Team Results Document

PariSens

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1. Abstract: Summary for the SensUs website

Our interdisciplinary team —students from ENS Paris-Saclay, CentraleSupélec, and AgroParisTech— took on the ambitious project of designing a continuous biosensor for creatinine, a kidney function key biomarker. We are divided into four specialized areas: electronics, biological experiments, microfluidics and entrepreneurship; we then brought together complementary expertises.

At the core of our biosensor lies a cascade of enzymatic reactions that converts creatinine into electrons, which are detected by a three-electrode system. The creatinine concentration can then be deduced from the electrochemical signal. We designed a custom printed circuit board (PCB) and developed a software interface that controls the devboard, performs measurements, and generates real-time graphs. In addition to that, we optimized the microfluidic system and characterized the enzymes.

Through rigorous teamwork, we are laying the groundwork for an innovative sensor for continuous kidney monitoring.

2. AP award: Biosensor developed for the Eindhoven Testing Event

2.1. Molecular recognition

The molecular recognition in our creatinine biosensor relies on a cascade of enzymatic reactions that convert creatinine into an electrochemically detectable compound (Fig.1 & Sup Fig.1).

The enzymatic sequence is as follow:

CAH: creatinine amidohydrolase

SOX: sarcosine oxidase

HRP: horseradish peroxidase

$$\text{Creatinine} \underset{\leftarrow}{\overset{\text{CAH}}{\longrightarrow}} \text{Creatine} + H_2O$$

$$\text{Creatine} + H_2O \underset{\leftarrow}{\overset{\text{creatinase}}{\longrightarrow}} \text{Sarcosine} + \text{Urea}$$

$$\text{Sarcosine} + O_2 + H_2O \underset{\leftarrow}{\overset{\text{SOX}}{\longrightarrow}} H_2O_2 + \text{Glycine} + \text{Formaldehyde}$$

$$H_2O_2 + \text{ABTS} \underset{\leftarrow}{\overset{\text{HRP}}{\longrightarrow}} 2\,H_2O + \text{ABTS}^{\bullet+}$$

***Figure 1*: Conversion of creatinine into H_2O_2 and electron production via peroxidase activity [1][2][3]**

H_2O_2 is the final enzymatic product. It generates electrons at the electrode surface through a redox reaction with ABTS [2][4].

To immobilize the enzymes and ensure their stability, we used chitosan hydrogel, which provides a biocompatible and porous matrix [5]. The enzymes are covalently crosslinked within this gel using glutaraldehyde, a commonly used

agent that forms stable connections between the enzymes and the chitosan. This process helps to maintain enzyme integrity while allowing diffusion of small molecules such as creatinine, creatine, sarcosine and H_2O_2 into the reaction zone.

This enzymatic cascade, thanks to crosslinked enzymes in the chitosan matrix, enables stable, specific, and sensitive detection of creatinine.

2.2. Physical transduction

We use electrochemical transduction to convert the biochemical (H_2O_2) into a measurable electrical signal. To do this, we use a three-electrode system: a gold working electrode, a gold counter-electrode and a Ag/AgCl coated gold reference electrode, all integrated on a customized printed circuit board (PCB).

At the end of the enzymatic cascade, the H_2O_2 produced is oxidized at the gold working electrode, generating a current from which we deduce the initial creatinine concentration. This electrochemical reaction is controlled by chronoamperometry [4][6][7] and cyclic voltammetry [4][8], via a dedicated software interface we have developed (Sup Fig.2).

The electrodes are made of gold, chosen for its high stability, conductivity and great electrochemical properties. As the electrode surface is already on a microscopic scale, sensitivity is sufficient without the need for nanostructures or nanoparticles. Gold provides a clean, reproducible signal with minimal background interference.

This approach guarantees accurate and reliable detection of H_2O_2, and therefore creatinine, without the need for additional signal amplification by nanomaterials.

2.3. Cartridge technology

The microfluidic architecture of our cartridge consists of (Fig.2 & Sup Fig.3):

1. An **inlet** for the entry of interstitial fluid (ISF),
2. A **narrow channel** guiding the fluid to the **detection zone**, where the enzyme-loaded hydrogel (chitosan with immobilized enzymes) is placed on the surface of the **three-electrode system**,
3. An **outlet** from which the analyzed interstitial fluid (ISF) exits, enabling recirculation.

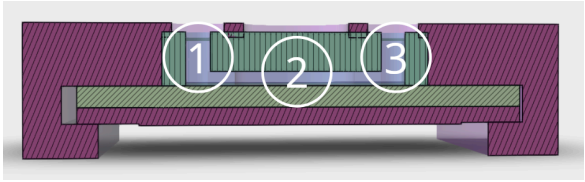


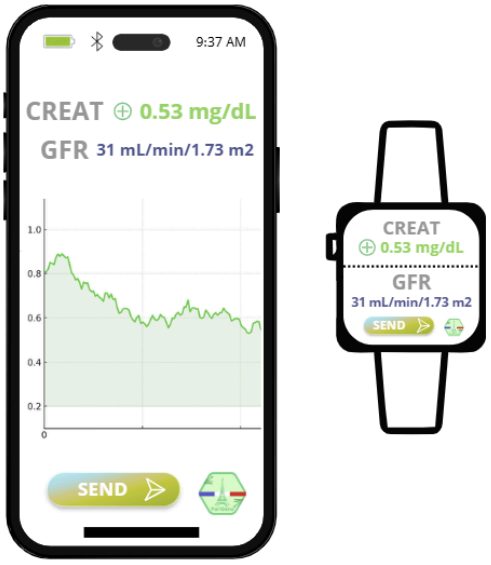
Figure 2 **: Microfluidic architecture of cartridge [9][10]**

The cartridge is therefore designed for **continuous sampling and detection** of creatinine from interstitial fluid (ISF). It features a **PDMS-based microfluidic chip** that channels the ISF through a reaction zone where enzymatic sensing occurs.

We designed our microfluidic system with a volume that is significantly inferior to the volume that we will have during the testing event. This allows the expulsion of the previous ISF Sample by the new ISF sample that also rinses off the narrow channel in which the detection of creatinine is happening.

2.4. Reader instrument and user interaction

Our biosensor, designed with our unique PCB (Sup Fig.6), can connect via bluetooth to smartphones and smartwatches [11]. These instruments serve as the reader instruments for both consumers and healthcare professionals, who could also use our website to manage their patients' data. The gel contained in the biosensor needs to be changed once every month by our team. This means the patient simply sends us the replaceable part of the sensor so we can reapply the gel. The patient only needs to connect their biosensor to their smartphone in order to keep track of their creatinine levels. At present, our biosensor measures 60 mm in length, 44 mm in width and 20 mm in height, which is not too invasive.



As you can see we want to design a customer-based interface in which patients decide what is shown in the app. In fact, they can decide whether or not they want figures and graphical data shown in their app or just a message that tells them if their creatinine levels are right. By using colors (green for healthy creatinine levels and red for not healthy ones) and figures (+ or -) the patient knows immediately how well their creatinine level is going (Fig.3).

***Figure 3*: Conceptual model of the customer-oriented interface**

3. IN award: Biosensor innovation

Regarding wearability, our team's objective is to reach that of market-available continuous glucose monitors. While working towards that goal, we thought of different ways to ensure that our prototype correctly embodies the ideas that we would like to further develop in regards to wearability.

3.1. Wearable sensor

The first aspect of our device that works towards maximizing its wearability potential is its size: spanning just a few square centimeters allows for it to be as little bothersome as possible (Fig.4.a).

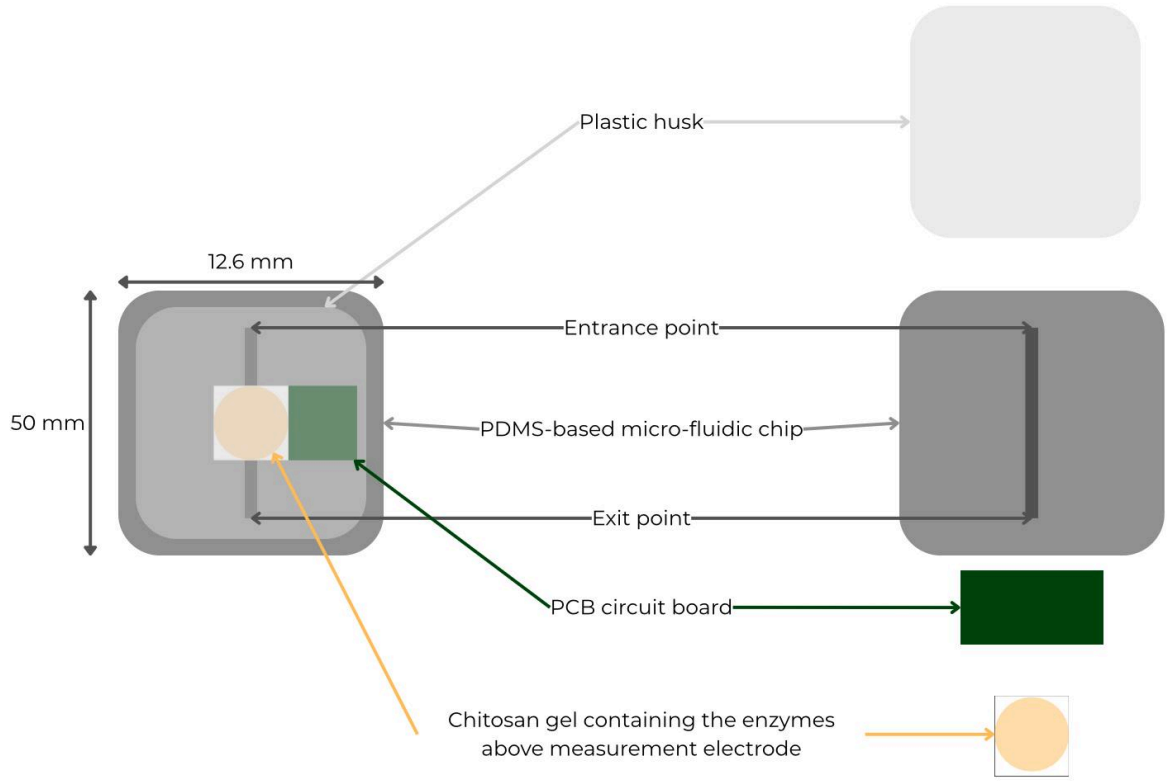


***Figure 4.a*: Portable sensor conceptual structure diagram, front view**

The second aspect was inspired by the design of most market-available continuous glucose monitors: our device uses a hypoallergenic adhesive patch to stick to the skin of the user, allowing for it to be worn casually for over a week.

The continuous measurement of creatinine levels is allowed by two thin filaments placed respectively at the entrance opening and at the exit opening of our device, allowing the interstitial fluid to circulate in a continuous flow inside our device, namely through the sensing zone, thus allowing for a continuous reading of creatinine levels (Fig.4.b).

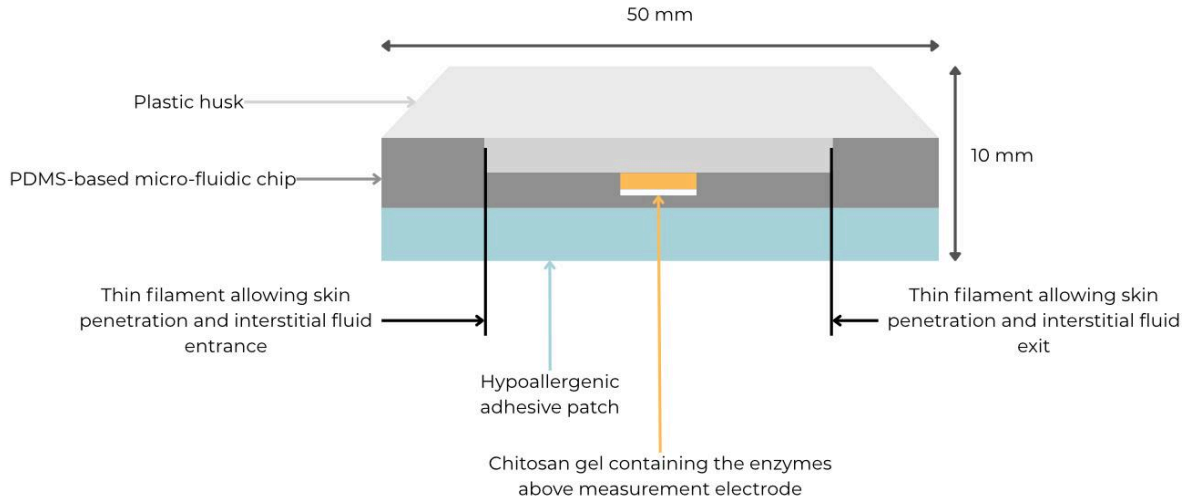


***Figure 4.b*: Portable sensor conceptual structure diagram, side view**

The entirety of the functional components are held inside a plastic husk, both for wearable practicality as well as for protection of these fragile components to accidental hits or harsh weather conditions. This includes the electronic components that allow the gathered readings to be sent via bluetooth to an app on the user's phone, thus making the task of keeping track of their creatinine levels much easier.

3.1.1. Technological novelty of wearable sensor

The main technological novelty behind our biosensor design rests in our chitosan gel, which we developed ourselves. Chitosan is a polysaccharide found in crustacean shells that has both antibacterial and filtration properties as well as being a gelling agent. Although gels made using only chitosan are not very resistant, we have taken inspiration from different sources to find a process that allows us to make a resistant chitosan-based gel that suits our needs.

Going beyond current known uses of chitosan gels in the biomedical field, we have developed, through thorough experimentation, our very own chitosan gel, perfectly suited to filter the interstitial fluid, hold the needed enzymes and allow for the creatinine, creatine, sarcosine and H_2O_2 to circulate freely. Indeed, most of our experimentation was angled towards finding the perfect combination of components to form a cohesive matrix that would still allow the circulation of small molecules while being a binding agent for our enzymes. Furthermore, our chitosan gel allows our sensing zone to work correctly at the same time as it protects our sensitive electrode from degradation.

3.1.2. Technical feasibility of wearable sensor

Although the different elements of our sensor have been tested separately, we have not yet managed to accurately test the technical feasibility of the whole

device: therein lies the most critical element in our opinion. The second most critical element is our experimental chitosan gel, since it is a product of our own making for which we can use little to no references. Our tests on its resistance to time and use yielded quite positive results.

However, we have not managed to accurately test the correct binding of our enzymes within the gel, which leaves a wide margin of improvement for our device.

Nonetheless, all the materials that we use for our biosensor such as the PCB, the electrode, the microfluidic circuit and the gel are all materials that could be easily miniaturised. Therefore, from all our components it could be quite easy to come up with a tiny biosensor that would be wearable for patients.

So, we would not be so bold as to say that our design and device are entirely feasible without further testing, but all of the elements that make up our biosensor have been heavily inspired by technologies that are already available and known, with the unique exception of our chitosan gel. Thus, it is our firm belief that with more time and testing, we could indeed reach our goal and fabricate the desired creatinine wearable and continuous biosensor.

3.2. Reliability of sensor output

While our device seems relatively simple, it holds multiple components and thus, multiple sources of variability and drift. We have thought of different ways to ensure maximum reliability from our sensor, but feel we still have a ways to go, especially since we can not have access to clinical trials.

3.2.1. Technological novelty of reliability concept

First of all, we use mathematical modeling of the chemical reactions happening within the chitosan gel to help tune our readings. It relies on a Physics-Informed Neural Network (PINN) which predicts the concentrations evolution of creatinine, creatine, sarcosine, H2O2 if the initial concentration of all relevant substances (creatinine, creatine, sarcosine, urea, glycine, formaldehyde, H2O2) and all four enzymes (creatinine amidohydrolase, creatininase, sarcosine oxidase and horseradish peroxidase) are given. We wrote several python scripts using reaction kinetics theory. This allowed us to have a basis to compare our results (Sup Fig.4) to and to adapt the mathematical model if needed.

Even though it works with approximative kinetic values, the more precise the used values are, the better the results. In order to achieve that, we used several references [12][13][14] and also tested the kinetic values (Sup Fig.5a & Sup Fig.5b) of the enzymes ourselves. Using this model, we can determine the best quantity and relative proportions of enzymes to be used in each gel to maximise the signal, thus improving the reliability of the measured creatinine concentration. It is indeed important that the enzyme concentrations do not limit the speed at

which the creatinine is converted into H2O2. However, using too many enzymes would result in higher cost as they are expensive. Striking the right balance is necessary to ensure reliable measures and affordable prices.

Second, although this year we chose to simplify the microfluidic circuit, last year our team presented a three-branch microfluidic circuit. This was meant to allow for a more precise reading of creatinine levels because it subtracted creatine level readings to total readings, thus taking away a source of error and maximizing reliability in the real use of our biosensor because creatine is found in our own interstitial fluids (Fig.5).

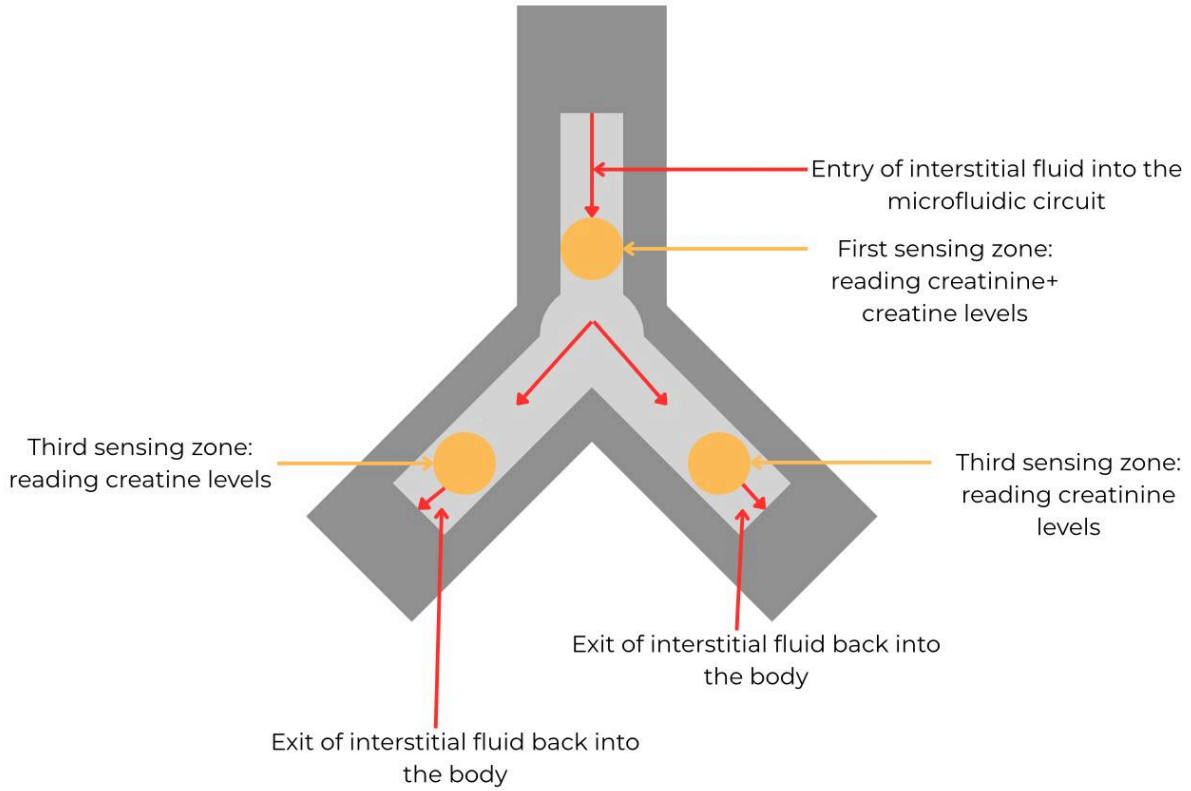


***Figure 5*: Schematic diagram of the microfluidic system structure**

Finally, as we explained in paragraph 3.1.2, the stability of enzymes in the chitosan gel is one of our most critical elements. While resistance tests have yielded good results, it is not fit to be used for long periods of time. To ensure readings as reliable as possible, it is important that the user changes the gel approximately every week. We have thought of a system to make this process of maintenance as easy as possible for the user: we shall fabricate two biosensors for each client, one to be worn first and then, when it is time to change the gel, one to be sent over to the customer while the first one is sent to us to have its chitosan gel changed. This rotation system allows us to avoid the extra cost of building a whole new device every time the gel has to be changed. It also lessens the user's responsibilities as much as possible.

3.2.2. Technical feasibility of reliability concept

As previously said the most critical part in the design of our biosensor is the chitosan gel including our enzymes. In fact the gel is not able to be preserved during long time periods. This makes the reliability of our wearable biosensor quite difficult. That's why we thought of the solution mentioned below in which the patient returns back the biosensor for us to change the gel while we provide him with another biosensor with fresh gel.

Also, we use enzymes in our biosensor, which are living materials so their life-spent is indeed a critical part of our biosensor. However we have conducted tests regarding the stability of the enzymes in gel that were really positive and allowed us to think that their life-spent is as long as the gel one. So this leads to the same strategy to tackle this problem as the gel.

The feasibility of the reliability of our biosensor will also be improved by the use by the patients. In fact, our PINN is trained by live data and gives best results with lots of data. So the network is a huge plus for improving our reliability.

So, to date, the feasibility of the reliability is still needing some improvement even though we have thought of some ways to tackle these problems.

3.3. Original contributions

Piece written by the team:

- Who conceived the novel ideas ?

As our team is composed of multiple units, we bring different expertises that allows us to conceive new ideas. Even though the enzymatic cascade was conceived by the members of last year's team, we aimed, this year, to characterise furthermore the characteristics of enzymes in our conditions (chitosan gel, microfluidic circuit). So the team helped to conceive a very unique enzymatic combo with adapted proportions giving us the best signal possible (we determined the best proportion with a python code). The microfluidic unit was conceived by some team members. And, the PCB and the electrodes allowing the transduction of the chemical signal were put together by our team.

- Who selected the novel ideas ?

The validation and the selection of this novel ideas were realised in collaboration between our team and some people outside the team. As a matter of fact, the novel idea regarding the enzymatic cascade was selected by the team and our supervisor that has expertise in this field. For the microfluidic unit, the validation process was realised by the team and a researcher that is outside the team. Finally, regarding the electronic system, the PCB part was selected by members

of the teal and the electrode part was validated by the team and someone else out of our team.

- Who adjusted the ideas ?

The protocols that we used to test our novel ideas were fully realised by the team and then corrected and adjusted by our team supervisor and other people that are specialised in microfluidic and electronics.

- Who scientifically tested the idea ?

All the ideas were tested by the team in labs under the supervision of our team supervisor.


<u>Piece written independently by the team's supervisor:</u>

- Who conceived the novel ideas ?

As part of the team is made up of students who were involved in the previous SensUs project (2024), the team decided to continue developing the prototype developed last year. However, this year the team undertook to further characterize the enzymatic activities in liquid medium and then on chitosan gel. The aim of these characterizations is to evaluate the stability of the enzymes in the gel and to generate a predictive mathematical model of the functioning of the cascade of enzymatic reactions. The new microfluidic circuit concept was devised by the team. The electronic board and data acquisition software were designed by the students in the team's electronics division.

- Who selected the novel ideas ?

The feasibility of the new ideas was assessed by the team through discussions with someone outside the team who had expertise in the targeted field (enzymology / microfluidics / electronics). As part of the assessment of enzymatic activities, the selection was made in consultation with the team supervisor. The validation of the microfluidic circuit idea and the validation of concepts related to electronics (electrode and signal integration) were carried out in collaboration with someone outside the team.

- Who adjusted the ideas ?

The experimental protocols were entirely conceptualized by the team and then adjusted following discussions with the supervisor for enzymology and with someone outside the team for microfluidic and electronic aspects.

- Who scientifically tested the idea ?

All experiments and tests were carried out by the team under the supervision of the team supervisor or someone outside the team.

4. TP award: Translation potential

4.1. Customer interviews

Daniel VASMANT (Rénif)

As part of our project, we had the opportunity to meet Dr. Daniel VASMANT, a nephrologist and coordinating physician at Rénif, an association that brings together both healthcare professionals and patients. Our discussion with Dr. Vasmant allowed us to assess the clinical relevance of our continuous monitoring biosensor in a variety of medical scenarios. He highlighted its potential value in intensive care units, particularly following cardiac surgery ; a procedure during which kidney function can be compromised due to reduced perfusion. He also pointed out the device's usefulness in post-transplant monitoring, where it could help detect early signs of complications or emerging renal dysfunction. Moreover, Dr. VASMANT expressed strong interest in the application of our biosensor for patients with end-stage renal disease (stage 5). He emphasized that such a tool could reduce unnecessary hospital visits by enabling medical teams to recall patients only when creatinine levels indicate the need for intervention. Also he expressed his concerns about making clear data accessible to some patients that could feel anxious about it.

In conclusion, this interview helped us to focus on very specific situations during which our biosensor could really help patients. The fact that Daniel VASMANT is a nephrologist and has a strong experience in hospitals made us identify clinical ways to use our biosensor. Even though we are more focused on the use of our biosensor from home, this interview opened up our minds to other ways to make our product useful.

Jean-Marc CHARREL (France Rein)

We also had the opportunity to discuss with Jean-Marc CHARREL, the leader of France Rein which is the leading association of patients and nephrologists in France. Jean-Marc CHARREL is also diagnosed with kidney failure which made our meeting with him really interesting because he has a close view on the challenges faced by both patients and health care professionals regarding kidney failure. This interview really helped us understand where we should go with our sensor

because France Rein could be one of our clients and Jean-Marc CHARREL a user of our biosensor. He was very enthusiastic about our project because it is really innovative and less costly than other detection tools of creatinine levels. However, he seemed to be not a big fan of the continuous part of the biosensor because he thought that it would have less impact than an easy-to-use and fast sensor that detects creatinine levels during screening tests. Since the development of a continuous biosensor is a requirement, we focused our discussion with him on the added value of the continuous monitoring aspect. He acknowledged that it could be quite useful after transplants to make sure it went well. Also, we were agreeing on the fact that it could be a useful tool to make sure that early stages of kidney failure do not deteriorate. But Jean-Marc CHARREL also pointed out the fact that creatinine levels do not really fluctuate in an hour or so. So we understood that our biosensor could be a very versatile tool for every person suffering or in risk of suffering from kidney failure. Indeed, we could use it as a less invasive tool to detect creatinine levels during screening tests (which does not really use the continuous part of the biosensor) and also as a tool to monitor creatinine levels after transplant or in very specific cases that require a huge surveillance of creatinine levels. We finally conclude on the fact that our biosensor could be really useful for research because we could know the influence of parameters (such as nutrition, sport, age...) on creatinine levels.

In conclusion, this interview was really enriching because we understood the needs of French patients regarding the monitoring of creatinine levels. Jean-Marc CHARREL showed genuine enthusiasm for our concepts and expressed strong interest in collaborating during the week of kidney that takes place every year during which screening tests are running in Paris. Thus, this interview gave us valuable insights into the kidney health market and helped us identify concrete opportunities for the commercialization and deployment of our prototype.

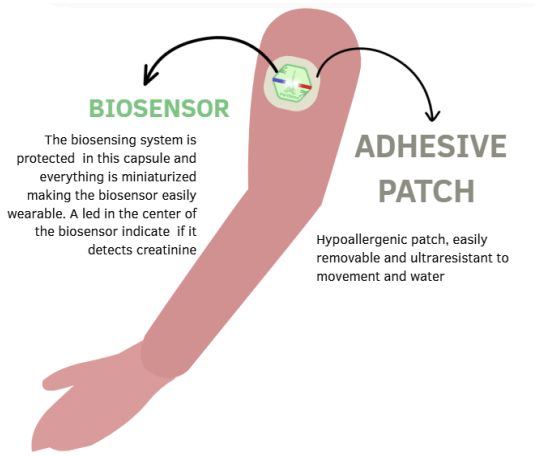
4.2. Design of validation study

When talking about our conceptual prototype we immediately said that we wanted a biosensor for and by the patients. In fact, we need to have a sensor centered around what we identified as the three big needs of patients : Simplicity, Reliability and Connectivity.

First reliability. We want to provide a 80 % accuracy for our results assuring a good enough precision for the sensing of creatinine. Also, we want the patients to have control over their condition by using this healthcare device from home in a secure place without the need of a healthcare professional. And finally we present to the patients multiple data in the interface such as the creatinine level and also the Glomerular Filtration Rate (GFR) which is a key figure in managing kidney failure.

Next connectivity. The biosensor is connected thanks to our PCB designed with a chip that is able to connect the sensor with the patient's smartphone or connected watch. Therefore the patients can have easy access to their creatinine

levels whenever they want. Moreover, the data collected by the sensor can be easily sent to health professionals and also to relatives in order to keep track of the health of our patient.



Finally, simplicity. We bring to the patient a very modular interface designed to adapt to their needs. In fact some want to have access to their data with lots of figures while others only want a simple message that lets them know if their creatinine levels are healthy or not. We also designed the interface with clear indicators to make it simple for patients. And, our biosensor is designed like a patch that just needs to be put on the arm making everyday life possible while monitoring creatinine.

Figure 6 **: Diagram of the conceptual representation of our wearable sensor, in the form of a patch**

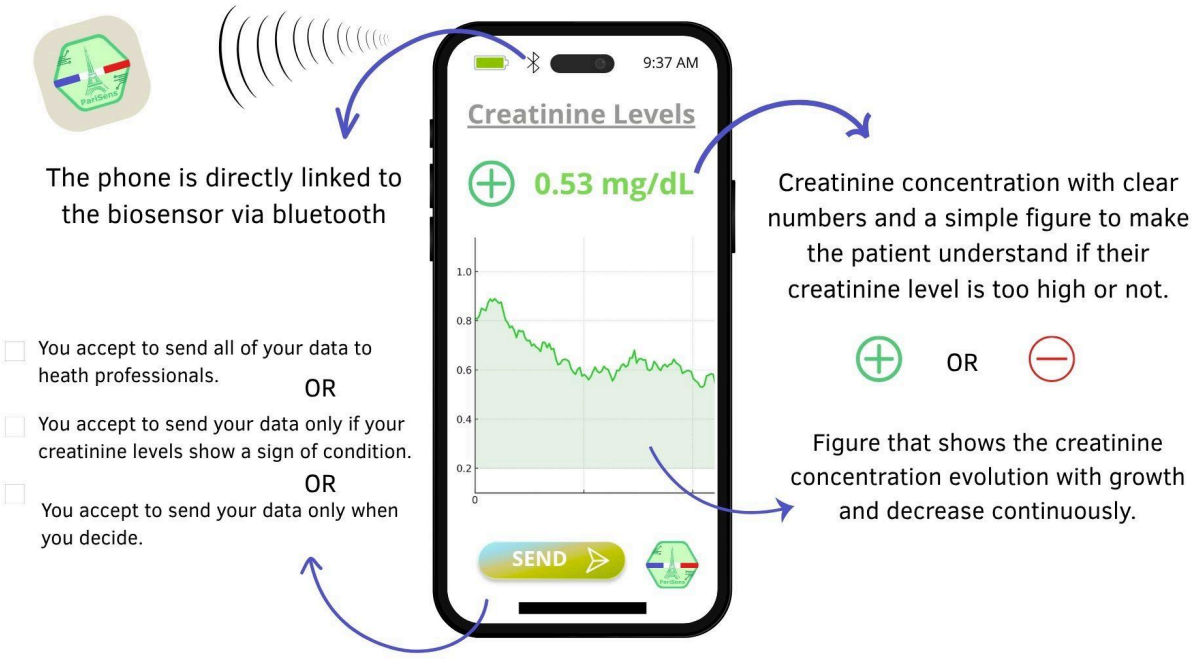


Figure 7 **: Schematic of the conceptual prototype of the smartphone patient interface**

We designed our prototype with an adhesive patch that should be strong enough to resist movement and water with our biosensor fixed in it with its microneedle that will be in the body of the patients (Fig.6). Also we would like to put a LED in

the PCB to indicate from the outside to the patient that the biosensor is really running. We finally would like our final product to be a square of 2.5 cm.

Finally, we decided to go with a simple interface showing whatever the patient wants. In fact everything is modular from the data the patient wants to send to a health professional to the details of the data shown (for example, showing only creatinine without the GFR). We are also able to show a graph to the patient that represents the evolution of the creatinine levels in the past hours or days. Easy figures are shown for it to be easily understandable for every one (Fig.7).

So, with our conceptual prototype we want something that is deeply adapted to each and every patient. Our goal is to make the most accessible biosensor as possible to help lots of people tackling kidney failure.

The critical aspects of this solution could be that the age group needing creatinine sensing is not really used to this kind of tools with smartphones. However, with other diseases such as diabetes, the sensors are linked to the smartphone and it seems to be something that does not bother the patients (especially the more aged ones).

For our validation study, we mentioned earlier (4.1) that Jean-Marc CHARREL would be interested in using our biosensor during the week of the kidney happening each year in Paris. This occasion could be a really good way to test in real life the feeling of patients wearing our biosensor and to see their reaction when they use our interface. This event gathers thousands of people each year so it is a very powerful way to test our product and also to gain visibility in the world of kidney health.

5. Team and support

5.1. Contributions of the team members

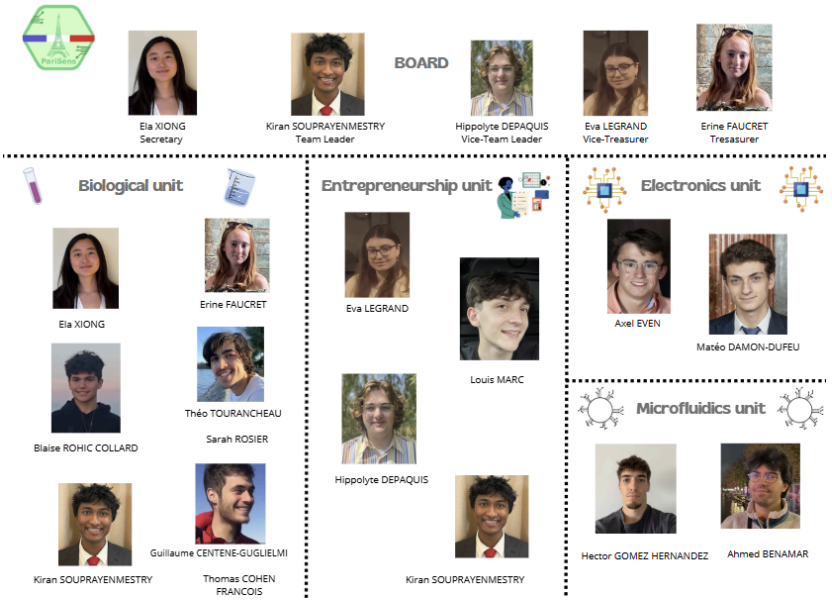


Figure 8 **: Team members diagram**

5.2. People who have given support

Vincent SAUVEPLANE: Assistant Professor at AgroParisTech and researcher in the UMR Micalis (INRAE). He guided us through the experimental aspects of our project as well as the more entrepreneurship aspects. Thanks to him we also got access to labs.

Jean-Frédéric AUDIBERT : Project engineer at the ENS Paris-Saclay. He helped us a lot with the modelisation of the microfluidic circuit and its printing.

Rasta GHASEMI : Platform engineer at the ENS Paris-Saclay. She designed and printed gold electrodes that were designed according to our needs.

Emmanuelle SCHMITT : Researcher at Polytechnique. She and her team received us in their labs during the summer break. It allowed us to continue to run crucial experiments at the end of our project.

5.3. Sponsors and partners

Fondation AgroParisTech : They financially helped when we won the maturation award (5,000 € award). They also helped us to understand the world of entrepreneurship and gave us contacts in the health field.

Compagnie financière de Haute-joux : This compagnie helped financially.

INRAE Micalis : This research institute helped us by giving us materials for our experiments.

6. Final remarks

Since we come from three different institutions, meeting in person was often difficult. However, staying in touch was essential, as each of us worked on different aspects of the biosensor. Bridging the gap between our areas of expertise was another challenge: some of us had a stronger background in mathematics, whereas others specialized in biology. At first, communication was a struggle—it was almost impossible to fully understand one another.

What notably lacked in our team was people who understood electrochemistry. That is why we are indebted to Jean-Frédéric AUDIBERT and Cédric TARD for explaining and showing us the path to better understanding of this complex field. For each field where we needed help, we contacted professors and researchers. We will be forever grateful for their kindness and their time and we were moved by the spirit of mutual support that thrives within the scientific community.

Yet another challenge was to always keep the big picture in mind, for no one but us would know in which direction our project needs to be driven. Our team

supervisor, Vincent SAUVEPLANE, really helped us with these challenges and we truly thank him for his presence. It is really impressive to see how fast the project is advancing now compared to the start. Although it was tough to juggle school and this project, we are glad to have made it so far.

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8. Appendix

For this international student competition, we have made the strategic choice to bring together students from three French “grandes écoles”, located in close proximity to each other on the Paris-Saclay plateau. The aim is to capitalize on the complementary nature of our educational backgrounds to take a multi-disciplinary approach to the project and reinforce its scientific and technical excellence.

AgroParisTech : specializing in life sciences, the environment and sustainable living, AgroParisTech provides expertise in biology, experimentation and life engineering. Students acquire a solid grounding in applied research, particularly valuable for the development of our project's experimental pole.

École normale supérieure Paris-Saclay (ENS Paris-Saclay): renowned for the excellence of its training in the basic sciences, ENS Paris-Saclay contributes to our project in particular in the areas of physics and microfluidics. Students at this school are trained for top-level research and scientific innovation.

CentraleSupélec: an engineering school specializing in advanced technologies, CentraleSupélec brings to the project skills in electronics, embedded computing and systems design. It is an essential pillar in the technological development of our solution.

All three institutions are highly selective, nationally and internationally recognized, and award engineering or master's degrees on completion. This collaboration enables us to strengthen our skills, enrich our approaches, and lead an ambitious project with high innovation potential.

$$\frac{d\text{creatinine}}{dt} = -\frac{V_{max,1} \cdot [\text{creatinine}]}{K_{M,1} + [\text{creatinine}]}$$

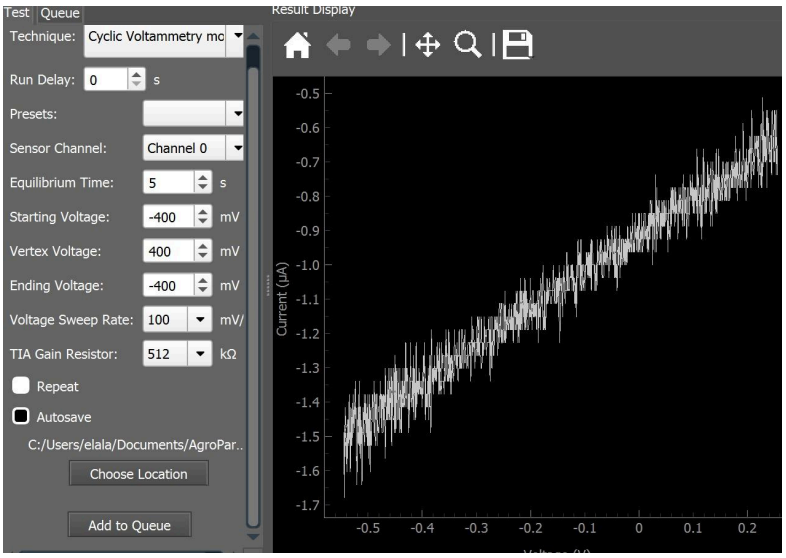
$$\frac{d\text{creatine}}{dt} = \frac{V_{max,1} \cdot [\text{creatinine}]}{K_{M,1} + [\text{creatinine}]} - \frac{V_{max,2} \cdot [\text{creatine}]}{K_{M,2} + [\text{creatine}]}$$

$$\frac{d\text{sarcosine}}{dt} = \frac{V_{max,2} \cdot [\text{creatine}]}{K_{M,2} + [\text{creatine}]} - \frac{V_{max,3} \cdot [\text{sarcosine}]}{K_{M,3} + [\text{sarcosine}]}$$

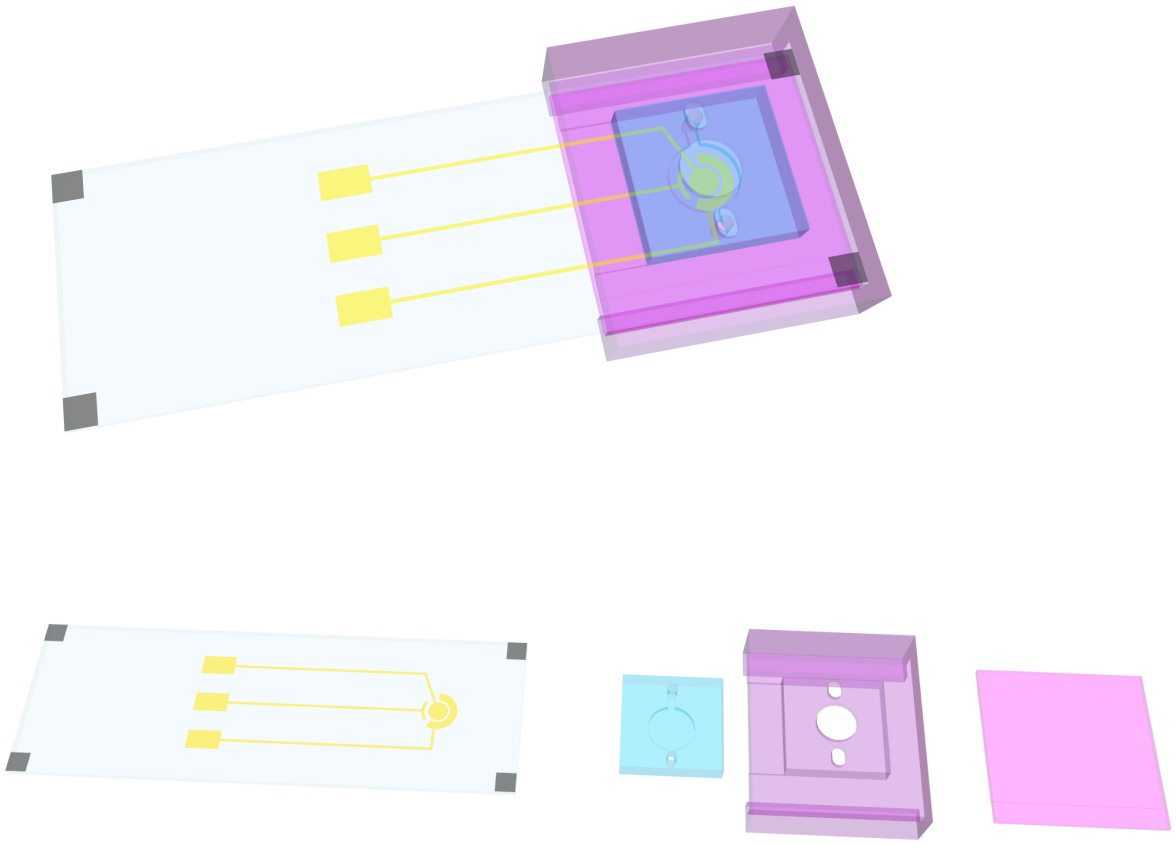
$$\frac{d\mathrm{H_2O_2}}{dt} = \frac{V_{max,3} \cdot [\text{sarcosine}]}{K_{M,3} + [\text{sarcosine}]} - \frac{V_{max,4} \cdot [\mathrm{H_2O_2}]}{K_{M,4} + [\mathrm{H_2O_2}]}$$

$$\frac{d\text{electrons}}{dt} = \frac{V_{max,4} \cdot [\mathrm{H_2O_2}]}{K_{M,4} + [\mathrm{H_2O_2}]}$$

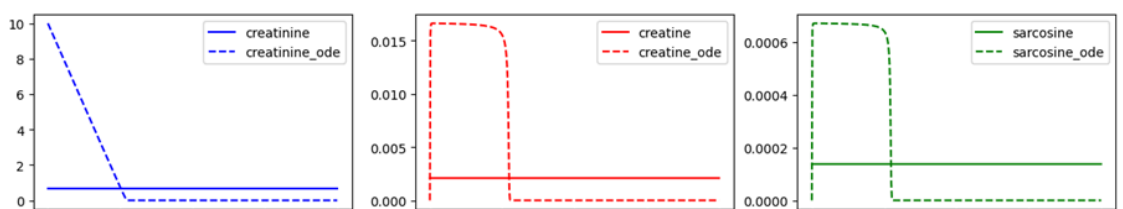
Sup Fig. 1: Differential equations in relation with our enzymatic chain reaction



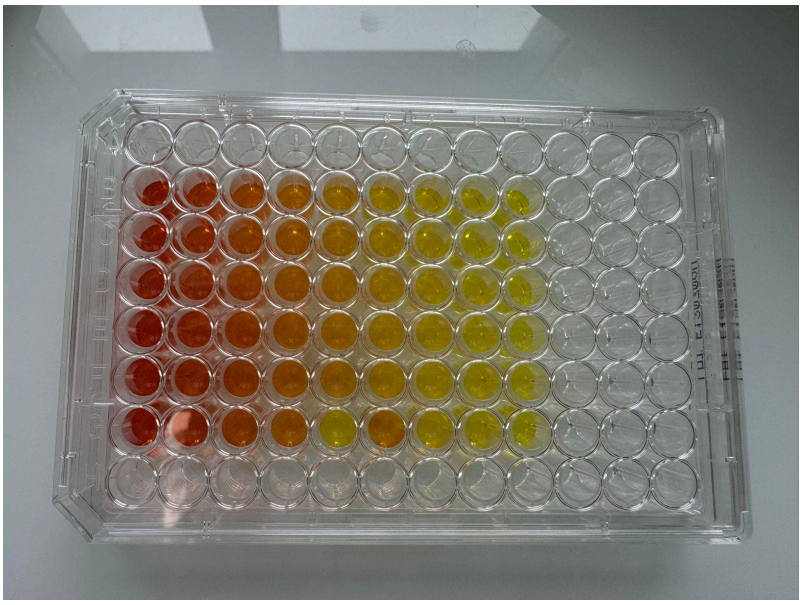
Sup Fig.2: Software interface developed by our electronic unit that helps us to see the results of our electrochemical signal



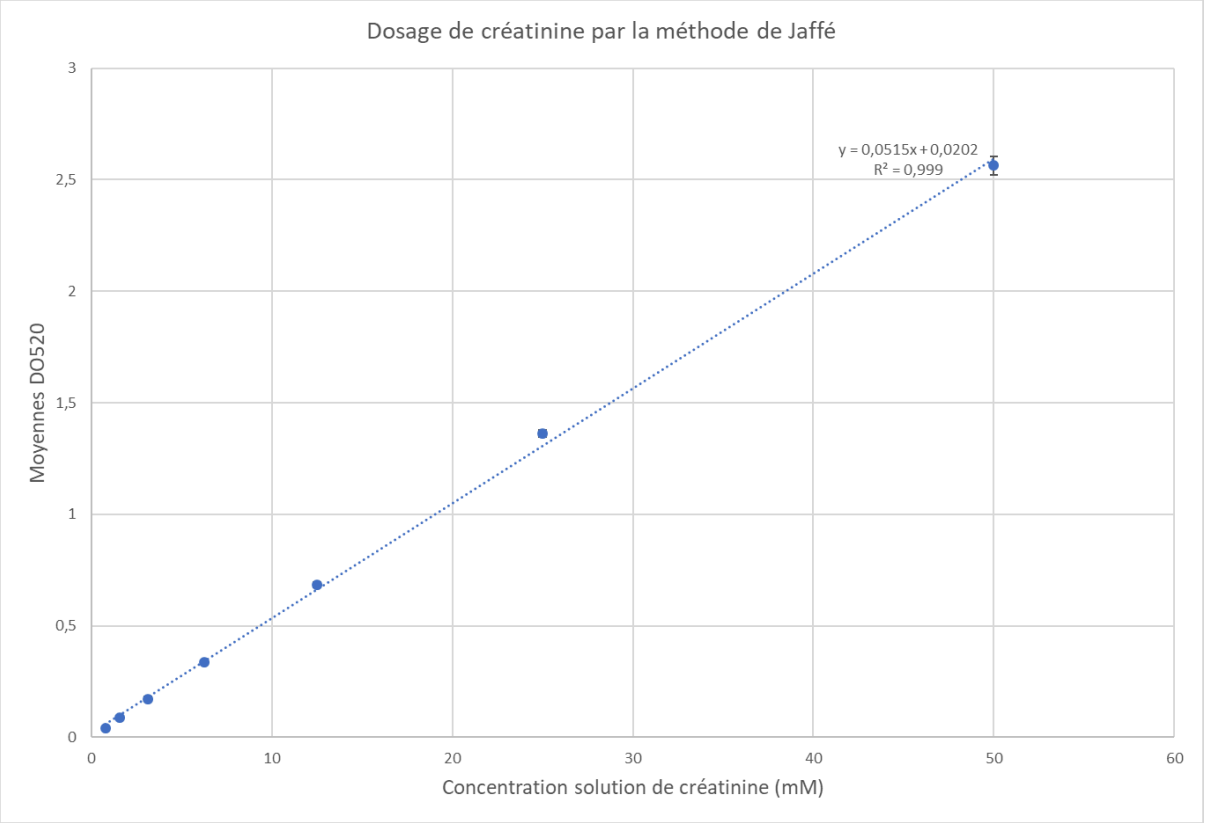
Sup Fig.3: Design of the microfluidic circuit



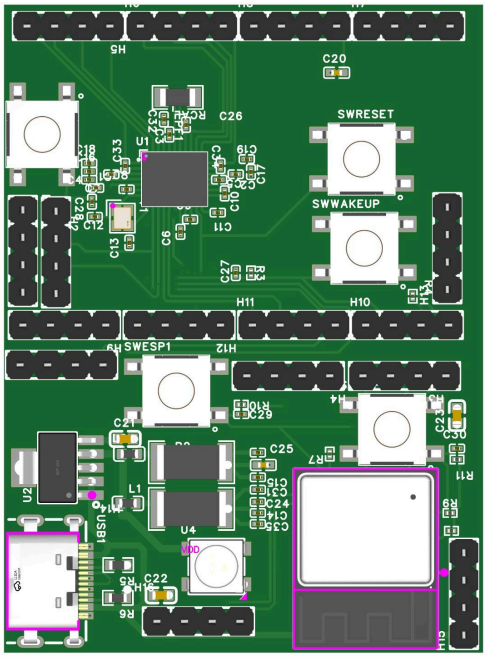
Sup Fig.4: Graphs showing the evolution of the molecules in the enzymatic cascade in time



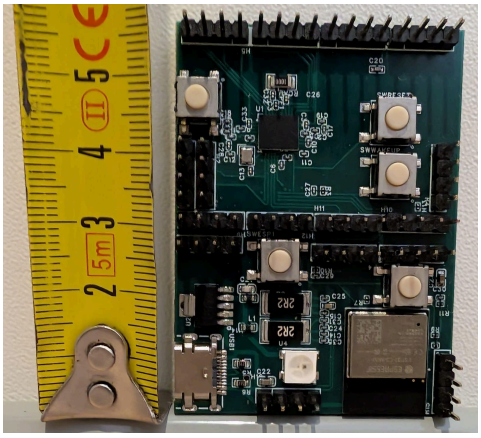
Sup Fig.5a: 96-well plate with different concentration in enzymes and substrate, the absorbance is tested to characterize the affinity



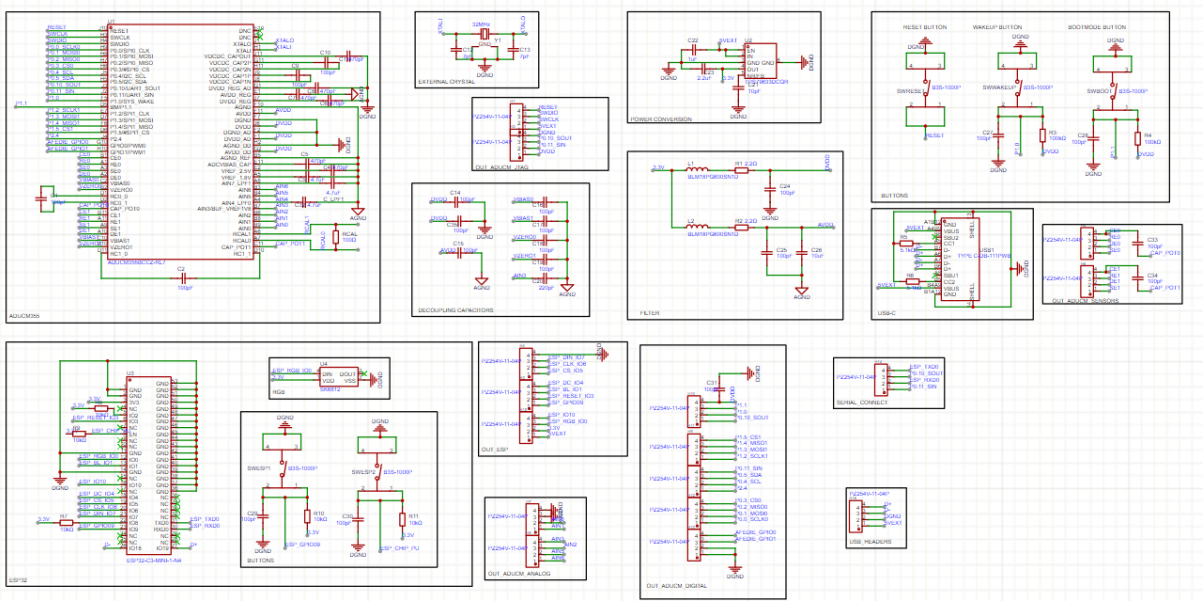
Sup Fig.5b: Graph showing the absorbance of the wells in function of the creatinine concentration



Sup Fig.6a: Render of the custom-made electronic board



Sup Fig.6b: Photos of the custom-made electronic board



Sup Fig.6c: Schematics of the custom-made electronic board