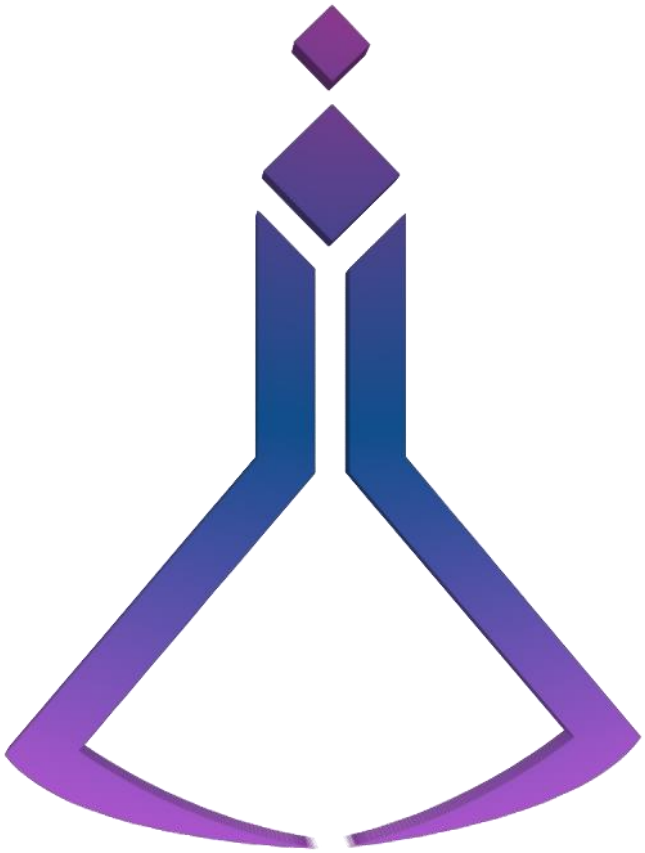
Team Result Document



UppSense

Uppsala University

Team SensUs Uppsala – UppSense

Anna Capria, Bono Jimmink, Carmen Abaurre, Cédric de Voghel, Daniel Friesen, Dianna Zeleskov, Florian Dietrich, Francesca Spagnuolo, Gustav Ahlström, Imali Ranatunge, Junyu Chen, Konstantinos Fragkoulis, Viktor Åkerfeldt

Supervisors

Gemma Mestres, Department of Materials Science and Engineering
Masood Kamali-Moghaddam, Department of Immunology, Genetics and Pathology

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Summary for the SensUs website

UppSense, our diverse team of young scientists, has developed a sensor for the detection of valproate in blood plasma. Sample transport, molecular recognition, physical transduction and data processing are facilitated by the sensor we propose.

A microfluidics chip is used to facilitate both sample clean-up and transport. Gold nanoparticles coated with a novel Molecular Imprinted Polymer (MIP) selectively bind valproate. By using Surface Enhanced Raman Spectroscopy – a Raman spectroscopy technique – any molecules that are close to the colloidal gold nanoparticles' surface will experience an enhancement in their Raman signal. Since valproate is selectively bound to the nanoparticles via the MIP, this translates into an enhanced valproate signal, with the background signals remaining unchanged. Quantification is achieved through linear regression and results are easily available on our user-friendly touchscreen display, as well as in a cloud-server.

Furthermore, by altering the MIP-coating on the gold nanoparticles, our sensor is capable of expanding its analyte portfolio, meaning that one device could potentially be used for detection of several analytes.

1. Biosensor system and assay reagents

1.1 Physical transduction

Raman scattering was originally theorised by Smekal in 1923[1] and later experimentally proven by Raman and co-workers.[2] In short, if a molecule is placed in an electromagnetic field, the electric field will induce a dipole in the molecule. The magnitude of the dipole is dependent on the electric field and the polarizability of the molecule. A fraction of light will be scattered, either elastically (Rayleigh) or inelastically (Raman). Raman scattering involves a change of frequency of the imposed light (Stokes shift) due to uptake of photon energy, which excites molecular vibrations, such as stretching or bending involved in the polarization.[3]

Raman spectroscopy utilises this scattering effect to measure the vibrational properties of molecules, but the weak signal is a limitation. There has been much improvement in methodology over the years[4] and one improved method is Surface Enhanced Raman Spectroscopy (SERS), which allows for an increase of sensitivity up to a factor of 10^{10} by having analytes close to a rough or colloidal, small size, metal surface, which is excited by a light source. This enables Raman spectroscopy to be suitable for trace analysis and biosensing applications. The exact mechanism of SERS is not entirely understood yet, but the general explanation is that the interacting light induces plasmons (i.e. collective motions of conduction electrons) on the metallic surface. These plasmons amplify the oscillating electromagnetic field, resulting in an increase of polarization of molecules close to the metal surface.[5] In the proposed sensor design, gold nanoparticles (AuNPs, ~12 nm in diameter) are utilised to generate the SERS effect. In order to facilitate the SERS effect, the UppSense sensor uses a 532 nm light source, which aligns with the absorption band of gold. SERS is only effective in the range of a few nanometers,[6] i.e. molecules closer to the surface experience more enhancement than those further from the surface. This makes SERS ideal for quantification of analytes in difficult matrices, such as plasma, but poses the additional challenge of selective adsorption on or near the metal surface.

1.2 Molecular recognition and assay reagents

To turn the SERS technique into a selective sensor, a molecularly imprinted polymer (MIP; an artificial antibody) was chosen as the molecular recognition element. The main motivation behind this choice was that the SERS effect decreases sharply with distance, making large molecules like antibodies a poor choice. Further, MIPs are rather robust, can be easily mass-produced, have multiple binding-sites and can be modified for different analytes.[7] A MIP is formed from three core components – the analyte (template), a crosslinker and a functional monomer. The crosslinker is the most abundant component, determining the macroscopic properties like flexibility, glass temperature etc. The functional monomer is a polymer building-block with one or more functional groups that can interact with the analyte. Monomers and crosslinkers are assembled into a polymer around the template analyte. This results in a scaffold selective for the corresponding analyte due to the steric cavity created by the chemical interaction of the analyte with the functional monomers.[7], [8]

In this project, the MIP was designed to work based on weak electrostatic interactions. This makes the reaction faster, easily reversible and simplifies the manufacturing process. To select the components a database of functional monomers used in literature was constructed. Then, a list of potential candidates was obtained by evaluating the electrostatic interaction of the valproate (VPA) carboxyl group with the functional groups of the monomer candidates. Ethylene glycol dimethacrylate (EGDMA) was chosen as crosslinker as it is the most commonly used crosslinker. Since all the reagents should be soluble in the same organic phase, 4-vinylpyridine (4-VP) was chosen as the functional monomer from our list of candidates.

The most important part of MIP development and functionality is the optimisation of the template-to-monomer-to-crosslinker ratio. This was done by optimising the monomer-to-crosslinker ratio against the VPA-to-total polymer ratio using two-dimensional factorial design.

Gold nanoparticles (AuNPs) were used as SERS-active metal substrates in this project. In order to accomplish the intensification of the Raman signal and the selectivity of the MIP, the AuNPs were coated with a MIP shell (AuNPs@MIP).[9] The AuNPs were in the size range of 12-15 nm in diameter as it gives ideal plasmonic radius. The AuNPs were prepared by reducing chloroauric acid ($HAuCl_4$) with sodium citrate ($Na_3C_6H_5O_7$) as capping agent, according to the Turkevich method.[10] In order to tie the coating to the AuNPs, they were functionalized with arginine. The positively charged guanidino group binds to the surface leaving the amino acid group to be implemented in the polymer network.[11][12] The polymerization starts by adding VPA, 4-VP, crosslinker (EGDMA) and initiator (AIBN) into the colloidal gold solution. The biggest challenge in this procedure is to avoid aggregation of the nanoparticles and to perform a uniform coating.

1.3 Cartridge technology

The current cartridge part consists of a PDMS microfluidic system, which through thin tubings is connected to two syringes. One syringe is for AuNPs-solution, while the other is for blood plasma. The syringes are mounted on a syringe pump that allows controlling the flow with precision. Future iterations of the sensor will combine these components into a single cartridge to make the device more user-friendly. In principle, the current setup and the future cartridge work the same, though the current setup requires the user to manually handle the three components, while the future setup will be a complete automatic sensor.

The microfluidics system has three functions: a cleaning step based on diffusion differences between VPA and larger blood plasma components such as proteins, a mixing step of VPA and AuNPs, and lastly a detection area for the laser. Appendix 8.1 depicts and explains the design of the microfluidic system.

1.4 Reader instrument and user interaction

The finalized diagnostic sensor reader instrument will be 60 x 46 x 40 cm and weigh approximately 5 kg. A power chord needs to be plugged in for the device to operate. Power consumption is estimated around 44 W when in use, 15 W when idle. A touch screen will be built into the casing for direct control. Further IT development will make the saved data accessible online for end-users with personal login credentials.

When turned on, the software will open the information page, displaying current state and health status of the device. In the measurement tab, the operator will be able to enter the sample reference number and choose or add a measurement protocol or profile. This protocol will contain measurement parameters: laser power, idle time, Raman integration time, average Raman scans, sample volume, cartridge selection, microfluidic flow rate etc. It will be possible to save and edit several protocols on a device. Once the measurement is completed, the result tab will automatically open while the device cleans the chip from the measurement. The results consist of different elements: a unique anonymized identification string shown in text and a QR-code format as reference.

It will be possible to view raw and processed data based on the protocol and sample. Raw data is useful when debugging and for device certification. Processed data can be shown with different levels of process to facilitate readout or include more information. Principally, these include measured concentration result and error range, reference concentration for the analyte and color code for either “too low”, “too high”, “critically high”, “OK”/”within therapeutic range” or “error”. The result tab can be closed and reopened within 5 minutes before next measurement, and other tabs will be accessed, for example to run the next sample. When cartridges are swapped, an automatic calibration will be prompted and confirmed by the technician.

Figure 1 depicts the whole sensor concept as described so far and in the following sections.

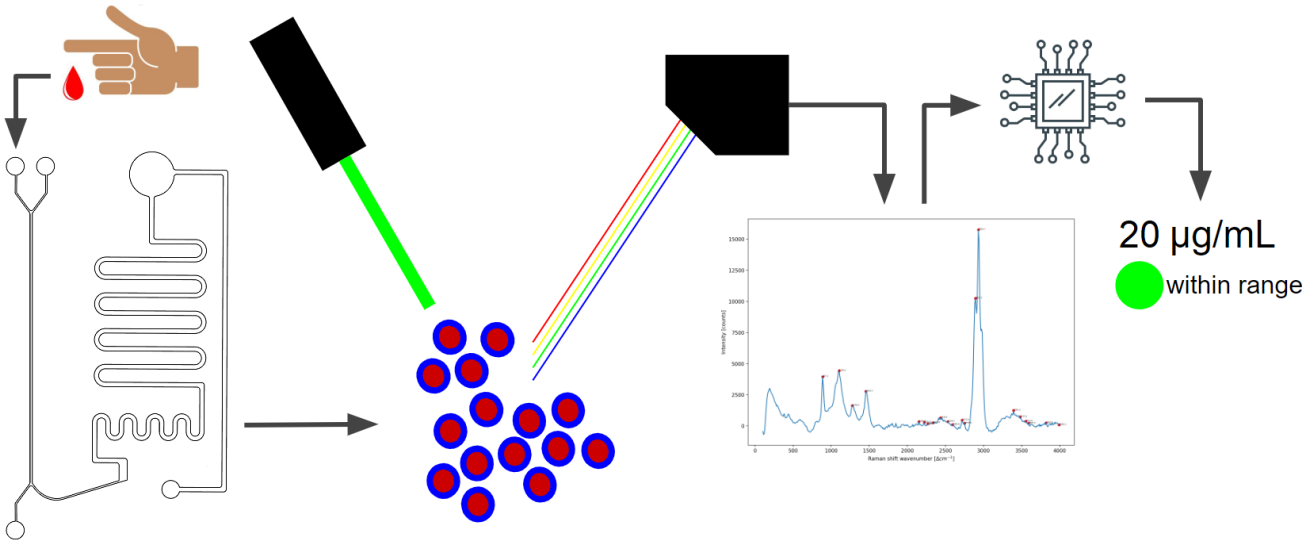

Figure 1. UppSense 2020 sensor. Blood serum is mixed with MIPs and AuNPs in a microfluidic system. The VPA concentration is detected by SERS. After data processing, the sensor software reports VPA concentration.

2. Technological feasibility

2.1 Proof of concept for the microfluidic chip

The microfluidic chip design was derived from Kamińska *et al.* 2017 supplementary materials,[13] who designed their chip for the purpose of a SERS-immunoassay.

Verification of the microfluidics system is based on testing each component separately.

The cleaning step is based on diffusion, where the VPA will quickly diffuse across the channel, while larger components of blood have a higher diffusion constant and will not have time to cross the channel. COMSOL simulations have shown this to be a viable way to clean the sample (see Appendix 8.2). Note that this will result in a 50% decrease in VPA concentration.

The mixing step was tested by mixing green dye (Brilliant Blue FCF + β,ε-carotene-3,3'-diol) and AuNPs (red color). A color change was observed, confirming that it is possible to mix small molecules and AuNPs.

2.2 MIP data

As mentioned previously, the most important part of MIP development is the optimisation of the monomer(s) and crosslinker(s) once suitable molecules have been chosen for both. The suitability of the chosen monomer, 4-vinylpyridine (4-VP), was firstly investigated with quantum chemical DFT-calculations at Perdew-Burke-Ernzerhof (PBE) level of theory. An interaction energy of $\Delta E = 125 \pm 50$ kcal•mol^{-1} was qualitatively estimated. This result places the binding strength somewhere in between a hydrogen- and a typical chemical bond, illustrating the suitability of 4-VP as a monomer for a functional MIP for VPA. As mentioned previously, the principle challenge in MIP manufacturing is the optimisation of the ratio of template-to-monomer-to-crosslinker.

According to the two-dimensional factorial design mentioned previously in '1.2 Molecular recognition and assay reagents', nine different polymer compositions (both imprinted and non-imprinted) were synthesised on a glass slide. The VPA used in the synthesis was removed from the polymers by means of heating the polymer in methanol to 50 °C for 45 min. To test the efficacy of the synthesized MIPs in binding VPA, the polymers (both MIPs and NIPs (Non-Imprinted Polymers)) were incubated overnight in a solution of 100 ppm VPA in methanol. After removing the polymers from the solution, the concentration of VPA left in the solution was measured quantitatively, using LC-MS, according to a method by Matsuura *et al*.[14] Due to the thickness of the synthesised polymers, it was not possible to obtain reliable data regarding the binding coefficient in this manner, although this could be solved by altering the (bulk) synthesis in various small ways. It was observed however, both by LC-MS and Raman measurements, that by heating the polymer, VPA is released, thus indicating the presence of weak chemical bonding, presumably within dedicated pockets of the polymer. This is observed both with bulk synthesized polymers, as well as with MIP-coated AuNPs (AuNPs@MIP).

2.3 Nanoparticle synthesis and coating protocol

The size and the surface composition of the gold nanoparticles represents a challenge in order to have a proper SERS signal.[15] When the surface is intentionally modified, it is important to ensure that the coating of the nanoparticles is homogeneous and complete.

The physicochemical characterization of the coated AuNPs with the molecular imprinted polymers (AuNPs@MIPs) was accomplished by thermogravimetric analysis (TGA). This technique only requires a simple preparation of the sample, i.e. drying it. For TGA, the samples are heated to high temperatures while the mass of the sample is recorded. The outcome is represented by a decomposition curve, which tells the purity yields, the composition of the material and the oxidation temperature (when the bulk of the material starts decomposing). The residual mass is due to the AuNPs and perhaps some impurities in the sample. The temperature range for heating the sample was between 25 °C and 750 °C. Two different samples were analysed – AuNPs directly polymerized with 4-VP (AuNPs@MIP) (Figure 2a) and AuNPs first functionalized with arginine and coated with 4-VP (Figure 2b). Figure 2a shows that the first transition at 100 °C is probably due to some residual water (3.8% weight loss) within the sample. At 150 °C, it can be observed a first degradation of the polymer shell, where the components are starting to degrade and changing the inner structure (7.5% weight loss). The second transition at 300 °C yields to almost a complete degradation of the polymer shell. The final part of the curve does not show changes by raising the temperature – the remaining sample is made by only AuNPs. Figure 2b depicts the TGA curve of AuNPs functionalized with arginine and then coated with 4-VP. There is a big variation at 100 °C, probably due to some water left in the sample. At 300 °C there is a drop,

due to the changes in the polymer structure and the degradation of the polyvinyl pyridine (PVP). After 400 °C, the curve remains steady in both graphs because there are no longer changes and the temperature does not affect the AuNPs. The variation in the curves is due to the differences in the weight of the samples, and in their manufacturing. From these two figures, one can assume that the change at 200-400 °C reflects the degradation of the polymer shell.

In Figure 2a, in the range between 100 °C and 280 °C (orange box), the average weight curve shows different peaks, which are not visible in Figure 2b at the same temperature ranges. These chemical changes could be dependent on the packing of the material or integrity of the coating. By looking at the derivative of the weight (green peaks) in the region between 100°C and 280°C, the peaks show that by increasing the temperature, there is a change in the structure of the polymer shell. These changes occur mostly in the AuNPs not functionalized with arginine. From these results, one can assert that in the AuNPs@MIP sample, first functionalized with arginine, the coating is much more homogeneous (see hypothesized drawings in each graph), underlying that the nanoparticles' functionalization with arginine yields a better coating.

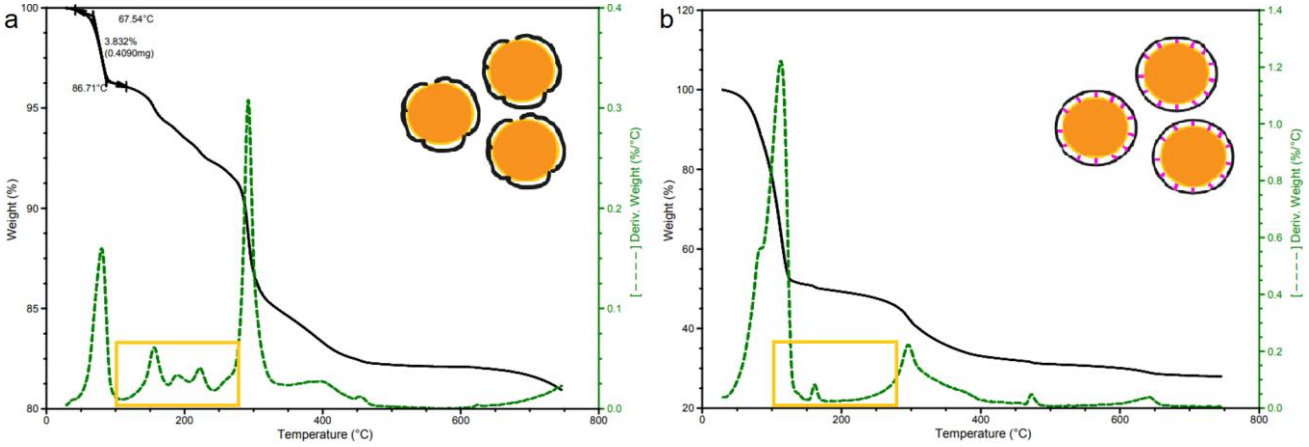

Figure 2. TGA graphs of the coated gold nanoparticles. a) The TGA curve of the AuNPs coated with MIP (illustrated in top right corner). b) The TGA curve of the MIP coated AuNPs previously functionalized with arginine (illustrated in top right corner). The black line represents the weight curve (%). In green it is shown the curve of the derivative of the weight.

2.4 Calibration of the Raman system

Due to time limitations, no effective calibration has yet been performed to be able to quantify VPA in the targeted therapeutic range. However, the software is set up and ready to record the corresponding information for further data analysis and then to use the obtained calibration curve during live measurements. For each analyte and its corresponding particle with different selectivity MIP coating, a different calibration has to be performed prior to use. Due to potential variation between each sensor device produced, these calibrations need to be performed and certified prior to shipment out of the production line. The next paragraph describes how the calibration is performed, applicable to a variety of different particles.

The operator starts by turning on the calibration mode on the device. Here, every measurement parameter has to be defined according to results obtained during the development of the particles. These parameters consist of both hardware and data analysis variables. Examples of these parameters are peak position, integration time, laser power etc. Samples are subsequently measured by our sensor, which then prompts for the corresponding concentration to be saved. Blank samples containing no analyte should be used in-between every measurement. Generally, no additional internal standard is necessary since the well characterized MIP coating itself is used for that. The concentration range should at least envelope the targeted concentration range to be measured by the device later on, otherwise an error will be raised. After completing the calibration measurement, the saved data is analyzed and a regression curve is drawn as reference for all future measurements. As a result, we obtain a calibration formula corresponding to this curve for analyte concentration as a function of Raman signal peak height. When creating a particular automated measurement protocol or profile, a regression curve has to be referenced to be used in those cases.

3. Originality

3.1 Team perspective

Our sensor can be segmented into four areas of innovation: The molecularly imprinted polymer, the coating protocol, the microfluidics chip and the signal enhancement through Surface Enhanced Raman Spectroscopy. Our team came up with a novel MIP selective for VPA and a corresponding synthesis protocol through literature research and quantum chemical calculations. There are several literature examples for MIPs selective for comparably sized molecules but none for valproic acid.

As this is a novel polymer composition we had to develop our own coating protocol by comparing similar examples from literature. The protocol is similar to that of Ren *et al.*[9] but uses a different crosslinker – arginine – as the one used in their research project was not compatible with our gold-based approach. Functionalization of AuNPs was achieved with arginine, inspired by a paper by Zhao *et al.*[16]

The microfluidic chip was derived from the design by Kamińska *et al.*[13] We adapted their mixing step and designed a separation step based on the common h-channel archetype. The length and pump-strength for the h-channel was optimised using our own COMSOL simulations and thereafter experimentally confirmed.

SERS is already a well-established measuring technique, so this aspect of the sensor is not innovative per se. However, the entire setup was assembled by our own team from parts either provided by our generous sponsors or ones 3D-printed in our own facilities.

Parts provided by sponsors include a green 532 nm laser (Cobolt o8-DPL) from Cobolt AB, a Raman probe (SPS-R) from Spectra Solutions Inc. and a micro-spectrometer (VersaPic) from Ibsen Photonics.

All the entrepreneurial aspects of this work were contrived solely by motivated members of our team. Of particular interest in regard to this aspect of our work, is our biosensors ability to be equipped for multiple analytes and the analysis of pure blood samples.

Anna Capria
Team Captain

Dianna Zeleskov
Team Captain

3.2 Supervisors' perspective

The team has come up with all the ideas themselves and the combination of the biomaterials and technology used to build their biosensor are novel. The students have been proactive discussing with some experts in different fields in order to be able to fabricate the different parts of the biosensor. However, the students can take all the credit for conceiving, recognizing, selecting, adjusting, and testing their biosensor. They have also been very successful getting partners, who contributed with small pieces of equipment that could be integrated together.

As mentioned in the team section, the students found an article by Ren *et al.*[9], where silver nanoparticles were coated with a molecularly imprinted polymer, and were used as SERS substrate for measuring bisphenol A. The students were inspired by the article to try the SERS technology to evaluate valproate. The use of MIP as the biorecognition element of valproate as well as the SERS technology to quantify valproate are novel, and to the best of our knowledge have not been previously reported.

The supervisors confirm hereby that the statements made by the team and by ourselves are true.

Gemma Mestres
Coach of UppSense

Masood Kamali-Moghaddam
Supervisor of the team

4. Translation potential

4.1 Business model canvas

Please find the charts and figures for our business model canvas in Appendix 8.3.

4.2 Stakeholder desirability

Inadequate drug dosage may negatively affect patients due to ineffective treatment or overdose and toxicity, leading to long-lasting health repercussions. This issue particularly affects patients whose well-being depends on the blood concentration of the active compound. High interindividual variability of this concentration makes it difficult for pharmaceutical companies and doctors to prescribe global treatment dosages. This represents a challenge for them in order to optimize their product or to efficiently treat the patients. As a result, monitoring is necessary on a regular basis and patients need to take blood tests at the hospital every few weeks or months, depending on their situation. Patient surveys and interviews with healthcare personnel about valproate treatment for epilepsy have confirmed that this is a strong inconvenience and they would like to improve this part of their treatment plan. However, these tests do not seem to be required frequently enough for them to justify costly at-home alternatives.

In order to facilitate small molecule drug monitoring, UppSense has developed a diagnostic tool to be used as a first instance in primary care units. Subsequently, we envision to sell our product to a broader market of diagnostic laboratories (such as those in hospitals and research facilities), but also to pharmacies in order to offer the patients a solution closer to home. Later, we would reach out to quality control, environmental control, and forensic laboratories for testing of other compounds as described further below. Unlike big assays currently performed at hospitals' analytical testing facilities, our device has several advantages. One is that it is user friendly – there is no need to train and invest in technicians since nurses can directly run the tests themselves. Our solution is fast, without the need for sample preparation or start-up delay and with a sample-to-result time of less than five minutes, so patients do not need to wait several hours for their results and can directly discuss these with the doctor while they are still in the hospital or primary care unit, shortening time to effective feedback. Results and data from the measurements can then easily and safely be saved, shared and communicated to all involved parties selected by the patient, which could include relatives and research groups. Some interviewees have pointed out this possibility in order to facilitate and promote research and development towards better treatments. The device is also small and can be a portable table-top instrument – it can easily be moved closer to the patient and where it is most needed, allowing for more flexibility.

Another asset of our approach is the use of custom-made MIP to achieve selectivity and specificity. By changing its nature and/or structure, the SERS particles can be designed to detect a versatile selection of biomarkers and compounds. Different cartridges will thus be produced according to diagnostic or measurement necessity. By either accepting different cartridges at once or by swapping between them through a plug and play interface, the device will become a modular and powerful platform. In the end, this advantage translates to enhanced value of the device because of the interest from pharmaceutical companies to be able to develop a new compound building on an existing platform of rapid and reliable diagnostics. Hence the important collaboration with MIP development companies and contract research organisations, but certainly also with the pharma industry. This synergy is beneficial for all parties involved, making it easier for the companies to penetrate the market, interesting for hospitals to have a single multi-functional device for different purposes and different patient profiles, and of course also valuable for the patients knowing that early on, they will be supported by an already established infrastructure. The benefits reach all the way to insurance companies and governmental institutions thanks to the increased accessibility to better and faster healthcare. According to our interviews with a professor in clinical chemistry (Anders Larsson), these points are considered by the hospital management and have an essential influence when analytical and diagnostic laboratories choose to buy new devices or invest in new equipment. By impacting the healthcare system at these levels, value is created in a much more direct and impactful manner.

4.3 Business feasibility

In order to be competitive and create value through collaborations with our partners, UppSense will focus on two main aims. First, we aim to create an impactful customer portfolio to penetrate the market, primarily focused on healthcare. Second, we want to diversify our market share offerings by expanding our product platform. These two approaches are described below.

A big part of research and development for the device is already done, but still needs to be refined. A crucial issue in that regard, though sometimes criticized by professionals in the health-tech sector we consulted with, will be to obtain the CE label. Most of our efforts in this phase will thus be focused on building a robust hardware for enabling later integration of new modules. After a round of small-scale testing, we can envision a first phase by selling our diagnostic sensor to 1% of the European hospitals. Reaching out to those clients will be outsourced to specialized hospital sales representatives. Financial resources will be the major challenge for this expansion, as described in the financial viability section. Regarding UppSense's human resources, the technical and scientific team will be retained from the current project to gain from their experience. These profiles include, but are not limited to, experts in polymer chemistry and biology for assay development, microfluidics engineers for cartridge development, spectroscopy experts for device hardware development, and also technological and biomedical experts, IT engineers, and a non-technical manager for support throughout. On the other hand, both sales, marketing, and financial personnel will be paramount to hire in order to grow the business and get into the market as intended. An advisory board will be assembled as well, probably based on the contribution section found in the next chapter of this report.

Collaborations and partners who are willing to further develop the product with us will enable us to fast-forward through the hardware development. Current partners are Ibsen Photonics, Cobolt Lasers and Spectra Solutions, who showed trust in us and gifted a spectrometer, a laser and a Raman probe, respectively. We would use their instrument technology to build and improve our device performance, while they have a regular customer with supplier exclusivity – a win-win situation. The envisioned sales target (see the financial viability section for details) seems reasonable in terms of production scale-up and supply chain capabilities. The relationships could eventually be strengthened by licencing out some of our technologies for them to develop their own product offer.

As device production was the central key activity described above, a second and long-term key activity will be under development of different MIP-coated SERS particles for different cartridges in order to bring a wide range of analyte detection possibilities to the market. In contrast to strong sales and marketing necessary in the first phase, most resources will subsequently be directed towards R&D. A greater share of scientific profiles will be necessary. Being located near a university and a scientific research park, recruitment will not be a difficult challenge. Furthermore, Uppsala University could support our company in its early stages through the university's holding company's support scheme for start-ups and spinouts, called UU Innovation. One particular aspect that is of interest to us is the collaboration with Cytiva to accommodate us at the Testa Center, providing infrastructure and support, helping to balance our expenditures and to scale up our production. Previous sales will steadily become sufficient to support the financial costs of R&D investment. With the existing platform, which is the sensor device itself, selling the cartridges will increase the overall value of the device. Accounting for the raise in pharmaceutical compounds, and the growing market for biosensors which is expected to reach $ 31.5 billion in 2025 globally with a CARG of 8%, from which more than 52 % was non-wearable in 2018, UppSense will be well positioned in that sector.[17] One major challenge besides fierce competition will be the strict regulatory context.

4.4 Financial viability

Our commercialization strategy will consist of two parts – a direct per-unit sale of the sensor device to the customers, including customer support services and necessary training, and a subscription-based service for the cartridges, allowing cartridge selection in terms of analyte and use frequency according to the customer's own specific needs. As mentioned above, we will target 1% of the European hospitals as a first instance. This still accounts for over 140 entities throughout Europe, mainly focused on the biggest hospitals in the countries with highest public health expenditures which includes Luxembourg, Netherlands, Sweden, Switzerland, Denmark, Germany, Austria, France, and Belgium.[18] We are conscious about the difficulty to sell a device to those parties which are more difficult to convince, but the advantage is the increased sales price we can charge them, and the continuous cartridge demand attached with this approach. A target of 140 units sold over a period of four years seems thus as a reasonable expectation, with an average of ten days of manufacturing and testing for conformance certification for every device and an average of three devices sold monthly. Being priced at €20,000 EUR with still 16 % benefit, our device could be considered low-cost, as medical diagnostic devices in analytical laboratories usually cost up to two times more according to our interviews. Further development is also expected to cut down production prices, increasing the benefit margin. Our significant income however will come from the cartridge sales. These multi-use consumables will be priced at €800 for

200 usages and 40% benefit for UppSense. For example, with VPA measurements at Uppsala University Hospital, the gross cost of the diagnostic will be reduced by 18% while roughly maintaining similar reimbursement policies for patients, going from €7.34 to €6.20. The calculations are in Appendix 8.4.

The development required for having different cartridges with different MIP particles represents most of our continuous R&D budget at UppSense. Maintenance of patents for IPR correspond to approximately €50,000 in expenses every year. Other recurrent and extraordinary costs for investments must be considered as well, such as outsourcing. On the other hand, private capitals and governmental or European grants for healthcare development can help us with initial investments. The treasury will stay positive and thus liquidity is assured if during the first round of financing we can raise a minimum of around € 700,000. A financial outlook with calculations is available in Appendix 8.5. Please note that these calculations are simplified and do not take into account initial funding and investments like property, laboratory buildings and instruments, fees etc. and have to be mentally shifted upwards – yet we hope they reflect the operational financial evolution of the company. Estimates consider a one-year period without sales while the product is refined, tested, and approved for commercialization.

Financial viability is assured after 2.5 years, while break-even point is difficult to estimate due to the lack of information about initial investments and funding. The principal revenue stream is obviously the cartridge consumable, which will exist in different types according to the analyte and thus boost the value as usage of our biosensor platform.

5. Team and support

5.1 Contributions of Team members

Anna Capria: Team captain, administration, patient surveys, note taking.
Bono Jimmink: Initial prototype development, MIP synthesis, computer simulations, measurement and verification, note taking.
Carmen Abaurre: Biosensor development, Raman hardware, note taking.
Cédric de Voghel: Raman hardware and software, sponsor recruitment, administration, business plan development.
Daniel Friesen: Microfluidics production and theoretical work.
Dianna Zeleskov: Team captain, administration, AuNPs production and development, MIP synthesis, microfluidics production.
Florian Dietrich: Initial prototype, MIP synthesis development, computer simulations, administration.
Francesca Diletta Spagnuolo: Originator of the sensor design, initial prototype development, AuNPs and MIP development. Design: the logo, business cards and t-shirts.
Gustav Ahlström: Microfluidic designs and production, social media, Instagram takeover, sponsor recruitment.
Imali Ranatunge: Patient surveys and patient contact, AuNPs production and development.
Junyu Chen: AuNPs production and development, social media,
Konstantinos Fragkoulis: AuNP production and development, social media, business plan development.
Viktor Åkerfeldt: Microfluidic designs and production. Design: social media, posters and logos.

All team members have actively contributed to literature search and brainstorming for different prototypes of biosensors, writing this report and preparing the pitches.

5.2 People who have given support

We at UppSense would like to show our gratitude towards all who gave us feedback and supported our endeavors, making our sensor a reality. We would like to highlight the following people: Abdul Raouf Atif for all his support and guidance throughout the whole project thanks to his previous experience in the SensUs competition and his implication and access to the university laboratories. Jacinto Sá for his advice and assistance, especially on SERS and Raman and the information he provided regarding nanoparticles as well as the guidance to improve our experiments. Ehsan Manouchehri Doulabi for his assistance in the biology lab, help with lab equipment and support provided during the Alternative Testing Event. Luke Robertson for providing expertise and instrumentation regarding LC-MS measurements. Jöns Hilborn and the Polymer Chemistry personnel for supporting us by providing the facilities for MIP synthesis and AuNP development. Ann Magnuson for providing facilities in her laboratory and use of the centrifuge. Anders Larsson for the background information provided in the hospital validation process and for support for the Alternative Testing Event. Federico Cantoni for help with clean-room access for microfluidics production. Gemma Mestres and Masood Kamali-Moghaddam for the project supervision and assistance provided in myriad ways – thank you for arranging lectures on epilepsy, team building and the hospital analytical lab background. As well as for the help given to us in ordering chemicals and the support provided during the entire project.

5.3 Sponsors

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6. Final remarks

For access to laboratories, devices and materials we thank Uppsala University for giving us the tools to turn our ideas into reality and the SensUs organization for creating a way for teams from different countries to connect with each other in order to reach the same target. We have learned the steps that should be taken in order to develop a biosensor – from the scientific and technical part to the business part. Science is the backbone, but without a business/entrepreneurship part, it would not be possible to share it, to let people exploit it and improve it. Thanks to this project, we had the opportunity to overcome our boundaries and to experience what we could not learn in a regular class. We learned new techniques, new methods and got the opportunity to make our own observations and turn them into prototypes. Thus, this gave us a starting point to improve our abilities and capacities even more and direct each of us in the path we will take in the future.

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8. Appendix

8.1 Microfluidics chip

The microfluidics system has two inlets (1 and 2 in Figure 1) and two waste outlets (3 and 4 in Figure 1). Inlet 1 is for patient sample and inlet 2 for AuNP-solution. The patient sample is first cleaned by diffusion (channel A, Figure 1) – separating the smaller, fast-diffusing VPA (into channel B, Figure 1) from larger proteins in the sample (outlet 3, Figure 1). Then, the cleaned plasma is mixed with the AuNP-solution (channel B, Figure 1). VPA mixed with AuNPs is led to a detection area (C in Figure 1), where the laser detects the level of VPA in the plasma sample.

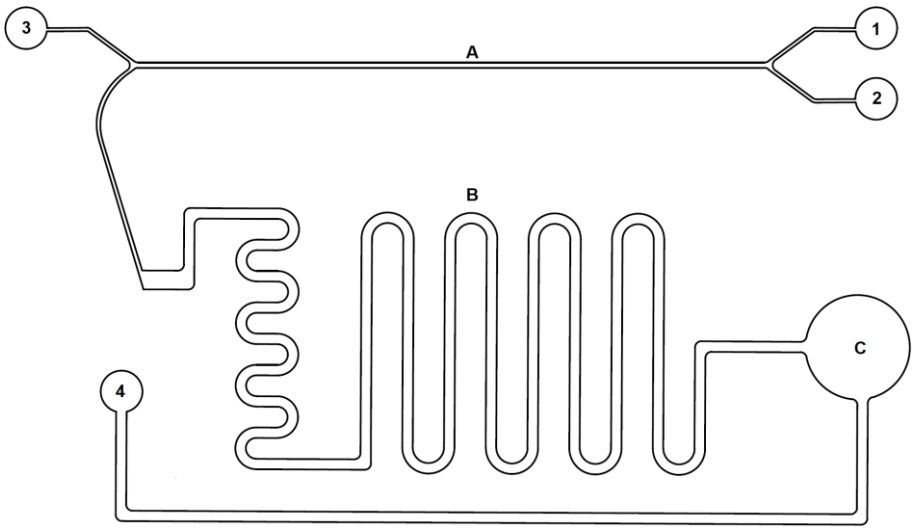


Figure 3. Microfluidics device design. 1) sample input; 2) NP input; 3) waste (proteins and large components in blood); 4) waste, AuNP outlet; A) cleaning of plasma sample; B) mixing step; C) laser detection area.

8.2 COMSOL simulated diffusion

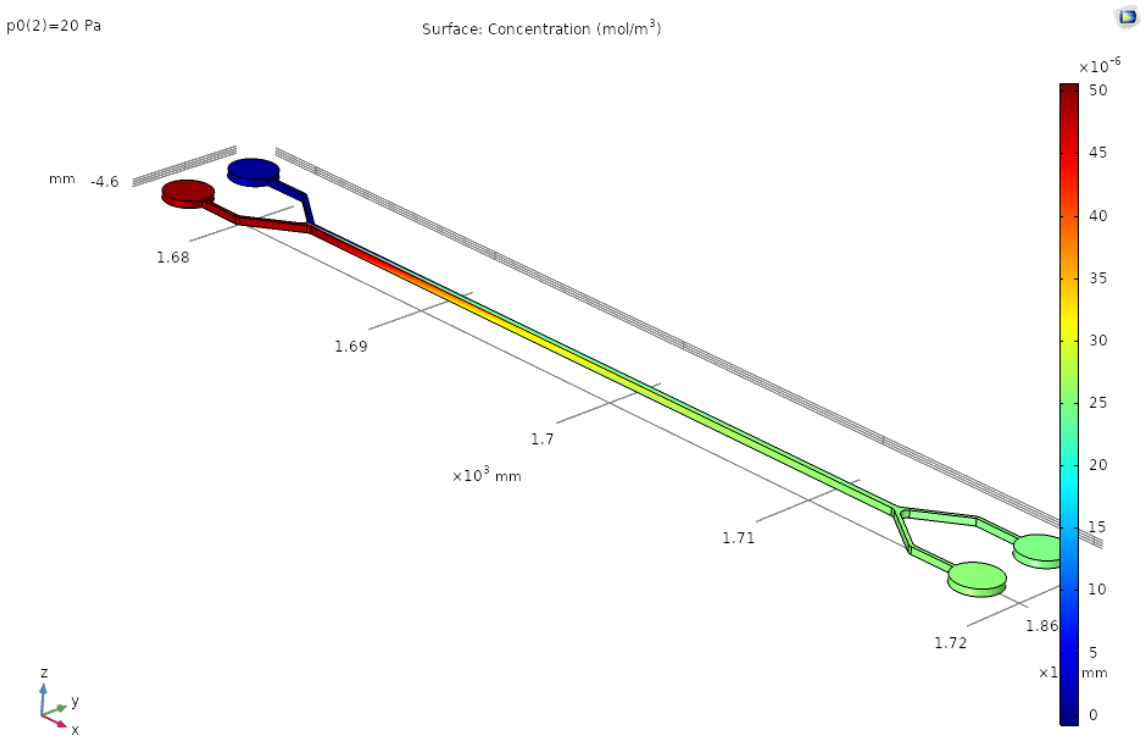
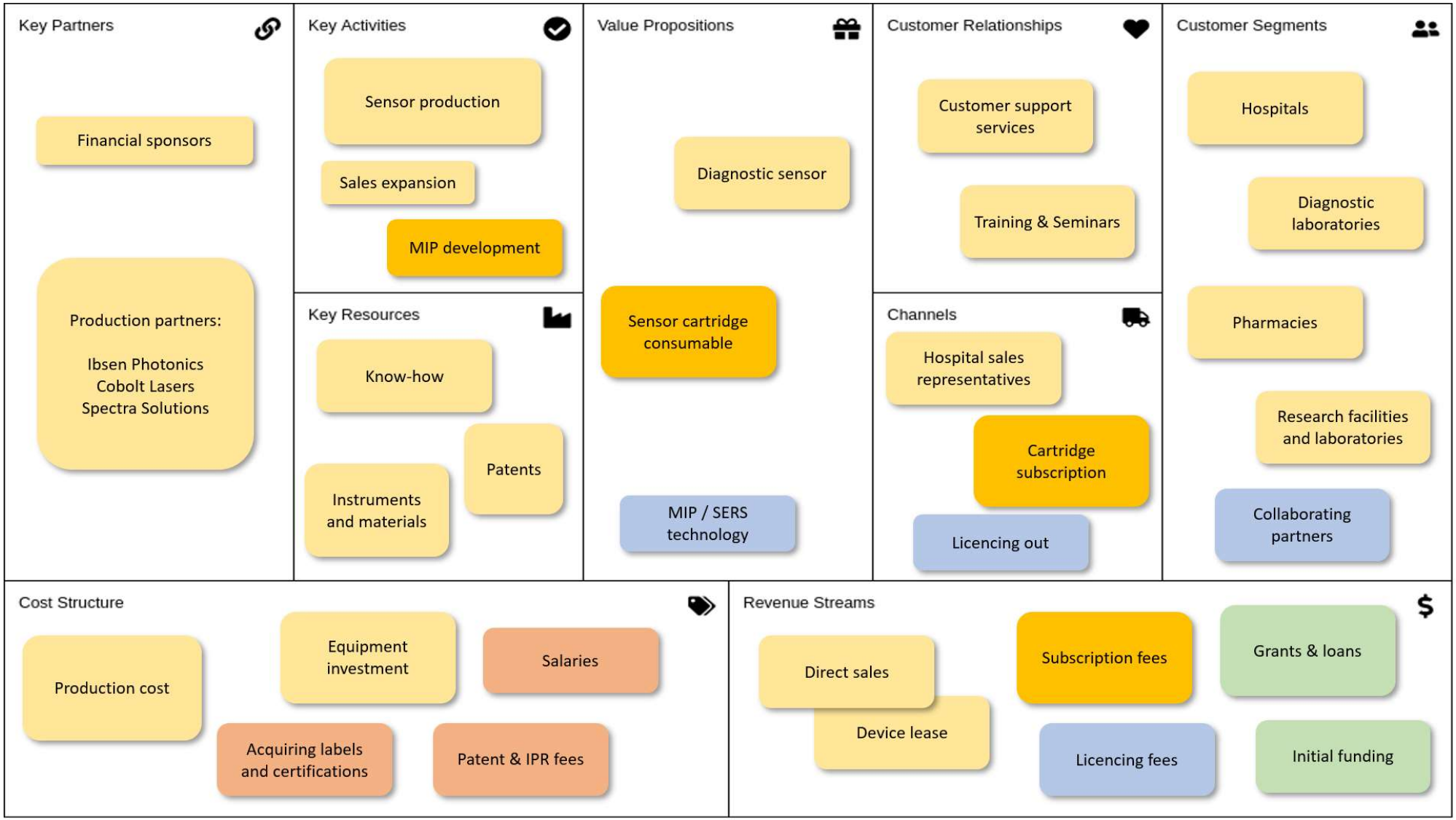


Figure 4. Excerpt of the parametric sweep showing that an applied pressure difference of 20 Pa to our chip results in a complete (50%) transfer of VPA from the serum to the buffer phase.

8.3 Translation potential: Business model canvas



8.4 Translation potential: Calculations for sensor device, cartridge, and measurement costs

Device cost

Reference	Supplier company	Instrument or device description/information	Price	Comment
Spectrometer	Ibsen Photonics	Freedom HR-VIS-NIR FHT-315 Spectrometer	4,295 €	unit order
			3,436 €	frame order x20
			3,093 €	frame order x50
			2,783 €	frame order x100
Laser	Cobolt Lasers	Cobolt 08-DPL 532 nm 160 mW Laser (with isolator and MM fibre coupling 80051)	9,850 €	unit order
Probe	Spectra Solutions	Standard Raman Probe 532 nm	2,565 €	unit order
			2,489 €	frame order x10
			2,437 €	frame order x25
			2,309 €	frame order x50
Microfluidic instruments	-	*Microfluidic PDMS chip and pump*	600 €	*unit estimation*
Computer and interface	Raspberry Pi Foundation	Raspberry Pi 4 Model B *and* 7" Touchscreen LCD	170 €	unit order
Other	-	*Includes casing, wiring, device fixation and others*	100 €	*unit estimation*
Work time			600 €	
Total			**18,180 €**	**estimated unit cost**
			16,721 €	**estimated batch x50 cost**
			20,000 €	final price (16 % benefit)

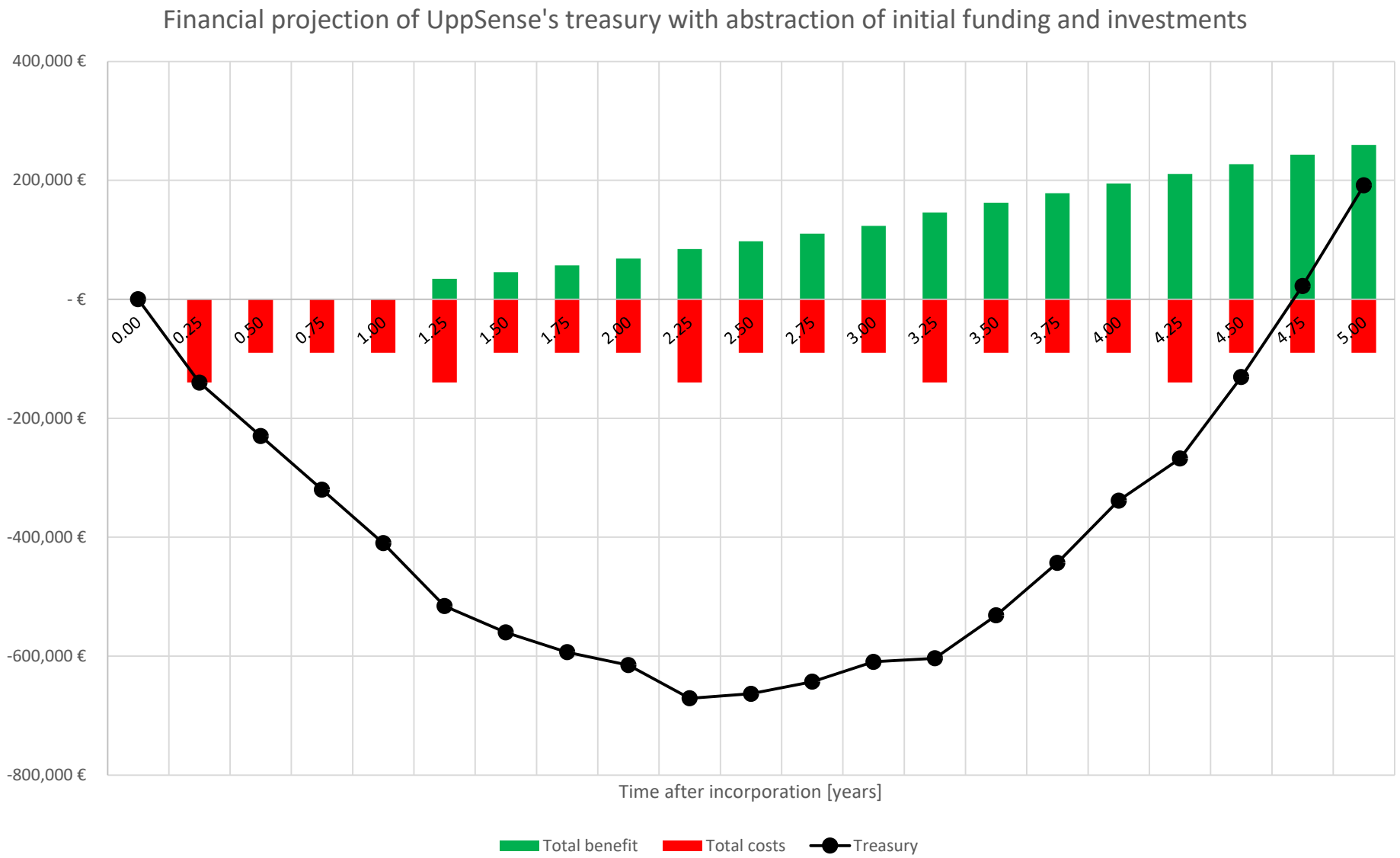
Cartridge cost (200 usages per cartridge)

Reference	Element description/information	Price	Comment
AuNP	Gold nanoparticle solution	22 €	*1 L estimation*
MIP	Molecularly imprinted polymer	5 €	*1 L estimation*
Synthesis chemicals	*For production and synthesis of particle solutions*	10 €	*1 L estimation*
Other	*Includes casing, flush solvents and others*	8 €	*unit estimation*
Work time		900 €	
Total		**477 €**	**estimated unit cost**
		800 €	final price (40 % benefit)

Measurement cost

Reference	Information	Price	Comment
Device	Expected device lifetime with warranty is 5 years (approx. 20,000 measurements)	10.96 €	*Daily device value loss*
	Expected average use per day is 10 measurements	1.00 €	*Cost per measurement*
Cartridge	Cartridges are expected sustain 200 measurements	4.00 €	*Cost per measurement*
Work time		1.20 €	
Total		**6.20 €**	**estimated single measurement cost**
		7.34 €	current price 18 % more expensive

8.5 Translation potential: Financial outlook graph (1/2)



8.5 Translation potential: Financial outlook table (2/2)

Year	Trimester	Sensor device				Cartridge					Total benefit	Funding	Investment	Salaries	IPR	Other costs	Total costs	Treasury
		Units sold	Cost	Revenue	Benefit	New clients	Units sold	Cost	Revenue	Benefit								
0.00	0	0	- €	- €	- €	0	0	- €	- €	- €	- €	- €	- €	- €	- €	- €	- €	- €
0.25	1	0	- €	- €	- €	0	0	- €	- €	- €	- €			- 75,000 €	- 50,000 €	- 15,000 €	- 140,000 €	- 140,000 €
0.50	2	0	- €	- €	- €	0	0	- €	- €	- €	- €			- 75,000 €	- €	- 15,000 €	- 90,000 €	- 230,000 €
0.75	3	0	- €	- €	- €	0	0	- €	- €	- €	- €			- 75,000 €	- €	- 15,000 €	- 90,000 €	- 320,000 €
1.00	4	0	- €	- €	- €	0	0	- €	- €	- €	- €			- 75,000 €	- €	- 15,000 €	- 90,000 €	- 410,000 €
1.25	5	7	117,050 €	140,000 €	22,950 €	35	35	16,678 €	28,000 €	11,323 €	34,273 €			- 75,000 €	- 50,000 €	- 15,000 €	- 140,000 €	- 515,727 €
1.50	6	7	117,050 €	140,000 €	22,950 €	35	70	33,355 €	56,000 €	22,645 €	45,595 €			- 75,000 €	- €	- 15,000 €	- 90,000 €	- 560,132 €
1.75	7	7	117,050 €	140,000 €	22,950 €	35	105	50,033 €	84,000 €	33,968 €	56,918 €			- 75,000 €	- €	- 15,000 €	- 90,000 €	- 593,214 €
2.00	8	7	117,050 €	140,000 €	22,950 €	35	140	66,710 €	112,000 €	45,290 €	68,240 €			- 75,000 €	- €	- 15,000 €	- 90,000 €	- 614,973 €
2.25	9	8	133,771 €	160,000 €	26,229 €	40	180	85,770 €	144,000 €	58,230 €	84,459 €			- 75,000 €	- 50,000 €	- 15,000 €	- 140,000 €	- 670,514 €
2.50	10	8	133,771 €	160,000 €	26,229 €	40	220	104,830 €	176,000 €	71,170 €	97,399 €			- 75,000 €	- €	- 15,000 €	- 90,000 €	- 663,115 €
2.75	11	8	133,771 €	160,000 €	26,229 €	40	260	123,890 €	208,000 €	84,110 €	110,339 €			- 75,000 €	- €	- 15,000 €	- 90,000 €	- 642,776 €
3.00	12	8	133,771 €	160,000 €	26,229 €	40	300	142,950 €	240,000 €	97,050 €	123,279 €			- 75,000 €	- €	- 15,000 €	- 90,000 €	- 609,497 €
3.25	13	10	167,214 €	200,000 €	32,786 €	50	350	166,775 €	280,000 €	113,225 €	146,011 €			- 75,000 €	- 50,000 €	- 15,000 €	- 140,000 €	- 603,485 €
3.50	14	10	167,214 €	200,000 €	32,786 €	50	400	190,600 €	320,000 €	129,400 €	162,186 €			- 75,000 €	- €	- 15,000 €	- 90,000 €	- 531,299 €
3.75	15	10	167,214 €	200,000 €	32,786 €	50	450	214,425 €	360,000 €	145,575 €	178,361 €			- 75,000 €	- €	- 15,000 €	- 90,000 €	- 442,937 €
4.00	16	10	167,214 €	200,000 €	32,786 €	50	500	238,250 €	400,000 €	161,750 €	194,536 €			- 75,000 €	- €	- 15,000 €	- 90,000 €	- 338,401 €
4.25	17	10	167,214 €	200,000 €	32,786 €	50	550	262,075 €	440,000 €	177,925 €	210,711 €			- 75,000 €	- 50,000 €	- 15,000 €	- 140,000 €	- 267,690 €
4.50	18	10	167,214 €	200,000 €	32,786 €	50	600	285,900 €	480,000 €	194,100 €	226,886 €			- 75,000 €	- €	- 15,000 €	- 90,000 €	- 130,803 €
4.75	19	10	167,214 €	200,000 €	32,786 €	50	650	309,725 €	520,000 €	210,275 €	243,061 €			- 75,000 €	- €	- 15,000 €	- 90,000 €	22,258 €
5.00	20	10	167,214 €	200,000 €	32,786 €	50	700	333,550 €	560,000 €	226,450 €	259,236 €			- 75,000 €	- €	- 15,000 €	- 90,000 €	191,495 €

5	Cartridge use per trimester
10	Employees
2,500 €	Average salary
5,000 €	Outsourcing
50,000 €	Patent rights cost