An aptamer-based SPR-polarization platform for high sensitive OTA detection

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ABSTRACT

Conventional methods used for the determination of mycotoxins are sensitive and give both qualitative and quantitative information, although they are greatly restricted by long assay time, high cost and limited portability. As a consequence, more rapid, low cost, highly specific and portable methods for detecting these analytes are the focus of a great deal of research. In this perspective, this work describes a label free, simple and reliable method using a specific sequence of ssDNA aptamer for detecting OTA, a toxic fungal metabolite frequently occurring in a variety of foodstuffs and feeds. A piezoelectric (QCM) based biosensor was used for real time monitoring of four ssDNA aptamers-OTA interactions to select the most efficient one. Based on these results, a lab-made plasmonic sensing platform based on sinusoidal gratings was fabricated and functionalized with the most efficient selected aptamer. The sensitivity of the biosensor was found to be dependent on the aptamer immobilization strategy. In the optimized experimental conditions the biosensor was demonstrated to detect down to 0.2 ng/ml of OTA with a LOD of 0.005 ng/ml. These findings sounds very promising to produce high sensitivity, fast and potentially portable biosensors for the detection of OTA in food commodities.

Keywords: *ochratoxin A; aptamers; quartz crystal microbalance; surface plasmon resonance; self-assembled monolayers*

Abbreviations

ochratoxin A (OTA), Quartz Crystal Microbalance (QCM), limit of detection (LOD), surface plasmon resonance (SPR), self-assembled monolayers (SAMs), polydimethylsiloxane (PDMS), Scanning Electron Microscopy (SEM), mercaptoundecanoic acid (MUA), N-hydroxysuccimide (NHS), N-(3-Dimethylaminopropyl)-N-ethylcarbodiimide hydro-chloride (EDC), 2-(2-pyridinyldithio)ethaneamine (PDEA), β-mercaptoethanol (BME)

1. Introduction

Ochratoxin A (OTA) - L-phenylalanine-N-[(5-chloro-3,4-dihydro-8-hydroxy-3-methyl-1-oxo-1H-2-benzopyrane-7-yl)carbonyl]-(R)-isocoumarin) is a mycotoxin produced by secondary metabolism of

many filamentous species belonging to the *Aspergillus* and *Penicillium* genera [1]. OTA is frequently found in a variety of foodstuffs, including wheat, maize, coffee, spices, beer, grape and derived products, products of animal origin and feeds [2-4]. OTA is a potent nephrotoxin that also displays hepatotoxic, teratogenic, immunosuppressive and carcinogenic effects, and it has been classified as a possible human carcinogen (class 2B) by the International Agency for Research on Cancer (IARC) [5]. Because of its toxic effects, the European Commission has set maximum permitted levels for OTA in the range 0.5-20 μg/kg depending on the food matrix [6].

Conventional methods commonly used for OTA identification and detection comprise high-pressure liquid chromatography (HPLC), coupled with fluorescence or mass spectrometry detectors and ELISA assays [7-10]. While these methods can be sensitive and give both qualitative and quantitative information about the presence of OTA, they are greatly restricted by assay time and their high cost. In addition, they do not allow analysis of mycotoxins in real time and are not portable. Fast acquisition and portability of biosensors would enable rapid detection outside laboratories [11, 12] and may improve food safety determining the presence of mycotoxins during the process of food and feed preparation [13]. As a consequence, the need for a more rapid, reliable, at low cost, specific and sensitive method for detecting these analytes joint with the development of a new technology for producing portable sensors, is the focus of a great deal of research. These methods are mainly based on the interaction between a recognition element and its target inducing a mechanism of molecular recognition. This interaction is then translated into a measurable signal by using a suitable transducer [14].

Surface plasmon resonance (SPR) is a modern analytical technique based on the changes in the refractive index of dielectric material at the metal-dielectric interface [16-17]. The wide diffusion of SPR sensing technology is due to its great advantages in term of rapidity, label-free and real time sensing and sensitivity. Recently a lot of portable SPR platforms were used for the detection of ricin [15], enterotoxin B [16], 2,4-dichlorophenoxyacetic acid [17] and atrazine [18] and over the past 10 years, great efforts have been made also in the development of SPR biosensors for rapid detection of several mycotoxins, including OTA, in both food and feed [19, 20]. In particular SPR biosensors have been developed for the determination of OTA in wheat, maize, oat, wine, fruit juice and milk with limits of detection ranging from 0.01 ng/ml to 0.5 ng/ml. Although many of these methods have been shown to be sensitive, accurate, easy to use and applicable to the monitoring of OTA in several matrices, there is still a lack of portable devices that could be used in the field, therefore there is a growing interest in the development of miniaturised devices.

In developing innovative label-free highly sensitive and selective OTA detection strategies, the stability of the capture probe layer immobilized on the biosensor surface is of crucial importance. The most used probes for OTA were monoclonal antibodies, even though the use of alternative probes like molecularly imprinted polypyrrole and polymerised pyrrole have also been reported [19]. Aptamers, which are synthetic, single-stranded DNA or RNA oligonucleotides, represent probes of great appealing in this respect. They can fold into secondary and tertiary structures that have the potential to bind certain targets with equal or higher affinity and specificity than their equivalent antibodies [21]. The *in-vitro* selection process is called systematic evolution of ligands by exponential enrichment (SELEX) [22, 23]. The aptamers can also be modified with a variety of fluorescent dyes or other tags, providing an extraordinary flexibility in assay development [24, 25]. In comparison to antibodies, aptamers are really stable and their production is easier and cost-effective. The first OTA-binding ssDNA aptamer was reported by Cruz-Aguado and Penner [26] and afterwards other two

research groups have selected other ssDNA aptamers that bind to OTA and display different sequence, structure and affinity toward it [27-29]. These OTA aptamers have been used in a large number of aptamer assays mainly based on electrochemical, fluorescence, chemiluminescence and colorimetric biosensors and at lesser extent on SPR biosensors [29-31] . It is well known that OTA is a small molecule and that small molecules are seldom detected by conventional SPR technique directly because the changes in the refractive index resulting from the binding processes of small biomolecules are often small. This could be the reason why very few reports have appeared on developing SPR biosensors based on aptamers for OTA detection [29, 31].

Aptamers can be easily labeled with thiol groups during their synthetic processes, which enable the direct immobilization of the aptamer on the surface of SPR gold film by greatly simplifying the procedure of the experiments.

In this perspective, here is reported the use of aptamers for OTA detection and the development of a plasmonic aptamer-based biosensor. Four thiolated ssDNA aptamers with different tails were screened by a commercial Quartz Crystal Microbalance (QCM) apparatus to select the more efficient one in the binding with OTA and optimize the working conditions to be used for the SPR analysis. The SPR system adopted was a custom equipment based on the azimuthally-controlled SPR under phase interrogation [32-34] leading to a refractive index sensitivity up to one order of magnitude higher than the classic grating-based SPR setup [32, 35] and to a sensing prototype promising for further miniaturization to make a portable device [34].

2. Materials and methods

2.1. Chemicals

Ochratoxin A (OTA) standard solution was supplied by Romer Labs (Tulln, Austria). Absolute ethanol was purchased from Carlo Erba (Italy). Mercaptoundecanoic acid (MUA), N-(3-Dimethylaminopropyl)-N-ethylcarbodiimide hydro-chloride (EDC), sodium chloride (NaCl), potassium chloride (KCl), calcium chloride ($CaCl_2$), β-mercaptoethanol (BME), monopotassium phosphate (KH_2PO_4), sodium dihydrogen phosphate (NaH_2PO_4), sodium phosphate dibasic (Na_2HPO_4), phosphate buffer, boric acid, cysteine, formic acid, *N*-hydroxysuccimide (98%) (NHS) and hydrogen peroxide (H_2O_2, 30%) were purchased from Sigma-Aldrich (Milan, Italy). *Tris*(hydroxymethyl)methylamine (TRIS) was purchased from AnalaR (England). Ammonium hydroxide (28-30%) was purchased from Baker (Holland). Sodium hydroxide (NaOH) was purchased from VWR International (West Chester PA). 2-(2-pyridinyldithio)ethaneamine (PDEA) was purchased from GE Healthcare (Italy). Ultrapure water with a resistivity of 18.2 MΩ was used (Purelab). The composition of the binding buffer was 10 mM TRIS,120 mM NaCl, 5 mM KCl, 20 mM $CaCl_2$, pH 8.5 [26].

SPR sensing substrates were fabricated by combining laser interference lithography (LIL) and soft lithography techniques at TASC-IOM-CNR laboratories (Basovizza, TS, Italy). S1805 photoresist was purchased from Microposit (Shipley European Limited, UK), while MF319 Developer and propylene glycol monomethyl ether acetate (PGMEA) were purchased from MicroChem Corp (Newton, MA, USA). Polydimethylsiloxane (PDMS) (Sylgard 184) was purchased from Dow-Corning Corp. (Midland, MI, USA), whereas the Norland Optical Adhesive (NOA 74) was purchased from Norland Products Inc. (Las Vegas, NV, USA).

2.2. DNA aptamers

The four ssDNA modified aptamers used in the present study were based on the OTA aptamer reported by Cruz-Aguado and Penner (2008) [26] and are shown in Table 1. Aptamers were modified with a thiol group at their 5' or 3' end and contained a carbon linker (C_3 or C_6) or a thymidine spacer (T_{15}). Aptamer solutions were prepared in KH_2PO_4 buffer at concentration of 1μM.

Table 1. *Oligonucleotide sequences of aptamers used.*

Aptamer sequences	
# 1	5'- SH-C_6-GATCGGGTGTGGGTGGCGTAAAGGGAGCATCGGACA - 3'
# 2	5'- SH-T_{15}-GATCGGGTGTGGGTGGCGTAAAGGGAGCATCGGACA - 3'
# 3	5'- GATCGGGTGTGGGTGGCGTAAAGGGAGCATCGGACA-C_3-HS - 3'
# 4	5'- GATCGGGTGTGGGTGGCGTAAAGGGAGCATCGGACA-T_{15}-HS - 3'

2.3 QCM apparatus

2.3.1. Functionalization of the QCM sensor surface and QCM measurements

The thiol-gold interaction was used to bind aptamers covalently onto sensor surfaces (Figure 1). After cleaning, the QCM gold chips were incubated into a 1 μM solution of each aptamer in KH_2PO_4buffer (1M, pH 7.4) in the dark overnight at 4°C and then washed in ultrapure water in order to remove weakly adsorbed molecules. The long incubation in controlled environment is expected to favor the formation of compact self-assembled monolayers (SAMs).

QCM measurements were performed using the Q-Sense E1 system (Q-Sense, Sweden). The QCM with dissipation monitoring technique was described elsewhere [36-39]. The sensor crystals used were 5MHz, 14mm diameter, AT-cut quartz discs with an evaporated gold surface (Q-Sense). The resonance frequency was measured at the fundamental frequency of the crystal (1^{st} harmonic at 5MHz) and six harmonics of the fundamental frequency (third, fifth, seventh, ninth, eleventh and thirteenth harmonic at 15 MHz, 25MHz, 35MHz, 45MHz, 55MHz, 65MHz, respectively). Measurements at natural frequency (5MHz) were not considered since the fundamental resonance is very sensitive to bulk solution changes and generates un-reliable data. For simplicity, only changes in the mass increase of the ninth overtone (45MHz) were presented in the reported graphs. Mass data were calculated applying the Sauerbrey equation since small dissipation changes ($\sim 10^{-6}$) observed (data not shown) indicated that the viscoelastic properties of the film formed at the sensor-liquid interface did not affect too much the frequency signal [40, 41]. The QCM data were analyzed by Origin8 software (OriginLab Corporation, Northampton, MA), Q-Soft and Q-Tools (Q-Sense). For each experiment, QCM measurements were repeated at least twice. Data are reported as an average over three overtones; the reported errors are calculated as standard deviation. Before each experiment, the QCM sensors were cleaned as reported in [42].

2.3.2 Aptamer screening via QCM

The QCM sensor chips functionalized with the four aptamers were screened for their ability to bind OTA and to select the most promising one to be used for SPR analyses. Each QCM measurement consisted of conditioning of the sensor surface with a phosphate buffer solution at 100 μl/m for 30

min to have a reliable baseline before changing the buffer (step A). Then, the sensor surface was incubated for 20 min in a 100 mM β-mercaptoethanol solution to block un-reacted gold sites and to avoid non-specific interactions (step B). This step was followed by washing with phosphate buffer for 10 min at 100µl/m to remove the molecules weakly adsorbed (step C) and by incubation with binding buffer for 30 min (step D). Successively, the sensor was incubated with OTA solution at a concentration of 40 ng/ml for 90 min (step E) followed by a final phosphate buffer washing for 30 min at 100µl/m (step F). A control run was also performed by repeating the same protocol from step A-D and then washing with phosphate buffer washing for 30 min at 100µl/m (step F).

2.4 SPR platform

2.4.1 Plasmonic sensor fabrication and characterization

S1805 (MICROPOSITTM):PGMEA (Microresist Technology) solution (2:3) was spun onto a silicon substrate with a spin speed of 4000 rpm for 30 s and the sample was nanopatterned using laser interference lithography in Lloyd's configuration. A 50 mW Helium Cadmium (He-Cd) laser emitting a TEM00 single mode at 325 nm light source with a beam incidence angle of 19° and an exposure dose of 67,7 mJ/cm^2 were adopted. Photoresist development was performed upon immersion in MF321 for 6 s and rinsing in milliQ water. A PDMS mold was realized curing a PDMS layer dropped onto the resist grating at 50° C for 4 h. The nano-pattern was imprinted onto a thiolene resin film (NOA 74) supported onto a previously cleaned (plasma oxygen, 90W for 2 min) microscope glass slide, illuminating the PDMS mold with ultra-violet (UV) light for 40 s (Hg 150 W lamp; Karl Lanz Reinraumtechnik E. K., Germany). Finally a gold (40 nm) layer was evaporated above the patterned resin and a chromium (5 nm) layer was used to improve gold adhesion onto the glass substrate. The SPR sample quality was evaluated by Scanning Electron Microscopy (SEM - SUPRA^{TM} 40 SEM facility, Carl Zeiss, Germany). MilliQ water static contact angle measurements were performed onto the nanostructured surface before and after sensing layer preparation in order to have a qualitative evaluation of the functionalization process achievement using a FM4200 serie (Krüss GmbH, Germany) contact angle measuring device. The sensing substrates were used immediately after gold deposition.

2.4.2 Phase-interrogation SPR measurements

A bench phase-SPR detection setup developed in our lab was adopted and the detailed description and working principle is reported elsewhere [33, 34]. The most efficient aptamer selected via QCM (i.e. aptamer #1) was used for SPR measurements for OTA detection in solution. Two different immobilization strategies of the aptamer onto SPR sensors surface, i.e. direct or in presence of an aliphatic spacer (named SAMs and mixed SAMs, respectively, Figure 1), were tested by SPR to check if the interaction of the aptamer probe #1 with the surface may influence the detection process. The direct immobilization consists of incubating the SPR sensor in an aptamer 1# solution as described in section 2.3.1 (Figure 1).

The mixed SAM immobilization procedure (occurring in presence of an aliphatic spacer) consisting of a MUA SAM deposition, its activation, coupling and blocking of residual reactive sites was carried out as described by Ayela et al. [43] (Figure 1). Briefly, a 10 mM ethanol solution of MUA was prepared. After cleaning, the SPR gratings were immersed in the mixture overnight, in the dark at room temperature. The modified gold surfaces were washed twice with absolute ethanol with gentle

stirring to remove the non-absorbed thiol compounds and washed twice with ultrapure water. Then the surface was incubated for 3 h into a freshly prepared aqueous mixture of 15 mM NHS and 75 mM EDC for activation of carboxyl groups of the SAM and then washed twice in borate buffer. Activated carboxyl groups were derivatized with 80 mM PDEA in a borate buffer (100 mM, pH 8.5) for 25 min at 4°C followed by washing twice in KH_2PO_4 buffer (1 M, pH 7.4). A 1 μM solution of each aptamer dissolved in KH_2PO_4 buffer was incubated in the dark on the functionalized gold surface overnight at 4°C and then washed with ultrapure water in order to remove weakly adsorbed molecules.

2.5 SPR protocols for OTA detection in solution

SPR sensors were incubated in a phosphate buffer solution for 30 min. Then the sensor surface was incubated for 20 min in a 100 mM BME solution to block un-reacted gold sites and to avoid non-specific interactions. This step was followed by washing with phosphate buffer for 10 min and with binding buffer for 30 min to rinse the surface and remove the molecules weakly adsorbed. Then, sensors were incubated with OTA solution (0.2, 0.4, 4, or 40 ng OTA/ml) for 90 min. Finally, sensors were washed with phosphate buffer for 30 min.

3 Results and discussion

3.1. DNA-aptamer screening by QCM measurements

QCM is a simple and fast tool to monitor in *real time* at nanoscale level interactions between molecules in solutions and functionalized surfaces [44]. Therefore it represents a useful apparatus to set working conditions to develop *lab-made* biosensors. In this work, QCM was used to perform a screening of modified OTA aptamers to find the most efficient one in the binding with OTA and then to be integrated in the custom bench portable SPR setup.

In the Figure 2 the mass shifts involved during aptamers/OTA interactions are shown. Being, the measurement in the initial A-C steps, affected by physisorbed molecules coming from the *ex-situ* functionalization, only steps C-F (reported in the Figure 2) have been considered for a comparison between the aptamers OTA bonding capabilities. The four different aptamers reported in Table 1 have been deposited on sensor surface by using the SAM functionalization approach and mass increases of 288 ± 74 ng/cm^2 for aptamer #1, 46 ± 19 ng/cm^2 for aptamer #2, 29 ± 8 ng/cm^2 for aptamer #3 and 46 ± 20 ng/cm^2 for aptamer #4 have been calculated after OTA incubation. These mass increases were measured subtracting the mass at the end of phosphate buffer flow before binding buffer injection and the mass at the end of final phosphate buffer washing (see red arrows in Figure figure2). From these data it was evident that the aptamer that is more efficient in the binding with OTA is aptamer #1.

However, from a quantitative point of view, the measured mass does not correspond to real amount of OTA molecules bonded to the surface; this value is, indeed, heavily affected by the mass of Ca^{2+} ions of the buffer chelated to the aptamers [26, 45, 46] as well as by the use of solutions with different viscosities.

With this aim, a control run was performed. The sensor surface was directly functionalized with aptamer #1 (Table 1) and the QCM curve was registered in real time without OTA (as described in 2.3.2). From steps C-F (data not shown) it was estimated a Ca^{2+} ions mass increase of 193 ng/cm^2 which corresponds to a density of 2.9x10^{15} Ca^{2+} ions/cm^2.

Therefore, considering that a part of the mass from Figure 2 in the case of aptamer 1# is due to Ca^{2+} adsorbed (193 ng/cm^2), it is possible to evaluate the number of OTA molecules (1.42 x 10^{14} molecules/cm^2) that bind the surface after incubation for 30 min into a 40 ng/ml solution. Roughly estimating that each OTA molecule may occupy a maximum area of 0.5 nm^2 (only from structural considerations, given that we don't have any information about the orientation of the molecule on the surface) and the sensor area is of about 1 cm^2, 2 x 10^{14} is the number of OTA molecules that can be linked to the sensor surface. In our working conditions, this could be approximately close to the full coverage of the sensor surface, while in the case of the other aptamers a negligible amount of OTA and Ca^{2+} ions are bound. This sounds reasonable, given the values of dissociation constant of the unmodified aptamer #1 reported in a previous work [26].

3.2. OTA detection by phase-SPR

3.2.1. SPR custom-made platform

The nanofabrication procedure developed allowed to obtain SPR substrates with high throughput and using standard micro- and nano-fabrication techniques. Indeed up to 40 replicas have been produced using the same PDMS mold. In addition, the strategy adopted allowed to easily tune the substrate geometry as the total number of SPR sensing areas onto the same substrate could be chosen by the user, according to the final sensor application. Our final plasmonic substrate consisted into a glass slide (3,5 x 2,5 cm) onto which four resin nano-patterns were imprinted so that a single chip could host up to four different experiments (Figure 3a and b). Two plasmonic substrates (four gratings each) were prepared by using both immobilization approaches previously described (Figure 1 par. 2.4.2). Grating geometrical parameters were assessed by Scanning Electron Microscopy (SEM). A pitch of 500 nm and an amplitude of 40 nm were obtained (Figure 3c). The nanostructure geometry was decided through theoretical simulations of Surface Plasmon Polaritons (SPPs) coupling on metallic gratings based on Chandezon's method [47, 48] with the aim of obtaining the optimal SPR response in terms of surface reflectivity, SPP resonance angle and sensitivity [49].

3.2.2. Phase-SPR OTA sensing

The sensing layers formed by aptamer #1 were prepared onto all the nanostructured sensing surfaces and the layer formations were qualitatively evaluated with water contact angle measurements (Figure 4a). Considering the long incubation in controlled environment, the direct immobilization is expected to form a compact SAM to the sensor surface whereas the spacer addition approach formed mixed SAMs on it (Figure 1).

After both aptamer and mixed aptamer SAMs deposition a surface hydrophobicity decrease was observed as both sensing layers expose polar functional groups. The water contact angle measurement was used as qualitative evaluation for the sensing layer successful formation monitoring, mainly in the first experimental tests, when the surface functionalization protocol was optimized. After sensing layer formation the experiments were performed by incubating each sensing area with different OTA concentrations (from 0.2 to 40 ng/ml) and then by rinsing the sensing areas with water. Phase-SPR measurements were performed after the sensing layer formation, OTA incubation and sensor rinsing. In Figure 4b all the results obtained with both SAMs and mixed SAMs functionalisation are shown as a function of concentration.

Three concentration regimes could be identified in our results: a high concentration regime (40 ng/ml), a medium concentration regime (4 and 0.4 ng/ml) and a low concentration regime (0.2 ng/ml). A drop in phase shift intensity (data not shown) was detected for all the concentration regimes after sample rinsing due to the removal of non-specific adsorptions. Upon 40 ng/ml OTA incubation the non-specific adsorption on the SAMs functionalization resulted higher than that of the mixed SAM (0.88±0.02° and 0.21±0.02°, respectively). In the medium concentration regime an average non-specific response of 0.11° was found for both sensing layer morphologies, while in the low concentration regime average responses of 0.05° and 0.09° were obtained for the aptamer and the mixed layer respectively. The evaluation of non-specific contribution to the final sensor response gives information about the sensing specificity of the adopted strategies. Our experiments showed that in the high concentration regime, i. e. for a large amount of available analyte molecules, the mixed SAM resulted more specific with respect to the SAMs layer, while in the low concentration regime the best specificity is given by the SAMs layer, thanks to the larger number of OTA binding sites grafted onto the sensor surface. In the medium concentration regime no differences were obtained between the two sensing surface morphology in term of protection from non-specific adsorptions. The limit of detection (LOD) was calculated from the slope of the concentration curve linear portion and the standard deviation of multiple measurements on the blank sample (n=10) [29, 50]. A LOD of 0.005 and 0.02 ng/ml was obtained for the SAMs and mixed SAMs with the sensor linearity of 81.7 and 92.5% respectively. SPR sensor sensitivities were evaluated as a ratio between the amount of molecules detected in term of ng/ml and the lowest phase shift detected; values of 2.5 and 4 (ng/ml)/° where obtained for the SAMs and mixed SAMs, respectively. Results showed that SAMs layer sensitivity was considerably higher than the mixed ones, probably due to the difference in the resulting sensing layer thickness [29] and to the available number of aptamer probes, which results significantly higher when a SAM is immobilized onto the surface.

The SPR-polarization platform with sensor functionalized with aptamer 1# using the SAMs strategy, tested in a detection range from 0.2 ng/ml to 40 ng/ml, exhibited a LOD of 0.005 ng/ml (corresponding to 0.01 nM). The developed platform can be applied to the detection of OTA in food matrices at levels in the range of the EU limits, nevertheless a purification/concentration of the extract is required before toxin detection. As demonstrated by data shown in Table 2, there are several methods in literature which are less sensitive. Few of them are label-free, but those that appear more sensitive use additional reagents to increase the signal/noise ratio and this means higher fabrication costs. The employment of gold-nanoparticles or graphene oxides is the most common method to decrease the LODs and improve sensor performances. However, for food safety purpose this is not meaningful, given that the law reference values are well higher than the limits that this system is able to detect [6]. Therefore, considering the scenario summarized in Table 2 and the results obtained, we believe that the system here proposed shows a high potential as portable label-free OTA sensor.

Table 2. *Summary table of aptamer based biosensors for the detection of OTA.*

Detection method	Enhancers	LOD	Reference
Colorimetric	-	20 nM	[51]
Evanescent all wave fibersensor	-	3 nM	[52]
Fluorescence		1 ng/ml	[53]
LSPR	-	1nM	[31]
SPR	Antibody-gold nanoparticle conjugates	60 pg/ml	[54]

Luminescence	Gold nanoparticles	0.007 ng/ml	[55]
Chemiluminescence	Upconversion nanoparticles	0.1 pg/ml	[56]
Electrochemical impedance	Graphene oxide, gold nanoparticles	0.74 pM	[57]
Electrochemical redox current	Gold nanoparticles	0.75 pM	[58]
Electrochemiluminescence	Loop-mediated isothermal amplification	10 fM	[59]

4. Conclusion

An SPR aptamer-based sensing platform for OTA detection was developed after performing an initial screening using a commercial QCM apparatus. The phase-interrogation SPR, based on a lab-made bench set up, was found to be a sensitive technique with potential use as portable device. The sensing architecture proposed allowed to detect down to OTA 0.2 ng/ml with a LOD of 0.005 ng/ml, comparable to state-of-the-art OTA SPR sensors. The employment of a high sensitivity phase-interrogation SPR setup demonstrated advantages in term of possible further scalability and compactness increase, as all the optical components could be further integrated and miniaturized in the future, and also in term of sensing platform versatility. Indeed custom nanostructured plasmonic surfaces can be fabricated through easy and high throughput lithographic techniques tuning the final sensor design depending on the final application and requirements.

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Figure 1

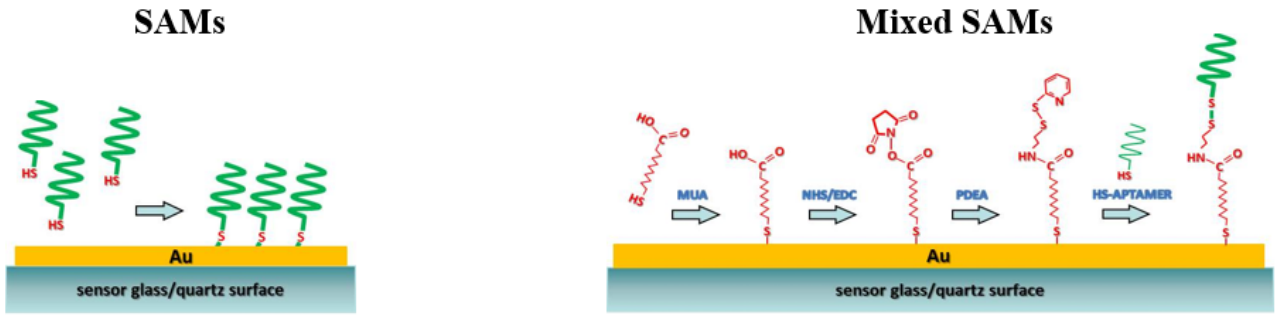


Figure 2

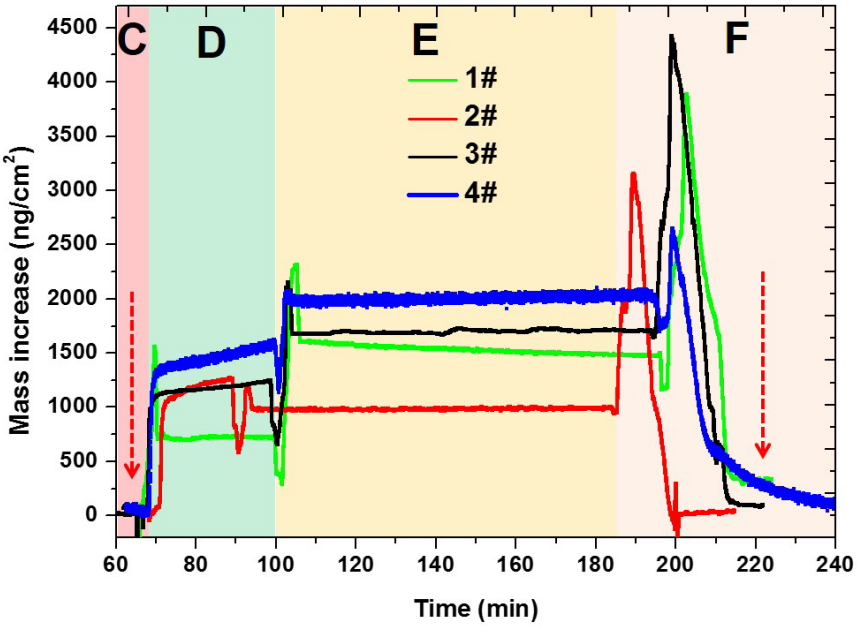
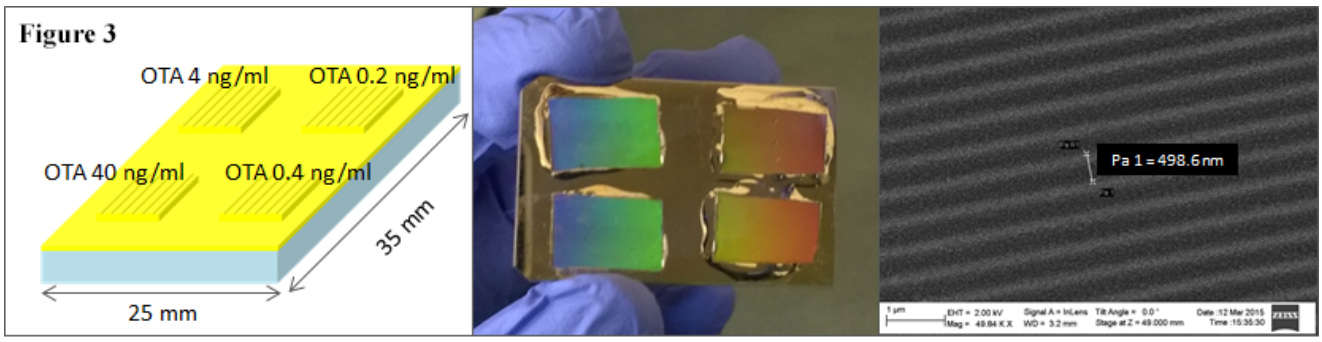
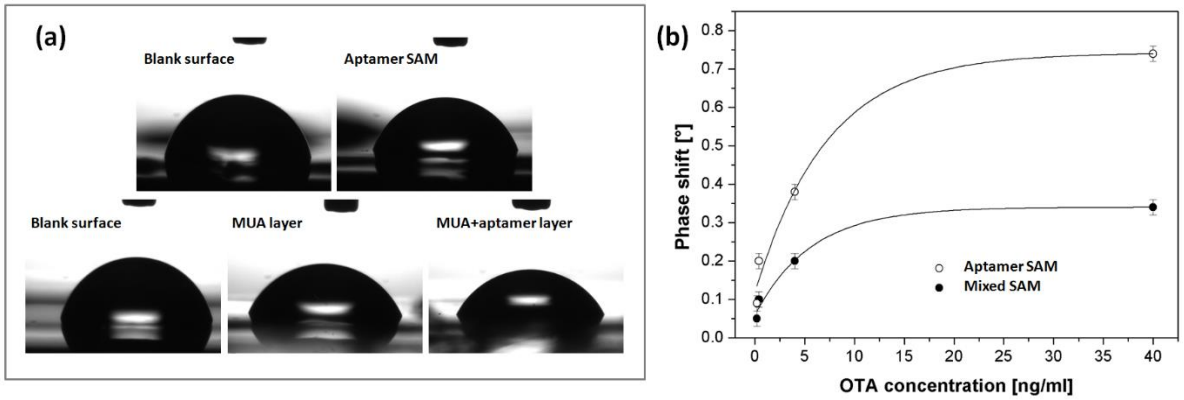


Figure 3



(a) (b) (c)

Figure 4


(a)
Blank surface
Aptamer SAM
Blank surface
MUA layer
MUA+aptamer layer
(b)
Phase shift [°]
OTA concentration [ng/ml]
Aptamer SAM
Mixed SAM
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0
5
10
15
20
25
30
35
40