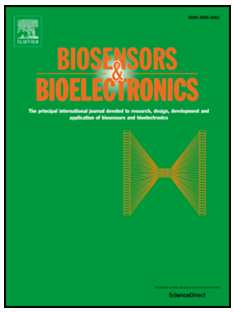

Point-of-care therapeutic drug monitoring of adalimumab by integrating a FO-SPR biosensor in a self-powered microfluidic cartridge

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CRediT authorship contribution statement

Jia-Huan Qu: Conceptualization, Methodology, Validation, Investigation, Writing – Original draft
Henry Ordutowski: Conceptualization, Methodology, Validation, Investigation, Writing – Original draft
Charlotte Van Tricht: Conceptualization, Methodology, Investigation, Writing – Review & editing
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Saba Safdar: Conceptualization, Methodology, Validation, Supervision, Writing – Review & editing
Dragana Spasic: Methodology, Writing – Review & editing, Supervision, Funding acquisition
Jeroen Lammertyn: Conceptualization, Methodology, Writing – Review & editing, Funding acquisition

Point-of-care therapeutic drug monitoring of adalimumab by integrating a FO-SPR biosensor in a self-powered microfluidic cartridge

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ABSTRACT (max 250 words)

Disease treatment with advanced biological therapies such as adalimumab (ADM), although largely beneficial, is still costly and suffers from loss of response. To tackle these aspects, therapeutic drug monitoring (TDM) is proposed to improve treatment dosing and efficacy, but is often associated with long sampling-to-result workflows. Here, we present an in-house constructed ADM-sensor, allowing TDM of ADM at the doctor's office. This biosensor brings fiber optic surface plasmon resonance (FO-SPR), combined with self-powered microfluidics, to a point of care (POC) setting for the first time. After developing a rapid FO-SPR sandwich bioassay for ADM detection on a commercial FO-SPR device, this bioassay was implemented on the fully-integrated ADM-sensor. For the latter, we combined (I) a gold coated fiber optic (FO) probe for bioassay implementation and (II) an FO-SPR readout system with (III) the self-powered iSIMPLE microfluidic technology empowering plasma sample and reagent mixing on the-cartridge as well as connection to the FO-SPR readout system. With a calculated limit of detection (LOD) of 0.35 µg/mL in undiluted plasma, and a total time-to-result (TTR) within 12 min, this innovative biosensor demonstrated a comparable performance to existing POC biosensors for ADM quantification in patient plasma samples, while requiring only 1 µL of plasma. Whereas this study demonstrates great potential for FO-SPR biosensing at the POC using ADM as a model case, it also shows huge potential for bedside TDM of other drugs (e.g. other immunosuppressants, anti-epileptics and antibiotics), as the bioassay is highly amenable to adaptation.

Keywords: adalimumab; fiber-optic surface plasmon resonance; self-powered microfluidics; point of care; therapeutic drug monitoring; patient blood plasma

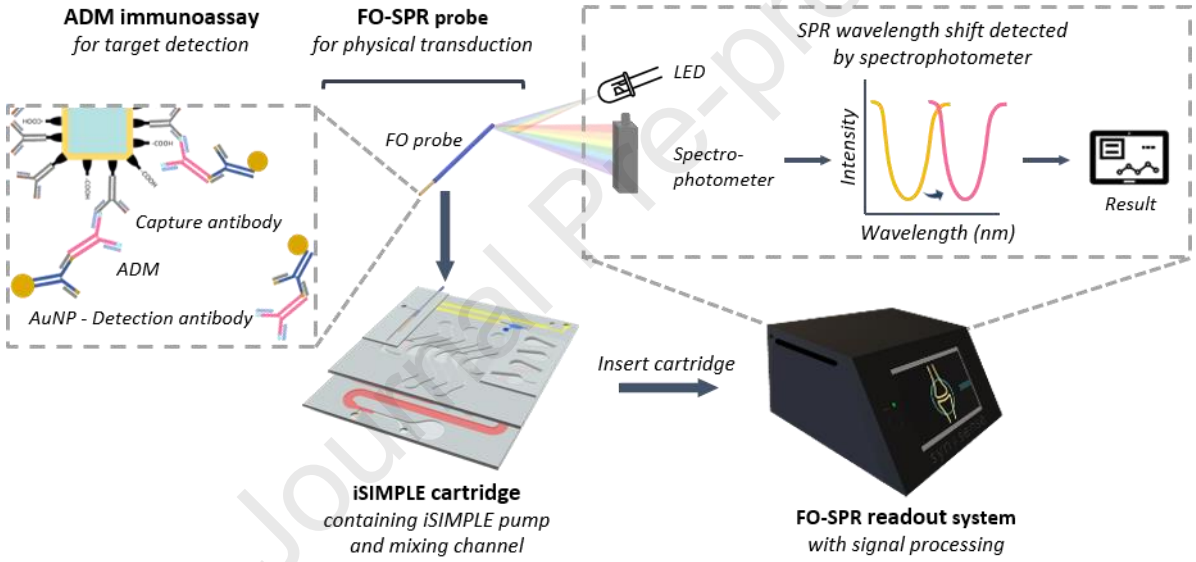
1. Introduction

Tumor necrosis factor alpha (TNFα), a pleiotropic cytokine, is involved in the pathogenesis of several autoimmune diseases as an inflammatory mediator and apoptosis inducer. The development of TNFα antagonists has greatly improved the treatment of several chronic autoimmune diseases, such as inflammatory bowel disease (IBD) (Hemperly et al., 2018), rheumatoid arthritis (Burmester et al., 2013), psoriasis (Pugliese et al., 2015), and ankylosing spondylitis (Arends et al., 2011). For instance, adalimumab (ADM, Humira®) is a fully human immunoglobulin G1 (IgG1) monoclonal antibody, which binds to TNFα with high specificity and affinity, thus being a safe and effective anti-TNFα agent in biological therapies. Biological therapies offer clear advantages over conventional treatment with e.g. corticosteroids to induce and maintain clinical remission, while the fully human makeup of ADM promises lower risk of immunogenicity and loss of response. The current treatment involves the subcutaneous administration of a standardized fixed ADM dose for all patients, leading to high variability in circulating drug concentrations between patients (Abbvie Inc., 2013; Ordás et al., 2012). As a consequence, subtherapeutic drug concentrations may result in ineffective treatment and immunogenicity, whereas supratherapeutic concentrations can lead to increased side effects and toxicity, as well as higher treatment expenses (Vande Casteele et al., 2017). To keep drug concentrations within the therapeutic window, maintain the efficacy of treatment and guide future personalized therapeutic decisions, therapeutic drug monitoring (TDM) of ADM in individual patients is crucial (Vande Casteele et al., 2014; Colombel et al., 2012). However, current procedures involve low throughput analysis by laboratory personnel (typically enzyme-linked immunosorbent assays, ELISA) in a centralized hospital setting (Bian et al., 2016; Hock et al., 2016), resulting in high turnaround times, thus discouraging TDM. Therefore, there is a clear need for a point-of-care (POC) TDM in the doctor's office or at the hospital bedside.

Several techniques have been proposed to improve TDM of ADM or other anti-TNF agents such as infliximab (IFX) (Lu et al., 2017, 2016), being radioimmunoassay (Ainsworth et al., 2008), cell-based reporter gene assay (Lallemand et al., 2011), homogeneous mobility shift assay (Wang et al., 2012), liquid chromatography tandem mass spectrometry method (Willeman et al., 2019), and lateral flow assays (LFAs) (BÜHLMANN Laboratories AG, 2021; R-Biopharm AG, 2021). More recently, a fiber optic surface plasmon resonance (FO-SPR) assay has been reported for quantification of ADM from 1 to 16 μg/mL in 400-fold diluted patient serum samples within 45 min (Bian et al., 2018). Furthermore, a label-free FO-SPR bioassay has been recently described for IFX quantification in 50-fold diluted serum in just over 10 min (Zeni et al., 2020). Even though these bioassays greatly shortened the time for ADM detection compared to ELISA, the time-to-result (TTR) for the former and sample handling for both were not optimized for POC testing.

In this paper, we demonstrate for the first time the potential of FO-SPR to be used outside centralized laboratories for TDM of ADM by combining it with microfluidic technology. Specifically, a portable ADM-sensor is developed for ADM detection in plasma samples within 12 min, combining three core elements as illustrated in Scheme 1: (I) FO sensing probe for bioassay implementation, (II) the FO-SPR readout system, and, (III) the disposable iSIMPLE microfluidic cartridge. The latter is based on our recently established self-powered, and low-cost microfluidic system that can work in two configurations: pulling (SIMPLE, stands for: self-powered imbibing microfluidic pump by liquid encapsulation) and pushing (iSIMPLE, where "i" stands for infusion) of liquids (Kokalj et al., 2014; Dal Dosso et al., 2018a, 2018b, 2018c, 2019b). To achieve this, we develop for the first time: (I) a one-step FO-SPR sandwich bioassay that allows direct measurement of ADM in a plasma sample,

premixed with functionalized gold nanoparticles (AuNPs) for signal amplification, thus minimizing the detection time and (II) successful reagent mixing on the iSIMPLE platform to support the implementation of the developed bioassay on the FO-SPR readout system. Eventually, we integrate all the components into a fully functional ADM-sensor, which requires only 1 µL of plasma sample and a single finger-press for its activation. Finally we validate this in-house developed biosensor with two commercialized POC tests for ADM detection, being the Quantum Blue® (BÜHLMANN Laboratories AG, 2021) and RIDA®QUICK (R-Biopharm AG, 2021) LFAs. Notably, this work brings great advancements both to FO-SPR and iSIMPLE technology by exploring new applications, which incorporates the strengths of FO-SPR (i.e. high sensitivity, real-time monitoring, measurement in complex matrices, miniaturized SPR configuration) and iSIMPLE (i.e. self-powered, portability, flexibility). More importantly, compared to LFAs, the ADM-sensor employs a controlled surface chemistry on a gold (Au)-coated FO surface, with a highly flexible microfluidic platform allowing advanced liquid manipulation (e.g. liquid shuttling by pushing and pulling). Such achievement validates the feasibility of implementing FO-SPR biosensing technology in a microfluidic system, thus offering new prospects for developing POC biosensors.



Scheme 1. Schematic depiction of the ADM-sensor components. Target detection occurs through a newly developed one-step ADM immunoassay on the functionalized FO probe surface. This probe, used for physical transduction, is integrated in the iSIMPLE cartridge (with mixing channels) that is subsequently inserted into the FO-SPR readout system. Here, an LED light source and spectrophotometer are connected to the FO probe through a bifurcated fiber. The measurement is controlled by a Raspberry Pi 3, which collects and processes the data and outputs the resulting SPR slope.

2. Materials and methods

2.1 Reagents and materials

ADM (Humira®) was purchased from AbbVie Inc. (North Chicago, IL, United States), while anti-ADM monoclonal antibodies MA-ADM28B8 and MA-ADM40D8 were produced in the Laboratory of Therapeutic and Diagnostic Antibodies (KU Leuven, Belgium) (Bian et al., 2016). Furthermore, albumin from bovine serum (BSA), fluorescein, glycine, 2-(N-Morpholino) ethanesulfonic acid (MES), phosphate buffered saline (PBS), sodium acetate (NaAc), trehalose, and Tween 20 were acquired from Sigma-Aldrich (Overijse, Belgium). Superblock™ PBS, 1-ethyl-3(3-

dimethylaminopropyl) carbodiimide (EDC), and N-Hydroxysuccinimide (NHS) were procured from Thermo Fisher Scientific (Merelbeke, Belgium). Food colorants were purchased from Vahiné (Avignon, France). The MES, EDC/NHS, NaAc, and glycine-HCl buffers were prepared as described previously (Lu et al., 2017). Carboxylic acid self-assembling monolayer (COOH-SAM) formation reagent was purchased from Dojindo laboratories (Kumamoto, Japan). AuNPs with 20 nm diameter were acquired from BBI Solutions (Cardiff, UK).

For the FO-SPR measurements, the custom precut FO probes (diameter: 400 µm; tip length: 0.6 cm) were purchased from FOx Biosystems (Diepenbeek, Belgium). For the iSIMPLE cartridges, Ibiclear polyvinyl chloride (PVC) transparent sheets (300 µm thick) were acquired from Ibico via Delbo (Maldegem, Belgium), and the double-sided pressure sensitive adhesive (PSA) tape (200MP-7945MP) was obtained from 3M (Machelen, Belgium). Porous materials for iSIMPLE pumps were Whatman qualitative filter paper grade 40 and grade 598, purchased from Sigma-Aldrich (Overijse, Belgium). The PVC glue used for cartridge sealing was purchased from Martens (Mol, Belgium).

For the reference tests, the RIDA®QUICK ADM monitoring LFA kits and RIDA®QUICK SCAN II device were acquired from R-Biopharm (Darmstadt, Germany). The sandwich ELISA was performed with the same antibodies as above, using Costar 96-well plates from Corning (Lasne, Belgium) and readout on an ELx808 Absorbance Microplate Reader from BioTek Instruments Inc. (Bad Friedrichshall, Germany).

2.2 Plasma samples collection, preparation and storage

The collection of plasma samples from healthy donors after signing an informed consent form was approved by the Ethics Committee Research UZ/KU Leuven, Belgium (S62134). Whole blood samples were collected in 4.5-mL BD Vacutainer tubes containing 3.2% buffered sodium citrate (Novolab, Geraardsbergen, Belgium) from which plasma was prepared by centrifugation (10 min, 1,500 RCF, 25°C) and samples were stored at -20 °C until further use. Venous blood samples from IBD patients treated with ADM (n=4) were collected at UZ Leuven. Written informed consent was provided by all patients in the framework of the Institutional Review Board approved IBD Biobank (B322201213950/S53684). The blood was collected in a 2.7 mL BD vacutainer containing 3.2% buffered sodium citrate, serving as an anticoagulant. Platelet poor plasma was obtained by centrifugation (20 min, 1,800 RCF, 4°C) and samples were stored at −80°C until analysis.

2.3 Preparation of the FO sensing surface

The Au sputtering of FO probes and their surface functionalization was carried out as previously described (Arghir et al., 2015; Lu et al., 2017). Briefly, after COOH-SAM formation on the Au-coated surface via thiol chemistry, EDC/NHS chemistry was used to activate the COOH groups. Subsequently, the activated FO probes were functionalized by immersion for 30 min in 20 µg/mL capture antibody (MA-ADM28B8) in NaAc buffer (10 mM at pH 5.5). After removal of non-covalently bound antibodies from the surface using regeneration buffer (10 mM glycine-HCl at pH 2.0), the surface was blocked with Superblock™ PBS. The storage of dry fibers for later use was performed by immersing the functionalized FO probes in 10% (w/v) trehalose dissolved in ultrapure water for 4 min, followed by drying at room temperature for 60 min. Afterwards, they were stored in nitrogen at 4 °C until further use. The whole functionalization process was performed on the white FOx 1.0 device, an FO-SPR biosensing platform commercialized by FOx Biosystems (Diepenbeek, Belgium) (Qu et al., 2022, 2021a, 2021b, 2020).

2.4 Developing one-step sandwich bioassay for ADM detection using FO-SPR platform

The AuNPs were functionalized with detection antibody (MA-ADM40D8) for achieving signal amplification in the sandwich bioassay, as previously described (Lu et al., 2017). Briefly, the functionalized AuNPs were resuspended in PBS with 0.5% (w/v) BSA (PBS/BSA) after centrifugation, with the concentration adjusted to an optical density (OD) of 2.0 at 530 nm.

To develop the one-step bioassay ('ADM immunoassay' in Scheme 1) using the white FOx 1.0, ADM was first spiked either in PBS buffer with 0.01% (v/v) Tween 20 (PBS/T) or 1 µL of undiluted plasma, at a concentration range from 0 to 16 µg/mL with two-fold dilutions, which corresponds to the expected ADM concentrations in clinical samples. To avoid high-dose hook effect occurring in immunoassays at high target concentrations (Amarasiri Fernando and Wilson, 1992), these samples were subsequently 20-fold diluted using premixed PBS/T-AuNPs (9:10) solution. This resulted in the final ADM concentration range to detect from 0 to 800 ng/mL (0 ng/mL as the negative control together with 25, 50, 100, 200, 400 and 800 ng/mL to establish the calibration curve). The sample was mixed with AuNPs for 4 min prior to measurement.

The FO probes, functionalized with capture antibodies (MA-ADM28B8) and stored as described in Section 2.3, were stabilized in the 1:1 mixture of PBS/T and PBS/BSA for 1 min, and afterwards submerged into the mixture of sample and AuNPs for 2 min of measurement. The calibration curves were established in both buffer and 20-fold diluted plasma based on the SPR slope signal within the first 2 min as a function of the ADM concentrations.

Once established on the FO-SPR platform, this one-step bioassay was transferred to a FO probe introduced into a simplified cartridge, driven by a syringe pump for liquid manipulation. Here, the reagents were still mixed outside the cartridge as depicted in Figure S1. The measurements were performed using our in-house built FO-SPR readout system, as described in Section 2.5.

2.5 Development of the portable FO-SPR readout system

The in-house assembled FO-SPR readout system, as illustrated in Scheme 1 (right side), consists of off-the-shelf, affordable components: a LUXEON C White 300K LED (Lumileds, San Jose, CA, USA) light source and a UV/VIS micro spectrophotometer (INSION GmbH, Obersulm, Germany), both connected to a bifurcated optical fiber and controlled by a Raspberry Pi 3 model B (Raspberry Pi Foundation, Cambridge, UK). All the components were integrated in a portable readout instrument (i.e. dimensions: 26 x 24.5 x 13 cm), connected to a power outlet, with a Raspberry Touch display for user interface, and a cartridge insertion slot to accommodate the disposable microfluidic cartridge. The light intensity measured by the spectrophotometer was processed by the Raspberry Pi 3 to detect the SPR wavelength shifts and to calculate the SPR slopes as the final signal readout.

2.6 Mixing of reagents on the iSIMPLE cartridge

For the mixing of the plasma sample with the PBS/T-AuNPs solution on the cartridge, different channel designs (Figure S2A-H) and mixing ratios were first compared. The microfluidic units were produced based on the iSIMPLE technology as previously reported (Dal Dosso et al., 2019b; Kokalj et al., 2014). Here, PSA layers were cut with a Trotec Speedy 100R laser cutter (Marchtrenk, Austria), whereas the inlets of the cartridges were connected to a Harvard Apparatus PHD 2000 syringe pump (Holliston, USA), driven at a flow rate of 10 µL/min. Based on the experiments with the external pump, the final design (Figure S2I, top view) was selected and further optimized for direct mixing on the cartridge of sample and PBS/T-AuNPs solution at a 1:19 ratio, by integrating an iSIMPLE pump in a separate layer of the cartridge. The mixing efficiency was first demonstrated by mixing blue and

yellow dyed solutions, followed by further evaluation by means of fluorescence microscopy. Herein, the changes in fluorescence intensity throughout the channel with 1:19 mixing of 0.01% fluorescein (prepared in PBS) and PBS buffer were monitored on an inverted fluorescence microscope (IX71, Olympus Corporation, Tokyo, Japan) with an EM-CCD camera (Hamamatsu Photonics K.K., Hamamatsu, Japan) by taking a series of images at different positions along the cartridge.

2.7 ADM detection in plasma samples using the ADM-sensor

Finally, to perform ADM detection using the in-house built ADM-sensor, we utilized a fully integrated and autonomous microfluidic cartridge, containing the FO probe-channel and a 1:19 mixing channel in the top layer, and an iSIMPLE pump in the bottom layer. First, the FO probes, pre-functionalized and pre-treated with trehalose as described in Section 2.3, were inserted into the foreseen cavity in the cartridge during assembly and sealed with PVC glue. After drying the glue for 20 min at room temperature, the assembled cartridges with FO probes were stored in nitrogen at 4 ^{o}C until further use. Prior to use, the cartridges were prefilled with working liquid in the corresponding channel and PBS/T-AuNPs solution in the reagent channel, after which their respective filling holes were closed, as shown in Section 3.3. The sample, either plasma from a healthy volunteer spiked with ADM or IBD patient plasma, was preloaded in the sample channel before measurement. Upon insertion of the cartridge in the FO-SPR readout system, the iSIMPLE pump was activated with a single finger press, initiating the mixing process and propagating the mixed sample towards the FO probe. Before contact with the FO probe, the recording script was initiated on the FO-SPR readout system to perform a reference measurement and start recording the SPR wavelength shift as a function of time once the FO probe was fully submerged. The obtained data were further analyzed as described below.

2.8 ADM detection in IBD plasma samples with RIDA®QUICK and traditional ELISA

The same IBD patient samples tested with the ADM sensor were also tested with two reference methods: (I) our in-house developed ELISA for ADM quantification, utilizing the same antibodies as the one-step bioassay on the FO probe as previously described by (Bian et al., 2016), and (II) a commercially available LFA, i.e. the RIDA®QUICK ADM Monitoring kit (R-Biopharm AG, 2021). Both are detailed in Supplementary Information (S1.1 and S1.2).

2.9 Data analysis

On the White FOx 1.0, data were collected by FOx software (FOx Biosystems), and analyzed to generate the calibration curves. The fitting was performed with non-linear regression ($y=Ax/(B+x)$) whereas the limits of detection (LOD), and the coefficients of variation (CV) were calculated as previously reported (Lu et al., 2017; Mahzabeen et al., 2021). The calibration curves were plotted by programming in Matlab 2019b (The MathWorks Inc., USA). The SPR slope was calculated based on the obtained SPR shifts within the first 30-120 s of measurement. Alternatively, for measurements with the in-house developed FO-SPR readout system, the SPR shift as a function of time was recorded and saved on the device by a Python script on a Raspberry Pi 3 (Raspberry Pi Foundation, UK). The saved files were exported to a computer and further processed through a script in JupyterLab (Project Jupyter), which calculated the slope of the SPR shift within the first 30-120 s of measurement. Although we performed the analysis in JupyterLab externally, this process can be easily integrated with the FO-SPR readout system.

To quantify the correlation of ADM concentrations in patient plasma samples that were

obtained by different platforms, Deming regression with 95% confidence intervals for the slope and intercept was performed in Matlab. We calculated the measurement errors orthogonal to the regression line to determine the correlation strength. For the methods to agree well, the 95% confidence intervals around the estimates of the slope and intercept were tested to include '1' and '0', respectively (Gleason et al., 2020; Lin, 1989). Additionally, Pearson correlation coefficient (PCC) and concordance correlation coefficient (CCC) were calculated in Matlab, with PCC used to quantify the correlation strength within the data pairs and CCC to indicate the agreement of the data pairs relative to the unity line. The microscopy images for determining reagent mixing efficiency on the cartridge were collected with HCImage Live software (Hamamatsu Photonics K.K., Japan) and analyzed in ImageJ (NIH).

3. Results and discussion

3.1 One-step FO-SPR bioassay for quantification of ADM using the FO-SPR platform

In order to rapidly detect ADM in samples, as required for POC testing, we first established an FO-SPR sandwich bioassay with short TTR, using the White FOx 1.0. To achieve this, we started from our previously reported FO-SPR sandwich bioassay for ADM detection in 400-fold diluted serum samples (Bian et al., 2018) and replaced the two-step by a one-step detection process. Hereto, 1 µL of the sample (i.e. buffer or undiluted plasma, spiked with ADM at 0 – 16 µg/mL) was premixed with 19 µL of PBS/T-AuNPs (9:10) solution, prior to measurement on a FO probe (Scheme 1, left), which significantly reduced the detection time from 45 to 2 min. Such pre-mixing resulted in a final ADM concentration to be detected in buffer or 20-fold diluted plasma samples ranging from 0 to 800 ng/mL (described in Section 2.4). The SPR slope was used to generate the calibration curves in both matrices (Figure 1, calibration curves: I and II) as described in Section 2.9 (the FO-SPR binding curves for each concentration can be found in Figure S3A-B). This bioassay resulted in calculated LODs of 42.47 and 38.81 ng/mL and CVs of 16% and 23% (i.e. averaged CV values of the obtained SPR slope signals) in buffer and 20-fold diluted plasma, respectively. Although the LOD values were comparable between buffer and 20-fold diluted plasma samples, the latter resulted in slightly lower signal values (for ADM concentrations ≥ 100 ng/mL) and slightly higher CVs. This might be due to the matrix effect, i.e. the significant amount of proteins still present in only 20-fold diluted plasma, which can interfere not only with the binding of ADM to the detection antibodies on AuNPs during the premixing, but might also hinder the binding of the conjugated complex to the sensing surface. Nevertheless, the theoretical LOD calculated for undiluted plasma of 0.78 µg/mL was highly comparable to the commercially available golden standard POC tests of ADM: Quantum Blue® Adalimumab (LOD of 0.80 µg/mL in serum) and RIDA®QUICK ADM Monitoring (limit of quantification (LOQ) of 0.32 µg/mL in serum/plasma) (BÜHLMANN Laboratories AG, 2021; R-Biopharm AG, 2021). Furthermore, this current bioassay developed on the White FOx 1.0 has sufficient sensitivity to perform TDM for ADM, as the sensitivity was comparable to that of the gold standard (i.e. ELISA performed in 1000-fold diluted serum samples with an LOD corresponding to 0.1 µg/mL in undiluted serum) (Bian et al., 2016). Therefore, these results pointed to the successful development of a fast (i.e. 2 min of measurement time) and sensitive bioassay for ADM detection in plasma samples, showing prospects for implementation in the microfluidic cartridge.

Next, the one-step ADM bioassay was transferred to the FO probe integrated in a simplified cartridge with the liquid flow driven by a syringe pump (Figure 2). This cartridge contained: (I) a

channel to load the manually mixed (i.e. before loading on cartridge) reagents via an inlet, (II) a connection to the pump, (III) a chamber to insert the FO probe, and (IV) an outlet after a hydrophobic barrier. Immediately after mixing 1 µL of undiluted plasma, spiked with ADM, and 19 µL of PBS/T-AuNP solution, the mixture was loaded into the channel. After 5 min of static incubation, the mixed solution was driven by the syringe pump to fully cover the FO probe. The SPR slope was plotted as a function of ADM concentration (0-800 ng/mL) in the diluted plasma sample to establish a calibration curve (Figure 1, III, with the FO-SPR binding curve shown in Figure S3C). The LOD and CV were calculated as 30.42 ng/mL and 11%, respectively. The theoretical LOD in undiluted plasma was calculated as 0.61 µg/mL, which was comparable to the obtained LOD of 0.78 µg/mL in undiluted plasma using the White FOx 1.0. Therefore, we demonstrated that the sensitivity of the developed one-step bioassay for ADM is maintained when being implemented on the FO probe inserted into a cartridge and measured using the in-house developed FO-SPR readout system.

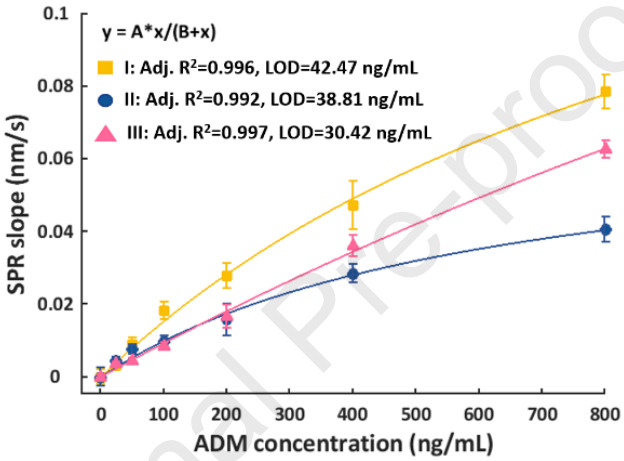


Figure 1. One-step FO-SPR bioassay for ADM detection. ADM was measured using the commercial FO-SPR device in buffer (I) and 20-fold diluted plasma (II), or measured using the in-house developed FO-SPR readout system in 20-fold diluted plasma (III). The fitting parameters are as follows (I: A=0.19 nm/s, B=1118.45 ng/mL; II: A=0.07 nm/s, B=627.12 ng/mL; III: A=0.36 nm/s, B=3718.67 ng/mL). Error bars represent one standard deviation (n_s=3).

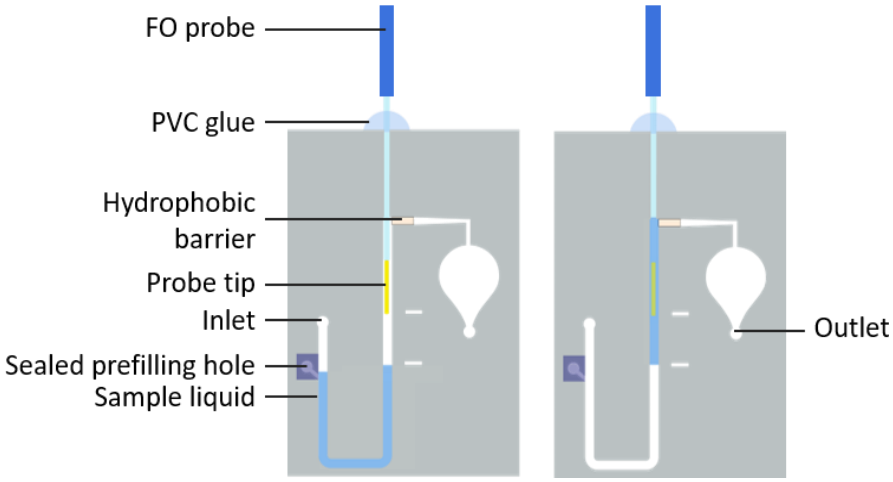


Figure 2. Schematic representation of the cartridge with inserted probe in both initiation (left) and termination (right) modes. Before initiation, the cartridge was prefilled with sample liquid (1:19 mixture of plasma spiked with ADM and PBS/T-AuNPs), after which the inlet was sealed with PSA. A syringe pump

propagated the sample liquid until it fully covered the probe tip, expelling air through the hydrophobic barrier, which allowed air to pass through the porous material and prevented the sample liquid to propagate too far and out of the cartridge through the outlet. The measurement started after the flow stopped, with the probe fully covered in the stationary sample liquid.

3.2 Mixing of sample with buffer and AuNPs on the cartridge

In order to realize sample dilution on the cartridge, we tested several different designs, before establishing a custom design for mixing sample and reagents at a 1:19 ratio. This was first optimized on a microfluidic cartridge connected with a syringe pump to drive the flow at 10 µL/min, followed by integration of the iSIMPLE pump in a separate layer on the cartridge to achieve fully autonomous mixing. With respect to the designs, we first created a custom 1:1 lateral mixing design based on literature (Cai et al., 2017; Chen et al., 2018; Lin, 2011), for which the mixing efficiency was tested colorimetrically by mixing dyed solutions (Figure S2A-G). Based on the comparisons among the different designs (Supplementary Information S2.1 and Figure S4, S5), the custom design combining expansion vortices in arrowhead-shaped chambers as well as Dean's vortices and low diffusion distance in the 300 µm wide curved channels (Figure S2G) enabled a successful homogeneous mixing across the channel width (i.e. lateral mixing). However, the Y-junction in this design was not feasible for a 1:19 dilution due to the high volume difference, and as such an alternative junction design (Figure S2H) was tested based on 'stretch and fold' droplet mixing. Although the mixing for this new design was sufficient along the length of the liquid plug (i.e. longitudinal mixing), the lateral mixing efficiency was proven insufficient (details in Supplementary Information S2.2 and Figure S6).

Eventually, both aforementioned mixing designs, suitable for lateral (Figure S2G) and longitudinal (Figure S2H) mixing, were combined in tandem to achieve homogeneous mixing of solution at 1:19 ratio (3D view in Figure 3A and schematic top view in Figure S2I). As illustrated in Figure 3, the blue and yellow dyed solutions were first prefilled in the liquid chamber at 1:19 prior to mixing (Figure 3A), after which an iSIMPLE pump was connected in a different cartridge layer. The pump was activated, flowing the blue and yellow solutions through the lateral mixing channel (Figure 3B) with visibly incomplete mixing, until the end of the longitudinal mixing channel (Figure 3C) with visually complete mixing. The 1:19 mixing efficiency was further validated and quantified by performing a fluorescence measurement with 1 µL fluorescein solution and 19 µL PBS with CV for the averaged fluorescence intensity of 3.4% and 1.9% for mixing on cartridge and manual mixing, respectively (detailed in Supplementary Information S2.2 and Figure S7). Considering both the colorimetric and fluorescent approach, it was evident that a complete mixing at 1:19 ratio was achieved by combining in a single cartridge two different mixing geometries responsible for longitudinal and lateral mixing, resulting in a homogeneous mixing on the cartridge comparable to manual mixing with a vortex. Our work thus demonstrated, for the first time, reagent mixing fully on the microfluidic cartridge and driven by the iSIMPLE pump, which can reduce and streamline the requisite sample pre-treatment prior to testing.

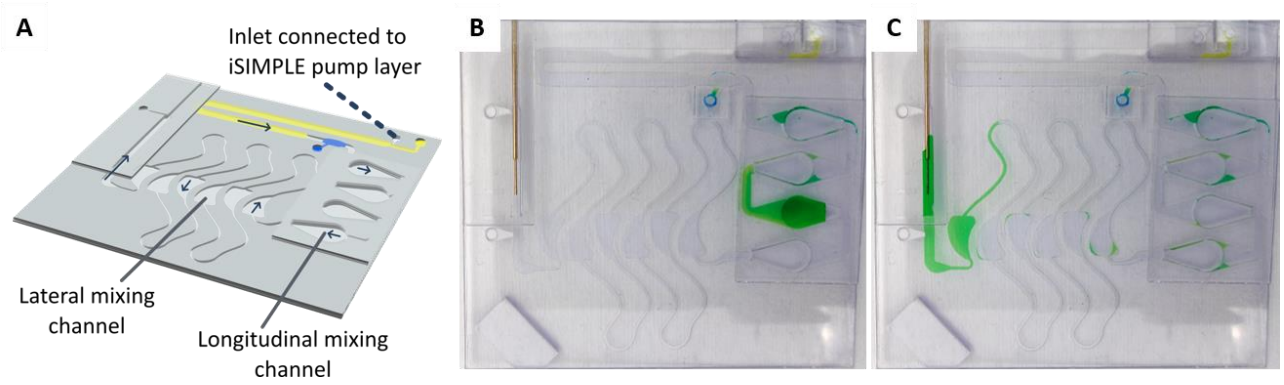


Figure 3. On-cartridge 1:19 mixing driven by the iSIMPLE pump. A) A schematic view of the prefilled cartridge with the longitudinal and lateral mixing designs in sequence. B-C) Photographs of mixing blue and yellow color dye, shortly after starting and towards the end of the mixing process, respectively. Whereas in B) the green color is heterogeneous, indicating mixing is not yet complete, in C) it is visually homogeneous.

3.3 Quantification of ADM in plasma samples using the ADM-sensor

Once robust mixing on the cartridge was established, we envisioned incorporation in a fully integrated cartridge comprising an FO probe, a mixing channel and an iSIMPLE pump for ADM measurement. In order to achieve completely self-powered actuation, the cartridge was designed with (I) a bottom layer, containing the iSIMPLE pump and the working liquid that is brought into contact with the pump, thus fueling the pump's pressure generation (Dal Dosso et al., 2019b), and (II) a top layer, containing the loading channel for sample and reagents, the mixing channel, and the chamber for the FO probe (Figure 4A). The measurement was performed as depicted in Figure 4B: (I) 1 µL of undiluted plasma sample, spiked with ADM, was loaded in the sample channel (highlighted with blue liquid), in front of the reagent channel (with yellow liquid) that was preloaded with 19 µL of PBS/T-AuNP solution; (II) The cartridge was connected to the FO-SPR readout system through the back end of the FO probe with the device connector. The iSIMPLE pump was activated with a single finger press, activating sample-reagent mixing on the cartridge. The sample-reagent mix was flowed to the FO probe with an average flow rate of 14.75 ± 2.09 µL/min (n=26) from start to finish. Notably, the iSIMPLE flow rate was in range of what was previously reported for iSIMPLE cartridges (Dal Dosso et al., 2019a, 2018c); (III) The measurement was initiated on the FO-SPR readout system, recording the reference signal before the sample-reagent mix got in contact with the FO probe. A recording of the complete process is depicted in Movie S1.

Eventually, the SPR slope between 30-120 s for each ADM concentration was calculated and used to generate a calibration curve (Figure 5A). The LOD and CV were determined to be 17.54 ng/mL and 9.3%, respectively, demonstrating that both aspects of the bioassay for the fully integrated ADM-sensor were in the same range as that of the White FOx 1.0 and the FO-SPR readout system when using manual mixing (described in Section 3.1). This can be translated to an LOD of 0.35 µg/mL and an LOQ of 0.93 µg/mL in undiluted plasma, which is comparable to the aforementioned Quantum Blue® and RIDA®QUICK tests (i.e. 0.8 µg/mL LOD and 0.32 µg/mL LOQ [LOD not available], respectively) (BÜHLMANN Laboratories AG, 2021; R-Biopharm AG, 2021). Finally, the established calibration curve of the ADM sensor was used to measure plasma samples of 4 ADM-treated patients. In parallel, the same patient samples were tested with the gold standard sandwich ELISA and the commercially available RIDA®QUICK LFA for comparison. Figure 5B-D shows the

correlation among the ADM-sensor and ELISA, ADM-sensor and LFA, and ELISA and LFA, respectively. The ADM-sensor and ELISA corresponded with a high correlation, as demonstrated by the high PCC, mediocre CCC, and the confidence intervals including slope '1' and intercept '0', indicating the interchangeability of the two methods. However, the agreement between ADM-sensor and LFA was insufficient, with only a high PCC. Furthermore, the two reference methods (i.e. ELISA and LFA) showed a good correlation, as illustrated by all three criteria. The regression slopes below 1 indicate that ADM concentration is underestimated using the ADM-sensor compared to the reference methods. It is hypothesized this is due to the different sample dilution factors (1:19 for ADM-sensor, 1:499 for LFA and 1:1999 for ELISA), which resulted in a different influence from the sample matrix. Further sample dilution in future research should be considered to alleviate these matrix effects. For the ADM-sensor, the average TTR was 710±88 s, i.e. approx. 12 min (n_s=26), which was lower than the existing commercially available alternatives (i.e. 15 and 20 min for Quantum Blue® and RIDA®QUICK tests, respectively). Furthermore, only 1 µL of plasma sample was used in the cartridge, enabling a much lower sample consumption than the existing alternatives (i.e. 10 µL serum, and 10 µL plasma/serum for the Quantum Blue® and RIDA®QUICK tests, respectively). A further comparison between the ADM-sensor and the commercial LFAs, including the detection ranges, cost and repeatability, has been summarized in Table S1. These results highlight the advantages of the in-house developed POC device when benchmarked against the commercial kits, and shine light on the future development of such devices by combining different technologies. The developed biosensor concept also has potential applications for POC TDM of other biological drugs, for instance IFX, which was tested using a bench-top FO-SPR platform in our previous work (Lu et al., 2017, 2016).

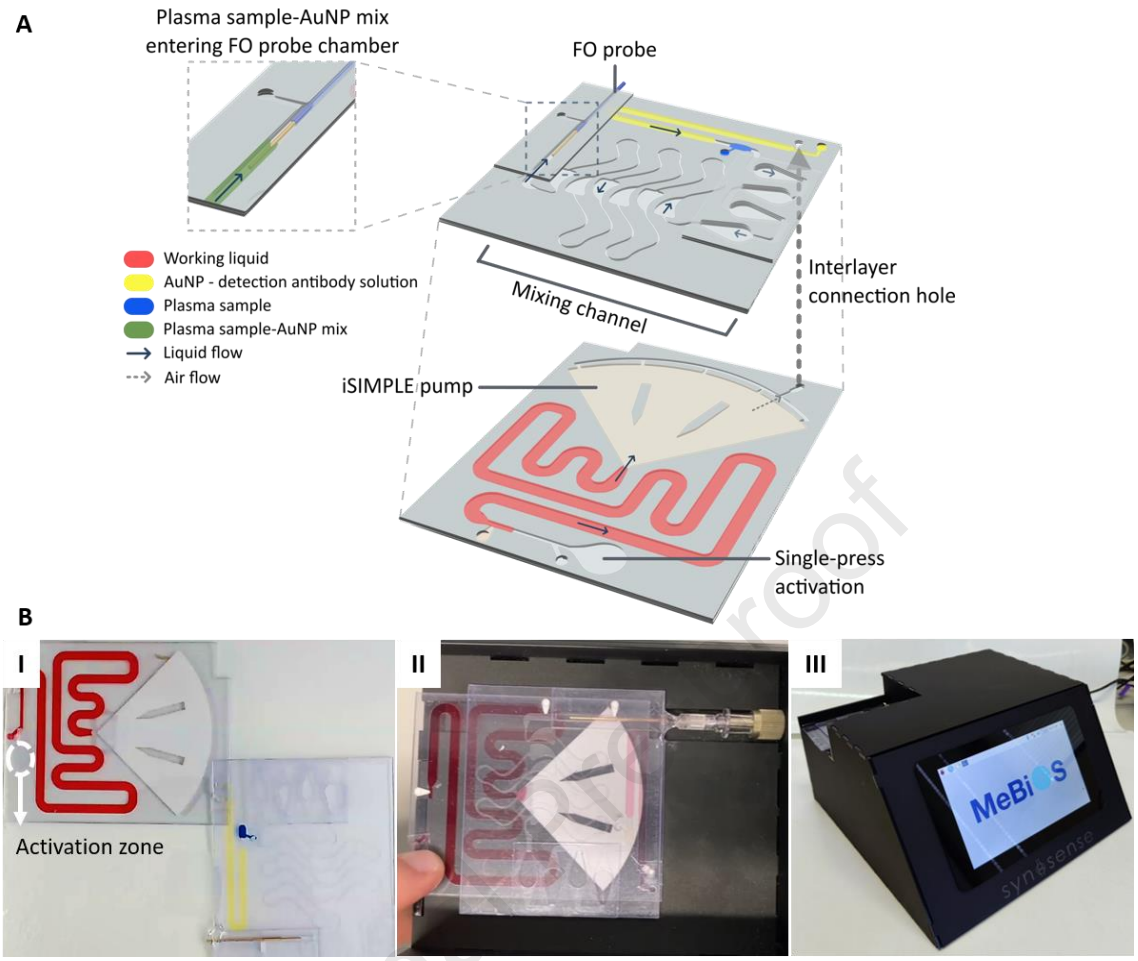


Figure 4. A) A 3D view of the cartridge design with the integrated FO probe and mixing channel in the top layer and the iSIMPLE pump in the bottom layer. Both layers were connected through the interlayer connection hole, where the air expelled from the iSIMPLE pump travelled to the top layer and pushed the PBS/T-AuNP solution and plasma sample through the mixing channel to the probe chamber. The pre-functionalized FO probe was integrated during cartridge assembly and sealed with glue. B) Steps of the measurement performed with the actual ADM-sensor (for illustration purposes, the cartridge in this figure was filled with colored liquids and the top and bottom layers have been placed next to each other). After cartridge assembly with the FO probe, I) the cartridge was prefilled with working liquid (red), 19 µL of PBS/T-AuNP solution (yellow), and 1 µL of plasma sample (blue) that was added just before use. II) Then, the cartridge was connected to the FO-SPR readout system and activated by a single finger press on the activation zone. III) Finally, the reader measurement was initiated right before the mixed solution arrived to the FO probe chamber.

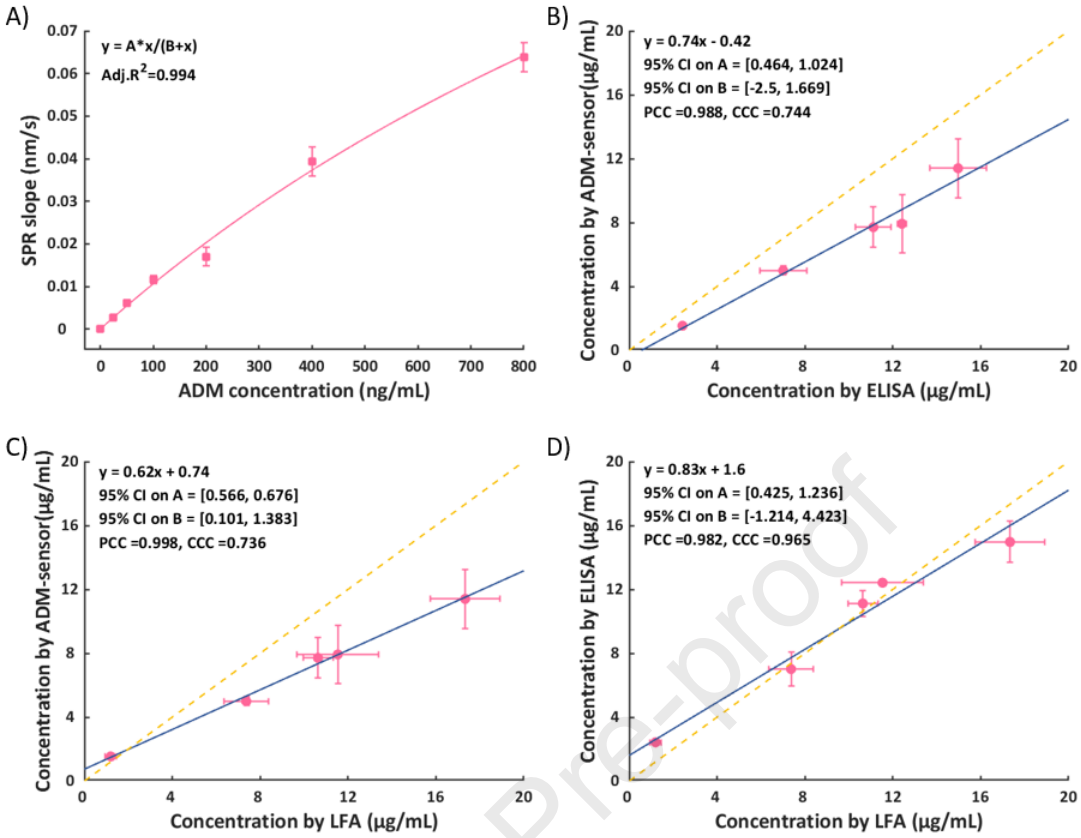


Figure 5. A) The calibration curve for quantification of ADM spiked in undiluted plasma and then further diluted 20-fold with PBS/T-AuNP solution using the one-step ADM bioassay on the ADM-sensor. The fitting parameters are as follows (A=0.23 nm/s, B=2064.76 ng/mL). The correlation plots of ADM concentrations (µg/mL) in patient plasma samples among: B) the ADM-sensor and ELISA, C) the ADM-sensor and LFA, and D) the LFA and ELISA. Error bars represent one standard deviation (n_s=3).

4. Conclusions

In this paper we have for the first time demonstrated the capacity of FO-SPR technology to be used as a POC device enabled by an in-house developed self-powered iSIMPLE microfluidic platform. To achieve this, we developed a fully integrated ADM-sensor, which combined (I) a FO sensing probe for implementing a rapid one-step FO-SPR sandwich bioassay, (II) a FO-SPR readout system and (III) a disposable iSIMPLE cartridge that can be activated by a single finger press to enable automatic sample-reagent mixing, thus simplifying sample pretreatment and reducing manual operations. This measurement enabled a 'plasma in – result out' POC test for TDM of ADM in 20-fold diluted plasma within 12 min. With only 1 µL plasma consumption, the sensitivity of the bioassay was competitive with the commercially available ADM POC tests. Moreover, we successfully applied the ADM-sensor for testing patient plasma samples, which was benchmarked against our in-house developed ELISA and a commercially available LFA. A good correlation was found between the ADM-sensor and the ELISA and LFA, however the observed underestimation of ADM concentrations by our sensor has to be further investigated. It is hypothesized that this underestimation was caused by the difference in dilution factor, and hence, the impact of the sample matrix on the measurements. Overall, this study has not only demonstrated the novel concept of mixing on the iSIMPLE cartridge, but also proved the feasibility of combining it with FO-SPR biosensing technology for POC TDM. Although ADM was selected for its relevance in TDM, the developed device can be used for TDM at the POC

of other biological drugs as well.

Conflicts of interests

Professor Jeroen Lammertyn is a board member of FOx Biosystems, a spin-off company of KU Leuven commercializing FO-SPR platforms, next to the principal investigator of the Biosensors group.

CRediT authorship contribution statement

Jia-Huan Qu: Conceptualization, Methodology, Validation, Investigation, Writing – Original draft
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Charlotte Van Tricht: Conceptualization, Methodology, Investigation, Writing – Review & editing
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Nick Geukens: Resources, Writing – Review & editing
Debby Thomas: Resources, Writing – Review & editing
Francesco Dal Dosso: Conceptualization, Methodology, Validation, Supervision, Writing – Review & editing
Saba Safdar: Conceptualization, Methodology, Validation, Supervision, Writing – Review & editing
Dragana Spasic: Methodology, Writing – Review & editing, Supervision, Funding acquisition
Jeroen Lammertyn: Conceptualization, Methodology, Writing – Review & editing, Funding acquisition

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Appendix A. Supplementary data

The supplementary information file contains the elaborate protocols about quantification of ADM by the in-house developed ELISA and RIDA®QUICK LFA, a detailed description about the design of the microfluidic cartridge driven by syringe pump, all the different designs of microfluidic cartridges for optimization of reagent mixing, as well as the intermediate results for 1:1 mixing and 1:19 longitudinal mixing on the cartridge, evaluated by colorimetric and fluorescence measurement, respectively.

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- A FO-SPR biosensor was combined with self-powered iSIMPLE technology
- The FO-SPR one-step sandwich bioassay was established for rapid adalimumab detection
- Reagent mixing on the microfluidic cartridge was realized by an optimized mixing design
- Plasma sample dilution was integrated on self-powered iSIMPLE microfluidic cartridge
- The ADM-sensor delivered measurement using only 1 µL of plasma within 12 min

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Jeroen Lammertyn reports a relationship with FOx BIOSYSTEMS that includes: board membership.
Jeroen Lammertyn has patent #US20180345288 issued to Katholieke Universiteit Leuven (Leuven, BE).
Jeroen Lammertyn has patent #WO/2019/025630 pending to Katholieke Universiteit Leuven (Leuven, BE).