SensUs

Team Results Document

BiosensUM

Team Members

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1. Summary for the SensUs website

The BiospectrUM is a simple device using an established technology adequate for quantifying antibodies (such as Humira) called Surface Plasmon Resonance (SPR). We produced a custom microfluidic chip fixed to a gold-coated glass prism functionalized with a receptor specific to adalimumab. Using the cellphone LED light as a light source; the specific binding of adalimumab to this receptor causes a SPR wavelength shift that can be detected as a pixel shift with the patient's cellphone camera by taking a quick picture and the images are then processed by our custom application. A patient can undergo a full analysis in under 4 minutes including the sample injection time, data processing and delivery of results. The data is automatically transferred to an external server, which can then be analysed by a health professional. The results are accessible in real-time and are archived for future consultation. Regarding our work and innovation, we filed a provisional patent application regarding our optical setup, cellphone application and bioassay. We truly believe that the rapid and simple analysis with our cellphone app and optical device will help patients around the world monitor their treatment, reduce adverse side effects and improve their overall health and lifestyle.

2. Biosensor System and Assay

2.1. Introduction to detection technology

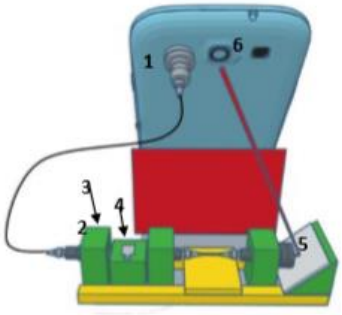
We developed a surface plasmon resonance (SPR) biosensor controlled by a custom smartphone application for the detection of adalimumab. Surface plasmon is a collective oscillation of electrons perpendicular to the surface of a thin metal layer causing an oscillation of the local electromagnetic field[1]. We chose gold as the metal because the SPR is located in red[1] wavelengths and can be detected by a cellphone camera. It is an optical technique that can be used to study the interaction between molecules at a distance up to 200nm from the surface[2]. Typical wavelength interrogation SPR instruments are composed of a polychromatic light source, a gold-coated glass prism covered by an aqueous solution, a spectrophotometer, a set of polarizers, and computer to process the data[3-4]. SPR occurs when an incident light travels through the gold-coated prism producing total internal reflection. P polarized light will penetrate the metal layer, called the evanescent wave, and the electromagnetic field will interact with the plasmon. The wavelength of the evanescent wave that is in phase with the plasmon will excite the plasmon and this results in an absorption of this wavelength energy[1]. The absorbed wavelength, λ_{SPR}, is dependant on the properties of the metal and of the medium around it. When molecules get close enough to the gold layer, the refractive index of the solution at the surface increases and the λ_{SPR} will shift to a higher wavelength (lower energy) proportionally to the change in refractive index.[1] We used this characteristic to develop our bioassay by attaching a specific receptor for adalimumab at the surface of the gold-coated prism, which allows us to selectively quantify the interaction of adalimumab with the receptor.

2.2. Molecular recognition and assay reagents

In order to specifically detect adalimumab, we functionalized the gold-coated prism according to an established protocol optimized for antibody sensing in plasma with a self-assembly monolayer (SAM)[5] to which we attached TNF alpha, the highly specific ligand of adalimumab. The molecule used for the SAM is a short hexa-peptide with a thiol functional group at the N-terminal extremity (3-MPA-LHDLHD-OH) (1mg/mL in DMF) that will covalently bond to the gold layer and will form a dense peptide layer after 16h of incubation. This SAM has shown great potential in decreasing non-specific adsorption of all contaminants present in plasma[5]. We then attached TNF-alpha (50µg/mL in PBS pH 4.0) to the C-terminal extremity of the SAM by standard EDC/NHS random amino coupling protocol[6]. This reaction causes a coupling between any primary amine on the protein with the EDC/NHS activated carboxylic acid of the SAM. This produces a statistically random immobilization of the receptor at the surface of the SAM. A schematic representation of the assay is represented in appendix 1. The prism is then passivated for 10 minutes with plasma to obtain a stable equilibrium at the surface and the assay can finally take place by injecting the plasma sample of unknown adalimumab concentration directly into the microfluidic cartridge.

2.3. Physical transduction

The refractive index increase caused by the binding of adalimumab with TNF-alpha at the gold-layer surface causes a shift in the λ_{SPR}[1]. A typical SPR instrument would be equipped with a spectrophotometer that can accurately measure wavelength shifts. However, such a detector is bulky, expensive and not user-friendly since it requires to be connected to a computer to process the data. We developed an SPR system using a cell phone LED light as the incident light source and the CCD camera as a detector by taking a simple picture. The CCD camera will not measure a wavelength shift; however, we can detect a corresponding pixel shift in the captured image with the custom algorithm integrated in our phone application. Figure 1 shows a simplified model of our instrument with the different parts outlined. The polarizer ensures a good orientation of the electromagnetic field while the grating prism separates wavelengths into a rainbow and allows the CCD camera of the cellphone to take a picture of the deconvoluted light. This design improves accessibility to the instrument by lowering the costs and allowing any user to use their cellphone to perform the analysis in the comfort of their own home.



1. Cellphone flashlight
2. Collimator to focus the light on the prism
3. Set of polarizer
4. Microfluidic chip and gold-coated prism
5. Diffraction grating
6. Cellphone camera

Fig. 1: Schematic representation of our instrument

2.4. Cartridge technology

We developed a microfluidic chip maximizing the area of detection while minimizing the volume of sample. Our cartridge is 2cm by 2cm by 1cm and made in polycarbonate and is sealed to the gold-coated prism. The sample is injected into the input channel and suction is then applied at the output channel using a small syringe to induce a controlled flow. This system allows multiple injections and washing steps required for the surface functionalization. Another important feature is the tight seal that forms between the microfluidic chip, the gold-coated prism and the syringe, which reduces the risk of leaks and contamination.

2.5. Reader instrument and user interaction

The image-processing algorithm was developed on MATLAB and translated in JAVA (see appendix 2 for screenshots of the app that calls the data processing). To obtain the SPR shift, which we associate to a pixel shift, the application must take three pictures. The first picture is taken of a blank sample (pure plasma) by using S-polarized light, which does not interact with the plasmon therefore does not produce SPR, and a second using P-polarized light, which produces an evanescent wave that produces SPR. The comparison of the two pictures allows us to localize low intensity pixels in the P-polarized picture caused by the wavelength that was absorbed by the plasmon (position 1, figure 2). The blank sample is then replaced by the testing sample of unknown adalimumab concentration and a third picture is taken using P-polarized light. The SPR wavelength (position 2, figure 2) will have shifted towards the red spectrum (lower energy) since the adalimumab that bound to the TNF alpha will have increased the refractive index at the gold surface. This will cause the band of low intensity pixels to also shift towards the red spectrum. The pixel shift is calculated by subtracting Position 2 and Position 1 and is proportional to the concentration of adalimumab in the testing sample. The exact concentration is obtained by inputting the shift in a calibration curve.

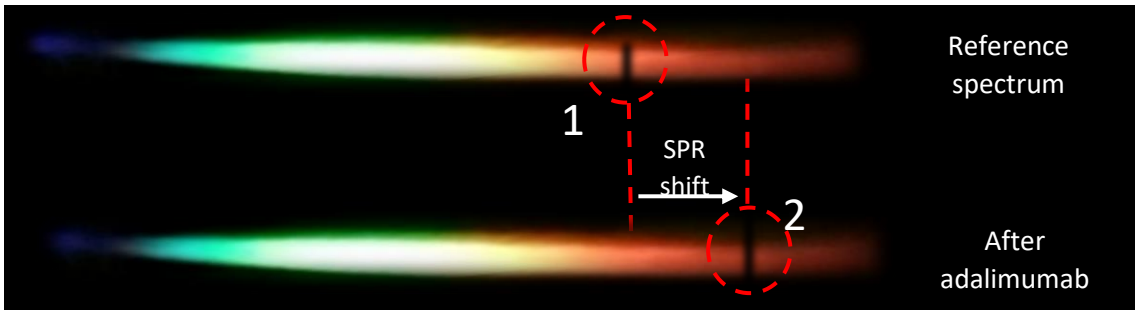


Fig. 2: Example of pictures taken by the cellphone before and after a sample injection. Black bands were added manually to facilitate comprehension. In reality, missing pixel bands cannot be visually detected.

3. Novelty and Creativity

3.1. Already available

- SPR clinical biosensors have been widely used for medical diagnostics[7] and a label free detection of adalimumab by SPR using TNF-alpha as a receptor has shown to be a valid strategy for real time analysis of adalimumab in plasma[8].
- Considering that the area analyzed by common SPR systems is around 200nm above the metal surface[1], low volume microfluidic cartridges are key. Microfluidic cartridges can be adapted to a multitude of SPR[9] instruments.
- The ability to miniaturize optical sensors makes them ideal candidates for rapid and easy therapeutic drug monitoring. Many researchers already reached the first milestone in developing analytical devices using cellphone applications[7-8].

3.2. New developments

To our knowledge, the BiospectrUM is the first instrument allowing the user to quantify an antibody directly from plasma in less than 4 minutes without dilution and with a sample volume of less than 20 µL using an Android smartphone. Other instruments in this category fall short on at least one of these parameters like Ulrich's group, back in 2018, that developed a portable device for Ebola monitoring that can assess concentration of the virus directly from a blood sample but requires 20 minutes for a complete run. To develop an application that is as user-friendly as possible, we created a simple interface and an analysis procedure requiring very few steps for the user. It is as easy as opening the application, activating the cellphone camera and placing the sample. We even created an interactive game that helps the user understand the science happening inside the device at a molecular level. Moreover, our microfluidic cartridge with the gold-coated prism was developed in a way to reduce sample manipulation by the user, to be easily disposable, to reduce possible contamination and to minimize sample volume and production costs.

3.3. Future developments

Since our current model requires the users to inject the sample themselves, we aim to develop a microfluidic chip relying solely on capillarity to eliminate user interaction with the microfluidic system. This development has already started in collaboration with one or our partners (CNRS). This technology will also increase reproducibility of flow rate injection from sample to sample. We are also currently investigating methods for long-term storage to allow the distribution of ready-to-use chips to our users. As of now, they can be stored in PBS at 4°C for 24h before use. Ultimately, we aim to achieve a 2 to 4-week storage time in synthetic plasma, so that the blanking reagent is already inside the chip. We believe this can be achieved by developing a packaging with optimal humidity and pressure conditions to conserve the integrity of the sensor throughout the shipping process. We are also planning on adding more complex functions in the application allowing health care professionals to access their patient's data remotely and privately, in real time. Following our innovation, we filed a provisional patent application regarding our instrument, cellphone application and bioassay. Work is ongoing to continue the development and fully protect the technology.

4. Analytical Performance

4.1. Optimisation process

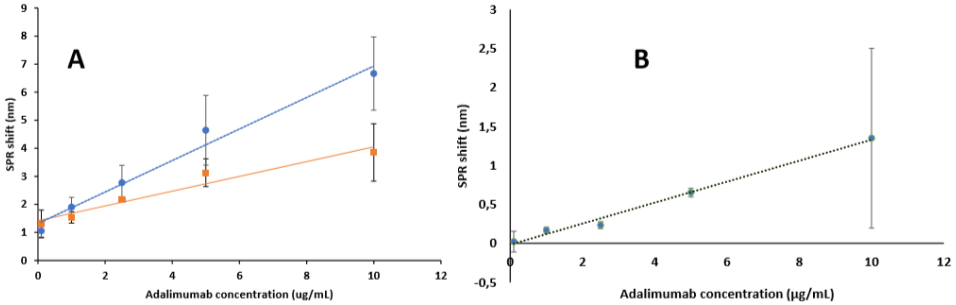


Fig. 3: SPR calibration curves for the detection of adalimumab in plasma performed on a commercially available SPR system. Functionalization of TNF-alpha (50µg/mL, PBS 1X pH 4.0) done by EDC/NHS random amino coupling to the SAM. **A) Blue curve**: Experiments performed by 3 different analysts on 3 different days. Error bars represent the standard deviation on n= 6 except for 2,5µg/mL (n=3). SPR shift = 0,5618[adalimumab] +1,3168, R^2 = 0,9781. **Orange**: Functionalized prisms kept at 4°C in PBS 1X pH 4,0 for 24h before analysis. Error bars represent the standard deviation on n= 2. SPR shift = 0,2634[adalimumab] +1,4185, R^2 = 0,9478. **B)** Same procedure as A) Blue. SPR shift (nm) = 0.1346[adalimumab]+0.1627, R^2= 0.9285. For A, measurements are done at equilibrium (20 mins) and for B, at initial velocity (3 mins). Instrument used: P4 from Affinité Instruments.

Figure 3A (blue line) shows the calibration curve of adalimumab spiked in a plasma sample. It shows great linearity with R^2 over 0.97 in the dynamic range which is over the threshold of the competition (0.1µg/mL to 10µg/mL). We then investigated the possibility of functionalizing our sensor chips 24h prior to the analysis storing them at 4°C in PBS (Figure 3A, Orange line). The sensitivity is a little bit lower than freshly prepared prisms, but it is still acceptable (R^2 = 0,9478). This is a promising result towards simplifying the procedure for the user. Storage in synthetic plasma was not possible due to the clogging of the microfluidic by plasma proteins. The chips therefore need to be incubated with plasma for 10 minutes prior to the injection of the testing sample to passivate the SAM and decrease non-specific adsorption. The data in figure 3A are acquired at equilibrium, which occurred 20 mins after sample injection. In order to reduce the time of the assay down to 5 minutes or less, we measured the data at 180s following injection and obtained a R^2 of 0.9285 (Figure 3B). The gain in precision is balanced by the loss in sensitivity, which is to be expected since the equilibrium has not yet been reached after 180s. Figure 3A and 3B were performed using random amino coupling to attach TNF alpha to the SAM, therefore the orientation of TNF-alpha is randomly distributed across the gold layer. We attempted to control the orientation by using a TNF-alpha with an N-terminal his-tag that attaches to the SAM via cobalt functionalization (Appendix 3). Unfortunately, the signal saturated at 1µg/mL. Protein engineering will be done to test different his-tag positions to optimally expose the epitope.

4.2. Final version of the BiospectrUM

Before moving on to using our hardware and phone application, we connected our device to a spectrophotometer and proved that our optical design does in fact produce an SPR signal (Appendix 4). Then all the optimization work was fully transferred to our instrument. Figure 4 shows the calibration curve of adalimumab spiked in plasma samples with our instrument showing good linearity in the dynamic range tested (R^2 = 0.9185). Work is ongoing to produce a triplicate to confirm reproducibility.

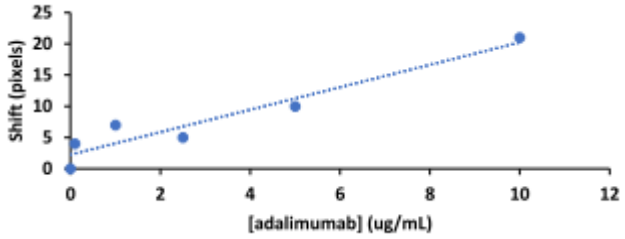


Fig. 4: SPR calibration curves for the detection of adalimumab in plasma performed on our instrument. Data were acquired after 180 sec on plasma incubation. Shift (pixels) = 1.7989[adalimumab] + 2.2567, R^2 = 0.9185

5. Translation Potential

5.1. Business model canvas

<table>
<tr>
<td rowspan="2">Problem
<u>Common</u>
•Finding the right prescription (combination of drug & dosage)

<u>Professional</u>
•Lack of reliable data to monitor given prescription

<u>Patient</u>
•Suffering caused by RA & secondary effects.
•Accessibility to professionals in rural area.

Existing Alternative
•Medical marijuana
•anti-inflammatories
•Slow-acting antirheumatic agents
•Modifiers of the biological response</td>
<td>Solution
•Biosensor giving objective results in 5 minutes
•Easier way to analyze the data by having access to our private database
•Customer service oriented towards client satisfaction
•Possibility of doing the test at home without the help of a professional</td>
<td rowspan="2">Unique Value Proposition
<u>Professionals</u>
The perfect device to find the right prescription quickly and efficiently in order to help their patient affected by RA.
<u>Patient</u>
A portable device that will help patients affected by RA to ease their pain by finding the right prescription faster.

High-Level Concept
Continuous diagnosis without the needs of a meeting with your healthcare professional</td>
<td>Unfair Advantage
•Accessibility
•Fast to use
•Easy to use
•Originality
•Inexpensive
• Heavily subsidized company</td>
<td rowspan="2">Customer Segments
1-Patients
Individual affected by RA

2-Professionals
Rheumatologist

3-Distributors
SigmaSanté
GACOQ
GACEQ
McKesson

Early Adopters
SigmaSanté: The principal distributor in the urban area of Montreal.</td>
</tr>
<tr>
<td>Key Metrics
•Having quick and accurate detection
•Having a good relationship with at least one strong distributor</td>
<td>Channels
•Call for tender
•Strategic publicity
•Presence in medical event
•Different distributors such as SigmaSanté & GACOQ (path to patient & professionals)</td>
</tr>
<tr>
<td colspan="3">Cost Structure
Raw material
Manufacturing overhead
Direct labor
Administration and sales cost
Taxes /Amortization</td>
<td colspan="2">Revenue Streams
Sales (device & microfluidic chips)
Leases (device)
Public subsidies
Returns on investments (short term liquidity management)</td>
</tr>
</table>

5.2. Stakeholder desirability

Rheumatoid arthritis is a serious problem for our society. Indeed, by the year 2026, it is estimated that over million Canadians 15 years of age and older will have arthritis. We need to address the situation with a better strategy, which includes a more profound understanding of the treatment effects on patient health. The various parties concerned by the situation all have their roles in the improvement of the situation. For the *professional*, they face many challenges such as the complexity of collecting enough data to understand and evaluate the response of the patient to treatment, the lack of reliable and objective tests and the fact that the compliance of the patients to their treatment is unknown. For the *patient*, there are also several issues that do not allow them to have adequate services and care to effectively deal with the effects of the disease. Indeed, with the technology currently available on the market, the geographic problematic regarding patients in rural areas, the shortage of specialized caregivers and the lack of psychological help received, it is extremely difficult to treat each patient properly. In regard to the *government*, the main issue is related to the cost of the different treatments related to the disease itself, including the side effects. In fact, RA costs approximately $ 38.3 billion in 2015 in direct and indirect costs[9]. Thereby, the product we propose grants a lot of *advantages* such as the portability of the sensor (at the point of care or at home), the accessibility of the results (patients can monitor the progress of their treatment from their cellphone), the rapidity of the test procedure (now in less than 4 minutes) and the user-friendliness (the biosensor works directly from an application in the cellphone of the client).

5.3. Financial viability

(Please note that many of the hypotheses and details relative to the estimation described in this section are available in the Business plan; Financial viability)

The business prosperity is mainly insured by the sales of both the device and the microfluidic chips. For each component, we started by calculating their unitary cost of production. Cost-wise, the greatest advantage of our sensor is the fact that it does not require an integrated detection system since it uses the users' cellphone. After adding up every component, we obtained a production cost of ***823$ CAD*** per device and ***5,50$ CAD*** per chip. The total economic burden of rheumatoid arthritis in Canada is estimated to be $76.6 billion and affects approximately around 378 259 Canadian adults[9]. We took those data into account when establishing the market size for RA as well as the market percentage we could possibly obtain. Then, in order to establish our price for the products, we based ourselves on how much money these tools would save for the patient, the health care professional and the public system. Pricing is also strategically determined by whom we're doing business with. We will price the device at ***4140$ CAD*** and the chips at ***66$ CAD***. Overall, our main source of income will be generated by continuous microfluidic chips replenishment from our clients. After the first two years (2022), we estimate our revenues to be around **$39 320 494 *CAD.***

5.4. Business feasibility

In our complete business plan, we describe the key resources in terms of assets to acquire in order to be fully operational. We will need a human resources and management department to insure operations run smoothly as well as experts in a variety of fields such as biochemistry, engineering, software development and industrial design to expand production and development. In order the elevate BiosensUM to an international level, we will establish partnerships with suppliers, distributors and other key actors involved in the business process. First, we aim to acquire a larger market share of the rheumatoid arthritis market in Canada and internationally. Then, we envision undertaking other disease markets that also use Humira for treatment, such as Crohn's disease. Finally, we will work to develop tests for other antibodies, for example, a test for anti-HER2, a monoclonal antibody used for treating HER2 positive breast cancer.

6. Team and Support

6.1. Contribution of the team members

Frédéric F. Team leader. Worked in the lab to develop the bioassay and establish the dose-response curve for the biosensor. Was also responsible of all the administrative tasks. Oversaw the ethical committee approval.

Elizabeth E. Science leader. Was responsible of keeping a good workflow in the lab and developing the bioassay. Worked on the hardware of the sensor.

Jean-Antoine G.C. Technology leader. Was responsible of organizing the technological development of the instrument. Worked on the hardware of the sensor and development of the fluidic cartridge.

Antoine N. Entrepreneurship leader**.** Oversaw the entrepreneurship development and establishment of the business model.

Javier G. Science team member Oversaw the synthesis and characterization of the peptide. Was involved in the lab for all manipulations from the very beginning of the project.

Malek B. Science team member: Was responsible of literature research and participated in the bioassay development. Oversaw public relationship and Instagram takeover.

Myriam C. Technology team member: Was involved in the software development for the cellphone application. Worked on the signal transduction from the optical signal to a concentration. Participated in the ethical committee approval process.

Antoine D.D. Technology team member: Computer expert, was heavily involved in the software development, particularly with the calibration and analysis workflow. Created the tools (database, server, etc.) to process and store the data.

Zoubaire M. Technology team member: Was the 3D printing expert and designed everything that was 3D printed. Also worked on the microfluidic cartridge design.

Jérémie G. Entrepreneurship team member: Was fully dedicated to the entrepreneurship program and was involved on all the steps from preliminary research to establishment of the business model.

Selma S. Entrepreneurship team member: Gave a scientific point of view in the entrepreneurship development. Participated to the ethical committee approbation process.

Clara G.B. Entrepreneurship team member: Participated to the ethical committee approbation process an on the establishment of the business plan.

6.2. People who have given support

Pr. Joelle Pelletier: Professor in the department of chemistry at the *U. de Montréal.*

Denis Deschênes: Chemist in the department of chemistry of *U. de Montréal*.

Karine Gilbert: Scientist at the Mass Spectrometry Center – *U. de Montréal*.

Martin Lambert: Mechanical engineering technician at *U. de Montréal*.

We give a special thanks to all the grad students in the Pr. Masson's lab, particularly **Benjamin Charron**, **Félix Lussier** and **Gregory Q. Wallace**.

6.3. Partners

We would like to thank TransMedTech, Affinité Instruments, NSERC, ASEQ foundation, National Research Council Canada, and of course *Université de Montréal*, *HEC Montréal* and SensUs for the help and support.

7. Final Remarks

After a year full of challenges, hard work, joy, achievements and endless ups and downs, we are proud of all the progress we made together as a team. Even though we started as 12 individual members, we are leaving Montreal for Eindhoven as one unstoppable team. Every member worked wholeheartedly towards the success of this project and produced something we can be very proud of. We believe that the BiospectrUM is an important addition to the field of medical biosensing, providing true innovation and scientific creativity. Over the last year, we learned new skills from each other that cannot be learned in conventional school projects. These invaluable skills will contribute to shaping each and every one of us into successful scientific entrepreneurs one day.

We would like to thank Pr. Jean-François Masson for supervising our team for a second year. His availability, his diplomacy and precious advice helped us keep a focussed direction for the project.

Again, we would like to thank all our partners. Without you, this project could not have been possible. By supporting scientific and entrepreneurial innovation, you also gave us the opportunity to better ourselves through the development of our instrument.

Best of luck to all the teams and see you in Eindhoven!

- BiosensUM

8. References

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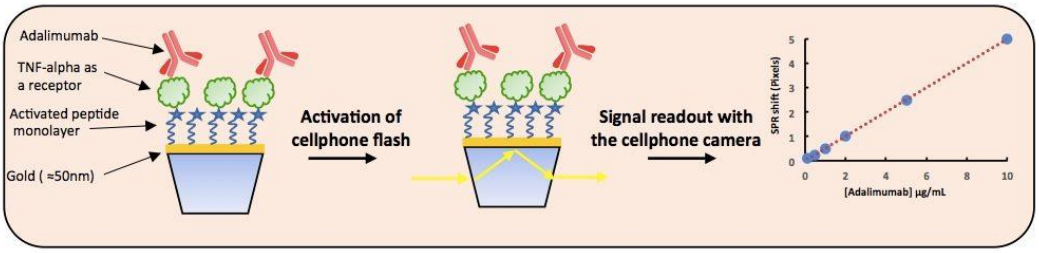
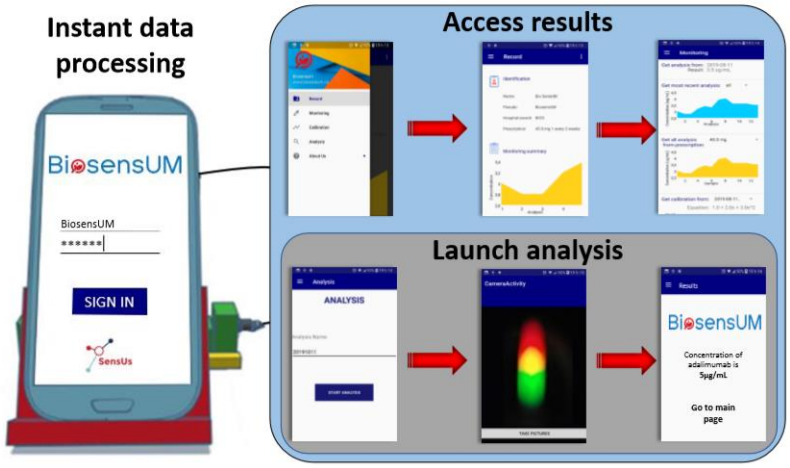
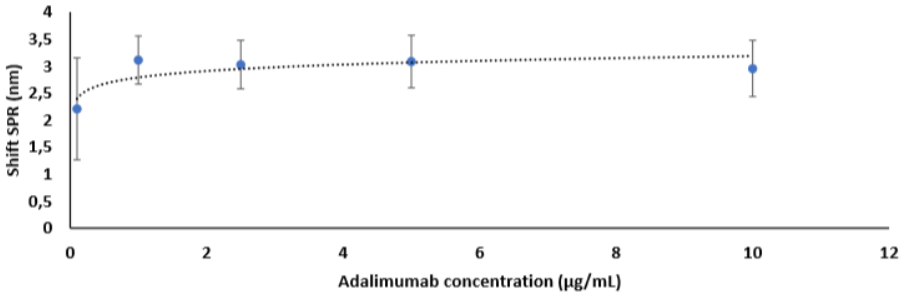
9. Appendix



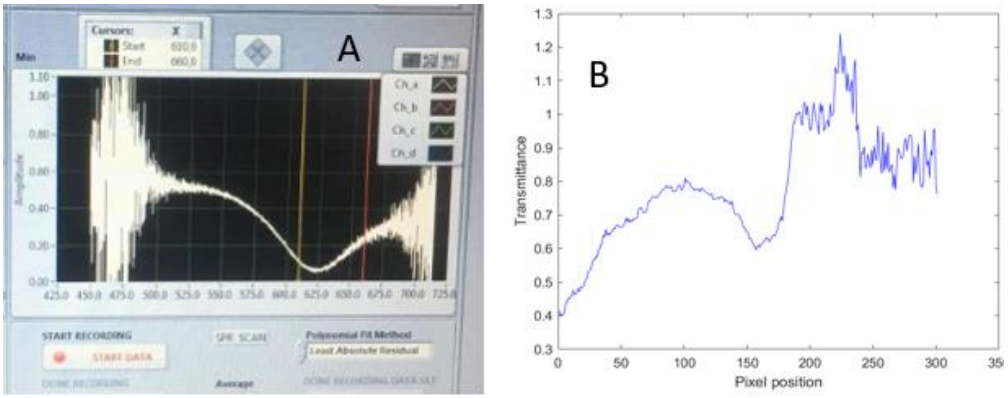
Appendix 1. Schematic representation of our bioassay.



Appendix 2. Screenshot of our application showing the data treatment procedure.



Appendix 3. SPR calibration curve for the detection of adalimumab in plasma performed on commercially available SPR system with his-tagged TNF alpha. TNF-alpha with N-terminal histag (50µg/mL, PBS pH 4.0) was linked to the SAM by cobalt functionalization. Errors bars represent the standard deviation on n= 3. Measurements are done at equilibrium (20 mins). Instrument used: P4 from Affinité Instruments



Appendix 4. Transmittance curve obtained with our device. We obtained Figure A by connecting the optic fibre directly to a spectrophotometer and processing the exiting with the P4 SPR system software (Affinité Instruments). Figure B was obtained after data processing through our cellphone application. Pixel 0 represents blue and pixel 300 represents red.