the wearable sensor follow the model of a fluid sample inserted into a microfluidic of reduced sized like we currently have or capillary or microneedle aptamer-based sensor? Microneedle aptamer functionalized used for continuous measurements have already been proved possible by Wu et al., 2022[)] using 3D printed poly(methyl methacrylate) materials in the shape of 1mm needles functionalized as references, working and counter electrodes. The needles shaped that way wan easily pierce the stratum corneum of the skin and access ISF for electronic sensing. Our team having access to printing equipment, such an innovation would be possible for the competition's iterations in the coming years.

The second key concept to achieve wearability is the capability of the system to take continuous measurements. Our electrochemical aptamer-based sensor is very similar to the one presented Swensen et al., 2009 who successfully measured real time detection of cocaine in continuously flowing serum. Using a multiport valve setup, they successfully detected the corresponding concentration relative to different input of serum in a short time period. Opening a valve of cocaine serum, the relative peak would be detected and after changing with a low concentration cocaine serum, the peak would disappear. The test could easily be replicated on our setup by adding a few valves and splitters to our current microfluidic. In our case, given our limited time, we assume that our setup will work in a similar way. Our sensor will be able to measure continuously and can be integrated into a wearable format.

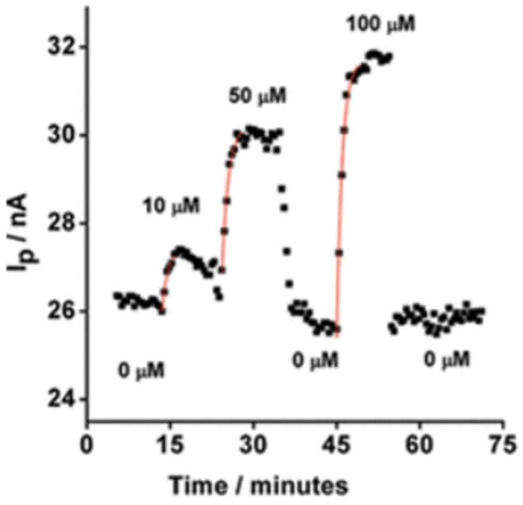


Figure 6. Real-time detection of cocaine in flowing serum.
The concentration is modulated over the course of the experiment. See article in reference [2].

2.1.1. Technological novelty of wearable sensor

In previous iterations of aptamer-based creatinine sensor designed by our team, we relied on screen-printed electrode on which microfluidic channels were seated directly. This prototype brings innovative ideas by relying on a functionalized gold wire as a working electrode, which increases the functionalized surface area in contact with the sample. This microfluidic design also allows for better flow through the recognition cell.

2.1.2. Technical feasibility of wearable sensor

Our sensor predictability is based on three parts : the feasibility of continuous measurement, the linearity between the biosensor's signal and the concentration of creatinine in a given sample, the accuracy and exactitude of the python code used for the voltammogram analysis. After the justification of the *section 3.1* we already know that the continuous measurement of our setup is well doable. Regarding the linearity of the signal with regards to the concentration of creatinine, the figure presented in *section 1.2* shows the linear trend between our signal and the concentration, with a R^2 value of 0.8661. Finally, the data processing algorithm guarantees the validity of the peak current value extracted

from the SWV measurements. This code was the same used for all our test, it proposes a fit of the voltammograms' data and a comparison between the minimum of the curve and the plateau induced by the baseline current. It is a well establish analysis method and very successful across each screened aptamer sequences.

2.2. Reliability of sensor output

Voltametric measurements can be inconsistent, presenting interfering signals, varying levels of measurement noise and artifacts which render some measurement unreliable. Our automated software handles this type of data and delivers consistent curve height extraction, rejecting or flagging abnormal data and providing a measure of data reliability based on iterations of experiments.

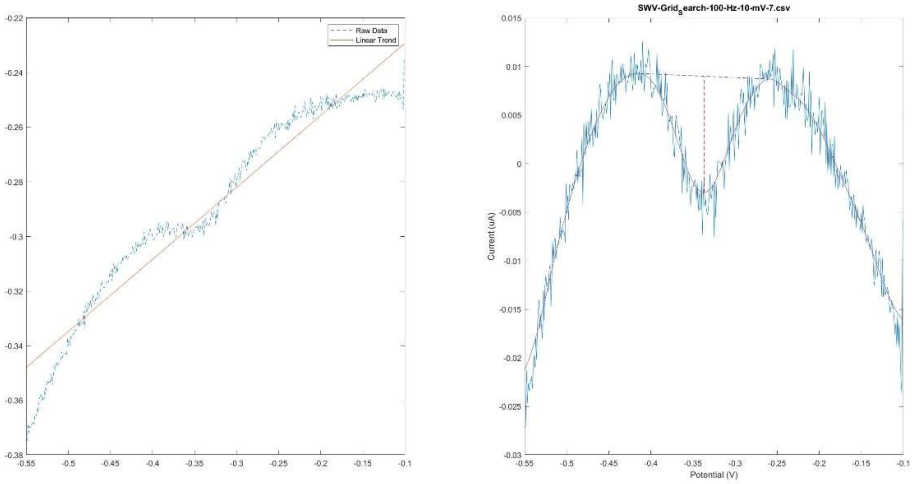


Figure 77. Software correction.

Software correcting for background signal by applying a 1st degree background correction automatically: (left) raw data and the background approximation, (right) background subtracted data, showing the curve calculated from smoothed data.

Specifically, the software performs the following steps : **(1)** linear background approximation and subtraction, **(2)** denoising using a gaussian filter, **(3)** curve detection using local extrema, resorting to inflection points if no suitable extrema are found, **(4)** data reliability approximation based on multiple factors including comparison of the peak height to the limit of detection, **(5)** output of results. The software generates a table which includes important metrics on the peak; specifically, peak height, position, width, and an approximation of data reliability. Reliability is based on iterations of experimental data and considers factors such as the height of the curve compared with the limit of detection.

In a recent study, Swensen et al., 2009 reported a novel technique for improving sensor measurements. They perform SWV measurements at two distinct frequencies, one which is responsive to the aptamer configuration and one that is non-responsive and acts a measurement of any other background variations (fig. 3B). This method is reliant on the physics of aptamer conformation which occurs in an equilibrium of folding and unfolding. The rate of binding state decay has a frequency at which it will result in the same current measurement regardless of analyte binding. This technique can correct for drift and device-to-device fabrication. Constants which are consistent across different setups can be calculated experimentally for a specific chemistry. These variables can then be used to calculate the analyte concentration according to the equation below (fig. 3A). We applied this technique to our sensor to enable reliable sensor results which are reproducible regardless of minor device fabrication variations.

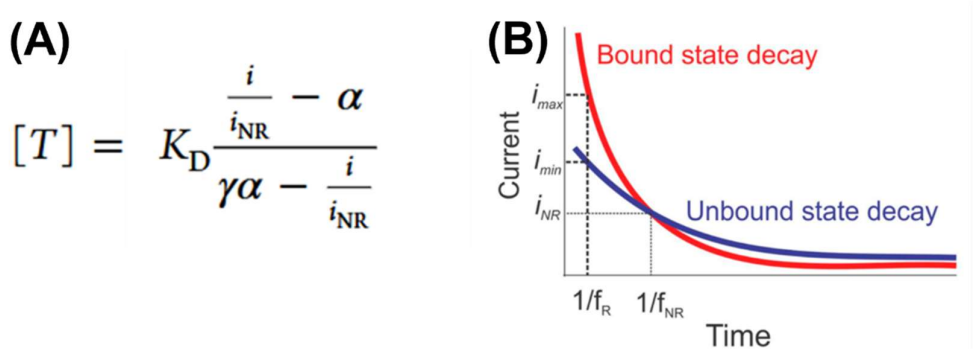


Figure 88. Calibration-free frequency normalization to improve sensor reliability.
(A) Relationship between concentration, constants, and measured responsive and non-responsive current. (B) illustration of the aptamer binding state release showing the phenomena that causes the non-responsive frequency to exist.

2.2.1. Technological novelty of reliability concept

Our sensor includes several novel aspects which contribute to measurement reliability. We applied the frequency normalization technique to a creatinine aptamer, which had not yet been done in literature to our knowledge.

Our data analysis software performs all curve property measurements automatically, which improves reproducibility by removing user variability. This software was written strictly for this purpose by a member of the team and is entirely novel, including many applications specific methods such as linear baseline correction and automatic anomaly detection (fig. 4.). This ensures that all calculated values are reliable and do not need to be manually parsed to verify fits.

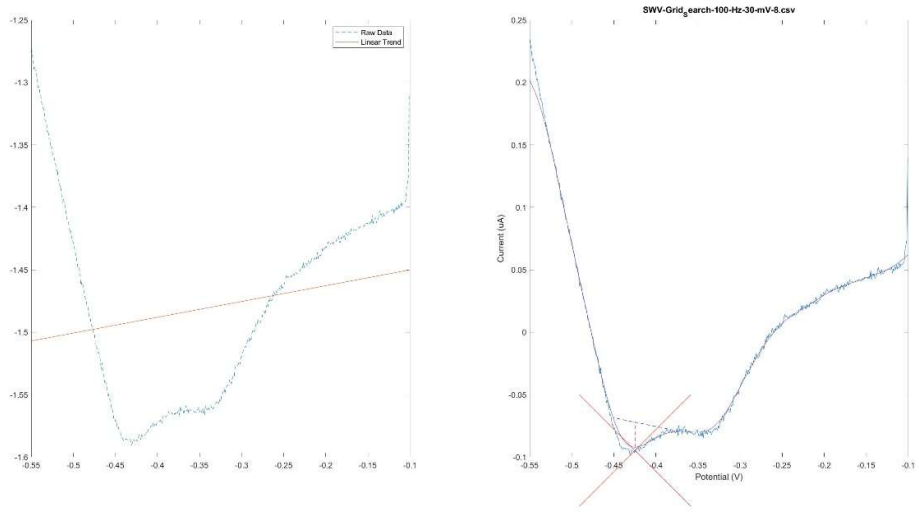


Figure 99. Automated curve analysis software rejecting anomalous data.
(Left) Linear baseline removal. (Right) Curve fitting and rejection.

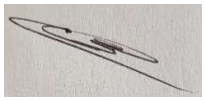
The software which controls our liquid pump has been calibrated to enable consistency in the liquid delivery. The sample enters the chamber at a fixed rate and is held for a fixed duration during the measurements, allowing reproducible measurements regardless of the volume placed at the inlet.

2.2.2. Technical feasibility of reliability concept

As seen in *section 2.2*, the exactitude of the creatinine concentration seen in ISF fall far below the required bar of 10% with a mean 0.002 of standard deviation. Furthermore, our aptamer was chosen within three potential candidates to optimize the free energy. In the appendix, we can see the 3 different aptamers selected with their different curves, the aptamer 288 has optimized the free Gibbs energy, thus being the one optimizing the electrochemical signal.

2.3. Original contributions

The team conceived the design of the microfluidic channel, the data processing algorithm and the integration of the technology. The aptamer sequences were extracted from publicly available literature and patents produced by people outside the team. Synthesis, functionalization, purification and quantification of those sequences were performed by members of the team, as well as the optimisation regarding gold wire functionalization and electrochemical parameters. The calibration algorithm was first reported in the public literature and adapted by the team members for the device and the use cases presented in the present document. The design of the potentiostat used in the proof-of-concept prototype belongs to PalmSens BV.



Jeanine Looman	Carl-Éric Gélinas	Jean-François Masson
Team Cocaptain	Team Cocaptain	Team Supervisor

3. Translation potential

3.1. Customer interviews

The development of our biosensor was made conjointly with the guidance of healthcare professionals who work with patients suffering from AKI, CKD, and diverse kidney conditions. Professionals from different areas of the healthcare system can offer different opinions on the matter; it was also important to talk to patients about the different steps of their journey. This is why we conducted interviews to be sure to design the best sensor possible, and for whom the sensor would be the most beneficial.

Doctors and clinical biochemists:

As what we are developing is a medical device, it was essential to get the opinions of nephrologists and clinical biochemists, and the possible applications. As we interviewed many nephrologists from Canada, France, and even Mauritius Island, we were first able to get a better understanding of the patient journey. Indeed, before diagnosing AKI, practitioners evaluate the patient's history especially prior creatinine levels to understand any rise. Urine or blood analysis and the monitoring of biomarkers like creatinine, potassium, and Cystatin C are also essential. During this process, the clinical biochemists estimate the glomerular filtration rate and notify the nephrologists when measuring critical values, indicative of kidney issues. A case of AKI is declared by the doctor when someone has an increase in serum creatinine levels by 0.3 mg/dl over 48 hours or by 50% considering the baseline levels of creatinine over seven days (*Acute Kidney Injury (AKI), 2025*). When informing a patient of a diagnosis, healthcare professionals prefer using simplified language to reduce anxiety. For treatment, home care can suit stable CKD patients with limited mobility, but not those with AKI who need close monitoring. Furthermore, when it comes to our biosensor, we heard healthcare professionals have various opinions about its possible application. Some argue that in the case of an AKI, such monitoring would be unnecessary as patients are already in the hospital, while other professionals commented that our biosensor could be useful in other medical fields such as oncology or radiography. Economically, neither nephrologists nor general practitioners are involved in the different costs related to a device that monitors creatinine. In Quebec, most medical expenses are covered by the *Régie d'assurance Maladie du Québec* (RAMQ) and private insurance companies without the intervention of the doctors. Finally, we concluded from our interviews with doctors and biochemists that such a biosensor could benefit healthcare professionals, but not in the way we first thought making us consider other markets.

Pharmacists:

Pharmacists are frontline actors in Canada's healthcare system, making their input essential. We understood that they play a key role in assessing their patients' current health situation pharmacists oversee follow-ups for patients who have glucose monitoring systems, for example, by communicating directly with MedTech companies to buy and sell biosensing devices; they also have a deep understanding of insurance coverage processes. Along with that, they prescribe biochemical evaluations such as creatinine levels to adjust medication dosages. Patients who have risk factors (high blood pressure, diabetes, cardiac problems, etc) are required to have their creatinine levels checked at least once per year (*Acute kidney injury, 2022*). Patients who are known for chronic kidney disease are tested more frequently, depending on the severity of their condition. However, the pharmacists reported that the timeframe for creatinine tests can lead to drastically different results in the 6 to 12-month span in which they get tested, leading to ICU admission for AKI. Therefore, some pharmacists argued that a point-of-care test, either continuous or discontinuous, could speed up treatment decisions, confirm if symptoms like fatigue or inability to urinate are kidney-related, and help guide patients to the right provider. To conclude, we perceived through those interviews how beneficial our biosensor would be, leading us to consider pharmacists as a strong potential market.

Patients

Finally, it was essential to understand the needs of the patients and their challenges. Alongside the difficulty to eliminate fluids, we understood that the major challenge for patients is the necessity to undergo a transplantation, as of today, it is the only solution to treat AKI. While not a cure, our biosensor could reduce stress related to AKI and improve quality of life. To summarize, these conversations confirmed that some patients would welcome such a tool, making them a relevant target market.

To conclude, as we considered the opinions of patients and healthcare professionals, we identified multiple potential customers among patients and healthcare professionals. In our opinion, the most viable plan is to sell the licence of our biosensing platform to companies for manufacturing and distribution; this way, it would provide us with a preexisting channel to reach out to target users, healthcare professionals, and patients. This strategy has many advantages, such as an easier entry into the market as well as reduced costs for manufacturing and marketing.

3.2. Design of validation study

Our wearable biosensor enables continuous monitoring of creatinine in human interstitial fluid. The device is a three-part system, namely the sample delivery mechanism (peristaltic pump and, in future iterations, microneedle arrays), the microfluidic and the electronic parts. The microneedles part, a third-party component, responsible for sampling the interstitial fluid of the user, will be connected to the microfluidic part where the aptamer and electrodes system will be housed. The electronic part will allow for the compilation of the measurement data and their transfer to external platforms. It will be detachable from the other components, as those will need to be replaced periodically. This device will be worn on the user's forearm and can be used to detect the early stages of an acute kidney injury (AKI) in high-risk patients for early intervention as well as to evaluate the nephrotoxicity of new or experimental drugs during clinical trials.

Figure 1010. BioSensUM's future wearable device.

In Canada, medical devices are legally classified into four classes (Branch, 2025). Based on the classification of the device, the requirements for its approval vary. For devices of classes I and II minimal to no testing is required before approval for commercialization. Whereas, for devices of class III and IV, extensive data and studies are necessary. A validation study will help us acquire the data and information requested by the regulatory bodies in Canada. We expect this device to be classified as a class III medical device due to its similarity in concept, method of use, and invasiveness to the already approved continuous glucose monitoring system of class III (Government of Canada, 2016). Due to the classification of the device, our validation study will be devised to start with an analytical validation phase then move on to clinical validation. The analytical portion of the validation will rely on in lab testing of human samples. It will specifically validate the reliability of the sensor data,

limit of detection of the device, correlation between serum and interstitial fluid creatinine levels, the accuracy of the device's measurements compared to the gold standard which is serum creatinine (SCr) assay using isotope dilution mass spectroscopy (IDMS) (Schwartz et al., 2009). After receiving the Investigational Testing authorization (ITA) from Health Canada, we will proceed to the clinical portion of the validation study where we address the impact of continuous creatinine testing using our device versus testing once every 7 days on the timely detection and treatment of AKI sensitivity (Oliveira et al., 2024). It will also address the specificity of the device for in vivo AKI monitoring, extended wear testing, interference testing, usability and comfort of the device. The study population will consist of 10 hospitalized patients, 10 at home high-risk patients and 10 patients in pharmaceutical trials of suspected highly nephrotoxic drugs, in order to prove statistical significance with current analytical gold standards in terms of creatinine detection. The patients admitted to the study must be adults (≥18 years old), who are either diagnosed with or at risk of an AKI due to surgery or use of known nephrotoxic drugs, undergo routine monitoring of their creatinine levels or participate in clinical trials involving new drugs. Exclusion criteria will include, the existence of skin conditions preventing sensor application of the device and the participation in concurrent clinical studies that could interfere with the evaluation of the biosensor's performance.

4. Team and support (max. 1 A4)

4.1. Contributions of the team members

This project would not have been possible without the support and participation of our entire team, starting with the co-captains. Carl-Eric Gélinas and Jeanine Looman excelled in leading the team and coordinating the sub-teams. While Carl-Eric was involved with the entrepreneurial and scientific side of the project, Jeanine was focused on the technological side of the sensor. She worked alongside Paul Vézina and Zackary Tardif – they developed the algorithm, the microfluidics, and the electronics of the sensor. They collaborated with the scientific team, primarily composed of Sai Ittoo, Sophie-Rose Boulanger, Dylan Ngoya, and Carl-Eric Gélinas. The scientific team was involved in the chemical development of the sensor, testing how every variable can change in the technology. Furthermore, this project would not exist without the financial and entrepreneurial team. Marc Al-Shikh, Axelle Drouard, and Aya Hussein were responsible for seeking funding, and developing a viable business model. Finally, it is very important to mention that even if each team member had the opportunity to get involved in the side of the project they wanted to explore, one could

and regularly did assist another team for a task. It is thanks to the dedication and passion that we were able to carry out this project.

4.2. People who have given support

During the journey of conception of the sensor, we received help from many coaches, teachers and professionals to guide us. Jean-François Masson, professor of the chemistry department in Université de Montréal, was of crucial help when it came to prioritizing tasks during the project and introducing us to biosensors. Our coach, Ryan Borotra – a previous SensUs contestant, who gave us precious advices on the do's and don'ts, especially for the entrepreneurial team. Finally, we would like to thank all the healthcare professionals that have given their precious time and wisdom about the state of acute kidney injury in the world: Dr Mark Lipman, Dr Vincent Deguire, Dre Elsa Vabret, Dr Lucien Rakoff, Dre Hugoline Boulay, Dre Véronique Beaunoyer, Dre Caroline Lamarche, Dr Thomas Weil, and the patients without who none of this would make sens. We would also like to thank Dominic Lauzon, Thibaut Babin Frédéric Leduc, Alexander Cunningham, Madline Savage, all the Anasens team, as well as all the members of the BiosensUM 2024 team for making this possible.

4.3. Sponsors and partners

We would like to thank our sponsors Millénium Québecor, Forces Avenirs, EduCoop for supporting us financially. We would also like to thank Université de Montréal, Polytechnique Montréal and the Institute for Research in Immunology and Cancer of the Université de Montréal for letting us use their campuses to develop the sensor. More specifically, we would like to thank the Biosensors and Nanomachine Laboratory of UdeM for the invaluable chance to have access to a high-quality lab for testing and optimizing our prototype.

5. Final remarks

Great challenges still lie ahead on the road towards continuous monitoring of vital organ functions. Biosensors, although being a new technology, may one day become the new standard approach at understanding human physiology in healthcare all around the world. We hope that our tiny contribution to this vast technological field will one day be the foundation for higher standards of living for the patients and their families.

We would like to thank the SensUs Organization and their collaborators, the Eindhoven University of Technology and Pr Menno Prins for this one-of-a-kind opportunity to tackle such a crucial, complex and important challenge that is developing a medical device as STEM students. This competition has been a unique learning experience giving us valuable insights into the development of biosensors, and we appreciate that we had this opportunity. We are infinitely grateful for the support and unwavering courage and passion SensUS has put into this that made it a reality.

6. References

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7. Appendix

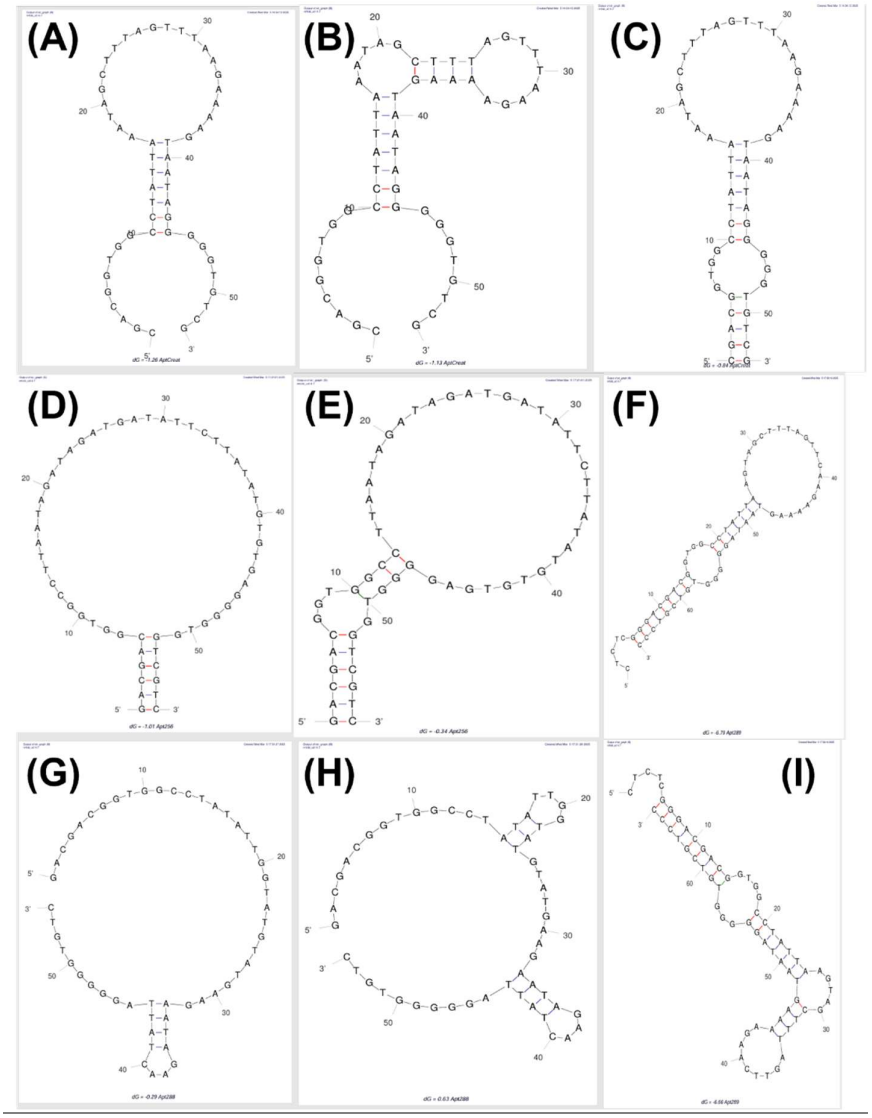

Figure 1111. *Mfold*-generated aptamer conformations.
These are the different secondary structures that the aptamers can take (Stojanovic et al., 2017). (A-C) refers to the sequence AptCreat; (D-E) refers to the sequence Apt256; (G-H) refers to the sequence Apt288; (F),(I) refers to the sequence Apt289

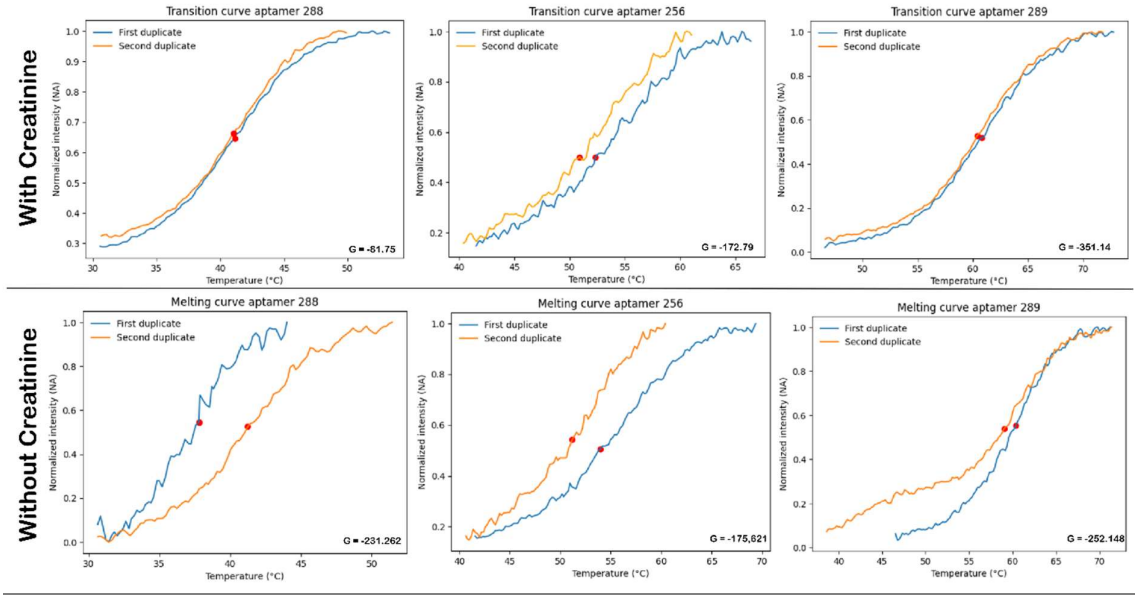

Figure 1212. Fluorimetry battery tests.

Those graphs were used to select the most stable aptamer candidate. From them, we could extract the following mid point as well as their respective Gibbs Free energy (G).

Aptamer 288 mid_point = 41.130, 0.646

Aptamer 256 mid_point = 50.910, 0.500

Aptamer 289 mid_point = 60.380, 0.529