



## Team Results Document

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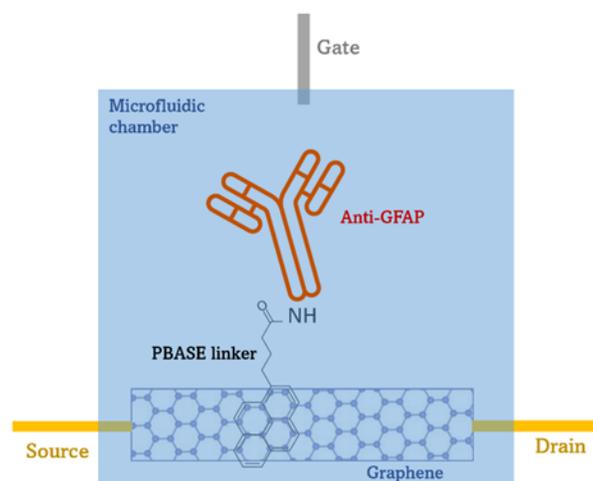
## Summary

To address the health concern of traumatic brain injuries (TBIs), we've developed a pioneering biosensor capable of detecting and quantifying glial fibrillary acidic protein (GFAP) concentrations—a biomarker intricately linked to TBI severity. Our biosensor is based upon the promising Graphene Field-Effect Transistor (GFET) technology. This biosensing platform combines the exceptional properties of graphene with the precision of field-effect transistors, resulting in a device that is compact, highly sensitive, and boasts a fast electrical response. Conjugating anti-GFAP antibodies on the graphene, we can specifically detect the protein of interest in under 10 minutes. This advancement carries significant implications, particularly within the professional sports context, where TBI occurrence has important consequences for players' health. Given the recurrent nature of such injuries and their repercussions, the need for accurate diagnostics is evident, necessitating innovative technologies that protect players from further complications. Guided by our commitment to advancing diagnostic capabilities, we aim to reshape TBI detection and provide medical practitioners the time they need to administer effective care to those in need.

## Biosensor system and assay

### Molecular recognition and assay reagents

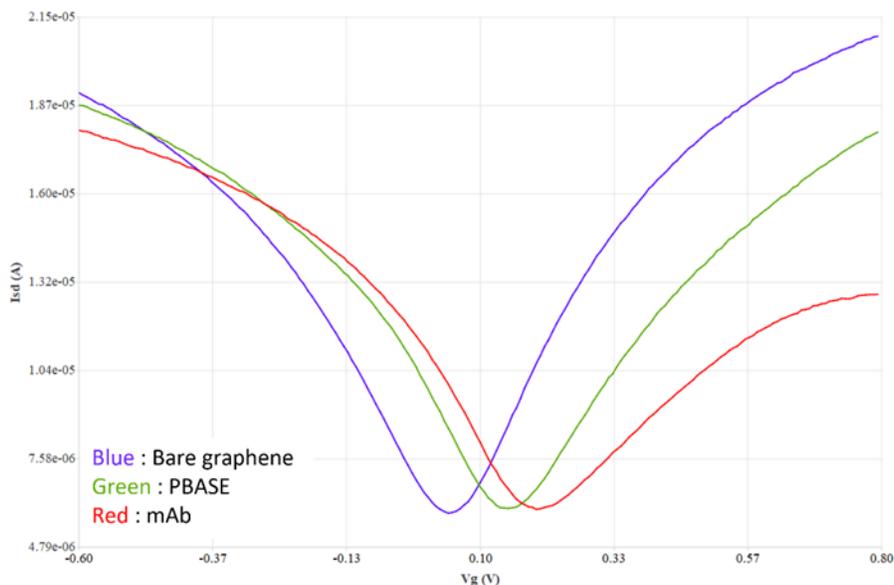
The molecular recognition mechanism we chose for our GFAP biosensor relies on the anti-GFAP monoclonal antibodies functionalized on the graphene surface using PBASE (Pyrenebutanoic Acid Succinimidyl Ester). This molecule has already been used by other researchers to introduce carboxyl groups onto graphene surfaces (Li H. et al., 2022). PBASE acts as a bridge molecule, possessing a pyrene moiety which interacts with graphene via non-covalent  $\pi$ - $\pi$  stack, and an active ester group which then reacts with amines, such as those found in antibodies, to create covalent bonds.



PBASE was chosen over other functionalization methods due to its ease of use and the expertise developed around it in our home laboratories.

### Physical transduction

Our biosensor is a simple electrical circuit composed of three electrodes: the source, the drain, and the gate. The source and drain are connected by a graphene ribbon. The gate plays a crucial role in controlling the current flowing through the device. When it is electrically charged, it creates an electric field that modifies the density of charge carriers in graphene. By varying the gate voltage, a change in graphene's conductivity is observed, depending on its surroundings. When the graphene's conductivity reaches its lowest point, it is referred to as the charge neutrality point voltage ( $V_{cnp}$ ). This point shifts to the left or right depending on whether the graphene's environment becomes more positive or negative (Beraud A. et al., 2021).

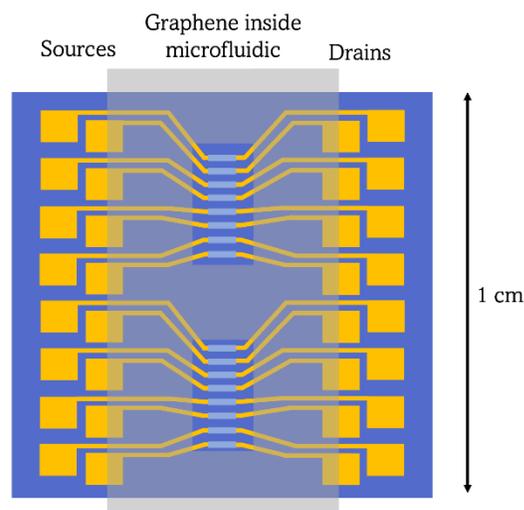


**Figure 1. Test chip representative device transfer curves.** The lowest source-drain current ( $I_{sd}$ ) point gives us the  $V_{cnp}$  of the measurement. Each curve was taken at a different assembly stage, namely initial or bare graphene (blue), post-PBASE functionalization (green) and post-mAb immobilization (red).

### Cartridge technology

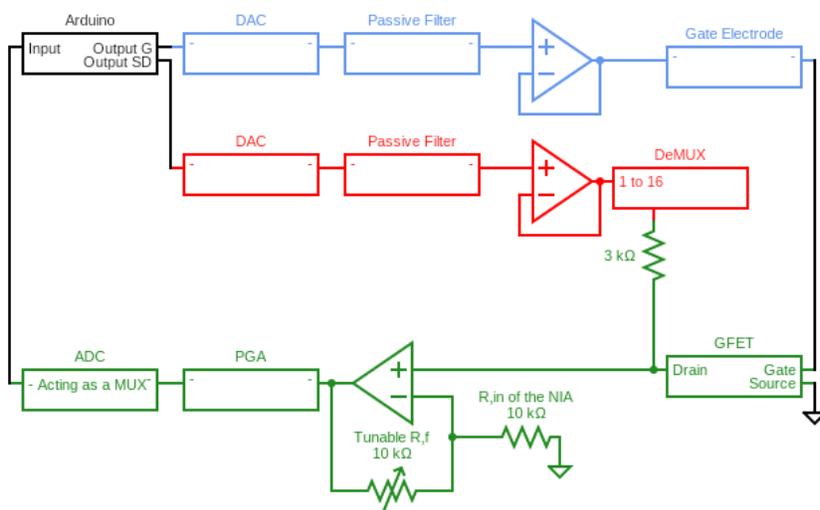
Our GFET chips, designed and built in our laboratories, have 16 devices. This ensures numerous replicates of electronic measurements. In addition, the graphene ribbons of the devices are small and clustered in the middle of the chip. This minimizes the amount of solute needed to cover the graphene and reduces the distance between the gate and the devices, thereby increasing the gate's reproducibility and efficiency. The gold electrode circuit is kept at its simplest and smallest, enabling chips of just  $1\text{cm}^2$  to be built.

For the gate, we chose to use an Ag/AgCl gate. The latter has the advantage of being able to detect biomolecules at lower voltages, which is necessary since higher voltages hydrolyze the water in the solution, thus disturbing the electrical environment (Bazan C.M. et al., 2022). This gate is fabricated in the laboratory using silver wire coated with silver chloride in a potassium chloride solution. When electrically charged, the gate releases ions into the KCl solution, which in turn releases ions into the KCl solution through a thin layer of agarose into the solution surrounding the graphene. To place the gate in the solution and near the graphene, we use a simple microfluidic cell. This enables a small volume of solution to be delivered to the graphene using a pump and tubing, and the gate to be attached to the top of the graphene ribbons.



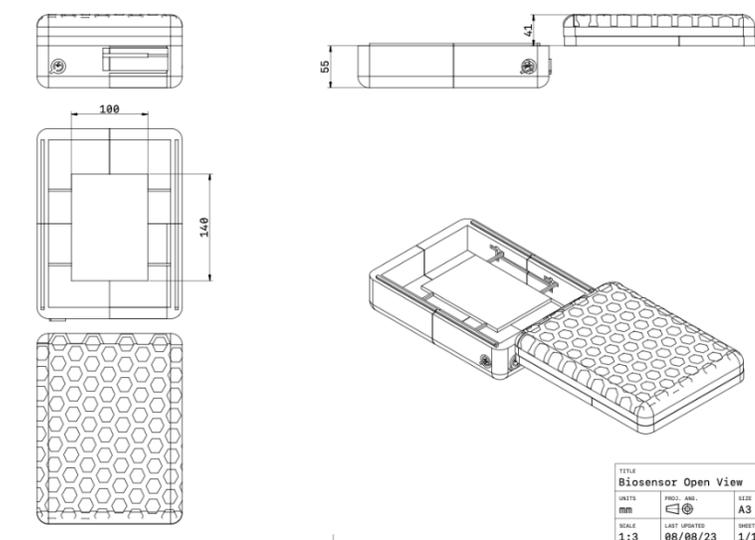
## Reader instrument and user interaction

Battery-powered, fully digital devices offer great user-friendly features to the public. The portable device designed for this biosensor is powered by 9V batteries and controlled entirely by an Arduino for maximum customisation even post-production. A 16-channel system connects each channel of the GFET chip to an electrode at its source and drain. Two voltages are generated from DACs (Digital-to-Analogue converters), combined with passive filters and buffers: one generates a sweep voltage between 0 to 1V for the gate (Fig 2, blue part), while the other generates a fixed voltage for the source-drain circuit (Fig 2, red part). Since the diagnosis relies on the shift of the  $V_{cnp}$ , the source and drain electrodes are connected in a manner that allows for the detection of current drops (Fig 2, green part). This digital design ensures adaptability and virtually eliminates the necessity for trained staff to interpret results, allowing for a very smooth usability and customisation based on the customer's needs. By design, the user interface consists of two sections: a test section and a maintenance section. The test section, suitable for the public, displays a loading test and result. An optional toggle button allows viewing of the transfer curve (Fig 1) – while the maintenance setting would allow for calibration and customisation.



**Figure 2. Electrical circuit.** Gate circuit in blue, source-drain circuit in red, and current drops detection in green.

The electrical circuit, as well as the cartridge and microfluidics, will be accessible from a casing consisting of three components: the base, the lid, and the movable platform. The electrical circuit will be housed beneath the movable platform, which features two "modes" for raising or lowering the platform. The platform serves to position the chip delicately and precisely for manipulation. The subsequent results will be displayed on the small screen situated on the front of the housing. This model has been created using 3D printing technology and is relatively compact for ease of handling (12.5 cm x 90 cm x 50 cm). While the current design facilitates manipulations, it will undergo significant modifications to enhance accessibility for the broader public.



**Figure 3. Biosensor casing and its cover**

### Signal enhancement with gold nanoparticles

Signal enhancement with gold nanoparticles is possible to obtain a greater signal when quantifying GFAP. The optical properties of metals like gold and silver can amplify the signals measured with GFET through plasmonic oscillations. This means that the application of a voltage will impact the movements of electrons on the nanoparticle (inducing redistribution), thus affecting the measured current. A movement of electrons between the graphene and the gold nanoparticle would be responsible for increasing the sensitivity of our measurements (and between themselves). The nanoparticle also provides an increased surface area and can improve the capture of GFAP, thus offering more ways to modify the surface of our biosensor. This will also reduce the non-specific interactions possible when working in a complex matrix, provided by a higher density of probes. Gold nanoparticles are known for their potential for multiple possibilities for biological conjugation and their stability. There are two ways: binding the gold nanoparticle to the graphene surface or binding the gold nanoparticle to the GFAP (previously binded to an antibody attached to PBASE). The first option can be achieved by using a thiol/amine linker on the nanoparticle to correctly bind with our surface chemistry (PBASE) and a GFAP specific antibody. The second option can be used by functionalizing the nanoparticle with the GFAP specific antibody and an anti-fouling linker like a thiolated PEG. Preferably, the first option would permit a better point-of-care aspect, since the chip can be previously coated with our surface chemistry and the nanoparticle, and be ready for on-site detection of our protein. This would not require a dilution by adding the nanoparticles following the introduction of our sample. Functionalization of the gold surface with a GFAP antibody can be electrostatically accomplished with the right pH. A positively charged antibody can easily interact, in the right environment, with the citrate capped nanoparticles. The gold will also easily interact with thiol groups. For GFET, smaller nanoparticles could ensure that the analyte is closer to the surface and as well

avoid steric hindrance between nanoparticles and GFAP. Combined with a deposit microfluidic system, the detection of GFAP can be fast and sensitive in a small volume of 20  $\mu$ L.

Gold nanoparticles were synthesized following the Bastùs method. Briefly, a gold solution of chloroauric acid was added dropwise to a boiling solution of sodium citrate and continuously stirred. After 10 mins, the vessel is cooled down to 93 degrees. After 30 minutes, a new addition of gold chloroauric acid is added and left to react for 30 minutes. This addition step is repeated 2 more times. The solution is then diluted by extracting 55 mL and adding 53 mL of milli-Q water and 2 mL of a more concentrated sodium citrate solution. Two more additions of gold bring us to our final product (of 40 nm) that is preferably stored in a solution of sodium citrate.

## Technological feasibility

The following describes the study we ran to validate anti-GFAP immobilization at the surface of our graphene field-effect transistor (GFET) sensors.

### **Anti-GFAP immobilization**

Two GFET sensors were used in this study, a test chip, and a control chip.

Functionalization with PBASE: the graphene surface of both chips was functionalized with PBASE.

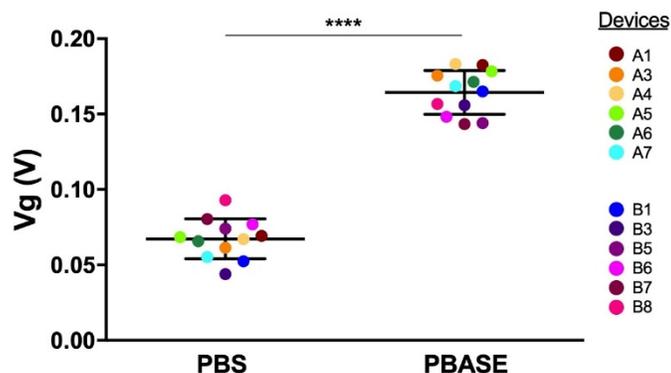
Antibody incubation: after PBASE treatment, the test chip was incubated with anti-GFAP monoclonal antibodies in PBS overnight. This facilitated covalent attachment of the antibodies to the graphene surface via a nucleophilic substitution reaction between the introduced carboxyl groups and the primary amines of the antibodies. On the other hand, the control chip was incubated with phosphate-buffered saline (PBS) to ensure that the changes observed were due to the immobilized antibodies.

Measurement protocols: To monitor and compare the electronic properties of both chips throughout the process, electrical measurements were carried out at various stages. Specifically, measurements were recorded:

1. Initially, before any functionalization (pre-PBASE stage).
2. Post-PBASE functionalization.
3. After immobilization of the monoclonal antibody (mAb) on the test chip and after PBS treatment on the control chip.

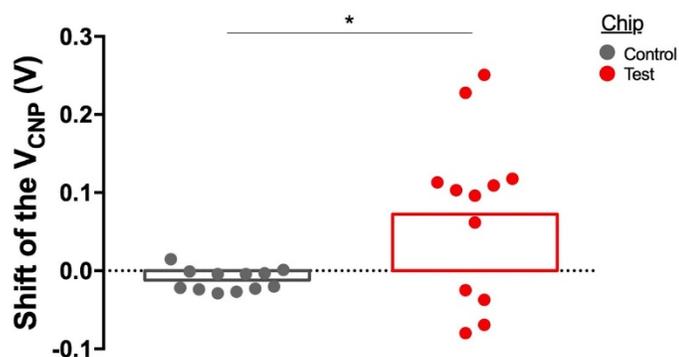
These measurements were performed in a phosphate buffered saline (PBS) concentration of 0.001X to assess the stability and repeatability of sensor readings and to determine any effect of ion concentration on the electrical properties of the device.

The figures below highlight our results. In the test chip, we notice a strong positive shift in the voltage charge neutrality point ( $V_{cnp}$ ) in the electrical measurements following PBASE functionalization (Fig. 1 and 3). This right shift in  $V_{cnp}$  is consistent with research into PBASE binding to graphene, which indicates a positive shift following successful functionalization (Nekrasov N. et al., 2022).



**Figure 4. Comparison of  $V_{cnp}$  measurements in PBS (bare graphene) and following PBASE functionalization on the test chip.** Electrical measurements were performed in PBS 0,001X before and after PBASE incubation. The graph shows the mean value of 12 devices. Error bars are standard errors.

In the test chip, we also notice a sharp increase in  $V_{cnp}$  between the PBASE measurements and the measurements taken following mAb incubation (Fig. 1 and 4). The reason for this positive shift still needs to be investigated, but from our understanding, a positive shift means that the antibodies were successfully anchored to the graphene surface and are blocking the PBS ions from interacting with the graphene, which normally dopes the graphene negatively. However, four devices don't seem to have antibodies attached to the graphene surface as they don't experience the strong positive shift found in the other devices at this stage. In the control chip, no sharp positive shift in  $V_{cnp}$  was noticed (Fig. 4) in the electrical measurements following incubation in PBS overnight.



**Figure 5. Antibody immobilization induces a significant right shift of the  $V_{cnp}$ .** Electrical measurements were performed in PBS 0,001X on 2 chips previously exposed to PBASE and exposed to PBS (control chip) or anti-GFAP antibody (test chip). The graph shows the mean value of 12 devices in each chip. Error bars are standard errors. Statistical analysis was performed on GraphPad Prism, Unpaired T-test comparing control and test condition was used, \*  $p=0,0135$ .

Figures 4 and 5 compare the mean shift in  $V_{cnp}$  between the PBASE stage and the antibody or control immobilization stage. As we can see, there is a clear difference in shift between the test and control chips. While the mAb incubated chip exhibits strong positive shift between post-PBASE electrical measurements and post-mAb measurements, the control chip results show that there is a slight negative  $V_{cnp}$  shift between these stages. Overall, these results indicate successful binding of the PBASE and anti-GFAP monoclonal antibodies onto our GFET surface. The right shift following mAb binding, as mentioned earlier, could be due to the antibodies physically “blocking” the effect of the PBS ions on the graphene surface. Usually, the ions found in PBS negatively dope the graphene surface, but since these are blocked, our  $V_{cnp}$  is found at a much higher gate voltage.

The stepwise process of surface modification and antibody immobilization has been meticulously designed to provide insight into the interaction of anti-GFAP antibodies with the graphene surface. By comparing the electrical properties of the test and control chips at each step, we can verify the specificity and efficiency of antibody immobilization. The control chip plays a crucial role in differentiating between changes brought about by the surface chemistry itself and those induced by anti-GFAP antibody binding. The design of this experiment thus guarantees a complete understanding of the functional properties of our GFET biosensor.

In theory, it is possible to detect GFAP at higher concentrations using graphene-based field enhanced transistors. A calibration curve was not measured since our focus was on the optimization of our biosensor and troubleshooting. We are confident that with further work on our surface chemistry, we can achieve the detection of GFAP in a complex matrix at various concentrations. Further optimization is needed for the signal enhancement with the gold nanoparticles and general functionalization. Another improvement would be the use of a different capture strategy like using an aptamer instead of an antibody. The stability of a GFAP specific aptamer would permit a more reliable functionalization than can be done prior to testing. Furthermore, for signal enhancement, it could be possible to do the growth of the nanoparticles directly on the graphene, but this would require a different strategy.

Another focus for our project was to downsize the instrument for GFET. We successfully made the apparatus portable and easier to manipulate. Combined with a 20  $\mu$ L drop microfluidic, our system works with an easy removal cartridge system.

# Translation potential

## Introduction

It is often noted that professional athletes' health is the object of fast-growing public attention. This is especially true for Sport Related Concussions (SRC). As documented by (Andersen & Kian, 2012), in 2009, the media coverage of National Football League players' attitudes has been shifting from its "warrior narrative", and towards the importance of maintaining good health conditions. This cultural change is not surprising considering the gravity of the situation: between 1.7 and 3.8 million sports- and recreational-related concussions happen each year in the US. Moreover, (Haarbauer-Krupa et al., 2018) found that 70% of concussed children were injured while playing sport. The chronic nature of SRC causes long term issues like chronic traumatic encephalopathy, depression and suicide, Alzheimer's disease, and Parkinson's disease (Abner et al., 2014; Goldman et al., 2006; VanItallie, 2019). SRC are therefore both more frequent and dangerous than non-sport-related concussions.

For these reasons, we think that addressing the concussion crisis from the sport perspective will be particularly effective. This will allow us to grow rapidly and to build our expertise with well-funded and incentivized partners while tackling a large part of the global concussion problem. We plan to generalize our technology in the long run. GFET is a new and promising sensing method, and we will eventually morph into a chip-customization business to take advantage of emerging possibilities. However, this document will mostly address the most urgent issue of concussions in sports.

## Business model canvas

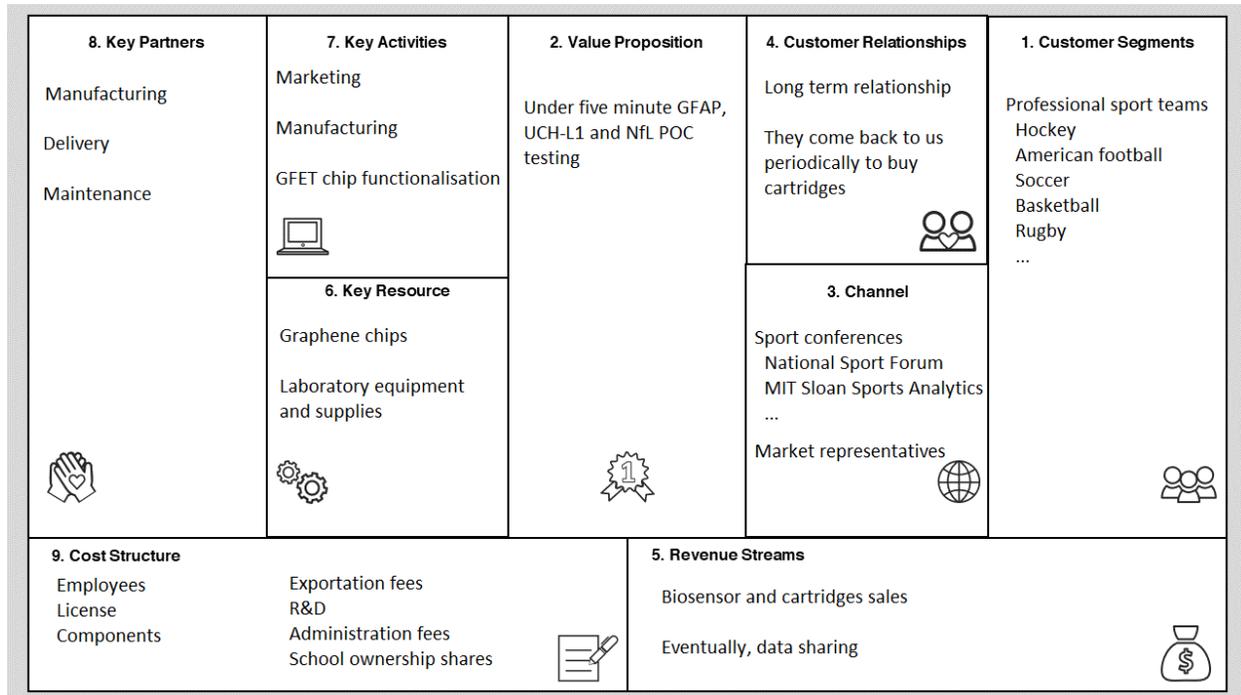


Figure 6. Business Model Canvas

## Market description

The demand for a rapid and precise Traumatic Brain Injury detection device within North American sports is substantiated by the frequent collisions and need for quick player participation decisions during games. Presently, these determinations rely on subjective, verbal and physical assessments, which, due to their overly cautious nature, result in significant expenses for both the team and the player. In the context of the global Sports Medicine market, which was valued at \$7 billion USD in 2022, North America accounts for 35%, amounting to approximately \$2.45 billion USD (*Global Sports Medicine Market Report and Forecast 2023-2031*). Since head injuries represent at most 10% of that amount, it leaves us with a \$245 million USD market (*Sports medicine market size, share & trends [2023 report]*).

## Stakeholder desirability

Our primary customers will be professional sport teams in North America. The product we propose will be used by the team's physicians or physical therapists following a positive sideline assessment. Sport teams are evolving in a peculiar market, benefiting from low concurrence in their home city and a devoted consumer base. These businesses are trying to optimize sporting as well as financial success. Profit maximization being highly correlated with sporting success suggests that technical-tactical investments might be the owner's best financial strategy (Simone & Zanardi, 2021).

GFAP peaks at 20h after impact and drops until 72h, after what the concentration stays low and is barely distinguishable from the baseline for at least a week (Papa et al., 2016). We will rapidly add multiplexing capabilities for other mTBI biomarkers with complementary temporal profiles to enlarge our testing window. Ubiquitin carboxyl terminal hydrolase L1 (UCH-L1) peaks shortly after the impact and will be useful for up to 2 or 3 days (Papa et al., 2016). It will be essential for the immediate Return to Play (RTP) decision. We plan to use the neurofilament light polypeptide (Nfl) for long term monitoring of the rehabilitation process (Shahim et al., 2016). Of course, further studies will be required to assess the usability of each biomarker, as well as their exact time window.

Our product will enhance the concussion procedures of sport teams in multiple ways. First, it will better inform the immediate RTP decision, preventing both unnecessary withdrawals of non-concussed players and the RTP of concussed ones, which could have devastating effects on the player's health, including the deadly second impact syndrome (McLendon et al., 2016). Second, it will prevent unnecessary CT scans, reducing the financial burden of TBIs and the negative effects of radiation on player's health. Finally, it will provide critical information on the evolution of the player's state. On the one hand, that will allow therapists to provide better care and to adjust the rehabilitation procedure, potentially reducing the RTP time. On the other hand, as stated by Tristan Castonguay, head therapist of the Alouettes - Montreal's Canadian Football League team - this will be crucial for sport analytics and team management, informing exchange and injured list placement operations.

(Donaldson & Cusimano, 2014) found that the cost of lost salaries due to a concussion for a NHL (hockey) team was 397,700 USD in 2012, which is equivalent to 579,979 USD in 2023 given the augmentation of salaries. We estimated the cost reduction associated with a reduction of the RTP time of one day for one in 5 concussions. On average, this rehabilitation time reduction would save NHL teams 13,611 USD per season, and NFL (American football) teams 20,659 USD per season [appendix TP.1]. These are indeed very

rough estimates, and the volatility is high given the high variance of the players' salaries and RTP time. The hypothesis of a reduction of RTP time of one day for one in 5 concussions would have to be empirically verified. Nonetheless, we believe these numbers to be conservative of the actual cost reduction, given that they do not consider the price of CT scan, the reduction of treatment costs, nor the reduced player's performance (Neustadtl et al., 2021).

Apart from sports teams, the stakeholders we must consider are insurers, sponsors, players, and leagues. Teams generally get insurance for their players' injuries. For example, NHL teams only pay injured players' complete salaries up to 30 games after the incident, after what insurance companies pay 80% of the salaries. This amounts to 7.2M USD yearly, according to (Donaldson & Cusimano, 2014). Sponsors also have incentives to keep their athletes healthy. Players of course will benefit from our business, and according to Dr. Josée Rainville, athletes are often reluctant to use new technologies. Extra care must be taken to ensure that they are willing to use our device by minimizing its invasiveness. Major sports leagues have concussion protocols that dictate what kind of tests are used for assessment, and the steps included in the rehabilitation process. We plan to work in close collaboration with the leagues' health teams to ensure that our technology is included in future amendments to these protocols.

These protocols are composed of multiple computerized and non-computerized neurocognitive tests that are in some way in competition with our product (Jacobi et al., 2023; Habli et al., 2022). These tests are well established, and the current state of TBI diagnosis and prognosis does not seem to indicate that it will completely replace them. The information each test provides will be used in conjunction with the others to dress a more accurate portrait of the injury. In direct competition, however, mTBI biosensors are already popping up on the market (Krausz et al., 2021). Abbott has a TBI plasma cartridge measuring GFAP and UCH-L1 for its i-STAT Alinity device, and NanoDx designed a POC device measuring GFAP and S100B. Our advantages over these competitors are the use of GFET, which has a strong accuracy-portability trade-off, and the choice of detecting NfL in addition to GFAP and UCH-L1.

### Business feasibility

We will be outsourcing most of our activities in the foreseeable future. This will allow us to take advantage of the expertise of well-established enterprises in the many areas we have few insights about, diminishing the rigidity of our organizational structure and concentrating our investments (Paek B. et al., 2019). We will keep manufacturing our graphene chips ourselves at Polytechnique Montreal's LMF until about 2026, when we'll buy our university's patent share. The Bureau Recherche-Développement-Valorisation will help us navigate intellectual property rules. After that, we plan on buying GFET chips from Graphinea. We also plan on partnering with StarFish for manufacturing, packaging, and delivery, and with PréviMed for maintenance. Our main activity will be electrode deposition and chip functionalization.

We want to continue the development of our product with a Design for Sustainability approach (Ongaro A.E. et al., 2022). An early commitment to this framework will put us on the right track to explore new and exciting alternatives. We will continue using PLA for our prototypes' shells, this material being the most easily available biodegradable material for now. However, we are aware of the limitations of this material (undegradable in natural environment, competition with agricultural food production, high water consumption), and will closely follow the development of promising materials like shellac and natural

fibers (Alfredo Eduardo Ongaro et al., 2022; Korol J. et al., 2019; Lausecker, Roland et al., 2016). We plan on performing a lifecycle assessment analysis that follows the ISO 14040 specification.

We will have to comply with multiple standards, including ISO 13485 and IEC 60601, for Health Canada clearance, and their equivalents for FDA clearance. Our product will be a FDA Class II device (*Code of Federal Regulations Title 21, Part 866, Subpart F, Section 866.5830*). We will communicate with FDA's Center for Devices and Radiological Health office to determine if we can use the 510(k) pathway for our device's clearance, predicated on the Bayan BTI or the Abbott i-STAT Alinity devices (*Evaluation Of Automatic Class III Designation For Banyan Brain Trauma Indicator; 510(k) Substantial Equivalence Determination Decision Summary Assay Only*), or if we must use the de novo pathway. Periodic regulatory landscape monitoring will be critical during the early phase of development to ensure we are designing a product in accordance with all regulations. Clinical trials will be conducted with Université de Montréal's sport teams.

Our marketing strategy will be centered on showcasing our product at sports events and conferences. We will hire or partner with market representatives like HTX Sports Tech. We should particularly attend The National Sport Forum, the biggest sport conference in North America, and the MIT Sloan Sports Analytics Conference. This product has the potential to generate data valuable to pharmaceuticals, sport teams and insurers. Partnering with a data analytics firm would help us investigate these opportunities.

### Financial viability

To ensure the strength of our business strategy, a comprehensive assessment of the financial viability of our product is essential. The biosensor is composed of four primary components: the casing, the electrical circuitry, the microfluidics and the GFET chips.

To optimize costs, our strategy is to buy circuit components in bulk quantities as soon as possible, since they are easy to store. The same goes for the material used for the casing and the microfluidics, leaving us with a cost of approximately 175\$ for the biosensor. Meanwhile, the GFET chips currently benefit from a cost advantage due to our collaborative arrangement with the school labs. Presently, these chips are valued at \$18 for a 80% success rate. However, as we transition away from our school affiliation in the fifth year, the cost of these chips is expected to increase to \$96. This potential loss of profit due to the price shift will be absorbed by the infusion of funds from investors. During that same year, we will be involved in pilot projects which will later help establish trust and secure significant clients, we will then be able to buy the chips in bulk to reach a cost closer to the first one. This financial support will also enable us to maintain a competitive market price for our biosensor and chips.

With these costs, we have established a final pricing for our products: the biosensor will be priced at \$2500, while the chips will be available at \$100. Our revenue model places a strategic emphasis on chip sales, as we anticipate selling more of them.

The choice to initially target North American sports companies is partly justified by the desire to minimize export fees. This decision is justified by our proximity to North American labs. In our first years of business, the revenue streams will primarily come from engagements with North American sports companies. Post-

pilot project, we will start a strategic expansion, reaching out to the global medical diagnosis and sports company sectors.

As we progress, our overarching objective is to capture 6% of the professional sports medicine market by the year 2032. Despite the anticipated price fluctuations that may arise in the fourth year, we plan on achieving profitability by the end of our fourth year of operation.

## Team and support

### Contributions of the team members

**Ryan Borotra:** Fabricated the GFET chips from bare silicon wafer to fully functional sensors and investigated the chosen graphene-PBASE-mAb surface chemistry with Jade. Also planned the protocols and ran the PBASE functionalization and mAb immobilization experiments on the GFETs with Jade. Analyzed the data following these experiments to validate probe immobilization. Lent support to the business portion of the project, notably in market size determination.

**Jade Cimmino:** Investigated the chosen graphene-PBASE-mAb surface chemistry, planned the protocols, ran the PBASE functionalization and mAb immobilization experiments on the GFETs and fabricated chips with Ryan. Conducted experiments to adapt the technology for GFAP and contributed to tests for signal enhancement. Took part in team coordination.

**Caroline Dubois (Team Captain):** Contributed on the surface chemistry and signal enhancement brainstorming. Fabricated gold nanoparticles of various sizes for signal enhancement testing. Optimized and did the functionalization of the nanoparticles. Participated in the fabrication of the signal drop microfluidic system. Participated in alternative routes of biodetection.

**Faten Idar (Team Captain):** Was in charge of coordinating the different parts of the project. Took care of the communications with the organization. Designed the casing of the biosensor and participated in the business portion of the project.

**Christopher Ledo:** Designed and built the electrical circuit, data interpretation algorithm and user interface of the portable readout device. Played an important role in enhancing team cohesion. Co-lead the research efforts on surface chemistry and signal enhancement. Developed alternative modes of bio-detection as back-up plans. Participated to the GFET test runs for calibration.

**Adrien Lamarche:** Assumed most of the business-related aspects of the project. Provided the team with valuable resources such as interviews, assistance from business experts and financial contributors.

### People who have given support

#### Millénium Québecor

In our pursuit of business, we found invaluable support from Millénium Québecor, a group dedicated to nurturing emerging entrepreneurs from the University of Montreal. They have, by offering us extensive consultations and informative business workshops, enriched our understanding of effective business practices. In addition to this guidance, Simon Cubillos-Renaud provided us with guidance and powerful financial tools. We are thankful for the support extended by all participants of this institution, along with its generous contributions.

#### Tristan Castonguay, Head Therapist for the Montreal Alouettes

Tristan took his personal time to walk us through the process of decision-making when a case of traumatic brain injury occurs. He described various tests performed and shared his experience on the field and provided us with data that were key to the development of our biosensor and financial plan.

#### Madline Sauvage

Assisted in planning experiments and protocols and provided feedback on analysis. Helped us greatly with the revision of our team results document and gave us advice all along the way for all aspects of the competition.

## Sponsors

We are grateful for all the assistance **Millénium Québecor** provided and their valuable financial support.

## Final remarks

In conclusion, our research this year has provided valuable insights into traumatic brain injuries. It has offered us a broader perspective on the significance of this issue, not only for professional athletes but also for the general population. This project has unlocked a realm of potential applications for our biosensor, for those who recognize the value of our innovation.

Our focus now shifts towards refining this technology by enhancing user-friendliness and implementing chip preservation techniques. It was a pleasure for us to use our knowledge to create something impactful and we are grateful for the opportunity the SensUs organization gave us.

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# Appendix

## 1. Reader Instrument and User Interaction

### 1.1 Technical drawings of the biosensor casing

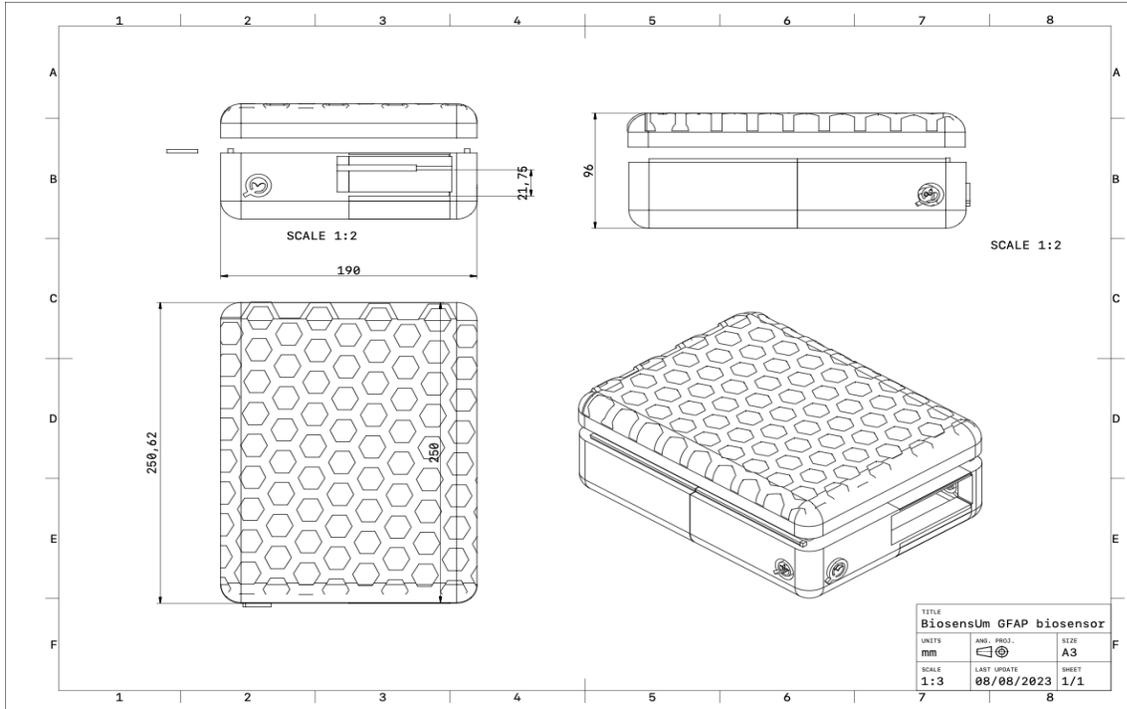


Figure 7. Technical drawing closed casing

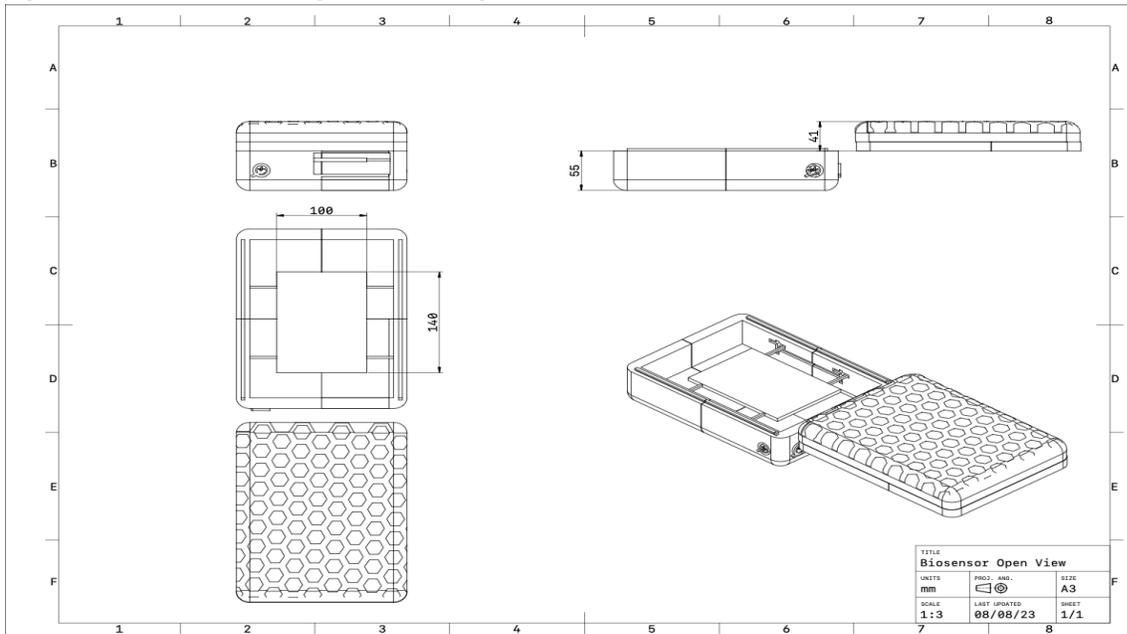


Figure 8. Technical drawing opened casing

## 2. Customer desirability

Concussion salary cost saved per season by reducing the RTP duration by one day for one in five concussions							
League	Mean salary per game <sup>1</sup>	Mean number of concussion per team <sup>2</sup>	Game occurrence (days) <sup>3</sup>	Mean RTP duration (days) <sup>4</sup>	Games missed per team	Concussion salary costs	Savings
NHL	\$42,683	4.4	2.74	18	28.70	\$1,225,000	\$13,611
				17.8	28.38	\$1,211,389	
NFL	\$158,824	6.3	9.71	15	9.76	\$1,549,432	\$20,659
				14.8	9.63	\$1,528,773	

**Figure 9.** Estimation of the customer savings

<sup>1</sup>Obtained by averaging season salary over number of games

NHL : BetMGM. *NHL Salaries : How Much Do Players Make?*

<https://sports.betmgm.com/en/blog/nhl/nhl-salaries-how-much-players-make-bm11/>

NFL : AS. *Who is the lowest paid player in the NFL? Is there a minimum salary in American football?*

<https://en.as.com/nfl/who-is-the-lowest-paid-player-in-the-nfl-is-there-a-minimum-salary-in-american-football-n-2/>

<sup>2</sup>These leagues have 32 teams each

NHL : Bruce JM, Thelen J, Meeuwisse W, et al. *Br J Sports Med* Epub ahead of print: 09/08/2023.

doi:10.1136/ bjsports-2020-102072

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NFL : NFL. *Player Health & Safety.*

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<sup>3</sup>Averaged from the number of games and the season length

NHL : Hockey Reference. *2023-23 NHL Schedule and Results.*

[https://www.hockey-reference.com/leagues/NHL\\_2023\\_games.html](https://www.hockey-reference.com/leagues/NHL_2023_games.html)

NFL : Pro Football Reference. *2023 NFL Weekly League Schedule.*

<https://www.pro-football-reference.com/years/2023/games.htm>

<sup>4</sup> NHL : Wennberg RA, Tator CH. Concussion incidence and time lost from play in the NHL during the past ten years. *Can J Neurol Sci.* 2008 Nov;35(5):647-51. doi: 10.1017/s031716710000946x. PMID: 19235451.

NFL : Mack CD, Herzog MM, Solomon G, Putukian M, Lee RY, Matava MJ, Cárdenas J, Theodore N, Sills A. Return to Full Participation Following Concussion in the National Football League, 2015 Through 2020. *Clin J Sport Med.* 2022 Nov 1;32(6):e605-e613. doi: 10.1097/JSM.0000000000001050. Epub 2022 Jun 17. PMID: 36315827.

### 3. Financial viability

#### 3.1 Biosensor and cartridge costs

Step	Tool/Material	Cost per unit/hour without school discount	Cost per unit/hour with school discount	Multiplier	Cost per 2 wafers (\$)
Materials	Bare wafer	680	26	2	52,00
	Graphene				
	Resist				
	PMMA				
	Remover PG				
<b>Step total:</b>		<b>52</b>			<b>52,00</b>
Electrodes patterning	YES Oven	115,5	47,25	0,5	23,63
	Hotte dev	115,5	21	1	21,00
	Spinbake Brewer	130	47,25	0,5	23,63
	MA6	231	47,25	2	94,50
	E-beam	231	47,25	2	94,50
<b>Step total:</b>		<b>1162,25</b>			<b>257,25</b>
Gate patterning	YES Oven	115,5	47,25	0,5	23,63
	Hotte dev	115,5	21	1	21,00
	Spinbake Brewer	130	47,25	0,5	23,63
	MA6	231	47,25	2	94,50
	E-beam	231	47,25	2	94,50
<b>Step total:</b>		<b>1162,25</b>			<b>257,25</b>
3x3 dicing	Spinbake Brewer	130	47,25	0,5	23,63
	Scie de découpe	231	47,25	2	94,50
<b>Step total:</b>		<b>527</b>			<b>118,13</b>
HMDS deposition	YES Oven		47,25	0,5	23,63
<b>Step total:</b>					<b>23,63</b>
Ribbons shaping	YES Oven	115,5	47,25	0,5	23,63
	Hotte dev	115,5	21	1	21,00
	Spinbake Brewer	130	47,25	0,5	23,63
	MA6	231	47,25	2	94,50
	RIE	231	47,25	2	94,50
<b>Step total:</b>		<b>1162,25</b>			<b>257,25</b>
Chip dicing	Spinbake Brewer	130	47,25	0,5	23,63
	Scie de découpe	231	47,25	2	94,50
<b>Step total:</b>		<b>527</b>			<b>118,13</b>
<b>Total / chip (80% succes)</b>					<b>96,43</b>
<b>Total / chip (80% succes) discount</b>					<b>18,81</b>

Figure 10. Cartridge costs

Components	Multiplier	Cost per unit	Cost per bisensor (\$)
<b>Electronics</b>			
Arduino / Shield	1	70	70
DAC	1	40	40
Resistors	6	0,2	1,2
Capacitors	3	0,5	1,5
Op amp	1	1,51	1,51
Gain amp	3	10	30
Screen	1	10	10
Solering kit	1	10	10
A - multiplexer	1	1,89	1,89
<b>Case</b>	<b>2</b>		<b>0</b>
PLA	0,33	25	8,25
<b>Total</b>			<b>174,35</b>

Figure 11. Biosensor costs

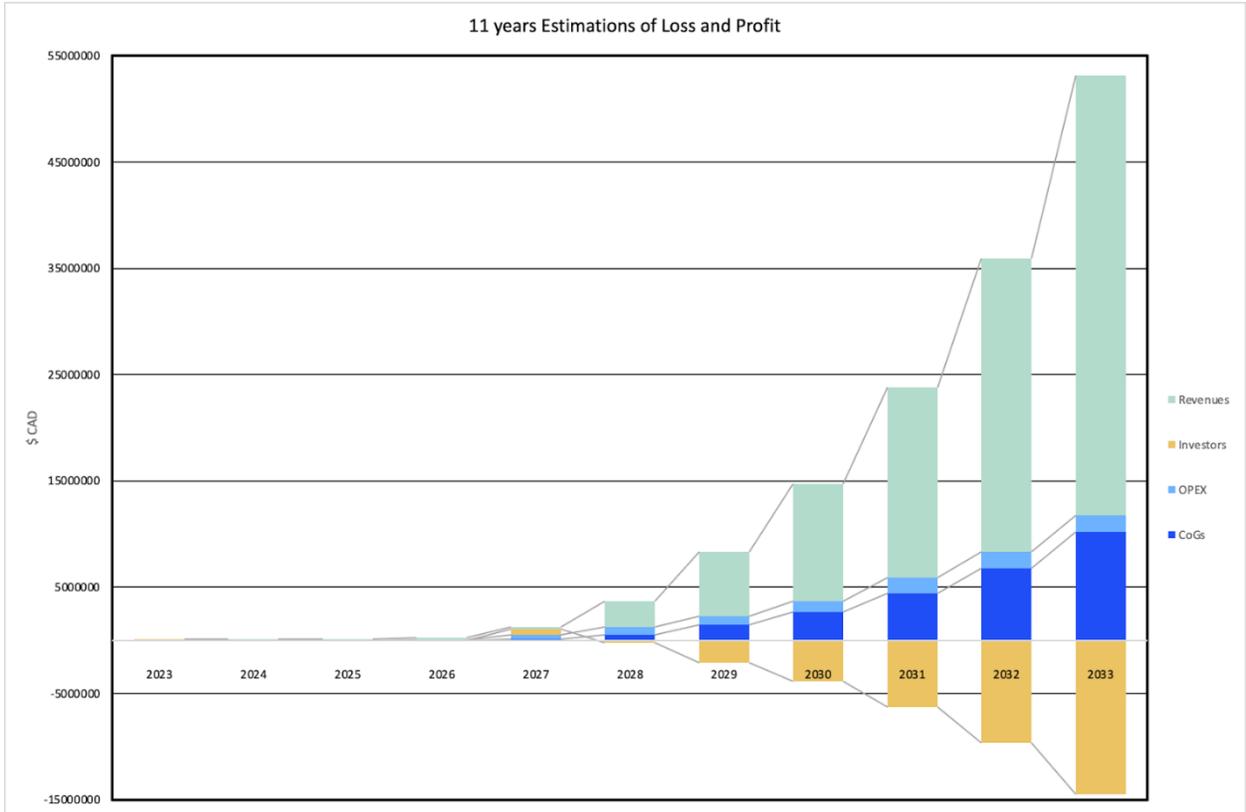


Figure 12. Profit and Loss of year 2023 to 2032