# UppSene Team Results Document



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UppSense

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## Summary for the SensUs website

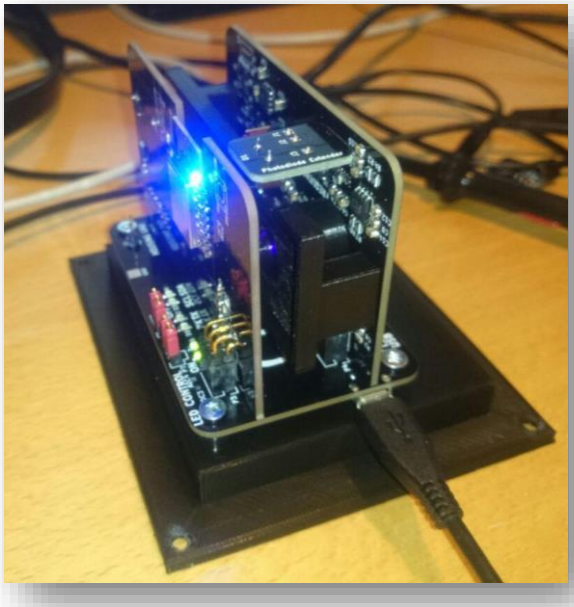

*Figure 1. Sensor device connected to computer via USB.*

Leveraging COTS (commercial of-the-shell) components for the biosensor device have driven the individual device manufacturing costs to a minimum of 35 € without neglecting precision and reliability demands. Furthermore, a user-friendly interface was an important design guideline. This device crafted in the forges of Uppsala University fits in a grandma's handbag. Ground-breaking IoT (Internet of Things) capabilities enable a doctor not having to stay in the same place where the measurement is carried out, but instead judging each single case remotely with a nurse send out to the patient.

UppSense does not only offer a readout device but a complete sensor system consisting basically of a newly developed assay, an in-house designed microfluidic system and finally a readout device. The assay is based on specific binding of two monoclonal antibodies to the biomarker, NT-proBNP. A disposable cartridge is used as a platform where the reaction and the measurement of fluorescence intensity take place. Within the device the sample is excited with one wavelength and the generated fluorescence in another wavelength is received and quantified. The output of the sensor system is the biomarker's concentration that is present in the plasma sample. A simple smartphone can be used to retrieve this value.

Albeit the in this document presented sensor is in a strict sense work in progress, UppSense already foresees in this early stage the potential, that this biosensor system could have an impact on society and health care due to its revolutionary aspects in terms of price, size and user-friendliness.

# 1. Biosensor System and Assay

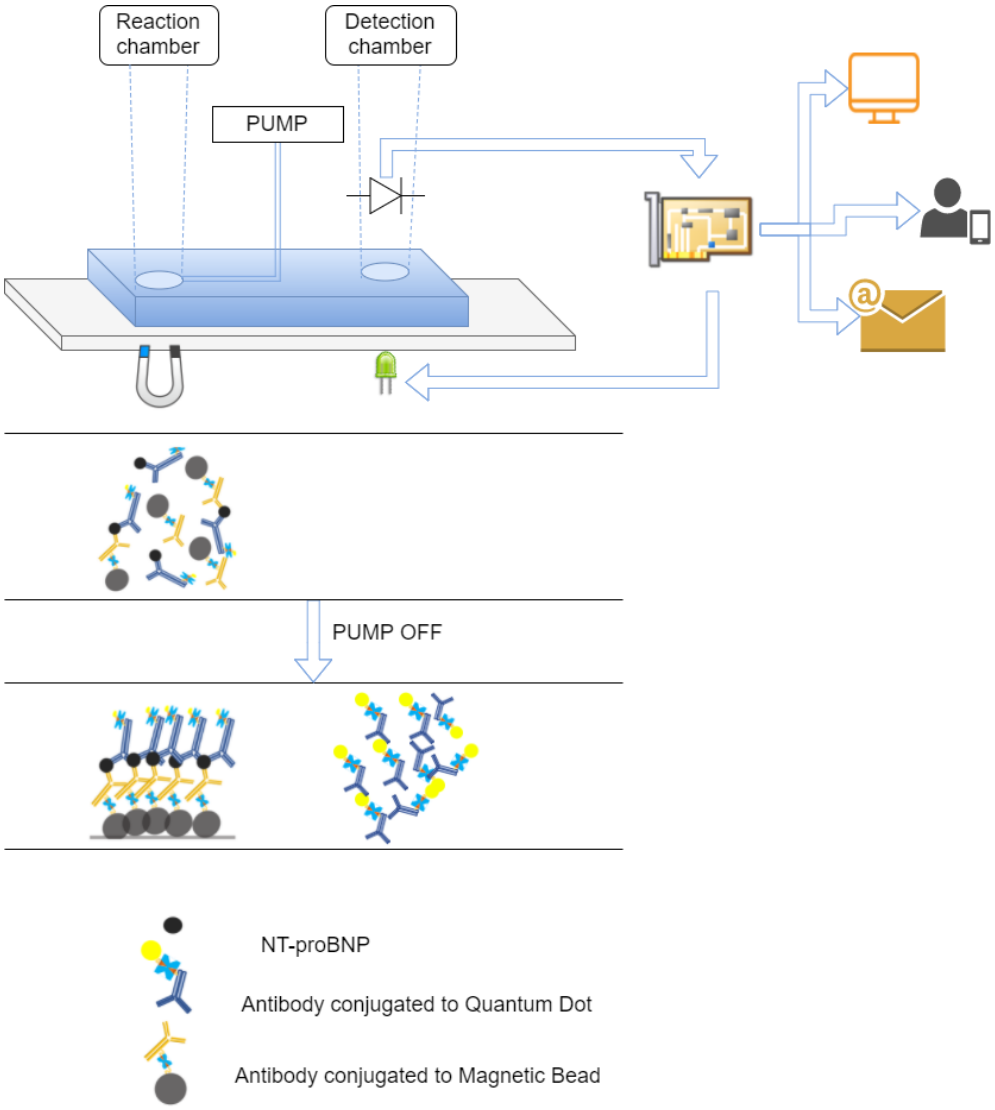


*Figure 2Full concept summarized in one picture.*

One of the major aspects of the biosensor, the assay, is based on specific binding of monoclonal antibodies and NT-proBNP. To provide high specificity in the detection, two antibodies have been used: 15C4 and 13G12 (#4NT1, HyTest).

The primary antibody 15C4 is conjugated with magnetic beads (#65001, Invitrogen); the secondary antibody 13G12 is conjugated with quantum dots (#Q10173MP, Invitrogen), which gives a fluorescence value with a peak at 800 nm (IR) when excited with 400 nm (UV) light.

In both cases, the conjugation between quantum dots / magnetic beads to the antibodies is achieved by biotin-streptavidin interaction.

Secondly, there is a need for a microfluidic system in order to integrate the assay and engineering part: It provides the infrastructure to mix and incubate antibodies with the biomarker and it furthermore is compliant to the fluorescence readout arrangement of the sensor device.

Polydimethylsiloxane (PDMS) and a glass slide are the two materials used for the microfluidic system which therefore makes it the solely disposable part of the sensor chain. It contains a reaction chamber, detection chamber, a channel and a small permanent magnet (located underneath the reaction chamber) (cf. Figure 2).

The sample and the antibodies are injected into the reaction chamber. Mixing is achieved by the propagation of air bubbles through the system. Once it is mixed, the pump is turned off and the solution remains untouched for the incubation time. During this time, proteins precipitate to the bottom. The particles which are conjugated to the magnetic beads are dragged to the bottom of the chamber due to the presence of the magnetic field. Once magnetic particles are at the bottom, the solution can be removed and conducted to the detection chamber, which consists of a small well.

Therefore, the sensor device makes use of the magnetic beads to enhance the precipitation of linked antibodies with biomarker and it also allows separating them from the non-binded antibodies conjugated with QD.

Thirdly, the engineering part comprises three major elements that harmonize perfectly well in order to quantify the biomarker by measuring the light intensity emitted by the quantum dots.

Customized electronics are the first major element. Counting all prototypes of the in-house developed hardware the current sensor device is equipped with the fourth electronics revision. Basically, the electronics can be divided in an analog part and a digital part. In principal, a photodiode is monitored with a transimpedance amplifier on the analog board. Moreover, there is a circuit to steer an excitation light precisely (µAmpere range). It is a well-known fact, that nowadays to leverage sophisticated analog Hardware like this digital processing is crucial. Hence the analog board is hooked up to a digital board capable of A/D (Analog / Digital) and D/A (Digital / Analog) converting. Moreover, it monitors the whole system including the battery status and the temperature to predict (mainly Johnson-Nyquist) noise effects. With an active backplane, the connection between the two boards and the battery or a USB host is established.

Software, as a second part of the engineering work, is where the magic happens. A powerful low-cost WiFi chip, the ESP8266 deployed on the digital board with a radio and a fast 32-bit RISC microcontroller under the hood is what enables countless possibilities for creative programmers. The device provides moreover a full TCP (Transmission Control Protocol) / IP (Internet Protocol) stack and therefore enables a wireless and easy-to-use interfacing to the

internet. This facilitates all kind of IoT (Internet of Things) applications. One possible configuration which has been mostly used during development is that the user connects to a hotspot which is spawn up by the device which provides a web- and DHCP (Dynamic Host Configuration Protocol) server in this configuration. Another is to use MQTT (Message Queue Telemetry Transport) on the sensor to push measured values to a cloud which can be accessed by patient and doctor the same way (all real-time and without the need of the patient having to leave his home).

Thirdly the mechanical structures of the sensor device is an engineering group achievement. One might come to a quick conclusion and state, that those are not so important in the context of the goal to build a sensor to detect proteins. Concluding so would be half-truth only as one has to keep in mind that light is measured. Therefore, the whole electronics need to be encapsulated to get the most precise results and it has been decided to use IEEE 802.11 instead of cables to communicate the value out of the device.

## 2. Analytical Performance

Direct ELISA was performed at the very beginning of our experiments to validate that there are no effects on antibody-antigen interactions by Ab biotinylation (15C4: P=0.56079; 13G12: P=0.6139). Afterwards, sandwich-ELISA-like assays were performed with spectrometer (Infinite 2000, Tecan) to reassure the analytical methods. However, as quantum dots will be quenched to a certain degree, the spectrometer used was not able to give a full view of analytical curve. Instead, we used our method to detect the NT proBNP at 10^6 pg/ml and 10^5 pg/ml, for which was sufficient for validations. Results are shown in Figure 1Figure 3.

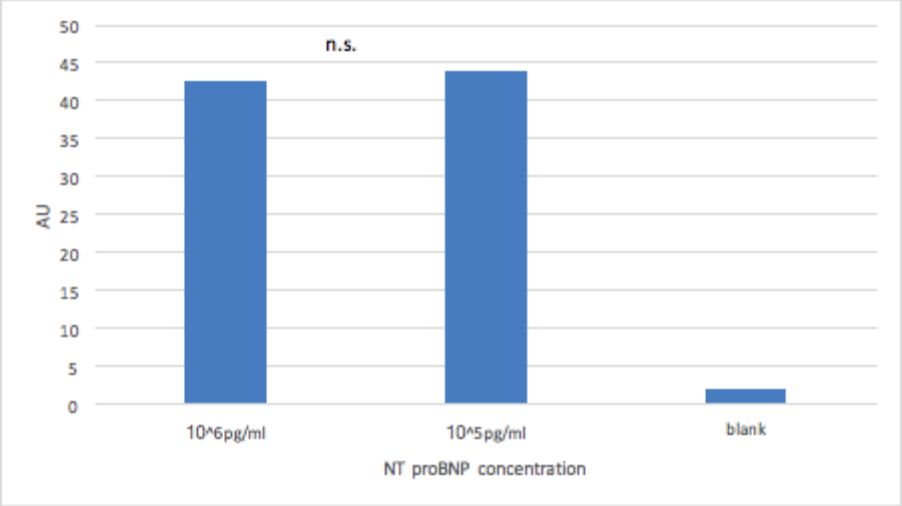


*Figure 3. Sandwhich-ELISA-like test readouts by spectrometer.*

Biotinylated 15C4 was coated onto the 96-well transparent plate; after overnight incubation and washing, certain amount of NT-proBNP in plasma was added in duplicates; same incubation and washing were applied after first adding biotinylated 13G12 and then quantum dots. The plate was performed by Infinite 2000 Tecan system. T test was performed, and there were no significant differences between 10^6 pg/ml and 10^5 pg/ml ($p = 0.423 > 0.05$), indicating that those two concentrations are reaching to limitation of detection of the normally used spectrometer.

The minimum detectable current is the femto ampere range since fancy guarding techniques have been used to avoid undesired coupling. So far, we can get down to a noise level of 2 pA without any signal processing techniques to filter out undesired frequencies. This noise is coming from undesired electric coupling. However, the actual most problematic noise contributor is the excitation light, which is coupling its signal into the receiver directly as well.

# 3. Novelty and Creativity

## 3.1. Already available

In the assay part of the in this document suggested method, the use of a pair of antibodies to capture and detect an analyte is applied in many assays, such as sandwich ELISA. Also, biotin-streptavidin interaction as a coupling mechanism between different molecules is widely spread.

The material used as disposable part of the sensor, PDMS with glass slide, is considered as the material of choice for fabrication of microfluidic devices due to its mechanical, low-cost and easy-handling features.

Whenever possible COTS (commercial off-the-shelf) components have been used to make an industrialization of the sensor device easier. As an example, one could mention a photodiode for remote control applications is leveraged. For this kind of applications, the photodiodes are already equipped with a IR filter.

## 3.2. New developments

Current immunoassays require long protocols, since they need time for immobilization and several washing steps. To shorten the incubation time, UppSense decided to take the approach to use magnetic beads for the assay. Hence the need of a matrix to immobilize antibodies is avoided.

One of the microfluidics requirements is a pump system that allows a specific flow speed inside the channel. Instead of using an injection syringe or high-pressure pumps that are very precise at high costs, a cheap and simple accessible aquarium air pump has been used. Together with the precisely calculated design of the channel, the right pressure inside the microfluidic system can be maintained.

Certain aspects of the sensor device introduce ground-breaking new approaches in comparison with available devices. Unlike traditional devices the readout circuitry is not based on a photomultiplier tube, but on a photodiode, which makes the device cheaper by a order of magnitude of four and also smaller. Moreover the IoT and digital processing capabilities are enabled through the low-cost chip the ESP8266 (roughly 1.5 €). Another aspect of the device where a lot of attention has been spend on is user-friendliness. This sensor is operable by amateurs who are monitoring the wellbeing of their heart at home. Coming to a conclusion one can state without hesitation, that the device can be produced very cheaply. One can even put a figure below this statement. 35 € is from our point of view a realistic cost estimation for the device and another argument to drive further development of the sensor.

# 4. Translation Potential

## 4.1. Healthcare application potential

Nowadays the period between taking a blood sample and getting a concentration value from requires a couple of days, according to Prof. Anders Larsson, Department of Medical Sciences, Clinical Chemistry, Uppsala University (Hospital). Prof. Larsson has worked with NT-proBNP based diagnosis for already for years and he sees the problem in the centralized NT-proBNP analysis in Sweden. It would be a better solution to have nurses equipped with this kind of devices allowing to measure the NT-proBNP concentration directly at home, suggests Prof. Larsson. Then a doctor which has his phone or computer connected to this device can send a message to the nurse to e.g. order her bringing the patient directly to the hospital for further treatment. An approach like this needs more cheap and small (handheld) device like the device introduced in this document. With the current system, the patient gets the result days later and must be picked up and carried to the hospital (worst-case) separately. Prof. Larsson believes a new approach with more devices can not only save lives but also money.

Two strong advantages of the device that UppSense has developed are the user-friendly interface and the low price for production. These are two features which make it the ideal product suitable for what the Swedish market needs according to experts in the field.

This biosensor has been developed to detect NT-proBNP protein. But only by changing the antibodies for another pair, the biosensor could be used for other protein quantifications. The user would only need to change the cartridge specific for the biomarker to detect.

## 4.2. Industrialization and commercialization potential

Using an industrial manufacturing process, a cartridge can be designed in a way, that the antibodies are already dry and pre-set in the reaction chamber.

The cartridge itself can be manufactured in a large scale using a technique called micro injection molding. Therefore, a change in the final material is a missing step towards industrialization. PDMS and the glass slide will be substituted by other materials which can be used in this process e.g. Poly(methyl methacrylate) or Polypropylene.

The sensor device was from the beginning developed to make it possible to launch a product on the market within the shortest period possible. We are convinced that the basic plastic parts can be produced easily in a injection molding process. The housing parts need to be assembled automatically or manually. A final device can probably work without screws to save money. The whole electronics can easily and very economically be manufactured in China. Component placement and mounting can be done completely with the help of machinery using a pick and place machine and a reflow oven. Before the device can be

launched on the market some teething problems have to be eradicated. Moreover a kind of interface needs to be developed to obtain blood from a patient in an easy fashion. The patient needs to be able to do this at home. Right now the device has no screen or buttons, however, steps have been taken already to make the device more accessible for a older client base without smartphone usage convinience.

# 5. Team and Support

## 5.1. Contributions of the team members

| Name | Study subject | Contributions |
|---|---|---|
| Blasi, Anna | Master in Bio and Nanomaterial's | Microfluidics subgroup |
| Kaffash Hoshiar, Aida | Master in Systems Biology | Coordinator |
| Lei, Linlin | Master in Biomedicine | Assay subgroup |
| Rachita, Ruth | Master in Medical Research | Microfluidics subgroup |
| Stiefel, Maximilian | Master in Embedded Systems | Engineering subgroup |
| Van Rijnswou, Elmar | Master in Embedded Systems | Engineering subgroup |
| Zhang, Hanzhao | Master in Biomedicine | Assay subgroup |

## 5.2. People who have given support

| People's support | Support description |
|---|---|
| Ola Söderberg | Dept. of Pharmaceutical Biosciences, Uppsala University, biotynilization kit |
| Javier Cruz | Uppsala University, Microfluidics, constant advice and manufacture of mask and final mold |
| Klas Hjort | Uppsala University, Microfluidics |
| Örjan Valling | Uppsala University, Lithography/cleanroom researcher/microfluidics |
| Uwe Zimmermmann | Uppsala University, Electronics |
| Svante Andersson | Uppsala University, 3D printer |
| Caroline Dahl | KTH Clinical Innovation Fellow and CTO & co-founder at Ortrud Medical, advice |
| Ove Ohman | Founder of Fiomi, advice |
| Peter Svendlidh | Uppsala University, professor of magnetism, advice |
| Hengameh Basir | Helped with the logo, and T-shirts |
| Uwe Zimmermann | Dept. of Engineering Sciences, Solid State Electronics, 3D printing, consulting regarding mechanics and (especially semiconductor) electronics |
| Philipp Rümmer | Dept. of Information Technology, Division of Computer Systems, Money, printing with a big plotter, provision of a lab |
| Yi Wang | Dept. of Information Technology, Division of Computer Systems, Money |
| Hugo Nguyen | Dept. of Engineering Sciences, 3D printing and designing |
| Anders Larsson | Dept. of Medical Sciences, Clinical Chemistry, advice on BNP and NT-proBNP |
| Ulf Landegren | Dept. of Immunology, Genetics and Pathology, UU, general advices |

## 5.3. Sponsors

| Sponsors | Support description |
|---|---|
| Uppsala University | The main sponsor, Biomedicinskt Centrum and Ångströmlaboratoriet department |
| Scilifelab-Uppsala University | Lab facilities in Immunology Genetics and Pathology (IGP) department as well as UppSense group meetings place, money |
| Myfab | Clean room facilities in Ångström |
| Q-linea | Free Magnetics Beads |
| Astrego | Advice and free material and reagents |
| Kingfocus | Advice on the assay and some free antibodies |
| Carpet Legend | T-shirts sponsor |







## 6. Final remarks

It has been a long way from the time that the team first met to now, a few days before the competition. We did not know each other, we did not know about NT-proBNP and only little about how to build up a biosensor from scratch.
After so many meetings and scientific discussions, we decided to investigate on several approaches for the challenge. A couple of them looked promising, and therefore the team decided to go for it. Of course, the result differs from the initial idea, but that's life: it does not always work as intended and time is limited. Concluding one could say SensUs gives students the opportunity to try out and learn new things on the playfield of real-life health care applications.